

Cotranscriptional Recruitment of the Pseudouridylsynthetase Cbf5p and of the RNA Binding Protein Naf1p during H/ACA snoRNP Assembly

Pok Kwan Yang,¹ Coralie Hoareau,² Carine Froment,³ Bernard Monsarrat,³ Yves Henry,² and Guillaume Chanfreau^{1*}

Department of Chemistry and Biochemistry and Molecular Biology Institute, UCLA, Los Angeles, California,¹ and Laboratoire de Biologie Moléculaire Eucaryote, UMR5099 CNRS-Université Paul Sabatier,² and Plate-forme protéomique, Institut de Pharmacologie et de Biologie Structurale (CNRS UMR 5089),³ Toulouse, France

Received 13 December 2004/Returned for modification 14 January 2005/Accepted 19 January 2005

H/ACA small nucleolar ribonucleoprotein particles (snoRNPs) are essential for the maturation and pseudouridylation of the precursor of rRNAs and other stable RNAs. Although the RNA and protein components of these RNPs have been identified, the mechanisms by which they are assembled in vivo are poorly understood. Here we show that the RNA binding protein Naf1p, which is required for H/ACA snoRNPs stability, associates with RNA polymerase II-associated proteins Spt16p, Tfg1p, and Sub1p and with H/ACA snoRNP proteins. Chromatin immunoprecipitation experiments show that Naf1p and the pseudouridylsynthetase Cbf5p cross-link specifically with the chromatin of H/ACA small nucleolar RNA (snoRNA) genes. Naf1p and Cbf5p cross-link predominantly with the 3' end of these genes, in a pattern similar to that observed for transcription elongation factor Spt16p. Cross-linking of Naf1p to H/ACA snoRNA genes requires active transcription and intact H/ACA snoRNA sequences but does not require the RNA polymerase II CTD kinase Ctk1p. These results suggest that Naf1p and Cbf5p are recruited in a cotranscriptional manner during H/ACA snoRNP assembly, possibly by binding to the nascent H/ACA snoRNA transcript during elongation or termination of transcription of H/ACA snoRNA genes.

Small nucleolar ribonucleoprotein particles (snoRNPs) have essential functions in many gene expression pathways. Most of these particles are required either for rRNA processing or for site-specific modification of nucleotides of the 35S pre-rRNA precursor and of other noncoding RNAs (4, 9, 27). A few snoRNPs are necessary for cleavage of the 35S, but the majority of them guide modifications of the bases or of the sugar phosphate backbone within the 35S (49). snoRNPs are made of the association of a small nucleolar RNA (snoRNA) with several snoRNP proteins. These particles are classified into two major structural families, according to the specific RNA motifs present in the snoRNA. These two families of snoRNPs perform different cellular functions. Box C/D motifs are present in snoRNAs that guide the methylation of the 2'-hydroxyl groups of the ribose moiety of some nucleotides in the 35S rRNA precursor (20). The box H and ACA motifs are found in snoRNAs that guide the pseudouridylation of nucleotides of the rRNA precursor or of other transcripts and in other stable RNAs such as the RNA component of vertebrate telomerase (20).

Box H/ACA snoRNPs contain four core proteins, Gar1p, Nhp2p, Nop10p, and Cbf5p, which is likely to be responsible for the pseudouridylsynthetase catalytic activity (4, 6, 18, 34). These proteins are strongly conserved from yeast to mammals (12) and have the same names, with the exception of rat Nap57 and human dyskerin, which are the mammalian homologues of

Cbf5p (37). Dyskerin has been the focus of intense genetic investigation, as mutations in the human gene encoding this protein have been linked to dyskeratosis congenita, an X-linked genetic disease with a predisposition to gastrointestinal cancers (16, 38, 39, 45).

The biogenesis of box H/ACA snoRNPs begins with the transcription and processing of the H/ACA snoRNAs. Most yeast H/ACA snoRNAs are generated from independent transcription units, while metazoan H/ACA snoRNAs are present within the introns of mRNA genes and are processed from the excised introns after splicing (5, 28, 29). Most independently transcribed yeast box H/ACA snoRNAs are processed at the 3' end by a complex that includes the Nrd1p and Sen1p proteins, presumably associated with RNA polymerase II (46). The packaging of box H/ACA snoRNAs into snoRNPs possibly occurs at an early stage of biogenesis, since 5'-unprocessed snoRNA precursors accumulation requires the presence of box H/ACA snoRNP proteins (18). However, it is not known when these proteins bind to the RNA during the biogenesis process. After or during the course of their maturation, the snoRNAs are targeted to the Cajal bodies, also named coiled bodies, where it is thought that some of the snoRNP assembly processes will occur (reviewed in reference 14). snoRNPs are then targeted to the nucleolus to function in rRNA processing and/or modification. Some RNPs remain in the Cajal bodies to guide the modifications of small nuclear RNAs (24, 43).

In an effort to understand the biogenesis pathway of box H/ACA snoRNPs, several studies have investigated the reconstitution of these particles in vitro or the RNA binding properties of their constituent proteins (12, 17, 19, 50). These studies have partially identified the RNA motifs and protein

* Corresponding author. Mailing address: Department of Chemistry and Biochemistry and Molecular Biology Institute, UCLA, Box 951569, Los Angeles, CA 90095-1569. Phone: (310) 825-4399. Fax: (310) 206-4038. E-mail: guillom@chem.ucla.edu.

domains or amino acids required for the association of the snoRNP proteins with the box H/ACA snoRNAs *in vitro*. However, little is known about the process by which the RNA and the protein components become assembled into mature snoRNPs *in vivo*. While reconstitution assays provide valuable tools to understand the molecular details of RNA-protein and protein-protein interactions, these studies do not provide an understanding of the dynamics of the association between RNA and proteins *in vivo* in the context of other cellular processes such as transcription and nuclear import and export.

Some *trans*-acting factors have been identified as putative H/ACA snoRNP assembly factors *in vivo*. A predicted RNA helicase was identified as important for biogenesis of both box C/D and H/ACA yeast snoRNAs (26). The Naf1p and Shq1p proteins have been suggested to function as yeast H/ACA snoRNP assembly factors (11, 13, 51). These essential proteins are connected to H/ACA snoRNP proteins Nhp2p and Cbf5p by a network of interactions on the basis of genomic two-hybrid studies (23) and proteomics studies, which showed that both Naf1p and Shq1p copurify with overexpressed Flag-tagged Cbf5p (22). Depletion of Naf1p or Shq1p leads to the destabilization of all H/ACA snoRNAs, and the absence of Naf1p also results in the depletion of some of the H/ACA snoRNP proteins, including Cbf5p (11, 13, 51). Shq1p and Naf1p are not integral components of mature box H/ACA snoRNPs, even though Naf1p shows a weak association with mature H/ACA snoRNAs *in vivo* (11, 13, 51). Shq1p and Naf1p are nucleoplasmic proteins (51), with Naf1p showing a minor nucleolar localization (11). Sequence analysis of Naf1p yielded a few clues regarding its potential functions. Naf1p contains a putative RNA binding domain (RBD) similar to that of the box H/ACA snoRNP protein Gar1p (3) and several regions of homology with the snoRNA 3'-processing factor-termination factor Nrd1p (46). Consistent with the presence of the RBD, Naf1p displays RNA binding activity *in vitro* (13). This activity is somehow specific to H/ACA snoRNAs; although Naf1p binds to a variety of RNA molecules *in vitro*, Naf1p-H/ACA RNA complexes cannot be competed away by U6 snRNA or other, unrelated molecules (13; C. Hoareau and Y. Henry, unpublished data). Naf1p is linked to the polymerase II transcriptional machinery, as shown by an interaction with the carboxy-terminal domain of RNA polymerase II (CTD) by two-hybrid analysis and *in vitro* pull-down experiments from yeast extracts (13). Interestingly, the *in vitro* interaction between Naf1p and the CTD is observed when the CTD has been phosphorylated (13). Both Shq1p and Naf1p have putative orthologs in higher eukaryotes (11, 13, 51), suggesting that their function might be conserved across eukaryotes. Despite these observations, the precise function of Naf1p and Shq1p in the biogenesis process of H/ACA snoRNPs is unknown. In this study, we have investigated the role of Naf1p in H/ACA snoRNP assembly *in vivo*. We found that Naf1p interacts with RNA polymerase II components and with snoRNP proteins and that it associates specifically with H/ACA snoRNA genes. The pseudouridylsynthetase Cbf5p is also found associated with H/ACA snoRNA genes. Our results suggest a functional model of snoRNP assembly in which the early stages of assembly are cotranscriptional and begin with the recruitment of Cbf5p and RNA binding protein Naf1p by the snoRNA substrate.

