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DNA topology and the global control of bacterial gene expression: implications for the regulation of virulence gene expression

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Overview

Bacterial cells are relatively simple entities with small, haploid genomes. Despite their simplicity, they are capable of coping with sudden and severe changes in the environment. This adaptability enables bacteria to colonize a wide range of ecological niches. When these niches are found on or within other living things, the bacteria may produce a pathological condition. The desire to obtain insights into the mechanisms by which bacteria cause disease has been a driving force in investigations of environmental adaptation by bacteria. The results of these investigations have revealed features which are common to many pathogenic and non-pathogenic organisms. These include an ability to sense changes in the external environment and to respond in an appropriate manner. This response frequently involves a change in the transcriptional profile of the cell as the expression of several genes is up- or down-regulated simultaneously. This phenomenon implies that programmes exist in the cell for coordinated control of changes in transcription. The research efforts of my laboratory have been aimed at elucidating the molecular basis of this coordinated gene regulatory programme.

Environmental sensing and response

Bacteria can detect changes in physical parameters (such as temperature or osmotic pressure) or chemical parameters (such as the presence or absence of specific compounds) and systems required for these sensing functions have been characterized in several species. It seems possible to classify these systems as belonging to 'families' whose members share mechanistic attributes. A simple case is one in which a small chemical binds to a protein, altering its ability to carry out a specific task, such as DNA binding. A group of proteins working in this way is the AraC family of transcription activators, many members of which have an amino-terminal carbohydrate-binding domain and a carboxy-terminal DNA-binding domain (Gallegos *et al.*, 1993). Here, sugar binding determines the efficiency of DNA binding. In such proteins, signal reception and the biological response are contained in a single polypeptide.

In other families there is a division of labour in which signal reception and the biological response are partitioned between two proteins. For example, the histidine protein kinase and response regulator (or 'two-component') family is composed of members which transmit environmental information between a sensing protein and a response regulating protein partner by phosphotransfer (Stock *et al.*, 1989). Reception of the environmental signal by the kinase results in autophosphorylation followed by phosphotransfer to the partner protein, which in turn elicits the required response within the cell. In some cases, such as in the control of chemotaxis in *Escherichia coli*, transcriptional control is not involved; protein–protein interactions alone alter the swimming pattern of the cell in response to the signals received (Stock *et al.*, 1989). More often, the response is a change in gene expression which allows the cell to adapt to the new environmental circumstances. This is true, for example, in the case of osmotic sensing in some members of the enterobacteriaceae by the EnvZ/OmpR kinase/response-regulator duo, where genes involved in expressing porin proteins are differentially expressed as a result of OmpR phosphorylation by EnvZ (Stock *et al.*, 1989). Appropriate expression of the porin profile on the cell surface is required for successful adaptation to certain high osmolarity environments, such as the human lower gut (Nikaido & Vaara, 1987).

Coordination of the transcriptional response

This review will concentrate on the contribution of transcriptional regulation to environmental adaptation. Classically, changes in transcription in bacteria have been thought to be elicited by the action of DNA-binding proteins acting at highly specific sites associated with

particular genes, as illustrated by the repression of the *lac* operon of *E. coli* by the Lac repressor, LacI, in the absence of inducer (reviewed in Beckwith, 1987). The LacI protein is specific for *lac* operator site binding and the effect of this is repression of *lacZYA* structural gene transcription. The arrangement of the three structural genes within an operon permits their coordinated regulation by the repressor. Of course, transcriptional regulation is not confined to negative influences, a point which can also be illustrated by reference to the *lac* operon. Activation of *lac* structural gene transcription is dependent not only on the removal of the repressor from the operator, but also on the action of the positive regulator CRP (catabolite regulatory protein), also known as CAP (catabolite gene activator protein). In the presence of its cofactor, cyclic-AMP (cAMP), CRP binds to a specific site at the *lac* promoter and activates structural gene transcription (Reznikoff, 1992).

Besides the rather obvious distinction of affecting *lac* transcription in opposite directions, LacI and CRP differ from one another in terms of their influence within the cell. LacI appears truly to be dedicated to *lacZYA* regulation whereas CRP regulates many other genes at other places on the *E. coli* chromosome. CRP is also more versatile in its effect on transcription; as well as being an activator, it can repress transcription in some cases (Botsford & Harman, 1992). Thus, the effects of a *lacI* mutation are quite local whereas a *crp* lesion is highly pleiotropic. In this way, genes whose expression must be adjusted in the event of a change in intracellular cAMP levels can be coordinately regulated if they possess a cAMP-CRP binding site at their promoters. Genes of this type which share a common regulator are said to constitute a regulon. Since several operons can belong to the same regulon, very large numbers of genes indeed can be controlled simultaneously in this fashion.

Bacteria also regulate groups of genes simultaneously in response to a common stimulus using independent regulators. Thus, a change in osmotic pressure will alter transcription of the *ompC* and *ompF* porin genes through the actions of the OmpR DNA-binding protein and its kinase, EnvZ, while transcription of the compatible-solute transport operon *proU* will also be altered but in a manner that is independent of the EnvZ/OmpR system (Csonka & Hanson, 1991). In this example, *ompC*, *ompF* and *proU* belong to the same group of co-regulated genes but they do not share a common protein regulator. Such a group is known as a stimulon.

By now it should be becoming apparent that regulatory hierarchies exist in the cell, with complexity increasing as one ascends the scale. This impression of complexity is compounded when one takes account of gene networking arrangements which allow individual genes or operons to belong to more than one regulatory group simultaneously. For example, the *ompC* and *ompF* porins belong to the EnvZ/OmpR regulon *and* to the osmotic stress stimulon. Since expression of *ompB*, the operon encoding the EnvZ and OmpR regulatory proteins, is controlled (in part) by cAMP-CRP (Huang *et al.*, 1992), *ompB* and its subservient genes *ompC* and *ompF* can be regarded as belonging to the CRP regulon (which includes the *lac* operon).

Global regulation of transcription

The identification of regulators having very widespread effects on transcription has given rise to the concept of global control (reviewed in Dorman & Ní Bhriain, 1992a). Proteins such as CRP and the related protein FNR (which regulates expression of several genes under anaerobic conditions) have been classified as global regulators. Although these proteins influence a large number of promoters, their effects are confined to those genes possessing the specific DNA sequence to which they can bind. Other regulatory factors have emerged which have a wider influence in controlling transcription and which also contribute to the organization of DNA topology in the cell. Investigations of these factors has shown that variations in DNA structure represent a form of global control which has the potential to influence the expression of any gene in the cell. This influence is so pervasive that, in comparison with the 'global' effects exerted by CRP or FNR, it may be said to be almost universal (reviewed in Dorman, 1994). The discovery that DNA topology is influenced by changes in environmental parameters (see later) raises the possibility that it can be exploited to reset the transcriptional profile as the cell moves from one environment to another. Before exploring these issues in detail, it is first necessary to review briefly the factors which influence the topology of bacterial DNA *in vivo*.

