Evolution of Eukaryotic rRNA: Constraints Imposed by RNA Interactions

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In this chapter we will discuss the effects of alterations within the ribosomal DNA (rDNA) genes. RNA interactions that are important for regions within rRNA impose selective constraints upon propagation of mutations within the gene. As a consequence, there are functionally important regions in rRNA that are highly conserved in primary sequence, even between different kingdoms. rRNA also has an evolutionarily conserved core secondary structure. Finally, in this chapter we will examine U3 small nuclear RNA (snRNA), and discuss whether its structure can support models of its putative interaction with the rRNA precursor.

Structure of *Xenopus* rDNA Transcription Unit

The rDNA of *Xenopus laevis*, the South African clawed toad, was the first eukaryotic gene to be cloned (Morrow et al. 1974), and we have used this model system to study its evolution. Figure 1 depicts a typical repeat unit of *X. laevis* rDNA; there are about 450 tandem copies of this rDNA repeat in the nucleolus organizer region (Brown and Weber 1968a,b). The stretch coding for the 40S RNA precursor alternates with the so-called nontranscribed spacer (NTS). Recent evidence suggests that the NTS is, in fact, also transcribed as part of a larger precursor that must be rapidly processed (DeWinter and Moss 1986; Labhart and Reeder 1986, 1987). Further processing events remove RNA from the external transcribed spacer (ETS) and internal transcribed spacers (ITS) to yield the mature molecules of 5.8S, 18S, and 28S rRNA. The sequence for the 11,580 nucleotides of *X. laevis* rDNA has been determined (Table 1).

Selection Superimposed on Molecular Drive

Mutations occur at essentially random positions within the rDNA, but the evolutionary consequences of each mutation depends on its position. For example, when the rDNA of *X. borealis* (Brown et al. 1977) was compared to *X. laevis* rDNA, it was found that the spacers differed greatly, whereas the rDNA coding regions were extremely similar (Brown et al. 1972; Furlong and Maden 1983; Furlong et al. 1983). Within any given individual, all repeated rDNA copies are virtually identical with one another. The coupling of intraspecific homogeneity with interspecific heterogeneity for sequences of a tandemly repeated gene family is called horizontal, coincidental, or concerted evolution (Brown et al. 1972; Brown and Sugimoto 1974). The constant turnover in rDNA sequence can be gradually corrected by "molecular drive," which includes the processes of unequal crossing-over, gene conversion, and transposition (Dover 1982; Dover and Flavell 1984). Molecular drive can spread variants through the multiple copies of rDNA, and could fix these changes within all individuals of a species under certain circumstances (discussed by Walsh 1985).

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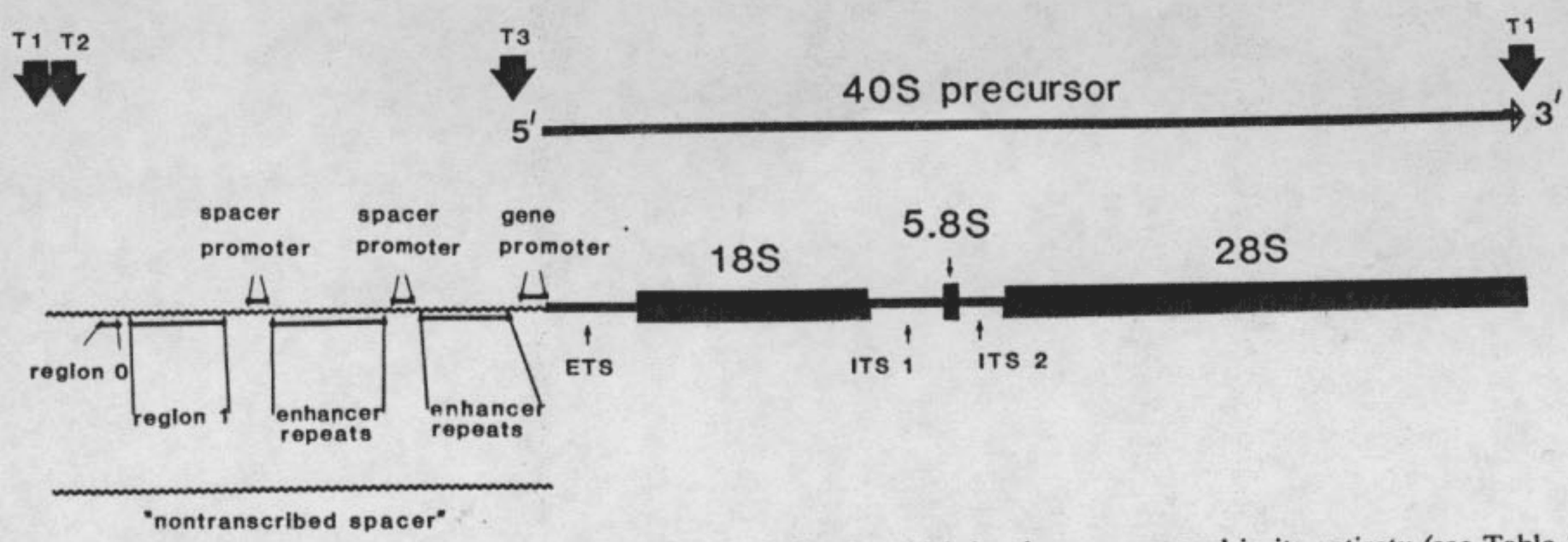

Figure 1. Structure of a typical repeat unit of rDNA from *X. laevis*, which has been sequenced in its entirety (see Table 1).

