BASIC AND APPLIED STUDIES ON MICROBIAL SYSTEMS: CONTRIBUTION TO RESEARCH PROGRAMMES IN BARC

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Abstract
This article provides a bird's eye view of long-standing programs in BARC employing microbial systems. These systems served as ideal models to address fundamental questions and to develop various applications. Microbial model systems were adopted early since the commencement of bioscience research in BARC. Over the years, these programs encompassed radiobiology, genetics, DNA recombination and repair, recombinant DNA technology, and stress biology. More recently, along with high class basic research, applications and translational aspects have gained traction. The programs have evolved to assimilate cutting edge technologies like next generation sequencing, omics, CRISPR-gene-editing and high-end imaging etc. Microbial research in BARC which carries a rich legacy of pioneering research is poised to take up new challanges through an interdisciplinary approach.

1. Introduction

Microbes are the natural choice for studying many fundamental processes of life. They provide the simplest yet elegant models for investigating most complex biological phenomena. The ease with which they can be cultivated requiring simple lab

infrastructure and their amenability to rapid, direct and detailed analysis of problems of wide interest makes them ideal model systems. The value of prokaryotic research is quite apparent now, and as many visionaries foresaw, because of the fundamental unity in conservation of the molecular processes throughout the biological kingdom. Many groundbreaking discoveries that have earned Nobel Prizes, such as restriction enzymes, transposons, DNA polymerases, genetic code for protein synthesis and genetic recombination, originated from fundamental microbial research. These innovations have not only deepened our understanding of molecular biology but also revolutionized fields like genetic engineering, biotechnology, and medicine, showcasing the immense value of studying microorganisms.

Studies employing bacteria and bacterial genetics started early in the life cycle of establishment of department of atomic energy. The bacterial research can be traced back to fifties and early sixties and have gone from strength to strength since then. No wonder bacterial research is of historical as well as contemporary interest. At BARC, the history of bacterial research can be divided into three eras; the first representing the early foundational studies employing bacterial model systems to study radio-sensitization using various radio-sensitizers and -modifiers and examination of post-irradiation biochemical processes. Then the natural progression to the second era where the power of genetics and molecular biology approaches was harnessed and finally the evolution to the modern era with the adoption of advanced genetic manipulation, next-generation sequencing and omics approaches. As the evolution was gradual and natural there is significant overlap between these eras and this chapter attempts to capture a glimpse of the glorious history of basic and applied microbial research made over seven decades without straining to be strictly chronological.

2. Early foundation of microbial research

The foundation of bacterial research within the atomic energy establishment was laid in its early years. In the fifties and early sixties, studies were initiated under the leadership of Dr. R. Gopal-Ayengar to understand the basis of radio-sensitivity in microorganisms. These studies which continued in the seventies and well into eighties mostly utilized microbial model systems such as *Escherichia coli* (*E. coli*), *Micrococcus radiodurans* (*M. radiodurans*) and *Hemophilus influenzae* (*H. influenzae*) for investigations of effects of radiation on biological systems. These studies were aimed at using microbes to understand the effects of gamma radiation on cellular components and the eventual outcome in terms of survival. Further, microbial models provided facile systems to test various radio-sensitizers such as iodoacetic acid, ascorbate, N-ethyl maleimide to name a few, which were studied for their capacity to cause radio-sensitization in microbes. Various hypotheses to explain the radio-sensitization in terms of hydroxyl radicals produced, repair capacity of the microorganisms and the interaction of the sensitizing chemicals to different cellular components like membrane, DNA and proteins were evaluated. Some of the notable findings of the various studies carried out in this area are summarized below.

In a breakthrough paper published in the journal Science in 1968 it was demonstrated that Iodine atoms are incorporated in bacterial membrane proteins when cells are irradiated in the presence of iodoacetic acid labelled with iodine-131. Such atoms are produced on reaction of iodoacetic acid with the gamma ray-induced hydroxyl radicals in the surrounding medium. This was one of the significant studies that formed the basis of investigation, in the later years, of incorporation of I-131 in higher cellular systems. Irradiation of cells in the presence of I^{131} showed a decrease in cell radioactivity with increasing concentration of ascorbate present during irradiation over a broad concentration range. It was hypothesized that ascorbate reduced the sensitizing effect by scavenging the iodine atoms which would have otherwise reacted with the cells. The sensitization by 2,2,6,6-tetramethyl-4-piperidone-N-oxyl (TAN) decreased with increasing concentration of ascorbate, but no net protective effect was noticed. Based on absorption studies and employing ESR signals an interaction between the two molecules was hypothesized and the effect of this interaction in net sensitization or protection was quantified.

