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A Crm1p-independent nuclear export path for the mRNA-associated protein, Npl3p/Mtr13p

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ABSTRACT **mRNA export involves association of mRNAs with nucleoplasmic proteins, delivery to the nuclear pore complex, translocation to the cytoplasm, and reimport of recycling components. Many yeast mutants inhibit mRNA export, but there is little information concerning the RNA carriers and steps of transport that they affect. The hnRNP/serine-arginine-rich-like protein, Npl3p/Mtr13p, binds poly(A)$^+$ RNA and shuttles between the nucleus and cytoplasm. Its export accelerates on inhibition of RNA synthesis. *In vivo* tests show that its export requires two proteins with putative leucine-rich nuclear export signals: Gle1p, Mex67p, and several additional nuclear and nuclear pore complex-associated proteins. Surprisingly, a nonnuclear pool of an import factor (the importin α homologue, Srp1p) is also required. Changes in the methylation status of Npl3p do not correlate with its nucleocytoplasmic distribution. A *crm1* mutant that inhibits export of proteins with leucine-rich nuclear export signals and mRNAs does not inhibit Npl3p export. Moreover, several proteins needed for Npl3p export are not needed for export of a typical Crm1p cargo. Thus, Npl3p export requires only a subset of proteins implicated in mRNA export, suggesting that more than one mRNA export path exists. A distinct group of mutants, including a mutation of a member of the importin β superfamily, inhibits Npl3p reimport from the cytoplasm.**

By contrast to many varieties of RNA, mRNAs exhibit immense structural diversity. Nevertheless, mRNA export can occur without recognition of either the 5′ m^{7}G cap or the poly(A) tail (1–3). mRNA export therefore may require more than one protein that binds mRNA. In animal cells, putative mRNA carriers such as heterogeneous nuclear ribonucleoprotein (hnRNP) A1, hnRNP K, and HIV-1 Rev, as well as several serine-arginine-rich (SR) proteins, shuttle between the nucleus and cytoplasm and relocate to the cytoplasm on inhibition of RNA synthesis (1, 3, 4).

The yeast hnRNP A1/SR-like protein, Npl3p/Mtr13p, binds poly(A)$^+$ RNA and also shuttles (Fig. 1*A*). Moreover, mutations in Npl3p cause nuclear accumulation of poly(A)$^+$ RNA (5–7). To define the mechanism by which this protein is transported, we have developed an *in vivo* assay to identify mutants that inhibit its export or import (see Table 1 for strains and specific references). Several export and import paths require proteins of the importin β superfamily (karyopherins), which interact with nucleoporins and the Ran GTPase (1, 3, 33, 34). Among the former is Crm1p, which binds leucine-rich nuclear export signals (LRNES) and therefore exports Rev and other proteins (8, 35, 36, 37). Some importin β superfamily proteins directly bind cargo, whereas importin β itself often requires the adaptor, importin α.

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MATERIALS AND METHODS

Yeast Strains and Media. Most cells were grown to midlog phase at 23°C in rich medium (yeast extract peptone dextrose), fixed in formaldehyde or shifted to 37°C for 1 hr ± 20 μg/ml thiolutin before fixation. *rsl1–1* was grown in synthetic uracil dropout medium to guarantee the presence of the *RNA1* plasmid (32). The *MTR4* depletion strain (YSL303) was grown at 30°C in yeast extract/peptone–galactose medium (22). Stocks (100x) of thiolutin in DMSO were kept at −20°C. Strains were transformed with pKW430 [=pNLS$_{LTAg}$NES$_{PKI}$(GFP)$_2$] (8) or pNLS$_{LTAg}$-GFP (green fluorescent protein) (38) and maintained on uracil dropout plates. ATP synthesis was inhibited as in ref. 38. Cells (>300) were routinely counted to quantitate Npl3p distributions.

Immunofluorescence Microscopy. Formaldehyde-fixed cells were carefully spheroplasted, adhered to polylysine-covered slides, dehydrated, and processed for antigen detection by indirect immunofluorescence (18). The monospecific rabbit anti-Npl3p/Mtr13p serum was raised against a synthetic peptide conjugate including the carboxyl-terminal 14 amino acids. Nab2p was detected with mAb, 3F2 (M. Swanson). For simultaneous detection of Npl3p and Srp1p, we used the anti-Npl3p mAb, 1E4 (M. Swanson) and a rabbit monospecific anti-Srp1p antibody (M. Nomura).

RESULTS

Inhibition of RNA Synthesis Accelerates Export of Npl3p/Mtr13p. In wild-type cells, Npl3p is abundant throughout the nucleoplasm and faintly visible in the cytoplasm at 23°–37°C, as judged by indirect immunofluorescence. Npl3p relocates to the cytoplasm on inhibition of RNA synthesis for 1 hr with thiolutin, one of the few inhibitors of RNA polymerases that is active on intact yeast (39) (Fig. 1*B*). Npl3p also relocates on inhibition of ATP synthesis and, more slowly, in a mutant that is temperature sensitive for RNA polymerase II (31). The titer of Npl3p does not change significantly during relocation, as judged by Western blotting. Extensive reversibility is seen after washing out thiolutin and reincubation in rich medium (not shown).

