Regulation of DNA topology in mycobacteria

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DNA topoisomerases catalyse essential DNA transaction processes in order to attain the balanced topology of the genome. Contrasting activities of DNA topoisomerase I and DNA gyrase result in the maintenance of topological homeostasis. The regulation of expression of different topoisomerases ensure steady state optimum level expression of the enzymes. Many aspects of their organization and regulation seem to be different in mycobacteria when compared with that of *Escherichia coli*. Here we present several aspects of the regulation of mycobacterial topoisomerases and discuss the significance.

Introduction

DNA topoisomerases have evolved to catalyse the topological alterations in DNA in order to ensure that DNA-transaction processes are completed without topological interruptions. Hence the enzymes play essential roles during replication, transcription, recombination, repair and chromosome segregation[1]. The genomes of bacteria are normally maintained in negative supercoiled state. Since all processes that involve DNA as a substrate either need to melt, bend or distort DNA; negative supercoiling modulates these cellular processes. For example, supercoiling influences recombination at two levels independently. First, supercoiling enhances recombination because the plectonemic winding of DNA facilitates the juxtapositioning of recombination sites and limits the extent of diffusion required for the sites to collide[2]. Second, the extent of supercoiling of the substrate determines the complexity of the product(s) since recombination converts the supercoil nodes into nodes of catenation or knots, depending on the relative orientation of the sites[3]. In a complementary manner, various DNA transactions alter the topology of DNA. The most obvious of these being the generation of catenated daughter duplexes after replication and activities of DNA tracking machineries. In eubacteria, the principal enzymes that influence the vital processes are topoisomerase I and DNA gyrase with substantial contribution from topoisomerase IV when present (see later section). Hence, the regulation of their expression and activities is an important determinant in the maintenance of balanced topological state and the global supercoiling of DNA is thus dependent on the balance of activities of various topoisomerases.

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Amongst the four topoisomerases found in *E. coli*, topoisomerase I and III belong to type IA group while DNA gyrase and topoisomerase IV are type II enzymes[4]. The key enzyme in all bacteria catalysing the formation of negatively supercoiled DNA in an ATP dependent reaction is DNA gyrase. The enzyme, encoded by *gyr*A and *gyr*B, is a heterotetrameric protein[5]. By virtue of its indispensability, the enzyme has been and continues to be a favourite drug target. As a consequence, several inhibitors and poisons, both natural and synthetic, have been characterized[6]. The second major player in influencing global topology is DNA topoisomerase I. In contrast to DNA gyrase, it comprises of a single polypeptide, encoded by *top*A gene. The enzyme catalyses the conversion of negatively supercoiled DNA into relaxed form in an ATP independent reaction[4].

Unlike *E. coli*, where four topoisomerases have been characterized, mycobacteria and many other organisms do not encode the full complement of topoisomerases. This was evident during our efforts to clone the genes for DNA gyrase from both *M. smegmatis* and *M. tuberculosis*[7,8]. Efforts to clone genes for other topoisomerases such as topoisomerase IV were unsuccessful hinting at the possibility of absence of these genes in *M. tuberculosis*. Genome sequencing efforts subsequently confirmed the presence of only single topoisomerase I and DNA gyrase in *M. tuberculosis*, while some other species such as *M. smegmatis*, *M. bovis* appear to have genes encoding for additional topoisomerases[9–11]. Amongst the two type IA enzymes found in *E. coli* and other bacteria, only topoisomerase I is present in mycobacteria[12].

Figure 1 depicts the organization of genes encoding DNA gyrase in *E. coli*, *M. smegmatis* and *M. tuberculosis*. Notably, *gyr*B and *gyr*A in *E. coli* are located far apart in the circular chromosome but present next to each other in both the species of mycobacteria. Furthermore, significant additional differences are observed in their primary sequences[13]. The genetic linkage between the *gyr* genes seems to correlate with the size of the *gyr*B gene. Species in which the genes are present far apart have 165 amino acids extra in the C-terminal half of *Gyr*B[14] and this insertion appears to be involved in DNA binding[15]. Our work over the last decade has revealed some of the distinctive characteristics of mycobacterial topoisomerase organization, function and regulation. In the following sections, the salient features of topoisomerase regulation in mycobacteria are presented and compared with that of *E. coli*.

