Mex67p Mediates Nuclear Export of a Variety of RNA Polymerase II Transcripts*

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Mex67p is essential for nuclear poly(A)+ RNA export in yeast, but which specific transcripts are transported by Mex67p is not known. We observed that thermosensitive mex67-5 cells do not produce a heat shock response at 37 °C but will induce heat shock proteins (Hsp) (e.g. Hsp104p and Hsp70p) when shifted back from the restrictive to permissive temperature (30 °C). This memory of a previous heat stress in mex67-5 cells could be explained if HSP mRNAs accumulated inside the nucleus during heat shock and were exported and translated in the cytoplasm on return to the permissive temperature. To test this hypothesis, nuclear export of heat shock mRNAs was directly analyzed by in situ hybridization using fluorescent-labeled oligonucleotide probes specific for SSA transcripts. This revealed that Mex67p is required for nuclear export of heat shock mRNAs. Furthermore, other polymerase II transcripts encoding the transcriptional repressor ASH1 and the glycolytic enzyme PGK1 are shown to require Mex67p for their export into the cytoplasm. Thus, Mex67p is an mRNA export factor for a broad range of polymerase II transcripts.

Transport of proteins and RNAs through nuclear pores depends on structural components of the nuclear pore complexes (NPCs)1 and soluble transport factors, which bind to the many different transport cargoes and shuttle between the nucleus and cytoplasm (reviewed in Refs. 1–4). Besides transport receptors, Ran and its modulating factors are essentially involved in nucleocytoplasmic transport, acting as a molecular switch for the vectorial transport of many if not all of the transport routes across the nuclear membrane (1, 5–7).

The export of different RNA transcripts from the nucleus to the cytoplasm is a major transport activity of the nuclear pores. If one assumes that of the 6,000 yeast genes on average 10–50 copies of a given mRNA are present in the cytoplasm and that the half-life of a transcript is in the 15-min range, 10,000 mRNA transcripts have to be exported to the cytoplasm per min. By taking into account that a yeast cell has about 150 NPCs, this translates to about 50–250 transcripts per pore per min. Furthermore, about 10–20 ribosomal subunits and probably 10–50 times more tRNAs are estimated to exit every minute through each nuclear pore. This shows that the NPCs have a high export capacity for the different classes of RNAs that are transcribed, processed, modified, and assembled inside the nucleus.

Many components that play a role in RNA export have been characterized. In yeast, mutants defective in mRNA export have largely been identified by screening for nuclear accumulation of poly(A)+ RNA using in situ hybridization with fluorescent-labeled oligo(dT) probes (8, 9). This revealed that nucleoporins, shuttling transport factors, human ribonucleoprotein proteins, helicases, and exonucleases are crucially involved in poly(A)+ RNA export (reviewed in Ref. 10). More recently, these analyses have been extended to follow the nuclear exit of individual mRNA transcripts using in situ hybridization. However, this required overexpression of the corresponding genes from high copy number plasmids to increase the level of transcripts in the cell. Among the transcripts analyzed so far were mRNAs encoding the abundant heat shock proteins (11, 12) and a low abundance transcription factor, Ash1p. Interestingly, it was shown that the ASH1 mRNA is further transported within the cytoplasm to the bud tip along actin fibers with the help of a myosin motor (13).

Two of the essential mRNA export factors in yeast are Mex67p and its associated co-factor Mtr2p (14, 15). The mammalian counterpart of the Mex67p-Mtr2p complex is called the TAP-p15 complex and is also implicated in nuclear exit of poly(A)+ RNA (16). The human TAP-p15 complex was able to complement the otherwise non-viable mex67/mtr2 null mutant (16), demonstrating that these are orthologs. The thermosensitive mex67-5 mutant revealed a rapid accumulation of poly(A)+ RNA inside the nucleus after shift to the restrictive temperature, suggesting that nuclear mRNA export is promptly inhibited. This analysis did not, however, determine whether the mex67-5 mutation reduces the export of all mRNAs or strongly inhibits the nuclear exit of specific mRNA transcripts. We therefore analyzed the nuclear export of heat shock mRNAs in mex67-5 cells at the restrictive temperature either indirectly by pulse labeling of heat shock proteins with [35S]methionine followed by SDS-PAGE and fluorography or directly by in situ hybridization using fluorescently labeled oligonucleotides specific for SSA transcripts. We further probed mex67-5 cells with antisense probes specific for ASH1 and PGK1, two other PolII transcripts. All this showed that Mex67p is an essential nuclear export factor for all three tested polymerase II-derived mRNA transcripts.

