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Maturation and Intranuclear Transport of Pre-Ribosomes Requires Noc Proteins

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Summary

How pre-ribosomes temporally and spatially mature during intranuclear biogenesis is not known. Here, we report three nucleolar proteins, Noc1p to Noc3p, that are required for ribosome maturation and transport. They can be isolated in two distinct complexes: Noc1p/Noc2p associates with 90S and 66S pre-ribosomes and is enriched in the nucleolus, and Noc2p/Noc3p associates with 66S pre-ribosomes and is mainly nucleoplasmic. Mutation of each Noc protein impairs intranuclear transport of 60S subunits at different stages and inhibits pre-rRNA processing. Overexpression of a conserved domain common to Noc1p and Noc3p is dominant-negative for cell growth, with a defect in nuclear 60S subunit transport, but no inhibition of pre-rRNA processing. We propose that the dynamic interaction of Noc proteins is crucial for intranuclear movement of ribosomal precursor particles, and, thereby represent a prerequisite for proper maturation.

Introduction

Most steps in eukaryotic ribosome synthesis take place in the nucleolus, a specialized subnuclear region. Generation of ribosomes starts with the synthesis of pre-rRNA transcripts, which are processed and base-modified to yield the mature 25S/28S, 18S, 5.8S, and 5S rRNAs, respectively. Before or during rRNA processing, about 80 ribosomal proteins assemble onto the rRNAs to yield preribosomal particles, which are exported into the cytoplasm (Woolford, 1991). In contrast to pre-rRNA processing and modification, very little is known about the assembly pathway for eukaryotic ribosomal subunits and the features that make them competent for nuclear exit (for recent reviews, see Kressler et al., 1999 and Venema and Tollervey, 1999).

The mechanisms of ribosome biogenesis appear to be well conserved throughout eukaryotes. Many components involved in this complex process were identified and characterized in the yeast *Saccharomyces cerevisiae*, where more than 60 *trans*-acting factors required to produce the mature 60S and 40S ribosomal subunits have been identified. These include rRNA modifying enzymes, endonucleases, exonucleases, RNA helicases, and proteins associated with the small nucleolar RNAs (snoRNAs) (Kressler et al., 1999). On the other hand, little is known about factors (e.g., nucleolar proteins) that are involved in ribosome assembly and transport of the preribosomal particles from the nucleolus to the cytoplasm. It is likely that processing and conformational rearrangement of preribosomal particles triggers release from the nucleolus and either exposes or allows the binding of nucleo-cytoplasmic transport factors. Whether the pre-ribosomes released from the nucleolus are already export competent, or require further nucleoplasmic assembly with transport factors, has not been assessed. Alternatively, it may be simply necessary to overcome retention of preribosomal particles to distinct intranucleolar and intranuclear sites so that the particles gain transport competence and that they can leave the nucleus. Both the *cis*-and *trans*-acting factors implicated in this process remain to be determined.

S. cerevisiae has proven useful for the analysis of the nuclear export of proteins, tRNA, and mRNA (Stade et al., 1997; Hellmuth et al., 1998; Sarkar et al., 1999; Kadowaki et al., 1992; Segref et al., 1997; Amberg et al., 1992). We previously reported a visual in vivo assay to analyze ribosomal export that uses a fusion protein between GFP and the large subunit ribosomal protein Rpl25p (Hurt et al., 1999). This assay showed that a subset of the nucleoporins and the Ran-system are required for 60S subunit export. An assay for the nuclear export of 40S subunits led to similar conclusions (Moy and Silver, 1999).

We have exploited this 60S subunit export assay to screen a bank of randomly generated ts mutants for ribosomal export defects (Gadal et al., 2001). One such mutant *noc2-1 (rix3-1)* was complemented by a gene designated *NOC2*. Noc2p was independently identified together with Noc1p and Noc3p (for *n*ucleo*l*ar *c*omplex associated *p*roteins) as components of a large complex that also contained many other nucleolar proteins (Fath et al., 2000). These three proteins can be isolated in two heterodimeric complexes, Noc1p-Noc2p and Noc2p-Noc3p, that are associated with different preribosomal particles. We suggest that Noc1p, Noc2p, and Noc3p participate in ribosome assembly, which is coupled to release of the preribosomal particles from the nucleolus and the acquisition of competence for nucleo-cytoplasmic transport.

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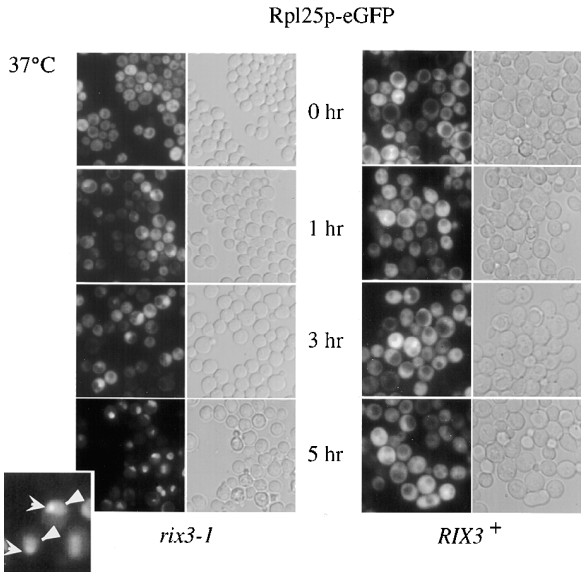

Figure 1. Time-Dependent Nuclear Accumulation of Rpl25p-eGFP in *rix3-1* Cells

rix3-1 cells harboring the empty plasmid pUN100 (*rix3-1*) or complemented by plasmid-borne *RIX3* (*RIX3*$^+$) were transformed with *RPL25-eGFP* (pRS316-RPL25-eGFP). The strains were grown in selective SDC-leu medium at 23°C, before dilution in fresh YPD-medium and incubation at 37°C. Cells were analyzed at the indicated time points by fluorescence microscopy, and Nomarski pictures were also taken. Triangles in the magnified picture (inset, 5 hr) point to nuclear staining, arrowheads to nucleolar staining.

Results

Isolation of the *rix3-1* Mutant that Is Impaired in the Export of Ribosomes from the Nucleolus to the Cytoplasm

To identify factors involved in nuclear export of pre-ribosomes, we expressed the large subunit protein Rpl25p, known to assemble early on pre-ribosomes, as an enhanced GFP-tagged reporter in a bank of randomly generated ts mutants. From this screen, we isolated 20 *ri*bosomal export (*rix*) mutants, which exhibit a ribosomal export defect at the restrictive temperature (Gadal et al., 2001). Notably, the pattern of accumulation of Rpl25-eGFP inside the nucleus was different in the various *rix* mutants. Some *rix* mutants exhibit an accumulation throughout the entire nucleoplasm, as observed in nucleoporin mutants, whereas other *rix* mutants show a stronger accumulation in the nucleolus than nucleoplasm. To this latter class belongs a mutant, *rix3-1* (see Figure 1). We conclude that transport of preribosomal particles is blocked in *rix3-1* cells at two stages, between the nucleolus and nucleoplasm, and between the nucleoplasm and cytoplasm. The wild-type *RIX3* gene was cloned by complementation of the ts phenotype of the *rix3-1* ts mutant and shown to correspond to gene locus YOR206W on chromosome 15, which encodes an essential and protein of 81.601 kDa.

RIX3 Is Identical to *NOC2*, which Encodes a Protein Present in a Large Nucleolar Subcomplex

A large nucleolar substructure, which contains many factors involved in rRNA transcription and rRNA processing and modification can be isolated from yeast (Fath et al., 2000). Components of this complex were identified by MALDI-TOF mass spectrometry, including three proteins termed Noc1p, Noc2p, and Noc3p (for *n*ucleolar *c*omplex associated proteins 1, 2 and 3). Noc2p is identical to Rix3p and Noc3p was previously uncharacterized, whereas Noc1p is identical to Mak21p, which is required for normal levels of 60S subunits (Edskes et al., 1998). Disruption of the *NOC1*, *NOC2*, and *NOC3* genes revealed that all three are essential for cell growth (data not shown). All three Noc proteins are well conserved during eukaryotic evolution, with homologs from yeast to human (Figure 2A). Interestingly, Noc1p and Noc3p have an evolutionary conserved domain in common (for dominant-negative phenotype of this overexpressed domain, see below), which is approximately 45 amino acids in length, and also found in another yeast protein termed Noc4p (Figure 2B). This all suggests that Noc proteins participate in a conserved nucleolar function, which is linked to nuclear export of pre-ribosomes.

