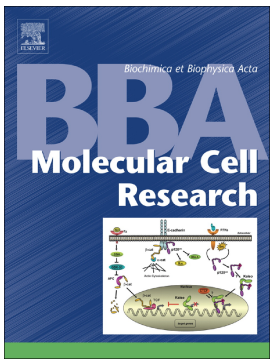

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A comprehensive overview of the complex world of the endo- and sarcoplasmic reticulum Ca^{2+}-leak channels

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ABSTRACT

Inside cells, the endoplasmic reticulum (ER) forms the largest Ca^{2+} store. Ca^{2+} is actively pumped by the SERCA pumps in the ER, where intraluminal Ca^{2+}-binding proteins enable the accumulation of large amount of Ca^{2+}. IP_3 receptors and the ryanodine receptors mediate the release of Ca^{2+} in a controlled way, thereby evoking complex spatio-temporal signals in the cell. The steady state Ca^{2+} concentration in the ER of about 500 µM results from the balance between SERCA-mediated Ca^{2+} uptake and the passive leakage of Ca^{2+}. The passive Ca^{2+} leak from the ER is often ignored, but can play an important physiological role, depending on the cellular context. Moreover, excessive Ca^{2+} leakage significantly lowers the amount of Ca^{2+} stored in the ER compared to normal conditions, thereby limiting the possibility to evoke Ca^{2+} signals and/or causing ER stress, leading to pathological consequences. The so-called Ca^{2+} leak channels responsible for Ca^{2+} leakage from the ER are however still not well understood, despite over 20 different proteins have been proposed to contribute to it. This review has the aim to critically evaluate the available evidence about the various channels potentially involved and to draw conclusions about their relative importance.

ABBREVIATIONS

2-APB	2-aminoethoxydiphenyl borate
a.a.	amino acid
AD	Alzheimer's disease
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma-2
Bcl-XL	Bcl extra-large
BI-1	Bax inhibitor-1
BiP	Binding immunoglobulin protein
$[Ca^{2+}]_{cyt}$	cytosolic Ca^{2+} concentration
CALHM1	calcium homeostasis modulator 1
CaMKII	Ca^{2+}/calmodulin kinase II
CISD2	CDGSH iron-sulfur domain-2
ER	endoplasmic reticulum
FAD	familial Alzheimer's disease
GAAP	Golgi anti-apoptotic protein
IP_3	inositol 1,4,5-trisphosphate
IP_3R	inositol 1,4,5-trisphosphate receptor
LRRC8	leucine-rich repeat-containing 8 protein
MEF cells	mouse embryonic fibroblasts
MOMP	mitochondrial outer membrane permeabilization
PKA	protein kinase A
RyR	ryanodine receptor
SERCA	sarco/endoplasmic reticulum Ca^{2+} ATPases
SOCE	store-operated Ca^{2+} entry
SR	sarcoplasmic reticulum
TMBIM	transmembrane Bax inhibitor motif containing
TMCO1	transmembrane and coiled-coil domain 1 (
TRP	transient receptor potential
TRPP2	polycystin-2
UPR	unfolded protein response

KEYWORDS

Ca^{2+} stores

ER Ca^{2+}-leak channels

IP_3 receptor

Ryanodine receptor

Presenilins

Translocon

1. INTRODUCTION

Ca^{2+} ions function in all cell types as important intracellular messengers. Ca^{2+} regulates fertilization, development, cell proliferation, secretion, contraction, metabolism, autophagy, cell death, etc. The exact effect exerted by Ca^{2+} in the cell depends on its actual concentration changes in time and space. In basal conditions, the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is between 50 and 100 nM, but will rapidly increase upon Ca^{2+} influx from the extracellular environment or Ca^{2+} release from intracellular stores through opening of Ca^{2+}-permeable channels. As the free $[Ca^{2+}]_{extracellular}$ is about 1.5 mM and the free $[Ca^{2+}]_{ER}$ about 0.5 mM, there is in both cases a large driving force for Ca^{2+} flux into the cytosol, thereby increasing $[Ca^{2+}]_{cyt}$ either globally or in specialized microdomains [1].

The ER forms the largest intracellular Ca^{2+} reservoir in the cell and therefore contains several proteins dedicated to Ca^{2+} handling/Ca^{2+} binding. The sarco/endoplasmic reticulum Ca^{2+} ATPases (SERCA) are responsible for active pumping of Ca^{2+} into the ER Ca^{2+} store [2]. The lumen of the ER contains a set of low-affinity, high-capacity Ca^{2+}-binding proteins of which calreticulin and calsequestrin are the most important [3]. Last but not least, dedicated Ca^{2+}-release channels are responsible for the controlled release of Ca^{2+} out of the ER. The two "classical" Ca^{2+}-release channels are the inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) and the ryanodine receptors (RyRs). The IP_3Rs are activated by IP_3, produced by phospholipase C after cell activation by extracellular agonists [4-6]. While IP_3Rs are ubiquitously expressed, the other main class of Ca^{2+}-release channels, the RyRs, have a more restricted tissue distribution, with very high expression levels in the sarcoplasmic reticulum (SR) of muscle cells as well as in the ER of certain brain areas. RyRs are in these tissues activated by either Ca^{2+} influx or by physical interactions with the dihydropyridine receptor upon membrane depolarization [7-9]. It should hereby be emphasized that the $[Ca^{2+}]_{cyt}$ has a bell-shaped effect on both IP_3Rs and RyRs, activating them at concentrations below ~1 µM while inhibiting them at higher ones. Moreover, Ca^{2+} release from the ER does not only affect $[Ca^{2+}]_{cyt}$ but can also affect the $[Ca^{2+}]$ in other organelles. A prime example are the mitochondria, which can be in close apposition with the ER so that a preferential transfer of Ca^{2+} can occur from the ER to the mitochondrial matrix [10, 11]. This transfer of Ca^{2+} is crucial for the regulation of mitochondrial energetics and thus the control of autophagy and of apoptosis [12-14]. Furthermore, the decrease in $[Ca^{2+}]_{ER}$ will also lead via a complex mechanism involving mainly the ER Ca^{2+} sensors STIM1 and 2 and the plasma membrane Orai channels to the phenomenon of store-operated Ca^{2+} entry (SOCE), which is necessary for the replenishment of the ER Ca^{2+} stores as well as for allowing sustained Ca^{2+} signals [15, 16].

In addition to the above mentioned classical Ca^{2+}-release channels (IP_3Rs and RyRs), it is however clear that several other channels contribute to the passive Ca^{2+} leak from the ER (Fig. 1). Many different proteins have been proposed to participate to this Ca^{2+} leak, but their respective roles were not yet clarified, in part because their expression levels as well as their regulatory mechanisms may

be cell type-dependent. This could also explain why the Ca^{2+} leak rate measured in various cell types can vary widely [17].

Hence, the steady-state $[Ca^{2+}]_{ER}$ will depend both on the actual activity of the SERCA pumps as well as of that of the ER Ca^{2+}-leak channels [17, 18]. This implies that the existence of a Ca^{2+}-leak pathway will become more evident upon SERCA inhibition. In most cases, thapsigargin is applied to inhibit SERCA activity with high selectivity and affinity in a non-competitive, irreversible manner [19]. Yet, also cyclopiazonic acid or 2,5-di-(tert-butyl)-1,4-benzohydroquinone have been used as inhibitors, although some caution should be exerted since these compounds can in addition to inhibiting SERCA pumps also inhibit the passive Ca^{2+} leak [20]. In contrast, other rather non-selective pharmacological treatments including glutathione [21], thimerosal [22], ATP [18], 2-aminoethoxydiphenyl borate (2-APB) [23] and its derivative DPB162-AE [24] can all increase the ER Ca^{2+} leak. Also some lipids have been shown to affect ER Ca^{2+}, and especially various sphingolipids and their derivatives [25, 26]. The apoptotic inducer ceramide, though not its dihydroceramide analog, causes a progressive release of Ca^{2+} leading to a full depletion of the ER [27]. Yet, its mechanism of action and whether ceramide itself or one of its metabolites is involved, remain elusive. In salivary adenoid cystic carcinoma, C6-ceramide so inhibits SERCA pumps [28] or activates IP$_3$R3 [29], depending on the cell model used. Moreover, sphingosine-1-phosphate, a downstream metabolite of ceramide, induces Ca^{2+} release from IP$_3$-sensitive Ca^{2+} stores [30-32]. Finally, also gangliosides affect ER Ca^{2+} handling. GM2, a type of ganglioside, increases Ca^{2+} leakage by inhibiting SERCA activity [33] while GM1 was proposed to act via the IP$_3$R [34]. Although the use of those various compounds in theory opens possibilities for further study of the ER Ca^{2+} leak, due to the promiscuous nature of those molecules, it is in most cases still difficult to accurately identify which channel or channels are involved.

Finally, it is important to realize that most Ca^{2+}-leak channels are probably either expressed at a quite low level and/or are, despite their name, not permanently open or at least not fully open (e.g. low open probability or low conductance) as this would lead to a rapid loss of the Ca^{2+} gradient between ER and cytosol, on the one hand prohibiting intracellular Ca^{2+} signaling, and at the other hand triggering ER stress and/or apoptosis. Regulation mechanisms dependent on the $[Ca^{2+}]_{ER}$ and/or the $[Ca^{2+}]_{cyt}$ are therefore likely, and will be discussed. Although some older reviews have already discussed some of the proteins putatively involved in the ER Ca^{2+} leak [17, 35-38], present review is, at the best of our knowledge, the first providing a comprehensive overview of all mammalian proteins that have emerged or have been proposed as potential ER Ca^{2+} leak channels.

2. DO THE CLASSICAL Ca^{2+} RELEASE CHANNELS CONTRIBUTE TO THE Ca^{2+} LEAK?

2.1. IP$_3$Rs

Three different genes (ITPR1-3) encode for IP_3R isoforms (IP_3R1-3), which have a length of about 2700 amino acids. Each of the three IP_3R isoforms form large homo- or heterotetrameric structures. These IP_3Rs form a hub for a multitude of modulatory proteins that thereby regulate the intracellular localization or the activity level of the former and thus its functional consequences [5, 6, 39]. Various kinases can phosphorylate the IP_3R, thereby regulating its activity [40]. Noteworthy are protein kinase A (PKA), since PKA-mediated phosphorylation of IP_3R1 at S^{1588} and S^{1755} (Fig. 2a) enhances its Ca^{2+}-releasing activity by sensitizing the channel to IP_3 [41-43], and the pro- and anti-apoptotic members of the B-cell lymphoma-2 (Bcl-2) protein family, which regulate IP_3R function and more generally affect ER Ca^{2+} handling [44].

An important property of the IP_3Rs is the high cooperativity of the IP_3 binding [45, 46] combined with the fact that channel opening requires IP_3 binding to the 4 subunits of the IP_3R [47]. This should normally protect the cell against ER Ca^{2+} release in conditions of low [IP_3], but however does not completely prohibit IP_3-induced Ca^{2+} release at basal [IP_3]. This Ca^{2+} release occurring at basal [IP_3] in intact cells has also be called an ER Ca^{2+} leak [21, 48, 49]. In support of this, IP_3R1 was identified in a siRNA-based screen in HEK293T cells as one of a few ER proteins contributing to the ER Ca^{2+} leak [50]. Depending on the intracellular conditions, including the occurrence of post-translational modifications, IP_3R sensitivity can be modulated, leading to increased Ca^{2+} leakage. A first way by which IP_3R function can be modulated is through phosphorylation (Fig. 2a). Indeed, mouse embryonic fibroblasts (MEF cells) lacking both pro-apoptotic Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist killer (Bak) displayed a lower steady-state ER Ca^{2+} content and an increased ER Ca^{2+} leak. This correlated with a higher phosphorylation level of IP_3R1 at the PKA–dependent phosphorylation sites [51]. This effect was linked to the higher level of association between Bcl-2 and IP_3R1 in the absence of Bax and Bak. This result fits with earlier studies demonstrating that Bcl-2 overexpression could lower ER Ca^{2+} levels (*see 4.1.*) as well as with a more recent study demonstrating that Bcl-2 can dock the protein phosphatase 1 inhibitor DARPP-32 to IP_3R1, leading to enhanced PKA-dependent IP_3R phosphorylation and Ca^{2+} release [52]. In the latter study, the increased Ca^{2+} release activates calcineurin eventually providing for a negative feedback on the IP_3R. However, in e.g. the absence of calcineurin it is possible that the IP_3R becomes hyperphosphorylated and so sensitive to IP_3 that in non-stimulated condition it effectively functions as an ER Ca^{2+}-leak channel. This process can be pathologically relevant as androgen deprivation of LNCaP prostate cancer cells led to increased PKA-mediated phosphorylation of IP_3R1 eventually leading to a decreased Ca^{2+}-store content [53]. As inhibition of IP_3R1 phosphorylation resensitized the cells to androgen deprivation-induced apoptosis while SERCA2b overexpression reduced resistance to androgen deprivation, the increased ER Ca^{2+} leak constitutes a protective mechanism by which LNCaP cells escape apoptosis.

