and plating onto both LB-Sm and LB-Nal plates. The media we used have been described [J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972)].

12. Cells from an aged ZK126 culture were mixed with a young ZK126 Nal^R culture. Dilutions made in M63 were plated on LB and LB Nal media to determine the total number of viable organisms and the number containing the Nal^R marker, respectively. After 8 days, the number of Nal^R CFU per milliliter dropped from 10^9 to 10^6, which indicates that cells from the aged ZK126 culture were taking over the population.
13. Aged cultures and cells that had overtaken young cells in a mixed culture were streaked out on LB plates with the appropriate antibiotic. Isolated colonies were then grown in liquid LB for 1 day. We then tested these cultures by mixing them as a minority with young cultures.
14. D. E. Bohannon *et al.*, *J. Bacteriol.* **173**, 4482 (1991).
15. To clone the wt *rpoS* and *rpoS819*, Kpn I–digested chromosomal DNA was ligated to pUC19 and used to transform a strain bearing the *rpoS::kan* mutation. Because cells develop an *rpoS*-dependent resistance to low pH [P. L. C. Small and S. Falkow, *ASM Abstr.* **B74**, 38 (1992)], ampicillin-resistant transformants (selected at 30°C) were incubated for 30 min in LB (pH 2.5) to select for plasmids that harbor the *rpoS* gene. Survivors were then screened for the *rpoS* gene by restriction enzyme analysis of plasmid DNA. Plasmid DNA was sequenced with Sequenase (U.S. Biochemical) and primers were synthesized on the basis of the published sequence of *rpoS* (*7*).
16. Our sequences of the wt and mutant alleles of *rpoS* have been submitted to GenBank (accession number X16400). The sequence of the wt allele differed slightly from a previously published sequence (*7*); the differences have been noted in the GenBank entry. The most significant change is the absence of a base near the end of the coding region (position 1020), which shortens the predicted protein product by 20 amino acids from its originally reported length. When we sequenced this region from both the *rpoS* gene obtained by Mulvey and Loewen (*7*) and the same gene from our strain, ZK126, we found them to be identical.
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18. DNA was amplified from whole-cell extracts with the use of AmpliTaq polymerase (Perkin-Elmer) and the following primers: 5′-GTTAACGACCATTCTCG-3′ and 5′-TCACCCGTGAACGTGTTC-3′.
19. Many laboratory strains of *E. coli* K-12 show reduced amounts of *rpoS*-regulated genes, which suggests that these strains may have inadvertently undergone selections similar to those of our experiments.
20. The defective transposon mini-Tn*10kan* [J. C. Way, M. A. Davis, D. Morisato, D. E. Roberts, N. Kleckner, *Gene* **32**, 369 (1984)] was used to generate random transposition events in the chromosome of *rpoS819* cells that were isolated from an aged culture. Phage P1 was grown on a pool of the kanamycin-resistant (Km^R) cells, and the lysate was used to transduce *rpoS819* cells to Km^R. Km^R transductants were grown in LB for 1 day and mixed as a minority with a young *rpoS819* culture. Cells that grew from the minority population were isolated and used to determine the linkage between the stationary phase growth advantage phenotype and the Km^R marker (50% cotransducible). We mapped the mini-Tn*10* insertion by cloning the Km^R marker in pUC19 and using this plasmid as a hybridization probe against filters that were blotted with Kohara's ordered phage library of the *E. coli* chromosome [Y. Kohara, K. Akiyama, K. Isono, *Cell* **50**, 495 (1987)].
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23. J. Mittler and R. Lenski [*Nature* **356**, 446 (1992)] have reported that single mutations in the *bgl* operon allow the slow growth of cells on salicin-containing medium, which can account for the observed high frequency of Sal^+ double mutants.
24. We thank D. Bohannon for suggestions, N. Hochberg for technical assistance, and M. Fox and L. Sonenshein for editorial comments. Supported by grants from the National Science Foundation and the American Cancer Society (R.K.), the Ryan Foundation (M.M.Z.), and Public Health Service (D.A.S.).

