Gle2p Is Essential to Induce Adaptation of the Export of Bulk Poly(A)+ mRNA to Heat Shock in *Saccharomyces cerevisiae* 

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The export of bulk poly(A)+ mRNA is blocked under heat-shocked (42 °C) conditions in *Saccharomyces cerevisiae*. We found that an mRNA export factor Gle2p rapidly dissociated from the nuclear envelope and diffused into the cytoplasm at 42 °C. However, in exponential phase cells pretreated with mild heat stress (37 °C for 1 h), Gle2p did not dissociate at 42 °C, and the export of bulk poly(A)+ mRNA continued. Cells in stationary phase also continued with the export of bulk poly(A)+ mRNA at 42 °C without the dissociation of Gle2p from the nuclear envelope. The dissociation of Gle2p was caused by increased membrane fluidity and correlated closely with blocking of the export of bulk poly(A)+ mRNA. Furthermore, the mutants gle2Δ and rip1Δ could not induce such an adaptation of the export of bulk poly(A)+ mRNA to heat shock. Our findings indicate that Gle2p plays a crucial role in mRNA export especially under heat-shocked conditions. Our findings also indicate that the nuclear pore complexes that Gle2p constitutes need to be stabilized for the adaptation and that the increased membrane integrity caused by treatment with mild heat stress or by survival in stationary phase is likely to contribute to the stabilization of the association between Gle2p and the nuclear pore complexes.

When exposed to various forms of stress, cells show adaptive responses such as changes in patterns of gene expression, in metabolism, and in other cellular processes. Adaptive response mechanisms aim to repair molecular damage and to protect cells against potentially adverse effects of stress, resulting in an increase in stress tolerance. Therefore, yeast cells pretreated with a comparatively mild and sublethal stress show increased resistance to subsequent lethal stress, since diverse adaptive responses are induced during the pretreatment (1–4). This phenomenon is termed adaptation. It is well known that the expression of heat shock proteins is induced during pretreatment with mild heat shock and contributes to an increased resistance to severe heat shock (3–6). Heat shock proteins play important roles in protecting other proteins against denaturation and in restoring their biological activities disrupted by stress (3, 7).

Under stressed conditions, yeast cells undergo changes not only in transcriptional patterns but also in the types of mRNA that are nuclear exported in order to adapt rapidly to the stress. It is known that both heat shock and ethanol affect the export of mRNA. Under heat-shocked (42 °C) conditions, yeast cells shut down the synthesis of most proteins with the exception of stress-responsive proteins such as heat shock proteins. This effect is caused in part by selective mRNA export. Stress-induced transcripts such as SSA4 encoding one of the Hsp70 proteins are efficiently exported through nuclear pore complexes (NPCs), whereas bulk poly(A)+ mRNA accumulates in the nucleus under heat-shocked conditions (8, 9). However, pretreatment of cells with a mild heat shock before a severe heat shock protects the mRNA export machinery and allows mRNA export to proceed unimpeded under subsequent severe heat-shocked conditions (10).

In *Saccharomyces cerevisiae*, the shut-off of bulk poly (A)+ mRNA export under stressed conditions involves the dissociation of Npl3p, a heterogeneous nuclear ribonucleoprotein, from mRNA (9). Additionally, the nucleoporin Rip1p/Nup42p was proposed to play an important role in the export of heat shock mRNA (hs mRNA) under heat-shocked conditions (11, 12). Initially, it was proposed that hs mRNA is exported through a specific pathway defined by the nucleoporin Rip1p/Nup42p (11, 12). However, it has been recently reported that Rip1p also participates in the export of non-hs mRNA at 42 °C (13). There is also a report that other general mRNA export factors are involved in the export of hs mRNA at elevated temperatures, suggesting that hs mRNA and non-hs mRNA are exported through similar pathways (13, 14). The pathways of mRNA export under stressed conditions seem to be quite complex and still controversial.

