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The STIM1-ORAI1 microdomain

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Graphical 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.

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 relocates to ER-plasma membrane junctions. STIM1 then recruits the plasma membrane Ca^{2+} channel ORAI1 to these sites and

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gates the channel. The process is rendered visible by following fluorescently labelled 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 relocate to clusters that appear by light microscopy to coincide [FIGURE 1A]. A schematic view of STIM1 and ORAI1 at an ER-plasma membrane junction is shown in cross-section in FIGURE 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.

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.

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 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 labelled STIM or ORAI observed in Jurkat T cells, HeLa cells, and HEK293T cells after stimulation [10,27,29,30-32]. 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.

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].

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 area is inferred from electrophysiological measurements of cell capacitance. Here the percentage of plasma membrane area occupied by junctions will be equated to the linear estimate from electron microscopy of the fraction of plasma membrane contacted, although this is only an approximation in the absence of a strict stereological analysis. Thus, Jurkat cells have ~ 360 junctions ($600 \mu\text{m}^2 \times 0.04 / 0.07 \mu\text{m}^2$) and HeLa cells, ~ 400 junctions ($1500 \mu\text{m}^2 \times 0.02 / 0.07 \mu\text{m}^2$). These values are in line with the totals that might be extrapolated from counting fluorescent ORAI clusters visible in the cell footprint of stimulated cells [27,29,30,31],

although none of the cited papers made a formal quantitative estimate. Extrapolation from clusters in the cell footprint gave an estimate of 300 total clusters in HEK293 cells overexpressing STIM and ORAI [34]. Unavoidably, all the fluorescence observations rely on overexpression of ORAI, but the agreement with estimates derived from electron microscopic observations on untransfected cells allays some concern on this point.

Substructure or subregions

A recent study has raised the possibility that ER-plasma membrane junctions are partitioned into regions subserving STIM signalling and regions subserving lipid metabolism or other functions [37]. The evidence on this point is discussed below. The existence of substructure within the ER-plasma membrane junction would not change the basic geometry of the STIM-ORAI microdomain or the broad conclusions drawn below regarding Ca^{2+} signalling in the microdomain, but it would have major implications for crosstalk between Ca^{2+} signalling and other processes that occur at ER-plasma membrane junctions.

Quantitating ORAI channels

The second step in the analysis of local signalling is to define the number of Ca^{2+} channels participating in a physiological response at a single junction. This number is perhaps lower than anticipated.

Human Jurkat T cells

The number of open ORAI channels in a cell following store depletion can be calculated from the ratio of the total cellular CRAC current to the single-channel CRAC current. The former is readily measured in voltage-clamp experiments, but the single-channel current is too small for recording above background electrical noise, and instead is inferred from fluctuation analysis (or 'noise analysis') of whole-cell CRAC currents [8,54-56]. Under assumptions that are believed to hold for the CRAC channel, the single-channel current is $\sigma^2/I(1-p)$, where σ^2 is the increased variance in whole-cell current attributable to CRAC channels, I is the mean whole-cell current through CRAC channels, and p is the open probability of an individual active channel. The variable p takes into account the fact that the channels actively contributing to current noise alternate between open and closed states. The form of this expression makes it clear that accurate determination of p is not necessary if it can be shown that p is small, but this situation does not hold for ORAI channels [55], and all three variables have to be determined experimentally.

The most reliable estimate for mammalian cells is that, in Jurkat cells with depleted stores, there are ~2200 active CRAC channels, with ~1700 channels open at any given time [54,55]. The measurements exploited the relatively large Na^+ current carried by CRAC channels when no divalent cations are present. The experimentally determined parameters for Na^+ current at a membrane potential of -110 mV were $\sigma^2/I(1-p) = -110$ fA, whole-cell CRAC current $I = -190$ pA, and $p = 0.8$ [55]. Perhaps the most striking conclusion from this experiment is that only a few channels are open per junction even under conditions designed to produce maximal store depletion. Combining the estimate of open channels in the cell

with the above estimate of the number of ER-plasma membrane junctions, there would be around 5 open channels per junction.

Current is carried entirely by Ca^{2+} when physiological levels of Ca^{2+} are present. Although noise analysis of the Ca^{2+} current in Jurkat cells is technically challenging, since both the current increase and the noise increase are very small, the single-channel Ca^{2+} current is more relevant to physiology than the single-channel Na^{+} current. Several measurements of the single-channel Ca^{2+} current in Jurkat cells have been made under slightly differing conditions [8,54]. For example, at -110 mV and with 20 mM external Ca^{2+} , σ^2/I was found to be -3.8 fA [54]. It can be plausibly argued from other data that p is roughly 0.27 in these conditions due to fast Ca^{2+} -dependent inactivation [55], which would make the single-channel current carried by Ca^{2+} -5.2 fA.

Drosophila S2 cells

In *Drosophila* S2 cells, the Ca^{2+} current and associated current noise proved somewhat more favorable for estimating the number of open channels [56]. At -110 mV and with 20 mM external Ca^{2+} , the measured σ^2/I was -6.9 fA, and whole-cell CRAC current was -67 pA. A value for p was not determined, because it was believed at the time that adequate evidence proved $p \ll 1$ for the mammalian ORAI channel. Since *Drosophila* Orai channels do not exhibit fast Ca^{2+} -dependent inactivation, the channel open probability might plausibly resemble that determined for CRAC current carried by Na^{+} in mammalian cells. On the assumption that $p = 0.8$, there would be ~ 2000 open channels (and 2500 active channels), with single-channel current around -35 fA; if $p = 0.5$, ~ 5000 open channels (and $10,000$ active channels), with single-channel current around -14 fA. Given that the transmembrane helices and particularly the pore-lining helices of the mammalian and *Drosophila* channels are very similar, it is not surprising that the single-channel currents are in reasonable agreement. The number of open channels per junction cannot be estimated for S2 cells, in the absence of a reliable count of junctions, but it is reasonable to expect that the number is of the same order of magnitude as in Jurkat cells.

