

## SURVEY AND SUMMARY

# Bacterial DNA topology and infectious disease

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### ABSTRACT

**The Gram-negative bacterium *Escherichia coli* and its close relative *Salmonella enterica* have made important contributions historically to our understanding of how bacteria control DNA supercoiling and of how supercoiling influences gene expression and vice versa. Now they are contributing again by providing examples where changes in DNA supercoiling affect the expression of virulence traits that are important for infectious disease. Available examples encompass both the earliest stages of pathogen–host interactions and the more intimate relationships in which the bacteria invade and proliferate within host cells. A key insight concerns the link between the physiological state of the bacterium and the activity of DNA gyrase, with downstream effects on the expression of genes with promoters that sense changes in DNA supercoiling. Thus the expression of virulence traits by a pathogen can be interpreted partly as a response to its own changing physiology. Knowledge of the molecular connections between physiology, DNA topology and gene expression offers new opportunities to fight infection.**

### INTRODUCTION

DNA gyrase was discovered in *Escherichia coli*, a bacterium that has played an important part in the foundation of modern molecular biology (1). DNA topoisomerase I was also discovered in *E. coli* (2), but the gene that encodes it, *topA*, was first identified as a suppressor of the *leu500* promoter mutation in *Salmonella enterica*, then called *S. typhimurium* (3). The genes that encode gyrase, *gyrA* and *gyrB*, have the interesting property of being up-regulated when DNA relaxes (4). In contrast, the *topA* gene is transcriptionally activated when DNA becomes more negatively supercoiled (5–7). This latter

response is intuitively appealing: a promoter must open for transcription to begin and the energy of negative supercoiling can be used to bring about the necessary breakage of the hydrogen bonds between the paired bases (8). The molecular mechanism responsible for the DNA-relaxation-dependent activation of *gyrA* and *gyrB* has yet to be fully explained (9).

Reciprocal regulation of the transcription of the *topA* gene and the *gyrA* and *gyrB* genes by DNA negative supercoiling and relaxation, respectively, is consistent with the maintenance of a homeostatic balance of DNA supercoiling that benefits the cell (10–13). As DNA becomes more negatively supercoiled expression of the *topA* gene is enhanced, leading to a higher level of DNA topoisomerase I, a DNA relaxing enzyme. DNA relaxation has the opposite effect because it enhances the transcription of the genes coding for DNA gyrase which can then correct the supercoiled-relaxed balance to a value in keeping with the physiological needs of the cell (10–13).

This simple picture of topoisomerase gene regulation neglects a number of additional influences. For example, the Fis protein is a regulator of *topA*, *gyrA* and *gyrB* (14–16). This protein is the factor for inversion stimulation, hence the name ‘Fis’. It was discovered originally as an important co-factor in the operation of invertible DNA switches that are catalyzed by members of the serine invertase family of site-specific recombinases (17). Fis is now known to play many regulatory roles in the cell, affecting the operation of several important DNA transactions such as bacteriophage integration and excision, expression of components of the translation machinery, DNA replication, and transposition (17,18). Fis represses the expression of its own gene, *fis*, and it has a highly characteristic expression pattern. The Fis protein is expressed to its maximum level in the early stage of exponential growth. Its intracellular concentration declines sharply thereafter and it is almost undetectable when the bacterial culture approaches the stationary phase of growth (19,20). This suggests that there is a window within which Fis-dependent molecular events can occur optimally. However, a straightforward correlation

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between Fis concentration, growth phase and the operation of Fis-dependent systems has been difficult to obtain. The picture is made complicated by the fact that Fis is not essential for any of the processes to which it contributes and by the fact that the classic pattern of Fis protein expression can be overridden by the manipulation of growth conditions (21).

The Fis protein represses the transcription of both *gyrA* and *gyrB* and has a bi-functional relationship with *topA*: at high concentrations, Fis represses *topA* transcription and at low concentrations Fis is an activator (14–16). Fis regulates transcription positively by acting both as a conventional transcription factor that makes protein–protein contact with RNA polymerase and by creating a micro-domain of negatively supercoiled DNA in the vicinity of the target promoter (22–25). Like the genes coding for the main topoisomerases, the *fis* gene is regulated by changes in DNA supercoiling: increased negative DNA supercoiling stimulates the *fis* promoter (26). The contribution of Fis to global regulation of DNA transactions through changes in DNA supercoiling is best appreciated in the context of the impact of growth phase on DNA superhelicity. DNA is more negatively supercoiled in bacteria that are growing exponentially than in those where growth has slowed or ceased (27). Fis is thought to play a valuable role in offsetting the negative effects of DNA that is too relaxed or too negatively supercoiled by acting as a topological buffer. It creates micro-domains of DNA where the degree of DNA supercoiling is optimal for promoter function and preserves the integrity of these micro-domains regardless of changes to global supercoiling levels (28). It can perform this role throughout the genome because its DNA sequence requirements for DNA binding are non-stringent (29). Thus Fis acts in intimate association with gyrase and DNA topoisomerase I to set and reset DNA supercoiling levels in the cell.

