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Molecular Mechanism Underlying Regulation of *tir* Promoter in the Locus of Enterocyte Effacement (LEE) in Enterohemorrhagic *Escherichia coli* O157:H7 by Global Regulator H-NS and Its Antagonist Ler

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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, and Ler, encoded by the gene ler of the LEE1 operon, induces the expression of these genes by counteracting the H-NS-mediated repression.

We report here the molecular mechanism of regulation by H-NS and Ler using the *tir* promoter (*tir*P) as a model based on *in vitro* studies carried out with purified components. We found that H-NS acted at or near the promoter and Ler at a sequence upstream of -198 of *tir*P. 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 was no longer subject to the repression by H-NS. Ler acting from the far upstream sequence negated the repression by H-NS. It was postulated that the Ler acts alone relying on its oligomeric nature although the factor determining the extent of oligomerization has yet to be elucidated. K_d determination of these proteins by Surface Plasmon Resonance (SPR) method suggested H-NS (K_d=2.21x10-^{5} M) would never be able

to sterically hinder binding of RNP with K_d in the range of nM. Thus, the H-NS could only repress transcription through the protein-protein interaction with the RNA polymerase. Whereas, Ler (K_d=4.59x10-^{8} M) should readily displace H-NS. Model explaining the mechanism will be discussed at the meeting.