Phage and Host Genetic Determinants
of the Specific Anticodon Loop Cleavages in
Bacteriophage T4-infected *Escherichia coli* CTr5X

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Anticodon loop cleavages of two host tRNA species occur in bacteriophage T4-infected *Escherichia coli* CTr5X, a host strain restricting phage mutants deficient in polynucleotide kinase (*pnk*) or RNA ligase (*rli*). The cleavage products accumulate with the mutants but are further processed in *wt* infection through polynucleotide kinase and RNA ligase reactions. Inactivating mutations in *stp* suppress *pnk*⁻ or *rli*⁻ mutations in *E. coli* CTr5X and, as shown here, also abolish the anticodon nuclease, implicating the *stp* product with this activity. We show also that there exist other suppressing mutations of a *pnk*⁻ (*pseT2*) mutation that appear not to affect the anticodon nuclease and are not in *stp*. It has been shown that a single locus in *E. coli* CTr5X, termed *prr*, determines the restriction of *pnk*⁻ or *rli*⁻ mutants. A transductant carrying *prr* featured upon infection the anticodon nuclease reaction products, suggesting that *prr* determines the specific manifestation of this activity. However, *prr* does not encode the tRNA species that are vulnerable to the anticodon nuclease.

1. Introduction

Polynucleotide kinase (Richardson, 1965) and RNA ligase (Silber *et al.*, 1972) have been investigated thoroughly *in vitro* and were applied in many synthetic and analytical reactions (Kleppe & Lillehaug, 1977; Higgins & Cozzarelli, 1979; Uhlenbeck & Gumport, 1982) but their physiological roles are not well-understood (cf. Snyder, 1983). Polynucleotide kinase catalyzes γ-phosphoryl transfer from ATP to 5′-OH polynucleotide termini. The protein also harbors a 3′-phosphatase function. The locus encoding these functions is termed interchangeably *pseT* or *pnk* (Becker & Hurwitz,

1967; Depew & Cozzarelli, 1974; Cameron & Uhlenbeck, 1977; Sirotkin *et al.*, 1978) but we shall refer to polynucleotide kinase deficient mutants as *pnk*⁻ for convenience.

RNA ligase catalyzes phosphodiester bond formation between 3′-OH (acceptor) and 5′-P (donor) termini of polynucleotides, single-stranded ribonucleotide acceptors being preferred (Uhlenbeck & Gumport, 1982). RNA ligase is the product of gene *63* (Snopek *et al.*, 1977), which also encodes a tail fiber attachment activity (TFA, Wood & Henninger, 1969). Despite their coresidence, RNA ligase and TFA are unrelated in their requirements (Snopek *et al.*, 1977) and can be differentiated by distinct mutations (Runnels *et al.*, 1982). We shall refer to RNA ligase deficient mutants as *rli*⁻.

It has been suggested that polynucleotide kinase

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and RNA ligase participate in host tRNA cleavage and reunion (Kaufmann & Kallenbach, 1975; David et al., 1979). This hypothesis was essentially confirmed by following the fate of host tRNA during phage infection of *Escherichia coli* CTr5X (David et al., 1982a,b; Kaufmann & Amitsur, 1985). This strain is restrictive to *pseT$^-$* (Depew & Cozzarelli, 1974), *pnk$^-$* (Sirotkin et al., 1978) or *rli$^-$* mutants (Runnels et al., 1982). A bacteriophage T4-induced nuclease that cleaves two tRNA species 3' to the anticodon is specifically manifested in infected *E. coli* CTr5X but not in other host strains that are permissive to *pnk$^-$* or *rli$^-$* mutants (David et al., 1982a). The CTr5X-specific cleavages are additional to the cleavage of the host leucine tRNA$_1$, which occurs also in the permissive strains (Kano-Sueoka & Sueoka, 1968; Yudelevich, 1971; David et al., 1982a) and depends on a different T4 protein (David et al., 1982a). Both leucine tRNA$_1$ and the CTr5X-specific tRNA fragments disappear in the course of wild-type infection but only the latter accumulate with *pnk$^-$* and *rli$^-$* mutants, suggesting that the products generated by the anticodon nuclease are normally processed by polynucleotide kinase and RNA ligase through terminal phosphoryl group rearrangement followed by religation (David et al., 1982b). Indeed, a tRNA species corresponding to the ligation product of the CTr5X-specific fragments *in vitro* is found in uninfected *E. coli* CTr5X as well as late in *wt* infection but is depleted in *rli$^-$* infection. Apparently, this tRNA species is first cleaved and then religated, without undergoing a net change (Kaufmann & Amitsur, 1985).

Inactivating mutations in another T4 gene (*stp*) allow the growth of *pseT$^-$* (*pnk$^-$*) and *rli$^-$* mutants in *E. coli* CTr5X (Depew & Cozzarelli, 1974; Runnels et al., 1982). We report here that *stp$^-$* mutants do not generate the anticodon nuclease reaction products, indicating that *stp* encodes a protein needed for the nuclease activity. In addition, we describe here another suppressor of at least one *pseT* mutation that is distinct from *stp*.

