The EMBO Journal vol.9 no.5 pp.1383 - 1389, 1990

The optional *E.coli prr* locus encodes a latent form of phage T4-induced anticodon nuclease

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Communicated by A.Rich

The optional *Escherichia coli prr* locus restricts phage T4 mutants lacking polynucleotide kinase or RNA ligase. Underlying this restriction is the specific manifestation of the T4-induced anticodon nuclease, an enzyme which triggers the cleavage-ligation of the host $tRNA^{Lys}$. We report here the molecular cloning, nucleotide sequence and mutational analysis of *prr*-associated DNA. The results indicate that *prr* encodes a latent form of anticodon nuclease consisting of a core enzyme and cognate masking agents. They suggest that the T4-encoded factors of anticodon nuclease counteract the *prr*-encoded masking agents, thus activating the latent enzyme. The encoding of a tRNA cleavage-ligation pathway by two separate genetic systems which cohabitate *E.coli* may provide a clue to the evolution of RNA splicing mechanisms mediated by proteins.

Key words: bacterial restriction system/polynucleotide kinase/RNA ligase/T4 *stp* gene/tRNA splicing

Introduction

Bacteriophage T4 provides a unique system of protein-mediated RNA splicing, exemplified by the cleavage-ligation (reprocessing) of the host $tRNA^{Lys}$ in reactions catalyzed by anticodon nuclease (ACNase), polynucleotide kinase and RNA ligase (David *et al.*, 1982; Amitsur *et al.*, 1987). Only *Escherichia coli* strains restricting T4 mutants devoid of polynucleotide kinase (*pnk*$^-$) or RNA ligase (*rli*$^-$) manifest the T4-induced ACNase (David *et al.*, 1982; Kaufmann *et al.*, 1986). The host locus *prr* encodes both this restriction (Abdul-Jabbar and Snyder, 1984) and the manifestation of ACNase (Kaufmann *et al.*, 1986). ACNase also depends on *stp* (Kaufmann *et al.*, 1986), the T4-suppressor of *prr*-encoded restriction (Depew and Cozzarelli, 1974; Sirotkin *et al.*, 1978; Runnels *et al.*, 1982) and on additional, yet unidentified T4 factor(s) (Amitsur *et al.*, 1989).

To gain insight into the biological meaning of the host tRNA reprocessing phenomenon, its underlying mechanism and relationship to other splicing systems, we have focused our studies in recent years on ACNase, the least understood among the reprocessing enzymes. The complexity of ACNase has impeded its isolation and characterization as one entity (Amitsur *et al.*, 1989). An alternative approach is to identify individual ACNase subunits by gene cloning and complementation assays. We have previously shown that *stp* coincides with an open reading frame (ORF) of 29 codons (Chapman *et al.*, 1988). A corresponding physiological Stp polypeptide has not been detected yet but its existence is strongly advocated by the ability of a synthetic Stp polypeptide to stimulate ACNase *in vitro*. The *in vitro* ACNase reaction requires additional T4-encoded factor(s) as well as *prr* encoded products (Amitsur *et al.*, 1989).

We report here the molecular cloning, nucleotide sequence and mutational analysis of *prr*-associated DNA. The data indicate that *prr* encodes a latent form of ACNase consisting of a core enzyme and cognate masking agents. Presumably, Stp and other T4-encoded ACNase factors counteract the masking agents, thus activating the latent enzyme.

Results

Molecular cloning of prr-associated DNA

Three phenotypes were used to select *prr*-clones: (i) the *prr*-linked restriction of T4 strains containing unmodified DNA (dC-DNA) (Abdul-Jabbar and Snyder, 1984), (ii) the restriction of T4 *pnk*$^-$ and *rli*$^-$ mutants, and (iii) the *in vivo* manifestation of ACNase. In the first cloning step, DNA of a restrictive (*prr*^{r}) transductant, *E.coli* BJMn10 (Abdul-Jabbar and Snyder, 1984; Kaufmann *et al.*, 1986), was partially cleaved with *Bam*HI restriction endonuclease and ligated into the Kan^R cosmid vector pWH4 (Herrero *et al.*, 1984). The restriction of T4-dC DNA served in initial screening for potential *prr* cosmids. A number of T4 (dC-DNA)-restricting cosmids were isolated but only one of them features the Prr^R (restrictive) phenotype. This clone, termed pW16, also manifested ACNase activity upon T4 infection. Cosmid pW16 contained ~28 kb of insert DNA unique to *prr*^{r} strains, as indicated by comparing Southern blots of *prr*^{r} and *prr*^{p} (permissive) cell DNAs with a pW16 probe (data not shown). Hence, *prr* resides in a DNA element whose presence in the *E.coli* chromosome is optional.

