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REVIEW

# Regulation by scaffolding proteins of canonical transient receptor potential channels in striated muscle

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**Abstract** Recent studies proposed a pivotal role of TRPC channels, in particular TRPC1, in the striated muscle tissue and in the development of calcium mishandling observed in dystrophin-deficient skeletal and cardiac muscle cells (Vandebrouck et al. in J Cell Biol 158:1089–1096, 2002; Williams and Allen in Am J Physiol Heart Circ Physiol 292:H846–H855, 2007; Stiber et al. in Mol Cell Biol 28:2637–2647, 2008). In skeletal muscle, TRPCs are proposed to function in a costameric macromolecular complex (Vandebrouck et al. in FASEB J 21:608–617, 2007; Gervasio et al. in J Cell Sci 121:2246–2255, 2008) in which scaffolding proteins and dystrophin are central components maintaining normal calcium entry (Stiber et al. in Mol Cell Biol 28:2637–2647, 2008; Sabourin et al. in J Biol Chem 284:36248–61, 2009). In this review, we shall summarize the roles played by scaffolding proteins in regulating the calcium entry through TRPC channels of skeletal muscle cells and the implications in muscle physiopathology. Interactions of TRPC1 with caveolin-3, Homer-1 and α-syntrophin will be addressed and these complexes will be compared with signalplex in other systems. The mechanosensitive function of scaffolding proteins will be discussed as well as interactions with TRPV2 channels regarding to calcium mishandling in Duchenne dystrophy.

**Keywords** TRPC channels · TRPV2 channels · Scaffolding proteins · Mechanosensitivity · Muscle · DMD

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## Introduction

The membrane family of transient receptor potential canonical (TRPC) channels are closely related channels of TRP, the transduction channel in *Drosophila* photoreceptor cells. A spontaneous mutation in the TRP locus leads to a decrease in calcium influx and photoreceptor cells depolarization. All TRP channels display intracellular $NH_2$ and COOH termini, as well as six putative transmembrane domains and an ion-selectivity P loop involved in the building of the pore. TRP proteins are thought to assemble as homo- or hetero-tetramers around a central ion-selectivity filter and gate to form cation selective channels, consistent with the structure of voltage-dependent $K^+$ channels. Based on amino acid homologies, the mammalian TRP channel superfamily can be divided into seven families including the TRPC subfamily. Of the TRP families, the *Drosophila* TRP channel exhibits the greatest homology to the TRPCs, the first member of which, TRPC1, was cloned in 1995 (Wes et al. 1995). The TRPC subfamily consists of seven channels (TRPC1–TRPC7). TRPC channels are constituted of six predicted transmembrane domains (TM1–TM6), including a putative region between TM5 and TM6 involved in the constitution of the pore. The cytosolic N-terminus is composed of three to four ankyrin repeats, a coiled-coil region involved in homo- or hetero-tetramerization of channels, and a putative caveolin binding domain. The cytoplasmic C-terminus region contains the TRP signature (EWKFAR), a highly conserved proline-riched motif (LPXPFXXXPSPK), the Calmodulin/$IP_3$ Receptor Binding (CIRB) region, and a coiled-coil region. Ankyrin-repeats that are homologue to the domains present in the N-terminal part of the scaffolding proteins ankyrins are also involved in homo- or hetero-tetramerization of the TRP channels (Schindl and Romanin 2007).

