Transient Inhibition of Translation Initiation by Osmotic Stress*

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Cells respond and adapt to changes in the environment. In this study, we examined the effect of environmental stresses on protein synthesis in the yeast Saccharomyces cerevisiae. We found that osmotic stress causes irreversible inhibition of methionine uptake, transient inhibition of uracil uptake, transient stimulation of glucose uptake, transient repression of ribosomal protein (RP) genes such as CYH2 and RPS27, and the transient inhibition of translation initiation. Rapid inhibition of translation initiation by osmotic stress requires a novel pathway, different from the amino acid-sensing pathway, the glucose-sensing pathway, and the TOR pathway. The Hog1 MAP kinase pathway is not involved in the inhibition of either methionine uptake or translation initiation but is required for the adaptation of translation initiation after inhibition and the repression of RP genes by osmotic stress. These results suggest that the transient inhibition of translation initiation occurs as a result of a combination of both acute inhibition of translation and the long-term activation of translation by the Hog1 pathway.

Translation is governed by the interaction of a number of different structural elements of mRNAs and the translation machinery. In eukaryotes, regulation of translation is carried out by distinct factors that interact with specific regions in the individual mRNA sequence, mostly the 5′ or the 3′ untranslated region. General regulation is exercised via the modulation of the components of the translation machinery, which interact with common regions including the 5′ cap, the coding region, and poly(A) tail structures (1, 2). For general regulation of translation initiation, many signals, such as serum, PDGF, insulin, phorbol ester, EGF, and others, stimulate translation in eukaryotic cells. Other signals, such as virus infection, vasopressin, heat shock, and deprivation of serum or amino acids, are known to inhibit translation initiation in eukaryotes (2, 3).

In the yeast Saccharomyces cerevisiae, nutrient limitation is known to be an strong general regulator of translation initiation (1, 4). Depriving yeast of amino acids or purines inhibits translation initiation through the phosphorylation pathway of the α-subunit of eIF2, a translation initiation factor (1, 5). The immunosuppressant rapamycin binds with the immunophilin FKBP and inhibits the TOR1 protein (a target of rapamycin), which is related to phosphatidylinositol-3 kinase. This results in the inhibition of translation initiation, and growth is arrested in early G1 phase. Loss of the TOR function also causes translational inhibition and induces physiological changes characteristic of nutrient-starved G0 cells. Thus, the TOR kinase controls cell growth by sensing environmental nutrients (6–8). Recently, it has been reported that glucose deprivation rapidly inhibits translation initiation through the glucose repression pathway or the protein kinase A pathway (4). However, signals other than the nutrient signals described above could also be involved in the general regulation of translation initiation.

S. cerevisiae cells respond to increases in external osmolarity by regulating amino acid uptake, protein synthesis (9), the activity of the solute transporter (10, 11), the expression of many genes (12–14) including the genes involved in solute accumulation (15), and the organization of actin (16) and tubulin (17). These reactions are thought to be required for adaptation to the new growth conditions. The HOG (high osmolarity glycerol) pathway is known to be rapidly stimulated by high osmolarity. The HOG pathway contains two transmembrane osmosensors, Sho1 and Sln1. Sho1 acts on Ste11 MAPKK (MAP kinase kinase kinase), and the two-component system that includes Sln1 stimulates Ssk2/Ssk22 MAPKK, resulting in the activation of Pbs2 MAPKK, which in turn activates Hog1 MAPK (18–21). The Hog1 MAPK pathway is necessary for the expression of specific genes for adaptation to high osmolarity, such as GPD1, which encodes cytosolic glyceral-3-phosphate dehydrogenase (15). Recently, genome-wide DNA chip analysis has shown that the Hog1 MAPK pathway regulates the expression of many genes in addition to GPD1 in response to high osmolarity (12).

It has been reported that osmotic stress inhibits nutrient uptake, protein synthesis, and the expression of many genes including ribosomal protein (RP) genes (9, 13, 22). However, it remains unclear which steps of translation are inhibited by osmotic stress, and it is not known whether the Hog1 MAPK pathway contributes to these phenomena. Here, we report the effects of osmotic stress on translation initiation and the role of the Hog1 MAPK pathway in the regulation of nutrient uptake and RP gene expression as well as translation initiation.