A second way by which the IP_3R could function as an ER Ca^{2+}-leak channel is after proteolytic cleavage (Fig. 2b). During apoptosis, caspase 3 cleaves IP_3R1 at a single site, ^{1888}DEVD^{1891}, leading to the formation of a 95 kDa C-terminal fragment containing the channel part but not the IP_3-binding domain of the receptor [54]. Subsequently, it was demonstrated that the caspase-cleaved channel domain of IP_3R1 functioned as a Ca^{2+}-leak pathway depleting the ER [55, 56] and that it was even required for optimal caspase 3 activation and apoptosis [57]. However, the magnitude of the Ca^{2+} leak varied between these studies, indicating that additional factors controlled the leak pathway. This may relate to the fact that the cleavage of IP_3R1 strongly depended on the apoptosis inducer used and the cell type under consideration [58] as well as on the presence of the Bcl-2 family member Bok, which interacts with a.a. 1895-1903 of IP_3R1 [59]. Calpain is a protease having a cleavage site on IP_3R1 26 a.a. downstream of the caspase 3 cleavage site [60]. Similarly to caspase 3, calpain cleaves IP_3R1 in such a way that the C-terminal 95 kDa part of the IP_3R remains intact and can act as a Ca^{2+}-leak channel. This protein demonstrated IP_3-independent gating together with a high open channel probability although it retained Ca^{2+}-dependent regulation [60]. As such calpain-mediated cleavage can occur after ischemic brain injury, these results suggest that the occurrence of such an ER Ca^{2+} leak mediated by a fragment of the IP_3R could be pathologically relevant. However, the simultaneous expression in IP_3R-deficient DT40 cells of the N-terminal 215 kDa fragment and the C-terminal 95 kDa fragment expected to result from caspase or calpain cleavage of IP_3R1, still exhibited robust IP_3-induced Ca^{2+} release [61]. The latter results indicate that caspase 3- or calpain-mediated IP_3R1 cleavage does not necessarily imply the formation of a Ca^{2+}-leak channel. Further research is warranted in order to determine to what extent the IP_3R1 channels cleaved during apoptosis remain sensitive or not to IP_3, as then also additional cleavages occur.

2.2. RyRs

Similarly as for the IP_3R, there are three different isoforms of RyR found in mammals, encoded by different genes, RyR1, RyR2 and RyR3. RyR1 is primarily found in skeletal muscles, cerebellum and Purkinje cells, RyR2 is expressed at high levels in cardiac muscle and in cerebral cortex, while RyR3 is expressed at low levels in several tissues including brain, smooth muscle, and slow-twitch skeletal muscle. RyR isoforms are also present at low levels in a variety of peripheral tissues, such as pancreas, kidneys and stomach [62]. RyR isoforms share 65% of their amino acid sequence, with the greatest homology in the C-terminal region, comprising the transmembrane and ion-conducting domain, while the N-terminal region form a large regulatory cytoplasmic region sensitive to Ca^{2+}, Mg^{2+}, ATP, phosphorylation, and redox state [63]. The RyRs function as macromolecular complexes whereby its cytoplasmic region serves as a scaffold for several regulatory proteins, including calstabin1/calstabin2 (FKBP12/FKBP12.6), calmodulin, protein kinases, and protein phosphatases [63].

Inherited RyR mutations and/or posttranslational modifications can provoke a RyR-mediated Ca^{2+} leak (Fig. 3), leading to profound deleterious effects. RyR mutations are predominantly present in four distinct clusters [64]. Mutations causing RyR hypersensitization and leakage of Ca^{2+} from the SR primarily correlate with a range of myopathies and cardiac disorders. Mutations in the RyR1 gene are associated with myopathies such as central core disease and malignant hyperthermia [65-67], while mutations in the gene encoding RyR2 are especially associated with arrhythmogenic syndromes, such as catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular cardiomyopathy type 2 [68-70].

Disturbances in the interaction of RyRs with various proteins have been related to Ca^{2+} leakage from the ER/SR. In fact, the interaction of RyRs with FKBP12/FKBP12.6 (Fig. 3) has been proposed as an essential regulatory mechanism to enhance the cooperativity of RyR channel opening, thereby preventing uncontrolled channel openings and thus Ca^{2+} leakage from the ER/SR [71, 72]. In addition, structural proteins that tether the ER/SR to the plasma membrane, such as junctophilins, calcium-binding proteins such as calsequestrin, and channels, such as trimeric intracellular cation channels and the Ca^{2+}-permeable polycystin-2 (TRPP2, *see 3.3.4*) can also stabilize the RyR and thus avoid excessive ER/SR Ca^{2+} release [73-77].

Post-translational modifications, including phosphorylation, induced by chronic adrenergic stimulation, ischemia-reperfusion and oxidative stress, can disturb RyR interaction with FKBP12/FKBP12.6, and are involved in the pathogenesis of neurodegenerative diseases, and in various cardiac and skeletal disorders [78-81]. For instance, PKA-mediated phosphorylation at S^{2844}, oxidation and S-nitrosylation decreases the interaction of RyR1 with FKBP12, resulting in an abnormal Ca^{2+} leakage from the SR in skeletal muscle [78, 82, 83].

Similarly, post-translational modifications of RyR2 that compromise the interaction of this channel with FKBP12.6 and results in ER Ca^{2+} leakage are involved in the genesis of Alzheimer's disease (AD) and Huntington's disease [79, 80, 84, 85], as well as in arrhythmias and heart failure [81]. With respect to these cardiac disorders, several studies have correlated chronic oxidation, and phosphorylation by PKA and/or by Ca^{2+}/calmodulin kinase II (CaMKII) with dissociation of FKBP12.6 from the RyR2 channel, resulting in a SR Ca^{2+} leak and a consequent reduction of the SR Ca^{2+} content in the cardiomyocytes [86-98]. In heart failure, the diastolic SR Ca^{2+} leak is also related to an aberrant increase of intracellular Zn^{2+} levels in cardiomyocytes, which provokes a higher open probability and longer mean open times of the RyR2 channels and thus their hyperactivation [99].

Conversely, RyR2 phosphorylation at S^{2367} by the muscle-specific SPEG kinase is responsible for stabilizing the channel and inhibiting the diastolic Ca^{2+} leak [100], while a basal level of RyR2 S-nitrosylation is required to avoid excessive diastolic Ca^{2+} leak, since S-nitrosylation decreases CaMKII-dependent phosphorylation at S^{2814} [101, 102].

In summary, a tight phosphorylation and redox balance are important mechanisms to control RyR-mediated Ca^{2+} release and to avoid Ca^{2+} leakage through the RyR.

3. A ROLE FOR OTHER ER Ca^{2+}-HANDLING PROTEINS IN THE ER Ca^{2+} LEAK

3.1. SERCA pumps

SERCA pumps are responsible for ATP-driven Ca^{2+} loading of the ER. Three different genes (ATP2A1-3) encode a SERCA pump and the gene products are known as SERCA1-3, each having a molecular mass of about 115 kDa. Alternative splicing further increases the number of isoforms, whereby SERCA2b is the housekeeping isoform in most tissues. SERCA1a and 1b are respectively expressed in adult and fetal fast-twitch skeletal muscle while SERCA2a and 2c are expressed in cardiac tissue and some other muscle and non-muscle tissues. SERCA3, of which 6 isoforms exist, has a low affinity for Ca^{2+} and is quite widely expressed [2].

Due to their high expression levels in muscles, a significant part of the SR Ca^{2+} leak is due to "slippage" of the SERCA1 pumps [103]. This Ca^{2+} leak can be blocked by using SERCA inhibitors or increasing the ADP concentration [104, 105). Sarcolipin, a single transmembrane α-helix of 31 amino acids exclusively present in the SR of striated muscle cells, increases the rate of slippage and the rate of Ca^{2+} leakage through SERCA1 [106]. This process could be important in non-shivering thermogenesis, whereby ATP hydrolysis in skeletal muscles is used to generate heat. Sarcolipin belongs to an increasing family of SERCA-regulatory micropeptides, also including phospholamban, dwarf open reading frame, myoregulin, endoregulin and another-regulin. However, the exact mechanism of action of these micropeptides on SERCA is still under investigation [107]. These results anyway indicate that slippage of the SERCA pumps can contribute to the ER/SR Ca^{2+} leak, certainly in cell types expressing high levels of SERCA. However, as this leak is per definition inhibited by SERCA inhibitors, it cannot contribute to the leak uncovered by using thapsigargin or other SERCA inhibitors as performed in a majority of studies.

Additionally, there exist two C-terminally truncated SERCA1 variants known as SERCA1T with a molecular mass of 43 and 46 kDa respectively. These truncated proteins are widely expressed though are inactive as Ca^{2+} pumps [108]. They preferentially localize at ER-mitochondrial sites where they, putatively by forming homodimers, increase Ca^{2+} leakage out of the ER [109]. Their expression leads to ER Ca^{2+}-store depletion, increased Ca^{2+} uptake in the mitochondria and consequently apoptosis.

3.2. Orai channels

Orai channels consist of small proteins (~33 kDa) with 4 transmembrane domains. They mediate Ca^{2+} entry via the SOCE pathway as well as via the arachidonate-regulated pathway. In mammals, they are encoded by 3 genes, leading to three distinct isoforms named Orai1-3, whereby alternative initiation-translation of the mRNA further leads to two Orai1 variants, α and β [110]. Orai proteins can

assemble to homo- and heteromeric channels. While Orai1 was most studied and its function as hexamer appears very clear in SOCE, the physiological functions of Orai2 and Orai3 are less well understood. They can participate to both canonical and non-canonical SOCE as well as to other forms of Ca^{2+} entry [110, 111]. Orai1/Orai3 heteropentamers have so been described as the channels responsible for arachidonate-regulated Ca^{2+} entry [112].

Interestingly, evidence exists that Orai channels are also expressed at the ER and consequently can also play a role in the ER Ca^{2+} leak (Fig. 1). First, the mRNA screening study [50] already mentioned above (*see 2.1.*), revealed Orai2 as another protein contributing to the ER Ca^{2+} leak, since its downregulation strongly decreased the ER Ca^{2+} leak while its overexpression had an opposite effect.

Second, it was proposed that Orai3 is responsible for the 2-APB-induced Ca^{2+} leak from the ER [113]. Indeed, it is well known that the pleiotropic compound 2-APB not only affects IP_3R and SOCE activity, but also at relatively high concentrations (>50 µM) increases the ER Ca^{2+} leak in cerebellar microsomes [23], permeabilized A7r5 smooth muscle cells [114], liver microsomes [115] and pancreatic acinar cells [116]. It should be noted that this effect is independent of the SERCA inhibition occurring at similar 2-APB concentrations, as it was observed in unidirectional Ca^{2+} flux experiments. Guerrero-Hernandez and collaborators found that 2-APB, at concentrations as low as 5 µM, activated the ER Ca^{2+} leak in HeLa cells [117]. This activation was, in contrast to the Ca^{2+} leak uncovered by thapsigargin, dependent on replete ER Ca^{2+} stores, indicating that at least two different Ca^{2+}-leak channels are present in HeLa cells [117]. In a follow-up study, the same authors demonstrated that Orai3 was necessary for the 2-APB-induced Ca^{2+} leak (Fig. 1), while Orai1 and Orai2 had an inhibitory effect [113]. Moreover, while lowering Orai3 expression inhibited the 2-APB-induced Ca^{2+} leak, it did not affect the ER Ca^{2+} leak uncovered by thapsigargin. These results relate to previous findings demonstrating that 2-APB is an activator of Orai3 in the plasma membrane [118].