E. coli B/r cells γ-irradiated under anoxic conditions and in the presence of N-ethyl maleimide showed enhanced damage in terms of their colony-forming ability. It was shown that irradiation leads to binding of N-ethyl maleimide with cellular macromolecules, particularly proteins. Binding of NEM to non-specifically to all amino acid residues was shown with in vitro irradiation of bovine serum albumin in aqueous solution. It was hypothesized that the binding of N-ethyl maleimide with cellular proteins may lead to inactivation of enzymes involved in post-irradiation biochemical processes giving rise to enhanced damage. A reaction between N-ethyl maleimide and amino acid radical was suggested. It was shown that N-ethyl maleimide reacts with amino acids in the absence of radiation also, which could partly explain the residual sensitization noticed in cells treated with N-ethyl maleimide. Using results obtained with ESR it was shown that part of the sensitizing effect of NEM was due to its electron affinic property. Similar studies were extended to recombination deficient mutants of *H. influenzae* to assess the role of recombination in cell protection from killing by gamma irradiation.

It needs to be placed on record that fifties were the nascent years of radio-biology research. Following the bombings of Nagasaki and Hiroshima worldwide interest emerged to understand the effects of radiation on biological systems. This was the period when radiobiology departments began to be established in atomic energy institutions and highly reputed universities in the west. It was during this period many pioneering studies on investigation of the mechanisms of radio-sensitization in microbes were carried out and it is a matter of pride that radio-biology research carried out in atomic energy establishment/ BARC contributed in no insignificant terms to these efforts.

3. Contribution of microbial research to advancement of genetics

The microbial genetic research which started in late sixties and seventies, was initially centered around studies of genetic transformation in *H. influenzae* but later expanded into investigations of recombination and repair pathways in *E. coli*, *Deinococcus radiodurans*

and cyanobacteria. Transformation was discovered by Fred Griffith in 1928. At the time, it was not known that DNA was the genetic material. It was not until 1944 that Avery, MacLeod and McCarty provided the proof that the transforming principle was DNA. This study along with the landmark study of Hershey and Chase in 1952 were pivotal in establishing DNA as the genetic material. While during fifties and sixties transformation was an actively studied area of research, the fate of DNA upon entry, processes of DNA degradation, recombination and integration were hardly understood. Several researchers, including M. S. Fox and Sol H. Goodgal pioneered the use of ^{32}P-labeled DNA to demonstrate the physical uptake of DNA in transformation.

The Hemophilus studies in BARC were led by Dr. N. K. Notani, who had been trained in the laboratories of R. A. Brink at University of Wisconsin–Madison and S. H. Goodgal in the University of Pennsylvania. Brink was a renowned plant geneticist and also doctoral advisor to Esther Lederberg a pioneer in bacterial genetics and molecular genetics. *H. influnzae* was an excellent choice for genetic studies because of its natural ability to take up external DNA. The research focused on elucidating molecular events following the entry of the DNA inside bacterial cell. Radioactive labelling of DNA facilitated quantitative measurements which proved invaluable for understanding molecular mechanisms of transformation. The fate of genetically marked, 32P-labeled, heavy transforming deoxyribonucleic acid (DNA) as opposed to 3-H-labelled host DNA was examined by sedimentation through sucrose gradients. Some of the notable findings were to show that transformation proceeds with insertion of single-stranded segments of donor DNA which displaces the resident homologous DNA. Further it was shown that either strand of DNA could transform. A method of digitonin lysis was developed to easily separate intracellular donor DNA from the resident DNA. This technique was utilized profitably to track the fate of donor DNA during transformation and subsequent recombination. Also, these techniques were combined with electron microscopy for direct visualization of the DNA. Two strains deficient in genetic recombination were studied by this method and also equilibrium density-gradient centrifugation to demonstrate that the two mutants were blocked in two different steps.

