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Pseudoknot and translational control in the expression of the S15 ribosomal protein

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Summary — Translational autocontrol of the expression of the ribosomal protein S15 proceeds through the transitory formation of a pseudoknot. A synopsis of the known data is used to propose a molecular model of the mechanism involved and for the role of the pseudoknot. This latter structure is able to recruit 30S ribosomal subunits to initiate translation, but also to bind S15 and to stop translation by trapping the ribosome on its loading site. Information on the S15 protein recognition of the messenger RNA site was deduced from mutational analyses and chemical probing. A comparison of this messenger site with the S15 ribosomal binding site was conducted by analysing hydroxyl radical footprintings of these two sites. The existence of two subsites in 16S RNA suggests that the ribosomal protein S15 might present either two different binding sites or at least one common subsite. Clues for the presence of a common site between the messenger and 16S RNA are given which cannot rule out that recognition specificity is linked to a few other determinants. Whether these determinants are different or not remains an open question.

S15 / pseudoknot / autocontrol / RNA binding / translation initiation

Introduction

Pseudoknots are known to be involved in protein expression and especially in the modulation of the translation rate of several messages. In most cases, polycistronic messengers of diverse origins (retroviruses, yeast retrotransposon *Ty*, several procaryotes) are affected. Translation responds to at least two sets of *cis*-acting elements, a short specific sequence and a highly ordered structure just downstream, which is very often a pseudoknot. As result, it allows the synthesis at a low yield of a specific protein either by inducing a frameshift upstream of a stop codon or by causing read-through at the termination step. As far as we know there is no regulation acting *in trans* and the translation rates observed are permanent, suggesting that the mechanisms involved can be considered as an integrated way to express several compacted messages. Despite intensive work to elucidate their precise role in the ribosome-mRNA complex, the mechanisms involved are still unknown. Sometimes the presence of a pseudoknot is absolutely necessary but at others sites it can be replaced by a stable stem-loop. Stability of stem 2 has also been invoked [1]. However, some nucleotides in loop 2 of the *gag-pol* pseudoknot were shown to be very important for the murine leukaemia virus [2] but not for coronavirus, another infectious bronchitis virus [3]. Thus, how pseudoknots fulfil their function remains to be understood.

In other cases, pseudoknots have been observed around the ribosome loading site and shown to be involved in the modulation of translation initiation. They constitute important signals *in cis* which are essential not only for ribosome binding and movement as noted above for the ribosomal frameshifting or the read-through of termination codons, but also for the interaction with a repressor molecule. Very often, the protein from the translated message corresponds to the repressor, thus inducing autoregulation by its translation rate. The study of *rpsO* expression is perhaps one of the most convenient tools to help understand these problems. The *rpsO* gene encodes a small ribosomal protein, of 10000 Da, S15, and constitutes the first gene of the *rpsO-pnp* operon, located at the 69th minute of the *E coli* chromosome. The expression of *rpsO* has been shown to be autoregulated through the formation of a pseudoknot. Taking into account the recent progress made in our knowledge of the operator site of *rpsO* mRNA and the general features of the pseudoknot structure by NMR [4–6], a tentative, comprehensive model of translation autoregulation is proposed.

Control of translation initiation

Initiation of translation can be modified by restricting the access of the initiating ribosome to the Shine-Dalgarno se-

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Abbreviations: CMCT, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluene sulfonate; DEPC, diethylpyrocarbonate; DMS, dimethylsulfate; ENU, ethylnitrosourea.

quence. This can occur in two ways: either reversibly, by masking the Shine-Dalgarno sequence or irreversibly by cutting this sequence with an endoribonuclease [7]. In most of the studied cases, the former solution has been observed. The Shine-Dalgarno binding site is often embedded in a more or less stable stem-loop created by alternative structure [8] or antisense RNA [9]. Proteins can also bind specifically to the Shine-Dalgarno region and inhibit ribosome loading by steric hindrance [10]. In this case, the ribosome loading site and the repressor binding site can overlap, but this is not absolutely necessary, as protein binding at a distant site is able to induce alternative RNA structures which, in turn, can entrap the Shine-Dalgarno sequence. In this classical model of autoregulation, occupancy of both sites is mutually exclusive and competition is observed between ribosomes and repressor. Another reversible way to modulate translation is to arrest the ribosome movement before the productive ternary initiation complex is formed. In this model, the ribosome is trapped by the repressor on mRNA at the preternary step. Interestingly, in the two messengers known to exhibit this feature (*ie* the messengers of the ribosomal protein S4 and S15 [11,12]), a pseudoknot is present, suggesting that it may serve as an energetic barrier to the ribosome.