An independent estimate of the total complement of Orai channels in *Drosophila* S2R+ cells, a subline of S2 cells, was made by quantitative mass spectrometry [57]. The number, ~ 800 Orai monomers or ~ 130 hexameric channels, is improbably low based on the CRAC current recorded in S2 cells. The discrepancy most likely reflects incomplete protein recovery in the cell fraction analyzed or incomplete trypsin digestion of *Drosophila* Orai, steps in the protocol for which no internal controls were included. Therefore, the quantitative mass spectrometry data can be considered valid for comparing the relative expression levels of Ca^{2+} signalling proteins under different experimental conditions, which was the purpose of the experiments, but not for the absolute quantitation of Orai.

Other cells

T cells, mast cells, and *Drosophila* S2 cells exemplify native CRAC current densities at the upper end of the range observed, although the actual whole-cell currents are small. Current densities in some other cells fall in the same range, but there are cells with demonstrably smaller CRAC currents. HEK293 cells are a prime example [12,58-60], even though they

have a comparable number of ER-plasma membrane junctions, at least when STIM and ORAI are overexpressed [34]. Proliferative vascular smooth muscle cells [61] and glioblastoma cells [62] were also found to have very small CRAC current densities, but the number of ER-plasma membrane junctions in these cells has not been reported. A reasonable conclusion, based on existing data, is that only a few ORAI channels per junction are active in mammalian cells, even under conditions of strong stimulation.

Experimental STIM-ORAI overexpression

It has been a common experimental approach to express STIM and ORAI at levels that are 10-fold to 100-fold above their native levels as assessed by evoked CRAC currents [13,60,63,64]. This has proven a powerful and even a necessary approach to visualize events at ER-plasma membrane junctions or to examine the currents of mutated channels, but a shortcoming is that STIM-ORAI overexpression may obscure certain endogenous mechanisms. One example is the involvement of the STIM1 polybasic tail in STIM1 recruitment to ER-plasma membrane junctions, which is masked in cells overexpressing ORAI1 [44], but which is likely to be important at physiological levels of STIM and ORAI expression [39,40,44]. Special care in controlling expression levels is warranted in studies of Ca^{2+} handling in the STIM-ORAI microdomain, where other functionally relevant proteins may be present at levels matched to the Ca^{2+} influx through native ORAI channels.

Experimental STIM-ORAI activation

Some caution is necessary in interpreting experiments even when STIM and ORAI are not overexpressed. Thus, the noise analysis experiments of native CRAC currents intentionally used strong stimulation to provide an adequate signal-to-noise ratio. These levels of stimulation cause massive relocation of STIM and ORAI to ER-plasma membrane junctions in experiments with the fluorescent proteins, but STIM-ORAI movement to junctions can be considerably less pronounced when physiological stimuli are used [50,65,66]. This raises the possibility that, at least in some cases of physiological stimulation, there are even fewer open channels per cluster than estimated above.

Local Ca^{2+} concentration

The key variables that will control physiological signalling are the rate at which Ca^{2+} is delivered by an individual channel, the number of active channels, and the amplitude and spatial extent of the resulting Ca^{2+} signal in the microdomain. The rate of Ca^{2+} delivery can be estimated with fair accuracy, and the number of active channels has been estimated above. The Ca^{2+} concentration profile in the microdomain can be described only qualitatively, mainly due to limited knowledge of the distribution of Ca^{2+} binding sites and the extent of Ca^{2+} clearance by plasma membrane Ca^{2+} ATPases (PMCA) and sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPases (SERCA) in the microdomain.

Single-channel current

How much Ca^{2+} current is carried by a CRAC channel under physiological conditions? The estimated -5.2 fA single-channel Ca^{2+} current in Jurkat cells refers to experiments at a membrane potential of -110 mV with 20 mM Ca^{2+} in the external solution. The current

should be adjusted downward by a factor 0.3 to correspond to physiological 1.2 mM Ca^{2+} , based on the Ca^{2+} concentration dependence of CRAC current in mast cells under conditions where Ca^{2+} -dependent inactivation is negligible [7]; and further reduced by half for physiological membrane potential, based on the typical CRAC current I–V relation. On the other hand, an upward adjustment by the factor 0.8/0.3 is called for, since there is little Ca^{2+} -dependent inactivation at physiological external Ca^{2+} concentration and physiological membrane potentials. The resulting estimate for physiological conditions is -2.1 fA ($-5.2 \text{ fA} \times 0.3 \times 0.5 \times (0.8/0.3)$), corresponding to an influx of 6300 Ca^{2+} ions/s. This admittedly rough approximation can be taken as a reference point for discussing Ca^{2+} concentrations in the STIM-ORAI microdomain. The effect of a different reference value would be to scale the steady-state concentration profile up or down linearly with the single-channel current.

