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RloC: A Translation-Disabling tRNase Implicated in Phage Exclusion During Recovery from DNA Damage

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1. Introduction

Bacteria respond to DNA damage by inducing the expression of numerous proteins involved in DNA repair and the reversible arrests of DNA replication and the cell division cycle (Fernandez De Henestrosa *et al*, 2000). This general rule may be violated by a conserved bacterial protein termed RloC (Davidov & Kaufmann, 2008). RloC combines structural-functional properties of two unrelated proteins (i) the universal DNA-damage-responsive/DNA-repair protein Rad50/SbcC (Williams *et al*, 2007) and (ii) the translation-disabling, phage-excluding anticodon nuclease (ACNase) PrrC (Blanga-Kanfi *et al*, 2006). These seemingly conflicting features may be reconciled in a model where RloC is mobilized as an antiviral back-up function during recovery from DNA damage (Davidov & Kaufmann, 2008), when DNA restriction, the cell's primary immune system is temporarily shut-off (Thoms & Wackernagel, 1984). Another intriguing feature of RloC is its ability to excise its substrate's wobble nucleotide (Davidov & Kaufmann, 2008). This harsh lesion is expected to encumber reversal by phage enzymes that repair the tRNA nicked by PrrC (Amitsur *et al*, 1987). Evaluating RloC's salient features and purported role requires prior description of its more familiar distant homolog PrrC and a DNA-damage-sensing device RloC shares with Rad50/SbcC. We conclude with an account of cellular RNA and DNA repair tools related to the phage tRNA repair mechanism that counteracts PrrC and may be frustrated by RloC.

2. PrrC – A potential phage-excluding tool counteracted by tRNA repair enzymes

2.1 A host-phage survival cascade yields an RNA repair pathway

RNA repair may seem unnecessary because damaged RNA molecules can be readily replenished by re-synthesis. Yet, there exist situations where RNA repair could be the preferred or only possible option. A case in point is presented by an RNA repair pathway triggered by the ACNase PrrC. This conserved bacterial protein was detected in quest of roles of two phage T4-encoded enzymes: 3'-phosphatase/5'-polynucleotide kinase (PseT/Pnk,

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henceforth Pnk) (Richardson, 1965;Becker & Hurwitz, 1967;Cameron & Uhlenbeck, 1977) and RNA ligase 1 (Rnl1, Silber *et al*, 1972;Ho & Shuman, 2002). The combined activities of Pnk and Rnl1 seemed tailored to fix RNA nicks, converting 3'-phosphoryl or 2',3'-cyclic phosphate and 5'-OH cleavage ends into 3'→5' phosphodiester linkages (Kaufmann & Kallenbach, 1975;Amitsur *et al*, 1987). Suggested alternative roles in DNA metabolism (Novogrodsky *et al*, 1966;Depew & Cozzarelli, 1974) were assigned in later years to a related eukaryal DNA kinase-phosphatase essential for genome stability and a possible therapeutic target in cancer cells rendered resistant to genotoxic drugs (Weinfeld *et al*, 2011).

Pnk and Rnl1 are dispensable for T4 growth on common *E. coli* laboratory strains but required on a rare host encoding the optional locus *prr* (*pnk* and *rnl1* restriction) (Depew & Cozzarelli, 1974; Sirotkin *et al*, 1978; Runnels *et al*, 1982; Jabbar & Snyder, 1984). Mutating a minuscule T4 orf termed *stp* (*s*uppressor of *t*hree-prime *p*hosphatase) abrogates *prr* restriction (Depew & Cozzarelli, 1974;Depew *et al*, 1975;Chapman *et al*, 1988;Penner *et al*, 1995). These facts reinforced the notion that Pnk and Rnl1 cooperate in RNA nick repair. They also led to the detection of the *prr*-encoded latent ACNase comprising the core ACNase PrrC and PrrC's silencing partner, the associated type Ic DNA restriction-modification (R-M) system EcoprrI (Levitz *et al*, 1990;Linder *et al*, 1990;Amitsur *et al*, 1992;Tyndall *et al*, 1994). EcoprrI and PrrC are also genetically linked, the ACNase core gene *prrC* is flanked by the genes encoding the three R-M subunit types *hsdMSR/prrABD* (Fig. 1A).

Type I R-M systems to which EcoprrI belongs recognize with their HsdS subunit a bipartite target containing a variable 6-8nt long spacer such as EcoprrI's $CCAN_7RTGC$ (Tyndall *et al*, 1994). HsdS associates with two HsdM protomers to form a site-specific DNA methylase ($HsdM_2S$). Further attachment of two HsdR protomers yields a full-fledged R-M protein ($HsdR_2M_2S$). The R-M protein ignores a fully methylated target and readily methylates a hemi-methylated one. A fully unmodified target, usually of foreign DNA, induces the helicase domains of the HsdR protomers to pump-in DNA flanking the target sequence at the expense of ATP hydrolysis. This translocation and consequent DNA looping go on until an obstacle is encountered and cleavage occurs, usually far away from the specific recognition site. The type I R-M proteins are divided into families by antigenic cross-reactivity, subunit interchangeability and sequence similarity. PrrC is invariably linked to type Ic family members while RloC may interact with type Ia or the distantly related type III R-M proteins. For detailed coverage of DNA restriction and anti-restriction the readers are encouraged to consult relevant reviews (Murray, 2000;Dryden *et al*, 2001;Youell & Firman, 2008;Janscak *et al*, 2001).

EcoprrI normally silences PrrC's ACNase activity in the uninfected cell (Fig. 1B). The significance of this masking interaction is indicated by the "double-edged" nature of the T4 encoded peptide Stp, mutations in which suppress *prr* restriction. Thus, Stp inhibits EcoprrI's DNA restriction, probably its intended function; and activates the latent ACNase, its host co-opted task (Penner *et al*, 1995). Once activated PrrC nicks cellular $tRNA^{Lys}$ 5' to the wobble base, yielding 2', 3'-cyclic phosphate and 5'-OH termini. Since T4 shuts-off host transcription (Mathews, 1994) and does not encode $tRNA^{Lys}$ (Schmidt & Apirion, 1983) the lesion inflicted by PrrC could disable T4 late translation and contain the infection (Sirotkin *et al*, 1978). However, T4 overcomes also this hurdle by using Pnk and Rnl1 to resuscitate the damaged $tRNA^{Lys}$. Pnk heals the cleavage termini, converting them into a 3'-OH and 5'-P pair that Rnl1 seals (Amitsur *et al*, 1987)(Fig. 1B). In other words, this host-phage survival cascade gave rise to an RNA repair pathway. The ability of the *prr*-encoded latent ACNase to restrict only tRNA repair-deficient phage invokes the possible

existence of a "smarter" ACNase able to encumber phage reversal. Later we ask if RloC could be one.

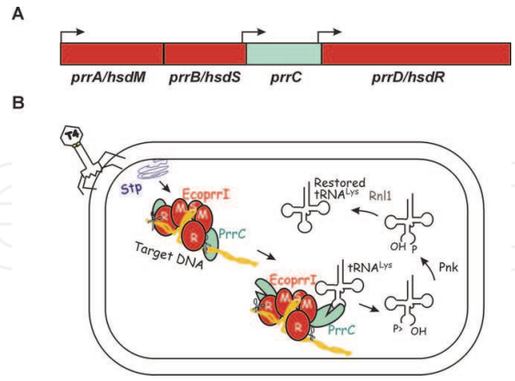


Fig. 1. A host-phage survival cascade gives rise to an RNA repair pathway. A. The optional host locus *prr* comprises the core ACNase gene *prrC* and flanking genes encoding the type Ic DNA R-M protein EcoprrI that silences PrrC's ACNase activity. Arrows mark transcription start sites. B. Cleavage-ligation of $tRNA^{Lys}$ in phage T4 infected *E. coli prr*$^{+}$. T4's anti-DNA restriction factor Stp inhibits EcoprrI and activates the latent ACNase. The resultant disruption of $tRNA^{Lys}$ is reversed by the T4's tRNA repair enzymes Pnk and Rnl1.