The S15 operator site

Identification and localisation

The regulation of *rpsO* expression has been studied *in vivo*, by creating a translational fusion between the proximal part of *rpsO* and the distal part of *lacZ* (fig 1). The fusion was transferred to a lambda phage and a strain deleted for the

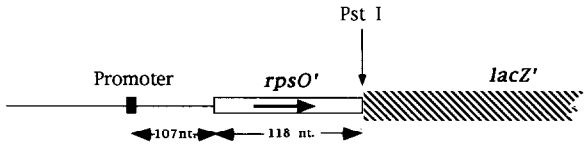

Fig 1. Translational fusion between *rpsO* and *lacZ*. *rpsO'* corresponding to the proximal part of *rpsO* is fused to the distal part of *lacZ* at the *Pst*I site of the polylinker carried by a M13mp8 derivative (see Portier *et al* [13, 14]). nt, nucleotide length of the leader and coding phase of *rpsO* cloned. The horizontal arrow indicates the direction of transcription.

lac operon was lysogenized. The effect of S15 overproduction was measured by introducing a multicopy plasmid into the lysogenized cell and measuring the β-galactosidase level. The repression ratio observed was used as an index of the regulatory capacity of the system. In the wild type strain, the repression was increased 22-fold in an overproducing strain compared to a cell carrying only the chromosomal copy of *rpsO* gene. Taking advantage of the low level of β-galactosidase synthesised in S15 overproducing strains, several deregulated mutants were isolated by selecting for growth on lactose medium. All the spontaneous mutations were localised just downstream from the initiation codon, suggesting that the S15 repressor site overlaps the ribosome loading site [13, 14].

Structure

The translational operator site covers approximately 80 nucleotides. Its secondary structure has been analysed by enzymatical (RNase T1 and V1) and chemical (CMCT,

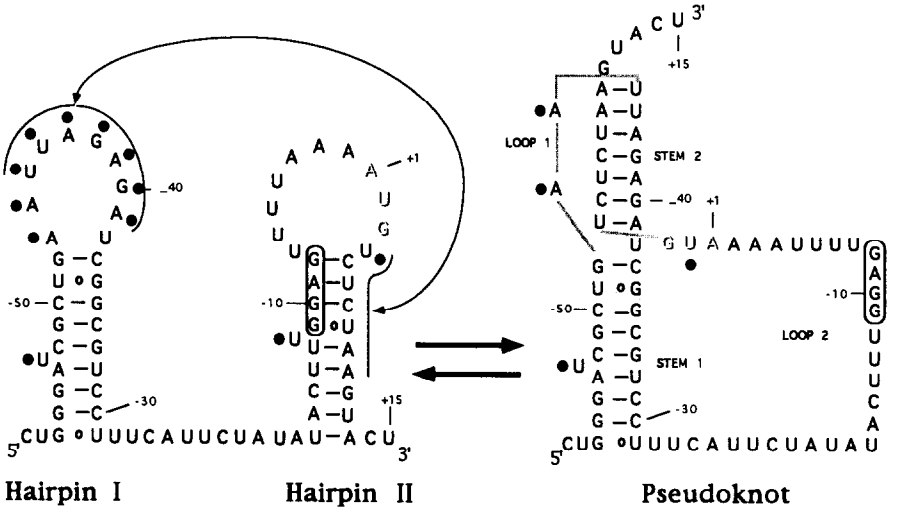

Fig 2. *In vitro* structure of the S15 operator site. The two structures observed in equilibrium are shown. On the left, a curved arrow connects the two stretches of complementary sequence. Black points indicate the bases which, fully reactive to DMS or CMCT in the absence of S15, become unreactive in its presence. Note that this change of reactivity is linked to base pairing for several of them. The Shine-Dalgarno sequence is framed and the initiation codon is shown in outlined characters.

DMS, DEPC, ENU) probes after *in vitro* synthesis of an RNA fragment containing the operator site [15]. It has been shown to consist of two hairpins, I and II. Hairpin II is less stable than hairpin I and carries the Shine-Dalgarno sequence embedded in the stem and the initiation codon in the loop (fig 2). However, an alternative structure is also present in equilibrium with hairpin II. The invariant core of this structure is formed by the stem of hairpin I, but its loop is paired with a stretch of seven nucleotides located just downstream the initiation codon (fig 2). When the pairing of the loop is achieved, a new stem (stem 2) is formed, creating a pseudoknot: both stems are separated by a spacer consisting of an unpaired U nucleotide. The two loops (1 and 2) connecting the stems are strongly dissymmetric: loop 1 carries two nucleotides crossing the deep major groove whereas loop 2 contains 31 residues and crosses the minor groove. Interestingly, the initiation codon and the Shine-Dalgarno sequence are carried by loop 2 with the initiation codon being adjacent to the junction of the stems.

In vivo pseudoknot formation

All spontaneous point mutations and deletions leading to the loss of autocontrol were located in the stretch of nucleotides complementary to the loop of the stable hairpin [13] (fig 3). At first glance, these mutations can be interpreted as disrupting hairpin II in which the Shine-Dalgarno sequence is embedded. Opening this stem would increase the ribosome loading efficiency and thereby increase the translational rate of the fusion. In this model, the ribosome binding site would be superimposed to the S15 binding site and deleting hairpin I would be expected not to change these sites and keep the regulation unaffected. However, when this deletion was analysed, the autocontrol indeed disappeared. Thus, although no spontaneous mutations were isolated in hairpin I, it could be concluded that this structure is necessary for the autocontrol because it is involved in the pseudoknot formation. More direct evidence for the existence *in vivo* of the pseudoknot was obtained by mutational analysis. It turns out that each time a point mutation disrupts the Watson-Crick base pairing in stem 2, autocontrol is lost. On the other hand, each time a complementary mutation is introduced in the other strand to restore Watson-Crick base-pairing, autocontrol is also restored [13, 14, 16]. Thus there is a perfect correlation between pseudoknot disruption *in vitro* and loss of autoregulation *in vivo* on the one hand and between restoration of autoregulation *in vivo* and restoration of stem 2 stability *in vitro* on the other.

