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PERSPECTIVES

Junctin – the quiet achiever

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Recent advances in understanding skeletal and cardiac muscle function have evolved with recognition of the active role played by the intracellular sarcoplasmic reticulum (SR) Ca^{2+} store in contraction. The key proteins in this store are the Ca^{2+} binding protein calsequestrin (CSQ), the ryanodine receptor (RyR) Ca^{2+} release channel and triadin and junctin (Beard *et al.* 2004). The CSQ–triadin–junctin–RyR complex (Fig. 1) in the SR lumen forms a 'Ca^{2+} transduction machine' that is central to EC coupling and to normal muscle development. Other proteins in the lumen of the SR, including the histidine rich calcium binding protein (HRC) (Suk *et al.* 1999), JP-45 (Anderson *et al.* 2003) and SRP-27 (Bleunven *et al.* 2008), must also contribute to control of SR intraluminal Ca^{2+} load, but the precise nature of their role remains undetermined. JP-45 in particular is ideally placed to communicate store load to the excitation–contraction (EC) coupling process as it binds to both CSQ and the dihydropyridine receptor (DHPR) in the surface/transverse tubule membrane. The importance of the luminal proteins has been underlined by the recent discovery that changes in Ca^{2+} signalling due to mutations in CSQ or to lack of its expression can result in sudden cardiac death (Viatchenko-Karpinski *et al.* 2004). Furthermore, studies in animal models show that changes in CSQ, junctin, triadin and HRC expression can lead to defective Ca^{2+} signalling. The review by Pritchard & Kranius (2009) examines the roles of junctin and HRC in Ca^{2+} cycling and their potential significance in heart failure. This commentary focuses on protein-protein interactions between CSQ, triadin, junctin and RyR proteins that may underlie their roles in Ca^{2+} cycling.

A brief background of the RyR, CSQ, HRC, junctin and triadin

Three RyR genes in mammals are the skeletal *RyR1*, the cardiac *RyR2* and *RyR3* first identified in brain. RyR1 in skeletal muscle has cleverly recruited the DHPR as a surface membrane voltage sensor and CSQ as an SR Ca^{2+} sensor as reviewed elsewhere (Dulhunty *et al.* 2002). Two CSQ genes encode type 1 CSQ (*CSQ1*), expressed in fast and slow twitch skeletal muscle, and type 2 CSQ (*CSQ2*) expressed in slow-twitch skeletal and cardiac muscle. The properties of isolated CSQ1 and CSQ2 differ and their opposite effects on RyR1 and RyR2 channels respectively in lipid bilayers suggest that CSQ1 may help conserve SR Ca^{2+} in skeletal muscle, but facilitate emptying of the SR in the heart (Wei *et al.* 2009*b*). HRC has been studied in less detail, however; although it has more acidic clusters than CSQ and a higher Ca^{2+} binding capacity, it constitutes only 1% of SR protein (Hofmann *et al.* 1989; Arvanitis *et al.* 2007). Like CSQ, HRC is associated with the RyR protein complex through triadin (Lee *et al.* 2001; Sacchetto *et al.* 2001; Arvanitis *et al.* 2007) and binds directly to sarco(endo)plasmic reticulum Ca^{2+}-ATPase type 2 (SERCA2) (Arvanitis *et al.* 2007).

Junctin and triadin are found in a range of tissues and thus may have a ubiquitous role in Ca^{2+} signalling. One isoform of junctin is expressed in all tissues and is a splice variant of the aspartate β-hydroxylase gene. Several isoforms of triadin are splice variants of one gene; the 94 kDa isoform binds to RyR1 in skeletal muscle and the 32 kDa isoform binds to RyR2 in the heart. Junctin and triadin both bind to CSQ and RyRs (Jones *et al.* 1995; Goonasekera *et al.* 2007) and are thought to anchor CSQ close to the RyR. Both proteins contain a short cytoplasmic tail, one trans-SR membrane domain and a

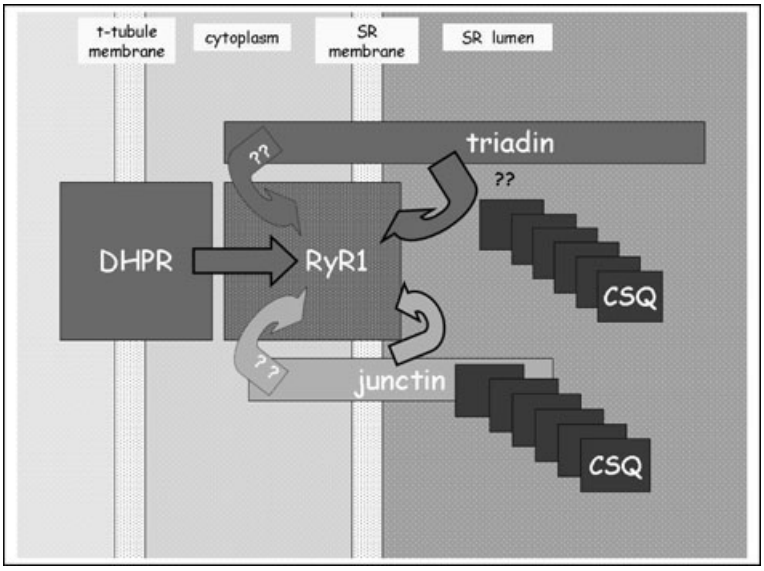

Figure 1. Illustration of the possible interactions between triadin, junctin, CSQ1 and RyR1 in the lumen of the SR
The question marks near CSQ and triadin indicate the uncertainty about simultaneous binding of CSQ1 and RyR1 to triadin. Question marks on the cytoplasmic side indicate the unknown nature of the cytoplasmic interaction between triadin and RyR1 and whether or not there is a cytoplasmic interaction between junctin and RyR1.