The *E. coli* CTr5X locus that restricts T4 *pnk$^-$* and *rli$^-$* mutants (*prr*) was previously mapped in the *E. coli* CTr5X chromosome (Abdul-Jabbar & Snyder, 1984). We show here that *prr* determines the specific manifestation of the anticodon nuclease activity upon T4 infection but does not encode the cleaved tRNA.

2. Materials and Methods

(a) Strains

The phage mutants in 3'-phosphatase and polynucleotide kinase. *pseT2* and *pseTΔ1*. were isolated by Depew & Cozzarelli (1974) and Sirotkin et al. (1978). respectively. The RNA ligase mutant *rli-13* was isolated by Runnels et al. (1982). Spontaneous revertants of *pseT* and *rli* mutants were isolated by plating the parent strains on *E. coli* CTr5X according to Depew & Cozzarelli (1974). *SaΔ3* and other *stp* deletion mutants were described by Depew et al. (1975). The bacterial strain *E. coli* CTr5X was constructed by Depew & Cozzarelli (1974) by crossing an *E. coli* K-12 strain with the clinical isolate CT196. *E. coli* B834 was isolated by Wood (1966). The isolation of a phage P1 transductant of *E. coli* B834 that is termed BJMn10 and carries the *prr* (polynucleotide kinase. RNA ligase restricting) locus of *E. coli* CTr5X and a nearby *tet* gene. as well as the isolation of Tet^s deletion mutants of *E. coli* BJMn10 without (BJMn11) or with (BJMn12) the *prr* function. were described by Abdul-Jabbar & Snyder (1984). Table 1 summarizes the relevant properties of these strains.

(b) Determination of anticodon nuclease activity *in vivo*

Host tRNA was pulse-labeled *in vivo*. extracted at various times of T4 infection. and analyzed according to

Table 1
Bacteriophage and bacterial strains

Strains, phage or bacteria	Relevant properties	Reference or source
A. T4 phage strains		
saΔ3	Deletion spanning *stp* and *ac*	Depew et al. (1975)
pseTΔ1	Deletion including *pseT*	Snyder et al. (1976)
pseT2	Point mutant in *pseT*	Depew & Cozzarelli (1974)
rli-13	Point mutant in *rli*	Runnels et al. (1982)
pseT2 revertants:		
Ra	Apparent Pnk$^+$	This study
Rb	*pseT2, stp$^-$*	This study
Rc	*pseT2*, mutated in a novel suppressor, distinct from *stp* and *pseT*	Thus study
B. E. coli strains		
CTr5X	Hybrid K-12 × CT196, restrictive to *pseT$^-$* or *rli$^-$* phage (*prr^r*)	Depew & Cozzarelli (1974)
B834	*E. coli* B *met$^-$ r$_B^-$ m$_B^-$*	Wood (1966)
BJMn10	Tc^r, *prr^r* transductant from the cross CTr5X × B834	Abdul-Jabbar & Snyder (1984)
BJMn11	Tc^s, *prr^o* deletion mutant derived from BJMn10	Abdul-Jabbar & Snyder (1984)
BJMn12	Tc^s deletion mutant derived from BJMn10	Abdul-Jabbar & Snyder (1984)

David et al. (1982a). To follow the kinetics of formation and further processing of host tRNA fragments during infection, the bands containing them were excised from the gel and counted.

(c) RNA fingerprint analysis

This was carried out according to Brownlee (1972) and Silberklang et al. (1979).

(d) RNA–DNA hybridization

DNA of the indicated bacteria was digested to completion with HindIII restriction endonuclease. The DNA fragments were blotted onto nitrocellulose (Southern, 1975) and probed with (5'-^{32}P)-labeled CTr5X-specific tRNA fragment 3'-A. The hybridization was carried out in 0·5% (w/v) sodium dodecyl sulfate, 0·02% (w/v) bovine serum albumin, 0·02% (w/v) polyvinylpyrrolidone, 0·02% (v/v) Ficoll, 0·01% (w/v) denatured salmon sperm DNA in 6 × SSC containing 10⁴ to 10⁵ cts min⁻¹ ml⁻¹ of the tRNA fragment (Denhardt, 1966; Maniatis et al., 1982) for approximately 4 h at 52°C (SSC is 0·15 M-NaCl, 0·015 M-trisodium citrate, pH 7). The filter was washed in 2 × SSC at 25°C and autoradiographed.

3. Results

(a) stp mutants are deficient in the anticodon nuclease

The T4-induced anticodon nuclease was monitored in vivo by following the appearance of host tRNA fragments in infected E. coli CTr5X cells. We previously designated these fragments by roman numerals, according to their electrophoretic mobility (David et al., 1982a,b). Here, we rename them 5'-L (I), 3'-L (IV), 3'-A (II), 3'-B (III), 5'-B (V) and 5'-A (VI), to denote, respectively, the 5' or 3' portions of leucine tRNA₁ (L) or the major (A) or minor (B) fragment pairs generated by the anticodon nuclease.