Nested *prr* loci where *prrC* intervenes a type Ic *hsd* locus (Fig 1A) appear sporadically in distantly related bacteria. They are present in some strains of a given species but not in others, as would a niche-function (Blanga-Kanfi *et al*, 2006). They abound among *Proteobacteria*, are less frequent in *Bacteroidetes* and *Firmicutes*, rare in *Actinobacteria* and apparently absent from *Cyanobacteria*. PrrC's phylogenic tree does not match the bacterial, unlike the associated type Ic R-M protein, which only rarely teams with PrrC. In contrast, a stand-alone *prrC* gene has not been detected so far. These facts hint that PrrC can be readily transmitted by horizontal gene transfer (HGT), possibly from a *prr* donor to an *hsd* acceptor. The dependence of PrrC's function on its detoxifying partner, the linked R-M system is indicated also by their coincident inactivation in a *Neisseria meningitidis* strain (Meineke and Shuman, pers. comm.). This addiction and the similar ACNase activities of various PrrC orthologs examined (Davidov & Kaufmann, 2008;Meineke *et al*, 2010) further suggest that PrrC acts in general as a translation-disabling, antiviral contingency mobilized when the linked R-M system is compromised.

The host-phage survival cascade depicted in Fig. 1B entails some caveats. Namely, the DNA of T4 and related phages incorporates 5-hydromethylcytosine (5-HmC) instead of cytosine and 5-HmC is further glucosylated at the DNA level (Morera *et al*, 1999). Due to this hyper-modification the phage DNA is refractory to many DNA restriction nucleases (Miller *et al*, 2003b) including EcoprrI and, hence, need not be protected from them by Stp. Moreover, a T4 mutant with unmodified cytosine in its DNA succumbs to EcoprrI's restriction, notwithstanding Stp's presence. The failure of Stp to protect this EcoprrI-sensitive mutant can be accounted for by the delayed-early schedule of its expression, a few minutes after the onset of the infection (Jabbar & Snyder, 1984;David *et al*, 1982). Due to these reasons EcoprrI's DNA restriction and Stp's anti-restriction activities were investigated using surrogate lambdoid phages (Jabbar & Snyder, 1984;Penner *et al*, 1995). Yet, the conservation of Stp's sequence among T4-like phages (Penner *et al*, 1995) http://phage.ggc.edu/,

indicates that this anti-DNA restriction factor provides selective advantage, e.g., preventing nucleases related to EcoprrI from cleaving nascent, not yet glucosylated progeny DNA.
The importance of Pnk and Rnl1 as PrrC's countermeasures is suggested by the following observations. First, docking tRNA on the crystal structure of T4 Pnk or Rnl1 places the anticodon loop at their respective active sites. These outcomes have been taken to indicate that both Pnk and Rnl1 evolved to repair a disrupted anticodon loop (Galburt *et al*, 2002;El Omari K. *et al*, 2006). Second, T4-related phages expected to infect *prr*-encoding bacteria feature both Pnk and Rnl1 (Miller *et al*, 2003a;Blondal *et al*, 2005;Blondal *et al*, 2003) whereas T4-related cyanophages, which are less likely to encounter *prr*, lack these tRNA repair proteins (http://phage.ggc.edu/).

2.2 PrrC's functional organization

PrrC comprises a regulatory motor domain occupying the N-proximal two thirds of its 396aa polypeptide (*Eco*PrrC). The remaining part constitutes the ACNase domain (Fig. 2A). The N-domain resembles ATP Binding Cassette (ABC) ATPases. These are universal motor components found in membrane-spanning transporters and in soluble proteins engaged in DNA repair, translation and related functions (Hopfner & Tainer, 2003). PrrC's N-domain differs from typical ABC ATPases in certain sequence attributes and in its unusual nucleotide specificity. The ABC ATPase motifs found in it partake in binding and hydrolysis of the nucleotide triphosphate moiety (Chen *et al*, 2003). However, the nucleobase recognizing motif of many transporter ABC ATPases termed A- or Y-loop (Ambudkar *et al*, 2006) is missing from PrrC. On the other hand, PrrC contains between its Walker A and Q-loop motifs a unique 16-residue motif rich in aromatic, acidic and other hydrophilic residues (Fig. 2A). This PrrC Box motif is highly degenerate (or rudimental) in RloC and is missing from other ABC ATPases and any other protein in the public database (Amitsur *et al*, 2003;Blanga-Kanfi *et al*, 2006). The PrrC Box candidates as a Y-loop substitute, responsible perhaps for PrrC's unusual specificity, the ability to simultaneously interact with its two different effector nucleotides GTP and dTTP (Blanga-Kanfi *et al*, 2006; unpublished data).
PrrC's ACNase domain harbors a catalytic ACNase triad (Arg^{320}-Glu^{324}-His^{356} in *Eco*PrrC) shared also by most RloC's orthologs except for a few cases where Glu is replaced by Asp. By analogy with the catalytic triad of RNase T1 (Gerlt, 1993;Steyaert, 1997), in the PrrC/RloC triad Glu and His could function as respective general base and acid catalysts while Arg could stabilize the pentameric transition state phosphate. The ACNase domain contains also residues implicated in recognition of the substrate's anticodon. Mutating one of them, *Eco*PrrC's Asp^{287} impairs the reactivity of the natural substrate and enhances that of analogs with a hypomodified or heterologous wobble base. These compensations hint that Asp^{287} interacts with the wobble base modifying side chain (Meidler *et al*, 1999;Jiang *et al*, 2001;Jiang *et al*, 2002).
When PrrC is expressed by itself it exhibits overt (core) ACNase activity. This core activity purifies with an oligomeric PrrC form, possibly a dimer of dimers. The N-domains of each dimer are expected to create two nucleotide binding sites (NBS) at their anti-parallel dimerization interfaces, as do typical ABC ATPases (Hopfner *et al*, 2000;Chen *et al*, 2003). In contrast, the ACNase C-domains are thought to dimerize in parallel, judged from the (i) behavior of a peptide mimic of a PrrC region implicated in the recognition of the tRNA substrate and (ii) ability of single to-Cys replacements in an overlapping PrrC region to induce disulphide-bond-dependent subunit dimerization (Klaiman *et al*, 2007).

Accordingly, the PrrC dimer of dimers assumes a phosphofructokinase-like topology (Schirmer & Evans, 1990) (Fig. 2B).

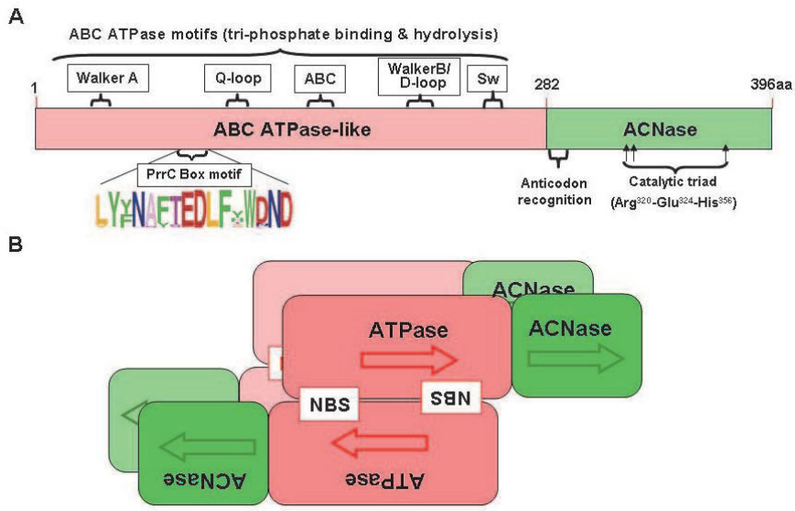


Fig. 2. Functional structure and possible quaternary organization of PrrC. **A**. PrrC's N-proximal ABC-ATPase domain features motifs involved in binding and hydrolysis of the nucleotide's triphosphate moiety (Walker A, Q-loop, ABC signature (ABC), Walker B, D-loop and linchpin Switch region (SW) but not the nucleobase recognizing Y-loop motif. The unique PrrC Box motif shown in WebLogo format, a putative functional substitute of the Y-loop, could confer the unusual GTP/dTTP specificity of PrrC. **B.** Antiparallel dimerization of the N-domains (Moody & Thomas, 2005) and anticipated parallel dimerization of the C-domains (Klaiman *et al*, 2007) suggest that PrrC assumes a phosphofructokinase-like quaternary topology (Schirmer & Evans, 1990). NBS – nucleotide binding site.

