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# Oxidant-Responsive Induction of the *suf* Operon, Encoding a Fe-S Assembly System, through Fur and IscR in *Escherichia coli*[▽]

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**The *suf* operon encoding a Fe-S assembly system is induced by peroxides through activators OxyR and IscR in *Escherichia coli*. For apo-IscR to bind, oxidation-mediated dissociation of Fur is required. Therefore, a peroxide-responsive signal is transduced through OxyR, IscR, and Fur to achieve oxidation-sensitive and maximal induction of this operon.**

Proteins with iron-sulfur (Fe-S) clusters are widely utilized in diverse metabolic reactions and gene regulation (1, 4, 5). The biogenesis of Fe-S clusters has been extensively studied in bacteria and in mitochondria and plastids of eukaryotes (2, 4, 8). Bacteria utilize at least one of the three systems known to assemble clusters, namely, the Nif, Isc, and Suf systems (14). In *Escherichia coli*, the Isc system, encoded from the *iscSUA-hscBA-fdx* gene cluster, is thought to serve as a housekeeping assembly system (11), whereas the Suf system, encoded from the *sufABCDSE* operon system, is regarded as an alternative system under oxidative and Fe-limiting conditions (10, 13).

Both the *isc* and *suf* operons are induced under oxidative stress conditions (6, 10, 18). The *isc* operon is regulated by an Fe-S-containing repressor, IscR, which loses its repressor activity upon oxidative stress (12). The *suf* operon is regulated by OxyR, integration host factor (IHF), Fur, and IscR (3, 6, 7, 10, 16). Its induction by peroxide stress is positively regulated by oxidized OxyR (10) and demetallated IscR (apo-IscR) (16), with nearly equal contributions from each, while induction by Fe limitation is proposed to be mediated through the inactivation of Fur (10).

Previous studies demonstrated that activation by OxyR from a distant binding site (nucleotides [nt] −236 to −197, as measured from the transcription start site) is assisted by IHF binding (at nt −156 to −127) that causes DNA bending (6, 10). Fur was estimated to bind at the promoter site between nt −32 and −3 (10), whereas apo-IscR binds between nt −60 and −26 (3, 16). Mutant IscR that lacks predicted Fe-S binding residues still allowed peroxide-sensitive induction of the *suf* operon in an Δ*oxyR* mutant, prompting us to propose a model that the concomitant dissociation of Fur and the binding of apo-IscR is needed to ensure the peroxide-sensitive induction of the *suf* operon by IscR (16) (Fig. 1). However, experimental evidence on the detailed interaction between Fur and IscR in the *suf* promoter region has been lacking.

In this study, we determined the exact binding site of Fur and demonstrated that it competes with IscR for binding. The dissociation of Fur under oxidative conditions is necessary for IscR to act as an activator. Iron depletion also caused derepression mediated through both the inactivation of Fur and the activation of IscR. Therefore, the activation of the *suf* operon by IscR occurs only under conditions where Fur loses its repressor activity, and those conditions at the same time can convert IscR to an ironless activator form.

Since the previously determined Fur binding site (nt −32 to −3 [10]) overlaps with the IscR binding site (nt −60 to −26) by several nucleotides, we examined whether IscR binding and Fur binding are mutually exclusive or not. Gel mobility shift assays were performed with Fur and IscR purified under ordinary atmospheric conditions as described previously (7, 16). Under this condition, the purified IscR exists as apo-IscR, lacking a Fe-S cluster. Proteins to be used for footprinting were stored in storage buffer without EDTA. Fur bound to the *sufA* promoter DNA probe (nt −70 to +30 from the transcription start site) in the absence of EDTA (Fig. 2, lanes 2 to 4). Treatment with 1 mM EDTA inhibited Fur binding, reflecting Fe-dependent binding (Fig. 2, lanes 5 to 7). IscR (500 nM) bound to the probe in the absence of Fur (lane 8) but was competed off by even 25 nM Fur (lanes 9 to 11). When we changed the order by adding 25 nM Fur first and then IscR (500 nM), Fur continued to stay bound (data not shown).

