Histidine Phosphorylation and Two-Component Signaling in Eukaryotic Cells

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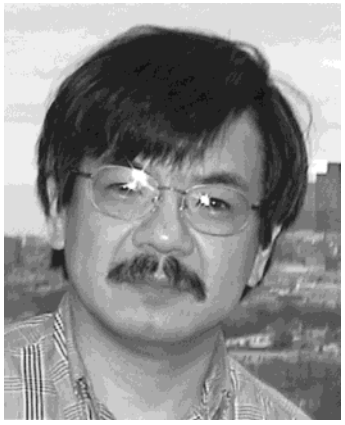
I. Introduction

Reversible protein phosphorylation is a major mechanism of signal transduction in both prokaryotes and eukaryotes. In eukaryotic cells, protein phosphorylation occurs mainly on serine, threonine, or tyrosine residues, as a quick scan of the articles in this issue of *Chemical Reviews* may attest. In contrast, prokaryotic cells use the so-called two-component mechanism, in which phosphorylation of a histidyl group and a subsequent transfer of the phosphoryl group to an aspartyl group serve a central role in intracellular signal transduction.[1] Thus, it was once believed that the specificities of protein kinases (serine/threonine/tyrosine vs histidine/aspartate) are defining characteristics of eukaryotes and prokaryotes. Recent findings, however, have eliminated such a dichotomy. Serine/threonine kinases were found in bacteria, and two-component proteins were found in eukaryotes in 1993.[2−4] Since then numerous two-component proteins have been identified in eukaryotic organisms, including yeasts, fungi, ameba, and higher plants. Because many of those proteins were initially identified through sequence similarity with bacterial two-component proteins, their functions and regulatory mechanisms were only matters of speculation. With rapidly accumulating functional data, however, it is now possible to draw pictures that are supported by experimental evidence. This review will first summarize the basic features of prokaryotic two-component systems that are relevant to the later discussion of eukaryotic two-component systems. [Note that this article is not intended as a comprehensive review of prokaryotic two-component systems. Interested readers are advised to consult the definitive book edited by Hoch and Silhavy[1] and a number of recent reviews on various aspects of two-component systems.[5−18]] I will then discuss recent



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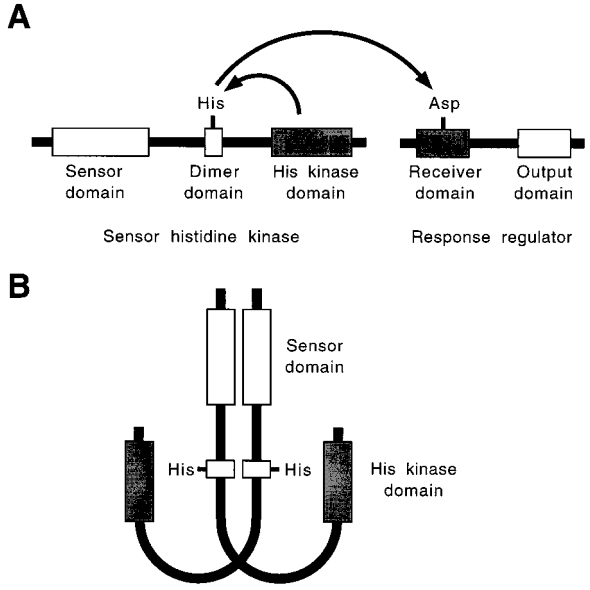

Figure 1. (A) Schematic model of a generic two-component system. Arrows indicate the transfer of a phosphoryl group. The His kinase domain phosphorylates a substrate histidine using ATP. Phosphoryl group on the histidine is then transferred to an aspartate residue in the receiver domain of the cognate response regulator. (B) Schematic model of a dimeric sensor histidine kinase. The histidine kinase domain of one subunit is juxtaposed to the histidine phosphorylation site of the other subunit.

findings in various eukaryotic organisms, with a particular emphasis on the studies published in the last three years.

II. Elementary Components in Two-Component Systems

Two-component systems are built around the phosphotransfer reaction between histidine and aspartate residues (sometimes called His-Asp phosphorelay), which is mediated by a sensor histidine kinase and a response regulator. A sensor histidine kinase autophosphorylates a histidine residue in the molecule and donates the phosphoryl group to a conserved aspartate in a cognate response regulator (Figure 1A). The His-to-Asp phospho-transfer reaction is catalyzed by the response regulator. For this reason, the response regulators are sometimes referred to as aspartate kinases. However, strictly speaking, they are phosphotransferases not kinases. Although the simplest of histidine/aspartate phosphorylation systems consist of only two proteins, many "two-component systems" contain more than two components.

A. Histidine Kinase

All sensor histidine kinases contain an ATP-binding catalytic domain, a dimerization domain, and a histidine phosphorylation site (Figure 1A). Often the histidine phosphorylation site is embedded within the dimerization domain, but there are exceptional cases such as chemotaxis CheA protein in which the histidine phosphorylation site and the dimerization domain are widely apart. The histidine phosphorylation site also serves as the donor domain of the phosphoryl group to the receiver domain in a cognate response regulator protein (see below). Most sensor histidine kinases contain an input (or sensor) domain covalently linked to the kinase domain. CheA is atypical also in this respect because its sensor domain is noncovalently associated.

Functional forms of sensor histidine kinases are homodimeric. Genetic and biochemical analyses both agree that the histidine phosphorylation in a dimer occurs in trans (Figure 1B). For example, two EnvZ mutants, one defective at the histidine phosphorylation site (His-243 to Val) and another defective in the kinase domain (C-terminal deletion), can complement each other when coexpressed in a single cell.[19] A similar inter-allelic complementation was observed between a CheA phosphorylation site mutant (His-48 to Gln) and a kinase-defective mutant (Gly-470 to Lys).[20] Inter-allelic complementation would be expected only if the kinase domain of one molecule phosphorylates the histidine residue of another molecule. It is believed that histidine kinase domain is constitutively active, but in its resting state the substrate histidine residue is out of register with the catalytic sites. When the input domain receives an appropriate stimulus (for example, ligand binding), conformation change brings the kinase domain of one subunit closer to the histidine phosphorylation site of the other subunit, allowing the phosphorylation of the substrate histidine residue.

B. Response Regulator

A typical response regulator protein contains a conserved receiver domain and a nonhomologous output domain (Figure 1A). An invariant aspartate in the receiver domain accepts a phosphoryl group from the donor domain of a cognate histidine kinase, resulting in a conformational change and modulation of output domain activity. The output domains of most bacterial response regulators are DNA-binding transcription regulators. In the case of the transcription factor Spo0A, the output domain becomes active, either when the receiver domain is phosphorylated or when the receiver domain is deleted.[21,22] Thus, the unphosphorylated receiver domain inhibits the output domain in the intact Spo0A protein and phosphorylation lifts such inhibition.

