Differential expression of heat shock proteins in proliferating and differentiating skeletal muscle cells

S.S. Thakur, N.J. Cranna, K. Swiderski, J.G. Ryall and G.S. Lynch, Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia..

Skeletal muscle has a remarkable regenerative capacity due to the presence of a population of adult stem cells termed satellite cells (SCs). SCs normally exist in a quiescent state within a distinct anatomical niche situated between the sarcolemma of the muscle fibre and the basal lamina, and express the paired homeobox protein Pax7 (Holterman & Rudnicki, 2005). In response to trauma or injury, SCs are activated, re-enter the cell cycle and become specified to the myogenic lineage (*via* expression of MyoD). They then proliferate rapidly, differentiate (marked by expression of myogenin) and ultimately undergo fusion and maturation (Yin *et al.*, 2013). During myogenesis there are dramatic changes in cell size, shape, metabolism, and the ability to migrate which can alter proteostasis and cause cellular stress.

The heat shock proteins (HSPs) are a family of highly conserved molecular chaperones that maintain proteostasis by regulating protein biogenesis, facilitating transport of polypeptides across intracellular membranes, preventing stress-mediated protein aggregation/unfolding, refolding denatured proteins and targeting aberrantly folded proteins for degradation (Clerico *et al.*, 2015). Given the dynamic demands of myogenic cells during the shift from proliferation to differentiation, HSPs may play a crucial role in maintaining proteostasis during this process. Interestingly, whole transcriptome analyses of quiescent and proliferating SCs have revealed differential expression of genes encoding various HSPs including HSP25, HSP40, HSP60, HSP70, HSP90 and HSP110 (Ryall *et al.*, 2015). However, the roles of specific HSPs in mediating myogenesis have yet to be determined. Therefore, the aim of the present study was to characterize the expression of HSPs in myogenic cells during proliferation and differentiation.

We utilised the immortal murine C2C12 cell line to characterize the expression of HSPs in proliferating myoblasts and differentiating myotubes. Cells from this cell line proliferate rapidly in conditions conducive for growth and undergo differentiation and fusion into myotubes upon removal of growth factors. Whole cell lysates prepared from proliferating C2C12 cells [50% and 100% confluent myoblasts (50%M and 100%M)] and C2C12 cells differentiated for 1-4 days (D1-D4), were subject to sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western immunoblotting to determine the protein expression of HSP25, HSP40, HSP60, HSP70, HSP90 and HSP110. Levels of HSP25, HSP40, HSP90 and HSP110 were highly elevated in 50% confluent proliferating myoblasts and declined 2- to 7-fold in 100%M ($P < 0.05$). The levels were similar to 100%M at all timepoints following differentiation, but, for HSP25 there was a further 2-fold decline at D4 relative to D1 and a trend towards lower levels relative to D2 ($P = 0.064$). HSP70 protein expression was low in proliferating C2C12 cells, increased 1.5-fold at onset of differentiation ($P < 0.05$), peaked at D2 ($P < 0.05$), and then returned to proliferation levels. HSP60 levels were also low in 50%M and increased progressively, peaking at D1 (3-fold higher relative to 50%M, $P < 0.05$), with levels declining progressively and returning to proliferation levels at D3 and D4.

These findings indicate that the roles of HSPs in regulating proliferation and differentiation are likely complex. Future studies will manipulate HSP expression to further investigate their role in myogenesis. Improved understanding of the roles of HSPs in myogenesis will aid development of therapeutic approaches to enhance muscle regeneration following injury and in muscle pathologies.

Clerico EM, Tilitsky JM, Meng W & Gierasch LM. (2015). *J Molec Biol* **427**, 1575-88.
Holterman CE & Rudnicki MA. (2005). *Semin Cell Dev Biol* **16**, 575-84.
Ryall JG, Dell'Orso S, Derfoul A, Juan A, Zare H, Feng X, Clermont D, Koulnis M, Gutierrez-Cruz G, Fulco M, Sartorelli V. (2015). *Cell Stem Cell* **16**, 171-83
Yin H, Price F & Rudnicki MA. (2013). *Physiol Rev* **93**, 23-67.

Supported by the National Health and Medical Research Council (Project grant APP1009114) and the Muscular Dystrophy Association (USA; Project grant 255153).