J. Biochem. 125, 1061-1066 (1999)

A Fission Yeast Gene (*prr1*$^+$) That Encodes a Response Regulator Implicated in Oxidative Stress Response[1]

Ryusuke Ohmiya, Chinatsu Kato, Hisami Yamada, Hirofumi Aiba, and Takeshi Mizuno[2]

Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464-8601

Received February 23, 1999; accepted March 10, 1999

An inspection of the *Schizosaccharomyces pombe* genome database revealed that this eukaryotic microorganism possesses a gene that may encode a bacterial type of histidine-to-aspartate (His-Asp) phosphorelay component, namely, a response regulator. The predicted gene, named *prr1*$^+$ (*S. pombe* response regulator), encodes a protein that contains a typical phospho-accepting receiver domain, preceded by a mammalian heat shock factor (HSF)-like DNA-binding domain. Inactivation of this *prr1*$^+$ gene resulted in mutant cells defective in some aspects of stress responses, including sensitivity to oxidative stress, cold-temperature, and heavy metal toxicity. It was also demonstrated that Prr1 is required for the transcription of some genes (*e.g.*, *trr1*$^+$, *ctt1*$^+$), which are induced by oxidative stress. These results suggest that a His-Asp phosphorelay system may be involved in a stress-activated signaling pathway in *S. pombe*.

Key words: His-Asp phosphorelay, response regulator, *Schizosaccharomyces pombe*, signal transduction, stress response.

In general, protein phosphorylation is one of the most commonly used mechanisms for regulating biological processes, including intracellular signal transduction. In eukaryotes, the cascades of protein phosphorylation events involving a number of protein tyrosine or serine/threonine kinases have been well documented. In contrast, bacteria have evolved a quite different phosphotransfer signaling mechanism for eliciting a variety of adaptive responses to their environment (for reviews, see Refs. *1-3*). The bacterial signal transduction mechanism is generally referred to as a "histidine-to-aspartate (His-Asp) phosphorelay system" (or a "two-component regulatory system") (for reviews, see Refs. *4-6*). The His-Asp phosphorelay is characterized by three types of common signal transducers, a sensor exhibiting histidine (His)-kinase activity, a response regulator containing a phospho-accepting receiver, and a histidine-containing phosphotransmitter (HPt). To date, numerous instances of His-Asp phosphorelay systems have been reported in many bacterial species (*6*).

The His-Asp phosphorelay system was once thought to be restricted to prokaryotes. However, many instances have recently been discovered in diverse eukaryotic species including yeast (*7, 8*), fungi (*9*), slim molds (*10-12*), and higher plants (*13, 14*). The best characterized is the osmo-responsive signal transduction of the budding yeast, *Saccharomyces cerevisiae* (for a review, see Ref. *15*). Three components, Sln1p (sensor kinase)-Ypd1p (HPt phosphotransmitter)-Ssk1p (response regulator), are involved together in the His-Asp phosphorelay signaling pathway. A striking fact is that this yeast signaling pathway is directly integrated into a classical MAPK (mitogen-activated protein kinase) signaling cascade termed the HOG1 (high osmolarity glycerol response) MAPK cascade. However, *S. cerevisiae* does not appear to make extensive use of such His-Asp phosphorelay systems. It has only one other response regulator, named Skn7p, which has been suggested to be involved in several distinct processes including cell wall assembly, oxidative stress response, and G1 cyclin gene expression (*16-22*). Although a possible link between the Sln1p-Ypd1p-Ssk1p phosphorelay and Skn7p function was recently demonstrated (*23, 24*), the underlying mechanism of this presumed cross phosphotransfer signaling is not fully understood.

The fission yeast, *Schizosaccharomyces pombe*, is an alternative model microorganism with which to gain an insight into how a bacterial type of signal transduction mechanism is integrated into the classical MAPK signaling cascades. In *S. pombe*, a stress-activated MAPK cascade has recently been well characterized; it includes MAPK Sty1 (also known as Spc1 and Phh1), MAPKK Wis1, and MAPKKK Wis4 (also known as Wik1 and Wak4) (*25-35*). The Sty1 MAPK cascade appears to be analogous to the *S. cerevisiae* HOG1 MAPK cascade. From a physiological viewpoint, however, the Sty1 MAPK cascade more closely resembles the mammalian stress-activated protein kinase (SAPK) cascade, in which c-Jun N-terminal kinases (JNKs) are involved (for reviews, see Refs. *36* and *37*). On the other hand, the presence of a His-Asp phosphorelay system in *S. pombe* is not yet recognized. However, the recent genome sequence project for *S. pombe* revealed the existence of several putative genes, each of which seems to encode a component of a His-Asp phosphorelay components. To gain insight into this His-Asp phosphorelay

[1] This work was supported by a Grant-in-Aid (09274102) for scientific research on a priority area from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Phone: +81-52-789-4089, Fax: +81-52-789-4091, E-mail: tmizuno@agr.nagoya-u.ac.jp

system, if present in *S. pombe*, we characterized one of the putative genes that may encode a response regulator, and demonstrate that this gene is essential for stress responses.