MATERIALS AND METHODS

Yeast strains. Tandem affinity purification (TAP)-tagged strains were purchased from Open Biosystems or constructed by homologous recombination (44). All of the strains used for chromatin immunoprecipitation (ChIP) experiments were TAP tagged at the C terminus of the corresponding proteins, except for Nhp2p, for which an Nhp2p-ZZ-tagged strain was used (17). The *ctk1Δ* strain was purchased from Open Biosystems, and a TAP tag was introduced into this strain as previously described (44). Insertion of a galactose-driven promoter in front of the *SNR32* snoRNA sequence was performed by homologous recombination with the cassette described in reference 35. Deletion of the *SNR44* sequence within the chromosomal *RPS22B* gene in the Naf1-TAP-tagged strain was performed by a two-step homologous recombination method known as the *delitto perfetto* method (47).

TAP tag purification. Naf1p-TAP and associated proteins were purified from extracts prepared from 4 liters of yeast cells grown in YPD medium (1% yeast extract, 1% peptone, 2% glucose) to an optical density at 600 nm of 0.6. Cells frozen in liquid nitrogen were broken with dry ice in a kitchen blender (Osterizer). The broken cell powder was resuspended in 10 ml of A200 buffer (20 mM Tris-HCl [pH 8.0], 5 mM MgCl₂, 0.2% Triton X-100, 200 mM KCl, 1 mM dithiothreitol) containing 0.5 U of RNasin (Promega) per μ l and protease and phosphatase inhibitors. Extracts were clarified by centrifugation in a Beckman Ti-50.2 rotor at 4°C for 15 min at 25,000 rpm. The supernatant was mixed for 2 h at 4°C on a shaking table with 200 μ l of immunoglobulin G (IgG)-Sepharose beads (Pharmacia) previously equilibrated with A200 buffer. After binding, IgG-Sepharose beads were washed with 80 ml of A200 buffer, followed by 30 ml of TEV protease cleavage buffer (10 mM Tris-HCl [pH 8.0], 1 mM dithiothreitol, 0.1% NP-40, 150 mM NaCl, 0.5 mM EDTA). The beads were then incubated for 2 h at 16°C with 100 U of TEV protease (Invitrogen) in 1 ml of TEV cleavage buffer. Eluted material was mixed with 6 μ l of 1 M CaCl₂, 1.2 μ l of 1 M Mg acetate (MgAc), and 1.2 μ l of 1 M imidazole and incubated for 1 h at 4°C with 200 μ l of calmodulin beads (Pharmacia) previously equilibrated with calmodulin binding buffer (10 mM Tris-HCl [pH 8.0], 10 mM β -mercaptoethanol, 0.1% NP-40, 150 mM NaCl, 1 mM MgAc, 2 mM EGTA, 1 mM imidazole). The beads were then washed with 40 ml of calmodulin binding buffer. The purified protein complexes were eluted from the calmodulin beads with six 200- μ l aliquots of calmodulin elution buffer (10 mM Tris-HCl [pH 8.0], 0.1% NP-40, 150 mM NaCl, 1 mM MgAc, 2 mM EGTA, 1 mM imidazole). The eluted proteins were precipitated with trichloroacetic acid, separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and identified by mass spectrometry as previously described (10).

ChIPs. ChIPs were performed as previously described (31), with minor modifications. The buffers used are described in reference 31. A 100-ml volume of cells was grown to an optical density of 0.4 to 0.5. Formaldehyde was added to a final concentration of 1%, and cells were cross-linked for 20 min at 25°C with shaking. The cross-linking reaction was quenched with glycine for 5 min at 25°C. Cells were then washed twice with Tris-buffered saline and once with FA150 lysis buffer. Cell extract was prepared by breaking the cells with glass beads in lysis buffer for 40 min. Extract was collected and centrifuged at 200,000 \times g with a Beckman SW50.1 rotor. The pellet was resuspended in 800 μ l of lysis buffer and sonicated to obtain DNA fragments of around 100 to 1,000 bp as analyzed with ethidium-stained agarose gel. Immunoprecipitation of TAP-tagged or ZZ-tagged protein was done overnight at 4°C with IgG beads. The reverse cross-linking procedure was carried out at 65°C overnight. Isolation of chromatin was done by phenol-chloroform extraction and ethanol precipitation. A list of the primers used for snoRNA gene amplification is available upon request. For each ChIP experiment, PCR samples were taken at various cycles from the input extracts or the immunoprecipitates to ensure that PCR samples were in the linear range. ³²P-labeled PCR products were loaded onto acrylamide gels, and the products were quantitated with a PhosphorImager.

RESULTS

H/ACA snoRNP proteins and RNA polymerase II-associated proteins copurify with Naf1p. To gain further insights into the functions of Naf1p in box H/ACA snoRNP biogenesis, we purified Naf1p from whole-cell extracts by the TAP method (44). Extracts were prepared from a yeast strain expressing Naf1p-TAP from the endogenous locus. Naf1p-TAP and associated proteins were purified, fractionated by SDS-polyacrylamide gel electrophoresis, and stained with Coomassie blue

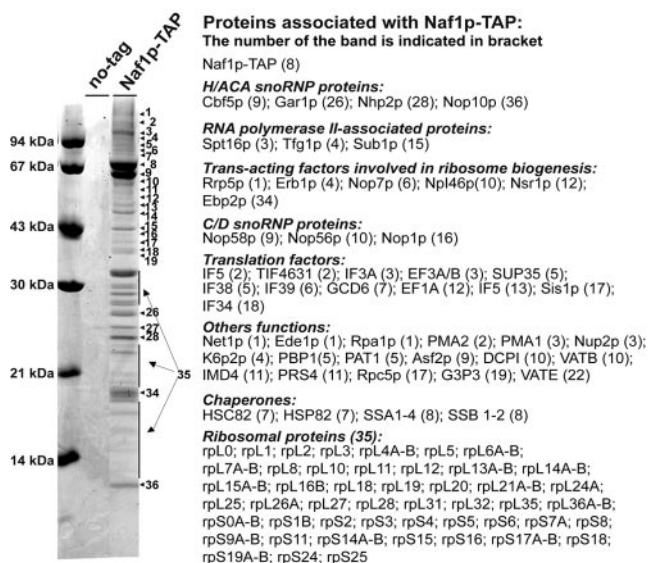


FIG. 1. Copurification of Naf1p with H/ACA snoRNP proteins and RNA polymerase II-associated proteins. Shown is a Coomassie-stained SDS gel obtained after two-step TAP tag purification from a Naf1p-TAP-tagged strain or from an untagged strain. Proteins identified by mass spectrometry are indicated. Also shown are the eluate obtained from an untagged strain and mass markers.

(Fig. 1). Proteins present in stained bands were then identified by mass spectrometry (Fig. 1). The predominant bands observed on this gel contained Naf1p (Fig. 1, band 8) and a degradation product of Naf1p (contained in band 9). Several minor bands correspond to factors very frequently found in large-scale TAPs, such as ribosomal proteins, protein chaperones, and translation factors (14a). Hence, their detection may not reflect a specific association with Naf1p. More interestingly, we identified all four H/ACA snoRNP proteins in the purified fractions, as well as several components of preribosomal particles. Cbf5p, the H/ACA snoRNP pseudouridylyltransferase, was identified in band 9 in a mixture with degradation products of Naf1p. While previous results suggest that Naf1p and Shq1p interact *in vivo* (23, 51), we did not find Shq1p in the Naf1p purification result. This negative result may be due to a lack of detection of Shq1p by mass spectrometry or to the fact that the Naf1p-Shq1p interaction may not resist the TAP tag purification procedure. To our surprise, three RNA polymerase II-associated proteins were also found in the purified fractions, namely, Spt16p, Sub1p, and Tfg1p. Unlike many of the less abundant proteins pulled down with Naf1p-TAP, Spt16p, Sub1p, and Tfg1p do not feature among the frequently observed “contaminant” proteins in large-scale TAP experiments. Spt16p is part of the yeast homologue of the human FACT complex and is important for linking transcriptional initiation to elongation (25, 32, 36). The Sub1p protein has been involved in linking transcription initiation, 3'-end processing, and termination (2, 7, 15, 30). Tfg1p is part of the general transcription factor TFIIF, which is involved in transcriptional initiation and elongation (32, 48). The association of Naf1p and H/ACA snoRNP-related proteins with the RNA polymerase II machinery, while surprising, is in agreement with recent observations linking Cbf5p and the transcriptional ma-

chinery. Cbf5p was found associated with Rtf1p, a member of the Paf1 complex involved in transcriptional elongation, which itself interacts with Spt16p (33). Furthermore, Cbf5p was recently shown to interact with the phosphorylated RNA polymerase II CTD (42). It is possible that the interaction among Naf1p, Cbf5p, and these transcription factors detected by our TAP tag purification is indirect, through multiple protein complexes. However, our proteomic approach reveals that H/ACA snoRNP proteins copurify with Naf1p and, taken together with recent results from other studies, suggest functional links among Naf1p, Cbf5p, and the actively transcribed chromatin.

