A Noc Complex Specifically Involved in the Formation and Nuclear Export of Ribosomal 40 S Subunits*

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Eukaryotic ribosome biogenesis is spatially organized into different subcellular compartments. Most steps in the pathway leading to mature ribosomes occur in the nucleolus, a specialized nuclear substructure, which includes transcription of the rDNA by RNA polymerase I, modification of the synthesized precursor RNA, and the assembly of both many ribosomal and non-ribosomal proteins with pre-ribosomal RNA (1). In the yeast Saccharomyces cerevisiae the resulting large ribonucleoprotein complex forms the 90 S pre-ribosome, which is split into precursor particles for the mature 40 S and 60 S ribosomal subunit (2). During or after their maturation the pre-ribosomes leave the nucleolus, move toward the nuclear pore, gain export competence, and are finally exported into the cytoplasm. Some maturation steps like processing of the 20 S rRNA intermediate within the 40 S subunit and the association of a few ribosomal proteins to the ribosomes occur rather late, even in the cytoplasm (3).

Many factors known to be involved in biosynthesis and maturation of ribosomes were identified and characterized in S. cerevisiae (4, 5). This organism represents a well suited model organism to study eukaryotic ribosome biogenesis, because homologues of the factors required are found in many eukaryotes. Of the more than 70 non-ribosomal proteins that participate in generation of ribosomes, most have been described to be required for modification of rRNA or removal of the external and internal spacer sequences from the precursor 45S pre-rRNA. End products of the rRNA processing pathways are the 18 S RNA, which is present in the 40 S subunit and the 25 S and 5.8 S rRNA, as well as the RNA polymerase III-encoded 5 S rRNA, which are the RNA constituents of the 60 S subunit. Among the transacting factors involved to produce mature 40 S and 60 S subunits are nuclease, putative RNA helicases, RNA modifying proteins, and proteins associated with small nucleolar RNAs (4, 5) (see also www.expasy.ch/linder/proteins.html).

Folding, processing, and maturation of the rRNA is coordinated with the association of ribosomal proteins, with the assembly and disassembly of transacting factors, and with the movement of the ribosomal particles toward the nuclear pore (6, 7). Different pre-ribosomal particles are generated, which differ in their sedimentation behavior on sucrose density gradients and in both their incorporated rRNA intermediates and (non-)ribosomal proteins. The 35S pre-rRNA, which is the primary rRNA transcript, is a constituent of the 90 S pre-ribosome. Precursor particles of the 40 S ribosomal subunit sediment with a size of ~43 S and contain 20 S pre-rRNA, whereas 60 S precursors co-sediment with ~66 S and contain 27 S or 25 S, 5 S, and 7 S pre-rRNA (2, 8, 9). The components associated with the different pre-ribosomal particles are thought to comprise the machineries required for ribosomal subunits formation and their regulation, as well as for quality control steps and for the movement of pre-ribosomes from the nucleolus to the cytoplasm. These protein complexes are transiently associated with nascent ribosomes. Recently, it became possible to purify large precursor assemblies employing (tandem-)affinity purification strategies under mild ionic strength using tagged non-ribosomal precursor subunits. Several 60 S and 40 S pre-ribosome intermediates could be isolated, which differ in their subunit composition (10–14) and probably reflect a snapshot of nascent ribosomes at a particular stage of development (15).

Biochemical purification of a subnucleolar structure and development of visual screens helped to identify factors that couple 60 S ribosome maturation to the nuclear export of the precursor particles (16–19). Recently, we have identified three novel nucleolar proteins that can be isolated in two heteromeric complexes; Noc1p-Noc2p is associated with 90 S and 66 S pre-ribosomes in the nucleolus, whereas Noc2p-Noc3p assembles with 66 S particles throughout the whole nucleus (20). The dynamic interaction of the Noc proteins appeared to be crucial for maturation and intranuclear movement of pre-
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ribosomes leading to the mature 60 S subunit. A common feature of Noc1p and Noc3p is a conserved stretch of 45 amino acids, which is also present in a third yeast protein, which we termed Noc4p (20). Here, we analyze the properties of Noc4p and show that it is required for maturation and transport of the 40 S, but not the 60 S, subunit. Noc4p localizes to the nucleolus and forms a stable heterodimer with Nop14, which was recently described to be involved in 40 S subunit biogenesis (21). Apparently, formation of different pairs of related Noc proteins represents a common theme in ribosome biogenesis; they participate in distinct steps of either pre-40 S or pre-60 S ribosome assembly, which is directly linked to ribosomal precursor transport.

