The Transcriptional Response of Yeast to Saline Stress*

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Francesc Posas‡‡§§, James R. Chambers‖, John A. Heyman‖, James P. Hoeffer‖, Eulalia de Nadal‡‡§§, and Joaquín Arinó**

From the ‡Departament de Bioquímica i Biologia Molecular, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra E-08193, Barcelona, Spain; §Cell Signaling Unit, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona E-08003, Spain; and Invitrogen Corp., Carlsbad, California 92008

Adaptation to changes in extracellular salinity is a critical event for cell survival. Genome-wide DNA chip analysis has been used to analyze the transcriptional response of yeast cells to saline stress. About 7% of the genes encoded in the yeast genome are induced more than 5-fold after a mild and brief saline shock (0.4 M NaCl, 10 min). Interestingly, most responsive genes showed a very transient expression pattern, as mRNA levels dramatically declined after 20 min in the presence of stress. A quite similar set of genes increased expression in cells subjected to higher saline concentrations (0.8 M NaCl), although in this case the response was delayed. Therefore, our data show that cells respond to saline stress by inducing the expression of a very large number of genes and suggest that stress adaptation requires regulation of many cellular aspects. The transcriptional induction of most genes that are strongly responsive to salt stress was highly or fully dependent on the presence of the stress-activated mitogen-activated protein kinase Hog1, indicating that the Hog1-mediated signaling pathway plays a key role in global gene regulation under saline stress conditions.

Yeast cells have been considered an excellent model for the study of the mechanisms underlying tolerance to saline stress, particularly because it has been shown that fungi and higher plants not only have similar ion transport systems at their plasma membranes (1), but they also share similar cation detoxification mechanisms (2) and, most probably, signal transduction pathways (3, 4).

Exposure of yeast cells to saline stress implies both exposure to specific cation toxicity and to osmotic stress. Certain ions such as Na⁺ or Li⁺ are toxic to cells due to their ability to inhibit specific metabolic pathways, probably through inhibition of specific targets. This has been shown to be the case for the yeast Hal2 protein and certain RNA-processing enzymes (5, 6). Therefore, regulation of intracellular ion contents represents an important response to ion stress. For instance, exposure to sodium increases the expression of the ENA1/PMR2A gene, encoding a P-type ATPase responsible for Na⁺ and Li⁺ ion efflux (7, 8).

Increases in extracellular osmolarity results in a transient induction of the expression of stress protective genes. A major outcome from this response is the accumulation of intracellular glycerol, which relies on the activation of the Hog1 (high osmolarity glycerol response) MAP kinase pathway (9, 10). MAP kinases play a key role in regulation of stress responses in many organisms from mammals to yeast (11). Hog1 MAP kinase is essential for the survival of yeast in high osmolarity environments (9) and is activated under osmotic-stress conditions by two independent osmo-sensors, a two-component system and the transmembrane protein Sho1 (12–14). These sensing mechanisms activate a kinase cascade that involves the Ssk2, Ssk22, and Ste11 MAPK kinase kinases (13, 15), the Pbs2 MAPK kinase, and finally, the Hog1 MAPK. Once phosphorylated, the Hog1 MAPK is translocated into the nucleus, where it induces diverse stress responses. It is worth noting that both phosphorylation and nuclear localization of Hog1 are very rapid and transient (13, 16).

The mechanism of gene regulation through activated Hog1 is still unknown because transcription factors under the control of this MAP kinase are not well characterized. Several candidates, however, have been described. These are the transcription factors Msn1, Msn2, and Msn4 (17, 18), the bZIP-type protein Sko1 (19), and Hoc1 (18). Although the requirement for the Hog1 kinase has been demonstrated for the osmotic up-regulation of a number of genes, an exhaustive list of the genes required for osmo-stress adaptation is far from complete.

