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Hypothesis

Variable base pairing in a helix of eubacterial 5 S ribosomal RNA points to the existence of a conformational switch

Hilde Van den Eynde and Rupert De Wachter

Department Biochemie, Universiteit Antwerpen (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium

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A survey of 160 published sequences of eubacterial 5 S rRNAs shows that there exists structural variability in one of the helices of the generally accepted secondary structure model. Four structural variants are found, which differ with respect to the position and the number of bulges present. Most eubacterial 5 S RNAs fit into at least two of these conformations. A reaction scheme connecting the four observed conformations by changes in the base pairing scheme is proposed. Each of the known 5 S RNA sequences fits into conformations interconvertible by the proposed reactions.

5 S ribosomal RNA; Secondary structure; Conformational switch

1. INTRODUCTION

The validity of a five-helix secondary structure model for 5 S ribosomal RNA [1-3] has been proven by its applicability to hundreds of sequences listed in recent compilations [4,5]. 5 S RNAs from eukaryotes, archaebacteria, eubacteria and organelles alike fit into this model, even though the size of the helices and the presence and location of bulges account for some variability among the primary kingdoms, as summarized in [6].

The model is illustrated in fig.1a with 5 S RNA from the eubacterium *Escherichia coli*. Helix B is drawn in a shape fitting the great majority of 5 S RNA sequences, with a single base bulging out on the 3'-strand, at a distance of two base pairs from the multibranched loop. A few eubacterial 5 S RNAs such as that of *Paracoccus denitrificans* (fig.1b) require a different shape for helix B, with a bulge on each strand. If one does not admit the presence of a second bulge, one has to settle for a much shorter helix B. It has been shown [6] that most 5 S RNAs from eubacteria and archaebacteria can actually be fitted into the model of fig.1b as well as in that of fig.1a. Moreover, a few recently published eubacterial sequences possess a helix B that does not fit in either of the shapes shown in fig.1a or b. Such is the case for *Rhodobacter capsulatus* 5 S RNA (fig.1c) and *Chlorella ellipsoidea* chloroplast 5 S RNA (fig.1d).

The hypothesis outlined below provides an explanation for the occurrence of apparently exceptional structures in helix B and links their existence to conformational switches taking place in the molecule.

Correspondence address: R. De Wachter, Departement Biochemie, Universiteit Antwerpen (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium

2. INVENTORY OF STRUCTURES FOUND IN HELIX B

We distinguish 4 possible shapes in helix B of eubacterial 5 S RNA, exemplified in fig.1a-d, which

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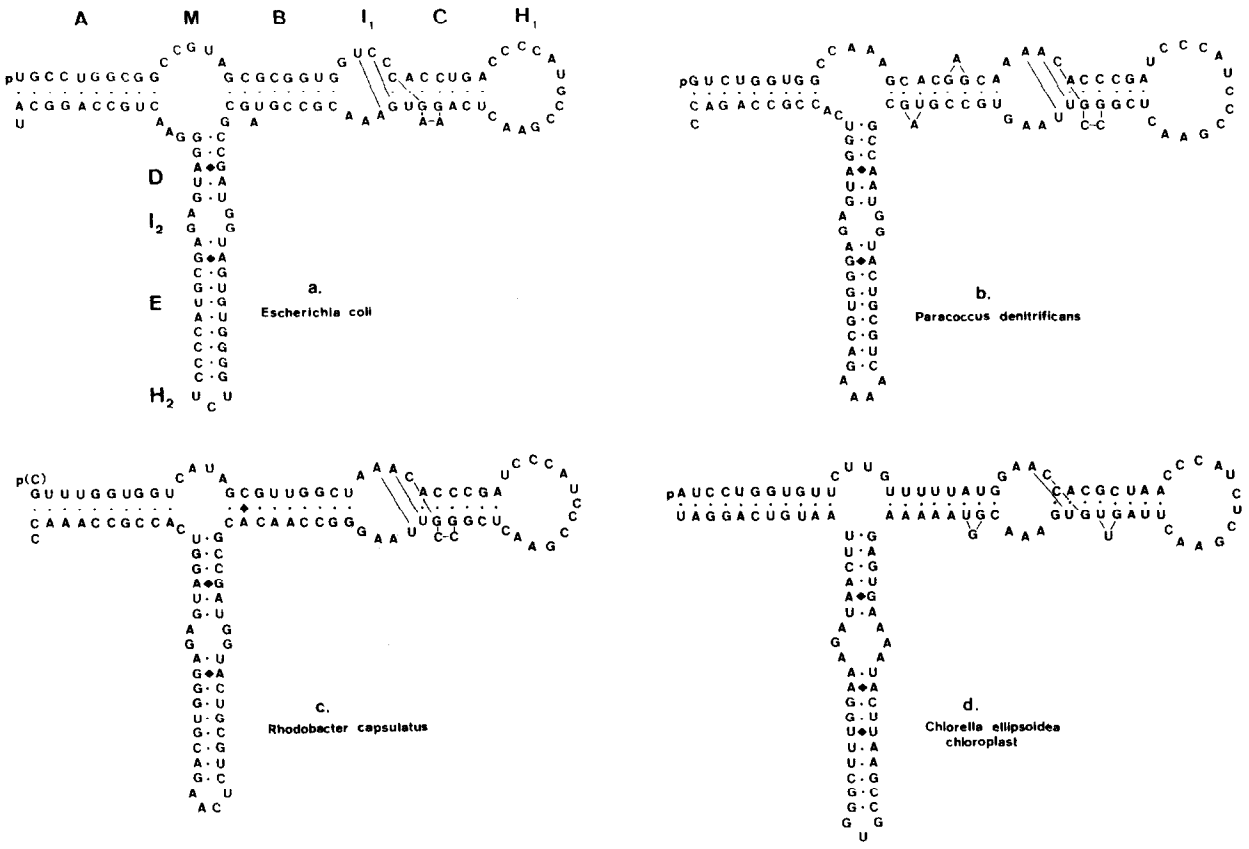

