Volume 283, number 1, 1–3 FEBS 09726 May 1991

ADONIS 001457939100435E

Discussion Letter

Aminoacyl-tRNA synthetases and DNA replication

Molecular mimicry between RNAII and $tRNA^{Lys}$

Marc Mirande

Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France

Received 14 January 1991

Recent data pertaining to different research areas, aminoacyl-tRNA synthetases and replication of ColE1 plasmids, have provided mutually attractive prospects. The gene encoding *Escherichia coli* lysyl-tRNA synthetase was first isolated as a host suppressor mutation that restores replication of a mutant ColE1 replicon. Comparison of RNAII and $tRNA^{Lys}$ suggests that lysyl-tRNA synthetase is involved in the formation of the displacement loop required for ColE1 plasmids replication and provides major identity elements of $tRNA^{Lys}$.

Lysyl-tRNA synthetase; ColE1 replicon; tRNA identity; Molecular mimicry

1. INTRODUCTION

Aminoacyl-tRNA synthetases play a key role in protein biosynthesis. They ensure the accuracy of aminoacylation of a tRNA species by the appropriate amino acid. Nucleic acid–protein interactions mediate specific recognition of a tRNA by the corresponding aminoacyl-tRNA synthetase. Molecular features governing the recognition process implicate nucleotide determinants which define tRNA identity [1]. The major identity determinant is a single base pair of $tRNA^{Ala}$ (G3:U70 in the acceptor stem [2,3]), the anticodon of $tRNA^{Met}$ or $tRNA^{Val}$ [4], or a combination of nucleotides [1,5].

The initiation of replication of ColE1 replicons is a complex, not fully understood process involving several proteins and RNA transcripts [6]. Transcription of RNAII starts at position −555 and proceeds towards the *ori* site where replication initiation takes place. The formation of a RNAII–DNA hybrid is required for the opening of a displacement loop, generating a single-strand DNA region suited for DNA replication. Several point mutations impairing ColE1 DNA replication affect ColE1 primer transcript (RNAII) conformation [7]. The replication of one of these ColE1 mutants, *cer114*, can be restored by a host mutation called *herC180* [8]. The wild-type *Escherichia coli herC* gene was cloned and sequenced [9] but the function of the HerC protein was not deciphered.

Correspondence address: M. Mirande, Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France. Fax: (1) (69) 823129

2. *herC* IS *lysS*; SIGNIFICANCE

Recently, the *lysS* gene, encoding the constitutive form of *Escherichia coli* lysyl-tRNA synthetase was sequenced [10] and shown to be identical to *herC* [10,11]. A second gene, *lysU*, encodes a heat-inducible lysyl-tRNA synthetase.

Interestingly, the *cer114* mutation, a single base pair substitution 95 bp upstream from the *ori* site [8], is found in a region of the non-transcribed strand of DNA that can be folded into a stem-loop structure closely mimicking that of a $tRNA^{Lys}$ molecule (Fig. 1). The T to C substitution destroys a TTT triplet. This anticodon-like structure is restored in a *rev20* revertant. In the same loop region, a *cer8* mutation [8] that creates a CTT anticodon (the other lysine anticodon) is very leaky. In addition, a mutation disrupting a base pairing in the anticodon stem (*pri9*: C to T) also affects replication of ColE1 replicons; a suppressor mutation (*spr91*: G to A) restores base pairing [7].

Collectively, these mutations lead to the proposal that the structural similarity between the clover leaf structure formed upstream of the *ori* site and $tRNA^{Lys}$ should be of functional significance.

The characteristic features of the *herC180* host suppressor mutation [8] provides compelling evidence in favor of the involvement of lysyl-tRNA synthetase in binding to this tRNA-like structure.

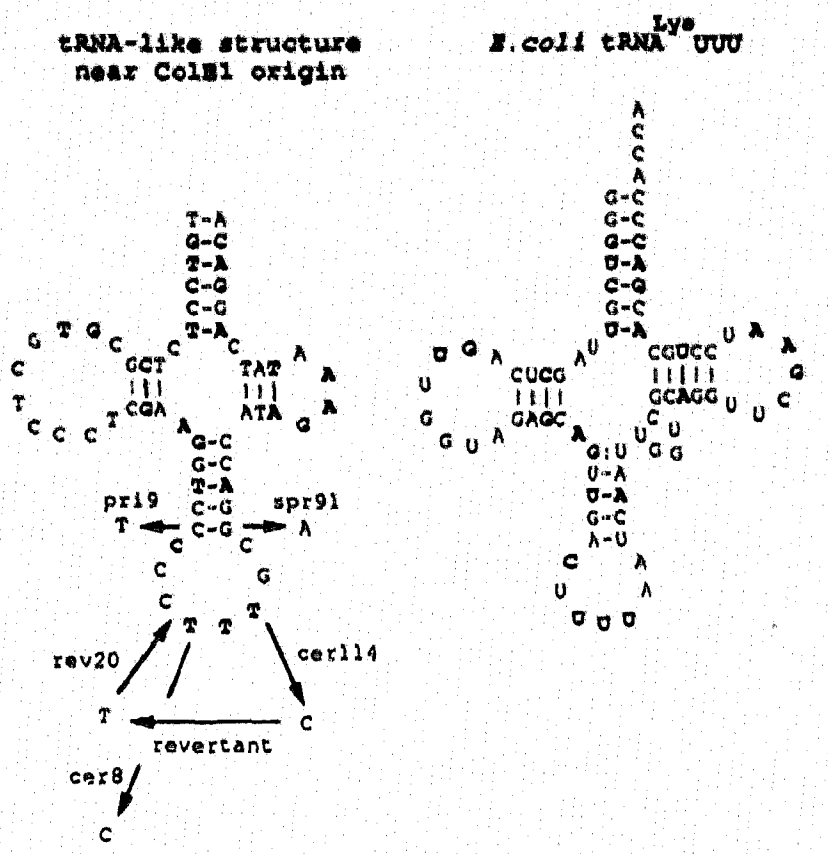

Fig. 1. Molecular mimicry between the non-transcribed strand of the ColE1 replication origin (position −71 to −129) and *E. coli* $tRNA^{Lys}$.