Heterodimeric Noc2p/Noc1p and Noc2p/Noc3p Subcomplexes

To identify proteins directly interacting with Noc2p, it was tagged with protein A to allow affinity purification from yeast whole-cell lysates (Grandi et al., 1993). ProtA-Noc2p was functional since it complemented a *noc2-*Δ null mutant (data not shown). Affinity purification was performed in the presence of high salt concentrations which were sufficient to disrupt the above mentioned large nucleolar subcomplex. Under these stringent conditions, ProtA-Noc2p copurified with only two further proteins in significant amounts, which were identified by mass spectrometry as Noc1p/Mak21p and Noc3p (Figure 3A, lane 4). To find out whether Noc1p, Noc2p, and Noc3p are present in a single complex, ProtA-Noc1p and ProtA-Noc3p fusion proteins were generated and affinity purified. Noc1p and Noc3p were each coprecipitated with Noc2p. However, Noc1p and Noc3p were not coprecipitated with each other (Figure 3A). We conclude that Noc2p organizes two distinct subcomplexes by directly interacting either with Noc1p or Noc3p.

To determine whether Noc1p, Noc2,p and Noc3p are functionally linked, we tested for genetic (i.e., synthetic lethal) interactions between the various Noc members (for an explanation of sl relationships see Doye and Hurt, 1995). Ts alleles of the three *NOC* genes (*noc1-1*, *noc2-1*, and *noc3-1*) were generated by PCR-directed random mutagenesis and combined pairwise in haploid cells. The single ts mutants *noc1-1*, *noc2-1*, and *noc3-1* are each viable at 23°C, whereas the combination *noc2-1* with *noc1-1* or *noc2-1* with *noc3-1* resulted in synthetic lethality (Figure 3B). In contrast, combining *noc1-1* with *noc3-1* did not cause a synthetic lethal phenotype (Figure 3B). This genetic relationship between *noc* ts alleles suggests that heterodimer formation is essential, but single mutations in the two separate complexes are tolerated.

Noc1p, 2p, and 3p Are Each Required for Synthesis of 60S Ribosomal Subunits

To determine whether the Noc proteins are involved in ribosome biogenesis, we analyzed pre-rRNA processing and ribosome synthesis in the *ts* mutant strains. It has

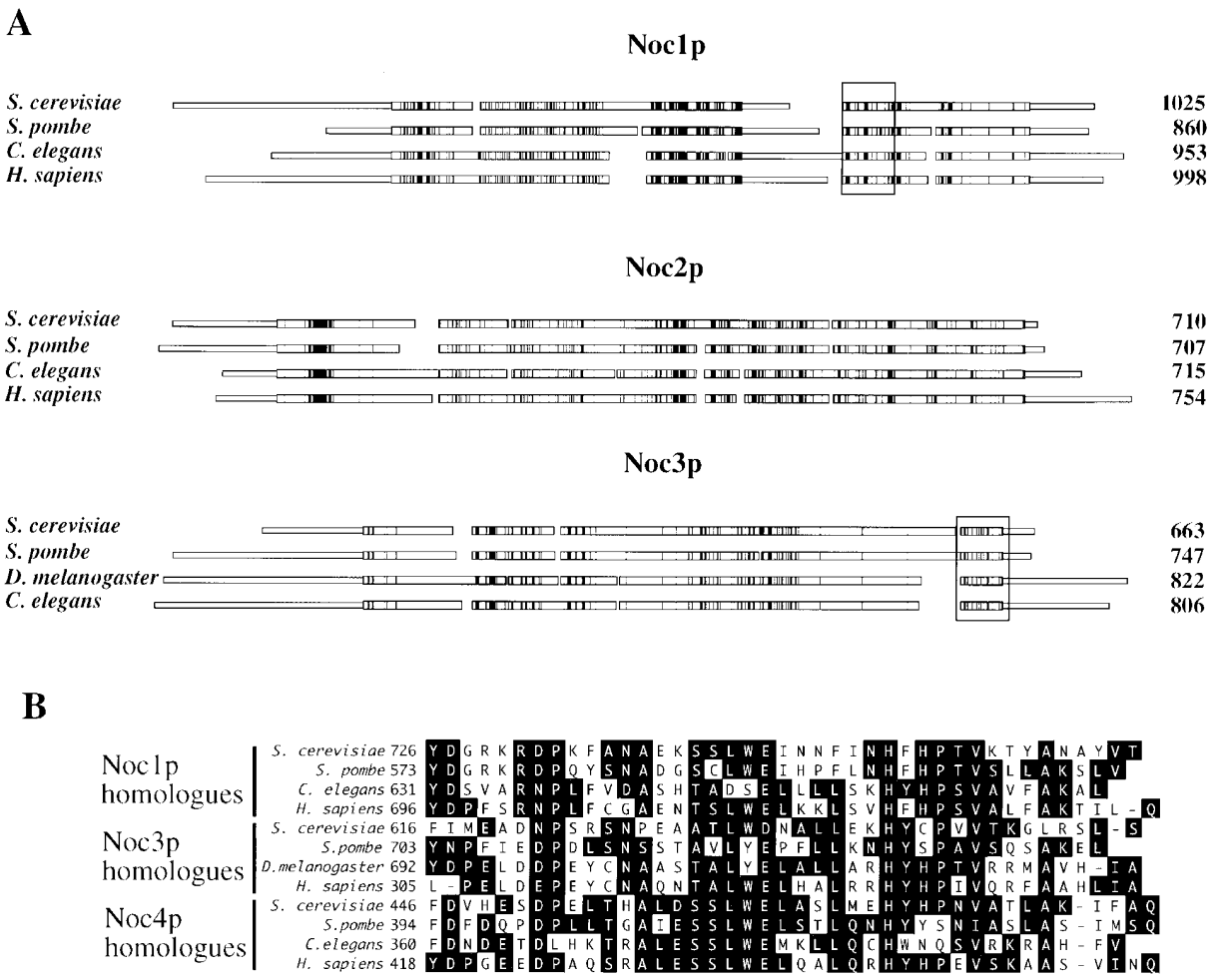

Figure 2. Noc Proteins Are Conserved in Evolution

(A) Schematic distribution of conserved amino acids within Noc1p, Noc2p, and Noc3p homologs. Black or gray rectangles denote invariant or homologous residues, respectively. Accession number Noc1p: *S. cerevisiae* (S54044), *S. pombe* (T38813), *C. elegans* (T21292), *H. sapiens* (A36368) and Noc2p: *S. cerevisiae* (S67098), *S. pombe* (T38027), *C. elegans* (T19060), *H. sapiens* (T08706); Noc3p: *S. cerevisiae* (S64824), *S. pombe* (T40728), *C. elegans* (T25614), *D. melanogaster* (locus: aaf54043). The large box in the Noc1p and Noc2p scheme represents the evolutionary conserved stretch of 45 amino acids.

(B) Sequence comparison of the conserved 45 amino acid long domain (Noc-domain; boxed in [A]) in Noc1p, Noc3p and Noc4p. Accession number for a partial cDNA of a Noc3p homolog in *H. sapiens* is FLJ12820. Accession numbers for Noc4p and homologs are: *S. cerevisiae* (S69032, systematic name: Ypr144c), *S. pombe* (T39508), *C. elegans* (T16903) and *H. sapiens* (mRNA: MGC3162). The numbers indicate the position of the domain within the respective protein.

been shown that in vivo depletion of Noc1p/Mak21p causes a reduction of 60S ribosomes (Edskes et al., 1998). Consistent with these data, we found that the ratio of the 60S to 40S subunits in polysome sucrose gradients is significantly reduced in *noc1-1*, *noc2-1*, and *noc3-1* ts mutants, when shifted to the restrictive temperature (Figure 4A). We conclude that Noc1-3p each function in the synthesis of the 60S subunit.