Does stem-loop II exist *in vivo*?

The *in vivo* formation of hairpin II was derived from deletion experiments: when hairpin I is deleted, the pseudoknot cannot form and only hairpin II is observed [13, 15]. Consequently, autocontrol is abolished (fig 4). However, the high level of β-galactosidase level expected to result from deregulation was not observed. Entrapment of the Shine-Dalgarno sequence in hairpin II which induces a drastic

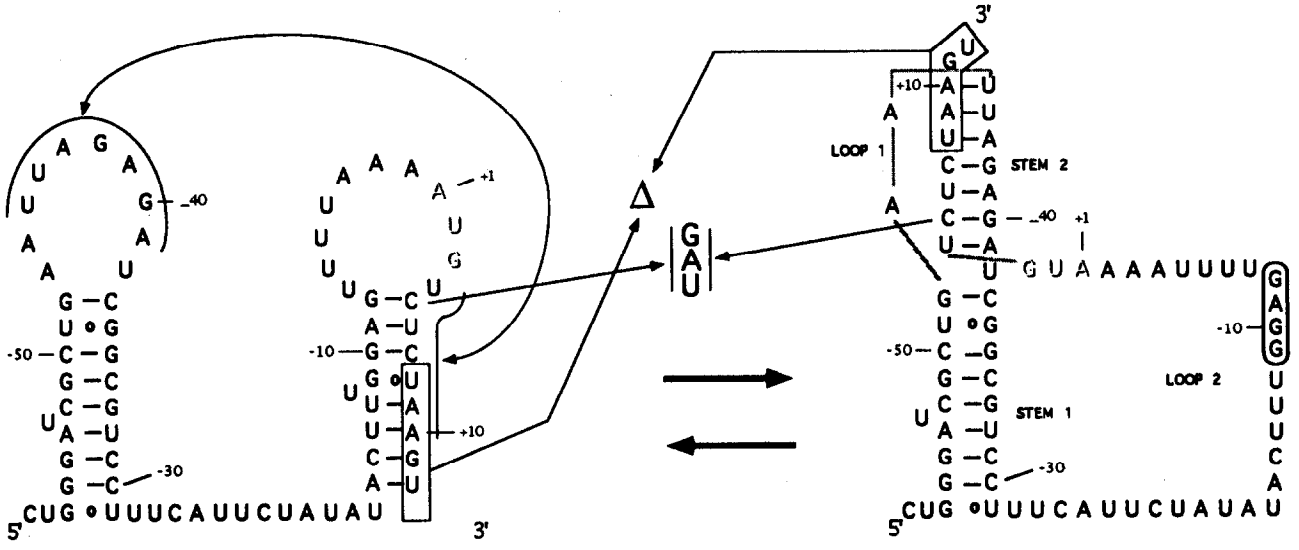

Fig 3. Location of the spontaneous mutations in one of the complementary stretches of the operator site. Point mutations were all located at +5 position. Deletions (one of which is shown in a square frame) cover the other end of the same stretch and are indicated by Δ. Both kinds of mutations affect the pseudoknot stability by destabilising stem 2. Other symbols as described in legend to figure 2.

decrease of the translation rate might account for this observation. This was further confirmed by a reduction of the *in vitro* binding of the ribosomes [17]. Conversely, G instead of C at position (–15) destabilises hairpin II and displaces the equilibrium towards the pseudoknot formation [16] (fig 4).

More indirect evidence for the existence of hairpin II was given by the concentration of spontaneous mutations in the stretch of nucleotides corresponding to one strand of stem 2. Mutations in the other strand, (in the loop of the stable hairpin), would have favoured the formation of hairpin II and thereby decrease the amount of β-galactosidase to an undetectable level for the screen used.

S15 binds to the pseudoknot

The best evidence for S15 binding to the pseudoknot was given *in vitro* by footprinting experiments using enzymatic and chemical probes. A major consequence of S15 binding is that all nucleotides corresponding to the loop of hairpin I (positions (–39) to (–47)) become unreactive to DMS and CMCT [17]. This reduction of reactivity is primarily due to the formation of stem 2 of the pseudoknot. Note that $A_{(-46)}$ and $A_{(-47)}$, which are not involved in base-pairing in the naked pseudoknot, are also protected. In addition, the bulged $U_{(-53)}$ and $U_{(+2)}$ become also unreactive. Further hydroxyl footprint experiments confirmed that S15 shields