The Fis protein is classified as a nucleoid-associated protein (NAP) and it is one of a number that belong to this group. Other abundant NAPs are HU and H-NS, two proteins that have the ability to constrain DNA supercoils (18). It is estimated that, during logarithmic growth, about half of the DNA in the bacterium is complexed with protein in ways that constrain negative supercoils (30,31). Thus the *effective* level of supercoiling, the portion that is available to do work in the cell and influence processes such as transcription, is only ~50% of the total detected when DNA superhelicity is measured with nucleic acid purified free of cellular components (32).

The physiological state of the cell is strongly influenced by the environment external to the bacterium (6,8). As the chemical and physical nature of the environment changes, the metabolic pathways of the microbe respond. DNA gyrase is intimately connected to these pathways by virtue of being an enzyme that requires ATP as an energy source and one that is inhibited by ADP: the ratio of the concentrations of ATP and ADP determines the level of gyrase activity (33,34). For this reason, shocks to the cell such as changes in osmolarity, temperature, pH, oxygen level, nutrient supply, etc. all potentially have an impact ultimately on the global level of DNA supercoiling (35–41). This is especially relevant in the cases of bacteria

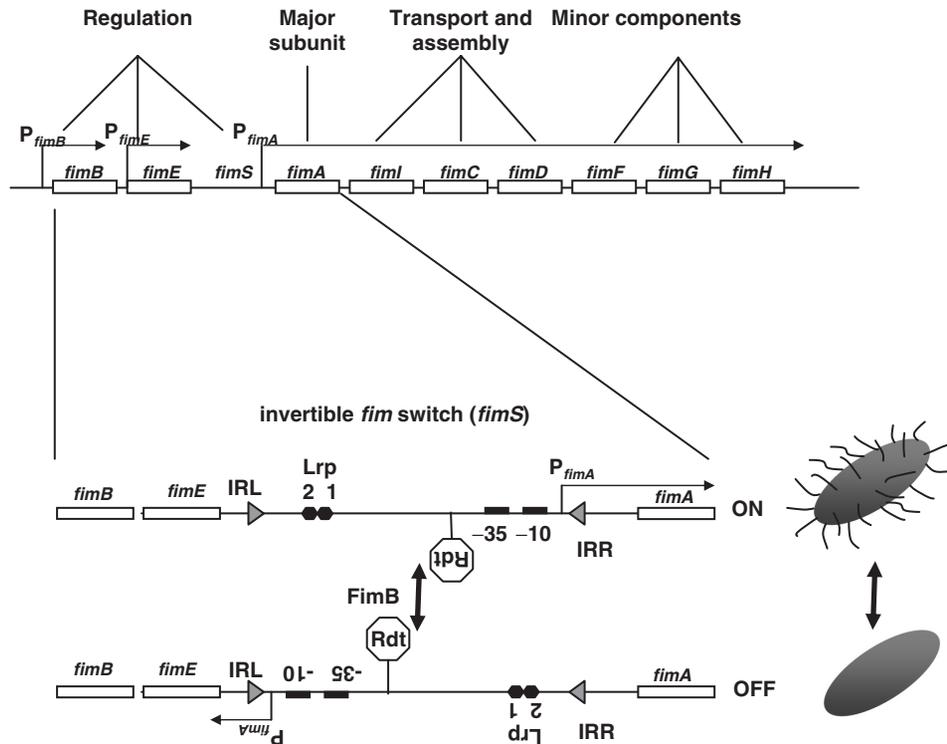
such as *E. coli* or *S. enterica* that can inhabit a wide range of environments. Thus, DNA supercoiling can be seen as a crude regulator of gene expression. It is variable in response to environmental signals and it has the potential to act widely within the genome (6,8). This leads to a model of global regulation in which the environment alters chromosome topology via topoisomerases and genes have evolved to respond to those environmentally determined changes (6). In addition to the influences of DNA supercoiling, further regulatory refinements are imposed by the multitude of locally acting transcription factors that are possessed by bacteria such as *E. coli* (42).