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An Osmosensing Signal Transduction Pathway in Yeast

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Yeast genes were isolated that are required for restoring the osmotic gradient across the cell membrane in response to increased external osmolarity. Two of these genes, *HOG1* and *PBS2*, encode members of the mitogen-activated protein kinase (MAP kinase) and MAP kinase kinase gene families, respectively. MAP kinases are activated by extracellular ligands such as growth factors and function as intermediate kinases in protein phosphorylation cascades. A rapid, *PBS2*-dependent tyrosine phosphorylation of *HOG1* protein occurred in response to increases in extracellular osmolarity. These data define a signal transduction pathway that is activated by changes in the osmolarity of the extracellular environment.

Cell growth requires the uptake of water, driven by an osmotic gradient across the plasma membrane. When the external osmolarity increases, many eukaryotic cells are capable of osmoregulation by increasing their internal osmolarity (*1*). The molecular mechanisms used by eukaryotic cells to sense changes in external osmolarity and transduce that information into an osmoregulatory response are poorly understood. The yeast *Saccharomyces cerevisiae* responds to increases in external osmolarity by increasing glycerol synthesis and decreasing glycerol permeability, thereby accumulating cytoplasmic glycerol up to molar concentrations (*2*, *3*).

We isolated osmoregulation-defective mutants of yeast (*4*) by first screening mutagenized cells for the failure to grow on high-osmolarity medium [YEPD (1% yeast extract, 2% bactopeptone, 2% dextrose) supplemented with 0.9 M NaCl or 1.5 M sorbitol]. Mutants that grew well on YEPD but not on high-osmolarity medium (Osm^S) were then assayed for cellular glycerol accumulation 1 hour after the addition of 0.4 M NaCl to the medium (*3*). Osm^S mutants with a reduction in the glycerol response were all recessive and fell into one of four complementation groups, identifying four *HOG* (high osmolarity glycerol response) genes, *HOG1* to *HOG4*. Of this collection of mutants, we further analyzed two mutants, *hog1-1* and *hog4-1*. The reduced glycerol response and Osm^S of *hog1-1* and *hog4-1* cosegregated 2:2 in tetrads resulting from a backcross to wild type and are thus the result of a single mutation.

Genomic DNA fragments were cloned (*5*) that complemented the Osm^S phenotype of *hog1-1* and *hog4-1*, respectively. To locate *HOG1* and *HOG4* on each genomic clone, we generated subclones and tested for complementation of the Osm^S phenotype of the respective *hog* mutant (*6*). The chromosomal locus of each clone was marked with a selectable marker and shown to be tightly linked to the original *hog* mutation (*7*), demonstrating that *HOG1* and *HOG4* (or closely linked genes) had been cloned.

The nucleotide sequence of the *hog1-1*–complementing DNA (*8*) revealed that *HOG1* (GenBank accession number L06279) is a member of the MAP (mitogen-activated protein) kinase family (*9*). The *HOG1* sequence contains a single, large open reading frame of 1.2 kb encoding a 416–amino acid protein with a molecular size of 47 kD. Northern (RNA) blot hybridization with the cloned *HOG1* gene as probe revealed a 1.4-kb transcript whose abundance was unaffected by exposure of the cells to increased osmolarity. Near the NH_2-terminus of the predicted amino acid sequence of *HOG1*, a stretch of 300 amino acids contains each of the strongly conserved amino acids found in protein kinases (*10*). This sequence is most similar to that of MAP kinase family members (*11*, *12*),

