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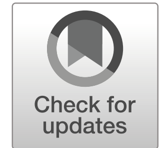

Excitation-contraction coupling in skeletal muscle: recent progress and unanswered questions

Dmitry Shishmarev[1]

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Abstract

Excitation-contraction coupling (ECC) is a physiological process that links excitation of muscles by the nervous system to their mechanical contraction. In skeletal muscle, ECC is initiated with an action potential, generated by the somatic nervous system, which causes a depolarisation of the muscle fibre membrane (sarcolemma). This leads to a rapid change in the transmembrane potential, which is detected by the voltage-gated Ca^{2+} channel dihydropyridine receptor (DHPR) embedded in the sarcolemma. DHPR transmits the contractile signal to another Ca^{2+} channel, ryanodine receptor (RyR1), embedded in the membrane of the sarcoplasmic reticulum (SR), which releases a large amount of Ca^{2+} ions from the SR that initiate muscle contraction. Despite the fundamental role of ECC in skeletal muscle function of all vertebrate species, the molecular mechanism underpinning the communication between the two key proteins involved in the process (DHPR and RyR1) is still largely unknown. The goal of this work is to review the recent progress in our understanding of ECC in skeletal muscle from the point of view of the structure and interactions of proteins involved in the process, and to highlight the unanswered questions in the field.

Keywords Excitation-contraction coupling · DHPR · $Ca_V1.1$ · RyR1 · STAC3

Overview of the excitation-contraction coupling in skeletal muscle

Excitation-contraction coupling (ECC) is an essential process in muscle physiology, responsible for linking electrical signals from the somatic nervous system (action potentials) to mechanical muscle contractions (Sandow 1952). In skeletal muscle, the ECC is initiated at the neuromuscular junction, where a motor neuron connects to a muscle fibre (a multinucleate cell), by the release of the neurotransmitter acetylcholine (ACh) from the axon terminal. Upon diffusing to the sarcolemma (muscle cell membrane), ACh binds to ligand-gated cation channels (ACh receptors) that initiate an action potential in the muscle fibre. The action potential travels down the specialised invaginations of the sarcolemma, called the transverse tubules (T tubules), causing a depolarisation of the T-tubular membrane. In skeletal muscle, the T tubules are flanked from their two sides by the terminal cisternae of the sarcoplasmic reticulum (SR), forming so-called triad junctions (Fig. 1). The rapid change in the electric potential across the T-tubular membrane is detected by the voltage-gated L-type Ca^{2+} channel $Ca_V1.1$ (also known as the dihydropyridine receptor, DHPR), which is embedded in the sarcolemma (Adams et al. 1990; Rios and Brum 1987; Tanabe et al. 1988). Upon changing its conformational state, DHPR communicates the contractile signal to the cation channel ryanodine receptor (RyR1), embedded in the membrane of SR, via mechanical interactions (Meissner and Lu 1995; Takeshima et al. 1994). This causes RyR1 to open up and release large amounts of Ca^{2+} from the SR into the sarcoplasm (Rebbeck et al. 2014). Finally, the released Ca^{2+} ions bind to troponin C, which changes conformation of the troponin complex. This, in turn, initiates the formation of cross-bridges between contractile proteins actin and myosin, causing them to slide along each other, leading to muscle contractions.

The molecular mechanisms of the ECC in skeletal muscle, in terms of protein-protein interactions involved in this process, have been previously covered in a number of excellent reviews (Bannister 2007; Bannister 2016; Calderón et al. 2014; Dulhunty et al. 2002; Rebbeck et al. 2014). Structurally, clusters of four DHPR channels (tetrads) are formed in the T-tubular sections of the sarcolemma, with each

✉ Dmitry Shishmarev
dmitry.shishmarev@anu.edu.au

[1] John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2601, Australia

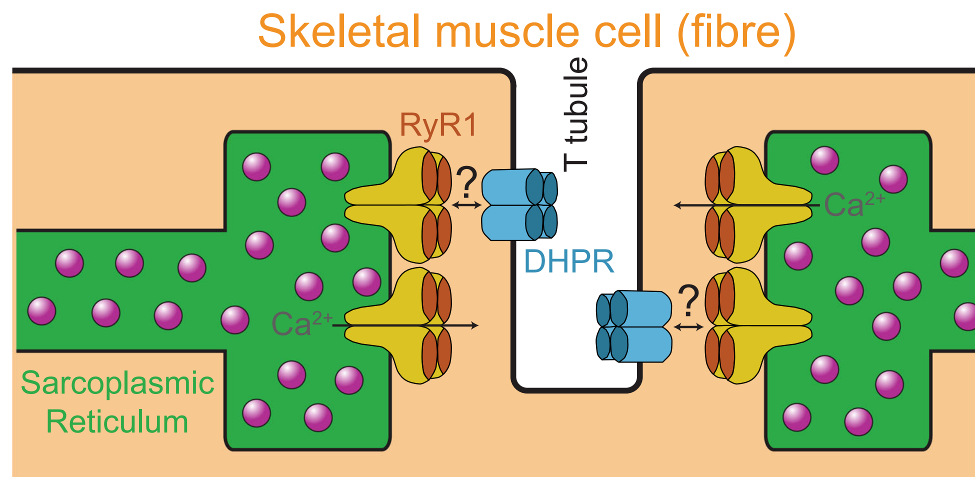


Fig. 1 Schematic of a triad junction in a skeletal muscle fibre. The interaction between the dihydropyridine receptor (DHPR) and type 1 ryanodine receptor (RyR1) is central to the molecular mechanism of the excitation-contraction coupling in skeletal muscle. Structurally, a single RyR1 tetramer attached to the membrane of the sarcoplasmic reticulum aligns opposite to a tetrad of DHPR channels embedded in the sarcolemma, suggesting a mechanical nature of the interactions between these two Ca^{2+} channels (Block et al. 1988; Paolini et al. 2004)

