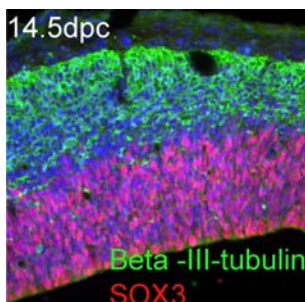


Laboratory of Developmental Genetics (A/Prof. Paul Thomas)

Development of the human brain into a complex structure containing one hundred billion cells is a fascinating process that requires thousands of genes. Recent advances in genomics technologies have facilitated rapid identification of causative genes for relatively common neurological disorders such as **mental retardation** and **epilepsy**. However, *understanding the function of these genes in the context of healthy and diseased brains remains a major challenge.*

Project 1: Investigating the molecular function of SOX3 in the brain and testis stem cells. Our laboratory has shown that duplication and mutation of the transcription factor gene *SOX3* is associated with the mental retardation syndrome X-linked Hypopituitarism (XH; Solomon et al 2004, Laumonier et al 2005). *Sox3* is expressed in the stem/progenitor cells of the developing brain and is a key regulator of neural differentiation. We have generated several mouse models with altered dosage of *Sox3*. These mice have abnormalities in brain development that resemble patients with XH (Rizzoti et al, 2004). However, the molecular and cellular basis of these defects is not understood. More recently, we have also shown that *Sox3* is expressed in the stem/progenitor cells of the testis from which the sperm are generated. Importantly, our unpublished work and that of others (Raverot et al, 2005) has shown that *Sox3* is essential for sperm production. However, the function of *Sox3* in testis stem/progenitor cells is not known.



Dual Fluorescence immunohistochemistry of 14.5 dpc mouse embryonic brain section showing expression of SOX3 in the Neural Stem cells and not in the committed neurons, which express Beta-III-tubulin.

The overall **aim** of this project is to understand how **SOX3 functions in neural and testis development at the molecular and cellular level**. The specific aim is **to identify molecular pathways that are regulated by Sox3 in the developing brain and testis**. Our approach will employ two cutting-edge molecular techniques: microarray analysis and next generation sequencing of Chromatin-immunoprecipitation libraries (ChIP-seq). Microarray analysis generates a snap-shot of gene expression that includes *all gene expression changes* associated with the mutation of a particular factor (in this case *Sox3*). In contrast, ChIP-seq will identify genes that are *directly* regulated by *Sox3*. Therefore, the use of both approaches will allow us to identify both direct and indirect targets of SOX3 and delineate genetic pathways that are downstream of this transcription factor. Comparison of wild type and *Sox3* null brain and testis tissue will be performed across several developmental time points. Differentially expressed genes (ie putative SOX3 target genes) will be validated using Realtime-PCR and in situ hybridisation analysis of wild type and knock-out *Sox3* embryos. To identify *direct* SOX3 targets, bioinformatics analysis of promoter sequences from putative target genes will be used to identify SOX3

consensus binding sites. These will be validated for SOX3 protein binding in vivo using Chromatin immunoprecipitation (ChIP) assays.

Project 2: Functional analysis of Protocadherin 19, a novel gene for Epilepsy and Intellectual Disability.

In collaboration with Prof. Jozef Gecz (SA pathology/University of Adelaide), we are investigating the function of Protocadherin 19 (*PCDH19*) which Prof Gecz's group recently identified as the causative gene for Epilepsy and Mental Retardation limited to Females (EFMR; Dibbins et al 2008). EFMR is an intriguing X-linked disorder because carrier females are affected while hemizygous males are spared. The molecular and cellular basis for this highly unusual mode of inheritance is not understood, although it is likely to involve the ability of PCDH19 to mediate homotypic cell-cell interactions. To investigate this hypothesis, we will compare the cell adhesion and synapse formation properties of wild type and EFMR-associated mutant PCDH19 proteins using cell culture techniques. In addition, we will utilise our *Pcdh19* KO mouse model to investigate the molecular, cellular and behavioural impact of *Pcdh19* mutation on brain development, structure and neuronal connectivity.



In situ hybridisation analysis of an embryonic mouse brain showing Pcdh19 expression (purple stain) in the cortex and hippocampus.

References:

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