Steady-state concentration profile

Consider, as a starting point, the constraints on local Ca^{2+} concentration imposed solely by the rate of Ca^{2+} influx and the geometry of an idealized STIM-ORAI microdomain with no Ca^{2+} binding sites. For simplicity, place a single CRAC channel at the center of the microdomain of typical dimensions. This case can be modelled approximately as two-dimensional diffusion of Ca^{2+} through an annular ring 15 nm deep, bounded by cylindrical surfaces 10 nm and 150 nm in radius, with a flux of 6300 Ca^{2+} ions/s entering at the inner face of the ring at steady state [FIGURE 2A]. The global Ca^{2+} concentration at the outer boundary is considered fixed, maintained unchanging at the level in the surrounding cytoplasm by Ca^{2+} buffering and Ca^{2+} uptake mechanisms. (The latter restriction simplifies the initial discussion, and is relaxed below.) The diffusion coefficient of Ca^{2+} in cytoplasm is set at $220 \mu\text{m}^2/\text{s}$ [67]. This simple model excludes the immediate vicinity of the channel opening because of the very small volume of that region, amounting to less than 0.5% of the total volume of the idealized microdomain; its very transient exposure to a Ca^{2+} ion entering the cell, typically less than 1 μs before the ion diffuses away if there is free diffusion; and the fact that Ca^{2+} movements near the channel are not adequately modelled as two-dimensional diffusion. The small region immediately surrounding the channel is considered separately below.

For the region more than 10 nm from the mouth of the channel, which constitutes the bulk of the microdomain, a graph of steady-state Ca^{2+} concentration versus radial distance from the channel is in FIGURE 2B. The concentration falls from 1.37 μM above global cytoplasmic Ca^{2+} concentration at 10 nm from the channel to cytoplasmic Ca^{2+} concentration at the boundary of the microdomain. The restriction that Ca^{2+} concentration is constant at the outer boundary can be relaxed to admit gradual changes in the global Ca^{2+} concentration. For example, the initial rate of free Ca^{2+} rise during store-operated Ca^{2+} entry or physiological Ca^{2+} oscillations in T cells is 30–40 nM/s [11,68], implying that cytoplasmic Ca^{2+} concentration can be regarded as effectively constant over the time required to reach steady state in the microdomain. With this minor modification, the graph depicts the increment above the global cytoplasmic Ca^{2+} concentration attributable to Ca^{2+} influx through the channel. The increment at a given radial position is the same for all levels of global Ca^{2+} , so that channel opening invariably elevates Ca^{2+} locally in the microdomain compared to Ca^{2+}

in the cytoplasm, but the relative difference is more pronounced at low cytoplasmic Ca^{2+} concentrations.

The picture is not changed dramatically by introducing a more realistic geometry or a few additional channels. A CRAC channel that is not located at the center of the microdomain will have a modified Ca^{2+} concentration profile, but the increments above global Ca^{2+} concentration will be of the same order of magnitude. The details need not be calculated here. When there is more than one open channel in the microdomain, the individual contributions to Ca^{2+} will be additive, as is characteristic of diffusion with multiple sources, but it is not a matter of summing the peak values. Unless channels are clustered, ‘additive’ will in general mean adding together values in the lower levels of the concentration profile. Likewise, fluctuations in channel opening and closing on the subsecond time scale are implied by the noise analysis, so that even Ca^{2+} influx through two channels that are close in space will be directly additive only during that fraction of time when both channels are open. The bottom line is that, with a single-channel current of -2.1 fA, a few channels per microdomain can be expected to elevate the local Ca^{2+} concentration above the global cytoplasmic Ca^{2+} concentration by only hundreds of nanomolar to several micromolar, in most of the microdomain.

It is worth noting here that the local free Ca^{2+} ‘concentrations’ in **FIGURE 2** can only be specified as averages over time. It is easy to calculate that even if the highest concentration depicted in the profile, 1.37 μM , were achieved uniformly in the microdomain, the free Ca^{2+} occupancy of the entire microdomain would be ~ 0.87 Ca^{2+} ions. With a little more effort, one can integrate the profile in **FIGURE 2** over volume, and show that the expected free Ca^{2+} content within the annular ring is only ~ 0.16 Ca^{2+} ions. Therefore, to the extent that a few channels can faithfully transmit a signal, it is because their signal is averaged over a large number of ER-plasma membrane junctions in the cell, or the local signal is averaged over time at a single ER-plasma membrane junction.

Ca^{2+} binding sites in the microdomain

The simplified picture presented thus far still needs to incorporate one critical feature of real cells. Ca^{2+} binding sites in a cell far outnumber free ions, and the free Ca^{2+} concentration near a site of Ca^{2+} influx can be profoundly shaped by the presence of Ca^{2+} binding sites. ‘Fixed’ sites, whether binding sites associated with effectors or other Ca^{2+} binding sites, can slow the rise and fall of Ca^{2+} concentration, but they do not alter the steady-state concentration profile of free Ca^{2+} . ‘Mobile’ sites can affect both the kinetics and the shape of the steady-state profile of free Ca^{2+} . Erwin Neher and colleagues developed a mathematical framework for analyzing the effect of mobile binding sites on local Ca^{2+} concentrations in a series of detailed papers. An accessible presentation of this framework [69] can be read either for the general conclusions or as an introduction to the technical side of the analysis.

A special class of ‘binding’ sites includes the plasma membrane Ca^{2+} ATPases (PMCA) and the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPases (SERCA), whose general effect will be to reduce local Ca^{2+} concentrations. PMCA has been shown to respond to local Ca^{2+} influx in Jurkat T cells and to shape Ca^{2+} signalling temporally [70,71]. The spatial

distribution of Ca^{2+} pumps in the microdomain may also be a critical variable, as addressed below for SERCA.

