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Evolutionary Origins of STIM1 and STIM2 within Ancient Ca2+ Signaling Systems

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Abstract

Human STIM (stromal interacting molecule) proteins are parts of elaborate eukaryotic Ca^{2+} signaling systems that include numerous plasma membrane (PM), endoplasmic reticulum (ER), and mitochondrial Ca^{2+} transporters, channels and regulators. STIM2 and STIM1 function as Ca^{2+} sensors with different sensitivities for ER Ca^{2+}. They translocate to ER-PM junctions and open PM Orai Ca^{2+} influx channels when receptor-mediated Ca^{2+} release lowers ER Ca^{2+} levels. The resulting increase in cytosolic Ca^{2+} leads to the activation of numerous Ca^{2+} effector proteins that in turn regulate differentiation, cell contraction, secretion and other cell functions. In this review, we use an evolutionary perspective to survey molecular activation mechanisms in the Ca^{2+} signaling system with a particular focus on regulatory motifs and functions of the two STIM proteins. We discuss the presence and absence of STIM genes in different species, the order of appearance of STIM versus Orai, and the evolutionary addition of new signaling domains to STIM proteins.

Introduction

Our review sheds light on the uses, mechanisms, and origins of the two vertebrate STIM proteins by taking an evolutionary approach that considers STIM1 and STIM2 as components of integrated Ca^{2+} signaling systems with ancient origins. Primordial cells had to regulate the contents of their cytoplasm to create an environment that was favorable for the basic chemical reactions needed for life. Ca^{2+} ions, which are highly abundant in seawater and on land, interact with phosphate and can interfere with nucleic acid metabolism[1]. This makes Ca^{2+} a prominent and ancient hazard. Eukaryotes addressed this challenge by efficiently pumping and transporting Ca^{2+} out of the cytoplasm into the extracellular space or into membrane-enclosed intracellular stores. The resulting steep concentration gradients across the plasma membrane (PM) and internal membranes provided cells with an opportunity to use these gradients as the driving forces to regulate Ca^{2+} influx and release from internal stores as part of different signaling mechanisms. Not surprisingly, the origins of such Ca2+ signaling pathways are very old. Use of Ca2+ as a signaling molecule is ubiquitous in all eukaryotes, and it is used as a signaling molecule in some bacteria as well[2].

Human cells use diverse sensors, Ca^{2+} buffering proteins, channels, pumps, and exchangers to regulate the transport of Ca^{2+} ions between the cytosol, internal organelles and the

extracellular space (Figure 1). Years of study have uncovered the identities of many of these genes. Two of the most recently discovered proteins are the endoplasmic reticulum (ER) Ca^{2+} sensor, STIM[3,4], and the PM Ca^{2+} channel regulated by STIM, Orai[5–7]. Together, they form the main components of a long hypothesized store-operated Ca^{2+} entry (SOCE) pathway[8]. This pathway includes a number of interesting new signaling concepts. First, it is one of only a few known "inside-out" signaling mechanisms. Specifically, it allows changes in luminal ER Ca^{2+} to regulate the opening of a PM Ca^{2+} channel. Second, STIM proteins have the interesting characteristic that they can directly bridge the ER to the PM at specialized junctions, the ER-PM junctions, that now appear to be ubiquitous in all eukaryotic cells. Third, the activation of STIM involves an oligomerization process that is different from other receptors that oligomerize, in that ligand dissociation (i.e., Ca^{2+} dissociation) rather than ligand binding triggers STIM oligomerization and activation. In this review, we survey existing knowledge about the evolutionary history of human Ca^{2+} signaling, with a particular emphasis on this intriguing STIM-controlled ER-to-PM Ca^{2+} signaling pathway.

Conserved generators of transmembrane Ca^{2+} gradients

The most fundamental task in setting up the Ca^{2+} signaling system is to maintain low basal cytoplasmic Ca^{2+} concentration. Long term reduction of cytoplasmic Ca^{2+} is primarily carried out by extruding Ca^{2+} out of the cell via active Ca^{2+} pumping (PMCA proteins) and by Na^+/Ca^{2+} exchange (NCX and NCKX proteins)[1,9] (Figure 1 and see Glossary for abbreviations used). As might be expected, homologs of these pumps or exchangers are widely present in eukaryotes (see Figure 2). The best known exchangers are homologs of the cardiac Na^+/Ca^{2+} exchanger NCX[9]. NCKX has an additional driving force from a co-transported K^+ ion, allowing cytosolic Ca^{2+} in retinal photoreceptor and other cells to be more effectively lowered into the tens of nanomolar range[10]. A likely reason for the prevalence of both pump- and exchange-based Ca^{2+} extrusion is that exchange can rapidly lower high cytoplasmic Ca^{2+} concentrations due to a low affinity and high capacity of the exchangers, whereas the ATP-consuming PMCA pump has a higher affinity but lower capacity that allows it to operate effectively at low cytosolic Ca^{2+} concentrations.

While long-term cytosolic Ca^{2+} levels are regulated at the PM, shorter term transient Ca^{2+} increases often result from release of Ca^{2+} stored inside the lumen of the ER[11]. The ER can typically release and reuptake Ca^{2+} ions on a time scale of seconds, often 10 to 100 times faster than extrusion out of the cell. This allows cells to terminate Ca^{2+} transients by rapidly pumping Ca^{2+} into ER stores with Ca^{2+} leaving the cell much more slowly. This transport from the cytoplasm into the ER is primarily mediated by SERCA Ca^{2+} pumps. A second related pump, the Ca^{2+} ATPase, SPCA[12], is more ubiquitously present and pumps Ca^{2+} into the Golgi, secretory vesicles, endosomes, and likely other compartments. Although not every organism has a homolog of both SERCA and SPCA (e.g., *Saccharomyces cerevisiae* lacks a SERCA homolog and *Arabidopsis thaliana* lacks an SPCA homolog[12]), a homolog of at least one internal pump appears to be present in almost every eukaryote. As will be discussed below, the ER is not only a Ca^{2+} store, but Ca^{2+} also has important regulatory roles inside the ER.

