**Student Opportunities**

The Neurogenetics Research Unit and Institute for Neuromuscular Research are affiliated with the Discipline of Paediatrics and Child Health, Faculty of Medicine, The University of Sydney.

The Unit offers opportunities for University enrolled students to conduct research projects over the summer university vacation. Scholarship or stipends enhancement are available for candidates with an excellent undergraduate record and interested students are encouraged to apply via the University of Sydney' s Faculty of Medicine's highly successful Summer Vacation Research Scholarship program. Information about the program, application forms and guidelines are available via the Faculty's website. All applications should be sent to:

Joanne Elliot
Research Support Officer
Faculty of Medicine
Edward Ford Building (A27)
University of Sydney NSW 2006

The Neurogenetics Research Unit and Institute for Neuromuscular Research are happy to offer the 2005/2006 summer projects listed below.

Preference will be given to students who are considering a later Honours project in this field.

**Project 1**

**Project Title**

Detecting expression of α-actinins in skeletal muscles of α-actinin-3 knockout mouse

**Brief outline of project**

The α-actinins are a highly conserved family of actin-binding proteins. There are four isoforms in human and mouse. α-Actinin-1 and α-actinin-4 are expressed widely in tissues with highest level in larynx (-1) and caudate nucleus (-4), and lower levels in skeletal muscles. Up to now, the functions of α-actinin-1 and -4 in skeletal muscles are remained largely unknown. α-Actinin-2 and -3 are highly expressed in muscles, with α-actinin-2 becoming the predominant isoform in heart muscle and oxidative skeletal muscle fibers, while α-actinin-3 expression is restricted mainly to fast glycolytic skeletal muscle fibers.

Due to homozygosity for a polymorphic stop codon (R577X) in the α-actinin-3 gene (*ACTN*3), about 18% of the general population (~ one billion people worldwide) lack α-actinin-3 in their skeletal muscles. We and others have demonstrated an association between *ACTN3* genotype and muscle strength and response to training. Our laboratory has generated an *Actn3* knockout mouse (*Actn*$3^{-/-}$) to further study its roles in skeletal muscle. There is an up-regulation of α-actinin-2 protein expression in glycolytic fibres in *Actn*$3^{-/-}$ muscles, demonstrating that α-actinin-2 is compensating the loss of α-actinin-3.

The aim of the project is to quantitatively detect transcript and protein levels of α-actinin-1, -2, and -4 in *Actn*$3^{-/-}$ mouse muscles compared to age-matched littermate controls. RNA will be isolated from wild-type and knockout mouse muscles. cDNA will be synthesized, and RT-PCR

and Real-time PCR will be performed to detect transcript levels. Protein expressions will be detected by immunohistochemistry and Western-blot.

**Project 2**

**Project Title**

The role of annexins I and II in muscular dystrophy and membrane repair

**Brief Outline of Project**

Many forms of muscular dystrophy are associated with a structural fragility of the muscle membrane, whereby membrane damage exceeds the ability of muscle to repair itself, resulting in the progressive degeneration of muscle fibres. We are studying a new form of muscular dystrophy, caused by mutations in the gene dysferlin. Rather than having a structural role, dysferlin has recently been shown to play a role in repairing the small sites of membrane damage caused through normal physical activity. In dysferlin patients, the structure of the membrane is normal, but membrane repair is impaired.

Our project seeks to more clearly define the role of dysferlin in skeletal muscle, and to study other proteins that interact with dysferlin and/or contribute to the muscle repair pathway. There are many patients with muscular dystrophy, in whom the genetic cause is unknown. We believe that some of these patients may have defects in other proteins that are also involved in the muscle repair process and may interact with dysferlin.

This summer research project will involved screening muscle biopsy samples from a group of muscular dystrophy patients for abnormalities in two proteins that interact with dysferlin, and play a role in the membrane repair process; annexin I and annexin II. We will use immunohistochemistry using fluorescent antibodies to stain muscle sections from the patients, and confirm any abnormalities by Western blot. In addition, we will damage cultured muscle cells, and examine the recruitment and association of the annexins with dysferlin to the site of membrane repair.

**Project 3**

**Project Title:**

The role of a novel form of clathrin in muscular dystrophy and membrane repair

**Brief outline of project**

Endocytosis of cell surface receptors and signalling molecules usually occurs via clathrin-coated pits, which form tiny vesicles that bud from the cell membrane and are trafficked to intracellular destinations. Recently, a new form of clathrin (CHC22) has been identified, that has a high degree of sequence similarity to the ubiquitously expressed clathrin isoform (CHC17), yet its expression pattern and function appear to be distinct from those of well-characterized clathrin-coated vesicles.

This new form of clathrin (CHC22) is found abundantly in muscle, with high levels of expression at the neuromuscular and myotendinous junctions, suggesting a role at sarcolemmal

contacts with extracellular matrix. CHC22 expression is also increased in regenerating muscle fibers with the same time course as embryonic myosin, indicating a role in muscle repair.

This research project aims to characterise the expression of this new clathrin isoform (CHC22) in human muscle samples and examine its expression in a group of patients with different subtypes of muscular dystrophy, whose muscle has been subjected to damage and repair. We will use fluorescent antibodies to define the localisation of CHC22, and determine what changes occur as part of the dystrophic process. In addition we examine the role of CHC22 in muscle membrane repair through analysis of cultured muscle fibres damaged using glass beads.

**Project 4**

**Project title:**

**Mutation detection in the muscle α-skeletal actin gene in patients with severe congenital onset myopathy and other related disorders**

**Brief outline of project**

Mutations in the muscle α-skeletal actin gene (*ACTA1*) were first identified in the inherited muscle disorder nemaline myopathy (NM). NM is characterised by muscle weakness and the presence of rod bodies within the muscle fibers of affected patients. Clinically, presentation of NM ranges from severe-congenital (lethal), to milder childhood- or adult-onset forms. Mutations in *ACTA1* are one of the most common causes of NM, accounting for ~20% of cases. Approximately 50% of these patients have severe-lethal congenital NM and died within the first year of life.

Recently, mutations in *ACTA1* have been identified in patients with a range of different pathological phenotypes including actinopathy (actin accumulations), central core disease and congenital fiber type disproportion. Mutations in *ACTA1* most commonly result in a severe lethal congenital-onset myopathy. We hypothesise that mutations in *ACTA1* may result in severe congenital onset disease in patients with non-specific “myopathic” changes on muscle biopsy.

This summer research project will involve screening of undiagnosed patients with severe congenital myopathy for mutations in the *ACTA1* gene. Upon identification of a mutation, the parents of the affected patients will also be screened to determine the mode of inheritance. We have a subset of NM patients in whom a diagnosis has not been made due to insufficient quantities of tissue to isolate DNA. We have recently developed a new technique to isolate DNA from very small amounts of tissue and even from paraffin embedded muscle. This newly developed technique will be employed to isolate DNA from these patients to screen for mutations in *ACTA1*.