There is insufficient information at present about the locations and properties of Ca^{2+} binding sites within the STIM-ORAI microdomain to model their effects on Ca^{2+} movements in detail. However, some qualitative lessons can be applied to the STIM-ORAI microdomain. Ca^{2+} binding can slow the rise and fall of the Ca^{2+} signal within the microdomain. Ca^{2+} binding to mobile sites can potentially narrow the Ca^{2+} peak in **FIGURE 2** so that the higher concentrations are focused in a smaller region surrounding each open channel. An important lesson is that Ca^{2+} binding is not expected to increase the concentration of free Ca^{2+} above the level in the steady-state profile calculated in the absence of binding, unless Ca^{2+} binding triggers the provision of Ca^{2+} from another source, for example by Ca^{2+} -induced Ca^{2+} release. Finally, unless the microdomain is insulated from global changes in cytoplasmic Ca^{2+} , the entire microdomain can serve as a locus of global Ca^{2+} sensing in parallel with the more localized sensing of Ca^{2+} influx through ORAI channels.

Baseline Ca^{2+} concentration

The treatment above is appropriate when the Ca^{2+} concentration difference between the boundary of the microdomain and the bulk cytoplasm is negligible or, equivalently, when there is negligible efflux of Ca^{2+} from the microdomain directly to the cytoplasm. This is likely to be a fair approximation under physiological conditions where either the local Ca^{2+} influx is low or the Ca^{2+} influx is largely balanced by local sequestration. However, there will be other physiological conditions in which Ca^{2+} efflux across the outer boundary of the microdomain results in a nonnegligible concentration gradient between the microdomain boundary and bulk cytoplasm. A detailed treatment of these cases is beyond the scope of this review, but it is possible to estimate the magnitude of the effect on Ca^{2+} concentrations by considering the boundary of the microdomain as a Ca^{2+} source in a three-dimensional diffusion problem.

Take, as above, an idealized microdomain of outer radius $R_0 = 150$ nm and depth $z = 15$ nm, having a single centrally located ORAI channel conducting 6300 Ca^{2+} ions/s, with 50% of the Ca^{2+} entering through the channel being captured by SERCA or extruded by PMCA before exiting the microdomain. This gives a symmetrical Ca^{2+} efflux of 3150 Ca^{2+} ions/s across the cylindrical surface bounding the microdomain. Solving for diffusion from this source into a half-space representing the cell interior, the steady-state Ca^{2+} concentration at the boundary of the microdomain would be less than 200 nM above the global Ca^{2+} concentration. The concentration increment is proportional to the net Ca^{2+} flux exiting the microdomain and indifferent to the details of how the flux arose, hence it would be the same with five centrally located open channels and 90% of the Ca^{2+} captured locally. These two examples are at one end of a physiological spectrum, and can fairly be modelled with the simplified treatment that ignores any concentration difference. On the other hand—and at the opposite end of the spectrum—with five open channels and no sequestration or extrusion, the Ca^{2+} concentration at the edge of the microdomain would be elevated into the range 500 nM–1 μM above the global Ca^{2+} concentration. This extreme example underlines

the point that the attainable Ca^{2+} concentrations in the immediate vicinity of an ER-plasma membrane junction are modest compared to those detected at ER-mitochondrial junctions or close to voltage-dependent Ca^{2+} channels.

The Ca^{2+} concentration at the boundary will set an elevated baseline Ca^{2+} concentration within the microdomain on which the steeper localized Ca^{2+} gradients around individual channels will be superimposed. Importantly, the steady-state analysis shows that any pronounced elevation of Ca^{2+} concentration falls off sharply within a few hundred nanometers from the boundary of the ER-plasma membrane junction. Thus the geometry and simple considerations of Ca^{2+} diffusion set the junction and its immediate vicinity apart as a preferred locus for Ca^{2+} signalling, even under conditions when cytoplasmic Ca^{2+} will be elevated globally.

A notable corollary of this analysis is that the baseline Ca^{2+} signal in an individual microdomain, and not just the local concentration profile, can depend on factors beyond the number of active channels in the microdomain. When there is appreciable Ca^{2+} flux across the boundary of the microdomain, the efflux and therefore the baseline Ca^{2+} concentration within the microdomain can be regulated by adjusting SERCA or PMCA activity within or at the edge of the microdomain. The analysis further reveals another point that is perhaps less obvious: that with a fixed number of open channels, the steady-state Ca^{2+} concentration at the edge of the microdomain is controlled by the geometry, and will increase with a decrease in R_0 or z . Thus the Ca^{2+} signal in an individual microdomain could, in principle, be regulated by cellular mechanisms that tune the fine geometry of the microdomain. This area needs further mathematical modelling and experimental study.

Mitochondria

Respiring mitochondria promote the initial activation of CRAC current in response to inositol 1,4,5-trisphosphate (IP3) in RBL-1 cells, and limit the slow Ca^{2+} -dependent inactivation of CRAC current in Jurkat T cells and RBL-1 cells, under physiological conditions of low cytoplasmic Ca^{2+} buffering [72,73]. The proposed mechanism in the case of CRAC channel activation is that mitochondria adjacent to IP3 receptor Ca^{2+} release sites buffer Ca^{2+} locally, reducing reuptake by SERCA, and thereby facilitate ER Ca^{2+} store depletion [72]. This mechanism might also reduce slow Ca^{2+} -dependent inactivation in T cells, which is due in part to store refilling [74].

