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# Conserved Composition of Mammalian Box H/ACA and Box C/D Small Nucleolar Ribonucleoprotein Particles and Their Interaction with the Common Factor Nopp140

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Small nucleolar ribonucleoprotein particles (snoRNPs) mainly catalyze the modification of rRNA. The two major classes of snoRNPs, box H/ACA and box C/D, function in the pseudouridylation and 2′-*O*-methylation, respectively, of specific nucleotides. The emerging view based on studies in yeast is that each class of snoRNPs is composed of a unique set of proteins. Here we present a characterization of mammalian snoRNPs. We show that the previously characterized NAP57 is specific for box H/ACA snoRNPs, whereas the newly identified NAP65, the rat homologue of yeast Nop5/58p, is a component of the box C/D class. Using coimmunoprecipitation experiments, we show that the nucleolar and coiled-body protein Nopp140 interacts with both classes of snoRNPs. This interaction is corroborated in vivo by the exclusive depletion of snoRNP proteins from nucleoli in cells transfected with a dominant negative Nopp140 construct. Interestingly, RNA polymerase I transcription is arrested in nucleoli depleted of snoRNPs, raising the possibility of a feedback mechanism between rRNA modification and transcription. Moreover, the Nopp140-snoRNP interaction appears to be conserved in yeast, because depletion of Srp40p, the yeast Nopp140 homologue, in a conditional lethal strain induces the loss of box H/ACA small nucleolar RNAs. We propose that Nopp140 functions as a chaperone of snoRNPs in yeast and vertebrate cells.

## INTRODUCTION

rRNA is heavily modified on specific nucleotides by 2′-*O*-methylation and pseudouridylation. These modifications occur cotranscriptionally in the nucleolus and number ~100 in vertebrates and 50 in yeast (Maden, 1990). The site specificity of these modifications is determined by a similar number of small nucleolar RNAs (snoRNAs). Two major classes of snoRNAs can be discerned based on short conserved sequence elements, box H/ACA and box C/D. The former guide the pseudouridylation of rRNA, whereas the latter determine the sites of 2′-*O*-methylation. The site specificity is achieved by base pairing of short stretches of the snoRNAs to complementary sequences of the rRNA flanking the nucleotides to be modified (for review, see Maxwell and Fournier, 1995; Smith and Steitz, 1997; Tollervey and Kiss, 1997). Although the function of these modifications is unknown, they cluster around the peptidyl transferase center of the ribosome, implying a role during peptide synthesis (Bakin *et al.*, 1994).

Although the snoRNA-mediated mechanism of site selection has been worked out elegantly, little is known about the proteins associated with the snoRNAs or the enzymes catalyzing the modifications. However, recent studies in yeast indicate that each of the major small nucleolar ribonucleoprotein particles (snoRNPs) is endowed with its distinct set of proteins, box H/ACA snoRNPs with Cbf5p, Gar1p, Nhp2p, and Nop10p and box C/D snoRNPs with Nop1p, Nop5/58p, and Sik1/Nop56p (see Figure 6) (Lübben *et al.*, 1995; Balakin *et al.*, 1996; Ganot *et al.*, 1997; Gautier *et al.*, 1997; Henras *et al.*, 1998; Lafontaine *et al.*, 1998; Watkins *et al.*, 1998a; Lafontaine and Tollervey, 1999). The best-characterized protein is Cbf5p, a candidate pseudouridylase of rRNA

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Abbreviations used: EST, expressed sequence tag; GAD, Gal4 activation domain; IgG, immunoglobulin G; RNA pol I, RNA polymerase I; RT, reverse transcription; snoRNA, small nucleolar RNA; snoRNP, small nucleolar ribonucleoprotein particle.

(Jiang *et al.*, 1993; Lafontaine *et al.*, 1998). Its mammalian homologue is rat NAP57 (Meier and Blobel, 1994), which was identified in humans as the gene mutated in the X-linked bone marrow failure disorder dyskeratosis congenita (Heiss *et al.*, 1998). Compared with yeast snoRNPs, even less is known about the composition of mammalian snoRNPs. In fact, aside from fibrillarin, the mammalian Nop1p orthologue, and a few bands on a gel, the composition of mammalian snoRNPs remains uncharacterized to date (Parker and Steitz, 1987; Tyc and Steitz, 1989; Caffarelli *et al.*, 1998; Watkins *et al.*, 1998b).

We previously identified rat Nopp140, a highly phosphorylated nucleolar and coiled-body protein that shuttles between the nucleolus and the cytoplasm on intranuclear tracks (Meier and Blobel, 1990, 1992, 1994). Using Nopp140 antibodies, we subsequently identified the Nopp140-associated protein NAP57, the putative pseudouridylase of rRNA (Meier and Blobel, 1994; see also Koonin, 1996). Because mammalian rRNA pseudouridylase activity is dependent on snoRNAs, Nopp140 may in fact be associated with entire snoRNPs. This idea was supported by subsequent studies that suggested a role for Nopp140 in snoRNP transport between the nucleolus and the coiled bodies (Isaac *et al.*, 1998).

To further investigate the role of Nopp140 in snoRNP function, we reexamined here in more detail the macromolecules that coprecipitate with Nopp140. We find that Nopp140 interacts with two discrete complexes corresponding to the mammalian box H/ACA and box C/D snoRNPs. Thus, GAR1 and box H/ACA snoRNAs specifically coprecipitate with NAP57. In addition to these box H/ACA snoRNP components, Nopp140 precipitates the newly identified NAP65 (the rat homologue of yeast Nop5/58), fibrillarin, and box C/D snoRNAs, all parts of box C/D snoRNPs. An in vivo interaction of Nopp140 with both major mammalian snoRNPs and its role in their intranuclear transport is indicated by the specific and exclusive depletion of snoRNP proteins from nucleoli of cells transfected with a dominant negative Nopp140 construct. The Nopp140-snoRNP interaction appears to be conserved in yeast, because genetic depletion of Srp40p, the yeast Nopp140 homologue (Meier, 1996), in a conditional lethal strain specifically reduces the level of box H/ACA snoRNAs. Based on these observations, we propose that Nopp140 functions as a chaperone of snoRNPs in yeast and vertebrate cells.

## MATERIALS AND METHODS

### *Immunoprecipitations*

Precipitations were performed from rat liver nuclei (Meier and Blobel, 1994) and from whole cell extracts of buffalo rat liver cells (Li *et al.*, 1997) for protein and RNA analysis, respectively. The antibodies used for the experiments were affinity-purified peptide antibodies directed against Nopp140 (Meier and Blobel, 1992) and NAP57 (Meier and Blobel, 1994). For the more stringent incubation conditions (see Figure 1A), the 25 mM Tris, pH 8.1, extracts were adjusted to 150 mM sodium chloride, 1% Triton X-100, and 0.2% SDS; under less stringent conditions (see Figure 2, A and B), the addition of sodium chloride and detergent was omitted. However, both immunoprecipitates were repeatedly washed with the same stringency, i.e., 150 mM sodium chloride, 0.1% Triton X-100, and 0.02% SDS. The precipitates were analyzed by SDS-PAGE and silver staining (Merril *et al.*, 1984) or transfer to nitrocellulose and subsequent amido black staining and immunodetection with ECL (Amersham Life Science, Arlington Heights, IL). The primary antibodies (and concentrations or dilutions) used for consecutive immunodetection were as follows: GAR1, Rab2B rabbit serum raised against human GAR1 (1:1000; Dragon, Pogačić, and Filipowicz, unpublished data); fibrillarin, D77 mouse monoclonal immunoglobulin G (IgG) (0.2 $\mu$g/ml; Aris and Blobel, 1988); CK2, polyclonal rabbit serum against the $\alpha$ subunit of casein kinase 2 (1:5000; Litchfield *et al.*, 1994); NAP57, RL12 affinity-purified rabbit IgG (0.4 $\mu$g/ml; Meier and Blobel, 1994); and Nopp140, RF11 affinity-purified rabbit IgG (0.1 $\mu$g/ml; Meier and Blobel, 1992).