Fig.1. Secondary structure models of four eubacterial 5 S RNAs. Helices and loops are labeled as in a previous compilation [4]. The 5 S RNAs are chosen such as to represent the four different conformations that we distinguish for helix B and the adjacent loop I_1. Presumed base pairs other than G·C, A·U, and G·U are indicated by lozenges instead of dots. Lines connecting bases at the I_1–C boundary reflect alternative base pairing that may cause structural equilibria described previously [1,7].

are further designated as forms, or conformations, 1–4. In a few 5 S RNAs, the structure of helix B is restricted to a single form, but in the majority two or even three of the structures are possible. This is illustrated in fig.2. As an example, helix B of *E. coli* 5 S RNA can be transformed from form 1 into form 2 if the complementary bases indicated at the boundary of helix B and the adjacent internal loop form pairs. The G residue originally terminating the 5′-strand of the helix then becomes a bulge. In *Thiobacillus acidophilus* 5 S RNA, a similar reaction can transform form 1 into form 2. In the latter case, the newly generated bulge can occupy 4 different positions alongside helix B and by migrating [7] into the 5′-direction until it meets the bulge on the opposite strand, give rise to the bulge-free helix designated as form 3. Other examples in fig.2 show cases where helix B can assume different combinations of structures 1–4.

A systematic survey of the structural combinations possible in 160 eubacterial 5 S RNA sequences published to our knowledge is listed in table 1. The survey includes 5 S RNAs from eubacteria as well as chloroplasts, but not from mitochondria since these lack the nucleotide responsible for the formation of the bulge present in form 1. A structural form was deemed acceptable if it contains at most one non-standard base pair intercalated between two Watson-Crick base pairs. The occasional occurrence of non-standard base pairs in places usually occupied by Watson-Crick pairs or G·U is extensively documented in 5 S RNA [8–10], large ribosomal RNAs [11], and transfer RNA [12]. Structures containing both a

