Modification and Editing of RNA
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Chapter 15

Regulatory Aspects of rRNA Modification and Pre-rRNA Processing

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In most eukaryotes, three out of the four mature rRNA species are produced from a single large RNA polymerase I transcript (designated the 35S pre-rRNA in yeast), which is processed in a complex pathway involving both endonuclease cleavage and exonuclease digestion (Fig. 1) (reviewed in Lafontaine and Tollervey, 1995b; Venema and Tollervey, 1995; Tollervey, 1996). The fourth mature species, 5S rRNA, is transcribed and processed independently. During pre-rRNA processing, many specific nucleotides within the rRNAs are covalently modified (Maden, 1990; Maden and Hughes, 1997). The major types of posttranscriptional modification are the isomerization of uracil to pseudouridine, 2′-O-methylation of the ribose moieties and base methylation (see accompanying Chapter 12 by Ofengand and Fournier and Chapter 13 by Bachellerie and Cavaillé). Concomitantly with the pre-rRNA processing and modification reactions, the ribosomal proteins assemble with the pre-rRNAs to form preribosomal particles; mechanisms are likely to exist that coordinate all of these activities.

Recent analyses have identified three rRNA-modifying enzymes in yeast. Dim1p carries out the conserved base dimethylation that converts two adenosine residues at the 3′-end of the small subunit rRNA (SSU-rRNA) to $m_2^6A_{1779}m_2^6A_{1780}$ (yeast numbering); Cbf5p is the presumed rRNA pseudouridine synthase, which potentially modifies 13 sites in 18S rRNA and 30 sites in 25S rRNA (see Chapter 12 by Ofengand and Fournier for their locations); and Pet56p is a 2′-O-methylase specific for G_{2270} in the 21S yeast mitochondrial rRNA (see Chapter 14 by Mason). In addition to their roles in pre-rRNA modification, both Dim1p and Cbf5p are required for pre-rRNA processing, and Pet56p is required for synthesis of mitochondrial large ribosomal subunits.

The data on Dim1p and Cbf5p have given us insights on the type of systems that may act to coordinate the many steps in ribosome synthesis and the origins of the snoRNA-directed rRNA modification systems present in eukaryotic cells. These will be discussed in this chapter.

Dim1p, A CASE OF QUALITY CONTROL

$m_2^6Am_2^6A$ is one of the few rRNA base modifications that have been conserved from bacteria to eukaryotes. The modification is almost universally conserved; the only known exceptions are the yeast mitochondrial ribosomes that are not dimethylated and the chloroplast ribosomes from *Euglena gracilis* that are partially dimethylated (Klootwijk et al., 1975; van Buul et al., 1984; reviewed in van Knippenberg, 1986). The site of modification lies at the 3′-end of the SSU-rRNA in the loop of a hairpin structure, which is highly conserved in sequence (Fig. 2). In the ribosome, the $m_2^6Am_2^6A$ residues are located at the interface between the subunits at a site where crucial recognition reactions occur during translation (Thamana and Cantor, 1978; Maden, 1990; Brimacombe et al., 1993).

Yeast Dim1p was identified by complementation of an *Escherichia coli* mutant defective in the rRNA dimethylase ksgAp (Lafontaine et al., 1994). Dim1p and ksgAp show substantial sequence homology (27% identity and 50% similarity) and Dim1p can dimethylate the *E. coli* SSU-rRNA in vivo, showing them to be orthologs (that is, the "same" gene from

