Cse1p functions as the nuclear export receptor for importin α in yeast

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Abstract ***CSE1*** **is essential for yeast cell viability and has been implicated in chromosome segregation. Based on its sequence similarity, Cse1p has been grouped into the family of importin β-like nucleocytoplasmic transport receptors with highest homology to the recently identified human nuclear export receptor for importin α, CAS. We demonstrate here that Cse1p physically interacts with yeast Ran and yeast importin α (Srp1p) in the yeast two-hybrid system and that recombinant Cse1p, Srp1p and Ran-GTP form a trimeric complex in vitro. Re-export of Srp1p from the nucleus into the cytoplasm and nuclear uptake of a reporter protein containing a classical NLS are inhibited in a** ***cse1*** **mutant strain. These findings suggest that Cse1p is the exportin of importin α in yeast.**

Key words: Nuclear protein export; Srp1p; Cse1p; CAS; Ran; *Saccharomyces cerevisiae*

1. Introduction

Transport of macromolecules across the nuclear envelope occurs through nuclear pore complexes (NPCs) and is an energy- and signal-dependent process depending on various transport factors (for review see [1–3]). Multiple transport pathways through the NPC have been identified whose specificity is ensured by nucleocytoplasmic transport receptors (collectively termed importins or exportins) which shuttle continuously between cytoplasm and nucleoplasm (for review see [4–6]). Binding and release of cargo to and from these receptors is regulated by the small Ras-like GTPase Ran which seems to be essential for most if not all nucleocytoplasmic transport pathways. Due to an asymmetric subcellular distribution of the only known RanGAP in the cytoplasm and the RanGEF in the nucleus, levels of the GTP-bound form of Ran are presumably rather low in the cytoplasm and high in the nucleus. This Ran-GTP gradient across the nuclear envelope together with intrinsic properties of nuclear import and export receptors (see below) is likely to ensure the directionality of nuclear transport (for review see [6,7]).

Nuclear import and export receptors identified to date show sequence similarity to importin β, the NPC-interacting subunit of the heterodimeric import receptor of proteins with a classical nuclear localization signal (NLS), importin α/β (for review see [4,5]). The similarity is mainly found in a Ran-GTP binding domain at the N-terminus of these proteins [8,9]. Binding of Ran-GTP has opposite effects on the binding of cargo by import and export receptors. Complexes between import receptors and cargo are dissociated by Ran-GTP, whereas binding of Ran-GTP and cargo to export receptors is cooperative. These properties of import and export receptors together with a high concentration of Ran-GTP in the nucleus trigger release of cargo from import receptors and association of cargo with export receptors in the nucleus. Release of export cargo and binding of import cargo in the cytoplasm is presumably dependent on another type of Ran-GTP binding domain present in a soluble Ran-GTP binding protein, RanBP1, and in some nucleoporins [10–13].

Characterized nuclear import and export receptors include, besides importin β (karyopherin β1) and its yeast homolog Kap95p [14–18], transportin (karyopherin β2) and yeast Kap104p [19,20]), the import receptor for some hnRNP proteins, karyopherin β3 (yeast Pse1p/Kap121p) and β4 (yeast Yrb4p/Kap123p) [21–23], conferring nuclear import of ribosomal proteins, Mtr10p [24,25], the nuclear import receptor for the yeast hnRNP protein Npl3p, Sxm1p [26], mediating nuclear import of the yeast La homolog, CRM1 (yeast Xpo1p) [27–30], the export receptor for proteins containing a leucine-rich nuclear export signal (NES), and hLOS1 and yeast Los1p [31–33]), an export receptor for tRNA (for more references see [4,5]).

Another member of this protein family, CAS, previously implicated in apoptosis [34] was recently shown to confer the re-export of importin α, the NLS binding subunit of the NLS receptor (importin α/β), from the nucleus into the cytoplasm [35]. The shuttling of importin α between nucleus and cytoplasm is thus mediated by two different members of the nuclear transport receptor family, importin β and CAS (for review see [6]). On its import route into the nucleus, importin α serves as an adaptor for proteins containing a classical NLS. It is not known, however, whether importin α is bound to any substrate on its way out of the nucleus. Based on the identification of a nuclear complex between yeast importin α, Srp1p, and the yeast cap binding protein complex (CBC), it was suggested that importin α plays a role in U snRNA export [36].

