Alternative base pairing between 5′- and 3′-terminal sequences of small subunit RNA may provide the basis of a conformational switch of the small ribosomal subunit+

Hans Kössel*, Brigitte Hoch and Patric Zeltz
Institut für Biologie III der Universität Freiburg, Schänzle-Straße 1, D-7800 Freiburg, FRG

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ABSTRACT

The compiled sequences of small subunit ribosomal RNAs have been screened for base complementary between 5′- and 3′-terminal regions. Highly conserved complementary sequences are found which allow formation of a helix between the two ends of 5 or 6 base pairs. This helix is composed of sequences from the loop region of the first 5′-terminal stem and from sequences immediately distal to the last stem (the Me_2A-stem) of the 3′ terminus and therefore allows a coaxial stacking with either of these two flanking stems. Formation of the 5′/3′-helical arrangement is, however, only possible at the cost of dissolving the 'pseudo-knot' helix between the 5′-terminal region and the internal region of small subunit RNA. It is postulated that the mutually exclusive conformational states are in dynamic equilibrium and that they correlate with distinct functional states of the small ribosomal subunit. The 'pseudo-knot' containing conformation with the 3′-terminal sequences more exposed is likely to represent the initiating state, whereas the 5′/3′ terminal paired 'closed' conformation may represent the elongating state in which interaction with fortuitous ribosomal binding sequences of mRNAs is avoided.

INTRODUCTION

Base pairing between terminal regions of RNAs is well documented for the secondary structures of tRNAs (1) 5S rRNAs (2), large subunit ribosomal RNAs (3, 4) and ribonuclease P RNA (5). With respect to small subunit RNAs earlier observations have led to the conclusion that the termini are positioned far apart on almost opposite sides of the small ribosomal subunit (for review see ref. 6), which would sterically prevent base pairing between the two termini. Furthermore, as the 3′ termini of small subunit rRNA from bacteria are involved in recognition of initiation sites of mRNA (7), an impairment of this function by intramolecular base pairing would be expected. On the other hand, precursors of bacterial 16S rRNAs are capable of forming long double stranded stems by base pairing of sequences immediately proximal and distal to the structural region (8), which is suggestive of, at least a temporary close proximity of the two mature ends during processing of 16S rRNAs. In an earlier proposal of such a processing stem for a 16S rRNA precursor from *Zea mays* chloroplasts, base pairing between the two terminal regions of mature 16S rRNA was also implied (9). Mapping of the two termini of *E. coli* 16S rRNA has been achieved by chemical labelling and immunoelectron microscopy of reconstituted 30S particles (for review see ref. 10). From this work a close neighbourhood of the two termini has emerged with the 5′ terminus at a region at the lower left of the 30S/50S interface side (11) and the 3′ terminus at the inner side of the large lobe (12, 13, 14).

This neighbourhood (though not immediate proximity) has gained support from several proposals of more detailed tertiary structure models of *E. coli* 16S rRNA (15, 16, 17). In these models neither the 5′- nor the 3′-terminal sequences are base paired and are, therefore, represented as flexible strings of 8 and 5 nucleotides, respectively, whereas the first two helices at the 5′ terminus and the last helix at the 3′ terminus are positioned in relatively close proximity to each other at the 30S/50S interface side near the cleft of the 30S particle. From this situation and by taking into account the possibility of rearrangements and/or partial melting of helical regions, an interaction of the two terminal regions by base pairing appears sterically possible. Therefore, and in view of the large amount of sequence information available from the compilation of more than one hundred small subunit RNA (DNA) primary structures (18), we have undertaken a systematic screening for complementary sequences between the two termini of small subunit rRNAs. This has led us to the detection of highly conserved sequences which allow formation of 5 or 6 base pairs between the two terminal regions. As this putative 5′/3′ helix is immediately adjacent to the first stem of the 5′ terminus and to the last (Me_2A containing) stem of the 3′ terminus, coaxial stacking with either of those two stems appears possible. Formation of this helical arrangement is, however, only possible at the cost of dissolving the 3 or 4 base pairs of the 'pseudo-knot' helix postulated by Pleij *et al.* (19), which connects sequences from the 5′-terminal

