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IscR-Dependent Gene Expression Links Iron-Sulfur Cluster Assembly to the Control of O_2-Regulated Genes in *Escherichia coli*

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Iron-sulfur (Fe-S) clusters are cofactors for many proteins across all three branches of life. Fe-S proteins function in a number of cellular processes, including electron transfer, gene regulation, photosynthesis, and nitrogen fixation, among others. Although Fe-S cluster formation into proteins can occur spontaneously *in vitro,* Fe-S clusters are synthesized *in vivo* by specialized Fe-S biogenesis proteins. In the housekeeping Isc system of *Escherichia coli,* IscS is a cysteine desulfurase that removes the sulfur from cysteine, which is the sulfur donor for Fe-S cluster biogenesis. IscU is postulated to be a scaffold upon which a transient Fe-S cluster is built and then transferred to an apo-protein, while IscA has been proposed to serve either as an Fe-S scaffold or as the Fe donor for the assembly process. HscA and HscB are homologs of the protein folding chaperones DnaK and DnaJ, respectively, and have been shown to specifically interact with IscU, perhaps to promote or stabilize a conformation suitable for assembly of an Fe-S cluster and transfer of the cluster to an apo-protein. In addition to the Isc system, a second Fe-S cluster biogenesis pathway, Suf (sulfur mobilization), has been identified in *E. coli,* which is encoded by *sufABCDSE.*

This study addresses how Fe-S biogenesis is regulated in *E. coli.* Previous work established that IscR (iron-sulfur cluster regulator) represses the transcription of *iscRSUA-hscBA-fdx,* the operon encoding the housekeeping Fe-S biogenesis system (Isc) and the regulator IscR. Electron paramagnetic resonance data shows that as-isolated IscR protein contains a [2Fe-2S] cluster that can be reversibly oxidized and reduced, a property characteristic of most Fe-S clusters. Furthermore, inactivation of the Isc Fe-S biogenesis pathway decreases repression of the *iscR* promoter, suggesting that the [2Fe-2S] cluster is required for IscR repressor function. Confining the function of IscR to the Fe-S form provides an attractive feedback mechanism for Fe-S biogenesis to be sensitive to the Fe-S cluster status of cells; when the Isc and Hsc proteins and the building blocks of Fe-S clusters (Fe^{2+}, cysteine) increase to the appropriate cellular concentrations, IscR itself will acquire an Fe-S cluster, thus enabling it to repress transcription of the

iscRSUA-hscBA-fdx operon. This feedback mechanism may also explain the increase in transcription observed from the *iscR* promoter upon treating cells with the oxidant H_2O_2 since oxidant-induced damage of Fe-S clusters would be expected to increase the demand for Fe-S biogenesis and thus result in the upregulation of the *isc* operon. In agreement with this notion, the induction of the *isc* operon by H_2O_2 is dependent upon IscR and is independent of OxyR, unlike the induction of the *suf* operon.

In this study, we investigated whether IscR regulates the expression of genes in addition to the *isc* operon. To identify additional members of the IscR regulon, we used global transcriptional profiling of strains grown under aerobic or anaerobic growth conditions that contain or lack IscR. Our results indicate that potentially 40 genes in 20 predicted operons are regulated by IscR. Of these 20 operons, DNase I footprinting and/or *in vitro* transcription reactions showed seven of these promoters were directly regulated by IscR. Among these were genes encoding known or proposed functions in Fe-S cluster biogenesis (*sufABCDSE, yadR,* and *yhgI*) and Fe-S cluster-containing anaerobic respiratory enzymes (*hyaABCDEF, hybOABCDEFG,* and *napFDAGHBC*). The finding that IscR repressed expression of the *hyaA, hybO,* and *napF* promoters specifically under aerobic growth conditions suggests a new mechanism to explain their upregulation under anaerobic growth conditions. Phylogenetic footprinting of the DNase I protected regions of the seven promoters implies that there are at least two different classes of IscR binding sites and these are conserved among many bacteria. The findings presented here indicate a more general role of IscR in the regulation of Fe-S cluster biogenesis and that IscR contributes to the O_2 regulation of several promoters controlling the expression of anaerobic Fe-S proteins.

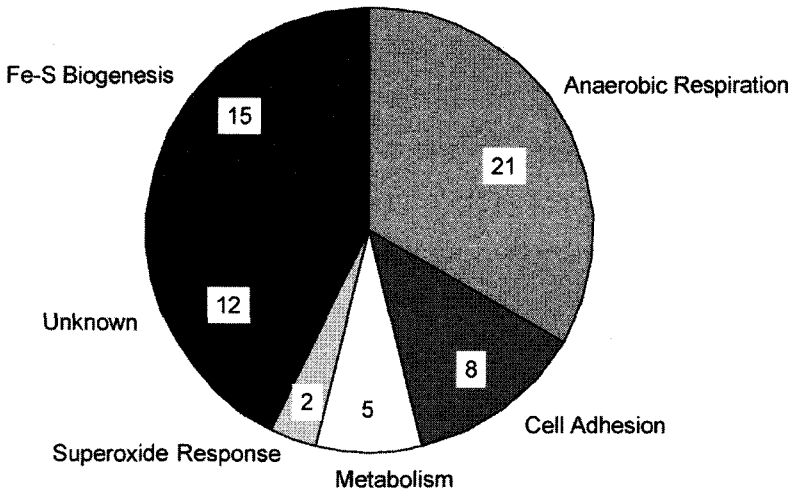

Fig. 1. Distribution of genes regulated by IscR from transcription profiling.

References

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