Genomics and Tuberculosis

"New hopes to conquer an ancient disease"

It is almost a decade since the World Health Organisation declared tuberculosis a global emergency and drew the world’s attention to the scale of TB in developing countries. Today, one person acquires TB every second on earth and around two billion people – one third of world’s population – are infected with latent TB. One person dies of TB every 15 minutes worldwide, whereas in India the number is as high as one death every minute. Although there are a large number of anti-tuberculosis drugs available, the number of multidrug resistant TB (MDR-TB) is also growing. Moreover in the backdrop of HIV/AIDS, the emergence of MDR-TB has reached an alarming proportion. The only vaccine available against tuberculosis is of limited utility and no new diagnostic test has been identified which is rapid or simple enough for global acceptance. At such a time, when the future of tuberculosis treatment and control appeared real bleak, the recent report of the complete genome sequence1 of M. tuberculosis generated new hopes and raised expectations of all those involved with the treatment or control of tuberculosis. There has been a revival of confidence and commitment to tackle the disease on multiple fronts with the power of genomics and bioinformatics. Armed with the state of the art techniques of genomics and proteomics2, the researchers are enthused to take on this ancient disease as a fresh challenge. It is being projected as the most exciting time "to open new fronts in the old war"3.

Genomics is the study of the molecular organization of genomes, their information content and the gene products they encode. M. tuberculosis genome is one of the largest found (4.40 Mb), exceeded only by E. coli (4.60 Mb) and Pseudomonas aeruginosa (6.26 Mb). While a complete genome sequence was the first step forward, extensive application of bio-informatic tool to the sequence data further widened the information database of M. tuberculosis. Using bio-informatics, more than 3920 genes of M. tuberculosis have been uncovered that encode proteins more than 80 amino-acids in length1. A large number of genes have been identified for further studies. However, only about 40% of the genes have been given precise function and 16% of the genes resemble no known proteins. It is possible that these genes code for unique mycobacterial proteins specific mycobacterial functions and are responsible for complex host-parasite interaction observed in tuberculosis. More than 250 genes are putative genes for lipid metabolism (E. coli has only about 50 such genes), and M. tuberculosis may obtain much of its energy by degrading host lipids, leading probably to cachexia in the patients. There are surprisingly large number of regulatory elements in the genome. This may mean that the infection process is more complex and sophisticated than previously thought. The structural and functional analysis of these genes has a potential to reveal the functionalities of the large number of genes unearthed. Elucidation of the three-dimensional structure of the proteins encoded by these genes with computer aided modelling and x-ray crystallography will help to predict the functionally active domains of these proteins. The knowledge gained from such detailed study will be useful to understand the parasites’ pathogenic potential to be translated in future into improved candidate vaccines or newer drug targets. Some of the genes and operons in focus are those responsible for fatty acid metabolism, the mce operons, the ESAT-6 loci and PE-PPE group of proteins. These are also being studied for their potential role as virulence factors.
Genomics and bio-informatics have helped identify PE-PPE proteins as a group of glycine rich novel proteins of \textit{M. tuberculosis}. These represent about 10\% of the genome and are being projected as putative virulence factors in addition to their possible role in imparting antigenic variation to otherwise conserved genome of \textit{M. tuberculosis}. Ramakrishnan et al\textsuperscript{5} studied \textit{M. marinum}, which causes granulomatous latent TB in frogs, closely resembling human tuberculosis. They identified gene promoters that were switched on only when the bacteria were encapsulated in frog's granulomas. Two of these genes were from PE-PGRS family encoding unusual proteins, unique to the genus mycobacteria. When these genes were knocked out, the mutated strains were unable to grow inside macrophages. Similar genes, if identified in \textit{M. tuberculosis}, will be putative virulence genes that could be studied in detail with a view to use the gene products as diagnostic antigens or vaccination material. \textit{In silico} analysis of PE-PPE proteins has demonstrated abundance of asparagine, a preferred nitrogen source for mycobacteria, in them. Interestingly, asparagine has long been identified as a growth factor for mycobacteria and is routinely incorporated in the growth media to culture \textit{M. tuberculosis} in the laboratory.

