



Exoribonucleases as modulators of virulence in pathogenic bacteria

Rute G. Matos, Cátia Bárria, Vânia Pobre, José M. Andrade and Cecília M. Arraiano*

Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal

*Correspondence: cecilia@itqb.unl.pt

A commentary on

Polynucleotide phosphorylase has an impact on cell biology of *Campylobacter jejuni*

Haddad, N., Tresse, O., Rivoal, K., Chevret, D., Nonglaton, Q., Burns, C. M., Prévost, H., and Cappelier, J. M. (2012). *Front. Cell. Inf. Microbio.* doi: 10.3389/fcimb.2012.00030

Pathogenic bacteria are responsible for severe diseases worldwide. RNA stability is a major player controlling the expression of virulence factors. Ribonucleases (RNases) are the enzymes responsible for the maturation and degradation of RNA molecules (Arraiano et al., 2010; Silva et al., 2011). Exoribonucleases have been implicated in virulence in an increasing number of pathogens such as *Salmonella enterica*, *Helicobacter pylori*, *Shigella flexneri*, and *Aeromonas hydrophila* (see Andrade et al., 2009; Matos et al., 2011 and references below). However, the mechanisms underlying virulence are still mostly elusive (Arraiano et al., 2010; Lawal et al., 2011).

The recently published paper by Haddad et al. (2012) adds to this list *Campylobacter jejuni*, one of the most important human foodborne pathogens. *Campylobacter* is recognized as the leading bacterial cause of gastroenteritis and even more severe clinical manifestations can arise. The present work shows that *C. jejuni* bacteria lacking an 3'-5' exoribonuclease called polynucleotide phosphorylase (PNPase) is significantly less virulent than the wild-type strain (Haddad et al., 2012).

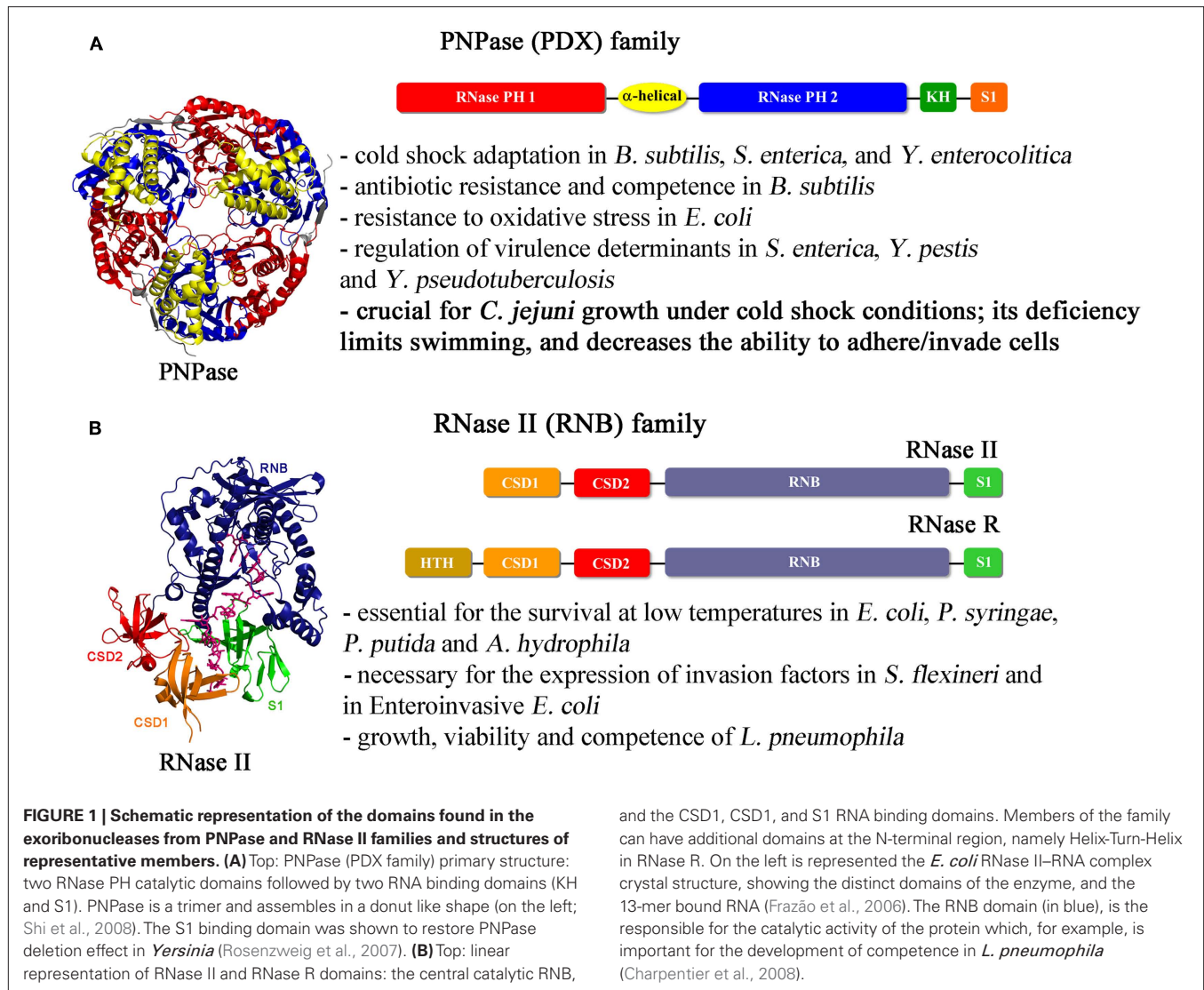
Different steps have been identified in the ability of different pathogenic bacteria to promote infection, namely motility, adherence, invasion, intracellular replication, or spreading to the neighboring cells. Inactivation of the *C. jejuni* PNPase is shown to affect many of these steps, with *pnp* mutants showing distinct phenotypes such as limitations in swimming, substantial delay in the colonization

