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Intermolecular base-paired interaction between complementary sequences present near the 3' ends of 5S rRNA and 18S (16S) rRNA might be involved in the reversible association of ribosomal subunits

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ABSTRACT

Highly conserved sequences present at an identical position near the 3' ends of eukaryotic and prokaryotic 5S rRNAs are complementary to the 5' strand of the m_2^6A hairpin structure near the 3' ends of 18S rRNA and 16S rRNA, respectively. The extent of base-pairing and the calculated stabilities of the hybrids that can be constructed between 5S rRNAs and the small ribosomal subunit RNAs are greater than most, if not all, RNA-RNA interactions that have been implicated in protein synthesis. The existence of complementary sequences in 5S rRNA and small ribosomal subunit RNA, along with the previous observation that there is very efficient and selective hybridization in vitro between 5S and 18S rRNA, suggests that base-pairing between 5S rRNA in the large ribosomal subunit and 18S(16S) rRNA in the small ribosomal subunit might be involved in the reversible association of ribosomal subunits. Structural and functional evidence supporting this hypothesis is discussed.

INTRODUCTION

Studies on the function of ribosome constituents have mainly tended to concentrate on ribosomal proteins. Ribosomal RNAs have largely been regarded as structural components despite the fact that base-pairing between complementary sequences in RNA molecules provides a mechanism for reversible intermolecular and intramolecular interactions. In recent years RNA-RNA interactions have been implicated in the recognition of mRNA by ribosomes (1-4), in the termination of protein synthesis (5), and in the binding of tRNA by ribosomes (6,7). Very specific and stable hybridization, in vitro, has been demonstrated between 5S and 18S rRNA from wheat embryo (8-10), barley and mouse sarcoma (11). 5S rRNA from wheat embryo (9), and from barley and mouse sarcoma (Azad, unpublished) can hybridize with 18S rRNA from various eukaryotes, suggesting that the sites of

interaction are probably conserved. Since 5S and 18S rRNA are present in the large and small ribosomal subunit, respectively, it has previously been suggested (8) that specific intermolecular base-pairing between 5S and 18S rRNA may be involved in the reversible association of ribosomal subunits.

A fragment of wheat embryo 5S rRNA obtained by RNase T_1 digestion of an in vitro hybrid formed between wheat embryo 5S and 18S rRNA has been sequenced (12). Since wheat embryo 5S rRNA efficiently hybridizes, in vitro, with heterologous 18S rRNAs (9), and with a view towards localizing the site(s) of interaction between 5S and 18S rRNA, it was of interest to see if the sequence of the wheat embryo 5S rRNA fragment (12) obtained from an in vitro 5S-18S rRNA complex is complementary to any of the known sequences of 18S rRNA. It was also of interest to see if such complementary sequences are present in prokaryotic 5S and 16S rRNA. A conserved sequence present near the 3' end of the eukaryotic 5S rRNA was found to be complementary to a sequence present near the 3' end of 18S rRNA. Conserved sequences present in prokaryotic 5S and 16S rRNA, at positions identical to that in eukaryotic RNA, are also complementary to each other. Structural and functional data, present in the literature, were examined to see if the complementary sequences in the small ribosomal subunit RNA and 5S rRNA are involved in ribosome function.

COMPLEMENTARITY BETWEFN EUKARYOTIC 5S AND 18S rRNA, AND BETWEEN PROKARYOTIC 5S AND 16S rRNA

The structure of the 3' end of yeast 18S rRNA (13) is shown in Fig. 1a. For descriptive purposes it is divided into regions (i) to (iv). The sequences of regions (i) and (ii) and the residues $m_2^6Am_2^6A$ are very highly conserved in eukaryotic 18S rRNA (4, 13-15). The sequence of region (iii) on the 5' side of the $m_2^6Am_2^6A$ residues is known in the case of yeast 18S rRNA (13) and rat liver 18S rRNA (15), and they are almost identical. The sequence of region (iv) and residues on the 5' side of it are known only for yeast 18S rRNA (13). The wheat embryo 5S rRNA fragment (12) that is protected from RNase T_1 digestion in the in vitro 5S-18S rRNA complex is complementary to regions (iii) and (iv) of yeast 18S rRNA, and a base-paired structure that can be constructed between the two is shown in Fig. 1b. The calculated free energy (16) of this proposed hybrid helix is -19 kilocalories

