Trans-acting factors in yeast pre-rRNA and pre-snoRNA processing

Denis Lafontaine and David Tollervey

Abstract: The major intermediates in the pathway of pre-rRNA processing in yeast and other eukaryotes were originally identified by biochemical analyses. However, as a result of the analysis of the effects of mutations in *trans*-acting factors, the yeast pre-rRNA processing pathway is now characterized in far more detail than that of other eukaryotes. These analyses have led to the identification of processing sites and intermediates that were either too close in size or too short lived to be detected by biochemical analyses alone. In addition, it was generally unclear whether pre-rRNA processing steps were endonucleolytic or exonucleolytic; analyses of *trans*-acting factors is now revealing a complex mixture of endonucleolytic and exonucleolytic processing steps. Many of the small nucleolar RNAs (snoRNAs) are excised from larger precursors. Analyses of *trans*-acting factors are also revealing details of pre-snoRNA processing in yeast. Interestingly, factors involved in pre-snoRNA processing turn out to be components that also function in pre-rRNA processing, suggesting a potential mechanism for the coregulation of rRNA and snoRNA synthesis. In general, very little is known about the regulation of pre-rRNA processing steps. The best candidate for a system regulating specific pre-rRNA processing reactions has recently been revealed by the analysis of a yeast pre-RNA methylase. Here we will review recent data on the *trans*-acting factors involved in yeast ribosome synthesis and discuss how these analyses have contributed to our current view of this complex process.

Key words: RNA processing, ribosome biogenesis, rRNA, exonuclease, methylation, yeast.

Résumé : Les intermédiaires majeurs de la voie de maturation du pré-ARNr ont, à l'origine, été identifiés à l'aide d'analyses biochimiques aussi bien chez la levure que chez d'autres eucaryotes. À présent, suite à l'analyse fonctionnelle de nombreux facteurs mutants agissant en *trans* lors de la maturation du pré-ARNr, c'est chez la levure que ce processus est le mieux caractérisé. Ces études ont permis d'identifier de nouveaux sites de clivage et de nouveaux intermédiaires qui, en raison de leurs tailles trop semblables ou de leurs durées de vie trop courtes échappaient aux analyses biochimiques. En outre, ces études ont également permis d'établir que la maturation du pré-ARNr se fait grâce à une série complexe d'étapes endo- et exonucléolytiques. Plusieurs (ARNpno) petits ARN nucléolaires sont excisés de précurseurs de plus grande taille. La caractérisation de facteurs agissant en *trans* lors de la maturation des pré-ARNpno chez la levure a révèlé que certains d'entre eux participent également à la maturation du pré-ARNr, ce qui suggère la possibilité d'une co-régulation entre la synthèse de l'ARNr et celle des ARNpno. Jusqu'à ce jour, la régulation de la maturation du pré-ARNr est très peu connue. Le meilleur système potentiel de régulation d'étapes spécifiques de maturation a récemment été découvert chez la levure lors de l'étude d'une méthylase spécifique du pré-ARNr. Dans cet article, nous faisons la revue des données les plus récentes relatives à la caractérisation fonctionnelle des facteurs agissant en *trans* lors de la synthèse du ribosome de levure et nous discutons des conséquences de ces études sur notre conception actuelle de ce processus complexe.

Mots clés : maturation de l'ARN, synthèse du ribosome, ARNr, exonucléase, méthylation, levure.

The pathway of yeast pre-rRNA processing

In all organisms, the synthesis of the mature rRNAs is not brought about by simple transcription but involves a complex series of post-transcriptional processing reactions. A current view of the major steps in pre-rRNA processing in *Saccharomyces cerevisiae* is shown in Fig. 1. As in other eukaryotes, the small subunit rRNA (18S rRNA in yeast) and two of the three large subunit rRNAs (5.8S and 25S rRNAs in yeast) are co-transcribed by RNA polymerase I and processed from a large pre-rRNA (the 35S pre-rRNA in yeast). In this precursor, the mature rRNA sequences are flanked by the 5′ and 3′ external transcribed spacers (5′ ETS and 3′ ETS) and separated by internal transcribed spacers 1 and 2 (ITS1 and ITS2) (Fig. 1A). The third rRNA of the large subunit, the 5S rRNA, is transcribed independently by RNA polymerase III (see Fig. 2).

The major steps in the pathway of pre-rRNA processing in yeast and other eukaryotes were initially identified by biochemical purification of the processing intermediates

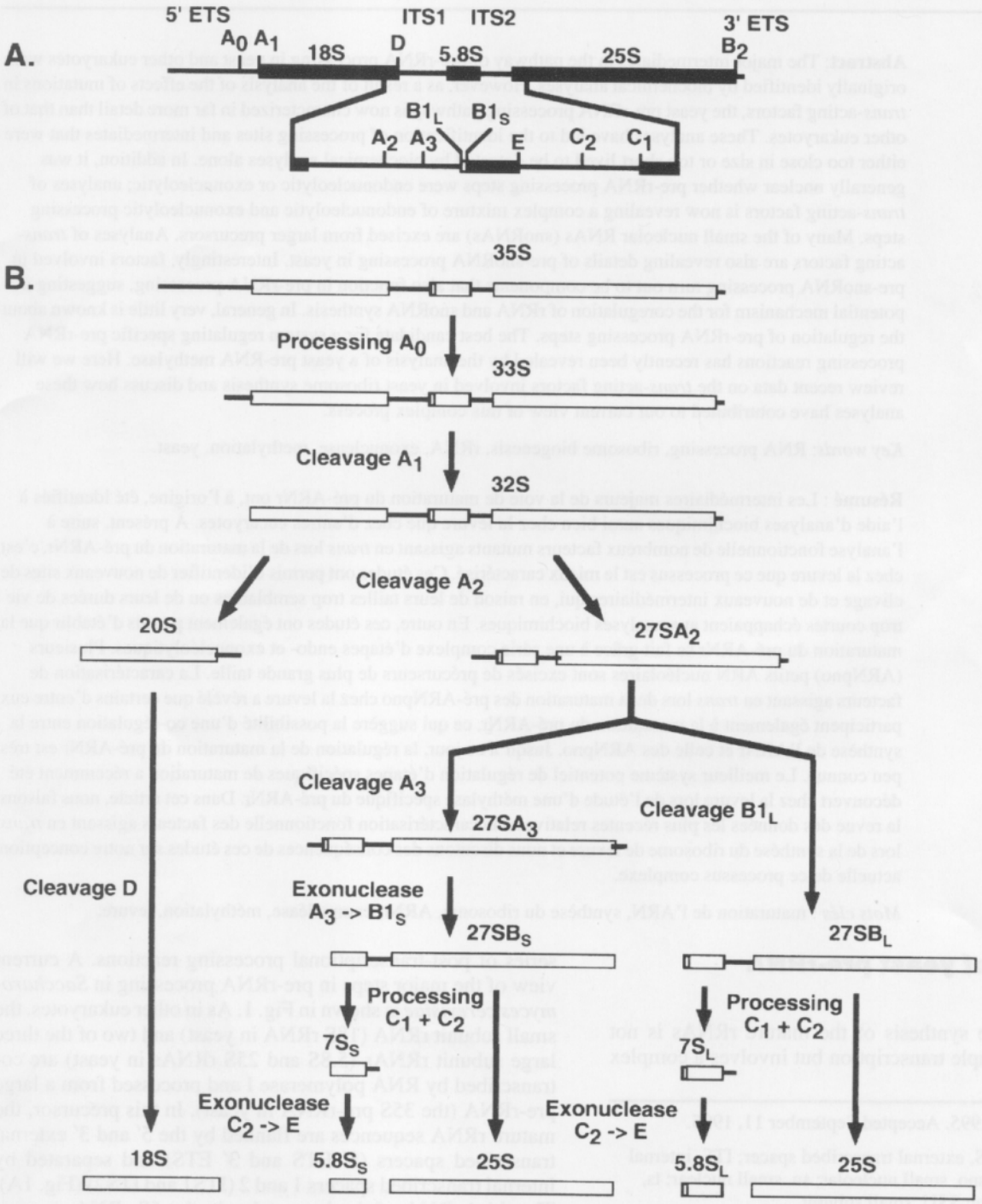
Received June 12, 1995. Accepted September 11, 1995.

Abbreviations: ETS, external transcribed spacer; ITS, internal transcribed spacer; sno, small nucleolar; sn, small nuclear; ts, temperature sensitive; SSU, small subunit.