of the chicken gut and a decreased ability to adhere and invade cells. Defects in motility are suggested to be responsible for many of the attenuation of the virulent traits of *C. jejuni* in the mutant *pnp* strain. Interestingly, the authors suggest that PNPase may be able to affect flagella-dependent motility by modulation of the NANA synthetase (*neuB*), involved in the post-translational modification of the flagellin subunit. Furthermore, proteomic studies also showed that PNPase affects the synthesis of proteins involved in virulence, such as LuxS and PEB3. This work confirms the importance of exoribonucleases, namely PNPase, in cell biology, and virulence (Haddad et al., 2012).

Bacterial pathogens rapidly adapt to environmental challenges. Adaptation requires a rapid adjustment in RNA levels, requiring not only transcriptional regulation, but also fine-tuning control of RNA stability. Stress-resistance plays an essential role in the capacity of many pathogenic bacteria to establish and maintain long-term intracellular residence in host cells. Many ribonucleases are regulated by stress conditions, being critical enzymes involved in the adaptation of bacteria to new environmental conditions. In particular, PNPase is a cold-shock protein in *Escherichia coli* being essential for growth at low temperatures (Zangrossi et al., 2000). Haddad et al. (2009) had previously shown that PNPase was also crucial for *C. jejuni* growth under cold-shock conditions. This was a relevant discovery especially when considering that this pathogen can persist and grow at refrigerated temperatures. PNPase also seems to be involved in *C. jejuni* resistance to acidic and oxidative stresses, as the *pnp* strain shows variations in the levels of the stress-response proteins KatA, DnaK, and Hsp90 (Haddad et al., 2012). In *S. enterica*, PNPase was shown to be important for acute infection and lethality in a murine model as result of increasing expression of the pathogenicity islands (Clements et al., 2002). In *Yersinia pseudotuberculosis* and *Y. pestis* PNPase was

shown to be essential for the function of the *Yersinia* type three secretion system (TTSS), an organelle that injects effector proteins directly into host cells (Rosenzweig et al., 2007). Interestingly, PNPase has been involved in the post-transcriptional regulation of small non-coding RNAs (Andrade and Arraiano, 2008; De Lay and Gottesman, 2011; Andrade et al., 2012). In *C. jejuni*, not much is known about this class of regulatory RNAs but transcriptomic studies have identified five candidate regions for harboring sRNAs (Chaudhuri et al., 2011). It is an exciting hypothesis that PNPase is able to regulate small RNAs possibly involved in the virulence traits of *C. jejuni* although this lacks experimental evidence at the time.

Together with PNPase, RNase II, and RNase R are the major exoribonucleases involved in RNA degradation in *E. coli* (Figure 1). Orthologs have been described in all domains of life (Arraiano et al., 2010). RNase R, a hydrolytic exoribonuclease, is also known to be involved in the virulence of several microorganisms. Like PNPase, RNase R is a cold-shock protein essential for the survival at low temperatures of several microorganisms, such as *E. coli*, *Pseudomonas putida*, *P. syringae*, and *A. hydrophila*. In some microorganisms RNase R was shown to be necessary for the expression of several invasion factors and mutations on its gene resulted in the reduced expression of virulence phenotypes in *S. flexneri* and in enteroinvasive *E. coli* (Tobe et al., 1992). *Legionella pneumophila* is an intracellular parasite of free-living protozoa which inhabits man-made water distribution systems, and is the most frequent cause of human legionellosis, community-acquired, and nosocomial pneumonia in adults. In this microorganism, RNase R is the only hydrolytic exoribonuclease present. Its activity was shown to be essential for growth and viability at low temperatures and induces competence (Charpentier et al., 2008). Similarly to what was shown in *E. coli* (Cairrão et al., 2003),



RNase R is also a cold-shock protein in *A. hydrophila*. In this highly toxic microorganism, which is resistant to multiple medications, chlorine, and cold temperatures, RNase R was shown to be essential for viability at lower temperatures and its absence leads to a reduction in motility. The infection of mouse cells with *A. hydrophila rnr* mutant strains showed that their virulence was attenuated in comparison to the wild-type, which confirms the role of RNase R in pathogenesis (Erova et al., 2008).

Considering the important functions that these proteins have in the establishment of virulence, ribonucleases (namely RNase II, RNase R, and PNPase) offer a new perspective for developing efficient compounds in clinical treatments: they can be potential targets to design compounds

able to kill specific microorganisms or to reduce their virulence ability. The further study of the function of exoribonucleases in the control of pathogenesis will certainly help in the comprehension of RNA-related processes involved in infection.

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