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GU receptors of double helices mediate tRNA movement in the ribosome

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ABSTRACT

A new RNA structural motif consisting of two double helices closely packed via minor grooves is found in many places in the ribosome structure. The packing requires that a GU base pair in one helix be packed against a Watson–Crick pair in the other helix. Two such motifs mediate the interaction of the P- and E-tRNA with the large ribosomal subunit. Analysis of the particular positions of these two motifs in view of the available data on occupancy of tRNA-binding sites and structural changes in the ribosome during the elongation cycle suggests a distinct role for each motif in tRNA translocation.

Keywords: double helix packing; GU base pair; ribosomal RNA; ribosomal translocation; ribosome structure

INTRODUCTION

Recent achievements in the X-ray crystallography of the whole ribosome (Yusupov et al., 2001) and its subunits (Ban et al., 2000; Schluenzen et al., 2000; Wimberly et al., 2000; Harms et al., 2001) are invaluable both for elucidating the mechanisms of the protein synthesis and for providing insight into how nucleotide sequence shapes RNA tertiary structure and how the latter determines the function. A necessary step toward these goals is a systematic analysis of the ribosome conformation, which has already succeeded with identification of new RNA structural motifs (Doherty et al., 2001; Klein et al., 2001; Nissen et al., 2001). One of the most common elements of the ribosome structure is the interaction of RNA double helices via minor grooves, which was acknowledged by all authors who determined X-ray structures of the ribosome or its subunits. This element has also been found in the structures of other RNA molecules (Pley et al., 1994; Strobel & Cech, 1995; Cate et al., 1996; Strobel et al., 1998) and thus should be considered as an important block of the RNA architecture in general. The presented analysis of the minor groove interactions existing in the ribosome structure shows that the close packing of two double helices imposes certain constraints on their nucleotide sequences, providing for a specific and stable complex. For two such complexes that mediate the interaction of the P- and E-tRNA with 23S rRNA, we suggest a distinct role in ribosome translocation.

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ALONG-GROOVE PACKING MOTIF

In the heterogeneous population of RNA helix–helix contacts existing in the crystal structures of both ribosomal subunits (Ban et al., 2000; Wimberly et al., 2000), we were looking for those where the minor grooves of two helices closely packed with each other. Because the minor groove in A-RNA has a slightly concave shape, we expected to find structures like that shown in Figure 1A, where the sugar–phosphate backbone of each helix packs along the minor groove of the other in the so-called along-groove packing. Analysis of the X-ray conformations of both ribosomal subunits reveals 12 such cases, 4 in 30S and 8 in 50S, having well-superimposed structures with root mean square deviation of 0.84 Å (Fig. 1B). In most cases, four base pairs from each helix are involved in contact with the other helix. The average contact area is about 150 $Å^2$ and includes more than 60 non-hydrogen atoms accountable for about 60 interhelix atom–atom interactions. Of course, because of the spiral character of the helices, it is impossible to keep the same pattern of base pair juxtaposition all along the helices. However, in all cases, one can identify two so-called “central” base pairs that stay close to the center of the contacting region and juxtapose in a manner resembling that seen in Figure 1A. Of the four strands forming the two double helices, two stay closer to the center (internal strands), whereas the other two are at the periphery of the struc-