Based on sequence similarity, a number of additional members of the nuclear transport receptor family have been suggested, but their function in nuclear transport remains to be shown and their substrate to be identified. One of these proteins, Cse1p, was previously identified in a genetic screen for mutations that affect chromosome segregation in *Saccharomyces cerevisiae* [37]. The *CSE1* gene is essential for yeast cell viability and encodes a 109 kDa protein with 36% overall identity to human CAS [34] as compared to 18% identity to importin β. Isolation of *SRP1* encoding yeast importin α, as a multicopy suppressor of a *cse1* mutation [37], localization of

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Abbreviations: NPC, nuclear pore complex; NLS, nuclear localization sequence; NES, nuclear export signal; GEF, guanine-nucleotide exchange factor; GAP, GTPase activating protein; GST, glutathione *S*-transferase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ORF, open reading frame; A, absorbance; CPRG, chlorophenol red-β-D-galactopyranoside; IPTG, isopropyl-β-D-thiogalactopyranoside; PCR, polymerase chain reaction; DBD, DNA binding domain; TAD, transcriptional activation domain

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Cse1p to the nuclear periphery (cited in [38]) and binding of Cse1p to Ran-GTP in an overlay assay [9] provided circumstantial evidence that the sequence similarity to CAS might be functionally relevant. Here, we show that Cse1p physically interacts with Srp1p and Ran in vivo and in vitro. Using recombinant proteins from *Escherichia coli*, a trimeric complex between Cse1p, Srp1p and Ran-GTP could be formed. In addition, a mutation in the *CSE1* gene leads to nuclear accumulation of Srp1p and cytoplasmic accumulation of a NLS-containing nuclear reporter protein. Taken together these results suggest that Cse1p is the functional homologue of CAS in *S. cerevisiae*.

2. Materials and methods

2.1. Strains and growth conditions

Yeast strains Y1709 (*MAT*a *ura3-52 his3-11,15 trp1-Δ901 ade2-101 cse1-1*) and JTY2500 (*MAT*a *ura3-52 his3-Δ200 leu2-3,112 trp1-Δ901 can*^{R} *gal4-542 gal80-538 ADE2::PGAL-URA3 LYS2::lexop-lacZ*) have been described earlier [37,39]. Unless indicated otherwise, yeast cells were grown at 30°C in YPD complete medium or SDC synthetic complete medium according to [40]. Yeast transformations were performed using a modified form of the lithium acetate method [41].

E. coli strain DH5α [42] was used for propagation of plasmids and protein expression controlled by the *tac* promoter (see below). Strain BL21(DE3) [43] was used for protein expression controlled by the bacteriophage T7 promoter (see below). Bacteria were cultivated and transformed using standard methods [44].

2.2. Plasmids

Standard techniques were used for the isolation and manipulation of recombinant DNA [44,45]. PCR amplifications were performed using Expand High Fidelity PCR system (Boehringer Mannheim) according to the manufacturer's recommendations.

The *CSE1* gene was subcloned as a genomic *Hin*dIII/*Sac*I fragment from plasmid 21-3 (provided by Stefan Irniger, Institut für Mikrobiologie und Genetik, Göttingen, Germany) on pRS316 yielding plasmid pMK330. *E. coli* expression plasmid pET9dt-*CSE1* encoding $(His)_6$ epitope tagged Cse1p under the control of the bacteriophage T7 promoter contains a PCR-generated *Nco*I/*Bam*HI fragment comprising the entire ORF on a modified version of plasmid pET9d (Gunter Stier, EMBL, Heidelberg, Germany). *SRP1* constructs were derived from plasmid pNOY162 [46]. Plasmid pMK286 containing a *SRP1-GFP* fusion was constructed by inserting a *Bam*HI *GFP* cassette [47] into a PCR-generated *Bgl*II site immediately upstream of the STOP codon of *SRP1* on pNOY162. *E. coli* expression plasmid pNOY3198 encoding a GST-Srp1p fusion protein under the control of the *tac* promoter has been described earlier [48]. *GSP1* constructs were derived from plasmid YEp352-*GSP1* [49]. A plasmid encoding a GFP-Npl3(284–414) fusion protein comprising the NLS of Npl3p under the control of the constitutive *NOP1* promoter has been described earlier [25]. The construction of a fusion gene between *GFP* and the entire open reading frame of *RPL25* encoding the ribosomal protein L25 on pRS314 will be described elsewhere (S. Hannus, B. Schmelzl, G. Simos and E. Hurt, in preparation). Plasmid pMK284 carrying a *YRB1-GFP* fusion on pRS314 has been described previously [33]. A *Pst*I/*Eco*RI fragment comprising a *ADH1-SV40*$_{NLS}$*-GAL4*$_{TAD}$*-GFP* fusion gene was recloned from plasmid pGADGFP [50] onto *ADE2*-vector pASZ11 [51] (Jun Katahira, unpublished).