Naf1p and Cbf5p cross-link specifically with H/ACA snoRNA genes. The copurification of RNA polymerase II factors with Naf1p and the requirement of Naf1p for the stability of H/ACA snoRNAs suggested that Naf1p might be associated with transcriptional complexes at H/ACA snoRNA genes. This hypothesis is consistent with previous results suggesting an association between Naf1p and the phosphorylated CTD of RNA polymerase II (13). The association of Cbf5p with the phosphorylated polymerase II CTD (42) and its association with the Paf1 complex (33) also suggested a link between Cbf5p and the actively transcribed chromatin at H/ACA snoRNA genes. To test whether Naf1p and/or Cbf5p are associated with the chromatin of snoRNA genes, we performed ChIP with Naf1-TAP- or Cbf5-TAP-tagged strains and tested the association of Naf1p and Cbf5p with various regions of an H/ACA snoRNA gene, *SNR32*. The sequence encoding the TAP tags is present at the 3' ends of the chromosomal loci encoding these proteins. Therefore, the genes coding for these proteins are expressed from their natural promoters in a normal chromosomal context. ChIP, followed by PCR amplification, showed that Naf1p and Cbf5p are enriched at the chromatin of the *snR32* gene (Fig. 2A and B). This association is specific, as it was not observed with the untagged strain. Surprisingly, Naf1p and Cbf5p cross-linked more efficiently to the middle and downstream regions of the *SNR32* gene (Fig. 2, regions B and C) than to the promoter region (Fig. 2, region A). This result suggests that Naf1p and Cbf5p associate with the *SNR32* gene during or immediately after transcription elongation rather than during transcription initiation. We performed similar ChIP experiments with the other core box H/ACA snoRNP proteins Gar1p, Nhp2p, and Nop10p; the H/ACA assembly factor Shq1p; and the three proteins identified by our TAP tag purification, Spt16p, Tfg1p, and Sub1p. In contrast to Naf1p and Cbf5p, Shq1p does not show a significant association with *SNR32*. Thus, it is likely that the described interaction between Naf1p and Shq1p does not occur when Naf1p is associated with the chromatin. Gar1p, Nhp2p, and Nop10p showed a weak association with this locus, only two-fold above the background. Given the low enrichment observed for these proteins, we do not know whether they are significantly present at the chromatin of the *SNR32* gene. This low enrichment cannot be attributed to inefficient immunoprecipitation of the corresponding proteins, as we consistently observed immunoprecipitation efficiencies for these proteins comparable to or better than the efficiency observed for Cbf5p (data not shown). We note, however, that the same gradient of association toward the 3' end of the gene can be observed for the core snoRNP proteins. Spt16p was highly enriched throughout the *SNR32* gene, while Sub1p and Tfg1p showed a

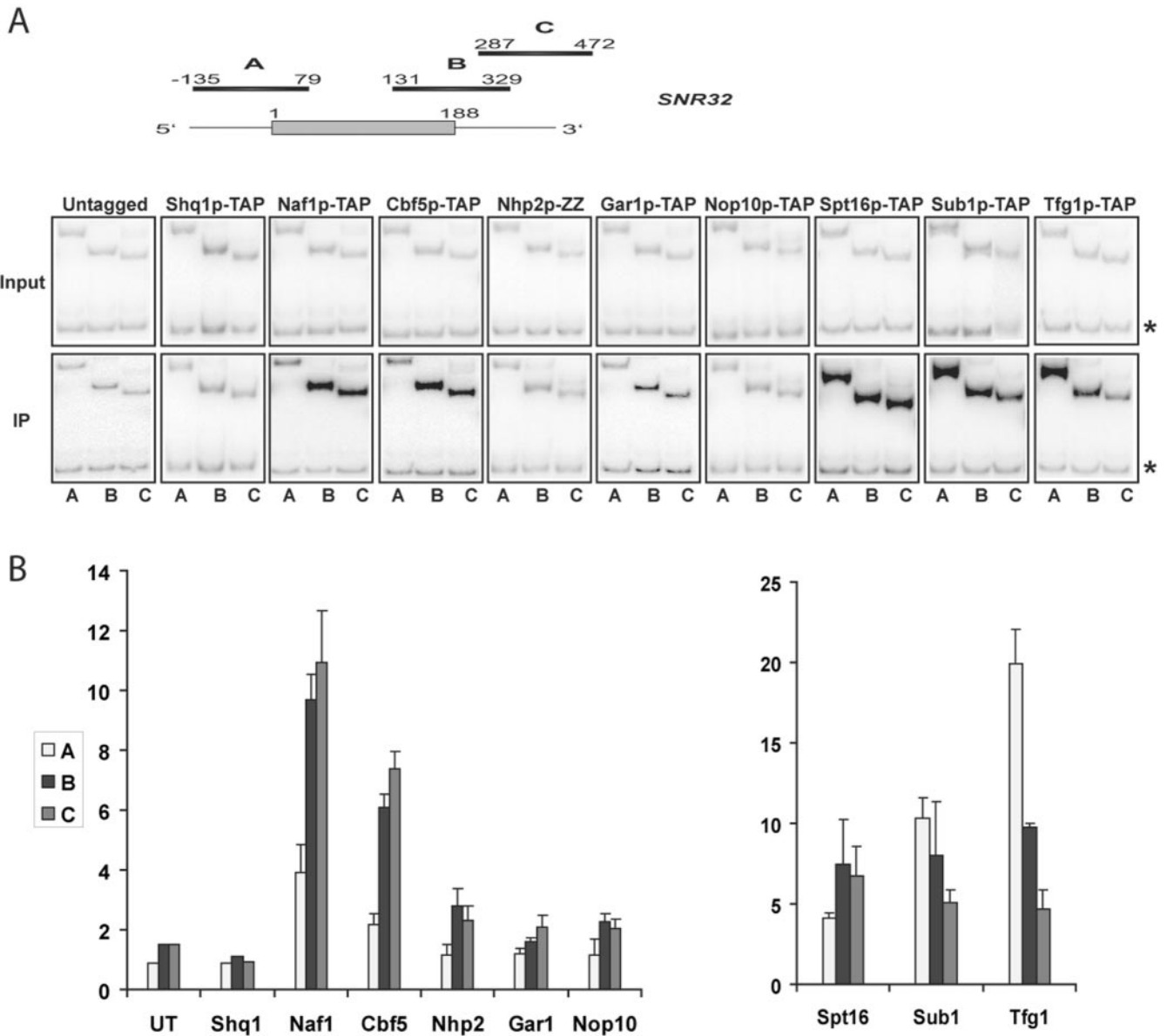


FIG. 2. Naf1p, Cbf5p, and transcription factors Spt16p, Sub1p, and Tfg1p cross-link in vivo with *SNR32*, a box H/ACA snoRNA gene. (A) Schematic diagram of box H/ACA snoRNA gene *SNR32* and ChIP analysis of Untagged (a wild-type strain with no tagged proteins; UT); H/ACA-related proteins Shq1p, Naf1p, Cbf5p, Nhp2p, Gar1p, and Nop10p; and the transcription factors Spt16p, Sub1p, and Tfg1p on the *SNR32* gene. The mature sequence of the gene is represented by a gray box. Bars above the gene show the positions of the PCR products used in the ChIP experiments. Numbers above the bars indicate the exact positions of the PCR products relative to the mature sequence of the snoRNA gene. The lower part of the panel represents PCR products obtained from the precipitated chromatin, while the upper part of the panel represents the PCR products obtained from the total input chromatin prior to precipitation. The upper band in each lane is the PCR product for *SNR32*, with the positions of the amplified region indicated by letters corresponding to those in the diagram at the top. The lower band (marked by an asterisk) is the PCR product for a telomeric (nontranscribed) region that acts as a negative control for the background. IP, immunoprecipitation. (B) Quantification of the ChIP experiments for *SNR32*. The y axis represents the level of enrichment for each region of the *SNR32* gene for each protein and relative to the telomeric region. A ratio of 1 indicates the signal for background association observed in the untagged strain. The level of enrichment of Naf1, Cbf5, Nhp2, Gar1, and Nop10 to various regions of *SNR32* is the average of three independent experiments. The level of enrichment of Spt16, Sub1p, and Tfg1 is the average of two independent experiments.

higher enrichment toward the promoter regions (probe A). The enrichment profile observed for these proteins in various regions of the *SNR32* H/ACA snoRNA gene is consistent with previously published studies on mRNA genes (15, 25, 32).