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Fig. 1. Similarity of *HOG1* to MAP kinase genes. Conserved subdomains present in all protein kinases are indicated by Roman numerals. *HOG1* was visually aligned with *FUS3*, *KSS1*, and *ERK1*, and gaps (- - -) were introduced to improve alignment. Identities and conservative substitutions (*32*) are boxed, and residues conserved by all four sequences are labeled. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

including those required for mating pheromone response in *S. cerevisiae* (*FUS3* and *KSS1*) and those that participate in growth factor responses in animal cells (for example, *ERK1*) (Fig. 1). Of note in the *HOG1* sequence are Thr^{174} and Tyr^{176} in the kinase subdomain VIII. These two residues in a comparable position in the MAP kinases encoded by *ERK2* and *FUS3* are phosphorylated in response to extracellular signals (*13, 14*); this modification is required for MAP kinase activity (*15*) and in vivo function (*14*).

HOG4 is identical to *PBS2*, a gene that when overexpressed confers resistance to the antibiotic polymyxin B (*16*). Two phenotypes of *hog4-1* were identical to those of a PBS2 disruption—an inability to grow in medium of high osmolarity and a terminal cell morphology (multibudded, highly extended, multinucleated cells) in high-osmolarity medium. *PBS2* and *HOG4* are both found on chromosome 10; *PBS2* has the same restriction map as *HOG4*; *PBS2* complemented a *hog4-1* mutant when carried on a centromere plasmid; and a partial DNA sequence of *HOG4* was identical to part of *PBS2* (*17*). *PBS2* is a member of a family of protein kinase genes that include a MAP kinase kinase. The kinase domain of *PBS2* is 65% identical to that of *wis1*, a dosage-dependent regulator of mitosis in *Schizosaccharomyces pombe* (*18*), 43% identical to that of the *S. pombe* gene *byr1* (*12*), 38% identical to that of *STE7* [a mating response pathway gene in *S. cerevisiae* (*19*)], and 48% identical to that of MEK, a mouse MAP kinase kinase (*20*).

HOG1 and *PBS2* are each required for the same cellular responses. First, a null mutant of *HOG1* had the same phenotype as a null mutant of *PBS2*. Second, a strain lacking both *HOG1* and *PBS2* had the same phenotype as a strain lacking only one gene. The *hog1-Δ1* and *pbs2-Δ1* mutants were constructed by one-step genomic sequence replacement (*21*). The *hog1-Δ1* mutant was created by the replacement of codons 62 to 199, a region that encodes several amino acids required for protein kinase function (*10*), with the genetic marker *TRP1*. The deletion of conserved residues together with the *TRP1* disruption make it likely that *hog1-Δ1* is a null mutant. The *pbs2-Δ1* mutant, created by the replacement of 92% of its protein-coding sequence with *URA3*, is almost certainly a null mutant. The *hog1-Δ1* and *pbs2-Δ1* mutants had three characteristics in common. Each grew as well as a congenic wild-type strain on YEPD but showed reduced growth on high-osmolarity medium such as YEPD with 0.9 M NaCl or 1.5 M sorbitol (*17*). Both mutants had an aberrant cell morphology on high-osmolarity medium, growing into large, multinucleated cells with multiple elongated buds. The glycerol response in *hog1-Δ1* and *pbs2-Δ1* null mutants was reduced by the same amount, to about half of that in wild-type strain (Fig. 2). The reduction in glycerol response in a *hog1-Δ1 pbs2-Δ1* mutant was the same as that observed in either the *hog1-Δ1* or *pbs2-Δ1* mutants.

HOG1 and *PBS2* are not required for two of the better characterized signaling pathways in yeast, the mating pheromone response (*22*) and nutrient assessment pathways (*23*). The *hog1-Δ1* and *pbs2-Δ1* mutants were tested for mating pheromone–induced growth arrest, recovery from arrest, and mating efficiency (*24*). No defects relative to a congenic wild-type strain were noted. The *hog1-Δ1* and *pbs2-Δ1* mutants showed no defects in response to nutrient deprivation; viability after prolonged nitrogen deprivation, heat shock sensitivity, accumulation of storage carbohydrates, and sporulation efficiency were the same as in wild-type controls (*23, 25*).