Mammalian TRPCs build non-selective $Ca^{2+}$-permeable cation channels that demonstrate variable $Ca^{2+}/Na^{+}$ permeability ratio, and can assemble both as homo-tetramers or hetero-tetramers complexes (Venkatachalam and Montell 2007). In many vertebrate cells, TRPCs contribute, in a widespread number of non-excitable and excitable cells, to non-voltage dependent cation entries. These cation channels were proposed to support both a calcium entry through the PhosphoLipase C (PLC)-activated channels, referred to Receptor-Operated $Ca^{2+}$ Entry (ROCE) and a calcium entry activated by depletion of internal $Ca^{2+}$ stores following calcium release, referred to Store-Operated Calcium Entry (SOCE). Depending on the signalling repertory of a given cell and on the complement TRPC channels co-expressed, the various stimuli activating TRPC may lead to complex changes in membrane-potential, various mode of calcium entry, slow sustained increase in intracellular calcium concentration and numerous $Ca^{2+}$-induced responses. Many cells and tissues coexpress several members of the seven TRPCs, which can result in various channels with significantly different current properties and the functional interactions of TRPCs with recently described proteins Orai1 and STIM1 (STromal Interaction Molecule) provide an additional possibility to form a store-operated complex resulting in SOCE (Nilius 2007) and also ROCE (Liao et al. 2009). Several studies also proposed that TRPC1, and also TRPC4 can constitute a store-operated channel when associated with STIM1 (Huang et al. 2006; Lopez et al. 2006; Yuan et al. 2007; Worley et al. 2007), a protein of the membrane reticulum, and another plasma membrane channel Orai1 (Liao et al. 2007, 2008, 2009; Cheng et al. 2008), while other reports proposed that Orai1 and STIM1 constitute the core component of SOCs independently of TRPCs (Lyfenko and Dirksen 2008; DeHaven et al. 2009).

Given the variety of cellular responses linked to the activation of these channels, many physiological or physiopathological functions have been shown to depend on TRPCs (Abramowitz and Birnbaumer 2009). Recent studies proposed a pivotal role of TRPC channels, in particular TRPC1, in the striated muscle tissue and in the development of calcium mishandling observed in dystrophin-deficient skeletal and cardiac muscle cells (Vandebrouck et al. 2002; Williams and Allen 2007; Stiber et al. 2008). In skeletal muscle, TRPCs are proposed to function in a costameric macromolecular complex (Vandebrouck et al. 2007; Gervasio et al. 2008) in which scaffolding proteins and dystrophin are central components maintaining normal calcium entry (Stiber et al. 2008; Sabourin et al. 2009). In this review, we shall summarize the roles played by scaffolding proteins in regulating the calcium entry through TRPC channels of skeletal muscle cells and the implications in muscle physiopathology.

## TRP in macromolecular signalling complex

The first evidence for a TRP-containing macromolecular complex was demonstrated in *Drosophila* photoreceptor, where the Inactivation No Afterpotential D (INAD) provides the central molecular scaffold (Montell 2005). INAD, which contains five PDZ (PSD95-Disc large-Zona occludens protein) domains, has the capacity of binding multiple proteins for constituting a signalling complex named "signalplex" consisting in INAD, TRP, PLC (NORPA) and PKC (Wang and Montell 2007). Other proteins interact with the core components of this signalplex such as TRPL, rhodopsin, calmodulin, FKBP59 and the nonconventional myosin III (NINAC), which is involved in efficiently terminating the photo-response. The clustering of signalling proteins with the scaffolding protein INAD into a single complex is thought to be necessary for the proper localization of the components in the light-sensitive structures, the rhabdomeres, and to provide efficient transmission and termination of the signals, which is extremely rapid in the case of phototransduction. The interaction of the TRP/TRPL signalplex with the cortical actin cytoskeleton is mediated by INAD and the non-conventional myosin NINAC, which also controls the gating of the channels (Wes et al. 1999) and regulates the translocation of TRPL (Bahner et al. 2002). Several evidences showed that mammalian TRP channels, similarly to *Drosophila* TRP, are linked to the membrane-associated protein and to scaffolding proteins, including the channels in a macromolecular complex. The recruitment of a macromolecular signalling complex was shown to be necessary for the proper activity, localization of mammalian TRPC channels and the constitution of "calcium microdomain" (Ambudkar et al. 2006). Within the cytosolic region of these channels, several domains, which could mediate multiple interactions with a macromolecular complex, have been identified, including coiled-coil domains and PDZ-binding domains (Venkatachalam and Montell 2007).