DHPR in the tetrad facing one monomeric unit of the tetrameric RyR1 embedded in the SR membrane at the triad junctions (however, interestingly, only half of all RyR1 tetramers face DHPR tetrads) (Fig. 1). This specific arrangement suggests direct mechanical interactions between DHPR tetrads and RyR1 channels (Block et al. 1988; Paolini et al. 2004). However, even though the main sequence of events in the ECC process has been known for several decades, the molecular mechanisms underlying the precise nature of the interactions between DHPR and RyR1 has remained a mystery for many years (Beam and Bannister 2010; Rebbeck et al. 2014).

Hereditary and acquired defects in the ECC proteins are known to compromise muscle development and performance, thereby causing pathological conditions such as congenital myopathies (Jungbluth et al. 2018; Pancaroglu and Van Petegem 2018). For example, several mutations in RyR1 have been linked to malignant hyperthermia, central core disease and multi-minicore disease (Jungbluth et al. 2018). Our lack of understanding of the precise molecular mechanisms of the skeletal ECC impedes our ability to develop new ways of mitigating detrimental effects of these inherited disorders as well as age-related muscle weakness (sarcopenia). Thus, there is a strong motivation to advance our understanding of the ECC on the molecular level and how it affects other aspects of skeletal muscle biology. Recent developments in the field have made significant progress in resolving this long-standing research problem. Nevertheless, there are still major gaps in our understanding of the molecular mechanism of the skeletal ECC. The aim of this work is to review the known molecular interactions that underpin the ECC process in skeletal muscle and highlight the remaining unanswered questions, with a focus on the most recent developments.

Proteins involved in the skeletal excitation-contraction coupling

DHPR and RyR1 have been known as the main players in the skeletal ECC for several decades (Rebbeck et al. 2014). However, multiple attempts to resolve the molecular details of the interactions underpinning the communication between these two Ca^{2+} channels have essentially failed (Bannister 2007; Bannister 2016). Thus, many researchers speculated about the involvement of other molecules in mediating this interaction. Until a few years ago, it was unknown whether there are any other proteins (apart from subunits of DHPR/RyR1) that play a crucial role in the signal transmission from DHPR to RyR1 that is central to the mechanism of skeletal ECC. This situation changed in 2013, when a new essential component of the skeletal ECC was uncovered — an adaptor protein STAC3 (Horstick et al. 2013; Nelson et al. 2013). This revived an interest in uncovering the precise nature of the interactions between DHPR, STAC3 and RyR1 in skeletal muscle.

Furthermore, in a recent study, Perni et al. (2017) managed to fully reconstruct functional skeletal ECC in non-muscle cells. This was achieved by heterologous expression of α_{1s} and β_{1a}-subunits of DHPR, RyR1, STAC3 and junctophilin2 in human embryonic kidney cells tsA201, thus demonstrating that these five components constitute the complete set of partners required for a fully functional ECC. This recent report suggested that a detailed study of the interactions between this set of five proteins (DHPR-α_{1s}, DHPR-β_{1a}, RyR1, STAC3 and junctophilin2) will allow full resolution of the detailed molecular mechanism of the ECC in skeletal muscle. While it is possible that other proteins endogenously present in tsA201 cells might also be required for the skeletal ECC, this set of five partners appears to be absolutely essential for the

process and thus each of them warrants an in-depth consideration. Accordingly, these individual ECC components are considered in turn in the following sections.

Dihydropyridine receptor

DHPR architecture

DHPR has been known as the critical component of the ECC machinery for > 30 years upon its identification as the ECC voltage sensor by Rios and Brum (1987). The main role of skeletal DHPR is to detect the action potential in the T-tubular part of the sarcolemma and communicate this signal to the RyR1, which then releases Ca^{2+} ions from the SR (Dulhunty et al. 2002; Paolini et al. 2004; Protasi 2002). Additionally, DHPR functions as a voltage-gated L-type Ca^{2+} channel ($Ca_V1.1$). However, its function as a Ca^{2+} channel has been shown to be irrelevant for skeletal ECC (Dirksen and Beam 1999) and, more recently, to muscle development and performance in general (Dayal et al. 2017). Unlike cardiomyocytes, in skeletal muscle, it is not the Ca^{2+} current via DHPR, but the conformational change of the protein upon depolarisation of the sarcolemma that is responsible for the transmission of the contractile signal to RyR1. This mechanical coupling between DHPR and RyR1 is bi-directional, as RyR1 is known to have an effect on Ca^{2+} currents via DHPR through the 'retrograde' coupling effect (Dirksen 2002; Nakai et al. 1996).

