Complexity in '2-component' signal transduction systems

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The '2-component' regulatory systems of bacteria are the predominant signal transduction mechanisms that bacteria utilise to modulate behaviours and metabolism in response to environmental changes. These systems classically involve two proteins – a membrane bound sensor histidine kinase and a soluble response regulator.

It is now clear, however, that there exists an enormous diversity in signal transduction proteins and pathways that are based upon the 2-component paradigm. This paper presents an overview of the complexity that exists in bacterial 2-component signal transduction systems.

Prototypical 2-component regulatory systems

The prototypical (Class I) sensory histidine kinase is comprised of a ligand binding domain coupled to an autokinase domain (Figure 1A). Ligand binding causes activation of the catalytic autokinase domain which hydrolyses ATP and autophosphorylates a conserved histidine residue located in a sub-domain of the histidine kinase. Transfer of this high energy phosphoryl group to a conserved aspartate residue in the receiver (REC) domain of the cognate response regulator protein causes a conformational change that results in activation (or relief of inhibition) of the output domain of the response regulator (Figure 1A) [1-3].

The majority of bacterial response regulator proteins are transcriptional activators which combine the REC domain with a DNA binding domain [4]. The biochemistry of signal transduction and response regulation will not be covered in this review; see references 1-3 for reviews that cover these aspects of 2-component signal transduction.

Multistep phosphorelays

In addition to the classic architecture of the 2-component regulatory systems, more complex versions involving multiple phosphotransfer events have now been described. In each of these systems, the phosphorylation cascade occurs via a His-Asp-His-Asp phosphorelay that is initiated with signal input and subsequent autophosphorylation of the conserved histidine in the autokinase domain of a histidine kinase. The phosphate group is then transferred to the conserved aspartate in an intermediary REC domain, then to a conserved histidine in an intermediary phosphotransfer domain, and finally to the conserved aspartate of the REC domain of the response regulator.

At least four distinct schemes of multistep His-Asp-His-Asp phosphorelays have been described (Figure 2). The first of these is exemplified by the KinA-SpoOF-SpoOB-SpoOA sporulation phosphorelay of *Bacillus subtilis* [5] in which each of the phosphorylated residues in the relay are located in separate proteins, including two intermediate stand-alone modules SpoOF and SpoOB (Figure 2A). In each of the three remaining systems (exemplified by the BvgS-BvgA system that controls transcription of virulence factors in *Bortedella pertussis* [6]; the Sln1p-Ypd1p-Ssk1 system that controls osmoregulation in the yeast *Saccharomyces cerevisiae* [7]; and the RcsC-RcsD-RcsB signalling pathway that controls capsular polysaccharide synthesis and cell division in *Escherichia coli* [5]) the intermediary histidine containing phosphotransfer modules all belong to the same domain class referred to as the HPT domain. This is distinct from that of SpoOB which has structural similarity to the phosphotransfer domain of Class I histidine kinases [8].

These final three systems also share the feature that the histidine kinase is a hybrid protein that combines (at least) the histidine kinase with a REC domain in a single polypeptide chain. The Sln1p-Ypd1p-Ssk1 phosphorelay of *Saccharomyces cerevisiae* is included in this review as it portrays a multistep phosphorelay scheme that is also likely to be found in prokaryotes [8]. Indeed, a recent census of the distribution of hybrid histidine kinases and HPT domains in 156 complete prokaryotic genomes found that hybrid kinases (containing both a histidine kinase domain and a REC domain) were present in 56 of the genomes and, of these, 32 also encoded putative proteins with HPT domains either as independent proteins or as a component of hybrid histidine kinases [8].

This study indicates that multi-step phosphorelays are likely to be reasonably common in prokaryotic signal transduction systems, particularly in those organisms with complex developmental systems, metabolic activities and/or cell-cell interactions [8].

Chemosensory systems

The bacterial chemotaxis systems that control swimming motility in response to chemo-attractants and repellents also belong to the family of 2-component signal transduction systems. The most extensively studied bacterial chemotaxis systems are those that control swimming chemotaxis in enteric species [9]. Related

systems have been found in many bacteria and control a number of motility and developmental phenotypes, and thus the more general 'chemosensory' terminology is often employed to describe these systems.

