आज़ादी का अमृत महोत्सव

वार्षिक प्रतिवेदन 2021-22
ANNUAL REPORT 2021-22

सी डी एफ डी
... नवीन शोध प्रक्रियाएं जनहित में

CDFD
... Innovating to benefit society

डीएनए फिंगरप्रिंटिंग एवं निदान केन्द्र
(जैव प्रद्योगिकी विभाग, विज्ञानं एवं प्रद्योगिकी भारत सरकार का स्वायत्त संस्थान)

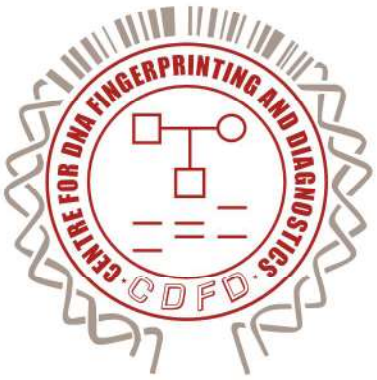
Centre for DNA Fingerprinting and Diagnostics
(An autonomous institute of the Dept. of Biotechnology, Ministry of Science and Technology, Govt. of India)

www.cdfd.org.in

सी डी एफ डी
CDFD

वार्षिक प्रतिवेदन
अप्रैल 2021 से मार्च 2022

ANNUAL REPORT
April 2021 to March 2022



डीएनए फिंगरप्रिंटिंग एवं निदान केन्द्र
उप्पल, हैदराबाद - 500 039

Centre for DNA Fingerprinting and Diagnostics
Uppal, Hyderabad - 500 039

Content

अधिदेश
Mandate

अधिदेश

सीडीएफडी सोसाइटी के समझौता ज्ञापन तथा नियम एवं विनियमों में बताए गए अनुसार डीएनए फिंगरप्रिंटिंग एवं निदान केंद्र (सीडीएफडी) की स्थापना जिन उद्देश्यों के लिए हुई वे निम्न प्रकार हैं :

I. पितृत्व विवाद, आप्रवास और अस्पतालों में नवजात शिशुओं की अदला-बदली जैसे मामलों में निजी पक्षों सहित विविध अभिकरणों के लिए पर्याप्त अदायगी पर डीएनए प्रोफाइलिंग और उससे संबंधित विश्लेषण का वैज्ञानिक अनुसंधान करना;

II. अपराध अन्वेषण अभिकरणों को डीएनए फिंगरप्रिंटिंग और उससे संबंधित विश्लेषण तथा सुविधाएं प्रदान करना;

III. अपराध अन्वेषण और परिवार मामलों में डीएनए प्रोफाइल विश्लेषण और उससे संबंधित तकनीकों के साक्ष्य संबंधी मूल्य को समझने में पुलिस कर्मियों, न्यायिक वैज्ञानिकों, वकीलों तथा न्यायपालिका की सहायता करना;

IV. आनुवंशिक अव्यवस्थाओं को संसूचित करने हेतु डीएनए नैदानिक विधियां सिद्ध करना और इस प्रकार के संसूचन के लिए संपरीक्षाएं विकसित करना।

V. पादप और जंतु कोशिका माल, कोशिका लाइनों के प्रमाणीकरण के लिए डीएनए फिंगरप्रिंटिंग तकनीकों का उपयोग करना और ऐसे प्रयोजनों के लिए आवश्यकतानुसार नई संपरीक्षाएं विकसित करना

VI. डीएनए फिंगरप्रिंटिंग तकनीकों पर प्रशिक्षण प्रदान करना;

VII. मूलभूत, अनुप्रयुक्त अनुसंधान एवं विकास कार्य करना;

VIII. देश में चिकित्सा संस्थाओं, जन-स्वास्थ्य अभिकरणों और उद्योग को परामर्शी सेवाएं प्रदान करना;

IX. केंद्र के उद्देश्यों से संगत क्षेत्रों में विदेशी अनुसंधान संस्थानों एवं प्रयोगशालाओं और अन्य अंतरराष्ट्रीय संगठनों के साथ सहयोग करना;

X. अनुसंधान छात्रों को स्नातकोत्तर उपाधियों के लिए पंजीकृत कर सकने के प्रयोजन हेतु उच्चतर अधिगम के मान्यता प्राप्त विश्वविद्यालयों एवं संस्थाओं के साथ संबंध स्थापित करना;

XI. भारत सरकार, राज्य सरकारों, देश में स्थित पूर्व संस्थाओं / न्यासों, व्यक्तियों और अन्य गतिविधियों के लिए अंतरराष्ट्रीय संगठनों सहित विदेशी स्रोतों से आर्थिक सहायता प्राप्त करना;

XII. केंद्र सरकार के पूर्व अनुमोदन से प्रशिक्षण कार्यक्रमों, वैज्ञानिक अनुसंधान और अन्य गतिविधिों के लिए अंतरराष्ट्रीय संगठनों सहित विदेशी स्रोतों से आर्थिक सहाता प्राप्त करना।

XIII. केंद्र की गतिविधियों को चलाने के लिए जैसा आवश्यक या सुविधाजनक हो, कोई भी संपत्ति चल या अचल या भवनों एवं निर्माणों को निर्मित करने, सुधार करने, परिवर्तित करने, गिरा देने या मरम्मत

करने हेतु उपहार, क्रय, विनियम, पट्टा, भाड़े पर लेने द्बारा या अन्था किसी भी तरह अर्जित करना।

XIV. केंद्र के प्रयोजन हेतु, भारत सरकार और अन्य प्रोनोटों, विनियम पत्रों या अन्य परक्राम्य लिखतों को आहरित करना और स्वीकार करना, तैयार करना और पृष्ठांकित करना, रियायत प्रदान करना और परक्रामण करना।

XV. केंद्र को सौंपी गई निधि के धन का निवेश करने के लिए, ऐसी प्रतिभूतियों को खोलना या ऐसे तरीके अपनाना, जो कि समय-समय पर शासी परिषद द्बारा निर्धारित किए जाते हैं, इस प्रकार के निवेश को विक्रय या पक्षांतरण करना।

XVI. उक्त सभी उद्देश्यों या उनमें से किसी उद्देश्य की प्राप्ति के लिए सभी ऐसे अन्य विधिसम्मत कार्य, जैसा आवश्क, प्रासंगिक या सहायक हो, करना।

XVII. केंद्र के उद्देश्यों को वास्तविक बनाने के लिए प्रोफेसरों, अन्य संकाय पदों, अभ्यागत अध्येतावृत्तियों सहित अध्येतावृत्तियों, अनुसंधान एवं संवर्ग पदों, छात्रवृत्तियों आदि को संस्थापित करना।

XVIII. केंद्र के वैज्ञानिक एवं प्रौद्योगिकी कार्य के लिए प्रयोगशालाओं, कार्यशालाओं, भंडार, पुस्तकालय, कार्यालय और अन्य सुविधाओं को स्थापित करना।

XIX. तकनीकी जानकारी को उद्यमकर्ताओं और उद्योगों से प्राप्त या उनको अंतरण करना, और

XX. पेटेंटों, डिजाइनों एवं तकनीकी जानकारी जो कि केंद्र द्बारा विकसित की गई हो, को पंजीकृत करना और केंद्र के हित में ऐसे पेटेंटों / डिजाइनों / तकनीकी जानकारी के किसी भाग को अंतरण करना।

MANDATE

The objectives for which the Centre for DNA Fingerprinting and Diagnostics (CDFD) was established, as enumerated in Memorandum of Association and Rules and Regulations of CDFD Society, are as follows:

I. To carry out scientific research pertaining to DNA profiling and related analysis in civil cases like paternity disputes, immigration, and exchange of newborns in hospitals, for various agencies including private parties, on appropriate payment;

II. To provide DNA fingerprinting and related analysis and facilities to crime investigation agencies;

III. To assist police personnel, forensic scientists, lawyers and the judiciary in understanding the evidential value of the DNA profile analysis and related techniques in crime investigation and family matters;

IV. To establish DNA diagnostic methods for detecting genetic disorders and to develop probes for such detection;

V. To use DNA fingerprinting techniques for the authentication of plant and animal cell material, cell lines and to develop new probes where necessary for such purposes;

VI. To provide training in DNA fingerprinting techniques;

VII. To undertake basic, applied and developmental R & D work;

VIII. To provide consultancy services to medical institutions, public health agencies and industry in the country;

IX. To collaborate with foreign research institutions and laboratories and other international organizations in fields relevant to the objectives of the Centre;

X. To establish affiliation with recognized universities and institutions of higher learning for the purpose of enabling research scholars to register for post-graduate degrees;

XI. To receive grants, donations and contributions in cash or in other forms from the Government of India, State Governments, Charitable Institutions/Trusts, individuals and industry within the country;

XII To receive, with the prior approval of the Central Government, monetary assistance from foreign sources including international organizations for training programmes, scientific research and other activities;

XIII. To acquire by gift, purchase, exchange, lease, hire or otherwise howsoever, any property movable or immovable or to construct, improve, alter, demolish or repair buildings and structures as may be necessary or convenient for carrying on the activities of the Centre;

XIV. For the purpose of the Centre, to draw and accept, make and endorse, discount and negotiate Government of India and other Promissory Notes, Bills of Exchange, Cheques or other Negotiable Instruments;

XV. For investing the funds of or money entrusted to the Centre, to open such securities or in such manner as may from time to time be determined by the Governing Council and to sell or transpose such investment;

XVI. To do all such other lawful acts as may be necessary, incidental or conducive to the attainment of all or any of the above objectives;

XVII. To institute Professorships, other faculty positions, fellowships including visiting fellowships, research and cadre positions, scholarships, etc. for realizing the objectives of the Centre;

XVIII. To establish, maintain and manage laboratories, workshops, stores, library, office and other facilities for scientific and technological work of the Centre;

XIX. To acquire or transfer technical know-how from/ to entrepreneurs and industries; and

XX. To register patents, designs & technical know-how that may be developed by the Centre and transfer any portion of such patents/designs/ technical know-how in the interest of the Centre.

निदेशक का संदेश
From the Director's Desk

निदेशक का संदेश

वर्ष 22-2021 के लिए डीएनए फ़िंगरप्रिंटिंग एवं निदान केंद्र (सीडीएफडी) की वार्षिक रिपोर्ट प्रस्तुत करते हुए मुझे वास्तव में बहुत खुशी हो रही है। सीडीएफडी जैव प्रौद्योगिकी विभाग, विज्ञान और प्रौद्योगिकी मंत्रालय, भारत सरकार के स्वायत्त संस्थानों में से एक है। सीडीएफडी के अस्तित्व के 26 साल सफलतापूर्वक पूरे हो गए हैं और केंद्र एक अद्वितीय हाइब्रिड मॉडल के रूप में उभरा है, जो न केवल अकादमिक उत्कृष्टता के लिए, आधुनिक जीव विज्ञान के अग्रणी क्षेत्रों में, बल्कि सामाजिक रूप से संगत कार्यों से भी जुड़ा हुआ है, यहां विशेष रूप से डीएनए फ़िंगरप्रिंटिंग और आनुवंशिक विकारों के निदान के क्षेत्रों में अनुसंधान करने के लिए प्रयास किए जाते हैं।

सीडीएफडी ने देश में बाल चिकित्सा दुर्लभ आनुवंशिक विकारों के आनुवंशिक आधार को डीकोड करने के लिए एक अंतःविषय दृष्टिकोण शुरू किया है। इस वर्ष के दौरान, जैव प्रौद्योगिकी विभाग (डीबीटी), विज्ञान और प्रौद्योगिकी मंत्रालय, भारत सरकार ने बाल चिकित्सा दुर्लभ आनुवंशिक विकारों (पीआरएजीईडी) पर एक मिशन कार्यक्रम को वित्त पोषित किया है। सीडीएफडी में दुर्लभ आनुवंशिक विकारों वाले बच्चों और उनके माता-पिता के नमूनों का विश्लेषण करने के लिए विभिन्न मेडिकल कॉलेजों के बाल रोग विभागों, डीबीटी-यूएमएमआईडी केंद्रों और भारत भर के 15 सहयोगी केंद्रों के साथ सहयोग से कार्य किया जाएगा। पीआरएजीईडी का लक्ष्य है; जागरूकता पैदा करना, आनुवंशिक निदान प्राप्त करना, नए जीन की खोज और विशेषता करना, परामर्श प्रदान करना और भारत में बाल चिकित्सा दुर्लभ आनुवंशिक रोगों के लिए नए उपचार विकसित करना। इसके अलावा, पीआरएजीईडी का उद्देश्य दुर्लभ आनुवंशिक रोगों के लिए नए और लागत प्रभावी निदान और छानबीन दृष्टिकोण विकसित करना है, जो स्वास्थ्य और परिवार कल्याण मंत्रालय की दुर्लभ बीमारियों के लिए राष्ट्रीय नीति 2021 के अनुरूप है, जो दुर्लभ बीमारियों के लिए भारत की उच्च उपचार लागत को कम करने का इरादा रखता है। अन्वेषकों का लक्ष्य है कि इस कार्यक्रम के माध्यम से भारत में दुर्लभ बीमारियों के बोझ को कम किया जाए।

सीडीएफडी में इस वर्ष के दौरान कोविड के निदान के लिए आरटी-पीसीआर आधारित परीक्षण करना जारी रखा गया और अब तक कुल 60,757 कोविड नमूनों का परीक्षण किया। सीडीएफडी भी सक्रिय रूप से कोविड 19- जीनोम अनुक्रमण में संलग्न है। उत्परिवर्तन स्पेक्ट्रम का अध्ययन करने के लिए 12,360 सार्स-कोव2- आरएनए नमूनों का पूरा जीनोम अनुक्रम किया गया है। सीडीएफडी भारतीय सार्स-कोव2- जीनोमिक्स कंसोर्शियम (आईएनएसएसीओजी) का एक हिस्सा है, जो सार्स-कोव2- में जीनोमिक विविधताओं की निगरानी के लिए 28 राष्ट्रीय प्रयोगशालाओं का एक संघ है। मुझे यह बताते हुए खुशी हो रही है कि हमने अपने सभी कर्मचारियों और छात्रों को कोविड19- के खिलाफ टीका और इसकी बूस्टर खुराक को सफलतापूर्वक लगाया है।

पिछले एक साल के दौरान सीडीएफडी ने केंद्र और विभिन्न राज्य सरकारों की न्यायपालिका और कानून लागू करने वाली एजेंसियों द्वारा अग्रेषित 50 मामलों के लिए डीएनए प्रोफाइलिंग सेवाएं प्रदान की हैं। कुछ प्रमुख मामले, जहां हमने डीएनए फिंगरप्रिंटिंग सेवाएं प्रदान की हैं, उनमें शामिल हैं; तमिलनाडु के कुन्नूर में आईएएफ एमआई17- वी5 हेलीकॉप्टर दुर्घटना में चीफ ऑफ डिफेंस स्टाफ, जनरल श्री बिपिन

रावत और उनकी पत्नी के साथ मारे गए 11 सशस्त्र बल कर्मियों के अवशेषों की पहचान, जहां हमने चौबीस घंटे के अंदर मृतकों की पहचान स्थापित की और रिपोर्ट सौंप दी। नैदानिकी प्रभाग में विभिन्न आनुवंशिक रोगों के लिए 3502 रोगियों को आनुवंशिक मूल्यांकन प्रदान किया गया। कुल 1130 साइटोजेनेटिक, 2024 आण्विक आनुवंशिकी और 348 जैव रासायनिक आनुवंशिक परीक्षण किए गए। निजाम के आयुर्विज्ञान संस्थान, हैदराबाद में स्थापित चिकित्सा आनुवंशिकी विभाग आनुवंशिक सेवाएं प्रदान करने के लिए सफलतापूर्वक कार्य कर रहा है और 8 छात्रों के प्रशिक्षण के साथ चिकित्सा आनुवंशिकी में एक डीएनबी प्रशिक्षण कार्यक्रम सफलतापूर्वक जारी है। मेडिकल जेनेटिक्स विभाग, एनआईएमएस, हैदराबाद में आनुवंशिक परामर्श में 2 वर्षीय एमएससी प्रशिक्षण कार्यक्रम ने दो छात्रों को प्रशिक्षित किया है। डीबीटी प्रायोजित «इनहेरिटेज डिसऑर्डर के प्रबंधन और उपचार के अनोखे तरीके» (यूएमएमआईडी) प्रोजेक्ट में ‹ट्रेनिंग ऑफ क्लिनिशियन› प्रोग्राम के तहत जेनेटिक डायग्नोस्टिक्स में छह माह की फेलोशिप ने 8 संकाय को प्रशिक्षित किया है। इसके अलावा, सीडीएफडी ने आकांक्षी जिलों में रोग जांच गतिविधियों के लिए यादगीर जिला अस्पताल और रायचूर आयुर्विज्ञान संस्थान में एक डीबीटी निदान केंद्र की स्थापना की है। एपीडा-सीडीएफडी सेंटर फॉर बासमती डीएनए विश्लेषण ने हमारे पेटेंट एसएसआर मार्कर पैनल का उपयोग करते हुए शुद्धता के लिए कुल 495 बासमती नमूनों का परीक्षण किया।

वर्ष के दौरान सीडीएफडी की अनुसंधान गतिविधियों का संक्षिप्त विवरण नीचे दिया गया है:

बैक्टीरियल जेनेटिक्स की प्रयोगशाला द्वारा किए गए अध्ययनों ने K+ ट्रांसपोर्टरों के मेम्ब्रेन बायोजेनेसिस की मध्यस्थता में एसईसीडी/एफ और मेम्ब्रेन इंसर्टेज़ वायआईडीसी को फंसाया है। उन्होंने एस्चेरिचिया कोलाई के जीवाणु मॉडल जीव में फैटी एसिड चयापचय और कोशिका विभाजन की प्रक्रियाओं के बीच परस्पर क्रिया की भी जांच की। उनके परिणामों से संकेत मिलता है कि संशोधित न्यूक्लियोटाइड्स (पी) पीपीजीपीपी जो बैक्टीरिया में संरक्षित हैं, दो प्रक्रियाओं के नियमन में महत्वपूर्ण भूमिका निभाते हैं।

कोशिका साइकल रेगुलेशन की प्रयोगशाला के परिणाम बताते हैं कि एमएलएल का नुकसान एक्टिन साइटोस्केलेटन को परेशान करता है जो खुद को विषम कोशिका आकार के रूप में प्रकट करता है।

कोशिका मृत्यु और कोशिका उत्तरजीविता प्रयोगशाला में विभिन्न फॉस्फेटेस के लिए नए कार्य सौंपे गए हैं। ध्यान दें, टीम ने टायरोसिन फॉस्फेट (एसएचपी 1-) और हिस्टोन के बीच एक नए कनेक्शन की पहचान की जिसकी यूकेरियोटिक ट्रांसक्रिप्शन में एक आवश्यक भूमिका है।

कोशिका में महत्वपूर्ण एंजाइम होने के बावजूद, विभिन्न कोशिकीय प्रक्रियाओं, मार्गों और रोगजनन में फॉस्फेटेस और उनकी आवश्यक भूमिकाओं का व्यापक विश्लेषण उपलब्ध नहीं है। अंत:क्रिया प्रोटिओमिक्स दृष्टिकोण के साथ मिलकर एक बंधुता-आधारित प्रोटीन शुद्धि के माध्यम से उत्पन्न सभी मानव फॉस्फेटेस के लिए इंटरएक्टिव डेटा का उपयोग करते हुए, कोशिका मृत्यु और कोशिका उत्तरजीविता प्रयोगशाला में विभिन्न फॉस्फेटेस के लिए नए कार्य सौंपे गए हैं। ध्यान दें, टीम ने टायरोसिन फॉस्फेट (एसएचपी 1-) और हिस्टोन के बीच एक नए कनेक्शन की पहचान की जिसकी यूकेरियोटिक ट्रांसक्रिप्शन में एक आवश्यक भूमिका है।

कोशिका सिग्नलिंग प्रयोगशाला में प्रदर्शित किया गया है कि आईपी6के1 द्वारा संश्लेषित इनोसिटोल पाइरोफॉस्फेट -5आईपी7 स्तनधारी कोशिकाओं में समरूप पुनर्संयोजन मध्यस्थता डीएनए मरम्मत को पूरा करने को बढ़ावा देने के लिए आरएडी51 और बीआरसीए2 के बीच अंत:क्रिया को नियंत्रित करता है।

मानव कोशिकाओं में इस नए नियामक तंत्र के संरक्षण को समझने की दिशा में काम कर रहे क्रोमैटिन बायोलॉजी और एपिजेनेटिस की

प्रयोगशाला और यह कैंसर में कैसे योगदान देता है। उनका अध्ययन मानव संसाधन को प्रभावित करने वाले एक नए तंत्र की व्याख्या करता है जो विभिन्न कैंसर चिकित्सा विज्ञानियों के लिए एक नया लक्ष्य हो सकता है।

कम्प्यूटेशनल और फंक्शनल जीनोमिक्स की प्रयोगशाला में ऑटोफैगी द्वारा पॉलीनेडिलेटेड एग्रीगेटेड मिसफोल्डेड प्रोटीन के क्षरण के समन्वय में हंटिंगिन अंत:क्रिया प्रोटीन के (एचवायपीके) की एक नई भूमिका दिखाई गई है। वे क्रमशः एसीबीपी कार्य के पैरालॉग और रासायनिक अवरोधक की कार्यात्मक भूमिका का अध्ययन करने में संक्रामक (तपेदिक) और परजीवी रोगों (मलेरिया) के क्षेत्र में भी काम कर रहे हैं।

ड्रोसोफिला तंत्रिका विकास की प्रयोगशाला से पता चलता है कि मूल-हेलिक्स-लूप-हेलिक्स टीएफ ग्रेनीहेड (जीआरएच) एक सामान्य हॉक्स कॉफ़ेक्टर के रूप में कार्य कर सकता है और विकास के दौरान उन्हें जीवे भूमिकाओं में प्रदर्शन करने में मदद कर सकता है।

कवक रोगजनन की प्रयोगशाला एक अवसरवादी मानव कवक रोगज़नक़ कैंडिडा ग्लेब्राटा की विकृति विज्ञान को समझने की दिशा में काम कर रही है। प्रयोगशाला में दिखाया गया कि मानव अवसरवादी कवक रोगज़नक़ कैंडिडा ग्लेब्राटा में कोशिका की सतह से जुड़े एस्पार्टिल प्रोटीज ग्लूकोज सेंसिंग और होमियोस्टेसिस तंत्र के लिए महत्वपूर्ण हैं।

यीस्ट और स्तनधारी सेल लाइनों दोनों में जीनोम आर्किटेक्चर प्रयोगशाला के डेटा जीनोम संगठन में डीएनए सुपरकोइल द्वारा निभाई गई महत्वपूर्ण भूमिका का सुझाव मिलता है।

जीनोम इंफॉर्मेटिक्स प्रयोगशाला, ऐमरैंथस हाइपोकॉन्ड्रिकस के लिए जीनोमिक संसाधनों के विकास की दिशा में काम कर रही है। साथ ही, भारतीय ब्लैक चिकन और केरैटाइटिस पैदा करने वाले कवक «फ्यूसैरियम सोलानी» के जीनोम डिकोडिंग से संबंधित उनका जारी अनुसंधान भारतीय ब्लैक चिकन «कड़कनाथ» के कई महत्वपूर्ण फिनोटाइपिक लक्षणों से संबंधित जीन की पहचान में मददगार होगा।

मानव और चिकित्सा आनुवंशिकी प्रयोगशाला की प्रयोगशाला गुणसूत्र और एकल जीन विकारों के लिए नए उत्परिवर्तन / जीन पहचान पर केंद्रित है। उन्होंने संपूर्ण एक्सोम/जीनोम अनुक्रमण विश्लेषण के विश्लेषण के लिए इन हाउस डेटा विश्लेषण पाइपलाइनों का विकास और उपयोग किया है। उन्होंने एंजाइम गतिविधि जैसे नैदानिक कार्यात्मक आमापनों की उपलब्धता के साथ जीन के लिए एक कम लागत वाली अगली पीढ़ी की अनुक्रमण आधारित आमापन विकसित किया है।

मानव आण्विक आनुवंशिकी प्रयोगशाला का कार्य मानव स्वास्थ्य और रोग में माइटोकॉन्ड्रियल शिथिलता को समझने पर केंद्रित है। यहां विशेष रूप से एक विशिष्ट उद्देश्य के साथ माइटोकॉन्ड्रियल विकारों से जुड़े नए जीनों का पता लगाने, आण्विक तंत्र को समझने और चिकित्सीय (निदान और उपचार) विकसित करने कार्य किया जाता है।

प्रतिरक्षा विज्ञान प्रयोगशाला में कई सिंथेटिक थियाज़ोल-पाइराज़ोल परिसरों के साथ-साथ ऑर्गेनो-टिन, -कॉपर, और - कोबाल्ट यौगिकों का परीक्षण किया और ट्यूमर कोशिकाओं की मृत्यु में संभावित कीमोथेराप्यूटिक एजेंटों के रूप में पूर्व जीवे और जीवे दोनों में कार्रवाई के संभावित तंत्र का परीक्षण किया। प्रोफिलिन की ट्यूमर शमन गतिविधि निर्धारित की गई है क्योंकि यह विभिन्न मार्गों में हस्तक्षेप करती है।

संक्रामक रोगों की प्रयोगशाला ने पहचाना कि ई. हिस्टोलिटिका वी-एटीपेस सबयूनिट परजीवी की कोशिकीय सिकुड़न के लिए संभावित रूप से महत्वपूर्ण हैं। उन्होंने यह भी पहचाना है कि ई. हिस्टोलिटिका आरएबी35 प्रोटीज की रुकावट के माध्यम से मेजबान बाह्य मैट्रिक्स के क्षरण में शामिल होने की संभावना है।

आण्विक कोशिका जीव विज्ञान की प्रयोगशाला द्वारा संकेत किया जाता है कि पीकेएनजी, आरएबी7।1 से आरजीडीआई1- के पृथक्करण को रोका जाता है और इस प्रकार आरएबी7।1 जीटीपेस गतिविधि को रोका जाता है जिसके परिणामस्वरूप मैक्रोफेज में P-L संलयन अवरुद्ध

हो जाता है। उनका मत है कि आरएबी7।1 एक संक्रमण के इंफ्लेमेटरी प्रतिक्रिया और परिणाम को निर्धारित करने में एक महत्वपूर्ण संकेतन अणु हो सकता है।

आण्विक ऑन्कोलॉजी की प्रयोगशाला में कोलोरेक्टल कैंसर में जीन फ्यूज़न और क्रोमैटिन वास्तुकला के बीच एक महत्वपूर्ण संबंध की पहचान की गई है, जो अन्य प्रकार के कैंसर में लागू होने की संभावना है। तेलंगाना राज्य से संक्रमित नमूनों में सार्स-कोव 2- जीनोम-वाइड न्यूक्लियोटाइड विविधताओं के मूल्यांकन से टीके की सफलता के मामलों के साथ दिलचस्प जुड़ाव का पता चला है।

पादप सूक्ष्म जीव अंत:क्रिया प्रयोगशाला में पहली बार रिपोर्ट किया गया कि एक्स ओरिजे पीवी में ओरिजे आरपीएफएफ फैटी एसिड संश्लेषण मार्ग में एक नियामक भूमिका निभाने के साथ झिल्ली की अखंडता के रखरखाव में शामिल है। प्रयोगशाला में पहली बार दिखाया गया है कि पैरेन्काइमल क्लोरोप्लास्ट के क्यूएस-सक्षम जीवाणु स्थानीयकरण से मेजबान मेसोफिल ऊतक पर आक्रमण किया गया, जिससे ट्रिगर लीफ क्लोरोसिस और प्रणालीगत संक्रमण हुआ।

प्रतिलेखन प्रयोगशाला में जीवे आरएचओ-आश्रित समाप्ति प्रक्रिया में आरएनए की भूमिका स्थापित की, प्रोफ़ेज टॉक्सिन-एंटीटॉक्सिन अभिव्यक्तियों के आरएचओ-मध्यस्थता विनियमन, और जीन अभिव्यक्ति के दमन में एंटी-आरएचओ पेप्टाइड्स की भूमिका को चित्रित करने में महत्वपूर्ण प्रगति की।

हमारी पशु बीएसएल3 सुविधा लगभग पूरी होने वाली है। इस सुविधा द्वारा रोगजनकों और संक्रामक रोगों के अध्ययन के लिए आवश्यक मूल सरंचना प्रदान किया जाएगा। उच्च प्रदर्शन सुपरकंप्यूटिंग सुविधा आगामी सुविधा है जिसमें एक अल्ट्रा-हाई-कैपेसिटी डेटा स्टोरेज सुविधा (2.2 पेंटाबाइट्स) है जो एक लाइटिंग फास्ट सीपीयू और जीपीयू-आधारित उच्च प्रदर्शन जीनोमिक डेटा सुपर कंप्यूटिंग सुविधा से जुड़ी है। यह सुविधा हाई-वॉल्यूम नेक्स्ट-जेनरेशन सीक्वेंसिंग (एनजीएस) डेटा विश्लेषण, स्टोरेज और हाई-स्पीड इंटरनेट के माध्यम से एक्सचेंज प्रदान करती है।

हमारे संकाय सदस्यों को कई पुरस्कार और सम्मान प्राप्त हुए हैं, जिनमें शामिल हैं; डॉ रंजन सेन और डॉ संगीता मुखोपाध्याय ने एसईआरबी, डीबीटी से जे. सी. बोस अध्येतावृत्ति प्राप्त की।

डॉ. एम सुब्बा रेड्डी ने जीव विज्ञान श्रेणी में दवा अनुसंधान में उत्कृष्टता के लिए सीडीआरआई पुरस्कार 2022 प्राप्त किया।डॉ। अश्विन दलाल को डॉ जी जयरामन एंडोमेंट अवार्ड मिला है। सीडीएफडी के छात्रों को बर्सरी अवार्ड सहित कई प्रतिष्ठित पुरस्कार, फेलोशिप और यात्रा अनुदान भी प्राप्त हुए। इस अवधि के दौरान कुल 12 छात्रों को पीएच डी की उपाधि से सम्मानित किया गया और कई को आधुनिक जीव विज्ञान के विभिन्न क्षेत्रों में प्रशिक्षण प्रदान किया गया।

अंत में, मैं इस अवसर पर अपने सभी सहयोगियों की ओर से जैव प्रौद्योगिकी विभाग, सीडीएफडी सोसाइटी के प्रतिष्ठित सदस्यों, शासी परिषद, वैज्ञानिक सलाहकार समिति, वित्त समिति और प्रबंधन समिति को उनके प्रोत्साहन, सलाह और निरंतर के लिए धन्यवाद देता हूं। समर्थन जिसके बिना हमारी अधिकांश उपलब्धियां संभव नहीं होतीं।

के थंगराज

31 मार्च 2022

From the Director's Desk

It is indeed a great pleasure for me to present the Annual Report of the Centre for DNA Fingerprinting and Diagnostics (CDFD) for the year 2021-22. CDFD is one of the autonomous institutes of the Department of Biotechnology, Ministry of Science and technology, Government of India. CDFD has successfully completed 26 years of its existence and the Centre has emerged as a unique Hybrid model that strives, not only for academic excellence, by undertaking research in frontier areas of modern biology, but is also knitted with socially relevant work, especially in the areas of DNA Fingerprinting and Diagnosis of genetic disorders.

CDFD has initiated an interdisciplinary approach to decode the genetic basis of Paediatric Rare Genetic Disorders in the country. During this year, the Department of Biotechnology (DBT), Ministry of Science and technology, Government of India has funded a Mission Programme on Paediatric Rare Genetic Disorders (PRaGeD). CDFD will be collaborating with paediatric departments of various medical colleges, DBT-UMMID centres, and 15 collaborating Centres across India to analyse the samples of children with rare genetic disorders, and their parents. PRaGeD aims to; create awareness, achieve genetic diagnosis, discover & characterize novel genes, provide counselling, and to develop novel therapies for paediatric rare genetic diseases in India. Furthermore, the PRaGeD aims to develop novel and cost-effective diagnostic and screening approaches for rare genetic diseases aligning with the Ministry of Health and Family Welfare's National Policy for Rare Diseases 2021, which intends to lower India's high treatment costs for rare diseases. Through this program, the investigators are aiming to minimize the burden of rare diseases in India.

During the year, CDFD continued performing RT-PCR based testing for diagnosis of COVID and so far, tested a total of 60,757 COVID samples. CDFD also actively engaged in COVID-19 genome sequencing. The complete genome sequence of 12,360 SARS-CoV-2 RNA samples have been carried out to study the mutation spectrum. CDFD is a part of Indian SARS-CoV-2 Genomics Consortium (INSACOG), a consortium of 28 National Laboratories to monitor the genomic variations in the SARS-CoV-2. I am happy to put it on record that we have successfully vaccinated all our staff and students against COVID-19 including its booster dose.

In the past year, CDFD provided DNA profiling services for 50 cases, forwarded by the judiciary and law enforcing agencies of the Union and different State Governments. Some of the prominent cases, where we provided DNA Fingerprinting services include; identification of remains of 11 armed forced personnel killed along with Chief of Defence Staff, General Shri Bipin Rawat and his wife in the IAF Mi-17 V5 helicopter crash at Coonoor, Tamil Nadu where we established the identification of the deceased within twenty four hours and submitted the report. The Diagnostics division provided genetic evaluation to 3502 patients for various genetic diseases. A total of 1130 cytogenetic, 2024 molecular genetics and 348 biochemical genetic tests were conducted. The Medical Genetics department established at Nizam's Institute of Medical Sciences, Hyderabad is functioning successfully to provide genetic services and a DNB training program in Medical Genetics is running successfully with training of 8 students. A 2 year MSc training programme in Genetic counselling at Department of Medical Genetics, NIMS, Hyderabad has trained two students. A six month Fellowship in Genetic Diagnostics under the 'Training of Clinicians' programme in DBT sponsored "Unique methods of management and treatment of inherited disorders" (UMMID) project has trained 8 faculty. In addition, CDFD has established a DBT Nidan Kendra at Yadgir District hospital and Raichur Institute of Medical Sciences, for disease screening activities in aspirational districts. The APEDA-CDFD Centre for Basmati DNA analysis tested a total of 495 Basmati samples for purity using our patented SSR marker panel.

A brief highlights of the research activities of CDFD during the year are given below:

Studies by Laboratory of Bacterial Genetics have implicated SecD/F and the membrane insertase YidC in mediating membrane biogenesis of K+ transporters. They also investigated the interplay between the processes of fatty acid metabolism and cell division in the bacterial model organism of Escherichia coli. Their results indicate that the modified nucleotides (p)ppGpp which are conserved across bacteria, play an important role in the regulation of the two processes.

The results by Laboratory of Cell Cycle Regulation shows that loss of MLL perturbs the actin cytoskeleton which manifests itself in the form of anomalous cell shape.

New functions for various phosphatases have been assigned in the lab of Cell Death & Cell Survival. Of note, the team identified a new connection between a tyrosine phosphatase (SHP-1) and histones that has an essential role in eukaryotic transcription.

Despite being critical enzymes in the cell, a comprehensive analysis of phosphatases and their essential roles in various cellular processes, pathways and pathogenesis is not available. By utilizing interactome data for all the human phosphatases generated via an affinity-based protein purification coupled with interaction proteomics approach, new functions for various phosphatases have been assigned in the Lab of Cell Death & Cell Survival. Of note, the team identified a new connection between a tyrosine phosphatase (SHP-1) and histones that has an essential role in eukaryotic transcription.

Laboratory of Cell Signalling has demonstrated that the inositol pyrophosphate 5-IP7 synthesized by IP6K1 modulates the interaction between RAD51 and BRCA2 to promote completion of homologous recombination mediated DNA repair in mammalian cells.

Laboratory of Chromatin Biology and Epigenetics is working towards understanding conservation of this novel regulatory mechanism in human cells and how it contributes to cancer. Their study deciphers a novel mechanism that affects HR which can be a novel target for various cancer therapeutics.

Laboratory of Computational and Functional Genomics have shown a novel role of the Huntingtin interacting protein K (HYPK) in coordinating degradation of polyneddylated aggregated misfolded proteins by autophagy. They are also working in the field of infectious (tuberculosis) and parasitic diseases (malaria) in studying the functional role of paralogs and chemical inhibitor of ACBP function respectively.

Laboratory of Drosophila Neural Development shows that basic-helix-loop-helix TF Grainyhead (Grh) can function as a generic Hox cofactor and help them perform their in vivo roles during development.

Laboratory of Fungal Pathogenesis is working towards understanding the pathobiology of an opportunistic human fungal pathogen Candida glabrata.. The lab showed that cell surface associated aspartyl proteases in the human opportunistic fungal pathogen Candida glabrata are pivotal to glucose sensing and homeostasis mechanisms.

The data from the Lab of Genome Architecture in both yeast and mammalian cell lines suggest important role played by DNA supercoil in genome organization.

Laboratory of Genome Informatics is working towards the development of genomic resources for Amaranthus hypochondricus. Also, their ongoing research related to the genome decoding of Indian black chicken and keratitis-causing fungus “Fusarium solani” will be helpful in the identification of genes related to many important phenotypic traits of the Indian black chicken “Kadaknath”.

Laboratory of Human and Medical Genetics Laboratory focusses on novel mutation/gene identification for chromosomal and single gene disorders. They have developed and used in house data analysis pipelines for analysis of whole exome/ genome sequencing analysis. They have developed a low-cost next generation sequencing based assay for genes with availability of diagnostic functional assays like enzyme activity.

Laboratory of Human Molecular Genetics focuses on understanding the mitochondrial dysfunction in human health and disease. In particular, with a specific aim to explore the new genes associated with mitochondrial disorders, understand the molecular mechanisms, and develop theragnostics (diagnosis and treatment).

The Laboratory of Immunology tested several synthetic thiazole-pyrazole complexes as well as organo-tin, -copper, and –cobalt compounds and its possible mechanism of action in the tumor cells' death as potential chemotherapeutic agents both ex vivo and in vivo. Tumor suppressor activity of Profilin has been determined as it interferes various pathways.

The Lab of Infectious Diseases identified that E. histolytica V-ATPase subunits are potentially important for cellular contractility of the parasite. They have also identified that E. histolytica Rab35 is likely to be involved in degradation of host extracellular matrix through trafficking of proteases.

Laboratory of Molecular Cell Biology indicates that PknG inhibits dissociation of RGDI-1 from Rab7l1 and thereby inhibits Rab7l1 GTPase activity resulting in blocking of P-L fusion in macrophages. They opine that Rab7l1 can be an important signalling molecule in dictating the inflammatory response and outcome of an infection.

The Laboratory of Molecular Oncology has pioneered the identification of a significant association between gene fusions and chromatin architecture in colorectal cancer, likely applicable across other cancer types. Evaluation of SARS-CoV-2 genome-wide nucleotide variations in infected samples from Telangana state has revealed interesting association with vaccine breakthrough cases.

The Laboratory of Plant-Microbe Interactions reported for the first time that in X. oryzae pv. oryzae RpfF is involved in the maintenance of membrane integrity by playing a regulatory role in the fatty acid synthesis pathway. The lab shows for the first time that QS-enabled bacterial localization of parenchymal chloroplast within heterogeneously invaded host mesophyll tissue, leading triggered leaf chlorosis and systemic infection.

Laboratory of Transcription established the role of RNA in the in vivo Rho-dependent termination process, Rho-mediated regulation of prophage toxin-antitoxin expressions, and made significant progress in delineating the role of anti-Rho peptides in the repression of gene expression.

Our animal BSL3 facility is near completion. This facility would provide necessary infrastructure to study pathogens and infectious diseases. High Performances Supercomputing Facility is upcoming facility which has an ultra-high-capacity data storage facility (2.2 Petabytes) connected with a lighting fast CPU and GPU-based high performances genomic data supercomputing facility. The facility provides high-volume next-generation sequencing (NGS) data analysis, storage, and exchange via high-speed internet.

Our faculty members have received several awards and honours, which includes; Dr Ranjan Sen and Dr Sangita Mukhopadhyay, received J.C. Bose fellowship from SERB, DBT. Dr. M Subba Reddy received CDRI Award 2022 for excellence in drug research in Life Science category. Dr. Ashwin Dalal has received Dr G Jayaraman Endowment Award. CDFD students also received several prestigious awards, fellowships and travel grants including Bursary Award. A total of 12 students were awarded PhD Degree during this period and several have been imparted with training in different fields of modern biology.

Finally, on behalf of all my colleagues, I take this opportunity and extend our sincere thanks to the Department of Biotechnology, distinguished members of the CDFD Society, Governing Council, Scientific Advisory Committee, Finance Committee and Management Committee for their encouragement, advice and unstinted support without which much of our achievements would not have been possible.

K Thangaraj
March 31, 2022

सेवाएँ
Services

Diagnostics Division

Faculty : **Ashwin Dalal**
Staff Scientist

Adjunct Faculty:

Prajnya Ranganath	Additional Professor, NIMS
Shagun Aggarwal	Additional Professor, NIMS

Other Members:

P. Rajitha	Technical Officer
Angalena R	Senior Technical Officer
Usha Rani Dutta	Technical Officer
M Muthulakshmi	Technical Officer
Jamal Md Nurul Jain	Technical Officer
Vasantha Rani	Technical Officer
C. Krishna Prasad	Technician I

Objectives:

1. To conduct genetic evaluation for patients/ families with genetic disorders
2. To develop new methods and assays for genetic analysis and engage in research on chromosomal and single gene disorders
3. To act as national referral center for analysis and quality control of genetic tests for few genetic diseases
4. To impart training in genetic evaluation of patients with genetic disorders

Services provided and Training programs during the year 2021-2022

Clinical Genetics

A total of 3502 patient samples were analyzed for genetic testing, during the year 2021-22 (1/4/2021 to 31/3/2022). These consisted of patients with chromosomal disorders, monogenic disorders, mental retardation, congenital malformations, inborn errors of metabolism, and other familial disorders. The Department of Medical Genetics established at Nizam's Institute of Medical Sciences, Hyderabad is functioning successfully. A total of 7248 patients, of which 3037 were new registrations, were examined and counseled in the department during April 2021- March 2022. In addition, antenatal ultrasonograms were done in 432 cases, antenatal invasive procedures (chorionic villus sampling and amniocentesis) in 346 cases, and fetal autopsies were conducted in 91 fetuses. A 3 year training program for Diplomate of National Board (DNB) in Medical Genetics initiated with affiliation to National Board of Examinations, New Delhi is running successfully and 8 students have completed the course and are placed at different institutions across the country.

MSc training programme in Genetic counseling

A MSc Genetic Counseling program has been initiated at Medical Genetics department established at NIMS, Hyderabad. It is a two year masters program and the course objective is to provide academic and vocational training to become professional genetic counselors. The students trained under this program will be able to cater to comprehensive clinical genetics clinics in tertiary level hospitals. Two students have completed the training.

Fellowship in Genetic Diagnostics

A six month Fellowship in Genetic Diagnostics has been started under the 'Training of Clinicians' programme in DBT sponsored "Unique methods of management and treatment of inherited disorders" (UMMID) project. Clinicians from government medical colleges/hospitals are being trained in cytogenetics and molecular genetics. Six faculty from Government medical colleges have completed training by March 2022. New batch of two faculty has joined in May 2022.

Outreach programme for Aspirational Districts

CDFD has established a DBT Nidan Kendra at Yadgir District hospital and Raichur Institute of Medical Sciences, Raichur, Karnataka under a DBT funded proposal called UMMID (Unique methods of management and treatment of inherited disorders). The plan of the DBT-UMMID initiative is to link the well-established centres of Medical Genetics in India to upcoming centres and to establish clinical genetics facilities in district hospitals. The activities being conducted under the programme include screening of 10,000 antenatal mothers annually attending the district hospital of the aspirational district for thalassemia followed by prenatal diagnosis for prevention of Thalassemia, screening of 5000 newborns annually for 5 common and treatable genetic diseases i.e. G6PD, Congenital hypothyroidism, Galactosemia, Biotinidase deficiency and Congenital adrenal hyperplasia and start early therapy, detection of high risk pregnancies for birth defects and genetic diseases using a questionnaire and referral for free prenatal diagnosis to CDFD and sensitization of school and college students by way of lectures/presentations in the identified schools /colleges regarding genetic diseases and new advancements.

Group of Diagnostics Services

Plant DNA Fingerprinting Services

Chairperson : Subhadeep Chatterjee

Scientist In-charge : K. Anupama

Other Members:

R. Lakshmi Vaishna
M. Sri Lalitha
P. Chandrashekar

Objectives:

1. Testing the purity of Basmati samples received from Export Inspection Council (EIC), Ministry of Commerce, Government of India, Basmati rice exporters from India, and other countries;
2. DNA fingerprinting of varieties and hybrids of rice and other crops.
3. To generate new panels of markers for varietal identification and accurate detection of adulteration in Basmati rice

Details of progress made in the current reporting year (April 1, 2021 - March 31, 2022)

Objective 1: Testing the purity of Basmati samples received from Export Inspection Council (EIC), Ministry of Commerce, Govt. of India, Basmati rice exporters from India and other countries.

During the current reporting year, a total of 495 samples were analyzed of which 50% of the samples were pure and remaining 50% of the samples were adulterated with non-Basmati rice (Figure 1).

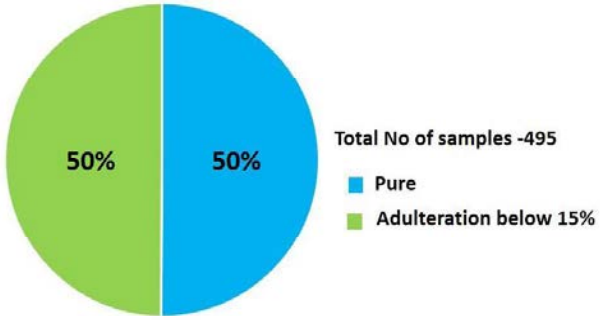


Figure 1. Basmati samples analyzed in the current reporting year.

Objective 2: DNA fingerprinting of varieties and hybrids of rice and other crops.

1. Fingerprinting of one rice hybrid from Pan Seeds, Kolkata was carried out with 15 SSR markers
2. Fingerprinting of 3 rice varieties from Pan Seeds, Kolkata was carried out with 15 SSR markers
3. Fingerprinting of five rice varieties from Dr. J. R. Diwan, College of Agriculture, Raichur was carried out with 10 SSR markers
4. Fingerprinting of 4 water chestnut varieties from Dr. B. R Jana, ICAR Research Complex for Eastern Region, RCM, Darbhanga, Bihar was performed with 10 RAPD, 10 ISSR and 12 SSR markers
5. Fingerprinting of 3 flowering plants, Nyctanthes arboristis, Thevatia peruviana and Adina cordifolia from Prof. Rudramuni.Kore, Associate Professor, D.S.T.S.Mandals College of Pharmacy, Solapur was done with 4 RAPD markers
6. Barcoding approach with matK and ITS regions was used to confirm that extract tablet and extract were made from Picrorhiza kurrooa
7. **Revenue generated:**

   An amount of ₹ 59,55,800/- which includes GST (18%) is received towards purity testing of Basmati samples and ₹ 2,04,453/- (including 18% GST) is received towards fingerprinting of varieties and hybrids of rice and other crops.

   Total revenue generated from April 1, 2020 - March 31, 2021 is ₹ 61,60,273/- which includes 18% GST as levied by the Govt. of India.

Objective 3: To Generate new panels of markers for varietal identification and accurate detection of adulteration in Basmati rice

Genotyping all the Basmati and few non-Basmati potential adulterants using gel-based approaches for SNPs present in the genes governing quality traits of Basmati rice such as in waxy, alk, Badh1, Os03g0717600 and InDel marker based on the 8-bp deletion in the exon 7 of badh2 gene, Os03g0717700 and GW5 genes have differentiated Basmati from potential adulterant non-Basmati varieties. These SNPs have provided unique profiles to all Basmati varieties except for Vallabh Basmati 22 and Vallabh Basmati 23 which are derived from the same cross PB1121 X Type 3. Now standardisation of method such as HRM or KASP assay for quantitative detection of adulteration in Basmati samples using these SNPs is under progress.

Publication:

1. Vaishna RL, Satyavathi VV and Anupama K* 2022 Single grain analysis of the complex Basmati rice samples to determine the nature of admixtures and accurate adulteration quantification. J Food Sci Technol 59(4):1658-1663

Group of Plant DNA Fingerprinting Services

Laboratory of DNA Fingerprinting Services

Scientist In-charge : **Dr. R. Harinarayanan**

Co-ordinator : **Dr. D. P. Kasbekar**

Other Members:

S P R Prasad	Senior Technical Officer
D S Negi	Technical Officer
Pooja Tripathi	Technical Officer
V A Girnar	Technical Officer
Shruti Dasgupta	Technical Assistant

Objectives

1. To provide DNA fingerprinting services in cases forwarded by law-enforcing agencies/ judiciary of State and Federal Governments, relating to murder, sexual assault, paternity, maternity, child swapping, deceased identification, organ transplantation, etc.;
2. To develop human resources skilled in DNA fingerprinting, to cater to the needs of State and Federal Government agencies;
3. To impart periodical training to manpower involved in DNA fingerprinting sponsored by State and Federal Government agencies;
4. To provide advisory services to State and Federal Government agencies in establishing DNA Fingerprinting facility;
5. To create DNA marker databases of different populations of India.

