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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* (*p*olynucleotide kinase-*R*NA ligase *r*estriction, 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*° 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 *Eco*R124 and *Eco*R124/3 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

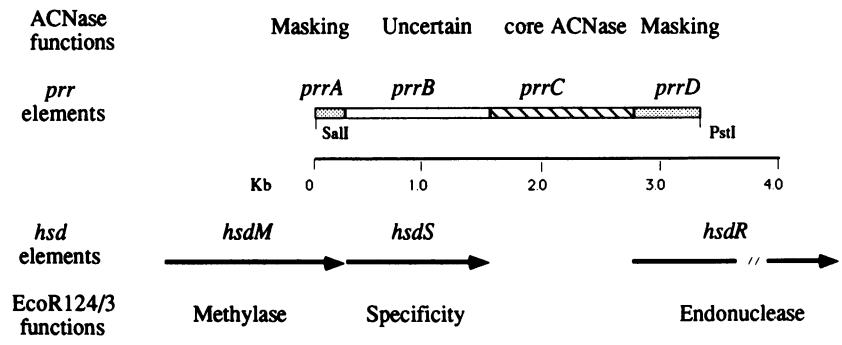

Fig. 1. Structure of the *prr–hsd* locus. This scheme shows the 3.4 kb *SalI–PstI* insert of the *prr* plasmid subclone pRR39 (Levitz *et al.*, 1990) aligned with the *Eco*R124/3 *hsd* genes (Linder *et al.*, 1990). The masking determinants of pRR39 *prrA* and *prrD* are homologous with C-terminal and N-terminal portions of *hsdM* and *hsdR*, respectively. *prrB*, whose role in ACNase is uncertain, is homologous with *hsdS*. The ACNase core gene *prrC* which is not homologous with *hsd* is inserted between the *hsdS* and *hsdM* analogues.

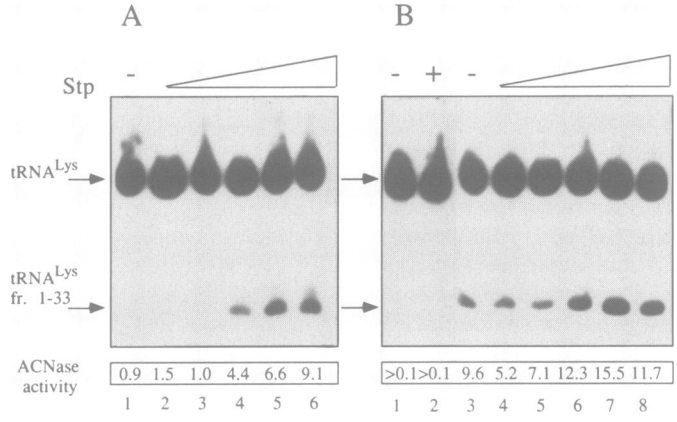

Fig. 2. *In vitro* activation of *prr*-encoded latent ACNase by synthetic Stp. ACNase activity was assayed and quantified as described in Materials and methods with the S-30 fraction of *E.coli* CTr5X (panel **A**), B834 (*prr*°) (panel **B**, lanes 1 and 2) and BJMn10 (*prr*$^+$) (panel B, lanes 3–8). Stp was added at 0.33 μM (lanes A2 and B4), 1 μM (A3 and B5), 3.3 μM (A4 and B6), 10 μM (A5 and B7) and 33 μM (A6, B2 and B8).

incubation temperature from 25 to 10°C stabilizes the enzyme. In addition, a purer and more active preparation of synthetic Stp was employed in the current study. Under the improved assay conditions, ACNase was reproducibly activated by Stp in the S-30 fraction of uninfected *E.coli* CTr5X (*prr*$^+$) cells. Addition of Stp augmented the basal level of ACNase in a dose-dependent manner (Figure 2A).

As expected, extracts from the *E.coli prr*° strains such as B834 exhibited no basal ACNase activity, and activity could not be elicited in them by adding Stp (Figure 1B, lanes 1 and 2). In contrast, the extract of the isogenic *prr*$^+$ derivative BJMn10 (Kaufmann *et al.*, 1986) featured a relatively high basal activity that was significantly augmented by Stp (Figure 2B, lanes 3–8). Since ACNase latency was more stringently maintained *in vitro* with CTr5X we used this strain as a standard source of enzyme in further studies.