Since stp⁻ mutations suppress the effects of pnk⁻ and rli⁻ mutations in E. coli CTr5X, it seemed possible that stp encodes the nuclease responsible for the anticodon cleavages. Accordingly, stp mutants were assayed for their ability to induce them. Among the strains investigated were mutants with deletions in stp and the nearby ac gene (Depew et al., 1975). Figure 1 shows a host tRNA cleavage pattern obtained during infection of E. coli CTr5X with saΔ3, a strain with a deletion of 230(±50) base-pairs that spans stp and ac only (Depew et al., 1975). This strain and other sa deletion mutants did not induce the cleavage of the CTr5X-specific tRNAs. This by itself does not prove that the stp gene is responsible for the anticodon cleavages, since more than one gene is deleted in saΔ3. Since spontaneous apparent revertants of pnk⁻ and rli⁻ mutants usually have point mutations in stp, we examined such mutants for their ability to induce the anticodon nuclease activity. We have isolated them from the following parent strains: pseTΔ1, pseT2 and rli-13 (see Table 1). The seven revertants of pseTΔ1 that were
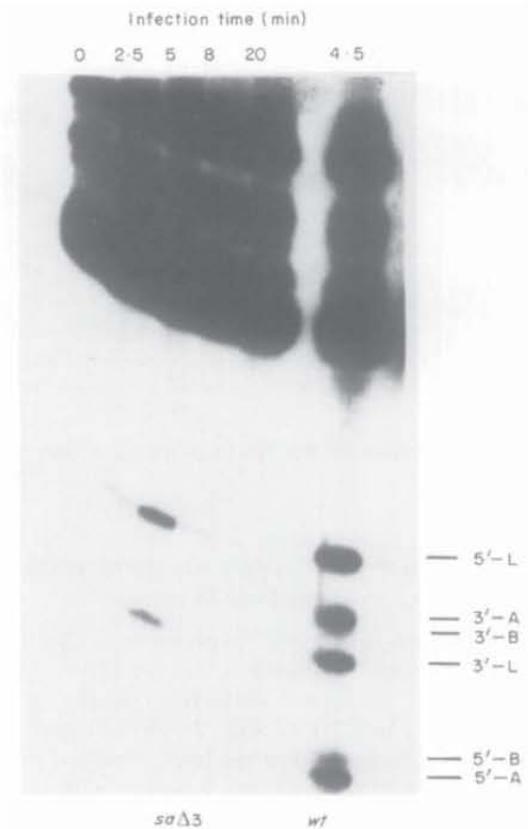
Figure 1. Host tRNA cleavage patterns during infection of E. coli CTr5X with a T4 stp deletion mutant. Host tRNA cleavage during infection of E. coli CTr5X with the T4 strain saΔ3 was followed as described in Materials and Methods. A time-point from wt infection was included to mark the positions of the anticodon nuclease reaction products. 5'-L and 3'-L denote leucine tRNA₁ fragments, while 5'-A, 3'-A, 5'-B and 3'-B denote the major (A) and minor (B) tRNA fragment pairs generated by the anticodon nuclease.

examined generated the leucine tRNA₁ fragments but not the anticodon nuclease reaction products similar to the stp deletion mutants. Also, of ten revertants obtained from the strain pseT2, two that were designated pseT2 Rb were specifically impaired in the anticodon nuclease, similar to saΔ3. When the Rb revertants were crossed with the pseT2, saΔ3 double mutant, no recombinants able to grow on E. coli CTr5X were obtained, indicating that the inactivating Rb mutations were also in stp. Among the rli-13 revertants characterized, one generated and further processed the anticodon nuclease reaction products, similar to wt phage. Apparently, this revertant regained the Rli⁺ phenotype. The second rli-13 revertant lacked only the anticodon nuclease reaction products, as found with defined stp⁻ mutants. Taken together, these results indicated that stp determined the expression of the anticodon nuclease. Incidentally, the leucine tRNA₁ fragments appeared after infection with stp⁻ mutant, confirming that this tRNA is cleaved by a different enzyme, distinct from the anticodon nuclease.
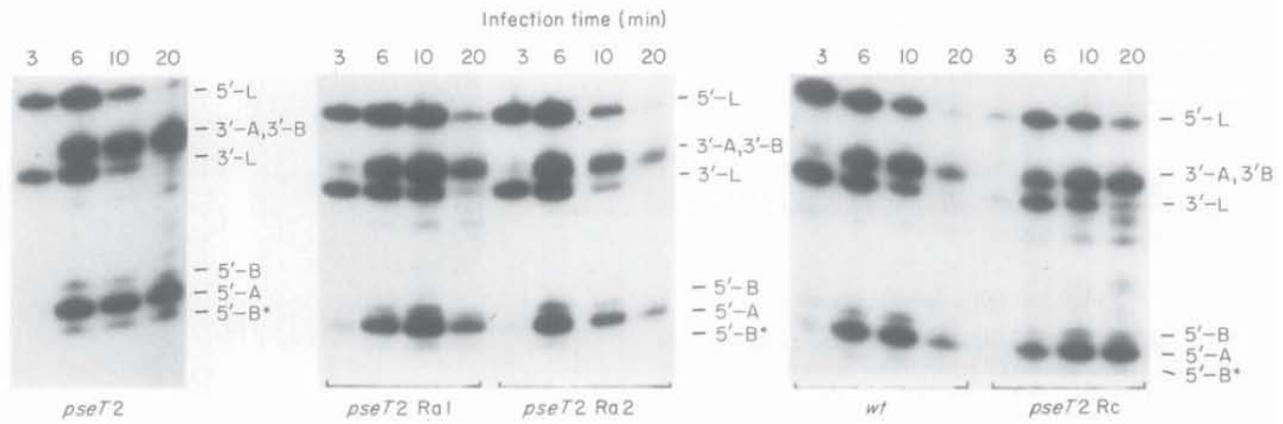
Figure 2. Host tRNA cleavage patterns during infection of *E. coli* CTr5X with some revertants of the phage strain *pseT*2.