Several years ago, the group of Nieng Yan determined the structure of rabbit DHPR at a resolution of 4.2 Å (Wu et al. 2015) and 3.6 Å (Wu et al. 2016) using single-particle cryo-electron microscopy (cryo-EM). This provided some important details about the principal architecture of the protein (Wu et al. 2016). Skeletal DHPR consists of four principal subunits: α_{1s}, $\alpha_2\delta$, β_{1a} and γ (Catterall 2011). α_{1s} is the major, membrane-embedded pore-forming subunit also referred to as $Ca_V1.1$. The other three subunits are auxiliary: γ is membrane-associated and forms contacts with one of the transmembrane domains of α_{1s} (Wu et al. 2016), $\alpha_2\delta$ is extracellular and interacts with the extended extracellular loops of α_{1s} (Wu et al. 2016), while β_{1a} is bound to α_{1s} from the intracellular side (Chen et al. 2004; Opatowsky et al. 2004; Van Petegem et al. 2004). Both α_{1s} and β_{1a} are known to be critical for the ECC (Coronado et al. 2004), while $\alpha_2\delta$ and γ subunits play roles in membrane targeting and regulatory functions of DHPR, but they are not essential for the ECC (Obermair et al. 2008).

DHPR-α_{1s}

α_{1s} subunit of DHPR is critical for the ECC as it contains the voltage sensor that changes its conformation upon sensing depolarisation of the membrane. The architecture of α_{1s} is similar to that of other Ca_V channels: it is composed of four homologous membrane-spanning motifs designated as I, II, III and IV (Fig. 2). Each of these motifs contains six transmembrane helices, denoted as S1–S6. It is the fourth helix S4 that contains several positively charged residues (Arg and Lys) participating in the voltage sensing mechanism. Additionally, there are five major cytosolic regions of $Ca_V1.1$: the N-terminal domain (NTD), the linkers between repeats I and II, II and III, III and IV, and the C-terminal domain (CTD) (Fig. 2).

Apart from the voltage sensor in the core of α_{1s}, the linker between domains II and III (II–III loop) is known to be critical for the skeletal ECC (Tanabe et al. 1990). The central portion of the II–III loop (designated as 'peptide C' (El-Hayek et al. 1995)) contains an amino acid sequence that was identified to have the stretch of residues (720–765) essential for skeletal ECC (Grabner et al. 1999; Nakai et al. 1998b; Wilkens et al. 2001). In fact, Kugler et al. (2004) demonstrated that substitution of only four critical residues in this portion of the loop (A739, F741, P742 and D744) suppresses ECC.

Later biophysical studies demonstrated that the N-terminal part of the II–III loop of DHPR-α_{1s} (residues 671–690) interacts with RyR1 (Cui et al. 2009; Tae et al. 2009). This part of the II–III loop ('peptide A') was previously shown to activate RyR1 channels in vitro (El-Hayek et al. 1995), and a later study determined the minimum motif (681–690) required for this interaction (El-Hayek and Ikemoto 1998). Subsequent studies showed that this N-terminal part of the II-III loop is not essential for the ECC (Bannister et al. 2009; Proenza et al. 2000b; Wilkens et al. 2001); nevertheless, the interaction of the N-terminal part of the II–III loop with RyR1 was reported to have at least some significance for the ECC process (Ahern et al. 2001). Thus, the physiological role of the N-terminal part of the II–III loop with respect to ECC remains ill-defined.

Overall, the fine details of how the II–III loop is involved in the ECC are still unknown. It is missing in the recent cryo-EM structures of DHPR (Wu et al. 2016; Wu et al. 2015), most likely due to its inherently disordered nature (Casarotto et al. 2006; Cui et al. 2009). It is possible, however, that the structure of the II–III loop changes upon the conformational change in the core α_{1s} during the action potential (Polster et al. 2012). Thus, a structural change in the II–III loop might be responsible for the transmission of the contractile signal to RyR1. However, despite an extensive search, the corresponding interacting residues of RyR1 have not been unambiguously identified (Rebbeck et al. 2014). In fact, there is still a lack of strong evidence that the II–III loop interacts directly with the RyR1 in a manner that is important for the ECC. This raised the possibility of other proteins directly participating in the coupling between DHPR and RyR1. Indeed, recent studies identified interactions of the critical peptide from the II–III loop with STAC3, which suggested a direct role of this

