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Symmetrical Interactions between the Translational Operator of the *thrS* Gene and Dimeric Threonyl Transfer RNA Synthetase

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Threonyl-tRNA synthetase from *Escherichia coli* represses the translation of its coding gene, *thrS*, by binding an operator located in the leader region of its messenger RNA. Published data on the structure of this leader region and on its interaction with threonyl-tRNA synthetase support a model in which each of two stem-and-loop structures mimics the anticodon arm of $tRNA^{Thr}$ and binds a different subunit of one synthetase dimer.

Keywords: regulation; translation; aminoacyl-transfer RNA synthetase; tRNA-like structure

The expression of the *thrS* gene, coding for threonyl-tRNA synthetase (ThrTS), is negatively controlled at the translational level in *Escherichia coli*. ThrTS binds a region of the *thrS* mRNA that is located upstream from the ribosome binding site. The binding of ThrTS to this leader region of the mRNA inhibits the initiation of translation (Brunel *et al.*, 1992).

An RNA fragment, covering the regulatory region of the *thrS* gene, has been synthesized *in vitro* and its structure has been analysed with enzymic and chemical probes. The leader mRNA folds into four well-defined domains of secondary structure (Fig. 1). Domain I comprises both ends of the leader mRNA (residues +3 to −12 and residues −118 to −131, taking the first nucleotide of the initiation codon of *thrS* as residue +1); these ends pair and form an unstable duplex of RNA. Domain I contains the ribosome binding site and the translation initiation codon of *thrS*. Domain II (residues −13 to −49) forms a stem-and-loop structure that is homologous to the anticodon stem-and-loop of $tRNA^{Thr}$ at the sequence level (Fig. 2). Domain III (−50 to −73) is a large loop, with little organization. Domain IV (−74 to −117) forms a stable stem-and-loop structure (Moine *et al.*, 1988).

ThrTS protects domains II and IV of the leader mRNA against enzymic or chemical attacks in footprinting experiments. $tRNA^{Thr}$ competes with the leader mRNA for the binding of ThrTS in experiments of retention on nitrocellulose filters. In turn, ThrTS competes with the ribosome for the binding of the leader mRNA (Moine *et al.*, 1990).

Similar experiments, performed with leader mRNAs that carry a constitutive mutation in domain II (i.e. a mutation abolishing the control of the *thrS* gene by ThrTS), have shown that the structure of domain IV is preserved when the structure of domain II is altered by the mutation (Moine *et al.*, 1988). Domain IV can still bind ThrTS, even though the mutant domain II is unable to do so. $tRNA^{Thr}$ still competes with the mutant mRNA for the binding of ThrTS but the synthetase does not compete any more with the ribosome for the binding of the mutant mRNA (Moine *et al.*, 1990).

A thorough genetic analysis of the leader mRNA has led to the following results and conclusions. Mutations of domain II eliminate or strongly diminish the control of the *thrS* gene by ThrTS. Domain II binds ThrTS or other aminoacyl-tRNA synthetases according to the same identity rules as the anticodon arm of tRNAs. The mutations of domains III and IV decrease but do not abolish the control of *thrS* by ThrTS. Domain III acts as an articulation between domains II and IV (Graffe *et al.*, 1992; Brunel *et al.*, 1992).

On the basis of the data summarized above and of sequence homologies between domain IV and the acceptor arm of $tRNA^{Thr}$, Springer, Ehresmann, Grunberg-Manago and co-workers have proposed that the leader mRNA of *thrS* folds into a structure that is similar to that of $tRNA^{Thr}$, domains II and IV being the equivalents of the anticodon and acceptor arms of $tRNA^{Thr}$, respectively (Graffe *et al.*, 1992; Brunel *et al.*, 1992). In this work, I show that the published data support a different model in

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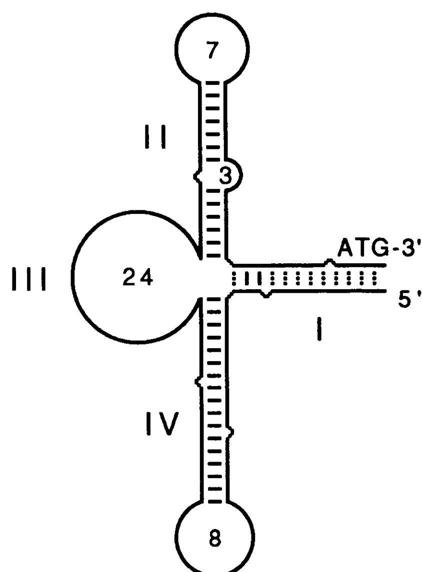