MATERIALS AND METHODS

Strains—The strains used in this study are listed in Table I. SS51A was constructed in the TM101 strain by replacing the resident SUT2 gene with the SUT2-S51A gene according to the method described by Shortle et al. (29).

Plasmids—CTF3M-27B, CTF3M-CYH2, CTF-GPD1, CTF-N4631, CTF-CAF20, CTR-PAB1, 903CU-3, 903CU-PBS2, 903CUA, and SK-CTT1 were constructed by inserting the PCR-amplified segments of KPS27B and CYH2 into CTF3M; those of GPD1, TIF4631, and CAF20 into CTF; that of PAB1 into CTR; those of CDC33, PBS2, and ADE2 into pTS903CU; and that of CTT1 into pBluescript II SK′. The CTF vector was constructed by inserting the 3xMYC epitope (donated by M. Shirayama) and the TDH2 terminator into YCplac22 (CEN, TRP1). CTF3M containing the 3xMYC tag was constructed by deleting the

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1 The abbreviations used are: TOR, target of rapamycin; HOG, high osmolarity glycerol; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; RP, ribosomal protein.

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TABLE I  
Yeast strains used in this study

| Strain     | Genotype                  | Source |
|------------|---------------------------|--------|
| TM100      | MATa ura3 leu2 trp1       | (20)   |
| TM101      | MATa ura3 leu2 his3       | (20)   |
| TM260      | MATa ura3 leu2 trp1 his3  | (20)   |
| TM232      | MATa ura3 leu2 his3       | (20)   |
| TM232-1    | MATa ura3 leu2 his3 hog1Δ | LEU2  |
| SS51A      | MATa ura3 leu2 his3       | This study |
| AIB83      | MATa ura3 leu2 trp1 his3  | This study |
| A1954      | MATa ura3 leu2 trp1 his3  | (23)   |
| W303-1A    | MATa ura3-1 leu2-3,112 trp1-1 his3-11,15 ade2-1 can1-100 | Our strain collection |
| yAS120     | MATa ura3 leu2 trp1 his3  | (24)   |
| yAS1898    | MATa ura3 leu2 trp1 his3  | (25)   |
| yAS1948    | MATa ura3 trp1 his3      | (27)   |
| Y262       | MATa ura3–52 his4–539 rpl1–1 | (26)   |
| yAS962 (SP1)| MATa ade6 ade5 leu2 trp1 his3 | (27)   |
| S18-1D     | MATa ade6 ade5 leu2 trp1 his3 | (27)   |
| yAS2399 (ASY62)| MATa ade6 ade5–52 leu2–7,112 trp1 his3 rpl1–1 | (29)   |

*Reference numbers are in parentheses.

6xMYC fragment of CTF. CTR is a derivative of CTF, pTS9305UC (CEN, URA3), containing the 2xHA 6xHis tag, was donated by T. Sasaki (30). The cloned genes, encoded by 903CU-PBS2, CTF-N4631, CTR-PAB1, and 903CU-33, were confirmed as functional by complementation of the strains TM260, the tif4631/tif4632 double mutant, the pab1 mutant, and the cdc35 mutant. The plasmid pSU12-S15A was constructed by inserting the PCR product of the SU12 open reading frame that has the mutation of Ser-51 to Ala in Sui2p and a deletion of the 5' portion into the pJJ215 vector (31). pGPD21 carrying F[rps2]-PBS2\(^{180}\) from a function of PBS2, was kindly donated by T. Maeda (Institute of Molecular and Cellular Biosciences, University of Tokyo).

