Feedback regulation of iron-sulfur cluster biosynthesis

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Iron-sulfur clusters ([Fe-S] clusters) represent one of nature's simplest, functionally versatile, and perhaps most ancient prosthetic groups (1). Among the familiar types of [Fe-S] clusters are [2Fe-2S] and [4Fe-4S] clusters, which usually are attached to their protein partners by four cysteine thiol ligands (Fig. 1). Proteins that contain one or more [Fe-S] clusters are commonly called [Fe-S] proteins, and they represent a large class of structurally and functionally diverse proteins that participate in many metabolic processes. For example, [Fe-S] proteins are essential players in the life-sustaining processes of respiration, nitrogen fixation, and photosynthesis with [Fe-S] clusters participating as agents of electron transfer, substrate activation, catalysis, and environmental sensing. Given the structural simplicity of [Fe-S] clusters and the participation of [Fe-S] proteins in so many metabolic processes it may be surprising that the pathway for biological formation of [Fe-S] clusters is only now beginning to emerge. Pioneering work in the laboratory of Berg and Holm (2) established that the chemical synthesis of structural analogs to many biological [Fe-S] clusters is achieved when $\text{Fe}^{3+/2+}$ and S^{2-} are combined under controlled conditions and in the presence of the appropriate thiolate donors. Along these same lines, Malkin and Rabinowitz (3) showed that purified [Fe-S] proteins, for which the cluster has been removed, are often reconstituted with the correct [Fe-S] cluster species by simple treatment with Fe^{2+} and S^{2-} under reducing conditions. Such *in vitro* "spontaneous" assembly, however, cannot represent the complete mechanism of biological [Fe-S] cluster formation because free Fe^{2+} and S^{2-} are metabolic poisons. Rather, it is now known that a group of highly conserved proteins are responsible for directing the controlled assembly of [Fe-S] clusters and the maturation of [Fe-S] proteins (4).

Although a significant portion of cellular Fe and S is contained within [Fe-S] proteins, these elements are also widely distributed among many other biomolecules. For example, Fe is a constituent of all heme proteins and S is found in cysteine, methionine, many cofactors, and certain tRNAs. For this reason, and be-

Fig. 1. Structure of a [2Fe-2S] cluster (Upper) and [4Fe-4S] cluster (Lower). Cysteiny l β -carbons are shown in gray, sulfur in yellow, and iron in green.

cause the accumulation of [Fe-S] clusters in excess of the amount necessary for maturation of [Fe-S] proteins would result in the deleterious formation of insoluble iron sulfides, all cells must control the formation of these clusters. An important aspect of how this is accomplished in *Escherichia coli* was recently discovered by Patricia Kiley and colleagues and is described in this issue of PNAS (5). The products of six linked genes, *iscS*, *iscU*, *iscA*, *hscB*, *hscA*, and *fdx*, use Fe and L-cysteine to form [Fe-S] cluster precursors, which subsequently are delivered to the appropriate target protein (ref. 4, Fig. 2). Schwartz *et al.* (5) report that one such target is a negative regulatory protein encoded by *iscR*, the first gene located within the [Fe-S] cluster biosynthetic gene transcription unit. Several lines of evidence support this suggestion. First, a fraction of isolated *IscR* contains a [2Fe-2S] cluster. Second, the *IscR* protein bears primary sequence identity with a family of transcription regulators. Third, deletion of *iscR* results in elevated expression of the [Fe-S] cluster assembly genes *in vivo* and isolated *IscR* inhibits their expression *in vitro*. Finally, inactivation of either *iscS* or *hscA*, both of which are required for full