To learn whether thiolutin inhibits Npl3p import, we have used a two-step assay in which we first relocate Npl3p to the cytoplasm by inhibiting ATP synthesis and then transfer cells to rich medium ± thiolutin (Fig. 1 *C* and *D*). When this is done with wild-type (wt) cells, Npl3p remains in the cytoplasm; however, when mutants are studied that inhibit relocation of Npl3p at 37°C (*mtr2–1, mtr3–1*; see below), Npl3p reconcentrates in the nucleus, showing that it can be imported. To learn whether thiolutin accelerates export of Npl3p, we have studied mutants that inhibit Npl3p reimport (see below) and observe

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Abbreviations: LRNES, leucine-rich nuclear export signal; wt, wild type; hnRNP, heterogeneous nuclear ribonuclearprotein; GFP, green fluorescent protein.
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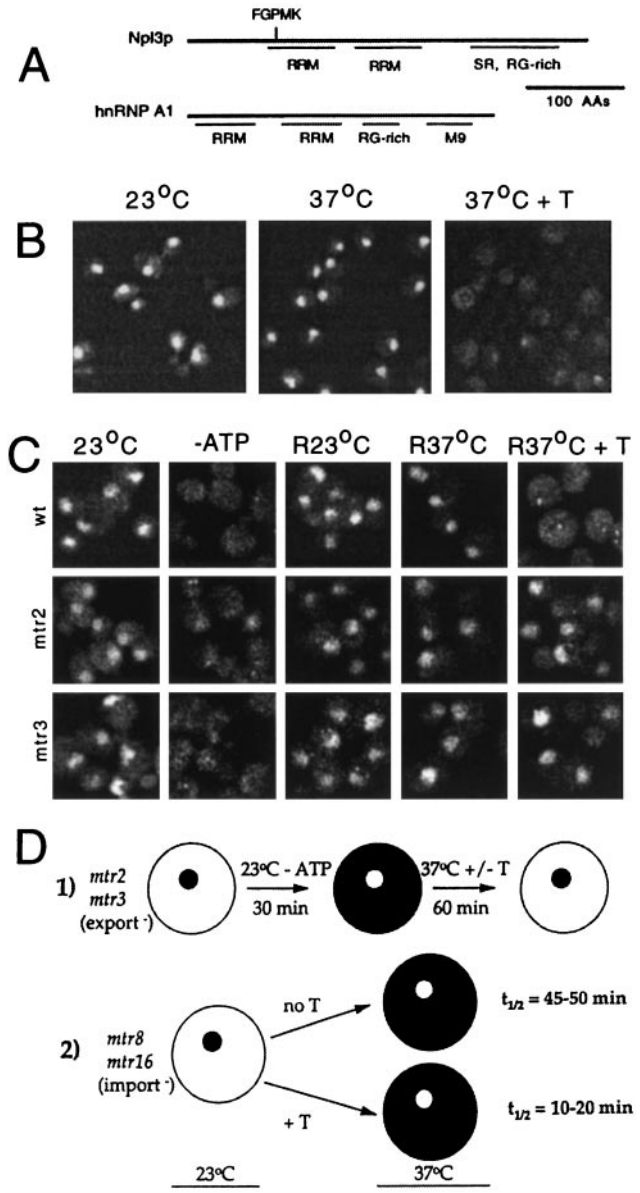

FIG. 1. Effect of inhibition of RNA synthesis on localization of Npl3p. (*A*) Structure of Npl3p compared with hnRNP A1. Note the RNA recognition motifs (RRM), the glycine-rich domains (SR, RG), and the FGPMK sequence, which is also found in hnRNP A1, where it is essential for function of the M9 import/export motif. (*B*) Localization of Npl3p by indirect immunofluorescence wt (YPH500) was incubated at 23°C or for 1 hr at 37°C ± 20 μg/ml thiolutin (T). Relocation is detected with the polyclonal Ab or mAb 1E4. It is not blocked by inclusion of 100 μg/ml cycloheximide. (*C*) To learn whether inhibition of RNA synthesis stops import of Npl3p, protocol 1 was used, as indicated in *D*: wt, *mtr2–1*, and *mtr3–1* were fixed at 23°C or incubated for 30 min at 23°C under conditions that deplete ATP (−ATP) to relocate Npl3p to the cytoplasm. Cells were then returned to yeast extract peptone dextrose and incubated 2 hr at 23°C or 37°C in the absence or presence of thiolutin (T). Because *mtr2–1* and *mtr3–1* inhibit relocation of Npl3p from the nucleus to the cytoplasm, if Npl3p can return to the nucleus in the presence of thiolutin at 37°C, it should remain there. Npl3p does reconcentrate in the nucleus of the mutants in all "return" (R) conditions, showing that thiolutin allows import to continue. (*D*) Protocol 1, used for the experiment illustrated in *C*. Protocol 2, used to evaluate whether relocation is accelerated in the presence of thiolutin. In this experiment, two mutants that appear not to allow import of Npl3p (*mtr8, mtr16*) were used to estimate the speed of relocation of Npl3p. Cells were incubated for increasing periods of time at 37°C ± thiolutin. The observations are summarized.

that the kinetics of relocation of Npl3p at 37°C accelerate in the presence of thiolutin [$t_{1/2}$ = 10–20 min vs. 45 min (Fig. 1*D*)]. Nevertheless, the release of Npl3p appears not to be by diffusion (see below).

An *in Vivo* Test to Identify Mutants That Affect Npl3p Transport. Because thiolutin relocates Npl3p in wt, thiolutin treatment provides a convenient way to learn whether a particular temperature-sensitive mutant inhibits Npl3p export: if it does, Npl3p will not relocate to the cytoplasm in a corresponding mutant even in the presence of thiolutin. Moreover, if a mutant inhibits Npl3p import, Npl3p will relocate to the cytoplasm in the corresponding mutant at 37°C. We therefore have surveyed the distribution of Npl3p in temperature-sensitive transport mutants, many of which rapidly cause nuclear accumulation of poly(A)$^+$ RNA (Fig. 2 *A* and B; Table 1).

Mutations That Affect Nucleocytoplasmic Transport but Not Npl3p Transport. If a given protein is not required for the transport of Npl3p, one would expect Npl3p to behave as in wt in the corresponding mutant strain [group (n)], i.e., concentrated in the nucleus both at 23°C and after 1 hr at 37°C, but in the cytoplasm when thiolutin is present at 37°C.

Strikingly, group (n) includes several mutants that cause rapid nuclear accumulation of poly(A)$^+$ RNA: two importin β superfamily mutants (*crm1–1, pse1–1*), a cytosolic RNA helicase mutant (*dbp5–2*), and a strain that carries a mutation in a protein that shows genetic interactions with nucleoporins (Gle2p).

