J4

Characterization of Protein Factor Regulating the Superoxide-Sensor SoxR in *Escherichia coli*

Mi-Sun Koo*, So-Yeon Rah, Kang-Lok Lee, and Jung-Hye Roe
Lab. of Molecular Microbiology, School of Biological Science and Institute of Microbiology, Seoul National University

Escherichia coli has developed *soxRS* regulon to defend against toxicity of superoxide radical. SoxR, superoxide sensor, is oxidized by superoxide-generating agents or nitric oxide and oxidized SoxR activates the transcription of *soxS* gene. In order to find out the trans-acting factors regulating SoxR activity *in vivo*, *soxS::lacZ* single copy operon fusion construct was prepared and random Tn10 insertional mutatons were performed. The four *soxS*-constitutive mutants showing elevated *soxS* transcription in the absence of oxidant were isolated. One mutation was mapped at 36.7 min on *E. coli* chromosome and function of *resA* and *resB* (putative reductase for SoxR) has not been characterized. The other mutation was localized at *rseB* of *rseABC* operon anti-sigma factor for SigE, extracytoplasmic factor. The effect of *resA* mutation on *soxS* transcription is mediated via SoxR. In addition, transduction of (*soxR4::cat*) marker in *soxR*-constitutive mutant into *resA:Tn10* mutant showed the additive increase at basal *soxS* transcription. Both ResB and ResC have two [4Fe-4S] clusters binding motif, $C\text{-}X_2\text{-}C\text{-}X_2\text{-}C\text{-}X_3\text{-}C$, characteristic of ferredoxin-type proteins which participate in electron transfer. Exogenous supply of multicopy *resB* in *ΔresAB::kan* mutant lowered the basal activity of *soxS::lacZ* expression to the level in wild type cell. EPR (Electron Paramagnetic Resonance) analysis of SoxR in intact cells revealed that most SoxR protein exist in oxidized form in *ΔresAB::kan* mutant in contrast to the reduced form in wild type cell. The inactivation kinetics of *soxS* mRNA after stopping aeration demonstrated that ResB indeed facilitates SoxR reduction. These results suggested that ResB might function as putative reductase to maintain SoxR as reduced form in the cytoplasm during normal aerobic growth.