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The abbreviations used are: NPCs, nuclear pore complexes; Hsp, heat shock protein; PAGE, polyacrylamide gel electrophoresis; Pol, polymerase; GFP, green fluorescent protein.

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EXPERIMENTAL PROCEDURES

Yeast Strains, DNA Recombinant Work, and Microbiological Techniques—Yeast strains used in this work are shown in Table I. Microbiological techniques, plasmid transformation, and plasmid recovery were done essentially as described earlier (14). DNA recombinant work was performed according to Ref. 17.

Construction of F_{SSA4}-NUP49-GFP—To construct a hybrid gene between the SSA4 promoter and the open reading frame of GFP-NUP49, the SSA4 promoter (−600 to +10) was amplified by polymerase chain reaction with appropriate primers from a plasmid containing the entire SSA4 gene (18) and fused to the GFP-NUP49 sequence (14). This created a fusion construct encoding the first 5 amino acids of Ssa4p created a fusion construct encoding the first 5 amino acids of Ssa4p.

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TABLE I

| Strain     | Genotype                                      |
|------------|-----------------------------------------------|
| MEX67*     | Mata,ade2,his3,leu2,tryp1,ura3,mex67HIS3 (pUN100-LEU2-MEX67) |
| mex67-5    | Mata,ade2,his3,leu2,tryp1,ura3,mex67HIS3 (pUN100-LEU2-mex67-5) (14) |
| nup85Δ     | Mata,ade2,his3,leu2,tryp1,ura3,HIS3:nup85Δ (24) |
| nsp1-5     | Mata,ade2,his3,leu2,tryp1,ura3,nspl::HIS3 (pSB32-LEU2-nsp1-5 [L640–5]) (23) |
| rat2-1     | Mata,tryp1,leu2,ura3,rs2-1 (30)               |

RESULTS

Heat Shock Proteins Are Not Induced in the mex67-5 Mutant—Following transfer of the thermosensitive mex67-5 mutant to the restrictive temperature (37 °C), poly(A)+ RNA accumulated strongly inside the nucleus after 5–10 min (14, 15). The fast onset of this phenotype suggested that mRNA export is rapidly inhibited in the mex67-5 strain at the non-permissive temperature. We have taken advantage of this observation to test the export of the heat shock-inducible transcripts. If the nuclear exit of these mRNAs is efficiently blocked in the mex67-5 cells following transfer from 30 °C (permissive temperature) to 37 °C (restrictive for mex67-5 cells and inductive for the heat shock response pathway), heat shock proteins should not be translated in the cytoplasm. In contrast, if Mex67p is not required for the nuclear exit of heat shock mRNAs or the export defect is only partial, it should be possible to follow the induction of heat shock proteins in mex67-5 cells at the restrictive temperature by metabolic pulse labeling. To test these possibilities, mex67-5 and wild-type cells were shifted from 30 to 37 °C, and the induction of the major heat shock proteins (e.g. Hsp70p and Hsp104p) was measured by pulse-labeling cells with [35S]methionine followed by SDS-PAGE and fluorography. MEX67+ cells exhibit the expected strong induction of the major heat shock proteins Hsp104p, Hsp90p, Hsp70p (Ssa1p, Ssa2p, Ssa3p, and Ssa4p), and Mex67p is not required for the nuclear exit of heat shock mRNAs or the export defect is only partial, it should be possible to follow the induction of heat shock proteins in mex67-5 cells at the restrictive temperature by metabolic pulse labeling. To test these possibilities, mex67-5 and wild-type cells were shifted from 30 to 37 °C, and the induction of the major heat shock proteins (e.g. Hsp70p and Hsp104p) was measured by pulse-labeling cells with [35S]methionine followed by SDS-PAGE and fluorography. MEX67+ cells exhibit the expected strong induction of the major heat shock proteins Hsp104p, Hsp90p, Hsp70p (Ssa1p, Ssa2p, Ssa3p, and Ssa4p), and Mex67p after a 15-min shift to 37 °C, which gradually disappeared when the duration of the heat shock was extended (Fig. 1A) (20). In contrast, no significant induction of the major heat shock proteins was observed when the mex67-5 strain was shifted to 37 °C for 15 min (Fig. 1A). This indicates that export of all of the major heat shock inducible mRNAs is strongly inhibited in the mex67-5 strain after shift to 37 °C.

