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Precursors to the U3 Small Nucleolar RNA Lack Small Nucleolar RNP Proteins but Are Stabilized by La Binding

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Almost all small eukaryotic RNAs are processed from transiently stabilized 3′-extended forms. A key question is how and why such intermediates are stabilized and how they can then be processed to the mature RNA. Here we report that yeast U3 is also processed from a 3′-extended precursor. The major 3′-extended forms of U3 (U3-I and -II) lack the cap trimethylation present in mature U3 and are not associated with small nucleolar RNP (snoRNP) proteins that bind mature U3, i.e., Nop1p, Nop56p, and Nop58p. Depletion of Nop58p leads to the loss of mature U3 but increases the level of U3-I and -II, indicating a requirement for the snoRNP proteins for final maturation. Pre-U3 is cleaved by the endonuclease Rnt1p, but U3-I and -II do not extend to the Rnt1p cleavage sites. Rather, they terminate at poly(U) tracts, suggesting that they might be bound by Lhp1p (the yeast homologue of La). Immunoprecipitation of Lhp1p fused to Staphylococcus aureus protein A resulted in coimmunoprecipitation of both U3-I and -II. Deletion of LHP1, which is nonessential, led to the loss of U3-I and -II. We conclude that pre-U3 is cleaved by Rnt1p, followed by exonuclease digestion to U3-I and -II. These species are stabilized against continued degradation by binding to Lhp1p. Displacement of Lhp1p by binding of the snoRNP proteins allows final maturation, which involves the exosome complex of 3′→5′ exonucleases.

Eukaryotic cells contain a large number of stable RNA species, nearly all of which are synthesized by posttranscriptional processing from larger precursors. This has long been known for the highly abundant cytoplasmic RNAs, tRNAs, and rRNAs, but more recently it has become clear that is also the case for the small nuclear RNAs (snRNAs), which participate in pre-mRNA splicing, and the small nucleolar RNAs (snoRNAs), which participate in rRNA processing and modification. It is a long-standing mystery why cells use such a strategy, rather than simply terminating transcription at the end of the mature RNA sequence. We will offer a potential explanation for this, at least in the case of the U3 snoRNA.

Analyses of the 3′ end processing of the 5.8S rRNA in Saccharomyces cerevisiae led to the identification of the exosome complex, composed of 11 different 3′→5′ exonucleases (6, 36, 37; E. Petfalski and D. Tollervey, unpublished data). Subsequent work showed that the exosome participates in the 3′ processing of other RNA substrates, including many snRNAs and snoRNAs (5, 55), and also participates in mRNA turnover (9). A homologous complex, designated the PM-Scl complex, is present in human cells and is a target for autoimmune antibodies (6).

In addition to the exosome, normal 3′ processing of the U1, U2, U4, and U5 snRNAs involves cleavage by the endonuclease Rnt1p (1, 5, 14, 45), the yeast homologue of Escherichia coli Rnase III (2). Rnt1p cleaves on both sides of extended stem-loop structures with closing AGNN tetraloops (15), and some complex, composed of 11 different 3′→5′ exonucleases.

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MATERIALS AND METHODS

Strains. Growth and handling of S. cerevisiae were by standard techniques. The transformation procedure was as described elsewhere (21). Yeast strains used and constructed in this study are listed in Table 1. Wild-type RNT1 and rnt1Δ sister strains (15) were used to prepare whole-cell extract. Strain ratl-I was kindly provided by C. Cole (7). The nonessential gene LHP1 was disrupted and tagged with Staphylococcus aureus protein A (“ProtA” in construct designations) at the carboxy-terminal end of Lhp1p by a PCR strategy (28) in the haploid strain YDL401, using the Kluyveromyces lactis URA3 (3) and ratl-I was used as a control strain. The oligonucleotides used to construct and test the gene disruption and protein A tagging were as listed in Table 1. Wild-type LHP1 and lhp1Δ strains were used to prepare whole-cell extract. Strain ratl-I was kindly provided by C. Cole (7). The nonessential gene LHP1 was disrupted and tagged with Staphylococcus aureus protein A (“ProtA” in construct designations) at the carboxy-terminal end of Lhp1p by a PCR strategy (28) in the haploid strain YDL401, using the Kluyveromyces lactis URA3 marker.