Fusions of various genes to the DBD of *E. coli* LexA protein and the TAD of yeast Gal4p were constructed by amplifying fragments comprising the entire ORFs including suitable cloning sites at the ends by PCR and inserting the PCR-generated fragments into the corresponding sites of two-hybrid vectors pBTM116 and pGAD424 [52], respectively. Resulting plasmids were pMK151 (pGAD424-*SRP1*), pMK178 (pGAD424-*GSP1*), pMK199 (pGAD424-*YRB1*) and pMK326 (pBTM116-*CSE1*). Plasmid pMK220 (pBTM116-*KAP95*) contains a *Bam*HI fragment comprising the entire ORF of *KAP95* from pGEX4T3-β_{1-861} [17].

2.3. Two-hybrid protein interaction assay

To assess interactions between $LexA_{DBD}$ and $Gal4_{TAD}$ fusion proteins, strain JTY2500 harboring the *E. coli lacZ* gene under the control of eight LexA binding sites was cotransformed with the appropriate pBTM116- and pGAD424-based plasmids. Transformants were grown in SDC-Leu-Trp medium to late exponential phase ($A_{600nm} \sim 2$) and assayed for β-galactosidase activity using the chromogenic substrate, CPRG (Boehringer Mannheim) [53]. The amount of CPR liberated was followed by the increase in absorbance at 574 nm and was normalized to cell number (A_{600nm}), culture volume, and reaction time. Values shown are means of three independent cultivations of three different transformants for each combination tested. The standard deviations of the mean were less than 25% and are indicated by error bars.

2.4. Expression and purification of recombinant proteins

For the expression of $(His)_6$-Cse1p and GST-Srp1p in *E. coli*, plasmids pET9dt-*CSE1* and pNOY3198 were transformed into BL31(DE3) or DH5α cells, respectively. Transformants were grown in 400 ml LB complete medium [44] at 30°C to OD_{600} 0.5, shifted to 23°C and induced by the addition of 0.5 mM IPTG. The bacterial cell pellet was lysed by sonication in 10 ml of universal buffer (20 mM HEPES-KOH pH 7.0, 100 mM KOAc, 2 mM $Mg(OAc)_2$, 0.1% Tween 20, 10% glycerol) containing 5 mM β-mercaptoethanol and 1 tablet of Complete EDTA-free protease inhibitor mix (Boehringer Mannheim) per 50 ml. In case of BL21(DE3) 20 mM imidazole was added to the buffer to reduce unspecific binding of *E. coli* proteins during subsequent application to the Ni^{2+}-NTA resin (Qiagen). The lysate was cleared by centrifugation at $100\,000 \times g$ for 1 h and applied to 200 μl of Ni^{2+}-NTA resin or glutathione-Sepharose (Pharmacia), respectively, in a 15 ml Poly-prep column (Bio-Rad). Binding was performed for 1 h at 4°C on a turning wheel. Bound proteins were washed three times with 5 ml universal buffer (+40 mM imidazole in case of the Ni^{2+}-NTA column) and eluted with 500 μl universal buffer containing 500 mM imidazole or 10 mM reduced glutathione (Sigma), respectively. Eluted proteins were stored at −70°C in 50 μl aliquots.

2.5. In vitro protein interaction assay

Per assay, 25 μl (~30 mg) purified GST-Srp1p in a total of 500 μl universal buffer were rebound to 20 μl glutathione-Sepharose in a spin column (Mobicol; MoBiTec) for 30 min at 4°C on a turning wheel. Beads were washed three times with 500 μl universal buffer before adding purified $(His)_6$-Cse1p (50 μl; ~10 mg) and human RanQ69L (2 μl; ~10 mg) either in its GDP- or GTP-bound form in a total volume of 200 μl universal buffer. Mixtures were incubated for 1 h at 4°C on a turning wheel. Unbound proteins were collected by centrifugation, beads were washed three times with 500 μl universal buffer and bound proteins were eluted by boiling the beads in 50 μl SDS sample buffer. Unbound (40 μl) and bound proteins (20 μl) were analyzed by SDS-PAGE and Coomassie blue staining.