Taken together these studies indicate that ER-localized Orai channels can play a role in the ER Ca^{2+} leak, though further study is needed to assess their exact physiological role.

3.3. Transient Receptor Potential (TRP) channels

The TRP channels form a very large family with a total of 27 members in humans, divided in 6 main families, i.e. TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPP (polycystin) and TRPML (mucolipin). All members of these various families form cation-permeable channels that can exhibit anything between either a very high and a very low Ca^{2+} selectivity. These channels are responsible for a large variety of biological functions and their dysfunction has been implicated in various pathological conditions [119, 120]. The majority of these channels are expressed at the plasma membrane but some are, at least partially, expressed at the ER/SR (Fig. 1). Endogenously expressed TRP channels proposed to contribute to the ER/SR Ca^{2+} leak are detailed in the paragraphs below. In addition, transient transfection studies showed that overexpressed TRPC3 channels also

localize to the ER and spontaneously decrease its Ca^{2+} content [121], supporting the idea that under the proper conditions, TRP channels can form Ca^{2+}-leak channels in the ER.

3.3.1. TRPC1

In skeletal muscle, TRPC1 is present in the plasma membrane as well as in the longitudinal SR [122]. In the latter, TRPC1 functions as a Ca^{2+}-leak channel, increasing the $[Ca^{2+}]_{cyt}$ at rest and during muscle activation [122]. The same function is described in cardiomyocytes, where TRPC1 is mainly expressed in the SR and is associated with a decreased SR Ca^{2+} content [123].

3.3.2. TRPC6

In myocytes, TRPC6 is overexpressed after myocardial infarction, which can induce hypertrophic signaling and SR Ca^{2+} leak [124]. In resting platelets, TRPC6 localizes to the ER, where it regulates the passive Ca^{2+}-leak rate from agonist-sensitive intracellular Ca^{2+} stores [125]. In neuroblastoma cell lines, STIM1 overexpression augmented TRPC6 at the ER membrane, resulting in an enhanced rate of passive Ca^{2+} leakage from the ER [126]. Findings by another group demonstrated in dedifferentiated liposarcoma that also the anti-ageing Klotho increased the ER Ca^{2+}-leak rate by reducing TRPC6 exocytosis and thus maintaining it at the ER [127].

3.3.3. TRPM8

TRPM8 is a non-selective cation channel activated by low temperatures (<28 °C), pressure, and cooling compounds as for instance menthol and icilin [128]. This channel is found in multiple organs and tissues, and regulates important processes such as cell proliferation, migration and apoptosis, inflammatory reactions, immunomodulatory effects, pain, and vascular muscle tension [129]. In the LNCaP human prostate epithelial cell line, TRPM8 is not present at the plasma membrane but is localized at the ER (Fig. 1), where its expression is androgen-dependent [130]. TRPM8 acts in those cells as an ER Ca^{2+}-release channel activated by cold, menthol and icilin. Subsequently, a shorter isoform of TRPM8 was described in keratinocytes [131]. At variance with canonical TRP channels with 6 transmembrane domains, this truncated TRPM8 displays only 4 transmembrane domains (4TM-TRPM8). ER-localized TRPM8 and 4TM-TRPM8 are expressed in multiple tissues, but in contrast to TRPM8, 4TM-TRPM8 expression is restricted to the ER and more in particular to the ER-mitochondria contact sites. Moreover, from prostate samples 3 distinct 4TM-TRPM8 isoforms were cloned [132]. All three isoforms function as ER Ca^{2+}-leak channel decreasing ER Ca^{2+}-store content while supporting mitochondrial Ca^{2+} uptake.

3.3.4. TRPP2

TRPP2, a.k.a. polycystin 2, is a non-selective cation channel of the TRP superfamily predominantly expressed at the ER, but also present in the plasma membrane and the primary cilia [133, 134]. Loss-of-function mutations in this channel are responsible for autosomal dominant polycystic kidney disease [135-137]. In the ER, TRPP2 can function as a Ca^{2+}-activated channel permeable to Ca^{2+} [138].

The open-state probability of TRPP2 follows a bell-shape Ca^{2+} response with low levels of Ca^{2+} (up to ~1 μM) increasing TRPP2 channel activity, whereas high Ca^{2+} levels (≥ 1 μM) are inhibitory [139]. Moreover, TRPP2 can interact with both the IP_3R and the RyR. The interaction of TRPP2 with the IP_3R prolongs IP_3-induced Ca^{2+} transients thereby establishing Ca^{2+} microdomains initiating Ca^{2+}-induced Ca^{2+} release through TRPP2 [140-143]. On the contrary, TRPP2 modulates the Ca^{2+} transients by binding to the RyR2 in its open state and inhibiting its channel activity [73], which minimizes the RyR2-mediated Ca^{2+} leak (*see 2.2*).

In MDCK kidney cells TRPP2 has been proposed to either regulate an ER Ca^{2+} leak that could be inhibited by polycystin 1 [144] or to directly contribute to the Ca^{2+} leak [145]. The latter conclusion was largely based on the fact that TRPP2 knockdown led to a decrease of the ER Ca^{2+}-leak rate and an increase in releasable Ca^{2+}. However, heterologous TRPP2 reexpression in TRPP2 knockout renal proximal tubule epithelial cells did neither affect the ER Ca^{2+}-leak rate nor the amount of Ca^{2+} that could be released from the ER [141], so that the role of TRPP2 as ER Ca^{2+}-leak channel remains unclear at best.

3.3.5. TRPV1

TRPV1 is a non-selective cation channel activated by noxious heat, protons, and vanilloids. TRPV1 is highly expressed in the central nervous system, where its localization in the plasma membrane is involved in the transduction of painful stimuli. In addition, TRPV1 is intracellularly located in various neuronal and non-neuronal tissues, and especially at the ER/SR, functioning as a non-selective Ca^{2+} channel [146]. Interestingly, both endogenously and heterologously expressed TRPV1 localized to a subcompartment of the ER, that although IP_3-sensitive, didn't seem to impact SOCE [147, 148].

Activation of TRPV1 localized in the ER of dorsal root ganglion neurons elicits an increase of cytosolic Ca^{2+} due to ER Ca^{2+} release [149]. However, Ca^{2+}/calmodulin decreased the sensitivity of the ER-located TRPV1 compared to the plasma membrane one, which may be important to avoid ER Ca^{2+}-store depletion, ER stress and cell death. In skeletal muscle cells, TRPV1 localized in the (predominantly longitudinal) SR acts as a Ca^{2+}-leak channel, responsible for SR Ca^{2+} leakage in the relaxed muscle and mediating a secondary SR Ca^{2+} liberation after RyR1 activation [150, 151].

3.4. Mitsugumin 23 (MG23)

MG23 is a small (23 kDa) protein with 3 putative transmembrane domains, originally identified in skeletal muscle in the outer nuclear membrane and the SR, including the terminal cisternae [152]. Unexpectedly, MG23 was not only expressed in skeletal, cardiac and smooth muscles, but also in many other, though not all, cell types. Affinity purification and particle analysis of MG23 led to the conclusion that it can form large homomultimeric complexes that can transiently assemble / disassemble [153]. Moreover, electrophysiological analysis demonstrated that MG23 functions as a voltage-dependent channel, equally permeable to K^+ and Ca^{2+}. Based on these characteristics it was

proposed that MG23 could function either as a Ca^{2+}-leak channel (Fig. 1) or as a channel playing a role in counterion movement during electrogenic Ca^{2+} uptake or release. More recent work supported a role for MG23 in the diastolic Ca^{2+} leak in heart. Increasing Zn^{2+} concentration to the nM range not only activated RyR2 (*see 2.2.*), but also affected MG23 function [99]. More specifically, H9C2 cardiac cells demonstrated under ischemic conditions increased Zn^{2+} levels and increased MG23 expression. Moreover, after incorporation of cardiac SR in lipid bilayers, the amplitude of the Ca^{2+} current passing through MG23 channels increased from non-observable in the absence of Zn^{2+} to -2 pA in the presence of 1 nM Zn^{2+}. As ischemic heart failure lead to Zn^{2+} dysregulation, these results strongly support MG23 as an additional Ca^{2+}-leak channel alongside RyR2 in this pathological situation. It is presently not known whether MG23 might have a similar role in other cell types as well, and it is also not clear whether its protective role against UVC-induced cell death is somehow related to its Ca^{2+}-transport properties [154].

4. PRO- AND ANTI-APOPTOTIC PROTEINS AND THE Ca^{2+} LEAK

It is widely accepted that various pro- and anti-apoptotic proteins perform in part their function by interfering with intracellular Ca^{2+} handling [155-158]. Some of them were also proposed as direct or indirect mediators of the ER Ca^{2+} leak.

4.1. Bcl-2

Bcl-2 is the founding member of a large family of pro-apoptotic and anti-apoptotic proteins characterized by the presence of one or several Bcl-2 homology domains. The balance between those various proteins and the dynamic interactions occurring between them provide for exquisite mechanisms to control apoptotic cell death [158-160]. Bcl-2 itself is a small (26 kDa) anti-apoptotic protein with one transmembrane domain. It is present both at the mitochondria and at the ER. Bcl-2 performs its anti-apoptotic function on the one hand through interaction with its pro-apoptotic family members, thereby preventing mitochondrial outer membrane permeabilization (MOMP) and on the other hand by modulating intracellular Ca^{2+} dynamics, thereby decreasing Ca^{2+} uptake in the mitochondria. The latter function is performed mainly via its regulatory action on various Ca^{2+} channels including the IP_3R and the RyR in the ER and the voltage-dependent anion channel in the outer mitochondrial membrane, as we recently reviewed [44].

Although many groups did not observe an effect of Bcl-2 on ER Ca^{2+}-store content [44, 161], a number of reports demonstrated that at least in some situations Bcl-2 could decrease the ER Ca^{2+}-store content [162-165]. This can be explained by either (i) an inhibitory effect of Bcl-2 on the Ca^{2+} uptake in the ER, (ii) a stimulation of Ca^{2+}-release channels of the ER, or (iii) a direct effect on ER permeability.

Related to the first mechanism (Fig. 4a), in LNCaP human prostate cancer cells it was shown that Bcl-2 overexpression led to a downregulation of SERCA2 and of calreticulin [164]. This decreased capacity for ER Ca^{2+} uptake and retention led to a decreased Ca^{2+} content in the ER, which could be potentially amplified by a parallel increase in Ca^{2+} leakage. The effect of Bcl-2 on SERCA activity was confirmed by the Schönheich group who demonstrated that the interaction of Bcl-2 with SERCA1 led in skeletal muscle to partial unfolding of the latter leading to its full inhibition [166]. Consequently, they demonstrated that this inhibition is accompanied by SERCA1 translocation from the caveolae-related domains to the higher density fractions of the SR [167] and that the same applies for SERCA 2b [168] and SERCA3b in HEK293 cells [169]. Moreover, this inactivation can be counteracted by HSP70 and other chaperones [168]. Some ambiguity however remains, as an older study performed in the MCF10A breast epithelial cell line demonstrated an increased expression level and activity of SERCA subsequently to heterologous Bcl-2 expression [170], suggesting that cell-specific elements could be involved.

Concerning the second mechanism (Fig. 4b), we already mentioned above (*see 2.1.*) the possibility that an interaction of Bcl-2 with the IP_3R can lead to its hyperphosphorylation and its sensitization to IP_3 [51]. Other studies have provided evidence that IP_3R can be sensitized by direct interaction with various Bcl-2-family members including Bcl-2 [171], although opposite results were found in other studies [172-175].