These approaches were extended to study the fate of phage DNA. A major finding was to show that after the entry of phage DNA into wild-type cells, the DNA is degraded at early times, but later some of the fragments are reassembled, resulting in molecules that sediment faster than the monomer length of phage DNA. This study demonstrated that phage DNA is fragmented after entry into host cell but is reassembled by recombination to form concatenates. Another significant finding was to demonstrate the requirement of rec-gene expression for chimeric plasmids.

Apart from the genetic studies, with the advancement of molecular tools, genetic engineering approaches were introduced and various cloning vectors were constructed in eighties. With gradual adoption of recombinant DNA technology, the foundation for transgenic research was laid. A plasmid borne larvicidal crystal protein gene from *B. thuringiensis* subsp. *kurstaki* was cloned in *E. coli* and high level of gene expression was demonstrated. Transgenic *E. coli* cells produced large irregular bodies which were

purified by sonic disruption of cells and were shown to be highly toxic to the larvae of the insect pest *Spodoptera litura, Helicoverpa armigera* (Gram pod borer) and *Bombyx mori* (Silkworm). While megaplasmids of Rhizobia were known, researchers in BARC were able to identify relatively, smaller-sized plasmids form Rhizobia and characterized the *nif* and *nod* genes important for nitrogen fixation. Similarly, efforts were made to characterize *nif* and *hut* operons of *Klebsiella pneumoniae*. A small endogenous plasmid from the Cyanobacterium *Plectonema boryanum* was characterized. These initial efforts paved the way for establishing advanced molecular biology research in DAE. Many of the studies listed above particularly those on bacterial transformation were indeed pioneering and fetched due recognition from national and international scientific community.

4. Pathways of Recombination and Repair

Faithful transmission of genetic material from one generation to the next is considered a sine qua non for preservation of life. The discovery of DNA as the genetic material and its replication as the basis of heritability triggered an avalanche of studies on processes that maintain its stability. Armed with the knowledge of deleterious effects of radiation and chemicals on DNA and tools of classical bacterial genetics, scientists were first to elucidate repair pathways in *E. coli*. The isolation of repair sensitive mutants in *E. coli* greatly facilitated the discovery of photo-repair of UV induced pyrimidine dimers, nucleotide excision repair and post-replication-repair (dependent on homologous recombination) all reported between 1962-1968. It was discovered that recombination serves as one of the most important mechanisms involved in DNA repair which ensures transmission of correct genetic information to offspring from bacteria to man. Soon after it was realized that the process of replication, recombination and repair are intricately linked and carefully orchestrated to maintain genomic integrity.

As pathways of repair and recombination were being elucidated in late sixties and seventies, a strong research program emerged in BARC and a glorious chapter was written with a flurry of exciting discoveries in this area of research. This burst of brilliance culminated in four back-to-back publications in the journal Nature with three of them appearing in 1977 alone. Remarkably, S.K. Bhattacharjee was the lead author in two of these publications and sole author in another. It is interesting to note that while these studies were focused on related aspects of 'light or dark repair' of UV induced DNA damage or pathways important in recombination and repair, three very different microbial model systems were used. The first paper which appeared in 1976, reported the variations in sensitivity to near-ultraviolet irradiation of amoebae grown in 12 h light/12 h dark and those grown in complete darkness. The study showed that in the dark-grown cells, as well as the S phase of the light-grown cells, the repair mechanism against the induced lethal damage might be lacking or non-functional. This study assumed significance as little was known of the light or dark repair of UV damage in eukaryotes. The second paper used cyanobacteria, believed to have been precursors to eukaryotes, as model system to study DNA repair. The study reported the existence of a very efficient

repair system against damage induced by UV in *Anacystis nidulans* and went on to demonstrate that the repair system was either inhibited or rendered less effective under aerobic conditions in the presence of light. This work was extended in the third Nature paper where further physiological evidence for the existence of a dark-repair (or protective) system in this organism was presented. It was also shown that a protein which was unstable in the light, appeared to be responsible for the resistance against lethal damage by ultraviolet light.