It is reasonable to imagine that changes in the processing of pre-mRNA would occur under stressed conditions, since the quality of mRNA affects competency for export (15–18). It has been reported that splicing is blocked following heat shock (19). Appropriate processing steps such as splicing, 5′ capping, 3′ cleavage, and polyadenylation are necessary for the efficient nuclear export of mRNA (7, 16, 20–23). Both hyperadenylation and defects in polyadenylation of the 3′-end of mRNA cause the blocking of mRNA export and the apparent accumulation of pre-mRNA at the site of transcription (21, 24). *Vice versa*, several mutant strains defective in mRNA export also show hyperadenylation of the 3′-end of mRNA (25). Changes in pre-mRNA quality caused by processing factors may regulate the export competency of mRNA under conditions of stress. Another possibility is that mRNA export factors have an

* This work was supported by the Koto Bio Science Research Foundation, Bio-oriented Technology Research Advancement Institution (BRAIN), and Japanese Ministry of Education, Culture, Sports, Science, and Technology Grant 14035229. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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+ The abbreviations used are: NPC, nuclear pore complex; hs mRNA, heat shock mRNA; GFP, green fluorescent protein; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; BA, benzyl alcohol; DAPI, 4′,6-diamidino-2-phenylindole.

This paper is available on line at http://www.jbc.org
effect on selective mRNA export under stressed conditions. In *S. cerevisiae*, mature mRNA is exported as messenger ribonucleoprotein complexes by mRNA export factors including RNA-binding proteins (Mex67p, Sub2p, Yra1p, Yra2p, and Npl3p), nucleoporins and NPC-associated proteins (Gle1p, Gle2p, Mtr2p, Rip1p/Nup42p, and Rat7p/Nup159p), and DEAD box RNA helicase Rat5p/Dhp5p. Several reviews describe well the functions and interactions of yeast mRNA export factors (26–28). It is still not clear how NPCs and mRNA export factors alter their functions in response to stress, except for the response of Npl3p and Hrp1p (9, 29). Therefore, it is quite difficult at present to estimate the contribution of mRNA export factors to selective mRNA export. The stress responses of mRNA processing factors and export factors still remain to be clarified.

In this study, we investigated the adaptive responses of mRNA export factors to heat shock. We found that most of the Gle2p dissociated from the NPCs and diffused into the cytoplasm under heat-shocked conditions, correlating well with the blocking of bulk poly(A)− mRNA export. Gle2p associates with the nuclear pores through interaction with Rip1p (30) and Nup116p (31). It has been reported that Gle2p plays a role in mRNA export in the NPCs (30). Intriguingly, pretreatment with mild heat stress precluded the dissociation of Gle2p and the accumulation of mRNA in the nucleus under subsequent severe heat-shocked conditions. Stationary phase cells also exported bulk poly(A)− mRNA without the dissociation of Gle2p under stressed conditions. Changes in the intracellular localization of Gle2p correlated closely with this adaptation of the export of bulk poly(A)− mRNA. Here we suggest the possibility that Gle2p regulates the export of bulk poly(A)− mRNA under conditions of stress through changes in its localization.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Medium**

*The S. cerevisiae* strains used in this study were W303-1A (MATα his3−11, 15 leu2−3, 112 trp1−1 ade2−1 ura3−1 can1−100, SWY1920 (MATα GLE2-GFP::HIS3 ade2−1 ADE2 his3−11, 15 leu2−3, 112 trp1−1 ura3−1 can1−100) (32), SWY1226 (MATα his3−11, 15 leu2−3, 112 trp1−1 ade2−1 ura3−1 can1−100 gle2::HIS3) (33), and FY17 (MATα his3−11, 15 leu2−3, 112 trp1−1 ade2−1 ura3−1 rip1−1 kanR) (12). The SW strains and FY17 were donated by Dr. S. P. Wentz and Dr. F. Stutz, respectively. Cells were cultured in 50 ml of SD minimal medium (2% glucose and 0.67% yeast nitrogen base without amino acids, pH 5.5) with appropriate amino acids and bases at 28 °C with reciprocal shaking in 300-ml Erlenmeyer flasks. Exponential phase cells were prepared by culturing until an *A*~600~ of 0.5–0.7, and stationary phase cells were prepared by culturing for over 48 h. A medium supplemented with palmitic acid was prepared with 1% Niaproof (tergitol) to solubilize the fatty acid (34). Palmitic acid-enriched cells were prepared as described by Mizoguchi and Harai (35, 36). To induce adaptation to heat shock stress at 42 °C, cells in exponential phase at 28 °C were immediately transferred into a water bath (37 °C) and then incubated with shaking at 37 °C for 1 h.