Finally, a direct action of Bcl-2 on the ER permeability (Fig. 4c) was proposed [163]. This idea is compatible with data obtained in planar lipid bilayers demonstrating that Bcl-2, the related Bcl extra-large (Bcl-XL) and the pro-apoptotic family member Bax can form cation-selective channels in artificial membranes [176-178]. In planar lipid bilayers Bcl-2, but not its deletion mutant missing helices α5 and α6, formed at neutral pH, under symmetric conditions, cation-selective channels with a primary conductance (18 pS) compatible with the formation of a Bcl-2 homodimer [176]. Greater conductance levels that were less frequently observed can be due to further oligomerization. A follow-up study by the Rizzuto group however demonstrated that the reduction of the ER Ca^{2+} content by Bcl-2 didn't depend on helices α5 and α6, which could be swapped by those from Bax, making one of the other mechanisms more likely [179].

Whatever the mechanism(s) involved, a further complexity arises, as Bcl-2's role in the ER Ca^{2+} leak can additionally depend on its interaction with other proteins (Fig. 4d). Firstly, Bcl-2 (and the related Bcl-XL) can interact with Bax inhibitor-1 (BI-1) [180]. As discussed further below (*see 4.3.1.*), BI-1 has been proposed to act as a Ca^{2+}-leak pathway. Moreover, in BI-1 knockout cells (HeLa and MEF cells), although Ca^{2+}-store content was increased, Bcl-XL was unable to lower $[Ca^{2+}]_{ER}$ showing that BI-1 acted downstream of Bcl-XL and, presumably, Bcl-2 [181]. Second, Bcl-2 interacts with CDGSH iron-sulfur domain-2 (CISD2, also named Naf-1) [182]. In the human non-small cell lung carcinoma H1299

cell line, ER-targeted Bcl-2 required the presence of CISD2 to reduces the ER Ca^{2+}-store level [182, 183]. These results would suggest that Bcl-2 can only elicit a Ca^{2+} leak from the ER in the presence of associated proteins. As both BI-1 [184] and CISD2 [182] also interact with the IP_3R, a role for the latter in this process can thus also not been excluded.

It must thereby be reminded that many other studies indicate that Bcl-2 and Bcl-Xl interact in a complex way with the IP_3R and affect the function of the latter in different ways, though generally without affecting ER Ca^{2+} store content [44].

4.2. Bax

Bax (21 kDa) is one of the pro-apoptotic members of the Bcl-2 family. It is in healthy cells predominantly located in the cytosol but translocates during apoptosis to the outer mitochondrial membrane where it forms, together with Bak, large proteinaceous pores that enable the release into the cytoplasm of mitochondrial apoptosis-inducing factors as e.g. cytochrome c. This MOMP process is considered the point of no return in apoptosis execution [159, 160]. However, a small fraction of Bax appear to localize at the ER, where it may be involved in the regulation of Ca^{2+} handling [185, 186]. Although several reports indicate that Bax/Bak knockout lead to a decreased ER Ca^{2+} content (e.g. [51], *see 2.1.*), Bax overexpression did not affect ER Ca^{2+} levels [179]. More recently, some evidence on the role of Bax in the ER Ca^{2+} leak in pancreatic acinar cells have been presented [187]. In particular, it was shown that loss of Bax (but not of Bak or Bcl-2) inhibited the Ca^{2+} release induced by BH3-mimetics and that the Ca^{2+} leak observed after thapsigargin application was slower in Bax knockout cells than in wild-type cells. However, additional work will be needed to clarify the exact role, direct or indirect, of Bax in the ER Ca^{2+}-leak process.

4.3. Transmembrane Bax inhibitor-1 motif-containing (TMBIM) family members

The TMBIM family consists of 6 proteins of between 25 and 40 kDa that share a very similar structure as well as physiological functions but have a distinct expression pattern and/or a different intracellular localization [188-190]. Their main characteristics are the presence of six or seven transmembrane domains, with the last one being only partially hydrophobic, their effects on intracellular Ca^{2+} handling and their anti-apoptotic action. In the framework of this review we will focus on BI-1 (also named TMBIM6), the founding member of this family, and on the Golgi anti-apoptotic protein (GAAP, also named TMBIM4). For both BI-1 and GAAP evidence was proposed for a structure with both the N- and C-termini in the cytosol and in between 6 transmembrane domains containing at the C terminus a reentrant loop that may form (part of) the pore [191, 192]. It should also be noted that these both proteins, together with TMBIM2 that is however predominantly present in the Golgi apparatus and the plasma membrane, are upregulated in many cancer types and shown to support several cancer hallmarks [193].

4.3.1. BI-1

In a yeast screening, BI-1 was originally described as a novel type of negative regulator of the Bax-dependent cell death pathway [180]. This evolutionary highly conserved protein protects the cells especially against ER stress and ischemia–reperfusion injury [194]. In spite of its name, BI-1 does not interact directly with pro-apoptotic Bax but, interestingly, was reported to reduce the ER Ca^{2+} level [181, 195, 196]. There is strong evidence that BI-1 forms a Ca^{2+}-permeable channel in the ER [191, 197, 198] causing a Ca^{2+} leak (Fig. 1). The C-terminal region of BI-1 contains its Ca^{2+}-channel pore domain, whereby D^{209} and D^{213} are critical for the Ca^{2+}-flux properties [191]. Moreover, the Ca^{2+}-flux properties of BI-1 are regulated in a bell-shaped way by the pH with a maximal permeability at neutral pH [199]. Additionally, BI-1 can interact with the IP_3R and sensitize it [184]. This function is however not dependent on the Ca^{2+} released by BI-1, as it could be mimicked by the channel-death mutant BI-1^{D213R}.

The Ca^{2+} ions released either directly by BI-1 or indirectly via sensitization of the IP_3R are physiologically as well as pathologically relevant, as they were shown to be involved in various important processes, as e.g. immune function [200], insulin secretion [201], or autophagy [202, 203].

4.3.2. GAAP

GAAP is an important anti-apoptotic protein expressed in eukaryotes but it is also present in prokaryotes as well as in a number of viruses [193]. Most studied were the human (hGAAP) and the viral (vGAAP) forms. hGAAP is expressed in all tissues in the Golgi apparatus, but in part also localize to the ER. Its overexpression in U2OS osteosarcoma cells decreased the ER Ca^{2+} content as well as the efficacy of agonist- or IP_3-induced Ca^{2+} release, while its knockdown led to the converse results [204]. Similarly to BI-1, GAAP interacted with the IP_3R though the sensitivity of the latter was not affected, strongly indicating that the decreased potency of IP_3-induced Ca^{2+} release was due to the lower Ca^{2+} store content. However, no SOCE activation was observed. The cation-conducting properties of vGAAP were confirmed after incorporation in planar lipid bilayers and amino acids E^{207} and D^{219} were identified as crucial for its conductance and/or ion selectivity [198].

In apparent contradiction with the study by de Mattia et al. [204], another study found in U2OS cells as well as in HeLa cells that the decrease in Ca^{2+} store content due to hGAAP elicited SOCE, resulting in enhanced cell adhesion and migration [205]. These effects were linked to the localized activation of the Ca^{2+}-dependent protease calpain 2 and an increased turnover of focal adhesions. More recent work indicated that the hGAAP-mediated Ca^{2+} release from ER and Golgi apparatus could via mitochondrial metabolism and ROS production also be more directly involved [206].

4.4. R-Ras

R-Ras belongs to the Ras family of small G-proteins and was shown to display, at least under some conditions, anti-apoptotic effects. A study performed in CHO cells demonstrated that overexpression of constitutively active V38R-Ras, but not of the dominant negative N43R-Ras, decreased both the

thapsigargin- and the agonist-induced Ca^{2+} transients [207.6]. As however the rate of rise of the thapsigargin-induced Ca^{2+} transient increased, it was concluded to an increased Ca^{2+} leak leading to a decreased Ca^{2+}-store content, which could explain the anti-apoptotic effect. How R-Ras mechanistically affects the ER Ca^{2+} leak was not elucidated, though the authors considered that the effect might be indirect, and related to the potential upregulation of Bcl-2.

5. CONNEXIN-LIKE PROTEINS

Connexins form a family of plasma membrane proteins with in humans 20 different genes coding for proteins with a molecular mass varying between 26 and 60 kDa. Those proteins are characterized by having their N- and C-termini in the cytosol and to contain four transmembrane domains, 2 extracellular and 1 large cytosolic loop [208, 209]. These proteins are well-known for their role in forming gap junctions between cells to allow intercellular communication of molecules up to a molecular mass of about 1 kDa. To this aim, 6 connexins assemble to form a connexon that interacts with a similar connexon located in the plasma membrane of a neighboring cell. In certain cases, the connexon does not associate and can function as a so-called hemichannel that plays a role in paracrine communication [208, 209].

5.1. Pannexins

Pannexins are structurally similar to connexins, though have evolved evolutionarily in a separate way, and are more related to the innexins, the proteins forming gap junctions in invertebrates. In contrast to the connexins, the pannexin family counts in mammals only three members: pannexin 1, 2 and 3 [210]. Pannexins and connexins can be co-expressed in the same cells and both are regulated independently and perform distinct cellular functions. Typically, pannexins have much longer half-time than connexins and preferentially form hemichannels. The latter are permeable for molecules less than 1 kDa, and allow passage of e.g. Ca^{2+}, IP_3 and amino acids, but especially the release of ATP has been described in multiple cell types [211]. In addition to these functions, a role for pannexins in ER Ca^{2+} homeostasis has been proposed (Fig. 1).

5.1.1. Pannexin 1

A study investigating the function of pannexin 1 in LnCaP prostate cancer cells and in HEK293 cells overexpressing the protein demonstrated not only the formation of Ca^{2+}-permeable gap junctions between adjacent cells but also pannexin 1 expression at the ER leading to an increased ER Ca^{2+} leakage and consequently a decrease in ER Ca^{2+} store content [212]. Downregulation of pannexin 1 in human prostate cancer cells led to the converse effect. Moreover, overexpression of two different connexin isoforms (Cx32 and Cx43) in the same cells did not led to an increased Ca^{2+} leak, demonstrating the specificity of the process.

5.1.2. Pannexin 3

Pannexin 3 was described as an important regulator of osteoblast differentiation acting via 3 distinct mechanisms: as hemichannel releasing ATP to the extracellular space, as part of gap junctions propagating Ca^{2+} waves and as ER Ca^{2+} channel [213]. In the latter function, pannexin 3 is activated by protein kinase B, subsequently to the activation of the phosphoinositide 3-kinase pathway by extracellular ATP. This kinase is responsible for the phosphorylation of S^{68} on pannexin 3, although this may occur indirectly. Interestingly, the S68A mutation inhibits Panx3-mediated osteoblast differentiation, and this uniquely via affecting the pannexin 3 in the ER [214]. This indicates that the gating of pannexin 3 as ER Ca^{2+} channel is differently regulated than its hemichannel and gap junctional properties, suggesting the existence of separate mechanisms.

5.2. Leucine-rich repeat-containing 8 protein (LRRC8) B

LRRC8 proteins are characterized by having an N-terminal region containing 4 transmembrane domains and a high similarity to pannexins to which they evolutionary relate, followed by up to 17 leucine-rich repeats in their C-terminus [215]. Five paralogs, LRRC8A-E, exist that all play a role in cellular communication. LRRC8A, in combination with one or more of its paralogs is involved in the formation of the volume-regulated anion channel, but the exact role thereby of LRRC8B remains to be clarified [216]. When overexpressed in HEK293 cells, LRRC8B leads to a decreased ER Ca^{2+}-store content, decreased Ca^{2+} signaling and an increased Ca^{2+} leak out of the ER [217]. These effects could successfully be counteracted by LRRC8B knockdown while not be mimicked by LRRC8A. Moreover, both endogenous and overexpressed LRRC8B predominantly localize at the ER (Fig. 1), in contrast to LRRC8A, which localize at the plasma membrane. Taken together, these results support a role of LRRC8B as an ER Ca^{2+}-leak channel.

