REGULAR REVIEW

Bacterial virulence: an environmental response

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On meeting their hosts, most bacteria manage to establish a harmless colonising relationship, but occasionally tissues are injured leading to the local or widespread damage that we recognise as disease. In the search for new ways to fight such disease, we need to understand the steps in which microbes adapt to changes in their surroundings, the better to be able to target novel vaccines and other antibacterial treatments on the so called virulence factors involved. The issue is complicated by the fact that organisms undergo extensive phenotypic variation during infection. Some bacterial products are *only* made in such circumstances and not at all in laboratory culture, where conditions are very different to those found in the host. This review summarises some recent advances in our understanding at the molecular level of how bacteria respond to the changes they encounter in the course of infection.

Many infections start at epithelial surfaces, and a whole range of host defences are correspondingly directed at preventing mucosal colonisation. Mucus blocks access to cell surfaces while the coordinated action of cilia wafts mucus and bacteria away. In their turn, bacteria derive selective advantage from attributes that allow them to avoid this physical removal, of which the best characterised are the protein adhesins that anchor organisms to host cell surfaces. In order to minimise adverse steric or electrostatic interactions between closely apposed cells, these adhesins are often located at the tips of hair-like projections, the pili. Their adhesive properties notwithstanding, pili carry the potential *disadvantage* for the organism of providing a prominent structure for antibody attachment and for interaction with phagocytes. This can be resolved by the processes of phase (on-off) and antigenic (A↔B) variation.

Spontaneous phase and antigenic variation
Escherichia coli produces a pilus that allows it to stick to the mannose molecules found on the surface of many epithelial cells. Its expression is regulated by a flip-flopping on-off genetic switch. No gene can be transcribed until the transcriptional apparatus—RNA polymerase and any regulatory factors—correctly engages the promoter region upstream of the coding region on the chromosome. The promoter for this pilus gene is located on a spontaneously invertible segment of DNA, and pili can only be produced when it is oriented the right way round (fig 1A).¹ Under colonising conditions, the flipping of this DNA segment occurs rapidly enough to ensure that the stock of piliated organisms can be constantly replenished from non-piliated cells, and the merits of both phenotypes exploited. *Neisseria gonorrhoeae* shows an extra sophistication. Pilus mediated attachment to host cells is important to protect organisms in the urinary tract from being washed away. In chronic and recurrent infections, gonococci also need to avoid antibody mediated host defences on the uroepithelium. Organisms not only show phase variation between piliated and non-piliated forms, but can also produce a whole range of different antigenic types of pili through shuffling a set of genes coding for the pilus subunit (pilin). All the genetic machinery needed for production of one type of pilus is found in one chromosomal region, the expression locus, but parts of more than a dozen variant pilin genes are found in non-expressing loci elsewhere. Two mechanisms have been identified by which pilus type can vary.² In a spontaneous process termed gene conversion, any of these partial pilin genes can become spliced into the expression locus to lead to variation in pilus type (fig 1B).^{3 4} Alterna-

Figure 1 Pilus phase and antigenic variation. (A) The spontaneously invertible promoter of the *E coli* pilin gene only directs transcription and pilin production when in the right orientation. (B) In *N gonorrhoeae* partial pilin genes lacking essential DNA are copied by gene conversion into the expression locus where they are transcribed, leading to production of different pilins.

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tively, the lysis of gonococci in the course of infection can provide DNA to be taken up and incorporated into the chromosome of neighbouring organisms, splicing a 'silent' pilin gene into the expression locus in the process.⁵

Sensor-regulator mechanisms to control gene expression

The pilus gene rearrangements help organisms to persist in a changing environment through natural selection. However, the production of some bacterial factors may be needed only at one point in the sequence of steps in which an organism adapts to new conditions. A more favourable arrangement in these circumstances is for gene expression to be responsive to relevant environmental stimuli such as changes in temperature, pH, osmolarity, or nutrient supply. For this, bacteria need mechanisms for sensing the environment, processing information and responding accordingly, most efficiently by an alteration in the pattern of gene transcription. Numerous examples can be identified where the production of bacterial factors, including those implicated in virulent behaviour, is indeed environmentally responsive. The molecular basis for signal transduction—by which changes in the surroundings lead to changes in gene expression—has recently been elucidated, and in many cases involves two interacting bacterial proteins, one an environmental sensor and the other a regulator of gene transcription (fig 2).⁶⁻⁸ The sensor protein spans the bacterial cytoplasmic membrane, its outward facing part responsive to a specific environmental signal, presumably through its affinity for some small ligand molecule. On binding the ligand, the protein is thought to undergo a substantial conformational change