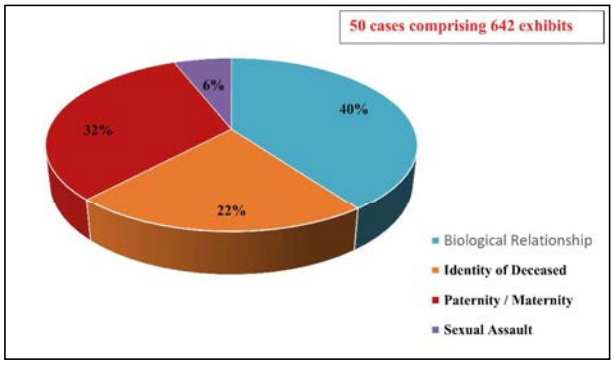
Details of services provided in the current reporting year (1st April 2021 to 31st March 2022):

Breakup of the types of cases received during this reporting period is given in Table – 1 and percentage (of the total) of each type of case is given in the pie chart (Figure – 1).

Table – 1

Identity of Deceased	11
Paternity/Maternity	16
Biological Relationship	20
Sexual Assault (Rape)	3
Total number of Cases	50

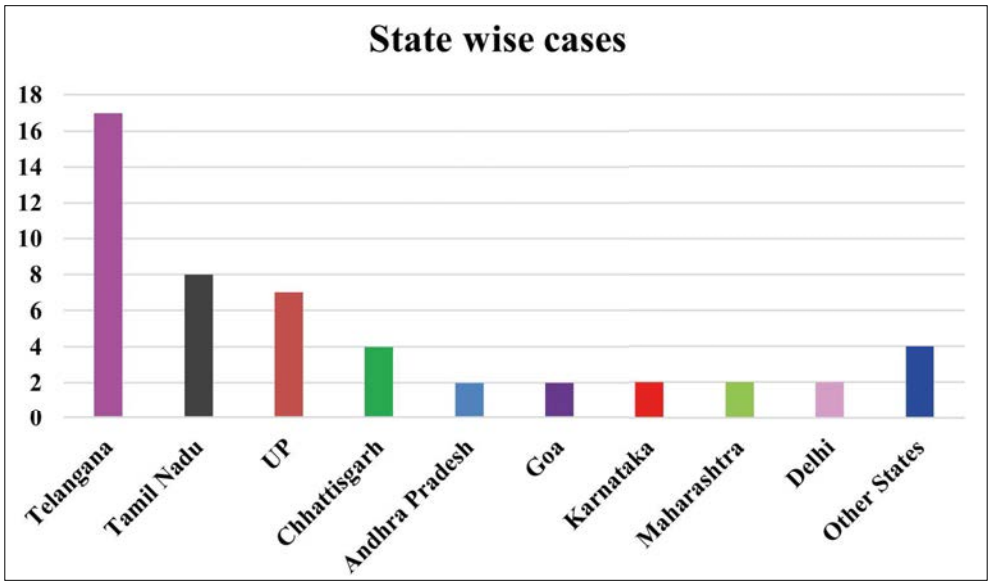
Figure – 1



Types of cases received in percentage

A total of 50 cases comprising of 642 exhibits were received for DNA fingerprinting examination in the current reporting period. Of these, 16 cases were related to maternity/paternity tests, 11 cases were related to identity of deceased, 20 cases were related to confirmation of biological relationship and 3 cases were of sexual assault. 11 States and 2 Union Territories of India have availed DNA fingerprinting services from CDFD during this period. Telangana State has forwarded the highest number of cases (17) followed by Tamil Nadu (8), Uttar Pradesh (7), Chhattisgarh (4), Andhra Pradesh, Goa, Karnataka, Maharashtra, Delhi (2 each), and Madhya Pradesh, Odisha, Puducherry, Tripura (1 each, referred together under “other states” in Figure 2). The nature of the cases with state-wise break-up is provided in Table2.

Figure – 2



State wise distribution of cases received

Table – 2: Summary of the State-wise breakup of DNA Fingerprinting cases

Name of the State	Biological Relationship	Identity of Deceased	Paternity/ Maternity	Sexual Assault (Rape)	No. of Cases
Andhra Pradesh	1	1	-	-	02
Chhattisgarh	-	-	4	-	04
Goa	-	-	2	-	02
Karnataka	-	-	2	-	02
Madhya Pradesh	-	-	1	-	01
Maharashtra	-	2		-	02
Odisha	-	1		-	01
Tamil Nadu	8	-	-	-	08
Telangana	11	1	5	-	17
Tripura	-	1	-	-	01
Uttar Pradesh		3	1	3	07
Delhi	-	1	1	-	02
Puducherry	-	1		-	01
Total No. of Cases	20	11	16	03	50

Prominent cases

1. Rape and murder of a girl student at Navodaya Vidyalaya forwarded by Mainipuri District of Uttar Pradesh.
2. Remains of 11 armed forced personnel killed along with Chief of Defence Staff, General Shri Bipin Rawat and his wife in the IAF Mi-17 V5 helicopter crash at Coonoor, Tamil Nadu, on 8th December 2021 were identified.
3. Mitochondrial DNA was amplified from 22-year-old bones forwarded by Tripura police for identification of the deceased.

Deposition of evidence in Courts of Law

During this reporting year, the DNA experts defended their reports in 6 cases in various Honorable Courts of Law throughout the country.

Training / Lectures / Workshops / Memorandum of Understanding

1. Hands-on Training course on Advancements in Forensic DNA Workflow was conducted at CDFD during 15-17 December 2021 in collaboration with M/s Thermo-Fischer Scientific.
2. A student carried out her M Sc dissertation work titled “Comparison of four different DNA extraction methods for forensic samples in case of human identification”.
3. “Mega Science and Technology Expo 2022, “Vigyan Sarvatra Pujyate”, Azadi ka Amrit Mahostav, held at Delhi, 22-28 Feb 2022.
4. MoU was signed between Uttar Pradesh government (represented by director general of police technical, Govt. of UP) and CDFD on 18th November 2021 to facilitate forensic DNA Fingerprinting services.

Revenue generated

During this reporting period, an amount of Rs. 33,01,348/- (Rupees Thirty-three lakhs one thousand three hundred and forty-eight only) has been received towards DNA fingerprinting analysis charges, which is inclusive of GST (18%) as levied by the Govt. of India.

Laboratory of DNA Fingerprinting Services

शोध
Research

Laboratory of Bacterial Genetics

Studies on integral membrane proteins of *Escherichia coli* involved in adaptive solute transport

Principal Investigator: Abhijit A. Sardesai
Staff Scientist

PhD Students:

Suchitra Upreti	Senior Research Fellow
Swati Dubey	Senior Research Fellow
Neeraj Kumar	Senior Research Fellow
Yogesh Patidar	Senior Research Fellow
Sayani Ghosh	Junior Research Fellow (Since 27-06-2022)

Collaborators:

B. Gopal Molecular	Biophysics Unit IISc Bangalore
Aravind Penmatsa	Molecular Biophysics Unit IISc Bangalore

Objectives

Research in the laboratory is broadly concerned with the study of integral membrane proteins of *E. coli* involved in adaptive solute transport with emphasis on proteins involved in potassium (K^+) uptake and efflux and amino acid exporters. Regulatory mechanisms concerned with the above are also being studied.

All life forms display a preference for the potassium ion (K^+) over the sodium ion (Na^+) as the predominant cytoplasmic cation. Uphill transport of K^+ into the cytoplasm and active extrusion of Na^+, contributes to this bias. *Escherichia* coli (*E. coli*) and members of Enterobacteriaceae utilize active K^+ uptake systems to mediate entry of K^+ into the cytoplasm. In bacteria K^+ is believed to exert regulatory effects on multiple physiological processes such as osmoregulation, pH homeostasis and regulation of enzyme activity. In E. coli the multi-subunit inducible Kdp transporter, the multi-component Trk transporter and the stand alone Kup transporter, member proteins of distinct protein families, constitute the active K^+ uptake (transporter) systems.

Prior studies from this laboratory implicated the activity of YcgO, a putative K^+/H^+ antiporter belonging to the CPA1 family of monovalent cation / proton exchangers, to be rendered cryptic by dephospho-PtsN. PtsN is the terminal phospho-acceptor of a three protein phosphorelay comprising PtsP-PtsO-PtsN. These studies offered an explanation for the paradoxical phenotype of K+ limited growth (K^L) of the Δ*ptsN* mutant in a synthetic medium with high (115 mM, K115) K^+ concentration ($[K^+]e$) but not in a medium with low (1 mM, K1) $[K^+]e$. We have proposed that in the Δ*ptsN* mutant K^+ limitation occurs due to hyperactivation of YcgO mediated K^+ efflux owing to absence of dephospho-PtsN. This is not seen in K1 medium because the inducible expression of the Kdp transporter, counteracts YcgO mediated K^+ limitation and repression of its expression and/or activity in K115 medium contributes to maintenance of the K^+ limitation.

During the course of the aforementioned studies, linkages between the auxiliary components of protein secretion namely the *YajC SecD* and *SecF* proteins, the membrane insertase YidC on one hand and K^+ metabolism on the other, have been identified. Our earlier studies with a particular lesion in yajC (*yajC**) the first gene of the *yajC secD secF* operon studies have shown that perturbed *SecD/F* function leads to a K^+ requirement (K^{Req}) in low [K+] e medium, whereas perturbed *YajC-SecD-SecF* function permits adaptation to K^+ limitation via a mechanism not involving active K^+ uptake transporters. Our recent studies have shown that the KReq occurs due to impaired K^+ transporter function and is alleviated by the absence of the protease HslVU.

The *yajC** mutation led to a modest reduction in the levels of TrkG and TrkH, the K^+ conducting component of the Trk transporter and Kup. Reduction in the levels of TrkG, TrkH and Kup caused by the *yajC** mutation were mitigated to some extent by the absence of HslVU. Furthermore, *yajC** led to reduced rates of K^+ uptake occurring via the Kup and the Trk K^+ transporters, that was alleviated by the absence of HslVU. Evidence from other studies indicated that alleviation of the K^{Req} by the Δ*hslV* mutation required the presence of K^+ transporters. Overexpression of the membrane insertase YidC was also found to alleviate the K^{Req} that was dependent upon the presence of K^+ transporters. Moreover, YidC overexpression partially mitigated the reduction in the levels of TrkH and Kup caused by the *yajC** mutation.

These studies indicate that perturbed SecD/F function directly or indirectly leads to impaired membrane biogenesis of K^+ transporters, leading to the K^{Req}. In mutants with perturbed SecD/F function the process of membrane biogenesis of K^+ transporters is attenuated that renders them prone to degradation by the HslVU protease. Removal of HslVU restores the process of membrane biogenesis that operates at an attenuated level. It is suggested that YidC overexpression might alleviate the attenuated process of membrane biogenesis of K+ transporters by establishing better linkage with the Sec translocon. Alternatively, it is possible that SecD/F and YidC may function in an overlapping manner for mediating membrane biogenesis of K^+ transporters such that a deficiency of SecD/F can be compensated by overexpression of YidC or that YidC overexpression leads to an increase in the efficiency of SecD/F function. To directly test the notion whether SecD/F and YidC participate in the membrane biogenesis of Kup and TrkH, we have constructed Kup and TrkH variants that bear the m-NeonGreen fluorescent protein abutted to their C-termini. We have constructed strains in which expression of components of inner membrane protein biogenesis namely SecE, Srp, YajC-SecD-SecF and YidC is under the control of the L-arabinose inducible P_{ara} promoter. Currently we are testing the effects of depletion of the aforementioned components of membrane protein biogenesis, on the localization of Kup and TrkH m-NeonGreen hybrid proteins.

Publication:

Dubey S, Majumder P, Penmatsa A, Sardesai AA. (2021) Topological analyses of the L-lysine exporter LysO reveal a critical role for a conserved pair of intramembrane solvent-exposed acidic residues. J. Biol. Chem. 4;297(4):101168.

Laboratory of Bacterial Genetics : Group of Dr Abhijit A. Sardesai

Laboratory of Bacterial Genetics

Studies on the physiological functions modulated by the stringent response factors (p)ppGpp/DksA in *Escherichia coli.*

Principal Investigator : R. Harinarayanan
Staff Scientist

Laboratory of Bacterial Genetics:

R. Harinarayanan	Staff Scientist
Vani Singh	SRF
Hafeezunnisa	Project Associate
Shaffiqu	Technical Officer

Escherichia coli is a model bacterium amenable to experimental manipulation. We are using it for addressing fundamental questions in bacterial physiology. We are studying processes regulated by the modified nucleotides (p)ppGpp and its protein co-factor DksA, popularly known as the stringent response factors. We are also investigating the metabolic significance of having a link between the pentose phosphate pathway and glycolysis. The objectives of our study in the present reporting period are,

1. To decipher the role of (p)ppGpp in the interplay between fatty acid metabolism, cell size and cell division in Escherichia coli.
2. To understand the physiological significance of the metabolic flexibility provided by the transketolase function in Escherichia coli.

Studies to understand the role of (p) ppGpp in the interplay between fatty acid metabolism and cell division.

In E. coli, metabolism of the modified nucleotides (p)ppGpp is primarily governed by three enzymes, namely, RelA, SpoT and GppA. RelA and SpoT are (p)ppGpp synthases and SpoT is also a (p)ppGpp hydrolase that converts ppGpp and pppGpp to GDP and GTP respectively. GppA is a hydrolase that converts pppGpp to ppGpp. Multiple studies have presented evidence that transcriptional regulation by (p)ppGpp was facilitated by the RNA polymerase binding protein DksA. In previous work, we had identified two phenotypes using mutant strains of E. coli, (i) the cell division defect of ftsZ84 mutant was accentuated by the loss of relA function (ii) the loss fadR function conferred synthetic growth defect in strains compromised for (p)ppGpp synthesis. Studies undertaken in this reporting period to characterize the molecular basis of these phenotypes are described below.

We had previously reported on the cell division defect that arose from the combined loss of Lon protease and (p)ppGpp during growth on rich medium. Our results indicated that the cell division defect was due to reduction in FtsZ protein concentration and consequent inhibition of FtsZ protein activity by SulA. However, the reduction in FtsZ level, as compared to wild type strain, was mild (~30 %). FtsZ protein function is essential for cell division, it forms a ring shaped polymeric structure at the site of cell division and is needed for generating the force necessary for cell constriction. We reasoned that phenotypes associated with mild reduction in FtsZ concentration may become more apparent in a genetic background with hypomorphic FtsZ allele, as the protein encoded by the allele would have reduced function. We used the ftsZ84 allele that exhibits conditional growth defect that follows from the cell division defect. Growth of the mutant can be impaired by increase in growth temperature and reducing the osmolarity of growth medium (achieved using salt or non-ionic solute such as sucrose). We observed that in strains with reduced or no (p)ppGpp, or in the absence of DksA, the growth defect was accentuated, that is, growth defect was now observed at normally permissive growth temperature. We are in the process of

characterizing genetic suppressors that rescue the growth defect to elucidate the role of (p)ppGpp/DksA in the process of cell division.

FadR is a protein involved in fatty acid metabolism. While the acronym FadR reflects the proteins role as a repressor of genes involved in fatty acid degradation, later studies have highlighted its role in the positive transcriptional regulation of overall fatty acid biosynthesis and especially the two genes involved in biosynthesis of unsaturated fatty acids, namely, fabA and fabB. The FadR protein activates transcription of all genes involved in fatty acid biosynthesis and represses genes involved in fatty acid degradation (β-oxidation). Recent reports have suggested a link between the fatty acid biosynthetic capacity of cells and its size - a decrease in fatty acid biosynthesis being associated with decreased cell size. Cell size control is also an intrinsic feature of the cell cycle. In bacteria, cell growth and division are thought to be coupled through a cell size threshold. We employed the ftsZ84 allele described above to delineate the roles of fatty acid metabolism and (p)ppGpp in the regulation of cell division and cell size.

We tested the effect of two mutations, namely, ΔfabH and ΔfadR both of which can be expected to lower fatty acid biosynthesis on the growth phenotypes of the ftsZ84 mutant. While the loss of fabH function did not affect growth, loss of fadR function rescued the growth defect under non-permissive growth conditions. These results suggested, limiting the fatty acid biosynthetic capacity in the cell by any means may not alleviate of division defect. While loss of fadR or fabH can lower synthesis of fatty acid, fadR function is uniquely required for the synthesis of unsaturated fatty acid (UFA) and it has 1/3rd the amount of unsaturated fatty acid as compared to the wild type strain. It is possible the altered UFA levels could be responsible for the rescue of division defect. Further studies to understand the role of UFA in the process of cell division are in progress.

Perturbation of fatty acid biosynthesis in (p)ppGpp deficient cells elicit synthetic cell division defect

We had earlier reported that, the (p)ppGpp deficient strain exhibited synthetic lethal phenotype following the loss of fabH function that can significantly slow down the rate of initiation of fatty acid biosynthesis. We therefore studied the effect of fadR deletion, which has been reported to lower the overall rate of fatty acid biosynthesis and as well as the composition of fatty acid in the membrane. Introduction of fadR deletion into the (p)ppGpp deficient strains conferred strong growth defect in the ppGpp0 strain at 30°C and in the ΔrelA mutant at 25°C. Both strains did not exhibit growth defect at 42° C. Based on the rescue of growth defect by the increase in growth temperature and the reduction in unsaturated fatty acid content of the membrane expected from loss of FadR function, we hypothesized the following. Growth defect followed from loss of membrane fluidity due to decrease in unsaturated fatty acid content and increase in the growth temperature rescued growth by increasing the fluidity of the membrane despite the lower level of unsaturated fatty acid. This implies, strains lacking (p)ppGpp or with lower level of (p)ppGpp are more sensitive to decrease in membrane fluidity.

To test this hypothesis, we asked if supplementation of unsaturated fatty acid (USF) to the growth medium, which can be expected to increase the USF content of the membrane would rescue the growth defect. Of the fatty acids tested, palmitoleic acid (16:1) but not palmitic acid (16:0) rescued the growth defect. Interestingly, oleic acid (18:1) did not rescue the growth defect. Since FadR is transcriptional activator of the FabA and FabB genes, the reduced expression of these genes, both of which are required uniquely for UFA synthesis, is believed to be primarily responsible for the reduction in UFA in fadR mutant. Expression of fabA or fabB from plasmid rescued the growth defect which further reinforced the evidence for growth defect being the consequence of decrease in unsaturated fatty acid.

Using an E. coli genomic DNA library made with multi-copy plasmid, we screened for genes that rescued the growth defect of ppGpp0 fadR strain. By sequencing the plasmid DNA flanking the insert, the genomic DNA fragment present in the plasmid clone that rescued growth defect was identified. This fragment among other genes carried gnsA that was reported to rescue the growth defect of fabA2 temperature sensitive allele at the non-permissive temperature. In addition to gnsA, its sequence homolog gnsB has also been reported to rescue the fabA2 allele. The mechanism of rescue by these genes is unclear, however, the sequence of the genes suggest they are unlikely to be directly involved in the biosynthesis of fatty acids. We found

the heterologous expression of gnsA or gnsB from plasmid rescued the growth defect of the ppGpp0 fadR strain. Measurement of fatty acid composition by fatty acid methyl ester (FAME) analysis showed that expression of each gene was able to restore the fatty acid composition of the fadR mutant to that seen in the wild type strain. This result once again reinforces the idea that decrease in UFA that is normally not detrimental to the growth of wild type strain confers growth inhibition in strains lacking or having reduced (p)ppGpp. This suggests that, (p)ppGpp may help E. coli adapt to conditions that lower UFA.

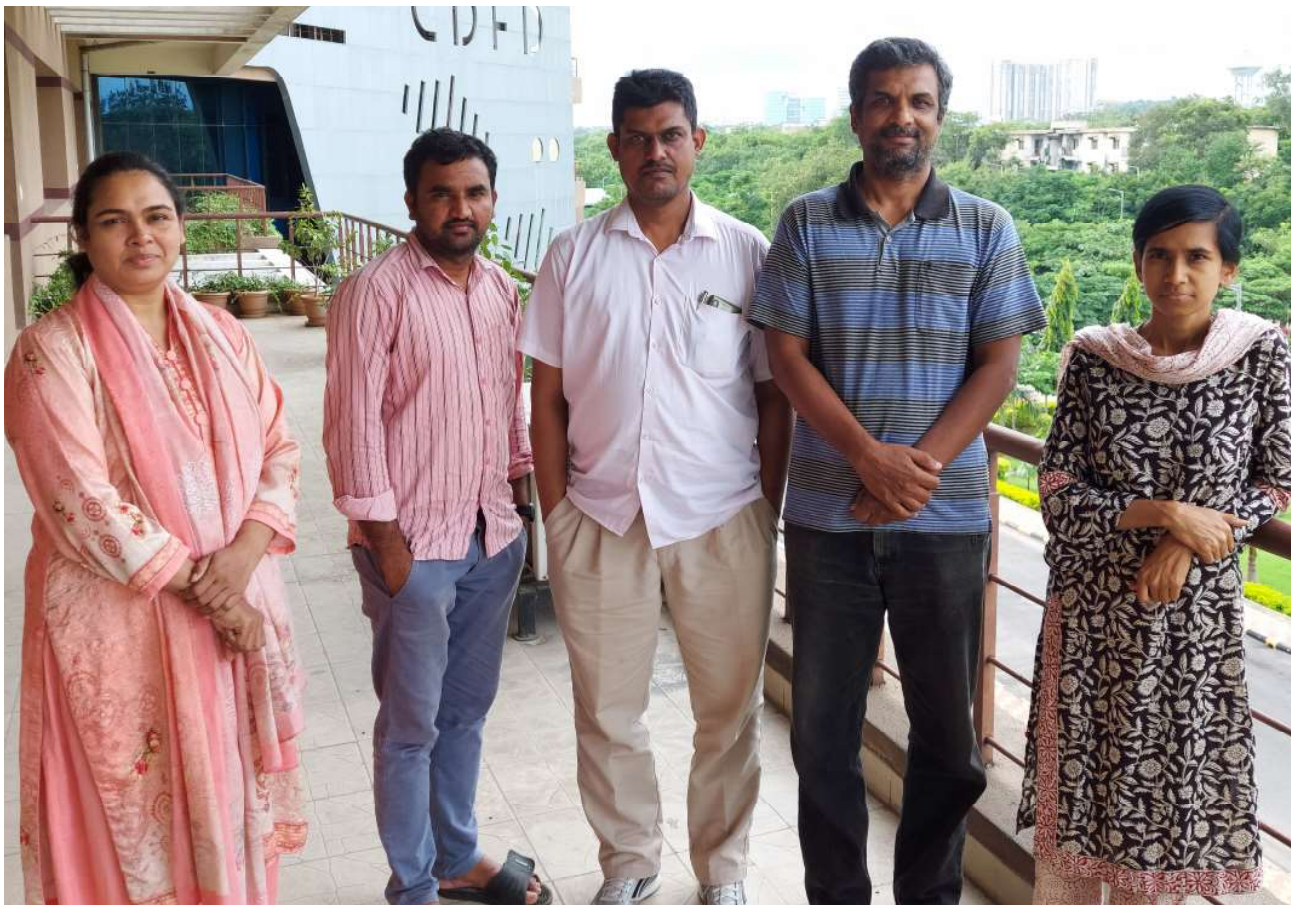
The experiments described above have primarily addressed the role of unsaturated fatty acid metabolism in the growth of (p)ppGpp deficient strains. In experiments describe below, we looked at (p)ppGpp mediated regulations that are important for the growth of strains having reduced unsaturated fatty acid. The modified nucleotides (p)ppGpp is a global regulator and studies have revealed the role of the molecules in the regulation of transcription, translation and protein activity. In transcription, the regulation is primarily exerted at the level of initiation, but evidence for regulation of elongation also exists and these regulations occur in concert with the RNA polymerase binding protein, DksA. There is evidence for regulation of translation at the level of initiation and elongation by (p)ppGpp. Regulation of protein activity is mediated mainly through the binding of (p)ppGpp to proteins with GTPase activity, which is believed to be due to the structural similarity between ppGpp/pppGpp and GDP/GTP. Multiple lines of genetic and biochemical evidence highlighting the role of (p)ppGpp in transcription initiation have been obtained by different laboratories. We used RNA polymerase mutations obtained in these studies to ask if the loss of (p)ppGpp mediated regulation of transcription was important for the growth defect of the ppGpp0 fadR and relA fadR strains.

Two kinds of RNAP mutations were used in the study, (i) those that rescued the multiple amino acid auxotrophy of the (p)ppGpp0 strain and (ii) RNAP mutations reported to lead to the loss of (p)ppGpp binding to RNAP. Generally mutations in the first class confer rifampicin resistance and do not map to the reported (p)ppGpp binding sites. These are expected to be gain-of-function mutations that mimic properties of (p)ppGpp bound RNAP in the absence of (p)ppGpp. Three mutations from this class were used and each one was found to rescue the ppGpp0 fadR synthetic lethality. RNAP mutations from the second class that abolish (p)ppGpp binding to RNAP in vitro when present in the fadR genetic background did not phenocopy the growth defect of the ppGpp0 fadR strain. Together these results indicate, (i) (p)ppGpp mediated transcriptional regulation was required for the viability of strains lacking FadR function (ii) the (p)ppGpp binding site mutants used in this study may not completely abolish the (p)ppGpp mediated transcriptional regulation in vivo.

Studies to understand the physiological significance of metabolic flexibility provided by transketolase function within the central carbon metabolic pathways of Escherichia coli.

Transketolase activity encoded by the tktA and tktB genes provides reversible link between glycolysis and pentose phosphate pathway, two major arms of central carbon metabolism. It supports synthesis of the glycolytic intermediates fructose-6-P and glyceraldehyde-3-P from ribose-5-P and xylulose-5-P, which are intermediates of the pentose phosphate pathway, and synthesis of xylulose-5-P and erythrose-5-P from the above-mentioned glycolytic intermediates, depending on the direction of metabolic flux. By using a method to deplete transketolase activity we studied the growth properties of transketolase mutant under glycolytic and gluconeogenic growth conditions and the genetic functions required for growth.

These studies showed, (i) transketolase deficient strains exhibit ribose sensitivity that was alleviated through the glycolytic catabolism of glucose, gluconate and glycerol; (ii) GAPDH (glyceraldehyde-3-P dehydrogenase) dependent NADH synthesis was required for growth of tranketolase deficient strain when glucose was catabolized solely through the oxidative pentose phosphate pathway; (iii) either transketolase or UdhA (soluble transhydrogenase that support NADH synthesis together with oxidation of NADPH) activity was required for growth when using amino acids as carbon source (gluconeogenic growth condition). These results provide genetic evidence that transketolase activity can contribute to maintenance of NADH pool depending on the growth condition.



Laboratory of Bacterial Genetics : Group of Dr. R. Harinarayanan

Laboratory of Cell Cycle Regulation

Elucidating the role of chromatin modifying proteins in cell cycle regulation

Principal Investigator: Shweta Tyagi
Staff Scientist &
DBT-Wellcome Trust
IA Senior Fellow

PhD Students:

Kausika Kumar Malik	Senior Research Fellow
Akash Nitin Chinchole	Senior Research Fellow
Kaiser Ahmed Lone	Senior Research Fellow
Aditi Arora	Senior Research Fellow
Payal Kataria	Senior Research Fellow
Avishek Katariya	Junior Research Fellow
Bijaya Ta	Junior Research Fellow

Other Members:

V N Sailaja	Technical Officer
Deepshika Pulimamidi	Project-JRF
Neeraja H	Project-JRF (since Jan. 2022)
Sree R C Sridhara	Research Associate
Geethanjali Ravindran	Research Associate

Collaborator:

Debabrata Biswas	Indian Institute of Chemical Biology, Kolkatta
Sanjeev Galande	Indian Institute of Science Education and Research, Pune
Himanshu Goel	Hunter Genetics, New South Wales, Australia

Objectives:

1. Role of MLL in regulation of repetitive non-coding regions.
2. Investigate how H3K4 HMTs regulate cell shape and cell migration.

Project 2: Investigate how H3K4 HMTs regulate cell shape and cell migration.

Leukemia or blood cancer can be caused due to multiple reasons. One such reason is when a gene called Mixed Lineage Leukemia (MLL) located on chromosome 11 breaks from between and both halves of this gene fuse with random regions of other chromosomes. This process is called translocation and it gives rise to 'unnatural' fusion proteins. These fusion proteins are believed to cause leukemia. Sadly, this type of leukemia is mostly found in infants and children. Often these children have poor prognosis and do not respond well to standard therapies of leukemia.

It has been puzzling the researchers how these random translocations with more than 100 different regions (in MLL-based leukemia) produce the same disease? The function assigned to MLL in 'normal' cell is transcription. It is believed that MLL-fusion protein also participates in transcription and deregulate it. The cure for this kind of leukemia will only be effective once we fully understand about the MLL protein and then apply that knowledge to appreciate which processes the MLL-fusions proteins are disturbing.

As cell division is intimately linked to cancer, we decided to look if MLL has any role in this process.

Details of the progress made in the current reporting year (April 1, 2021 –March 31, 2022)

MLL is present in most cells of the body. Hence to study its function, we artificially create cells where MLL is destroyed by siRNA technology. After siRNA treatment, the levels of MLL are very low (20-30%) and observing these cells can help us understand which processes are disturbed. By correlation, MLL is required in those processes.

Attainment of proper cell shape and regulation of cell migration are essential processes in development of an organism. Same processes, if deregulated,

are associated with disease conditions like chronic inflammation and cancer metastasis. In a recently initiated project, we investigate the role of H3K4 HMTs in determining cell shape.

Mammalian cells attain various complex shapes ranging from flat, tightly adhered epithelial cells to round leukocytes. To understand the role of H3K4 HMTs in regulating cell shape, we performed RNAi experiments using specific MLL siRNA. Cell shape is governed primarily by actin cytoskeleton and intermediate filaments. Therefore, we stained the cells with rhodamine dye-conjugated-phalloidin, a peptide toxin known to bind filamentous actin (F-actin) with high-affinity. Phalloidin-stained actin stress fibers could be clearly visualized in control siRNA-treated cells (Figure 1, panel a-c). In contrast, in MLL depleted cells, we observed a dramatic loss of visually discernible actin stress fibers (Figure 1). Upon MLL knock down, we observed: i) elongated cells with both long, prominent as well as multiple

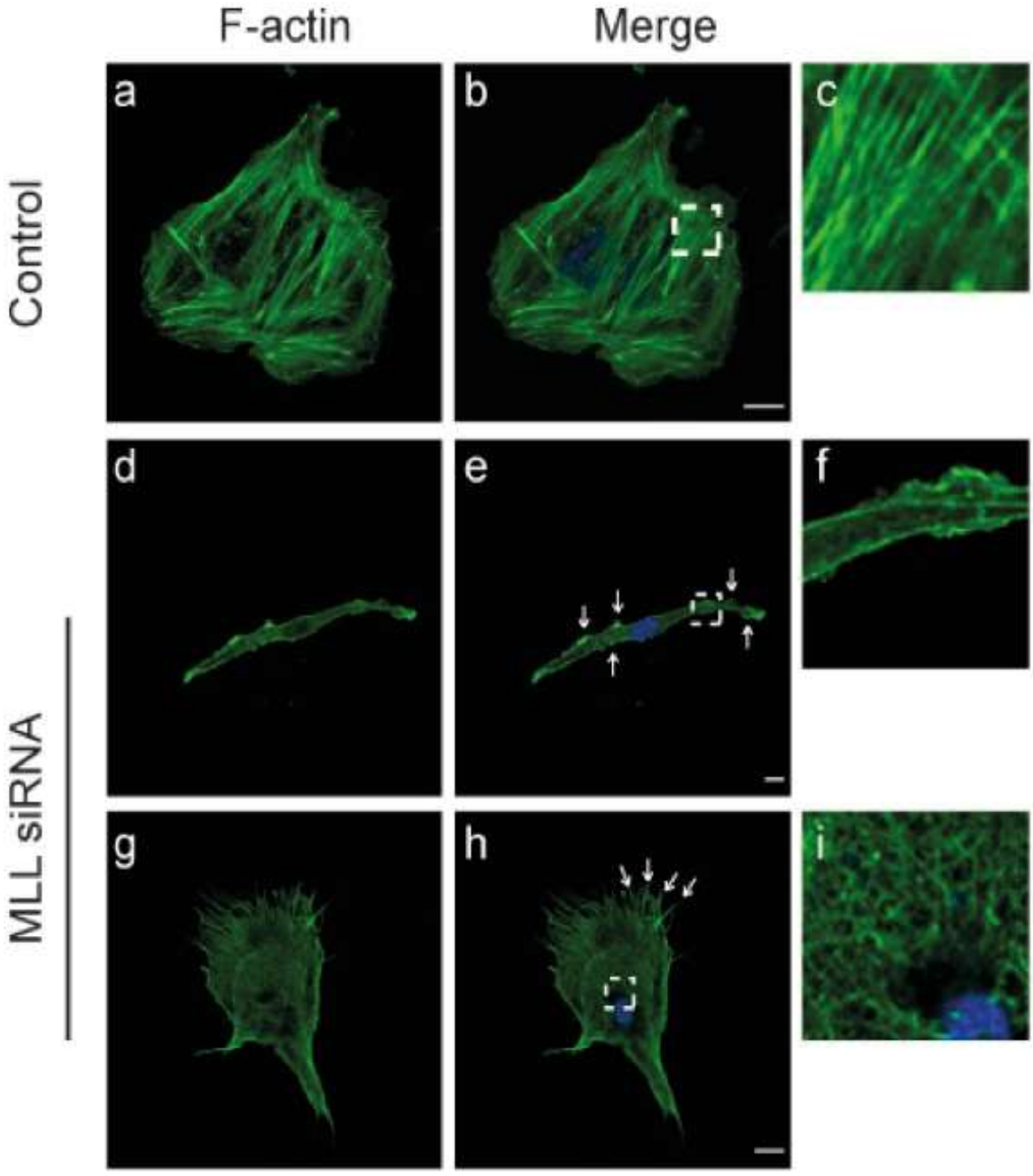


Figure 1. Loss of MLL results in impaired actin stress fiber formation. Cells show actin stress fibers upon staining with phalloidin peptide (see Control). These actin stress fibers are one of the key determinants of cell shape and cell migration. Upon treatment with MLL siRNA, the actin stress fibers are lost. Also note the shape of cell in presence and absence of MLL and formation of small protrusions in latter.

small protrusions (Figure 1, middle panel), or ii) triangular cells with number of filopodia (Figure 1, bottom panel). In both types of cell shapes we could not detect any actin fibers. As the cell shape and actin cytoskeleton were dramatically perturbed upon knock down of MLL, we suspected that loss of MLL would affect cell migration as well.

Publication:

1. Sugeedha J*, Gautam J* and Tyagi S. (2021) SET1/MLL Family of Proteins: Functions beyond histone methylation. Review. (*equal authors). Epigenetics. 16(5); 469-487, doi: 10.1080/15592294.2020.1809873.PMID: 32795105

2. Malik K K, Sridhara S C, Lone K A, Katariya P D and Tyagi S. (2022) KMT2 family members regulate H3K4 methylation to ensure kinetochore activity at human centromeres. BioRxiv doi: https://doi.org/10.1101/2022.06.20.496844

Laboratory of Cell Cycle Regulation

Laboratory of Cell Death & Cell Survival

Functional protein networks controlling cellular pathways and their role in human diseases

Principal Investigator: Maddika Subba Reddy
Staff Scientist &
Wellcome Trust-DBT IA
Senior Fellow

PhD Students:

Prajakta Tathe	Senior Research Fellow
Vaishna V	Senior Research Fellow
Hilal A Reshi	Senior Research Fellow
Devanshi Gupta	Senior Research Fellow
Rahul Baroi	Senior Research Fellow
Keshav Gupta	Senior Research Fellow
Himanshu Darji	Senior Research Fellow
Dhruv Gohil	Senior Research Fellow

Other Members:

Dev Ashish Giri	Postdoctoral fellow
Adithya Pallepati	Project Associate II
Devika Prakash	Project Associate I
Meera Mahendran	Dissertation trainee
Nanci Rani	Technical Assistant

Objectives of the lab

1. To identify new cellular functions for phosphatases and assess their role in human diseases
2. To map the functions of ubiquitin system in cells and evaluate its aberrations in human diseases

Research Summary

Theme 1: Functional phosphatase network in cells

Proteins in general are synthesized as inactive molecules in the cells. Once synthesized, they need to be modified to mediate their functions. Phosphorylation (attachment of a chemical group of phosphate) is one such protein modification required for them to function in the cell. Kinases are the enzymes, which add phosphate group to the proteins, while phosphatases are enzymes that oppose this process. Phosphatases play a crucial role in biological functions and controls nearly every cellular process, including metabolism, gene transcription, translation, cell-cycle progression, protein stability, signal transduction, and apoptosis. Phosphatases are so far studied in isolation to assess their function in the cell, but in reality, they work in a network of protein complexes. In this theme, we aim to map the functional phosphatase network with the identification of interacting partners of every phosphatase in human cell. By using a biochemical and proteomic approach we identified the associated protein complexes of more than 140 phosphatases. During earlier years, we assigned several novel cellular functions to different phosphatases based on their interacting partners. During this year, we characterized the role of phosphatases associated with chromatin. In this work, we demonstrated that a tyrosine phosphatase SHP-1 dephosphorylates histone H2B and plays a critical role during transition of initiation to elongation stage of transcription (EMBO J 2022). SHP-1 associates with Paf1 complex at chromatin and dephosphorylates H2B at tyrosine 121 residue. Knock out of SHP-1 or presence of constitutively phosphorylated Y121 on H2B leads to reduction in genome wide H2B ubiquitination that subsequently causes defects in RNA Pol II dependent transcription. Functionally, we show that SHP-1 dependent H2B dephosphorylation maintains basal autophagic flux in cells through efficient transcription of autophagy and lysosomal genes. Collectively, our study revealed an important modification of histone H2B regulated by SHP-1 that has an essential role during eukaryotic transcription (Figure 1).

Theme 2: Network of ubiquitin system

Ubiquitin is a small protein that attaches to

other proteins via a covalent addition. Similar to phosphorylation, ubiquitin attachment to substrate proteins acts as a regulatory protein modification. Ubiquitin attaches to target proteins through the activity of three different sets of enzymes: ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). Ubiquitin E3 ligases are the most critical enzymes in this pathway where they facilitate the activation and transfer of ubiquitin either directly to the target protein or to other ubiquitin proteins that already have been attached to the target protein. Ubiquitin linked to the substrates serves as a molecular tag that marks proteins for either degradation by proteasome (a multi-subunit complex that degrades proteins in cells) dependent pathway or to function in wide variety of processes in a proteasome independent manner. When a chain of more than one ubiquitin molecule attaches to the same target protein, that protein is said to be poly-ubiquitinated. Poly-ubiquitin chains appear to serve multiple purposes, of which the best understood is marking target proteins for degradation through the proteasome. However, seven different kinds of ubiquitin-ubiquitin attachments are possible in the cell that can provide wide variety of topologies, each of which signal a different outcome. In this theme, we are interested in identifiying new functions for ubiquitin system by mapping the interaction network of different E3 ligases as well as various ubiquitin chain types in cells. We have reported several new complexes in this pathway during previous years. In the current reporting year, we characterized a new function for ubiquitin linkage in cells. We established that addition of a non-canonical ubiquitin linkage (K63 type) plays an essential role in liquid-liquid phase separation of Dvl2 protein, a central regulator of Wnt signaling. We identified that liquid-liquid phase separation of Dvl2 mediated by E3 ligase WWP2 as a major driving mechanism for promoting Wnt signaling.

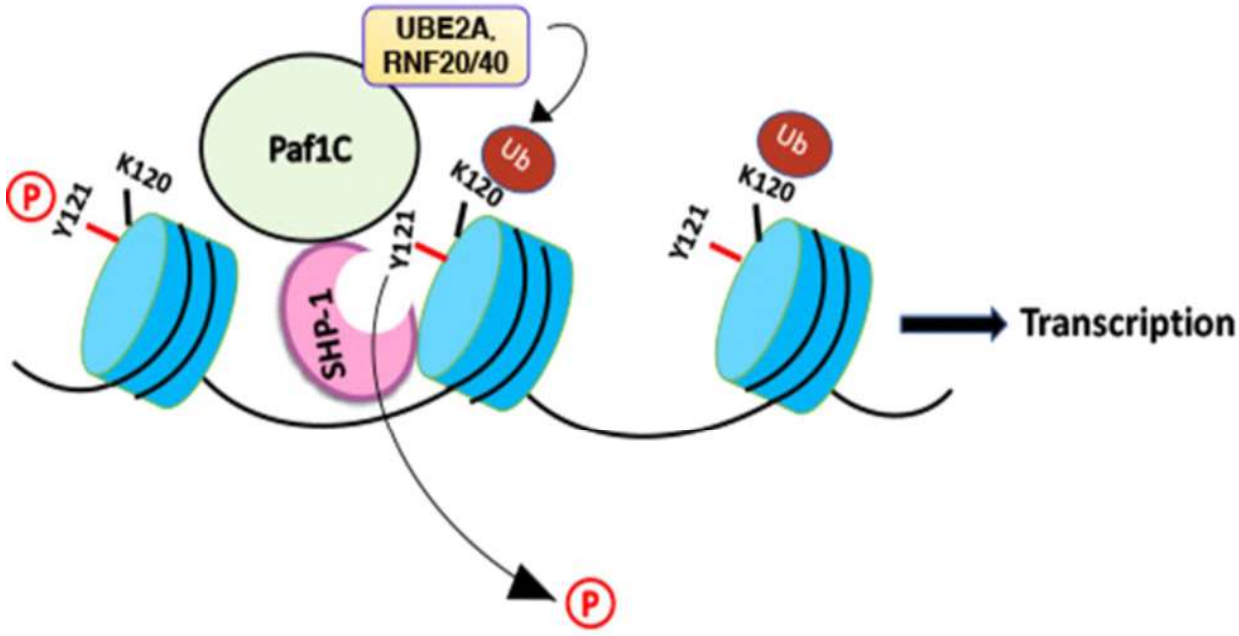


Figure-1: A model depicting the role of tyrosine phosphatase SHP-1 at chromatin during transcription

Publications

1. Palicharla VR, Gupta D, Bhattacharya D, Maddika S (2021). Ubiquitin-independent proteasomal degradation of Spindlin-1 by the E3 ligase HACE1 contributes to cell-cell adhesion. FEBS Lett. 595(4): 491-506.

Laboratory of Cell Death & Cell Survival

Laboratory of Cell Signalling

Investigating the functions of phosphate-rich biomolecules in eukaryotic cells

Principal Investigator: Rashna Bhandari
Staff Scientist

PhD Students:
Shubhra Ganguli
Akruti Shah
Jayraj Sen
Arpita Singh
Jayashree S.Ladke
Manisha Mallick
Tanmay Mohanty
Anindita
Shrutika S Padwal
(joint student with Ashwin B Dalal)

Other Members:
Ruth Manorama R
Suma Katta

Collaborators:

Henning Jessen	University of Freiburg, Germany
Dorothea Fiedler	FMP, Berlin, Germany
Kana M. Sureshan	IISER, Thiruvananthapuram
Manish Jaiswal	TCIS-TIFR, Hyderabad

Our laboratory studies the biochemical, cellular and physiological functions of two phosphate-rich biomolecules: (i) the inositol pyrophosphate, 5-IP7 (5PP-IP5), and (ii) inorganic polyphosphate (polyP). Our broad objectives are (a) to understand the cellular processes by which the levels of these small molecules are regulated, and (b) investigate the cellular and physiological processes that these phosphate-rich molecules influence.

Cellular functions of inositol pyrophosphates

5-IP7 is synthesised from IP6 and ATP by a family of enzymes known as inositol hexakisphosphate (IP6) kinases, of which there are three isoforms in mammals – IP6K1, 2, and 3. We utilise mammalian cell lines and knockout mouse strains as model systems to investigate the signalling and metabolic pathways that are altered when 5-IP7 levels are perturbed.

We have previously reported that IP6K1 supports homologous recombination-mediated DNA repair in mouse embryonic fibroblasts, and that this effect is dependent on 5-IP7 synthesis by IP6K1 (Jadav *et al.*, J. Biol. Chem. 2013). We used U-2 OS cells expressing shRNA directed against IP6K1 (shIP6K1) as a model system to investigate the molecular mechanism by which 5-IP7 regulates homologous recombination (HR) mediated DNA repair. We found that synthesis of 5-IP7 by IP6K1 is necessary for cells to recover from DNA damage induced by the inter-strand crosslinker mitomycin C. It is known that a reduction in the interaction between the C-terminal domain (CTD) of BRCA2 and the HR marker protein RAD51 helps dislodge RAD51 from the sites of DNA damage post-repair. We observed that expression of active, but not inactive, IP6K1 in cells reduces the BRCA2 CTD – RAD51 interaction. RAD51 obtained from mammalian cells lacking active IP6K1 displayed enhanced binding with GST-tagged BRCA2 CTD expressed in bacteria. Conversely, the enzymatic activity of IP6K1 did not influence the binding of BRCA2 CTD obtained from mammalian cells to GST-tagged RAD51 expressed in bacteria. These data suggest that 5-IP7 synthesized by IP6K1 post-translationally modifies RAD51 to reduce its interaction with BRCA2 CTD, perhaps ensuring the removal of RAD51 from the DNA damage foci after repair.

Cellular and physiological functions of IP6K1

We have shown that male mice lacking IP6K1 display

spermiogenesis failure which is attributed to a defect in the formation of spermatid ribonucleoprotein (RNP) granules called chromatoid bodies (Malla and Bhandari, J. Cell Sci. 2017). The functional analogue of chromatoid bodies in somatic cells are called processing (P)- bodies. These cytoplasmic RNP granules are sites for mRNA storage and harbour proteins involved in suppression of translation. We noted that P-body granules are nearly absent from the bronchiolar epithelia of Ip6k1 knockout mice, and from IP6K1 depleted U-2 OS cells. Expression of either active or catalytically inactive versions of IP6K1 was essential to form P-bodies. Since IP6K1 did not co-localize with P-bodies, we probed whether protein-protein interactions by IP6K1 maintains P-bodies. We found that IP6K1 binds to the mRNA decapping complex on ribosomes. We observed that IP6K1 also interacts with the translation initiation complex eIF4F, which is known to bind to the mRNA cap. An early event in the formation of P-bodies is the exchange of proteins bound to translationally stalled mRNA – the eIF4F complex bound to the mRNA cap is replaced with the mRNA decapping complex. Our data suggests that IP6K1 facilitates this proteome exchange on the mRNA cap to promote the formation of P-bodies (Shah and Bhandari, J. Cell Sci. 2021).

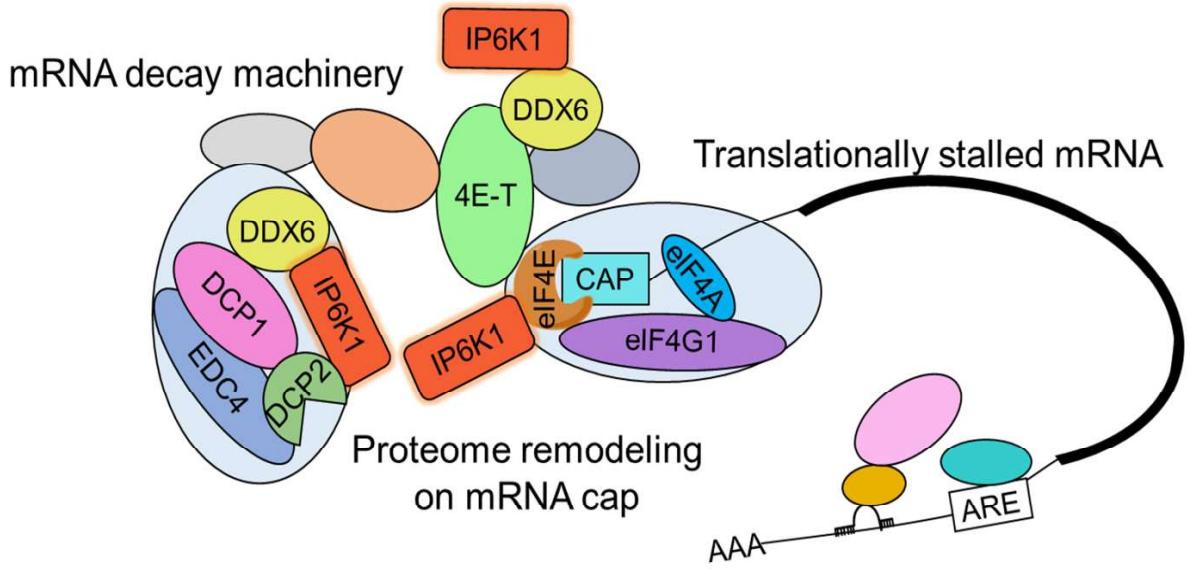


Figure 1. IP6K1 associates with the mRNA decapping complex via DCP2 and DDX6, and with the translation initiation complex via eIF4E. Other proteins that constitute the mRNA decapping complex and the translation initiation complex (shown inside light blue capsules) interact directly or indirectly with IP6K1.

Publications

1. Shah A and Bhandari R† (2021). IP6K1 upregulates the formation of processing bodies by influencing protein-protein interactions on the mRNA cap. Journal of Cell Science 134: jcs259117
2. Shukla A, Kaur M, Kanwar S, Kaur G, Sharma S, Ganguli S, Kumari V, Mazumder K, Pandey P, Rouached H, Rishi V, Bhandari R, and Pandey A.K.† (2021) Wheat inositol pyrophosphate kinase TaVIH2-3B modulates cell-wall composition and drought tolerance in Arabidopsis. BMC Biology 19: 1-23
3. Mohanrao R, Manorama R, Ganguli S, Madhusudhanan MC, Bhandari R† and Sureshan KM† (2021) Novel substrates for kinases involved in the biosynthesis of inositol pyrophosphates and their enhancement of ATPase activity of a kinase. Molecules 26: 3601
4. Lolla P*, Shah A*, Unnikannan CP, Oddi V and Bhandari R† (2021) Inositol pyrophosphates promote MYC polyubiquitination by FBW7 to regulate cell survival Biochemical Journal 478: 1647-1661. (Cover image).

