Thesis abstract

Title- A study of mycobacteriophage D29 growth under hypoxic condition

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Mycobacteriophages have been considered as corner stones of mycobacterial research. They have been used in various ways to understand the molecular genetics of mycobacteria. Although more than 4500 mycobacteriophages have been identified to date, and the genomes of many of them sequenced, only a few have been characterized. One such mycobacteriophage, D29 is the subject matter of thesis. Mycobacteriophage D29 possess in its genome genes encoding several nucleotide metabolism related enzymes. These include Gp50, a ribonucleotide reductase, Gp48, a thymidylate synthase. The ribonucleotide reductase encoded by this phage is however not the commonly found Class I type which functions only in the presence of oxygen. On the other hand it is of the class II type which functions in an oxygen independent manner. Also the thymidylate synthase encoded by this phage is of the ‘X’ type (and not the usual ‘A’ type) which is found in bacteria that can grow under microaerobic environments. The presence of genes encoding class II type ribonucleotide reductase and also the X type thymidylate synthase in the genome of mycobacteriophage D29 and related phages, suggest that these phages may have evolved mechanisms by which they can survive and grow in their mycobacterial hosts, under oxygen deficient conditions.

Since mycobacteriophage D29 encodes both a ribonucleotide reductase (Gp50) as well as a thioredoxin family protein (Gp56) therefore it was hypothesized that the latter may act as the reducing partner for the former. In order to address this and related issues Gp56, the thioredoxin family protein encoded by D29 was analyzed in silico. The recombinant version of the protein, synthesized in E. coli and isolated in the purified form was also subjected to biochemical characterization. The results indicated that Gp56 and related proteins, found in mycobacteriophages, form a unique cluster with the NrdH family of thioredoxin like proteins. Gp56 was found to have a lower standard redox potential as compared to Thioredoxin. It could function as an efficient reductant for Gp50 particularly under highly reducing conditions. It is speculated that Gp56 is likely to play a key role in phage growth in mycobacterial cells that have been deprived of oxygen.

In the second part of the thesis the growth of mycobacteriophage D29, in cells exposed or pre-exposed to hypoxic conditions was addressed. It was found that if aeration was reduced, phage bursts increased. Moreover it was found that pre-exposure of host cells to hypoxic conditions leads to higher bursts. The phenotypes observed were found to be dependent on the hypoxia sensitive regulator DevR. Interestingly it has been shown that there are DevR binding sites in mycobacteriophage D29. This is the first report of the demonstration of DevR binding sites in mycobacteriophage genomes. Overall the investigations shows that mycobacteriophage D29 has the ability to respond to hypoxic conditions utilizing the host derived DevR-DevS two component system.

Attested

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