Not all response regulators are, however, transcription factors. Some receiver domain proteins either activate or inhibit enzymes. Chemotaxis regulatory protein CheY, for example, controls the behavior of the bacterial flagellar motor depending on its phosphorylation status. Another example is the *Dictyostelium* RegA protein, which has a catalytic cAMP phosphodiesterase domain whose activity is regulated by its own receiver domain.[23]

III. Examples of Bacterial Two-Component Systems

Most of the functional speculation about eukaryotic two-component systems relies heavily on their similarity to the bacterial counterparts. Therefore, I will first survey three well-characterized examples from bacterial two-component systems. This will also serve

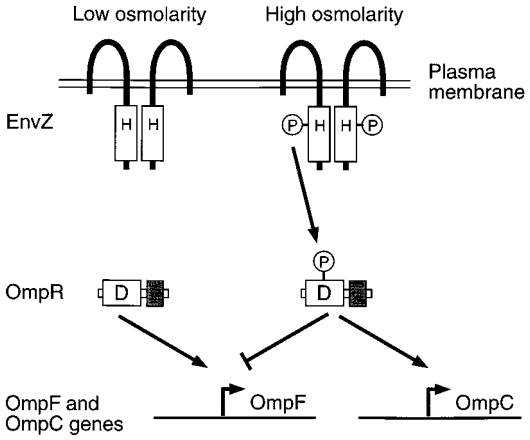

Figure 2. Bacterial EnvZ-OmpR osmosensing pathway. At high extracellular osmolarity, the activated histidine kinase domain of the dimeric EnvZ protein phosphorylates a specific histidine (H) residue. The phosphoryl group (P) is then transferred to an aspartate (D) residue in a receiver domain of the cytoplasmic OmpR protein. The DNA-binding domain (gray) of OmpR interacts differentially with the promoter regions of the *OmpF* and *OmpC* genes, depending on the OmpR phosphorylation state.

to illustrate how the two elementary components are combined to create ever more complex signaling pathways.

A. EnvZ-OmpR Osmosensing Pathway

The *E. coli* EnvZ-OmpR osmoregulatory signal transduction pathway is an example of simple two-component systems in which the entire signals transduction pathway is composed of only two proteins: the sensor histidine kinase EnvZ and the response regulator OmpR (Figure 2). The extracellular domain and the two flanking transmembrane segments (TM1 and TM2) of EnvZ serve to detect the extracellular osmotic conditions. Although the 3D structure of the EnvZ extracellular sensor domain is unknown, mutational studies have revealed the importance of the interaction between TM1 and TM2.[24] Because the functional form of EnvZ is dimeric, a bundle of four α helices ($TM1_2$ $TM2_2$) formed in the lipid bilayer may be important for transmitting a signal across the plasmamembrane.

The cytoplasmic region of EnvZ is subdivided into two functionally distinct domains: a dimerization domain and a kinase domain. When the two domains are separately purified and mixed in the presence of ATP, they reconstitute the phosphorylation reaction at His-243.[25] The structures of the EnvZ kinase domain and its dimerization domain, though separately, are now known. The solution structure of the EnvZ kinase domain determined by NMR has a completely distinct topology than that of any serine/threonine or tyrosine kinases.[26] However, its α/β sandwich fold has similarities to the ATP-binding domains of a chaperone (Hsp90) and a type II DNA topoisomerase (Gyrase B). Because the histidine kinase, Hsp90, and Gyrase B share the very rare left-handed $\beta\alpha\beta$ conformation, it seems likely that these proteins have a common evolutionary ancestor.

The relatively short (~60 amino acids) EnvZ dimerization domain has a hairpin-like helix−turn−helix topology.[27] In an EnvZ dimer, two hairpins are juxtaposed to form a four-helix bundle with a 2-fold symmetry. Amphipathic Helix I of one subunit intimately interacts with equally amphipathic Helix II of the same subunit as well as Helix II of the other subunit. The phosphorylation site His-243 is located near the middle of Helix I; two phosphorylation sites stick out from opposite sides of the four-helix bundle.

A phosphoryl group on EnvZ His-243 is transferred to a conserved aspartyl group in the receiver domain of OmpR. OmpR is composed of an N-terminal receiver domain and a C-terminal DNA-binding domain.[28,29] Depending on its phosphorylation status, the OmpR receiver domain modulates the DNA-binding properties of the output domain; the output domain then either activates or represses the expression of the major outer membrane proteins OmpC and OmpF. The [OmpC]/[OmpF] ratio is adjusted by the EnvZ-OmpR system to fit optimally to the environmental osmotic conditions.[30]

B. CheA-CheY Chemotactic Pathway

The *E. coli* chemotactic signaling pathway is a sophisticated but still relatively a straightforward extension of the two-component theme. The histidine kinase component of this pathway, CheA, is a cytoplasmic protein without its own sensor domain. It forms a complete sensor histidine kinase by binding to one of several specialized transmembrane chemosensors (aspartate receptor, serine receptor, etc). This arrangement allows the same CheA kinase domain to be regulated by diverse chemical entities including both attractants ant repellents. These receptors are homodimeric, and because each of these receptors has two transmembrane segments (TM1 and TM2), a four-helix bundle similar to that of EnvZ formed in the lipid bilayer.[31] Ligand binding does not affect the dimerization state of the receptors, but it causes a downward piston-like movement of TM2 relative to TM1 in the lipid bilayer.[32] This relatively subtle (1 Å) movement transmits the conformational change of the extracellular ligand-binding domain to its cytoplasmic tail, which is linked to the CheA histidine kinase. Surprisingly, however, a dimeric chemotactic sensor with only one cytoplasmic domain has been shown to signal properly.[33,34] How such a hemi-truncated dimer induces a ligand-binding-dependent conformational change in the attached CheA dimer is unknown. One possible solution might be offered by hypothesizing a transient formation of a tetramer, in which at least one pair of cytoplasmic dimer is formed.

The crystal structure of a large segment of the CheA protein including the dimerization domain and the catalytic domain was recently solved.[35] The topology of these two domains is essentially identical to those of the corresponding EnvZ domains. Furthermore, the CheA structure clearly shows that the two domains are relatively independent and are connected by a flexible hinge region. Because a mutation at the hinge region reduces autophosphorylation activity, it seems likely that a movement of the kinase domain relative to the dimerization domain is important in kinase activation, perhaps by

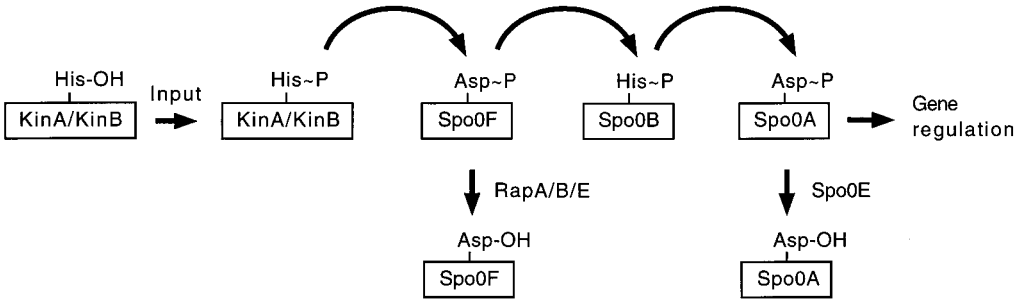

Figure 3. Spo0 multistep phosphorelay pathway. Sensor histidine kinases, KinA and KinB, autophosphorylate it histidine (His) residue when they are activated by a respective input signal. Phosphoryl group is then transferred, in steps, to an aspartate (Asp) residue in the receiver domain of Spo0F, to a histidine in Spo0B, and then to an aspartate in Spo0A. Spo0A is DNA-binding transcription factor that regulates genes essential for sporulation. RapA, RapB, RapE, and Spo0E are specific aspartate phosphatases. Wavy line (~) indicates a high-energy phosphate bond.

allowing the kinase domain to reach the substrate histidine. The known CheA structure, however, does not include the histidine phosphorylation site (His-45), precluding any further speculation on the kinase activation mechanism.

The response regulator protein CheY receives a phosphoryl group from CheA and regulates the rotation of flagellar motor. In another response regulator, CheB, a receiver domain is covalently linked to a methylesterase domain, which is activated when CheA phosphorylates the CheB receiver domain. CheB and the methyltransferase CheR regulate the sensitivity of the chemosensors.[36] Methylation state of chemosensors serves as a kind of molecular memory, so that a moving bacterium can determine whether it is moving up or down concentration gradients of chemoattractants or chemorepellents.