†Corresponding author

*Equal author

Group of Laboratory of Cell Signalling

Laboratory of Chromatin Biology and Epigenetics

Understanding the functions and regulation of sirtuins in maintaining genomic integrity

Principal Investigator: Devyani Haldar
Staff Scientist

PhD Students:
Arijit Mallick Senior Research Fellow

Other Members:
Paresh Priyadarshan Rana
Sobhan Babu
Ghouse Sharif

Collaborators:
Viji Sarojini University of Auckland, New Zealand
Kuljeet Sandhu Assistant Professor, IISER Mohali

Objectives

Research in the laboratory is broadly aimed at understanding the molecular functions and mechanisms of regulation of Sirtuins during normal growth, proliferation of cells as well as under stress such as DNA damage. We use fission yeast, Schizoschharomyces pombe and human cell lines as model systems. Reversible acetylation/ deacetylation of proteins regulates numerous important cellular processes. The Sirtuin family NAD+ dependent protein/histone deacetylases (HDAC) are conserved from yeast to human cells carry out a broad range of crucial cellular functions ranging from transcriptional silencing to DNA damage response, cell cycle regulation, metabolism and aging etc. During DNA metabolic processes such as DNA replication and DNA repair, the expression level of specific Sirtuins are known to alter, indicating conditional regulation of these proteins. However, the molecular functions and mechanisms of regulation of sirtuins under many of these conditions remain elusive. There is a need to study these regulatory mechanisms as sirtuins are often deregulated in various diseases including cancer.

We are currently focused on the following objectives:

1) Investigation of novel molecular mechanisms by which sirtuins family protein deacetylases regulate DNA metabolic processes such as DNA replication and repair. We are also studying regulation of sirtuins during DNA replication stress response in fission yeast.
2) Understanding the molecular functions and regulation of human sirtuins in DNA Double Strand Break Repair pathways
3) Discovery of new epigenetic therapeutics targeted to sirtuin family histone deacetylases.

To understand the molecular functions and mechanisms of regulation of fission yeast sirtuin Hst4 in replication stress response.

DNA replication stress is one of the hallmarks of cancer. The DNA replication machinery encounters variety of obstacles during the unperturbed DNA replication including damaged template DNA and various difficult to replicate chromosome regions due to the presence of DNA secondary structures. These conditions stall the replication fork, generating replication stress. Recent studies have indicated that chromatin regulators may play active part in replication stress response. In fission yeast, Schizosaccharomyces pombe, a sirtuin family histone deacetylase (HDAC), Hst4, functions in the maintenance of genome stability by promoting cell survival upon replication stress. We have earlier reported that sirtuin hst4 deficient cells are sensitive to replication stress generated on methyl methanesulfonate (MMS) treatment and Hst4 is

downregulated during replication stress. However, the molecular mechanism and significance of this regulation is not known. The aim of this study is to decipher the molecular mechanism of regulation of Hst4 upon replication stress and significance of this degradation. We have discovered that DDK kinase phosphorylates and targets Hst4 for degradation by SCF complex upon replication stress. This degradation increase histone H3K56ac (target of Hst4) which is required for stabilization and recovery of stalled replication forks (Figure) through recruitment and stable association of fork protection complex (FPC) components Swi1 (Timeless, human homolog) and Mcl1 (hAND1) to the chromatin. In this work, we have discovered a novel mechanism for maintenance of genomic integrity during replication stress via induction of degradation of histone deacetylase Hst4 to stabilize the fork protection complex (FPC) for protecting stalled replication forks and promoting recovery of stalled forks following stress. Our results indicate that this mechanism is conserved in human cells. It is known that sirtuins and FPC components (Timeless and Claspin) are deregulated in cancer, therefore, these could be potential targets for anti-cancer therapeutics. This work has been published this year in high impact international journal eLife. Currently, we are working towards understanding the role of this novel regulatory mechanism human cells and how it contributes to cancer. We are also investigating how the increase in H3K56ac due to degradation of Hst4 stabilize the FPC.

Understanding the molecular functions and regulation of Human Sirtuins in DNA Double Stranded Break Repair Pathway

DNA double strands breaks are deleterious in nature, if not repaired can result in diseases such as Cancer. Histone modifications, especially, acetylation of various histones has been linked to DNA Double Strand break (DSB) Repair pathways. Nuclear sirtuins, SIRT1, SIRT3, SIRT6 and SIRT7 are known to function is DNA repair. How DNA repair pathway is selected for repair of specific types of DNA damage still remains elusive. End resection is a crucial step in the DNA repair of DSB repair pathway which is crucial for choice of DSB repair pathway. This is an important step which directs DNA repair to the pathway called homologous recombination (HR). In response to DNA damage, H3K56Ac is rapidly deacetylated by SIRT6, thereby reducing the level of this modification and facilitating the recruitment of other DNA repair enzymes to the damaged foci. We have observed that, absence of H3K56Ac hinders the recruitment of early DNA damage sensors. Our results indicate that in absence of ASF1, a chaperone needed for H3K56Ac, the process of end resection is severely affected. U2OS Cells lacking H3K56ac shows reduced number of foci of HR pathway proteins and poor ssDNA formation as a result of faulty End Resection during S phase of the cell cycle. This study deciphers a novel mechanism that affects HR which can be a target for various cancer treatments.

Discovery of new epigenetic therapeutics targeted to sirtuin family histone deacetylases.

Discovery of new epigenetic anti-cancer therapeutics targeted to sirtuin family histone deacetylases. Epigenetic therapeutics of cancer such as inhibitors of DNA methyltransferases and histone deacetylases (class I and classII) are already being used in combination with the standard cytotoxics with encouraging results. The Sirtuins (class III NAD-dependent deacetylases) are being considered as important targets for cancer therapeutics as they are up-regulated in many cancers. Inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to reduced growth of cancer cells. However, very few sirtuin inhibitors have entered into the clinic yet as an anticancer agent. In this project, we are working towards identifying novel small molecule inhibitors of Sirtuins and characterize their potential as anti-cancer agents using budding yeast as model system for compound screening. Our have discovered 4bb, a new class of human SIRT1 inhibitor and results suggest that inhibition of SIRT1 by 4bb induces apoptosis of colon cancer cells at least in part via activating p53 by preventing p53 deacetylation, increasing Bax expression and inducing caspases. Therefore, this molecule provides an opportunity for lead optimization and may help in development of novel, non-toxic epigenetic therapeutics for colon cancer. We have also identified very potent hit peptide inhibitors for sirtuins using yeast cell based reporter silencing assay. Our data indicates these peptides can inibit human SIRT1 and SIRT2. We are currently investigating mechanism of inhibition and testing the effect these peptides on different types of cancer cells and also working towards understanding their mechanism of action.

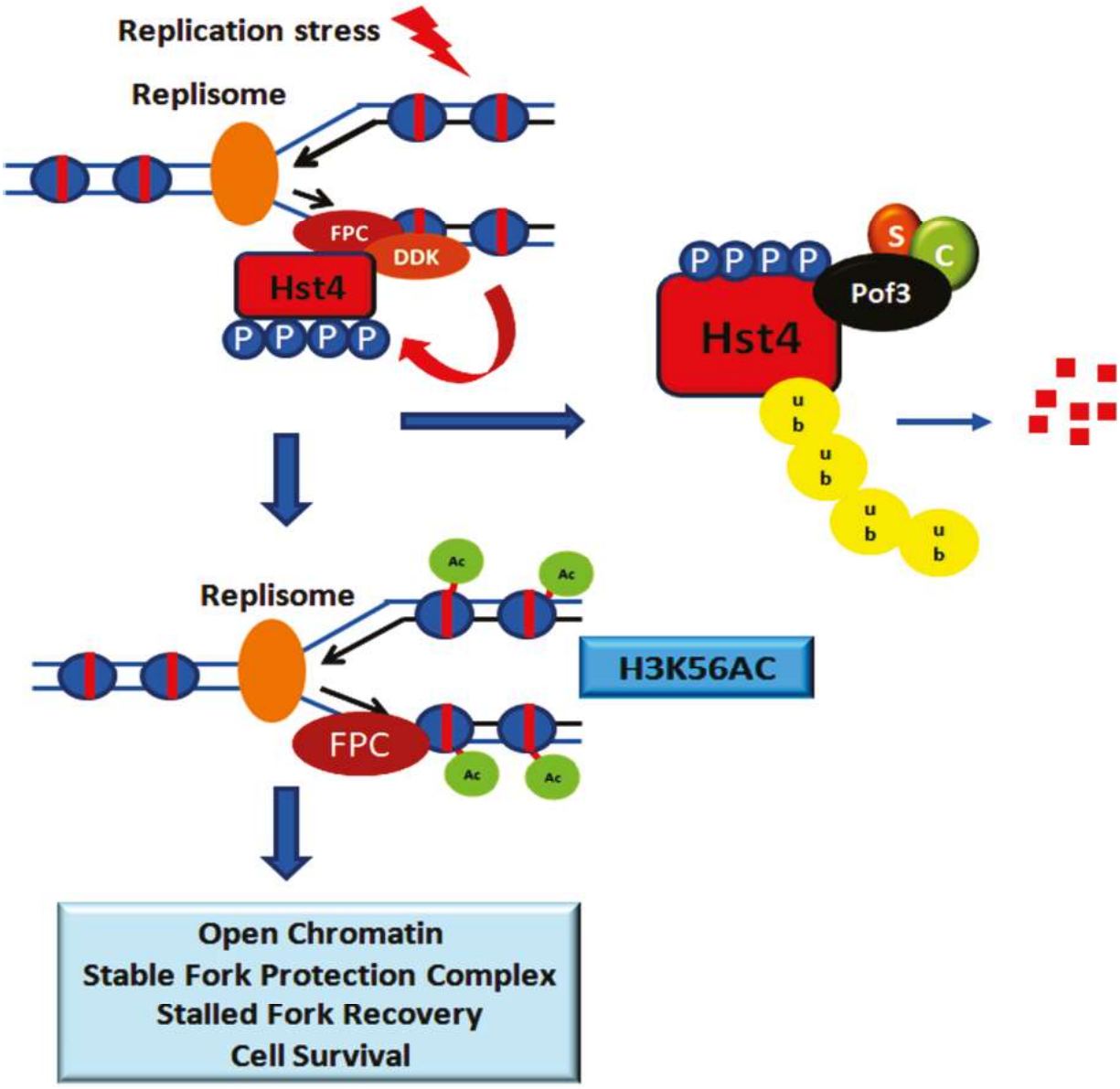
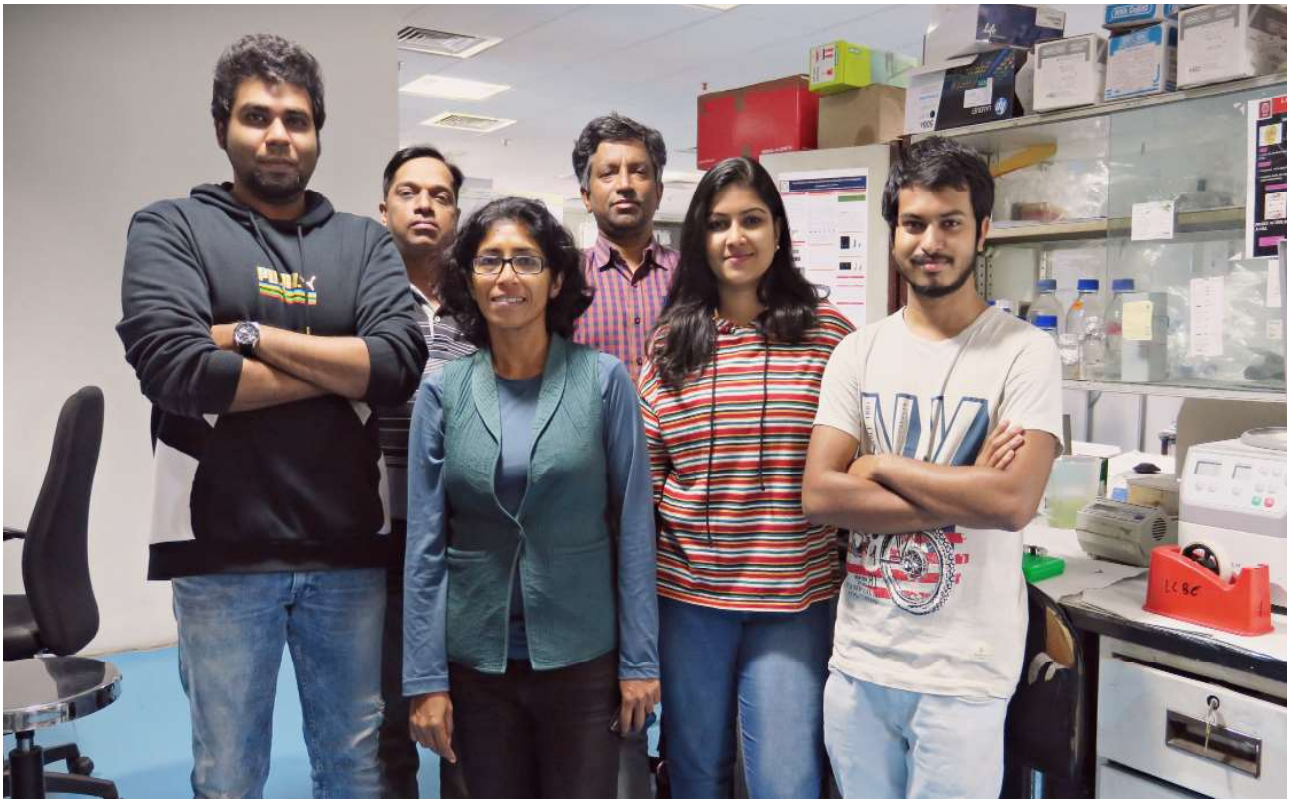


Figure - 1. The function of dynamic regulation of Hst4 in maintenance of FPC stability via H3K56ac during replication stress response. Upon replication stress, DDK/Hsk1 phosphorylates Hst4 at serine residues at its the C-terminus. The phosphorylated Hst4 is recognized and ubiquitinated by SCFpof3 ubiquitin ligase and degraded via proteasome. Degradation of Hst4 increases H3K56ac levels leading to stable maintenance of FPC Swi1 and Mcl1 at the chromatin which helps in fork recovery and cell survival.

Publications

Shalini Aricthota and Devyani Haldar (2021) DDK/Hsk1 phosphorylates and targets fission yeast histone deacetylase Hst4 for degradation to stabilize stalled DNA replication forks. eLife 10, e70787.



Laboratory of Chromatin Biology and Epigenetics

Laboratory of Computational and Functional Genomics

Computational and functional genomics of human diseases

Principal Investigator: Akash Ranjan
Staff Scientist

PhD Students:
Abhishek Kumar (Till May 2021)
Ch Gangi Reddy
S Akshaykumar Nanaji
Ch Kiranmai
Smita Saha
Rupa Chowdhury (Since March 2022)

Other Members:
G Rajalingam
J Aravindh Kumar

Collaborators

Ashwin Dalal	CDFD, Hyderabad India
Rohit Joshi	CDFD, Hyderabad India
Sailu Yellaboina	AIIMS, Bibinagar, India
KM Girisha	KMC, Manipal, India
Debashish Ghosh	KMC, Manipal, India
Vijay K Muley	UNAM, Mexico.

The primary objective of our group is to understand the structural and functional roles of proteins in protein-protein and protein-ligand interactions responsible for the biology of human diseases. Specially, we study the molecular structure, function, and interactions associated with infectious/parasitic diseases and the human neurodegenerative diseases.

Molecular structure, function, and interactions associated with the biology of parasitic diseases

We have screened small molecule compounds from the PubChem database and validated the shortlisted compounds for their target binding through in silico methods. We have initiated the testing of the screened novel compound for its potential to serve as an PfACBP inhibitor. Towards this goal, we have initiated the study at a molecular level through molecular dynamics simulation studies on the stability and the dynamics of this novel compound upon target binding.

In addition, we are investigating a moonlighting function of Circumsporozoite Protein (CSP) of Plasmodium falciparum in host remodelling. It is generally believed that the parasite remodels their host environment upon liver infection. In order to understand the molecular mechanisms involved in host remodelling, we investigated the role of parasite exported proteins in host remodelling. Finding from such studies could assist in designing novel chemotherapeutics strategies to interrupt transmission between mosquitoes and humans by interfering at the initial stage of parasite infection. One of these proteins is a well-known immunodominant antigen and is an essential constituent of the sporozoite surface coat, circumsporozoite protein (CSP). Plasmodium secretes CSP inside the cytoplasm of the host cell and its presence in the hepatocyte enhances the growth of liver stage parasite.

Our preliminary study shows that the CSP of P.falciparum has at least two nuclear localization signal (NLS). We predicted NLS sequences by using the NLS Mapper computer algorithm. The algorithm identified two sequences in CSP, one is of monopartite type and the other is bipartite (Figure 1). To experimentally prove this, we fused these predicted NLS sequences with Pf Aldolase that normally localised within cytoplasm and expressed them in human hepatoma cell line HepG2 cells. We observed that individually both monopartite and bipartite NLS are functional NLS but show weak nuclear localization, whereas when used

together results in synergistically enhanced nuclear accumulation of Pf Aldolase. The previous studies have shown the presence of a monopartite type NLS in P. yoelii CSP, which is different from the two NLS we have identified in P. falciparum CSP.

In order to understand what effect these parasite-secreted PfCSP protein may have on the host nuclear targeted/localised proteins, we tested the ability of host nucleus-targeted host protein to localise in nucleus in presence and absence of PfCSP NLS signal containing parasite protein Pf Aldolase. Using this approach, we observe that the parasite with NLS from PfCSP protein disturbs/perturbs the de novo histone deposition in HepG2 cells. Upon analysis, we have observed that the de novo deposition of histone H3 & H4 and not H2B was disturbed. Our results support the hypothesis that PfCSP interference with transport and localisation of histone H3 & H4 in mammalian cells by binding to one of the importin α. On the contrary, histone H2B transport to the nucleus is unaffected as it is an energy-dependent transport mechanism different from the importin α/β-mediated process.

Molecular structure, function, and interactions associated with the biology of infectious diseases: Functional role of mycobacterial transcriptional regulators in physiology and pathology associated with tuberculosis

The genome of M. tuberculosis consists of three annotated IclR like proteins including Rv1719, Rv1773c, and Rv2989. Among these, Rv2989 was previously characterized to induce dormancy-like features. In the current study, we have carried out sequence-based clustering (phylogenetic study) of all published IclR like proteins of various species, along with Rv1719 and Rv1773c. The sequence-based clustering revealed that Rv1719 and Rv1773c cluster with other IclR like proteins involved in antibiotic resistance (Figure 2). Rv2989 is clustered with a protein involved in biosynthesis and previous reports from our lab has shown that Rv2989 regulates leuCD operon. Further, we have checked the changes in the promoter activity of Rv1719 and Rv1773c in the presence and absence of Isoniazid and Rifampicin. The β-galactosidase reporter assay showed high promoter activity of both Rv1719 and Rv1773 in the presence of Rifampicin, whereas only Rv1719 has higher promoter activity in the presence Isoniazid, but not Rv1773c.

Some of the functionally-characterized IclR family proteins are reported to be auto-regulated. Therefore, we looked into the activity of Rv1719 and Rv1773c promoters, with and without ectopic expression of Rv1719 and Rv1773c respectively. The β-galactosidase reporter assay showed that Rv1773c is autoregulatory, whereas Rv1719 is not autoregulatory. Further, we performed an electrophoretic mobility shift assay (EMSA) and observed that autoregulation of Rv1773c is through direct interaction of Rv1773c protein with DNA element upstream of the gene.

Molecular structure, function, and interactions associated with the biology of human neurodegenerative/proteostasis diseases

Here we report an additive role of HYPK in the clearance of protein aggregates by neddylation-dependent autophagy, resulting in a cytoprotective effect during proteotoxic stress. HYPK is the receptor in neddylation-dependent autophagy where it interacts with Nedd8 through its C-terminal UBA domain. Our findings suggested that the neddylation of cytosolic protein aggregates and their inclusion in autophagosomes by the receptor function of HYPK are parts of the defensive response to control aggrephagy during proteotoxicity. In conditions of intrinsic or extrinsic proteotoxic stress, intracellular protein aggregates are progressively neddylated. HYPK binds to the Nedd8 of the polyneddylated protein aggregates, followed by recruitment of LC3 to the site by using its LIR domain. This promotes autophagosomal enclosing of the neddylated protein aggregates for their subsequent degradation upon delivery to the lysosome. Further through a bioinformatics approach, we identified a homolog of HYPK in Drosophila. It would be exciting to know whether this Drosophila homolog share similar role in sensing, regulating, and clearing protein aggregation.

In addition, we have also examined the dynamic nature of structural segments of N-α acetyltransferase 10 (NAA10) protein by using molecular dynamics simulation. The HYPK is also reported to interact with the human N-terminal acetyltransferase A (NatA) complex. This complex predominantly acetylates newly synthesized peptides co-translationally through association with the ribosome. HYPK plays the role of intrinsic regulator of this complex. The

missense variants in NAA10 (subunits of hNatA complex) have been identified in individuals with various neurodevelopmental disorders. Deleterious mutants of NAA10 such as F128I and F128L show loss of cellular stability and functional activity. Our data suggest that the NAA10F128I shows a loss of flexibility at the substrate binding groove of the protein. We also found that NAA10F128L mutant shows a loss of backbone interaction between the mutated residue (L128) and an active site residue Y122. Further, NatA complex is also conserved across multiple species. It would be exciting to know how this complex evolved its sequence, structure, and function.

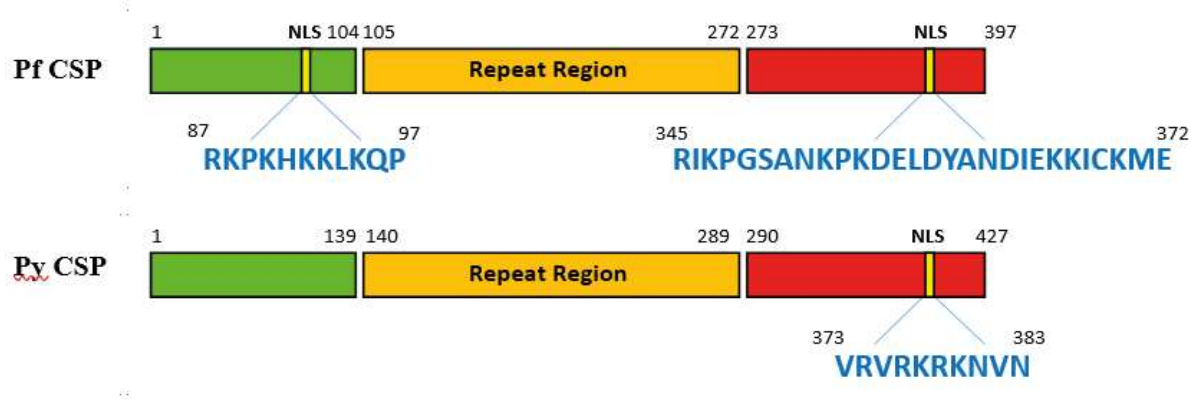


Figure 1. Localization of monopartite and bipartite type NLS sequences in P.falciparum and and monopartite type NLS sequence in P.yeolii

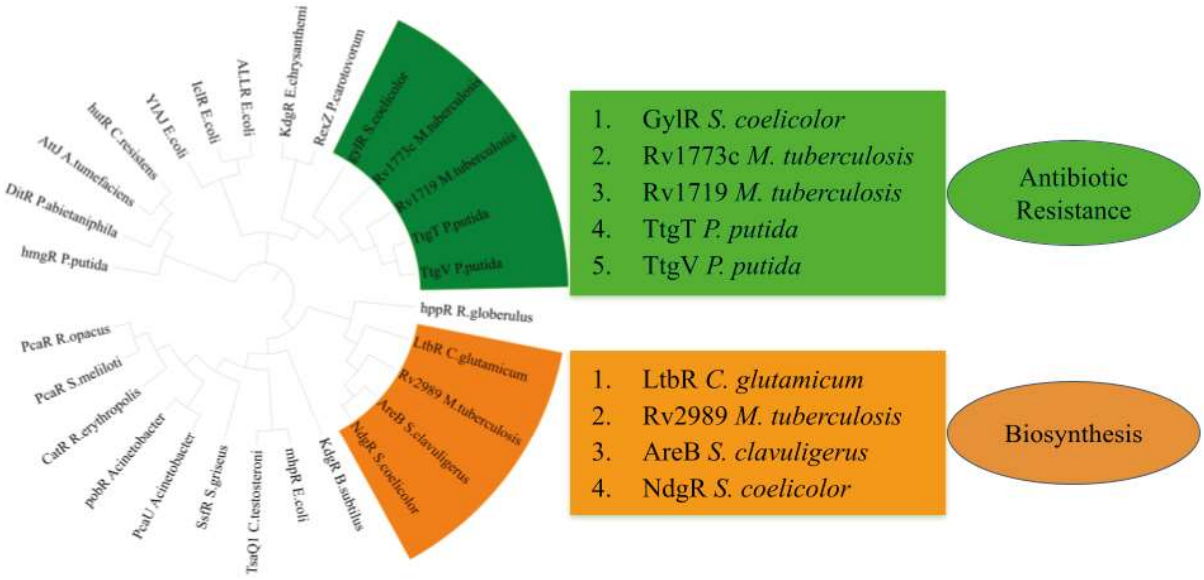


Figure 2. Phylogenetic analysis of IclR like proteins of various species. Rv1719 and Rv1773c clusters with functionally characterized IclR proteins reported to be involved in antibiotic resistance, whereas Rv2989 clusters with proteins reported to be involved in biosynthesis.

Publications

Ghosh DK, Ranjan A (2021) HYPK coordinates degradation of polyneddylated proteins by autophagy. Autophagy, 1-22 [Epub]

Ghosh DK, Kumar A, Ranjan A (2021) Cellular targets of mefloquine. Toxicology 464: 152995.

Deshpande D, Gupta SK, Sarma AS, Ranganath P, Jain S JMN, Sheth J, Mistri M, Gupta N, Kabra M, Phadke SR, Girisha KM, Dua Puri R, Aggarwal S, Datar C, Mandal K, Tilak P, Muranjan M, Bijarnia-Mahay S, Rama Devi A R, Tayade NB, Ranjan A, Dalal AB (2021) Functional characterization of novel variants in SMPD1 in Indian patients with acid sphingomyelinase deficiency. Hum Mutat. 42(10):1336-1350.

Computational and Functional Genomics Group

Laboratory of Drosophila Neural Development

Central Nervous System development in *Drosophila melanogaster*

Principal Investigator: Rohit Joshi
Staff Scientist

PhD Students:

Rashmi Sipani	Senior Research Fellow (Till April 2022)
Asif Ahmad Bakshi	Senior Research Fellow (Till Feb 2022)
Yamini Rawal	Senior Research Fellow
Punam Bala	Senior Research Fellow
Jiban Barman	Senior Research Fellow
Savita	Junior Research Fellow

Other Members:

Chandra Shekhar Singh	Technical Assistant
Aishwarya Kunchur	Project Assistant (till Feburary 2021)

Collaborator:

Anuradha Ratnaparkhi	Agarkar Research Institute, Pune
Deepti Jain	Regional Center for Biotechnology, Faridabad

Two significant features of bilaterian organisms (like insects, vertebrates and mammals-humans) are the head-to-tail axis and the complex central nervous system (CNS). A highly conserved family of transcription factors (TFs) called Hox genes; express segmentally along the head-to-tail axis, and play a critical role in determining both of these features. The long-term goal of our lab is to understand how neural stem cells (NSCs) generate a variety of different cell types and cell numbers along the head-to-tail axis of the developing CNS. To this end, studying the region-specific coordination of NSC proliferation, differentiation, and apoptosis by Hox genes will give insights into the generation of such cellular and numerical diversity. Expectedly, misregulation of any of these processes will result in developmental disorders and malignancies. An alternative but less common mode used to regulate neuronal numbers is the apoptosis of NSCs itself. In Drosophila CNS, Hox-mediated NSC apoptosis is one of the primary modes of regulating neuronal numbers during the development. Understanding the molecular basis of this apoptosis in CNS development has been the research focus of our group.

Objectives:

1. **Understanding the integration of spatial, temporal, and sex-specific inputs in proliferation and apoptosis of NSCs.**

   Background: The generation of sexually dimorphic CNS is vital for animal reproduction and propagation. While establishing sex-specific neuronal circuity has been studied and explored, the molecular basis of sex-specific proliferation and apoptosis of NSCs in developing CNS is not well understood. Highly conserved DM-domain containing transcription factors (Doublesex / MAB-3 / DMRT1) are responsible for generating sexually dimorphic features. In the terminal region of Drosophila larval CNS, a set of Doublesex (Dsx) expressing NSCs undergo apoptosis in females. At the same time, their male counterparts proliferate and give rise to serotonergic neurons crucial for adult mating behavior. The molecular mechanism of the female-specific cell death of NSCs and the generation of serotonergic neurons in males is not entirely understood. We study Dsx expressing NSCs in male and female CNS to understand how these cells coordinate spatial-temporal and sex-specific input during development.

   Result: Our work shows for the first time that DM-domain containing non-classical Zn finger TF Dsx can function as a cooperative cofactor for

HD containing Hox gene Abdominal-B (Abd-B). This cooperation helps Abd-B select and activate the RHG family of apoptotic genes resulting in female-specific NSC apoptosis. The capacity of Abd-B to utilize the sex-specific isoform of Dsx as a cofactor underlines the possibility that two classes of proteins can cooperate in the selection and regulation of target genes in tissue and sex-specific manner. We propose that this interaction could be a common theme in generating sexual dimorphism in different tissues across different species.

Future plan: We are working to understand the molecular basis of the continued proliferation of Dsx expressing NSCs in male CNS and how these cells generate neurons responsible for male mating behavior. We are focusing on how temporal series TFs facilitate the generation of neuronal diversity in these lineages. Furthermore, we are also investigating how a homeodomain-containing TF like Abd-B forms a complex with DM-domain containing factor Dsx to regulate the target genes. To this end, we are collaborating with Dr. Deepti Jain to crystallize the Abd-B and Dsx on DNA motifs found on the apoptotic enhancer.

2. **Understanding the molecular collaboration of Hox genes with Grainyhead and Notch signaling in developmental apoptosis of NSCs.**

Background: NSCs in terminal segments of Drosophila larval CNS are subdivided into two groups based on the expression of TF Dsx. While the sex-specific fate of Dsx-positive NSCs has been characterized (discussed above), the fate of Dsx-negative NSCs was not known so far. In addition to this, our previous work with abdominal NSCs shows that these cells undergo apoptosis by coordinating an increase in the levels of Hox factor Abdominal-A (Abd-A) (apoptotic trigger) with Notch signaling and helix-loop-helix TF Grainyhead (Grh). However, the molecular details of Dsx-negetive NSCs apoptosis in terminal segments have not been completely understood.

Results: Our studies with Dsx-negative NSCs suggest that these cells, like their counterparts in abdominal segments, use Hox, Grh, and Notch to undergo cell death during larval development. However, we find that, unlike abdominal NSCs, Dsx-negative NSCs keep the levels of resident Hox gene Abd-B constant. Instead, these cells utilize increasing levels of Grh and rise in Notch activity to activate the apoptotic genes through common enhancer to undergo cell death. These results highlight that region-specific Hox-dependent NSC apoptosis utilizes overlapping molecular players but seems to have evolved different molecular strategies to pattern CNS.

Continuing on the theme of abdominal NSC apoptosis, we have also shown that Abd-A and Grh interact through their highly conserved DNA binding domains, and the DNA binding specificity of AbdA-HD is vital for interacting with Grh and essential for executing NSC apoptosis. We further establish that two other regions of the CNS also require Grh for Hox-mediated NSC apoptosis, and Grh can physically interact with all the Hox proteins in vitro, supporting the idea that Grh can function as a generic Hox cofactor during development (Sipani and Joshi, Genetics (in press)).

Future plan: We intend to investigate why abdominal and terminal NSCs use common players but utilize different molecular mechanisms to undergo apoptosis.

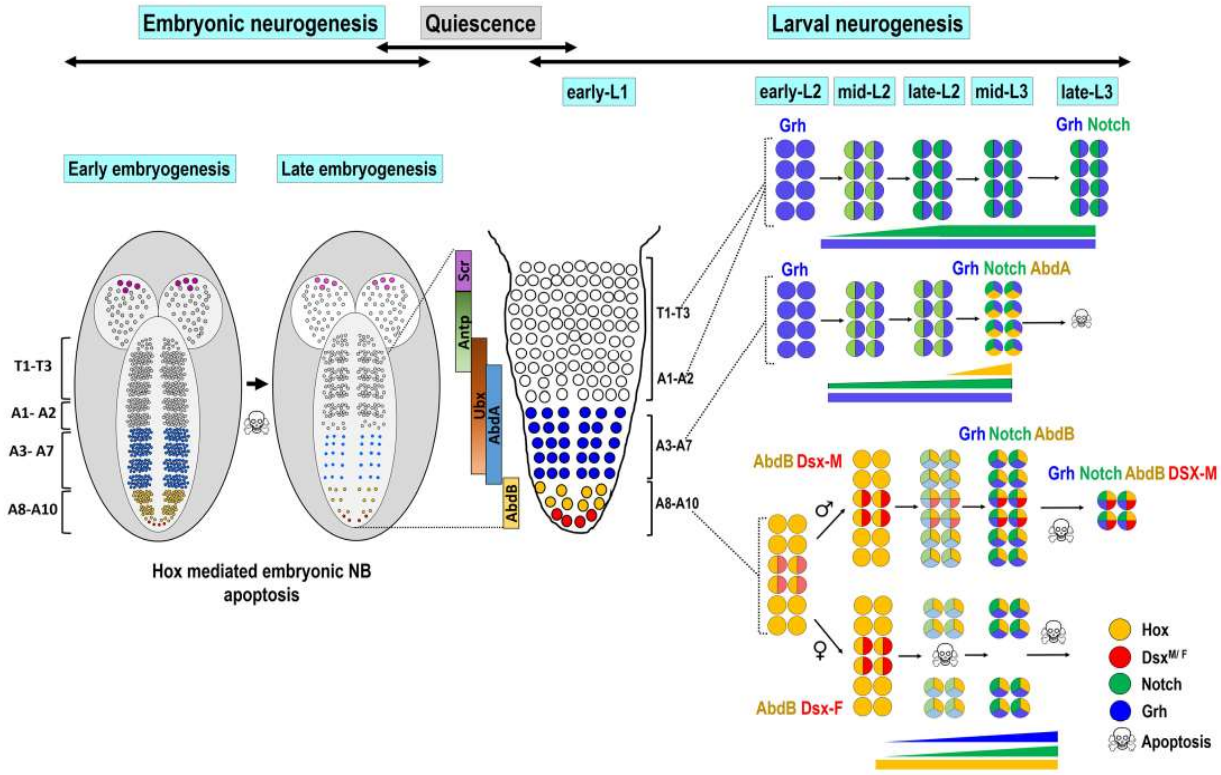


Fig 1: Mechanism of abdominal and terminal NSC apoptosis. Schematic of embryonic CNS showing that NSC (Neuroblasts-NBs) in T1-T3 and A1-A10 segments undergo Hox dependent apoptosis in late embryonic stages. The surviving NBs undergo quiescence and start dividing in the early larval stages. Larval NBs in T1-A2 segments express Grh (shown in blue color) and Notch (shown in green color) but never express the resident Hox gene (*antp, Ubx, or abd-A*) and hence continue dividing till late larval stages. The abdominal NBs (A3-A7 segments) express near-constant levels of Grh (shown in blue color) and Notch (shown in green color). In these cells, a mid-L3 pulse of Abd-A (shown in yellow color) results in their apoptosis by making them Abd-A+ Grh+ Notch+ (Khandelwal et al., 2017). In terminal segments, Abd-B expresses in A8-A10 NBs (shown in yellow color). Four of these NBs incrementally express Dsx (shown in a deepening shade of red) and die in the mid-L2 stage by becoming Abd-B+ DsxF+ in females VNC (Ghosh et al., 2019), while their male counterparts (which become Abd-B+ DsxM+) continue dividing till pupal stages. The Dsx-negative NBs, on the other hand, show an increase in Notch activity and Grh expression (indicated by deepening shades of green and blue color), coupled with constant levels of Abd-B (shown in yellow color), and undergo apoptosis (by becoming Abd-B+ Grh+ Notch+) at mid-L3 stage (Bakshi et al., 2020).

Publications:

1. Rashmi Sipani and Rohit Joshi. “Hox genes collaborate with helix-loop-helix factor Grainyhead to promote neuroblast apoptosis along the anterior-posterior axis of the Drosophila larval central nervous system.” Genetics (In press, 2022).
2. Bakshi A., Sipani S., Ghosh N., Joshi R. Sequential activation of Notch and Grainyhead gives apoptotic competence to Abdominal-B expressing larval neuroblasts in Drosophila Central nervous system. PLoS Genetics (2020), 16(8): e1008976.
3. Ghosh N., Bakshi A., Khandelwal R., Govinda Rajan S., Joshi R (2019). Hox gene Abdominal-B uses DoublesexF as a cofactor to promote neuroblast apoptosis in Drosophila central nervous system. Development (2019) 146, dev175158.
4. Risha Khandelwal, Rashmi Sipani, Sriivatsan Govinda Rajan, Raviranjan Kumar, Rohit Joshi. Combinatorial action of Grainyhead, Extradenticle and Notch in regulating Hox mediated apoptosis in Drosophila larval CNS. PLoS Genet. (2017) Oct 12;13(10): e1007043.

Laboratory of Drosophila Neural Development

Laboratory of Fungal Pathogenesis

Understanding the pathobiology of the human opportunistic fungal pathogen *Candida glabrata*

Principal Investigator: Rupinder Kaur
Staff Scientist
DBT/Wellcome Trust India Alliance Senior Fellow
(Till 28 February 2022)

PhD Students:

Anamika Battu	Senior Research Fellow (Till 15 December 2021)
Fizza Askari	Senior Research Fellow
Mahima Sagar Sahu	Senior Research Fellow
Sandip Patra	Senior Research Fellow
Aditi Pareek	Junior Research Fellow
Mayur Raney	Junior Research Fellow
Asmita Sarowgi	Junior Research Fellow (Since 13 August 2021)

Other Members:

S Surya Vamshi	Technical Officer
Priyanka Bhakt	Research Associate (Till 30 April 2021)
Kundan Kumar	Research Associate (Since 17 June 2021)
Anamika Battu	Research Associate (Since 16 December 2021)
Rajaram Purushotham	Project Associate-II (Till 31 January 2022)
Bhogadi Vasavi	Project Associate-II
Adarsh Goel	Project-JRF (Since 28 March 2022)

Collaborator:

Rajendra Prasad	Amity University Haryana Gurgaon
CV Srikanth	RCB, Faridabad
Arunaloke Chakrabarti	PGIMER, Chandigarh
Debasis Biswas	AIIMS-Bhopal, Bhopal
Suman S Thakur	CCMB, Hyderabad

Candida species are the most prevalent cause of bloodstream fungal infections, with *Candida glabrata* being the second to fourth most frequently isolated *Candida* species depending upon the geographical location. Evolutionarily, *C. glabrata* is closer to the non-pathogenic yeast Saccharomyces cerevisiae than to the most common *Candida* species, *C. albicans*. Research in our laboratory is aimed at a better understanding of pathogenesis and antifungal drug resistance mechanisms in *C. glabrata*.

Objectives:

1. Characterization of glycosylphosphatidylinositol-linked aspartyl proteases in *Candida glabrata:* role in pathogenicity
2. Elucidating the role of histone H3 lysine methylation in antifungal drug resistance

Research summary

Details of the progress made in the current reporting year (1st April 2021 – 31st March 2022)

Project 1: Characterization of glycosylphosphatidylinositol-linked aspartyl proteases in *Candida glabrata*: role in pathogenicity

A family of eleven putative glycosylphosphatidylinositol-linked, cell surface-associated aspartyl proteases is essential for pathogenesis of *C. glabrata*. These proteases, also referred to as yapsins, are encoded by *CgYPS1-11* genes. We had previously found elevated expression of the high-affinity glucose transport genes, including the high-affinity glucose sensor-encoding gene *CgSNF3*, in the *Cgyps1-11Δ* (lacks eleven CgYapsins) mutant. Notably, Snf3 and its paralog Rgt2 in *S. cerevisiae* are known to generate signal for glucose-induced hexose transporter gene expression, through their C-terminal signalling tails, in response to low and high glucose concentration, respectively.

Therefore, to determine if CgYapsins modulate the cellular response to extracellular glucose in *C. glabrata,* we first checked whether CgSnf3-mediated signalling of glucose-limited environment is impaired in the *Cgyps1-11Δ* mutant. For this, we generated and characterized the *Cgsnf3Δyps1-11Δ* mutant (deleted for 12 genes, *CgSNF3* and *CgYPS1-11*). Time-course analyses in YNB medium containing varied glucose concentrations [0.03 and 0.3% (low), 2% (regular) and 5% (high)] revealed that, compared to *wt* cells, the *Cgyps1-11Δ* and *Cgsnf3Δ* mutants exhibited 1.3- and 1.2-fold higher doubling time in YNB medium-containing 2% and 0.03% glucose (Fig. 1A and B), respectively. Contrarily, the growth rate of *Cgyps1-11Δ* and *Cgsnf3Δ* mutants was same as that of the wt strain in glucose-limited (0.3%) and glucose-rich (5%) medium, respectively (Fig. 1C and D). Intriguingly, the doubling time of *Cgsnf3Δyps1-11Δ* mutant population was substantially lower and higher than the *Cgyps1-11Δ* mutant population in medium-containing 2% and 0.03% glucose, respectively, indicating that the elevated environmental glucose levels lead to growth retardation in *Cgyps1-11Δ* mutant. These results also implicate CgYapsins in glucose homeostasis probably via regulation of CgSnf3-dependent glucose sensing and signalling pathway.

To probe deeper into CgSnf3-mediated glucose signalling, we next examined the transcript levels of *CgSNF3,* three hexose transporter genes [(*CgHXT1, CgHXT2/10*) *(I)* and *CgHXT3* (*CAGL0A02321g*)], CgRGT1 (codes for a glucose-responsive transcription factor, that is regulated by the low affinity-glucose sensor CgRgt2) and *CgMIG1* (encodes transcription factor involved in glucose repression) genes under low (0.03%), regular (2%) and high (5%) glucose conditions. We found *CgMIG1*, *CgRGT1*, *CgSNF3*, and *CgHXT2/10 (I)* gene expression to be upregulated, while the transcription of *CgHXT1* and *CgHXT3* genes was downregulated in YNB medium (contains 2% glucose)-grown *Cgyps1-11Δ* cells, compared to YNB medium-grown *wt* cells (Fig. 1E). Contrarily, transcript levels of *CgMIG1* and *CgHXT2/10* genes were lower in *Cgsnf3Δ* mutant, compared to *wt* cells (Fig. 1E). Intriguingly, the *Cgsnf3Δyps1-11Δ* mutant displayed transcriptional profiles, that were in-between the two mutants *Cgsnf3Δ* and *Cgyps1-11Δ,* with higher and lower transcript levels of *CgRGT1,* and *CgMIG1, CgHXT1, CgHXT2/10 (I)* and *CgHXT3* genes, respectively (Fig. 1E). Further, transcriptional response of the *Cgyps1-11Δ* mutant to glucose starvation and abundance was very distinct from that of the *wt* strain, suggesting that CgYapsin loss impairs the cellular ability to sense environmental glucose, with the high-glucose environment probably being perceived as a low-glucose environment.

Next, to elucidate the link among CgYapsins, CgSnf3 and glucose homeostasis, we measured glucose uptake using the fluorescent glucose analog 2-NBDG, and found 1.8-fold higher glucose uptake in *Cgyps1-11Δ* mutant, compared to *wt* cells (Fig. 1F). Importantly, *CgSNF3* gene deletion in *Cgyps1-11Δ* mutant led to diminished glucose uptake (Fig. 1F), suggesting that elevated glucose uptake in the *Cgyps1-11Δ* mutant is largely dependent upon CgSnf3. We also found diminished JC-1 dye aggregate formation in *Cgyps1-11Δ* mutant, indicating decreased mitochondrial membrane potential in the mutant. Consistently, we found 1.6-fold higher levels of ethanol in the culture supernatant of *Cgyps1-11Δ* cells, suggestive of an increased flux through glycolysis in *Cgyps1-11Δ* cells, which may lead to higher production of ethanol from the glycolysis-derived pyruvate. The respiratory metabolism was also found to be impaired in *Cgyps1-11Δ* cells. Altogether, our data unveil an unanticipated role of fungal GPI-anchored aspartyl proteases in regulating extracellular glucose sensing mechanisms (Fig. 1G), that may partially account for their essentiality for virulence. The substrate proteins, via which CgYapsins could regulate glucose homeostasis, are currently being identified.

Project 2: Elucidating the role of histone H3 lysine methylation in antifungal drug resistance

We had recently reported that the loss of histone H3 lysine 36 methyltransferase confers growth advantage to *C. glabrata* in the presence of ergosterol biosynthesis-inhibitory azole antifungals. The intrinsic low susceptibility to widely used, cost-effective azole antifungals, and a high propensity of *C. glabrata* to acquire resistance towards azole and echinocandin (targets cell wall biosynthesis) drugs, render antifungal therapy unsuccessful in many cases. Herein, we are elucidating the link between histone H3 lysine methylation and antifungal resistance via identification of drug-

resistance-conferring genes, whose regulation is impacted by chromatin architectural changes. In this regard, we had shown that the SET domain-containing, nucleus-localized protein CgSet4 acts as a negative regulator of resistance towards azole and echinocandin drugs. Studies are currently underway to determine whether CgSet4 possesses lysine methyltransferase activity.

