The localization of nuclear exporters of the importin-β family is regulated by Snf1 kinase, nutrient supply and stress

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Abstract

In the budding yeast Saccharomyces cerevisiae, four members of the importin-β family of nuclear carriers, Xpo1p/Crm1p, Cse1p, Msn5p and Los1p, function as exporters of protein and tRNA. Under normal growth conditions GFP-tagged exporters are predominantly associated with nuclei. The presence of Snf1 kinase, a key regulator of cell growth and a metabolic sensor, controls the localization of GFP-exporters. Additional glucose-dependent, but Snf1-independent, mechanisms regulate carrier distribution and a switch from fermentable to non-fermentable carbon sources relocates all of the carriers, suggesting a link to the nutritional status of the cell. Moreover, stress controls the proper localization of GFP-exporters, which mislocalize upon exposure to heat, ethanol and starvation. Stress may activate the MAPK cell integrity cascade, and we tested the role of this pathway in exporter localization. Under non-stress conditions, the proper distribution of GFP-Cse1p and Xpo1p/Crm1p-GFP requires kinases of the cell integrity cascade. By contrast, Msn5p-GFP and Los1p-GFP rely on the MAPK module to relocate to the cytoplasm when cells are stressed with ethanol. Our results indicate that the association of nuclear exporters with nuclei is controlled by multiple mechanisms that are organized in a hierarchical fashion and linked to the physiological state of the cell.© 2007 Elsevier B.V. All rights reserved.

Keywords: Nuclear export carriers; Snf1/AMP kinase; Stress; Cell integrity pathway

1. Introduction

Transport of molecules between cytoplasmic and nuclear compartments is a fundamental biological process conserved amongst eukaryotes. Nuclear trafficking is required for proper growth, development, and the adaptation to environmental changes, all of which depend on nuclear export of transcriptional regulators and other components [1,2]. Trafficking between nucleus and cytoplasm is mediated by specialized carriers that translocate proteins and RNAs across the nuclear envelope [3–6]. The budding yeast Saccharomyces cerevisiae has 14 members of the importin-β, or karyopherin-β, family which are classified into importins and exportins, depending on whether they move cargo in or out of the nucleus. Four members of the importin-β family function as nuclear exporters; Xpo1p/Crm1p, Cse1p, Msn5p and Los1p, function as exporters of protein and tRNA. Under normal growth conditions GFP-tagged exporters are predominantly associated with nuclei. The presence of Snf1 kinase, a key regulator of cell growth and a metabolic sensor, controls the localization of GFP-exporters. Additional glucose-dependent, but Snf1-independent, mechanisms regulate carrier distribution and a switch from fermentable to non-fermentable carbon sources relocates all of the carriers, suggesting a link to the nutritional status of the cell. Moreover, stress controls the proper localization of GFP-exporters, which mislocalize upon exposure to heat, ethanol and starvation. Stress may activate the MAPK cell integrity cascade, and we tested the role of this pathway in exporter localization. Under non-stress conditions, the proper distribution of GFP-Cse1p and Xpo1p/Crm1p-GFP requires kinases of the cell integrity cascade. By contrast, Msn5p-GFP and Los1p-GFP rely on the MAPK module to relocate to the cytoplasm when cells are stressed with ethanol. Our results indicate that the association of nuclear exporters with nuclei is controlled by multiple mechanisms that are organized in a hierarchical fashion and linked to the physiological state of the cell.© 2007 Elsevier B.V. All rights reserved.

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Abbreviations: BSA, bovine serum albumin; DAPI, 4',6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide; GFP, Aequorea victoria green fluorescent protein; MAPK, mitogen-activated protein kinases; NE, nuclear envelope; PKC, protein kinase C; SFN1, sucrase non-fermenting; TOR, target of rapamycin

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Msn5p are several transcription factors that regulate nutrient-dependent gene expression, like Mig1p and Pho4p, the cell cycle regulator Swi6p and the hsp70, Ssa4p [11–14]. The importin-β family member Los1p promotes nuclear export of tRNAs [15,16].