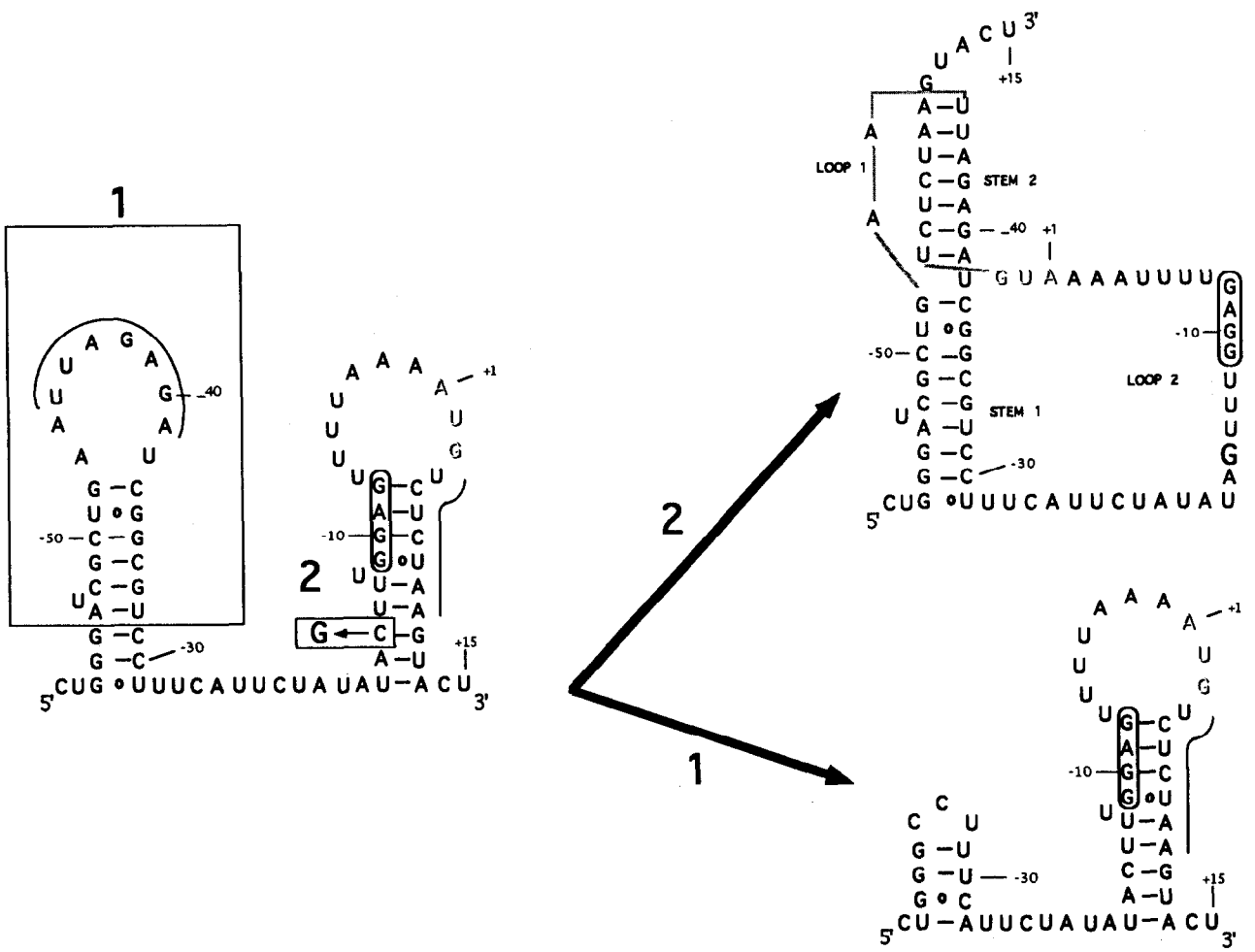

Fig 4. Mutations modifying the equilibrium between the two forms of the operator site. Deletion of the stable hairpin, (framed nucleotides in 1) displaces the equilibrium towards hairpin II as proved by the structure obtained from *in vitro* probing experiments (arrow 1). Displacement of the equilibrium towards the reverse direction can be observed by changing $C_{(-15)}$ to G. *In vitro* only the pseudoknot is formed. Other symbols as described in legend to figure 2.

stems 1 and 2 of the pseudoknot ([18], see below). Additional evidence for the stabilisation of the pseudoknot by S15 was provided by toeprinting experiments. Indeed, the primer extension of a complementary oligonucleotide at the 3′ end of the pseudoknot fragment by reverse transcriptase was analysed in the presence and in the absence of the S15 protein. Faint bands corresponding to stops around +10, +11, +13, +14 were detected only in the presence of S15 and were shown to increase with S15 concentration, suggesting that the binding of S15 induces stabilisation of the pseudoknot [12]. Moreover, a direct correlation between the formation of the pseudoknot and S15 binding was shown by *in vitro* binding experiments using retention on nitro-cellulose filters. Indeed, mutations preventing pseudoknot formation induce a drop of the binding affinity of at least 10-fold [18].

Cooperativity of S15 and ribosome 30S subunits binding

When toe-print experiments were performed in the presence of 30S ribosomal subunits and of $tRNA_f^{Met}$, a clear stop appeared at the +17 position, called toeprint, which corresponds to the position of the ribosomal subunit on the initiation codon when a productive ternary initiation complex is formed. Thus, in the ternary complex, the pseudoknot structure must be open. When $tRNA_f^{Met}$ was omitted, the stop corresponded to +10, at the edge of the pseudoknot, suggesting that in the binary complex, the pseudoknot is present and able to stop reverse transcriptase [12]. In the presence of S15, the signal at +10 increases with S15 concentration and does not change in position even when $tRNA_fMet$ is added. These observations indicate that S15 does not prevent 30S subunit binding, as was observed in competition mechanisms. On the contrary, it stabilises the pseudoknot in the presence of 30S subunits. Direct evidence for the presence of both 30S and S15 on the same RNA molecule was provided by footprint experiments [12]. In the presence of S15, specific protection around $G_{S(-42,-43)}$ in stem 2 was clearly detected whereas protection of the Shine-Dalgarno sequence appeared in the presence of 30S subunits. When both components were present, protection in the Shine-Dalgarno sequence and in stem 2 were present indicating that both kind of molecules are bound simultaneously to the pseudoknot. Participation of pseudoknots in ribosome loading has already being described [20, 21].

