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SYMPOSIUM REVIEW

New and notable ion-channels in the sarcoplasmic/endoplasmic reticulum: do they support the process of intracellular Ca^{2+} release?

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Abstract Intracellular Ca^{2+} release through ryanodine receptor (RyR) and inositol trisphosphate receptor (IP_3R) channels is supported by a complex network of additional proteins that are located in or near the Ca^{2+} release sites. In this review, we focus, not on RyR/IP_3R, but on other ion-channels that are known to be present in the sarcoplasmic/endoplasmic reticulum (ER/SR) membranes. We review their putative physiological roles and the evidence suggesting that they may support the process of intracellular Ca^{2+} release, either indirectly by manipulating ionic fluxes across the ER/SR membrane or by directly interacting with a Ca^{2+}-release channel. These channels rarely receive scientific attention because of the general lack of information regarding their biochemical and/or electrophysiological characteristics makes it difficult to predict their physiological roles and their impact on SR Ca^{2+} fluxes. We discuss the possible role of SR K^+ channels and, in parallel, detail the known biochemical and biophysical properties of the trimeric intracellular cation (TRIC) proteins and their possible biological and pathophysiological roles in ER/SR Ca^{2+} release. We summarise what is known regarding Cl^- channels in the ER/SR and the non-selective cation channels or putative 'Ca^{2+} leak channels', including mitsugumin23 (MG23), pannexins, presenilins and the transient receptor potential (TRP) channels that are distributed across ER/SR membranes but which have not yet been fully characterised functionally.

Since studying for his PhD at Kyoto University, Japan, **Hiroshi Takeshima** has been focusing on identifying new sarcoplasmic reticulum (SR) components including the ryanodine receptor (RyR), junctophilin and TRIC proteins. He has developed many knockout mice models to shed light on the physiological roles of the various SR proteins. After his appointment as Professor at Kurume University and Tohuko University, his group moved to the Graduate School of Pharmaceutical Sciences at Kyoto University where he is now based. **Elisa Venturi** investigated the single-channel properties of RyR and other cation channels in the SR to earn her PhD in 2011 from the University of Bristol. She is now a post-doctoral research assistant in the Department of Pharmacology at the University of Oxford where she is purifying novel SR membrane proteins such as TRIC for subsequent structure–function studies. **Rebecca Sitsapesan** obtained her PhD at the University of Strathclyde and following an appointment as British Heart Foundation Basic Science Lecturer at Imperial College, London, she moved to Bristol University and then to the University of Oxford where she is currently Professor of Pharmacology. Her group investigates the biophysical properties of RyR and other ion channels present on intracellular organelles and that are involved in the process of intracellular Ca^{2+} release, particularly in regard to cardiac physiology and pathophysiology.

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Abbreviations ADPKD, autosomal dominant polycystic kidney disease; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CFTR, cystic fibrosis transmembrane conductance regulator; CLC, Cl^- channel family; CLIC, intracellular Cl^- channel family; CLCA, $Ca2^+$-activated Cl^- channel; CPVT, catecholaminergic polymorphic ventricular tachycardia; ER, endoplasmic reticulum; GDD, gnathodiaphyseal dysplasia; GST, glutathione-S-transferase; IP3R, inositol trisphosphate receptor; KO, knockout; LGMD2L, limb girdle muscular dystrophy 2 L; MG23, mitsugumin23; MMD3, Distal Miyoshi-like myopathy 3; PanX, pannexin; PKA, protein kinase A; RyR, ryanodine receptor; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SNPs, single nucleotide polymorphisms; SR, sarcoplasmic reticulum; TRIC, trimeric intracellular cation channel; TRP, transient receptor potential; VRAC/LRRC, volume-regulated anion channel/leucine-rich repeat-containing.

Introduction

The sarcoplasmic reticulum (SR) is a highly specialised intracellular Ca^{2+} store that controls the contractile cycle in striated muscle and provides a traditional model system for Ca^{2+} signalling (Fig. 1). In striated muscle, Ca^{2+} release from and uptake into the SR during the contraction cycle are mediated by the ryanodine receptor (RyR) and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), respectively. In contrast, the Ca^{2+} released via the inositol trisphosphate receptor (IP_3R), which is activated by cytokine- and hormone-induced signalling pathways, primarily regulates cellular metabolic processes including gene expression. There are many unexplained aspects to the Ca^{2+} release events of muscle SR that occur via these two types of Ca^{2+} release channel. This should not be surprising since the SR contains a plethora of incompletely characterised proteins and these may regulate the activity of RyR or IP_3R, directly or indirectly.

