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TMBIM-mediated Ca^{2+} homeostasis and cell death☆

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Abstract

Ca^{2+} is a ubiquitous intracellular messenger that regulates numerous physiological activities in humans, animals, plants, and bacteria. Cytosolic Ca^{2+} is kept at a low level, but subcellular organelles such as the endoplasmic reticulum (ER) and Golgi apparatus maintain high-concentration Ca^{2+} stores. Under resting conditions, store Ca^{2+} homeostasis is dynamically regulated to equilibrate between active Ca^{2+} uptake and passive Ca^{2+} leak processes. The evolutionarily conserved Transmembrane BAX Inhibitor-1 Motif-containing (TMBIM) proteins mediate Ca^{2+} homeostasis and cell death. This review focuses on recent advances in functional and structural analysis of TMBIM proteins in regulation of the two related functions. The roles of TMBIM proteins in pathogen infection and cancer are also discussed with prospects for treatment.

Keywords

Ca^{2+} homeostasis; Ca^{2+} signaling; Cell death; Apoptosis; Bax inhibitor-1; TMBIM; Membrane proteins; Cancer; Cellular stress; Ca^{2+} channel structure

1. Ca^{2+} homeostasis

Ca^{2+} is an essential signaling messenger in regulation of normal physiology and development. In solution, calcium exists as Ca^{2+} with two positive charges. This property allows it to interact with proteins, lipids and other biomolecules carrying negative charges. During the evolution of living organisms, from prokaryotes to plants and mammals, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{Cyto}$) has been restricted to and constantly maintained at a very low concentration of about 100 nM. In eukaryotes, cytosolic high Ca^{2+} concentration triggers cell death [1]. In order to prevent the accumulation of Ca^{2+} in cytosol, cells use a variety of Ca^{2+} toolkits such as pumps and channels to mobilize cytosolic Ca^{2+} to intracellular Ca^{2+} stores (Fig. 1). Endoplasmic reticulum (ER) and Golgi apparatus are two major Ca^{2+} stores [2–4] where $[Ca^{2+}]$ can be at the 0.3–0.5 mM range which is 3000–5000 fold higher than that in cytoplasm. At physiological conditions, $[Ca^{2+}]$ is balanced between cytosol and subcellular organelles so that Ca^{2+} stores are not overloaded and the cytosolic buffering capability is sufficient; and stress-induced Ca^{2+} release will trigger appropriate signaling events for cytoprotection [5–7]. However, under prolonged stress conditions, for

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example ER stress [7,8], Ca^{2+} could be released from ER, transferred into mitochondria, and causes Ca^{2+} -mediated cell death [1,9–11].

2. Apoptosis and its regulators

Apoptosis, or programmed cell death, controls the fate of cells in multi-cellular organisms during development and in response to cellular signals and stresses [12]. Appropriate apoptosis protects organisms from cellular damage and diseases. While uncontrolled apoptosis leads to malfunction and disrupted apoptosis leads to abnormity or carcinogenesis [13]. Two pathways regulate apoptosis: intrinsic and extrinsic [12,14]. In the intrinsic pathway, proapoptotic protein Bax or Bak is activated and translocates to the mitochondrial outer membrane. It subsequently undergoes a conformational change and oligomerizes to form a pore through which mitochondrial apoptotic factors are released [15, 16]. These mitochondrial apoptotic factors, including cytochrome c, apoptosis induce factor (AIF) and Smac/DIABLO, flux into the cytosol and trigger cell death through caspase activation [17]. In the extrinsic pathway, extracellular death signals activate cell surface receptor CD95 (also known as Fas) or tumor necrosis factor receptor (TNFR1) and induce an across-the-membrane signaling cascade leading to caspase activation and, ultimately, apoptosis.

Apoptosis is precisely regulated by at least two groups of regulators, e.g. proapoptotic regulators and antiapoptotic regulators related to B-cell lymphoma 2 (Bcl-2) family [18–20]. Proapoptotic regulators of the Bcl-2 family contain a BH3 domain (Fig. 2). Among these, Bim, Bad, Puma, Bid, Bik, Bmf, Hrk and Noxa have a BH3-only domain [21]; and Bax, Bak, and Bok have multiple domains (BH1–3) [22]. Antiapoptotic regulators of the Bcl-2 family include Bcl-2, Bcl-XL, Bcl-w, and Mcl-1 [23]. In addition to having the BH1–3 domains, antiapoptotic Bcl-2 regulators have an N-terminal BH4 domain that can bind directly or indirectly to the proapoptotic Bax to inactivate it [24,25]. Comparing to antiapoptotic regulators, proapoptotic regulators can function by either directly promoting the formation of the permeation transition pore (Bax or Bak) or competitively binding to antiapoptotic proteins to release Bax or Bak for initiation of apoptosis (BH3-only). Proapoptotic Bok is persistently active without apparent regulators except Mcl-1 [26]. It is proposed that Bok is regulated at expression levels through ER-associated degradation pathways [22,27].