Group (n) also includes several mutants that are important for other aspects of nucleocytoplasmic transport but do not cause rapid accumulation of poly(A)$^+$ RNA: the importin β superfamily mutants, *los1–1* and *cse1*, two *nup49* nucleoporin mutants, and the yeast importin β mutant, *rsl1–1.*

Mutations That Inhibit Npl3 Export. If a protein is required for Npl3p export, Npl3p should remain in the nucleus at 23°C and at 37°C ± thiolutin in corresponding mutants. Such behavior is characteristic of group (ex) mutants that cause nuclear accumulation of poly(A)$^+$ RNA: for example, on depletion of the nuclear DEAD-box protein, Mtr4p, which has several sequences that match the LRNES consensus; for strains that carry mutations in the nucleoplasmic and nucleolar proteins, Mtr2p and Mtr3p; and for a strain that carries a mutation in the yet uncloned distinct gene, *MTR14*. Relocation is also slowed in four nucleoporin mutants (*nsp1, nup85*(*rat9–1*), *nup145–10, nup159*(*rat7–1*). These nucleoporins may constitute docking sites for export. Npl3p export is also inhibited in two strains that have mutations in proteins with putative LRNESs (*gle1–4, mex67–5*), the latter of which binds Mtr2p and RNA. Control [^{3}H]uridine labeling experiments show that the (ex) mutants are still sensitive to thiolutin (not shown). The lack of relocation that we observe therefore is not a reflection of intrinsic resistance to this inhibitor.

We have inquired whether selected (ex) mutants inhibit protein diffusion through the NPC. For these experiments, *gle1–4, mex67–5, mtr2–1, mtr3–1,* and *srp1–31* carrying a NLS_{LTAg}-GFP expression plasmid (38) were shifted to glucose-free medium supplemented with 2-deoxyglucose and sodium azide at 23°C vs. 37°C, and the speed of GFP diffusive efflux was followed over 20 min. In no case was efflux of this small protein (28 kDa) slower at 37°C than at 23°C. The relocation characteristics of Npl3p therefore do not match those expected for diffusion.

A Role for the Importin α Homologue, Srp1p, in Npl3p Export. The importin α homologue, Srp1p, cycles between the cytoplasm and the nucleus as it delivers cargo to the nucleoplasm in conjunction with importin β (2, 3). Strikingly, Npl3p export is inhibited in *srp1–31* and, judging from our observations of an importin β mutant (above), this does not reflect a need for import along the importin α/β path. To learn whether the function of Srp1p in Npl3p export can occur when Srp1p is in the nucleus, we have studied an importin β superfamily mutant, *cse1,* which inhibits export of Srp1p (9, 10, 11, 40). Significant nuclear accumulation of Srp1p is seen in *cse1* at 37°C ± inclusion of thiolutin. Double-label experiments show that there is a close correlation between the localization of

Table 1. Yeast strains

Relevant genotype; name*†	(Phenotypic group††); comment	Refs.
wt; YPH500		
crm1-1; Δ*XPO1*[pKW457]*	(n); importin β superfamily member; binds LRNES	8
cse1; AGSY54	(n); importin β superfamily member; homologue of CAS; needed for export of Srp1p	9–11
dbp5-2; DBP5-2*	(n); cytoplasmic DEAD-box helicase	12
gle1-4; SWY1191*	(ex); putative LRNES, synthetic lethal with *nup100*	13
gle2-1; SWY1016*	(n); homologue of Rae1p; synthetic lethal with *nup100*	14
los1-1; 201-1-5	(n); importin β superfamily member; needed for pre-tRNA export/splicing	15, 16
mex67-5; Y881*	(ex); putative LRNES, synthetic lethal with *nup85* binds RNA and Mtr2p	17
mtr1-1; T136*	(imb); Ran–GEF (= Prp20p)	18, 19
mtr2-1; T6*	(ex); nuclear protein, binds Mex67p	18, 20
mtr3-1; T34*	(ex); nucleolar protein	18, 21
GALMTR4 mtr4::*TRP1*; YSL303§	(ex); allows depletion of nuclear DEAD-box protein; has putative LRNESs	18, 22
mtr8-1; T301*	(im); nc	18
mtr10-1; T255*‡	(im); importin β superfamily member	18
mtr14-1; T263*¶	(ex); nc	18
mtr16-1; T179*	(im); nc	18
nsp1(ala6); nsp1(ala6)‖	(ex); nucleoporin with FG repeats	23
nup49-313; *nup49-313**	(n); nucleoporin with FG repeats	24
nup85/rat9-1; AG195.204A*	(ex); essential nucleoporin	25
nup145-10; TDY105*	(ex); nucleoporin with FG repeats	26
nup159/rat7-1; LGY101*	(ex); nucleoporin with FG repeats	27
*pse1-1*Δ*kap123**,**	(n); importin β superfamily member; imports some ribosomal proteins	28, 29
rna1-1; EE1b-6*	(im); Ran-GAP	30
rpb1-1; RY260	polymerase II ts strain	31
rsl1-1; PSY876††	(n); Kap95p; importin β homologue	32
srp1-31; NOY612	(ex); Kap60p; importin α homologue	33

nc, the corresponding gene has not been cloned.
*The indicated strains carry recessive ts mutations and accumulate poly(A)$^+$ RNA in the nucleus at 37°C within 1 hr.
†The genetic background of most of the mutants is YPH500 or W303.
‡This ts strain accumulates poly(A)$^+$ RNA throughout the nucleoplasm at 37°C after 2 hr, as judged by FISH. It was obtained from M. Fitzgerald-Hayes.
§This strain allows interruption of transcription of *MTR4* by transfer from galactose to glucose-containing medium. Since poly(A)$^+$ RNA accumulates conspicuously in the nucleolus of these cells within 5 hr, for the present experiments the cells were grown at 30°C in galactose medium and then fixed at once or first shifted to glucose medium for 5 hr ± inclusion of thiolutin for the final hour.
¶*MTR14* has not been cloned, but it is distinct from *GLE1, MEX67, NSP1, NUP145, RAT7, RAT9*, and *SRP1*, since *mtr14-1* complements the corresponding mutant strains (not shown).
‖This strain accumulates poly(A)$^+$ RNA as a focal small intranuclear spot (not shown).
**The *PSE1* gene is essential only in a Δ*KAP123* background. The *pse1-1* mutant strain therefore carries the *KAP123* deletion.
††This strain carries an *rna1* ts mutation covered by a *URA3 RNA1* plasmid. It is studied in uracil dropout medium.
‡‡See text.