It is widely believed that oxygen limitation, amino-acid starvation and carbon source restriction are involved in establishing and maintaining \textit{M. tuberculosis} in a dormant state. Correspondingly, emergence from dormancy is related to a partial or complete amelioration of these conditions. Hectic search is on to identify the genetic and enzymatic proteins that could be responsible for dormancy of \textit{M. tuberculosis}. Mycobacterial sigma factors (\textit{sig}), mce proteins (\textit{mce}) and isocitrate lyase (\textit{aceA}) genes are some of the candidate genes\textsuperscript{4,6,7}.

Isocitrate lyase is an enzyme crucial to the survival of \textit{M. tuberculosis} in granulomas. This enzyme allows the bacteria to get energy and build carbohydrates from fatty acids in the absence of oxygen. Search is on to look for compounds that would function as specific inhibitors of isocitrate lyase as future drugs. The proteins encoded by the \textit{mce} (mammalian cell entry) operons may mediate initial interactions between \textit{M. tuberculosis} and host cells. Similarly, ESAT-6 loci encode small proteins, very early in the growth cycle of \textit{M. tuberculosis}. These are a group of highly potent T-cell antigens that offer great diagnostic potential, as its gene, \textit{esx}, together with extensive flanking sequences, has been lost from the genome of the vaccine strain \textit{M. bovis} BCG as part of the deletion of region RD-1. Using tools of genomics and bio-informatics all these genes are being dissected to understand the putative proteins encoded by them, with a view to develop powerful diagnostic/protective antigens or drug targets.

There has been no new treatment for tuberculosis in the last three decades. But there is now the potential for a radical resurgence of new drug development as can be felt from the news coverage of various journals and the number of national and international meetings to discuss this thrust area. The Global Alliance for TB Drug Development has put a further thrust in the area of new drug development. In addition, the complete genome sequence of \textit{M. tuberculosis} has made it possible, for the first time, a comprehensive genomics approach to the discovery of newer drugs to treat tuberculosis. Micheal Wilson et al\textsuperscript{8}, used DNA microarray technique to monitor changes in \textit{M. tuberculosis} gene expression in response to the anti tuberculosis drug isoniazid. They demonstrated that isoniazid induced several genes that encode proteins physiologically relevant to the drug's mode of action. They have identified an operonic cluster of five genes encoding type II fatty acid synthase enzymes and \textit{fepC} gene that encodes trehalose dimycoclyl transferase, an enzyme necessary for the synthesis of mycobacterial cell wall material.

By using similar and more techniques it will be possible in future to define newer drug targets and suggest new methods for identifying compounds that could inhibit these targets. Research in this area will help in developing more potent drugs to combat the multi-drug resistant-TB (MDR-TB) which has reached an alarming proportion globally. Currently four different classes of anti tuberculosis drugs,
namely rifamycins, fluoroquinolones, nitroimidazopyrans and oxazolidines are being re-examined extensively to develop improved molecules with an aim to further shorten the course of chemotherapy. It may be mentioned here that nitroimidazopyrans seem to kill mycobacteria including persistent, slowly dividing ones.

To reiterate, availability of the genome sequence data of *M. tuberculosis* along with the tools of bio-informatics and techniques of genomics and proteomics like DNA microarray, 2-D Gel electrophoresis and MALDI mass spectrometry, there is renewed hope to tackle tuberculosis on a war footing. New knowledge acquired from the genome studies could be of great importance to fight and control the spread of tuberculosis including MDR-TB. From identifying newer drug targets in the tubercle bacillus to the ultimate goal of a multivalent vaccine, the tuberculosis control programme will receive a shot in the arm from the genomics effort.

**Mridula Bose**  
*Mycobacteria Cell*  
*Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110 007*  
*Tel/fax : 91-11-7667420  
E-mail : mridulabose@hotmail.com*

**REFERENCES**


NATIONAL COLLEGE OF CHEST PHYSICIANS (INDIA)

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