The His-Asp phosphorelay signal transduction system has been identified in most organisms, including bacteria, yeasts, fungi, and plants, except for animals. This system is important in adaptation to stress, control of cell growth, and induction of development in response to environmental changes. On the basis of genomic information, it has been found that Aspergillus nidulans, a model species of fungi, includes 15 histidine kinases (HKs), one histidine-containing phosphotransmitter protein (HPt), and four response regulators (RRs) as factors related to the signal transduction system. In this review, it is explain that the His-Asp phosphorelay system is important in controlling cell growth (responses to fungicides, the induction of asexual and sexual development, and so on) under different growth conditions with reference to A. nidulans.

Key words: His-Asp phosphorelay signal transduction; histidine kinase; Aspergillus nidulans; stress response

His-Asp phosphorelay signal transduction systems were originally studied as bacterial systems (Fig. 1A) that control the expression of genes in response to environmental stresses.\(^1,2\) Sensor histidine kinases (HKs) are usually localized in the inner membranes of bacterial cells, and sense some environmental changes, autophosphorylate their histidine residues, and transfer phosphate to the aspartic acid residues of cognate response regulators (RRs). Osmo-regulation of outer membrane proteins OmpF and OmpC by EnvZ (HK)/OmpR (RR), chemotaxis by CheA (HK)/CheY (RR), and gene regulation of nitrogen metabolism by NtrA (HK)/NtrC (RR) are better understood mechanisms at the molecular level in Escherichia coli. Determination of the entire genome sequence of E. coli has indicated the existence of 28 HKs (including for eukaryotic HKs, see below) and 32 RRss, each of which pairs up with one of the HKs or RRss, meaning that E. coli can respond to about 30 different environmental signals.\(^3\)

In eukaryotes, the factors involved in His-Asp phosphorelay systems are more complicated than in prokaryotes.\(^1,2,4\) (Fig. 1B). Eukaryotic HKs are localized in the cytosol of cells, except for a few HKs (Sln1 of Saccharomyces cerevisiae, TcsB of A. nidulans, and so on). HKs are a hybrid type, because they include an RR domain in the C-terminal part of the protein, receiving phosphate signals from the HK domain in the N-terminal part. Thus the phosphate is transferred to aspartic acid residues in other RR proteins through histidine-containing phosphotransmitter proteins, called HPts. This means that the continual phosphotransfer of His (HK) to Asp (RR) occurs in the transmission of signals inside the cells. This review explains that His-Asp phosphorelay systems in filamentous fungi, A. nidulans, are important to the control of cell growth, including development. Comprehensive networks of phosphotransfers among the signal transduction factors might be constructed to achieve responses to various environmental conditions.

I. Osmo-Regulation in S. cerevisiae through the His-Asp Phosphorelay System

Before discussing the systems of A. nidulans, the system of S. cerevisiae should be considered, because the first eukaryotic His-Asp phosphorelay system reported comprised osmo-regulation in S. cerevisiae.\(^4–6\) (Fig. 2). In S. cerevisiae, Sln1 is the sole sensor histidine kinase protein. It is localized in the cytoplasmic membrane. Several in vivo studies have indicated that Sln1 exhibits autophosphorylation activity under normal growth conditions and transfers the phosphate signals to downstream regulatory factors (Fig. 2A), but under high osmolarity conditions Sln1 loses this activity, and this results in increased amounts of unphosphorylated forms of Ssk1 (RR), which activates a mitogen-activated protein pathway (the HOG pathway) (Fig. 2B). This transient activation of the HOG pathway is necessary for adaptation to osmotic stress, but continual abnormal activation under high osmolarity can cause the cell death in S. cerevisiae. Sln1, Ypd1 (HPt), and Ssk1 have also been purified from E. coli, and phosphotransfer among them has been observed in vitro.\(^7\)

II. Involvement of the His-Asp Phosphorelay Signal Transduction System in Susceptibility to Fungicides in Fungi

Of filamentous fungi, it is known that HKs classified as type III, are involved in sensitivity to some fungicides, viz., dicarboximides and phenylpyrroles.
Phenylpyrrole fungicides are derived from the natural bacterial antibiotic pyrrolnitrin produced by *Pseudomonas pyrrocinia*. It has been found that these molecules interfere with osmoregulation in filamentous fungi. Many different mutations of *Neurospora crassa* NIK-1 (OS-1)\(^{8,9}\) and related HK genes in other fungal pathogens lead to resistance to fungicides.\(^{10–13}\) This indicates that NIK-1 HKs are involved in the sensitivity in the wild types. Recent studies have also identified NikA as an HK that responds to the presence of a fungicide in *A. nidulans* (as described below).\(^{14,15}\)

**III. Control of Asexual and Sexual Development in *A. nidulans* through His-Asp Phosphorelay Systems**

*A. nidulans* is a homothallic ascomycete that has two major reproductive cycles, sexual and asexual. In

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**Fig. 1.** His-Asp Phosphorelay Signal Transduction Systems in Prokaryotes (A) and Eukaryotes (B).