### *Protein Analysis*

Amino-terminal peptide sequences of NAP65 and fibrillarin were obtained exactly as described for NAP57 (Meier and Blobel, 1994). The full-length cDNA of NAP65 was derived from the overlapping expressed sequence tags (ESTs) 108064, 111317, 105505, and 105838 and was constructed from EST105838 and EST108064, which were obtained from the American Type Culture Collection (Manassas, VA) on plasmids designated pTM131 and pTM135, respectively. The two ESTs were ligated with the use of PCR-based splicing by the overlap extension method (Vallejo *et al.*, 1995) on an 870-nucleotide *Sph*I/*Xho*I fragment, which was subsequently cloned into those sites of pTM131 to generate pTM136 (full-length NAP65 in pBluescript SK$^-$ [Stratagene, La Jolla, CA]). DNA sequencing was used to verify all sequences and discovered a base change in the EST105838 sequence at nucleotide 1356 of the full-length cDNA from A to G, resulting in the amino acid switch of lysine 396 to arginine. Indeed, arginine 396 is conserved in all metazoan homologues analyzed (see Figure 1C). The sequence for full-length NAP65 was deposited in GenBank (accession number AF194371). NAP65 was transcribed/translated from pTM136 as described (Isaac *et al.*, 1998). The NAP57 plasmid (pTM575) was generated previously (Meier and Blobel, 1994). Multiple protein sequence alignments were produced with the use of the CLUSTAL alignment method and were presented with the use of the BOXSHADE program through the Baylor College of Medicine Search Launcher (Smith *et al.*, 1996).

### *RNA Analysis*

Total RNAs were extracted from whole cell extracts and immunoprecipitates by phenol/chloroform and digested with RNase-free DNase I (Sigma Chemical, St. Louis, MO) to remove any contaminating traces of genomic DNA. Reverse transcription (RT)-PCR was performed with the use of the DNase I–treated RNA as template in the SuperScript One-Step RT-PCR system (BRL-Life Technologies, Grand Island, NY), as described by the manufacturer. The amplified DNAs were analyzed by 4% agarose (NuSieve, FMC BioProducts, Rockland, ME) gel electrophoresis, and ethidium bromide staining. Control amplifications with the use of *Taq* polymerase (Perkin Elmer-Cetus, Norwalk, CT) alone confirmed the absence of any genomic snoRNA sequences. The following snoRNA-specific primer pairs were used in the RT-PCR experiments: 5′-ACTCTCCCGGGCTCTGT-3′ and 5′-TAGGAATATGCAGGCGCAGA-3′ for U17/E1; 5′-GAGAATTCTAAGCAGGATTTTACTACAATAT-3′ and 5′-CTCAGTGAGCTCATGTATGAGACCAAGCGT-3′ for E3; 5′-NNNNNNGAATTCCAAAACCATTCGTAG-3′ and 5′-NNNNNNGAGCTCATCCAAGGAAGGAACTAGCCAAC-3′ for U14; and 5′-CCAGAGCCTGAAAAGGTGAA-3′ and 5′-CTCAGACAGTTCCTTCTGGA-3′ for U22.

### *Yeast Strains and Plasmids*

Yeast cell growth (Ausubel *et al.*, 1993; Meier, 1996; Lafontaine *et al.*, 1998) and DNA manipulation (Maniatis *et al.*, 1989; Meier, 1996) were performed according to standard procedures and as described previously. The plasmids used for the complementation studies of

yeast Cbf5p by rat NAP57 were based on pACT2 (Clontech Laboratories, Palo Alto, CA), a yeast vector expressing the protein of interest as a carboxyl-terminal fusion of the Gal4 activation domain (GAD) under the *ADH* promoter. pTM113 contained full-length NAP57 amplified and cloned into the *Nco*I and *Eco*RI sites of pACT2. The haploid yeast strains YDL401 (*CBF5*) and YDL521-1 (GAL::cbf5; Lafontaine *et al.*, 1998) and the diploid strains YCC130 (*CBF5/cbf5::TRP1*) and YWJ64-ts (cbf5-1/cbf5-1; Cadwell *et al.*, 1997) were obtained from the indicated sources. From these strains, we generated the following transformants carrying the indicated plasmids (in parentheses): YYY64, YWJ64-ts (pTM113); YYY66, YWJ64-ts (pACT2); YYY68, YCC130 (pTM113); YYY69, YCC130 (pACT2); YYY138, YDL521-1 (pTM113); YYY139, YDL521-1 (pACT2); and YYY143, YDL401 (pACT2).

The synthetic lethal screen that generated YYY206 (*Matα TRP1 lys2 ade2 ade3 ura3 can1 Δsrp40::HIS3 les2::LEU2*), the *srp40Δ les2* mutant carrying pGAL-SRP40 (pYY38), will be described elsewhere. pYY38 was constructed by cloning the *Sal*I fragment containing *SRP40* under the *GAL10* promoter from pTM41 (Meier, 1996) into pRS317, which carries a *LYS2* marker (Sikorski and Boeke, 1991). To allow growth of the wild-type strain (*wt*, YCH128) and the singly disrupted strains *srp40Δ* (YYY7) and *les2* (YYY216) in lysine-free medium, they were transformed with pRS317 to generate YYY231, YYY232, and YYY236, respectively. Growth in lysine-free medium was required for the maintenance of pYY38 (pGAL-SRP40) in YYY206 (*srp40Δ les2*). The genetic backgrounds of the strains were as follows: *wt*, YCH128 (*Matα TRP1 lys2 ade2 ade3 ura3 leu2 his3 can1*; a kind gift from Susan Wente and Chris Hardy [Washington University School of Medicine, St. Louis, MO]); *srp40Δ*, YYY7 (*Matα TRP1 lys2 ade2 ade3 ura3 leu2 can1 Δsrp40::HIS3*); and *les2*, YYY216 (*Matα TRP1 lys2 ade2 ade3 ura3 his3 can1 les2::LEU2*).

For the Cbf5p and Srp40p depletion experiments, we followed essentially the protocol described for Cbf5p (Lafontaine *et al.*, 1998). Total RNA was prepared (Schmitt *et al.*, 1990), and 9 μg was loaded in each lane for Northern blot analysis. The snoRNAs U3, U14, snR3, snR11, and snR33 were detected by hybridization with the following $^{32}$P-labeled oligonucleotides: 5′-GGATTGCGGACCAAGCTAA-3′, 5′-CGAATGTTAAGGAACCAG-3′, 5′-TCGATCTTCGTACT-GTCT-3′, 5′-GACGAATCGTGACTCTG-3′, and 5′-GATTGTCCAC-ACACTTCT-3′, respectively. To detect the *CBF5* mRNA, *CBF5* was amplified from genomic yeast DNA (Meier, 1996) and random prime labeled as described (Meier and Blobel, 1992). Strains YYY68 and YYY69 were used for tetrad analysis (Meier, 1996).

### *Transfection and Indirect Immunofluorescence Experiments*

COS-1 cells were transiently transfected with the HA-tagged dominant negative Nopp140 carboxyl-terminal construct HA-NoppC (pWG13) and processed for indirect double immunofluorescence exactly as described (Isaac *et al.*, 1998). The following primary antibodies were used at the dilutions given in parentheses: anti-recombinant Nopp140 serum (RH10 at 1:1000; Meier, 1996); anti-GAR1 serum (Rab2B at 1:50; Dragon, Pogačić, and Filipowicz, unpublished data); anti-RNA polymerase I serum (αPolIβ at 1:50 from Larry Rothblum [Geisinger Clinic/Weis Center for Research, Danville, PA]); and anti-HA ascites fluid (12CA5 at 1:200; Wilson *et al.*, 1984). Secondary antibodies were rhodamine-labeled goat anti-rabbit IgG and fluorescein-labeled goat anti-mouse IgG antibodies, both from Boehringer Mannheim (Indianapolis, IN).

