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Global regulators and environmental adaptation in Gram-negative pathogens

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Abstract

A powerful combination of single-gene studies and whole genome approaches has provided a wealth of information about the regulatory circuits used by bacteria to adapt to the environmental changes that are encountered during infection. The facultative intracellular pathogen *Salmonella enterica* will be used to illustrate how global regulators such as the nucleoid-associated proteins Fis and H-NS collaborate with fluctuations in the superhelicity of the DNA template to modify the gene expression profile of the bacterial cell outside and inside the host.

Keywords: DNA supercoiling, Escherichia coli, Fis, H-NS, nucleoid-associated protein, *Salmonella enterica*, serovar Typhimurium

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Understanding how bacteria manage their gene expression programmes in response to environmental change is an important goal of modern molecular microbiology. Among the Gram-negative bacteria, the field is perhaps best advanced in *Escherichia coli* K-12, an organism that has been studied at the genetic and physiological levels for many decades [1]. *Salmonella enterica* is a close relative of this model bacterium, and its Typhimurium serovar has been used with great success as a model for bacterial pathogenesis for many years. *S.* Typhimurium offers many of the advantages of *E. coli* as a model organism, but has the additional benefit of being a pathogen whose relationship with its natural host, the mouse, is understood in great detail. In the course of a single round of infection, the bacterium must adapt from an existence in an external environment, such as contaminated food or drink, to the digestive tract of the mammalian host (including the highly acidic environment of the stomach), to an intracellular milieu in the epithelium, to the aggressive environment that characterizes the interior of macrophage, and finally to the deeper tissues of the host. There is also the possibility that the host may shed the bacterium, forcing it to begin the process all over again. From the perspective of research in cellular microbiology, a particularly attractive aspect of *S.* Typhimurium pathogenesis is its ability to invade and replicate within the cells of its mammalian host [2].

It is clear that *S.* Typhimurium employs a large number of genes to survive and thrive in its relationship with the host. Intensive research efforts have identified scores of virulence genes that are required for one or more steps in the infection process. Some of the most important are grouped together in the bacterial chromosome in so-called pathogenicity islands. Early genetic work had identified some of these genes, but it was the genome sequencing revolution that led to the discovery of the islands. In *S.* Typhimurium, these are large contiguous sections of the chromosome that have DNA with an A + T content that is higher than that of the remainder of the chromosome. This mismatch in DNA sequence composition was part of the reasoning behind the proposal that the islands have been acquired by horizontal gene transfer from a source outside the *Enterobacteriaceae* [3].

The two most important and best-studied *Salmonella* pathogenicity islands are SPI1 and SPI2. Each of these encodes a distinct type III secretion system and associated effector proteins. SPI1 is required for host cell invasion, and the effector proteins that it secretes induce bacterial uptake by the non-professional phagocytic cells lining the intestinal lumen [4]. SPI2 is necessary for intracellular survival, and plays an important role in manipulating events within host defense cells, such as the macrophage, in ways that ensure the survival of the bacterium [4]. Other virulence genes are located elsewhere on the *Salmonella* chromosome or on a virulence plasmid that is a characteristic feature of the non-typhoid serovars, of which *S.* Typhimurium is one [3].

Our understanding of the cell biology of *S.* Typhimurium infection is well advanced. However, a detailed understanding of the regulation of its many virulence genes has lagged behind. In particular, it is still not completely clear how genes that are acquired by lateral transfer mechanisms can become integrated with the existing gene regulatory circuits of the bacterium that receives them, so that they do not compromise the organism's competitive fitness. An attractive hypothesis proposes that a global repressor protein, H-NS, downregulates the horizontally acquired A + T-rich genes, avoiding their inappropriate expression [5–7]. However, this still leaves the problem of how to activate them when they are required. A combination of whole genome and single-gene studies has suggested that the bacterium has evolved a multitude of solutions to this problem.

Transcriptomic investigations using DNA microarrays have identified a striking overlap between the memberships of the H-NS and Fis regulons [5]. Similarly, chromatin immunoprecipitation on chip studies have revealed a remarkable correspondence between the binding sites of the H-NS and Fis proteins in the *E. coli* genome [8]. Fis is the factor for inversion stimulation, an 11.2-kDa DNA-binding protein that was discovered originally as a cofactor in DNA inversion-based genetic switches that are catalysed by members of the serine DNA invertase family of site-specific recombinases. It is now known to have a multitude of roles in the cell; it contributes to transposition, DNA replication, bacteriophage excision, and the control of transcription [9,10] (Fig. 1). In particular, it makes a positive contribution to the transcription of genes coding for components of the translation machinery. Fis can be either a repressor or an activator of transcription. It binds to an A + T-rich DNA element of degenerate nucleotide sequence, something that is also a feature of H-NS-binding sites. However, H-NS has not been described as activating any promoter directly; its primary direct effect on transcription is one of repression [5].