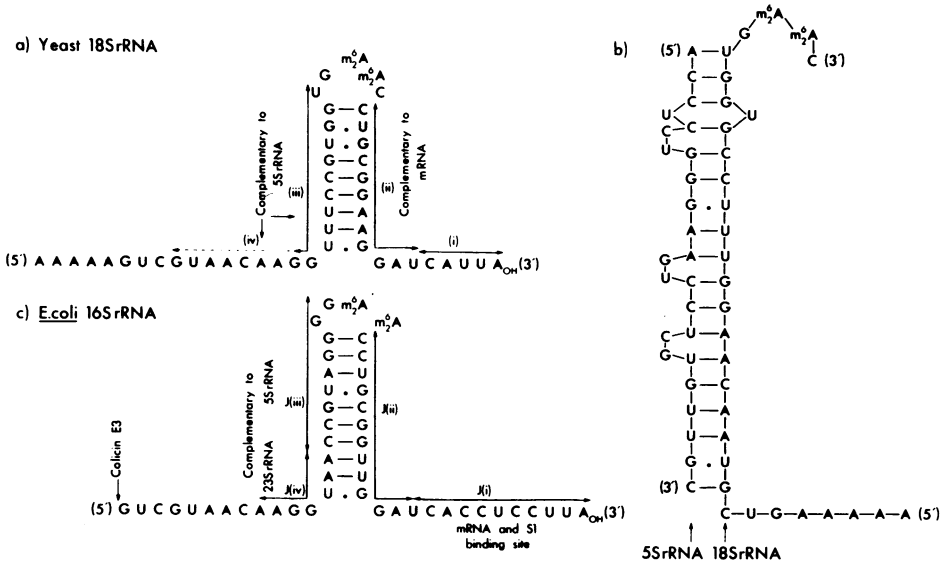


Fig. 1 (a) The sequence and predicted secondary structure of the 3' end of yeast 18S rRNA (13). Different regions are designated (i) to (iv) for descriptive purposes. (b) A hybrid structure that can be constructed between regions (iii) and (iv) of yeast 18S rRNA and a fragment of wheat embryo 5S rRNA (12) obtained by RNase T_1 digestion of a hybrid formed, in vitro, between wheat embryo 5S rRNA and 18S rRNA. (c) The sequence and predicted secondary structure of the 3' end (Section J) of E. coli 16S rRNA (17). For descriptive purposes different regions are designated J(i) to J(iv).

as compared to -7 kilocalories for the predicted m_2^6A hairpin structure at the 3' end of 18S rRNA. The primary sequence and the predicted secondary structure (17) of the 3' end (Section J) of E. coli 16S rRNA (Fig. 1c) is very similar to that of 18S rRNA (Fig. 1a) and as shown later region J (iii) of E. coli 16S rRNA is complementary to prokaryotic 5S rRNA.

The sequence of the wheat embryo 5S rRNA fragment that is complementary to 18S rRNA is identical to sequences (18) present near the 3' end of 5S rRNA from rye (residues 89-113) and dwarf bean (residues 88-112). In fact, the sequences of identical regions, starting around residue 90 with the residues ACC, of all eukaryotic 5S rRNAs (18) are very similar and relatively stable base-paired hybrids can be constructed between these

eukaryotic 5S rRNA sequences and region (iii), and in the case of plant 5S rRNAs also region (iv), of yeast 18S rRNA (Fig. 2a). Because the sequence of rat liver 18S rRNA (15) on the 5' side of the $m^6_2Am^6_2A$ residues is almost identical to that of yeast 18S rRNA, similar base-paired hybrids can also be constructed between region (iii) of rat liver 18S rRNA and the conserved sequences in eukaryotic 5S rRNAs. Very similar sequences present near the 3' end of most prokaryotic 5S rRNAs (18), at a position identical to that in eukaryotic 5S rRNAs, are complementary to region J (iii) of E. coli 16S rRNA (Fig. 2b). The calculated free energies (16) of the proposed eukaryotic 5S-18S rRNA hybrids, and prokaryotic 5S-16S rRNA hybrids are very similar and range from -15 to -22 kilocalories.

