

Pumping Iron with Zebrafish

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Yesterday in the Plenary Scientific Session, medical student George Shaw presented a fascinating story about a rare disorder, erythropoietic protoporphyria (EPP), that demonstrates a fundamental pathway for normal iron metabolism. They found that the mitoferrin (MFRN) protein was mutated in zebrafish with hypochromic anemia and was aberrantly spliced in 6/6 children with EPP who did not have the more common mutation in ferrochelatase. The aberrant spliceform leads to an early nonsense codon that prematurely truncates the MFRN protein. The aberrant transcript was neither present in patients with classic EPP, nor in normal humans. Furthermore, it fails to complement mutant yeasts in low-iron media, as the wild-type MFRN protein does, demonstrating that it has lost functional activity.

In a previous paper (Wingert RA, et al. *Nature* 2005; 436: 1035-39), the same group showed that the MFRN protein localizes to the mitochondria like other SLC25 solute carriers. Ectopic expression of murine MFRN rescues the mutant zebrafish, and zebrafish MFRN complements the yeast mutant, indicating that gene function is conserved, and raises the question about whether this protein may be the principal mitochondrial iron transporter. This was underscored by the finding that murine embryonic stem cells null for MFRN show maturation arrest and impaired incorporation of ⁵⁵Fe into heme.

These findings underscore the importance of mitochondrial processing for heme synthesis. Cobbold (*Mol. Genet Metab* 89 (3): 227, 2006) found that ferrochelatase is synthesized with a 62 amino acid cleavable aminoterminal sequence, and this leader sequence is required to target the enzyme to mitochondria. In the absence of normal heme synthesis, protoporphyrin IX accumulates in erythrocytes, plasma, liver, and skin, the latter of which causes a severe photosensitivity. However, determinants of function of ferrochelatase once targeted to the mitochondria is still under investigation.

Erythropoietic protoporphyria (EPP) was originally described 45 years ago. This rare disease causes light-sensitive dermatitis and can lead to pigmented gallstones, polyneuropathy, and liver failure. Increased production of protoporphyrin was identified as the cause of these severe and life-threatening symptoms, but, obviously, our understanding of the genetics of this disorder continues to evolve.

In most cases of EPP ferrochelatase (the mitochondrial enzyme responsible for the final step in heme synthesis), the enzyme has reduced activity to only 10-25 percent of normal. The genetic mechanisms underlying the greater reductions seen in EPP are complex. Initial investigations into the genetics of EPP pointed to an autosomal recessive inheritance. Fifty percent of the members of affected families had occasional fluorescent red cells — biochemical evidence of carrier status — but with normal protoporphyrin levels. However, with further investigation a genetically informative family was found. In this family, both parents had occasional fluorescent red cells, as did eleven of their children. However, none of their 13 children had EPP.

It is now appreciated that in most cases of EPP, the mode of inheritance is autosomal dominant with incomplete penetrance. One loss-of-function allele is inherited, and clinical disease occurs when a common hypomorphic allele is also present. In certain ethnic populations, over 40 percent of individuals have just such an allele — a polymorphism that doubles the rate of aberrant splicing. This change reduces gene expression through a nonsense-mediated decay mechanism and leads to disease if a loss-of-function allele is also inherited. Less frequently, EPP can also be inherited as an autosomal recessive disease with two loss-of-function alleles.

Thus erythropoietic protoporphyria is a rare disorder that can be induced by at least two mutations: ferrochelatase or, infrequently, mitoferrin.