An example of a debated aspect of SR function is how the membrane potential across the SR is maintained. Physiological SR Ca^{2+} release through RyR and IP_3R channels can persist efficiently for several milliseconds and therefore counterion movements are required to balance the movement of positive charge that occurs as Ca^{2+} leaves the SR. Counter currents of ionic species such as K^+, Mg^{2+} or Cl^- are expected but there is controversy over which ion channels within the SR are involved (Yazawa *et al.* 2007; Gillespie & Fill 2008; Guo *et al.* 2013). It has, for decades, been assumed that the physiological role of SR K^+ channels and/or SR Cl^- channels is to enable rapid counter-ion fluxes across the ER/SR to compensate for the charge movements associated with Ca^{2+} release and re-uptake processes. More recently it has been suggested, however, that RyR can pass sufficient counter current to balance its own Ca^{2+} release and that other SR ion channels would not be required for this function (Gillespie & Fill 2008). Instead, it is argued that there are always some SR K^+ channels opening irrespective of the membrane potential across the SR. These channels would allow the excess K^+ (built up during Ca^{2+} release) to leave the SR following termination of a Ca^{2+}-release event (hence equilibrating SR membrane potential). This would be important because RyR channels would be closed and not able to pass K^+. This does not take into account the possible involvement of anion fluxes in controlling ER/SR membrane potential and leaves open the question as to the physiological role/s of the various types of SR Cl^- channel. Also unknown, but perhaps related, is how the SR maintains luminal pH. SERCA catalyses the extrusion of protons (Fig. 1) as Ca^{2+} is pumped into the SR (Inesi & Tadini-Buoninsegni 2014) and therefore it is expected that a mechanism/s exists to balance the change in intraluminal pH. Acidification of isolated skeletal SR vesicles is observed during the release of Ca^{2+} from those vesicles and it has been suggested that proton movements could contribute a small proportion (<10%) towards the countercurrent required for charge compensation during SR Ca^{2+} release (Kamp *et al.* 1998). The pathway/s for rapid proton influx during the Ca^{2+} release process has not been identified but there is evidence for localisation of a K^+–H^+ exchanger in the ER/SR of some tissues which could be involved in pumping protons into the SR in exchange for K^+ out (Kuum *et al.* 2012). A number of Ca^{2+}-leak channels have been localised to the ER/SR and as these tend to show little specificity among cations, perhaps protons could be permeable in one or more of these channels. The identification and characterisation of SR ion channels and transporters remains a relatively unexplored area of muscle biology. This short review discusses recent progress in the field and highlights what we do and do not know about the ion channels, other than RyRs and IP_3Rs, in ER/SR membranes.

TRIC and K^+ channels in the SR; voltage is the main regulator

TRIC (trimeric intracellular cation) channel subtypes, namely TRIC-A and TRIC-B, are derived from distinct genes in the mammalian genome, and their counterparts are also detected in the fruit fly and nematode genomes (Yazawa *et al.* 2007). In the gene-expression profile among mouse tissues, TRIC-A is preferentially

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detected at high levels in excitable tissues, while relatively low levels of TRIC-B are ubiquitously observed in various tissues. In muscle cells, TRIC proteins are localised in the SR and nuclear membranes. TRIC proteins are composed of ∼300 amino acid residues and contain three putative membrane-spanning segments to form a bullet-shaped homo-trimeric structure. When either native or recombinant TRIC-A and TRIC-B proteins were incorporated into artificial membranes under voltage-clamp conditions, monovalent cation-selective channel events were observed, suggesting that both TRIC subtypes function as K^+ channels in cells (Yazawa *et al.* 2007; Pitt *et al.* 2010). These single channel current fluctuations share certain characteristics with those of the previously reported SR K^+ channels obtained from muscle SR from a variety of species (Miller 1978; Labarca & Miller 1981; Shen *et al.* 1993) and tissues (Gray & Williams 1985; Picher *et al.* 1996) thus indicating, since muscle SR contains abundant TRIC-A, that the TRIC subtypes form the SR K^+ channel.