Pre-rRNA processing in the ts noc mutant strains was analyzed by Northern hybridization (Figure 4B), primer extension, and pulse-chase labeling experiments (data not shown). All three mutants showed similar but not identical phenotypes 2 hr after transfer to 37°C, which underlines that the rRNA pocessing pathway leading to the mature 25S rRNA/5.8S rRNA of the 60S ribosomal subunit was impaired. In each mutant, there was a mild accumulation of the 35S pre-rRNA, accompanied by a low level of the 23S rRNA. Synthesis of the $27SA_2$ pre-rRNA and 20S pre-rRNAs continued, although at a reduced rate. These phenotypes demonstrate a delay in the three early cleavages, at sites A_0, A_1, and A_2. The precursors on the pathway of 25S and 5.8S rRNA synthesis, 27SB, 7S, and 6S pre-rRNA, are strongly reduced, as seen by Northern (Figure 4B) and pulse-chase analysis (data not shown). Primer extension analysis indicated that cleavage at sites A_3 continued, and $27SB_L$ and $27S_B$ short were still detected, at reduced levels but with no clear change in their relative efficiencies. Cleavage at site A_0 also continued as judged by primer extension, and all cleavages were accurate at the nucleotide level. We conclude that processing of the pre-rRNA at sites A_3, $B1_L$, and $B1_S$ continues in the *noc1*, *noc2*, and *noc3* mutant strains though with reduced efficiency. Together, these data indicate that the *noc1* to *noc3* strains are not pri-

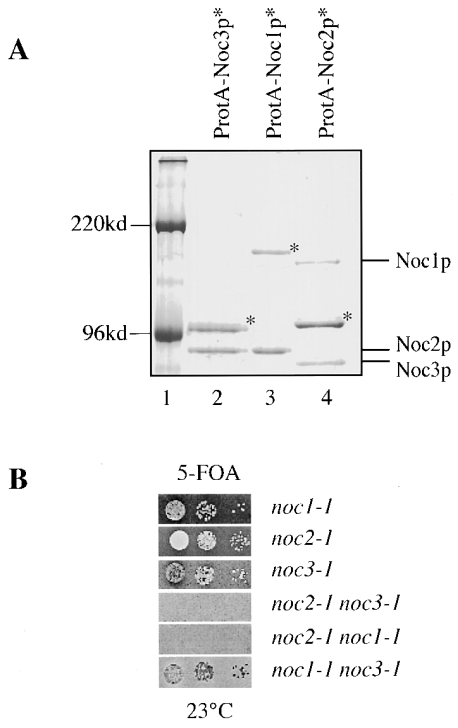

Figure 3. Physical and Genetic Interactions between *NOC1*, *NOC2*, and *NOC3*

(A) Affinity purification of Protein A-Noc fusion proteins. Functional fusion proteins ProtA-Noc1p, ProtA-Noc2p, and ProtA-Noc3p, expressed in the corresponding null strains, were affinity-purified on IgG-Sepharose beads. Copurifying proteins were separated by SDS-PAGE, visualized by Coomassie staining, and identified by mass spectrometry.

(B) Synthetic lethal relationship between *noc1-1*, *noc2-1*, and *noc3-1* alleles. Synthetic lethality between *noc* mutants was analyzed by the ability to lose wild-type *NOC1*, *NOC2*, and *NOC3* genes in a mutant background of either single mutants *noc1-1*, *noc2-1*, and *noc3-1* or double mutants *noc2-1/noc3-1*, *noc1-1/noc2-1*, and *noc1-1/noc3-1* (see Table 1). Wild-type *NOC* genes were present on plasmids harboring the *URA3* marker. Growth was analyzed after 4 days at 23°C on 5-FOA-containing plates.

marily defective in pre-rRNA cleavage, suggesting that Noc1-3p may be involved in correct assembly and/or transport of 60S ribosomal subunits (see below).

The Noc1p/Noc2p and Noc2p/Noc3p Complexes Localize to Different Intranuclear Compartments

Since upon biochemical purification, Noc1p, Noc2p, and Noc3p were significantly enriched in a nucleolar substructure (see above), all three proteins should be found in the nucleolus. To determine their exact subnuclear localization, Noc1p, Noc2p, and Noc3p were tagged with GFP and expressed in the corresponding null mutants. Noc1p-GFP exhibited a predominant crescent-like nuclear stain, which is typical of a nucleolar location (Figure 5A) and colocalized with the nucleolar marker Nop1p (data not shown). GFP-Noc2p was also concentrated in the nucleolus, but exhibited an additional, although weaker, nucleoplasmic staining (Figure 5A). In contrast, GFP-Noc3p was distributed throughout the entire nucleus (nucleolus plus nucleoplasm), although nucleolar enrichment was observed in a few cells (Figure 5A).

Immunoelectron microscopy was used to show the ultrastructural localization of all Noc proteins and to quantify the distribution of protein A-tagged Noc proteins (Figures 5B–5E) (Léger-Silvestre et al., 1997, 1999). Noc1p, Noc2p, and Noc3p were all detected in the nucleolus along the dense fibrillar component (DFC), but with different distribution in the nucleoplasm. Consistent with light microscopy, ProtA-Noc1p was concentrated in the nucleolus (80%) (Figure 5B), as was RNA polymerase I (85%). In contrast, ProtA-Noc3p was more evenly distributed between the nucleolus (40%) and nucleoplasm (60%) (Figure 5D). ProtA-Noc2p exhibited an intermediate distribution (60% nucleolus and 40% nucleoplasm) (Figure 5C). These data are consistent with the association of Noc2p with both Noc1p and Noc3p. We conclude that Noc2p acquires a nucleolar location by binding to Noc1p, but partitions to the nucleoplasm due to binding to Noc3p (see Discussion).

To find out whether a ts mutation in a given Noc protein affects the intranuclear distribution of the other two Noc members, we expressed GFP-Noc proteins in different *noc* ts mutants. The most striking change in distribution was observed when the location of Noc1p-GFP was analyzed in the *noc2* ts mutant. Instead of being mainly nucleolar as seen in wild-type cells, Noc1p-GFP was distributed throught the entire nucleoplasm in *noc2* ts cells (Figure 5A). On the opposite, Noc1p-GFP and Noc2p-GFP concentrate strongly in the nucleolus in both *noc1 and noc3* ts mutants (Figure 5A). This analysis shows that complex formation with Noc2p is important for the intranuclear location of Noc1p, whereas localization of Noc2p depends on the functionality of its interaction partners Noc1p and Noc3p, respectively. This all suggests that Noc proteins exhibit a dynamic intranuclear distribution with the potential to shuttle between the nucleolus and nucleoplasm.

Association of the Noc proteins with Preribosomal Particles

The difference in the subnuclear distribution of Noc1p/Noc2p and Noc2p/Noc3p suggests that these complexes perform distinct functions in ribosome synthesis which are spatially and temporally separated. Three types of ribosomal precursor particles, which significantly differ in size, have been described in yeast (Trapman et al., 1975). An early 90S particle contains the 35S pre-rRNA and many early assembling ribosomal proteins, while 66S and 43S pre-ribosomes are the precursors to the mature 60S and 40S subunits, respectively. The 66S preribosomal particle contains the 27S and 7S pre-rRNAs, whereas the 43S particle contains the 20S pre-rRNA.

Sucrose gradient centrifugation in combination with Western and Northern blotting was used to assess cofractionation of Noc1p, 2p, and 3p with the preribosomal particles. The subunit protein S8 served as a marker for 40S subunits, 80S ribosomes, and polysomes (Figure 6A), while Northern hybridization was used to determine the distribution of the 35S, 27S, and 7S pre-rRNAs. As expected, 35S pre-rRNA sedimented at ~90S, while the 27S and 7S pre-rRNAs sedimented at ~66S (Figure 6A). Strikingly, Noc3p cosedimented with the 27S and 7S pre-rRNAs and thus appears to be associated