To ensure that the association of Naf1p and Cbf5p with the chromatin is not restricted to the *SNR32* snoRNA gene, we

performed ChIP experiments and probed for the presence of Naf1p, Cbf5p, Nhp2p, Nop10p, Gar1p, Shq1p, Spt16p, Tfg1p, and Sub1p on four additional H/ACA snoRNA genes, *SNR3*, *SNR30*, *SNR37*, and *SNR42* (Fig. 3). To control for the specificity of the association of Naf1p and Cbf5p with H/ACA snoRNA genes, we also investigated the presence of these

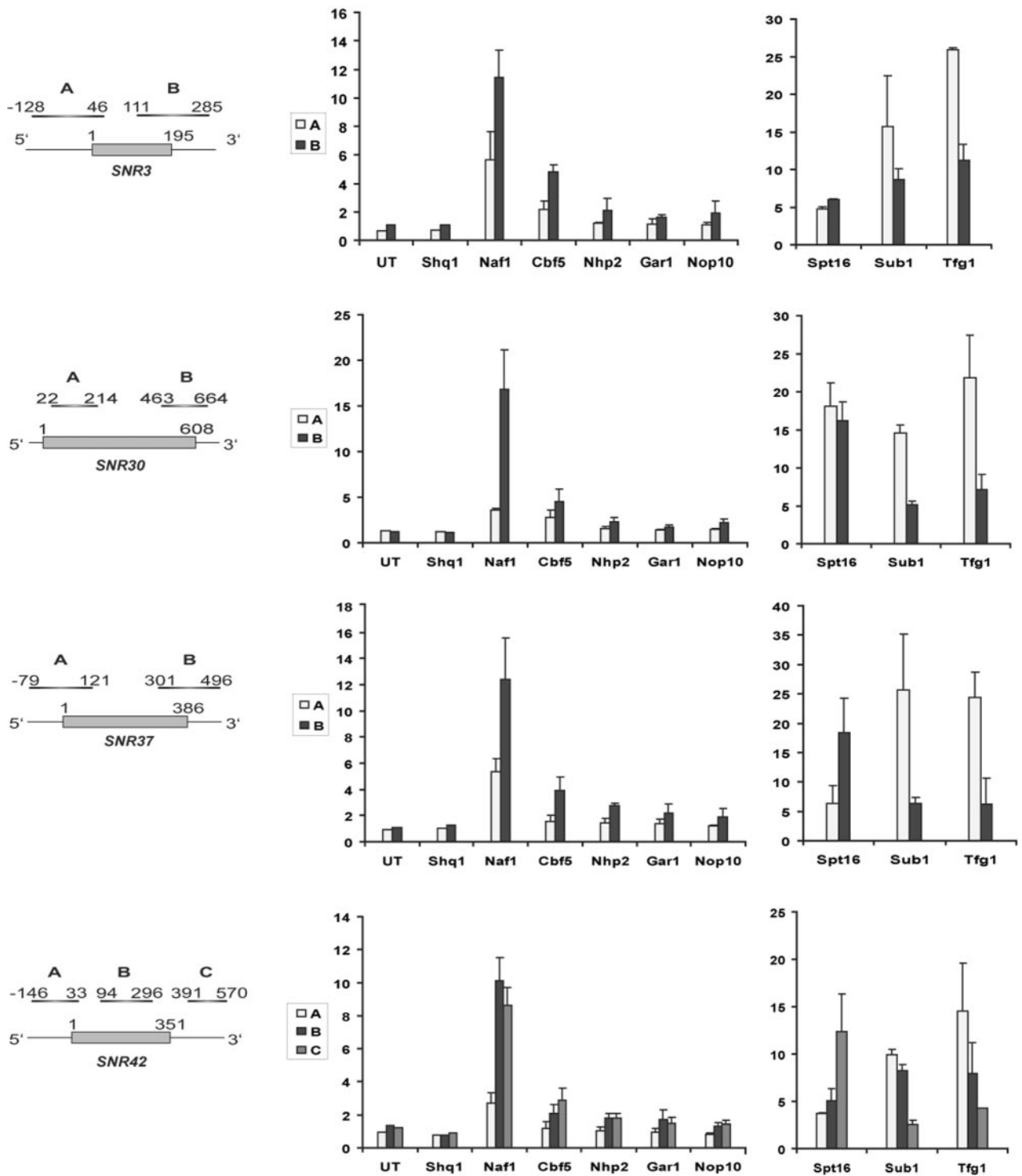


FIG. 3. Naf1p and Cbf5p cross-link to the chromatin of H/ACA snoRNA genes and are enriched in their 3' regions. Shown are quantifications of the ChIP results obtained with H/ACA snoRNA genes. On the left side are schematic diagrams of the box H/ACA snoRNA gene analyzed, with the bars (lettered A, B, and C) above the gene indicating the regions chosen for PCR amplification. On the right is the quantification of the ChIP results for each protein in various regions of each gene. The level of enrichment of Naf1p, Cbf5p, Nhp2p, Gar1p, and Nop10p is the average of three independent experiments. The level of enrichment of Spt16p, Sub1p, and Tfg1p is the average of two independent experiments. UT, untagged.

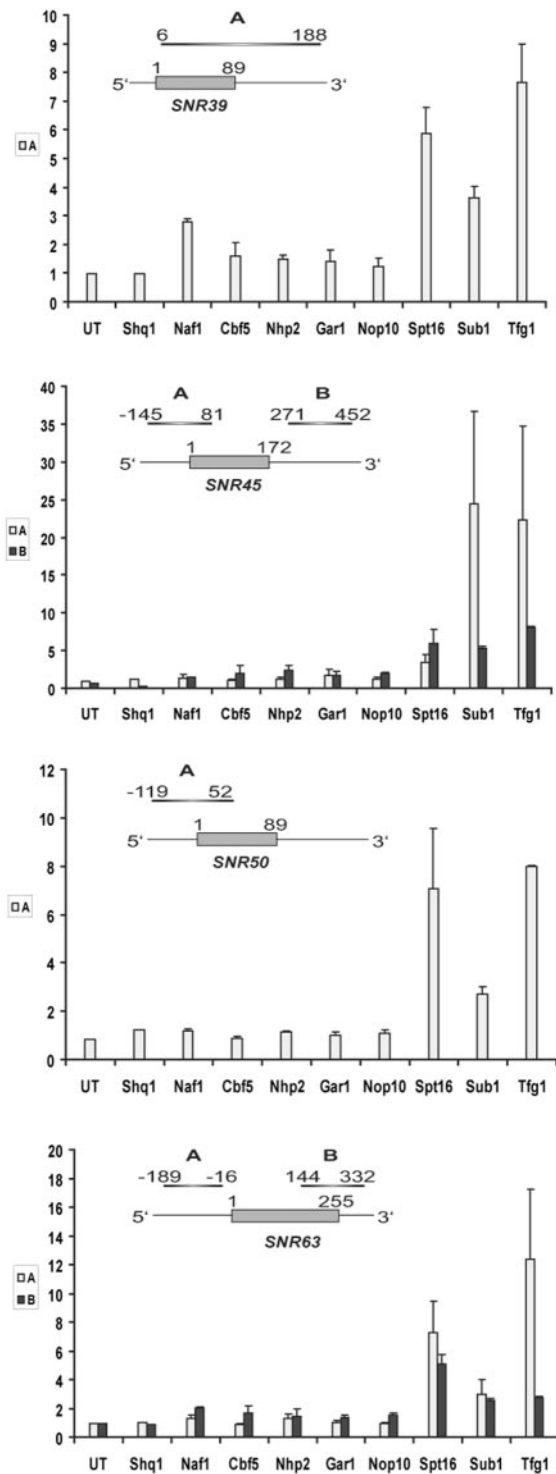


FIG. 4. Naf1p and Cbf5p do not cross-link to the chromatin of C/D snoRNA genes. Shown are quantifications of the ChIP of C/D snoRNA genes and the schematic diagram of each box C/D snoRNA gene with the various regions tested by ChIP. The number of independent experiments is the same as in Fig. 3. UT, untagged.