DNA supercoiling

As in most living cells, DNA in bacteria is maintained in an underwound or negatively supercoiled state which imparts free energy to the DNA. Underwound DNA experiences torsional stress and this can be relieved by (i) writhing of the DNA duplex in three-dimensional space, (ii) binding of proteins which constrain supercoils or (iii) a structural transition, such as strand separation, within a discrete segment of the duplex. In bacteria, all three solutions appear to be applied. The first approximates to the intuitive notion of supercoiling, the second results in the formation of nucleoprotein complexes, including bacterial chromatin, while the third illustrates the ability of duplex underwinding to potentiate reactions of DNA such as transcription (reviewed by Drlica, 1984, 1992).

Topoisomerases and the control of DNA supercoiling

DNA gyrase is a bacterial protein with the unique ability to introduce negative supercoils into DNA; it can also remove positive supercoils (Table 1). Gyrase is a topoisomerase which operates by a type-2 mechanism; it alters the linking number of DNA molecules in steps of two. This involves the introduction of a double-stranded break in the substrate, the passage of an intact section of the duplex through this gap and then religation. Gyrase must

Table 1. DNA topoisomerases of *E. coli*

Protein name	Function (mechanism)	Subunit structure	Subunit molecular mass (kDa)	Gene(s)
Topoisomerase I	DNA relaxation (type-1)	α	105	*topA*
DNA gyrase (or topoisomerase II)	DNA supercoiling and relaxation (type-2)	$\alpha_2\beta_2$	105 (α) 95 (β)	*gyrA* (α) *gyrB* (β)
Topoisomerase III	Decatenation (type-1)	α	73·2	*topB*
Topoisomerase IV	Decatenation; DNA relaxation (type-2)	$\alpha_2\beta_2$	83·7 (α) 70·2 (β)	*parC* (α) *parE* (β)

hydrolyse ATP to reset itself after each round of activity (Drlica, 1984, 1992).

Highly supercoiled DNA becomes a target for the relaxing activity of DNA topoisomerase I, an enzyme which operates by a type-1 mechanism. This involves the cleavage of one strand of the duplex, followed by passage of the intact strand through the gap by a swivelling action (Table 1) (Drlica, 1984, 1992). An important function of topoisomerase I is to counteract the influence of gyrase; mutants deficient in topoisomerase I activity have over-supercoiled DNA and grow very poorly, having many physiological defects. Compensatory mutations, many of which make gyrase less potent, allow the supercoiling in these strains to approach that in wild-type cells and promote survival (DiNardo *et al.*, 1982; Dorman *et al.*, 1989; Pruss *et al.*, 1982; Raji *et al.*, 1985; Richardson *et al.*, 1984). This finding has led to the proposal that supercoiling in bacteria is controlled by a self-regulating or homeostatic mechanism. This hypothesis is supported by the observation that the promoter of *topA*, the topoisomerase I structural gene, is activated by increases in supercoiling while those of *gyrA* and *gyrB* (encoding the subunits of gyrase) are activated by DNA relaxation (Menzel & Gellert, 1983, 1987; Tse-Dinh, 1985; Tse-Dinh & Beran, 1988).

E. coli possesses at least two other topoisomerases (Table 1). Topoisomerase III is a type-1 enzyme thought to contribute to decatenation reactions, although it is not essential for survival (Dean *et al.*, 1983; DiGate & Marians, 1988, 1989; Srivenugopal *et al.*, 1984). Topoisomerase IV is a type-2 enzyme with significant homology to gyrase but without the ability to supercoil DNA. It is essential for survival and seems to be involved in the decatenation of daughter chromosomes following DNA replication (Adams *et al.*, 1992; Kato *et al.*, 1990, 1992; McNairn *et al.*, 1995; Peng & Marians, 1993).

DNA supercoiling and transcription

Supercoiling of DNA might be expected to assist the local unwinding of DNA required for transcription initiation at promoters. Several studies have shown that supercoiling can have a strong influence on this process *in vitro* and *in vivo* (Borowiec & Gralla, 1987; Dorman *et al.*, 1988; Free & Dorman, 1994; Friedman *et al.*, 1984; Kreuzer & Cozzarelli, 1979; Sanzey, 1979; Yang *et al.*, 1979). The *in vivo* experiments have usually involved variations in supercoiling achieved by artificial means, such as gyrase inhibition by drugs or the use of topoisomerase gene mutations. The *in vitro* situation is of necessity artificial and sometimes data obtained under these conditions cannot be reproduced when the same system is studied *in vivo* (Free & Dorman, 1994; Lamond, 1985). Thus the relationship between changes in supercoiling and effects on transcription has often been a difficult one to interpret accurately.

Transcription and DNA supercoiling

Several lines of evidence show that transcription influences the topology of the DNA template. Data from *in vitro* and from *in vivo* experiments have shown that movement of the transcription complex along the DNA partitions this into a domain of negatively supercoiled DNA behind and a domain of positively supercoiled DNA ahead (Fig. 1) (Cook *et al.*, 1992; Liu & Wang, 1987; Pruss & Drlica, 1986; Rahmouni & Wells, 1992; Tsao *et al.*, 1989; Wu *et al.*, 1988). For this to happen the DNA must be incapable of rotating rapidly to allow diffusion of the accumulated supercoils and the transcription complex must not be able to rotate around the

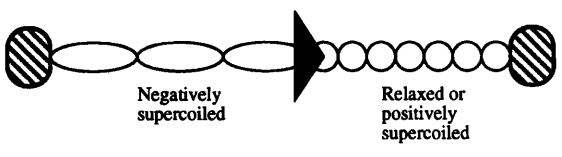

Fig. 1. Transcriptionally induced supercoiling in the DNA template. A segment of DNA bounded by non-rotatable supports (hatched) is shown. A transcription complex (large arrowhead), which is unable to rotate about the template, moves through the DNA, dividing it into positively and negatively supercoiled domains.

DNA. Such effects become exaggerated when the transcribed gene encodes a membrane-located protein; here, the coupled transcription and translation of the prokaryotic cell produces a moving complex incapable of rotation around the DNA duplex. If the differentially supercoiled domains so-generated are not dealt with by topoisomerases, they can influence other events taking place nearby in the DNA. Thus, transcriptionally induced negative supercoils associated with one transcription unit can modulate a supercoiling-sensitive promoter in a second, neighbouring, transcription unit *in vivo* in the absence of topoisomerase I activity (Bowater *et al.*, 1994; Chen *et al.*, 1992).

Environmental stimuli and DNA supercoiling

In vivo studies have shown that several environmental factors can influence the supercoiling of bacterial DNA. In particular, growth of bacterial cells under anaerobic conditions, or at high osmolarity, at different temperatures, or in a nutrient-poor medium can alter significantly DNA supercoiling levels (Balke & Gralla, 1987; Dorman *et al.*, 1988; Goldstein & Drlica, 1984; Higgins *et al.*, 1988; Yamamoto & Droffner, 1985). Aerobic-to-anaerobic shifts and transitions from low to high osmolarity result in increases in the [ATP]/[ADP] ratio and in negative supercoiling levels in DNA. This has been interpreted as being due to enhanced gyrase activity (Hsieh *et al.*, 1991a, b). Consistent with this hypothesis, the increased supercoiling observed in plasmids isolated from anaerobic cultures is not seen if the culture is treated with a gyrase-inhibiting antibiotic (Dorman *et al.*, 1988). Changes in temperature are thought to cause an alteration in DNA structure (such as the helical pitch of the DNA) which is sensed and compensated for by the action of topoisomerases (Goldstein & Drlica, 1984). Starvation of *E. coli* cultures produces a decline in negative supercoiling and the recovery of supercoils which accompanies a nutrient upshift is ATP-dependent (Balke & Gralla, 1987). Most data point to gyrase as being the key activity contributing to environmental responsiveness at the level of DNA supercoiling. However, several other DNA-topology-altering proteins also contribute to environmental responses; these are the so-called architectural proteins of the genome (see next section).