2.3 Players in PrrC's silencing and activation

As mentioned, PrrC's toxic activity is normally silenced, being unleashed only during phage infection. The requisite switches are provided in the case of *Eco*PrrC by its silencing partner EcoprrI, the phage T4-encoded anti-DNA restriction factor Stp and the motor domains of the ACNase protein itself. Insights into the underlying mechanisms were provided by the discrepant behaviors of the latent ACNase holoenzyme and the core ACNase activity of the unassociated PrrC. Thus, *in vitro* activation of the latent ACNase requires besides the Stp peptide, the DNA tethered to EcoprrI, GTP hydrolysis and the presence of dTTP. In contrast, the overt activity of the core ACNase is refractory to Stp, DNA and GTP but rapidly decays without dTTP (Amitsur *et al*, 2003;Blanga-Kanfi *et al*, 2006). These differences have been taken to indicate that Stp triggers the activation of the latent ACNase, GTP hydrolysis drives conformational changes needed to turn it on while the binding of dTTP stabilizes the ACNase once activated. The possible role of EcoprrI's DNA ligand is discussed later in this section.

GTP and dTTP probably exert their respective ACNase activating and stabilizing functions by interacting with PrrC's N-domains. This is suggested by their binding to

full-sized PrrC protein or PrrC's isolated N-domains with vastly differing affinities (mM- and μM-range, respectively) and without displacing each other (Amitsur *et al*, 2003;Blanga-Kanfi *et al*, 2006; and unpublished data). This unusual specificity distinguishes PrrC from its distant homolog RloC and other ABC ATPase-containing proteins, which bind and hydrolyze ATP or GTP (Guo *et al*, 2006) and are not expected to avidly bind dTTP (our unpublished data).

The biological significance of PrrC's idiosyncratic interaction with dTTP has been hinted at by the dramatic increase in the cellular level of dTTP early in phage T4 infection, when the ACNase is induced (Amitsur *et al*, 2003;Blanga-Kanfi *et al*, 2006). The increased level of dTTP benefits the phage by safeguarding effective and faithful replication of its AT-rich DNA. In fact, delaying dTTP's accretion by mutating T4's dCMP deaminase (Cd) elicits a mutator phenotype indicated by increased frequency of AT→GC transitions (Sargent & Mathews, 1987). The Cd deficiency, and, by implication, the consequent delay in dTTP's accretion, also reduce 2-3 fold the extent of the PrrC-mediated cleavage of $tRNA^{Lys}$. This partial inhibition does not suffice to suppress *prr* restriction but is synthetically suppressive with a leaky *stp* mutation that also fails to suppress *prr* restriction by itself (Klaiman & Kaufmann, 2011). Thus, dTTP's accretion is another T4 contraption expatiated by the bacterial host, in that case to stabilize the activated ACNase.

PrrC's ability to "gauge" changes in dTTP's level could benefit its host also by precluding the toxicity of any free PrrC molecules that could arise in the uninfected cell due to their translation in excess over EcoprrI or dissociation from the latent holoenzyme. Their excessive translation may be stochastic or programmed to saturate the silencing partner. PrrC's dissociation from the latent holoenzyme may be accidental or due to EcoprrI's disruption in response to DNA damage (Restriction Alleviation, RA) (Makovets *et al*, 2004) (see also section 3.6). Free PrrC's cytotoxicity has been indicated by the coincident inactivation of *prrC* and linked *hsd* genes, by the self-limiting expression of free PrrC (Meidler *et al*, 1999;Blanga-Kanfi *et al*, 2006) and the rapid *in vivo* inactivation of the core ACNase (Amitsur *et al*, 2003). The ACNase enhancing effects of dTTP's accretion during phage T4 infection (Klaiman & Kaufmann, 2011) and *in vitro* stabilization of the core ACNase by dTTP (Amitsur *et al*, 2003) suggest that the *in vivo* instability of the core ACNase owes to the relatively low dTTP level in the uninfected cell. Although this level far exceeds that needed to stabilize the core ACNase *in vitro*, the actual level availed to PrrC in the cell could be prohibitively low due to localization of the nucleotide pools (Wheeler *et al*, 1996). In sum, we propose that PrrC's ability to gauge dTTP's level not only stabilizes its activated form but also confines the toxicity of this ACNase to the viral target.

Yet another player in PrrC's regulation is the DNA tethered to EcoprrI (Amitsur *et al*, 2003). Its possible role is suggested by three observations. First, short nonspecific ssDNA oligonucleotides avidly bind PrrC and competitively inhibit its ACNase activity (Fig. 3A and unpublished results), hinting that ssDNA encountered by PrrC in the uninfected cell helps silence the ACNase. Second, the type Ic DNA R-M protein EcoR124I unwinds short DNA stretches flanking its target sequence (van Noort *et al*, 2004;Stanley *et al*, 2006), suggesting a possible source for the putative ACNase-inhibiting ssDNA. Third, within a latent ACNase complex tethered to an EcoprrI DNA ligand PrrC was UV-crosslinked to DNA regions flanking EcoprrI's recognition site (Fig. 3B). These facts underlie a model where DNA unwound by EcoprrI helps silence PrrC and its rewinding due to Stp's interaction with EcoprrI unleashes the ACNase (Fig. 3C).

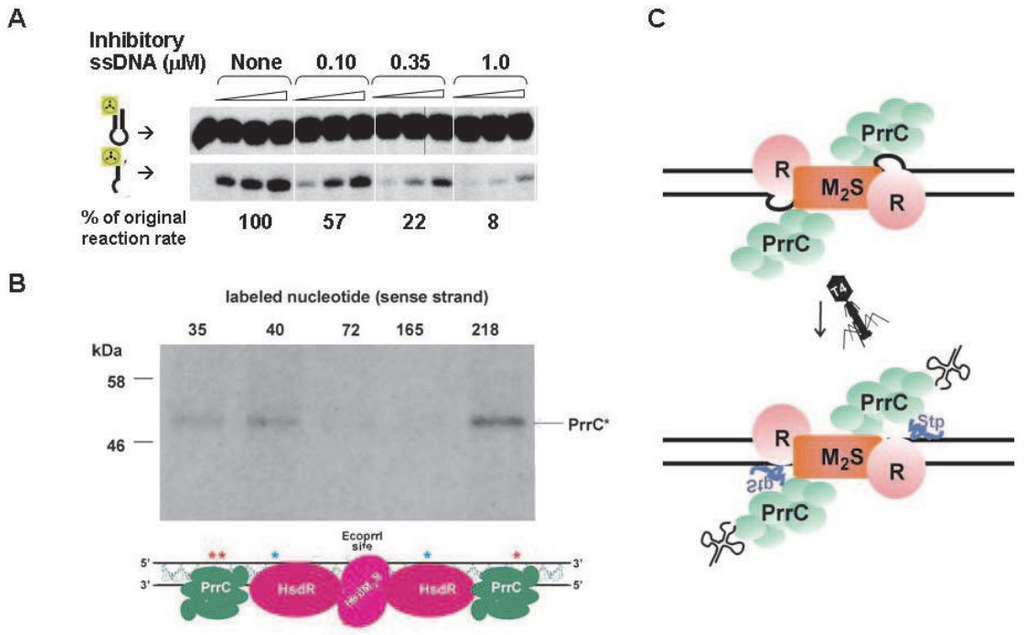


Fig. 3. DNA tethered to EcoprrI could figure in PrrC's regulation. A. ssDNA inhibits PrrC ACNase. PrrC ACNase was assayed using a 5'-^{32}P labeled anticodon stem loop substrate and increasing levels of a nonspecific 17nt PCR primer. B. PrrC contacts DNA regions flanking EcoprrI's target. A 249bp DNA fragment with a near-central EcoprrI site was singly ^{32}P-labeled at specific sites and tethered to the EcoprrI-PrrC complex. Following UV-irradiation, DNase I digestion, the photo-labeled PrrC was immunoprecipitated, separated by SDS-PAGE and monitored by autoradiography. Brown and blue asterisks indicate sites PrrC did or did not crosslink to, respectively. C. In this model DNA unwound by EcoprrI silences PrrC and its rewinding due to Stp's interaction with EcoprrI unleashes the ACNase.