(b) *Other* pnk⁻ *suppressor mutations that are distinct from* stp

There were eight *pseT*2 revertants that did not feature the *stp*⁻ phenotype, i.e. selective loss of the anticodon nuclease activity. Most of these revertants (*pseT*2 Ra, Fig. 2) yielded and further processed the anticodon nuclease reaction products, similar to wild-type phage. Another wild-type trait in some of these revertants was the lack of the 5'-B* derivative (V*). This derivative is characteristic of *pseT* and *rli* lesions (David *et al.*, 1982*b*). Therefore, it is conceivable that the Ra reversions restored the Pnk⁺ phenotype to varying degrees. One of the eight revertants that did not feature the *stp*⁻ phenotype yielded and apparently accumulated the anticodon nuclease reaction products (Fig. 2, *pseT*2 Rc), similar to the *pseT*2 parent strain. The

revertant *pseT*2 Rc yielded recombinants that were restricted on *E. coli* CTr5X when crossed with *sa*Δ3 phage. Thus, the suppressing mutation in *pseT*2 Rc seems to reside in a gene other than *stp*, and is probably too far removed from *pseT* to be an intracistronic suppressor.

(c) *Cotransduction of CTr5X* prr *function and ability to manifest the anticodon nuclease activity*

To determine whether *prr* is related to the specific manifestation of the anticodon nuclease activity in *E. coli* CTr5X, we examined *E. coli* BJMn10, a *prr*-transductant of the permissive recipient strain *E. coli* B834 (Abdul-Jabbar & Snyder, 1984) for host tRNA cleavage patterns. Indeed, we found that *E. coli* BJMn10, but not B834, featured a transient appearance of tRNA
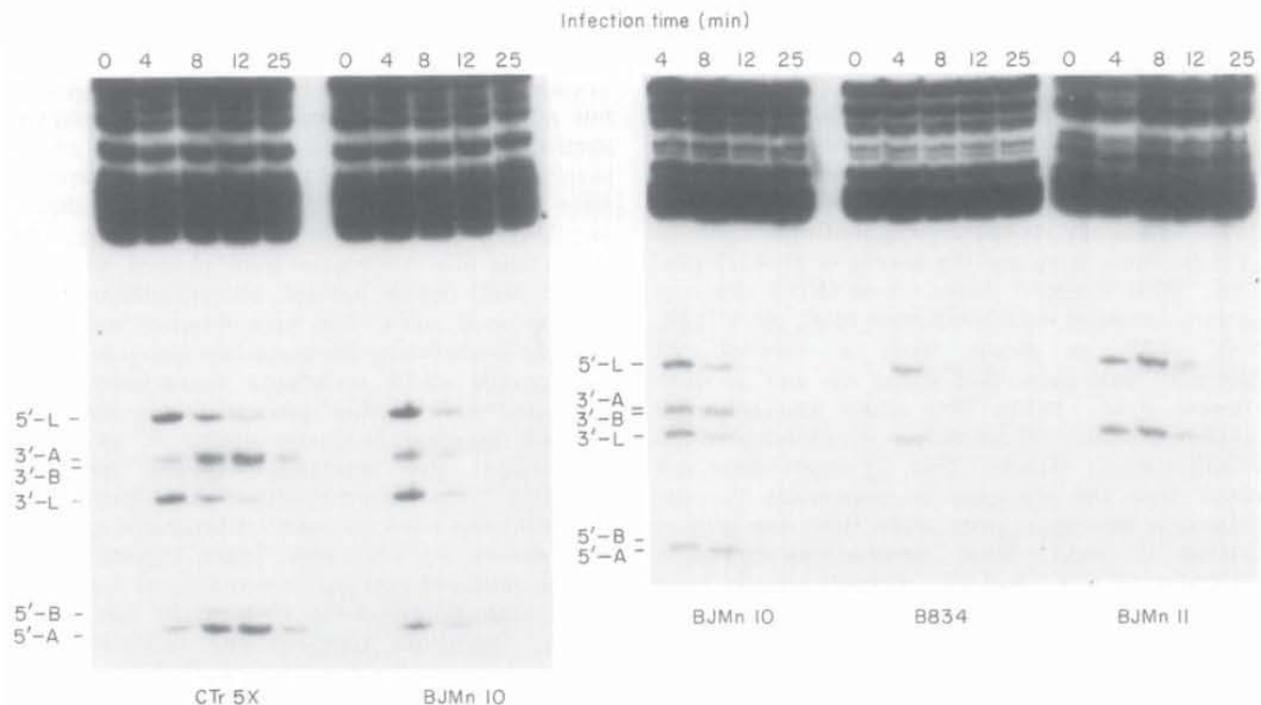
Figure 3. Comparison of host tRNA cleavage patterns of T4-infected *E. coli* B834, BJMn10, BJMn11 and CTr5X.
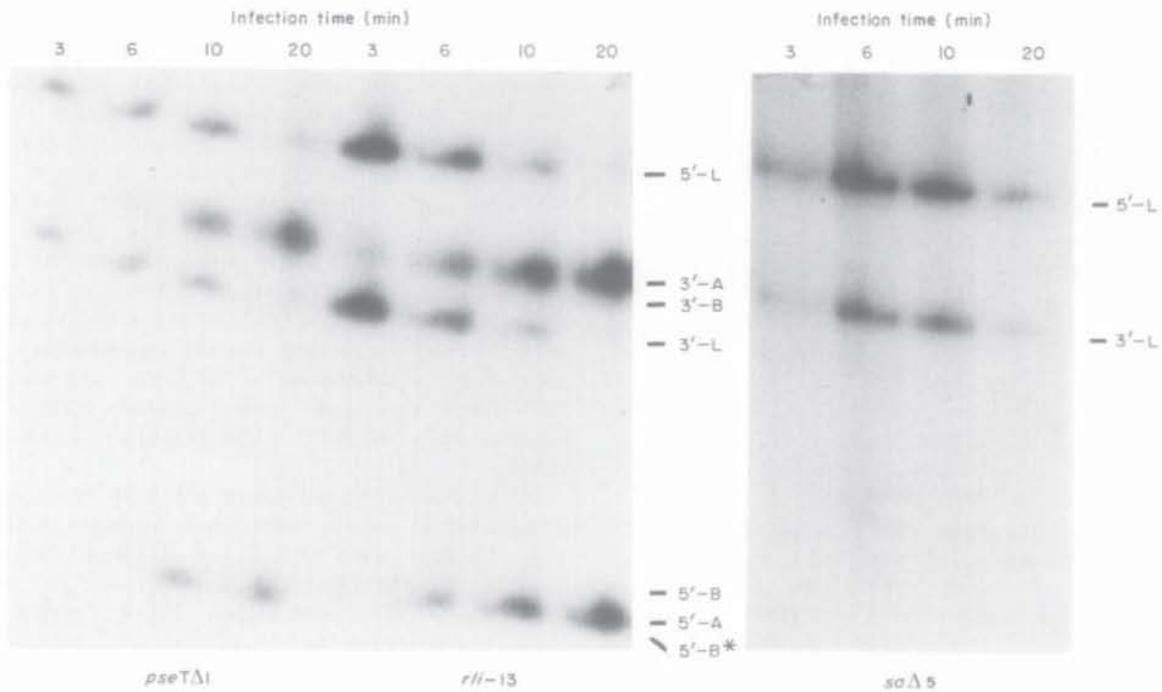
Figure 4. Host tRNA cleavage patterns of *E. coli* BJMn10 infected with various T4 mutant strains