If not only heat shock mRNA, but also bulk mRNA is impaired in nuclear exit in mex67-5 cells, de novo synthesis of proteins should gradually decrease during incubation at the restrictive temperature. Indeed, incorporation of [35S]methionine into newly synthesized protein decreases significantly during the first 3 h of incubation at the restrictive temperature in mex67-5 cells but not in MEX67+ cells (Fig. 1B, see also Fig. 1C). This suggests that bulk mRNA export is blocked in mex67-5 cells, and due to the time-dependent cytoplasmic degradation of mRNA a gradual inhibition of protein synthesis takes place.

Other yeast mutants reported to exhibit nuclear transport
defects were also tested for the induction of heat shock proteins at the restrictive temperature. Strains carrying the thermosensitive nucleoporin mutations nsp1-5 (23), nsp85Δ (27), and rat2-1 (24, 25) were shifted to 37 °C for 15 min before [35S]methionine was added, and cells were pulse-labeled for a further 10 min. In each of these strains substantial synthesis of heat shock proteins was observed at the restrictive temperature (Fig. 1C). The nsp1-5 strain was reported to have a nuclear protein import defect, whereas the nsp85Δ (8, 27) and rat2-1 (9) mutants exhibit a less strong mRNA export defect with a delayed onset compared with mex67-5. There appears to be a good correlation between the nuclear poly(A)+ RNA accumulation and heat shock mRNA export phenotypes.

To show in a different way that mRNA export is immediately inhibited in mex67-5 cells after shift to 37 °C, a time course of nuclear poly(A)+ RNA export was performed using in situ hybridization with fluorescently labeled oligo(dT) probes. This revealed that export of bulk poly(A)+ RNA must be very strongly inhibited in mex67-5 cells, since the cytoplasmic poly(A)+ RNA pool begins to decrease already after a 5-min shift to 37 °C and is further diminished within 1 h of incubation at the restrictive temperature (Fig. 2). Concomitantly, after 5 min incubation at 37 °C, poly(A)+ RNA begins to accumulate inside the nucleus in mex67-5 cells (Fig. 2). This shows that nuclear export of bulk poly(A)+ RNA is immediately and strongly inhibited in mex67-5 cells after shift to the restrictive temperature.

mex67-5 Cells Exhibit Reduced Thermotolerance—Exposure of yeast cells to a brief, non-lethal heat shock (e.g. 37 °C) induces heat shock proteins and allows them to survive temperatures (e.g. 52 °C) that are otherwise lethal (20). Since mex67-5 cells failed to induce heat shock proteins at 37 °C, we predicted that mex67-5 cells are more efficiently killed at 52 °C, even if they have gone through a non-lethal incubation at 37 °C. Isogenic mex67-5 and MEX67+ strains were grown at room temperature before the cultures were split; one-half was directly shifted for 10 min to 52 °C, whereas the other half was incubated for 50 min at 37 °C, before a 10-min shift to 52 °C. Cells were plated at 23 °C, and the survival rate was measured. This revealed that MEX67+ cells were much better protected...
against a killing heat shock at 52 °C (about 50-fold) than mex67-5 cells (about 8-fold), when previously exposed to 37 °C (Table II). The lack of the heat shock response observed in mex67-5 cells therefore correlates with a decreased thermotolerance when briefly exposed to killing temperatures.

Expression of a Heat-inducible SSA4::GFP-NUP49 Reporter Construct in mex67-5 Cells—To test whether another heat-inducible mRNA not encoding a heat shock protein is translatable in mex67-5 cells at the restrictive temperature, a hybrid gene was made consisting of the SSA4 promoter fused to the open reading frame encoding GFP-Nup49p (see “Experimental Procedures”). This SSA4::GFP-NUP49 construct was inserted into an ARS/CEN/ADΔ2 plasmid and transformed into mex67-5 and isogenic MEX67 cells. Cells were grown at permissive temperature (23 °C) or shifted for 15 min to 39 °C before whole cell extracts were prepared and analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting using antibodies against GFP (Fig. 3). GFP-Nup49p was expressed only at low levels in wild-type cells at 23 °C but was strongly induced under the control of the SSA4 promoter by shifting cells for 15 min to 39 °C (Fig. 3, lanes 1 and 2). In contrast, mex67-5 cells do not reveal induction of GFP-Nup49p translation at 39 °C (Fig. 3, lanes 3 and 4); the basal expression of GFP-Nup49p was, however, enhanced at 23 °C as compared with wild-type cells (see also “Discussion”).