Figure 1. Structure of the leader region of the *thrS* mRNA. The structure can be divided into 4 distinct domains (I to IV). The intra-strand base-pairings are represented by plain or dotted bars, according to their stability. The sizes of the loops are indicated in Arabic numerals. Adapted from Moine *et al.* (1988).

which both domains II and IV mimic the anticodon arm of $tRNA^{Thr}$ and interact with the two subunits of the ThrTS dimer.

First, a comparison of the mutagenesis data on the *thrS* mRNA (Graffe *et al.*, 1992; Brunel *et al.*, 1992) with those on $tRNA^{Thr}$ (Hasegawa *et al.*, 1992) disproves the model of functional equivalence between domain IV of the leader mRNA and the acceptor arm of $tRNA^{Thr}$ that was proposed by Springer and co-workers. Mutation JC(5+6) of the leader mRNA includes the change of base-pair $C^{-79}\cdot G^{-112}$ into $G^{-79}\cdot C^{-112}$. According to the model, this change in domain IV is equivalent to the change of base-pair $C^2\cdot G^{71}$ into $G^2\cdot C^{71}$ in the acceptor arm of $tRNA^{Thr}$. In fact, mutation JC(5+6) has no effect on the control of *thrS* (Brunel *et al.*, 1992), whereas mutation $G^2\cdot C^{71}$ strongly affects the kinetic parameters for the threonylation of $tRNA^{Thr}$ by ThrTS: K_M is increased 34 times by the mutation and V_{max} is decreased 17 times (Hasegawa *et al.*, 1992). According to the model, mutations X-18-II-1, BS3-2 and BS3-3 of the leader mRNA change residues of domain IV that have no equivalent in the acceptor arm of $tRNA^{Thr}$. In fact, these three mutations affect the control of *thrS* by ThrTS (Fig. 2; Brunel *et al.*, 1992). The data on $tRNA^{Thr}$ show that mutations of both acceptor and anticodon arms strongly increase the K_M parameter in the aminoacylation reaction and that the mutations of the acceptor arm have a lesser effect on V_{max} than the mutations of the anticodon arm (Hasegawa *et al.*, 1992). Thus, the data do not support the hypothesis that was put forward by Springer and co-workers to explain the lack of effect of mutation JC(5+6) on the control of *thrS*: these authors proposed that the identity elements of $tRNA^{Thr}$ that are located in the acceptor arm would be important for catalysis but not for binding in the interaction between $tRNA^{Thr}$ and ThrTS (Brunel *et al.*, 1992).

In contrast, the mutagenesis data are compatible with an equivalence between domain II of the leader mRNA and the anticodon arm of $tRNA^{Thr}$ (Fig. 2). Mutations X-18-2, VII-5 and M1-11 of the leader mRNA, which change the central nucleotide G^{-32} in the loop of domain II, abolish the control of *thrS* (Brunel *et al.*, 1992). The equivalent mutations of $tRNA^{Thr}$, which change the central nucleotide G^{35} of the anticodon, decrease at least 2000 times the k_{cat}/K_M parameter for the threonylation of $tRNA^{Thr}$ by ThrTS (Hasegawa *et al.*, 1992).

Instead of an equivalence between domain IV of the *thrS* mRNA and the acceptor arm of $tRNA^{Thr}$, I propose an equivalence between domain IV and the anticodon arm (Fig. 2). The main argument against my proposal comes from the size of the domain IV loop. It has eight residues when the anticodon loops of tRNAs always comprise seven residues. The following arguments support my proposal.

