Parallel dimerization of a PrrC-anticodon nuclease region implicated in tRNA^Lys recognition

Daniel Klaiman^1, Michal Amitsur^1, Shani Blanga-Kanfi^1, Michal Chai^1, Darrell R. Davis^2 and Gabriel Kaufmann^1,*

^1Department of Biochemistry, Tel Aviv University, Tel Aviv 69978, Israel and ^2Department of Medicinal Chemistry, University of Utah, Salt Lake City, 84112 UT, USA

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ABSTRACT

The optional Escherichia coli restriction tRNase PrrC represents a family of potential antiviral devices widespread among bacteria. PrrC comprises a functional C-domain of unknown structure and regulatory ABC/ATPase-like N-domain. The possible involvement of a C-domain sequence in tRNA^Lys recognition was investigated using a matching end-protected 11-meric peptide. This mimic, termed here LARP (Lys-anticodon recognizing peptide) UV-cross-linked tRNA^Lys anticodon stem-loop (ASL) analogs and inhibited their PrrC-catalyzed cleavage. Trimming LARP or introducing in it inactivating PrrC missense mutations impaired these activities. LARP appeared to mimic its matching protein sequence in ability to dimerize in parallel, as inferred from the following results. First, tethering Cys to the amino- or carboxy-end of LARP dramatically enhanced the ASL-cross-linking and PrrC-inhibiting activities under suitable redox conditions. Second, Cys-substitutions in a C-domain region containing the sequence corresponding to LARP elicited specific intersubunit cross-links. The parallel dimerization of PrrC’s C-domains and expected head-to-tail dimerization of its N-domains further suggest that the NTPase and tRNA^Lys-binding sites of PrrC arise during distinct assembly stages of its dimer of dimers form.

INTRODUCTION

PrrC, the optional Escherichia coli anticodon nuclease (ACNase) (1,2) represents potential antiviral devices widespread among bacteria (3). PrrC’s activity is silenced by the genetically linked type Ic DNA restriction endonuclease EcoprrI (4–7) and is unleashed by Stp, the phage T4-encoded peptide inhibitor of EcoprrI (8). The activation of PrrC causes specific cleavage of tRNA^Lys 5’ to the wobble base (2) and, consequently, could block T4-late translation and contain the infection (9). However, the T4-coded RNA healing and sealing enzymes 3’-phosphatase/5’-polynucleotide kinase (Pnk) and RNA ligase 1 (Rnl1) (10) normally restore the intact form of tRNA^Lys (1,2), exercising perhaps their intended functions (11,12). Known PrrC homologs are invariably linked to EcoprrI homologs and, hence, could also act as secondary defenses mobilized when an associated DNA restriction endonuclease is compromised (3,8,13).

When PrrC is expressed by itself it exhibits overt (core) ACNase activity (14) that purifies with a homo-oligomeric PrrC form, possibly a dimer of dimers (3). PrrC’s N-proximal ~265 amino acids are thought to constitute an NTPase domain that mediates the activation of the latent ACNase (3,13) (Figure 10A). This region contains ABC ATPase-like motifs (15), albeit, sufficiently different from the typical to justify classifying PrrC’s N-domain as a distinct subtype. The divergent sequence of PrrC’s N-domain could account for the unusual nucleotide requirements of the ACNase activation reaction and PrrC’s idiosyncratic nucleotide binding attributes. Namely, the activation depends on the cooperation of GTP and dTTP and is inhibited by ATP. Moreover, dTTP exhibits higher affinity for PrrC than GTP or ATP (µM versus mM-range); and dTTP but not GTP or ATP stabilizes PrrC’s core ACNase activity. PrrC differs from the typically dimeric ABC ATPases (16) also in its apparent dimer of dimers structure (3). The remaining ~130 amino acid region of PrrC harbors residues implicated in tRNA^Lys recognition (17–19) and cleavage (3) and does not resemble a known protein structure. The main cues PrrC recognizes in tRNA^Lys map to the anticodon stem-loop (ASL). They comprise the anticodon sequence, base modifications and base-pairing interactions (17–20).

Peptide mimicry and Cys-mediated intersubunit cross-linking data reported here suggest that the tRNA^Lys-binding motif of PrrC is shared by C-domain portions interfacing in parallel. The proposed parallel dimerization of the C-domains can be reconciled with the

*To whom correspondence should be addressed. Tel: +1 972 3 642 6213; Fax: +1 972 3 640 6834; Email: gabika@tauex.tau.ac.il

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expected head-to-tail dimerization of the N-domains (15) by further suggesting that the regulatory and functional sites of PrrC arise at distinct assembly stages of its dimer of dimers form.