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TABLE 1. Yeast strains used in this work

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TC; 84 (5′ LHP1::ProtA); 5′-GAGGAGCTTCTTCGCCATGGCCAGTGAGCA GGAGCACAGAGGGGGGTCAACAAATTCC; and 843 (3′ LHP1::ProtA); 5′-TCTATCTTAAAACATGGAATTTATACCTAA AAGAAGGCTGTGAGCAGTGTCGCCTGTCGC. RNA extraction, Northern hybridization, and primer extension. For depletion of Rrp41p and Rrp45p, cells were harvested at intervals following the shift from YSO medium (2% galactose, 2% sucrose, 2% raffinose) to medium containing 2% glucose. Otherwise, strains were grown in YPD medium. RNA was extracted as described previously (52). Northern hybridization and primer extension were as described previously (12, 51). Standard 6 or 8% acrylamide gels were used to analyze low-molecular-weight RNA species and primer extension reactions. For RNA hybridization and primer extension, the following oligonucleotides were used: 200 (U3), 5′-AAACGAAATAAATTCTTTGTAAA; 203 (5′U3), 5′-CAAAAATGTTGTTAACTTGTCA; 252 (U3ADS), 5′-TTCTTTTTTGAAGGGAT; and reverse primers 253 (U3DS), 5′-GCGAATTCTAATACGACTCACTATAGGTAC. The synthesis of U3 from cDNA constructs was analyzed by expression of the ARS-CEN pU3-wt plasmid carrying an ARS1-A-intron, which hybridizes with the pre-mRNA splicing machinery (38). The size and hybridization pattern of U3-A-int 3′ are identical except that the oligo(dT) was omitted. Immunoprecipitation. For immunoprecipitation of ProtA-Nop1p, ProtA-Nop58p, ProtA-Nop56p, Lhp1p-ProTa, and m3G-capped RNAs, yeast whole-cell extracts were prepared as described elsewhere (46) except that for immunoprecipitation of m3G-capped RNAs, cells were resuspended in buffer A (150 mM potassium acetate [KAc; pH 7.5], 20 mM Tris–Ac, 5 mM MgAc) with 1 mM dithiothreitol, 0.5% Triton X-100, and 5 mM phenylmethylsulfonyl fluoride. Immunoprecipitation of ProtA-Nop1p, ProtA-Nop58p, and Lhp1p-ProTa with rabbit immunoglobulin G (IgG) agarose beads (Sigma) was performed as previously described (33) at 150 mM salt (KAc) concentration. For immunoprecipitation with m3G-capped specific serum (R1131; kindly provided by R. Lührmann), 30 μl of suspension of protein G-Sepharose was washed with phosphate-buffered saline buffer and incubated on a rotating wheel with extract equivalent to 4 units of optical density at 600 nm of cells in 120 μl of buffer A for 2 h at 4°C. The pellet was washed in buffer A, bound m3G-capped RNAs were eluted in 10 μl of 5 M guanidium thiocyanate (5 M G (Pharmacia) in 30 μl of buffer A. The RNAs were extracted with GTC phenol–chloroform and ethanol precipitated.

RESULTS

Yeast cells contain 3′-extended forms of U3. Yeast U3 is encoded by two genes, SNR17A, encoding U3A, and SNR17B, encoding U3B (25). U3A is approximately 10-fold more abundant than U3B (25), and all analyses have been performed for U3A. On Northern hybridization, probe 200, to mature U3A, was observed to hybridize to two RNA species of slower gel mobility (U3-3′ and U3-3′ II) in total yeast RNA preparations (Fig. 1A, lane 1) that were estimated to be approximately 10 and 20 nucleotides (nt), respectively, longer than the mature U3 (333 nt). A probe complementary to the sequence across the 3′ end of the mature U3A (probe 251), which hybridizes specifically to 3′-extended species, also detected these RNA species as well as a longer species (U3-int 3′) of approximately 470 nt. Both SNR17A and SNR17B contain introns that are excised by the pre-mRNA splicing machinery (38). The size and hybridization pattern of U3-int 3′ indicates that it corresponds to a 3′-extended precursor that retains the intron (Fig. 1D and 6B).
It is not clear whether U3-int 3' has 3' ends identical to those of U3-3'I and U3-3''I. Synthesis of the U3-3'I and U3-3''I RNAs was not affected by the presence or absence of the intron in the pre-snoRNA, since identical species were observed in strains expressing U3 cDNA constructs (see Materials and Methods) (data not shown).