(A) Bacterial His-Asp phosphorelay signal transduction systems control the expression of genes in response to environmental stresses. Sensor histidine kinases recognize certain environmental changes as signals, autophosphorylate on their own histidine residues, and transfer the phosphates to aspartic acid residues of the cognate response regulators. (B) In eukaryotes, HKs are of hybrid type and include the RR domain in the C-terminal part of the proteins, receiving phosphate signals from the HK domain in the N-terminal part of the proteins. Then the phosphates are transferred to aspartic acid residues on other RR proteins through histidine-containing phosphate transmitter proteins, called HPts. This means that continual phosphotransfer of His (HK) Asp (HK) His (HPt) Asp (RR) occurs to transmit the outside signals inside the cells.

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**Fig. 2.** Osmo-Regulation of *Saccharomyces cerevisiae*.

In *S. cerevisiae*, Sln1 is an osmosensing histidine kinase protein, that has autophosphorylation activity under normal growth condition and transfers phosphate signals to downstream regulatory factors (A). Under conditions of high osmolarity (B), Sln1 stops the activity, resulting in increased amounts of unphosphorelated forms of Ssk1 (RR), which activate the mitogen-activated protein pathway (the HOG pathway).
Asexual development, specialized reproductive structures called conidiophores are formed and millions of asexual spores called conidia are produced. In sexual development, mycelia aggregate to yield hülle cells, and ascospore-bearing organs called cleistothecia are generated. Initiation of the sexual reproductive cycle takes place shortly after conidiation begins. Environmental factors including light, CO$_2$, surface exposure, nutritional state, amino acids, and hormones also influence sexual development, but the details of the mechanisms by which these signals are perceived and transduced have not been well elucidated.

Several groups have reported that His-Asp phosphorelay signaling proteins are involved in the control of cell growth and the asexual and sexual development of *A. nidulans*, in addition to fungicide sensing, as described above. On the other hand, whole-genome sequencing offers an opportunity to identify all the HK, HPt, and RR genes in a given organism. The recently sequenced model filamentous fungus *A. nidulans* possesses 15 putative HK genes, four putative RR genes, and one HPt gene. Next for the detailed function of signaling factors, it will be explained that complicated regulation with combinations of His-Asp phosphorelay systems and other regulatory proteins exists.

1. **TcsA**

*A. nidulans* TcsA (a two-component signaling protein) can regulate the formation of asexual spores (conidia). TcsA encodes both a histidine kinase domain and a response regulator domain similar to those found in bacterial, lower eukaryotic, and plant members of the two-component family of proteins, while two PAS domains in the amino-terminal region of the predicted TcsA product might monitor the signal that regulates the tcsA histidine kinase-response regulator phosphorelay. While tcsA is nonessential for vegetative growth, the tcsA deletion mutant is unable to produce normal forms and numbers of conidia, differently from the wild-type strain. One group has reported that a basic-region helix-loop-helix (bHLH) protein-encoding gene (*devR*) is involved in the development of *A. nidulans*. The phenotype of the *devR* deletion strain is similar to that of the tcsA deletion strain. Localization of DevR is dependent on TcsA, because the DevR-Egfp protein fusion has been detected in the cytoplasm of the tcsA deletion strain, whereas DevR is exclusively located in the nuclei of the tcsA wild-type strain. These results indicate that DevR is part of the TcsA signal transduction network and that it is required for development under standard growth conditions.

2. **TcsB**

*tcsB*, an HK gene related to yeast osmosensor *sln1* in *A. nidulans*, was isolated from an *A. nidulans* cDNA library and introduced into a temperature-sensitive osmosensing-defective *sln1-ts* yeast mutant. Overexpression of the *tcsB* cDNA suppressed the lethality of the *sln1-ts* mutant. In addition, introduction of the *tcsB* cDNA into an *sln1 sho1* yeast double mutant, which lacked two osmosensors, suppressed lethality in high-salinity media and activated HOG1 MAPK. These results suggest that TcsB functions as an osmosensor histidine kinase in yeast. An *A. nidulans* strain lacking the *tcsB* gene was constructed and examined, but unexpectedly, the *tcsB* deletion mutant did not exhibit a detectable phenotype for either hyphal development or morphology on standard or stress media. This suggests that *A. nidulans* has more complex, robust osmoregulatory systems than the yeast Sln1-HOG MAPK cascade, suggesting the possibility that others among the 15 histidine kinases also work as osmosensors in *A. nidulans*. 