Requirements of the Stp-mediated activation of ACNase

Activation of latent ACNase by Stp was refractory to inhibitors of transcription and translation (Table IA), suggesting that neither activation of ACNase nor its activity depend on these processes. ATP and GTP, routinely included in the reaction mixture, were both required for maximal ACNase activity. Omission of one of them severely inhibited the reaction, and the absence of both abolished it altogether (Table IIA). The S-150 supernatant derived from the S-30 fraction displayed no basal ACNase activity, and activity could not be elicited by addition of Stp to this fraction (Figure 3, compare lanes 1 with 3 and 2 with 4). The S-150

Table I. Effect of inhibitors on ACNase activation

Reaction conditions[a]	Extent of tRNA^{Lys} cleavage[b]	% of standard reaction mixture
A. Stp-mediated activation[c]		
Standard mixture	9.4	100
Rifampicin	9.0	95
Puromycin	9.5	101
Chloramphenicol	11.0	117
Kasugamycin	8.3	88
Kanamycin	8.9	95
Streptomycin	11.0	117
B. X-mediated activation[d]		
Standard mixture	21.0	100
Rifampicin	24.0	114
Puromycin	21.8	103
Chloramphenicol	29.4	140
Kasugamycin	20.7	98
Kanamycin	21.0	100
Streptomycin	18.0	86

[a]Antibiotics were employed at 100 μM.
[b]Percentage of input tRNA^{Lys} converted into fragment 1–33.
[c]Stp-mediated activation was carried out on S-30–*prr*$^+$ fractions.
[d]Performed with 1:1 mixture of S-150 *prr*$^+$ and heated S-150 *prr*°.

Table II. Nucleotide requirements of ACNase activation

Reaction conditions[a]	Extent of $tRNA^{Lys}$ cleavage[b]	% of standard reaction mixture
A. Stp mediated activation[c]		
Complete	14.6	100
Without ATP	0.5	3
Without GTP	2.8	19
Without both ATP and GTP	0.0	0
B. X-mediated activation[d]		
Complete	44.5	100
Without ATP	15.9	35
Without GTP	21.4	48
Without both ATP and GTP	1.0	2

[a]Standard reaction mixtures contained 2 mM ATP and 0.1 mM GTP.
[b]Percentage of input $tRNA^{Lys}$ converted into fragment 1–33.
[c]Stp-mediated activation was carried out on S-30–*prr*$^+$ fractions.
[d]Performed with 1:1 mixture of S-150 *prr*$^+$ and heated S-150 *prr*°

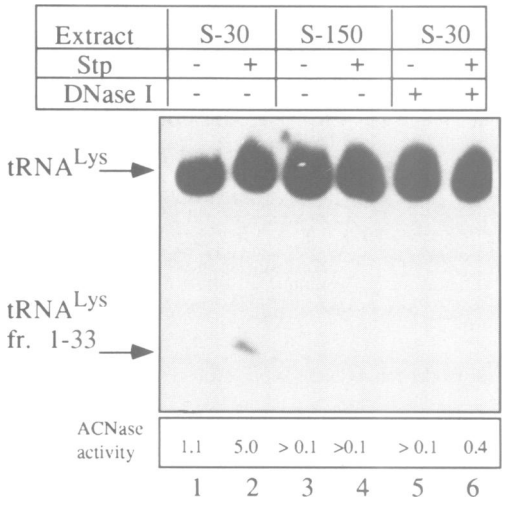

Fig. 3. Stp-mediated ACNase activation may depend on DNA. ACNase was assayed in the absence or presence of Stp, as described in Figure 1, in mixtures containing untreated S-30 (lanes 1 and 2) or S-150 (lanes 3 and 4) fractions of the *prr*$^+$ strain *E.coli* CTr5X, or the S-30 CTr5X fraction treated with DNase I (lanes 5 and 6).

pellet was also inactive. However, the Stp-mediated activation was restored by recombining the supernatant and pellet fractions (Amitsur *et al.*, 1989; and data not shown), suggesting involvement of components from both fractions in the process. Treatment of the S-30 fraction with DNase I rendered it inactive (Figure 3, lanes 5 and 6). This result and the detection of ACNase in the S-150 fraction following activation by other means (see below) suggest that endogenous DNA was a necessary component of the complementing S-150 pellet. However, a requirement for endogenous DNA could not reflect a dependency of ACNase activation on *prrC* gene expression as indicated by the failure of inhibitors of transcription and translation to affect ACNase (Table I).

