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Minireview

Control of virulence gene transcription by indirect readout in *Vibrio cholerae* and *Salmonella enterica* serovar Typhimurium

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Summary

Indirect readout mechanisms of transcription control rely on the recognition of DNA shape by transcription factors (TFs). TFs may also employ a direct readout mechanism that involves the reading of the base sequence in the DNA major groove at the binding site. TFs with winged helix–turn–helix (wHTH) motifs use an alpha helix to read the base sequence in the major groove while inserting a beta sheet 'wing' into the adjacent minor groove. Such wHTH proteins are important regulators of virulence gene transcription in many pathogens; they also control housekeeping genes. This article considers the cases of the non-invasive Gram-negative pathogen *Vibrio cholerae* and the invasive pathogen *Salmonella enterica* serovar Typhimurium. Both possess clusters of A + T-rich horizontally acquired virulence genes that are silenced by the nucleoid-associated protein H-NS and regulated positively or negatively by wHTH TFs: for example, ToxR and LeuO in *V. cholerae*; HilA, LeuO, SlyA and OmpR in *S.* Typhimurium. Because of their relatively relaxed base sequence requirements for target recognition, indirect readout mechanisms have the potential to engage regulatory proteins with many more targets than might be the case using direct readout, making indirect readout an important, yet often ignored, contributor to the expression of pathogenic phenotypes.

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Introduction

Gram-negative enteric pathogens have acquired many of the virulence genes used for infection of their hosts through horizontal gene transfer (Ochman *et al.*, 2000; Dorman, 2009; Bliven and Maurelli, 2016; Navarre, 2016). The expression of these genes is usually restricted to sites on or in the host where their products can contribute to a specific stage of the infection process. Signals associated with those sites are detected by the bacterium and transduced to the virulence genes (Skorupski and Taylor, 1997; Rhen and Dorman, 2005; Erhardt and Dersch, 2015; Ayala *et al.*, 2017). Gene activation at the level of transcription typically involves DNA-binding proteins that recruit and/or activate RNA polymerase at specific promoters and/or remove factors that silence transcription (Stoebel *et al.*, 2008; Browning and Busby, 2016).

The evidence that lateral transfer has played a role in the acquisition of important virulence genes has come, in part, from an analysis of the base composition of the genes. Virulence genes have a higher A + T base content than the average for the core genome. They are frequently grouped in clusters called pathogenicity islands that possess features normally associated with mobile genetic elements (Hazen *et al.*, 2010; Syvanen, 2012; Rodriguez-Valera *et al.*, 2016). A combination of regulatory proteins encoded by the core and the horizontally acquired genome controls virulence gene transcription (Rhen and Dorman, 2005). Further evidence has come from observing horizontal transfer taking place in contemporary pathogens: for example, the transduction of the genes encoding cholera toxin by the CTXΦ filamentous bacteriophage in *Vibrio cholerae* (Waldor and Mekalanos, 1996).