2.6. Miscellaneous

SDS-PAGE and immunoblotting were done using standard protocols [54]. Immune complexes on blots were visualized using a commercial chemiluminescence detection system (ECL; Amersham) and X-ray film (Biomax; Kodak). Fluorescence microscopy of living yeast cells expressing GFP fusion proteins was done according to [55].

3. Results

3.1. Yeast importin α (Srp1p) and Ran (Gsp1p) bind to Cse1p in vivo

If Cse1p acts as the nuclear export receptor for importin α in yeast, it should interact with its cargo and the regulatory factor Ran. To assess an interaction between these proteins in vivo, we employed the yeast two-hybrid system. The entire open reading frame of the *CSE1* gene was fused to the DNA binding domain (DBD) of LexA (see Section 2). This fusion protein was assayed for interaction with fusions of full-length Srp1p (yeast importin α) and Gsp1p (yeast Ran) to the transcription activation domain (TAD) of Gal4p. An analogous fusion of Yrb1p (yeast RanBP1) to the $Gal4_{TAD}$ and the empty vector expressing the $Gal4_{TAD}$ alone served as controls. The same $Gal4_{TAD}$ constructs were tested for interaction with

a fusion protein of the entire open reading frame of Kap95p (yeast importin β) to the $LexA_{DBD}$. Plasmids encoding the various fusion proteins were introduced into a reporter strain harboring a chromosomal *E. coli lacZ* gene under the control of eight LexA binding sites. Interaction between the various fusion proteins was monitored by determining the β-galactosidase activities of transformants harboring the corresponding plasmids. The results are summarized in Fig. 1. Using this assay, a distinct interaction of Cse1p with Srp1p and Gsp1p was detected. It should be noted that the $Gal4_{TAD}$-Yrb1p fusion protein used here as a negative control shows a strong interaction with a $LexA_{DBD}$-Gsp1p fusion protein under the same conditions (data not shown). Significantly, Kap95p shows essentially the same interaction pattern as Cse1p in this assay. In summary, like Kap95p, Cse1p specifically interacts with Srp1p and Gsp1p in the two-hybrid system.

3.2. *Cse1p, Srp1p and Ran-GTP form a trimeric complex in vitro*

All nuclear export receptors tested so far, CRM1 [27], CAS [35] and hLOS1 [31,32], form trimeric export complexes between receptor, cargo and Ran-GTP. Only weak binding or no binding of the export cargo to the export receptor was observed in the absence of Ran-GTP. In order to test whether Cse1p, Srp1p and Ran can form such a trimeric complex in vitro, we performed solution binding assays using recombinant proteins. For this purpose, we purified $(His)_6$-Cse1p and GST-Srp1p from *E. coli* using respective affinity matrices (see Section 2). For the binding assay, GST-Srp1 was rebound to glutathione-sepharose beads and incubated with approximately equal amounts of affinity-purified $(His)_6$-Cse1p and human RanQ69L (Fig. 2). RanQ69L is a mutant form of Ran whose GTP-bound form is stabilized due to a defect in GTP hydrolysis. The protein can, however, be loaded like wild-type Ran with either GDP or GTP (kindly provided by Ralf Bischoff, Deutsches Krebsforschungszentrum, Heidelberg). After incubation of the protein mixtures for 1 h at 4°C, unbound and bound fractions were analyzed by SDS-PAGE and Coomassie blue staining. Clearly, binding of Srp1p to Cse1p was only observed in the presence of Ran-GTP, but not Ran-GDP (Fig. 2, lanes 5 and 9). In the bound fractions, Ran was detected by immunoblotting using a polyclonal anti-human Ran antiserum. This indicates the formation of a trimeric complex between Cse1p, Srp1p and Ran-GTP.