MATERIALS AND METHODS

Materials

Purified *E. coli* tRNA<sup>Lys</sup> labeled with <sup>32</sup>P at the 33p34 junction (13,21) and synthetic tRNA<sup>Lys</sup> ASL analogs (22,23) were prepared as previously described. The analogs included ASL-3 that matches mammalian tRNA<sup>Lys</sup> in RNA sequence and base modifications but contains an extra <sup>30</sup>-dT that facilitated its synthesis; and ASL-C that has the same RNA sequence but base modifications of *E. coli* tRNA<sup>Lys</sup> (Figure 1); both were [5'-<sup>32</sup>P] labeled as described (19). The synthetic end-protected peptides used (Figure 2) were purchased from Genscript Corporation and were over 80% pure. Diaminecarboxylic acid (diamide) was purchased from Sigma.

**PrrC mutagenesis**

The PrrC forms used contained a C-terminal His<sub>6</sub> tag and, except where indicated, also the leaky D222E mutation that allows high level expression of the protein (3). The triple mutant D222E/C268A/C385A termed PrrC<sup>*</sup>, its Cys replacement derivatives and the H356A/F292S double mutant were generated by Quick Change (24). Other PrrC mutants have been described (3,17).

**PrrC-expression plasmids and bacterial hosts**

The PrrC proteins were expressed under the control of the T7-Lac promoter and Shine–Dalgarno sequence of plasmid pRRC11 (17) in *E. coli* Rosetta (DE3)pLysS (Novagen, UK) encoding T7 RNA polymerase, T7 lysozyme (25) and rare tRNAs from plasmid pRARE. The cells were grown in LB medium at 37°C to a density of ~6.10<sup>8</sup> cells/ml. They were shifted then to 30°C and PrrC’s expression induced by adding 1 mM IPTG. After further incubation for 2 h, the cells were harvested and the PrrC proteins purified by immobilized metal affinity chromatography as described (3).

**Diamide treatment of cells expressing PrrC-to-Cys mutants**

*Escherichia coli* cells induced to express the indicated PrrC forms were harvested by high-speed centrifugation, re-suspended in 0.01 vol of 10 mM diamide in water and incubated at 10°C for the indicated time. The cells were then lysed by heating them for 1 min at 100°C in SDS–PAGE sample buffer, their proteins were separated by SDS–PAGE and PrrC monitored by immunoblotting using purified polyclonal anti-PrrC antibodies (17).

**ACNase isolation and assays**

The standard core ACNase form used in the *in vitro* assays was a His<sub>6</sub>-tagged derivative of the leaky PrrC mutant D222E. It was purified by TALON<sup>®</sup> (Clontech) affinity chromatography followed by Superdex-200 gel filtration (3). The standard ACNase reaction mixture (10 μl) contained 1 fmol of the <sup>32</sup>P-labeled *E. coli* tRNA<sup>Lys</sup> or the indicated [5'-<sup>32</sup>P] labeled ASL, both at 3000 Ci/mmol; 2 μM dTTP, 4 mM Na-HEPES buffer, pH 7.5; 0.5 mM MgCl<sub>2</sub>, 15 mM NaCl, 5% glycerol and 0.5 M trimethylamine-N-oxide (TMAO). The reaction was
performed at 10°C. It was initiated by adding the enzyme and terminated at the indicated time by adding 10 M urea, 0.01% bromphenol blue and 0.01% xylene cyanol. The products were separated by polyacrylamide–urea gel electrophoresis and quantified by densitometry or counting.

Peptide-RNA UV-cross-linking

Peptide-RNA UV-cross-linking was performed in mixtures (10 μl) containing 0.1–2.0 nmol of Lys-anticodon recognizing peptide (LARP) or indicated LARP derivative, 5 mM Na-HEPES buffer, pH 7.5; 1 mM MgCl2; 30 mM NaCl, 0.5–1.0 M TMAO; and 10% glycerol. The mixture was placed in a 35-mm polystyrene culture dish and irradiated for 5 min at 4°C over a TFX-20 M transilluminator providing radiation spectrum with a peak at 312 nm. Aliquots were mixed with 1.5 vol of 10 M urea containing 0.01% each of xylene cyanol and bromphenol blue and separated by denaturing gel electrophoresis.

