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Hypothesis

A conformational switch involving the 915 region of *Escherichia coli* 16 S ribosomal RNA

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A novel alternative conformation, which involves an interaction between the 5' terminal and 915 regions (*E. coli* numbering), is proposed after a screening of compiled sequences of small subunit ribosomal RNAs. This conformation contains a pseudoknot helix between residues 12–16 and 911–915, and its formation requires the partial melting of the 5' terminal helix and the disruption of the 17–19/916–918 pseudoknot helix of the classical 16 S rRNA secondary structure. The alternate pseudoknot helix is proximal to the binding site of streptomycin and various mutations in rRNA which confer resistance to streptomycin have been located in each strand of the proposed helix. It is suggested that the presence of streptomycin favours the shift towards the alternate conformation, thereby stabilizing drug binding. Mutations which destabilize the novel pseudoknot helix would restrict the response to streptomycin.

16 S ribosomal RNA; Streptomycin; Translational accuracy; Conformational switch

1. HYPOTHESIS

Models have been proposed to account for the secondary structure of 16 S rRNA and its folding in the 30 S subunit of *Escherichia coli* [1,2] (see also [3]. However, it is generally agreed that multiple allosteric forms of the ribosome occur during the translation process and that a better understanding of the mechanisms of protein synthesis requires the characterization of these forms. Conformational switches affecting the 16 S rRNA have been proposed previously (see [1,4]). In this report, we propose a novel conformational switch which involves the 5' end region and the 915 region of 16 S rRNA, and suggest that this switch could be related to the control of translational accuracy.

The region encompassing nucleotides 906 to 915 in *E. coli* 16 S rRNA does not contain any base involved in a recognized secondary structural interaction (see [2]). However, the low reactivity of several bases in this region [5] suggests that they could be involved in a higher order interaction. The 900 region has been positioned near the 9–13/21–25 5' terminal helix and 17–19/916–918 pseudoknot helix (see Fig. 1). Bases 911 to 915 are complementary to the neighbouring bases 12 to 16, and we propose that pairing between segments 12–16 and 911–915 during protein synthesis would lead to the formation of a novel 12–16/911–915 pseudoknot helix (Fig. 2). Such base-pairing would require the partial melting of the 5' terminal helix and the disruption of the 17–19/916–918 pseudoknot helix. That these two latter helices are labile is supported by their reactivity to single-strand specific probes [5] (reviewed in [8]). We present here observations which support the novel conformational switch and discuss its possible relationship with ribosome function.

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2. PHYLOGENETIC ANALYSIS

Phylogenetic comparison is a useful approach to infer higher order structure in the large rRNAs [9]. Table I presents the results of a screening of the compiled sequences of the small subunit rRNAs, using the collection of Neefs et al. [10]. The alignment of these authors was used to examine sequence variations involving base positions 12 to 16 and 911 to 915 (*E. coli* numbering) of the small subunit rRNAs. Out of 214 organisms, 209 were able to form four base pairs involving residues 12 to 16 and 911 to 915 and out of them 122 could form five base pairs. The number of organisms having the potential to form four and five pairs could be increased to 214 and 197 out of 214, respectively, when one includes the non-canonical A · G pairs which have been shown to occur in rRNAs [8,11]. These observations are therefore consistent with the proposed alternate conformation. However, the high conservation of the two regions involved in the novel helix restricts the use of the phylogenetic approach, since there are very few base changes which can conclusively prove or disprove the

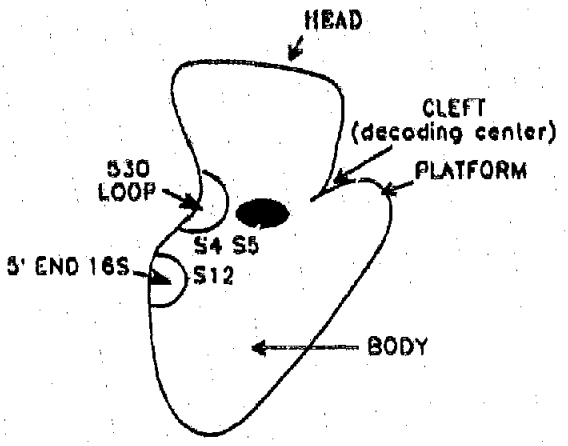

Fig. 1. The Lake model of *E. coli* 30 S subunit, as seen from the interface between the subunits [6]. The dashed area indicates the localization of the 5' terminal and the pseudoknot helices of 16S rRNA, between the decoding site in the cleft and the 530 loop. They are at the junction between the head and the body of the 30 S subunit, proximal to proteins S4, S5 and S12 (see [1,2]). The 900 helix is located beneath the middle of the pseudoknot, pointing to the left, as viewed from the 50 S subunit side.

existence of this helix. An independent analysis by Robin Gutell, using a larger sequence collection led to the same conclusion (personal communication). As stressed by Brimacombe [12] and Kössel et al. [13], sequences which are involved in alternate helical conformations are obviously constrained more than helical regions which are not involved in such switches. Additional support for the proposed switch is however provided by experimental data relating this conformational rearrangement to the action of streptomycin, an error-promoting drug.