The mature U3 carries a 5' trimethylguanosine (TMG) cap structure (25) and was precipitated with anti-TMG antibodies (Fig. 1A, lane 3) (generously provided by R. Lührmann, University of Marburg). In contrast, the U3-3'I, U3-3''I, and U3-3''II RNAs were not precipitated with anti-TMG and were recovered exclusively in the immune supernatant (Fig. 1A, lane 2). Mature yeast U3, like all box C+D snoRNAs, is associated with Nop1p, Nop56p, and Nop58p (30, 31, 44) and was coprecipitated with protein A-tagged fusion proteins (Fig. 1B, lanes 3, 6, and 9). No association of U3-3'I, U3-3''I, or U3-3''II with these proteins was observed, and the RNAs were again recovered exclusively in the immune supernatants (Fig. 1B, lanes 2, 5, and 8).

Genetic depletion of Nop58p leads to the loss of all tested box C+D snoRNAs including U3 (30). The GAL::nop58 strain was pregrown on permissive, galactose medium (0-h sample) and then transferred to glucose to repress synthesis of Nop58p (Fig. 1C). Mature U3 was codepleted with Nop58p, whereas the levels of the U3-3'I and U3-3''II RNAs were increased. The U3-int 3' species was unaffected.

We conclude that the U3 snoRNA is synthesized from 3' extended precursors that lack the TMG cap structure. The pre-U3 species are not associated with snoRNP proteins and, unlike the mature snoRNA, do not require Nop58p for stability. Indeed, the accumulation of U3-3'I and U3-3''II in strains depleted of Nop58p indicates that their normal maturation to U3 requires Nop58p binding.

3' processing of U3 involves cleavage by Rnt1p. Rnt1p cleaves 3'-extended precursors to the U1, U2, U4, and U5 snRNAs and processes polycistronic pre-snoRNAs. We therefore determined whether it is also involved in the 3' processing of pre-U3 species. In strains carrying a complete deletion of the RNT1 gene, the level of mature U3 was reduced approximately threefold (Fig. 1D, I; see also Table 2). Strains carrying rnt1::Δ lacked the U3-3'I and U3-3''II RNAs (Fig. 1D, II) and we observed a heterogeneous group of RNAs extending to approximately 600 nt (see Fig. 6A, lane 16, where more RNA is loaded). In addition, the intron-containing precursor was found to be 3' processed in the rnt1::Δ strain, in contrast to the 3'-extended form seen in the wild type (Fig. 1D, III, lane 2; see also Fig. 6C, lanes 12 to 14). The reduced levels of U3 in the rnt1::Δ strain were initially postulated to be due to impaired splicing (15). However, subsequent work indicated that splicing was not defective in the rnt1::Δ strain (45) and, as shown in Fig. 1D, there is no overall accumulation of intron-containing forms of U3.