+This article is dedicated to the memory of H.G.Wittmann

* To whom correspondence should be addressed

stem with internal sequences at the border between the central and major 3'-terminal domains (positions 915–918 in *E. coli* 16S rRNA). We propose that the two mutually exclusive helices of small ribosomal subunit RNA reflect alternative conformational states of the small ribosomal subunit, one with a more accessible 3' terminus, the other with a base paired 3' terminus,less accessible for mRNA recognition. It is postulated that the two states represent an initiating and a non-initiating functional state of the small ribosomal subunit and that the latter state is maintained during the elongation (and perhaps termination) phase of mRNA translation.

MATERIALS AND METHODS

The compilation of 106 small ribosomal subunits RNAs of Dams *et al.* (18) was used for visual screening of complementary sequences between the 5'- and 3'-terminal regions. GU pairs and single mismatches were allowed for alignment as indicated in Table 1. The nomenclature for the various base paired regions in the secondary structure model of small subunit ribosomal RNAs (see Fig. 1, helices 1, 3, 19, 30, 47 and 48 and the 'pseudo-knot' 2) also is according to Dams *et al.* (18). It should, however, be noted that helices 30, 47 and 48 are termed helices 28, 44 and 45, respectively in the tertiary structure model of Brimacombe *et al.* (16). For designation of complementary sequences the nomenclature 1/1', 2/2', 2a/2a' etc. is used irrespectively of whether the sequences are unpaired or part of a helix (see Figures 1–3).

RESULTS

Complementarity between 5'- and 3'-terminal sequences of small subunit ribosomal RNAs is observed for all the 80 species listed in Table I. The 5'-terminal sequences (designated 2a) consist of loop sequences from the first stem (1/1') structure, which overlap with the positions involved in the 'pseudo-knot'-helix (designated 2/2'). The 3'-terminal sequences (designated 2a') consist of the positions immediately following the 3'-terminal stem structure (designated 48/48') but in most cases also includes the last G residue from the base of this stem which has to be melted out from the conserved UG pair in order to allow a CG pair in the helix 2a. The position and possible rearrangement of the 5'- and 3'-terminal regions with the helices 1, 2, 2a and 48 in the general secondary structure model are illustrated in Fig. 1 for *E. coli* 16S rRNA. The two terminal regions with their respective helices including the 'pseudo-knot'-helix are depicted with the actual sequences of *E. coli* 16S rRNA in Fig. 2.

As summarized in Table I, a majority of 60 species which include 32 eukaryotes, all archaebacteria, 15 eubacteria and one plastid species shows a 2a/2a' complementarity of six contiguous Watson-Crick base pairs (section A of Table I) with only few cases (sections B and C) containing also one UG pair. A small number of species (including *E. coli*) shows only a 5 base pair complementarity (Table I, section D and E) or a 5 or 6 base pair complementarity with one AA or CU mismatch in an interior position (sections F, G and H). However, interruption or weakening of helical regions by variable numbers of non classical base pairs and/or by looped out nucleotides is not a rare feature in many of the conserved helices of small subunit ribosomal RNA (see Fig. 1) as well as of the other ribosomal RNAs (2, 4) and tRNAs (1). Therefore we consider the 5 species showing a non-classical base pair merely as examples with reduced strength of the 5'/3'-terminal base pairing.

Mitochondrial rRNA sequences are not included in Table I. Our compilation has, however, shown (data not presented) that a large portion of the 26 mitochondrial small subunit rRNA sequences listed by Dams *et al.* (18) also contain complementary sequences corresponding to the positions 2a and 2a', although reduced to lengths of 4 or 3 base pairs. We cannot explain the few remaining mitochondrial species which either show no apparent or only highly disrupted complementarities between the two ends or which even lack positions distal to the 3'-terminal stem. In view of this and because of other known bizarre features of mitochondrial genetic systems we prefer not to include mitochondrial small subunit rRNAs in the present considerations.

