Commentary

His kinase or mine? Histidine kinases through evolution

Sensing environmental changes and responding to them is the key to any organism's survival. The simplest example of a sensor-response system is seen in bacteria in the form of what is known as the two-component system. This system involves proteins in which the sensor or component I detects the stimulus via its input or sensor domain and is trans-autophosphorylated on a conserved histidine. The sensor then transfers this phosphate to the response regulator, or component II, on a conserved aspartic acid residue (Hoch 2000). This His-Asp signal transduction pathway, though ubiquitous in prokaryotes, is rarely encountered in higher eukaryotes.

A host of bacterial signalling pathways involve two-component systems. For example, the EnvZ-OmpR osmosensing system is a simple two-component system seen in *Escherichia coli* (see figure 1). EnvZ is the membrane bound sensor and the response regulator, OmpR, as in many other two-component pathways, is a transcription factor. Oxygen tension is sensed by the cytoplasmic FixL-FixJ proteins in *Bradyrhizobium japonicum* (Bauer *et al* 1999). The *com*CDE operon in *Streptococcus pneumoniae* encodes a competence stimulating peptide, its cognate receptor histidine kinase and the response regulator. All three are variable in different species, but the operon always consists of the cognate peptide and receptor-regulator genes (Whatmore *et al* 1999).

The sensor and the autokinase domains of the first component of the two-component system are sometimes present in two different proteins. In the chemotaxis pathway in *E. coli*, the methyl accepting receptor proteins are the chemosensors, and CheA, containing the histidine kinase domain, is a different protein. Autophosphorylation of the histidine residue in CheA requires the linker protein CheW. The second components that receive the phosphate on an aspartate residue are CheY and CheB. The response regulator in this case is not a transcription factor since CheY interacts with flagellar proteins and elicits a response by changing the direction of flagellar rotation. Phosphorylation on the aspartate in CheB enhances its methylesterase activity that is required during the adaptation process (Parkinson 1993). Thus, in bacteria, we see that the two-component pathway involving protein phosphorylation is also a 'protein-activity' regulating pathway. The chemotaxis machinery in *E. coli* is complex, in that it accommodates other regulatory circuits, either by using more than one protein as a substrate for the histidine kinase CheA, or by the requirement of an associated protein that is essential for CheA kinase activity. Interestingly, CheA and CheW associate via SH3-like domains, which are commonly used in eukaryotes to regulate signalling pathways (Hoch 2000).

In *Bacillus subtilis*, the sporulation pathway involves a four step sequential phosphorelay from a histidine to an aspartate to another histidine to another aspartate, all present on different proteins (Hoch 2000 and see figure 1). A similar relay is seen in the aerobic/anaerobic status sensing ArcB/ArcA regulon of *E. coli*, where an entire set of operons is regulated (Bauer *et al* 1999). The ArcB protein is a hybrid kinase with an intrinsic receiver domain, that accepts the phosphate on a conserved aspartate from the histidine present in the primary active site. The phosphate is then transferred to a second histidine residue in the histidine-containing phosphotransferase (HPt) domain (see figure 1). Such modifications have occurred in the basic plan, possibly to incorporate more regulatory components and fine-tune the signalling. This argument is supported by the discovery of a novel histidine kinase inhibitor protein in the *Bacillus* sporulation pathway, which inhibits the autokinase activity (Wang *et al* 1997). In addition, the sporulation pathway can be triggered by two additional histidine kinases, KinB and KinC, providing for additional inputs into the signalling pathway (Appleby *et al* 1996).

Another interesting two-component pathway is seen in light-sensitive protein kinases, bacteriophytochromes, in bacteria. These have been found even in non-photosynthetic bacteria such as *Deinococcus* sp. and *Pseudomonas aeruginosa*. The proteins BphB-BphR, constituting a typical two-component system, were discovered when a plant histidine kinase homologue was identified in the bacterium, along with a closely linked gene resembling a typical response regulator. This pathway is now known to regulate the synthesis of the light protective pigment deinoxanthin (Davis *et al* 1999). What draws attention is that the two genera *Deinococcus* (Gram-positive cocci) and *Pseudomonas* (Gram-negative rods) are only distantly related to each other, and even more distant evolutionarily from cyanobacteria, which have such bacteriophytochromes. Moreover, neither of the two genera is photosynthetic. The only proposed mechanism for such a distribution of bacteriophytochromes is horizontal transfer from a cyanobacterium, since many genes in *Deinococcus* have similarity to cyanobacterial genes (Davis *et al* 1999). This still does not explain their presence or their role in *P. aeruginosa*. However this bacterium is known to produce a host of coloured pigments, which appear to be correlated with the virulence of the organism.