![Fig. 3. Members of the His-Asp Signal Transduction Systems in *A. nidulans* and Their Structural Domain Based on Estimated Amino Acids.](http://smart.embl-heidelberg.de/)

Analysis of the functional domain was performed with the SMART (Simple Modular Architecture Research Tool) program (http://smart.embl-heidelberg.de/). Among the four RR proteins, SrrB is an ortholog of *S. cerevisiae* Rim15p, and does not include the estimated phosphate accepting site (the aspartic acid residue) in the RR domain of SrrB. Domain H includes a histidine kinase A domain and a histidine kinase ATPase domain. D represents a receiver domain. PAS, PAS domain; PAC, C-terminal domain of PAS motif; STKc, unidentified protein kinase domain; GAF, GAF domain; HAMP, histidine kinase-adenylcyclase-methyl binding protein-phosphatase domain; HPt, histidine-containing phosphate transmitter protein; HSF, heat shock factor domain; STKc, Ser/Thr protein kinase domain.
3. FphA
Light sensing is very important for organisms in all biological kingdoms to adapt to changing environmental conditions. In photosynthetic organisms, including cyanobacteria, phytochrome photoreceptors sense red and far-red light, and regulate the time of flow and other responses, including the germination of seeds, elongation of seedlings, the size, shape, and number of leaves and so on. It has been found previously in *A. nidulans* that asexual sporulation is stimulated and sexual development is repressed by red light. The effect is reminiscent of a phytochrome response, indeed phytochrome-like proteins have been detected in several fungal genomes. All fungal homologs are more similar to bacterial than to plant phytochromes, and have multifunctional domains in which the phytochrome region and the histidine kinase domain are combined in a single protein with a C-terminal response-regulator domain. The latest finding is of *A. nidulans* phytochrome FphA, which binds a biriderin chromophore, acts as a red-light sensor, and represses sexual development under red-light conditions. It has also been found that FphA is part of a protein complex containing LreA and LreB, the orthologs of two central components, WC-1 and WC-2, of the *N. crassa* blue-light-sensing system. Surprisingly, FphA interacts with LreB and with VeA, another regulator involved in light sensing and mycotoxin biosynthesis. LreB also interacts with LreA. All protein interactions occur in the nucleus. This light sensing mechanism of *A. nidulans* is the best example of regulatory mechanisms in which His-Asp phosphorelay systems are correlated with another regulatory factor.

4. NikA
It has been reported that the *A. nidulans* signaling pathway, including NikA (HK), SskA (RR), and SrrA (RR), is implicated in responses to fungicides (a dicarboximide-derivative and a phenylpyrrole-derivative) and other several stress conditions. As the sensing mechanism of this light responses, including the germination of seeds, elongation of seedlings, the size, shape, and number of leaves and so on. It has been found previously in *A. nidulans* that asexual sporulation is stimulated and sexual development is repressed by red light. The effect is reminiscent of a phytochrome response, indeed phytochrome-like proteins have been detected in several fungal genomes. All fungal homologs are more similar to bacterial than to plant phytochromes, and have multifunctional domains in which the phytochrome region and the histidine kinase domain are combined in a single protein with a C-terminal response-regulator domain. The latest finding is of *A. nidulans* phytochrome FphA, which binds a biriderin chromophore, acts as a red-light sensor, and represses sexual development under red-light conditions. It has also been found that FphA is part of a protein complex containing LreA and LreB, the orthologs of two central components, WC-1 and WC-2, of the *N. crassa* blue-light-sensing system. Surprisingly, FphA interacts with LreB and with VeA, another regulator involved in light sensing and mycotoxin biosynthesis. LreB also interacts with LreA. All protein interactions occur in the nucleus. This light sensing mechanism of *A. nidulans* is the best example of regulatory mechanisms in which His-Asp phosphorelay systems are correlated with another regulatory factor.