**Plasmids**

*Ylp-GLE1-GFP*—A 1698-bp fragment encoding the open reading frame of GLE1 was amplified using the primers 5′-CTAGATAAT-GCTAAGAGGCTCAAGATTGCCAAC-3′ and 5′-GTTCCAGAATTTTCTCA-GAGACATTCCC-G-3′. The amplicon was digested with SacI/Xhol and cloned into the SacI/Xhol site of pVS1630 (37) to construct Ylp-GLE1-GFP. To integrate the GLE1-GFP gene at the chromosomal GLE1 locus, Ylp-GLE1-GFP was linearized by NheI and introduced into yeast cells. The amplicon was digested with SacI/BglII and cloned into the SacI/BglII site of pKW-1106-GFP. To integrate the GLE2-GFP gene at the chromosomal GLE2 locus, Ylp-GLE2-GFP was linearized by Clal and introduced into yeast cells.

*Ylp-NUP116-GFP*—A 1215-bp fragment encoding the open reading frame of NUP116 was amplified using 5′- TCTCAACGGGT-TCACCGGGGCTTGGTAG-3′ and 5′-AACCTCAGGCTGCTGCTGCTC-GAGCGCTTGTTC-3′. The amplicon was digested with SpeI/Xhol and cloned into the SpeI/Xhol sites of pVS1630 to construct Ylp-NUP116-GFP. To integrate the NUP116-GFP gene at the chromosomal NUP116 locus, Ylp-NUP116-GFP was linearized by EcoRI and introduced into yeast cells.

*Ylp-2K-GLE1-GFP*—A 1832-bp fragment encoding the promoter region and open reading frame of GLE2 was amplified using 5′-GACCGGAT- TGGACCTAGATATTCTCGGAT-3′ and 5′-CCGAAGAAGCATTATC- TACCACTATTTCGTTAC-3′. The amplicon was digested with SacI/BglII and cloned into the SacI/BglII site of pKW-1106-GFP.

*pCS835 (GFP-RAT7)* was donated by Dr. C. N. Cole (39). pBS315-GFP-MTR2 was provided by Dr. E. Hurt (40). *pTS-RIP1-GFP* was provided by Dr. Y. Kikuchi (41). *pFS2146 (pha-YRA1)* and *pFS2262 (pMYC-YRA2)* were provided by Dr. F. Stutz (42).

**In Situ Hybridization and Oligonucleotide Probes**

The oligo(dT)~20~ probe was labeled at its 3′-end with digoxigenin using a DIG Oligonucleotide Tailing Kit (Roche Applied Science). In situ hybridization assays to detect poly(A)− mRNA were performed as described previously (43, 44).

**Isotopic Labeling of Yeast Cells under Heat-shocked Conditions**

Cells pretreated at 37 °C and cells without pretreatment were collected and washed and then transferred to prewarmed fresh SD medium (42 °C). After incubation for 10 min at 42 °C, 10 μl of [35S]methionine/cysteine solution (Pro-mix [35S] in situ cell labeling mix; Amersham Biosciences) was added per 10 ml of medium, and cells were labeled for 20 min at 42 °C. Subsequently, protein extract was prepared immediately by the method of Blomberg (45).

**Two-dimensional PAGE**

Immobilized polyacrylamide gel dry strips (11 cm) with pH 4.0–7.0 gradients (Bio-Rad) were used for the first dimension separation. Soluble protein from whole-cell lysates was mixed with rehydration buffer (8 M urea, 2% CHAPS, 115 mM dithiothreitol, 0.1% Bio-Lytes, and 0.001% bromphenol blue), and 250 μl of mixed solution (80 μg of protein/ml) was added to individual lanes of a rehydration tray (Bio-Rad). The strips were allowed to rehydrate at 20 °C for 12 h. The samples were run at 250 V for 15 min, then the voltage was raised to 8000 V over a period of 3 h and kept at 8000 V for 4.5 h in a PROTEIN IEF Cell (Bio-Rad). Subsequently, the immobilized polyacrylamide gel strips were reequilibrated in equilibration buffer I (6 μl urea, 2% SDS, 0.375 μM Tris-HCl, pH 8.8, 20% glycerol, and 130 mM dithiothreitol) for 15 min and in equilibration buffer II (6 μl urea, 2% SDS, 0.375 μM Tris-HCl, pH 8.8, 20% glycerol, and 135 mM iodoacetamide) for 15 min at 20 °C. Following reequilibration, the strips were subjected to SDS-PAGE using 12.5% polyacrylamide gels.