D. Lafontaine and D. Tollervey.[1] European Molecular Biology Laboratory (EMBL), Postfach 10 22 09, Meyerhofstrasse 1, D-69117 Heidelberg, Germany.

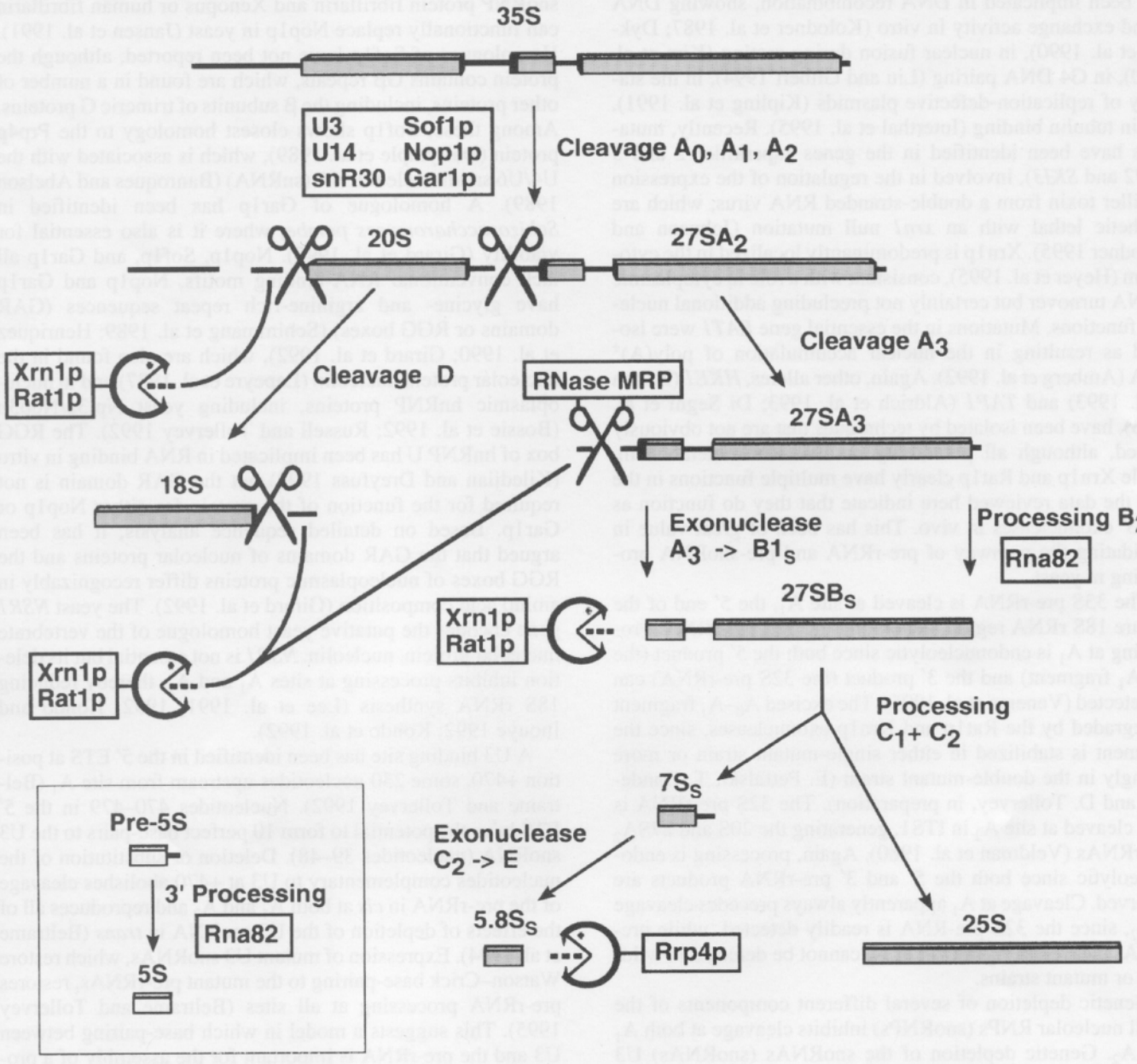
[1] Author to whom all correspondence should be addressed.

Fig. 1. (A) Structure of the yeast pre-rRNA and (B) processing pathway. Processing sites are indicated as A_0 to E. In an effort to make the names of the processing intermediates more systematic, some of these have been renamed (see also the accompanying review by van Nues et al.). The 27S pre-rRNA species have been named according to the processing sites that form their 5′ ends. Species with 5′ ends at sites A_2 and A_3 are designated $27SA_2$ and $27SA_3$, respectively; these were previously named 27SA and 27SA′. The B1(L) and B1(S) processing sites have been designated $B1_L$ and $B1_S$, respectively; RNAs with 5′ ends at these sites are designated $27SB_L$, $7S_L$, and $5.8S_L$ and $27SB_S$, $7S_S$, and $5.8S_S$, respectively.



(reviewed in Klootwijk and Planta 1989). Recently, however, much more detailed information has become available on processing in yeast as a result of analyses of the effects of mutations in *trans*-acting factors. Many processing steps and intermediates can only be readily detected in strains that are specifically defective in their processing. In other eukaryotes, the processing pathway remains poorly characterized, and it is not generally apparent whether processing is endonucleolytic

Fig. 2. Endonucleolytic and Exonucleolytic steps in yeast pre-rRNA processing. The major processing pathway of the yeast 35S pre-rRNA is outlined, with endonuclease steps indicated by scissors and exonuclease steps indicated by monsters. Components in boxes are required for the processing steps indicated. For simplicity, the 33S and 32S pre-rRNA, the products of the A_0 and A_1 cleavages, respectively, are not shown and only the major processing pathway from $27SA_2$ to $5.8S_S$ is shown. The inset depicts the processing of the 5S rRNA.



or exonucleolytic. In yeast, it is now clear that both endonucleolytic and exonucleolytic processing occurs at different steps in the processing pathway. The role that the analysis of the effects of mutations in *trans*-acting factors has played in the development of our current models for yeast pre-rRNA processing will be a major theme of this review. The analysis of the role of *cis*-acting elements will not be addressed here as it is the subject of the accompanying review by van Nues et al.

Processing reactions on the pathway of 18S rRNA synthesis

The earliest, well-characterized pre-rRNA processing event is at site A_0 in the 5′ ETS, which generates the 33S pre-rRNA (Hughes and Ares 1991). This processing has not been demonstrated to be endonucleolytic, although this is probable as several discrete fragment can be detected on Northern blots hybridized with probes upstream of A_0. Moreover, no 5′ extension at A_0 can be detected in the *rat1*, *xrn1* mutant strains, which are defective in 5′→3′ exonuclease activity (E. Petfalski, T. Dandekar, and D. Tollervey, in preparation).

Xrn1p and Rat1p, which contain a region of homology (Kenna et al. 1993), have both been shown to have 5′→3′ exonuclease activity in vitro (Larimer et al. 1992; Stevens 1980; Stevens and Maupin 1987; Johnson and Kolodner 1991; Kenna et al. 1993). Xrn1p has also been shown to be required