Srp1p and Npl3p: the very cells that strongly accumulate Srp1p in the nucleus after 2–3 hr also retain nuclear Npl3p at 37°C + thiolutin (Fig. 2*D*). Furthermore, after these longer incubations (but not after 1 hr), nuclear accumulation of poly(A)$^+$ RNA can be detected (not shown).

We therefore consider it most likely that the function of Srp1p for Npl3p export is exerted in the cytoplasm or during its reexport, which might occur in association with Npl3p. As controls, we have localized Srp1p in *gle1–4, mex67–5, mtr2–1, mtr3–1,* and on depletion of Mtr4p. In all cases, Srp1p remains concentrated in the cytoplasm (not shown).

Mutations That Inhibit Npl3p Reimport. If a given protein is not needed for Npl3p export but is required for its import, Npl3p should concentrate in the cytoplasm of the corresponding mutant. This behavior is seen for several mutants that cause nuclear accumulation of poly(A)$^+$ RNA. These are an importin β superfamily mutant, *mtr10–1*, which shows extensive relocalization even at 23°C, and several strains that have nuclear Npl3p at 23°C: two affect the Ran GTPase cycle [*mtr1–1*(*Ran-GEF*)*, rna1–1*(*Ran-GAP*)] and two carry mutations in yet uncloned genes (*mtr8–1, mtr16–1*). The corresponding proteins are thus important for nuclear localization of Npl3p. Western blotting shows that these strains do not cause substantial reduction of Npl3p levels at 37°C (not shown). One-hour [^{3}H]uridine-labeling experiments show that (im) strains do not inhibit RNA synthesis nearly to the same extent as thiolutin (not shown). It is therefore unlikely that inhibition of RNA synthesis in these strains causes Npl3p relocation.

Methylation Status of Npl3p Does Not Correlate with Its Localization. Because Npl3p methylation or phosphorylation may facilitate Npl3p transport (58), we have used both our polyclonal antibody and an antibody that reacts only with methylated Npl3p (1E4, from M. Swanson) for Western blotting to look for alterations of SDS/PAGE mobility or differential reactivity in the strains that affect Npl3p transport. No such changes are seen; however, we repeatedly observe that Npl3p cannot be detected in lysates of *mtr2–1* and *mtr10–1* (not shown). We therefore suspect that Mtr2p and Mtr10p are needed to stabilize Npl3p in cell lysates.

Distinct Requirements for the Transport of a Protein with a LRNES. We have compared the requirements for export of Npl3p to the requirements of NES_{PKI}-NLS_{LTAg}-$(GFP)_2$, which normally resides in the cytoplasm and shuttles. On inhibition of its export in *crm1–1*, this protein becomes concentrated in the nucleus (8). By contrast, this protein is concentrated in the cytoplasm in each of the (ex) and (im) mutants, both at 23°C and after 15- to 60-min incubation at 37°C. To learn whether this concentration in the cytoplasm results from inhibition of its import, we have also studied the distribution of NLS_{LTAg}-GFP in wt and all but the nucleoporin mutants. This reporter concentrates in the nucleus of wt, relocates to the cytoplasm on inhibition of ATP synthesis, and reenters the nucleus in less than 10 min on