5.3. Calcium homeostasis modulator 1 (CALHM1)

CALHM1 is a multipass transmembrane glycoprotein showing structural similarity with the members of the connexin, pannexin and innexin families [218]. It possesses 4 transmembrane domains, cytosolic N- and C-termini and assembles into hexamers, having a pore diameter of about 14 Å, enabling permeation of large, charged molecules. It was first described as a neuronal plasma membrane Ca^{2+} channel that controls amyloid β levels and susceptibility to late-onset AD [219]. As CALHM1 is also expressed in the ER it was investigated whether it could affect ER Ca^{2+} handling. Overexpression of CALHM1 in HEK293T cells indicated that it not only increased the Ca^{2+} leak from the ER (Fig. 1) but also reduced transport capacity and the Ca^{2+} affinity of the SERCA pumps [220]. The resulting 7-fold reduction in ER Ca^{2+} store content triggered ER stress and the unfolded protein response (UPR). As CALHM1 is predominantly expressed in neurons it is not likely to play a universal role as Ca^{2+}-leak channel, though it may be involved in the development of neurodegenerative diseases.

6. ER PROTEINS ASSOCIATED TO THE RIBOSOMES

6.1. Translocon

The translocon plays an essential role in protein synthesis by allowing the transport of nascent proteins into the ER as well as by facilitating numerous co-translational processes [221, 222]. The core of the translocon is formed by the heterotrimeric Sec61 protein-conducting channel complex with its α, β and γ subunits. To perform the various co-translational functions, several other proteins interact transiently or stably with the translocon. These include the translocon-associated protein complex, the oligosaccharyl transferase complex, various chaperones/Ca^{2+}-binding proteins including calreticulin, calnexin and binding immunoglobulin protein (BiP, also named GRP78), or inositol-requiring enzyme 1, which is one of the initiators of the UPR [221, 223].

Important in the context of this review, it was convincingly shown that the translocon with its large aqueous channel could, in its protein-free form, provoke Ca^{2+} leakage out of the ER [115, 223, 224], leading to a sufficient Ca^{2+} depletion to activate SOCE [225, 226].

As a continuous leak of Ca^{2+} out of the ER would disturb the cellular Ca^{2+} homeostasis and thus impair cell function, regulatory mechanisms exist to control the Ca^{2+} leak through the translocon in the absence of protein translation. Hence, translocon-mediated Ca^{2+} leakage is counteracted by the cytosolic Ca^{2+}-binding proteins calmodulin [227], Sec62 [228] and the intraluminal BiP [229, 230] (Fig. 5).

Although a meaningful role for the translocon as Ca^{2+}-leak channel under physiological conditions has been questioned (i.e. in the absence of puromycin to keep the translocon open) [231], different studies indicate that uncontrolled Ca^{2+} leakage through the translocon results in patho(physio)logical consequences, especially in situations of ER stress. The translocon inhibitor anisomycin thus not only blocked the Ca^{2+} leak, but also antagonized apoptosis, indicating that during ER stress the Ca^{2+} leakage out of the ER through the translocon contributes to cell death [230]. An interesting feature is that anisomycin reduces BiP expression both under control conditions and under ER stress conditions, underscoring their interrelation. The importance of the translocon-mediated Ca^{2+} leak for the UPR was at about the same time confirmed for Xenopus oocytes [232]. Interestingly, the translocon can be involved in type 2 diabetes. First, a mutation affecting BiP binding to the translocon or knockdown of BiP co-chaperones leading to increased Ca^{2+} leakage induced β-cell failure and diabetes [229, 233]. Second, while palmitate treatment reduced in β cells ER Ca^{2+} content, thereby inducing ER stress and decreasing insulin secretion, translocon inhibition by anisomycin reversed these dysfunctions [234], indicating in all those conditions the importance of controlling Ca^{2+} leakage through the translocon for maintenance of cellular health. In cardiomyocytes, translocon activation led to Ca^{2+} release from intracellular stores without interfering with excitation–contraction coupling. Puromycin pretreatment affected mitochondrial Ca^{2+} handling and slowed

down mitochondrial permeability transition pore opening [235]. Moreover, this treatment protected the cardiomyocytes in ischemia-reperfusion conditions, demonstrating that proper modulation of the translocon can have therapeutical implications.

A new perspective on the role of the translocon as Ca^{2+}-leak channel was obtained by the observation that in dedifferentiated liposarcomas the anti-ageing Klotho not only maintains TRPC6 at the ER (*see 3.3.2.*) but also enhances Ca^{2+} leakage through the translocon, leading to subsequent apoptosis [127]. This process appears at least in part mediated by the IGF1 receptor and ERK inhibition but the exact mechanism must still be elucidated.

Moreover, the translocon and its regulation by BiP allows for a connection between ER Ca^{2+} handling, protein folding capacity, and UPR induction [236]. Furthermore, the translocon may regulate ATP import in the ER, which is essential for BiP function. Zimmerman and coworkers propose that low $[ATP]_{ER}$ can cause a decrease in BiP activity and an increased Ca^{2+} leakage out of the ER. The decreased $[Ca^{2+}]_{ER}$ and the increased $[Ca^{2+}]_{cyt}$ would activate the ER ADP/ATP exchanger allowing for a recovering of $[ATP]_{ER}$ and binding of BiP to the translocon, inhibiting the Ca^{2+} leak [221, 237].

Finally, a number of pathogenic mutations in the Sec61 α subunit have been reported, leading to various clinical phenotypes [236]. Interestingly, some of those mutations affect Ca^{2+} leakage through the translocon [238, 239]. Especially V67G, V85D and Q92R have been shown to increase the Ca^{2+} leak through the translocon (Fig. 5a), to contribute to ER stress and the development of plasma cell deficiency [238], and to severe congenital neutropenia [239].

6.2. Transmembrane and coiled-coil domain 1 (TMCO1)

TMCO1 is a 21 kDa protein associated with the ribosomes and the translocon and which is evolutionary highly conserved. A mutation resulting in a truncated protein led to a form of cerebrofaciothoracic dysplasia [240]. TMCO1 contains two transmembrane domains in its N-terminal half with further downstream a hydrophobic stretch while both N- and C-termini are located in the cytosol [241].

Interestingly, ER Ca^{2+}-store overfilling induces the formation of a tetrameric TMCO1 complex acting as a Ca^{2+}-selective channel. This channel releases Ca^{2+} from the ER, thereby counteracting the overfilling of the store, earning it the name of a Ca^{2+} load-activated Ca^{2+} channel (Fig. 1). In this process the tetrameric structure rapidly disassembles. As in resting conditions already 20% of TMCO1 is tetrameric, this protein participates to a small proportion of the basal Ca^{2+} leak, but its main function appear to be to protect the cell from abnormal Ca^{2+} signaling related to ER Ca^{2+}-store overfilling [241]. Moreover, TMCO1 appeared essential for ovarian follicle development [242] as well as osteoblast function [243]. In the latter cells the TMCO1-mediated ER Ca^{2+} leak activated CaMKII, subsequently promoting via its phosphorylation the nuclear exit of HDAC4 so that the RUNX2 transcription factor, a master regulator of osteogenesis, remained acetylated and thus active.

7. PRESENILINS

Presenilin 1 and 2 are 50 kDa proteins with 9 transmembrane domains that are for 67% identical. Both are predominantly expressed in the ER and the Golgi apparatus. They are best known for forming the catalytic core of the γ-secretase complex, an intramembrane-cleaving protease responsible for cleavage of multiple proteins including e.g. the amyloid precursor protein and the Notch receptors. The catalytic aspartates D^{257} and D^{385} responsible for the cleaving are resp. located in the 6^{th} and 7^{th} transmembrane domains (Fig. 6). As such, presenilin 1 and 2 mutants form the main genetic risk factors for familial Alzheimer's disease (FAD). In addition to this function, presenilins perform various γ-secretase-independent roles, such as modulation of intracellular Ca^{2+} handling [244-248].

Presenilins can regulate Ca^{2+} loading as well as Ca^{2+} release from the intracellular Ca^{2+} stores by affecting SERCA [249], IP_3R [48, 250-252] and RyR [253-255] expression and/or activity. Moreover, presenilins can also interact with BI-1 [256], which itself forms a Ca^{2+}-leak channel (*see 4.3.1.*).

A general finding, reviewed by Frank LaFerla already in 2002 was that the expression of presenilin FAD mutants led to ER Ca^{2+}-store overloading [244]. This is compatible with the presenilins acting as ER Ca^{2+}-leak channels but does not exclude effects via other ER Ca^{2+}-handling proteins. The first report indicating that presenilins can themselves function in a direct way as Ca^{2+}-leak channels showed that wild-type presenilins, but not FAD mutants, form low-conductance divalent-cation-permeable channels when incorporated in planar lipid bilayers [257]. Moreover, comparison between wild-type MEFs and MEFs in which both presenilins were knocked out indicated that 80% of the Ca^{2+} leak depends on the presenilins. In a subsequent study, these results were confirmed and extended by showing that the various presenilin 1 FAD mutants corresponded to inactive channel activity, while presenilin 1 mutations associated with frontal temporal dementia did not affect channel activity [258]. Interestingly, presenilins do not play an identical role in the ER Ca^{2+} leak in all neurons [259]. For example, they have a much more important role in hippocampal than in striatal neurons.

The situation for presenilin 2 seemed however different than that for presenilin 1, since both wild-type and FAD mutants of presenilin 2 can lower the ER Ca^{2+}-store content [260, 261]. A further study from the same group, investigating a larger number of both presenilin 1 and 2 FAD mutations demonstrated that mutations in both presenilin isoforms led to a reduced ER Ca^{2+} content, but that the effect was the largest for the presenilin 2 mutants [262]. Interestingly, presenilin 2 emerged as a third candidate ER Ca^{2+}-leak channel from the previously mentioned (*see 2.1. and 3.2.*) siRNA screening study [50]. Moreover, in the same study, knockdown of presenilin 2 in HEK293T cells significantly decreased the ER Ca^{2+} leak and increased the ER Ca^{2+} load, while its overexpression led

to the opposite result. Taken together these results indicate a certain, but complex, role for presenilins in ER Ca^{2+} handling.

However, there are also studies that do not support the role of presenilins as Ca^{2+}-leak channels. Work in DT40 cells indicated that the presenilin 1-induced Ca^{2+} leak could be inhibited upon IP_3R inhibition or knockout, pointing to an indirect action of presenilins via the IP_3R [251]. In subsequent work from the same group, various intraluminal indicators were used to measure $[Ca^{2+}]_{ER}$ in primary neurons, fibroblasts and conditional presenilin double knockout B cells [263]. No consistent differences in ER Ca^{2+}-leak rates, ER Ca^{2+}-loading rates or steady-state $[Ca^{2+}]_{ER}$ were found between cells overexpressing wild-type or mutant presenilin 1 or presenilin-deficient cells. Noteworthy, presenilin 2 as such was not investigated in this study.

Finally, it should be pointed out that recent work on the one hand demonstrated that the presenilin 1-mediated Ca^{2+} leak can occur in more cell types than only in neurons or cellular model systems and on the other hand that it can be subject to critical regulation mechanisms. Indeed, the presenilin 1-mediated Ca^{2+} leak was observed in pancreatic islets as well as β-cell lines [264]. This leak was directed towards the mitochondria, where it could modulate respiration and the ATP production. Moreover, it appeared that phosphorylation of presenilin 1 by glycogen synthase kinase 3 β at S^{353} and S^{357} (Fig. 6) was critical for presenilin 1's ER Ca^{2+}-leak properties [264]. Interestingly, these phosphorylation sites are located in the same cytosolic loop as the endoproteolytic cleavage site [265]. The endoproteolytic cleavage site leads to the formation of a N-terminal fragment and a C-terminal fragment (Fig. 6). This cleavage is important for the correct maturation of the presenilins and for obtaining proper γ-secretase activity. It is therefore remarkable that the phosphorylation sites regulating the Ca^{2+} leak through presenilin 1 are exactly located in the cytosolic loop between transmembrane domains 6 and 7 containing the cleavage site. This may indicate that the region around transmembrane domains 6 and 7 are not only important for γ-secretase activity but also for the Ca^{2+}-leak pathway.