It is important to understand that responses to ion stress require the activity of several pathways and that a single gene can receive different inputs (20). For instance, the expression of the ENA1 ATPase is regulated by both a calcium signaling pathway, which involves the protein phosphatase 2B (calcineurin), and the HOG signaling pathway (21). We felt that better understanding of the yeast response to saline stress could be achieved from the use of DNA microarrays (reviewed in Ref. 22) to perform a genome-wide analysis of the transcriptional response under this type of stress. This technology has been used to accurately analyze whole genome expression in several organisms, including yeast (23–26). Because a number of examples suggested that different conditions (such as time of exposure to salt) might result in a different pattern of expression, we decided to test two different NaCl concentrations at different time points. In addition, the role of the Hog1 MAP kinase has been investigated by testing the transcriptional response of a hog1 mutant strain under stress.

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‡‡ To whom correspondence should be addressed; Dept. Bioquímica i Biologia Molecular, Facultat de Veterinària, Ed. V, Universitat Autònoma de Barcelona, Bellaterra 08193, Barcelona, Spain. Tel.: 34-93-5512182; Fax: 34-93-5512006; E-mail: J.Arino@cc.uab.es.

1 The abbreviations used are: HOG, high osmolarity glycerol; ORF, open reading frame; kbp, kilobase pairs; STRE, stress-responsive element; MAP, mitogen-activated protein; MAPK, MAP kinase.

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Generation of Open Reading Frame (ORFs) DNA for Deposition in Microarrays—Saccharomyces cerevisiae genes were reamplified from 6,035 polymerase chain reaction-amplified full-length ORFs provided by Research Genetics (Huntsville, AL). Each of these originally amplified ORFs of S. cerevisiae contained the sequence 5'-GGATTCGAGCT- GACCAC immediately 5' of the start ATG, thereby making it possible to re-amplify each ORF with the common primer YAMP1 (5'-GCAGTGGTAGTTCCGAGTCA) and the appropriate gene-specific 3' primer, which was also provided by Research Genetics. Amplified ORFs were purified by using Qiagen (Valencia, CA) 96-well polymerase chain reaction clean-up kits. This amplification and purification process resulted in the production of deposition targets for approximately 85% of the S. cerevisiae ORFs.

Production of Microarrays, Hybridization, and Scanning—The yeast gene targets were arrayed in a 12-tip format using a quill-type pin by an Intelligent Automation Systems (Cambridge, MA) microarrayer. The microarrayer tip delivered approximately 4 nl per spot on silylated aldehyde-coated glass slides (CEL Associates, Houston, TX). Yeast microarrays were hybridized for 4 h under cover slides with a Cy5-dCTP (Amersham Pharmacia Biotech)-labeled cDNA probe. After hybridization, the labeled microarrays were washed and dried. The fluorescently labeled microarrays were then subsequently scanned using a confocal laser ScanArray 3000 (General Scanning Inc.) system. Data was collected using ImaGene software (BioDiscovery Inc.).

Yeast Strains—The following strains were used: TM141 (MATα ura3 leu2 trp1 his3) and TM233 (MATa ura3 leu2 trp1 his3 hog1:TRP1). RNA Preparation—Cells were grown in YEPD (10 g/l yeast extract, 20 g/l peptone, and 20 g/l dextrose) at 28 °C to an optical density of 0.7 at 600 nm. RNA was obtained from untreated cells or cells treated with 0.4M NaCl or 0.8 M NaCl for 10 min and 20 min, respectively. Cells were centrifuged for 5 min at 7000 rpm, and total RNA was extracted by using hot phenol and glass beads as described (27). Poly(A)+ RNA was purified from total RNA with an mRNA isolation kit (Roche Molecular Biochemicals) based on biotin-labeled oligo(dT) probes and streptavidin bound to magnetic particles. Hybridization buffer (700 mM NaCl, 40 mM NaH2PO4, pH 7.6, 4 mM EDTA, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.1% SDS, 0.2 mg/ml salmon sperm DNA) and 105 cpm/ml appropriate 32P-labeled DNA fragment. RNA fragments containing the entire ORF of the following genes were used as probes: SLTI (1.7 kb), GLKI (1.5 kb), GSY2 (2.1 kb), YDR057c (1.6 kb), RPS10B (0.7 kb), RPL28A (0.95 kb), and CTT1 (1.7 kb). Probes were labeled using the random-primed DNA labeling kit (Roche Molecular Biochemicals). Filters were washed in 0.1× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 0.1% SDS at 55 °C.