3. RloC - A translation-disabling and potential DNA-damage-sensing protein

3.1 Functional organization

RloC is a conserved bacterial protein that shares PrrC's overall organization into a motor N-domain and ACNase C-domain (Fig. 4) (Davidov & Kaufmann, 2008). However, RloC is about twice as large, its orthologs ranging in size between 650 to 900 residues compared to 350-420 with PrrC. This increase is mainly due to a long coiled-coil forming sequence inserted between RloC's Walker A and ABC signature motifs. This coiled-coil sequence contains near its center a loop featuring the conserved zinc-hook motif CXXC. A similar coiled-coil insert in an ABC ATPase head-domain characterizes the universal DNA-damage-checkpoint/DNA-repair protein Rad50/SbcC (Hopfner *et al*, 2002;Connelly *et al*, 1998). Rad50's insert protrudes from the ATPase head-domain as an antiparallel coiled-coil presenting the zinc-hook motif at its apex. The apical ends of two such protrusions dimerize by coordinating Zn^{++} to their four cysteines. This zinc-hook linkage can arise intra-molecularly, connecting the two coiled-coil protrusions of the same Rad50 dimer. Alternatively, when Rad50's ATPase head-domains are bound to DNA the two protrusions straighten. In this form they can dimerize only inter-molecularly, bridging in this manner

distant DNA molecules (Moreno-Herrero *et al*, 2005). Other proteins belonging to the SMC (Structure Maintenance of Chromosomes) super-family exhibit similar DNA bridging activity but link their coiled-coil protrusions via apical hydrophobic domains (Hirano, 2005). RloC is the only known protein other than Rad50/SbcC with a coiled-coil/zinc-hook containing ABC-ATPase domain. Therefore, cellular functions imparted by Rad50/SbcC may provide clues to RloC's.

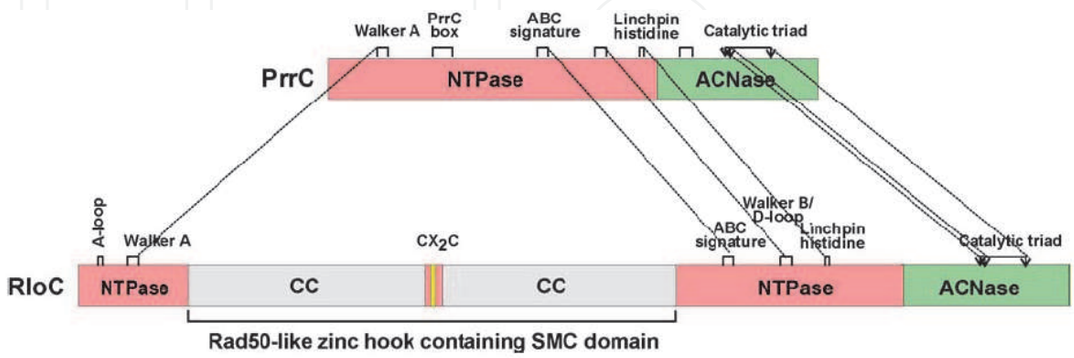


Fig. 4. RloC and PrrC share the same functional organization. The alignment of *Gka*RloC and *Eco*PrrC sequences reveals shared ABC ATPase and ACNase motifs and presence in RloC's N-domain of a large coiled-coil (CC) stretch interrupted by a loop containing the zinc hook motif CXXC (adapted from ref. 4).

3.2 RloC's occurrence and genomic attributes

RloC genes appear in major bacterial phyla except for *Cyanobacteria*. They are often encased within a cryptic mobile element as a single cargo gene. This pattern and a phylogenic tree not matching the bacterial suggest that RloC is readily transmitted by HGT, like PrrC. RloC's genes are also sporadically distributed but they occur ~3-fold more frequently than PrrC's. These facts suggest that the niche function RloC provides is more beneficial to its bacterial host.

RloC was originally identified as one of various open reading frames that intervene type Ia *hsd* loci in different *Campylobacter jejuni* strains (Restriction Linked Orf, Miller *et al*, 2005). This fact and the overall resemblance to PrrC could be taken to indicate that RloC is a related ACNase also silenced by an associated Hsd protein (Davidov & Kaufmann, 2008). Yet, only ~10% of the identified RloC orthologs turned out to be linked to type Ia or the distantly related type III DNA R-M system. Nonetheless, other genomic attributes suggested that the majority of the non-linked RloC orthologs team with an R-M system *in trans*. First, most bacteria encoding them encode also a suitable R-M system while in those lacking it RloC often features poor ATPase or ACNase motifs, as if inactivated. Second, some *rloC* genes are flanked by a cryptic *hsd* locus, a full-fledged homologue of which exists elsewhere in the genome, hinting that a past Hsd-RloC interaction *in cis* was superseded by one *in trans*. Third, RloC is occasionally linked to an ArdC-like anti-DNA restriction factor (Belogurov *et al*, 2000) with or without an adjacent R-M system, suggesting its possible regulation by an R-M system in either case. Fourth, non-linked *rloC* and *hsd* genes of one species, but not their respective flanking genes can be missing both from related, syntenic species [e.g., *Acinetobacter* sp. ADP1 *rloC* and *hsd* (ACIAD0152, ACIAD3430-2) but not flanking genes are missing from various *A. baumannii* strains] (http://www.cns.fr/agc/microscope/mage/viewer.php?S_id=36&wwwpkgdb =aa12fda27bb61b62ac34913acfd35916.)

The role ascribed to the R-M proteins in RloC's ACNase regulation need not contradict the existence of additional or alternative switches provided by the coiled-coil/zinc-hook insert. For example, silencing of the ACNase function by the latter device could be advantageous when RloC is introduced by HGT into a new host. Namely, silencing by a pre-existing R-M system could require a highly promiscuous interaction between the two partners. The possibility that RloC is endowed with an internal ACNase silencing mechanism agrees with properties of the ortholog encoded by the thermophile *Geobacillus kaustophilus* (*Gka*RloC) to be described in the following sections.

3.3 RloC wobble-nucleotide-excising activity

Due to its potential toxicity, RloC's ACNase activity was expected to be as unstable as PrrC's (Blanga-Kanfi *et al*, 2006). Indeed, among several RloC orthologs investigated, only *Gka*RloC proved sufficiently stable to warrant its *in vitro* characterization (Davidov & Kaufmann, 2008). Yet, even *Gka*RloC's ACNase is intrinsically unstable. Its *in vitro* activity is highest at 25°C and undetectable at 45°C (our unpublished results) although *G. kaustophilus* grows optimally at 65°C (Takami *et al*, 2004). When expressed in *E. coli* *Gka*RloC preferentially cleaved $tRNA^{Glu}$. However, identifying RloC's natural substrate must await physiological studies. This reservation is based on the experience gained with PrrC, the over-expression of which results in cleavages of secondary substrates that overwhelm the natural (Meidler *et al*, 1999).