proteins at the chromatin of four box C/D snoRNA genes, *SNR39*, *SNR45*, *SNR50*, and *SNR63* (Fig. 4). Because of space limitation, only the quantifications of the ChIP assays are shown. These experiments revealed that Naf1p and Cbf5p are

present in the vicinity of all of the H/ACA snoRNA genes tested and that the same gradient of enrichment can be observed toward the 3' end of all H/ACA snoRNA genes. In contrast, no strong enrichment was observed for most of the box C/D snoRNA genes tested (Fig. 4). A slightly higher enrichment was observed for *SNR39* (Fig. 4), but for most C/D snoRNA genes, the enrichments observed were close to background levels, suggesting that Naf1p and Cbf5p associate specifically with H/ACA snoRNA genes. As observed previously for the *SNR32* gene, Nhp2p, Gar1p, and Nop10p showed only marginal or no enrichment, which could not be attributed to a low immunoprecipitation efficiency (see above). In contrast, Spt16p, Tfg1p, and Sub1p were found associated with all of the H/ACA and C/D snoRNA genes tested (Fig. 3 and 4). Spt16p was found sometimes enriched toward the 3' end (*SNR37*, *SNR42*), in a pattern similar to that of Naf1p and Cbf5p, while Sub1p and Tfg1p were predominantly associated with the promoter regions. The pattern of cross-linking of these RNA polymerase II-associated proteins with both types of snoRNA genes is consistent with previously published ChIP profiles for mRNA genes and supports a general role for these factors in RNA polymerase II transcription. Overall, these results demonstrate that Naf1p and the H/ACA pseudouridylyltransferase Cbf5p are present near the chromatin of box H/ACA snoRNA genes, particularly at the 3' end. This suggests that the assembly of the snoRNP begins during transcription elongation or transcription termination of the snoRNA genes.

Cross-linking of Naf1p to the chromatin of H/ACA snoRNA genes requires active transcription but is independent of Ctk1p. To test whether transcription is required for Naf1p association with H/ACA snoRNA genes, we replaced the endogenous *SNR32* gene promoter with a galactose-inducible promoter (*GAL::SNR32*) by homologous recombination (35). We then tested the association of Naf1p with the *SNR32* gene by ChIP in the *GAL::SNR32/NAF1-TAP* strain. ChIP experiments were performed on this strain grown in the presence of galactose, when transcription of *SNR32* is active, and then shifted to a medium containing glucose to repress transcription of the *SNR32* gene. The same experiment was performed in the context of the *SNR32* endogenous promoter (*SNR32* on Fig. 5A) in order to monitor the effects of the carbon source on the association of Naf1p with the normal gene. This experiment showed that the association of Naf1p with the *GAL::SNR32* gene was reduced to background levels when the cells were shifted from galactose to glucose (Fig. 5A), indicating that transcription is required for Naf1p recruitment. We also note that the association of Naf1p with box H/ACA snoRNA genes (*SNR3* and *SNR32*) under the control of their natural promoters is weaker in the presence of galactose than in the presence of glucose. This suggests that H/ACA snoRNA genes are more actively transcribed in glucose than in galactose and that the level of association of Naf1p as monitored by ChIP reflects the levels of transcription. Overall, these experiments demonstrate that the association of Naf1p with the H/ACA snoRNA genes requires active transcription.

Previous reports have shown that Naf1p associates preferentially with the phosphorylated CTD *in vitro* (13), suggesting that Naf1p may associate with the phosphorylated CTD *in vivo*. Ctk1p is the protein kinase involved in phosphorylating the C-terminal domain of RNA polymerase II at serine 2 of the

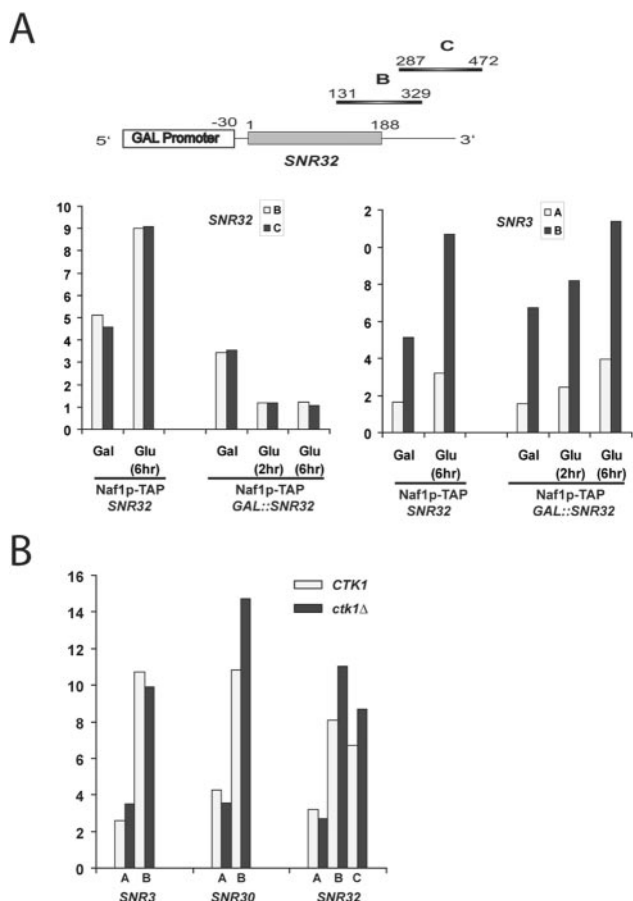


FIG. 5. Cross-linking of Naf1p to H/ACA snoRNA genes requires active transcription but does not require the CTD kinase Ctk1p. (A) Quantification of the ChIP results upon transcriptional repression of the *SNR32* gene. Expression of *SNR32* was placed under the control of a galactose-inducible promoter (Gal promoter) 30 bp upstream of the mature sequence, as indicated in the schematic diagram. Naf1p-TAP *SNR32* (*SNR32* under the control of its endogenous promoter), and Naf1p-TAP *GAL::SNR32* strains were grown in galactose-containing medium (Gal) and then shifted to glucose-containing medium (Glu). For Naf1p-TAP *SNR32*, ChIP experiments were done with cells grown in galactose-containing medium 6 h after a shift to glucose-containing medium. For Naf1p-TAP *GAL::SNR32*, ChIP experiments were done with cells grown in galactose-containing medium and cells shifted to glucose-containing medium for 2 and 6 h. The left bar graph indicates the enrichment level of Naf1p for the *SNR32* gene in the two strains in various media. The right bar graph indicates the enrichment level of Naf1p for the *SNR3* gene, which is under the control of its endogenous promoter. (B) Quantification of the ChIP results of Naf1p-TAP strains containing (*CTK1*) or lacking (*ctk1Δ*) Ctk1p. The graph indicates the level of enrichment for various regions of three box H/ACA snoRNA genes, *SNR3*, *SNR30*, and *SNR32*.

CTD repeats, and this phosphorylation event is a hallmark of late transcriptional elongation (31). The requirement for active transcription and the gradient of association of Naf1p with the downstream regions of H/ACA snoRNA genes suggested that Naf1p associates with H/ACA snoRNA genes in the context of the elongating RNA polymerase. To investigate if Naf1p association with H/ACA snoRNA genes requires phosphorylation of the CTD at serine 2, we performed ChIP experiments on Naf1p in a *ctk1Δ* strain. Deletion of the gene encoding Ctk1p

has been shown to abolish recruitment of mRNA 3'-end processing factors (1). If Naf1p associates with the phosphorylated CTD during transcriptional elongation similarly to mRNA 3'-end processing factors, one might expect that depletion of Ctk1p would inhibit recruitment of Naf1p. ChIP experiments in a *ctk1Δ* deletion background showed that Ctk1p is not required for Naf1p association with H/ACA snoRNA genes (Fig. 5B), suggesting that phosphorylation of the CTD at serine 2 is not a prerequisite for association of Naf1p. This result is reminiscent of data obtained with some RNA polymerase II elongation factors, in particular Spt16p, which are not dependent upon Ctk1p for their association with the chromatin of mRNA genes (1).