RESULTS AND DISCUSSION

Transcriptional gene induction has been recognized as an important mechanism for adaptation of yeast cells to saline stress. We have performed a DNA microarray analysis of the global transcriptional response of the yeast genome under NaCl stress. Because of the very large amount of information generated, only the most relevant aspects will be presented in this report, although the whole set of data generated from this work can be retrieved from the Universidad Autonoma de Barcelona web site. Our results revealed that expression of a quite large number of genes increased after a short exposure (10 min) to a relatively low concentration of NaCl (0.4 M). As shown in Table I, the mRNA level of more than 1300 genes increased at least 3-fold under these conditions. In some cases the increase was rather dramatic, with a ratio of expression between stressed and non-stressed conditions higher than 10 (these genes are denoted as very highly induced genes). Interestingly, exposure of the cells for a longer period (20 min) reduced the number of responsive genes by a factor of about 10-fold. When cells were exposed for 10 min to a higher NaCl concentration (0.8 M), the number of genes with at least a 3-fold increase in its mRNA level was much lower (about 400). However, the number of responsive genes doubled when cells were stressed for 20 min. This figure clearly indicate that, at the genome level, time of exposure to NaCl and the concentration of salt used markedly affect the results obtained.

One of the most interesting aspects derived from our data is that at 0.4 M NaCl, a concentration of salt widely used to test responses to osmotic shock, the transcriptional response is rather early and largely transient. As shown in Table II, about 95% of the induced genes shows an early response. We consider here early response genes whose signal increases at least 3-fold after 10 min of stress and is at least 80% of the signal at 20 min. Among them, about 85% display a transient response, defined as the case in which the level of expression at 10 min at least doubles the level of expression at 20 min. This implies that many transcriptional responses, when tested at the mRNA level, could be missed if cells are exposed for too long to stress. This situation is reflected in Fig. 1. Genes were listed on the basis of their increase in expression under a stress of 0.4 M NaCl for 10 min. The top 50 genes were selected, and their induction levels at 0.4 M and 0.8 M NaCl (after exposure of the cells for 10 and 20 min) were plotted. As can be observed, many genes that showed a strong increase in expression after 10 min at 0.4 M sharply decreased after 20 min. Fig. 1 also presents another interesting fact. Most of the genes that are highly induced after 10 min at 0.4 M NaCl remain almost silent when cells are challenged with 0.8 M NaCl (Fig. 1, left panel). However, in many cases, a strong response is triggered after 20 min of exposure to high NaCl concentration (Fig. 1, right panel). This observation indicates that exposure to a high concentration of salt results in a delayed transcriptional response. A similar conclusion has been recently reached regarding the kinetics of induction of the gene GPD1, a key player in the response to osmotic stress in yeast (28). Our data indicates that this delayed response affects most genes that are induced at high salinity, as it can be deduced from the observation that a majority of these genes (about 62%) showed a response that can be defined as moderately late or late, whereas in cells treated with 0.4 M NaCl this behavior is observed in less than 7% of the

| Level of induction | No. of Genes |
|--------------------|--------------|
| 0.4 M [NaCl]       | 315         |
| 0.8 M [NaCl]       | 1022        |
TABLE II
Timing of the transcriptional response of yeast genes to saline stress

Induction level of genes showing at least a 3-fold increase in mRNA after saline stress was scored, and the response was classified with respect to the ratio of induction at 10 and 20 min ($R_{10/20}$) as follows: early transient ($R_{10/20} > 2$), early sustained (0.8 $< R_{10/20} < 0.8$), moderately late (0.2 $< R_{10/20} < 0.8$), and late ($R_{10/20} < 0.2$). Figures in parentheses denote the percentage of each class with respect of the total number of scored genes.

| Response           | No. of Genes |
|--------------------|--------------|
| 0.4 M [NaCl]       | 1330 (93.2%) |
| 0.8 M [NaCl]       | 332 (38.2%)  |
| Early              | 1127         |
| Transient          | 203          |
| Sustained          | 75 (5.3%)    |
| Moderately late    | 22 (1.5%)    |
| Total number       | 1427         |
|                    | 870          |

Fig. 1. Effect of NaCl concentration on the response of highly induced genes. The 50 genes showing the highest induction after a 10-min exposure to 0.4 M NaCl were selected, and the intensity of their response after 10 min (left panel) and 20 min (right panel) to 0.4 M (○) and 0.8 M (○) NaCl was plotted.