Around the same time, Mahajan and Datta, utilized a genetic approach to study the nature of intermediate products utilized by RecBCD and RecF pathways of recombination in *E. coli*. These pathways are important for the repair of DNA damage induced by gamma and UV radiation respectively. It is to be noted that like DNA repair, most of the recombination pathways were originally discovered in *E. coli*. Mahajan and Datta used results from conjugation crosses of *E. coli* K 12 to demonstrate that the viable recombinants produced by the RecBC and the RecF pathways were significantly different in terms of the density of genetic exchanges present in them. These experiments built upon the previous work of Dr. Mahajan carried out in University of Pennsylvania, where a mathematical formulation of *E. coli* conjugation system visualized the recombinational process as consisting short regions of recombination (RRs), each of which may contain several genetic exchanges. Increase in the probability of initiation of the RRs was reflected in increased values of two-point recombination frequencies (Rl) between pairs of markers, while increase in the mean number of exchanges per RR, was reflected in increased values of R2(1), which was a measure of the frequency of additional exchanges close to a selected exchange. The study used R1 and R2 values to measure differences in final products of RecBCD and RecF pathways. With remarkable foresight this work proposed a model which postulated that the RecBC pathway preferentially promotes integration of double-stranded donor DNA segments which may have short single-stranded ends, whereas the RecF pathway mostly promotes integration of single-stranded donor segments.

Another notable study that needs mention is the investigation of the role of polynucleotide phosphorylase (*pnp*) in repair of UV damage. The involvement of DNases in recombination repair pathways was well established but it was difficult to envisage a role for RNases. The study stemmed from the observation that deletion of *pnp* conferred increased UV sensitivity in *E. coli*. By analysing various DNA repair pathway mutants and employing classical genetic approaches an interaction between recombination repair pathway and *pnp* was established. Pulse labelling and examination of nascent DNA synthesis showed that restoration of replication fork and replication re-start were unaffected in *pnp* mutant. Interaction of the repair helicase RecG with PNPase was shown at genetic level. Subsequently, studies at other labs with Bacillus corroborated these findings.

In addition to various prokaryotes, the single-celled fungi *Saccharomyces cerevisiae* served as a suitable eukaryotic model to study repair pathways particularly to study the effect of environmental stress on radiation response. Altered UV and gamma radiation

response under various stress conditions, such as osmotic shock, heat shock, and mild chemical treatments was studied. The investigations of stress-inducible DNA repair in *Saccharomyces cerevisiae* identified a general response enhancing repair and a particular response where the DNA damage may act as a signal for enhancement of the DNA repair.

5. Microbial Stress biology research

While studying a genetic conjugational cross in *E. coli*, low yield of recombinants at lower non-optimal temperature led to serendipitous discovery of a cold sensitive mutant MD1157. This sparked investigations into the linkage between cold stress tolerance and recombination pathways. While a linkage could be not be firmly established, these studies gave a fillip to research in cold stress tolerance in *E. coli*. At that time, heat stress response in bacteria and other organisms had been well studied but molecular mechanisms governing cold stress tolerance were less known. The original mutation in MD1157 mapped to a gene *gicA* (growth in cold), which was later renamed as *cspE* (cold shock protein). Interestingly, though *gicA* from MD1157 was cloned and a mutation in the promoter was identified the cold sensitive phenotype could not be complemented with a wild type allele. Contemporaneous, to these studies Masayori Inouye at Rutgers University identified a highly cold inducible gene *cspA*. Analysis of the genome sequence showed that *E. coli* K-12 strains had eight other genes, including *cspE*, with high homology to *cspA* and the gene family was called 'CspA' family.

The quest to understand how *E. coli* responds to cold stress began with a seemingly simple question: why does this microorganism possess not just one, but nine different cold shock proteins (CSPs)? The presence of these highly conserved proteins suggests an ancient and essential role in the survival of this enteric organism—a role that may include functions still hidden from our understanding. Though these RNA/DNA binding proteins seem redundant at the first glance, they are not all regulated in the same way. This hints at a complex, finely tuned system where these proteins play distinct roles in helping the bacterium cope with different environmental stresses. The RNA chaperone activity of these proteins seemed to be crucial for cold-stress as well as virulence. One of the key players in this regulatory network is CspE, a nucleic acid-melting protein. Studies in BARC showed that CspE is regulated by Ribonuclease E post-transcriptionally through temperature-dependent secondary structures, demonstrating a quick and dynamic response of *E. coli* to the cold environments.