PrrC-RNA UV-cross-linking

The PrrC mutant proteins H356A and H356A/F292S were cross-linked to ASL-3 in 12 μl ACNase reaction mixtures essentially as described above but containing 0.3 pmol of ASL-3 and ~1 μg of the indicated PrrC allele purified by TALON cobalt affinity chromatography. The mixture was irradiated for 5 min at 4°C as described for the LARP-ASL-3 cross-linking. An equal volume of 2x SDS–PAGE gel sample buffer devoid of reducing agent was added and the products separated by 10% SDS–PAGE. The proteins were transferred then to a nitrocellulose membrane, PrrC monitored by immunoblotting using anti-PrrC antibodies (17) and the PrrC/ASL-3 cross-linking product detected by autoradiography.

Glutaraldehyde (GA)-mediated protein–protein cross-linking

The indicated PrrC forms were subjected to GA-mediated protein–protein cross-linking as previously described and the products monitored by immunoblotting (3).

RESULTS AND DISCUSSION

The synthetic 11-mer LARP matching PrrC residues 284–294 inhibits ACNase activity

Mutations that alter ACNase cleavage specificity have implicated PrrC’s Asp287 in tRNA1lys recognition (17–19). Asp287 maps to a consensus sequence shared by a subset of the known PrrC homologs. This sequence comprises a predicted coil region (284–292) and the essential aromatic residue Phe285 and Tyr294 (Figure 3) (3,17). Its possible role in tRNA1lys recognition was investigated using an end-protected mimic (Ac-KYGDSNKFSY-NH2). This mimic was termed LARP to indicate its anticipated role in tRNA1lysoccluding the RNA substrate and/or competing with the substrate within the range examined (Figure 4F and G). These results hinted that LARP acted by qualifying the RNA substrate and/or competing with the substrate over the protein.
LARP UV-cross-links ASL-3

The possible interaction of LARP with the ACNase substrate was tested by attempting to UV-cross-link the peptide to ASL-3. The cross-linking was performed at 312 nm where the tRNA Lys wobble base is highly photoreactive (26). Several cross-linking products were obtained in amounts proportional to the dose of LARP (Figure 5A and B). The most abundant (designated a) was retarded in gel electrophoresis relative to ASL-3 by the equivalent of ~4 nt, possibly due to a single LARP adduct. Slower migrating products designated b-d could contain additional LARP moieties, judged from their incremental retardations and dependence on LARP. Presumably, they arose through peptide–peptide cross-links since data shown below suggested that LARP could also self-interact (Figures 8 and 9). The non-irradiated mixture yielded weaker bands over a continuous distribution trailing behind ASL-3 (lane 2), probably non-covalent complexes that partially dissociated during the fractionation.

Figure 4. LARP inhibits ACNase activity. (A) Effect of LARP on the PrrC-catalyzed cleavage of E. coli tRNA Lys. The ACNase assay was performed in the presence of the indicated LARP levels and aliquots analyzed at the indicated time points. (B) Time course of the reactions of panel A. (C and D) Effect of LARP on the respective PrrC-catalyzed cleavages of ASL-3 or ASL-C. Only 15-min time points are shown. (E) ASL-3 or ASL-C cleavage by PrrC versus LARP’s level. (F and G) Effect of LARP on ACNase activity at different ASL-3 levels. tRNA Lys fr. 1–33, ASL-3 fr. 1–7 and ASL-C fr. 1–7, respective labeled ACNase cleavage products of tRNA Lys, ASL-3 and ASL-C.
Mutating LARP impairs the ASL-cross-linking and ACNase-inhibiting activities

LARP was mutated to evaluate the importance of its sequence to the ASL-cross-linking and ACNase-inhibiting activities. Trimmed derivatives lacking Lys1 (Δ1) or Tyr11 (Δ11) formed UV-cross-links to ASL-3 with the respective levels of LARP: 8.25, 16.5, 33, 82.5, 165 or 330 μM. XC, dye marker corresponding in position to a 22 nt oligoribonucleotide; a–d indicate cross-linking products assumed to contain one, two, three or four LARP moieties per ASL-3, respectively. (B) Relative levels of the primary (a), secondary (b) and tertiary (c) conjugates versus that of LARP. The amount of primary conjugate formed at the highest LARP level is assigned a value of 1.0.