**Yeast Subcellular Fractionation**

Yeast cells were converted to spheroplasts by incubation with 10 mg/ml Mzymolase 20T (Seikagaku Co., Tokyo, Japan) in 100 mM potassium phosphate buffer with 1.2 mM sorbitol (pH 7.5). Cytoplasmic fraction and nuclei fraction were prepared from the spheroplasts by the method of Bécana et al. (46). Each fraction (20 μg of protein) was subjected to SDS-PAGE, and proteins were electrically transferred onto polyvinylidenefluoride membrane (Immobilon-P; Millipore Corp., Bedford, MA). Nop1p and Ena1p were used as a nuclear protein marker and a cytoplasmic protein marker, respectively. Anti-Nop1p antibody and anti-Ena1p antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The secondary antibodies and anti-GFP antibody were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

**RESULTS**

*Change in the Localization of Gle2p under Stressed Conditions*—We first investigated the localization of mRNA export factors under heat-shocked conditions (42 °C) in exponential phase cells, using various GFP fusion proteins and immunofluorescence techniques. The intracellular localization of most of the export factors we investigated did not change. Rip1p and Nup116p remained at the nuclear envelope following heat shock (Fig. IA). The distribution of other factors such as Rat7p, Rat6p, Yra1p, Yra2p, Gle1p, and Mtr2p did not change either (data not shown). However, the localization of Gle2p clearly
changed under heat-shocked conditions. Gle2p is one of the constituents of NPCs, and Gle2p-GFP usually shows a fluorescent signal around the nuclear rim with a punctate pattern of expression (30), a finding we reconfirmed here (Fig. 1A). Although a certain amount of Gle2p-GFP still remained around the nuclear rim, most was dissociated from the nuclear envelope and moved into the cytoplasm under heat-shocked conditions (Fig. 1A). The dissociation of Gle2p from the nucleus was verified by cell fractionation experiments (Fig. 1B). Gle2p-GFP was detected by immunoblotting in the nuclei fraction but not in the cytoplasmic fraction of cells without stress treatment (Fig. 1B, lanes 1 and 2). However, in cells treated with heat shock at 42°C, Gle2p-GFP was detected in both fractions (lanes 3 and 4). Compared with nonstressed cells, the level of Gle2p-GFP in the nuclei fraction was decreased in these cells (lanes 2 and 4). The localization of Gle2p was changed quickly (within 5 min) by the heat shock. We examined the levels of total Gle2p-GFP by Western blotting analysis using anti-GFP-antibody, but found no significant change under the conditions (data not shown). In the analysis in Fig. 1, the GLE2-GFP gene was integrated into the genomic DNA (33). This strain (SWY1920) did not show the phenotypes of the gle2/H9004 mutant such as slow growth, indicating that this Gle2p-GFP was functional. We confirmed that Gle2p-GFP is functional using pKW-GLE2-GFP. A gle2/H9004 mutant (SWY1226) carrying pKW-GLE2-GFP recovered the growth rate, and Gle2p-GFP dissociated from the nuclear envelope in this mutant at 42°C (data not shown).