With the possible exception of the latter, the strong conservation of complementarity between the two termini of small subunit rRNAs leads us to the conclusion, that the potential base pairing is highly unlikely to be fortuitous but may rather determine a certain structural and functional state of the small ribosomal subunit during the translational process.

DISCUSSION

Proposal of a functional two state model of the small ribosomal subunit

Alternative base pairings for certain parts of small subunit rRNAs or base pairings for tertiary interactions have been proposed

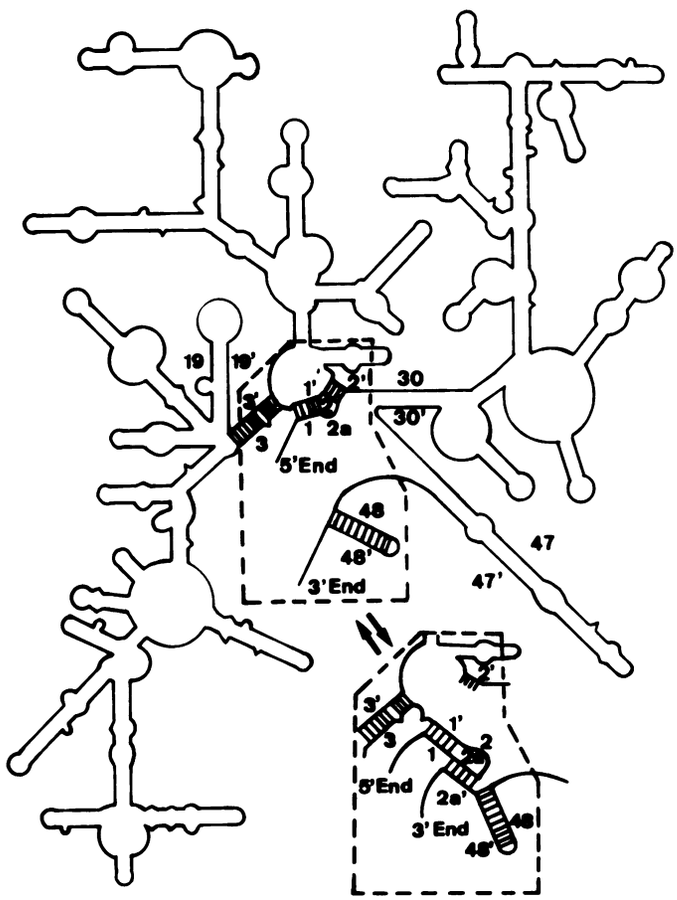


Figure 1. A diagram of the secondary structure of *E. coli* 16S rRNA (modified according to ref. 41). The helices 1, 2, 3, 19, 30, 47 and 48 (base paired regions 1/1', 2/2', 3/3', 19/19', 30/30', 47/47', 48/48') and the 3'-terminal region 2a' proposed for alternative pairing with the 5'-terminal sequence 2a are indicated. The section involved in the conformational switch is marked by framing and a diagram showing the alternative conformation of this region is given within the separate frame.

earlier (20, 21, 22, 23), for review see also ref. (16), but none of the proposals include sequences from the two termini or the 'pseudo-knot' helix. As shown in Figs. 1 and 2 for the *E. coli* 16S rRNA, the two alternative secondary structures proposed for a 5'/3'-terminal base pairing are open or closed states of the 3'-terminal sequences, respectively. As these sequences are known to function in the selection of initiator regions of at least prokaryotic mRNAs (7, 24), the two conformational states are likely to represent two functional states of the small ribosomal subunit especially when the region immediately flanking the 48 stem are included in the mRNA selection of highly expressed genes (25). It is, therefore, reasonable to hypothesize, at least for the eubacterial, chloroplast and archaebacterial 30S ribosomal subunits, whose RNAs contain at their 3' termini the conserved mRNA binding sequences AUCACCUCC (anti-Shine-Dalgarno-sequence), that the conformational state with the open 3' end (Fig. 2A) represents a functional state of the free 30S particle for mRNA recognition and perhaps for subsequent steps of translational initiation. The closed conformation (Fig. 2B), on the other hand, would represent the state of a 30S particle which in conjunction with the 50S particle is involved in the elongation and termination process of mRNA translation in which interaction with fortuitous mRNA binding sites has to be avoided. It is also tempting to speculate that initiation factor IF3, which is known to interact with the 3'-terminal region of bacterial 16S rRNA (26, 27) stabilizes the open form of the free 30S subunit or even actively causes a switch from the closed to the open form by melting out specifically the helix between the two RNA ends.