Mitochondria handle Ca^{2+} very differently from the ER. They take up Ca^{2+} instead of releasing it upon cell stimulation[13]. This Ca^{2+} uptake involves a mitochondrial uniporter channel and an as of yet poorly understood process where Ca^{2+} released from the ER directly enters mitochondria[14]. Due to high Ca^{2+} cooperativity in uniporter activation, Ca^{2+} uptake into mitochondria also plays a safety role in limiting peak cytosolic Ca^{2+} levels. Inside mitochondria, Ca^{2+} is stored as a phosphate precipitate due to the high pH and high phosphate content in the mitochondrial inner matrix[13,15]. This precipitation limits

mitochondrial free Ca^{2+} concentration to levels much lower than those in the ER. When cytosolic Ca^{2+} signals are terminated, mitochondria slowly transport Ca^{2+} back out into the cytosol. A primary role of this transient increase in mitochondrial Ca^{2+} is to link cell activation to an enhancement of ATP production and other metabolic activities[16].

While the molecular identity of the uniporter is not yet known, a mitochondrial H^+/Ca^{2+} antiporter (LETM1)[17], a mitochondrial Na^+/Ca^{2+} exchanger (NCLX)[18], and a Ca^{2+} binding protein that regulates uniporter activity (MICU1) have all been recently discovered[19]. LETM1 may provide cells with a mechanism to link mitochondrial Ca^{2+} to the mitochondrial proton gradient and aerobic ATP production by participating in both Ca^{2+} uptake and extrusion[17]. All three of these genes are widely conserved in eukaryotes, suggesting that mitochondrial Ca^{2+} regulation became important in the earliest protozoans but was lost in some lineages such as fungi (Figure 2). Indeed, the presence of MICU1 homologs in distant protozoan lineages and absence of a homolog in *Saccharomyces cerevisiae* were key observations that allowed for its identification[19].

Thus, the stage for Ca^{2+} signaling in humans is set by a conserved transport system that generates Ca^{2+} gradients across the PM as well as the ER, mitochondrial and other internal membranes, and provides a driving force for Ca^{2+} influx or release when channels are opened. Markedly, subsets of these ancient transporters were lost in multiple eukaryotic lineages[12,19] (Figure 2) but each species has at least some of these Ca^{2+} gradient generators.

Ancient roots, but great plasticity of PM Ca^{2+} channels

Cytosolic Ca^{2+} signals are almost exclusively generated by regulated opening and closing of Ca^{2+} channels and many of these channels are in the PM[1]. Humans have diverse PM Ca^{2+} conducting channels to respond to an array of stimuli with a particular importance in highly specialized cells such as muscle cells and sensory neurons. Given that many of these specialized cells are unique to animals, it is remarkable that representatives of most human PM Ca^{2+} channels are found in distant eukaryotic lineages (Figure 2). This implies that single-celled eukaryotes, living over 1 billion years ago, already used influx of extracellular Ca^{2+} as a major signaling mechanism to respond to a variety of external cues.

Humans have gene families encoding PM Ca^{2+} channels that are gated by different mechanisms, including voltage-gated Ca^{2+} channels (VGCC) that are activated by PM depolarization1, transient receptor potential (TRP) channels that are often regulated by different sensory inputs such as heat and mechanical stress[1,20], cyclic nucleotide-gated channels (CNGCC) that are typically opened by the intracellular second messengers cAMP or cGMP[21], and glutamate receptor channels (GLURCC) that respond to the extracellular presence of the amino acid glutamate. The VGCC, CNGCC, and GLURCC families each have apparent homologs in the *Naegleria gruberi* genome[22] and in either plants or algae[23]. The presence of these distant homologs (Figure 2) suggests that they were likely present in the earliest eukaryotes. The TRP family has homologs in *Saccharomyces cerevisiae*[24] and in the algae *Chlamydomonas reinhardtii*[23]. The polycystic kidney disease (PKD) channels, which are a subtype of TRP channel often associated with specialized cell extensions called primary cilia[25], are widely represented. These observations suggest that TRP channels also arose early. Finally, the ATP-regulated purinergic Ca^{2+} channels (PurRCC) and the cholinergic Ca^{2+} channels (ChoRCC), which have been largely implicated in cell-cell communication in higher eukaryotes, may be more recent inventions as homologs of these channels appear to be less widespread. However, PurRCC homologs have been identified in *Dictyostelium discoideum*[26] and the *Chlamydomonas reinhardtii* genome contains a ligand-gated ion channel with similarity to ChoRCCs[23,27]. As a note of caution, the ion selectivity and subcellular localization of Ca^{2+} channel homologs is difficult to discern from sequence

homology alone and some of these homologs may conduct other cations and may not act in the PM[28]. Nevertheless, evolutionary analyses argue that PM Ca^{2+} channels of many subtypes arose early in eukaryotic evolution, though particular subtypes were also often lost in different eukaryotic lineages.

Evolution of the intracellular Ca^{2+} release system

Humans have three major known routes for Ca^{2+} release from internal stores. The most prominent mechanism is based on inositol trisphosphate (IP_3)-mediated opening of IP_3 receptor (IP3R) Ca^{2+} channels[29], a process that is further amplified by Ca^{2+} binding to IP3Rs themselves[30]. A second ER release mechanism is based on Ca^{2+}-triggered opening of ryanodine receptor (RyR) Ca^{2+} channels[31], a process that can be modulated by cyclic adenosine diphosphate (cADP)-ribose and, in the case of skeletal muscle, by a physical interaction with a PM-localized voltage-gated Ca^{2+} channel. A third release mechanism involves the opening of two-pore Ca^{2+} channels, endosome- and vacuole-localized proteins that can be regulated by nicotinic acid adenine dinucleotide phosphate (NAADP) but have otherwise largely unresolved functions[32].

Both the IP3R and the two-pore channel have distant homologs in the plant kingdom and protozoan lineages while the RyR appears more recently[23,33,34]. It is also interesting that the most common regulation of human IP_3 production, via G-protein (GαQ) activation of phospholipase C β (PLCβ)[35], is likely to be a relatively recent phenomenon (appearing after the IP3R) while the Ca^{2+} regulated phospholipase C δ (PLCδ), which also produces IP_3, likely arose earlier and is more broadly present than IP3R Ca^{2+} channels (Figure 2)[36]. This suggests that hydrolysis of phosphoinositide lipids by phospholipase C has older roles than triggering Ca^{2+} release, possibly in the production of IP_6 and other higher phosphorylated inositol products[35,37]. It is then conceivable that the IP3R may have initially contributed to an indirect Ca^{2+} amplification process whereby IP3R-mediated release of Ca^{2+} increased PLCδ activity which in turn produced more IP_3, more Ca^{2+} release and more polyinositol phosphates. However, there is also evidence for IP_3-mediated Ca^{2+} signaling in organisms that lack IP3R homologs[38,39], although the channels responsible have not been identified and the mechanism may be indirect. Thus, an alternative possibility is that IP_3 generation was initially linked to Ca^{2+} release from internal stores by multiple mechanisms, and different mechanisms remain in different modern organisms.

