Dr. Jonathan Barasch

Project Abstract
The transport of iron poses a significant problem because free ferric iron is insoluble (< 10^{-18} M) in aerobic solutions at physiologic pH, while upon solubilization by some chelators, a reactive form of iron is created that can produce toxic oxygen species. Specialized mechanisms are consequently required to traffic iron and these specialized mechanisms are found in proteins which utilize conserved motifs to directly bind iron (transferrin and ferritin) or utilize embedded cofactors. While extracellular iron transport is largely mediated by transferrin, mice carrying deletions of these genes displayed surprisingly limited phenotypes (Barasch, Developmental Cell, 2009). We found that a member of the lipocalin superfamily called Ngal acted as a high affinity iron carrier (Barasch, Molecular Cell, 2002) when binding a family of novel cofactors called the catechols or else the related bacterial siderophores constructed from catechol. In the presence of iron, formation of the Ngal:siderophore:FeIII complex occurred at subnanomolar affinity (Barasch, NatureChemical Biology, 2010) forming a bright red protein, which was stable for many days in solution and stable in vivo for transport of its tightly bound iron. Ngal is expressed in vivo, but a number of "damage" stimuli raise its concentration by orders of magnitude. Thereafter, Ngal traffics in the serum and is thought to be captured by the kidney receptor megalin, where Ngal clears the siderophore:Fe complex. While we have learned a great deal about the metabolism of the urinary form of Ngal (it is expressed from the distal nephron and is excreted in the urine as a full length protein), we know much less about this clearance system and the role of the megalin receptor, which is the only confirmed receptor for Ngal. To study this process in depth we will examine a conditional mutant of megalin, and for studies in wild type mice we are creating a series of Ngal mutants, some of which bypass the proximal tubule where megalin is located, resulting in their presence in the urine. During these studies we realized that the mutants could still bind to siderophore:FeIII at high affinity (they were red colored proteins), and that they could definitely excrete iron, we speculate in a redox inactive manner. Indeed, rather than donate iron to micro-organisms, which is a major concern for small molecule chelators, the Ngal:siderophore:Fe complexes sequester iron from bacteria. In sum, in this proposal, we test the hypothesis that megalin is the key recycling receptor for Ngal and as a result of this idea, we propose that when the megalin-Ngal complex is inhibited, Ngal can carry tightly bound iron in the urine, hence serving as a safe, novel therapeutic for the common syndromes of iron overload diseases.