We previously identified Msn5p as the nuclear export carrier for Ssa4p in budding yeast and demonstrated that Msn5p localization is controlled by stress and carbon source [14]. It is not known whether other exporters share these properties. The presence of nutrients, including the carbon source, regulates protein synthesis, gene expression and the activities of many proteins. Snf1 kinase, a highly conserved eukaryotic serine/threonine protein kinase, is an essential regulator of these processes [17–19]. Snf1 kinase not only controls cell growth and transcription, but also the response to nutrient limitation, heat and osmotic stress [17]. Interestingly, the presence of Snf1, even in the absence of Snf1 kinase activity, contributes to some of the regulatory effects of the protein, including the proper localization of Gal83p, one of the Snf1 β-subunits [20]. Like Snf1, the TOR (target of rapamycin) kinase pathway is at the center of metabolic and growth control in response to nutrient availability [21–24]. TOR complex 1 can be inhibited with the antifungal antibiotic rapamycin, which interferes with growth in yeast and higher eukaryotes [24]. Moreover, the TOR and Snf1 kinase pathways converge to respond to nitrogen or carbon source availability [25,26].

One of the key regulators that are downstream of TOR function and also essential for the response to many forms of stress is the Pck1p-cell integrity pathway [27–30]. Following rapamycin treatment or plasma membrane stress, sensors in the plasma membrane, including Wsc1p and Mid2p, promote the activation of Pck1p and thereby of the downstream cell integrity MAPK cascade. This MAPK module consists of the four kinases Bck1p (MAPKKK), Mkk1p/Mkk2p (MAPKK) and Mpk1p (MAPK), which can interact with phosphatases or other kinases [reviewed in 28–30]. Although Pck1p is the most prominent activator of this MAPK cascade, input from other pathways may regulate this signaling route [28–31].

Snf1 kinase, the cell integrity pathway and nuclear transport are essential components necessary to respond to nutrient availability or stress. We explored here a possible role of these protein kinases as well as nutrient supply in regulating nuclear exporters of the importin-β family.

2. Materials and methods

2.1. Strains, plasmids and growth conditions

Wild type and mutant yeast strains used in this study are shown in Table 1. Plasmids encoding GFP fused to the N-terminal end of Cse1p or the C-terminal end of Xpo1p, Msn5p or Los1p and ribosomal protein Rpl25p or Yrb1p were kindly provided by K. Weis, G. Schlenstedt, L. Huang, D. Mangroo and E. Hurt. Snf1p wild type and mutant cells were a gift of M. Johnston [32]. Yeast cells were transformed using standard procedures; transformants were selected and grown on complete minimal medium lacking the amino acids or nutrients used for selection. The growth media of pck1Δ yeast strains mutated in components of the cell integrity pathways and control strains used in these experiments were supplemented with 10% sorbitol. Except for starvation experiments (see Section 2.5); cells were in logarithmic phase for all experiments.

### Table 1

<table>
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<tr>
<th>Strain</th>
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</tr>
<tr>
<td>reg1Δ</td>
<td>MATa lys2–801::BM1499 snf1Δ [32]</td>
</tr>
<tr>
<td>BY474</td>
<td>MATa his3Δ leu2Δ met15Δ ura3Δ wsc1Δ</td>
</tr>
<tr>
<td>BY474</td>
<td>BY4741 wsc1Δ</td>
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<td>BY474</td>
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2.2. Localization of GFP-fusion proteins

Fusion proteins containing the GFP-tag were localized as described [14]. In brief, cells were fixed at room temperature for 10 min with 3.7% formaldehyde, washed in 1.1 M sorbitol/0.1M potassium phosphate pH 6.5 and immobilized on polylysine coated multwell slides. Cells were permeabilized for 1 min with methanol (–20 °C) followed by a 10-s rinse with acetone. Samples were air-dried at room temperature, stained with DAPI and images were taken with a Nikon Optiphot microscope or Zeiss LSM 510 at 1,000 X magnification for 0.5 μm sections. The localization of proteins was monitored by visual inspection of the specimens using a Nikon Optiphot microscope. To avoid the non-linear range of fluorescence signals, cells highly overexpressing one of the GFP-tagged carriers were excluded from further analyses. A minimum of 100 cells were scored in each experiment, and at least three independent experiments were performed for each condition. N>C denotes nuclear accumulation of the GFP-tagged reporter protein; C>N, fluorescence signals in the cytoplasm were brighter than in the nuclei; N+C, similar fluorescence intensities for nuclei and cytoplasm. Within each experiment, identical settings were used for imaging. Negatives and confocal images were processed with Adobe Photoshop 8.0.