The enteric chemotaxis system serves as a paradigm model for bacterial chemosensory systems. Unlike the class I histidine kinases which possess a ligand binding module in the same polypeptide chain as the histidine kinase, the chemosensory systems have separated these functions into distinct proteins (Figure 1B). The membrane bound chemoreceptors are referred to as methyl-accepting chemotaxis proteins (MCPs) and interact with the class II histidine protein kinase CheA and the monomeric protein CheW in large receptor-signalling complexes which modulate the kinase activity of CheA in response to chemical stimuli. CheA possesses conserved motifs required for nucleotide binding in domains that are similar to those found in Class I histidine kinases.

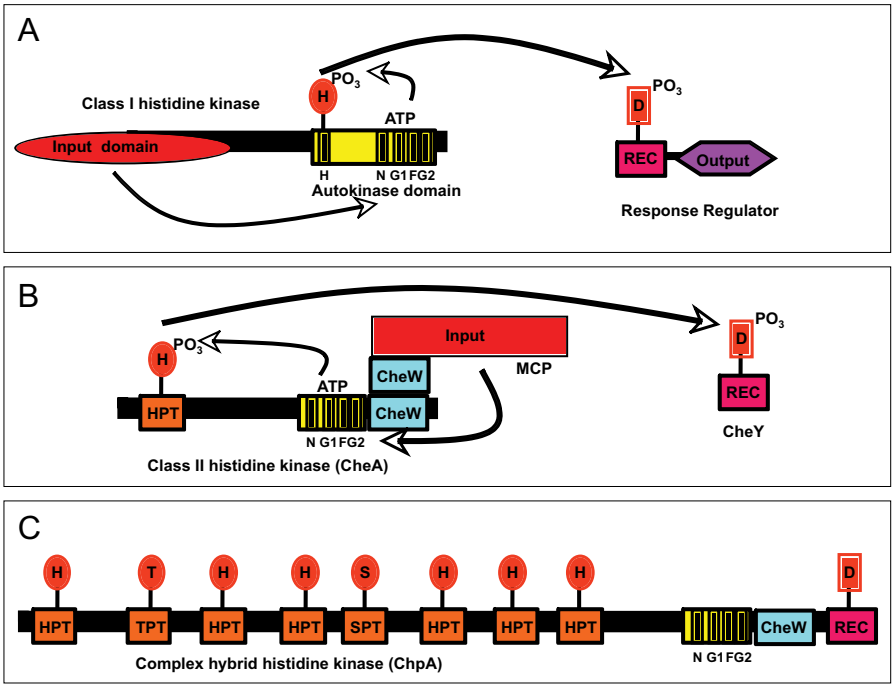

Figure 1. Comparison of domain architecture and phosphotransfer pathways of '2-component'" signal transduction systems.

The conserved sub-domains involved in ATP binding (N,G1, F,G2 boxes) and phosphate acceptance (H box) of Class I and Class II histidine kinases are indicated. Upon ligand binding, the autokinase domain of the histidine kinase is activated and phosphorylation occurs at a conserved histidine in either the H box of the Class I histidine kinases or the HPT domain of the class II (CheA) histidine kinase. The phosphoryl group is then transferred to the conserved aspartate in the REC domain of the response regulator.

(A) Prototypical '2-component' signal transduction scheme involving a Class I histidine kinase and a REC domain containing response regulator which also includes an output domain. The output domain is commonly a DNA binding domain involved in transcriptional regulation.

(B) The membrane bound methyl-accepting chemotaxis protein (MCP) is a chemoreceptor that interacts with the CheW domain of the class II histidine protein kinase CheA as well as the monomeric protein CheW in large receptor-signalling complexes which modulate the kinase activity of CheA in response to chemical stimuli. CheA autophosphorylates at a conserved residue in the N-terminal histidine phosphotransfer (HPT) domain. The phosphoryl group is then transferred to the stand-alone REC domain protein CheY.

(C) ChpA of *P. aeruginosa* is a Class II hybrid histidine kinase with multiple HPT domains, novel serine (SPT) and threonine (TPT) containing phosphotransfer domains, and a C-terminal REC domain. Phosphorylation of these residues in each of the conserved domains is yet to be demonstrated.