Thus, very similar sequences present near the 3' end of

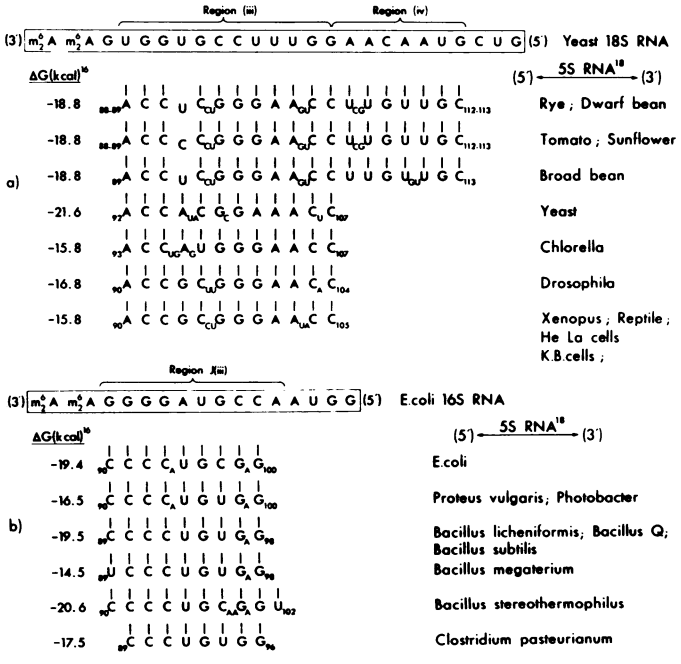


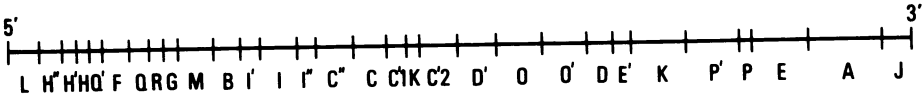
Fig. 2 Intermolecular base-paired structures that can be constructed between sequences near the 3' ends of: (a) eukaryotic 5S rRNAs and yeast 18S rRNA, and (b) prokaryotic 5S rRNAs and E. coli 16S rRNA. Vertical lines indicate 5S rRNA residue that can base-pair with the small ribosomal subunit RNA. The numbered residues indicate position from the 5' end of 5S rRNA.

eukaryotic and prokaryotic 5S rRNAs are complementary to sequences present 5' proximal to the $m_2^6Am_2^6A$ residues near the 3' ends of 18S and 16S rRNA, respectively, Since 5S and 18S(16S) RNA are present in the large and small ribosomal subunits, respectively, base-pairing between the complementary sequences in 5S and 18S(16S) rRNA may be involved in the reversible association of ribosomal subunits. In the case of E. coli, complementarity between region J (iv) of 16S rRNA and a sequence present near the 3' end of 23S rRNA has been implicated in ribosomal subunit association (19). The binding of 5S rRNA and 23S rRNA to adjacent regions J (iii) and J (iv) at the 3' end of 16S rRNA would make the interaction between the ribosomal subunits even stronger. The following sections of this communication examine structural and functional evidence in support of the hypothesis that the complementary sequences in 5S and small ribosomal subunit RNA have physiological significance at the level of ribosomal subunit association.

SITE(S) IN THE SMALL RIBOSOMAL SUBUNIT RNA COMPLEMENTARY TO 5S rRNA

If the sequence in the small ribosomal subunit RNA complementary to 5S rRNA has any functional role in the association of ribosomal subunits, then this sequence should be conserved, be exposed in the small ribosomal subunit but protected in the 70S or 80S ribosomes, and should be able to assume a single stranded conformation in order to base-pair with 5S rRNA. Most of the evidence along these lines comes from E. coli 16S rRNA, but wherever possible the structure of 18S rRNA is also disucssed.

The entire sequence of E. coli 16S rRNA is known (20), and for descriptive purposes it is divided into a number of lettered sections (17).