While Ca^{2+} release from internal stores is an ancient signaling mechanism prominent in all human cells, each of these types of release channels has also been lost during the evolution of a number of modern organisms (Figure 2 and Box 2). For example, the IP3R is absent from the genome of the diatom *Thalassiosira pseudonana*[40], and the two-pore channel was lost during the evolution of *Caenorhabditis elegans*[41]. A number of species, including the yeast *Saccharomyces cerevisiae*[42], lack all three (IP3R, RyR, and two-pore) of these channel types. However, the *Saccharomyces cerevisiae* uses a TRP-family channel (YVC1) to release Ca^{2+} from vacuolar stores[24]. Thus, while internal Ca^{2+} release mechanisms are present in almost every eukaryote, the channels used, and presumably the current and activation properties of those channels, vary widely.

Placing STIM proteins into the eukaryotic evolutionary map

In addition to having cytosolic signaling roles, Ca^{2+} also plays an important role inside the ER where it regulates a host of Ca^{2+}-dependent chaperones that assist protein folding[43]. A requirement of Ca^{2+} for protein folding and processing continues throughout the secretory system[44,45]. Several Ca^{2+}-dependent chaperone proteins (such as calnexin and calreticulin) are highly conserved[46] (Figure 2), and even in yeast where ER Ca^{2+} levels are lower (~10 μM) and the calnexin homolog does not appear to be Ca^{2+}-dependent, depletion of secretory

pathway Ca^{2+} results in defects in protein processing and secretion[47]. This raises the question of how cells can maintain sufficient ER Ca^{2+} concentration (~400 μM in humans) to prevent ER stress in spite of variable Ca^{2+} signaling activity. It is also not obvious how cells can generate long-term cytosolic Ca^{2+} signals since cells with elevated cytosolic Ca^{2+} are expected to slowly lose all Ca^{2+} from the ER and cytosol by PMCA-mediated extrusion from the cell.

STIM proteins solve this problem by providing a functional connection between the regulation of ER and cytosolic Ca^{2+}. STIM1 was first identified[3,4] as the elusive ER Ca^{2+} sensor that can control ER Ca^{2+} levels and enable long-term increases in cytosolic Ca^{2+}upon receptor stimulation. Initial studies showed that ER-localized STIM1 proteins are kept inactive by binding of Ca^{2+} to an EF hand located in the ER luminal part of STIM1[3,48]. Furthermore, Ca^{2+} dissociation induced by ER Ca^{2+} depletion led to the translocation of STIM1 to sites in the ER close to the PM without insertion of STIM1 into the PM[3]. Imaging of fluorescent protein conjugated STIM1 using total internal reflection fluorescence microscopy (TIRF), which allows monitoring specifically of events close to the PM, suggested that dedicated ER-PM junctions exist where STIM1 remains on the ER side in close proximity to the PM, enabling potential direct regulation of PM Ca^{2+} channels[3]. This provided evidence for the now well established model that STIM proteins have a direct ER to PM signaling role. While the existence of an ER store-operated Ca^{2+} influx process (SOCE) was hypothesized to exist in 1986[8], it took close to 20 years to identify STIM1 as the first molecular component of this SOCE pathway. A second component, the PM Ca^{2+} channel Orai[5–7] was then identified and found to be directly activated by STIM at these ER-PM junctions[36] and STIM2 was found to have similar roles as STIM1 in regulating Ca2+ influx[49]. Furthermore, accessory proteins were identified such as calmodulin (CALM)[50], the Ca^{2+} regulated adaptor protein CRACR2[51] and possibly other STIM-regulated proteins such as voltage-gated Ca2+ channels[52,53], Trp channels[54,55] and adenylyl cyclases[56].

The physiological roles of STIM proteins and SOCE have been extensively characterized in T-cells where they are critically required for allowing sustained Ca^{2+} signaling, and long-term activation of CALM, the protein phosphatase calcineurin (CALNA and CALNB) and the transcription factor NFAT, which in turn leads to T-cell differentiation and activation[57]. Recent studies further showed that mutations or deletion of STIM or Orai result in severe immunodeficiencies[5,58]. STIM and Orai have been found in every mammalian cell type examined thus far and in a number of model organisms, and regulate many additional processes such as neuronal excitability[59] and muscle activity[60], suggesting that they are likely core elements of all human Ca^{2+} signaling systems.

At what point along the trajectory of human evolution did this STIM signaling system evolve? One can imagine two scenarios for the ancient roles of the SOCE system. STIM-mediated signaling could initially have served to stabilize ER Ca^{2+} levels and regulate the function of ER chaperones. Alternatively, the main role of the ancient STIM pathway could have been to sustain cytosolic Ca^{2+} signals when receptor stimuli persistently increased IP_3 levels. A survey of the evolutionary record provides some insights. First, STIM and Orai homologs are present in the genome of the single-celled choanoflagellate *Monosiga brevicollis*[61] (see also[62]), which diverged from the human line prior to the apparent origins of the RyR, the muscle ER Ca^{2+} storage protein calsequestrin (CASQ)[63], or the T-cell differentiation transcription factor NFAT (Figure 2b)[57]. A second notable observation is that a number of species that have the IP3R lack STIM and Orai. Examples include *Paramecium tetraurelia*[33] and *Naegleria gruberi*[22]. Therefore, the presence of STIM and Orai is not a universal complement to IP_3-mediated Ca^{2+} signaling.

Remarkably, distant but recognizable Orai homologs appear in the genomes of green algae (*Chlamydomonas reinhardtii*)[27], moss (*Physcomitrella patens*)[64], and of the diatom *Thalassiosira pseudonana*[40]. While it is impossible to determine by sequence analysis alone whether these homologs function as store-operated Ca^{2+} channels, it is interesting to note that putative homologs in each of these species have conserved the E106 residue (Figure 2c), which has been identified as the main Ca^{2+} binding site controlling ion selectivity in the pore of Orai1[65–67]. Several of these species with Orai ancestors lack the IP3R (*Physcomitrella patens* is an example[23,64]). The distant Orai homologs are suggestive of the presence of SOCE without IP_3-mediated signaling. This could imply an initial evolutionary role for SOCE in regulating ER Ca^{2+} levels before SOCE became critical for prolonging IP_3-mediated Ca^{2+} signals. However, the co-appearance of clear STIM homologs with PLCβ and GαQ in *Monosiga brevicollis* and animals, supports the alternative model that STIM arose as a complement to receptor-operated IP_3 signaling, and that this system co-opted an existing Orai channel for SOCE. Additionally, the appearance of Orai before STIM could indicate that the earliest Orai proteins were controlled by a different gating mechanism. Indeed, there is evidence that human Orai1 and Orai3 isoforms may have a second role as part of an arachidonic acid-gated Ca^{2+} channel[68]. Thus, this question remains until some of the functions of these ancestral homologs are better characterized. (See Box 1 for additional food for thought on why ER Ca^{2+} levels are below the extracellular levels and a putative alternative SOCE mechanism to regulate Ca^{2+} influx in fungi.)