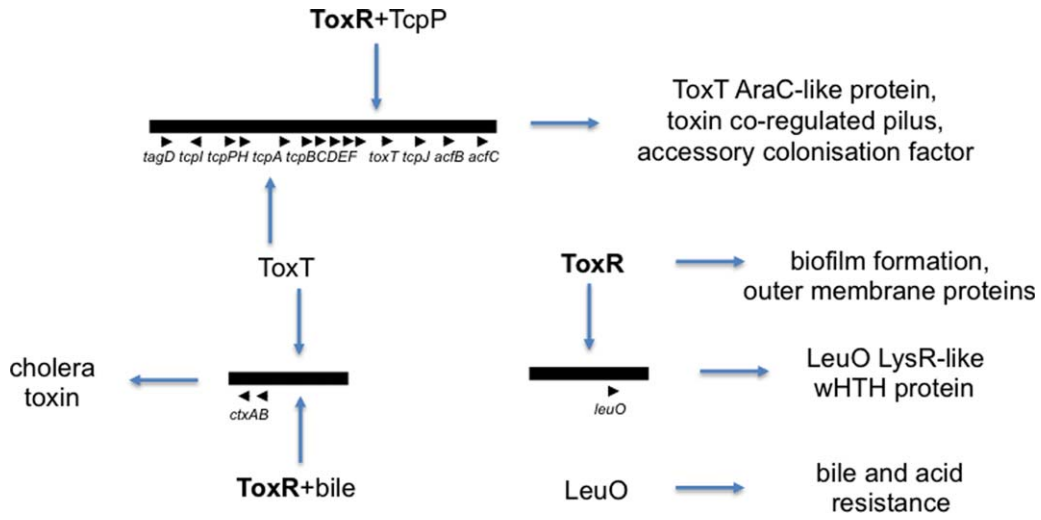

Fig. 1. ToxR-associated virulence gene control circuits in *Vibrio cholerae*. Genes and phenotypes governed by the wHTH TF ToxR (bold type) are shown. ToxR works in association with another wHTH protein, TcpP, to activate transcription of the *toxT* gene. ToxT positively autoregulates *toxT* transcription from an upstream promoter at *tcpA*. The AraC-like ToxT protein is the principal activator of the cholera toxin operon, *ctxAB*, although ToxR can also activate its transcription when over-expressed or in the presence of bile. Among the genomic targets of ToxR are genes contributing to biofilm formation; ToxR also activates the expression of the *leuO* gene whose LysR-like protein product is involved in resistance to bile and acid. Genomic segments are represented by horizontal bars and individual genes by arrowheads (not to scale).

Transcription silencing by the H-NS nucleoid-associated protein is a common feature of virulence genes in enteric pathogens (Porter and Dorman, 1994; Hromockyj *et al*., 2002; Beloin and Dorman, 2003; Lucchini *et al*., 2006; Baños *et al*., 2008; Zwir *et al*., 2014; Dorman, 2015; Hüttener *et al*., 2015; Prieto *et al*., 2016). This protein targets genes with a high A + T base content and prevents their transcription by RNA polymerase (Dame *et al*., 2001; 2002; Beloin and Dorman, 2003; Dorman, 2004; Lucchini *et al*., 2006). The relief of H-NS silencing is achieved by an impressively wide range of mechanisms, many of which rely on DNA binding proteins that target H-NS-silenced promoters and either remove H-NS or remodel the nucleoprotein complex in ways that facilitate the activation of gene expression (Prosseda *et al*., 2004; Stoebel *et al*., 2008).

It has been proposed that the transcription silencing activity of H-NS assists in the evolution of pathogens by allowing them to acquire new genes and then to incorporate them into the genome at a regulatory level as well as integrating them physically (Dorman, 2007; Ali *et al*., 2014). Silencing prevents the inappropriate expression of new genetic information that could, if not properly controlled, compromise the competitive fitness of the bacterium (Lucchini *et al*., 2006; Dorman, 2007; Navarre, 2016). The requirement by H-NS for an A + T-rich DNA substrate on which to construct its silencing complex provides the basis for a crude-yet-effective regulatory switch. In this switch, H-NS imposes transcriptional silence. For the switch to be reversed, an H-NS antagonist with similar requirements in its binding substrate would be required so that the opposing proteins can encounter one another on the DNA (Stoebel *et al*., 2008; Dorman and Kane, 2009; Kane and Dorman, 2011).

Here it will be proposed that DNA binding proteins which use a winged helix–turn–helix motif (wHTH) to bind to A + T-rich DNA are particularly well suited to this task (Brennan, 1993; Schell, 1993). The point will be illustrated with reference to the intensively studied enteric pathogens *Vibrio cholerae* (Fig. 1) and *Salmonella enterica* serovar Typhimurium (Fig. 2). These organisms use very different strategies for infection, yet have similar mechanisms for virulence gene regulation. The similarities include a reliance on wHTH transcription factors to overcome H-NS-mediated silencing of A + T-rich genes that have been acquired by lateral gene transfer (Bajaj *et al*., 1995; Nye *et al*., 2000; Kenney, 2002; Dolan *et al*., 2011; Quinn *et al*., 2014; Kazi *et al*., 2016).

Direct versus indirect readout in gene regulation

Transcription factors that select their binding sites by detecting specific base sequences are said to use a direct readout mechanism for the regulation of their target genes (Travers, 1997; Mendieta *et al*., 2007). Base sequence information is most readily accessible in the major groove of DNA with transcription factors that use direct readout typically possessing a motif for insertion into that groove (Steffen *et al*., 2002). In the case of proteins that use a helix–turn–helix (HTH) motif, that

