Two-component signal transduction systems: the adapt and survive response

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In order to survive and proliferate, bacteria have evolved to sense and adapt to the changes in their surroundings. One of the major ways in which this adaptive regulatory response is achieved is through a two-component signal transduction system.

In essence, these regulatory systems involve the detection of an external stimulus, transmission of that signal across the cell membrane, propagation of the signal through a phosphorelay cascade and, ultimately, the regulation of target gene expression. Two-component signal transduction systems have been shown to be involved in the regulation of a range of cellular processes, including virulence, osmolarity, quorum sensing, chemotaxis and general house-keeping, to name but a few.

Over the past two decades, the number of two-component systems identified has increased exponentially. In a recent study, approximately 4000 systems were identified in 145 sequenced bacterial genomes [1]. The identification of so many regulatory networks has been accompanied by the revelation of novel information regarding the way bacteria adjust to their environments.

As the name suggests, most two-component regulatory systems consist of two elements – a sensor histidine kinase and its cognate response regulator.

The cascade starts with the sensor histidine kinase. Proteins in this family are generally membrane bound and usually contain at least two transmembrane domains, with the number of membrane-spanning regions varying from kinase to kinase (Figure 1A). It is postulated that these N-terminal transmembrane domains are responsible for the detection and propagation of the external signal. As a result, the N-terminal domains of sensor histidine kinases are highly variable, enabling these proteins to detect a diverse range of different environmental or growth phase signals. Upon detection of a specific stimulus by this input domain, the sensor histidine kinase undergoes ATP-dependent autophosphorylation at a conserved histidine residue, which is located in the conserved cytoplasmic C-terminal transmitter domain.

In addition to the essential histidine residue, this region also contains motifs that are involved in ATP binding and protein dimerisation [2] (Figure 1A). Dimerisation is an essential part of the phosphorylation process, with the conserved histidine residue in one monomer of the homodimer being phosphorylated by a kinase domain located on the other monomer.

Response regulators are soluble proteins that generally have a conserved N-terminal domain and a variable C-terminal domain that usually has a specific DNA binding region. The phosphorylated sensor histidine kinase donates its phosphoryl group to an invariant aspartate residue, located in the conserved N-terminal receiver domain of its cognate response regulator (Figure 1B), in a reaction that is catalysed by the latter protein. Specific interactions between the amino acid residues surrounding the phosphorylation sites of both proteins ensure that phosphotransfer occurs between cognate pairs [3].

This phosphotransfer reaction is the hallmark of a two-component signal transduction system. Cross talk, or phosphorylation of a response regulator by a non-cognate sensor histidine kinase, has been demonstrated *in vitro*, but this phenomenon rarely occurs *in vivo* [4].

Upon phosphorylation, the response regulator is believed to alter its

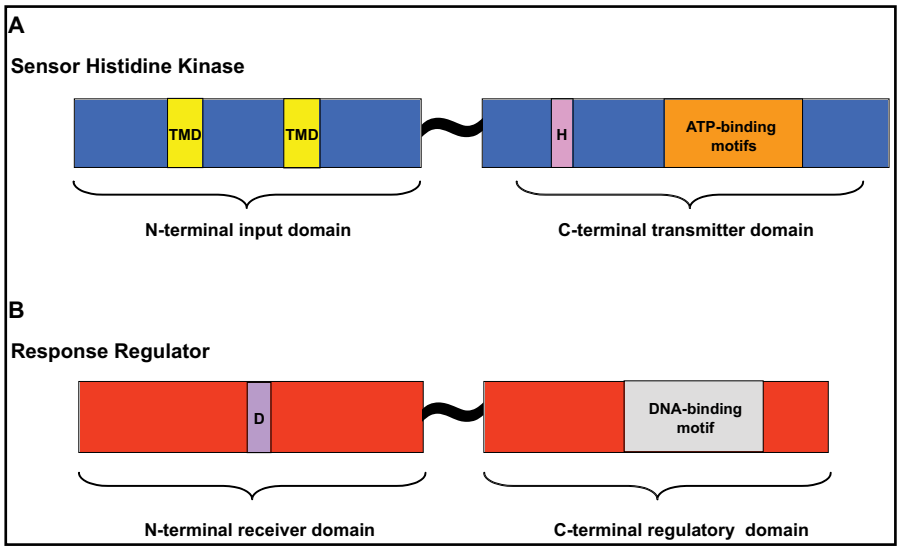

Figure 1. Schematic domain model of sensor histidine kinases (A) and response regulators (B).

conformation to an active form [4], with the effects of these alterations differing from system to system. These effects include increasing the binding affinity of the response regulator for its target DNA binding site [5], inducing multimerisation of the response regulator [6] and relieving structural autoinhibition [7]. Note that these effects are not mutually exclusive, since most response regulators require one or more of the above to achieve regulation.

In contrast, inactivation or 'switching off' the response regulator involves the dephosphorylation of the protein. In some cases, this process is carried out by the phosphatase function of the cognate sensor kinase when the signal is no longer detected. In other systems, dephosphorylation is achieved via an intermediary protein or occurs spontaneously. Whilst phosphorylation has been found to be essential for the function of most response regulators, there are exceptions to the rule. Some response regulators have been shown to function in a phosphorylation-independent manner [8], while others are functional in their phosphorylated or non-phosphorylated forms depending on the target gene [9].