Proapoptotic and antiapoptotic proteins were originally thought to be only for soluble proteins or membrane anchored single-transmembrane proteins. Recently multi-pass transmembrane proteins of the TMBIM family were found to either suppress or promote cell death depending on whether there is a BH3-domain at the C-terminus [28,29]. Thus far, the majority of the TMBIM family is only comprised of antiapoptotic proteins, which inhibit apoptosis of both intrinsic and extrinsic pathways [30]. The only known proapoptotic TMBIM protein is the yeast homolog Ynl305cp in which a BH3 domain at the C-terminus promotes cell death [31] (Fig. 2).

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3. TMBIM proteins in mediating cell death and Ca^{2+} homeostasis

In humans, there are six TMBIM homologs named as TMBIM1–6 based on the HUGO gene nomenclature. TMBIM proteins are defined by sequence conservations to Bax inhibitor-1 (BI-1, or TMBIM6), which was discovered as a human gene product capable of blocking Bax-induced cell death in yeast [32]. Human TMBIM proteins contain multiple predicted transmembrane helices varying mainly in their N-terminal extensions [29,30,33,34] (Fig. 3). The TMBIM family is essentially ubiquitous, present in humans, animals, plants, fungi and prokaryotes [29] (Fig. 3). Based on phylogenetic analysis, TMBIM1–3 are clustered together and TMBIM4–6 are separately clustered and remotely related [29,34].

TMBIM6 (BI-1 or TEGT) is an ER membrane protein with seven predicted transmembrane helices. TMBIM6 regulates apoptosis sensitive to ER-stress triggers such as etoposide and staurosporine, but not to extrinsic apoptosis ligand FasL (also known as CD95L), consistent with its sole ER localization [32,35]. Reduced $[Ca^{2+}]_{ER}$ is cytoprotective [36, 37]; and TMBIM6 reduces resting $[Ca^{2+}]_{ER}$ as well as $[Ca^{2+}]_{Cyto}$ upon treatment with ER-stress inducers such as thapsigargin or tunicamycin [35,38]. TMBIM6 regulates Ca^{2+} flux in a pH-dependent manner [39–41].

TMBIM1 (RECS1, LFG3, PP1201, or PSEC0158) is localized to lysosome and endosome membranes in addition to ER and Golgi [42]. Relative to TMBIM6, TMBIM1 has a long N-terminal extension, presumably in which residing the localization information. TMBIM1 was identified from screening for genes responsive to centrifugal force and shear stress [43]. Deficiency of TMBIM1 in mice induced mice susceptibility to cystic medial degeneration and aortic dilation [44,45]. TMBIM1 suppresses Fas-mediated extrinsic apoptosis by interacting with Fas receptor [46] and mediates Ca^{2+} homeostasis by reducing $[Ca^{2+}]_{ER}$ [42].

TMBIM2 (FAIM2, LFG, LFG2, NMP35, or KIAA0950) is localized to plasma membrane in addition to ER and Golgi [47]. TMBIM2 also suppresses Fas-induced apoptosis by interfering with caspase-8 activation [47]. Like TMBIM1, TMBIM2 has an N-terminal extension for localization. TMBIM2 is highly expressed in neurons and is rich at postsynaptic sites in dendrites [48]. The overexpression of TMBIM2 improved axon growth [49] and protected brain neurons from transient ischemia [50]. TMBIM2 is required for survival and maintenance of Purkinje and granule neuron cells [51]. TMBIM2 decreases releasable ER Ca^{2+} [42,52].

TMBIM3 (GRINA, LFG1, NMDAR1, or PM02) is localized to ER and Golgi apparatus and is mainly expressed in neuron cells [53,54]. TMBIM3 suppresses ER-stress induced cell death and reduces $[Ca^{2+}]_{ER}$. TMBIM3's function is correlated with TMBIM6 [54]. In fact, TMBIM3 interacts with TMBIM6 in ER membrane and synergizes its function in Ca^{2+} homeostasis and cell death. The binary interaction is also linked to unfolded protein response (UPR) and autophagy [54].

TMBIM4 (LFG4, S1R, Z-protein, or GAAP) is also localized to ER and Golgi where it mediates intracellular Ca^{2+} homeostasis and cell death [42,55,56]. Through modulation of Ca^{2+} homeostasis and Ca^{2+} signaling, TMBIM4 can mediate cell adhesion and migration

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[57,58]. TMBIM4 suppresses cell death of both intrinsic pathways induced by ceramide and H_2O_2, and extrinsic pathways induced by Fas ligand and TNF. TMBIM4 is essential for cell survival; and RNA interference induced spontaneous cell death [56].

TMBIM5 (GHITM, MICS1, or DERP2), also known as growth hormone-inducible transmembrane protein (GHITM), is localized to the mitochondrial inner membrane where it is required for maintenance of mitochondrial structure and release of apoptotic factor cytochrome c [59]. Different from other TMBIM proteins, TMIBM5 has eight predicted transmembrane helices, seven TMBIM-conserved plus an additional one at the N-terminus. The N-terminal helix is hypothesized to be involved in the translocation and positioning of the protein to mitochondrial inner membrane. Whether TMBIM5 can mediate mitochondrial Ca^{2+} homeostasis is unknown. However, premature cleavage of the protein may end up with a localization to ER where it indeed mediates Ca^{2+} homeostasis [42].