The physiological importance of the TRIC subtypes is gradually becoming realised through the study of TRIC-knockout (KO) mice and there are now several reports of TRIC mutations in human disease. In TRIC-KO mice, we have observed impaired ER/SR Ca^{2+} release in several cell types. The *Tric-a*-KO mice developed hypertension due to vascular hypertonicity (Yamazaki *et al.* 2011). In the mutant vascular smooth muscle cells, insufficient RyR-mediated Ca^{2+} sparks for inducing hyperpolarisation were detected (Yamazaki *et al.* 2011). The *Tric-b*-knockout mice exhibited neonatal respiratory failure, demonstrating the essential physiological role of this TRIC subtype in the lungs. In the mutant alveolar epithelial cells devoid of TRIC-B, the IP_3R-mediated Ca^{2+} release which controls surfactant production and handling was markedly reduced (Yamazaki *et al.* 2009). The double-knockout mice lacking both TRIC subtypes developed embryonic heart failure, and SR Ca^{2+} handling was severely impaired in the mutant cardiac myocytes (Yazawa *et al.* 2007). These studies demonstrate the indispensable role of the TRIC proteins to the Ca^{2+} release process in a variety of tissues and show that the proteins are important both for RyR- and IP_3R-mediated Ca^{2+} release.

Recent studies have also identified two distinct associations between TRIC channels and human diseases. In Japanese population-based studies, single nucleotide polymorphisms (SNPs) in the *TRIC-A* gene are associated with hypertension risk and sensitivity to common anti-hypertensive medications (Yamazaki *et al.* 2011). In the TRIC-A risk variant, gene expression may be restricted to vascular smooth muscle, thus elevating resting vascular tone. It has also been found that two deletion mutants in the *TRIC-B* gene are responsible for the autosomal recessive disease osteogenesis imperfecta, a condition which leads to bone fragility and increased susceptibility to fractures (Shaheen *et al.* 2012; Rubinato *et al.* 2014). Both mutations lead to severe truncation of the TRIC-B protein

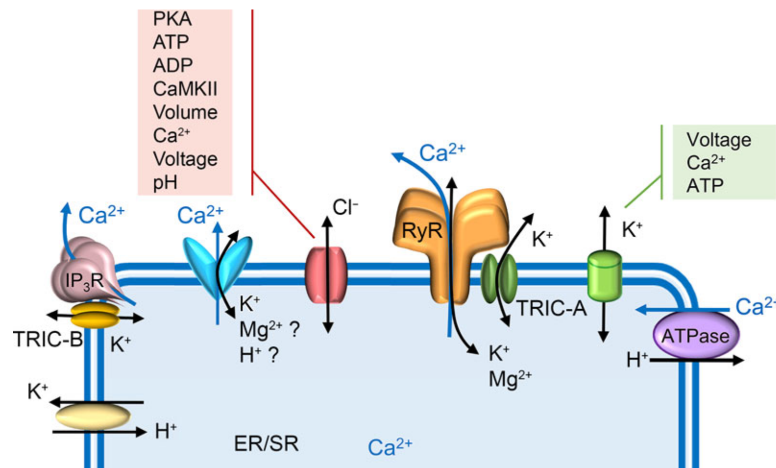

Figure 1. Ionic fluxes across the ER/SR compartment
RyR and IP_3R are the acknowledged Ca^{2+} release channels of the ER/SR. The sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps Ca^{2+} into ER/SR with counter movement of H^+ out. There are also numerous unidentified ionic pathways that are predicted from experimental work. These comprise the various SR anion and cation channels which appear to be triggered by many distinct activators. In addition, a putative ER/SR-residing K^+–H^+ exchanger may be responsible for pumping protons into the SR in exchange for K^+ moving out. Many recognised ion channels are also located on ER/SR membranes although their physiological roles in this location have not been clearly assigned. These include the monovalent cation-conducting TRIC channels and several non-selective cation channels (for example, MG23, pannexins, presenilins and the TRP channels, TRPV1, TRPP2 and TRPM8), often described as Ca^{2+} leak channels.

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but the pathological mechanisms leading to this novel type of osteogenesis imperfecta are not yet understood.

