

Potential Honours Projects in the Laboratory of Protein and DNA Interactions

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The basic Helix-Loop-Helix (bHLH) transcription factors have a basic DNA binding sequence adjacent to the helix-loop-helix dimerisation region, which allows homo- or hetero-dimerisation amongst bHLH proteins to form functional DNA binding complexes. The bHLH.PAS sub-group of bHLH proteins has the bHLH domain contiguous with a second domain, the PAS (Per-Arnt-Sim homology) domain, which regulates dimerisation [1] has a critical influence on DNA binding, contributing to affinity and protein-bound DNA conformation [2, 3], and defines partner choice [4] and target gene specificity [5]. The bHLH.PAS proteins are often coexpressed and dimerisation behaviour is tightly regulated. The various protein dimers in the family bind to closely related DNA sequences, but are functionally distinct, activating specific and discrete sets of target genes. The molecular properties that drive this specific dimer formation and DNA recognition, which are critical to maintain the precision of gene expression networks, are poorly understood.

Our work is aimed at understanding specificity of function of the bHLH and PAS domains at the molecular level, for protein-protein and protein-DNA interactions. Current projects focus on the Aryl hydrocarbon Receptor (AhR), the Hypoxia Inducible Factors, HIF-1 α and HIF-2 α and their common partner Aryl hydrocarbon Receptor Nuclear Translocator (Arnt).

Projects will be offered in two areas:

1. Characterising the role of key amino acids in the PAS domains of Arnt and AhR that control specific protein dimerization and DNA binding

Dimerization: We have developed a reverse bacterial two-hybrid system that selects for loss of protein interactions, and have identified mutations in the PAS domains of Arnt and AhR that disrupt heterodimerization [6]. The project will involve purification of wildtype and mutant Arnt or AhR PAS domains from *E. coli* expression systems, and biophysical and biochemical characterisation of the purified proteins. The aim is to understand how mutation of critical amino acids has an effect on dimerization through the PAS domain.

DNA binding: Both the basic DNA binding regions and the PAS domains are required for specific and high affinity DNA binding. The PAS domains also alter local DNA conformation in the bound complex, in a manner not well understood. We will investigate the role of specific amino acids in the bHLH and PAS domains in achieving high affinity DNA binding and altering DNA conformation. The project will involve site-directed mutagenesis, purification of wildtype and mutant bHLH.PAS dimers from *E. coli* expression systems, and biophysical and biochemical characterisation of the purified proteins.

Techniques include:

- common molecular biology techniques, such as DNA cloning, PCR and sequencing
- bacterial protein expression and purification
- SDS poly-acrylamide gel electrophoresis and western blotting
- biochemical and biophysical techniques such as circular dichroism, surface plasmon resonance, dynamic light scattering
- electrophoretic mobility shift assays (gel shifts)
- small angle X-ray scattering (SAXS) data collection at the Australian Synchrotron in collaboration with A/Prof Matthew Wilce

2. Identifying amino acids in the PAS domains of Arnt and the HIF- α s that regulate specific dimerization using the reverse bacterial two-hybrid system (RevB2H).

This system differs from standard two-hybrid systems, in that it *selects* for *loss* of interaction, since protein-protein interaction results in cell death. It was developed in collaboration with Dr Keith Shearwin's lab and is based on the coliphage 186 cl repressor protein. The RevB2H can easily detect loss of interaction between Arnt and AhR, and we are at present working to reconfigure the system to increase the sensitivity. The project will continue with the construction and assessment of changes to the RevB2H system to determine conditions where we can reliably detect the interaction between the HIF- α and Arnt PAS domains. This modified RevB2H will then be used to select mutations in these PAS domains that disrupt the interaction.

Techniques include:

- conceptual understanding of bacterial protein expression systems, and the effects modifying components
- two-hybrid protein-protein interaction assays in bacterial cultures
- common molecular biology techniques, such as DNA cloning, PCR and sequencing
- PCR-based random mutagenesis
- bacterial protein expression and purification
- SDS poly-acrylamide gel electrophoresis and western blotting
- electrophoretic mobility shift assays (gel shifts)

Related reading

1. Kewley, R.J., M.L. Whitelaw, and A. Chapman-Smith, *The mammalian basic helix-loop-helix/PAS family of transcriptional regulators*. Int J Biochem Cell Biol, 2004. **36**(2): p. 189-204.
2. Chapman-Smith, A., J.K. Lutwyche, and M.L. Whitelaw, *Contribution of the Per/Arnt/Sim (PAS) domains to DNA binding by the basic helix-loop-helix PAS transcriptional regulators*. J Biol Chem, 2004. **279**(7): p. 5353-62.
3. Chapman-Smith, A. and M.L. Whitelaw, *Novel DNA binding by a basic helix-loop-helix protein. The role of the dioxin receptor PAS domain*. J Biol Chem, 2006. **281**(18): p. 12535-45.
4. Pongratz, I., et al., *Role of the PAS domain in regulation of dimerization and DNA binding specificity of the dioxin receptor*. Mol Cell Biol, 1998. **18**(7): p. 4079-88.
5. Zelzer, E., P. Wappner, and B.Z. Shilo, *The PAS domain confers target gene specificity of Drosophila bHLH/PAS proteins*. Genes Dev, 1997. **11**(16): p. 2079-89.
6. Hao, N., et al., *Identification of residues in the N-terminal PAS domains important for dimerization of Arnt and AhR*. Nucleic Acids Res, 2011.