A more striking difference between RloC and PrrC is the ability of the former to cleave its tRNA substrates successively, first 3' and then 5' to the wobble position (Davidov & Kaufmann, 2008). Such an excision reaction using as a substrate yeast $tRNA^{Glu}$ radiolabeled 3' to the wobble base is shown in Fig. 5. The incision of this substrate 3' to the wobble base yields a labeled 5' fragment containing residues 1-34. This intermediate is further cleaved immediately upstream, yielding the labeled wobble-nucleotide. Under these *in* vitro conditions *Gka*RloC inadvertently incises the substrate also 5' to the wobble base but this reaction yields a dead-end product that is not further cleaved. This is indicated by the accumulation of this product when the overall reaction declines; and of RloC to cleave it when generated by PrrC, which normally cleaves its substrates 5' to the wobble position. Such a 5' incision product of GkaRloC is not detected *in vivo* and, therefore, is considered an *in vitro* artifact. The excision of the wobble nucleotide has been observed with different tRNA and anticodon-stem-loop substrates and was catalyzed also by a mesophilic RloC species of *E. coli* APECO1 (Davidov & Kaufmann, 2008; unpublished data).

3.4 RloC may frustrate phage reversal

The harsh lesion inflicted by *Gka*RloC could render this ACNase a more potent antiviral device than PrrC. Namely, RloC could perform the successive cleavages of its substrate in a processive manner, i.e., without releasing the incision intermediate. The phage tRNA repair enzymes would in that case process and ligate back the fragments lacking the wobble nucleotide and yield a defective product. Conversely, if *Gka*RloC's incision intermediate were accessible, the repair enzymes would faithfully restore the original tRNA substrate. Simulated *in vitro* encounters between *Gka*RloC and T4 Pnk or both tRNA repair enzymes indicated that a sizable fraction of its incision intermediate was occluded from the repair enzymes (Davidov & Kaufmann, 2008; and unpublished data). It is possible that under physiological conditions RloC's would more effectively occlude its incision intermediate.

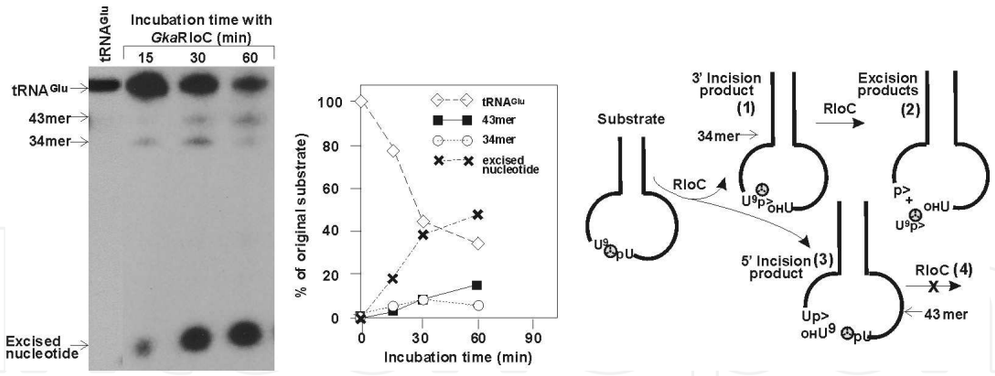


Fig. 5. RloC excises the wobble nucleotide. Yeast tRNA^{Glu} ^{32}P-labeled 3' to the wobble base was incubated with *Gka*RloC. The 34mer resulting from incision 3' to the wobble base is further cleaved, yielding the wobble nucleotide. The 43mer resulting from incision 5' to the wobble base is a dead-end product that is not further cleaved. It is considered an *in vitro* artifact, as explained in the text. In the cartoon depicting these reactions the substrate is schematically represented by the anticodon stem loop outline. ☢ marks the labeled phosphate. U^{9} is the modified wobble base 5-methoxycarbonylmethyl-2-thiouridine. (mcm^{5}s^{2}U).

Moreover, repeated cleavage-ligation cycles would diminish the proportion of any incision intermediate ligated back by phage enzymes. On the other hand, the existence of tRNA repair enzymes that more efficiently extract RloC's incision intermediate and generate perhaps repair products immune to re-cleavage (Chan *et al*, 2009b) cannot be excluded. Clearly, whether RloC does frustrate phage reversal remains to be examined in situations closer to the natural.

3.5 RloC's DNA bridging domain regulates its ACNase

RloC's second striking feature is the coiled-coil/zinc-hook insert in its ABC ATPase head-domain. The presence of this structure raised the possibility that RloC is endowed with Rad50-like DNA bridging activity and uses such a faculty to respond to DNA damage cues by turning on its ACNase. That RloC is in fact endowed with DNA bridging activity is indicated by an electrophoresis mobility shift experiment and by scanning force microscopy (AFM) imaging. In the first experiment we compared *Gka*RloC constructs with an intact or mutated zinc-hook. The first protein aggregated a dsDNA probe that the second only bound (Fig. 6). Their discrepant behavior suggests that the aggregation was due to the formation of zinc-hook-dependent DNA bridges. Preliminary AFM imaging data reinforce this assumption (Fig. 7).

That RloC's ACNase is regulated by the protein's coiled-coil/zinc-hook and ATPase head-domain is indicated by several observations. First, mutating RloC's zinc-hook dramatically enhances its ACNase activity *in vivo* and *in vitro* (Davidov & Kaufmann, 2008). Second, *Gka*RloC's ACNase activity is modestly enhanced by ATP and further stimulated when the protein is also tethered to DNA (Fig. 8). In contrast, DNA alone has no effect on the ACNase and the residual ACNase activity seen without added ATP is abolished by the non-hydrolyzable analog AMP-PNP. Presumably, RloC's interaction with DNA turns on its ATPase to drive conformational changes that activate the ACNase. Interestingly, mutating the zinc-hook renders the ACNase refractory to these various agents, uncoupling the ACNase from the protein's internal controls (not shown). Together, these facts suggest that RloC's mode of interaction with DNA, which is sensed by its coiled-coil/zinc-hook monitoring device and relayed by the ATPase (Fig. 9), determines if the protein's ACNase will be silenced or turned on.

The ability to activate *Gka*RloC's ACNase by ATP hydrolysis in the presence of tethered DNA is in stark contrast with the behavior of PrrC's ACNase. As mentioned, PrrC's ACNase is activated by nucleotide hydrolysis only when associated with its silencing partner EcoprrI. However, its unassociated form exhibits overt ACNase activity refractory to nucleotide hydrolysis. This discrepancy raises the possibility that RloC's ACNase can be regulated by the internal device of the protein, the coiled-coil/zinc-hook and the ATPase domain that harbors this structure.

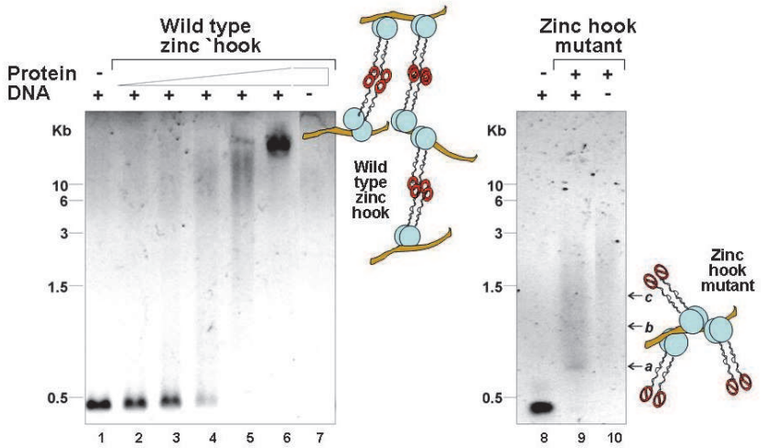


Fig. 6. *Gka*RloC aggregates DNA in a zinc-hook-dependent manner. A 485bp DNA fragment was incubated with increasing levels of GkaRloC's ACNase-null mutant E696A (lanes 2-6) or with its ZH mutant derivative E696A-C291G (lane 9). Lanes 1 and 8 contain only DNA, 7,10 only the indicated protein. The cartoons depict the assumed bridged DNA aggregate formed by E696A (right) and the simpler complex formed by E696A-C291G (left). The ACNase-null mutation allows high level expression and facilitates the isolation of the RloC proteins.

