Damionne C Bell

New Orleans Science and Math High School, New Orleans. Louisiana
Dr. Ben Kelly
LSUHSC

Generation of a *Leishmania* plasmid to express a mutated cytochrome c oxidase subunit in *Leishmania*.

Leishmania is kinetoplastid parasite transmitted by phlebotomine sand flies and causes the parasitic disease leishmaniasis. The major forms of the disease are cutaneous, mucosal, and visceral. The most common form is cutaneous leishmaniasis which causes skin sores. Mucosal leishmaniasis causes sores in the nose, mouth, and throat. Visceral leishmaniasis affects the internal organs and can be deadly. The World Health Organization estimates that between 600,000 and 1 million people get cutaneous leishmaniasis each year. Leishmania is found in South Africa, Middle East, East Africa, East Asia, Central America, South America, Mexico, and Texas.

Following sandfly bite-mediated entry into the mammalian host, *Leishmania* must first survive an abrupt increase in temperature as it transitions from the sandfly (~27^{o}C) to the mammalian (33-37 ^{o}C) host environment. Our previous studies indicate that, unexpectedly, upon initial exposure to mammalian temperature, expression of *Leishmania* mitochondrial cytochrome c oxidase subunit IV (LmCOX4) is transiently down-regulated. Further studies from our laboratory indicate this transient loss of LmCOX4 is important for parasite viability at mammalian temperature. We hypothesize that LmCOX4 down-regulation in response to elevated temperature is caused via a transient decrease in mitochondrial-membrane potential that occurs following heat stress. Most eukaryotic proteins are imported into the mitochondrion via a positively charged mitochondrial targeting sequence (MTS) in their N-termini.

Mitochondrial membrane potential is typically required to drive the import of such proteins into the mitochondrion. Such import usually relies upon the opposite charges of the positively charged MTS and the negative charge within the matrix of metabolically active mitochondria. We propose that the transient loss of *Leishmania* mitochondrial membrane potential, following heat stress during entry into the mammalian host, results in a temporary decrease in MTS-mediated mitochondrial import. This may lead to an accumulation of mitochondria-destined proteins, such as LmCOX4, in the cytosol, followed by their immediate degradation to maintain homeostasis. Our current research focus is to confirm the importance of the positively charged amino acid residues in the MTS of LmCOX4 in mediating its mitochondrial localization and stability at 27 vs. 35 ^{o}C. We therefore sought to generate a mutant form of LmCOX4 whereby the four positively charged arginine residues of the WT LmCOX4 MTS were replaced by non-charged alanine residues. We used PCR followed by plasmid cloning to generate a *Leishmania* plasmid allowing for episome-based expression of this mutant in *Leishmania*. To track the expression and cellular localization of this mutant protein at 27 vs. 35 ^{o}C, we also incorporated an HA-epitope tag toward its C-terminus. A better understanding of *Leishmania* mitochondrial protein targeting throughout its parasitic lifecycle may identify new therapeutic targets to guide the development of better drugs against this disease.