It is worth mentioning that while CSPs were cloned, purified and characterized *in vitro* across labs, cold sensitive mutants in any gene of *cspA* family could not be obtained. The *in vivo* role of CSPs was a matter of scientific debate for a long time and it is in this context the subsequent studies carried out in BARC assume significance. Studies in BARC led to identification of loss-of-function alleles of CspC in related strains of *E. coli* K-12. Sequencing of *cspC* alleles showed that the gene suffered mutations frequently and by diverse molecular processes which included deletion, transposon insertion and point mutations. Occurrence of *cspC* mutations in independent strains suggested that they had

an evolutionary advantage. A combination of genetic crosses and competitive fitness studies demonstrated that loss of CspC conferred a selective growth advantage to the cells. Significantly, the selective advantage was manifested irrespective of the molecular mechanism that led to loss of *cspC* function.

The research in cold-shock response extended further with the finding that the cyclic AMP receptor protein (CRP), traditionally known as a master regulator of metabolism, contributed to cold adaptation. Its unexpected role in cold shock gene regulation, particularly in governing the expression of CspD, a bacterial toxin, and CspE revealed that its function encompassed more than just metabolism. When CRP is absent, *E. coli* struggles to grow in cold conditions, emphasizing its vital role in the survival of this bacterium at low temperatures. Further studies highlighted that the role of CRP in stress response goes beyond cold adaptation. It was shown to play a significant role in helping *E. coli* withstand antibiotic treatment by regulating MqsRA, the toxin-antitoxin pair, which is crucial for the formation of persister cells—tough, resilient cells that can survive antibiotic exposure causing recurrent infections. These findings suggested that CRP is central to the *E. coli* overall stress response strategy. Notably, other labs have continued to build on our work, further exploring CRP involvement in antibiotic persistence.

In a parallel approach that focused beyond the CSP-centric narrative, cold sensitive mutations were identified and studied in *E. coli*. One such mutation identified in MD1157 strain, *gicD1*, which was extremely interesting because of its association with UV and gamma sensitivity was investigated intensely. It was demonstrated that *gicD* locus was allelic to *infB* gene coding for translation initiation factor IF2 and this led to identification of a novel mutation in IF2. The mutation completely abolished the streptomycin resistance by *rpsL31* mutation in S12 ribosomal subunit showcasing the temperature dependent fine-tuning of the tripartite interaction between ribosome, IF2 and streptomycin in *E. coli*. As MD1157 carried both *gicA1* and *gicD1* mutations their individual contribution to the cold sensitive phenotype was clarified. Employing a series of genetic crosses and construction of genetic backgrounds devoid of one or the other mutation and sequencing of the alleles it was demonstrated that cold sensitivity in MD1157 was primarily governed by the *gicD1* mutation and the *gicA1* mutation in *cspE* had little contribution.

In addition to research on bacterial persistence, significant progress has been made in understanding the antibacterial mechanisms of various common and important antibiotics. It was found that antibiotics like ciprofloxacin kill bacteria by damaging their DNA and inducing oxidative stress through the production of reactive oxygen species (ROS). It was discovered that common antioxidants, such as glutathione (GSH), ascorbic acid, and N-acetylcysteine (NAC), can protect bacteria from this oxidative damage. The studies revealed that antioxidants neutralize ROS, shielding bacteria from the full impact of antibiotics. Furthermore, GSH lead to activation of bacterial "efflux pumps," which help bacteria expel antibiotics from their cells, thereby increasing their resistance to ciprofloxacin. This means that these antioxidants when taken along with antibiotics might actually hinder the effectiveness of the antibiotics. In stark contrast, while GSH

protected bacteria from ciprofloxacin and aminoglycosides, it made them more susceptible to the β-lactam class of antibiotics. This finding indicates that antioxidants may have different effects depending on the type of antibiotic used. Utilizing advanced techniques like transcriptomic profiling, researchers discovered that GSH triggers multiple stress responses in bacteria, aiding their survival in challenging conditions. This research demonstrates that while antioxidants are beneficial for human health, they may inadvertently contribute to antibiotic resistance in bacteria. This insight could have significant implications for the future use of antioxidants with antibiotics.