Table 1. Sequence of *Xenopus laevis* rDNA

Region	Length (nucleotides)	Reference
ETS	712	Maden et al. (1982)
18S	1825	Salim and Maden (1981)
ITS 1	557	Hall and Maden (1980)
5.8S	162	Hall and Maden (1980)
ITS 2	262	Hall and Maden (1980)
28S	4110	Ware et al. (1983)
NTS	3952	Sollner-Webb and Reeder (1979); Moss et al. (1980); Labhart and Reeder (1986 and pers. comm.)
Total:	11,580	

If molecular drive were the only force acting on rDNA to homogenize the multiple copies after random changes, then the variation between species should be uniform throughout the whole rDNA gene. In fact, this is not the case. Not only do spacers vary more than the rRNA coding regions, but even within the coding regions some sequences are more highly conserved than others. This suggests that selection pressures are superimposed on the products of molecular drive. Negative selection will drive downward the number of rDNA copies with a deleterious alteration, and positive selection will result in the spread of useful changes. In rDNA, much selection is influenced by RNA interactions necessary for ribosome biogenesis and ribosome function. Examples of such RNA interactions will be reviewed in the following sections.

CONSERVED PRIMARY SEQUENCE WITHIN rRNA

Heterologous hybridization experiments have demonstrated that portions of rRNA sequence have been highly conserved between different eukaryotic species (Sinclair and Brown 1971; Birnstiel and Grunstein 1972; Gerbi 1976). Southern blot hybridization showed that the evolutionarily conserved regions are scattered throughout 18S and 28S rRNA at distinct locations (Cox and Thompson 1980; Gourse and Gerbi 1980a). With the accumulation of rDNA sequences for many different species (see Tables II and III in Gerbi 1985; Gutell et al. 1985; Huysmans and DeWachter 1986), it has became possible to align sequences to determine regions of conservation at the nucleotide level (for early examples, see Olsen et al. 1983 for 16S–18S rRNA and Ware et al. 1983 for 23S–28S rRNA alignments). When used judiciously, rRNA sequence comparisons between different taxa can provide a powerful molecular approach for phylogenetics (Lane et al. 1985; Pace et al. 1986; Rothschild et al. 1986).

Certain regions within rRNA are conserved even between kingdoms. Selection must be very strong at these areas to prevent changes from being perpetuated. We will now describe the functions attributed to some of these highly conserved areas in rRNA.

Intermolecular Interactions: RNA-protein

rRNA interacts with proteins in ribosome biogenesis and also for ribosome function. Initially it was thought that perhaps the main function of rRNA was to act as a scaffold on which the early binding ribosomal proteins were laid during ribosome biogenesis. Although this is certainly one function for rRNA, we know now that rRNA has several other roles as well for ribosome function during protein synthesis.

L1 ribosomal protein provides a good example of the scaffold function for rRNA. We found that *Escherichia coli* ribosomal protein L1 can bind to *Dictyostelium discoideum* 26S rRNA (Gourse et al. 1981). The L1 protected regions in *E. coli* 23S, *D. discoideum* 26S, and the counterpart region in *X. laevis* 28S rRNA all share similarities in secondary structure and also share two stretches of conserved primary sequence (Gourse et al. 1981). These similarities also extend to other bacteria (Branlant et al. 1981; P. Cahill et al., pers. comm.). Presumably these are features that must be maintained in the rRNA in order for it to be able to bind ribosomal protein L1. The L11-L1 polycistronic mRNA of *E. coli* lacks the majority of the two conserved stretches found in rRNA but retains some of the same secondary structure features (Gourse et al. 1981; Baughman and Nomura 1983, 1984), and this may explain why L1 protein binds to its own message less well than to rRNA for L1 autoregulation.

Another area containing evolutionarily conserved sequence is the GTPase center located one third of the way in from the 5′ end of 23S rRNA. This region associates with the protein EF-G, as demonstrated by cross-linking (Sköld 1983). The antibiotic thiostrepton blocks the interaction of EF-G with the ribosome, thereby stopping EF-G-dependent GTPase activity and inhibiting translocation (Thompson et al. 1982). The bacterium that makes thiostrepton is *Streptomyces azureus;* it may be resistant itself to thiostrepton because it methylates an A residue in the putative GTPase center (Thompson et al. 1982). *Xenopus* and other eukaryotes have a G instead of an A at this position, but site-directed mutagenesis shows that this cannot be the sole explanation for the decreased sensitivity of eukaryotes to thiostrepton (J. Thompson et al., pers. comm.).