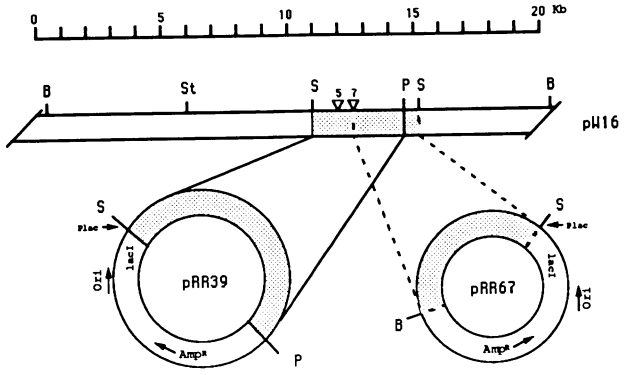

Fig. 1. Generation of *prr* plasmid subclones. The indicated fragments of pW16 were cloned between the appropriate sites of pBluescript KS^+ as detailed in the text. Triangles mark miniTet insertion sites of pW16-5 (5) and pW16-6 (7). B, P, St and S denote *Bam*HI, *Pst*I, *Sst*II and *Sal*I restriction sites, respectively.

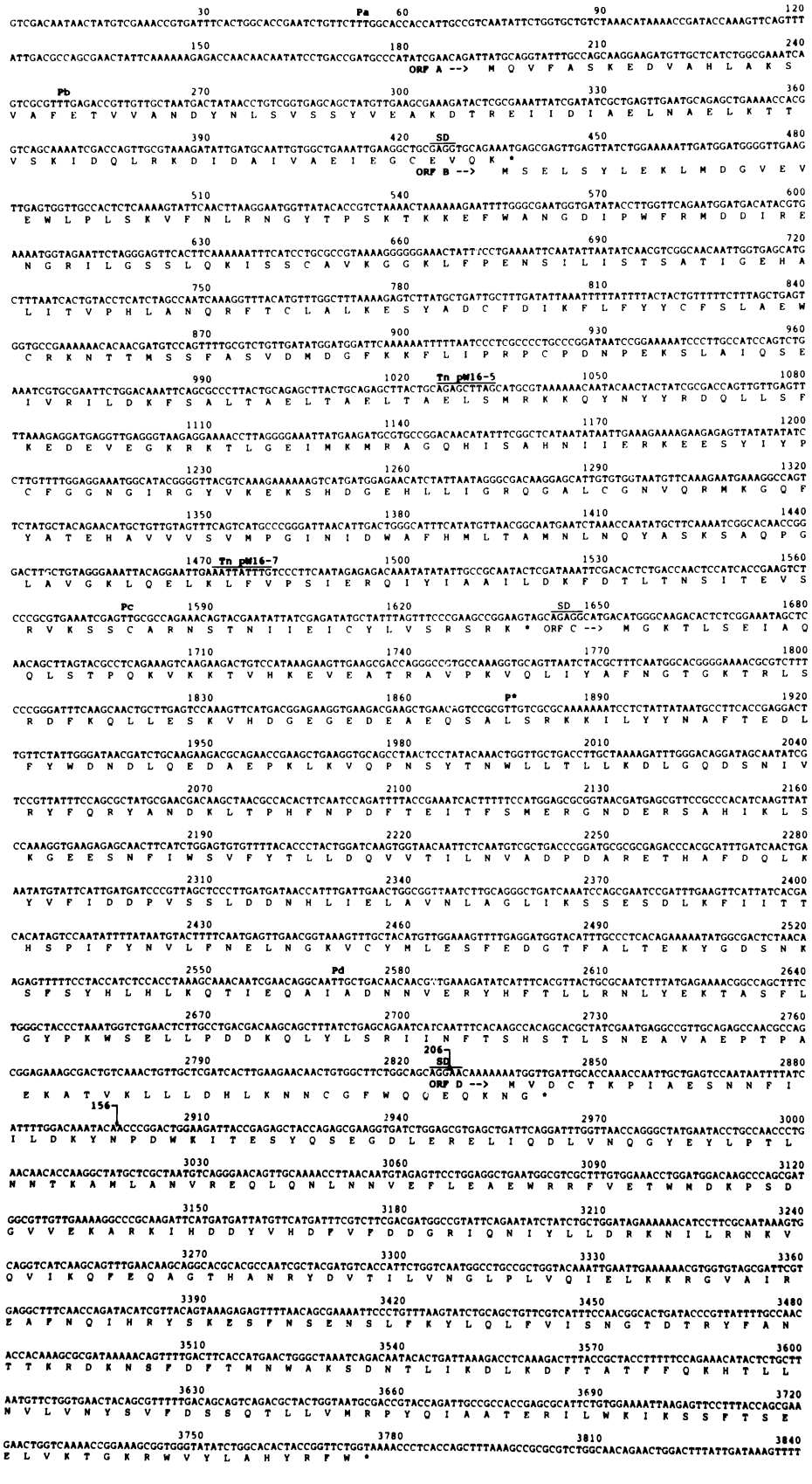

Fig. 2. Nucleotide and deduced amino acid sequences of *prr* DNA. SD indicates suspected ribosome binding sites. Pa, Pb, etc. indicate the beginning of sequences resembling the *E.coli* consensus promoter. The end points of deletions beginning at the *Pst*I end of the pRR39 insert (position 3430) are indicated by clone number and downpointing arrow. The *prr*-inactivating transposon target sites are overlined.

The location of *prr* within cosmid pW16 was approximated by transposon mutagenesis, using a Tn*10* derivative (miniTet) launched from plasmid pNK861 (Way *et al.*, 1984). Twenty-five Tet^R derivatives of pW16 were examined. Three of them lost the ability to restrict T4 *pnk* and *rli* mutants and to manifest ACNase upon T4 infection. The sites of these inactivating insertions were clustered within a 2 kb region of cosmid pW16. Two of the tet^r and prr^p cosmid clones (pW16-5 and pW16-7) retained the original restriction pattern about the miniTet target sites. A pW16 DNA fragment containing both sites was subcloned in two orientations into the plasmid vectors pBluescript KS^+ and SK^+, yielding the respective clones pRR39 (Figure 1) and pRR93. In addition, the two *Bam*HI sites at the ends of miniTet (Way *et al.*, 1984) were utilized to subclone flanking pW16-5 and pW16-7 fragments. Particularly useful were clones pRR67 (Figure 1) and pRR65 whose inserts partially overlap that of pRR39. The pRR65/67 inserts range from the end of the respective miniTet to a *Sal*I site in the cosmid, ~450 bp beyond the *Pst*I end of the pRR39 insert (Figure 1). The *prr* plasmid subclones imparted no *prr* restriction. Yet, they contained relevant ACNase genes, as shown below.

Nucleotide and deduced amino acid sequences of prr DNA