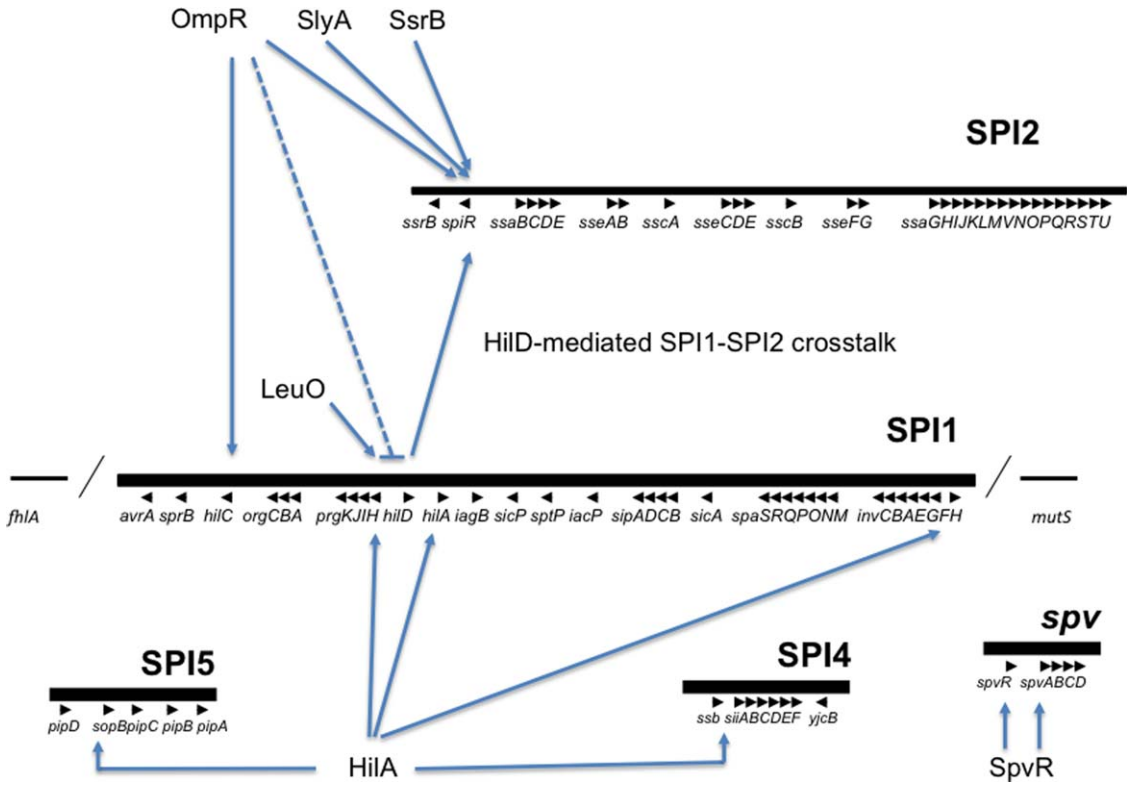

Fig. 2. Virulence gene control and wHTH TFs in *Salmonella enterica* serovar Typhimurium. Pathogenicity islands in the chromosome and the *spv* locus on the *Salmonella* Virulence Plasmid are represented by horizontal bars with arrowheads representing individual genes (not to scale). Regulatory inputs by various wHTH TFs mentioned in the text are indicated by arrows (positive inputs) or dotted 'T' bars (negative inputs). HilA autoregulates *hilA* transcription in addition to controlling other genes in SPI1, SPI4 and SPI5. HilD is an AraC-like TF, it regulates *hilA* in association with AraC-like activators HilC and RstA; SsrB is a member of the NarL/FixJ response regulator subfamily (Carroll *et al*., 2009; Walthers *et al*., 2011).

feature is an alpha helix (Brennan and Matthews, 1989; Harrison and Aggarwal, 1990) (Fig. 3).

Indirect readout refers to regulatory mechanisms where the transcription factor reads the shape of the DNA molecule rather than relying on the base sequence as the main source of information (Rohs *et al*., 2010; Slattery *et al*., 2014; Yang *et al*., 2017). Base content and sequence both affect DNA shape but they work in the context of other influences (Rohs *et al*., 2009). These include changes to shape that arise from modifications to the parameters of DNA topology: linking number, twist and writhe (Bauer *et al*., 1980; Mathelier *et al*., 2016). Other factors that have an impact include intrinsic DNA curvature and variations in DNA flexibility and persistence length (Travers, 1997). In addition to DNA shaping influences that are intrinsic to the polymer, there are also modifications to shape that are imposed by DNA binding proteins: nucleoid-associated proteins bend, wrap or bridge DNA and most other proteins that bind to DNA will cause some distortion to its topology (Dillon and Dorman, 2010). Molecular crowding within the cytoplasm may also impose change on DNA shape (Zimmerman, 2006) and very localised modifications can be produced by the methylation of particular bases (Casadesús, 2016). This constellation of influences creates the possibility of a wide variety of modifications to local and global DNA shape without the need for any changes to the base sequence of the DNA.