ACNase activation in the absence of Stp

Latent ACNase could be activated in the absence of Stp by complementing the *prr*$^+$ S-30 fraction with the inactive *prr*° counterpart (Figure 4, lanes 1–3). The *prr*° extracts of strains K-10 and B834 functioned equally well in this complementation (not shown). The *prr*$^+$/*prr*° complementation could be effected with the corresponding S-150 fractions (Figure 4, lanes 4–6), although the resulting ACNase activity was weaker than that seen with the S-30 fractions. Heating the complementing *prr*° fraction (either from strain B834 or K-10) for 5 min at 90°C increased its activation potential (Figure 4, compare lanes 6 and 7), suggesting that there was a heat stable ACNase-activating factor (henceforth called substance X) in this fraction. Mixing the original *prr*$^+$ fraction with the heated counterpart also activated ACNase, albeit to a far lesser extent (Figure 4, compare lanes 7 and 8). Apparently, the native *prr*$^+$ extract contained less substance X and in a form released by the heat treatment. The heated S-150 fractions could not be replaced by buffer (Figure 4, lane 9), indicating that the activation was not caused by non-specific dissociation due to dilution of a latent ACNase complex. Substance X activity was partially retained after passing the heated S-150 *prr*° fraction through a membrane with a 3 kDa cut-off (Figure 4, lane 10) and resisted digestion with DNase I (Figure 4, lane 11). However, when the latent ACNase-containing *prr*$^+$ S-150 fraction was itself treated with DNase I, it no

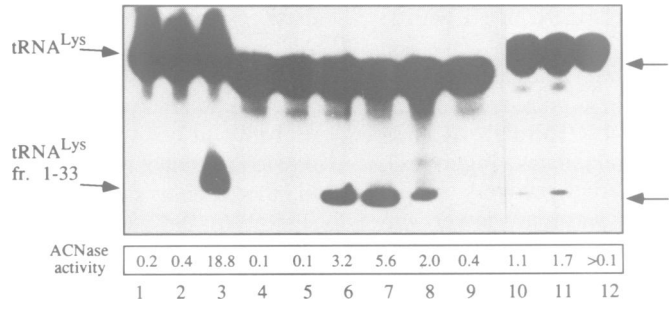

Fig. 4. Activation of ACNase by *prr*$^+$/*prr*° complementation. ACNase was assayed as described in Figure 2 in mixtures containing the following S-30 (lanes 1–3) or S-150 (lanes 4–12) fractions: fraction 1, *E.coli* K-10 (*prr*°); 2, *E.coli* CTr5X (*prr*$^+$); 3, 1:1 mixture of the CTr5X and K-10 fractions. The remaining reaction mixtures contained S-150 fractions as follows: fraction 4, *E.coli* K-10 (*prr*°); 5, *E.coli* CTr5X (*prr*$^+$); 6, 1:1 mixture of the CTr5X and K-10 fractions; 7, 1:1 mixture of the CTr5X fraction and heat-treated (5 min at 90°) K-10 fraction; 8, 1:1 mixture of original and heated CTr5X fractions; 9, 1:1 mixture of the CTr5X fraction and buffer A; 10, 1:1 mixture of the CTr5X fraction and the heated K-10 fraction which has been filtered through an Amicon 3C membrane; 11, 1:1 mixture of the CTr5X fraction and DNase I-treated, heated and filtered K-10 fraction; and 12, 1:1 mixture of the CTr5X fraction treated with DNase I for 90 min at 10°C and the heated and filtered K-10 fraction. Lanes 10–12 are from a separate experiment in which the relative activities of the original and filtered *prr*° S-150 fractions were comparable to that shown.