Topology and transcription

Topological organization of DNA is known to have important influence in regulation of gene expression. Movement of RNA polymerase along the helical axis results in an increase in twist ahead of the tracking machinery and decrease behind, introducing positive and negative supercoils respectively. This is termed as twin domain of supercoiling[16]. As a result, DNA gyrase and topoisomerase I have to function ahead and behind the transcription bubble to remove positive and negative supercoils respectively (Figure 2). The twin domain model of supercoiling has several biological implications. (i) DNA transactions may prove to be a major determinant of local DNA topology; (ii) transcription of adjacent genes could significantly influence expression of a particular gene; (iii) Most importantly, for the first time, there appeared to be a necessity to expect efficiency in topoisomerases. Supercoiling influences transcription of many genes in the cell[17–19], modulating by several ways. Directly, it can realign promoter elements or facilitate open complex formation. Indirectly, it can stabilize loops, bends or other non-B-DNA structures in DNA.

In majority of the promoters, negative supercoiling facilitates isomerization of closed complex to open complex. However, failure to remove negative supercoils generated behind the transcription elongation complex would lead to the accumulation of R-loops and as a consequence, inhibition of transcription[20]. In addition, study of regulation of topoisomerase expression in mycobacteria is important especially since the genome lacks full complement of topoisomerases. Furthermore, in many pathogenic bacteria, expression of virulence genes is dependent on topological status of the genome[21,22]. As sensor of supercoils, the topoisomerases influence the specific gene expression.

Transcription of topoisomerases and regulation of topology

The net supercoiling of intracellular DNA is maintained by the relaxation activities of DNA topoisomerases I and IV opposing the supercoiling activity of DNA gyrase. Thus, by modulating the expression of any one of these genes, the cell can bring about rapid changes in supercoiling as well as compensate for sudden changes in supercoiling. As the sole supercoiling activity in the cell, DNA gyrase faces the daunting task of opposing the relaxation activities of both topoisomerases I and IV[23] and regulates its expression by a unique mechanism termed relaxation-stimulated transcription (RST). In general, transcription of most genes is induced by increased negative supercoiling. In contrast, negative supercoiling represses transcription of the gyrase genes in *E. coli*[24]. Increased gyrase levels lead to an increase in supercoiling, which, in turn, represses the expression of gyrase and allows other topoisomerases to bring the topology of the DNA back to its optimum state. Following observations led to the discovery of RST in *E. coli*. Cell-free transcription showed that transcription was dependent directly on the DNA topology, being maximal on a relaxed template[24]. Deletion analysis of the promoter regions of both *gyr*A and *gyr*B genes defined a short region around the transcriptional start site, including the –10 region, that is necessary and sufficient for conferring RST to a reporter gene[25,26]. Extensive mutagenesis of the

