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DBT-CDFD

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डीबीटी- डीएनए फिंगरप्रिंटिंग एवं निदान केन्द्र
उप्पल, हैदराबाद - 500 039

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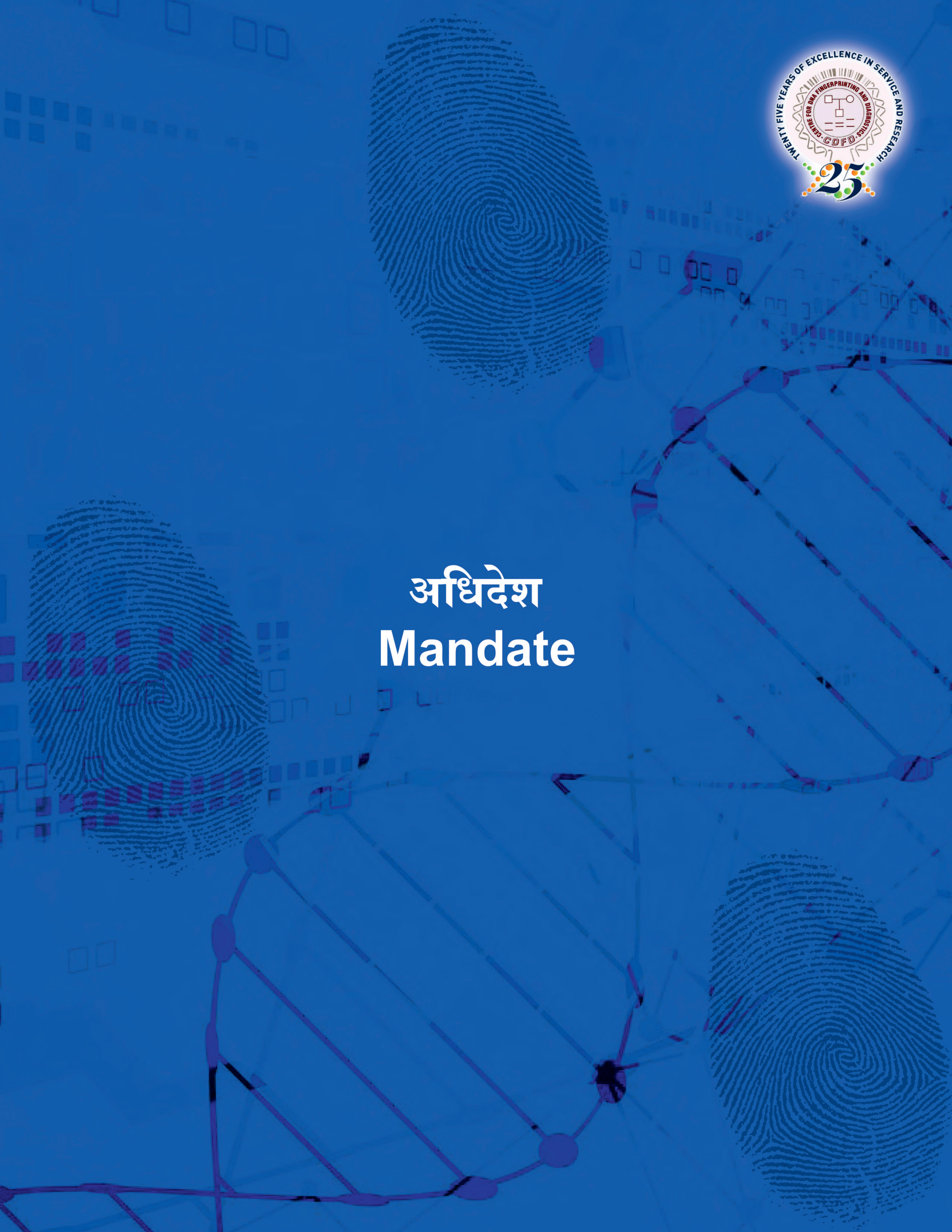


अधिदेश
Mandate

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।

XII. 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।

XIII. 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।

XIV. 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।

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

XVI. 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।

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

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

XIX. 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;

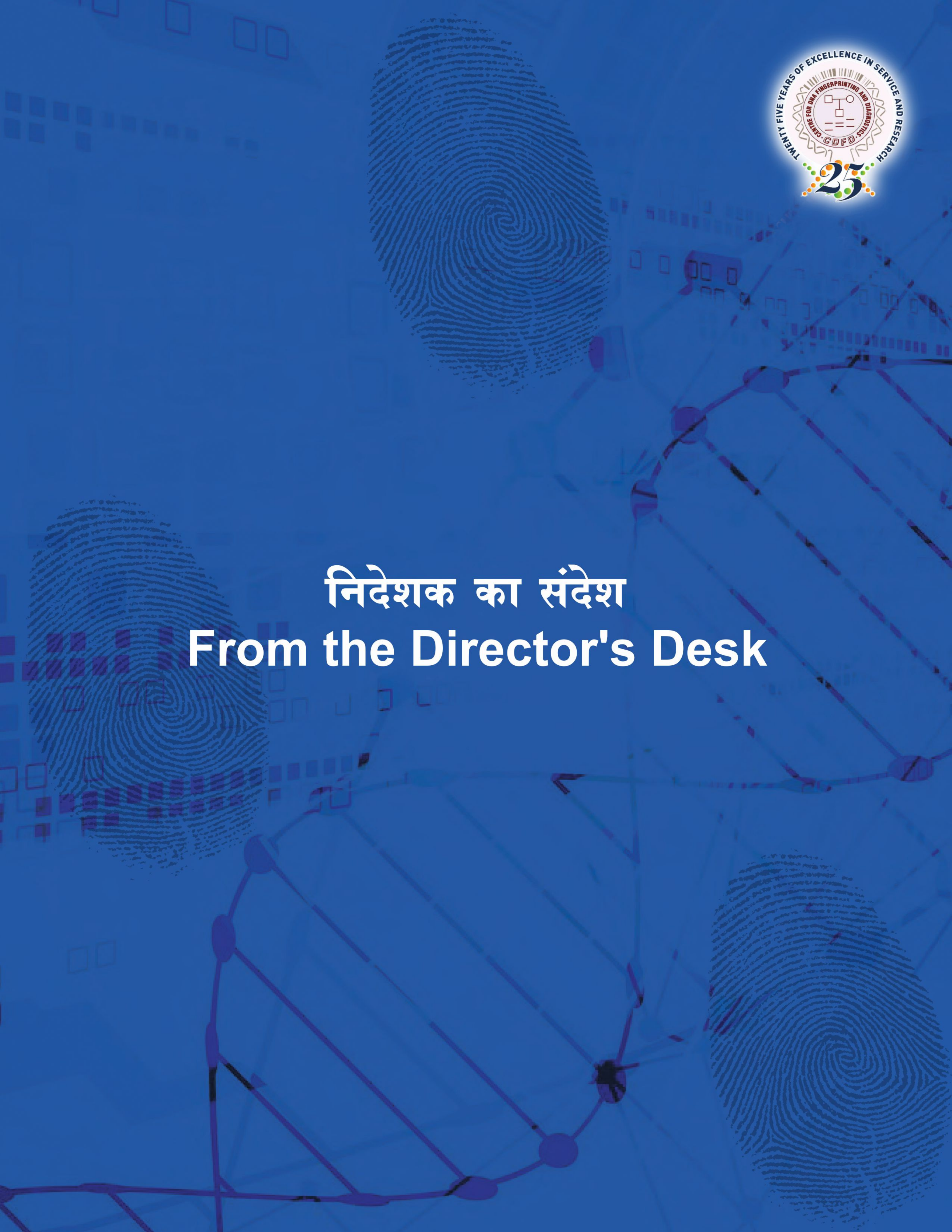
XVI. To do all such other lawful acts as may be necessary, incidental or conducive to the attainment of all or any of the above objectives;

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 knowhow in the interest of the Centre.



निदेशक का संदेश
From the Director's Desk

निदेशक का संदेश

मुझे वर्ष 2020-21 के लिए सेंटर फॉर डीएनए फ़िंगरप्रिंटिंग एंड डायग्नोस्टिक्स (सीडीएफडी) की वार्षिक रिपोर्ट प्रस्तुत करते हुए मुझे बहुत खुशी हो रही है। सीडीएफडी भारत सरकार के जैव प्रौद्योगिकी विभाग (डीबीटी) का एक स्वायत्त संस्थान है, और डीएनए फिंगरप्रिंटिंग और आनुवंशिक निदान सेवाएं प्रदान करने और इसे आधुनिक जीव विज्ञान के क्षेत्रों में मौलिक बुनियादी अनुसंधान करने के लिए एक अद्‌वितीय जनादेश हासिल है। इस वार्षिक रिपोर्ट में, हम वर्ष २०२०-२१ के दौरान सीडीएफडी द्‌वारा की गई कुछ प्रमुख गतिविधियों, सामाजिक योगदान और वैज्ञानिक उपलब्धियों को प्रस्तुत कर रहे हैं।

सीडीएफडी के अस्तित्व के 25 साल सफलतापूर्वक पूरे हो गए हैं और विज्ञान और समाज में महत्वपूर्ण योगदान दिया गया है। इस अवसर को चिह्नित करने के लिए हमने डीबीटी की सचिव, डॉ. रेणु स्वरूप की पावन उपस्थिति में 25वें स्थापना दिवस व्याख्यान के रूप में इंस्टीट्यूट ऑफ़ न्यूरोलजी यूसीएल यूके के निदेशक, प्रोफेसर मिशेल हन्ना द्‌वारा आमंत्रित वार्ता के साथ एक वर्ष तक चलने वाले रजत जयंती समारोह की शुरुआत की।

माननीय उपराष्ट्रपति, श्री एम वेंकैया नायडू ने 20 फरवरी २०२१ को "बाल चिकित्सा दुर्लभ रोग प्रयोगशाला" का उद्‌घाटन करने के लिए दौरा किया। उन्होंने जीनोम आधारित सार्वजनिक स्वास्थ्य अनुसंधान को बढ़ावा देने में डीबीटी की भूमिका की सराहना की। इस अवसर पर तेलंगाना के माननीय गृह मंत्री मोहम्मद महमूद अली भी उपस्थित थे।

यहां 17 अप्रैल 2020 को प्रति दिन कोविड के 450 नमूनों की अधिकतम परीक्षण क्षमता के साथ अत्याधुनिक प्रयोगशाला की स्थापना की गई है। हमने रिपोर्टिंग अवधि के दौरान कोरोना के लगभग ४४,००० संदिग्ध रोगियों के लिए आरटी-पीसीआर आधारित परीक्षण सेवाएं प्रदान की हैं। धनात्मक नमूनों की पहचान से राज्य सरकार को संपर्क ट्रेसिंग और रोकथाम उपायों में मदद मिली है।

हम सक्रिय रूप से कोविड-19 जीनोमिक्स अनुसंधान में भी कार्य कर रहे हैं और मार्च से जुलाई, 2020 की शुरुआत के दौरान देखे गए सार्स-कोव-2 जीनोमिक विकास की गतिशीलता पर तेलंगाना राज्य से पहला व्यापक अध्ययन किया। सार्स-कोव-2 के 200 से अधिक आरएनए नमूनों का संपूर्ण जीनोम अनुक्रम अद्‌वितीय उत्परिवर्तन की पहचान करने के व्यापक उद्देश्य के साथ निर्धारित किया गया था। सीडीएफडी भारतीय सार्स-कोव-2 जीनोमिक्स कंसोर्शियम (आईएनएसएसीओजी) का एक हिस्सा है, जो सार्स-कोव-2 में जीनोमिक विविधताओं की निगरानी के लिए 28 राष्ट्रीय प्रयोगशालाओं का एक संघ है। हमें यह घोषणा करते हुए खुशी हो रही है कि हमने अपने सभी कर्मचारियों और छात्रों को कोविड-19 के खिलाफ सफलतापूर्वक टीका लगाया है।

पिछले वर्ष में सीडीएफडी ने ५७ मामलों के लिए मानव डीएनए प्रोफाइलिंग सेवाएं प्रदान कीं, जो केंद्र और विभिन्न राज्य सरकारों की न्यायपालिका और कानून लागू करने वाली एजेंसियों द्‌वारा अग्रेषित की गईं। एपीडा-सीडीएफडी केंद्र ने हमारे आंतरिक एसएसआर मार्कर पैनल का उपयोग करके शुद्धता के लिए कुल ८८१ बासमती नमूनों का परीक्षण किया है। चावल की १७ किस्मों, ३ चावल संकरों और २४ भिंडी संकरों की डीएनए फिंगरप्रिंटिंग भी की गई है। चावल की 17 किस्मों, 3 चावल संकरों और 24 भिंडी संकरों का डीएनए फिंगर प्रिंटिंग कार्य भी किया गया है।

नैदानिक प्रभाग द्‌वारा विभिन्न आनुवंशिक रोगों हेतु 1835 रोगियों को आनुवंशिक मूल्यांकन प्रदान किया गया। निज़ाम के आयुर्विज्ञान संस्थान, हैदराबाद में स्थापित चिकित्सा आनुवंशिकी विभाग आनुवंशिक सेवाएं प्रदान करने के लिए सफलतापूर्वक कार्य कर रहा है और चिकित्सा आनुवंशिकी में एक डीएनबी प्रशिक्षण कार्यक्रम सफलतापूर्वक जारी है। चिकित्सा आनुवंशिकी विभाग, एनआईएमएस, हैदराबाद में इस वर्ष आनुवंशिक परामर्श में 2 वर्षीय एमएससी प्रशिक्षण कार्यक्रम शुरू किया गया है। डीबीटी प्रायोजित "आनुवंशिक विकार के प्रबंधन और उपचार के अनोखे तरीके" (उम्मीद) प्रोजेक्ट में 'ट्रेनिंग ऑफ क्लिनिशियन' प्रोग्राम के

तहत जेनेटिक डायग्नोस्टिक्स में छह माह की अध्येतावृत्ति शुरू की गई है। इसके अलावा, सीडीएफडी ने यादगीर जिला अस्पताल, कर्नाटक में एक डीबीटी निदान केंद्र की स्थापना की है और आकांक्षात्मक जिला रोग जांच गतिविधियों हेतु रायचूर, कर्नाटक में एक और निदान केंद्र स्थापित करने की प्रक्रिया में है।

हमने चार वैज्ञानिकों की भर्ती की है, जिनमें से एक 3डी जीनोम और क्रोमैटिन वास्तुकला के क्षेत्र में, एक माइटोकॉन्ड्रियल जीनोमिक्स और काय चिकित्सा के क्षेत्र में, एक संक्रामक रोग के क्षेत्र में, और एक कम्प्यूटेशनल बायोलॉजी और जैव सूचना विज्ञान सेवाओं के क्षेत्र में हैं।

बैक्टीरियल जेनेटिक्स की प्रयोगशाला एक पोटेशियम *(K+)* एफ्लक्स प्रोटीन *YcgO* और एक *K+* अपटेक सिस्टम *Trk* पर डीफॉस्फो-पीटीएसएन द्वारा लगाए गए विचलन नियामक प्रभावों के पीछे तंत्र को चित्रित करने की कोशिश कर रही है। प्राप्त साक्ष्य संकेत करते हैं कि डीफॉस्फो-पीटीएसएन, Y c g O के साइटोप्लाज्मिक सी-टर्मिनल क्षेत्र (सीटीआर) और YcgO गतिविधि को लक्षित कर सकता है और YcgO कार्य के लिए सीटीआर आवश्यक है। उन्होंने साक्ष्य प्रस्तुत किया है कि प्रमुख (पी) पीपीजीपीपी हाइड्रोलेस स्पॉट की अनुपस्थिति में भी, इस अणु का धीमा टर्न-ओवर होता है और इस तरह पीपीजीपीपी की न्यूनतम मात्रा (जीटीपी के अनुपात के रूप में) की पहचान की जाती है जो विकास अवरोध प्रदान करती है।

कोशिका चक्र नियमन की प्रयोगशाला में ट्रांसक्रिप्शनल और सेंट्रोसोम आमापन करने के लिए विडेमैन-स्टीनर सिंड्रोम के रोगियों से प्राप्त लिम्फोब्लास्टोइड कोशिकाओं का निर्माण किया है और यह भी दिखाया है कि एमएलएल और एसईटीडी *1* ए सेंट्रोमियर लोकस से जुड़ते हैं तथा इन प्रोटीनों का नुकसान सेंट्रोमियर से ट्रांसक्रिप्शन को प्रभावित करता है।

कोशिका मृत्यु और कोशिका उत्तरजीविता प्रयोगशाला, सभी मानव फॉस्फेटेस के लिए अंत:क्रियात्मक डेटा का उपयोग करते हुए, एक एफिनिटी आधारित प्रोटीन शुद्धिकरण के माध्यम से अंत:क्रिया प्रोटियोमिक्स दृष्टिकोण के साथ उत्पन्न, विभिन्न फॉस्फेटेस के लिए नए कार्यों को प्रयोगशाला में सौंपा गया है। ध्यान दें, उन्होंने कोशिकाओं में एक गैर-रिसेप्टर टाइरोसिन फॉस्फेट (एसएचपी-१) हेतु एक नए परमाणु भूमिका की पहचान की।

कोशिका संकेतन प्रयोगशाला में प्रदर्शित किया गया है कि *IP6K1* द्वारा *5-IP7* संश्लेषण स्तनधारी कोशिकाओं में सजातीय पुनर्संयोजन मध्यस्थता डीएनए सुधार के पूरा होने का समर्थन करता है। उन्होंने यह भी दिखाया कि *IP6K1*, प्रसंस्करण निकायों के रखरखाव के लिए आवश्यक है, जो एमआएनए भंडारण में शामिल साइटो प्लाज्मिक राइबो न्यूक्लियो प्रोटीन ग्रैन्यूल हैं।

कम्प्यूटेशनल और कार्यात्मक जीनोमिक्स प्रयोगशाला में एक Msmeg ट्रांसक्रिप्शन रेगुलेटर-MSMEG_2386 की भूमिका का अध्ययन किया गया है, जो तुलनात्मक ट्रांसक्रिप्टोमिक दृष्टिकोण का उपयोग करते हुए एम. ट्यूबरकुलोसिस डॉसआर रेगुलॉन जीन के कुछ होमोलॉग्स को वृद्धि में रुकावट और रेगुलेट करने जैसी डॉर्मेंसी को प्रेरित करता है। उन्होंने मेफ्लोक्वीन-बंधन ह्यूमन और प्लाज्मोडियम फाल्सीपेरम एसिल-सीओए-बंधनकारी प्रोटीन की विभेदक स्थिरता के आण्विक आधार की जांच की है।

ड्रोसोफिला तंत्रिका विकास की प्रयोगशाला में सीएनएस के एब्डोमिनल क्षेत्र में हॉक्स-मध्यस्थता एनएससी एपॉप्टॉसिस के आण्विक आधार की जांच की गई। उन्होंने इस एपॉप्टॉसिस में 717bp बढ़ाने वाले के महत्व को स्थापित किया।

कवक रोगजनन प्रयोगशाला एक अवसरवादी मानव कवक रोगजनक कैंडिडा ग्लेब्रेटा के रोगविज्ञान को समझने की दिशा में कार्य कर रही है। आईपी-एमएस विश्लेषण के माध्यम से, उन्होंने कैंडिडा ग्लेब्रेटा में सीजीवाईपीएस1 एस्पार्टिल प्रोटीज के 19 प्रोटीन अंत:क्रिया की पहचान की।

मानव और चिकित्सा आनुवंशिकी की प्रयोगशाला में अनुसंधान गुणसूत्र और एकल जीन विकारों के लिए नए उत्परिवर्तन / जीन पहचान पर केंद्रित है। हमने दुर्लभ पुनरावर्ती मेंडेलियन विकारों वाले परिवारों और क्रोमोसोमल पुनर्व्यवस्था वाले रोगियों में संपूर्ण एक्सोम और संपूर्ण जीनोम अनुक्रमण विश्लेषण के विश्लेषण हेतु आंतरिक डेटा विश्लेषण पाइप लाइनों में विकसित और उपयोग किया है। उन्होंने नए रोग के कारण उत्परिवर्तन और तंत्र के साथ-साथ दुर्लभ फिनोटाइप के लिए संभावित नए जीन की ओर नए नेतृत्व का प्रकट किया।

क्रोमैटिन जीवविज्ञान और एपि जेनेटिक्स प्रयोगशाला में विखंडन यीस्ट एस पॉम्बे के एचएसटी 4 के सिर्टुइन के सी-टर्मिनस में पहले से खोजे गए फोफोडेग्रोन में मौजूद सेरीन को उत्परिवर्तित किया गया है और दिखाया गया है कि ये द्विगुणन तनाव के तहत

अप्रभावित कोशिका चक्र में यीस्ट के अस्तित्व के लिए भी महत्वपूर्ण हैं।

प्रतिरक्षा विज्ञान प्रयोगशाला साक्ष्य प्रदान करने की दिशा में काम कर रही है जो सुझाव देती है कि एजीई का ऊंचा स्तर न्यूरो डीजेनेरेशन, मोटापा, एपॉप्टॉसिस आदि को कई तरह से बढ़ाता है और एजीई-मध्यस्थता संकेतन के नियमन से इन बीमारियों को दूर करना चाहिए जिन्हें जीवे में और अधिक मान्य करने की आवश्यकता है।

आण्विक कोशिका जीव विज्ञान प्रयोगशाला से अध्ययन हमारे अध्ययनों से संकेत मिलता है कि पीपीई 2 प्रोटीन मेजबान की जन्मजात प्रतिरक्षा प्रतिक्रिया को दबाता है और अस्थि मज्जा हिमेटोपोइजिस को प्रभावित करने के साथ-साथ मुक्त कणों (पहले के अध्ययन) को प्रभावित करके बैक्टीरिया की उत्तरजीविता का सहायता प्रदान करता है।

आण्विक ऑन्कोलॉजी प्रयोगशाला में ईओएसआरसी में जीन फ्यूज़न के एक संग्रह की पहचान की गई है और उसकी विशेषता बताई है। एचएनएससीसी और ईएससीसी के लिए संगतता के साथ भारत विशिष्ट टीपी53 उत्परिवर्ती के नए ऑन्कोजेनिक ट्रांसक्रिप्शनल लक्ष्यों की पहचान की गई थी। उन्होंने तेलंगाना और अन्य भारतीय राज्यों में कोविड-19 नमूनों से सार्स-कोव-2 उत्परिवर्तन परिदृश्य की भी पहचान की।

पादप-सूक्ष्म जीव अंत:क्रिया की प्रयोगशाला में दिखाया गया है कि ज़ैंथोमोन सोरीज़ा से एक बैक्टीरियो फाइटोक्रोम (XooBphP), प्रकाश संकेत को पहचानता है और अपनी ईएएल-मध्यस्थता वाले फॉस्फो डिएस्टरेज़ गतिविधि के माध्यम से ट्रांसड्यूस करता है, जो सर्वव्यापी बैक्टीरिया के दूसरे संदेशवाहक चक्रीय-डी-जीएमपी के अंत: कोशिकीय स्तर को संशोधित करता है। यह विविध कोशिकीय प्रक्रिया के समन्वय के लिए दूसरे संदेशवाहक को संशोधित करके सामाजिक व्यवहार, आयरन चयापचय और विषाणु के बैक्टीरियो फाइटोक्रोम मध्यस्थता विनियमन की पहली रिपोर्ट है।

ट्रांसक्रिप्शन प्रयोगशाला में आरएचओ अवरोधक पेप्टाइड्स-आरएचओ अंत:क्रिया के लाक्षणीकरण को पूरा किया गया है, प्रोफेज टॉक्सिन-एंटी टॉक्सिन अभिव्यक्तियों में आरएचओ-निर्भर समाप्ति की भूमिका स्थापित की है, जीवे आरएचओ-उपयोग साइटों के व्यवहार को चित्रित करने और आरएचओ-ईसी कार्यात्मक अंत:क्रिया सतहों के लाक्षणीकरण में महत्वपूर्ण प्रगति की है।
पिछले एक वर्ष में सीडीएफडी ने हाइ प्रोफाइल पीयर रिव्यूड इंटरनेशनल जर्नल्स में 56 अनुसंधान पत्र प्रकाशित किए हैं। हमारे संकायों को इस वर्ष कई पुरस्कार और सम्मान प्राप्त हुए, जिनमें शांति स्वरूप भटनागर पुरस्कार, राष्ट्रीय अकादमियों की अध्येतावृत्ति और प्रतिष्ठित पत्रिकाओं में संपादकीय शामिल हैं।

अंत में, मैं अपने सहयोगियों की ओर से इस अवसर का लाभ उठाता हूं और जैव प्रौद्योगिकी विभाग, सीडीएफडी संस्था, शासी परिषद, अनुसंधान क्षेत्र पैनल्स-वैज्ञानिक सलाहकार समिति, प्रबंधन समिति, और वित्त समिति के विशिष्ट सदस्यों को उनके प्रोत्साहन, सलाह और निरंतर समर्थन के लिए धन्यवाद देते हैं जो हमारी बहुत सी उपलब्धियां संभव नहीं होती।

के थंगराज
31 **मार्च**, 2021

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 2019-20. CDFD is an autonomous institute of the Department of Biotechnology (DBT), Government of India, and has a unique mandate to provide DNA fingerprinting and genetic diagnostic services, and to perform fundamental basic research in areas of modern biology. In this annual report, we are presenting some of the key activities, societal contributions and scientific achievements made by CDFD during the year 2020-21.

CDFD has successfully completed 25 years of its existence and contributed significantly to science and society. To mark this occasion, we initiated a yearlong Silver Jubilee Celebration with an invited talk by Prof. Michel Hanna, Director, Institute of Neurology, UCL, London as 25th Foundation Day Lecture in the august presence of Dr. Renu Swarup, Secretary, DBT.

Hon'ble Vice President, Shri M Venkaiah Naidu visited CDFD on 20th February, 2021 and inaugurated "Pediatric Rare Disease Laboratory". He appreciated the role of DBT in promoting genome-based public health research. Hon'ble Home Minister of Telangana, Mohd Mehmood Ali was also present on the occasion.

A state of Art COVID testing laboratory was established on 17th April 2020 with a maximum testing capacity of 450 samples per day. We have provided RT-PCR based testing services for about 44,000 suspected patients for corona during the reporting period. Identification of positive samples has helped the State Government in contact tracing and containment measures.

We also actively engaged in COVID-19 Genomics Research and performed the first comprehensive study from the state of Telangana on the dynamics of SARS-CoV-2 genome evolution. The complete genome sequence of >200 SARS-CoV-2 samples was determined with the overarching objective of identifying unique mutations. CDFD is a part of Indian SARS-CoV-2 Genomics (INSACOG) Consortium, a consortium of 28 National Laboratories to monitor the genomic variations in the SARS-CoV-2. We are happy to announce that we have successfully vaccinated all our staff and students against COVID-19.

In the past year, CDFD provided *Human DNA profiling services* for 57 cases, forwarded by the judiciary and law enforcing agencies of the Union and different State Governments. The *APEDA-CDFD* Centre has tested a total of 881 Basmati samples for purity using our in-house SSR marker panel. DNA fingerprinting of 17 rice varieties, 3 rice hybrids and 24 okra hybrids have also been carried out.

The *Diagnostics division* provided genetic testing to 1835 patients for various genetic disorder. 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. A 2 year MSc training programme in Genetic counselling has been initiated at Department of Medical Genetics, NIMS, Hyderabad. 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. In addition, CDFD has established a DBT Nidan Kendra at Yadgir District hospital, Karnataka and we are in the process of establishing another Nidan Kendra at Raichur, Karnataka for aspirational district disease screening activities.

We have recruited four scientists, one in the fields of 3D genomes and chromatin architecture, one in the area of Mitochondrial Genomics and Medicine, one in the area of infectious disease, and one in the area of Computational Biology and Bioinformatics Services.

Laboratory of Bacterial Genetics is trying to delineate the

mechanism behind the divergent regulatory effects exerted by dephospho-PtsN on a potassium (K+) efflux protein YcgO and a K+ uptake system Trk. The evidence obtained indicates that dephospho-PtsN may target the cytoplasmic C-terminal region (CTR) of YcgO and fetter YcgO activity and that the CTR is required for YcgO function. They have presented evidence that even in the absence of the major (p)ppGpp hydrolase SpoT, there is a slow turn-over of this molecule and thereby identified the minimum amount of ppGpp (as a ratio of GTP) the confers growth inhibition.

Laboratory of Cell Cycle Regulation has generated lymphoblastoid cells from patients of Wiedemann-Steiner Syndrome to carry out transcriptional and centrosome assays and also showed that MLL and SETD1A binds to centromere locus and loss of these proteins affect the transcription from the centromere.

Laboratory of Cell Death & Cell Survival, 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 note, they identified a novel nuclear role for a non-receptor tyrosine phosphatase (SHP-1) in cells.

Laboratory of Cell Signalling has demonstrated that 5-IP7 synthesis by IP6K1 supports the completion of homologous recombination mediated DNA repair in mammalian cells. They also showed that IP6K1, is essential for the maintenance of processing bodies, which are cytoplasmic ribonucleoprotein granules involved in mRNA storage.

Laboratory of Computational and Functional Genomics has studied the role of a Msmeg transcription regulator-MSMEG_2386 in inducing dormancy like growth arrest and regulating some of the homologs of *M. tuberculosis dosR* regulon genes using comparative transcriptomic approach. They have examined the molecular basis of differential stabilities of mefloquine-bound human and *Plasmodium falciparum* acyl-CoA-binding proteins

Laboratory of Drosophila Neural Development investigated the molecular basis of Hox-mediated NSC apoptosis in the abdominal region of the CNS. They established the importance of a 717bp enhancer in this apoptosis.

Laboratory of Fungal Pathogenesis is working towards understanding the pathobiology of an opportunistic human fungal pathogen *Candida glabrata.* Through IP-MS analysis, they identified 19 protein interactors of the CgYps1 aspartyl protease in *Candida glabrata*.

The research in *Laboratory of Human and Medical Genetics* focusses on novel mutation/gene identification for chromosomal and single gene disorders. We have developed and used in house data analysis pipelines for analysis of whole exome and whole genome sequencing analysis in families with rare recessive mendelian disorders and patients with chromosomal rearr-angements. They revealed novel disease causing mutations and mechanisms as well as new leads towards putative novel genes for rare phenotypes.

Laboratory of Chromatin Biology & Epigenetics has mutated the serines present in the earlier discovered phophodegron in the C-terminus of sirtuin of Hst4 of fission yeast S. pombe, and shown these are critical for the survival of the yeast in unperturbed cell cycle as well as under replication stress.

Laboratory of Immunology is working towards providing evidences which suggest the elevated level of AGE increases neurodegeneration, obesity, apoptosis, etc. in multiple ways and regulation of AGE-mediated signalling should ameliorate these ailments which needs to be further validated in vivo.

The studies from *Laboratory of Molecular Cell Biology* Our studies indicate that PPE2 protein suppresses innate immune response of host and favour bacterial survival by affecting bone marrow hematopoiesis as well as inhibiting free radicals (earlier studies).

Laboratory of Molecular Oncology has identified and characterized a compendium of gene fusions in EOSRC. Novel oncogenic transcriptional targets of India specific *TP53* mutations were identified with relevance for HNSCC and ESCC. They also identified SARS-CoV-2 mutation landscape from COVID-19 samples in Telangana and other Indian states

Laboratory of Plant-Microbe Interactions have shown that a bacteriophytochrome (*Xoo*BphP) from *Xanthomonas oryzae*, perceives light signal and transduce through its EAL-mediated phosphodiesterase activity, modulating the intracellular level of the ubiquitous bacterial second

messenger cyclic-di-GMP. 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.

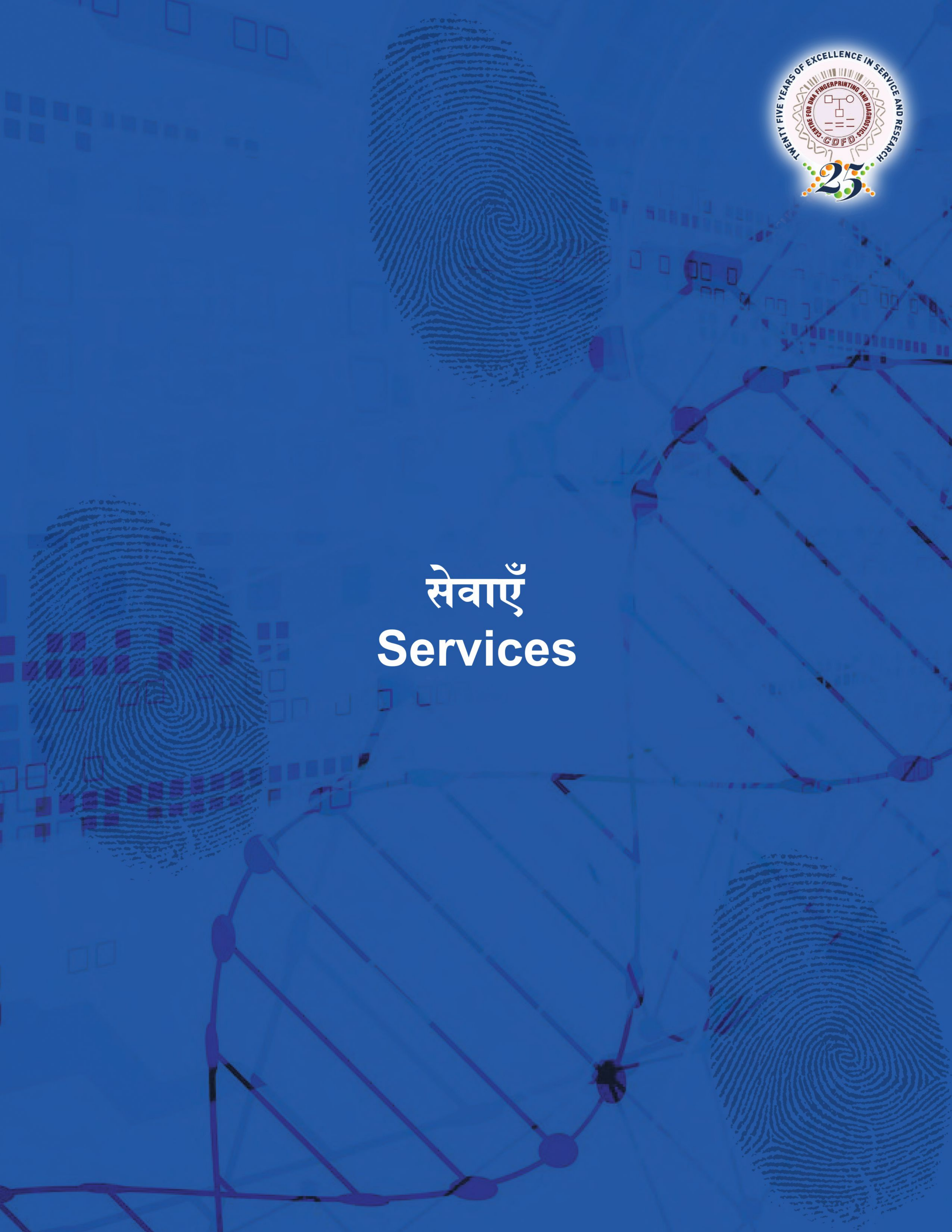
Laboratory of Transcription has completed the characterizations of Rho inhibitor peptides-Rho interactions, established the role of Rho-dependent termination in prophage toxin-antitoxin expressions, made significant progress in delineating the behaviour of Rho-utilization sites *in vivo* and characterization of the Rho-EC functional interaction surfaces.

In the past year CDFD has published 56 research papers in high profile peer reviewed international journals. Our faculties were recipients of several awards and honours, including Shanti Swarup Bhatnagar prize, fellowship of national academies and editorship in prestigious journals. A total of 6 students were awarded PhD Degrees during this period.

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

K Thangaraj

March 31, 2021



सेवाएँ
Services

DIAGNOSTICS DIVISION

Faculty

Ashwin Dalal	Staff Scientist

Adjunct Faculty

Prajnya Ranganath	Associate Professor, NIMS
Shagun Aggarwal	Associate 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

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 2020-2021

Clinical Genetics

A total of 1835 patient samples were analyzed for genetic testing, during the year 2020-21 (1/4/2020 to 31/3/2021). 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 3970 patients, of which 1814 were new registrations, were examined and counseled in the unit during 2020-21. In addition, antenatal ultrasonograms were done in 287 cases, antenatal invasive procedures (chorionic villus sampling and amniocentesis) in 204 cases, and foetal autopsies were conducted in 139 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; five batches of students (total 8 students) have joined so far and the next batch is due to join in May 2021.

MSc training programme in Genetic counseling

A MSc Genetic Counseling program has been initiated at Medical Genetics department established a 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 joined and are undergoing 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. Four faculty from Government medical colleges have completed training by March 2021. New batch of two faculty is expected to join in July 2021.