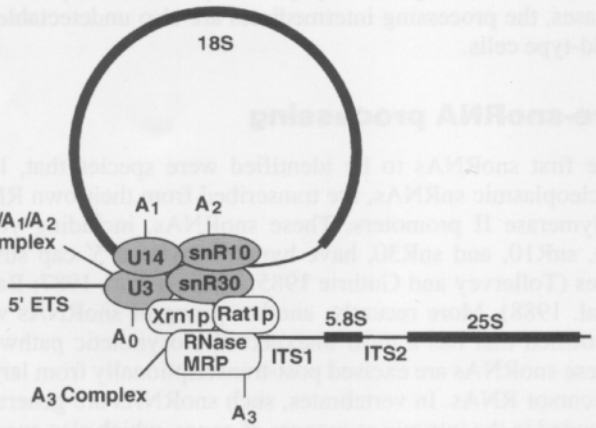
for 5′→3′ digestion of mRNA in vivo (Hsu and Stevens 1993; Muhlrad et al. 1994; Muhlrad and Parker 1994). In addition to the roles described below, *XRN1* (also known as *SEP1*, *DST2*, *KEM1*, and *RAR5*, reviewed in Kearsey and Kipling 1991) has also been implicated in DNA recombination, showing DNA strand exchange activity in vitro (Kolodner et al. 1987; Dykstra et al. 1990), in nuclear fusion during mating (Kim et al. 1990), in G4 DNA pairing (Liu and Gilbert 1994), in the stability of replication-defective plasmids (Kipling et al. 1991), and in tubulin binding (Interthal et al. 1995). Recently, mutations have been identified in the genes superkiller 2 and 3 (*SKI2* and *SKI3*), involved in the regulation of the expression of killer toxin from a double-stranded RNA virus, which are synthetic lethal with an *xrn1* null mutation (Johnson and Kolodner 1995). Xrn1p is predominantly localized in the cytoplasm (Heyer et al. 1995), consistent with a role in cytoplasmic mRNA turnover but certainly not precluding additional nucleolar functions. Mutations in the essential gene *RAT1* were isolated as resulting in the nuclear accumulation of poly(A)$^+$ RNA (Amberg et al. 1992). Again, other alleles, *HKE1* (Kenna et al. 1993) and *TAP1* (Aldrich et al. 1993; Di Segni et al. 1993), have been isolated by techniques that are not obviously related, although all potentially involve RNA metabolism. While Xrn1p and Rat1p clearly have multiple functions in the cell, the data reviewed here indicate that they do function as 5′→3′ exonucleases in vivo. This has been of great value in elucidating the pathway of pre-rRNA and pre-snoRNA processing in yeast.

The 33S pre-rRNA is cleaved at site A_1, the 5′ end of the mature 18S rRNA region, generating the 32S pre-rRNA. Processing at A_1 is endonucleolytic since both the 5′ product (the A_0–A_1 fragment) and the 3′ product (the 32S pre-rRNA) can be detected (Venema et al. 1995). The excised A_0–A_1 fragment is degraded by the Rat1p and Xrn1p exonucleases, since the fragment is stabilized in either single-mutant strain or more strongly in the double-mutant strain (E. Petfalski, T. Dandekar, and D. Tollervey, in preparation). The 32S pre-rRNA is then cleaved at site A_2 in ITS1, generating the 20S and 27SA_2 pre-rRNAs (Veldman et al. 1980). Again, processing is endonucleolytic since both the 5′ and 3′ pre-rRNA products are observed. Cleavage at A_1 apparently always precedes cleavage at A_2, since the 32S pre-RNA is readily detected, while pre-rRNAs cleaved at A_2 but not at A_1 cannot be detected in wild-type or mutant strains.

Genetic depletion of several different components of the small nucleolar RNPs (snoRNPs) inhibits cleavage at both A_1 and A_2. Genetic depletion of the snoRNAs (snoRNAs) U3 (Hughes and Ares 1991), U14 (Li et al. 1990), or snR30 (Morrissey and Tollervey 1993) or of the snoRNP proteins Nop1p (Tollervey et al. 1991), Sof1p (Jansen et al. 1993), or Gar1p (Girard et al. 1992) prevents A_1 and A_2 cleavage, while deletion of the gene encoding the snoRNA, snR10, delays cleavage (Tollervey 1987). Nop1p is associated with all known yeast snoRNAs with the exception of RNase MRP (Schimmang et al. 1989; Morrissey and Tollervey 1993; Dandekar and Tollervey 1993), Sof1p is associated with the U3 snoRNA (Jansen et al. 1993), and Gar1p is associated with snR30 and snR10 (Girard et al. 1992). It is therefore likely that it is the U3, U14, and snR30 snoRNP particles that are required for A_1 and A_2 processing. Yeast U3 and U14 are clearly homologous to vertebrate snoRNAs (Hughes et al. 1987; Li et al. 1990); hybrid mouse – yeast U14 constructs can provide U14 function in yeast (Li and Fournier 1992). In contrast, homologues of snR10 and snR30 have not yet been identified in other organisms. Nop1p is highly homologous to the vertebrate snoRNP protein fibrillarin and Xenopus or human fibrillarin can functionally replace Nop1p in yeast (Jansen et al. 1991). Homologues of Sof1p have not been reported, although the protein contains Gβ repeats, which are found in a number of other proteins, including the β subunits of trimeric G proteins. Among these, Sof1p shows closest homology to the Prp4p protein (Dalrymple et al. 1989), which is associated with the U4/U6 small nuclear RNA (snRNA) (Banroques and Abelson 1989). A homologue of Gar1p has been identified in *Schizosaccharomyces pombe*, where it is also essential for viability (Girard et al. 1993). Nop1p, Sof1p, and Gar1p all lack conventional RNA-binding motifs. Nop1p and Gar1p have glycine- and arginine-rich repeat sequences (GAR domains or RGG boxes) (Schimmang et al. 1989; Henriquez et al. 1990; Girard et al. 1992), which are also found in the nucleolar protein nucleolin (Lapeyre et al. 1987) and in nucleoplasmic hnRNP proteins, including yeast Npl3p/Nop3p (Bossie et al. 1992; Russell and Tollervey 1992). The RGG box of hnRNP U has been implicated in RNA binding in vitro (Kiledjian and Dreyfuss 1992) but the GAR domain is not required for the function of the protein for either Nop1p or Gar1p. Based on detailed sequence analysis, it has been argued that the GAR domains of nucleolar proteins and the RGG boxes of nucleoplasmic proteins differ recognizably in amino acid composition (Girard et al. 1992). The yeast *NSR1* gene encodes the putative yeast homologue of the vertebrate nucleolar protein, nucleolin. *NSR1* is not essential but its deletion inhibits processing at sites A_1 and A_2, thereby reducing 18S rRNA synthesis (Lee et al. 1991, 1992; Kondo and Inouye 1992; Kondo et al. 1992).

A U3 binding site has been identified in the 5′ ETS at position +470, some 230 nucleotides upstream from site A_1 (Beltrame and Tollervey 1992). Nucleotides 470–479 in the 5′ ETS have the potential to form 10 perfect base-pairs to the U3 snoRNA (nucleotides 39–48). Deletion or substitution of the nucleotides complementary to U3 at +470 abolishes cleavage of the pre-rRNA in *cis* at both A_1 and A_2 and reproduces all of the effects of depletion of the U3 snoRNA in *trans* (Beltrame et al. 1994). Expression of mutant U3 snoRNAs, which restore Watson–Crick base-pairing to the mutant pre-rRNAs, restores pre-rRNA processing at all sites (Beltrame and Tollervey 1995). This suggests a model in which base-pairing between U3 and the pre-rRNA is important for the assembly of a processing complex that subsequently cleaves sites A_0, A_1, and A_2. The exact roles of the snoRNPs in the cleavage reactions are obscure. It is tempting to speculate that multiple snoRNAs assemble together with the pre-rRNA to form an RNA-based enzyme that cleaves the pre-rRNA (see Fig. 3). This would be analogous to the role proposed for snRNAs in pre-mRNA processing. There is, however, no evidence at present that these snoRNPs function as ribozymes in the catalysis of cleavage. In the case of U14, the snoRNA contains two regions of extended complementarity to the mature 18S rRNA sequence (Jarmolowski et al. 1990) and at least one of these regions can be cross-linked to the mature 18S rRNA region of the 35S pre-rRNA in vivo (Morrissey 1994). This suggests that the role of the U14 snoRNP is more likely to be in establishing the pre-

Fig. 3. Model for the early steps in the yeast pre-rRNA processing. The A_0–A_1–A_2 processing complex (snoRNPs, snR30, U3, U14, and snR10, shaded) initially assembles on the 5′ ETS at sites including the characterized U3-binding site. This complex brings together A_0, A_1, and A_2 and then cleaves these sites in a coordinated manner. The A_3 processing complex (RNase MRP and the exonucleases, in white) binds at or near A_3, its site of action. Because of their proximity, components of the two processing complexes may interact.



ribosomal particle in the correct conformation or structure for processing than in the catalysis of the reaction.