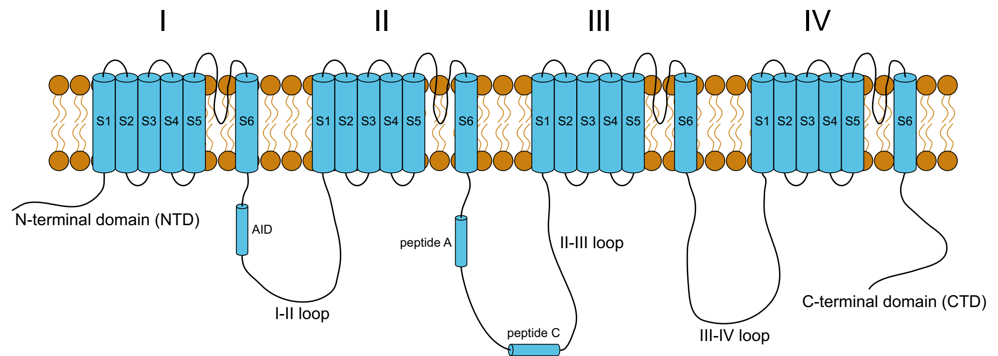


Fig. 2 Topology of the membrane-embedded pore-forming α_{1s}-subunit of the skeletal muscle dihydropyridine receptor ($Ca_V1.1$). Each of the membrane-spanning motifs I–IV is composed of six transmembrane helices S1–S6. The five cytosolic regions of the protein include the N-terminal domain (NTD), I–II loop, II–III loop, III–IV loop and the C-terminal domain (CTD)

adaptor protein in the transmission of the ECC signal in skeletal muscle (see below the section for STAC3 protein).

The linker between domains I and II of α_{1s} binds another ECC-critical subunit of DHPR (β_{1a}) (Pragnell et al. 1994), and this interaction between β_{1a} and the I–II loop is required for targeting of β_{1a} to the triad junctions (Neuhuber et al. 1998). The III–IV linker forms a globular domain in the complex with the C-terminal domain (CTD) of α_{1s} (Wu et al. 2016) and influences ECC indirectly through its ability to modulate channel gating (Bannister et al. 2008; Weiss et al. 2004), while the CTD is also required for targeting of DHPR to the triad junctions (Flucher et al. 2000; Proenza et al. 2000a). The N-terminal cytosolic part of α_{1s} is not essential for the ECC (Bannister and Beam 2005).

DHPR-β_{1a}

As introduced above, the cytosolic β_{1a} subunit binds with a high affinity to a sequence in the I–II loop of DHPR-α_{1s} (residues 357–374) that forms an α-helix designated as the AID (alpha-interacting domain). Multiple reports identified that β_{1a} is critical for the ECC process (Beurg et al. 1999; Gregg et al. 1996; Schredelseker et al. 2009; Strube et al. 1996). β_{1a}-knockout mice die perinatally due to their inability to breathe (Gregg et al. 1996), as β_{1a} is required for correct assembly of the DHPR arrays in the triad junction (Schredelseker et al. 2005) and β_{1a}-null muscles do not elicit RyR1-mediated Ca^{2+} transients upon electric stimulation (Strube et al. 1996).

In mammals, there are four distinct isoforms for the β-subunits (β_1–β_4) (Buraei and Yang 2010), but β_{1a} is specific to skeletal muscle and the only isoform capable of mediating the skeletal-type ECC (Schredelseker et al. 2009). Structurally, β-subunits belong to a family of membrane-associated guanylate kinase (MAGUK) proteins (Karunasekara et al. 2009; Norris et al. 2017; Van Petegem et al. 2004). They consist of conserved and structured Src homology 3 (SH3) and guanylate kinase (GK) domains, which interact with each other via hydrogen bonds and van der Waals interactions, thus forming the stable core of the protein (Opatowsky et al. 2003). β-Subunits also contain less conserved and mostly disordered N-terminal and C-terminal domains, and the so-called HOOK region which forms a long loop within the SH3 domain (Dolphin 2003) (Fig. 3).

The SH3 domains are known to bind proline-rich motifs thus mediating protein-protein interactions (Mayer 2001). However, the canonical binding site in the SH3 domains of β-subunits is occluded by an α-helix leading to the HOOK region, thus making direct interactions of this domain with other proteins unlikely (Chen et al. 2004; Van Petegem et al. 2004). Nevertheless, the SH3 domain and the proximal C-terminus of β_{1a} were found to work cooperatively to enable α_{1s} to act as a voltage sensor, thus playing an important role in the ECC (Dayal et al. 2013). The GK domain is structurally related to the nucleotide monophosphate kinase family of proteins; however, in β-subunits, this domain is catalytically inactive. It is the GK domain that contains a hydrophobic groove that binds the AID sequence of the I–II loop of the DHPR-α_{1s} with a nanomolar affinity (Richards et al. 2004; Van Petegem et al. 2004), with the AID domain being sandwiched in between the α_{1s} and β_{1a} domains of the DHPR complex (Wu et al. 2016).

While it is possible that the ECC signal is transmitted from DHPR-α_{1s} to RyR1 via the DHPR-β_{1a}/AID complex, it is