EXPERIMENTAL PROCEDURES

Yeast Strains, DNA Recombinant Work, and Microbiological Techniques—Yeast strains used in this study are given in Table I. Microbiological techniques, plasmid transformation and recovery, mating, sporification of protein A-tagged Noc proteins was performed as described previously (11).

Affinity Purification of Protein A-tagged Noc Proteins—Affinity purification of protein A-tagged Noc proteins was performed as described previously (20). Preparation of yeast cell extracts was according to Ref. 27. Briefly, 20 liters of yeast cultures were grown in YPD to an OD600 of 1–2 (2 × 10^11 cells), harvested by centrifugation, washed with ice-cold distilled water, resuspended in ice-cold lysis buffer (0.5 ml/g of cell paste) (0.15 M Hepes, pH 7.8, 60% glycerol, 0.5 M (NH4)2SO4, 1 mM diithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine), and frozen in liquid nitrogen. Subsequent manipulations were done at 4 °C. After thawing the cells, the equal volume dilution buffer (0.1 M Hepes, pH 7.8, 20 mM MgCl2, 200 mM (NH4)2SO4, 1 mM dithiothreitol), was added, and cells were broken with glass beads as described (28), using four cycles of bead beating for 20 s. Glass beads and cellular debris was removed by centrifugation at 14000 × g for 20 min, and the supernatant was clarified by centrifugation at 100,000 × g for 90 min.

Ribosomes Purification by Sucrose Density Gradient Centrifugation Analysis—Analysis of polysomes by sucrose density gradient centrifugation was performed as described (20). To disrupt the ribosomal subunits, cells were not incubated with cycloheximide before breakage, and cell breakage and sucrose gradient analysis were performed in 20 mM Hepes, pH 7.8, 20 mM MgCl2, 200 mM (NH4)2SO4, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine) was added, and

| NAME | Genotype | Origin |
| --- | --- | --- |
| BY4743 | MATa/α, his3Δ1, leu2Δ, ura3Δ, lys2Δ0/LYS2, MEL15/mets5Δ0, ypr144c::kanMX4/YPR144c | Euroscarf |
| Noc4 shuffle | MATa, his3Δ1, leu2Δ0, ura3Δ0, ypr144c::kanMX4 + YCplac33-NOC4 (ARS/ CEN URA3 NOC4) | Offspring of BY4743 |
| ProtA-NOC4 | MATa, his3Δ1, leu2Δ0, ura3Δ0, ypr144c::kanMX4 + pNOPPAIL-NOC4 (ARS/CEN LEU2 ProtA-NOC4) | Isogenic to Noc4 shuffle |
| GFP-NOC4 | MATa, his3Δ1, leu2Δ0, ura3Δ0, ypr144c::kanMX4 + pNOPPAIL-NOC4 (ARS/CEN LEU2 GFP-NOC4) | Isogenic to Noc4 shuffle |
| NOCA-GFP | MATa, ura3, trpl, his3, leu2, NOC4-GFP: HIS3 | Offspring of FY23 × FY6 |
| NOP14-ProtA | MATa, ura3, trpl, his3, leu2, NOP14-ProtA: TRP1 | Offspring of FY23 × FY6 |
| NOP14-GFP | MATa, ura3, trpl, his3, leu2, NOP14-GFP: HIS3 | Isogenic to NOC4 shuffle |
| noc4–1 | MATa, his3Δ1, leu2Δ0, ura3Δ0, ypr144c::kanMX4 + pNOPPAIL-noc4–1 (ARS/CEN LEU2 ProtA-noc4–1) | (20) |
| noc4–2 | MATa, his3Δ1, leu2Δ0, ura3Δ0, ypr144c::kanMX4 + pNOPPAIL-noc4–2 (ARS/CEN LEU2 ProtA-noc4–2) | (20) |
| noc1–1 | MATa, ade2, can1, leu2, ura3, his3, trpl, noc1Δ::HIS3 + pNOPPAIL-noc1–1 (ARS/CEN LEU2 ProtA-noc1–1) | (20) |
| noc2–1 | MATa, ura3, his3, leu2, noc2–1 | (20) |
| noc3–1 | MATa, ade2, can1, leu2, ura3, his3, trpl, noc3Δ::HIS3 + pNOPPAIL-noc3–1 (ARS/CEN LEU2 ProtA-noc3–1) | (20) |
| ProtA-NOC1 | MATa, ade2, can1, ura3, leu2, his3, trpl, noc1Δ::HIS3 + pNOPPAIL-NOC1 (ARS/CEN LEU2 ProtA-NOC1) | (20) |
| noc4–1 NOP14-GFP | MATa, his3Δ1, leu2Δ0, ura3Δ0, NOP14-GFP: HIS3, ypr144c::kanMX4 + pNOPPAIL-noc4–2 (ARS/CEN LEU2 ProtA-noc4–2) | Offspring of noc4–1 α × NOP14-GFP |

