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Regulation of virulence gene expression

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The bacterial pathogen that finds itself, either by accident or design, within the human or animal host, senses and adapts to the prevailing conditions by modulating its gene expression on a global scale.[1-3] A subset of these genes will be key players in the ability of the bacterium to cause disease. The products of such genes that facilitate the successful colonisation and survival of the bacterium in or cause damage to the host are considered as virulence or pathogenicity determinants. For an individual bacterial pathogen, the total number of genes that can be categorised as virulence genes (i.e. the 'virulome') ranges from the low hundreds to more than one thousand depending upon the system under investigation and the approach used to identify such genes.[4-7] However, while the expression profile and dynamics of any particular virulome may suggest a dependence on, or dominance by, a single regulatory player (as with some quorum sensing regulons) this view is far too simplistic. In many cases, the expression of the virulome depends on the ability of the pathogen to simultaneously sense several of the multiple environmental cues (e.g. temperature, availability of key metal ions, concentration of anions such as bicarbonate or phosphate, pH, oxygen tension, osmolarity etc.) encountered upon interaction with and within the host and to initiate the appropriate and coordinate the adaptive response in gene expression. This ability underpins the pathogenic success of well-adapted gastrointestinal pathogens such as *Escherichia coli* or *Salmonella* that differentially coordinate the expression of sets of genes as they pass from one host environment to another in their passage through the gut, including transition through the gastric barrier and survival within macrophages or intestinal epithelial cells.

Moreover, whereas in some cases the trancriptional activity of a virulence gene or operon may respond to a single environmental signal (this is very often the case with genes encoding iron acquisition systems that characteristically respond only to iron availability [see forthcoming article by Maddox and Andrews]), in many cases they respond (either directly or indirectly) to more than one environmental input such as occurs in the regulation of the *ctxAB* genes of *V. cholerae*, encoding cholera toxin, which involves a highly complex cascade of two- and three-component systems and quorum sensing that coalesces on the gene encoding ToxT, the direct regulator of *ctxAB*.[8] Other well-studied but by no means fully elucidated systems that serve as the hub for multiple regulatory inputs include the *agr* locus which acts as the regulatory nexus for controlling virulence factor gene expression in *Staphylococcus aureus* and the *Bordetella pertussis* virulence sensory protein BvgS, a hybrid sensor kinase that contains at least 3 putative extracytoplasmic perception domains and multiple cytoplasmic phosphotransfer domains that allow for additional regulatory inputs.[9-10] This ability of virulence gene regulatory loci to integrate multiple environmental cues into a coherent and coordinated response reveals a high degree of sophistication. It also makes constructing an overall picture of the regulatory circuits governing the virulome of a bacterial pathogen a daunting task.

Rather than considering individual virulence regulatory systems as exemplars or paradigms of virulence, such as those mentioned above, for which many high quality reviews are already available, we have chosen to update the interested reader on the latest advances in our understanding of some of the mechanisms employed by the bacterial pathogen to respond to particular changes in its environment. The various environmental signals to which pathogenic bacteria are attuned and the mechanisms by which they sense and respond to such signals and thereby mobilise their virulence functions are far too many to comprehensively review here. Rather, we have selected areas in which recent advances have either uncovered a new mechanism for regulating virulence gene expression in response to a particular environmental signal or where an established mechanism has recently been revealed to have a previously unrecognised relevance, or an increased relevance for control of virulence gene expression.

The employment of two-component systems and ECF sigma factor-dependent systems as signal transduction mechanisms for regulating virulence gene expression is well established.[11-13] In contrast, eukaryotic-like serine-threonine kinase/phosphatase-dependent (eSTK/eSTP) systems are now beginning to be widely recognised as important components of bacterial signal transduction arsenal. However, the full extent to which eSTKs/eSTP systems modulate virulence gene expression has yet to be elucidated. The article by Wright and Ulijasz in this Special Focus issue provides a comprehensive mechanistic survey of these systems in *S. aureus*, pathogenic Streptococci and *Mycobacterium tuberculosis* which, serves as a useful primer for anyone interested in this important area of bacterial signal transduction.[14]