Association of Naf1p with box H/ACA snoRNA genes requires an intact H/ACA snoRNA sequence. The previous results showed that transcription is required for association of Naf1p with the H/ACA snoRNA genes. This requirement could be explained either by the recruitment of Naf1p to the transcribed snoRNA genes through an interaction with the transcribing RNA polymerase II machinery or by a recruitment of Naf1p through a direct interaction with the nascent H/ACA snoRNA transcript. The former hypothesis is consistent with the interaction between Naf1p and the CTD of RNA polymerase II observed in vivo by two-hybrid analysis and in vitro by glutathione *S*-transferase pull-down assays (13). It is also consistent with the observed copurification of Spt16p with Naf1p (Fig. 1). The latter model is consistent with the RNA binding properties of Naf1p (13). If Naf1p was recruited by the RNA transcript, one would expect that deletion of the H/ACA snoRNA sequence would inhibit the recruitment of Naf1p to the chromatin. However, such an experiment is problematic. In the case of independently transcribed snoRNA genes, deletion of the H/ACA snoRNA sequence would result in the deletion of almost the entire transcription unit, prohibiting any conclusion with respect to the role of the RNA. To circumvent this problem, we decided to investigate the association of Naf1p with the gene encoding the snR44 H/ACA snoRNA. In contrast to independently transcribed snoRNAs, snR44 is encoded within the second intron of the *RPS22B* gene (Fig. 6A) (8). Therefore, deletion of the snR44 snoRNA sequence in the *RPS22B* long transcription unit is not expected to affect transcription. We deleted the *SNR44* sequence from the chromosomal *RPS22B* locus in the Naf1-TAP-tagged strain by delitto perfetto (Fig. 6A) (47). Northern analysis showed that the steady-state levels of the *RPS22B* mRNA were similar in a wild-type strain and in the *snr44* deletion strains (data not shown), suggesting that deletion of the *SNR44* sequence does not perturb the levels of transcription of the *RPS22B* gene. We then investigated the association of Naf1p with the *RPS22B* transcription unit by ChIP in strains containing the normal *RPS22B* gene or containing the *RPS22B* gene lacking the *SNR44* intronic snoRNA sequence ($\Delta 44$). This experiment showed that Naf1p is associated with the chromatin of the *RPS22B* wild-type gene and that a 5' \rightarrow 3' gradient of enrichment can be observed, with the strongest enrichment observed immediately after the *SNR44* snoRNA sequence (Fig. 6B). This cross-linking profile is consistent with the results that we obtained for independently transcribed snoRNA genes (Fig. 2 and 3). This result shows that Naf1p associates with H/ACA snoRNA genes, regardless of whether the snoRNAs are inde-

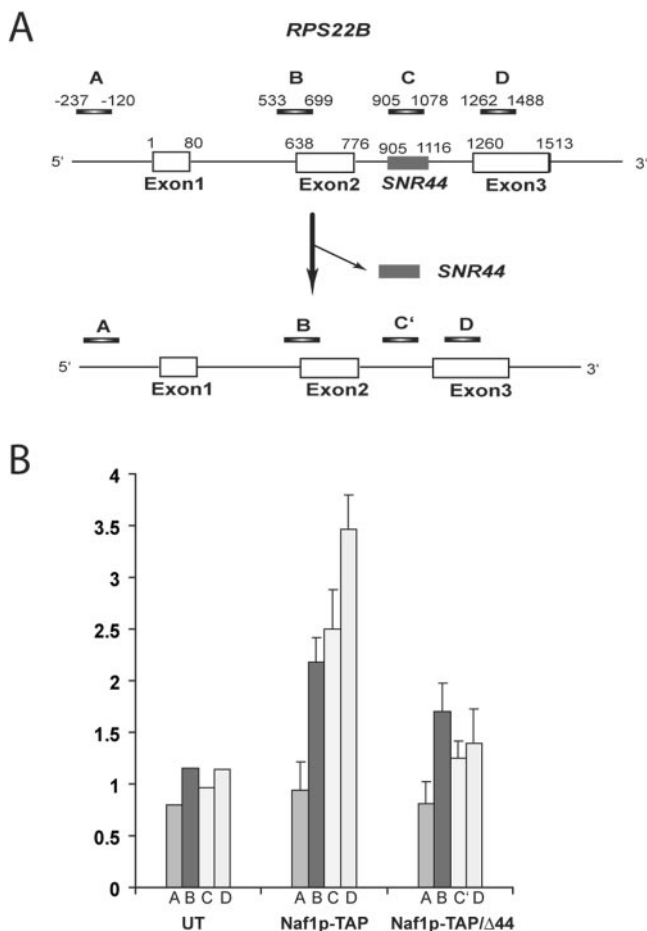


FIG. 6. Cross-linking of Naf1p to the *SNR44* H/ACA snoRNA gene requires an intact H/ACA snoRNA sequence. (A) Genomic organization of the chromosomal *RPS22B* gene and of the *RPS22B* gene lacking the *SNR44* sequence. Also shown are the PCR products corresponding to the primer pairs used in the ChIP assays. (B) Quantification of the ChIP results for Naf1p on the *RPS22B* transcription unit. ChIP experiments were carried out with strains with the *RPS22B* gene containing or lacking the *SNR44* sequence. Various regions of *RPS22B*, shown as bars (lettered A, B, C or C', and D) above the gene diagram, were analyzed by ChIP for Untagged (UT), Naf1p-TAP, or Naf1p-TAP with or without *SNR44* (Naf1p-TAP/ Δ 44). Region C' produces a PCR product that starts 69 bp upstream and ends 104 bp downstream of the mature *SNR44* sequence. The levels of enrichment of Naf1p-TAP and Naf1p-TAP/ Δ 44 to various regions of the gene are derived from three independent experiments.

pendently transcribed or intron encoded. In contrast, the cross-linking of Naf1p with the *RPS22B* gene was reduced to background levels when the *SNR44* sequence was deleted (Fig. 6B). This result strongly suggests that the binding of the RNA by Naf1p mediates the association of Naf1p with the chromatin of H/ACA snoRNA genes and that the H/ACA RNA plays a major role in the cotranscriptional recruitment of Naf1p. To further investigate this finding, we performed ChIP assays and treated the extract after cross-linking but prior to immunoprecipitation with RNase A. RNase treatment had no effect on Naf1p association with H/ACA snoRNA genes (data not shown). However, this negative result does not show that the snoRNAs are not responsible for the recruitment of Naf1p.

Formaldehyde addition results in the formation of protein-protein and protein-DNA cross-links. Therefore, recruitment of Naf1p to the chromatin by the nascent snoRNAs could result in covalent cross-linking of Naf1p to chromatin proteins and to the DNA that cannot be removed by RNase treatment.

DISCUSSION

In this study, we have shown that Naf1p and the H/ACA snoRNP pseudouridylyltransferase Cbf5p can be cross-linked to the 3' region of H/ACA snoRNA genes (Fig. 2 and 3). Moreover, H/ACA snoRNP proteins and the RNA polymerase II factors Spt16p, Tfg1p, and Sub1p copurify with Naf1p (Fig. 1). These data support a model of cotranscriptional assembly of H/ACA snoRNPs. Given the ChIP data obtained for Cbf5p and the interaction between Naf1p and Cbf5p, Naf1p may be responsible for recruiting Cbf5p to the vicinity of the nascent H/ACA snoRNA transcripts. We could not test a direct role for Naf1p in Cbf5p recruitment to the chromatin, since depletion of Naf1p results in codepletion of Cbf5p (11). Alternatively, interaction of Cbf5p with the Paf1 complex (33) or with the phosphorylated RNA polymerase II CTD (42) may be responsible for recruiting Cbf5p. Whatever the details of the mechanisms of the recruitment of Cbf5p, the results obtained in this study are consistent with a model in which the nascent RNA is bound by Naf1p and Cbf5p. We were unable to determine the exact sequence of the interactions among Naf1p, Cbf5p, and the nascent snoRNA. Naf1p could interact with Cbf5p before interacting with the nascent snoRNA or alternatively bind the nascent RNA and then recruit Cbf5p. Direct binding of the nascent snoRNA by Naf1p is consistent with the RNA binding properties of Naf1p (13) and with the results showing that deletion of the *snR44* snoRNA sequence in the *RPS22B* transcription unit abolishes Naf1p recruitment (Fig. 6). The association of Naf1p and RNA polymerase II-associated proteins, in particular Spt16p, could be due to the binding of Naf1p to the nascent transcript in the context of the elongating RNA polymerase. In this respect, it is worth noting that the ChIP profile of Spt16p on H/ACA snoRNA genes is sometimes similar to those of Naf1p and Cbf5p (Fig. 2 and 3). Binding of Cbf5p to the nascent RNA, possibly recruited by Naf1p, would nucleate the assembly of the snoRNP. Binding of the other H/ACA snoRNP proteins may occur at a later stage, as the chromatin enrichment observed for these proteins is often marginal. However, it is also possible that some of these proteins join Cbf5p early during transcriptional elongation but that the epitopes used for the ChIP assays for these other proteins are masked when the proteins are cross-linked to the chromatin, resulting in only marginal enrichment. The gradient of enrichment observed for these proteins is consistent with early recruitment, but in the absence of higher values, it is impossible for us to draw conclusions about their actual presence at the chromatin of H/ACA snoRNA genes.