Indirect readout by transcription factors has received a great deal of attention in studies of eukaryotes (Joshi *et al*., 2007; Gordân *et al*., 2013; Abe *et al*., 2015; Mathelier *et al*., 2016; Yang *et al*., 2017). In bacterial systems there has been historically more emphasis on direct readout, perhaps because regulatory interactions that rely on the reading of DNA base sequence have provided important regulatory paradigms (e.g., the Lac repressor, the lambda CI repressor and the cyclic AMP–Crp complex), and the presence of matches to protein-binding-site consensus sequences in DNA is easier to study *in silico* (Chen *et al*., 1971; Johnson *et al*., 1978; Steitz *et al*., 1982). Arguably, the difficulty in developing bioinformatic tools that can interrogate protein–DNA interactions guided by DNA shape has caused a significant bottleneck in the development of the field (Chiu *et al*., 2014).

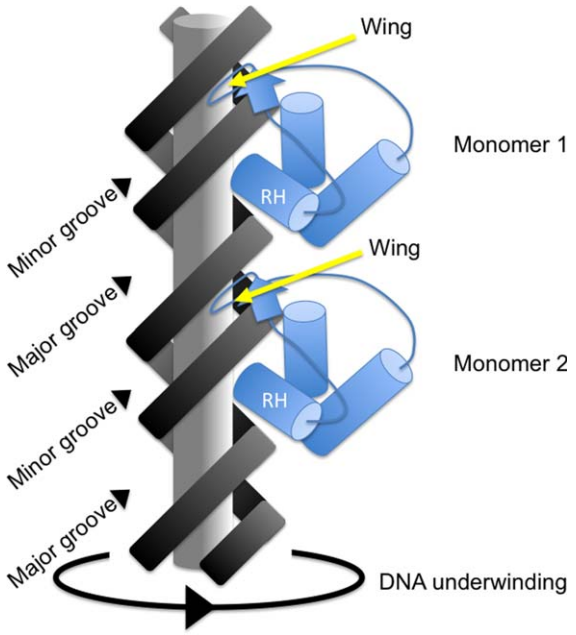

Fig. 3. Interaction of a wHTH TF with DNA. A diagram of a vertical segment of B-DNA is shown with the major and minor grooves labelled. The wHTH motifs of each monomer of a dimeric DNA binding protein are shown in blue. Cylinders represent alpha helices with the recognition helix labelled 'RH' in each case. Yellow arrows point to the wings inserted into the minor groove of the DNA. (Not to scale.) Rotating the DNA in the direction of the curved arrow at the bottom while holding the top of the duplex immobile would mimic the effect of underwinding the DNA as a result of negative supercoiling.

Having a narrow minor groove is an important feature of A + T-rich DNA; the higher the A + T content, the narrower the groove becomes (Rohs *et al*., 2009). The minor groove width narrows over a three-fold range as one moves from G + C-rich to A + T-rich sequences (Rohs *et al*., 2009). This may have implications for the binding to DNA of proteins that engage the minor groove (Fig. 3). Furthermore, changes to the supercoiling of the DNA alter the minor groove width with compensatory adjustments to the width of the major groove (Vologodskii and Cozzarelli, 1994), introducing another variable to modulate DNA–protein interactions. In this context, it is interesting to note that several gene regulatory proteins that antagonise H-NS-mediated silencing in A + T-rich DNA rely on a wHTH motif to interact with both the major and the minor groove of DNA. Among these are ToxR, the master regulator of virulence gene expression in *Vibrio cholerae* (Fig. 1), HilA, a key regulator of virulence gene expression in the SPI1 pathogenicity island of *Salmonella enterica* serovar Typhimurium, and OmpR, a regulator of housekeeping genes together with SPI1- and SPI2-located virulence genes in *S*. Typhimurium (Fig. 2) (Bajaj *et al*., 1995; Martínez-Hackert and Stock, 1997). In the case of OmpR, changes to DNA supercoiling alter the affinity of binding of the OmpR protein to its targets *in vivo* and *in vitro* (Cameron and Dorman, 2012; Quinn *et al*., 2014).

ToxR in *V. cholerae*

V. cholerae is a non-invasive pathogen of humans and has an aquatic reservoir in the environment. Toxigenic strains of *V. cholerae* are the cause of Asiatic cholera, and these express a potent enterotoxin together with secondary virulence factors. Cholera toxin, encoded by the cholera toxin operon *ctxAB*, is produced in the small intestine where it causes a dysregulation of human adenylate cyclase, disturbing intestinal epithelial physiology leading to loss of water and electrolytes accompanied by severe watery diarrhoea that is characteristic of the disease (Robins and Mekalanos, 2014).