F9S and Y11S impaired the two activities in a correlated manner. The milder Y11S mutation reduced the yield of the primary UV-cross-linking conjugate with ASL-3 nearly 2-fold (Figure 7A, lane 3 versus 4) and partially impaired the inhibition of ACNase (Figure 7B, lane 3 versus 4). It is noteworthy that this mutation also prevented the formation of a stable secondary conjugate, as inferred from the absence of the expected band b and appearance instead of a smear trailing behind band a. The more drastic mutation F9S reduced the cross-linking to ASL-3 8-fold (Figure 7A, compare lanes 8 and 9) and severely impaired ACNase inhibition (Figure 7B, compare lanes 8 and 9). The corresponding PrrC mutation F292S could also abrogate the UV-cross-linking of PrrC to ASL-3. This was inferred from the observation that the active site PrrC mutant H356A, which lacks ACNase activity (3) but efficiently binds tRNA<sup>Lys</sup> (data not shown), formed UV-cross-links to ASL-3 ~30-fold more efficiently than F292S/H356A (Figure 7C).

The Y11F mutation conferred a different phenotype. It nearly abolished the ACNase-inhibiting potential of CLARP (Figure 7B, compare lanes 2, 3 and 5) yet doubled the yield of the primary UV-cross-linking conjugate obtained with ASL-3 (Figure 7A, compare lanes 3 and 5). In part this increase could be ascribed to the failure of Y11F to form stable higher conjugates, as with Y11S. Nonetheless, the overall cross-linking yield obtained with Y11F suggested that this mutant bound ASL-3 at least as efficiently as CLARP. The discrepant behavior...
of Y11F hinted that the ACNase-inhibiting forms of LARP exerted their effect not only by occluding the RNA substrate but also by forming a binary complex with PrrC. Since LARP was employed in excess over PrrC and ASL-3, it could exert these two ACNase-inhibiting modes independently. The possibility that LARP interacted with PrrC directly was reinforced by data shown below suggesting that both LARP and its matching PrrC sequence can self-interact (Figures 8 and 9). The failure of the Y11S and Y11F mutants to form stable conjugates containing additional peptide moieties could not be ascribed to a critical role of Tyr 11 in peptide–peptide UV-cross-linking since deleting this residue did not elicit such an effect (Figure 6A, lane 5).

Extending LARP with Cys augments its activities under suitable redox conditions

LARP derivatives extended at the N- or C-end with Cys (CLARP and LARPC, respectively) were intended for further modification with Fe-EDTA that rendered them artificial nucleases (M. Amitsur, D. Klaiman and G. Kaufmann; unpublished data). Unexpectedly, CLARP and, to a lesser extent, LARPC were far more potent than LARP in UV-cross-linking ASL-3 and inhibiting ACNase. Thus, when CLARP, LARP or LARPC were UV-cross-linked to ASL-3 at 1 mM DTT and identical peptide levels, similar product patterns were obtained. However, the product yields with CLARP or LARPC were about an order of magnitude higher than with LARP (Figure 8A). Employing the Cys-containing peptides at a level 10-fold lower than that of LARP resulted in comparable yields (Figure 8B). CLARP and LARPC UV-cross-linked ASL-3 more efficiently than LARP also without DTT, LARPC yielding under these conditions a relatively high proportion of the secondary conjugate (Figure 8C). However, the cross-linking efficiency of CLARP and LARP to ASL-3 was relatively weak at 10 mM DTT (Figure 8D). CLARP and LARPC inhibited ACNase more strongly than LARP at 1 mM DTT (Figure 8E) but were less effective than LARP at 10 mM DTT (Figure 8F).

Thus, tethering Cys to one or the other end of LARP enhanced the ACNase-inhibiting and ASL-3 cross-linking activities of the peptide although not under highly reducing conditions. Moreover, LARPC yielded under oxidizing conditions higher proportions of conjugates likely to contain two peptide moieties per ASL-3. These facts may be accounted for by the stabilization of a common functional form of LARP both by a C-terminal or N-terminal S-S bond, a requirement satisfied by a parallel but not a head-to-tail LARP dimer. An alternative explanation is that mere dimerization of LARP by a disulfide bond at either end increased the probability of non-specific electrostatic, hydrogen bonding and stacking interactions between the peptide and the RNA.

The tethered Cys residue enhanced the UV-cross-linking efficiency of LARP to ASL-3 optimally at intermediate redox conditions (1 mM) rather than oxidizing conditions. This outcome suggested that reversible formation of the disulfide link facilitated the
fixation of original LARP–LARP and LARP–RNA contacts by UV-cross-linking. In other words, a stable S-S bond could impede the UV-cross-linking by constraining the dimer.