Figure 2 shows the nucleotide and deduced amino acid sequences of a *Sal*I fragment of cosmid pW16 containing the pRR39 insert and an adjacent *Pst*I–*Sal*I fragment found in pRR67. This region spans 3853 nucleotides numbered from the *Sal*I end of the pRR39 insert down to the *Sal*I end of the pRR67 insert. The 9 bp target sites of the *prr*-inactivating miniTet insertions begin at positions 1025 and 1471 of the sequence. A contiguous array of four ORFs read in one direction can be deduced from the sequence. These ORFs are oriented with the vector's *lac* promoter in pRR39 and against it in pRR93, pRR65 and pRR67. Their general properties are summarized in Table I. Plasmids pRR39 and pRR93 contain the complete ORFs A–C and the 200 N-terminal codons (out of 313) of ORF D. Plasmid pRR67 encodes the intact ORFs C and D. Each of the four ORFs is preceded by a sequence (designated Pa through Pd) resembling the *E.coli* consensus promoter. An additional potential promoter sequence (P*) is found within the N-terminal portion of ORF C, upstream to a cluster of GATC *dam* methylation sites.

Expression of ACNase from the prr plasmid subclones

The *prr* plasmid subclones described above and some of their mutant derivatives were examined for the ability to confer ACNase activity *in vivo*. In this assay, ^{32}P-labeled host tRNA isolated prior to or during T4 infection is separated by gel electrophoresis to monitor $tRNA^{Lys}$ fragments generated by ACNase. The results indicated that plasmids pRR65, pRR67 and certain mutant derivatives of pRR39 and pRR93 encode a form of ACNase which does not depend on T4 infection. Cells expressing this 'phage-less' ACNase (PL-ACNase) contained products migrating identically with the $tRNA^{Lys}$ fragments which accumulate in T4 mutant *pnk*-infected *E.coli* CTr5X (prr^r) (Figure 3). Specific hybridization of the PL-ACNase products with ssDNA of an M13-$tDNA^{Lys}$ recombinant ascertained that they originated from $tRNA^{Lys}$ by an authentic ACNase cleavage (Figure 4). The amount of the intact $tRNA^{Lys}$ in the PL-ACNase expressing cells was comparable to that of the fragments derived from it. When the PL-ACNase expressing cells were infected with a T4 pnk^- strain the level of the $tRNA^{Lys}$ fragments was not augmented (Figure 3) nor was the level of the remaining intact $tRNA^{Lys}$ reduced (not shown). In contrast, the parent *E.coli* prr^r strain features the ACNase activity only after T4 infection and $tRNA^{Lys}$ is cleaved in it to completion if the repair reactions are impaired by *pnk* or *rli* lesions (Amitsur *et al.*, 1987). The cleavage of $tRNA^{Leu}1$ (Kano-Sueoka and Sueoka, 1968) which is mediated by a different T4-induced nuclease (David *et al.*, 1982) was normally expressed in T4-infected PL-ACNase containing cells (Figure 3), indicating that the infection was effective under the assay conditions.