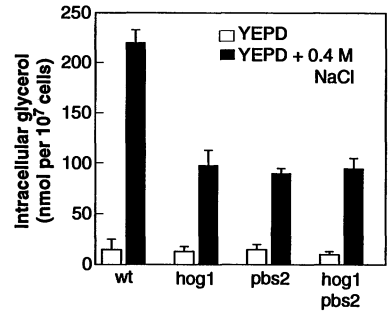

Fig. 2. High osmolarity–induced glycerol accumulation in *hog1-Δ1* and *pbs2-Δ1* mutants. Intracellular glycerol was measured (*4*) after a 1-hour incubation of cells in either YEPD or YEPD + 0.4 M NaCl. Strains (*28*) are as follows: wt, YPH499; hog1, JBY10; pbs2, JBY40; hog1 pbs2, JBY1041.

MAP kinases in animal and yeast cells are phosphorylated on tyrosine and threonine in response to extracellular signals (*13, 14*). The sequence similarity of *HOG1* to members of the MAP kinase family raised the possibility that HOG1 (the protein product of *HOG1*) would be tyrosine-phosphorylated in response to an increase in external osmolarity. To test this idea, we exposed strains containing 0, 1, or multiple

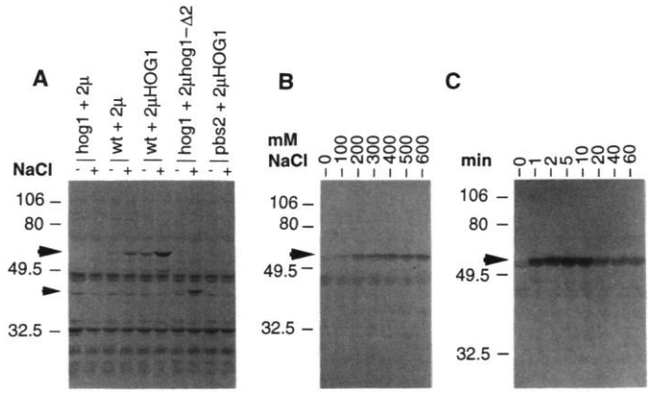

Fig. 3. Osmotic stress–induced tyrosine phosphorylation of HOG1. Anti-phosphotyrosine immunoblot analysis of cells exposed to increased osmolarity. (**A**) Comparison of extracts prepared from control (−) and 0.4 M NaCl–treated (+) cultures. Samples were prepared by mechanical disruption of cells, followed by ammonium sulfate precipitation (*28*). All strains contain high-copy 2μ-based control plasmids or identical plasmids containing *HOG1* or the truncated *hog1-Δ2* as indicated. Strains (*28*) are as follows: hog1+2μ, JBY10 containing control plasmid pRS426; wt+2μ, YPH499 containing pRS424; wt+2μHOG1, YPH499 containing pJB17; hog1+2μhog1-Δ2, JBY10 containing pJB12T; and pbs2+2μHOG1, JBY40 containing pJB17. Full-length HOG1 and the *hog1-Δ2* product are indicated by a large and small arrow, respectively. The position of prestained molecular size markers is given at the left (in kilodaltons). (**B**) NaCl dependence of HOG1 tyrosine phosphorylation. NaCl was added at the indicated concentration to growing cultures for 10 min. Cells were collected by centrifugation and then boiled in sample buffer, and cell extracts were analyzed by immunoblot analysis. Molecular size markers are indicated on the left (in kilodaltons). (**C**) Time course of HOG1 phosphotyrosine accumulation. Cells were exposed to 0.4 M NaCl for the time indicated before phosphotyrosine accumulation was analyzed as described in (B). The strain in (B) and (C) was YPH499 containing pJB17. Molecular size markers are indicated on the left (in kilodaltons).