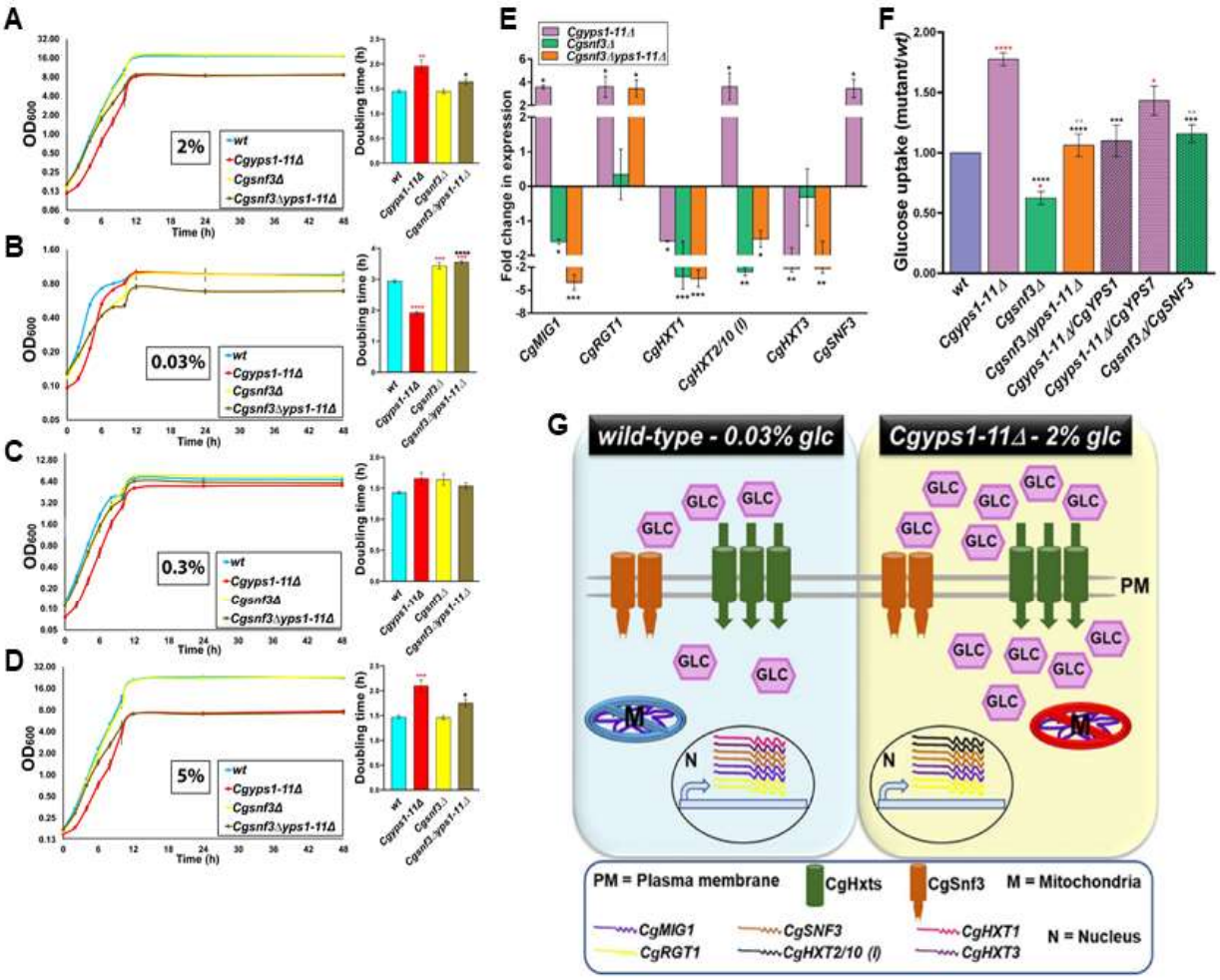


Figure 1: CgYapsins regulate glucose homeostasis. Time course analysis of wt, *Cgyps1-11Δ*, *Cgsnf3Δ* and *Cgsnf3Δyps1-11Δ* strains. *C. glabrata* strains were grown overnight in YPD medium, and inoculated, at an initial OD_{600} of 0.1, in YNB medium containing 2% (A), 0.03% (B), 0.3% (C) and 5% (D) glucose. Cultures were incubated at 30˚C with continuous shaking (200 rpm), and absorbance was monitored at regular intervals till 48 h. The absorbance (OD_{600}) values are plotted against time, and the growth period, corresponding to the log-phase (between 2 and 6 h), was used to determine the doubling time. Data represent mean ± SEM (n = 3-4). The one-way ANOVA with Tukey's test was employed to determine the statistical significance of doubling time differences between strains. Red and black asterisks denote differences in doubling time between *wt* and mutants, and *Cgyps1-11Δ* and *Cgsnf3Δyps1-11Δ* mutants, respectively. *, p≤0.05; **, p≤0.01; ***, p ≤ 0.001; ****, p ≤ 0.0001. (E) qRT-PCR-based expression analysis of indicated genes in YNB-medium-grown log-phase *wt, Cgyps1-11Δ*, *Cgsnf3Δ* and *Cgsnf3Δyps1-11Δ* cells. Data (mean ± SEM, n = 3-4) were normalized against *CgACT1* mRNA control, and represent fold change in expression in mutant cells, compared to *wt* cultures (considered as 1.0). *, p≤0.05; **, p≤0.01; ***, p≤0.001, one-way ANOVA with uncorrected Fisher's LSD test. (F) Uptake of 2-NBDG [2-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose in indicated *C. glabrata* strains, as determined by spectrofluorimetry. Glucose-starved cells were incubated with 2-NBDG (100 µM) for 1 h at 30˚C, and the fluorescence emission was recorded at 540 nm, under excitation at 465 nm. Data (mean ± SEM, n = 3-5) were normalized against *wt* fluorescence values (considered as 1.0), and represent fold change in NBDG uptake in mutant strains, compared to the *wt* strain. Red asterisks denote differences in

glucose uptake between *wt* and indicated strains, black asterisks denote differences between *Cgyps1-11*Δ and indicated strains, while grey asterisks denote differences between *Cgsnf3*Δ and indicated strains. *, p≤0.05; **, p≤0.01; ***, p≤0.001; ****, p≤0.0001, one-way ANOVA with Tukey's test. (G) The loss of CgYapsins impairs the ability of *C. glabrata* cells to sense the external glucose concentration. The *Cgyps1-11Δ* mutant perceives the 2% glucose environment (YPD/YNB medium) as glucose-poor environment, which results in transcriptional activation of genes coding for CgSnf3 glucose sensor, CgMig1 and CgRgt1 transcription factors, and CgHxt2/10 (I) (CAGL0I00286p) hexose transporter, that possibly leads to higher glucose uptake and perturbed glucose homeostasis. Contrarily, *wt* cells respond to a low-glucose environment (0.03% glucose) by elevating the expression of *CgSNF3, CgMIG1, CgRGT1,* and *CgHXT1* and *CgHXT3* (code for hexose transporters) genes, which probably facilitates glucose import, and glucose homeostasis is maintained. Of note, *CgHXT2/10* (I) is transcriptionally repressed in glucose-starved wild-type cells while *CgHXT1* and *CgHXT3* genes are transcriptionally repressed in 2% glucose-grown Cgyps1-11*Δ* cells. Additionally, proteins belonging to glycolysis and oxidative phosphorylation are over- and under-represented, respectively, in total membrane proteome of the *Cgyps1-11Δ* mutant, as compared to *wt* cells, that may contribute partly to depolarized mitochondria and elevated ethanol production in the mutant. Altogether, these data underscore a critical requirement for CgYapsins in glucose metabolism in *C. glabrata.*

Publications

1. Askari, F.¶, Rasheed, M.¶ and Kaur, R. (2022) The yapsin family of aspartyl proteases regulate glucose homeostasis in Candida glabrata. Journal of Biological Chemistry 298 (2): 101593. ¶Equal contribution.
2. Battu, A., Purushotham, R., Dey, P., Vamshi, S.S. and Kaur, R. (2021) An aspartyl protease-mediated cleavage regulates structure and function of a flavodoxin-like protein and aids oxidative stress survival. PLoS Pathogens 17: e1009355.
3. Battu, A.¶, Purushotham, R.¶, and Kaur, R. (2021) An assay to determine NAD(P)H: quinone oxidoreductase activity in cell extracts from Candida glabrata. Bio-protocol 11 (21): e4210. ¶Equal contribution.
4. Moirangthem, R.¶, Kumar, K.¶ and Kaur, R. (2021) Two functionally redundant FK506-binding proteins regulate multidrug resistance gene expression and govern azole antifungal resistance. Antimicrobial Agents and Chemotherapy 65 (6): e02415-20. ¶Equal contribution.

Laboratory of Fungal Pathogenesis

Laboratory of Genome Architecture

Impact of DNA topology in genome organization and functional regulation

Principal Investigator: Yathish J. Achar
Staff Scientist

PhD Students:

Md. Altamash	Junior Research Fellow
Nilay Bhowal	Junior Research Fellow

Other Members:

Pooja Tripathi	Technical Officer-I

Chromatin structural transitions play a crucial role in facilitating numerous biological functions. DNA supercoiling is one such transition playing an important role in compacting DNA, regulating protein-DNA association and gene expression. Supercoiling is a fundamental property of DNA and is modulated by polymerases, topoisomerases and DNA-bound protein complexes. Supercoiling, bending and twisting of DNA, along with alternative DNA structures (cruciform, R-loop and Z-DNA) define the mechanical and physical properties of chromatin. Recently, by using yeast, we described the first genome-wide map of DNA supercoil in a eukaryotic cell. The scenario emerging from the study provides a novel perspective on topology where chromatin is imprinted with 'negative supercoiled' structures at gene boundaries and does not depend on transcription per se, implicating that genes retain a "topological memory" even when transcription is repressed. Interestingly, cancer related genes including p53, BRCA1, DEK, PARP1, WRN, Top2 and HMG family proteins, all show preferential binding to negative supercoiled structures, suggesting their role in expression of oncogenes and tumour suppressors.

According to our working model, DNA within chromosomes is dynamic and shows deformation, where two strands can be pulled apart in the middle and let each strand twist about itself, forming a cruciform-like structure (Figure 1). These negative supercoiled cruciform-like structures not only provide a mechanically simpler and energetically easier option to open up DNA, but also traps supercoil waves within the domain. Moreover, our recent finding suggests that these structures also trap sliding cohesin rings, thereby playing an active role in three-dimensional architecture of the genome. Based on these principles, research in our lab aims to understand DNA supercoil and the role of supercoil-induced structures in:

(A) Deregulation of Genome organization in cancer genome

(B) Transcription control and alternative splicing

Research Summary

Theme I: DNA topology and genome organization in cancer genome

Interphase chromosomes are spatially arranged and folded into topologically associated domains (TADs) which will allow effective control of gene expression and DNA replication. TADs are megabase-scale domains, limited by boundaries enriched with CCCTC-binding factor (CTCF) and cohesion protein complexes. In general, TADs are regarded as structural and functional units of the genome, which will promote 3D spatial organization by bringing proximity of regulatory elements to the targeted genes. Alterations to TADs, either by disruption or abnormal fusion of boundary has severe consequences on genome stability which will elevate to disorders and diseases. Recent advancement in imaging and chromatin conformation capture techniques (3C/Hi-C), have revealed that TADs are hierarchical in nature, where TADs and sub-TADs form a complex nested structure. Several studies suggest that hierarchical architecture in TAD correlates with epigenetic modifications and gene expression landscape. However, functional

and biological significance of these hierarchies remains elusive. Particularly, how these structures are altered in cancer conditions are of greater interest as genome organization in human tumors are known to be highly deregulated. The major striking aspect of topological domain organization is that enhancer-promoter pairs are always located in the same domain. This suggests that TADs enhance contacts between regulatory elements and promoters and they act as structural foundation for the regulatory landscape of the genome. However, the rudimentary mechanism enhancing the contacts between enhancers and promoters within the same domain remained unanswered. Earlier studies suggested DNA supercoil as a primary force facilitating functional communication between promoter and regulatory elements particularly over a larger distance. Simulation studies supported this hypothesis and provided evidence for synergy between DNA supercoil and promoter-enhancer association. Additionally, psoralen based supercoil mapping also advocated that chromatin domains can be supercoiled due to transcriptional activities.

According to our working model, chromatin is imprinted with 'negative supercoiled' cruciform-like structures at gene boundaries (Figure 1). These structures are dependent on a negative supercoil and are formed when two strands are pulled apart in the middle and let each strand twist about itself, boundaries a cruciform-like structure. These negative supercoil structures are vital to the formation of TAD boundaries and to control long-range interactions. Our previous data with yeast suggest the same as these negative supercoiled structures trap sliding cohesin molecules, hence playing a vital role during loop extrusion. We will be probing for negative supercoil regions using bTMP (biotinylated 4,5,8-trimethylpsoralen) in cell lines to understand supercoil related changes occurring in cancer genome. Our working hypothesis is that during cancer transformation, stable TADs metamorphose into unstable ones (Figure 2). Major reason for such a disarray could be due to alteration in DNA mechanics, where negative supercoiled structures could drastically change. This change in DNA mechanics results in transcriptional reprogramming, epigenetic modifications, loop extrusion adaptations and variations in boundary elements. These modifications switch chromatin-chromatin interaction into dynamic mode, where stable interactions are lost and new interactions are formed and lost at regular intervals. We aim to identify TADs which are prone to alter its DNA mechanics and further study how hierarchical genome architecture is altered during cancer development.

Theme II: DNA mechanics in transcription control and alternative splicing

Introns have been has to enhance gene expression at different stages of transcription and also translation. However, the cost and benefits of having introns is yet to be fully understood. In humans and yeast intron-harbouring genes produce more copies of RNA than intronless genes, and removal of intron from intron-containing genes lowered their transcriptional rate. Highly expressed genes contain elevated intron densities when compared to weakly expressed genes. Such positive effects of introns on transcriptional rate are usually attributed to coordination between spliceosome complexes and elongating RNA pol2 complexes. However, by large there is still no unanimity in how introns and their removal can increase transcription efficiency.

Introns also contribute to alternative splicing, a phenomenon by which many varieties of mRNAs can be produced from a single gene by either including or excluding a particular intron. Alternative splicing is now considered as a trait in most of the tumors and are salient features during the onset and progression of cancer. Generally accepted model for alternative splicing is that the lower elongation rates increase chances of splicing compared to higher elongation rates. Proteins such as Poly [ADP-ribose] polymerase-1 (PARP1) found at exon/intron boundaries also slow down RNA pol2 at boundaries, suggesting that elongation rate is tightly regulated at the boundaries. Alternative splicing can have prognostic value as most cancer cells show general as well as cancer-specific variation in splicing events. Our hypothesis is that PARP1, along with its partners might play a crucial role in supercoil distribution at exon/intron boundaries. PARP1 regulate Top1 activity though PARylation. Top1 acts as kinase for splicing complex SF2/ASF, which upon phosphorylation, reversely inhibits topoisomerase activity of Top1, suggesting that splicing and topoisomerase activities are antagonist to each other. Hence, interrelation between PARP1/ antagonistic, Top1 and SF2/ASF is the key for understanding the machnics of DNA transitions at exon/intron boundaries.

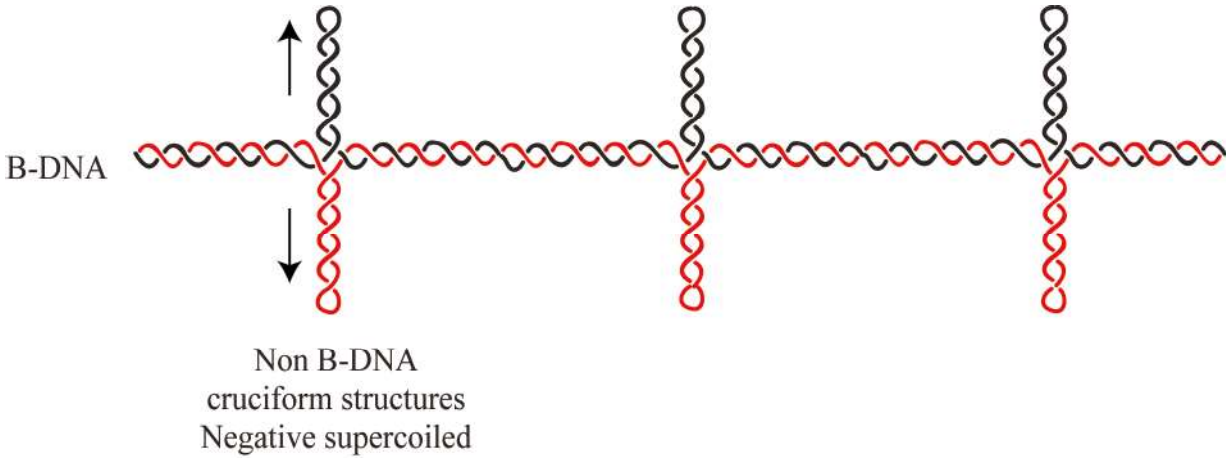


Figure 1. Alternative model for DNA, where negative supercoiled regions are melted and single strand regions are intertwined to form cruciform-like structures.

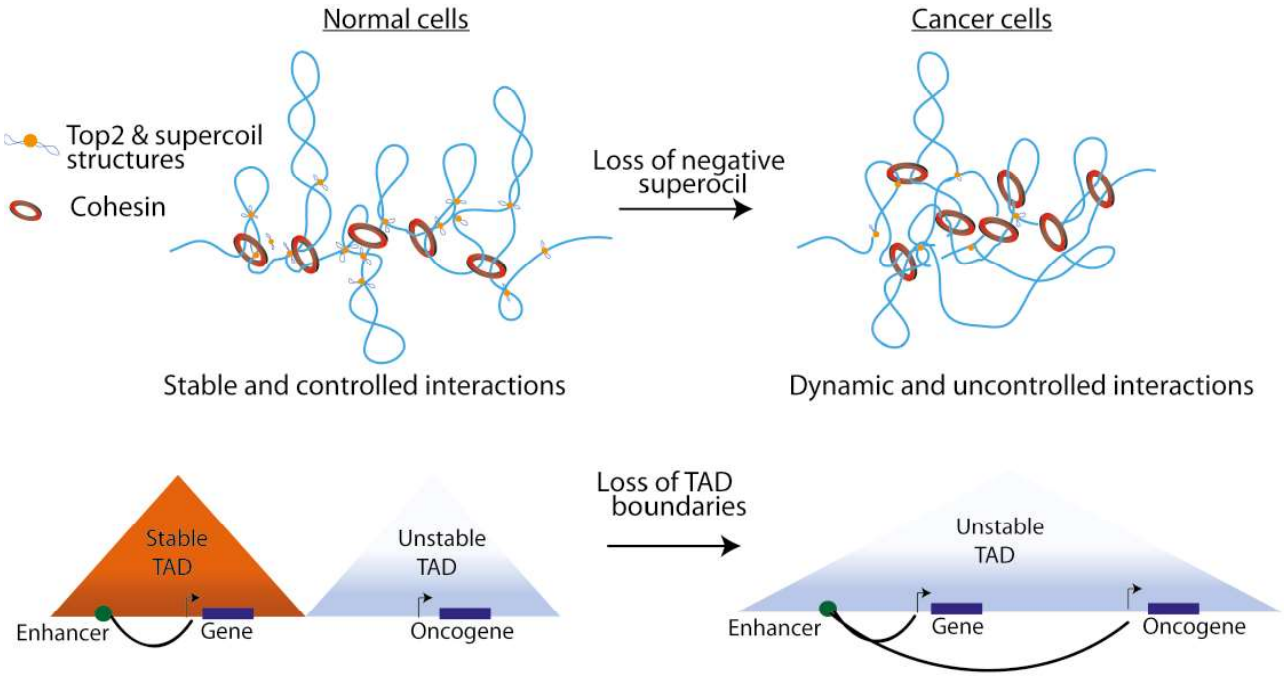


Figure 2. Loss of Negative supercoil reduces Top2 complexes, which stabilize and lock cohesin ring (upper panel). This suggests a possibility of loss of stable TAD by either transforming into unstable one or merging with neighbouring unstable TADs. Super-enhancer can activate oncogene as a result of merging of two independent TADs (lower panel).

Publication:

Topology of RNA:DNA Hybrids and R-Loops in Yeast. Yathish Jagadheesh Achar and Marco Foiani Methods Mol Biol 2022 Vol. 2528 Pages 317-328

Laboratory of Genome Architecture

Laboratory of Genome Informatics

Application of big data, artificial intelligence, and deep learning in medical and agricultural genomics

Principal Investigator: Ajay Kumar Mahato
Staff Scientist

PhD Students:
E. Ramesh Junior Research Fellow

Other Members:
Satyam Shrivastava Computer programmer

Collaborators:
National
Rakesh Singh ICAR-NBPGR, Delhi
Mamta Sharma ICRISAT, Hyderabad
Satya Pal Yadav ICAR-DPR, Hyderabad
Devarshi Gajjar The Maharaja Sayajirao **University of Baroda** Vadodara.

International
Fei Zhao Shanghai Institute of Plant Physiology and Ecology, Shanghai

Objectives:

Big data, artificial intelligence, and deep learning applications in the area of genomics

Our dedicated In-silico laboratory focuses on Big-data science, artificial intelligence, and deep learning in the area of genomics (humans, Plants, pathogens, etc.). with a specific aim of big-data mining and extracting novel information via exploring the novel genes that are associated with several phenotypic traits especially disease-causing in the human, plant, pathogen, etc. Also, decoding the new genome of species which are having national importance in terms of food security, nutrition, and human health. These new genomic resources will be an essential source for the Indian and other global scientific research communities to develop better/ improved cultivars and breeds, via QTL mapping and genome-wide SSR/SNP mining for marker development, linkage map, GWAS, and other analysis. We use next-generation sophisticated open-source software to investigate and conduct the above exercise on genomics Big-data.

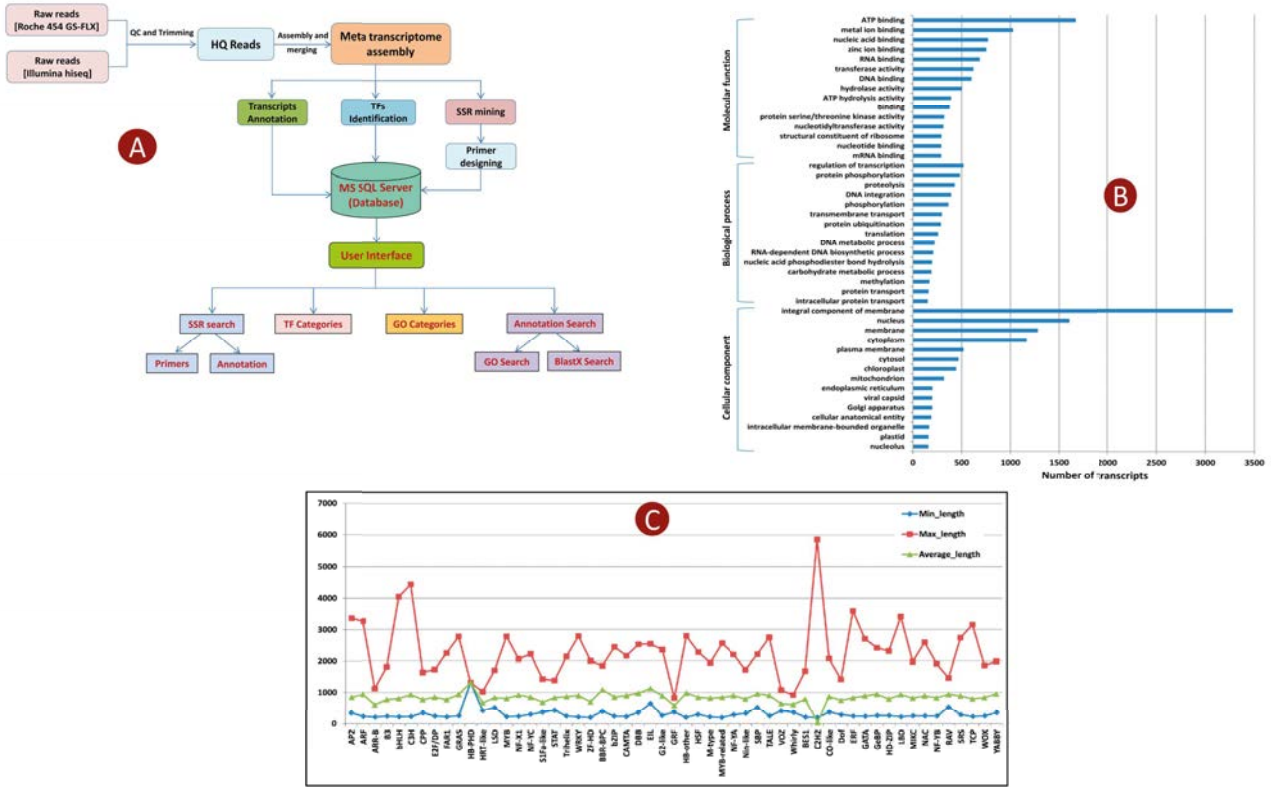
Our lab will also focus on the large genomic data sets we generate in-house and gather from the public repository to develop new algorithms/methods that are further used for artificial intelligence-based or deep learning-based model generation using an object-oriented programming language. After several rounds of in-silico training, refining, and benchmarking, the generated models on our on-prime 5 Petaflop GPU server will be further developed into a web application and genomic resources that will be freely available for the global research community.

Project : Development of genomic resources for Amaranthus hypochondricus (A cultivated leafy vegetable, pseudocereal, and ornamental plant)

In India, Tinospora cordifolia, commonly known as “Giloe,” is a shrub belonging to the family Menispermacea an important medicinal plant known for its antipyretic, anti-inflammatory, antispasmodic, and anti-diabetic properties and is used in the treatment of jaundice, gout, and rheumatism. Despite its economic importance, the limited information about its genomic resources prohibits its judicious exploitation through molecular breeding or biotechnological approaches. This study generated a meta-transcriptome assembly of 43,090 non-redundant transcripts by merging the RNASeq data obtained from Roche 454 GS-FLX and Illumina platforms. Report the first transcriptome-based database for simple sequence repeats and transcription factors (“TinoTranscriptDB” (Tinospora cordifolia Transcriptome Database)). We annotated 26,716 (62%) of the total transcripts successfully from

the National Center for Biotechnology Information non-redundant protein (NCBI-NR) database, gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Swiss-Prot, and Pfam databases. This database contains information on 2,620 perfect simple sequence repeats (P-SSRs) with a relative abundance of 340.12 (loci/Mb) and a relative density of 6309.29 (bp/Mb). Excluding mono-nucleotides, the most abundant SSR motifs were tri-nucleotides (54.31%), followed by di-nucleotides (37.51%), tetra-nucleotides (4.54%), penta-nucleotides (3.16%) and hexanucleotides (0.45%). We also identified 4,311 transcription factors (TFs) and categorized them into 55 sub-families. This database is expected to fill the gap in genomic resource availability in T. cordifolia and thus accelerate molecular breeding and related functional and other applied studies aimed toward genetic improvements of T. cordifolia and related species.

Figure: (A) Process flow diagram of the complete materials and methodology used for the development of "TinoTranscriptDB"; (B) Functional classification of transcripts based on GO terms, distributed in three major categories: molecular function, biological process, and cellular component.; (C) Functional classification of transcripts based on GO terms, distributed in three major categories: molecular function, biological process, and cellular component.



Details of the progress made in the current reporting year (29th April 2021 – March 2022)

During the year, we have initiated several collaboration and research MOU; below are the brief details of each collaborative research work under progress.

(1) We signed an MOU with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, in patho-genomics. In this project, we are working on constructing the Pan-genome of one of the economically important pathogens of pulses (chickpea-Fusarium oxysporum f. sp. ciceris), genome-wide identification of SNPs, and diversity analysis of 60 isolated Fusarium oxysporum collected from pan India germplasm collection.

We received ~70 Gbp of resequencing data of 60 isolates, and the data filtration has been completed. The software parameter customization for the construction of the Pan-genome is in progress.

(2) The second collaboration we had was with the ICAR-Directorate of Poultry Research, Hyderabad. This collaboration aims to generate a reference genome of the Indian black chicken "Kadaknath," which is the best alternative for health-conscious meat eaters. This mainly focused on developing the reference-level genome of the Indian black chicken "Kadaknath." The Kadaknath breed has evolved through natural selection in the indigenous milieu. This breed was conserved and reared by tribals/ Adivasis of Madhya Pradesh. Kadaknath chicken breed Geographical Indicator (G.I.) This Kadaknath breed has entirely black externally and internal organs. In this study, the whole genome sequence of the Kadaknath chicken was assembled with the combination of long at low depth PacBio Sequel II platform and the high-depth Illumina short reads platform. We received the data at our lab, and data processing, assembly, and other secondary analysis are in progress.

(3) Decoding of keratitis-causing fungus "Fusarium solani" genome and identifying virulence factors and antifungal resistance genes.

The third collaboration with the Department of Microbiology and Biotechnology Centre, The Maharaja Sayajirao University of Baroda, Vadodara, on keratitis-causing rare fungus.

Eye infections are the second leading cause of blindness worldwide, only surpassed by cataracts. Fusarium solani is a keratitis-causing filamentous fungus that can cause this rare but essential eye infection. Fungal keratitis is a dangerous condition that can cause sight problems and even blindness and accounts for 5-10% of eye infections and is commonly caused by yeast such as Candida and filamentous fungi such as Aspergillus and Fusarium.

This study aims first to decode the genome of the fusarium solani and identify the virulence factors and genes which are antifungal resistant. We received the data from 5 isolates of the fungus fusarium solani.

Publications:

1. Fei Zhao, Shilong Tian, Qiuhong Wu, Zijuan Li, Luhuan Ye, Yili Zhuang, Meiyue Wang, Yilin Xie, Shenghao Zou, Wan Teng, Yiping Tong, Dingzhong Tang, Ajay Kumar Mahato, Moussa Benhamed, Zhiyong Liu, Yijing Zhang (2022). Utility of Triti-Map for bulk-segregated mapping of causal genes and regulatory elements in Triticeae. Plant Communications. Volume 3, Issue 4,2022,100304. ISSN 2590-3462, https://doi.org/10.1016/j.xplc.2022.100304.

2. Singh, R.; Mahato, A.K.; Singh, A.; Kumar, R.; Singh, A.K.; Kumar, S.; Marla, S.S.; Kumar, A.; Singh, N.K. TinoTranscriptDB: A Database of Transcripts and Microsatellite Markers of Tinospora cordifolia, an Important Medicinal Plant (2022). Genes, 13, 1433. https://doi.org/10.3390/genes13081433.

Laboratory of Genome Informatics

Laboratory of Human and Medical Genetics

Genomic studies in chromosomal and single gene disorders

Principal Investigator: Ashwin Dalal
Staff Scientist

Adjunct Faculty:

Prajnya Ranganath	Additional Professor, NIMS
Shagun Aggarwal	Additional Professor, NIMS

PhD Students:

Dipti Deshpande	Senior Research Fellow (Until 30/08/2021)
A Sandeep	Senior Research Fellow
Shruthika Padwal	Junior Research Fellow (From 13/08/2021)
Upasana	Senior Research Fellow (From 10/01/2022)

Other Members:

Anjana Kar	Research Associate
Mugdha Singh	Research Associate
Pragna Lakshmi	Research Associate (From 11/10/2021)
Mohini Annapurna	Project Assistant (From 15/04/2021)
Shivangi Wagh	Project Assistant

Objectives

1. To conduct genetic evaluation for patients/ families with genetic disorders
2. To develop new methods and assays for genetic analysis and engage in research on chromosomal and single gene disorders
3. To act as national referral center for analysis and quality control of genetic tests for few genetic diseases
4. To impart training in genetic evaluation of patients with genetic disorders

Genetic studies on Congenital Hypothyroidism

Congenital hypothyroidism (CH) is one of the most common preventable causes of intellectual disability in the world, with an estimated prevalence of 1 in 3000 to 4000 live births. CH could be either permanent or transient. Permanent CH can result from primary or secondary dysfunction of thyroid gland. It can occur in isolation or as part of a syndromic association. Primary CH results from defects of thyroid gland development (thyroid dysgenesis – 80-85%), defects of thyroid hormone synthesis (thyroid dyshormonogenesis – 10-15%), and defects of Thyroid Stimulating Hormone (TSH)-binding or signal transduction. Secondary CH occurs due to defects of thyrotropin releasing hormone (TRH) formation or binding and defects of TSH production. Disorders associated with thyroid dysgenesis and secondary CH present with non-goitrous CH while thyroid dyshormonogenesis is usually associated with goiter. Many different genes are known to be associated with congenital hypothyroidism, but the genetic/ molecular etiological basis in a significant proportion of cases remains unknown. The genetic basis has been identified in only around 2-3% of cases of thyroid dysgenesis (TTF2, NKX2.1, NKX2.5 and PAX8). Most cases of thyroid dyshormonogenesis, on the other hand, are known to be caused by specific genetic mutations including those associated with thyroid peroxidase deficiency (TPO), sodium-iodide symporter defects (SLC5A5), pendrin defect (SLC26A4), hydrogen peroxide generation defects (DUOX2 and DUOXA2), thyroglobulin defect (TG) and iodotyrosine deiodinase defects (DEHAL1 and SECISBP2). TSHR gene mutations lead to resistance to TSH and result in primary CH. Causative genes associated with secondary CH include TSHB and TRHR.

A total of 134 (Dysgenesis 90, Dyshormonogenesis 40 and four syndromic CH) cases underwent exome sequencing. Of these, a final genetic diagnosis could be achieved in 42 (3 Dysgenesis, 37 Dyshormonogenesis and two syndromic) cases wherein a pathogenic/likely pathogenic variant causative of the phenotype was identified.

Of the 40 patients with TDH, exome sequencing identified rare disease-causing variants in 37 patients (92.5%). Homozygous or compound heterozygous variants were detected in 29 individuals with TDH (72.5%), putative digenic variants were identified in five patients (12.5%), and monoallelic variants were identified in three patients (7.5%). In three patients (7.5%), we could not find any obvious disease-causing variants in known TDH causing genes. Among the 29 patients with monogenic etiologies, DUOX2 was the most frequently mutated gene 34.5% (10/29). Along with the DUOX2 gene, TG and TPO gene mutations were frequently observed in TDH patients' TG 31% (9/29) and TPO 31% (9/29). SLC5A5 gene mutations were found in one patient with TDH (3.4%) (1/29). Recurrent mutations were identified in TG (c.475C>T:p.(Arg159Ter)) and DUOX2 (c.1709A>T:p.(Gln570Leu). Further we performed series of invitro functional studies to characterize the TPO missense variants. All the missense variants except p.(Gly860Arg) were analyzed using in silico methods and five variants [p.(Arg206Gln), p.(Glu413Gly), p.(Asp536Glu), p.(Arg648Trp), p.(Gly860Arg)] were studied using in vitro experiments. In silico experimental data showed that the three variants p.(Arg206Gln), p.(Glu413Gly), and p.(Leu444Pro) have the severe destabilizing effect on protein compared to other missense variants. Further molecular modelling studies showed that the observed missense variants disrupted the multiple hydrogen bonding with neighboring residues that may have destabilizing effect on protein structure and its function. In vitro immunofluorescence studies showed that all the five missense variants studied, were expressed on plasma membrane, similar to wildtype. Further TPO enzyme assay using AmplexRed substrate showed that enzymatic activities of these mutants were remarkably reduced.

A total of 90 dysgenesis cases underwent exome sequencing. Among these, disease-causing variants were identified in three patients. In two patients with TD, we identified TSHR gene variants and in one patient we identified FOXE1 gene variant. In remaining 87 cases, we could not find any significant variants in known causative genes. A total of four patients with syndromic CH underwent exome sequencing and disease disease-causing variants were identified in two cases. In one patient we identified a homozygous missense variant in the ALMS1 gene causing Alstrom syndrome and in second patient we identified a denovo frameshift variant in the SPEN gene causing Radio-Tartaglia syndrome.

Development of genomic technologies for predictive genetic health and forensic profiling

India, with unique genetic composition, owing to large population and practice of consanguineous marriage, provides interesting opportunities to identify novel genes for unknown and unexplained inherited phenotypes. Advancement in genomic technologies is helpful for diagnosis, counselling, prenatal testing and better management of the disease. This project aims for investigating indigenous rare disease-causing variants using exome sequencing and use of Long Amplicon PCR NGS in cases with diagnosed single gene disorder based on a functional assay like enzyme assay, factor assay etc. We have developed in-house sample to report pipeline for providing NGS based test at lower cost and higher accuracy for rare genetic diseases testing.

Patients with unknown genetic etiology were recruited for exome sequencing

Patients with clinical features suggestive of a genetic disease with unknown diagnosis were recruited for the study (n =98 individuals from 78 families). Exome sequencing yielded the diagnosis for 44 individuals from 39 families. Confirmatory investigation based on NGS findings are ongoing for 6 families whereas further analysis is ongoing for 33 families. In this study we have identified 23 novel and 16 known rare deleterious variants (24 homozygous, 2 hemizygous, 3 compound heterozygous, 10 heterozygous variants) in 39 families. Among the rare variants 19 were missense and 1 synonymous, 22 LOF (loss of function variants: insertion, deletion, stop gain & exon deletion).

A family was referred for evaluation to the outpatient clinic of the Medical Genetics department. The two affected sibs were 8.5 and 6 years of age, respectively. The parents were third-degree consanguineous. Based on the clinical features, a

possible diagnosis of complicated hereditary spastic paraplegia (HSP) was considered for the siblings.

Exome sequencing identified a homozygous synonymous variant NM_033505.4:c.126G>A:44(p. Lys42Lys) in exon two of SELENOI gene. The variant was present in both affected siblings and correlated with the clinical features. Mendelian segregation analysis confirmed that both the parents were heterozygous for this synonymous variant consistent with the autosomal recessive inheritance. Although the identified variant does not cause any amino acid change in the protein but it is adjacent to the SELENOI exon two donor splice site. The effect of this synonymous variant on pre mRNA splicing was analysed using fresh cDNA from patients' blood samples by performing PCR and sanger sequencing. cDNA analysis revealed partial skipping of entire exon two in the patient, that causes an in-frame deletion of N-terminal 21 amino acids of the EPT1 protein. Therefore, we classified this variant as Pathogenic based on the ACMG/ AMP criteria PVS1, PM2, PP1, PP3, and PP4.

Development and application of high throughput sequencing-based assay for affordable diagnosis of known monogenic disorders: NGS based assays were mostly available for sequencing of large genomic regions i.e. whole exome or genome. However, for sequencing targeted regions of the genome such as single gene or set of genes associated with specific group of disorders there are limitations in terms of sequencing time, cost, and scalability. A combination of target enrichment system using long amplicon PCR followed by next generation sequencing was developed at our centre that can help in elucidation of pathogenic rare variants in cases with suspected diagnosis. We have developed this unique testing method for 29 genes (diseases with relatively high frequency in Indian population) including mitochondrial genome. This method will be extended to 50 comprehensive genes testing. We have performed targeted amplicon sequencing in 188 patients with provisional diagnosis (majorly Cystic fibrosis, Haemophilia A, Metachromatic Leukodystrophy, Mucopolysaccharidosis, Mucolipidosis, Thalassemia etc.). Long amplicon NGS yielded confirmed diagnosis in 126 patients. 62 patients with negative diagnosis are being investigated for other genetic causes. In this study we have identified 28 novel variants and 98 known variants.

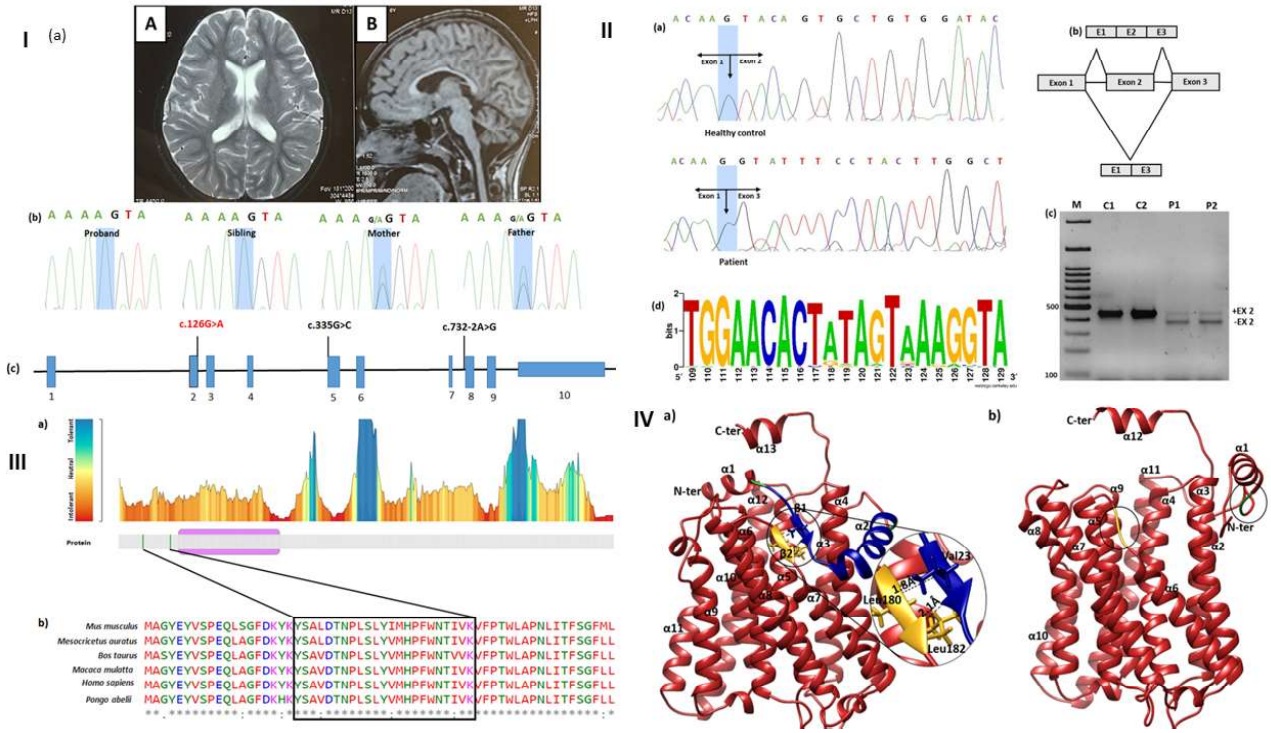


Figure 1: I: (a) Magnetic resonance imaging (MRI) of the brain [T2-weighted axial view (A) and T1-weighted sagittal view (B)] showing diffuse cerebral and cerebellar atrophy, with thinning of the corpus callosum; (b) Mendelian segregation analysis of SELENOI:c.126G>A variant; (c) SELENOI gene mutation spectrum,

previously reported two variants and variant reported in the present study. II (a) Sanger sequencing of wildtype and mutant transcripts; (b) Graphical representation of the exon two skipping in patient; (c) SELENOI cDNA analysis in two control individuals (C1, C1) and patents (P1, P2); (d) Nucleotide conservation analysis of the c.126G>A variant. III: Schematic presentation of SELENOI protein using Metadome tool for visualization of conserved regions. IV: Structural modeling using ITASSER and Pymol: 3D Structure of SELENOI wild and variant. α-helix – Maroon; β-sheets – Yellow; Deleted exon – Blue; Joining residue – Green (J) Schematic presentation of SELENOI gene mutation spectrum

Publication

Research papers published in 2021:

1. Kausthubham N, Shukla A, Gupta N, Bhavani GS, Kulshrestha S, Das Bhowmik A, Moirangthem A, Bijarnia-Mahay S, Kabra M, Puri RD, Mandal K, Verma IC, Bielas SL, Phadke SR, Dalal A, Girisha KM. (2021) A data set of variants derived from 1455 clinical and research exomes is efficient in variant prioritization for early-onset monogenic disorders in Indians. Human Mutation 42(4):e15-e61.
2. Gupta A, Sabarinathan R, Bala P, Donipadi V, Vashisht D, Katika MR, Kandakatla M, Mitra D, Dalal A, Bashyam MD. (2021) A comprehensive profile of genomic variations in the SARS-CoV-2 isolates from the state of Telangana, India. Journal of General Virology 102(3):001562.
3. Endrakanti M, Saluja S, Ethayathulla AS, Sapra S, Dalal A, Palanichamy JK, Gupta N. (2021) A patient with POLA1 splice variant expands the yet evolving phenotype of Van Esch O'Driscoll syndrome. European Journal of Medical Genetics 64(8):104261.
4. Sait H, Srivastava P, Gupta N, Kabra M, Kapoor S, Ranganath P, Rungsung I, Mandal K, Saxena D, Dalal A, Roy A, Pabbati J, Phadke SR. (2021) Phenotypic and genotypic spectrum of CTSK variants in a cohort of twenty-five Indian patients with Pycnodysostosis. European Journal of Medical Genetics 64(7):104235.
5. Knapp KM, Fellows B, Aggarwal S, Dalal A, Bicknell LS. (2021) A synonymous variant in a non-canonical exon of CDC45 disrupts splicing in two affected sibs with Meier-Gorlin syndrome with craniosynostosis. European Journal of Medical Genetics 64(4):104182.
6. Mukherjee S, Roy M, Ghosh S, Guha G, Prasad Saha S, Dalal A. (2021) Rare mutation in ELOVL4 gene in SCA34 and cognitive affection: Expounding the role of cerebellum. Clinical Neurology and Neurosurgery 210:106983.
7. Dhar MS, Marwal R, Vs R, Ponnusamy K, Jolly B, Bhoyar RC, Sardana V, Naushin S, Rophina M, Mellan TA, Mishra S, Whittaker C, Fatihi S, Datta M, Singh P, Sharma U, Ujjainiya R, Bhatheja N, Divakar MK, Singh MK, Imran M, Senthivel V, Maurya R, Jha N, Mehta P, A V, Sharma P, Vr A, Chaudhary U, Soni N, Thukral L, Flaxman S, Bhatt S, Pandey R, Dash D, Faruq M, Lall H, Gogia H, Madan P, Kulkarni S, Chauhan H, Sengupta S, Kabra S; Indian SARS-CoV-2 Genomics Consortium (INSACOG)â€¡, Gupta RK, Singh SK, Agrawal A, Rakshit P, Nandicoori V, Tallapaka KB, Sowpati DT, Thangaraj K, Bashyam MD, Dalal A, Sivasubbu S, Scaria V, Parida A, Raghav SK, Prasad P, Sarin A, Mayor S, Ramakrishnan U, Palakodeti D, Seshasayee ASN, Bhat M, Shouche Y, Pillai A, Dikid T, Das S, Maitra A, Chinnaswamy S, Biswas NK, Desai AS, Pattabiraman C, Manjunatha MV, Mani RS, Arunachal Udupi G, Abraham P, Atul PV, Cherian SS. (2021) Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. Science 374(6570):995-999.
8. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira IATM, Datir R, Collier DA, Albecka A, Singh S, Pandey R, Brown J, Zhou J, Goonawardane N, Mishra S, Whittaker C, Mellan T, Marwal R, Datta M, Sengupta S, Ponnusamy K, Radhakrishnan VS, Abdullahi A, Charles O, Chattopadhyay P, Devi P, Caputo D, Peacock T, Wattal C, Goel N, Satwik A, Vaishya R, Agarwal M; Indian SARS-CoV-2 Genomics Consortium (INSACOG); Genotype to Phenotype Japan (G2P-Japan) Consortium; CITIID-NIHR BioResource COVID-19 Collaboration, Mavousian A, Lee JH, Bassi J, Silacci-Fegni C, Saliba C, Pinto D, Irie T, Yoshida I, Hamilton WL, Sato K, Bhatt

S, Flaxman S, James LC, Corti D, Piccoli L, Barclay WS, Rakshit P, Agrawal A, Gupta RK. (2021) SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature 599(7883):114-119.

9. Ranganath P, Ranganath P, Vineeth VS, Dalal A, Patil SJ. (2021) Report of an Asian-Indian patient with Okur-Chung Syndrome and comparison of the clinical phenotype in different ethnic groups. Clinical Dysmorphology 30(4):209-212.

10. Deshpande D, Gupta SK, Sarma AS, Ranganath P, Jain S JMN, Sheth J, Mistri M, Gupta N, Kabra M, Phadke SR, Girisha KM, Dua Puri R, Aggarwal S, Datar C, Mandal K, Tilak P, Muranjan M, Bijarnia-Mahay S, Rama Devi A R, Tayade NB, Ranjan A, Dalal AB. (2021) Functional characterization of novel variants in SMPD1 in Indian patients with acid sphingomyelinase deficiency. Human Mutation 42(10):1336-1350.

11. Agrawal N, Verma G, Saxena D, Kabra M, Gupta N, Mandal K, Moirangthem A, Sheth J, Puri RD, Bijarnia-Mahay S, Kapoor S, Danda S, H SV, Datar CA, Ranganath P, Shukla A, Dalal A, Srivastava P, Devi RR, Phadke SR. (2022) Genotype-phenotype spectrum of 130 unrelated Indian families with Mucopolysaccharidosis type II. European Journal of Medical Genetics 65(3):104447.

12. Puri RD, Dalal A, Moirangthem A. (2022) Indian Undiagnosed Diseases Program (I-UDP) - The Unmet Need. Indian Pediatrics 59(3):198-200.

13. Narayanan DL, Majethia P, Shrikiran A, Siddiqui S, Dalal A, Shukla A. (2022) Further evidence of affected females with a heterozygous variant in FGF13 causing X-linked developmental and epileptic encephalopathy 90. European Journal of Medical Genetics 65(1):104403.

14. Chaudhary AK, Gholse A, Nagarajaram HA, Dalal AB, Gupta N, Dutta AK, Danda S, Gupta R, Sankar HV, Bhavani GS, Girisha KM, Phadke SR, Ranganath P, Bashyam MD. (2022) Ectodysplasin pathogenic variants affecting the furin-cleavage site and unusual clinical features define X-linked hypohidrotic ectodermal dysplasia in India. American Journal of Medical Genetics A 188(3):788-805.

Research papers in press (as on 31st March 2022):

1. Kemp SA, Cheng MTK, Hamilton WL, Kamelian K; Indian SARS-CoV-2 Genomics Consortium (INSACOG), Singh S, Rakshit P, Agrawal A, Illingworth CJR, Gupta RK. (2022) Transmission of B.1.617.2 Delta variant between vaccinated healthcare workers. Scientific Reports (In Press)

2. Saini N, Venkatapuram VS, Vineeth VS, Kulkarni A, Tandon A, Koppolu G, Patil SJ, Dalal A, Aggarwal S. (2022) Fetal phenotypes of Mendelian disorders: A descriptive study from India. Prenatal Diagnosis (In Press)

3. Ranganath P, Vs V, Rungsung I, Dalal A, Aggarwal S. (2022) Next Generation Sequencing in a Case of Early Onset Hydrops: Closing the Loop on the Diagnostic Odyssey! Fetal and Pediatric Pathology (In Press)

4. Nerakh G, Vineeth VS, Tallapaka K, Nair L, Dalal A, Aggarwal S. (2022) Microcephalic primordial dwarfism with predominant Meier-Gorlin phenotype, ichthyosis, and multiple joint deformities-Further expansion of DONSON Cell Cycle-opathy phenotypic spectrum. American Journal of Medical Genetics A (In Press)

Other publications like patents, Book chapters, etc.(01.04.2021 to 31.03.2022)

1. Usha Dutta. (2022) Essentials of Cytogenetic and Molecular Cytogenetic Laboratory Testing. Cambridge Scholars Publishing.

Laboratory of Human and Medical Genetics

Laboratory of Human Molecular Genetics

Understanding the mitochondrial dysfunction in human health and disease

Principal Investigator: P. Govindaraj
Staff Scientist

PhD Students:
Rohan Peter Mathew
B Disha

Other Members:
Lallu Nepali

Collaborators

Madhu Nagappa	NIMHANS, Bangalore
Sireesha Yareeda	NIMS, Hyderabad

Objectives

Our laboratory focuses on understanding the mitochondrial dysfunction in human health and disease. In particular, with a specific aim to explore the new genes that are associated with mitochondrial disorders, understand the molecular mechanisms, and develop theragnostics (diagnosis and treatment). We use next-generation sequencing to investigate the interaction between mitochondrial DNA and nuclear DNA. Further, we use patient-derived cell lines (fibroblasts) for generating transmitochondrial cybrids for mtDNA mutations and other cellular models to delineate the molecular mechanism leading to neuronal loss and neurological defects. In addition, our group is also involved in identifying the novel genetic cause of other rare genetic disorders.

Project: The Identification and characterization of newer pathogenic variants associated with mitochondrial diseases of the nervous system

Last decade of biomedical research, there has been a remarkable convergence of interest in the powerhouse of cells, the mitochondria. Mitochondrial dysfunction is associated with a broad spectrum of human disorders, ranging from rare, inborn errors of metabolisms to common, age-related conditions, including cardiovascular and neurodegenerative diseases. However, the emerging field of mitochondrial medicine is hindered by the complexity of these organelles and the breadth of implication in disorders, leading to a lack of mechanistic insights, biomarker discovery, and therapeutic targets.