3. RELATIONSHIP BETWEEN THE ALTERNATE CONFORMATION OF 16 S rRNA AND EXPERIMENTAL OBSERVATIONS

The 915 region of 16 S rRNA is known to be related to the action of streptomycin. A previous study from our laboratory indicated that streptomycin can be crosslinked to a fragment spanning residues 892 to 917 [14]. Footprinting studies from Moazed and Noller [7] showed that the binding of streptomycin protects residues 911 to 915 against chemical probes, and, in line with these results, mutations affecting positions 912 to 915 in *E. coli* 16 S rRNA or in the chloroplast 16 S rRNA of *Chlamydomonas reinhardtii* were found to restrict the response to streptomycin [15–17] (Leclerc, Melançon and Brakier-Gingras, unpublished). The protection of residues 911–915 could result from a direct interaction between the drug and these bases. However, a mutation in the 5' terminal helix, at a position equivalent to position 13 of *E. coli* 16 S rRNA also causes resistance to streptomycin in the chloroplast [17]. A different interpretation of the footprinting studies relating the protection afforded by streptomycin to an allosteric effect could account for this latter fact. We and others have previously shown that the binding

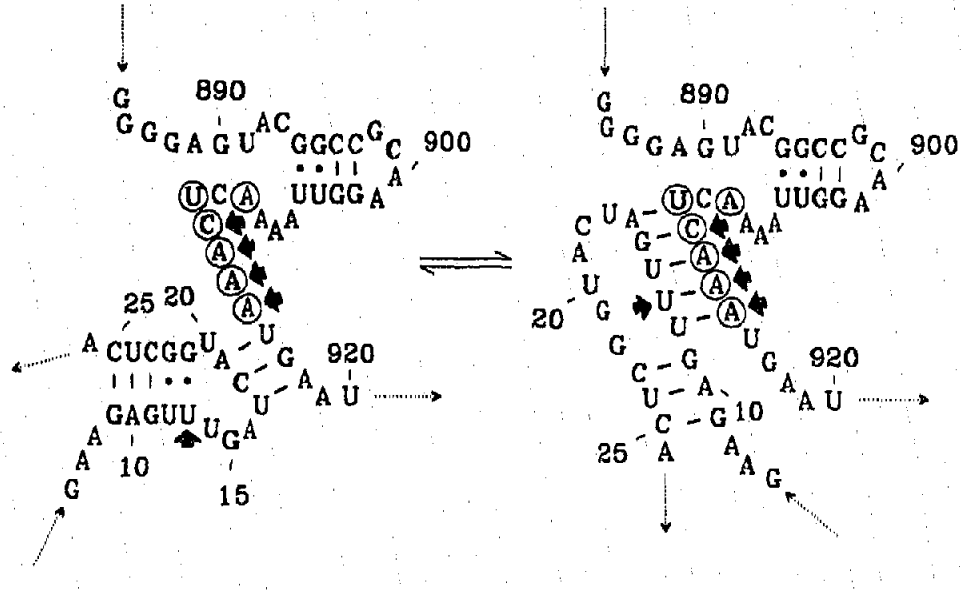

Fig. 2. Proposed equilibrium between two secondary structures of a portion of *E. coli* 16 S rRNA. The left structure, which encompasses the 5' terminal helix, the 17–19/916–918 pseudoknot helix and the 900 irregular helix, corresponds to the classical secondary structure described by Noller and collaborators [2]. The conformer to the right is the alternative structure which we suggest to be promoted by streptomycin and S4 mutations (see the text). Circled residues are footprinted by streptomycin [7]. Arrowheads point to sites where mutations confer resistance to streptomycin.

Table I

Examples of sequence variation associated with base positions 12 to 16 and 911 to 915 (*E. coli* numbering) of small subunit rRNAs.