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long luminal tail in the SR. These structural similarities suggested that the proteins may be functionally redundant. However, recent evidence indicates that their functions are in fact vastly different and that junctin plays a distinct and important role in Ca^{2+} homeostasis (see below).

The distinct role of skeletal junctin

The activity of RyR1 and RyR2 channels is increased after addition of either junctin and/or triadin (purified from skeletal muscle) to the luminal side of purified RyR1 channels in lipid bilayers (Goonasekera *et al.* 2007; Wei *et al.* 2009*a*). However, the skeletal proteins behave quite differently in the presence of CSQ1 (Beard *et al.* 2008; Wei *et al.* 2009*a*). The junctin–RyR1 complex is inhibited when CSQ1 is added to the luminal solution (containing 1 mM Ca^{2+}), while the triadin–RyR1 complex is unaffected by luminal CSQ1 addition (Wei *et al.* 2009*a*). Equivalent studies of HRC interactions with junctin or triadin and effects on RyR activity have yet to be performed. Different roles of junctin and triadin are also seen in intact skeletal myotubes where disruption of the triadin–RyR1 association depresses EC coupling, while junctin (which remains bound to RyR1) is unable to support EC coupling (Goonasekera *et al.* 2007).

These separate functions of junctin and triadin are reflected in changes in Ca^{2+} signalling in skeletal C2C12 myotubes after triadin and/or junctin knockdown, which indicate that junctin plays a major role in SR Ca^{2+} load, while triadin assists in depolarization-induced Ca^{2+} release (Wang *et al.* 2009). A role of triadin in skeletal EC coupling is similarly observed when over-expression of triadin reduces depolarisation-dependent Ca^{2+} transients (Fodor *et al.* 2008). Conversely, pan-triadin knockout does not appear to affect EC coupling (Shen *et al.* 2007), but numerous compensatory changes could mask effects of triadin depletion (Goonasekera *et al.* 2007).

The fact that junctin is more involved than triadin in mediating Ca^{2+}-dependent signals between CSQ1 and RyR1 (Goonasekera *et al.* 2007; Wei *et al.* 2009*a*; Wang *et al.* 2009) may be explained if CSQ1 and RyR1 bind to a single KEKE motif between residues 200 and 232 in the C-terminal tail of triadin (Kobayashi *et al.* 2000; Lee *et al.* 2004). Although this site was determined for CSQ2 binding to cardiac triadin, and skeletal triadin binding to RyR1, the same sites are present in each of the isoforms of the proteins and are likely to have the same function. It is yet to be determined whether CSQ can also bind to the extended C tail of skeletal triadin which contains five additional KEKE motifs and several other clusters of acidic residues. If the binding site is unique, either CSQ cannot regulate the RyR through triadin or dynamic swapping between triadin binding to CSQ and the RyR would be required for CSQ to have an influence on RyR. In contrast to triadin, at least two of the KEKE-like motifs in the C-tail of junctin are involved in its binding to CSQ, so that it may bind to CSQ and the RyR simultaneously and transmit signals between the proteins.

Junctin in the heart

As with the skeletal proteins, junctin and triadin each increase the activity of RyR2 channels in the absence of CSQ2 (Gyorke *et al.* 2004). The *in vivo* roles of junctin and triadin in the heart have been explored in knockdown, knockout and over-expression models. The results are difficult to interpret in terms of protein–protein interactions because the expression of other proteins and the regulatory links between CSQ2 and RyR2 can be affected even after transient knockdown (Wang *et al.* 2009). However the results again support a hypothesis that junctin and triadin have separate roles. Junctin knockout and knockdown lead to an enhancement of Ca^{2+} release (Fan *et al.* 2008), suggesting that either junctin or junctin–CSQ2 inhibit RyR2. Similarly, the reduced Ca^{2+} release in response to β-adrenergic stimulation with triadin over-expression (Kirchhefer *et al.* 2007), suggests an inhibitory role for triadin. In contrast, enhanced EC coupling (Terentyev *et al.* 2005) and caffeine-induced Ca^{2+} release (Kirchhefer *et al.* 2007) with triadin over-expression indicate an excitatory action of triadin. The role of triadin in skeletal muscle is further addressed in this issue in the speakers review by Marty *et al.* (2009) and the perspective by Allen (2009).

Cytoplasmic RyR–junctin interactions

An unexplored possibility is that the cytoplasmic N-terminal tail of junctin interacts with the cytoplasmic domain of RyR1 or RyR2 to allow junctin to regulate cytoplasmic functions of the RyR. A curious observation is that the cytoplasmic tail of triadin can modulate RyR1 activity by binding to the cytoplasmic domain of RyR1 (Ohkura *et al.* 1998; Groh *et al.* 1999). It is possible that this cytoplasmic interaction is involved in the influence of triadin on EC coupling.

In conclusion, despite the numerous gaps that remain in the CSQ–junctin–RyR story, the unfolding picture is one of a complex integration of signals arising from these proteins that regulates Ca^{2+} homeostasis in skeletal muscle and the heart. Yet to be addressed are the functional consequences of molecular interactions between HRC, triadin, junctin, CSQ and the RyR and the SR Ca^{2+} pump, which suggest that the protein may facilitate communication between the Ca^{2+} release and uptake function in the SR (Arvanitis *et al.* 2007).

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