## Characteristics of MMP-9 protein in rat skeletal muscle

*X. Ren,[1] G.D. Lamb[2] and R.M. Murphy,[1] [1]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC 3086, Australia and [2]School of Life Sciences La Trobe University, Melbourne, VIC 3086, Australia.*

In skeletal muscle, matrix metalloproteinases (MMPs) are zinc and calcium-dependent enzymes which are implicated in maintaining the integrity and homeostasis of muscle extracellular matrix (ECM) (Vu & Werb, 2000). MMP-9 (also referred to as gelatinase B) levels are elevated in muscle wasting diseases such as mdx mice (Li *et al.*, 2009). However, the characteristics of MMP-9 in healthy resting muscle are still not understood. In this study, the absolute amounts of MMP 9 and its diffusibility and fibre type dependency were examined in resting rat skeletal muscles.

*Extensor digitorum longus* (EDL) and *soleus* (SOL) muscles were excised from male Sprague Dawley rats (4-6 months old) which had been sacrificed by lethal overdose of isoflurane in accordance with the La Trobe University Ethics Committee. Portions of muscles were homogenized in $K^+$ based physiological buffer. To determine absolute amounts of MMP-9 in skeletal muscle, known amounts of latent and active MMP-9 pure protein were run alongside rat muscle homogenates on Western blots. Type I and type II muscle fibres were dissected from SOL and EDL muscles respectively. To clarify MMP-9 localization inside skeletal muscle, some fibre segments were skinned by mechanically peeling back the sarcolemma and washed in physiological intracellular solution for a set time. Additional skinned fibres were similarly washed and then also treated with Triton X-100 to solubilise membranes. The intact muscle fibres, skinned fibres, and sets of skinned fibres with wash solutions and Triton-wash solutions were analysed by Western blotting.

The relative amounts of MMP-9 in SOL and EDL muscle homogenates were $1.0 \pm 0.13$ and $0.3 \pm 0.04$ (mean ± SD, n=5, $P<0.001$). Preliminary data indicated the absolute amounts of MMP-9 in SOL and EDL to be of the order of ~1 and ~0.12 μmol/kg wet weight muscle, respectively (n=1 each). Most of the MMP-9 was seemingly localized intracellularly, as the amount of MMP-9 detected in skinned fibres was similar to that in intact fibres (n=7 each). No MMP-9 was detected in wash solutions of skinned SOL and EDL muscle fibres (n=7 each), even after treatment with Triton X-100, indicating that the MMP-9 in skeletal muscle is neither freely diffusible in the cytosol nor membrane bound. This work highlights that MMP-9 localization in resting healthy skeletal muscle is quite different from that generally assumed. Future work will ascertain these properties in diseased muscle.

Li H, Mittal A, Makonchuk DY, Bhatnagar S, Kumar A. (2009). Matrix metalloproteinase-9 inhibition ameliorates pathogenesis and improves skeletal muscle regeneration in muscular dystrophy. *Hum Mol Genet* **18**, 2584-98.

Vu, TH & Werb Z. (2000). Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* **14**, 2123-33.