Outreach programme for Aspirational Districts

CDFD has established a DBT Nidan Kendra at Yadgir District hospital, Karnataka under a DBT funded proposal call UMMID (Unique methods of management and treatment of inherited disorders". The plan of the 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 Yadgir 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. In addition, CDFD has obtained approval of Department of Biotechnology to establish the second Nidan Kendra at Government Medical College, Raichur, Karnataka for similar activity.

Diagnostics Division

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, 2020 - March 31, 2021)

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 881 samples were analyzed of which 57% of the samples were pure and 43% of the samples were adulterated with non-Basmati rice (Figure 1).

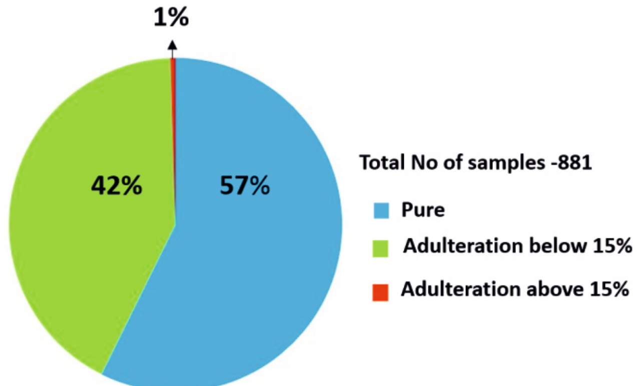


Figure 1. Basmati samples analyzed in the current reporting year.

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

1. Fingerprinting of three rice hybrids from Pan Seeds, Kolkata was carried out with 9 SSR markers.
2. Fingerprinting of 14 rice varieties from Pan Seeds, Kolkata was carried out with 20 SSR markers.
3. Fingerprinting of five rice varieties from Dr. J. R. Diwan, College of Agriculture, Raichur was carried out with 5 SSR markers.
4. Fingerprinting of 24 okra hybrids from Advanta Seeds, UPL Ltd, Hyderabad was performed with 13 SSR markers. Forward primers of all the 13 markers were tagged with 6-carboxyfluorescein (6FAM) and genotyping of all the hybrids was performed using ABI3730 genetic analyzer (Applied Biosystems).

Revenue generated:

An amount of **₹ 1,03,95,800/-** which includes GST (18%) is received towards purity testing of Basmati samples and **₹ 9,54,186/-** (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 **₹ 1,13,49,986/-** 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 varieties which are potential adulterants with 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 has clearly differentiated all the Basmati varieties from non-Basmati varieties except for Pusa Basmati 1121 and Pusa Basmati 1509. Few more SNPs present in *GW7*, *GW8* and GW2 genes governing seed length phenotype are being tested to see if they can separate out these two varieties from non-Basmati varieties.

Publication:

Anupama K*, Pranathi K and Sundaram RM (2020) Assessment of genetic purity of bulked-seed of rice CMS lines using capillary electrophoresis. ***Electrophoresis*** 41:1749-1751.

Plant DNA Fingerprinting Services

LABORATORY OF DNA FINGERPRINTING SERVICES

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

Other Members

SPR Prasad	Senior Technical Officer
Devinder Singh Negi	Technical Officer
Pooja Tripathi	Technical Officer
Vijay Amrutarao Girnar	Technical Officer
Shruti Dasgupta	Technical Assistant

Co-ordinator
Dr D. P. Kasbekar

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.

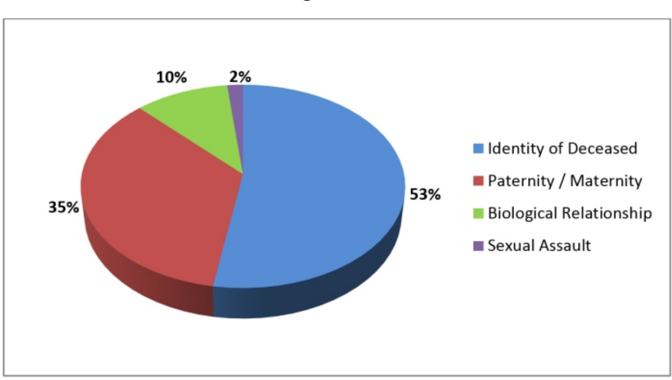
Details of services provided in the current reporting year (1st April 2020 to 31st March 2021):

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	30
Paternity/Maternity	20
Biological Relationship	6
Sexual Assault (Rape)	1
Total number of Cases	57

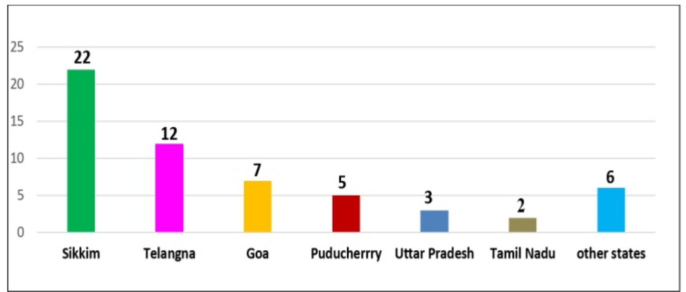
Figure – 1



Types of cases received in percentage

A total of 57 cases were received for DNA fingerprinting examination in the current reporting period. Of these, 30 cases were related to maternity/paternity, 20 cases were related to identity of deceased, 6 cases were related to biological relationship and 1 case was related to sexual assault. 10 States and 2 Union Territories of India have availed DNA fingerprinting services from CDFD during this period. Sikkim State has forwarded the highest number of cases (22) followed by Telangana (12), Goa (7), Puducherry (5), Uttar Pradesh (3), Tamil Nadu (2). Himachal Pradesh, Odisha, Andhra Pradesh, Chhattisgarh, Bihar and Andaman & Nicobar Islands have contributed one case each, and these have been clubbed together as shown in Figure 2. A summary of the state-wise break-up of cases received is shown in Table 2.

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	Maternity/ Paternity	Sexual Assault (Rape)	No. of Cases
Andhra Pradesh	-	-	1	-	1
Andaman & Nicobar	-	1	-	-	1
Bihar	-	-	1	-	1
Chhattisgarh	-	-	1	-	1
Goa	-	4	2	1	7
Himachal Pradesh	-	1	-	-	1
Odisha	-	1	-	-	1
Puducherry	-	2	3	-	5
Sikkim	-	7	15	-	22
Tamil Nadu	-	1	1	-	2
Telangana	6	-	6	-	12
Uttar Pradesh	-	3	-	-	3
Total No. of Cases	6	20	30	1	57

Prominent case

Identification of the body of India Navy pilot killed in MiG-29 air crash into the Arabian sea.

Deposition of evidence in Courts of Law

During this reporting year, the DNA experts defended their reports in 5 cases in various Honorable Courts of Law throughout the country.

Training/Lectures/Workshops: 2020 – 2021

1. Participated in IISF 2020 – Mega Science and Technology Expo, India International Science Festival during 22 -25 December 2020.
2. Participated by virtual mode in Global Bio India 2021 during 1-3 March 2020.
3. A Talk delivered by Director, CDFD, on the topic "DNA Fingerprinting and its use in Medico Legal issues" to the trainee junior civil judges who have visited CDFD as part of their XXIV Basic Course Part – II Mid-Term Practical Training.
4. Hosted a three-member team from Chennai FSL. The purpose of the team's visit was to obtain external validation for the FORENSIC DNA PROFILE SEARCH TOOL developed at the Forensic Sciences Department, Government of Tamil Nadu, using the DNA profiles available at CDFD.
5. Dr Shruti Dasgupta discussed the subject and explained DNA fingerprinting procedures to a student interested in writing an Investigatory project on "A study on DNA Fingerprinting and its applications.

Revenue generated:

During this reporting period, an amount of Rs. 7,92,286/- (Rupees seven lakhs ninety-two thousand two hundred and eighty-six only) has been received towards DNA fingerprinting analysis charges, which is inclusive of GST (18% at present) as levied by the Govt. of India.

Laboratory of DNA Fingerprinting Services

शोध
Research

Laboratory of Bacterial Genetics

RESEARCH

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

Principal Investigator: **Abhijit A. Sardesai**
Staff Scientist

Ph D Students:

Suchitra Upreti	Senior Research Fellow
Swati Dubey	Senior Research Fellow
Neeraj Kumar	Senior Research Fellow
Yogesh Patidar	Senior Research Fellow

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. In this regard the interplay between a three protein phosphorelay comprising the proteins PtsP-PtsO-PtsN and potassium ion (K^+) metabolism is being studied. The second project deals with studies on basic amino acid export with an emphasis on functional studies on amino acid exporters. Regulatory mechanisms concerned with the above are also being studied. The following projects are being pursued;

1. The PtsP-PtsO-PtsN phosphorelay and its interplay with K^+ metabolism.
2. Studies on basic amino acid export.

The PtsP-PtsO-PtsN phosphorelay and its interplay with potassium K^+ ion metabolism:

In earlier studies pertaining to this topic, our genetic and physiological studies have implicated dephospho-PtsN, as an inhibitor and as an activator of the K^+ efflux protein YcgO and the K^+ uptake transporter Trk respectively. PtsN is the terminal phospho-acceptor protein of the PtsP-PtsO-PtsN phosphorelay. Towards delineating the mechanistic basis behind the divergent regulatory effects of dephospho-PtsN on two K^+ transporters namely YcgO and Trk, we have initiated multiple studies. We have noted in our earlier studies that the *ptsN* mutant displays a phenotype of K^+ limited growth (K^L) in a medium with high external K^+ concentration ($[K^+]_e$, 115 mM K^+) but not in a medium with low (1 mM K^+) $[K^+]_e$. The K^L of the *ptsN* mutant is suppressed by the absence of YcgO. We have shown that in the *ptsN* mutant, K^+ efflux mediated by YcgO is activated leading to K^+ limitation in a medium of high $[K^+]_e$, an effect that is attributed to the absence of dephospho-PtsN. We have reported the isolation of dominant mutations in YcgO that are rendered constitutively active ($YcgO^{Con}$), that is they yield a phenotype identical to that of a Δ*ptsN* mutant, but in a wild type strain. Amino acid substitutions in two distinct regions of YcgO namely, the putative TMD (transmembrane domain) and the CTR (C-terminal region) lead to the $YcgO^{Con}$ phenotype. The isolation of $YcgO^{Con}$ mutants supports the notion that that dephospho-PtsN is a negative regulator of YcgO activity. In this year we performed a limited analyses of the topology of YcgO in the membrane which indicated that YcgO adopts an N_{out}-C_{in} configuration in the membrane and that the CTR of YcgO is located in the cytoplasm. This has allowed for a finer localization of $YcgO^{Con}$ substitutions in the two putative domains of YcgO. Our prior studies have implicated a requirement of the CTR for YcgO activity since expression of YcgO but not of its derivative YcgO403Δ bearing a deletion of the CTR (from position 403 to 578), leads to the K^L in the Δ*ptsN* mutant. Additional studies have shown that the two $YcgO^{Con}$ mutations in the TMD represent versions of YcgO, that do not require the CTR for activation. Overall these studies are compatible with the notion that dephospho-PtsN may target the CTR of YcgO to fetter its activity and that in its absence the CTR activates YcgO mediated K^+ efflux.

With regard to the stimulatory effect of dephospho-PtsN on the Trk transporter, we have isolated mutations in TrkA, the protein that gates the K^+ channels TrkH and TrkG, that alleviate the attenuated Trk activity in absence of PtsN. We also noted that the same substitutions also alleviated impaired Trk activity that occurs in the absence of SapD and SapF, two proteins that are known to present ATP to the Trk ring. Additional studies in this regard support the notion that dephospho-PtsN may exert its stimulatory effects on the Trk transporter at the step of ATP presentation by SapD/F to the TrkA gating ring. Future studies in this regard are directed towards obtaining *in vitro* correlates of our genetic studies.

Project 2: Studies on basic amino acid export:

Earlier we had reported work directed towards obtaining structure function relationships of of the L-lysine (Lys) exporter LysO in *E. coli*. In this regard we have investigated functional aspects of LysO in terms of obtaining its membrane topology. Additionally, other aspects of LysO function were reported, namely, identification of functionally important amino acid residues, its monomeric state *in vivo* and obtaining insights into its export mechanism.

During the course of our studies on the topology of LysO involving *in situ* scanning Cys accessibility, we noted that some Cys substitutions in LysO spanning the amino acids 182 to 266 (182-266 region), yielded anomalous accessibility. Thus assigning a cytoplasmic, intramembrane or periplasmic location to them was not straightforward. We have performed detailed studies of LysO topology in this region. Our analyses indicate that this region constitutes an intramembrane solvent exposed region comprising the TMS7 (transmembrane segment 7) linked to an intramembrane segment that is linked in turn to TMS8 from the cytoplasmic side. A pair of conserved acidic residues essential for LysO function, are located in this region The intramembrane segment does not appear to form a conventional TMS. Over all the topology of LysO is consistent with a model in which both its N and C-termini face the periplasm, with a TM domain comprising 8 TMSs and the model conforms to the positive inside rule. Future studies in this regard will involve determining how the various TMSs of LysO coalesce in the membrane.

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

Laboratory of Bacterial Genetics

RESEARCH

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

Principal Investigator: R. Harinarayanan
Staff Scientist

Lab Members:
Rajeshree Sanyal SRF
Vani Singh SRF
Vimala Allada Project Associate
Shaffiqu Technical Officer – I

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 reporting period are,

1. To study regulations in the metabolic turnover of global regulator and modified nucleotide (p)ppGpp.
2. To understand the interplay between nucleotide metabolism and stringent response.

Compensation of SpoT Requirement in (p)ppGpp turnover by Over-Expression of Nudix hydrolases MutT or NudG

Previously, we reported that the growth of Δ*rlmD* Δ*spoT* strain was dependent on GppA function (Sanyal and Harinarayanan, 2020). In work carried out in the current reporting period, using a multi-copy plasmid library of *E. coli* genes, we identified and sequenced plasmid clones that suppressed the growth defect of the Δ*rlmD* Δ*spoT* Δ*gppA* strain. This led to the identification of *nudG* and *mutT* genes as multi-copy suppressors of the growth defect and suggested, the nudix hydrolases may be capable of degrading (p)ppGpp. We qualitatively compared the (p)ppGpp hydrolase proficiency of the two nudix hydrolases to that of SpoT, by comparing their ability to alleviate stringent response to amino acid starvation following over-expression from plasmid.

While a wild type like stringent response was observed in the presence of plasmid vector, (p)ppGpp accumulation was almost completely abolished after SpoT expression (Figure 1A). This indicated the (p)ppGpp synthesized could be completely hydrolyzed due to net increase in hydrolase activity following over-expression of SpoT. Stringent response to isoleucine starvation was alleviated during the over-expression of nudix hydrolases NudG or MutT as well (Figures 1B, C). However, unlike SpoT over-expression, some residual (p)ppGpp accumulation was observed. Although this seems to suggest (p)ppGpp is less efficiently hydrolyzed by the nudix hydrolases than by SpoT, since the expression level of the proteins have not been determined, definitive conclusions cannot be drawn.

Since *mutT* or *nudG* over-expression was able to hydrolyze (p)ppGpp like SpoT, we asked, if the absence of pppGpp pool associated with reduced SpoT hydrolase activity could be rescued by the expression of the nudix hydrolases. In the *spoT1* strain carrying the plasmid vector pCA24N, when stringent response was induced by isoleucine starvation, as expected, there was accumulation of ppGpp but not pppGpp (Figure 2A, lanes 1–4). In the *spoT1* strain carrying either pCAnudG or pCAmutT and grown in the presence of IPTG to induce expression of the nudix hydrolases, isoleucine starvation resulted in the accumulation of ppGpp and pppGpp (Figure 2A, lanes 5–12). As compared to the *spoT1*/pCA24N strain, the concentration of ppGpp relative to GTP decreased in the strains induced for nudG or mutT expression, and this would be expected due to enhanced hydrolysis of ppGpp. The decrease in ppGpp was more

pronounced with MutT over-expression than with NudG. This may be attributed to differences in the expression/activity of the two proteins under our experimental conditions or possibly more efficient hydrolysis of ppGpp by MutT than by NudG. Given that pppGpp was not detected during stringent response in the spoT1/pCA24N strain, accumulation of this nucleotide in the *spoT1* strain over-expressing a nudix hydrolase indicated a positive correlation between pppGpp level and cellular (p)ppGpp hydrolase activity.

Since *nudG* or *mutT* over-expression rescued the growth defect of Δ*spoT* strain, we studied the stringent response in Δ*spoT*/pCAnudG and ΔspoT/pCAmutT strains and compared it to that seen in the ΔspoT/P_{lac}-spoT+/pCA24N strain after *spoT* expression was reduced by IPTG withdrawal. As compared to the vector, over-expression of NudG or MutT lowered the ppGpp pool relative to GTP following amino acid starvation (Figure 2B). This effect of NudG or MutT over-expression was similar to that observed in the wild type or *spoT1* background (Figures 1B, 1C, 2A) and can be expected from the constitutive degradation of ppGpp to GTP. On the other hand, there was an increase in pppGpp level relative to ppGpp following the expression of nudix hydrolases, once again revealing a positive correlation between pppGpp level and (p)ppGpp hydrolase activity. Since SpoT is also a (p)ppGpp synthase, it may be argued that, reducing the spoT expression in Δ*spoT*/Plac-spoT+ strain lowered the pppGpp pool due to reduction in the synthase activity. The recovery of pppGpp pool following the expression of nudix hydrolase would rule out this possibility.

These results suggest, the unexpected decline in pppGpp pool in strains deficient for SpoT hydrolase activity is the consequence of decrease in (p)ppGpp hydrolase activity and not the presence of pppGpp hydrolase such as GppA. Further studies will be needed to address the molecular basis of this phenotype.

Studies to delineate the interplay between nucleotide metabolism and stringent response by using the *gsk3* allele

In *Escherichia coli*, there are two pathways for generating guanosine – *de novo* synthesis or salvage pathway. The major salvage pathway involves the phosphorolysis of guanosine by DeoD and subsequent phosphoribosylation to GMP of the resulting guanine molecule by either Gpt or Hpt enzymes. A secondary pathway is catalyzed by the enzyme guanosine-inosine kinase or Gsk that phosphorylates exogenous guanosine to GMP which can be further phosphorylated to GTP. The Gsk enzyme is feedback inhibited by GTP and thus, contributes less to the salvage pathway than the DeoB/Gpt/Hpt pathway (Hove-Jensen and Nygaard, 1989). The *gsk3* allele was isolated as a suppressor of the purine auxotroph (*purE deoD* mutant) that was unable to grow even after supplementation with guanosine. This allele codes for an enzyme that is resistant to feedback inhibition by GTP (Petersen, 1999). In a wild type strain, *gsk3* allele conferred sensitive to guanosine supplementation (Hove-Jensen and Nygaard, 1989) and caused an increase in the guanosine and adenine nucleotide pools. It was proposed the increased in purine nucleotides feedback inhibited Prs (ribose-phosphate diphosphokinase), and restricted phosphoribosyl pyrophosphate (PRPP) synthesis. PRPP is a precursor required for synthesis of purine and pyrimidine nucleotides, the amino acids histidine and tryptophan, and pyridine nucleotide NAD. Reduction in histidine and tryptophan levels was proposed to trigger RelA-dependent (p)ppGpp accumulation and growth inhibition - *relA* mutation suppressed guanosine sensitivity (Petersen, 1999). However, it is not clear if reducing (p)ppGpp pool was sufficient to overcome pyrimidines, histidine, tryptophan, and NAD requirements. We have obtained evidence that elimination of RelA-mediated (p)ppGpp synthesis was not sufficient to overcome the guanosine sensitivity of the *gsk3* mutant and that the expression level of the *pyrE* gene plays a role in the growth rescue.

Publication

1. Rajeshree Sanyal, Allada Vimala and R. Harinarayanan (2020). Studies on the Regulation of (p)ppGpp Metabolism and Its Perturbation Through the Over-Expression of Nudix Hydrolases in *Escherichia coli*. Front. Microbiol. doi: 10.3389/fmicb.2020.562804.

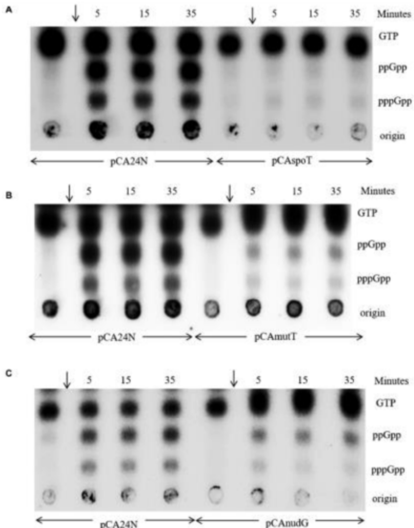


Fig. 1. Increased expression of *spoT* or *mutT* or *nudG* alleviates the stringent response to isoleucine starvation. A representative TLC of MG1655 Δ*lacZYAI*::FRT strain carrying the ASKA plasmids indicated below each panel was cultured in MOPS minimal medium containing glucose Cm and 0.1 mM IPTG. The culture was labeled with P^{32} to follow the accumulation of stringent nucleotides after isoleucine starvation by the addition of valine (arrow). Samples were collected immediately before the addition of valine or at the time points indicated and subjected to PEI-TLC.

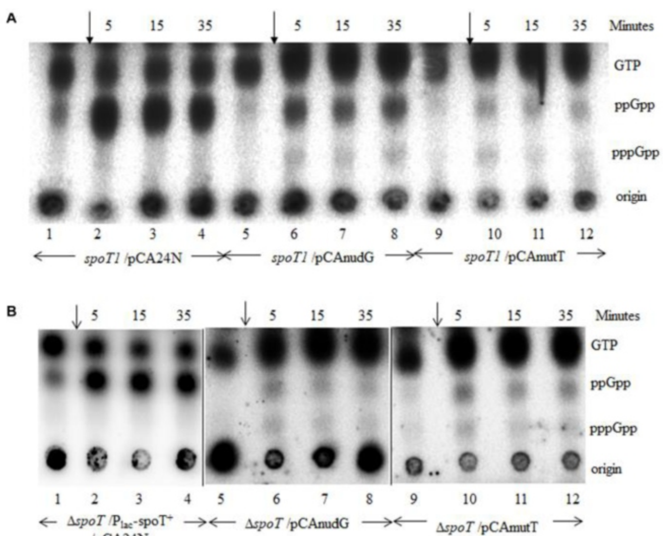


Fig. 2. Increased expression of mutT or nudG lowers the ppGpp pool and elevates pppGpp pool during stringent response in strains with reduced SpoT. hydrolase activity. (A) Isoleucine starvation was induced by the addition of valine (arrow) to cultures of the spoT1 strain carrying the vector pCA24N (lanes 1–4), the vector with nudG (lanes 5–8), or mutT (lanes 9–12). (B) Isoleucine starvation was induced in the 1spoT/Plac-spoTC/pCA24N strain after reducing spoT+ expression by growth in the absence of IPTG (lanes 1–4), in the 1spoT/pCAnudG (lanes 5–8), and 1spoT/pCAmutT (lanes 9–12) strains cultured in the presence of 0.1 mM IPTG. The accumulation of stringent nucleotides was followed with P32 labeled cultures as described in the methods.

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
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Ph D Students

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Geethanjali Ravindran	Research Associate (since Feb. 2021)

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Objectives

1. Study of non-canonical roles of MLL in cell cycle.
2. Role of MLL in regulation of repetitive non-coding regions.

Project 1: Study of non-canonical roles of MLL in cell cycle.

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, 2020 –March 31, 2021)

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.

While trying to understand the functions of MLL, we observed that it localized on the centromere with CENPA protein (Figure 1A). CENPA is a variant of histone H3, found only on the centromere. It is thought to be essential for proper segregation of chromosomes during cell division. Earlier it was thought that no transcription occurs at centromere but recently it has been shown that centromere transcription is necessary for its chromosome segregation function.

The current hypothesis states that H3K4 me2 deposition makes centromere permissive to transcription, which in turn facilitates CENPA deposition at the end of G1 in each cycle by facilitating the recruitment of CENPA-chaperone—Holliday Junction Recognition Protein (HJURP). It was also shown that transcription may affects the deposition of other centromere proteins like CENPC (Figure 1B). On the basis of data generated in our lab, we propose that as the enzyme depositing H3K4 me2 marks, the MLL/SETD1A lie at the top of this regulatory chain (Figure 1B). According to this hypothesis, loss of MLL/SETD1A will also affect the levels of HJURP and CENPC proteins. Therefore, we performed siRNA knock down of MLL/SETD1A proteins, followed by immuno staining and imaged cells for HJURP, or CENPC signals. The quantification from these experiments (data not shown) indicates that levels of both HJURP and CENPC are significantly down regulated upon MLL and SETD1A knockdown.

Taken together, our findings strongly indicate that MLL and SETD1A bind at the centromeres and their loss affects centromeric transcription. This loss of transcription adversely affects recruitment of key proteins at centromere. In our future experiments, we will interrogate the status of H3K4me 2 and CENPA at these loci upon loss of MLL.

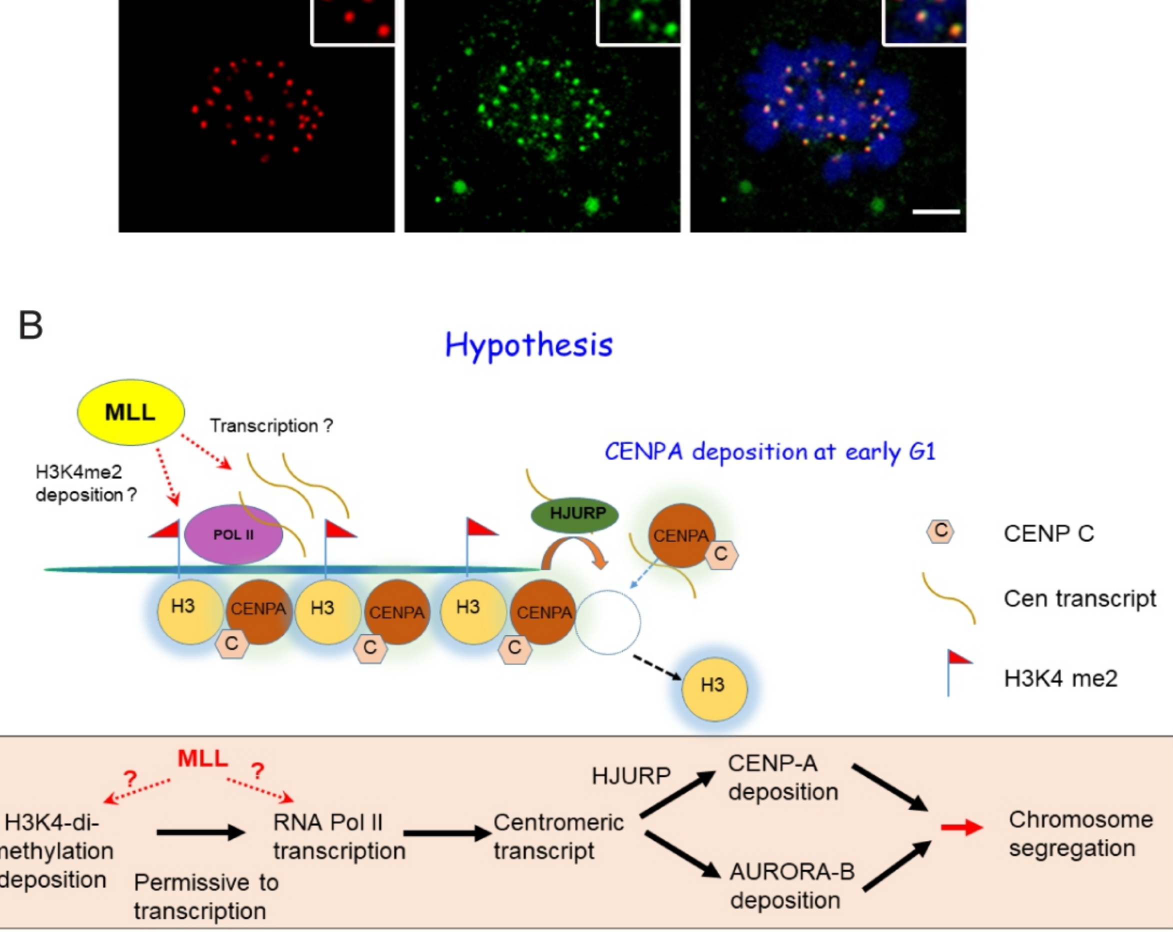

Figure 1. MLL regulates epigenetic status of centromeres.

(A) U2OS cells were fixed with acetone, followed by staining with anti-MLL (green) and CENP-A (red) antibody. Cells are shown at pro-metaphase stage of mitosis. Zoomed version is shown inset to highlight that MLL shows localization pattern similar to the centromere protein CENP-A. Scale bar is equal to 5μm.

(B) A model depicting events that lead to CENPA deposition on the centromere in early G1 are shown. H3K4 me2 makes the centromeres permissive to transcription. The centromeric transcripts interact with CENPC and facilitate the recruitment of CENPA chaperone HJURP. In early G1 phase place-holder H3 is evicted and CENPA is deposited on the centromere. We propose that MLL/SETD1A are the writer proteins responsible for deposition of H3K4 me2 mark on the centromere.

Publication:

J Sugeedha *, Jyoti Gautam* and Shweta Tyagi. (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.

Laboratory of Cell Cycle Regulation

From left to right (back row): Akash Chinchole, V.N. Sailaja, Geetanjali Ravindran, Shweta Tyagi, Kaisar Lone, Kausika Malik, Bijaya Ta.
(Front row): Babu Rao, Payal Katariya , Avishek Katariya, Aditi Arora, Anam.

Laboratory of Cell Death & Cell Survival

RESEARCH

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

Principal Investigator: **Maddika Subba Reddy**
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Parveen Kumar	Project Associate II
Sunu Joseph	Project Associate II
Adithya Pallepati	Project Associate I
Devika Prakash	Technical Assistant
Nanci Rani	Junior Research Fellow

Objectives:

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. As an old saying "Show me your friends, and I will know who you are", finding interaction partners for these proteins can reveal their function better. 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 143 phosphatases so far. During earlier years, we assigned several novel cellular functions to different phosphatases based on their interacting partners. During this year, we identified novel nuclear functions associated with cytoplasmic tyrosine phosphatases. We discovered a new histone modification - H2B Y121 phosphorylation - that exhibits a functional crosstalk with H2B ubiquitination during transcription. We identified SHP1 as a phosphatase that regulates this modification and promotes transcription. SHP-1 is a non-receptor tyrosine phosphatase that predominantly exist in cytoplasm of hematopoietic cells. We have shown that SHP-1 localizes to nucleus in various epithelial cells where it interacts with Paf1 complex and histone H2B (Fig. 1A). By using various biochemical assays, we demonstrated that nuclear SHP-1 dephosphorylates H2B at Y121 residue. We demonstrated that the cross talk between Y121 dephosphorylation and another modification on H2B (ubiquitination) is essential for productive transcription as SHP-1 loss led to defects in Pol II transition from initiation to elongation (Fig. 1B &1C). Since phosphatases are involved in various human diseases such as cancer, and

neurodegenerative disorders, finding their partners will help us in designing better future therapies for these diseases.

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

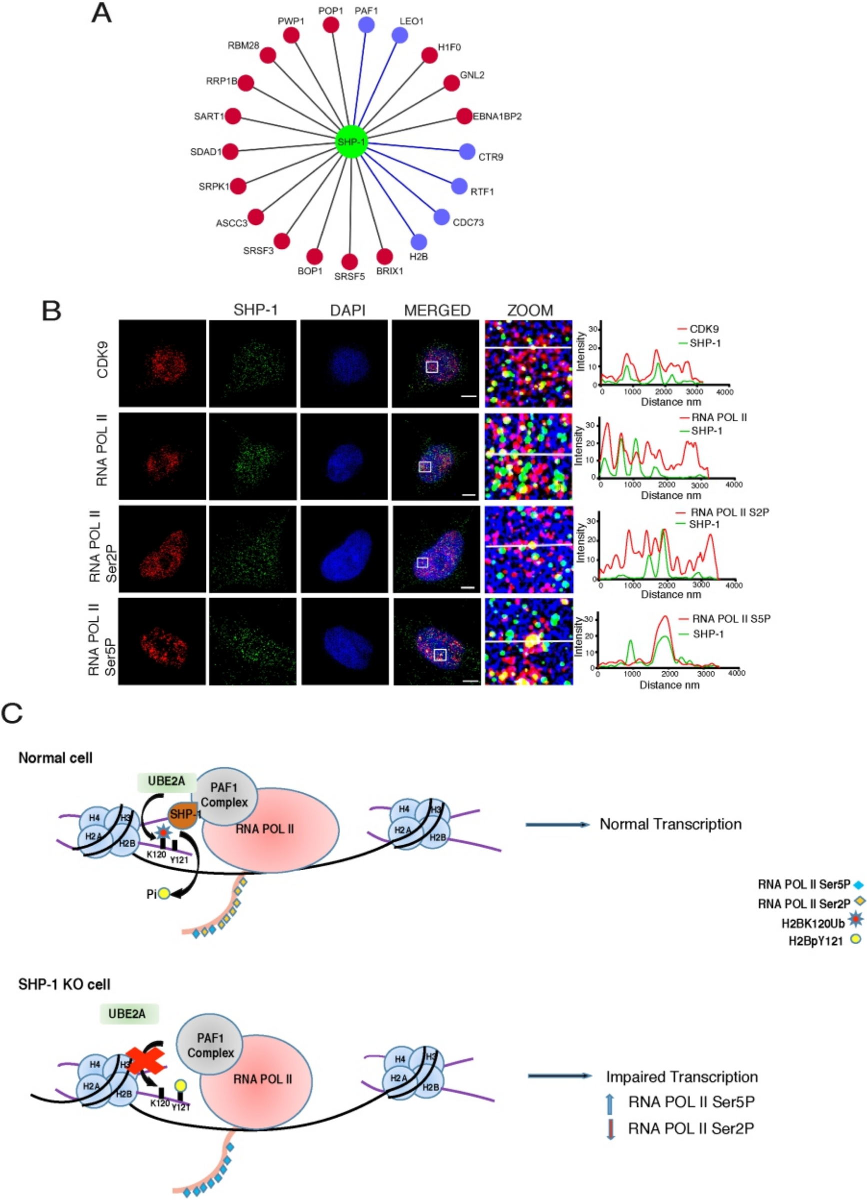

Figure-1: Nuclear role of SHP-1. (A) Interaction network of SHP-1 in complex with PAF1 components and histone H2B identified through affinity proteomics. (B) Co-localization of SHP-1 with transcription machinery shown by super-resolution imaging (C) A proposed model to depict the role of nuclear SHP-1 during active transcription.

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 identified a new function for ubiquitin linkage in cells. We found 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.

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

RESEARCH

Investigating the functions of phosphate-rich biomolecules in eukaryotic cells

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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-IP_7 (5PP-IP_5), 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-IP_7 is synthesised from IP_6 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-IP_7 levels are perturbed. Protein pyrophosphorylation is a unique attribute of inositol pyrophosphates such as 5-IP_7, wherein the β-phosphate moiety can be transferred from 5-IP_7 to a pre-phosphorylated serine residue in a protein to generate pyrophosphoserine. We have recently demonstrated that pyrophosphorylation of the oncoprotein MYC in its central PEST domain creates a novel "pyrophosphodegron", which promotes its polyubiquitination and degradation (Lolla *et al*., Biochem. J. 2021).

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 (sh*IP6K1*) as a model system to investigate the molecular mechanism by which 5-IP7 regulates homologous recombination (HR) mediated DNA repair. Upon treatment with the inter-strand crosslinker mitomycin C, U-2 OS cells expressing either non-targeting shRNA (shNT) or sh*IP6K1* accumulate DNA damage foci marked by the key HR protein RAD51. However, when cells were allowed to recover for 8 h after removal of mitomycin C, we observed a higher number of DNA damage foci persisting in U-2 OS shIP6K1 cells compared with control cells. Expression of active IP6K1 was able to restore the ability of U-2 OS shIP6K1 cells to clear RAD51 foci following an 8 h recovery, but catalytically inactive IP6K1 did not have the same effect, indicating that synthesis of 5-IP7 by IP6K1 is necessary to complete HR-mediated DNA repair in U-2 OS cells. Following mitomycin C treatment, we observed that IP6K1 co-localises with the DNA damage marker γH2AX and interacts with RAD51. We are currently investigating the molecular mechanism by which protein-protein interactions and 5-IP7 synthesis by IP6K1 at the DNA damage site promote the completion of HR repair.

Cellular and physiological functions of IP6K1

We have shown that male mice lacking IP6K1 display spermiogenesis failure and consequent azoospermia. We observed high levels of IP6K1 expression in round spermatids, where it was found to be essential for the formation of perinuclear ribonucleoprotein (RNP) granules called chromatoid bodies (Malla and Bhandari, J. Cell Sci. 2017). The absence of chromatoid bodies in *Ip6k1* knockout mice leads to premature translation of key spermiogenic proteins, resulting in defects in spermiogenesis, and concomitant infertility.