ACNase mutational analysis

Certain deletions beginning from the *Pst*I end of pRR39 or introduced at the *Sal*I end of both pRR39 and pRR93

Table I. Open reading frames of the *prr* locus

ORF	Nucleotides	Codons	Promoter[a]	Proposed role
A	191– 433	81	50	Masking agent
B	435–1637	401	248	T4 interface?
C	1652–2840	396	1577	Core ACNase
D	2836–3774	313	2569	Masking agent

[a]Beginning of sequences resembling the *E.coli* consensus promoter.

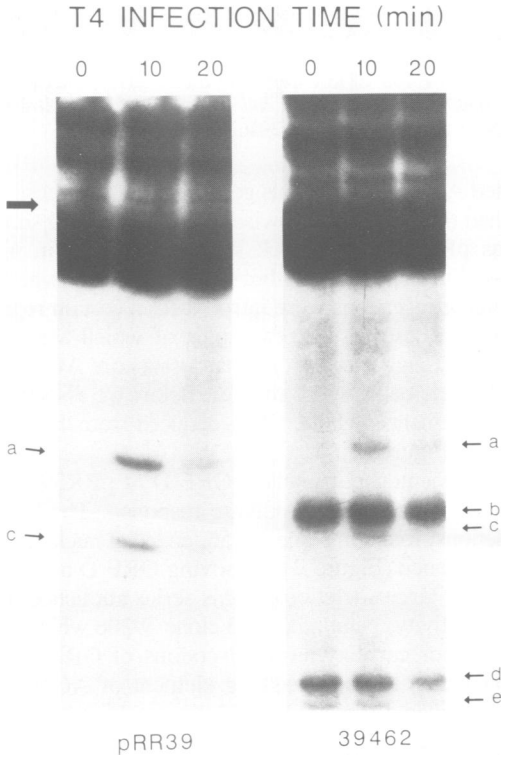

Fig. 3. Expression of PL-ACNase by a *prr* plasmid subclone. ^{32}P-Labeled host tRNA was isolated from *E.coli* 1046 transformed with pRR39 (non-expressing) or 39462 (a PL-ACNase expressing derivative), before or at the indicated infection time. The RNA was separated by gel electrophoresis as detailed in Materials and methods. (**a**) $tRNA^{Leu}1$ fragment 1–48, (**b**) $tRNA^{Lys}$ fragment 34–76, (**c**) $tRNA^{Leu}1$ fragment 49–87, (**d**) $tRNA^{Lys}$ fragment 1–33, (**e**) fragment 1–32 of a minor $tRNA^{Lys}$ species cleaved by ACNase. The thick arrow indicates bulk tRNA.

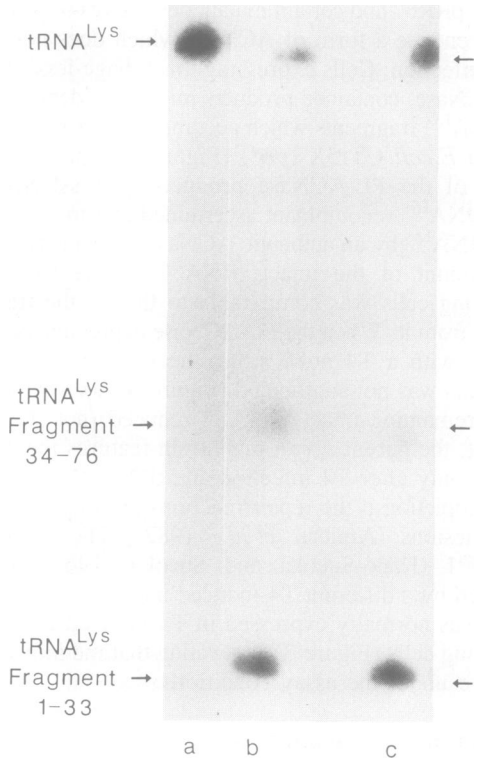

Fig. 4. Identification of PL-ACNase reaction products. Labeled host tRNA isolated from the indicated source was hybridized with mRL8601 ssDNA (Materials and methods) and separated by agarose gel electrophoresis. RNA was released from the hybrid in formamide and separated by polyacrylamide gel electrophoresis as in Figure 3. (**a**) Uninfected *E.coli* CTr5X (*prr*$^+$), (**b**) *E.coli* CTr5X infected with T4 *pseTΔ1* (*pnk*$^-$), (**c**) uninfected *E.coli* 1046 transformed with the PL-ACNase expressing plasmid 39462.