MATERIALS AND METHODS

Strains and Media—The *S. pombe* strains used in this study are listed in Table I. The strains were grown in YES medium composed of 0.5% yeast extract (Difco) and 3% glucose, supplemented with appropriate growth requirements in standard amounts (*38*), unless otherwise noted.

Recombinant DNA Techniques—The conditions used for the DNA-manipulating enzymes, such as restriction endonucleases, a DNA-ligation kit, and *Escherichia coli* DNA polymerase I, were those recommended by the suppliers (Takara Shuzo or Toyobo). Other recombinant DNA techniques including polymerase chain reaction (PCR), were carried out according to standard laboratory manuals (*39*).

Yeast Genetics—Standard procedures for *S. pombe* genetics were those described previously (*40, 41*).

Gene Disruption—The one-step gene disruption method was employed to construct the *prr1* deletion strains (*42*). A 2.0 kb PCR-fragment containing the *prr1*$^+$ gene was cloned onto the *Sma*I-*Sal*I sites of pUC119. The *S. pombe ura4*$^+$ gene was inserted into the *Eco*T22I site located at the center of the *prr1*$^+$ coding sequence (see Fig. 1A). Stable Ura$^+$ transformants were selected for a deploid strain (JY741×JY746), and then the existence of the *prr1*::*ura4*$^+$ allele was confirmed by Southern hybridization with an appropriate probe. After sporulation, Ura$^+$ haploid-segregants were analyzed.

Northern Hybridization Analysis—For Northern hybridization analysis, exponentially growing cells in YES medium were collected and re-suspended in fresh YES medium or the same medium containing 1.0 M KCl or 0.2 mM H_2O_2, and then incubated for 30 min at 30°C. Total RNA was prepared from the collected cells (*43*). RNA (10 μg) was analyzed in a 1.4% agarose gel containing formaldehyde, and then alkaline-blotted onto Hybond-N+ (Amersham Int.). Hybridization was carried out for 2 h at 65°C in Rapid-hyb buffer with appropriate ^{32}P-labeled probes, containing either the *gpd1*$^+$, *ctt1*$^+$, *trr1*$^+$, or *leu1*$^+$ coding sequences, as recommended by the supplier (Amersham Int.).

RESULTS

Existence of an S. pombe Gene That May Encode a Response Regulator—As shown schematically in Fig. 1A, an inspection of a recently released genome sequence of *S. pombe* (chromosome I, consmid c8C9, GenBank accession no. Z99168) revealed a 36,310 bp sequence that appears to encompass a putative gene (named SPAC8C9.14), which may encode a response regulator-like protein. The predicted coding sequence is separated by four introns, and presumably encodes a protein of 539 amino acids. The inspection of the GenBank database by the BLAST and FASTA programs showed this amino acid sequence to be similar to that of the *S. cerevisiae* Skn7p transcription factor (622 amino acids) (*16-24*), as shown schematically in Fig. 1B (32.8% identity in 524 amino acid overlap). Both contain a receiver-like domain in their C-terminal sequence that might serve as a phospho-acceptor in a His-Asp phosphorelay. In general, a typical receiver of about 120 amino acids exhibits a phospho-accepting ability to be modified at a certain aspartate residue (D1), located near the center (*3, 6*, and see Fig. 1B). Together with this critical aspartate residue, two other amino acids are conserved exclusively among the receiver family of proteins, another aspartate residue (D2) at the N-terminal end, and a lysine residue (K) at the C-terminal end. All these characteristics are found in the *S. pombe* gene product (Fig. 1B, D1=D418, D2=D375, K=K468). Furthermore, the *S. pombe* protein contains an N-terminal sequence highly similar to those of mammalian heat shock factors (HSFs) (*e.g.*, the mouse HSF-2, SwissProt accession no. P38533, 24.7% identity in 352 amino acid overlap), which contain HSF-type DNA-binding domains (Fig. 1B). Thus, this inspection prompted us to characterize the putative gene (hereafter named *prr1*$^+$, *pombe* response regulator), as a first step in gaining insight into the presumed His-Asp phosphorelay signal transduction in *S. pombe*.