The gene pair *tod*S-*tod*T, of the toluene degradation pathway in *Pseudomonas putida*, encodes two-component proteins. The TodS protein has an additional leucine zipper-type dimerization domain that assists the phosphorylated dimer to bind to sites on DNA. These sites are very similar to the DNA-binding site of the eukaryotic Fos-Jun dimer that is activated via the mitogen activated protein kinase (MAPK) or the c-jun N-terminal kinase (JNK) pathways in eukaryotes (Lau *et al* 1997).

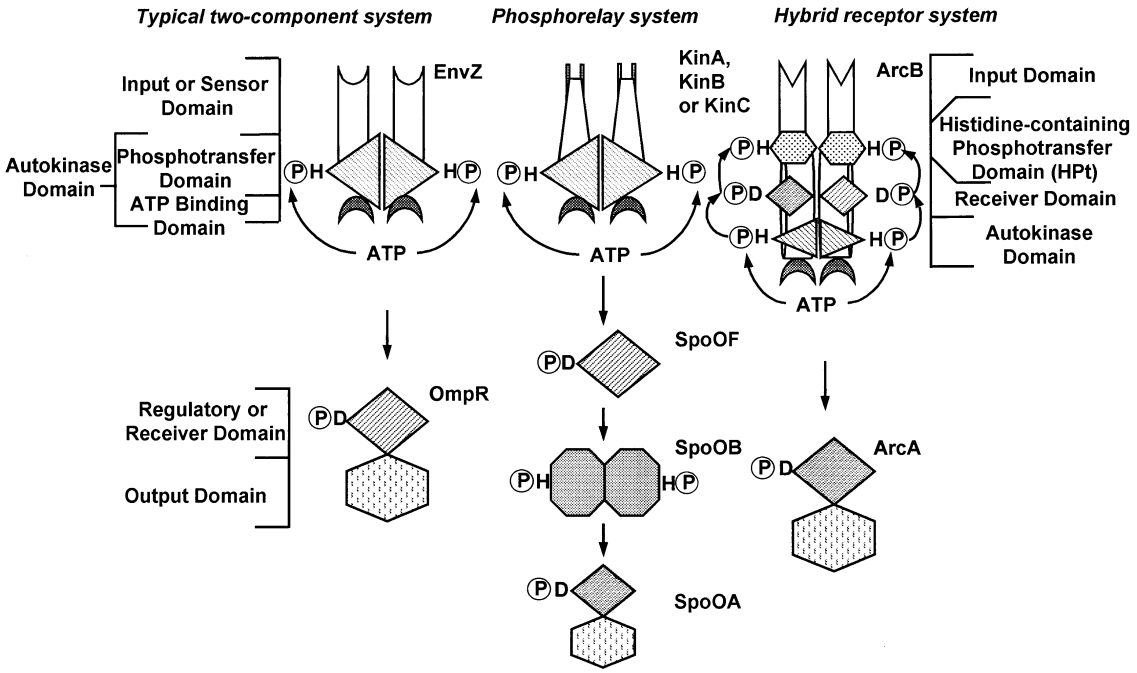

Figure 1. A schematic representtion of the different architectures of two-component systems showing the domain structures of the proteins involved. 'Typical two-component system' as seen in the EnvZ-OmpR pair of *E. coli*. The first transfer step is the transfer of the *g*-phosphate of ATP on to the histidine residue as a result of *trans* phosphorylation in receptor dimers. 'Phosphorelay system' as seen in the *Bacillus subtilis* sporulation pathway involves sequential transfers on four different proteins KinA/B/C-SpoOF-SpoOB-SpoOA. In eukaryotic phosphorelays, the role of SpoOB is played by different types of proteins, the HPt proteins e.g., Ypd1p in *Saccharomyces cerevisiae* and RdeA in *Dictyostelium discoideum.* However, the first two transfer steps occur on the same protein. The 'hybrid receptor system' is the more common type of phosphorelay in prokaryotes where the HPt domain is fused to the sensor kinase protein, forming a hybrid receptor as seen in ArcB. Here three transfer steps occur on the same protein. ArcA is the response regulator. Similar shapes indicate homologous domains. Input domains are specific to the stimuli they sense and hence are variable. H, Histidine; D, aspartic acid; circled P, phosphate.