The RNA polymerase I (RNA pol I) run-on assays were performed as described previously (Moore and Ringertz, 1973) with the modifications described by Savino *et al.* (1999). The cells were post-fixed with 2% paraformaldehyde for 15 min, and 5-bromo-uridine-triphosphate (BrUTP) (Sigma Chemical) incorporation was detected with a mouse anti-5-bromo-2′-deoxyuridine (BrdU) mAb, F(ab′)$_2$ fragments conjugated with FLUOS (Boehringer Mannheim) at a dilution of 1:5.

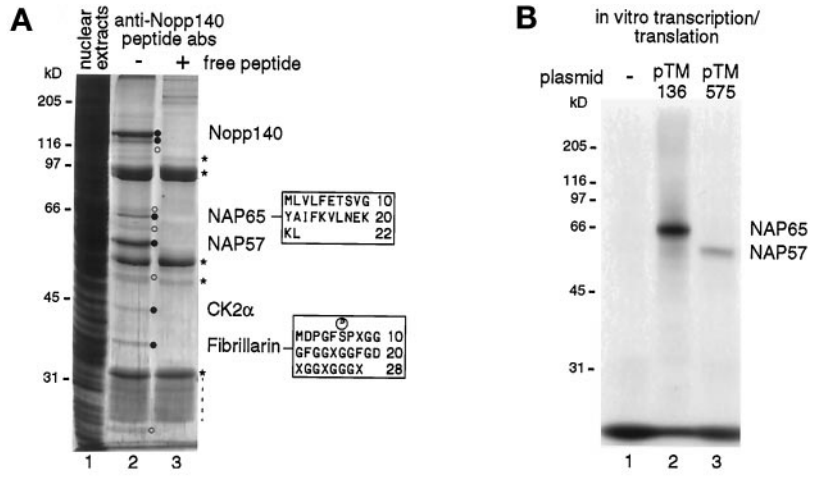
## RESULTS

### *Identification of Nopp140-associated Proteins*

We previously used coimmunoprecipitation with Nopp140 to identify NAP57, a putative component of box H/ACA snoRNPs and pseudouridylase of rRNA (Meier and Blobel, 1994; Nurse *et al.*, 1995; Lafontaine *et al.*, 1998). To test if additional proteins coprecipitated with Nopp140, we reexamined the original Nopp140 precipitates with a more sensitive method, silver staining. Thus, after precipitation of Nopp140 with anti-peptide antibodies from rat liver nuclear extracts, the associated proteins were visualized by silver staining after separation by SDS-PAGE (Figure 1A). Careful comparison of the proteins precipitated in the absence (Figure 1A, lane 2) and presence (lane 3) of free competing peptide revealed the proteins specifically associated with Nopp140. In this way, eight minor bands of 110, 66, 65, 61, 50, 42, 38, and 24 kDa were detected in the Nopp140 precipitates in addition to the stoichiometric amounts of NAP57 (Figure 1A, lane 2, dots). Here we report on the identities of three of these proteins, one novel and two known.

The 42-kDa protein band was previously shown to correspond to the α subunit of casein kinase 2 by antibody reactivity (see Figure 2B) (Li *et al.*, 1997). The 38- and 65-kDa proteins were identified by amino-terminal peptide sequencing after precipitation of increased amounts of proteins and transfer to an Immobilon membrane, as described for NAP57 (Meier and Blobel, 1994). GenBank searches showed the 28 amino-terminal residues of the 38-kDa protein to be identical to those of rat fibrillarin, an integral component of box C/D snoRNPs (Figure 1A) (Tyc and Steitz, 1989; Balakin *et al.*, 1996; Ganot *et al.*, 1997). The positions of the unidentified residues in the fibrillarin peptide sequence (Figure 1A, X) matched exactly those of the arginines modified by $N^G$,$N^G$-dimethyl groups (Lischwe *et al.*, 1985). In addition, the serine in position 6 was identified as a dehydroserine, indicating that it was phosphorylated (Figure 1A, circled P). Interestingly, fibrillarin purified from Novikoff hepatoma nucleoli was not modified at that position (Lischwe *et al.*, 1985). Therefore, only a fraction of the total cellular fibrillarin appears to be phosphorylated at that residue, perhaps only the fraction associated with Nopp140.

Comparison of the amino-terminal peptide sequence of the 65-kDa protein band with the GenBank sequences revealed it to be an uncharacterized EST (EST105839; Lee *et al.*, 1995). The full-length sequence was derived from overlapping ESTs and constructed by linking two ESTs together (EST105839 and EST108064). It encoded a protein of 534 amino acids with a calculated molecular mass of 60,043 Da and a theoretical isoelectric point of 8.7. Its high charge density (34% of all amino acids were charged) likely accounted for its slightly reduced mobility on SDS-PAGE. According to its mobility and association with Nopp140, we termed the protein NAP65. To ascertain that the cDNA constructed from the two ESTs was indeed full length, it was in vitro transcribed/translated in the presence of [$^{35}$S]methionine and [$^{35}$S]cysteine and analyzed by SDS-PAGE (Figure 1B, lane 2). Indeed, the resulting protein migrated as a single band of 65 kDa compared with NAP57 (lane 3), and no product was observed in the absence of exogenous DNA (lane 1).