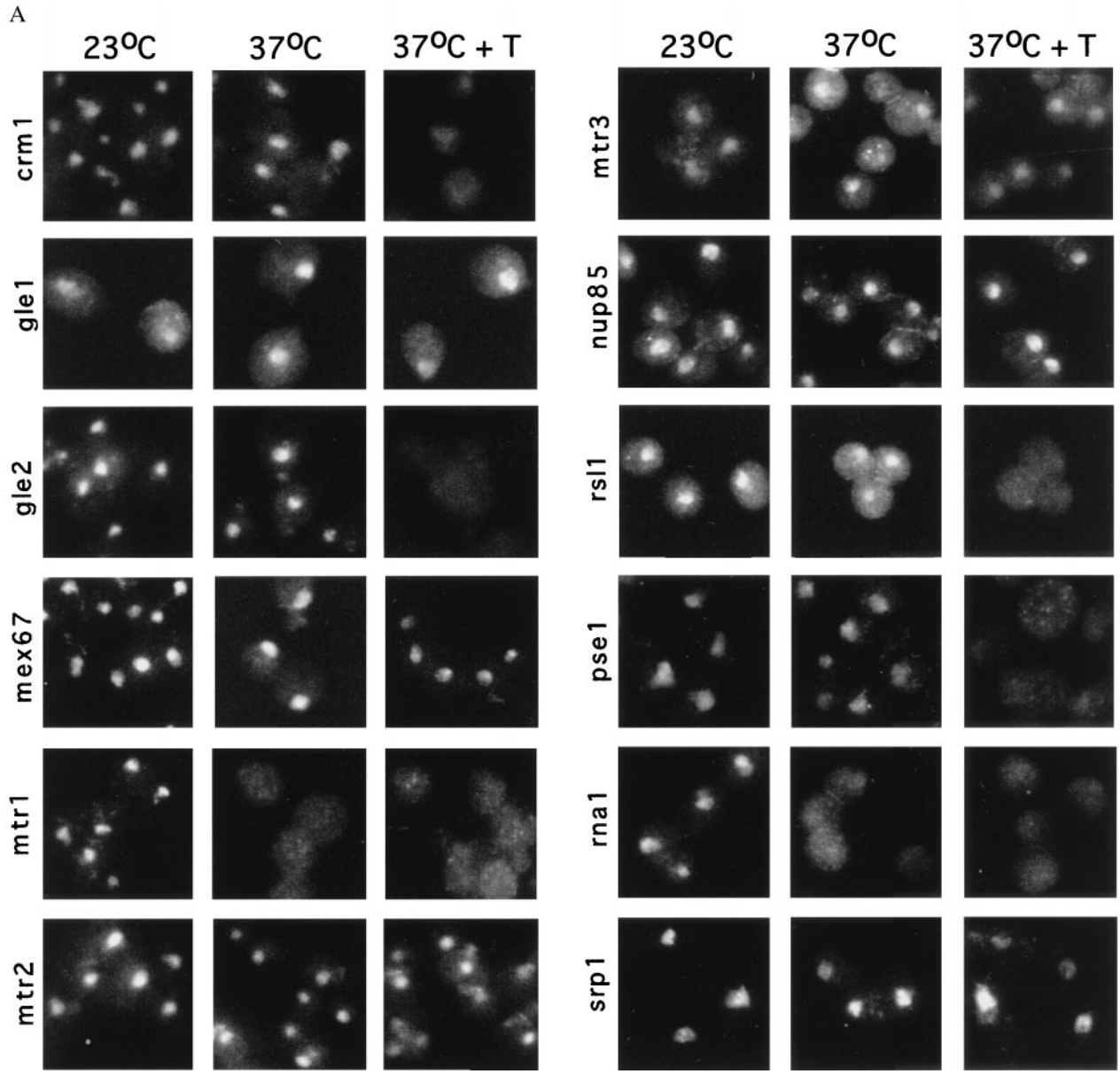

FIG. 2. (*Figure continues on the opposite page.*) Localization of Npl3p in transport mutants. (*A*) Each temperature-sensitive mutant was incubated at 23°C, at 37°C, or at 37°C with 20 μg/ml thiolutin for 1 hr, fixed and immunostained to localize Npl3p. To evaluate the contribution of newly synthesized Npl3p to the cytoplasmic signal detected at 37°C, we have also conducted these incubations $\pm$100 μg/ml cycloheximide. In no case does cycloheximide reduce the cytoplasmic signal. (*B*) Graphical summary of data from experiments comparable to those illustrated in *A*. Each set of three columns indicates the percentage of cells that show a cytosolic Npl3p signal and no obvious nuclear signal. (*C*) Summary of groupings of mutants with regard to the distribution of Npl3p. (*D*) Influence of *cse1* on the distribution of Srp1p and Npl3p. *cse1* was incubated 3 hr at 37°C with inclusion of thiolutin for the final hour. Npl3p and Srp1p are concentrated in the same nuclei.

reincubation in rich medium at 23°C. *srp1–31* slows, but does not stop, return at 37°C (38).

In each case, we have therefore inhibited ATP synthesis at 23°C, verified that NLS_{LTAg}-GFP shifts to the cytoplasm, and then reincubated the cells at 23°C vs. 37°C in rich medium for 0–10 min. Only *srp1–31* reduces import of NLS_{LTAg}-GFP (not shown). Thus, none of the other mutants has a major effect on export of NES_{PKI}-NLS_{LTAg}-$(GFP)_2$.

DISCUSSION

Parallels with Higher Eukaryotic Proteins. Several aspects of the structure and transport of Npl3p resemble what is known for the higher eukaryotic hnRNP A1 protein (1). Both proteins

(*i*) have RNA-recognition motifs, glycine-rich domains, and the sequence FGPMK;

(*ii*) bind poly(A)$^+$ RNA and shuttle;

(*iii*) are reimported by members of the importin β superfamily (transportin, Mtr10p);

(*iv*) relocate to the cytoplasm on inhibition of RNA synthesis.

Concerning the final item, observations of the impact of thiolutin on GFP-tagged Npl3p have previously been interpreted to indicate that Npl3p relocates to the nucleolus and actually requires RNA synthesis to be exported (7). The discrepancy between this view and our evidence may result from differences in the behavior of the normal vs. the overexpressed tagged protein. We also suspect that partial relocation of GFP-Npl3p to the cytoplasm occurs in the presence of thiolutin but is difficult to detect because of dilution in the large cytoplasmic volume. This hypothesis is especially probable because we observe that on treatment of wt with thiolutin,