A third example of an evolutionarily conserved region that is possibly important for rRNA-protein interactions is the peptidyltransferase center, located one quarter of the way inward from the 3′ end of 23S–28S rRNA. Peptidyl-tRNA and aminoacyl-tRNA must be positioned appropriately in the ribosome so that transpeptidation can occur. Note that a protein with peptidyltransferase activity has not yet been purified, and it is conceivable that this activity is not protein based. The peptidyltransferase center includes the sites of base mutation rendering mitochondrial ribosomes resistant to erythromycin (Sor and Fukuhara 1982, 1984) and chloramphenicol (Dujon 1980; Blanc et al. 1981a,b; Kearsay and Craig 1981; Slott et al. 1983); the same is true for eubacterial ribosomes (Skinner et al. 1983; Sigmund et al. 1984; Ettayebi et al. 1985). Also in archaebacterial ribosomes the sites of anisomycin resistance help to define the peptidyltransferase center (Hummel and Böck 1987). These studies suggest that this area of rRNA has been conserved to maintain a conformational pocket that holds the 3′ ends of the aminoacyl- and peptidyl-tRNAs in appropriate orientation to allow transpeptidation to occur. Cross-linking experiments demonstrate directly that tRNA is associated with this region of rRNA (Barta et al. 1984; Hall et al. 1985).

Intermolecular Interactions: RNA-RNA

Some conserved regions within rRNA interact with other RNA molecules during translation. The first example of such an RNA-RNA interaction was the finding of Shine and Dalgarno (1974) that the sequence CUCC adjacent to the 3′ end of prokaryotic 16S rRNA has a complementary region upstream of the AUG initiation codon in mRNA. The reality of this mRNA-rRNA interaction has been supported by several studies (Steitz and Jakes 1975; reviewed in Kozak 1983; Hui and deBoer 1987; Jacob et al. 1987). Although the Shine-Dalgarno sequence is found in the 16S rRNAs of eubacteria, archaebacteria, and chloroplasts, it is missing in mitochondria and in the cytoplasmic ribosomes of eukaryotes (Hagenbüchle et al. 1978). Even though the Shine-Dalgarno sequence is not present in eukaryotes, psoralen cross-linking has implicated association of mRNA with the 3′ end of 18S rRNA (Nakashima et al. 1980). An additional 18S mRNA-rRNA interaction has been hypothesized by Thompson and Hearst (1983), whereby the hypermodified base amψ found one third of the way in from the 3′ end of 18S rRNA may be base-paired with the 3′ end of 18S rRNA, allowing it to interact specifically with the m^7G cap found at the 5′ end of eukaryotic mRNAs.

During translation, tRNAs also come into close association with rRNA. A conserved 17-mer is found slightly inward from the 3′ end of 16S–18S rRNA in all known cases. This 17-mer includes C_{1400} in *E. coli* 16S rRNA and the equivalent in other species, which have been cross-linked to tRNA (Ofengand et al. 1982; Prince et al. 1982; Ehresmann and Ofengand 1984; Gornicki et al. 1984; Ciesiolka et al. 1985). Mutation to paromomycin resistance maps to this region of rRNA, supporting the view that it is part of the decoding site (Li et al. 1982; Spangler and Blackburn 1985), as do site-directed mutagenesis studies (Krzyzosiak et al. 1987). It should be noted that no base pairing seems to be involved for the association of tRNA to the conserved 17-mer area of 16S–18S rRNA. Furthermore, DNA hybridization electron microscopy has shown that this region of 16S–18S rRNA that interacts with tRNA is exposed in the cleft of the small ribosomal subunit (Keren-Zur et al. 1979; Oakes et al. 1986). tRNA spans the interface between the two ribosomal subunits, and also associates with the peptidyltransferase center in 23S–28S rRNA as described in the preceding section (Barta et al. 1984; Hall et al. 1985).

Intramolecular Interactions: RNA Switches

It is conceivable that a stretch of sequence within rRNA might have more than one possible pairing partner within rRNA. At different stages of translation, one stem might open up and a new stem might be formed with an alternate stretch of complementary sequence. It has been speculated that such an RNA switch mechanism may be central to the process of translation (reviewed by Brimacombe et al. 1983); a chain of RNA switches that could cycle the conformation of the ribosome back to its ground state has been formulated for *E. coli* 16S rRNA (Thompson and Hearst 1983). RNA pairing interactions important for switching could be intramolecular (between two stretches of the same molecule) or intermolecular (e.g., between rRNA and a small RNA pairing partner such as 5S RNA, tRNA, or 5.8S RNA). In either case, when more than one pairing partner is involved, mutation at one position would favor compensatory mutations at the complementary nucleotides of both of its alternate pairing partners. Since such simultaneous multiple compensatory mutations are unlikely to occur, there would be negative selection against mutation of just one of the three interacting partners. The net effect would be evolutionary conservation of sequences utilized for RNA switches. It remains to be seen if data support the hypothesis of RNA switches. Instead of breaking and remaking stems, an alternate model is that changes in coaxial alignments of helices might drive functionally important changes in tertiary conformation of rRNA.

NONCONSERVED PRIMARY SEQUENCE WITHIN rRNA

Co-evolution to Retain a Core Secondary Structure of rRNA

As discussed above, functional constraints may preserve certain sequences within rDNA, since alteration of these sequences would be deleterious for rRNA function. Other regions within rRNA do not seem to have the same requirement for preservation of the

actual nucleotide sequence, but may still be subject to other structural constraints.