While Ca^{2+} buffering near IP3 receptors will affect local Ca^{2+} signals in the microdomain only indirectly, other evidence points to a separate direct effect of mitochondria on these local Ca^{2+} signals. Full development of CRAC current requires respiring mitochondria even when ER Ca^{2+} stores are emptied by passive leakage following treatment with the SERCA inhibitor thapsigargin [72,73,75,76]. There is no store refilling under these conditions, and the effect on CRAC channels in this case has been attributed to the ability of mitochondria to prevent slow Ca^{2+} -dependent inactivation of CRAC current by lowering the local Ca^{2+} concentration near the CRAC channels [72,73,75]. Giacomello *et al* have expressed reservations about this interpretation, on the grounds that a mitochondrially targeted D1cpv probe does not report 'hot spots' of Ca^{2+} near the plasma membrane during store-dependent Ca^{2+} entry comparable to those at ER-mitochondrial contacts during Ca^{2+} release from ER

stores [77]. However, their data are consistent with the analysis of the previous section, which indicates that the local elevations of Ca^{2+} concentration in the vicinity of ER-plasma membrane junctions are very much lower than those observed at ER-mitochondrial contacts and might be poorly detected by the D1cpV probe. Mitochondrial Ca^{2+} uptake can occur at these modestly elevated levels of Ca^{2+} [reviewed in 78], and would, if anything, be more effective under the conditions of enhanced Ca^{2+} entry normally employed to study slow Ca^{2+} -dependent inactivation. There is a lingering question, though, whether mitochondria are specifically positioned to take up Ca^{2+} at ER-plasma membrane junctions [79]. It may be that respiring mitochondria act mainly as a check on the global cytoplasmic Ca^{2+} concentration and its additive contribution to junctional Ca^{2+} concentration.

Ca^{2+} near the channel opening

Signalling in the immediate vicinity of the channel was first examined thoroughly for fast Ca^{2+} -dependent inactivation, a process in which Ca^{2+} influx through a single CRAC channel feeds back to decrease current through the same channel [80]. Initial experiments established that fast Ca^{2+} -dependent inactivation is dependent on the single-channel Ca^{2+} current, but independent of the membrane potential V_m . The single-channel current was then manipulated by adjusting V_m , in order to make the degree of inactivation equal for cells dialyzed with 12 mM EGTA or with 12 mM BAPTA, Ca^{2+} chelators chosen for their very different binding kinetics. A larger single-channel current was required to produce a given level of inactivation in cells dialyzed with BAPTA, consistent with its rapid binding of Ca^{2+} , which would narrow the Ca^{2+} peak. Steady-state Ca^{2+} concentrations near the channel were modelled assuming free diffusion, with the corrections described by Neher for binding to the mobile buffers EGTA and BAPTA. The predicted Ca^{2+} concentration profiles with EGTA and BAPTA under the conditions that produced half-maximal inactivation in each case were distinct, but the curves crossed at a radial distance 3–4 nm from the opening of the channel, defining the distance to the relevant Ca^{2+} binding site. For comparison with the discussion of Ca^{2+} concentrations in the broader microdomain, the predicted Ca^{2+} concentration at this radial distance was 3–4 μM .

More recent experiments in various cells have shown that activation of the Ca^{2+} -responsive adenylyl cyclase 8 (AC8), STAT5-dependent transcription of the *Fos* gene, activation of the transcription factor NFAT, production of leukotriene C_4 , and incorporation of reserve TRPC1 channels into the plasma membrane depend on local Ca^{2+} influx through ORAI channels [81–87]. In each case, physiological evidence places the immediate target of the Ca^{2+} signal in close proximity to the ORAI channel, although the distance is less precisely defined than for the fast Ca^{2+} -dependent inactivation site. Complementary biochemical evidence indicates that AC8 is constitutively a part of the ORAI channel complex, and that the Ca^{2+} -dependent phosphatase calcineurin, which controls NFAT activation, is recruited transiently to the complex [88,89].

The fact of Ca^{2+} signalling near the channel is established, but the underlying Ca^{2+} signal is not well characterized. The assumption that Ca^{2+} diffuses freely near the channel opening is simply an admission that no information is available. It is more realistic to expect that Ca^{2+} follows restricted paths through the channel complex, whose cytoplasmic part is thought to

measure at least 9 nm in the direction perpendicular to the plasma membrane [38], but proper modelling or simulation will then require some knowledge of the diffusion paths and the distribution and characteristics of Ca^{2+} binding sites.

Ca^{2+} diffusion

It is instructive to think about the fate of a single Ca^{2+} ion after it emerges from the channel into a microdomain modelled with no Ca^{2+} binding sites and unhindered diffusion. The overwhelming fact is that diffusion of free Ca^{2+} on the length scale of interest is rapid. The expected radial dispersion r of a single Ca^{2+} ion after it leaves the mouth of the channel, separating out the radial component of diffusion from the component perpendicular to the plasma membrane, is given by $\langle r^2 \rangle = 4Dt$. Taking the cutoff at $r = 10$ nm, a free Ca^{2+} ion lingers in the vicinity of the channel, on average, only ~100 ns if it is not detained by binding.

Extending this line of thought to the entire STIM-ORAI microdomain, the probability density for finding an individual Ca^{2+} ion at radial distance r after time t is plotted for a series of time points in **FIGURE 3**. Again in the absence of binding, an individual Ca^{2+} has nearly a 50% chance of exiting the microdomain in 30 μs , more than a 90% chance by 300 μs , and a 99% chance by 1 ms. The fact is that this unceremonious exit almost certainly will not occur, at least not for every Ca^{2+} ion delivered by the channel, precisely because Ca^{2+} will encounter binding sites or perhaps transporters as it traverses the microdomain. Indeed, it could make sense to view the STIM-ORAI microdomain not as a region of high local Ca^{2+} concentration, but rather as a region organized for local delivery of Ca^{2+} to effectors stationed there.