2.3. Growth on different carbon sources

The expression of genes encoding GFP-Cse1p and Los1p-GFP is under control of the GAL1 promoter; for comparison all strains synthesizing GFP-tagged carriers were cultured overnight in selective media with 2% galactose. Under these conditions, transformants derived from snf1Δ cells show slow growth. To monitor the effect of different carbon sources, cells were collected by centrifugation and resuspended in fresh medium containing 2% glucose, 2% ethanol or 3% glycerol. After transfer to fresh medium, cells were grown for 4 h, fixed and further treated as described [14]. For the shift from high to low glucose cells grown overnight in medium containing 2% galactose were shifted for 4 h 4% glucose (high glucose) followed by a 10 min incubation with 0.05% glucose (low glucose). Aliquots of cells were fixed following the incubation in high and low glucose medium and the distribution of carriers was monitored.

2.4. Rapamycin or cycloheximide treatment

Yeast cells synthesizing one of the GFP-tagged exporters were grown overnight in selective medium with 2% galactose. Rapamycin was added from freshly prepared stocks (50 μM) to a final concentration of 500 nM, and controls were incubated with solvent only. Cycloheximide was present at 100 μg/ml. After 0.5-, 1- or 3-h incubations cells were fixed and processed for microscopy.
2.5. Stress assays

Yeast cells were cultured overnight at room temperature in selective medium containing 2% galactose and exposed to different forms of stress; heat: 6 h 37 °C or 1 h 42 °C; ethanol: 10 min 10% (final concentration); osmotic stress: 10 min 0.4 M NaCl (final concentration); oxidative stress: 10 min or 1 h 0.3 mM H2O2 (mild), and 10 min 2 mM H2O2 (severe). Cells were starved by continuous growth for 4 days in the same medium. For analyses of the cell integrity pathway (Figs. 4 and 5) all growth media contained 10% sorbitol.

2.6. Western blot analysis

Western blotting with antibodies against GFP was carried out as described previously [14].

3. Results

3.1. The localization of nuclear exporters is controlled by Snf1 kinase, by carbon source, and is sensitive to rapamycin

To identify a possible link between the nutritional state of the cell and the regulation of nuclear exporters, we analyzed yeast cells that carry a knockout of the \textit{SNF1} gene. In mutant as well as isogenic wild type cells, GFP-tagged exporters were localized by fluorescence microscopy. For wild type cells under non-stress conditions all of the exporters were predominantly associated with nuclei (Fig. 1A). However, their distribution was altered in a
significant number of snf1Δ cells. For instance, Xpo1p-GFP nuclear accumulation decreased from 91% (wild type) to about 47% of the cells in the mutant strain, with relocation to the cytoplasm. Similar results were obtained for Los1p-GFP, GFP-Cse1p and Msn5p-GFP (Fig. 1A).

Since Snf1 kinase is crucial for the regulation of glucose-sensitive gene expression, we further investigated the effects of different carbon sources on nuclear exporters. After overnight growth in medium containing galactose, cells were shifted to glucose, glycerol or ethanol as carbon source, and GFP-tagged carriers were located 4 h after the shift. At this time point, cells were growing exponentially (Suppl. Fig. 1). In the presence of glucose all of the GFP-carriers were mostly nuclear, whereas incubation with glycerol or ethanol induced relocation to the cytoplasm in a significant number of cells (Fig. 1B). As previously reported, the subcellular distribution of the GFP-tag alone was not affected by the carbon source, GFP is both nuclear and cytoplasmic under all conditions [14, Fig. 6 and data not shown].

We further tested the possibility that the association of GFP-exporters with nuclei is sensitive to the changes in glucose concentration, by signaling events that are independent of Snf1 (Fig. 1C). Gal83p is a target of such a regulatory event that involves Snf1 kinase without requiring its enzymatic activity [20]. To achieve this, cells grown overnight on 2% galactose were incubated for 4 h in high glucose medium (4% glucose, H in Fig. 1C) and shifted to low glucose (0.05%, L) for 10 min. GFP-tagged carriers were located after incubation with high glucose and following the 10 min shift to low glucose. For comparison, this experiment was carried out in wild type, snf1Δ and a reg1Δ strain with the same genetic background. (Note that these reg1Δ cells also carry a mig1Δ2::LEU2 marker.) In reg1Δ, Snf1 kinase is always active, as cells fail to inactivate Snf1 by dephosphorylation. For all strains, the number of cells that concentrated GFP-tagged carriers at nuclei was reduced upon shift from high to low glucose. In snf1Δ and reg1Δ strains the decreased number of cells that accumulate GFP-tagged carriers at the nucleus upon shift to low glucose suggest glucose-sensitive control mechanisms which do not rely on the activity of Snf1 kinase.