To understand the specific physiological roles of the TRIC subtypes in various tissues and how mutations can lead to human disease, comprehensive biophysical characterisation of each TRIC channel subtype is required. This is complicated, however, since both isoforms are present in most cell types and since purification of recombinant proteins has proved difficult (Venturi *et al.* 2013). The *Tric-b*-KO mouse dies at birth and neonatal SR is not sufficiently developed for the study of SR ion channels. The *Tric-a*-KO mouse survives. Tissue from *Tric-a*-KO mice has enabled characterisation of the native K^+ channels that remain when the SR is devoid of TRIC-A (Venturi *et al.* 2013). These channels are selective for monovalent cations and are sensitive to voltage, being activated at voltages where the SR luminal side of the channels is negative with respect to the cytosolic side of the channels. Single-channel studies reveal complex sub-conductance gating behaviour. The channels appear to gate to at least four sub-conductance open states which are approximately 80%, 60%, 46% and 30% of the full open single-channel conductance (200 pS). Multiple sub-conducting states were not previously reported in early SR K^+ channel studies; instead a single 'noisy' sub-level at 60–70% of the full conductance was repeatedly reported (for example, Tomlins *et al.* 1984; Fox 1985; Tomlins & Williams 1986). This discrepancy could be attributed to the fact that fast gating between different conductance levels would not be resolved at the filtering (100–200 Hz) applied in their analysis. It is notable that different investigators described slightly different current amplitudes for the 'noisy' sub-conductance state (for example, Tomlins & Williams 1986; Hill *et al.* 1989, 1990; Rousseau *et al.* 1992) and that, occasionally, markedly different sub-conductance state amplitudes were reported (Labarca & Miller 1981; Picher *et al.* 1996).

As described above, the main functional modulator of SR K^+ channels appears to be voltage. The majority of the single SR K^+ channels (82%) observed after incorporating vesicles of skeletal muscle SR from *Tric-a*-KO mice into bilayers, display voltage-dependent gating behaviour with increased open probability (P_o) at positive holding potentials (cytosol relative to SR lumen) compared to negative holding potentials(Venturi *et al.* 2013). Thus, if the SR were to become negatively charged with respect to the cytosol, this would lead to more opening of SR K^+ channels. A proportion of the channels (12%) do not appear to be sensitive to voltage and exhibit higher than usual activity at positive and negative voltages and an increased propensity for sub-conductance state gating behaviour (Venturi *et al.* 2013). The heterogeneity in gating behaviour is likely to ensure that there will always be a proportion of SR K^+ channels opening irrespective of the potential across the SR membrane. This gating behaviour together with the voltage sensitivity of SR K^+ channels suggests that these channels would be extremely effective at dissipating any asymmetry in $[K^+]$ that could occur across the SR membrane and in preventing SR membrane potential moving far from 0 mV. Apart from voltage, there is no other obvious physiological regulator of SR K^+ channels. SR K^+ channels do exhibit sensitivity to cytosolic and luminal pH (Miller 1978; Labarca *et al.* 1980; Bell 1985) and to membrane lipid composition (Bell & Miller 1984) but perhaps these factors become more important in pathological conditions such as ischaemia. It has been reported that SR K^+ channel gating is regulated by cytosolic and luminal $[Ca^{2+}]$ but this is not observed by all investigators (Rousseau *et al.* 1992; Uehara *et al.* 1994; Wang & Best 1994; Picher *et al.* 1996). Luminal Ca^{2+} could also cause some degree of block of outward K^+ flux if the luminal $[Ca^{2+}]$ was raised high enough (Liu & Strauss 1991). It is possible that TRIC-A and TRIC-B may have additional or different physiological roles to that of maintaining $[K^+]$ equilibrium across the SR or of permitting charge-compensating ionic fluxes. For example, there is evidence that TRIC-A may bind to or indirectly interact with RyR2 to enhance RyR2 opening (Zhou *et al.* 2014). It will be important to investigate this and other putative regulatory functions of TRIC-A and TRIC-B.

There are isolated reports of other types of K^+ channels in ER/SR membranes and these include small (Kuum *et al.* 2012) and large (Yamashita *et al.* 2006) conductance Ca^{2+}-activated K^+ channels and ATP-sensitive K^+ channels (Zhou *et al.* 2005). Unfortunately there is sparse information regarding the physiological relevance of these channels for ER/SR Ca^{2+} stores, and again, speculation on the role of the small conductance Ca^{2+}-activated K^+ channels is that of providing counter-current of K^+ during the Ca^{2+}-uptake process (Kuum *et al.* 2012).