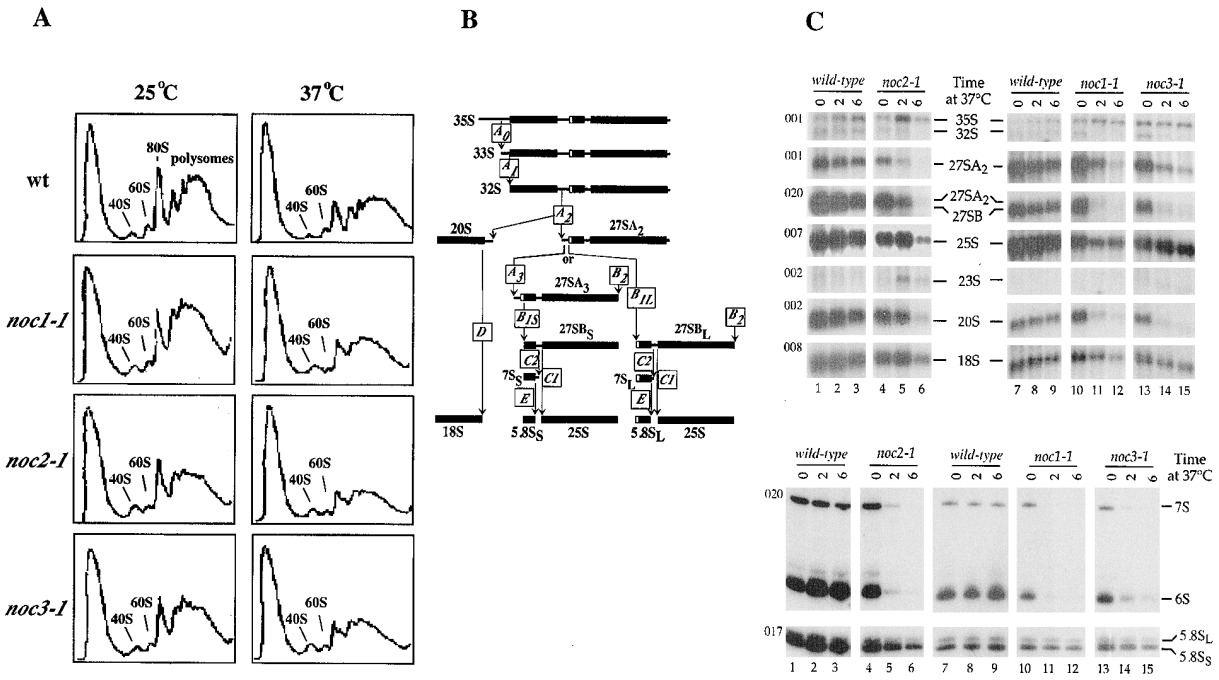

Figure 4. *noc* ts Mutants Are Impaired in Ribosome Biogenesis

(A) UV profiles. (OD_{260}) of polysome sucrose gradients derived from *noc1-1*, *noc2-1*, and *noc3-1* ts mutants at permissive (25°C) and 3.5 hr after shift to restrictive (37°C) temperature. Note that *noc2-1* cells show a reduced ratio of free 60S to 40S ribosomal subunits at the permissive temperature.

(B) Northern analysis of pre-rRNA processing. Wild-type and *noc2-1* (lanes 4–6), *noc1-1* (lanes 10–12), and *noc3-1* strains (lane 13–15) were analyzed following growth at 25°C (0 hr samples) and 2 and 6 hr after transfer to 37°C. Pre-rRNA species detected are indicated, as are the oligonucleotides used.

with 66S pre-ribosomes (Figure 6A). In contrast, Noc1p and Noc2p are seen not only in the 66S peak, but also in lower fractions of the gradient, which contain 35S pre-rRNA and may correspond to 90S pre-ribosomes (Figure 6A). When wild-type cells were grown at higher temperature (37°C), the segregation of Noc1p into the 90S peak became more evident on the sucrose gradient (Figure 6A), most likely due to a slow down in ribosome biogenesis. When preribosomal particles were analyzed in the presence of high salt, Noc1p, 2p, and 3p completely dissociated from pre-ribosomes and were recovered in the upper part of the sucrose gradient, in which soluble proteins partition, whereas mature ribosomal subunits were unaffected (Figure 6B). We conclude that Noc1p and Noc2p associate with 90S pre-ribosomes and remain associated with the 66S pre-ribosomes, whereas Noc3p (presumably in complex with Noc2p) associates predominantly with 66S preribosomal particles. Under high salt conditions, the proteins are dissociated from the preribosomal particles, presumably as the dimers observed by immunoprecipitation.

GFP-Tagged 60S Subunit Reporter Rpl25p Accumulates in Different Intranuclear Compartments in *noc1-1* and *noc1-3* Strains and in Cells Overexpressing the Noc Domain

In *noc2-1* cells, the 60S subunit reporter, Rpl25p-eGFP, was distributed between the nucleolus and nucleoplasm, with stronger accumulation in the nucleolus (see Figure 1). The *noc1-1* and *noc3-1* strains were also tested for intranuclear accumulation of Rpl25p-eGFP. Notably, Rpl25p-eGFP accumulated predominantly in the nucleolus in *noc1-1* cells upon shift to the restrictive temperature (the red fluorescently labeled Nop1p marker was used to visualize the nucleolus), whereas *noc3-1* cells exhibit accumulation of Rpl25p-eGFP throughout the nucleolus and nucleoplasm (Figures 7A and 7B). The accumulation of the 60S subunit reporter in different subnuclear regions correlates with the localization of Noc1p (nucleolus) and Noc3p (nucleoplasm and nucleolus). Apparently, Noc1p (presumably in complex with Noc2p) is required for an early transport step of ribosomal precursor particles from the nucleolus to the nucleoplasm. In contrast, Noc3p (presumably complexed with Noc2p) appears to participate in a later step, which coincides with transport of pre-ribosomes from the nucleoplasm into the cytoplasm.

When a short and conserved domain (3xNoc-domain) found in Noc1p and Noc3p (as well as in another Noc protein called Noc4p; see also Figure 2) was overexpressed in yeast, a dominant-negative effect on cell growth was observed with a nucleolar accumulation of Rpl25p-eGFP (Figure 7C). Strikingly, no defect in pre-rRNA processing was detected in the first six hours after induction of the 3xNoc-domain (Figure 7C). The simplest interpretation of these experiments is that the 3xNoc-domain interferes with the ribosomal transport machinery, thereby inhibiting the movement of ribosomal precursors out of the nucleolus without primarily affecting pre-rRNA processing. In contrast, a dominant-negative

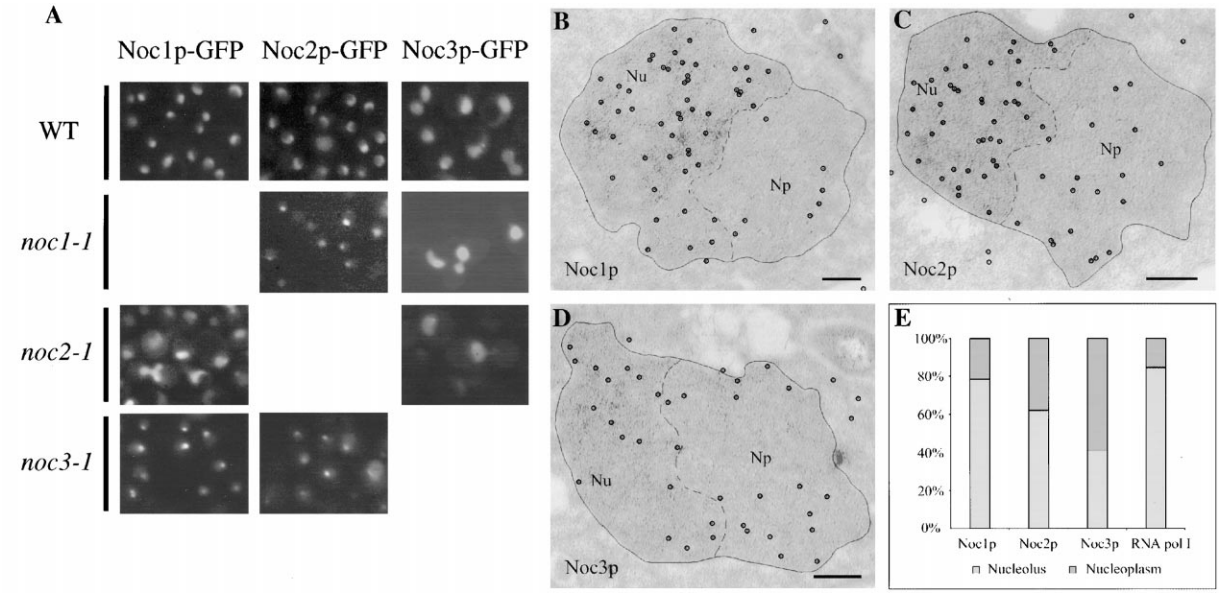

Figure 5. Nuclear Distribution of Noc Proteins

(A) In vivo localization of Noc1p-GFP, Noc2p-GFP, and GFP-Noc3p fusion proteins in wild-type cells and in *noc* ts mutants as determined by fluorescence microscopy.

(B–D) Localization of ProtA-tagged Noc1p, Noc2p, and Noc3p as determined by immuno-EM. Gold particles are highlighted by thick circles; the nuclear membrane and nucleolus by a thin and dashed line, respectively. Representative examples of each Noc distribution are depicted. Scale bar, 300 nm.