Co-evolution can occur when RNA-RNA interactions are at stake. For instance, if it is important to retain a base-paired stem as part of the rRNA secondary structure, then mutation of a base on just one side of the stem would be selected against; only when a compensatory mutation occurs for its complementary base-pairing partner would selection permit fixation of the mutations. There is an increasing body of experimental data on the secondary structure of rRNAs; compensatory base changes are taken as additional evolutionary support for the existence of specific stems in rRNA. Covariation may also be found to maintain the tertiary structure of rRNA (Gutell et al. 1986).

Interruptions within the Core Secondary Structure of rRNA

Compensatory base changes suggest that the experimentally derived secondary structures for *E. coli* 16S and 23S rRNAs also are conserved as core structures in ribosomes from all bacteria, plants, animals, and organelles (summarized in Tables IV and V of Gerbi 1985; see also Brimacombe et al. 1983; Woese et al. 1983; Noller 1984). Can mutations occur that do not disrupt the core secondary structure of rRNA? Introns are one such example. These intervening sequences interrupt highly conserved sequences of some rDNAs (summarized in Gerbi et al. 1982), the most striking of which are the 87 bases in a row with no mismatch that are conserved beween *Xenopus* 28S rDNA (which lacks an intron) and the region surrounding the intron of *Tetrahymena* rDNA (Gourse and Gerbi 1980b). This conserved region doubtless represents an area of important function for rRNA, and disruption of this region would be harmful. *Tetrahymena* copes with such interruptions by removing introns via self-splicing so that the mature rRNA is no longer interrupted (Kruger et al. 1982). In other cases (such as *Drosophila*), where there are both intron-plus and intron-minus copies of rDNA in the genome, only the intron-minus copies of rDNA seem to be transcribed (reviewed by Beckingham 1982). Introns have not yet been found in the rDNA of vertebrates.

Eukaryotes carry additional sequences ("expansion segments"; Clark et al. 1984) that are not present in the core structure of *E. coli* rRNA, and are not usually removed by RNA processing. The location of expansion segments within regions of rRNA of little primary sequence conservation supports the idea that they can be tolerated in the mature rRNA molecules because they do not disrupt a region of functional importance. Since the length and sequence of expansion segments is quite variable between species (though some secondary structure features are preserved within but not between kingdoms; Michot and Bachellerie 1987), it seems plausible that they may not have any role in the ribosome. Indeed, it appears that if an expansion segment is too large and might create a steric hindrance to the ribosome, it can be removed during rRNA maturation. One example of such RNA processing is the excision of the 3′-most expansion segment in higher plant chloroplast 23S rRNA. Unlike intron removal, subsequent splicing does not occur, so a separate 4.5S RNA molecule results that corresponds to the 3′ end of *E. coli* 23S rRNA (Edwards et al. 1981; Machatt et al. 1981; Mackay 1981; Clark and Gerbi 1982).

A second example of removal of an expansion segment is found during rRNA processing in insects and many lower eukaryotes, thereby subdividing 28S rRNA into 28S α and 28S β halves (Delanversin and Jacq 1983; Ware et al. 1985; Fujiwara and Ishikawa 1986). In yeast 26S rRNA the counterpart expansion segment is not removed; it is smaller and apparently does not interfere with binding of yeast ribosomal protein L25 to this area (El-Baradi et al. 1985). Interestingly, yeast ribosomal protein L25 binds even tighter to the homologous region of *E. coli* 23S rRNA, which lacks an expansion segment altogether (El-Baradi et al. 1985).

In contrast to the expanded structure of eukaryotic rRNA, the rRNA of mammalian mitochondria appears to be decreased in size relative to *E. coli* rRNA, due to several "amputations" of blocks of sequence. Sometimes these amputations coincide with positions at which eukaryotic expansion segments are found inserted into the core structure (e.g., Mankin and Kopylov 1981).

DOES U3 snRNA INTERACT WITH rRNA PRECURSOR?

As described above, some regions within rRNA are highly conserved in primary sequence because they represent areas of functional importance for RNA-protein or RNA-RNA interactions. Other regions within rRNA are not conserved in primary sequence, but co-evolve by compensatory base changes to retain base-paired stems necessary for the conserved core secondary structure of rRNA. Let us now see whether these rules of RNA evolution can give information about another case of intermolecular RNA-RNA interaction, namely the postulated association of U3 snRNA with rRNA precursor.

Previous Work on U3 snRNA

snRNAs are present in eukaryotic nuclei and have been highly conserved in size and sequence throughout evolution. These RNAs are U-rich, and so were initially named U1–U6 snRNA; they exist associated with proteins in ribonucleoprotein particles (snRNPs). snRNAs are transcribed by RNA polymerase II (Gram Jensen et al. 1979; Reddy and Busch 1981), and lack a poly(A) tail at their 3′ end. A unique trimethylguanosine cap occurs at the 5′ end of all snRNAs except U6 (Reddy et al. 1972; Reddy and Busch 1981).