Mitochondrial diseases are multi-systemic, heterogeneous group of disorders affecting children and adults with 1 in 5000 individuals. They are due to mutation in mitochondrial DNA (mtDNA) or nuclear DNA that can affect the assembly of mitochondrial components and function. Because of the clinical heterogeneity and tissue-specificity, the molecular basis of which is largely unknown. The typical mitochondrial syndromes include mitochondrial encephalomyopathy with lactate acidosis and stroke-like episodes (MELAS), Leigh syndrome (LS), Leber hereditary optic neuropathy (LHON), chronic progressive external ophthalmoplegia (CPEO), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), and myoclonic epilepsy with ragged red fibers (MERRF) and non-syndromic groups that have diverse clinical manifestations. The genetic diagnosis (>50%) has been transformed by the next-generation sequencing (NGS) technologies. However, novel variants and variants of unknown significance (VUS) identified by whole-exome sequencing (WES) and whole-genome sequencing (WGS) remain a challenge while interpreting in the context of the clinical phenotype, pending functional evidence. Recent studies have highlighted that RNA sequencing (RNAseq) is an essential companion of genomic sequencing to address undiagnosed

genetic disorders. Both mitochondrial and nuclear genome sequencing methods have limitations due to the tissue-specificity of mitochondrial diseases. However, the recent advances in genomics and transcriptomics provide solutions to overcome these challenges. Therefore, we aim to work on the pathobiology of mitochondrial disorders focusing on the nervous system to understand the impact of mtDNA and nuDNA mutation on disease pathogenesis and tissue-specific manifestations (Figure 1).

During the current year (April 2021- March 22), we initiated a new collaboration with the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, and Nizam's Institute of Medical Sciences (NIMS), Hyderabad, for clinical samples. A total of 35 patients suspected of mitochondrial disorders and their relatives were recruited after the ethical clearance (January 2022). Complete mitochondrial sequencing revealed several known and novel variants. The whole-exome sequencing will be carried out for patients that fail to detect mtDNA variants to identify the causative mutations in nuclear DNA. Further, patient-derived fibroblasts will be established from the skin punch and used for studying mitochondrial functions.

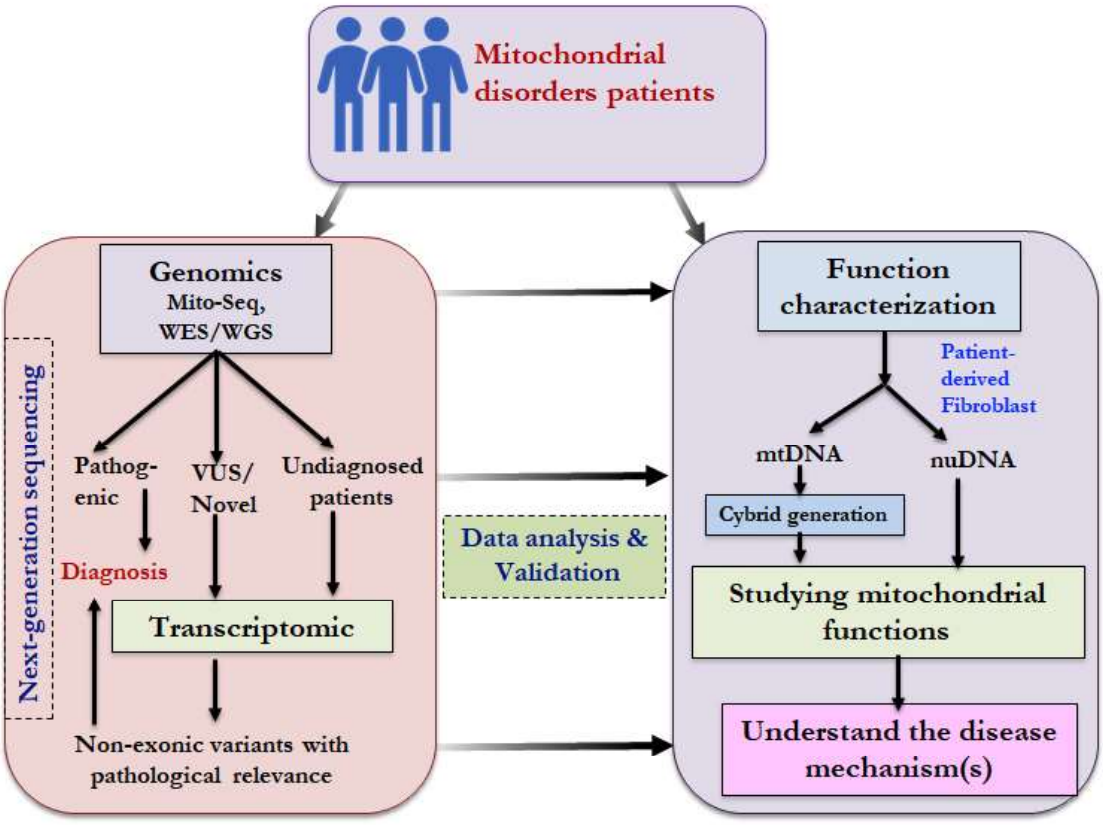


Figure 1: Summary of the identification and characterization of newer pathogenic variants associated with mitochondrial diseases of the nervous system.

Publications

1. Shivaram S, Nagappa M, Seshagiri DV, Saini J, Govindaraj P, Sinha S, Bindu PS, Taly AB (2021). Leukodystrophy Due to eIF2B Mutations in Adults. Can J Neurol Sci. doi: 10.1017/cjn.2021.202. (Inpress).

2. Huddar A*, Govindaraj P*, Chiplunkar S, Deepha S, Jessiena Ponmalar JN, Philip M, Nagappa M, Narayanappa G, Mahadevan A, Sinha S, Taly AB, Parayil Sankaran B (2021). Serum fibroblast growth factor 21 and growth differentiation factor 15: Two sensitive biomarkers in the diagnosis of mitochondrial disorders. Mitochondrion, 60:170-177. *Equal Contribution.

3. Guo Le, Govindaraj P, Kievit M, de Coo IFM, Gerards M, Hellebrekers DMEI, Gayathri N, Taly AB Sankaran BP, Smeets HJM (2021). Whole exome sequencing reveals a homozygous C1QBP deletion as the cause of progressive external ophthalmoplegia and multiple mtDNA deletions. Neuromuscular Disorders, 31: 859-864.

Laboratory of Human Molecular Genetics

Laboratory of Immunology

An emerging Tumor Suppressor

Principal Investigator: Sunil K Manna
Staff Scientist

PhD Students:

Shashank Saurav	Senior Research Fellow
Aher Abhishek Taterao	Senior Research Fellow
Saphy	Senior Research Fellow
V Chandana Praneetha	Senior Research Fellow
Bindi Goradia	Senior Research Fellow
Homagni Dey	Junior Research Fellow

Other Members:

T Navaneetha	Technical Assistant

Collaborators:

Tushar Basu Baul	NEHU, Shilong
Pulakesh Bera	Vidyasagar University, WB
Sudit Mukhopadhyay	NIT, Durgapur, WB

Objectives

1. Understanding the role of Profilin in regulation of tumorigenesis.
2. Understanding and regulation of advanced glycation end products (AGE)-mediated deleterious effects.
3. Understanding and regulation of inflammatory and tumorigenic responses.

Research Summary

Advanced glycation end (AGE) products are formed by covalently attaching reducing sugars or its reactive carbonyl metabolites such as methylglyoxal (MGO) and glycolaldehyde, to amino group of the basic amino acids present in the proteins. The mechanism for AGE formation involves the formation of Schiff base between amino terminal of the basic amino acids and the carbonyl group of sugar moiety. AGEs are known to interact with their specific receptors, Receptors for AGE (RAGE), members of super immunoglobulin family. The signal induced by AGE-RAGE binding is tissue and disease specific. Depending on the intensity and duration of AGE-RAGE ligation, various pathways get activate such as ERK1/2, P38MAPK, CDC42/RAC, SAPK/JNK and NF-κB. During natural aging, AGEs get accumulated inside the human body triggering various pathological consequences ranging from retinopathy, diabetes, kidney failure to Alzheimer. Senescence is a biological process involving the un-programmed cell death or growth arrest leading to aging of human body. Neuroblastoma cells IMR32 were treated with different concentration of AGE for 48 h and then SA-beta-gal assay was performed to check the occurrence of senescence. The number of senescent cells increases with increasing the dose of AGE which was further supported by FACS data. The same result was supported by the overexpression of p21 after treatment. Overall, AGE-mediated increase in senescence suggests – i) inactivation or degeneration of cells that finally move for apoptosis; ii) the increased number of senescent cells may liberate toxic superoxide radicals (RNI and ROI) and proteolytic enzymes that promote ageing. All these events need to be proven experimentally.

Details of progress in the current reporting year (April 1, 2021 - March 31, 2022) Upregulated Profilin induces autophagy through stabilization of AMP-activated protein kinase

Profilin, a 15 kDa globular protein regulates several biological responses including organ development, wound healing, immune functions, etc. upon interaction with cellular proteins, like actin. Most of the cancers express a lower amount of profilin, which results in reduction of focal adhesion and increased

malignancy. Although reduced expression of profilin increases cancer aggressiveness, complete ablation of this protein results in compromised growth and viability. Profilin expression was found to be very low in a triple-negative breast cancer (TNBC), MDA MB-231 cells and its overexpression results in inhibition of tumor initiation and growth. Autophagy is a well-organized, multi-step cellular recycling event, which is controlled by more than 18 autophagy regulating genes (ATGs). During autophagy, microtubule light chain-3 (LC3) also known as LC3A/B-I, is a cytosolic protein that conjugates with phosphatidylethanolamine (LC3A/B-II) and is recruited to the autophagosomal membrane. Autophagosome maturation is important step to renew energy especially in the rapid growing tumor cells. As Profilin interacts with several biological proteins to regulate diverse cellular functions, we have determined its role in autophagy in the triple negative breast tumor. Since, genetic manipulation is not a viable option to overexpress any protein in terms of prognosis, clinically safe molecule can be used as a potent inducer. We have used ATRA, a potent inducer of profilin through RAR-RXR mediated transcriptional activation in our study. ATRA treatment of MB-231 cells for 24 h, exhibited autophagy induction in a dose-dependent manner as determined by the increased amounts of LAMP 2 degradation, LC3A/B-I to LC3A/B-II conversion along with increased profilin expression (A) and was further validated by the increased amount of MDC fluorescence (2-6 fold), which was almost 3-fold at 20 μM of ATRA (B). Increased puncta numbers of LC3A/B and p62 along with increased profilin expression have been observed upon ATRA treatment (C). The number of puncta from 60 individual cells was counted and the scatter plot exhibited an approximately 2.5-fold increase in puncta number per cell upon ATRA treatment (D). Together with these, data infer that ATRA induces autophagy along with profilin and inhibits autophagic clearance resulting in impaired autophagy. MB-231 and profilin-stable cells were treated with ATRA (20 μM) for 24 h followed by cycloheximide (50 μg/ml) treatment in a time-dependent manner. Since cycloheximide inhibits protein synthesis, decrease in the basal level of AMPKα was observed in MB-231 cells. Whereas ATRA pretreated MB-231 or profilin-stable cells (high profilin background) showed protection of AMPKα degradation (G). To find out the cross-talk between profilin and AMPKα, MB-231 cells were seeded on coverslip and prepared for immunofluorescence microscopy using anti-profilin and anti-AMPKα antibodies. Co-localization of profilin and AMPKα was observed (F), which was further validated with super-resolution microscopic observation. To find out the physical interaction between these molecules, endogenous AMPKα was immuno-precipitated with the Protein A/G PLUS-Agarose beads linked with mice raised non-specific IgG or IgG against AMPKα and immunoblot was probed with anti-AMPKα (raised in rabbit) or anti-profilin (raised in rabbit) antibodies. An intense profilin band was detected in the pull-down product, which suggests the physical interaction between AMPKα and profilin (E). Altogether, data infer that profilin interacts with AMPKα and protect its' degradation. We have analyzed 'in silico' interaction of AMPK holo-complex (4RER) and profilin (1PFN) using the docking tool ClusPro 2.0 and the best model represented AMPK catalytic subunit (AMPKα, green) interaction with profilin (cyan). Amino acid residues of AMPKα (red) and profilin (magenta) are shown as interacting members (H). To translate these findings in vivo, tumor sections from the given animal model study were examined for profilin, AMPKα, p62, LC3, LAMP 2 and mTOR by immunofluorescence microscopy and Western blot. MB-231 borne tumor in xenograft nude mice upon ATRA treatment exhibited increased amounts of profilin, AMPKα, p62 and LC3 but reduced amount of mTOR (I). ATRA treatment of MB-231 born tumor-bearing mice resulted in decrease of mTOR and LAMP 2 but increase in AMPKα, p62, profilin and LC3A/B-I to LC3A/B-II conversion in tumors as determined from tumor lysates by Western blot (J). Altogether, ATRA treatment studies on animal model validated the 'in vitro' data.

In summary, this study shows that ATRA-mediated profilin expression increases anti-tumor potential by impairing autophagy through AMPK stabilization. Taken this as proof of concept from both cell-based and 'in vivo' data, ATRA may be a potent and safe agent which can be utilized for future combination therapeutics. Therapeutic utilization of ATRA-induced cytotoxic autophagy to drive cancer cell death especially for the triple-negative cancer, could be an emerging paradigm for cancer therapy.

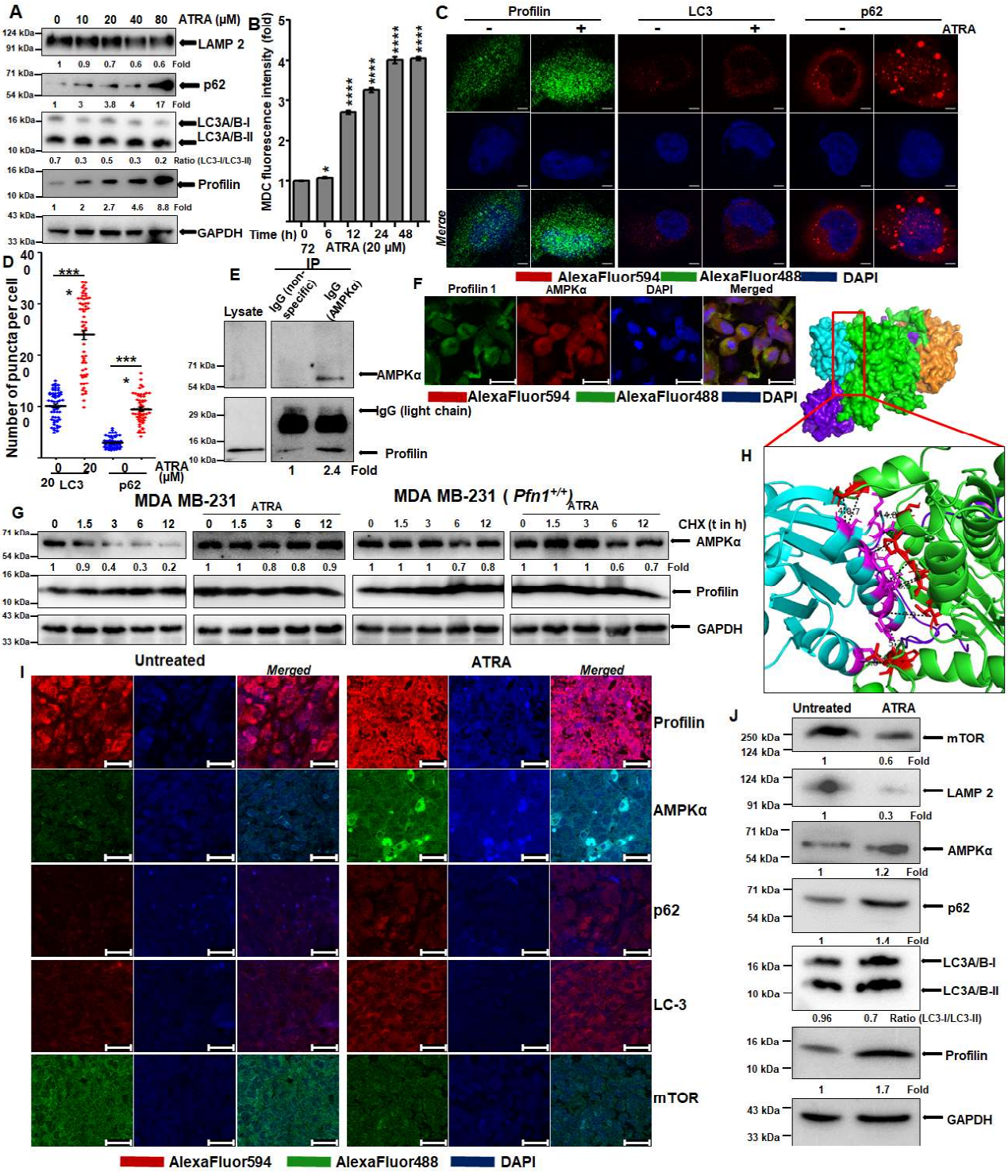


Figure Legend: Profilin upregulation induces autophagy through stabilization of AMP-activated kinase E. Western blot analysis to determine the amount of LAMP 2, LC3A/B and profilin in MB-231 cells upon treatment with different concentrations of ATRA for 24 h (A). MB-231 cells, treated with ATRA in a dose dependent manner for 24 h were stained with MDC. Samples were washed and excited at 335 nm. The fluorescence intensity was measured at 518 nm wavelength (n=9) and indicated as fold, considering untreated cells' value as 1 fold (B). MB-231 cells were cultured on coverslip, treated with 20 μM of ATRA for 24 h, and analyzed in immunofluorescence (LSM700) microscope. The images showed the amounts of profilin, LC3A/B and p62 (scale bar=5 μm) (C), scatter plot showed LC3A/B and p62 puncta per cell (each dot represents single cell) (n=60) (D). Endogenous AMPKα was immuno-precipitated using anti-IgG (mice raised) or anti-AMPKα (mice raised) –Agarose beads from WCE of MB-231 cells, followed by immunoblotting with anti-AMPKα (rabbit

raised) or anti-Profilin 1 (rabbit raised) antibody (E). MB-231 cells were cultured on coverslip and prepared for immunofluorescence microscopy. Microscopic images showing co-localization of profilin (AF-488) and AMPKα (AF-594) (F). Parental MB-231 and profilin-stable MB-231 cells (1x106), treated with ATRA (20 μM) for 24 h were incubated with cycloheximide (50 μg/ml) for indicated time periods. Western blot images show the amount of AMPKα and profilin (G). Crystal structure of the phosphorylated human holo-AMPK (containing α1, β2 and γ1 subunits) complex bound to AMP and cyclodextrin (4RER) PDB structure was processed by removing AMP and cyclodextrin, docked with refined solution structure of human profilin 1 (1PFN) using ClusPro 2.0 docking tool. Structure of best balanced model of AMPK (green) with profilin (cyan) is depicted (H, upper panel) and detailed interaction structure showing AMPK (red) and profilin (magenta) amino acid residues (H, lower panel). MB-231 borne tumor bearing nude mice treated with ATRA (0.15 mg/Kg body weight/mice/twice in a week) for 47 days, mice were sacrificed and tumors were collected. Tumor tissue sections showing immunofluorescence imaging of profilin, AMPKα, p62, LC3 and mTOR (scale bar = 25 μm) (I). Western blot images showing amount of mTOR, LAMP 2, AMPKα, p62, LC3 and profilin in tumor lysates (J).

Publications:

1. Bera P, Aher A, Brandao P, Manna SK*, Bhattacharyya I, Mondal G, Jana A, Santra A and Bera P* (2021). Anticancer activity, DNA binding and docking study of M(II)-complexes (M = Zn, Cu and Ni) derived from a new pyrazine-thiazole ligand: synthesis, structure and DFT. New Journal of Chemistry 45 (27): 11999-12015 (https://doi.org/10.1039/D0NJ05883A).
2. Saurav S and Manna SK* (2022). Increased expression of Profilin potentiates chemotherapeutic agent-mediated tumour regression. British Journal of Cancer (In press). (https://doi.org/10.1038/s41416-021-01683-5).
3. Jana A, Aher A, Brandão P, Bera P, Sharda S, Phadikar U, Manna SK, Mahapatra AK and Bera P* (2022). Evaluation of anticancer activities varying ligand's substituents in Co(II/III)-picolyl phenolate derivatives: Synthesis, characterization, DFT, DNA cleavage and molecular docking studies. Dalton Transactions (In press). (https://doi.org/10.1039/D1DT02825A).
4. Bera P, Aher A, Brandao P, Debnath U, Dewaker V, Manna SK*, Jana A, Pramanik C, Mandal B and Bera P* (2022). Instigating the In Vitro Anticancer Activity of New Pyridine–Thiazole-Based Co(III), Mn(II), and Ni(II) Complexes: Synthesis, Structure, DFT, Docking, and MD Simulation Studies. Journal of Chemical Information and Modeling (In press). (DOI: 10.1021/acs.jcim.1c01280).
5. Jana A, Aher A, Brandao P, Sharda S, Bera P, Phadikar U, Manna SK, Mahapatra AP and Bera P* (2022). Dissociation of a tripodal pyridyl-pyrazole ligand and assortment of metal complex: Synthesis, structure, DFT, thermal stability, cytotoxicity, DNA cleavage, and molecular docking studies. Journal of Molecular Structure (In press). (https://doi.org/10.1016/j.molstruc.2022.132479).

Laboratory of Immunology

Laboratory of Infectious Diseases

Understanding the biology of human pathogens *Entamoeba histolytica* and *Naegleria fowleri*

Principal Investigator: Kuldeep Verma
Staff Scientist

Lab Members:

Bhagyashree Chordiya	Junior Research Fellow (since May 2021)
P Navyaka	Junior Research Fellow (since September 2021)

The pathogenic amoeba, *Entamoeba histolytica* and *Naegleria fowleri*, are a class of human pathogens that causes life threatening infections such as amoebic colitis, hepatic abscess, and primary amoebic meningoencephalitis (PAM). The long-term goal of our lab to understand how host cues modulate the invasive nature of pathogenic amoeba and how it helps in tissue destruction in a complex host environment.

Research Summary

Details of the progress made in the current reporting year (17th May 2021-31st March 2022)

Project 1: Understanding the functional role of vacuolar ATPases in trogocytosis and tissue invasion mediated by *E. histolytica*

Trogocytosis is an evolutionary conserved process from ancient amoebas to higher eukaryotic cells. Trogocytosis (from Greek: trogo means nibble or chew) is a cellular process in which the target cell physically captures and engulfs a piece of cellular components from donor cells. *E. histolytica* trophozoite employs a similar process to kill the host cell as well as acquires the immune cell protein and displays it on their own surface. In a previous study, it has been identified that amoebic trophozoite treated with V-ATPase pharmacological inhibitor showed reduced trogocytosis and phagocytosis of host cells. Based on our recent investigation, we have identified that amoebic V-ATPase subunit/s recruited on early-stage of phagocytosis and nibbling the host cell. Further, we are investigating the molecular mechanism of how amoebic V-ATPase subunit/s recruited in cellular nibbling for host tissue destruction.

Project 2: Understanding the spatiotemporal dynamics and ultrastructure details of ECM degrading device "amoebic invadosomes" and their crosstalk with Rab GTPases and cell surface proteases trafficking machinery in *E. histolytica*

Podosome and invadopodia are actin rich concerted foci observed in mammalian cells. These structures are localized to the ventral surface of the cell and important for degradation of the extracellular matrix (ECM) by cell surface proteases. Amoebic trophozoite also displayed the actin dot-like structure upon the contact with ECM and fibronectin. Further, it has been shown that amoebic F-actin dot-like structures resemble podosomes or invadosomes and are important for ECM degradation. We have identified that some of the Rab proteins regulate F-actin dot dynamics and transport of membrane proteases in amoebic parasite. Future study will establish the crosstalk between Rab proteins, F-actin and membrane proteases dynamics at the amoebic invadopodial sites.

Laboratory of Infectious Diseases

Laboratory of Molecular Cell Biology

Signal transduction pathways in macrophages and host-pathogen interaction in tuberculosis

Principal Investigator: Sangita Mukhopadhyay
Staff Scientist

PhD Students:

KM Rohini	Senior Research Fellow
Ravi Pal	Senior Research Fellow
Manoj Kumar	Senior Research Fellow
Priyanka Dahiya	Junior Research Fellow
S. Brahmaji	Junior Research Fellow
G. Akshay	Junior Research Fellow
Pooja Kushwaha	Junior Research Fellow
Shahid Aziz	Junior Research Fellow
Abhishek Dutta	Junior Research Fellow (upto Sep 24, 2021)
Sajal Dey	Junior Research Fellow (since Sep 09, 2021)
Ruhi Gupta	Junior Research Fellow (since Mar 09, 2022)

Other Members:

Niteen Pathak	Senior Technical Officer
B. Srikanth	DST-INSPIRE Faculty (upto July 01, 2021)
Faiza Nazar	Senior Research Fellow (upto October 07, 2021)
Sivapriya Pavuluri	Research Associate-III
Swapnila Pramanik	Junior Research Fellow (upto March 09, 2022)

Collaborator:

Prof. K N Balaji	IISc, Bangalore
Sudip Ghosh	NIN, Hyderabad
Gaddam Sumanlatha	Osmania University, Hyderabad
Vinay K. Nandicoori	CCMB, Hyderabad
Sunil K Manna	CDFD, Hyderabad
S. Aparna	BPHRC, Hyderabad

Objectives:

i) Signal transduction pathways in macrophages regulating its innate-effector functions and how various candidate proteins of *Mycobacterium tuberculosis* (M.tb) interfere with macrophage signaling cascades to modulate host's protective responses against the bacilli.

ii) Identification of therapeutics against tuberculosis and inflammatory diseases.

PknG protein of M.tb targets RGDI-1 in the regulation of Rab7l1 GTPase activity

Summary of the work done until the beginning of this reporting year

Inhibition of phagosome-lysosome (P-L) fusion is the hall-mark for survival of M.tb inside macrophages and its successful infection. The mycobacterial secretory protein kinase G (PknG), a serine/threonine kinase is a unique protein that blocks P-L fusion in macrophages. In a recent study, we have shown that PknG interacts with a Rab GTPase, Rab7l1 (which plays a crucial role in P-L fusion) and inhibits its GTPase activity, thereby blocking recruitment of active Rab7l1 and the subsequent P-L fusion markers to phagosomes and arrests phagosome maturation process (Pradhan et al[2018]J. Immunol.201:1421). In the present study, it was aimed to identify the Rab7l1 interacting GDI and studied if PknG targets this GDI(s) to inhibit Rab7l1 GTPase activity to inhibit phagosome-lysosome fusion.

Details of progress made in the current reporting year (April 1, 2021 - March 31, 2022).

Rab7l1 but not PknG interacts with Rho-GDP dissociation inhibitor-1 (RGDI-1): The Yeast two-hybrid (Y2H) assay indicates that Rab7l1 interacts specifically with RGDI-1 (Fig. 1A). The physical interaction between Rab7l1 and RGDI-1 was confirmed by the GST pull-down assay and with a His pull-down assay. The Y2H assay indicates that

though Rab7l1 interacts specifically with RGDI-1, PknG does not directly interact with any GDI protein (Fig. 1A). To understand in details the interaction between Rab7l1 and RGDI-1, Rab7l1 structure was next predicted and based on the possible and similar orientations developed in Protein – Protein docking, which were related to the other Rab7l1 like structures such as Rac1 and Cdc42 interacting with RGDI-1 were taken into consideration for the final lead development in the interaction studies. Based on the understating, N-terminal Switch 1 (S1) and Switch 2 (S2) domains of Rab7l1 are observed to interact with N-terminus regulatory arm of RGDI-1 (Fig. 1B) as similar as Cdc42 and Rac1 in the revealed Crystal structures. To confirm the result shown in Fig. 1B, various deletion mutants of Rab7l1 were next generated; mainly, deletion of Switch 1 domain (ΔSwitch 1), deletion of Switch 2 domain (ΔSwitch 2), deletion of N-terminus (N) + Switch 1 + Switch 2 domains (ΔN + Switch 1 + Switch 2) and His pull-down assay was carried out, which indicated that both Switch 1 and Switch 2 regions of Rab7l1 are crucial for its binding with RGDI-1 (Fig. 1C).

PknG phosphorylates RGDI-1: It was hypothesized that PknG phosphorylates RGDI-1 to inhibit dissociation of RGDI-1 from Rab7l1 and thereby preventing the GTPase activity of Rab7l1. As PknG does not directly interact with RGDI-1 (Fig 1A), and since PknG, Rab7l1 and RGDI-1 were found to form a tri-molecular complex, it is possible that Rab7l1 acts as a scaffold which anchors both PknG and RGDI-1 and thus facilitates PknG to access RGDI-1 for phosphorylation. Next, an in vitro kinase assay was carried out using His-PknG-WT along with recombinantly purified GST-RGDI-1 and His-Rab7l1-WT. The result indicates that PknG does not phosphorylate Rab7l1 but phosphorylates specifically RGDI-1. As expected, a kinase-deficient mutant PknG (PknG-K181M, contains a mutation in the kinase domain of PknG) protein failed to phosphorylate RGDI-1. These results indicate that in a tri-molecular complex of PknG-Rab7l1-RGDI-1, PknG gets physical access to phosphorylate RGDI-1.

PknG causes accumulation of higher amount of Rab7l1-RGDI-1 complex in infected macrophages and prevents Rab7l1-GTPase activity: It is suggested that interaction of GDI with Rab GTPase keeps Rab GTPase in its inactive form (Rab-GDP) and inhibits its GTPase activity. In the earlier section, we have shown that PknG phosphorylates RGDI-1, which is probably responsible for forming a stable RGDI-1-Rab7l1 complex that can result in inhibition of Rab7l1 GTPase activity. Thus, more amounts of Rab7l1-RGDI-1 complex was expected in macrophages infected with M. bovis BCG that carries an intact PknG as compared to macrophages infected with PknG knock-out M. bovis BCG (M. bovis BCGΔPknG). It could be observed that macrophages infected with M. bovis BCG had truly more Rab7l1-RGDI-1 complex than macrophages infected with M. bovis BCGΔPknG (Fig. 1D) indicating that PknG probably inhibits dissociation of RGDI-1 from Rab7l1 and is responsible for the formation of a stable Rab7l1-RGDI-1 complex. To further confirm that PknG-mediated phosphorylation of RGDI-1 is responsible for formation of RGDI-1-Rab7l1 complex, we next infected macrophages with Msmeg-PknG-WT or Msmeg-PknG-K181M (defective for PknG kinase activity) and the Rab7l1-RGDI-1 complex level was compared between these groups. Macrophages infected with Msmeg-pVV16 served as control group. It could be observed that more amount of Rab7l1 was complexed with RGDI-1 in macrophages infected with Msmeg-PknG-WT as compared to macrophages infected with Msmeg-PknG-K181M (Fig. 1E) indicating that kinase activity of PknG is responsible for formation of stable Rab7l1-RGDI-1 complex. When GTPase activity in these groups was measured at 4 hours time point post-infection, a reduction of Rab7l1-GTPase activity was observed in macrophages infected with Msmeg-PknG-WT when compared with macrophages infected with Msmeg-PknG-K181M (Fig. 1F). These data together indicate that the kinase activity of PknG was responsible for decreased GTPase activity of Rab7l1, which is probably due to phosphorylation of RGDI-1 by PknG, resuls in formation of a stable Rab7l1-RGDI-1 complex and prevention of Rab7l1-GTPase activity.

Future studies: Since P-L fusion is important for regulation of autophagy and MHC class II antigen presentation our future studies are aimed at understanding if M.tb PknG inhibits these processes in macrophages for its better survival.

Project 2: Rab7l1 plays a role in regulating surface expression of Toll like receptors and cytokine secretion in activated macrophages

In our previous study, we have identified a novel role of Rab7l1 in phagosome–maturation (Pradhan et al[2018]J. Immunol.201:1421). However, its role

in regulating macrophage innate-effector signaling and cytokine response is not clearly understood, which is being addressed in the present study. Since cytokine plays an important role in the regulation of macrophage innate functions, the role of Rab7l1 in cytokine signaling was investigated. The cytokine response was compared between Rab7l1 knock-down (Rab7l1-KD) THP-1 macrophages and control THP-1 macrophages that received scramble shRNA. Cells were activated with LPS (TLR4 agonist) and Pam3CSK4 (TLR2 agonist). Interestingly, it could be observed that the induction of TNF-α and IL-10 was higher in Rab7l1-KD as compared to the control THP-1 macrophages (Figure 2). The ERK 1/2 and p38 MAPK signaling cascades were higher in Rab7l1-KD THP-1 macrophages and were found to be responsible for induction of TNF-α and IL-10 cytokines respectively (Figure 2). Interestingly, though there is no change in total expression, the surface levels of TLR2, TLR4 and CD14 receptors were higher in Rab7l1-KD THP-1 macrophages in contrast to control cells while intracellular levels of these receptors are in opposite order for both the cells indicating that Rab7l1 is involved in the recycling process of these receptors. The LPS-induced TLR4 signaling is known to predominantly trigger activation of NF-κB transcription factor and subsequent transcription of various proinflammatory cytokines like TNF-α. Hence, the nuclear levels of NF-κB in control and Rab7l1-KD macrophages and its contribution in TNF-α induction in LPS-stimulated Rab7l1-KD THP-1 macrophages was investigated. The results indicated that Rab7l1-KD THP-1 macrophages had more NF-κB translocation and was responsible for increased induction of TNF-α as inhibition of NF-κB activity by the specific pharmacological inhibitors suppressed induction of TNF-α cytokine in Rab7l1-KD THP-1 macrophages. Thus, Rab7l1 plays an important role in TLR transport and cytokine production in macrophages and can be an important signaling molecule influencing the inflammatory response and outcome of an infection (Figure 2).

Future study: It would be interesting to further examine the role of Rab7l1 in the endocytosis and receptor recycling.

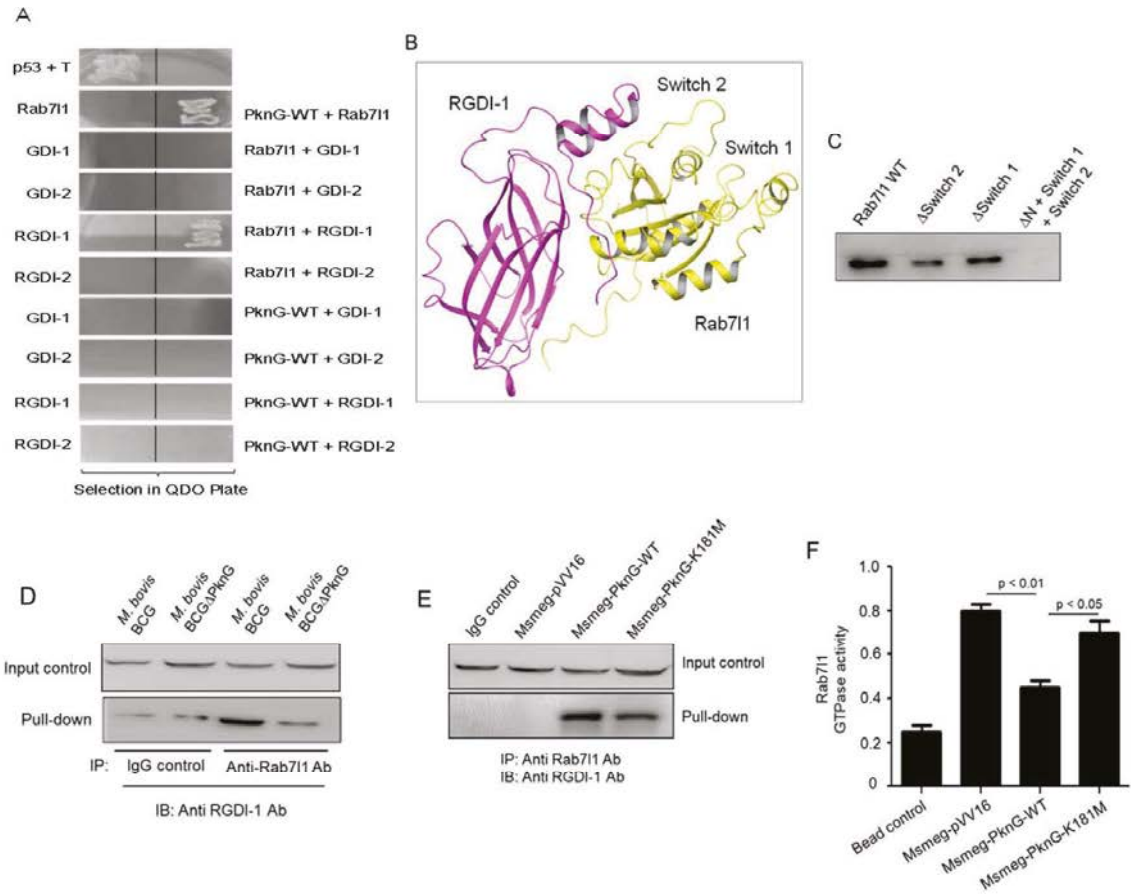


Figure 1: RGDI-1 interacts with Rab7l1 and makes stable complex with Rab7l1 in presence of PknG and eventually reduces the Rab7l1 GTPase activity. (A) Rab7l1 was cloned in pGADT7 vector in frame with GAL4-AD and different GDI (GDI-1, GDI-2, RGDI-1 and RGDI-2) were cloned in pGBKT7 vector in frame with

GAL4-BD and were co-transformed in AH109 yeast strain and activation of reporter genes were observed by selecting them on QDO plates. The plasmids pGBKT7-p53 and pGADT7-T were used as a positive interaction control. Activation of reporter genes Adenine (Ade) and Histidine (His) were monitored by selecting them on quadruple dropout (QDO) (Supplement dropout (SD) /-Ade, -His, -Leu, -Trp) plates. (B) Ribbon illustration of Rab7l1-RGDI-1 structure complex was shown in yellow and pink respectively. Protein-Protein docking analysis represented the docked pose of the molecules at the N terminus S1 and S2 regions of Rab7l1 interaction with RGDI-1. (C) Ni-NTA beads based-immunoprecipitation assay indicate that RGDI-1 interacts with Ni-NTA beads bound Rab7l-WT or Switch 1 and Switch 2 region of Rab7l1. PMA-differentiated THP-1 macrophages were infected with M. bovis BCG/M. bovis BCGΔPknG (D) or Msmeg-pVV16/Msmeg-PknG-WT/Msmeg-PknG-K181M (E) for 1 h at 4°C followed by another 1 h at 37°C. Cells were harvested and lysates were prepared using lysis buffer. Cell lysates (1000 µg) were incubated with 10 µg of anti-Rab7l1 Ab (IP) overnight at 4°C. Next, about 25 µl of pre-equilibrated Sepharose A/G beads were added to each sample and kept on rotor at 4°C for 2 h. Immune-complexes were run on a 12% SDS-PAGE and transferred onto nitrocellulose membrane for immunoblotting (IB) using anti-RGDI-1 Ab (1:2500 dilution). (F) PMA-differentiated THP-1 macrophages were infected for 1 h at 4°C followed by another 3 h at 37°C with Msmeg-pVV16/Msmeg-PknG-WT/Msmeg-PknG-K181M at 1:10 MOI. Cell lysates were prepared and used to measure Rab7l1 GTPase activity using GTPase assay kit. Results are mean ± SEM of 3 different experiments.

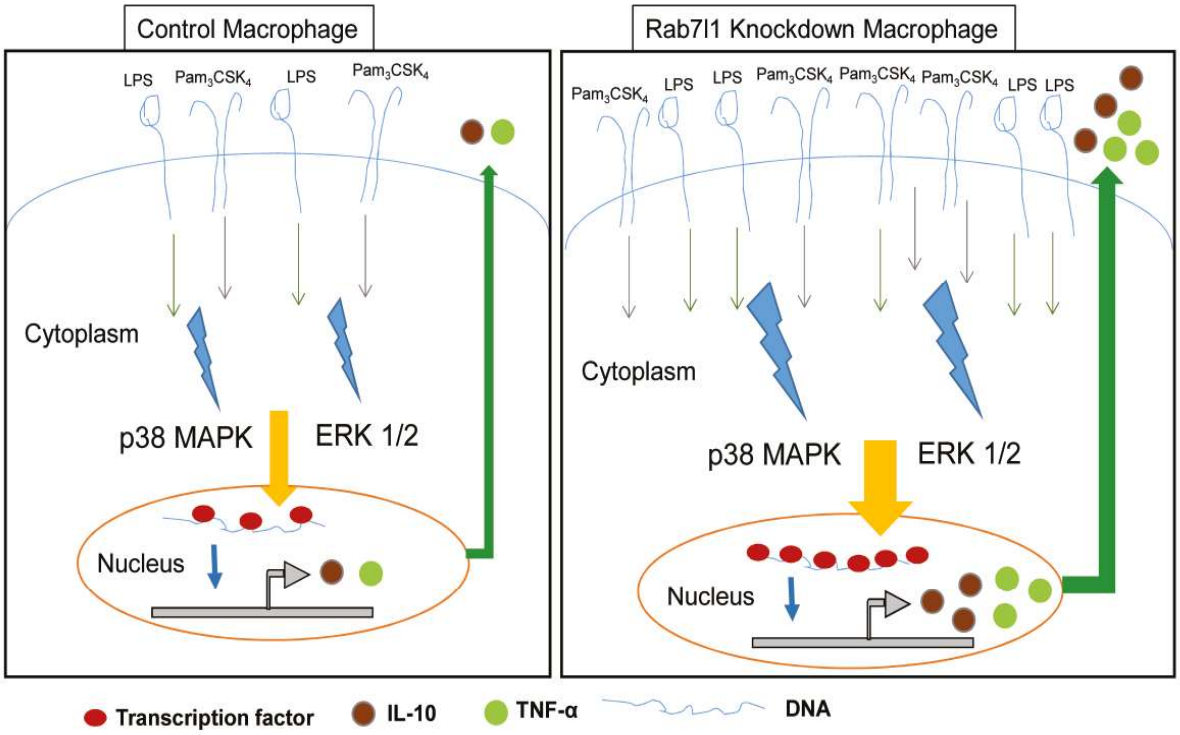


Figure 2 : Rab7l1 plays a role in TLR mediated innate-effector signaling and cytokine responses in macrophages. Rab7l1 is involved in receptor recycling and surface levels of TLR2, TLR4 and CD14 are higher in Rab7l1 knock-down macrophages. These results in more activation of TLR4- (LPS) and TLR2- (Pam3CSK4) triggered signaling resulting in enhanced stimulation of p38 MAPK and ERK 1/2 and cytokine (TNF-α and IL-10) secretion.

Publications

i) Research papers published in the calendar year 2020-2021

1. Shrivastava R, Pradhan G, Ghosh S and Mukhopadhyay S (2022). Rabaptin5 acts as a key regulator for Rab7l1-mediated phagosome maturation process. Immunology 165:328-340
2. Sontyana B, Shrivastava R, Battu S, Ghosh S and Mukhopadhyay S (2022). Phagosome maturation and modulation of macrophage effector functions by intracellular pathogens: targets for therapeutics. Future Microbiology. 17:59-76.
3. Pal R, Bisht MK, and Mukhopadhyay S (2022). Secretory proteins of Mycobacterium tuberculosis and their roles in modulation of host immune responses: Focus on therapeutic targets. The FEBS Journal. doi: 10.1111/febs.16369.
4. Srivastava S, Abraham P and Mukhopadhyay S (2021). Aptamers: An apt reply towards the fundamental battle against tuberculosis. Frontiers in Cellular and Infection Microbiology 11:656421.
5. Srivastava S and Mukhopadhyay S (2021). Mycobacterium tuberculosis protein PPE2 binds to DNA region containing promoter activity. Biochemical and Biophysical Research Communications. 567:166-170.
6. Pal R, Ghosh S and Mukhopadhyay S (2021). Moonlighting by PPE2 protein: Focus on mycobacterial virulence. The Journal of Immunology. 207:2393-2397.
7. Pal R and Mukhopadhyay S (2021). PPE2 protein of Mycobacterium tuberculosis affects myeloid hematopoiesis in mice. Immunobiology 226:152051.

Laboratory of Molecular Cell Biology

Laboratory of Molecular Oncology

Genomics and molecular genetics of cancer

Principal Investigator: Murali Dharan Bashyam
Staff Scientist

PhD Students:

Sara Anisa George	Senior Research Fellow
Pradipta Hore	Senior Research Fellow
Shaily Agrawal	Senior Research Fellow
Sanjana Sarkar	Senior Research Fellow
Mudodi Devaunshi Sadanand	Junior Research Fellow
Sumaiya Sabnam	Junior Research Fellow
Rinita Dutta	Junior Research Fellow (Since August 2021)
Rikita Karar	Junior Research Fellow (Since August 2021)
Bambhaniya Sandipkumar Mohanlal	Junior Research Fellow (Since March 2022)

Other Members:

Ajay Kumar Chaudhary	Technical Officer-II
Asmita Gupta	Research Associate
Neetu Sharma	Research Associate (Till December 2021)
Padmavathi Kavadipula	Project SRF
Shivani Yadav	Project JRF
Barsha Bharati	Project JRF (Till December 2021)
Mandla Vasanth Kumar	Project JRF (Till October 2021)
Rupin Gangadhar Shelke	Project JRF (From November 2021)

Collaborators:

Ashwin Dalal	CDFD, Hyderabad
HA Nagarajaram	UoH, Hyderabad
Neerja Gupta	AIIMS, New Delhi
Sumita Danda	CMC Vellore
Rekha Gupta	MGMCH, Jaipur
Hariharan V Sankar	Medical Collage, Trivandrum
KM Girisha	MAHE, Manipal
SR Phadke	SGPGIMS, Lucknow
Prajnya Ranganath	NIMS, Hyderabad

Objectives

Identification and characterization of important deregulated genes/pathways in cancers prevalent in India.

Research Summary

Project title: Characterization of Gene Fusions (GFs) in early-onset sporadic rectal cancer (EOSRC).

Concise report: We identified and validated several gene fusions (GFs) by analyses of RNA-Seq data generated from early-onset sporadic rectal cancer (EOSRC) samples. We detected a higher correlation between the GF 5' (compared to 3') breakpoints (BPs) and common chromosomal fragile sites (as determined by FANCD2 ChIP Seq and Mitotic DNA Synthesis (MiDAS) seq data). Scrutiny of CRC Hi-C data revealed that a majority of EOSRC GFs arose from genes located in 'open' chromatin regions (the 'A' compartment). Interestingly, GFs between 5' 'open' and 3' 'closed (the 'B' compartment)' partners often resulted in transcriptional activation of the latter revealing a novel mode of oncogenic activation in cancer (Figure 1).

Future plans and directions:

Functional studies on novel EOSRC gene fusions.

Project title: Genomics of SARS-CoV-2.

Concise report: We analyzed genome-wide variations in more than 3500 SARS-CoV-2 infected samples collected from the state of Telangana as well as from other Indian states. Specific nucleotide variations including V1104L (S), S26L (ORF3a),

V82A (ORF7a), R203M (N), and T3646A (ORF1ab) exhibited significant association with vaccination breakthrough cases. We also identified intra host variation (iSNV) in 15% of the total dataset and surprisingly identified 11 samples harboring iSNVs at >9 genomic positions suggesting possible mixed infection.

Future plans and directions:

Analyses of SARS-CoV-2 genomes from various Indian states to study viral evolution in various categories of patients and to track emergence of new mutations/lineages of concern.

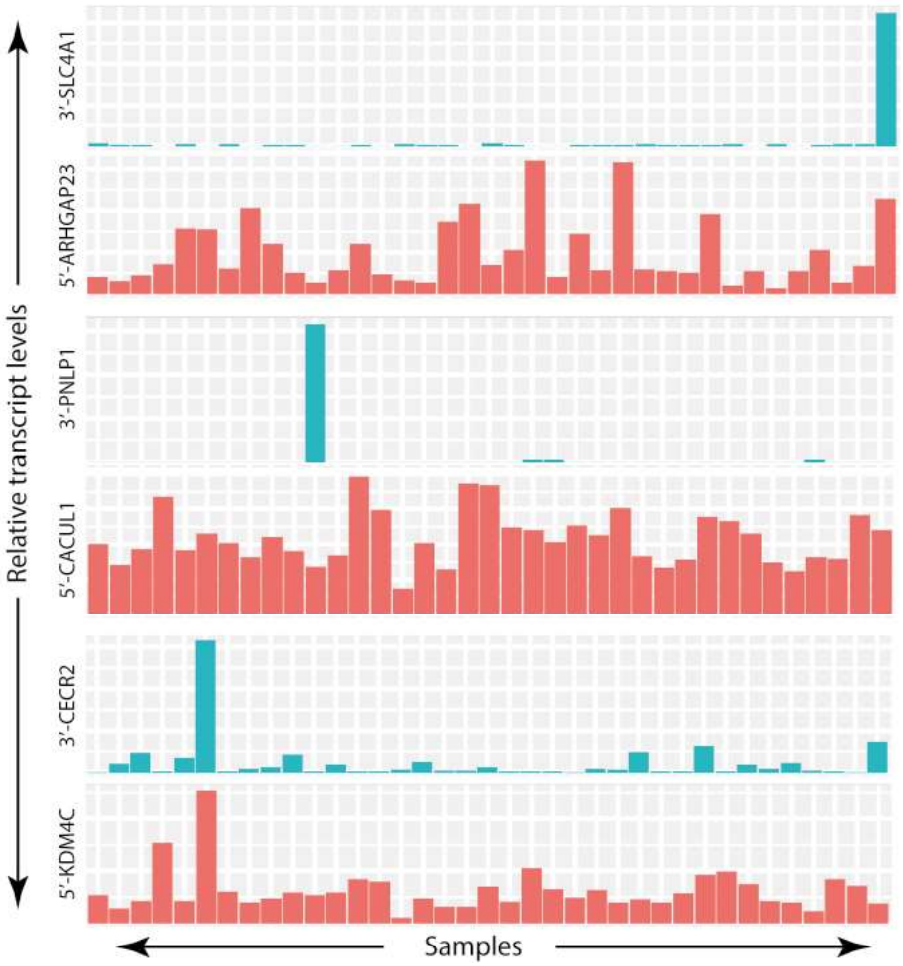


Figure 1 Transcript levels of genes involved in three EOSRC GFs showing induction of the 3' fusion partner gene in the sample that habors the GF.