In the absence of complementary sequences between the 3'-terminal regions of eukaryotic small ribosomal subunit RNAs and eukaryotic mRNA initiator regions (for reviews see refs. 28, 29) the suggestion that the conformation with the open 3' end represents the initiation small ribosomal subunit is less compelling. However, taking into account the strong conservation of the 5'/3'-terminal pairing also in the eukaryotic small subunit RNAs, it is reasonable to extrapolate that the conformational switch here too is paralleled by a corresponding functional switch between an initiating and elongating (and terminating) ribosomal particle.

Experimental evidence supporting the model

Wollenzien and Cantor (30) have observed that 5'- and 3'-terminal sequences of 16S rRNA in the free 30S subunit of *E. coli* can be crosslinked to a certain low extent by psoralen. This 5'/3' crosslink was also observed in studies with free 16S rRNA (31). Although the exact positions of the 5'/3' crosslinked nucleotides were not identified in these studies, a close proximity of the two terminal regions is clearly evident and supports our proposal of the 5'/3' terminal base pairings. The lower frequency observed for the 5'/3' terminal crosslink may reflect the fact that the equilibrium between the two conformational states within the isolated 30S particle is in favour of the open form, which is in accordance with the function of the free 30S particle in mRNA selection and initiation.

Studies with colicin E3 endonuclease provide evidence for a conformational switch of the 3'-terminal sub-domain of bacterial 16S rRNA during the transition from free 30S subunit to the 70S particle. Whereas 16S rRNA of free 30S subunits is resistant against colicin E3, cleavage of the 3' terminal 49nt fragment of 16S rRNA which contains helix 48 together with adjacent single stranded regions (see Fig. 2) is observed in 70S particles (32, 33). Further experimental evidence for the flexibility of 3' terminal sequences comes from studies of protein S21 which is positioned at the cleft of 30S particle (10, 34, 17) close to the