The sequence of the 3' terminal section J is shown in Fig. 1c. Region J (iii) is complementary to prokaryotic 5S rRNA. On the basis of comparative sequence analysis of RNase T_1 fragments of 16S rRNA from 27 different prokaryotes, Woese et al. (21) have shown that sequences present within section J, which includes the

site complementary to 5S rRNA, are highly conserved and present on the surface of the 30S ribosomal subunit. The sequence of the 3' end of yeast 18S rRNA equivalent to section J of E. coli 16S rRNA is shown in Fig. 1a. Sequences up to and including the $m_2^6Am_2^6Am$ residues (regions (i) and (ii)) are very highly conserved in eukaryotic 18S rRNA (4,13-15) and are very similar to the same region in E. coli 16S rRNA. Region (iii) is complementary to eukaryotic 5S rRNA. If the hairpin structure in which regions (ii) and (iii) are base-paired is universal, as seems likely, then region (iii) is also likely to be conserved since region (ii) is highly conserved. Region (iii) of yeast 18S rRNA and rat liver 18S rRNA have almost identical sequences. The conservation of the 5S rRNA binding site in 18S rRNA has also been predicted from studies that show that wheat embryo 5S rRNA (9) and 5S rRNA from barley and mouse sarcoma (Azad, unpublished) can efficiently hybridize, in vitro, with 18S rRNA from various heterologous sources.

A hairpin structure with the $m_2^6Am_2^6A$ residues in the loop can be predicted from the primary sequences of section J of E. coli 16S rRNA (17,20), and equivalent regions of yeast 18S rRNA (13), rat liver 18S rRNA (15), and Zea mays chloroplast 16S rRNA (22). The presence of a hairpin structure within section J of E. coli 16S rRNA is supported by high-resolution proton magnetic resonance study (23). There are a number of base exchanges within regions J (ii) and J (iii) when E. coli 16S rRNA is compared to yeast 18S rRNA, and yet in both instances region (ii) and (iii) can be base-paired to form the stem of the $m_2^6Am_2^6A$ hairpin loop (Fig. 1a and 1c). There are two transversions at the base of the hairpin stem but two A-U base-pairs are maintained. Since these two A-U base-pairs at the base would not significantly increase the thermodynamic stability of the hairpin structure, their maintenance during evolution might be related to a universal physiological role of the hairpin stem and loop. When the 3' terminal hairpin structure in Zea mays chloroplast 16S rRNA (22) is compared to that in E. coli 16S rRNA, there are six base exchanges within the stem region but they exactly match each other, so that the hairpin structures at the 3' ends of E. coli 16S rRNA and Zea mays chloroplast 16S rRNA have identical shapes and very

similar stabilities. This again suggests a functional role for the hairpin structure.

Colicin E3 cleaves at a site at the junction of sections J and A of E. coli 16S rRNA and therefore this region of the molecule must be present on the surface of the 30S ribosomal subunit. The sequence of section J of E. coli 16S rRNA on the 3' side of the colicin E3 cleavage site is homologous to the same region of yeast 18S rRNA (Fig. 1c and 1a), and therefore colicin E3 might be expected to cleave 18S rRNA also at this site. In fact colicin E3 treatment has similar inhibitory effects in prokaryotic (24, 25) and eukaryotic (26,27) protein synthesis. Thus, the 3' end of 18S rRNA is also probably present on the surface of the 40S ribosomal subunit. This is supported by the presence in rat liver cytoplasm of a fragment comprising the 3' end of 18S rRNA (15).

Woese et al. (21) have shown that the universal and highly conserved RNase T_1 fragments of 16S rRNA are clustered in about nine different regions mainly concentrated in the 3' half of the molecule. About six of these conserved regions, including section J, have been shown to be present on the surface of E. coli 30S ribosomal subunit by chemical modification with kethoxal (28) and susceptibility to RNase T_1 (29). The 3' end of E. coli 16S rRNA in the 30S subunit is also accessible to periodate oxidation and the fluorescent dye proflavine semicarbazide (30). In the 70S ribosomes section J remains inaccessible to kethoxal modification (28). The 3' terminal residue of 16S rRNA can be oxidized with periodate in the 70S ribosomes but the fluorescent dye does not bind (30) suggesting that the site near the 3' end of 16S rRNA that binds the dye is not accessible in 70S ribosomes. RNase T_1 digestion studies (28) show that within section J of E. coli 16S rRNA only the 5' half, which contains the sequences complementary to 5S (and 23S) rRNA, is protected in the 70S ribosomes. This suggests that the sequences complementary to 5S and 23S RNA are present at the ribosome interface. Electron microscopic examination also shows the $m_2^6Am_2^6A$ residues adjacent to 5S RNA binding site is present at the ribosome interface (31). Section A which is adjacent to section J is also present at the ribosome interface. RNase T_1 digestion studies also show that the 3' half of section J of E. coli 16S rRNA which