Conventionally, blue-green algae or cyanobacteria, are placed higher in the evolutionary scale than eubacteria. Therefore, the question arises, if eubacteria have phytochromes, do the cyanobacteria, which have a host of phytochrome proteins, have histidine kinases? Indeed they do. Some examples are the phytochromes RcaE and Cph1 in *Synechocystis* sp. and *Fremyella diplosiphon*. The Cph1-Rcp1 system of *Synechocystis* has been speculated to regulate its circadian rhythm, since both genes are co-regulated by light-dark transitions (Garcia-Dominguez *et al* 2000). A purple bacterium *Rhodospirillum centenum* also has such phytochromes (Davis *et al* 1999). Interestingly, the existence of histidine kinases in archaebacteria is supported by the discovery of an *E. coli* CheA protein homologue in *Halobacterium salinarium* that is involved in chemotaxis and phototaxis (Rudolph and Oesterhelt 1995)

Thus eubacteria, cyanobacteria and archaebacteria have two-component systems. What is known about the structures of these proteins? Autophosphorylation of sensor proteins in two-component systems occurs in *trans*, in a manner similar to that seen in the receptor tyrosine kinases that regulate mammalian signal transduction pathways (Swanson and Simon 1994). However, histidine kinases have a catalytic domain that differs in structure from the well-known serine/threonine or tyrosine kinases in eukaryotes. In the *E. coli* EnvZ osmosensor and the *Bacillus* SpoOB, a four-helix bundle is formed by homodimerization, using two helices from each subunit. This region also contains the active site histidine and the phosphotransferase function. However, CheA uses a homologous region only for dimerization and not for the phosphotransferase activity (Hoch 2000). The ATP-binding domains in sensors are well conserved, and are similar in structure to those found in functionally unrelated proteins, such as Hsp90 and gyraseB that also bind ATP (Robinson and Stock 1999). The response regulators in two-component systems share a structure similar to CheY, and have a conserved threonine residue (in the vicinity of the active site aspartate) that is crucial for the conformational change-mediated regulation of their down-stream effects (Hoch 2000).

Have histidine kinases been modified into the serine/threonine/tyrosine kinase pathways in higher eukaryotes, or have the latter pathways evolved independently? There are very few serine/threonine/tyrosine kinases in bacteria, and very few histidine/aspartate kinases in the higher organisms (Bakal 2000). But, there are indications that histidine kinase pathways might have evolved into the serine/threonine/tyrosine pathways in higher organisms. The best evidence for such a change would be obtained if a good 'archaeopteryx' is identified. Are there any such examples in the context of histidine kinases? I think there are two.

The budding bacterium *Caulobacter cresentus* introduces more molecular complexity in regulating its cell cycle than a typical eubacterium and undergoes an asymmetric cell division (and differentiation). This gives rise to a stalk cell and a swarmer cell. CtrA, a response regulator protein, is the key regulator in this process and its activity in the two cells is regulated by differential transcription, phosphorylation and proteolysis. This type of regulation is analogous to mammalian cell cycle control, where cyclin and cyclin-dependent kinase complexes are the key players (Amon 1998). CtrA phosphorylation occurs on a conserved aspartate. The kinase domain of another protein involved in the cell cycle, DivL, being 24% identical to the H motif in the kinase domain of EnvZ (data not shown), is a homologue of histidine kinases. However, in DivL, phosphorylation occurs on a tyrosine, present in place of the conserved histidine residue seen in other histidine kinases, and hence DivL is a tyrosine kinase. The importance of this observation cannot be overstated. The phosphate is then transferred to the aspartate in CtrA, which is reminiscent of the two-component system. In biochemical terms, it is known that histidine-phosphate bonds and tyrosine-phosphate bonds are of high-energy and capable of transferring the phosphate residue to an aspartate. The presence of a tyrosine in place of histidine is not a rare event and is seen in many strains of this genus and related family of bacteria. A DivL mutant, generated by site directed mutagenesis of the active site tyrosine residue to a histidine residue could autophosphorylate, and also phosphorylate the response regulator, CtrA. However, both activities were observed at levels about six times lower than the wild type DivL protein. Importantly, a mutation of the active site tyrosine to phenylalanine did not autophosphorylate, emphasizing the importance of the active site tyrosine or histidine (in case of the mutant). In a similar study, substitution of the histidine residue in EnvZ of *E. coli* with a tyrosine residue abolished the kinase activity (Wu *et al* 1999). Therefore, DivL is a kinase specifically optimized to function with an active site tyrosine. This perhaps can thus be considered an 'archaeopteryx' between the prokaryotes, which mainly use His-Asp phosphory-