Follow-up work in pancreatic islets and β cells revealed that glucose-triggered $[Ca^{2+}]_{cyt}$ oscillations and subsequent insulin secretion depended on the Ca^{2+}-leak activity of presenilin 1 [266]. As ER-mitochondria Ca^{2+} transfer is important for adequate insulin release [267, 268], disturbances in the presenilin 1-dependent ER Ca^{2+} leak towards the mitochondria may contribute to postprandial hyperglycemia and a subsequent development of type 2 diabetes mellitus. In addition, the dependence of the ER Ca^{2+}-leak properties of presenilin on post-translational modification may explain at least part of the divergent results found in the literature about the properties of presenilins. These findings warrant that further work should be pursued to fully understand the regulatory mechanisms acting on presenilin 1 and 2.

8. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Hence, it is clear that multiple proteins can function as ER Ca^{2+}-leak channels, depending on the cell type and/or the intracellular conditions. Each of those Ca^{2+}-leak channels will participate in controlling the steady-state Ca^{2+} level in the ER thereby impacting intracellular Ca^{2+} signaling. Such changes in ER Ca^{2+} dynamics will affect the function of downstream effectors and of ER-associated organelles such as the mitochondria. A proper exchange of Ca^{2+} between ER and mitochondria is vital for proper cell function [14, 269]. Moreover, several of the Ca^{2+}-leak channels appear to be preferentially located at this ER-mitochondria interface (e.g. truncated SERCA1T, the 4TM-TRPM8 isoforms and presenilin 1) and thus be uniquely positioned to impact/control mitochondrial metabolism and behavior (Fig. 7). Adequate Ca^{2+} flux from the ER to the mitochondria will support mitochondrial bioenergetics as observed for presenilin 1 in pancreatic islets and in β-cell lines [264] (Fig. 7a). However, exaggerated Ca^{2+} flux to the mitochondria, will lead to mitochondrial permeability transition pore opening and thus cell death (Fig. 7b) as reported for SERCA1T [109] and for the 4TM-TRPM8 isoforms [270]. Effects of Ca^{2+}-leak channels on mitochondrial metabolism and function may however also be indirect, and related to the filling state of the ER. Higher ER Ca^{2+} levels will lead to larger Ca^{2+} signals to cytosol and/or mitochondria. This risk is alleviated when TMCO1 is expressed. The activity of this channel depends on the Ca^{2+} load of the ER and thus protects the cell from ER overfilling [241]. BI-1, which is not preferentially located at ER-mitochondria contact sites, protects cell from apoptosis by decreasing the $[Ca^{2+}]_{ER}$ [191, 195]. Additionally, by sensitizing the IP_3R [184], it can lower the $[Ca^{2+}]_{ER}$ down to a level that the Ca^{2+} flux between ER and mitochondria is so low that mitochondrial bio-energetics is compromised, thereby inducing autophagy [202] (Fig 7c).

Several of the Ca^{2+}-leak pathways mentioned in this review are mostly recognized for their canonical cell biological functions, as e.g. the translocon and the presenilins. However, also bona fide Ca^{2+}-leak channels may perform additional functions. Sitsapesan and collaborators speculate that the large number of potential non-specific Ca^{2+}-leak channels expressed in the ER, is related to their conductance for other ions than Ca^{2+} [38]. H^+ transport to stabilize intraluminal pH, or ion transport for charge compensation belongs to the possibilities, as well as the importance of maintaining a particular ionic composition in (subdomains of) the ER to allow optimal functioning of the various metabolic pathways present in the ER. Further work will be needed to clarify these various, not exclusive, possibilities.

Although probably everyone will agree that in most cells several Ca^{2+}-leak channels can be expressed simultaneously, this does not mean that all are active at the same time. The activity of the translocon will be largely dependent on the translational process, but also other Ca^{2+}-leak channels are subject to regulation. A prime example of this forms presenilin 1, which Ca^{2+}-leak activity seems to depend on its prior phosphorylation [264]. Another example is TMCO1 that only can form a tetrameric

structure acting as a Ca^{2+}-selective channel at very high $[Ca^{2+}]_{ER}$ [241]. Finally, the putative role of Bcl-2 as Ca^{2+}-leak channel is anyway of complex nature, due to its numerous possible interactions with other proteins also involved in ER Ca^{2+} handling which may or may not mediate or contribute to the leak (e.g. IP_3R, SERCA, BI-1, CISD2) [44, 157, 158, 271].

Apart from their function as regulator of ER Ca^{2+} handling in healthy cells, dysregulation of most, if not all, of the ER Ca^{2+} leak will either affect proper differentiation of the cells and/or plainly lead to pathological situations. Therefore, differences in expression, localization, regulation and/or activity of the Ca^{2+}-leak channels exist between healthy and diseased states. Moreover, pathological mutations have been described for several of those channels including the RyR, pannexin 3 and the translocon. The RyR is mostly important in muscle, including cardiac muscle, where in diseased state it strongly contributes to Ca^{2+} leakage, thereby supported by MG29 under ischemic conditions.

It is always difficult to make assessments on which protein would be the most important for a given function, but based on their expression patterns and their properties, one could propose that IP_3Rs, the translocon and probably also presenilins, should be considered as the most prevalent Ca^{2+}-leak pathways. It, however, remains important to consider that for specialized cell types the other channels discussed in this review may be of prime importance for the regulation of ER Ca^{2+} homeostasis and that their dysfunction can lead to developmental problems or severe pathologies.

Therefore, in our opinion, future work should especially focus on (i) the development of drugs that can act in a specific way on well-determined Ca^{2+}-leak channels, (ii) the understanding of the expression patterns, also during development, of the various Ca^{2+}-leak channels, (iii) the elucidation of the dynamic regulation mechanisms acting on those Ca^{2+}-leak channels, including by posttranslational modifications, and last but not least (iv) the further identification and characterization of human mutations in Ca^{2+}-leak channels and their patho(physio)logical consequences.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Legends to the Figures

Figure 1. Ca^{2+} handling by the ER is performed by a large variety of Ca^{2+} transporters. Ca^{2+} uptake in the ER is performed by ATP-driven Ca^{2+} pumps (SERCA). This uptake is balanced by controlled Ca^{2+} release by the "classical" Ca^{2+}-release channels, the inositol 1,4,5-trisphosphate receptors (IP_3Rs) and the ryanodine receptors (RyRs), but also by Ca^{2+} leakage out of the ER by a variety of other Ca^{2+}-permeable channels as depicted in the figure consisting of (starting from upper right in clockwise direction) calcium homeostasis modulator 1 (CALHM1), mitsugumin 23 (MG23), presenilins 1 and 2, the translocon and transmembrane and coiled-coil domain 1 (TMCO1) located in the rough ER, leucine-rich repeat-containing 8 protein B (LRRC8B), pannexins 1 and 3, Orai 3 (Orai 2 is also proposed as Ca^{2+}-leak channel but is not depicted), TMBIM-family proteins as BI-1 and a panoply of TRP channels (TRPC1, TRPC6, TRPP2, TRPV1 and TRPM8) of which for simplicity only the latter was drawn. All these proteins are subjected to cell-type dependent expression, splicing, cleavage, post-translational modifications and various other types of regulation and their leak activity therefore widely varies between cells, tissues and developmental stage. Furthermore, some leak channels are activated by (exogenous) compounds: the activation of Orai 3 by 2-APB and of MG23 by Zn^{2+} are indicated. Also shown is SERCA inhibition, e.g. by thapsigargin, that will uncover the part of the Ca^{2+} leak that under normal physiological conditions is compensated by the SERCA pump activity. The mechanisms of Ca^{2+} leakage through the IP_3R, the RyR, the translocon and the presenilins are explained in more details in Figs. 2, 3, 5 and 6 resp. while the possible modes of action of anti-apoptotic Bcl-2 in the Ca^{2+}-leak pathway are presented in Fig. 4. See text for more information.

Figure 2. IP_3R1 as ER Ca^{2+}-leak channel. IP_3Rs contain at their N-terminus the ligand-binding domain which is subdivided in a suppressor domain (SD) and an IP_3-binding core (IBC) to which IP_3 (*in turquoise*) binds. Binding of IP_3 triggers a conformational change leading to opening of the channel, located near the C-terminus. Two mechanisms are depicted. (a) Phosphorylation by PKA at S^{1588} and S^{1755} (*in green*) increases the sensitivity of IP_3R1 for IP_3. It had been proposed that binding of the anti-apoptotic Bcl-2 protein to IP_3R1 can affect the phosphorylation state of the receptor. The three binding sites for Bcl-2 identified on IP_3R1 are depicted (*in blue*). The Bcl-2 binding in the regulatory domain at a.a. 1389-1408 has been proposed to act as a scaffold for DARPP-32, a protein phosphatase 1 (PP1) inhibitor when phosphorylated at T^{34} by PKA (*in green*), leading thus to IP_3R1 hyperphosphorylation and hypersensitization. (b) Cleavage of IP_3R1 by caspase 3 or calpain (*cleavage sites represented by scissors*) can uncouple the channel domain from IP_3-dependent activation, though Bcl-2-related Bok (*in orange*) can protect IP_3R1 against such cleavages by binding at a.a. 1895-1903. See text for more information.

Figure 3. RyR1 and RyR2 as ER/SR Ca^{2+}-leak channels. Linear representation of RyR1 (*upper panel*) and RyR2 (*lower panel*) with their channel domain in their C-terminal part. RyR channel activity can be stabilized by associated proteins of which FKBP12 and FKBP12.6 (*in dark blue*) are the most important. Destabilization of RyR channel activity can occur via pathological mutations, predominantly occurring in the 4 indicated clusters, or via posttranslational modifications. Phosphorylation by PKA of RyR1 (S^{2844}) or RyR2 (S^{2030} and S^{2808}) (*phosphorylation sites depicted in green*) as well as of RyR2 by CaMKII (S^{2808} and S^{2814}; *in orange*) act in a destabilizing way. Such destabilization of the RyR channel will increase Ca^{2+} leakage. In contrast, phosphorylation of RyR2 by the SPEG kinase on S^{2367} (*in purple*) has a stabilizing effect and thus decreases Ca^{2+} leakage. See text for more information.

Figure 4. Possible mechanisms by which Bcl-2 can activate the ER Ca^{2+} leak. Bcl-2 has been proposed to participate to Ca^{2+} leakage from the ER by (a) inhibiting SERCA pumps, (b) sensitizing the IP_3R, (c) forming itself a Ca^{2+} pathway through the ER membrane or (d) acting via another protein serving as Ca^{2+}-leak channel as e.g. BI-1. See text for more information.

Figure 5. Structure of the translocon as ER Ca^{2+}-leak channel. Sec61α is the key component of the translocon, but can also function as an ER Ca^{2+}-leak channel. (a) Graphical representation of Sec61α and its interactions with BiP, calmodulin (CaM) and Sec62. The latter three proteins can limit Ca^{2+} leakage through Sec61α. In contrast, the pathogenic V67G, V85D and Q92R mutations (*yellow stars*) increase the Ca^{2+} leakage out of the ER. (b) Closed conformation of Sec61α (PDB 4CG7) [272] and (c) open conformation of Sec61α (PDB 3JC2) [273]. Each time a side view (left) and a top view (right) is presented. In panels b and c the following Sec61α domains are indicated as follows: cytosolic and luminal domains (*in green*), transmembrane domains (*in red*), the region interacting with both Sec62 and CaM (*in dark blue*), and the region interacting with BiP (*in cyan*). As one can appreciate, when the regulatory proteins BiP, CaM, and/or Sec62 are bound to their respective binding sites they are located at a strategic place to control Ca^{2+} flux through the open translocon. See text for more information.