C. Spo0 Sporulation Regulatory Pathway

The sporulation regulation in the Gram-positive bacterium *Bacillus subtilis* is a more complex example of complex two-component systems.[37] In the Spo0 pathway (KinA/B−Spo0F−Spo0B−Spo0A), the His-Asp phosphotransfer reaction is repeated twice in the His-Asp-His-Asp sequence (for simplicity, this will be abbreviated as H1-D1-H2-D2). Thus, this and similar two-component systems are called multistep phosphorelays (Figure 3). The multistep phosphorelay reaction starts, like in conventional two-component systems, with the autophosphorylation of a histidine residue (H1) in a sensor histidine kinase (KinA or KinB). The phosphoryl group is then transferred to the conserved aspartate (D1) in the receiver domain of Spo0F. The phosphoryl group is then transferred to a specific histidine residue (H2) in the intermediary protein Spo0B, from which the phosphoryl group is further transferred to an aspartyl group (D2) in another receiver domain protein Spo0A. Spo0A has, like many other prokaryotic response regulators, a DNA-binding domain.

The structure of Spo0B (H2 protein) is reminiscent of the dimerization/histidine-phosphorylation domain of the EnvZ kinase.[38] The N-terminal domain of Spo0B is composed of a hairpin-like helix−turn−helix structure, and the site of phosphorylation (His-30) is located in the middle of the second helix. This domain mediates, like in EnvZ, dimerization of Spo0B by forming a four-helix bundle. The phosphorylation sites, His-30, of the two subunits protrude from the opposite faces of the helix bundle. Because the side chain of the His-30 does not interact with other parts of the molecule, its phosphorylation is unlikely to cause any major structural alteration. This may be important because both Spo0B−OH and Spo0B~P interact with Spo0F and Spo0A, allowing very rapid transfer of phosphoryl group between Spo0F and Spo0A in both directions.

In the multistep phosphorelay system (H1-D1-H2-D2), the D1 and H2 components appear to be unnecessary because in theory phosphoryl group can be directly transferred from H1 to D2. However, there is no or little direct interaction between the H1 and D2 components. In the Spo0 pathway, the extra steps are used to integrate multiple input signals. For example, protein aspartate phosphatases (RapA, RapB, and RapE) that specifically dephosphorylate Spo0F and are inhibited by secreted peptides whose concentration reflects cell density.[39,40] Another protein aspartate phosphatase (Spo0E) specifically dephosphorylates Spo0A.[41] Thus, the multistep mechanism can combine diverse environmental conditions to control its output signal.

IV. Eukaryotic Two-Component Systems

Eukaryotic organisms also use the same two-component principle for their signal transduction. It is, however, often adapted to regulate other eukaryotic signaling machinery, such as MAP kinase cascade and cAMP-dependent protein kinase (PKA). It is also notable that most eukaryotic two-component systems are multistep phosphorelays rather than simple two protein systems. Two-component systems seem to play particularly important roles in fungi, yeasts, ameba, and higher plants. Not every eukaryotic organism, however, uses two-component signaling. The complete genomes of the fruit fly *Drosophila melanogaster* and the nematode *C. elegans*, for example, do not have any two-component elements. In this section, I will summarize the current state of the investigations in the eukaryotic two-component systems.

A. *Saccharomyces cerevisiae*

The genome of the budding yeast *Saccharomyces cerevisiae* encodes only one histidine kinase, Sln1,

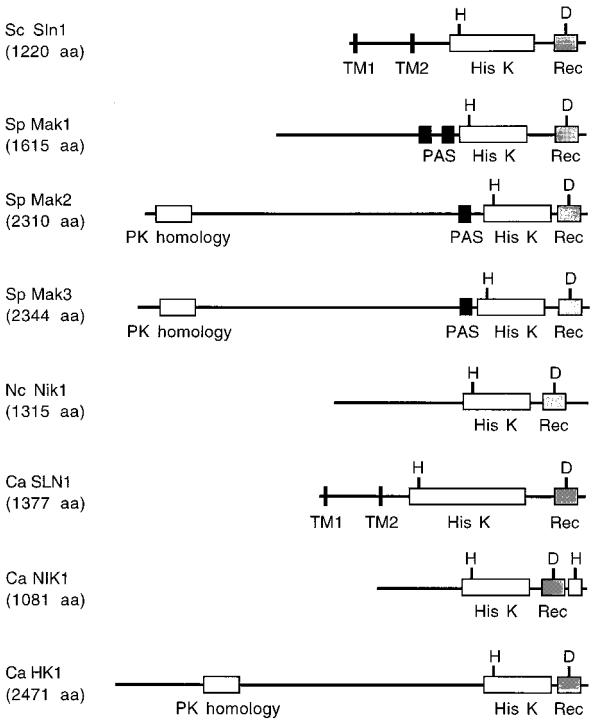

Figure 4. Schematic structures of sensor histidine kinases in *Saccharomyces cerevisiae* (Sc), *Schizosaccharomyces pombe* (Sp), *Neurospora crassa* (Nc), and *Candida albicans* (Ca). The size of each molecule is indicated in parentheses. Abbreviations used in this figure are as follows: His K, Histidine kinase domain including dimerization domain; Rec, receiver domain; TM, transmembrane segments; PAS, PAS domain; PK homology, serine/threonine protein kinase homology domain; H, histidine phosphorylation site; D, aspartate phosphorylation site.

and two receiver domain proteins, Ssk1[4,42] and Skn7.[43,44] Sln1 and Ssk1 are involved in sensing of high-osmolarity stress, whereas Skn7 is responsible for oxidative stress signaling.

1. Sln1-Ypd1-Ssk1 Osmosensing Pathway

Sln1 is an example of hybrid histidine kinases in which both a histidine kinase domain and a receiver domain are covalently encoded in a single protein. N-terminus of Sln1 is topologically similar to the bacterial osmosensor EnvZ in that it has an extracellular domain flanked by two transmembrane segments, TM1 and TM2 (Figure 4). Indeed, Sln1 is an osmosensor.[42] Sln1 functions in a multistep phosphorelay mechanism in which Sln1 provides both the H1 and D1 sites and two other proteins, Ypd1 and Ssk1, provide, respectively, the H2 and D2 sites (Figure 5).[45] It is believed that the Sln1 histidine kinase is active under normal (nonstressful) osmotic conditions; when the environmental osmolarity is increased, its kinase activity is inhibited.[45] The Sln1 osmosensing mechanism is not yet fully understood, but it is probably similar to that of EnvZ. Deletion of the first transmembrane segment (TM1) constitutively activates the Sln1 kinase, whereas removal of both TM1 and extracellular domain inactivates Sln1.[46] Replacement of the Sln1 extracellular domain with dimerization-promoting leucine zipper motif restores the kinase activity (but not the capacity as an osmosensor). Thus, it seems likely that transmembrane segments are essential for an osmosensing mechanism and the extracellular domain is required for Sln1 dimerization.

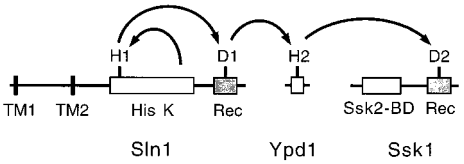

Figure 5. Sln1-Ypd1-Ssk1 multistep phosphorelay pathway. Binding of unphosphorylated Ssk1 activates the Ssk2 MAPKKK. Arrows indicate the movement of a phosphoryl group. H1 and H2, histidine phosphorylation sites; D1 and D2, aspartate phosphorylation sites; Ssk2-BD, Ssk2-binding domain.