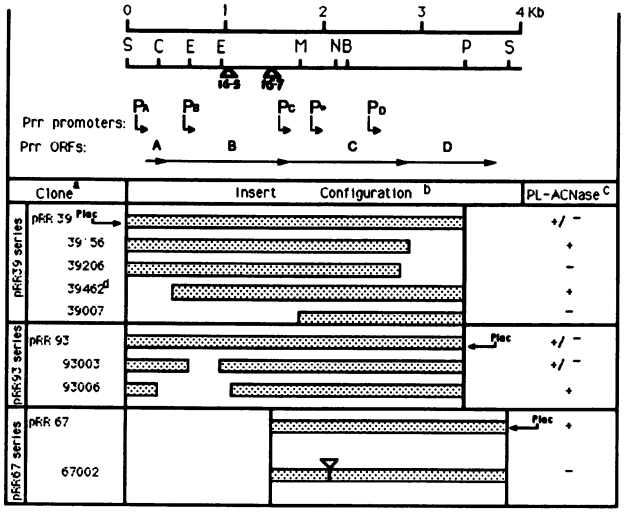

Fig. 5. Mutation analysis of *prr*-associated DNA. (**a**) Clone construction is described in Materials and methods. (**b**) Insert DNA is indicated by a grey bar, discontinuities represent deletions. Triangles pointing upwards indicate transposon insertion sites, that pointing downwards a 4 bp insertion. (**c**) PL-ACNase activity is expressed as follows: (+) indicates levels of tRNA^{Lys} fragment 34–76 ranging between 0.1 and 0.8% of the total tRNA fraction; (±) usually barely detectable or undetectable (0.05–0.1%). However, cells transformed with these plasmids occasionally yielded (+) levels due to spontaneous changes in the plasmid DNA. (−) Invariably undetectable (<0.05%, as background of non-specific RNA fragments). (**d**) This deletion ranges also upstream into vector sequences including the *lac* promoter.

activated ACNase. Deletions going further into these inserts abolished the activity. Likewise, original plasmid subclones such as pRR67, which lack sequences found in pRR39, expressed the PL-ACNase but lost this activity when small deletions or insertions were introduced in certain regions of the insert. These results, examples of which are depicted in Figure 5, suggested that *prr* harbors a core ACNase gene flanked by elements which mask it. Below we associate these ACNase elements with the ORFs deduced from the *prr* DNA sequence (Figure 2).

Deletions entering truncated ORF D of pRR39 from the *PstI* end elicited the PL-ACNase response. The largest of the deletions, found in clone 39156, ended at nucleotide 2896 of the sequence (Figure 2), removing ORF D almost completely. All larger deletions in this series abolished the PL-ACNase activity, beginning with clone 39206 which is missing only four carboxy-terminal codons of ORF C. Thus, ORF D seems to be a masking element of ACNase even in its truncated form while ORF C is associated with the core ACNase.

ORF A could not be inactivated without inactivating ORF B. Thus, an *Acc*I−*Cla*I deletion (base pairs 1–323) entering ORF A and removing promoters Pa and Pb could be stably introduced only into plasmid pRR93 in which the *prr* ORFs cannot be expressed from the vector's *lac* promoter. In contrast, cells transformed with a corresponding pRR39 construct contained smaller plasmids which had probably arisen by further deletions. Since ORF B can be expressed from the pRR39 derivative in the absence of the indigenous *prr* promoters it seems that inactivating ORF A but not ORF B is lethal. Derivatives of pRR93 lacking the C part or all of ORF A and the N part of ORF B (such as clone 93006, Figure 5) expressed the PL-ACNase. A similar behavior was exhibited by stable pRR39 derivatives which lost ORF A and suffered an additional loss of vector sequences containing the *lac* promoter (such as clone 39462). In contrast, several deletions removing internal parts of ORF B (such as clone 93003, base pairs 609–969), or causing early termination of translation (clone 93004, base pairs 719–1036) did not induce the PL-ACNase. This leaves ORF A and vicinal upstream sequences and/or a small N portion of ORF B as candidates for the upstream masking elements of ACNase.

A 4 bp insertion introduced at the *Nco*I site (base pair 2115) of the PL-ACNase expressing plasmid pRR67, causing early termination of ORF C (clone 67002), also abolished ACNase. A pRR39 derivative lacking ORF A, ORF B and the N portion of ORF C but which retained the potential P* promoter found within ORF C (clone 39007) was also ACNase negative. These data suggest that the entire ORF C is needed for the core ACNase function. Furthermore, core ACNase activity cannot be mediated by a shorter alternative product expressed from promoter P*.