Figure 6. Structure of presenilin 1 as ER Ca^{2+}-leak channel. Ca^{2+} leakage through presenilin 1 has been proposed to depend on its phosphorylation by glycogen synthase kinase 3 β at S^{353} and S^{357} (*in blue*). Endoproteolytic cleavage of presenilin 1 leads to the production of two fragments, the N-terminal fragment (NTF) and the C-terminal fragment (CTF), which are needed for its correct maturation and γ-secretase activity. The location of the endoproteolytic site (*scissors*) and of the two catalytic

aspartates D^{257} and D^{385} (*in red*) located in transmembrane domain 6 and 7 resp. are represented. It is tempting to speculate that these two transmembrane domains may also delineate the Ca^{2+}-leak pathway. See text for more information.

Figure 7. The interrelation between the ER Ca^{2+} leak and the mitochondria in various situations. The $[Ca^{2+}]_{ER}$ depends on the balance between Ca^{2+} uptake via SERCA and Ca^{2+} release via Ca^{2+}-release channels and Ca^{2+}-leak channels. Ca^{2+}-release channels located at the ER-mitochondria contact sites (e.g. the truncated SERCA1T, the 4TM-TRPM8 isoforms and presenilin 1) can deliver Ca^{2+} to the mitochondria. (a) Ca^{2+} taken up by the mitochondria can stimulate its metabolism leading to ATP production. (b) Excess Ca^{2+} release through the Ca^{2+}-leak channels located at the ER-mitochondria contact sites will lead to mitochondrial Ca^{2+} overload, mitochondrial outer membrane permeabilization and apoptosis. (c) If only, or mainly, Ca^{2+}-leak channels outside the ER-mitochondria contact sites are open, the ER can become depleted, leading in extreme cases to ER stress while also cytosolic Ca^{2+} signals occur, but mitochondrial metabolism will not be stimulated and autophagy may be triggered. See text for more information.

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A comprehensive overview of the complex world of the endo- and sarcoplasmic reticulum Ca^{2+}-leak channels

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Graphical abstract

HIGHLIGHTS

- ER Ca^{2+} levels impact ER biology and intracellular Ca^{2+} signaling
- Over 20 proteins have been proposed to act as ER Ca^{2+}-leak channels
- Ca^{2+}-leak channels play an important role in maintaining ER Ca^{2+} levels
- Dysregulated IP_3Rs, translocon and presenilins form the main candidates as ubiquitously expressed ER Ca^{2+}-leak channels
- Other ER Ca^{2+}-leak channels may be of crucial importance in specialized cells and/or under specific conditions

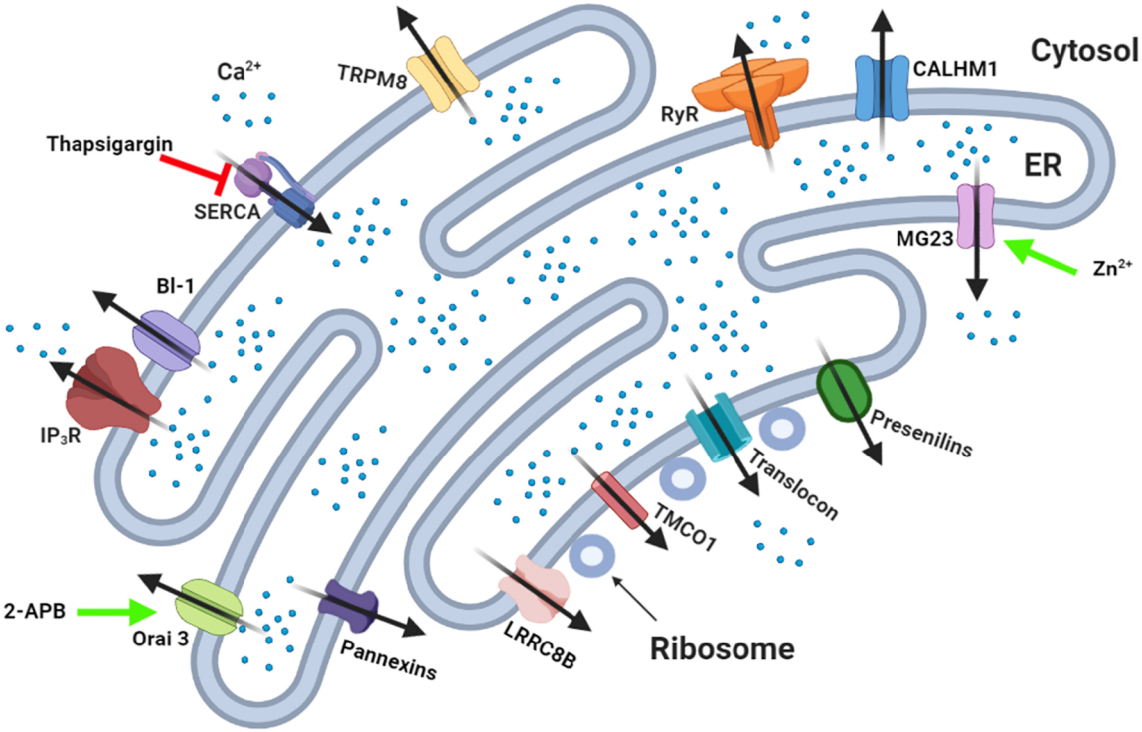
Cytosol
ER
Ca^{2+}
TRPM8
RyR
CALHM1
Thapsigargin
SERCA
MG23
Zn^{2+}
Bl-1
IP_3R
Presenilins
Translocon
TMCO1
2-APB
Orai 3
Pannexins
LRRC8B
Ribosome

Figure 1

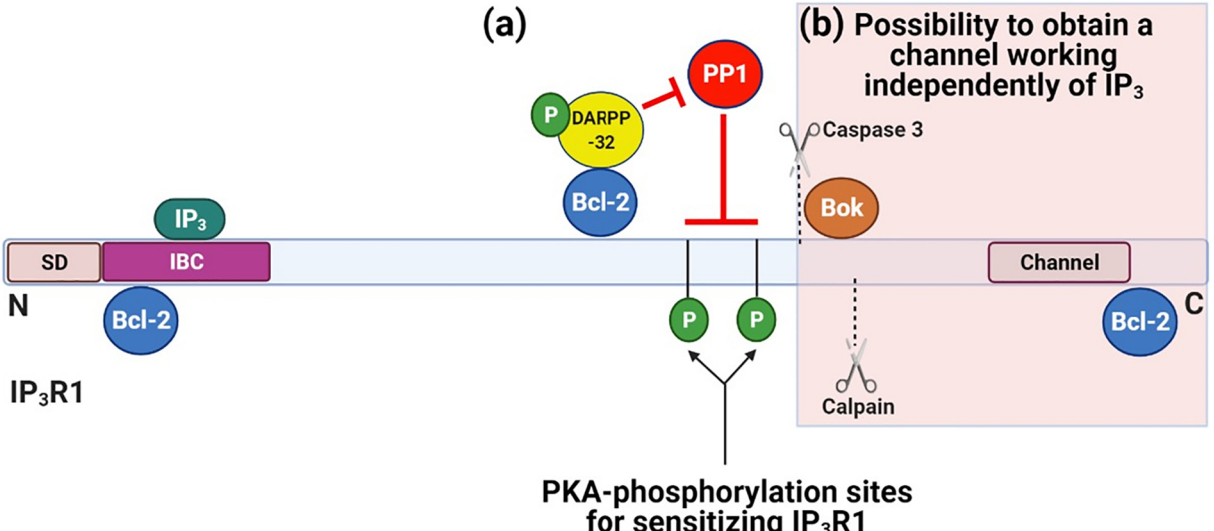
(a)
(b) Possibility to obtain a channel working independently of IP3
PP1
P
DARPP -32
Bcl-2
Caspase 3
Bok
IP3
SD
IBC
Channel
N
Bcl-2
P
P
Bcl-2
C
IP3R1
Calpain
PKA-phosphorylation sites for sensitizing IP3R1

Figure 2

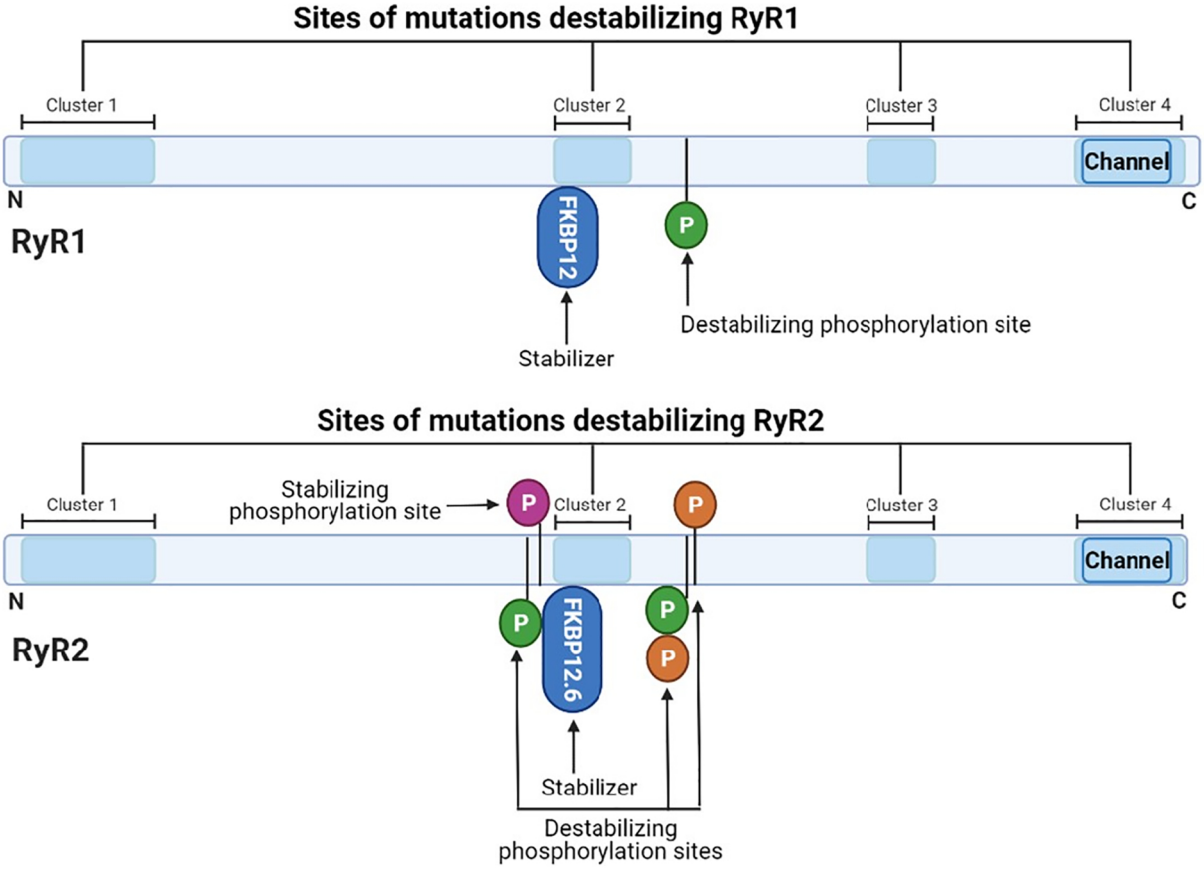
Sites of mutations destabilizing RyR1
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Channel
N
C
RyR1
FKBP12
P
Stabilizer
Destabilizing phosphorylation site
Sites of mutations destabilizing RyR2
Cluster 1
Stabilizing phosphorylation site
P
Cluster 2
P
Cluster 3
Cluster 4
Channel
N
C
RyR2
P
FKBP12.6
P
P
Stabilizer
Destabilizing phosphorylation sites

