Exoribonucleases as modulators of virulence in pathogenic bacteria

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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 essential for the survival at low temperatures 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, 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. Like PNPase, RNase R is a cold shock protein essential for the survival at low temperatures of several microorganisms.

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.
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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**REFERENCES**

Andrade, J. M., and Arraiano, C. M. (2008). PNPase is a key player in the regulation of small RNAs that control the expression of outer membrane proteins. *RNA* 14, 543–551.

Andrade, J. M., Pobre, V., Matos, A. M., and Arraiano, C. M. (2009). The role of 3′ to 5′ exoribonucleases in RNA degradation. *FEMS Microbiol. Rev.* 34, 883–923.

Cairrão, F., Cruz, A., Mori, H., and Arraiano, C. M. (2003). Cold shock induction of RNase R and its role in the maturation of the quality control mediator SsrA/tmRNA. *FEMS Microbiol. Lett.* 190, 8126–8136.

Charpentier, X., Faucher, S. P., Kalachikov, S., and Shuman, H. A. (2008). Loss of RNase R induces competence development in *Legionella pneumophila*. *J. Bacteriol.* 190, 8126–8136.

Chaudhuri, R. R., Yu, L., Kanji, A., Perkins, T. T., Gardner, P. P., Choudhary, J., Maskell, D. J., and Grant, A. J. (2011). Quantitative RNA-seq analysis of the *Campylobacter jejuni* transcriptome. *Microbiology* 157, 2922–2932.
Clements, M. O., Eriksson, S., Thompson, A., Lucchini, S., Hinton, J. C., Normark, S., and Rhen, M. (2002). Polynucleotide phosphorylase is a global regulator of virulence and persistence in *Salmonella enterica*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8784–8789.

De Lay, N., and Gottesman, S. (2011). Role of polynucleotide phosphorylase in sRNA function in *Escherichia coli*. *RNA* 17, 1172–1189.

Erova, T. E., Kosykh, V. G., Fadl, A. A., Sha, J., Horneman, A. J., and Chopra, A. K. (2008). Cold shock exoribonuclease R (VacB) is involved in *Aeromonas hydrophila* pathogenesis. *J. Bacteriol.* 190, 3467–3474.

Frazão, C., Mcvey, C. E., Amblar, M., Barbosa, A., Vonrhein, C., Arraiano, C. M., and Carrondo, M. A. (2006). Unravelling the dynamics of RNA degradation by ribonuclease II and its RNA-bound complex. *Nature* 443, 110–114.

Haddad, N., Burns, C. M., Bolla, J. M., Prevost, H., Federighi, M., Drider, D., and Cappelier, J. M. (2009). Long-term survival of *Campylobacter jejuni* at low temperatures is dependent on polynucleotide phosphorylase activity. *Appl. Environ. Microbiol.* 75, 7310–7318.

Haddad, N., Tresse, O., Rivoal, K., Chevret, D., Nonglaton, Q., Burns, C. M., Prevost, H., and Cappelier, J. M. (2012). Polynucleotide phosphorylase has an impact on cell biology of *Campylobacter jejuni*. *Front. Cell. Infect. Microbiol.* 2:30. doi: 10.3389/fcimb.2012.00030

Lawal, A., Jejelowo, O., Chopra, A. K., and Rosenzweig, J. A. (2011). Ribonucleases and bacterial virulence. *Microb. Biotechnol.* 4, 558–571.

Matos, R. G., Pobre, V., Reis, F. P., Malecki, M., Andrade, J. M., and Arraiano, C. M. (2011). “Structure and degradation mechanisms of 3’ to 5’ exoribonucleases,” in Ribonucleases (Nucleic Acids and Molecular Biology), (Heidelberg: Springer-Verlag), 26,193–222.

Rosenzweig, J. A., Chromy, B., Echeverry, A., Yang, J., Adkins, B., Plano, G. V., McCutchen-Maloney, S., and Schesser, K. (2007). Polynucleotide phosphorylase independently controls virulence factor expression levels and export in *Yersinia* spp. *FEMS Microbiol. Lett.* 270, 255–264.

Shi, Z., Yang, W. Z., Lin-Chao, S., Chak, K. F., and Yuan, H. S. (2008). Crystal structure of *Escherichia coli* PNPase: central channel residues are involved in processive RNA degradation. *RNA* 14, 2361–2371.

Silva, I. J., Saramago, M., Dressaire, C., Domingues, S., Viegas, S. C., and Arraiano, C. M. (2011). Importance and key events of prokaryotic RNA decay: the ultimate fate of an RNA molecule. *Wiley Interdiscip. Rev. RNA* 2, 818–836.

Tobe, T., Sasakawa, C., Okada, N., Honma, Y., and Yoshikawa, M. (1992). vacB, a novel chromosomal gene required for expression of virulence genes on the large plasmid of *Shigella flexneri*. *J. Bacteriol.* 174, 6359–6367.

Zangrossi, S., Briani, E., Ghisotti, D., Regonesi, M. E., Tortora, P., and Dehò, G. (2000). Transcriptional and post-transcriptional control of polynucleotide phosphorylase during cold acclimation in *Escherichia coli*. *Mol. Microbiol.* 36, 1470–1480.