The apparent binding cooperativity observed between S15 and 30S subunits might be the result of an increased binding of 30S after S15 binding exposing the Shine-Dalgarno sequence in loop 2. On the other hand, an increased binding rate of S15 only in the presence of 30S cannot be excluded because toeprints are clearly detected at position (+10) in the presence of 30S subunits, whereas, in their absence, only very faint bands are observed around this position in the mutant CFP5517 [12,17]. Thus, the repressor might bind to the 30S-mRNA complex more tightly than to the mRNA as proposed by Draper [19] and this increased affinity might account for the cooperativity observed. If this last hypothesis is correct, a true entrapment mechanism (and not competition) would exist, in which S15 would bind and prevent the preternary complex to form the ternary complex. Unfortunately, neither the binding constant affinities of S15 and 30S subunits for the messenger, either naked or in a binary complex (see fig 8), nor the *in vivo* concentration of free components, which would allow to deduce the order of binding, are precisely known and no definitive conclusion can be drawn actually about the origin of this cooperativity.

Structural determinants recognised by S15

What are the determinants recognised by S15 in the pseudoknot? The fact that S15 protects bases against the chemical probes cannot be used as a definitive indicator that changes in conformation linked to the S15 binding may alter their reactivities. In fact, protected nucleotides such as the bulging $U_{(-53)}$ or the initiation codon can be deleted or displaced without effect on autoregulation. On the other hand, genetic data provide some clues derived from compensatory mutations or deletions not altering the autocontrol. All the base-pair changes in the pseudoknot which do not alter the autocontrol indicate that the corresponding wild type nucleotides do not constitute sequence specific determinants. Unexpectedly, this means that despite the necessity of a pseudoknot for recognition, no determinants are present in the sequence of stem 2 (fig 5) [14, 16]. On the other hand, loop 2 is likely not to be involved in recognition, being protected by the ribosomal 30S subunit. As for the two nucleotides of loop 1, chemical and genetic data show they occupy different positions in the pseudoknot. $A_{(-47)}$ is highly reactive in the absence of S15, but unreactive after S15 binding, suggesting S15 protection. However, genetic data show that it can be exchanged for G without loss of autoregulation as long as base pairing with the nucleotide at position (–38) is satisfied. Thus, the protection observed corresponds to A-U base-pairing and not from S15 protection. Adjacent $A_{(-46)}$ is always moderately reactive, even in the absence of S15, suggesting it is buried in the major groove. Its protection by S15 might correspond to shielding. It can be exchanged for a G but not for a pyrimidine or deleted because the extension of stem 1 and the reduction of the loop of hairpin I prevent pseudoknot formation [18].

Unexpectedly, mutations in stem 1 changing $U_{(-49)}$●G to C-G abolish the autoregulation [16] (fig 5). Thus, restoring perfect base pairing induces loss of autoregulation, suggesting that U●G is specifically recognized by S15. However, to explain that this base-pair is not recognised in strains carrying mutations preventing pseudoknot formation, it is necessary to admit that the formation of a pseudoknot is required because either it carries another binding site or/and it provides a correct adjustment of the U●G base-pair.

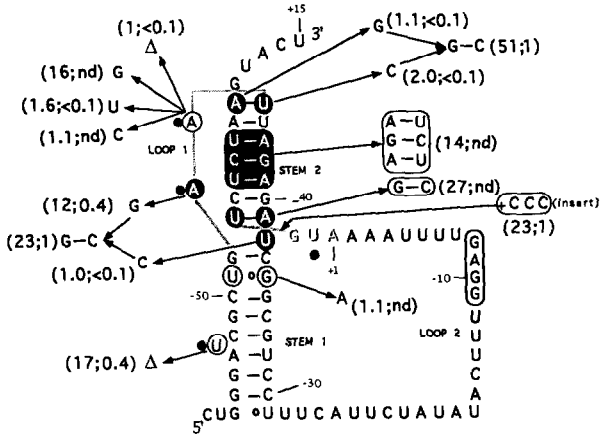

Fig 5. Mutational analysis of the pseudoknot. Several mutations were introduced in the pseudoknot and their effects analysed *in vivo* by measuring the repression level in the presence of an excess of S15 and *in vitro* by measuring the dissociation constant between S15 and the mutated RNA. Repression ratio for the wild type corresponds to 22 and the binding constant to 1. Arrows indicates the nucleotide(s) changed or deleted (Δ). Between brackets are written respectively the repression ratio and the binding strength. The mutated nucleotides are circled and the nucleotides shown to be base-paired by complementary mutations are circled in grey. A triplet insertion is indicated by +.