We conclude that 3' processing of U3 normally involves cleavage by Rnt1p. In the absence of cleavage, long 3'-extended forms are synthesized. The time required for these to be synthesized and then processed may allow assembly of the mature snoRNP proteins, and processing proceeds directly to the 3' end of the mature snoRNA. This processing is, however,
Rnt1p cleaves on both sides of extended stem-loop structures with a closing AGNN tetraloop (15). Inspection of the 3' flanking sequences revealed the presence of good matches to consensus Rnt1p cleavage sites 3' to both SNR17A and SNR17B, the genes encoding U3A and U3B, respectively (shown for SNR17A in Fig. 2D). To confirm that these are authentic cleavage sites, the cleavage of the SNR17A site was tested in vitro. The U3(−60/+139) in vitro transcript, which spans the region between positions −60 and +139 with respect to the mature U3 3' end including the predicted stem-loop structure, was used to map the cleavage site by primer extension (Fig. 2A). Incubation with recombinant His<sub>6</sub>-Rnt1p (Fig. 2A, lane 5) resulted in the appearance of two primer extension stops that were not detected after incubation in the absence of Rnt1p (Fig. 2A, lane 6). The primer extension stops were at nt +22 and +59, corresponding to cleavage between nt +21/22 and +58/+59, and are in good agreement with the consensus sites of Rnt1p cleavage (Fig. 2D). To demonstrate that in vitro processing is by endonuclease cleavage, a longer transcript was labeled internally; U3(−60/+177) spans the 3' region of the U3A precursor between positions −60 and +177. Incubation with either recombinant His<sub>6</sub>-Rnt1p (Fig. 2B, lanes 3 to 5) or an extract from a wild-type (RNT1<sup>+</sup>) strain of yeast (Fig. 2B, lane 6) led to the appearance of a set of discrete cleavage products that were not observed with the no-enzyme control reaction (Fig. 2B, lane 2) or with an extract from an <i>rnt1</i>Δ strain of yeast (Fig. 2B, lane 7). The substrate is 237 nt, and comparison to size markers (Fig. 2B, lanes 1 and 8) indicated that the sizes of the three smaller species were in good agreement with the predicted cleavage products: from +59 to the 3' end of the transcript (predicted size, 119 nt) (band a), from the 5' end to +21 (predicted size, 81 nt) (band b), and from +22 to +58 (predicted size, 37 nt) (band c).

The 3' fragments generated by Rnt1p cleavage of the pre-U4 snRNA and the pre-rRNA are strongly stabilized by mutation of the nuclear 5'→3' exonuclease Rat1p (5, 28), indicating that it normally degrades these regions. The sites of in vivo cleavage of pre-U3 were identified by primer extension using probe 252, which hybridizes in the SNR17A flanking sequence 3' to the stem-loop structure. In the <i>rat1</i>Δ strain (Fig. 2C, lane 5), primer extension stops were observed at +22 and +59, identical to the in vitro cleavage sites. These were absent from RNA extracted from the <i>rnt1</i>Δ strain (Fig. 2C, lane 6) but were also not detectable in the wild-type strain (Fig. 2C, lane 7). The stop corresponding to the position of the 3' end of mature U3 may be a consequence of the stem structure at this position. The level of this stop is unaltered in the <i>rnt1</i>Δ strain, suggesting that it is not a cleavage site. We cannot, however, exclude the possibility that a fraction of U3 is processed by endonucleolytic cleavage at the mature 3' end. RNase MRP was shown not to be involved in this process (data not shown).

We conclude that Rnt1p cleaves the 3' extended pre-U3 at +21/+22 and +58/+59. Following cleavage the 3' fragment is inefficient since mature U3 levels are strongly reduced (Fig. 2D; see also Table 2).

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The level of the mature U3 is reduced in strains lacking Rnt1p, indicating that this is normally the major degradation pathway.

The major 3′-extended forms of U3 do not extend to the Rnt1p cleavage sites. High-resolution Northern hybridization showed that the U3-3′I band was too small to extend to the Rnt1p cleavage sites, and even the larger U3-3′ II species appeared to be slightly smaller than expected. The 3′ ends of these species were therefore determined by RNase protection. For this, the region of SNR17A from 295 to +36 was amplified by PCR using a primer that incorporated a T7 promoter (see Materials and Methods). RNA was extracted from wild-type (WT), rnt1 Δ, and lhp1 Δ strains grown at 30°C and from GAL:rep41 and GAL:rep41/rnt1 Δ strains following transfer from permissive, RSG medium to repressive, glucose medium at 30°C for 24 and 48 h, respectively. Total E. coli tRNA was used as a control RNA. Positions of the Rnt1p-dependent protected species at +12 and +18 are indicated. (B) Schematic of the U3 3′ flanking region showing the ends of the protected regions and the Rnt1p cleavage sites.