SSA1/SSA4 Transcripts Are Synthesized at Normal Levels in mex67-5 Cells at the Restrictive Temperature—The possibility remained that the lack of heat shock response in mex67-5 strains was due to the inhibition of heat shock mRNA induction or mRNA instability. This question was addressed by Northern analysis using probes specific for the SSA1 and SSA4 transcripts (Fig. 4). SSA1 and SSA4 were expressed at similar, low levels in the mutant and wild-type at 23 °C (Fig. 4, lanes 1 and 7). Following transfer to 42 °C, SSA1 and SSA4 were strongly and comparably induced in both MEX67+ and mex67-5 cells (Fig. 4, compare lanes 1 and 7 with lanes 2 and 8). The degradation of the SSA1 and SSA4 mRNAs was monitored during a time course following transcription inhibition with thiolutin and transfer to 37 °C (Fig. 4, lanes 3–6, and lanes 9–12). Decay of both mRNAs was slightly slower in the mex67-5 strain than in the MEX67+ strain, possibly reflecting differences between the nuclear and cytoplasmic RNA degradation pathways. When cells were transferred directly from 23 to 37 °C, lower induction was observed for both strains but was similar for the MEX67+ and mex67-5 cells (data not shown). We conclude that the failure of mex67-5 cells to produce the major heat shock proteins at 37 °C or higher temperatures is not due to the inhibition of synthesis or stability of the mRNAs and is therefore most likely due to inhibition of nuclear export of heat shock mRNAs.

mex67-5 Cells Have a Memory of a Previous Heat Stress—If the heat shock mRNAs accumulated inside the nucleus in mex67-5 cells at the restrictive temperature, it seemed possible that they would be exported, following shift of the cells to the permissive temperature. Moreover, the mex67-5 mutant is highly thermoreversible for growth (14), and the decay of accumulated heat shock mRNAs is not too fast in mex67-5 strain at 37 °C (see Fig. 4). As shown above, mex67-5 cells do not significantly induce the major heat shock proteins Hsp70p and Hsp104p when shifted for 15 min to 37 °C or higher temperatures (e.g. 39 °C). However, when the mex67-5 cells were returned to 30 °C for 20 min, induction of Hsp104p and Hsp70p was observed (Fig. 5A). In contrast, MEX67+ cells exhibited a strong heat shock response at 39 °C, and the major heat shock proteins gradually disappeared upon a 20-min incubation time at the permissive temperature (Fig. 5A). To exclude that de novo transcription of SSA mRNA occurred in mex67-5 cells when shifted from restrictive to permissive conditions, the PolII inhibitor thiolutin was added to the cells after heat shock to stop any PolII transcription in the following permissive phase. Basically, the same results were obtained in the pres-
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SSA, ASH1, and PGK1 mRNA Transcripts Accumulate Inside the Nucleus in mex67-5 Cells—To demonstrate that SSA mRNAs accumulate in the nucleus in the mex67-5 mutant at the non-permissive temperature, in situ hybridization against SSA transcripts was performed before and after temperature shift of wild-type, mex67-5, and nsp1-5 cells. For SSA mRNA detection, Cy3-coupled antisense oligonucleotides were chosen that hybridize to sequences in the SSA1, SSA2, and SSA3 transcripts (see “Experimental Procedures”). This oligonucleotide-based hybridization procedure has previously been reported to detect only the corresponding mRNA targets (22).

Wild-type cells (Fig. 6A, Mex67+) showed a weak and diffuse cytoplasmic in situ signal for SSA transcripts when grown at 25 °C. This corresponds to the low abundance of SSA transcripts in cells grown at non-heat shock conditions (see also Fig. 4). The cytoplasmic in situ signal for SSA transcripts significantly increased after cells were shifted to 38 °C, and no nuclear staining was seen. This is consistent with previous findings that heat shock mRNAs are efficiently exported from the nucleus at elevated temperatures (e.g. 42 °C) (11). In mex67-5 cells an increased SSA in situ signal is seen at 25 °C compared with the wild type. This possibly reflects the partial induction of a stress pathway in mex67-5 cells at the permissive temperature due to the mex67-5 mutation (see also Fig. 3, lanes 3 and 4). When mex67-5 cells were shifted to 38 °C for 10–20 min, the cytoplasmic in situ signal did not increase, but instead SSA mRNAs rapidly accumulated inside the nucleus, indicating a failure of SSA mRNA export (Fig. 6A, mex67-5). Strikingly, the intranuclear heat shock mRNA signal was concentrated in several discrete spots, which varied in number, as described previously for bulk poly(A)+ RNA (14). No clear defect in the export of the heat shock mRNAs was observed in a strain carrying a thermosensitive mutation in the nucleoporin Nsp1p (Fig. 6A, nsp1-5).