Anion channels of the ER/SR and their multiple mechanisms of activation

SR Cl^- channel current fluctuations were first observed after incorporation of rabbit skeletal SR vesicles into artificial membranes (Miller 1978). Subsequent investigations of the biophysical properties of the SR Cl^- channels have shown that there are multiple types of Cl^- channel in the SR and that they exhibit a wide range of conductance and gating properties (for example, Tanifuji *et al.* 1987; Rousseau *et al.* 1988; Townsend & Rosenberg 1995). Several channels show Ca^{2+} dependence, some are regulated by pH and many are voltage sensitive (Kawano & Hiraoka 1993; Townsend & Rosenberg 1995). Some SR Cl^- channels are regulated by ATP (for example, Ahern & Laver 1998; Kawano *et al.*

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1999), some by protein kinase A (PKA; Kawano *et al.* 1992, 1999) or by Ca^{2+}-calmodulin-dependent protein kinase (CaMKII; Kawano & Hiraoka 1993). Anion selectivity and single-channel conductance of the various SR Cl^- channels also varies widely and even adenine nucleotides are reported to be permeant in certain channels (Kawano *et al.* 1999). It seems clear that, unlike the SR K^+ channels, where voltage is the main regulator and no specific or potent ligand has been identified, the SR Cl^- channels are functionally diverse and are regulated by different conditions and specific regulators. Thus, the SR Cl^- channels may be involved in multiple functions. Unfortunately, the majority of SR Cl^- channels that can be observed following incorporation of SR vesicles into bilayers appear to gate to multiple sub-conductance levels and this makes it extremely difficult to distinguish between the different types of channel, especially since multiple channels usually incorporate into the membrane.

The observed functional SR Cl^- channel behaviour has not yet been linked to any identified protein. Several classes of Cl^- channel are known and these include the CLC family of chloride channels and transporters (Stauber *et al.* 2012). In the mammalian genome, there are nine CLC genes, four of which encode cell-surface Cl^- channels and five encode intracellular Cl^-–H^+ exchangers. The Cl^-–H^+ exchangers (ClC3–7) mainly reside on components of the endosomal–lysosomal pathway and influence vesicular acidification. There is sparse information regarding the possible localisation of ClC transporters in the ER/SR although one report indicates that ClC4 resides on this compartment (Okkenhaug *et al.* 2006). In addition to CLC proteins, members of the CLCA (Ca^{2+}-activated Cl^- channel; Patel *et al.* 2009), CLIC (intracellular Cl^- channel; Jiang *et al.* 2014) and VRAC/LRRC (volume-regulated anion channel/leucine-rich repeat-containing; Voss *et al.* 2014) families could potentially also form functional SR Cl^- channels. The CLIC proteins show sequence homology with glutathione-S-transferase (GST) and can function both as Cl^- channels or as soluble, globular proteins that interact with and regulate the function of other proteins (Dulhunty *et al.* 2001). In this regard, it is interesting that CLIC2 has been shown to bind directly to RyR1 (Meng *et al.* 2009) and inhibit channel opening (Dulhunty *et al.* 2001; Meng *et al.* 2009).

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated Cl^- channel expressed on the apical surface of epithelial cells in lungs, pancreas and intestine. CFTR is also found in muscle cells and there is recent evidence for its localisation in human skeletal muscle SR (Divangahi *et al.* 2009). It has been suggested that the dysregulation of this Cl^- channel, which is seen in cystic fibrosis sufferers, could explain certain abnormalities in muscle function (Lamhonwah *et al.* 2010). No obvious similarity in the biophysical properties of any of the reported SR anion channels and those of CFTR is apparent although experimental conditions were not specifically designed for activation of CFTR.

The physiological role/s of the different SR Cl^- channels and their involvement in Ca^{2+} signalling remains a subject of speculation. It has been assumed that they provide charge compensation during Ca^{2+} release and uptake processes. But if this was their only role, then why does the SR contain so many different types of anion channel? An interesting report of a Cl^- channel from liver rough ER that was activated by Mg^{2+}-ADP and inhibited by Mg^{2+}-ATP, suggested that this Cl^- channel could be activated by metabolic stress (Ashrafpour *et al.* 2012). The need for further study in this area is obvious and it must begin with identification of the proteins that constitute the SR Cl^- channels so that structure-function studies can begin.