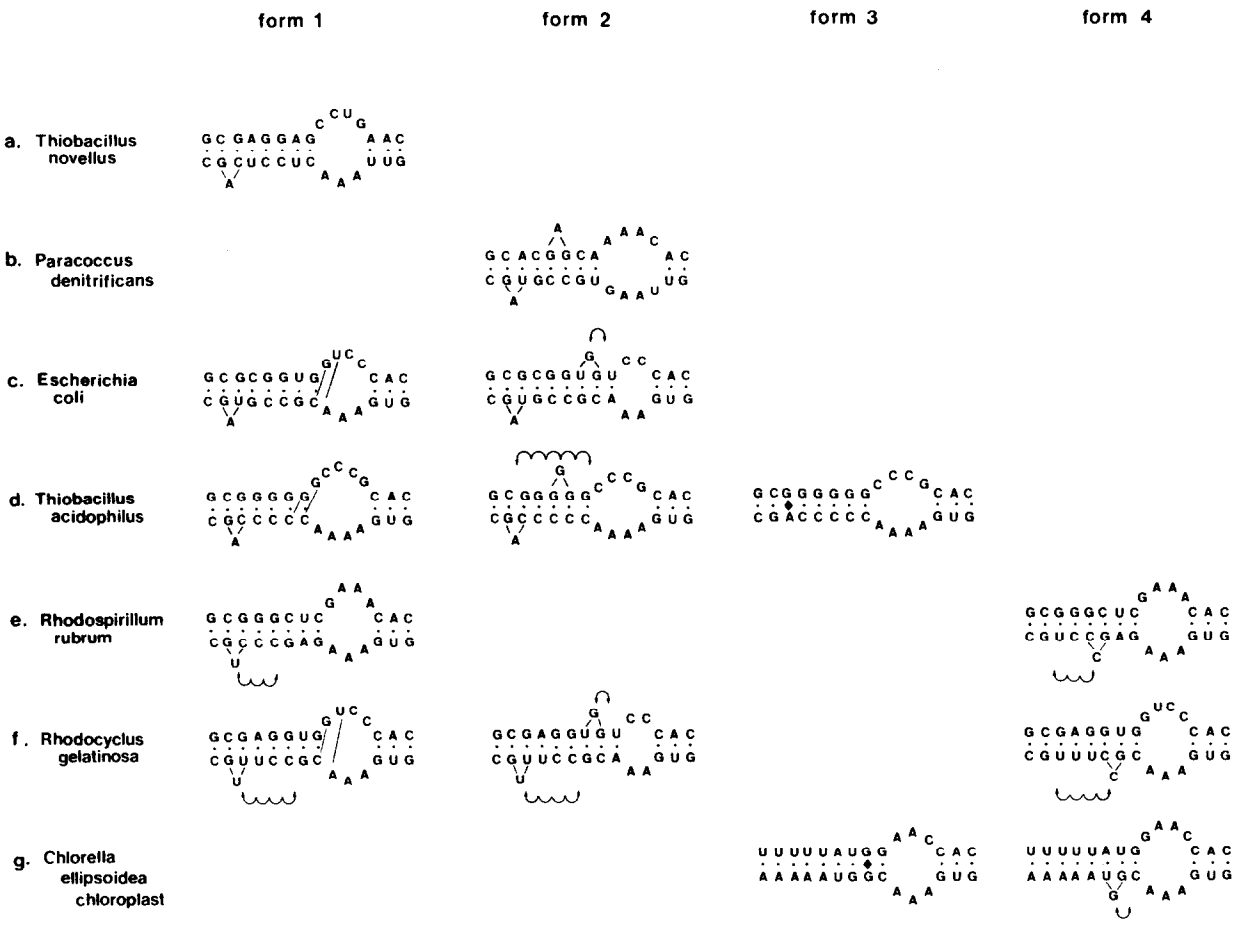

Fig.2. Alternative conformations possible in area B–I_1 of eubacterial 5 S RNAs. Each species chosen for illustration represents a different combination of possible conformations. A complete list of the combinations found in each presently known eubacterial 5 S RNA sequence appears in table 1. In conformation 1, the lines connecting bases at the boundary of helix B and loop I_1 designate alternative base pairing leading to conformation 2. Two-headed curled arrows alongside helices indicate alternative positions that can be occupied by a migrating bulge.

non-standard base pair and a bulge were rejected if a simpler form comprising a non-standard base pair in a bulge-free helix is possible.

Table 1 mentions all the species for which a combination of structures, different from the combination 1-2, which is the most common one, was found. For each different combination listed, an example of the structures is given in fig.2.