We wanted to test whether other PolII transcripts are also blocked in nuclear export in the mex67-5 strain. Specifically, we were interested to see whether not only the abundant SSA transcripts, but a rare and cell cycle-regulated mRNA is exported by Mex67p. We chose ASH1 as a reporter transcript, which encodes a daughter cell-specific transcriptional repressor, whose mRNA is transcribed only in cells that have completed anaphase (26). It was recently shown that the ASH1 mRNA transiently accumulates at the tip of the large bud and serves as a localized source for Ash1p protein synthesis (13). In the wild-type and nsp1-5 strains, post-anaphase cells show a bud tip localized signal for ASH1 mRNA at both 23 and 38 °C (Fig. 6B). In contrast, the ASH1 mRNA accumulates inside the nucleus of both mother and daughter mex67-5 cells shifted to 38 °C for 10–20 min, and the bud-specific signal was completely absent (Fig. 6B). Nuclear ASH1 transcripts also accumulate in spots located close to the nuclear periphery but less pronounced as compared with SSA mRNAs. Finally, a third mRNA transcript corresponding to an abundant glycolytic enzyme (phosphoglycerate kinase) was tested in this assay. As revealed by in situ hybridization, also PGK1 mRNAs encoding phosphoglycerate kinase accumulate inside the nucleus in mex67-5 cells when shifted to the non-permissive temperature (Fig. 6C).

In summary, the low abundance, cell cycle-regulated ASH1 mRNA and the abundant mRNAs encoding the glycolytic enzyme phosphoglycerate kinase and HSP70 proteins accumulate inside the nucleus in mex67-5 cells at the restrictive temperature. Mex67p is therefore a nuclear export factor for different types of mRNA transcripts.
FIG. 6. In situ hybridization using Cy3-labeled oligonucleotides specific for heat shock, ASH1, and PGK1 mRNAs. The in situ hybridization to detect mRNAs encoding SSA1, SSA2, and SSA3 as well as ASH1 and PGK1 transcripts in MEX67+, mex67-5, and nsp1-5 cells was done as described under “Experimental Procedures.” A, nuclear accumulation of SSA mRNAs (SSA1, SSA2, and SSA3) encoding heat shock proteins in mex67-5 but not MEX67+ or nsp1-5 cells at the restrictive temperature. B, nuclear accumulation of ASH1 mRNAs in mex67-5 but not MEX67+ or nsp1-5 cells at the restrictive temperature. C, nuclear accumulation of PGK1 mRNAs in mex67-5 but not in MEX67+ cells at the restrictive temperature. Cells were shifted for 30 min to the restrictive temperature. DNA staining is also shown.
In the thermosensitive mex67-5 mutant strains the major heat shock proteins including the Hsp70p family and Hsp104p are no longer synthesized under conditions of heat shock induction. In consequence, mex67-5 cells show a strongly decreased thermostolerance. By using a variety of different assays, we have shown that Mex67p is required for the nuclear export of the heat shock mRNAs. The inhibition of export of the heat shock mRNAs to the cytoplasm in mex67-5 cells at 37 °C is responsible for the lack of a heat shock response. Strikingly, the heat shock proteins were partly induced when the mex67-5 strain was returned from heat shock conditions to the permissive temperature. We assume that this is due to the transport and translation of the “stored” nuclear heat shock mRNAs. It has been demonstrated previously that the mex67-5 mutant is highly thermoreversible (14). The accumulated heat shock mRNAs in mex67-5 cells thus do not represent a dead-end product but rather a functional intermediate, which can be chased into the cytoplasm upon transfer to permissive temperatures. Thus, nuclear export of mRNA does not require ongoing PolII transcription.