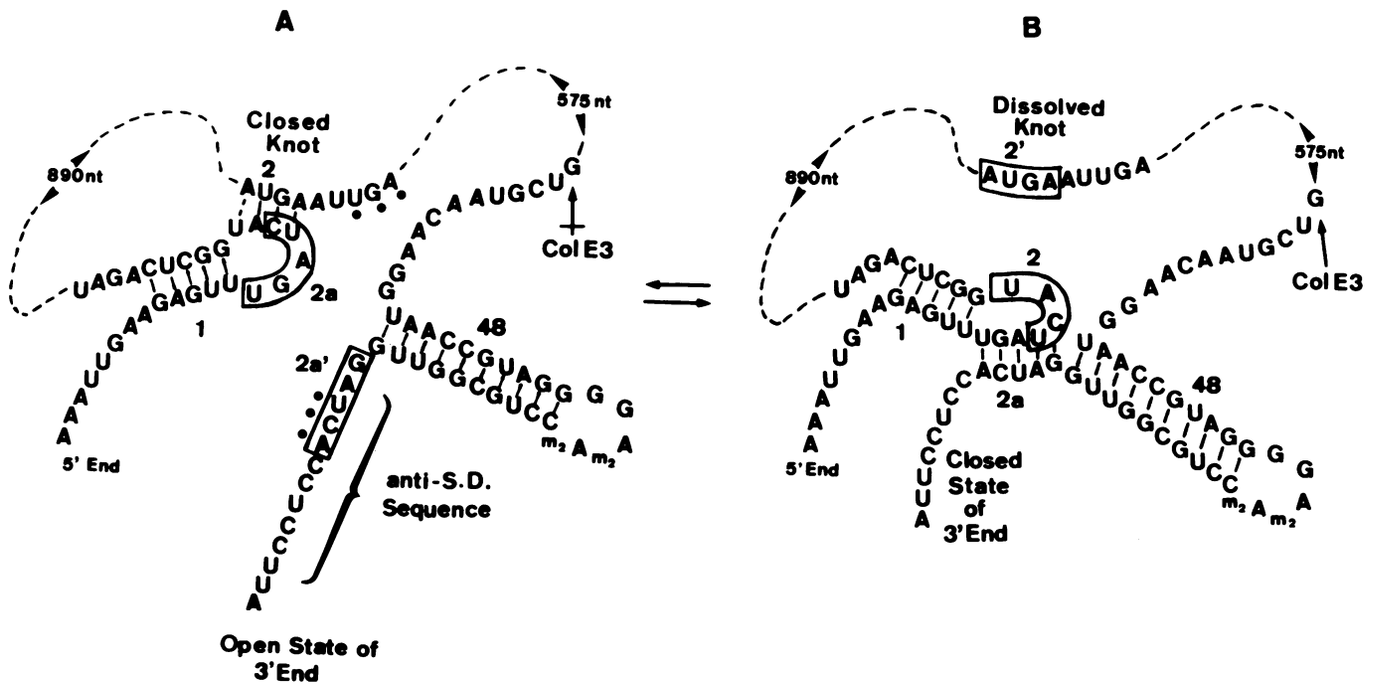
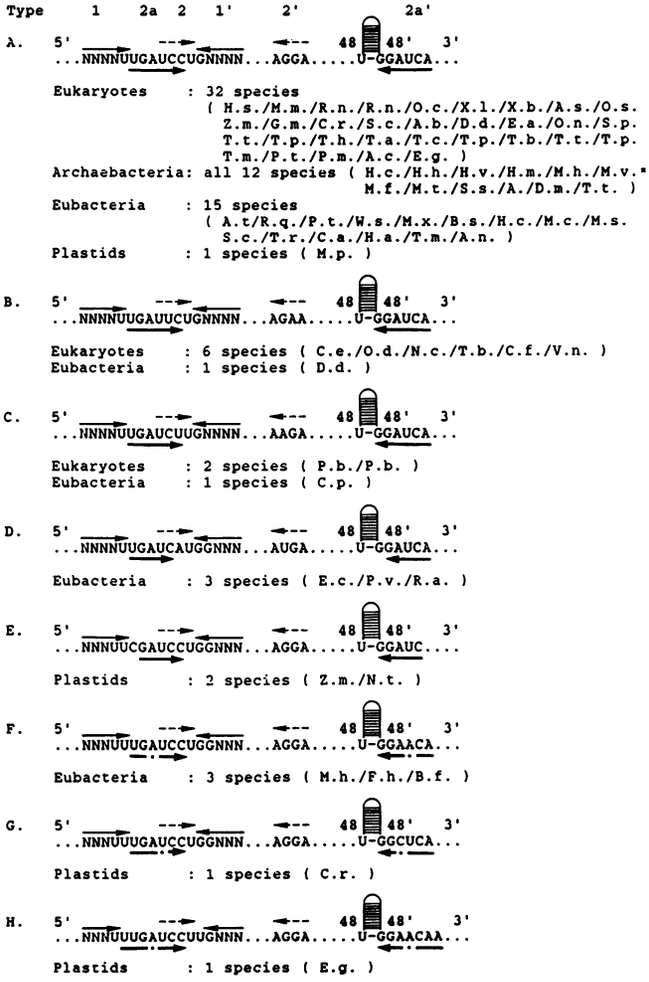


