Battle with a Bug: The Legacy of Prof. T Ramakrishnan

Kavitha Sarvepalli and M E Sandeepa


(left) Kavitha Sarvepalli is a PhD student in the Microbiology and Cell Biology Department, IISc, Bangalore. She works in the area of plant development.
(Right) M E Sandeepa is a post-doctoral research fellow at the Cleveland Clinic Foundation, USA. He is investigating the molecular mechanisms involved in cell migration in endothelial cells.

Professor T Ramakrishnan, fondly remembered as TR, is celebrated as one of the outstanding scientists associated with tuberculosis research not just in India but in the world. He is best remembered for his pioneering work on *Mycobacterium tuberculosis* (*M. tuberculosis*) metabolism, elucidation of the action of anti-tuberculosis drugs and the discovery of mycobacteriophages. It was largely his efforts which kept the spirit of inquiry and research alive at a time when tuberculosis was considered a third-world disease, unworthy of attention in developed countries. He also spearheaded the advent of molecular biology as a modern research tool at the Indian Institute of Science (IISc), Bangalore.

TR's tryst with IISc began in the mid-1940s. After completing his graduation in Chemistry from Madras University, he joined the Department of Biochemistry in 1945 as a post-graduate student. Seven years later, he left Indian shores to pursue higher studies in Canada, supported by the Government of India under international science exchange programme. There he joined J J R Campbell in the Department of Dairying, University of British Columbia, Vancouver. After obtaining his doctoral degree in microbial metabolism and biochemistry, TR applied to the Indian National Science Academy (erstwhile National Institute of Sciences of India) for a postdoctoral fellowship. In 1957 he re-joined his *alma mater*, as a lecturer in the Pharmacology Laboratory, then housed within the Department of Biochemistry and commenced work on the carbohydrate metabolism of *M. tuberculosis*.

The decision to work on *M. tuberculosis* was by no means a random choice. It was a well thought out move. Though his PhD work was on the metabolism of *Pseudomonas aeruginosa*, he was always interested in the physiology of the *M. tuberculosis*. Since centuries, this dreaded pathogen had been the cause of immeasurable misery to mankind. In the pre-independence era, attempts to

Keywords
Mycobacterium tuberculosis, mycobacteriophages, I3.

treat and contain tuberculosis were woefully inadequate – rest at a sanatorium with a normal diet supplemented with calcium tablets, and occasional injections of gold suspensions were the only recourse left to patients. Thousands perished to this wasting disease every year. In fact, as a child, TR himself underwent the trauma of seeing his mother succumb to tuberculosis. The advent of anti-TB drugs such as streptomycin and the launch of mass BCG vaccination after independence helped in curbing this menace to some extent. Sadly, even this respite was short-lived. It soon became clear that the BCG vaccine could not afford protection beyond childhood; moreover the physicians now had tougher adversaries to contend with – emerging strains of drug-resistant bacilli. The need of the hour was to develop new chemotherapeutic agents. More importantly, it was essential to understand the mechanism of resistance to the existing drugs and to use this knowledge to synthesize drugs that can specifically inhibit the metabolic activity of the pathogen without affecting the host. The onus was now on basic science.

With these ideas, TR initiated work on H37Rv, a virulent strain of mycobacterium. At that time, carrying out research in India, that too on a virulent pathogen, was easier said than done. M Sirsi, the head of the Pharmacology Laboratory at that time and incidentally a physician by training, had set up some facilities to test the action of anti-mycobacterial drugs. Apart from that, the department did not have the basic facilities for conducting experiments on metabolism. The only equipments available were a ramshackle shaker and a Beckman UV spectrophotometer. There was no provision for getting imported chemicals; to get a standard chemical like ATP, one had to isolate it from rabbit muscle after giving repeated injections of magnesium sulfate solution to the anaesthetized animal. Micropipettes were considered a luxury.

Such difficult circumstances, however, did not deter TR; they only strengthened his resolve. Backed by his departmental head who gave him unstinting support, TR set about the formidable task of organizing his research. Since funding from Indian agencies was meager, he approached foreign agencies. The first sonicator, a refrigerated centrifuge and a fraction collector in the department were in fact bought with the money that TR obtained from the Rockefeller Foundation, USA. He obtained chemicals from Sigma and also arranged for the import of radioactive isotopes for use in metabolic tracing. His first PhD students were M Indira, P S Murthy and V G Malathi. They formed a diligent team who were constantly motivated by their mentor. The quiet dedication and passion in TR served as a bouyant force which kept everybody's spirits up. Within a year of joining the institute, he made an astonishing discovery which was published in *Nature* [1]. His group showed, by simple experiments, that tender coconut water contains a growth-promoting factor for *Mycobacterium tuberculosis* which could shorten the incubation time required for full growth of the organism by almost half, without affecting its virulence.