(E) Quantitation of gold particles corresponding to ProtA-Noc1p, ProtA-Noc2p, ProtA-Noc3p, and RNA polymerase I, according to the immuno-EM data from (B)–(D). The number of gold particles in nucleolus and nucleoplasm was counted on 20 micrographs of independent cells taken at random. These quantitative numbers are expressed in % of gold particles present in the nucleolus (light bars) and nucleoplasm (dark bars).

mutant expressing a truncated form of Nmd3p, a protein involved in late ribosomal export steps (Gadal et al., 2001) exhibits accumulation of Rpl25p-eGFP throughout the nucleoplasm (Figure 7C). These data show that it is possible to uncouple maturation of ribosomal precursor particles from nuclear transport reactions and that Noc proteins play a direct role in intranuclear movement of pre-ribosomes.

Discussion

We report here the characterization of three proteins Noc1p, Noc2p, and Noc3p, all of which are components of a machinery that participates in maturation of preribosomal particles and their subsequent transport from the nucleolus via the nucleoplasm into the cytoplasm. Since homologs for each of the Noc1-3p proteins exist in higher eukaryotes, it is likely that their functions in ribosome synthesis are conserved in evolution.

What could be the mechanism by which Noc proteins participate in the coupled maturation/transport steps of intranuclear pre-ribosomes? It has been assumed for a long time that ribosomal precursor particles are transiently associated with different snoRNAs and a vast number of nonribosomal proteins that mediate and coordinate rRNA and ribosome biogenesis. Since some of these proteins shuttle between the nucleus and cytoplasm, it was suggested they could be involved in nucleocytoplasmic transport of ribosomal proteins or ribosomal particles (Borer et al., 1989). Besides, proteins involved in the correct assembly of pre-ribosomes could be also required for the vectorial movement of pre-ribosomes through the nucleus, if quality control is a prerequisite for transport competence.

There is a general agreement that the cascade of pre-rRNA processing and ribosome assembly steps is spatially organized in different, highly ordered nucleolar substructures, which have been designated as fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC) (Scheer and Hock, 1999). Accordingly, precursor particles could migrate from the site of transcription (FC or DFC) through the sites of processing/modification (DFC) and assembly (GC) and, finally, gain export competence through the nuclear pore. It is thought that nonribosomal proteins (e.g., nucleolar components) are successively removed from these particles during maturation since ribosomal precursor particles mature in a time-dependent way from large particles (90S or 35S pre-rRNA) to smaller particles (e.g., 66S or 27S rRNA; 43S or 20s rRNA) (Trapman et al., 1975).

Our data show that size maturation of ribosomal precursor particles and their release from stable association with the nucleolus is coupled with a change in association with distinct nonribosomal components. It thus appears that during maturation of 90S to 66S pre-ribosomes, Noc1p dissociates from Noc2p and is replaced by Noc3p, which triggers movement of pre-ribosomes from the nucleolus to the nucleoplasm. Alternatively, the Noc1p/Noc2p and Noc2p/Noc3p complexes do not dissociate, but accompany as stable complexes the different preribosomal particles from the nucleolus to the nucleoplasm in a temporally and spatially ordered fash-

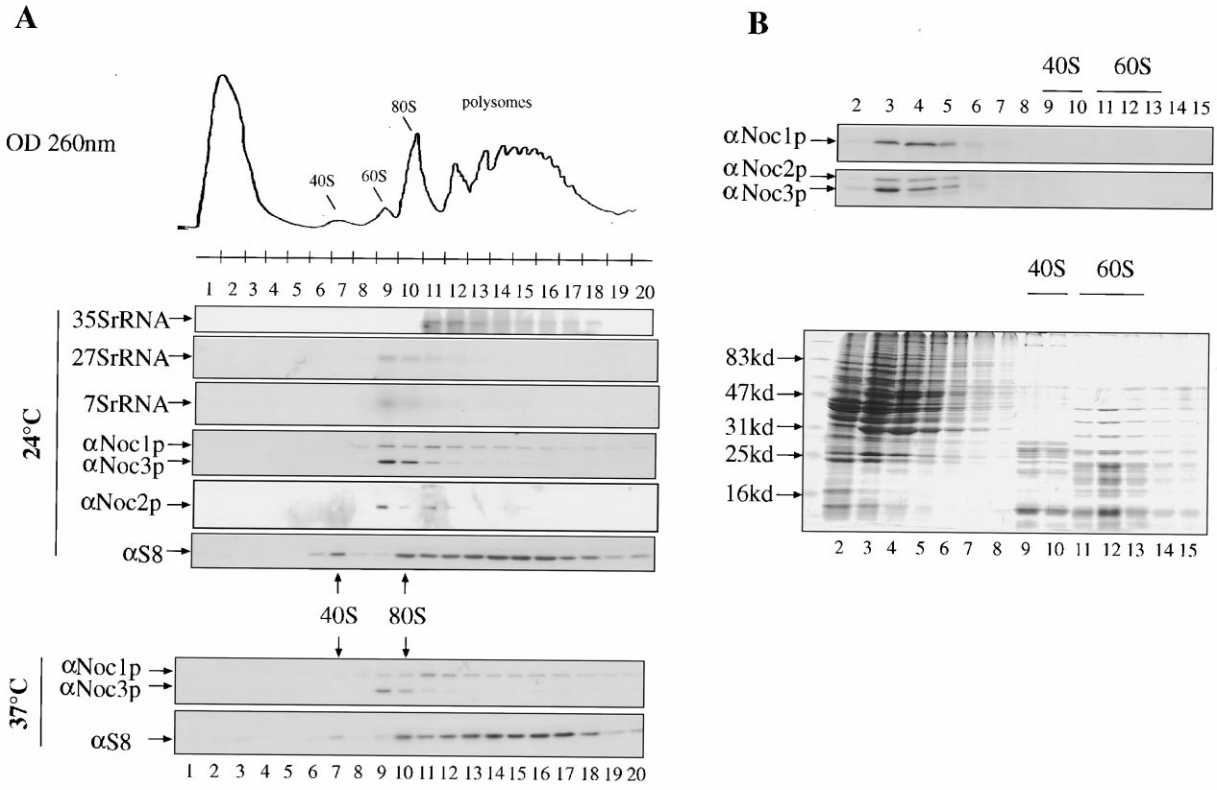

Figure 6. Sedimentation Behavior of Noc proteins and (Pre)-Ribosomal Particles on Sucrose Gradients

(A) Analysis of polysomal fractions derived from wild-type cells grown at 24°C (upper panel) and 37°C (lower panel) was performed as described in the Experimental Procedures. Aliquots of the fractions were analyzed by Western blot analysis with polyclonal antisera directed against ribosomal protein S8 or against Noc proteins. To detect pre-rRNAs, total RNA was extracted from the same fractions and analyzed by Northern blot analysis (see Experimental Procedures).

(B) Noc proteins are released from pre-ribosomes by high salt treatment. Whole-cell yeast lysates were separated on a sucrose gradient in the presence of 0.8 M KCl. Aliquots of the fractions were analyzed by Western blotting (upper panel) or by SDS-PAGE/silver staining (lower panel).The fractions containing 40S and 60S ribosomal subunits are marked.

ion. Independent of which model is correct, the Noc1p/Noc2p complex appears to be crucial to enable 66S precursor particles to leave the nucleolus. This could be brought about by inactivating nucleolar retention sites or by active movement to "downstream" sites. Whether intranuclear movement of pre-ribosomes from the site of transcription to the site of processing and assembly and further on to the nuclear pores and cytoplasm is an active process or is regulated by retention and release from retention sites is not known. Accordingly, it is possible that resident nucleolar proteins transiently and sequentially associate with preribosomal precursor particles inside the nucleolus, as long as they are not fully processed or assembled, thereby coordinating the ordered array of processing and assembly steps with transport through the nucleolus and to the nucleoplasm. Interesting in this context is the observation that Noc proteins influence each other in terms of their distinct intranuclear location, since a given noc ts mutant alters the steady-state distribution of another Noc protein (e.g., noc2-ts changes the distribution of Noc1p, and noc3-ts changes those of Noc1p and Noc2p). Consequently, Noc proteins exhibit a dynamic intranuclear location and can shuttle between the nucleolus and nucleoplasm, which might represent a necessary prerequisite for a Noc protein mediated intranuclear movement of preribosomal particles.