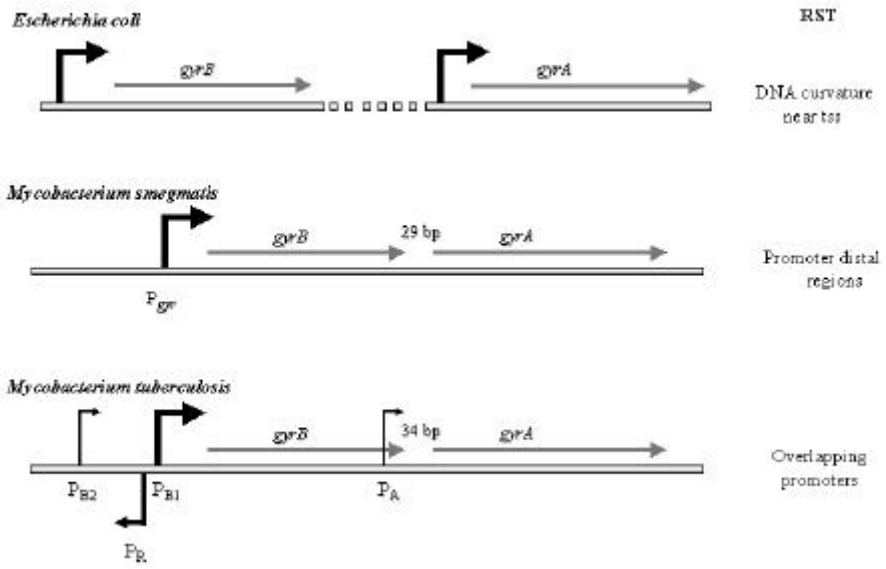

Figure 1. Transcriptional organization and regulation of *gyr* genes in *E. coli* and two species of mycobacteria. In *E. coli*, the genes are transcribed independently. A strong promoter drives the transcription of *gyr*BA operon in both *M. smegmatis* and *M. tuberculosis*. The vertical arrows and their thickness depict the promoters and their strength. tss; transcription start site.

*gyr*A promoter showed that the –10 region is responsible for both promoter strength and supercoil-sensitive behaviour[27]. Paradoxically, the promoter region harbours a sequence that matches the *E. coli* consensus for extended –10 promoters[28]. Since most extended –10 promoters do not show RST, it appears unlikely that the sequence of the –10 region alone is responsible for RST. Our recent analysis suggests that DNA curvature around the transcription start point plays a role in RST in *E. coli*[29].

The *gyr* operon in *M. smegmatis* is induced by novobiocin at the transcriptional level. However, unlike *E. coli*, minimal promoter of *gyr* operon do not confer RST in this case[30]. The presence of a strong CHPS (cruciform/hairpin potential sequence) with an 8 base pair stem and a 4 base loop in the 5′ untranslated region suggested a potential mechanism for RST in *M. smegmatis* and found to have a positive effect on promoter activity but is not sufficient for the operon to respond to novobiocin. The induction of the genomic copy and the results with the constructs harbouring upstream and downstream DNA sequences show the essential role played by promoter distal elements. DNA elements that are present 600 bp downstream of the promoter are necessary for RST to occur in the plasmid context[30]. This suggests the involvement of long range interactions and formation of repressor loops which could either prevent the binding of the polymerase to the promoter or prevent its release. Since such repressor loops are stabilized by negative supercoiling[31–33], the repression would occur in a supercoil sensitive manner. However, the downstream element along with the minimal promoter region is not sufficient to respond to novobiocin. Sequential deletions of both upstream and downstream distal regions reveal interesting novel features of the regulation. Therefore, RST appears to operate in *M. smegmatis* by some sort of de-repression rather than direct induction and a distal element has a negative influence on transcription[30].

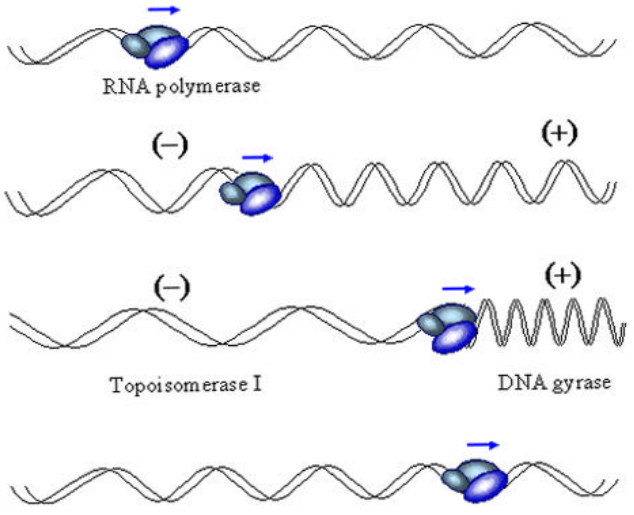

Figure 2. Twin domain of supercoiling. During transcription, RNA polymerase movement results in positive (+) in front and negative (–) supercoils behind the tracking machine. Action of the topoisomerases relieve the excessive supercoiling ensuring the further movement of RNA polymerase.

