

Aminoacyl-tRNA synthetase genes of *Bacillus subtilis*: organization and regulation

Martin Pelchat and Jacques Lapointe

Abstract: In *Bacillus subtilis*, 14 of the 24 genes encoding aminoacyl-tRNA synthetases (aaRS) are regulated by tRNA-mediated antitermination in response to starvation for their cognate amino acid. Their transcripts have an untranslated leader mRNA of about 300 nucleotides, including alternative and mutually exclusive terminator-antiterminator structures, just upstream from the translation initiation site. Following antitermination, some of these transcripts are cleaved leaving at the 5'-end of the mature mRNAs, stable secondary structures that can protect them against degradation. Although most *B. subtilis* aaRS genes are expressed as monocistronic mRNAs, the *gltX* gene encoding the glutamyl-tRNA synthetase is cotranscribed with *cysE* and *cysS* encoding serine acetyl-transferase and cysteinyl-tRNA synthetase, respectively. Transcription of *gltX* is not controlled by a tRNA, but tRNA^{Cys}-mediated antitermination regulates the elongation of transcription into *cysE* and *cysS*. The full-length *gltX-cysE-cysS* transcript is then cleaved into a monocistronic *gltX* mRNA and a *cysE-cysS* mRNA.

Key words: regulation, aminoacyl-tRNA synthetase, T-Box, processing.

Résumé : Chez *Bacillus subtilis*, 14 des 24 gènes d'aminoacyl-ARNt synthétases sont réglés par un mécanisme d'antitermination fonction du niveau d'aminoacylation de leur ARNt, en réponse à un manque de leur acide aminé substrat. Leurs transcrits ont une séquence 5' non traduite d'environ 300 nucléotides, qui inclut des structures alternatives et mutuellement exclusives de type terminateur-antitermineur situées juste en amont du site d'initiation de la traduction. Dans le cas d'une antitermination, plusieurs de ces ARNm sont coupés, ce qui laisse des structures secondaires stables à leur extrémité 5' pouvant les protéger contre la dégradation. Alors que plusieurs gènes d'aminoacyl-ARNt synthétases de *B. subtilis* sont exprimés sous la forme d'ARNm monocistroniques, le gène *gluX* codant la glutamyl-ARNt synthétase est cotranscrit avec *cysE* et *cysS*, codant respectivement la sérine acétyltransférase et la cystéinyl-ARNt synthétase. La transcription de *gluX* n'est pas réglée par son ARNt, mais une antitermination médiée par l'ARNt^{Cys} règle l'élongation de la transcription de *cysE* et *cysS*. Le transcrit pleine longueur de *gluX-cysE-cysS* est ensuite coupé en un ARNm monocistronique de *gluX* et en un ARNm de *cysE-cysS*.

Mots clés : régulation, aminoacyl-ARNt synthétase, boîte T, maturation.

Introduction

Protein biosynthesis plays a central role in the metabolism of all living organisms, consuming a major portion of their cellular energy (Ingraham et al. 1983). The numerous factors that catalyze this process include ribosomal proteins and RNAs, tRNAs, and aminoacyl-tRNA synthetases. In bacteria, the expression of these factors, encoded by more than 200 genes, must be efficient and coordinated. However, the mechanisms coordinating the expression of these genes and those involved in other metabolic pathways remain poorly

understood. The aminoacyl-tRNA synthetases (aaRS) play an important role in protein biosynthesis by catalyzing the accurate charging of tRNAs with their cognate amino acids. As with most macromolecules involved in protein biosynthesis, the aaRS are generally present in high levels, and these levels increase with growth rate, a phenomenon known as metabolic regulation (Neidhardt et al. 1977).

Organisation of aaRS genes in *Bacillus subtilis*

The determination of the nucleotide sequence of the *Bacillus subtilis* genome (4 214 814 bp) by Kunst et al. (1997) allowed the identification of 24 aaRS genes specific for 19 amino acids (Putzer 1997). In this organism, there is no glutaminyl-tRNA synthetase, but a single glutamyl-tRNA synthetase is responsible for aminoacylating both tRNA^{Glu} and tRNA^{Gln} with glutamate (Lapointe et al. 1986; Pelchat et al. 1998); tRNA^{Gln} is first charged with glutamate, which is subsequently converted to glutamine by an amidotransferase while it is attached to tRNA (Wilcox and Nirenberg 1968;

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Abbreviation: aaRS, aminoacyl-tRNA synthetases.

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Curnow et al. 1997). Two heterodimeric aaRSs are encoded by distinct but cotranscribed genes: glycyl-tRNA synthetase by *glyQ* and *glyS* and phenylalanyl-tRNA synthetase by *pheS* and *pheT*. There are also two distinct aaRSs for each of the following amino acids: threonine (encoded by *thrS* and *thrZ*), tyrosine (encoded by *tyrS* and *tyrZ*) and histidine (encoded by *hisS* and *hisZ*; *hisZ* is known only from sequence comparison).