When active, Sln1 autophosphorylates its H1 site near the histidine kinase domain and transfers the phosphoryl group to the D1 site in the Sln1 C-terminal receiver domain and then to the H2 site in Ypd1. The primary sequence of Ypd1 does not have any significant similarity to other H2 domain proteins except for a short segment (~10 amino acids) around the histidine phosphorylation site. Nonetheless, the crystal structure of Ypd1 is surprisingly similar to the H2 domain (called HPt domain) of the *E. coli* ArcB protein.[47−49] Both are composed of a four-helix bundle with the phospho-accepting histidine in the middle of one helix. It is interesting to note that another H2 domain protein, Spo0B, has only two α helices but also forms a four-helix bundle by dimerization.[38] The phosphoryl group is transferred from the H2 site of Ypd1 to the D2 site in the receiver domain of Ssk1.

Ssk1 is an activator of a protein kinase termed Ssk2 (and a redundant kinase Ssk22).[50] Binding of unphosphorylated Ssk1 to the Ssk2 N-terminal regulatory domain activates (or un-inhibits) Ssk2 kinase activity.[51] Ssk2 and two additional kinases, Pbs2 and Hog1, constitute a so-called MAP kinase cascade in which one kinase activates the next kinase by phosphorylation. Phosphorylated Hog1 is imported into the nucleus, where it activates yet unidentified transcription factor(s) to induce genes that are required for osmoadaptation.[52] Both *pbs2*Δ and *hog1*Δ mutants fail to grow under a hyperosmotic environment, indicating that the Hog1 activity is essential for osmoadaptation. Interestingly, however, excessive and constitutive activation of Hog1 MAP kinase is as detrimental as its absence. Thus, in *sln1*Δ or *ypd1*Δ mutant, where Ssk1 is never phosphorylated, constitutive activation of the Hog1 pathway occurs, resulting in cell death.[42,45]

Purified Ssk1~P has an unusually long half-life of 42 h (in comparison, the D1 site in the Sln1 receiver domain has a more typical half-life of 13 min).[53] The extra stability of Ssk1~P is functionally important to maintain the levels of dephosphorylated Ssk1-OH sufficiently low in resting cells, so that an inadvertent firing of the Ssk2-Pbs2-Hog1 kinase cascade is avoided. When yeast is stimulated with high osmolarity, however, activation of the kinase cascade occurs within minutes, indicating that a rapid dephosphorylation of Ssk1~P takes place.[50] In cell, therefore, the dephosphorylation of Ssk1~P might be

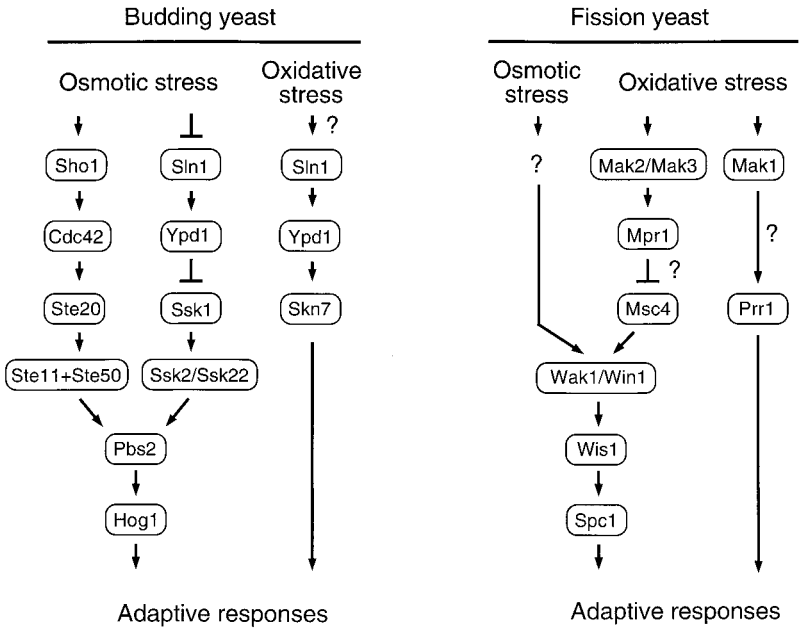

Figure 6. Comparison of the stress responsive signal transduction pathways between the budding and fission yeasts. Sho1 is a membrane anchorage protein that interacts with Pbs2 and is essential for osmotic activation of the Ste11 MAPKKK. Ste50 is a Ste11-binding protein and is also essential for osmotic activation of Ste11. Ste11, Ssk2, Ssk22, Wak1, and Win1 are MAPKKKs; Pbs2 and Wis1 are MAPKKs; Hog1 and Spc1 are MAPKs. Cdc42 is a small GTPase, and Ste20 is a protein kinase of the PAK family. Other proteins are discussed in the text. Arrows indicate activation, whereas T-bars indicate inhibition.

accelerated by specific aspartate phosphatases, perhaps in an analogous manner as in the bacterial Spo0 pathway. So far, however, no phosphatases have been identified. Ypd1 may also have a role in the accelerated dephosphorylation of Ssk1~P in cell, because Ypd1 can mediate retrograde phosphotransfer reaction, from Ssk1 to Sln1.[53] It is possible that a rapid shuttling of the phosphoryl group occurs from Ssk1 to Sln1 via Ypd1, when the Sln1 kinase activity is suppressed under high osmolarity.

S. cerevisiae has a second osmosensing mechanism that also activates the Pbs2 and Hog1 kinases (but not the Ssk2 and Ssk22 kinases). The second osmosensing machinery does not use the two-component mechanism but instead is known to involve a transmembrane protein Sho1, a small GTPase Cdc42, a PAK-like protein kinase Ste20, a MAPKKK Ste11, and a Ste11-binding protein Ste50.[50,54−57]

2. Skn7 Response Regulator

The Skn7 response regulator protein is composed of an N-terminal DNA-binding domain and a C-terminal receiver domain, suggesting that it is an OmpR-like transcription factor.[43] It has been shown that Skn7 governs the induction of oxidative stress-responsive genes, including the genes that encode thioredoxin, thioredoxin reductase, and heat shock proteins.[58,59] Consistently, *skn7* mutants are hypersensitive to oxidative stresses, such as exposure to hydrogen peroxide.[60] The transcription inducing activity of Skn7 is abolished when the phospho-accepting aspartate (Asp-427) is mutated to asparagine, indicating that it is dependent on aspartate-phosphorylation.[61] Interestingly, the Skn7 activity is also abolished in *sln1*Δ or *ypd1*Δ mutants but not in *ssk1*Δ mutants, suggesting that Sln1-Ypd1 phosphorelay may be responsible for the phosphorylation of Skn7.[61] The predicted phosphotransfer reaction from Ypd1 to Skn7 has been demonstrated in vitro.[62] It is not known, however, whether Sln1 is a sensor for oxidative stress or not.