Measurement of Uptake and Protein Synthesis by the Pulse Labeling Method—Cells were grown in SD-Met medium (0.67% yeast nitrogen base, 2% glucose, with appropriate supplements omitting methionine) or SRA-Ura (0.67% yeast nitrogen base, 2% raffinose, with appropriate supplements omitting uracil) at 25 °C or 30 °C. When the \(A_{600}\) of cultures reached 0.15, NaCl was added to the final indicated concentrations. After cells were collected at the indicated times, the rates of both uptake of methionine and protein synthesis were measured by the pulse labeling method (6). Briefly, 0.02 \(A_{600}\) equivalents of cultures grown in SD medium without methionine were removed at each time point and pulse-labeled for 5 min at 25 °C with 2 \(\mu\)Ci of \(^{35}\)Smethionine (Amersham Biosciences). For measuring trichloroacetic acid-precipitated counts as a gross protein synthesis rate, half of the reaction mixtures were mixed with the same amount of 20% trichloroacetic acid and 903CU-33, were confirmed as functional by complementation of the strains were added to the culture to a final concentration of 100 \(\mu\)g/ml, and the mixture was immediately chilled on ice. Extracts for polysome and monosome subunit analysis were prepared, and fractionation of ribosomes was performed by sucrose gradient centrifugation with 32) or without Mg\(^{2+}\) (33). Each sample, containing 10 or 5 \(A_{600}\) units, was layered onto 12 ml of a continuous 7–47% sucrose gradient made by Gradient Mate (Towa Kagaku), and ultracentrifugation was performed using a Beckman rotor SW41 at 40,000 rpm for 2.5 h (polysomes) or 3.5 h (monosome subunit) at 4 °C. Gradients were fractionated using an ALC-20 automatic liquid charger (Advantec) and an Amersham Biosciences FPLC system at 254 nm (34). The DNA content in the extract was determined by a method using Hoechst 33258 which specifically binds DNA (35). The enhancement of fluorescence was analyzed by F-2000 spectrofluorometer (Hitachi). The polysome/monosome ratio was determined as the ratio of the areas of 2–4–mer polysomes to the areas of 80 S monosomes using NIH Image (developed and maintained by the National Institutes of Health, Bethesda, MD).

Northern Blot Analysis—Total RNAs were isolated at the indicated time points, separated by electrophoresis as described previously (36), and probed with RPS27B, CYH2, GPD1, CTT1, and ACT. The signals were detected using a phosphorimaging device (Fuji BAS-1000). The following gene probes were used: a 1.2-kb SpIHI-Sali RPS27B fragment, a 1.5-kb SpIHI-SalI CYH2 fragment, a 1.8-kb SpIHI-BanHI GPD1 fragment, an 0.6-kb CTT1 EcoRI fragment, and a 0.7-kb ACT1 XhoI-HindIII fragment, prepared from the plasmids CTF3M-27B, CTF3M-CYH2, CTF-GPD1, SK-CTT1, and pACT1 (donated by K. Kamada), respectively.

RESULTS

Osmotic Stress Inhibits Nutrient Uptake and Protein Synthesis—To find a novel signal that regulates the general translation machinery, we first investigated the effects of several environmental stresses on protein synthesis by pulse labeling. As shown in Fig. 1A (left), the rates of methionine uptake were decreased by a shift to 37 °C and by treatment with 0.4 mM H2O2 or 1 mM NaCl, and it did not recover to the initial level during the experimental periods. Because the uptake of \(^{35}\)Smethionine decreased, decreases in the trichloroacetic acid-precipitated counts in an aliquot of cells might reflect this decrease in uptake rather than a decrease in protein synthesis during stress. For this reason, we instead used the ratio of trichloroacetic acid-precipitated \(^{35}\)S to cell-associated \(^{35}\)S (trichloroacetic acid/uptake) as the measure of protein synthesis. The initial ratio of methionine converted from the trichloroacetic acid/uptake) in each aliquot as the rate of protein synthesis. The initial ratio of methionine converted from the trichloroacetic acid-precipitated \(^{35}\)S to cell-associated \(^{35}\)S (trichloroacetic acid/uptake) in each aliquot as the rate of protein synthesis. The initial ratio of methionine converted from the trichloroacetic acid/uptake) in each aliquot as the rate of protein synthesis.
Among the stresses examined, NaCl treatment was more prominent than other stresses in having an affect on protein synthesis. We therefore further examined the effect of NaCl concentration on methionine uptake and protein synthesis. The rates of methionine uptake decreased as the concentration of NaCl increased and did not recover to the initial level during further incubation (Fig. 1B, left), indicating that NaCl inhibits the uptake of methionine irreversibly. In contrast, the ability to take up [3H]uracil decreased at 30 min after NaCl treatment and recovered to the initial level at 120 min (Fig. 1C), indicating that the inhibition of uracil uptake by NaCl is transient. Similarly, the rates of protein synthesis also decreased rapidly but recovered to their initial levels irrespective of the concentration of NaCl (Fig. 1B, right). Because methionine uptake decreases upon stress and does not recover, the inhibition of amino acid uptake by stress may participate in the inhibition of protein synthesis but not in its recovery. Varela et al. (9) also reported a decrease in methionine uptake by moderate osmotic stress such as 0.7 M NaCl. However, they reported that the inhibition was transient, whereas our results indicate that the inhibition of methionine uptake by moderate osmotic stress such as 0.6 or 0.8 M NaCl is irreversible.

