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Phage T4-induced dTTP accretion bolsters a tRNase-based host defense

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ABSTRACT

The anticodon nuclease (ACNase) PrrC is silenced in *Escherichia coli* by an associated DNA restriction-modification protein, activated by the phage T4-encoded anti-DNA restriction factor Stp and counteracted by T4's tRNA repair enzymes polynucleotide kinase and RNA ligase 1. Hence, only tRNA repair-deficient phages succumb to PrrC's restriction. PrrC's ABC-ATPase motor domains are implicated in driving its activation by hydrolyzing GTP and in stabilizing the activated ACNase by avidly binding dTTP. The latter effect has been associated with dTTP's accumulation early in T4 infection when PrrC is activated. In agreement, delayed dTTP accumulation caused by dCMP deaminase deficiency coincided with impaired manifestation of PrrC's ACNase activity. This impairment did not suffice to suppress the PrrC-mediated restriction of tRNA repair deficient phage but was synthetically suppressive with a leaky *stp* mutation that only partly impairs PrrC's activation. Presumably, ability to gauge dTTP's changing level helps confine PrrC's toxicity to its viral target.

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Introduction

During their everlasting struggle, bacteria and phages evolved numerous survival measures and countermeasures (Labrie et al., 2010). In some cases, a defensive tool of one protagonist is co-opted as an offensive device of the other. A case in point is the coupled bacterial DNA–tRNA *prr* restriction system (Jabbar and Snyder, 1984; Levitz et al., 1990; Tyndall et al., 1994; Penner et al., 1995). *prr* loci are found in distantly related bacterial groups (mainly in *Proteobacteria*, *Bacterioidetes*, less in *Firmicutes,* hardly among *Actinobacteria* and apparently not at all in *Cyanobacteria*). These loci appear sporadically present in some strains of a given species but not in others, as would a niche function. A *prr* locus comprises four open reading frames. Three of them, *prrABD*/*hsdMSR* encode respective methylase, specificity and restriction nuclease subunits of a type Ic DNA restriction-modification (R-M) protein. The fourth, *prrC* encodes a $tRNA^{Lys}$ specific anticodon nuclease (ACNase) (Levitz et al., 1990; Tyndall et al., 1994). In *prr* encoding *Escherichia coli* strains, PrrC's ACNase activity is normally kept inactive by PrrC's physical association with the linked R-M protein EcoprrI (Amitsur et al., 1992; Tyndall et al., 1994). The latent ACNase holoenzyme (PrrC-EcoprrI complex) is activated during phage T4 infection by a minuscule peptide encoded by the 26-codon T4 *stp* gene. Stp exhibits also anti-DNA restriction activity, probably its intended function. Mutational analysis indicated that the conserved N-proximal 18 residue region of Stp is critical both for its anti-DNA restriction and ACNase activating functions (Penner et al., 1995). Once activated, the PrrC ACNase incises $tRNA^{Lys}$ 5′ to the wobble base, yielding 2′, 3′-cyclic phosphate and 5′-OH termini (Amitsur et al., 1987). The lesion inflicted by PrrC could disable T4 late protein synthesis and contain the infection (Sirotkin et al., 1978) since T4 shuts-off host transcription (Mathews, 1994) and does not encode its own $tRNA^{Lys}$ (Schmidt and Apirion, 1983). However, T4 overcomes this hurdle using the 3′-phosphatase/5′-polynucleotide kinase (PseT/Pnk) and RNA ligase 1 (Rnl1) proteins it encodes. These proteins heal and seal in respective order the cleavage termini generated by PrrC, restoring the intact form of $tRNA^{Lys}$ (Amitsur et al., 1987). Consequently, only tRNA repair-deficient phage mutants succumb to PrrC's mediated restriction. This restriction and the locus encoding it were consequently termed *prr* (*pnk*, *rnlA* restricting; Jabbar and Snyder, 1984). Not surprisingly, mutating PrrC's activator Stp suppresses *prr* restriction (Depew and Cozzarelli, 1974; Depew et al., 1975; Kaufmann et al., 1986; Penner et al., 1995). Table 1 lists the various gene products directly or indirectly involved in this restriction/anti-restriction cascade.

