Modeling Ca\(^{2+}\) signaling differentiation during oocyte maturation

Ghanim Ullah \(^{a}\), Peter Jung \(^{a,\ast}\), Khaled Machaca \(^{b,\ast\ast}\)

\(^{a}\) Department of Physics and Astronomy and Quantitative Biology Institute, Ohio University, Athens, OH 45701, USA
\(^{b}\) Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

Received 13 December 2006; received in revised form 19 January 2007; accepted 25 January 2007

Available online 8 March 2007

Abstract

Ca\(^{2+}\) is a fundamental intracellular signal that mediates a variety of disparate physiological functions often in the same cell. Ca\(^{2+}\) signals span a wide range of spatial and temporal scales, which endow them with the specificity required to induce defined cellular functions. Furthermore, Ca\(^{2+}\) signaling is highly plastic as it is modulated dynamically during normal physiological development and under pathological conditions. However, the molecular mechanisms underlying Ca\(^{2+}\) signaling differentiation during cellular development remain poorly understood. Oocyte maturation in preparation for fertilization provides an exceptionally well-suited model to elucidate Ca\(^{2+}\) signaling regulation during cellular development. This is because a Ca\(^{2+}\) signal with specialized spatial and temporal dynamics is universally essential for egg activation at fertilization. Here we use mathematical modeling to define the critical determinants of Ca\(^{2+}\) signaling differentiation during oocyte maturation. We show that increasing IP\(_3\) receptor (IP\(_3\)R) affinity replicates both elementary and global Ca\(^{2+}\) dynamics observed experimentally following oocyte maturation. Furthermore, our model reveals that because of the Ca\(^{2+}\) dependency of both SERCA and the IP\(_3\)R, increased IP\(_3\)R affinity shifts the system’s equilibrium to a new steady state of high cytosolic Ca\(^{2+}\), which is essential for fertilization. Therefore our model provides unique insights into how relatively small alterations of the basic molecular mechanisms of Ca\(^{2+}\) signaling components can lead to dramatic alterations in the spatio-temporal properties of Ca\(^{2+}\) dynamics.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Calcium signaling; Oocyte maturation; Mathematical modeling; Xenopus; IP3 receptor; Plasma membrane Ca-ATPase

1. Introduction

Ca\(^{2+}\) is a fundamental second messenger that mediates a plethora of cellular functions ranging from neurotransmitter release to fertilization. Specificity in Ca\(^{2+}\) signaling is encoded in the spatial, temporal and amplitude features of cytoplasmic Ca\(^{2+}\) (Ca\(_{\text{cyt}}^{2+}\)) dynamics [1]. That is, in the same cell Ca\(^{2+}\) signals of disparate duration, amplitude or frequency result in different cellular responses. For example, localized Ca\(^{2+}\) release through ryanodine receptors in vascular smooth muscle leads to vasodilation [2], whereas global sustained Ca\(^{2+}\) signals lead to vasoconstriction [3]. Ca\(^{2+}\) signals achieve this specificity by differentially activating Ca\(^{2+}\)-dependent effectors based on their frequency, location, duration and amplitude.

Although, much is known about Ca\(^{2+}\) signaling specificity, the mechanisms regulating developmental acquisition of this specificity are poorly defined. At different stages of cellular differentiation, the cell’s competence to produce specific Ca\(^{2+}\) dynamics varies. Therefore, Ca\(^{2+}\) signals differentiate concomitantly with cellular development to serve the need of the cell throughout its growth and differentiation. Furthermore, Ca\(^{2+}\) signaling components remodel during
pathological conditions, such as congestive heart failure, to maintain Ca\(^{2+}\) homeostasis [4].

Oocyte maturation provides an exceptional model system to elucidate the mechanisms regulating Ca\(^{2+}\) signaling differentiation during cellular development, because Ca\(^{2+}\) signaling differentiation during oocyte maturation is essential for the egg to acquire developmental competence at fertilization [5,6]. In fact Ca\(^{2+}\) is the universal signal for egg activation in all sexually reproducing species [5,7], and the fertilization-induced Ca\(^{2+}\) transient has specialized spatial and temporal dynamics that are crucial for egg activation [5,6]. This specialized Ca\(^{2+}\) signal takes the form of a single, or multiple Ca\(^{2+}\) transients depending on the species [5]. Ca\(^{2+}\) mobilization in immature Xenopus oocytes (oocyte) produces relatively transient Ca\(^{2+}\) signals or Ca\(^{2+}\) oscillations [8,9]. In contrast, Ca\(^{2+}\) mobilization in mature fertilization competent eggs (Egg) produces a sustained Ca\(^{2+}\) transient [10,11]. This sustained Ca\(^{2+}\) signal is essential for crucial early steps of egg activation, including the block to polyspermy, completion of meiosis and the transition into the embryonic mitotic cell cycle [10–14].