In conclusion, evaluation of transcriptional responses after saline stress must take into account the timing of the response. For instance, a recent survey (29) of the transcriptional response of 250 novel yeast genes from chromosome XIV after exposure of cells to 0.7 M NaCl for 1 h revealed only five regulated genes (YNL274c, YNL195c, YNL194c, YNL066c and YNL051w). Our screening also revealed three of them (YNL274c, YNL195c, and YNL066c) as increased at least 3-fold, but in addition, 27 more genes were identified as regulated after a 20-min exposure to 0.8 M NaCl. In light of our data, it seems reasonable to assume that the observed difference is the result of the fact that, after 1 h of exposure, most of the genomic transcriptional response vanished.

Quite a few genes ranking in the very highly induced and highly induced categories after exposure to 0.4 M NaCl for 10 min do not have a recognized functional role, and for many of them, the response described here represents the first clue on functional data available. In other cases, increases in the mRNA level after salt stress had been previously documented, indicating that the data generated by the genomic chip analysis was reliable and can be safely compared with reported data. As far as we could detect, the CTTI gene was the only one not showing the expected behavior, since no induction was detected from the microarray hybridization experiments (despite the strong induction observed in our preliminary Northern blot experiments). Subsequent analysis showed that this specific target was among the few that failed to amplify or purify, and therefore, no signal could be expected.

Table III presents a list of highly induced genes after exposure to 0.4 M NaCl for 10 min that can be ascribed to known functional families. Genes encoding proteins involved in carbohydrate metabolism are well represented. As mentioned above, synthesis of glycerol is a major response of yeast cells to osmotic stress (see Ref. 30 for a review). In addition to the well characterized genes GPD1 and GPD2, responsible for the synthesis of glycerol, we have detected a strong increase in expression in a number of genes encoding plasma membrane sugar transporters, such as STL1, HXT10, HXT7, and HXT5. Of particular interest is STL1, a gene encoding a putative hexose transporter still to be functionally characterized (31). This gene shows the strongest induction after 10 min at 0.4 M NaCl, whereas after 20 min its mRNA level has declined to reach almost basal levels. Genes encoding glucose-phosphorylating enzymes, such as GLK1 and the related ORF YDR316c, as well as HXK2 were also strongly induced.

A close correlation between trehalose content and stress tolerance, including osmotic stress (32, 33), has been established in many cases, although both circumstances can be uncoupled (34). Our results indicated that expression of trehalose-metabolizing enzymes as Ts1, Tps1, and Tps3 (encoding subunits of the trehalose 6-phosphate synthase-phosphatase complex) was increased, as previously documented (32, 34), although under our conditions, Tps2, the remaining member of the complex, was only slightly affected. The neutral trehalase encoded by NTH1, which contains three STRE (stress-responsive element) sequences in its promoter, was also greatly induced, despite that it was previously reported as insensitive to NaCl exposure (35).

Another interesting set of genes that quickly respond to a mild NaCl stress are those related to glycogen metabolism. It has been shown that GSY2 (encoding the major isofrom of glycogen synthase) and GPH1 (encoding glycogen phosphorylase) are induced under saline stress (32). We show here that this can be considered within the frame of a more general effect, since virtually every gene involved in the synthesis or degradation of glycogen shows a positive response under saline stress. We include in this group genes such as UGP1, encoding UDP-glucose pyrophosphorylase (the enzyme that forms UDP-glucose, the direct substrate for glycogen synthase), the less prominent isoform of glycogen synthase (GSY1), the branching enzyme GLC3, and even the self-glucosylating initiator of glycogen synthesis, GLG1. Genes encoding components known to modulate glycogen synthase activity can be also included, as the type 1 protein phosphatase Glc7 (36) and its glycogen-specific regulatory subunit Gac1 (previously reported as inducible by saline stress in Ref. 32) as well as the type 2A phosphatase Pph21 (37). A remarkable feature is that, in contrast with the observation that the induction of a large number of genes after short term exposure to 0.4 M NaCl is substantially decreased (34), our results indicated that expression of trehalose-metabolizing enzymes is very intriguing, because although salt stress results in a mild glycogen accumulation (32), the role of this polysaccharide in stress protection is still obscure.