The requirement for the snoRNPs in A_0 cleavage is less simple. The excised A_0–A_1 fragment is detected in wild-type strains (Venema et al. 1995) and the A_0-cleaved 33S pre-rRNA accumulates in cells genetically depleted of Dim1p (Lafontaine et al. 1995) or carrying mutations in the pre-rRNA at site A_1 (Venema et al. 1995), showing that A_0 is a major cleavage site in the wild-type pre-rRNA. The level of the 33S pre-rRNA is strongly reduced by genetic depletion of the U3 snoRNA or the U3–associated proteins Nop1p or Sof1p, indicating that the U3 snoRNP is required for cleavage at A_0 (Beltrame et al. 1994). Indeed, it was the loss of the primer extension stop at A_0 in strains genetically depleted of U3 that led to the identification of this processing site (Hughes and Ares 1991). Deletion or substitution of the U3–binding site at +470 also eliminates formation of the 33S pre-rRNA, consistent with a requirement for the U3 snoRNP in A_0 cleavage (Beltrame et al. 1994; Beltrame and Tollervey 1995). The role of the other snoRNPs is less clear; 33S pre-rRNA is not clearly reduced during genetic depletion of snR30 or Gar1p, and mildly accumulates on depletion of U14 (Beltrame et al. 1994). However, in all of these mutants the 35S pre-rRNA accumulates (Morrissey and Tollervey 1993; Girard et al. 1992; Li et al. 1990), indicating that A_0 cleavage is at least delayed. The simplest explanation is that depletion of any of these snoRNP components delays cleavage at site A_0 and that the relative inhibition of cleavage at A_0 and A_1 determines the level of 33S in the mutants. The level of 33S pre-rRNA is also strongly reduced by deletion of the *NSR1* gene (I. Russell and D. Tollervey, unpublished) but not by deletion of *SNR10* (Beltrame et al. 1994).

The 20S pre-rRNA is processed at site D to yield the mature 18S rRNA (Figs. 1 and 2). This processing step, which is reported to occur after transport of the pre-ribosomal particle to the cytoplasm (Udem and Warner 1973), is also endonucleolytic, since the excised D–A_2 fragment is detected. This fragment is very strongly accumulated in *xrn1* mutant strains (Stevens et al. 1991), although more of the full length fragment is observed in rat1, *xrn1* double-mutant strains (E. Petfalski, T. Dandekar, and D. Tollervey, in preparation). No mutants specifically defective in cleavage at site D have been reported.

Processing pathway leading to the synthesis of the 5.8S and 25S rRNAs

Recent analyses of *trans*-acting factors have revealed that the processing of the yeast ITS1 spacer region is much more complex than would have been anticipated. Yeast 5.8S rRNA is heterogeneous at the 5′ end with a major short form, designated $5.8S_S$, and a minor (about 15%) form 7–8 nucleotides longer. It transpires that these arise because the $27SA_2$ pre-rRNA can be processed by two alternative pathways (Henry et al. 1994) (see Fig. 1). The major fraction of the $27SA_2$ pre-rRNA is cleaved endonucleolytically at site A_3 by the snoRNP RNase MRP generating the $27SA_3$ pre-rRNA (Henry et al. 1994; Lygerou et al. 1994). Cleavage at site A_3 is inhibited by temperature-sensitive (ts) mutations in the MRP RNA (Shuai and Warner 1991; Lindahl et al. 1992; Chu et al. 1994; Schmitt and Clayton 1993) or the MRP snoRNP protein Pop1p (Lygerou et al. 1994). The excised A_2–A_3 fragment can be detected and accumulates at higher levels in rat1, *xrn1* mutant strains (E. Petfalski, T. Dandekar, and D. Tollervey, in preparation). The $27SA_3$ pre-rRNA is the substrate for 5′→3′ exonuclease digestion from site A_3 to site $B1_S$, generating the $27SB_S$ pre-rRNA (Henry et al. 1994). This processing mechanism was identified because it is inhibited in *rat1*, *xrn1* mutant strains, leading to the accumulation of 5′ extended forms of the 5.8S rRNA (Henry et al. 1994). The cleavage of site A_3 and the subsequent exonuclease digestion appear to be tightly coupled because the steady-state level of the $27SA_3$ pre-rRNA is very low in wild-type cells. Site A_3 was initially identified because it forms the 3′ end of a 23S pre-rRNA that is generated by direct cleavage of the 35S pre-rRNA at site A_3, in mutants defective in the U3, U14, snR10, and snR30 snoRNPs. The $27SA_3$ pre-rRNA accumulates in strains genetically depleted of snR30 (Henry et al. 1994), allowing the clear identification of its 5′ end. This accumulation is presumably due to a delay between A_3 cleavage and the subsequent exonuclease digestion. The basis of this effect remains obscure, but it may indicate that an snoRNP processing complex assembled at sites A_0–A_1–A_2 interacts with an RNase MRP – exonuclease complex assembling at site A_3 (see Fig. 3). A minor proportion of the $27SA_2$ pre-rRNA is processed at site $B1_L$, yielding the $27SB_L$ pre-rRNA, which is processed to $5.8S_L$. Nothing is known about the mechanism of, or components required for, $B1_L$ processing.

At about the same time as the 5′ ends of the 27SB pre-rRNAs are being formed, the 3′ ends are processed at site B_2 (reviewed in Raué and Planta, 1991). This processing may require the product of the *RNA82* gene (Piper et al. 1983) since *rna82* mutants are defective in 3′ end formation on pre-rRNA transcribed from mini-gene reporter constructs in vivo (Kempers-Veenstra et al. 1986). In vitro processing at site B_2

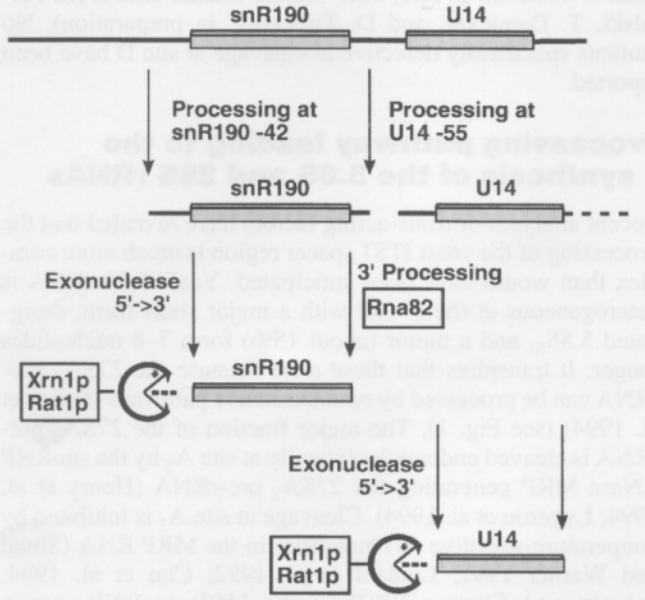


Fig. 4. Model for processing of pre-snR190–pre-U14. Proposed processing pathway for the common precursor to the yeast snoRNAs snR190 and U14. It is likely that a 3′ extension is present on U14 in the pre-snoRNA, but this has not yet been demonstrated.

has been described (Yip and Holland 1989), but it has not been determined whether this in vitro activity is due to the product of the *RNA82* gene. The cloning of *RNA82* has not been reported.

Subsequently, both the $27SB_L$ and $27SB_S$ pre-rRNAs appear to follow the same processing pathway. Processing at sites C_1 and C_2 in ITS2 generates the 5′ end of the 25S rRNA and the 3′ ends of both the $7S_L$ and $7S_S$ pre-rRNAs, respectively. No mutants specifically defective in *trans*-acting factors required for processing at C_1 or C_2 have been reported and no mutants defective in processing of the 5′ ETS or ITS1 have been found to be impaired in ITS2 processing. This suggests that the processing of ITS2 is distinct from ETS–ITS1 processing.

Processing of the 7S pre-rRNA to the mature 5.8S rRNA requires Rrp4p. In ts *rrp4–1* mutants, 3′ extended forms of 5.8S rRNA accumulate and the mature rRNA is depleted (Mitchell et al. 1996). Affinity-purified Rrp4p is associated with a 3′→5′ exonuclease activity in vitro and it is therefore very likely that the 7S pre-rRNA is processed exonucleolytically to 5.8S rRNA in vivo. Yeast Rrp4p is 43% identical over its whole length with an expressed human ORF of unknown function, making it likely that human pre-rRNA processing follows the same pathway. There is a pleasing symmetry to the synthesis of the major form of the 5.8S rRNA; both ends are produced by exonucleases digesting in from endonuclease cleavage sites within the spacers.