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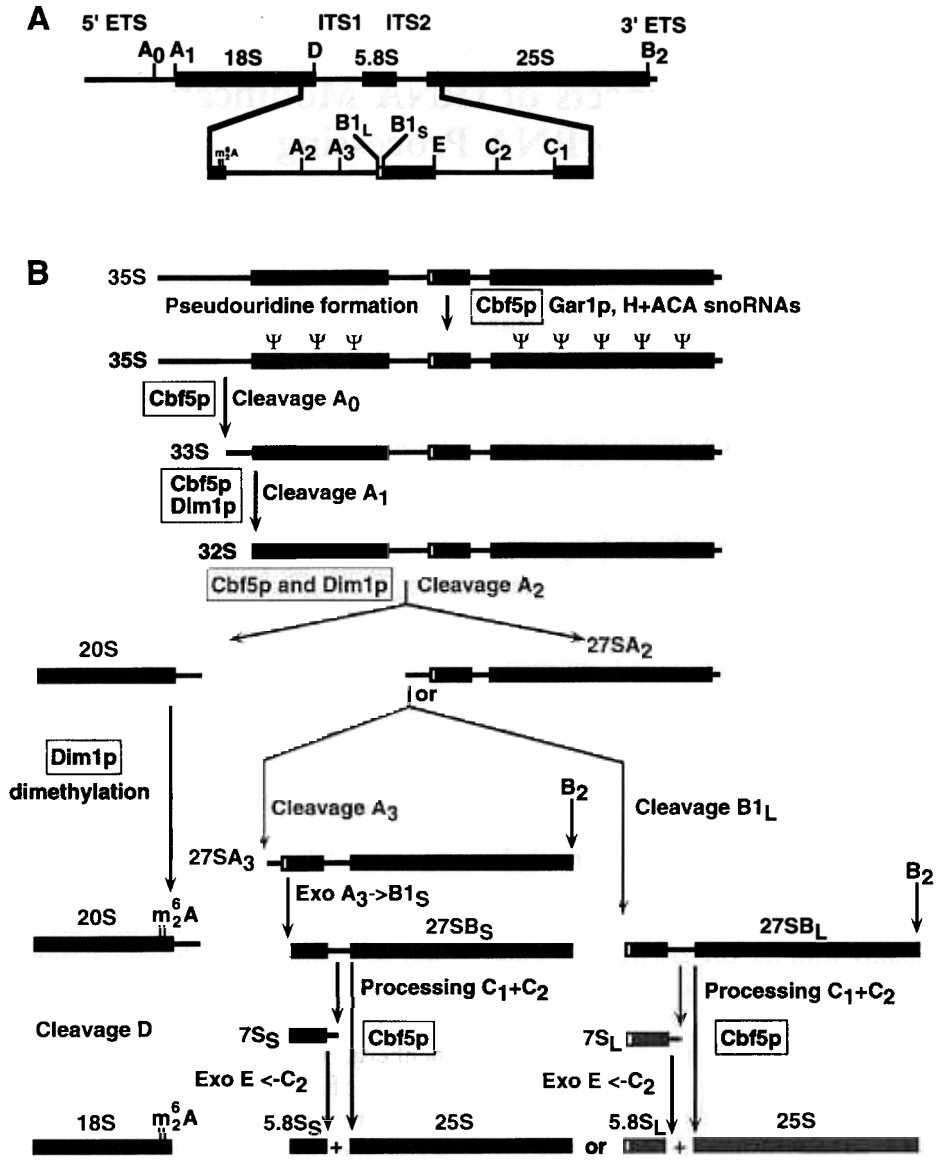

Figure 1. Structure of the yeast pre-rRNA and its processing pathway. (A) The 35S pre-rRNA. The sequences encoding the mature 18S, 5.8S and 25S rRNAs (thick lines) are flanked by the 5′ and 3′ external transcribed spacers (5′ ETS and 3′ ETS) and separated by internal transcribed spacers 1 and 2 (ITS1 and ITS2). Sites of pre-rRNA processing are indicated with uppercase letters (A_0 to E). The site of dimethylation is represented by m_2^6A. (B) The pre-rRNA processing pathway. Pseudouridine formation occurs shortly after or during transcription. Pseudouridine synthesis is targeted by the H + ACA snoRNAs and requires Cbf5p assisted by Gar1p. Processing of the primary 35S precursor starts at site A_0, yielding the 33S pre-rRNA. This molecule is subsequently processed at sites A_1 and A_2, giving rise successively to the 32S pre-rRNA and to the 20S and 27SsA$_2$ precursors. Cleavage at A_2 separates the pre-rRNAs destined for the small and large ribosomal subunit. The 20S precursor is dimethylated by Dim1p and then cleaved endonucleolytically at site D to yield the mature 18S rRNA. The 27SA$_2$ precursor is processed by two alternative pathways to form the mature 5.8S and 25S rRNAs. The major pathway involves cleavage at a second site in ITS1, A_3, rapidly followed by exonucleolytic digestion to site $B1_S$, generating the 27SB$_S$ precursor. Approximately 15% of the 27SA$_2$ molecules are processed by an alternative pathway at site $B1_L$, producing the 27SB$_L$ pre-rRNA. At the same time as processing at B1 is completed, the 3′-end of mature 25S rRNA is generated by processing at site B2. The subsequent processing of both 27SB species appears to follow a similar pathway. Cleavage at sites C_1 and C_2 releases the mature 25S rRNA and the 7S pre-rRNAs, which undergo rapid 3′→5′ exonuclease digestion to site E, generating the mature 3′-end of 5.8S rRNA. Cbf5p and Dim1p are required for the early cleavages at sites A_1 and A_2; loss of these cleavages inhibits formation of the 20S and 27SA$_2$ pre-rRNAs, preventing synthesis of 18S rRNA. In addition, Cbf5p is required for efficient processing at site A_0 and efficient processing of the 27SB and 7S pre-rRNAs in ITS2.