Position numbers	12	13	14	15	16	911	912	913	914	915		
Frequence of pairing	134/214	208/214	212/214	209/214	214/214	214/214	207/214	213/214	208/214	134/214		
	U	U	U	G	A	U	C	A	A	A	*Escherichia coli*	(91)
4 bp 5 (122 organisms)	U	U	U	G	G	U	C	A	A	A	*Homo sapiens* (mitochondria)	(11)
	U	U	U	G	A	U	U	A	A	A	*Methanobacterium Formicicum*	(18)
	U	U	U	G	G	C	C	A	A	A	*Xenopus laevis* (mitochondria)	(2)
	g	U	U	G	A	U	U	A	A	a	*Homo sapiens*	(72)
	a	U	U	G	A	U	U	A	A	a	*Caenorhabditis elegans*	(1)
	U	U	U	u	A	U	c	A	A	A	*Pimelobacter simplex*	(1)
	g	U	U	G	A	U	C	A	A	a	*Desulfurococcus mobilis*	(1)
4 bp 5 (87 organisms)	U	U	U	G	g	a	C	A	A	A	*Rattus norvegicus* (mitochondria)	(1)
	U	U	U	g	G	U	g	A	A	A	*Podospora anserina* (mitochondria)	(1)
	U	U	c	G	A	U	U	a	A	A	*Asteroleplasma anaerobium*	(1)
	U	U	U	u	A	U	u	A	A	A	*Drosophila virilis* (mitochondria)	(2)
	U	U	U	G	a	c	C	A	A	A	*Schizosaccharomyces pombe* (mitochondria)	(1)
	U	U	U	g	A	U	g	A	A	A	*Aspergillus nidulans* (mitochondria)	(1)
	g	U	C	G	A	U	U	G	A	a	*Giardia lamblia*	(1)
	U	a	U	G	A	U	C	A	a	A	*Saccharomyces cerevisia* (mitochondria)	(4)
	u	g	U	G	G	U	U	A	a	c	*Tetrahymena pyriformis* (mitochondria)	(1)
3 bp 5 (6 organisms)	u	g	U	G	A	U	U	A	a	c	*Paramecium primaurelia* (mitochondria)	(3)
	g	U	c	G	A	U	U	a	A	a	*Thermoplasma acidophilum*	(1)

Sequence data are from Neefs et al. [10]. Residues with potential base pairing (the Watson-Crick pairs G-C and A-U and the wobble pair G-U) are in bold capital letters. An example is given for each particular sequence shown. The number of organisms with the same sequence is indicated between parentheses. Residues 11 and 916 were not included, since these two positions may pair in only 10 organisms of the collection. Sequences where some of the residues at positions 12–16 and 911–915 are absent or unknown were not considered.

of streptomycin to the 30 S subunits occurs in two steps [18,19]: an initial interaction, followed by a conformational rearrangement which stabilizes the binding of streptomycin. An appealing hypothesis would be that this rearrangement corresponds to the conformational switch which we propose. Disruption of the 5′ terminal and 17–19/916–918 pseudoknot helices and formation of the 12–16/911–915 helix upon binding of strep-

tomycin would protect residues 911 to 915. Furthermore, any mutation destabilizing the proposed helix would restrict the binding of streptomycin and this applies to the mutations in the 915 area as well as to the mutation at position 13.

Streptomycin perturbs the control of translational accuracy [20,21], and one must consider the relationship between the proposed alternate conformation and the effect of the antibiotic. According to Moazed and Noller [22], the binding of tRNA induces conformational changes in the 900 irregular helix proximal to the streptomycin binding site, which results in a local tightening of the ribosome. Noller and collaborators have suggested that streptomycin would strengthen this effect and would therefore stabilize the binding of tRNA and decrease the probability of rejecting noncognate tRNAs [2]. The rearrangement promoted by streptomycin which we propose, is quite compatible with a tightening around the 900 helix region, resulting in a more error-prone ribosome.

Allen and Noller [23] have proposed that an equilibrium exists between two conformational states of the 30 S subunit, which affects the reactivity of some residues in the 900 irregular helix. Their studies show that the balance between these two conformations is controlled by S4 and S12, two proteins known to be involved in the control of translation accuracy and in the response to streptomycin (reviewed in [20,21,24]). Mutations in S4 favour one conformation, whereas mutations in S12 favour the other one. Mutations in S4, which make the ribosome more error-prone, facilitate the binding of streptomycin [25] whereas mutations in S12, which make the ribosome more accurate, decrease the binding of streptomycin [18]. We suggest that the conformation favoured by S4 mutations could correspond to the alternate conformation of the 16 S rRNA which we have proposed whereas the S12 mutations would promote the classical conformation.

An alternative base pairing between bases 14 to 18 and 1530 to 1534 of 16 S rRNA has recently been proposed by Kössel et al. [13]. This conformation, which also requires the disruption of the classical 17–19/916–918 pseudoknot helix, cannot coexist with the alternate one which we propose here, and each of these conformations could correspond to a distinct step of the translation process.

The suggested conformational rearrangement for the 915 region lends itself to experimental verification. The use of site-directed cross-linking reagents, as recently developed by Teare and Wollenzien [26], could demonstrate whether this alternate structure occurs and whether it is promoted by streptomycin. Additional mutations could also be introduced in the 16 S rRNA, to determine whether mutations stabilizing the alternate helix favour the binding of streptomycin while mutations stabilizing the classical 5′ terminal and pseudoknot helices have the opposite effect.

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