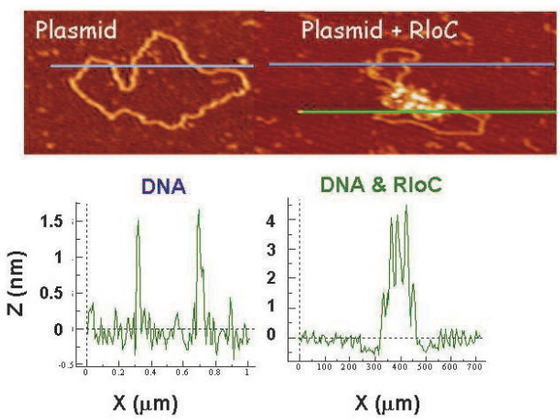


Fig. 7. AFM images of plasmid pUC19 (DNA) and its complex with RloC-E696A (DNA and RloC). Blue lines stretch over pure DNA regions, green lines also over regions containing the bound protein. Regions transected by the green line feature virtual heights both of the DNA alone (~1.5nm) and of the presumptive RloC-DNA complexes (~4.5nm).

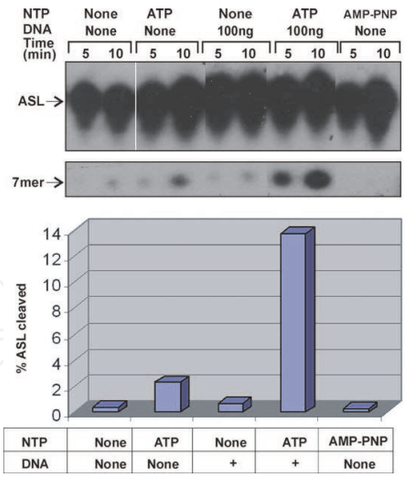


Fig. 8. *Gka*RloC's ATPase and tethered DNA cooperatively regulate its ACNase function. *Gka*RloC's ACNase activity was assayed using as a substrate a 5'-^{32}P labeled anticodon-stem-loop analog corresponding to mammalian tRNA^{Lys3} (ASL). The reaction was performed in the absence or presence of 2mM of ATP and/or 10ng/µl of BstE II digested λ DNA, or in the presence of the non-hydrolyzable ATP analog AMP-PNP. The 7mer is a radiolabeled fragment resulting from the final excision reaction

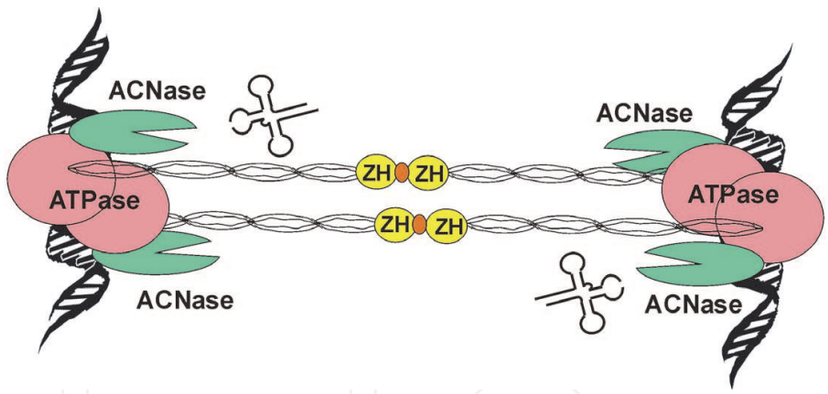


Fig. 9. RloC's anticipated DNA bridging activity. By analogy with Rad50, RloC bridges DNA through Zn^{++} (orange circles) coordinated at zinc-hook (ZH) dimerization interfaces (yellow circles) at the apical tips of the coiled-coils protruding from the DNA-borne ATPase head domains (pink circles). The status of the bound DNA sensed by RloC determines if its ATPase will be activated and drive structural changes needed to switch on the ACNase domains (split green ovals) toward tRNA cleavage.

3.6 Is RloC a suicidal DNA-damage-responsive device?

If RloC can be regulated by its internal devices, what role plays the anticipated interaction of RloC with a DNA R-M protein? Do these external and internal devices cooperate or act separately, responding to the same or different environmental cues? The present state of RloC's research does not permit us to distinguish between these possibilities, let alone assign to this protein specific biological functions. However, cues provided by Rad50/SbsC, the only other known coiled-coil/zinc-hook containing entity, may facilitate the formulation of

useful guiding hypotheses. Here it will suffice to briefly summarize pertinent features of this universal DNA-damage-responsive, DNA-repair protein. For comprehensive coverage several recent reviews are suggested (Hirano, 2006;Stracker & Petrini, 2011;Williams *et al*, 2010;Paull, 2010) as well as relevant chapters in this book.

Archaeal Rad50 and the bacterial SbcC counterparts associate with the respective dimeric DNases Mre11 or SbcD. The eukaryal Rad50-Mre11 complex (MR) further associates with an adapter protein termed Nbs1 (Xrs2 in yeast), which links the ternary complex to key DNA damage checkpoints. The ternary MRN complex controls key sensing, signaling, regulating, and effecter responses triggered by DNA double-strand breaks (DSB). These responses include the activation of master regulators such as ATM as well as roles in homologous recombinational repair (HRR), microhomology-mediated end joining (MMEJ) and, occasionally, non-homologous end-joining (NHEJ). Rad50 figures in these transactions as a DNA-bridging SMC protein, using its coiled-coil/zinc-hook and ATPase to properly orient the DNA molecules it bridges and its associated protein partners (Hirano, 2005;Stracker & Petrini, 2011;Williams *et al*, 2010;Paull, 2010;Stracker & Petrini, 2011). As mentioned, Rad50's coiled-coils bend when the ATPase domains are free and stretches when tethered to DNA (van *et al*, 2003;Moreno-Herrero *et al*, 2005). This flexibility also allows the linked ATPase domain to communicate nucleotide binding and DNA ligand signals across distances and between components of the complex. These transmissions depend, among others, on the binding of Mre11 to the coiled-coil portion closest to the ATPase domain, which positions the DNase to resect DSB ends (Williams *et al*, 2011).

Rad50's bacterial homologue SbcC may likewise exert its function as a DNA bridging protein, directing SbcD to cleave hairpin structures that impede DNA replication and initiate DSB that drive HRR (Darmon *et al*, 2010;Storvik & Foster, 2011;White *et al*, 2008). Interestingly, over-expressed in *E. coli, SbcC* co-localizes with the replication factory whereas SbcD is dispersed throughout the cytoplasm. Their discrepant behaviors underlie the proposal that at its low, natural level SbcC constantly checks the replication fork for misfolded DNA, recruiting SbcD only when repair is required. A different distribution in *B. subtilis* suggests that in this organism SbcCD partakes also in NHEJ (Mascarenhas *et al*, 2006; Darmon *et al*, 2007).

*Gka*RloC could use its DNA bridging activity (Figs. 5, 6) to monitor the status of cellular DNA molecules like Rad50 and SbcC. However, there is no evidence that RloC associates with a DNase corresponding to Mre11 or SbcD. On the other hand, RloC's regulatory domain, Rad50/SbcC's counterpart is uniquely appended to the translation-disabling ACNase domain. It is tempting to speculate therefore that the ACNase C-domain interacts with the regulatory N-domain in a manner analogous to Mre11's, i.e., tethers to the proximal portion of the coiled-coil fiber emerging from the ATPase head-domain. Such a contact could help transduce DNA damage signals sensed by RloC's DNA monitoring device and relayed by the ATPase to the ACNase effecter domain. The existence of such a signal transduction pathway agrees with the effects of RloC's zinc-hook mutations, ATPase and tethered DNA on its ACNase function (Davidov & Kaufmann, 2008) (Fig 8).