However, unlike the Class I histidine kinases, autophosphorylation occurs at a conserved histidine in the N-terminus of the protein in a conserved HPT domain which is similar to those that participate on multistep phosphorelays [1, 2]. The high-energy phosphoryl group is then transferred from the histidine of the CheA to a conserved aspartate in the response regulator CheY which is comprised solely of a REC domain. Phospho-CheY then interacts directly with the flagellar motor to switch the direction of flagellar rotation.

The rate of autocatalytic dephosphorylation of CheY-P is enhanced through interaction with CheZ. Sensory adaptation which allows temporal control of swimming motility occurs through methylation of specific glutamate residues on the MCP to reset it into a non-signalling state. The methylation status of the MCP is adjusted via competing activities of the methyltransferase CheR and the methylesterase CheB. CheB also possesses a REC module and is competitively phosphorylated by CheA [9].

Numerous variations on this theme have now been described. Interestingly, many of these systems lack a homologue of the phosphatase CheZ but instead include multiple CheY (REC domain) proteins which are thought to act as phosphate sinks to 'dephosphorylate' the active CheY[10], whereas the *B. subtilis* CheC protein combines the CheY (REC) domain with a phosphoaspartate phosphatase domain to dephosphorylate phospho-CheY [11].

The bacterial chemosensory systems demonstrate that the REC domain can be combined with protein modules other than DNA binding domains. For example, the CheB proteins combine the REC domain with a methylesterase enzyme; CheC of *B. subtilis* combines REC with a

phosphatase domain; CheV of *B. subtilis* combines the REC domain with the protein interaction CheW domain [12]; FrzZ of *Myxococcus xanthus* combines 2 REC domains [13]; and hybrid Class II histidine kinases such as FrzE of *M. xanthus* combine a CheA like histidine kinase with a REC domain at its C-terminus [14].

Chp chemosensory system of *Pseudomonas aeruginosa*

We are currently characterising a complex chemosensory system that controls type IV pili biogenesis and twitching motility in *Pseudomonas aeruginosa*. This chemotaxis-like system, referred to as the Chp system, is comprised of the *P. aeruginosa* proteins PilG, PilH, PilI, PilJ, PilK, ChpA, ChpB, and ChpC [15-18].

The CheA-like protein of the system (ChpA) is an extremely complex hybrid histidine kinase that possesses nine putative sites of phosphorylation: six histidine-containing phosphotransfer (HPT) domains, two novel serine- and threonine-containing phosphotransfer domains (SPT, TPT) and a REC domain at its C-terminus (Figure 1C). The Chp chemosensory system also consists of two stand-alone CheY (REC) domain proteins (PilG, PilH), a putative methyl-accepting chemoreceptor (PilJ), a methyltransferase CheR-like protein (PilK), a methylesterase CheB homologue (ChpB), and two CheW homologs (PilI and ChpC). It is likely that ChpA is responsible for phosphotransfer reactions with its C-terminal REC domain as well as PilG, PilH and the REC domain of ChpB. Furthermore, given the complexity of the protein, ChpA may well feed into other as yet unidentified REC and HPT modules.

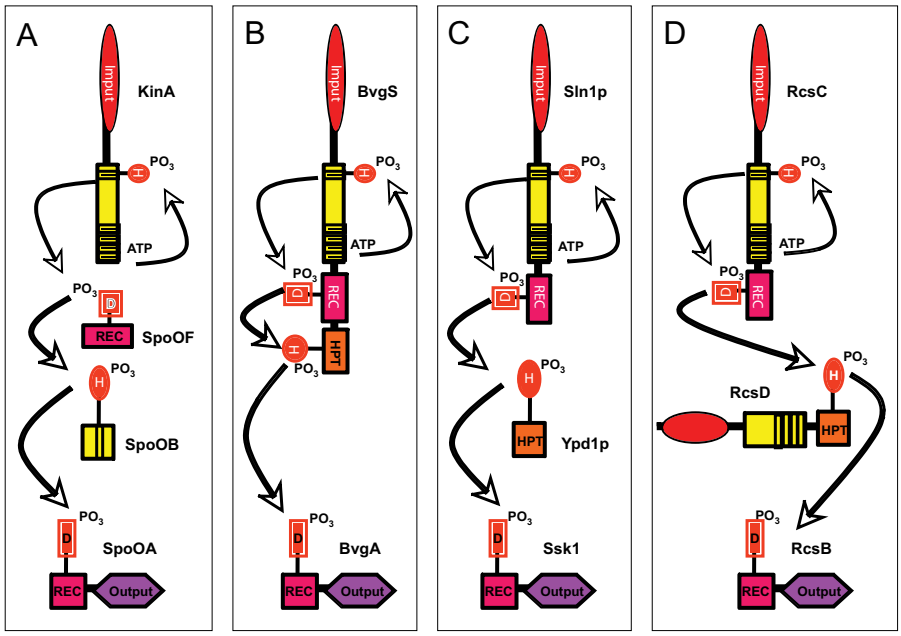

Figure 2. Multistep phosphorelay signal transduction systems.