copies of *HOG1* to increases in external osmolarity, and cell proteins were assayed for tyrosine phosphorylation by immunoblot analysis with an antibody to phosphotyrosine (*26*, *27*). One minute after we raised the osmolarity of the medium with 0.4 M NaCl, an increase in tyrosine phosphorylation of a single band with a molecular size of 55 kD was observed (Fig. 3). The magnitude of the tyrosine phosphorylation of the 55-kD band was proportional to *HOG1* copy number, with no detectable signal in the *hog1-Δ1* strain (Fig. 3A). A truncated *HOG1*, *hog1-Δ2*, created by the deletion of 73 codons at the COOH-terminal end (outside the kinase domain) of *HOG1* (*28*), was introduced on a high-copy plasmid into a *hog1-Δ1* mutant. Raising the osmolarity stimulated the tyrosine phosphorylation of a faster migrating band in the *hog1-Δ2* strain (Fig. 3A), showing that it is HOG1 that is phosphorylated in cells exposed to an increase in external osmolarity. Tyrosine phosphorylation of HOG1 was not detected in *pbs2-Δ1* cells, suggesting that tyrosine phosphorylation of HOG1 requires *PBS2*. This result could also be explained by *PBS2*-dependent expression of HOG1. However, immunoblot analysis with an antibody to bacterially expressed HOG1 showed that the amount of HOG1 in yeast was independent of *PBS2* (*17*). The addition of 0.7 M sorbitol to the medium elicited identical changes in tyrosine phosphorylation of HOG1, indicating that a change in osmolarity rather than the concentration of a specific solute is the stimulus that elicits this response. The amount of HOG1 phosphorylation depended on the concentration of NaCl and was maximal at 300 mM NaCl (Fig. 3B). After an increase in osmolarity, maximal phosphorylation of HOG1 on tyrosine was reached within 1 min, was maintained for roughly 15 to 20 min, and then declined to a new steady-state level (Fig. 3C). This steady-state amount of HOG1 phosphorylation was rapidly reversed to that of untreated cultures when the salt was removed from the medium.

These results raise questions as to how yeast cells sense osmotic stress. A decrease in turgor pressure resulting from the collapse of the osmotic gradient might be the initial signal. Yeast cells possess mechanosensitive ion channels in their plasma membrane (*29*) that could function in sensing changes in turgor pressure. The similarity between mating response genes and *HOG1* and *PBS2* suggest another possibility. Mating responses are triggered by interaction between mating pheromone, a polypeptide ligand, and its receptor on the plasma membrane. If the similarity between the two signaling pathways extends to the triggering event, the osmotic stress signaling pathway could also involve a ligand-receptor interaction. For example, turgor pressure changes could alter the interaction between a cell wall–bound ligand and a specific plasma membrane receptor. Elucidation of the mechanism of osmosensing will require the isolation and characterization of mutants defective in upstream functions of the osmotic response pathway.