The inhibition of the heat shock proteins is readily detected by pulse labeling with [35S]methionine followed by SDS-PAGE and fluorography. This provides a simple assay that could be applied to other mutants with potential mRNA export defects. It is predicted that nuclear export mutants with a fast and complete onset of intranuclear mRNA accumulation (within the range of a few minutes after shift to the restrictive temperature) will not express heat shock proteins. Previous estimates of the severity of mRNA export mutant have generally been based on in situ hybridization using probes for poly(A)+ RNA. However, the level of residual mRNA export in these mutants was difficult to evaluate. With the assay described here, it is possible to estimate in a semi-quantitative way how efficiently mRNA export is blocked, simply by measuring de novo synthesis of heat shock proteins (by pulse labeling) or other SSA4 promoter-dependent reporter constructs (e.g. GFP-Nup49p; by Western blotting). Other yeast mutants, which gave a weaker nuclear poly(A)+ RNA accumulation, did not show a clear inhibition of heat shock protein induction at 37 °C. Thus, in contrast to mex67-5 cells, nup85Δ and rat2-1 cells apparently are not tightly blocked in heat shock mRNA export within the first 15–30 min after shift to the restrictive temperature.

Although several mRNA export factors have been identified, it is not known whether they function for all yeast mRNA transcripts or are specialized for different types of mRNAs. Interesting in this context is the report that heat-inducible SSA1 and SSA4 mRNAs are exported from the nucleus into the cytoplasm at heat shock temperatures (e.g. 42 °C), whereas the bulk of poly(A)+ RNA is not exported and accumulates inside the nucleus (11). Furthermore, the SSA1/SSA4 mRNA export is not Ran-dependent as mutants of the Ran cycle can still export heat shock mRNAs at 42 °C. However, the nucleoporin Rip1p, which is dispensable for cell growth, and Gie1p, a nuclear export sequence-containing mRNA export factor, were found to be important for nuclear export of heat shock mRNAs (12, 27). By in situ hybridization with fluorescently labeled antisense oligonucleotides, we could show that Mex67p is involved in the nuclear export of both the heat shock mRNAs and the ASH1 transcription factor mRNA. Although this does not demonstrate that all yeast mRNAs are exported via the Mex67p-dependent export pathway, it is likely that many (if not all) PolII transcripts follow this route.

In contrast to assays reported previously (11), the in situ assay used here for mRNA detection does not require transformation of strains with high copy number plasmids that overexpress the transcript to be studied. Accordingly, we have shown for the first time that specific endogenous transcripts accumulate in the nucleoplasm in an mRNA export mutant. In the mex67-5 strain, the SSA transcripts accumulated in discrete nucleoplasmic spots, preferentially located close to the nuclear periphery. A similar picture was seen for bulk poly(A)+ RNA in this strain (14). These foci most likely correspond to the several electron-dense aggregates scattered throughout the nucleoplasm that were previously seen in electron micrographs of mex67-5 cells (14). Whether these are clustered ribonucleoprotein particles is not clear. The observation that the mRNAs do not accumulate at the NPCs suggests that a transport step somewhere between the site of mRNA transcription and the nuclear envelope is inhibited.

It is interesting to note that in the case of ASH1, its mRNA accumulates in both mother and daughter nuclei. This is the first evidence that ASH1 is indeed transcribed in both nuclei before being transported to the bud tip. This observation might help to understand better the function of one of the proteins involved in ASH1 mRNA localization, Bni1p (13, 28). Mutations in BNI1 lead to an accumulation of ASH1 mRNA at the bud neck between mother and daughter cell (13). This phenotype has been explained by the synthesis of ASH1 mRNA in the mother cell followed by a block of ASH1 mRNA export from the mother cell at the bud neck (29). Alternatively, a misorientation of the localization machinery to the bud neck in bni1 mutants could result in the same ASH1 mRNA mislocalization pattern. Our finding that ASH1 mRNA is produced in mother and daughter nuclei is not compatible with the first hypothesis since ASH1 mRNA synthesized in daughter cell nuclei should be properly transported in the case of Bni1p functioning at the bud neck. Therefore, the failure of bni1 mutants to localize ASH1 mRNA is most likely due to an improper orientation of the localization machinery.

In summary, we have shown that Mex67p is a mRNA export factor for at least three different mRNA transcripts that differ in abundance, function, and induction. It is therefore likely that Mex67p serves as a general mRNA export factor for PolII-derived transcripts.

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