The anticodon loops of tRNAs and their size are important for their interactions with the mRNAs and the ribosome but not necessarily for their interactions with the aminoacyl-tRNA synthetases. Synthetic mini-tRNAs (for Ala, His or Gly), comprising only the acceptor arm of the normal tRNAs, can be specifically charged by the cognate aminoacyl-tRNA synthetases (Francklyn *et al.*, 1992). The genomic RNA of Brome mosaic virus is charged with tyrosine by yeast tyrosyl-tRNA synthetase even though this RNA has no equivalent of the anticodon loop (Dreher & Hall, 1988) and the anticodon is an identity element for yeast $tRNA^{Tyr}$ (Bare & Uhlenbeck, 1985). An *E. coli* $tRNA^{Gly}$ can become a suppressor of missense mutations by insertion of an extra nucleotide in its anticodon loop and shifting of the anticodon triplet (Murgola *et al.*, 1983).

All the nucleotides in the loop of domain IV (residues −92 to −99) are highly reactive towards chemical probes, except G^{-92} and A^{-99} at N-1 and N-7 respectively. Domain IV is cleaved by RNase V1, which is specific for the paired or stacked regions of RNA, between residues U^{-98} and A^{-99}, and between A^{-99} and C^{-100}. These data are compatible with the existence of either a non-canonical base-pair, involving A^{-99} (N-7, N-6) and G^{-92} (N-1, O-6), or with a stacking of base A^{-99} on the helix of domain IV (Moine *et al.*, 1988). The abnormal pairing of G^{-92} with A^{-99} or the stacking of A^{-99} could confer a structure to the loop of domain IV, close to that of an anticodon loop, and enable a proper interaction of the domain IV loop with ThrTS. Compatible with this hypothesis, the interaction of the leader mRNA with ThrTS increases the above-mentioned cleavages by RNase V1 (Moine *et al.*, 1990).

As recalled above, the loop of domain II (residues

tRNA^Thr^		5'	***	3'		k_{cat}/K_M (relative)
Thr1(wt):	27	CACCC	UU**GGU**AA	GGGUG	43	-
Thr2(wt):		GCGCA	UU**CGU**AA	UGCGA		-
Thr4(wt):		ACUGA	CU**UGU**AA	UCAGU		-
Thr3(wt):		CACCC	UU**GGU**AA	GGGUG		1.0
Thr3(GUU):		.....	...**U**...	.....		4.6×10^{-4}
Thr3(GAU):		.....	...**A**...	.....		$<1.0\times10^{-5}$
Thr3(CAU):		.....	..**CA**...	.....		$<1.0\times10^{-5}$
Thr3(UUU):		.....	..**UU**...	.....		$<1.0\times10^{-6}$
Thr3(CCU):		.....	..**CC**...	.....		$<1.0\times10^{-6}$
Domain II						**Control**
II(wt):	-40	GAUCU	UU**CGU**GU	GGGUC	-24	wt
II(X-18-2):		.....	...**A**...	.....		eliminated
II(M1-11):		.....	...**U**...	.....		eliminated
II(VII-5):		.....	...**C**...	.....		eliminated
II(B3):		....A	....	.....		str. decreased
II(X-18-II-4):		.....	.......G	.....		str. decreased
Domain IV						**Control**
IV(wt):	-104	UUAGC	AUU**UGU**UG	GCUAG	-87	wt
IV(BS3-2):		.....	....**A**...	.....		decreased
IV(X-18-II-1):		.....	....**U**...	.....		decreased
IV(BS3-3):		.....	....	A....		decreased

Figure 2. Comparison of domains II and IV with the anticodon stem-and-loop of tRNA^Thr^. The anticodon sequences are written in bold letters and their positions are indicated by the symbol ***. The nucleotides belonging to the stem structures are underlined. For the mutant molecules, only the mutated residues are written and the unchanged residues are represented by dots. wt, wild-type. *Upper part*: The sequences for the 4 isoaccepting species of *E. coli* tRNA^Thr^ are listed (Komine *et al.* 1990; Komine & Inokuchi, 1992). The relative k_{cat}/K_M parameters for the threonylation of mutated $tRNA_3^{Thr}$ by ThrTS are given in the last column (Hasegawa *et al.*, 1992). *Middle part*: Stem-and-loop of domain II. *Lower part*: Stem-and-loop of domain IV. The effects of the mutations in domains II and IV on the control of the *thrS* gene by ThrTS are given in the last column (Springer *et al.*, 1986; Brunel *et al.*, 1992).