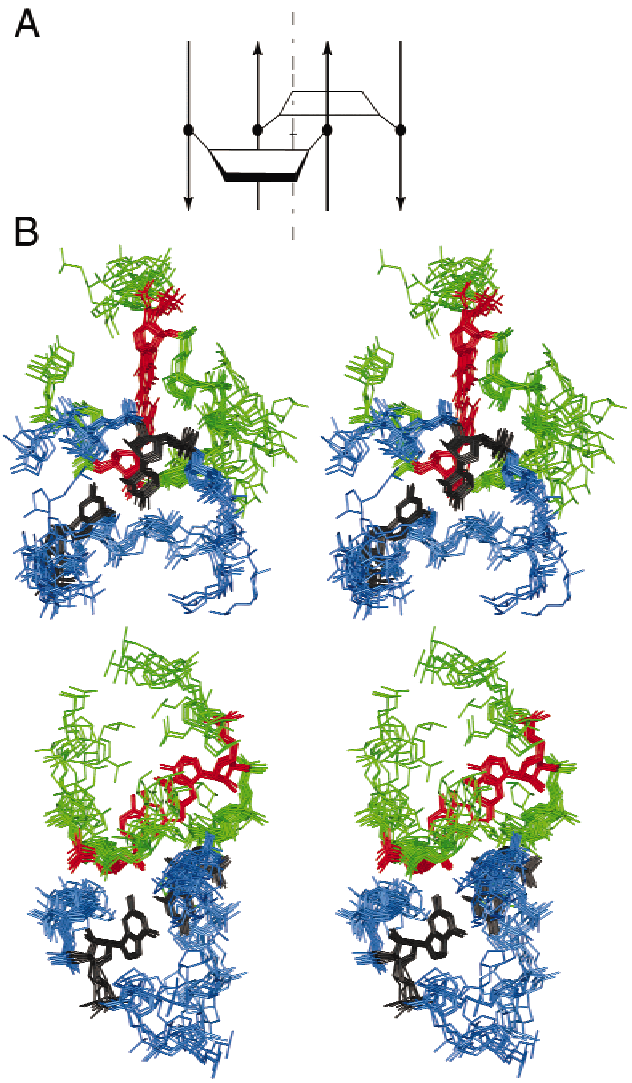

FIGURE 1. Along-groove packing of double helices. **A:** Schematic representation. Trapezoids represent base pairs opened toward the minor grooves. Arrows represent backbones directed 5′ → 3′. The internal strand of each helix interacts with the minor groove of the other helix. Rotation of a helix 180° around the symmetry axis (dash-dotted line) superimposes it with the other helix. **B:** Two different orientations of the superimposition of 10 motifs having a GU central base pair. For clarity, only backbones and central base pairs are shown. The GU and WC central base pairs are black and red, respectively. Their helices are blue and green.

ture (external strands). The arrangement is characterized by an axial symmetry shown in Figure 1A.

ROLE OF THE GU BASE PAIR

In view of this symmetry, it was therefore surprising to see that in the region of contact, the two helices are in fact asymmetric. Thus, we noticed among the identified cases a strong tendency to have one central base pair GU, which was observed in 10 out of 12 cases (Fig. 2A), whereas the other central base pair was always of the Watson–Crick (WC) type. Moreover, in all GU base pairs, G occupied exclusively the external position. This nonrandomness suggests that GU serves a unique role in the along-groove packing. Analysis of the juxtaposition of the central base pairs in the identified cases suggests an explanation for this asymmetry. As one can see in Figure 3, the close packing of the central base pairs GU and GC (the first letter in each base pair corresponds to the external position) is stabilized by a unique interhelix network of five H-bonds, which is a variation of the well-known ribose zipper motif (Schindelin et al., 1995; Shah & Brunger, 1999; Doherty et al., 2001; Nissen et al., 2001). The existence of this network reflects a perfect complementarity between the two interacting surfaces, which includes the correspondence of the shapes as well as of the donors and acceptors of H-bonds. Analysis shows that only the asymmetric combination of GU versus WC would allow this packing. Any other combination including two WC base pairs would create a crack between the helices clearly seen in the two exceptional cases, SC549C501 and LC2291C2374. Such a crack destabilizes the packing by eliminating several interhelix atom–atom contacts in the middle of the contact region, which, in some cases, may become critical. As to the WC base pair, it would prefer GC or CG because G in both cases can make a direct H-bond with the nearby O3′ atom, which in AU and UA has to be replaced by a water bridge. On average, GU versus WC as two central base pairs is observed in 85% of the corresponding regions of rRNAs from other organisms (De Rijk & De Wachter, 1993; Wuyts et al., 2001, 2002). In view of the asymmetry between the two helices, we can consider the GU-containing helices as receptors of WC helices able to bind them almost indiscriminately.