Pathogenic bacteria possess virulence genes that their commensal counterparts lack completely or they express virulence genes that are inactive due to mutation or crypticity in the commensal. The evolution of bacterial pathogens has involved the lateral transfer of virulence genes and their integration into the regulatory regime of the bacterium (43,44). Studies in a number of pathogens have provided evidence that the expression of many virulence genes is influenced by changes in DNA supercoiling (45–48). Given the impressive correspondence between the environmental stresses that pathogens must endure during infection, and the known impact of these stresses on the degree of DNA supercoiling in bacteria, this is perhaps unsurprising.

The infection process may be regarded as a series of relationships between the pathogen and the host of ever-deepening intimacy. Preliminary contact often involves attachment to the host by bacterial surface structures called fimbriae. The genes that encode these are often subject to complex regulation that includes a role for DNA supercoiling.

### THE *fim* GENETIC SWITCH IN *E. coli* K-12

Type 1 fimbriae are important virulence factors in many bacterial species (49). They are expressed by most members of the *Enterobacteriaceae* and were the first bacterial fimbriae to be described (50,51). Type 1 fimbriae attach bacteria to mannoseylated glycoproteins on a variety of eukaryotic cells. In *E. coli* K-12, these fimbriae are expressed phase-variably with bacterial populations containing fimbriate (phase-ON) and afimbriate (phase-OFF) members (Figure 1). Moreover, the two cell types are interchangeable. This is because the transcriptional promoter for the *fim* structural genes is part of an invertible DNA segment known as the *fim* switch, *fimS* (52). This 314 bp DNA segment is bounded by 9 bp perfect inverted repeats within which DNA cleavage and religation occur during the site-specific recombination reactions that invert the switch (53). Inversion is catalyzed by two tyrosine integrase site-specific recombinases that act independently and have distinct activities. FimB inverts the switch in both the ON-to-OFF and the OFF-to-ON directions with approximately equal efficiency and does this at a frequency of about  $10^{-2}$  per cell per generation (54,55). The FimE protein has a marked preference for inverting the switch in the ON-to-OFF direction and its activity is dominant to the OFF-to-ON activity of FimB (53,55).



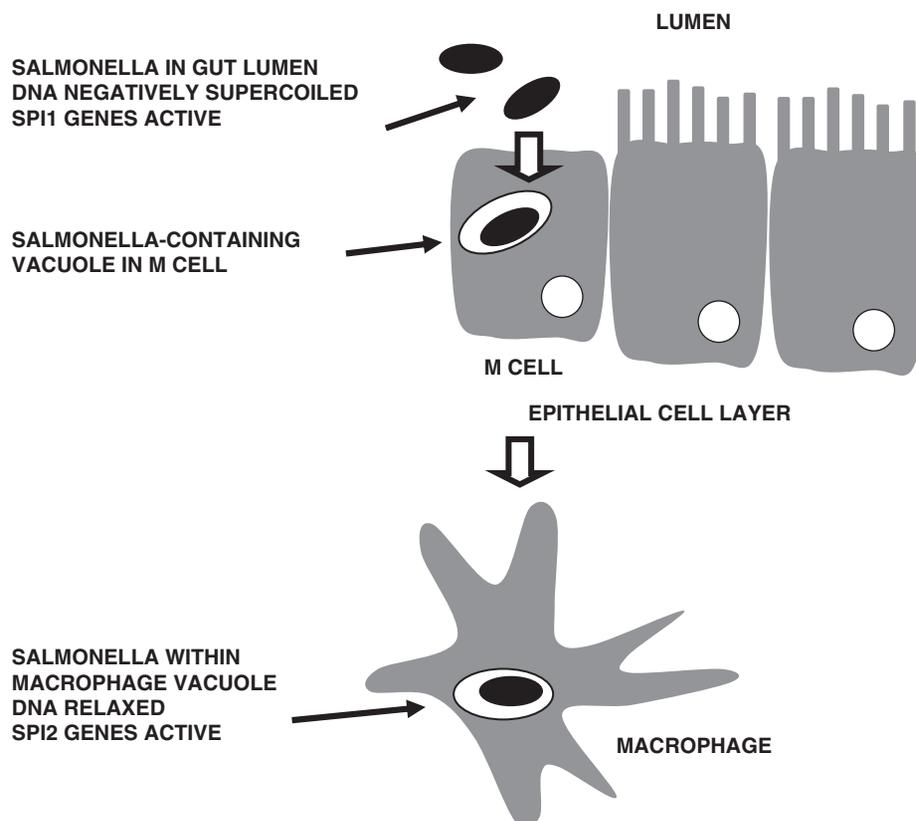
**Figure 1.** The invertible genetic switch in the *fim* operon of *E. coli*. The structure of the complete *fim* operon is summarized at the top of the figure. The positions and directions of transcription of each of the nine genes are shown, together with their functions. The positions of the transcription start sites associated with the three main promoters are represented by angled arrows. The invertible genetic element, *fimS*, that harbours the promoters for transcription of the structural genes, is shown in an expanded form in the centre and bottom of the figure. In the ON orientation, the  $P_{fimA}$  promoter is directed towards the *fimA* gene, resulting in an ON phenotype and a fimbriated bacterium. In the OFF orientation, the *fimS* element has inverted and the  $P_{fimA}$  promoter has been disconnected from the *fimA* gene. This results in an OFF phenotype and an afimbriated bacterium. The 314 bp *fimS* element is bounded by 9 bp inverted repeats (grey arrowheads labelled IRL and IRR) that encompass the  $P_{fimA}$  promoter (–10 and –35 boxes and transcription start site shown) and a Rho-dependent transcription terminator, Rdt. This terminator reduces the length and stability of the *fimE* transcript only when *fimS* is in the OFF orientation. In the ON orientation, the *fimE* gene reads across the switch into the *fimA* gene (56,57).