Construction of a Mutant with prr1::ura4$^+$—To confirm the existence of the sequence in question in the *S. pombe* genome, a region of about 2.0 kb extending from the predicted ATG initiation codon of *prr1*$^+$ to the TAA termination codon was amplified by polymerase chain reaction (PCR), and then the product was cloned onto an *E.*

TABLE I. **List of *S. pombe* strains relevant to this study.**

Strain	Genotype/relevant remarks	Source
JY333	*h*$^-$ *leu1 ade6-M216*	M. Yamamoto
JY741	*h*$^-$ *leu1 ade6-M216 ura4-D18*	M. Yamamoto
JY746	*h*$^+$ *leu1 ade6-M210 ura4-D18*	M. Yamamoto
JM544	*h*$^-$ *leu1 ura4-D18 wis1::ura4*$^+$	P. Russell
JM1160	*h*$^-$ *leu1 ade6-M216 ura4-D18 sty1::ura4*$^+$	J. Millar
JX305	*h*$^-$ *leu1 ade6-M216 ura4-D18 gad7(atf1)::ura4*$^+$	M. Yamamoto
TP108-3C	*h*$^-$ *leu1 his2 ura4-D18 pap1::ura4*$^+$	M. Yanagida
DP1	*h*$^-$ *leu1 ade6-M216 ura4-D18 prr1::ura4*$^+$	This work

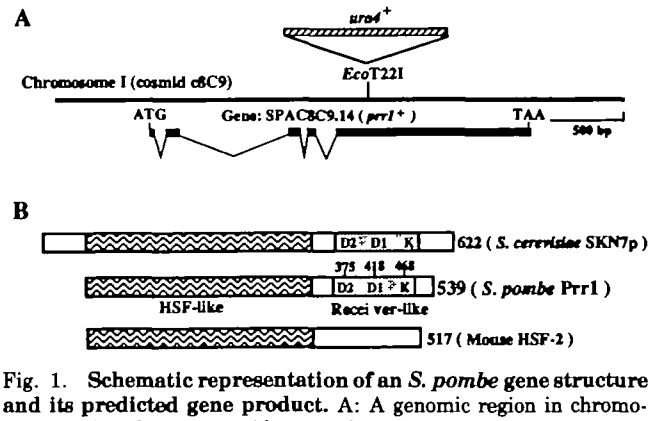

Fig. 1. **Schematic representation of an *S. pombe* gene structure and its predicted gene product.** A: A genomic region in chromosome-I, cloned onto cosmid no. c8C9, containing a putative gene (named SPAC8C9.14 in the database, and hereafter named *prr1*$^+$) is shown schematically (GenBank accession no. Z99168), where a putative gene is located. The gene was deduced to comprise six exons (bold lines) and five introns (thin lines). B: The structure of the *S. pombe* gene product (named Prr1) was compared schematically with those of the Skn7p transcription factor of *S. cerevisiae* and the HSF-2 heat shock factor of mouse. Other details are described in the text.

coli plasmid (see Fig. 1A). Identity was confirmed by nucleotide sequencing and restriction mapping with various endonucleases (data not shown). Since this putative gene was predicted to contain several introns, we also needed to isolate its corresponding cDNA in order to confirm that this is indeed a transcribable gene. The *prr1*$^+$-cDNA was isolated from an *S. pombe* cDNA bank by means of PCR with appropriate primers, and its identity and integrity were confirmed by nucleotide sequencing. The results showed that the predicted *prr1*$^+$ gene is functional, and also that its exon-intron assignment is correct.

The cloned genomic DNA segment was used to carry out a one-step gene disruption utilizing a diploid parental strain (JY741 × JY746) and the *ura4*$^+$ marker in order to construct a *prr1::ura4*$^-$ mutant allele (Fig. 1A). The heterozygous diploids were sporulated, and tetrads were dissected. The resulting haploid cells carrying the presumed *prr1::ura4*$^+$ allele, confirmed by Southern hybridization analysis, were found to be viable on both YES and SD standard agar plates. On the assumption that the *prr1*$^+$ gene was knocked out in segregants by *ura4*$^+$ insertion, its genotype is referred to as "*prr1Δ*" for clarity. The phenotypes of the *prr1Δ* mutant were then characterized extensively.

prr1Δ Cells Exhibit Pleiotropic Phenotypes in Response to Environmental Stress—As mentioned above, the stress-activated Sty1 MAPK cascade appears to respond to a range of environmental stresses, including osmotic stress, oxidative stress, UV light, heat shock, and nutrient limitation (*25-35*). As a consequence, *sty1Δ* mutants exhibit highly pleiotropic phenotypes: osmosensitive, H_2O_2 sensitive, heat sensitive, UV sensitive *etc*. A set of transcription factors of the basic-leucine zipper (bZIP) type are known to function downstream of the Sty1 MAPK cascade. The first bZIP to be discovered was Atf1 (also known as Gad7), which shares homology with the mammalian transcription factor ATF-2 (Refs. *43-45*). The second more recently discovered was Pap1, whose structure and DNA-binding properties are similar to those of mammalian c-Jun (*46, 47*). Since *atf1Δ* mutants and *pap1Δ* mutants show non-overlapping subsets of the phenotypes observed in *sty1Δ* mutants, the occurrence of parallel signaling pathways downstream of the Sty1 MAPK cascade was proposed (*47*). For this reason, here we characterized *prr1Δ* cells in comparison with *sty1Δ*, *atf1Δ*, and *pap1Δ* cells with special reference to responses to various environmental stresses.