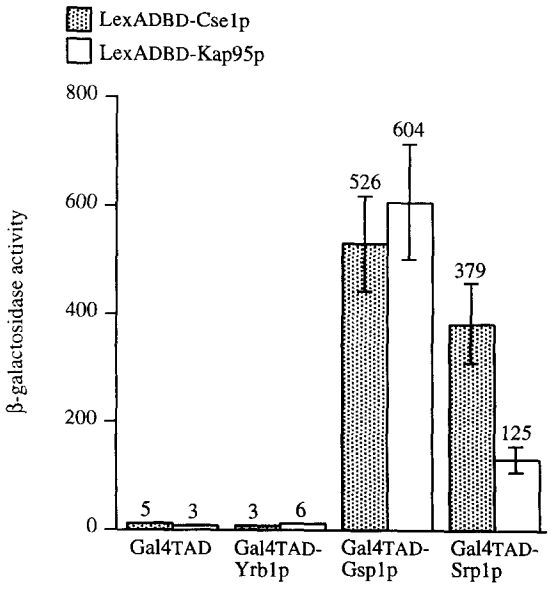


Fig. 1. Cse1p and Kap95p interact with Srp1p and Gsp1p in the yeast two-hybrid system. The complete ORFs of *CSE1*, *KAP95*, *YRB1*, *GSP1* and *SRP1* were fused to the DNA binding domain (DBD) of LexA and the transcriptional activation domain (TAD) of Gal4p, respectively. Plasmids encoding the respective fusion proteins were transformed in various combinations into a yeast strain harboring a chromosomal *lacZ* gene under control of eight LexA binding sites. Transformants were assayed for β-galactosidase activity as described in Section 2. Yrb1p served as a negative control.

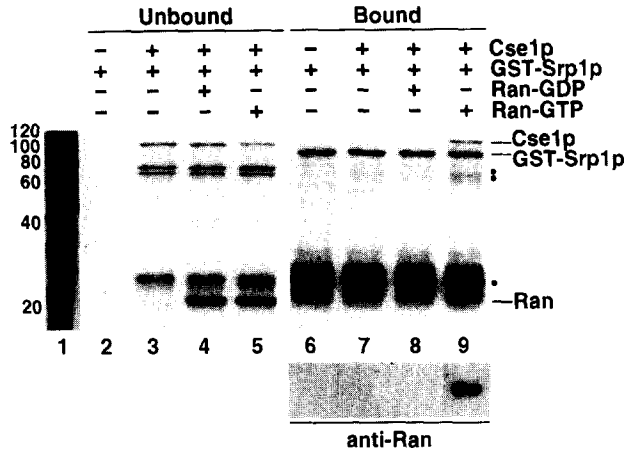
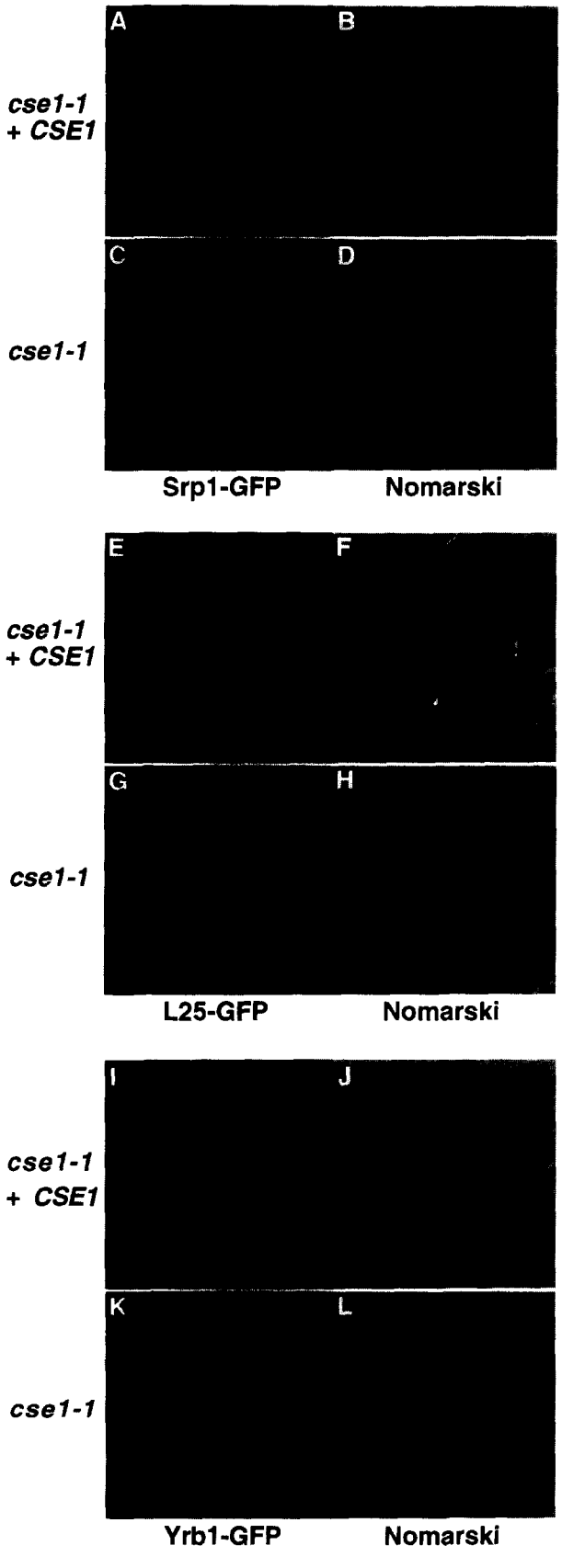


