Virology 427 (2012) 144–150

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Structure–function relations in the NTPase domain of the antiviral tRNA ribotoxin *Escherichia coli* PrrC

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ARTICLE INFO

Article history:
Received 21 January 2012
Returned to author for revision
8 February 2012
Accepted 11 February 2012
Available online 2 March 2012

Keywords:
Anticodon nuclease
Antiviral defense
Structure-guided mutagenesis

ABSTRACT

Breakage of tRNA by *Escherichia coli* anticodon nuclease PrrC (*Eco*PrrC) underlies a host antiviral response to phage T4 infection. Expression of *Eco*PrrC is cytocidal in yeast, signifying that PrrC ribotoxicity crosses phylogenetic domain boundaries. *Eco*PrrC consists of an N-terminal NTPase module that resembles ABC transporters and a C-terminal nuclease module that is sui generis. PrrC homologs are prevalent in many other bacteria. Here we report that *Haemophilus influenzae* PrrC is toxic in *E. coli* and yeast. To illuminate structure–activity relations, we conducted a new round of mutational analysis of *Eco*PrrC guided by primary structure conservation among toxic PrrC homologs. We indentify 17 candidate active site residues in the NTPase module that are essential for toxicity in yeast when *Eco*PrrC is expressed at high gene dosage. Their functions could be educed by integrating mutational data with the atomic structure of the transition-state complex of a homologous ABC protein.

Introduction

The *Escherichia coli* tRNA anticodon nuclease PrrC (*Eco*PrrC) mediates an RNA-damaging innate immune response to bacteriophage T4 infection (Kaufmann, 2000). The "ribotoxin" activity of *Eco*PrrC is normally suppressed by its association with its cognate "antitoxin", a type I DNA restriction–modification enzyme (*Eco*prrI) encoded by neighboring ORFs in the *prr* operon (Levitz et al., 1990; Tyndall et al., 1994). The latent *Eco*PrrC nuclease is activated by the virus-encoded Stp peptide synthesized early during T4 infection (Amitsur et al., 1989, 1992; Penner et al., 1995). The activated form of *Eco*PrrC incises the $tRNA^{Lys(UUU)}$ anticodon loop at a single site 5′ of the wobble uridine, leaving 2′,3′ cyclic phosphate and 5′-OH ends at the break (Amitsur et al., 1987). Depletion of the $tRNA^{Lys}$ pool inhibits the synthesis of T4 late proteins and blunts spread of the virus through the bacterial population. In effect, the initially infected host bacteria altruistically commit suicide to protect the community. However, phage T4 thwarts the host defense strategy by encoding a tRNA repair system that restores late viral protein synthesis (Amitsur et al., 1987; Sirotkin et al., 1978).

*Eco*PrrC consists of two domains: an N-terminal nucleoside triphosphate phosphohydrolase (NTPase) module (aa 1–264) related to the ABC transporter NTPase family and a distinctive C-terminal ribonuclease module (aa 265–396) (Blanga-Kanfi et al., 2006; Kaufmann, 2000) (Fig. 1). The NTPase and nuclease domains must be linked in *cis* for *Eco*PrrC to exert its toxicity (Meineke et al., 2011). The NTPase domain of *Eco*PrrC activates the latent anticodon nuclease via a mechanism entailing both GTP hydrolysis and the binding of dTTP, a putative allosteric effector (Amitsur et al., 2003; Klaiman and Kaufmann, 2011). Physical and genetic studies, and analogy to ABC family proteins, support a model in which *Eco*PrrC toxicity requires head-to-tail dimerization of the NTPase modules to form two composite sites for NTP binding and hydrolysis (Blanga-Kanfi et al., 2006; Klaiman et al., 2007; Meineke et al., 2011).

PrrC homologs are present in the proteomes of many other bacteria (Davidov and Kaufmann, 2008), though their biological activities in their native environments are uncharted. The ribotoxicity of bacterial PrrC is portable to eukarya. Induced expression of *Eco*PrrC or *Streptococcus mutans* (*Smu*) PrrC in budding yeast cells is fungicidal, signifying that PrrC can be toxic in a eukaryon in the absence of any other bacterial or viral proteins (Meineke et al., 2011). The apparent lack of toxicity of *Neisseria meningitidis* (*Nme*) PrrC was attributed to a nuclease-inactivating arginine substitution for an essential tryptophan (highlighted in purple in Fig. 1); a single back-mutation of this arginine to tryptophan sufficed to make *Nme*PrrC toxic when expressed in yeast or *E. coli* (Meineke and Shuman, 2012).

An extensive survey of the effects of alanine and conservative mutations on *Eco*PrrC toxicity in yeast has so far identified 22 functionally important residues in the NTPase domain and 15 in the nuclease domain (Meineke and Shuman, 2012; Meineke et al., 2011) (see Fig. 1; positions highlighted in green without a ▼ symbol). Replacing these important side chains by alanine resulted in loss of toxicity in yeast when the *prrC* gene was expressed from a *CEN* plasmid. Nineteen residues were defined by the alanine scan as inessential for

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doi:10.1016/j.virol.2012.02.008