prr1Δ cells grow normally on YES standard agar plates at both 30°C and 37 C, but not at 20°C, showing a cold-sensitive phenotype (Fig. 2A). As also shown, *atf1Δ* cells exhibit cold-sensitivity for growth on YES agar plates (*43*). It was also found that *prr1Δ* cells are hypersensitive to heavy metal toxicity elicited by cadmium ($CdSO_4$), as has been reported for *sty1Δ* and *pap1Δ* cells (Fig. 2A) (*47*). In contrast to *sty1Δ* cells and *atf1Δ* cells (*48, 49*), however, both *prr1Δ* cells and *pap1Δ* cells do not show an osmosensitive phenotype on agar plates containing 2.2 M sorbitol (Fig. 2A). Also, *prr1Δ* cells are not hypersensitive to UV irradiation (data not shown). Another striking phenotype observed for *prr1Δ* cells is their hypersensitivity to H_2O_2-treatment, as in the case of *pap1Δ* cells (Fig. 2B). It should be noted that these mutational lesions observed for *prr1Δ* cells are suppressed by the introduction of *prr1*$^+$-cDNA on a plasmid (data not shown).

Thus, the inactivation of *prr1*$^+$ results in mutant cells sensitive to certain set of environmental stresses, and these pleiotropic phenotypes overlap somewhat with those observed for *pap1Δ* cells. In particular, it was revealed that both *prr1Δ* cells and *pap1Δ* cells are highly sensitive to oxidative stress.

Prr1 Is Required for Stress-Activated Gene Expression—Because *prr1Δ* cells were found to exhibit a clear phenotype, H_2O_2 sensitivity for growth, we tested whether or not Prr1 is directly responsible for the transcriptional induc-

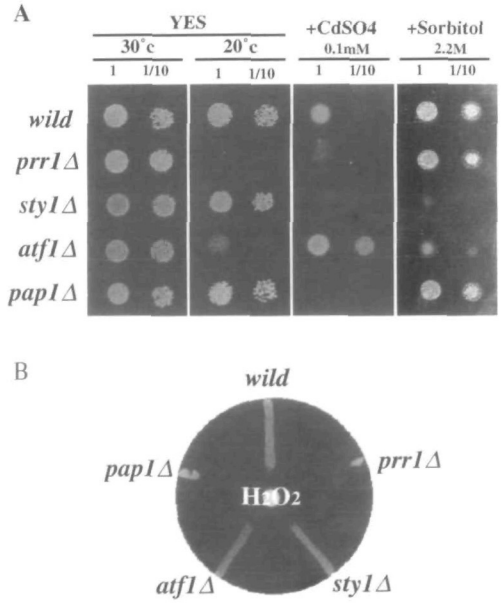

Fig. 2. **Growth of *S. pombe* cells on YES agar plates.** A: The *S. pombe* mutant strains characterized in this study were wild-type (JY333), *prr1Δ*, *sty1Δ*, *atf1Δ*, and *pap1Δ* (see Table I). Appropriate numbers of cells (1 = 10^4 and 1/10 = 10^3 cells) were spotted on YES agar plates, under the different conditions indicated, and the plates were incubated for 5 days. B: The same set of cells were streaked on YES agar plates, at the center of which a piece of Whatmann 3MM paper, previously soaked in H_2O_2 (30%), was placed. The plate was incubated at 30 C for 5 days.

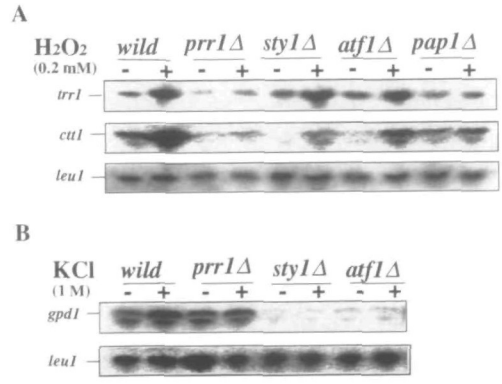

Fig. 3. **Northern hybridization analysis.** Total RNAs were isolated from the indicated strains. The cells were grown in YES medium, treated with either H_2O_2 (0.2 mM) or KCl (1.0 M) for 30 min as indicated (panels A and B, respectively), and analyzed by Northern hybridization with the indicated probes (*i.e., trr1, ctt1, gpd1,* and *leu1*). Note that the *leu1* probe was used for a loading control.