[Fe-S] cluster formation, also results in a marked elevation in expression of the assembly genes. These observations lead to a feedback regulation model where a demand for the maturation of [Fe-S] proteins controls expression of genes whose products are necessary for [Fe-S] cluster formation. In this model proteins destined to contain [Fe-S] clusters establish synthetic homeostasis by effectively competing with *IscR* for the available clusters produced by the biosynthetic machinery. Namely, when the capacity to produce [Fe-S] clusters exceeds the demand, the maturation of *IscR* shuts down further expression of the assembly machinery. This model suggests that the cellular pool of immature [Fe-S] proteins is more effective than immature *IscR* in sequestering [Fe-S] clusters formed by the biosynthetic machinery. How this is accomplished has not yet been evaluated, although it might be significant there are only three cysteine residues located within the *E. coli* *IscR* primary sequence, whereas four cysteines are usually involved in the coordination of a [2Fe-2S] cluster. This situation could make it either more difficult to assemble the [2Fe-2S] cluster within *IscR* relative to other [Fe-S] proteins or lead to instability of the cluster once inserted. These possibilities are supported by the observation that the population of as-isolated *IscR* does not contain a full complement of [2Fe-2S] clusters.

The feedback model proposed by Schwartz *et al.* (5) provides an elegant way for the cell to synchronize [Fe-S] cluster formation with a demand for the maturation of [Fe-S] proteins. However, detailed features of this regulatory circuit are likely to be more complicated because the function of at least one member of the [Fe-S] cluster biosynthetic machinery, *IscS*, is not strictly limited to [Fe-S] cluster formation as the end product. As indicated in Fig. 2, the intracellular source of S for [Fe-S] cluster formation is L-cysteine. One of the [Fe-S] cluster gene products is *IscS*, a pyridoxal-phosphate-dependent enzyme that mobilizes S through the desulfurization of L-cysteine to yield

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Fig. 2. Model for feedback regulation of [Fe-S] cluster biosynthetic gene expression. The gene cluster (Upper) and the corresponding products (Lower) are shown. The products of the *iscSUA*, *hscBA*, and *fdx* genes are indicated as forming a biosynthetic complex but the presence of this complex has not been demonstrated experimentally. This representation is for convenience and because interaction between IscU and IscS, among HscB, HscA, and IscU, and between IscA and Fdx has been reported.

alanine and an IscS-bound persulfide (4, 6). Current evidence indicates that this persulfide is directly donated to IscU (7, 8), which, in turn, provides a scaffold for [Fe-S] cluster assembly (9). Such a direct transfer pathway prevents the release of toxic S^{2-} during the [Fe-S] cluster assembly process and the same mechanism also might apply to the mobilization of S for biosynthesis of certain other S-containing biomolecules. As it turns out, IscS is an amazingly versatile player in cellular S trafficking because both genetic and biochemical studies have indicated that, in addition to its role in [Fe-S] cluster assembly, IscS could provide S for biotin, thiamine, thiolated tRNA, and perhaps Mo cofactor (10–14). Although there is one situation where such delivery appears to involve the participation of an [Fe-S] cluster as an intermediate S carrier (10), current genetic and biochemical evidence indicates that this is not always the case (15, 16). Thus, the expression of *iscS* must be regulated, or the activity of IscS controlled, so that the correct distribution of S among various S-containing biomolecules is accomplished without the release of toxic S^{2-} . Another interesting situation is evidence that IscS can be recruited to use selenocysteine as a substrate for the ultimate formation of selenophosphate (17). Finally, because L-cysteine is a substrate for IscS, the formation or activity of this enzyme must be controlled so that futile cycling of cysteine biosynthesis and desulfurization does not occur. It appears that for some organisms such regulation is accomplished, in part, by recruiting a cysteine biosynthetic enzyme as part of the [Fe-S] cluster assembly machinery (4).

The principles of chemical [Fe-S] cluster assembly described by Holm and Berg (2) will apply to the biological system. Nevertheless, several features of the biological process are not yet understood. For

example, it appears that both [2Fe-2S] and [4Fe-4S] cluster-containing proteins obtain their clusters from the same biosynthetic machinery (18). It has been shown that both cluster types can be formed on the IscU scaffold *in vitro* (19), but whether either or both of these clusters types can be transferred intact to a particular target protein is not clear. The IscA protein is also able to provide an *in vitro* scaffold for [Fe-S] cluster assembly (20, 21), although genetic studies show that IscA is not as functionally important as IscU (18). It is not yet known whether this feature might reflect that IscU and IscA provide alternative assembly scaffolds involved in the maturation of different [Fe-S] proteins. A related possibility is that IscU and IscA operate separately as alternative assembly scaffolds under different physiological conditions.