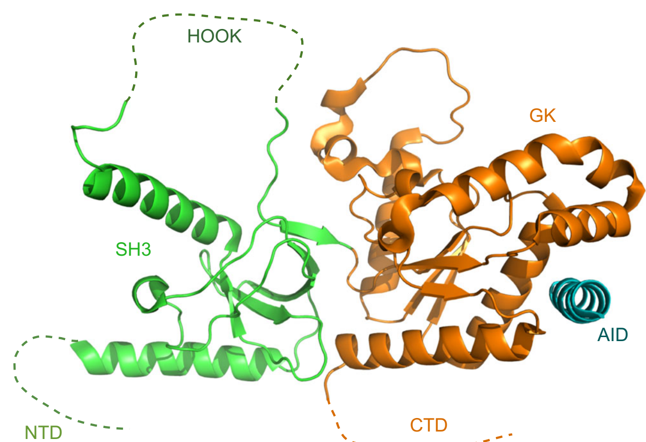


Fig. 3 Structure of the β_{1a}-subunit of the skeletal muscle dihydropyridine receptor in complex with the alpha-interacting domain (AID) peptide. The five principal domains of β_{1a} are indicated: the N-terminal domain (NTD), Src homology 3 (SH3), the HOOK region, guanylate kinase (GK) and the C-terminal domain (CTD). Adapted from the PDB structure 4ZW2 (Norris et al. 2017)

unknown whether the structural change in α_{1s} might confer an allosteric change in DHPR-β_{1a} via its interaction with AID. Even though crystal structures of homologous β_2–β_4 were determined > 15 years ago (Chen et al. 2004; Opatowsky et al. 2004; Van Petegem et al. 2004), a high-resolution structure of the β_{1a}-subunit in a complex with AID was determined only recently (Fig. 3), and the detailed structure of β_{1a} in the absence of AID still remains unknown (Norris et al. 2017). In fact, it is quite likely that β_{1a} is distinct among the β-subunits in that its three-dimensional fold is significantly altered by the interaction with AID, as it has an 8 °C lower melting point in the absence of the peptide (Norris et al. 2017). A perturbation in the conformation or position of AID during the ECC is quite likely as it is separated from the transmembrane helix S6 of the DHPR repeat I by only a small number of residues (Fig. 2). In turn, any perturbation in the conformation or position of AID during the ECC might cause an allosteric change in β_{1a} that is subsequently transmitted to RyR1. However, as a high-resolution structure of DHPR-β_{1a} on its own (without AID) has not been determined yet, the structural role of the DHPR-β_{1a}/AID interaction in the ECC process remains unclear.

Several studies suggested that the C-terminal domain of β_{1a} binds directly to RyR1 in an ECC-relevant interaction. In particular, the distal C-terminal part of β_{1a} has been proposed to be critical (Coronado et al. 2004), as deletion of the 35-residue C-terminal tail (residues 490–524) resulted in a 5-fold reduction in the ECC efficiency (Beurg et al. 1999). In later studies, affinity chromatography showed that this 35-residue peptide can pull-down the whole RyR1 and activate the channel in planar lipid bilayers (Karunasekara et al. 2012; Rebbeck et al. 2011). Moreover, more recent studies showed that a shorter β_{1a} peptide from the same region (490–508) was able to activate the RyR1 (Hernández-Ochoa et al. 2014), and residues 489–503 were determined to be critical for communication between DHPR and RyR1 (Eltit et al. 2014); nevertheless, the corresponding interacting interface of RyR1 has not been determined. In another study, Sheridan et al. (2004) reported that simultaneous mutation of the three hydrophobic residues forming a 'heptad repeat' within the C-terminal sequence of β_{1a} (L478A, V485A, V492A) suppressed the skeletal ECC. However, a later study showed that the same combined mutations did not affect the ECC in β_{1a}-null zebrafish myotubes (Dayal et al. 2010). Overall, despite a consensus regarding the importance of the C-terminal portion of DHPR-β_{1a} in the ECC process, the corresponding interacting residues in RyR1 have not been determined, and the exact structural function of the β_{1a}-subunit in the signal transmission to RyR1 remains unresolved (Bannister 2016).