tion of two known *S. pombe* gene, *trr1*$^+$ and *ctt1*$^+$, in response to oxidative stress (*47, 50*). In *S. pombe*, the *trr1*$^+$ gene encodes thioredoxin, whereas the *ctt1*$^+$ gene encodes catalase, both of which have been reported to be induced at the transcriptional level by oxidative stress (*e.g.*, by H_2O_2) in a Sty1- or Pap1-dependent manner (*47*). The results of Northern hybridization analysis showed that both *trr1*$^+$ and *ctt1*$^+$ transcriptions are remarkably induced by external H_2O_2 oxidative stress in wild-type cells, but this induction is greatly reduced in *prr1Δ* cells (Fig. 3A). As reported previously (*47*), the levels of oxidative stress-induced *trr1*$^+$ and *ctt1*$^+$ transcription are significantly reduced in *pap1Δ* cells, but the extent seems to be less than in the case of *prr1Δ* cells, as shown in Fig. 3A. The results also show that Atf1 appears not to be required for the oxidative stress-induced *trr1*$^+$ and *ctt1*$^+$ expression (this observation is consistent with that reported previously) (*47*). Surprisingly, *trr1*$^+$ expression in *sty1Δ* cells is indistinguishable from that in wild-type cells in contrast to the previous data (*47*), although the expression of *ctt1*$^+$ is markedly lower in *styΔ* cells, as reported previously (*27*). Thus, to evaluate *atf1Δ* cells and *sty1Δ* cells more critically, it was necessary to examine osmotic stress-induced *gpd1*$^+$ transcription in our *atf1Δ* and *sty1Δ* mutants (Fig. 3B). Both *sty1Δ* and *atf1Δ* cells are known to exhibit osmo-sensitivity for growth (see Fig. 2), because one of the target of the Sty1 kinase and the Atf1 transcription factor is the *gpd1*$^+$ gene (*48, 49*), which is responsible for osmo-adaptive glycerol production. The results showed that *gpd1*$^+$ transcription does indeed depend on both Sty1 and Atf1, while Prr1 appears to do nothing about the osmotic induction of the *gpd1*$^+$ gene.

These results indicate that the expressions of *trr1*$^+$ and *ctt1*$^+$ are induced by oxidative stress in a manner that is largely dependent on both the Prr1 response regulator and the Pap1 transcription factor, but not on the Atf1 transcription factor. Note also that the importance of the Sty1 kinase for the oxidative stress-induced transcription of *trr1*$^+$ and *crr1*$^+$ is less clear, although this MAP kinase is critically required for the osmotic stress-induced expression of *gpd1*$^+$.

DISCUSSION

The results obtained in this study show that *S. pombe* possesses a gene (named *prr1*$^+$) that encodes a bacterial type of response regulator, which contains a potential phospho-accepting receiver implicated to be a His-Asp phosphorelay signaling domain. Furthermore, this *prr1*$^+$ gene was demonstrated to be crucially involved in a range of stress responses. *S. pombe* cells deficient in Prr1 show pleiotropic phenotypes, namely, hypersensitivity to oxidative stress (H_2O_2), heavy metal treatment (Cd^{2+}), and cold temperatures (20°C). In particular, the expression of both the *trr1*$^+$ and *ctt1*$^+$ genes is not fully induced in these mutant cells even when the cells are treated with H_2O_2. It was thus concluded that Prr1 plays an important role in oxidative stress response, most likely by regulating the transcription of a certain set of stress-activated genes including *trr1*$^+$ and *ctt1*$^+$.

The structural design of the Prr1 protein is similar to that of the *S. cerevisiae* transcription factor Skn7p (see Fig. 1B). In addition to their bacterial-type receiver domains, both contain mammalian heat shock factor (HSF)-like DNA-binding domains, followed by a nuclear localization signal (NLS). Skn7p was previously demonstrated to function as a DNA (promoter)-binding transcription factor localized predominantly in the nucleus (*16-24*). Because of its ability, shown here, to enhance the transcription of *trr1*$^+$ and *ctt1*$^+$, Prr1 also appears to be a DNA-binding transcription factor. As compared with Skn7p, however, Prr1 appears to be more deeply involved in stress responses in *S. pombe*, because the only phenotype observed for *skn7Δ* cells is a moderate growth sensitivity to oxidative stress (*16-24*). Thus, extensive and comparative studies on these proteins will shed light on the physiological significance of bacterial type of signal transducers in eukaryotic cells.

As described above, at least two bZIP-type transcription factors, Atf1 and Pap1, function downstream of the stress-activated Sty1 MAPK cascade (Fig. 4). In this respect, *prr1Δ* and *pap1Δ* (but not *atf1Δ*) mutants show an overlapping phenotype (*e.g.*, hypersensitivity to oxidative stress). This suggests the occurrence of parallel (Prr1- and Pap1-dependent) signaling pathways for oxidative stress response in *S. pombe*, which may converge on the downstream target genes for *trr1*$^+$ and *ctt1*$^+$ (Fig. 4). This should be tested by asking whether or not both Prr1 and Pap1 are capable of binding directly to the *trr1*$^+$ and *ctt1*$^+$ promoter region, and also by asking how the Prr1 and Pap1 activities are modulated through distinct signaling pathways in response to oxidative stress. In this respect, it was previously proposed that Pap1 activity is directly regulated by Sty1 kinase in response to oxidative stress (*47*), as in the case of Atf1 in response to osmotic stress. However, our laboratory-stocked *sty1Δ* cells, used in this study, are not as sensitive to H_2O_2 as *pap1Δ* cells, although the *sty1Δ* mutant cells are clearly osmo-sensitive (Figs. 2 and 3). This raises the question of whether or not Pap1 activity is regulated solely by Sty1 kinase in response to oxidative stress. In any event, the bZIP-type Pap1 transcription factor appears to be under the control of the classical MAPK cascade signaling pathway. Then, what about Prr1?