1 The abbreviations used are: GFP, green fluorescent protein; WT, wild-type; ts, thermosensitive; ProtA, protein A.

References

1. The abbreviations used are: GFP, green fluorescent protein; WT, wild-type; ts, thermosensitive; ProtA, protein A.
Noc4p Is a Nucleolar Protein with a "Noc Domain"—The short and conserved Noc domain (~45 amino acids in length) is found in Noc1p and Noc3p, two nuclear proteins with a role in transport and maturation of ribosomal 60 S subunits. Interestingly, an uncharacterized yeast protein named Noc4p also exhibits such a Noc domain (20). To find out whether Noc4p is involved in ribosome biogenesis, we sought to characterize Noc4p, which is an essential protein of 63 kDa. Together with the other Noc proteins, Noc4p is enriched in the previously isolated large nucleolar subcomplex (data not shown) that contains many factors involved in rRNA transcription and ribosome biogenesis (16). Consistent with this biochemical data, chromosomally encoded Noc4p-GFP is located in the nucleolus (Fig. 1A). Because Noc4p not only has the short Noc domain (residue 447–468 in Noc4p) but shows an extended homology throughout a large part of the Noc1 sequence (Fig. 1B), Noc4p and Noc1p could perform a related function. Moreover, Noc4p orthologs exist in other organisms including Schizosaccharomyces pombe and human (Fig. 1B). Taken together these data suggest that Noc4p is a further member of the Noc protein family with a role in ribosome biogenesis. Noc4p Stably Associates with Nop14p—Previous work showed that Noc1p and Noc2p form a stable heterodimeric complex, which is associated with 60 S pre-ribosomes and required for ribosome maturation and nuclear export. When functional ProtA-tagged Noc4p was affinity-purified under the same stringent conditions that yielded the Noc1p-Noc2p heterodimer, another protein of ~98 kDa was co-enriched (Fig. 2, lane 3). Mass spectrometry analysis identified this protein as Nop14p, which was shown previously to play a role in 40 S subunit biogenesis (21). Subsequently, we generated a chromosomally integrated Nop14p-ProA and affinity-purified it under the same stringent conditions. This revealed that the major band co-purifying with Nop14p-ProA is Noc4p (Fig. 2, lane 4). Other bands found in the Nop14p-ProA eluate were Noc4p or Nop14p breakdown products and heat shock proteins (possible contaminants). Notably, antibodies raised against the N-terminal domain of Noc2p detect Nop14p on Western blots, suggesting a structural relationship between both proteins, despite the fact that Nop14p and Noc2p do not exhibit an apparent sequence homology (data not shown). Taken together, our data show that Noc4p and Nop14p form a heterodimeric complex, reminiscent of the previously characterized Noc1p-Noc2p and Noc3-Noc3p complexes. Noc4p Is Required for 40 S Subunit Biogenesis—Previous work showed that Nop14p plays a role in 40 S subunit biogenesis (21), yet Noc1p, which is related to Noc4p (see Fig. 1B), is involved in 60 S subunit biogenesis (20, 35). Therefore, we sought to generate temperature-sensitive noc4 mutants to test them for defects in rRNA processing, ribosome formation, and nuclear export. Two noc4 ts mutants, noc4–1 and noc4–2, which grow well at 24 °C, but not at 37 °C, were obtained (Fig. 3A). The mutated noc4–1 protein has amino acid exchanges at position 283 (Ser → Pro), 344 (Ile → Val), 463 (Leu → Gln), and 550 (Val → Ala). Strikingly, the amount of free 40 S subunits significantly decreased in the noc4–1 mutant upon shift to the restrictive temperature, whereas the amount of 60 S subunits increased. In contrast, the noc1–1 mutant shows opposite effects with a loss of 60 S and an increase of 40 S subunits (Fig. 3, B and C). Similar results were obtained when a noc4 ts mutant was used that exhibits a mutation in the Noc domain (data not shown). Noc4p Is Required for Processing of 18 S rRNA—To test whether and at which specific steps Noc4p is involved in the processing pathway leading to 18 S rRNA, the rRNA component of the 40 S subunit, we performed Northern analysis (Fig. 4, B and C) and pulse-chase experiments (Fig. 4D). After a 4-h shift to restrictive temperature (37 °C), the total amount of mature 18 S rRNA was significantly reduced in the noc4–1 mutant, whereas the 25 S rRNA level remained almost unaffected (Fig. 4C). Fig. 4B also depicts that noc4 ts mutants are defective in the pathway leading to mature 18 S rRNA but not to 25 S and 5.8 S rRNA; the 20 S pre-rRNA (the immediate precursor to mature 18 S rRNA) was significantly decreased in the noc4–1 mutant, whereas the 25 S pre-rRNA was more pronounced after shift to the restrictive temperature; in contrast, after an initial reduction briefly after the temperature shift, the amounts of 27 S and 7 S pre-rRNA (precursors of the mature 25 S and 5.8 S rRNA, respectively) raised again to...
wild-type levels (Fig. 4B). To compare the precise processing steps that are affected in noc4 and in noc1 mutants, we used different probes complementary to certain regions of the rRNA transcript. The reduction in the 20 S, 32 S, and 27 S A2 rRNA species in the noc4 mutants clearly indicates blocks at the early cleavage steps at A0, A1, and A2, respectively. Furthermore, an intermediate could be detected that corresponds in size to the 23 S product, a presumably aberrant species when processing at the early sites A0-A2 are blocked. Interestingly, this 23 S species was only slightly accumulated when compared with other yeast strains defective in 18 S processing. This could be because of a reduced stability of this intermediate in noc4 ts.
mutants. Appearance of the 27SA/27SB rRNA intermediate underlines that later cleavage steps still occur and that the processing of 25S and 5.8S rRNA is not affected in the noc4–1 mutant. By contrast, the noc1–1 mutant showed cleavage at sites A1 and A2 (the 20S species is still detectable) but is impaired in 25S processing, because all 27S intermediates are clearly reduced.