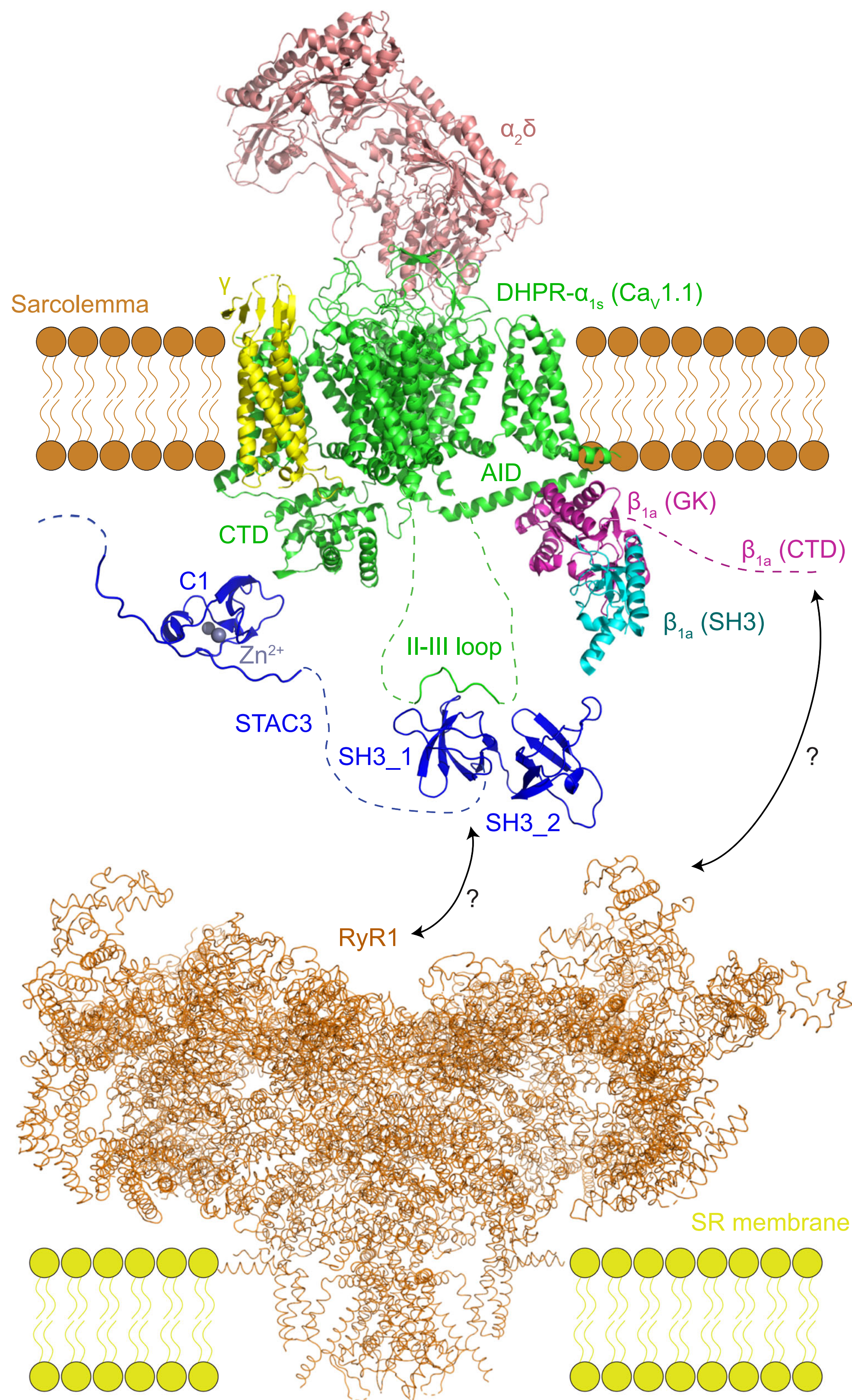
In summary, while the role of the core DHPR-α_{1s} as the voltage sensor in the ECC is well established, it is still unknown whether DHPR-β_{1a} or the II–III loop plays an important role in the transmission of the signal to RyR1 in the ECC process.

Ryanodine receptor

Ryanodine receptor is the largest known ion channel in mammals: it is composed of four identical subunits, each with the molecular weight of ~ 560 kDa (Lanner et al. 2010). In 2015, three groups simultaneously reported cryo-EM structures of RyR1 with an overall resolution of < 5 Å (Efremov et al. 2015; Yan et al. 2015; Zalk et al. 2015). RyR1 has a pore structure similar to that of K^+ channels, and it is permeable to both monovalent and divalent cations. The channel has a mushroom-like appearance, with the N-terminal domain forming 90% of the structure which is located in the sarcoplasmic gap between the terminal cisternae of the SR and the sarcolemma, while the remaining C-terminal domain is embedded in the SR membrane and forms the channel pore (Fig. 4).

In mammals, there are two other isoforms of ryanodine receptors called RyR2 and RyR3 which are primarily expressed in the cardiac and neuronal tissues, respectively (Franzini-Armstrong and Protasi 1997). However, the three isoforms are sufficiently different and only RyR1 is capable of mediating the skeletal-type ECC (Nakai et al. 1997). This allowed RyR1/RyR2 and RyR1/RyR3 chimaera studies using dyspedic myotubes (naturally lacking RyR) to identify regions in RyR1 that are important for the ECC in skeletal muscle. In an early study, Nakai et al. (1998a) suggested that residues 1635–2636 of RyR1 are important for the bi-directional coupling with DHPR, while Yamazawa et al. (1997) reported that deletion of a small region of RyR1 that is poorly conserved between the three RyR isoforms (1303–1406) abolishes the ECC in skeletal muscle. In line with the latter study, Sheridan et al. (2006) demonstrated that residues 1272–1455 of RyR1 were important for DHPR tetrad formation; however, a chimaera containing residues 1–1681 of RyR1 on the background of RyR3 only partially restored the ECC, indicating the importance of multiple interactions between DHPR and RyR1 (Sheridan et al. 2006).

In another study, a weak interaction between the critical region of the II–III loop of DHPR-α_{1s} and residues 1835–2154 of RyR1 was identified (Proenza et al. 2002). However, the chimaera of these residues of RyR1 on the background of RyR2 restored only a weak ECC (Proenza et al. 2002), suggesting a requirement for additional sequences in RyR1 for the proper ECC. Indeed, further studies showed that a much larger sequence of residues 1635–3720 was necessary for full restoration of skeletal ECC, again leading to a conclusion that multiple regions of RyR1 are essential for this process (Perez et al. 2003; Protasi et al. 2002; Sheridan et al. 2006). Furthermore, an ECC-enhancing interaction between DHPR-β_{1a} and a short sequence of RyR1 (3495–3502) was reported (Cheng et al. 2005). Nevertheless, when the proposed interacting residues in RyR1 have been substituted, the interaction persisted (Rebbeck et al. 2014).