Several different snRNA molecules are utilized during mRNA splicing. The function of U3 snRNA is less

clear. U3 snRNA is localized in the nucleolus, where it has been shown to be associated with nucleolar RNA sedimenting at 28S–32S (Prestayko et al. 1970; Zieve and Penman 1976; Reddy et al. 1981). In addition to U3 snRNA-rRNA interaction by hydrogen bonding, much U3 snRNP is bound to preribosomal RNP by protein interactions (Epstein et al. 1984). The observations above led to the belief that U3 snRNP plays a role in the processing removal of ITS 2 to convert 32S pre-rRNA into 28S rRNA.

How might U3 snRNP function in rRNA processing? It has been noticed that there is extensive primary sequence and secondary structure conservation between eukaryotic 5.8S RNA and the 5′ end of prokaryotic 23S rRNA (Nazar 1980; Jacq 1981; Clark and Gerbi 1982), suggesting that the 5.8S gene has become separated from the main body of the 28S gene by the insertion of the ITS 2 sequence (Fig. 1). The ITS 2 resembles the introns found in mRNA genes, since the ITS 2 is a sequence inserted into what was once probably a contiguous gene for the large rRNA. However, unlike intron splicing, the processing of the ITS 2 transcript does not entail religation of the 5.8S and 28S RNA products. Instead of being spliced together, 5.8S and 28S RNA are joined together by hydrogen bonds (Pene et al. 1968; Weinberg and Penman 1968; Prestayko et al. 1970), involving both termini of 5.8S RNA (Pace et al. 1977; Sitz et al. 1981; Peters et al. 1982; Walker et al. 1982; for review, see Walker and Pace 1983). The analogy between ITS 2 and introns prompted the hypothesis that U3 snRNA plays a role in the excision of the ITS 2 transcript (Bachellerie et al. 1983; Crouch et al. 1983; Tague and Gerbi 1984). As shown in Figure 2, we speculated that U3 snRNA base-pairs with a sequence found at the 5′ end of ITS 2, which is conserved in those vertebrates studied thus far (Tague and Gerbi 1984). However, this U3-ITS 2 interaction does not appear in nonvertebrates (Tague and Gerbi 1984).

Primary Sequence of *Xenopus* U3 snRNA

We used comparative sequence analysis to test the hypothesized interaction of U3 snRNA with ITS 2. We chose *X. laevis* and *X. borealis* as model systems because previously Furlong and Maden (1983) had compared the ITS 2 sequences from these two species. Figure 3 summarizes their results, which show that conserved tracts are interspersed between divergent sequences. We predicted that if any of the conserved tracts in ITS 2 base-pair with U3 snRNA, then both *X. laevis* and *X. borealis* U3 snRNA should have an identical sequence that is complementary to the conserved tract of ITS 2. Alternatively, if co-evolution has occurred, then compensatory base changes should be found between a nonconserved stretch of ITS 2 sequence and the complementary region of U3 snRNA.

We prepared cDNA clones of U3 snRNA from both *X. laevis* and *X. borealis*, using a synthetic oligonucleotide complementary to the 3′ end of the molecule to prime first strand synthesis. The DNA sequence of both strands of these two cDNA clones was determined by the method of Maxam and Gilbert (1980), and subsequently confirmed by dideoxy primer extension off U3 snRNA templates. The very 3′ end was deduced from RNA sequencing of *X. laevis* U3 snRNA. As can be seen in Figure 4, the primary sequence of U3 snRNA is almost identical between these two species of *Xenopus*; both have a U3 snRNA sequence of 219 nucleotides with only a few positions differing between the two. With these data we can rule out compensatory base changes between nonconserved sequences in ITS 2 and U3 snRNA. Therefore, if U3 snRNA hydrogen bonds to ITS 2, such an association must be with one or more of the conserved tracts in ITS 2 (Fig. 3). When we compared the sequence of the conserved tracts in ITS 2 to that of *Xenopus* U3 snRNA (Fig. 4), only tract 0 of ITS 2 showed any appreciable complementarity to the U3 sequence.

How widely conserved are U3 snRNA sequences? We have aligned the U3 snRNA sequences of both species of *Xenopus* with the complete U3 snRNA sequences now available from rat (Reddy et al. 1979; Stroke and Weiner 1985), human (Suh et al. 1986), *Dictyostelium* (Wise and Weiner 1980), and the yeast *Saccharomyces cerevisiae* (Hughes et al. 1987). Regions of evolutionary conservation between these species for U3 snRNA are shown by enclosed boxes in Figure 4. Note that the last box contains much of the stretch of U3 snRNA hypothesized to base pair with tract 0 of ITS 2 (Fig. 2).