Figure 2. The two alternative secondary structures A (open form) and B (closed form) of the terminal regions and of the 'pseudo-knot' region of *E. coli* 16S rRNA. The structures A and B correspond to the segments of the 16S rRNA secondary structure marked by the central and lower frames of Fig. 1, respectively. The 3'-terminal sequences which are known to interact with initiation sites of mRNAs according to the proposal of Shine and Dalgarno (7) are indicated in structure A as anti-S.D. sequence. The triplets involved in alternative base pairing as proposed by Ericson and Wollenzien (39) are marked by dots in structure A. The cleavage site for colicin E3 is marked by Col E3.

Table 1 :



Base complementarity between the 5'- and 3'-terminal regions of small subunit ribosomal RNAs. Pairs of apposing arrows above each sequence indicate complementary sequences for the formation of helices 1 (solid arrows, sequences 1 and 1') and helix 2 (hatched arrows, sequences 2 and 2'). The pair of solid arrows below each sequence indicates complementary sequences 2a and 2a' between the 5'- and 3'-terminal region for formation of helix 2a. Helices 48 are not presented in the form of nucleotide sequences except for the conserved UG pair at the base of this helix from which the G (after melting from the U) is included in the 2a/2a' pairing (with the exception of the 3 species listed under D). Helices 1 are not presented in the form of specific nucleotides except for conserved UG pairs at the positions close to the loop region. With respect to more detailed sequence information and abbreviations of the species the compilation of Dams *et al.* (18) should be consulted. One archaebacterial helix 2 (M.v.) shows only 3 complementary bases. It is however, included in part A, as this species shows a 6 bp complementarity of helix 2a.

area where the conformational switch is likely to take place. An increased distance between the 3' end of 16S rRNA and protein S21 is observed after binding of the 50S subunit (35). A different less accessible conformation of the 3'-terminal sequences results in protein S21 deficient 30S subunits as opposed to S21 complemented 30S subunits, which show accessible 3' ends (36).

In addition to this experimental support for a flexible orientation of the 3'-terminal region, the single stranded region between helices 47 and 48 comprises a long string of 14 nucleotides which appears large enough to allow sufficient freedom for either permanent or temporary reorientation of helix 48.

Suggestive support for a neighbourhood of 5'- and 3'-terminal sequences comes from the proposal of Peterson *et al.* (37), that in addition to the 3'-terminal region, sequence positions 1 to 18 of the 5' end of bacterial 16S rRNA may also be involved in mRNA recognition. This extended type of mRNA recognition with 3' *and* 5'-terminal sequences of 16S rRNA pairing on adjacent regions of mRNA would imply neighbourhood of the two termini, but in contrast to our model would also imply melting of helices 1 (positions 9–13) and 2 (positions 18–20) during mRNA recognition.

Why does the 5'-/3'-terminal helix not show compensating base changes?

As evident from Table I the sequences forming the postulated helix 2a are highly conserved except for several C to U transitions in sequence 2a, which lead to weakening of the helix by single UG pairs (B and C), or for a reduction from a 6 to a 5 base pair helix (D and E) or introduction of one mispair (F, G and H). However, truly compensating base changes between sequences 2a and 2a' are not observed. As an explanation for this rigidity an alternative pairing of the sequence 2a' with sequences at the 3' side of the 'pseudo-knot' sequence, as has been proposed earlier, can be offered. Hui and Cantor (38) were able to identify by oligodeoxynucleotide mediated S_1 mapping a psoralen induced crosslink between the sequence UGA, which occurs immediately at the 3' side of the base pairing sequence 2', with the complementary sequence UCA, which is part of the single stranded region 2a' of the open 3' terminus (see Fig. 2A). This conclusion has been confirmed by direct identification of this crosslink in the inactive state of the 30S subunit and the base pairing between the two complementary triplets was proposed as a reason for trapping 30S subunits in the inactive state (39). However, in view of the absence of compensating base substitution and of a very limited number of mismatches, this base pairing was interpreted as due to a fortuitous complementarity and consequently the inactive state of the 30S particle resulting from this base pairing was not regarded as functionally significant.