B. *Schizosaccharomyces pombe*

In the fission yeast *Schizosaccharomyces pombe*, the stress-activated Sty1 MAPK (also called Spc1, Phh1) is activated not only by hyperosmotic stress but also by oxidative and heat shock stresses.[63−66] Activation of the Sty1 MAPK at least partly depends on a two-component system that is very similar to the Sln1-Ypd1-Ssk1 multistep phosphorelay in the budding yeast (for a comparison of the budding and fission yeasts, see Figure 6). In the nearly finished fission yeast genome sequences, we can find genes that encode three histidine kinases (Mak1, Mak2, and Mak3),[67] one Ypd1-like H2 domain protein (Mpr1; also called Spy1),[68,69] and two response regulators (Msc4 and Prr1).[70−72]

The response regulator Prr1 is similar to the budding yeast Skn7 in that both contain a heat shock factor (HSF)-like DNA-binding domain in their N-terminus and a receiver domain in C-terminal region. *prr1*Δ mutants are sensitive to cold temperature and oxidative stress but not to high osmolarity. In contrast, mutants of the Sty1 MAPK are sensitive to high osmolarity but not to cold temperature.[72] Thus, the role of the Prr1 response regulator in stress response seems independent of the Sty1 MAPK pathway (as the Skn7 response regulator is independent of the Hog1 MAPK pathway). The other response regulator, Msc4, is structurally more closely related to the budding yeast Ssk1.[70,71] In *msc4*Δ

mutant cells, activation of the Syt1 MAPK by high osmolarity or oxidative stress is severely impaired.[70,73]

The Ypd1-like protein Mpr1 interacts with the Msc4 response regulator in response to oxidative stress.[65] Furthermore, in the *mpr1*Δ mutant cells, the Sty1 MAPK cascade is constitutively activated.[65] These observations suggest that Mpr1 and Msc4 are the H2 and D2 components of a multistep phosphorelay that regulates the stress-responsive MAPK cascade. By analogy, it can be predicted that the Wak1 and Win1 MAPKKKs (homologues of SSK2) are activated by the unphosphorylated response regulator Msc4, as in the Sln1 pathway. Unlike the budding yeast *ypd1*Δ mutants, however, *mpr1*Δ mutants are viable, probably because a strong negative feedback by protein phosphatases prevents a runaway activation of the Sty1 kinase.[74]

The major difference between the budding yeast and the fission yeast two-component pathways is that the latter responds strongly to oxidative stress (for example, hydrogen peroxide) whereas the former does not. This seems to be due to an interesting difference in the structure of respective sensor histidine kinases (Figure 4). Each of the three fission yeast histidine kinases (Mak1, -2, and -3) is a cytoplasmic protein and contains one or two so-called PAS domain.[67] The PAS domain is a sensory motif that was originally identified in the *Drosophila* Period protein, the vertebrate Aryl hydrocarbon receptor nuclear translocater, and the *Drosophila* Single-minded protein. More important, PAS domain has been identified in a variety of bacterial sensors of oxygen or redox,[75] and in the case of the oxygen sensor FixL, the PAS domain was shown to bind a heme group.[76] Thus, it is possible that a bound heme group serves as the oxygen-sensing mechanism in the Mak proteins too, although no heme binding has been demonstrated. The absence of a PAS domain in the budding yeast osmosensor Sln1 might explain why oxidative stress does not activate the Hog1 MAPK cascade in *cerevisiae*.

In the absence of either Mak2 or Mak3, no activation of Sty1 MAPK occurs by oxidative stimulation. The requirement for both Mak2 and Mak3 suggests that the two histidine kinases act as a heterodimer. Mak1 deletion does not affect Sty1 activation. Because Mak1 also contains PAS domains, it is possible that it is involved in activation of the Prr1 response regulator.

The fission yeast histidine kinases are cytoplasmic proteins without any transmembrane segments, making them unlikely candidates for osmosensors. Indeed, disruption of these kinase genes does not influence the osmotic activation of the Sty1 MAPK cascade.[67] In fission yeast, therefore, osmosensing may to be effected by a mechanism that does not involve a sensor histidine kinase. Because no Sho1 homologue is found in the current *S. pombe* sequence database, the fission yeasts may employ an entirely novel osmosensing mechanism.

C. *Neurospora crassa*

Neurospora crassa, the light red mold that might be found on old bread, grows as branched multicellular network of long filamentous hyphae. By a PCR-based approach, two histidine kinase genes (*nik1* and *nik2*) have been identified, although nothing has been reported about *nik2*.[77] The *nik1* gene product (Nik1) is a hybrid histidine kinase that contains both a histidine kinase domain and a receiver domain. The kinase and receiver domains are most closely related to bacterial BarA (except, of course, the Nik1 homologues in other fungi). The N-terminal region of Nik1 has six tandem repeats of 90-amino acids that are predicted to form a coiled-coil structure (these repeats are not found in the bacterial BarA).

In *nik1* mutant strains, hyphal development is aberrant and cells become swollen and lyzed.[77] These mutant cells form protoplasts by secreting glucan and chitin polymers, instead of assembling them into a cell wall.[78] Thus, Nik1 signaling may regulate cell wall assembly. *nik1*Δ mutants are also highly osmosensitive and fail to grow on media containing 1M sorbitol or 0.7 M NaCl, and one of osmosensitive mutants (*os1*) was found to have a defect in the *nik1* gene.[78] *os1* mutants are also resistant to dicarboximide, a widely used fungicide. Interestingly, some dicarboximide resistant mutants of other fungi, such as *Aspergillus nidulans*, *Penicillium expoansum*, and *Botrytis cinerea*, are also osmosensitive,[78] suggesting that Nik1-like histidine kinases are present in those fungi, too.

D. *Candida albicans*

Candida species are opportunistic pathogenic fungi frequently present on the normal mucous membranes of the mouth, vagina, and gastrointestinal tract of healthy humans, but it may cause severe diseases in immunologically compromised individuals. *C. albicans* is dimorphic, that is, it grows as oval budding yeast cells at the surface of a rich agar medium but also invades deep in the agar medium as filamentous hyphae. Because the invasive hyphal form establishes various legions in the mucous membranes, the yeast-to-hyphae transition is important for *C. albicans* virulence. This transition is controlled by complex interaction of cells and their environment, in which two-component signaling seems to serve an important role.

Three histidine kinase genes *CaSLN1*, *CaNIK1* (also called *COS1*), and *CaHK1* have been cloned from *C. albicans*, either by functional complementation of *S. cerevisiae* mutants or by sequence-based methods.[79−83] Furthermore, a gene that encodes a receiver domain protein (*CaSSK1*) has been cloned by a PCR-based method.

1. *CaSLN1*

The structural organization of the CaSLN1 protein is identical to *S. cerevisiae* Sln1, with two transmembrane segments, a histidine kinase domain, and a C-terminal receiver domain (Figure 4). The *CaSLN1* gene can complement the defect of *S. cerevisiae sln1*Δ mutations, implying that CaSLN1 functions in an Sln1-like phosphorelay pathway.[81] Unlike *S. cerevisiae sln1*Δ mutants, however, the homozygous *casln1*Δ null mutant cells grew under both normal and high osmotic conditions.

2. CaNIK1/COS1

CaNIK1 is a homologue of *Neurospora crassa* Nik1/Os1; the amino acid sequences of CaNIK1 and Nik1 are 83% identical. In addition to the histidine kinase domain and a receiver domain near its C-terminus, the very C-terminus (120 amino acids) of CaNIK1 is similar to the C-terminal HPt (or H2) domain of the bacterial BarA protein (Figure 4). This region may serve as the H2 domain in a multistep phosphorelay. In contrast, the *N. crassa* Nik1 protein does not contain the conserved histidine residue. The N-terminal region of CaNIK1 has five (rather than six) tandem repeats of 90-amino acids that may form a coiled-coil structure. Homozygous (*canik1*Δ/*canik1*Δ) deletion mutants grow normally at 30 °C, but they cannot form hyphae when grown at 37 °C.[79] Thus, both *N. crassa* Nik1 and *C. albicans* CaNIK1 are essential for hyphal growth. Unlike *N. crassa*, however, *C. albicans canik1*Δ mutants are not osmosensitive.