We then determined the binding sites of Fur and IscR in the *sufA* promoter region by DNase I footprinting. IscR (0 and 2.5 mM) and Fur at different concentrations were incubated in either the absence or the presence of 1 mM EDTA. Results in Fig. 3A demonstrate that Fur binds at a site encompassing nt −39 to −11 in the absence of EDTA (lanes 3 to 4 and 9 to 11). IscR bound to three sites, as observed before (16). IscR binding at the promoter-proximal site (site 1; nt −60 to −32) was observed only in the absence of Fur binding (Fig. 3A, lanes 7, 8, and 12 to 14), whereas binding to upstream sites was independent of Fur. The Fur binding site we determined (nt −39 to −11) overlapped significantly with site 1 (nt −60 to −26), in contrast with the previously determined Fur site (nt −32 to −3 [10]) (Fig. 3B). These results clearly indicate that Fur and IscR bindings are mutually exclusive. The intracellular concen-

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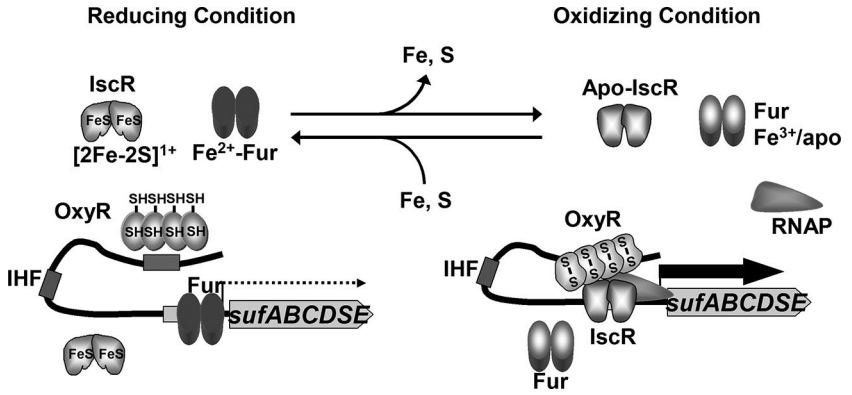


FIG. 1. A proposed model for regulation of the *suf* operon by IscR and Fur (modified from Fig. 8 of reference 16 with permission).

trations of Fur and IscR under ordinary aerobic growth conditions have been estimated to be about 5,000 and 9,000 molecules per cell, respectively (17; P. Kiley, personal communications). Considering the large difference in the binding affinities of apo-IscR (apparent dissociation constant of ~200 nM [16]) and Fur (~20 nM), we can predict that Fur will inhibit IscR from binding at the *sufA* promoter in vivo under ordinary nonstressed growth conditions. Binding of Fur in the promoter region will inhibit transcription by RNA polymerase, keeping the basal level of the *suf* transcripts low, which is consistent with S1 nuclease mapping results (16) (Fig. 4). However, since the DNA binding affinities of Fur and IscR can be modulated by the status of oxidation and metallation, which depends on redox and metal physiology in the cell, more systematic investigation is necessary to obtain a better understanding of the in vivo status.