Although many data presented here point to a tight coupling between pre-rRNA processing, maturation of pre-ribosomes and nuclear export, one experiment suggests that both processes indeed may become uncoupled. Overexpression of a short domain found in Noc1p and Nop3p causes a dominant-negative phenotype of cell growth with an accumulation of pre-ribosomes inside the nucleolus, but no impairment at all in pre-rRNA processing. We suggest that the dynamic interaction of Noc proteins forming distinct Noc complexes is crucial for intranuclear movement of ribosomal precursor particles. When these movements occur, precursor particles can proceed in their proper maturation, which occurs in a spatially and temporally ordered way.

The pre-rRNA processing phenotype and instability of rRNA processing intermediates observed in the *noc* mutant strains is typical of defects seen in many other mutants that are defective in 60S synthesis and nuclear export (O.G., E.H., and D.T., unpublished observation). In almost all cases, the early pre-rRNA cleavages at sites A_0–A_2 show at least some inhibition, even when there is no clear or direct relation between the mutant product and these cleavages (reviewed in Venema and

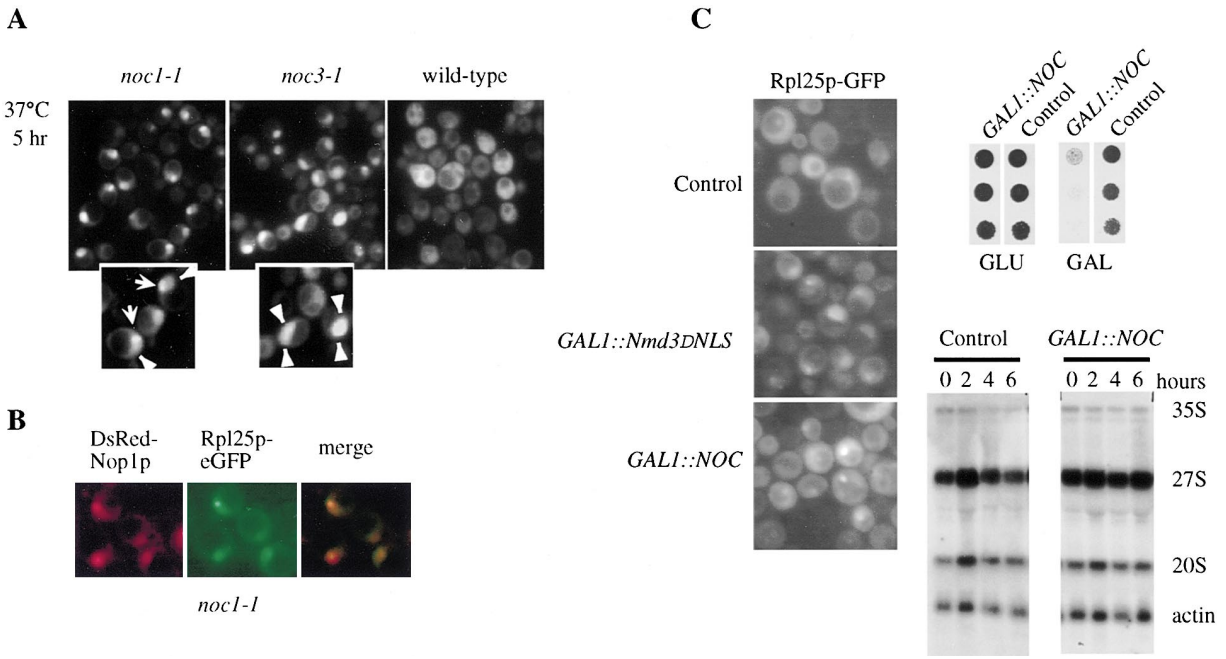

Figure 7. Rpl25p-eGFP Accumulates in Different Parts of the Nucleus in *noc* Mutants

(A) Rpl25p-eGFP accumulates in the nucleolus in *noc1-1* and nucleoplasm in *noc3-1* cells. *noc1-1*, *noc3-1*, and wild-type cells were transformed with *RPL25-eGFP* (pRS316-RPL25-eGFP). Strains were grown in SDC-leu medium at 23°C, before dilution in fresh YPD-medium and incubation for 5 hr at 37°C. Samples of the cultures were analyzed by fluorescence microscopy. Triangles in the magnified picture (inset) point to nuclear staining, arrowheads to nucleolar staining.

(B) *noc1-1* cells transformed with *RPL25-eGFP* (pRS316-RPL25-eGFP) and DsRed-Nop1p (pUN100-DsRed-NOP1) were shifted for 5 hr to 37°C and analyzed by fluorescence microscopy. The Rpl25p-eGFP and DsRed-Nop1p pictures were merged to observe colocalization.

(C) Overexpression of the short and conserved domain found in Noc1p and Noc3p (3xNoc domain) from the strong *GAL1* promoter leads to both accumulation of Rpl25p-eGFP in the nucleolus and to growth inhibition, but does not affect pre-rRNA processing. Wild-type cells bearing a control plasmid with the *GAL1* promoter (pYX243), cells expressing either the Noc-domain in triplicate (3xNoc-domain), or Nmd3ΔNES under the galactose inducible promoter were transformed with *RPL25-eGFP* and grown in the presence of raffinose. Two hours after shift to galactose, the strains were analyzed by fluorescence microscopy (left). Growth phenotype of cells overexpressing 3xNoc-domain and control cells were analyzed on glucose (GLU) and galactose (GAL) plates (upper right). Fifty milliliter aliquots of the cultures were removed at the time points indicated after shift to galactose and analyzed by Northern blotting (lower right). Pre-rRNA species and actin mRNA detected are indicated.

Tollervey, 1999). This has led to the proposal that all pre-rRNA processing factors must assemble together prior to the first pre-rRNA cleavage step. The present data suggest the possibility that normal pre-rRNA processing requires ongoing ribosome export. The high rate of ribosome synthesis is likely to require disassembly of the preribosomal processing factors either following, or concomitant with, nuclear export. We suggest that the common processing defect seen for many 60S synthesis and export mutants, including those reported here, arises from impaired disassembly of processing factors. In actively growing yeast cells, around 2000 ribosomes are synthesized per minute, so even a modest reduction in the rate of recycling of processing factors will lead to a rapid depletion of the available pool. It is well established that pre-rRNA processing and pre-ribosome assembly are intimately linked. We propose that these processes are also closely linked to release the pre-ribosomes from the nucleolus and to export them from the nucleus.

In summary, we could demonstrate that pre-ribosomes associate with different Noc proteins in a temporally and spatially ordered way during ribosome biogenesis, which is required for intranuclear transport of precursor particles. Thus, maturation and intranuclear transport of pre-ribosomes requires the function of Noc proteins.

Experimental Procedures

Yeast Strains, DNA Recombinant Work, and Microbiological Techniques

Yeast strains used in this study are given in Table 1. Microbiological techniques, plasmid transformation and recovery, mating, sporulation of diploids, and tetrad analysis were done essentially as described (Santos-Rosa et al., 1998). DNA recombinant work was performed according to Maniatis et al., 1982.

Plasmid Constructions

Plasmids pUN100-DsRed-Nop1, pRS316-Rpl25-eGFP, pNOPPA1L, pNOPGFP1L, and pFA6a-GFP(S65T)-kanMX6 were described previously (Gadal et al., 2001; Hellmuth et al., 1998; Longtine et al., 1998).

Noc1, with its authentic 5′ and 3′UTR was amplified by PCR from yeast genomic DNA using 60w3f and 60w6rb oligonucleotides as primers. The derived fragment was cut with HindIII and SacI and cloned into YCplac33 using the same sites, generating YCplac33-NOC1. Ycplac33-NOC2 and Ycplac33-NOC3 were generated similarly, using oligonucleotides 206w1f and 206w13r for NOC2 (sites PstI and SacI) and oligonucleotides 002c3f and 002c2r for NOC3 (sites SacI and SphI), respectively.

