The cytoskeletal tropomyosin, Tm5NM1, associates with T-tubules in skeletal muscle and has a role in skeletal muscle contractile function

Anthony Kee[1], Nicole Vlahovich[1], Gordon Lynch[2], Bernadette Vhrovski[3], Frances Lemckert[3], Robert Parton[4], Peter Gunning[3], Edna Hardeman[1]. [1]Muscle Development Unit, Children's Medical Research Institute, Westmead, Australia, [2]Department of Physiology, University of Melbourne, Australia, [3]Oncology Research Unit, Children's Hospital at Westmead, Australia and [4]Institute for Molecular Biosciences, University of Queensland, Brisbane, Australia.

There are a large number of tropomyosin (Tm) isoforms associated with actin microfilaments in eukaryotic cells. Cytoskeletal Tms confer distinct properties on actin filaments, defining functional domains within cells. The three muscle-specific Tm isoforms associate with sarcomeric actins to form the thin filaments of the sarcomere. Recently, we identified a novel Tm/actin filament system adjacent to the Z-line that contains a nonmuscle, cytoskeletal Tm isoform, Tm5NM1. Further immunofluorescent studies with Tm isoform-specific antibodies show that this Tm closely associates with the T-tubule system (co-localises with the dihydropyridine receptor). To understand the function of the Tm5NM1-defined filament system in skeletal muscle we created a knockout (KO) mouse eliminating Tm5NM1 expression. Analysis of this mouse has revealed no overt muscle phenotype (whole body and forearm weakness, running gait or histopathology). To assess the functional effects of the absence of Tm5NM1, *in vitro* whole muscle contractile measurements were performed on the extensor digitorum longus (EDL) and soleus muscles of KO and WT mice. Absolute and specific tetanic force, twitch contraction and relaxation times, and fatigue resistance were not different between WT and KO mice for either muscle. However, in the EDL there was a leftward shift in the frequency-force relationship in the KO mice such that the force at low stimulation frequencies (1-50Hz) was significantly greater ($P < 0.05$). This effect was not due to fibre-type shift as myosin heavy-chain composition (by gel electrophoresis) was similar in KO and WT mice. These findings are consistent with an alteration in the KO mouse of Ca^{2+} release/uptake or Ca^{2+} sensitivity. Taken together these results suggest that Tm5NM1 is necessary to maintain normal muscle contraction function. Whether this is due to altered T-tubule function is under investigation.