

HSD restriction – modification proteins partake in latent anticodon nuclease

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Phage T4-induced anticodon nuclease triggers cleavage–ligation of the host tRNA^{Lys}. The enzyme is encoded in latent form by the optional *Escherichia coli* locus *prr* and is activated by the product of the phage *stp* gene. Anticodon nuclease latency is attributed to the masking of the core function *prrC* by flanking elements homologous with type I restriction–modification genes (*prrA*–*hsdM* and *prrD*–*hsdR*). Activation of anticodon nuclease in extracts of uninfected *prr*⁺ cells required synthetic Stp, ATP and GTP and appeared to depend on endogenous DNA. Stp could be substituted by a small, heat-stable *E.coli* factor, hinting that anticodon nuclease may be mobilized in cellular situations other than T4 infection. Hsd antibodies recognized the anticodon nuclease holoenzyme but not the *prrC*-encoded core. Taken together, these data indicate that Hsd proteins partake in the latent ACNase complex where they mask the core factor PrrC. Presumably, this masking interaction is disrupted by Stp in conjunction with Hsd ligands. The Hsd–PrrC interaction may signify coupling and mutual enhancement of two prokaryotic restriction systems operating at the DNA and tRNA levels.

Key words: *Escherichia coli* *prr* locus/phage T4 *stp* gene/polynucleotide kinase/RNA ligase/tRNA^{Lys}

Introduction

Phage T4 induces, in certain *Escherichia coli* strains, cleavage of the host tRNA^{Lys} by anticodon nuclease (ACNase). The damaged tRNA^{Lys} is normally resuscitated by the phage RNA repair enzymes polynucleotide kinase and RNA ligase (David *et al.*, 1982; Amitsur *et al.*, 1987). However, deficiency in one of these enzymes blocks T4 late protein synthesis (Sirotkin *et al.*, 1978; Runnels *et al.*, 1982), probably due to tRNA^{Lys} depletion (Amitsur *et al.*, 1987).

Manifestation of ACNase depends on host and phage genes (Kaufmann *et al.*, 1986). The optional host locus *prr* (polynucleotide kinase–RNA ligase restriction, Abdul-Jabbar and Snyder, 1984) encodes a latent form of ACNase (Levitz *et al.*, 1990). Mutations in the phage *stp* gene suppress *prr* restriction (Depew and Cozzarelli, 1974; Sirotkin *et al.*, 1978; Runnels *et al.*, 1982) and abolish the induction of ACNase (Kaufmann *et al.*, 1986). *stp* coincides with a short basic open reading frame (ORF) (Chapman *et al.*, 1988). A synthetic Stp polypeptide fashioned

accordingly stimulates ACNase in extracts of T4-infected *E.coli* *prr*⁺ cells, suggesting that a natural counterpart activates ACNase upon T4 infection (Amitsur *et al.*, 1989).

Mutational analysis of a *prr* plasmid subclone revealed a contiguous array of four ORFs (*prrA*–*D*) of which at least three are relevant to ACNase (Levitz *et al.*, 1990; and Figure 1). *prrC* is the active core gene. When expressed over a *prr*⁰ background, *prrC* elicits ACNase activity in uninfected *E.coli* (I.Morad, M.Amitsur, D.Chapman-Shimshoni and G.Kaufmann, in preparation). The activity of *prrC* is somehow masked by *prrA* and *prrD* but it is not certain what *prrB*'s contribution is to ACNase (Levitz *et al.*, 1990; D.Chapman-Shimshoni, unpublished results).

A striking similarity has been noticed between the DNA sequences of *prrA*, *B* and *D* (but not *prrC*) and *EcoR124* and *EcoR124/3* type Ic *hsd* restriction–modification (R–M) systems of conjugative plasmids (Linder *et al.*, 1990). Type I R–M enzymes contain three polypeptides designated HsdM, R and S. Two of them (M and S) are needed for methylase activity and all three for the endonuclease activity. The specific recognition sequence directs the endonuclease to cleave DNA at a distant site, at the expense of ATP hydrolysis (Bickle, 1987). The ORFs designated previously by Levitz *et al.* (1990) as *prrA* and *prrD* are in fact truncated: the sequenced portion of *prrA* is 98% identical with the 145 C-terminal amino acids of *hsdM*, that of *prrD* is 84% identical with the N-terminal third of *hsdR*. The similarity between *prrB* and *hsdS* does not include the two DNA recognizing domains, as expected for HsdS proteins specific for different DNA sequences. The non-homologous *prrC* sequence is inserted into the 120 bp that normally separate *hsdS* and *hsdR* (Linder *et al.*, 1990; and Figure 1). Association of *prr* with a DNA restriction system was been pointed out early on by Abdul-Jabbar and Snyder (1984) and has been confirmed by more recent data showing that *prr* encodes type Ic R–M activity (C.Tyndall and T.Bickle, personal communication).

Here we show that ACNase can be activated in extracts of uninfected *E.coli* *prr*⁺ cells in the absence of *prr* gene expression. The activation depended on synthetic Stp and additional effector molecules, some of which may function as Hsd ligands. Moreover, anti-Hsd antibodies recognized latent ACNase but not the core enzyme expressed from a *prrC* plasmid. The data suggest a model (Figure 6) in which Hsd proteins determine ACNase latency by masking PrrC while Stp abolishes this interaction.

Results

Synthetic Stp activates latent ACNase in extracts of uninfected *E.coli* *prr*⁺ cells

ACNase has been detected previously in an extract of T4-infected *E.coli* *prr*⁺ cells but not in an extract of the uninfected cells, even when supplemented with synthetic Stp (Amitsur *et al.*, 1989). Later we noticed that lowering the