The enrichment profiles of Naf1p and Cbf5p for H/ACA snoRNA genes show a strong cross-linking gradient toward the 3' ends of these genes. This suggests that Naf1p and Cbf5p are recruited only when most of the transcript has been produced, possibly during the late stages of transcription elongation or transcription termination. Because of the low resolution of the ChIP technique, it is impossible to tell whether the gradient of

enrichment observed toward the 3' ends of the genes is representative of progressive loading of the Naf1p and Cbf5p proteins during elongation or whether loading of Naf1p and Cbf5p occurs only at the very termini of the snoRNA genes.

Recent results have shown that mammalian and yeast H/ACA snoRNP proteins can form a stable complex in the absence of a snoRNA (19, 50). These results have suggested that a preformed cytoplasmic snoRNP protein complex directly binds the snoRNAs. The results presented here show that yeast H/ACA snoRNP assembly occurs cotranscriptionally and that at least one of the H/ACA core snoRNP proteins, Cbf5p, associates with the H/ACA snoRNA gene during transcription elongation or termination. We do not know whether this mode of assembly is also conserved in the case of mammalian H/ACA snoRNPs. Although their protein components are conserved, the mechanisms of snoRNP assembly may differ significantly between yeast and metazoan cells, consistent with a difference in their modes of expression. While most yeast H/ACA snoRNAs are independently transcribed, mammalian snoRNAs are generated from excised lariat introns by debranching and exonucleolytic digestion or by endonucleolytic cleavage. While we detected significant enrichment of Naf1p in the vicinity of an intronic yeast snoRNA sequence (Fig. 5), the binding of biogenesis factors and/or mammalian H/ACA snoRNP proteins may occur only after some of the splicing steps have occurred, and not necessarily in a cotranscriptional manner. This hypothesis is supported by the observation that binding of another class of mammalian C/D snoRNP proteins to the snoRNA transcript occurs only at a late stage of spliceosome assembly (21).

Overall, our results strongly suggest that the yeast H/ACA snoRNP assembly process begins cotranscriptionally. Previous studies have demonstrated a functional linkage between transcription and yeast C/D snoRNP formation. Mutations in the conserved D box perturb C/D snoRNA gene transcription (40). In addition, the box C/D snoRNP protein Nop1p interacts with the chromatin of C/D snoRNA genes (40), suggesting that yeast C/D snoRNP assembly also begins cotranscriptionally. In the case of C/D snoRNPs, a good candidate for a factor whose role is similar to that of Naf1p is Bcd1p, which is not a core component of box C/D snoRNPs, but whose depletion leads to a specific decrease in the levels of yeast C/D snoRNAs (41). Interestingly, Nop1p is thought to carry the methyltransferase activity of C/D snoRNPs. Our results obtained with Cbf5p and those described for Nop1p (40) suggest that the enzyme responsible for the catalytic activity of the snoRNPs is involved in the earliest steps of snoRNP assembly *in vivo*. The enzymes are likely loaded onto the guide RNA cotranscriptionally, with the possible assistance of assembly factors such as Naf1p. Whether or not other snoRNP subunits are also assembled at this stage remains to be determined.

ACKNOWLEDGMENTS

We thank A. Henras, D. Robyr, and S. Buratowski for advice on ChIP experiments; K. Sakurai for help and support; and A. Henras for critical reading of the manuscript. We thank Michèle Caizergues-Ferrer for fruitful discussion and Christophe Dez for assistance with TAP tag purification.

P.K.Y. was supported by a UCLA dissertation year fellowship. C.H. was supported by a Ph.D. fellowship from the Ministère Délégué à la Recherche et aux Nouvelles Technologies. This research was sup-

ported by NIH grant GM61518 (G.C.); the CNRS, Université Paul Sabatier, and La Ligue Nationale contre le Cancer (Equipe Labelisée) (Y.H.); and the Région Midi-Pyrénées and the Gépole Toulouse Midi-Pyrénées (B.M.).

REFERENCES

- Ahn, S. H., M. Kim, and S. Buratowski. 2004. Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3' end processing. *Mol. Cell* **13**:67–76.
- Archambault, J., R. S. Chambers, M. S. Kobor, Y. Ho, M. Cartier, D. Bolotin, B. Andrews, C. M. Kane, and J. Greenblatt. 1997. An essential component of a C-terminal domain phosphatase that interacts with transcription factor IIF in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**:14300–14305.
- Bagni, C., and B. Lapeyre. 1998. Gar1p binds to the small nucleolar RNAs snR10 and snR30 *in vitro* through a nontypical RNA binding element. *J. Biol. Chem.* **273**:10868–10873.
- Balakin, A. G., L. Smith, and M. J. Fournier. 1996. The RNA world of the nucleolus: two major families of small RNAs defined by different box elements with related functions. *Cell* **86**:823–834.
- Bortolin, M. L., and T. Kiss. 1998. Human U19 intron-encoded snoRNA is processed from a long primary transcript that possesses little potential for protein coding. *RNA* **4**:445–454.
- Bousquet-Antonelli, C., Y. Henry, P. G'Elugne, J., M. Caizergues-Ferrer, and T. Kiss. 1997. A small nucleolar RNP protein is required for pseudouridylation of eukaryotic ribosomal RNAs. *EMBO J.* **16**:4770–4776.
- Calvo, O., and J. L. Manley. 2001. Evolutionarily conserved interaction between CstF-64 and PC4 links transcription, polyadenylation, and termination. *Mol. Cell* **7**:1013–1023.
- Danin-Kreiselman, M., C. Y. Lee, and G. Chanfreau. 2003. RNase III-mediated degradation of unspliced pre-mRNAs and lariat introns. *Mol. Cell* **11**:1279–1289.
- Decatur, W. A., and M. J. Fournier. 2002. rRNA modifications and ribosome function. *Trends Biochem. Sci.* **27**:344–351.
- Dez, C., C. Froment, J. Noaillac-Depeyre, B. Monsarrat, M. Caizergues-Ferrer, and Y. Henry. 2004. Npa1p, a component of very early pre-60S ribosomal particles, associates with a subset of small nucleolar RNPs required for peptidyl transferase center modification. *Mol. Cell. Biol.* **24**:6324–6337.
- Dez, C., J. Noaillac-Depeyre, M. Caizergues-Ferrer, and Y. Henry. 2002. Naf1p, an essential nucleoplasmic factor specifically required for accumulation of box H/ACA small nucleolar RNPs. *Mol. Cell. Biol.* **22**:7053–7065.
- Dragon, F., V. Pogacic, and W. Filipowicz. 2000. *In vitro* assembly of human H/ACA small nucleolar RNPs reveals unique features of U17 and telomerase RNAs. *Mol. Cell. Biol.* **20**:3037–3048.
- Fatica, A., M. Dlakic, and D. Tollervy. 2002. Naf1p is a box H/ACA snoRNP assembly factor. *RNA* **8**:1502–1514.
- Filipowicz, W., and V. Pogacic. 2002. Biogenesis of small nucleolar ribonucleoproteins. *Curr. Opin. Cell Biol.* **14**:319–327.
- Gavin, A. C., et al. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**:141–187.
- He, X., A. U. Khan, H. Cheng, D. L. Pappas, Jr., M. Hampsey, and C. L. Moore. 2003. Functional interactions between the transcription and mRNA 3' end processing machineries mediated by Ssu72 and Sub1. *Genes Dev.* **17**:1030–1042.
- Heiss, N. S., S. W. Knight, T. J. Vulliamy, S. M. Klauck, S. Wiemann, P. J. Mason, A. Poustka, and I. Dokal. 1998. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat. Genet.* **19**:32–38.
- Henras, A., C. Dez, J. Noaillac-Depeyre, Y. Henry, and M. Caizergues-Ferrer. 2001. Accumulation of H/ACA snoRNPs depends on the integrity of the conserved central domain of the RNA-binding protein Nhp2p. *Nucleic Acids Res.* **29**:2733–2746.
- Henras, A., Y. Henry, C. Bousquet-Antonelli, J. Noaillac-Depeyre, J. P. Gelugne, and M. Caizergues-Ferrer. 1998. Nhp2p and Nop10p are essential for the function of H/ACA snoRNPs. *EMBO J.* **17**:7078–7090.
- Henras, A. K., R. Capeyrou, Y. Henry, and M. Caizergues-Ferrer. 2004. Cbf5p, the putative pseudouridine synthase of H/ACA-type snoRNPs, can form a complex with Gar1p and Nop10p in absence of Nhp2p and box H/ACA snoRNAs. *RNA* **10**:1704–1712.
- Henras, A. K., C. Dez, and Y. Henry. 2004. RNA structure and function in C/D and H/ACA s(no)RNPs. *Curr. Opin. Struct. Biol.* **14**:335–343.
- Hirose, T., M. D. Shu, and J. A. Steitz. 2003. Splicing-dependent and -independent modes of assembly for intron-encoded box C/D snoRNPs in mammalian cells. *Mol. Cell* **12**:113–123.
- Ho, Y., A. Gruhler, A. Heilbut, G. D. Bader, L. Moore, S. L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutillier, L. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A. R. Willems, H. Sassi, P. A. Nielsen, K. J. Rasmussen, J. R. Andersen, L. E. Johansen, L. H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen,

