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Review

The STIM1–ORAI1 microdomain

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ABSTRACT

The regulatory protein STIM1 controls gating of the Ca^{2+} channel ORAI1 by a direct protein-protein interaction. Because STIM1 is anchored in the ER membrane and ORAI1 is in the plasma membrane, the STIM–ORAI pathway can support Ca^{2+} influx only where the two membranes come into close apposition, effectively demarcating a microdomain for Ca^{2+} signalling. This review begins with a brief summary of the STIM–ORAI pathway of store-operated Ca^{2+} influx, then turns to the special geometry of the STIM–ORAI microdomain and the expected characteristics of the microdomain Ca^{2+} signal. A final section of the review seeks to place the STIM–ORAI microdomain into a broader context of cellular Ca^{2+} signalling.

1. STIM–ORAI signalling

Store-operated Ca^{2+} influx is a cellular mechanism downstream of a variety of cell surface receptors, in which Ca^{2+} release from intracellular stores indirectly triggers Ca^{2+} influx through plasma membrane Ca^{2+} channels. The latter influx is utilized both for sustained cytoplasmic Ca^{2+} signalling and for refilling of the internal Ca^{2+} stores. The notion of a store-operated Ca^{2+} channel was formulated based on a body of experimental evidence from many cell types [1,2], and electrophysiologically validated by the identification of the Ca^{2+} release-activated Ca^{2+} current, or CRAC current, in mast cells and T lymphocytes [3–8].

Store-operated Ca^{2+} influx gained a tangible connection to cellular proteins with the discovery that STIM and ORAI are essential components of the store-operated influx pathway [9–13], and with subsequent detailed analyses of how these proteins function [reviewed in 14–18]. In brief, the ER Ca^{2+} sensor STIM1 is activated when a decrease in Ca^{2+} concentration in the ER lumen leads to dissociation of Ca^{2+} from its EF-hand. STIM1 undergoes a conformational change, oligomerizes, and relocalizes to ER-plasma membrane junctions. STIM1 then recruits the plasma membrane Ca^{2+} channel ORAI1 to these sites and gates the channel. The process is rendered visible by following fluorescently labeled STIM1 and ORAI1 before and during a stimulus that causes depletion of ER Ca^{2+} stores. STIM1 is present throughout the ER in unstimulated cells, and ORAI1 is more or less uniformly distributed in the plasma membrane. Following store depletion, both proteins relocalize to clusters that appear by light microscopy to coincide (Fig. 1A). A schematic view of STIM1 and ORAI1 at an ER-plasma membrane junction is shown in cross-section in Fig. 1B.

Apart from the interactions at the ER-plasma membrane junctions considered here, there is evidence of STIM–ORAI interaction at other sites where cellular membranes are in close contact. Examples are ER-secretory granule junctions [19], ER-phagosome junctions [20], and the specialized variant of ER-plasma membrane contact where sarcoplasmic reticulum is apposed to transverse tubule in skeletal muscle [21–26]. These STIM–ORAI microdomains may require individualized treatment. For example, both the dimensions and the internal free Ca^{2+} concentration of PC12 cell secretory granules are likely to require revisions to the discussion below.

2. ER-plasma membrane junctions

STIM–ORAI microdomains are restricted regions of the cell where STIM and ORAI come together and the ORAI channel is gated. A first step in analyzing signalling in the STIM–ORAI microdomain is to establish the microdomain dimensions and the typical number of STIM–ORAI microdomains in a cell.

2.1. Lateral dimensions

The geometry of ER-plasma membrane junctions has been defined most precisely by electron microscopy. In thin sections, the close appositions of ER and plasma membrane in Jurkat T cells

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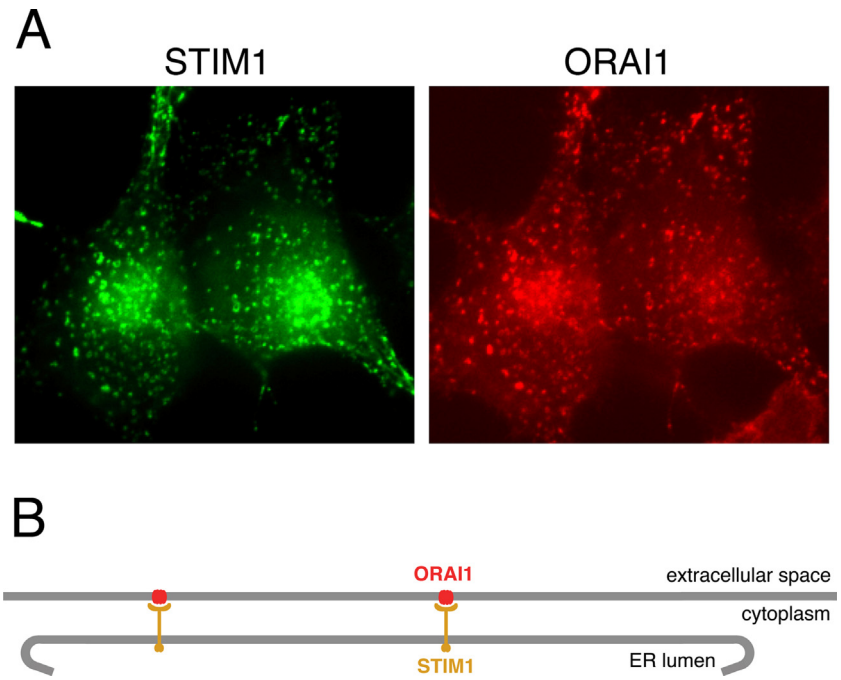