Fig. 2. Cse1p, Srp1p and Ran-GTP form a trimeric complex in vitro. Recombinant Cse1p, GST-Srp1 and human Ran in its GDP- and GTP-bound form were purified from *E. coli* as described in Section 2. GST-Srp1p was immobilized on glutathione-Sepharose beads (Pharmacia), washed and incubated with buffer (lanes 2 and 6), Cse1p (lanes 3 and 7), Cse1p+Ran-GDP (lanes 4 and 8) and Cse1p+Ran-GTP (lanes 5 and 9). Unbound proteins were collected, beads were washed and bound proteins were eluted by boiling the beads in SDS sample buffer. Unbound and bound protein fractions were analyzed by SDS-PAGE and Coomassie blue staining (see Section 2). To visualize bound Ran, an immunoblot of the bound fractions (10 μl) was performed using a polyclonal rabbit antiserum against human Ran. Bands marked with filled circles in the unbound fractions are copurifying proteins from *E. coli*, whereas analogously marked bands in the bound fraction represent degradation products of Srp1p. The sizes of the molecular weight marker used in lane 1 are given in kDa.

3.3. *Srp1p and a nuclear reporter protein containing a classical NLS are mislocalized in a cold-sensitive cse1 mutant*

In order to test whether Cse1p acts as a nuclear export receptor for importin α in vivo, we analyzed the subcellular localization of a functional, plasmid-encoded Srp1-GFP fusion protein in a cold-sensitive *cse1-1* mutant strain carrying in addition either the wild-type *CSE1* gene on a plasmid or the corresponding empty vector (see Section 2). Cells were grown in liquid medium at semi-permissive temperature (23°C) and viewed directly by fluorescence microscopy. Clearly, the cold-sensitive mutation in *CSE1* caused a strong mislocalization of Srp1p-GFP to the nucleoplasm (Fig. 3A–D). In a wild-type strain, Srp1p-GFP is found both in the cytoplasm and nucleoplasm, with an increased, punctate labeling at the nuclear envelope. This intracellular distribution of Srp1p-GFP differs slightly from published localization studies using indirect immunofluorescence microscopy which detected Srp1p either exclusively cytoplasmic, nucleoplasmic or at the