is resistant in 30S ribosomes is cleaved in the middle (region J (ii)) in 70S ribosomes (29). This is consistent with the view that in the 30S ribosomes, regions J (ii) and J (iii) are base-paired to form a hairpin structure, while in the 70S ribosomes region J (iii) is involved in intermolecular base-pairing leaving region J (ii) single-stranded.

Chemical modification (28) and enzymatic digestion (29) studies show that section D' near the middle of the E. coli 16S rRNA is also present at or very near the ribosome interface. Section D' contains a sequence complementary to the same region of 5S rRNA that is complementary to region J (iii) of 16S rRNA.

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 5'————————————Part of section D' of 16S rRNA————————————3'
---G A A A G C G U G G G G A G C A A A C A G G A U U A G A U A C---
         C G U A C C C C U C           U C C C A A U U
                                          C
       3'————5S rRNA————5'       3'——23S rRNA——5'
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Section D' of 16S rRNA also contains a sequence complementary to the same region of 23S rRNA that is complementary to region J (iv).

It is perhaps no coincidence that the only two regions of the 16S rRNA molecule present at or near the ribosome interface, namely sections A-J and D', contain sequences complementary to 5S rRNA and 23S rRNA of the large ribosomal subunit. Sections A-J and D'-O bind ribosomal proteins S21 (32) and S18 (33), respectively, and these two proteins can be crosslinked in the intact 30S subunit (32,34). Though sections A-J and D'O are separated by about 700 nucleotides the crosslinking experiments suggest that sections J and D' are in close proximity at the ribosome interface.

In order to hybridize with 5S rRNA region J (iii) of 16S rRNA has to be single-stranded. Helix unwinding proteins, similar to ribosomal protein S1 (35) which binds to region J (i) of 16S rRNA (36) may be involved in opening up the hairpin structure. Ribosomal proteins S7 and S8 which bind to sequences in section J and D', respectively, have also been shown to bring about conformational changes in 16S rRNA by disrupting helical structures (37). The disruption of the hairpin structure would allow region J (iii) to interact with 5S rRNA. Initiation factor IF3, which promotes dissociation of ribosomal subunits (38), binds near the 3' end of 16S rRNA (39), and antibodies against the $m^6_2Am^6_2A$ residues inhibit binding of IF3 to 30S subunits (40).

Van Duin et al. (19) have suggested that interaction of IF3 with sites near the 3' terminus of 16S RNA could account for the dissociation activity of this factor. IF3 might promote dissociation of ribosomal subunits by being involved in the disruption of intermolecular base-pairing between region J (iii) of 16S rRNA and 5S rRNA and/or in the stabilization of the hairpin structure at the 3' end of 16S rRNA.

Eukaryotic ribosomal and factor proteins, similar to those in prokaryotes, could bring about conformational changes in the 18S rRNA. The 5' noncoding regions of at least 20 different eukaryotic mRNAs have been shown to contain sequences complementary to parts of region (ii) at the 3' end of eukaryotic 18S rRNA (4,41). Since 5S rRNA and the 5' noncoding regions of eukaryotic mRNAs are complementary to opposite strands of the $m_2^6Am_2^6A$ hairpin stem in 18S rRNA (Fig. 1a) which can base-pair with each other, it has been suggested (42) that the binding of a complementary sequence in the 5' noncoding region of eukaryotic mRNA to region (ii) of 18S rRNA might prevent the reformation of the hairpin structure and thus facilitate base-pairing between the single-stranded region (iii) of 18S rRNA and 5S rRNA.