Exposure of cells to 0.4 M for 10 min also results in the induction of a rather large number of components of the protein biosynthesis machinery. This includes 31 genes encoding ribosomal proteins (both cytosolic and mitochondrial), a figure that represents about one-fourth of the 137 genes encoding ribosomal proteins found in yeast (38). It is worth noting that transcriptional regulation of the expression of ribosomal proteins has been recognized as an important mechanisms to control ribosome assembly and function. Although a general pattern
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The asterisks denote the existence of previously reported experimental data, proving that the transcription of the specific gene was induced by saline stress. In some cases, the intensity of the hybridization signal was out of the linear range of the detection system, resulting in an increase in expression probably higher than the one calculated. This is denoted by a > symbol. The total number of ORFs within the VHI and HI classes was 461. The complete set of data for all the conditions tested will be accessible through the Universitat Autonoma de Barcelona web page. 

| Carbohydrate metabolism | Sugar transport and phosphorylation |
|-------------------------|-----------------------------------|
| ORF code                | Gene name                        | Induction (-fold) | Gene description                          |
| YDR536W                 | STLI                             | 89.8              | Plasma membrane sugar transporter         |
| YCL040W                 | GLKI(†)                          | 52.4              | Glucokinase                               |
| YFL011W-A               | HXT10                           | 46.6              | Hexose transporter                        |
| YDR516C                 | HXT7                            | 27.7              | Protein with similarity to Glk1p          |
| YDR342C                 | HXT7                            | 9.6               | High affinity hexose transporter          |
| YHR094C                 | HXT1(†)                         | >9.1              | High affinity hexose (glucose) transporter |
| YGL253W                 | HXK2                            | 7.6               | Hexokinase II (PII)                       |
| YHR096C                 | HXT5                            | 6.6               | Hexose transporter                        |
| Glycol metabolism       |                                    |                   |                                        |
| YDL022W                 | GPD1(†)                          | 76.4              | Glyceraldehyde-3-P dehydrogenase          |
| YOL059W                 | GPD2(†)                          | 16.3              | Glyceraldehyde-3-P dehydrogenase (NAD+)   |
| YML070W                 | DAK1(†)                          | 9.4               | Dihydroxyacetone kinase                   |
| Trehalose metabolism    |                                    |                   |                                        |
| YML100W                 | TSL1(†)                          | 46.5              | Trehalose-6-P synthase/phosphatase        |
| YBR126C                 | TPS1(†)                          | 32.7              | Trehalose-6-P synthase/phosphatase        |
| YMR261C                 | TPS3(†)                          | 14.6              | Trehalose-6-P synthase/phosphatase        |
| YDR001C                 | NTH1                            | 12.0              | Neutral trehalase                         |
| Glycogen metabolism     |                                    |                   |                                        |
| YLR258W                 | GSY2(†)                          | 24.8              | Glycogen synthase                         |
| YKL035W                 | UGP1                             | 20.8              | UDP-glucose pyrophosphorylase             |
| YOR178C                 | GAC1(†)                          | 9.1               | Regulatory subunit for Glc7p              |
| YPR160W                 | GPH1(†)                          | 9.1               | Glycogen phosphorylase                    |
| YFR015C                 | GSY1                             | 8.3               | Glycogen synthase                         |
| YKL127W                 | PGM1                             | 6.5               | Phosphogluco mutase                       |
| YKR133W                 | GLC7                             | 6.3               | Ser/Thr protein phosphatase 1             |
| YKL193C                 | SD52                            | 5.5               | Regulator of GLC7                         |
| YDL134C                 | PPH21                           | 5.2               | Ser/Thr protein phosphatase 2A            |
| YEL011W                 | GLC3                             | 5.1               | Branching enzyme                          |
