Structural and biochemical characterization of a defence system protecting bacteria from predation by a temperate bacteriophage

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Integrative and conjugative elements or ICEs are mobile genetic elements found in host bacterial chromosomes and typically carry cargo genes that benefit the host [1]. At least two such mobile genetic elements can be observed in certain strains of *Bacillus subtilis* – the integrative and conjugative element ICE*Bs1* [2,3], and the temperate phage SPß [4]. The *spbK* gene in ICE*Bs1* inhibits the production of active SPß. Its gene product, BsSPBK1, contains a TIR domain that is necessary for function [5]. TIR domains are found in plants, animals, archaea, and bacteria, and are known to show NADase activity [6]. This suggests that BsSPBK1 could also be an NADase effector, eliciting abortive infection that leads to cell death. The abortive infection depends on the SPß gene *yonE*, where co-expression of *spbK* and *yonE* inhibits the growth of host cells [5]. The exact function of *yonE* is not known yet. Its encoded protein, YonE, shares similarity with viral capsid portal proteins. Portal proteins are important components of many dsDNA viruses as they control genome packaging and capsid assembly [7]. They usually assemble as dodecamers and provide a channel for bidirectional passage of viral DNA.

The mechanism of how YonE and BsSPBK1 trigger an antiphage response is poorly understood. In this study, we want to confirm if BsSPBK1 has NADase activity and characterize if this is modulated by YonE. To this end, we have successfully expressed full-length, N-, and C-terminally truncated constructs of YonE, and preliminary cryoEM data suggest that YonE assembles into a dodecamer as expected for portal proteins. BsSPBK1 has proven challenging to express, and currently we are trying solubility tags such as Maltose-binding protein (MBP) to facilitate the process. Co-expression strategies with YonE are also being explored.

[1] Johnson CM, Grossman AD. Integrative and conjugative elements (ICEs): what they do and how they work. Annu Rev Genet. 2015; 49:577–601.
[2] Auchtung JM, Lee CA, Monson RE, Lehman AP, Grossman AD. Regulation of a Bacillus subtilis mobile genetic element by intercellular signaling and the global DNA damage response. Proc Natl Acad Sci U S A. 2005 Aug 30; 102(35):12554–9.
[3] Auchtung JM, Aleksanyan N, Bulku A, Berkmen MB. Biology of ICEBs1, an integrative and conjugative element in Bacillus subtilis. Plasmid. 2016; 86:14–25.
[4] Warner FD, Kitos GA, Romano MP, Hemphill HE. Characterization of SPβ: a temperate bacteriophage from Bacillus subtilis 168M. Can J Microbiol. 1977 Jan 1; 23(1):45–51.
[5] Johnson CM, Harden MM, Grossman AD (2022) Interactions between mobile genetic elements: An anti-phage gene in an integrative and conjugative element protects host cells from predation by a temperate bacteriophage. PLOS Genetics. 2022; 18(2): e1010065.
[6] Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, Chen J, Qi T, Gilley J, Lai JS, Rank MX, Casey LW, Gu W, Ericsson DJ, Foley G, Hughes RO, Bosanac T, von Itzstein M, Rathjen JP, Nanson JD, Boden M, Dry IB, Williams SJ, Staskawicz BJ, Coleman MP, Ve T, Dodds PN, Kobe B. NAD^+ cleavage activity by animal and plant TIR domains in cell death pathways. Science. 2019 Aug 23;365(6455):793-799.
[7] Bayfield OW, Steven AC, Antson AA. Cryo-EM structure in situ reveals a molecular switch that safeguards virus against genome loss. Elife. 2020; Apr 14(9): e55517.