The Hog1 MAPK Pathway Is Required for Adaptation of Protein Synthesis after Osmotic Stress—Next we examined the roles of the Pbs2 and Hog1 kinases in the NaCl-induced inhibition of nutrient uptake and protein synthesis. The rates of methionine uptake in both the pbs2Δ and the hog1Δ disruptants decreased after NaCl treatment and did not recover during further incubation, similar to the results seen in the wild-type strain (Fig. 2A). In contrast to the case of methionine, the rates of glucose uptake in the wild type increased transiently after NaCl treatment, whereas those in the hog1Δ disruptant decreased gradually (Fig. 2C). These results indicate that NaCl stress inhibits the uptake of methionine independently of the Hog1 MAPK pathway, whereas it stimulates the uptake of glucose in a Hog1 MAP kinase-dependent manner.

The rate of protein synthesis decreased after NaCl treatment but recovered to the initial level in the wild-type strain after further incubation in the presence of NaCl (Fig. 2B). This resumption of protein synthesis after its inhibition by NaCl represents adaptation. Protein synthesis was inhibited in the pbs2Δ and hog1Δ disruptants, as it was in the wild type after NaCl treatment, but the rate of protein synthesis in both mutant strains only recovered to about half of that in wild type (Fig. 2B). These results indicate that the Hog1 MAPK pathway is not involved in the stress-induced inhibition of protein synthesis but seems to stimulate the recovery of protein synthesis after it is inhibited.

Next, we examined whether the Hog1 MAPK pathway can stimulate protein synthesis in the absence of stress. The pbs2Δ disruptant expressing PBS22AD, an active form of PBS2, showed no remarkable change in the rate of protein synthesis in comparison with cells expressing PBS2 (Fig. 2D). This result suggests that the Hog1 MAPK pathway does not simply activate protein synthesis but is involved in the adaptation of protein synthesis after stress-induced inhibition.

The Hog1 MAPK Pathway Is Required for Adaptation of Translation Initiation after Osmotic Stress—To distinguish whether the NaCl treatment inhibited the initiation or the elongation step of translation, we investigated polysome profiles when cells were exposed to osmotic stresses. After cycloheximide was added to the culture to arrest translation elongation and preserve the polysomes during preparation of samples, sucrose sedimentation analysis of extracts was car-
grown in SD-Met were exposed to 1 M NaCl at time zero.

The slow response of cells to 1 M NaCl as described below (Fig. 3, B) but did not recover to the initial level even at 180 min (Fig. 3, C). The polysome/monosome ratios decreased as in wild-type cells that the Hog1 MAPK pathway is not involved in stress-induced translation inhibition but is required for adaptation of trans-

Thus, we concluded that osmotic stress transiently inhibits translation initiation. The hog1 disruptant was exposed to 0.6 or 1 M NaCl, the peak of inhibition detected by the analysis of polysome profiles was at 30 min (Fig. 3C), whereas that detected by pulse labeling was around 5–15 min (Fig. 3A, right panel; Fig. 2B). This time difference is possibly because of the different media used for the experiments: YPD complete medium was used for the analysis of polysome profiles, and SD synthetic medium was used for pulse labeling.

It is also possible that the adaptation defect in the hog1 disruptant is a secondary effect resulting from the partial inhibition of the glucose-sensing pathway that tightly regulates translation initiation, as described in Fig. 4A. The uptake rate of glucose in the hog1 disruptant was about half that in the wild-type strain after 1 M NaCl treatment (Fig. 2C). However, the polysome/monosome ratios of wild-type cells did not decrease at 2 h as the hog1 disruptant did, even if the glucose content in the medium was changed from 2% to 0.2% (Fig. 3D).

Therefore, the defect in adaptation of translation initiation seen in the hog1 disruptant is not due to a decrease in glucose uptake.