To confirm this, a C-terminal fusion between Lhp1p and two copies of the Z domain of S. aureus protein A was constructed and integrated at the chromosomal LHP1 locus by a one-step PCR approach (29) (see Materials and Methods). Western blotting confirmed that the fusion protein was expressed and could be efficiently immunoprecipitated with IgG agarose (data not shown). Immunoprecipitation was performed on two independently isolated Lhp1p-ProtA strains; data are presented for only one strain in Fig. 5. Processing of pre-tRNA35S appeared to be the same in the strain expressing only Lhp1p-ProtA and the wild type (Fig. 5D); however, some accumulation of the shorter 3′-extended pre-U3 species was visible (Fig. 5A), suggesting that the Lhp1p-ProtA fusion protein is under-expressed or otherwise not fully functional.

As expected, the tRNA35S primary transcript (Fig. 5D) and the U6 snRNA (Fig. 5E) were immunoprecipitated on IgG agarose from the strain expressing Lhp1p-ProtA (lane 6) but not from the wild type (lane 3). Both U3-3′I and U3-3′II were coprecipitated with Lhp1p-ProtA (Fig. 5A), as were U3-int 3′ and a species of approximately 800 nt designated U3-3′III (Fig. 5B). The species shorter than U3-3′I seen in the Lhp1p-ProtA strain was not coprecipitated and remained in the immune degraded by Rat1p. The level of the mature U3 is reduced in strains lacking Rnt1p, indicating that this is normally the major synthesis pathway.

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To confirm this, a C-terminal fusion between Lhp1p and two copies of the Z domain of S. aureus protein A was constructed and integrated at the chromosomal LHP1 locus by a one-step PCR approach (10) using the K. lactis URA3 marker (see Materials and Methods). RNAse protection analysis of RNA from the lhp1 Δ strain showed the loss of the major 3′-ended ends at +18 and +12 and the appearance of shorter, heterogeneous protected fragments corresponding to RNAs from U3-3′ to U3-3′I (Fig. 3A, lane 7). This result was confirmed by Northern hybridization (Fig. 4). The U3-3′I and U3-3′II species were absent from the lhp1 Δ strain (Fig. 4A), and a species slightly shorter than U3-3′I was detected. The level of mature U3 was unaffected in the lhp1 Δ strain (Figs. 3A and 4B), as were the sizes of the truncated U3 degradation intermediates seen in wild-type cells (see Fig. 6; data not shown). These data suggested that both U3-3′I and U3-3′II were stabilized by binding to Lhp1p.

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supernatant (Fig. 5A, lane 5). Mature U3 (Fig. 5B and C) and the 3'-processed, intron-containing pre-tRNA Tyr (Fig. 5D) were recovered at the same low levels in the wild-type and Lhp1-ProtA precipitates. The pre-U3 and pre-tRNA species were more efficiently precipitated than U6, presumably because only the newly synthesized U6 is associated with Lhp1p (35, 39).

We conclude that Lhp1p binds and stabilizes the major 3'-extended forms of U3.

The exosome participates in 3'-processing of U3. The levels of 3'-extended precursors to other snoRNAs and snRNAs are elevated in strains carrying mutations in the exosome complex (5, 55). To assess the effects of genetic depletion of exosome components on the 3'-extended forms of U3, Rrp41p and Rrp45p were depleted by transfer of GAL::rrp41 and GAL::rrp45 strains (6, 36) from permissive RSG medium (0-h samples) to repressive, glucose medium for the times indicated. A strain deleted for the gene encoding the Rrp6p component of the exosome (6) was also analyzed. In the strains lacking Rrp41p (Fig. 6A and C, lanes 5), Rrp45p (lanes 10), or Rrp6p (lanes 2), the levels of U3-3'1 and U3-3'II were higher than in the isogenic wild-type control strains (lanes 3 and 14); these results are quantitated in Table 2. For the GAL::rrp41 strain, this increase was confirmed by RNase protection (Fig. 3A, lane 4), which showed that the accumulated precursors were identical to U3-3'1 and -II. Rrp41p is underexpressed in the GAL::rrp41 strain in RSG medium and therefore shows some accumulation of the extended species in the 0-h sample (6, 36).

