

essential for correct fibre-type specification and emergent stem cell function. These data plug a significant gap in the natural history of muscular dystrophy and will be invaluable in establishing an earlier diagnosis for DMD/LGMD and in designing earlier treatment protocols, leading to better clinical outcome for these patients.

**P29** Poster  
**Preventing dystroglycan phosphorylation as a route to therapy in DMD**

G. Miller<sup>1</sup>, C. Moore<sup>1</sup>, L. Lipscomb<sup>1</sup>, A. Mitchell<sup>1</sup>, S. Winder<sup>1</sup>.  
<sup>1</sup>Department of Biomedical Science, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN, UK

Dystroglycan is a central component of the dystrophin glycoprotein complex (DGC) of striated muscle, mediating essential connections between the extracellular matrix and the actin cytoskeleton of muscles. No muscle disease involving mutations in dystroglycan itself have so far been described, however mutations in genes that post-translationally modify dystroglycan give rise to a class of diseases known as the dystroglycanopathies. All dystroglycanopathies currently characterised arise from proteins involved in the glycosylation of  $\alpha$ -dystroglycan, a post-translational modification that is essential for dystroglycan function in binding to laminin in the ECM. However  $\beta$ -dystroglycan is also post-translationally modified, by glycosylation and by phosphorylation. *In vitro* analyses have demonstrated that phosphorylation of  $\beta$ -dystroglycan on tyrosine targets  $\beta$ -dystroglycan for degradation and this could be part of the mechanism that underlies loss of dystroglycan and the whole DGC in conditions such as DMD. Restoration of dystroglycan in DMD could prevent loss of DGC components and allow other compensatory proteins such as utrophin and plectin to bind to and stabilise the complex, thus ameliorating the DMD phenotype. We are using dystroglycan-null zebrafish and a new knock-in dystroglycan mutant in mouse to test the restorative effect of preventing dystroglycan phosphorylation on a key regulatory tyrosine. The homozygote dystroglycan knock-in mice are phenotypically normal with no obvious signs of muscle pathology. The knock-in has been crossed with *mdx* and analyses are in progress to determine any affect on pathology.

**P30** Poster  
**The integrin effectors talin 1 and 2 are essential for skeletal muscle development and integrity**

F.J. Conti<sup>1</sup>, S.J. Monkley<sup>2</sup>, M.R. Wood<sup>1</sup>, D.R. Critchley<sup>2</sup>, U. Müller<sup>1</sup>.  
<sup>1</sup>The Scripps Research Institute, Department of Cell Biology and Institute of Childhood and Neglected Diseases, La Jolla, CA, USA;  
<sup>2</sup>University of Leicester, Department of Biochemistry, Leicester, UK

Integrins are essential for development and maintenance of skeletal muscle. Mutations in  $\alpha$ 7-integrin cause congenital myopathy in humans, and integrin ablation causes muscular dystrophy and defects in myofibre development in mice. Talin 1 and talin 2 mediate a connection between integrins, actin and signaling proteins, and in muscle, they concentrate at the myotendinous junction (MTJ). We have used genetically modified mice to identify the specific functions of the talin genes in muscle development. Ablation of either talin 1 or talin 2 leads to a myopathy characterized by the detachment of myofilaments from the sarcolemma at the MTJ. Defects are more pronounced in talin 2-null mice, which present with centrally nucleated fibers, and appear not to be caused by an increase in sarcolemmal damage as observed for example in *mdx* mice. Interestingly, the phenotype of talin2-null mice resembles that of  $\alpha$ 7-integrin-null mice, which also present centrally nucleated fibres with only a moderate increase in serum creatine kinase. Ablation of both talin isoforms causes severe developmental defects, with impaired myoblast fusion and myofibrillogenesis. Together, the data reveal an essential function for talin 1 and talin 2 in muscle development and integrity. The similarity of the phenotype of talin 2- and  $\alpha$ 7-integrin-null mice

suggests that mutations in talin 2 could lead to a congenital myopathy similar to that caused by  $\alpha$ 7-integrin mutations, and suggests that this gene should be considered as a candidate in patients without an identified genetic defect.

**P31** Poster  
**Myofibrillar myopathy caused by a mutation in the mouse Myh4 gene**

R. Kuraparti<sup>1</sup>, C. McKenna<sup>2</sup>, J. Baker<sup>1</sup>, S. Laval<sup>2</sup>, H. Lochmüller<sup>2</sup>, G. Blanco<sup>1</sup>. <sup>1</sup>MRC Mammalian Genetic Unit, Harwell OX11 0RD, UK; <sup>2</sup>Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne NE1 3BZ, UK

*Ariel* is a mouse mutant selected from a cohort of ENU mutagenized mice owing to the onset of hind-limb paralysis at post-natal day 12 in homozygotes. Histopathology showed the presence of large protein aggregates and myofibrillar degeneration in skeletal muscle. The mutation, identified by positional cloning, causes a L349G change within the motor domain of MYH4 (MyHC IIb), the most abundant skeletal muscle myosin in the adult. Biochemical analysis of the aggregates indicated the presence of proteins found to be mutated in myofibrillar myopathies (FilaminC, ZASP and A/B-Crystallin) and ultrastructural analysis showed predominantly disorganized filamentous material. Transfections *in vitro* using GFP tagged versions of the proteins, showed that over-expression of MYH4(L349G) in a non-sarcomeric cell line produced twice as many cells with intracellular aggregates compared to MYH4, suggesting that the pathogenic mechanism involves a change in the folding of the motor domain. In fully differentiated myotubes, overexpressed MYH4 is incorporated into the A-band effectively, but MYH4L349G forms aggregates. Intriguingly, the purely recessive nature of the mutation indicates that dimers of wild type and mutant MYH4 assemble into thick filaments and that this is sufficient to prevent the onset of molecular pathogenesis. This mouse model represents a useful resource to elucidate the mechanisms of myofibrillar degeneration and to study the prevention of protein aggregates formation.

**P32** Poster  
**Investigating novel mutant mouse models of motor neuron disease**

P. McGoldrick<sup>1</sup>, J. Dick<sup>1</sup>, T. Ricketts<sup>1</sup>, A. Acevedo-Aroza<sup>1</sup>, E. Fisher<sup>1</sup>, L. Greensmith<sup>1</sup>. <sup>1</sup>MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

Mutations in Tar DNA binding protein (TDP-43) have been identified as causes of both sporadic and familial motor neuron disease. TDP-43 is a ubiquitous, multi-domain and multifunctional nuclear protein and is crucially involved in gene expression, development and RNA metabolism. It remains unclear as to why mutations in the protein selectively cause motor neuron death. However since it has been identified as a cause of motor neuron disease, interest has heightened as to whether aberrant RNA metabolism could lead to selective motor neurone degeneration.

We have access to two lines of mutant TDP-43 mice (K160R, Q101STOP), produced by ethylnitrosourea (ENU) mutagenesis. We will initially investigate these mice with a combination of *in vitro* and *in vivo* techniques. Firstly, embryonic motor neuron cultures will be prepared and stress granule formation will be assessed. Stress granules are discrete cytoplasmic granules, containing untranslated mRNAs, dynamically formed following cellular insult as an anti-apoptotic mechanism. We will determine whether the TDP-43 mutations affect stress granule formation and cell viability. Secondly, muscle strength and number of functional motor units of the Tibialis Anterior and Extensor Digitalis Longum will be assessed in anaesthetised adult animals to determine whether the TDP-43 mutations cause motor neuron degeneration and muscle wasting.