Architectural proteins and DNA topology

E. coli possesses several low-molecular-mass proteins which contribute to the higher-order organization of the bacterial nucleoid and to the expression of genetic information (Drlica & Rouvière-Yaniv, 1987; Pettijohn, 1988; Schmid, 1990). One of these is protein HU, which is abundant and binds to DNA in a sequence-independent manner, wrapping it into nucleosome-like structures. It influences a range of functions, including DNA replication, recombination (general and site-specific), transcription and transposition (Table 2) (Broyles & Pettijohn, 1986; Craigie *et al.*, 1985; Dixon & Kornberg, 1984; Johnson *et al.*, 1986; Kohno *et al.*, 1990; Morisato & Kleckner, 1987; Pontiaggia *et al.*, 1993).

A related protein is the integration host factor, IHF. Originally identified for its role in the life cycle of bacteriophage lambda (Miller *et al.*, 1979; Nash & Robertson, 1981), IHF is now known to regulate several host functions, including DNA replication, recombination, transcription and transposition. IHF is a sequence-specific DNA-binding protein and it bends DNA at the binding site (Table 2). This bending activity seems to be central to its biological role (Freundlich *et al.*, 1992; Friedman, 1988; Hoover *et al.*, 1990).

Protein H-NS is an abundant component of the bacterial genome (Table 2) (Pon *et al.*, 1988). It shows a preference for binding to curved DNA and has been shown to compact DNA *in vitro* (Spassky *et al.*, 1984; Yamada *et al.*, 1990). H-NS usually exerts a negative influence on transcription, perhaps by converting DNA in the vicinity of the affected promoter into a nucleoprotein complex which is transcriptionally inert (Dersch *et al.*, 1993; Falconi *et al.*, 1993; Owen-Hughes *et al.*, 1992). This effect does not seem to be indiscriminate, as not all promoters are H-NS repressible (Ueguchi *et al.*, 1993).

FIS (Factor for Inversion Stimulation) is a small heat-stable protein identified originally as playing a key role in enhancing the efficiency of site-specific recombination systems of the invertase type (Table 2) (Haffter & Bickle, 1987; Johnson *et al.*, 1986; Koch & Kahmann, 1986). It is now recognized as playing a much wider role in the cell, although it is not essential for cell survival (Ball & Johnson, 1991; Filutowicz *et al.*, 1992; Gille *et al.*, 1991; Gosink *et al.*, 1993; Nilsson *et al.*, 1990; Weinreich & Reznikoff, 1992). Like IHF, FIS is a DNA-bending protein (Finkel & Johnson, 1992; Thompson & Landy, 1988).

Frequently, these small, architectural proteins contribute to the control of transcription of genes whose products play a role in environmental adaptation. In this way they link together aspects of control of a large number of genes coding for disparate functions which, when appropriately expressed, enhance the chances that the cell will survive and prosper in its new environmental circumstances (reviewed in Dorman, 1994). A corollary to this is that such control mechanisms should help improve the fitness of pathogenic bacteria when these encounter novel environments during infection of their hosts (Dorman & Ní Bhriain, 1993). To appreciate how such complex regulation operates, it is necessary to examine some specific examples.

The virulence gene regulatory cascade of *Shigella flexneri*

Shigella flexneri is a facultative intracellular pathogen and is the causative agent of bacillary dysentery. The invasive phenotype depends on the expression of genes carried on a high-molecular-mass virulence plasmid (Sansonetti *et al.*, 1982). These genes are regulated at the level of transcription in response to changes in temperature and osmolarity: when the bacteria are cultured at 37 °C in growth media exerting osmotic pressure equivalent to

Table 2. 'Histone-like' proteins of *E. coli*

Protein name	Function	Subunit structure	Subunit molecular mass (kDa)	Gene(s)
FIS	DNA bending (bend angle 40°–90°)	α_2	11·2	*fis*
H-NS	DNA-curve-binding	α_2	15·4	*hns*
HU	DNA wrapping	$\alpha_2\beta_2$	9·5 (α) 9.5 (β)	*hupA* (β) *hupB* (α)
IHF	DNA bending (bend angle 140°)	$\alpha_2\beta_2$	11·2 (α) 10·5 (β)	*himA* (α) *himD* (β)

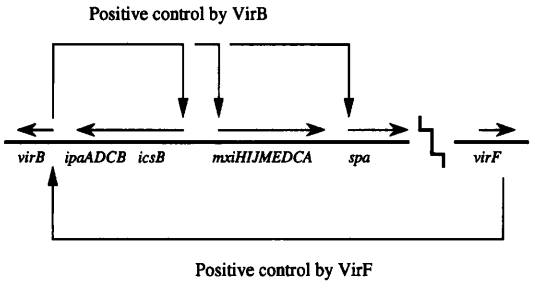

Fig. 2. Partial genetic map of the *S. flexneri* virulence gene regulon. The product of the *virF* gene activates transcription of the intermediate regulatory gene *virB*, whose product activates transcription of the structural gene operons. All of the genes shown are located on the 230 kb virulence plasmid. Other regulatory genes (discussed in the text) are located on the chromosome.

that of physiological saline, the invasion genes are expressed; at lower temperatures or osmolarities they are repressed (Bernardini *et al.*, 1990; Maurelli *et al.*, 1984; Porter & Dorman, 1994).

Genes coding for regulatory proteins concerned specifically with controlling expression of the invasive phenotype are located on the plasmid. One of these is the transcription activator VirF, which shows homology to members of the AraC family of DNA-binding proteins (Dorman, 1992). VirF belongs to a sub-group of these proteins whose members are concerned with thermoregulation of transcription rather than with controlling transcription in response to a specific carbohydrate (as in the case of AraC and the arabinose operon) (Gallegos *et al.*, 1993). The *S. flexneri* VirF protein is required to activate transcription of a second regulatory gene, *virB*, whose product is a positive regulator of the structural genes encoding the factors necessary for host cell invasion and for certain post-invasion events (Fig. 2) (Adler *et al.*, 1989). VirF binds just upstream of the *virB* promoter and plays a central role in transmitting the thermal signal to it, although how it does this is unclear (Tobe *et al.*, 1993).