We next investigated the effects of other forms of stress. Ethanol stress (10%, v/v) as well as heat shock caused changes in the localization of Gle2p (Fig. 1A), although osmotic stress (Fig. 1, A and B, lanes 7 and 8) and treatment with various drugs such as tunicamycin and diamide (data not shown) did not cause significant changes.
not. The distribution of Rip1p-GFP and Nup116p-GFP did not change under conditions of ethanol stress (Fig. 1A). Ethanol stress also induced a rapid change in the localization of Gle2p-GFP (within 5 min). It is known that heat shock and 10% ethanol cause a blocking of the export of bulk poly(A)$^+$ mRNA (8, 9). Therefore, we further investigated the concentration-dependent effects of ethanol on the changes in the localization of Gle2p and the export of bulk poly(A)$^+$ mRNA. As shown in Fig. 2, 6% ethanol caused a partial dissociation of Gle2p-GFP from the nuclear envelope, and 9% ethanol caused the dissociation of most Gle2p-GFP, as did 10% ethanol. Corresponding to the dissociation of Gle2p-GFP, a partial accumulation of bulk poly(A)$^+$ mRNA in the nucleus was observed with 6% ethanol, and a complete accumulation was seen with 9% ethanol. The minimum concentration of ethanol (6%) causing the accumulation of bulk poly(A)$^+$ mRNA was consistent with the minimum concentration needed to cause the dissociation of Gle2p-GFP. The dissociation of Gle2p from the nuclear envelope correlated well with the blocking of the export of bulk poly(A)$^+$ mRNA. It was reported that Gle2p plays a role in mRNA export in the NPCs (30). It has been also reported that Gle2p requires physical association with Nup116p to function in vivo (31). Therefore, it seems that the functional activities of Gle2p were decreased by the dissociation from the NPCs under heat-shocked and ethanol-stressed conditions. These results suggest that the dissociation of Gle2p is presumably one of the reasons for the blocking of the export of bulk poly(A)$^+$ mRNA under heat-shocked conditions.

Membrane Fluidity Affects the Localization of Gle2p—Next we investigated how the dissociation of Gle2p from the nuclear envelope was caused by heat shock. It is known that heat shock as well as ethanol increases the fluidity of the plasma membrane, followed by changes in the lipid composition of the membranes, including the saturation level and chain length of unsaturated fatty acids (47–49). There is no information about the fluidity of the nuclear membrane. However, the same effects on the nuclear membrane can be expected for the following reasons: (i) the thermal gradient in each yeast cell seems to be negligible, and (ii) the intracellular and extracellular concentrations of ethanol in yeast cells are comparable, since the cells are permeated by ethanol (50). Therefore, we examined whether membrane fluidity affects the localization of Gle2p.

Benzyl alcohol (BA), commonly used as a membrane fluidizer, precludes any selective interaction with charged lipid species and fluidizes bilayer membranes (49, 51, 52). We examined whether BA affects the localization of Gle2p in the same manner as heat shock and ethanol. Gle2p-GFP dissociated from the NPCs following the addition of BA (0.8%, v/v) as well as under heat-shocked and ethanol-stressed conditions (Fig. 3). Furthermore, treatment with 0.8% BA for 5 min also blocked the export of bulk poly(A)$^+$ mRNA (Fig. 3). No change in the localization of Rip1p or Nup116p resulted from the treatment with BA (data not shown). We further investigated the concentration-dependent effects of BA on the changes in the localization of Gle2p and the export of bulk poly(A)$^+$ mRNA. As shown in Fig. 3, 0.6% BA caused a partial dissociation of Gle2p-GFP from the nuclear envelope, and 0.7% BA caused the dissociation of more Gle2p-GFP than 0.6% BA. Corresponding to the dissociation of Gle2p-GFP, a partial accumulation of bulk poly(A)$^+$ mRNA in the nucleus was observed with 0.6% BA and an almost complete accumulation with 0.7% BA (Fig. 3).

In contrast to the effects of BA, palmitic acid enrichment is known to rigidify membranes. It has been reported that culture in a medium with palmitic acid resulted in a striking increase in the palmitic acid content of phospholipid fatty acids and that such palmitic acid-enriched cells show a decrease in membrane fluidity and an increase in membrane integrity (35, 36). We
investigated the effects of membrane rigidification on the localization of Gle2p-GFP. The membrane rigidification caused by the enrichment of palmitic acid affected the localization of Gle2p under heat-shocked conditions (Fig. 4), although no effect of the enrichment of palmitic acid was observed under nonstressed conditions (data not shown). Palmitic acid-enriched cells showed a delay in the dissociation of Gle2p after the shift to heat-shocked conditions (Fig. 4). Gle2p-GFP remained around the nuclear envelope in the palmitic acid-enriched cells for a somewhat longer period (more than 15 min) at 42 °C and under 10% ethanol-stressed conditions (Fig. 4), whereas Gle2p-GFP rapidly dissociated in normal cells (within 5 min). In the palmitic acid-enriched cells, the export of bulk poly(A)^+ mRNA was carried out even at 42 °C, whereas Gle2p-GFP remained around the nuclear envelope (data not shown). These results strongly indicate that membrane fluidity is associated with changes in the localization of Gle2p.