Discussion

The prr locus encodes a latent anticodon nuclease

The optional *E.coli* locus *prr*, previously defined by restriction of phage T4 mutants lacking polynucleotide kinase or RNA ligase (Abdul-Jabbar and Snyder, 1984) and ability to manifest ACNase upon T4 infection (Kaufmann *et al.*, 1986) is shown here to encode a latent form of ACNase.

Below we discuss the derivation of this conclusion and some of its implications.

Transposon insertions inhibited both *prr* restriction and T4-induced ACNase, reinforcing previous conclusions about the causal connection between the two traits (Kaufmann *et al.*, 1986). These mutations also located a DNA region encoding an aberrant form of ACNase (PL-ACNase), resembling the native form in cleavage specificity but being expressed without T4 infection. In fact, PL-ACNase ceases to function during T4 infection, contrary to the native enzyme which is detected only after the onset of infection (David *et al.*, 1982). Nevertheless, the origin of the clones encoding PL-ACNase and the cleavage specificity exhibited by this activity implicate it with an altered form of native ACNase. Such an alteration could be due to the absence of original *prr* sequences from the plasmid subclones or loss of normal ACNase controls when expressed from a multicopy plasmid.

Possible roles of the T4-encoded ACNase factors

The T4 *stp* gene is absolutely required for the manifestation of ACNase *in vivo* (Kaufmann *et al.*, 1986). Recent *in vitro* studies suggest that a polypeptide encoded by *stp* is directly involved in the ACNase reaction and invoke the existence of additional T4-encoded ACNase factor(s) (Amitsur *et al.*, 1989). It was surprising, therefore, to find that *prr* itself encodes an ACNase activity which can be expressed without T4 infection. This apparent discrepancy is reconciled by the existence of *prr* DNA sequences which somehow silence the PL-ACNase. Presumably, Stp and other T4 ACNase factor(s) counteract these masking agents and thereby activate the latent enzyme.

Proposed mechanism of ACNase shielding and activation

How is ACNase shielded in the uninfected prr^r cells and then activated during T4 infection? Could the core ACNase gene be transcriptionally repressed by *prr*-encoded masking agents and be derepressed by the T4 ACNase factors? Such a mechanism is contradicted by the cessation of host translation early in T4 infection (cf. Wiberg and Karam, 1983) and the delayed-early schedule of ACNase appearance (David *et al.*, 1982). Moreover, ACNase can be reconstituted *in vitro* by combining one extract of uninfected *E.coli* prr^r cells with a second extract of T4-infected prr^p cells (Amitsur *et al.*, 1989). The first extract which is a source of Prr remains active in complementation even when treated with DNase I, i.e. in the absence of *prr* gene expression. We also note that ACNase activity can be elicited at low temperature in uninfected prr^r cell extracts, in the absence of any T4 factors. Our data further indicate that, under these conditions, it is possible to resolve a ribosome-associated core ACNase from a cognate masking activity found mostly in the S-150 supernatant (M.Amitsur and G.Kaufmann, unpublished results).

These facts and considerations lead us to propose that ACNase shielding and activation occur at a post-translational level, as depicted in a hypothetical scheme (Figure 6). Accordingly, *prr* encodes several components of a latent ACNase complex including a core enzyme, cognate masking factors and, perhaps, a T4 interface (see below). Upon infection, Stp and other T4-encoded factors alter the latent complex in a way which exposes the core enzyme. However, the core enzyme can also be artificially exposed in the uninfected cells through genetic manipulations which inactivate the masking agents, as demonstrated above.

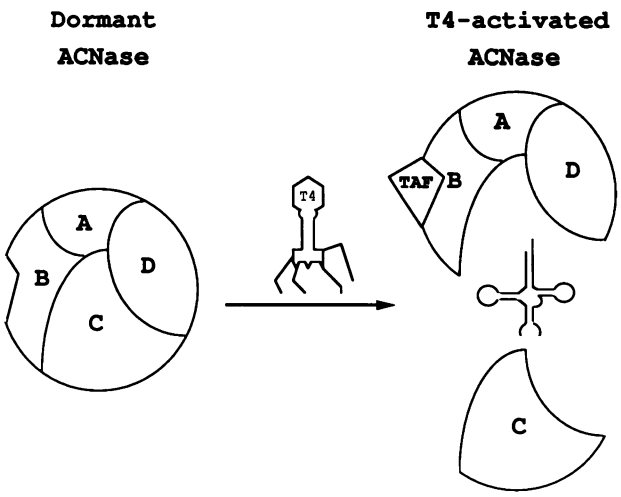

Fig. 6. Proposed scheme for ACNase shielding and activation. Prr proteins designations are as follows. Subunit C is the core ACNase. Subunits A and D are masking agents. Subunit B interfaces T4 ACNase factors (TAF).