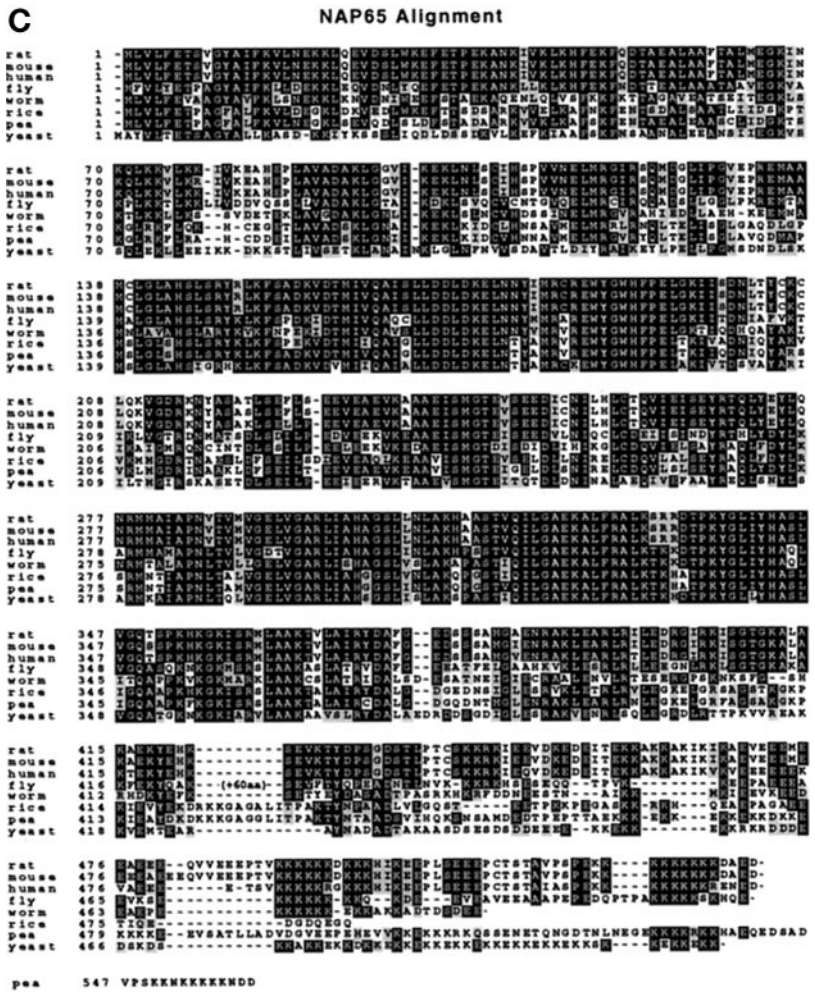




**Figure 1.** Identification of Nopp140-associated proteins, including the novel, conserved NAP65. (A) Immunoprecipitates were separated by SDS-PAGE and visualized by silver staining. Nopp140 was precipitated from rat liver nuclear extracts (lane 1) with peptide antibodies in the absence (lane 2) and presence (lane 3) of competing peptide. The identified polypeptides (closed dots) are listed on the right. The top two dots correspond to Nopp140, as confirmed by immunoreactivity. Open dots indicate unidentified protein bands that specifically coprecipitate with Nopp140. The amino-terminal protein sequences, in single-letter amino acid code, that served to identify NAP65 and fibrillarin are shown in boxes to the right. The circled P above the serine residue in the fibrillarin sequence indicates that it was identified as a dehydroserine, suggesting that this serine was phosphorylated. Xs refer to unidentified residues, all of which correspond to $N^G$,$N^G$-dimethylarginines in fibrillarin. The asterisks indicate bands that are not competed for by free peptide representing the Nopp140 IgGs. (B) In vitro transcription/translation of the assembled NAP65 cDNA (pTM136) in the presence of [$^{35}$S]methionine and [$^{35}$S]cysteine that was analyzed by SDS-PAGE and fluorography (lane 2). Control reactions contained either no DNA (lane 1) or a plasmid encoding NAP57 (pTM575; lane 3). (C) Protein sequence alignment of NAP65 to the closest homologues of other species. The following lists the species and the sequence sources (EST or GenBank accession numbers) from which the sequences were derived: rat, *Rattus* species (EST105839 and EST108064); mouse, *Mus musculus* (AA065550 and AF053232); human, *Homo sapiens* (AF123534); fly, *Drosophila melanogaster* (AC004277; 60 amino acids were omitted at position 423 and a frame shift was inserted to align this to the other sequences); worm, *Caenorhabditis elegans* (AF043704); rice, *Oryza sativa* (AB015431); pea, *Pisum sativum* (AF061962); and yeast, *Saccharomyces cerevisiae* Nop5/58p (AF056070).

GenBank searches further revealed NAP65 to be a highly conserved protein exhibiting sequence identities across its entire sequence of 98% (mouse), 95% (human), 58% (*Drosophila melanogaster*), 52% (*Caenorhabditis elegans* and *Pisum sativum*), 51% (*Oryza sativa*), and 45% (*Saccharomyces cerevisiae*). The alignment of these sequences is depicted in Figure 1C. NAP65, like NAP57, even displayed homology to ORFs of archaea, e.g., 28% identity to the entire gene MJ0694 of

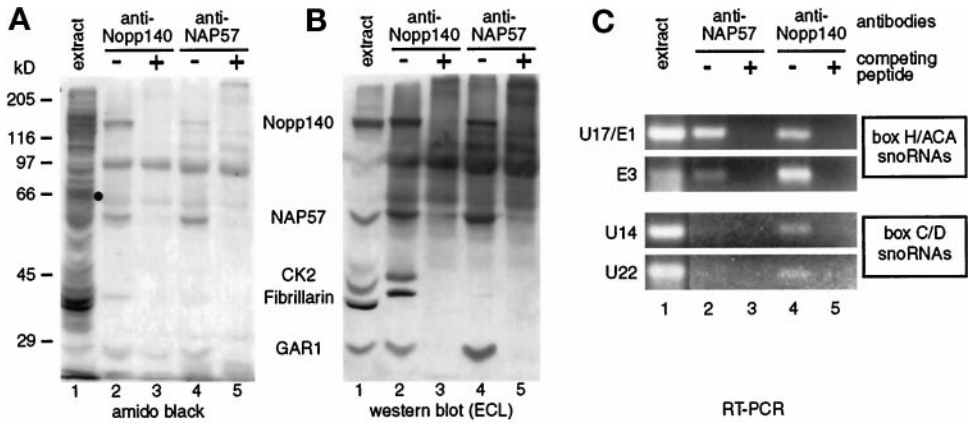



**Figure 2.** NAP57 is a specific component of box H/ACA snoRNPs, whereas Nopp140 associates with both box H/ACA and box C/D snoRNPs. (A) Immunoprecipitates were separated by SDS-PAGE, transferred to nitrocellulose, and visualized by amido black staining. Nopp140 (lanes 2 and 3) and NAP57 (lanes 4 and 5) were precipitated from rat liver nuclear extracts (lane 1) with peptide antibodies in the absence (lanes 2 and 4) and presence (lanes 3 and 5) of competing peptide. Proteins that precipitated exclusively in the absence of competing peptide are listed on the right. NAP65 is indicated by a dot because it stains very poorly by amido black. (B) The membrane in A was probed consecutively with antibodies specific for the antigens listed on the left, and the antibody reactivity was detected by ECL. All of the films showing the ECL results of the individual antibodies were overlaid and are displayed simultaneously. (C) Box H/ACA (top two panels) and box C/D (bottom two panels) snoRNAs were immunoprecipitated from whole cell extracts (lane 1), and their RT-PCR products were separated on agarose gels and detected by ethidium bromide. NAP57 (lanes 2 and 3) and Nopp140 (lanes 4 and 5) were precipitated in the absence (lanes 2 and 4) and presence (lanes 3 and 5) of competing peptide, total RNAs were extracted, and the snoRNAs were amplified by RT-PCR with primers specific for the snoRNAs indicated on the left. To improve the visibility of the precipitated box C/D snoRNA RT-PCR products, the contrast of lanes 2–5 of the bottom two panels was selectively increased.

*Methanococcus jannaschii*. In contrast to NAP57, however, no NAP65-related sequences were identified in eubacterial genomes. In addition, homologous ESTs were present in most other organisms whose genomes are being sequenced, but they did not amount to full-length sequences and therefore have been omitted from the alignment. Of all these putative NAP65 homologues, only the yeast Nop5/58p has been characterized to date (Gautier *et al.*, 1997; Wu *et al.*, 1998). Nop5/58p is an integral part of box C/D snoRNPs (Lafontaine and Tollervey, 1999). In summary, therefore, Nopp140 associates with NAP57, CK2, and two integral components of box C/D snoRNPs, fibrillarin and NAP65.

### *Association of Nopp140 with Box H/ACA and Box C/D snoRNP Components*

Because in our previous Nopp140 precipitations only minute amounts of proteins aside from NAP57 were detected (Figure 1A) (Meier and Blobel, 1994), we repeated the precipitations under slightly less stringent conditions (see MATERIALS AND METHODS). In addition, we performed the precipitations with peptide antibodies directed against NAP57 to test its association with snoRNPs. The precipitates were separated by SDS-PAGE, transferred to nitrocellulose, and stained with amido black (Figure 2A). The membrane was incubated consecutively with antibodies to Nopp140, NAP57, CK2, fibrillarin, and GAR1 (an integral component of box H/ACA snoRNPs) (Balakin *et al.*, 1996; Dragon, Pogačić, and Filipowicz, unpublished data), and immunoreactivity was detected by secondary antibodies and ECL. The combined results of all of the antibody reactions generated by overlaying the individual films are depicted in Figure 2B. Under the less stringent conditions, stoichiometric amounts of not only NAP57, the 57-kDa protein band, but also of fibrillarin and GAR1, the 38- and 27-kDa protein bands, precipitated with Nopp140 (Figure 2, A and B, compare lanes 2). Amido black, however, stained only smaller amounts of NAP65, the 65-kDa band, in the Nopp140 precipitates (Figure 2A, dot). In contrast, precipitations with peptide antibodies against NAP57 yielded only stoichiometric amounts of GAR1 compared with NAP57 and smaller quantities of Nopp140 (Figure 2, A and B, compare lanes 4). The presence of the $\alpha$ subunit of casein kinase 2 in the Nopp140 precipitates was detectable only by immunoreactivity (Figure 2B, lane 2) (Li *et al.*, 1997). The specificity of the peptide antibody precipitations was established by competition with free peptide (Figure 2, A and B, lanes 3 and 5). Together, these data indicate that Nopp140 coprecipitated protein components of both box C/D (fibrillarin and NAP65) and box H/ACA (NAP57 and GAR1) snoRNPs. NAP57, however, exclusively precipitated GAR1, a specific component of box H/ACA snoRNPs.