**Adaptation in mRNA Export and Localization of Gle2p to Heat Shock Stress**—Cells pre-exposed to a comparatively mild and sublethal stress show various adaptive responses and acquire resistance to subsequent lethal stress, a phenomenon termed adaptation (1–4, 53–55). In *Schizosaccharomyces pombe*, pretreatment with mild heat stress also protects the mRNA transport machinery at 42 °C and allows mRNA export to proceed unimpeded, indicating the existence of adaptation in mRNA export (10). We investigated whether pretreatment with mild heat stress affects the localization of Gle2p-GFP at 42 °C. Cells in exponential phase at 28 °C were pretreated at 37 °C for 1 h and then subjected to heat shock at 42 °C. Such a pretreatment affected the localization of Gle2p and the export of bulk poly(A)^+ mRNA at 42 °C (data not shown). The export of poly(A)^+ mRNA was still detected in the pretreated cells (Fig. 5A), whereas in the normal cells, poly(A)^+ mRNA was not detected in the cytoplasmic fraction after heat shock (Fig. 5B, lanes 5 and 6). These results clearly demonstrated that adaptation to heat shock was induced in the localization of

**FIG. 3. Effects of the membrane fluidizer, BA, on mRNA export and the localization of Gle2p.** Cells in exponential phase at 28 °C were treated with BA (0.6–0.8%, v/v) for 5 min, and then the intracellular distribution of Gle2p-GFP and bulk poly(A)^+ mRNA was monitored.

**FIG. 4. Effects of decreased membrane fluidity on the localization of Gle2p.** Palmitic acid-enriched cells were prepared by culture in SD medium containing 1 mM palmitic acid. Cells were treated with heat shock (42 °C) and ethanol (10%, v/v) for 15 min, and then the intracellular localization of Gle2p-GFP was monitored.
Gle2p is essential to induce adaptation in mRNA export to heat shock stress. We further investigated whether Gle2p is essential or not to induce the adaptation in the export of bulk poly(A)$^+$ mRNA to heat shock. Pretreatment with 37 °C for 1 h did not affect the export of bulk poly(A)$^+$ mRNA of gle2Δ cells at 42 °C (Fig. 6, left). Bulk poly(A)$^+$ mRNA accumulated in the nucleus at 42 °C in the pretreated gle2Δ cells, indicating that adaptation in the export of mRNA was not induced (Fig. 6, left). Additionally, the gle2Δ cells in stationary phase did not undergo adaptation to heat shock in terms of the export of bulk poly(A)$^+$ mRNA (Fig. 6, right). On the other hand, we were not able to detect a strong defect in the export of bulk poly(A)$^+$ mRNA in gle2Δ cells under nonstressed conditions at 28 °C, as
Adaptive Responses in the rip1Δ Mutant—Murphy et al. (30) found that Gle2p interacts with Rip1p in a yeast two-hybrid assay. Rip1p is proposed to be important for the export of hs mRNAs under heat-shocked conditions (11, 12). Additionally, rip1Δ cells are defective in mRNAs at 42 °C (11, 13, 21). We investigated whether the deficiency of Rip1p affects the induction of adaptation in both bulk poly(A)+ mRNA export and Gle2p localization. Although exponential phase rip1Δ cells at 28 °C showed a normal distribution of Gle2p-GFP and exported bulk poly(A)+ mRNA (data not shown), these cells did not exhibit adaptation to heat shock (Fig. 8). Pretreatment at 37 °C for 1 h did not mitigate the blocking of the export of bulk poly(A)+ mRNA at 42 °C in the exponential phase rip1Δ cells, whereas bulk poly(A)+ mRNA was still exported in the pre-treated wild-type cells (Figs. 5A and 8A). Gle2p-GFP dissociated from the nuclear envelope at 42 °C in the pretreated rip1Δ cells (Fig. 8A) but not in the pretreated wild-type cells (Fig. 5A). Gle2p-GFP dissociated from the nuclear envelope even at 37 °C in the rip1Δ cells but not in wild-type cells (Fig. 8B), indicating that Gle2p easily dissociates from NPCs with a deficiency of Rip1p. Furthermore, the stationary phase rip1Δ cells did not adapt to heat shock in terms of mRNA export. Bulk poly(A)+ mRNA accumulated in the nucleus, and Gle2p-GFP dissociated from the nuclear envelope at 42 °C in the stationary phase rip1Δ cells (Fig. 8C). These results indicate that Rip1p assists Gle2p to remain in the NPCs in cells pretreated with mild heat stress and in stationary phase cells.