STRUCTURE OF 5S rRNA

The sequences of different eukaryotic 5S rRNAs, that are complementary to regions (iii) and (iv) of the 3' end of 18S rRNA are identical or very similar to each other (Fig. 2a). The prokaryotic 5S rRNA sequences that are complementary to region J (iii) at the 3' end of 16S rRNA are also very similar to each other. These sequences are present at an identical position near the 3' ends of both eukaryotic and prokaryotic 5S rRNA molecules. In order to hybridize with the small ribosomal subunit RNA, the region of the 5S rRNA containing the complementary sequence probably has to exist as a single-stranded structure.

Innumerable models for the secondary structure of 5S rRNA have been proposed (for review see ref. 43) on the basis of chemical modification, enzymatic digestion, oligonucleotide binding, computer analyses, and physical methods. The only aspect common to all 5S rRNA models is that the 3' end and 5' end of the molecule are base-paired to each other. None of the above methods has definitely proved the correctness of any one model,

suggesting that 5S rRNA probably has a flexible structure which might be related to biological function. Different stable conformational forms of 5S rRNA having different electrophoretic mobilities have been observed (43) but it remains to be seen whether any of these are equivalent to the functional conformations within the ribosome.

One way of defining functionally significant secondary structure would be to look for base-paired regions that are conserved (44), a method similar to the one used to substantiate the clover leaf model of tRNA. On the basis of such comparative analyses of 5S rRNA primary sequences, Fox and Woese (45) have suggested a universal model which is applicable to prokaryotic as well as eukaryotic 5S rRNAs. Partial enzymatic digestion studies on 5S rRNA from a number of prokaryotes and eukaryotes support this model (46). The sequence CCCCAUGC, that is complementary to region J (iii) of E. coli 16S rRNA, occurs twice in E. coli 5S rRNA (regions I and III, Fig. 3). Residue G_{41} of E. coli 5S rRNA is modified by kethoxal both in the 50S subunit (47) and in the 70S ribosome (48,49), suggesting that though present on the 50S subunit surface it is not present at the ribosome interface. Moreover, in reconstitution experiments in which the 5S rRNA has been modified with kethoxal (48) or formylated (50) at position 41, the reconstituted 50S subunits are still active. These results suggest that the region of 5S rRNA containing G_{41} may not be involved in ribosome function.

It is not known whether the other CCCCAUGC sequence (region I) is accessible in the 50S subunit. All prokaryotic and eukaryotic 5S rRNAs contain sequences at this very position that are complementary to the small ribosomal subunit RNA. However, according to the universal secondary structure model (45), region I of prokaryotic 5S rRNA is not single-stranded. Weidner et al. (51) have argued on the basis of comparative sequence analysis that the prokaryotic 5S rRNA can exist in two thermodynamically stable forms (Fig. 3) and 5S rRNA can function by a switch between these forms. This has relevance to the interaction between 5S and 16S rRNA. In the A-form, which is analogous to the universal model (45), region I is base-paired with region II to form the stem of the prokaryotic loop and therefore not

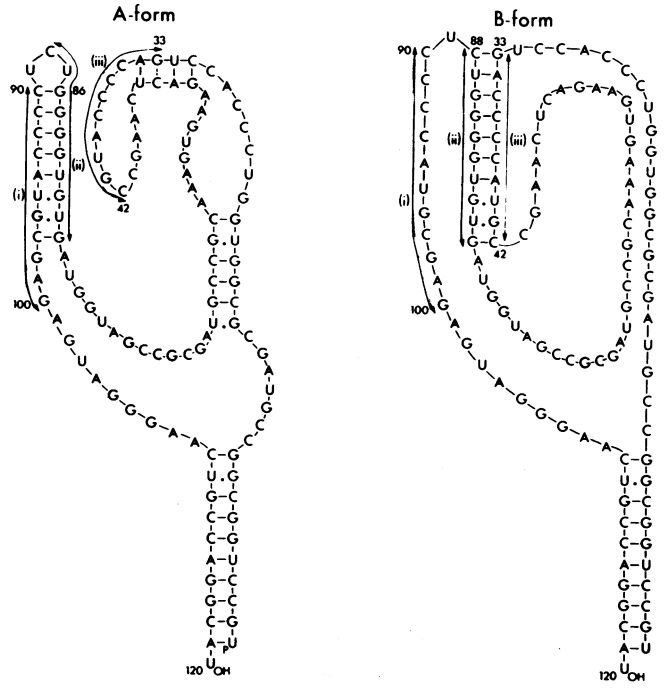

Fig. 3 Two conformations of prokaryotic 5S rRNA based on ref. 51. Regions I and III of E. coli 5S rRNA contain the homologous sequence CCCCAUGC and are complementary to region II, and also to region J(iii) of E. coli 16S rRNA.