## TRPC channels and macromolecular complexes

TRPC channels function within calcium signalling complexes that are assembled in plasma membrane microdomains. Assembly and retention of calcium signalling proteins in the complexes is achieved by interaction of the proteins with scaffolds. TRPC4 and TRPC5 have an extended C-terminal tail containing a PDZ-binding motif. Through this VTTRL motif, TRPC4 and TRPC5 binds to the PDZ domain of the scaffolding protein $Na^{+}/H^{+}$ Exchanger Regulatory Factor, NHERF (Tang et al. 2000) which links the channels to PLC$\beta$ (in this regard, the NHERF is a functional homolog of INAD in mammalian

cells) and to the human orthologue EBP50 (Mery et al. 2002). The interaction of TRPC4 via the last three amino acids (TRL) with the cytoskeleton through adaptor protein EBP50 have been shown to control the localization of the channel at the plasma membrane, and the Ezrin/Radixin/Moesin (ERM) binding domain of EBP50 seems to play a role in protein insertion of the channel into the plasma membrane (Mery et al. 2002). NHERF, PLC$\beta$ and TRPC4 (Tang et al. 2000), TRPC1, TRPC5, and PLC$\beta$1 (Strubing et al. 2001) were co-immunoprecipitated suggesting the channels are part of a macromolecular signalling complex, in which the scaffolding protein could play a pivotal role. Indeed, the PDZ domain of NHERF has been shown to bind several PLC$\beta$ isoforms (Tang et al. 2000; Reczek and Bretscher 2001). Moreover, NHERF interacts with the proteins of the ERM family through its C-terminal 30 amino-acids and may link the TRPC4/5 and PLC$\beta$ to the cytoskeleton. Furthermore, the double-PDZ-domain-containing protein NHERF can multimerize, allowing to cluster additional protein such as G-protein-coupled $\beta$2-adrenergic receptor. In endothelial cells, TRPC4 was also found to interact with protein 4.1, which is linked to spectrin, anchoring the TRPC4-complex to actin cytoskeleton (Cioffi et al. 2005). Disruption of the interaction between protein 4.1 and spectrin was shown to inhibit SOCE (Cioffi et al. 2005).

Another type of interaction in cholesterol enriched membrane microdomains, named caveolae, is involving the scaffolding protein caveolin. Caveolin-1 contains two membrane anchoring domains, which interacts with many signalling proteins. Co-immunoprecipitations and co-localizations with caveolins have been demonstrated for TRPC1 and TRPC3 (Lockwich et al. 2000, 2001; Brazer et al. 2003). Brazer and collaborators have shown the binding of caveolin-1 to N- and C-termini of TRPC1 and suggested a role for caveolin-1 interaction in membrane translocation of TRPC1 (Brazer et al. 2003). Caveolin-1 binding usually occurs through consensus motifs containing aromatic rich regions with a specific spacing, and all TRPC members conserve a similar motif at the N-terminal part close to the first transmembrane domain TM1. Expression of N-terminal-truncated TRPC1 or a dominant-negative caveolin mutant (lacking its protein scaffolding and membrane anchoring domains) inhibited the targeting of TRPC1 to the plasma membrane (Brazer et al. 2003) and exerted a dominant negative effect on endogenous SOCE. Using mice deficient in caveolin-1, Murata and collaborators demonstrated that the scaffolding protein governs, in endothelial cells, the localization and the interactions of TRPC1 and TRPC4 and its requirement for calcium entry (Murata et al. 2007). More generally, caveolae are thought to organize a calcium signalling complex containing TRPC1 anchored to caveolin-1 where it associates with signalling proteins including $IP_3R$, calmodulin, plasma membrane calcium pump (PMCA), and $G_{\alpha q/11}$.(Lockwich et al. 2000; Ambudkar et al. 2006). Similarly, TRPC3 is assembled in macromolecular complex containing $G_{\alpha q/11}$, PLC$\beta$, $IP_3R$, SERCA, Ezrin and caveolin-1 (Lockwich et al. 2001).