Many laboratory strains of *E. coli* K-12 lack an active *fimE* gene and invert the switch using FimB alone (54). Posttranscriptional control of *fimE* gene expression plays a key role in controlling *fimS* inversion in the complete wild-type *fim* operon. This is because the *fimS* element harbours a Rho-dependent terminator in addition to the promoter for *fim* structural gene transcription (56,57) (Figure 1).

Although the FimB integrase inverts the switch in a relatively unbiased manner, its activity becomes strongly biased in favour of the ON phase when DNA gyrase is inhibited (58). Inhibition of gyrase activity with the antibiotic novobiocin results in a clear dose-dependent preference for the ON orientation of *fimS* (58). This is not explained by changes in the expression of the *fimB* gene but is related to the quality of the FimB substrate. If the *topA* gene is inactivated by transposon insertion, the switch ceases to be invertible. It maintains thereafter the switch orientation (ON or OFF) that obtained at the moment that the *topA* gene was mutated. Again, this is not due to changes in the expression of the *fimB* gene or to global changes in DNA supercoiling. Instead it is due to a requirement for topoisomerase I activity in the immediate vicinity of the switch (58).

The simplest interpretation of the experimental data is that the switch becomes trapped in the ON orientation because this form of the switch is a poor substrate for FimB. This is not due to the creation of differentially supercoiled domains by the activity of the  $P_{fimA}$  promoter that might distinguish phase-ON from phase-OFF switches; complete inactivation of this promoter has no influence on switch biasing in the wake of DNA relaxation (59). Instead the trap is composed of a nucleoprotein complex that involves the left inverted repeat, two binding sites for the leucine-responsive regulatory protein within *fimS* and a reference site in the flanking, invariant DNA (Figure 1). Removal of the Lrp protein or abrogation of Lrp binding to the switch eliminates the OFF-to-ON bias that accompanies DNA relaxation; in fact, the switch now acquires a strong bias in the ON-to-OFF direction (59).

What is the physiological significance of inversion-biasing? DNA relaxation accompanies cessation of growth and a shift in the [ATP]/[ADP] ratio that is unfavourable for DNA gyrase activity (27,33,34). In addition, the Lrp protein is a barometer of the metabolic status of the cell and an indicator of nutrient depletion (60). It is tempting to speculate that by evolving sensitivities to these factors, the cell has developed a mechanism to override the



**Figure 2.** The intracellular life of *S. enterica* in the mammalian gut. A summary of the main steps in invasive disease in the murine gut caused by *S. enterica* serovar Typhimurium is presented. In the lumen of the gut the bacterium experiences environmental stresses that are known to result in a reduction in the linking number of its DNA. This increase in negative DNA supercoiling is part of the mechanism by which the SPI1 pathogenicity island genes are up-regulated. The bacteria traverse the antigen-sampling M cells in a *Salmonella*-containing vacuole. Following release on the basolateral surface, the bacterium may be engulfed by macrophage. *S. Typhimurium* undergoes DNA relaxation within the macrophage and this is part of the mechanism by which the SPI2 pathogenicity genes are activated. The products of these genes prevent the macrophage from killing the microbe, which is then able to establish a systemic disease.

stochastic DNA inversion behaviour of FimB in favour of a fimbriate phenotype. This may enhance the ability of the bacterium to participate in biofilm formation as a means to ride out physiologically unfavourable circumstances.