The remaining rRNA species, 5S rRNA, is not processed from the same pre-rRNA molecule as the other rRNAs. 5S rRNA is transcribed independently by RNA polymerase III as a precursor, which, in yeast, is 3′ extended by up to 10 nucleotides. Processing of the pre-5S rRNA requires the product of the *RNA82* gene (Fig. 2); in *rna82* mutants, 3′ processing of pre-5S rRNA is slowed and the mature 5S rRNA carries short 3′ extensions (Piper et al. 1983). These 3′ extensions do not appear to interfere with the function of the 5S rRNA since *rna82* mutants are not detectably impaired in growth (Piper et al. 1983). The processing of pre-5S RNA can be reproduced in vitro (Tekamp et al. 1980), but it has not been determined whether the in vitro activity requires the product of the *RNA82* gene. This 3′ processing has been ascribed to an endonuclease activity, based on the failure to detect processing intermediates in vitro. However, the excised 3′ fragment was also not detected and it should be noted that in pre-rRNA processing reactions involving the Rrp4p, Rat1p, and Xrn1p exonucleases, the processing intermediates are also undetectable in wild-type cells.

Pre-snoRNA processing

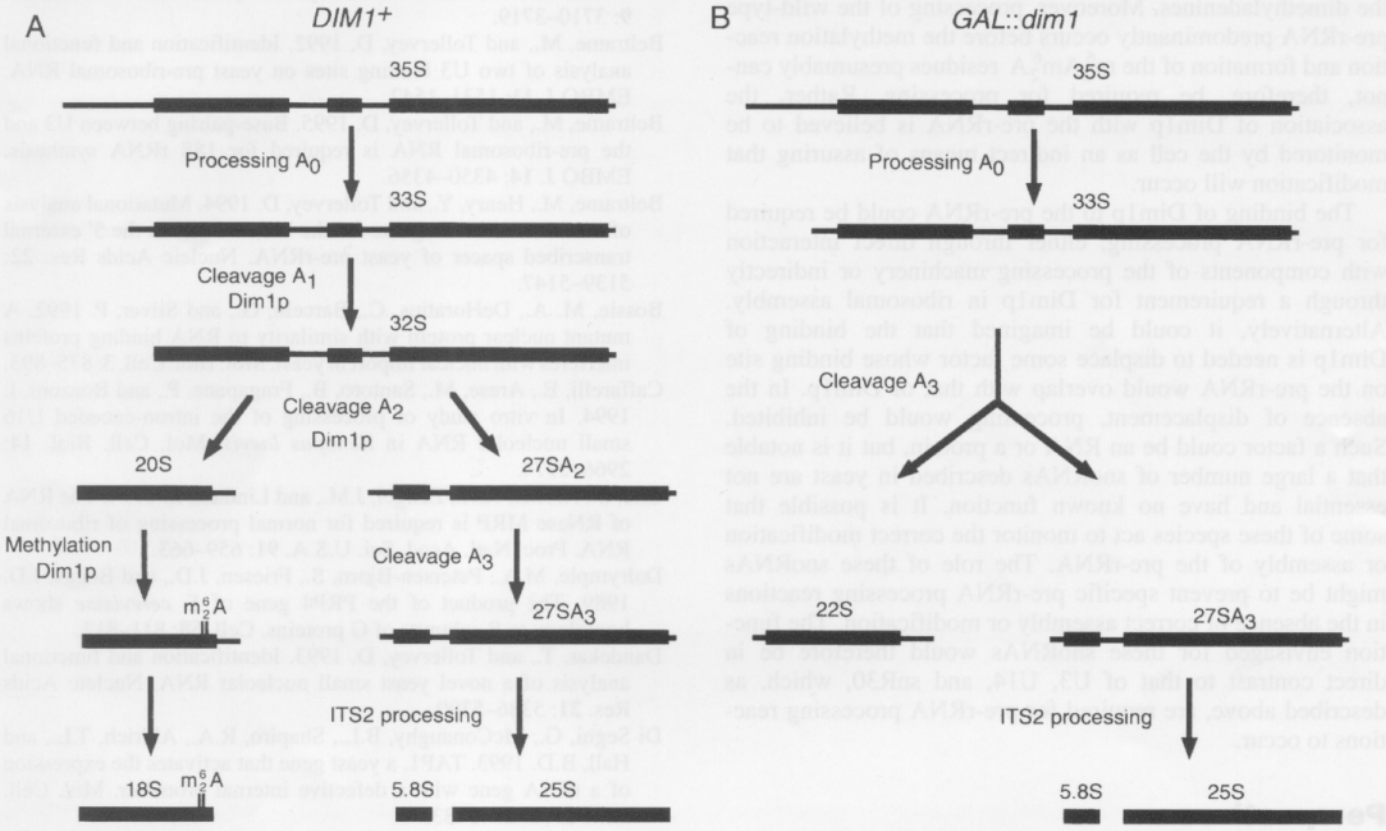
The first snoRNAs to be identified were species that, like nucleoplasmic snRNAs, are transcribed from their own RNA polymerase II promoters. These snoRNAs, including yeast U3, snR10, and snR30, have hypermethylated 5′ cap structures (Tollervey and Guthrie 1985; Hughes et al. 1987; Bally et al. 1988). More recently, another group of snoRNAs was identified that has a most unexpected biosynthetic pathway. These snoRNAs are excised post-transcriptionally from larger precursor RNAs. In vertebrates, such snoRNAs are generally encoded in the intronic sequences of genes, which also encode proteins in their exonic sequences (Caffarelli et al. 1994; Fragapane et al. 1993; Tycowski et al. 1993; Qu et al. 1994; Nicoloso et al. 1994).

In yeast, the first snoRNAs of this class to be identified, were snR190 and U14. These snoRNAs are co-transcribed as a larger pre-snoRNA, which does not, however, appear to also encode a protein product (Zagorski et al. 1988). Analysis of mutants defective in pre-snoRNA processing are now providing some details on the processing of snR190 and U14 from their common precursor (Fig. 4). In the rat1, *xrn1* mutant strains, 5′ extended forms of both snR190 and U14 accumulate; these extend to positions 42 nucleotides 5′ to snR190 and 55 nucleotides 5′ to U14, respectively (E. Petfalski, T. Dandekar, and D. Tollervey, in preparation). In addition, in *rna82* mutant strains, forms of snR190 accumulate with short 3′ extensions. From these data, we propose the pathway of processing shown in Fig. 4. Cleavage, by as yet unidentified components, at snR190 −42 and U14 −55 is followed by 5′ maturation of both snoRNAs by the Rat1p and Xrn1p exonucleases. For both snoRNAs, it is probable that the exonucleases are arrested by snoRNP proteins bound to the conserved box C and box D regions, which are located close to the 5′–3′ terminal stem (Huang et al. 1992). Normal 3′ maturation of snR190 requires the product of the *RNA82* gene. We have not yet detected a 3′ extended form of pre-U14. However, as the 5′ and 3′ flanking sequences of U14 have the clear potential to extend the base-pairing of the terminal stem, it seems likely that a 3′ extended precursor does exist.

The processing pathways of the $27SA_2$ pre-rRNA and of snR190 show interesting similarities. In both cases, 5′ maturation involves processing at an upstream site followed by 5′→3′ exonuclease digestion requiring Rat1p and Xrn1p, while normal 3′ maturation requires the *RNA82* gene product.

In vertebrates, the host genes for the excised snoRNAs generally encode proteins with roles in ribosome synthesis or function (Caffarelli et al. 1994; Fragapane et al. 1993; Tycowski et

Fig. 5. Effects of Depletion of Dim1p. (A) The major yeast pre-rRNA processing pathway in *DIM1*$^+$ strains. Dim1p-dependent steps, methylation and cleavage at sites A_1 and A_2, are indicated. (B) The pathway of pre-rRNA processing seen in cells depleted of Dim1p by growth of a *GAL::dim1* mutant on glucose medium. Processing of the 35S pre-rRNA at site A_0 is not affected by depletion of Dim1p but cleavage at A_1 and A_2 is inhibited, leading to the accumulation of the 33S pre-rRNA. This is processed at site A_3 yielding the $27SA_3$ pre-rRNA, which is processed to 5.8S and 25S rRNA, and a 22S species, which does not appear to be a substrate for processing to 18S rRNA. For simplicity, only the major processing pathway in ITS1 is shown and steps between $27SA_3$ and the mature 5.8S and 25S rRNAs are omitted.



al. 1993; Qu et al. 1994; Nicoloso et al. 1994) (see accompanying review by J.-P. Bachellerie). This suggests that there is significance to the selection of host genes for the snoRNAs, perhaps promoting the co-regulation of the transcription of the snoRNAs and other components required for ribosome synthesis and function. The involvement of common factors in pre-snoRNA and pre-rRNA processing suggests a further post-transcriptional mechanism for the co-regulation of rRNA and snoRNA synthesis. This potential for co-regulation may have been a factor leading to the selection for the synthesis of snoRNAs by excision and may explain why the snoRNAs are the only small RNA species known to be synthesized by such a pathway.