The scaffolding protein Homer contains a highly conserved EVH1 (Enabled/Vasodilatator-stimulated phosphoprotein Homology) domain (Xiao et al. 1998) which could interact with TRPC1. A proline-rich motif (PPXXF and PPXF) flanked by phenylalanine, downstream of the TRP domain (EWKFAR) is conserved in all members of the TRPC subfamily and is thought to mediate the interaction with Homer in TRPC1 (Beneken et al. 2000). Homer is able to multimerize through coiled-coil and leucine zipper domains to generate a lattice that is used to assemble and regulate calcium signalling complexes. TRPC1 is associated with Homer in brain protein extracts (Yuan et al. 2003), and localizes in a complex containing $IP_3R$ and group 1 metabotropic Glutamate Receptors (mGluR1). Yuan and collaborators found a second binding site of Homer in the N-terminus of TRPC1 (LPSSP) which does not include a phenylalanine, unlike the previous Homer binding sites (PPXXF). Homer expression was found to be crucial for mediating a TRPC1-$IP_3R$ complex necessary for responses to G-protein-coupled receptor activation (Yuan et al. 2003). On the contrary, expression of TRPC1 with mutation of the proline-rich motif disrupted Homer binding and resulted, interestingly, in a constitutive activity of channels with reduced agonist regulation. Homer was thus proposed to permit the assembly of an agonist responsive TRPC1–$IP_3R$ complex. Furthermore, measurements of $Ca^{2+}$ influx in acini from Homer1$^{-/-}$ mice confirmed, in native cells, the role of Homer in assembling SOC (Yuan et al. 2003). Deletion of Homer1 also resulted in the increase of spontaneous $Ca^{2+}$ entry in pancreatic acinar cells. TRPC3 was also found in complex with IP3R and Homer1b, which is suggested to mediate both gating and trafficking of TRPC3 by IP3R (Kim et al. 2006).

## TRPC1 and macromolecular complexes in skeletal muscle

TRPC1 was the first member of the TRPC family to be cloned (Wes et al. 1995) and the first shown to form a calcium-permeable cation channel (Zitt et al. 1996). The function of TRPC1 is generally thought to be as an element of non-voltage-gated $Ca^{2+}$ and $Na^{+}$ influx pathways. The first evidence of a functional involvement of TRPC channels in skeletal muscle was provided by the study of Vandebrouck and collaborators (Vandebrouck et al. 2002). Using the cell-attached configuration of the patch-clamp

technique, voltage-independent calcium channels were recorded at the sarcolemma of mouse fibres. These channels with a low conductance of 8 pS (with 110 mM $CaCl_2$ in the pipette), had the same properties when recorded at rest in normal fibres or in dystrophic fibres from *mdx* mice lacking dystrophin, but with a greater occurrence in the latter fibres. This suggested that the activity of this channel might be directly affected by the absence of dystrophin, a large spectrin-like cytoskeleton protein associated to the sarcolemma through a glycoprotein complex, and providing the scaffold for multiple cytosolic proteins such as dystrobrevins and syntrophins. The open probability of the channels was increased by treatment with thapsigargin and caffeine, which were thought to deplete calcium stores in the sarcoplasmic reticulum, suggesting that these channels could be store-operated. Using an antisens strategy directed against the TRP box, a motif conserved among TRPC members, this study showed that when TRPC1, TRPC4 and TRPC6 channels were decreased at the protein level, the occurrence of channel activity was drastically lowered in both normal and dystrophic fibres. A recent work from Zanou et al. (2009) confirmed the importance of TRPC1 in skeletal muscle by studying $TRPC1^{-/-}$ mice. This study suggested that the entry of calcium dependent on TRPC1 represents a minor part of calcium influx into fibres at rest, but that the channels contribute to calcium entry during repeated contractions and to maintain the force during the sustained contractile activity. However, the current lost in $TRPC1^{-/-}$ displayed a conductance of 13 pS and were not activated by thapsigargin, which suggest these channels are not store-dependent in fibres. On the contrary, repression of TRPC1 in C2C12 myoblast (Louis et al. 2008), and also in SoD6 myotubes (Sabourin et al. 2009), confirm that TRPC1 participates to store-dependent cation entry in developing skeletal muscle cells, which raised the question of the trigger mechanism in adult fibres. In developing mouse myotubes, repression of TRPC1 or TRPC4 separately induced a decrease in store-dependent cation entry to the same extent, which suggests that both proteins may work together in a hetero-tetrameric channel (Sabourin et al. 2009). Indeed, TRPC1 and TRPC4 could be co-immunoprecipitated from whole lysates isolated from mouse myotubes and limb muscles (Sabourin et al. 2009).