Transcriptional regulation of specific target genes is achieved by the recognition and binding of the activated response regulator to specific DNA binding sites. The majority of these recognition sites are located in the promoter regions of the target genes and are generally in the form of direct repeats [10] or indirect repeats [11]. Binding to these sites is accomplished through the various DNA binding motifs located in the C-terminal domain of the response regulator [4] (Figure 1B). Regulation of gene expression is generally achieved through direct protein-protein interactions with RNA polymerase subunits [12]. Note that not all response regulators act at the transcriptional level – some proteins such as CheY act directly on specific protein targets to exert their phenotypic effects.

To date, two-component signal transduction systems have mainly been identified in prokaryotes, but some have been found in lower eukaryotes such as yeast and fungi, and also in plants like *Arabidopsis thaliana*. In contrast, no such systems have been identified in mammals and it is this feature that has made two-component systems, in particular the sensor histidine kinases, potential targets for antimicrobial therapy [13].

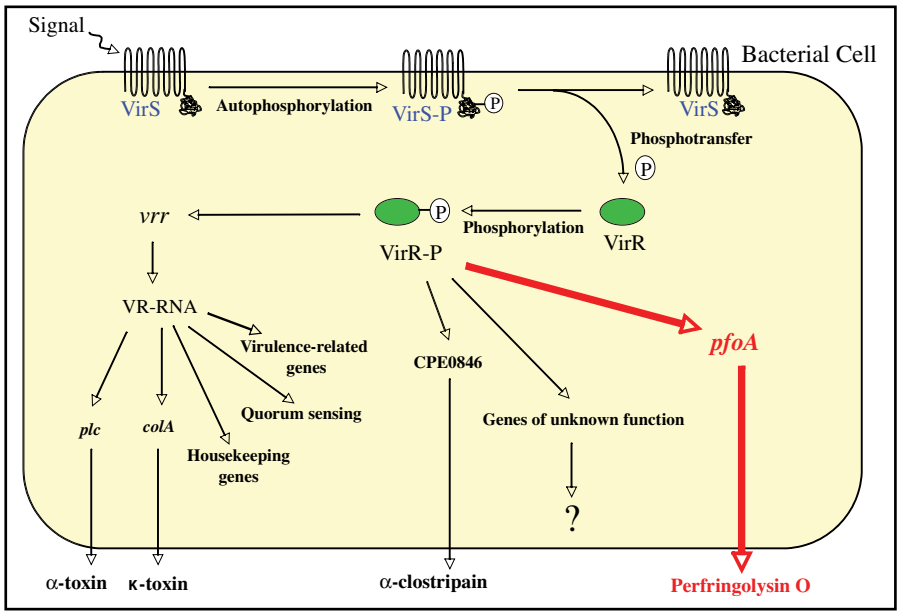

Figure 2. Model of the VirS/VirR two-component signal transduction system from *Clostridium perfringens*.

In our laboratory we have been studying the VirS/VirR two-component signal transduction system from *Clostridium perfringens*. This Gram-positive anaerobe is the causative agent of gas gangrene (clostridial myonecrosis) and food poisoning in humans [14] and of several enterotoxaemic diseases of domestic animals [15]. It produces an array of extracellular toxins and enzymes, of which α-toxin (phospholipase C) and perfringolysin O (θ-toxin) have been demonstrated to act synergistically in gas gangrene [16]. The production of these toxins, as well as many other gene products, is regulated by the VirS/VirR system, where VirS is the sensor histidine kinase and VirR its cognate response regulator.

Early work on this system showed that mutation of the genes encoding either component resulted in reduced levels of α-toxin and several other toxins, whereas the production of perfringolysin O was totally eliminated [14]. Since then, we have shown that the VirS/VirR regulatory network directly activates perfringolysin O production by VirR binding independently to two imperfect direct repeats, designated as VirR boxes, located in the promoter region of the *pfoA* gene, which encodes perfringolysin O [17]. Further work demonstrated that the correct sequence and spatial arrangement of the VirR boxes are critical for optimal transcriptional activation [18].

Additional VirR boxes have also been identified in the published *C. perfringens* genome [17] and we have shown that VirR is also able to bind to these sites and activate transcription [18]. An extensive site-directed mutagenesis study has shown that two novel domains in the C-terminal region of VirR are responsible for its stable binding to DNA [17].

In addition to toxin production, the VirS/VirR system is involved in the regulation of housekeeping genes, a regulatory RNA molecule, virulence-related genes and genes involved in quorum sensing [17].

The current model for the VirS/VirR regulatory cascade (Figure 2) involves the detection of an as yet unidentified signal by VirS, VirS autophosphorylation at a conserved histidine residue and phosphotransfer from VirS-P to VirR, to produce VirR-P. This activated response regulator then directly initiates the production of perfringolysin O and a protease called α-clostripain. VirR-P also indirectly regulates the expression of housekeeping genes, virulence-related genes, genes involved in quorum sensing and the genes encoding α-toxin and κ-toxin (collagenase), through its direct activation of the *vrr* gene, which encodes the regulatory RNA molecule VR-RNA.

Although VirS/VirR is the best studied two-component signal transduction system in the clostridia, there are still many aspects that are yet to be deciphered; in particular the signal that leads to VirS activation and the mechanism by which VirR interacts with RNA polymerase to activate transcription.

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