Figure 2. Time course of the rates of nutrient uptake and protein synthesis. Left panels (A and C) indicate the relative rates of [35S]methionine (A) or [14C]glucose (C) uptake, and right panels (B and D) indicate the ratios of trichloroacetic acid-precipitated counts to cell-associated counts (TCA/uptake) as measures of protein synthesis rates. A and B, TM100 (Wild), TM260 (pbs2Δ), or TM232-1 (hog1Δ) grown in SD-Met were exposed to 1 M NaCl at time zero. C, TM100 (Wild) and TM232-1 (hog1Δ) grown in YPD were exposed to 1 M NaCl at time zero. D, 60 cells containing 903CU-PBS2 (a centromeric plasmid that carries a functional PBS2 gene and URA3) or pGPD21 (2 μm plasmid that carries P(gal1)-PBS2Δα, an active form of PBS2, and URA3, α) were grown in S Raf-Ura to mid-log phase, and galactose was added to a final concentration of 0.5% at time zero. The relative rates of uptake are plotted as a percentage of the control, the wild type at 0 min (A and C). The data shown are representative of three independent experiments.

Figure 3. Changes of polysome profiles by osmotic stress. A, polysome profiles (A254 nm = 10) of TM100 cells (Wild) grown in YPD after exposure to the indicated concentrations of NaCl for 15 min. Gradient fractions were collected from top (left) to bottom (right) in each unit. M and P indicate 80 S monosomes and polyribosomes, respectively. The thin line indicates the level of 80 S monosomes of untreated cells. Polysome profiles of TM100 (Wild) or TM232-1 (hog1Δ) at indicated times after treatment with 0.6 M NaCl (B) or 1 M NaCl (C). D, polysome profiles of TM100 cells grown for 2 h after a shift to YP containing 0.5 or 0.2% glucose from YP containing 2% glucose. E, monosome subunits profiles (A254 nm = 5) of TM100 at indicated times after treatment of 1 M NaCl. The arrows indicate a fraction containing the 43–48 S initiation complex. The polysome/monosome ratios are indicated in parentheses as a percentage of the controls: 0 M (A); each strain at 0 min (B and C); 2% glucose (D).
Adaptation of Translation Initiation

Osmolarity-induced Translation Inhibition Is Not a Secondary Effect Caused by Nutrient Limitation—Both depletion of amino acids or glucose from the medium and rapamycin treatment are known to cause the inhibition of translational initiation (1, 4). To investigate whether osmotic stress inhibits translation initiation through these pathways, we examined polysome profiles using mutant strains in these pathways (Fig. 4A). Indeed, because osmotic stress decreases the uptake of methionine, as described in Fig. 2A, it is possible that amino acid starvation inhibits translation initiation as a secondary effect of the osmotic stress. To examine this possibility, we used the su12-S51A disruptant, which has the mutation of Ser-51 to Ala in Su12p, the α-subunit of eIF2, making translation initiation resistant to the inhibition caused by the deprivation of amino acids. When the su12-S51A strain was exposed to 1 M NaCl or transferred to a medium without glucose for 30 min, the inhibition of translational initiation occurred to the same extent as in the wild-type strain (Fig. 4A, su12-S51A, TM100, TM101), whereas the translation initiation was not completely inhibited when the su12-S51A strain was transferred to medium without amino acids (Fig. 4A, su12-S51A) (4). This indicates that the inhibition of translation by osmotic stress occurs through a mechanism other than the phosphorylation of Su12p. Moreover, the osmotic stress inhibited the translation initiation in a prototrophic cell that does not undergo amino acid starvation upon a shift to SD medium, indicating that the inhibition of translation by osmotic stress occurs through a different pathway than amino acid starvation (Fig. 4, TM100 (U, L, T)). However, the inhibition level in the prototrophic cell treated with 1 M NaCl was slightly lower than in the auxotrophic cell (TM100), suggesting that the inhibition of translation initiation by osmotic stress does depend in part on the amino acid starvation caused by osmotic stress.