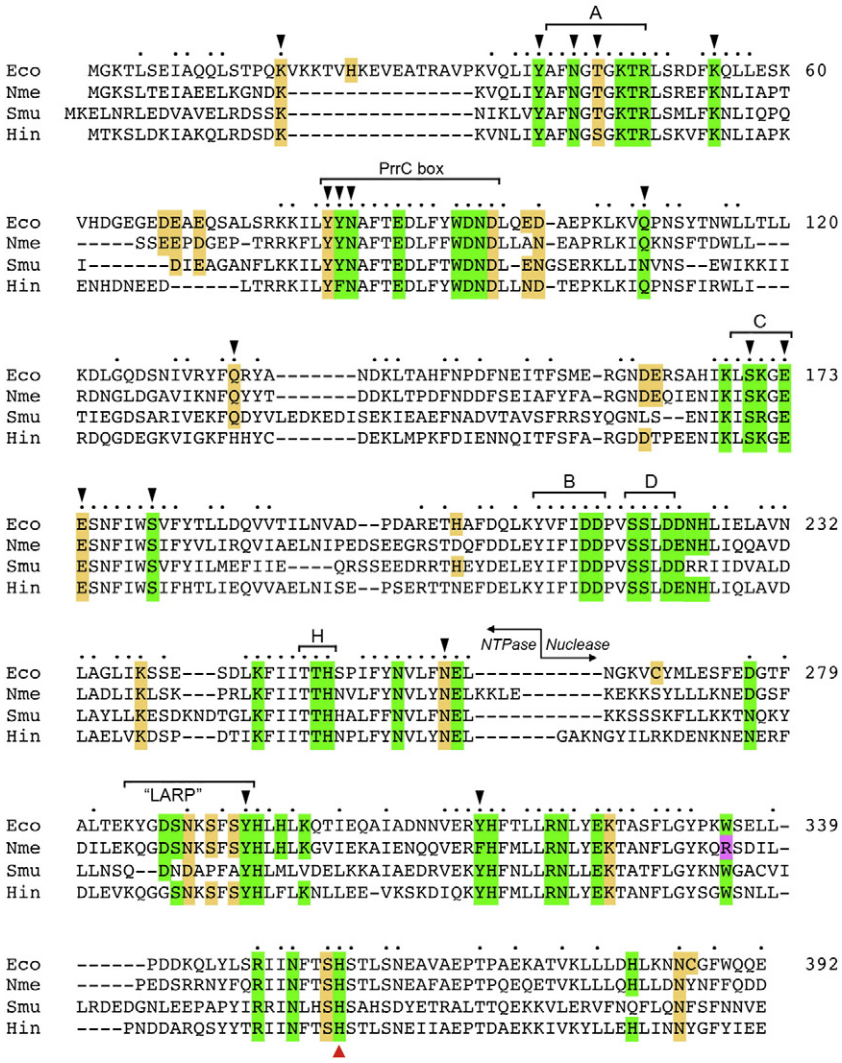

Fig. 1. Homology-guided mutagenesis of *Eco*PrrC. The amino acid sequence of *Eco*PrrC is aligned to sequences of the *Nme*PrrC, *Smu*PrrC, and *Hin*PrrC proteins. Positions of side chain identity/similarity in all four proteins are indicated by • above the alignment. The conserved peptide motifs of the N-terminal ABC-type NTPase domain (labeled A, C, B, D and H) are demarcated by brackets. The PrrC-box of the NTPase domain and the lysine anticodon recognition peptide ("LARP") motif of the nuclease domain are labeled and demarcated by brackets. *Eco*PrrC residues defined as important for yeast toxicity by alanine mutagenesis of the *CEN*-based *prrC* gene are shaded green; nonessential residues are shaded yellow. The new residues mutated in the present study are denoted by ▼ above the alignment.

*Eco*PrrC toxicity; i.e., alanine changes did not affect toxicity when the mutants were expressed from a *CEN* plasmid (Fig. 1; positions highlighted in yellow without a ▼ symbol). As one might expect, many (though not all) of the important residues in *Eco*PrrC are conserved in the toxic *Smu*PrrC and latently toxic *Nme*PrrC proteins (Fig. 1). As noted previously (Meineke et al., 2011), and discussed in detail below, the roles of some of the NTPase domain residues can be surmised from their location within the defining peptide motifs of structurally characterized ABC proteins (Fig. 1) (Oldham and Chen, 2011). By contrast, the nuclease domain has no discernible primary structure similarity to any known ribonucleases or tRNA-binding proteins, which makes it difficult to guess which essential residues might be directly involved in catalysis versus substrate recognition versus PrrC folding/stability. By testing the 15 nontoxic alanine mutants in the *Eco*PrrC nuclease module for restoration of toxicity by increasing gene dosage, we could distinguish between hypomorphs and potential nuclease nulls (Meineke and Shuman, 2012). This criterion highlighted eight essential residues (Asp276, Asp287, His295, His315, Arg320, Glu324, Arg349, and His356) as candidate active site constituents. Among these, Arg320, Glu324 and His356 had been proposed by the Kaufmann lab to comprise a catalytic triad for transesterification chemistry (Blanga-Kanfi et al., 2006).

Here, we extend our studies of bacterial PrrC on two fronts. First, we interrogated the in vivo activity of the PrrC homolog encoded by *Haemophilus influenzae* strain 86-028NP (Harrison et al., 2005) and thereby found that *Hin*PrrC was toxic in yeast and growth suppressive in *E. coli*. Second, we used the primary structure conservation among confirmed ribotoxic PrrC homologs to guide a further round of mutagenesis of *Eco*PrrC (Fig. 1). We thereby identified 9 new side chains in the NTPase module and two in the nuclease domain that are important for toxicity in yeast. We interpret the sum of mutational data for the NTPase domain in light of recently reported atomic structures of an exemplary ABC protein that illuminate the Michaelis complex and the transition state of the phosphohydrolase reaction (Oldham and Chen, 2011).

Results and discussion

H. influenzae PrrC is toxic in vivo

The 384-aa *Hin*PrrC polypeptide (GenBank accession YP_247935) has 274 positions of side-chain identity/similarity to *Eco*PrrC in a pairwise alignment. Whereas most of the essential residues in *Eco*PrrC are conserved in *Hin*PrrC, there is a conspicuous exception whereby the