which allows it to interact with the second, cytoplasmic, component. The latter protein then becomes an active gene regulator, able to bind to the promoters of target genes and alter their level of transcription. There are many examples of virulence genes being controlled in this way. In *Salmonella typhimurium*, such a two component system regulates production of an outer membrane protein thought to confer resistance to toxic host factors in phagolysosomes.⁹ In *Bordetella pertussis* a single two component system activated by temperature changes and other environmental signals governs the expression of more than half a dozen virulence genes—including those coding for pertussis toxin, fimbrial haemagglutinin, haemolysin, and adenyl cyclase toxin.¹⁰

Chromosomal supercoiling and global regulation of gene expression

Through the action of two component sensor/response-regulator systems, environmental changes lead to altered gene expression through the activation of regulators that go on to bind to specific promoters. The importance of another signal transduction process has recently been emphasised, in which environmental stresses lead, through changes in DNA topology, to alterations in the accessibility of promoters to the transcriptional apparatus. This provides a mechanism for organisms to respond to environmental changes with alterations in gene expression all round the chromosome.¹¹⁻¹³ The double helix of the bacterial chromosome is packed as a coiled coil—supercoiled—within the cell. The degree of supercoiling fluctuates constantly during bacterial growth through the shifting balance between the actions of enzymes continually twisting and untwisting the double

Figure 2 Environmentally responsive gene regulation. The roles of response regulator proteins and histone-like proteins on the expression of a virulence gene. Sensor proteins are shown spanning the bacterial membrane, transmitting information to inactive cytosolic proteins. Once activated, the latter bind to appropriate sites on the DNA, leading to conformational changes that allow the RNA polymerase to engage the gene promoter and start transcription.

helix, and through variation in the production of DNA binding proteins analogous to the histones that are involved in the shaping of the eukaryotic chromosome. Studies on pathogens subjected to fluctuations in temperature, osmolarity and other stresses that occur on host colonisation have shown that DNA supercoiling levels are responsive to these changes.^{12 14–16} Transduction of environmental signals into a supercoiling response can be envisaged through the action of the histone-like proteins, some binding to specific target sequences and others associating with the chromosome in a more non-specific way. One environmentally regulated histone-like protein, OsmZ, seems to bind DNA in a non-specific manner, altering its topology^{12 17} and with this the expression of many genes. An important control function relating to virulent behaviour is suggested by the observations that mutations in the OsmZ gene accelerate the flip-flop regulation of *E coli* pili, increase production of *E coli* capsular polysaccharide, and derepress the invasion system of *Shigella flexneri*.^{12 13 18} Another, known as IHF, binds to specific target sequences scattered through the *E coli* chromosome with profound effects on local DNA structure and gene expression, again including pilus gene regulation.¹⁹

Conclusion

As host-bacterial interactions are dissected at the molecular level, the hierarchical nature of the bacterial side of the equation is clear to see. Environmental responsiveness dominates the scene. Fluctuations in temperature, osmolarity, pH, etc are detected by the sensor molecules of two component systems. Conformational changes in these proteins allow information about the environment to be transmitted to gene regulators, which then attempt to activate their target genes. The effectiveness of the regulators will vary with the accessibility of gene promoters, modulated by the level of DNA supercoiling. The topology of the DNA will be influenced both generally and in a site specific manner by histone-like proteins, whose number and activity will also be environmentally controlled. The moment to moment coordination of gene expression—one aspect of which is virulent behaviour—represents the interplay of these overlapping factors. The molecular approach to the development of bacterial vac-

cines and other treatment strategies must take this into account. The study of organisms under laboratory conditions cannot be relied upon for the identification of critical determinants of virulent behaviour.

I am grateful to many colleagues, and in particular to Charles Dorman and Richard Moxon, for stimulating my thoughts on bacterial virulence.

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