In strains genetically depleted of other exosome components, Rrp4p, Rrp40p, Rrp46p, or Csl4p, increased levels of U3-3'1 and -II were also observed (data not shown). In addition, an RNA species that comigrated with the U3-3'III RNA, seen on Lhp1p-ProtA precipitation (Fig. 5), was accumulated in the exosome mutants. On prolonged exposure, this species could also be detected at low levels in wild-type cells. Depletion of the exosome components did not lead to depletion of the mature U3. Indeed, as was previously observed for the U4 and U5 snRNAs, depletion of exosome components led to an increase in the mature U3 snoRNA of approximately twofold (Table 2).
In strains lacking exosome components, the 3′ processed, intron-containing precursor is clearly detected. This is most visible for Rrp6p (Fig. 6B, lane 2) but was also seen for several other exosome mutants (Fig. 6B and data not shown). This species is not detected in the wild type, and we speculate that this processing intermediate is normally a dead-end product that is degraded by the exosome. 3′ processing appears to be dependent on snoRNP protein binding, but assembly with the mature snoRNP proteins may be incompatible with assembly of a functional spliceosome. The exosome also degrades other stalled, intron-containing pre-mRNAs (C. Bousquet-Antonelli, C. Presutti, and D. Tollervey, submitted for publication).

The combination of the deletion of both RNT1 and RRP6 (Fig. 6, lane 1) partially restored synthesis of species with the same gel mobility as the U3-3′I and U3-3′II RNAs. Depletion of Rrp41p or Rrp45p from the strain lacking Rnt1p (Fig. 6A and C, lanes 7, 8, 12, and 13) led to the appearance of heterogeneous RNA species slightly smaller than U3-3′I, similar in size to the species seen in the lhp1-Δ strain (Fig. 4). Consistent with this, RNase protection analysis in the GAL::rrp41/rnt1 strain reveals a ladder of protected RNA fragments extending from mature U3 to position U3+12 (Fig. 3A, lane 6); due to the location of the hybridization probe, the only longer RNAs were detected by Northern hybridization (Fig. 6). A stronger ladder of RNA species extending up to the position of U3-3′III was observed by Northern hybridization (Fig. 6, lanes 7, 8, 12, and 13), which was reflected by the strong protection of the full-length antisense probe (Fig. 3A, lane 6). The combination of each of exosome mutants with rnt1-Δ partially restored the mature U3 levels compared to the rnt1-Δ single mutant strain (Table 2).

We conclude that the exosome complex of 3′→5′ exonucleases participates in the 3′ processing of U3. This processing pathway closely resembles that of the U1, U4, and U5 snRNAs (5, 14, 45, 55). In each case, synthesis of the mature RNA continues in strains depleted of single components of the exosome, indicating that either different components of the complex are partially functionally redundant or that other exonucleases can largely substitute for the exosome.

The level of the mature U3 is elevated in the exosome mutants, indicating competition between the synthetic pathway and degradation of the pre-U3. This was also seen for the U4 and U5 snRNAs (5). Consistent with this model, a truncated U3 species (U3**) was observed in wild-type strains (Fig. 7, lanes 1 and 12) (24, 35). The U3** species was 5′ and 3′ truncated, as shown by its failure to hybridize to probes directed against either the 3′ end of U3 (Fig. 7B) or the 5′ end of U3 (Fig. 7C). In contrast, the U3* species that is accumulated in rrp6-Δ, GAL::rrp41, and GAL::rrp45 strains was truncated only at the 5′ end, indicating that U3 is normally 3′ degraded by the exosome. The level of U3** is further elevated in exosome mutants that also lack Rnt1p, consistent with the model that degradation of pre-U3 is increased in rnt1-Δ strains. The 5′ degradation activity has not been further characterized but is likely due to the 5′→3′ exonuclease Rat1p, which 5′ processes other snoRNAs and degrades pre-rRNA spacer fragments (41).

