Frataxin, Iron and Oxygen

Yan Zhang (UPenn), Elise Lyver (UPenn), Simon Knight (UPenn), Debkumar Pain (UMDNJ, NJ), Timothy Stemmler (Wayne State, Detroit), Emmanuel Lesuisse (CNRS, Paris), Andrew Dancis* (UPenn, Phila)

Heme, like Fe-S clusters, requires iron, and the iron substrate for the processes of heme synthesis and Fe-S cluster formation must be maintained in reduced (ferrous) and bioavailable form. How exactly this is accomplished in cells has not been determined; the mitochondrial protein frataxin may be involved.

In the yeast S. cerevisiae, deletion of the yeast frataxin homolog (*YFH1*) was combined with deletions of *MRS3* and *MRS4*, mitochondrial carrier proteins implicated in iron homeostasis. A "synthetic" slow growth phenotype in air was noted, with the combined (ΔΔΔ) mutant characterized by a much more severe defect than the individual Δ*mrs3/4* or Δ*yfh1* mutants. However, under anaerobic conditions, the growth defect was minimally noticeable if at all. Using a real-time assay of heme biosynthesis, porphyrin precursor and iron were presented to mitochondria within permeabilized cells and the appearance and disappearance of fluorescent porphyrins were followed. The Mrs3/4p carriers were required for rapid iron transport into mitochondria for heme synthesis, whereas, there was also evidence for an alternative slower system. A different role for Yfh1p was observed under conditions of low mitochondrial iron and aerobic growth (revealed in the ΔΔΔ), acting to protect bioavailable iron within mitochondria and to facilitate its use for heme synthesis.

Heme synthesis does not occur anaerobically, because of requirement of porphyrin oxidases for oxygen. However, Fe-S cluster synthesis does occur and aconitase activity was assayed anaerobically. Even under strictly anaerobic conditions, the ΔΔΔ mutant showed more severely deficient aconitase activity than Δ*yfh1* or Δ*mrs3/4* mutants. After air exposure, the rank order in activities reversed, with Δ*yfh1* showing more severe aconitase deficiency than Δ*yfh1* perhaps due to toxicity from mitochondrial iron accumulation. We are currently endeavoring to use this permeabilized cell system and manipulation of air exposure to characterize the role of frataxin in iron delivery for new Fe-S cluster formation. We are studying the interactions of frataxin mutants with iron and partner proteins to better understand this process.