Molecular nature of ACNase

The intact ORFs B, C and D can be translated into polypeptides with apparent mol. wts of 43, 45 and 36 kd, respectively (D.Chapman, I.Morad and G.Kaufmann, unpublished results); close to the corresponding predicted values of 45 399, 45 517 and 36 994. However, the expected 9 kd translation product of ORF A has not been detected so far. Therefore, the alternative possibility that this region does not constitute an independent cistron remains open. It also remains to establish which of the *prr* gene products actually participates in ACNase functions, either directly or by activating indigenous *E.coli* constituents. Meanwhile, however, we shall assume that these functions are mediated by polypeptides encoded by ORFs A through D (henceforth Prr A–D).

The results of the mutation analysis (Figure 5) suggest that Prr C is a core ACNase protein. This conclusion is supported by the ability of a *prr*C plasmid to sustain the expression of the core ACNase both *in vivo* and *in vitro* (I.Morad and G.Kaufmann, unpublished results). However, it should be noted that the *in vitro* requirements of ACNase parallel those of protein synthesis (Amitsur *et al.*, 1989). Therefore, it is possible that Prr C is not the sole constituent of the core ACNase. Rather, ubiquitous components of the translation apparatus, proteins or RNA molecules, might also form part of the ACNase enzyme. Thus, catalysis of $tRNA^{Lys}$ cleavage by RNA constituents encoded by indigenous *E.coli* genes cannot be excluded at this stage.

The masking of ACNase is attributed to Prr D and Prr A. These components may interact directly with the core ACNase. Alternatively, one of them plays a regulatory role. The N portion of Prr B could be yet another masking element since the effects of specific mutations in this region have not been determined. However, since at least the C-terminal bulk of Prr B is needed neither for core ACNase nor for cognate masking activities, Prr B may serve in a different capacity. Noteworthy here is that the transposon insertions which interrupted ORF B inactivated the T4-induced

ACNase. Taken together, these results raise the possibility that Prr B constitutes yet another type of ACNase element. Namely, Prr B could interface the T4 ACNase factors and relay their unshielding effect to other Prr subunits. Alternatively, Prr B is not related to ACNase, the inactivating transposon mutations being attributed to polar effects on neighboring ORFs.

Physiological functions of host tRNA reprocessing

The reprocessing pathway can be viewed as a mere outcome of host restriction and phage rebuttal. Thus, loss of vital host tRNAs in the absence of *pnk*- and *rli*-mediated repair can by itself account for *prr* restriction. Similarly, suppression of *prr* restriction by *stp* mutations is due to the inactivation of ACNase, obviating the need for tRNA repair (Kaufmann *et al.*, 1986; Amitsur *et al.*, 1987). These interpretations portray Stp as a co-factor of the host restriction system. They raise intriguing questions about the manner by which *stp* benefits T4 and about the roles *pnk* and *rli* might serve other than to rebut *prr* restriction. It has been proposed that *stp*, *pnk* and *rli* may adapt the $tRNA^{Lys}$ ensemble to suit T4 codon usage (Amitsur *et al.*, 1987). However, this explanation leaves open the question about the general role these genes might exercise in the absence of Prr. Their involvement in RNA cleavage-ligation reactions in the prr^{p} context seems now to be conditioned by the existence of a nuclease substituting that encoded by *prr*. Regarding the primary physiological role of *prr*, one can envisage cellular circumstances which lead to the selective induction of the core ACNase in the absence of T4 infection. In other words, ACNase might have evolved originally as a function which benefited the uninfected host cell or the optional DNA element which encodes it. The involvement of *prr* in phage restriction could be a later adaptation.

Reprocessing and pre-tRNA splicing

Phage T4-induced host tRNA reprocessing resembles in many of its attributes the splicing of nuclear pre-tRNA (cf. Greer *et al.*, 1983), suggesting a common descent. In this regard, the encoding of the latent ACNase by a gene which is not indigeneous in *E.coli* raises the possibility that homologs of this enzyme might be found in eukaryotic systems. Finally, the host tRNA reprocessing system may illustrate how protein-catalyzed RNA splicing evolved. Namely, this pathway could emerge by the coalescence of two separate gene systems, one encoding RNA cleavage, the other RNA repair, both parasitizing the same host cell.