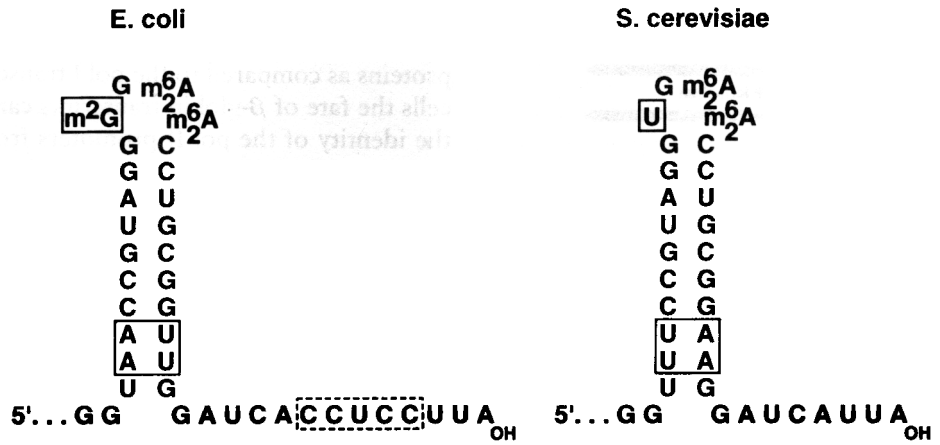

Figure 2. 3'-end of the SSU-rRNA in *E. coli* and yeast cytoplasmic ribosomes. Divergent nucleotides are boxed. A precise deletion of the anti-Shine-Dalgarno box (CCUCC) has occurred in the eukaryotic SSU-rRNA. The twin adenosine substrates (1518–1519 in *E. coli* and 1779–1780 in yeast) of Dim1p are universally conserved.

different organisms). Genetic depletion of Dim1p under the control of a regulated *GAL* promoter led to the inhibition of dimethylation, confirming that it is the only dimethylase in yeast. Deletion of the *DIM1* gene in yeast was found to be lethal, in contrast to *E. coli* in which *ksgA* mutants are viable although mildly impaired in growth. Surprisingly, however, the lack of the dimethylation was not the cause of this lethality. Genetic depletion of Dim1p inhibited pre-rRNA processing, preventing the synthesis of the mature SSU-rRNA, and it is the loss of this rRNA that is responsible for the lethality of *dim1* mutants (Lafontaine et al., 1995a).

Depletion of Dim1p inhibited the cleavage of sites A_1 and A_2 (see Fig. 1 and 3). These cleavages generate the 20S pre-rRNA, which is the substrate for the dimethylation reaction in wild-type cells. Because the processing reactions normally occur before dimethylation of the pre-rRNA (Fig. 1), it seemed unlikely that processing was dependent on dimethylation. This conclusion was supported by the observation that replacement of the A residues at the site of dimethylation with G residues that cannot be modified did not interfere with pre-rRNA processing (Lafontaine et al., 1995a). This was subsequently confirmed by the finding that point mutations in Dim1p could uncouple the pre-rRNA processing defect from the dimethylation defect (Lafontaine et al., 1998b).