In strains lacking Rnt1p, 3′ extended forms of U1 and U2 snoRNAs undergo a low level of polyadenylation (1, 45), and the precursors to several snoRNAs and snoRNAs are polyadenylated in exosome mutants (5, 55). To determine whether this was also the case for the 3′ extended U3, RNA was treated in vitro with oligo(dT) and RNase H. Following this deadenylation treatment, the longer 3′-extended species detected in the rnt1-Δ strain became shorter and more discrete (data not shown), indicating that a low level of polyadenylation had indeed occurred.

**DISCUSSION**

**How is U3 processed?** A model for 3′ processing of the U3A snoRNA is presented in Fig. 8. We postulate that processing is normally initiated by cotranscriptional cleavage by Rnt1p across a stem structure at positions +21 and +58 with respect to the 3′ end of U3. The released 3′ fragment is degraded by the 5′→3′ exonuclease Rat1p, as shown by its accumulation in the rnt1-Δ strain. The 3′ extended pre-snoRNA is rapidly processed to +12 and +18, since the species extended to +21 is not readily detected in total RNA. The products of Rnt1p cleavage of pre-U4 and pre-U5 are elevated in strains deleted for components of the exosome (5), and we think it probable that the exosome complex also carries out the initial shortening of the pre-U3. We cannot, however, exclude the participation of other exonucleases, such as the Rex1-3p family that carry out the final trimming of several small RNA species (54). The pre-U3 is stabilized against further 3′ degradation by binding of Lhp1p to the 3′ poly(U) tracts at +19 and +13; whether Lhp1p binds to internal poly(U) tracts prior to the start of digestion, or binds to free 3′ poly(U) tracts generated during digestion, cannot be determined at present. The larger U3-3′III species is bound by Lhp1p, suggesting that Lhp1p does bind to internal poly(U) sequences prior to processing, but the endpoints of this have not been mapped and we cannot exclude the possibility that it has a terminal poly(U) tract. It is likely that the poly(U) tracts at +19 and +13 can each bind Lhp1p, although binding may be mutually exclusive.

The box C+D snoRNAs, including U3, bind a set of common proteins, Nop1p, Nop56p, and Nop58p (13, 30–32, 40, 44, 53) that probably bind to the box D sequence close to the 3′ end of the snoRNA and the 3′-terminal stem (13, 57). These proteins are not associated with the 3′-extended U3 species, and we propose that their binding displaces Lhp1p from the 3′

**TABLE 2. PhosphorImager quantification of Northern hybridization data from Fig. 6a**

<table>
<thead>
<tr>
<th>Construct</th>
<th>Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GAL::rrp41</td>
</tr>
<tr>
<td>U3</td>
<td>2.1</td>
</tr>
<tr>
<td>scR1</td>
<td>0.83</td>
</tr>
<tr>
<td>U3(scR1)</td>
<td>2.53</td>
</tr>
<tr>
<td>U3-3′I + -II</td>
<td>2.3</td>
</tr>
<tr>
<td>U3-3′I + -II(scR1)</td>
<td>2.77</td>
</tr>
</tbody>
</table>

*a* The U3-3′I and -II doublet was quantified as one species. The GAL::rrp41 and GAL::rrp41/rnt1-Δ data are from the 24-h time points; the GAL::rrp45 and GAL::rrp45/rnt1-Δ data are from the 40- and 48-h time points, respectively. Values are relative to the wild-type level, assigned a value of 1.