Contrary to this, we would like to propose that this base pairing and the inactive state of the 30S particle represents a functionally inactive conformation only for the initiation process but represents one of the functionally active conformations during the elongation phase of translation. Accordingly, the open conformation depicted in Fig. 2A is essential for mRNA recognition during initiation, whereas during the elongation cycle conformation B is alternating with a modified conformation A in which due to the UGA/UCA base pairing the anti-Shine-Dalgarno sequences are also in a closed state (not depicted in Fig. 2 as a separate state but marked by the UGA/UCA complementary of Fig. 2A). In addition to offering a more satisfying explanation of the 'inactive' state of the 30S particle, this would also explain the absence of compensating base changes of the helical regions involved, as the necessity of conserving two alternative functional helices would impose a much higher constraint as compared to helical regions not involved in alternative base pairing. It is noteworthy in this connection that a strong protection by peptidyl-tRNA is observed for the position G925, which is in close neighbourhood of the UGA sequence (40). This p-site protection may be the result of an induced conformational change rather than of a direct tRNA interaction and may therefore, according to our proposal, reflect the switch to the UGA/UCA pairing during the elongation phase.

Compatibility of the 5'/3'-terminal base pairing with the three-dimensional models proposed for the *E. coli* 30S subunit

Three-dimensional models for the arrangement of the 16S rRNA secondary structure in relation to subunit shape and ribosomal proteins have been proposed independently by Brimacombe *et al.* (16) and by Stern *et al.* (17) more recently and an earlier version was proposed by Expert-Bezancon and Wollenzien (15). The former two models show large accordance with each other but as evident from various differences in details (also with the earlier model of Expert-Bezancon and Wollenzien 15) they must still be considered as approximations, open to further refinement and improvement.

As depicted schematically in Fig. 3 the helices 1, 2, 3, 30 and 48 are located at the side of the 30S/50S interface on both sides of the cleft of the 30S particle. However, with respect to the orientations of these helices the two models show differences: a coaxial stack of helices 1, 2 and 3 in horizontal orientation is proposed by Stern *et al.* (17) (as depicted in Fig. 3), whereas Brimacombe *et al.* (16) suggest a coaxial stack of only helices 1 and 2, which are orientated in an almost vertical orientation separate from helix 3, which is positioned in a more horizontal orientation and penetrates into the internal region of the 30S particle. With respect to the orientation of helix 48 the model of Stern *et al.* (17), poses little constraint, whereas Brimacombe *et al.* (16), propose an orientation of this helix on the platform of the 30S particle, in which the loop rather than the base would be closer to the stacked helices 1 and 2 (as is also implied in Fig. 3A). Expert-Bezancon and Wollenzien (15) in their earlier model place helix 48 and the 3'-terminal single stranded region at the base of the cleft more towards the centre of the particle, which would be in good accordance with our proposal. Neither of the models contradict our proposal of the open form (Fig. 3A) in which the two terminal regions are placed in proximity but not in direct contact. However, as depicted in Fig. 3B a different orientation of helix 48 has to be postulated for the closed form, in order to allow formation of helix 2a. A certain degree of flexibility of helix 48 and its flanking single stranded sequences is therefore necessary for accommodation into the alternative conformation. However, as discussed in the previous section, there is experimental evidence supporting such flexibility.

It may also be argued that opening of the knot helix should result in an increased reactivity of the sequence 2' (AUGA, s. Fig. 2B) which is not observed during the various elongation steps tested (40). This apparent discrepancy may, however, be solved by assuming that the open knot sequence, though unpaired, may be hidden in a more interior cleft of the 30S particle or may be protected by interaction with a protein such as S12 (17).