An insulated microdomain

There has been tacit acceptance that fast Ca^{2+} -dependent inactivation and other cellular processes activated locally by store-operated Ca^{2+} influx are insensitive, or relatively insensitive, to cytoplasmic Ca^{2+} signals merely because the cytoplasmic signal does not reach the Ca^{2+} concentration delivered locally through the ORAI channel. There is an alternative view. Willoughby *et al* have proposed that the ORAI1 partner AC8— and by implication ORAI1 itself— occupies a plasma membrane compartment in HEK293 cells that is insulated from changes in Ca^{2+} concentration in the cytoplasm [90]. The proposal is based on the failure of the Ca^{2+} sensor GCaMP2, expressed as a GCaMP2-AC8 fusion, to register elevated cytoplasmic Ca^{2+} , and on the unresponsiveness of AC8 itself to elevated cytoplasmic Ca^{2+} .

Certain points related to the proposal remain to be clarified. First, if AC8 is constitutively associated with ORAI1 [88], and given that ORAI1 is distributed throughout the plasma membrane at the time of carbachol-evoked Ca^{2+} release from the ER, why does GCaMP2-AC8 not ‘see’ the initial sharp rise in cytoplasmic Ca^{2+} prior to ORAI1 movement into the microdomain? Second, is GCaMP2 accurately reporting on local Ca^{2+} in the STIM-ORAI microdomain? The observed complete block of the GCaMP2-AC8 response to store-dependent Ca^{2+} influx in cells preloaded with BAPTA and the delayed block in cells preloaded with EGTA are not in line with the inferred localization of AC8 in the ORAI1

channel complex, unless local factors other than Ca^{2+} concentration influence the GCaMP2 signal. Willoughby *et al* suggested that the GCaMP2 signal is quenched by local acidification [90], which would also damp down AC8 responsiveness to Ca^{2+} [91]. Thus, third, could local acidification or another quenching process account for the lack of GCaMP2 sensor and cyclic AMP signals in response to elevated cytoplasmic Ca^{2+} ?

Resolution of these questions, or parallel lines of inquiry suggested by the GCaMP2-AC8 data, could potentially give new insight into the organization of the STIM-ORAI microdomain. Whether the discovery of a barrier would necessitate revision of the above discussion of local Ca^{2+} signals would depend on the nature of the insulation. Restricting diffusion of free Ca^{2+} at the boundary of the microdomain, for example, could lead to higher Ca^{2+} concentrations than predicted throughout the microdomain. However, an insulating mechanism that works purely through a reduction of Ca^{2+} diffusion is unlikely, since such a mechanism must be compatible with STIM and ORAI diffusion into the junction, and since even a decrease in the diffusion coefficient of Ca^{2+} by several orders of magnitude would not by itself prevent a cytoplasmic Ca^{2+} elevation to 1 μM for 10 s from spilling into the microdomain. A more plausible mechanism that combines some restraints on Ca^{2+} diffusion with efficient local Ca^{2+} sequestration need not change the discussion of local Ca^{2+} signals substantially.

Processes downstream of Ca^{2+} influx

The STIM-ORAI microdomain interfaces with cellular Ca^{2+} signalling in the refilling of Ca^{2+} stores, in its contribution to cytoplasmic Ca^{2+} signals, and in the provision of a local Ca^{2+} signal to effectors at ER-plasma membrane junctions. Its crosstalk with other signalling pathways may be modified by dynamic rearrangements of ER-plasma membrane junctions. This section outlines some issues and open questions related to these processes.

Refilling of Ca^{2+} stores

The defining role of store-operated Ca^{2+} influx is to balance an increased Ca^{2+} efflux from the cell that occurs during signalling. Because STIM-ORAI signalling is closely attuned to the filling state of ER Ca^{2+} stores, it might be expected to serve in this role even in cells where there is substantial Ca^{2+} influx through other Ca^{2+} channels outside the STIM-ORAI microdomain. Indeed, STIM1 and ORAI1 are necessary to maintain sarcoplasmic reticulum Ca^{2+} stores in muscle in the face of continued high-frequency stimulation [21,26]. STIM1 is also required in other cells, at least under specified conditions, for the continuing Ca^{2+} oscillations that reflect the maintenance of Ca^{2+} stores during an ongoing response to physiological stimulation [65,92,93]. STIM2-dependent activation of ORAI channels maintains ER Ca^{2+} stores in unstimulated cells [94], and in some conditions STIM2 contributes to maintenance of stores during sustained Ca^{2+} signalling [93,95,96]. There is evidence that STIM2 may have more importance at low levels of physiological stimulation [96].

What has been unclear is whether ‘privileged’ coupling between store-operated Ca^{2+} influx and refilling of ER Ca^{2+} stores [97-100] reflects a specialized function of ER-plasma membrane junctions, occurring within the STIM-ORAI microdomain, or simply the efficient

refilling of ER Ca^{2+} stores by SERCA after Ca^{2+} has diffused into the surrounding cytoplasm. The measured V_{max} of SERCA2b is $\sim 36 \text{ Ca}^{2+}$ ions per second, based on its ATPase activity and a coupling ratio of two Ca^{2+} transported per ATP hydrolyzed [101], so five ORAI channels each capable of delivering 6300 Ca^{2+} per second and having open probability $p = 0.8$ would keep at least 700 SERCA pumps occupied. In fact the estimate is a lower limit, since it is unlikely that all the pumps will operate at V_{max} at ambient Ca^{2+} concentrations. This is a larger number of pumps than might be expected in or near an individual ER-plasma membrane junction if the pumps were randomly distributed within the ER. On the other hand, the resting distribution of SERCA pumps need not be random; sufficient SERCA pumps are probably available for deployment—for example, RBL-2H3 basophilic leukemia cells possess 1.6 million SERCA pumps per cell [102]; and it is in fact reported that overexpressed SERCA2a and SERCA2b redistribute upon stimulation to the circumference of STIM-ORAI clusters [32,98,103]. It will be a serious challenge to quantitate the local pumping capacity at or surrounding the STIM-ORAI microdomain at native levels of SERCA and under typical signalling conditions.