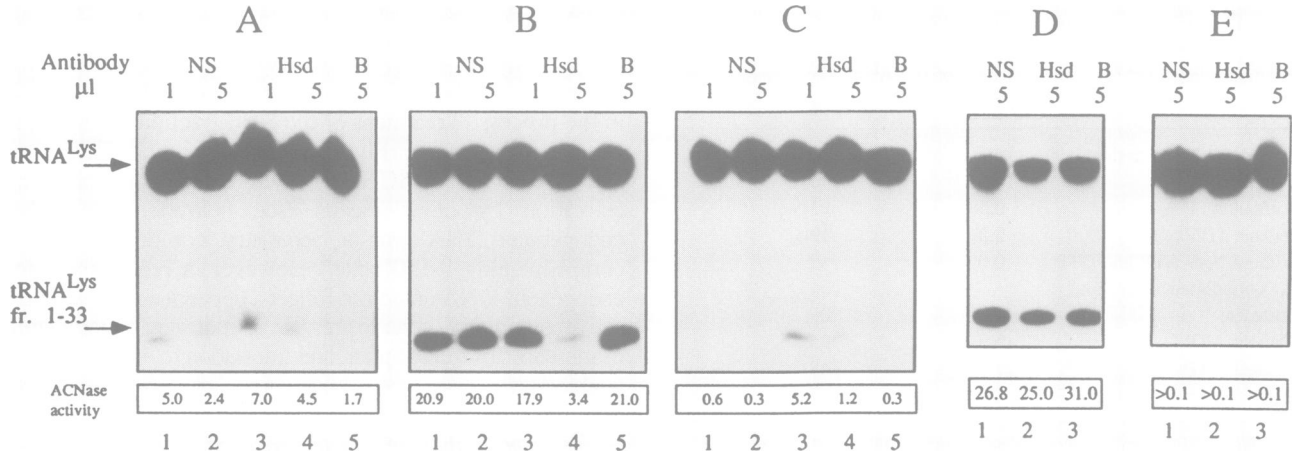

Fig. 5. Immunoprecipitation of latent ACNase by Hsd antiserum. The S-30 extract of *E.coli* CTr5X (*prr*$^+$) was treated with Hsd antiserum as described in Materials amd methods. The supernatants were assayed for ACNase activity as described in Figure 1, either as such (**A**) or following activation by *prr*$^+$/*prr*° complementation (**B**). Immunoprecipitates were assayed suspended in the activating *prr*° S-30 fraction (**C**). The core ACNase-containing S-150 fraction from *prrC*-overexpressing cells (I.Morad, M.Amitsur and G.Kaufmann, in preparation) was treated with Hsd antiserum and ACNase assayed in the immunosupernatant (**D**) or in the immunoprecipitate (**E**). NS, non-specific rabbit serum; Hsd, rabbit *Eco*R124/3 antiserum; B, buffer A.

longer responded to activation by substance X (Figure 4, lane 12). Hence, as with Stp, the X-mediated activation seemed to depend on endogenous DNA although the smaller amount of DNA remaining in the S-150 fraction sufficed to prompt it. The X-mediated activation was also refractory to inhibitors of transcription and translation (Table IB) and required ATP and GTP (Table IIB).