3. TRANSITIONS BETWEEN STRUCTURAL FORMS

Neither of the 5 S RNAs examined possesses a B-helix that can adopt all four conformations. Nevertheless, it is possible to summarize the structural transitions connecting the four conformations in a single reaction scheme, shown in fig.3. Conformation 1 can give rise to conformation 2 if a base in the 3′-strand releases its partner in order to pair with the following base on the opposite strand. As a consequence, bulge 'a' arises on the 5′-strand. Depending on the local sequence, bulge a can move one or several positions to the left, eventually arriving in front of bulge 'b'. If this happens then conformation 3, which is devoid of bulges but contains a non-standard base pair,

Table 1

Conformations possible for area B–I_1 in eubacterial 5 S RNAs of known sequence

Species[a]	Phylum[b]	Conformation[c]			
		1	2	3	4
Thiobacillus novellus	purple and relatives	+	−	−	−
Rhodopseudomonas palustris [14]	purple and relatives	+	−	−	−
Paracoccus denitrificans	purple and relatives	−	+	−	−
Rhodobacter sphaeroides	purple and relatives	−	+	−	−
Thiobacillus versutus	purple and relatives	−	+	−	−
Escherichia coli[d]	purple and relatives	+	+	−	−
Herpetosiphon strain Senghas Wie 2[d] [15]	green non-sulfur and relatives	+	+	−	−
Clostridium pasteurianum[d]	gram-positive	+	+	−	−
Desulfovibrio vulgaris[d] [16]	sulfur-dependent and relatives	+	+	−	−
Thermus aquaticus[d]	radio-resistant and relatives	+	+	−	−
Anacystis nidulans[d]	cyanobacteria–plastids	+	+	−	−
Thiobacillus acidophilus	purple and relatives	+	+	+	−
Octopus Spring isolate 3	radio-resistant and relatives	+	+	+	−
Rhodospirillum rubrum	purple and relatives	+	−	−	+
Alcaligenes faecalis ATCC 8750	purple and relatives	+	+	−	+
Alcaligenes sp. [17]	purple and relatives	+	+	−	+
Achromobacter xylosoxidans [17]	purple and relatives	+	+	−	+
Achromobacter cycloclastes [17]	purple and relatives	+	+	−	+
Rhodocyclus gelatinosa	purple and relatives	+	+	−	+
Rhodobacter capsulatus [14,18]	purple and relatives	−	−	+	+
Chlorella ellipsoidea chloroplast [19]	cyanobacteria–plastids	−	−	+	+

[a] References are mentioned only after sequences not listed in the last compilation [5]

[b] As defined in [13]

[c] The four possible conformations are illustrated in figs 1 and 2. (+) Indicates that the conformation is possible, (−) indicates its absence

[d] The combination + + − − is the most common one. It applies not only to the 6 examples given here but also to the remaining 139 eubacterial 5 S RNAs of known structure not mentioned in this table

results. If at this point the original base pairing scheme at the boundary of the helix and the internal loop is restored, then bulge b is formed anew, resulting in conformation 4. If the local sequence permits a leftward migration of bulge b, then conformation 1 is finally restored. In this circular reaction scheme, each potential reaction is reversible and consists of a bulge migration in the direction opposite to the one described above.

A glance at table 1 shows that each of the sets of conformations encountered in eubacterial 5 S RNAs consists of structures adjacent in the reaction scheme of fig.3 and interconvertible by the postulated reactions. If the faculty of the RNAs to fit into alternative base pairing schemes were just a fortuitous consequence of the local sequence, then one would expect to find some cases fitting only in forms 1 and 3, or in forms 2 and 4, i.e. forms that are not interconvertible. Sequences having this property are perfectly imaginable, but not a single instance is found among the 160 presently known eubacterial 5 S RNAs. We postulate that the reason is that the different conformations are actually connected by the reactions described in