Bacillus subtilis aaRS genes are generally grouped on the chromosome. This is in contrast to what is found in *Escherichia coli*, where aaRS genes are scattered around the chromosome (Grunberg-Manago 1996; Blattner et al. 1997). In *B. subtilis*, half of these genes (*glyQS*, *alaS*, *aspS*, *hisS*, *valS*, *pheST*, *tyrS*, *leuS*) are between 223° and 268° (0°/360° being the replication origin), five (*serS*, *metS*, *lysS*, *gltX*, *cysS*) are near the replication origin between 2° and 10°, and three (*argS*, *thrZ*, *tyrZ*) are situated between 328° and 337°. Furthermore, all *B. subtilis* aaRS genes, except *trpS* and *tyrZ*, are in the same orientation as the replication fork, which is different from what is found in *E. coli* (Grunberg-Manago 1996; Blattner et al. 1997). This orientation is typical of highly expressed genes, probably because there is less interference between their transcription and genome replication (French 1992).

Coexpression of aaRS genes

In prokaryotes, the genes encoding enzymes involved in the same pathway are often cotranscribed. There have been few known cases of cotranscribed aaRS genes. Two of them are the *gltX-cysE-cysS* operons of *B. subtilis* and *Staphylococcus xylosum*, in which the genes encoding two aaRSs specific for glutamate and cysteine, respectively, are separated by a gene encoding the first enzyme of cysteine biosynthesis (Gagnon et al. 1994; Fiegler and Brückner 1997). Other reported cases are the *Rhizobium meliloti gltX-lysS* operon (Pelchat et al. 1999), in which two aaRS genes (specific for glutamate and lysine respectively) are separated by 10 bp, and the *thrS-infC-rpmL-rplT-pheS-pheT-himA* cluster of *E. coli* in which the genes encoding the aaRSs specific for threonine (*thrS*) and phenylalanine (*pheS-pheT*), respectively, are separated by three genes (Springer et al. 1985; Butler et al. 1986; Lesage et al. 1990; for a review, see Grunberg-Manago 1996).

Transcriptional and posttranscriptional regulation of some *B. subtilis* aaRS genes

In contrast to what is found in *E. coli*, where different mechanisms are used to regulate the expression of various aaRS genes (Grunberg-Manago 1996), in *B. subtilis* and other Gram-positive bacteria, many of these genes and many amino acid biosynthetic operons are regulated by a common mechanism. Among the 24 aaRS genes in *B. subtilis*, 14 are controlled by tRNA-mediated antitermination of transcription in response to starvation for their cognate amino acid (Henkin et al. 1992; Grundy and Henkin 1993; Putzer et al. 1992, 1995b). Their transcripts have an untranslated leader mRNA of about 300 nucleotides, including a ρ -independent terminator just upstream from the translation initiation site (Grundy and Henkin 1994; Henkin 1994; Putzer et al. 1992,

1995a). A highly conserved sequence, called the T-box, is situated just upstream from the terminator structure and can form, with the 5'-strand of the terminator stem, an alternative and mutually exclusive antiterminator structure (Grundy and Henkin 1993). The much weaker antiterminator conformation is thought to be stabilized by base-pairing of the cognate uncharged tRNA with its NCCA-3' end to a UGGN sequence in the T-box (Grundy et al. 1994; Putzer et al. 1995b) and with its anticodon to a specifier codon present in a loop near the 5'-end of the leader (Grundy and Henkin 1993; Luo et al. 1998) (Fig. 1). This model proposes that two contacts between uncharged tRNA and the leader may be sufficient to allow specific antitermination. When the regulatory tRNA, whose anticodon is complementary to the specifier codon is aminoacylated, it cannot interact with the T-Box, which allows the formation of the ρ -independent terminator structure and causes transcription termination. From this model, the over-expression of any aaRS having a T-Box leader in its mRNA should inhibit its gene expression via the aminoacylation of the regulatory tRNA. This is true for *B. subtilis tyrS* (Grundy and Henkin 1993), *thrS*, *thrZ* (Putzer et al. 1992), *valS* (Luo et al. 1997), and *cysS* (Gagnon et al. 1994), but not for *pheST*, where an overproduction of PheRS has no influence on the expression of a *pheS-lacZ* fusion (Putzer et al. 1995b). This observation and studies of *B. subtilis thrS*, *thrZ*, *leuS*, and *valS* suggest that the cognate aaRSs could be involved in the regulation of their genes by interacting directly with mRNA (for a review, see Putzer 1997).