Rapamycin treatment mimics amino acid starvation, thereby signaling stress and causing growth inhibition [21–23]. As shown in Fig. 2 incubation with rapamycin for 0.5, 1 or 3 h induced a significant mislocalization of GFP-tagged nuclear exporters; albeit to a lesser extent than glycerol or ethanol. To test whether the effect of rapamycin on carrier localization can be explained by an inhibition of protein synthesis, experiments were repeated in the presence of 100 μg/ml cycloheximide. A small percentage of GFP-exporters relocated to the cytoplasm in the presence of cycloheximide, but this effect was always less pronounced than the redistribution following rapamycin treatment.

Taken together, our results suggest that the nucleocytoplasmic distribution of export carriers is regulated by the presence of Snf1 kinase, the carbon source and TOR.

3.2. Stress relocates nuclear exporters

Results described above show the importance of nutrients for the proper distribution of nuclear exporters. Thus, we further investigated the effect of starvation and other forms of stress. Mostly nuclear in unstressed wild type cells (Figs. 1A and 3), exposure to certain stressors increased the number of cells with GFP-carriers in the cytoplasm. This redistribution of carriers cannot simply be attributed to stress-induced degradation and relocation of fluorescent proteolytic products, since similar amounts of intact transporters were detected in crude extracts of control and stressed cells (Fig. 3A–C, and data not shown).

As depicted in Fig. 3, individual carriers differ in sensitivity towards a particular form of stress. For instance, when exposed

![Fig. 2. Rapamycin treatment changes the localization of nuclear exporters. (A) Wild type cells synthesizing GFP-tagged carriers were incubated with solvent (control) or rapamycin. After a 3-h incubation, cells were fixed and exporters localized by fluorescent microscopy. (B) Cells synthesizing GFP-fused exporters were treated with rapamycin for 0.5, 1 and 3 h. For comparison, the effect of 100 μg/ml cycloheximide on carrier distribution was analyzed. Nuclear accumulation (N>C) was quantified in control and drug-treated cells. Means and standard deviations are shown for at least three independent experiments for each exporter, **p<0.01, *p<0.05.](image-url)
to heat shock or ethanol, GFP-exporters mislocalized to the
 cytoplasm in a substantial number of cells (Fig. 3A–C and Ref. [14]). Starvation had a strong effect on GFP-Cse1p and Msn5p-
GFP (Fig. 3B and Ref. [14]), but was less efficient in relocating
Xpo1p-GFP and Los1p-GFP (Fig. 3A, C). The distribution of
all carriers was somewhat altered by osmotic and oxidative
stress, with NaCl having its greatest effect on Xpo1p-GFP
(Fig. 3).

Taken together, for all GFP-tagged yeast importin-β like
exporters the nuclear versus cytoplasmic localization can be

Fig. 3. Xpo1p-GFP, GFP-Cse1p and Los1p-GFP relocate to the cytoplasm upon stress. Yeast cells synthesizing GFP-tagged Xpo1p (A), Cse1p (B) or Los1p (C) were
exposed to the different stress conditions described in Materials and methods. Carrier association with nuclei (N>C) was determined in three independent experiments
(means and S.D.); a minimum of 100 cells was scored in each experiment. For Western blotting equal amounts of protein from control and stressed cells were separated
by SDS-PAGE and nuclear exporters were detected with antibodies against GFP.
Fig. 4. The cell integrity MAPK pathway controls the nuclear accumulation of Xpo1p-GFP and Cse1p-GFP in unstressed cells. (A) GFP-tagged Xpo1p, Cse1p, Los1p and Msn5p were located under non-stress conditions in wild type (wt) and mutant strains, missing different components of the Pkc1p-cell integrity pathway as indicated in the figure. Wt1 is the isogenic wild type for all cells except mid2Δ, wsc1Δ and pkc1Δ cells, for which wt2 serves as control. Part (A) shows the localization of GFP-Cse1p in different strains, (B) the quantification of results obtained for all carriers.
modulated by stress. However, individual exporters display distinct patterns of sensitivity towards specific types of stress.