In *dim1-2* mutant strains, dimethylation of the pre-rRNA is strongly inhibited with little effect on pre-rRNA processing at permissive temperature (Lafontaine et al., 1998b). In consequence, the *dim1-2* strains accumulated normal levels of small ribosomal subunits, but these lacked the $m^6_2Am^6_2A$ dimethylation. The strain grew normally, demonstrating that the dimethylation is not required for ribosome function in vivo. However, extracts prepared from the *dim1-2* mutant strain did not support in vitro translation. This suggested that the $m^6_2Am^6_2A$ dimethylation "fine-tunes" the function of the ribosome in vivo, but becomes much more important for function in the suboptimal in vitro conditions. Related observations have been made for pre-mRNA splicing; mutations that drastically inhibit in vitro splicing in cell extracts frequently have little effect on splicing activity in vivo (Jacquier et al., 1985; Séraphin et al., 1988).

The uncoupling of the dimethylation and pre-rRNA processing defects showed that Dim1p rather than the dimethylation activity is required for pre-rRNA processing. This observation did not, however, determine whether Dim1p is itself directly required for pre-rRNA processing. This question was resolved in an unexpected manner by the observation that when the transcription of an rDNA unit was driven by an RNA polymerase II (pol II) *PGK* promoter, processing of the pre-rRNAs became insensitive to temperature-sensitive mutations in *DIM1* or depletion of Dim1p (Lafontaine et al., 1998b). Dim1p is not, therefore, directly required for pre-rRNA processing.

These observations led to the conclusion that an active repression system blocks pre-rRNA processing in the absence of the binding of Dim1p to the pre-rRNA. According to this model, Dim1p binds to the pre-rRNA in the nucleolus at an early stage in ribosome synthesis. This step is monitored by a component of the processing machinery such that processing at sites A_1 and A_2 occurs only if Dim1p has bound to the pre-rRNA. In mutant strains that lack Dim1p this leads to the synthesis of a dead-end intermediate, the 22S pre-rRNA, and prevents synthesis of unmodified 18S rRNA (Fig. 3). The pre-rRNAs that are