We previously observed that the *suf* operon in an Δ*oxyR*

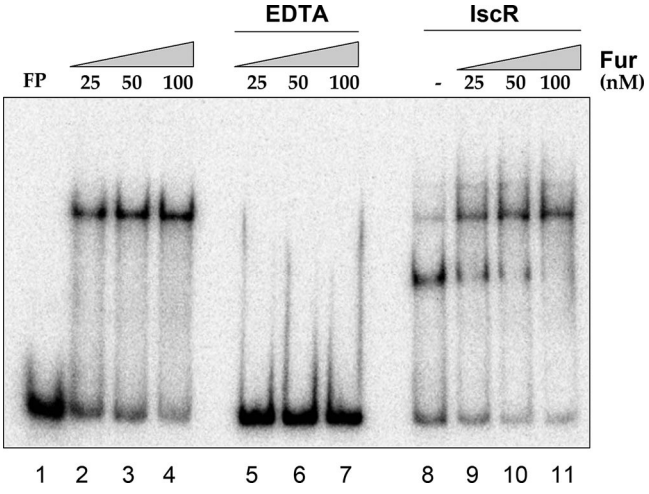


FIG. 2. Competitive binding of IscR and Fur to the *sufA* promoter region. Increasing concentrations of Fur protein (0 to 100 nM) were incubated at room temperature for 10 min with $^{32}$P-end-labeled DNA probes (~3 nM) containing a *sufA* promoter region from nt −70 to +30 relative to the transcriptional start site. Either EDTA (1 mM; lanes 5 to 7) or IscR (500 nM; lanes 8 to 11) was included in the binding buffer. FP, free probes. The reaction mixture was electrophoresed on an 8% polyacrylamide gel with 0.5× Tris-borate buffer at room temperature.

*iscR* double mutant was not activated at all by phenazine methosulfate, which generates superoxide and hydrogen peroxide, or by $H_2O_2$ (16), suggesting that the peroxide-responsive induction of this operon is mediated through two activators, OxyR and IscR. The contribution of Fur was further analyzed through examining the effect of a Δ*fur* mutation and treatment with iron-specific chelator 2,2′-dipyridyl. As demonstrated in Fig. 4, the uninduced level of *sufA* transcript in the Δ*fur* mutant was about sixfold higher than that in the wild type (lane 1 versus lane 5). In a Δ*fur iscR* double mutant, the uninduced level of *sufA* transcript was lowered to the wild-type level (Fig. 4, lane 9), suggesting that the elevation of the uninduced *sufA* level in the Δ*fur* mutant is due to transcription activation by IscR. Treatment with dipyridyl slightly increased the induction in the Δ*fur* mutant, suggesting that Fe chelation of IscR might have increased its activator function (Fig. 4, lane 7). Consistent with this interpretation, for the Δ*fur iscR* mutant background, dipyridyl did not elevate *suf* expression (Fig. 4, lane 11). In the Δ*fur iscR* mutant background, the remaining oxidative induction of *sufA* is contributed by OxyR (Fig. 4, lanes 10 and 12). We previously observed that the contribution from either OxyR or IscR alone is much lower than that from both together (16). Similarly, we observed that activation by OxyR alone in the Δ*fur iscR* background produced only a 17-fold induction by phenazine methosulfate compared with an over-80-fold induction through the combined effect of OxyR and IscR (Fig. 4, lane 10 versus lane 6).

We investigated the effect of IscR in this mutant background. The provision of wild-type IscR on multiple-copy plasmid pTac1 (16) restored the maximal induction by oxidant treatment, as expected (Fig. 4, lanes 14 and 16). The increased basal level of *sufA* expression (about ninefold induction [Fig. 4, lane 13 versus lane 9]) reflects the contribution from overproduced IscR under an unstressed condition. The mutant IscR (3CA), whose three cysteine ligands (C92, C98, and C104) predicted for Fe-S binding were replaced with alanines, also produced a pattern of induction similar to that seen for the wild-type IscR, with a slightly lower extent of activation (Fig. 4, lanes 17 to 20). Taken together, these results indicate that in the absence of Fur, IscR can exert its activator function under nonstressed as well as oxidative stress conditions. Whether the activation is primarily due to apo-IscR and whether Fe-S form

