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PERSPECTIVES

Calcium entry in skeletal muscle

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It was established decades ago that excitation–contraction (EC) coupling relies on the depolarization-dependent release of stored calcium for skeletal muscle contraction and since that time considerable effort by many groups have detailed the molecular mechanism of calcium release underlying EC coupling (Edman & Grieve, 1964; Caputo & Gimenez, 1967; Luttgau & Oetliker, 1968). More recently, growing evidence suggests that alternative calcium signalling pathways exist in skeletal muscles that rely on calcium entry (Hopf *et al.* 1996; Kurebayashi & Ogawa, 2001). In this symposium, R. T. Dirksen provided an important overview of calcium entry in skeletal muscle (Dirksen, 2009). Two forms of Ca^{2+} entry have been characterized in skeletal muscle fibres: excitation-coupled calcium entry (ECCE) and store-operated calcium entry (SOCE) (Williams & Rosenberg, 2002; Cherednichenko *et al.* 2004). ECCE is activated in muscle cells following prolonged membrane depolarization that is independent of the calcium stores. ECCE requires functioning L-type calcium channel (LTCC) and ryanodine receptor (RYR1) channels, but the molecular identity of the pore remains undefined although it is likely to involve the LTCC (Hurne *et al.* 2005; Bannister *et al.* 2008, 2009). ECCE is altered in malignant hyperthermia (MH) and may contribute to the disordered calcium signalling found in muscle fibres of MH patients (Cherednichenko *et al.* 2008). SOCE on the other hand requires depletion of the internal stores and has been best characterized in non-excitable cells (Putney, 1986, 2007). SOCE in skeletal muscle was described some time ago in myotubes (Hopf *et al.* 1996), but it was not until the discovery of two important molecules, stromal interaction molecule 1 (STIM1) and Orai1 in non-excitable cells, that the importance of SOCE was recognized in muscle (Stiber *et al.* 2008*a*). SOCE is likely to be important for sustaining calcium stores to prevent muscle weakness and contribute calcium needed to modulate muscle-specific gene expression. Key questions raised during this symposium include the identity of the molecular components of these pathways, the interrelationship of ECCE, SOCE and EC coupling, and finally, the relevance of these pathways to muscle performance and disease.

STIM1 is a single-pass, transmembrane phosphoprotein that was initially cloned from stromal cells involved in pre-B cell differentiation, and has been implicated as a tumour suppressor for rhabdoid tumours and rhabdomyosarcoma cell lines (Oritani & Kincade, 1996; Manji *et al.* 2000). STIM1 contains several domains that include an EF-hand domain, a sterile-α-motif (SAM) domain at the N-terminus, and two coiled-coil regions and a proline-rich region at the C-terminus (Putney, 2007). The EF-hand domain of STIM1 has a high affinity for calcium (200–600 μM range) and is located in the lumen of the endoplasmic reticulum (ER), where it is thought to sense changes in calcium store content (Stathopulos *et al.* 2006). The coiled-coil domains are located in the cytosolic C-terminus and are important in the oligomerization and punctae formation described for STIM1 and consequent activation of store-operated calcium (SOC) channels (Liou *et al.* 2007). Orai1 was identified simultaneously by high throughput screening and is the mutated gene responsible for a familial form of severe combined immunodeficiency (SCID). The Orai channel family consists of three family members that form a highly selective calcium channel by tetramerization. STIM1 and Orai1 are both expressed in skeletal muscle, and mice lacking STIM1 and Orai1 display reduced muscle mass. An important aspect of future work will need to focus on why these mice with defective SOCE manifest reduced muscle mass and early lethality.