Based on the well-established scenario of the His-Asp phosphorelay system (see "Introduction"), it is tempting to speculate that the Prr1 activity may be regulated by phosphorylation at a certain aspartate residue (possibly D418) in its receiver domain through a presumed His-Asp phosphorelay signaling pathway (Fig. 4). If so, *S. pombe*

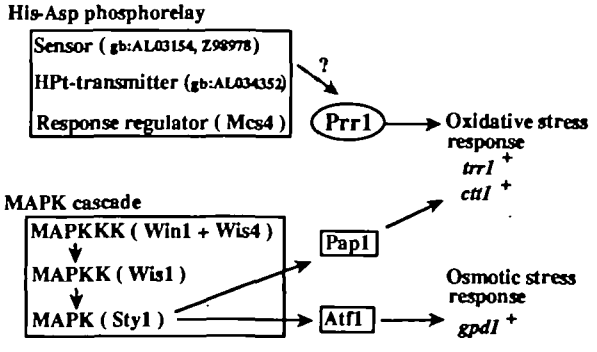

Fig. 4. **Schematic views of the presumed signaling pathways relevant to stress responses in *S. pombe*.** These views are based on a number of previous report, as cited in the text. Details are given in the "Introduction" and "DISCUSSION."

should possess other His-Asp phosphorelay components, such as sensor kinases, and HPt-transmitters (*6*). The only component reported so far for *S. pombe* is the Mcs4 response regulator, which has been suggested to play a role in osmotic stress response upstream of the Sty1 MAPK cascade, in osmotic stress response (Fig. 4) (*51, 52*). We then inspected the current genome database for *S. pombe* in order to search for genes encoding His-Asp phosphorelay components. This inspection revealed the presence of two typical sensor genes and one HPt-transmitter gene: sensor-1 with 2344 amino acids, GenBank accession no. AL031543 (gene SPCC70.04); sensor-2 with 2310 amino acids, GenBank accession no. Z98978 (gene SPAC227E.09); and HPt-1 with 295 amino acids, GenBank accession no. AL034352 (gene SPBC725.02) (Fig. 4). Each of these predicted sensors has a typical His-kinase domain (note that these may correspond to those described and named Mak1 and Mak2 in previous reports, see Ref. *51*). The predicted HPt-transmitter is similar to the *S. cerevisiae* Ypd1p HPt-transmitter (*15*). Thus, one can reasonably assume that a His-Asp phosphorelay signaling pathway(s) operates in *S. pombe* cells, as summarized in Fig. 4. The Prr1 response regulator may be linked to one or more of these uncharacterized components. This issue remains to be addressed. In short, the *prr1*$^+$ gene, characterized in this study, provides the first insight into the significance of His-Asp phosphorelay implicated in stress-activated responses in *S. pombe*.