The ToxR protein is a cytoplasmic-membrane-associated DNA binding protein that controls, *inter alia*, the transcription of the *toxT* gene, a positive regulator of *ctxAB* and several other virulence genes in *V. cholerae* (Fig. 1) (Miller *et al*., 1987; Higgins and DiRita, 1994; Yu and DiRita, 1999). The amino terminal portion of ToxR contains a wHTH motif within a segment that is related to the DNA binding domain of the OmpR protein (Miller *et al*., 1987). ToxR binds to DNA that has a very high A + T content and many of the sites in the *V. cholerae* genome that are bound by ToxR are also targets for the *V. cholerae* orthologue of H-NS (Kazi *et al*., 2016). This protein, sometimes called VicH, is approximately 50% identical in amino acid sequence to H-NS from *Escherichia coli* (Tendeng *et al*., 2000; Cerdan *et al*., 2003). VicH can substitute for H-NS in an *E. coli hns* mutant, showing that the proteins share functional similarity in gene regulation (Tendeng *et al*., 2000). The primary role of the *V. cholerae* ToxR protein is to de-repress genes that are silenced by H-NS/VicH; in the absence of the nucleoid-associated protein, ToxR becomes redundant for *V. cholerae* virulence gene expression (Nye *et al*., 2000; Kazi *et al*., 2016). The H-NS protein also plays a central role in controlling the wider virulence programme of *V. cholerae* beyond the ToxR regulon (Ayala *et al*., 2015; 2017; Dorman, 2015).

ToxR activation of the *toxT* promoter involves an interaction with a second membrane-associated wHTH protein, TcpP (Krukonis and DiRita, 2003) (Fig. 1). An unusual role has been proposed for the wing in the wHTH motif of TcpP in promoting protein–protein interaction with ToxR: together the proteins control *toxT* transcription through a division of labour with ToxR acting to overcome H-NS-mediated silencing while TcpP recruits RNA polymerase to the promoter without TcpP having to bind to DNA (Krukonis and DiRita, 2003). This represents an interesting example of repurposing of a

component in a TF normally associated with DNA binding through partnership with a second protein that provides a DNA binding function (Haas et al., 2015). ToxR seems only to form this partnership with TcpP at a very small subset of ToxR targets in the genome, one of which is the *toxT* promoter (Kazi *et al*., 2016).

The SPI1-encoded HilA virulence regulator in *S.* Typhimurium

The HilA protein is encoded by the *hilA* gene in the SPI1 pathogenicity island of *S*. Typhimurium where it regulates the transcription of genes required for invasion of the host epithelium in the small intestine (Fig. 2) (Rhen and Dorman, 2005; Thijs *et al*., 2007). The positively autoregulated *hilA* gene has been acquired through lateral gene transfer and so have its most important target genes (Lucchini *et al*., 2006). The protein binds to A + T-rich DNA using a wHTH motif to activate SPI1, SPI4 and SPI5 promoters that are subject to H-NS-mediated transcription silencing (Ahmer *et al*., 1999; Lostroh *et al*., 2000; Main-Hester *et al*., 2008). Expression of the *hilA* gene is under multifactorial control. In addition to positive regulation by its own product, *hilA* is silenced by a complex consisting of the Hha and H-NS nucleoid-associated proteins (Fahlen *et al*., 2001; Queiroz *et al*., 2011; Ali *et al*., 2013). Transcription of the *hilA* gene is also stimulated by the SPI1-encoded AraC/XylS-like regulatory proteins HilC, HilD and RtsA (Eichelberg and Galán, 1999; Lostroh *et al*., 2000; Rhen and Dorman, 2005). These proteins resemble ToxT from *V. cholerae* in that they are members of the AraC-like TF family and use HTH motifs to bind to A + T-rich DNA. A further point of similarity concerns the sensitivity of their target genes to changes in DNA supercoiling in *S*. Typhimurium (Cameron and Dorman, 2012), *V. cholerae* (Parsot and Mekalanos, 1992) and other pathogens, such as *Shigella flexneri* (Tobe *et al*., 1995).