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4. Mutant isolation and analysis will be described more fully elsewhere. Parental strains MG159A and MG159B (*MATa trp1* and *MATα ura3*) and YPH98 and YPH102 (*30*) were derived from S288C. Growth was at 30°C. Colonies grown from ethyl methanesulfonate–mutagenized cells (*31*) were tested by replica-plating for growth on YEPD containing 0.9 M NaCl or 1.5 M sorbitol. Osm^S mutants that failed to grow on both plates were tested for glycerol accumulation after transfer to YEPD containing 0.4 M NaCl. Cell extracts were prepared (*3*), and the amount of glycerol was measured in a colorimetric assay (*17*) based on the specific oxidation of glycerol by glycerol dehydrogenase in the presence of oxidized β-nicotinamide adenine dinucleotide [C. S. Frings and H. L. Pardue, *Anal. Chim. Acta* **34**, 225 (1966)]. Three independent cultures of each strain were tested for glycerol response. Nineteen Osm^S mutants, the *hog* mutants, had a statistically significant reduction in 0.4 M NaCl–induced glycerol accumulation as compared to the parental strain and fell into four complementation groups. Linkage analysis showed that each group corresponded to a separate gene, *HOG1* through *HOG4*. The reduced glycerol response in the *hog* mutants was due to a decrease in glycerol synthesis (*17*). To test for cosegregation of *hog* phenotypes, we assayed five tetrads from a third backcross to wild type for Osm^S and glycerol response.
5. *HOG* genes were cloned from a yeast genomic library on the centromere plasmid YCp50 [M. Rose, P. Novick, J. Thomas, D. Botstein, G. Fink, *Gene* **60**, 237 (1987)]. Yeast were transfected by the LiCl or spheroplast procedure (*31*). Other methods are as described by J. Sambrook, E. F. Fritsch, and T. Maniatis [*Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989)].
6. Restriction fragments of the *hog1-1*–complementing and *hog4-1*–complementing genomic clones were introduced into pRS316 (*30*), transformed into a *hog* mutant, and the transformants tested for growth on plates containing YEPD + 0.9 M NaCl. The putative *HOG1* and *HOG4* genes were localized to a 2.5-kb Cla I–Bam HI and a 3.7-kb Kpn I–Spe I fragment, respectively.
7. M. Rose and J. R. Broach, *Methods Enzymol.* **194**, 195 (1991). A 5.5-kb Hind III–Bgl II fragment of *hog1-1*–complementing DNA was subcloned into an integrating vector, pRS306 (*30*). This plasmid was linearized at a unique Hpa I in the insert and used to transform a *hog1-1 ura3* strain to Ura^+ Osm^R. In 23 complete tetrads from crosses between two transformants and MGY159A, only one of the haploid progeny was Osm^S, indicating tight linkage of the complementing gene to *hog1-1*. In the eight complete tetrads from a genetic cross between one transformant and a *hog1-1 ura3* strain, only the parental ditype (2:2 Ura^+Osm^R:Ura^-Osm^S) was observed. The tight linkage between *HOG1* and *URA3* genes is consistent with integration of the plasmid by homologous recombination at the *hog1-1* locus. A 5.5-kb Sal I–Spe I fragment of *hog4-1*–complementing DNA was subcloned into pRS306. This plasmid was linearized at a unique Eco RI site in the insert and used to transform a *hog4-1 ura3* strain to Ura^+ Osm^R. In 12 complete tetrads from crosses between two transformants and MGY159A, no Osm^S progeny appeared, indicating tight linkage of the complementing gene to *hog4-1*. In 11 complete tetrads from a cross between one transformant and a *hog4-1 ura3* strain, only the parental ditype (2:2