Core regulatory motifs of human STIMs with common evolutionary roots

After STIM1 and STIM2 proteins were discovered in 2005, a burst of research led to the characterization of domains and sequence motifs that define the molecular signaling steps in the activation of these two luminal ER Ca^{2+} sensors (Figure 3a). Key parts of the activation mechanisms are shared between STIM1 and STIM2[49,69]. In short, activation begins with Ca^{2+} dissociation from a conserved EF-SAM module in the lumen of the ER[3,70] that triggers STIM oligomerization followed by translocation of the oligomers to ER-PM junctions where they directly interact with PM-localized Orai channels and induce Ca^{2+} influx.

A detailed description of this signaling process begins with the luminal EF-SAM module of both STIM1 and STIM2, which each bind a single Ca^{2+} ion (Figure 3b)[70], suppressing their homo or hetero-oligomerization[49,70–72]. Several studies suggest that STIM may already be present as an inactive dimer in unstimulated cells[73–75] and then undergo higher order oligomerization upon Ca^{2+} dissociation. Independent of the basal state, upon depletion of ER Ca2+ stores, STIM proteins undergo a rapid increase in their oligomeric state, and this increase triggers their translocation to the PM where they bind and activate Orai. Live cell fluorescence resonance energy transfer (FRET) experiments show that oligomerization occurs within seconds after a drop in ER Ca^{2+} while STIM is still in ER membranes away from ER-PM junctions[71]. In turn, this rapid oligomerization process is sufficient for STIM to translocate to ER-PM junctions and activate Orai[76]. Post-oligomerization, the translocation process likely involves a local passive diffusion of STIM proteins over a few micrometers[71] to reach nearby ER-PM junctions.

The evolutionarily conserved minimal functional motifs of STIM1 and STIM2 (Figure 3c) consist of the EF-SAM Ca^{2+} sensor in the lumen of the ER, a single transmembrane spanning domain and a cytosolic coiled-coil domain that likely serves as a ~15 nm spacer (estimated as 0.15 nm per amino acid for an alpha helix[77]) to bridge the space between the ER and PM (in STIM homologs, this domain is often broken into two segments). A subsequent CRAC activation domain (CAD; also termed a STIM1 Orai activating region, SOAR) also contributes to the oligomerization[73,78,79] and acts as the main mediator of Orai recruitment and activation. The formation of a CAD tetramer is sufficient for binding to

Orai[79] and, within the native protein, the same CAD is required to recruit and activate PM-localized Orai at ER-PM junctions.

Evolutionary and functional separation of STIM1 and STIM2

STIM1 and STIM2 proteins appeared by gene duplication from a common ancestral STIM protein during the evolution of vertebrates approximately 500 million years ago[62] (Figure 3c). The conservation of both types of STIM proteins in all currently sequenced vertebrate species suggests that they have non-redundant important roles. A main molecular difference between STIM1 and STIM2 is an approximately 2-fold difference in their respective Ca^{2+} sensitivity. The ER Ca^{2+} level where translocation starts to be triggered is approximately 400 μM for STIM2 and 200 μM for STIM1[49]. In cells where both proteins are present, this difference in Ca^{2+} affinity makes STIM2 more likely to be a main regulator of basal ER and cytosolic Ca^{2+} levels. In addition, STIM2 can also generate Ca^{2+} signals for weak receptor stimuli that cause only a small reduction in ER Ca^{2+}. STIM1, which is typically present at much higher concentrations than STIM2, is only activated for stronger receptor stimuli. The activation of both STIM isoforms is very steep, changing cooperatively with ER Ca^{2+}[49]. STIM2 has further been shown to have a lower relative activity than STIM1 in triggering Orai activation[49,69], suggesting that it is well suited for basal Ca^{2+} homeostasis, which typically requires small Ca^{2+} fluxes. Nevertheless, in T-cells, STIM2 is strongly upregulated following stimulation and prolongs the long-term Ca^{2+} signals[80], possibly allowing ER Ca^{2+} levels to remain sufficiently high to prevent an ER stress response while still driving long term SOCE Ca^{2+} influx. STIM2 also has important functions in neuronal and muscle Ca^{2+} homeostasis[59,81], consistent with a ubiquitous role in long term Ca^{2+} homeostasis and signaling.

Evolution of additional regulatory features of STIM1 and STIM2

In addition to the four core domains, all vertebrates and some invertebrates, but not insects, have a polybasic charged motif at the C-terminus of STIM that is reminiscent of an electrostatic phosphoinositide lipid binding motif[82]. Indeed, this motif has been shown to bind to phosphoinositide lipids *in vitro*[83], and phosphoinositide lipids are highly enriched in the PM. While the eight charges in a STIM1 monomer are too weak to induce PM binding on their own[82], it is interesting that the translocation of oligomerized STIM1 to ER-PM junctions in the absence of Orai requires this motif[71]. The motif is not required to trigger Orai activation in cells where both proteins are overexpressed[79]. Since STIM and Orai are present physiologically at much lower levels, the polybasic region likely enhances the retention of STIM at ER-PM junctions and thereby increases the kinetics and cooperativity of STIM-mediated Orai binding and activation. The same motif may play a subsequent additional role by participating in the interaction with Orai[84]. The Orai-independent lipid interaction of STIM is likely a result of an oligomerization-mediated exposure of a large polybasic surface (consisting of the combined polybasic tails) that then becomes sufficiently strong to bind to phosphoinositide lipids in ER-PM junctions[71,85]. In other words, each polybasic C-terminal peptide may only contribute a weak binding activity that generates in the oligomer a high affinity phosphoinositide interaction and drives the translocation. While the polybasic motif is conserved in STIM proteins in vertebrates[62] and in *Monosiga brevicollis*[86], it is not always present. STIM proteins in *Drosophila melanogaster* do not have the polybasic motif[62] and may instead rely on direct regulation of Orai by STIM without support by this intriguing lipid facilitation mechanism.