The functional analogue of chromatoid bodies in somatic cells are called processing bodies or P-bodies. These cytoplasmic RNP granules are sites for mRNA storage and harbour proteins involved in suppression of translation. As IP6K1 is indispensable for the assembly of chromatoid bodies in round spermatids, we investigated whether IP6K1 depletion also affects the formation of P-bodies. We noted that P-body granules are nearly absent from the bronchiolar epithelia of *Ip6k1* knockout mice. We used U-2 OS sh*IP6K1* cells as a model system to probe the mechanism by which IP6K1 regulates P-bodies. Immunostaining revealed a drastic reduction in the number of P-bodies in IP6K1 depleted cells (sh*IP6K1*) compared with non-targeted (shNT) control cells (Figure 1). We did not observe any co-localization between IP6K1 and DCP1A in P-bodies, indicating that unlike most proteins involved in P-body assembly, IP6K1 does not localize to P-bodies. Expression of either active or catalytically inactive versions of IP6K1 restored the ability of U-2 OS sh*IP6K1* cells to form P-bodies, indicating that IP6K1 is required for the presence of P-bodies independent of its catalytic activity. We conclude that IP6K1 protein, irrespective of its enzymatic activity, is sufficient for the formation of P-bodies. Overexpression of either IP6K2 or IP6K3, which share significant sequence similarity with IP6K1, failed to rescue the depletion of P-bodies in U-2 OS sh*IP6K1* cells. We are currently investigating the protein-protein interactions by which IP6K1 maintains P-bodies.

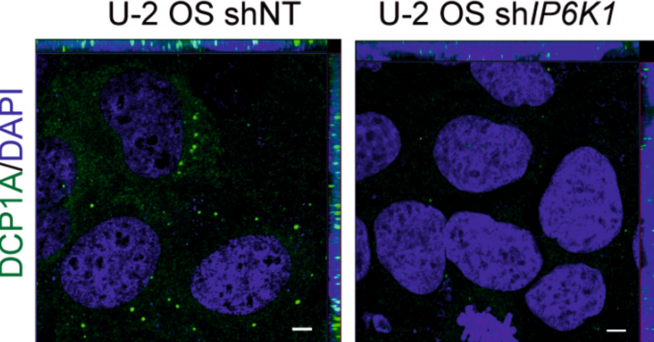


Figure 1. IP6K1 upregulates P-bodies independent of its catalytic activity. Asynchronous U-2 OS shNT and shIP6K1 cells were stained with anti-DCP1A antibody (green). Nuclei were stained with DAPI (blue). Scale bar, 5 µm.

Publication

Manasa Chanduri and Rashna Bhandari (2020). Back-pyrophosphorylation assay to detect in vivo InsP7-dependent protein pyrophosphorylation in mammalian cells Methods in Molecular Biology 2091: 93-105.

Group of Laboratory of Cell Signalling

Laboratory of Chromatin Biology and Epigenetics

RESEARCH

Understanding the functions and regulation of sirtuins in maintaining genomic integrity

Principal Investigator: Devyani Haldar
Staff Scientist

PhD Students: Shalini Aricthota
Arijit Mallick

Other Members: Sobhan Babu
Gouse Sharif

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

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) Discovery of novel molecular mechanisms by which sirtuins family protein deacetylases regulate DNA metabolic processes such as DNA replication and DNA repair. We are studying regulation of sirtuins during DNA replication stress response now.
2) Study functional link and cross-talk between chromatin modifications and cell metabolism and their implication in cancer progression
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 upon replication stress.

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. Stalled forks are prone to collapse, which leads to DNA damage, genomic instability, a hallmark of cancer. Several mechanisms to detect, prevent and counter the deleterious effects of replication stress have been reported. We use fission yeast as model system in this study. In fission yeast, the replisome components are targeted for degradation upon replication stress, in absence of fork protection complex, to maintain genome stability. 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 fission yeast 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 the current study is to decipher

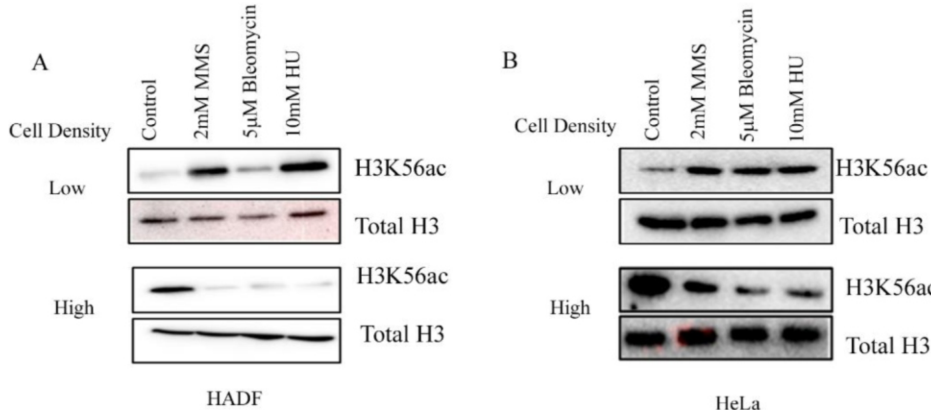
the molecular mechanism of regulation of Hst4 upon replication stress. We have discovered that phosphorylation of Hst4 make it a target for degradation by SCF complex upon replication stress. In this work have found a novel mechanism to maintain the integrity of the replicating DNA during replication stress via induction of degradation of histone deacetylase Hst4 to stabilize the fork protection complex (FPC). Currently, we are working towards understanding how the increase in H3K56ac due to degradation of Hst4 stabilize the FPC.

Study functional link and cross-talk between chromatin modifications and cell metabolism and their implication in cancer progression. The epigenome is crucial for sensing and responding to fluctuations in the cellular environment. However, little is known about mechanisms by which the chromatin machinery signal, interact and respond to the alterations in the cellular micro-environment. Histone acetylation is affected by the levels of acetate in yeast and that of glucose and lactate in mammals. In response to changes in the extracellular and intracellular environment, chromatin modification patterns are altered to regulate gene expression.

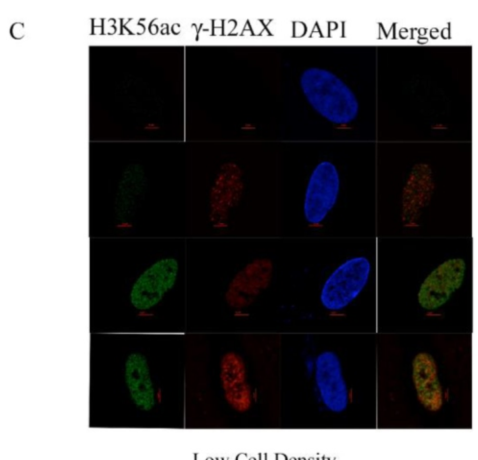
Our previous work on histone acetylation profile on DNA damage indicated cellular metabolic status and mircro-environment may alter DNA damage signalling. Our recent work revealed that environmental changes and accumulation of metabolites during growth of cells in culture affects the epigenetic states during normal and stress conditions. The regulation of H3K56ac by metabolic factors such as lactic acid suggests that environmental changes directly affects histone modifications. Accumulation of lactic acid at high cell density reflect the conditions similar to tumor micro environment. As it has been reported that levels of H3K56ac is increased in tumors, lactic acid and low pH might affect H3K56ac in tumors leading to deregulated gene expression. We will study, how metabolic changes in the environment affect epigenome and effects of these alteration on DNA damage signalling and DNA repair. This will be useful to understand how cells maintain genomic integrity under various stressful conditions. The level of H3K56ac changes in response to DNA damage. It also co-localizes with DNA damage marker g-H2AX (Das et al, 2009 and Vempati et al. 2010). However, conflicting results from various groups have been reported with respect to the changes in levels of this acetylation upon DNA damage. Increase in H3K56ac on DNA damage was observed by three groups (Das et al., Vempati et al. 2010, Yuan et al. 2011) and it was shown to decrease in experiments carried out by other groups (Tjeertes et al, 2010).

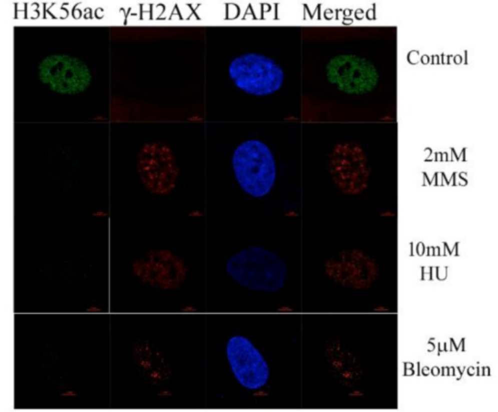
The contradictory results reported earlier and our observation that H3K56ac varies with cell density, prompted us to hypothesise that dynamics of H3K56 acetylation upon DNA damage is dependent on cell density and initial acetylation levels. To test this hypothesis, we induced DNA damage under two different cell density conditions a) in very low cell density in fresh medium, where the initial acetylation levels were observed to be low and b) in high cell density where the initial acetylation levels are generally high. Initially, we treated HADF and HeLa cells seeded at low and high density with various DNA damaging agents like methyl methane sulphonate (MMS), hydroxyurea (HU) and bleomycin and checked the levels of H3K56ac by Western blot. Our results indicated that in the low cell density cells, which had low levels of H3K56ac initially, acetylation increased in response to DNA damage. On the contrary, in medium or high cell density cells, which had higher initial acetylation, H3K56ac levels decreased upon DNA damage (Figure panel A and B). To further confirm the observed difference in H3K56ac levels in response to DNA damage, we also performed immunofluorescence experiments in HeLa and HADF cells on treatment with DNA damaging agents at low and high density and observed the same differential H3K56ac dynamics by Western blot (Figure panel C, D, E).

Figure. Initial levels of H3K56ac determine its dynamics upon DNA damage. A. H3K56ac and total H3 levels in primary human fibroblast HADF seeded at low and high cell density treated with 5μM bleomycin, 2mM MMS and 10mM HU. B. H3K56ac and total H3 levels in cervical cancer line HeLa seeded at low and high cell density and high cell density treated with 5μM bleomycin, 2mM MMS and 10mM HU. C. H3K56ac levels by immuno-fluorescence in HADF cells seeded at low and high cell density (control and cells treated with 2mM MMS, 5μM Bleomycin and 10mM HU). g-H2AX staining was done to verify DNA damage. D and E. Box and whisker plot representing the Mean Fluorescence Intensity of H3K56ac in control and different treatment groups of HADF in low and high density.

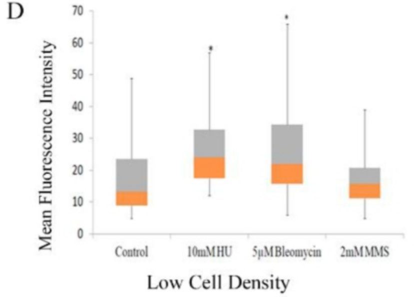


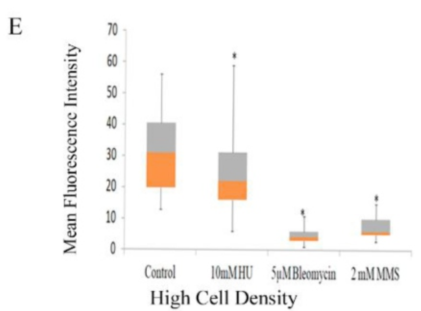
HADF





High Cell Density





Discovery of new epigenetic therapeutics targeted to sirtuin family histone deacetylases

Epigenetic therapeutics of disease such as inhibitors of DNA methyltransferases and histone deacetylases (class I and classII) are already being used in combination with the standard therapeutics with encouraging results. The Sirtuins (class III NAD-dependent deacetylases) are being considered as important targets therapeutics for various diseases as they are up-regulated in many disease conditions. Inhibition of sirtuins allows restores the normal cellular conditions, leading to recovery from disease. However, very few sirtuin inhibitors have entered into the clinic yet as therapeutic agents.

To address the problem of bacterial resistance, substrate-based sirtuin inhibitors targeting the bacterial genome provide a promising approach. N-tri fluoroacetyl lysine and N-thioacetyl lysine peptides (KP 13, KP 15 and KP 24) and their cell-penetrating peptide conjugates Tat KP 13, Tat KP 15 and Tat KP 24 were designed and tested for their ability to inhibit sirtuins. The anti-bacterial and anti-fungal activity of the identified peptide inhibitors were checked. The conjugated peptides were successfully internalised and showed signs of bacterial transcription inhibition resulting in enhanced antibacterial potency against model Gram negative and Gram positive pathogens. Synergistic activity in combination with streptomycin and polymyxin B has also been established. These peptides were effective in inhibiting biofilm formation and eradicating preformed biofilms. Morphological analysis using both SEM and TEM showed bacterial membrane disruption. Calcein dye leakage analysis established the selectivity of these peptides to bacterial membranes. This study is the first report of the application of substrate-based sirtuin inhibitors as antimicrobial therapeutics.

Publications

Kamal D.Patel , Sk. Abdul Mohid, Arkajyoti Datta , Shalini Aricthota , Anirban Bhunia, Devyani Haldar, and Vijayalekshmi Sarojini (2021) Synthesis and Antibacterial Study of Cell-Penetrating Peptide Conjugated Trifluoroacetyl and Thioacetyl Lysine modified Peptides. Eur. J. Med. Chem 219, 113447.

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

Ph D Students:

Mr. Abhishek Kumar	Senior Research Fellow
Mr. Shailesh Kumar Gupta	Senior Research Fellow (till Sep 2020)
Mr. Ch Gangi Reddy	Senior Research Fellow
Mr. S. Akshaykumar Nanaji	Senior Research Fellow
Ms. Ch. Kiranmai	Junior Research Fellow
Ms. Smita Saha	Junior Research Fellow (since Oct 2020)

Other Members:

Mr. G. Rajalingam	Skilled Work Assistant
Mr. J. Aravindh Kumar	Technical Assistant

Collaborators:

Ashwin Dalal	CDFD, Hyderabad, India
Rohit Joshi	CDFD, Hyderabad, India
Sailu Yellaboina	AIIMS, Bibinagar, India
Vijay K Muley	UNAM, Mexico.

Objectives:

The primary objective of our group is to understand the structural and functional roles of genome encoded proteins in protein-protein and protein-ligand interactions responsible for the biology of human diseases and as well as their causative agents. Specially, we study the molecular structure, function, and interactions associated with the biology of tuberculosis, malaria, and human neurodegenerative diseases.

Project 1

Role of mycobacterial transcriptional regulators in physiology and pathology associated with tuberculosis

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

Previously, we have characterised FadR (Rv0494, Rv0586, Rv0043c) and IclR (Rv2989) family of transcription regulators. We have shown Rv0494 to be autoregulatory, lipid-responsive, and have induced expression during starvation. Rv0586 was reported to be the functional homolog of *E. coli* FadR. We have also investigated the role of Rv2989 in inducing dormancy-like growth arrest in *M. smegmatis.* Further, we identified Rv0023 (an Mtb protein belonging to the xenobiotic response element (XRE) family of transcription regulators) has a role in generating higher tolerance toward INH and ETH in a surrogate model system - *Mycobacterium smegmatis* (Msmeg). Enhanced expression of Rv0023 in Msmeg leads to the development of INH- and ETH-tolerant strains. The strains expressing Rv0023 have a higher ratio of NADH/NAD+ and this physiological event is known to play a crucial role in the development of INH/ETH co-resistance in Msmeg. Gene expression analysis of some target genes revealed a reduction in the expression of the *ndh* gene, but no direct interaction was observed between Rv0023 and the ndh promoter region. The gene Rv0023 is divergently transcribed to whiB5 (Rv0022c) and we observed a direct interaction between the recombinant Rv0023 protein with the upstream region of Rv0022c, confirmed using reporter constructs of Msmeg. However, we found no indication that this interaction might play a role in the development of INH/ETH drug tolerance.

Details of the work done in the current reporting period (April 1, 2020- March 31, 2021)

In the current study, we have carried out comparative transcriptomics of *M. smegmatis* with ectopically expressed *MSMEG_2386* and identified 129 genes (53 upregulated; 76 downregulated) to be differentially

expressed (DE) (Figure 1). To identify the function of these DE genes, enrichment of gene ontology (GO) terms (biological processes and molecular functions) was performed using DAVID functional annotation tool. The functional annotations returned for upregulated and downregulated genes were performed separately. The upregulated genes were reported to be associated with biological processes involving Cellular response to stress and DNA damage; and were annotated with molecular functions involving helicase and peptidase activities (Figure 2A, 2B). The downregulated genes were associated with biological processes of localization and transport; and were annotated with symporter activity, monooxygenase, and oxidoreductase activities (Figure 2C, 2D). We have also identified that five genes (viz., *MSMEG_3932*, *MSMEG_3939*, *MSMEG_3942*, *MSMEG_3947* and *MSMEG_3955*) of 25 *M. smegmatis* homologues of *dosR* regulon to be differential regulated (Table 1). This differential expression of the aforementioned five *dosR* regulon genes was validated by quantitative real-time PCR, and results were concordant with computational analysis (Figure 3).

Project 2

Role of parasite and human Acyl-CoAs binding proteins in antimalarial chemotherapeutics

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

In our previous studies, we had characterized the biophysical and the lipid-binding properties of the three *Plasmodium falciparum* acyl-CoAs binding proteins (*pf*ACBPs), naming ACBP16, ACBP99, and ACBP749. We had shown that the *pf*ACBPs are globular proteins that are composed mainly of α-helical structures. These *pf*ACBPs could bind to long-chain fatty acyl-CoAs, like myristoyl-CoA, and conjugated lipids, like phosphatidylcholine. However, the *pf*ACBPs could not bind the fatty acids (like palmitic acid) and phosphatidic acid. All the *pf*ACBPs showed high sequence conservation and two specific tyrosine residues (Y30 and Y33) were identified as the critical residues that determined the interaction stability with the fatty acyl-CoAs. We have conducted studies to find the small molecule modulators of *pf*ACBPs. To identify the potential binding molecules, we used high-throughput virtual screening of the chemicals of Pubchem and Tres Cantos antimalarial compound set (TCAMS) against the modeled structures of ACBP16, ACBP99, and ACBP749. We identified several small molecules that could interact with *pf*ACBPs with high-affinity scores. The FDA-approved drug, mefloquine, appeared as one promising candidate. We confirmed the high-affinity binding of mefloquine with *pf*ACBPs by isothermal titration calorimetry experiments. Mefloquine appeared to be a competitive inhibitor for *pf*ACBPs against their substrates, like myristoyl-CoA. Mefloquine engaged and blocked the essential Y30 and Y33 amino acid residues to prevent the fatty acyl-CoA binding to *pf*ACBPs. Mefloquine showed high toxicity against the *P. falciparum*, resulting in slower multiplication and increased death of the organism in presence of mefloquine. Further, we examined the role of a human homolog of acyl-CoAs binding protein (hACBP) in binding antimalarial mefloquine and its potential adverse effects in humans. Though mefloquine is known to be neurotoxic, the molecular mechanism associated with this phenomenon was still obscure. We showed that mefloquine binds to and inactivates the human acyl-CoA binding protein (hACBP) to potentially inducing redox stress in human neuroblastoma cells (IMR-32). Mefloquine occupies the acyl-CoA binding pocket of hACBP by interacting with several of the critical acyl-CoA binding amino acids, which leads to the competitive inhibition of acyl-CoA(s) binding to hACBP and the accumulation of lipid droplets inside the IMR-32 cells. The accumulation of cytosolic lipid globules and oxidative stress finally correlates with the apoptotic death of cells. Collectively, our study provides a mechanistic detail of how mefloquine leads to the death of human cells by perturbing the activity of hACBP and lipid homeostasis.

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

We used molecular dynamics simulation and other computational biology approaches to investigate the differences in stabilities of mefloquine-PfACBP749 and mefloquine-hACBP complexes. The stability of mefloquine in the binding pocket of PfACBP749 is less than its stability in the binding pocket of hACBP. Although the essential tyrosine residues (Y-30 and Y-33 of PfACBP749 and Y-29 and Y-32 of hACBP) mediate the initial binding of mefloquine to the proteins by π-stacking interactions, additional temporally longer interactions between mefloquine and aspartate-22 and methionine-25 of hACBP result in stronger binding of mefloquine to

hACBP. The higher fluctuation of mefloquine-binding residues of PfACBP749 contributes to the instability of mefloquine in the binding pocket of the protein. On the contrary, in the mefloquine-bound state, the stability of hACBP protein is less than the stability of PfACBP749. The helix-to-coil transition of the N-terminal hydrophobic region of hACBP has a destabilizing effect on the protein's structure. This causes the induction of aggregation properties in the hACBP in the mefloquine-bound state.

Project 3

Computational and functional studies on the aggregation-prone metastable proteins

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

Huntingtin Interacting Protein K (HYPK) is a small protein that acts as a stabilizing switch to prevent protein aggregation in at least three human neurodegenerative disorders. Using a proteomics experiment, we have shown that this protein forms an interactive network with some other proteins like VCP, LC3, and others. The HYPK mRNA is differentially translated from an internal start/initiation codon to generate an amino terminal-truncated isoform (HSPC136) of HYPK protein. In the current period of study, we showed that IRES-dependent translation initiation of HYPK mRNA results in the formation of the HSPC136/HYPK-ΔN isoform of HYPK protein. The IRES-driven translation product, HYPK-ΔN, lacks the N-terminal tri-arginine motif that acts as the nuclear localization signal (NLS) in the full-length HYPK protein. While the full-length HYPK protein translocates to the nucleus and prevents the aggregation of the mutant p53 (p53-R248Q) protein, the HYPK-ΔN lacks this activity. The NLS of HYPK is not evolutionarily conserved. It is exclusive present in the HYPK of higher eukaryotic animals and possibly imparts additional advantage to the HYPK protein in tackling both cytosolic as well as nuclear protein aggregates. The presence of the NLS in full-length HYPK also allows this protein to modulate the cell cycle. Our results provide mechanistic insight on the HYPK mRNA's translation initiation control by an IRES that dictates the formation of HYPC136/HYPK-ΔN, which lacks nuclear localization and other functional abilities. In addition, we have also examined the dynamic nature of structural segments in the modulation of SOD1 intrinsic stability. Mutants of superoxide dismutase 1 (SOD1) protein, like SOD1G85R and SOD1G93A, adopt misfolded states that undergo aggregation in motor neuron cells. We had used correlative computational studies to investigate the temporal flux of structural alterations in SOD1G85R and SOD1G93A that increased the instability and aggregation tendency of these proteins. Molecular dynamics simulation studies showed that the G85R and G93A mutations caused localized transitions of the edge-strands of beta-sheets to disordered structures near the mutation regions. Though this structural perturbation did not alter the conformation of the catalytic zinc and copper-binding residues, it could dislocate the electrostatic loop of SOD1G85R. This had rendered the electrostatic loop of SOD1G85R to be incapable of guiding the substrate to the catalytic cleft. The beta sheet-to-disorder transitions near the mutations had caused the induction of steric clashes in the edge-strand residues, resulting in the loss of several intra-molecular interactions in the mutant SOD1 proteins. These had affected local structural destabilization and increased aggregation potential in SOD1G85R and SOD1G93A. Mutant SOD1 proteins adopted energetically less favourable states, with some changes in the residue-level conformation entropy and solvent-exposed surfaces of the mutation neighbouring residues. Collectively, our study demonstrated that the two mutations, G85R and G93A, did not have global effects in changing the SOD1 structure. Instead, the instability-associated aggregation of these mutants arose due to the local structural alterations in the edge-strands of specific beta-sheets.

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

This year, we have analysed the properties of metastable proteins in the context of their stability and capability of undergoing atypical aggregation in physiological conditions. The metastable state of a protein is represented by specific conformational characteristics, which place the protein in a local free energy minimum state of the energy landscape. The native-to-metastable structural transitions are governed by either the transient or the long-lived thermodynamic and kinetic fluctuations of the intrinsic interactions of the protein molecules. Depiction of the structural and functional properties of metastable proteins will help us understand the complexity of folding patterns as well as comprehend the mechanisms of anomalous aggregation of different proteins.

Publications

1. Kumar A, Ghosh DK, Ranjan A (2021). Differential Stabilities of Mefloquine-Bound Human and *Plasmodium falciparum* Acyl-CoA-Binding Proteins. *ACS Omega*, 6(3):1883-1893.
2. Ghosh DK, Ranjan A (2020). The metastable states of proteins. *Protein Sci,* 29(7):1559-1568.

Figure 1. Heat map of normalized counts for 129 differentially expressed genes with logFC of 2, p-value <= 0.05 and FDR <= 0.05. DE genes - Differentially expressed genes; U1 and U2-genes in uninduced samples; I1 and I2- genes in induced samples.

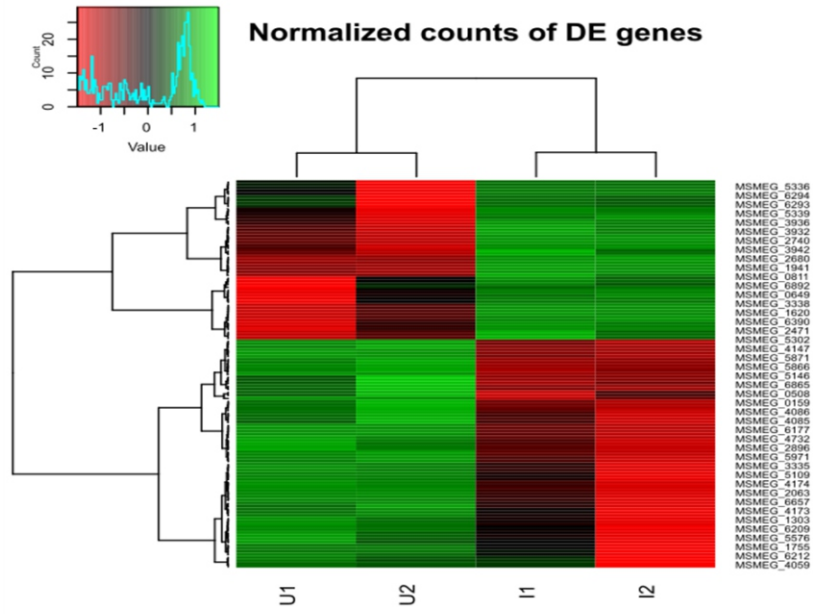


Figure 2. Preliminary analysis of RNASeq data of DEGs. GO term enrichment associated with DEGs (A, B) induced and (C, D) repressed upon ectopic expression of MSMEG_2386 in *M. smegmatis* categorized by (A, C) biological processes (BP) or (B, D) molecular function (MF).

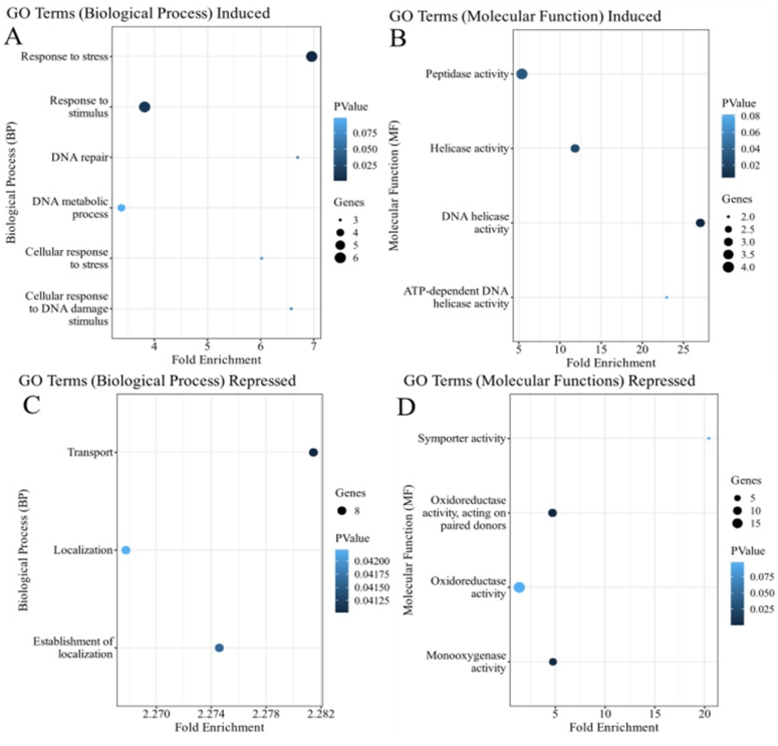


Figure 3. Quantitative real-time PCR of dosR regulon ortholog genes in *M. smegmatis* with ectopically expressed MSMEG_2386.

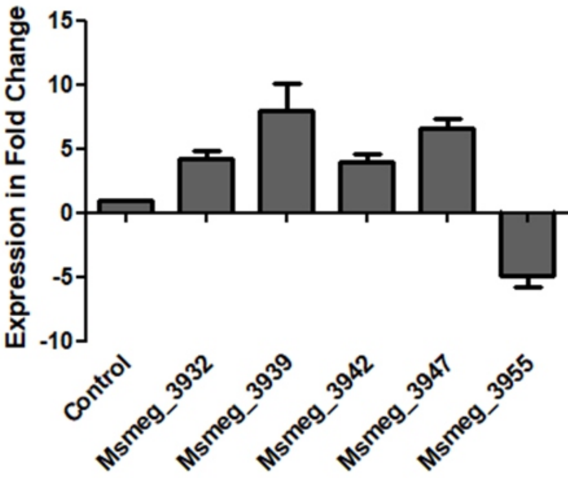


Table1. Orthologs of dosR regulon genes differentially expressed upon *MSMEG_2386* ectopic expression

	logFC	logCPM	P Value	FDR	*M. tuberculosis homolog*
MSMEG_3942	2.058	9.350	6.7E-04	0.02	Rv2004c
MSMEG_3955	-2.386	7.733	1.7E-04	0.008	Rv3131
MSMEG_3932	2.260	8.715	9.9E-06	0.001	Rv2031c (hspX, acr)
MSMEG_3947	2.300	7.587	9E-06	9.4E-4	Rv2029c (pfkB)
MSMEG_3939	3.444	7.213	4.2e-10	1.4E-7	Rv2624c

Laboratory of Computational and Functional Genomics

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
Asif Ahmad Bakshi	Senior Research Fellow
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 to regulate neuronal numbers during the development of CNS. Understanding the molecular basis of this apoptosis is the primary focus of this report.

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 are capable of cooperating in the selection and regulation of target genes in a 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 facilitates the generation of neuronal diversity in these lineages. 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.

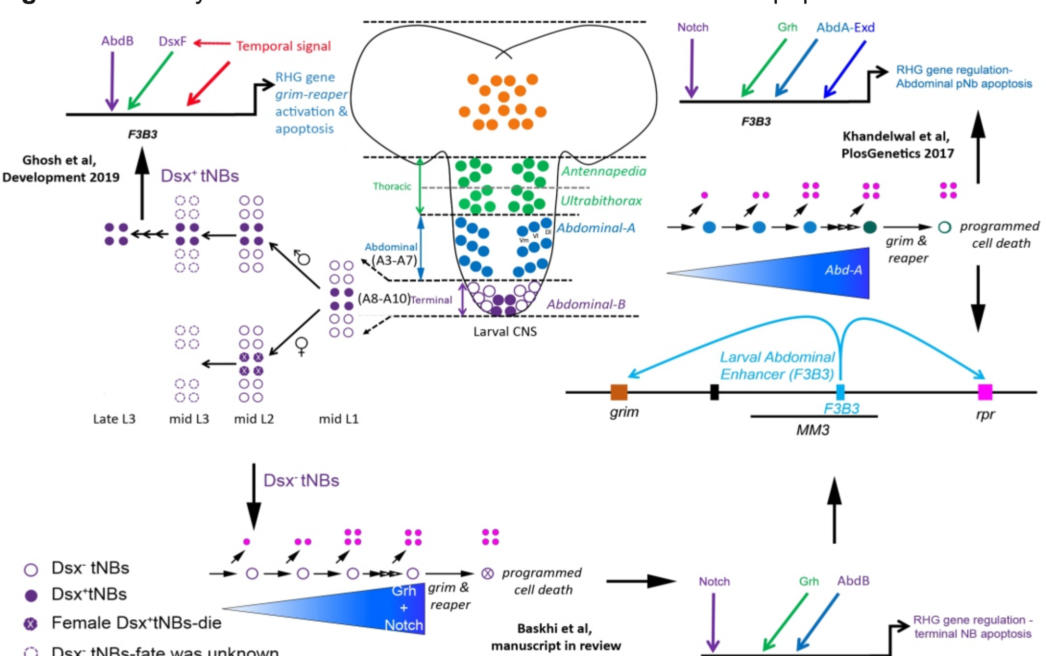
Background:

Neural circuitry for mating and reproduction resides within the terminal segments of the CNS. 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 is not known so far. Our previous work with abdominal NSCs shows that these cells undergo apoptosis by coordinating increasing Abd-A levels (apoptotic trigger) with Notch signaling and helix-loop-helix TF Grainyhead (**Grh**). In continuation of this theme, we have recently focused on Dsx-negative NSCs in the most terminal segments of developing CNS.

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 *RHG* family of genes through the abdominal apoptotic enhancer to undergo cell death. The deletion of this enhancer by the CRISPR-Cas9 method blocks the apoptosis of both abdominal and Dsx-negative NSCs. 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. Our recent studies with abdominal NSCs show that abdominal Hox gene AbdA and helix-loop-helix TF Grh interact through their highly conserved DNA binding domains to execute NB apoptosis in CNS.

Figure: Summary of the molecular mechanism of Hox mediated apoptosis in larval CNS



Future plan:

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

Publications:

Ghosh N., Bakshi A., Khandelwal R., Govinda Rajan S., Joshi R (2019). Hox gene Abdominal-B uses Doublesex^F as a cofactor to promote neuroblast apoptosis in *Drosophila* central nervous system. Development (2019) **146**, dev175158. doi:10.1242/dev.175158.

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..

Drosophila larval NSCs (or neuroblasts-**NBs**) undergo Hox-dependent apoptosis in different regions of developing CNS. In abdominal segments (A3-A7), Abdominal-A (Abd-A) mediated apoptosis relies on transcriptional activation of apoptotic *RHG* genes through a 1Kb *F3B3* enhancer. This enhancer integrates inputs from Abd-A, Extradenticle (Exd), Grainyhead (Grh), and Notch signaling (**Khandelwal et al., 2017, PLoS Genetics**).

In Dsx-positive NSCs (or **Dsx$^+$tNBs**) in terminal segments (A8-A10) of female CNS, it is seen that NSC apoptosis is independent of Grh and Notch signaling. Instead, we find that cooperative interaction between Hox gene Abdominal-B (**Abd-B**) and female-specific isoform of transcription factor Dsx^F is central for this apoptosis and generates sexually dimorphic CNS (**Ghosh et al., 2019, Development**).

Lastly, in Dsx-negative NSCs (or **Dsx$^-$ tNBs**) of the terminal segment, it is observed that these cells die at the same stage as their abdominal counterparts and use the Hox gene (Abd-B in this case), Grh and Notch signaling. However, the mechanism for inducing death is fundamentally different from that used in abdominal NSCs. These cells use an increase in Grh and Notch signaling while keeping the resident Hox gene Abd-B at constant levels to trigger apoptosis (**Bakshi et al., PLoS Genetics, 2020**).