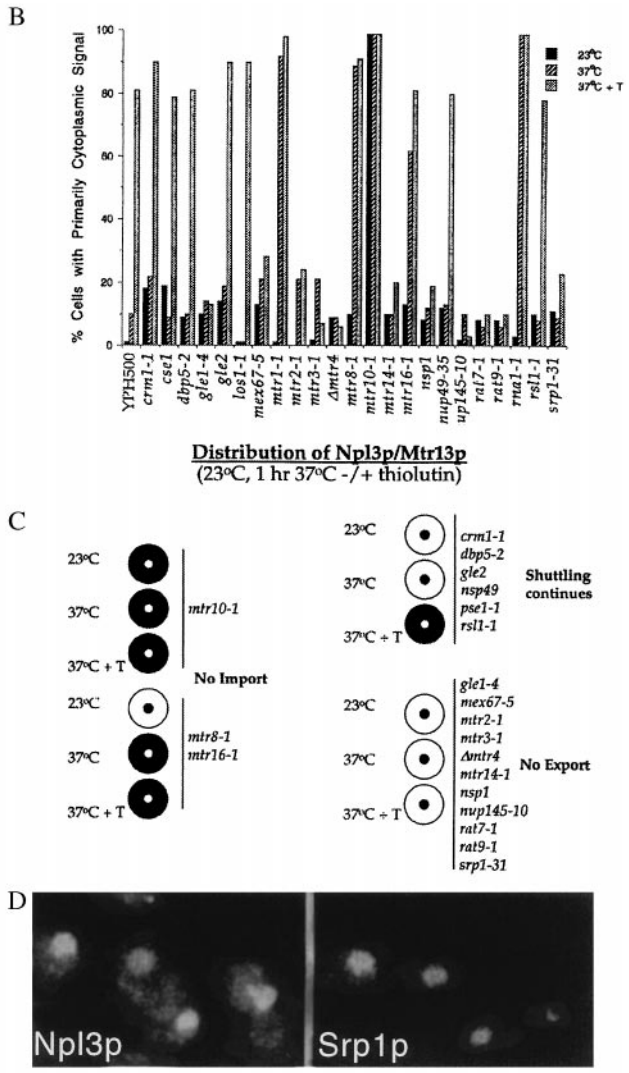

FIG. 2. *Continued.*

the endogenous protein gives a residual nucleolar signal at an intermediate time point when the cytoplasm is not yet bright.

Relocation on inhibition of RNA synthesis is rare among yeast proteins, judging from a survey of 40 fusion proteins that at steady state are concentrated in the nucleus (41). Even a second hnRNP-like protein, Nab2p, remains concentrated in the nucleus of wt cells in the presence of thiolutin (not shown). Nab2p does relocate in the importin β superfamily mutant, *kap104–16* (42), and in *Ran-GAP* and *Ran-GEF*; however, Nab2p relocation is not seen for other *mtr* mutants at 23 or 37°C ± thiolutin, including the (im) mutants (not shown).

Why does export of Npl3p (and possibly all "RNA carriers") accelerate on inhibition of transcription? Perhaps the duration of transit of these proteins through the nucleus is prolonged by association with newly synthesized RNA. For example, when newly synthesized RNA is present, Npl3p may obligatorily associate with early RNA processing intermediates and await their maturation. A minor alteration of the relative export and import rate constants might then be sufficient to shift the net distribution of Npl3p.

A Role for Npl3p in mRNA Export? Npl3p binds poly(A)$^+$ RNA and shuttles between the nucleus and cytoplasm. Moreover, mutations in Npl3p cause nuclear accumulation of poly(A)$^+$ RNA. Taken together with the evidence of relocation on inhibition of RNA synthesis, it is likely that Npl3p normally accompanies RNA cargo to the cytoplasm (1, 43).

The mechanism and path of Npl3p export might depend on whether it is associated with newly synthesized RNA. Moreover, addition of inhibitors of RNA synthesis may provoke unwanted changes, including a heat-shock response (44). Nevertheless, Npl3p does continue to be both exported and imported in the absence of newly synthesized RNA. Moreover, multiple proteins implicated in mRNA export also inhibit Npl3p export in the absence of RNA synthesis.

A further indication that RNA carriers use the same export equipment both when RNA synthesis occurs and when it does not comes from investigation of Rev (13 kDa): both normal Rev-dependent RNA export and Rev export on inhibition of RNA synthesis are sensitive to the Crm1p inhibitor, leptomycin B (45). Moreover, nuclear microinjection of hnRNP A1 inhibits the export of selected mRNAs, presumably because it interacts with downstream components that normally mediate the export of hnRNP A1-mRNA complexes (46, 47).

The Path of Npl3p-Dependent Export. We suggest (Fig. 3*A*) that mRNA cargo is recognized by Npl3p and Mex67p, perhaps with help from Mtr4p, and that this unit interacts directly or indirectly, with Gle1p. Export of Npl3p may or may not require a member of the importin β superfamily; however—given our observations—it does not require Crm1p, Cse1p, Los1p, Mtr10p, or Pse1p. Gle1p and Mex67p actually localize to the nuclear pore complex. It is not known whether they are exported. Because their higher eukaryotic homologues do not include putative LRNESs (although they are implicated in RNA export), there is question as to whether the putative LRNESs of these yeast proteins are functional (13, 17, 48, 49).

Export also requires the nuclear proteins Mtr2p and Mtr3p. Mtr2p interacts with Mex67p, and overexpression of Mtr2p suppresses *mex67–5* (17). Considering that nuclear Ran(GTP) is required for most export, the apparent lack of export requirement for the two Ran regulatory proteins likely reflects

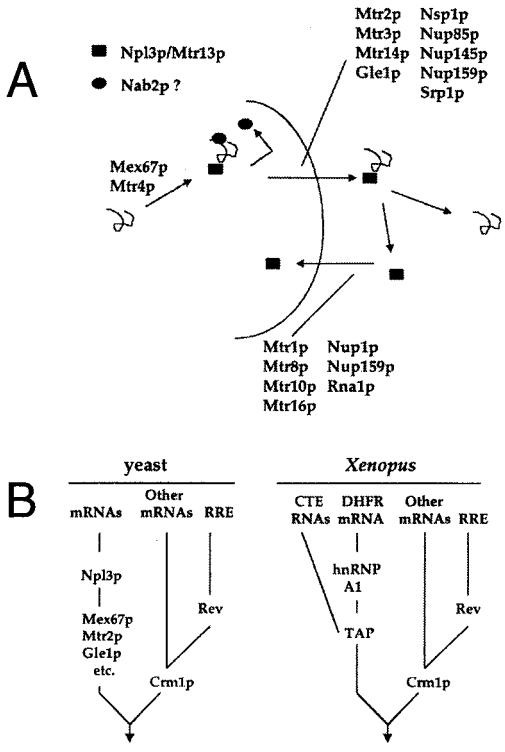

FIG. 3. Models of nuclear export. (*A*) Suggested sites of interruption of transport of Npl3p. Mex67p and Mtr4p are positioned inside the nucleus because they both bind RNA and therefore may be needed to constitute an export mRNP. (*B*) Comparison of the paths of nuclear export in yeast and *Xenopus* oocytes. Conserved transport element (CTE) RNAs are retroviral mRNAs that include the "conserved transport element."

there being sufficient Ran(GTP) at the moment of temperature shift to allow most Npl3p to exit the nucleus, whereupon it becomes trapped in the cytoplasm. Alternately, a Ran-independent export path may be involved (50–52). The nucleoporins indicated on the export path in Fig. 3*A* are those that are needed for Npl3p relocation.