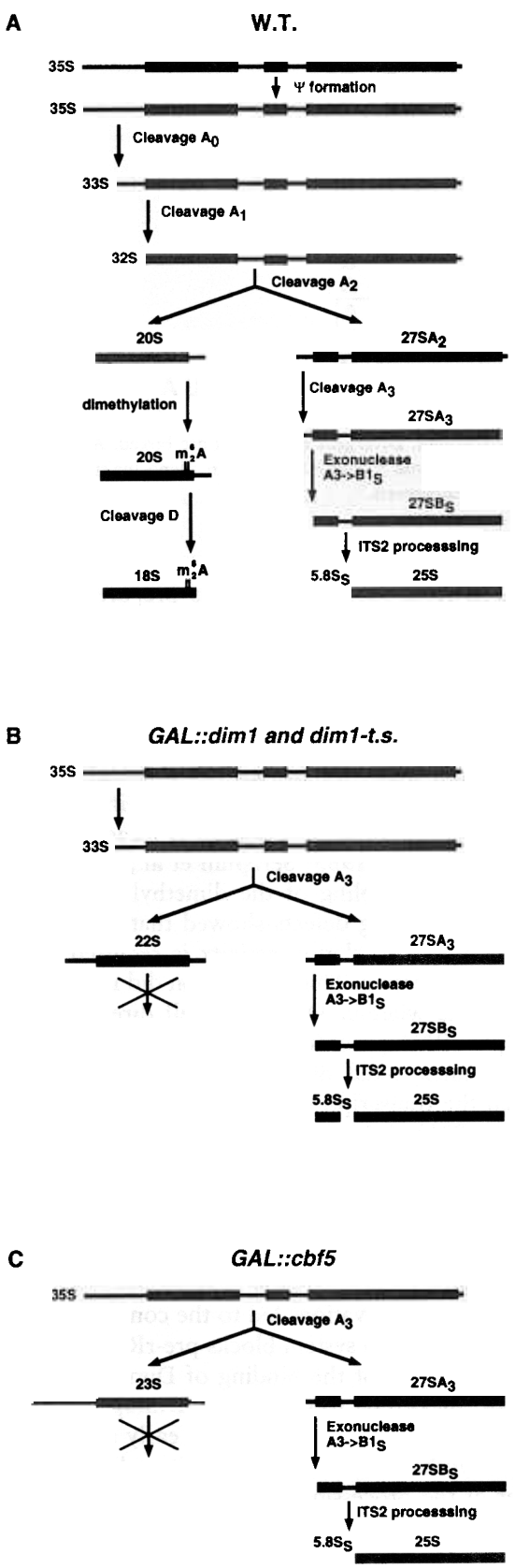

Figure 3. Pre-rRNA processing in the wild type (A) and *dim1* (B) and *cbf5* (C) mutants. (A) Simplified version of the wild-type pre-rRNA processing pathway, as described in Fig. 1. (B) *GAL::dim1* strains and *dim1-t.s.* strains are inhibited in cleavage at sites A_1 and A_2. Consequently, the 22S pre-rRNA accumulates and no 18S rRNA is made. The $27SA_3$ pre-rRNA is normally processed to 25S and 5.8S rRNAs. (C) *GAL::cbf5* strains are inhibited in cleavage at sites A_0, A_1 and A_2. In this case the 23S pre-rRNA is accumulated and no 18S rRNA is made. The $27SA_3$ pre-rRNA can be processed to 25S and 5.8S rRNAs, although processing of the 27SB and 7S pre-rRNAs is delayed.

transcribed from the pol II *PGK* promoter are presumably associated with a different set of hnRNP proteins as compared to the pol I transcripts. In HeLa cells the fate of β-globin transcripts can be altered by the identity of the pol II promoters from which they are transcribed (Enssle et al., 1993). We speculate that in the *PGK*-driven pre-rRNAs an hnRNP protein occupies the Dim1p binding site and is detected by the pre-rRNA processing machinery, thus alleviating the need for authentic Dim1p.

In *E. coli*, mutations in ksgAp block the $m_2^6Am_2^6A$ dimethylation of the 16S rRNA, but do not interfere with processing of the rRNA. In consequence, the mutants synthesize small ribosomal subunits that lack the modification. These allow the growth of the mutant strains, although at a reduced rate, and the unmodified ribosomes are defective in several aspects of translation in vitro (reviewed in van Knippenberg, 1986). This contrasts with the situation in yeast, where mutations in Dim1p are lethal because pre-rRNA processing is blocked. On first inspection the situation in *E. coli* appears preferable because the mutants are at least alive. However, wild-type cells are unlikely to be faced with the situation in which the dimethylase is actually absent or defective. The regulatory system in yeast presumably evolved to deal with those preribosomal particles to which Dim1p binds late in the process. In this situation, pre-rRNA processing would simply be delayed until Dim1p binding occurs and processing would then resume. This is desirable because the unmodified ribosomal subunits are impaired in function.

An obvious question that arises from these observations is why the dimethylation is not directly monitored. One possible explanation is that a system that detected the binding of the Dim1p protein to the preribosome developed more readily than a system that could detect the presence or absence of the methyl groups themselves. An alternative explanation was offered by the observation that all *dim1* mutants tested were hypersensitive to the aminoglycoside antibiotics paromomycin and neomycin B, even under conditions where neither dimethylation nor processing was clearly affected (Lafontaine et al., 1998b). This suggested that Dim1p plays an additional role in ribosome assembly. The binding of Dim1p to the

pre-rRNA may therefore be monitored to ensure that it fulfills its functions in both modification and assembly.

Ribosome synthesis is a highly dynamic process during which a vast number of processing, modification, and assembly reactions occur simultaneously. How can all of these reactions be coordinated? In any given preribosomal subunit there must be a significant chance that some components will bind late. This is a particular problem in eukaryotes because the assembly, modification, and processing reactions proceed very quickly with the preribosomal particles being rapidly exported to the cytoplasm, where they are likely to be inaccessible to nucleolar assembly and processing factors. We anticipate that many other quality control systems of the type proposed for Dim1p exist to coordinate assembly, modification, processing, and export to ensure that no irrevocable steps occur before all major components are assembled. It is notable that the depletion or mutation of any of several different ribosomal proteins has been found to inhibit pre-rRNA processing (Moritz et al., 1990; Moritz et al., 1991; Deshmukh et al., 1993; Vilardell and Warner, 1997). It is very likely that such inhibition also reflects the activity of quality control systems, which would normally simply delay processing to allow time for the assembly of the missing protein.