Some invertebrate and all vertebrate STIMs also have a stretch of negative charges referred to as a CRAC modulatory domain (CMD) or inactivation domain of STIM (ID_{STIM}) that mediates delayed Orai inactivation[87,50]. This domain appears to be absent in *Monosiga*

brevicollis, and the *Monosiga brevicollis* genome[86] lacks an apparent homolog of the recently characterized SOCE regulator CRACR2A or its paralog CRACR2B (grouped together as CRACR2 in Figure 2). CRACR2A is an EF-hand containing protein that interacts with both STIM and Orai. Upon ER Ca^{2+} release, CRACR2A first enhances STIM-Orai interaction in its Ca^{2+} free form and then suppresses STIM-Orai interaction following Ca^{2+} binding[51]. The absence of CMD domains and CRACR2 in *M. brevicollis* suggests that Ca^{2+}-dependent feedback mechanisms to inactivate Orai may have been elaborations only advantageous in some multicellular organisms. Nevertheless, the role of this region in different multicellular species is at this point not yet fully understood.

STIM also has an [S/T]xIP sequence, a transport motif that mediates STIM binding to the microtubule plus-end tracking protein EB1 and directs STIM movements within the ER membrane[88,89]. An [S/T]xIP motif is present in STIM1 and STIM2 in many vertebrate genomes including those of *Xenopus laevis* and *Gallus gallus*, but it is absent in *Danio rerio* and in the invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans*[90–94]. The EB1 binding interaction is apparently lost upon oligomerization[88], suggesting that microtubule binding helps STIM proteins localize to regions in the ER closer to the periphery of cells without directly mediating translocation to ER-PM junctions. As discussed above, STIM oligomers may then translocate to ER-PM junctions over short distances by a diffusion and capture mechanism. In addition, the STIM-mediated coupling of ER and microtubules may also support, together with other coupling mechanisms, the well known role of microtubules in ER morphology and tubule formation[88,89]. Finally, phosphorylation sites matching a cyclin-dependent kinase consensus sequence have been identified in STIM1[95]. Phosphorylation of these sites suppresses STIM1-mediated Ca^{2+} influx during mitosis[95]. They are specific to STIM1, and the predicted phosphorylation sites are only conserved in higher vertebrates. It is unclear whether a similar mechanism is used in other organisms. Thus, while the pathway includes a number of important modulators, the main function of STIM1 and STIM2 proteins is to monitor ER luminal Ca^{2+} levels and enhance Ca^{2+} influx across the PM when ER Ca^{2+} levels drop below critical respective thresholds.

The above discussion argues that STIM proteins are part of interconnected cytosolic, ER and mitochondrial Ca^{2+} signaling systems that differ between organisms and cell types. The purpose of such complex systems with Ca^{2+} as a single nodal point is likely to integrate multiple upstream receptor and sensory inputs that merge onto Ca^{2+}, which then controls in parallel a large number of mediators that in turn coordinate processes such as cell contraction, secretion, translation, transcription and differentiation to generate an organized cell specific physiological response.

Conclusions

We have discussed STIM proteins using an evolutionary perspective that provides valuable molecular insights into the activation and role of STIM in the context of other Ca^{2+} channels, transporters and regulatory processes. This led to insights into the functions and origins of the SOCE signaling pathway. Our considerations suggest that the ER Ca^{2+} sensor STIM may have arisen in evolution after an existing Orai PM Ca^{2+} channel and co-opted Orai for two purposes: first, to stabilize ER Ca^{2+} levels and, second, to support increases in amplitude and duration of IP3R signaling. These increases in IP3R signaling may have coincided with the appearance of receptor coupling to PLCβ for IP_3 production. Duplication of STIM in an early vertebrate ancestor allowed specialization of two regulatory functions with STIM2 specializing as a basal homeostatic Ca^{2+} regulator that also responds to weak receptor stimuli (sensing small decreases in ER Ca^{2+}) and STIM1 playing a primary role in

prolonging and increasing cytosolic Ca^{2+} signals following strong receptor stimulation that triggers larger drops in ER Ca^{2+}.

Box 1

ER Ca^{2+} homeostasis without STIM and Orai

Eukaryotic cells need to keep a high Ca^{2+} concentration (~400 μM) in the ER to regulate chaperones and enzymes that control protein folding, modification, and secretion. How do organisms that lack STIM and Orai regulate ER Ca^{2+}? One mechanism has been uncovered in the yeast *Saccharomyces cerevisiae*. In this organism, depletion of Ca^{2+} from the secretory pathway results in Ca^{2+} influx across the plasma membrane through a voltage-gated Ca^{2+} channel homolog Cch1 (which has no homology to Orai)[104] and this leads indirectly to refilling of ER Ca^{2+} stores. This regulatory process involves two stress response pathways. One is the unfolded protein response (UPR)[105] that is triggered by low ER and Golgi Ca^{2+} which leads to incorrect folding, glycosylation, and sorting of secreted proteins. This in turns activates the cell wall integrity MAP kinase pathway which protects cells from lysis during polarized growth and in response to hypo-osmotic shock[105,106]. This suggests that yeast employ a STIM-independent ER Ca^{2+} homeostasis mechanism that is triggered indirectly by defects in the secretory system. While there is no evidence for a similar UPR- related Ca^{2+} influx mechanism in humans, a close link between ER Ca^{2+} homeostasis and ER protein folding and cell stress has also been found in humans and other animals[107]. It is therefore conceivable that STIM or other sensors may, in addition to their signaling and homeostatic roles, have roles related to the ER stress response.

If high ER Ca^{2+} levels prevent ER stress, this raises the question of why Ca^{2+} levels in the ER are not much higher and are instead relatively close to the threshold for triggering a stress response (basal ER Ca^{2+} is ~ 400 μM [Ca^{2+}] compared to ~ 1.5 mM outside of the cell). A plausible reason is that ER Ca^{2+}-regulated chaperones also have a regulatory role so that the level of ER Ca^{2+} may adjust the rate of secretion of selected proteins. Furthermore, the level of ER Ca^{2+} may have to be kept below that of the extracellular environment to reduce the chance that adhesion proteins that have weak Ca^{2+} binding sites[108] inadvertently polymerize along the secretory path before reaching the outside of cells (Ca^{2+} is needed for many secreted proteins to bind to each other). Thus, several open questions remain concerning how ER Ca^{2+} levels are regulated, how STIM signaling is connected to protein folding and secretion and how these processes are related to ER stress and human disease.

