

Honours Projects 2012

Deciphering the mechanisms and functions of bHLH/PAS transcription factors in development, stress response and disease

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Basic-Helix-Loop-Helix/Per-Arnt-Sim (bHLH/PAS) transcription factors function broadly throughout life, playing key roles during embryonic development and then subsequently aiding homeostasis control mechanisms in the adult. Some family members are critical for sensing and responding to various forms environmental and/or physiological stress, such as toxin invasion, oxygen deficiency or hyperactivation of neurons. Defects in bHLH/PAS protein signalling can initiate or exacerbate several diseases, including cancer, stroke, mental disorders and early onset obesity. Members of the bHLH/PAS family which we study include:

1) Single Minded 1 (SIM1), which functions in hypothalamic neuron development and satiety signalling. Mutation in Sim1 results in hyperphagia (excessive eating syndrome) and early onset obesity

2) Single Minded 2 (SIM2) is essential for postnatal survival but its aberrant expression increases tumour development in a colon, pancreatic and prostate cancers.

3) NPAS4 is brain specific, induced by neuron activity, and aids inhibitory synapse formation. NPAS4 is neuroprotective and prevents excitotoxicity during neuron signalling.

4) Aryl Hydrocarbon (Dioxin) Receptor is critical for fetal liver angiogenesis, fertility and toxin degradation.

5) Hypoxia Inducible Factors (HIF-1a & HIF-2a) are essential for embryonic angiogenesis, glycolytic metabolism and adaption to low oxygen stress.

Our projects use gene knock out and point mutant knock-in mouse models, in addition to genetic manipulation of cultured cells, to elucidate normal and pathogenic functions of the bHLH/PAS proteins. We seek to understand the signalling mechanisms which control activity of the bHLH/PAS transcription factors and define their direct target genes. Mechanistically, we aim to discover the mechanisms by which they either activate or repress their target genes and hope to gain insights which will enable us to design novel strategies to treat disease.

Projects available for Honours in 2012

1. Sim1 is essential for terminal differentiation of distinct neuroendocrine secreting cells of the hypothalamus. Sim1 deficiency is linked to severe childhood obesity, resultant from defects in satiety signalling in the hypothalamus. In collaboration with a Cambridge University team studying the genetics of obesity, we are analysing several point mutants of the Sim1 gene that have been found in morbidly obese children. Our studies include creating Sim1 point mutant knock-in mice for physiological assays and analyses of protein malfunction in cultured cells and in cell free translation systems. We also aim to study the role of Sim1 in neuron maturation, using in vitro protocols for differentiating ES cells to neurons. The Honours project will involve analysis of wild type and obesity linked Sim1 missense variants in one or more of the above experimental systems.

2. Sim2 null mice die from an ill-defined breathing defect soon after birth. Recent evidence suggests that Sim2 is involved in terminal differentiation of certain of neuronal subtypes and development of tubules in the kidney. This project will aim to generate kidney specific Sim2 knock-out mice, which we predict will allow the mice to survive to adulthood and reveal homeostatic functions in the kidney. While the natural functions of Sim2 remain to be fully established, it is one of the most commonly dysregulated genes in prostate cancer and high Sim2 levels correlate with poor prognosis. We have found that Sim2 can function as a transcription activator of some genes, but a repressor of other genes. A second project will seek to understand these dual functions of Sim2 and discover novel Sim2 regulated genes which are involved in the cancer process.

3. NPAS4 is highly induced during epileptic seizure to dampen the hyperexcitation of neurons, and also induced during stroke to function as a neuroprotective agent. The role of NPAS4 in maintaining homeostasis between excitatory and inhibitory synapses has lead to the hypothesis that defects in NPAS4 may be a component of autism spectrum disorders. The project will explore the signalling pathways that induce NPAS4 and the downstream mechanisms by which NPAS4 functions in synapse development and neuroprotection.

The projects in our lab use many techniques of modern cell and molecular biology, eg point mutant knock-in and knock-out gene targeting in mice, generation of stable cells lines which exhibit inducible expression of cDNAs for ectopic expression of proteins, inducible expression of shRNA for gene knockdown, RNA analysis by real time PCR and microarray, isolation of protein complexes by Ab bound resins and mass spec identification of proteins residing in the complexes, ChIP assay to determine DNA sites bound by bHLH/PAS proteins (including ChIP-sequencing for global analysis of DNA binding sites), bacterial expression and purification of proteins, dissection and culture of primary neurons from rodent embryos, FACS sorting of cell populations, analysis of point mutant proteins in cell based and in vitro biochemical assays.

Please contact Murray Whitelaw for any further details