Fig. 1. (A) STIM1 and ORAI1 colocalize after ER Ca^{2+} store depletion. The micrographs are optical sections at the footprint of two HeLa cells expressing GFP-STIM1 and mCherry-ORAI1. ER Ca^{2+} stores had been depleted by treatment with thapsigargin. Images provided by GM Findlay. (B) STIM1 and ORAI1 at an ER-plasma membrane junction. This schematic view conveys the arrangement and relative dimensions of STIM1, ORAI1, and the apposed membranes that define the junction. The detailed structure and stoichiometry of the active STIM–ORAI complex have not been determined.

are, with few exceptions, under 300 nm in length – with most being under 200 nm – and occupy ~4% of the plasma membrane in linear profile [27]. In HeLa cells the junctions average 100–200 nm in length and occupy <1% of the plasma membrane [28]. Both reports are consistent with a maximum linear dimension of ~300 nm, comparable in size to the clusters of fluorescently labeled STIM or ORAI observed in Jurkat T cells, HeLa cells, and HEK293T cells after stimulation [10,27,29–32]. Note that in T cells, this dimension refers to what has been called the 'elementary unit' of store-operated Ca^{2+} entry [29], not to the T cell synapse.

Fluorescent ORAI clusters of somewhat larger diameter have been observed in some mammalian cells. The larger clusters have been particularly well documented for HEK293 cells, where single-particle tracking indicated that ORAI1 channels become confined after store depletion to domains ~700 nm in diameter, and direct measurement of the associated fluorescent STIM1 clusters gave a diameter ~1.1 μm [33]. Additionally, separate measurements of the average area of ORAI1 clusters in HEK293 cells [34] translate to a diameter ~1.4 μm. The observed variations in diameter may reflect in part the cell types or experimental conditions. However, a caveat is that overexpression of STIM1, or overexpression of STIM1 together with store depletion, has been found to increase the extent of ER-plasma membrane contacts in electron microscopic measurements [27,28]. Indeed, in the second HEK293 case cited, 30% of plasma membrane in the TIRF microscopy footprint corresponded to clusters [34], indicating a likelihood that STIM1 overexpression perturbed the ER-plasma membrane contacts.

2.2. ER-plasma membrane spacing

The gap between ER and plasma membrane in Jurkat T cells has been measured from electron micrographs as 17 ± 10 nm [27]. In pancreatic acinar cells, it averaged ~13 nm [35]. The measurement for HeLa cells averaged ~8 nm in epon sections and ~11 nm in cryosections, with a maximum separation of 17 nm [28,36]. Junctions were visible in control HeLa cells, but were measured in cells overexpressing STIM1, which increases the extent of junctions. In HeLa cells overexpressing the extended synaptotagmin E-Syt3, which also increases junctions, the average spacing was 10 nm [37]. Since deviation of the plane of a 50-nm section by a few degrees from perpendicular to the apposed membranes could cause an apparent narrowing of the gap by several nanometers, it is conceivable that the actual distances are nearer to the reported maximum measurements. An independent series of experiments in which ER and plasma membrane were artificially tethered at defined distances indicated that a separation >9 nm is required for ORAI1 to enter the junction [38]. Collectively, these data support a conclusion that the ER-plasma membrane spacing is 10–20 nm.

The narrow gap is intrinsic to the mechanism of store-operated Ca^{2+} influx. STIM remains anchored in the ER during STIM–ORAI signalling, but it is clear that the STIM cytoplasmic domain spans the gap. The early proposal that the C-terminal polybasic tail of STIM1 contacts the plasma membrane [39,40] is supported by evidence that this segment of STIM1 interacts with phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3) [41–43] and evidence suggesting that the interaction contributes to STIM1 targeting to ER-plasma membrane junctions [39,40,44,45]. In parallel, abundant evidence has established that STIM1 interacts with the ORAI channel complex in the plasma membrane and, indeed, interacts directly with ORAI to gate the channel [31,44,46–52]. It is generally accepted that it is the CC1 region of STIM that spans the distance, either as a coiled coil or as a partially α-helical extended form. Measurements of intramolecular FRET using suitable labels have shown that the STIM1 cytoplasmic domain is physically extended in its active conformation [42,53].

2.3. Number of junctions

A working estimate of the number of junctions in a cell can be obtained from the total plasma membrane area, the percentage of plasma membrane area occupied by junctions, and an assumed junction diameter of 300 nm. Total plasma membrane

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