Maurelli & Sansonetti (1988) isolated a mutation on the chromosome which permitted invasion gene expression at the non-permissive temperature. At first, this gene appeared to code for a thermally controlled repressor of invasion gene transcription. We succeeded in demonstrating by genetic means that this locus, *virR*, was an allele of *osmZ*, a gene now known to be equivalent to *hns*, the structural gene for the nucleoid-associated protein H-NS (Dorman *et al.*, 1990). This was a significant discovery because it hinted at a role for DNA topology in controlling the expression of an important virulence trait in a bacterial pathogen. It shows that in addition to its 'private' regulatory system consisting of VirF and VirB, the invasion gene control mechanism includes a 'shared' regulator, H-NS, which also contributes to a large (possibly very large) number of other gene control circuits. In principle, this means that this virulence regulon will be coregulated with other H-NS-dependent genes (Dorman & Ní Bhriain, 1993).

H-NS binding to the *virB* promoter has been demonstrated *in vitro* and it has been proposed that VirF is required to counteract the negative influence of H-NS on transcription initiation here (Tobe *et al.*, 1993). Consistent with this hypothesis, *in vitro* footprinting data show that the VirF- and H-NS-binding sites overlap. What is lacking at the time of writing are *in vivo* data on site occupancy under different environmental conditions. We find it difficult to demonstrate significant regulation of *virF* gene expression in response to environmental change (M. E. Porter & C. J. Dorman, unpublished results). This may mean that the VirF protein is constantly available in the cell, perhaps bound to the *virB* promoter, awaiting its activation signal. We have also found that the thermal signal is completely ineffective when the cell is maintained in a low osmolarity medium (Porter & Dorman, 1994). Thus, a combination of thermal and osmotic signals is required for optimal expression of the invasion genes. This may be a mechanism for ensuring that expression occurs only in the lower gut of the host.

Virulence gene expression in *S. flexneri* has also been shown to be influenced by factors which vary the level of DNA supercoiling (Dorman *et al.*, 1990; McNairn *et al.*, 1995; Ní Bhriain & Dorman, 1993). The *virB* promoter has been shown to be sensitive to such variations *in vivo* and *in vitro* (Tobe *et al.*, 1993). Thus, this sequence appears to be the major signal reception centre of the system. Consistent with this hypothesis, it has been shown that

the invasion regulon can be decoupled from its normal regulatory functions if *virB* is expressed from a heterologous promoter (Tobe *et al.*, 1991).

Regulatory systems of the type controlling expression of invasion genes in *S. flexneri* operate according to stereotypic principles; all of the cells in the population can be expected to perform identically in response to the same environmental cues each time they encounter them. This may pose risks for the long-term survival of the species unless a mechanism for varying the response in at least a few members of the population can be evolved. To look at this issue of variation in more detail, we have studied the phase-variable system controlling expression of type-1 fimbriae in *E. coli*.

The type-1 fimbrial system of *E. coli*

Type-1 fimbriae mediate attachment of bacteria to host cells. This is advantageous to the bacterium because colonization of the host is assisted but the fimbriae are highly immunogenic, making their deployment on the bacterial cell surface potentially hazardous to the survival of the microbe when it encounters host defences. Expression of type-1 fimbriae in *E. coli* is phase-variable, meaning that any given population of cells is likely to contain both fimbriate and afimbriate members. Presumably, the environment then selects the sub-population which is best fitted to the prevailing circumstances. In principle, any fimbriate cell can become afimbriate and vice versa. The rate of phase switching is approximately 5×10^{-2} and the switching process is believed to act at random within the population (Abraham *et al.*, 1985).

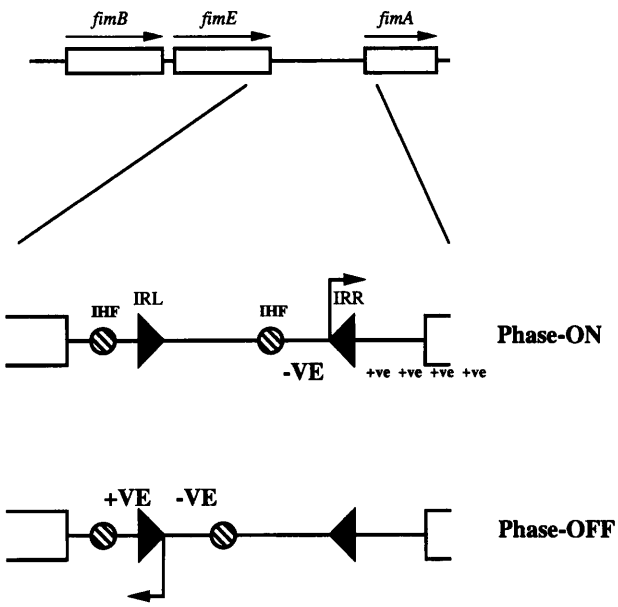

Fig. 3. Regulatory region of the *E. coli* type-1 fimbrial operon. The upper part shows a partial genetic map of the operon, indicating the relative locations and directions of transcription of the *fimB* and *fimE* regulatory genes and the *fimA* gene encoding the fimbrial subunit protein. The 314 bp invertible segment carrying the *fimA* promoter is located between *fimE* and *fimA* (shown enlarged in Phase-ON and Phase-OFF orientations). The promoter is represented by the angled arrow and the 9 bp inverted repeats (IRL and IRR) by the filled triangles. IHF-binding sites are represented by the hatched circles. Transcriptionally generated domains of DNA supercoiling are indicated by −VE (negative domain) and +VE (positive domain).

The control mechanism of the type-1 fimbrial system shares certain general features with the *S. flexneri* invasion gene regulatory system, such as the possession of 'private' regulators and a sensitivity to 'shared' control elements. The molecular basis of fimbrial phase variation has been elucidated and shown to concern the inversion of a 314 bp segment of DNA flanked by 9 bp inverted repeats. This DNA segment carries the promoter required for transcription of the *fimA* gene, coding for the type-1 fimbrial subunit. Site-specific recombination across the 9 bp inverted repeats is believed to invert the segment (Abraham *et al.*, 1985). The products of two closely linked genes, *fimB* and *fimE*, contribute to inversion (Fig. 3). These show a high degree of amino acid sequence identity (48%) and both have a region of homology to the catalytic site of members of the integrase family of site-specific recombinases (Dorman & Higgins, 1987; Eisenstein *et al.*, 1987; Klemm, 1986). The FimB protein inverts the segment in either direction with equal efficiency; FimE has a preference for the ON-to-OFF direction (McClain *et al.*, 1991). In many *E. coli* K12 strains, including those in use in our laboratory, the *fimE* gene is inactive (Blomfield *et al.*, 1991). Apart from this role in *fimA* regulation, FimB and FimE have no other known role in the cell.