Placing Cys at or near the PrrC region matching LARP triggers intersubunit cross-links

The suspicion that LARP exerted its ASL-3 cross-linking and ACNase inhibiting activities as a parallel dimer prompted us to examine if the matching PrrC sequence dimerizes similarly. To this end, a Cys residue was introduced in this sequence instead of Ser 288, Ser 291 or Ser 293. We expected that the mutant Cys residues will self-interact and form intersubunit disulfide cross-links if the region containing them dimerizes in parallel.

Each of the three mutations was placed over the D222E/C268A/C385A background termed PrrC/C3. This facilitated the isolation of the mutant proteins (3,17) and precluded

Figure 8. Cys extensions enhance LARP activities under suitable redox conditions. (A) Terminal Cys extensions enhance the UV-cross-linking of LARP to ASL-3. The indicated peptides employed each at 0.2 mM were UV-cross-linked to ASL-3 in the presence of 1mM DTT. (B–D) Effect of the DTT level on the cross-linking efficiencies of LARP, CLARP and LARPC on their ability to form UV-cross-links to ASL-3. LARP, CLARP and LARPC employed at the indicated concentrations were UV-cross-linked to ASL-3 at 1 mM DTT (panel B), without DTT (panel C) or at 10 mM DTT (panel D). (E) Effect of LARP, CLARP and LARPC on ACNase activity at 1 mM DTT. The incubation times were 2 (lanes 2, 5, 8, 11, 14 and 17), 5 (lanes 3, 6, 9, 12, 15 and 18) and 10 min (lanes 4, 7, 10, 13, 16 and 19). (F) Effect of 10 mM DTT on the ACNase-inhibiting potential of LARP, CLARP or LARPC. The incubation times were 2 (lanes 2, 5, 8, 11, 14, 17 and 20), 5 (lanes 3, 6, 9, 12, 15, 18 and 21) and 10 min (lanes 4, 7, 10, 13, 16, 19 and 22). a–c indicate respective cross-linking products assumed to contain one, two or three LARP moieties per ASL-3; a'–c', corresponding products of CLARP and LARPC. ASL-3 fr. 1–7, labeled ACNase cleavage product of ASL-3.
the formation of non-specific S-S cross-links due to the wild-type Cys residues. The PrrC* control and to-Cys derivatives exhibited comparable in vivo ACNase activities and protein levels (data not shown).

However, only PrrC* and S288C/PrrC* retained ACNase activity in vitro (Figure 9A).

To induce the formation of S-S cross-links the cells expressing the to-Cys mutants or the PrrC* control were
exposed to diamide (27). The cellular proteins were separated then by SDS–PAGE under non-reducing conditions and PrrC visualized by immunoblotting. As shown, the three mutants, but not PrrC<sup>C</sup> yielded products that migrated in SDS–PAGE slower than the 47.5-kDa PrrC monomer (Figure 9B). These products included a major form designated X that migrated below the 175-kDa size marker as well as several less pronounced and faster migrating forms. These various products appeared also without diamide, perhaps due to partial in vitro oxidation. However, their amounts increased considerably with diamide, consistent with their formation by disulfide cross-linking.

To determine if any of the diamide-dependent products arose by intra-PrrC cross-links, the four PrrC forms were isolated by immobilized metal affinity chromatography in the absence of a reducing agent. Subsequently they were fractionated by SDS–PAGE as such, after prior treatment with 1 mM DTT or also with 2 mM diamide. Western analysis revealed that the purified Ser→Cys mutants, but not PrrC<sup>C</sup>, yielded a single product with mobility similar to that of band X of the diamide treated cells (compare Figure 9C, lanes 1, 4, 7 and 10 to Figure 9B lanes 4, 8, 12 and 16). Moreover, this band was abolished by DTT (Figure 9C, lanes 2, 5, 8 and 11) and restored by excess diamide (lanes 3, 6, 9 and 12), indicating it depended on disulfide cross-linking. Corresponding protein staining (Figure 9D) ascertained that the various PrrC forms were not contaminated by significant amounts of other proteins and, consequently, that band X was unlikely to contain proteins other than PrrC. The failure of a considerable fraction of the to-Cys mutant proteins to generate band X could be attributed to its misfolding since PrrC<sup>C</sup> is thermally unstable. Alternatively, this fraction could represent a functionally relevant conformation incompatible with the S-S cross-linking; e.g. due to a substrate or inhibitor bound to the tRNA<sup>Lys</sup> site.