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

Ph.D Students

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Anamika Battu	Senior Research Fellow
Fizza Askari	Senior Research Fellow
Mahima Sagar Sahu	Senior Research Fellow
Sandip Patra	Junior Research Fellow
Aditi Pareek	Junior Research Fellow (since 08 October 2020)
Mayur Raney	Junior Research Fellow (since 28 October 2020)

Other Members

S Surya Vamshi	Technical Officer
Mubashshir Rasheed	Research Associate (Till 30 September 2020)
Priyanka Bhakt	Research Associate
Rajaram Purushotham	Project SRF
Romila Moirangthem	Project SRF (Till 23 October 2020)
Partha Dey	Project JRF (Till 28 February 2021)
Bhogadi Vasavi	Project JRF (Since 17 July 2020)

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CV Srikanth	RCB, Faridabad
Arunaloke Chakrabarti	PGIMER, Chandigarh
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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. Identification and molecular characterization of the CgHog1 kinase interactome: impact on iron homeostasis and Candida pathogenesis.
3. Delineation of iron transport and antifungal drug resistance mechanisms in Candida glabrata

Research summary

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

Project 1: Characterization of glycosylphospha tidylinositol-linked aspartyl proteases in Candida glabrata: role in pathogenicity

A family of eleven putative glycosylphosphatidy linositol-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 are currently trying to delineate cellular processes regulated by the proteolytic activity of CgYapsins and examine their centrality to virulence. Towards this end, we have identified interactors of the CgYps1 aspartyl protease through co-immuno-

precipitation and mass spectrometry analysis, and demonstrated the flavodoxin-like protein, CgPst2, to be a substrate of the CgYps1 protease. Notably, flavodoxins are conserved electron-carrier proteins, which convert quinone to hydroquinone through two-electron reduction, thereby bypassing the formation of unstable and reactive semi-quinone species, and implicated in the oxidative stress response. We also showed that C. glabrata possesses four Fld-LPs, CgPst2, CgRfs1, CgPst3 and CgYcp4, which are required for survival in kidneys in the murine model of systemic candidiasis. We further reported that CgPst2 contributes to cellular NADH:quinone oxidoreductase activity, and is uniquely essential for menadione-induced oxidative stress survival. We also showed that CgYps1 cleaves CgPst2 at the C-terminus, and the arginine-174 (R174) residue in CgPst2 is essential for this cleavage. We further demonstrated that the menadione (MD) treatment led to increased CgPst2 and CgYps1 protein levels, diminished CgYps1-CgPst2 interaction, and enhanced CgPst2 cleavage and activity, suggesting that removal of the C-terminal region may stimulate CgPst2 activity. We tested this possibility by generating the C-terminally truncated CgPst2 (CgPst2-$C^{R174-F198}$), that lacked last 25 amino acids from R174 onwards. We found CgPst2-$C^{R174-F198}$ to rescue MD sensitivity of CgPST2-deleted strains including Q-KO (Cgrfs1pst3ycp4pst2), which lacked all four flavodoxin-like proteins, CgPst2, CgRfs1, CgPst3 and CgYcp4. CgPst2-$C^{R174-F198}$ was also more active than CgPst2 protein in the penta mutant (Cgrfs1pst3ycp4pst2yps1; denoted as penta-KO), that lacked CgYps1 as well as four flavodoxin-like proteins (**Fig. 1A**). This result was consistent with the diminished capability of CgPst2 to restore MD sensitivity of penta-KO, as compared to CgPst2-$C^{R174-F198}$. Further, native PAGE analysis revealed about a 90 kDa band, probably representing CgPst2 tetramer, in CgPST2-expressing Q-KO cells, whose intensity was significantly increased upon MD treatment (**Fig. 1B**). Intriguingly, this CgPst2 oligomer species was neither present in untreated nor MD-treated CgPST2-expressing penta-KO cells (**Fig. 1B**). Instead, a higher-order CgPst2 band of about 130 kDa was observed in untreated and MD-treated CgPST2-expressing penta-KO cells (**Fig. 1 B**), which may represent the non-functional aggregated form of CgPst2. Altogether, these data suggest that CgPst2 exists in a homo-oligomeric form, and CgYps1 modulates homo-oligomerization as well as CgPst2 activity (**Fig. 1C**). Overall, our findings have established CgYps1-mediated proteolytic cleavage as a key regulatory determinant of CgPst2.

Project 2: Identification and molecular characterization of the CgHog1 kinase interactome: impact on iron homeostasis and Candida pathogenesis.

CgHog1 MAPK (mitogen-activated protein kinase), a terminal MAPK of the HOG (high osmolarity glycerol) response pathway, regulates iron homeostasis in C. glabrata. The mutant lacking CgHog1 kinase (Cghog1Δ) showed high intracellular iron and elevated susceptibility to surplus iron. We have previously identified protein interactors of the CgHog1 MAPK, through the affinity purification-mass spectrometry approach, under regular-, high- and low-iron conditions. We have now shown an effect of the environmental iron content on the CgHog1 interactome, and uncovered an exquisite control for the surplus-iron-dependent relief of subtelomeric gene silencing, that may be pivotal to colonization of the gastrointestinal tract of the mammalian host by C. glabrata.

Project 3: Delineation of iron transport and antifungal drug resistance mechanisms in Candida glabrata.

Successful treatment of C. glabrata infections is hampered due to intrinsic low susceptibility of C. glabrata to azole antifungals, which inhibit ergosterol biosynthesis pathway, and emerging resistance to azoles and cell wall-targeting echinocandin antifungals in C. glabrata. Gain-of-function mutations in the Zn-finger pleiotropic drug resistance transcriptional activator-encoding gene, CgPDR1, are the most prevalent cause of azole resistance in clinical settings. CgPDR1 is also upregulated upon azole exposure. During the reporting period, we have uncovered a novel role for two FK506-binding proteins, CgFpr3 and CgFpr4, in CgPDR1 regulation. We showed that CgFpr3 and CgFpr4 possess peptidyl-prolyl cis-trans isomerase domain at their C-termini, and act redundantly to regulate CgPDR1 gene expression. We found increased expression of the CgPDR1 gene as well as its target genes, CgCDR1, CgCDR2 and CgSNQ2, that code for ATP-binding cassette multidrug transporters, in the Cgfpr34 mutant. Further, we observed an increase in histone H3 and H4

protein levels in both Cgfpr34 and azole-treated wild-type cells. Consistent with a role for histone proteins in azole resistance, disruption of genes coding for the histone demethylase CgRph1 and the histone H3K36-specific methyltransferase CgSet2 led to increased and decreased susceptibility towards fluconazole, respectively, with the Cgrph1 mutant also displaying significantly lower basal expression of CgPDR1 regulon genes. Altogether, these data point towards an epigenetic control of azole antifungal resistance in C. glabrata.

Additionally, we have uncovered a pivotal role for the phosphoinositide 3-kinase CgVps34, that converts phosphatidylinositol to phosphatidylinositol 3-phosphate, in functions and localization of key components of the high-affinity ion acquisition systems. Studies are currently underway to examine the effect of perturbed localization of ion-uptake proteins on pathogenesis-related traits in C. glabrata.

Publications

1. 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 **(In press)**. [¶]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. Rasheed, M.[¶], Battu, A.[¶] and **Kaur, R.** (2020) Host-pathogen interaction in Candida glabrata infection: Current knowledge and implications for antifungal therapy. Expert Review of Anti-infective Therapy **18:** 1093-1103. [¶]Equal contribution.
4. Kumar, K.[¶], Moirangthem, R.[¶] and **Kaur, R.** (2020) Genome protection: Histone H4 and beyond. Current Genetics **66:** 945-950. [¶]Equal contribution.
5. Kumari, S., Kumar, M., Khandelwal, N.K., Pandey, A.K., Bhakt, P., **Kaur, R.**, Prasad, R., and Gaur, N.A. (2020) A homologous overexpression system to study roles of drug transporters in Candida glabrata. FEMS Yeast Research **20:** foaa032.
6. Kumar, K., Moirangthem, R. and **Kaur, R.** (2020) Histone H4 dosage modulates DNA damage response in the pathogenic yeast Candida glabrata via homologous recombination pathway. PLoS Genetics **16:** e1008620.
7. Sahu, M.S., Patra, S., Kumar, K. and **Kaur, R.** (2020) SUMOylation in human pathogenic fungi: Role in physiology and virulence. Journal of Fungi **6:** pii: E32.
8. Rasheed, M., Kumar, N. and **Kaur, R.** (2020) Global secretome characterization of the pathogenic yeast Candida glabrata. Journal of Proteome Research **19:** 49-63.

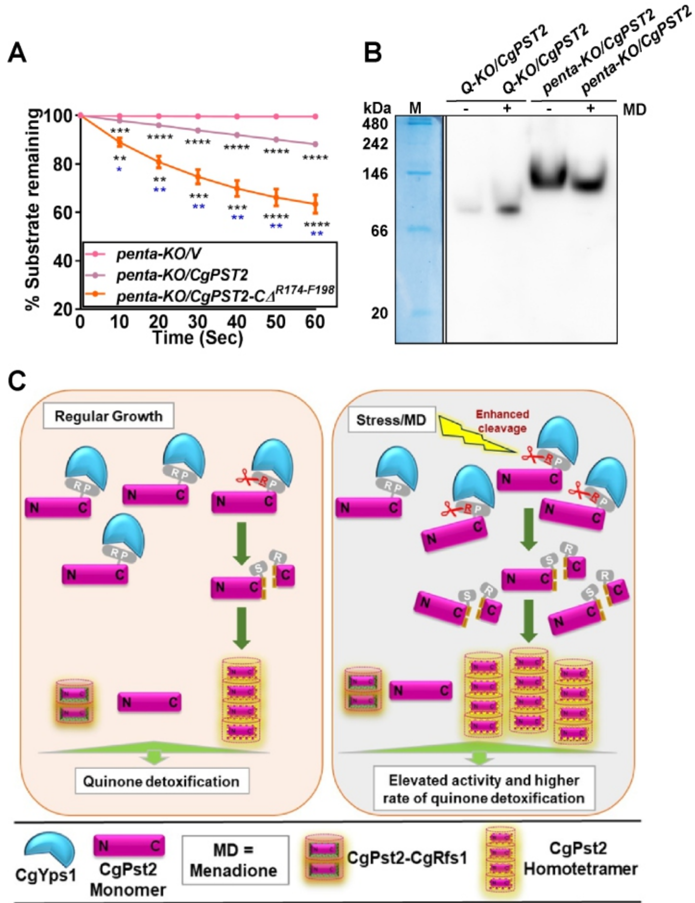


Figure 1: CgYps1-mediated cleavage is a key regulatory determinant of CgPst2. (A) NADH:quinone oxidoreductase activity in cell extracts of *penta-KO* expressing *CgPST2* or *CgPST2-C*$^{R174-F198}$, as measured using menadione (500 μM) and NADH (500 μM)

substrates. Absorbance of the substrate NADH was considered as 100 at 0 h time point, and NADH oxidation was deduced from the formula: [(absorbance at each time point/0 h absorbance) X 100]. Data represent mean ± SEM (n= 3). Black and blue asterisks represent statistically significant activity differences in indicated strains compared to *penta-KO/V* and *penta-KO/CgPST2*, respectively. *, p < 0.0332; **, p < 0.0021; ***, p < 0.0002; ****, p < 0.0001; Grouped multiple *t*-test. V, pRK74 vector. (B) Native PAGE analysis showing enhanced oligomer formation upon menadione (MD) treatment. 200 g whole cell lysates of untreated and MD (90 M for 90 min)-treated *Q-KO* and *penta-KO* expressing *CgPST2* were resolved in a discontinuous Tris-glycine buffer system under non-denaturing conditions, and probed with anti-CgPst2 antibody. The native protein molecular weight marker (M) was stained with coomassie brilliant blue. (C) A schematic summarizing key findings of the study. Arginine-174 (R) and Proline-176 (P) residues of CgPst2 are predicted to interact with CgYps1 at the plasma membrane, and CgYps1 processes R174 residue in the C-terminus of CgPst2. This cleavage, which is elevated upon menadione treatment, leads to removal of the C-terminal domain, resulting in CgPst2 homo-tetramerization, higher activity and efficient quinone detoxification. CgPst2 also interacts with CgRfs1, however, CgPst2-CgRfs1 association is not dependent on CgYps1, and occurs under both regular and MD treatment conditions. Altogether, CgPst2 functions are regulated at multiple levels, and CgYps1-mediated cleavage of CgPst2 reflects one of many mechanisms controlling CgPst2 activity.

Laboratory of Fungal Pathogenesis

Laboratory of Human and Medical Genetics

Genomic studies in chromosomal and single gene disorders

Principal Investigator: **Ashwin Dalal**
Staff Scientist

Adjunct Faculty

Prajnya Ranganath	Associate Professor, NIMS
Shagun Aggarwal	Associate Professor, NIMS

PhD Students

Dipti Deshpande	Senior Research Fellow
A Sandeep	Senior Research Fellow
Arijita Mitra	Senior Research Fellow

Other Members

Anjana Kar	Research Associate (From 8/3/2021)
Vineeth VS	Research Associate (Until 7/6/2020)
Amrita Bhattacherjee	National Postdoctoral Fellow (Until 3/8/2020)
Ikrormi Rungsung	Research Associate (Until 2/12/2020)
P Divya	Project Assistant (Until 11/1/2021)
Shivangi Wagh	Project Assistant
Arjita Jaiswal	Project Assistant (Until 12/2/2021)

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 in fetal malformations

Non-chromosomal syndromes and Mendelian disorders are emerging as an important cause of birth defects and fetal malformations. This study aims to identify copy number defects and single gene abnormalities in fetuses which undergo a post mortem examination following pregnancy termination on ultrasound detection of anomaly/malformation or suffer intrauterine death/ stillbirth and have morphological abnormalities. Cases with unexplained phenotypes and possible novel genetic disorders are selected with the objective of discovering new genotype-phenotype associations. After detailed post-mortem evaluation including radiographs and histopathology, the cases satisfying inclusion criteria undergo DNA extraction from available biological samples. These include fetal blood samples, amniotic fluid samples, cord mesenchymal tissue or skin samples as per availability. After quantification and qualitative assessment of DNA, chromosomal microarray or whole exome sequencing experiments are performed based on the inheritance pattern and/or clinical presentation. RNA sequencing studies were also planned as a continuation of this work to study the functional outcomes of the identified variants on the transcripts of the specific gene as well as the whole transcriptome. These studies would help in assessing the pathophysiology of various Mendelian disorders during the crucial period of organogenesis and foetal development.

Whole exome sequencing was performed in 57 fetuses fitting the inclusion criteria of the study. Of these, a pathogenic/likely pathogenic variant was identified in 28 cases, with a diagnostic yield of 49%. In addition, eight cases had a variant of uncertain significance. 26/36 foetuses had an autosomal recessive disorder, while others had an autosomal dominant condition. In three cases with *LOX, SERPINA11* and *CDK8* variants, we

have identified a novel perinatal lethal phenotype. Few foetuses harbor more than one pathogenic variant indicating Dual Mendelian phenotype. This indicates the possibility of Dual Mendelian diagnoses being more abundant in complex fetal phenotypes as compared to postnatal cohorts, where this phenomenon is reported in 5-7% individuals.

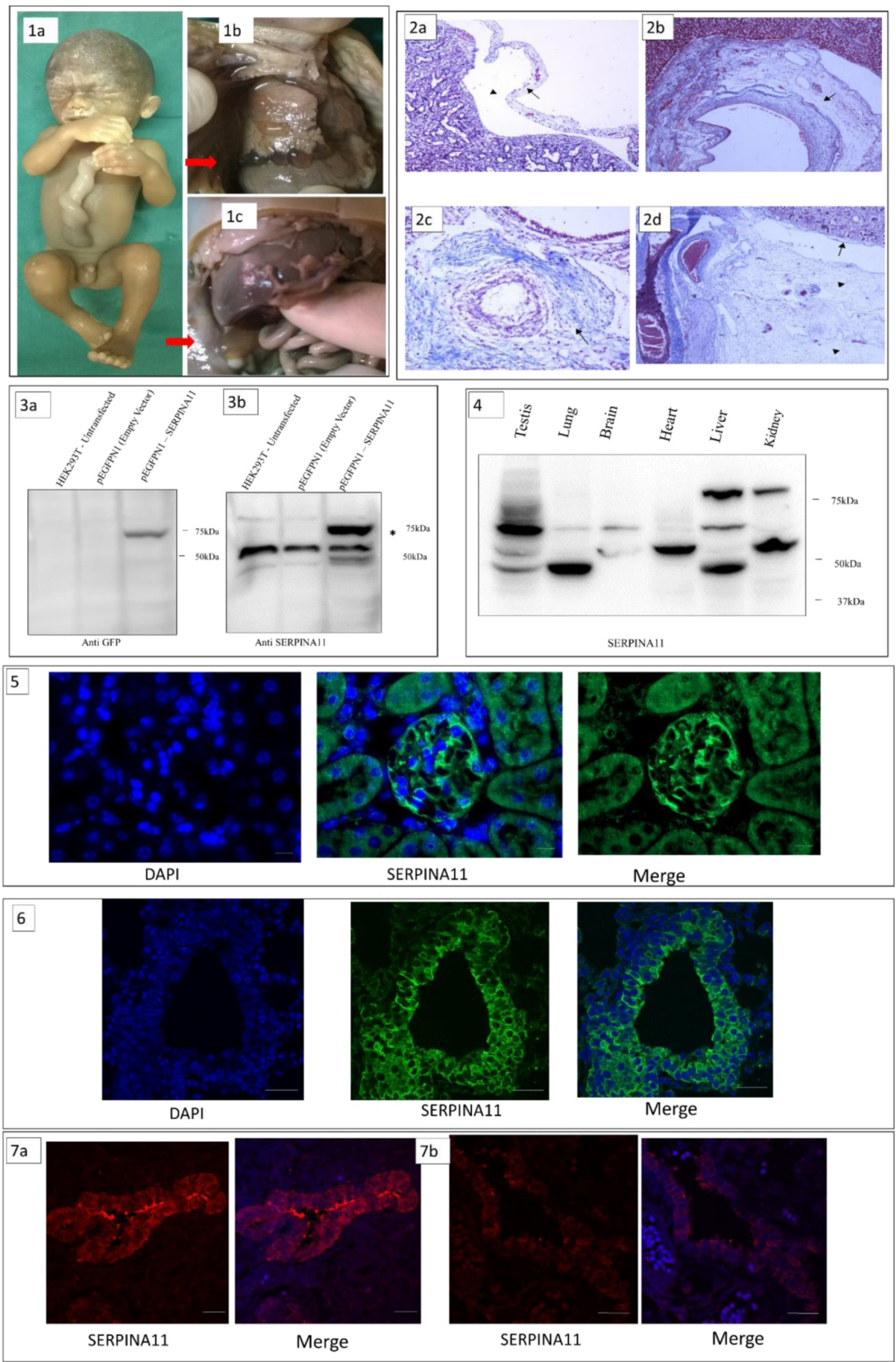
An interesting case where a novel Mendelian gene was identified is discussed in more detail. This was a 22 week fetus conceived of consanguineous parents and found to have pericardial effusion on ultrasound. There was history of a previous sibling death in neonatal period with intractable pleural effusion. Following pregnancy termination, the postmortem examination of the fetus revealed subtle external dysmorphology, but striking internal findings. All the visceral surfaces in abdominal and thoracic cavity were studded with thin wall cysts containing thin gelatinous material. These cysts involved the peritoneal membrane, mesentry, pleura and pericardium. No other structural defects were apparent. Whole exome sequencing was performed on fetal DNA from cultured amniocytes and bioinformatics analysis done as per standard protocols. Fetus was found to harbor homozygous nonsense variant in a novel candidate gene, *SERPINA11* NM_001080451: exon3:c.C672A:p.Y224X. *SERPINA11* belongs to the family of SERPINs which function as serine protease inhibitors, thereby maintaining extracellular matrix homeostasis. As the SERPINA11 protein has been uncharacterised till now, we further attempted to study it in cell lines and mice. For this expression studies of C terminal GFP tagged *SERPINA11* constructs were done in HEK293T cell lines. This showed overexpression of SERPINA11 using anti GFP and anti SERPINA11 antibodies. Western blot experiments were carried out on wild mice tissues using anti-SERPINA11 antibody. These experiments revealed presence of SERPINA11 protein in various tissues. Immunofluorescence studies using SERPINA11 antibody were carried out on wild type mice, normal gestation matched fetal tissues and affected fetal tissues. These showed expression of SERPINA11 in mice and human kidneys and lungs, with the affected fetus showing reduced expression compared to the normal. Finally, site directed mutagenesis was carried out followed by study of transformed HEK293T cell lines by Western blot. This revealed reduced SERPINA11 expression confirming pathogenicity of the variant. The findings of this case led us to conclude that *SERPINA11* biallelic variants are responsible for a perinatal lethal phenotype and this is a novel serpinopathy.

Figure 1

Figure 1a: Postmortem photograph of fetus with subtle dysmorphism; 1b &c : Multiple, thin gelatinous material filled blebs 2a: Cystic enlargement of pleural space-bullae formation, Expanded stroma, disrupted collagen and elastin fibers, Masson Trichome 10 X ; 2b: Liver: expansion of the extracellular matrix of the hilum (arrow). Masson Trichrome, 4xl; 2c: Expanded matrix and disrupted collagen fiber architecture in peribronchiolar region, Masson Trichome 20 X; 2d: Normal hepatocyte morphology and arrangement, PAS negative 3a: Western blot with GFP antibody shows expression of SERPINA11 with a C-terminal GFP tag- GFP with linker (c terminus) - 28kDa, SERPINA11 - 47kDa, GFP tagged SERPINA11 ~ 75kDa; 3b: Western blot with rabbit polyclonal SERPINA11 antibody detects overexpressed SERPINA11-GFP (as well as non-specific proteins) 4:Western blot analysis of mice tissue lysate with SERPINA11 antibody showing expected band of 47KDa in lung and liver. Brain,kidney and heart may have glycosylated forms 5:Immunofluorescence staining on kidney sections of 2 month old mice- Expression observed in glomerulus capillary network 6: Immunofluorescence staining on lung sections of 2 month old mice- Expression observed in bronchiolar epithelium 7: Immuno-fluorescence staining with SERPINA11 antibody on lung sections of human fetuses; Normal(7a) vs affected(7b)- Reduced SERPINA11 staining in bronchioles of affected fetus compared with control.

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)-


1a
1b
1c
2a
2b
2c
2d
3a
HEK293T - Untransfected
pEGFPN1 (Empty Vector)
pEGFPN1 – SERPINA11
75kDa
50kDa
Anti GFP
3b
HEK293T - Untransfected
pEGFPN1 (Empty Vector)
pEGFPN1 – SERPINA11
*
75kDa
50kDa
Anti SERPINA11
4
Testis
Lung
Brain
Heart
Liver
Kidney
75kDa
50kDa
37kDa
SERPINA11
5
DAPI
SERPINA11
Merge
6
DAPI
SERPINA11
Merge
7a
SERPINA11
Merge
7b
SERPINA11
Merge

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 goitre. 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 110 (Dysgenesis,70 and 40 Dyshor monogenesis) cases underwent exome sequencing. Of these, a final genetic diagnosis could be achieved in 39 (4 Dysgenesis, 35 Dyshormonogenesis) cases wherein a pathogenic/likely pathogenic variant causative of the phenotype was identified. Of the 35 cases of dyshormonogenesis most of the patients, harbored likely pathogenic/pathogenic variants in three major known causative genes i.e. Thyroid Peroxidase (*TPO*), Dual Oxidase 2 (*DUOX2*), and Thyroglobulin (*TG*), and one patient harbored a variant in *SLC5A5* gene. A total of 70 dysgenesis cases underwent exome sequencing and only in 4 cases we were able to find likely pathogenic/ pathogenic variants. Among these 4 cases two of them are isolated congenital hypothyroidism cases harboring pathogenic/pathogenic variants in two different known causative genes *PAX8* and *TSHR*, and the other two cases are syndromic Alstrom and Bamforth-Lazarus syndrome harboring likely pathogenic variants in *ALMS1* and *FOXE1* genes respectively. Remaining 66 cases we could not find any significant variants in known causative genes.

Publications

Research papers published in 2020:

1. Nampoothiri S, Yesodharan D, Bhattacherjee A, Ahamed H, Puri RD, Gupta N, Kabra M, Ranganath P, Bhat M, Phadke S, Radha Rama Devi A, Jagadeesh S, Danda S, Sylaja PN, Mandal K, Bijarnia-Mahay S, Makkar R, Verma IC, Dalal A, Ramaswami U. (2020) Fabry disease in India: A multicenter study of the clinical and mutation spectrum in 54 patients. *Journal of Inherited Metabolic Diseases Reports* 15;56(1):82-94.
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3. Girisha KM, Pande S, Dalal A, Phadke SR. (2020) Untapped opportunities for rare disease gene discovery in India. *American Journal of Medical Genetics A*. 182(12):3056-3059.
4. Dutta UR, Suttur MS, Venugopal VS, Posanapally LP, Gopalasetty S, Talwar S, Anand S, Billapati S, Jesudasan RA, Dalal A. (2020) Cytogenetic and molecular study of 370 infertile men in South India highlighting the importance of copy number variations by multiplex ligation-dependent probe amplification. *Andrologia* 52(10):e13761.
5. Arora V, Setia N, Dalal A, Vanaja MC, Gupta D, Razdan T, Phadke SR, Saxena R, Rohtagi A, Verma IC, Puri RD. (2020) Sialidosis type II: Expansion of phenotypic spectrum and identification of a common mutation in seven patients. *Molecular Genetics and Metabolism Reports* 22:100561.
6. Aggarwal S, Vineeth VS, Das Bhowmik A, Tandon A, Kulkarni A, Narayanan DL, Bhattacherjee A, Dalal A.(2020) Exome sequencing for perinatal phenotypes: The significance of deep phenotyping. *Prenatal Diagnosis* 40(2):260-273.
7. Shetty K, Sarma AS, Devan M, Dalal A, Dash GK, Jannabhatla A, Patil SJ. (2020) Recurrent ADCY5 Mutation in Mosaic Form with Nocturnal Paroxysmal Dyskinesias and Video Electroencephalography Documentation of Dramatic Response to Caffeine Treatment. *Journal of Movement Disorders* 13(3):238-240.
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IC, Puri RD, Bhavsar R, Mistry M, Sankar VH, Gowrishankar K, Agrawal D, Nair M, Danda S, Soni JP, Dalal A. (2020) Identification and characterization of 30 novel pathogenic variations in 69 unrelated Indian patients with Mucolipidosis Type II and Type III. *Journal of Human Genetics* 65(11):971-984.

9. Ranganath P, Perala S, Nair L, Pamu PK, Shankar A, Murugan S, Dalal A. (2020) A newly recognized multiple malformation syndrome with caudal regression associated with a biallelic c.402G>A variant in TBX4. *European Journal of Human Genetics* 8(5):669-673.

Research papers in press (as on 31st March 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* (In Press)
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* (In Press)
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* (In Press)
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* (In Press)
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* (In Press)
6. *Aggarwal S. (2020) Role of whole exome sequencing for unidentified genetic syndromes. *Current Opinion in Obstetrics and Gynecology* (In Press)
7. *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. (2021) Missense mutations in CASK, coding for the calcium-/calmodulin-dependent serine protein kinase, interfere with neurexin binding and neurexin-induced oligomerization. *Journal of Neurochemistry* (In Press)

Other publications like patents, Book chapters, etc.(01.04.2020 to 31.03.2021)

1. Amrita Bhattacherjee, Ashwin Dalal, Prajnya Ranganath. Ghosal Hematodiaphyseal Dysplasia: An Unusual but Easy-to-Diagnose Genetic Cause of Anemia. *Genetic Clinics* 2020; 14 (4): 3-6. *Work done elsewhere.

Laboratory of Human and Medical Genetics

Laboratory of Immunology

RESEARCH

Understanding and regulation of advanced glycation endproducts (AGE) mediated deleterious effects

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Staff Scientist

Ph D Students:

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

Other Members:

T Navaneetha	Technical Assistant
Praseeda Vamadevan	Project JRF

Collaborators:

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

Objectives:

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

Research Summary:

Profilin1 basically involved in actin sequestering as well as facilitates its polymerization, so regulates cytoskeleton maintenance and in consequence cytokinesis (actin driven process). Profilin1 overexpression in MDA-MB 231 cells stabilizes p27kip1 and inhibits its growth by cell cycle arrest at G1 phase. Profilin1 activates AMP-activated protein kinase (AMPK) and AMPK phosphorylates p27 on T198 residue, a post-translational modification results in increased p27 stabilization. Profilin1 activates AMPK through phosphorylation. However, activated AMPK is known to promote autophagy through p27 phosphorylation. Advance Glycation End products (AGE) induce autophagy through activation of RAF protein kinase and NF-κB. AGE is also able to induce Profilin1. We have shown here all-trans retinoic acid (ATRA) induces prolonged autophagy in breast tumor cells. Cytoplasmic level of AMPK and p27 are also increasing along with Profilin1 upon treatment with ATRA. Profilin1 physically interacts with AMPK as determined by affinity purification of endogenous AMPK and Profilin1 immunoprecipitation. Stabilization of cytoplasmic AMPK was further investigated by Cycloheximide-Chase experiment and as data suggests AMPK is getting stabilized in presence of high Profilin1 level. These data infer that Profilin1 physically interacts with AMPK and prevents its degradation which further stimulates autophagy.

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

Role of Advanced Glycation End products in inducing Senescence

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. 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. Senescence is a biological process involving the un-programmed cell death or growth arrest leading to aging of human body. Senescence provides immunity for tumour suppression, wound healing, embryonic and placental development. But, its deleterious role in various

pathological conditions related to age has been reported. One study has shown that removal of senescent cells in mice led to increased resistance against aging associated disorders. In diabetic patients, the deleterious effects of senescence in presence of glycated HSA are more pronounced. The Senescence associated (SA)-beta- gal assay is widely used to check the occurrence of senescence as the senescent cells overexpress beta-galactosidase which give blue colour in presence of X-gal. In present study, 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 senescent cells impart blue colour which was visualised under light microscope (A). The number of senescent cells increases with increasing the dose of AGE and cells treated with 0.75 µm of etoposide was kept as positive control (B). Further, occurrence of senescence was confirmed with Fluorescence-activated cell sorting (FACS) based assay using florescent analogue C_{12}FDG of X-gal. The senescent get stained with C_{12}FDG and can be quantified with FACS assay. The results obtained from FACS were in accordance with beta-gal assay (C). In addition to over expression of beta-galactosidase, senescent cells differentially express other molecular markers like p16, p21, Rb, p-RB. The p21 is cyclin-dependent kinase inhibitor which causes cell cycle arrest by inhibiting the activity of cyclin. The level of p21 was varying with changing the different concentration of AGE. The expression of p21 was significantly increased with 50 µg of AGE (D) and for further experiments 50 µg was used. Then, the level of p21 was assessed in AGE treated IMR32 for different time point with AGE and after 3 day of treatment, there was no significant increase in the level of the p21 (E). Thus, 50 µg AGE induces senescence in neuroblastoma cell line which was confirmed with qualitative beta-gal assay and quantitative FACS based assay. The same result was supported by the overexpression of p21 after treatment. The possible pathway for AGE-mediated senescence involving the molecules in the cells are indicated in the cartoon (F).

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.

Publications :

1. Bera P, Aher A, Brandao P, Manna SK, Mondal G, Jana A, Santra A, Jana H and Bera P (2020). Induced apoptosis against U937 cancer cells by Fe (II), Co (III) and Ni (II) complexes with a pyrazine-thiazole ligand: Synthesis, structure and biological evaluation. *Polyhedron* 182: 114503.
2. Bose K JC, Kapoor B, Mandal K, Ghosh S, Mokhamatam R, Manna SK and Mukhopadhyay S (2020). Loss of mitochondrial localization of human FANCG causes defective FANCJ helicase. *Molecular and Cellular Biology* 40: e00306-20.
3. Jana A, Aher A, Brandao P, Ali SS, Samanta SK, Mondal G, Bera P, Santra A, Manna SK, Mahapatra AK and Bera P (2020). Picoline based fluorescence 'turn-on' chemosensor for zinc(II) ion recognition, cell imaging and cytotoxicity study: Synthesis, crystal structure, spectroscopy and DFT. *Polyhedron* 192: 114815.
4. Bera P, Aher A, Brandao P, Manna SK, Bhattacharyya I, Pramanik C, Mandal B, Das S and Bera P (2021). Synthesis, structure elucidation and DFT study of a new thiazole-pyridine anchored nnn donor and it's cobalt(II) complex: In-vitro antitumor activity against U937 cancer cells, DNA binding property and molecular docking study. *Journal of Molecular Structure* 1224: 129015.

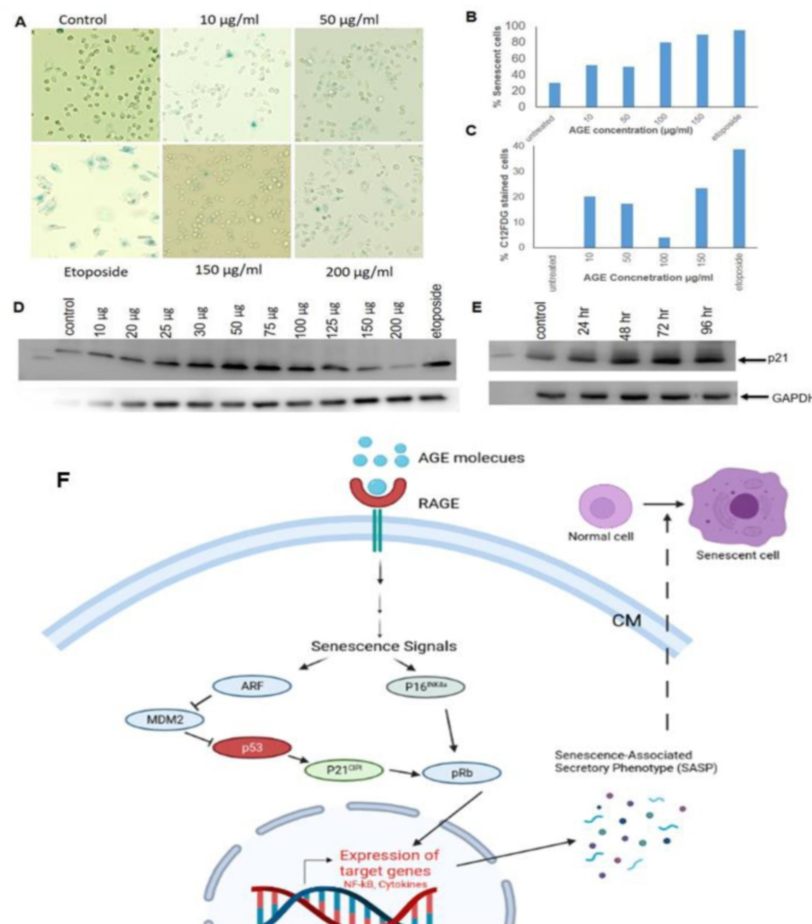

Figure Legend: Effect of AGE in inducing senescence. IMR32 cells were treated with different concentration of AGE and stained for B-Gal. The senescence cells giving blue color were visualized under microscope (A). Quantitative representation of senescence cells, counted and presented in bar graph (B). Senescent IMR32 cells were quantified with FACS using florescent C_{12}FDG analogue of X-Gal (C). The amount of p21 was determined by Western blot using cells' lysate collected from different concentrations of AGE-treated cells (D). The amount of p21 was checked by Western blot from cells stimulated with AGE for different time €. The possible mechanism of AGE-mediated senescence involving several molecules are depicted in the cartoon (F).

Laboratory of Immunology

Laboratory of Molecular Cell Biology

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

Principal Investigator: **Sangita Mukhopadhyay**
Staff Scientist

PhD Students:

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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 (Since October 09, 2020)
Abhishek Dutta	Junior Research Fellow (Since October 29, 2020)

Other Members:

Niteen Pathak	Senior Technical Officer
B. Srikanth	DST-INSPIRE Faculty
Shruti Srivastava	Research Associate-I (upto August 17, 2020)
Faiza Nazar	Project- Senior Research Fellow
Sivapriya Pavuluri	Research Associate-III (Since February 01, 2021)
Swapnila Pramanik	Junior Research Fellow (Since March 09, 2021)

Collaborators

Prof. Seyed E Hasnain	IIT, New Delhi
Prof. D. Sriram	BITS, Hyderabad
Dr. Sudip Ghosh	NIN, Hyderabad
Dr. Gaddam Sumanlatha	Osmania University, Hyderabad
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Dr. Sunil K Manna	CDFD, Hyderabad
Dr. 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.

Project I: PPE2 protein of *M. tuberculosis* affects myeloid hematopoiesis in mice

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

Occurrence of anemia and thrombocytopenia is common in TB patients. Also, there are reports indicating occurrence of pancytopenia in TB patients. In adults, bone marrow hematopoiesis is the major source of macrophages, neutrophils, mast cells, dendritic cells, platelets and red blood cells. These sentinel cells are involved in mounting the primary immune responses against mycobacterial infection. Any reduction in population of these cells may create an immuno-suppressive environment inside host and therefore, might increase the predisposition towards TB infection. Our previous work reveals that *M.tb* PPE2 is a secretory protein that affects innate immunity of the host, and plays a role in the virulence process of *M.tb*. Since innate immunity is dictated by various bone marrow-derived cells and since during infection, *M.tb* is shown to be disseminated to mice bone marrow, we next investigated whether PPE2 influences bone marrow hematopoiesis to facilitate better survival of the bacilli.

PPE2 protein of *M. tuberculosis* induces myeloid cytopenia in blood, and confers survival advantage to the bacterium in mice: To understand how PPE2 protein of

M.tb influences adult hematopoiesis, we expressed *PPE2* gene in a non-pathogenic *M. smegmatis* mc^2155 (M. smeg-PPE2), which is widely used as a surrogate bacterium to characterize *M.tb* proteins. *M. smegmatis* harboring the empty vector (pVV16) was used as control (M. smeg-pVV16). About 8-10 weeks old BALB/c mice were either left uninfected or infected with 10×10^7 colony forming units (CFUs) of either M. smeg-pVV16 or M. smeg-PPE2 by intravenous injection. Mice were sacrificed 3, 5 and 7 days after infection, and the blood samples were collected and analyzed for complete blood profiles. As expected, mice infected with M. smeg-pVV16 had increased blood cells in peripheral circulation when compared with uninfected healthy mice at all the time points investigated (Fig. 1A) as infections are known to be associated with increased blood cells in peripheral circulation to fight the invading pathogens. However, significant reduction of blood cell population, mainly of myeloid origin (monocyte, neutrophil, eosinophil, basophil, platelets, reticulocytes and mast cells) was observed in mice infected with M. smeg-PPE2 when compared with mice infected with M. smeg-pVV16 at all the time points (Fig. 1A). Lymphocyte population remained unperturbed (Fig. 1A). Mice infected with M. smeg-PPE2 were found to be anemic. Loss in body weight was noted in mice infected with M. smeg-PPE2 as compared to mice infected with M. smeg-pVV16 and uninfected mice. Since, at day 5 post-infection, we observed significant suppression of most of the blood cell counts, subsequent experiments were carried out at day 5 post-infection. We also observed a higher bacterial load of M. smeg-PPE2 in lung, liver and spleen tissues compared to M. smeg-pVV16 (Fig. 1B). Together, these observations reveal that PPE2 induces myeloid cytopenia, along with better survival of bacilli in the host.