Protein A-tagged versions of NOC1, NOC2, and NOC3 were generated by cloning Noc genes amplified by PCR with their authentic 3′

Table 1. Yeast Strains

Name	Genotype	Origin
FY23	*MATa, ura3, trp1, leu2*	derived from S288C
FY86	*MAT*α, *ura3, his3, leu2*	derived from S288C
W303a	*MATa, ade2, his3, leu2, trp1, ura3*	derived from ATCC 201239
rix3-1	*MAT*α, *ura3, his3, leu2, noc2-1*	isolated from Ts collection, Amberg et al., 1992
RIX3$^+$	*MAT*α, *ura3, his3, leu2, noc2-1* +*pUN100-NOC2* (*ARS/CEN LEU2 NOC2*)	derived from *rix3-1*
BSY420	*MATa/*α, *ade2/ade2, can1/can1, leu2/leu2, ura3/ura3, his3/his3, trp1/trp1*	autodiploid BMA38 gift from B. Séraphin
BSY420a	*MATa, ade2, can1, leu2, ura3, his3, trp1*	offspring of BSY420
NOC1 shuffle	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc1*Δ*::HIS3* + *YCplac33-NOC1* (*ARS/CEN URA3 NOC1*)	offspring of BSY420
NOC2 shuffle	*MAT*α, *ade2, can1, leu2, ura3, his3, trp1, noc2*Δ*::HIS3* + *YCplac33-NOC2* (*ARS/CEN URA3 NOC2*)	offspring of BSY420
NOC3 shuffle	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc3*Δ*::HIS3* + *YCplac33-NOC3* (*ARS/CEN URA3 NOC3*)	offspring of BSY420
ProtA-NOC1	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc1*Δ*::HIS3* + *pNOPPAIL-NOC1* (*ARS/CEN LEU2 ProtA-NOC1*)	isogenic to NOC1 shuffle
ProtA-NOC2	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc2*Δ*::HIS3* + *pNOPPAIL-NOC2* (*ARS/CEN LEU2 ProtA-NOC2*)	isogenic to NOC2 shuffle
ProtA-NOC3	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc3*Δ*::HIS3* + *pNOPPAIL-NOC3* (*ARS/CEN LEU2 ProtA-NOC3*)	isogenic to NOC3 shuffle
NOC1-GFP	*MATa, ura3, trp1, his3, leu2, NOC1-GFP::KANMX4*	offspring of FY23 × FY86
NOC2-GFP	*MATa, ura3, trp1, his3, leu2, NOC2-GFP::KANMX4*	offspring of FY23 × FY86
GFP-NOC3	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc3*Δ*::HIS3* + *pNOPGFPIL-NOC3* (*ARS/CEN LEU2 GFP-NOC3*)	isogenic to *NOC3* shuffle
noc1-1	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc1*Δ*::HIS3* + *pNOPPAIL-noc1-1* (*ARS/CEN LEU2 ProtA-noc1-1*)	isogenic to *NOC1* shuffle
noc3-1	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc3*Δ*::HIS3* + *pNOPPAIL-noc3-1* (*ARS/CEN LEU2 ProtA-noc3-1*)	isogenic to *NOC3* shuffle
noc1-1 noc2-1	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc2-1, noc1*Δ*::HIS3* + *pNOPPAIL-noc1-1* (*ARS/CEN LEU2 ProtA-noc1-1*) + *YCplac33-NOC1* (*ARS/CEN URA3 NOC1*)	offspring of *rix3-1* × *noc1-1*
noc3-1 noc2-1	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc2-1, noc3*Δ*::HIS3* + *pNOPPAIL-noc3-1* (*ARS/CEN LEU2 ProtA-noc3-1*) + *YCplac33-NOC2* (*ARS/CEN URA3 NOC2*)	offspring of *rix3-1* × *noc3-1*
noc1-1 noc3-1	*MATa, ade2, can1, leu2, ura3, his3, trp1, noc2-1, noc3*Δ*::HIS3, noc1*Δ*::HIS3* + *pRS314-noc3-1* (*ARS/CEN TRP1 ProtA-noc3-1*) + *pNOPPAIL-noc1-1* (*ARS/CEN LEU2 ProtA-noc1-1*)	offspring of *noc3-1* × *noc1-1*

into pNOPPA1L. *NOC1* was amplified using oligonucleotides 60w1f and 60w6rc and cloned using HindIII, *NOC2* was amplified using oligonucleotides 206w3f and 206w2r and cloned through Pst1 and Xho1 sites, and *NOC3* was amplified using oligonucleotides Ylr002c-2f and Ylr002c-3r and cloned after Klenow treatement using the SalI-ApaI sites of pNOPPA1L. pNOPGFP1L-NOC3 was generated by replacing the PstI-SphI fragment of pNOPPA1L-NOC3 encoding Protein A by the PstI-SphI fragment of pNOPGFP1L encoding GFP. pUN100-NOC2 was recovered from the genomic library. The 3xNoc domain was generated by PCR amplification of the Noc3 fragment from nucleotide1629 to 1992 downstream of the start codon using the oligonucleotides 002-CT-5′ and 002-CT-3′. The resulting PCR fragment was cloned in pQE30 (Quiagene) using the HindIII site, sequenced, and cloned in frame into the galactose inducible yeast shuttle vector pYX243 which carries a C-terminal HA-tag (Novagene). Plasmid DNA of a clone encoding the Noc-domain in triplicate was isolated and transformed in yeast strain w303-1a. Significant overexpression of the 3xNoc-domain as determined by immunodetection of the HA tag was observed 1 hr after shift to galactose. Yeast strains expressing Nmd3ΔNES, which lacks the nuclear export signal of Nmd3 were constructed cloning the EcoRI-BamHI fragment of pRS315-NMD3ΔNESall (Gadal et al., 2001) into plasmid Yep351:gal1. The resulting construct was transformed into strain w303-1a.

Strain Construction

Genes *NOC1* (Ydr060w), *NOC2* (Yor206w), and *NOC3* (Ylr002c) were disrupted by homologous recombination using HIS3 in the diploid strain BSY420. The disruptions in the resulting strains were confirmed by PCR analysis. Strains *NOC1 shuffle*, *NOC2 shuffleI*, and *NOC3 shuffle* were obtained by transforming plasmids Ycplac33-NOC1, Ycplac33-NOC2, and Ycplac33-NOC3 into their respective heterozygote strains. After tetrade analysis, HIS$^+$ URA$^+$ spores were selected.

Strains *ProtA-NOC1*, *ProtA-NOC2*, *ProtA-NOC3I*, and *GFP-NOC3* were obtained by transforming *NOC1 shuffle*, *NOC2 shuffle*, and *NOC3 shuffle*, respectively, with plasmids pNOPPA1L-NOC1, pNOPPA1L-NOC2, pNOPPA1L-NOC3, and pNOPGFP1L-NOC3 and shuffling out of the *URA3*-containing vector using 5-FOA. Functionality of these tagged version of noc proteins were tested from 16 to 37°C and no growth defects were observed.

Genomic integration of GFP in frame with *NOC1* and *NOC2*, respectively, was obtained as described previously (Longtine et al., 1998).

Generation of *noc1 and noc3* temperature-sensitive strains was performed by random PCR-mediated mutagenesis using plasmids pNOPPA1L-NOC1 and pNOPPA1L-NOC3 and oligonucleotide o_nocup and o_nocdo as described previously (Sträßer and Hurt, 2000). pRS314-noc3-1 was generated by subcloning noc3-1 from pNOPPA1L-noc3-1 into pRS314.

Mass Spectrometry and Protein Identification

Mass spectrometry and protein identification were performed as previously described (Fath et al., 2000; Shevchenko et al., 1996a, 1996b).

Cloning of RIX3/NOC2

A yeast genomic library in a *LEU2*-containing *ARS/CEN* plasmid was transformed into the *rix3-1* strain. From colonies growing at

the restrictive temperature (37°C), plasmids with genomic inserts were isolated. Complementing plasmids always contained the *YOR206w* gene. pUN100-NOC2 harboring only the *YOR206w* gene was generated and shown to complement the ts growth defect of the *rix3-1* mutant.

Affinity Purification of Protein A-Tagged Noc Proteins

Protein A purifiaction was performed as described previously with some modification (Siniossoglou et al., 1996). Yeast cells were grown in 20 liter YPD to an OD of 2. Harvesting and breakage of yeast cells and preparation of 100.000 g supernatant was done as described previously (Milkereit et al., 1997) with the exception that DTT was omitted in the dilution buffer (100 mM HEPES [pH 7.8], 20 mM $MgCl_2$, 200 mM NH_4SO4, 1 mM PMSF, and 2 mM Benzamidine). Triton X-100 was added to the 100.000 g supernatant to a final concentration of 0.05%. One milliliter human IgG-Sepharose (Pharmacia), which was subsequently washed with 15 ml H_2O, 30 ml dilution buffer, 15 ml 5 mM NH_4 acetate (pH 5), 30 ml elution buffer (100 mM glycine [pH 2.8], 150 mM NaCl, and 0.05% Triton X-100), and 50 ml buffer TST (50 mM Tris [pH 7.5],150 mM NaCl, 0.05% TritonX-100, 1 mM PMSF, and 2 mM Benzamidine), was added to the extract and the suspension was incubated at 8°C on a turning wheel. After 2 hr, the suspension was transferred to a column and washed with 50 ml buffer TST and 6 ml 5 mM NH_4-acetate (pH 5). Bound proteins were eluted with 5× 2 ml elution buffer. Five percent of eluted fractions were analyzed by SDS-PAGE and Coomassie staining.