-29 to -35) is composed of seven residues and is equivalent to the anticodon loop of tRNA^Thr^. The insertion of an additional residue into the loop of domain II, between G^{-28} and U^{-29} as in mutation X-18-II-4, lengthens the loop to eight residues. The change of pair $G^{-28}\cdot U^{-36}$, which closes the loop of domain II, into $G^{-28}\cdot A^{-36}$ as in mutation B3, lengthens the loop to nine residues. Both mutations strongly diminish but do not abolish the control of *thrS* by ThrTS (Fig. 2; Brunel *et al.*, 1992).

If one disregards the additional residue, the stem and the loop of domain IV are strongly homologous with the anticodon arm of tRNA^Thr^ at the sequence level. In particular, domain IV carries the anticodon for threonine, $U^{-94}G^{-95}U^{-96}$, in the middle of its loop (Fig. 2).

The equivalence between domain IV of the leader mRNA and the anticodon of tRNA^Thr^ is further supported by the properties of the mutations of this domain (Fig. 2). The changes of residue G^{-95}, which is equivalent to the central nucleotide of the anticodon, into A or U as in mutations BS3-2 and X-18-II-1, decrease the control of *thrS* by ThrTS. These two mutations have as strong an effect on the control of *thrS* as a large deletion, Δ2, of domain IV, which removes residues -83 to -105 (Brunel *et al.*, 1992). As mentioned above, the changes of G^{35}, the central nucleotide in the anticodon of tRNA^Thr^, strongly affect the kinetic parameters for the threonylation of tRNA^Thr^ by ThrTS (Hasegawa *et al.*, 1992). Thus, the mutations in the loop of domain IV and in the anticodon of tRNA^Thr^ have analogous effects on the regulation of *thrS* and on the charging of tRNA^Thr^.

The similarities between domains II and IV suggest a common mode of interaction with ThrTS, whereas their different roles in the regulation of the *thrS* gene seem mainly related to their respective positions relatively to the site of translation initiation for this gene. Domain II and domain IV each form a stem-and-loop structure. The loops of domains II and IV have seven and eight residues, respectively, and both resemble the anticodon loop of tRNA^Thr^. The stems of domains II and IV have similar numbers of base-pairs: 13 for the stem of domain II, which is interrupted by a loop of three residues, and 17 for the stem of domain IV. The two domains can be arranged symmetrically with respect to domains I and III (Fig. 1). ThrTS protects both domains II and IV against enzymic and chemical attacks. The protections of the two loops have exactly the same dependence towards