lation, and eukaryotes which use Ser/Thr/Tyr phosphorylation for signalling events. Evidence for another such 'missing link' is described below.

Two-component systems are not restricted to the prokaryotes. They appear in fungi, both in yeasts and moulds. In *Saccharomyces cerevisiae*, a two-component pathway interacts with the 'eukaryotic' MAPK pathway. The HOG pathway for osmosensing is triggered by a sequential His-Asp-His-Asp phosphorelay. The first His-Asp phosphotransfer occurs on Sln1p. The phosphate is then passed on to the histidine on Ypd1p and finally to an aspartate in the Ssk1p response regulator. Here the 'old' system has fused with the 'newer' one and generated a novel hybrid pathway (Loomis *et al* 1998). Ypd1p is the first eukaryotic two-component protein whose structure is known at 1·8 Å resolution. The structure has revealed it to be similar to the HPt domain of *E. coli* ArcB protein, both of which have a single polypeptide coding for the four-helix bundle in which lies the active site histidine (Song *et al* 1999). However, this type of a phosphorelay architecture might have evolved earlier, since it is seen in at least one prokaryotic pathway, the Lux pathway for quorum sensing in *Vibrio harveyi* (Thomason and Kay 2000). Two-component systems also regulate the sporulation cascade in the fungi *Aspergillus nidulans* and in *Candida albicans* (CaSln1p-CaNik1p). The *C. albicans* genes are also known to be involved in virulence. Osmosensing in *Neurospora crassa* too involves the two-component system (Virginia *et al* 2000).

In *Dictyostelium discoideum*, the RdeA protein (with only a histidine phosphotransfer domain) and RegA may constitute a phosphorelay system amenable to control by a number of upstream sensor kinases (Thomason and Kay 2000). But that is not all. RegA in addition has a phosphodiesterase activity domain. Such an activity is seen typically in eukaryotic cells where the concentrations of cAMP and cGMP regulate many functions. In *D. discoideum*, development is regulated by cAMP via the activation of protein kinase A. Here, therefore, is a linking of the His-Asp pathway to the cAMP pathway, exclusively and extensively used in eukaryotes (Loomis 1998).

The existence of two-component systems in plants is not surprising since, as mentioned earlier, a plant protein had aided in homology-based identification of a bacterial two-component system. Ethylene and cytokinin signalling in plants involve two-component proteins, and plants so far are known to have five sensors for the growth regulator ethylene, possibly having arisen by gene duplication and divergence. In addition, typical eukaryotic phytochrome proteins in plants are observed, similar in sequence to light-regulated cyanobacterial proteins. Interestingly, autophosphorylation in plant sensors occurs on a conserved serine residue (Yeh and Lagarias *et al* 1998), or in some cases glutamate or aspartate residues (Urao *et al* 2000). Is this another 'archaeopteryx'?

Finally we come to animals. Two known examples are present in mitochondrial enzymes and perhaps are not considered in a sense 'truly eukaryotic', due to the acceptance of the mitochondrial endosymbiont theory. The discoveries of histidine phosphorylation in the rat mitochondrial enzymes-branched chain alpha ketoacid dehydrogenase kinase (BCKDH) and pyruvate dehydrogenase kinase – could represent remnants of early histidine kinases. In case of BCKDH kinase, the conserved histidine residue has of date not shown to be phoshorylated. Instead autophosphorylation occurs on a serine reside, and BCDKH kinase phosphorylates its substrate on a pair of serine residues (Kennelly and Potts 1996).