The OmpR wHTH regulatory protein

OmpR is a DNA binding protein of the response regulator family and uses a wHTH motif to bind to its targets in DNA (Martínez-Hackert and Stock, 1997; Rhee *et al*., 2008). The DNA binding activity of OmpR is triggered by phosphorylation of an aspartic acid at position 55 in its 'receiver' domain (Delgado *et al*., 1993). EnvZ is the sensor-kinase that specifically phosphorylates OmpR (Cai and Inouye, 2002). EnvZ first becomes phosphorylated on a histidine at position 243 within its 'transmitter' domain in response to an environmental signal and subsequently transfers the phosphate to OmpR (Foo *et al*., 2015). The OmpR protein can act as a repressor or an activator of transcription; its regulatory mode is dictated by the positions of its binding sites relative to the promoter(s) of the target gene and their relative affinities for OmpR (Pratt *et al*., 1996).

In *E. coli*, osmotic stress is a major signal for the EnvZ-OmpR phosphorelay but acid pH is also known to be an important signal (Stincone *et al*., 2011). In *S*. Typhimurium, acid pH is particularly important for OmpR activation and for the adaptation of the bacterium to low pH experienced during the stationary phase of the growth cycle (Bang *et al*., 2002). OmpR binds to over 200 genomic targets in *S*. Typhimurium, far more than in *E. coli*, and many of the genes that it regulates have been imported by horizontal gene transfer and play a role in infection (Quinn *et al*., 2014). Among these horizontally acquired genes are those that *S*. Typhimurium uses to adapt to the hostile environment within the vacuole of a macrophage. These genes are located in the SPI2 pathogenicity island and are under the control of the SpiR-SsrB two-component regulatory system: SpiR (sometimes called SsrA) is a cytoplasmic membrane located sensor kinase and SsrB is a response regulator (Fig. 2) (Feng *et al*., 2003; Fass and Groisman, 2009). This is an interesting example of the integration of regulatory inputs that are fed through one two-component system (EnvZ-OmpR) encoded by the core genome and a second system (SpiR-SsrB) that is encoded by the horizontally acquired portion of the genome (Rhen and Dorman, 2005). The OmpR protein regulates the transcription of the *spiR* and *ssrB* genes, partly by acting there as a conventional TF and partly by acting as an anti-repressor that overcomes H-NS-mediated transcription silencing (Walthers *et al*., 2011). The OmpR protein also controls the transcription of key regulatory genes in the SPI1 island including *hilC* and *hilD*, allowing OmpR to contribute to the coordination of expression of these major virulence genetic elements (Cameron and Dorman, 2012; Quinn *et al*., 2014).

The OmpR protein is influenced in its binding activity by the topological state of the target DNA. Overall, OmpR will bind more avidly to a DNA sequence in a relaxed template than to the same DNA sequence in a more negatively supercoiled conformation (Cameron and Dorman, 2012; Quinn *et al*., 2014). This allows the DNA to play an allosteric role in determining the binding activity of the protein. Why might this be useful? Negative supercoiling is introduced to DNA by the type II topoisomerase, DNA gyrase (Gellert *et al*., 1976; Higgins *et al*., 1978). It can also be introduced at a local level as a by-product of the processes of transcription and DNA replication (Liu and Wang, 1987). In the case of gyrase, the enzyme hydrolyses ATP to complete each cycle of DNA underwinding. One of the products of this reaction, ADP, is an inhibitor of the supercoiling activity of gyrase. Thus, shifts in the ratio of the intracellular concentration of ATP to ADP in favour of ADP result in a global shift in

DNA topology toward a more relaxed template. This happens when metabolic flux rates in the cell decline, leading to an extension in doubling time and eventually, the onset of stationary phase (Conter *et al*., 1997; Dorman *et al*., 2016; Dorman and Dorman, 2016). Topological shifts in DNA shape throughout the growth cycle present to DNA binding proteins the same sets of binding site sequences in a variety of conformations. This variety may become manifest across different cells in the population, leading to distinct readouts from the same genetic elements. The resulting physiological heterogeneity allows the population to 'hedge its bets' regarding the environmental challenges and opportunities with which it may be faced. Although the importance of physiological heterogeneity to overall population fitness is generally understood, the contribution of variable DNA topology to achieving this heterogeneity is less well appreciated (Dorman *et al*., 2016).