Fis can also modulate the topology of the genetic material in the cell, especially its degree of negative supercoiling. Most cells, including bacterial cells, maintain their DNA in an underwound state; that is to say, the DNA has a deficit in linking number (the number of times that one strand of the DNA duplex winds around the other). This deficiency places the DNA duplex under torsional stress, enhancing its tendency to form bubbles of unpaired bases that allow a return to a more energetically favourable conformation. This tendency facilitates those transactions of DNA that depend on the formation of locally unpaired bases, such as transcription. Negative supercoiling is introduced to bacterial DNA by the ATP-dependent type II topoisomerase DNA gyrase, and the activity of gyrase is opposed by topoisomerase I, a type I enzyme that removes the negative supercoils [11]. The result is thought to be a homeostatic balance that keeps the degree of negative supercoiling within limits that are appropriate for the biological functions of DNA, including its replication and the expression of the genes contained within it. The Fis protein can influence this process at several levels. It is a transcriptional regulator of the genes encoding DNA gyrase and topoisomerase I, allowing it to influence the supply of these

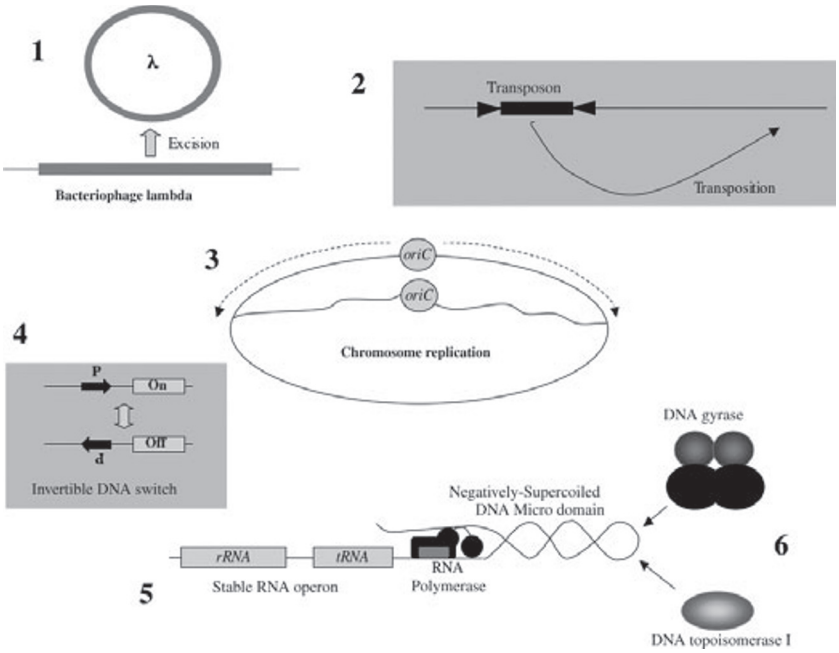

Fig. 1. Regulatory roles of the Fis protein. Fis plays multiple roles in Gram-negative enteric bacteria, including: (1) excision of bacteriophage lambda from the *attλ* site in the *Escherichia coli* chromosome; (2) intramolecular and intermolecular transposition of insertion sequences and transposons; (3) initiation of bidirectional chromosome replication from *oriC*; (4) operation of invertible DNA switches involving site-specific recombination reactions catalysed by serine invertase recombinases; (5) sustained transcription of stable RNA operons through action as a conventional transcription factor and as a topological homeostat; and (6) expression and activity of DNA gyrase and DNA topoisomerase I.

supercoiling-controlling enzymes (Fig. 1). The degenerate nature of the Fis-binding site facilitates the promiscuous interaction of the protein with DNA, and Fis has been shown to bind best to DNA with intermediate levels of DNA supercoiling, which it then helps to preserve in the face of the activities of gyrase and topoisomerase I. The Fis protein can perform this supercoil-preservation function in the vicinity of certain promoters, allowing them to continue functioning after the superhelicity of the remainder of the genome may have become unfavourable for transcription. It should be pointed out that Fis can also act as a conventional transcription factor, influencing RNA polymerase activity at promoters through protein–protein interactions [12].

Mutations in the *fis* gene have pleiotropic effects, as one might expect, given the many systems that it influences. Loss of Fis protein expression results in reduced expression not only of the SPI1 and SPI2 virulence genes of *S.* Typhimurium, but also the genes involved in motility, many of which contribute to the expression of the third type III secretion system in this species, which which is responsible for the deployment and activity of flagella [9]. Fis has also been found to play a role in governing the expression of housekeeping functions that are required for the normal expression of the metabolic pathways that *S.* Typhimurium needs when growing in the gut lumen. Thus, Fis contributes to the management of the gene expression programmes involved in the transition from the gut lumen to an intracellular niche. None of these genes displays an absolute requirement for Fis, but by acting to optimize the expression of them all, Fis plays the role of a strategic modulator in these important environmental transitions.