A comparison of the expression of DNA gyrase in *M. smegmatis* and *M. tuberculosis* reveals an amalgamation of conserved and divergent features (Figure 1). In addition to conserved genomic arrangement and dicistronic organization, the primary promoter in *M. tuberculosis*, P_{B1}, is located upstream of the *gyrB* gene at a position similar to that of the *M. smegmatis gyr* promoter[34]. Furthermore, the promoter region of P_{B1} shows extensive conservation with P_{gyr}, the promoter driving the *gyr* genes of *M. smegmatis*, indicating the evolutionarily relationship. Apart from the primary promoter, the *gyr* locus in *M. tuberculosis* employs at least three other promoters[34]. These additional promoters are weak and appear to play a regulatory role. P_A, the internal promoter for *gyr*A is 70-fold weaker than P_{B1} in exponentially growing *M. tuberculosis*, possibly employs an *M. tuberculosis*-specific sigma factor. Moreover, P_A may be induced under specific conditions which require the production of excess GyrA. Induction of GyrA alone in *E. coli* in response to treatment with GyrA inhibitors has been demonstrated earlier[35]. The other weak promoter, P_R, is divergently oriented and almost completely overlaps P_{B1}. Therefore, the binding of RNA polymerase to one of them would prevent binding in the opposite orientation. There are no identifiable coding sequences upstream of *gyrB* that P_R could be involved in transcribing, suggesting the function of P_R to be regulatory. Overlapping, mutually exclusive promoters are one of the mechanisms for regulating gene expression[36]. Recruitment of the polymerase to P_R would decrease expression of DNA gyrase by reducing transcription initiation at P_{B1}. In the converse scenario, as in relaxation of the template, P_R is repressed and P_{B1} gets induced to almost the same extent.

These studies highlight the importance of regulation of constituitively expressed, housekeeping, essential functions. While RST is a convenient mechanism to attain steady state levels of the enzyme, the complete molecular details of its operation vary and are not yet understood. Analysis of the promoter region of *M. smegmatis* and *M. tuberculosis* reveals a distinct lack of any axial distortion upstream of the +1 start site unlike *E. coli*[29]. To further substantiate the demarcation, all known *gyr*ase promoters were analysed for the presence of curvature in the vicintiy of the –10 region (+5 nucleotides). It is noteworthy that roughly half of these show a significant curvature in this region while others do not[29]. Interestingly, both the position and the extent of curvature are conserved between the *E. coli* and *Klebsiella pnemoniae gyr*A promoters[29]. How a promoter distal (downstream) element located within the ORF contributes for RST in *M. smegmatis* is not clear. Although it appears that trans factor/s could be involved in promoting the long range interactions, the molecular mechanism is still elusive. *M. tuberculosis*

genome unlike that of *M. smegmatis*, has its own variant mechanism of RST. The RST response in case of *M. tuberculosis* is extremely slow. The gyrases from both the species are very similar (about 90% identity) and hence the difference in the response is not likely due to catalytic properties. On the other hand, rate of transcription is varied between the species. *M. tuberculosis* RNA polymerase is at least 3–5 times slower than *M. smegmatis* enzyme[37]. Lower transcriptional rates in conjunction with yet unknown features may contribute for the difference in the response.

