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Mechanism of Redox- and Metal-dependent Modulation of RsrA, an Anti-sigma Factor for Redox-dependent Regulation of Thioredoxin Operons in *Streptomyces coelicolor*

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SigR (σ^R) is a sigma factor responsible for inducing the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor*. RsrA specifically binds to σ^R and inhibits σ^R-directed transcription under reducing conditions. Exposure to H_2O_2 or thiol-specific oxidant diamide dissociates σ^R-RsrA complex. RsrA contains 7 cysteine residues in 105 total amino acid residues. Using single and multiple cysteine substitution mutagenesis of cysteines into serines, we found that 4 cysteines at 11, 41, 44, and 62th position are necessary for redox-dependent modulation of RsrA activity, as judged by σ^R–binding and inhibition of σ^R-directed transcription *in vitro*. The mutation at each of these positions caused loss of σ^R-binding ability, suggesting that the free thiols, not the absence of disulfide bonds among them, are necessary for RsrA function. MALDI-TOF Mass Spectroscopy revealed that disulfide bonds form between Cys-11 and Cys41/44 as well as between Cys-62 and Cys41/44, upon oxidation. We measured the content of metals in recombinant RsrA purified from *E. coli.* Ca, Mg, and Zn were detected in varying amounts depending on the batch of preparation. The content of Zn was systematically lowered under oxidized condition, suggesting that it is released when disulfide bonds form in RsrA. Zn protected free thiols in RsrA from alkylating agent such as AMS. The substitution mutagenesis of cysteines to histidines is underway to examine their effect on RsrA function and Zn binding.