The very early evolution of protein phosphorylation/dephosphorylation for regulation of critical cellular responses may account for the presence of such mechanisms in all life forms. What mutation event can lead to substitutions of the active site histidine in kinases in higher eukaryotes? Histidine is coded by CAY, serine by UCN and AGY, and threonine by ACN (where, Y = pyrimidine and N = any nucleotide). Tyrosine is coded by UAY. A missense mutation of CAY to UAY, which would result in a histidine to tyrosine substitution, would require a cytosine to thymine transition. However, a change from histidine to serine or threonine requires a transversion type of mutation in the DNA as well, which is a rare occurrence. Did such changes occur as rare events in an ancestor and get carried along as a 'frozen accident' in evolution, or did such amino acid substitutions occur repeatedly in many organisms and then spread as organisms diverged?

Why are tyrosine and serine/threonine phosphorylations more prevalent in higher organisms? One explanation is that the N-P phosphoramidate bond in the histidine phosphate has a high phosphate transfer potential and is hence unstable. Therefore the phosphate is transferred immediately to aspartate in the acceptor domain (Robinson and Stock 1999). The reversibility of this reaction may be its most

favoured feature. But then, how does the aspartate transfer its phosphate to another histidine residue in the case of hybrid sensors? The acyl-phosphate bond in residues like aspartate or glutamate does indeed have a high phosphate transfer potential. In contrast, the phosphate bond on serine, threonine and tyrosine residues is comparatively more stable, and therefore better suited for the protein to remain phosphorylated, and in an altered conformational state for longer, and advantage exploited to the full in eukaryotic signalling pathways (Stock *et al* 1990).

The crucial question is whether the His-Asp pathway has been modified to the serine/threonine or tyrosine pathways in eukaryotes? Arguing against this is the observation that serine/threonine or tyrosine kinases are not exclusively present in eukaryotes. Eubacteria also possess these kinases and their cognate phosphatases. Even bacteriophages code for Ser/Thr phosphatases, but the possibility of horizontal gene transfer cannot be, and has not been, ruled out. For example the virulence plasmid of *Yersinia pseudotuberculosis* is now believed to have acquired a eukaryotic tyrosine kinase by horizontal gene transfer (Kennelly and Potts 1996).

What are the other differences one sees in using histidine/aspartate or serine/threonine/tyrosine pathways? Firstly, phosphorelays in prokaryotes are very different from the phosphoprotein cascades seen in eukaryotes. In the two-component system, the same phosphate is transferred at each step, always maintaining exact stoichiometry. However, in the eukaryotic protein kinase cascades, phosphorylation drives the conformational change which activates/deactivates the kinase activity of an upstream kinase, which in turn phosphorylates the next protein in the pathway, using ATP. Secondly, bacterial response regulators are usually transcription factors, unlike what is seen in some eukaryotic pathways, that involve a string of sequential kinases or other enzymes, before a transcription factor is regulated. It is thus evident that the two-component system does not allow for any signal amplification. Interestingly, such amplification is also not seen in the eukaryotic MAPK pathway, following the discovery and characterization of scaffolding proteins (Burack and Shaw 2000).

The complexity of prokaryotic pathways has been increased by utilizing multiple kinases (as in *B. subtilis* sporulation) or multiple response regulators (as in *E. coli* chemotaxis). The use of separate HPt proteins seems to be rare in prokaryotes, and predominates in eukaryotes. This may allow increased flexibility in the signalling network, since the active site histidine is more accessible to multiple response regulators (Thomason and Kay 2000). The inherent complexity of eukaryotic cells compels them to have a host of other interacting proteins, feeding in different signals, or regulating a response. Such fine-tuning and control of a cellular response may be limiting in the simple His-Asp system. The apparent absence of two component systems in higher organisms may be simply because they have yet to be discovered, and such information may be forthcoming in future following whole genome sequence analyses. However, the ultimate aim of every organism remains the same, regardless of the type of pathway involved. At the molecular level, it is altering gene expression, and in a social context, its survival.

Acknowledgement

I am grateful to Dr Sandhya S Visweswariah for a critical reading of the manuscript.