available for interaction with 16S rRNA. In the B-form region II is base-paired with region III to form the central helix leaving region I single-stranded and free to interact with 16S rRNA. The central helix of the B-form is conserved over all prokaryotic 5S rRNA sequences (51), and is consistent with the enzymatic degradation pattern of the 'denatured' form of 5S rRNA reported by Jordan (52). Region I of prokaryotic 5S rRNA may not be accessible in the 50S subunit because it is involved in intramolecular base-pairing (A-form), while in the 70S ribosome it may not be accessible because it is involved in intermolecular base-pairing with 16S rRNA. The reason why only the A-form has been isolated from native ribosomes may be because the B-form might exist only in the context of ribosomal subunit association,

and during isolation the 5S-16S RNA base-pairs may be disrupted and the 5S rRNA might very readily switch back to the A-form. Specific ribosomal proteins such as L25, L18 and L5 have been shown to bring about conformational changes in E. coli 5S rRNA (53), and such proteins might be involved in the switches between the two stable conformations proposed by Weidner et al.(51).

Comparative sequence analyses (45) and partial enzymatic digestion studies (46) of eukaryotic 5S rRNAs suggest a universal model (54) in which the sequence complementary to 18S rRNA is single-stranded. However, there is no data available to suggest that this conformation of eukaryotic 5S rRNA exists within the ribosome. It is possible that additional and variable secondary structures might exist in 5S rRNA from different eukaryotic sources, and some conformational changes may be required in order for the 5S RNA within the 60S subunit to interact with 18S rRNA. Such conformational changes may be dependent on specific eukaryotic ribosomal and factor proteins.

EVIDENCE SUGGESTING A FUNCTIONAL ROLE FOR THE BASE-PAIRED INTERACTION BETWEEN 5S and 18S rRNA

There is quite extensive complementarity between 5S rRNA sequences and small ribosomal subunit RNA sequences in both eukaryotes and prokaryotes. This together with the fact that there is efficient and selective hybridization, in vitro, between 5S and 18S rRNA (8-11) strongly suggests that base-paired interaction between 5S rRNA and small ribosomal subunit RNA might have physiological significance. Because 5S rRNA is present in the 60S subunit but hybridizes selectively with 18S rRNA from the 40S subunit, it has previously been proposed (8) that specific base-pairing between eukaryotic 5S and 18S rRNA may be involved in the reversible association of ribosomal subunits. This is supported by the presence of complementary sequences near the 3' ends of 18S rRNA and eukaryotic 5S rRNA (Fig. 2a). On the basis of extensive complementarity between sequences present near the 3' end of prokaryotic 5S rRNAs and 16S rRNA (Fig. 2b), it is suggested that ribosomal subunit association in prokaryotes may also involve base-pairing between 5S and 16S rRNA.

The extent of base-pairing and the calculated stabilities of the hybrids that can be constructed between 5S rRNA and small ribosomal subunit RNA are greater than most, if not all, RNA-RNA

