

Linking physiological functions of iron

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Iron-sulfur clusters and hemes are two iron-containing prosthetic groups involved in important physiological functions. Identification of the gene responsible for anemia in a mutant zebrafish has revealed an unexpected link between iron-sulfur cluster assembly and heme synthesis in red blood cells.

Virtually all aerobic organisms synthesize heme, an iron protoporphyrin complex that functions as an oxygen transporter and electron carrier in multiple enzymes and mitochondrial respiratory chain complexes. Heme is synthesized by a highly conserved multistep process, beginning with the condensation of glycine and succinyl-CoA by ALA synthase (ALAS) to form 5-aminolevulinic acid, and continuing through seven successive enzymatic steps that culminate with insertion of iron into the porphyrin ring to form heme. It is advantageous to inhibit heme biosynthesis in iron-depleted cells at the first step of the pathway, because the final step of heme synthesis is catalyzed by ferrochelatase, an iron-sulfur protein that inserts iron into protoporphyrin IX. If cells are iron deficient and cannot complete the final step of synthesis, later heme biosynthetic intermediates such as uroporphyrin and protoporphyrin IX can accumulate and cause toxicity¹. Thus, it is of great interest that Wingert *et al.*, in the 18 July issue of *Nature*, have discovered a functional connection between initiation of heme biosynthesis in red cells and an enzyme important for activity of proteins that contain iron-sulfur clusters².

Shiraz mutants are zebrafish mutants that have a heritable disorder of hemoglobin synthesis. Using positional cloning to identify the gene responsible for *shiraz* anemia in zebrafish, the authors established that the anemia was due to loss of *glutaredoxin 5* function, a gene first identified as important for the activity of iron-sulfur cluster proteins in yeast³. Iron-sulfur clusters are prosthetic groups composed of inorganic sulfur and iron that are of fundamental importance in respiration, photosynthesis and nitrogen fixation⁴. Thus, this result suggested a previously unrecognized connection between two fundamental biochemical processes, the iron-sulfur cluster assembly and the heme biosynthetic pathways of higher eukaryotes.

To clarify the mechanistic connection between glutaredoxin 5 deficiency and ane-

mia, the authors extrapolated from insights gained over the past decade about regulation of mammalian erythropoiesis. Mammalian red blood cells contain a distinct form of ALA synthase (eALAS) in which the 5' untranslated region (UTR) of the transcript contains an RNA stem-loop element known as an iron-responsive element (IRE)⁵. The IRE is a binding site for iron-sensing proteins known as iron regulatory proteins (IRPs)⁶, which repress heme synthesis in iron-depleted cells by binding to the IRE of eALAS, thereby preventing initiation of translation. Mammalian cells contain two IRPs, each of which can bind to IREs found in mRNAs of important iron-metabolism proteins, including the iron-storage protein, ferritin and the transferrin receptor (TfR), an iron-uptake protein. IRP1 is a bifunctional protein that functions as a

cytosolic aconitase in iron-replete cells, or as an RNA-binding protein that inhibits translation of transcripts such as ferritin with IREs at the 5' end of the transcript, while it stabilizes the TfR transcript by binding to IREs in the 3' UTR in iron-depleted cells. The function of IRP1 is determined by the presence or absence of a cubane iron-sulfur cluster ligated to the enzymatic active site⁷ (Fig. 1). Much has been learned about bacterial iron-sulfur cluster assembly enzymes⁴, and the process of iron-sulfur cluster assembly seems to be highly conserved in mammals⁸.

In the zebrafish genome, Wingert *et al.* discovered that, as in mammalian cells, there are two IRPs, and there are also two forms of ALAS, one of which, ALAS2, contains an IRE at the 5' end and is expressed uniquely in erythroid cells⁹. Recognizing that glutaredoxin 5 is required for