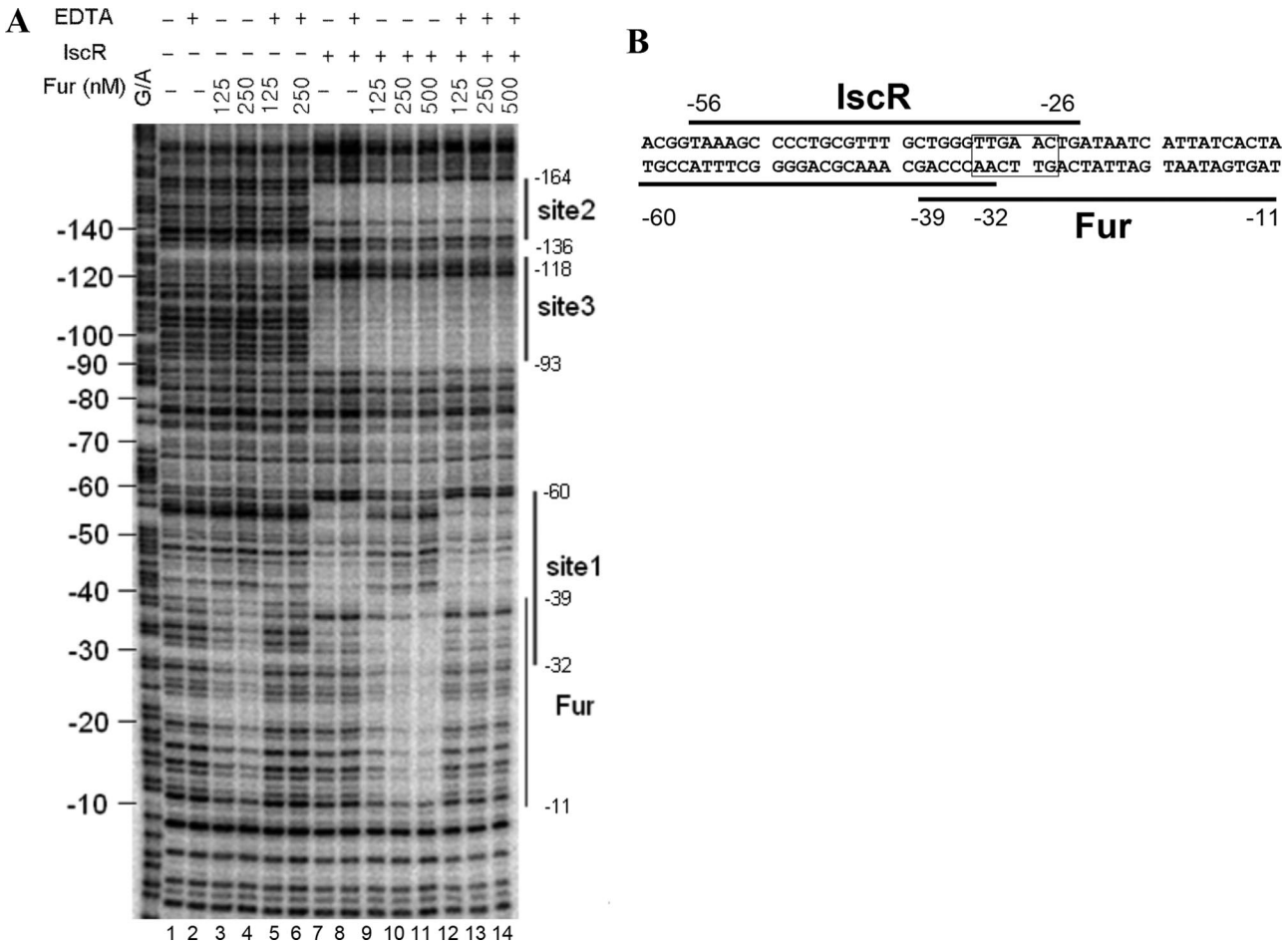


FIG. 3. Precise determination of Fur and IscR binding sites. (A) DNase I footprinting analysis. The *sufA* DNA probe was labeled at the 5′ end of the bottom strand and incubated with increasing amounts of Fur (0, 125, 250, and 500 nM) with or without IscR (2.5 μM) in the binding buffer with or without EDTA (1 mM). Following DNase I treatment, samples were run on an 8% polyacrylamide sequencing gel with Maxam-Gilbert G+A sequencing ladders. (B) Overlapping locations of IscR and Fur binding sites within the *sufA* promoter region. The boundary of the Fur binding site on the bottom strand (nt −39 to −11) is indicated relative to IscR binding sites on both strands (16). The location of the −35 hexamer is boxed.

also contributes as an activator in vivo require further investigation.

The apparent contradiction between the observations that IscR mediates the oxidant-responsive induction of the *suf* gene and that the constitutively active variants of IscR (C92, C98, and C104 mutants) still require oxidative treatment for *suf* induction (16) is now resolved by incorporating the contribution from Fur, which is sensitive to both oxidants and iron limitation and acts through an overlapping binding site. The inactivation of Fur by peroxides and metal depletion is reflected in the induction behavior of *ryhB*, a small RNA whose synthesis is regulated by Fur (Fig. 4) (6). Recent work from the Imlay group (15) showing that submicromolar hydrogen peroxide inactivates Fur is consistent with our observation.