REFERENCES

1. Stock, J.B., Ninfa, A.D., and Stock, A.M. (1989) Protein phosphorylation and regulation of adaptive response in bacteria. *Microbiol. Rev.* **53**, 450-490
2. Bourret, R.B., Borkovich, K.A., and Simon, M.I. (1991) Signal transduction pathways involving protein phosphorylation in prokaryotes. *Annu. Rev. Biochem.* **60**, 401-441
3. Parkinson, J.S. and Kofoid, E.C. (1992) Communication modules in bacterial signalling proteins. *Annu. Rev. Genet.* **26**, 71-112
4. Hoch, J.A. and Silhavy, T.J. (1995) *Two-Component Signal Transduction*, American Society for Microbiology, Washington, DC
5. 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
6. Mizuno, T. (1998) His-Asp phosphotransfer signal transduction. *J. Biochem.* **123**, 555-563
7. Maeda, T., Wurgler-Murphy, S.M., and Saito, H. (1994) A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* **369**, 242-245
8. Maeda, T., Takekawa, M., and Saito, H. (1995) Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. *Science* **269**, 554-558
9. Alex, L.A., Borkovich, K.A., and Simon, M.I. (1996) Hyphal development in *Neurospora crass*: involvement of a two-component histidine kinase. *Proc. Natl. Acad. Sci. USA* **93**, 3416-3421
10. Wang, N., Shaulsky, G., Escalante, R., and Loomis, W.F. (1996) A two-component histidine kinase gene that functions in *Dictyostelium* development. *EMBO J.* **15**, 3890-3898
11. Schuster, S.C., Noegel, A.A., Oehmen, F., Gerisher, G., and Simon, M.I. (1996) The hybrid histidine kinase DokA is part of the osmotic response system of *Dictyostelium*. *EMBO J.* **15**, 3880-3889
12. Chang, W.-T., Thomason, P.A., Gross, J.D., and Newell, P.C. (1998) Evidence that the RdeA protein is a component of a multistep phosphorelay modulating rate of development in *Dictyostelium*. *EMBO J.* **17**, 2809-2816
13. Chang, C., Kwok, S.F., Bleeker, A.B., and Meyerowitz, E.M. (1993) *Arabidopsis* ethylene-response gene ETR1: similarity of product to two-component regulators. *Science* **262**, 539-544
14. Kakimoto, T. (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science* **274**, 982-985
15. Wurgler-Murphy, S.M. and Saito, H. (1997) Two-component signal transducers and MAPK cascades. *Trend Biol. Sci.* **22**, 172-176
16. Alberts, A.S., Bouquin, N., Johnston, L.H., and Treisman, R. (1998) Analysis of PhoA-binding proteins reveals an interaction domain conserved in heteromeric G protein β subunits and the yeast response regulator protein Skn7. *J. Biol. Chem.* **273**, 8616-8622
17. Morgan, B.A., Banks, G.R., Toone, W.M., Raitt, D., Kuge, S., and Johnston, L.H. (1997) The Skn7 response regulator controls gene expression in the oxidative stress response of the budding yeast *Saccharomyces cerevisiae*. *EMBO J.* **16**, 1035-1044
18. Krems, B., Charizanis, C., and Entian, K.D. (1996) The response regulator-like protein Pos9/Skn7 of *Saccharomyces cerevisiae* is involved in oxidative stress resistance. *Curr. Genet.* **29**, 327-334
19. Johnston, L.H., Morgan, B.A., and Bouquin, N. (1996) A yeast transcription factor activating G1 cyclin expression has similarity to bacterial signal transduction protein. *Biochem. Soc. Trans.* **24**, 49-54
20. Morgan, B.A., Bouquin, N., Merrill, G.F., and Johnston, L.H. (1995) A yeast transcription factor bypassing the requirement for SBF and DSC1/MBF in budding yeast has homology to bacterial signal transduction protein. *EMBO J.* **14**, 5679-5689
21. Brown, J.L., Bussey, H., and Stewart, R.C. (1994) Yeast Skn7 function in a eukaryotic two-component regulatory pathway. *EMBO J.* **13**, 5186-5194
22. Brown, J.L., North, S., and Bussey, H. (1993) SKN7, a yeast multicopy suppressor of a mutation affecting cell wall beta-glucan assembly, encodes a product with domains homologous to prokaryotic two-component regulators and to heast shcok transcritpion factors. *J. Bacteriol.* **175**, 6908-6915
23. Li, S., Ault, A., Malone, C.L., Raitt, D., Dean, S., Johnston, L.H., Deschenes, R.J., and Fassler, J.S. (1998) The yeast histidine protein kinase, Sln1p, mediates phosphotransfer to two response regulators, Ssk1p and Skn7p. *EMBO J.* **17**, 6952-6962
24. Ketela, T., Brown, J.L., Stewart, R.C., and Bussey, H. (1998) Yeast Skn7 activity is modulated by the Sln1p-Ypd1p osmosensor and contributes to regulation of the HOG pathway. *Mol. Gen. Genet.* **259**, 372-378
25. Millar, J.B., Buck, V., and Wilkinson, M.G. (1995) Pyp1 and Pyp2 PTPases dephosphorylate an osmosensing MAP kinase controlling cell size at division in fission yeast. *Genes Dev.* **9**, 2117-2130
26. Shiozaki, K. and Russell, P. (1995) Cell-cycle control linked to extracellular environment by MAP kinase pathway in fission yeast. *Nature* **378**, 739-743
27. Degols, G., Shiozaki, K., and Russell, P. (1996) Activation and regulation of the Spc1 stress-activated protein kinase in *Schizosaccharomyces pombe*. *Mol. Cell Biol.* **16**, 2870-2877
28. Kato, T., Okazaki, H., Murakami, S., Stetter, P., Fantes, P., and Okayama, H. (1996) Stress signal, mediated by a Hog1-like MAP kinase, controls sexual development in fussion yeast. *FEBS Lett.* **378**, 207-212
29. Degols, G. and Russell, P. (1997) Discrete roles of the Spc1 kinase and the Atf1 transcription factor in the UV response of *Schizosaccharomyces pombe*. *Mol. Cell Biol.* **17**, 3356-3363
30. Samejima, I., Mackie, S., and Fantes, P.A. (1997) Multiple modes of activation of the stress-responsive MAP kinase pathway in fission yeast. *EMBO J.* **16**, 6162-6170
31. Gaits, F., Degols, G., Shiozaki, K., and Russell, P. (1998) Phosphorylation and association with the transcription factor Atf1 regulate localization of Spc1/Sty1 stress-activated kinase in fission yeast. *Genes Dev.* **12**, 1464-1473
32. Samejima, I., Mackie, S., Warbrick, E., Weisman, R., and Fantes, P.A. (1998) The fission yeast mitotic regulator *win1*$^+$ encodes an MAP kinase kinase kinase that phosphorylates and