fragments matching in electrophoretic mobility those specific to *E. coli* CTr5X. That these products were not seen with the permissive recipient *E. coli* B834 (Fig. 3) agrees with results reported for other *pnk*⁻ permissive strains (David *et al.*, 1982a). Likewise, during infections of *E. coli* BJM10 with *pseT*⁻ or *rli*⁻ strains, the matching products accumulated while with *stp*⁻ strains they did not appear (Fig. 4), as found with the corresponding

infections of *E. coli* CTr5X (David *et al.*, 1982b; and Fig. 1). Comparisons of RNase T₁ fingerprints indicated that the BJMn10 tRNA fragments were similar to, if not identical with, the CTr5X counterparts (shown for 5′-A in Fig. 5). Thus, the host tRNA cleavage phenotype exhibited by the transductant BJMn10 was qualitatively similar to that of CTr5X.

Despite this qualitative similarity, the transient
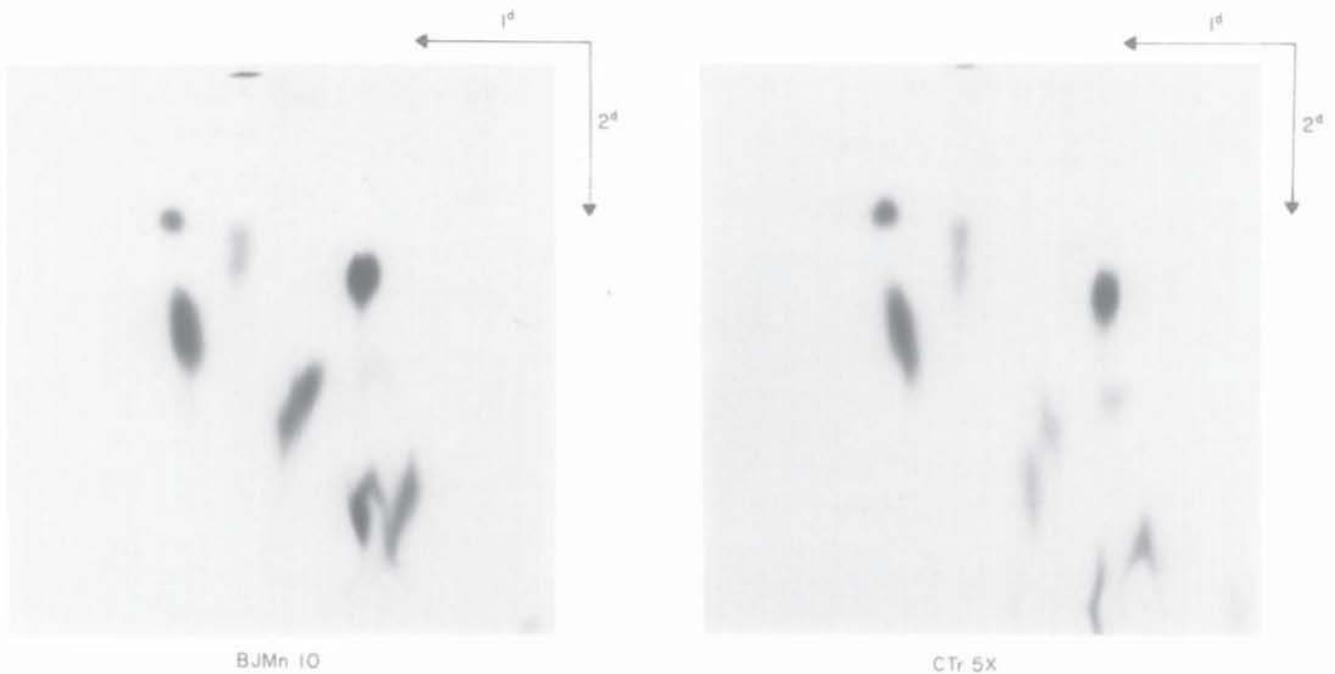
Figure 5. Comparison of RNase T₁ fingerprints of the anticodon nuclease product 5′-A from *E. coli* CTr5X and BJMn10.
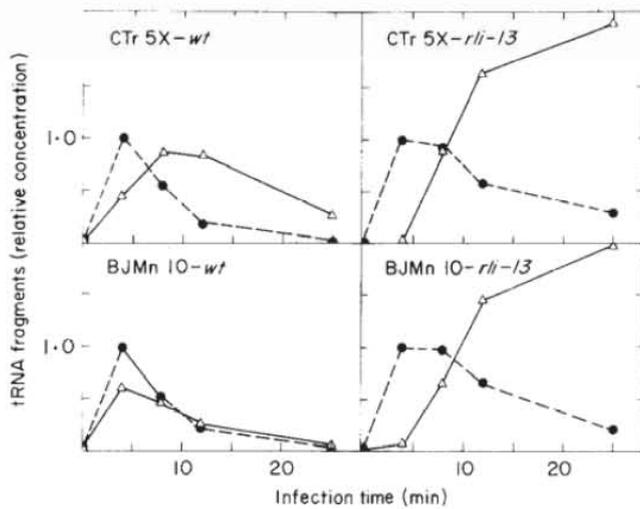
Figure 6. Kinetics of host tRNA cleavage and further processing in T4-infected *E. coli* CTr5X and BJMn10. The amounts of leucine $tRNA_1$ (●) and CTr5X-specific (△) tRNA fragments were normalized relative to that of leucine $tRNA_1$ fragments at 4 min infection time.

level of the anticodon nuclease reaction products (relative to that of leucine $tRNA_1$ fragments) during *wt* infection of *E. coli* BJMn10 was found to be about twofold lower than in the corresponding CTr5X infection (Fig. 6). In *rli⁻* (and probably *pnk⁻*) infections of BJMn10, the maximal extent of

anticodon nuclease reaction products was similar to that seen with *E. coli* CTr5X but was attained at a somewhat slower rate. These data suggested a decreased rate of anticodon loop cleavage but an unaltered rate of further processing of the cleavage products in the *prr* transductant strain *E. coli* BJMn10 and, furthermore, that the vulnerable tRNA levels are similar in the donor and transductant strains. In agreement with these observations, there are slight differences between *E. coli* BJMn10 and CTr5X in the strength of the *prr*-mediated restriction. On the transductant, the restriction is alleviated at a lower temperature (37°C) and some *pnk⁻* point mutants form small plaques even at 30°C (Abdul-Jabbar & Snyder, 1984).

Other evidence connecting *prr* with the specific manifestation of the anticodon nuclease activity was obtained with the *E. coli* BJMn10 deletion derivative BJMn11, which has lost the *prr* locus (see Table 1). The host tRNA cleavage pattern of BJMn11 resembled that of permissive *E. coli* strains (Fig. 3).