In summary, multiple interactions between DHPR and RyR1 during the ECC process might be at play, as several regions of RyR1 appear to be essential for the ECC. Moreover, as introduced above, STAC3 might also play an important role in mediating these interactions, which is discussed in the next section.

◀ **Fig. 4** Overview of the proteins and interactions involved in the excitation-contraction coupling in skeletal muscle. The cartoon is based on the cryo-EM structure of the DHPR complex (PDB 5GJV) (Wu et al. 2016), cryo-EM structure of RyR1 (PDB 3J8E) (Zalk et al. 2015), X-ray structure of the tandem SH3 domain of STAC2 in the complex with the II–III loop peptide (PDB 6B27) (Yuen et al. 2017) and the NMR structure of the C1 domain of STAC3 (PDB 2DB6). The established interactions include those between the GK domain of DHPR-β_{1a} and the AID peptide of the DHPR-α_{1s} I–II loop (Norris et al. 2017), between the C1 domain of STAC3 and the C-terminal domain (CTD) of DHPR-α_{1s} (Campiglio et al. 2018a; Campiglio and Flucher 2017), and the interaction between the central part of the II–III loop and the first SH3 domain of STAC3 (Polster et al. 2018; Yuen et al. 2017). Interactions of RyR1 with either DHPR or STAC3 remain ambiguous

STAC3

STAC3 is a member of a small group of STAC (SH3 and cysteine-rich domain-containing) proteins, consisting of STAC1, STAC2 and STAC3 (Flucher and Campiglio 2018). STAC1 and STAC2 are predominantly located in neural tissues, while the STAC3 isoform is specific to skeletal muscle (Nelson et al. 2013). In the context of muscle physiology, STAC3 was initially reported as an important regulator of myotube formation and myogenic differentiation (Bower et al. 2012; Ge et al. 2014), with homozygous STAC3-knockout mice dying at birth (Reinholt et al. 2013). In 2013, the knockout studies in zebrafish (Horstick et al. 2013) and mice (Nelson et al. 2013) revealed STAC3 as an essential component of the skeletal muscle ECC.

STAC3 is a 364-residue protein consisting of a mostly disordered N-terminal domain which contains a sequence of 12 consecutive glutamate residues, a protein kinase C-like domain (PKC C1) which is rich in cysteines, the flexible linker, and the tandem-SH3 domains at the C-terminus (designated as SH3_1 and SH3_2) (Fig. 4). The structure of the Zn^{2+}-binding PKC C1 domain was determined by NMR spectroscopy and deposited to the PDB database in 2006 (PDB ID: 2DB6). Recently, crystal structures of STAC1 and STAC2 tandem-SH3 domains were determined by X-ray crystallography to the resolution of 2.4 and 1.2 Å, respectively (Yuen et al. 2017). However, STAC3 tandem-SH3 resisted crystallisation, and only the structure of the individual 2nd SH3 domain of STAC3 (SH3_2) was determined (Yuen et al. 2017). The structure of SH3_2 was similar for all three STAC proteins, and it was speculated that the structure of the tandem SH3 domains of STAC3 would be quite similar to those of STAC1 and STAC2 (Yuen et al. 2017).

Several recent reports strongly suggested that the DHPR-RyR1 interactions in the ECC process are in fact mediated by STAC3 (Flucher and Campiglio 2018). The initial zebrafish study showed that STAC3 co-immunoprecipitates with both DHPR and RyR1 (Horstick et al. 2013), and it is required for voltage-dependent Ca^{2+} release from the SR (Nelson et al. 2013). Moreover, a mutation in SH3_1 of STAC3 (W284S) results in misregulation of the DHPR channels (Linsley et al. 2017), disruption of ECC (Polster et al. 2016) and the direct cause of a severe muscle disease known as Native American myopathy (NAM) (Horstick et al. 2013).

Several years ago, Polster et al. (2015) demonstrated that STAC3 facilitates expression and membrane targeting of $Ca_V1.1$ in tsA201 cells, and later, this function of STAC3 was shown to be due to the interaction of the PKC C1 domain of STAC3 with the C-terminal domain of the DHPR-α_{1s} (Campiglio et al. 2018a; Campiglio and Flucher 2017). This interaction was also shown to play a role in the inhibition of calcium-dependent inactivation of Ca_V channels (Campiglio et al. 2018a; Niu et al. 2018a; Niu et al. 2018b); however, it does not appear to be directly relevant to the ECC. In a follow-up work, Polster et al. (2016) showed that the STAC3 is not absolutely required for membrane targeting of $Ca_V1.1$ in tsA201 cells, as this role could also be accomplished by the γ-subunit of DHPR; however, STAC3-null myotubes with properly membrane-targeted $Ca_V1.1$ did not have functional ECC, thus implicating the direct role of STAC3 in mediating the interactions between DHPR and RyR1 (Polster et al. 2016).