Following this tRNA-mediated antitermination, the leaders of *tyrS*, *serS*, *trpS*, and *ilv-leu* mRNAs are cleaved between the T-Box consensus sequence and the terminator structure leaving a stable secondary structure at the 5'-end of the processed aaRS mRNA (Condon et al. 1996). For the *B. subtilis thrS* mRNA this processing increases its half-life and was reported to be catalyzed in vivo and in vitro by *E. coli* RNase E (Condon et al. 1996, 1997). Moreover, the ratio of the amounts of processed and full-length *thrS* transcripts is influenced by threonine starvation, indicating that this processing participates to *thrS* regulation.

Processing of the *B. subtilis gltX-cysE-cysS* transcript

In *B. subtilis*, the *gltX* gene is cotranscribed with *cysE* and *cysS* encoding, respectively, the serine acetyl-transferase (the first enzyme in the pathway for cysteine biosynthesis from serine) and the cysteinyl-tRNA synthetase (Gagnon et al. 1994; Pelchat and Lapointe 1999). The mRNA of the intergenic region between *gltX* and *cysE* may adopt secondary structures similar to those found in the leader of several other *B. subtilis* aaRS mRNAs (Fig. 1). We previously demonstrated that the expression of *cysE* and *cysS* is partly uncoupled from that of *gltX* by the presence of a tRNA^{Cys}-dependent transcriptional attenuator between *gltX* and *cysE* (Gagnon et al. 1994). Moreover the mRNA of the intergenic region between *B. subtilis gltX* and *cysE* is cleaved in vivo and in vitro immediately downstream from the ρ -independent terminator structure (Pelchat and Lapointe 1999), which differs by its position and its mechanism from that of the *B. subtilis thrS* transcript, reported to be catalyzed by *E. coli*

Fig. 1. Putative secondary structure of the transcript of the *B. subtilis* *glx-cysE* intergenic region. A part of this structure was proposed by Gagnon et al. (1994) following the model of Grundy and Henkin (1993). The T-Box is indicated by a line, whereas the specifier codon for cysteine is surrounded by a line. The alternative terminator–antiterminator secondary structures are presented.

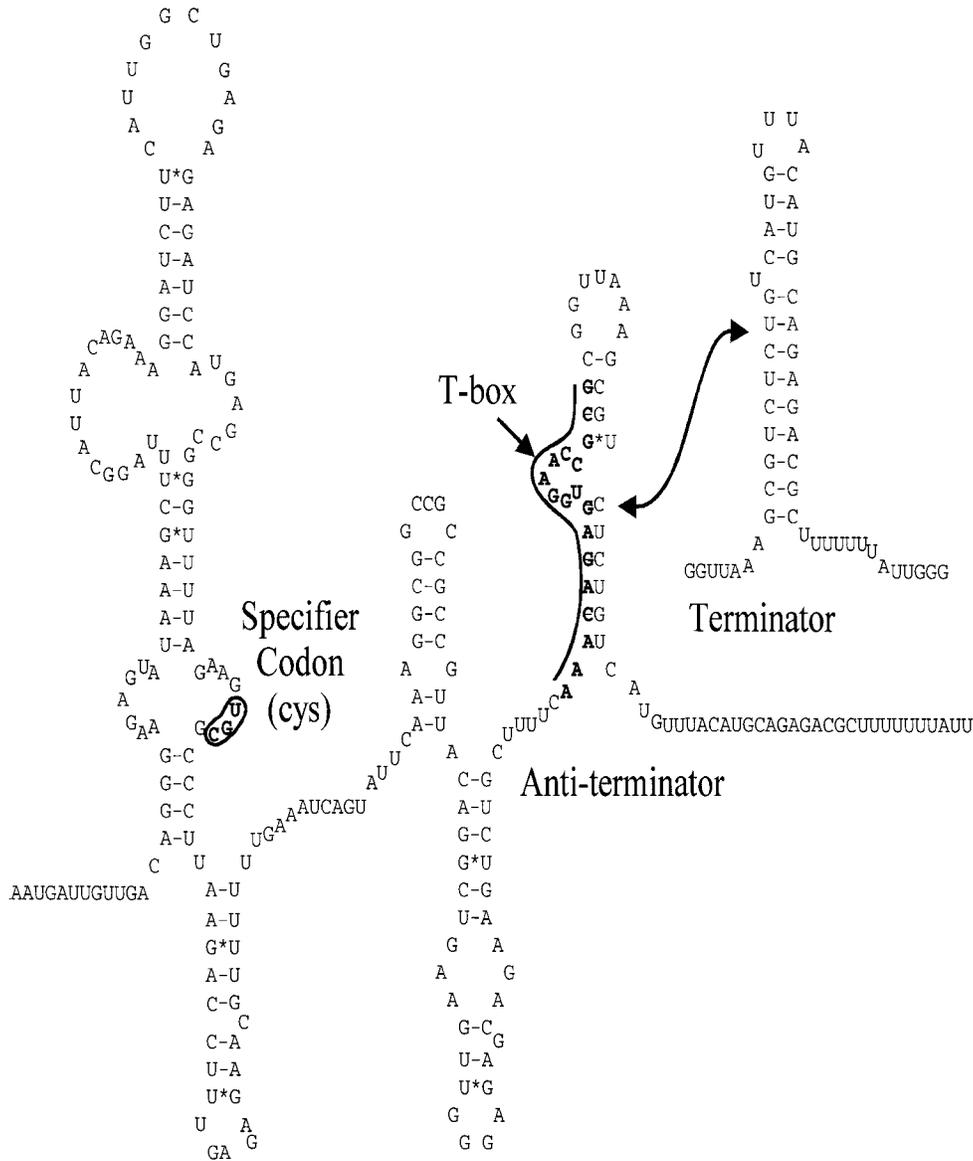
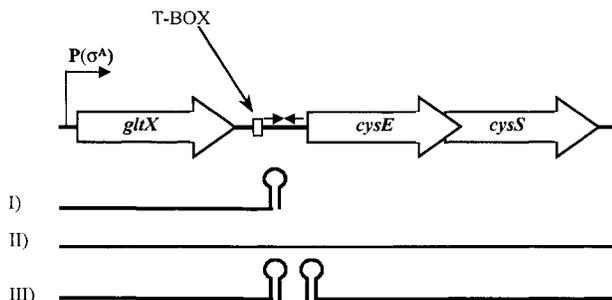


