

**Dr Bryan Haines** Leucine rich repeat transmembrane proteins- regulators of cell signalling during development

My current research involves studying the function and mechanism of action of leucine rich repeat transmembrane proteins, focusing on their regulation and action during muscle formation and neural development *in vitro* and *in vivo*.

This work initiated with the identification of Nlrr1, a protein containing 12 extracellular leucine rich repeats, an immunoglobulin domain, a fibronectin domain and a short intracellular tail, as a gene with unique expression in a subset of the earliest embryonic myoblasts (muscle cells). This gene is a member of a highly conserved three gene family whose members show distinct, highly regulated expression patterns during development particularly in the nervous system. Many multimember gene families exist that are highly conserved in sequence and secondary structure (Fig.1).

Our research has shown that one of these families, the Flrts, is a member of the Fgf signalling pathway. Flrts can interact with Fgf receptors (Fgfrs), be phosphorylated in response to Fgf signalling and effect signalling pathways downstream of the Fgfr. This, combined with the restricted expression pattern of Flrt3 during muscle and neural development, suggests that Flrts are important regulators of Fgf signalling during development (Fig.1).

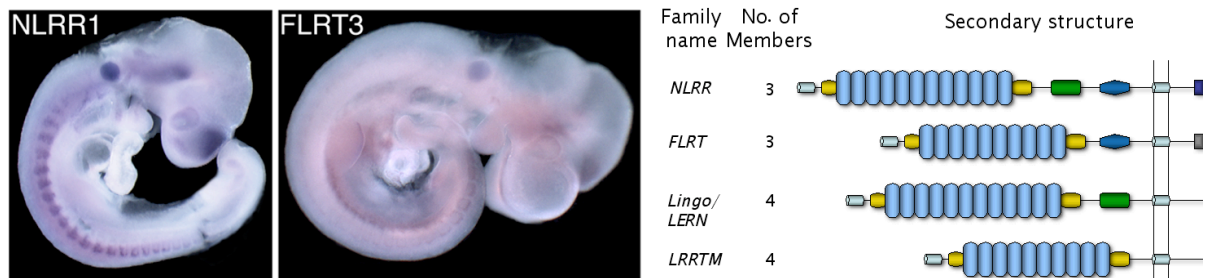


Fig.1 a) Expression of Nlrr1 and Flrt3 transcripts in mouse embryos. b) Schematic diagram of the protein secondary structures of four leucine rich repeat transmembrane protein families.

These data suggest that leucine rich repeat transmembrane proteins are novel regulators of cell signalling by interaction with cell surface receptors. Our laboratory is interested in understanding how the specific expression of these genes is regulated during development. Furthermore, we aim to determine the cellular function of these proteins and how they interact with and regulate cell signalling pathways.

The fact that these molecules contain an extracellular component and can interact with and affect signalling from receptors has led to investigations into possible therapeutic applications. Understanding the mechanism of action and function of these molecules may allow us to modulate the signalling pathways that they regulate which may be valuable in altering cellular function in processes like neural regeneration, muscle repair and stem cell differentiation.

### Projects

1. Studying Nlrr1 function in muscle differentiation. We will use lentiviral vectors to analyse the effect of over- and under-expression of Nlrr1 on the differentiation of the C2C12 skeletal muscle cell line.

2. We have identified Flrt3 as a gene up-regulated by the signalling molecule retinoic acid in stem cell differentiation. We will use overexpression and siRNA knockdown to analyse the role of Flrt3 in stem cells?

3. Analysis of Flrt3 dependent regulation of Fgf signalling. We will use Fgf signalling assays to analyse the effect of Flrt3 and various Flrt3 mutants on the ability to activate FGF signalling in cell culture and embryos.
4. Analysis of Nlrr1 intracellular trafficking. Nlrr1 is rapidly internalised from the cell surface into intracellular vesicles mediated by protein-protein interaction domains in the cytoplasmic tail. We will determine the sub-cellular localisation of Nlrr1 and identify the mechanism that drives this localisation.
5. Do other leucine rich repeat transmembrane proteins interact with cell surface receptors? We will analyse the interaction of leucine rich repeat transmembrane proteins with various membrane localised signalling receptors in cell culture.