M.tb PPE2 protein affects bone marrow cellularity, and induces loss of myeloid-progenitor cells in the bone marrow of mice during infection: Based on the differences in blood profile of M. smeg-pVV16- *versus* M. smeg-PPE2-infected mice, we next compared cellularity/cytology of bone marrow. Comparison of the Myeloid:Erythroid (M:E) ratio derived from the bone marrow provides information regarding changes in the myeloid population and myeloid hematopoiesis. Femur bones were collected from all the groups at day 5 post-infection. Bone marrow cells were isolated, and analyzed for Myeloid:Erythroid (M:E) ratio (Table 1). Approximately 500 myeloid and erythroid cells were counted, and ratio for myeloid to erythroid cells was calculated by counting the total myeloid precursors in proportion to the total erythroid precursors. The M:E ratio is higher in M. smeg-pVV16 infected mice as compared to uninfected control which is probably due to infection (Table 1). However, this ratio is found to be lower in mice infected with M. smeg-PPE2 as compared to mice infected with M. smeg-pVV16 suggesting that myeloid cell numbers were reduced in mice infected with M. smeg-PPE2 (Table 1). For further understanding any altered pattern of cellular maturation in the bone marrow, Maturity index was calculated in the bone marrow of mice infected with either M. smeg-pVV16 or M. smeg-PPE2. Proliferating myeloid cells represent the population of precursor cells (immature) undergoing proliferation to maintain a constant supply of myeloid cells. Whereas non-proliferative myeloid cells represent a population of mature myeloid cells. Maturity index refers to the ratio of proliferating (immature) cells to the non-proliferating cells (mature cells). In our results, the maturity index of M. smeg-PPE2-infected mice was found to be 1:0.9428, whereas it was 1:3.171 and 1:2.268 in case of M. smeg-pVV16-infected mice and uninfected healthy controls, respectively (Table 2). Increased number of proliferating cells but decreased number of non-proliferating cells in M. smeg-PPE2-infected mice as compared to M. smeg-pVV16-infected mice suggests a halt in the development of myeloid precursor cells by PPE2.

To study the cellularity of the bone marrow, femurs from all the groups were collected, fixed and stained with H&E. It was observed that mice infected with M. smeg-pVV16 had a marked increment in mature myeloid cells with mild hyperplasia of myeloid cells whereas M. smeg-PPE2 had a mildly hypo-cellular bone marrow with increased immature myeloid cells (Fig. 2A). This shows that infection with M. smeg-pVV16 increases the number of myeloid cells, while PPE2 lowers the myeloid cell population in the bone marrow of mice. Additionally, there was no sign of cell death in the bone marrow derived from M. smeg-PPE2-infected mice indicating that lower myeloid cells population in M. smeg-PPE2-infected mice were not due to cell death.

In adults, hematopoietic stem cells (HSCs) proliferate and produce heterogeneous populations of different blood

cells. When cultured in a semi-solid medium along with specific cytokines, progenitor cells proliferate and differentiate to form discrete cell clusters or CFUs. Based on the number of circulating white blood cells (WBCs) and red blood cells (RBCs) in peripheral blood, we next examined the bone marrow function during infection of mice with M. smeg-PPE2. Five day post-infection, bone marrow cells of uninfected, M. smeg-pVV16- and M. smeg-PPE2-infected mice were harvested from femur, and cultured in cytokines enriched MethoCult™ GF M3434 medium. Bone marrow cells of uninfected mice showed consistent colonies derived from granulocyte/ monocyte progenitor cells (CFU-GM) and multi-potential progenitor cells for myeloid cells [granulocyte, erythroid, macrophage, megakaryocyte progenitor cells (CFU-GEMM)]. Total and granulocyte colonies (CFU-GM and CFU-GEMM) were higher in number in M. smeg-pVV16-infected mice as compared to control, which was expected (Fig. 2B). However, interestingly, total as well as granulocyte colony numbers were significantly reduced in mice infected with M. smeg-PPE2 as compared to mice infected with M. smeg-pVV16 (Fig. 2C-E). These results indicate that in the presence of PPE2, myeloid hematopoiesis of the bone marrow is greatly impaired which is correlated with the observed blood cytopenia. We further stained bone marrow population using Ab against CD11b (granulocyte surface marker) as a representative of myeloid lineage. We found that mice infected with M. smeg-PPE2 showed significant reduction in CD11b+ population at day 5 post-infection as compared to mice infected with M. smeg-pVV16 (Fig. 2F, G). This further confirms that PPE2 suppresses the myeloid lineage.

PPE2 activates induction of IFN-γ in mice during infection*:* Interestingly, not M. smeg-pVV16, but M. smeg-PPE2 were found to be present in the bone marrow of infected mice. Since IFN-γ is shown to inhibit HSC activity, we next examined the levels of IFN-γ in the sera of infected mice and we found that mice infected with M. smeg-PPE2 had increased levels of IFN-γ as compared to mice infected with M. smeg-pVV16 and uninfected control mice. We also examined the transcript levels of IFN-γ in the bone marrow microenvironment using real-time PCR, and found higher levels of IFN-γ transcripts in M. smeg-PPE2-infected mice when compared with M. smeg-pVV16-infected mice. These results suggest that PPE2 probably influences bone marrow hematopoiesis *via* IFN-γ. Thus, our study reveals that higher bacterial burden of M. smeg-PPE2 in the lung, liver and splenic tissues could be due to suppression of adult bone marrow hematopoiesis (in the present study) as well as inhibition of nitric oxide and reactive oxygen species by PPE2, as demonstrated by us earlier (*Bhat et al [2017]Scientific Reports 7:39706; Srivastava et al [2019] Journal of Immunology. 203:1218*). Together, it can be inferred that PPE2 acts in a pleotropic manner to support bacterial survival (*Published in Immunobiology [2021]*).

Table 1. Myeloid : Erythroid (M:E) ratio

Animal ID	Myeloid cells (M) (Mean ± SEM)	Erythroid cells (E) (Mean ± SEM)	Other cells (Mean ± SEM)	M:E ratio (Mean ± SEM)
Uninfected	243 ± 8.363	182 ± 3.787	74.60 ± 11.15	1.335 ± 0.0354
M. smeg-pVV16	422 ± 10.76	58 ± 6.904	20.20 ± 6.053	7.916 ± 1.242
M. smeg-PPE2	302.0 ± 17.72	102 ± 8.240	96.00 ± 22.91	3.003 ± 0.1813*

Table 1. Myleoid to Erythiod (M:E) ratio in bone marrow of mice infected with either M. smeg-pVV16 or M. smeg-

PPE2

BALB/c mice were infected with 10 × 10^7 CFUs of either M. smeg-pVV16 or M. smeg-PPE2 *via* intravenous route. Uninfected mice were kept as healthy control. After 5 days of infection, femur bone marrow cells were collected from each group, and analyzed for M:E ratio. Total of 500 cells were counted. Results are shown as mean ± SEM of 5 mice per group. Unpaired t-test was used to calculate p values. (*p < 0.05 as compared with M. smeg-pVV16 group)

Table 2. Maturation index (MI) of bone marrow

Category		Uninfected	M. smeg-pVV16	M. smeg-PPE2
1. Myeloid cells	Proliferating (Mean ± SEM)	79 ± 6.120	108.0 ± 3.521	180 ± 4.261
	Non-proliferating (Mean ± SEM)	172 ± 6.592	341 ± 13.98	170.0 ± 8.319
Myeloid maturation index		1:2.268 ± 0.2732	1:3.171 ± 0.04329	1:0.9428 ± 0.04329*

Table 2. Maturity index of bone marrow of mice infected with either M. smeg-pVV16 or M. smeg-PPE2 BALB/c mice were infected with 10 × 10^7 CFUs of either M. smeg-pVV16 or M. smeg-PPE2 *via* intravenous route. Uninfected mice were kept as healthy control. After 5 days of infection, femur bone marrow cells were collected from each group, and analyzed for Maturity index. Total of 500 cells were counted. Results are shown as mean ± SEM of 5 mice per group. Unpaired t-test was used to calculate p values. (*p < 0.001 as compared with M.

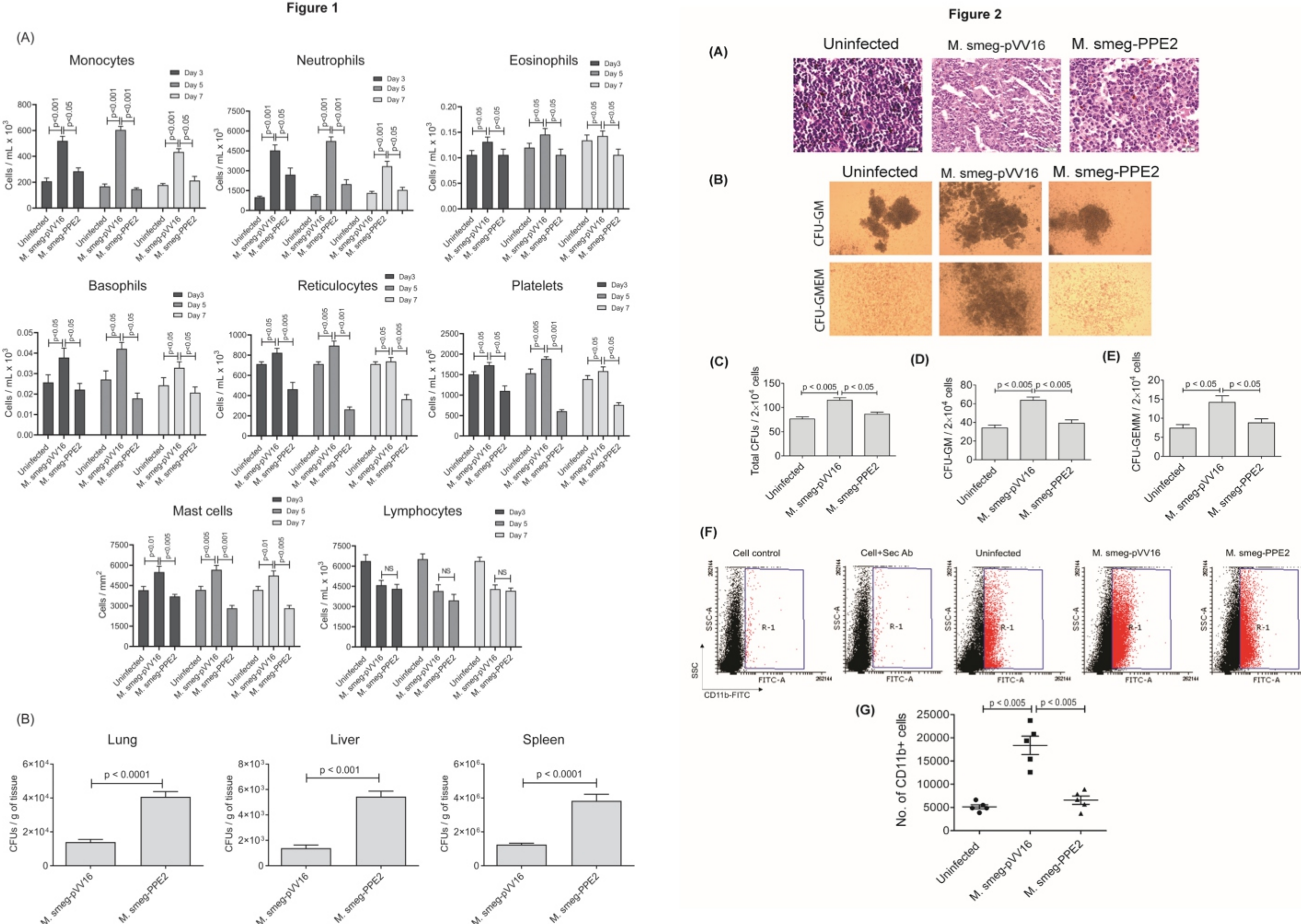


Figure 1. PPE2 protein of *M.tb* induces blood cytopenia of myeloid cells, and confers survival advantage to the bacterium in mice. BALB/c mice were infected with 10 × 10^7 CFUs of either M smeg-pVV16 or M. smeg-PPE2 *via* intravenous route. Uninfected mice were kept as healthy control. After day 3, 5 and 7, blood samples were collected, and analyzed for complete blood profiles (A). Data represent mean ± SEM of 5 mice per group. In another experiment, BALB/c mice were infected with 10 × 10^7 CFUs of M smeg-pVV16/M. smeg-PPE2 *via* intravenous route and at day 5 post-infection, lung, liver and spleen tissues were harvested and homogenates were prepared for CFU determination (B). CFUs were counted as per gram of the tissue. Data represent mean ± SEM of 7 mice per group.

Figure 2. *M.tb* PPE2 protein affects bone marrow cellularity and induces loss of myeloid progenitor cells in the bone marrow of mice during infection. BALB/c mice were infected with 10 × 10^7 CFUs of either M smeg-pVV16 or M. smeg-PPE2 *via* intravenous route. Uninfected mice were kept as healthy control. After 5 days of infection, femur bone was collected and paraffin sections were prepared and stained with hematoxylin and eosin (H&E). Photographs of representative sections were visualized at 40X magnification. Scale bar = 20 µm (A). Also, femur bone marrow cells were harvested, and 2 × 10^4 cells were cultured. Morphology (B) and population (C-E) of the colonies were analyzed under phase contrast microscope after 9 days of culture. Photographs of representative sections were visualized at 5X magnification. Around 0.5-1.0 million bone marrow cells were stained with anti-CD11b Ab, and surface expression of CD11b was analyzed by flow cytometry (F). Area outlined as R1 represents gating strategy. $CD11b^+$ cells were counted and plotted for all experimental groups (G). Results shown are mean ± SEM of 5 mice per group.

Publications:

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

1. Pal R and Mukhopadhyay S (2021). PPE2 protein of *Mycobacterium tuberculosis* affects myeloid hematopoiesis in mice. *Immunobiology* 226: 152051 (Impact factor 3.18).
2. Dolasia K, Nazar F and Mukhopadhyay S (2021). *Mycobacterium tuberculosis* PPE18 protein inhibits MHC class II antigen presentation and B cell response in mice. *European Journal of Immunology* 51: 603-619. (Impact Factor – 5.179)
3. Jha V, Pal R, Kumar D and Mukhopadhyay S (2020). ESAT-6 protein of Mycobacterium tuberculosis increases holotransferrin mediated iron uptake in macrophages by downregulating surface hemochromatosis protein HFE. *Journal of Immunology* 205: 3095-3106 (Impact factor – 5.05).

ii) Patent filed

Mukhopadhyay S, Pal R and Battu MB. Therapeutic composition for Inflammation/Tissue Injury.

Indian Patent has been filed on January 7, 2020 (Priority date – January 8, 2019); Patent No. 201941000876

The US patent application has been filed on January 8, 2020 at the US Patent Office (USPTO) and the application number accorded is '16737012'.

Laboratory of Molecular Cell Biology

Laboratory of Molecular Oncology

RESEARCH

Genomics and molecular genetics of cancer

Principal Investigator: **Murali Dharan Bashyam**
Staff Scientist

Ph D Students

Srinivas Animireddy	Senior Research Fellow (Till October 2020)
Sara Anisa George	Senior Research Fellow
Diya Sarkar	Junior Research Fellow (Till January 2021)
Pradipta Hore	Senior Research Fellow
Sanjana Sarkar	Junior Research Fellow
Shaily Agrawal	Junior Research Fellow
Devaunshi Mudodi	Junior Research Fellow (Since October 2020)
Sumaiya Sabnam	Junior Research Fellow (Since October 2020)

Other Members

Ajay Kumar Chaudhary	Technical Officer
Asmita Gupta	Research Associate
Raju Kumar	Research Associate (Till November 2020)
Neetu Sharma	Research Associate
Padmavathi Kavadipula	Project SRF
Indra Sara Lama	Project JRF (Till November 2020)
Shivani Yadav	Project JRF
Barsha Bharati	Project JRF
Mandla Vasanth Kumar	Project JRF (Since January 2021)

Collaborators

Ashwin Dalal	CDFD, Hyderabad
R Sabarinathan	NCBS, Bengaluru
K Madhumohan	NIMS, Hyderabad

Objectives

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

Research Summary:

Project title: Characterization of novel oncogenic role(s) of rare but India-specific *TP53* mutant forms.

Concise Report: Functional assays performed in cell lines, following ectopic expression of non-hotspot p53 mutant proteins identified in ESCC tumours, led to the confirmation of pro-oncogenic property of the mutants. RT-qPCR analysis performed on ESCC tumours revealed significantly higher expression of the targets *ARF6*, *C1QBP* and *TRIM23* in tumour samples harbouring mutant p53. The expression levels of the targets were also observed to be elevated in cell lines upon ectopic expression of p53 mutants as well as in ESCC tumours versus normal samples. shRNA-based knockdown of the targets resulted in a significant reduction in the proliferative and migratory potential of cancer cells. Thus, our work has revealed novel oncogenic transcriptional targets for non-hotspot mutant p53 relevant for ESCC.

Future plans and directions:

Characterization of mode of activation of novel transcriptional targets of *TP53* mutants.

Project title: Genomics of SARS-CoV-2

Concise report: The work focused on analysing genome-wide nucleotide variations in the SARS-CoV-2 isolates collected from Telangana as well as from other Indian states. Our analysis revealed the dominance of the B.1 lineage during March to August 2020 in Telangana (Figure 1). Thereafter, there appeared to be an absence of a clear dominant lineage during August 2020 till February 2021

(Figure 1). However, in March, 2021, the emergence of B.1.617.1 (Kappa) variant as the dominant lineage was observed along with a few cases of the B.1.617.2 (Delta) variant, a trend that was also seen in other Indian states (Figure 1). The Kappa and Delta variants were marked by the presence of characteristic spike protein mutations including T19R, G142D, del156, P681R, E484Q, L452R, T478K, D614G.

Future plans and directions:

Analyses of SARS-CoV-2 genomes to track emergence of new variants of concern and to identify new mutations in functionally relevant viral proteins.

Publications

Research papers published in 2020

Adduri RSR, George SA, Kavadipula P, Bashyam MD. SMARCD1 is a transcriptional target of specific non-hotspot mutant p53 forms. **J Cell Physiol,** 2020; 235:4559-4570.

Research papers published in 2021

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.

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.

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

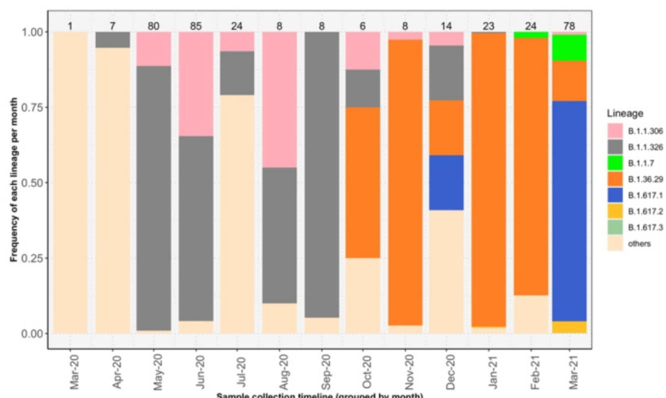


Figure :1. Trends in dominant SARS-CoV-2 lineages in Telangana (top) and India (bottom) plotted over time.

Laboratory of Molecular Oncology

Laboratory of Plant Microbe Interaction

RESEARCH

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

Principal Investigator:	**Subhadeep Chatterjee**
	Staff Scientist
PhD Students:	
Biswajit Samal	Senior Research Fellow
Prashantee Singh	Senior Research Fellow
Yasobanta Padhi	Senior Research Fellow
Chayan Bhattacharjee	Junior Research Fellow
Kanishk Saraf	Junior Research Fellow
Project:	
Raj Kumar Verma	Senior Research Fellow
Dayakar	Senior Research Fellow
Parimala Gundu	Junior 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

Bacteria coordinate their social behavior in a density dependent manner by production of diffusible signal molecules by a process known as quorum sensing (QS). Sensing and adaptation to changing environmental conditions were traditionally attributed to two-component sensors and response regulators. Increasing volume of work now suggest that coordination of responses to fluctuating environment is very complex, as many of the microbial species live in community under natural condition. We are using *Xanthomonas* and *Pseudomonas* group of plant pathogens which makes diverse quorum sensing signaling molecule to address the mechanism of integration and adaptation to changing environmental condition. Our work has shown that fine tuning of QS regulatory circuits in closely related members of the *Xanthomonas* group of phytopathogens contribute to their lifestyle change inside the host. We are also trying to understand how the QS-mediated social structure and individuality in the bacteria coexists to improve their fitness in fluctuating environments. We are using genetics and molecular tools to understand the mechanism of switching of lifestyle of bacteria from a planktonic to sessile biofilm. In this we are trying to understand the role of adhesions, virulence factors, nutrient and environmental sensing which plays a role in coordinating the lifestyle switch. Iron is required for virulence of several animal and plant pathogenic bacteria. The availability of iron within the host plays a critical role in the growth and survival of the pathogens. Ability of the pathogens to sequester host iron and respond to host iron status has been proposed to be critical for virulence and survival of plant pathogens. Although iron has been implicated in the virulence of pathogenic bacteria, very little is known about how pathogens acquire complex iron source and maintain iron homeostasis inside host. It has been proposed that pathogens modulate their metabolism and virulence associated functions depending on iron availability, wherein, iron availability act as a signal for coordinated regulation of different cellular functions. We are trying to understand the mechanism of how iron plays a major role in regulating diverse cellular process and production of virulence associated functions. We are trying to understand the mechanism of iron dependent regulation of virulence associated function and would like to address how iron and virulence associated functions are co-

ordinately regulated in host-pathogen interactions. We are also trying to understand the mechanism by which novel pathogen molecules belonging to Pathogen associated molecular patters are able to induce innate immune response in host using plant and *Xanthomona*s interaction as a model system.

Research summery (April 1, 2019 – March 31, 2020)

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

Pathogenic bacteria exhibit tight regulation of virulence associated functions such as iron homeostasis, production of virulence factors in order to meet rapid changes in the environmental conditions to adapt to living in different environmental conditions, including in the host. Bacteria must adapt in quick time against fluctuating environmental condition as well as it has to coordinate several social task in response to its density. Sensing and adaptation to changing environmental conditions were traditionally attributed to two component sensors and response regulators, which are involved in regulating expression of genes required for adaptation to changing environment or stress. 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.

In this study we show that a bacteriophytochrome (*Xoo*BphP), from a non-photosynthetic phytopathogen of rice, *Xanthomonas oryzae*, perceives light signal and transduce through its EAL-mediated phosphodiesterase activity, modulating the intracellular level of the ubiquitous bacterial second messenger cyclic-di-GMP. We discover that the XooBphP integrate photo-sensing and fine-tune intracellular second messenger c-di-GMP level in response to different wavelength of light, which plays a crucial role in transition of sessile to planktonic motile lifestyle and regulation of several virulence associated functions. Here we show that a bacteriophytochrome (*Xoo*BphP), 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 *Xoo*BphP 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. *Xoo*BphP 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 (Fig. 1)

Project 2: Bacterial QS expands the *Xanthomonas campestris* pv. *campestris* invasion of host tissue to trigger host-chlorophagy and maximize disease symptom.

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.

Quorum Sensing (QS) helps the *Xanthomonas* group of phytopathogens to infect several crop plants. In the case of vascular phytopathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) infection on leaves of *Brassicaceae*, the black rot disease. The symptom often develops with a typical V-shaped lesion spanning both vascular and mesophyll regions that proceeds with progressive leaf chlorosis and drying of the host-leaf tissue from the infection site. In general, the role of autophagy in triggering host-chlorosis during a pathogen attack has been widely demonstrated. Recently, the QS-benefits in the *Xcc* population have been elucidated within-host vasculature during early infection. However, detailed insight into the possible role of QS-regulated bacterial invasion in host-chlorophagy during the late infection stages remained elusive to date. Here, using QS-responsive whole-cell bioreporters of *Xcc* and cabbage (*Brassica oleracea*) as a model system and confocal microscopy, we show a detailed chronology of QS-

facilitated *Xcc* colonization in the host mesophyll region. We report the QS-enabled bacterial localization of parenchymal chloroplast within heterogeneously invaded host mesophyll tissue, leading triggered leaf-chlorosis and systemic infection. 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. Altogether, our results reveal that QS-response in the *Xanthomonas* group of vascular phytopathogens maximizes the population fitness across host tissues to trigger stage-specific host- chlorophagy and establish a systemic infection

Publications:

(I) Research papers published in the calendar year 2020:

1. Chatterjee S, Samal B, Singh P, Pradhan B.B, and Verma R.K. (2020). Transition of a solitary to a biofilm community life style in bacteria: Social cooperation and conflict in a unison response. Int. J. Dev. Biol. 64:269-275.
2. Verma RK, Biswas A, Kakkar A, Lomada SK, Pradhan BB, Chatterjee S. (2020). A Bacteriophytochrome Mediates Interplay between Light Sensing and the Second Messenger Cyclic Di-GMP to Control Social Behavior and Virulence. Cell Rep. 32(13):108202. doi: 10.1016/j.celrep.2020. 108202. PMID: 32997993.

(ii) Research papers in press as on 31st March 2021
Nil

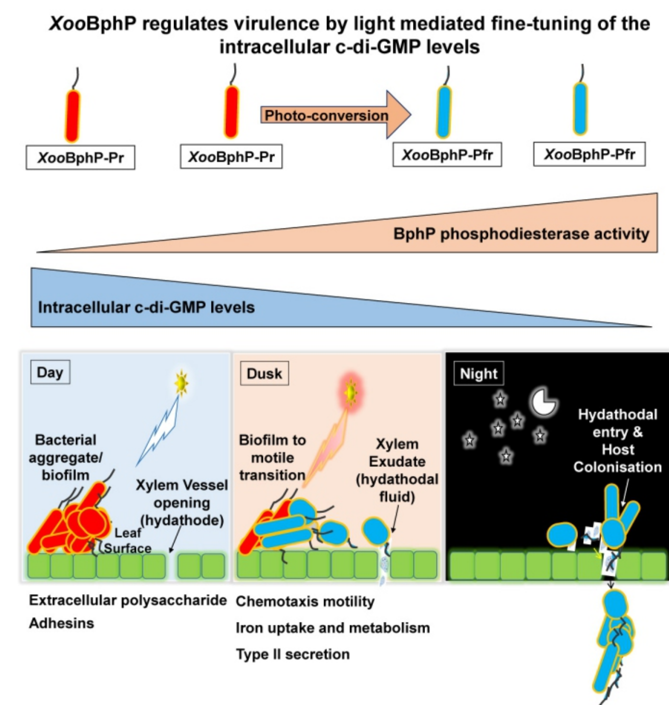


Figure 1. A bacterial photoreceptor mediates light sensing and modulates transition of social behavior from sessile to motile lifestyle. Light. Photo-sensing stimulate dynamic change in intracellular second messenger c-di-GMP, which regulates several cellular and virulence associated functions. At day time, c-di-GMP level is higher due to Pfr conformational form of the photo sensor, triggering biofilm formation and night, the c-di-GMP level falls, triggering chemotaxis driven motility which promotes bacterial entry, production of virulence factor and colonization inside host

Laboratory of Plant Microbe Interaction

Laboratory of Transcription

RESEARCH

Bacterial transcription terminator Rho and mycobactericidal proteins from mycobacteriophages

Principal Investigator: **Ranjan Sen**
Staff Scientist

Names and designations of Ph.D. students:

Md. Hafeezunnisha	Senior Research fellow
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
Amit Kumar	Postdoctoral Fellow
Naveen Kumar	Postdoctoral Fellow
Sapna Godavarthi	Technical Officer (till November 2020)
B. Yogesh	Technical Assistant-I (since Feb 2021)

Collaborators names and brief affiliation:

Prof Markus Wahl	Freie Universität Berlin, Germany.
Prof. Udayditya Sen	SINP, Kolkata, India.
Agneiszka Szaleskewa	Palasz University of Gdnask, Poland.

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.

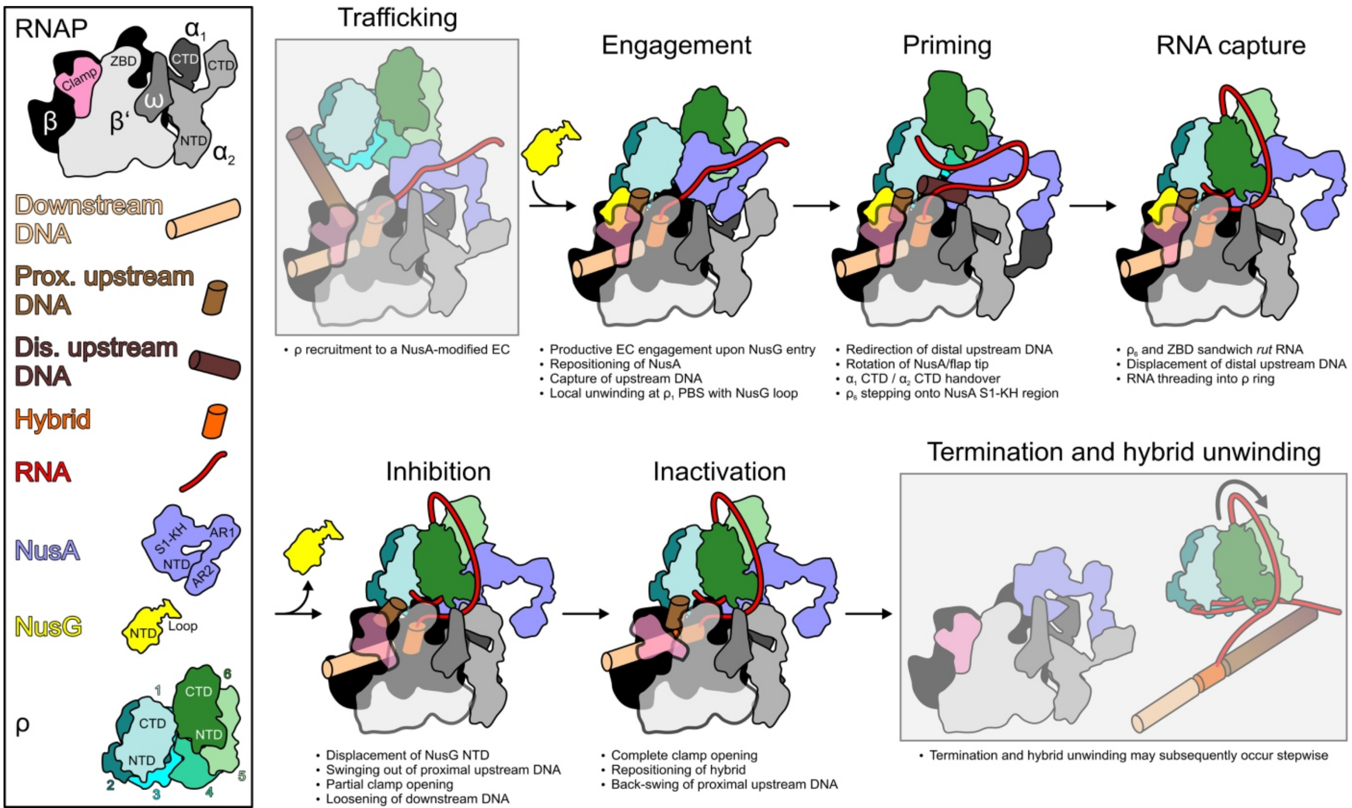
Details of the progress made in the current reporting year (1st April 2020- 31st March 2021):

Design of novel peptide-inhibitors against the conserved bacterial transcription terminator, Rho. The transcription terminator Rho regulates many physiological processes in bacteria, such as antibiotic sensitivity, DNA repair, RNA-remodelling, etc, and hence, is a potential antimicrobial target, which is unexplored. The bacteriophage P4 capsid protein, Psu, moonlights as a natural Rho antagonist. Here, we report the design of novel peptides based on the C-terminal region of Psu using phenotypic screening methods. The resultant 38-mer peptides, in addition to containing mutagenized Psu sequences, also contained plasmid sequences, fused to their C-termini. Expression of these peptides inhibited the growth of *E. coli* and specifically inhibited Rho-dependent termination *in vivo*. Peptides 16 and 33 exhibited the best Rho-inhibitory properties *in vivo.* Direct high-affinity binding of these two peptides to Rho also inhibited the latter's RNA-dependent ATPase and transcription termination functions *in vitro*. These two peptides remained functional even if 8-10 amino acids were deleted from their C-termini. *In-silico* modeling and genetic and biochemical evidence revealed that these two peptides bind to the primary RNA binding site of the Rho hexamer near its subunit interfaces. Additionally, the gene expression profiles of these peptides and Psu overlapped significantly. These peptides also inhibited the growth of *Mycobacteria* and inhibited the activities of Rho proteins

from *M. tuberculosis*, *Xanthomonas*, *V. cholerae,* and *S. enterica*. Our results showed that these novel anti-Rho peptides mimic the Rho-inhibition function of the ~42 kDa dimeric bacteriophage P4 capsid protein, Psu. We conclude that these peptides and their C-terminal deletion derivatives could provide a basis on which to design novel anti-microbial peptides (AMP).

***E. coli* cryptic prophage expressions are controlled by Rho-dependent transcription termination primarily to regulate their toxin-antitoxin modules.** Bacterial Rho-dependent transcription termination regulates many physiological processes. Here, we report that it controls the expressions 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. The *in vivo* termination efficiency and the mRNA sequence analyses 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 concluded that Rho-dependent termination silences specifically the TA modules of prophages thereby augmenting the bacterial innate immunity.

RNA Polymerase-Rho interaction during the termination process*:* While the nascent RNA plays important role in recruiting Rho to the elongating RNA polymerase (RNAP), Rho can interact with regions of RNAP, especially near the RNA exit channel. In an international collaborative effort between our lab and those from Germany and the USA, we have solved structures of different RNAP-Rho complexes formed during the termination process. Using extensive mutagenesis of the RNAP-beta prime subunit followed by in vivo assays performed in our laboratory functionally validated Rho-RNA exit channel interaction. Figure 1 depicts a multi-step pathway for Rho-EC interaction during the termination process.



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 and iv) characterization of the Rho-RNAP-NusA-NusG interaction during the transcription termination process.