Pulse-chase experiments confirmed the results obtained by Northern analysis. After a 3.5-h shift to non-permissive temperature, cells were labeled with 3H-uracil and chased for certain time periods with an excess of cold uracil. As expected, comparison of the time course of rRNA processing between wild-type and noc4–1 mutant cells revealed a delayed cleavage of the 35S rRNA and a strong reduction of the 18S rRNA in the mutant strain, whereas processing to the mature 25S rRNA still occurs although it is delayed. Thus, our data demonstrate that Noc4p and Noc1p, although structurally related, participate in two different ribosome biogenesis pathways that lead to 40S and 60S subunits, respectively.

The Noc4p-Nop14p Heterodimer Associates with 40S Pre-ribosomal Particles—As Noc4p and Nop14p participate in 40S
subunit biogenesis, we wanted to know whether they associate with pre-ribosomal particles to the 40 S subunit. Therefore, we performed sucrose gradient centrifugation of yeast lysates containing ribosomal and pre-ribosomal particles and looked for co-fractionation with Noc4p-ProtA and Nop14p-ProtA. This revealed that Noc3p co-sediments with 66 S pre-ribosomes, and Noc1p co-sediments with 66 S and 90 S pre-ribosomes (see also Ref. 20). Apparently, Noc4p does not co-peak with 66 S particles but is detected in fractions of higher density, which could correspond to 90 S particles (Fig. 5, upper panel). Moreover, a small fraction of ProtA-tagged Noc4p is present in the part of the gradient that contains 43 S pre-ribosomes and 40 S subunits, which becomes evident upon a longer exposure of the Western blot (data not shown). A similar sedimentation behavior on sucrose gradients was also observed for Nop14p-ProtA, although this fusion protein tends to be partly degraded during overnight centrifugation in fractions with rather high protein concentrations (data not shown). We conclude that Noc4p and Nop14p are associated with precursor particles to the 40 S subunit.