The suggestions that RloC's ACNase is activated in response to DNA damage and, consequently, arrests translation may seem self-contradictory. After all, bacteria normally respond to DNA insults by enhancing the synthesis of DNA repair and other stress responsive proteins (Fernandez De Henestrosa *et al*, 2000). This apparent contradiction may be reconciled by considering the phenomenon of DNA restriction alleviation (RA) (Thoms & Wackernagel, 1984). RA is enacted in response to genotoxic stress as a protective measure

intended to prevent degradation of self DNA. In the best documented RA case, the restriction subunit HsdR of the type Ia R-M protein EcoKI is degraded by the protease ClpXP (Makovets *et al*, 2004). In the case of the type Ic protein EcoR124I, RA may entail dissociation or functional occlusion of the HsdR subunit (Youell & Firman, 2008). RA prevents the degradation of fully unmodified portions of the cellular DNA synthesized during the recovery from DNA damage, mainly by HRR. In fact, exposure of an RA-deficient mutant to DNA damage causes DSB and eventual cell death (Cromie *et al*, 2001;Makovets *et al*, 2004;Blakely & Murray, 2006).

RA exacts also a price. Namely, inactivation of the cell's primary immune system renders it highly vulnerable to phage infection (Yamagami & Endo, 1969;Blakely & Murray, 2006). In theory, RloC could benefit its host in this situation by acting as an antiviral back-up device, mobilized when the cell is infected by a phage during recovery from DNA damage. The activation of RloC under these circumstances would prevent the spread of the phage to other members of the vulnerable bacterial population. In this regard RloC could resemble PrrC, which fails to rescue the cell in which it is turned on but can contain the infection. However, the proposed mode of RloC's activation calls for combined inputs of DNA damage and phage infection. Namely, phage infection alone would be offset by the functional DNA restriction nuclease while DNA damage alone would be effectively dealt with by the SOS response (Friedberg *et al*, 2006). It is noteworthy that exposure of an RloC encoding species to mytomycin C did not induce detectable ACNase activity (unpublished results).

Clearly, the above model raises more questions than it attempts to answer. For example, how does the anticipated RloC-Hsd interaction fit in this scheme? Do the genotoxic and viral stress signals cooperate or act separately? Can RloC frustrate phage encoded tRNA repair? To address these issues it will be necessary to employ experimental systems based on natural RloC-encoding hosts and cognate T4-like phages that activate RloC and encode a tRNA repair system.

4. RNA damage repair

4.1 Why repair damaged RNA?

The emergence of an RNA cleavage-ligation pathway in the wake of a host-parasite encounter (Fig. 1) brought to the fore the rather overlooked subject of RNA damage repair. RNA is susceptible to the same agents that threaten DNA. Radiation and chemicals that break the DNA backbone and modify its bases have similar effects on RNA and its precursors. RNA is also attacked by stress responsive RNases (Thompson & Parker, 2009) and various secreted ribotoxins (Wool *et al*, 1992;Masaki & Ogawa, 2002;Lu *et al*, 2005). What is more, its backbone is more sensitive to spontaneous hydrolysis than DNA's. Yet, the repair of damaged RNA seems necessary only in cases where its replenishment by re-synthesis is not possible, e.g., when a DNA template to transcribe from is missing.

Thus, it is conceivable that RNA repair tools played a critical role in sustaining the genomes of the hypothetical RNA and RNA/Protein Worlds (Cech, 2009). One may further speculate that some of these tools could have evolved into extant devices with similar RNA repair tasks or expatiated roles in other RNA transactions (Abelson *et al*, 1998;Sidrauski *et al*, 1996) or even in DNA repair (Aas *et al*, 2003;Tell *et al*, 2010).

RNA repair can be the only option also in extant situations, especially when a DNA template to transcribe from is missing. A relevant example already given here is the reliance

of phage T4 on its tRNA repair proteins as a means to overcome the disruption of $tRNA^{Lys}$ by the host's ACNase PrrC (section 2.1). Another relevant example is the AlkB RNA demethylase of certain single stranded plant RNA viruses. The intended role of this demethylase is probably the removal of toxic methyl groups from the viral genomic RNA (van den *et al*, 2008). Homologous bacterial and human RNA-specific AlkB methylases could save the resources and/or time needed to re-synthesize damaged RNAs. In fact, these enzymes have been found able to resuscitate damaged RNA models while distinguishing between natural base modifications and toxic ones. However, the biological relevance of these findings remains uncertain (Aas *et al*, 2003;Ougland *et al*, 2004). Another DNA repair protein with possible roots in RNA metabolism is the abasic DNA endonuclease APE1 (Tell *et al*, 2010). Below we focus on recently discovered cellular tools able to repair nicked RNA, as do the phage T4-encoded proteins Pnk and Rnl1 that counteract PrrC and are frustrated perhaps by RloC.

4.2 Cellular RNA nick repair systems

The RNA phosphodiester linkage is vulnerable to nucleophilic attack. Deprotonation of its adjacent 2' oxygen, subsequent formation of a pentameric phosphate intermediate and 5'-O protonation disrupt it, yielding 2', 3' cyclic phosphate and 5'-OH cleavage ends. This reaction occurs spontaneously and nonspecifically under physiological conditions but is also catalyzed at critical target sites by stress-responsive tRNases (Thompson & Parker, 2009) and secreted ribotoxins (Wool *et al*, 1992;Masaki & Ogawa, 2002;Lu *et al*, 2005;Jablonowski *et al*, 2006;Klassen *et al*, 2008). Some of the small self-cleaving ribozymes that catalyze it also catalyze the reverse reaction, converting 2',3'-cyclic-P and 5'-OH ends into a 3'-5' phosphodiester linkage (Ferre-D'Amare & Scott, 2010). A similar RNA ligase activity involved in tRNA splicing was detected early on in HeLa cell extracts (Filipowicz & Shatkin, 1983) and later in an archaeon (Gomes & Gupta, 1997). The protein catalyzing it termed RtcB has been recently identified in an archaeon, human cells and bacteria (Englert *et al*, 2011;Popow *et al*, 2011;Tanaka & Shuman, 2011). The archaeal and human proteins join 5' and 3' exons of tRNAs and the human possibly also those of the mRNA of an unfolded-protein-response factor (Englert *et al*, 2011;Popow *et al*, 2011). A role for the bacterial RtcB has not been assigned yet. However, its possible participation in an RNA-nick-repair pathway is suggested by the operon RtcB shares with the RNA 3'-P cyclase RtcA. RtcA turns the 3'-P end into 2', 3'-P> through an adenylated intermediate, analogous to the manner in which RNA and DNA ligases activate 5'-P termini (Genschik *et al*, 1998). Thus, combined, RtcAB could convert a 3'-P and 5'-OH pair into a 3'-5' phosphodiester linkage. Unlike RtcA, the RtcB mediated transesterification reaction does not require an energy source although it may be allosterically directed by bound GTP (Tanaka & Shuman, 2011). Given their ability to repair such RNA nicks, RtcAB or RtcB alone could mend accidentally broken RNAs, restore RNAs temporarily inactivated by stress-responsive RNases (Neubauer *et al*, 2009;Zhang *et al*, 2005) or counteract ribotoxins secreted by rival cells (Masaki & Ogawa, 2002). Moreover, the existence of both RtcA and RtcB in all three domains of life (Tanaka & Shuman, 2011;Englert *et al*, 2011;Popow *et al*, 2011) suggests that their cooperation could be rather widespread.