3.3. The nuclear association of Xpo1p-GFP and GFP-Cse1p, but not Los1p-GFP and Msn5p-GFP, depends on kinases of the cell integrity pathway under normal growth conditions

Some forms of stress, in particular heat, lead to the activation of Pkc1p and the cell integrity MAPK pathway. However, these signaling pathways may also regulate nuclear exporter function under non-stress conditions. To address this question, we located GFP-tagged exporters in strains mutant in components of the Pkc1p-cell integrity pathway. To keep all strains viable, wild type and mutant cells were grown on medium containing the osmolyte sorbitol. Surprisingly, even in the absence of stress, in comparison to the wild type strain, Xpo1p-GFP was mislocalized in a larger number of bck1Δ, mkk1Δ, mkk2Δ and mpk1Δ cells. Similarly, GFP-Cse1p required kinases of the MAPK module, Rom1p, (GDP/GTP exchanger of the small GTPase Rho1p), the plasma membrane sensor Mid2p, and Pmt2p, an O-mannosyl transferase that modifies Mid2p. None of the GFP-carriers required Pkc1p for their association with nuclei (Fig. 4B) and none of the mutant strains mislocalized Msn5p-GFP or Los1p-GFP in unstressed cells (Fig. 4A, B).

3.4. Ethanol-induced mislocalization of Msn5p-GFP and Los1p-GFP requires protein kinases of the cell integrity MAPK module

Ethanol stress may alter the fluidity of the plasma membrane and thereby activate the cell integrity kinase cascade. We therefore determined whether the ethanol-induced relocation of transporters to the cytoplasm (Fig. 3) is altered in mutants that cannot signal through this pathway. The liberation of Msn5p-GFP and Los1p-GFP from nuclei was drastically reduced in mutants bck1Δ, mkk1Δ, mkk2Δ and mpk1Δ, but not in pkc1Δ cells (Fig. 5A, B). By contrast, Xpo1p-GFP and GFP-Cse1p efficiently redistributed to the cytoplasm in all of the kinase mutants (Fig. 5B), suggesting that their release from the nuclei of ethanol-stressed cells is regulated differently. It should be noted that in Fig. 5 the extent of the response to ethanol in wild type cells differs from Fig. 3. This is possibly caused by the different strain background and/or the addition of sorbitol to the growth medium for all of the experiments shown in Fig. 5.
Fig. 6. Effect of stress on the nucleocytoplasmic distribution of Xpo1p cargoes Rpl25p-GFP and Yrb1p-GFP. Wild type cells synthesizing Rpl25p-GFP (A), Yrb1p-GFP (B) or the GFP-tag (C) were exposed to different types of stress as described for Fig. 3. DAPI staining, green fluorescence and the overlay of both signals is shown. The distribution of GFP signals was quantified for three independent experiments (C > N for Rpl25p-GFP and Yrb1p-GFP; N + C for GFP), each scoring at least 100 cells. **p < 0.005, *p < 0.05.
3.5. Effect of stress on export of Xpo1p cargo

Our previous studies showed a correlation between the nuclear association of Msn5p and the concentration of its cargo Ssa4p in nuclei, supporting the idea that carrier mislocalization can alter the distribution of its cargo [14]. We further tested this model with two cargoes that are exported by Xpo1p. Rpl25p-GFP, a tagged ribosomal protein is transported to the cytoplasm as part of the 60S ribosomal subunit [33]. Nuclear export of Rpl25p-GFP as component of the large ribosomal subunit to the cytoplasm depends not only on the function of Xpo1p, but relies on several preceding transport and processing steps; this includes the nuclear import of Rpl25p-GFP and subsequently a large number of assembly and processing steps in the nuleolus and nucleoplasm [reviewed in 34]. Export of the large ribosomal subunit also requires the adaptor Nmd3p which connects the 60S subunit to Xpo1p [33]. Fig. 6A shows fluorescence signals for DAPI, Rpl25p-GFP and the overlay of both signals after exposure to different stresses. Under normal growth conditions Rpl25p-GFP is cytoplasmic, with little fluorescence associated with nuclei and no fluorescence present in vacuoles. No drastic changes were obvious for Rpl25p-GFP in cells incubated at 37 °C for 6 h, similar to reports from others for a 5-h shift to 37 °C [34]. Likewise, we could not detect nuclear accumulation of Rpl25p-GFP after a 1-h heat shock at 42 °C. These results are consistent with a model that links nuclear association of Xpo1p-GFP to the efficient export of its cargo. However, we cannot rule out that an inhibition of Rpl25p-GFP nuclear import by heat prevents the detection of potential defects in 60S ribosomal export.

