Michelle Bradley

C. Clarke Group

Characterization of Coq11, a novel protein involved in the biosynthesis of coenzyme Q in *Saccharomyces cerevisiae*

Coenzyme Q, also known as ubiquinone or Q, is a redox-active lipid component of the electron transport chain that functions in cellular energy metabolism. Due to its redox capabilities, reduced Q (or QH$_2$) also serves as an important lipid-soluble antioxidant effective in alleviating lipid peroxidative damage. In *Saccharomyces cerevisiae*, thirteen known mitochondrial proteins (Coq1-Coq11, Yah1, and Arh1) drive Q biosynthesis. Many of these Coq polypeptides required for Q production are localized to the matrix side of the mitochondrial inner membrane where they form a high molecular weight, multi-subunit complex known as the ‘CoQ synthome’. Absence of individual Coq proteins causes severe defects in Q biosynthesis, and such coq null mutants lose the ability to respire. However, the functional roles of some Coq polypeptides as well as several steps in the Q biosynthetic pathway remain unknown. Here, we present the identification of a novel mitochondrial protein that associates with the CoQ synthome encoded by the open reading frame *YLR290C*, which we renamed Coq11. Co-immunoprecipitation and lipid analyses demonstrate that Coq11 interacts with other Coq polypeptides as well as Q and Q-intermediates, and is required for efficient de novo Q biosynthesis in the BY4742 yeast genetic background. We have begun work towards understanding the function of Coq11 in CoQ synthome formation as well as the effect of its absence on the steady-state levels of other CoQ synthome proteins. Further, we have started investigation of the relationship between Coq11 and Coq10, which are found as protein fusions in five fungal genomes. Taken together, this project will provide powerful insights into Q biosynthesis and regulation towards the objective of designing effective therapeutics for diseases caused by Q deficiencies.

Scott McConnell

Clubb Group

Structural and Mechanistic Insights into Pilus Construction by Specialized Sortase Enzymes

Pathogenic Gram-positive bacteria use pili to adhere to host tissues during infections. These long protein filaments project from the cell surface and can endure high levels of tensile stress due to robust isopeptide linkages between subunits. I am studying the assembly mechanism of the archetypal SpaA-pilus from *Corynebacterium diphtheriae*, the causative agent of diphtheria. Using an integrated approach that employs structural, molecular and cellular biology I am determining how the pilin polymerase recognizes its substrates and catalyzes lysine isopeptide bonds. A crystal structure reveals that the polymerase is held in an inactive state by an auto-inhibitory “lid” structure. Targeted mutations introduced into the lid activate the enzyme, enabling in vitro reconstitution of the pilus assembly reaction and facilitate its biochemical characterization. Using this assay we have discovered that pilin polymerases in Gram-positive bacteria contain a functionally important sequence motif, which is critical for transpeptidation. My ongoing research is studying the mechanism of substrate recognition of the polymerase and developing the enzyme into a chemoenzymatic tool to create isopeptide linked bioconjugates.