In contrast to *gyrB* and *gyrA* genes which are transcribed by single promoters, topoisomerase I expression appears to be regulated by multiple promoters in *E. coli*[35,38]. Two of them seem to be dependent on sigma-70, the major sigma factor of *E. coli* RNA polymerase. Amongst the others, one promoter is active during heat shock response while the other directs transcription at stationary phase of the growth[38–40]. As a consequence, the overall topoisomerase I expression is the net result of combined activation of various promoters[38]. Although the underlying mechanisms of regulation of *top*A and *gyr* genes by supercoiling are not completely understood, these represent an efficient homeostatic mechanism for the maintenance of supercoiling within physiological limits. For example, when global supercoiling goes down, *top*A is repressed while *gyr* genes are induced, compensating for the deficit in supercoiling. Instead of transfactor/s, DNA topology, the substrate/product for topoisomerases, directly modulates the levels of the enzymes in a manner reminiscent of product mediated inhibition in metabolic pathways.

Post-transcriptional regulation: mRNA stability

The half-life of the bulk of the mRNA in *E. coli* is 2.4 min at 37°C[41]. This short half-life could reflect the fastgrowing nature of *E. coli*, possibly facilitating rapid adaptation to environmental changes[42]. Thus, one would expect mycobacteria and other slow-growing organisms would have more stable messages. In addition, the regulation of degradation of these messages would be different. Based on this hypothesis, stability of the DNA *gyr*ase mRNA in *M. smegmatis* was analysed[43]. A secondary structure near the 5′ end of mRNA that protects the message against degradation was identified (Figure 3). The stabilization effect is significantly pronounced in nutrient-deprived conditions. In addition to the transcriptional regulation discussed earlier, the nutrient-dependent stabilization of the gyrase message, represents a second, hitherto unexplored, level of regulation of the *gyr* genes in any organism. While, in general, stabilization of a housekeeping message would be important for slow-growing organisms like mycobacteria, in the specific case of DNA gyrase, it probably has additional significance due to the operonic arrangement and for all known biological functions both proteins are required in equimolar amounts. Since genes present downstream in an operon are usually underrepresented at the protein level, it would be useful for the organism to evolve methods to prevent this discrepancy. The mycobacterial *gyr* operon attempts to circumvent this problem by subtle changes in its primary sequence (see later section). In such a context, the presence of a stabilizing secondary structure is probably an additional mechanism to ensure that the downstream message is maintained long enough to be translated efficiently. Thus, *M. smegmatis* appears to rely on two distinct sensors: a promoter-proximal sensor for nutrient levels and a promoter-distal sensor for DNA topology. Although not experimentally verified for its function, similar secondary structure is found upstream of *gyr*BA operon in *M. tuberculosis*.

Enhanced stability of mRNA upon starvation has been reported in many organisms[42,44,45] which would allow the cells to utilize the already synthesised messages to their fullest and conserve resources when they are scarce. This is arguably more important for organisms like mycobacteria that grow slowly even under nutrient-rich conditions. In these organisms, the lower rate of transcription elongation is probably compensated for by enhanced stability of the message. Secondary structures at or near the 5′ end of the mRNA in *E. coli* are believed to function by preventing access of RNase E[46]. Furthermore, cleavage by RNase E appears to be the primary rate

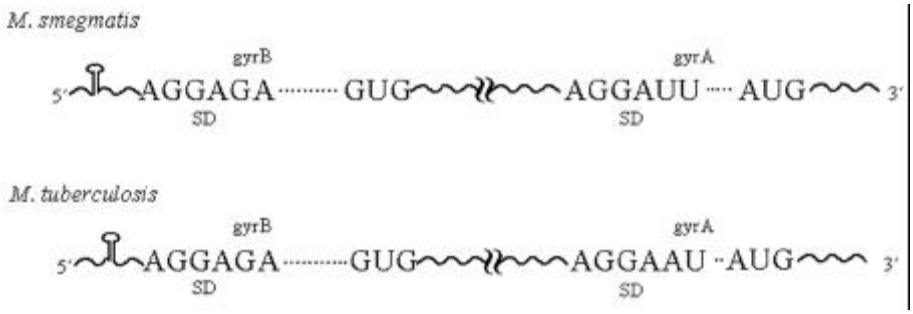

Figure 3. Translational regulation of *gyr* mRNA. The stabilizing stem loop structure is shown at 5′ end of the *gyr*BA mRNA. The SD and start codon sequences of the individual cistrons are shown which suggest translational optimization. The dots between SD sequence and start codon represent the actual number of nucleotides.