Interestingly, with an ER localization, all TMBIM proteins mediate ER Ca^{2+} homeostasis and suppress intrinsic apoptosis pathways by reducing $[Ca^{2+}]_{Cyto}$ upon ER stress [42]. In addition to suppressing the intrinsic pathway, TMBIM1, 2 and 4 suppress extrinsic pathways originating from CD95 or TNF receptor which may accumulate at the Golgi prior to their traffic to plasma membrane for signaling [60,61]. In light that TMBIM1, 2 and 4 also have a Golgi localization [42,62,63], it is likely interactions between Golgi TMBIM proteins and CD95 or TNF receptor may block membrane trafficking and thus suppress extrinsic pathways [46]. TMBIM6 has only the ER localization [35], consistent with it being only sensitive to ER-stress induced intrinsic pathways. It is intriguing that TMBIM3 has both ER and Golgi localizations; while it is only sensitive to ER-stress induced intrinsic pathways [54]. All together, we can envision that the functions of TMBIM proteins in cell death and Ca^{2+} homeostasis are closely correlated with their subcellular localizations.

To explain how TMBIM proteins mediate Ca^{2+} homeostasis, two models were proposed based on TMBIM6. One is a Ca^{2+}/H^+ antiporter in which a proton gradient drives the Ca^{2+} flux from ER to cytoplasm. At the C-terminus of TMBIM6, there is a lysine-rich motif “EKDKKKEKK” which has certain similarity to a sodium channel and was thus proposed to form a pH-sensitive Ca^{2+} selectivity filter [32,39,40]. The other one is a channel in which the structure opens as a pore to allow the flow of Ca^{2+} along its gradient [64,65]. For both models, mediation of Ca^{2+} flux by TMBIM6 is pH dependent [41,66].

4. Ca^{2+}-leak mechanism inspired by bacterial TMBIM structures

TMBIM proteins are conserved in almost the entire life kingdom (Fig. 2), suggesting that TMBIM-mediated cellular protection is an ancient trait, concomitant with the formation of single-cell organisms. The recent crystal structures of a bacterial TMBIM protein, YetJ from *Bacillus subtilis* (BsYetJ), provide substantial structural insights for mechanistic understanding the Ca^{2+} flux and homeostasis mechanisms [34].

The sequence alignment of BsYetJ with human TMBIM proteins are quite well in particular for the C-terminal region (TM6-7) where two aspartic acid residues (Asp171 on TM6 and Asp195 on TM7 in BsYetJ) are highly conserved (Fig. 3). BsYetJ functionally uptakes Ca^{2+}

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in *E. coli* and mediates a pH-dependent Ca^{2+} flux in proteoliposomes [34]. The BsYetJ structure was solved at three difference pH conditions, each corresponding to a different conformational state: closed at pH 8, open at pH 6 and in equilibrium at pH 7 (Fig. 4A–F). In these structures, BsYetJ is a monomer and contains seven transmembrane helices as predicted. The structure has a novel fold consisting of two inversely assembled triple-helix-sandwich repeats that wraps the TM7 in the middle. At pH 7, both the pH 8 and pH 6 structures coexist in equilibrium (Fig. 4E–F), with TM2 underwent conformational changes by as large as 14 Å. Consisting of only 14 residues in the closed-state structure, TM2 is short, with relatively long connecting loops to TM1 and TM3, consistent with it being flexible for conformational changes.

Structurally, TM2 can regulate the Ca^{2+} flux. In the structure, the C-terminal residues Asp171 and Asp195 form an H-bond (Fig. 5A). At pH 8 the carboxylate group of Asp171 is negatively charged and forms a hydrogen-bonded salt bridge with Arg60 from TM2 and latches TM2 in the closed conformation. At pH 6, a predicted protonation of Asp171 disrupts its interaction with Arg60 and thus opens the structure (Fig. 4D). Asp171 and Asp195 are TMBIM-family conserved (Fig. 3) and may form a pH sensor that can sense environmental pH and regulate the pH-dependent Ca^{2+} flux.

Based on the BsYetJ structure and the sequence alignment of the TMBIM family (Figs. 2 and 3), insights into the molecular mechanisms of human TMBIM-mediated Ca^{2+} homeostasis may be proposed. First, all six human TMBIM proteins have the two conserved aspartic acid residues; and they should necessarily form an H-bond and allow the aspartic acid residue on TM6 to latch/release a positively charged residue from TM2 for regulation of Ca^{2+} leak. Sequence alignment shows most homologs have an arginine at the latch position, suggesting a conserved pH-dependent Ca^{2+} leak mechanism. However, TMBIM5 has an aspartic acid and TMBIM6 has a histidine, suggesting existence of alternative latch mechanisms and sensitivity to environmental pHs. For example, in the TMBIM homology model, deprotonated Asp209 (Ser191 in BsYetJ) on TM6 favors an H-bond with His78 from TM2 (Fig. 5B). Asp209 is unique to TMBIM6 and is in close proximity to the D188–D213 pH sensor (Asp171–Asp195 in BsYetJ). Asp 209 and Asp213 in TMBIM6 have been demonstrated critically important in TMBIM6-mediated Ca^{2+} homeostasis and cell death [64]. It is likely that the three aspartic residues will sense pH and regulate the Ca^{2+} flux with a bell-shaped pH-dependence [34,67]. At pH 8, TMBIM6 would be closed due to deprotonation of Asp209 and formation of an H-bond with His78 (Fig. 5B). At a more neutral pH of 7, the pKa of Asp209 would increase; and Asp209 will be partially protonated which disrupts its interaction with His78 and allows for formation of a negatively charged pore for Ca^{2+} passage. At pH 5–6, the pore is open; while the complete protonation of Asp209, Asp188 and Asp213 makes the pore hydrophobic and unfavorable for Ca^{2+} flux. In addition, lipid hydrocarbon tails might also fill up the hydrophobic pore which further seals the pore to prevent Ca^{2+} to pass through. This model fits with the experimental data obtained for TMBIM6-mediated Ca^{2+} release [41].