While nested *prr* loci where *prrC* is inserted between the R-M genes abound, stand-alone *prrC* genes have not been detected so far. This fact and the similar ACNase activities of several PrrC orthologs examined (Davidov and Kaufmann, 2008; Meineke et al., 2010)

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Table 1
Direct and peripheral components of the *prr* restrictions/anti-restrictions cascade.

Component	Function
E. coli EcoprrI	DNA restriction-modification, silences PrrC's ACNase activity
E. coli PrrC	$tRNA^{Lys}$ ACNase, potential disabler of phage protein synthesis
T4 Stp	Phage anti-DNA restriction factor and host co-opted activator of PrrC
T4 PseT/Pnk	3'-phosphatase/5'-polynucleotide kinase, heals termini formed by PrrC
T4 Rnl 1	RNA ligase, ligates back the termini healed by PseT/Pnk
T4 Cd	dCMP deaminase, contributes to dTTP's accretion, thus safeguards faithful replication of the phage DNA and stabilizes the activated PrrC ACNase

suggest that PrrC functions in general as an antiviral back-up function mobilized when an associated DNA restriction nuclease is compromised. The importance of PseT/Pnk and Rnl 1 as PrrC's countermeasures is suggested by their widespread occurrence among T4-like phages. An exception are the five known T4-like cyanophages, which may not encounter a *prr* encoding host (Miller et al., 2003; Blondal et al., 2005; Blondal et al., 2003; and http://phage.ggc.edu/). Moreover, docking tRNA onto the crystal structure of PseT/Pnk or Rnl1 suggested that both could have evolved to repair a disrupted anticodon loop (Galburt et al., 2002; El Omari et al., 2006). Hence, PseT/Pnk and Rnl1 are collectively referred to as T4's tRNA repair enzymes.

Activating the latent ACNase *in vitro* requires apart from Stp, the DNA tethered to EcoprrI, GTP hydrolysis and presence of dTTP (Amitsur et al., 2003). Expressing PrrC ectopically in the absence of EcoprrI elicits overt (core) ACNase activity that is refractory to Stp, DNA and GTP but exceedingly unstable without dTTP (Morad et al., 1993; Amitsur et al., 2003; Blanga-Kanfi et al., 2006). These facts suggest that the protection of the core ACNase by dTTP reflects the role of this nucleotide in stabilizing the activated ACNase. GTP and dTTP exert their respective activating and protecting functions via PrrC's ABC-ATPase domains, to which they bind with vastly differing affinities (mM- and μM-range, respectively) and without displacing each other (our unpublished results). This versatile nucleotide specificity and PrrC's unique aromatic/acidic-rich PrrC Box motif implicated in conferring it (Blanga-Kanfi et al., 2006; and our unpublished results) distinguish PrrC from typical ABC ATPase containing proteins (Chen et al., 2003; Moody and Thomas, 2005; Guo et al., 2006).

The level of dTTP increases about five fold early in phage T4 infection, possibly to safeguard effective and faithful replication of the AT-rich T4 DNA (Greenberg et al., 1994; Sargent and Mathews, 1987). Interestingly, dTTP's accumulation occurs in parallel with the induction of PrrC's ACNase activity. This coincidence and the *in vitro* stabilization of the ACNase by dTTP have suggested that dTTP's accretion during the infection and the induction of PrrC's ACNase activity are causally related (Amitsur et al., 2003; Blanga-Kanfi et al., 2006). Data shown here lend credence to the notion that dTTP's accretion is another phage device co-opted by the bacterial host, in that case to bolster its ACNase-mediated defense and confine it to the viral target.