activates Wis1 MAP kinase kinase in response to high osmolarity. *Mol. Biol. Cell* **9**, 2325-2335
33. Shieh, J.C., Martin, H., and Millar, J.B. (1998) Evidence for a novel MAPKKK-independent pathway controlling the stress activated Sty1/Spc1 MAP kinase in fission yeast. *J. Cell. Sci.* **111**, 2799-2807
34. Shieh, J.C., Wilkinson, M.G., and Millar, J.B. (1998) The Win1 mitotic regulator is a component of the fission yeast stress-activated Sty1 MAPK pathway. *Mol. Biol. Cell.* **9**, 311-322
35. Shiozaki, K., Shiozaki, M., and Russell, P. (1998) Heat stress activates fission yeast Spc1/Sty1 MAPK by a MEKK-independent mechanism. *Mol. Biol. Cell.* **9**, 1339-1349
36. Robinson, M.J. and Cobb, M.H. (1997) Mitogen-activated protein kinase pathways. *Curr. Opin. Cell Biol.* **9**, 180-186
37. Wilkinson, M.G. and Millar, J.B. (1998) SAPKs and transcription factors do the nucleocytoplasmic tango. *Genes Dev.* **12**, 1391-1397
38. Moreno, S., Hayles, J., and Nurse, P. (1991) *Methods Enzymol.* **194**, 795-823
39. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (2nd ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
40. Gutz, H., Heslot, H., Leupold, U., and Lopreno, N. (1974) *Schizosaccharomyces pombe* in *Handbook of Genetics* (King, R.C., ed.) Vol. 1, pp. 395-446, Plenum Press, New York
41. Rothestein, R.J. (1983) One-step gene disruption in yeast. *Methods Enzymol.* **101**, 202-211
42. Elder, R.T., Loh, E.Y., and Davis, R.W. (1983) RNA from the yeast transposable element TY1 has both ends in the direct repeats, a structure similar retrovirus RNA. *Proc. Natl. Acad. Sci. USA* **80**, 2432-2436
43. Kanoh, J., Watanabe, Y., Ohsugi, M., Iino, Y., and Yamamoto, M. (1996) *Schizosaccharomyces pombe gad7*$^+$ encodes a phosphoprotein with a bZIP domain, which is required for proper G1 arrest and gene expression under nitrogen starvation. *Genes Cells* **1**, 391-408
44. Shiozaki, K. and Russell, P. (1996) Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. *Genes Dev.* **10**, 2276-2288
45. Wilkinson, G.M., Samuels, M., Takeda, T., Toone, M.W., Shieh, J., Toda, T., Millar, J.B., and Jones, N. (1996) The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. *Genes Dev.* **10**, 2289-2301
46. Toda, T., Shimanuki, Y., and Yanagida, M. (1991) Fission yeast genes that confer resistance to staurosporine encode an AP-1-like transcription factor and a protein kinase related to the mammalian ERK1/MAP2 and budding yeast FUS3 and KSS1 kinases. *Genes Dev.* **5**, 60-73
47. Toone, W.M., Kuge, S., Samuels, M., Morgan, B.A., Toda, T., and Jones, N. (1998) Regulation of the fission yeast transcription factor Pap1 by oxidative stress: requirement for the nuclear export factor Crm1 (Exportin) and the stress-activated MAP kinase Sty1/Spc1. *Genes Dev.* **12**, 1453-1463
48. Aiba, H., Yamada, H., Ohmiya, R., and Mizuno, T. (1995) The osmo-inducible *gpd1*$^+$ gene is a target of the signaling pathway involving Wis1 MAP-kinase kinase in fission yeast. *FEBS Lett.* **376**, 199-201
49. Ohmiya, R., Yamada, H., Nakashima, K., Aiba, H., and Mizuno, T. (1995) Osmoregulation of fission yeast: cloning of two distinct genes encoding glycerol-3-phosphate dehydrogenase, one of which is responsible for osmotolerance for growth. *Mol. Microbiol.* **18**, 963-973
50. Nakagawa, C.W., Yamada, K., and Mutoh, N. (1998) Two distinct upstream regions are involved in expression of the catalase gene in *Schizosaccharomyces pombe* in response to oxidative stress. *J. Biochem.* **123**, 1048-1054
51. Shieh, J.C., Wilkinson, M.G., Buck, V., Morgan, B.A., Makino, K., and Millar, J.B. (1997) The Mcs4 response regulator coordinately controls the stress-activated Wak1-Wis1-Sty1 MAP kinase pathway and fission yeast cell cycle. *Genes Dev.* **11**, 1008-1022
52. Shiozaki, K., Shiozaki, M., and Russell, P. (1997) Mcs4 mitotic catastrophe suppressor regulates the fission yeast cell cycle through the Wik1-Wis1-Spc1 kinase cascade. *Mol. Biol. Cell* **8**, 409-419