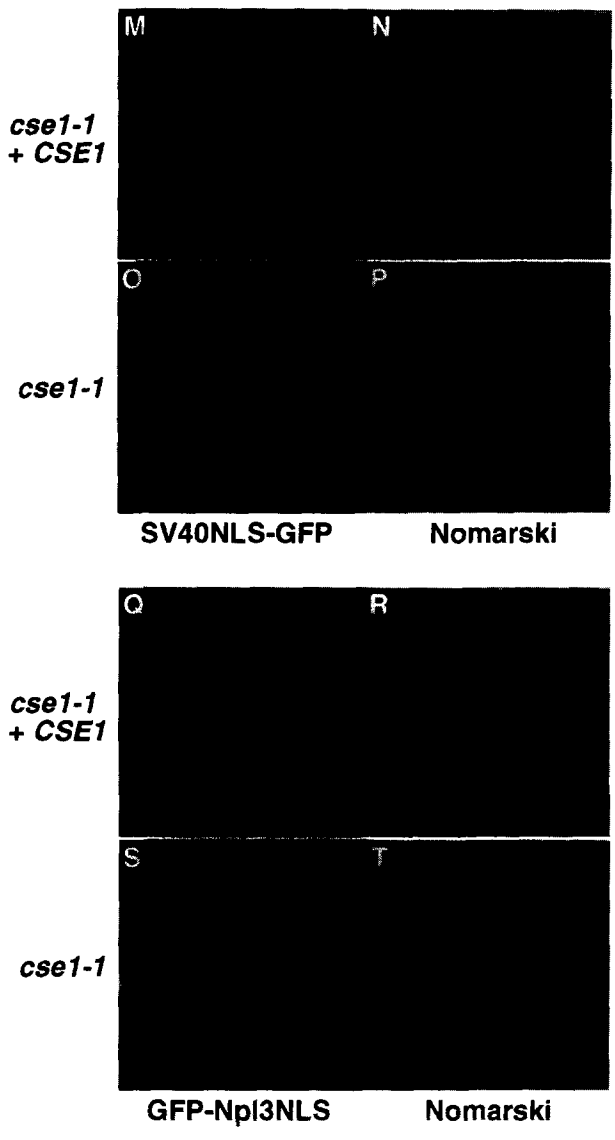


Fig. 3. Srp1p-GFP and $SV40_{NLS}$-GFP, but not L25-GFP, Yrb1-GFP or GFP-$Npl3_{NLS}$ are mislocalized in a *cse1-1* mutant. The *cse1-1* mutant strain Y1709 containing the wild-type *CSE1* gene on a low copy number plasmid or the corresponding vector without insert were transformed with plasmids coding for the respective GFP fusion proteins. Transformants were grown in liquid medium at semi-permissive temperature (23°C) to mid-exponential phase and viewed by fluorescence microscopy and Nomarski optics.

nuclear envelope depending on the experimental conditions [18,56–59]. The differences maybe due to different fixation procedures for immunofluorescence or the method of detection. In a *cse1-1* mutant strain, the cytoplasmic and nuclear envelope staining were clearly reduced and the nucleoplasmic signal accordingly increased. As controls for the specificity of this nuclear export defect, we analyzed, under the same conditions, the steady-state localization of ribosomal protein L25 fused to GFP (L25-GFP) (Fig. 3E–H) and of a Yrb1-GFP (Fig. 3I–L) fusion protein. Both fusion proteins complement, like Srp1-GFP, respective null mutants and exhibit a cytoplasmic localization in a wild-type strain ([33]; S. Hannus, B. Schmelzl, G. Simos and E. Hurt, in preparation). Yrb1-GFP was used since there is evidence that Yrb1p shuttles continuously, like Srp1p, between nucleus and cytoplasm [22], but exploits other exportins for exit from the nucleus (M. Künzler and E. Hurt, in preparation). L25-GFP was used since this ribosomal protein is first imported into the nucleolus where it

assembles into ribosomal particles which are then exported into the cytoplasm [60]. L25 uses distinct importins for its nuclear uptake [22] and the exportins for ribosomal particles are not yet known. The localization of both reporters, L25-GFP and Yrb1-GFP, was completely unaffected by the *cse1-1* mutation. In addition to these controls, we also performed in situ-hybridization of poly(A)$^+$ RNA in the *cse1-1* mutant cells and could not detect any increase in the number of cells showing nuclear accumulation as compared to wild-type cells (data not shown). Poly(A)$^+$ RNA normally gives a cytoplasmic signal under steady-state conditions and is presumably exported from the nucleus via pathways different from Srp1p (for review see [3,61]). It should be noted in addition that, in contrast to GFP fusion proteins containing leucine-rich NESs, the Srp1-GFP fusion protein is not mislocalized in strains mutated in the gene encoding the nuclear export receptor for NESs of this type, *XPO1* [28,62] (data not shown).

In agreement with a mislocalization of the receptor for classical NLSs, Srp1p, a GFP fusion protein containing such a NLS, $SV40_{NLS}$-$Gal4_{TAD}$-GFP ($SV40_{NLS}$-GFP), was significantly mislocalized to the cytoplasm in the *cse1-1* mutant strain (Fig. 3M–P). The nuclear pool of $SV40_{NLS}$-GFP observed in a wild-type background was reduced and the cytoplasmic pool accordingly increased. By contrast, the localization of GFP-Npl3(284–414) (GFP-$Npl3_{NLS}$) (Fig. 3Q–T) was unaffected by the *cse1-1* mutation as compared to the wild-type situation. This control was chosen since Npl3p shuttles continuously, like Srp1p, between nucleus and cytoplasm [63], but exploits a different importin for its nuclear import, Mtr10p [24,25]. For recognition by Mtr10p the C-terminal 130 amino acids (residues 284–414) were shown to be necessary and sufficient [25].