Publications

Research papers published in 2021

1. Dhar MS, et al., 2021. Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. Science, 2021; 374:995-999.
2. Mlcochova P, et al., SARS-CoV-2 B.1.617.2 Delta variant replication and immune invasion. Nature, 2021; 599:114-119.
3. M Callea*, F C Scalisi, I Yavuz, M S Dogan, C E Willoughby, M D Bashyam*, “HED (hypohidrotic ectodermal dysplasia): a review”. J Int Dent Med Res, 2021; 14:785-789 (*combined corresponding authors).
4. Bala P, Singh A, Padmavathi K, Kotapalli V, Sabarinathan R and Bashyam MD. Exome sequencing identifies ARID2 as a novel tumor suppressor in early-onset sporadic rectal cancer. Oncogene, 2021; 40:863-874.
5. Srinivas A, Padmavathi K, Viswakalyan K, Swarnalata G, Satish R and MD Bashyam. Aberrant cytoplasmic localization of ARID1B activates ERK signaling and promotes oncogenesis. J Cell Sci, 2021; 134:jcs251637.
6. Gupta A, Sabarinathan R, Bala P, Donipadi V, Vashisht D, Katika MR, Kandakatla M, Mitra D, Dalal A, Bashyam MD. A comprehensive profile of genomic variations in the SARS-CoV-2 isolates from the state of Telangana, India. J Gen Virol, 2021;102:001562.

Research papers published in 2022

1. Desai, S; Dharavath, B; Manavalan, S; Rane, A; Redhu, A; Sunder, R; Butle, A; Mishra, R; Joshi, A; Togar, T; Apte, S; Bala, P*; Chandrani, P; Chopra, S; Bashyam, MD; Banerjee, A; Prabhash, K; Nair, S; Dutt, A. Fusobacterium nucleatum is associated with inflammation and poor survival in early-stage HPV-negative tongue cancer. NAR Cancer, 2022; 4:zcac006. *, from laboratory of molecular oncology, CDFD.
2. Chaudhary, AK, A Gholse, HA Nagarajaram, AB Dalal, N Gupta, AK Dutta, S Danda, R Gupta, HV Sankar, GS Bhavani, KM Girisha, SR Phadke, P Ranganath, and MD Bashyam. Ectodysplasin pathogenic variants affecting the furin-cleavage site and unusual clinical features define X-linked hypohidrotic ectodermal dysplasia in India. American Journal of Medical Genetics Part A, 2022; 188A:788-805.

Laboratory of Molecular Oncology

Laboratory of Plant Microbe Interaction

Understanding virulence mechanisms of *Xanthomonas* plant pathogens and interaction with host plants

Principal Investigator : Subhadeep Chatterjee
Staff Scientist

PhD Students:

Yasobanta Padhi	Senior Research Fellow
Chayan Bhattacharjee	Junior Research Fellow
Kanishk Saraf	Junior Research Fellow
Arkaprabha China	Junior Research Fellow

Project:

Dayakar	Senior Research Fellow (Till June 2022)
Parimala Gundu	Junior Research Fellow
Biswajit Samal	Senior Research Fellow

Other Members:

Binod Bihari Pradhan	Technical officer
Krishnamurty	Tradesman

Objectives

1. Identification and characterization of virulence factors of Xanthomonas
2. Role of cell-cell communication in Xanthomonas colonization and virulence
3. Function of protein secretion system in Xanthomonas and its role in virulence
4. Role of PAMP in pathogen recognition and plant defense response

Summary of work done until the beginning of this reporting year (up to March 31, 2020)

Light is one among the most abundant environmental signal which is sensed by diverse forms of life. Bacteria respond to light signal and modulate several social behaviors. Bacteriophytochrome are ubiquitous light sensing photo-receptors in bacteria, however, their role in regulating diverse cellular processes is poorly understood outside some prominent model photosynthetic bacteria. In non-photosynthetic bacteria, very little is known about the mechanism by which bacteriophytochrome transduce the photo-sensing to the downstream intracellular signal transduction cascade to coordinate diverse cellular processes and bacterial social behaviors. we show that a bacteriophytochrome (XooBphP), from the plant pathogen Xanthomonas oryzae pv. oryzae, perceives light signal and transduces a signal through its EAL-mediated phosphodiesterase activity, modulating the intracellular level of the ubiquitous bacterial second messenger c-di-GMP. We discover that the light mediated fine-tuning of the intracellular c-di-GMP levels by XooBphP regulates the production of virulence functions, iron metabolism and the transition of sessile to a free-swimming motile lifestyle, contributing to its colonization of the host and virulence. XooBphP thus plays a crucial role in integrating photo-sensing with intracellular signaling to control the pathogenic lifestyle and social behaviors. This is the first report of a bacteriophytochrome mediated regulation of social behavior, iron metabolism and virulence by modulating second messenger to coordinate diverse cellular process.

Details of progress made in the current reporting year (April 1, 2020 - March 31, 2021)

Project 1: Understanding the Mechanisms of quorum sensing mediated gene regulation and environmental adaptation in bacteria.

The diffusible signal factor synthase, RpfF, in Xanthomonas oryzae pv. oryzae is required for the maintenance of membrane integrity and virulence.

The Xanthomonas group of phytopathogens communicate with a fatty acid-like

cell–cell signalling molecule, cis-11-2-methyl-dodecenoic acid, also known as diffusible

signal factor (DSF). In the pathogen of rice, Xanthomonas oryzae pv. oryzae, DSF is involved in the regulation of several virulence-associated functions, including production and secretion of several cell wall hydrolysing type II secretion effectors. To understand the role of DSF in the secretion of type II effectors, we characterized DSF synthase-deficient

(rpfF) and DSF-deficient, type II secretion (xpsE) double mutants. Mutant analysis by expression analysis, secretion assay, fatty acid analysis, and physiological studies indicated that rpfF mutants exhibit hypersecretion of several type II effectors due to a perturbed membrane and DSF is required for maintaining membrane integrity. The rpfF mutants exhibited significantly higher uptake of 1-N-phenylnapthylamine and ethidium bromide, and up-regulation of rpoE (σE).

Increasing the osmolarity of the medium could rescue the hypersecretion phenotype of the rpfF mutant. The rpfF mutant exhibited highly reduced virulence. We report for the first time that in X. oryzae pv. oryzae RpfF is involved in the maintenance of membrane integrity by playing a regulatory role in the fatty acid synthesis pathway (Fig. 1).

Project 2: Bacterial quorum sensing facilitates Xanthomonas campesteris pv. campestris invasion of host tissue to maximize disease symptoms

Quorum sensing (QS) helps the Xanthomonas group of phytopathogens to infect several crop plants. The vascular phytopathogen Xanthomonas campestris pv. campestris (Xcc) is the causal agent of black rot disease on Brassicaceae leaves, where a typical v-shaped lesion spans both vascular and mesophyll regions with progressive leaf chlorosis. Recently, the role of QS has been elucidated during Xcc early infection stages. However, a detailed insight into the

possible role of QS-regulated bacterial invasion in host chlorophagy during late infection stages remains elusive. In this study, using QS-responsive whole-cell bioreporters of Xcc, we present a detailed chronology of QS-facilitated Xcc colonization in the mesophyll region of cabbage (Brassica oleracea) leaves. We report that QS-enabled localization of Xcc to parenchymal chloroplasts triggers leaf chlorosis and promotion of systemic infection. Our results indicate that

the QS response in the Xanthomonas group of vascular phytopathogens maximizes their population fitness across host tissues to trigger stage-specific host chlorophagy and establish a systemic infection (Fig .2).

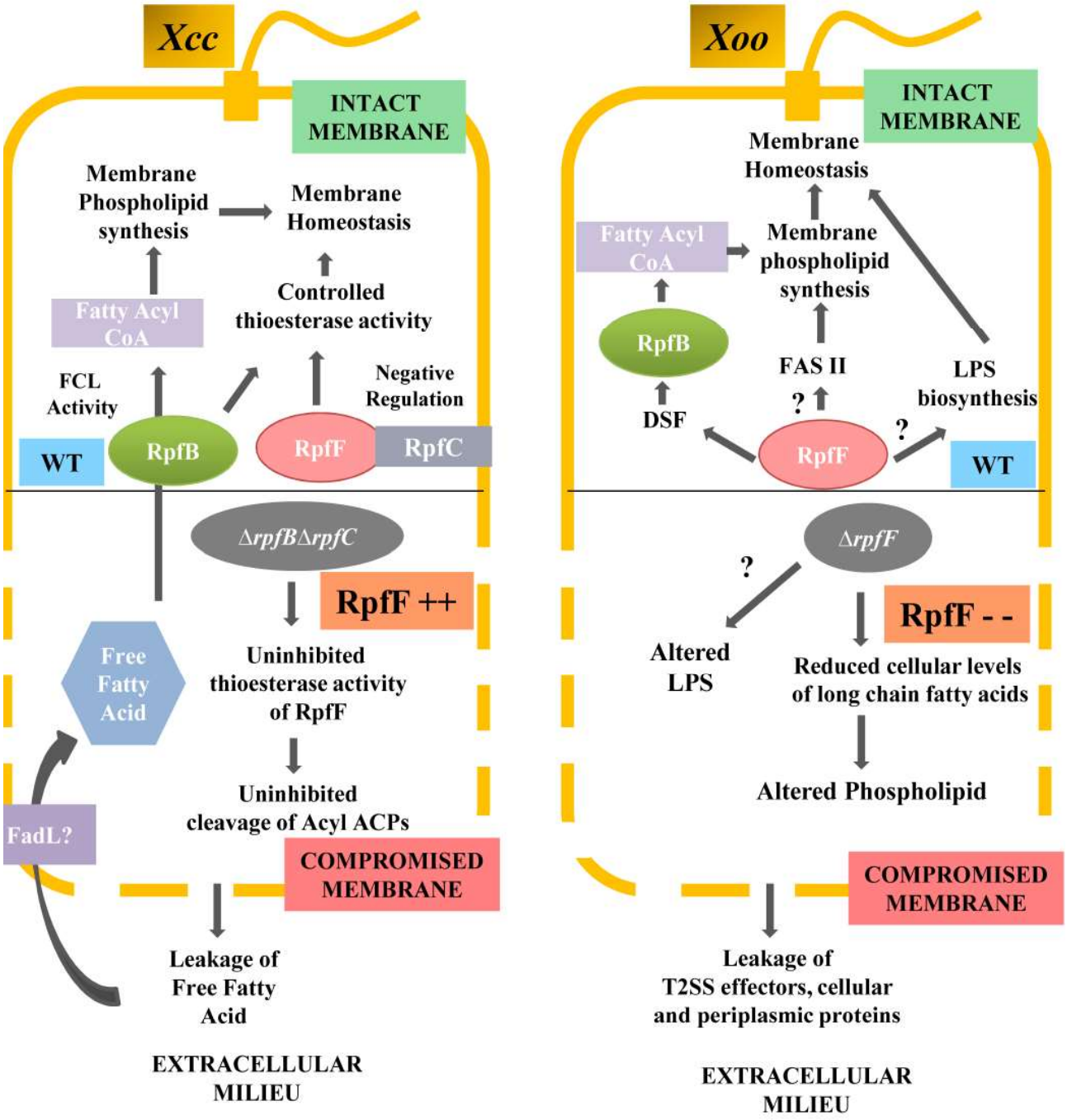


Figure 1. Proposed model for the antagonistic mechanism of membrane homeostasis by rpfF in Xcc and Xoo. In Xcc, the unregulated RpfF thioesterase activity in the rpfB-rpfC double mutant leads to uninhibited cleavage of acyl ACP intermediates resulting in the release of free fatty acids in the extracellular medium, which eventually causes cell membrane damage. This uninhibited thioesterase activity of RpfF has been proposed to be counteracted by the fatty acyl-CoA ligase (FCL) activity of RpfB in the wild type strain, it does that by sequestering free fatty acids back into the cell to produce acyl-CoAs that is probably rerouted back to the membrane phospholipid biosynthesis pathway for the maintenance of membrane integrity. In Xoo lack of RpfF activity causes reduced cellular fatty acid and altered phospholipid, and LPS profile which could be the likely cause for the hyper-release of the T2SS effectors and intracellular proteins in the rpfF mutant. rpfB expression and possibly its FCL activity in Xoo is regulated by the quorum molecule-DSF which could be one of the plausible explanation for lack of membrane stability in the DSF synthase- rpfF mutant. Other likely explanations for the presence of a compromised membrane in rpfF mutant could be regulation of the FAS II pathway, and/or the LPS biosynthesis pathway.

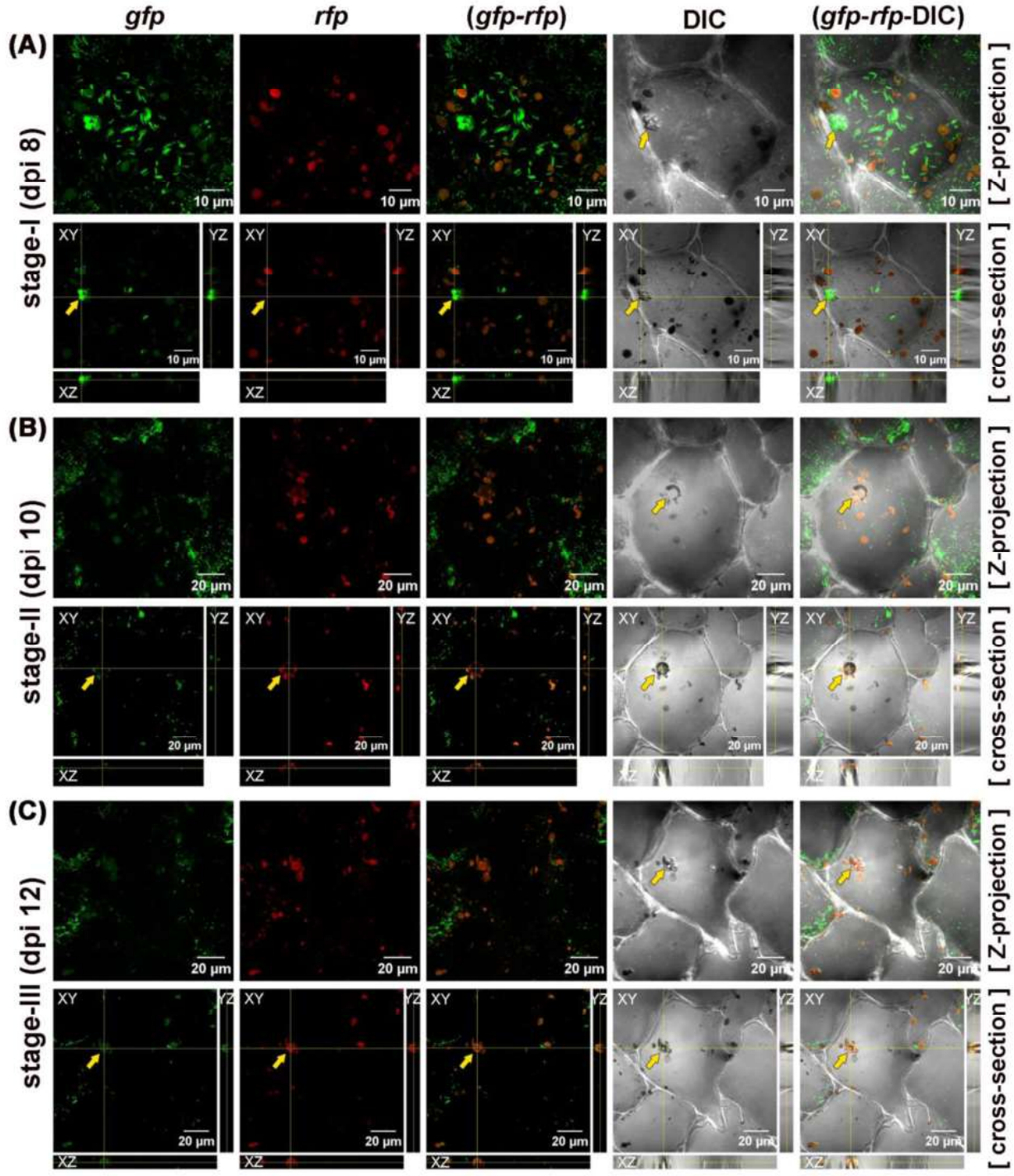


Figure 2. Host parenchymal chloroplast and cell-wall degradation followed by complete cell-distortion with QS induced Xcc invasion during disease phenotype progression. The above figure shows the representative individual and merged CLSM images of green and red fluorescence and bright field for different stages of successful disease establishment. The panels from left to right represent gfp, rfp, gfp-rfp merged, DIC, and gfp-rfp-DIC merged images, respectively. (A) Initiation of chloroplast degradation within a host parenchymal cell; that is invaded with QS-induced wild-type Xcc 8004 single-bioreporter cells on dpi 8 (i.e. stage-I). Scale bars on each panel, 10 µm. (B) An infected host parenchymal cell indicating the cell-wall degradation (i.e. formation of hole in cell-wall) with loss of chloroplast on dpi 10 (i.e. stage-II). Scale bars on each panel, 20 µm. (C) An infected host parenchymal cell, indicating cell-wall shrinkage initiation along with hole formation in cell-wall and loss of chloroplast on dpi 12 (i.e. stage-III). Scale bars on each panel, 20 µm. Upper panel; Maximum intensity projection view for the Z-stacks, Bottom panel; cross-sectional view at particular Z-plane for the respective upper panel. Yellow arrows; chloroplast infection and/or cell-wall degradation within infected parenchymal

cells. Bacterial localization and fluorescence were analyzed using ZEN software. Images were prepared using FIJI (Image J) software. The day of plant inoculation was considered as dpi 0. Data representation is based on experimental repeat of at least thrice.

We report for the first time that in X. oryzae pv. oryzae RpfF is involved in the maintenance of membrane integrity by playing a regulatory role in the fatty acid synthesis pathway We show for the first time that QS-enabled bacterial localization of parenchymal chloroplast within heterogeneously invaded host mesophyll tissue, leading triggered leaf chlorosis and systemic infection.

Publications:

i) Research papers published in the calendar year 2021:

1. Samal, B and Chatterjee, S. (2021). Bacterial quorum sensing facilitates Xanthomonas campesteris pv. campestris invasion of host tissue and to maximize disease symptom. Journal of Experimental Botany. 72: 6524–6543, https://doi.org/10.1093/jxb/erab211.

2. Kakkar A, Verma RK, Samal B, Chatterjee S. (2021). Interplay between the cyclic di-GMP network and the cell-cell signalling components coordinates virulence associated functions in Xanthomonas oryzae pv. oryzae. Environmental Microbiology. 23: 5433–5462. doi: 10.1111/1462-2920.15664.

3. Pandey SS, Chatterjee S. Insights into the cell-cell signaling and iron homeostasis in Xanthomonas virulence and lifestyle. Phytopathology. 2021 Jul 21. doi: 10.1094/PHYTO-11-20-0513-RVW.

ii) Research papers in press as on 31st March 2022

Singh, P., Verma, R.K., & Chatterjee, S. (2022) The diffusible signal factor synthase, RpfF, in Xanthomonas oryzae pv. oryzae is required for the maintenance of membrane integrity and virulence. Molecular Plant Pathology. 23: 118–132. 2. https://doi.org/10.1111/mpp.13148

Laboratory of Plant Microbe Interaction

Laboratory of Transcription

Bacterial transcription terminator Rho and mycobactericidal proteins from mycobacteriophages

Principal Investigator : Ranjan Sen
Staff Scientist

Names and designations of Ph.D. students:

Passong Immanual	Senior Research Fellow
Ajay Khatri	Senior Research Fellow
Saddam Ansari	Senior Research Fellow
Pankaj Sharma	Junior Research Fellow
Ankita Bhosale	Junior Research Fellow
Abhijeet Behera	Junior Research fellow

Names and designations of other members, including only those who are considered bench workers:

Shriyans Jain	Postdoctoral fellow
Naveen Kumar	Postdoctoral Fellow
B. Yogesh	Technical Assistant-I

Collaborators' names and brief affiliation:

Markus Wahl Freie Universität Berlin, Germany.

Udayditya Sen SINP, Kolkata, India.

Prof. Agneiszka Szaleskewa-Palasz University of Gdnask, Poland.

Objectives:

Our laboratory is at present focused to understand the mechanism of action, physiology, and inhibition of the conserved bacterial transcription terminator, Rho. The following studies are underway in our laboratory. 1) Mechanism of action of transcription termination factor, Rho both in vivo and in vitro. 2) Molecular basis of Rho-NusG interaction. 3) Designing peptide inhibitors of Rho from the bacteriophage protein, Psu. 4) Involvements of Rho in different physiological processes. In a translational project on synthetic biology, we are characterizing novel mycobactericidal proteins from the genomes of mycobacteriophages.

Details of the progress made in the current reporting year (1st April 2021-31st March 2022):

Rho-dependent transcription termination regulates the toxin-antitoxin modules of cryptic prophages to silence their expression in Escherichia coli. Bacterial Rho-dependent transcription termination regulates many physiological processes. Here, we report that it controls the expression of toxin-antitoxin (TA) modules of cryptic prophages in E. coli. Microarray profiles of Rho mutants showed upregulation of genes of the CP4-6 and CP4-44 prophages, including their TA modules, that were validated by RT-qPCR. Analysis of the in vivo termination efficiency and the mRNA sequences of these prophages revealed the presence of many Rho-dependent terminators. The prophage TA modules exhibited synthetic lethality with the Rho mutants, indicating functional involvement of Rho-dependent termination in controlling these modules. Rho-dependent termination does not regulate most of the chromosomal TA modules. We conclude that Rho-dependent termination specifically silences the TA modules of prophages, thereby augmenting bacterial innate immunity.

In vivo regulation of bacterial Rho-dependent transcription termination by the nascent RNA. Bacterial Rho is an RNA-dependent ATPase that functions in the termination of transcription. The in vivo nature of the bacterial Rho-dependent terminators, as well as the mechanism of the Rho-dependent termination process, are not fully understood. Here, we measured the in vivo termination efficiencies of 72 Rho-dependent terminators in Escherichia coli by systematically performing qRT-PCR analyses of cDNA prepared

from mid-log phase bacterial cultures. We found that these terminators exhibited a wide range of efficiencies, and many behaved differently in vivo compared to the predicted or experimentally determined efficiencies in vitro. Rho-utilization sites (rut sites) present in the RNA terminator sequences are characterized by the presence of C-rich/G-poor sequences or C > G bubbles. We found that weaker terminators exhibited a robust correlation with the properties (size, length, density, etc.) of these C > G bubbles of their respective rut sites, while stronger terminators lack this correlation, suggesting a limited role of rut sequences in controlling in vivo termination efficiencies. We also found that in vivo termination efficiencies are dependent on the rates of ATP hydrolysis as well as Rho translocation on the nascent RNA. We demonstrate that weaker terminators, in addition to having rut sites with diminished C > G bubble sizes, are dependent on the Rho auxiliary factor, NusG, in vivo. From these results, we concluded that in vivo Rho-dependent termination follows a nascent RNA-dependent pathway, where Rho-translocation along the RNA is essential and rut sequences may recruit Rho in vivo, but Rho-rut binding strengths do not regulate termination efficiencies (see adjacent figure).

Future plans/directions:

The following projects, being pursued in my lab, are in different stages of completion. 1) Characterizations of Rho-inhibitor peptide-DNA interactions, iii) characterization of different myco-bacteriocidal factors from mycobacteriophages, iv) characterization of the Rho-RNAP-NusA-NusG functional interaction during the transcription termination process and v) involvement of Rho in RNase pathways.

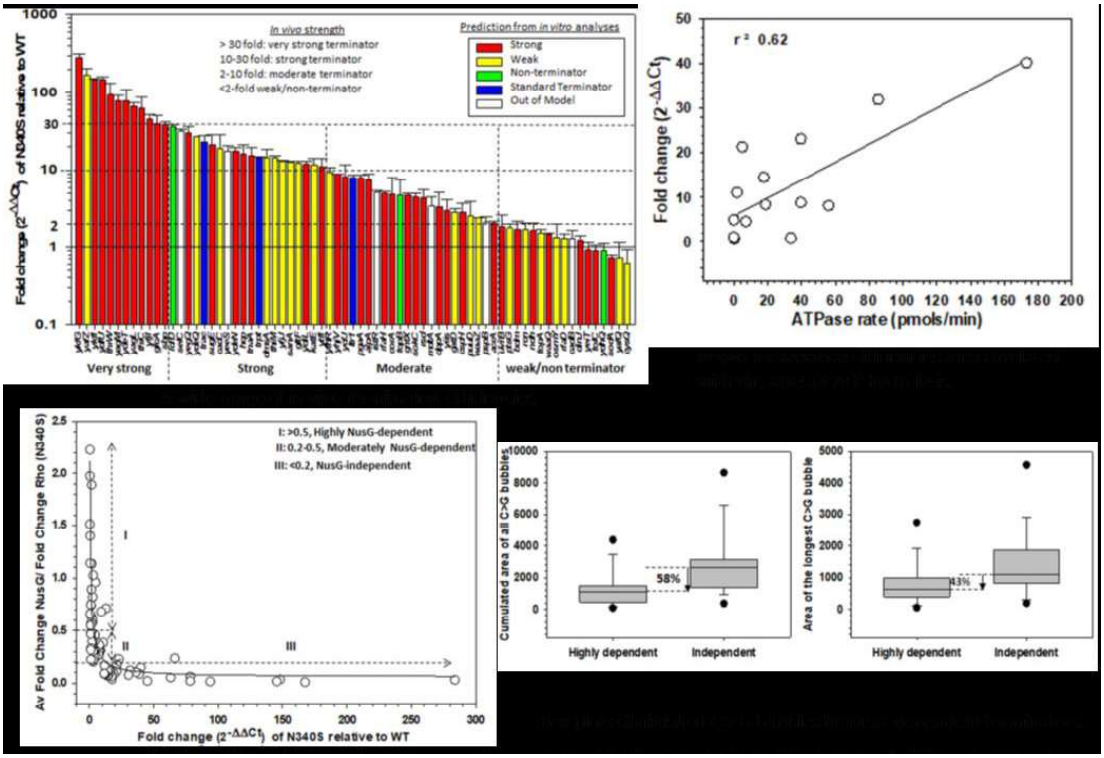


Figure 1: Regulation of *in vivo* Rho-dependent termination.

Publications:

Publications in press:

1. Chhakchhuak, P. I. R. and Sen, R. (2022). In vivo regulation of bacterial Rho-dependent transcription termination by the nascent RNA. Journal of Biological Chemistry, in press.

Publications 2021-2022:

1. Hafeezunnisa, M., Chhakchhuak, P. I. R., Krishnakumar, J. and Sen, R. (2021) E. coli cryptic prophage expressions are controlled by Rho-dependent transcription termination primarily to regulate their toxin-antitoxin modules. FEBS Letters, 595, 2057-2067.
2. Ghosh, G., Sharma, P. V., Kumar, A., Jain, S., and Sen, R. (2021). Design of novel peptide - inhibitors against the conserved bacterial transcription terminator, Rho. Journal of Biological Chemistry, Jan-Jun;296:100653. doi: 10.1016/j.jbc.2021.100653.

Laboratory of Transcription

अन्य वैज्ञानिक सेवाएँ/सुविधाएं

Other Scientific Services/ Facilities

Bioinformatics

In-charge:

Ajay Kumar Mahato : Staff Scientist
M Kavita Rao : Staff Scientist
[On leave from 02/07/2020 till date]

Members :

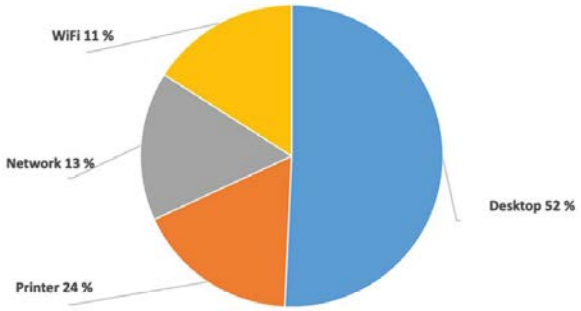
R Chandra Mohan : Technical Officer
Prashanthi Katta : Junior Assistant
Murali Mohan : Silked Work Assistant
B Laxminarayan : IT Engineer

Objectives

This section provides critical IT services to all the users in CDFD. The primary job is to manage and maintain various servers, workstations, PCs, printers, and other peripherals devices; to support multiple servers, workstations, PCs, printers, and other peripheral devices; to maintain regularly update the CDFD website. To provide web-based and e-mail services, Institute-web LAN/WAN, as well as internet connectivity, securing CDFD network from cyber security threats. Integration of the institute's network into National and International grid computing networks. Coordinate the procurement and installation process of servers, workstations, PCs, laptops, printers, and other devices with requisite software /license.

Details of progress made in the current reporting year (April 1, 2021 - March 31, 2022)

Activities were related to installing, administering, and maintaining high-end servers, which provide various services, databases, and computational jobs, as well as installing newly procured PCs with antivirus software.



Internet, web, e-mail, and other intranet services are being maintained in-house and provided to users with upgraded functionalities. We have also been participating in creating the NGC website. Further, we have been involved in redesigning the CDFD website as per the Government of India Guidelines. We have initiated procurement of over 50 new PCs to replace old, outdated ones. In addition, we have also commenced procurement of high-end servers and new Data canter racks for the NGC project. The AMC support renewal of existing high-end servers domain services and SSL certificate renewal was also carried out.

We have developed CDFD intranet services e-portal for online complaint registration related to IT work, e-mail registration for mail server migration to NIC server, Committee hall booking management system, and an In-house project information management portal is under development.

BIoinformatics Team



Covid 19 Testing Laboratory

Contributions of the Centre for DNA Fingerprinting and Diagnostics (CDFD) towards Diagnostics and Genomics research on COVID-19

COVID-19 Diagnostics:

Principal Investigators :

Kumarasamy Thangaraj	Director
Murali Dharan Bashyam	Staff Scientist
Ashwin Dalal	Staff Scientist

Present Members :

Arunkumar Karunanidhi	Project Scientist
Prajakta Meshram	Project Associate II
Salava Hymavathy	Project Associate II
Sivakumar Pandian	Project Associate II
Rajeshwar Rao M	Data Analyst
Shankar Lavudia	Lab Assistant

Past Members :

Reelina Basu	Project Scientist
S Sharan Ratnam	Project Associate II
Mounika Challapalli	Project Associate II
Dhanraj Adey	Data Analyst
Shivashanker	Lab Assistant
Laxma Reddy	Lab Assistant
Lavanya Banda	Lab Assistant
Bathula Siddartha	Lab Assistant
Narender Eslavath	Lab Assistant
Shaik Nasarvali	Lab Assistant
Shaik Ayaz	Lab Assistant

- CDFD initiated RT-PCR based diagnostics of SARS-CoV-2 causing COVID-19 infection from 19th April 2020 by establishing a state of the art laboratory with a maximum testing capacity of 450 samples per day.

- 7th April: CDFD decided to set-up Covid-19 testing lab
- 13th April: Approval to set-up facility received
- 18th April: Facility set-up on with maximum testing capacity of 450 samples per day
- Till date ~60,000 Covid-19 patient samples tested and reported to Telangana state

- Almost 60,757 suspected patient samples obtained from various districts of Telangana have been analyzed so far. Identification of positive samples has helped the State Govt. in contact tracing and containment measures.

COVID-19 Genomics Research:

- We performed the first comprehensive study from the state of Telangana on the dynamics of SARS-Cov-2 genomic evolution observed during the period March 2020 till to March, 2022.
- As part of the Indian SARS-CoV-2 genomics consortium (INSACOG) initiative, CDFD has sequenced 12,360 SARS-CoV-2 genomes, collected from the states of Tamil Nadu, Rajasthan, Himachal Pradesh, Punjab, Andhra Pradesh, Telangana, Goa, Uttar Pradesh, and Manipur with the overarching objective of identifying unique mutations, in addition to determining the dominant viral lineages circulating in the population. These sequences have been submitted to the national data hub maintained at NIBMG, Kalyani, WB as well as to the GISAID international data base. The sequencing strategy included sentinel surveillance as well as evaluation of samples from sudden clusters/surge events and international travellers tapped from Airports. In addition, special efforts have been undertaken to meticulously monitor and collect samples which are suspected and/or confirmed to be vaccination breakthroughs and reinfection cases. The genomic analyses of such samples are expected to shed light into possible mechanisms of viral immune escape.
- The analysis performed on Telangana SARS-CoV-2 samples collected by CDFD internally has revealed a consistent increase in the B.1.617 lineage since March 2021 onwards. Especially the B.1.617.2 (Delta) lineage has risen sharply since April 2021.
- More recently, we have reported several Omicron sub-variants as well.
- Science Outreach and Popularisation:

Publications:

1. PP Singh, A Srivastava, GNN Sultana, N Khanam, A Pathak, P Suravajhala, R Singh, P Shrivastava, G van Driem, K Thangaraj, G Chaubey. The major risk factor for severe COVID-19 does not show any association among South Asian populations. Sci Rep; 2021, 11: 12346.
2. PP Singh, P Suravajhala, CB Mallick, R Tamang, AK Rai, P Machha, R Singh, A Pathak, VN Mishra, P Shrivastava, KK Singh, K Thangaraj, G Chaubey. COVID-19: Impact on linguistic and genetic isolates of India. Genes Immun; 2021, 23: 47-50.
3. A Gupta, R Basu, MD Bashyam. Monitoring SARS-CoV-2 genome evolution in a localized population. medRxiv; 2022, https://doi.org/10.1101/2022.01.19.22269572

Covid 19 Testing Laboratory

Experimental Animal Facility

Scientist In-charge : **Pranjali Pore**

Other Members : **Arikothan Sheeba**
Kadingula Pavan

Faculty Co-coordinators:

Rashna Bhandari Staff Scientist, CDFD

Murali Bashyam Staff Scientist, CDFD

Objectives

Our objective of the Experimental Animal Facility (EAF) is to (i) breed, maintain and supply laboratory animals to CDFD and other institutional scientists. Breeding and experimentation of all strains of mice is undertaken in individually ventilated caging systems;(ii)to support research programmes that promote the health and wellbeing of people and animals by facilitating high quality and scientifically sound research with animals; (iii)to comply with regulatory government body (CPCSEA) requirements for animal breeding and experimentation.

Details of the progress made in the current reporting year (April 1, 2021 - March 31, 2022)

During this reporting year, the CDFD Experimental Animal Facility was working smoothly in compliance with regulatory government body CPCSEA for animal experimentation as well as strictly following GOI COVID-19 lockdown regulations. All the mice and rats were housed in IVC caging system. The rabbits are maintained for generation of polyclonal antibody in SPF environment. The CDFD Institutional Animal Ethics Committee (IAEC) was held virtually on August 9th 2021, for explaining the updated rules of CPCSEA to conduct of an experiment and regular maintenance of breeding animals during emergency pandemic situations. As per these rules, CDFD Experimental Animal Facility went completely under CCTV surveillance and all the procedures for better experimentation and wellbeing of the animals. The eighth meeting of CDFD Institutional Animal Ethics Committee (IAEC) was electronically connected on 8th November 2021 for review and approval of all ongoing and new studies conducted by CDFD scientists and the annual inspection was held on March 28th 2022 for yearly inspection and review.

Standard Operating Procedures (SOPs) were prepared, revised for the CDFD EAF as per new CPCSEA guidelines and all EAF staff were trained accordingly. The EAF was fumigated periodically. All the essential equipments of Experimental Animal facility were validated annually for better performance. Rat studies were initiated in rat Individually Ventilated caging system. Mice were kept in a specially designed cages called “Metabolic cages” which allow measurements of fluid intake, and to separate and collect feces and urine of numerous qualitative and quantitative determinations for specialized experiments. Breeding colonies were continuing to expand for all the five strains of mice (Table 1), all mice are breeding well.

Mice were bred to expand the colonies and 1433 mice were supplied to users for IAEC approved experimentation. Rats and Rabbits were brought from CPCSEA authorized vendor and housed for further experimentation.

Table 1. Strain-wise break up of adult mice, rats and rabbit housed at CDFD Experimental Animal Facility during 1st April 2021 to 31st March 2022, and supplied to users during 1st April 2021 to 31st March 2022.

Strain	Breeding (Male + Female)	Supplied
BALB/c	89 + 178	1008
C57BL/6	40+80	154
Ip6k1	27+54	58
NnatΔNEO/ΔI2	06 +12	Only Maintenance
Foxn1nu	84+168	213
Sprague Dawley Rats	Only Supply	06
NZW Rabbits	Only Supply	17

The experiments conducted during this period are listed below:

- 118 BALB/c mice were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.
- 58 Ip6k1 mice were used for histopathological and physiological analyses of testes and gastrointestinal tract.
- 381 BALB/c mice were injected intravenously with Candida glabrata for
- Studies on comparative bio-burden of different Candida strains.
- 100 BALB/c mice were used to study the efficacy of PPE2 protein in treatment of inflammation and tissue injury.
- 213 FoxN1nu athymic mice were injected with oncogenic cell lines to study tumor progression and metastasis.
- 220 BALB/c mice were used to study comparative vaginal bio burden analysis of Candida glabrata strains.
- 05 BALB/c mice were used to study the in vivo anti-inflammatory roles of recombinantly purified PPE2 and PPE18 proteins of Mycobacterium tuberculosis
- 51 C57BL/6 and 82 BALB/c mice were injected with thioglycolate by intra-peritoneal route for the generation of macrophages.
- 36 C57BL/6 mice were used to study comparative bio burden analysis of Candida glabrata strains.
- 45 C57BL/6 mice were used to rational design and structure and function of tailored antimicrobial peptides to treat fungal ocular infections.
- 22 C57BL/6 mice were used study molecular mechanisms involved in the antitumerigenic effects of PPE2 protein from Mycobacterium tuberculosis against B16F10 melanoma.
- 102 BALB/c mice were used to study molecular characterization of CgHog 1 kinase interactome impact on iron hemostasis and Candida pathogenesis.
- 06 SD rats were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.
- 17 NZW rabbits were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.

Future direction

The CDFD EAF is fully functional after lockdown, we plan to expand our breeding colonies, and additional transgenic mouse strains to add to the repertoire of experimental animal research being conducted at CDFD. We also aim to collaborate with academic institutions for research and experimentation, and to develop cryopreservation, archiving and retrieval of transgenic mouse strains in EAF for future use.

Fig.1 : Blood collection in NZW rabbit from marginal ear vein.

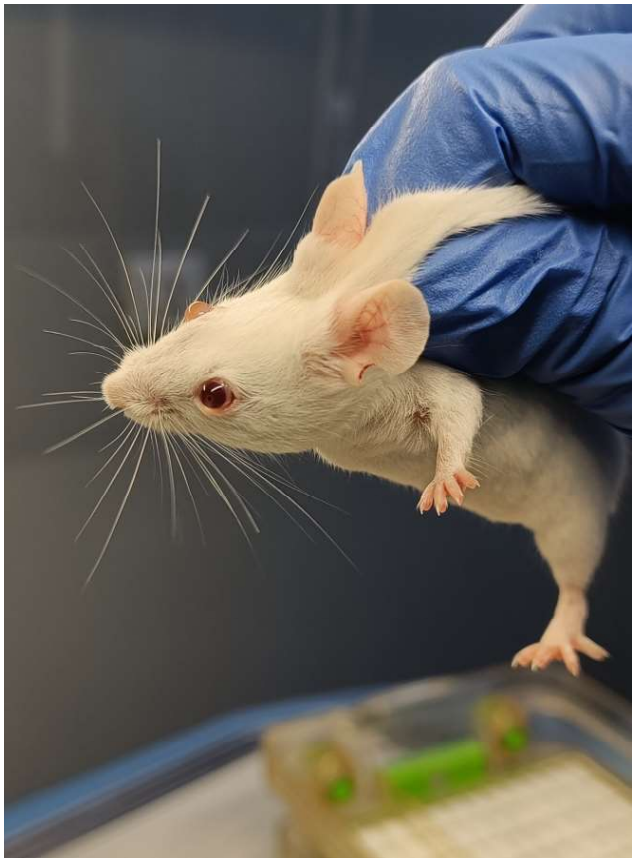


Fig.2 : Ear punching in BALB/c mouse as an identification method.

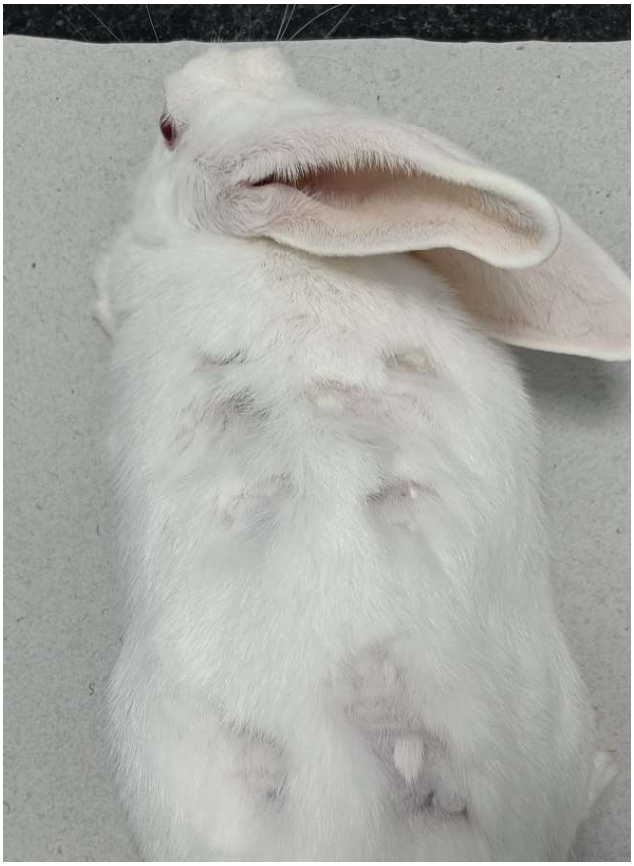


Fig.3 : Markings of subcutaneous injections in rabbit for Polyclonal Antibody Generation.

Fig.4 : Metabolic cage for mouse experimentation.

Group of Experimental Animal Facility

Instrumentation

In-charge : **R.N.Mishra**
Members : **S D Varalaxmi**
M Laxman
R M K Satyanarayana
T Ramakrishna Reddy

Objectives

To upkeep all the equipments in the laboratory by preventive maintenance, breakdown maintenance, repair and calibration. To provide technical specifications as per end user research requirements for the newly purchased equipments. Technical comparative statement along with ordering information. To provide pre-installation requirements for the newly purchased instruments and to co-ordinate with the manufacturer/ local agents in installation and warranty service of the new instruments. Also to provide test/ installation reports for newly installed instruments.

Work undertaken during 2021-22

During the year 2021-22, we have installed 108 Nos new equipment including Electrophoresis units, Biosafety Cabinets, 3730 Genetic Analyzer, 3X32 Well PCR Systems, Water Bath Shaker, Power Pack, Real Time PCRs, Digital Dry Bath, Analytical Balances, Laboratory Refrigerators, Gel Documentation System, Multi-Channel Pipettes, Video Conferencing System, Electroporation System, Laminar Hoods, Inverted Fluorescence Microscope, Bench top Centrifuges, 2-Color IR Imaging System, ULT Freezer, CO2 Incubator, Digital Notice Board, Ice Machine, Thermal Cycler, Orbital Incubator Shaker, Gel Rocker, Surface Plasmon Resonance System, Super Resolution Microscope, Speed Vac Concentrator etc.

Adding Instruments in CDFD GeM Cart with technical specifications. We have completed more than 350 maintenance work orders, 198 Pipette calibrations, processed 109 Purchase Indents for purchase of new equipments, maintaining the communication system etc. We have maintained most of the Instruments for maximum uptime in the Laboratory by replacing the local compatible electronics and electromechanical components. Most of the instruments are maintained by our Instrumentation Engineers, thereby saving on expensive AMCs and with very little downtime. In addition to above, we have involved in organizing the audio visual requirements for presentation in various seminar, lectures and workshops.

Instrumentation Team


National Genomics Core

Principal Investigator : K. Thangaraj
Director

Co-Principal Investigator : Ashwin Dalal
Staff Scientist

Chief Executive Officer:
Divya Vashisht Staff Scientist

Experimental Laboratory Manager:
Priyanka Kambli
Srinivas Kovvali Until 30.09.21

Technical Associate :
Vinay D
Mobeen Shaik Until 30.04.2022
Sonal Kale Until 28-02-2022

Computational Laboratory Manager :
Jibin John Until 20-04-2022

Technical Associate :
Divya B
Avinash Dhar Until 04-07-2022
Harish Kothandaraman Until 06-09-2021

Project co-ordinator Administration :
Swetha G
Project co-ordinator Finance :
G V S Manoj Kumar Until 17-02-2022

About NGC

National Genomics Core (NGC), is the establishment of Department of Biotechnology (DBT), India to act as a facilitator of genomics-driven discovery and application, and to accelerate the ushering in of a vibrant bio economy in our nation. South-Central regional core at CDFD, Hyderabad has been established along with central core-NIBMG (National Institute of Biomedical Genomics, Kolkata) and North-Central regional core (University of Allahabad, Prayagraj) to provide genomics services such as genome-scale DNA and RNA sequencing, genome-wide microarrays and gene-panel assays to institutes and the industry. The Core is intended to be a one-stop shop for all genomics services.

NGC Objectives

- Provide high-throughput platform facilities and expertise for generation of genome-scale data, using massively-parallel nucleic acid sequencing platforms
- Provide facilities and expertise for big data analysis, storage, management and access.
- Develop genomics skills using a pyramidal approach and taking advantage of India's recent membership of international molecular biology organizations (e.g., EMBO)

Summary of work done from project start until March 31, 2021

- Accomplished infrastructure development of NGC office space and laboratories
- Procurement, installation and training of experimental and computational staff has been achieved for Massively parallel DNA sequencer and accessory equipments (details of instruments in Figure 1).
- Standardization and optimization of different genomics experiments
- More than 140 different genomics services have been offered to various research scientist from CDFD, ISSER etc.

- Around 13000 samples have been sequenced generating 6.3 Tb of data and business ~4.2 Cr INR.

Highlights of NGC-CDFD's work in COVID-19 pandemic

NGC-CDFD has actively in Nation's initiative against on-going pandemic of COVID-19 by performing whole genome sequencing of SARS-COV-2 under

a) DBT-PAN-INDIA 1000 genome SARS-CoV-2 RNA consortium (more than 210 samples)*

b) Indian SARS-COV-2 Genome Consortium (INSACOG) (under which ~10,000 samples are sequenced)*

*The sequenced samples are submitted to openly available database GISAID (Global Initiative on Sharing Avian Influenza Data)

c) Strengthening COVID task force for various state institutes by training for COVID-genomics protocols

Skill development program

More than 700 researchers have been trained during courses conducted by various institutes such as National Academy of Agricultural Research Management (NAARM) and Central Institute of Freshwater Aquaculture for utilizing tools of analyzing Next Generation Sequencing data analysis.

Three rounds of five days' hands-on workshop have been organized for Next Generation Sequencing Data Analysis for Clinical Diagnostics (01-05 March, 2021; 21-26 June, 2021; 25-29 October, 2021) during which medical professionals from across the nation (AIIMS- Delhi, JIPMER-Pondicherry, SGPGIMS- Lucknow) joined to attend the workshop.

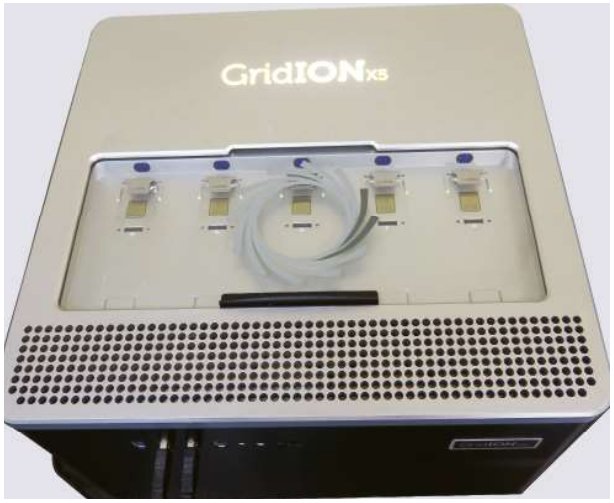
Next Generation Sequencing instruments at NGC-CDFD

Illumina MiSeq FGx

- First fully validated, next-generation sequencing (NGS) solution designed for forensic science
- FBI NDIS approved
- Analysing over 230 genetic markers using a single, streamlined workflow

Illumina Nextseq 2000

- First in South-Asia, Illumina's latest cost-effective, high-throughput system for diverse genomics applications
- Reduced sequencing costs and deeper exploration for ground breaking discoveries in basic biology, health, agriculture, oncology, microbiome and many more



Oxford Nanopore GridION X5

- Long reads sequencing with enhanced analysis of repetitive regions, structural variation, phasing, metagenomics, and more
- As much as 150 Gb of data — streamed in real time for immediate analysis
- Automate sample and library preparation using VolTRAX- enabling reproducible and portable sample preparation

Group of National Genomics Core

Science Communication

Head : **Varsha**
Staff Scientist

Other Members : **K Shirisha**
Junior Assistant

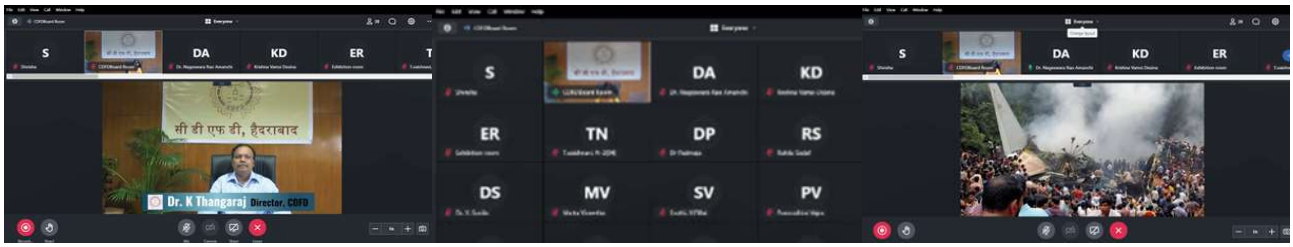
Science communication and outreach is communicating scientific research and its outcome to general public. It is very essential to connect the common man with science. With this pespective, CDFD organises many institutional visits and outreach activities to create awareness and encourage curiosity about science among school and college students. These activities include:

Open days:

During these days, school and college students, educators or anyone from general public can visit our labs and interact with our scientists / researchers to learn more about the world of research.