Results

Coincident impairment of the T4-induced dTTP accretion and PrrC ACNase activity

To examine if dTTP's accumulation and manifestation of PrrC's ACNase activity during phage T4 infection are causally related we exploited the T4 dCMP deaminase (Cd) deletion mutant *pseTΔ4*. The Cd lesion prevents the conversion of dCMP to dUMP and, consequently, significantly delays dTTP's accumulation. This, in turn, confers a mutator phenotype indicated by an increased A:T→G:C transition rate (Sargent and Mathews, 1987). The Cd deficient strain *pseTΔ4* lacks also the adjacent *pseT/pnk* gene. The isogenic control *pseTΔ5* used in this study lacks *pseT/pnk* but is cd^+. Due to the lack of PseT/Pnk both phage strains cannot reverse the lesion inflicted by PrrC and are susceptible to *prr* restriction (Jabbar and Snyder, 1984; Depew and Cozzarelli, 1974; David et al., 1982). The lack of the tRNA repair enzyme also facilitates monitoring the ACNase during the infection because the $tRNA^{Lys}$ fragments generated by PrrC accumulate (David et al., 1982). We assumed that dependence of PrrC's activation or stability of its activated form on dTTP's accretion will be revealed by delayed and/or weaker induction of the ACNase during infection of a prr^+ host by the cd^- T4 strain *pseTΔ4*. It should be pointed out that *pseTΔ4*'s infection efficiency is lower than *pseTΔ5*'s, possibly due to pleutropic effects of dTTP's deficiency. Therefore, to calibrate the infection efficiencies of the two strains we used as a yardstick the degradation of the host $tRNA^{Leu1}$, which occurs in T4-infected *E. coli* irrespective of PrrC (Kano-Sueoka and Sueoka, 1968; Schmidt and Apirion, 1983). Comparing the $tRNA^{Lys}/tRNA^{Leu1}$ fragment ratios during the infection of the prr^+ host *E. coli* CTr5X (Depew and Cozzarelli, 1974) by either phage strain indicated that the ACNase activity manifested with *pseTΔ4* was significantly weaker than with *pseTΔ5*, whether the infections were performed at 25 °C (Fig. 1) or at 30 °C (not shown).

A. leaky T4 stp mutation suppresses prr restriction over the cd^- background

The partial ACNase-attenuating effect observed with *pseTΔ4* did not suffice to suppress the restriction of this tRNA repair-deficient strain by the prr^+ host. However, *pseTΔ4* yielded ~5000-fold more pseudorevertants able to escape *prr* restriction than *pseTΔ5* (Table 2). This excess could be accounted for at least in part by *pseTΔ4*'s cd^- mutator phenotype, indicated by a 5-fold or ~1,000-fold increase in the A:T→G:C transition rate at different genomic sites (Sargent and

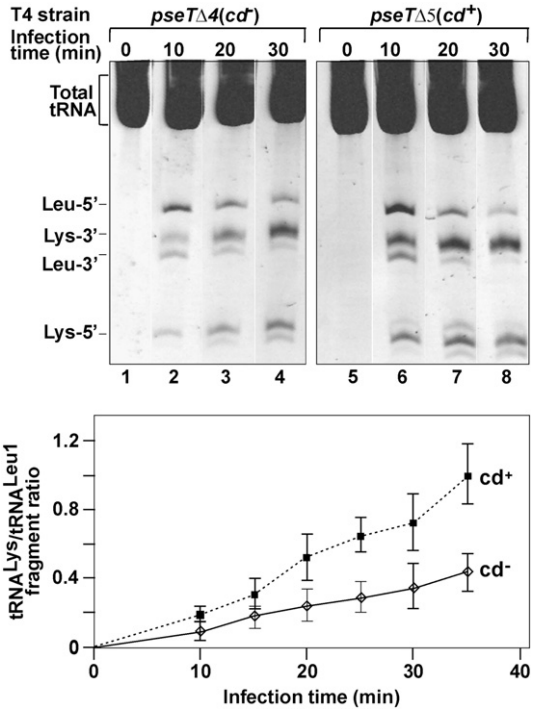

Fig. 1. Attenuated ACNase phenotype of a T4 cd^- mutant. *E. coli* CTr5X (prr^+) was infected by the Cd^- strain *pseTΔ4* (lanes 1–4) or the isogenic Cd^+ strain *pseTΔ5* (lanes 5–8). Low weight RNA aliquots were isolated at the indicated infection times, separated by denaturing PAGE and stained with ethidium bromide. Leu-5′, Leu-3′, Lys-5′ and Lys-3′ are respective 5′ and 3′ cleavage products of $tRNA^{Leu1}$ or $tRNA^{Lys}$. The graph depicts the ratios of the 5′ fragments of the two tRNA species versus infection time.

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