Box 2

Brief description of model organisms

Xenopus laevis. African clawed frog.

Gallus gallus. Chicken.

Danio rerio. The zebrafish is a tropical freshwater fish and a common model organism for studying vertebrate development.

Caenorhabditis elegans. This is a millimeter-long roundworm that serves as a popular model for genetics and development.

Drosophila melanogater. Fruit fly.

Hydra magnipapillata. This organism is a small (millimeter-scale), radially symmetric freshwater invertebrate. It is also known as a freshwater polyp.

Nematostella vectensis. Like Hydra, the sea anemone belongs to the phylum Cnidaria which are among the simplest animals with neurons, muscle fibers, and epithelial cells.

Monosiga brevicollis. This aquatic protozoan is one of the closest known single-celled relatives of multicellular animals. It is also known as a type of choanoflagellate.

Saccharomyces cerevisiae. The common budding yeast is a popular model organism.

Dictyostelium discoideum. Also known as a slime mold, *D. discoideum* is a species of amoeba that has both single-celled and multicellular states, and serves as a model for motility, chemotaxis and differentiation.

Chlamydomonas reinhardtii. This single-celled green alga swims by the use of two flagella. It lives in both soil and fresh water.

Physcomitrella patens. This multicellular moss is used as a model system for plant genetics and development. Mosses are estimated to have had a common ancestor with flowering plants approximately 200–400 million years ago.

Arabidopsis thaliana. This flowering plant is a popular model for genetics, and its genome was the first plant genome to be sequenced.

Thalassiosira pseudonana. These are single-celled organisms commonly known as diatoms. They live in oceans and derive energy from photosynthesis.

Paramecium tetraurelia. Paramecium are single-celled protozoan that live in freshwater environments, move by use of cilia that cover the cell surface, and prey on other single-celled organisms such as bacteria and algae.

Naegleria gruberi. These are single-celled protozoans that are capable of transitioning between a state in which they move in an amoeboid process to a state in which they move through flagellar motion.

Glossary

Important Ca^{2+}-related transport and signaling proteins.

CALM	Calmodulin. Most ubiquitous and important mediator of cytosolic Ca^{2+} signaling. Binds four Ca^{2+} ions.
CANX	Calnexin. Ca^{2+}-regulated ER chaperone.
CALNA, CALNB	Calcineurin (catalytic and regulatory subunits, respectively). Ca^{2+}/CALM-regulated Ser/Thr phosphatase.
CALR	Calreticulin. Ca^{2+}-regulated ER chaperone.
CASQ	Calsequestrin. Ca^{2+}-binding protein that enhances storage capacity of the ER.
CholRCC	PM-localized cholinergic Ca^{2+} channels (e.g., acetylcholine receptors).
CNGCC	PM cyclic nucleotide-gated Ca^{2+} channels.
CRACR2	Ca^{2+}-regulated adapter for Orai and STIM. CRACR2A has a characterized role in regulating SOCE[51], but we also include here its less characterized paralog CRACR2B.
EB1	Microtubule plus end binding protein.
GluRCC	PM-localized glutamate-regulated Ca^{2+} channels (nicotinic).

GαQ	G-protein alpha subunit that regulates PLCβ.
IP3R	IP_3-regulated ER-localized Ca^{2+} channel.
LETM1	Mitochondrial Ca^{2+}/H^+ exchanger.
MICU1	Ca^{2+} sensor that regulates the Ca^{2+} uniporter.
NCKX	(SLC24A1–5) PM-localized $Na^+/K^+/Ca^{2+}$ exchanger.
NCLX	(SLC24A6). PM-localized $Na^+/Li^+/Ca^{2+}$ exchanger.
NCX	(SLC8A). PM-localized Na^+/Ca^{2+} exchanger.
NFATc	Nuclear factor for activated T-cell, key regulator of T-cell differentiation.
Orai	PM Ca^{2+} channel regulated by the ER Ca^{2+} sensor STIM
PKD	Polycystic kidney disease Ca^{2+} channel, TRP subfamily.
PLCβ,δ	Phospholipase C, hydrolyzes phosphatidylinositol 4,5-bisphosphate, produces inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol.
PMCA	(ATP2B). PM Ca^{2+} pump regulated by Ca^{2+}/CALM.
PurRCC	ATP-regulated purinergic PM Ca^{2+} channels.
RyR	ER-localized Ca^{2+} channel regulated by Ca^{2+}.
SERCA	(ATP2A) ER-localized Ca^{2+} pump.
SPCA	(ATP2C). Golgi- and endosome-localized Ca^{2+} pump.
STIM	ER luminal Ca^{2+} sensor that controls store-operated Ca^{2+} influx into cells.
TPCC	Two-pore channel. Internal endosomal NAADP-regulated Ca^{2+} channel.
TRP	(TRPc, TRPv …) PM Ca^{2+} channels often involved in sensory signal transduction.
VGCC	PM-localized voltage-gated Ca^{2+} channels.

