For decades, the bacterial ferric uptake regulator (Fur) has been thought to respond to ferrous iron to transcriptionally regulate genes required for balancing iron uptake, storage, and utilization. Because iron binding to Fur has never been confirmed in vivo, the physiological iron-sensing mechanism remains an open question. Fontenot et al. now show that Fur purified from *Escherichia coli* binds an all-Cys-coordinated [2Fe-2S] cluster. This finding opens the exciting possibility that Fur may join numerous well-studied bacterial, fungal, and mammalian proteins that use FeS clusters for cellular iron regulation.

Despite the abundance of iron on earth, this transition metal is difficult to acquire by living cells. This problem has led to the development of sophisticated cellular iron uptake systems that are adapted to the lifestyle of the particular organisms. Conversely, iron overload of cells or cell compartments is known to be toxic because iron can generate reactive oxygen species via Haber–Weiss and Fenton chemistry. To precisely balance intracellular iron levels to physiological needs, cells utilize numerous transcriptional and post-transcriptional regulatory systems that tightly control iron acquisition, intracellular delivery, and storage. Many decades ago, the *Escherichia coli* global transcription factor Fur was identified as the founding member of a large family of bacterial regulators of metal (Fe, Zn, Ni, and Mn) homeostasis (1, 2). Numerous studies on Fur and related proteins have suggested that Fur is regulated by ferrous iron (Fe^{2+}) to repress genes coding for, for example, iron uptake systems and, vice versa, induce genes of, for example, iron storage proteins (3) (Fig. 1a). Despite its small size of 18 kDa, Fur is a complex dimeric or tetrameric DNA-binding protein containing up to three different metal binding sites that *in vitro* bind various metals, including Fe^{2+} and Zn^{2+} ions. However, despite several attempts, the iron-binding holo-form of Fur has never been isolated from living cells, leaving open the question of whether the *in vitro* experiments with reconstituted Fe^{2+}-binding Fur faithfully reflect the physiological situation (Fig. 1a).

Moreover, the situation is further complicated by the fact that iron is not only inserted into mono- and dinuclear iron-binding proteins, but also needed for heme and iron-sulfur (FeS) cofactors. The levels of these compounds are also tightly regulated, frequently by using these cofactors themselves as a co-regulatory device (4). Finally, Fur regulation is known to integrate oxidative stress conditions, but the molecular mechanism behind this connection remains unclear (2).

Work by Fontenot et al. (5) now brings a new twist into how Fur may be regulated *in vivo*. Surprisingly, the authors find a [2Fe-2S] cluster bound to Fur purified from *E. coli* cells, leading them to suggest that this cofactor, rather than Fe^{2+}, may be the iron-regulatory device (Fig. 1b). What was the trick to identify a Fur-bound FeS cluster after such a long time studying this protein? Fontenot et al. (5) took advantage of increased iron levels present in ΔiscA/sufA *E. coli* cells lacking the proteins IscA and SufA. Together with the related ErpA, these factors specifically mature [4Fe-4S] proteins, whereas [2Fe-2S] proteins are made independently (6, 7). Fontenot et al. (5) reasoned that labile iron may have accumulated in ΔiscA/sufA cells because the [4Fe-4S] subset of FeS proteins is no longer made, thus providing a chance to isolate Fur with bound iron. To test this assumption, Fur was overexpressed in ΔiscA/sufA cells and purified as a red-colored protein. Unexpectedly, Fur contained both iron and acid-labile sulfur, indicating the presence of an FeS cluster. Several methods, including UV-visible, EPR, and Mössbauer spectroscopy, provided unequivocal evidence for a [2Fe-2S] type. WT cells overexpressing Fur also contained Fur with a bound FeS cluster, yet about 8-fold less compared with ΔiscA/sufA cells, providing a likely explanation for why this cofactor has been missed previously.

*E. coli* Fur contains three metal-binding sites. Fontenot et al. (5) used site-specific mutagenesis to identify the precise binding site of the [2Fe-2S] cluster. Amino acid exchanges at sites 1 and 2 (numbered as defined in Ref. 5) had virtually no effect on FeS cluster association to Fur, whereas exchanges of any of the three conserved Cys residues of the C-terminal site 3 to Ala completely abolished binding. The fourth coordinating residue may be another nonconserved C-terminal Cys. This finding fits perfectly with the Mössbauer data, suggesting an all-Cys coordination of the Fur-bound [2Fe-2S] cluster. Independent support for a possible physiological relevance of the [2Fe-2S] moiety has come from earlier mutational studies showing that two Cys residues of site 3, but none of the tested residues of sites 1 and 2 are crucial for the iron-regulatory activity of Fur *in vivo* (8). In clear contrast, previous biochemical studies analyzing Fe^{2+} binding to Fur *in vitro* had indicated histidines and carbonylates at site 2 as iron ligands (Fig. 1b) (2, 9). Finally, Fontenot et al. (5) overexpressed the Fur ortholog from the Gram-negative bacterium *Haemophilus influenzae* in ΔiscA/sufA *E. coli* cells. This protein bound even 2-fold more [2Fe-2S] cluster, implying that cluster binding may be a general feature of Fur proteins.

The new study by Fontenot et al. (5) convincingly shows that bacterial Fur proteins isolated from *E. coli* can bind an all-Cys-
Iron regulator Fur binds FeS clusters

Figure 1. Simplified model for transcriptional regulation of iron homeostasis by bacterial Fur. a, the dimeric transcriptional ferric uptake regulator Fur can act in multiple ways to modulate gene expression (3). Upon binding to DNA promoter regions, holo-Fur acts as a repressor of genes for various iron uptake systems and the regulatory RNA rhyB (top right). Additionally, holo-Fur induces genes for iron storage and utilization (bottom right). The co-regulatory species of Fur that senses intracellular iron levels in vivo (indicated by a question mark) is not known with certainty yet. b, for a long time, ferrous iron (Fe^{2+}) bound to histidine and acidic residues (site 2, left) was thought to be the iron sensor, yet the new study by Fontenot et al. provides evidence for an all-cysteine-bound [2Fe-2S] cluster (site 3, right) raising the exciting possibility that intracellular iron is sensed and regulated indirectly via FeS clusters. For simplicity, the cartoon omits the transcription-regulatory activity of apo-Fur lacking a bound iron cofactor.

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