determining step in the degradation of most messages in *E. coli*[47]. Genomes of *M. tuberculosis*[10] and *Mycobacterium leprae*[48] encode for a homologue of this enzyme. Genome-wide analysis of the distribution of secondary structures indicates that genes in slow-growing organisms like *M. tuberculosis* are more likely to have a strong secondary structure ahead of them than those in fast-growing organisms like *E. coli*[49]. This probably protects a majority of the messages against the degradative activities. Such a strategy would make economic sense for a slow growing organism that does not necessarily need to respond to environmental changes rapidly[29,34,50].

Post-translational regulation

The *gyr*BA dicistron in both *M. smegmatis* and *M. tuberculosis* exhibits additional interesting regulatory features (Figure 3). The *gyr*B has a near perfect Shine-Dalgarno (SD) sequence upstream of a weak start codon while *gyr*A has relatively weak SD sequence and an efficient start codon. Such an arrangement suggests a translational regulation that could facilitate the production of equimolar amounts of the two subunits that constitute the heterotetrameric functional holoenzyme.

Notably, there is a remarkable difference in the activities of DNA gyrase from *E. coli* and mycobacteria. *M. smegmatis* DNA gyrase has 3–5 fold weaker ATP hydrolyzing activity compared to that of *E. coli*[51]. However, the enzyme is a potent decatenase suggesting a more important role during segregation of daugther chromosomes. Since *M. tuberculosis* genome encodes only for topoisomerase I and DNA gyrase[10], the DNA gyrase is likely to possess strong decatenase activity to take care of added responsibility of daughter genome segregation.

Additional post translational measures seem to play a role in the expression of functional gyrase in mycobacteria. The mycobacterial recombinant DNA gyrase expressed in *E. coli* do not show high specific activity when analysed for supercoiling or ATPase activities in contrast to the enzymes isolated from wild type cells or overexpressed in mycobacteria (unpublished). This suggests a role for post-translational modification of the enzyme. Furthermore, the presence of two *gyr*B in *M. smegmatis* raises interesting possibilities regarding the intracellular functions[11]. The GyrB encoded by *gyr*BA operon is known to be associated with GyrA in a tetrameric holoenzyme that carries out the DNA supercoiling reaction[51]. From our comparative analysis it appears that the additional *gyr*B, termed as orphan *gyrB* is a functional allele and hence retained in the genome at a different location. Considering the difference in the growth rates of *M. smegmatis* and *M. tuberculosis*, the orphan GyrB could be contributing to the higher levels of enzymatic activity required during exponential growth phase. Alternatively, it is expressed differentially under certain conditions as an immediate requirement for cellular function. Another point to be noted is that GyrB is intrinsically less stable than GyrA in *E. coli*[52] and also in *M. smegmatis* (unpublished results).

Conclusions

Diverse topoisomerases influence the topological state of the genome. Although topoisomerases are essential housekeeping functions, the fine tuning of their expression is important in order to maintain the balanced topological state. Our analysis of regulation of gyrase expression between two species of mycobacteria has revealed an amalgamation of several concepts with important species specific differences. The conserved features include dicistronic organization, mycobacteria specific promoters, RNA stability etc. However, autoregulation of transcription appears to have species specific variation. In *M. smegmatis* promoter distal downstream elements and possibly transfactors have a role in RST while in *M. tuberculosis*, overlapping mutually exclusive divergently organized promoters regulate the process. The organization as an operon in order to assemble heterotetrameric enzyme rapidly and extraordinary stability of the dicistronic mRNA are some of the measures taken by these group of bacteria to compensate for slower growth rates. The studies on regulation of topoisomerase I expression now underway would reveal other facets of regulation which contribute to attain cellular homeostasis in DNA topology.

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