Intuitively, Ca^{2+} influx due to STIM2-ORAI1 signalling is the strongest candidate to be contained locally by SERCA pumps, if the limited ability of STIM2 to gate ORAI channels [65] reflects a lower channel open probability and therefore a lower rate of Ca^{2+} delivery per channel. The influx due to STIM1-ORAI1 signalling would also be a plausible candidate in cases where it is modulated so that Ca^{2+} influx into an individual microdomain is low and intermittent. The pronounced movement of STIM2 to ER-plasma membrane junctions observed in HEK293 cells with modest physiological stimulation [65], if it is recapitulated in other cells, might provide a mechanism to damp down STIM1 signalling [104]. Intermittent brief pulses of Ca^{2+} might be intercepted by local Ca^{2+} binding sites and released gradually over time, effectively smoothing out the rate of Ca^{2+} delivery and so reducing the required rate of SERCA pumping. Even these tentative conclusions will require experimental validation. It may be that the main role of microdomain-associated SERCA pumps is to insulate the microdomain from cytoplasmic Ca^{2+} signals.

Cytoplasmic Ca^{2+} signalling

Store-operated Ca^{2+} influx contributes directly to the cytoplasmic Ca^{2+} signal if a fraction of the Ca^{2+} delivered into the STIM-ORAI microdomain is not intercepted by SERCA, PMCA, or—in some cells— Na^+ - Ca^{2+} exchangers. This contribution is best seen in ‘overshoot’ experiments where external Ca^{2+} is added back after stores have been depleted in the absence of external Ca^{2+} , either by treatment with the SERCA inhibitor thapsigargin or by exposure to a physiological agonist. However, overshoot experiments are an extreme case. Physiological Ca^{2+} oscillations typically reflect Ca^{2+} release from ER stores [105], with store-operated Ca^{2+} influx playing a supporting role. There are some clear examples where Ca^{2+} oscillations are sustained when store-dependent Ca^{2+} influx is blocked, provided that efflux of Ca^{2+} through the plasma membrane has also been blocked using millimolar concentrations of Gd^{3+} or La^{3+} [106,107]. These examples indicate that spillover of Ca^{2+} from the microdomain into the cytoplasm does not make a major contribution to physiological Ca^{2+} oscillations in these cells and under the specific conditions of stimulation

examined. The extent to which ORAI channels contribute directly to the cytoplasmic Ca^{2+} signal at physiological levels of signalling in other cells or conditions is an open question.

Effectors at ER-plasma membrane junctions

A conceptually simple, and technically very challenging, experiment has suggested an alternative locus of action for Ca^{2+} coming through ORAI channels. Bird *et al* were able to monitor cytoplasmic Ca^{2+} with a ratiometric dye, and movement of EYFP-STIM1 close to the plasma membrane in the same cells by TIRF microscopy, during methacholine-elicited Ca^{2+} oscillations [65]. Many cells exhibited a delayed increase in STIM1 in the TIRF layer after the onset of oscillations, and in a fraction of cells periodic increases in STIM1 in the TIRF layer were coordinated with the cytoplasmic Ca^{2+} oscillations. These changes in the TIRF signal were typically only a small fraction of that elicited by full store depletion, but, tellingly, in each case the STIM1 movements lagged behind the cytoplasmic Ca^{2+} signal in time. An appealing interpretation is that Ca^{2+} release from ER stores directly provides a signal to effectors in the cytoplasm, whereas the associated partial depletion of ER stores drives a Ca^{2+} influx through ORAI channels that provides signals to a distinct set of effectors in the STIM-Orai microdomain.

The notion that physiological Ca^{2+} oscillations might indirectly drive STIM-Orai signalling localized to ER-plasma membrane junctions is consonant with the examples cited above of effectors or pathways preferentially coupled to store-dependent Ca^{2+} influx. One practical outcome of such a division of signalling domains would be a richer palette of options for modulating cellular Ca^{2+} signalling, since store-dependent influx is linked to cytoplasmic Ca^{2+} oscillations, but can be subject to additional independent regulation. Along the same lines, it will be worth asking how the localization of effectors within the microdomain itself affects Ca^{2+} signalling. In principle, direct targets of Ca^{2+} closely associated with the ORAI channel complex might be able to sense altered patterns of opening of individual channels, even when targets elsewhere in the microdomain detect the same average Ca^{2+} elevation over time.

Dynamic rearrangements of ER-plasma membrane junctions

Electron microscopy may give an impression of permanence, but ER-plasma membrane junctions are changing during signalling in important ways. Store depletion by treatment with thapsigargin or by a physiological stimulus triggers a rapid rearrangement of septins at existing junctions [30]. This rearrangement is necessary for efficient STIM-Orai interaction, and for efficient gating of store-dependent Ca^{2+} entry [30]. The fact that septins serve as filamentous scaffold proteins at other sites and the finding that their rearrangement at ER-plasma membrane junctions leads to a local redistribution of PIP2 [30] both suggest that dynamic changes in other proteins and lipids of the junctions may occur in conjunction with store-dependent Ca^{2+} influx. There is evidence that productive STIM-Orai interaction is regulated by lipid nanodomains within the junction [108-110]. In addition, electron microscopy shows that new junctions form following store depletion [27,28,36]. These observations point to an adaptive component of signalling in the STIM-Orai microdomain, whose place in cellular signalling requires further study.