(i) *herC180* confers cold-sensitivity in growth. This mutation should result in a modified LysS protein unable to aminoacylate $tRNA^{Lys}$. At low temperature, the level of expression of the heat-inducible *lysU* gene must not be appropriate to provide protein synthesis with a sufficient amount of aminoacylated $tRNA^{Lys}$.

(ii) *herC180* is recessive to its wild-type allele, as assessed by complementation tests using a plasmid bearing the *herC* gene. Overproduction of *lysS* (*herC*) should result in the formation of three species of dimeric lysyl-tRNA synthetase: LysS-LysS, the major species, able to aminoacylate $tRNA^{Lys}$ and to maintain the wild-type replicon; LysS-HerC180, inactive for aminoacylation and plasmid maintenance, since a single tRNA molecule binds to the dimeric wild-type enzyme [12]; HerC180-HerC180, a minor species, present in a too low amount to ensure maintenance of the mutant replicon.

(iii) Both wild-type and *cer114* replicons are maintained in a *herC180* mutant. HerC180 (LysS mutant) should ensure replication of the mutant replicon and LysU that of the wild-type one.

3. *herC* IS *lysS*; IMPLICATIONS

The above observations are quite in accordance with the following proposals.

(1) Lysyl-tRNA synthetase is involved in ColE1 plasmid replication through binding to a tDNA clover leaf structure located in the lagging strand of the displacement loop. Its fixation is necessary for the opening of double-stranded DNA. In that connection, it must be noted that the *herC180* mutation restores replication of *cer114* replicons only in *rnh*⁻ strains. In the light of the modes of ColE1 replication in *rnh*⁺ and *rnh*⁻ strains [6], it seems likely that the HerC180 protein is less effective than HerC in the opening of a displacement loop, thus requiring a more extensive elongation of the RNAII-DNA hybrid.

(2) This model implies *de facto* that a $tDNA^{Lys}$ is efficiently recognized by lysyl-tRNA synthetase. That a $tDNA^{Lys}$ can be aminoacylated by *E. coli* lysyl-tRNA synthetase has already been demonstrated [13].

(3) The major determinants of $tRNA^{Lys}$ identity are shared with the clover leaf structure formed near the replication origin. As shown in Fig. 1, they are mainly clustered in the anticodon and in the amino acid acceptor stem. In particular, the G3:C70 base pair is conserved. A missense suppressor with a C70 to U70 substitution [14] is misacylated with alanine.

A similar case of molecular mimicry has been observed between $tRNA^{Thr}$ and the non-translated region of *thrS* mRNA [15]. It was clearly established that translational regulation of threonyl-tRNA synthetase

expression is controlled through binding of this enzyme to its mRNA.

In *Saccharomyces cerevisiae*, lysyl-tRNA synthetase is encoded by a single gene [16]. The reason for maintenance of a gene duplication in *Escherichia coli* could be due to the multiple cellular functions of the prokaryotic enzyme.

REFERENCES

[1] Normanly, J. and Abelson, J. (1989) Annu. Rev. Biochem. 58, 1029–1049.
[2] McClain, W.H. and Foss, K. (1988) Science 240, 793–796.
[3] Hou, Y.M. and Schimmel, P. (1988) Nature 333, 140–145.
[4] Schulman, L.H. and Pelka, H. (1988) Science 242, 765–768.
[5] Schimmel, P. (1989) Biochemistry 28, 2747–2759.
[6] Kües, U. and Stahl, U. (1989) Microbiol. Rev. 53, 491–516.
[7] Matsuka, H. and Tomizawa, J. (1986) Cell 44, 125–136.
[8] Kawakami, K., Naito, S., Inoue, N., Nakamura, Y., Ikeda, H. and Uchida, H. (1989) Mol. Gen. Genet. 219, 333–340.
[9] Kawakami, K., Jonsson, Y.H., Bjork, G.R., Ikeda, H. and Nakamura, Y. (1988) Proc. Natl. Acad. Sci. USA 85, 5620–5624.
[10] Lévêque, F., Plateau, P., Dessen, P. and Blanquet, S. (1990) Nucleic Acids Res. 18, 305–312.
[11] Gampel, A. and Tzagoloff, A. (1989) Proc. Natl. Acad. Sci. USA 86, 6023–6027.
[12] Ryme, L., Lagerkvist, U. and Wonacott, A. (1970) J. Biol. Chem. 245, 4308–4316.
[13] Khan, A.S. and Roe, B.A. (1988) Science 241, 74–79.
[14] Prather, N.E., Murgola, E.J. and Mims, B.H. (1984) J. Mol. Biol. 172, 177–184.
[15] Springer, M., Graffe, M., Dondon, J. and Grunberg-Manago, M. (1989) EMBO J. 8, 2417–2424.
[16] Mirande, M. and Waller, J.P. (1988) J. Biol. Chem. 263, 18443–18451.