To test if Nopp140 and NAP57 associated only with the snoRNP proteins or also with their snoRNAs, the two proteins were precipitated from whole cell extracts and the coprecipitating RNAs were analyzed by RT-PCR with snoRNA-specific primers. The amplified cDNAs were visualized with ethidium bromide after agarose gel electrophoresis (Figure 2C). The presence of two representative box H/ACA snoRNAs, U17/E1 and E3, and two box C/D snoRNAs, U14 and U22, for which the rat or rodent sequences were available, was tested (Liu and Maxwell, 1990; Tycowski *et al.*, 1996; Selvamurugan *et al.*, 1997). All primers amplified DNA bands of the expected size from whole cell extracts (Figure 2C, lane 1). NAP57 exclusively coprecipitated the two box H/ACA snoRNAs, whereas Nopp140 coprecipitated the box H/ACA snoRNAs and lesser but distinct amounts of the box C/D snoRNAs (Figure 2C, lanes 2 and 4, respectively). No snoRNAs were detected if precipitation of NAP57 or Nopp140 was competed for with free peptide (Figure 1C, lanes 3 and 5, respectively). We conclude that Nopp140 and NAP57 associate with the protein and

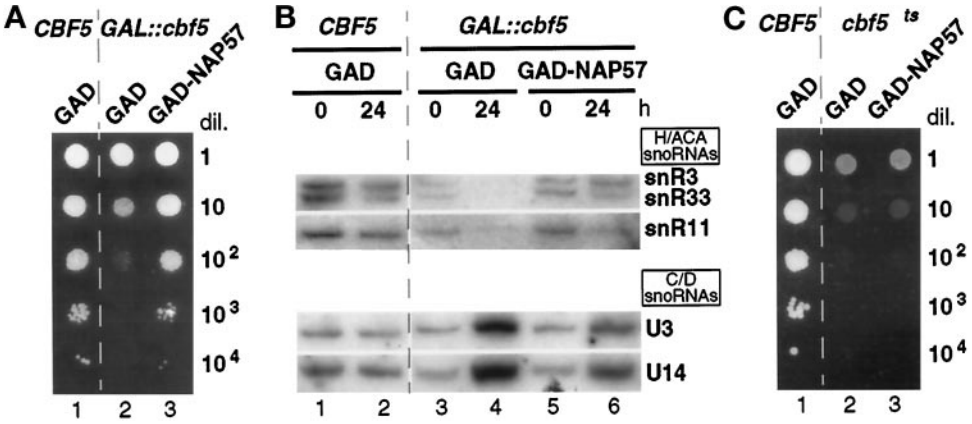



**Figure 3.** Rat NAP57 partially complements the function of yeast *CBF5*. (A) A wild-type yeast *CBF5* strain (lane 1) and a conditional strain with *CBF5* under the conditional *GAL10* promoter (*GAL::cbf5*; lanes 2 and 3) were grown in permissive medium followed by spotting at the dilutions indicated (dil.) on a restrictive, glucose-containing plate. The strains carried plasmids expressing the indicated proteins. The colonies were imaged after 2 d of growth at 30°C. (B) The same strains shown in A were grown for 24 h in liquid glucose-containing medium, and total RNAs were prepared at 0 h (lanes 1, 3, and 5) and 24 h (lanes 2, 4, and 6). The RNAs were separated on a standard 8% acrylamide gel, transferred to a nylon membrane, and detected by Northern hybridization with $^{32}$P-labeled primers specific for the indicated snoRNAs. (C) A wild-type *CBF5* strain (lane 1) and strains with a temperature-sensitive mutation in *CBF5* (*cbf5*$^{ts}$; lanes 2 and 3) carrying plasmids expressing the indicated proteins were spotted on a plate as in A. The colonies were imaged after 2 d of growth at 38°C, the nonpermissive temperature.

RNA components of snoRNPs, indicating that they associate with the intact particles.

### *A Conserved Function for NAP57*

The high sequence conservation between rat NAP57 and yeast Cbf5p (Meier and Blobel, 1994), together with their specific association with box H/ACA snoRNPs, prompted us to test if NAP57 could functionally complement the essential Cbf5p. For this purpose, we obtained a yeast strain whose genomic copy of *CBF5* was placed under the control of the conditional *GAL10* promoter (GAL::cbf5; Lafontaine *et al.*, 1998). Genetic depletion of Cbf5p in this strain by growth on glucose-containing medium leads to significantly reduced levels of pseudouridines in rRNA, instability of box H/ACA snoRNAs, and growth arrest (Lafontaine *et al.*, 1998). First, we tested for the ability of rat NAP57 to complement the growth-arrest phenotype of Cbf5p depletion in this strain (Figure 3A). Expression of a GAD-NAP57 fusion construct, in contrast to GAD alone, fully restored the growth of the Cbf5p-depleted strain (Figure 3A, compare lanes 2 and 3). Expression of two unrelated nuclear proteins fused to GAD, however, failed to restore growth (data not shown). The fusion of GAD to NAP57 was necessary to stabilize NAP57, because it was detected on immunoblots only when expressed as a fusion protein with GAD. In conclusion, NAP57 complemented the growth-arrest phenotype of a Cbf5p-depleted strain.

In addition to growth arrest, Cbf5p depletion caused a specific loss of box H/ACA but not box C/D snoRNAs (Figure 3B, lane 4) (Lafontaine *et al.*, 1998). To test whether the rat NAP57 was able to stabilize the yeast box H/ACA snoRNAs, total RNAs were extracted from wild-type *CBF5* and mutant *GAL::cbf5* strains after growth in glucose-containing medium for 0 and 24 h and were analyzed for the presence of the snoRNAs by Northern blotting (Figure 3B). Indeed, GAD-NAP57 stabilized the box H/ACA snoRNAs (Figure 3B, lane 6), whereas GAD alone had no effect (lane 4). Surprisingly, the amount of box C/D snoRNAs appeared increased under the conditions in which the box H/ACA snoRNAs were lost (Figure 3B, lane 4). This was most likely caused by a relative overload of non-rRNAs when applying equal amounts of total RNAs, because Cbf5p depletion led to a decrease in rRNAs compared with non-rRNAs (Lafontaine *et al.*, 1998). In agreement with this interpretation, expression of GAD-NAP57 restored the levels of box C/D snoRNAs (Figure 3B, compare lanes 4 and 6). This indicated that NAP57 functionally replaced Cbf5p in the box H/ACA snoRNPs and consequently restored normal rRNA synthesis in the Cbf5p-depleted strain. We did not test if the pseudouridylation of rRNA was restored. However, to ascertain that the complementation was indeed caused by GAD-NAP57 and not by Cbf5p through spurious release from glucose repression, we confirmed by Northern blotting that Cbf5p mRNA remained depleted in glucose-containing medium (data not shown). Therefore, rat NAP57 was able to functionally complement a yeast strain genetically depleted of Cbf5p.