Type 1 fimbriae do not contribute exclusively to early phases of the host–pathogen interaction: they have been identified as important factors in the establishment of more intimate associations with the host during urinary tract infection by uropathogenic *E. coli* (61) and *Klebsiella pneumoniae* (62). Here, the fimbriae are expressed within bacterial communities living within epithelial cells of the bladder lining. The invertible *fim* switch in these bacteria is maintained in the ON phase, showing that DNA inversion in the ON-to-OFF direction is suppressed in this niche (63).

### THE INTRACELLULAR LIFE OF *S. enterica*

Like *E. coli* K-12, *S. enterica* serovar Typhimurium (*S. Typhimurium*) uses type 1 fimbriae to interact with its host, although it controls their expression through mechanisms that are independent of DNA inversion (64). Unlike *E. coli* K-12, *S. Typhimurium* has the ability

to invade mammalian epithelial cells and to survive engulfment by macrophage (Fig. 2). This is due to its possession of two separate type III secretion systems (TTSS) with separate sets of effector proteins that *S. Typhimurium* can use to modify the mammalian cells to its advantage (65–67). The TTSS that is encoded by the genes of the SPI1 pathogenicity island confer an invasive phenotype on the bacterium. The promoters of the SPI1 genes are up-regulated by negative DNA supercoiling (68). In this respect they resemble the TTSS genes of the dysentery bacillus *Shigella flexneri* (69). The TTSS that is encoded by the SPI2 pathogenicity island of *S. Typhimurium* is essential for the survival of the bacterium in the otherwise hostile environment of the macrophage. The effector proteins secreted via the SPI2 TTSS prevent phagolysosome fusion through modification of the macrophage vacuole that contains the engulfed bacterium (68). Interestingly, the promoters of the genes in the SPI2 island are up-regulated by DNA relaxation (70), which is the opposite of the SPI1 genes. This differential dependency on the state of DNA topology is likely to represent a key distinguishing factor between these two sets of virulence genes that ensures that each is active in the correct environment and repressed elsewhere. The lumen of the

mammalian gut exposes the bacterium to a range of stresses that have been shown to shift DNA supercoiling to more negative values (48). Indeed the recommended growth conditions for the induction of SPI1 genes in the laboratory involve low aeration and growth in a high-osmolarity medium (71). In contrast, SPI2 gene activation is favoured by a low-osmolarity growth regime (72). Measurements of plasmid topoisomer distributions have shown that bacterial DNA becomes more relaxed during growth of *S. Typhimurium* in the vacuole of cultured macrophage, which is consistent with SPI2 gene upregulation (70). Both SPI1 and SPI2 have a requirement for the Fis protein for optimal gene expression (20,70). This is in keeping with the role of Fis as a topological buffer (28). Fis is just one of the NAPs that has been shown to influence transcription within the major pathogenicity islands of *S. Typhimurium*. Like Fis, the HU NAP has a positive influence on SPI1 gene expression (73). In contrast, the H-NS protein represses the transcription of the genes of both SPI1 and SPI2 and it is assisted in this process by the Hha protein, a partial paralogue of H-NS (74).

The SPI1 and SPI2 pathogenicity islands possess genes coding for dedicated regulators of their own structural virulence genes (75). These operate in a regulatory environment in which DNA supercoiling and the NAPs set the regulatory background, in tune with signals coming from the external environment that modulate the metabolism of the bacterium.

## CONCLUSIONS

DNA supercoiling has been identified as a factor that modulates the expression of virulence genes in pathogenic bacteria at different phases of the host–pathogen relationship. This is by no means confined to the four Gram-negative pathogens discussed above; DNA supercoiling has been identified as an important factor influencing gene expression in many other bacteria (45–48). It should also be emphasized that these effects on gene expression are not relevant only to pathogens but are also involved in the physiology of bacteria pursuing commensal or symbiotic lifestyles. The model that best describes the role of DNA supercoiling in bacterial gene regulation is one that takes a hierarchical view of the gene regulatory network of the cell. DNA supercoiling has a place at or near the apex of the hierarchy due to its potential to influence the activities of so many promoters simultaneously. The NAPs also have a high position in the hierarchy, but below that occupied by DNA supercoiling. Their widespread influences on transcription arise because each governs a large regulon of genes and the memberships of the different regulons overlap in ways that are conditional on environmental conditions. This form of flexible networking provides a backdrop for the activities of the conventional transcription factors, DNA binding proteins that regulate few, or possibly just one, promoters. As we come to appreciate the subtle sophistication of bacterial gene regulation and the complexity of its networks the task of intervening in infection by targeting the gene control programmes of the pathogen can indeed appear

daunting. It is to be hoped that our ever-deepening knowledge of how bacteria manage their physiology at the level of gene expression will improve our position in this struggle.

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