

THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Jordan Craig Evans

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE

Department of Microbiology and Immunology

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A conserved cholesterol-dependent cytolysin motif directs prepore and pore formation and reveals a new class of pore-forming proteins.

COMMITTEE IN CHARGE: Rodney Tweten, PhD, Chair; Jimmy Ballard, PhD; Zachary Dalebroux, PhD, David Dyer, PhD; Augen Pioszak, PhD

ABSTRACT: The cholesterol-dependent cytolysins (CDCs) are bacterial, β -barrel, pore-forming toxins. The secreted soluble toxins bind to eukaryotic cells, then oligomerize into a prepore complex of 35-40 monomers, which then assembles and inserts a large β -barrel pore. The conversion of the prepore to the pore is associated with a 40Å vertical collapse relative to the membrane of the prepore complex. A central enigma of the CDCs is the structure(s) that senses the completion of the prepore and initiates its conversion to the pore. The structures of CDC monomers and pore complex revealed that a short loop comprised of a short α -helix and β -strand ($\alpha\beta$ loop) assume a helix-turn-helix (HTH) structure in the pore. We have identified a conserved motif which makes critical stabilizing interactions that are important in the function of the $\alpha\beta$ loop and show that the formation of the β -barrel pore is highly sensitive to the timing of the transition of the $\alpha\beta$ loop to the HTH. Furthermore, specific residues of the conserved motif directly stabilize the pore-forming domain of the soluble monomers. This motif but little else is conserved in a diverse family of nearly 300 uncharacterized proteins present in over 220

species that span at least 10 microbial and 2 eukaryotic phyla. Crystallography, single particle cryo-electron microscopy and biochemical studies of one of these CDC-like (CDCL) proteins from *Elizabethkingia anophelis*, a commensal of the malarial mosquito midgut, reveals a high degree of structural similarity to the CDC monomer structure and their large oligomeric pores. Hence, these studies reveal the critical nature of this motif in the CDC pore-forming mechanism, which has led to the discovery of a large and diverse family of CDC-related proteins.