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H-NS Silencing of the *Salmonella* Pathogenicity Island 6-Encoded Type VI Secretion System Limits *Salmonella enterica* Serovar Typhimurium Interbacterial Killing

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The secretion of bacterial toxin proteins is achieved by dedicated machineries called secretion systems. The type VI secretion system (T6SS) is a widespread versatile machine used for the delivery of protein toxins to both prokaryotic and eukaryotic cells. In *Salmonella enterica* serovar Typhimurium, the expression of the T6SS genes is activated during macrophage or mouse infection. Here, we show that the T6SS gene cluster is silenced by the histone-like nucleoid structuring H-NS protein using a combination of reporter fusions, electrophoretic mobility shift assays, DNase footprinting, and fluorescence microscopy. We further demonstrate that derepression of the *S.* Typhimurium T6SS genes induces T6SS-dependent intoxication of competing bacteria. Our results suggest that relieving T6SS H-NS silencing may be used as a sense-and-kill mechanism that will help *S.* Typhimurium to homogenize and synchronize the microbial population to gain efficiency during infection.

During the course of infection, bacteria produce and secrete bacterial toxins. Secretion of these toxin proteins is achieved by dedicated, specialized machineries called secretion systems. The type VI secretion system (T6SS) is required for the virulence of several Gram-negative pathogens. In *Vibrio cholerae*, the T6SS translocates VgrG1, a toxin that carries a domain responsible for actin cross-linking in eukaryotic cells (1–4). However, the role of the T6SS is not limited to virulence toward eukaryotes; an increasing number of reports demonstrate that the T6SS is also involved in interbacterial intoxication (5–10). With bacteriocins and contact-dependent inhibition (CDI), the T6SS is therefore involved in shaping bacterial communities (11–14) by delivering antibacterial toxins, including murein hydrolases, DNases, and phospholipases, directly into the target recipient bacterial cell (5, 6, 15; for recent reviews see references 14, 16 and 17). The attacker cell is protected from these toxins by the coproduction of cognate proteins that confer immunity (15, 18). The T6SS therefore confers a growth advantage to bacteria in mixed cultures and has been suggested to be important in environmental niches where bacterial competition for nutrients is critical for survival or for the uptake of DNA for transformation and acquisition of new traits (14, 19). However, while the role of the T6SS in interbacterial competition has been evidenced and characterized under laboratory conditions, its role in shaping bacterial communities of the microbiota is not yet clearly elucidated.

At the molecular level, the T6SS is assembled from 13 proteins, called core components, that form a transenvelope apparatus anchoring a cytoplasmic tubular structure to the membrane (20–24). Based on structural homologies with bacteriophage tail components, this tubular structure has been proposed to be constituted of an inner tube assembled by stacked hexameric rings of the Hcp protein, resembling the tail tube of bacteriophages, tipped by the VgrG protein (21, 25–27). The current model describes the internal tube as a conduit for the secretion of the effector toxins (13, 28–30). This model has been recently supported by data demonstrating direct contacts between the Hcp protein and effectors (31). However, additional mechanisms have recently been reported, such as the tip protein, VgrG, or an adaptor protein, PAAR (proline, alanine, alanine, arginine), serving as a carrier for effectors (17, 32, 33). The Hcp internal tube is wrapped into a coating cylinder resembling the sheath of contractile phages (21, 26, 34). Cryo-electron and fluorescence microscopies showed that this sheath-like structure is dynamic and undergoes cycles of elongation and contraction (21). Similarly to the bacteriophage infection mechanism, it has been proposed that upon contact with a bacterial neighbor, contraction of the T6SS sheath propels the Hcp tube toward the target cell (2, 9, 35, 36). Because of the T6SS function in bacterial virulence and interbacterial competition, the expression of T6SS genes needs to be tightly controlled. A broad variety of transcriptional, translational, and posttranslational regulatory mechanisms have been identified and characterized (13, 37, 38).

In *Salmonella enterica* serotypes, T6SS gene clusters are encoded within different pathogenicity islands (SPIs), the most

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