Altogether our proposal of a 5'/3'-terminal base pairing does not violate any structural constraints imposed by the three-dimensional models. The vicinity of the helices 1, 2 and 48 at the 30S/50S interface side proposed in all three models even adds support for a 5'/3'-terminal interaction and for our proposal of a conformational switch.

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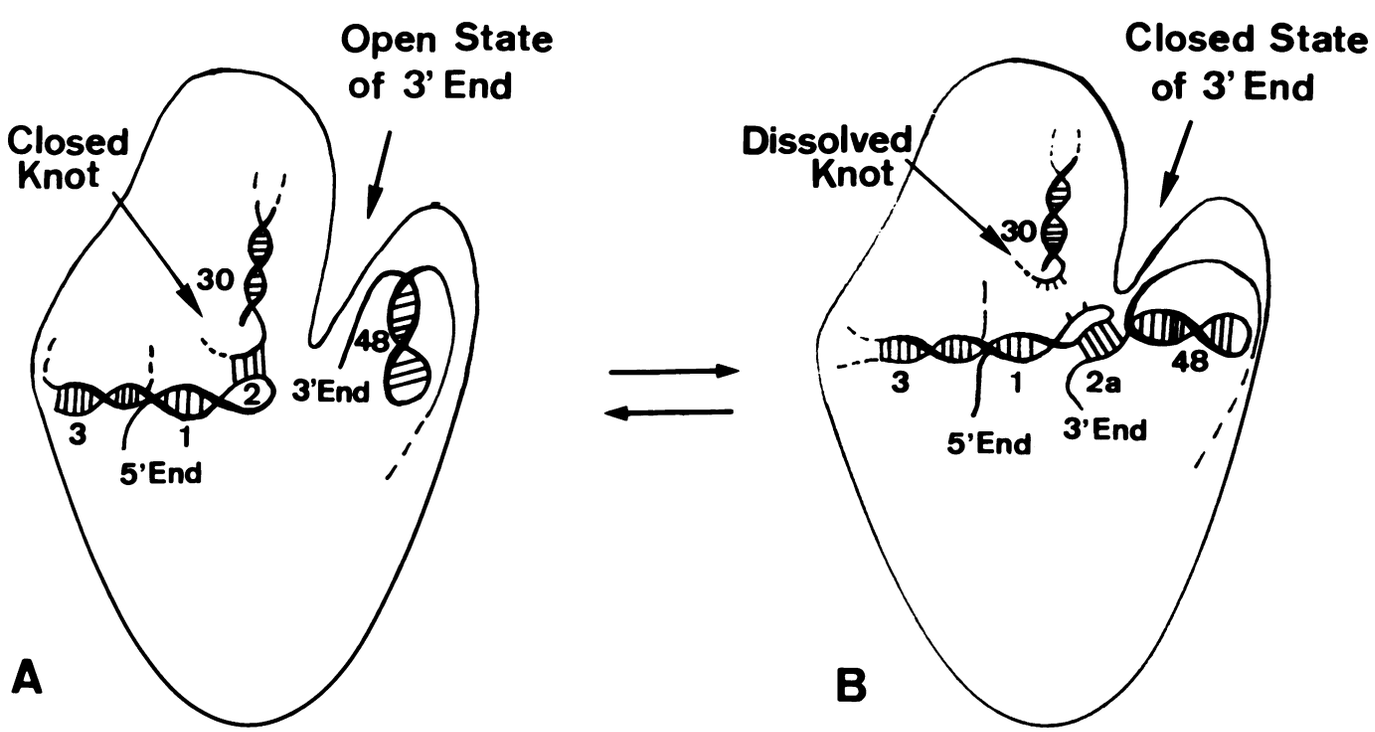

Figure 3. A. Position and orientation of helices 1, 2, 3, 30 and 48 in the three-dimensional structure of the *E. coli* 30S subunit as proposed by Stern *et al.* (17). **B.** Position and orientation of the same elements after the conformational switch to the closed state of the 3'-terminus. In both cases the 30S/50S interface side of the subunit is depicted.

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