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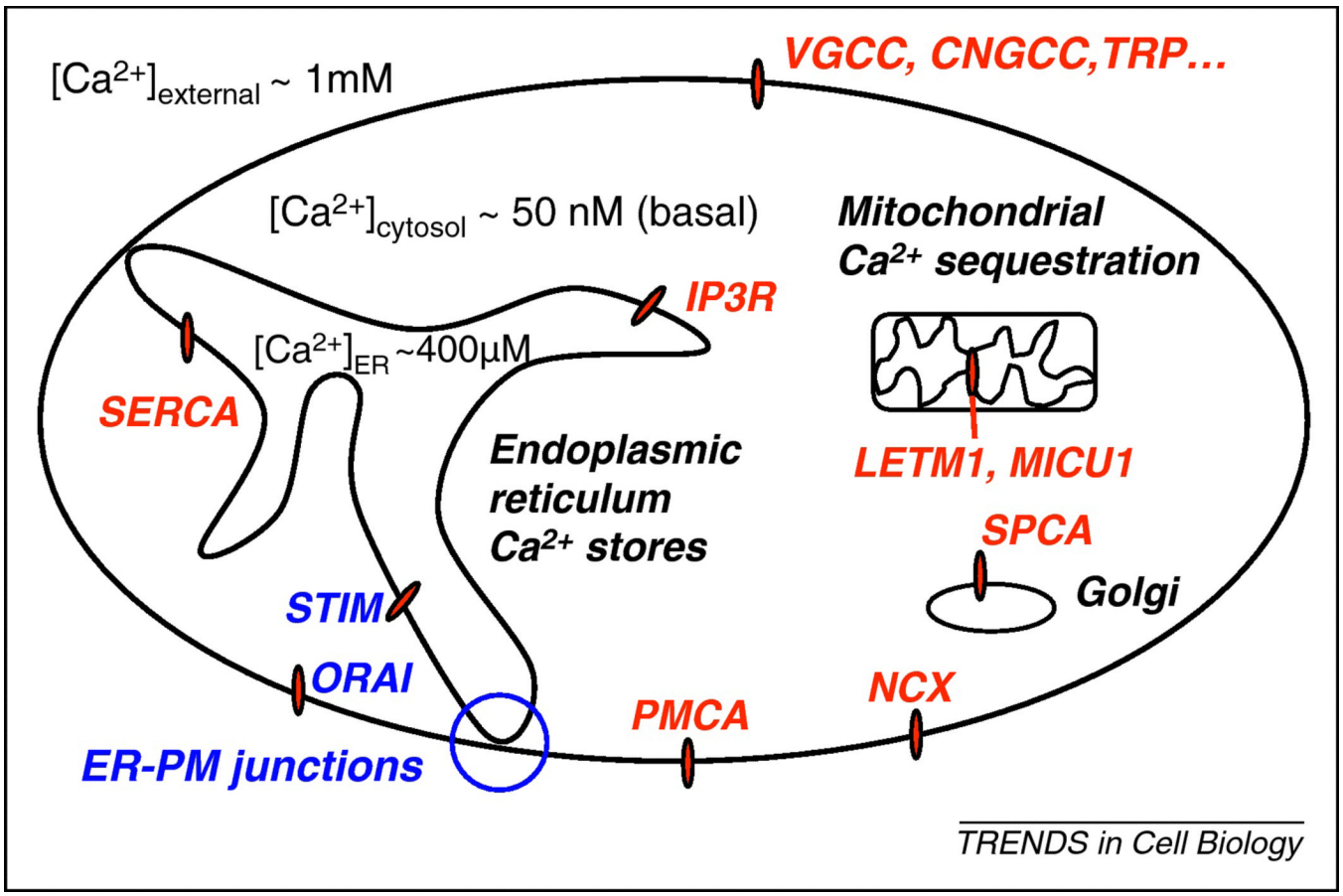

Figure 1.

Schematic representation of relevant Ca^{2+} regulators. Shown is a diagram of a eukaryotic cell with the localization of PM channels (VGCC, CNGCC, TRP, ORAI), Ca^{2+} pumps (PMCA, SERCA, SPCA), internal channels (IP3R), Ca^{2+} exchangers (NCX, LETM1), and key regulatory proteins (STIM, MICU1) indicated. Also indicated are approximate values for the Ca^{2+} concentration in the extracellular space (~1 mM), the cytoplasm (~50 nM under basal conditions), and the ER (~400 μM).

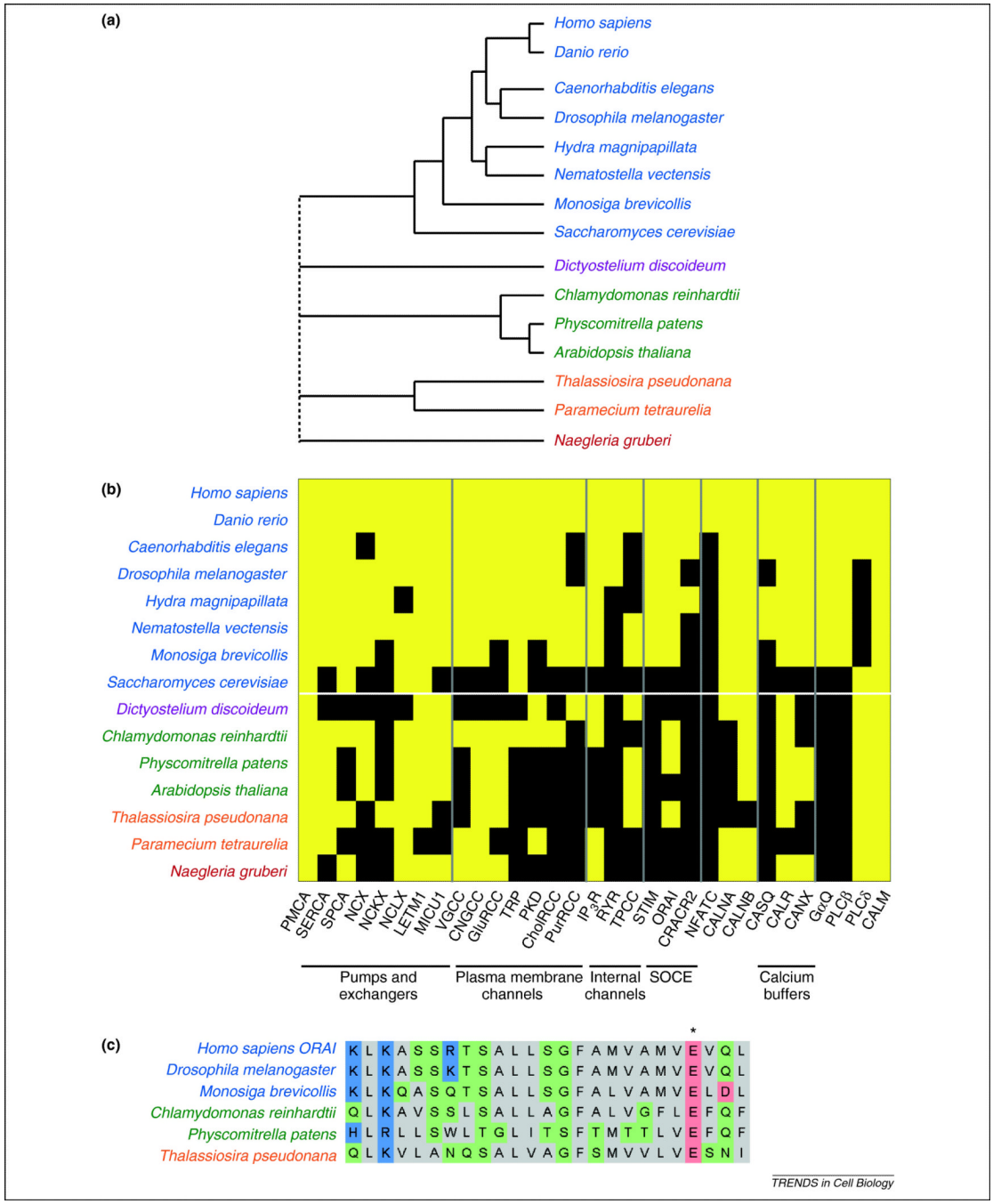

Figure 2.