The Importance of Srp1p. Srp1p is needed for export of Npl3p, and this function does not require import along the importin α/β path. Srp1p may act in the cytoplasm, perhaps by dissociating export complexes on their arrival. The involvement of Srp1p in Npl3p export is reminiscent of a report of importin α function in export of U1 snRNA (53). The absence of nuclear accumulation of poly(A)$^+$ RNA in *srp1–31* is likely to reflect the profound and rapid inhibition of [^{3}H]uridine incorporation that occurs in this mutant (not shown). Moreover, the involvement of Srp1p in Npl3p export allows rationalization of the observation that *npl3* mutants inhibit protein import (5–7).

The Reimport Path. Both our observations and other reports indicate that return of Npl3p to the nucleus requires the importin β superfamily member, Mtr10p (56, 57). The Ran cycle and the Ran import factor, Ntf2p, are also required, as *ntf2* causes Npl3p to relocate to the cytoplasm (54, 55). The nucleoporins that are indicated on the reimport path in Fig. 3*A* bind Mtr10p under *in vitro* conditions that may simulate the docking step of import (56).

Evidence for Multiple mRNA Export Paths (Fig. 3*B*). In yeast, the export of mRNAs and several cargoes with LRNESs requires Crm1p (8, 59), yet *crm1–1* does not stop Npl3p export under the conditions assayed. Moreover, several proteins that are needed for Npl3p export are not required for export of NLS_{LTAg}-NES_{PKI}-$(GFP)_2$. There therefore appear to be at least two export paths. They may function in export of distinct subsets of mRNAs.

In *Xenopus* oocytes, Rev requires Crm1p for export. There is also a Crm1p-independent path that exports mRNAs of simple retrovirus, which includes a conserved transport element and DHFR mRNA (which also requires hnRNP A1). It may share several characteristics with the yeast Crm1p-independent Np13p-dependent path (36, 60–62). For example, it depends upon the Mex67p homologue, TAP (48). Surprisingly, competition studies in which LRNES conjugates are microinjected into *Xenopus* oocytes suggest that this path also involves LRNESs (63). Thus, LRNESs might function along multiple export paths, independent of the participation of Crm1p.