Another model organism that generated worldwide interest for its extraordinary resistance to gamma radiation was *Deinococcoccus radiodurans*. This bacterium can reassemble its genome even after it is completely shattered by gamma irradiation. Interestingly, a highly radio-resistant member of Deinococcaceae family, *Micrococcus radiophilus* later renamed as *Deinococcus radiophilus*, was first isolated in BARC by N. F. Lewis in Bombay duck (*Harpadon nehereus*) in 1971. Lewis along with his co-workers in BARC went on to record extreme UV resistance of this organism as well as reported presence of characteristic pigments of lycopene family. They proceeded to deposit the culture with the National Collection of Type Cultures, Colindale, London. However, it was the publication of the genome sequence of *Deinococcoccus radiodurans* in 1999 that created a renewed vigour in the scientific community to unravel molecular mechanisms underpinning its extreme resistance to radiation. Immediately after the publication of genome sequence, in around year 2000, programs were started in BARC to explore the potential of this organism for remediation of low-level radioactive waste as well as to understand the basis of radioresistance. Extensive genetic and biochemistry studies were carried out on repair pathways, signalling and protein complexes involved in such pathways. A series of studies involving a proteomic approach demonstrated protein recycling during post irradiation recovery which was first time reported from BARC. Further it was shown that a common set of proteins are involved in response to gamma radiation and desiccation exposure.

In summary, these findings revealed the intricate link between various stress responses offering new insights into how these bacteria adapt and survive under different adverse conditions. This research opens up new avenues for further exploration, hinting at the complex interplay between metabolism, stress adaptation, and bacterial resilience.

6. Journey of CRISPR-Cas

In recent years CRISPR/Cas based systems have emerged as the most advanced and very powerful technology for genome manipulation. The rapidly emerging CRISPR-Cas toolbox has ushered in a revolution of sorts with versatile applications in all areas of biomedical sciences. Since the first demonstration of the potential of type II *S. pyogens* CRISPR/Cas9, by Jennifer Doudna and Emmanuelle Charpentier labs in 2012, for genome editing, innovative applications of this technology are continuously emerging. In addition to gene editing, CRISPR-Cas tools have expanded into genetic/epigenetic regulations, imaging and genome-scale screens. CRISPR-Cas tools are also facilitating

new discoveries in food, medical and plant biotechnology research. Exciting avenues such as gene therapy, creation of transgenic models, development of new age anti-microbials, diagnostics and vaccine development are being explored with renewed vigour. The Bioscience group realized the potential of this technology as early as 2014 and programs were launched with an overarching goal of establishing the CRISPR technology in-house. Several approaches to engineer natural CRISPR components in various host systems and exploit them for a broad range of biological problems were explored as described below.

CRISPR/Cas systems can introduce double-strand breaks in target DNA, which, if not repaired, can be fatal to the bacteria, making them promising candidates for antimicrobial strategies. This feature was used to demonstrate the antimicrobial action of CRISPR-Cas9 in *Mycobacterium* which was further extended to targeted killing of drug resistant mycobacteria. As gene knockouts are difficult to achieve in Anabaena, Cas9-based system was engineered to develop a gene silencing tool for Anabaena PCC 7120. This tool was used to knockdown a specific gene which was crucial to prove its role in maintaining redox homeostasis. This demonstrated the potential of this tool in advancing cyanobacterial research. It was observed that extending the applications of Cas9 was constrained by cellular toxicity it conferred in different species of bacteria. Investigation of Cas9 toxicity showed that expression of *cas9* caused plasmid instability and reduced frequency of genetic transformation. In order to utilize the full potential of CRISPR-Cas systems, alternative Cas effectors with complementary features were explored. Research in BARC and few labs elsewhere have pioneered the use of type I Cascade for various applications. Additionally, type V Cas12 systems were explored for specific applications. Engineering of these systems, however, requires careful consideration of parameters to select optimal crRNA and effector properties. In-depth exploration of influence of attributes such as GC content of crRNA, secondary structure of crRNA, strand bias, target location and extent of off targets has been carried out through experimental and computational approaches. The findings provided a 'practical guidebook' for determining the Cas effector that would be most optimum for a particular application to achieve expected outcome.