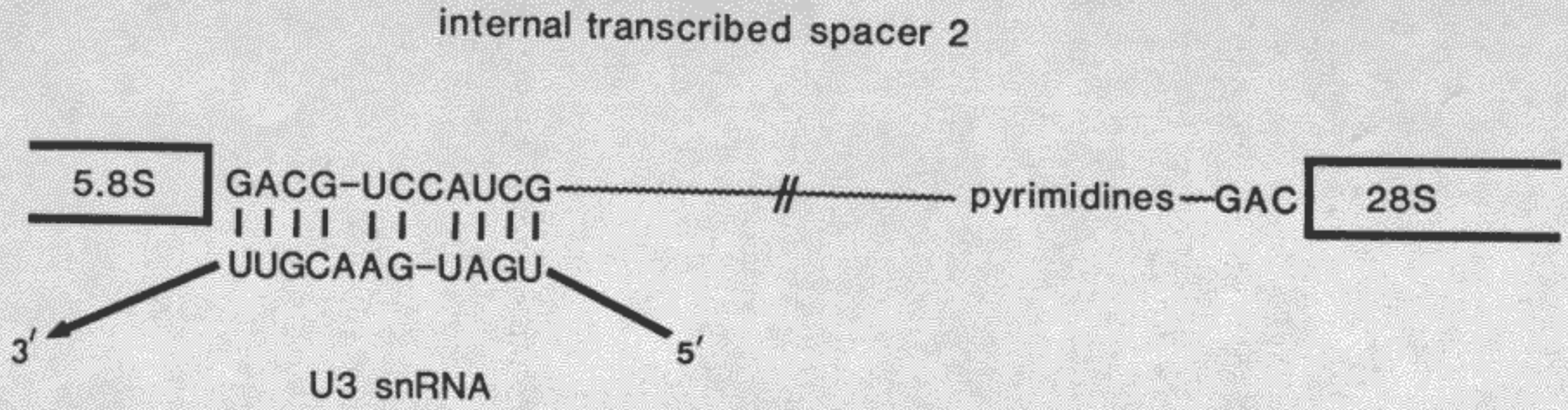

Figure 2. A hypothetical model for pairing vertebrate U3 snRNA to ITS 2 (Bachellerie et al. 1983; Tague and Gerbi 1984) is depicted here for *X. laevis*.

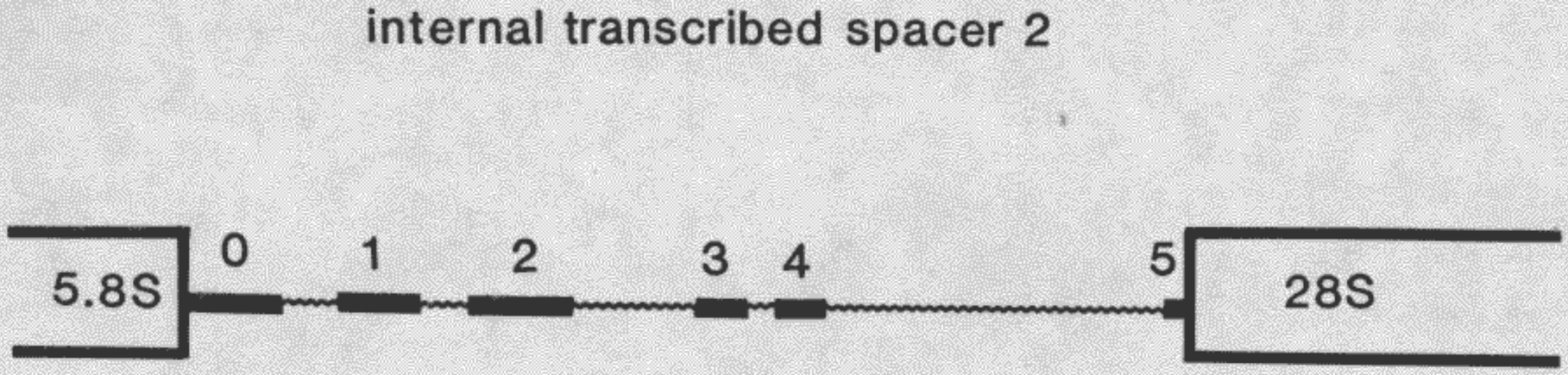

Figure 3. ITS 2 tracts that are conserved between *X. laevis* and *X. borealis* are depicted by blackened boxes (modified from Furlong and Maden 1983).

Secondary Structure of U3 snRNA

We used chemical modification (Inoue and Cech 1985; Lempereur et al. 1985; Moazed et al. 1986) to determine if nucleotides within the conserved boxes of U3 snRNA are single stranded and therefore available for hydrogen bonding to rRNA precursor. Nuclei were isolated from *X. laevis* livers and U3 snRNP was modified in situ; only those accessible nucleotides that are not base paired will be reactive with the chemical reagent. Subsequently, the modified unbound U3 snRNA was purified and used as a template for synthetic oligonucleotide-directed primer extension (variation of method of Qu et al. 1983). Reverse transcriptase pauses or stops one nucleotide before the modified residue (Hagenbüchle et al. 1978; Youvan and Hearst 1979); therefore, positions of chemical modification can be read from a sequencing gel.