Publications / patents:

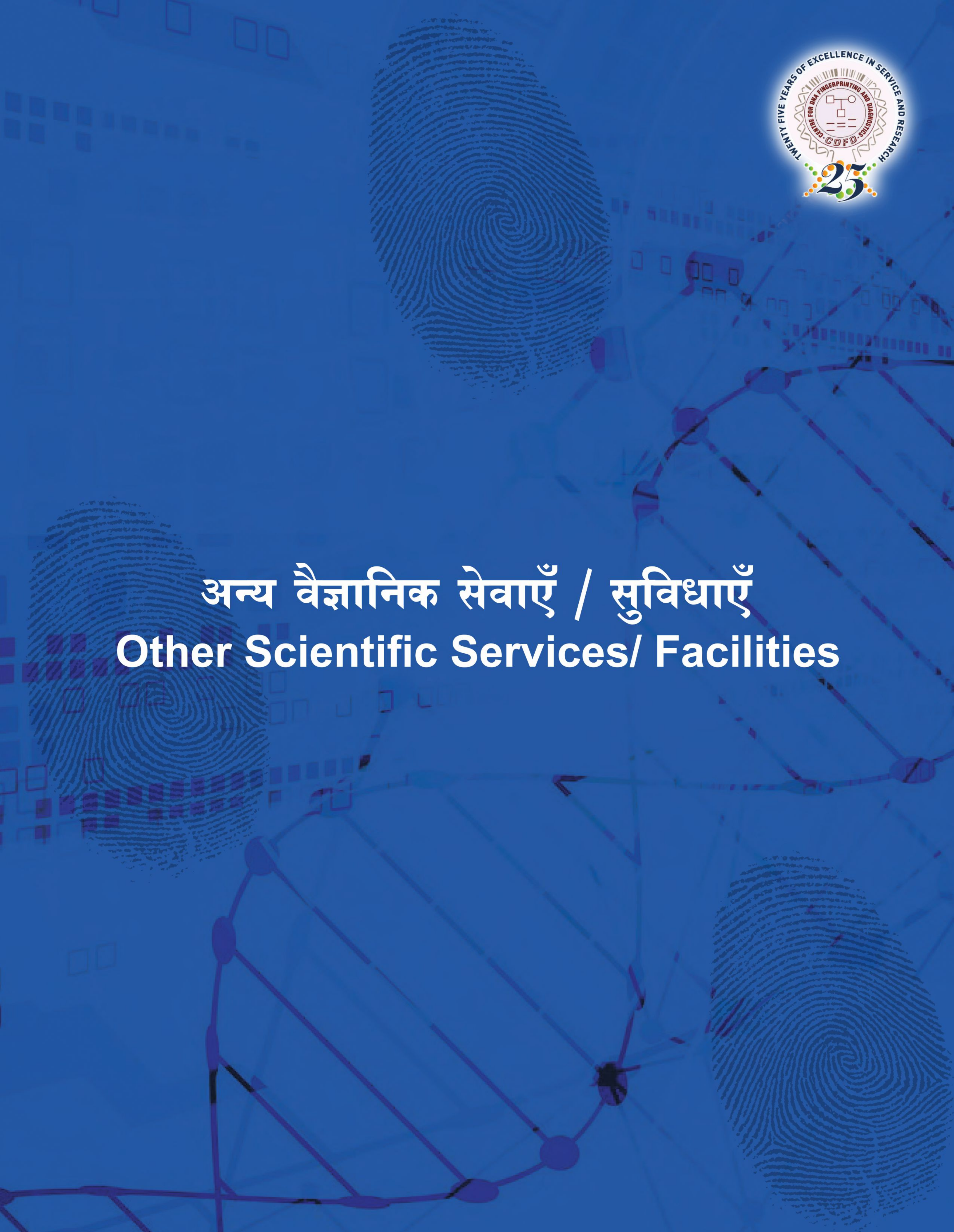
1) **Publications in press:**

1. 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.*** **In press.**

Publications 2020-2021

1. Said, N., Hilal, T., Sunday, N. D., Khatri, A., Bürger, J., Mielke, T. Belogurov, G. A., Loll, B., **Sen, R.**, Artsimovitch, I. and Wahl, M. C. (2021). Steps toward translocation-independent RNA polymerase inactivation by terminator ATPase ρ. ***Science,*** Jan 1;371(6524): eabd1673.
2. Hafeezaunnisa, M. and **Sen, R.** (2020). The Rho-Dependent Transcription Termination Is Involved in Broad-Spectrum Antibiotic Susceptibility in Escherichia coli. ***Front. Microbiol.*** 11:605305. doi: 10.3389/fmicb.2020.605305.

Laboratory of Transcription



अन्य वैज्ञानिक सेवाएँ / सुविधाएँ
Other Scientific Services/ Facilities

Laboratory of Experimental Animal Facility

Understanding and regulation of advanced glycation endproducts (AGE) mediated deleterious effects

Principal Investigator: **Dr. Pranjali Pore**
Staff Scientist

Officer-In-Charge (Consultant): Dr. S. Harinarayana Rao

Other Members:
Arikothan Sheeba
Kadingula Pavan

Faculty Co-coordinators: Dr. Rashna Bhandari,
Staff Scientist

Dr. Murali Bashyam,
Staff Scientist

Objectives:

Our objective of the Experimental Animal Facility (EAF) is to(i)breed, maintain and supply laboratory animals to 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 experimentation and breeding.

Service

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

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 CDFD Institutional Animal Ethics Committee (IAEC) was held on July,6th 2020, 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 sixth meeting of CDFD Institutional Animal Ethics Committee (IAEC) was held on 10th November 2020 for review and approval of all ongoing and new studies conducted by CDFD scientists and the annual inspection was held on January, 9th 2021 for yearly inspection and review.

After permission from CPCSEA, CDFD Experimental Animal facility brought Sprague Dawley rats and New Zealand White rabbits for polyclonal Antibody Generation. As per Standard Operating Procedures, rats were quarantined for 7 days and rabbits were quarantined for 14 days and then shifted to Experimental rooms for further procedures. No health-related issues and no mortalities were noticed during the transfer and the quarantine period. 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 IVCs were brought and installed for rat experimentation. 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 had been brought and installed for specialized experiments in mice. 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 750 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 2020 to 31st March 2021, and supplied to users during 1st April 2020 to 31st March 2021.

Strain	Breeding (Male + Female)	Supplied
BALB/c	189+380	1138
C57BL/6	36 + 56	99
Ip6k1	56 + 105	53
NnatΔNEO/ΔI^{2}	06 +12	Only Maintenance
Foxn1^{nu}	32 + 64	214
SD rats	Only Supply	06
NZW Rabbits	Only Supply	08

The experiments conducted during this period are listed below:

- 542 BALB/c mice were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.
- 53 *Ip6k1* mice were used for histopathological and physiological analyses of testes and gastrointestinal tract.
- 140 BALB/c mice were injected intravenously with *Candida glabrata* for studies on comparative bio-burden of different *Candida* strains.
- 86 BALB/c mice were used to study the effect of *Mycobacterium tuberculosis* protein PPE18 on caecal ligation and puncture-induced sepsis.
- 214 *FoxN1*^{nu} athymic mice were injected with oncogenic cell lines to study tumor progression and metastasis.
- 84 BALB/c mice were used to study the effect of *Mycobacterium tuberculosis* protein PPE18 coated nanoparticles on microbial sepsis.
- 86 BALB/c mice were used to study the *in vivo* anti-inflammatory roles of recombinantly purified PPE2 and PPE18 proteins of *Mycobacterium tuberculosis*
- 99 C57BL/6 and 200 BALB/c mice were injected with thioglycolate by intra-peritoneal route for the generation of macrophages.
- 06 SD rats were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.
- 08 NZW rabbits were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.

Future direction

The CDFD EAF is 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 - Retro-Orbital blood collection in BALB/c mouse.
Fig.2 – Intra-peritonial injection in C57BL/6 mouse.
Fig.3 – Markings of subcutaneous injections in rabbit for Polyclonal Antibody Generation.
Fig.4 – Metabolic cage for mouse experimentation.

Group of Experimental Animal Facility

BIOINFORMATICS

In-charge

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

Members

R Chandra Mohan	Technical Officer
Prashanthi Katta	Junior Assistant
Dinesh Thakur	Junior Assistant (till Oct 2020)
B.Vijaykumar	Computer Programmer (Project)
B Laxminarayana	Computer Technician
V. Murali Krishna	SWA (till Oct 2020)

Objectives

We provide critical IT services for the institute is to maintain various servers, workstations, PCs, printers and other peripheral devices; to maintain and regularly update the CDFD website; to provide web based services and e-mail services, institute-wide LAN/WAN as well as internet connectivity; to secure CDFD network from network security threats; to integrate institute's network into National and International grid computing networks; and to coordinate the procurement and installation process of servers, workstations, PCs, laptops, printers, other peripheral devices with requisite software/licenses.

Details of works undertaken during the period April 1, 2020-March 31, 2021:

Activities related to installation, administration and maintenance of high-end servers which provide various services, databases and computational jobs as well as installation of newly procured PCs with anti-virus software were undertaken.

Internet, web, email and other intranet services are being maintained in-house and provided to users with upgraded functionalities.

We have also been participating in creating NGC website. Further, we have been involved in and redesigning CDFD website as per the Government of India Guideline.

We have initiated procurement of over 50 new PCs to replace old outdated ones.

In addition, we have also initiated procurement of high end servers and a new Data centre racks for NGC project.

The AMC support renewal of existing high-end servers; domain service and SSL certificate renewal was also carried out.

Bioinformatics Group

INSTRUMENTATION

In-charge: **R.N.Mishra**

Member:
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 2020-21

During the year 2020-21, we have installed 110 Nos. new equipments including Gen ASI's High Band System, Refrigerated Centrifuge, Nucleic Acid Separation System, Sorval Centrifuge, Hemoglobin Variant Analyzer, Fast Prep -24, Digital Dry Bath, 4200 Tape Station, Next Seq 2000 Sequencing System, Biorupter Plus, De Humidifier FFB-170, Cold Cabinets, Elisa Microplate Reader, Semi Automated Microtome, BD FACS Aria Fusion System, AB 3500XL Genetic Analyser, PCR Work Station, Cell Density Meter, Animal Facility IVC System & Cage Changing System Etc.

Setup the COVID – 19 Testing Laboratory with Equipments. We have completed 201 maintenance work orders, 122 Pipette calibrations, Completed 87 NOC's for purchase of new equipments, maintaining the communication system. We have maintained most of the Instruments maximum uptime in the Laboratory. 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 Group

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 (Out sourcing)
Mohd. Mudassir	Technical Officer (Out sourcing)
Abhijeet	Technical Officer (Out sourcing)
Viswa Kalyan	Technical Officer (Out sourcing)
Tripti Sharma	Technical Assistant (Out sourcing)

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 leads to better utilization of instruments.

Summary of work done till March 2020

- Activities related to installation, administration and maintenance of various sophisticated equipments 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.

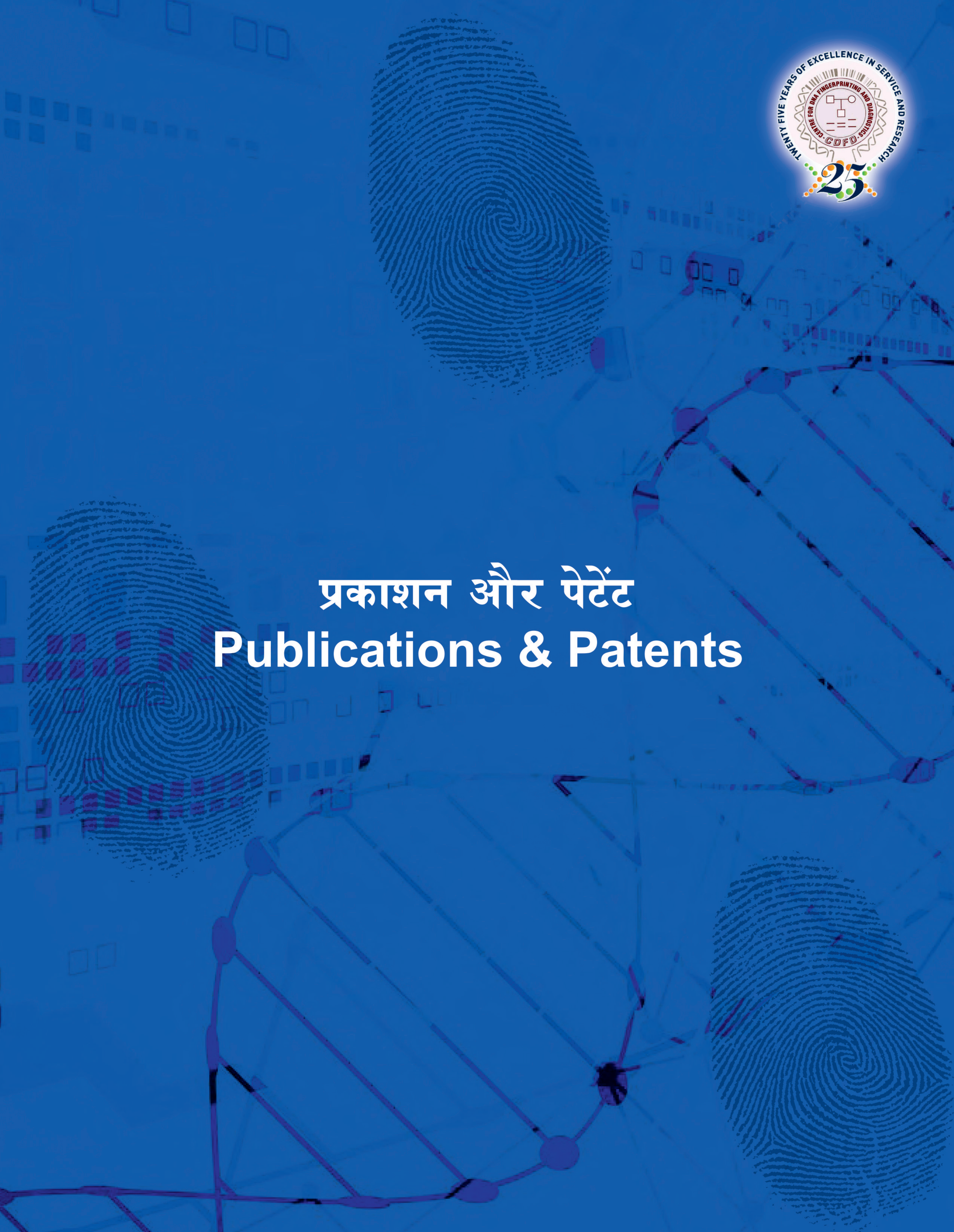
Details of Progress made in the current reporting year April 1, 2020 to March 31, 2021)

- New additions- 3500 XL genetic analyzer, Confocal Super Resolution (LSM 980), atomic absorption spectrophotometer (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 sent to various 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	15747 Samples
Confocal LSM 700	861 hours
Confocal Leica SP-8	591 hours
Super Resolution LSM 980	259 hours
FACS ARIA III	291 Samples
FACS ARIA Fusion	425 Samples
LSR Fortessa	337 Samples
CD Spectropolarimeter	18 Users
RT-PCR	637 Users
Histopathology	258 Samples

- Revenue generated for the year April 2020 –March 2021 was Rs. 2682494/- (Rupees twenty-six lakhs eighty-two thousand four hundred nighty four).

Sophisticated Equipment Facility Group



प्रकाशन और पेटेंट
Publications & Patents

CDFD Publications FY 2020-2021

(1 April 2020 to 31 March 2021)

1. Adduri, Raju S. R.; George, Sara A.; Kavadipula, Padmavathi; Bashyam, Murali D (2020). SMARCD1 is a transcriptional target of specific non-hotspot mutant p53 forms. ***Journal of Cellular Physiology***. 235(5): 4559-4570.
2. Agrahari, Raj Kishan; Singh, Prashantee; Koyama, Hiroyuki; Panda, Sanjib Kumar (2020). Plant-microbe Interactions for Sustainable Agriculture in the Post-genomic Era. ***Current Genomics***. 21(3): 168-178.
3. Agarwal, Rachna; Trivedi, Jay; Mitra, Debashis (2020). High yield production of recombinant cyanovirin-N (antiviral lectin) exhibiting significant anti-HIV activity, from a rationally selected Escherichia coli strain. ***Process Biochemistry***. 93: 1-11.
4. Afroz, Sumbul; Battu, Srikanth; Giddaluru, Jeevan; Khan, Nooruddin (2020). Dengue Virus Induced COX-2 Signaling Is Regulated Through Nutrient Sensor GCN2. ***Frontiers in Immunology***. 11: (1831).
5. Anupama, Kornepati; Pranathi, Karnati; Meenakshi Sundaram, Raman (2020). Assessment of genetic purity of bulked-seed of rice CMS lines using capillary electrophoresis. ***Electrophoresis***. 41(20): 1749-1751.
6. Bakshi, Asif; Joshi, Rohit (2020). Role of glial niche in regulating neural stem cell proliferation in Drosophila central nervous system. ***Journal of Neuroscience Research***. 98(12): 2373-2375.
7. Bakshi, Asif; Sipani, Rashmi; Ghosh, Neha; Joshi, Rohit (2020). Sequential activation of Notch and Grainyhead gives apoptotic competence to Abdominal-B expressing larval neuroblasts in Drosophila Central Nervous System. ***Plos Genetics***. 16(8): (e1008976).
8. Bera, Pradip; Aher, Abhishek; Brandao, Paula; Manna, Sunil Kumar; Mondal, Gopinath; Jana, Abhimanyu; Santra, Ananyakumari; Jana, Harekrishna; Bera, Pulakesh (2020). Induced apoptosis against U937 cancer cells by Fe(II), Co(III) and Ni(II) complexes with a pyrazine-thiazole ligand: Synthesis, structure and biological evaluation. ***Polyhedron***. 182: (114503).
9. Bose, Jagadeesh Chandra K.; Kapoor, Bishwajit Singh; Mandal, Kamal; Ghosh, Shubhrima; Mokhamatam, Raveendra B.; Manna, Sunil K.; Mukhopadhyay, Sudit S (2020). Loss of Mitochondrial Localization of Human FANCG Causes Defective FANCJ Helicase. ***Molecular and Cellular Biology***. 40(23): (e00306-20).
10. Chatterjee, Subhadeep; Samal, Biswajit; Singh, Prashantee; Pradhan, Binod B.; Verma, Raj K (2020). Transition of solitary to biofilm community life style in bacteria: a survival strategy with division of labour. ***International Journal of Developmental Biology***. 64(4-6): 259-265.
11. Dolasia, Komal; Nazar, Faiza; Mukhopadhyay, Sangita (2020). Mycobacterium tuberculosis PPE18 protein inhibits MHC class II antigen presentation and B cell response in mice. ***European Journal of Immunology***. 51(3):603-619.
12. Dutta, Usha R.; Suttur, Malini S.; Venugopal, Vineeth S.; Posanapally, Laxmi Priyanka; Gopalasetty, Sravani; Talwar, Sangamesh; Anand, Suhana; Billapati, Sushmita; Jesudasan, Rachel A.; Dalal, Ashwin (2020). Cytogenetic and molecular study of 370 infertile men in South India highlighting the importance of copy number variations by multiplex ligation-dependent probe amplification. ***Andrologia***. 52(10): (e13761).
13. Ghosh, Debasish Kumar; Ranjan, Akash (2020). The metastable states of proteins. ***Protein Science***. 29(7): 1559-1568.
14. Girisha, Katta Mohan; Pande, Shruti; Dalal, Ashwin; Phadke, Shubha R (2020). Untapped opportunities for rare disease gene discovery in India. ***American Journal of Medical Genetics Part A***. 182(12): 3056-3059.
15. Hafeezunnisa, Md; Sen, Ranjan (2020). The Rho-Dependent Transcription Termination Is Involved in Broad-Spectrum Antibiotic Susceptibility in Escherichia coli. ***Frontiers in Microbiology***. 11: (605305).
16. Jana, Abhimanyu; Aher, Abhishek; Brandao, Paula; Ali, Syed Samim; Samanta, Sandip Kumar; Mondal, Gopinath; Bera, Pradip; Santra, Ananyakumari; Manna, Sunil Kumar; Mahapatra, Ajit Kumar; Bera, Pulakesh (2020). Picoline based fluorescence 'turn-on' chemosensor for zinc(II) ion recognition, cell

imaging and cytotoxicity study: Synthesis, crystal structure, spectroscopy and DFT. ***Polyhedron***. 192: (114815).

17. Jha, Vishwanath; Pal, Ravi; Kumar, Dhiraj; Mukhopadhyay, Sangita (2020). ESAT-6 Protein of Mycobacterium tuberculosis Increases Holo-transferrin-Mediated Iron Uptake in Macrophages by Downregulating Surface Hemochromatosis Protein HFE. ***Journal of Immunology***. 205(11):3095-3106.
18. Kasbekar, Durgadas P (2020). Fungal senescence induced by the Neurospora sen mutation and mitochondrial plasmids - the contributions of Ramesh Maheshwari. ***International Journal of Developmental Biology***. 64(1-3): 29-34.
19. Kasbekar, Durgadas P (2020). A cross-eyed geneticist's view VI. Segregation distortion in Drosophila melanogaster: Recent progress in solving 'an esoteric puzzle'. ***Journal of Biosciences***. 45(1): (139).
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(A) PATENTS

DETAILS OF PATENT APPLICATIONS FILED (NATIONAL & INTERNATIONAL) :

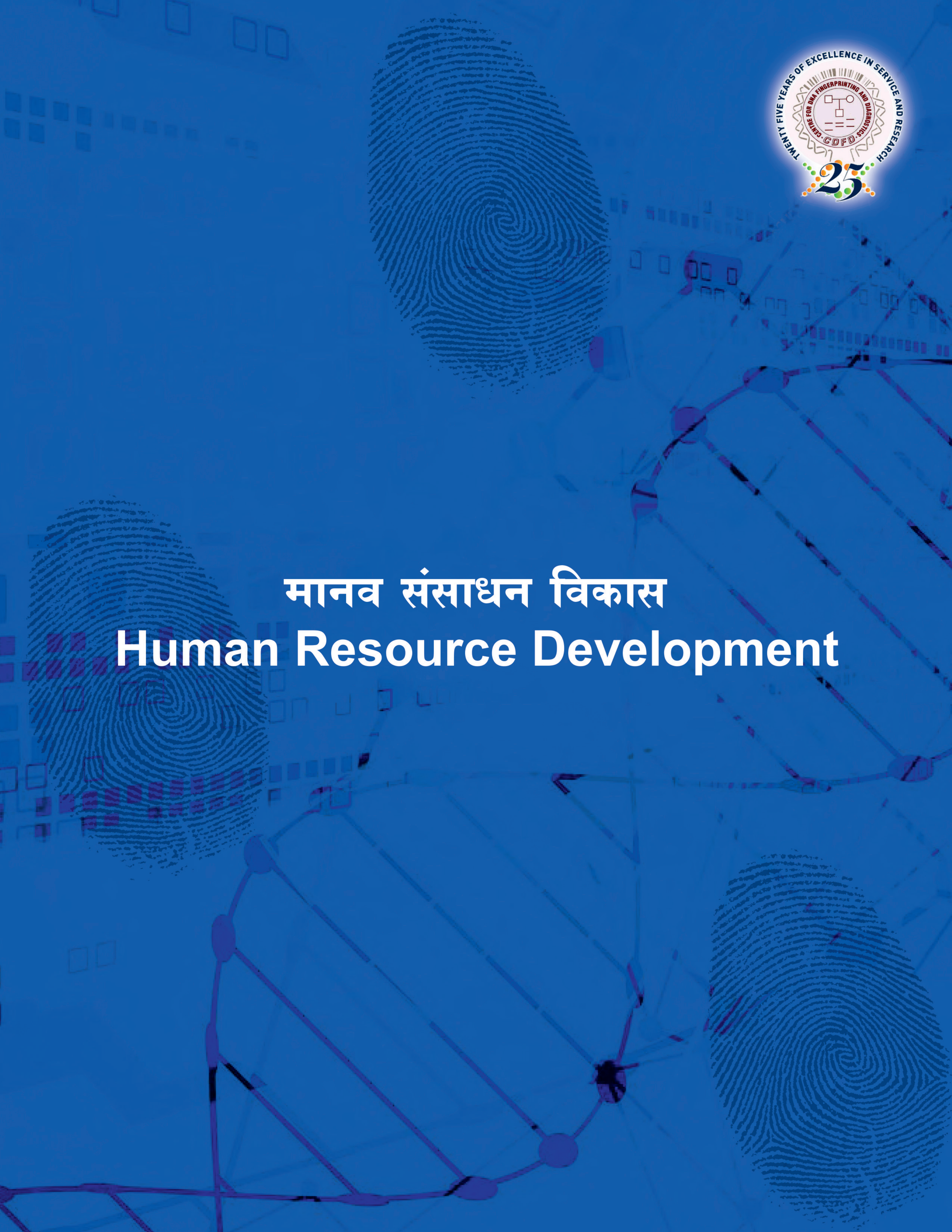
Period	No. of Patent Applications Filed	Sl. No.	Country of filing	Application No.	Date of filing	Title of Patent Application
01.04.2020 **to** 31.03.2021	**01**	1	**India**	**202041028231**	**02.07.2020**	Xanthoferrin producing mutant strain and process thereof **(PI : Dr Subhadeep Chatterjee)**

DETAILS OF PATENT APPLICATIONS GRANTED (NATIONAL & INTERNATIONAL) :

Period	No. of Patent Applications Granted	Sl. No.	Country of filing	Patent No.	Grant Date	Title of Patent Application
01.04.2020 **to** 31.03.2021	Nil					

(B)TECHNOLOGIES DEVELOPED / TRANSFERRED / COMMERCIALIZED

Sl. No.	Period	Technologies		
		Developed	Transferred	Commercialized
1	01.04.2020 **to** 31.03.2021	Nil	Nil	Nil


TWENTY FIVE YEARS OF EXCELLENCE IN SERVICE AND RESEARCH
CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
CDFD
25
मानव संसाधन विकास
Human Resource Development

Ph.D. 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, or AcSIR. 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, 2021 the Centre has 93 Research Scholars working for their doctorates in different areas of research. In the reporting year, 6 of the Research Scholars have completed PhD and are pursuing careers in science elsewhere in India or abroad.

Postdoctoral Program

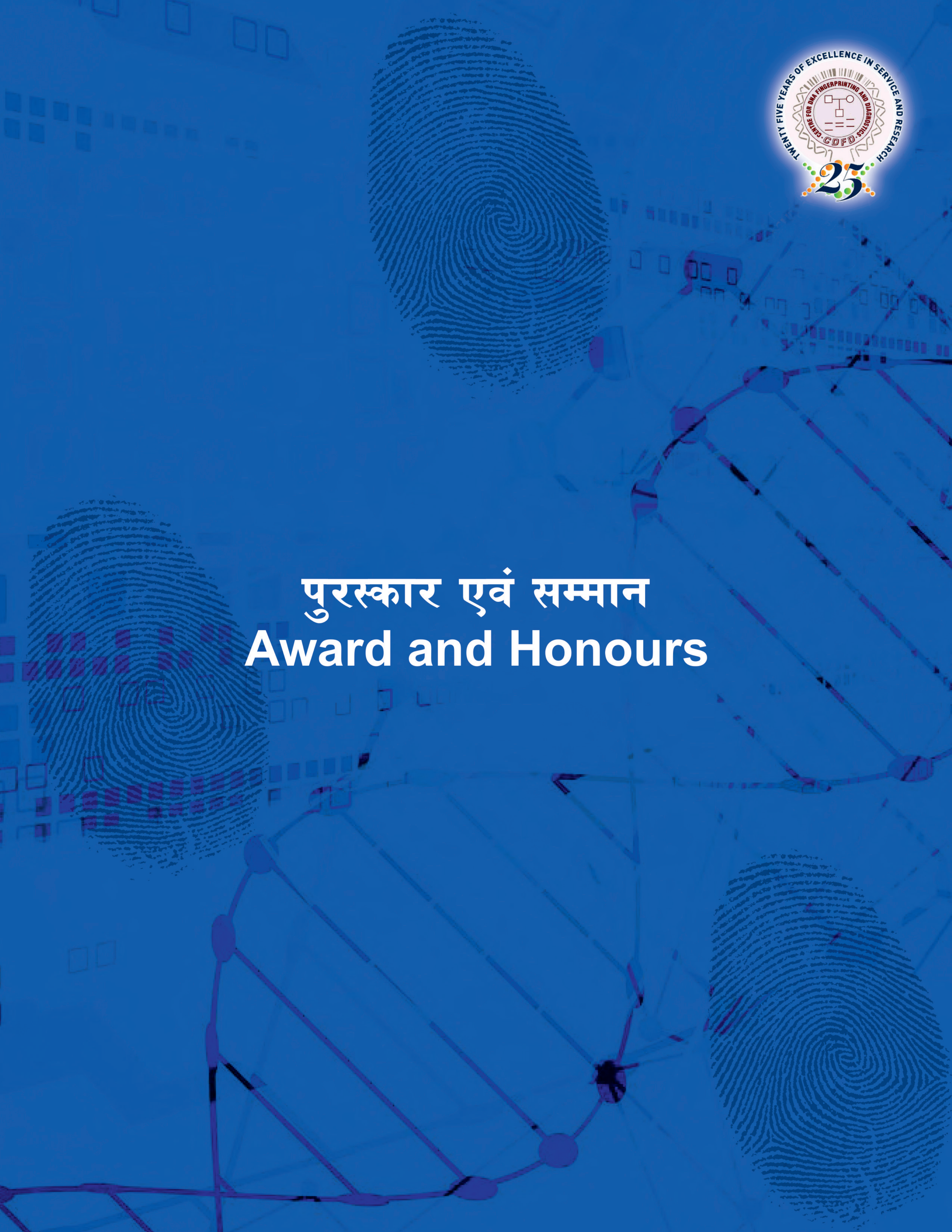
In addition to the JRF program, the Centre also carries out training at the post-doctoral level. The post-doctoral fellows are funded through extramural grants that CDFD receives. Some are also selected competitively by various schemes of Government of India such as the DST fast track young scientist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

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 NIL students received summer training at the Centre (due to the ongoing pandemic).

Dissertation based Research Training for students

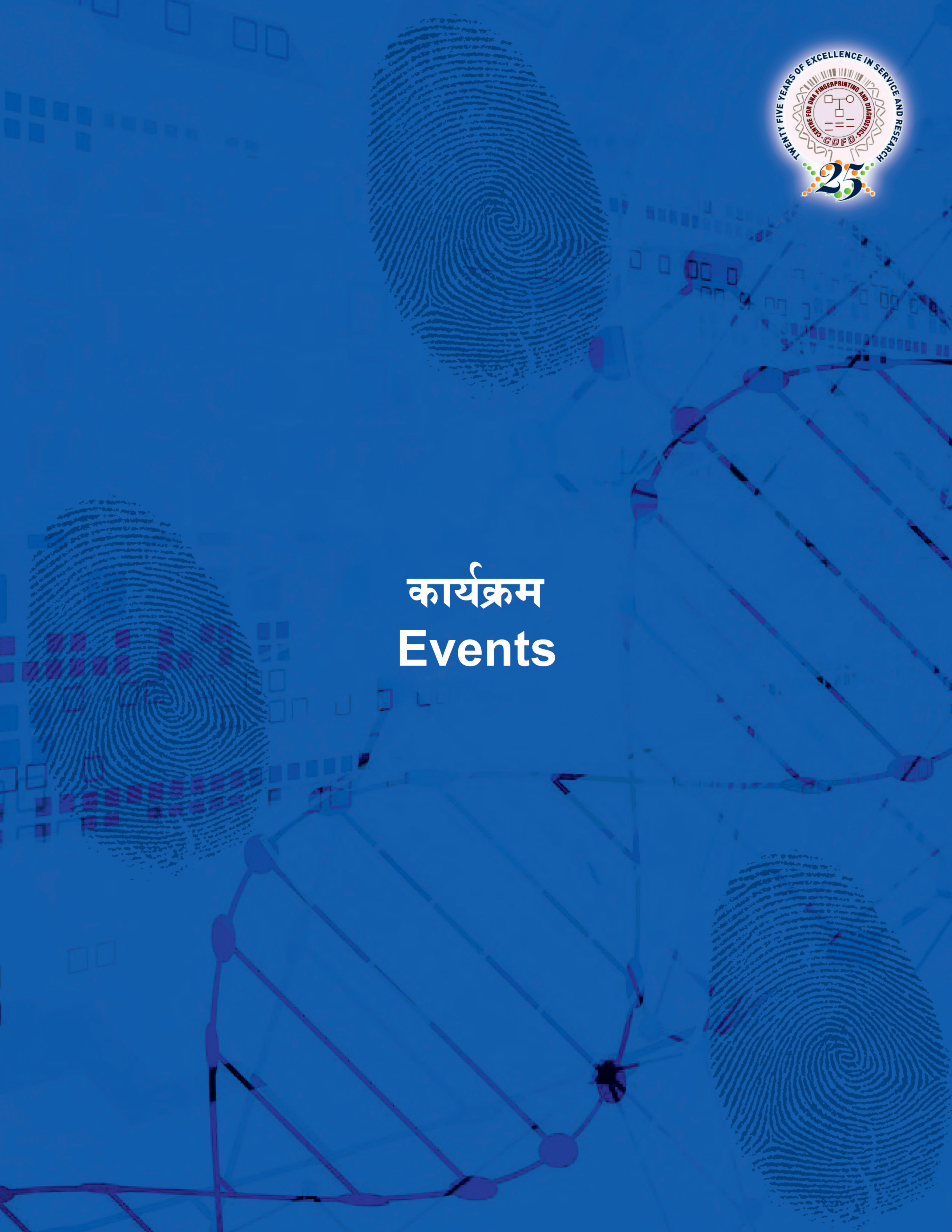
Under this programme, the students spend 4 - 6 months at CDFD and work on active projects being carried out by CDFD faculty. The project work helps the students in gaining hands-on experience in modern biology. In the reporting year, NIL students were given the opportunity to avail training under this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



पुरस्कार एवं सम्मान
Award and Honours

AWARDS & HONOURS

	FACULTY & STAFF	
1.	Dr Subhadeep Chatterjee	1) Shanti Swarup Bhatnagar prize for the year 2020 in Biological Sciences by the Council of Scientific and Industrial Research (CSIR). 2) Assigned as Senior Editor in Journal Phytopathology, An International Journal of the American Phytopathological Society (APS). 3) Elected as fellow of the Indian National Science Academy (INSA) for the year 2021.
2.	Dr. Rupinder Kaur	Appointed as Associate Editor of the journal “Frontiers in Fungal Biology” for the section ‘Fungal Genomics and Evolution’.
3.	Dr. M. Subba Reddy	Elected as fellow of the Indian Academy of Sciences (IAS) for the year 2021.