A mutation at a distant locus, *pilG*, which resulted in accelerated inversion of the *fimA* promoter segment was shown by us to be an allele of *hns* (Higgins *et al.*, 1988). Thus, the fimbrial phase-variation system shares with the *S. flexneri* virulence gene regulatory cascade a sensitivity to negative control by H-NS. It also requires IHF for normal function; in the absence of this second genome architectural protein, inversion of the promoter fragment ceases (Dorman & Higgins, 1987; Eisenstein *et al.*, 1987) and the *fimA* promoter operates at a reduced efficiency (one seventh the value of the wild-type) (Dorman & Higgins, 1987). A further *trans*-acting regulator has been described for the *fim* system. This is the leucine-responsive regulatory protein (LRP), an 18·8 kDa DNA-binding protein which alters transcription of a wide range of operons. Depending on the individual system, the effect of LRP can be either positive or negative and its influence can be potentiated, antagonized or unaffected by the presence of L-leucine (Calvo & Matthews, 1994). In the case of *fim* inversion, LRP has a positive role and this is potentiated by leucine (Blomfield *et al.*, 1993). The status of LRP as a regulator is not completely clear. It certainly has pleiotropic effects and probably ranks between proteins such as CRP, which is a dedicated transcription factor with widespread effects on transcription and has a cofactor requirement, and structural elements such as HU, which has a secondary role in transcriptional control.

All of the available evidence suggests that the contributions of IHF, H-NS and LRP are made through interactions with the *fim* inverting segment itself, and probably involve the formation of a nucleoprotein

complex which facilitates formation of a recombinational synapse (S. L. Dove & C. J. Dorman, unpublished data; Gally *et al.*, 1994). Most site-specific recombination systems require precise management of local DNA topology and the same is likely to be true of the *fim* system. We have investigated the possibility that DNA supercoiling contributes to the control of *fim* expression (Dove & Dorman, 1994). Removal of DNA topoisomerase I from the cell results in a strong reduction in the rate of inversion in both the ON-to-OFF and the OFF-to-ON directions. Loss of this DNA-relaxing activity produces an increase in the supercoiling of reporter plasmids but compensatory mutations, which reset the level of supercoiling to near-wild-type levels, do not restore wild-type inversion frequencies. This apparent paradox can be resolved by proposing that the role of topoisomerase I is in relaxing locally generated domains of negative supercoiling at *fim*. These domains may be generated by transcriptional activity initiating at the *fimA* promoter, which is located close to one of the inverted repeats (Fig. 3). The moving transcription complex might be expected to generate domains of differentially supercoiled DNA here, with the more negatively supercoiled DNA being a target for topoisomerase I and common to both the Phase-ON and Phase-OFF orientations (Fig. 3). Transcriptionally generated domains of positively supercoiled DNA may not be common to both orientations of the invertible segment due to the presence of an IHF-binding site (and its associated bend in DNA when it is occupied by IHF) which lies on the *fimE* side of the inverting segment (Fig. 3). In the Phase-ON orientation, the positive supercoils can diffuse into the *fimA* ORF by rotational diffusion whereas in the Phase-OFF orientation diffusion is blocked by the occupied IHF site. For these reasons, the two orientations are not equivalent from the point of view of locally generated positive supercoils. Positive supercoils are relaxed by DNA gyrase and when gyrase is inhibited by antibiotic treatment, the effect on inversion is found to be strongly phase-dependent (Dove & Dorman, 1994). Phase-OFF cells turn ON rapidly whereas Phase-ON cells stay ON. This is consistent with a difference in the distribution of the gyrase target (relaxed or positively supercoiled DNA) between Phase-ON and Phase-OFF.

To employ topoisomerase gene mutations or inhibitors of the proteins themselves clearly creates an artificial situation in the cell. However, work from our laboratory and elsewhere shows that environmental influences can modulate topoisomerase function, and hence supercoiling of DNA (see above). This suggests that the data obtained using artificial means may hint at situations which can arise as cells adapt to novel ecological circumstances. Several studies have already indicated that this system is sensitive to environmental changes: *fim* expression varies with temperature, L-leucine levels and during transitions between growth in liquid medium and on solid surfaces (Dorman & Ní Bhriain, 1992b; Gally *et al.*, 1993; Schwan *et al.*, 1992). It is likely that these features increase the probability that fimbriation occurs in the most appropriate environment. Thus, the outcome (Phase-ON or Phase-OFF) of the *apparently* random inversion system may be subject to significant environmental influence.

Conclusions and perspective

These examples help to illustrate the sophistication of gene regulation in prokaryotes and to raise questions about why things are so complicated. There is very probably a major advantage to be gained in not expressing genetic information when it is not needed. Similarly, to fail to express such information when it is required could be very disadvantageous to the cell. To be successful, the cell requires an efficient means of environmental sensing and response and a good system for coordinating the response at the level of transcription. Global regulatory systems involving variations in DNA topology can provide a crude mechanism for the coordination of gene expression by influencing the structure of the genetic material. This influence can be exerted in response to environmental stimuli such as osmotic stress or anaerobic growth, both of which change the supercoiling of DNA, or by growth phase, which varies the intracellular concentrations of proteins such as FIS or IHF and changes the supercoiling of DNA. Fine-tuning of the genetic response depends on specific factors, such as DNA-binding proteins which act as transcription factors for just single operons or regulons. Systems like these contribute to a stereotypic response to environmental change; all of the cells in the population may be expected to respond in much the same way. In addition, there are apparently random events within the genome which add a layer of variability to the response. Inversion of the *fimA* promoter segment is an example of this sort of random event. Its apparent 'randomness' can be influenced by the same topological factors which are at work within the stereotypic response network. Thus H-NS, which regulates negatively the *virB* promoter in *S. flexneri*, also regulates negatively *fimA* promoter segment inversion in *E. coli*. Since these systems share this common control factor, their expression is crudely coordinated: any increase in H-NS levels will affect both negatively. Factors such as the VirF transcription activator or the FimB recombinase are unique to each system and have no known role beyond the virulence cascade and the fimbrial operon, respectively. This pattern of shared and private regulators is an emerging theme in studies of bacterial gene regulation. It points to the integrated nature of the cellular gene expression system and the perpetual tension between forces attempting to activate transcription units and those which repress them. The cell as it exists at any moment in time is an expression of the outcome of these countervailing forces.

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References

Abraham, J. M., Freitag, C. S., Clements, J. R. & Eisenstein, B. I. (1985). An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. *Proc Natl Acad Sci USA* **82**, 5724–5727.

Adams, D. E., Shekhtman, E. M., Zechiedrich, E. L., Schmid, M. B. & Cozzarelli, N. R. (1992). The role of topoisomerase IV in partitioning bacterial replicons and the structure of catenated intermediates in DNA replication. *Cell* **71**, 277–288.

Adler, B., Sasakawa, C., Tobe, T., Makino, S., Komatsu, K. & Yoshikawa, M. (1989). A dual transcriptional activation system for the 230 kb plasmid genes coding for virulence-associated antigens of *Shigella flexneri*. *Mol Microbiol* **3**, 627–635.

Balke, V. L. & Gralla, J. D. (1987). Changes in the linking number of supercoiled DNA accompany growth transitions in *Escherichia coli*. *J Bacteriol* **169**, 4499–4506.

Ball, C. A. & Johnson, R. C. (1991). Efficient excision of phage λ from the *Escherichia coli* chromosome requires the FIS protein. *J Bacteriol* **173**, 4027–4031.

Beckwith, J. (1987). The lactose operon. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 1444–1452. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.

Bernardini, M. L., Fontaine, A. & Sansonetti, P. J. (1990). The two-component regulatory system OmpR-EnvZ controls the virulence of *Shigella flexneri*. *J Bacteriol* **172**, 6274–6281.