Fig. 2. Proposed model for the processing of the *glx-cysE-cysS* mRNA in *B. subtilis*. As previously described (Gagnon et al. 1994), the intergenic region between *glx* and *cysE* responds to a terminator–antiterminator mechanism (I and II). In the case of antitermination, the mRNA is cleaved immediately downstream from the terminator secondary structure, which leaves putative stable secondary structures at the 3′-end of the *glx* mRNA and at the 5′-end of the *cysE/S* RNA (III) (Pelchat and Lapointe 1999).



RNase E in vivo and where no processing was observed in vitro in the absence of this enzyme (Condon et al. 1997).

The mechanism of this cleavage is not fully characterized, but the fact that it can be observed in vitro, as well as in vivo, suggests self-cleavage. However, we cannot rule out the possibility that this highly specific cleavage is due to the RNA polymerase (Surratt et al. 1991) or to the presence of one fragile phosphodiester bound in this long transcript (Watson et al. 1984; Hosaka et al. 1991; Kierzek 1992). This processing leaves stable putative secondary structures at the 3′-end of the *glx* transcript and at the 5′-end of the *cysE/S* mRNA (Fig. 2) (Pelchat and Lapointe 1999). The presence of these hairpins at the extremities of these processed mRNAs may protect them against degradation from their ends (see Emory et al. 1992), and is consistent with the observation of the longer half-life of the proximal part of *cysE* than that of *glx* mRNA, which contains no such secondary structure in its short leader (Pelchat and Lapointe 1999).

This result also suggests that this specific cleavage of the *gltX-cysE-cysS* transcript may allow distinct regulation of these cotranscribed genes.

Conclusion and future directions

Among the 22 aaRS genes identified on the *B. subtilis* chromosome, 14 contain in their 5'-flanking region a T-box and sequences whose identified or putative secondary structures suggest a common regulatory mechanism at the level of tRNA-dependent transcription antitermination (reviewed by Putzer 1997). Little is known about the regulation of the other eight aaRS genes, but it is likely that they are subject to metabolic regulation, as are all *E. coli* aaRS genes (Grunberg-Manago 1996). In the case of the *B. subtilis* *gltX-cysE-cysS* operon, it is unlikely that metabolic regulation could be mediated via a tRNA-dependent transcriptional regulation or via autoregulation because of the short length (about 45 nt) of the *gltX* 5'-leader (Gagnon et al. 1994). It is tempting to speculate that for *gltX* and maybe other members of this second group, metabolic regulation could depend on the energy charge of the cell via the intracellular levels of ATP and (or) GTP, as shown for the promoters of *E. coli* rRNA genes (Gaal et al. 1997).

Cleavage of the *gltX-cysE-cysS* transcript takes place downstream of the ρ -independent terminator near the end of the transcript of the *gltX-cysE* intergenic region (Pelchat and Lapointe 1999), whereas it takes place upstream of this terminator in the other studied T-Box containing aaRS transcripts. This difference may allow a stabilization of the upstream *gltX* mRNA, and the presence of a putative hairpin immediately following this terminator appears to stabilize the downstream *cysE-cysS* transcript (Pelchat and Lapointe 1999). These observations suggest that these processing sites may have been selected by their stabilizing influence on the adjacent aminoacyl-tRNA synthetase mRNA and may have a general importance in the regulation of this family of genes.

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