The P0P2/CAF1 gene is known to be a component of the Ccr4-NOT transcription complex (40) and to be involved in poly(A) shortening (41) and the glucose derepression pathway (42). Recently, Moriya et al. (43) have reported that P0P2/Caf1 is rapidly phosphorylated upon glucose removal. Because the timing of the Pop2/Caf1 phosphorylation was close to the same extent as the translation inhibition upon glucose removal, we examined whether Pop2/Caf1 is involved in the translation inhibition upon glucose removal. As shown in Fig. 4A (pop2Δ), deprivation of glucose for 30 min did not completely inhibit the translation initiation in the pop2 disruptant, suggesting that Pop2/Caf1 contributes to the translation inhibition upon the removal of glucose as in the case of the reg1 or hxx2 mutants, which are also involved in the glucose-sensing pathway (4). However, the severe condition of 30-min glucose deprivation used in this experiment almost completely inhibited the translation initiation even in the reg1 mutant (data not shown), suggesting that the Pop2/Caf1 has a more crucial role than Reg1 in the inhibition of translation upon glucose removal. In contrast, osmotic stress did inhibit translation initiation in the pop2 disruptant to the same extent as in the wild type (Fig. 4A, pop2Δ). In addition, osmotic stress also inhibited initiation in the tpk1Δ mutant (tpk1Δ tpk2Δ tpk3A), which has a low level of the protein kinase A activity that is required for glucose sensing, whereas deprivation of glucose or amino acids did not inhibit initiation in the tpk1Δ mutant at all, as reported previously (Fig. 4A, tpk1Δ) (4). The inhibition of translation by these stresses occurred normally in the parental strains, A1634 for the pop2 disruptant and SP1 for the tpk1Δ mutant, as was the case for the TM100 strain (data not shown). These results also suggest that the inhibition of translation by osmotic stress is...
caused by a different mechanism from that caused by glucose or amino acid removal.

Because the extent of inhibition of translation initiation caused by osmotic stress in the \( tpk1^{\mu} \) mutant was lower than that in the wild-type cells, it is possible that osmotic stress-induced translation inhibition would not occur if the protein kinase A activity were completely lost. To exclude any effect of the remaining protein kinase A activity, we used the \( tpk \) msn2/4 strain (\( tpk1^{\Delta} \) \( tpk2^{\Delta} \) msn2/4) (28). Translation inhibition by osmotic stress occurred in the \( tpk^{\Delta} \) msn2/4 strain to the same extent it had in the wild-type strain. Translation inhibition by deprivation of amino acids or glucose also occurred, to some extent, as reported previously (Fig. 4A, \( tpk^{\nu} \)) (4). Thus, the inability of the \( tpk1^{\mu} \) mutant to inhibit translation may depend on the gene expression regulated by Msn2/4 rather than on protein kinase A activity directly.

Rapamycin treatment (0.5 \( \mu \)g/ml) only partially inhibited the translation initiation of the auxotrophic TM100 strain (Fig. 4A, TM100, 30 min, 2 h), whereas 0.2 \( \mu \)g/ml rapamycin inhibited the growth of TM100 on YPD plates (data not shown). This incomplete inhibition of translation initiation by rapamycin essentially coincides with previous reports (6, 44). We also found that the TM101 strain (\( Mat^{a} \) ura3 leu2 his3 \( TRP1 \)) resistant to rapamycin, although the isogenic TM100 strain (\( Mat^{a} \) ura3 leu2 \( trp1^{HIS3} \)) was sensitive to rapamycin (Fig. 4B). Therefore, it is possible that the translation inhibition initiated by the cell is required for sensitivity to rapamycin. Indeed, the TM101 strain carrying YCplac22 (\( TRP1 \)) resistant to rapamycin, whereas the strain carrying YCplac33 (\( URA3 \)) or YCplac111 (\( LEU2 \)) was not (Fig. 4B). This finding is consistent with the fact that rapamycin inhibits translation initiation via the Tor pathway (45). However, the W303 strain carrying YCplac22 (\( TRP1 \)) was still sensitive to rapamycin (data not shown), indicating that these phenomena are specific to the TM101 strain.

Osmotic stress inhibited the translation initiation completely even in the translation-proportrophic TM101 strain in which translation is resistant to rapamycin (Fig. 4A, TM101). These results suggest that the inhibition of translation initiation by osmotic stress does not result from inactivation of the rapamycin-sensitivity pathway.