Pet56p, A MITOCHONDRIAL rRNA-MODIFYING ENZYME

Pet56p is a mitochondrial rRNA 2′-*O*-methylase specific to position G_{2270} in the peptidyl transferase center of the 21S yeast mitochondrial rRNA. The equivalent position in the 23S rRNA of *E. coli*, position G_{2251}, is also ribose methylated (see Chapter 14 by Mason).

Like Dim1p, Pet56p is required for ribosome synthesis. Strains depleted of Pet56p or carrying a deletion of *PET56* strongly underaccumulate large ribosomal subunits (Sirum-Connolly and Mason, 1993). Extragenic mutations were isolated that weakly suppress the *pet56* loss-of-function mutations, and this was interpreted as showing that neither the Gm_{2270} modification nor the Pet56p protein itself is absolutely required for the synthesis of functional ribosomes (Sirum-Connolly and Mason, 1995) (also see Chapter 14 by Mason). Pet56p may therefore be the target of a quality control system that functions in a manner analogous to that postulated for Dim1p.

Cbf5p, A COMPONENT OF MULTIPLE snoRNPs

Yeast Cbf5p is a nucleolar protein that shows strong sequence homology to the prokaryotic tRNA: Ψ55 synthase truBp (Nurse et al., 1995) and to the rat nucleolar protein Nap57p (Meier and Blobel, 1994). The latter is tightly associated with Nop140p, a protein that is reported to shuttle between the nucleolus and the nuclear envelope (Meier and Blobel, 1992). These observations suggested that Cbf5p might be a nucleolar pseudouridine synthase. Genetic depletion of Cbf5p impaired total rRNA Ψ formation with no clear effect on formation of Ψ in tRNA (Lafontaine et al., 1998a). As with depletion of Dim1p, pre-rRNA processing was also strongly impaired, leading to the loss of the SSU-rRNA (Fig. 1 and 3).

Formation of Ψ in the rRNA is guided by the H + ACA class of snoRNAs (Ni et al., 1997; Ganot et al., 1997; see Chapter 12 by Ofengand and Fournier and recent reviews by Maden, 1997; Peculis, 1997; and Smith and Steitz, 1997) and Cbf5p is associated with this class of snoRNA. Epitope tagged Cbf5p was able to efficiently coprecipitate all tested H + ACA snoRNAs, while genetic depletion of Cbf5p led to the loss of these snoRNAs (Lafontaine et al., 1998a). Gar1p is also associated with the box H + ACA snoRNAs (Girard et al., 1992; Lübben et al., 1995) and depletion of Cbf5p led to the loss of Gar1p. Cbf5p is therefore an integral component of the box H + ACA class of small nucleolar ribonucleoproteins (snoRNPs). Depletion of Gar1p also leads to a global defect in rRNA Ψ formation (Bousquet-Antonelli et al., 1997), but it does not show any of the known pseudouridine synthase motifs and is unlikely to act enzymatically. Gar1p is not required for the stability of the box H + ACA snoRNAs tested, snR10, snR30, and snR36 (Girard et al., 1992; Bousquet-Antonelli et al., 1997), but it may play an important role in their association with the pre-rRNA. In Gar1p-depleted cells, snR36 snoRNPs are unable to associate with higher order nucleolar particles (Bousquet-Antonelli et al., 1997). The function of Gar1p in rRNA pseudouridine formation is therefore likely to be the stabilization of Cbf5p-containing snoRNP complexes at the sites of Ψ formation.