Otherwise, it has been shown that the formation of the pseudoknot *per se* does not allow S15 binding as illustrated by a mutant exhibiting C instead of $U_{(-38)}$ in the spacer between the stem. This mutant is able to form a pseudoknot *in vitro*, but does not autoregulate [18]. The pseudoknot formed is probably different because the spacer nucleotide cannot form a canonical Watson-Crick base-pair and presumably does not allow correct stacking of the two stems. Thus, the pseudoknot conformation must satisfy several rules before S15 can recognise its target and bind: 1) the U•G determinant must be present; 2) a pseudoknot has to form, accounting for the presence of stem 2; and 3) complementarity between position (–47) of loop 1 and (–38) of the spacer is required leading to stacking of the stems and to the formation of loop 1 restricted to only one nucleotide. This last condition predicts that a pseudoknot exhibiting only seven nucleotides in stem 2 is needed to allow only one nucleotide to cross the deep major groove. Then, a constrained quasi-continuous helix of 17 base pairs would result which would carry the determinants recognised by S15 (fig 6).

Model of S15 binding to the pseudoknot

From the rules derived above, it appears that some mutations which do not comply to these rules can be tolerated even if they have as a consequence to induce the formation of slightly different pseudoknots. This conclusion accounts for the detection *in vitro* of several conformations of the pseudoknot, with either one or two nucleotides in loop 1 and correlative variation of ± 1 base pairs in stem 1 and 2. However, S15 binding is able to stabilise the formation of a unique conformation constituting two stacked stems of 17

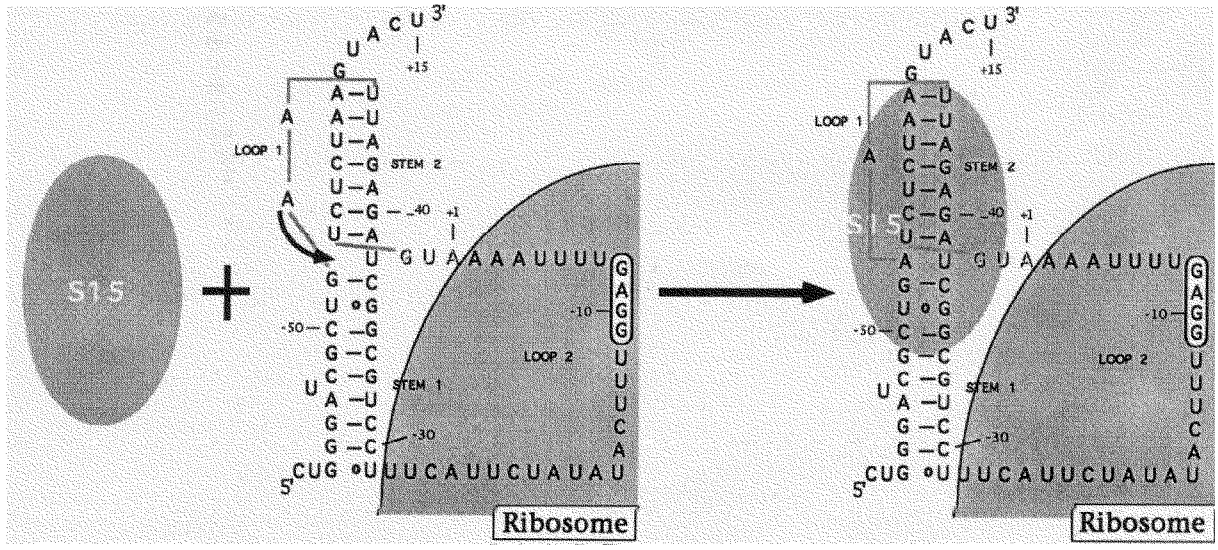

Fig 6. Conformational change in the pseudoknot linked to S15 binding. Genetic evidence indicates that $A_{(-47)}$ in loop 1 pairs with $U_{(-38)}$ in the spacer.

base pairs long, with only one nucleotide crossing the major groove. Fe(II)-EDTA footprinting experiments, which generate free hydroxyl radicals able to attack the ribose moiety of the backbone show that S15 protects essentially one strand from positions (–42) to (–49), a region encompassing the upper part of stem 1, stem 2 and loop 1. Further protection was detected at +10 in the other strand, corresponding to the upper part of stem 2 and also minor protection around the initiation codon in loop 2 [18]. As this probe is insensitive to secondary structure, it can be concluded that the protection observed does not correspond to some rearrangement linked to S15 binding but to a true shielding by the ribosomal protein. Protection at (–49) means that $U_{(-49)}$ of the predicted U•G determinant might interact with the protein or at least that it is within its close proximity as is also $U_{(-45)}$ which is known not to interact with S15. No clear answer can be given for $A_{(-46)}$ either. It might interact directly or might be simply buried in the deep narrow groove of stem 2. A 3D model was derived from these data [18]. In this model, S15 sits in the deep groove of the coaxial stack, especially in the region of the loop that displays the sharp turn of the ribose phosphate backbone making contacts with both strands [18]. S15 might recognise a specific and unique conformation of the sugar-phosphate backbone that is provided by the pseudoknot or interact non-specifically in this area. Interactions in the minor groove may occur with base $U_{(-49)}$, but likely not with the bulging $U_{(-53)}$. Thus, the apparent protection of this residue by S15 should be considered as a local rearrangement.