In order to provide information regarding the job options in science and to give students and educators an experiential understanding of research, we conduct visits for them on our campus. During the reporting period students from various schools and colleges visited us including RBVRR Women's College, Nizam College, St. Anns College for Women, Keshav Memorial Degree College, Kendriya Vidyalayas, Aurora degree college and many more.

Science Setu: Our scientists will visit various schools/colleges and educational institutions and deliver talk and interact with students. It gives them a chance to get exposed to the cutting edge research which is being carried out at the Centre and also inspires them to opt science as a career. Our scientists also visit the schools and colleges in the twin cities under 'Bridge', 'Jigyasa' and 'Vigyan- Jyothi' programs and teach the students. The webinars have been arranged for DBT STAR Colleges and virtual Open Days have been organised for the benefit of the students.



Virtual Open Days for DBT Star Colleges

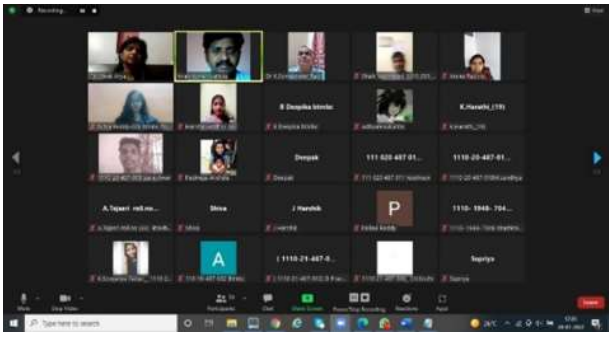
Physical Open Day

Institutional visits:

Jigyasa program with Kendriya Vidyalaya-2, Uppal

Vigyan Jyothi program with Jawahar Navodaya Vidyalaya

Lecture at RBVRR College, Hyderabad



Webinar for Keshav Degree College on 28.01.2022

Popular science talks and lecture series: Popular talks are being organised on different occasions like the Foundation Day, National Science Day, the India International Science Festival, Lalji's birth anniversary, visits of eminent scientists etc. This is always an opportunity to the staff and students to interact with such eminent personalities of scientific fraternity. Lecture series by eminent scientists are also being organised for the benefit of the students.

Other Outreach activities:

CDFD takes part in science exhibitions like the annual "India International Science Festival"

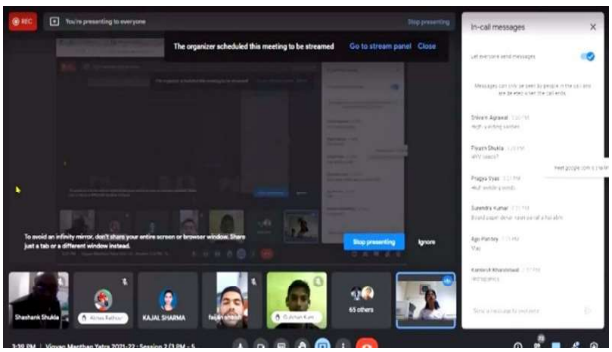

Organ donation awareness programme under Jeevandhan Scheme with Gandhi Medical College under our social outreach programme

(IISF), Global Bio-india, Vigyan Yatra, Science Showcase Mega Expo, Vigyan Sarvatra Pujyate -- Festival of Science & Technology under Azadi ka Amrit Mahotsav, Science Week Festival, Vigyan Manthan Yatra under Mission Excellence Program with Madhya Pradesh Council of Science and Technology, Science Congress organised by Kendriya Vidyalaya and so many others. We keep posting our important research outcomes on all our social media handles (Facebook, Twitter, Instagram, Linkedin and YouTube). Also, we disseminate the scientific knowledge through other media, including science articles in magazines and newspapers, TV programmes with Rajya Sabha TV, Yadgiri TV Channel etc. We are also initiating popular science talks/ podcasts etc with our scientists and students on our social media handles.

Under the societal outreach activities, we have initiated an organ donation awareness programme under “Jeevandhan Scheme” with Gandhi Medical College, Secunderabad.


Participation in Nation-Wide Science Week Festival under Azadi ka Amrit Mahotsav at ICMR-NIN, Hyderabad from 22-28 February 2022


Participation in Vigyan Sarvatra Pujyate -- Festival of Science & Technology under Azadi ka Amrit Mahotsav: Mega Expo, organized by PSA office, New Delhi from 22-28 February 2022



Virtual Open Day- Advance celebrations on the occasion of Science Day 2022 -under Vigyan Manthan Yatra - Mission Excellence Program 2021-22 with Madhya Pradesh Council of Science and Technology

National Scienec Conference at Kendriya Vidyalaya-2, Uppal

Media coverage

Science Communication Team

Sophisticated Equipment Facility (SEF)

Head : **Vinod Kumar Mishra**
Staff Scientist

Other Members :

Ch V Goud	Technical Officer
K Sreethi Reddy	Technical Officer
Bala Maddileti C	Technical Officer
Mohd. Mudassir	Technical Officer
Abhijeet Singh	Technical Officer
Viswa Kalyan	Technical Officer
Tripti Sharma	Technical Officer

Objectives

- In order to maximize the utilization of all high end equipments and their better management, these equipments are brought under one umbrella “Sophisticated Equipment Facility” (SEF).
- To extend testing and analysis facility to research personnel, doctoral students and faculty members of CDFD
- To extend its facilities to other academic institutions, R & D laboratories and industries.
- To organize short term courses/workshops on the use and application of various instruments and analytical techniques.
- To train technicians for maintenance and operation of sophisticated instruments.
- The initiative minimizes duplication of expensive equipment and lead to better utilization of instruments.

Summary of work done till March 2022

- Activities related to installation, administration and maintenance of various sophisticated equipment’s in the facility.
- The list of services offered with the major equipments available under the scope of this Sophisticated Equipment Facility (SEF) are as follows:
- Genomics Services: DNA Sequencers and Real-Time PCR Machines.
- Proteomics Services: HPLC System, Circular Dichroism spectropolarimeter.
- Cellomics Services Confocal Microscope with multiphoton laser, Live Cell Imaging and FACS ARIA Flow cytometer with Sorter.
- Tissue processing unit: Microtome
- We have carried out outreach programmes for educating children of various schools and colleges regarding the services offered by us and efficient use of such high end equipments
- Efficiently propagated the idea of using centralized facility for various R & D activities within CDFD as well as various academic institutes and private research organizations.
- Various companies had the opportunity to display their high end equipment in CDFD.

Details of Progress made in current reporting year April 1, 2021 to March 31, 2022)

- New additions- 3500 XL genetic analyzer, Confocal Super Resolution (LSM 980), Atomic Absorption Apectrophotometer (AAS), Fermentor, Biacore X 100, FACS ARIA Fusion and LSR analyzer with sorter were installed in the facility and is being efficiently used by internal as well as external people for their research work.
- An Outreach activity was done to promote the

use of centralized facility by making the SEF Flyer. The Flyer was given to various visitors from academic and R&D labs and corporate companies.

- Many schools and outside personnel visited the facility for acquiring knowledge of various equipment in the facility.
- Co-ordination with various users and the instrumentation department for AMC/ CMC requirements for smooth functioning of SEF facility.
- The facility was used by various inside as well as outside users and the list are as follows:

Sequencing and Genotyping	1818 users (19145 Samples)
Confocal LSM 700/Leica SP-8	1230 users
Super Resolution LSM 980	980 users
FACS	190 users
CD Spectropolarimeter	6 users
RT-PCR	445 users
Histopathology	15 users

- Revenue generated for the year April 2021 – March 2022 was Rs. 4070844/- (Rupees Forty lakhs seventy thousand eight hundred forty four).

Sophisticated Equipment Facility (SEF) Group

प्रकाशन
Publications

Publications 2021-22 (April 2021 to March 2022)

1. Aggarwal S. Role of whole exome sequencing for unidentified genetic syndromes. ***Current Opinion in Obstetrics and Gynecology.*** 33(2) : 112-122.
2. Aggarwal S. Expanding spectrum of PCDH12 related phenotype begs exploration of multipronged pathomechanisms. European ***Journal of Paediatric Neurology.*** 36 : A2-A3. 2022 Jan 4.
3. Agrawal N, Verma G, Saxena D, Kabra M, Gupta N, Mandal K, Moirangthem A, Sheth J, Puri RD, Bijarnia-Mahay S, Kapoor S, Danda S, H SV, Datar CA, Ranganath P, Shukla A, Dalal A, Srivastava P, Devi RR, Phadke SR. Genotype-phenotype spectrum of 130 unrelated Indian families with Mucopolysaccharidosis type II. ***European Journal of Medical Genetics.*** 65 (3) : 104447.
4. Alpaslan-Roodenberg S, Anthony D, Babiker H, Bánffy E, Booth T, Capone P, Deshpande-Mukherjee A, Eisenmann S, Fehren-Schmitz L, Frachetti M, Fujita R, Frieman CJ, Fu Q, Gibbon V, Haak W, Hajdinjak M, Hofmann KP, Holguin B, Inomata T, Kanzawa-Kiriyama H, Keegan W, Kelso J, Krause J, Kumaresan G, Kusimba C, Kusimba S, Lalueza-Fox C, Llamas B, MacEachern S, Mallick S, Matsumura H, Morales-Arce AY, Matuzeviciute GM, Mushrif-Tripathy V, Nakatsuka N, Nores R, Ogola C, Okumura M, Patterson N, Pinhasi R, Prasad SPR, Prendergast ME, Punzo JL, Reich D, Sawafuji R, Sawchuk E, Schiffels S, Sedig J, Shnaider S, Sirak K, Skoglund P, Slon V, Snow M, Soressi M, Spriggs M, Stockhammer PW, Szécsényi-Nagy A, Thangaraj K, Tiesler V, Tobler R, Wang CC, Warinner C, Yasawardene S, Zahir M. Ethics of DNA research on human remains: five globally applicable guidelines. ***Nature.*** 599(7883) : 41-46.
5. Aricthota S, Haldar D (OCT-2021). DDK/Hsk1 phosphorylates and targets fission yeast histone deacetylase Hst4 for degradation to stabilize stalled DNA replication forks. ***eLife.*** 10 : e70787.
6. Askari F, Rasheed M, Kaur R. The yapsin family of aspartyl proteases regulate glucose homeostasis in Candida glabrata. ***Journal of Biological Chemistry.*** 298(2):101593.
7. Badarukhiya JA, Tupperwar N, Nizamuddin S, Mulpur AK, Thangaraj K. Novel FCN2 Variants and Haplotypes are Associated with Rheumatic Heart Disease. ***DNA and Cell Biology.*** 40(10) : 1338-1348.
8. Basak N, Norboo T, Mustak MS, Thangaraj K. Heterogeneity in Hematological Parameters of High and Low Altitude Tibetan Populations. ***Journal of Blood Medicine.*** 12 : 287-298.
9. Battu A, Purushotham R, Kaur R. An Assay to Determine NAD(P)H: Quinone Oxidoreductase Activity in Cell Extracts from Candida glabrata. ***Bio Protocol.*** 11(21) : e4210.
10. Bera P, Aher A, Brandao P, Debnath U, Dewaker V, Manna SK, Jana A, Pramanik C, Mandal B, Bera P. Instigating the In Vitro Anticancer Activity of New Pyridine-Thiazole-Based Co(III), Mn(II), and Ni(II) Complexes: Synthesis, Structure, DFT, Docking, and MD Simulation Studies. ***Journal of chemical information and modeling.*** doi: 10.1021/acs.jcim.
11. Chakrabarty S, Govindaraj P, Sankaran BP, Nagappa M, Kabekkodu SP, Jayaram P, Mallya S, Deepha S, Ponmalar JNJ, Arivinda HR, Meena AK, Jha RK, Sinha S, Gayathri N, Taly AB, Thangaraj K, Satyamoorthy K. Contribution of nuclear and mitochondrial gene mutations in mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. ***Journal of Neurology.*** 268(6) : 2192-2207.
12. Chaudhary AK, Gholse A, Nagarajaram HA, Dalal AB, Gupta N, Dutta AK, Danda S, Gupta R, Sankar HV, Bhavani GS, Girisha KM, Phadke SR, Ranganath P, Bashyam MD (MAR-2022). Ectodysplasin pathogenic variants affecting the

furin-cleavage site and unusual clinical features define X-linked hypohidrotic ectodermal dysplasia in India. ***American Journal of Medical Genetics Part – A.*** 188 (3) : 788-805.

13. Deepha S, Govindaraj P, Sankaran BP, Chiplunkar S, Kashinkunti C, Nunia V, Nagappa M, Sinha S, Khanna T, Thangaraj K, Taly AB, Gayathri N. Clinico-pathological and Molecular Spectrum of Mitochondrial Polymerase γ Mutations in a Cohort from India. ***Journal of Molecular Neuroscience***. 71(11) : 2219-2228.

14. Desai, S; Dharavath, B; Manavalan, S; Rane, A; Redhu, A; Sunder, R; Butle, A; Mishra, R; Joshi, A; Togar, T; Apte, S; Bala, P*; Chandrani, P; Chopra, S; Bashyam, MD; Banerjee, A; Prabhash, K; Nair, S; Dutt, A. Fusobacterium nucleatum is associated with inflammation and poor survival in early-stage HPV-negative tongue cancer. ***NAR Cancer,*** 2022; 4:zcac006.

15. Deshpande D, Gupta SK, Sarma AS, Ranganath P, Jain S JMN, Sheth J, Mistri M, Gupta N, Kabra M, Phadke SR, Girisha KM, Dua Puri R, Aggarwal S, Datar C, Mandal K, Tilak P, Muranjan M, Bijarnia-Mahay S, Rama Devi A R, Tayade NB, Ranjan A, Dalal AB. Functional characterization of novel variants in SMPD1 in Indian patients with acid sphingomyelinase deficiency. ***Human Mutation.*** 42(10) : 1336-1350.

16. Dhar MS, Marwal R, Vs R, Ponnusamy K, Jolly B, Bhoyar RC, Sardana V, Naushin S, Rophina M, Mellan TA, Mishra S, Whittaker C, Fatihi S, Datta M, Singh P, Sharma U, Ujjainiya R, Bhatheja N, Divakar MK, Singh MK, Imran M, Senthivel V, Maurya R, Jha N, Mehta P, A V, Sharma P, Vr A, Chaudhary U, Soni N, Thukral L, Flaxman S, Bhatt S, Pandey R, Dash D, Faruq M, Lall H, Gogia H, Madan P, Kulkarni S, Chauhan H, Sengupta S, Kabra S; Indian SARS-CoV-2 Genomics Consortium (INSACOG)‡, Gupta RK, Singh SK, Agrawal A, Rakshit P, Nandicoori V, Tallapaka KB, Sowpati DT, Thangaraj K, Bashyam MD, Dalal A, Sivasubbu S, Scaria V, Parida A, Raghav SK, Prasad P, Sarin A, Mayor S, Ramakrishnan U, Palakodeti D, Seshasayee ASN, Bhat M, Shouche Y, Pillai A, Dikid T, Das S, Maitra A, Chinnaswamy S, Biswas NK, Desai AS, Pattabiraman C, Manjunatha MV, Mani RS, Arunachal Udupi G, Abraham P, Atul PV, Cherian SS. (2021) Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. ***Science 374*** (6570):995-999.

17. Dixit S, Shrivastava P, Dash HR, Kaitholia K, Sahajpal V, Sahoo S, Srivastava V, Rani HS, Mishra A, Choudhary SK, Thekkatavan A, Chaubey G, Kumawat RK. Assessment of significance and forensic relevance of SE33 (ACTBP2) locus in five Indian populations. ***Gene Reports*** 24 (2021) 101293

18. Dubey S, Majumder P, Penmatsa A, Sardesai A. Topological analyses of the L-lysine exporter LysO reveal a critical role for a conserved pair of intramembrane solvent-exposed acidic residues. ***Journal of Biological Chemistry.*** 3 : 101168.

19. Endrakanti M, Saluja S, Ethayathulla AS, Sapra S, Dalal A, Palanichamy JK, Gupta N. A patient with POLA1 splice variant expands the yet evolving phenotype of Van Esch O'Driscoll syndrome. **European Journal of Medical Genetics**. 64(8) : 104261.

20. Ghosh DK, Kumar A, Ranjan A. Cellular targets of mefloquine. ***Toxicology.*** 464 : 152995.

21. Ghosh DK, Ranjan A. HYPK coordinates degradation of polyneddylated proteins by autophagy. ***Autophagy.*** 26 : 1-22.

22. Ghosh G, Sharma PV, Kumar A, Jain S, Sen R. Design of novel peptide-inhibitors against the conserved bacterial transcription terminator, Rho. ***Journal of Biological Chemistry.*** 296 : 100653.

23. Guo L, Govindaraj P, Kievit M, de Coo IFM, Gerards M, Hellebrekers DMEI, Stassen APM, Gayathri N, Taly AB, Sankaran BP, Smeets HJM. Whole exome sequencing reveals a homozygous C1QBP deletion as the cause of progressive external ophthalmoplegia and multiple mtDNA deletions. ***Neuromuscular disorders(NMD).*** 31(9) : 859-864.

24. Hafeezunnisa M, Chhakchhuak PIR, Krishnakumar J, Sen R. Rho-dependent transcription termination regulates the toxin-antitoxin modules of cryptic prophages to silence their expression in Escherichia coli. ***FEBS Letters.*** 595(15) : 2057-2067.

25. Huddar A, Govindaraj P, Chiplunkar S, Deepha

S, Jessiena Ponmalar JN, Philip M, Nagappa M, Narayanappa G, Mahadevan A, Sinha S, Taly AB, Parayil Sankaran B. Serum fibroblast growth factor 21 and growth differentiation factor 15: Two sensitive biomarkers in the diagnosis of mitochondrial disorders. ***Mitochondrion.*** 60 : 170-177.

26. Jain PK, Jayappa S, Sairam T, Mittal A, Paul S, Rao VJ, Chittora H, Kashyap DK, Palakodeti D, Thangaraj K, Shenthar J, Koranchery R, Rajendran R, Alireza H, Mohanan KS, Rathinavel A, Dhandapany PS. Ribosomal protein S6 kinase beta-1 gene variants cause hypertrophic cardiomyopathy. ***Journal of Medical Genetics.*** jmedgenet-2021-107866.

27. Jana A, Aher A, Brandao P, Bera P, Sharda S, Phadikar U, Manna SK, Mahapatra AK, Bera P. Evaluation of the anticancer activities with various ligand substituents in Co(II/III)-picolyl phenolate derivatives: synthesis, characterization, DFT, DNA cleavage, and molecular docking studies. ***Dalton Transactions.*** 51 (6) : 2346-2363.

28. Jha RK, Dawar C, Hasan Q, Pujar A, Gupta G, Vishnu VY, Kekunnaya R, Thangaraj K. Mitochondrial Genetic Heterogeneity in Leber's Hereditary Optic Neuropathy: Original Study with Meta-Analysis. ***Genes (Basel).*** 12(9) : 1300.

29. Joshi R, Sipani R, Bakshi A. Roles of Drosophila Hox Genes in the Assembly of Neuromuscular Networks and Behavior. ***Frontiers in Cell and Developmental Biology.*** 7 9 : 786993.

30. Kakkar A, Verma RK, Samal B, Chatterjee S. Interplay between the cyclic di-GMP network and the cell-cell signalling components coordinates virulence-associated functions in Xanthomonas oryzae pv. oryzae. ***Environmental Microbiology.*** 23(9) : 5433-5462.

31. Kausthubham N, Shukla A, Gupta N, Bhavani GS, Kulshrestha S, Das Bhowmik A, Moirangthem A, Bijarnia-Mahay S, Kabra M, Puri RD, Mandal K, Verma IC, Bielas SL, Phadke SR, Dalal A, Girisha KM. A data set of variants derived from 1455 clinical and research exomes is efficient in variant prioritization for early-onset monogenic disorders in Indians. ***Human Mutation.*** 42(4) : e15-e61.

32. Knapp KM, Fellows B, Aggarwal S, Dalal A, Bicknell LS. A synonymous variant in a non-canonical exon of CDC45 disrupts splicing in two affected sibs with Meier-Gorlin syndrome with craniosynostosis. ***European Journal of Medical Genetics.*** 64(4) : 104182.

33. Kumar L, Farias K, Prakash S, Mishra A, Mustak MS, Rai N, Thangaraj K (OCT-2021). Dissecting the genetic history of the Roman Catholic populations of West Coast India. ***Human Genetics.*** 140(10) : 1487-1498.

34. Kuthethur R, Prasad K, Chakrabarty S, Kabekkodu SP, Singh KK, Thangaraj K, Satyamoorthy K. Advances in mitochondrial medicine and translational research. ***Mitochondrion.*** 61 : 62-68.

35. Leela JK, Raghunathan N, Gowrishankar J. Topoisomerase I Essentiality, DnaA-Independent Chromosomal Replication, and Transcription-Replication Conflict in Escherichia coli. ***Journal of Bacteriology***. 203(17) : e0019521.

36. Lolla P, Shah A, Unnikannan CP, Oddi V, Bhandari R. Inositol pyrophosphates promote MYC polyubiquitination by FBW7 to regulate cell survival. ***Biochemical Journal..*** 478(8) : 1647-1661.

37. Mehta P, Singh P, Gupta NJ, Sankhwar SN, Chakravarty B, Thangaraj K, Rajender S. Mutations in the desert hedgehog (DHH) gene in the disorders of sexual differentiation and male infertility. ***Journal of Assisted Reproduction and Genetics.*** 38(7) : 1871-1878.

38. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira IATM, Datir R, Collier DA, Albecka A, Singh S, Pandey R, Brown J, Zhou J, Goonawardane N, Mishra S, Whittaker C, Mellan T, Marwal R, Datta M, Sengupta S, Ponnusamy K, Radhakrishnan VS, Abdullahi A, Charles O, Chattopadhyay P, Devi P, Caputo D, Peacock T, Wattal C, Goel N, Satwik A, Vaishya R, Agarwal M; Indian SARS-CoV-2 Genomics Consortium (INSACOG); Genotype to Phenotype Japan (G2P-Japan) Consortium; CITIID-NIHR BioResource COVID-19 Collaboration, Mavousian A, Lee JH, Bassi J, Silacci-Fegni C, Saliba C, Pinto D, Irie T, Yoshida I, Hamilton WL, Sato K, Bhatt S, Flaxman S, James LC, Corti D, Piccoli L, Barclay WS, Rakshit P, Agrawal A, Gupta RK. (2021) SARS-CoV-2 B.1.617.2 Delta

variant replication and immune evasion. ***Nature*** 599(7883):114-119.

39. Mohanrao R, Manorama R, Ganguli S, Madhusudhanan MC, Bhandari R, Sureshan KM. Novel substrates for kinases involved in the biosynthesis of inositol pyrophosphates and their enhancement of ATPase activity of a kinase. ***Molecules.*** 26(12) : 3601.

40. Moirangthem R, Kumar K, Kaur R. Two functionally redundant FK506-binding proteins regulate multidrug resistance gene expression and govern azole ***Antifungal resistance. antimicrobial agents and chemotherapy.*** 65(6) : e02415-20.

41. Mukherjee S, Roy M, Ghosh S, Guha G, Prasad Saha S, Dalal A. Rare mutation in ELOVL4 gene in SCA34 and cognitive affection: Expounding the role of cerebellum. ***Clinical Neurology and Neurosurgery.*** 210 : 106983.

42. Narayanan DL, Majethia P, Shrikiran A, Siddiqui S, Dalal A, Shukla A (JAN-2022). Further evidence of affected females with a heterozygous variant in FGF13 causing X-linked developmental and epileptic encephalopathy 90. ***European Journal of Medical Genetics.*** 65(1) : 104403.

43. Nerakh G, Vineeth VS, Tallapaka K, Nair L, Dalal A, Aggarwal S. Microcephalic primordial dwarfism with predominant Meier-Gorlin phenotype, ichthyosis, and multiple joint deformities-Further expansion of DONSON Cell Cycle-opathy phenotypic spectrum. ***American Journal of Medical Genetics Part – A.*** doi: 10.1002/ajmg.a. 62725.

44. Pal R, Ghosh S, Mukhopadhyay S. Moonlighting by PPE2 Protein: Focus on Mycobacterial Virulence. ***Journal of Immunology.*** 207(10) : 2393-2397.

45. Pal R, Kumar Bisht M, Mukhopadhyay S. Secretory proteins of Mycobacterium tuberculosis and their roles in modulation of host immune responses: Focus on therapeutic targets. ***FEBS Journal.*** doi: 10.1111/febs.16369

46. Pan YE, Tibbe D, Harms FL, Reißner C, Becker K, Dingmann B, Mirzaa G, Kattentidt-Mouravieva AA, Shoukier M, Aggarwal S, Missler M, Kutsche K, Kreienkamp HJ. Missense mutations in CASK, coding for the calcium-/calmodulin-dependent serine protein kinase, interfere with neurexin binding and neurexin-induced oligomerization. ***Journal of Neurochemistry.*** 157(4) : 1331-1350.

47. Pandey SS, Chatterjee S. Insights into the cell-to-cell signaling and iron homeostasis in xanthomonas virulence and lifestyle. ***Phytopathology.*** 112 (2) : 209-218.

48. Patel KD, Mohid SA, Dutta A, Arichthota S, Bhunia A, Haldar D, Sarojini V. Synthesis and antibacterial study of cell-penetrating peptide conjugated trifluoroacetyl and thioacetyl lysine modified peptides. ***European Journal of Medicinal Chemistry.*** 219 : 113447.

49. Phanindranath R, Sudhakar DVS, Thangaraj K, Sharma Y. Conformational scanning of individual EF-hand motifs of calcium sensor protein centrin-1. Biochemical and ***Biophysical Research Communications.*** 570 : 67-73.

50. Puri RD, Dalal A, Moirangthem A. Indian Undiagnosed Diseases Program (I-UDP) - The unmet need. ***Indian pediatrics.*** 59 (3) : 198-200.

51. Rajeev R, Dwivedi AP, Sinha A, Agarwaal V, Dev RR, Kar A, Khosla S. Epigenetic interation of microbes with their mammalian hosts. ***Journal of Biosciences.*** 2021;46(4):94.

52. Ranganath P, Vineeth VS, Dalal A, Patil SJ. Report of an Asian-Indian patient with Okur-Chung Syndrome and comparison of the clinical phenotype in different ethnic groups. ***Clinical dysmorphology.*** 30(4) : 209-212.

53. Rani DS, Vijaya Kumar A, Nallari P, Sampathkumar K, Dhandapany PS, Narasimhan C, Rathinavel A, Thangaraj K. Novel Mutations in β-MYH7 Gene in Indian Patients With Dilated Cardiomyopathy. ***Canadian Journal of Cardiology*** - CJC Open. 4(1) : 1-11.

54. Sait H, Srivastava P, Gupta N, Kabra M, Kapoor S, Ranganath P, Rungsung I, Mandal K, Saxena D, Dalal A, Roy A, Pabbati J, Phadke SR. Phenotypic and genotypic spectrum of CTSK variants in a cohort of twenty-five Indian patients with pycnodysostosis. ***European Journal of Medical Genetics.*** 64(7) : 104235.

55. Samal B, Chatterjee S. Bacterial quorum sensing facilitates Xanthomonas campesteris

pv. campestris invasion of host tissue to maximize disease symptoms. ***Journal of Experimental Botany.*** 72(18) : 6524-6543.

56. Saurav S, Manna SK. Increased expression of Profilin potentiates chemotherapeutic agent-mediated tumour regression. ***British Journal of Cancer.*** 2022 June; 126(10):1410-1420

57. Shah A, Bhandari R. IP6K1 upregulates the formation of processing bodies by influencing protein-protein interactions on the mRNA cap. ***Journal of Cell Science.*** 134(24) : jcs259117.

58. Sharma S, Yadav R, Sahajpal V, Singh M, Ranga S, Kadian L, Yadav C, Patial A, Devi N, Ahuja P. Y-23 mediated genetic data analysis of endogamous Brahmin population of Rajasthan, India. ***Data in brief.*** 42 : 108061. doi: 10.1016/j.dib.2022.108061.

59. Shivaram S, Nagappa M, Seshagiri DV, Saini J, Govindaraj P, Sinha S, Bindu PS, Taly AB. Leukodystrophy Due to eIF2B Mutations in Adults. ***Canadian Journal of Neurological Sciences (CJNS).*** 2 : 1-5.

60. Shrivastava R, Pradhan G, Ghosh S, Mukhopadhyay S (MAR-2022). Rabaptin5 acts as a key regulator for Rab7l1-mediated phagosome maturation process. ***Immunology.*** 165 (3) : 328-340.

61. Shukla A, Kaur M, Kanwar S, Kaur G, Sharma S, Ganguli S, Kumari V, Mazumder K, Pandey P, Rouached H, Rishi V, Bhandari R, Pandey AK. Wheat inositol pyrophosphate kinase TaVIH2-3B modulates cell-wall composition and drought tolerance in Arabidopsis. ***BMC Biology.*** 19(1) : 261

62. Singh PP, Suravajhala P, Basu Mallick C, Tamang R, Rai AK, Machha P, Singh R, Pathak A, Mishra VN, Shrivastava P, Singh KK, Thangaraj K, Chaubey G. COVID-19: Impact on linguistic and genetic isolates of India. ***Genes and Immunity.*** 23(1):47-50.

63. Singh PP, Srivastava A, Sultana GNN, Khanam N, Pathak A, Suravajhala P, Singh R, Shrivastava P, Van Driem G, Thangaraj K, Chaubey G. The major genetic risk factor for severe COVID-19 does not show any association among South Asian populations. ***Scientific Reports.*** 11(1) : 12346.

64. Singh PP, Suravajhala P, Basu Mallick C, Tamang R, Rai AK, Machha P, Singh R, Pathak A, Mishra VN, Shrivastava P, Singh KK, Thangaraj K, Chaubey G. COVID-19: Impact on linguistic and genetic isolates of India. ***Genes and immunity.*** 11 : 1-4.

65. Singh, P., Verma, R.K., & Chatterjee, S. (2022) The diffusible signal factor synthase, RpfF, in Xanthomonas oryzae pv. oryzae is required for the maintenance of membrane integrity and virulence. ***Molecular Plant Pathology.*** 23: 118–132. 2. https://doi.org/10.1111/mpp.13148

66. Sontyana B, Shrivastava R, Battu S, Ghosh S, Mukhopadhyay S. Phagosome maturation and modulation of macrophage effector function by intracellular pathogens: target for therapeutics. ***Future Microbiology.*** 17 : 59-76.

67. Srivastava S, Abraham PR, Mukhopadhyay S. Aptamers: An emerging tool for diagnosis and therapeutics in tuberculosis. ***Frontiers in Cellular and Infection Microbiology.*** 11 : 656421.

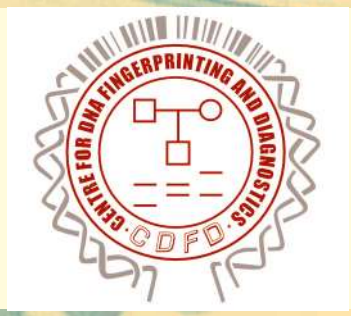
68. Srivastava S, Mukhopadhyay S. Mycobacterium tuberculosis protein PPE2 binds to DNA region containing promoter activity. ***Biochemical and Biophysical Research Communications.*** 567 : 166-170.

69. Sugeedha J, Gautam J, Tyagi S. SET1/MLL family of proteins: functions beyond histone methylation. ***Epigenetics.*** 16(5) : 469-487.

70. Venkatapuram VS, Aggarwal S, Kulkarni AD, Vineeth VS, Bhikaji Dalal A, Bhat V, Kiran L, Patil SJ. Fetal presentation of chondrodysplasia with joint dislocations, GPAPP type, caused by novel biallelic IMPAD1 variants. ***American Journal of Medical Genetics Part A.*** 188(4) : 1287-1292.

मानव संसाधन विकास

Human Resource Development Programme



PhD Program

The students admitted as Junior Research Fellows (JRFs) are encouraged to take admission in the PhD program of Manipal Academy of Higher Education, Regional Centre of Biotechnology, or University of Hyderabad. Keeping in view the interdisciplinary nature of scientific research, the Centre especially encourages persons from different scientific disciplines to take up challenges in various areas of modern biology.

The eligibility for the program is Masters degree in any branch of Science, Technology or Agriculture from a recognized University / Institute or MBBS. Candidates must have cleared National Eligibility Test (NET) with a valid fellowship. Eligible candidates are invited for a written examination followed by interviews of shortlisted candidates.

As of March 31, 2022 the Centre has 96 Research Scholars working for their doctorates in different areas of research. In the reporting year, 12 Research Scholars have completed PhD and are pursuing careers elsewhere in India or abroad.

Postdoctoral Program

In addition to the JRF program, the Centre also carries out training at the post-doctoral level. The post-doctoral fellows are funded through extramural grants that CDFD receives. Some are also selected competitively by various schemes of Government of India such as the DST WoS-A program, the DST N-PDF program, the DBT post-doctoral fellowship program and others.

Summer Training Program

CDFD provides admissions to summer training program to those students who are supported either by the Indian Academy of Science, Bangalore or Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore or the Kishore Vigyanik Protsahan Yojna, New Delhi. In the reporting year 04 students received summer training at the Centre.

Dissertation based Research Training for students

Under this programme, the students spend 4 - 6 months at CDFD and work on active projects being carried out by CDFD faculty. This training helps the students in gaining hands-on experience in modern biology. In the reporting year, 18 students were given the opportunity to avail training under this programme.

SERB-SSR Training for students

Under this programme, the students spend 2 months at CDFD and work on active projects being carried out by CDFD faculty. This training helps the students in gaining hands-on experience in frontier areas of modern biology. In the reporting year, 04 students availed training under this programme.

पुरस्कार एवं सम्मान
Awards and Honours

Awards & Honours

Sl No	Faculty & Staff	
1.	Dr. Ranjan Sen	J.C. Bose fellowship by SERB
2.	Dr. Sangita Mukhopadhyay	J.C. Bose fellowship by SERB
3.	Dr. M Subba Reddy	CDRI Awards 2022 for Excellence in Drug Research in life science category.
4.	Dr. Ashwin Dalal	Dr G Jayaraman Endowment Award at Department of Genetics, Dr.ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai on 11.03.2022
	PhD Students & Project Personnel	
1	Ms Faiza Nazar	1. e-poster presentation (Research) - 1st Prize. 2. Dr. Awtar Krishan Award - 3rd position in the Quiz competition in 22nd INDO-US Flow Cytometry Workshop organized by Trust for Education and Training in Cytometry (TETC) from 22nd – 28th February, 2021.
2	Ms. Devanshi Gupta	1. Bursary award to attend Wellcome Connecting Science Conference on Organoids, Conference held during 28th-30th September 2021 2. DST AWSAR award 2021 by DST, Govt. of India
3	Ms. Akruti Shah	First person interview published in Journal of Cell Science | The Company of Biologists
4	Dr Asmita Gupta	Department of Science and Technology, Government of India-Women Scientist Scheme -A and also received Best Poster Presentation Award Annual in SBC(I)meeting.
5	Ms Payal Katariya	Poster presentation award - 5th position in the 3rd National Biomedical Research Competition-2021 (NBRCCOM 2021) organised by the Society of Young Biomedical Scientists
6	Ms Anamika Battu	Award of appreciation for oral presentation (Category-Life Science) in the 3rd National Biomedical Research Competition-2021 (NBRCCOM 2021), Department of Biotechnology
7	Ms Sara Anisa George	Poster presentation award in the 3rd National Biomedical Research Competition-2021 (NBRCCOM 2021) organised by the Society of Young Biomedical Scientists, India from 6th-10th December 2021

कार्यक्रम
Events

Important Events

Sl.No.	Event	Date
1	Observance of Anti-Terrorism Day	21.05.2021
2	Vaccination at CDFD	15.06.2021
3	International Yoga Day	21.06.2021
4	NGC-CDFD workshop on Hands on workshop on Next Generation Sequencing Data Analysis for Clinical Diagnostics	21.06.2021 to 25.06.2021
5	Dr. Lalji Singh Memorial Lecture: Talk by Dr. Subbaya Subramanian, Associate Professor, University of Minnesota on “Tunor-intrinsic Immune Regulation in Colorectal Cancer”	05.07.2021
6	Vaccination of staff and students against COVID 19 at CDFD	27.07.2021
7	Webinar by Dr. Ashwin Dalal entitled "The story of ATCG" for DBT Star colleges.	04.08.2021
8	Visit of AIIMS, Bibinagar for collaborative work	12.08.2021
9	Independence Day celebrations	15.08.2021
10	Observance of Sadbhavana Diwas	19.08.2021
11	Open Day for DBT Star Colleges on virtual platform	23.08.2021
12	Organ donation awareness programme under Jeevandhan programme by Gandhi Medical College	02.09.2021
13	Webinar on DNA fingerprinting technology - its success and future	02.09.2021
14	Hindi Day Celebrations	14.09.2021
15	MoU between CDFD and All India Institute of Medical Sciences, Bibinagar.	29.09.2021
16	MoU between CDFD and University of Jammu, Jammu	20.10.2021
17	SAC meeting	22.10.2021 to 23.10.2021
18	Observance of Vigilance Awareness Week	26.10.2021 to 01.11.2021
19	Hands on Workshop on “Next Generation Sequencing (NGC) Data Analysis for Clinical Diagnosis”	25.10.2021 to 29.10.2021
20	MoU between CDFD, Hyderabad and Asian Healthcare Foundation, Hyderabad	27.10.2021
21	Observance of Rashtriya Ekta Diwas (National Unity Day)	01.11.2021
22	Lecture by Shri U Ramamohan , Commandant, AP Kurnool under Vigilance Awareness Week	01.11.2021
23	MoU between CDFD, Hyderabad and BCIL for IP Management and Technology Transfer Services.	11.11.2021

24	Training workshop on BioVia Discovery Studio (From Genome to Proteome) (Virtual mode) in association with ALTEM Technologies.	11.11.2021 to 12.11.2021
25	MoU between CDFD, Hyderabad and FSL-UP for DNA fingerprinting services to Govt.of U.P.	18.11.2021
26	Hindi workshop by DBT Officials	25.11.2021
27	Observance of “Constitution Day”	26.11.2021
28	Open Day for DBT Star Colleges on virtual platform	30.11.2021
29	Open Day for DBT Star Colleges on virtual platform	01.12.2021
30	Hands-on training course on Advancements in Forensic DNA Workflow	15.12.2021 to 17.12.2021
31	MoU between CDFD, Hyderabad and Amity University for Collaborative academic activities	22.12.2021
32	Talk in the scientific seminar on organized jointly by NGRI, CCMB and IICT laboratories of CSIR on the occasion of World Hindi Day (Vishwa Hindi Diwas)	13.01.2022
33	Booster dose vaccination for CDFD Staff and Students against COVID 19 at CDFD	18.01.2022
34	Republic Day Celebrations	26.01.2022
35	One-day virtual Symposium on Current Trends in Bioscience	27.01.2022
36	CDFD Silver Jubilee celebrations 2022	28.01.2022
37	Vishwa Hindi Diwas Samaroh. Webinar by Dt. Monika Goel, Head Dietician delivered an online talk on “COVID महामारी में उचति पोषण का महत्त्व"	09.02.2022
38	An interactive virtual meeting on Molecular Intricacies of Plant Associated Microorganisms (MIPAM-2022)	17-20 February 2022
39	Participation in Nation-Wide Science Week Festival under Azadi ka Amrit Mahotsav at ICMR-NIN, Hyderabad	22-28 February 2022
40	Participation in Vigyan Sarvatra Pujyate -- Festival of Science & Technology under Azadi ka Amrit Mahotsav: Mega Expo, organized by PSA office, New Delhi	22-28 February 2022
41	Virtual Open Day for Madhya Pradesh Council of Science and Technology (MPCS&T), Govt. of Madhya Pradesh, Bhopal.	23.02.2022
42	International Women’s Day celebrations	08.03.2022
43	MoU between CDFD and Foundation for Advancing Science and Technology (FAST)	11.03.2022
44	MoU between CDFD and Semantic Web India Limited	17.03.2022
45	Awareness program on protection of women from sexual harassment (POSH Act, 2013)	31.03.2022

Outreach Activities

Sl.No.	Activity	Date
1	Opportunities and career advice for PhD and Postdocs in India with a special focus on Women in Science." during a JK Scientists Outreach session.	21.05.2021
2	Webinar and interaction with students of DBT Star colleges under BRIDGE Programme	15.06.2021
3	Open Day for DBT Star Colleges on virtual platform	21.06.2021
4	Organ donation awareness programme under Jeevandhan Scheme with Gandhi Medical College under our social outreach programme	21.06.2021 to 25.06.2021
5	Webinar on DNA fingerprinting technology - its success and future	05.07.2021
6	Talk on “Recent Trends in chemistry” at One-day international webinar jointly organized by Dept. of Chemistry & IQAC, Kandi Raj college, Murshidabad.	27.07.2021
7	Talk and interaction with students at RBVRR Women’s College in Hyderabad	04.08.2021
8	Talk for MSc Biotechnology and MSc Microbiology students at Kannur University, Kerala	12.08.2021
9	Open Day on virtual platform for Nizam College (Autonomous), a constituent college of Osmania University,	15.08.2021
10	Open Day on virtual platform for St. Anns College for Women	19.08.2021
11	Talk in the scientific seminar organized jointly by NGRI, CCMB and IICT laboratories of CSIR on the occasion of World Hindi Day	23.08.2021
12	Talk and interaction with the students of Keshav Memorial Degree College	02.09.2021
13	Azadi Ka Amrit Mahotsav-Science Showcase Mega Expo in Hyderabad, Warangal and Vizag. Talk by three CDFD scientists.	02.09.2021
14	Nation-Wide Science Week Festival at ICMR-NIN, Hyderabad	14.09.2021
15	Dr. G Jayaraman Endowment Lecture 2022 on the topic “Identification of novel genes in Indian patients with rare disease” at Department of Genetics, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai.	29.09.2021
16	Talk on “Population genomics and personalized medicine” under International Hybrid Workshop on “Integrating Pharma Education & Research in Developmental Therapeutics” organizing by RBVRR College of Pharmacy, Narayanguda, Hyderabad	20.10.2021
17	Talk on “DNA fingerprinting: Forensic & medico-legal perspectives” at International Webinar lecture Series 2021-22 at Institute of Forensic Science & Criminology, Panjab University, Chandigarh	22.10.2021 to 23.10.2021
18	Talk on “Fundamentals of Bioinformatics: tools and databases” for Society for Research and Initiatives for Sustainable Technologies and Institutions (SRISTI), Ahmedabad, Gujarat.	26.10.2021 to

संकाय एवं अधिकारी
Faculty and Officers

Scientific Group Leaders (Faculty)

Dr. K Thangaraj
Dr. Ranjan Sen
Dr. Sangita Mukhopadhyay
Dr. Murali Dharan Bashyam
Dr. Sanjeev Khosla
Dr. Sunil Kumar Manna
Dr. Akash Ranjan
Dr. Rupinder Kaur
Dr. Ashwin B Dalal
Dr. Rashna Bhandari
Dr. Devyani Halder
Dr. N Madhusudan Reddy
Dr. Shweta Tyagi
Dr. M V Subba Reddy
Dr. Subhadeep Chatterjee
Dr. Rohit Joshi
Dr. Sardesai Abhijit Ajit
Dr. Yathish Jagadheesh Achar
Dr. R Harinarayanan
Dr. P Govindaraj
Dr. Kuldeep Verma
Dr. Ajay Kumar Mahato

Adjunct Faculty

Prof. Anuradha Lohia,	VC of Presidency University
Dr. Renu Wadhwa,	National Institute of Advanced Industrial Science & Technology
Dr. Prajnya Ranganath,	Nizam's Institute of Medical Sciences
Dr. Shagun Aggarwal,	Nizam's Institute of Medical Sciences

Other Service Group Leaders

Dr. Varsha
Dr. Pore Pranjali Milind
Mr. Vinod Kumar Mishra
Ms. M Kavita Rao
Dr. V Punnaiah
Mr. K Arun Kumar
Mr. Rabinarayan Mishra

Administrative Group Leaders

Mr. G Ravindar
Mr. E V Rao

Directors Office

Administration Section

DDO Section

Estate Section

Security Section

Finance and Accounts Section

EMPC and Academics Section

Stores and Purchase Section

Library Section

Electrical Engineering Section

Civil Engineering Section

Transport Section

Canteen Section

केंद्र की समितियाँ
Committees of the Centre

Members of CDFD Society :

1	Dr. Jitendra Singh	The Hon'ble Union Minister of Science & Technology	President
2	Sri Allola Indra Karan Reddy	The Hon'ble Forests & Environment and Science & Technology Minister, Telangana State	Member – Ex-officio
3	Dr. Rajesh S Gokhale	Secretary, DBT	Member – Ex-officio
4	Prof. Balram Bhargava	Secretary, DHR & DG, ICMR	Member – Ex-officio
5	Dr. Shekhar C Mande	Secretary, DSIR & DG, CSIR	Member – Ex-officio
6	Dr. Rajat Kumar	IAS, Special Chief Secretary Environment, Science & Technology Department, Telangana State	Member – Ex-officio
7	Shri Sunil Kumar	JS (Admin), DBT	Member – Ex-officio
8	Shri Vishvajit Sahay	Additional Secretary & Financial Advisor, DBT	Member – Ex-officio
9	Dr. K Thangaraj	Director, CDFD	Member – Secretary
10	Dr. J M Vyas	Vice-Chancellor, National Forensic Sciences University	Nominated members
11	Dr. Vineet Ahuja	Professor, Department of Gastroenterology, AIIMS, New Delhi	Nominated Member
12	Dr. M R S Rao	Honorary Professor, Chromatin Biology Laboratory, Neuroscience Unit (NSU), Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru	Nominated Member
13	Prof. V Nagaraja	Former President, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), and Hon. Professor, IISc, Bengaluru	Nominated Member
14	Prof. P Appa Rao	Former Vice-Chancellor, University of Hyderabad, Hyderabad	Nominated Member
15	Shri Dilip S Shanghvi	Managing Director, Sun Pharma, Goregaon, Mumbai	Nominated Member

Members of CDFD Governing Body :

1	Dr. Rajesh S Gokhale, Secretary, DBT	Chairperson
2	Shri Sunil Kumar, JS (Admin), DBT	Member – Ex-officio
3	Shri Vishvajit Sahay, Additional Secretary & Financial Advisor, DBT	Member – Ex-officio
4	Dr. Suchita Ninawe, Scientist 'G'/Advisor, DBT	Member – Ex-officio
5	Dr. K Thangaraj, Director, CDFD	Member – Ex-officio
6	Dr. Ranjan Sen, Staff Scientist – VII, CDFD	Member – Ex-officio

7	Dr. Onkar N. Tiwari, Scientist 'F', DBT	Member – Ex-officio
8	Shri G. Ravindar, Head – Administration, CDFD	Member – Secretary
9	Dr. Sanjeev Khosla, Director, CSIR-Institute of Microbial Technology (CSIR-IMTech), Chandigarh	Nominated Member
10	Dr. Anurag Agrawal, Director, CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi	Nominated Member
11	Lieutenant General (Dr.) Madhuri Kanitkar, Vice Chancellor, Maharashtra University of Health Sciences, Nasik	Nominated Member
12	Dr. Subeer S Majumdar, Distinguished Professor, National Institute of Animal Biotechnology (NIAB), Hyderabad	Nominated Member

CDFD Scientific Advisory Committee (SAC) – Oct. 2021

1	Prof M R S Rao, JNCASR, Bangalore	Chairperson
2	Dr. Suchita Ninawe DBT, New Delhi (DBT Representative)	Member
3	Dr. Sanjeev Khosla CSIR-IMTech, Chandigarh	Member
4	Dr. Anurag Agrawal CSIR-IGIB, New Delhi	Member
5	Lieutenant General (Dr.) Madhuri Kanitkar Maharashtra University of Health Sciences, Nashik	Member
6	Dr. Subeer S Majumdar NIAB, Hyderabad	Member
7	Dr. Rajan Sankaranarayanan CSIR-CCMB, Hyderabad	Member
8	Prof. Usha Vijayaraghavan IISc., Bengaluru	Member
9	Prof. Suman Kumar Dhar JNU, New Delhi	Member
10	Dr. Eric Green NHGRI, NIH, USA	Member
11	Dr K Thangaraj Director, CDFD	Member Secretary

Members of CDFD Finance Committee :

1	Shri Vishvajit Sahay	Additional Secretary & Financial Advisor, DBT	Chairperson
2	Dr. Suchita Ninawe	Scientist 'G'/Advisor, DBT	Member – Ex-officio
3	Dr. K Thangaraj	Director, CDFD	Member – Ex-officio
4	Shri G Ravindar	Head – Administration, CDFD	Member – Ex-officio
5	Shri E V Rao	I/c – Finance & Accounts, CDFD	Member – Secretary
6	Dr. Nagendra R. Hegde	Director in-charge Director, NIAB, Hyderabad	Nominated Member
7	Ms. Kapavarapu Ganga	IA & AS (1981) (Retired), Former Deputy Comptroller and Auditor General, Government of India	Nominated Member
8	Shri Atul Kumar Gupta	Former President, Institute of Chartered Accountants of India	Nominated Member

Institutional Ethics Committee

1	Prof. G B Reddy, University College of Law, Osmania University, Hyderabad	Chairperson

2	Prof. Sheela Prasad, Associate Professor, Centre for Regional Studies, School of Social Sciences, University of Hyderabad	Member
3	Dr. Mahtab S Bamji, Emeritus Scientist, Dangoria Charitable Trust, Hyderabad	Member
4	Mrs. Amita Kasbekar, VP, Deloitte Consulting India Pvt. Ltd., RMZ, Hitech City, Hyderabad	Member
5	Dr. M D Bashyam, Staff Scientist – VII, CDFD	Member
6	Dr. P Govindaraj, Staff Scientist – IV,CDFD	Member
7	Dr. Ashwin B Dalal, Staff Scientist – VII, CDFD	Member Secretary

Institutional Biosafety Committee (IBSC)

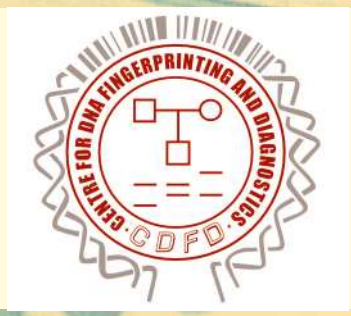
1	Dr. Sangita Mukhopadhyay, Staff Scientist – VII, CDFD	Chairperson
2	Dr. Arvind Kumar, Principal Scientist, CCMB	DBT Nominee
3	Dr. Ashwin B Dalal, Staff Scientist – VII, CDFD	Biosafety Officer
4	Dr. Shweta Tyagi, Staff Scientist – VI, CDFD	Member Secretary
5	Prof. Krishnaveni Mishra, Professor, UoH	Outside Expert
6	Dr. Sardesai Abhijit Ajit, Staff Scientist – V, CDFD	Internal Expert
7	Dr. P Govindaraj, Scientist – IV, CDFD	Internal Expert

Sexual Harassment Complaints Committee (SHCC)

1	Dr. Sangita Mukhopadhyay, Staff Scientist – VII	Chairperson
2	Dr. Rupinder Kaur, Staff Scientist – VII	Member
3	Dr. M V Subba Reddy, Staff Scientist – VI	Member
4	Mr. G Ravindar, Head – Administration	Member
5	Ms. M V Sukanya, Technical Officer – II	Member
6	Ms. V Naga Sailaja, Technical Officer – II	Member
7	Ms. P Padmavathi, Pratinidhi, Kasturba Gandhi National Memorial Trust, Telangana State	External Member

सूचना अधिकार अधिनियम, 2005 का परिपालन

Implementation of RTI Act, 2005



Implementation of RTI Act, 2005

We maintain transparency in the system and in order to achieve this we have provided following information in our website:

1. CDFD Society: Memorandum of association and rules and regulations
2. Particulars of organisation, functions and duties
3. Powers and duties of officers and employees
4. Norms for discharge of functions
5. Categories of documents held or under control
6. Formulation of policy or implementation thereof
7. Statement of the boards, councils, committees and other bodies
8. Directory of scientists, officers and employees
9. Monthly remuneration of scientists, officers and employees and system of compensation
10. Budget allocations (all plans, proposed expenditures and reports on disbursements made)
11. Execution of subsidy programmes (including amounts allocated, details and beneficiaries)
12. Names, designations and other particulars of the Public Information Officers
13. CDFD Recruitment Rules 2018-19 & Bye laws 2019.
14. Recipients of concessions, permits or authorisations granted
15. Particulars of facilities available to citizens for obtaining information (library/reading room)
16. Procedure followed in decision making process
17. Monthly RTI Returns
18. Immovable property returns statement
19. Details of CDFD purchase orders valuing more than Rs. 10 lakh
20. CDFD Policy on research misconduct
21. Procedure for handling of complaints under Public Interest Disclosure and Protection of Informers (PIDPI) Resolution to be followed by Chief Vigilance Officer (CVO)
22. Vigilance Manual
23. Below table gives a detailed description of the receipt of RTI cases at CDFD and their disposal.