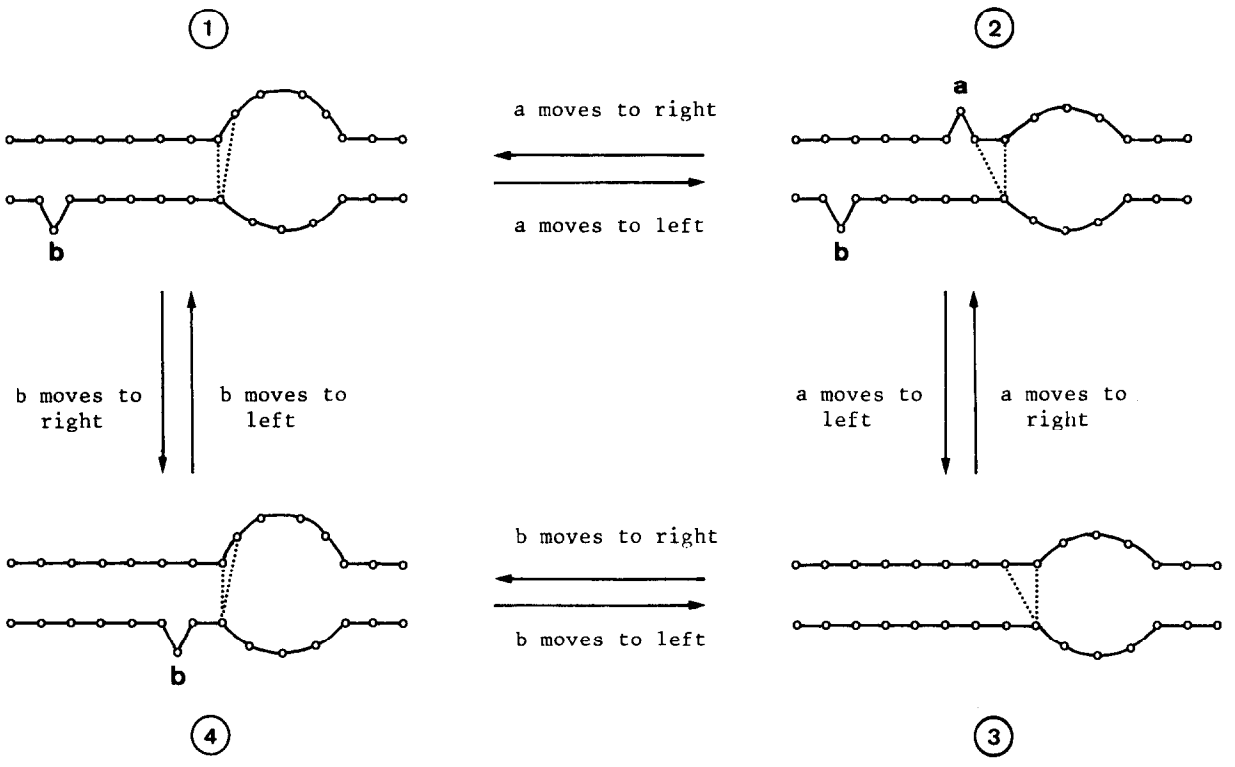

Fig.3. Equilibrium transitions connecting the four conformations possible for area B-I_1. Bulges on the 5'- and 3'-strands of helix B are labeled a and b. Dotted lines at the boundary of the helix and the adjacent internal loop symbolize alternative base pairings usually possible at this site.

fig.3, in other words that this area of the molecule is subject to structural switches.

4. DISCUSSION

The hypothesis formulated here is that subsets of a set of four possible conformations for helix B and the adjacent internal loop I_1 are adopted during switches in the tertiary structure of eubacterial 5 S RNA. The occurrence of such switches, probably a functional requirement for 5 S RNA, has already been suggested [1,7] for area I_1-C in all 5 S RNAs and for helix E in the case of eukaryotic 5 S RNA. The present availability of many additional eubacterial sequences allows us to extend the hypothesis to helix B. In the latter case the evidence is not purely comparative, but there is the additional argument that only combinations of structures are observed, interconvertible via the reactions of fig.3.

The following objection could be raised. If helix B should, for some functional reason, be flexible, what happens in the few species such as *Thiobacillus novellus* and *P. denitrificans*, where only one conformation for helix B seems plausible? Possibly such 5 S RNAs rely on structural switches solely involving area I_1-C to achieve the required change in shape. The reverse situation seems to prevail in even rarer cases, such as the 5 S RNA of a *Herpetosiphon* strain [15] where helix B can take alternative conformations, but area I_1-C cannot.

What is the situation in eukaryotic and archaebacterial 5 S RNAs? In eukaryotes, area B-I_1 seems to be constantly in conformation 1. The fact that the molecule is 'stiff' at this point may be compensated by flexibility in helix E which, in contrast to the eubacterial helix E, contains bulges that can migrate [7]. In most archaebacterial 5 S RNAs, helix B seems to be most stable in conformation 2, but conformation 1 is possible as well [6].

It is often said that a hypothesis is useful only if it can be tested. The present hypothesis could be

tested by examination of additional eubacterial 5 S RNA sequences to be determined in the future. If the molecule switches between the conformations 1–4 via the postulated reactions (fig.3), then only subsets of conformations that are adjacent in the reaction scheme should be found, as has hitherto been the case. The collection of known eubacterial 5 S RNAs, although numerous, only contains representatives of 6 out of 10 eubacterial phyla [13], so there remains room for discovery of variant structural types.

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