Regulation of pre-rRNA processing

Little is known about the regulation of pre-rRNA processing reactions and, indeed, there was little evidence for specific regulation until recently. Analyses of *trans*-acting factors are now providing hints that such regulation may occur.

The mechanism(s) that stop the exonucleases involved in pre-rRNA processing are not known but by analogy to the role envisaged for snoRNP proteins bound to boxes C and D in snoRNA synthesis, digestion could be blocked by r-proteins bound to mature rRNA sequence. Such a mechanism would offer an opportunity to regulate pre-rRNA processing reactions in response to assembly. In the absence of correct assembly, the exonucleases would degrade the pre-rRNA preventing the synthesis of incorrectly assembled ribosomes. Strains with mutations in individual proteins are impaired in processing reactions leading to the formation of the mature rRNA(s) of the ribosomal subunit affected (Moritz et al. 1990, 1991; Ripmaster et al. 1993, Fabian and Hopper, 1987; Sachs and Davis, 1990). This suggests that some steps in assembly may act as check points that must be passed for pre-rRNA processing reactions to occur.

The best candidate for a system specifically regulating pre-rRNA processing reactions has recently been revealed by the analysis of a yeast pre-RNA methylase. The pre-rRNAs from all organisms are modified through formation of pseudouridine and base and 2'-*O*-ribose methylation, but the pattern of modification is generally not conserved between bacteria and eukaryotes (reviewed in Raué et al. 1988). One of the few exceptions is the $m_2^6A_{1779}m_2^6A_{1780}$ dimethylation (yeast numbering), occurring in the loop of the hairpin helix 45 at the very 3′ end of the SSU-rRNA (van Knippenberg et al. 1984). In yeast, this dimethylation is carried out by Dim1p, which shows significant homology to the *E. coli* KsgAp dimethylase (Lafontaine et al. 1994). Genetic depletion of Dim1p, under the control of a regulated *GAL* promoter, not only inhibits dimethylation of the pre-rRNA but also prevents cleavage at

sites A_1 and A_2 (see Fig. 5). This blocks the synthesis of the 20S pre-rRNA thereby preventing the production of non-methylated 18S rRNA (Lafontaine et al. 1995). Mutation of both A residues at the site of methylation to G residues does not, however, interfere with processing, showing that the processing of this pre-rRNA is not dependent on the formation of the dimethyladenines. Moreover, processing of the wild-type pre-rRNA predominantly occurs before the methylation reaction and formation of the $m^6_2Am^6_2A$ residues presumably cannot, therefore, be required for processing. Rather, the association of Dim1p with the pre-rRNA is believed to be monitored by the cell as an indirect means of assuring that modification will occur.

The binding of Dim1p to the pre-rRNA could be required for pre-rRNA processing, either through direct interaction with components of the processing machinery or indirectly through a requirement for Dim1p in ribosomal assembly. Alternatively, it could be imagined that the binding of Dim1p is needed to displace some factor whose binding site on the pre-rRNA would overlap with that of Dim1p. In the absence of displacement, processing would be inhibited. Such a factor could be an RNA or a protein, but it is notable that a large number of snoRNAs described in yeast are not essential and have no known function. It is possible that some of these species act to monitor the correct modification or assembly of the pre-rRNA. The role of these snoRNAs might be to prevent specific pre-rRNA processing reactions in the absence of correct assembly or modification. The function envisaged for these snoRNAs would therefore be in direct contrast to that of U3, U14, and snR30, which, as described above, are required for pre-rRNA processing reactions to occur.

Perspectives

While much remains to be understood, some of the steps in the yeast pre-rRNA processing pathway have become clearer. This information has brought a number of questions into sharp focus. Some of the unresolved questions are as follows. (*i*) What are the mechanisms of cleavage in the early, snoRNP-dependent processing steps? Particularly, are these RNA-catalyzed reactions in which the snoRNAs directly participate in the cleavage reactions, or is the role of the snoRNAs to present the pre-rRNA in the correct conformation for cleavage by (proteinaceous) endonucleases? (*ii*) Related to this, how does the base-pairing interaction between U3 and the 5′ ETS relate to subsequent cleavage reactions? (*iii*) How is Dim1p binding sensed and how does the absence of Dim1p block processing? (*iv*) Are other modification and assembly reactions similarly controlled? These questions will not be easily resolved, but in yeast, the tools at least now exist to directly address them.

References

Aldrich, T.L., Di Segni, G., McConaughy, B.L., Keen, N.J., Whelen, S., and Hall, B.D. 1993. Structure of the yeast TAP1 protein: dependence of transcription activation on the DNA context of the target gene. Mol. Cell. Biol. **13**: 3434–3444.

Amberg, D.C., Goldstein, A.L., and Cole, C.N. 1992. Isolation and characterization of *RAT1*: an essential gene of *Saccharomyces cerevisiae* required for the efficient nucleocytoplasmic trafficking of mRNA. Genes & Dev. **6**: 1173–1189.

Bally, M., Hughes, J., and Cesareni, G. 1988. snR30: a new essential small nuclear RNA from *Saccharomyces cerevisiae*. Nucleic Acids Res. **16**: 5291–5303.

Banroques, J., and Abelson, J.N. 1989. PRP4: a protein of the yeast U4/U6 small nuclear ribonucleoprotein particle. Mol. Cell. Biol. **9**: 3710–3719.

Beltrame, M., and Tollervey, D. 1992. Identification and functional analysis of two U3 binding sites on yeast pre-ribosomal RNA. EMBO J. **11**: 1531–1542.

Beltrame, M., and Tollervey, D. 1995. Base-pairing between U3 and the pre-ribosomal RNA is required for 18S rRNA synthesis. EMBO J. **14**: 4350–4356.

Beltrame, M., Henry, Y., and Tollervey, D. 1994. Mutational analysis of an essential binding site for the U3 snoRNA in the 5′ external transcribed spacer of yeast pre-rRNA. Nucleic Acids Res. **22**: 5139–5147.

Bossie, M. A., DeHoratius, C., Barcelo, G., and Silver, P. 1992. A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast. Mol. Biol. Cell, **3**: 875–893.

Caffarelli, E., Arese, M., Santoro, B., Fragapane, P., and Bozzoni, I. 1994. In vitro study of processing of the intron-encoded U16 small nucleolar RNA in *Xenopus laevis*. Mol. Cell. Biol. **14**: 2966–2974.

Chu, S., Archer, R.H., Zengel, J.M., and Lindahl, L. 1994. The RNA of RNase MRP is required for normal processing of ribosomal RNA. Proc. Natl. Acad. Sci. U.S.A. **91**: 659–663.

Dalrymple, M.A., Petersen-Bjørn, S., Friesen, J.D., and Beggs, J.D. 1989. The product of the PRP4 gene of *S. cerevisiae* shows homology to β subunits of G proteins. Cell, **58**: 811–812.

Dandekar, T., and Tollervey, D. 1993. Identification and functional analysis of a novel yeast small nucleolar RNA. Nucleic Acids Res. **21**: 5386–5390.

Di Segni, G., McConaughy, B.L., Shapiro, R.A., Aldrich, T.L., and Hall, B.D. 1993. TAP1, a yeast gene that activates the expression of a tRNA gene with a defective internal promoter. Mol. Cell. Biol. **13**: 3424–3433.

Dykstra, C.C., Hamatake, R.K., and Sugino, A. 1990. DNA strand transfer protein β from yeast mitotic cells differs from strand transfer protein α from meiotic cells. J. Biol. Chem. **265**: 10 968 – 10 973.

Fabian, G.R., and Hopper, A.K. 1987. *RRP1*, a *Saccharomyces cerevisiae* gene affecting rRNA processing and production of mature ribosomal subunits. J. Bacteriol. **169**: 1571–1578.