Phylogenetic profiles of eukaryotic Ca^{2+} signal generating genes. (a) A consensus cladogram showing the topology (branch lengths are not intended to be to scale) of the phylogenetic relationships between the species shown in b[22,96–98]. Colored type is used to indicate membership in different major branches of the eukaryotic tree. The dotted line joining the major branches indicates an unclear phylogenetic relationship. (b) A representative set of species with sequenced genomes are labeled on the left. Human gene families are listed below (e.g., “STIM” includes the genes STIM1 and STIM2). Yellow indicates evidence for the presence of a homolog of a human gene family in a particular species, and black indicates the lack of an apparent

homolog[12,17,22–24,26,27,33,34,36,40,42,61,64,99,86,100–103,90,91,94]. (c) Shown is a portion of a multiple sequence alignment of Orai1 homologs. The organism corresponding to each sequence is indicated to the left. Only one sequence is given for human Orai, because the sequences of Orai1, Orai2, and Orai3 are identical in this region. The E106 residue in human Orai1 is indicated with an asterisk. This residue has been implicated as a key determinant of ion specificity.

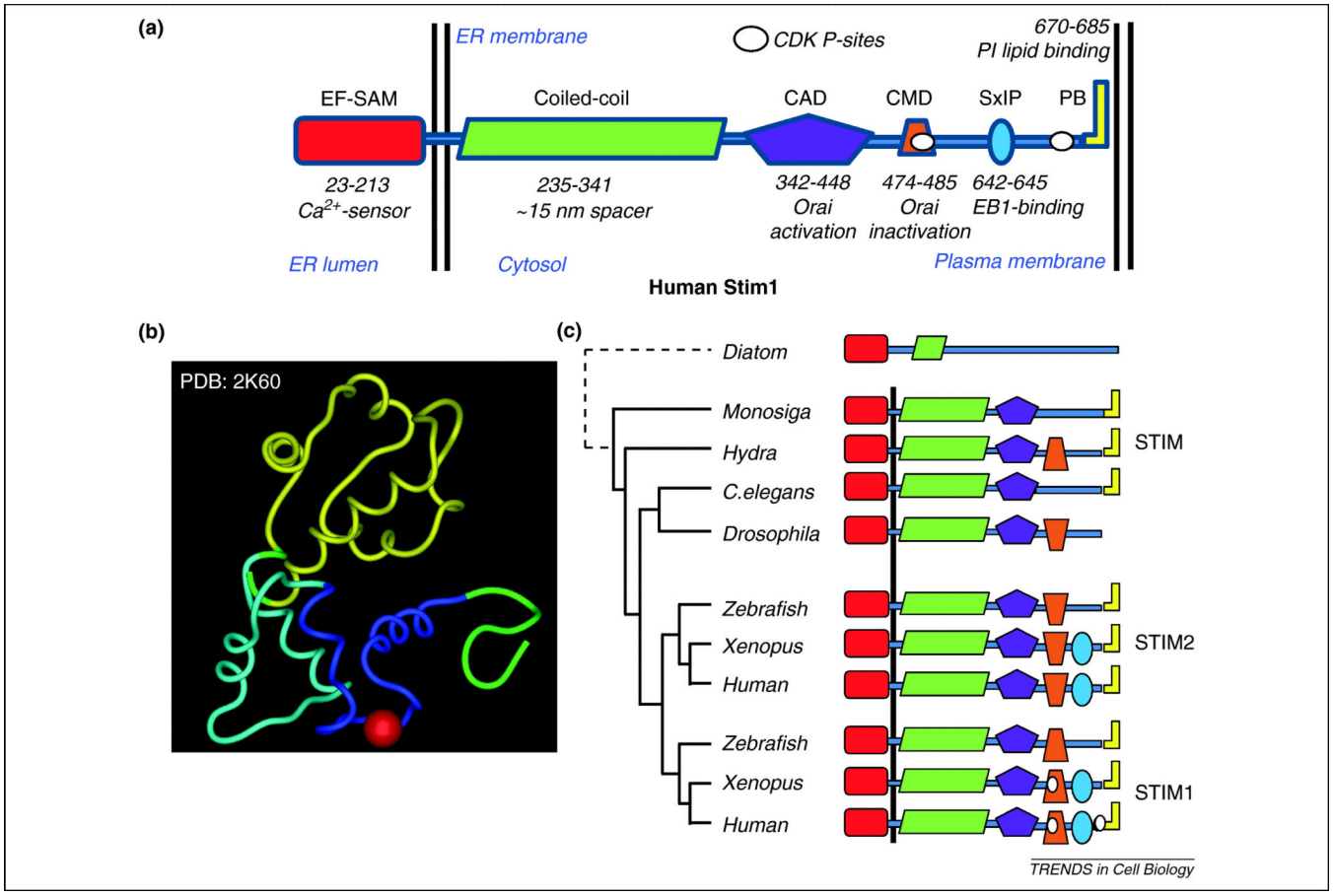

Figure 3.

Evolution of regulatory motifs in STIM proteins. (a) A diagram of the domain organization of human STIM1. The domains are annotated with residue numbers and brief functional descriptions. (b) High-resolution structure of the EF-SAM domain of STIM1 in Ca^{2+}-bound form[70]. (c) Diagrams of the domain organization of STIM homologs in *Monosiga brevicollis* (Monosiga), *Hydra magnipapillata* (Hydra), *Caenorhabditis elegans* (C. elegans), *Drosophila melanogaster* (Drosophila), *Danio rerio* (Zebrafish), *Xenopus laevis* (Xenopus), and *Homo sapiens* (Human). Diagrams for both STIM1 and STIM2 are shown for the vertebrate organisms. Invertebrates and *Monosiga brevicollis* have only a single STIM homolog. Also included is a distant STIM-related protein in *Thalassiosira pseudonana* (Diatom). The tree indicates the topology of the phylogenetic relationships between the included STIM proteins.