Two independent studies (Krüger et al. 2008; Zanou et al. 2009) have shown by Real-time RT-PCR that TRPC1, C3, C4, C5, C6 and C7 are expressed in skeletal muscle, with different patterns of expression depending on muscle type. According to Krüger et al. (2008), TRPC3 and TRPC6 ARNm are the most expressed in Tibialis anterior (TA) muscle, but Zanou et al. (2009) described a higher expression of TRPC4, TRPC1 and TRPC6 ARNm than TRPC3 ones in Tibialis Anterior, EDL and Soleus muscles. TRPC4 and TRPC6 proteins were found to be expressed at the sarcolemma of skeletal muscles (Vandebrouck et al. 2002, Krüger et al. 2008; Sabourin et al. 2009), but TRPC3 was described with an intracellular distribution (Krüger et al. 2008), which could be in accordance with the interaction of TRPC3 with triadic proteins (Lee et al. 2006; Woo et al. 2008). TRPC1 was found to co-interact with both TRPC3 and RYR1 in mouse myotubes (Woo et al. 2008), which suggests that TRPC1 and TRPC3 could form heteromeric channels in triadic compartments. In fibers expressing TRPC1-yellow fluorescent protein (YFP), a striated expression pattern was observed compatible with a longitudinal SR localization (Berbey et al. 2009), but this could be due to forced expression of the channel. Previous reports showed that the movement of TRPC1 to the plasma membrane is dependent on co-expression with TRPC4 or TRPC5 (Hoffman et al. 2002) and that, when expressed alone, TRPC1 forms functional endoplasmic reticulum (ER) homotetrameric channels (Salgado et al. 2008). In the work of Berbey and collaborators, immunostaining for endogenous TRPC1 revealed the same pattern of expression that matched sarcoplasmic reticulum (SR) $Ca^{2+}$ pump immunolabeling. However, this striated pattern was recently shown to be conserved in fibers from TRPC1 deficient mice, which strongly suggests that this labeling with anti-TRPC1 Chemicon antibody is not specific of TRPC1 when used in these conditions (Tajjedine et al. 2010).

TRPC1 was mainly found at the sarcolemma of adult mouse fibres (Vandebrouck et al. 2002; Gervasio et al. 2008; Sabourin et al. 2009; Zanou et al. 2009) and of developing mouse myotubes (Vandebrouck et al. 2007; Sabourin et al. 2009), co-localizing with dystrophin (Vandebrouck et al. 2007) and caveolin-3 (Gervasio et al. 2008). This suggests that these sarcolemmal cationic channels could be anchored to the subsarcolemmal cytoskeleton including spectrin and/or dystrophin. Indeed, TRPC1 isolated from adult mouse skeletal muscle and from developing myotubes was found to co-immunoprecipitate with dystrophin (Vandebrouck et al. 2007). This suggested for the first time that TRPC1 could be a constituent of a costameric macromolecular complex anchored to the dystrophin-based cytoskeleton (Fig. 1). Dystrophin is providing, in striated muscle, the scaffold for multiple dystrophin-associated proteins (DAPs) such as cytosolic adaptors like syntrophin, which may confer to the complex a role in cell signalling (Ervasti and Sonneman 2008). Through syntrophins, the DAP-complex is thought to anchor various signaling molecules such as enzymes and channels near their functional site at the membrane. The α1-syntrophin is the predominant syntrophin isoform in skeletal and cardiac muscles (Adams et al. 1993). This adaptor protein binds transmembrane channels through a PDZ domain such as voltage-gated sodium channels

(Gee et al. 1998; Gavillet et al. 2006) or the potassium channel Kir4.1 (Connors et al. 2004). We demonstrated that TRPC1 could be co-immunoprecipitated with endogenous and recombinant α1-syntrophin, and GST-pull down assays showed that the PDZ domain of the α1-syntrophin could be involved in this association (Vandebrouck et al. 2007). These observations were providing the idea that TRPC1 association with scaffolding protein and DAPs in skeletal muscle could constitute a signalling complex at costameres, which regulates the localization and function of the channel. In accordance with a costameric DAPs complex including TRPC1 at the sarcolemma, Gervasio and collaborators reported the association of TRPC1 with the scaffolding protein caveolin-3, by showing co-localization at the sarcolemma and co-immunoprecipitation of endogenous proteins (Gervasio et al. 2008). Moreover, this work suggests, by FRET assay and coexpression of TRPC1-CFP with caveolin-3-YFP, that the latter is necessary for localization of TRPC1 at the plasma membrane of myoblasts. In accordance with this idea, we observed that TRPC1-myc expressed in developing muscle cells was intracellular in myoblasts and targeted to the plasma membrane later at the myotubes stage (personal observations), suggesting that associated proteins are necessary along development for including the channel at the surface membrane with DAPs (Fig. 1).