The fertilization induced Ca\(^{2+}\) transient in Xenopus eggs persists for ~10 min, and includes a sustained plateau (5–6 min) [10]. Every aspect of Ca\(^{2+}\) signaling, both elementary (Ca\(^{2+}\) puffs) and global events (Ca\(^{2+}\) waves), have specialized dynamics in eggs as compared to oocytes. Ca\(^{2+}\) waves in eggs are significantly slower (~9 μm/s) than in oocytes (~20 μm/s) [15,16]. The mode of wave propagation is salatory in oocytes [16,17], whereas it is continuous in eggs [12,15,16]. IP\(_3\)-dependent elementary Ca\(^{2+}\) release events (Ca\(^{2+}\) puffs) are spatially separate in oocytes, whereas they are short lived and cluster dramatically in eggs [18]. Therefore, Ca\(^{2+}\) signals differentiate in a dramatic fashion during oocyte maturation to endow the egg with the capacity to respond to sperm and initiate development. In this study we define the most critical determinants of Ca\(^{2+}\) signaling differentiation during oocyte maturation. Because this problem is not tractable experimentally we used a mathematical model that successfully reproduces Ca\(^{2+}\) signaling dynamics over a wide range of spatial and temporal dimensions. This model not only identified IP\(_3\)R affinity as the most critical determinant of the sustained Ca\(^{2+}\) signal in eggs, but also provided unique insights into our understanding of Ca\(^{2+}\) signaling differentiation at the systems level during cellular development.

2. Results and discussion

Before simulation approaches can be undertaken a basic understanding of the system based on experimental results is needed. A working model of Ca\(^{2+}\) signaling differentiation based on our experimental data is summarized in Fig. 1A [16,19]. In oocytes, following Ca\(^{2+}\) mobilization from intracellular stores, Ca\(_{\text{cyt}}^{2+}\) levels decay back to baseline due to Ca\(^{2+}\) extrusion out the cell through the plasma membrane Ca\(^{2+}\) ATPase (PMCA), and Ca\(^{2+}\) reuptake into the ER through the sarcoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) (Fig. 1, oocyte). Ca\(^{2+}\) mobilization depletes Ca\(^{2+}\) stores and activates Ca\(^{2+}\) influx through the store-operated Ca\(^{2+}\) entry (SOC) pathway (Fig. 1A, oocyte) [20–22]. In contrast, in eggs fertilization or Ca\(^{2+}\) mobilizing agents, produce a sustained Ca\(^{2+}\) rise, in the absence of Ca\(^{2+}\) influx through SOC, which is blocked in eggs due to MPF activation (Fig. 1A, egg) [22,23]. The Ca\(^{2+}\) rise in eggs differs from that in oocytes as it holds at a high Ca\(^{2+}\) plateau to fully induce egg activation (Fig. 1, egg). We previously hypothesized that the sustained Ca\(^{2+}\) plateau involves Ca\(^{2+}\) recycling between the ER lumen and the cytosol [19]. Ca\(^{2+}\) that is taken up into stores by SERCA leaks back into the cytosol through open IP\(_3\) receptors (Fig. 1A). This Ca\(^{2+}\) recycling is expected to maintain Ca\(_{\text{cyt}}^{2+}\) levels high for several minutes using limited store Ca\(^{2+}\). Moreover, PMCA internalization [19] removes the Ca\(^{2+}\) extrusion pathway, which prevents Ca\(^{2+}\) from exiting the cell thus contributing to the sustained Ca\(^{2+}\) signal in eggs (Fig. 1A).

One of the most important features of Ca\(^{2+}\) signals in eggs is the sustained Ca\(^{2+}\) rise in response to Ca\(^{2+}\) store mobilization. The traces in Fig. 1B show Ca\(^{2+}\) dynamics in response to a 1 s UV uncaging pulse in cells injected with caged-IP\(_3\) and oregon-green-BAPTA-1 as previously described [16,19]. This leads to a sustained Ca\(^{2+}\) rise in the region of uncaging in eggs compared to a transient Ca\(^{2+}\) signal in oocytes (Fig. 1B). Furthermore, as we have previously shown, elementary Ca\(^{2+}\) release events (Ca\(^{2+}\) puffs) in eggs are short lived compared to oocytes with an oocyte/egg duration ratio of 1.31 (Fig. 1C).