Immunoprecipitation of ACNase by Hsd antiserum

Antiserum raised against the *Eco*R124/3 Hsd system was expected to cross-react with *prr*-encoded Hsd proteins and thus show whether they form part of ACNase. The *prr*$^+$ S-30 fraction was treated with the Hsd antiserum or control serum. Subsequently, the immuno-supernatant was assayed, either directly for basal ACNase activity (Figure 5A), or by X-mediated activation following complementation with the *prr*° fraction (Figure 5B). The immunoprecipitate was suspended in the activating *prr*° extract and then assayed (Figure 5C). Recognition of ACNase by the Hsd antiserum was demonstrated in each case. First, the antibodies slightly increased the basal activity above the background seen with the non-specific serum (Figure 5A, compare lane 1 with 3, and lane 2 with 4). Secondly, the X-mediated activation assay demonstrated that Hsd antibodies remove the bulk of ACNase from the supernatant when employed at the appropriate dose (Figure 5B, lane 4). Thirdly, ACNase appeared in the activated immunoprecipitates (Figure 5C, lanes 3 and 4). However, the relative recovery of the immunoprecipitated ACNase activity was much lower with the higher dose of antibodies (compare lanes 3 and 4 in panels B and C), indicating that these antibodies may also inhibit ACNase. An active form of ACNase (core ACNase) is expressed from a *prrC* plasmid over a *prr*° background (I.Morad, M.Amitsur and G.Kaufmann; in preparation). The *prrC*-encoded core enzyme was not affected by Hsd antiserum. Its level in the supernatant remained unaltered (Figure 5D) and none of it was detected in the immunoprecipitate (Figure 5E). Hence, the Hsd antibodies do not recognize ACNase through PrrC.

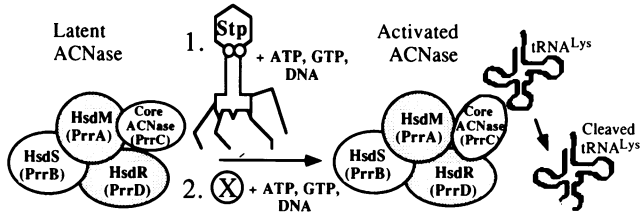

Fig. 6. A model for ACNase latency and activation.

Discussion

ACNase exists in latent form in uninfected E.coli prr$^+$ *cells*

Mutational analysis has suggested that *prr* encodes a latent form of ACNase, comprising the core factor PrrC and the cognate masking functions PrrA and PrrD (Levitz *et al.*, 1990). Comparison of *prr* and *Eco*R124/3 *hsd* DNA sequences has implicated type I restriction proteins with the masking function (Linder *et al.*, 1990). We have confirmed these assumptions by demonstrating the existence of a latent ACNase complex recognized by Hsd antibodies. We conclude that Hsd proteins cooperate with PrrC in the ACNase holoenzyme and that Stp modifies this interaction, probably in conjunction with Hsd ligands. However, the possibility cannot be excluded that ACNase may be activated in other cellular situations. Evidence underlying this model (Figure 6) and general implications are discussed below.

Stp may activate ACNase in conjunction with Hsd ligands

A polypeptide encoded by T4 *stp* has been proposed to be necessary for activating ACNase during phage T4 infection (Chapman *et al.*, 1988; Amitsur *et al.*, 1989). The ability of synthetic Stp to activate the latent enzyme *in vitro* in the absence of other T4 gene products (Figures 2 and 3) confirms this proposal and also suggests that Stp is the only T4 factor needed to mediate this function. Further support to this

conclusion is lent by manifestation of ACNase activity in uninfected *E.coli prr*$^+$ cells transformed by an *stp* plasmid (I.Morad, D.Chapman-Shimshoni and G.Kaufmann, unpublished results). We show elsewhere that core ACNase activity expressed from a *prrC* plasmid clone over the *prr*° background is not stimulated by synthetic Stp in an *in vitro* assay (I.Morad, M.Amitsur and G.Kaufmann, in preparation). Combined, these facts suggest that Stp activates ACNase indirectly, probably by counteracting the masking elements.

The Stp-mediated activation of ACNase depended on additional effector molecules including ATP, GTP (Table II) and, by implication, also endogenous DNA (Figures 3 and 4). None of these components is needed for $tRNA^{Lys}$ cleavage by core ACNase (I.Morad, M.Amitsur and G.Kaufmann; in preparation). Hence, these effector molecules may also contribute to the unmasking of ACNase. DNA and ATP, which are substrates and allosteric effectors of the HsdRMS enzyme complex (Bickle, 1987), could alter the conformation of the Hsd-masking proteins and, consequently, their ability to sequester the core factor PrrC. Preliminary experiments suggest that DNA isolated from stationary *prr*° cells is more effective in ACNase activation than the *prr*$^+$ counterpart (M.Amitsur, unpublished results). The role of GTP in ACNase activation is less clear. A clue to its role is perhaps provided by the finding that McrBC restriction nuclease activity depends on GTP (Sutherland *et al.*, 1992). Since *hsd* and *mcrBC* map closely in *E.coli* K-12 within the immigration control region that is optional in *E.coli* (Raleigh *et al.*, 1989), they may also be linked in the optional DNA element that harbours *prr*. In this regard it is noteworthy that an ACNase-activating deletion maps in the *prr*-containing element several kilobases outside the *prr-hsd* locus (D.Chapman-Shimshoni and G.Kaufmann, unpublished results).