Different from other known Ca^{2+} channels in which there is a defined filter selectively for Ca^{2+} [68,69], TMBIM proteins are unique in that there is no clear Ca^{2+} filter inside, consistent with it being functioning as a monomer and with TM2 opening and closing

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laterally. Although a higher TMBIM oligomer does exist, it is not required for regulation of either Ca^{2+} homeostasis or cell death [70]. The unique structures of TMBIM proteins make them suitable as intrinsic Ca^{2+} leak channels to prevent the overload of intracellular Ca^{2+} stores. Necessarily, the leaky capacity of TMBIM proteins must be regulated to reach an equilibrium with Ca^{2+} loading capacity by pumps as depleted Ca^{2+} stores also cause cell death.

5. Functional conservation of TMBIM proteins in plant and bacteria

TMIBM proteins, originally identified in humans and mammals, were also identified to be present ubiquitously in plants and bacteria [34,71–74].

In plants, the first TMBIM protein was identified by functional screening [72,75] in a way similar to TMBIM6. Plant TMBIM proteins mediate Ca^{2+} homeostasis [76] and have resistance to various biotic and abiotic stresses including Bax protein, heat and cold shock, fungal toxin, tunicamycin, fungal pathogen, sucrose starvation, and H_2O_2 [77]. The growing plant TMBIM functions include suppression of Bax-induced cell death [73,77], regulation of calmodulin binding [76], enhanced sphingolipid synthesis upon cold stress [78], and enhanced drought tolerance [79].

In bacteria, TMBIM proteins are classified as BI-1 family in the uniprot database (www.uniprot.org). However, so far, the physiological functions of these bacterial TMBIM proteins are not clear. In *Escherichia coli* (strain K12), there are three TMBIM proteins YccA, YbhL and YbhM. Among these, YccA was identified to be an inhibitor of FtsH AAA^+ protease that can otherwise degrade translocon subunit SecY under stress conditions [80]. As BsYetJ can uptake Ca^{2+} when overexpressed in *E. coli* [34], likely these bacterial TMBIM proteins have similar Ca^{2+} uptake activities. The consequence of Ca^{2+} uptake in bacteria remains to be defined in particular under physiological and stress conditions. It is noted that *ybhL* and *ybhM* genes are physically clustered in the *E. coli* genome, suggesting that their expression might be regulated by a common promotor and transcription factor(s). In analogy to their eukaryotic relatives in cytoprotection, it may be hypothesized that under stress conditions such as heat and cold shock, bacterial TMBIM proteins might be involved in cytoprotection through an as-yet-unknown mechanism.

6. TMBIM proteins hijacked for invasion

In eukaryotic cells, most TMBIM proteins are located in subcellular membranes rather than plasma membranes. Due to their critical roles in playing with the life and death, TMBIM proteins have been hijacked by certain pathogens for infection. Below are a few examples of demonstrating how the TMBIM pathway is being jeopardized. Likely new ways of TMBIM-mediated pathogen attacks are to be identified.

Camelpox virus (CMLV), cowpox virus (CPXV) and certain vaccinia viruses (VACV) encode virulent membrane proteins of TMBIM4 like [56,57]. These viral TMBIM4 proteins share ~70% sequence identity with human TMBIM4 and can mimic TMBIM4 to prevent host cell death during their infection processes. During the virus infection, viral TMBIM4 proteins are dumped into host cells together with other virulence factors. The viral TMBIM4

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proteins then translocate to ER and Golgi to suppress the cell death so that viruses can replicate in host cells without killing them. These viral TMBIM proteins regulate host cell Ca^{2+} homeostasis and suppression of cell death indistinguishable to that of human TMBIM4 [55,70].

In addition to the TMBIM4 pathway, TMBIM6 is also the target for attack by some pathogen bacteria enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) through an effector protein NleH [81]. NleH, after injection into host cells through the bacterial type II secretion system, can recognize and bind to the N-terminus of TMBIM6 to gain antiapoptosis function. It is intriguing that the binding of NleH to TMBIM6 increased $[Ca^{2+}]_{Cyto}$, while TMBIM6 alone reduced $[Ca^{2+}]_{Cyto}$. Except TMBIM5, which is mainly in the mitochondrial inner membrane and less likely to be attacked by cytosolic pathogenic effectors, TMBIM1–4 could also be possible targets to be hijacked by bacterial pathogens for assisting their infection and survival. Future studies on pathogen-TMBIM1–4 interactions may be expected.