Ethanol mislocalizes efficiently Xpo1p-GFP (Fig. 3); if export to the cytoplasm is the limiting factor for Rpl25p-GFP localization one would predict a redistribution of Rpl25p-GFP in ethanol-stressed cells. In line with this idea, treatment with ethanol increases the fluorescence signals in nuclei when compared to untreated controls. In most ethanol-incubated cells cytoplasmic and nuclear fluorescence was similar in intensity, but nuclear accumulation of Rpl25p-GFP was detected as well (Fig. 6A). We were unable to determine the effect of starvation on Rpl25p-GFP localization since the fluorescence signals were drastically reduced (Fig. 6A). Since a strong signal was obtained in starving cells for the GFP-tag (Fig. 6C), reduced synthesis and/or increased degradation of Rpl25p-GFP during starvation may diminish cellular levels of the tagged ribosomal protein.

Given that the localization of Rpl25p-GFP in stressed cells depends on nuclear import and a large number of processing events in the nucleus, we wished to test another cargo that is subject to complex regulation on different levels. Our studies identify new links between the presence of the metabolic sensor Snf1 kinase or nutrient supply and the proper localization of GFP-tagged export carriers. In particular, results with snf1Δ and reg1Δ strains support the idea that glucose-dependent, but Snf1-independent, pathways regulate the carrier distribution. This scenario is reminiscent to the localization of Gal83p, which is controlled by the presence of Snf1 kinase and glucose-dependent mechanisms that do not require the kinase activity [20]. Future experiments will have to determine whether Snf1 kinase activity is dispensable for carrier distribution as well. The regulatory links identified by us provide a general control switch that contributes to the distribution of all GFP-exporters. A common denominator of this control mechanism could be the cell’s metabolic status [17–19,21–27], which is likely to impinge on energy-consuming processes like nuclear export. Relocation of nuclear exporters to the cytoplasm may then reduce export efficiency by making only a portion of the carriers available in the nucleus.

On another level, signaling through the MAPK cell integrity pathway [28,29] regulates different classes of carriers. We defined Xpo1p-GFP and GFP-Cse1p as well as Msn5p-GFP and Los1p-GFP, respectively, as two groups that are similarly affected by this kinase module. For Xpo1p-GFP and GFP-Cse1p the kinase cascade is required for nuclear association under non-stress conditions, whereas Msn5p-GFP and Los1p-GFP rely on this pathway for their redistribution upon stress. Although Pkc1p is the most important upstream regulator of the MAPK cell integrity cascade, our results suggest that input from Pkc1p is not essential to localize nuclear exporters. This could suggest that other upstream regulator(s) are involved in these signaling events. An example of Pkc1p-independent activation of the cell integrity pathway was recently reported in Schizosaccharomyces pombe [31], and similar mechanism(s) may control the localization of exporters in S. cerevisiae as well. A possible model to explain the effect of the MAPK module would propose that factors interacting with either group of carriers are regulated by the cell integrity pathway. Candidate components for this control are cytoplasmic anchors for Xpo1p-GFP and GFP-Cse1p or nuclear anchors that prevent the stress-induced release of Msn5p-GFP and Los1p-GFP. Alternatively, the cell integrity cascade may control the function of nucleoporins that are necessary for either group of carriers to move in and out of the nucleus.

Furthermore, we demonstrate that environmental stresses alter the association of exporters with the nucleus. Although all exporters are sensitive to stress, they show unique sensitivities towards specific insults, suggesting that some stresses control carriers on an individual basis. Here a testable hypothesis is that individual regulation is achieved by reversible posttranslational modifications of a particular carrier.
Our results are consistent with the idea that exporter localization is controlled in a hierarchical fashion. This complex regulation may provide a platform to adjust the efficiency of nuclear export according to the changes in cellular metabolism or environmental conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamacr.2007.04.014.

References