Figure 5 shows our secondary structure model for *X. laevis* U3 snRNA. Base pairing is indicated by bars only if phylogenetic comparisons yield two or more compensatory base changes per stem. No nucleotides that are susceptible to strong modification by chemicals are located in base-paired stems of this model. The evolutionarily conserved sequences in U3 snRNA indicated by boxes in Figure 4 are depicted by wavy line brackets

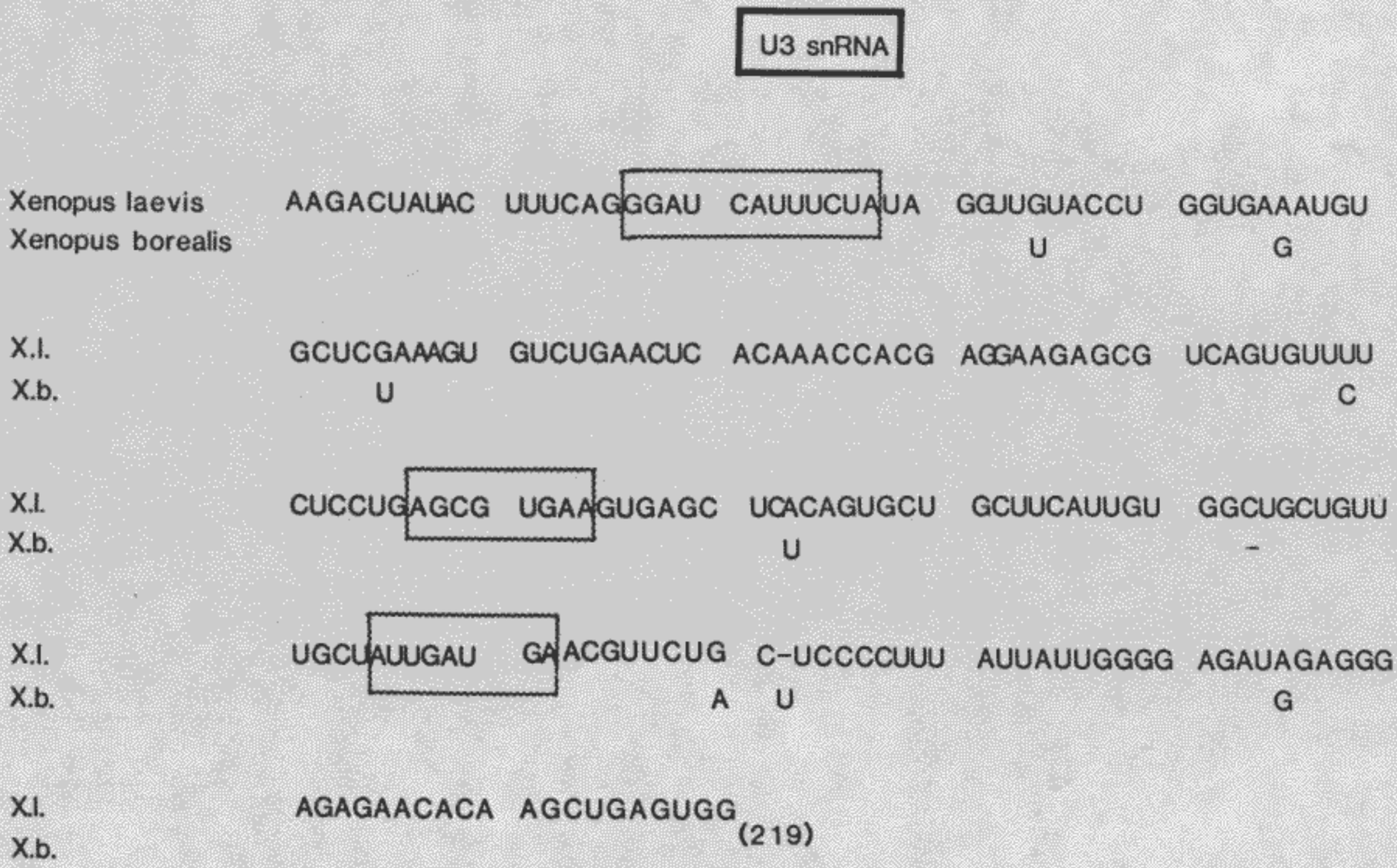

Figure 4. Primary sequence of U3 snRNA from *X. laevis* and *X. borealis*; the two sequences are identical except where differences are indicated. There seems to be population polymorphism for residue 99 in *X. laevis*: some frogs have a U at this position (as indicated in the figure), and other frogs have a C (identical to the *X. borealis* sequence at this region). RNA sequencing ambiguities occurred for residues A_{210} and U_{213}. The wavy lines enclose boxes 1, 2, and 3 that are conserved in sequence in all organisms studied so far (see text).