**DISCUSSION**

**Dissociation of Gle2p Correlates with Blocking of mRNA Export**—We have demonstrated here that heat shock and ethanol stress lead to changes in the localization of Gle2p. Gle2p is involved in mRNA export and associates with Nup116p and Rip1p in the NPCs (30, 31). It has been reported that the Gle2p-binding sequence within Nup116p is required for the association of Gle2p with nuclear pores (31). Most Gle2p-GFP dissociated from the nuclear envelope and diffused into the cytoplasm under both heat-shocked and ethanol-stressed conditions (Fig. 1), whereas the distribution of Nup116p and Rip1p did not change under these conditions. The dissociation of Gle2p correlated closely with blocking of the export of bulk poly(A)+ mRNA. Stressed conditions that block the export of bulk poly(A)+ mRNA caused the dissociation of Gle2p (Figs. 1 and 2), and adapted cells that can export poly(A)+ mRNA at 42 °C did not show a dissociation of Gle2p (Fig. 5). Since Gle2p requires contact with Nup116p in order to function in vivo (30, 31), the dissociation of Gle2p from the nuclear envelope indicates a decrease in functional activity. Therefore, it is reasonable to assume that the dissociation of most Gle2p caused by heat shock and ethanol stress leads to a decrease in efficiency of mRNA export and results in accumulation of bulk poly(A)+ mRNA in the nucleus. Blocking the export of bulk poly(A)+ mRNA under heat shock may partly arise from the dissociation of Gle2p. Since most of the Gle2p diffused into the cytoplasm quickly (within 5 min) after the exposure to heat shock, it is likely that Gle2p contributes to the regulation of the export of bulk poly(A)+ mRNA under heat-shocked conditions through...
the stress responsive changes in its localization. In other words, Gle2p may function as a stress-responsive regulator for the export of bulk poly(A)⁺ mRNA.

Under nonstressed conditions, the gle2Δ cells are viable (30) and still can export bulk poly(A)⁺ mRNA (Fig. 7A) (31). On the other hand, the null mutant of the GLE2 gene cannot grow at 37 °C (data not shown), and Gle2p was essential to induce the adaptation in the export of bulk poly(A)⁺ mRNA to heat shock stress (Fig. 7). The cells pretreated with mild heat shock or stationary phase cells retained Gle2p-GFP around the nuclear rim without any dissociation and exported bulk poly(A)⁺ mRNA at 42 °C (Fig. 5). These results indicate that Gle2p probably plays a crucial role in mRNA export especially under conditions of heat shock. Retaining a functional Gle2p or not may be critical to whether yeast cells survive and adapt to heat shock.

Membrane Fluidity Affects the Localization of Gle2p—The intracellular localization of Gle2p seems to be affected by membrane fluidity. It is known that membrane lipid composition and fluidity change in response to environmental temperature and that heat shock causes an increase in the fluidity of membranes (49). Ethanol also causes an increase of membrane fluidity that adversely affects many membrane-associated processes (48, 58). BA, an effective membrane fluidizer, had similar effects to heat shock at low concentrations (Fig. 3), and membrane rigidification caused by saturated fatty acid (palmitic acid) attenuated the effects of heat shock and ethanol (Fig. 4). The mechanisms by which membrane fluidity can affect Gle2p are still little understood. However, some evidence of a correlation between membrane fluidity and the functions of membrane-bound proteins has emerged. It has been reported that membrane fluidity affects the topology and conformation of proteins.
of a channel formed by *Staphylococcus aureus* α-toxin protein in vitro (59) and also the function of an ABC transporter in rats (60). Additionally, Shulga and Goldfarb (61) very recently reported that an aliphatic alcohol, 1,6-hexanediol, induces the reversible dissociation of several structural nucleoporins, resulting in disruption of the permeability barrier of NPCs and facilitation of the nucleocytoplasmic transport of nuclear localization sequence-GFP and nuclear export sequence-GFP. These reports support the possibility that the conformation and functions of NPCs may be influenced by nuclear membrane fluidity.