The S15 ribosomal site

The S15 ribosomal binding site is located in the 16S RNA central domain and is constituted essentially by the 655–672/734–751 irregular helix (fig 7). Interestingly, this helix contains 17 base pairs, the same number as the pseudoknotted operator. The nucleotides specifically protected from hydroxyl radicals are located in two subsites: a major, at the bottom of the stem and a minor one, in the centre of the helix, overlapping a site specifically protected from base specific chemicals probes (residues 666–668/740–741) [22]. In addition, no other ribosomal protein binding interferes with these regions, suggesting that S15 might interact specifically with these two subsites. Comparisons between the messenger and 16S RNA sites show several striking features. A U•G pair is common to both sites near the centre of the helix, adjacent to either the non-canonical base pairs in the 16S site or the stem junction in the pseudoknot. It might correspond to a common subsite for both sites. In addition, in the messenger, some nucleotides protected ($U_{(-44,-45)}$) are located at the same distance from the common U•G motif as $G_{(656)}$,$U_{(657)}$ in the ribosomal site (fig 7). Whether it is coincidental or if it corresponds to similar points of interaction is an open question. No features similar or identical between

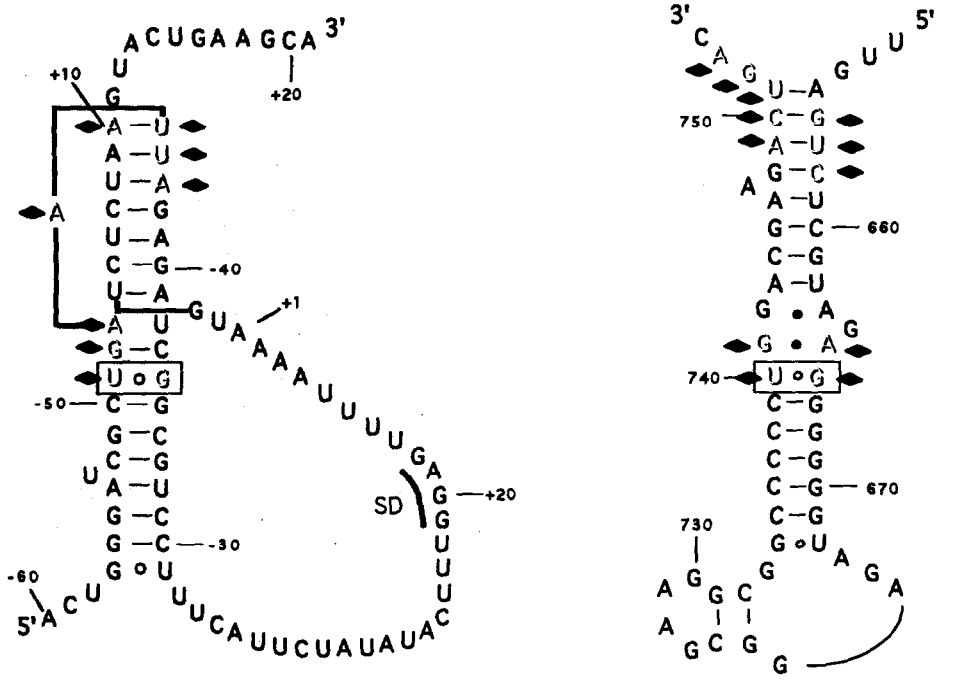

Fig 7. Comparison between the stacked pseudoknot (at the left) and the 16S ribosomal binding site (on the right). For easier comparison, the ribosomal fragment has been set upside down. Black diamonds show the ribose residues protected by S15 after hydroxyl radical footprinting. For the messenger, protections common to mutants CFP5517 and LB6 are indicated [18]. Data for the 16S were taken from Noller's work [22]. The U•G pair common to both sites is framed.