Fragapane, P., Prislei, S., Michienzi, A., Caffarelli, E., and Bozzoni, I. 1993. A novel small nuclear RNA (U16) is encoded inside a ribosomal protein intron and originates by processing of the pre-mRNA. EMBO J. **12**: 2921–2928.

Girard, J.P., Lehtonen, H., Caizergues-Ferrer, M., Amalric, F., Tollervey, D., and Lapeyre, B. 1992. GAR1 is an essential small nucleolar RNP protein required for pre-rRNA processing in yeast. EMBO J. **11**: 673–682.

Girard, J.-P., Caizergues-Ferrer, M., and Lapeyre, B. 1993. The *SpGAR1* gene of *Schizosaccharomyces pombe* encodes the functional homologue of the snoRNP protein GAR1 of *Saccharomyces cerevisiae*. Nucleic Acids Res. **21**: 2146–2155.

Henriquez, R., Blobel, G., and Aris, J.P. 1990. Isolation and sequencing of NOP1. A yeast gene encoding a nucleolar protein homologous to a human autoimmune antigen. J. Biol. Chem. **265**: 2209–2215.

Henry, Y., Wood, H., Morrissey, J.P., Petfalski, E., Kearsey, S., and Tollervey, D. 1994. The 5′ end of yeast 5.8S rRNA is generated by exonucleases from an upstream cleavage site. EMBO J. **13**: 2452–2463.

Heyer, W.-D., Johnson, A.W., Reinhart, U., and Kolodner, R.D. 1995. Regulation and intracellular localization of *Saccharomyces*

cerevisiae strand exchange protein 1 (Sep1/Xrn1/Kem1), a multifunctional exonuclease. Mol. Cell. Biol. **15**: 2728–2736.

Hsu, C.L., and Stevens, A. 1993. Yeast cells lacking 5′→3′ exoribonuclease 1 contain mRNA species that are poly(A) deficient and partially lack the 5′ cap structure. Mol. Cell. Biol. **13**: 4826–4835.

Huang, G.H., Jarmolowski, A., Struck, J.C.R., and Fournier, M.F. 1992. Accumulation of U14 small nuclear RNA in *Saccharomyces cerevisiae* requires box C, box D, and a 5′,3′ terminal stem. Mol. Cell. Biol. **12**: 4456–4463.

Hughes, J.M.X., and Ares, M.J. 1991. Depletion of U3 small nucleolar RNA inhibits cleavage in the 5′ external transcribed spacer of yeast pre-ribosomal RNA and impairs formation of 18S ribosomal RNA. EMBO J. **10**: 4231–4239.

Hughes, J.M.X., Konings, D.A.M., and Cesareni, G. 1987. The yeast homologue of U3 snRNA. EMBO J. **6**: 2145–2155.

Interthal, H., Bellocq, C., Bähler, J., Bashkirov, V. I., Edelstein, S., and Heyer, W.-D. 1995. A role of Sep1 (= Kem1, Xrn1) as a microtubule binding protein in *Saccharomyces cerevisiae*. EMBO J. **14**: 1057–1066.

Jansen, R.P., Hurt, E.C., Kern, H., Lehtonen, H., Carmo-Fonseca, M., Lapeyre, B., and Tollervey, D. 1991. Evolutionary conservation of the human nucleolar protein fibrillarin and its functional expression in yeast. J. Cell. Biol. **113**: 715–729.

Jansen, R., Tollervey, D., and Hurt, E.C. 1993. A U3 snoRNP protein with homology to splicing factor PRP4 and Gβ domains is required for ribosomal RNA processing. EMBO J. **12**: 2549–2558.

Jarmolowski, A., Zagorski, J., Li, H.V., and Fournier, M.J. 1990. Idenfication of essential elements in U14 RNA of *Saccharomyces cerevisiae*. EMBO J. **9**: 4503–4509.

Johnson, A.W., and Kolodner, R.D. 1991. Strand exchange protein 1 from *Saccharomyces cerevisiae*. A novel multifunctional protein that contains DNA strand exchange and exonuclease activities. J. Biol. Chem. **266**: 14 046 – 14 054.

Johnson, A.W., and Kolodner, R. 1995. Synthetic lethality of *sep1* (*xrn1*) *ski1* and *sep1* (*xrn1*) *ski3* mutants of *Saccharomyces cerevisiae* is independent of killer virus and suggests a general role for these genes in translational control. Mol. Cell. Biol. **15**: 2719–2727.

Kearsey, S., and Kipling, D. 1991. Recombination and RNA processing: a common strand? Trends Cell Biol. **1**: 110–112.

Kempers-Veenstra, A.E., Oliemans, J., Offenberg, H., Dekker, A.F., Piper, P.W., Planta, R.J., and Klootwijk, J. 1986. 3′-End formation of transcripts from the yeast rRNA operon. EMBO J. **5**: 2703–2710.

Kenna, M., Stevens, A., McCammon, M., and Douglas, M.G. 1993. An essential yeast gene with homology to the exonuclease-encoding *XRN1/KEM1* gene also encodes a protein with exoribonuclease activity. Mol. Cell. Biol. **13**: 341–350.

Kiledjian, M., and Dreyfuss, G. 1992. Primary structure and binding activity of the hnRNP U protein: binding through RGG box. EMBO J. **11**: 2655–2664.

Kim, J., Ljungdahl, P.O., and Fink, G.R. 1990. *kem* mutations affect nuclear fusion in *Saccharomyces cerevisiae*. Genetics, **126**: 799–812.

Kipling, D., Tambini, C., and Kearsey, S.E. 1991. *rar* mutations which increase artificial chromosome stability in *Saccharomyces cerevisiae* identify transcription and recombination proteins. *Nucleic Acids Res.* **19**: 1385–1391.

Klootwijk, J., and Planta, R.J. 1989. Isolation and characterization of yeast ribosomal RNA precursors and preribosomes. Methods Enzymol. **180**: 96–109.

Kolodner, R., Evans, D.H., and Morrison, P.T. 1987. Purification and characterization of an activity from *Saccharomyces cervisiae* that catalyzes homologous pairing and strand exchange. Proc. Natl. Acad. Sci. U.S.A. **84**: 5560–5564.

Kondo, K., and Inouye, M. 1992. Yeast NSR1 protein that has structural similarities to mammalian nucleolin is involved in pre-rRNA processing. J. Biol. Chem. **267**: 16 252 – 16 258.

Kondo, K., Kowalski, L.R.Z., and Inouye, M. 1992. Cold shock induction of yeast NSR1 protein and its role in pre-rRNA processing. J. Biol. Chem. **267**: 16 259 – 16 265.

Lafontaine, D., Delcour, J., Glasser, A.-L., Desgrès, J., and Vandenhaute, J. 1994. The *DIM1* gene responsible for the conserved $m^6 2Am^6 2A$ dimethylation in the 3′ terminal loop of 18S rRNA is essential in yeast. J. Mol. Biol. **241**: 492–497.

Lafontaine, D., Vandenhaute, J., and Tollervey, D. 1995. The 18S rRNA dimethylase Dim1p is required for pre-ribosomal RNA processing in yeast. Genes & Dev. **9**: 2470–2481.

Lapeyre, B., Bourbon, H., and Amalric, F. 1987. Nucleolin, the major nucleolar protein of growing eucaryotic cells: an unusual protein structure revealed by nucleotide sequence. Proc. Natl. Acad. Sci. U.S.A. **84**: 1472–1476.

Larimer, F.W., Hsu, C.L., Maupin, M.K., and Stevens, A. 1992. Characterization of the XRN1 gene encoding a 5′→3′ exoribonuclease: sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells. Gene (Amsterdam), **120**: 51–57.

Lee, W.-C., Xue, Z., and Mélèse, T. 1991. The *NSR1* gene encodes a protein that specifically binds nuclear localization signals and has two RNA recognition motifs. J. Cell Biol. **113**: 1–12.

Lee, W.C., Zabetakis, D., and Mélèse, T. 1992. *NSR1* is required for pre-rRNA processing and for the proper maintenance of steady-state levels of ribosomal subunits. Mol. Cell. Biol. **12**: 3865–3871.

Li, H.V., and Fournier, M.J. 1992. U14 function in *Saccharomyces cerevisiae* can be provided by large deletion variants of yeast U14 and hybrid mouse-yeast U14 RNAs. EMBO J. **11**: 683–689.