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1. Nakielny, S., Fisher, U., Michael, M. & Dreyfuss, G. (1997) *Annu. Rev. Neurosci.* **20,** 269–301.
2. Corbett, A. & Silver, P. (1997) *Microbiol. Mol. Biol. Rev.* **61,** 193–210.
3. Mattaj, I. & Englmeier, L. (1998) *Annu. Rev. Biochem.* **67,** 265–306.
4. Caceres, J., Screaton, G. & Krainer, A. (1998) *Genes Dev.* **12,** 55–66.
5. Flach, J., Bossie, M., Vogel, J., Corbett, A. & Silver, P. (1994) *Mol. Cell Biol.* **14,** 8399–8407.
6. Singleton, D. Chen, S., Hitomi, M., Kumagai, C. & Tartakoff, A. (1995) *J. Cell Sci.* **108,** 265–272.
7. Lee, M., Henry, M. & Silver, P. (1996) *Genes Dev.* **10,** 1233–1246.
8. Stade, K., Ford, C., Guthrie, C. & Weis, K. (1997) *Cell* **90,** 1041–1050.
9. Xiao, Z., McGrew, J., Schroeder, A. & Fitzgerald-Hayes, M. (1993) *Mol. Cell Biol.* **13,** 4691–4702.
10. Solsbacher, J., Maurer, P., Bischoff, F. & Schlenstedt, G. (1998) *Mol. Cell Biol.* **18,** 6805–6815.
11. Kunzler, M. & Hurt, E. (1998) *FEBS Lett.* **433,** 185–190.
12. Tseng, S., Weaver, P., Liu, Y., Hitomi, M., Tartakoff, A. & Chang, T.-H. (1998) *EMBO J.* **17,** 2651–2662.
13. Murphy, R. & Wente, S. (1996) *Nature (London)* **383,** 357–360.
14. Murphy. R., Watkins, J. & Wente, S. (1996) *Mol. Cell Biol.* **7,** 1921–1937.
15. Sarker, S. & Hopper, A. (1998) *Mol. Biol. Cell* **9,** 3041–3055.
16. Hellmuth, K., Lau, D., Bischoff, F., Kunzler, M., Hurt, E. & Simos, G. (1998) *Mol. Cell Biol.* **18,** 6374–6386.
17. Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Pante, N. & Hurt, E. (1998) *Mol. Cell Biol.* **18,** 6826–6838.
18. Kadowaki, T., Chen, S., Hitomi, M., Jacobs, E., Kumagai, C., Liang, S., Schneiter, R., Singleton, D., Wisniewska, J. & Tartakoff, A. (1994) *J. Cell Biol.* **126,** 649–659.
19. Kadowaki, T., Spitz, L., Goldfarb, D., Tartakoff, A. M. & Ohno, M. (1993) *EMBO J.* **12,** 2929–2937.
20. Kadowaki, T., Hitomi, M., Chen, S. & Tartakoff, A. (1994) *Mol. Biol. Cell* **5,** 1253–1263.
21. Kadowaki, T., Schneiter, R., Hitomi, M. & Tartakoff, A. (1995) *Mol. Biol. Cell* **6,** 1103–1110.
22. Liang, S., Hitomi, M., Hu, Y-H., Liu, Y. & Tartakoff, A. (1996) *Mol. Cell Biol.* **16,** 5139–5146.
23. Mutvei, A., Sihlmann, S., Herth, W. & Hurt, E. (1992) *Eur. J. Cell Biol.* **59,** 280–295.
24. Wimmer, C., Dopye, V., Grandi, P., Nehrbass, U. & Hurt, E. (1992) *EMBO J.* **11,** 5051–5061.
25. Goldstein, A., Snay, C., Heath, C. & Cole, C, (1996) *Mol. Cell Biol.* **7,** 917–934.
26. Dockendorff, T., Heath, C., Goldstein, A., Snay, C. & Cole, C. (1997) *Mol. Cell Biol.* **17,** 906–920.
27. Gorsch, L., Dockendorff, T. & Cole, C. (1995) *J. Cell Biol.* **129,** 939–955.
28. Seedorf, M. & Silver, P. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 8590–8595.
29. Rout, M., Blobel, G. & Aitchison, J. (1997) *Cell* **89,** 715–725.
30. Traglia, H. M., Atkinson, N. & Hopper, A. (1989) *Mol. Cell. Biol.* **9,** 2989–2999.
31. Nonet, M., Scafe, C., Sexton, J. & Young, R. (1987) *Mol. Cell. Biol.* **7,** 1602–1611.
32. Koepp, D., Wong, D., Corbett, A. & Silver, P. (1996) *J. Cell Biol.* **133,** 1163–1176.
33. Yano, R., Oakes, M. L., Tabb, M. M. & Nomura, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6880–6884.
34. Gorlich, D., Dabrowski, M., Bischoff, F., Kutay, U., Bork, P., Hartmann, E., Prehn, S. & Izaurralde, E. (1996) *J. Cell Biol.* **138,** 65–80.
35. Fornerod, M., van Deursen, J., van Baal, S., Reynolds, A., Davis, D., Murti, K., Fransen, J. & Grosveld, G. (1997) *EMBO J.* **16,** 807–816.
36. Fornerod, M., Ohno, M., Yoshida, M. & Mattaj, I. (1997) *Cell* **90,** 1051–1060.
37. Ossareh-Nazari, B., Bachellerie, F. & Dargemont, C. (1997) *Science* **278,** 141–144.
38. Shulga, N., Roberts, P., Gu, Z., Spitz, L., Tabb, M., Nomura, M. & Goldfarb, D. (1996) *J. Cell Biol.* **135,** 329–339.
39. Tipper, D. (1973) *J. Bacteriol.* **116,** 245–256.
40. Kutay, U., Bischoff, F., Kostka, S., Kraft, R. & Gorlich, D. (1997) *Cell* **90,** 1061–1071.
41. Burns, N. & Snyder, M. (1994) *Genes Dev.* **8,** 1087–1105.
42. Aitchison, J., Blobel, G. & Rout, M. (1996) *Science* **274,** 624–627.
43. Visa, N., Alzhanova-Ericsson, A., Sun, X., Kiseleva, E., Bjorkroth, B., Wurtz, T. & Daneholt, B. (1996) *Cell* **84,** 253–264.
44. Adams, C. & Gross, D. (1991) *J. Bacteriol.* **173,** 7429–7435.
45. Wolff, B., Sanglier, J.-J. & Wang, Y. (1997) *Chem. Biol.* **4,** 139–147.
46. Izaurralde, E., Jarmolowski, A., Beisel, C., Mattaj, I., Dreyfuss, G. & Fischer, U. (1997) *J. Cell Biol.* **137,** 27–35.
47. Saavedra, C., Felber, B. & Izaurralde, E. (1997) *Curr. Biol.* **7,** 619–628.
48. Gruter, P., Tabernero, C., von Kobbe, C., Schmitt, C., Saavedra, C., Bachi, A., Wilm, M., Felber, B. & Izaurralde, E. (1998) *Mol. Cell* **1,** 649–659.
49. Watkins, J., Murphy, R., Emtage, J. & Wente, S. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6779–6784.
50. Saavedra, C., Tung, K-S., Amberg, D., Hopper, A. & Cole, C. (1996) *Genes Dev.* **10,** 1608–1620.
51. Kose, S., Imamoto, N., Tachibana, T., Shimamoto, T. & Yoneda, Y. (1997) *J. Cell Biol.* **139,** 841–849.
52. Nakielny, S. & Dreyfuss, G. (1998) *Curr. Biol.* **8,** 89–95.
53. Gorlich, D., Kraft, R., Kostka, S., Vogel, F., Hartmann, E., Laskey, R., Mattaj, I. & Izaurralde, E. (1996) *Cell* **87,** 21–32.
54. Corbett, A. & Silver, P. (1996) *J. Biol. Chem.* **271,** 18477–18484.
55. Ribbeck, K., Lipowsky, G., Kent, H., Stewart, M. & Görlich, D. (1998) *EMBO J.* **17,** 6587–6598.
56. Pemberton, L., Rosenblum, J. & Blobel, G. (1997) *J. Cell Biol.* **139,** 1645–1653.
57. Senger, B., Simos, G., Bischoff, F., Podtelejnikov, A., Mann, M. & Hurt, E. (1998) *EMBO J.* **17,** 2196–2207.
58. Shen, E., Henry, M., Weiss, M., Valentini, S., Silver, P. & Lee, M. (1998) *Genes Dev.* **12,** 679–691.
59. Neville, M., Stutz, F., Lee, L., Davis, L. & Rosbash, M. (1997) *Curr. Biol.* **7,** 767–775.
60. Fischer, U., Huber, J., Boelens, W., Mattaj, I. W. & Luhrmann, R. (1995) *Cell* **82,** 475–483.
61. Pasquinelli, A., Ernst, R., Lund, E., Grimm, C., Zapp, M., Rekosh, D., Hammarskjold, M.-L. & Dahlberg, J. (1997) *EMBO J.* **16,** 7500–7510.
62. Bogerd, H., Echarri, A., Ross, T. & Cullen, B. (1998) *J. Virol.* **72,** 8627–8635.
63. Pasquinelli, A., Powers, M., Lund, E., Forbes, D. & Dahlberg, J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 14394–14399.