It should be noted that the observed mislocalization of Srp1p and $SV40_{NLS}$-GFP in a *cse1-1* mutant strain at 23°C did not change significantly by shifting the cells to lower (16°C) or higher temperatures (30°C, 37°C) (data not shown).

4. Discussion

Importin α/β is the heterodimeric nuclear import receptor for proteins that contain a classical NLS [15,16]. Both subunits shuttle continuously between the nucleus and the cytoplasm. Recently, CAS, a protein previously implicated in apoptosis [34], was identified as the nuclear export receptor for importin α [35]. The data presented here suggest that Cse1p is the functional homologue of CAS in the yeast *S. cerevisiae* and thus is the exportin for yeast importin α.

(i) Cse1p associates, both in vivo and in vitro, with its export cargo Srp1p and the regulator of export, Gsp1p or Ran, respectively; (ii) the in vitro binding studies show that binding of Cse1p to Ran is specific for the GTP-bound form of Ran as shown previously by overlay assays [9]; (iii) the binding of Cse1p to Ran-GTP is cooperative with the binding of Cse1p to Srp1p demonstrated by the formation of a trimeric complex between Cse1p, Srp1p and Ran-GTP. Analogous complexes have been demonstrated for CRM1 [27], the human Los1p homologue [31,32] and CAS [35]; (iv) subcellular localization studies in living yeast cells show that Srp1p, which under steady-state conditions is found in the cytoplasm, nucleoplasm and at NPCs, accumulates in the nucleus of a cold-sensitive *cse1-1* mutant at semi-permissive temperature. Under the same conditions, a reporter protein containing a classical NLS is mislocalized to the cytoplasm suggesting that Srp1p becomes limiting in the cytoplasm for import of such NLSs. Previous isolation of the *SRP1* gene as a multicopy suppressor of the *cse1-1* mutation is in accordance with such an interpretation [37]. Other nuclear export and import pathways such as exit of L25-GFP and Yrb1-GFP from the nucleus, and nuclear uptake of Npl3p which uses Mtr10p as importin [24,25], respectively, are not affected in a *cse1-1* mutant.

Although our data suggest that Cse1p functions as a specific nuclear export receptor for Srp1p, it is possible that Srp1p is not the only export substrate of Cse1p and that additional proteins are exported via this pathway. Such proteins could possibly be identified by their primary sequence once the NES in Srp1p is characterized. Preliminary results suggest that the N-terminal IBB-domain of Srp1p which is necessary and sufficient for interaction with Kap95p [64–66] is dispensable for binding to Cse1p (M. Künzler and E. Hurt, unpublished results) consistent with a recent study in higher eukaryotes [67]. This suggests that the NLS and NES are in separate domains of Srp1p. Candidate alternative substrates of Cse1p are proteins involved in mitosis given the strong defects of a *cse1-1* mutation in chromosome segregation [37]. In accordance with these defects, another mutation, *cse1-22*, was reported to inhibit degradation of B-type cyclins [38]. However, this mutation turned out to be located in *APC10*, a neighboring gene of *CSE1* encoding a component of the anaphase-promoting complex [68]. Consistent with the phenotype of the *cse1-1* mutation, however, CAS was also implicated in mitosis of higher eukaryotic cells [69]. Alternatively to a defective nuclear export, the observed defects of mutations in Cse1p/CAS in mitosis could also be the consequence of a defective nuclear import of proteins involved in mitosis that contain a classical NLS. This explanation seems reasonable given the fact that the mitotic spindle in *S. cerevisiae* remains intranuclear due to the absence of nuclear envelope breakdown [70]. Indeed, point mutations in *SRP1* that reduce NLS binding [46,50] and depletion of Srp1p have been reported to cause similar defects in mitosis such as stabilization of B-type cyclins, cell cycle arrest at G2/M, aberrant spindles and chromosome segregation [57,71]. Formally, as a third explanation for the observed mitotic defects, we cannot exclude that, in addition to its role as a nucleocytoplasmic export factor, Cse1p/CAS has additional roles in the cell, e.g. in mitosis. In this regard, it might be interesting that CAS was found in association with the mitotic spindle in higher eukaryotes [72].

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