ACNase can be activated by a small heat-stable E.coli molecule

Latent ACNase could be activated *in vitro* in the absence of Stp by complementation with an extract from *prr*° cells (Figure 4). This activation resembled that mediated by Stp in its dependence on ATP and GTP (Table II) and, probably, endogenous DNA (Figure 4). The putative factor associated with this alternative mode of ACNase activation, substance X, appears to be a small, heat-stable *E.coli* molecule. The scarcity of X in the *prr*$^+$ extract suggests that it is dissipated or sequestered by a product encoded by a *prr* gene.

Physical interaction of PrrC and the Hsd-masking factors

Hsd antibodies effectively depleted the *prr*$^+$ extract of latent ACNase (Figure 5B and C). In contrast, core ACNase encoded by *prrC* over a *prr*° background was refractory to these antibodies (Figure 5D and E). It follows that PrrC, which is essential for ACNase activity, was affected by the antibodies indirectly, most probably due to its interaction with Hsd protein(s) within the latent ACNase. Likely partners in such an interaction are PrrA−HsdM and PrrD−HsdR since portions of their genes function as masking elements in a plasmid construct (Levitz *et al.*, 1990). Whether PrrA and PrrD mask PrrC directly or relay their influence through additional subunits of ACNase encoded by other, as yet unidentified *prr* genes or indigenous *E.coli* genes remains an open question. Although the role of the PrrB−HsdS subunit in ACNase is not certain, we include it in the ACNase holoenzyme scheme to indicate its likely participation in a nestled ternary HsdRMS complex. These considerations and the alternative modes of ACNase activation by Stp and X are integrated in the scheme shown in Figure 6. Whether PrrC remains attached to Hsd proteins (as shown arbitrarily in Figure 6) or dissociates away remains an open question.

Physiological implications

ACNase is a potential bacterial restriction system operating at the tRNA level. Its existence as a latent protein complex ensures rapid response, even when host gene expression is turned off by a parasitic genome such as phage T4. This utility is illustrated in *E.coli prr*$^+$ cells infected by T4 *pnk* or *rli* mutants. In these infections the host $tRNA^{Lys}$ is depleted (Amitsur *et al.*, 1987), T4 late protein synthesis comes to a halt and the infection is consequently contained (Sirotkin *et al.*, 1978; Runnels *et al.*, 1982). Although natural activation of ACNase has so far been observed only with phage T4-infected *E.coli*, it cannot be ruled out that ACNase is mobilized under other cellular circumstances. Such a possibility is hinted at by the ability of common *E.coli* metabolites to activate ACNase *in vitro* (Figure 4). Thus, for example, one can envisage a situation in which invading genomes recognized by the *prr*-associated *hsd* system trigger in turn ACNase. ACNase could serve perhaps as a second defence line, enhancing restriction by preventing expression of genomes that escaped Hsd.

Materials and methods

Materials

Synthetic Stp (residues 2−29) was custom-synthesized by Multiple Peptide Systems Inc. (San Diego) and purified by HPLC as described by Amitsur *et al.* (1989). Its sequence was ascertained by protein sequencing on a 470A gas phase protein sequencer (Applied Biosystems).

Bacteria, phage and plasmids

Most strains specific to this study have been described previously (Kaufmann *et al.*, 1986; Amitsur *et al.*, 1987). Bacterial strains and plasmids used for expression of *prrC* in the T7 RNA polymerase/promoter system (Tabor and Richardson, 1985) will be described elsewhere (I.Morad, M.Amitsur and G.Kaufmann; in preparation).