After this, there was no looking back. The laboratory made significant advances in understanding the metabolism of *M. tuberculosis* over the next decade. *M. tuberculosis* was found to have not only the glycolytic pathway and the TCA cycle but also the glyoxylate shunt [2]. One of the enzymes of the glyoxylate shunt, isocitrate lyase, was found to be highly active and TR had the foresight to suggest exploiting this enzyme as a potential drug target – a prediction which came true in the year 2000. In addition it was shown that the virulent strain metabolized glucose predominantly by the glycolytic pathway while the avirulent strain did so by the oxidative pathway [3]. This finding shed light on a long-standing puzzle of how the virulent strain alone could survive successfully in anaerobic conditions of the developing tissues. Simultaneously, work was also carried out on the biosynthesis of nucleic acid in mycobacterium [4]. About this time, studies were initiated on the Nicotinamide Adenine Dinucleotidase (NADase) enzyme, which controls the levels of oxidized and the reduced forms of NAD, a key cofactor in oxidative metabolism and its protein inhibitor was also isolated [5]. The inhibitor gained in significance when it was proposed by another group that it is the target of the antitubercular drug isoniazid. Subsequently, the mode of action of isoniazid was worked out in detail by TR's group and it was shown that it was, in fact, a protein with catalase-peroxidase activities which was responsible for isoniazid sensitivity [6,7]. Similarly, the mode of action of streptomycin was investigated [8].

Apart from being the hotspot of intensive research on mycobacterial metabolism, TR's laboratory also had the distinction of being the "cradle of modern molecular biology" in the country. The idea to introduce molecular biology tools in the department took seed from a fortuitous meeting with one of the founders of molecular biology, Jacques Monod, at the Society of Biological Chemists' conference at Delhi in 1961. Monod convinced TR that the field of molecular biology, i.e., isolating genes responsible for a particular phenotype and manipulating them to alter the properties of organisms, held the key to solving the problem.

The implementation of this idea, however, was another Herculean task. Molecular Biology was a new concept in India. To improve his understanding of and expertise in molecular genetics, TR went to Yale University and worked in Adelberg's laboratory for two years. Even there, since no one worked on *Mycobacterium*, he worked on *E. coli*, with the hope of applying the methods learnt on *Mycobacterium* back home. He even took courses to strengthen his hold on the subject. After returning to India in 1964, he applied for grants from the Wellcome Trust, to buy modern equipments like liquid scintillation counter and ultracentrifuge.

Besides paving way for modern research at the Institute, TR also had to fight unwillingness to accept an emerging area of biology into the fold by some scientists. Incredible, though, it may sound today, especially when each and every laboratory in the department employs molecular

biology tools extensively, there was a time when this new discipline was seen as a threat to the dominance of the old tried-and-tested magic of "pure" biochemistry! TR however did not care about such fears but went ahead with a great deal of enthusiasm in fostering modern research. Keeping in mind the benefits he accrued from attending courses during his stint at the Yale University, he started giving similar courses in this subject to students of both the Department of Biochemistry and the Fermentation Technology and Pharmocology Laboratories. Later on, when he took over as the Chairman of this Department in 1973 (then known as the Microbiology and Pharmacology Laboratory), he helped in founding an interdepartmental Molecular Biology Group and became its convener. The aim of this group was to organize lectures in molecular biology and also to train select PhD students in research work, specifically in this area of biology. All these contributed immensely to the growth of molecular biology and genetic engineering at the Institute in later years.

The molecular biology-based approaches provided impetus for novel studies in TR's laboratory. The first of the landmark achievements was the isolation of mycobacteriophage, I3, the first transducing phage for this genus to be characterized. This finding, which was published in *Nature*, greatly simplified genetic studies of mycobacteria and their drug resistance [9]. Subsequently, amber mutants of I3 were studied to understand the genetics of this phage [10]. Simultaneously work was initiated on transcription and replication in mycobacteria – work that is being carried out in the department to this day. The aim was same as the one for the work on metabolism, i.e., to study the differences between the virulent and avirulent strains to exploit those differences in designing new drugs. Prior to this TR went on a sabbatical for two years and spent some time in Harrison Echol's laboratory in University of California, Berkeley, where he worked on transcriptional control in *E. coli*. In 1976, it was shown that RNA polymerase of *M. tuberculosis* was 1000 times more sensitive to rifampicin than the enzyme in *E. coli* [11,12]. This finding formed the basis of the most highly-cited article for mycobacterial molecular biologists and also led to the development of a new antitubercular drug, N-napthylglycine hydrazide which inhibits the mycobacterial enzyme at low concentrations [13]. In the area of DNA replication, it was shown that the slow growth of *M. tuberculosis* was reflected in the slow rate of its DNA synthesis [14].

Though mycobacterial research was his chief interest, TR was actively involved in other research problems as well. He was instrumental in initiating many projects where he led the way and then passed on the baton. Both the animal and the plant tissue culture facilities at the Institute were started by him. The work on animal tissue culture came into existence in 1973 in collaboration with W E Levinson, who had the expertise in working with Rous Sarcoma Virus. The techniques learnt and practiced on this virus were then used to study rinderpest virus, which causes extensive disease in cattle of India and other tropical countries. Simultaneously, plant

tissue culture was initiated in collaboration with C S Vaidyanathan of the Department of Biochemistry. He also started work on serotyping of rotaviruses as part of a project funded by the World Health Organization.