INVOLVEMENT IN THE tRNA ASSOCIATION WITH THE RIBOSOME

The analysis of the 70S ribosome (Yusupov et al., 2001) revealed two more cases of the motif. Their identification, however, was not as straightforward as in the previous cases due to the 5.5 Å resolution of the structure with only phosphorus positions given for all ribosomal RNAs. Nevertheless, the knowledge of the exact conformations of the motifs already identified allowed us to superimpose them with the conformations of the candidates and thus select the structures in which the chains were arranged closely enough to those in the known cases to guarantee the same type of helix–helix arrangement. Also, the knowledge of the sequence requirements for the formation of the motif, including the position and orientation of the central GU base pairs, served as an additional criterion for selection. The two new motifs dealt with intermolecular complexes formed between elements of helices 69 and 68 of 23S rRNA and the tRNA molecules bound to the P- and E-sites,

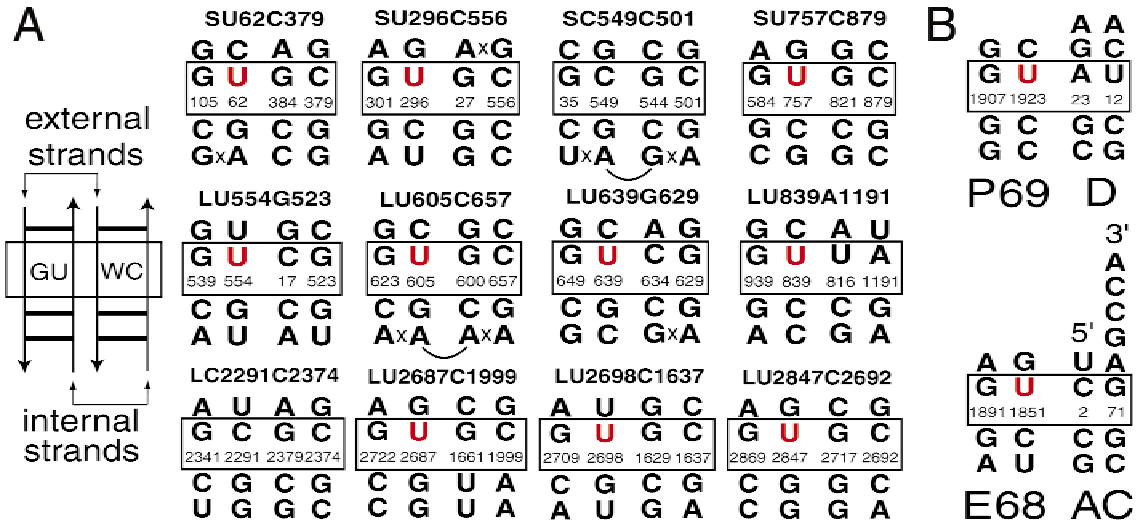

FIGURE 2. Nucleotide sequences of the along-groove packing motifs identified within ribosomal subunits (**A**) and between 23S rRNA and tRNA (**B**). The positions and orientations of the GU- and WC-containing helices correspond to those in the schematic representation on the left. Central base pairs are boxed. U in a central internal position is red. x designates the absence of nucleotide–nucleotide interactions. The nucleotide numbering in rRNA and tRNA is taken from Yusupov et al. (2001) and Sprinzl et al. (1998), respectively. **A:** The name of each motif starts with letter S or L, reflecting the small or large subunit in which it is found, followed by the identity and the number of the internal central nucleotides. **B:** Helices 69 and 68 of 23S rRNA pack, respectively, with the D and acceptor (AC) stems of the tRNAs in the P- and E-sites.

respectively (Figs. 2B and 4). In the tRNAs, the central base pairs were, respectively, 12–23 in the D stem and 2–71 in the acceptor stem, which allowed us to refer to the corresponding tRNA-binding elements as the D and AC receptors.

Although tRNAs at any ribosomal site make different contacts with the ribosome (Yusupov et al., 2001), their interactions with the D and AC receptors are overwhelmingly more stable than all other tRNA–ribosome interactions outside the peptidyl transferase center. This can be deduced from comparison of the potential number of van der Waals contacts and H-bonds as well as of the estimated sizes of the contact areas. Analysis shows that these interactions occur in all types of organisms. First, in both cases, the GU-containing helix is a part of rRNA. In the absence of conservative GU base pairs in tRNA, only GU from rRNA can guarantee that most tRNAs fit these interactions. Both GU base pairs are very conservative: We have found only one (D receptor) and no (AC receptor) exception in the 595 available nucleotide sequences of the large subunit rRNA from all three major branches of evolution (De Rijk & De Wachter, 1993; Wuyts et al., 2001). Finally, the WC character of base pairs 12–23 and 2–71 is observed in 98% and 97% of cytosolic tRNAs, respectively (Sprinzl et al., 1998).