TWENTY FIVE YEARS OF EXCELLENCE IN SERVICE AND RESEARCH
CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
CDFD
25
कार्यक्रम
Events

IMPORTANT EVENTS

Sl. No.	Event	Date
1.	Observance of Anti-Terrorism Day and administering of pledge	21.05.2020
2.	Observance of Sadbhavana Diwas	20.08.2019
3.	22nd CDFD RAP-SAC meeting	24-25 August 2020
4.	Online Hindi Day celebrations	14.09.2020
5.	A Webinar by Dr. Ashwin Dalal on "NGS technology and data analysis". Training Programme on Advanced Bioinformatics Tools and its Applications in Agriculture organized by National Academy of Agricultural Research Management (NAARM), Hyderabad.	19.09.2020
6.	Finance Committee meeting	28.09.2020
7.	Governing Council meeting	29.09.2020
8.	Visit of Junior Civil Judges from TSJA, Secunderabad as a part of Training programmes-XXIV Basic Course Part-II Mid Term Practical Training.	21.10.2020
9.	Vigilance Awareness Week	27.10.2020 to 02.11.2020
10.	Observance of Rashtriya Ekta Diwas (National Unity Day)	02.11.2020
11.	Observance of Constitution Day-Preamble Reading alongside with Hon'ble President of India on DD National Channel and also a talk by Dr. Repalle Shiva Praveen Kumar, IPS, Secretary, Social Welfare and Tribal Welfare Residential Schools, Govt. of Telangana under the Constitution Day Celebrations.	26.11.2020
12.	'Vigyan Yatra' was conducted at DBT-CDFD as a part of IISF-2020 Celebrations.	14.12.2020
13.	25th Foundation Day Lecture in DBT-CDFD on "Genomic Medicine in Neuromuscular Diseases: from Molecular Genetics to Diagnosis and Treatment" by Prof. Michael G Hanna, Director UCL Institute of Neurology London, UK.	28.01.2021
14.	Program on "Awareness on Organ Donation" along with Gandhi Hospital on virtual platform.	29.01.2021

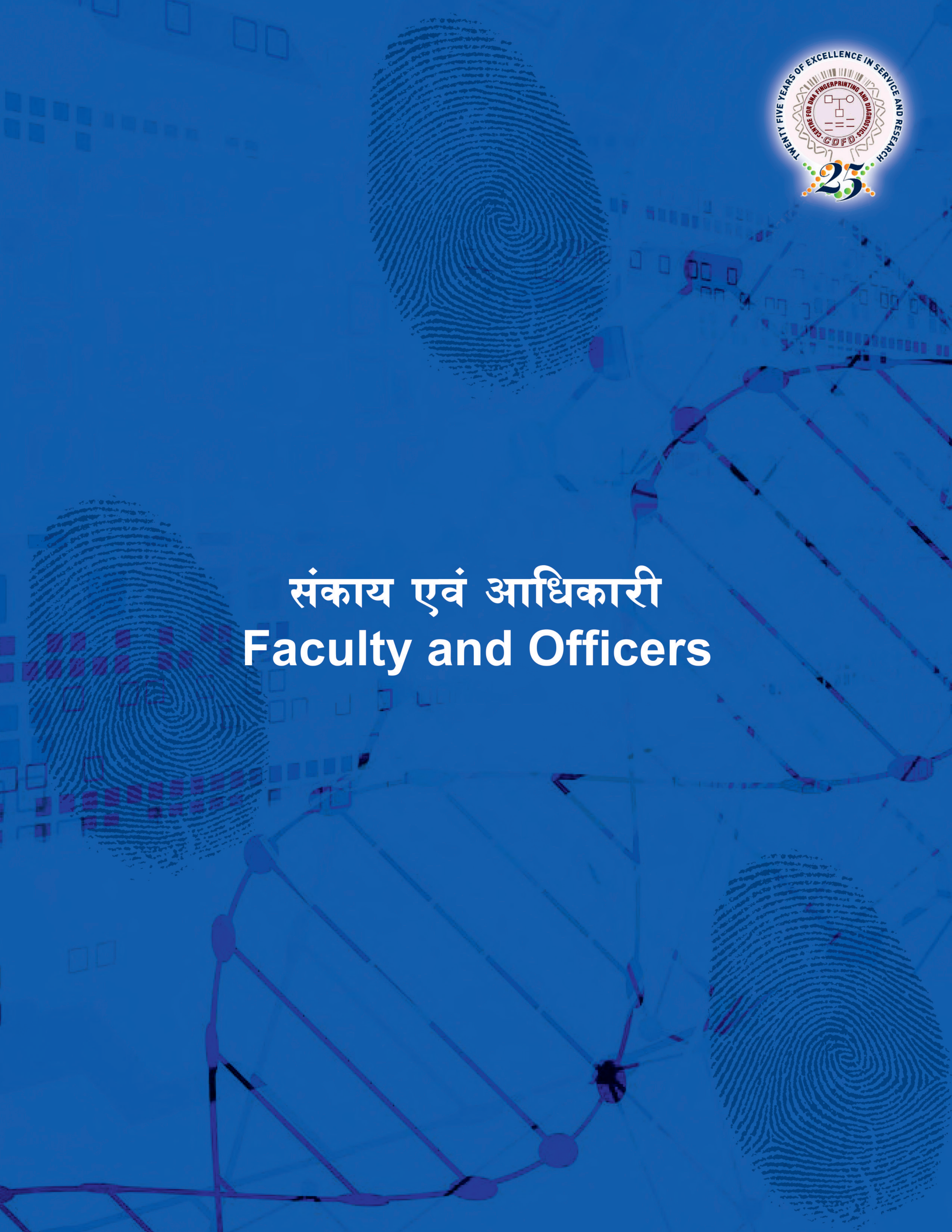
15.	Vaccination of 75 Corona Warriors against COVID 19 at CDFD	03.02.2021
16.	Observance of 32nd National Road Safety Month	15.02.2021
17.	Visit of Hon'ble Vice President of India Shri M. Venkaiah Naidu and Hon'ble Home Minister of Telangana Shri Mohammed Mahmood Ali to DBT-CDFD and Hon'ble Vice President of India inaugurated the Paediatric Rare Genetic Disorders Laboratory	20.02.2021
18.	National Science Day, Dr. K. Thangaraj, Director CDFD delivered a talk on "Where did we come from and why are we unique" under the Science Outreach Program "BRIDGE" for DBT star Colleges.	25.02.2021
19.	Participation in Global Bio India – 2021 (Virtual)	01.03.2021 to
20.	Hands on Workshop on Next Generation Sequencing Data Analysis for Clinical Diagnostics	01.03.2021 to
21.	2nd Dose of Vaccination of Corona Warriors against COVID 19 at CDFD	03.03.2021
22.	25th Society meeting of CDFD	05.03.2021
23.	International Women's Day celebrations	08.03.2021
24.	43rd Finance Committee meeting	11.03.2021
25.	MoU between CDFD, Hyderabad and Genome Foundation, Hyderabad to supplement, support and strengthen resources of both the organizations for patients suffering from genetic disorders.	22.03.2021
26.	50th Governing Council meeting	23.03.2021

Virtual Lectures/ talks by CDFD Scientists

Sl.No.	Talk	Date
1	Talk by Dr. K. Thangaraj, on “Mitochondrial diseases: an integrative approach to diagnosis and treatment ”	25.09.2020
2	Talk by Dr. Rashna Bhandari for Biology Departmental Webinar series for Monsoon 2020	29.09.2020
3	Talk by Dr. K. Thangaraj on “The Chronicle of Our Past and Present" during IISF-2020 Celebrations.	14.12.2020
4	Talk by Dr K. Thangaraj on DNA Technology Regulation Bill.	17.12.2020
5	Dr. Rashna Bhandari delivered Popular lecture hosted by Visvesvaraya Industrial & Technological Museum on "Women scientists at the forefront of fight against COVID-19" on account of International Day of Women and Girls in Science.	11.02.2021
6	Online Interaction with Experts of MSc Biochemical Technology, Team, Scigupshup - Interview talk on Bhatnagar prize work by Dr. Subhadeep Chatterjee.	11.02.2021
7	Talk by Dr. Subhadeep Chatterjee on account of Researchers’ Day - 2021 on 22.02.2021 organised by Gitam Institute of Science, Visakhapatnam.	22.02.2021
8	Talk by Dr. K. Thangaraj on “Neuromuscular disorders of mitochondrial origin”	24.02.2021
9	Talk by Dr. K. Thangaraj delivered a talk on "Where did we come from and why are we unique” under the Science Outreach Program “BRIDGE” for DBT star Colleges on the occasion of National Science Day.	25.02.2021

Virtual Lectures/ talks by outside Scientists and Professors

Sl.No.	Talk	Date
1.	Talk by Prof. Jay Gopalakrishnan, Laboratory for Centrosome and Cytoskeleton Biology | Institute of Human Genetics, Universitätsstr, Universitätsklinikum | Heinrich-Heine-Universität Düsseldorf on “Human brain organoids; From development to diseases modeling of COVID19”	01.10.2020
2.	Talk by Dr. Hilary Martin, Wellcome Sanger Institute, Cambridge on "Genetics architecture of developmental disorders and population genetics of British Pakistanis".	16.11.2020
3.	25th Foundation Day Lecture on “Genomic Medicine in Neuromuscular Diseases: from Molecular Genetics to Diagnosis and Treatment” by Prof. Michael G Hanna, Director UCL Institute of Neurology London, UK.	28.01.2021
4.	Talk by Dr. Sreedhar Chinnaswamy, Associate Professor, NIBMG, Kalyani on “Genetics, transcription and immunobiology of human lambda interferons and my future interests”	02.03.2021



संकाय एवं आधिकारी
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. R Harinarayanan

ADJUNCT FACULTY

Dr. E A Siddiq, Prof. Jayashankar Telangana State Agricultural University
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

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Mr. Vinod Kumar Mishra
Ms. M Kavita Rao
Dr. V Punnaiah
Mr. K Arun Kumar
Mr. Rabinarayan Mishra

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Mr. E V Rao

Director’s Office

Science Communication Section

Administration Section

DDO Section

Estate Section

Security Section

Transport Section

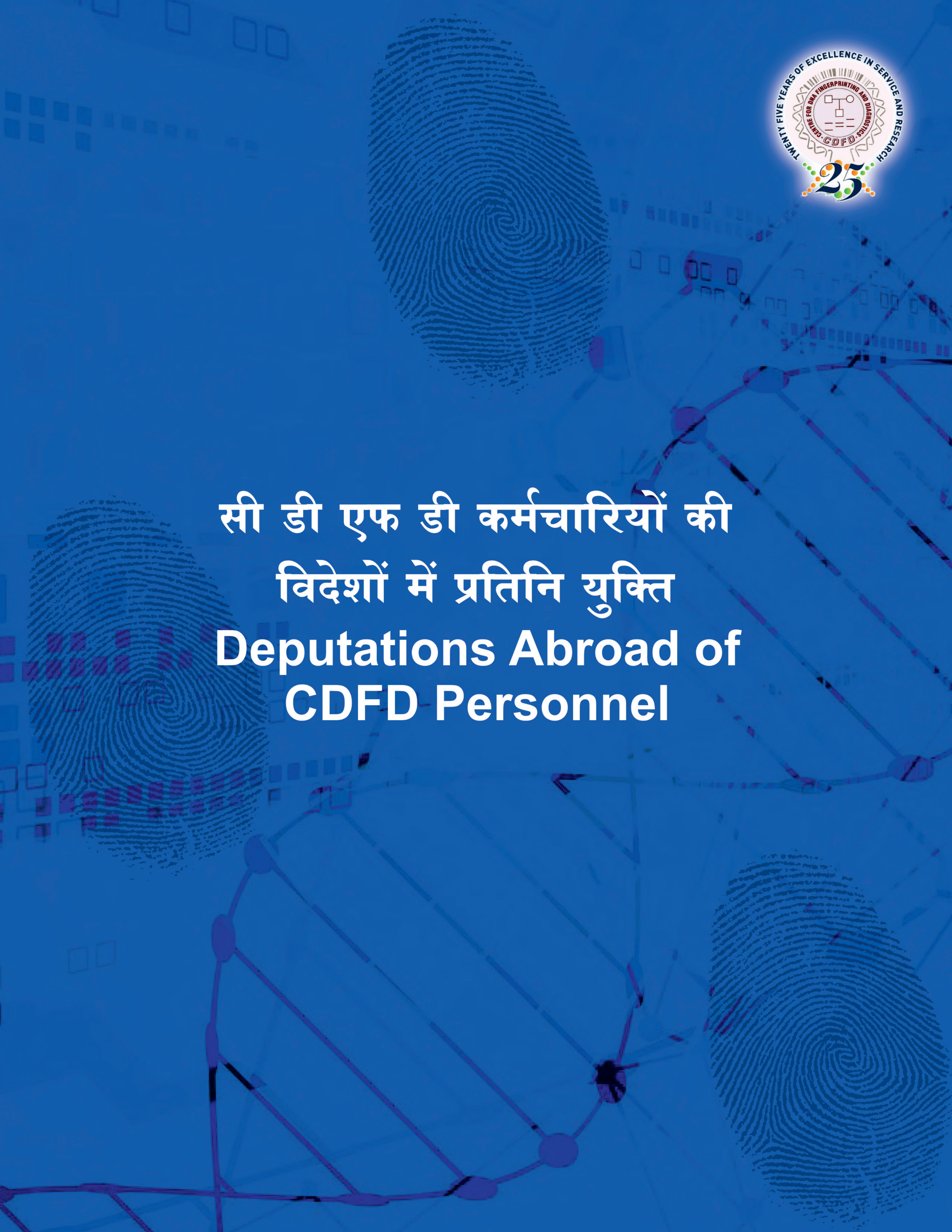
Finance and Accounts Section

EMPC and Academics Section

Stores and Purchase Section

Library Section

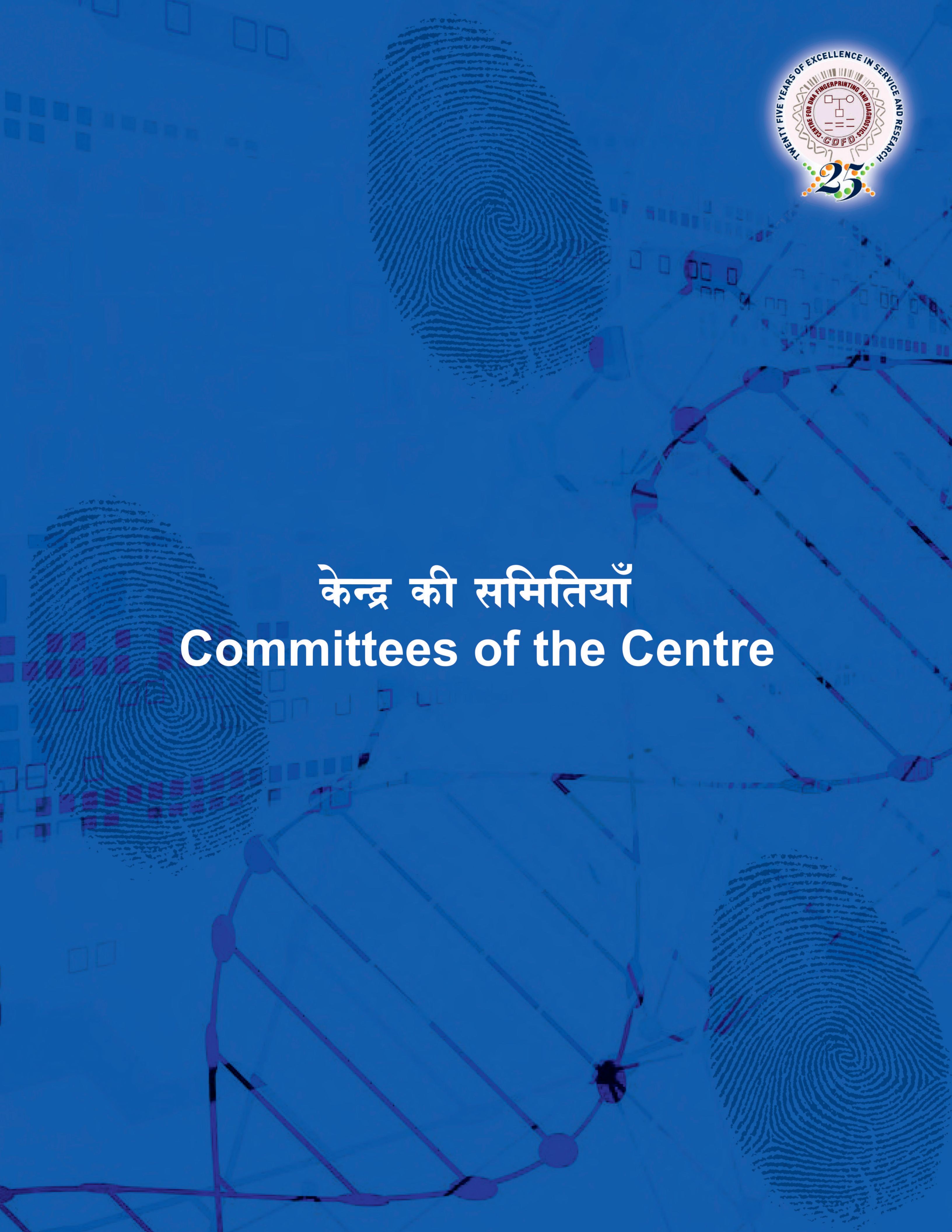
Engineering Section



सी डी एफ डी कर्मचारियों की विदेशों में प्रतिनि युक्ति

Deputations Abroad of CDFD Personnel

Sl. No.	Name of the Employee & Designation	Duration of visit / conference		Conference attended through online
1.	Dr. Rupinder Kaur Staff Scientist – VII	21.03.2021	27.03.2021	Online conference titled " Candida and Canidiasis 2021" organized by the Microbiology Society, London, UK held during 21-27 March 2021.
2.	Dr. Rashna Bhandari Staff Scientist – VI	10.05.2021	13.05.2021	Online FEBS Advanced Course on "the new biology of polyphosphate in Health and Disease" organized by Federation of European Societies, UK held during 10-13 May, 2021.



केन्द्र की समितियाँ
Committees of the Centre

(a) Members of CDFD Society :

1	Hon'ble Dr. Harsh Vardhan Hon'ble Minster for Science & Technology and Earth Sciences	President
2	Dr. Renu Swarup Secretary, DBT, New Delhi	Chairperson
3	Dr. Shekhar C Mande Director General, Council of Scientific & Industrial Research or his nominee	Member (Ex-officio)
4	Dr. Suchita Ninawe, Scientist – G/Adviser, DBT Scientist Co-ordinator, DBT-CDFD	Member (Ex-officio)
5	Shri Vishvajit Sahay Additional Secretary & Financial Adviser, DBT	Member (Ex-officio)
6	Shri C P Goyal Joint Secretary (Admin.,), Department of Biotechnology	Member (Ex-officio)
7	Joint Secretary (PM), Ministry of Home Affairs, GoI	Member (Ex-officio)
8	Joint Secretary or his nominee Ministry of Law, Justice & Company Affairs	Member (Ex-officio)
9	Director General or his nominee Bureau of Police Research and Development (BPR&D)	Member (Ex-officio)
10	Dr. Rakesh K Mishra Director, Centre for Cellular and Molecular Biology (CCMB), Hyderabad	Member (Ex-officio)
11	Prof. Partha P Majumder Chairman of the Scientific Advisory Committee of the Centre	Member (Ex-officio)
12	Prof. V S Chauhan, Visiting Scientist, ICGEB, Delhi	Member
13	Prof. Dipankar Chatterji, Honorary Professor, IISc, Bangalore	Member
14	Dr. K Thangaraj, Director, CDFD	Member-Secretary

(b) Members of CDFD Governing Council :

1	Dr. Renu Swarup Secretary, Department of Biotechnology (DBT)	Chairperson
2	Dr. Shekhar C Mande Director General, Council of Scientific & Industrial Research or his nominee	Member (Ex-officio)
3	Dr. Suchita Ninawe, Scientist – G/Adviser, DBT Scientist Co-ordinator, DBT-CDFD	Member (Ex-officio)
4	Shri Vishvajit Sahay Additional Secretary & Financial Adviser, DBT	Member (Ex-officio)
5	Shri C P Goyal Joint Secretary (Admin.,), Department of Biotechnology	Member (Ex-officio)
6	Joint Secretary (PM), Ministry of Home Affairs, GoI	Member (Ex-officio)
7	Joint Secretary or his nominee Ministry of Law, Justice & Company Affairs	Member (Ex-officio)
8	Director General or his nominee Bureau of Police Research and Development (BPR&D)	Member (Ex-officio)
9	Dr. Rakesh K Mishra Director, Centre for Cellular and Molecular Biology (CCMB), Hyderabad	Member (Ex-officio)

10	Prof. Partha P Majumder Chairman of the Scientific Advisory Committee of the Centre	Member (Ex-officio)
11	Prof. V S Chauhan, Visiting Scientist, ICGEB, Delhi	Member
12	Prof. Dipankar Chatterji, Honorary Professor, IISc, Bangalore	Member
13	Dr. K Thangaraj, Director, CDFD	Member-Secretary

(c)MEMBERS OF CDFD RESEARCH AREA PANELS –SCIENTIFIC ADVISORY COMMITTEE (RAP-SAC):

1	Prof Partha P Majumder NIBG, West Bengal	Chairman
2	Dr Arun Kumar Rawat DBT, New Delhi (DBT Representative)	Member
3	Dr Rajiv Giroti CFSL, Hyderabad (MHA Representative)	Member
4	Dr Manisha Madkaikar Natl. Inst. of Immunohaematology, Mumbai (ICMR Representative)	Member
5	Dr Sunil Archak Natl. Bureau of Plant Genetic Resources New Delhi (ICAR Representative)	Member
6	Dr Rakesh Mishra CCMB, Hyderabad (CCMB Representative)	Member
7	Dr Anurag Agrawal CSIR- IGIB, New Delhi	Member
8	Dr Rajan Sankaranarayanan CCMB, Hyderabad	Member
9	Prof. B.K. Thelma University of Delhi (South Campus), New Delhi	Member
10	Prof Jaya Sivaswami Tyagi AIIMS, New Delhi	Member
11	Prof Usha Vijayraghavan IISc., Bangalore	Member
12	Prof V Nagaraja IISc, Bangalore	Member
13	Dr Shekhar C Mande Secretary, DSIR and Director General, CSIR New Delhi - 110001,	Member
14	Prof Samit Chattopadhyay CSIR –IICB Kolkata	Member
15	Prof Tapas K Kundu CSIR-CDRI, Lucknow	Member
16	Prof Suman Kumar Dhar JNU, New Delhi	Member
17	Prof Amitabha Mukhopadhyay IIT, New Delhi	Member

18	Dr. Anand K Bachhawat IISER Mohali	Member
19	Dr. Shantanu Chowdhury CSIR-IGIB, New Delhi	Member
20	Dr. Manjula Reddy CCMB, Hyderabad	Member
21	Dr. K Thangaraj, Director, CDFD	Member Secretary

(d) Members of CDFD Finance Committee :

1	Shri Vishajit Sahay Additional Secretary & Finance Adviser, DBT (Ex-Officio)	Chairman
2	Joint Secretary and Financial Adviser MHA or his nominee	Member
3	Dr. Suchita Ninawe, Scientist – G/Adviser, DBT Scientist Co-ordinator, CDFD	Member
4	Dr. K Thangaraj Director, CDFD	Member
5	Prof. V S Chauhan, Visiting Scientist, ICGEB, Delhi	Member
6	Prof. Dipankar Chatterji, Honorary Professor, IISc, Bangalore	Member
7	Shri S K Roy Finance & Accounts Officer of the CCMB	Member
8	Mr. E V Rao, I/c – Finance & Accounts, CDFD	Member Secretary & Convenor

(e) Members of the Institutional Bio-safety Committee (IBSC) :

1.Dr. Sangita Mukhopadhyay, Staff Scientist - VII, CDFD-- Chairperson

2.	Dr. Arvind Kumar, Principal Scientist, CCMB	- DBT Nominee
3.	Dr. Rashna Bhandari, Staff Scientist – VI, CDFD	- Member Secretary
4.	Dr. Krishnaveni Mishra, Asso. Professor, Department of Biochemistry, SLS, University of Hyderabad, Hyderabad	-Outside Expert
5.	Dr. Ashwin B Dalal, Staff Scientist – VI, CDFD	- Biosafety Officer
6.	Dr. M D Bashyam, Staff Scientist – VII, CDFD	- Internal Expert
7.	Dr. Sanjeev Khosla, Staff Scientist – VII, CDFD	- Internal Expert
8.	Dr. Rupinder Kaur, Staff Scientist – VII, CDFD	- Internal Expert

(f) Members of CDFD Management Committee :

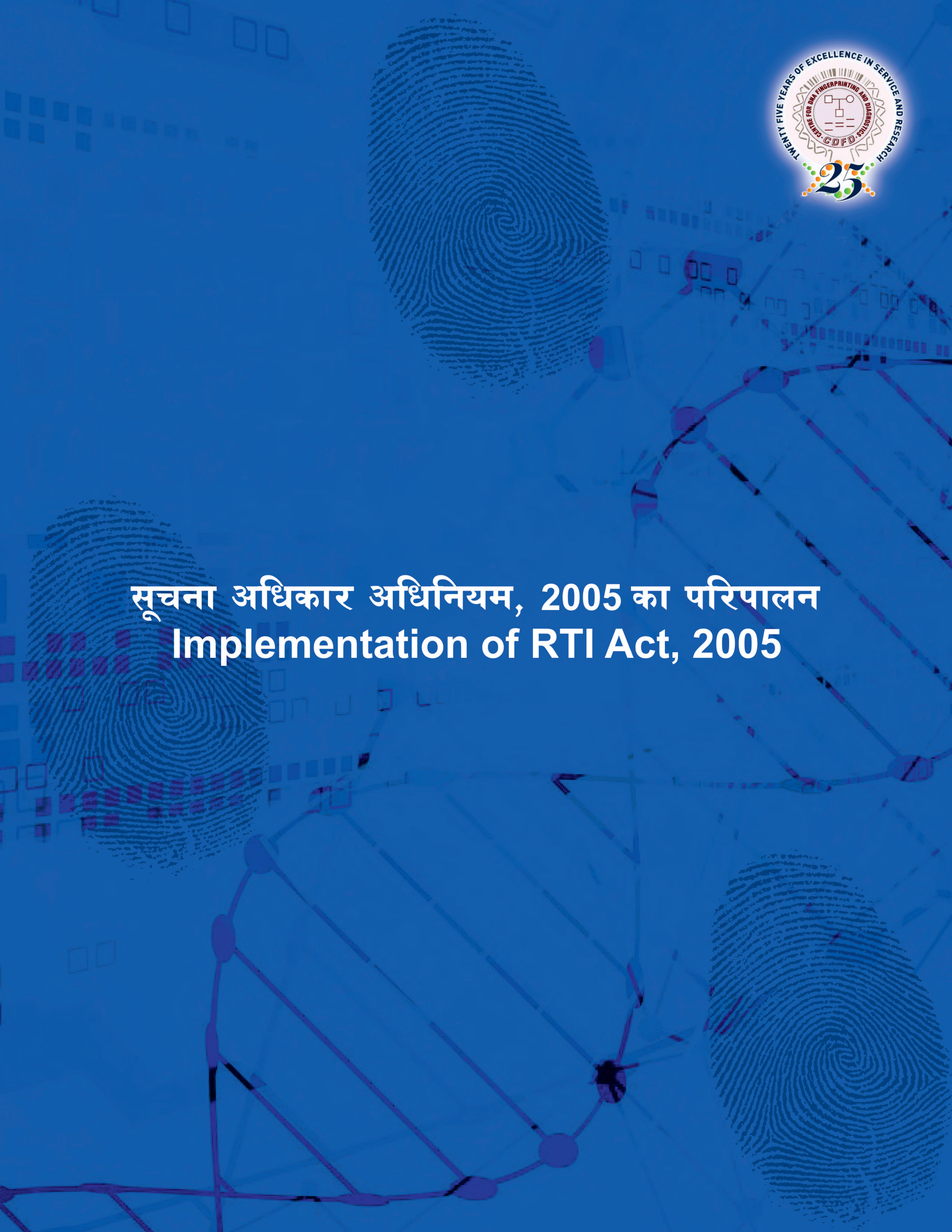
1.	Director, CDFD	- Chairperson
2.	Director, NIAB, Hyderabad	- Member
3.	Dr. Ranjan Sen, SS – VII	- Member
4.	Dr. Sangita Mukhopadhyay, SS – VII	- Member
5.	Dr. M D Bashyam, SS – VII	- Member
6.	Dr. Devyani Halder, SS – VI	- Member
7.	Dr. V Punnaiah, Executive Engineer	- Member
8.	Dr. Usha Dutta, Technical Officer – II	- Member
9.	I/c – Finance & Accounts	- Member
10.	Head – Administration	- Member & Convenor

(g) Members of Sexual Harassment Complaints Committee :

(I) Dr. Sangita Mukhopadhyay, SS – VII	- Chairperson
(ii) Dr. Rupinder Kaur, Staff Scientist – VII	- Member
(iii) Dr. M V Subba Reddy, Staff Scientist – VI	- Member
(iv) Mr. G Ravindar, Head – Administration	- Member
(v) Ms. V Naga Sailaja, TO – II	- Member
(vi) Ms. M V Sukanya, TO – II	- Member
(vii) Ms. P Jamuna Gramya Resource Centre for Women (Representing as NGO)	- External Member

(h) Members of Institutional Bio-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. Rupinder Kaur, Staff Scientist – VII, CDFD	- Member
7. Dr. Ashwin B Dalal, Staff Scientist – VI, CDFD	- Member Secretary


TWENTY FIVE YEARS OF EXCELLENCE IN SERVICE AND RESEARCH
CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
CDFD
25
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

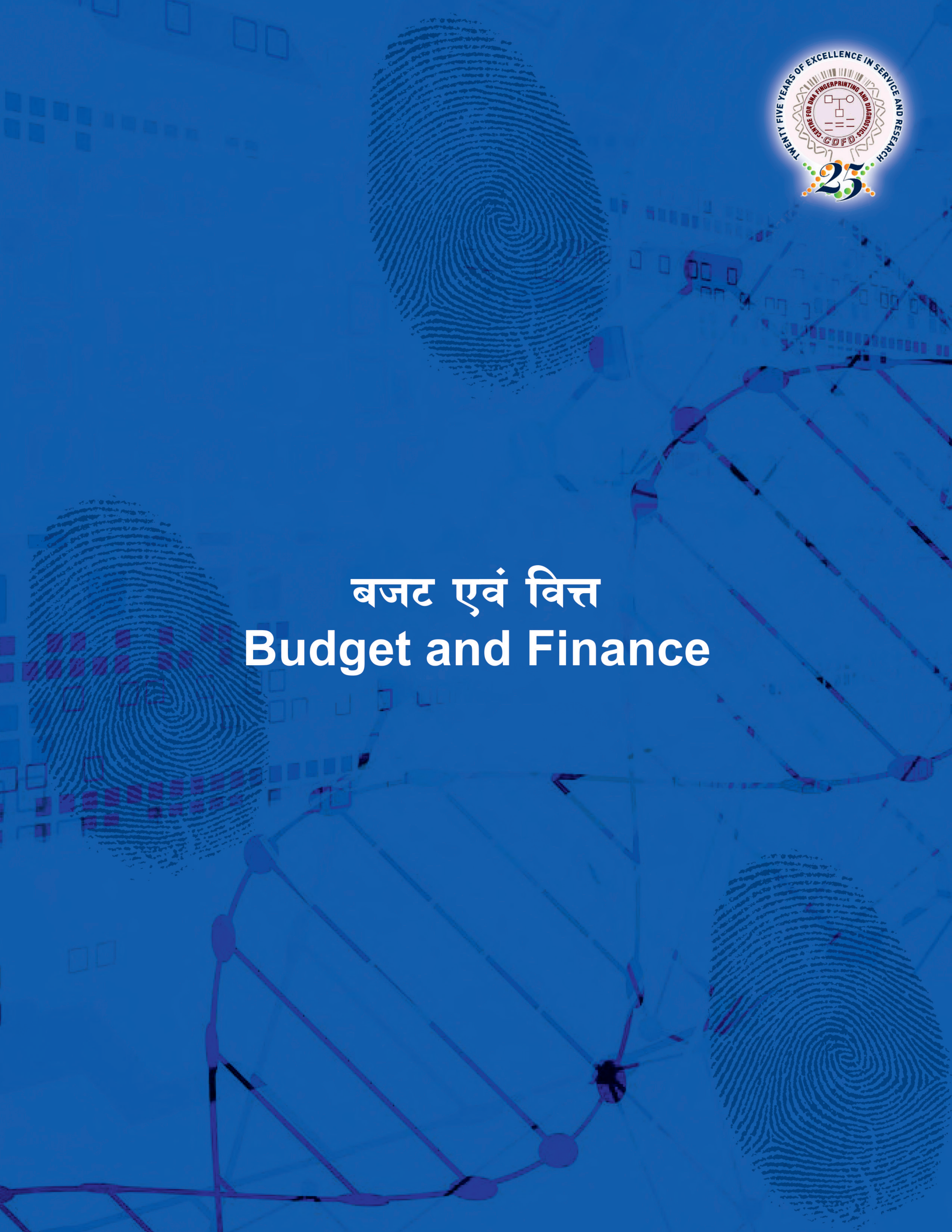
Dr. M.D. Bashyam
Appellate Authority

Ms. Varsha
Central Public Information Officer (till 20.10.200)

Mr. G Ravindar
Central Public Information Officer (from 21.10.2020)

Details about the RTI applications and appeals received in CDFD

As received under the RTI Act 2005	Opening Balance as on 01-04-2020	Received during the year 2020-21			Disposed off during the year 2020-21				Closing Balance as on 31-03-2021
		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	05	15	36	51	56	NIL	NIL	56	06
Appeals	NIL	NIL	01	01	01	NIL	NIL	01	NIL



बजट एवं वित्त
Budget and Finance

लेखा परीक्षक की रिपोर्ट
Auditor's Report

M.S. Appala Chary FCA
9441490545
ca.msachary@gmail.com

AUDITOR'S REPORT

To

Date: 01-10-2021

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

We have audited the attached Balance Sheet of **CENTRE FOR DNA FINGER PRINTING AND DIAGNOSTICS**, Hyderabad, as at 31st March 2021 and also the Income & Expenditure Account for the year ended on that date annexed there to. These Financial Statements are the responsibility of the organization management. Our responsibility is to express an opinion on these Financial Statements based on our audit.

We report that:

1. We have obtained all the information and explanations, which to the best of our knowledge and belief were necessary for the purpose of our audit.
2. In our opinion, the organization has kept proper books of account as required by law so far, as appears from our examination of these books.
3. The Balance Sheet and Income & Expenditure account dealt with by this report is in agreement with the books of account.

a) In our opinion and to the best of our information and according to the explanations given to us, the said Balance Sheet and the Income & Expenditure account read together with the notes there on **subject to following reservations:**

1. T*he CDFD CP Fund account and the corresponding Bank accounts including fixed deposits are to be reconciled by cross verifying the CPF records and deposit accounts. Further the legal formalities regarding CPF fund formation are to be complied with. The compliance of IND AS -19 Employee Benefits be considered. .(Refer Note No 8 to notes to accounts).*

2. *We observed during the course of our audit verification, that there are considerable adjustment entries to be made in respect of bank reconciliation statement.* Long outstanding un reconciled Debits/ credits to the extent of Approx. Rs.18.90 Crores are

to be reconciled. *The management needs to take immediate steps to pass necessary entries to clear the pending bank reconciliation adjustments since reconciliations may bring to notice any wrong classifications or missing entries / un identified entries which may in turn have an effect on other individual accounts including expenditure / assets of Core or project grants.(Refer Note No 9 to notes to accounts)*

3. *We observed from objection register that advances to the tune of Rs.16.03 crores (Refer Annex-H & K) 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 to initiate immediate steps to clear the same. It has observed that even after the related receipt and consumption of the materials/consumables or receipt and use of the related equipment(s), the concerned advances are not reversed. Hence the concerned expenditure or asset account are not reflecting at its appropriate balances. (Refer note no 7 to Notes to Accounts).*

4. *The details regarding individual project wise grants & expenditure there on are not maintained and hence could not comment on the individual project wise statement of grants and expenditure. (Refer note no 10 to Notes to Accounts).*

gives the required information in the manner so required and gives a true and fair view

a) In so far it relates to the Balance Sheet as at 31st March 2021 and

b) In so far as it relates to the Income & Expenditure account excess of income over expenditure for the year ended on 31st March 2021.,

for CHARY AND CO
Chartered Accountants
F R No-014102S

M S APPALA CHARY
M.No -221442
UDIN: 21221442AAAAEL9864
Place : HYDERABAD
Date : 01-10-2021.

2

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

BALANCE SHEET AS ON 31st MARCH 2021

(Amount - ₹.)

	Schedule	Current Year	Previous Year
CORPUS/CAPITAL FUND AND LIABILITIES			
Corpus / Capital Fund	1	2,22,79,02,694.00	2,09,37,36,336.00
Reserves and Surplus	2	6,84,52,844.00	5,37,16,479.00
Earmarked / Endowment funds	3	15,05,56,541.00	24,19,86,387.00
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	22,18,84,578.00	20,56,26,036.00
TOTAL		2,66,87,96,657.00	2,59,50,65,238.00
ASSETS			
Fixed Assets	8	1,69,84,68,080.00	1,55,76,49,796.00
Investments- From Earmarked / Endowment Funds	9	0.00	0.00
Investments - Others	10	12,07,78,393.00	12,92,01,312.00
Current Assets, Loans, Advances etc.	11	84,95,50,184.00	90,82,14,130.00
Miscellaneous Expenditure		0.00	0.00
TOTAL		2,66,87,96,657.00	2,59,50,65,238.00
Significant Accounting Policies	24		
Contingent Liabilities and Notes on Accounts	25		

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

Place: Hyderabad
Date : 01-10-2021

For CHARY AND CO
CHARTERED ACCOUNTANTS
F R No - 014102S

M S APPALA CHARY
M.No - 221442
UDIN : 21221442AAAAEL9864

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

3.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

INCOME & EXPENDITURE FOR THE YEAR ENDED 31st MARCH 2021

(Amount - Rs.)

	Schedule		Current Year		Previous Year
INCOME					
Income from Sales/Services	12		1,47,36,365.00		1,41,08,833.00
Grants/Subsides	13		35,50,00,000.00		39,50,00,000.00
Fees/Subscriptions	14		0.00		0.00
Income from Investments	15		95,07,124.00		1,60,44,467.00
Income from Royality, Publications etc.	16		0.00		0.00
Interest Earned	17		35,19,229.00		65,66,680.00
Other Income	18		18,25,592.00		17,55,144.00
Increase/(decrease) in stock of Finished goods and works-in-progress	19		0.00		0.00
TOTAL (A)			38,45,88,310.00		43,34,75,124.00
EXPENDITURE					
Establishment Expenses	20		17,45,18,131.00		15,82,69,725.00
Administrative Expenses	21		18,33,46,920.00		20,06,58,809.00
Expenditure on Grants, Subsides etc.	22				0.00
Interest	23				0.00
Depreciation (Net Total at the year-end -corresponding to Schedule 8)		7,38,41,917.00		4,90,89,537.00	
Less:Transferred to Grants-in-Aid		7,38,41,917.00	0.00	4,90,89,537.00	0.00
Provision For Salaries			82,63,277.00		88,83,130.00
TOTAL (B)			36,61,28,328.00		36,78,11,664.00
Balance being excess of Income over Expenditure (A-B)			**1,84,59,982.00**		**6,56,63,460.00**
Transfer to Special Reserve (Specify each)					
Transfer to/from General Reserve			1,47,36,365.00		1,41,08,833.00
BALANCE BEING SURPLUS/(DEFLICT) CARRIED TO CORPUS/CAPITAL FUND			37,23,617.00		5,15,54,627.00
SIGNIFICANT ACOUNTING POLICIES	24				
CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS	25				

I/c - FINANCE & ACCOUNTS
CDFD

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

Place: HYDERABAD
Date : 01-10-2021

For CHARY AND CO
CHARTERED ACCOUNTANTS
F R No - 014102S

CHARY AND CO ★ Chartered Accountants FRN: 014102S M.No. 221442

M S APPALA CHARY
M.No - 221442
UDIN : 21221442AAAAEL9864

DIRECTOR
CDFD

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

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS (CDFD)
RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2021

(Amount - Rs.)

