Investigating the role of the N-terminal domain unique to eukaryotic class III Biotin Protein Ligases

L.M. Sternicki,[1] T.L. Pukala,[2] N.R. Pendini,[1,3] M.C. Wilce,[3] G.W. Booker,[1] K.L. Wegener[1] and S.W. Polyak,[1]
[1]School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia, [2]School of Physical Sciences, The University of Adelaide, Adelaide, SA 5005, Australia and [3]School of Biomedical Sciences, Monash University, Clayton, VIC 3168, Australia.

Biotin protein ligase (BPL) catalyses the covalent attachment of the biotin co-factor onto essential biotin-dependent enzymes involved in vital metabolic processes including lipogenesis and energy transduction. As a result BPL is essential for the viability of all forms of life, thereby making it an attractive target for the design of novel antimicrobials. Bacterial BPLs have been well characterized structurally, and have been selectively targeted over the human homologue for the development of antibacterials. Eukaryotic BPLs are now beginning to be targeted for the design of anti-fungal chemotherapeutics. However, the lack of structural data for eukaryotic BPLs impedes this process. In particular, structural information is required to allow the selective targeting of pathogenic eukaryotic BPLs over the human counterpart.

The structure of the BPL from the prototypical yeast *Saccharomyces cerevisiae* (*Sc*BPL) is currently under investigation as a model for other eukaryotic BPLs. Limited proteolysis studies revealed *Sc*BPL contains a 27 kDa N-terminal domain (NTD) that is separated from the 50 kDa C-terminal catalytic domain by a protease sensitive linker. This catalytic domain, responsible for the biotinylation activity, is homologous between all BPLs. The NTD, only found in eukaryotic class III BPLs, is involved in stabilising the interaction of the BPL with the substrate targeted for biotinylation. It is also hypothesized that this interaction helps select appropriate substrates through a proof-reading function. Cross-linking mass spectrometry and nuclear magnetic resonance are being used to map the interaction between *Sc*BPL and different substrate biotin domains. This will help elucidate the role of the NTD and how it helps to select between different substrates. Future studies include X-ray crystallography, which will help deliver structural information to inform anti-fungal drug design efforts.