References

Amon A 1998 Controlling cell cycle fate: common strategies in prokaryotes and eukaryotes; *Proc. Natl. Acad. Sci. USA* **95** 85–86

Appleby J L, Parkinson J S and Bourret R B 1996 Signal transduction via the multi-step phosphorelay: not necessarily a road less traveled; *Cell* **86** 845–848

Bakal C J 2000 No longer an exclusive club: eukaryotic signalling domains in bacteria; *Trends Cell Biol* **10** 32–38

Bauer C E, Elsen S and Bird T H 1999 Mechanisms for redox control of gene expression; *Annu. Rev. Microbiol.* **53** 495–523

Burack W R and Shaw A S 2000 Signal transduction: hanging on a scaffold; *Curr. Opin. Cell Biol.* **12** 211–216

Davis S J, Vener A V and Vierstra R D 1999 Bacteriophytochromes: Phytochrome like photoreceptors from non-photosynthetic eubacteria; *Science* **286** 2517–2520

Garcia-Dominguez M, Muro-Pastor, Reyes J C and Florencio F J 2000 Light-dependent regulation of cyanobacterial phytochrome expression; *J. Bacteriol.* **182** 38–44

Hoch J A 2000 Two component phosphorelay signal transduction; *Curr. Opin. Microbiol.* **3** 165–170
Kennelly P J and Potts M 1996 Fancy meeting you here! a fresh look at prokaryotic protein phosphorylation; *J. Bacteriol.*, **178** 4759–4764
Lau P C, Wang Y, Patel A, Labbe D, Bergeron H, Brousseau R, Konishi Y and Rawlings M 1997 A bacterial basic region leucine zipper histidine kinase regulating toluene degradation; *Proc. Natl. Acad. Sci. USA* **94** 1453–1458
Loomis W F, Kuspa A and Shaulsky G 1998 Two-component signal transduction systems in eukaryotic microorganims; *Curr. Opin. Micobiol.* **1** 643–648
Parkinson J S 1993 Signal transduction schemes of bacteria; *Cell* **73** 857–871
Robinson V L and Stock A M 1999 High energy exchange: proteins that make or break phosphoramidate bonds; *Structure Fold Des.* **7** R47–R53
Rudolph J and Oesterhelt D 1995 Chemotaxis and phototaxis require a CheA histidine kinase in the archaeon *Halobacterium salinarium*; *EMBO J.* **14** 667–673
Song H K, Lee J Y, Lee M G, Moon J, Min K, Yang J K and Suh S W 1999 Insights into eukaryotic multistep phosphorelay signal transduction revealed by the crystal structure of Ypd1p from *Saccharomyces cerevisiae*; *J. Mol. Biol.* **293** 753–761
Stock J B, Stock A M and Mottonen J M 1990 Signal transduction in bacteria; *Nature* (*London*) **344** 395–400
Swanson R V and Simon M I 1994 Signal Transduction. Bringing the eukaryotes up to speed; *Curr. Biol.* **4** 234–237
Thomason P and Kay R 2000 Eukaryotic signal transduction via histidine-aspartate phosphorelay; *J. Cell Sci.* **113** 3141–3150
Urao T, Yamaguchi-Shinozaki K and Shinozaki K 2000 Two-component systems in plant signal transduction; *Trends Plant Sci.* **5** 67–74
Virginia M, Appleyard C L, McPheat W and Stark M J 2000 A novel 'two-component' protein containing histidine kinase and response regulator domains required for sporulation in *Aspergillus nidulans*; *Curr. Genet.* **37** 364–372
Wang L, Grau R, Perego M and Hoch J A 1997 A novel histidine kinase inhibitor regulating development in *Bacillus subtilis*; *Genes Dev.* **11** 2569–2579
Whatmore A M, BarcusV A and Dowson C G 1999 Genetic diversity of the streptococcal competence (com) gene locus; *J. Bacteriol.* **181** 3144–3154
Wu J, Ohta N, Zhao J L and Newton A 1999 A novel bacterial tyrosine kinase essential for cell division and differentiation; *Proc. Natl. Acad. Sci. USA* **96** 13068–13073
Yeh K C and Lagarias J C 1998 Eukaryotic phytochromes: Light regulated serine/threonine protein kinases with histidine kinase ancestry; *Proc. Natl. Acad. Sci. USA* **95** 13976–13981

AVINASH R SHENOY
Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560 012 India
(Email, avirs@hamsadvani.serc.iisc.ernet.in)