

**Molecular Ageing Laboratory, Freemasons Foundation Centre for Men's Health
Discipline of Medicine, Hanson Institute Building, Frome Road
PhD Project Opportunities**

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Dr Grant Buchanan & Dr Eleanor Need

Primary Research Interest - The androgen receptor

Androgens such as testosterone are essential in men for development, growth and continued health as an adult. However, as men age the capacity of androgens to maintain male sexual function and prostate health, muscle strength and mental capacity declines, and is associated with unacceptable levels of morbidity and mortality. Androgens mainly mediate their effects by acting through the androgen receptor (AR), a ligand dependent transcription factor that activates or suppresses a large number of target genes. Nevertheless, we do not yet fully understand how the AR works functionally at a genomic level to affect these changes in a temporal and spatial manner in different tissues, and as yet have few effective ways of intervening with androgen/AR signaling as therapeutic options. We are utilizing a broad array of contemporary molecular, genomic and computational approaches (i.e. ChIP-Chip, ChIP-sequencing, cloning, microarray analysis, drug screening, confocal microscopy, cellular signaling) to (i) define the determinants of AR function in target tissues, (ii) investigate the role of androgens in male and female physiology, and in ageing and disease (i.e. breast and prostate cancers), and (iii) undertake drug screening and development for novel ways of modulating AR function. We have a range of projects suitable for both Honours and PhD students.

Title and short description of projects offered for 2009 (maximum 150 words each):

Project 1. Tissue specific determinants of AR transcriptional activity

The AR functions by interacting in a temporal and sequential manner with an array of specialized coregulator proteins, resulting in productive activation and/or repression of transcription throughout the genome. Many AR coregulators have been identified, yet we don't yet know the expression profile that will define tissue-specific AR function. This project involves the elucidation of the expression of many AR coregulators in an array of androgen target tissues (prostate, breast, muscle, bone, brain) and the sequential interactions that the AR may make with them during transcriptional activation. This project will involve the several molecular techniques including the isolation of RNA from microdissected fresh frozen tissue sections, PCR array platform technology, chromatin immunoprecipitation and molecular cloning. Understanding the actions of the AR in different tissues will allow us to develop novel tissue specific modulators of AR signaling.

Project 2. Molecular interaction of the AR with cochaperones

We have previously reported an interaction between the AR and the cochaperones α SGT and FKBP52. These two proteins act in an opposing manner to retain AR in the cytoplasm or promote nuclear transfer respectively. This represents an unappreciated level of control that may be utilized in the treatment of AR associated diseases. This project is aimed at providing insights into how FKBP52, α SGT and the cochaperone system in general modulate AR function. Techniques utilized in this project include mutagenesis, GST-pulldown, yeast and mammalian 2-hybrid interaction assays, and kinetic analysis of the AR-cochaperone interactions in prostate, breast and muscle cells utilising confocal microscopy, fluorescence resonance energy transfer (FRET), and photobleaching. We are currently developing knockout mice and undertaking drug screening for small molecules that inhibit these interactions.

Project 3. Defining bioactive androgen levels in ageing men

Current androgen measures focus on the gross levels of serum in men. However, there is evidence that the metabolic conversion of androgens within target cells will be more informative to measure androgen action. It was recently demonstrated that polymorphisms in genes involved in androgen inactivation via glucuronidation are associated with physiological parameters associated with androgen status (i.e. bone mineral density, BMI, body fat), indicating that these genes are pivotal in defining the actual amount of androgen in circulation that can be regarded as bioactive. The aim of this project is to analyse 1200 samples from the Florey Adelaide Male Aging Study (FAMAS), a longitudinal cohort study investigating the reproductive, physical and psychological health of males as they age, for single nucleotide polymorphisms pivotal to androgen status. This project will involve PCR, SNP analysis and statistical techniques to compare androgen status with a panel of over 40 independent biological measures of health and wellbeing in the FAMAS cohort.

Project 4. Structural and functional determinants of nuclear receptor signaling

Transcriptional regulation by nuclear receptors (e.g. AR, estrogen receptor, progesterone receptor) stems from recruitment of cofactors that mediate nuclear-cytoplasmic movement, response element recognition, chromatin remodeling, and ultimately recruitment of basal transcription factors. As nuclear receptors share highly

conserved domains for hormone and DNA binding, the evolution of different enhancer/promoter and cell-specific regulation has necessitated divergence of their amino-terminal domains (NTD) for distinct regulatory roles. This project aims to define what elements in the NTD and/or interacting proteins determine specificity, function and diversity of nuclear receptor signaling, and will involve cellular and molecular biology, chromatin immunoprecipitation, microarray and genomic technology, confocal microscopy, cloning and protein interaction assays. This will define how these proteins regulate aspects of ageing and diseases such as breast and prostate cancer.

Key references :

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2. Buchanan G, Yang M, Cheong A, Harris JM, Irvine RA, Lambert PF, Moore NL, Raynor M, Neufing PJ, Coetzee GA, Tilley WD (2004) Structural and functional consequences of glutamine tract variation in the androgen receptor. *Human Molecular Genetics* 13:1677-1692
3. Han G, Buchanan G, Ittmann M, Harris JM, Yu X, DeMayo FJ, Tilley W, Greenberg NM (2005) Mutation of the androgen receptor causes oncogenic transformation of the prostate. *Proc Natl Acad Sci U S A* 102:1151-1156
4. Scher HI, Buchanan G, Gerald W, Butler LM, Tilley WD (2004) Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. *Endocr Relat Cancer* 11:459-476
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