| YKR058W                 | GLO1                             | 5.0               | Glycogen-like protein                     |
| Others                  |                                    |                   |                                        |
| YMR170C                 | ALD2(†)                          | 66.7              | Alddehyde dehydrogenase (NAD(P)+)         |
| YMR318C                 | ALD6(†)                          | 44.4              | Similarity to alcohol dehydrogenases      |
| YPL061W                 | ALD6(†)                          | 38.9              | Cytosolic aldehyde dehydrogenase          |
| YOR374W                 | ALD4                             | 27.1              | Mitochondrial aldehyde dehydrogenase      |
| YDL178W                 | AJP2                             | 12.5              | n-Lactate dehydrogenase                   |
| YBR289W                 | SNF5                            | 10.2              | Component of SWI-SNF complex              |
| YDL066W                 | IDP1                             | 8.6               | Mitochondrial isocitrate dehydrogenase    |
| Stress response         |                                    |                   |                                        |
| YL028W                  | SED5                             | 49.8              | Heat shock protein 104                     |
| YMR174C                 | PAI3                             | 33.4              | Cytoplasmic inhibitor of proteinase Pep4p  |
| YDR171W                 | HSP42(†)                         | 26.3              | Similar to HSP26                         |
| YMR173W                 | DDR48                            | >25.5             | Induced by different stresses             |
| YOL151W                 | GRE2(†)                          | 22.0              | Induced by osmotic stress                 |
| YMR251W-A               | HOR7(†)                          | 18.7              | Hyperosmolarity-responsive gene           |
| YML131W                 | GRE3(†)                          | 15.7              | Aldose reductase                          |
| YHR104W                 | GRE3(†)                          | 15.7              | Aldose reductase                          |
| YKR066C                 | CCR1                             | 13.0              | Cytochrome c oxidase                      |
| YKL100C                 |                                  | 12.7              | Unknown, increased 7.7-fold by MMS        |
| YNR014W                 |                                  | 12.7              | Unknown                                    |
| YNL116W                 |                                  | 12.5              | Unknown, increased 4-fold by MMS          |
| YDR077W                 | SED1                             | 12.1              | Abundant cell surface glycoprotein        |
| YNL077W                 |                                  | (†)               | Similar to Escherichia coli DnaJ          |
| YDR258C                 | HSP78(†)                         | 11.0              | Heat shock protein                        |
| YLR180W                 | SAM1                             | 10.0              | S-Adenosylmethionine synthetase           |
| YBR054W                 | YRG2                             | 7.6               | Homolog to HSP39 heat shock protein       |
| YOR020C                 | HSP10                            | 5.1               | 10-kDa mitochondrial heat shock protein    |
| YGR020C                 | TRX2                             | 5.0               | Thioredoxin                               |

Table III

Classification into functional families of ORFs whose transcripts are induced more than 5-fold (VHI plus HI) after 10 min of treatment with 0.4 m NaCl

Include the well characterized gene EDA1/PMR2A, encoding a P-type, salt-inducible ATPase, which is the major determinant for Na⁺ efflux (7, 8). Interestingly, a remarkable induction is observed for several components of the vacuolar H⁺-ATPase complex, such as VMA6, VPH1, VMA7, VMA5, and TFP1. The role of this complex is pivotal in creating the electrochemical gradient of protons required for sequestration of sodium into the vacuole (39). In plant cells, tonoplasts are fundamental for ion compartmentation in the vacuole. It is remarkable that, in

cannot be established, in many cases the induction of the ribosomal genes is largely or fully independent of the presence of the HOG1 gene. In addition to specific ribosomal genes, response to saline stress involves increases in the level of several aminocyl-tRNA synthetases and a number of translation initiation factor mRNAs, including TIF1 and TIF2, PRT1, TIF11, HYP2, and CDC95. Saline stress results in transcriptional induction of a number of genes encoding proteins related to ion homeostasis. These
tobacco cells, an increase in the mRNA levels for the 70-kDa catalytic subunit of the tonoplast H\(^+\)-ATPase has been reported after a short term exposure to NaCl (40).