Further experiments in a different background, however, revealed that the rat NAP57 only partially complemented Cbf5p function. Thus, we tested for the NAP57 complementation of a *CBF5* temperature-sensitive strain (*cbf5*$^{ts}$) and a *CBF5* null strain (Cadwell *et al.*, 1997). Surprisingly, GAD-NAP57, like GAD alone, failed to rescue growth of the *cbf5*$^{ts}$ strain at the nonpermissive temperature (Figure 2C, lanes 2 and 3). Because this finding was in contrast to our observations with the Cbf5p-depleted strain, we tested whether GAD-NAP57 was able to rescue a *CBF5* null strain. For this purpose, a diploid strain heterozygous for *CBF5* (CBF5/cbf5::TRP1; Cadwell *et al.*, 1997) was transformed with pTM113 (GAD-NAP57) and sporulated, and the tetrads were dissected and allowed to germinate at room temperature and 30°C. All tetrads yielded only two viable spores, all harboring the wild-type *CBF5* gene and many still carrying pTM113 (data not shown). This result indicated that NAP57 failed to complement a *CBF5* null mutant. The difference in behavior of the *GAL::cbf5* strain and the *cbf5*$^{ts}$ and *CBF5* null strains toward NAP57 complementation is apparently caused by residual expression of *CBF5* from the *GAL10* promoter even when grown in glucose, as was observed previously for *GAR1* in a *GAL::gar1* strain (Girard *et al.*,

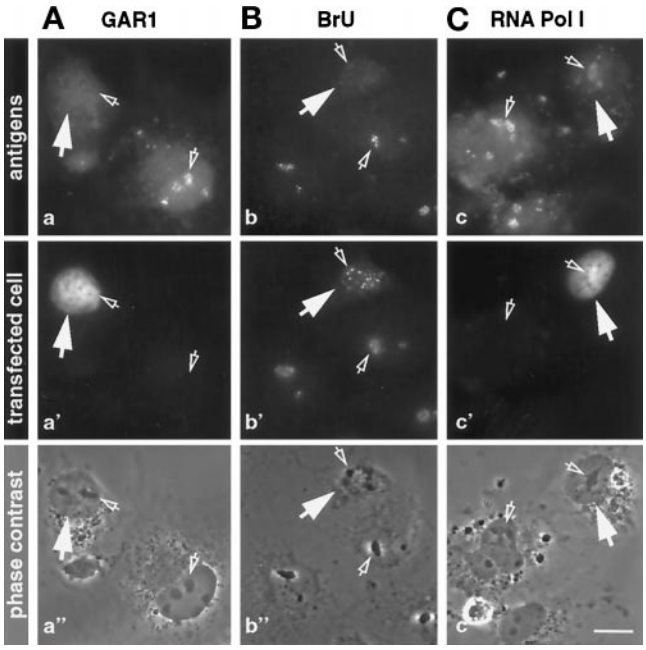



**Figure 4.** Transient expression of the dominant negative Nopp140 carboxyl terminus chases snoRNPs out of the nucleolus (A) and arrests RNA pol I transcription (B) without affecting the localization of RNA pol I (C). Cells were transfected with the HA-tagged carboxyl terminus of Nopp140, and subsequently endogenous GAR1 (a) and RNA pol I (c) were labeled by indirect double immunofluorescence or run-on transcription was detected by BrUTP incorporation and visualization with anti-BrdU antibodies (b). The transfected cells (solid arrows) were identified with anti-HA antibodies (a′ and c′) or by the aberrant nucleoplasmic distribution of endogenous Nopp140 detected with Nopp140-specifc antibodies (b′). All nucleoli can be clearly identified in the phase-contrast images (a′′, b′′, and c′′). One nucleolus of a transfected and an untransfected cell is marked in each panel (small open arrows). Bar, 10 $\mu$m.

1992). Therefore, NAP57 can complement the function of Cbf5p only in the presence of residual amounts of Cbf5p, indicating the existence of an additional function or of high-affinity binding sites for Cbf5p that NAP57 cannot rescue. Nevertheless, the mammalian NAP57 restored growth and stabilized the box H/ACA snoRNAs of a yeast strain genetically depleted of Cbf5p, supporting its function as a box H/ACA-specific snoRNP protein and putative pseudouridylase.

### *In Vivo Interaction of Nopp140 with snoRNPs and Their Relationship to RNA Polymerase I Transcription*

To test if Nopp140 interacted with snoRNPs in vivo, we took advantage of a dominant negative Nopp140 construct identified previously (Isaac *et al.*, 1998). We had found that expression of the conserved carboxyl terminus of Nopp140, NoppC, chased endogenous Nopp140, NAP57, and fibrillarin out of the nucleolus but left the nucleolar localization of all other antigens tested (nucleolin, B23/NO38, UBF, and RNA pol I) unaffected. Figure 4A, a, shows that GAR1 is also depleted from the nucleoli (open arrows) in NoppC-transfected cells (solid arrow). Therefore, the localization of all snoRNP proteins to which antibodies are currently available (NAP57, fibrillarin, and GAR1) is affected by the expression of NoppC but not the localization of other nucleolar proteins. This strongly indicates an interaction of Nopp140 with box H/ACA and box C/D snoRNPs in the cell.

Surprisingly, despite the depletion of the snoRNPs, the nucleolus appeared unaltered by phase-contrast imaging (Figure 4, a′′, b′′, and c′′). Therefore, we tested if RNA pol I transcription, one of the main nucleolar functions, was affected in NoppC-transfected cells (Figure 4B). For this purpose, we studied run-on transcription in permeabilized cells by BrUTP incorporation and subsequent indirect fluorescent detection with anti-BrdU antibodies that cross-react with BrU (Wansink *et al.*, 1993). In untransfected cells, BrUTP is incorporated into rRNA, as indicated by the bright fluorescence in the nucleoli (Figure 4B, compare b and b′′). Transfected cells in this case were identified by the mislocalization of endogenous Nopp140 in a punctate pattern in the nucleoplasm, as described previously (Figure 4B, solid arrow in b′) (Isaac *et al.*, 1998). In all of these transfected cells, BrUTP incorporation was completely absent (Figure 4B, solid arrow in b), indicating an inhibition of RNA pol I transcription. Interestingly, the nucleolar localization of RNA pol I itself remained unaffected in NoppC-transfected cells (Figure 4C, compare c and c′). These results support the idea that the presence of snoRNPs in the nucleolus is required for transcription to occur. For example, the snoRNAs could coat the rRNA during transcription by hybridization to its complementary sequences. Finally, neither snoRNPs nor transcription seems to be required for the phase-dense appearance of the nucleolus in light micrographs.