(d) prr *does not encode the vulnerable tRNAs*

We examined whether the specific manifestation of the anticodon nuclease was due to the encoding of the vulnerable tRNA species by *prr*. We compared the hybridization patterns of a $(5'-^{32}P)$-labeled vulnerable tRNA fragment (5'-A) to
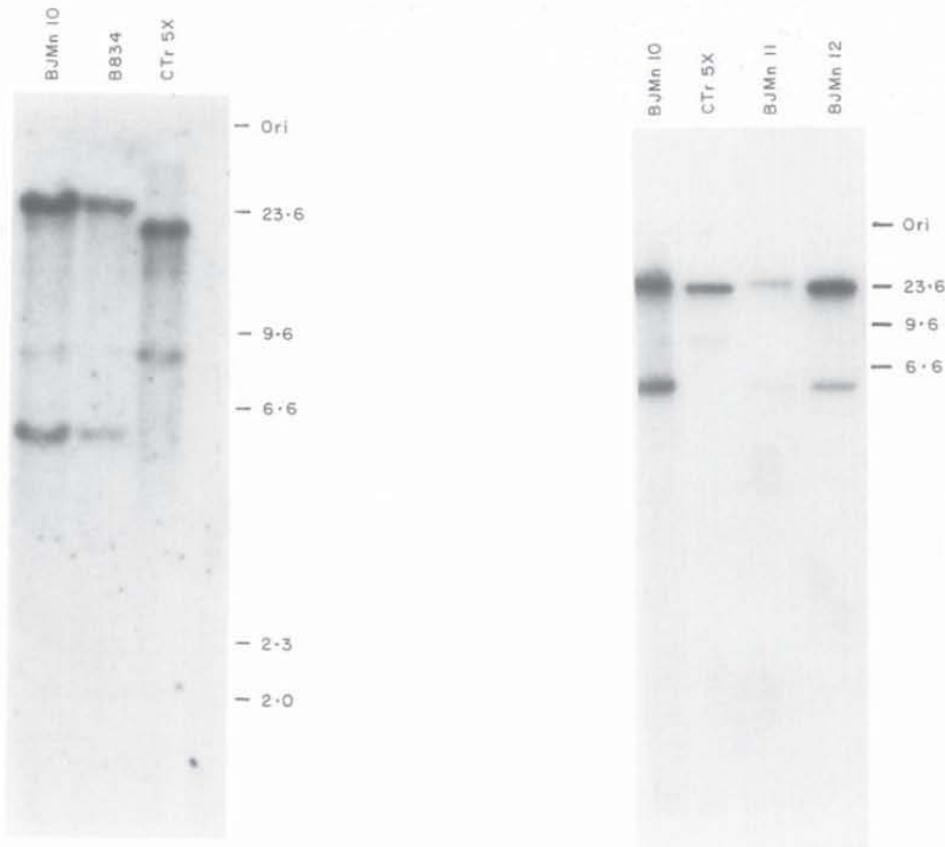
Figure 7. Hybridization of $(5'-^{32}P)$-labeled CTr5X-specific tRNA fragment 3'-A to Southern blots of *Hind*III restricted DNAs from *prr*ᵖ or *prr*ᶠ *E. coli* strains. Sizes $(\times 10^{-3}$ base-pairs) are indicated.

Southern blots of restricted genomic DNAs from *E. coli* B834, BJMn10, BJMn11, BJMn12 and CTr5X. Strikingly, the same *Hind*III restriction fragments were lit up by the probe in the four B strains, whether *prr* permissive or *prr* restrictive (Fig. 7). The identity of *E. coli* BJMn10 and BJMn11 patterns, despite the large deletion that removes *prr* from the latter (Green & Snyder, unpublished results) suggested by itself that the vulnerable tRNA genes exist in both *prr*^{r} and *prr*^{p} strains, and that *prr* does not encode the vulnerable tRNA species. The difference observed between *E. coli* CTr5X and the other strains probably indicates a corresponding difference between the organization of the region in the genome encoding the vulnerable tRNA species in *E. coli* B and K-12 strains, since the CTr5X pattern resembled that of the permissive K-10 strain (not shown).

4. Discussion

(a) stp *probably encodes an anticodon nuclease protein*

We have shown that the *stp* gene of T4, previously defined as an extracistronic suppressor of *pnk*$^-$ and *rli*$^-$ mutations (Depew & Cozzarelli, 1974; Depew *et al.*, 1975; Sirotkin *et al.*, 1978; Runnels *et al.*, 1982), is required for the anticodon nuclease activity (Fig. 1). *stp* is transdominant in mixed infections, indicating that it encodes a diffusable product (Depew & Cozzarelli, 1974), while the anticodon nuclease depends on a translation event set on two to three minutes post-infection (David *et al.*, 1982*a*), commensurate with the expected time needed by RNA polymerase to travel from early promoters at 164,500 base-pairs to *stp* (Gram *et al.*, 1984). Hence, *stp* is probably a structural gene of the anticodon nuclease or a subunit of it. We have found at least one other extracistronic suppressor that is distinct from *stp* for at least one *pnk* mutant. However, it may be unrelated to the anticodon nuclease.