Materials and methods

T4 and E.coli strains

The T4 pnk^{-} strain employed was *pseTΔ1* (Snyder *et al.*, 1976). The following *E.coli* strains were employed: BJMn10 (Abdul-Jabbar and Snyder, 1984; Kaufmann *et al.*, 1986) is a prr^{r} transductant derived from strain B834 (*E.coli* B *met*, r_B, m_B; Wood, 1966). Strain BJMn10 was a source of *prr* DNA; strain 1046 (*recA,supE,supF,hsdS,met*; Cami and Kourilski, 1978) served as a recipient of *prr* cosmids and *prr* plasmids; strain JM109 (*recA1,endA1,gyrA96,thy1,hsdR17,supE44,relA1*,λ^{-},Δ(*lac,proAB*),[F, *traD36,proAB*$^{+}$,*lacI*^{q},ZΔM15], Yanisch-Perron *et al.*, 1985). Strain JM107 was transformed with *prr* plasmid derivatives, providing a source of ssDNAs for sequencing.

Cloning vectors

Cloning vectors employed were the Kan^{R} and λ-*ori* containing cosmid vector pWH4 (Herrero *et al.*, 1984) and phagemids pBluescript KS^{+} and SK^{+} (Stratagene Inc., San Diego).

Transposon mutagenesis

Transposon mutagenesis was carried out using the high transposition frequency plasmid pNK861 (Way *et al.*, 1984). The transposase gene of plasmid pNK861 lies outside a 2.8 kb element derived from Tn*10* (miniTet). Transposition of miniTet does not usually cause deletions/inversions adjacent to the target site. *E.coli* 1046 double transformants containing the Kan^{R} target cosmid and pNK861 were exposed to IPTG to induce the transposase. Cosmid DNA was then isolated and used to retransform *E.coli* 1046. Kanamycin (50 μg/ml) and tetracycline (10 μg/ml) resistances served to select cosmids which had acquired the miniTet insertions.

Plasmid constructions

Original *prr* plasmid subclones (Figure 1 and text) are of the following descriptions: pRR39 contains a 3.4 kb *SalI*–*PstI* fragment of cosmid pW16, ligated between the appropriate sites of plasmid pBluescript KS^{+}; pRR93 contains the same insert but in pBluecript SK^{+}; pRR65 and pRR67 contain the respective 2.9 and 2.5 kb *Bam*HI–*Sal*I fragments ranging from the end of miniTet to a *SalI* site within the cosmid derivatives pW16-5 and pW16-7. Controlled deletions such as in clones 39156 and 39206 were generated by the exonuclease III procedure (Henikoff, 1987) by digestions from the *PstI* end of pRR39. Thirteen specimens of this series, covering ~2000 bp were sequenced and analyzed for ACNase activity. Small deletions generated in a similar manner from the *SalI* end of pRR39 usually resulted in selection of derivatives which underwent additional deletions *in vivo* and lost vector sequences as well (exemplified by clone 39462). Other deletions were created by using appropriate restriction sites as follows: clone 39003 and 93003, *Eco*RI (612–969); clone 93004, *Sph*I (719–1036); clones 39006 and 93006, *Nru*I (313–1062), clone 39007, *SalI*–*Mlu*I (1–1789). Clone 67002 was derived from pRR67 by filling the *Nco*I overhangs (2115) in a Klenow polymerase reaction. This created a new *Nsi*I site. General DNA manipulation procedures were as described by Maniatis *et al.* (1982).

DNA sequencing

DNA was sequenced by the dideoxy chain termination procedure (Sanger *et al.*, 1977), using the Sequenase system (USB Corporation, Cleveland) and utilizing a shotgun library of the pRR39 insert, nested deletion derivatives, miniTet target sites, synthetic oligonucleotide primers corresponding to internal sequences and various restriction sites within the insert.

Determination of in vivo ACNase activity

E.coli 1046 cells transformed with the indicated plasmid clone were labeled with [^{32}P]inorganic phosphate and chased with non-labeled Pi. Low mol. wt RNA was extracted from the cells before or during infection with T4 *pseTΔ1* and separated by electrophoresis on 15% polyacrylamide–7 M urea gels in Tris–borate–EDTA, as described by David *et al.* (1982). Intact $tRNA^{Lys}$ and its fragments were purified away from bulk tRNA by hybridization to recombinant phage mRL8601 which contains the $tRNA^{Lys}$ template (Amitsur *et al.*, 1987).

Acknowledgements

We thank Dr Phillip A.Sharp for his comments. The gift of synthetic *prr* oligonucleotides from Dr Phillip Gottlieb is gratefully acknowledged. This work was supported by grants from the National Institutes of Health and from the Israeli National Academy of Sciences to G.K. and by a grant from the National Institutes of Health to L.S.

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Received on December 15, 1989; revised on January 31, 1990

Note added in proof

The nucleotide sequence of *prr* DNA has been submitted to the EMBL data base and has been assigned the accession number X52284 E.coli prr ABCD.