The involvement of the Fis protein in *S.* Typhimurium pathogenicity island gene expression led to the hypothesis that these genes might also respond to changes in negative supercoiling of the DNA. Variations in the degree of DNA supercoiling have been proposed previously as potential global regulators of transcription of both housekeeping and virulence genes [13]. There is ample experimental evidence that the environmental stresses encountered by bacteria during infection can modulate DNA supercoiling, most likely through an alteration of the activity of DNA gyrase as a result of fluctuations in the ratio of ATP and ADP in the cell [11].

Reporter plasmid DNA supercoiling assays have shown that DNA becomes more relaxed in *S.* Typhimurium as the bacteria adapt to life in macrophages [14] (Fig. 2). Furthermore, the Fis protein is required to manage this relaxation process. Interestingly, the SPI2 virulence genes, which are required for survival in the macrophage, have promoters that are stimulated by DNA relaxation, as can be shown by artificially relaxing DNA in bacteria growing in laboratory media by treating the culture with the DNA gyrase-inhibiting drug novobiocin [14].

These observations led to an integrated view of gene regulation in response to environmental stress. In this view,

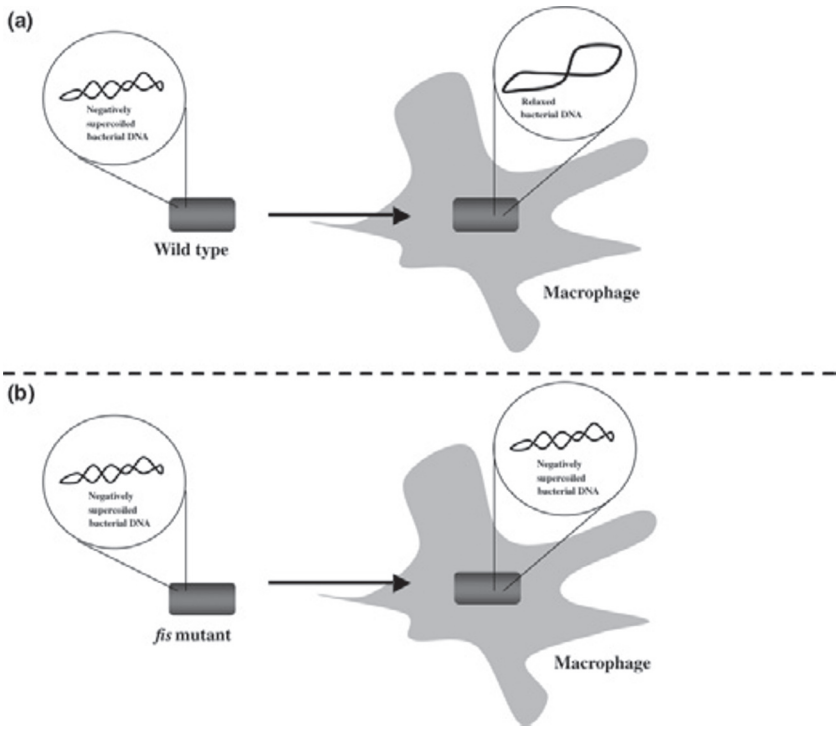

Fig. 2. The effect of adapting to the intracellular environment on the negative supercoiling of DNA in *Salmonella enterica* serovar Typhimurium bacteria. (a) DNA in wild-type bacteria is negatively supercoiled when the microbes are growing outside the macrophage. However, it becomes relaxed soon after engulfment of the bacteria by the host defence cells. (b) A *fis* mutant deficient in the expression of the Fis protein can still be engulfed by macrophage, but its DNA does not show the rapid relaxation that is seen in the wild type. Similarly, the relaxation-dependent genes of the SPI2 pathogenicity island that are required for full adaptation to the macrophage fail to be upregulated, leading to long-term sensitivity of the *fis* mutant bacteria to killing by the host defence cells [14].

crude control operates at the level of the genetic material itself as a result of changes in DNA topology, caused by alterations in the activity of DNA gyrase in combination with the modulating activity of the nucleoid-associated protein Fis. These events occur against a backdrop of transcriptional repression by the H-NS nucleoid-associated protein [5]. More refined control is then imposed by the action of 'conventional' transcription regulators, usually DNA-binding proteins, whose activities are controlled by elements of the dynamic environment. In the case of the pathogenicity islands, these consist of a mixture of proteins encoded by the islands themselves and others that are encoded by genes located in the ancestral genome [15]. These fine-tuning regulators can respond to individual stimuli, such as the presence or absence of specific signalling molecules, allowing fine adjustments to be made to the gene expression programme, optimizing it to suit the complex environment.

Transparency Declaration

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