ER/SR Ca^{2+} leak channels: a growing community

Several non-selective cation channels are also found in the ER/SR membranes of a variety of cell types and may function as Ca^{2+} leak channels. These include mitsugumin23 (MG23), pannexin channels (PanX1 (Vanden Abeele *et al.* 2006), PanX2 (Ambrosi *et al.* 2010), and PanX3 (Ishikawa *et al.* 2011)), presenilins (Tu *et al.* 2006; Zhang *et al.* 2010) and several ion-channels belonging to the transient receptor potential (TRP) family: TRPV1, TRPP2 and TRPM8 (Dong *et al.* 2010; Taylor & Dale 2012). Why so many different types of non-selective cation channel are expressed on ER/SR Ca^{2+} stores is not known but the possible physiological consequences of activating such channels are numerous. Passive Ca^{2+} leak from intracellular compartments is observed in many cell types (Lomax *et al.* 2002; Giunti *et al.* 2007) and the ionic pathway is usually expected to be that of RyR or IP_3R. In cardiac cells, diastolic SR Ca^{2+} leak is facilitated in certain pathophysiological conditions including heart failure and catecholaminergic polymorphic ventricular tachycardia (CPVT) and may lead to fatal cardiac arrhythmias (Bers, 2014). There is evidence, however, for RyR-independent mechanisms of SR Ca^{2+} efflux, and that these ionic fluxes may be more strongly activated in disease states (Zima *et al.* 2010). The participation of one or more of the newly discovered Ca^{2+}-permeable ion channels located on ER/SR membranes in generating the ER/SR Ca^{2+} leak from various cells is a subject that deserves detailed investigation. We describe those Ca^{2+} leak channels that have been identified and discuss what little is known regarding their biophysical properties. Figure 2 summarises some of the putative cellular roles of these channels.

Mitsugumin 23. MG23 is a 23 kDa transmembrane protein found in ER/SR and nuclear membranes of striated muscle and other tissues including epithelial

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cells, secretory organs and the brain (Nishi *et al.* 1998). Electron microscopy and 3D particle reconstruction revealed the unstable and peculiar morphology of MG23 (Venturi *et al.* 2011). Two types of particle were consistently observed; small asymmetric particles and large bowl-shaped particles of hexameric symmetry. It was hypothesised that the MG23 bowl configuration is constructed from six asymmetric particles and can be readily constructed and disassembled.

Recombinant purified MG23 proteins reconstituted into planar lipid bilayers behave as voltage-sensitive cation-conducting channels which are equally permeable to Ca^{2+} and K^+ (Venturi *et al.* 2011). MG23 exhibits very unusual gating behaviour characterised by brief 'flickery' opening events and apparently co-ordinated gating of multiple channels; possibly the instability of the bowl-shaped assembly formed by MG23 molecules underlies the observed unusual ion-channel activity observed in bilayer experiments. The biophysical properties of MG23 may allow its involvement in charge compensation and/or in SR Ca^{2+} leak. The use of the MG23 KO mouse (Yamazaki *et al.* 2010) may help to expose the functional roles of MG23. It is interesting that two recent studies have reported MG23 to play a role in generating lethal signals from the ER after stressed-induced DNA damage (Yamazaki *et al.* 2010; Yamashita *et al.* 2013). It is known that changes in the Ca^{2+} content and leak from ER/SR compartments can affect the incidence of apoptotic cell death (Pinton *et al.* 2008) but the molecular identity of this pathway remains unknown. The cation permeability of MG23 suggests that it could be implicated in this phenomenon.

TRP channels. TRPV1 is highly expressed in the plasma membrane of a variety of cellular components of the central nervous system but is also present in non-neuronal tissues, for example in kidney, liver and vascular smooth muscle (Veronesi & Oortgiesen, 2006). TRPV1 has also been detected in the ER and the Golgi system (Veronesi & Oortgiesen, 2006; Dong *et al.* 2010). In particular, in skeletal muscle, it has been proposed to act as a functional SR Ca^{2+} leak channel (Xin *et al.* 2005; Lotteau *et al.* 2013), and that this particular mechanism for intracellular Ca^{2+} mobilisation may be directly involved in RyR1 activation (Lotteau *et al.* 2013).

TRPP2 (polycystin-2) is widely expressed in various cell types, with highest levels found in kidney. Loss-of-function mutations of the gene encoding TRPP2 are associated with autosomal dominant polycystic kidney disease (ADPKD; Koulen *et al.* 2002). This cation-selective channel is mainly located on the plasma membrane but it is also abundant in the ER of kidney epithelial and smooth muscle cells (Cai *et al.* 1999; Koulen *et al.* 2002; Geng *et al.* 2008). The specific physiological functions of TRPP2 in the distinct cellular compartments where it is located are still not fully understood. It has been suggested that, in the ER, TRPP2 functions as a Ca^{2+}-release channel which can interact with IP_3Rs and that it is triggered to open by local rises in Ca^{2+} concentration (Koulen *et al.* 2002;