Nucleolar Location of Nop14p Depends on Intact Noc4p

Previous work showed that the Noc proteins Noc1–3 exhibit a dynamic intranuclear distribution (20). To find out whether the Noc4-Nop14p complex also has the capability to migrate be-

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**Fig. 4.** noc4 ts mutants are impaired in processing of 18 S rRNA. **A**, scheme of rRNA processing. The probes for Northern analysis are indicated in the upper two levels. **B**, Northern analysis of pre-rRNA processing. Left panel, noc4–1 was analyzed following growth at 24 °C (0 and 4-h samples) and 1, 2, and 4 h after transfer to 37 °C. Pre-rRNA species detected are indicated. The probes for hybridization were used simultaneously (oligos 5′A2, 7, and actin). Right panel, quantification of the noc4–1 pre-rRNAs after shift to 37 °C. The signals were normalized using actin mRNA as an internal standard. **C**, comparison of pre-rRNA cleavages in WT cells and noc1–1 and noc4–1 mutants. rRNA species were analyzed at time points 0 and 4 h after shift to 37 °C for WT strains and noc1–1 mutants and at 1, 2, and 4 h for noc4–1, respectively. rRNA species used and probes used are indicated. D, pulse-chase labeling of rRNA (intermediates) with 3H-uracil for 3 min. The cells were chased for the time points indicated with an excess of unlabeled uracil. Equal amounts of extracted RNA were loaded on each lane of a denaturing 1.2% agarose gel. Positions of mature and intermediate forms of rRNA are indicated.

**Fig. 5.** Sedimentation behavior of Noc4p and Nop14p proteins and (pre-)ribosomal particles on sucrose gradients. Analysis of polysomal fractions derived from strain ProtA-NOC4 grown at 30 °C was performed as described under “Experimental Procedures.” Aliquots of the fractions were analyzed by Western blot analysis with polyclonal antibodies directed against ribosomal protein S8 or affinity-purified antibodies directed against either Noc1p or Noc3p. The fractions containing 40 S, 60 S, and 90 S ribosomal subunits are marked.
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Fig. 6. In vivo localization of Nop14p-GFP in wild-type cells and in the noc4–1 ts mutant after shift to the non-permissive temperature. Shown are fluorescence microscopy photographs of Nop14p-GFP in wild-type (NOC4+) and noc4–1 ts cells shifted for 4 h to 37 °C. Cells were also stained for DNA, and the GFP- and 4',6-diamidino-2-phenylindole-stained pictures were merged.

Between the nucleolus and nucleoplasm, we sought to analyze the intranuclear location of Nop14p-GFP in noc4 ts mutants. In wild-type cells, Nop14p-GFP like Noc4p-GFP is mainly located in the nucleolus (Fig. 6). However, Nop14p-GFP is significantly found in the nucleoplasm in the noc4–1 mutant upon shift to the restrictive temperature (Fig. 6; see merge between the Noc4-eGFP and DNA staining). This suggests that an intact Noc4p is required for steady-state nuclear location of Nop14p.

A Fluorescence-based In Vivo Assay Reveals That noc4 ts Mutants Are Defective in 40 S Subunit Export—Previous studies revealed that the Noc1-Noc2 and Noc2-Noc3 complexes are involved in intranuclear transport and nuclear export of 60 S pre-ribosomal subunits (20). We sought to analyze whether the Noc4p-Nop14p complex plays a role in the export of 40 S subunits from the nucleus into the cytoplasm. To this end, we developed a visual in vivo assay for 40 S small subunit export exploiting a GFP-tagged version of a ribosomal protein of the 40 S subunit. This assay, together with the previously established 60 S subunit export assay (18), allows us to study under comparable conditions which factors are involved in ribosomal export and which affect large and small subunit export differently. Furthermore, it can be determined whether and which (mal)function in 40 S biogenesis is coupled to 40 S transport. Recently, another test system for 40 S small subunit export was reported; however, this assay was based on in situ hybridization of rRNA (19).