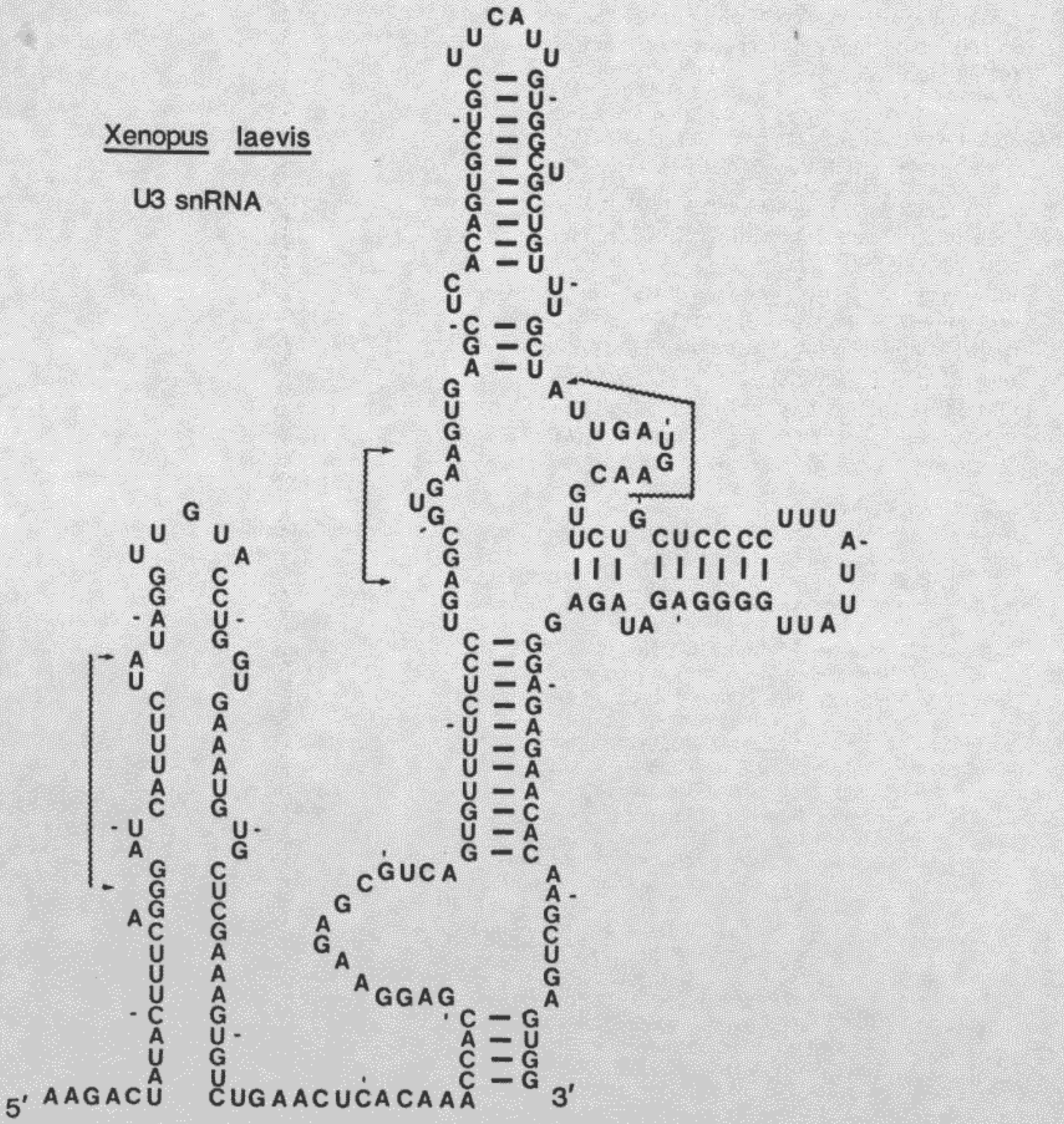

Figure 5. Secondary structure model of *X. laevis* U3 snRNA, supported by chemical modification data and by compensatory base changes in various organisms. Base-pairing bars are not drawn in for the 5′-most stem because phylogenetic comparisons and chemical modification data do not support their existence.

in Figure 5. There is no evidence for base pairing for any of these three conserved boxes. Chemical modification can be found at residues within all three conserved boxes of U3 snRNP. Their accessibility for chemical reaction suggests that these nucleotides in U3 snRNA could be available for base pairing with precursor rRNA.

Do any of the conserved boxes in U3 snRNA interact with rRNA precursor? It has been proposed that a region near box 1 of U3 snRNA might interact with the ETS of rRNA precursor (I.L. Stroke and A.M. Weiner, pers. comm.), that box 2 might base-pair with a termination processing region of rRNA precursor (Parker and Steitz 1987) and that box 3 includes much of a region that might base-pair with ITS 2 (Fig. 2). It is possible that U3 snRNA is used for some or all of these roles. However, arguments can be raised against each of the three proposed interactions, as will be discussed more fully elsewhere. None of the models for base pairing of U3 snRNA to rRNA precursor fare well when phylogenetic comparisions are made. Proteins of the U3 snRNP particle have already been shown to play a major role for U3 snRNP binding in the nucleolus (Epstein et al. 1984), and perhaps proteins are sufficient for U3 snRNP binding. Alternatively, there may be other forces needed for RNA-RNA association besides hydrogen bond base pairing, as suggested earlier in this chapter by the conserved sequence at the end of 16S–18S rRNA that closely associates with tRNA despite a lack of sequence complementarity in the latter. Finally, U3 snRNP might play a structural role for nucleolar organization rather than have an enzymatic function for

cleavage events in rRNA processing. The role of U3 snRNP in the nucleolus still awaits further investigation for its elucidation.

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