Recently, it has been revealed that the II–III loop of DHPR interacts with STAC3 (Yuen et al. 2017), and this interaction appears to be critical for the ECC (Polster et al. 2018). According to the determined crystal structure of STAC2 with a peptide from the II–III loop of DHPR-α_{1s} (residues 747–760), the interaction is mostly with SH3_1. Notably, mutation of the three critical residues in the II–III loop peptide (I752A, P753A and R757A) abolished the interaction (Yuen et al. 2017). The binding also involves the critical tryptophan residue which corresponds to the residue in STAC3 mutated in NAM (W284) (Yuen et al. 2017). Nevertheless, more recent co-immunoprecipitation studies showed that the NAM mutation does not significantly compromise association between STAC3 and DHPR (Zaharieva et al. 2018), possibly due to an intact interaction via the C1 domain of STAC3. As the canonical binding site of SH3_2 remains unoccupied, it might potentially interact with other proteins, including RyR1 (Yuen et al. 2017). Indeed, recent indirect evidence hints that STAC3 might interact with RyR1 as it incorporates into triad junctions independently from the DHPR (Campiglio et al. 2018b). Nevertheless, a direct interaction between STAC3 and RyR1 is yet to be reported.

In summary, there are several established interactions of STAC3 with DHPR (PKC C1 domain of STAC3 with the CTD of DHPR-α_{1s} and SH3_1 with the II–III loop of DHPR-α_{1s}). There is good amount of evidence suggesting that the interaction of STAC3 with the II–III loop of DHPR-α_{1s} is critical for the ECC, confirming an essential role of STAC3 in mediating the interactions between DHPR and RyR1 (Campiglio et al. 2018b; Yuen et al. 2017). However, the lack of the available structural information on the tandem-

SH3 domains of the STAC3 isoform (either in the absence or presence of the II–III loop peptide) prevents the understanding of any direct implication of this interaction in the ECC process. Moreover, any unambiguous evidence for the interaction between STAC3 and RyR1 is still missing. Thus, despite these significant recent advances, the exact functional role of STAC3 in skeletal ECC remains unknown.

Junctophilins

While DHPR, RyR1 and STAC3 appear to be directly responsible for the transduction of the action potential to the release of Ca^{2+} from the SR in the ECC process, the primary role of junctophilins (JPs) is to maintain the structure of junctions between the plasma membrane and sarcoplasmic reticulum (Takeshima et al. 2000). There are two isoforms of junctophilins (JPs) expressed in skeletal muscles — JP1 and JP2 (Nishi et al. 2000). The N-terminal part of the JPs contains the so-called MORN (membrane occupation and recognition nexus) motif that binds the sarcolemma, while the C-terminal part of JPs binds to the membrane of the SR. This allows formation of junctions between the T-tubular membranes and the terminal cisternae of the SR, and proteolysis of JPs was shown to cause disruption of the triad junctions and suppression of ECC (Murphy et al. 2013). JP1 has been reported to co-immunoprecipitate with RyR1 (Phimister et al. 2007), and both JP1 and JP2 were reported to interact with the DHPR (Golini et al. 2011). Recently, the interaction of junctophilins with the C-terminus of DHPR has been shown to be essential for muscle contraction (Nakada et al. 2018). Overall, these interactions of JPs appear to be important for the formation of the triad junctions, and thus, they are relevant for the maintenance of the functional ECC.

Summary and outlook

In summary, it has been recently revealed that there are five core requisite components in the skeletal ECC machinery: α_{1s} and β_{1a} subunits of DHPR, RyR1, STAC3 and junctophilin2 (Perni et al. 2017). The major established and putative interactions are summarised in Fig. 4. While binding of β_{1a}-subunit to DHPR-α_{1s} via the AID peptide is well established (Norris et al. 2017; Wu et al. 2015), its functional relevance in the transduction of the ECC signal remains ambiguous. The other interaction that appears to be important for the ECC is the interaction between the first SH3 domain of STAC3 and the II–III loop of DHPR-α_{1s}. However, there are still no definitive data clearly showing ECC-relevant interactions of RyR1 with either STAC3 or any of the subunits of DHPR. Thus, overall, despite ~30 years of research after the identification of DHPR and RyR1 as key ECC partners, the precise nature of molecular interactions between these two proteins in skeletal muscle remains mostly unresolved (Bannister 2016; Rebbeck et al. 2014; Yuen et al. 2017). As discussed, one of the major hurdles for further progress in our understanding of the ECC mechanism is the fact that the exact three-dimensional structures of DHPR-β_{1a} (without AID) and STAC3 (tandem-SH3 domains) are still unavailable. Another factor that makes this research problem difficult is the fact that ryanodine receptor is a huge protein and multiple interactions of RyR1 appear to be at play, making it difficult to dissect the exact interface or amino acid residues that participate in the ECC. Future structural, biophysical and physiological studies should provide better understanding of the molecular mechanism underlying the interactions between the ECC proteins. The holy grail of the field is to determine a high-resolution structure of a complex containing DHPR and RyR1, potentially together with STAC3 and any of the junctophilins or other components. The rapidly evolving field of cryo-EM might be instrumental in resolving this long-standing issue. Delineation of the molecular details of the communications between DHPR and RyR1 in the ECC process will not only answer a major outstanding question in field, but will have a strong impact in designing rational therapies for the ECC-related pathological conditions.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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