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DISSERTATION DEFENSE SEMINAR OF

Kristin B. Linscott

"Discovering a Novel Antifungal Target in Downstream Sterol Biosynthesis using a Squalene Synthase Functional Motif"

Doctor of Philosophy Candidate

Thursday, April 27th, 2017
9:00 AM
115 College of Nursing

BIOGRAPHY

Kristin grew up in Cincinnati, Ohio. She obtained her B.A. in Biology and in Religious Studies from Agnes Scott College in Atlanta, Georgia in 2010. She entered the MD/PhD program at the University of Kentucky in 2010 and joined Joe Chappell's lab in 2012.

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ABSTRACT OF DISSERTATION

The sterol biosynthetic pathway is essential for growth of all eukaryotic cells and the main target of antifungal agents. The emergence of resistance to these antifungals in an already ill patient population indicates a need to identify novel therapeutic targets and develop drugs that have a broad spectrum of activity among pathogenic fungi and have minimal patient toxicity. Squalene synthase is the first committed step in the sterol pathway and has been studied intensively for development of antifungal agents. While the overall architecture of this enzyme is identical throughout eukaryotes, it was shown that plant and animal genes cannot complement a squalene synthase knockout mutation in yeast unless the carboxy-terminal domain is swapped for one of fungal origin. This implies that there is a component of the fungal carboxy-terminal domain that is responsible for the complementation phenotype and so is unique to the fungal kingdom of life.

To determine the role of the carboxy-terminal domain of squalene synthase in the sterol pathway, we used the yeast *Saccharomyces cerevisiae* with a squalene synthase knockout mutation and expressed squalene synthases originating from fungi, plants, and animals. In contrast to previous observations, all enzymes tested could partially complement the knockout mutation when the genes were weakly expressed. When induced, non-fungal squalene synthases could not complement the knockout mutation and instead led to the accumulation of carboxysterol intermediates, suggesting an interaction between squalene synthase and the downstream C4-sterol decarboxylase. Overexpression of a C4-sterol decarboxylase from any kingdom of life both decreased the accumulation of carboxysterol intermediates and allowed non-fungal squalene synthases to complement the *SQS* knockout mutation.

Using chimeric squalene synthases from each kingdom of life, the motif in the C-terminal domain of SQS responsible for preventing this toxicity was mapped to a kingdom-specific 26-amino acid hinge motif of fungal squalene synthase adjacent to the catalytic domain. Furthermore, over-expression of the carboxy-terminal domain alone containing a hinge motif from fungi, not from animals or plants, led to growth inhibition of wild-type yeast. Since this hinge region is unique to and highly conserved within each kingdom of life, this data provides evidence for the development of an antifungal therapeutic as well