According to our current model, the transcription of the *sufA* operon responds more sensitively to oxidant (peroxide) stress than it does to iron limitation. Peroxide stimulates the induction of *sufA* through activating OxyR, deactivating Fur, and activating IscR. Iron limitation only partially activates the *sufA* operon, as indicated by the effect of a *fur* mutation and chelation with dipyridyl. In *suf* regulation, Fur serves as a repressor that blocks RNA polymerase to bind but also acts simultaneously as an antiactivator. IHF binding in the OREII region (nt −169 to −113 [6]) appears to ensure the action of OxyR only. Even though IHF shares its binding site with apo-IscR (nt −132 to −164, site 2 [16]), as revealed upon examination in vitro, its binding affinity far exceeds that of apo-IscR and therefore it is most likely unaffected by IscR in vivo (data not shown). IHF does not support the action of IscR either, since the site-specific mutagenesis of the IHF binding site still allows activation by IscR (data not shown). In summary, all four transcriptional regulators (OxyR, IHF, Fur, and IscR) participate in inducing the *sufA* operon in response to the oxidative condition. This contrasts with the induction of the *isc* operon, which also responds to aerobic and iron-limited conditions but primarily through modulating the repressor activity of Fe-S-IscR (10, 12). The multiplex pathway of response to oxidative stress and iron limitation in *sufA* regulation agrees with the specialized role

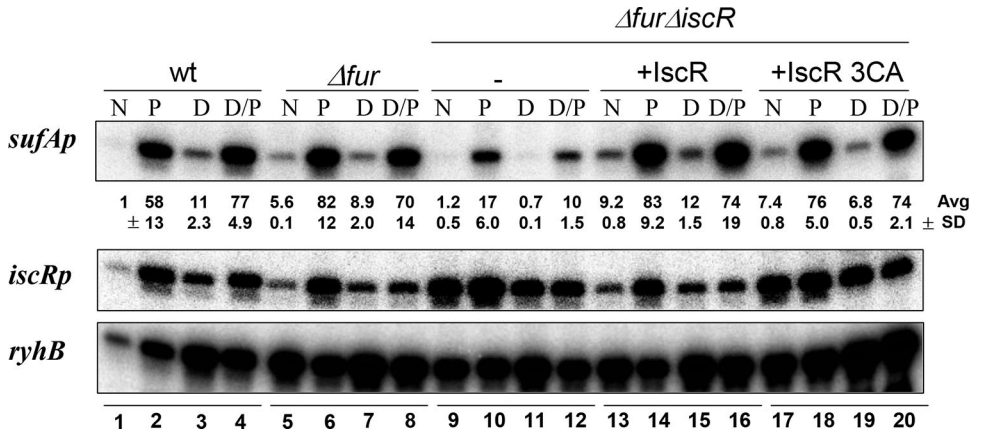


FIG. 4. Contribution of Fur inactivation in *sufA* induction by IscR. *sufA* transcripts were analyzed by S1 nuclease mapping of RNA samples from the exponentially grown wild-type (wt), Δ*fur* mutant, and Δ*fur iscR* mutant strains treated with the peroxide generator phenazine methosulfate (P; 10 min at 0.1 mM), the iron chelator 2,2-dipyridyl (D; 20 min at 0.2 mM), both (D/P; for 20 min) or none (N) in LB medium. To assess the effect of IscR, the Δ*fur iscR* mutant was exogenously provided with the wild-type (+IscR) or with the triple cysteine (C92/94/108A) mutant (+IscR 3CA) *iscR* gene cloned in the pTac1 plasmid or the parental plasmid, pTac1 (-) (16). Transcripts from *iscR* and *rhyB* promoters were analyzed in parallel as described previously (16). Quantitation by phosphorimage analyzer for three independent experiments is presented with average values and standard deviations (Avg ± SD).

of the Suf system under oxidative and iron-limited conditions (9, 10, 14).

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