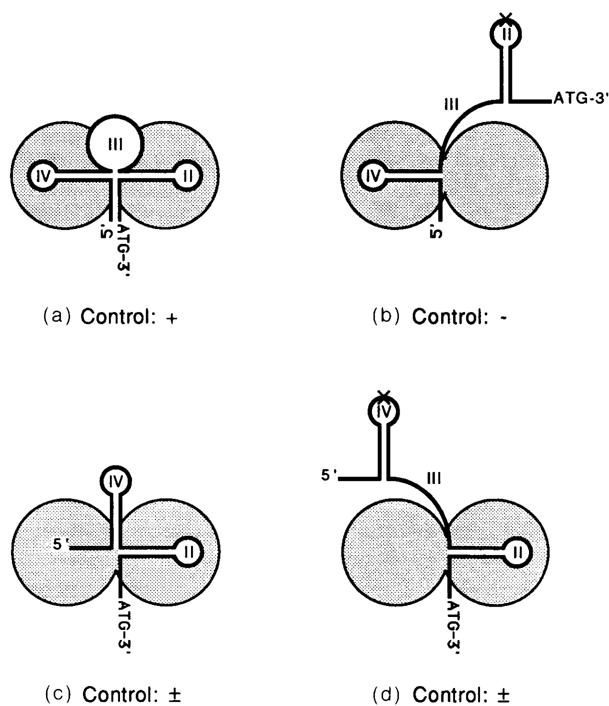

Figure 3. A model of interaction between the leader mRNA of *thrS* and threonyl-tRNA synthetase. The 2 identical subunits of ThrTS are represented by 2 shaded spheres. The stem-and-loop structures of domains II and IV mimic the anticodon stem and loop of $tRNA^{Thr}$ and interact symmetrically with the 2 subunits of the dimeric ThrTS. In (a), the leader mRNA has the wild-type; in (b), it carries a constitutive mutation in the loop of domain II, indicated by the symbol X; in (c), it carries a deletion of loop III and, in (d), a mutation in the loop of domain IV. The symbols +, ± and −, indicate the effects of the mutations on the control of *thrS* by ThrTS. Note that this representation is schematic and makes no hypothesis on the symmetries really at play. For simplicity, the synthetase and leader mRNA are represented with 2-fold symmetry or pseudo-symmetry axes perpendicular to the plane of the Figure, and with the 2 active sites of the synthetase on the same face of the molecule, as in tyrosyl-tRNA synthetase from *B. stearothermophilus*.

the concentration of ThrTS (Moine *et al.*, 1990), which suggests that the energies of interaction between ThrTS and the two loops are identical. Mutations in equivalent positions of domains II and IV have similar effects on the control of *thrS* by ThrTS, except that the mutations of domain II have stronger effects than those of domain IV (Fig. 2).

Toeprint experiments have shown that, when domain II is altered by mutation and cannot bind anymore ThrTS but domain IV is intact and active for binding, ThrTS does not compete anymore with the ribosome for the binding of the mutant mRNA (Moine *et al.*, 1990). Thus domain II, which is closer to the site of translation initiation, seems to prevail on domain IV for the control of *thrS*. A mutation in domain II can abolish the control of *thrS*, whereas a mutation in domain IV only decreases but does not abolish the control. Thus, domain IV seems to strengthen the activity of domain II. Finally, large alterations of domain III, in particular deletions, have similar effects on the control of *thrS* as mutations of domain IV, even though domain III does not directly bind ThrTS. Thus, domain III could constitute an articulation between domains II and IV, responsible for their relative positionings (Moine *et al.*, 1990; Brunel *et al.*, 1992).

The model of interaction between the leader mRNA of *thrS* and ThrTS that I propose, is a direct consequence of the above considerations and of the quaternary structure of ThrTS (Fig. 3). This aminoacyl-tRNA synthetase is a dimer of type $\alpha 2$ (Hennecke *et al.*, 1977). One molecule of ThrTS thus possesses two identical sites for binding the anticodon arm of $tRNA^{Thr}$, one on each subunit. In the model, the stem-and-loop of domain II binds one of these two sites and the stem-and-loop of domain IV binds the other one. Domains II and IV of the same mRNA molecule thus interact with the two subunits of one ThrTS molecule (Fig. 3(a)). The simultaneous binding of both domains II and IV to the two subunits of one ThrTS molecule strengthens the affinity of the leader mRNA for ThrTS and thus the repression of the *thrS* gene. When a mutation of domain II prevents its binding to one subunit of ThrTS, domain IV remains able to interact with the anticodon binding site of the other subunit, in competition with authentic $tRNA^{Thr}$. The mutated domain II remains unbound and free, and thus ThrTS does not prevent anymore the access of the ribosome to the adjacent site of translation initiation: repression is abolished (Fig. 3(b)). When a mutation of domain IV prevents its binding to ThrTS, domain II can still bind but with a lower overall affinity: repression is decreased (Fig. 3(d)). The deletions of domain III prevent the correct positioning of domain IV relative to domain II. In this case, the two stem-and-loop structures cannot simultaneously bind the two subunits of ThrTS and the control is also decreased (Fig. 3(c)).

The three-dimensional structure of ThrTS is unknown and thus also the spatial relationship that links its two subunits and the two corresponding active sites. The loop of domain III, which has 24 residues and little structural organization, could constitute a hinge sufficiently long and flexible to let domains II and IV reach the two anticodon binding sites, whether these sites are located on the same face of the synthetase molecule, as in tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* (Bedouelle & Winter, 1986; see Fig. 3), or on different faces, as in yeast aspartyl-tRNA synthetase (Ruff *et al.*, 1991).

This new model of interaction does not involve the binding site for the acceptor arm of $tRNA^{Thr}$ at the surface of ThrTS. It is compatible with the existing data, the observation that most regulatory proteins are dimeric and most operators, symmetrical, and with the general principal that natural evolution prefers to duplicate existing structures than create more complex ones. The model suggests

simple predictions that could be tested by mutagenesis of the leader mRNA.

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