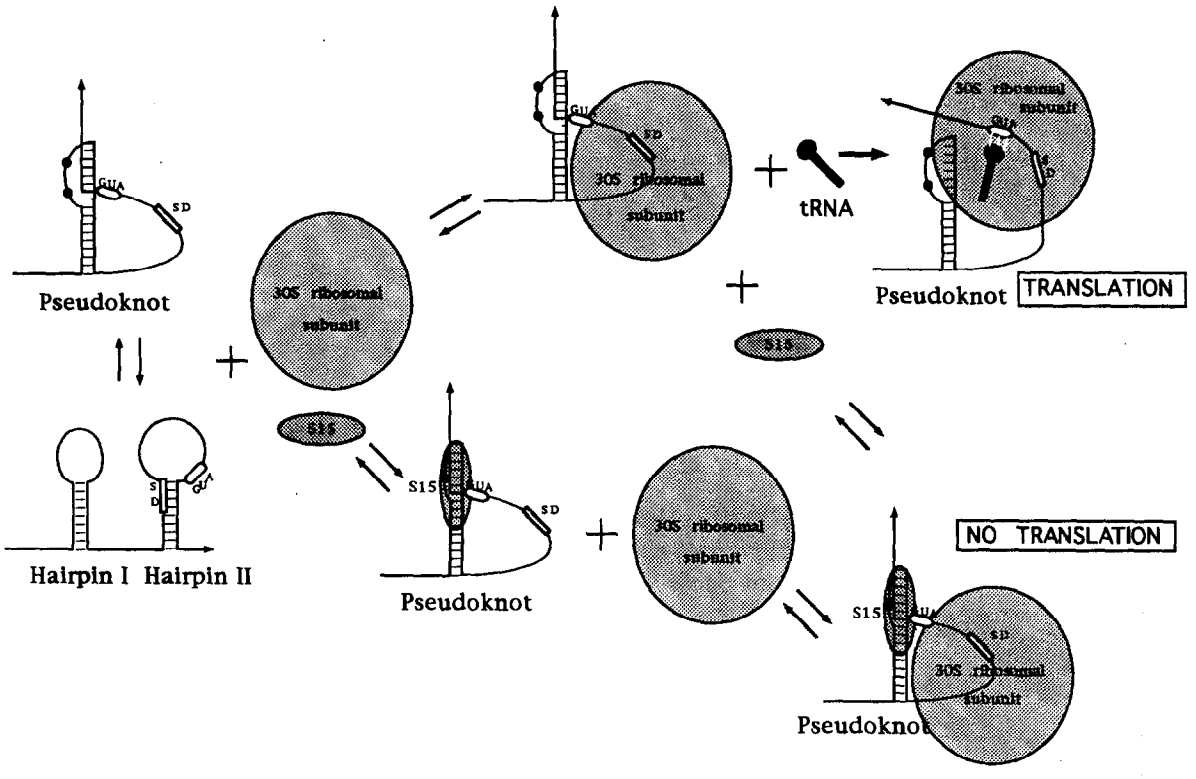

Fig 8. Model of the mechanism used for autoregulation of S15 synthesis. At the left, the messenger operator in its two conformations. The 30S subunit binds efficiently to the pseudoknot because the Shine-Dalgarno sequence is presumed to be fully exposed. One can imagine that, after binding, if the concentration of free S15 is low, the pseudoknot opens, the initiation complex is formed and translation can proceed. In the presence of S15, the pseudoknot stability is increased drastically by S15 binding and pseudoknot transconformation and translation initiation is blocked. Note that once the active ternary complex is formed (after melting the pseudoknot), S15 is unable to bind and inhibit translation.

the corresponding regions are apparent, except for their position relative to U•G. Site directed mutagenesis on both 16S RNA and the S15 protein might help to clarify this point. It is worth noting that although two sets of RNA contacts can exist in S4 protein for proper messenger and 16S RNA binding, only one would specifically interact with RNA [23, 24].

Conclusion

Model of translational control

After its synthesis, the *rpsO* message can adopt two conformations exhibiting either a pseudoknot or two hairpins. The pseudoknot appears to be the fully active species for translation because it recruits efficiently 30S ribosomal subunits thanks to its presumed fully exposed Shine-Dalgarno sequence. What is the function of hairpin II? A possible function is to increase the translation rate and autoregulation by preventing the ribosome from binding outside of the pseudoknot by masking the Shine-Dalgarno sequence. One can imagine that an equilibrium between a pseudoknot and an unstructured messenger would permit the formation of an initiation complex independently of the pseudoknot formation, thus escaping from regulation. By combining an optimal stability of the pseudoknot with high efficiency of ribosomal binding, this pseudoknot is able to initiate translation actively because after 30S binding and the ternary initiation complex can be formed easily through pseudoknot disruption. On the other hand, by keeping the same structure for ribosome and S15 binding, a cooperativity is developed which gives a high translational control efficiency. Thus, the messenger reconciles contradictory requirements for two opposed functions and a precise equilibrium must be conserved for correct regulation. If the binding rate of ribosomes to the Shine-Dalgarno sequence is altered, all the regulation is perturbed. For example, increasing the binding of 30S by improving the complementarity of the Shine-Dalgarno sequence with 16S RNA stabilises the binary com-

plex and stimulates translation whereas repression is decreased. Conversely, increasing the ribosome dissociation rate increases autoregulation efficiency by allowing a decrease in the rate of the ternary initiation complex.

In the entrapment model proposed, the S15 message is actively translated until the free concentration of S15 reaches a concentration high enough to bind to the pseudoknot. S15 is able to recognise a specific structure created on the pseudoknot, probably even after ribosome binding. The two unstacked stems would stack after base-pairing at the spacer position, forming a seventeen base-pair quasi-continuous helix and it is this structure, stabilised by S15, which would prevent the productive initiation complex formation. The role of the junction between the two helices appears crucial as already observed for frameshift pseudoknots. It probably provides a correct positioning of the sugar phosphate backbone, allowing specific recognition of the U•G base pair.

The efficiency of autoregulation is linked to the free concentration of S15, which in turn is dependent of the ribosomal RNA synthesis rate. Competition for ribosomal or messenger binding is essential for the co-ordination of ribosome synthesis. How this competition does occur, involving a more or less extended interaction for each binding site or two different subsites remains still undetermined.

From this model, it can be concluded that autoregulation is a finely tuned mechanism which requires not only specific and limited interactions between quite different molecules but also sophisticated changes of RNA conformation illustrating the high plasticity of this nucleic acid and the importance of the kinetics for RNA folding [25]. The pseudoknot formation plays a central role in this mechanism, being able both to recruit ribosome for translation initiation and to create a specific binding site for S15. Whether the determinants carried by this site share identity with the ribosomal site remains a tantalising unsolved question.

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