5. HysA
Recently, the expression levels of all HKs, HPt, and RRs were determined at various stages of cell development in *A. nidulans* to confirm the pathway of phosphorelay signal transduction among them (unpublished data). Based on the results of expression analysis, we attempted to determine the function of hysA, which encodes a histidine kinase that is much expressed in growth under low-oxygen conditions. Analysis of hysA deletion and overexpression and phosphotransfer mutant strains indicated that HysA is involved in the low-oxygen growth of *A. nidulans*. The function of HysA and the signal transduction system has not yet been reported in detail.

IV. Evidence of *in Vitro* Phosphotransfer among His-Asp Phosphorelay Signaling Proteins in *A. nidulans*
It is important to document direct phosphotransfer from the 15 HKs to YpdA, and from YpdA to four RRs, all of which are derived from *A. nidulans*. Such direct evidence would be helpful in order to establish the entire networks of phosphotransfer among them. It has been reported that there are obvious results of phosphotransfer from *A. nidulans* HPt, YpdA to two RRs, SskA and SrrA, with the use of *E. coli* HK ArcB *in vitro*. It is uncertain which *A. nidulans* HKs phosphorylate SskA, SrrA through YpdA, without any active *A. nidulans* HKs *in vitro*, but these *in vitro* experiments were the first evidence that indicated direct phosphotransfer among His-Asp signaling proteins in filamentous fungi. Recently, the recombinant FphA (fungal phytochrome) protein was purified, and autophosphorylation activity dependent on the light signal was observed. Our groups succeeded in purifying enough active HysA protein, which is assumed to be a redox sensor controlling *A. nidulans* development. Specific phosphotransfer from HysA to RR proteins through YpdA could be observed under redox conditions.
V. Regulation of Signal Transduction Networks

It is important to consider how *A. nidulans* regulates signal transduction networks among the 15 HKs, YpdA, and four RRs dependent on signals (Fig. 3), because *A. nidulans* has only one YpdA to transmit the phosphate signals of the 15 HKs to the four RRs and must cause appropriate responses to these stress signals. To understand the control of the signal transduction networks, all the protein expression levels involved in the signal transduction systems were analyzed at different growth stages of *A. nidulans*. RNA samples were prepared with vegetative cells cultivated in liquid minimal medium and were used for quantitative real-time PCR. It was found that most of HKs are not expressed in vegetative growth, but expression of them was induced after vegetative cells were laid on minimal medium plates in order to induce asexual development. In the transformants containing the *tcsBp* and *nikAp-gfp* genes, GFP fluorescence was observed in all areas of the conidial heads and stalks. On the other hand, for the *fphA* construct, fluorescence was observed in the conidial heads but not in the stalks. For the *hysA* and *hk8-5* constructs, stronger fluorescence was observed in the conidia. It must be noted, however, that these analyses are too qualitative.

VI. Characteristic Aspects of the His-Asp Phosphorelay Signal Transduction Systems in *A. nidulans*

As explained above, bacterial His-Asp phosphorelay systems include about 30 different pairs of sensor histidine kinases and response regulators in order to respond to similar counts of environmental stresses. Most bacterial response regulators are transcriptional factors that directly regulate target gene expression under stress conditions. Inside bacterial cells, phosphate signals are never disordered or confused as between different pairs, even if these signaling proteins have high amino acid homology to transmit phosphate signals.

Eukaryotes, His-Asp phosphorelay signal transduction is done by upstream regulatory systems, and their signals are transmitted to other downstream pathways. For example, *Saccharomyces cerevisiae* has one HK (Sln1), one HPh (Ypd1), two RRs (Ssk1, and Skn7). The osmotic signal of *S. cerevisiae* is transmitted from the His-Asp phosphorelay system (Sln1-Ypd1-Ssk1) to the HOG MAPK pathway. On the other hand, Skn7 is a direct transcriptional regulator receiving osmotic stress signals from Sln1 through Ypd1. Skn7 also plays important role in oxidative stress resistance, but the role of Skn7 in oxidative stress is not dependent on Sln1, although Skn7 cooperates with redox-responsive transcription factor Yap1 in activating many of the key oxidative stress response genes.

The His-Asp phosphorelay systems in *A. nidulans* might be more complicated than in *S. cerevisiae*. The orthologs of Sln1 (HK), Ssk1 (RR) and Skn7 (RR) are conserved in *A. nidulans*. Moreover, in all, *A. nidulans* includes 15 HKs, one YpdA, and four RRs. The functions of some HKs and RRs remain unclear. It should be considered that there are comprehensive networks of signal transduction dependent on *A. nidulans* developments. Many data are needed to conclude that the His-Asp phosphorelay signal transduction system in *A. nidulans* is essential for higher adaptation to environmental stresses.

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