npj | Biofilms and Microbiomes

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ARTICLE OPEN

Ser/Thr protein kinase PrkC-mediated regulation of GroEL is critical for biofilm formation in *Bacillus anthracis*

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PrkC is a conserved Ser/Thr protein kinase encoded in *Bacillus anthracis* genome. PrkC is shown to be important for *B. anthracis* pathogenesis, but little is known about its other functions and phosphorylated substrates. Systemic analyses indicate the compelling role of PrkC in phosphorylating multiple substrates, including the essential chaperone GroEL. Through mass spectrometry, we identified that PrkC phosphorylates GroEL on six threonine residues that are distributed in three canonical regions. Phosphorylation facilitates the oligomerization of GroEL to the physiologically active tetradecameric state and increases its affinity toward the co-chaperone GroES. Deletion of *prkC* in *B. anthracis* abrogates its ability to form biofilm. Overexpression of native GroEL recovers the biofilm-forming ability of *prkC* deletion strain. Similar overexpression of GroEL phosphorylation site mutants (Thr to Ala) does not augment biofilm formation. Further analyses indicate the phosphorylation of GroEL in diverse bacterial species. Thus, our results suggest that PrkC regulates biofilm formation by modulating the GroEL activity in a phosphorylation-dependent manner. The study deciphers the molecular signaling events that are important for biofilm formation in *B. anthracis*.

npj Biofilms and Microbiomes (2017)3:7 ; doi:10.1038/s41522-017-0015-4

INTRODUCTION

Bacillus anthracis (*B. anthracis*) is a bacterial pathogen that causes anthrax and has been historically used as a model organism to understand the bacterial response during infection.[1] The lifecycle of *B. anthracis* comprises of vegetative and sporulation phases and possess the ability to form capsules and biofilms.[2, 3] *B. anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis* are the three major species comprising the pathogenic *Bacillus cereus* group. These bacteria are difficult to eradicate, both in the environment and during infection, mainly due to the efficient development of spores and biofilms. *B. anthracis* cells readily form biofilms under stagnant conditions in environment and protect the vegetative cells, which continue to divide within biofilm communities.[4] The cells can eventually sporulate and disseminate, thus causing exponential increase in bacterial cell number under favorable conditions. During infection, pathogens tend to sporulate or form biofilms on epithelial cells, enabling the bacteria to escape innate immune responses and become antibiotic-resistant.[5–8] Thus, entering the sporulation phase or forming biofilm is a survival strategy for bacteria.[3, 9–11] The mechanism of biofilm formation remains poorly understood in *B. anthracis*, although biofilm-forming cells are suggested to be particularly resistant to high levels of antibiotic treatments.[3] Further, *Bacillus* forms communities that have both biofilm and spores, and the biofilm disassembly leads to spore release. It is plausible that for planktonic cells biofilm-associated growth provides fitness advantage.

Bacterial cells sense extracellular signals and respond in a concerted manner during morphogenesis. The role of molecular signaling events occurring inside *B. anthracis* remains unknown. The cascades initiate at cellular surface and culminate in the modulation of defined set of proteins, leading to specific cellular response, such as biofilm formation.[12] In *B. anthracis*, PrkC is the only known Ser/Thr protein kinase (STPK) with a sensor domain capable of receiving external signals.[13–16] PrkC senses muropeptides through the extracellular C-terminal PASTA domains,[17–20] but its downstream signaling events are not yet known. Homologs of PrkC are conserved in many bacteria such as *Mycobacterium*, *Streptomyces*, *Staphylococcus*, *Corynebacterium*, and other diverse gram-positive bacteria along with its cognate Ser/Thr phosphatase (PrpC), and is known to regulate vital functions.[16, 19, 21–28] PrkC plays an important role in virulence of *B. anthracis* and is important for survival in macrophages during infection.[15, 16] PrkC is capable of transducing signals efficiently during environmental stress conditions and has been proposed to function even in the spores.[14, 17]

In view of these facts, we aimed to explore the importance of *B. anthracis* PrkC in the cellular signaling events. To identify its role(s) in vivo, we searched for proteins phosphorylated by PrkC. Further, using a *B. anthracis* deletion strain of *prkC* (*BasΔprkC*), we investigated its role in regulation of biofilm formation. In accordance to our hypothesis, deletion of *prkC* resulted in complete loss of biofilm formation and possibly altered cell-to-cell adherence properties. Our results indicated that PrkC may regulate biofilm formation by activating an essential chaperone GroEL. Thus, this study describes the novel signaling pathway involving the conserved STPK and chaperone in *B. anthracis* biofilm development.

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Received: 23 May 2016 Revised: 29 November 2016 Accepted: 15 December 2016
Published online: 07 March 2017

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