**Osmotic Stress Reduces RP mRNA via the Hog1 MAPK Pathway**—Osmotic stress causes transient repression of many genes, including RP genes (22, 13). Thus, it is possible that the inhibition of translational initiation by osmotic stress occurs as a result of the transcriptional inhibition of more than one gene. To know the effect of gene expression on translation inhibition by osmotic stress, we examined the expression of the RP genes as an example of osmotic stress-repressed genes. In wild-type cells the amounts of mRNA of \( CYH2 \) or \( RPS27B \) started to decrease at 45 min after treatment with 0.6 \( \mu \)M NaCl, whereas the \( GPD1 \) mRNA peaked at 30 min and returned to the basal level by 120 min (Fig. 5A). Following treatment with 1 \( \mu \)M NaCl, the peaks of both the decrease of the RP mRNAs and the increase of the \( GPD1 \) mRNA were delayed to 120 min in the wild type (Fig. 5B). These results indicate that osmotic stress causes a transient decrease of the RP mRNAs. As described previously, the peaks of translational inhibition of the wild-type strain treated with 0.6 and 1 \( \mu \)M NaCl were 15 min (Fig. 3B) and 30 min (Fig. 3C), respectively, indicating that the translation inhibition precedes the decrease of the RP mRNAs. In addition, the translation initiation recovered to its initial level at 120 min in the wild-type cells treated with 1 \( \mu \)M NaCl (Fig. 3C), whereas the RP mRNAs were not detected at 120 min (Fig. 5B, wild). These results indicate that the translation initiation does not correlate with the decrease of the RP mRNAs.

When the \( hog1^{\Delta} \) disruptant was treated with 0.6 \( \mu \)M NaCl or 1 \( \mu \)M NaCl, the rapid decrease of the RP mRNAs and rapid increase of the \( GPD1 \) mRNA were not observed (Fig. 5, A and B). In contrast, the expression of \( PBS2^{DD} \) reduced the level of \( RPS27B \) mRNA to about half of that in cells carrying \( PBS2 \) at 120 min (Fig. 5C). This indicates that the \( Hog1 \) MAPK pathway contributes to the transient decrease of the RP mRNA as well as to the induction of many genes, including \( GPD1 \), in response to osmotic stress (12). The translation inhibition of the \( hog1^{\Delta} \) disruptant by osmotic stress (Fig. 3C) occurred without an apparent decrease of the RP or actin mRNAs (Fig. 5B). These results also suggest that the translation inhibition by osmotic stress is a primary rather than a secondary effect of the depression of gene expression by osmotic stress. Indeed, the polysome/monosome ratio of the \( rpb1-1 \) mutant, defective in the largest subunit of RNA polymerase II, decreased gradually for 2 to 3 h after a shift to a restrictive temperature that leads to rapid cessation of mRNA synthesis (data not shown) (4), as in the case of rapamycin-treated cells or the \( tor \) mutant (6, 44).

This response was apparently slower than that of the osmotic stress-induced inhibition of translation initiation. Therefore, it is not likely that the rapid decrease of the polysome/monosome ratio in response to osmotic stress is due to rapid repression of the expression of many genes. In contrast, rapamycin treatment of yeast cells causes repression of the RP genes before the inhibition of translation initiation (6, 44). These facts also suggest that translational inhibition by osmotic stress may be caused by a mechanism distinct from that of rapamycin treatment.
Adaptation of Translation Initiation

eIF4G Is Not Degraded under Osmotic Stress Conditions—In S. cerevisiae, the translation initiation factor eIF4G, encoded by the redundant genes TIF4631 and TIF4632, has been shown to serve as an adapter between Pab1, a poly(A) tail-binding factor, and eIF4E, a cap-binding factor (46). The eIF4G protein has been reported to be degraded following the addition of rapamycin or nutrient deprivation (47). However, no significant changes in the levels of eIF4G, eIF4E, Pab1, and Caf20, a yeast homologue of 4E-BP (48), were observed after addition of 1 m NaCl. Furthermore, the facts that rapamycin treatment causes slow and weak inhibition of translation initiation as compared with osmotic stress, that osmotic stress can inhibit translation initiation in rapamycin-resistant protocytic cells (Fig. 4), and that osmotic stress can inhibit the translation initiation before the repression of the RP genes (Fig. 5) also indicate that osmotic stress inhibits the translation initiation by a mechanism other than the TOR pathway.

DISCUSSION

We have confirmed the previous report by Varela et al. (9) that osmotic stress causes the inhibition of methionine uptake and found that the Hog1 MAP kinase pathway is not required for this inhibition. Furthermore, we have demonstrated that there are several mechanisms controlling nutrient uptake under osmotic stress leading to transient inhibition of uracil uptake, transient stimulation of glucose uptake, and irreversible inhibition of methionine uptake (Figs. 1 and 2). The growth rate of yeast cells is reduced irreversibly in proportion to an increase in external osmolarity (49). This phenomenon appears to resemble the irreversible decrease of methionine uptake. Therefore, the uptake of some nutrients necessary for growth may be inhibited irreversibly following an increase in the NaCl concentration (Fig. 1B). The uptake inhibition may result in the reduction of growth rate because of an insufficient supply of nutrients for cell growth.