Blomfield, I. C., McClain, M. S., Princ, J. A., Calie, P. J. & Eisenstein, B. I. (1991). Type 1 fimbriation and *fimE* mutants of *Escherichia coli* K-12. *J Bacteriol* **173**, 5298–5307.

Blomfield, I. C., Calie, P. J., Eberhardt, K. J., McClain, M. S. & Eisenstein, B. I. (1993). Lrp stimulates phase variation of type 1 fimbriation in *Escherichia coli* K-12. *J Bacteriol* **175**, 27–36.

Borowiec, J. A. & Gralla, J. D. (1987). All three elements of the *lac* p^s promoter mediate its transcriptional response to DNA supercoiling. *J Mol Biol* **195**, 89–97.

Botsford, J. L. & Harman, J. G. (1992). Cyclic AMP in prokaryotes. *Microbiol Rev* **56**, 100–122.

Bowater, R. P., Chen, D. & Lilley, D. M. J. (1994). Modulation of *tyrT* promoter activity by template supercoiling *in vivo*. *EMBO J* **13**, 5647–5655.

Broyles, S. & Pettijohn, D. E. (1986). Interaction of the *E. coli* HU protein with DNA: evidence for formation of nucleosome-like structures with altered DNA helical pitch. *J Mol Biol* **187**, 47–60.

Calvo, J. M. & Matthews, R. G. (1994). The leucine-responsive regulatory protein, a global regulator of metabolism in *Escherichia coli*. *Microbiol Rev* **58**, 466–490.

Chen, D., Bowater, R., Dorman, C. J. & Lilley, D. M. J. (1992). Activity of a plasmid-borne *leu500* promoter depends on the transcription of an adjacent gene. *Proc Natl Acad Sci USA* **89**, 8784–8788.

Cook, D. N., Ma, D., Pon, N. G. & Hearst, J. E. (1992). Dynamics of DNA supercoiling by transcription in *Escherichia coli*. *Proc Natl Acad Sci USA* **89**, 10603–10607.

Craigie, R., Ardnt-Jovin, D. & Mizuuchi, K. (1985). A defined system for the DNA strand-transfer reaction at the start of bacteriophage Mu transposition: protein and DNA substrate requirements. *Proc Natl Acad Sci USA* **82**, 7570–7574.

Csonka, L. N. & Hanson, A. D. (1991). Prokaryotic osmoregulation: genetics and physiology. *Annu Rev Microbiol* **45**, 569–606.

Dean, F., Krasnow, M. A., Otter, R., Matzuk, M. M., Spengler, S. J. & Cozzarelli, N. R. (1983). *Escherichia coli* type-1 topoisomerases: identification, mechanism, and role in recombination. *Cold Spring Harbor Symp Quant Biol* **47**, 769–777.

Dersch, P., Schmidt, K. & Bremer, E. (1993). Synthesis of the *Escherichia coli* K-12 nucleoid-associated DNA-binding protein H-NS is subjected to growth-phase control and autoregulation. *Mol Microbiol* **8**, 875–889.

DiGate, R. J. & Marians, K. J. (1988). Identification of a potent decatenating enzyme from *Escherichia coli*. *J Biol Chem* **263**, 13366–13373.

DiGate, R. J. & Marians, K. J. (1989). Molecular cloning and DNA sequence analysis of *Escherichia coli topB*, the gene encoding topoisomerase III. *J Biol Chem* **264**, 17924–17930.

DiNardo, S., Voekel, K. A., Sternglanz, R., Reynolds, A. E. & Wright, A. (1982). *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. *Cell* **31**, 43–51.

Dixon, N. E. & Kornberg, A. (1984). Protein HU in the enzymatic replication of the chromosomal origin of *Escherichia coli*. *Proc Natl Acad Sci USA* **81**, 424–428.

Dorman, C. J. (1992). The VirF protein from *Shigella flexneri* is a member of the AraC transcription factor superfamily and is highly homologous to Rns, a positive regulator of virulence in enteropathogenic *Escherichia coli*. *Mol Microbiol* **6**, 1575.

Dorman, C. J. (1994). *The Genetics of Bacterial Virulence*. Oxford: Blackwell.

Dorman, C. J. & Higgins, C. F. (1987). Fimbrial phase variation in *Escherichia coli*: dependence on integration host factor and homologies with other site-specific recombinases. *J Bacteriol* **169**, 3840–3843.

Dorman, C. J. & Nĺ Bhriain, N. (1992a). Global regulation of gene expression during environmental adaptation: implications for bacterial pathogens. In *Molecular Biology of Bacterial Infection: Current Status and Future Perspectives, Society for General Microbiology Symposium*, vol. 49, pp. 193–230. Edited by C. E. Hormaeche, C. W. Penn & C. J. Smyth. Cambridge: Cambridge University Press.

Dorman, C. J. & Nĺ Bhriain, N. (1992b). Thermal regulation of *fimA*, the *Escherichia coli* gene coding for the type 1 fimbrial subunit protein. *FEMS Microbiol Lett* **99**, 125–130.

Dorman, C. J. & Nĺ Bhriain, N. (1993). DNA topology and bacterial virulence gene regulation. *Trends Microbiol* **1**, 92–99.

Dorman, C. J., Barr, G. C., Nĺ Bhriain, N. & Higgins, C. F. (1988). DNA supercoiling and the anaerobic and growth phase regulation of *tonB* gene expression. *J Bacteriol* **170**, 2816–2826.

Dorman, C. J., Lynch, A. S., Nĺ Bhriain, N. & Higgins, C. F. (1989). DNA supercoiling in *Escherichia coli*: *topA* mutants can be suppressed by DNA amplifications involving the *tolC* locus. *Mol Microbiol* **3**, 531–540.

Dorman, C. J., Nĺ Bhriain, N. & Higgins, C. F. (1990). DNA supercoiling and environmental regulation of virulence gene expression in *Shigella flexneri*. *Nature* **344**, 789–792.

Dove, S. L. & Dorman, C. J. (1994). The site-specific recombination system regulating expression of the type 1 fimbrial subunit gene of

Escherichia coli is sensitive to changes in DNA supercoiling. *Mol Microbiol* **14**, 975–988.

Drlica, K. (1984). The biology of bacterial deoxyribonucleic acid topoisomerases. *Microbiol Rev* **48**, 273–289.

Drlica, K. (1992). Control of bacterial DNA supercoiling. *Mol Microbiol* **6**, 425–433.

Drlica, K. & Rouvière-Yaniv, J. (1987). Histonelike proteins of bacteria. *Microbiol Rev* **51**, 301–319.

Eisenstein, B. I., Sweet, D. S., Vaughn, V. & Friedman, D. I. (1987). Integration host factor is required for the DNA inversion that controls phase variation in *Escherichia coli*. *Proc Natl Acad Sci USA* **84**, 6506–6510.

Falconi, M., Higgins, N. P., Spurio, R., Pon, C. L. & Gualerzi, C. O. (1993). Expression of the gene encoding the major bacterial nucleoid protein H-NS is subject to transcriptional autoregulation. *Mol Microbiol* **10**, 273–282.