IMPLEMENTATION OF RIGHT TO INFORMATION (RTI) ACT, 2005

Dr. M.D. Bashyam
Appellate Authority

Dr. Varsha
Central Public Information Officer

As received under the RTI Act 2005	Opening Balance as on 01-04-2021	Received during the year 2021-22			Disposed off during year the 2021-22				Closing Balance as on 31-03-2022
		Received directly	Received as transfer from other Public Authorities [U/s 6(3) of Act]	Total	Decisions where applications accepted/ appeals upheld	Decisions where applications accepted/ appeals rejected	Transferred to other Public Authorities [U/s 6(3) of Act]	Total	
Applications	06	43	19	62	64	NIL	NIL	64	04
Appeals	NIL	09	NIL	09	08	NIL	NIL	08	01

बजट एवं वित्त

Budget and Finance

लेखा परीक्षक की रिपोर्ट
Auditor's Report

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

AUDITOR'S REPORT

To

The Director,
Centre for DNA Fingerprinting and Diagnostics,
Hyderabad.

We have audited the attached Financial statements of **CENTRE FOR DNA FINGER PRINTING AND DIAGNOSTICS**, Hyderabad, which comprises of Balance Sheet as at 31st March 2022 and also the Income & Expenditure Account and the Receipts and Payments Account for the year ended on that date annexed there to. These Financial Statements are the responsibility of the organization's management. Our responsibility is to express an opinion on these Financial Statements based on our audit.

ORGANIZATION'S RESPONSIBILITY FOR FINANCIAL STATEMENTS

The management of the organization is responsible for the preparation of these Financial Statements. This responsibility includes the design, implementation and maintenance of Internal Control relevant to the preparation of the Financial Statements that are free from material misstatement.

AUDITOR'S RESPONISBILITY

Our responsibility is to express an opinion on these financial statements based on our Audit. We conducted our audit in accordance with the Standards on Auditing specified by ICAI. Those standards require that we comply with ethical requirements and plan and perform the Audit to obtain reasonable assurance about whether the financial statements are free from material misstatement.

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

As part of an audit in accordance with SAs, we exercise professional judgment and maintain professional skepticism throughout the Audit. We also:

Identify and assess the risks of material misstatement of the Financial Statements, whether due to fraud or error, design and perform audit procedures responsive to those risks, and obtain audit evidence that is sufficient and appropriate to provide a basis for our opinion. The risk of not detecting a material misstatement resulting from fraud is higher than for one resulting from error, as fraud may involve collusion, forgery, intentional omissions, misrepresentations, or the override of internal control.

Evaluate the appropriateness of accounting policies used and the reasonableness of accounting estimates and related disclosures made by management.

Conclude on the appropriateness of management's use of the going concern basis of accounting and, based on the Audit evidence obtained, whether a material uncertainty exists related to events or conditions that may cast significant doubt on the Company's ability to continue as a going concern. If we conclude that a material uncertainty exists, we are required to draw attention in our auditor's report to the related disclosures in the Financial Statements or, if such disclosures are inadequate, to modify our opinion. Our conclusions are based on the Audit evidence obtained up to the date of our Auditor's report. However, future events or conditions may cause the Company to cease to continue as a going concern.

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

We communicate with those charged with governance regarding, among other matters, the planned scope and timing of the audit and significant audit findings, including any significant deficiencies in internal control that we identify during our Audit.

Report on the Audit of the standalone Financial Statements

Qualified Opinion

We have audited the financial statements of "Centre for DNA Fingerprinting and Diagnostics", which comprises the Balane Sheet as at 31St March 2022, and the Income and Expenditure Account for the year then ended, and notes to the financial statements, including a summary of significant accounting policies.

In our opinion and to the best of our information and according to the explanations given to us, except for the effects of the matter described in the Basis for Qualified Opinion section of our report, the accompanying financial statements give a true and fair view of the financial position of the institute as at 31St March 2022, and of its financial performance for the year ended in accordance with the Accounting Standards issued by the Institute of Chartered Accountants of India (ICAI).

Basis for Qualified Opinion is based on the following reservations:

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

1. We observed during the course of our audit that there are considerable adjustment entries to be made in respect of bank reconciliation statement. Suspense Asset amounting to Rs.82,94,906 and Suspense Liabilities of Rs. 85,33,220 are outstanding and are to be identified swiftly to more accurate presentation of financial statements.

2. We have observed that Objection Register (OB) has an outstanding amount of Rs. 8.61 Crores in respect of from advances for equipment, consumables and other advances and they have been reconciled. As per the last year audit report objection register that advance to the tune of Rs.16.03 crores as on 31-03-2021 in respect of advances for equipment, consumables and other advances are pending for clearance and some adjustments are outstanding since more than three years. Management has initiated steps to clear such outstanding balances and to the tune of Rs. 8.8 Crores have been reconciled and identified. However, there are still long outstanding advances are not reconciled yet, hence unable to comment on the expenditure and asset account balances.

3. Bank reconciliations are not completed in SBI Current Account, and there are still unidentified entries to be reconciled, to an extent of a minor percentage.

4. We are unable to comment on Fixed Assets as physical verification of assets is not done by the management and there are differences found in the Fixed Asset Register maintained by the Stores department with the Fixed Asset schedule in the books of accounts.

5. We have observed that interest accrued has not been recognized properly with respect to deposits made in the Banks and STDRs are stated at book values.

6. We have found that there are differences in revenues between GST and Books of accounts.

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

7. Canteen Receipts & Payments are not considered in books of accounts.

For K. Prahlada Rao & Co.,
Chartered Accountants
F R No-002717S

K. PRAHLADA RAO & Co. Chartered Accountants FRN No: 002717S Kurnool

K. Prahlada Rao
M.No -018477
UDIN: 22018477AUHNCD8043
Place : HYDERABAD
Date : 23/09/2022

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

BALANCE SHEET AS ON 31st MARCH 2022

(Amount - ₹.)

	Schedule	Current Year	Previous Year
CORPUS/CAPITAL FUND AND LIABILITIES			
Corpus / Capital Fund	1	2,26,41,72,167.23	2,22,79,02,694.00
Reserves and Surplus	2	8,28,93,791.00	6,84,52,844.00
Earmarked / Endowment funds	3	12,99,85,300.00	15,05,56,541.00
Secured Loans & Borrowings	4	0.00	0.00
Unsecured Loans & Borrowings	5	0.00	0.00
Deffered Credit Liabilities	6	0.00	0.00
Current Liabilities and Provisions	7	17,95,31,233.00	22,18,84,578.00
TOTAL		2,65,65,82,491.23	2,66,87,96,657.00
ASSETS			
Fixed Assets	8	1,70,68,03,510.23	1,69,84,68,080.00
Investments- From Earmarked / Endowment Funds	9	0.00	0.00
Investments - Others	10	12,07,78,393.00	12,07,78,393.00
Current Assets, Loans, Advances etc.	11	82,90,00,588.00	84,95,50,184.00
Miscellaneous Expenditure		0.00	0.00
TOTAL		2,65,65,82,491.23	2,66,87,96,657.00
Significant Accounting Policies			
Contingent Liabilities and Notes on Accounts			

PLACE : HYDERABAD
DATE : 23/09/2022

I/c - FINANCE & ACCOUNTS
CDFD E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

For K.PRAHLADA RAO & CO
CHARTERED ACCOUNTANTS
F R No - 002717S

K.PRAHLADA RAO
Partner
FRN : 002717S
M.No - 018477
UDIN : 22018477AUHNCD8043

For CDFD
Director
डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

INCOME & EXPENDITURE FOR THE YEAR ENDED 31st MARCH 2022

(Amount - Rs.)

	Schedule		Current Year		Previous Year
INCOME					
Income from Sales/Services	12		1,44,40,947.00		1,47,36,365.00
Grants/Subsides	13		42,41,00,000.00		35,50,00,000.00
Fees/Subscriptions	14		0.00		0.00
Income from Investments	15		83,63,715.00		95,07,124.00
Income from Royality, Publications etc.	16		0.00		0.00
Interest Earned	17		34,37,793.00		35,19,229.00
Other Income	18		1,24,36,093.00		18,25,592.00
Increase/(decrease) in stock of Finished goods and works-in-progress	19		0.00		0.00
TOTAL (A)			46,27,78,548.00		38,45,88,310.00
EXPENDITURE					
Establishment Expenses	20		18,58,41,038.00		17,45,18,131.00
Administrative Expenses	21		22,62,57,347.00		18,33,47,020.00
Expenditure on Grants, Subsides etc.	22				0.00
Interest	23				0.00
Depreciation (Net Total at the year-end -corresponding to Schedule 8)		7,31,31,719.78		4,90,89,537.00	
Less:Transferred to Grants-in-Aid		7,31,31,719.78	0.00	4,90,89,537.00	0.00
Provision For Salaries			94,91,937.00		88,83,130.00
TOTAL (B)			42,15,90,322.00		**36,67,48,281.00**
Balance being excess of Income over Expenditure (A-B)			**4,11,88,226.00**		**1,78,40,029.00**
Transfer to Special Reserve (Specify each)					
Transfer to/from General Reserve			1,44,40,947.00		1,47,36,365.00
BALANCE BEING SURPLUS/(DEFLICT) CARRIED TO CORPUS/CAPITAL FUND			2,67,47,279.00		31,03,664.00
SIGNIFICANT ACOUNTING POLICIES					
CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS					

PLACE : HYDERABAD
DATE : 23/09/2022

I/c - FINANCE & ACCOUNTS
CDFD
E.V.RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

For K.PRAHLADA RAO & CO
CHARTERED ACCOUNTANTS
F R No - 002717S

K.PRAHLADA RAO
Partner
FRN : 002717S
M.No - 018477
UDIN : 22018477AUHNCD8043

For CDFD
Director

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2022

(Amount - Rs.)

RECEIPTS	Current Year	Previous Year	PAYMENTS	Current Year	Previous Year
1.Opening Balances			1. Expenses		
a) Cash in hand	0	0	a) Establishment Expenses (SCH 20)	18,58,41,038	17,45,18,131
b) Bank Balances			b) Administrative Expenses (SCH 21)	22,62,57,347	18,33,46,920
i) In current accounts	9,60,19,063	7,74,49,083			
ii) In deposit accounts	27,43,99,614	31,43,99,614			
iii) Savings accounts	32,81,44,381	25,97,89,587			
2. Grants Received			2. Payments made against funds for various projects		
a) From Government of India	44,06,00,000	43,50,00,000	(Name of the fund or project should be shown along with		
b) From State government		0	the particulars of payments made for each project)		
c) From other sources (details)		0	Projects	15,53,01,888	16,91,17,418
(Grants for capital & revenue			Projects - CB Clearances	0	5,12,20,240
exp. To be shown seperately)			Reaearch Fellow Associates Payments	0	23,38,295
Research Fellow Associates Receipts	5,09,600	92,98,039	Grant in Aid Capital	1,65,00,000	0
Project Grants	16,91,53,163	7,76,87,572			
Project Grants - OB Clearances	0	5,12,20,240			
			3. Investments and deposits made		
			a) Out of Earmarked/Endowement funds		
			b) Out of Own Funds (Investments-Others)		
3. Income on Investments from			c) Deposit made in CPF A/C		
a) Earmarked/Endow. Funds	83,63,715	95,07,124	4. Expenditure on Fixed Assets & Capital Work-in-Progress		
b) Own Funds (Oth. Investment)			a) Purchases of Fixed Assets:		
Investments EnCashed			Books & Journals		
			Equipment -Lab/Office/Furniture	7,58,86,514	21,33,47,940
4. Interest Received			b) Expenditure on Capital Work-in-Progress:	0	13,12,261
a) On Bank deposits	34,37,793	35,19,229			
b) Loans, Advances etc		0	5. Refund of surplus money/Loans		
c) Interest on Computer Advance, Conveyance advance and HBA	29,612	6,783	a) To the Government of India	0	0
d) Interest on CPF		0	b) To the State Government	0	0
			c) To other providers of funds	0	0
5. Other Income(Specify)					
a) Analysis Charges	1,44,40,947	1,47,36,365	6. Finance Charges (Interest)		
b) Other Income (SCH 28)					
6. Any Other Receipts(Give Details)			7. Other Payments (Specify)		
I-Remittances (Annexure-A)	2,99,88,122	5,07,22,861	Advances (Annexure-D)	10,84,85,564	27,92,87,576
CPF-SUB,Arrears and adv.Refund	53,08,643	1,40,02,555	I-Remittances (Annexure-E)	3,43,89,680	4,75,69,800
Sundry Receipts	15,29,022	13,43,596	CPF A/c	3,40,40,902	10,68,600
Application Fee	22,251	96,643	New Pension Scheme	2,34,46,854	61,85,093
Sale OF Tender Forms	14,500	41,500			
Contingencies Students	66,150	0	8. Closing Balances		

E.V.RAO
I/C - F & A
Centre for DNA Fingerprinting and Diagnostics

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

NGC Charges	15,25,341	0	a) Cash in hand	0	0
NPS	0	61,85,093	b) Bank Balances		
Advance/Refunds/Recovery/Adj	6,21,19,078	49,17,76,847	i) In current accounts	12,67,51,547	9,60,19,063
NIMS	0	0	ii) In deposit accounts	8,43,99,614	27,43,99,614
Income Tax Refund	0	3,37,070	iii) Savings accounts	36,43,70,047	31,73,88,850
TOTAL	**1,43,56,70,995**	**1,81,71,19,801**	**TOTAL**	**1,43,56,70,995**	**1,81,71,19,801**

PLACE : HYDERABAD
DATE : 23/09/2022

I/c - FINANCE & ACCOUNTS
CDFD

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

For K.PRAHLADA RAO & CO
CHARTERED ACCOUNTANTS
F R No - 002717S

K.PRAHLADA RAO
Partner
FRN : 002717S
M.No - 013477
UDIN : 22013477AUHNCD8043

For CDFD
Director

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
BALANCE SHEET AS ON 31st MARCH 2022

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 1 - CORPUS/CAPITAL FUND :				
Balance as at the begining of the year		2,22,79,02,694.00		2,09,37,36,336.00
Add : Contribution towards Corpus/Capital Fund				
CDFD Core - Plan (Non-Recurring)	0.00		8,00,00,000.00	
Capitalised portion of Capital Expenditure of projects	8,26,53,914.00	8,26,53,914.00	12,42,84,658.00	20,42,84,658.00
Less : Depreciation For the Year		7,31,31,719.78		7,38,41,917.00
Less : Fund returned to DBT		0.00		0.00
Add : Excess of Income over Expenditure		2,67,47,279.00		37,23,617.00
BALANCE AS AT THE YEAR - END		**2,26,41,72,167.23**		**2,22,79,02,694.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN 002727S Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 2 -RESERVES AND SURPLUS :				
1.Capital Reserve :				
As per last Account	0.00		0.00	
Addition during the year	0.00		0.00	
Less : Deductions during the year	0.00	0.00	0.00	0.00
2.Revolution Reserve :				
As per last Account	0.00		0.00	
Addition during the year	0.00		0.00	
Less : Deductions during the year	0.00	0.00	0.00	0.00
3.Special Reserves :				
As per last Account	0.00		0.00	
Addition during the year	0.00		0.00	
Less : Deductions during the year	0.00	0.00	0.00	0.00
4.General Reserve - Lab Reserve :				
As per last Account	6,84,52,844.00		5,37,16,479.00	
Addition during the year	1,44,40,947.00		1,47,36,365.00	0.00
Less : Deductions during the year	0.00	8,28,93,791.00	0.00	6,84,52,844.00
Total		**8,28,93,791.00**		**68452844.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN 007175 Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 3 - EARMARKED/ENDOWMENT FUNDS :	Current Year		Previous Year	
(Refer Annexures)				
(a) Opening balance of the Funds		**15,05,56,541.00**		**24,19,86,387.00**
(b) Additions to the Funds :				
i. Donations /grants (net)	16,91,53,163.00		7,76,87,572.00	
ii. Income from investments made on account of funds	0.00		0.00	
iii. Other additions (OB clearances)	-	16,91,53,163.00	5,12,20,240.00	12,89,07,812.00
TOTAL (a+b)		31,97,09,704.00		37,08,94,199.00
(c) Utilisation/Expenditure towards objective of funds				
(i) Capital Expenditure (Refer Annexures I & II)				
- Fixed Assets	8,26,53,914.00		12,42,84,658.00	
- Others	0.00	8,26,53,914.00	0.00	12,42,84,658.00
- Total				
(ii) Revenue Expenditure (Refer Annexures I & II)				
- Salaries, Wages and allowances etc.	3,94,33,914.00		3,24,27,095.00	
- Rent/ REFUNDS	0.00		0.00	
- Project Consumales & Other Expenses	6,26,98,364.00	10,21,32,278.00	6,12,87,610.00	9,37,14,705.00
Total				
(iii) Refund of Project grants		49,38,212.00		23,38,295.00
TOTAL (c)		18,97,24,404.00		22,03,37,658.00
NET BALANCE AS AT THE YEAR-END [(a + b)-c]		**12,99,85,300.00**		**15,05,56,541.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO.

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 5 - UNSECURED LOANS AND BORROWINGS :	Current Year		Previous Year	
1. Central Government		0		0
2. State Government (Specify)		0		0
3. Financial Institutions		0		0
4. Banks :				
a) Terms Loans	0		0	
b) Other Loans	0	0	0	0
5. Other Institutions and Agencies		0		0
6. Debentures and Bonds		0		0
7. Fixed Deposits		0		0
8. Others (Specify)		0		0
TOTAL		**0**		**0**
Note: Amount due within one year				

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 7 - CURRENT LIABILITIES AND PROVISIONS :	Current Year		Previous Year	
A. CURRENT LIABILITIES				
1. Acceptances			0.00	
2. Sundry Creditors		7,49,361.00	0.00	
3. Advances Received			0.00	
4. Interest accured but not due on:			0.00	
5. Statutory Liabilities:			0.00	
TDS on salaries		18,02,993.00	13,20,250.00	
TDS others		4,25,246.00	2,98,760.00	
Service Tax		24,325.00	24,325.00	
Works Tax		16,80,631.00	16,80,631.00	
PM Cares Fund Payable		5,95,935.00	6,04,318.00	
6. Other current Liabilities		0.00	0.00	
CDFD.CP Fund A/C		13,94,08,534.00	17,34,49,436.00	
Contract Staff security deposit		5,78,049.00	50,974.00	
Diagnostics Collabration With NIMS				
ECCS		9,520.00	4,81,241.00	
EMD		21,73,734.00	22,18,734.00	
Festival Advance		450.00	450.00	
GSLI		2,596.00	3,796.00	
House Building Advance		1,29,831.00	1,29,831.00	
Lab Security Deposit & Hostel Security Deposit		15,29,741.00	14,73,747.00	
LIC		20,22,145.00	2,16,068.00	
Performance Guarantee Deposit		39,436.00	22,436.00	
Others (I-Remittances)		0.00	0.00	
Other Out Standing Liabilities		64,37,523.00	1,92,56,329.00	
PT Payable		43,650.00	39,500.00	
Public Provident Fund		3,91,158.00	3,91,158.00	
Royalty & Consultancy		15,31,642.00	15,31,642.00	
Security Deposit		1,03,13,709.00	1,02,60,783.00	
STAFF BENEVOLENT FUND		1,15,333.00	86,983.00	
TA Abroad [Advance]		0.00	0.00	
TA-DA-Hon within India [Advance]		33,754.00	79,909.00	21,36,21,301.00
TOTAL (A)		17,00,39,296.00		21,36,21,301.00
8.PROVISIONS				
1. For Taxation		0.00		0.00
2. Gratuity		0.00		0.00
3. Superannuation/Pension		0.00		0.00
4. Accumulated Leave Encashment		0.00		0.00
5. Trade Warranties/Claims		0.00		0.00
6. Others - Salary & Other Provisions		94,91,937.00		82,63,277.00
TOTAL (B)		94,91,937.00		82,63,277.00
TOTAL (A+B)		**17,95,31,233.00**		**22,18,84,578.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 8 - FIXED ASSTES :	GROSS BLOCK					DEPRECIATION				NET BLOCK	
	Cost/valuation As at begining of the year	Addition during Before September	After September	Deductions during the year	Cost/valuation at the year end	As at the begining of the year	during the year	On Deducti during the y	Total up to the year end	As at the Current year end	As at the Previous year end
INTANGIBLE ASSETS											
1. Firewall Cord	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0
TANGIBLE ASSETS											
A. FIXED ASSETS:											
1. LAND:											
a) Freehold	39,00,000.00	0.00			39,00,000.00	0.00	0.00	0.00	0.00	39,00,000.00	39,00,000.00
b) Leasehold	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
2. BUILDINGS											
a) On Freehold Land	22,00,52,369.00	0.00			22,00,52,369.00	14,18,96,663.00	78,15,570.60	0.00	14,97,12,233.60	7,03,40,135.40	7,81,55,706.00
b) On Leasehold Land	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
c) Ownership Flats/Premises	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
d) Superstructures on Land not belongs to the entity	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. PLANT MACHINERY & EQUIPMENT	99,39,21,289.00	2,36,35,434.00	4,83,51,695.00		1,06,59,08,418.00	63,11,30,574.00	6,17,02,393	0.00	69,28,32,966.83	37,30,75,451.18	36,35,38,004.00
4. VEHICLES	41,53,026.00		14,70,420.00		56,23,446.00	39,40,074.00	1,42,224	0.00	40,82,298.30	15,41,147.70	2,12,952.00
5. FURNITURE, FIXTURES	1,72,33,625.00	2,29,176.00	17,17,254.00		1,91,80,055.00	1,34,70,393.00	4,85,612	0.00	1,39,56,004.80	52,24,050.20	37,68,315.00
6. OFFICE EQUIPMENT	1,31,13,077.00	3,81,387.00	2,58,998.00		1,37,53,462.00	1,12,01,838.00	3,63,319	0.00	1,15,65,156.75	21,88,305.25	19,11,239.00
7. COMPUTER/PERIPHERALS	19,76,435.00	23,31,659.00	34,45,079.00		77,53,173.00	9,04,134.00	21,47,000	0.00	30,51,134.20	47,02,038.80	13,13,302.00
8. SOFTWARE	17,71,823.00	12,094.00	4,19,221.00		22,03,138.00	13,21,286.00	3,47,290	0.00	16,68,575.80	5,34,562.20	6,46,520.00
9. ELECTRIC INSTALLATIONS	0.00	0.00			0.00	0.00	0	0.00	0.00	0.00	0.00
10. LIBRARY BOOKS	2,13,35,865.00	5,104.00	5,743.00		2,13,46,712.00	2,13,35,865.00	9,272	0.00	2,13,45,136.50	1,575.50	1,296.00
11. TUBEWELLS & WATER SUPPLY	0.00	0.00			0.00	0.00	0	0.00	0.00	0.00	0.00
12. OTHER FIXED ASSETS	88,87,898.00	3,90,650.00			92,78,548.00	84,84,948.00	1,19,040	0.00	86,03,988.00	6,74,560.00	4,02,950.00
Airconditioning works	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aluminium partition work	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
DG Set	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
Paintings	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
Typewriters	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
Miscellaneous non consumables	46,400.00	0.00			46,400.00	0.00	0.00	0.00	0.00	46,400.00	42,511.00
Other Assets	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
EMB Net	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	1,28,63,91,807.00	2,69,85,504.00	5,56,68,410.00	0.00	1,36,90,45,721.00	83,36,85,775.00	7,31,31,719.78	0.00	90,68,17,494.78	46,22,28,226.23	45,38,92,795.00
B. CAPITAL WORK-IN-PROGRESS	1,24,45,75,284.00			0.00	1,24,45,75,284.00	0.00	0.00	0.00	0.00	1,24,45,75,284.00	1,24,45,75,284.00
TOTAL	**2,53,09,67,091.00**	**2,69,85,504.00**	**5,56,68,410.00**	0.00	**2,61,36,21,005.00**	**70,95,67,558.00**	**7,31,31,719.78**	**0.00**	**90,68,17,494.78**	**1,70,68,03,510.23**	**1,69,84,68,079.00**

E.V. RAO
I/C - F&A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

PRAHLADA RAO & CO Chartered Accountants

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 10 - INVESTMENTS - OTHERS :	Current Year	Previous Year
(Annexure-J)		
1. In Government Securities	0.00	0.00
2. Other approved securities	0.00	0.00
3. Shares	0.00	0.00
4. Debentures and Bonds : UTI Bonds		
5. Subsidiaries and Joint Ventures	0.00	0.00
6. Others (to be specified) - STDRs,(CPF),CDFD CP FUND A/C	12,07,78,393.00	12,07,78,393.00
TOTAL	**12,07,78,393.00**	**12,07,78,393.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 11 - CURRENT ASSETS AND LOANS , ADVANCES & OTHER ASSETS :	Current Year	Previous Year	Current Year	Previous Year
A. CURRENT ASSETS				
1. Inventors				
a) Stores and Spares	0.00		0.00	
b) Loose Tools	0.00		0.00	
c) Stock-in-trade				
Finished Goods	0.00		0.00	
Work-in-progress	0.00		0.00	
Raw Materials	0.00	0.00	0.00	0.00
2. Sundry Debtors:				
a) Debts Outstancing for a period exceeding six months	0.00	0.00	0.00	
b) Others-Life Membership Fees	1,69,236.00	1,69,236.00	1,69,236.00	1,69,236.00
3. Cash balances in hand (including cheques/drafts and imprest)				
4. Bank Balances:				
a) With Scheduled Banks:				
-On Current Accounts	12,67,51,547.00		9,60,19,063.00	
-On Deposit Accounts (includes margin money)	8,43,99,614.00		27,43,99,614.00	
-On Savings Accounts	36,43,70,047.00	57,55,21,208.00	31,73,88,850.00	68,78,07,527.00
b) With non-Schedules Banks:				
-On Current Accounts	0.00	0.00	0.00	
-On Deposit Accounts	0.00	0.00	0.00	
-On Savings Accounts	0.00	0.00	0.00	0.00
5. Post Office-Savings Accounts				
TOTAL (A)		57,56,90,444.00		68,79,76,763.00
B.LOANS, ADVANCES AND OTHER ASSETS				
a) Staff (Annexure-L)	4,38,162.00		438162.00	
b) Other Entities engaged in activities/objectives similar to that of the Entity	0.00	438162.00	0.00	4,38,162.00
2. Advances and other amounts recoverable in cash or in kind or for value to be received				
a) On Capital Account (Annexure-H)	12,24,75,096.00		5,03,99,356.00	
b) Prepayments - Deposits (Annexure-I)	2,19,55,233.00		2,01,39,580.00	
c) TDS Receivable	9,43,109.00		7,64,579.00	
d) Others (Annexure-K)	9,17,52,127.00		8,98,31,744.00	
e) GST on Purchases (Schedule 21B)		23,71,25,565.00		16,11,35,259.00
3. Income Accured:				
a) On Investments from Earmarked/Endowments Funds	0.00		0.00	
b) On Investments - Others	0.00		0.00	
c) On Loans and Advances	0.00		0.00	
d) Others	0.00	0.00	0.00	0.00
4. NPS Contribution		1,57,46,417.00		0.00
TOTAL (B)		25,33,10,144.00		16,15,73,421.00
TOTAL (A+B)		82,90,00,588.00		84,95,50,184.00

E.V.RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

V. PRAHLADA RAO

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 12 - INCOME FROM SALES/SERVICES :	Current Year	Previous Year
1) Income from sales		
a) Sale of Finished Goods	0.00	0.00
b) Sale of Raw Material	0.00	0.00
c) Sale of Scraps	0.00	0.00
2) Income from Services		
a) Labour and Processing Charges	0.00	0.00
b) Professional/Consultancy Services (Analysis & Diagnostics Charges)	1,44,40,947.00	1,47,36,365.00
c) Agency Commission and Brokerage	0.00	0.00
d) Maintenance Services (Equpiment/Property)	0.00	0.00
e) Others (Specify)	0.00	0.00
TOTAL	**1,44,40,947.00**	**1,47,36,365.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN No. 002[illegible] Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 13 - GRANTS/SUBSIDES : (Irrevocable Grants & Subsides Received)		
1) Central Government (DBT Plan Grant-in-Aid)	42,41,00,000.00	35,50,00,000.00
2) State Government(s)	0.00	0.00
3) Government Agencies	0.00	0.00
4) Institutions/Welfare Bodies	0.00	0.00
5) International Organisations	0.00	0.00
6) Others (Specify)	0.00	0.00
TOTAL	**42,41,00,000.00**	**35,50,00,000.00**

E.V. RAO
I/C- F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K.PRAHLADA RAO
Chartered Accountants
Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 15 - INCOME FROM INVESTMENTS :				
(Income on Invest from Earmarked/Endowment Funds transferred to Funds)				
1) Interest:				
a) On Govt. Securities			0.00	
b) Other Bonds/Debentures		0.00	0.00	0.00
2) Dividends:				
a) On Shares		0.00	0.00	0.00
b) On Mutual Fund Securities		0.00	0.00	0.00
3) Rents		0.00	0.00	0.00
4) Others (Specify) STDRs	83,63,715.00		95,07,124.00	0.00
TOTAL	**83,63,715.00**	**0.00**	**95,07,124.00**	**0.00**
TRANSFERRED TO EARMARKED/ENDOWMENT FUNDS				

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN No: 007175 Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 18 - OTHER INCOME :	Current Year	Previous Year
1) Profit on Sale/disposal of Assets:	0.00	0.00
a) Owned assets	0.00	0.00
b) Assets acquired out of grants, or received free of cost	0.00	0.00
2) Export Incentives realized	0.00	0.00
3) Fees for Miscellaneous Services	0.00	0.00
4) Miscellaneous Receipts		
5) Other Receipts	29,612.00	
Sundry Receipts	15,27,832.00	13,43,596.00
Application Fee and collaboration with UCL	22,251.00	96,643.00
Sales Of Tender Forms	14,500.00	41,500.00
Income tax refund		3,37,070.00
Contingencies(Students)	66,150.00	6,783.00
NGC CHARGES	1,07,75,748.00	0.00
TOTAL	**1,24,36,093.00**	**18,25,592.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN 003... Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 20 - ESTABLISHMENT EXPENSES :	Current Year	Previous Year
a) Salaries and Wages	10,83,64,486.00	13,81,50,779.00
b) Allowances and Bonus	37,82,204.00	81,88,547.00
c) Contribution to Provident Fund	48,65,960.00	55,37,473.00
d) Contribution to Other Fund (NPS)	6,34,03,715.00	61,85,093.00
e) Staff Welfare Expenses - Medical charges	44,68,946.00	44,28,947.00
f) Expenses on Employees Retirement and Terminal Benefits	0.00	1,18,98,613.00
g) Others (specify) -	9,55,727.00	0.00
h) EPF Employer Contribution		1,28,679.00
TOTAL	**18,58,41,038.00**	**17,45,18,131.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO
Chartered Accountants
Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2022

(Amount - Rs.)

SCHEDULE 21 - OTHER ADMINISTRATIVE EXPENSES :	Current Year	Previous Year
1) Purchases	5,93,10,036.00	6,94,45,077.00
2) Electricity and power	2,93,10,318.00	3,00,64,628.00
3) Water charges	46,01,208.00	43,39,673.00
4) Insurance	1,02,030.00	1,02,260.00
5) Repairs and maintenance	3,74,09,337.00	1,71,44,632.00
6) Rent, Rates and Taxes	2,42,50,837.00	32,43,141.00
7) Vehicles Running and Maintenance	19,89,713.00	68,39,214.00
8) Postage, Telephone and Communication Charges	37,25,061.00	18,19,013.00
9) Printing and Stationary	3,12,896.00	1,86,415.00
10) Travelling and Conveyance Expenses	4,194.00	66,610.00
11) Expenses on Seminar/Workshops	8,33,600.00	12,53,738.00
12) Subscription Expenses	0.00	56,000.00
13) Expenses on Fees & Renewals	3,94,623.00	4,94,089.00
14) Auditors Remuneration	96,000.00	37,500.00
15) Hospitality Expenses	4,75,973.00	3,41,887.00
16) Professional Charges	9,333.00	3,07,393.00
17) Advertisement and Publicity	14,30,354.00	37,52,174.00
18) Bank Charges	27,486.00	1,27,145.00
19) Security & Cleaning Contract Charges	2,63,32,162.00	2,42,70,857.00
20) CDFD Contract Staff Salaries	57,89,267.00	0.00
21) Other Contingencies	6,39,392.00	13,83,841.00
22) AMC	21,29,949.00	0.00
23) Other Research Expenses	80,73,940.00	71,77,710.00
24)Office Books	4,368.00	56,104.00
26)Contract Staff	16,42,699.00	18,74,710.00
27)Manpower Outsourcing(Staff)	81,39,376.00	75,41,193.00
28) Prior Period Expenses	92,23,195.00	14,22,016.00
TOTAL	22,62,57,347.00	18,33,47,020.00

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2022

Annexure: A Forming part of Receipts and Payment a/c

RECEIPTS

Previous Year	Particulars	Current Year
	I-Remittances	
50,42,467.00	TDS other than Salaries	44,32,029.00
1,41,45,332.00	TDS on Salaries	20,66,779.00
13,37,534.00	Works Tax	1,76,170.00
17,55,463.00	LIC	81,000.00
1,73,120.00	GSLI	3,336.00
0.00	CPF ADVANCE FUND	0.00
4,42,350.00	Professional Tax	2,01,250.00
26,64,167.00	Service Tax	0.00
2,89,925.00	Others (I-Remittances)	2,15,15,752.00
1,29,638.00	Health Insurance	0.00
30,13,664.00	ECCS	0.00
15,000.00	Contract Staff security deposit	15,11,806.00
30,104.00	STAFF BENEVOLENT FUND	0.00
1,05,999.00	EPF	0.00
58,788.00	GST	0.00
2,92,03,551.00		**2,99,88,122.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN No. 0[illegible]S Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2022

Annexure: B Forming part of Receipts and Payment a/c

RECEIPTS

Previous Year	Particulars	Current Year
	Advance refunds/recovery/Adjst.	
4,26,739.00	Advance for Expenses- purchases by Staff	1,31,397.00
0.00	Other Research Expenses	0.00
0.00	Computer Advance [Research Fellows]	0.00
1,14,541.00	Computer Advance [Staff]	0.00
0.00	Consumables, glassware and Spares [Advance]	0.00
0.00	Debtors	90,48,414.00
79,256.00	Conveyance Advance	0.00
0.00	Margin Money	96,03,050.00
2,08,000.00	EMD	0.00
22,14,182.00	Equipment [Advance]	1,62,77,008.00
42,300.00	Festival Advance	0.00
16,000.00	GDA [Others]	4,56,202.00
19,29,593.00	General Deposits And Advances	4,30,402.00
0.00	Human Resource Develpment - Training of Staff - Conferences [Ad	0.00
9,84,84,058.00	Inter Bank Transfer	0.00
1,78,000.00	Lab Security Deposit & Hostel Security Deposit	2,36,822.00
4,17,780.00	LTC [Advance]	1,96,136.00
95,678.00	Miscellaneous Salary [Advance]	0.00
0.00	Diagnostic Collab with NIMS	1,04,14,549.00
40,821.00	Pay of Establishment [Advance]	0.00
2,84,057.00	Revolving Advance	0.00
33,67,370.00	Security Deposit	0.00
73,778.00	TA Abroad [Advance]	0.00
44,000.00	TA-DA-Hon within India [Advance]	0.00
10,000.00	Trainee Security Deposit	4,000.00
0.00	Misc Advances	1,53,04,098.00
0.00	Workshop & Conference	0.00
0.00	Leave Salary & Pension	0.00
0.00	Performance Guarantee Deposit	17,000.00
10,80,26,153.00		**6,21,19,078.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2022

Annexure: D Forming part of Receipts and Payment a/c

PAYMENTS

Amount in Rs.

Previous Year	Particulars	Current Year
	Advances	
5,45,167.00	Advance for Expenses- purchases by Staff	13,28,021.00
35,35,441.00	Chemicals [Advance]	
0.00	Computer Advance [Research Fellows]	50,000.00
0.00	Computer Advance [Staff]	
5,12,655.00	Consumables, glassware and Spares [Advance]	1,57,79,739.00
0.00	Conveyance Advance	
4,34,270.00	EMD	45,000.00
5,42,30,746.00	Equipment [Advance]	8,32,17,078.00
0.00	GST	91,704.00
1,000.00	GDA [Others]	2,11,684.00
9,84,84,058.00	Inter Bank Transfer	
1,26,380.00	Lab Security Deposit & Hostel Security Deposit	
0.00	Liveries & Blankets [Advance]	
5,73,984.00	LTC [Advance]	1,14,800.00
0.00	Margin Money LC	46,85,525.00
33,904.00	Others [Advances]	5,55,035.00
17,453.00	Others [Contingencies Advance]	
1,88,800.00	Printing & Stationery [Advance]	
2,87,000.00	Transport Advance	
0.00	Diagnostic Services CCMB	5,12,390.00
4,18,515.00	Security Deposit	7,61,737.00
3,75,400.00	Software [Advance]	
2,18,267.00	TA Abroad [Advance]	
30,000.00	TA-DA-Hon within India [Advance]	26,025.00
0.00	TDS Receivable	1,83,293.00
10,500.00	Trainee Security Deposit	4,000.00
0.00	Student CF	7,60,585.00
0.00	TA Arrears	56,088.00
1,70,564.00	Workshop & Conference	1,02,860.00
16,01,94,104.00		**10,84,85,564.00**

E. V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2022

Annexure: E Forming part of Receipts and Payment a/c

PAYMENTS

Amount in Rs.

Previous Year	Particulars	Current Year
	I-Remittances	
3,21,745.00	Contract Staff security deposit	18,000.00
21,40,898.00	ECCS	42,78,473.00
0.00	NPS Payable	0.00
2,59,987.00	GSLI	0.00
0.00	HRA DA Arrears	9,74,008.00
8,35,000.00	Health Insurance	1,45,000.00
81,61,043.00	TDS on Salaries	2,04,70,891.00
18,65,076.00	LIC	17,15,092.00
7,08,678.00	Others (I-Remittances)	7,20,000.00
5,08,250.00	Professional Tax	4,43,700.00
11,58,742.00	Public Provident Fund	0.00
41,34,084.00	GST	23,09,012.00
0.00	CPF advance recovery	3,30,014.00
52,71,099.00	TDS on Others	29,85,490.00
1,74,000.00	Works Tax	0.00
2,55,38,602.00		**3,43,89,680.00**

E.V. RAO
I/C - F&A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K. PRAHLADA RAO & CO. Chartered Accountants FRN No. 002475S Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2022

Annexure: H Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
9,00,669.00	Advance for Expenses- purchases by Staff	4,310.00
0.00	Advances [Previous Years]	0.00
0.00	Chemicals [Advance- Proj Consumables)	0.00
0.00	Computer Advance [Research Fellows]	0.00
0.00	Computer Advance [Staff]	0.00
4,79,26,685.00	Consumables, glassware and Spares [Advance]	1,55,33,582.00
0.00	Conveyance Advance	0.00
12,63,556.00	Equipment [Advance]	10,43,28,715.00
3,08,446.00	Festival Advance	1,446.00
0.00	Health Insurance	0.00
0.00	Liveries & Blankets [Advance]	0.00
0.00	LTC [Advance]	26,07,043.00
0.00	Magzines [Advance]	0.00
0.00	Miscellaneous Salary	0.00
0.00	NPS Subscription	0.00
0.00	Office Equipment [Advance]	0.00
0.00	Others [Advances]	0.00
0.00	Pay of Establishment	0.00
0.00	Rent [Advance]	0.00
0.00	Research Fellows-Associates	0.00
0.00	Revolving Advance	0.00
0.00	Scientific Workshops - Symposiums - Seminars [Advance]	0.00
0.00	Telephone [Advance]	0.00
0.00	Trainee Security Deposit	0.00
0.00	Transport maintenance [Advance]	0.00
0.00	Workshop & Conference	0.00
5,03,99,356.00		**12,24,75,096.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2022

Annexure: I Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	DEPOSITS	
1,91,69,454.00	General Deposits And Advances	2,09,85,107.00
9,70,126.00	GDA[Others]	9,70,126.00
2,01,39,580.00		**2,19,55,233.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2022

Annexure: K Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
4,310.00	Advances [Previous Years]	4,310.00
1,14,35,274.00	Chemicals [Advance]	2,14,35,274.00
1,18,83,068.00	Consumables, glassware and Spares [Advance]	1,14,49,940.00
1,00,65,134.00	Diagnostics Collabration With NIMS	96,50,585.00
1,92,678.00	ECCS	1,92,678.00
0.00	GST on Reverse Charge	0.00
6,63,909.00	Health Insurance	6,63,909.00
1,58,200.00	Liveries & Blankets [Advance]	1,58,200.00
26,87,643.00	LTC [Advance]	26,53,205.00
854.00	Magzines [Advance]	854.00
1,54,433.00	Others (I-Remittances)	1,54,333.00
74,69,531.00	Others [Advances]	0.00
17,453.00	Others [Contingencies Advance]	17,453.00
1,63,800.00	Printing & Stationery [Advance]	1,63,800.00
3,04,569.00	Rent [Advance]	3,04,569.00
4,37,58,727.00	Research Fellows-Associates	4,37,58,727.00
1,00,482.00	Revolving Advance	1,00,482.00
8,000.00	Scientific Workshops - Symposiums - Seminars [Advance]	8,000.00
3,75,400.00	Software [Advance]	3,75,400.00
34,913.00	TA Abroad [Advance]	84,913.00
50,000.00	Telephone [Advance]	50,000.00
25,000.00	Trainee Security Deposit	25,000.00
11,510.00	Transport maintenance [Advance]	11,510.00
2,66,856.00	Workshp & Conference	4,88,985.00
8,98,31,744.00		**9,17,52,127.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

K.PRAHLADA RAO & CO
Chartered Accountants
Kurnool

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2022

Annexure: L Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
2,36,923.00	Advance for Expenses- purchases by Staff	2,23,011.00
1,35,445.00	Computer Advance [Research Fellows]	1,35,445.00
33,195.00	Computer Advance [Staff]	46,528.00
46,678.00	Conveyance Advance	33,178.00
4,52,241.00		**4,38,162.00**

E.V. RAO
I/C - F & A
Centre for DNA Fingerprinting
and Diagnostics
Uppal, HYDERABAD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad

फोटो गैलरी
Photo Gallery

Photo Gallery of some important events held during the period

Vaccination to staff and students against COVID -19 at CDFD

Dr. Lalji Singh Memorial Lecture by Dr. Subbaya Subramanian, Associate Professor, University of Minnesota on “Tumor-intrinsic immune regulation in colorectal cancer” on 05.07.2021

International Yoga Day – Talk by Dr. Dr Akshay Anand, Professor, Neuroscience Research lab, Department of Neurology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarhon 21.06.2021

Independence Day celebrations on 15.08.2021

NGC-CDFD workshop on Hands on workshop on Next Generation Sequencing Data Analysis for Clinical Diagnostics from 21.06.2021 to 25.06.2021

Organ donation awareness programme under “Jeevandhan”, a societal outreach activity along with Gandhi Medical College on 02.09.2021

Photo Gallery of some important events held during the period

Hindi Day Celebrations – A talk by Dr Arun Tiwari, DRDO Scientist (Retd.) and co -author of “Wings of Fire” on 14.09.2021”

Hands on Workshop on “Next Generation Sequencing (NGC) Data Analysis for Clinical Diagnosis” from 25.10.2021 to 29.10.2021

Pledge ceremony on National Unity Day on 01.11.2021

Lecture by Shri U Ramamohan , Commandant, AP Kurnool under Vigilance Awareness Week on 01.11.2021

Photo Gallery of some important events held during the period

Hindi workshop by DBT Officials on 25.11.2021

"Constitution Day lecture" by Dr. P J Sudhakar, Professor and Social Scientist on 26.11.2021

Hands-on training course on Advancements in Forensic DNA Workflow from 15.12.2021 to 17.12.2021

Participation in Vishwa Hindi Diwas Samaroh on 13.01.2022

Foundation Day lecture by Prof. B.J. Rao, Vice - Chancellor, University of Hyderabad on 28.01.2022

Photo Gallery of some important events held during the period


One-day virtual Symposium on Current Trends in Bioscience on the occasion of CDFD Foundation Day celebrations on 27.01.2022

Vishwa Hindi Diwas, Webinar by Dt. Monika Goel on 09.02.2022

An interactive virtual meeting on Molecular Intricacies of Plant Associated Microorganisms (MIPAM-2022) from 17.02.2022 to 20.02.2022

Photo Gallery of some important events held during the period

Participation in Nation-Wide Science Week Festival under Azadi ka Amrit Mahotsav at ICMR-NIN, Hyderabad from 22.02.2022 to 28.02.2022

Participation in Vigyan Sarvatra Pujyate -- Festival of Science & Technology under Azadi ka Amrit Mahotsav: Mega Expo, organized by PSA office, New Delhi from 22.02.2022 to 28.02.2022

International Women's Day celebrations on 08.03.2022

Awareness program on protection of women from sexual harassment (POSH Act, 2013) on 31.03.2022

Photo Gallery of some important events held during the period

Outreach activities under “BRIDGE Program” with DBT STAR college

डीएनए फिंगरप्रिंटिंग एवं निदान केन्द्र

(जैव प्रद्योगिकी विभाग, विज्ञानं एवं प्रद्योगिकी भारत सरकार का स्वायत्त संस्थान)
कार्यालय ब्लॉक इनर रिंग रोड, उप्पल, हैदराबाद - 500039, तेलंगाना, भारत
दूरभाष: +91 40 2721 6000 / 6011 / 6012 **फैक्स :** +91 40 2721 6006 **वेबसाइट :** www.cdfd.org.in

Centre for DNA Fingerprinting and Diagnostics

(An autonomous institute of the Dept. of Biotechnology, Ministry of Science and Technology, Govt. of India)
Office Block: Inner Ring Road, Uppal, Hyderabad - 500 039, Telanganga, India.
Tel: +91 40 2721 6000 / 6011 / 6012 **Fax:** +91 40 2721 6006, **Website:** www.cdfd.org.in