Another unexpected and poorly understood aspect of [Fe-S] cluster assembly is the participation of molecular chaperones (4, 22). The products of the *hscA* and *hscB* genes contained within the [Fe-S] cluster assembly transcription unit are members of the molecular chaperone family, exhibiting sequence identity when compared with their corresponding homologs, DnaK and DnaJ. Vickery and colleagues (22) have shown that IscU, the [Fe-S] cluster assembly scaffold protein, interacts with HscBA, causing a remarkable 480-fold stimulation in the intrinsic ATPase activity of the complex. Whether or not the involvement of molecular chaperones is related directly to [Fe-S] cluster assembly, maintaining the integrity of the assembly scaffold, or delivery of [Fe-S] clusters to their cognate proteins remains to be determined. The system described by Schwartz *et al.* (5) might prove valuable toward addressing these issues. For exam-

ple, the activation of IscR could be used to assess *in vitro* transfer of [Fe-S] clusters from the assembly machinery to the immature form of IscR. The system also holds promise for the development of *in vitro* DNA-directed formation of IscR as a way to test whether molecular chaperones or other components are involved in the maturation of [Fe-S] proteins posttranslationally or cotranslationally. Finally, because active IscR can be abundantly produced in *E. coli* by using recombinant DNA methods, and the active form contains an [Fe-S] cluster, it should be possible to examine the role of each [Fe-S] cluster assembly protein *in vivo* by using whole-cell Mossbauer and electron paramagnetic resonance spectroscopies to monitor the assembly, and perhaps, disassembly of the [2Fe-2S] cluster contained in IscR.

There appears to be an extraordinary conservation in higher organisms with respect to the basic features and primary sequences of bacterial proteins that directly participate in [Fe-S] cluster biosynthesis. In the case of [Fe-S] cluster biosynthesis in *Saccharomyces cerevisiae* the emerging story is a fascinating one (23–28). For this organism there is strong evidence that proteins involved in [Fe-S] cluster biosynthesis are imported from the cytosol into the mitochondrion where [Fe-S] cluster assembly initially occurs. Some of the assembled clusters are used for the maturation of mitochondrial [Fe-S] proteins with the balance being exported for the maturation of cytosolic [Fe-S] proteins. This system appears to represent the major pathway for [Fe-S] cluster formation and distribution in all higher organisms, although there is evidence for some partitioning of the IscS homolog to the cytosol and nucleus (29, 30). Lill and Kispal (31) have advanced the hypothesis that sequestration of [Fe-S] cluster assembly machinery within the mitochondrion occurs so that [Fe-S] formation proceeds in the favorable reducing environment provided by this organelle. This suggestion is also relevant to questions concerning the origin of the mitochondrion and why this organelle is essential (31). Eukaryotic proteins associated with mitochondrial [Fe-S] cluster export are not generally conserved in bacteria. Similarly, the IscR protein is not conserved in eukaryotic organisms. Nevertheless there is a clear connection between eukaryotic iron homeostasis and [Fe-S] cluster assembly because inactivation of the *S. cerevisiae* [Fe-S] cluster assembly and trafficking proteins results in the accumulation of Fe deposits within the mitochondrion (23–28). This phenotype has a striking parallel to a variety of human iron storage diseases, highlighting the current interest within the scientific community concerning the mechanism and control of [Fe-S] cluster assembly.