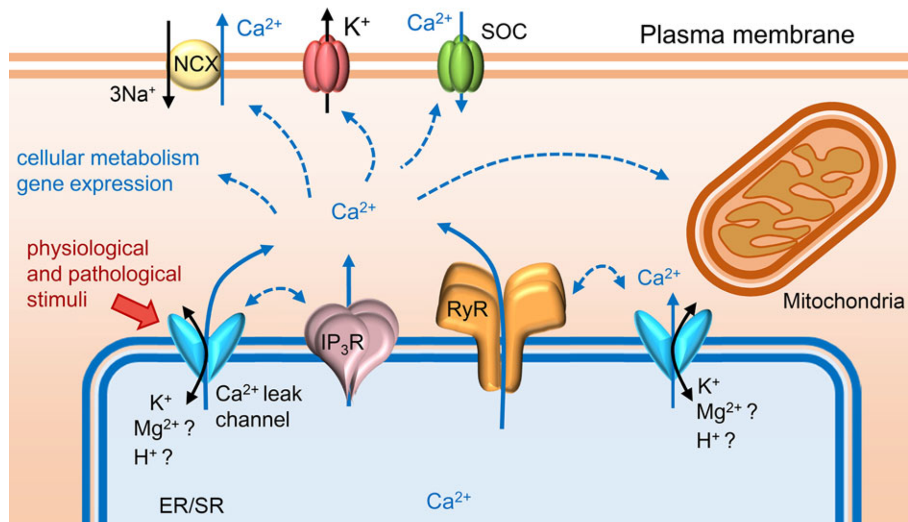

Figure 2. ER/SR Ca^{2+} leak
Ca^{2+} signals from the ER/SR of different cell types can originate through distinct mechanisms and can also be mediated/enhanced by Ca^{2+} leak channels located on the ER/SR network. Some of these non-selective cation channels, thought to be novel pathways for Ca^{2+} release, have also been shown to directly affect the function of the main Ca^{2+}-release channels, RyR and IP_3R. Certain pathological stimuli, such as accumulated reactive oxygen and nitrogen species, might be involved in upregulation of ER/SR Ca^{2+} leak, but usually the mechanisms underlying the activation of Ca^{2+} leak channels are unclear. Rises in cytosolic Ca^{2+} influence many cellular processes and can lead to altered cellular metabolism, deranged gene expression and mitochondrial-induced apoptosis.

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Geng *et al.* 2008; Sammels *et al.* 2010). It has also been proposed that TRPP2 could be involved in protecting cells from apoptosis by reducing the releasable ER Ca^{2+} pool in response to apoptotic stimuli (Wegierski *et al.* 2009).

TRPM8 is predominantly found in the sarcolemma of somatosensory neurons but it is also abundant in the ER membranes of human prostate epithelial cells, where has been identified as a novel pathway for the release of Ca^{2+} (Thebault *et al.* 2005). TRPM8 is activated by cold temperatures and menthol and is sensitive to pH (Mahieu *et al.* 2010). Again, the physiological relevance of the expression of this particular channel in the ER is not understood.

Pannexin channels. PanX1, a member of the ubiquitously expressed pannexin family of channels (Bruzzone *et al.* 2003), has been shown to form mechanosensitive, cation- and ATP-permeable channels which enable intercellular Ca^{2+} mobilisation (Bao *et al.* 2004; Vanden Abeele *et al.* 2006; Penuela *et al.* 2014). It has been reported that overexpression of PanX1 drastically increases ER Ca^{2+} permeability implicating a role in ER Ca^{2+} leak (Vanden Abeele *et al.* 2006; Penuela *et al.* 2014). PanX1 is not the only pannexin channel residing in intracellular membranes; the PanX2 (Ambrosi *et al.* 2010; Wicki-Stordeur *et al.* 2013) and PanX3 (Ishikawa *et al.* 2011) isoforms have also been detected in the ER network. In particular, PanX3 is suggested to contribute to the ER Ca^{2+} leak mechanism that is required to stimulate osteoblast differentiation (Ishikawa *et al.* 2011).

Presenilins. The Ca^{2+} permeability of the presenilins, gene mutations of which have been linked to familial Alzheimer diseases, has been extensively documented in several studies (Tu *et al.* 2006; Nelson *et al.* 2010; Zhang *et al.* 2010). In these reports, a speculative role for presenilins as participants in ER Ca^{2+} leak has been proposed although these conclusions are disputed (Shilling *et al.* 2012).