The essential ribosomal protein Rps2p served as a suitable reporter for the in vivo small subunit assay. Importantly, GFP-tagged Rps2p (Rps2p-eGFP) efficiently complements the non-viable rps2 null mutant (Fig. 7A). Furthermore, Rps2p-eGFP is exclusively located in the cytoplasm, with nuclear and vacuolar exclusion, as revealed by fluorescence microscopy (Fig. 7B). Moreover, Rps2p-eGFP effectively assembles into 40 S subunits and is also found in 80 S ribosome and polysome fractions (Fig. 7C). Finally, it was tested whether Rps2p-eGFP accumulates in the nucleus of a bona fide export mutant. Recently, it was shown that Xpo1p, which is the export receptor for nuclear export signal-containing export cargoes (36), is involved in 60 S (17, 37) and 40 S subunit export (19). As expected, the xpo1–1 ts mutant exhibits a strong nuclear accumulation of the Rps2p-eGFP reporter after shift to the restrictive temperature (Fig. 7D).

We next tested whether nuclear export of 40 S and 60 S subunits is impaired in the noc4 ts mutant, using the fluorescence-based in vivo export assays for 40 S (Rps2-eGFP) and 60 S subunits (Rpl25-eGFP) (Fig. 8). Whereas Rps2p-eGFP significantly accumulates in the nucleus of two different noc4 ts mutants upon shift to the non-permissive temperature, nuclear export of the large subunit reporter Rpl25p-eGFP is not impaired (Fig. 8A). To test whether Noc1p, Noc2p, and Noc3p are required for 40 S subunit export, the ts mutants noc1–1, noc2–1, and noc3–1 were transformed with the Rps2p-eGFP reporter. However, no nuclear accumulation of the small subunit reporter was seen in these noc mutants after shift to the non-permissive temperature (Fig. 8B). Previous work showed nuclear accumulation of Rpl25p-eGFP in noc1, noc2, and noc3 ts mutants (20). To find out whether the Rps2-GFP reporter remains associated with pre-ribosomal particles at the restrictive condition, we performed sucrose gradient centrifugation of whole cell lysates derived from the noc4–1 (Fig. 8C) and noc4–2 ts mutants (20). To test whether Rps2p-eGFP reporter remains associated with 40 S pre-ribosomes upon shift to the non-permissive temperature. Moreover, the amount of 40 S subunits is decreased whereas that of 60 S subunits is increased in both noc4 ts mutants upon shift to the restrictive temperature (see also Fig. 3). All ts mutants yet analyzed that are impaired in 40 S biogenesis also exhibited a defect in the small subunit export assay (data not shown). Apparently, 40 S subunit maturation is closely linked to its transport, and it is not yet distinguishable whether accumulation of the Rps2-GFP reporter construct in the nucleus is because of the blockage of 40 S biogenesis, the missing relief of a retention signal, or the direct inhibition of transport. We conclude that maturation and nuclear export of 40 S pre-ribosomes requires the Noc4-Nop14p complex, whereas 60 S subunit maturation and export depends on the Noc1p/2p and Noc2p/3p complexes.

Discussion

To date little is known about how 40 S subunits assemble in the nucleolus and are exported in the cytoplasm. We could demonstrate that Noc4p is part of the stable Noc4p-Nop14p heterodimer and is specifically involved in maturation of the 40 S subunit, which is closely linked to pre-40 S subunit transport.
from the nucleolus to the cytoplasm. In contrast, the related Noc1p-Noc2p and Noc2p-Noc3p complexes have specific roles in biogenesis and transport of the 60 S subunits. In particular, we have developed a fluorescence-based \textit{in vivo} assay, which allows us to monitor specifically 40 S nuclear export and to directly compare it to 60 S subunit export. Both \textit{in vivo} export assays use functional GFP-tagged ribosomal proteins of the small (Rps2p-eGFP) and large subunit (Rpl25p-eGFP). In agreement with its specific role in 40 S subunit export, Noc4p is involved in rRNA processing of 18 S, but not of 25 S and 5.8 S, rRNA. Thus, the Noc4p-Nop14p complex adds to a growing list of related complexes that play a role in maturation-coupled transport processes of different pre-ribosomal particles.