A conserved central domain of Gar1p is sufficient to allow nucleolar localization and fulfill the essential function of the protein (Girard et al. 1994), and is both necessary and sufficient for in vitro binding to the H + ACA snoRNAs snR30 and snR10 (Bagni and Lapeyre, personal communication). We speculate that the central domain of Gar1p specifically binds to the H + ACA snoRNAs, while the two external glycine and arginine rich (GAR) domains

(Girard et al., 1992) stabilize the snoRNA and pre-rRNA interactions. GAR domains have been reported both to confer RNA-binding activity (Kiledjian and Dreyfuss, 1992) and to destabilize RNA structures (Ghisolfi et al., 1992). An interesting possibility is that the GAR domains could both open up the complex structure of the pre-rRNAs and stabilize the interaction between the H + ACA snoRNAs and the pre-rRNA at the sites of modification. Such an activity may be crucial because the sequence complementarity between the H + ACA snoRNAs and the rRNA consists only of two short motifs of 3–10 nucleotides (see Chapter 12 by Ofengand and Fournier; Ganot et al., 1997). Rok1p, a putative ATP-dependent RNA helicase that functionally interacts both with Gar1p and the H + ACA snoRNA snR10 (Venema et al., 1997) may also be involved in this process.

The pre-rRNA processing defects observed on depletion of Cbf5p, inhibition of cleavage at sites A_1 and A_2 and delay of cleavage at site A_0 (Fig. 1 and 3), closely resemble those observed on depletion of the box H + ACA snoRNA, snR30 (Morrissey and Tollervey, 1993). snR30 is lost on depletion of Cbf5p, suggesting that the pre-rRNA processing defect is due to the lack of snR30. Depletion of Gar1p leads to a similar pre-rRNA processing defect (Girard et al., 1992), but the effects of Gar1p depletion on the association of snR30 and the pre-rRNA have not been reported.

The requirement for Cbf5p in pre-rRNA processing appears to be quite different from the requirement for Dim1p. In no case has the pseudouridine guide activity of a box H + ACA snoRNA been found to be required for pre-rRNA processing. In the absence of Cbf5p or Gar1p, 25S rRNA synthesis continues, leading to the synthesis of highly undermodified 60S subunits. Similarly, no 2′-*O*-methylation is known to be required for pre-rRNA processing and *nop1-3* mutants that are globally inhibited for 2′-*O*-methylation show little inhibition of processing (Tollervey et al., 1993). This raises an obvious question: why do quality control systems not exist to ensure that pseudouridine formation and 2′-*O*-methylation also occur? In this case we speculate that the sheer number of modifications (43 Ψ and 55 2′-*O*-methyl groups are present in the yeast rRNAs) has made the development of a regulatory system for each site of modification impractical. A predicted consequence of the absence of such regulatory systems is that some proportion of ribosomal subunits will lack one or more pseudouridine or 2′-*O*-methyl modifications. This in turn would be expected to apply selective pressure for the absence of individual modifications, not to result in any major impairment in ribosome function. This appears to be the case because deletion of individual Ψ guide or methylation guide snoRNAs has no detectable effect on growth (see Chapter 12 by Ofengand and Fournier and Chapter 13 by Bachellerie and Cavaillé and references therein).

During pseudouridine formation in the rRNA, snoRNAs base paired to the rRNA provide a common signal, allowing a single pseudouridine synthase to recognize multiple sites. This system shows some resemblance to the pseudouridylation of positions 34 and 36 in the anticodon loop of minor-tRNA^{Ile}. In yeast, modification of these sites by Pus1p is dependent on the presence of the tRNA intron; the mature tRNA is not a substrate for modification (Szweykowska-Kulinska et al., 1994; Simos et al., 1996). The intron therefore acts in *cis* as an internal guide sequence, allowing recognition of sites in the mature tRNA region (Grosjean et al., 1997).

MODEL FOR THE EVOLUTIONARY ORIGIN OF MODIFICATION GUIDE snoRNAs

Comparison of the number and distribution of Ψ residues in the rRNA of bacteria and eukaryotes raises two questions. First, why is there such a large (~10-fold-higher) number of Ψ residues in eukaryotic rRNA compared to bacterial rRNA? Second, why is there such a poor correspondence between the actual sites that are modified in each kingdom? The work on Cbf5p suggests possible explanations for these puzzling observations.