Each of the multi-step phosphotransfer cascades is initiated by signal input to a membrane bound histidine kinase that subsequently autophosphorylates the histidine residue located in a conserved sub-domain of the autokinase domain. The phosphate group is then transferred to the conserved aspartate in an intermediary REC domain, then to a conserved histidine in an intermediary phosphotransfer domain, and finally to the conserved aspartate of the REC domain of the response regulator.

(A) The KinA-SpoOF-SpoOB-SpoOA sporulation phosphorelay of *Bacillus subtilis*. Each of the phosphorylated residues in the relay are located in separate proteins.

(B) The BvgS-BvgA system that controls transcription of virulence factors in *Bortedella pertussis*. This system involves a complex multi-domain hybrid histidine kinase BvgS which combines the histidine kinase, a REC domain and a histidine phosphotransfer (HPT) domain in a single protein.

(C) The Sln1p-Ypd1p-Ssk1 system that controls osmoregulation in the yeast *Saccharomyces cerevisiae*. This system is comprised of a hybrid histidine kinase containing a REC domain in its C-terminus (Sln1p). The intermediary HPT domain is present as a stand-alone module in the form of the protein Ypd1p.

(D) RcsC-RcsD-RcsB signalling pathway that controls capsular polysaccharide synthesis and cell division in *Escherichia coli*. In this system the intermediary HPT domain is located at the C-terminus of RcsD which shares many of the conserved features of histidine kinases but lacks the critical histidine residue required for autophosphorylation.

Diversity in architecture

With the availability of numerous bacterial genome sequences, it is now clear that 2-component signal transduction proteins can possess a diversity of modules and complex domain architectures. A recent census of response regulator proteins of 200 bacterial and archeal species has demonstrated enormous diversity of modular architecture of proteins containing REC domains [4].

In its simplest form, the REC domain is functional as a stand-alone module (e.g. SpoOB, CheY) participating in protein-protein interactions, as intermediates in phosphorelays or as phosphate sinks. REC domains are most commonly found associated with DNA binding domains, but may also be associated with protein modules with other functions including RNA binding, a variety of enzymatic activities, protein binding, ligand binding, histidine kinase domains and others [4].

Outside of DNA binding and methylesterase domains, the most common modules found to be associated with REC domains are enzymatic domains associated with cyclic di-GMP production and turnover. These include the diguanylate cyclase GGDEF domain, and the EAL and HD-GYP cyclic-di-GMP phosphodiesterases [4].

Histidine kinases also show an impressive diversity of domain architectures. The histidine kinase domain may be

associated with a variety of domains, including those associated with environmental sensing such as PAS (heme and flavin binding, oxygen, light, redox sensing), FliY (amino acid binding), Cache (small ligand binding), and GAF (cGMP binding) domains, as well as domains associated with signal transduction such as HPT (histidine phosphotransfer) and HAMP (linker) domains [19].

Bioinformatic analyses have also demonstrated that histidine kinases and response regulator proteins can possess a multitude of additional domains to form extremely complex multi-domain proteins [4]. Thus it seems that we are really just at the tip of the iceberg when it comes to understanding the functionality and complexity of 2-component signal transduction systems of bacteria.

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ASM joins APACE

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Recently the Australasian Association of Clinical Biochemists (AACB) and the Australian Society for Microbiology (ASM) signed a memorandum of understanding with AIMS to allow AACB and ASM members to also participate in the APACE programme.

AIMS will continue to administer the APACE programme. ASM members will be able to enrol in the programme for an annual fee of $25.00 (non-member rate $184.80). Applications from ASM members wishing to enrol in APACE will be processed initially by the ASM National Office. Application forms will be available shortly on the ASM website and joining APACE will be an option for members when paying their annual membership subscription.

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