interactions that have been suggested to have biological functions (1-7). Earlier on in this communication it has been shown that the complementary sequences are highly conserved and are present at identical positions within the 5S rRNA and small ribosomal subunit RNA molecules. It has also been shown that the E. coli 16S rRNA sequence that is complementary to 5S rRNA is present at the ribosome interface. The 16S and 18S rRNA sequences complementary to 5S rRNA constitute the 5' strand of a hairpin structure that seems to be conserved. The $m^6_2Am^6_2A$ residues in the loop of the hairpin structure are unmethylated in certain kasugamycin strains (55), and since kasugamycin has been shown to inhibit initiation of protein synthesis, the hairpin structure may be essential for the formation of the initiation complex (56). However, the $m^6_2Am^6_2A$ residues themselves may not be directly involved in this process since cells in which they are unmethylated are viable (57). The removal of a 3' terminal 49 nucleotide fragment, which contains the sequence complementary to 5S rRNA, of 16S rRNA by colicin E3 completely abolishes protein synthesis in E. coli (24,25). The structure of the 3' end of 18S rRNA, which contains the sequence complementary to eukaryotic 5S rRNA, is very similar to the 3' end of E. coli 16S rRNA, and colicin E3 also abolishes protein synthesis in eukaryotes (26,27). There is also evidence to show that the 3' end of 16S rRNA is directly involved in the association of ribosomal subunits. When 16S rRNA within the 30S subunit is modified with kethoxal, the 30S subunits lose their ability to associate with 50S subunits under tight couple conditions (58). As discussed earlier there are only two regions in the 16S rRNA that are present at (section J at the 3' end) or near (section D' in the middle of the molecule) the ribosome interface, and both sections J and D' contain sequences complementary to 5S rRNA. The fluorescent dye proflavine semicarbazide binds to a sequence near the 3' end of 16S rRNA in 30S subunits. 30S subunits reconstituted with 16S rRNA labelled with this dye are inactive in polypeptide synthesis apparently due to the inability of these 30S subunits to form tight couples with 50S subunits (30). From studies on the cleavage, in situ, of a 3' terminal fragment of 16S rRNA by cloacin DF13, Baan et al. (59) have also concluded

that the 3' end of 16S rRNA is involved in ribosomal subunit interaction.

Erdmann et al. (60) have shown that 5S rRNA is essential for protein synthesis since 50S subunits reconstituted without 5S rRNA are inactive in polypeptide synthesis. 5S rRNA modified with monoperphthalic acid, which is reactive towards unpaired adenines, can be incorporated into Bacillus stereothermophilus 50S subunits, but these reconstituted 50S subunits retain only about 40% of their activity in a poly(U)-directed polyphenylalanine system (6). This experiment does not localize the reactive sites in 5S rRNA but suggests that unpaired adenine residues present on the surface of the 50S subunits are necessary for their activity. There are a number of studies that suggest that 5S rRNA is directly involved in ribosomal subunit association. Yu and Wittmann have shown that reconstituted E. coli 50S subunits in which 5S RNA is structurally disoriented are unable to combine with native 30S subunits to form 70S ribosomes (61), suggesting that the secondary structure of 5S rRNA is very important in the process. Avadhani and Buetow have shown (62) that Euglena gracilis 60S subunits can combine with the 40S initiation complex to form the 87S initiation complex only if the 5S rRNA within the 60S subunit is physically intact. The hypothesis that base-paired interaction between 5S and 18S rRNA is responsible for the association of the 60S subunit and 40S intitiation complex would predict that the addition of free 5S rRNA to a cell-free protein synthesizing system would inhibit protein synthesis by inhibiting the formation of the 80S initiation complex and therefore polysomes. Wreschner (63,64) has recently shown that the addition of free 5S rRNA to wheat germ and reticulocyte cell free systems completely abolishes protein synthesis. In the wheat germ system no polysomes are formed on added globin mRNA in the presence of free 5S rRNA (63). In the reticulocyte system, the polysomes formed on endogenous mRNA are frozen in the presence of free 5S rRNA and there is accumulation of 60S and 40S subunits at the expense of 80S ribosomes (64). According to the hypothesis proposed in this paper free 5S rRNA would exert its inhibitory effect by competing with 5S RNA in the large ribosomal subunit for the same 5S RNA binding site on 18S RNA in the small ribo-

somal subunit thus preventing the formation of the 80S initiation complex.

In conclusion, it is suggested that base-paired interaction between 5S rRNA in the large ribosomal subunit and 18S(16S) rRNA in the small ribosomal subunit might provide a mechanism whereby the ribosomal subunits reversibly associate during protein synthesis. The association and dissociation of ribosomal subunits through base-pairing between 5S rRNA and small ribosomal subunit RNA would probably require the agency of specific ribosomal and factor proteins that bring about conformational changes in RNA molecules and help to stabilize or destabilize intermolecular and intramolecular RNA-RNA base-pairs.

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