ER-plasma membrane junctions themselves exhibit dynamic rearrangements dependent on the extended synaptotagmins E-Syt1, E-Syt2, and E-Syt3 [37,111]. E-Syts are affixed to the ER through a membrane-embedded hairpin segment, and tether the ER to the plasma membrane through their C-terminal C2 domains [37]. E-Syt binding to the plasma membrane requires PIP2 and, in the case of E-Syt1, elevated cytoplasmic Ca^{2+} [37]. Elevation of cytoplasmic Ca^{2+} induces a rapid movement of E-Syt1 to ER-plasma membrane junctions, and an E-Syt1 dependent increase of cortical ER that has been measured empirically using TIRF microscopy of an ER marker or of a genetically encoded marker of close ER-plasma membrane contacts [37,111]. The dual sensitivity of E-Syt proteins to local Ca^{2+} concentration and to local PIP2 levels in the plasma membrane will shape the acute remodelling of junctional contacts during Ca^{2+} entry.

Despite their function as ER-plasma membrane tethers, E-Syts do not have a primary role in STIM-ORAI signalling, since depletion of the three E-Syt proteins and the corresponding loss of junctional area does not decrease the initial amplitude of store-operated Ca^{2+} influx [37]. E-Syt depletion does impact Ca^{2+} -dependent inactivation of ORAI1 channels and sustained Ca^{2+} signalling, indicating that there is crosstalk between the primary processes controlled by E-Syts and STIM-ORAI signalling [110,111]. The specialized function most closely associated with E-Syt dependent contacts is lipid transfer between the apposed membranes [111,112]. Lipid transfer is thought to support normal Ca^{2+} influx by providing phosphatidylinositol to replenish PIP2 locally during ongoing signalling [111] and may contribute to maintaining the PIP2-rich junctional subdomains that favor Ca^{2+} -dependent inactivation of ORAI channels [110]. Although STIM1 has been associated with morphologically distinct 'thin' cortical ER in some circumstances [28,36], and although STIM1, E-Syt1, and E-Syt3 overexpressed individually in cells can promote the formation of extensive junctional contacts [27,28,37,113], STIM-ORAI complexes and E-Syt proteins are likely to function together in native ER-plasma membrane junctions. Close intermingling of the proteins at these sites could be a part of the physical basis for the observed crosstalk between Ca^{2+} and phospholipid signalling pathways.

Conclusion

This review has highlighted three central points. First, a low density of ORAI channels at ER-plasma membrane junctions is sufficient for the observed Ca^{2+} signalling and store refilling, a conclusion that must be considered in any analysis of physiological STIM-ORAI signalling. Second, the ORAI single-channel Ca^{2+} current, the number of channels contributing current, local Ca^{2+} clearance by SERCA and PMCA, and the geometry of the STIM-ORAI microdomain will shape signalling downstream of ORAI. Third, some information that is needed for a complete description of the STIM-ORAI microdomain is still lacking. Important parameters that remain to be defined are the organization of the ORAI channel complex itself, the identities and numbers of binding sites and effectors in the STIM-ORAI microdomain, the activity and regulation of SERCA and PMCA in the microdomain, and the possible presence of a diffusion barrier at the boundary of the microdomain. With a more complete description of the STIM-ORAI microdomain, it will become possible to model its local Ca^{2+} signalling at the level of detail already achieved for neuronal dendritic spines and the sites of vesicle release in neurons and pancreatic β cells.

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Highlights

The STIM-Orai pathway of store-operated Ca^{2+} influx

The special geometry of the STIM-Orai microdomain

The expected characteristics of the microdomain Ca^{2+} signal

The STIM-Orai microdomain in a broader context of cellular Ca^{2+} signalling

FIGURE 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.

FIGURE 2.

(A) Idealized STIM-ORAI microdomain. The compartment delimited by the apposed membranes is taken as 15 nm in depth and 150 nm in outer radius. For convenience in calculation, a single ORAI channel is placed at the center of the idealized microdomain, though placing the channel elsewhere in the microdomain would not alter the qualitative conclusions. The region less than 10 nm from the mouth of the channel is not included in the model for reasons noted in the text.

(B) Steady-state concentration profile. The increment in Ca^{2+} concentration over the Ca^{2+} concentration at the outer boundary of the microdomain is plotted against radial distance r from the mouth of the channel. Small concentration differences across the depth of the microdomain that may exist near its inner boundary are not considered. Other caveats are stated in the text. The increment in Ca^{2+} concentration at radial distance r , obtained as the steady-state solution of the diffusion equation under the stated conditions, is

$$K (\Phi/2 \pi D z) \ln (R_0/r),$$

where the flux of Ca^{2+} $\Phi = 6300$ ions/s, the diffusion coefficient for Ca^{2+} $D = 220 \mu\text{m}^2/\text{s}$, the depth of the annulus $z = 15$ nm, and the outer radius of the annulus $R_0 = 150$ nm. $K = 1.66 \times 10^{-9}$ is the factor required to convert the units from ions/ μm^3 to M.

FIGURE 3.

Free diffusion of Ca^{2+} is rapid. The probability density for finding an individual Ca^{2+} ion is plotted against radial distance r for times $t = 1 \mu\text{s}$, $10 \mu\text{s}$, $100 \mu\text{s}$, and 1 ms , assuming that the Ca^{2+} ion emerges from a channel at the central location $r = 0$ at $t = 0$. Formally, the variable plotted is P/r , where $P(r,t)$ is the probability that Ca^{2+} is found at a radial distance less than r at time t . Its value, which can be derived from the diffusion equation, is

$$(r/2\sqrt{Dt}) \exp(-r^2/4Dt).$$

The model used in this case differs slightly from that in Figure 2A, in that the model compartment extends to infinity and the region near the channel is not excluded. Note that this example of free diffusion is shown as an explicit counterpoint to the situation in cells, where binding sites and transporters will detain or contain Ca^{2+} .