*b* Species shorter than U3-3′I that appears in the GAL::rrp41/rnt1-Δ and GAL::rrp45/rnt1-Δ strains.
flanking sequence. Since the snoRNP proteins bind at the very 3′ end of the snoRNA, this displacement may be steric. Removal of Lhp1p is envisaged to allow the exosome to resume processing, generating the mature snoRNA 3′ end. This is followed by cap trimethylation; in vertebrates this snoRNA modification requires the conserved box C1D snoRNAs (50), probably acting via binding the mature snoRNP proteins. The yeast U3 genes are unusual in that they contain an intron that is excised by the normal pre-mRNA splicing machinery. In wild-type cells this is spliced from the 3′-extended pre-U3, since only the 3′-extended, intron-containing species is detected. The endpoints of the U3-int 3′ species have not been determined, but these species are associated with Lhp1p, suggesting that they may have been largely processed to +18 and +12.

Deletion of Rnt1p strongly reduces synthesis of mature U3 (Table 2). Processing of the long 3′-extended pre-U3 species generated in the absence of Rnt1p cleavage involves the exosome, as shown by their increased levels in rnt1Δ strains lacking exosome components. We speculate that a processive exosome complex assembles on the long 3′-extended pre-U3, which is able to substantially displace bound Lhp1p and/or the snoRNP proteins and therefore degrades most of the pre-U3 population. Consistent with this model, depletion of exosome components from rnt1Δ strains restored mature U3 to the wild-type level (Table 2).

In the absence of Lhp1p, the U3 snoRNA was still 3′ pro-
The mature regions of U3, U2, and U5 snoRNAs are in uppercase. For U1, U4, U2, U4, and U5 snRNAs. In panel A, the Rnt1p cleavage sites (\) have been not addressed for U2. Also in each case, shorter 3' ends have not yet been accurately mapped. In the case of pre-U4 and pre-U5, the Rnt1p cleavage site is adjacent to a poly(U) tract (Fig. 9A). For the U3-3' end cleavage site is adjacent to a poly(U) tract (Fig. 9B). Lhp1p is associated with yeast pre-U1, U2, U4, and U5 (58). However, in contrast to the model presented here for U3, Lhp1p is proposed to function as a cofactor for the assembly of the spliceosomal snRNAs with the Sm proteins. The human and plant U3 snoRNAs also have 3' flanking poly(U) tracts, suggesting that this feature may be conserved throughout eukaryotes (27, 49).

**Why is U3 processed?** The 3' ends of almost all RNAs from all organisms are generated by 3' processing rather than transcription termination, but the reasons for this have largely remained obscure. The data presented here provide a possible explanation, at least for U3. The binding sites for the common snoRNP proteins, the box D element and the terminal stem structure, define the 3' end of the mature U3 snoRNA. Transcription termination at this site would generate an RNA with a monomethylguanosine cap structure and lacking the snoRNP proteins. This could not readily be distinguished from the products of premature termination or failed pre-mRNA splicing. It is likely that these are normally very rapidly degraded by the exosome complex and Rat1p (C. Bouquet-Antonelli, C. Presutti, and D. Tollervey, unpublished data). Delaying or reducing these degradative activities might allow sufficient time for snoRNP assembly and cap trimethylation, but at the expense of allowing greater accumulation of aberrant RNAs. Such a strategy might also allow a greater level of accidental protection of inappropriate RNA species by RNA-binding proteins. Instead, the cell has adopted a mechanism to specifically delay 3' processing of the snoRNA. Transcription continues beyond the 3' end of the mature snoRNA, with the transcript normally being cleaved by Rnt1p and protected by binding of Lhp1p. This leaves the mature 3' end free for binding of the snoRNP proteins. Such a system has the additional advantage of acting as a quality control system. We envisage that the snoRNP proteins, or at least Nop58p, must displace Lhp1p to allow final maturation of the snoRNA. In the absence of Nop58p binding, the 3' extended pre-U3 accumulates to low levels and is then degraded. Binding of La to pre-tRNAs has also been proposed to function as a quality control system (19), and binding of Lhp1p to the U6 snoRNA and pre-tRNAs\textsuperscript{5\textasciitilde} is also likely to antagonize rapid 3' degradation (8, 39).

We propose that 3' processing acts as a quality control system in the synthesis of many RNA species and that this underlies its ubiquitous occurrence.

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