Figure 3

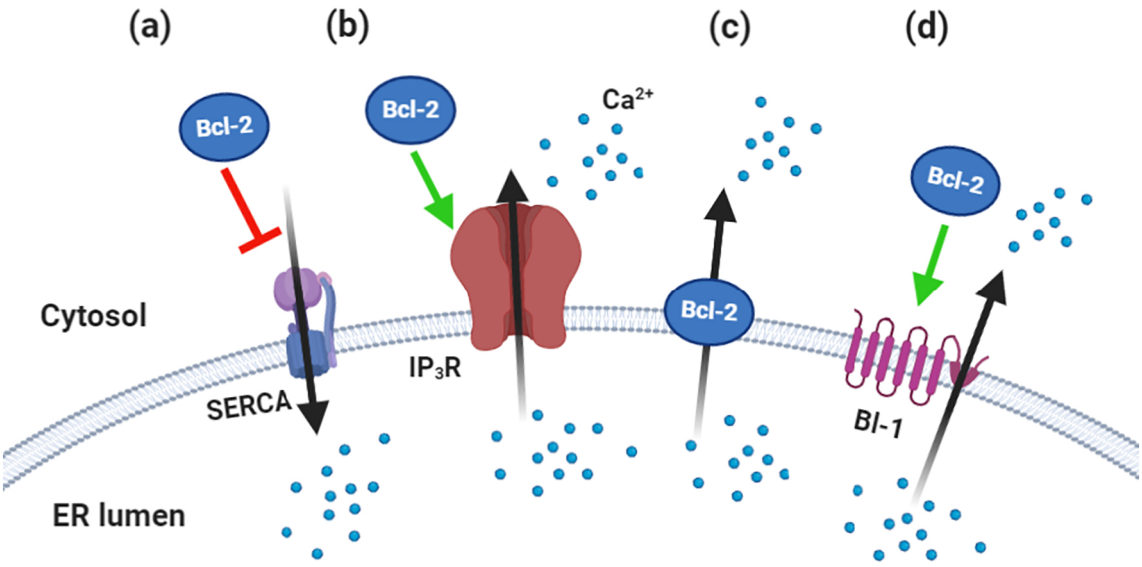

Figure 4

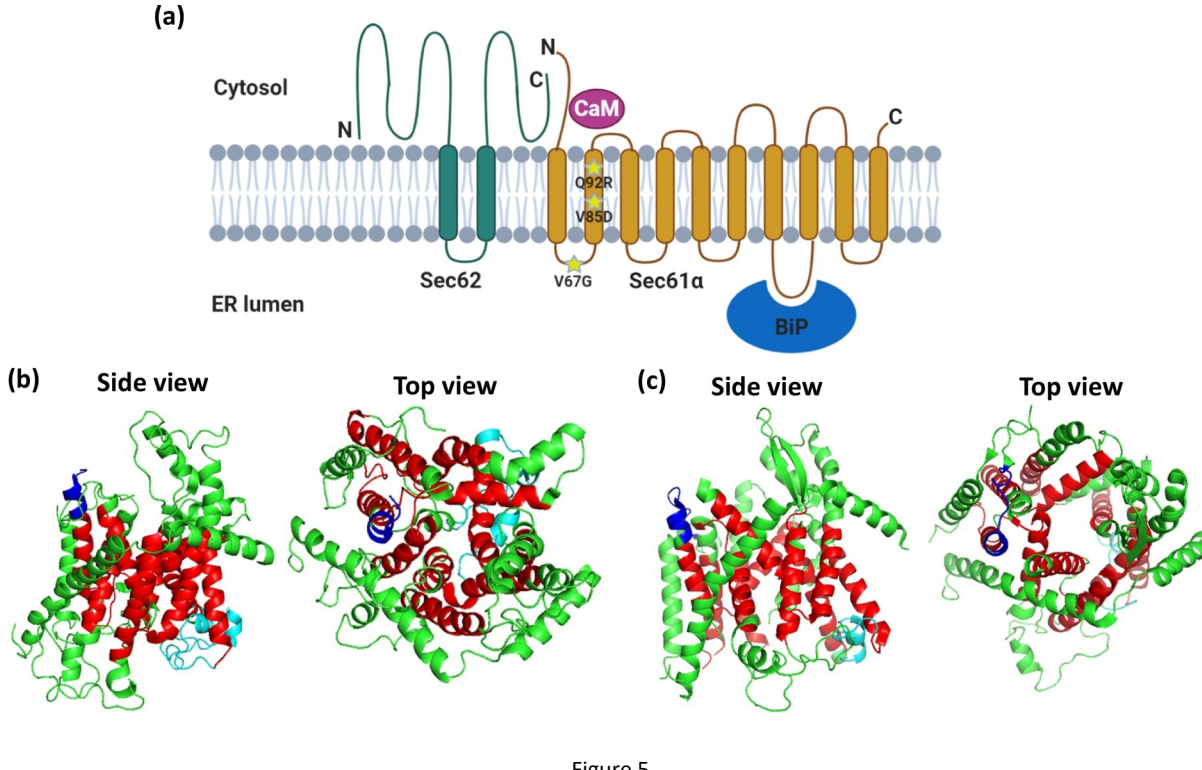
(a)
Cytosol
N
C
N
CaM
C
Q92R
V85D
V67G
Sec62
Sec61α
ER lumen
BiP
(b)
Side view
Top view
(c)
Side view
Top view

Figure 5

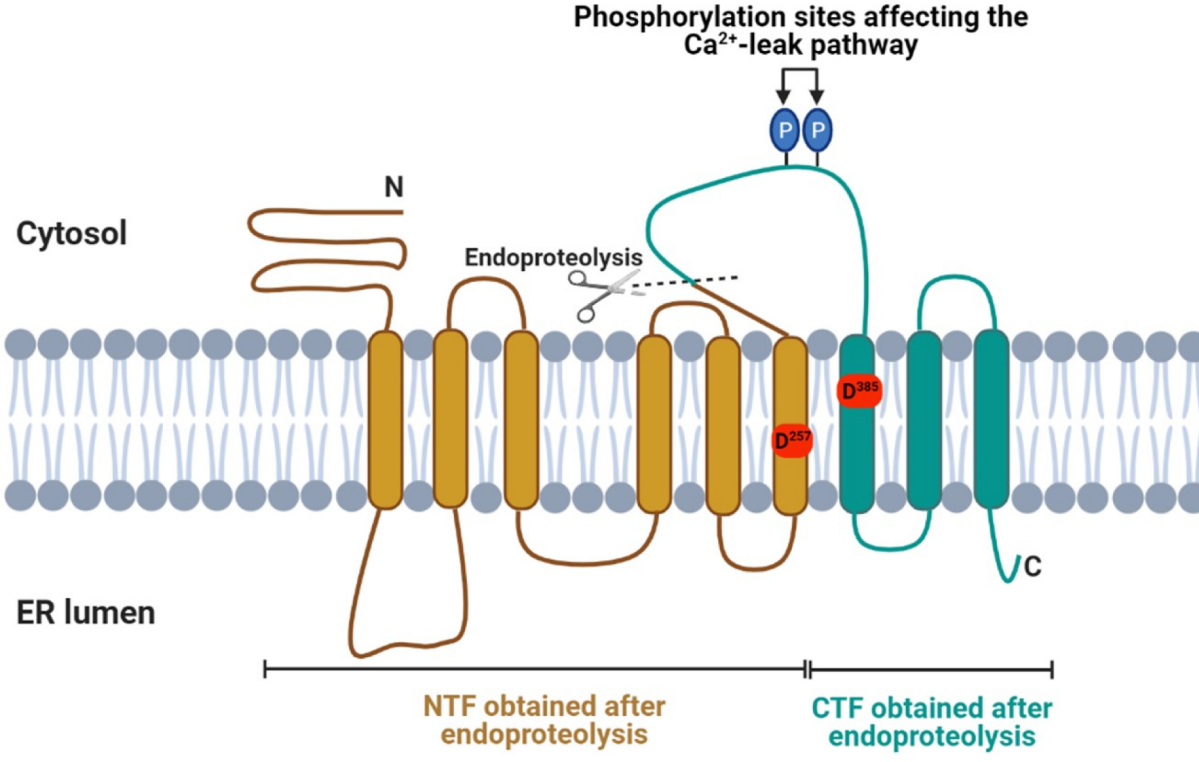
Phosphorylation sites affecting the
Ca2+-leak pathway
P
P
N
Cytosol
Endoproteolysis
D385
D257
C
ER lumen
NTF obtained after
endoproteolysis
CTF obtained after
endoproteolysis

Figure 6

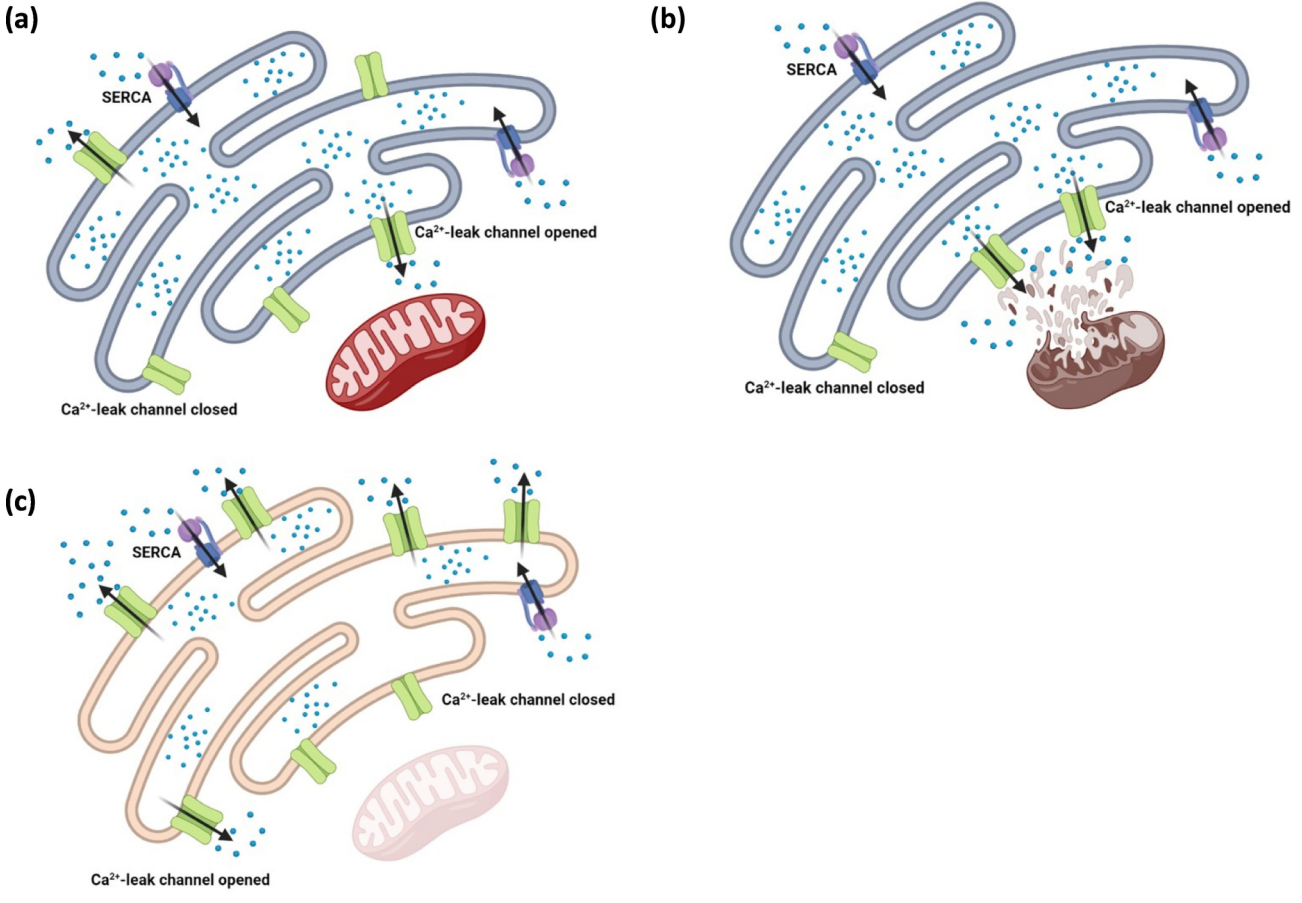
(a)
SERCA
Ca2+-leak channel opened
Ca2+-leak channel closed
(b)
SERCA
Ca2+-leak channel opened
Ca2+-leak channel closed
(c)
SERCA
Ca2+-leak channel closed
Ca2+-leak channel opened

Figure 7