1. Beinert, H., Holm, R. H. & Munck E. (1997) *Science* **277**, 653–659.
2. Berg, J. M. & Holm, R. H. (1982) in *Iron-Sulfur Proteins*, ed. Spiro, T. G. (Wiley, New York), pp. 1–66.
3. Malkin, R. & Rabinowitz, J. C. (1966) *Biochem. Biophys. Res. Commun.* **23**, 822–827.
4. Zheng, L., Cash, V. L., Flint, D. H. & Dean, D. R. (1998) *J. Biol. Chem.* **273**, 13264–13272.
5. Schwartz, C. J., Giel, J. L., Patschkowski, T., Luther, C., Ruzicka, F. J., Beinert, H. & Kiley, P. J. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 14895–14900. (First Published December 11, 2001; 10.1073/pnas.251550898).
6. Zheng, L., White, R. H., Cash, V. L. & Dean, D. R. (1994) *Biochemistry* **33**, 4714–4720.
7. Urbina, H. D., Silberg, J. J., Hoff, K. G. & Vickery, L. E. (2001) *J. Biol. Chem.*, in press.
8. Smith, A. D., Agar, J. N., Johnson, K. A., Frazzon, J., Amster, J., Dean, D. R. & Johnson, M. K. (2001) *J. Am. Chem. Soc.* **123**, 11103–11104.
9. Agar, J. N., Zheng, L., Cash, V. L., Dean, D. R. & Johnson, M. K. (2000) *J. Am. Chem. Soc.* **122**, 2136–2137.
10. Kiyasu, T., Asakura, A., Nagahashi, Y. & Hoshino, T. (2000) *J. Bacteriol.* **182**, 2879–2885.
11. Lauhon, C. T. & Kambampati, R. (2000) *J. Biol. Chem.* **275**, 20096–20103.
12. Skovran, E. & Downs, D. M. (2000) *J. Bacteriol.* **182**, 3896–3903.
13. Schwartz, C. J., Djaman, O., Imlay, J. A. & Kiley, P. J. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 9009–9014. (First Published July 25, 2000; 10.1073/pnas.160261497)
14. Leimkuhler, S. & Rajagopalan, K. V. (2001) *J. Biol. Chem.* **276**, 22024–22031.
15. Tokumoto, U. & Takahashi, Y. (2001) *J. Biochem.* **130**, 63–71.
16. Mueller, E. G., Palenchar, P. M. & Buck, C. J. (2001) *J. Biol. Chem.* **276**, 33588–33595.
17. Lacourciere, G. M., Mihara, H., Kurihara, T., Esaki, N. & Stadtman, T. C. (2000) *J. Biol. Chem.* **275**, 23769–23773.
18. Nakamura, M., Saeki, K. & Takahashi, Y. (1999) *J. Biochem.* **126**, 10–18.
19. Agar, J. N., Krebs, C., Frazzon, J., Huynh, B. H., Dean, D. R. & Johnson, M. K. (2001) *Biochemistry* **39**, 7856–7862.
20. Ollagnier-de-Choudens, S., Mattioli, T., Takahashi, Y. & Fontecave, M. (2001) *J. Biol. Chem.* **276**, 22604–22607.
21. Krebs, C., Agar, J. N., Smith, A. D., Frazzon, J., Dean, D. R., Huynh, B. H. & Johnson, M. K. (2001) *Biochemistry*, in press.
22. Hoff, K. G., Silberg, J. J. & Vickery, L. E. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 7790–7795. (First Published June 27, 2000; 10.1073/pnas.130201997)
23. Strain, J., Lorenz, C. R., Bode, J., Garland, S., Smolen, G. A., Tall, D. T., Vickery, L. E. & Culotta, V. C. (1998) *J. Biol. Chem.* **273**, 31138–31144.
24. Kispal, G., Csere, P., Prohl, C. & Lill, R. (1999) *EMBO J.* **18**, 3981–3989.
25. Lill, R., Diekert, K., Kaut, A., Lange, H., Pelzer, W., Prohl, C. & Kispal, G. (1999) *Biol. Chem.* **380**, 1157–1166.
26. Schilke, B., Voisine, C., Beinert, H. & Craig, E. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 10206–10211.
27. Garland, S. A., Hoff, K., Vickery, L. E. & Culotta, V. C. (1999) *J. Mol. Biol.* **294**, 897–907.
28. Li, J., Kogan, M., Knight, S. A. B., Pain, D. & Dancis, A. (1999) *J. Biol. Chem.* **274**, 33025–33034.
29. Tong, W. H. & Rouault, T. (2000) *EMBO J.* **19**, 5692–5700.
30. Nakai, Y., Nakai, M., Hayashi, H. & Kagamiyama, H. (2001) *J. Biol. Chem.* **276**, 8314–8320.
31. Lill, R. & Kispal, G. (2000) *Trends Biochem. Sci.* **25**, 352–356.