A more intricate RNA-nick-repair pathway is catalyzed by the bacterial proteins PnkP and Hen1. PnkP and Hen1 share the same operon and form a tetrameric P_2H_2 complex (Martins & Shuman, 2005;Chan *et al*, 2009b). The reactions catalyzed by the PnkP component of the complex resemble those mediated by phage T4 Pnk and Rnl1 (section 1) and the yeast and

plant tRNA splicing ligase (Abelson *et al*, 1998). What makes this repair system unique is its ability to render the restored phosphodiester linkage immune to re-cleavage by virtue of the 2'-O methylase activity of Hen1 (Chan *et al*, 2009b). PnkP comprises an N-terminal kinase domain, a central metallophosphoesterase domain and a C-terminal ligase domain. Thus, it comprises functions similar to those of the yeast tRNA splicing ligase but differs in domain order and different origin of the phosphoesterase domain (Apostol *et al*, 1991;Martins & Shuman, 2005). Interestingly, by itself the bacterial PnkP heals 2', 3'-cyclic P and 5'-OH termini pairs and undergoes the first step in the RNA ligase reaction, its auto-adenylation, but does not proceed to activate the 5'-P end and generate the phosphodiester linkage (Martins & Shuman, 2005). This deficiency is corrected by expressing PnkP with the 2'-O methylase Hen1. Within the resultant PnkP/Hen1 complex PnkP heals and seals the cleavage termini while Hen1 2'-O methylates the dephosphorylated 3'-end prior to the ligation step. This modification renders the restored ligation junction immune to re-cleavage (Chan *et al*, 2009b). The bacterial Hen1 is so named because it resembles in sequence and structure the methylase domain of eukaryal miRNA methyltransferase Hen1 (Chan *et al*, 2009a). The eukaryal Hen1 protects the 3'-terminal ribose of miRNA from exonucleolytic degradation or utilization as replication primer (Chen, 2005).

As with bacterial RtcAB, the biological role of the PnkP/Hen1 is not known. Noteworthy in this regard is that PnkP/Hen1 is most abundant among *Actinobacteria.* In contrast, RtcAB is more prevalent among *Proteobacteria* and has not been detected yet in *Actinobacteria*. This coincidence raises the possibility that the two systems provide similar benefits to their respective hosts. In theory, PnkP/Hen1 complexes could defend their host cells from secreted ribotoxins more efficiently than RtcAB due to the ability to prevent re-cleavage of the susceptible RNA. It is noteworthy though that colicin-like ribotoxins that target rRNA (Bowman *et al*, 1971;Senior & Holland, 1971) or tRNA anticodon loops (Masaki & Ogawa, 2002) have not been identified yet in bacteria likely to accommodate PnkP/Hen1.

If PnkP/Hen1 were to counteract an ACNase that cleaves its substrate 3' to the wobble base like colicin E5 (Ogawa *et al*, 1999), then the repaired tRNA would contain a 2'-O methylated wobble nucleotide. Such a protective modification need not impair the tRNA's function since it exists in some natural bacterial tRNAs (Juhling *et al*, 2009). However, it cannot be excluded that PnkP/Hen1 plays additional or other roles and may be exploited differently in different bacterial hosts. One example of such a different role is hinted at by the juxtaposition of the PnkP/Hen1 and CRISPR-Cas loci of *Microscilla marina*. The CRISPR-Cas system confers adaptive immunity against foreign nucleic acids. During its antiviral interference activity specific RNA portions of the CRISPR transcript are used to target a Cas protein to cleave the invasive nucleic acid (Deveau *et al*, 2010). Hence, it may be asked if *M. marina* PnkP/Hen1 catalyze some RNA processing and/or modification steps during CRISPR RNA maturation. Finally, in a reversal of roles, one could envisage PnkP/Hen1 encoding phage able to prevent re-cleavage of a tRNA by the ACNase they counteract.

4.3 An essential eukaryal DNA repair protein is related to T4 Pnk

There are a number of examples of DNA repair devices that could have originated from RNA-specific progenitors, some of them already alluded to above. Here it will suffice to describe just one of them, related to the phage T4-encoded end healing protein Pnk. This conserved eukaryal protein termed interchangeably PNKP and Pnk1 contains 5'-kinase and 3'-phosphatase domains resembling those of T4 Pnk but arranged in the reverse order, the phosphoesterase domain preceding the kinase domain. The mammalian PNKP is also

endowed with an N-terminal FHA (Fork Head Associated) phosphopeptide binding domain that links PNKP to the scaffold proteins XRCC1 and XRCC4 (Bernstein *et al*, 2009). The latter recruit PNKP to exercise its functions in base excision repair (Hegde *et al*, 2008) or NHEJ (Lieber, 2008). PNKP's essential role in these ssDNA and DSB repair pathways is to convert 3'-P and 5'-OH DNA termini into 3'-OH and 5'-P pairs that are ligatable or fit for gap-filling by a DNA polymerase. A wide DNA binding cleft accounts for the ability of this protein to prefer nicked duplexes and recessed 5'-termini over ssDNA substrates and distinguishes it from the RNA end healing phage counterpart. The 3'-P and 5'-OH DNA termini are caused by ionizing radiation, genotoxic chemicals and enzymatic reactions. Specific examples include excision of abasic sites (Hazra *et al*, 2002), DSB generated by DNase II (Evans & Aguilera, 2003) and release of camptothecin- trapped topoisomerase I-DNA adducts by a tyrosine-DNA specific phosphodiesterase (Pouliot *et al*, 1999). Failure to repair such lesions underlies several inborn neural disorders. Conversely, PNKP can render cancer cells resistant to certain genotoxic drugs and, therefore, is considered itself a potential therapeutic target (Weinfeld *et al*, 2011).

5. Conclusions

In this chapter we addressed the possible biological role of the conserved bacterial anticodon nuclease RloC that combines two seemingly conflicting properties. One, predicted by resemblance of its regulatory region to the universal DNA-damage-checkpoint/DNA repair protein Rad50/SbcC is monitoring DNA insults. The second, predicted by its tRNase activity is disabling the translation apparatus. The co-existence of such functions in the same molecule and the regulation of one by the other suggests that RloC is designed to block translation in response to DNA damage. Such a response is suicidal since it prevents recovery from DNA damage. Hence, it must be executed only under special circumstances where cell death is advantageous. One possibility considered here is that RloC benefits its host cell by acting as an antiviral contingency during recovery from DNA damage. Under these conditions bacterial cells may shut off their primary antiviral defense, i.e., their DNA restriction activity. RloC's suicidal activity would not rescue the infected cell but would prevent the spread of the infection to other vulnerable members of the population recovering from DNA damage.

Another unique property, which could make RloC particularly suited to thwart phage infection, is the ability of this ACNase to excise its substrate's wobble nucleotide. In this regard RloC differs from its distant homologue the ACNase PrrC, which only incises its tRNA substrate and is counteracted by phage tRNA repair enzymes. Therefore, it seems conceivable that the harsher lesion inflicted by RloC will encumber such phage reversal. The possibility that RloC is a more efficient antiviral device than PrrC is also hinted at by its ~3-fold more frequent occurrence among bacteria.

While these notions are supported by some demonstrated properties of RloC, testing them and identifying RloC's true call requires studying this protein under physiological conditions; ideally, using a natural host encoding it and cognate phages endowed with tRNA repair enzymes.

The RNA repair pathway instigated by PrrC and possibly avoided by RloC brings to the fore the rather overlooked issue of RNA-damage-repair. Such repair would seem necessary only under circumstances such as the absence of a DNA template to transcribe from. Nonetheless, recent discoveries of various cellular RNA repair devices distributed in the three domains of life suggest that RNA damage repair is more prevalent, exercised perhaps also during

responses to nutritional, pathogenic and other forms of stress. RNA repair is also of interest because many of its devices seem to have evolved to serve in other RNA transactions and even in DNA repair. Conversely, the vast repertoire of DNA repair, RNA splicing and RNA editing reactions may be exploited by investigators to discover novel RNA repair phenomena.

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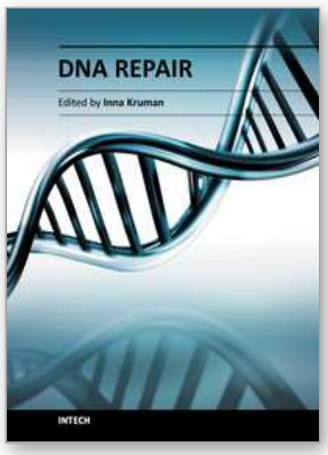
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