QTHA QUEENSLAND TROPICAL HEALTH ALLIANCE

2013 Seminar Series

JAMES COOK UNIVERSITY AUSTRALIA

School of Public Health, Tropical Medicine and Rehabilitation Sciences presents

"Functional Roles of Conserved Structural Features in α-Defensins"

Dr K. Johan Rosengren, The University of Queensland

Friday 18th October 2013, 11am

JCU Cairns A21.001 Teaching Annex; Videolinked to JCU Townsville Bldg 9 Multi Purpose AV Services room 001 (DA 9001)

All welcome!

Mammalian defensins are cationic, cysteine-rich peptides with broad-spectrum antimicrobial activities. α-Defensins consist of 32-40 amino acids including six cysteines that form three disulfide bonds in a CysI-CysVI, CysII-CysIV, CysIII-CysV arrangement. In addition to the invariant cysteines a salt-bridge is highly conserved in the family. We have investigated the role of these features for the structure, function, stability and folding of a mouse Paneth cell α-defensin cryptdin-4 (Crp4), which is an important mediator of immunity in the small intestine. Mutations removing the disulfides result in a highly compromised structure, evident from NMR spectroscopy studies, but interestingly do not affect the antimicrobial activity. However, unstructured variants are highly susceptible to degradation by proteolytic enzymes. Removal of the salt-bridge by substitution of either Arg7 or Glu15 yields a similar result. Mutated variants retain antimicrobial activity, and although all variants adopt native-like folds the structures are more flexible, translating into a decrease in proteolytic stability. α-Defensins are produced as inactive propeptides with a prodomain rich in acidic residues followed by the highly basic defensin domain. Comparisons of refolding of the precursors proCrp4 and (E15D)-proCrp4 showed that even the most conservative salt-bridge disrupting replacement Glu15Asp impaired refolding of the peptide precursor in vitro, also highlighting a role for the salt-bridge in folding. NMR studies of proCrp4 reveal that the prodomain does not adopt an ordered structure, but is motionally restricted based on 15N relaxation measurements. Mutations to the acidic residues of the prodomain restores antimicrobial activity and results in it becoming fully flexible, suggesting the autoinhibition of the propeptide is due to a charge neutralizing interaction between the domains. We conclude that both the disulfide array and the salt-bridge are important for in vivo function by conferring proteolytic stability, including during the precursor processing. The salt bridge also facilitates adoption of the characteristic α-defensin fold. The studies on Crp4 highlight how protein features evolve not only to optimise a particular bioactivity, but also to ensure efficient production, sufficient stability, and minimal toxicity.

Johan Rosengren completed his PhD under Prof David Craik at the Institute for Molecular Bioscience, in 2003. He has held appointments at Institute for Molecular Bioscience, Linnaeus University and Uppsala University and since 2011 he is a group leader at the School of Biomedical Sciences, University of Queensland and is funded by an NHMRC Career Development Award. Dr Rosengren's research interests are in NMR spectroscopy and protein structure in particular peptides with unusual structural restraints. He was awarded the Sir Paul Callaghan Medal by the Australian & New Zeeland Society for Magnetic Resonance for contributions to the field of magnetic resonance.

www.qtha.org.au/news-events