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## Binase-like guanyl-preferring ribonucleases are new members of *Bacillus* PhoP regulon

Vera Ulyanova<sup>a,b,1</sup>, Valentina Vershinina<sup>b</sup>, Olga Ilinskaya<sup>b</sup>, Colin R. Harwood<sup>a,\*</sup><sup>a</sup> Institute for Cell and Molecular Biosciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom<sup>b</sup> Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, 420008, Russian Federation

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### ABSTRACT

Extracellular low-molecular weight guanyl-preferring ribonucleases (LMW RNases) of *Bacillus* sp. comprise a group of hydrolytic enzymes that share highly similar structural and catalytic characteristics with barnase, a ribonuclease from *Bacillus amyloliquefaciens*, and binase, a ribonuclease from *Bacillus intermedius*. Although the physical-chemical and catalytic properties of *Bacillus* guanyl-preferring ribonucleases are very similar, there is considerably more variation in the environmental conditions that lead to the induction of the genes encoding these RNases. Based on structural differences of their genes the guanyl-preferring ribonucleases have been sub-divided into binase-like and barnase-like groups. Here we show the ability of the key regulator of phosphate deficiency response, PhoP, to direct the transcription of the binase-like RNases but not barnase-like RNases. These results, together with our demonstration that binase-like RNases are induced in response to phosphate starvation, allow us to categorise this group of ribonucleases as new members of *Bacillus* PhoP regulon. In contrast, the barnase-like ribonucleases are relatively insensitive to the phosphate concentration and the environmental conditions that are responsible for their induction, and the regulatory elements involved, are currently unknown.

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### 1. Introduction

Representatives of the soil-living bacterial genus *Bacillus* secrete a variety of macromolecular hydrolases that allow them to utilize the complex carbohydrates, proteins and nucleic acids in their environment as sources of nutrients. Nucleic acids provide sources of carbon, nitrogen and phosphate and consequently, bacteria often secrete nucleases to facilitate the recovery of these essential nutrients from environmental DNA and RNA. Several species of *Bacillus*, including *Bacillus amyloliquefaciens* (Bam RNase, barnase), *Bacillus pumilus* (Bpu RNase), *Bacillus thuringiensis* (Bth RNase), *Bacillus intermedius* (Bin RNase, binase) and *Bacillus circulans* (Bci RNase) [Hartley and Barker, 1972; Aphanasenko et al., 1979; Struminskaia et al., 1992; Dement'ev et al., 1993a; Dementiev et al., 1993b], secrete low-molecular weight guanyl-preferring ribonucleases

(LMW RNases), that belong to N1/T1 ribonuclease family (Fig. 1). These basic proteins of *Bacillus* are between 109 and 110 amino acid residues in length with a molecular mass of about 12 kDa. Their primary structures share more than 80% identity, with similar secondary and tertiary structures [Bycroft et al., 1991; Reibarkh et al., 1998]. Although the physical-chemical and catalytic properties of *Bacillus* guanyl-preferring ribonucleases are similar, there is considerable variation in the environmental conditions that lead to the induction of the genes encoding these RNases [Ulyanova et al., 2011]. This variability is reflected in differences in their upstream regulatory sequences (Fig. 2A). Based on these and other features, LMW RNases of *Bacillus* can be divided into two groups – binase-like RNases that include Bin, Bpu and Bth and barnase-like RNases that include Bam and Bci (Fig. 1). As an example, these RNases respond to phosphate availability differently. Generally, binase-like RNases are repressed at high phosphate concentrations and strongly induced at low concentrations. In contrast barnase-like RNases are less sensitive to the concentration of phosphate in the medium, indicating that they may have an ecological role that is distinct to that of binase-like RNases [Znamenskaya et al., 1995; Znamenskaia et al., 1998; Shul'ga et al., 2000; Ulyanova et al., 2011].

The adaptation of *Bacillus subtilis* to phosphate-starvation conditions involves the activation of the Pho stimulon, comprising at least the specific PhoP regulon, regulated by the PhoP-PhoR

\* Corresponding author at: The Centre for Bacterial Cell Biology, Baddiley-Clark Building, Newcastle University, Richardson Road, Newcastle upon Tyne, NE2 4AX, United Kingdom. Tel.: +44 (0) 191 208 3221; fax: +44 (0) 191 208 3205.

E-mail addresses: [ulyanova.vera@gmail.com](mailto:ulyanova.vera@gmail.com) (V. Ulyanova), [valentina.vershinina@kpfu.ru](mailto:valentina.vershinina@kpfu.ru) (V. Vershinina), [ilinskaya.kfu@mail.ru](mailto:ilinskaya.kfu@mail.ru) (O. Ilinskaya), [colin.harwood@ncl.ac.uk](mailto:colin.harwood@ncl.ac.uk) (C.R. Harwood).

<sup>1</sup> Permanent address: Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, 420008, Russian Federation.