### *Genetic Interaction of the Yeast Nopp140 Homologue Srp40p with snoRNPs*

Because Nopp140 interacted in mammalian cells with snoRNPs and NAP57 could partially complement the function of its snoRNP protein counterpart in yeast, we tested if Srp40p, the yeast Nopp140 homologue (Meier, 1996), also interacted with snoRNPs. For this purpose, we took advantage of a strain in which the nonessential *SRP40* gene was rendered essential by the mutation of a gene, *LES2*, which caused synthetic lethality with the SRP40 deletion (*srp40*Δ). *LES2* was identified in a synthetic lethal screen with *SRP40* with the use of random *lacZ LEU2* insertions throughout the genome by transformation with a mutagenized yeast library (Burns *et al.*, 1994; our unpublished observations). The *LEU2* marker inserted in *LES2* allowed us to determine that only a single additional gene, aside from *SRP40*, was mutated in the *srp40*Δ *les2* strain and to segregate the single *les2* mutation from the *srp40*Δ deletion. Growth of the *srp40*Δ *les2* double mutant was dependent on the presence of *SRP40* supplied on a plasmid under its own promoter (data not shown) or under the conditional *GAL10* promoter (pGAL-SRP40; Figure 5A). Thus, the *srp40*Δ *les2* (pGAL-SRP40) strain grew as well as wild-type yeast when Srp40p was expressed (Figure 5A, top, compare lanes 1 and 4) but not when Srp40p expression was repressed by glucose (Figure 5A, bottom, lane 4). Therefore, we generated a conditional lethal strain whose growth was dependent on the expression of Srp40p. The singly disrupted *srp40*Δ and *les2* strains showed no or little growth defect, respectively, on glucose

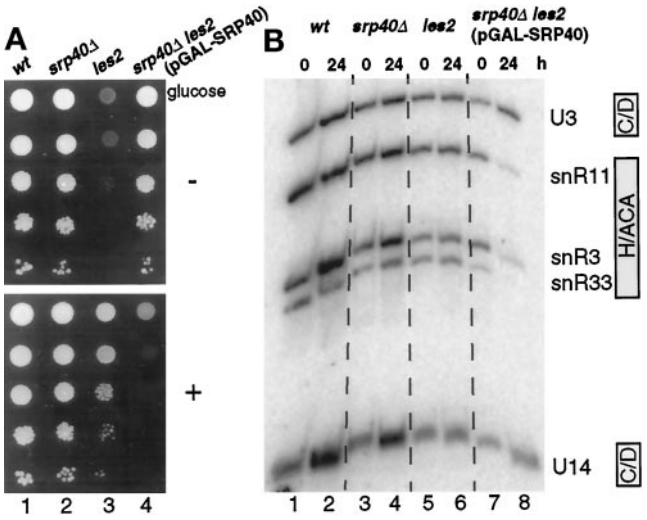



**Figure 5.** A genetic interaction between Srp40p, the yeast Nopp140 homologue, and box H/ACA snoRNAs. (A) Wild-type yeast (*wt*), a strain disrupted in *SRP40* and in the synthetically lethal gene *LES2* but carrying *SRP40* on a multicopy plasmid under the control of the conditional *GAL10* promoter (*srp40*Δ *les2* [pGAL-SRP40]), and the singly disrupted strains *srp40*Δ and *les2* were spotted at various dilutions on glucose-containing plates (bottom panel) or on plates containing instead raffinose, galactose, and sucrose (top panel). (B) Northern blot of total RNAs extracted from the various strains described in A and indicated on top after growth for 0 and 24 h in glucose-containing medium (odd and even lanes, respectively). The blot was probed consecutively with $^{32}$P-labeled oligonucleotides specific for the snoRNAs listed on the right. The respective class of the snoRNAs is indicated.

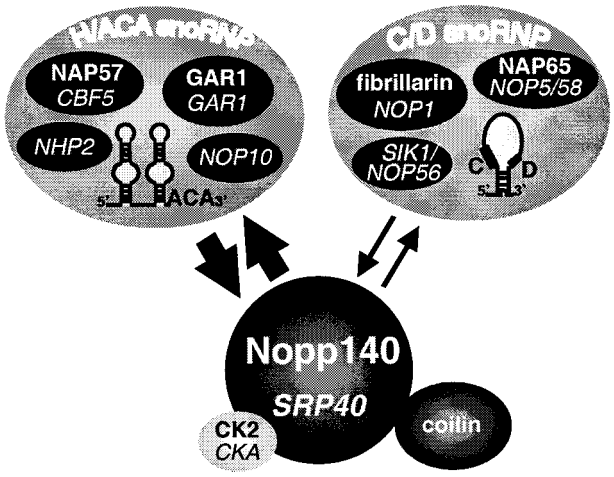



**Figure 6.** Scheme of Nopp140 interactions summarizing our present and previous results and incorporating data from studies in yeast (see INTRODUCTION). The names of the mammalian proteins are printed in bold, and those of the homologous yeast genes are printed in italic. Box H/ACA and box C/D snoRNPs contain not only a separate class of snoRNAs (represented by their conserved secondary structures) but also a distinct set of proteins. Uniquely, Nopp140 interacts with both, although it exhibits a stronger affinity for box H/ACA than for box C/D snoRNPs, as illustrated by the different thickness of the arrows. In addition, Nopp140 appears to be constantly accompanied by small amounts of casein kinase 2 (see also Li *et al.*, 1997) and to interact with the coiled-body protein p80 coilin (Isaac *et al.*, 1998).

compared with wild-type yeast (Figure 5A bottom, compare lanes 1, 2, and 3). However, *les2* growth was severely diminished in the absence of glucose in medium containing raffinose, galactose, and sucrose (Figure 5A, top, lane 3). The latter phenotype was rescued by additional *SRP40* expression from its own promoter on a low-copy-number *CEN* plasmid, indicating a tight relationship between the *SRP40* and *LES2* genes (data not shown, but see Figure 5A, top, lane 4).

To test if depletion of Srp40p in the conditional strain affected the stability of snoRNAs, as in the case of Cbf5p (Figure 3B), the various strains were grown for 0 and 24 h in glucose-containing medium. The stability of the snoRNAs was tested on Northern blots with antisense oligonucleotide probes to two box C/D (U3 and U14) and three box H/ACA (snR3, snR11, and snR33) snoRNAs (Figure 5B). As expected from their undiminished growth under these conditions, the levels of all tested snoRNAs remained unaffected in the wild-type and single-mutant strains (Figure 5B, lanes 1–6). However, depletion of Srp40p in the double mutant led to the specific reduction of the tested box H/ACA but not the box C/D snoRNAs (Figure 5B, compare lanes 7 and 8). Therefore, Srp40p depletion exhibited the same phenotype as that of the integral box H/ACA proteins Cbf5p, Nhp2p, and Nop10p (Henras *et al.*, 1998; Lafontaine *et al.*, 1998; Watkins *et al.*, 1998a). These findings demonstrated that Srp40p genetically interacts with box H/ACA snoRNAs and suggested that Srp40p in yeast associates with snoRNPs, such as Nopp140, in mammalian cells. Therefore, these data add to the previously presented evidence that Srp40p is the bona fide Nopp140 homologue (Meier, 1996) and suggest that Srp40p and consequently Nopp140 play a role in snoRNP function.

## DISCUSSION

The previously determined and the novel associations of Nopp140 described here can be distilled into a model of Nopp140 interactions (Figure 6). Nopp140 is the first protein shown to interact with both major classes of snoRNPs, box H/ACA and box C/D. This is in contrast to the integral components of snoRNPs, which are specific for each class. In addition, a Nopp140-snoRNP interaction appears to be conserved in evolution, because Srp40p, the yeast Nopp140 homologue, genetically interacts with at least one class of snoRNPs.