3. CaHK1

CaHK1 is also a member of hybrid histidine kinase with a histidine kinase domain and a receiver domain near its C-terminus.[80,83] Two histidine kinases (Mak2 and Mak3) from *S. pombe* are most closely related to CaHK1, but CaHK1 does not seem to have any PAS domain (Figure 4). CaHK1 is likely to be a soluble protein because it does not have any transmembrane segment. Interestingly, a 180-amino acid segment near the CaHK1 N-terminus (460 to 640) has a significant similarity to the protein serine/threonine kinase PknB from *Mycobacterium tuberculosis*.[83] The same kinase homology domain is found in the *S. pombe* Mak2 and Mak3. This kinase similarity, however, only extends over a part of the kinase catalytic domain. Thus, it is unlikely that CaHK1, Mak2, and Mak3 are active serine/threonine kinases. Homozygous *cahk*1Δ/*cahk*1Δ null mutants show extensive flocculation (aggregation of filamentous hyphal cells) when grown at neutral pH but not at acidic pH. Altered expression of hyphal-cell-surface molecules is likely to be responsible for the flocculation phenotype, but its exact nature is unknown.

Disruption of *CaHK1* genes results in a complete loss of the serum-induced hypha-forming ability, while disruption of either the *CaSLN1* or *CaNIK1* gene results in partial loss.[84] Consistent with this observation, the virulence of the *casln1*Δ or *canik1*Δ mutant is intermediate between the wild-type (virulent) and *cahk*1Δ (avirulent). Interestingly, *casln1*Δ *cahk*1Δ and *canik1*Δ *cahk*1Δ double mutants develop hyphae more efficiently than *cahk*1Δ single mutant, and the double mutants are more virulent than the *cahk*1Δ single mutant. Thus, even though the three histidine kinases are involved in hyphal development and virulence, their functional interaction is complex.

4. CaYPD1 and CaSSK1

The structures of the three hybrid histidine kinases in *C. albicans* suggest that they are parts of multistep phosphorelay pathways. They all contain the H1 and D1 domains, and CaNIK1 may also contain the H2 domain. Thus, it can be predicted that Ypd1-like H2 domain proteins and Ssk1-like D2 domain proteins exist in *C. albicans*. Indeed, two genes (CaYPD1 and CaSSK1) that encode, respectively, an Ypd1-like protein and an Ssk1-like protein have been cloned.[85,86] Because *CaYPD1* complements a yeast *ypd1*$^-$ mutant, CaYPD1 is likely to be a functional H2 protein.

The receiver domain at the C-terminus of CaSSK1 is highly homologous to both the receiver domains of *S. cerevisiae* Ssk1 and *S. pombe* Mcs4.[87] In contrast, the N-terminal region of CaSSK1 has only a weak similarity to Mcs4 and none to Ssk1. The limited similarity, as well as the failure of the *CaSSK1* gene to complement *ssk1*Δ and *mcs4* mutants, suggests that *CaSSK1* is probably not a functional homologue of *SSK1* and *mcs4*$^+$.[85] Homozygous *cassk1*Δ/*cassk1*Δ mutants have a complex phenotype concerning hyphal development. In liquid media, *cassk1*Δ mutant cells develop hyphae identical to those of the wild-type cells. At neutral pH, however, *cassk1*Δ mutant cells flocculated extensively in the same manner as *cahk*1Δ mutants. Homozygous *cassk1*Δ mutants are totally defective in hyphal development on nitrogen-rich solid media. In contrast, on nitrogen-poor solid media, *cassk1*Δ mutants develop extensive hyphae that hyper-invade the solid agar. Clearly, CaSSK1 is required not for the hyphal development itself, but for its proper regulation. Because the *cassk1*Δ phenotype is similar to a superposition of those of *canik1*Δ and *cahk*1Δ mutations, CaSSK1 may function as a common downstream element of both CaHK1 and CaNIK1 histidine kinases.

E. *Dictyostelium discoideum*

When *Dictyostelium* cells, growing as a population of amoeba, have depleted their nutrient, they aggregate into a migratory slug. It eventually initiates a complex multicellular developmental program to form a fruiting body that is composed of a stalk and a sorus packed with spores. Various stages of *Dictyostelium* development are governed by the inter- and intracellular mediator, cAMP, and its target, protein kinase A (PKA). Several histidine kinases and phosphorelay components have been identified and implicated in this complex regulatory mechanism. For more details on the *Dictyostelium* differentiation program, consult recent review articles.[88−91]

Five histidine kinases (DokA, DhkA, DhkB, DhkC, and DhkD) have been identified through gene cloning.[92−94] All of these five proteins are hybrid histidine kinases containing both a histidine kinase domain and a receiver domain (Figure 7). DhkA and DhkB have two and six potential transmembrane sequences, respectively, and are likely to be transmembrane proteins, whereas DokA and DhkD are without any transmembrane segment. The very N-terminal sequences of DhkC are hydrophobic, but it is not certain whether DhkC is membrane associated or not.[95] In addition to these histidine kinases, an Ypd1-like H2 domain protein (RdeA) and a receiver domain protein (RegA) have been identified in *Dictyostelium*.

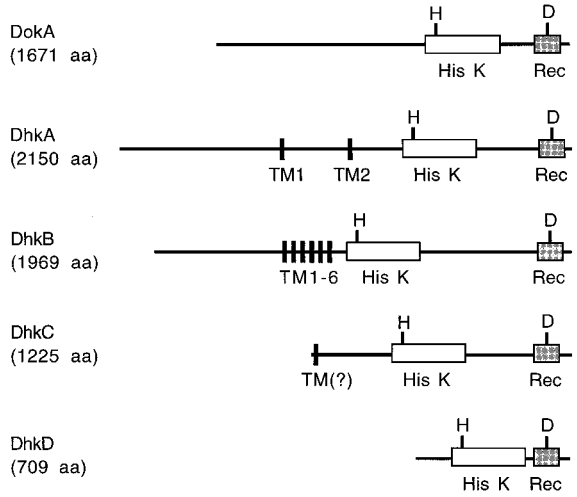

Figure 7. Schematic structures of sensor histidine kinases in *Dictyostelium discoideum*. DhkA, DhkB, and DhkC might be receptors for, respectively, SDF-2, discadenine, and ammonia. For abbreviations used in this figure, see Figure 4.

1. DokA

dokA$^-$ mutants grow and differentiate normally, although their spores have slightly reduced (30%) viability.[92] The most conspicuous phenotype of the mutants is their sensitivity to high osmolarity. Cells that have been exposed to 0.4 M sorbitol die in 100 min. If DokA is an osmosensor, however, its osmosensing mechanism must be distinct from that of the yeast Sln1 osmosensor because DokA does not have transmembrane segments.

2. DhkA

The early development of *dhkA*$^-$ mutants appears normal, but their fruiting body formation is aberrant, and a severely reduced number of viable spores are formed.[93] During normal culmination process, prestalk cells secrete the sporulation-inducing peptide Spore Differentiation Factor-2 (SDF-2), which stimulates pre-spore cells to encapsulate. A *dhkA*$^-$ mutant in which the DhkA extracellular domain is modified by an insertion of MYC_6 epitope requires 100-fold higher levels of SDF-2 to induce sporulation, raising the possibility that DhkA is a sensor for SDF-2.[96] Overexpression of PKA or exogenous addition of cAMP, on the other hand, induces encapsulation even in *dhkA*$^-$ mutants. Thus, when activated by ligand binding, DhkA up-regulates (probably indirectly) PKA to stimulate spore formation.

3. DhkB

In *dhkB*$^-$ mutants, the fruiting bodies appear to develop normally but their spores precociously germinate within the sori.[94] The intracellular concentration of cAMP in *dhkB*$^-$ spores is only 40% of that of the wild-type spores. Normally, germination of spores in sori is inhibited by the autoinhibitor discadenine or by other yet unidentified inhibitors. Activated PKA is also known to suppress the germination in sori. Thus, a plausible model is that DhkB, in response to discadenine, activates PKA by increasing the concentration of cAMP, to inhibit premature germination.