Expression of a number of genes is transiently increased or decreased in response to osmotic stress. The Hog1 pathway and several transcription factors are known to function in the transient increase (12, 14, 50). However, there have been no reports describing the factors involved in the transient suppression of gene expression by osmotic stress. Our finding is the first example indicating that the Hog1 pathway is required for the transient decrease of expression of genes such as the RP genes (Fig. 5).

Our finding that osmotic stress causes the transient inhibition of bulk protein synthesis essentially coincides with previous reports (Figs. 1 and 2) (9). However, it was not previously known whether osmotic stress inhibits the initiation or the elongation of translation. In this study, sucrose density gradient sedimentation analysis clearly indicated that the inhibition caused by osmotic stress is at the initiation step (Fig. 3). In addition, our results indicate that the Hog1 MAP kinase pathway is not required for the rapid inhibition of translation initiation but is required for adaptation of the translation initiation after osmotic shock. In terms of translation initiation, the responses to osmotic stress are divided into two opposite reactions: the inhibition by an unidentified mechanism and the activation that might be stimulated by the Hog1 MAPK pathway because the hog1 disruptant shows insufficient adaptation of translation initiation (Fig. 3, B, C, and E). Thus, it is possible to explain the mechanism of transient inhibition as follows. The inhibition starts after ~2 min as shown in Fig. 3B (0.6 m NaCl). Because the tyrosine phosphorylation of Hog1 occurs at 1 and 5 min after treatment with 0.4 and 0.7 m NaCl, respectively (18, 51), the Hog1 MAPK pathway is activated during the inhibition of translation by the treatment of cells with 0.6 m NaCl (Fig. 3B). Therefore, two opposite reactions possibly start at nearly the same time. The activation of translation may last for a long time through the gene expression regulated by the Hog1 MAPK pathway, whereas the inhibition may last for only a short time. As a result of a combination of both the short-term reaction of inhibition and the long-term reaction of activation, the translation is inhibited only transiently.

Polysome formation after the osmotic stress-induced inhibition of translation initiation (Fig. 3, B and C) might occur at or after the time of the expression of GPD1 or CTT1 encoding the cytoplasmic catalase in the wild-type strain (Fig. 5, A and B). Polysome formation did not occur fully in the hog1 disruptant (Fig. 3, B and C) that is defective in the expression of those genes (Fig. 5, A and B). These results cannot exclude the possibility that the adaptation of translation occurs as a result of gene expression activated by the Hog1 MAPK pathway. However, protein synthesis and polysome formation continued to increase successively (Figs. 2B and 3B, Wild) after the end of the transient expression of many genes, including GPD1 (12), suggesting that the adaptation of translation may not simply depend on the transient expression of the Hog1 MAPK pathway-induced genes. We think that the Hog1 MAPK pathway might also directly contribute to the adaptation of translation as well as to gene expression.

Sudden exposure of growing yeast cells to osmotic shock causes a temporal pause in cell growth, after which the cells resume growing. It has been interpreted that the pause in cell growth is because of a transient disappearance of actin cables or microtubules (16, 17). The timing of the transient disappearance of actin cables upon exposure to osmotic stress closely resembles that of the transient inhibition of translation initiation by osmotic stress, suggesting that the transient inhibition of translation initiation may also be a mechanism contributing to the pause in growth after osmotic stress.

What is the biological significance of the transient inhibition of translation initiation? It has been reported that the production pattern of proteins is changed dramatically by osmotic stress (49). One of the possibilities is that shutdown of translation initiation but not elongation increases intracellular free ribosomes. Consequently, mRNAs, which are now not protected by ribosomes, may be attacked by ribonuclease. Therefore, the cells can easily change the intracellular pattern of mRNAs by changes in stability or transcriptional regulation of mRNAs during the shutdown. Subsequently, the cell may produce the proteins corresponding to the newly changed pattern of mRNAs by the restarted translation using free ribosomes. It is thus likely that the cell can efficiently change the intracellular pattern of its proteins in response to an environmental change.

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