A homology observed between Noc1p and a novel and uncharacterized protein termed Noc4p was the basis to identify the novel Noc4p-Nop14p heterodimer. Thus, several Noc-complexes (Noc1p-Noc2p, Noc2p-Noc3p, Noc4p-Nop14p) have now been characterized. The capability to form separate Noc heterodimers points to an interesting principle in ribosome biogenesis; Noc complexes can either participate in subsequent steps during biogenesis of a distinct precursor species (e.g. 60 S pre-ribosomes) or function in the biogenesis of different pre-ribosomal particles (i.e. 60 S and 40 S subunits). Thus, Noc complexes may perform both common and different functions, thereby coupling intranuclear assembly and movement of pre-ribosomal particles with export into the cytoplasm.

Possible functions of Noc complexes are to accompany their cognate pre-ribosomal particle during maturation, actively mediate maturation, actively promote transport from the nucleolus to the nuclear pore, or overcome intranucleolar/intranuclear retention sites. A common structural element within several Noc proteins is the Noc domain, which could trigger some of these events. Notably, a change in Noc complex composition, which correlates with association to different particles and with different nuclear locations, was observed for the Noc2p-containing complexes. When Noc2p is associated with Noc1p, it is predominantly nucleolar and interacts both with 66 S and 90 S pre-ribosomes; however, when Noc3p is bound to Noc2p, the complex is also found in the nucleoplasm and exclusively associated with 66 S pre-ribosomes (20). In analogy, the Noc4p-Nop14p heterodimer could function in distinct steps during 40 S subunit biogenesis/transport like the Noc1p-Noc2p complex that performs its role in 60 S subunit formation.

Whether a second stable Nop14p-containing complex exists that is analogous to the Noc2p-Noc3p complex and functions in a later step during 40 S subunit biogenesis remains unknown.

The association of the Noc4p-Nop14p complex with 90 S particles might suggest that it is involved in an early step during 40 S subunit biogenesis. This conclusion is also supported by the observation that early pre-rRNA processing at sites $A_0$, $A_1$, and $A_2$ is inhibited in both noc4 and nop14 ts mutants (see also Ref. 21). Moreover, rRNA processing and
FIG. 8. Temperature-sensitive noc4 mutants are defective in 40 S subunit, but not 60 S subunit, export. A, analysis of 40 S and 60 S subunit export in noc4-1 and noc4-2 mutants expressing the Rps2p-GFP and Rpl25p-GFP reporter constructs. Cells were shifted for 5 h to 37 °C before pictures were taken in the fluorescence microscope. B, Rps2p-GFP does not accumulate in the nucleus in thermosensitive noc1, noc2, and noc3 mutants. ts strains noc1-1, noc2-1, and noc3-1, transformed with the Rpl25p-GFP large subunit reporter, were shifted for 5 h to 37 °C before they were viewed in the fluorescence microscope and under Nomarski optics. C, Rps2p-GFP remains associated with pre-ribosomal particles in noc4 ts mutants upon shift to the restrictive temperature. Sucrose gradient centrifugation of whole cell lysates derived from the noc4-1 mutants grown at the permissive temperature or shifted for 4 h to the restrictive temperature. A260 nm profiles of sucrose gradients (40 S, 60 S, and 80 S ribosomes and polysomes) are indicated. The fractions from the sucrose gradient were analyzed by SDS-PAGE and Western blotting using an anti-GFP-antibody to detect Rps2p-GFP.
transport events leading to mature 60 S subunits are not significantly inhibited in noc4 ts mutants. This suggests that the cleavages leading to the release of pre-rRNA to 25 S rRNA are not dependent on the Noc4-Nop14p complex. It appears that the activities of the different Noc protein are clearly separated between Noc4p and Nop14p (14). Moreover, these 90 S pre-ribosomes include proteins associated with U3 small nucleolar RNA (14). Among these core components are also Noc4p and Nop14p (14).

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