Integrated regulatory circuits and evolution

The OmpR/SsrB example of gene regulation in the *S*. Typhimurium SPI2 pathogenicity island is an excellent illustration of cooperation between gene regulators encoded by the core and 'accessory' parts of the genome (Fig. 2). What is it about OmpR that makes it such a useful protein for controlling the expression of so many genes in both parts of the genome? The answer seems to include its tolerance of base sequence variety in the DNA targets to which it binds. These are characterised by a high A + T content and we have already seen that this property is a feature of the horizontally acquired components of genomes in Gram-negative bacteria, especially enteric pathogens. Thus, OmpR, with its wHTH DNA binding motif, is literally a good fit for laterally acquired genes with this high A + T profile. The same DNA is also an attractive target for the transcription-silencing H-NS nucleoid-associated protein (Lucchini *et al*., 2006; Navarre, 2016). This protein shares with OmpR sensitivity to DNA features other than base sequence: although an optimal sequence for H-NS binding has been described, its chief characteristics are a high A + T content and intrinsic DNA flexibility (Lang *et al*., 2007). Conflicts between OmpR and H-NS at the same A + T-rich DNA targets provide a basis for a simple genetic switch, allowing those environmental signals that flow to OmpR (osmotic and acid stress) to influence transcription by overcoming H-NS-mediated repression. By relying on features of DNA other than just the presence of very close matches to a consensus base sequence for binding, wHTH proteins can participate in, and drive, the rapid evolution of control circuits. Because of their relatively relaxed base sequence requirements for target recognition, indirect readout mechanisms have the potential to engage regulatory proteins with many more targets than might be the case using direct readout. Changes to DNA shape brought about through alterations in the parameters of DNA supercoiling (linking number, twist and writhe) in response to fluctuations in metabolic flux create a very dynamic profile of DNA targets. A target that is proficient for protein binding at a given superhelical density (σ) may be much less proficient at another value of σ as is the case for the OmpR protein in *S*. Typhimurium (Cameron and Dorman, 2012; Quinn *et al*., 2014).

Other wHTH proteins and *V. cholerae* and *S.* Typhimurium pathogenesis

The SlyA protein of *S*. Typhimurium and *E. coli* uses a wHTH motif to bind to DNA and is frequently found to overcome H-NS-mediated transcription silencing (Westermark *et al*., 2000; Corbett *et al*., 2007; Lithgow *et al*., 2007; Perez *et al*., 2008; Weatherspoon-Griffin and Wing, 2016). Its counterpart in *Yersinia*, RovA, has a similar role in gene regulation and both belong to the ancient family of MarR-like wHTH proteins (Heroven et al., 2004; Ellison and Miller, 2006; Lawrenz and Miller, 2007; Wang *et al*., 2014).

Many of the genes that are regulated by SlyA or RovA are A + T-rich, horizontally-acquired virulence genes that are silenced by H-NS (Navarre *et al*., 2005; Colgan *et al*., 2016). The antiquity of these MarR-like proteins and the simplistic nature of the genetic switches involving H-NS antagonism suggest that they are part of a very early form of transcription control that does not have to rely on physical contact between the TF and RNA polymerase. Instead, the positively acting TF achieves its goal by removal of H-NS or by repositioning H-NS, permitting transcription to be initiated. In other words, these proteins operate as anti-repressors rather than as conventional TFs (Perez *et al*., 2008).

The use of indirect readout by the MarR-like proteins liberates them from the need to observe strictly the base sequence of their DNA targets. Instead, they are guided by DNA shape, something that can be set and reset by environmental conditions. The result is, potentially, an exquisitely versatile gene regulatory mechanism that can be adapted quickly to encompass new genes that have been acquired by horizontal transfer. With the basic on-and-off elements of the switch provided by the wHTH protein and H-NS, respectively, additional specificity can be imposed by signal-controlled TFs operating through a DNA-sequence-dependent direct readout mechanism. The pH- and osmotic-stress-modulated OmpR protein, with its wHTH DNA binding motif, seems to lie at the nexus of the DNA-sequence-dependent and DNA-shape-dependent worlds, combining features of both.

Interestingly, several SlyA target genes are also controlled by OmpR, showing that the potential for the integration of the two regulators has been realised in several instances (e.g., the virulence genes of *Salmonella)* (Feng *et al*., 2004; Linehan *et al*., 2005).