Band X migrated in SDS–PAGE less than expected of a cross-linked PrrC dimer of ~95 kDa. This fact could be attributed to its particular shape or irreversible entanglement of two such dimers within the PrrC tetramer. To distinguish between these possibilities, we subjected PrrC<sup>C</sup> and S293/C<sup>PrrC</sup> to GA-mediated protein–protein cross-linking. PrrC<sup>C</sup> yielded the familiar pattern (3) where the monomer is gradually converted into apparent GA-cross-linked tetramer forms via dimeric and less pronounced trimeric intermediates (Figure 9E, lanes 1–4). The monomeric fraction of the mutant behaved similarly (lanes 5–8). Band X, which coincided with the most retarded GA-cross-linked dimers, was also gradually converted into higher forms superimposed over those generated by the fraction that failed to form the S-S cross-links. These data suggested that band X was a dimer that was retarded due to its particular shape.

We analyzed in a similar fashion PrrC<sup>C</sup> derivatives containing Cys in the predicted α-helix found immediately downstream to the LARP-like region (Figure 3). It replaced L298, I302 or I306 of the α-helix hydrophilic face or the hydrophilic Q300. These mutants also yielded the S-S cross-linked form designated X, L298C yielding the highest proportion (Figure 9F, lanes 1–3), possibly due to closer contact of the dimerizing α-helices at position 298. Q300C yielded a variant (designated Xb) that migrated slightly faster than the form X generated by the other mutants designated here Xa (Figure 9F, compare lanes 3 and 4, 6 and 7). The slight mobility difference may be attributed to the presence of Cys<sup>300</sup> at the hydrophilic and, hence, outward-pointing face of the dimerizing α-helix. Consequently, an S-S cross-link mediated by it could constrain the dimer into a more compact, faster migrating form. The PrrC mutant D222E containing the wild-type residues Cys<sup>268</sup> and Cys<sup>385</sup> failed to generate Band X (Figure 9F, lanes 16–18). This result underscored the specificity of the intersubunit cross-links triggered by the mutant Cys residues placed in the 288–306 range.

**Peptide mimicry of tRNA<sup>Lys</sup> recognition by PrrC**

LARP formed UV-cross-links to ASL analogs of tRNA<sup>Lys</sup> and inhibited their PrrC-catalyzed cleavage...
The tRNA^Lys^ site of PrrC may arise between C-domains interfacing in parallel

The functional relevance of LARP to its matching PrrC region was suggested also by the apparent ability of both to dimerize in parallel. We deduced that LARP acted as a parallel dimer in forming UV-cross-links to ASL-3 and inhibiting ACNase from the ability of a single Cys residue tethered to either the N- or C-end of LARP to dramatically enhance these activities and abolition of these enhancements under highly reducing conditions (Figure 6). These coincidences could be accounted for by the stabilization of a common functional dimeric structure of LARP by a disulfide bond formed at one of the other end of its protomers, a requirement met by a parallel but not anti-parallel dimer. Alternatively, the similar effects of the N- and C-proximal Cys extensions reflected the increased probability of non-specific peptide–RNA interactions caused by mere dimerization of the peptide, whether caused by the N- or C-terminal S-S bond. However, we favor the first explanation in view of the intersubunit S-S cross-links triggered by all seven Cys substitutions placed along the C-proximal PrrC 288–306aa region overlapping the sequence matching LARP (Figure 9). Such an outcome is consistent with parallel dimerization of the mutated region and lends support to the assumption that LARP could also dimerize in this orientation. The PrrC region that dimerizes in parallel could be confined to a portion of the ACNase domain since the regulatory N-domain is expected to dimerize in a head-to-tail fashion, like typical ABC/ATPases where the nucleotide binding pocket arises by the interaction of the Walker A motif of one subunit with the ABC signature motif of the second (15). Moreover, the wild-type Cys^268^ and ^385^ at the edges of the C-domain (Figure 10A) did not trigger intersubunit cross-links (Figure 9F). The relation of the PrrC region that dimerizes in parallel to the tRNA^Lys^ -binding motif may be revealed by accurately mapping and functionally characterizing it.

Finally, as illustrated in Figure 10B, the opposite orientations in which PrrC's N- and C-domains dimerize dictate a phosphofructokinase-like topology (30) where the NTPase and tRNA^Lys^ sites arise at distinct assembly stages of the PrrC dimer of dimers (3).

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Conflict of interest statement. None declared.

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