Filutowicz, M., Ross, W., Wild, J. & Gourse, R. L. (1992). Involvement of FIS protein in replication of the *Escherichia coli* chromosome. *J Bacteriol* **174**, 398–407.

Finkel, S. E. & Johnson, R. C. (1992). The FIS protein: it's not just for DNA inversion anymore. *Mol Microbiol* **6**, 3257–3265.

Free, A. & Dorman, C. J. (1994). *Escherichia coli tyrT* gene transcription is sensitive to DNA supercoiling in its native chromosomal context: effect of DNA topoisomerase IV overexpression on *tyrT* promoter function. *Mol Microbiol* **14**, 151–161.

Freundlich, M., Ramani, N., Mathew, E., Sirko, A. & Tsui, P. (1992). The role of integration host factor in gene expression in *Escherichia coli*. *Mol Microbiol* **6**, 2557–2563.

Friedman, D. I. (1988). Integration host factor: a protein for all reasons. *Cell* **55**, 545–554.

Friedman, D. I., Plantefaber, L. C., Olson, E. J., Carver, D., O'Dea, M. H. & Gellert, M. (1984). Mutations in the DNA *gyrB* gene that are temperature sensitive for lambda site-specific recombination, Mu growth, and plasmid maintenance. *J Bacteriol* **157**, 490–497.

Gallegos, M.-T., Michán, C. & Ramos, J. L. (1993). The XylS/AraC family of regulators. *Nucleic Acids Res* **21**, 807–810.

Gally, D. L., Bogan, J. A., Eisenstein, B. I. & Blomfield, I. C. (1993). Environmental regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media. *J Bacteriol* **175**, 6186–6193.

Gally, D. L., Rucker, T. J. & Blomfield, I. C. (1994). The leucine-responsive regulatory protein binds to the *fim* switch to control phase variation of type 1 fimbrial expression in *Escherichia coli*. *J Bacteriol* **176**, 5665–5672.

Gille, H., Egan, J. B., Roth, A. & Messer, W. (1991). The FIS protein binds and bends the origin of chromosomal DNA replication, *oriC*, of *Escherichia coli*. *Nucleic Acids Res* **19**, 4167–4172.

Goldstein, E. & Drlica, K. (1984). Regulation of bacterial DNA supercoiling: plasmid linking numbers vary with growth temperature. *Proc Natl Acad Sci USA* **81**, 4046–4050.

Gosink, K. K., Ross, W., Leirmo, S., Osuna, R., Finkel, S. E., Johnson, R. C. & Gourse, R. L. (1993). DNA binding and bending are necessary but not sufficient for FIS-dependent activation of *rrnB* P1. *J Bacteriol* **175**, 1580–1589.

Haffter, P. & Bickle, T. A. (1987). Purification and DNA-binding properties of FIS and Cin, two proteins required for P1 site-specific recombination system, *cin*. *J Mol Biol* **198**, 579–587.

Higgins, C. F., Dorman, C. J., Stirling, D. A., Waddell, L., Booth, I. R., May, G. & Bremer, E. (1988). A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. *Cell* **52**, 569–584.

Hoover, T. R., Santero, E., Porter, S. & Kustu, S. (1990). The integration host factor stimulates interaction of RNA polymerase with NifA, the transcriptional activator for nitrogen fixation operons. *Cell* **63**, 11–22.

Hsieh, L.-S., Burger, R. M. & Drlica, K. (1991a). Bacterial DNA supercoiling and [ATP]/[ADP]: changes associated with a transition to anaerobic growth. *J Mol Biol* **219**, 443–450.

Hsieh, L.-S., Rouvière-Yaniv, J. & Drlica, K. (1991b). Bacterial DNA supercoiling and [ATP]/[ADP] ratio: changes associated with salt shock. *J Bacteriol* **173**, 3914–3917.

Huang, L., Tsui, P. & Freundlich, M. (1992). Positive and negative control of *ompB* transcription in *Escherichia coli* by cyclic AMP and the cyclic AMP receptor protein. *J Bacteriol* **174**, 664–670.

Johnson, R. C., Bruist, M. F. & Simon, M. I. (1986). Host protein requirements for *in vitro* site-specific inversion. *Cell* **46**, 531–539.

Kato, J.-I., Hishimura, Y., Imamura, R., Niki, H., Hiraga, S. & Suzuki, H. (1990). New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* **63**, 393–404.

Kato, J.-I., Suzuki, H. & Ikeda, H. (1992). Purification and characterization of DNA topoisomerase IV in *Escherichia coli*. *J Biol Chem* **267**, 25676–25684.

Klemm, P. (1986). Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli*. *EMBO J* **5**, 1389–1393.

Koch, C. & Kahmann, R. (1986). Purification and properties of the *Escherichia coli* host factor required for inversion of the G segment in bacteriophage Mu. *J Biol Chem* **261**, 15673–15678.

Kohno, K., Wada, M., Kano, Y. & Imamoto, F. (1990). Promoters and autogenous control of the *Escherichia coli hupA* and *hupB* genes. *J Mol Biol* **213**, 27–36.

Kreuzer, K. N. & Cozzarelli, N. R. (1979). *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. *J Bacteriol* **140**, 424–435.

Lamond, A. I. (1985). Supercoiling response of a bacterial tRNA gene. *EMBO J* **4**, 501–507.

Liu, L. F. & Wang, J. C. (1987). Supercoiling of the DNA template during transcription. *Proc Natl Acad Sci USA* **84**, 7024–7027.

Maurelli, A. T. & Sansonetti, P. J. (1988). Identification of a chromosomal gene controlling temperature-regulated expression of *Shigella* virulence. *Proc Natl Acad Sci USA* **85**, 2820–2824.

Maurelli, A. T., Blackmon, B. & Curtiss, R., III (1984). Temperature-dependent expression of virulence genes in *Shigella flexneri*. *Infect Immun* **43**, 195–201.

McClain, M. S., Blomfield, I. C. & Eisenstein, B. I. (1991). Roles of *fimB* and *fimE* in site-specific DNA inversion of type 1 fimbriae in *Escherichia coli*. *J Bacteriol* **173**, 5308–5314.

McNairn, E., Ní Bhriain, N. & Dorman, C. J. (1995). Overexpression of the *Shigella flexneri* genes coding for DNA topoisomerase IV compensates for loss of DNA topoisomerase I: effect on virulence gene expression. *Mol Microbiol* **15**, 507–517.

Menzel, R. & Gellert, M. (1983). Regulation of the genes for *E. coli* DNA gyrase: homeostatic control of DNA supercoiling. *Cell* **34**, 105–113.

Menzel, R. & Gellert, M. (1987). Fusions of the *Escherichia coli gyrA* and *gyrB* control regions to the galactokinase gene are inducible by coumermycin treatment. *J Bacteriol* **169**, 1272–1278.

Miller, H. I., Kikuchi, A., Nash, H. A., Weisberg, R. A. & Friedman, D. I. (1979). Site-specific recombination of bacteriophage λ: the role of host gene products. *Cold Spring Harbor Symp Quant Biol* **43**, 1121–1126.

Morisato, D. & Kleckner, N. (1987). Tn*10* transposition and circle formation *in vitro*. *Cell* **51**, 101–111.