Among the genes that are depressed by ToxR in *V. cholerae* is *leuO*, encoding the LysR-like wHTH DNA binding protein LeuO (Bina *et al*., 2013; 2016). LysR-type transcription factors (LTTRs) have very weak consensus DNA binding site sequences: T-N_{11}-A, where N is any base. They also represent the largest family of TFs in bacteria (Schell, 1993; Alanazi *et al*., 2013). LeuO is an H-NS antagonist that regulates positively genes that contribute to bile and acid resistance in *V. cholerae* (Ante *et al*., 2015a,b). In *Salmonella*, the *leuO* gene is best expressed when the bacterium is grown in a medium that mimics the conditions found in a macrophage vacuole (Dillon *et al*., 2012). Here, LeuO controls a large regulon of genes, including virulence genes, many of which are silenced by H-NS (Dillon *et al*., 2012; Cordero-Alba and Ramos-Morales, 2014). The *leuO* gene is regulated positively by its own product and, at least in *E. coli*, by another wHTH protein called LrhA (Breddermann and Schnetz, 2017). The *leuO* gene is located within an unusual regulatory cascade that involves transcription activation by locally generated changes in DNA negative supercoiling: a promoter relay (Fang and Wu, 1998; Dorman and Dorman, 2016). The LeuO protein relieves H-NS-mediated repression and cooperates with DNA topological dynamism in this relay to regulate transcription of genes involved in branched chain amino acid synthesis (Chen *et al*., 2003). This intriguing blend of *cis*- and *trans*-acting regulators may hint at gene control mechanisms that predate the contributions of direct-readout-dependent TFs. The SpvR TF is a LysR-like protein that is encoded by the principal virulence plasmid of non-typhoidal strains of *S. enterica*. It regulates the *spv* operon whose products are secreted by the SPI2 secretion apparatus and alter host cell cytoskeletons (Guiney and Fierer, 2011). The *spv* operon displays sensitivity of changes in DNA supercoiling and is silenced by H-NS (O'Byrne and Dorman, 1994; Sheehan and Dorman, 1998). Its location on a large and mobilisable plasmid illustrates the importance of horizontal gene transfer, H-NS-mediated silencing and sensitivity to DNA topology to the assembly and control of virulence gene clusters in pathogenic organisms.

Concluding remarks

Variable DNA topology is, perhaps, an underappreciated contributor to the collective control of transcription in bacteria of all kinds, including pathogens (Dorman *et al*., 2016). Bacteria strive to control DNA supercoiling homeostatically, but the supercoiling set points change from one stage of growth to another (Conter *et al*., 1997) and as a result of environmental stresses such as acid stress (Bang *et al*., 2002), osmotic stress (Higgins *et al*., 1978), thermal stress (Goldstein and Drlica, 1984), etc. The influence of DNA supercoiling on transcription (and *vice versa*) is well established, but the potential for the changing transcriptional landscape to modulate the *binding* of proteins is sometimes overlooked in studies of gene control. Here we have emphasised the connection between DNA supercoiling and the binding to DNA of wHTH TFs. The principles that have been outlined concerning the impact of altered DNA twist on major and minor groove width, especially in A + T-rich DNA illustrate the potential of DNA supercoiling to act allosterically on TF function (Fig. 3). The link between changes in DNA twist and adjustments to the minor and major groove width was established some time ago (Vologodskii and Cozzarelli, 1994). We suggest that these adjustments play a role in modulating the binding of proteins to the DNA. In the case of wHTH-dependent DNA binding, the need to engage two DNA grooves of variable width provides a mechanism to enhance or to limit TF binding to target sequences that meet the requirements of the protein on base sequence criteria alone. Put crudely, this is an example of indirect readout trumping direct readout. It is an opportunity for DNA supercoiling, which is set and reset in response to the global physiological state of the cell, to influence a process that is otherwise driven by protein recognition of DNA sequence, an invariant feature of the DNA target. Is this mechanism unique to wHTH-dependent proteins? No. Proteins relying on HTH motifs are also sensitive to variation in DNA groove width and many virulence genes are controlled by members of the large HTH-dependent TF super-family. The nucleoid-associated protein Fis (Factor for Inversion Stimulation) relies on an HTH motif for DNA binding and it binds as a dimer. Docking the Fis dimer successfully to its target involves an induced fit mechanism in which minor groove compression accompanied by bending of the target DNA accommodates the side-by-side protein monomers (Stella *et al*., 2010). Fis binding is influenced strongly by indirect readout and minor groove conformation (Hancock *et al*., 2016). Changing the supercoiling of the DNA also exerts an effect: Fis prefers DNA that is negatively supercoiled, a requirement that is easily met during the early stages of logarithmic growth when Fis is abundant. As the cell runs out of energy and DNA relaxes, Fis binding declines (and so does Fis production) (Schneider *et al*., 1997; Cameron and Dorman, 2012). The sensitivity of DNA gyrase to the ATP/ADP ratio of the cell links DNA topology intimately to bacterial

metabolism, which is in turn linked to the composition of the environment of the microbe (Hsieh *et al*., 1991a,b; Van Workum *et al*., 1996). In this way, the environment can modulate in important ways the interactions of TFs of all kinds with their gene targets, adding a regulation coordination function to the genetic-information-carrying function of DNA.

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