In accordance with the presence of Homer-binding motif at C-terminal and N-terminal domains of TRPC1, this channel could also be co-immunoprecipitated with endogenous Homer protein from mouse gastrocnemius muscle protein lysates (Stiber et al. 2008). Homer1 was found to be the predominant isoform in skeletal muscle, and Homer1$^{-/-}$ mice exhibited a myopathy characterized by decreased muscle fibre cross-sectional area and decreased muscle force generation (Stiber et al. 2008). Interestingly myotubes from Homer1 KO mice also exhibited alterations suggesting that Homer1 can regulate cation channels. Firstly an increased outwardly rectifying current was observed in Homer KO myotubes. This current could be blocked by the GsMTx4 peptide suggesting it could be carried by a stretch-activated channel. Secondly, a drastic increase in a spontaneous barium influx was observed in Homer KO myotubes, which could be blocked by forced expression of Homer1b and also by transfection of shRNA construct for silencing of TRPC1. This strongly supported that Homer1 association with TRPC1 channels was regulating the cation entry through TRPC1 which may be partly responsible for the increase in basal cytosolic calcium observed in Homer KO myotubes (Stiber et al. 2008). It is known for a long time that dystrophin-deficient myotubes also exhibit an increased level in resting cytosolic calcium (Turner et al. 1988; Imbert et al. 1996), which can be restored to normal levels with expression of a functional mini-dystrophin (Marchand et al. 2004).

As stated above, in adult muscle and developing myotubes, TRPC1 was found to co-localize and co-immunoprecipitate with dystrophin and α1-syntrophin (Vandebrouck et al. 2007). Interestingly, we also demonstrated that mini-dystrophin expression also restored normal store-operated cation entry in myotubes (Vandebrouck et al. 2006), as well as forced expression of α1-syntrophin (Vandebrouck et al. 2007). Moreover, both regulating proteins were forming a complex with endogenous TRPC1 in transfected myotubes (Vandebrouck et al. 2007), which strongly supports that the association of TRPC1 with the α1-syntrophin/dystrophin complex regulates the cation entry in mouse myotubes. The store-dependent cation influx regulated by expression of mini-dystrophin were shown to decrease upon application of siRNA against TRPC1 and TRPC4 and to increase at abnormal levels after silencing of α1-syntrophin (Sabourin et al. 2009). Moreover, the elevated cation influx recorded in α1-syntrophin-deficient myotubes could also be decreased by repression of TRPC1 or TRPC4. TRPC1 channels in skeletal myotubes were thus proposed to function in association with TRPC4 and to be regulated by the presence of α1-syntrophin in the dystrophin-associated protein complex. Forced expression of α1-syntrophin deleted of its N-terminal part also suggested that the PDZ domain was essential for restoring normal cation entry in dystrophin deficient myotubes (Sabourin et al. 2009). The absence of dystrophin in dystrophic muscles leads to a dramatic reduction in sarcolemmal α1-syntrophin, although the syntrophins remain concentrated at the NMJ (Peters et al. 1997; Compton et al. 2005). We thus suggested that the absence of dystrophin leading to a reduction of sarcolemmal α1-syntrophin may be responsible for enhanced activity of cation channels constituted at least of TRPC1. Homer1 expression was also shown to decrease in dystrophin-deficient muscle (Stiber et al. 2008) suggesting that a reduction in both scaffolding proteins may participate in deregulation of TRPC1 channels, leading to increased calcium entries in dystrophic muscle cells. On the contrary, expression of caveolin-3 was shown to increase together with TRPC1 in dystrophic muscles, which should maintain the incorporation of TRPC1 at the sarcolemma and may contribute to the elevated activity.

