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Molecular Mechanism Underlying Regulation of *tir* Promoter in the Locus of Enterocyte Effacement (LEE) in Enterohemorrhagic *Escherichia coli* O157:H45 by Global Regulator H-NS

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, a Shiga toxin-producing *E. coli*, causes a broad spectrum of diseases, including uncomplicated diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. In addition to Shiga toxins, EHEC O157:H7 harbors genes mediating its adherence to intestinal epithelial cells by a characteristic attaching-and-effacing mechanism. The attaching and effacing phenotype requires concerted action of several genes contained within a pathogenicity island, called the locus of enterocyte effacement (LEE). The LEE comprises 41 open reading frames organized in five major operons, LEE1, LEE2, LEE3, *tir* (LEE5), and LEE4, which encode a type III secretion system, the intimin adhesin, the translocated intimin receptor (Tir), and other effector proteins. Strict control of LEE gene expression is mediated by the coordinated activities of several regulatory elements. The global regulator H-NS, the most abundant DNA-binding proteins (20,000 molecules per cell), represses the expression of several LEE genes, if not all.

We report here the molecular mechanism of regulation by H-NS using the *tir* promoter (*tir*P) as a model based on various *in vitro* studies carried out with purified components. We found that H-NS acted at immediately upstream of the promoter. Various *in vitro* measurements revealed that the H-NS blocks open promoter complex formation (RP_O) through a protein-protein interaction with RNA polymerase – RNA polymerase lacking the C-terminal domain of α subunit was no longer subject to the repression by H-NS. This was further verified *in vivo* using the plasmid-borne *rpoA* variants encoding alanine substitutions in the C-terminal domain of the α subunit. In an attempt to identify the amino acid determinants on H-NS, mutant H-NS were generated by dirty-PCR method and screened using a strain carrying *tirP::lac* and *hdeABp::cat*. Phenotype of interesting mutant H-NS will be discussed at the meeting in view of specific interaction with RNA polymerase that leads to transcription repression.