Ura$^+$Osm^R:Ura$^-$Osm^S) was observed. Tight linkage between the *HOG4* and *URA3* genes is consistent with plasmid integration at the *hog4-1* locus.
8. The DNA sequence of *HOG1* on a 2.5-kb Cla I–Bam HI genomic DNA fragment was determined [F. Sanger, S. Nicklen, A. R. Coulsen, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)] by Lofstrand Labs (Gaithersburg, MD) on both strands by primer walking.
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27. Tyrosine phosphorylation of proteins was analyzed by immunoblot analysis with immunoglobulin 2b$_k$–affinity purified monoclonal antibody (Upstate Biotech, Lake Placid, NY). Cells were grown in liquid SC medium lacking the appropriate supplement (*31*), pelleted, resuspended in YEPD, and incubated for 2 hours before the addition of NaCl or sorbitol in an equal volume of 0.8 M NaCl of 1.4 M sorbitol in YEPD. Preparation of cell extracts, electrophoresis, transfer conditions, and incubation with antibodies were done as described (*26*). For ammonium sulfate precipitations of extracts, cells were incubated for 1 min after the addition of NaCl, collected by centrifugation at 4°C, and lysed by agitation with 1 volume of glass beads and 3 volumes of buffer [20 mM tris (pH 7.4), 20 mM Na_3VO_4, 1 mM EDTA, 10% glycerol, 0.1% Triton X-100, and leupeptin, aprotinin, and pepstatin A (each 10 μg/ml)]. The extract was clarified by centrifugation and mixed with an equal volume of saturated ammonium sulfate. The precipitate formed after 2 hours at 4°C was collected by centrifugation and washed with 50% ammonium sulfate and 1 mM EDTA, and a portion (60 μg of protein) was subjected to immunoblot analysis. The antibody recognized phosphotyrosine on HOG1 protein because phosphotyrosine (1 mM), but not phosphoserine or phosphothreonine, competed for immunoreactivity (*26*). Addition of 0.4 M NaCl to control cultures after boiling in sample buffer had no effect on the immunoreactivity of cell proteins.
28. Mutant strains were in a YPH499 background. Strains JBY10, JBY40, and JBY1041 contain *hog1-Δ1*, *pbs2-Δ1*, and *hog1-Δ1 pbs2-Δ1*, respectively. The pJB17 plasmid was constructed by insertion of *HOG1* into the polylinker of the high-copy 2μ-based pRS424 plasmid (*30*). The pJB12T plasmid was constructed by deletion of a 1.1-kb Bst XI–Bst XI fragment extending from within the 3′-end of *HOG1* to the polylinker of pRS316 and insertion of this truncated allele, *hog1-Δ2*, into the 2μ-based plasmid pRS426 (*30*). The *hog1-Δ2* mutant on a centromere plasmid complemented the Osm^S of JBY10.
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PAC-1: A Mitogen-Induced Nuclear Protein Tyrosine Phosphatase

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Tyrosine phosphorylation of proteins is required for signal transduction in cells and for growth regulation. A mitogen-induced gene (*PAC-1*) has been cloned from human T cells and encodes a 32-kilodalton protein that contains a sequence that defines the enzymatic site of known protein phosphotyrosine phosphatases (PTPases). Other than this sequence, PAC-1 is different from several other known related PTPases exemplified by PTP-1b. PAC-1 is similar to a phosphatase induced by mitogens or heat shock in fibroblasts, a yeast gene, and a vaccinia virus–encoded serine-tyrosine phosphatase (VH1). PAC-1 was predominantly expressed in hematopoietic tissues and localized to the nucleus in transfected COS-7 cells and in mitogen-stimulated T cells.

The activation of quiescent peripheral blood T cells by antigens or mitogens stimulates proliferation and the expression of effector functions. Progression through the cell cycle requires the expression of a set of genes that are induced within minutes to hours after binding of the mitogenic agent (*1*, *2*). Immediate early genes have been identified that are transcribed in response to phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) in human peripheral blood T (PBT) cells (*3*, *4*). One such cDNA clone, pAT 120, now referred to as *PAC-1* (phosphatase of activated cells), is transiently expressed during the G1 phase in mitogen-activated PBT cells and in antigen-activated T cell clones (*4*, *5*). The PAC-1 gene is constitutively expressed in T cells infected with the human retrovirus human T cell leukemia virus (HTLV-I), the etiological agent of adult T cell leukemia (*5*).

Structural characterization of two independent, nearly full-length PAC-1 cDNA clones from human PBT cells revealed an encoded polypeptide of 314 amino acids (Fig. 1A) (GenBank accession number L11329). The predicted protein sequence was confirmed by in vitro transcription and translation and by the production of three antibodies to peptides derived from the predicted amino acid sequence that precipitate an endogenous protein of the expected molecular size. A murine cDNA, cloned by cross-hybridization, encodes a very similar protein (Fig. 1A) (GenBank accession number L11330). The PAC-1 protein has 13 cysteines in humans and 14 cysteines in mice, 12 of which are conserved in position between the two species.

A search of GenBank revealed similarity between amino acids 250 and 270 of the PAC-1 protein sequence to several protein tyrosine phosphatases (PTPases) in a region that is the most highly conserved among

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