Ribosome Purification by Sucrose Density Gradient Centrifugation Analysis

Isolation of ribosomes under low salt by sucrose gradient centrifugation was performed as described, with some modifications (Tollervey et al., 1993). Two hundred milliliters of yeast culture were grown in YPD to an OD of 0.5 and cycloheximide was added to a final concentration of 100 μg/ml. After 10 min incubation, yeast cells were harvested by centrifugation and washed in 20 ml ice-cold buffer A (20 mM HEPES [pH 7.5], 10 mM KCl, 2.5 mM $MgCl_2$, 1 mM EGTA, 100 ug/ml cycloheximide, and 1 mM DTT). 1.4 g glass beads (0.5 mm) were added to 0.8 ml of cell suspension in a 2ml reaction tube. Cells were broken by 15 min of shaking at 8°C on a shaking device (IKA Vibrax VXR). One hundred fifty microliters buffer A was added and the suspension was centrifuged for 5 min at 10,000 rpm. The supernatant was loaded on a 10.5 ml 10%–50% sucrose gradient in buffer A without DTT and cycloheximide and centrifuged for 8 hr at 27,000 rpm in a SW41 rotor. A gradient collector (Labconco Auto Densi-Flow), in combination with a Pharmacia UV-detector UV-1, was used to record the UV profile. 0.5 ml fractions were collected and 25% of the fractions were TCA-precipitated for Western analysis. For Northern blot analysis, 30 μl 10% SDS in 1M Tris (pH 7.5), 700 μl phenol (Roth, RothiPhenol) and 700 μl chloroform was added to 250 μl of the fractions. After mixing and heating to 65°C for 2 min, the samples were centrifuged for 1 min at 14,000 g at room temperature and the supernatant was extracted again with 450 μl phenol and 450 μl chloroform. 1/10 volume 3 M Na-acetate and 2.5 volumes ethanol were added to the supernatants, and after 20 min, incubation at −20°C the precipitated RNA was recovered by 10 min centrifugation at 4°C. RNA was blotted onto a Hybond-N membrane (Amersham) according to Maniatis et al. (1982). Hybridization with radiolabeled oligonucleotides was carried out overnight at 30°C in 50% formamide, 5×SSC, 50 mM Na-phosphate (pH 6.5), 0.4% SDS, 0.1 mg/ml salmon sperm DNA, and 5× Denhardts solution. To detect 27S rRNA and 7S rRNA, endlabeled oligonucleotide 5′C2-site was used, and after hybridization, the blot was washed two times with 2× SSC and two times with 0.1% SDS in 2× SSC for 30 min at 30°C. To detect 35S rRNA, a PCR fragment created with oligos 5′A1(REV) and 3′IS(FOR) and yeast genomic DNA as a template was labeled by random priming (BioRad, Random Primer DNA labeling kit). After hybridization, the blot was briefly washed twice with 2× SSC at room temperature and twice with 2× SSC at 65°C for 30 min.

Fluorescence Microscopy

pRS316-RPL25-eGFP was introduced by transformation and selected on SDC-leu medium. Individual transformants were picked and grown in liquid SDC-leu medium at 23°C to OD_{600} of about 1, before shift to 37°C in liquid YPD medium. After centrifugation, cells were resuspended in water, mounted on a slide, and viewed in the fluorescence microscope. In vivo, the GFP signal was examined in the fluorescein channel; the DsRed used in fusion with Nop1p was examined in the rhodamine channel of a Zeiss Axioskop fluorescence microscope and pictures were obtained with a Xillix Microimager CCD camera. Digital pictures were processed by software program Improvision (Open lab) and Photoshop 4.0.1 (Adobe).

Electron Microscopy

The strains were prepared for electron microscopy according to Trumtel et al., 2000. For immunolocalization of the Protein A-tagged Noc proteins, we used anti-Protein A antibodies from Sigma. This antibody was labeled with gold-conjugated protein A (British Bio Cell). No labeling was detected when the immunolocalization was performed on cells devoid of Protein A-tagged protein, or when the anti-Protein A antibody was omitted.

Semiquantitative Analysis of Noc1p, Noc2p, and Noc3p Distribution

Micrographs were analyzed after immunolocalization of Noc1p, Noc2p, and Noc3p. The number of gold particles in nucleolus and nucleoplasm was hand counted on 20 micrographs of independent cells taken at random. Areas of the nucleolus and nucleoplasm were estimated by cutting up the micrograph and weighing the pieces. The minimum sample size for each condition and cell component considered was determined by the progressive mean technique (confidence limit 10%) (Williams et al., 1977).

Northern Blot Analysis

Northern hybridization in noc mutants was performed as described (Tollervey, 1987).

Oligonucleotides used were: 003: TGT TAC CTC TGG GCC C, 004: CGG TTT TAA TTG TCC TA, 007: CTC CGC TTA TTG ATA TGC, 008: CAT GGC TTA ATC TTT GAG AC, 013: GGC CAG CAA TTT CAA GTT A, 017: GCG TTG TTC ATC GAT GC, 020: TGA GAA GGA AAT GAC GCT, 219: GAA GCG CCA TCT AGA TG. 5′ A_0L GGT CTC TCT GCT GCC GG.

Miscellaneous

Polyclonal antibodies against a peptide composed of the 20 carboxy terminal aminoacids of Noc1p, a fragment containing the 120 aminoterminal aminoacids of Noc2p, and a fragment containing the 211 aminoterminal aminoacids of Noc3p were raised in rabbits and affinity purified with the appropriate immobilized antigen. SDS-PAGE and Western blot analysis were performed according to Siniossoglou et al., 1996. Polyclonal antiserum against S8, L16 was a kind gift and G. Dieci (Parma).

Oligonucleotides used for cloning and PCR: 60w3f: TTT TTT AAG CTT CCT CCA CCA CAG CCA TGC TTC AAA; 60w6rb: TTT TTT GAG CTC TTG TCG CTA CAA ATG TGC CTA TAT; 60w1f: TTT TTT AAG CTT ATG AGT GAG AAC AAC GGC AAT CCG C; 60w6rc: TTT TTT AAG CTT TTG TCG CTA CAA ATG TGC CTA TAT; o_nocup: CAA TAA CTC CGA TCA AAT TAA CTC AAA TCA AC; o_nocdo: GCA GTG AGC GCA ACG CAA TTA ATG TGA G; 206w1f: TTT TTT CTG CAG TCC CGC ATA TTG AAT TGT TCA AAG TAA TTA; 206w13r: TTT TTT GAG CTC GAT CCA TCG AAC GTT TGA CG; 206w3f: TTT TTT CTG CAG ATG GGT AAA GTT TCT AAA TCG ACC AAG; 206w2r: TTT TTT CTC GAG TTA AGC GTC TGA CAT TTC AAC ATC TTC; Ylr002-2f: TTT TTT GTC GAC ATG GCT AAG AGA AAT AGA TCT CAA TTT C; Ylr002-3r: TTT TTT GGT ACC CTA TTT AGA ACA CTC TTT AGA TCT AGA TG; Ylr002-3f: TTT TTT GAG CTC CCT AGG CGC GCA GCA AAC TCC A; Ylr002-2r: TTT TTT GCA TGC AGG CCC TTA ATA TAC GCC TAA TGG GG; 5′C2-Site: GGC CAG CAA TTT CAA GTT A; 5′A1(REV): GAA GAA GCA ACA AGC AGT; 3′IS(FOR): GGC CTC GTC AAA CGG TGG; 002-CT-5́: TTT TTT AAG CTT GCA GAA CTT CTG CTA AAG GCT CTG; 002-CT-3́: TTT TTT AAG CTT TTT AGA ACA CTC TTT AGA TCT AGA.

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Accession Numbers

GenBank accession numbers for the genes reported in this paper are as follows: Noc1p: *H. sapiens* (A36368); Noc2p: *S. cerevisiae* (S67098), *S. pombe* (T38027), *C. elegans* (T19060), *H. sapiens* (T08706); and Noc3p: *S. cerevisiae* (S64824), *S. pombe* (T40728), *C. elegans* (T25614), and *D. melanogaster* (locus: aaf54043).