Based on a BLAST search against human TMBIM proteins, there are ten human cytomegalovirus (HCMV) viral proteins of the US12 family (US12–21) with appreciable homology to TMBIM proteins [82–84]. Like TMBIM proteins, these US12 proteins all have a predicted 7TM topology. These proteins are essential for virulence through yet a not clear pathway. Nevertheless, based on their similarities to TMBIM proteins, it can be hypothesized that these remote TMBIM homologs may localize to subcellular organelles and contribute to the modulation of Ca^{2+} homeostasis and cell death pathways for their virulence.

7. TMBIM proteins as regulators of Ca^{2+} signaling

In addition to functioning as standalone proteins in mediation of Ca^{2+} homeostasis, TMBIM proteins interact with other Ca^{2+} signaling proteins for regulation of cell life and death.

TMBIM6 inhibits Bax-induced cell death through the intrinsic pathway. It is puzzling that TMBIM6 does not interact with Bax. Rather, it interacts with antiapoptotic proteins Bcl-2 and Bcl-XL involving the BH4 domain [32]. Whether TMBIM6 interacts with other BH4-domaining containing antiapoptotic proteins, e.g. Bcl-w and Mcl-1 (Fig. 2) is waiting for experiments. Recently TMBIM2 and TMBIM4 were also shown to interact with Bcl-2 and Bcl-XL, thus broadening the generality of the Ca^{2+} signaling mediated by the two families of apoptosis regulators [52]. It is noted that the BH4 domain is amphiphilic; and the TM6 and TM7 in TMBIM proteins are also amphiphilic and contribute to formation of a transmembrane pore. Hence the interactions between Bcl-2 and TMBIM proteins might have the amphiphilic BH4 domain helix in the TMBIM pore [34]. Because Bcl-2 and Bcl-XL mediate Ca^{2+} homeostasis upstream of TMBIM6 [85], such interactions should allow a Ca^{2+} leak to reduce $[Ca^{2+}]_{ER}$ and to suppress cell death [39,85].

TMBIM proteins, atleast TMBIM3, TMBIM4 and TMBIM6, have interactions with IP_3 activated Ca^{2+} channels (IP_3Rs) for regulation of ER stress and autophagy [86–89]. Bcl-2 family members have been longtime thought to mediate Ca^{2+} signaling pathways [90,91].

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For example, Bcl-2 and Bcl-XL can directly interact with IP_3R channels to regulate their Ca^{2+}-flux properties [92–99]. It is possible that TMBIM proteins function as docking proteins for Bcl-2- and Bcl-XL-mediated IP_3R function. However, a challenging concept for this is that the BH4 domain of Bcl-2 implicated in interaction with TMBIM6 is also responsible for binding IP_3Rs. Therefore, it is also possible that TMBIM6 negatively regulates the interaction of Bcl-2 with IP_3Rs and vice versa. Future *in vivo* and *in vitro* studies of interplays between TMBIM, Bcl-2/Bcl-XL and IP_3R proteins would be pivotal to understand the associated Ca^{2+} signaling and cell death.

8. TMBIM proteins in cancer

Apoptosis plays a crucial role in carcinogenesis as well as in resistance to chemotherapy and radiotherapy [100]. Despite differences in genetic background and lineage among cancer cells, they all share characteristic hallmarks such as evasion from apoptosis surveillance [13, 101]. TMBIM6 expression is upregulated in certain cancers including prostate, breast, glioma, uterine, ovarian and lung [86,102–104]. Knockdown of TMBIM6 expression by RNA interference induced spontaneous cell death of cancer cells in prostate and breast [102,103]. Clearly these cancers have escaped the surveillance of apoptosis by overexpression of antiapoptotic proteins including TMBIM6.

In addition to TMBIM6, TMBIM3 expression is up regulated in breast cancer, colorectal cancer, gastric cancer, head and neck cancer; and TMBIM4 expression is upregulated in colorectal cancer and leukemia [28]. Conversely, TMBIM1 is significantly down regulated in breast cancer, leukemia and sarcoma; and TMBIM2 is down regulated in brain and CNS cancers [105]. TMBIM5, although with an inner mitochondrial membrane localization, has an expression that is up regulated in head and neck cancer and down regulated in leukemia [28]. Because TMBIM proteins regulate Ca^{2+} homeostasis, abnormal TMBIM expression (up or down) in cancer cells may have changed the Ca^{2+} homeostasis toward favoring cancer cell survival and progression. Altered Ca^{2+} homeostasis mediates many hallmarks of cancer [13,106,107]. Therefore, remodeling of the Ca^{2+} homeostasis in cancer mediated by TMBIM proteins might provide a novel strategy for treatment.