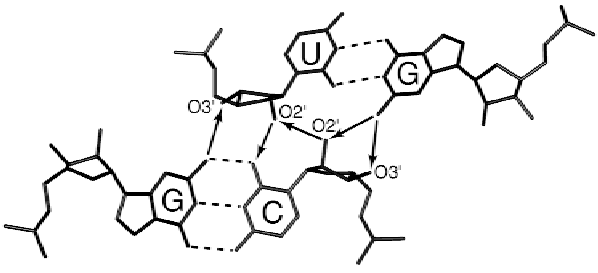

FIGURE 3. Juxtaposition of the central base pairs. Arrows designate interhelix H-bonds directed from the donor to the acceptor atom. The presence of a central base pair GU in one helix makes the close packing possible and the arrangement asymmetrical.

POSSIBLE ROLE IN TRANSLOCATION

Because the D and AC receptors bind tRNAs, they have to change partners each elongation cycle and, therefore, must be somehow involved in the ribosomal translocation. Analysis of the particular positions of both receptors within the ribosome in view of the available experimental data on occupancy of the tRNA-binding sites and structural changes in the ribosome during the elongation cycle suggests for each of them a very distinct and active role in this process.

There have already been indications of the involvement of the AC receptor in translocation. Indeed, modifications of the 2′-hydroxyl group of ribose 71 that block the tRNA interaction with this receptor severely affect

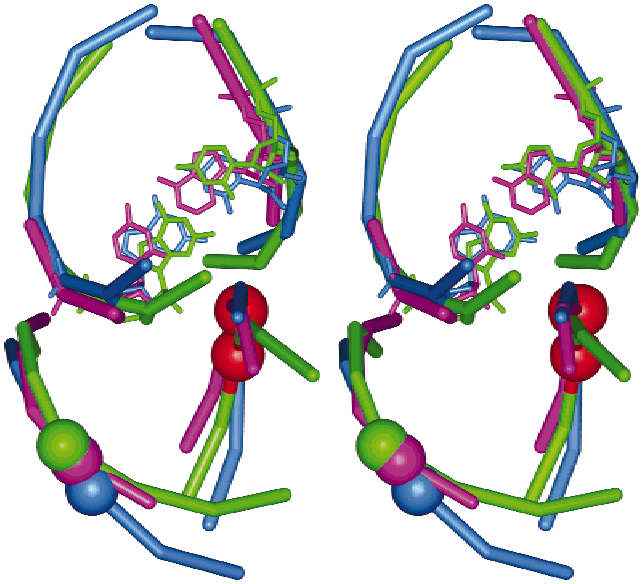

FIGURE 4. Superimposition of the rRNA-tRNA intermolecular along-groove packing motifs [D receptor–P-tRNA] (magenta) and [AC receptor–E-tRNA] (green) with motif LU554G523 (blue). In all structures, the WC central base pair is shown explicitly, and the rest of each complex is represented by lines connecting the consecutive phosphorus atoms. The phosphorus atoms of the nucleotides comprising all GU central base pair are shown as spheres. For uridines, these spheres are red. In both rRNA-tRNA complexes, the WC central base pair belongs to a tRNA, and the GU pair is a part of 23S rRNA. Even with the 5.5 Å resolution of the 70S ribosome conformation, the superimposition of the rRNA-tRNA complexes with LU554G523 is high enough to tell with certainty that they exemplify the close along-groove packing motif.