The mechanisms underlying the regulation of TRPC1 channels by scaffolding proteins is not yet known, and could directly modulate the gating of the channel or involve the recruitment of other signaling molecules changing the activity of the channel. The regulation of TRPC1 by homer was proposed to be mechanosensitive (Stiber et al. 2008), and TRPC1 has also been proposed to be mechanosensitive in myoblasts (Formigli et al. 2009). TRPC1 channels could be anchored to the actin cytoskeleton via syntrophin, which has been proposed to be an actin-binding protein (Iwata et al. 2004), and also via dystrophin, which binds actin through actin-binding sites at

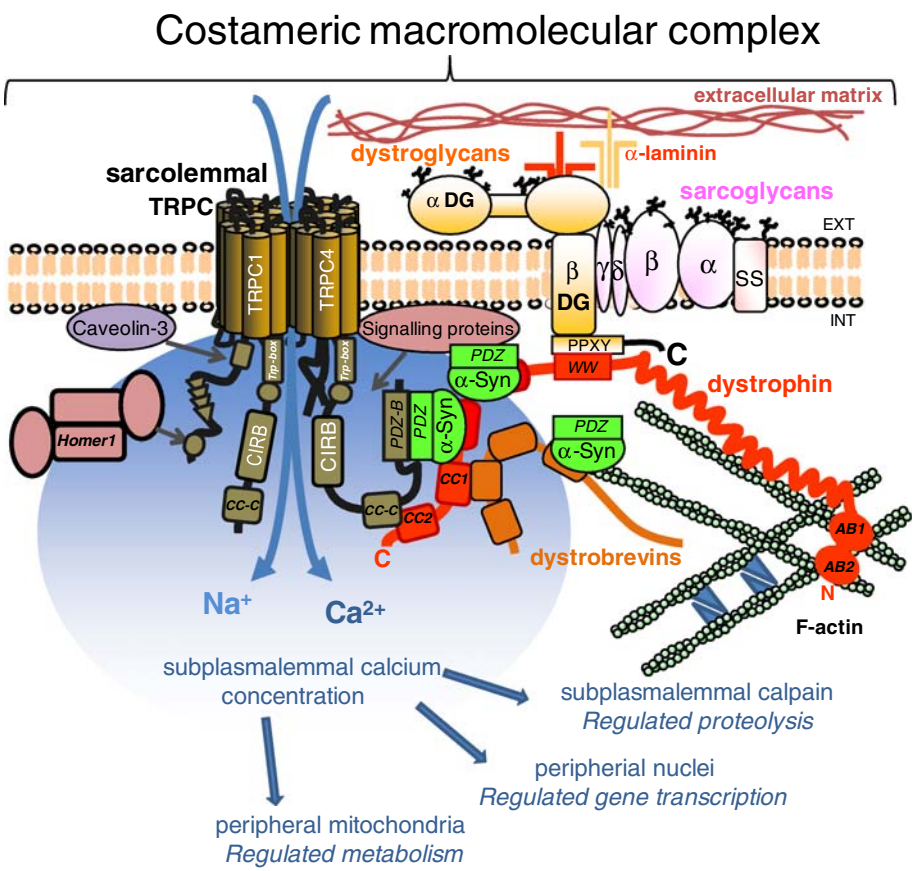


**Fig. 1** The figure represents a hypothetical model for a costameric macromolecular complex incorporating TRPC1/TRPC4 cationic channels. The link to the complex can be achieved by multiple interactions with scaffolding proteins such as caveolin-3, Homer1 and the PDZ-containing α-syntrophin bound to dystrophin and dystrobrevin. Caveolin-3 could bind TRPC1 through a motif at the N-terminal part close to the first transmembrane domain TM1, and Homer through binding motifs at the N-terminal part and at C-terminal part close to the trp-box. The TRPC4 could bind directly to α-syntrophin through a C-terminal consensus PDZ-binding domain. The dystrophin scaffold anchors the signaling complex to actin cytoskeleton and to the transmembrane glycoprotein complex involved in the binding of the extracellular matrix. The cytosolic α-syntrophin can also recruit various signaling proteins near the non-voltage-gated channel for modulating its activity, and the integrity of this scaffold is necessary for maintaining normal cation entry through the sarcolemma. This regulation participates to normal calcium homeostasis in the subsarcolemmal microdomain, which may have functional consequences in peripheral nuclei and mitochondria or for calcium-dependent enzymes such as proteases