Global changes in virulence gene expression can also be implemented in response to fluctuations in a single environmental parameter and can involve a

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single regulatory mechanism, the most notable example of which occurs with some quorum sensing regulon.[15] However, it is becoming increasingly apparent that large scale alterations in the transcriptional profile of the bacterium that occur in response to other environmental signals can also include groups of virulence genes. In this Special Focus issue Green and colleagues discuss how bacteria sense and adapt to low oxygen environments, the presence of reactive oxygen species (ROS) and the presence of nitric oxide, each of which may be encountered by the pathogen upon infection of the host and can lead to global changes in gene expression.[16-20] Adaptive responses that result in increased resistance to the action of ROS and nitric oxide are key to the survival of several pathogens in the host.[21-22] Moreover, hypoxia or anoxia is used as a signal not only to trigger changes in expression of genes that allow adaptation to the lack of oxygen but also upregulate the activity of genes that result in obvious damaging consequences for the cell: they encode toxins.[23]

Perhaps it would not be surprising to learn that bacterial pathogens use perturbations of their membrane(s) induced by certain offensive external stimuli not only as a signal to elicit appropriate responses to maintain the integrity of the cell envelope ('envelope stress response', ESR) but also to mobilise components of their pathogenic armoury, as such distress may be interpreted as a signal that they have encountered hostile elements of the host immune system. Moreover, perturbations of the bacterial cell envelope may be self-inflicted and can occur through the assembly and/or activity of protein secretion systems.[24] In this situation, the pertinent system for sensing membrane disruption is playing an auxiliary offensive role, although one that is also essential to survival of the pathogen while engaged in subverting the host. Accordingly, in the article by Darwin and colleagues we see how perturbations or damage to the cell envelope of bacterial pathogens are sensed by different mechanisms, which in some cases modulate the expression of more overt virulence functions.[25]

An often-overlooked molecule when considering possible modes of sensing and adaptation to the host niche is phosphate. In the article by Dozois and colleagues in this Special Focus issue,[26] the pathways for uptake of this key nutrient and its regulation by the bacterial cell are discussed before the authors consider examples where virulence genes have effectively 'plugged in' to the ancestral phosphate homeostatic control system in Gram-negative bacteria. The net result of this is that the extracellular phosphate concentration can exert quite profound effects on virulence gene expression.

In contrast, temperature and iron availability have long been recognised as triggers for modulating gene expression in bacterial pathogens. Induction of virulence gene expression as a result of a shift to 37°C or a depletion of extracellular iron is a common theme in bacterial pathogenicity. Many readers will be familiar with the temperature-dependent Bvg system of the *Bordetellae* and the *yop* genes of *Yersinia* spp that are activated upon entry into mammalian or human hosts, or the role of the Fur repressor in regulating iron acquisition systems.[27-29] While some mechanisms for signal detection and response are highly conserved in bacteria (the use of Fur or Zur to orchestrate the responses to iron and zinc availability, respectively [see forthcoming article by Maddox and Andrews]), in some cases bacteria have evolved multiple distinct mechanisms to regulate genes in response to changes in a single environmental parameter. For example, the mechanisms by which bacteria regulate gene expression in response to changes in temperature can take many forms. This is illustrated in the article by Tang and colleagues, which highlights the recent advances in thermoregulation of virulence gene expression.[30] Here, we see that bacteria have taken advantage of the base pairing property of RNA to evolve mechanisms for regulating virulence gene expression in response to temperature at the post-transcriptional level.

Although only scratching the surface, this series of review articles in this Special Focus issue highlights the sheer diversity of mechanisms employed by bacteria to regulate expression of their virulence genes in response to the environmental conditions that prevail in the host niche. While the evolution and spread of antibiotic resistance in bacterial pathogens continues to pose a serious threat, efforts to unravel the fundamental regulatory mechanisms that are responsible for expression of virulence factors that compromise the host or enable the bacterial pathogen to evade host defense strategies will continue to be of paramount importance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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