The unique protein composition of box H/ACA and box C/D snoRNPs depicted in Figure 6 was derived from recent experiments in yeast (Lübben *et al.*, 1995; Balakin *et al.*, 1996; Ganot *et al.*, 1997; Gautier *et al.*, 1997; Henras *et al.*, 1998; Lafontaine *et al.*, 1998; Watkins *et al.*, 1998a; Lafontaine and Tollervey, 1999) and from the specific precipitation of human box H/ACA snoRNAs with GAR1 antibodies (Dragon, Pogačić, and Filipowicz, unpublished data). All of these proteins are highly conserved and exhibit sequence identities that range from 45 to 70% between yeast and mammals, including the comparison of the novel rat NAP65 and yeast Nop5/58p (Figure 6, mammalian proteins in bold, yeast

homologues in italic). Our studies demonstrate that not only are the protein components conserved from yeast to mammals but that the protein compositions of both major classes of snoRNPs are conserved as well. Thus, based on their coprecipitation, NAP57, GAR1, and box H/ACA snoRNAs form a specific complex analogous to yeast box H/ACA snoRNPs. Nopp140, however, in addition to these box H/ACA snoRNP components, precipitates fibrillarin, the novel NAP65, and box C/D snoRNAs. Because the box H/ACA snoRNP proteins and RNAs form a distinct complex, the other components coprecipitating with Nopp140 likely constitute a separate complex, as they do in yeast, i.e., box C/D snoRNPs. In fact, the snoRNP composition is so highly conserved between mammals and yeast that rat NAP57 functionally replaced its yeast counterpart in at least one strain genetically depleted of its homologue Cbf5p. This finding is similar to the functional complementation of the yeast box C/D snoRNP protein Nop1p by its human orthologue fibrillarin (Jansen *et al.*, 1991). The establishment of the composition of mammalian snoRNPs has gained clinical importance as a result of the identification of dyskerin, the human NAP57 homologue, as the protein mutated in the X-linked recessive bone marrow failure syndrome dyskeratosis congenita (Heiss *et al.*, 1998).

At present, there are antibodies available against only three mammalian snoRNP proteins: fibrillarin, NAP57, and GAR1. All of these proteins associate with Nopp140 and behave according to the emerging model for snoRNP composition (Figure 6). In addition, Nopp140 coprecipitated the novel NAP65 and several unidentified bands (Figure 1A, open dots). It is likely that some of these bands correspond to additional snoRNP proteins. For example, the 24-kDa band is a good candidate for the mammalian NHP2 homologue, whereas the 66- or 61-kDa band could correspond to the vertebrate Sik1p/Nop56p homologue. GAR1 is not visible because it comigrates on that gel with the IgG light chains (Figure 1A, asterisks), and NOP10 is expected to migrate with the gel front. Analysis of the residual bands will possibly identify additional snoRNP proteins, particularly those of the box C/D class, because NAP57, GAR1, NHP2, and NOP10 are predicted to make up the complete complement of box H/ACA snoRNP proteins (Henras *et al.*, 1998; Watkins *et al.*, 1998a). Interestingly, a 65/68-kDa protein band was previously identified to specifically cross-link to box C/D snoRNAs (Caffarelli *et al.*, 1998; Watkins *et al.*, 1998b). This protein likely corresponds to the NAP65 characterized here. Therefore, those findings support our evidence that NAP65 is a specific part of box C/D snoRNPs, as demonstrated for its yeast counterpart Nop5/58p (Lafontaine and Tollervey, 1999).

Our data demonstrate that Nopp140 interacts with both major classes of snoRNPs. Thus, we show that proteins and RNAs of box H/ACA and box C/D snoRNPs coprecipitate with Nopp140 under low-stringency conditions (Figure 2). Furthermore, proteins of both classes are specifically chased out of the nucleolus by the dominant negative Nopp140 carboxyl terminus (Figure 4A). Interestingly, the association of Nopp140 with box H/ACA snoRNPs appears tighter than that with the box C/D class (depicted by the thick arrows in Figure 6). This is supported by the coimmunoprecipitation of stoichiometric amounts of NAP57, a box H/ACA snoRNP protein, under conditions that precipitate only small amounts of box C/D proteins (Figure 1A). A preferred interaction with box H/ACA snoRNPs is also suggested by the genetic depletion studies of Srp40p, the yeast Nopp140 homologue (Figure 5B).

What is the nature of the Nopp140-snoRNP interaction? Is it transient or is Nopp140 an integral component of snoRNPs? We suggest that Nopp140 only transiently associates with snoRNPs. Thus, Nopp140 is easily isolated as a single species (away from snoRNPs) under low-ionic-strength or high-salt (500 mM sodium chloride) conditions (Meier and Blobel, 1990). Moreover, most box C/D snoRNP proteins already dissociate from Nopp140 at physiological salt concentrations (Figure 1), and purified yeast box H/ACA snoRNPs appear to lack the yeast Nopp140 homologue Srp40p (Lübben *et al.*, 1995; Watkins *et al.*, 1998a). It is likely that snoRNPs remain intact under conditions in which Nopp140 dissociates, because intra-snoRNP interactions survive even the harsh conditions of cesium chloride gradients (Lübben *et al.*, 1995). Such a transient and reversible association of Nopp140 with snoRNPs is consistent with it functioning as a chaperone of snoRNPs, either for their intranuclear transport or during snoRNP-rRNA association and dissociation (see below).

It is unclear why Nopp140 exhibits a preferred affinity for NAP57 and if all of this NAP57 is snoRNP associated. Surprisingly, extensive yeast two-hybrid analysis with both proteins, full-length and individual domains, failed to reveal any interaction (our unpublished results). Although this could be a peculiarity of the yeast two-hybrid system, it indicates that Nopp140 may not bind to box H/ACA snoRNPs through NAP57. Alternatively, the highly and mostly positively charged carboxyl terminus of NAP57 would appear to be a good interacting partner with the highly phosphorylated repeat domain of Nopp140. This possibility is supported by the fact that such a charged carboxyl terminus is also present in NAP65, which could provide the Nopp140 handle for box C/D snoRNPs. Furthermore, these charged carboxyl termini are conserved in Cbf5p and Nop5/58p, the yeast counterparts of NAP57 and NAP65, respectively. In summary, however, the components of snoRNPs that bind directly to Nopp140 remain to be identified and/or confirmed. It will be interesting to determine what governs the Nopp140-snoRNP association and what fraction of Nopp140 at any given time is associated with these particles.

rRNA modification in vertebrate cells occurs cotranscriptionally (Maden, 1990). This is supported by our finding that the lack of snoRNPs in nucleoli leads to transcription arrest. Although it cannot be ruled out that other factors contribute to our observation, it is interesting to speculate that newly transcribed rRNA emerging from RNA pol I is immediately covered by snoRNPs. Consequently, the absence of these snoRNPs could induce a negative feedback mechanism of rRNA transcription. It is possible that transcription is physically restrained by the accumulation of misfolded rRNA or that there is a direct interaction between the snoRNPs and RNA pol I. For example, Nopp140 itself could provide such a link because it has been reported to function as a transcription factor in another system (Miau *et al.*, 1997). Although the interaction between snoRNPs and the RNA pol I transcription machinery is an intriguing possibility, it needs to be further investigated, e.g., in in vitro transcription systems.

In conclusion, we report here on the interaction of Nopp140 with both major classes of mammalian snoRNPs.

What is the function of this interaction? Three possibilities come to mind. First, Nopp140 shuttles between the nucleolus, the coiled bodies, and the cytoplasm and may as such facilitate the transport of snoRNPs through the nucleoplasm, e.g., between the nucleolus and the coiled bodies. A snoRNP transport role for Nopp140 is supported by the specific depletion of snoRNPs from the nucleolus upon expression of the dominant negative NoppC construct. Second, Nopp140 may aid in the biogenesis of snoRNPs, similar to the function of the SMN and SIP proteins in the assembly of spliceosomal snRNPs (Fischer *et al.*, 1997). Third, Nopp140 may be directly involved in the function of snoRNPs, the modification and processing of rRNA. As such, it may aid the binding or release of the snoRNPs to or from the nascent rRNA. Therefore, its function could be analogous to that of SR proteins, which are required for the assembly and function of the spliceosome (for review, see Valcarcel and Green, 1996). These possibilities are not mutually exclusive and may be partially overlapping. Nopp140 appears to exhibit a conserved function as a chaperone of snoRNPs.

***Note Added in Proof.*** In agreement with our data, hNop5/Nop58, the human homologue of rat NAP65 and yeast Nop5/58p, was now also shown to be a specific component of box C/D snoRNPs (Lyman *et al.* [1999]. RNA *5*, 1597–1604).

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