RECEIPTS	Current Year	Previous Year	PAYMENTS	Current Year	Previous Year
1. Opening Balances			1. Expenses		
a) Cash in hand	-	-	a) Establishment Expenses (corresponding to Schedule 20)	174,518,131.00	158,269,725.00
b) Bank Balances			b) Administrative Expenses (corresponding to Schedule 21)	183,346,920.00	200,658,809.00
i) In current accounts	77,449,083.00	42,266,046.00	c) Schedule 22	-	-
ii) In deposit accounts	314,399,614.00	260,711,420.00			
iii) Savings accounts	259,789,587.00	102,330,604.00			
			2. Payments made against funds for various projects		
2. Grants Received			(Name of the fund or project should be shown along with		
a) From Government of India	435,000,000.00	490,000,000.00	the particulars of payments made for each project)		
b) From State government	-	-	Projects	169,117,418.00	49,098,207.00
c) From other sources (details)	-	-	Projects - OB Clearances	51,220,240.00	-
(Grants for capital & revenue			Research Fellow Associates Payments	2,338,295.00	16,752,532.00
exp. to be shown seperately)					
Research Fellow Associates Receipts	9,298,039.00	10,737,899.00	3. Investments and deposits made		
Project Grants	77,687,572.00	232,589,036.00	a) Out of Earmarked/Endowment funds	-	-
Project Grants - OB Clearances	51,220,240.00	-	b) Out of Own Funds (Investments-Others)		-
3. Income on Investments from			c) Deposit made in CPF A/C		-
a) Earmarked/Endowment Funds	9,507,124.00	16,044,467.00			
b) Own Funds (Oth. Investment)					
Investments encashed		-	4. Expenditure on Fixed Assets & Capital Work-in-Progress		
			a) Purchases of Fixed Assets:		
4. Interest Received			Books & Journals	-	-
a) On Bank deposits	3,519,229.00	6,566,680.00	Equipment -Lab/Office/Furniture	213,347,940.00	19,113,023.00
b) Loans, Advances etc	-	-	b) Expenditure on Capital Work-in-Progress:	1,312,261.00	851,029.00
c) Interest on Computer Advance, Conveyance advance and HBA	6,783.00	-			
d) Interest on LC	-	-			
			5. Refund of surplus money/Loans		
5. Other Income(Specify)			a) To the Government of India		-
a) Analysis Charges	14,736,365.00	14,108,833.00	b) To the State Government		-
b) Scrap Sales	-	-	c) To other providers of funds	-	87,900,000.00
6. Any Other Receipts (Give Details)			6. Finance Charges (Interest)	-	-
I-Remittances (Annexure-A)	50,722,861.00	25,376,899.00			
CPF-SUB,Arrears and adv.Refund	14,002,555.00	19,996,521.00	7. Other Payments (Specify)		
Sundry Receipts	1,343,596.00	1,434,647.00	Advances (Annexure-D)	279,287,576.00	277,544,233.00
Application Fee	96,643.00	245,997.00	I-Remittances (Annexure-E)	47,569,800.00	25,785,663.00
Sale OF Tender Forms	41,500.00	74,500.00	CPF A/c	1,068,600.00	9,741,733.00
Leave Salary-Pension Contribution	-	-	New Pension Scheme	6,185,093.00	4,268,488.00
License Fee	-	-	NIMS	-	8,000,000.00
NPS	6,185,093.00	4,268,488.00			
Advance/Refunds/Recovery/Adj(Annexure-B)	491,776,847.00	277,183,018.00			
NIMS	-	5,686,671.00	8. Closing Balances		
Project Travel Grant Refund	-	-	a) Cash in hand		-
Income Tax Refund	337,070.00	-	b) Bank Balances		
			i) In current accounts	96,019,063.00	77,449,083.00
			ii) In deposit accounts	274,399,614.00	314,399,614.00
			iii) Savings accounts	317,388,850.00	259,789,587.00
TOTAL	**1,817,119,801.00**	**1,509,621,726.00**	**TOTAL**	**1,817,119,801.00**	**1,509,621,726.00**

DIRECTOR
CDFD

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

CHARY AND CO
CHARTERED ACCOUNTANTS

CHARY AND CO ★ Chartered Accountants ★ FRN: 014102S M.No. 221442

I/c - FINANCE & ACCOUNTS
CDFD

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

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

BALANCE SHEET AS ON 31st MARCH 2021

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 1 - CORPUS/CAPITAL FUND :				
Balance as at the begining of the year		2,09,37,36,336.00		2,08,10,61,597.00
Add : Contribution towards Corpus/Capital Fund				
CDFD Core - Plan (Non-Recurring)	8,00,00,000.00		9,50,00,000.00	
Capitalised portion of Capital Expenditure of projects	12,42,84,658.00	20,42,84,658.00	31,09,649.00	9,81,09,649.00
Less : Depreciation For the Year		7,38,41,917.00		4,90,89,537.00
Less : Fund returned to DBT		0.00		8,79,00,000.00
Add : Excess of Income over Expenditure		37,23,617.00		5,15,54,627.00
BALANCE AS AT THE YEAR - END		**2,22,79,02,694.00**		**2,09,37,36,336.00**

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

6.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2021

(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	5,37,16,479.00		3,96,07,646.00	
Addition during the year	1,47,36,365.00		1,41,08,833.00	0.00
Less : Deductions during the year	0.00	6,84,52,844.00	0.00	5,37,16,479.00
Total		**6,84,52,844.00**		**53716479.00**

	Current Year	Previous Year
DNA Fingerprinting Receipts	1,00,41,838.00	91,40,617.00
Diagnostics Receipts	43,49,978.00	46,79,225.00
	3,47,849.00	2,88,991.00
Total Receipts	**1,47,39,665.00**	**1,41,08,833.00**

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

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2021

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 3 - EARMARKED/ENDOWMENT FUNDS :				
(Refer Annexures)				
(a) Opening balance of the Funds		**24,19,86,387.00**		**5,84,95,558.00**
(b) Additions to the Funds :				
i. Donations /grants (net)	7,76,87,572.00		23,25,89,036.00	
ii. Income from investments made on account of funds	0.00		0.00	
iii. Other additions (OB clearances)	5,12,20,240.00	12,89,07,812.00	0.00	23,25,89,036.00
TOTAL (a+b)		37,08,94,199.00		29,10,84,594.00
(c) Utilisation/Expenditure towards objective of funds				
(i) Capital Expenditure (Refer Annexures I & II)				
- Fixed Assets	12,42,84,658.00		31,09,649.00	
- Others	0.00	12,42,84,658.00	0.00	31,09,649.00
- Total				
(ii) Revenue Expenditure (Refer Annexures I & II)				
- Salaries, Wages and allowances etc.	3,24,27,095.00		1,86,12,180.00	
- Rent/ REFUNDS	0.00		0.00	
- Project Consumales & Other Expenses	6,12,87,610.00	9,37,14,705.00	2,66,02,983.00	4,52,15,163.00
Total				
(iii) Refund of Project grants		23,38,295.00		7,73,395.00
TOTAL (c)		22,03,37,658.00		4,90,98,207.00
NET BALANCE AS AT THE YEAR-END [(a + b)-c]		**15,05,56,541.00**		**24,19,86,387.00**

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(Amount - Rs.)

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

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(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				

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(Amount - Rs.)

SCHEDULE 6 - DEFFERED CREDIT LIABILITIES :	Current Year		Previous Year	
a) Acceptances secured by hypothecation of capital equipment and other assets		0		0
b) Others		0		0
TOTAL		**0**		**0**
Note: Amount due within one year				

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(Amount - Rs.)

SCHEDULE 7 - CURRENT LIABILITIES AND PROVISIONS :	Current Year		Previous Year	
A. CURRENT LIABILITIES				
1. Acceptances	0.00		0.00	
2. Sundry Creditors	0.00		0.00	
3. Advances Received	0.00		0.00	
4. Interest accured but not due on:	0.00		0.00	
5. Statutory Liabilities:	0.00		0.00	
TDS on salaries	13,20,250.00		12,36,450.00	
TDS others	2,98,760.00		1,92,140.00	
Service Tax	24,325.00		24,325.00	
Works Tax	16,80,631.00		16,80,631.00	
PM Cares Fund Payable	6,04,318.00		0.00	
6. Other current Liabilities	0.00		0.00	
CDFD.CP Fund A/C	17,34,49,436.00		16,05,15,481.00	
Contract Staff security deposit	50,974.00		1,25,594.00	
Diagnostics Collabration With NIMS			0.00	
ECCS	4,81,241.00		1,95,156.00	
EMD	22,18,734.00		20,52,644.00	
Festival Advance	450.00		450.00	
GSLI	3,796.00		3,796.00	
House Building Advance	1,29,831.00		1,29,831.00	
Lab Security Deposit & Hostel Security Deposit	14,73,747.00		14,72,741.00	
LIC	2,16,068.00		69,583.00	
Performance Guarantee Deposit	22,436.00		22,436.00	
Others (I-Remittances)	0.00		0.00	
Other Out Standing Liabilities	1,92,56,329.00		1,72,86,826.00	
	39,500.00		28,500.00	
Public Provident Fund	3,91,158.00		3,91,158.00	
Royalty & Consultancy	15,31,642.00		15,31,642.00	
Security Deposit	1,02,60,783.00		96,36,500.00	
STAFF BENEVOLENT FUND	86,983.00		67,113.00	
TA Abroad [Advance]	0.00		0.00	
TA-DA-Hon within India [Advance]	79,909.00	21,36,21,301.00	79,909.00	19,67,42,906.00
TOTAL (A)		21,36,21,301.00		19,67,42,906.00
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		82,63,277.00		88,83,130.00
TOTAL (B)		82,63,277.00		88,83,130.00
TOTAL (A+B)		**22,18,84,578.00**		**20,56,26,036.00**

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SCHEDULE 8 - FIXED ASSTES :

	GROSS BLOCK				DEPRECIATION				NET BLOCK	
	Cost/valuation As at begining of the year	Addition during the year	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
A. FIXED ASSETS:										
1. LAND:										
a) Freehold	3,900,000.00			3,900,000.00	0.00	0.00	0.00	0.00	3,900,000.00	3,900,000.00
b) Leasehold	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
2. BUILDINGS	0.00			0.00					0.00	0.00
a) On Freehold Land	220,052,369.00			220,052,369.00	133,212,696.00	8,683,967.00	0.00	141,896,663.00	78,155,706.00	86,839,673.00
b) On Leasehold Land	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
d) Superstructures on Land	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
not belongs to the entity	0.00			0.00	0.00			0.00	0.00	0.00
3. PLANT MACHINERY & EQUIPMENT	784,426,508.00	209,494,781.28		993,921,289.28	567,108,683.00	63,274,602.00	0.00	630,383,285.00	363,538,004.28	217,317,825.00
4. VEHICLES	4,153,026.00			4,153,026.00	3,902,494.00	37,580.00	0.00	3,940,074.00	212,952.00	250,532.00
5. FURNITURE, FIXTURES	16,725,072.00	508,553.00		17,233,625.00	13,052,256.00	413,054.00	0.00	13,465,310.00	3,768,315.00	3,672,816.00
6. OFFICE EQUIPMENT	12,154,882.00	958,195.00		13,113,077.00	10,864,560.00	337,278.00	0.00	11,201,838.00	1,911,239.00	1,290,322.00
7. COMPUTER/PERIPHERALS	266,023.00	1,710,412.34		1,976,435.34	189,266.00	473,867.00	0.00	663,133.00	1,313,302.34	76,757.00
8. SOFTWARE	1,344,886.00	426,937.00		1,771,823.00	819,936.00	305,367.00	0.00	1,125,303.00	646,520.00	524,950.00
9. ELECTRIC INSTALLATIONS	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
10. LIBRARY BOOKS	21,133,204.00	202,661.30		21,335,865.30	21,093,365.00	241,204.00	0.00	21,334,569.00	1,296.30	39,839.00
11. TUBEWELLS & WATER SUPPLY	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
12. OTHER FIXED ASSETS	8,887,898.00			8,887,898.00	8,413,839.00	71,109.00	0.00	8,484,948.00	402,950.00	474,059.00
Airconditioning works	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
DG Set	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
Typewriters	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
Miscellaneous non consumables	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
Other Assets	0.00	46,400.00		46,400.00	0.00	3,888.75	0.00	3,888.75	42,511.25	0.00
EMB Net	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	1,073,043,868.00	213,347,939.92	0.00	1,286,391,807.92	758,657,095.00	73,841,916.75	0.00	832,499,011.75	453,892,796.17	314,386,773.00
B. CAPITAL WORK-IN-PROGRESS	1,243,263,023.00	1,312,261.00	0.00	1,244,575,284.00	0.00	0.00	0.00	0.00	1,244,575,284.00	1,243,263,023.00
TOTAL	**2,316,306,891.00**	**214,660,200.92**	**0.00**	**2,530,967,091.92**	**758,657,095.00**	**73,841,916.75**	**0.00**	**832,499,011.75**	**1,698,468,080.17**	**1,557,649,796.00**
P Y	2,296,342,839.00	19,964,052.00	0.00	2,316,306,891.00	709,567,558.00	49,089,537.00	0.00	758,657,095.00	1,557,649,796.00	1,586,775,281.00
	Equipment - Core	85,210,123.70								
	Equipment - Projects	124,284,657.50								
	Total	209,494,781.20								

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(Amount - Rs.)

SCHEDULE 9 - INVESTMENTS FROM EARMARKED/ENDOWMENT FUNDS :	Current Year	Previous Year
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	0.00	0.00
5. Subsidiaries and Joint Ventures	0.00	0.00
6. Others (to be specified)	0.00	0.00
TOTAL	**0.00**	**0.00**

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(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 10 - INVESTMENTS - OTHERS :		
(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	12,92,01,312.00
TOTAL	**12,07,78,393.00**	**12,92,01,312.00**

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(Amount - Rs.)

	Current Year	Previous Year	Current Year	Previous Year
SCHEDULE 11 - INVESTMENTS - OTHERS :				
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 Outstanding for a period exceeding six months	0.00		0.00	
b) Others-Life Membership Fees	1,69,236.00	169236.00	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	9,60,19,063.00		7,74,49,083.00	
-On Deposit Accounts (includes margin money)	27,43,99,614.00		31,43,99,614.00	
-On Savings Accounts	31,73,88,850.00	68,78,07,527.00	25,97,89,587.00	65,16,38,284.00
b) With non-Schedules Banks:				
-On Current Accounts	0.00		0.00	
-On Deposit Accounts	0.00		0.00	
-On Savings Accounts	0.00	0.00	0.00	0.00
5. Post Office-Savings Accounts				
TOTAL (A)		68,79,76,763.00		65,18,07,520.00
B.LOANS, ADVANCES AND OTHER ASSETS				
a) Staff (Annexure-L)	4,38,162.00		452241.00	
b) Other Entities engaged in activities/objectives similar to that of the Entity	0.00	438162.00	0.00	4,52,241.00
2. Advances and other amounts recoverable in cash or in kind or for value to be received				
a) On Capital Account (Annexure-H)	5,03,99,356.00		11,96,48,762.00	
b) Prepayments - Deposits (Annexure-I)	2,01,39,580.00		20111572.00	
c) TDS Receivable	7,64,579.00		4,74,140.00	
d) Others (Annexure-K)	8,98,31,744.00		10,16,12,419.00	
e) GST on Purchases (Schedule 21B)		16,11,35,259.00	1,41,07,476.00	25,59,54,369.00
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. Claims Receivable		0.00		0.00
TOTAL (B)		16,15,73,421.00		25,64,06,610.00
TOTAL (A+B)		**84,95,50,184.00**		**90,82,14,130.00**

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(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)	1,47,36,365.00	1,41,08,833.00
c) Agency Commission and Brokerage	0.00	0.00
d) Maintenance Services (Equipment/Property)	0.00	0.00
e) Others (Specify)	0.00	0.00
TOTAL	**1,47,36,365.00**	**1,41,08,833.00**

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(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 13 - GRANTS/SUBSIDES :		
(Irrevocable Grants & Subsides Received)		
1) Central Government (DBT Plan Grant-in-Aid)	35,50,00,000.00	39,50,00,000.00
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	**35,50,00,000.00**	**39,50,00,000.00**

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(Amount - Rs.)

SCHEDULE 14 - FEES/SUBSCRIPTIONS :	Current Year	Previous Year
1) Entrance Fees	0	0
2) Annual Fees/Subscriptions	0	0
3) Seminar/Program Fees	0	0
4) Consultancy Fees	0	0
5) Others (Specify)	0	0
TOTAL	**0**	**0**

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(Amount - Rs.)

SCHEDULE 15 - INCOME FROM INVESTMENTS :	Current Year		Previous Year	
(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	95,07,124.00		1,60,44,467.00	0.00
TOTAL	**95,07,124.00**	**0.00**	**1,60,44,467.00**	**0.00**
TRANSFERRED TO EARMARKED/ENDOWMENT FUNDS				

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(Amount - Rs.)

SCHEDULE 16 - INCOME FROM ROYALITY, PUBLICATION ETC. :	Current Year	Previous Year
1) Income from Royality	0	0
2) Income from Publications	0	0
3) Others (Specify)	0	0
TOTAL	**0**	**0**

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(Amount - Rs.)

SCHEDULE 17 - INTEREST EARNED :	Current Year	Previous Year
1) On Term Deposits		
a) With Schedule Banks	0.00	0.00
b) With Non-Scheduled Banks	0.00	0.00
c) With Institutions	0.00	0.00
d) Others	0.00	0.00
2) On Saving Accounts		
a) With Schedule Banks	35,19,229.00	65,66,680.00
b) With Non-Scheduled Banks	0.00	0.00
c) post Office Savings Accounts	0.00	0.00
d) Others	0.00	0.00
3) On Loans		
a) Employees/Staff	0.00	0.00
b) Others	0.00	0.00
	0.00	
4) Interest on Debtors and Other Receivables	0.00	0.00
TOTAL	**35,19,229.00**	**65,66,680.00**
Note :- Tax deducted at source to be indicated		

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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		
Sundry Receipts	13,43,596.00	14,34,647.00
Application Fee	96,643.00	2,45,997.00
Sales Of Tender Forms	41,500.00	74,500.00
Income tax refund	3,37,070.00	0.00
Interest On Computer Advance,Conveyance Advance And HBA	6,783.00	0.00
Leave Salary - Pension Contribution	0.00	0.00
TOTAL	**18,25,592.00**	**17,55,144.00**

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SCHEDULE 19 - INCREASE/(DECREASE) IN STOCK OF FINISHED GOODS & WORK IN PROGRESS :	Current Year	Previous Year
a) Closing stock		
-Finished Goods	0	0
-Work-in-progress	0	0
Total (a)	0	0
b) Less: Opening stock		
-Finished Goods	0	0
-Work-in-progress	0	0
Total (b)	0	0
NET INCREASE/(DECREASE) [a-b]	**0**	**0**

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(Amount - Rs.)

SCHEDULE 20 - ESTABLISHMENT EXPENSES :	Current Year	Previous Year
a) Salaries and Wages	13,81,50,779.00	13,58,02,419.00
b) Allowances and Bonus	81,88,547.00	60,55,067.00
c) Contribution to Provident Fund	55,37,473.00	51,48,946.00
d) Contribution to Other Fund (NPS)	61,85,093.00	42,68,488.00
e) Staff Welfare Expenses - Medical charges	44,28,947.00	36,19,588.00
f) Expenses on Employees Retirement and Terminal Benet	1,18,98,613.00	26,38,811.00
g) Others (specify) -	0.00	0.00
h) EPF Employer Contribution	1,28,679.00	7,36,406.00
TOTAL	**17,45,18,131.00**	**15,82,69,725.00**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2021

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 21 - OTHER ADMINISTRATIVE EXPENSES :		
1) Purchases	6,94,45,077.00	4,97,77,594.00
2) Electricity and power	3,00,64,628.00	3,80,58,518.00
3) Water charges	43,39,673.00	36,69,428.00
4) Insurance	1,02,260.00	3,498.00
5) Repairs and maintenance	1,71,44,632.00	2,70,86,202.00
6) Rent, Rates and Taxes	32,43,141.00	33,17,019.00
7) Vehicles Running and Maintenance	68,39,214.00	28,76,055.00
8) Postage, Telephone and Communication Charges	18,18,913.00	46,81,677.00
9) Printing and Stationary	1,86,415.00	1,14,114.00
10) Travelling and Conveyance Expenses	66,610.00	86,40,384.00
11) Expenses on Seminar/Workshops	12,53,738.00	3,07,299.00
12) Subscription Expenses	56,000.00	60,462.00
13) Expenses on Fees & Renewals	4,94,089.00	6,20,677.00
14) Auditors Remuneration	37,500.00	0.00
15) Hospitality Expenses	3,41,887.00	10,88,607.00
16) Professional Charges	3,07,393.00	1,54,208.00
17) Advertisement and Publicity	37,52,174.00	17,94,683.00
18) Bank Charges	1,27,145.00	2,26,307.00
19) Security & Cleaning Contract Charges	2,42,70,857.00	1,26,78,169.00
20) Training Course /Symposia	0.00	22,56,908.00
21) Other Contingencies	13,83,841.00	25,52,085.00
22) Paytent Application Fee	0.00	3,19,531.00
23) Other Research Expenses	71,77,710.00	6,17,200.00
24)Office Books	56,104.00	16,13,142.00
26)Contract Staff	18,74,710.00	1,42,63,314.00
27)Manpower Outsourcing(Staff)	75,41,193.00	2,38,81,728.00
28) Prior Period Expenses	14,22,016.00	0.00
TOTAL	**18,33,46,920.00**	**20,06,58,809.00**

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Centre for DNA Fingerprinting
and Diagnostics

CHARY AND CO ★ Chartered Accountants ★ FRN: 014102S M.No. 221442

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2021

(Amount - Rs.)

SCHEDULE 22 - EXPENDITURE ON GRANTS, SUBSIDES ETC. :	Current Year	Previous Year
a) Grants given to Institutions/Organisations	0	0
b) Subsidies given to Institutions/Organisations	0	0
TOTAL	**0**	**0**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2021

(Amount - Rs.)

SCHEDULE 23 - INTEREST :	Current Year	Previous Year
a) On Fixed Loans	0	0
b) On Other Loans (including Bank Charges)	0	0
c) Others	0	0
TOTAL	**0**	**0**

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Schedule 24:Significant Accounting Policies &Schedule 25: Contingent Liabilities & Notes on Account for the period ended 31/03/2021

1. **Method of Accounting:**

   a. The accounting system adopted by the organization is on "accrual basis".
   b. The organization has been getting plan Grant-In-Aid under the "Non-recurring" & "Recurring" heads.

2. **Revenue recognition:**

   Income comprises of Grant-in-Aid, Internal Resources through services and interest from short term deposits. Income accounted on the basis of the Cash/DD/Cheques/Cr notes/ on line transfers received.

3. **Fixed Assets:**

   (a) Fixed assets are stated at cost. Cost includes freight, duties, and taxes etc.,

   (b) Depreciation: Depreciation Account on Fixed Assets has since been prepared at the rate prevailing to the concerned Fixed Assets as specified in the Income Tax Act, 1961 on Written Down Value Method of Depreciation.

   (c) Capital work in progress has been entered to the extent of the last running account bills paid.

   (d) Realization on sale of obsolete/surplus fixed assets which is not required for the purpose of research activities are adjusted against capital cost.

4. **Inventories:**

   All purchases of chemicals, glassware and other consumables have been charged to consumption at the time of purchase.

5. **Foreign Currency transactions:**

   Foreign Currency transactions are recognized in the books at the exchange rates prevailing on the date of transaction.

6. **Investments:**

   Investments in STDR's are stated at book values.

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7. **Advances:**

   Advances 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 necessary entries to be made in the books of accounts.

8. The CDFD CP Fund Account and the corresponding Bank accounts including Deposit accounts are to be cross verified the CPF records and deposit accounts necessary adjustments may be made to ensure reconciliation of CP Fund and the corresponding bank accounts.

9. Adjustment entries to be made in respect of bank reconciliation statements of bank accounts. Long outstanding un reconciled Debits/ credits to the extent of Approx. Rs.18.90 Crores are to be reconciled.

10. ***Project Grants :*** *During the year the institute had received a sum of* ***Rs. 7.76*** *crores as project grants. The individual project wise details of Grants received and expenditure made there on are under compilation.*

11. As this is a non profit organization with no major output GST, Input Tax Credit availed as per GST Returns not recorded in Books of Accounts.

12. The previous year balances have been regrouped/rearranged, wherever necessary.

I/c F&A **CDFD**	**Director** **CDFD**	**For CHARY AND CO** **Chartered Accountants**
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.	**M S APPALA CHARY**
Place: Hyderabad **Date: 01-10-2021.**		**UDIN: 21221442AAAAEL9864**

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

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS (CDFD)
RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2021

Annexure: A Forming part of Receipts and Payment Account

RECEIPTS

Previous Year	Particulars	Current Year
	I-Remittances	
192,140.00	TDS other than Salaries	2,035,732.00
16,870,050.00	TDS on Salaries	17,720,206.00
0.00	Works Tax	0.00
1,512,061.00	LIC	1,945,009.00
155,151.00	GSLI	176,376.00
0.00	Public Provident Fund	0.00
422,200.00	Professional Tax	449,600.00
0.00	PM Cares Fund	980,132.00
241,969.00	Others (I-Remittances)	8,298,297.00
0.00	Health Insurance	0.00
3,645,603.00	ECCS	3,412,119.00
0.00	Contract Staff security deposit	0.00
0.00	STAFF BENEVOLENT FUND	29,870.00
736,406.00	EPF	13,989,623.00
1,601,319.00	GST	1,685,897.00
25,376,899.00		**50,722,861.00**

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Uppal, HYDERABAD

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS (CDFD)

RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2021

Annexure: B Forming part of Receipts and Payment Account

RECEIPTS

Previous Year	Particulars	Current Year
	Advance refunds/recovery/Adjst.	
543,721.00	Advance for Expenses- purchases by Staff	265,241.00
0.00	Chemicals [Advance]	0.00
0.00	Computer Advance [Research Fellows]	0.00
0.00	Computer Advance [Staff]	27898.00
164,668.00	Consumables, glassware and Spares [Advance]	75,323,722.00
0.00	Conveyance [Advance]	0.00
0.00	Conveyance Advance	0.00
0.00	DA [Advance]	0.00
0.00	EMD	175,000.00
51,917,200.00	Equipment [Advance]	165,154,196.00
0.00	Festival Advance	33000.00
16,505.00	GDA [Others]	5000.00
122,477.00	General Deposits And Advances	191551.00
0.00	Human Resource Develpment - Training of Staff - Conferences [Advance]	0.00
209,224,103.00	Inter Bank Transfer	125,510,325.00
0.00	Lab Security Deposit & Hostel Security Deposit	1,248,223.00
32,115.00	LTC [Advance]	603,436.00
0.00	Miscellaneous Salary [Advance]	0.00
493,000.00	Others [Advances]	168,858.00
0.00	Pay of Establishment [Advance]	0.00
17,879.00	Revolving Advance	9924.00
5,726,518.00	Security Deposit	234,044.00
0.00	TA Abroad [Advance]	0.00
0.00	TA-DA-Hon within India [Advance]	0.00
0.00	Trainee Security Deposit	0.00
0.00	Water [Advance]	0.00
8,883,130.00	Leave Salary & Pension	122,826,429.00
41,702.00	Performance Guarantee Deposit	0.00
277,183,018.00		**491,776,847.00**

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Uppal, HYDERABAD

CHARY AND CO ★ Chartered Accountants ★ FRN: 0[illegible]02S M.No. 221442

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS (CDFD)

RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2021

Annexure: D Forming part of Receipts and Payment Account

PAYMENTS
Amount in Rs.

Previous Year	Particulars	Current Year
	Advances	
451,000.00	Advance for Expenses- purchases by Staff	1,175,910.00
0.00	Chemicals [Advance]	0.00
0.00	Computer Advance [Research Fellows]	0.00
0.00	Computer Advance [Staff]	41,231.00
4,974,698.00	Consumables, glassware and Spares [Advance]	10,981,805.00
0.00	Conveyance Advance	0.00
155,302.00	EMD	8,910.00
48,052,890.00	Equipment [Advance]	16,081,842.00
0.00	Festival Advance	341,446.00
285,501.00	GDA [Others]	25,000.00
209,224,103.00	Inter Bank Transfer	125,510,325.00
46,500.00	Lab Security Deposit & Hostel Security Deposit	765,615.00
0.00	Liveries & Blankets [Advance]	0.00
451,500.00	LTC [Advance]	484,274.00
0.00	Magzines [Advance]	0.00
1,411,822.00	Others [Advances]	333,543.00
0.00	Others [Contingencies Advance]	0.00
0.00	Printing & Stationery [Advance]	0.00
15,000.00	Revolving Advance	0.00
0.00	Scientific Workshops - Symposiums - Seminars [Advance]	0.00
1,598,071.00	Security Deposit	91,393.00
0.00	Software [Advance]	0.00
0.00	TA Abroad [Advance]	0.00
0.00	TA-DA-Hon within India [Advance]	0.00
0.00	Telephone [Advance]	0.00
0.00	Trainee Security Deposit	0.00
10,779,150.00	March salaries provision discharged	123,446,282.00
0.00	Water [Advance]	0.00
98,696.00	Workshop & Conference	0.00
277,544,233.00		**279,287,576.00**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS (CDFD)
RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2021

Annexure: E Forming part of Receipts and Payment Account

PAYMENTS

Amount in Rs.

Previous Year	Particulars	Current Year
	I-Remittances	
0.00	Contract Staff security deposit	74,620.00
3,645,603.00	ECCS	3,126,034.00
736,406.00	EPF/GPF	13,488,873.00
155,151.00	GSLI	176,376.00
1,601,319.00	GST TDS	1,682,773.00
0.00	Health Insurance	0.00
16,779,200.00	TDS on Salaries	17,636,406.00
1,499,573.00	LIC	1,798,524.00
272,614.00	Others (I-Remittances)	6,829,544.00
422,200.00	Professional Tax	438,600.00
0.00	Public Provident Fund	0.00
0.00	PM Cares Fund	375,814.00
0.00	STAFF BENEVOLENT FUND	10,000.00
673,597.00	TDS on Others	1,932,236.00
0.00	Works Tax	0.00
25,785,663.00		**47,569,800.00**

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2021

Annexure: H Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
0.00	Advance for Expenses- purchases by Staff	9,00,669.00
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
0.00	Consumables, glassware and Spares [Advance]	4,79,26,685.00
0.00	Conveyance Advance	0.00
11,96,26,062.00	Equipment [Advance]	12,63,556.00
0.00	Festival Advance	3,08,446.00
0.00	Health Insurance	0.00
0.00	Liveries & Blankets [Advance]	0.00
0.00	LTC [Advance]	0.00
0.00	Magzines [Advance]	0.00
0.00	Miscellaneous Salary	0.00
0.00	NPS Subscription	0.00
22,700.00	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
	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
11,96,48,762.00		**5,03,99,356.00**

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CHARY AND CO Chartered Accountants FRN: 014102S M.No. 221442

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2021

Annexure: I Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	DEPOSITS	
1,91,61,446.00	General Deposits And Advances	1,91,69,454.00
9,50,126.00	GDA[Others]	9,70,126.00
2,01,11,572.00		**2,01,39,580.00**

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Centre for DNA Fingerprinting
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Uppal, HYDERABAD

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2021

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]	1,14,35,274.00
1,42,00,773.00	Consumables, glassware and Spares [Advance]	1,18,83,068.00
1,00,65,134.00	Diagnostics Collabration With NIMS	1,00,65,134.00
1,92,678.00	ECCS	1,92,678.00
9,200.00	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
28,06,805.00	LTC [Advance]	26,87,643.00
854.00	Magzines [Advance]	854.00
1,54,433.00	Others (I-Remittances)	1,54,433.00
73,04,846.00	Others [Advances]	74,69,531.00
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
5,30,56,766.00	Research Fellows-Associates	4,37,58,727.00
1,10,406.00	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]	34,913.00
50,000.00	Telephone [Advance]	50,000.00
	Trainee Security Deposit	25,000.00
11,510.00	Transport maintenance [Advance]	11,510.00
4,58,186.00	Workshp & Conference	2,66,856.00
10,15,87,419.00		**8,98,31,744.00**

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Uppal, HYDERABAD

CHARY AND CO ★ Chartered Accountants ★ FRN: 014102S M.No. 221442

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2021

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**

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Uppal, HYDERABAD

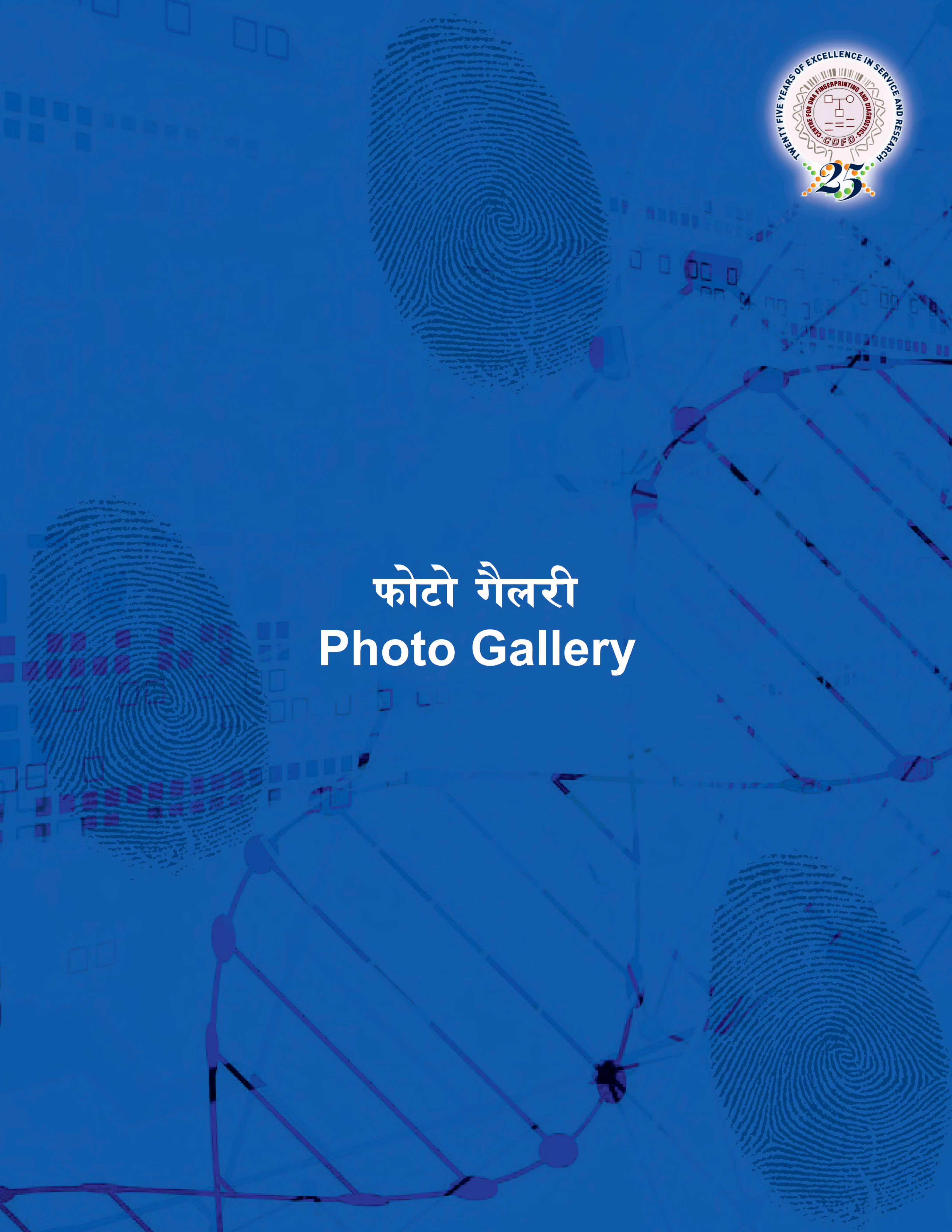

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Photo Gallery

Photo Gallery of some important events held during the period

Hindi Day Celebrations on 14.09.2020

Visit of Junior Civil Judges from TSJA, Secunderabad as a part of Training programmes-XXIV Basic Course Part-II Mid Term Practical Training on 21.10.2020

Observance of Constitution Day-Preamble Reading along side with Hon'ble President of India on DD National Channel and also a talk by Dr Repalle Shiva Praveen Kumar, IPS, Secretary, Social Welfare and Tribal Welfare Residential Schools, Govt. of Telangana under the Constitution Day Celebrations on 26.11.2020

Photo Gallery of some important events held during the period

Dr. Renu Swarup
Secretary, DBT

Prof. Michael Hanna,
Director, UCL Institute of Technology

25th Aniversary of CDFD Foundation, a lecture by Prof. Machael Hanna, Director, UCL Institute of Technology in August presence of Dr. Renu Swarup, Secretary DBT

Vaccination of 75 Corona Warriors against COVID 19 at CDFD on 03.02.2021

Observance of 32nd National Road Safety Month on 15.02.2021

Photo Gallery of some important events held during the period

Visit of Hon'ble Vice President of India Shri M. Venkaiah Naidu and Hon'ble Home Minister of Telangana Shri Mohammed Mahmood Ali to DBT-CDFD, Hon'ble Vice President of India inaugurated the Paediatric Rare Genetic Disorders Laboratory on 20.02.2021

Photo Gallery of some important events held during the period

Hands on Workshop on Next Generation Sequencing Data Analysis
for Clinical Diagnostics from 01.03.2021 to 05.03.2021

International Women's Day celebrations on 08.03.2021

Photo Gallery of some important events held during the period

CDFD Silver Jubilee Run (3k & 5k) on 15.02.2021

Cricket Tournament arranged during Foundation Day Celebrations - 2021