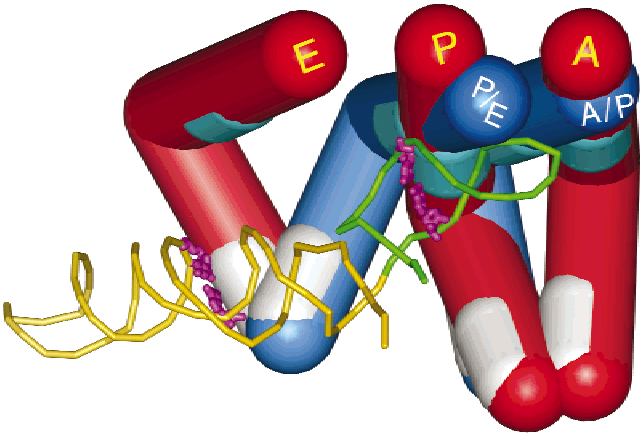

FIGURE 5. Positions of the binding sites in the acceptor (white patches) and D stems (light blue patches) of tRNAs in different pure (red) and hybrid (dark blue) states with respect to the D and AC receptors in helices 69 (green) and 68 (yellow) of 23S rRNA. tRNAs are shown as L-shapes with anticodons marked by the name of the state. The hybrid tRNAs are positioned according to Moazed & Noller (1989a). Other elements are positioned as in Yusupov et al. (2001). The central GU base pairs of both receptors are purple. The D and AC receptors are close to their binding sites in the P/E-tRNA, and the D receptor is also close to its binding site in the A/P-tRNA. Loop 1915, which closed helix 69, interacts with helix 44 of the 30S subunit forming bridge B2a (not shown).

the translocation (Feinberg & Joseph, 2001). The fact that the AC receptor binds to the very end of the E-tRNA acceptor stem at the side farthest from the P-site (Fig. 5) allows this complex to form even when the tRNA is in the P/E hybrid state. This state was postulated by Moazed and Noller (1989a, 1989b) as one of two intermediate states (together with A/P) during translocation (Rodnina et al., 2000). In fact, the end of the acceptor stem together with the four 3′-terminal nucleotides seems to be the only part of the deacylated tRNA that can touch the E-site in the P/E hybrid state. In view of the expected high stability of the complex between the acceptor stem and the AC receptor, we can identify the latter with the hypothetical E-based tRNA-binding site for the deacylated tRNA that has long been thought to provide the thermodynamic driving force for the first spontaneous step of translocation (Bergemann & Nierhaus, 1983; Spirin, 1985; Noller et al., 2000, 2002; Rodnina et al., 2000).

As to the D receptor, several observations can give clues to its functional role. First, the position of helix 69 and, therefore, of the D receptor is rather flexible, which was acknowledged previously (Yusupov et al., 2001). This would allow a tRNA to assume the P/E hybrid state without dissociation from the D receptor (Fig. 5). Second, we found that the D stem of a tRNA in either the A/P or P/E hybrid position is notably closer to the D receptor than in the pure A and E positions. This would make easier the exchange of the D receptor between the two tRNAs at some moment while they are in the hybrid positions. In other words, the hybrid state of the tRNAs, due to a particular arrangement of the two tRNA-binding receptors on the ribosome, facilitates both the formation of the complex of the AC receptor with the P/E tRNA and the redirection of the D receptor interaction from the P/E to A/P tRNA. This will force the translocation to take a pathway via the [A/P–P/E] intermediate. It will also make the D receptor bound to a tRNA for most of the time, and not only when the tRNA is in the pure P-site. The latter aspect becomes especially important in view of the fact that loop 1915, which closes helix 69, forms bridge B2a with helix 44 in 30S subunit (Moazed & Noller, 1989b; Mitchell et al., 1992; Yusupov et al., 2001). The simultaneous binding of helix 69 and its closing loop to tRNAs and to helix 44 of the 30S subunit, respectively, mechanically couples the position of tRNAs to a particular arrangement of helix 44 within the whole ribosome. We suggest that at the first spontaneous step of translocation, the changes in the tRNA position induce rearrangements in the 30S subunit. At further steps, the same communication line can be used to transmit a signal from this subunit to the tRNAs to accomplish the translocation (VanLoock et al., 2000). When this communication line is affected by mutations in loop 1915, the tRNAs are no longer able to move properly, which results in frameshift (O'Connor

& Dahlberg, 1995). The detailed mechanism of this communication is, however, a matter of future analysis.

CONCLUDING REMARKS

This is only an example of how the along-groove packing of double helices can affect the ribosome function. The other cases of this motif can also play important structural or functional roles, which, however, are still to be discovered.

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