Research in BARC contributed to the first demonstration of type I Cascade-based CRISPRi in any model system. Its utility in investigating difficult to study essential genes was demonstrated in *E. coli*. Employing the Cascade-based CRISPRi an uncharacterised essential gene *racR* was shown to be a negative regulator of toxins, Ydas and YdaT. Further, Cascade-based CRISPRi was extended to *Salmonella* species another important microbial model system. Another significant milestone achievement was to develop CRISPR-based gene silencing in *Deinococcus radiodurans.* It is worthwhile to mention that even though the organism carries a multiploid genome, knockdown efficiency of around 90% was achieved. The expertise in type I system was utilized to develop a CRISPRi vector with several useful features such as easy targeting and inducible, reversible, multiplexed and titrable gene silencing in *E. coli* and *Salmonella*. It is the only vector available with such advanced features for bacterial CRISPRi.

Further extending the applications of CRISPR technology, a type V CRISPR/Cas12 system was employed to investigate essential genes in *M. smegmatis*. The CRISPRi screen revealed several essential genes including a conserved gene cluster involved in cell wall synthesis. Extensive characterization of MSMEG_0311, a gene in the cluster identified it as a potential drug target. The CRISPR-based in house capabilities were extended to manipulate higher organisms. In a significant feat, the expression and interference phases of type IE CRISPR system was reconstituted in the yeast, *Saccharomyces cerevisiae*. CRISPR-based antiviral and gene editing potential was demonstrated as proof of concept. For eukaryotic applications vectors were designed for mammalian and plant genome editing. A challenging task of generating *rag1* gene knockout mouse was accomplished in collaboration with IISER, Pune. The CRISPR-based transgenic mice technology is available only in highly advanced laboratories and select countries across the world. This endeavour supports the department's need for transgenic mice required for disease modelling, cancer research and drug screening.

In response to global SARS-CoV-2 pandemic, a CRISPR-based sensitive detection method for molecular diagnosis of the pathogen was established. Towards point-of-care application, particularly in low resource settings, the assay was coupled with a portable, battery-operated device, CRISPR-Cube which was developed in collaboration with EmA&ID, for rapid and visual detection of the results. The assay was validated on patient samples and the technology was transferred to the industry. This versatility of this platform technology has been demonstrated by single copy detection of Mpox virus in contrived samples, highlighting its potential for broader application in infectious disease diagnostics.

In the ten years since inception of the work in the department, a strong expertise in CRISPR-Cas technology has been developed for addressing various biological problems of fundamental and applied nature as showcased by quality publications and technology transfers. The endeavor to adopt, adapt and improvise the technology for a plethora of applications will continue. It can be anticipated that in future the power of CRISPR-technology will be widely harnessed in almost all programs of Bio-Science group.

7. Future Outlook

The foundation of the journey of microbial research in BARC was erected on strong pillars. Along the way it imbibed new ideas and generally set an illustrious standard of academic scholarship. As we enter the modern era of research, the strive for excellence to remain in the forefront continues. Building upon the core strength of microbiology, genetics, biochemistry, molecular biology and genetic engineering, the new era ushers next-gen approaches that includes omics, informatics, genome engineering and super-resolution imaging that seeks to break the barriers of magnification and limits of resolution. It is also the time to see biology in action, live inside the cells, to catch molecular machines in the act.

CRISPR-Cas tools and next generation sequencing have already hugely impacted all the areas of bioscience research and technology. It is likely that innovations in sequencing

technologies will continue to increase the throughput and will play a bigger role in all areas of research necessitating increased adoption of powerful bioinformatics tools. More and more CRISPR-based gene therapies are likely to cross clinical trials and reach clinics. The two complementary technologies together provide a very powerful combination for genome scale interrogation of structure and function. For instance, it is becoming increasingly common to employ large-scale CRISPR screens for identifying new drug targets for cancer. Together these technologies are likely to usher in an era of personalized medicine. The tools and approaches like AI, machine learning, high throughput sequencing, omics, CRISPR-gene-editing, high-end imaging place unprecedent power in the hands of researchers to venture and explore unchartered territories. The traditional boundaries between biological, physical and chemical science are increasingly getting blurred as different scientific disciplines collaborate and coalesce to create new science and technologies.

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