Modern eukaryotes are equipped with a huge array of pseudouridine guide H + ACA snoRNAs (see Chapter 12), but this system clearly did not spring into its existence fully formed, and it is reasonable to assume that a single snoRNA originally acquired the ability to select a site of pseudouridine formation. The yeast tRNA:Ψ55 pseudouridine synthase Pus4p (Becker et al., 1997) and Cbf5p both show high homology to the *E. coli* tRNA:Ψ55 pseudouridine synthase truBp (Koonin, 1996). This suggests that an early eukaryote (or archaea) had a single "truBp-like" tRNA pseudouridine synthase which, like truBp, recognized its substrate via structural features in the RNA. This enzyme then acquired the ability to recognize an RNA structure comprised of an snoRNA base paired in *trans* to the rRNA (Lafontaine et al., 1998a). Gene duplication and divergence of function would then lead to two forms of the pseudouridine synthase: one specialized for Ψ formation in the pre-rRNA (Cbf5p), and one for the tRNA (Pus4p). Over time new box H + ACA snoRNAs would arise, allowing the system to modify new sites in the rRNA.

This would slowly replace the preexisting system of "bacterial-like" rRNA pseudouridine synthases.

According to this model, new sites of Ψ formation in the eukaryotic rRNA arose when complementarity to new rRNA sequences was generated by mutations in the H + ACA snoRNAs. Only two short stretches of 3–10 nucleotides in the snoRNAs are base paired to the rRNA, and it is evident that new snoRNA and rRNA interactions will arise more often than will protein enzymes with specificity for a new site in the rRNA. The snoRNA-directed system, therefore, offered much greater flexibility in generating new sites. Moreover, the requirement that each site be recognized both as a binding site for the snoRNA and as a substrate for a structure-dependent pseudouridine synthase allowed greater selectivity, reducing the problems of misrecognition that would be associated with the presence of a large number of distinct pseudouridine synthases. These observations may explain why the eukaryotic rRNAs have many more modified sites than their bacterial counterparts.

This model also predicts that the new sites would have been generated by mutations in the guide snoRNAs independently of the preexisting proteinaceous system. This leads to the conclusion that the Ψ sites in eukaryotic rRNA are related to the sites in the bacterial rRNA by convergent, not divergent, evolution. This potentially explains why there is so little correspondence between the sites of Ψ in bacteria and eukaryotes. A more complete description of this model can be found in Lafontaine and Tollervey (1998c).

CONCLUSIONS AND PROSPECTS

Eukaryotic ribosome synthesis involves very complex pre-rRNA processing and assembly pathways. These include numerous steps occurring in different cellular compartments and requiring a plethora of RNA and proteins, many of them in the form of snoRNPs that only transiently associate with the preribosomal particles. Such a complex process would be expected to be highly regulated, and there is increasing evidence that this is the case. Some processing steps appear to occur in an obligatory order, and there are several examples of coupling between processing reactions that occur at sites that are distant in the primary sequence. Presumably these interactions help to ensure the coordinated processing of the pre-rRNA. Moreover, the pre-rRNAs are extensively modified with the bulk of modification (2′-O-methylation and pseudouridylation) being snoRNP dependent and occurring at an early stage in the ribosome synthesis, but with some positions, mostly bases and a few sugars, being specifically methylated at later stages. $m_2^6Am_2^6A$ belongs to this latter class and it will be interesting to determine whether the other late modifications are also subject to quality control systems of the type proposed here for Dim1p.

All tested Ψ and 2′-O-methyl residues are dispensable for ribosome function in vivo. This is not evidence that these modifications do not make important contributions to ribosome function. The $m_2^6Am_2^6A$ dimethylation is also dispensable in vivo but is required for translation in vitro, showing that it does play an important role in the normal function of the ribosome. It also remains possible that a small number of individual Ψ or 2′-O-methyl modifications are essential for ribosome function, and it seems likely that the absence of multiple modifications will not be tolerated.

The archaea may hold the final clues to understanding the origins of the snoRNA-directed systems of rRNA modification. The pattern of Ψ formation and of 2′-O-methylation in archaeal rRNAs would be a usefull indication of the time at which the guide snoRNA system developed. The archaea have a homolog of the box C + D snoRNA-associated protein Nop1p (fibrillarin) (Amiri, 1994); it remains to be determined whether they also have homologs of Cbf5p, Gar1p, and, particularly, the modification guide snoRNAs.

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