4. DhkC

In wild-type *Dictyostelium*, aggregated cells form a migratory slug, which culminates to form the fruiting body only when its nitrogen source is depleted. Ammonia is the endogenous regulator of the slug versus culmination choice. It is also known that activated PKA prevents culmination. In *dhkC*$^-$ mutants, however, aggregated cells immediately commence culmination in the presence or absence of ammonia.[95] In contrast, a slug of cells that overexpress active DhkC continues to migrate until their energy source is depleted. This "slugger" phenotype is reversed by exogenous application of cAMP. From these observations, it seems likely that DhkC, when activated by ammonia, prevents precocious culmination by inhibiting PKA, possibly by lowering the levels of cAMP.

5. RdeA and RegA

Although each *Dictyostelium* histidine kinase has a distinct functional role in development, they all seem to regulate activity of PKA. The two additional phosphorelay proteins, RdeA and RegA, are also responsible for regulating PKA activity. Both *rdeA*$^-$ and *regA*$^-$ mutants have high cAMP levels and as a consequence high PKA activities. RegA has two domains: the N-terminal receiver domain and a C-terminal cAMP phosphodiesterase, which hydrolyzes cAMP. Phosphorylation at the conserved aspartate in the RegA receiver domain enhances the phosphodiesterase activity at least 20-fold.[23,97] RdeA, an activator of RegA, is a homologue of Ypd1. Indeed, the yeast *YPD1* gene can complement an *rdeA*$^-$ mutant.[98]

It is tempting to speculate that at least one of the known histidine kinases is an upstream regulator of the RdeA-RegA module. For this role, DhkC is a good candidate because the phenotypes of *dhkC*$^-$ and *rdeA*$^-$ are similar in that both mutants culminate precociously. Furthermore, there is genetic evidence that RegA is responsible for the PKA down-regulation caused by DhkC overexpression.[95] In contrast, DhkA and DhkB, which up-regulate PKA activity, are unlikely to be the upstream activator of the RdeA-RegA module. Thus, DhkC-RdeA-RegA might be a unit of signal transduction analogous to yeast Sln1-Ypd1-Ssk1. Even so, however, there must be additional upstream elements for the RdeA-RegA module because *dhkC*$^-$ mutants do not completely reproduce the more severe phenotype of *rdeA*$^-$ mutants.

E. Higher Plants

Numerous sensor histidine kinases and response regulators have been identified from higher plants. On the basis of their input stimuli, three functional subgroups can be recognized: ethylene receptors, cytokinin receptors, and osmosensors. There are many excellent review articles on this subject.[99−108]

1. Ethylene Receptors

Ethylene is a gaseous plant hormone that regulates various aspects of plant growth and development,

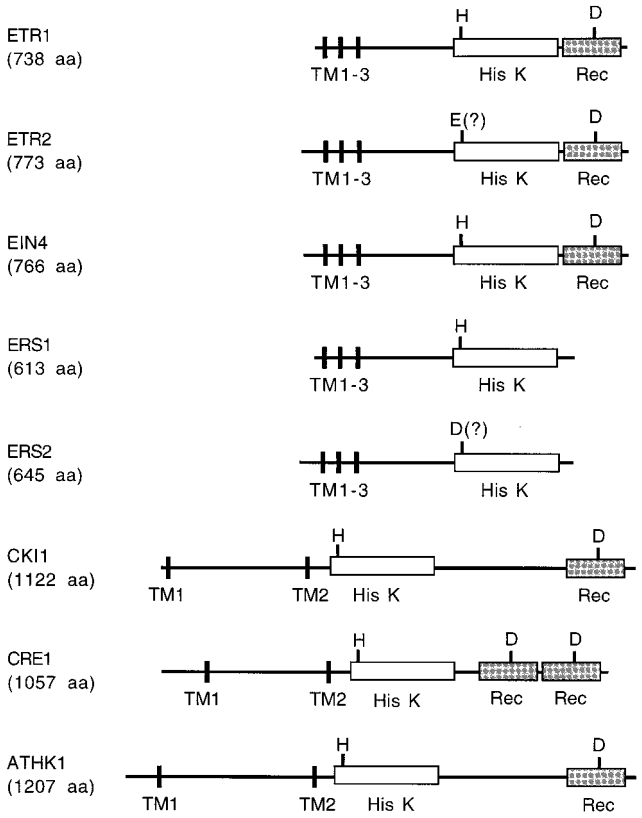

Figure 8. Schematic structures of sensor histidine kinases in *Arabidopsis thaliana*. TM1-TM3 in the ethylene receptors ETR1, ETR2, EIN4, ERS1, and ERS2 form an ethylene-binding site. CKI1 and CRE1 are putative cytokinin receptors, and ATHK1 is an osmosensor. For abbreviations used in this figure, see Figure 4.

including fruit ripening, seed germination, seedling growth, leaf expansion, senescence, and abscission.[99,109] The first ethylene receptor gene, *ETR1*, was cloned in 1993 from *Arabidopsis thaliana* by chromosome walking using ethylene-insensitive *etr1* (*ethylene response 1*) mutants.[3] It was also one of the first eukaryotic histidine kinases to be discovered. Since then, four additional ethylene receptor genes (*ETR2*, *EIN4*, *ERS1*, and *ERS2*) have been isolated from *Arabidopsis* by positional method and/or homology to the *ETR1* gene.[110−112] Homologues of these ethylene receptors have been also isolated from tomato,[113−116] *Rumex palustris* (sorrel),[117] and *Cucumis melo* (muskmelon).[118]

Each ethylene receptor contains a homologous histidine kinase domain (Figure 8). Histidine phosphorylation activity of the ETR1 kinase domain has been demonstrated in vitro.[119] Surprisingly, the position of the conserved histidine residue is occupied by a glutamate residue (Glu-377) in ETR2 and by an aspartate residue (Asp-392) in ERS2.[111,112] Because the sequences around these residues are very well conserved in ETR2 and ERS2, these kinases might use the acid residues as their phosphorylation site. Phosphorylation of aspartate and glutamate by "histidine kinase" is a clear violation of the two-component canon, but energetically speaking it seems possible. Alternatively, a nearby histidine residue (His-384) might serve as a substitute phosphorylation site in ETR2, but there is no histidine at this position or within a reasonable distance in ERS2. It should be also noted that mutational analyses clearly exclude the possibility that *ETR2* and *ERS2* are nonfunctional pseudogenes.[120]

In addition to the histidine kinase domain, three of the ethylene receptors (ETR1, ETR2, and EIN4) also contain a C-terminal receiver domain. The functional form of the ethylene receptors is a homodimer covalently linked through two extracellular cysteine residues near their N-terminus (Cys-4 and Cys-6 in ETR1, for example).[121,122] The ethylene-binding site is composed of three hydrophobic transmembrane α helices that are also located near their N-terminus. A copper (Cu^+) ion, which is an essential cofactor, is coordinated within the three transmembrane α helices.[123] The *etr1−1* mutation (Cys-65 to Tyr), which renders the mutant plant insensitive to ethylene, eliminates both ethylene binding and copper binding.[123] On the basis of these observations, it is likely that the hydrophobic pocket formed by the three transmembrane α helices provides a favorable environment for copper binding, which in turn binds an ethylene molecule, perhaps by the interaction of ethylene π-electrons and copper *d*-orbital electrons.[100] Binding of ethylene may then alter the coordination chemistry and thus induce a conformational change of the ethylene receptor molecule.