(b) prr *determines the specific manifestation of the anticodon nuclease*

We have shown that the presence of the *prr* locus causes the appearance of the anticodon nuclease reaction products after infection (Figs 3 to 5). Regarding the nature of *prr*, based on the similar hybridization patterns of the vulnerable tRNA fragment to *prr*^{r} and *prr*^{p} genomes, it appears not to encode the vulnerable tRNAs (Fig. 7). Moreover, a *prr*-cosmid probe lights up homologous DNA restriction fragments in the restrictive genomes of *E. coli* CTr5X and BJMn10 but not in the permissive B834 and BJMn11 (Green & Abdul-Jabbar, unpublished results). Therefore, we consider other possible roles of *prr*. One is to encode an activity connected with tRNA modification. According to this view, the vulnerable BJMn10 or CTr5X tRNAs may differ from their putative counterparts in the permissive host in a post-transcriptional modification. To examine this possibility we are currently comparing the major vulnerable tRNA species from *E. coli* CTr5X and BJMn10 with a corresponding B834 species.

A second possible role of *prr* is to encode a subunit or factor of the anticodon nuclease. This view predicts that an anticodon nuclease activity may be found only in infected *prr*^{r} cells. The reduced rate of anticodon cleavages in *E. coli* BJMn10, compared to CTr5X (Fig. 6), can be fitted in each of the above two schemes by assuming in the first case a weaker reactivity of the vulnerable BJMn10 tRNA due to a different degree of base modification, or a lower level of the anticodon nuclease host component in the transductant, in the second.

The third possibility is that *prr* encodes an inhibitor of a putative host ligation pathway. Accordingly, the vulnerable tRNA species are also cleaved in *prr*^{p} strains but cannot be detected because they are rapidly processed. By contrast, in *prr*^{r} strains the host pathway is inhibited and the phage depends on its *pnk* and *rli* products for repair of the vulnerable tRNAs. Hence, the vulnerable tRNA fragments may appear transiently before sufficient levels of these enzymes have accumulated, if the *stp* product appears first.

(c) *Functional relation of anticodon nuclease, polynucleotide kinase and RNA ligase and possible mechanism of* prr *restriction and its suppression*

The suppression of *pnk*$^-$ or *rli*$^-$ mutations in *E. coli* CTr5X by a second mutation that inactivates the anticodon nuclease reinforces previous conclusions that the anticodon nuclease reaction products must be further processed in this host, presumably through phosphoryl group rearrangement followed by religation (David *et al.*, 1982*a,b*). We have shown elsewhere that at least one *E. coli* CTr5X tRNA species is cleaved and religated during T4 infection without a detectable net change (Kaufmann & Amitsur, 1985).

For the sake of simplicity, we assume that the cleavage and religation products that we have monitored, and not others that might have escaped detection, are responsible for the phage restriction phenomenon. We attribute *prr* restriction to the cleavage of the vulnerable tRNA species which, in turn, may cause the previously observed lesions in T4 DNA replication and late gene expression (Depew & Cozzarelli, 1974; Sirotkin *et al.*, 1978; Runnels *et al.*, 1982). It is noteworthy that the inhibition of late protein synthesis is indiscriminate, i.e. both true late gene products and early gene products whose syntheses are not turned off normally even late in infection are affected (Runnels *et al.*, 1982), lending support to the proposal of a primary *prr* effect at the level of translation. However, *prr* has been shown to restrict mutants in *mot* (D. Hall, personal communication), a T4 gene that modulates middle

mode transcription (see Brody *et al.*, 1983). Therefore, interference with a transcriptional process cannot be excluded.

We propose two general hypotheses to explain the relation between the cleavage of the vulnerable tRNAs and inhibition of T4 development. According to one, the anticodon nuclease reaction products themselves are inhibitory to a phage process, perhaps by acting as tRNA analogs. According to the second, damage is caused by depletion of one or more vulnerable tRNA species. Although we cannot disprove either hypothesis, we can try to evaluate them by considering relevant data.

The inhibition of late protein synthesis and *prr* restriction are alleviated at 42°C (Runnels *et al.*, 1982) but the anticodon nuclease reaction products still accumulate at this temperature (unpublished results). It is possible that the cleaved tRNAs are partially melted at the elevated temperature, with consequent reduction of their inhibitory potential. Alternatively, the lack of the depleted tRNA may be compensated by another pathway that functions at the higher temperature.

Another problem posed by these models is that the anticodon nuclease reaction products persist in *wt* infection for a considerable time, during which they are "tolerated". This problem is accentuated by the existence of a *pseT2* revertant (Fig. 2, *pseT2* Rc) that still accumulates the anticodon nuclease reaction products. Thus, on the one hand, to exert an inhibitory effect, the products must reach a high threshold level at a critical time point in infection. On the other hand, the depletion model requires that the false revertant with the undetectable anticodon nuclease impairment allows sufficient levels of the intact vulnerable tRNA, or bypasses the need for it.

The existence of the cleavage repair pathway raises a question about the advantage provided by phage genes whose products interfere with its own development. Clearly, indicator host strains on which *stp* is essential should be sought in a quest for possible advantages of the anticodon nuclease or other functions that the *stp* product may assume. Additional roles of the anticodon nuclease, polynucleotide kinase and RNA ligase in host tRNA inactivation, conversion into new species or replacement by phage-encoded tRNAs do not seem to apply in *E. coli* CTr5X, since inactivation of the anticodon nuclease restores growth on the *prr*[r] host. Nonetheless, such roles may be relevant in other hosts.

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