9. Conclusion and prospects

TMBIM proteins are evolutionary conserved membrane proteins regulating both Ca^{2+} homeostasis and cell death. TMBIM proteins have novel 7TM-fold structures that allow the leak of Ca^{2+} for regulating Ca^{2+} homeostasis. Their pH-dependence and interactions within TMBIM proteins and with Bcl-2 and IP_3R family proteins add another level of regulation of Ca^{2+} signaling and cell death. For the future, it is needed to study the detailed mechanisms of TMBIM proteins in organelle-specific cell death mechanisms, pathogen-host interactions, plant and bacteria stress resistance, pH-dependent regulation of Ca^{2+} signaling, and remodelling of Ca^{2+} signaling in cancer. The work will pave the way to harness the highly conserved and ubiquitously expressed TMBIM proteins for biological and biomedical applications.

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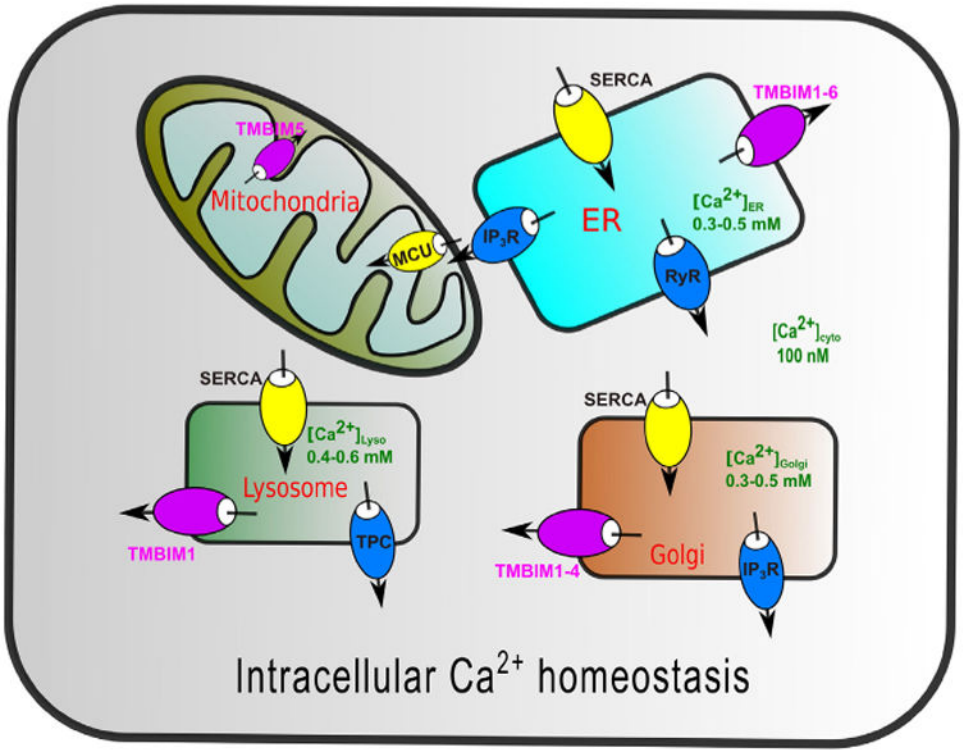

Fig.1.

Intracellular Ca^{2+} homeostasis. Intracellular organelles are calcium stores where Ca^{2+} pumps and Ca^{2+} channels dynamically regulate intracellular Ca^{2+} homeostasis. Overloaded store Ca^{2+} causes Ca^{2+} transfer to mitochondria and induces Ca^{2+} -mediated cell death.

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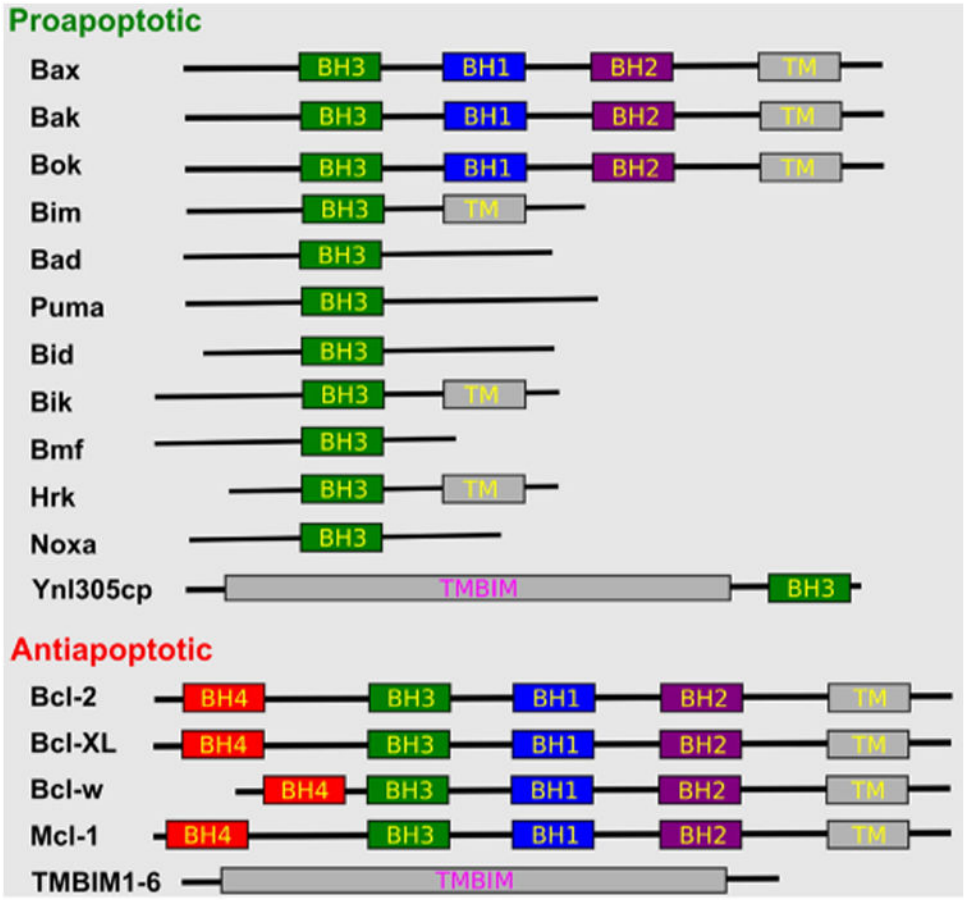