- B. D. Sorensen, J. Matthieson, R. C. Hendrickson, F. Gleeson, T. Pawson, M. F. Moran, D. Durocher, M. Mann, C. W. Hogue, D. Figeys, and M. Tyers. 2002. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* **415**:180–183.
23. Ito, T., T. Chiba, R. Ozawa, M. Yoshida, M. Hattori, and Y. Sakaki. 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. USA* **98**:4569–4574.
24. Jady, B. E., E. Bertrand, and T. Kiss. 2004. Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. *J. Cell Biol.* **164**:647–652.
25. Kim, M., S. H. Ahn, N. J. Krogan, J. F. Greenblatt, and S. Buratowski. 2004. Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. *EMBO J.* **23**:354–364.
26. King, T. H., W. A. Decatur, E. Bertrand, E. S. Maxwell, and M. J. Fournier. 2001. A well-connected and conserved nucleoplasmic helicase is required for production of box C/D and H/ACA snoRNAs and localization of snoRNP proteins. *Mol. Cell. Biol.* **21**:7731–7746.
27. Kiss, T. 2001. Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs. *EMBO J.* **20**:3617–3622.
28. Kiss, T., and W. Filipowicz. 1995. Exonucleolytic processing of small nucleolar RNAs from pre-mRNA introns. *Genes Dev.* **9**:1411–1424.
29. Kiss, T., and W. Filipowicz. 1993. Small nucleolar RNAs encoded by introns of the human cell cycle regulatory gene *RCC1*. *EMBO J.* **12**:2913–2920.
30. Kobor, M. S., L. D. Simon, J. Omichinski, G. Zhong, J. Archambault, and J. Greenblatt. 2000. A motif shared by TFIIF and TFIIB mediates their interaction with the RNA polymerase II carboxy-terminal domain phosphatase Fcp1p in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **20**:7438–7449.
31. Komarnitsky, P., E. J. Cho, and S. Buratowski. 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. *Genes Dev.* **14**:2452–2460.
32. Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, and J. F. Greenblatt. 2002. RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. *Mol. Cell. Biol.* **22**:6979–6992.
33. Krogan, N. J., W. T. Peng, G. Cagney, M. D. Robinson, R. Haw, G. Zhong, X. Guo, X. Zhang, V. Canadian, D. P. Richards, B. K. Beattie, A. Laley, W. Zhang, A. P. Davierwala, S. Mnaimneh, A. Starostine, A. P. Tikuisis, J. Grigull, N. Datta, J. E. Bray, T. R. Hughes, A. Emili, and J. F. Greenblatt. 2004. High-definition macromolecular composition of yeast RNA-processing complexes. *Mol. Cell* **13**:225–239.
34. Lafontaine, D. L., C. Bousquet-Antonelli, Y. Henry, M. Caizergues-Ferrer, and D. Tollervey. 1998. The box H + ACA snoRNAs carry Cbf5p, the putative rRNA pseudouridine synthase. *Genes Dev.* **12**:527–537.
35. Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle. 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* **14**:953–961.
36. Mason, P. B., and K. Struhl. 2003. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol. Cell. Biol.* **23**:8323–8333.
37. Meier, U. T., and G. Blobel. 1994. NAP57, a mammalian nucleolar protein with a putative homolog in yeast and bacteria. *J. Cell Biol.* **127**:1505–1514.
38. Mitchell, J. R., E. Wood, and K. Collins. 1999. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* **402**:551–555.
39. Mochizuki, Y., J. He, S. Kulkarni, M. Bessler, and P. J. Mason. 2004. Mouse dyskerin mutations affect accumulation of telomerase RNA and small nucleolar RNA, telomerase activity, and ribosomal RNA processing. *Proc. Natl. Acad. Sci. USA* **101**:10756–10761.
40. Morlando, M., M. Ballarino, P. Greco, E. Caffarelli, B. Dichtl, and I. Bozoni. 2004. Coupling between snoRNP assembly and 3' processing controls box C/D snoRNA biosynthesis in yeast. *EMBO J.* **23**:2392–2401.
41. Peng, W. T., M. D. Robinson, S. Mnaimneh, N. J. Krogan, G. Cagney, Q. Morris, A. P. Davierwala, J. Grigull, X. Yang, W. Zhang, N. Mitsakakis, O. W. Ryan, N. Datta, V. Jovic, C. Pal, V. Canadian, D. Richards, B. Beattie, L. F. Wu, S. J. Altschuler, S. Rowles, B. J. Frey, A. Emili, J. F. Greenblatt, and T. R. Hughes. 2003. A panoramic view of yeast noncoding RNA processing. *Cell* **113**:919–933.
42. Phatnani, H. P., J. C. Jones, and A. L. Greenleaf. 2004. Expanding the functional repertoire of CTD kinase I and RNA polymerase II: novel phosphoCTD-associating proteins in the yeast proteome. *Biochemistry* **43**:15702–15719.
43. Richard, P., X. Darzacq, E. Bertrand, B. E. Jady, C. Verheggen, and T. Kiss. 2003. A common sequence motif determines the Cajal body-specific localization of box H/ACA scaRNAs. *EMBO J.* **22**:4283–4293.
44. Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann, and B. Seraphin. 1999. A generic protein purification method for protein complex characterization and proteome exploration. *Nat. Biotechnol.* **17**:1030–1032.
45. Ruggero, D., S. Grisendi, F. Piazza, E. Rego, F. Mari, P. H. Rao, C. Cordon-Cardo, and P. P. Pandolfi. 2003. Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. *Science* **299**:259–262.
46. Steinmetz, E. J., N. K. Conrad, D. A. Brow, and J. L. Corden. 2001. RNA-binding protein Nrd1 directs poly(A)-independent 3'-end formation of RNA polymerase II transcripts. *Nature* **413**:327–331.
47. Storic, F., L. K. Lewis, and M. A. Resnick. 2001. In vivo site-directed mutagenesis using oligonucleotides. *Nat. Biotechnol.* **19**:773–776.
48. Sun, Z. W., and M. Hampsey. 1995. Identification of the gene (*SSU71/TFG1*) encoding the largest subunit of transcription factor TFIIF as a suppressor of a TFIIB mutation in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **92**:3127–3131.
49. Tollervey, D., and T. Kiss. 1997. Function and synthesis of small nucleolar RNAs. *Curr. Opin. Cell Biol.* **9**:337–342.
50. Wang, C., and U. T. Meier. 2004. Architecture and assembly of mammalian H/ACA small nucleolar and telomerase ribonucleoproteins. *EMBO J.* **23**:1857–1867.
51. Yang, P. K., G. Rotondo, T. Porras, P. Legrain, and G. Chanfreau. 2002. The Shq1p.Naf1p complex is required for box H/ACA small nucleolar ribonucleoprotein particle biogenesis. *J. Biol. Chem.* **277**:45235–45242.