AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology®

RESEARCH ARTICLE

Fur-Dam Regulatory Interplay at an Internal Promoter of the Enteroaggregative *Escherichia coli* Type VI Secretion *sci1* Gene Cluster

Yannick R. Brunet,[a]* Christophe S. Bernard,[a]* Eric Cascales[a]

[a]Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), Institut de Microbiologie de la Méditerranée (IMM), CNRS–Aix-Marseille Université UMR7255, Marseille, France

ABSTRACT The type VI secretion system (T6SS) is a weapon for delivering effectors into target cells that is widespread in Gram-negative bacteria. The T6SS is a highly versatile machine, as it can target both eukaryotic and prokaryotic cells, and it has been proposed that T6SSs are adapted to the specific needs of each bacterium. The expression of T6SS gene clusters and the activation of the secretion apparatus are therefore tightly controlled. In enteroaggregative *Escherichia coli* (EAEC), the *sci1* T6SS gene cluster is subject to a complex regulation involving both the ferric uptake regulator (Fur) and DNA adenine methylase (Dam)-dependent DNA methylation. In this study, an additional, internal, promoter was identified within the *sci1* gene cluster using +1 transcriptional mapping. Further analyses demonstrated that this internal promoter is controlled by a mechanism strictly identical to that of the main promoter. The Fur binding box overlaps the −10 transcriptional element and a Dam methylation site, GATC-32. Hence, the expression of the distal *sci1* genes is repressed and the GATC-32 site is protected from methylation in iron-rich conditions. The Fur-dependent protection of GATC-32 was confirmed by an *in vitro* methylation assay. In addition, the methylation of GATC-32 negatively impacted Fur binding. The expression of the *sci1* internal promoter is therefore controlled by iron availability through Fur regulation, whereas Dam-dependent methylation maintains a stable ON expression in iron-limited conditions.

IMPORTANCE Bacteria use weapons to deliver effectors into target cells. One of these weapons, the type VI secretion system (T6SS), assembles a contractile tail acting as a spring to propel a toxin-loaded needle. Its expression and activation therefore need to be tightly regulated. Here, we identified an internal promoter within the *sci1* T6SS gene cluster in enteroaggregative *E. coli*. We show that this internal promoter is controlled by Fur and Dam-dependent methylation. We further demonstrate that Fur and Dam compete at the −10 transcriptional element to finely tune the expression of T6SS genes. We propose that this elegant regulatory mechanism allows the optimum production of the T6SS in conditions where enteroaggregative *E. coli* encounters competing species.

KEYWORDS type VI secretion, epigenetism, methylation, microbial communities, regulation, repression

The fate of microbial communities is governed by communication, cooperation, and competition mechanisms between microorganisms (1–9). Bacteria have therefore developed an arsenal of signaling, sensing, and antagonistic activities. To eliminate competitors, bacteria evolved distinct mechanisms for release of antibiotics or bacteriocins in the extracellular medium, as well as delivery of toxins directly into the target cell (10–12). One of the delivery apparatuses, the type VI secretion system (T6SS),

Citation Brunet YR, Bernard CS, Cascales E. 2020. Fur-Dam regulatory interplay at an internal promoter of the enteroaggregative *Escherichia coli* type VI secretion *sci1* gene cluster. J Bacteriol 202:e00075-20. https://doi.org/10.1128/JB.00075-20.

Editor Thomas J. Silhavy, Princeton University

Address correspondence to Eric Cascales, cascales@imm.cnrs.fr.

* Present address: Yannick R. Brunet, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA; Christophe S. Bernard, Laboratoire de Chimie Bactérienne (LCB), Institut de Microbiologie de la Méditerranée (IMM), CNRS–Aix-Marseille Université UMR7283, Marseille, France.

Received 6 February 2020
Accepted 28 February 2020
Accepted manuscript posted online 9 March 2020
Published 27 April 2020