Fig.2.

Apoptosis regulators of Bcl-2 and TMBIM families. Bcl-2 family regulators are either soluble or membrane anchored; while TMBIM family regulators are multi-pass membrane proteins. The BH domains, transmembrane domains (TMs), and multi-pass TMBIM domains (TMBIM) were colored differently.

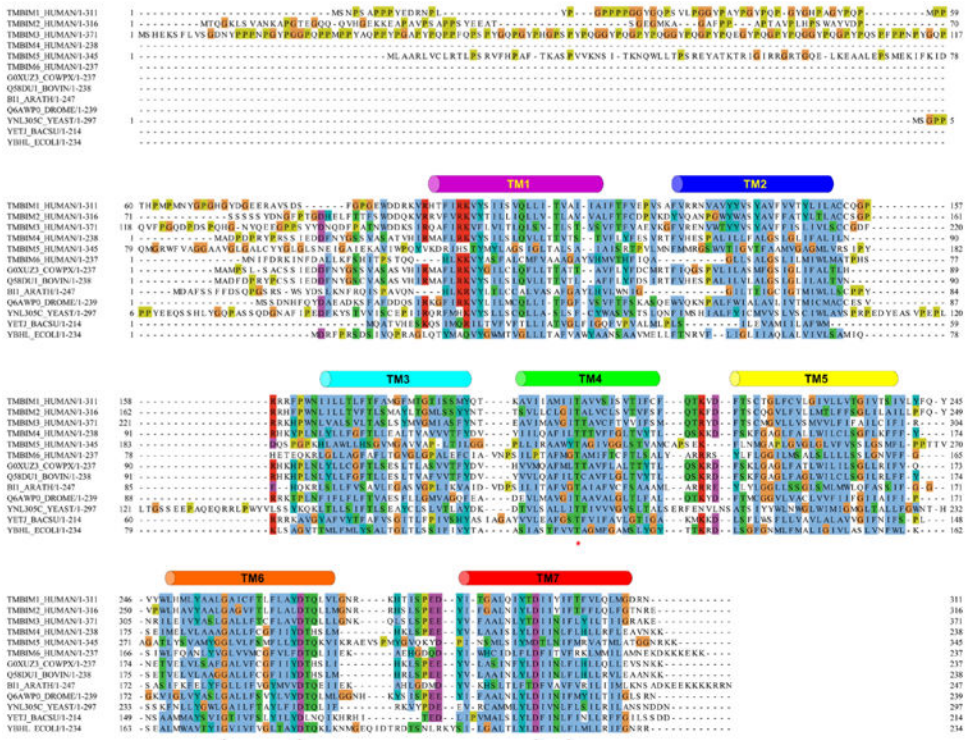

Fig.3.

Sequence alignment of TMBIM proteins. The TMBIM family is highly conserved in humans, animals, worms, plants, fungi, bacteria, and certain viruses. The conserved residues were marked as "*". The seven transmembrane helices based on the BsYetJ structure were drawn as cylinders and were colored differently.