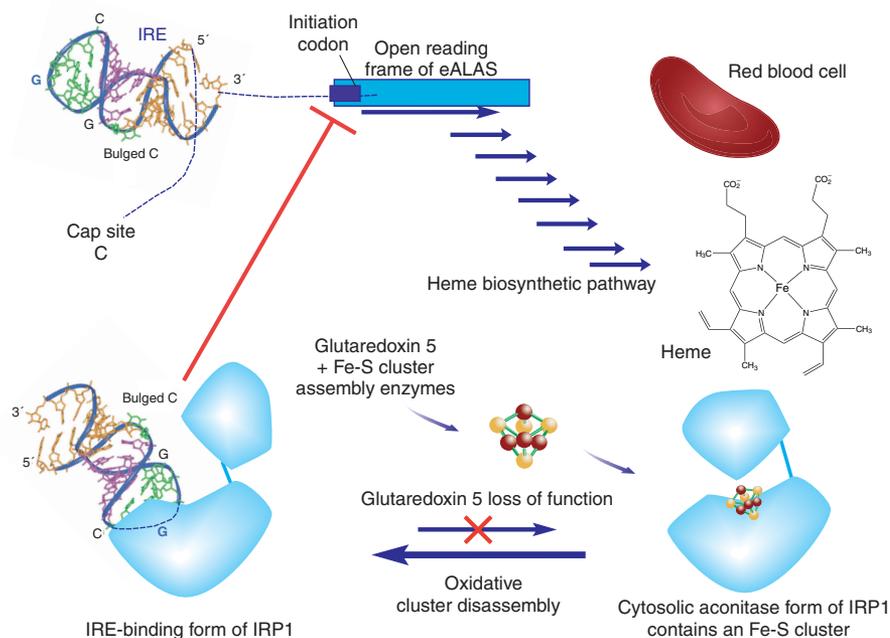


Figure 1 IRP1 that lacks an iron-sulfur cluster because of glutaredoxin 5 deficiency represses heme synthesis by repressing translation of the first enzyme of the heme biosynthetic pathway. IRP1 is a bifunctional protein in which function is determined by the presence or absence of an iron-sulfur cluster. In iron-replete cells, iron-sulfur cluster assembly enzymes, including glutaredoxin 5, participate in cluster formation and insertion of a [4Fe-4S] cluster into IRP1. Iron-sulfur clusters are readily disassembled by oxidative stress. When cells are deficient in iron, or iron-sulfur cluster assembly enzymes, IRP1 binds to IREs. When IRP1 binds to the IRE in the 5' UTR of ALAS2, the zebrafish erythroid ALAS, heme biosynthesis is impaired in red blood cells and the fish become anemic.

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activity of iron-sulfur enzymes in yeast, they reasoned that perhaps the iron-sulfur cluster of IRP1 was absent in shiraz mutants, and that as a result IRP1 constitutively repressed ALAS2 translation and heme biosynthesis.

To investigate the hypothesis that IRP1 was binding to the IRE of erythroid ALAS2 and repressing heme synthesis, the authors injected early zebrafish embryos with ALAS2 expression constructs or antisense sequences that eliminated expression of IRP1 as the embryo developed. When ALAS2 without its 5' IRE was overexpressed, normal erythroid development was restored. Moreover, when ALAS2 constructs with IRE loop mutations were overexpressed, the anemia was also rescued. When they used antisense techniques to eliminate IRP1 in shiraz mutants, they again rescued the anemia, whereas antisense elimination of IRP2 expression had no effect. These findings provided convincing evidence that loss of glutaredoxin 5 in zebrafish converts IRP1 to a constitutive IRE-binding protein, which represses heme biosynthesis in red blood cells by preventing the first step of heme biosynthesis (Fig. 1).

Glutaredoxin has not been implicated in iron-sulfur cluster biogenesis in bacteria, where most iron-sulfur assembly enzymes have been identified. One of the notable findings of this study is that glutaredoxin 5 appears to be important in yeast and zebrafish iron-sulfur cluster protein activity and is conserved in the mouse and human genomes. Zebrafish glutaredoxin 5 restores activity of iron-sulfur proteins in glutaredoxin 5-deficient yeast, demonstrating that function is conserved. Although the exact mechanism of glutaredoxin 5 action is not yet known, possibilities include glutathione removal from the active site cysteine of the sulfur donor needed in early iron-sulfur cluster biogenesis, or from scaffold proteins important in the assembly process¹⁰.

In mammals, IRP2 has thus far emerged as the dominant regulator of intracellular iron metabolism¹¹ and of ALAS expression in erythroid cells¹², but the discovery that loss of glutaredoxin 5 activity can activate IRP1, with important physiologic consequences in zebrafish, raises the possibility that the process of iron-sulfur cluster biogenesis is regulated, and its regulation affects red blood cell

heme biosynthesis through its effect on IRP1 activity. Thus, the fundamental pathways of iron-sulfur cluster assembly and heme biosynthesis are linked by IRP1, which emerges as a nexus of potential regulatory power that also connects and perhaps regulates intermediary metabolism through its other role as a cytosolic aconitase and regulator of citrate levels.

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