TR has contributed immensely to the overall growth of the Department of Microbiology and Cell Biology, not just through his scientific work but also through his other activities. For instance, he organized many international courses in the department where experts were invited to give lectures to students. He obtained the funding for these courses from International Cell Research Organization (ICRO) in collaboration with UNESCO. The first of such courses covered the topic of molecular biology of viruses and bacteriophages. Many distinguished scientists were invited to teach students and among them was M Levine, who along with F Stahl, had discovered the molecular nature of the genetic material. Participating students came even from outside the country. The second international workshop, again funded by ICRO/ UNESCO, was held a few years later. This time the topic was 'Animal Virus and Tissue Culture'. During his stay at the Institute, the department grew from strength to strength – be it starting of new courses, diversification into unchartered but exciting areas and induction of right people at right times. Even when he retired (as the Chairman of the department) in 1982, after a tenure of ten years, his association with the Department continued. TR was one of the conveners of the Genetic Engineering (GE) group and was entrusted with the job of supervising the construction of the new GE building, for which he had co-written the grant to the Department of Science and Technology.

TR received many awards, such as Watmull Award in Microbiology (1964), FIE Foundation Award in Microbiology (1983) and Ranbaxy Research Award in Medical Sciences (1985) in appreciation of his invaluable work on mycobacteria. Though his achievements in a career spanning four decades went largely unnoticed by the Government of India, his calibre and dedication were hailed by scientists all over the world. In fact, Barry Bloom, author of the book '*Tuberculosis*: *Pathogenesis*, *Protection* and *Control* has made a dedication in the preface, to five scientists and physicians from all over the world who have made lasting contributions to research on tuberculosis and TR is one of them.

In many respects, TR was a man who was ahead of his times. He was respected and admired not only as a dedicated scientist but also as a visionary. His passion for research on *Mycobacterium tuberculosis* and for science, in general, was awe-inspiring. One small and little-known fact is that he had built a model of I3, the mycobcteriophage that was discovered in his laboratory, on the lawn in front of the department. His colleagues and ex-students remember him as a strict disciplinarian (to put it mildly!) who did not condone lax behaviour, be it from his own students or from any other department member. Over and above, he strove hard to provide every facility

needed by the students. His generosity extended beyond his laboratory. As one of the ex-students of the department recollects, anybody was free to approach TR and ask for any reagent for his/her experiments and he never hesitated to help. Though he received world-wide fame and repute as a scientist, all the adulation never touched his selfless and humble persona. As Nayak, an ex-student and the former chairman of MCB, puts it, "Prof. Ramakrishnan was a brilliant scientist and a compassionate human being, and mind you…these qualities are not mutually exclusive. His life has inspired and will continue to inspire generations of students".

Suggested Reading

[1] **T Ramakrishnan, M Indira and M Sirsi, *Nature*, Vol.179, p.1357, 1957.**

[2] **P Suryanarayana Murthy, M Sirsi and T Ramakrishnan, *Biochem. J.*, Vol.84, p.263, 1962.**

[3] **T Ramakrishnan, M Indira and R K Maller, *Biochim. Biophys. Acta.*, Vol.59, p.529, 1962.**

[4] **V G Malathi and T Ramakrishnan, *Biochem. J*, Vol.98, p.594, 1966.**

[5] **K P Gopinathan, T Ramakrishnan and C S Vaidyanathan, *Arch. Biochem. Biophys.*, Vol.113, p.376, 1966.**

[6] **K S Sriprakash and T Ramakrishnan, *J. Gen. Microbiol.*, Vol.60, p.125, 1970.**

[7] **B G Devi, M S Shaila, T Ramakrishnan and K P Gopinathan, *Biochem. J.*, Vol.149, p.187, 1975.**

[8] **M S Shaila, K P Gopinathan and T Ramakrishnan, *Antimicrob Agents Chemother.*, Vol.4, p.205, 1973.**

[9] **C V Sunderaj and T Ramakrishnan, *Nature*, Vol.228, p.280, 1970.**

[10] **T Ramakrishnan and M S Shaila, *Arch. Microbiol.*, Vol.120, p.301, 1979.**

[11] **R M Harshey and T Ramakrishnan, *Biochim. Biophys. Acta.*, Vol. 432, p.49, 1976.**

[12] **R M Harshey and T Ramakrishnan, *J. Bacteriol.*, Vol.129, p.616, 1977.**

[13] **Rekha Prabhu, G Ramananda Rao, M Jamaluddin and T Ramakrishnan, *J. Biosci.*, Vol.10, pp.163–166, 1986.**

[14] **K T Hiriyanna and T Ramakrishnan, *Arch. Microbiol.*, Vol.144, p.105, 1986.**

Address for Correspondence

Kavitha Sarvepalli, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India. Email: kavitha@mcbl.iisc.ernet.in

M E Sandeepa, Post Doctoral Research Fellow, Department of Cell Biology, Lerner Research Institute / NC10, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195, USA
Email: eswaras@ccf.org.