The presence of many ethylene receptor genes might be a reflection of the diverse roles of ethylene during the entire plant life cycle. However, their functions seem to be at least partially redundant. That would explain why no recessive loss-of-function mutants have been isolated from functional genetic screenings. All the isolated mutants were dominant, gain-of-function mutants. What is more difficult to understand, however, is the fact that those dominant mutants are insensitive to ethylene: when one receptor is mutated, even though there are four other intact ethylene receptors, plant loses its ability to respond to ethylene stimulus and behaves as if there is no ethylene. To make the puzzle even deeper, one of the dominant mutants, *etr1−1*, was shown to have lost its ability to bind ethylene.[124] These observations could be explained, however, if ETR1 negatively regulates (i.e., inhibits) the ethylene response in the absence of ethylene binding. Although this model has a somewhat artificial flavor, it was proved to be correct by the isolation of loss-of-function mutants in ethylene receptor genes.[120] Loss-of-function mutants in any one of the *ETR1*, *ETR2*, *EIN4*, and *ERS2* genes had no ethylene-response defect. However, quadruple loss-of-function mutants had constitutive ethylene responses, which is a phenotype opposite to that caused by the dominant alleles. Thus, each of these ethylene receptors negatively regulates ethylene responses and the induction of ethylene response is through inactivation of the inhibitors rather than activation of activators. This double-negative mode of action is reminiscent of the yeast Sln1 osmosensor, which is also a negative regulator of the downstream MAP kinase cascade.[42,45]

The presence of the C-terminal receiver domain in several ethylene receptors (ETR1, ETR2, EIN4) suggests that they might function as a part of multistep phosphorelay mechanism analogous to the yeast

Sln1-Ypd1-Ssk2 pathway. Indeed, Ypd1-like HPt proteins have been identified from *Arabidopsis* (ATHP1-ATHP3; also known as AHP1-AHP3)[125,126] and from maize (ZmHP2).[127] Furthermore, as many as 14 response regulator proteins have been identified from *Arabidopsis* alone (ARR1-ARR14).[128−133] It is not yet known, however, which of these proteins work in the ethylene signaling.

Ethylene receptor regulates the activity of the Raf-like protein kinase CTR1.[134] By analogy with the yeast Sln1 pathway, it is likely that one or more of the receiver domain ARR proteins interact with and activate CTR1. Alternatively, however, a direct interaction between CTR and the histidine kinase domain of ethylene receptors might be involved.[135]

2. Cytokinin Receptors

Cytokinin, a derivative of adenine, is another important plant hormone. Cytokinin stimulates cell growth in culture and in the intact plant it has diverse effects, including promotion of lateral bud growth, delay of senescence in leaves, and control of nutrient metabolism. Even though the hormone was known for 45 years, cytokinin receptor was not known until recently. Now there are three candidate cytokinin receptors, two of which are sensor histidine kinases. Using T-DNA-mediated insertional mutagenesis, Kakimoto isolated several cytokinin-independent (*cki1*) mutants.[136] The rescued *cki1−1* DNA contained T-DNA inserted upstream of the *CKI1* gene, which encodes a transmembrane histidine kinase. Apparently, *cki1* phenotype is caused by overexpression of the *CKI1* gene. The architecture of CKI1 is more closely related to the yeast Sln1 than to ethylene receptors: both CKI1 and Sln1 have two transmembrane segments that flank a relatively large extracellular domain (Figure 8). The CKI1 extracellular region could provide a binding site for cytokinin, although it has not yet been demonstrated. CKI1 also contains a C-terminal response regulator domain, suggesting that it might interact with one or more of the Ypd1-like HPt proteins (AHP1-AHP3).

A seven-transmembrane protein GCR1, which might interact with a G-protein, has been proposed as another candidate for cytokinin receptor because inhibition of *GCR1* expression by antisense RNA causes specific cytokinin unresponsiveness.[137] It is possible that both CKI1 and GCR1 are physiologically relevant cytokinin receptors. However, because no direct cytokinin binding has been demonstrated in these cases, it is still possible that either or both are modulators of cytokinin response but not the cytokinin receptor itself.

More recently, another sensor histidine kinase, CRE1 (also known as WOL and AHK4), has been identified as a cytokinin receptor.[138] *cre1* (*cytokinin response 1*) mutants are defective in cytokinin-induced greening and shoot formation and are less sensitive to cytokinin-induced inhibition of root elongation. CRE1/WOL1/AHK4 is a hybrid histidine kinase which is composed of two transmembrane segments flanking a putative extracellular cytokinin-binding domain, a histidine kinase domain, and two tandem receiver domains[139,140] (Figure 8). In addition to the *cre1* mutant phenotype, an elegant functional study using the yeast *sln1* mutant suggests that CRE1 is indeed a cytokinin receptor.[138] As explained earlier, budding yeast *sln1* mutants are nonviable because of constitutive activation of the Hog1 pathway. Expression of CRE1 by itself did not suppress the lethality of *sln1*Δ mutants. However, addition of cytokinins to the growth media allowed the CRE1-expressing *sln1*Δ mutants to survive, indicating that the CRE1 histidine kinase is activated by binding of cytokinin.[138] Perhaps important, suppression of *sln1*Δ by CRE1 requires functional Ypd1 protein, suggesting that phosphoryl transfer occurs between one of the CRE1 receiver domains and Ypd1. It thus implies that a homologue of Ypd1 is involved in cytokinin response in plants.

Two additional hybrid histidine kinases, AHK2 and AHK3, share extensive sequence similarity with CRE1 (52% and 54%, respectively, over their entire proteins and 61% and 60% over their extracellular domain).[140] It will not be too surprising if these two also turn out to be cytokinin receptors. The presence of multiple cytokinin receptors may explain the diverse physiological effects of cytokinins.

3. Osmosensor

Water deficit and high salinity are among the most serious environmental stresses that limit plant growth. To test if an Sln1-like osmosensor is involved in the plant water stress response, Urao et al. isolated a histidine kinase gene, *ATHK1*, from dehydrated *Arabidopsis* plants.[141] Structurally, ATHK1 is similar to Sln1. More important, the plant *ATHK1* gene could complement the yeast *sln1*Δ defective mutants. Successful complementation required expression of the entire ATHK1 protein, including the extracellular domain flanked by the two transmembrane segments, the histidine phosphorylation site in the histidine kinase domain, and the aspartate phosphorylation site in the C-terminal receiver domain. Thus, ATHK1 can serve as an osmosensor in yeast and is likely to do so in plant cells as well. In plants, as in yeast, MAP kinases are activated by osmotic stress.[142,143] Thus, it will be very interesting to find out how similar the osmoregulatory signaling pathways are between plant and yeast.

V. Conclusion

In a relatively short time since the discovery of the first eukaryotic sensor histidine kinases in 1993, the two-component system has firmly established itself as an important signaling mechanism in the eukaryotic world. Initially, their functions could be only speculated by analogy with their bacterial counterpart. The situation is rapidly improving by isolation and characterization of mutants and by biochemical and structural investigations. Nonetheless, it is obvious that we have just scratched the surface of eukaryotic two-component systems. There will be many surprises as more and more eukaryotic two-component systems are studied in depth.

One notable aspect of the eukaryotic two-component system is its versatility. It is used to detect such

a diverse stimuli as oxygen, ethylene, cytokinin, cAMP, high osmolarity, peptide factors, and perhaps ammonia. Even more fascinating, however, is the manner by which the two-component mechanism is seamlessly incorporated into the intricate eukaryotic signaling networks including the MAPK cascade and the PKA pathway. Finding the evolutionary route that brought those disparate signaling mechanisms together is but one of many challenges facing the future studies of eukaryotic two-component systems.

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