Three basic models for SOCE have developed in recent years: two of these involve conformational coupling between the Transient Receptor Potential channels (TRPC) and either the inositol trisphosphate receptor (IP3R) and/or RYR1 and a third that involves physical interaction of STIM1 and Orai1 (Kiselyov *et al.* 2000; Lee *et al.* 2006). Dr Dirksen presented data developed in his lab that tested each of these models as the mechanism for SOCE. He determined that TRPC3 channels do not contribute to SOCE in myotubes (Lyfenko & Dirksen, 2008). Here, TRPC3 channel fragments that interrupt the TRPC3/RYR1 interaction did not prevent SOCE. Whether additional TRPC channels function as SOC channels in muscle remains to be determined, particularly since TRPC1 is expressed in muscle as well (Stiber *et al.* 2008*b*). Data were also presented that indicated SOCE in myotubes did not require calcium release from the RYR1 channels as $RYR1^{-/-}$ myotubes displayed intact SOCE. It is clear from this work that SOCE is not mediated through TRPC3/RYR1 conformational coupling, but it remains to be determined if STIM1 in cooperation with TRPC channels provides calcium entry in muscle (Liao *et al.* 2007).

The importance of STIM1 in mediating SOCE in muscle fibres is clear from studies of STIM1 knockout mice where loss of STIM1 in muscle leads to a profound reduction in SOCE. Muscles from mice lacking a functional STIM1 manifest skeletal muscle weakness and neonatal lethality (Stiber *et al.* 2008*a*). We observed both reduced force from tetanic contractions and loss of force when the muscle was stimulated under fatiguing conditions. Several possible mechanisms may account for the reduced force generation and weakness observed in STIM1-deficient mice (Allen *et al.* 2008). For example, muscles from STIM1-deficient mice display reduced expression of contractile proteins and the calcium pump SERCA1. It is possible that a reduction in the number of sarcomeres may account for the reduced force generation. Likewise the reduced SERCA1 expression may lead to an inability to generate force as seen in muscles of patients with Brody's disease and mice lacking SERCA1a (Pan *et al.* 2003). On the other hand, proliferation of abnormal mitochondria in muscles lacking STIM1 may limit the available energy supply needed to maintain contractile force. Finally, given the ubiquitous expression of STIM1 it is possible that the muscle weakness seen in STIM1-null

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animals results from defective SOCE in cells other than muscle, e.g. cells of the vessel wall (Abdullaev *et al.* 2008). Therefore strategies designed to delete the STIM1 gene in skeletal muscle will probably help determine how much of the pathology observed in these mice results from the loss of muscle SOCE.

What are the SOC channels governing calcium entry in muscle? The best evidence to date suggests that Orai1 is the muscle SOC channel in part because patients carrying a loss of function mutation in the Orai1 gene exhibit a skeletal myopathy (Feske *et al.* 2006). While the precise location of Orai1 within skeletal muscle membranes remains to be defined (e.g. T-tubules or sarcolemma), Orai1 can be detected in skeletal muscle with Orai1-specific antibodies (Gwack *et al.* 2008). The presence of Orai1 in the muscle membranes is supported by imaging studies measuring SOCE from isolated flexor digitorum brevis (FDB) fibres of wild-type mice. Here, skinned muscle fibres with sealed T-tubules were imaged with a novel technique (SEER) and revealed the rapid onset of SOCE following store depletion (Launikonis & Ríos, 2007). Four Orai1 subunits assemble into tetramers of the channel making a dominant negative approach useful for loss of function studies. In fact, expression of the Orai E108Q mutant channel in cultured myotubes and isolated FDB fibres by the Dirksen group effectively blocked SOCE in muscle (Lyfenko & Dirksen, 2008). Given the obvious impairment of SOCE in muscle fibres expressing Orai1 E108Q mutants, it will be important to determine if mice carrying the Orai1 E108Q mutant channel only in skeletal muscle develop muscle growth defects that phenocopy those defects observed in STIM1- and Orai-null mice (Stiber *et al.* 2008*a*; Vig *et al.* 2008).

Accumulating evidence suggests that calcium entry does influence calcium transients in skeletal muscle through distinct mechanisms involving either membrane depolarization (ECCE) or internal calcium store depletion (SOCE). Recent findings implicate these calcium entry pathways in distinct skeletal muscle diseases. Further work will be needed to identify all of the molecular components of these complexes. But it is likely that a better understanding of these pathways will provide novel therapeutic targets for muscle atrophy and disease.

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