At present, we infer that the increasing nuclear membrane fluidity caused by heat shock and ethanol probably leads to unstable conditions in the interaction of Gle2p with Nup116p or Rip1p and finally causes the dissociation of Gle2p from NPCs. This hypothesis may be supported by the finding that Gle2p easily dissociates from the nuclear envelope in the rip1Δ cells (Fig. 8). Since Rip1p is one of the phenylalanine-glycine-nucleoporins (11, 62) and interacts with Gle2p (30), a deficiency of Rip1p may lead to constitutively unstable conditions in NPCs under stress and facilitate the dissociation of Gle2p from NPCs. In fact, Gle2p-GFP diffused into the cytoplasm even at 37°C in the rip1Δ cells but not in wild type cells (Fig. 8).

Furthermore, only heat shock and ethanol stress have been reported to induce the blocking of bulk poly(A)^+ mRNA export (8, 9). We investigated the effects of osmotic stress with sorbitol and NaCl but found no dissociation of Gle2p (Fig. 1) and no accumulation of mRNAs in the nucleus (data not shown). As far as we know, there is no report about the effects of osmotic stress on membrane fluidity, and actually osmotic stress did not induce the dissociation of Gle2p (Fig. 1). This might be why...
osmotic stress does not induce blocking of the export of bulk poly(A)\(^+\) mRNA and also might support the idea that the dissociation of Gle2p is induced by increased membrane fluidity under heat-shocked and ethanol-stressed conditions.

**Gle2p in the Adaptation of Bulk Poly(A)\(^+\) mRNA Export**—In this study, we demonstrated that Gle2p is essential to induce the adaptation to heat shock in mRNA export (Fig. 6). Gle2p-GPP did not dissociate from NPCs at 42 °C in the cells pretreated with mild heat shock or stationary phase cells (Fig. 6), indicating that the conformation of NPCs is probably held in a stable state in these adapted cells. To export bulk poly(A)\(^+\) mRNA under conditions of stress, it seems indispensable that Gle2p be present in NPCs and functional. This idea seems to be supported by results of the experiments using BA and the rip1Δ cells. BA treatment fluidized membranes and invalidated the adaptation in the pretreated exponential phase cells and in stationary phase cells (Fig. 7). The rip1Δ cells did not show adaptive responses in Gle2p localization and bulk poly(A)\(^+\) mRNA export (Fig. 8). These results indicate that a change in the nuclear membrane integrity is probably part of the mechanism of adaptation. It seems appropriate to consider that membranes including the nuclear membrane are rigidified during pretreatment at 37 °C and during survival in the stationary phase. Both the treatment with mild heat stress and the survival in stationary phase probably improves the nuclear membrane integrity and stabilizes the conformation of NPCs. It seems that stationary phase cells have greater membrane integrity and more stable NPCs than pretreated exponential phase cells, since a higher concentration of BA was needed for the stationary phase cells (1.0%) than exponential phase cells (0.1%).

To summarize, blocking of the export of bulk poly(A)\(^+\) mRNA under stressed conditions may be caused by stress-responsive changes in the localization of Gle2p. Our findings also indicate that increased membrane integrity and functional activity of Gle2p were required for the adaptation in the export of bulk poly(A)\(^+\) mRNA to heat shock. These results may aid in understanding of the contribution of mRNA export factors to the export of mRNA under stressed conditions.

**Acknowledgments**—We are sincerely grateful to Drs. C. N. Cole, E. Hurt, Y. Kikuchi, P. A. Silver, F. Stutz, K. Weis, and S. R. Wente for providing plasmids and strains.