The subcellular distribution of these different types of Ca^{2+}-permeable channels is, without doubt, fascinating, even though their physiological roles are primarily topics for speculation. Further investigation of their single-channel properties, their possible modulation by endogenous ligands and the development of specific pharmacological regulators are needed to clarify their contribution to physiological and pathophysiological ER/SR Ca^{2+} fluxes.

TMEM16E: a novel SR protein of unknown function but possible role in ion transport

As yet, TMEM16E cannot be listed as an anion channel, K^+ channel, or Ca^{2+} leak channel and so must belong to its own unique class of putative SR ion-channel. Ten members of the TMEM16/anoctamin family were identified from the mammalian genome and share a homologous structure containing eight putative transmembrane segments with cytoplasmic amino and carboxyl-terminal tails (Pedemonte & Galietta, 2014). TMEM16 family members are expected to be involved in ion transport and phospholipid scrambling based on the functional properties of TMEM16A, TMEM16B and TMEM16F (Pedemonte & Galietta, 2014). TMEM16A and TMEM16B function as cell-surface Ca^{2+}-activated Cl^- channels, and contribute to apical Cl^- secretion of epithelial cells and membrane potential regulation of smooth muscle. TMEM16F seems to function as both a Ca^{2+}-dependent lipid scramblase and as a Ca^{2+}-activated Cl^- channel in blood cells. In Scott syndrome patients, TMEM16F mutations impair the phospholipid scrambling of the platelet cell membrane leading to a bleeding disorder (Suzuki *et al.* 2010). TMEM16E is an unusual family member as it is immunochemically detected in the ER/SR of skeletal and cardiac muscle cells, chondrocytes and osteoblasts (Mizuta *et al.* 2007). The expression profile suggests that TMEM16E plays multiple biological roles in the musculoskeletal system. TMEM16E was originally identified as the gene *GDD1*; missense mutations in this gene cause gnathodiaphyseal dysplasia (GDD), a rare bone disorder (Tsutsumi *et al.* 2004). *TMEM16E* nonsense mutations are associated with proximal limb-girdle muscular dystrophy (LGMD2L) and distal Miyoshi myopathy (MMD3), both of which are recessive types of muscular dystrophy (Bolduc *et al.* 2010). Linkage of *TMEM16E* mutations with muscular dystrophies, together with the subcellular distribution of this protein has led to suggestions that TMEM16E may mediate SR Cl^- currents or phospholipid scrambling and may be important for muscle-specific cellular functions such as efficient SR Ca^{2+} handling and membrane repair after cell-membrane wounding. Aside from speculation, however, the function of TMEM16E in the ER/SR is still totally unknown.

Concluding remarks

We have described the biophysical properties of the various ion-channels of the SR where known, and tried to relate these properties to possible physiological roles. We have particularly been considering how the ionic fluxes would be important to the process of ER/SR Ca^{2+} release (Figs 1 and 2). However, the ER/SR is a multi-functional organelle and controls other cellular processes including secretory protein folding and modification, sterol and fatty acid biosynthesis, and ER stress signalling. Such divergent functions require specific ionic conditions within luminal ER/SR domains and changes to those conditions may cooperatively modulate more than one process. For example, SERCA inhibitors not only diminish Ca^{2+}-release signalling, but also disrupt protein folding

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to induce the ER stress response because major ER chaperones are Ca^{2+} dependent. Moreover, the ER/SR functionally communicates with the cell membrane, mitochondria and nuclei. The use of SERCA inhibitors to reduce Ca^{2+} store content also triggers store-operated Ca^{2+} entry via the cell membrane and possibly reduces Ca^{2+}-dependent mitochondrial metabolism and nuclear transcription. This diverse range of functions may explain the need for multiple types of ER/SR anion channel and the variety of the so-called Ca^{2+} leak channels.

Since the ER/SR controls a number of crucial cellular functions and since precise, spatiotemporal Ca^{2+} release events from ER/SR are also required to initiate or regulate many processes within cells, it is important to understand the molecular basis of ionic fluxes across the ER/SR. Functional investigations of ER/SR ion channels are difficult because of their inaccessibility for *in situ* voltage-clamp studies hence adding to the challenges of protein purification and reconstitution procedures. However, since there remain many fundamental, unanswered questions regarding intracellular Ca^{2+} signalling, it is essential to persist in the identification and characterisation of ER/SR channels.

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Additional information

Competing interests

None of the authors has any conflicts of interests.

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