Latent anticodon nuclease assays

The preparation of the substrate and the conditions of the *in vitro* ACNase assay were essentially as previously described (Amitsur *et al.*, 1989). The $tRNA^{Lys}$ substrate labelled with $[^{32}P]P_i$ in the cleavage junction is converted by ACNase into labelled $tRNA^{Lys}$ fragment 1−33 carrying a 2′:3′-cyclic phosphate end-group. The standard source of latent ACNase was the S-30 extract or the derived S-150 supernatant fraction prepared from uninfected *E.coli* CTr5X (*prr*$^+$) similar to the protocol of Chambliss *et al.* (1983), except that DNase I treatment was omitted. Other *E.coli* strains were used for extract preparation as indicated. The latent enzyme was activated either with synthetic Stp or by complementation with extracts of uninfected *prr*° cells. The Stp-mediated ACNase activation mixture (10 μl) contained 5 μl of the S-30 fraction (~80 μg protein and ~100 ng endogenous $tRNA^{Lys}$ substrate), 5000 c.p.m. of $[^{32}P]tRNA^{Lys}$ (3000 Ci/mmol), 60 mM NH_4Cl, 7.5 mM $MgCl_2$, 4% polyethylene glycol, 2 mM ATP, 0.1 mM GTP, 1 mM dithiothreitol and up to 1 μg of synthetic Stp. The extract was added to the remaining components and the mixture incubated at 10°C for 2 h. The reaction was stopped by adding an equal volume of 2% SDS in 0.6 M sodium acetate pH 5.5. RNA was extracted with phenol and precipitated with ethanol. The RNA was dissolved in 10 μl formamide containing 0.01% xylene cyanol and separated by electrophoresis on 15% polyacrylamide in 7 M urea, 25 mM Tris−borate buffer (pH 8.3), 2.5 mM EDTA. Following

autoradiography, intact $tRNA^{Lys}$ and fragment 1–33 bands were excised from the gel and counted in liquid scintillation fluid. ACNase activity was expressed as the percent $tRNA^{Lys}$ radioactivity converted into fragment 1–33. Activation of ACNase by the *prr*$^+$/*prr*° extract complementation was performed similarly except that the reaction mixture contained 2.5 μl of the *prr*$^+$ cell extract (S-30 or S-150, as indicated) supplemented with 2.5 μl of the corresponding *prr*° fraction. The latter was used as such or after subjecting it to one of the following treatments: (i) 5 min incubation at 90°C followed by 1 min centrifugation at 10 000 *g* to remove denatured proteins, (ii) incubation for 30 min at 37°C with 0.2 U/ml DNase I followed by the above heat treatment, (iii) heat treatment followed by filtration through Amicon 3C membrane (3 kDa cut-off). To degrade DNA in the *prr*$^+$ S-30 and S-150 fractions they were incubated with 0.2 U/ml of DNase I for 90 min at 10°C.

Immunoprecipitation of latent ACNase

Rabbit antiserum raised against purified *Eco*R124/3 R–M complex was obtained from Thomas Bickle (Biozentrum, Basel). The conditions of immunoprecipitation were adapted from a laboratory manual (Harlow and Lane, 1988). 50 μl of S-30 extracts of *E.coli* CTr5X were incubated with up to 5 μl of Hsd antiserum or corresponding amounts of non-specific rabbit serum for 1 h at 0°C followed by 30 min incubation at 0°C with 2 μg protein A-bearing *Staphylococcus aureus* Cowan I, equilibrated with buffer A [10 mM Tris–HCl buffer (pH 7.5), 15 mM $MgCl_2$, 60 mM NH_4Cl, 10% glycerol, 5 mM β-mercaptoethanol, 3.3 phenylmethyl sulfonyl chloride and 0.2 mM diisopropylfluorophosphate]. The suspension was centrifuged, the supernatant removed and aliquots were assayed for basal ACNase activity. Alternatively, supernatant aliquots were mixed with the *prr*° S-30 fraction to assay activated ACNase. The pellet was washed twice with 0.5 ml of buffer A and resuspended in 50 μl of the S-30 fraction of *E.coli* K-10 (*prr*°) cells to activate any immunoprecipitated ACNase. This suspension was cleared by centrifugation prior to the assay.

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