the N-terminal domain and the central domain. It is thus possible that the link to syntrophin also confers a mechanical regulation of TRPC1 as well. It was indeed shown by Ou et al. (2003) that γ2 syntrophin determines mechanosensitivity of SCN5A $Na^+$channels in intestinal smooth muscle. It is interesting to note that the activity of mechanosensitive channels has been shown to be higher in dystrophic muscle cells from *mdx* (Franco and Lansman 1990) and human DMD patients (Vandebrouck et al. 2001), as well as calcium responses to hypoosmotic chocs (Imbert et al. 1996). TRPV2, which is a component of osmotically sensitive cation channels in murine aortic myocytes (Muraki et al. 2003) could also participate to the increased mechanosensitive calcium influx in dystrophic muscles. Indeed, TRPV2 was shown to be activated in response to stretch and to be responsible for enhanced calcium influx in myotubes prepared from δ-sarcoglycan-deficient BIO14.6 hamsters (Iwata et al. 2003) and in *mdx* FDB fibers (Iwata et al. 2009). In *mdx* mice, calcium abnormality and muscle dysfunction were ameliorated by expression of a dominant negative TRPV2 (Iwata et al. 2009). To our knowledge, no interaction has been reported between TRPV2 and TRPC or muscle scaffolding proteins. TRPV2 normally localizes in the intracellular membrane compartments but translocates to the plasma membrane in dystrophic muscle fibers (Iwata et al. 2003, 2009). Interestingly, the translocation of TRPV2 to the sarcolemma and TRPV2-dependent calcium influx required the entry of external calcium (Iwata et al. 2003, 2009). This triggering $Ca^{2+}$ could enter via low level of TRPV2 at the sarcolemma as proposed by Iwata and collaborators but also via other channels such as TRPC1. A sustained increase of TRPC-dependent calcium entry could

trigger the surface translocation of TRPV2 in dystrophin-deficient myotubes and fibers as well. Further studies could help to understand if TRPC channels and scaffolding proteins are also involved in TRPV2 translocation.

## Conclusion

Several independent observations show that skeletal muscle TRPC1 channels are incorporated in a costameric macromolecular complex based on dystrophin (Fig. 1), in which the presence of scaffolding proteins such as α1-syntrophin is essential for the regulation of slow cation entry. The mechanism of this regulation is not yet understood, although the PDZ domain of α1-syntrophin appeared to be essential probably by binding the channels or by recruiting PDZ-binding signalling proteins, which modulate the activity of the channel. By analogy with the TRP signalplex originally described in *Drosophila* photoreceptor, one can expect that the adaptor proteins anchored by the scaffold of dystrophin may maintain a functional signalling complex at specialised membrane domain containing TRPC1 and assuring the normal activation and modulation of the cation channels. Future research may discover new members of this muscle signalplex, which could include membrane associated enzymes that could be recruited by syntrophin for moderating the activity of TRPC channels. This shows that the integration of channels in a signalling complex is necessary for maintaining ion homeostasis. In skeletal muscle, this regulation of slow non-voltage-activated cation entry may be crucial for calcium and sodium homeostasis in the subsarcolemmal compartment. Modulation of a subsarcolemmal calcium microdomain will have consequences on subsarcolemmal proteins such as calcium-activated enzymes, on intramitochondrial calcium homeostasis and functions as well as on transcription activity in peripheral nuclei through the calcineurine/NFAT pathway (Fig. 1). Although, it is not yet known if this TRPC-signalling complex may be involved in muscle differentiation and plasticity, the study of muscle from TRPC$^{-}$/$^{-}$ mice have suggested an involvement in maintaining force during prolonged stimulation. On the contrary, the exploration of muscle from dystrophin-deficient mice suggests that an elevated activity may be related with activation of calcium-activated proteolysis and necrosis.

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