Li, H.V., Zagorski, J., and Fournier, M.J. 1990. Depletion of U14 small nuclear RNA (snR128) disrupts production of 18S rRNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**: 1145–1152.

Lindahl, L., Archer, R.H., and Zengal, J.M. 1992. A new rRNA processing mutant of *Saccharomyces cerevisiae*. Nucleic Acids Res. **20**: 295–301.

Liu, Z., and Gilbert, W. 1994. The yeast *KEM1* gene encodes a nuclease specific for G4 tetraplex DNA: implication of in vivo functions for this novel DNA structure. Cell, **77**: 1083–1092.

Lygerou, Z., Mitchell, P., Petfalski, E., Séraphin, B., and Tollervey, D. 1994. The *POP1* gene encodes a protein component common to the RNase MRP and RNase P ribonucleoproteins. Genes & Dev. **8**: 1423–1433.

Mitchell, P., Petfalski, E., and Tollervey, D. 1996. The 3′ end of yeast 5.8S rRNA is generated by an exonuclease processing mechanism. Genes & Dev. In press.

Moritz, M., Paulovitch, A.G., Tsay, Y., and Woolford, J.L. 1990. Disruption of yeast ribosomal proteins L16 or rp59 disrupts ribosome assembly. J. Cell Biol. **111**: 2261–2274.

Moritz, M., Pulaski, B.A., and Woolford, J.L. 1991. Assembly of 60S ribosomal subunits is perturbed in temperature-sensitive yeast mutants defective in ribosomal protein L16. Mol. Cell. Biol. **11**: 5681–5692.

Morrissey, J.P. 1994. The role of small nucleolar ribonucleoprotein particles (snoRNPs) in pre-rRNA processing in *Saccharomyces cerevisiae*. PhD. Thesis, National University of Ireland, Cork.

Morrissey, J.P., and Tollervey, D. 1993. Yeast snR30 is a small nucleolar RNA required for 18S rRNA synthesis. Mol. Cell. Biol. **13**: 2469–2477.

Morrissey, J.P., and Tollervey, D. 1995. Birth of the snoRNPs: the evolution of RNase MRP and the eukaryotic pre-rRNA processing system. Trends Biochem. Sci. **20**: 78–82.

Muhlrad, D., and Parker, R. 1994. Premature translation termination triggers mRNA decapping. Nature (London), **370**: 578–581.

Muhlrad, D., Decker, C.J., and Parker, R. 1994. Deadenylation of the unstable mRNA encoded by the yeast *MFA2* gene leads to

decapping followed by 5′→3′ digestion of the transcript. Genes & Dev. **8**: 855–866.

Nicoloso, M., Caizergues-Ferrer, M., Michot, B., Azum, M., and Bachellerie, J. 1994. U20, a novel small nucleolar RNA, is encoded in an intron of the nucleolin gene in mammals. Mol. Cell. Biol. **14**: 5766–5776.

Piper, P.W., Bellatin, J.A., and Lockheart, A. 1983. Altered maturation of sequences at the 3′ terminus of 5S gene transcripts in a *Saccharomyces cerevisiae* mutant that lacks a RNA processing endonuclease. EMBO J. **2**: 353–359.

Qu, L.-H., Nicoloso, M., Michot, B., Azum, M.-C., Caizergues-Ferrer, M., Renalier, M.-H., and Bachellerie, J.-P. 1994. U21, a novel small nucleolar RNA with a 13 nt. complementarity to 28S rRNA, is encoded in an intron of ribosomal protein L5 gene in chicken and mammals. Nucleic Acids Res. **22**: 4073–4081.

Raué, H.A., and Planta, R.J. 1991. Ribosome biogenesis in yeast. Prog. Nucleic Acid Res. Mol. Biol. **41**: 89–129.

Raué, H.A., Klootwijk, J., and Musters, W. 1988. Evolutionary conservation of structure and function of high molecular weight ribosomal RNA. Prog. Biophys. Mol. Biol. **51**: 77–129.

Ripmaster, T.L., Vaughn, G.P., and Woolford, J.L. 1993. *DRS1* to *DRS7*, novel genes required for ribosome assembly and function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13**: 7901–7912.

Russell, I.D., and Tollervey, D. 1992. NOP3 is an essential yeast protein which is required for pre-rRNA processing. J. Cell Biol. **119**: 737–747.

Sachs, A.B., and Davis, R.W. 1990. Translation initiation and ribosomal biogenesis: involvement of a putative rRNA helicase and RPL46. Science (Washington, D.C.), **247**: 1077–1079.

Schimmang, T., Tollervey, D., Kern, H., Frank, R., and Hurt, E.C. 1989. A yeast nucleolar protein related to mammalian fibrillarin is associated with small nucleolar RNA and is essential for viability. EMBO J. **8**: 4015–4024.

Schmitt, M.E., and Clayton, D.A. 1993. Nuclear RNase MRP is required for correct processing of pre-5.8S rRNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13**: 7935–7941.

Shuai, K., and Warner, J.W. 1991. A temperature sensitive mutant of *S. cerevisiae* defective in pre-rRNA processing. Nucleic Acids Res. **19**: 5059–5064.

Stevens, A. 1980. Purification and characterization of a *Saccharomyces cerevisiae* exoribonuclease which yields 5′-mononucleotides by a 5′→3′ mode of hydrolysis. J. Biol. Chem. **255**: 3080–3085.

Stevens, A., and Maupin, M.K. 1987. A 5′→3′ exoribonuclease of *Saccharomyces cerevisiae*: size and novel substrate specificity. Arch. Biochem. Biophys. **252**: 339–347.

Stevens, A., Hsu, C.L., Isham, K.R., and Larimer, F.W. 1991. Fragments of the internal transcribed spacer 1 of pre-rRNA accumulate in *Saccharomyces cerevisiae* lacking 5′→3′ exoribonuclease 1. J. Bacteriol. **173**: 7024–7028.

Tekamp, P. A., Garcea, R. L., and Rutter, W. J. 1980. Transcription and *in vitro* processing of yeast 5S rRNA. J. Biol. Chem. **255**: 9501–9506.

Tollervey, D. 1987. A yeast small nuclear RNA is required for normal processing of pre-ribosomal RNA. EMBO J. **6**: 4169–4175.

Tollervey, D., and Guthrie, C. 1985. Deletion of a yeast small nuclear RNA gene impairs growth. EMBO J. **4**: 3873–3878.

Tollervey, D., Lehtonen, H., Carmo-Fonseca, M., and Hurt, E.C. 1991. The small nucleolar RNP protein NOP1 (fibrillarin) is required for pre-rRNA processing in yeast. EMBO J. **10**: 573–583.

Tycowski, K.T., Shu, M., and Steitz, J.A. 1993. A small nucleolar RNA is processed from an intron of the human gene encoding ribosomal protein S3. Genes & Dev. **7**: 1176–1190.

Udem, S.A., and Warner, J.R. 1973. The cytoplasmic maturation of a ribosomal precursor ribonucleic acid in yeast. J. Biol. Chem. **248**: 1412–1416.

van Knippenberg, P.H., van Kimmenade, J.M.A., and Heus, H.A. 1984. Phylogeny of the conserved 3′ terminal structure of the RNA of small ribosomal subunits. Nucleic Acids Res. **12**: 2595–2604.

van Nues, R.W., Venema, J., Rientjes, J.M.J., Dirks-Mulder, A., and Raué, H.A. 1995. Processing of eukaryotic pre-rRNA: the role of the transcribed spacers. Biochem. Cell Biol. **73**. This issue.

Veldman, G.M., Brand, R.C., Klootwijk, J., and Planta, R.J. 1980. Some characteristics of processing sites in ribosomal precursor RNA of yeast. Nucleic Acids Res. **8**: 2907–2920.

Venema, J., Henry, Y., and Tollervey, D. 1995. Two distinct recognition signals define the site of endonucleolytic cleavage at the 5′ end of yeast 18S rRNA. EMBO J. **14**: 4883–4892.

Yip, M.T., and Holland, M.J. 1989. *In vitro* RNA processing generates mature 3′ termini of yeast 35S and 25S ribosomal RNAs. J. Biol. Chem. **264:** 4045–4051.

Zagorski, J., Tollervey, D., and Fournier, M.J. 1988. Characterization of an *SNR* gene locus in *Saccharomyces cerevisiae* that specifies both dispensible and essential small nuclear RNAs. Mol. Cell. Biol. **8**: 3282–3290.