Finally, a number of genes encoding proteins required for signal transduction are also induced. Interestingly, these include a catalytic (SRA3) and regulatory (SRA1) subunit of the cAMP-dependent protein kinase as well as cytosolic adenylate kinase. This is remarkable because a role for cAMP signaling in general stress response as well as in regulation of sodium extrusion (20) has been postulated. Known components of signal transduction pathways related to stress response appear also induced. Examples are the MSN2 transcriptional activator (17, 41), the HOP1 transcription factor (18), the HOG1 MAP kinase (42), and the RCK2 kinase (47), which appears to be a direct substrate for the HOG1 MAPK (47), as well as the BMHI gene, encoding a protein of the 14-3-3 family essential for Ras/MAPK cascade signaling during pseudohyphal development (43).

Transcriptional response to stress is often mediated through the STRE system (core consensus AG \(4^{+}T\)) subunit of the 14-3-3 family essential for Ras/MAPK cascade signaling during pseudohyphal development (43).
tested for a variety of stresses, although the response of the majority (54 genes) remained still uncharacterized from an experimental point of view. We have analyzed the expression levels of this subset of genes and found that 27 of them (50%) increase their expression at least 3-fold in wild type cells under at least one of the conditions tested. In fact, an interesting aspect derived from the availability of data on the transcriptional response of the yeast genome to a defined set of conditions is that this data allows for computer analysis in search of motifs present in the promoters of responsive genes that could be candidates for mediating such response. Although a detailed analysis remains to be done, we have used the method described by van Helden et al. (45) to detect over-represented oligonucleotides within the promoter regions of salt-responsive genes. When this kind of analysis was performed by selecting the region comprising nucleotides 600 to −1 for the 100 genes that showed a stronger induction after exposure to 0.4 M NaCl for 10 min, the sequences AG4 and C4T appeared heavily over-represented. An equivalent set of promoters dissected from genes that did not show any response to salt stress failed to produce this (or any other) significant consensus pattern.

Induction of a number of genes after osmotic shock has been documented to be mediated at least in part by the Hog1 signal transduction pathway (42). Here we have analyzed the contribution of the Hog1 pathway by comparing the level of induction of responsive genes in wild type cells and in a strain lacking the Hog1 MAP kinase pathway. It is worth noting that, as far as we know, of responsive genes in wild type cells and in a strain lacking the Hog1 pathway by comparing the level of induction documented to be mediated at least in part by the Hog1 signal transduction pathway.

### Table IV

**Dependence of gene induction upon saline stress on the presence of the Hog1 MAP kinase**

| Hog1 dependence | 0.4 M (10 min) | 0.8 M (20 min) |
|-----------------|---------------|---------------|
| Fully dependent | 12.4 43.9     |               |
| Strongly dependent | 20.6 31.6 |               |
| Weakly dependent | 30.7 21.6     |               |
| Non-dependent   | 36.3 2.9      |               |

In conclusion, in this report we show that exposure of yeast cells to saline stress results in a substantial transcriptional response. A relevant outcome of our global analysis is that the magnitude of the changes is defined in many cases by both the timing of the response and the intensity of the stress. A clear example of this can be found in Fig. 3, in which the responses of a number of genes identified in our global microarray analysis and representative of different functional families, have been tested by conventional Northern blot analysis. We can observe, for instance, the response of gene STLI, which is extremely intense after both mild and severe saline stress. Interestingly, the response is early and very transient in the first case and late under the second condition, although always fully dependent of the presence of the Hog1 kinase. In contrast, the ORF YDR057w, encoding a protein of unknown function, shows a late response under both stress conditions, which is essentially independent of the Hog1-mediated signal transduction pathway.
FIG. 2. Relationship between level of induction and dependence of the HOG pathway. Genes classified according their dependence of the HOG pathway, as indicated in Table IV, were grouped on the basis of the intensity of their response to saline stress as described in Table I. Open bars denote fully or strongly Hog1-dependent genes, hatched bars indicate weakly dependent genes, and crossed bars account for Hog1-independent genes.

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