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THE POST-TRANSLATIONAL REGULATION OF σ^S INVOLVES ClpPX PROTEOLYTIC ACTIVITY DURING EXPONENTIAL PHASED Escherichia coli

Lee, Kyu-Ho, Dept. Environ. Sci., Hankook Univ. of Foreign Studies. σ^{S} plays important roles in starvation survial of E. coli. Based on fusion studies, its systhesis is thought to be controlled at the transcriptional and post-transciptional levels, resulting in increased levels in starved cells. The post-transciptional regulation has been ascribed to rpoS mRNA secondary structure formation. A series of rpoS::lacZ translational fusions containing different lengths rpoS coding resion fell into three classes, based on the basal level during the exponential phase and the degree of increase upon starvation. These findings were inconsistent with the secondary structure hypothesis. In search of an alternative explanation, we studied σ^{S} regulation in several protease null mutants. The heat shock protease, ClpP along with ClpX ATPase activity, degraded σ^{S} longer than 188 amino acid residues during the exponential phase, but did not degrade those shorter than 160 amino acid residues. Western blot analysis confirmed that in such protease subunit backgrounds their basal σ^{S} levels reached to the induced amount of σ^{S} in wild-type. This increase was revealed to be due to 10-times increased half-life of σ^{S} under ClpP mutant. We conclude that the ClpP and ClpX-mediated degradation of σ^{S} in exponential phase plays a major role in lowering its levels in exponential phase.

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AUTOINDUCTION OF A TI PLASMID CONJUGAL TRANSFER INDUCED BY MANNOPINE (MOP)

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MOP is an opine produced from crown galls induced by octopine/mannitylopine type Agrobacterium tumefaciens. Octopine, but not MOP, is an inducer for the conjugal propagation of Ti plasmids in such strains as 15955 and R10. When coincubated with MOP, these strains produce agrobacterial autoinducer (AAI), which is absolutely necessary and a good indication for the induction of a Ti plasmid conjugal transfer. This observation suggested that Ti plasmids from these strains may also encode a MOP-associated autoinduction activator corresponding to the octopine-associated activator traR. DNA nucleotide sequence analysis revealed that Ti plasmids in these strains encode traR-like gene as a part of MOP transport operon, which showed more than 90% identity with traR. However, the trlR (traR-like regulator) gene has a frame-shift mutation at its 3'-end, which results in the absence of helix-turn-helix motif necessary for positive regulator activities, suggesting that those strains may have encoded MOP-specific AAI genes and that the trlR gene acquired a mutation in their evolution. We tested various MOP-type isolates for their ability to conjugate their Ti plasmids upon the induction with MOP. We could find several such strains, suggesting that these strains encode functional activators associated with MOP induction.