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## Researchers Discover New Route to Hemoglobin Synthesis

Researchers studying zebrafish that die from anemia have discovered a new pathway for the synthesis of heme, the deep red, iron-containing molecule that is a component of hemoglobin and myoglobin. The research suggests that defects in this pathway may be an overlooked cause of anemia in humans.

A research team led by Leonard I. Zon, a Howard Hughes Medical Institute investigator at Children's Hospital Boston and Harvard Medical School, published its findings in the August 18, 2005, issue of the journal *Nature*. Zon and his colleagues in Boston collaborated on the studies with researchers from the University of Rochester Medical Center and the University of Utah School of Medicine.

The researchers began their studies hoping to learn why a zebrafish mutant known as *shiraz* (*sir*) failed to produce hemoglobin. The *sir* mutant zebrafish, which were first isolated by Zon and colleagues in the Tübingen Screen Consortium in Germany, intrigued the researchers because they die from anemia caused by lack of hemoglobin.

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Over the years, Zon and his colleagues have discovered many zebrafish mutants that fail to make hemoglobin because of defects in iron metabolism. As they have teased out the causes of these defects, they have learned that the biochemical pathway involved in hemoglobin synthesis in zebrafish has been largely conserved over the 300 million years of evolution between fish and humans. According to Zon, the easily manipulable fish constitutes an excellent model organism for studying the regulation of heme formation.

In the current study, the researchers traced the hemoglobin defect to the gene for an enzyme known as glutaredoxin 5 (*grx5*). But the researchers found early on that the enzyme was not directly connected to hemoglobin production. “Nobody had worked on this gene in vertebrates before, but we found in the scientific literature that this gene in yeast was required for the production of iron-sulfur clusters in the mitochondria,” said Zon. Iron-sulfur clusters are incorporated into certain proteins to enable their enzymatic functions. In further experiments, the researchers confirmed that versions of *grx5* in zebrafish, yeast, mice and humans are functionally equivalent.

“It seemed like the whole process was evolutionarily conserved,” said Zon. “But the difference is that yeast do not make hemoglobin. So we needed to figure out a mechanism that would explain why these fish that have problems making iron-sulfur clusters could not make hemoglobin.”

Other researchers' studies had indicated that the presence of iron-sulfur clusters in the cell is important for controlling an enzyme called iron regulatory protein 1 (IRP1). In turn, IRP1 regulates another enzyme called ALAS2 that plays a key role in heme synthesis. Indeed, experiments by Zon and his colleagues demonstrated that the loss of *grx5* in the mutant zebrafish inappropriately activates IRP1, which blocks the synthesis of ALAS2, and thus heme production. For example, when they restored ALAS2 by injecting into the *sir* mutants a truncated form of ALAS2 that lacked the portion of the molecule sensitive to IRP1, they completely restored the mutant zebrafish hemoglobin production.

“People have always thought that hemoglobin synthesis required only enough iron in the cell for heme production to proceed and then just the addition of the globin protein to form hemoglobin,” said Zon. “Now, we've added a fourth component, iron-sulfur clusters, which are required for heme production. This is a very interesting and unpredicted finding from what we had known before, and our experiments have really defined a new pathway for hemoglobin production,” he said.

Zon said that the findings could apply to developing new treatments for a rare form of anemia, known as sideroblastic anemia, in which elevated IRP1 activity causes a deficiency of ALAS2. In most cases, an increase in IRP1 is likely caused by a mutation in a transporter for iron-sulfur clusters that traps them in mitochondria, where they cannot interact with IRP1 to control it.

In a search for possible treatments for the anemia, Zon and his colleagues are exploring the genetic machinery of hemoglobin production in zebrafish for targets of drugs that could restore normal levels of iron-sulfur clusters. “The pathway that we have found is very sensitive, so our findings might be extended to enable treatments for other forms of anemia,” said Zon.