Nash, H. A. & Robertson, C. A. (1981). Purification and properties of the *Escherichia coli* protein factor for λ integrative recombination. *J Biol Chem* **256**, 9246–9253.

Ní Bhriain, N. & Dorman, C. J. (1993). Isolation and characterization of a *topA* mutant of *Shigella flexneri*. *Mol Microbiol* **7**, 351–358.

Nikaido, H. & Vaara, M. (1987). Outer membrane. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 7–22. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.

Nilsson, L., Vanet, A., Vijgenboom, E. & Bosch, L. (1990). The role of FIS in *trans* activation of stable RNA operons of *E. coli*. *EMBO J* **9**, 727–734.

Owen-Hughes, T. A., Pavitt, G. D., Santos, D. S., Sidebotham, J. M., Hulton, C. S. J., Hinton, J. C. D. & Higgins, C. F. (1992). The chromatin-associated protein H-NS interacts with curved DNA to influence DNA topology and gene expression. *Cell* **71**, 255–265.

Peng, H. & Marians, K. J. (1993). Decatenation activity of topoisomerase IV during *oriC* and pBR322 DNA replication *in vitro*. *Proc Natl Acad Sci USA* **90**, 8571–8575.

Pettijohn, D. E. (1988). Histone-like proteins and bacterial chromosome structure. *J Biol Chem* **263**, 12793–12796.

Pon, C. L., Calogero, R. A. & Gualerzi, C. O. (1988). Identification, cloning, nucleotide sequence and chromosomal map location of *hns*, the structural gene for *Escherichia coli* DNA-binding protein H-NS. *Mol & Gen Genet* **212**, 199–202.

Pontiaggia, A., Negri, A., Beltrame, M. & Bianchi, M. E. (1993). Protein HU binds specifically to kinked DNA. *Mol Microbiol* **7**, 343–350.

Porter, M. E. & Dorman, C. J. (1994). A role for H-NS in the thermo-osmotic regulation of virulence gene expression in *Shigella flexneri*. *J Bacteriol* **176**, 4187–4191.

Pruss, G. J. & Drlica, K. (1986). Topoisomerase I mutants: the gene on pBR322 that encodes resistance to tetracycline affects plasmid DNA supercoiling. *Proc Natl Acad Sci USA* **83**, 8952–8956.

Pruss, G. J., Manes, S. H. & Drlica, K. (1982). *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. *Cell* **31**, 35–42.

Rahmouni, A. R. & Wells, R. D. (1992). Direct evidence for the effect of transcription on local DNA supercoiling *in vivo*. *J Mol Biol* **223**, 131–144.

Raji, A., Zabel, D. J., Laufer, C. S. & Depew, R. E. (1985). Genetic analysis of mutations that compensate for loss of *Escherichia coli* DNA topoisomerase I. *J Bacteriol* **162**, 1173–1179.

Reznikoff, W. S. (1992). Catabolite gene activator protein activation of *lac* transcription. *J Bacteriol* **174**, 655–658.

Richardson, S. M. H., Higgins, C. F. & Lilley, D. M. J. (1984). The genetic control of DNA supercoiling in *Salmonella typhimurium*. *EMBO J* **3**, 1745–1752.

Sansonetti, P. J., Kopecko, D. J. & Formal, S. B. (1982). Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infect Immun* **35**, 852–860.

Sanzey, B. (1979). Modulation of gene expression by drugs affecting deoxyribonucleic acid gyrase. *J Bacteriol* **138**, 40–47.

Schmid, M. B. (1990). More than just 'histone-like' proteins. *Cell* **63**, 451–453.

Schwan, W. R., Seifert, H. S. & Duncan, J. L. (1992). Growth conditions mediate differential transcription of *fim* genes involved in phase variation of type 1 pili. *J Bacteriol* **174**, 2367–2375.

Spassky, A., Rimsky, S., Garreau, H. & Buc, H. (1984). H1a, an *E. coli* DNA-binding protein which accumulates in stationary phase, strongly compacts DNA *in vitro*. *Nucleic Acids Res* **12**, 5321–5340.

Srivenugopal, K. S., Lockshon, D. & Morris, D. R. (1984). *Escherichia coli* DNA topoisomerase III: purification and characterization of a new type I enzyme. *Biochemistry* **23**, 1899–1906.

Stock, J. B., Ninfa, A. J. & Stock, A. M. (1989). Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol Rev* **53**, 450–490.

Thompson, J. F. & Landy, A. (1988). Empirical estimation of protein-induced DNA bending angles: applications to λ site-specific recombination complexes. *Nucleic Acids Res* **16**, 9687–9705.

Tobe, T., Nagai, S., Adler, B., Yoshikawa, M. & Sasakawa, C. (1991). Temperature-regulated expression of invasion genes in *Shigella flexneri* is controlled through the transcriptional activation of the *virB* gene on the large plasmid. *Mol Microbiol* **5**, 887–893.

Tobe, T., Yoshikawa, M., Mizuno, T. & Sasakawa, C. (1993). Transcriptional control of the invasion regulatory gene *virB* of *Shigella flexneri*: activation by VirF and repression by H-NS. *J Bacteriol* **175**, 6145–6149.

Tsao, Y.-P., Wu, H.-Y. & Liu, L. F. (1989). Transcription-driven supercoiling of DNA: direct biochemical evidence from *in vitro* studies. *Cell* **56**, 111–118.

Tse-Dinh, Y.-C. (1985). Regulation of the *Escherichia coli* topoisomerase I gene by DNA supercoiling. *Nucleic Acids Res* **13**, 4751–4763.

Tse-Dinh, Y.-C. & Beran, R. K. (1988). Multiple promoters for transcription of the *Escherichia coli* DNA topoisomerase I gene and their regulation by DNA supercoiling. *J Mol Biol* **202**, 735–742.

Ueguchi, C., Kakeda, M. & Mizuno, T. (1993). Autoregulatory expression of the *Escherichia coli hns* gene encoding a nucleoid protein: H-NS functions as a repressor of its own transcription. *Mol & Gen Genet* **236**, 171–178.

Weinreich, M. D. & Reznikoff, W. S. (1992). FIS plays an active role in Tn*5* and IS*50* transposition. *J Bacteriol* **174**, 4530–4537.

Wu, H.-Y., Shyy, S., Wang, J. C. & Liu, L. F. (1988). Transcription generates positively and negatively supercoiled domains in the template. *Cell* **53**, 433–440.

Yamada, H., Muramatsu, S. & Mizuno, T. (1990). An *Escherichia coli* protein that preferentially binds to sharply curved DNA. *J Biochem* **108**, 420–425.

Yamamoto, N. & Droffner, M. L. (1985). Mechanisms determining aerobic or anaerobic growth in the facultative anaerobe *Salmonella typhimurium*. *Proc Natl Acad Sci USA* **82**, 2077–2081.

Yang, H.-L., Heller, K., Gellert, M. & Zubay, G. (1979). Differential sensitivity of gene expression *in vitro* to inhibitors of DNA gyrase. *Proc Natl Acad Sci USA* **76**, 3304–3308.