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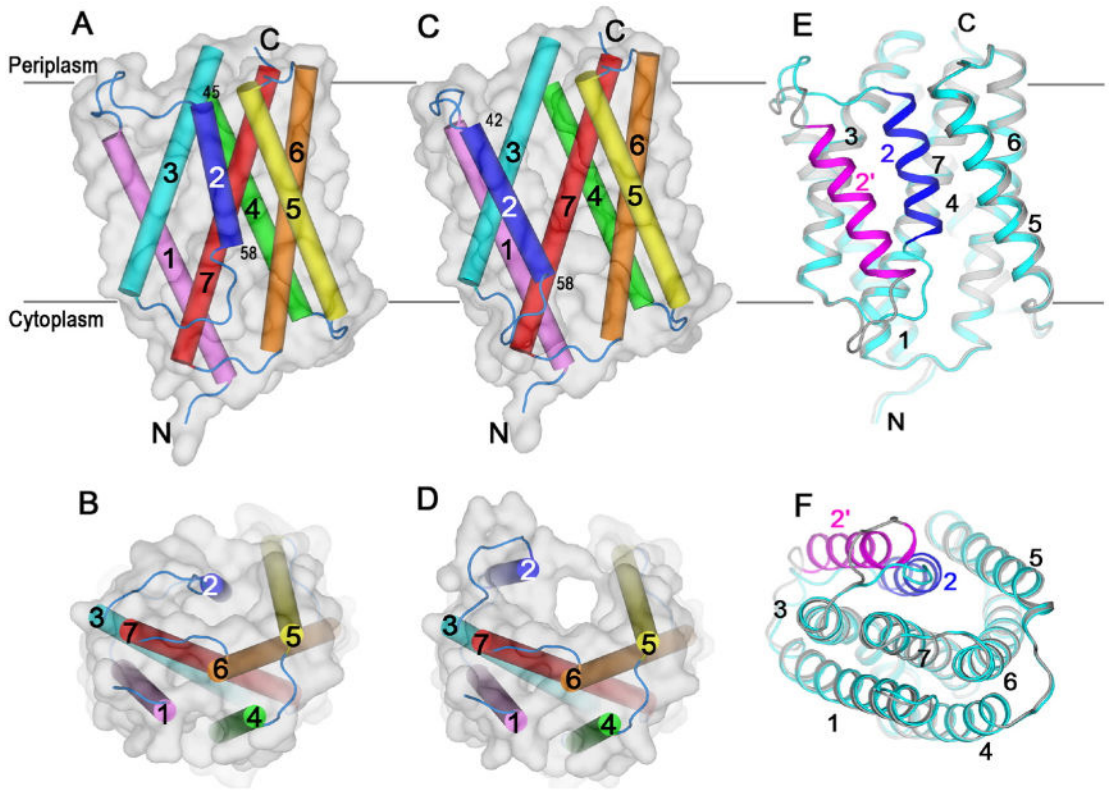

Fig.4.

Structures of BsYetJ in multiple conformational states. (A–B) Closed conformation structure at pH 8 in two orientations. (C-D) Open conformation structure at pH 6 in two orientations. (E-F) Equilibrated conformations of closed and open structures at pH 7 in two orientations. (A), (C) and (E) are side views and (B), (D), and (F) are top views from the periplasm. The seven transmembrane helices were color-coded. The grey transparent surfaces in (A-D) show the overall shapes of these structures. In (E) and (F), the closed conformation was colored in blue for TM2 and cyan for the rest; and the open conformation was colored in magenta for TM2 and grey for the rest.

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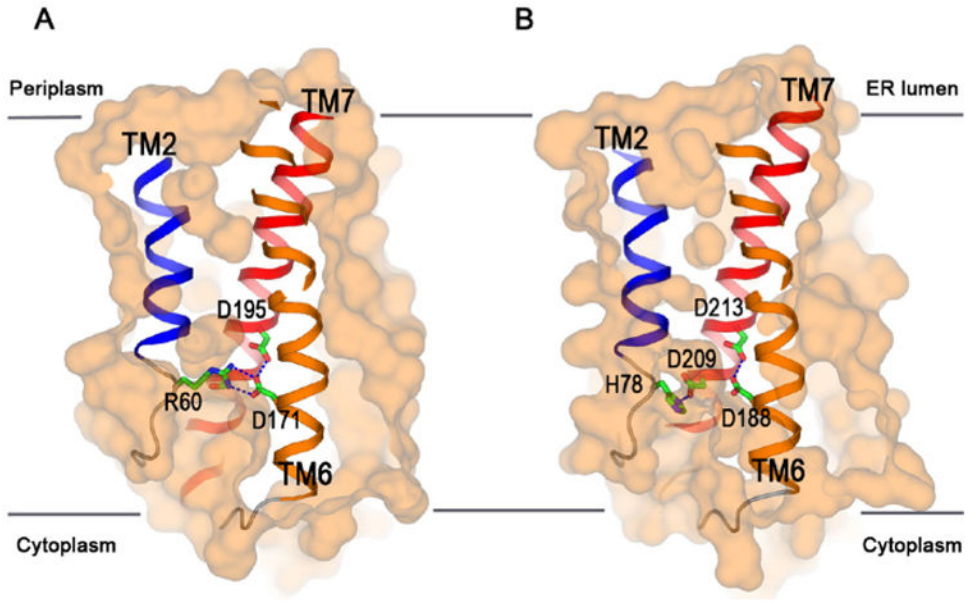

Fig.5.

Molecular basis for Ca^{2+} leak. (A) BsYetJ structure in the closed conformation with the Asp171-Asp195 pH sensor. The sensor forms two H-bonds with Arg60 from TM2 and locks the structure in a closed conformation. The protonation of Asp171 at a neutral pH would disrupt TM2 and allow a Ca^{2+} leak. (B) Homology model of human TMBIM6 in the closed conformation with an equivalent pH sensor comprising of Asp188, Asp209 and Asp213. It is proposed that the protonation states of these aspartate residues will regulate Ca^{2+} leak in a pH-dependent manner.