Based on these experimental results we were interested in determining the most critical determinants of Ca\(^{2+}\) signaling differentiation during oocyte maturation in terms of the sustained plateau observed in eggs. Experimentally this is an intractable problem given the wide spatial and temporal scale of Ca\(^{2+}\) signals, and the interdependence of Ca\(^{2+}\) signaling components (channels and transporters) on Ca\(^{2+}\). We thus resorted to mathematical modeling. Numerous modeling studies of intracellular Ca\(^{2+}\) signaling have been pursued in recent years (for a detailed review see Falcke [24]). The observed spiral-waves in Xenopus oocytes have led to mathematical models in the realm of excitable systems, while the tidal-type Ca\(^{2+}\) waves in eggs have been modelled based on the explicit assumption of bistable kinetics [25–27]. While bistable models are appropriate for modeling of the sweeping wave front, they cannot capture the kinetics of Ca\(^{2+}\) after the wave has passed, which is of primary interest in this current study. For simulating Ca\(^{2+}\) signaling differentiation during oocyte maturation we need a model that (a) is adequate at describing Ca\(^{2+}\) signals at both stages of cellular differentiation and (b) accurately reproduces elemental Ca\(^{2+}\) signaling processes, since we correlate Ca\(^{2+}\) signaling differentiation during oocyte maturation on both, the cell and the elemental level. The model described in Section 4 fits these criteria, and is thus well suited to explore the critical molecular alterations in Ca\(^{2+}\) signaling modulators (transporters
and channels) responsible for Ca\(^{2+}\) signaling differentiation during oocyte maturation.

2.1. Determinants of the Ca\(^{2+}\) plateau in eggs

We used this model in conjunction with conventional expressions for the other fluxes [28], to explore what could be responsible for the long-lasting Ca\(^{2+}\) response in eggs versus oocytes. The model faithfully replicates the kinetics of the transient Ca\(^{2+}\) signal observed in oocytes in response to IP\(_3\) (Fig. 2A, oocyte). Note that we have ignored gradients of IP\(_3\) and Ca\(^{2+}\) completely, and hence do not attempt to model accurately the rising phase of the Ca\(^{2+}\) signal. We then systematically altered different Ca\(^{2+}\) fluxes to determine which changes will reproduce the sustained Ca\(^{2+}\) plateau observed in eggs (Fig. 1B). Knowing that both SOC and PMCA are
not functional in eggs [19,22,23], we began by deleting these fluxes from the model (Fig. 2A, egg). Although this extends the duration of the Ca\(^{2+}\) signal it does not replicate the sustained plateau observed experimentally in eggs (Fig. 2, egg).

We were then interested in whether increasing the number of IP3Rs could reproduce the Ca\(^{2+}\) plateau given a recent report arguing that the number of functional IP3Rs increases during oocyte maturation [29]. However, the number of IP3Rs as determined by immunoblots is only slightly higher in eggs as compared to oocytes [19,30,31]. Nonetheless, increasing the number of IP3Rs five-folds increased peak Ca\(^{2+}\) amplitude with little effects on the duration of the Ca\(^{2+}\) plateau (Fig. 2A, egg-IP3R).

We then tested the effects of altering IP3R dynamics based on the model shown in Fig. 2B. Decreasing IP3R inactivation from the R, A or O states, that is decreasing \(\phi_1\), \(\phi_3\) or \(\phi_5\) five-folds, did not reproduce the sustained Ca\(^{2+}\) plateau (Fig. 2A). The only way, the experimentally observed long-lasting plateau can be generated, is to increase the affinity of the IP3 receptor to IP3 (increase \(\phi_2\) five-folds) or to increase IP3R Ca\(^{2+}\)-dependent gating (increase \(\phi_4\) five-folds) (Fig. 2A). In fact, we have previously shown that IP3R affinity is significantly higher in eggs as compared to oocytes [19].

2.2. Modeling Ca\(^{2+}\) puffs

To differentiate whether IP3R affinity to IP3 (\(\phi_2\)) or Ca\(^{2+}\)-dependent gating (\(\phi_4\)) is the primary determinant of the Ca\(^{2+}\) plateau in eggs, we stochastically modelled elemental Ca\(^{2+}\) release events by a single cluster of 20 IP3Rs. Our
rationale was that alterations that reproduce the sustained 
Ca^{2+} plateau must also replicate the experimentally observed 
shorter duration of Ca^{2+} puffs observed in eggs (Fig. 1C), 
since Ca^{2+} puffs underlie global Ca^{2+} signals. We simulated 
the duration of Ca^{2+}-puffs generated by our models for the 
oocyte and for the egg with a five-fold increase in $\phi_2$ or $\phi_4$ 
(Fig. 3). Analysis of the statistical properties of the in-silico 
puffs [32], shows that puff duration is shorter following an 
increase in $\phi_2$ (Fig. 3). The average durations of the modelled 
puffs are larger than those collected experimental, which is 
fully expected since long puffs in vivo are likely to lead to 
global Ca^{2+} waves thus precluding their observation exper-
imentally as Ca^{2+} puffs. Nonetheless, fully consistent with 
experimental data the ratio of the oocyte/egg puff duration 
when $\phi_2$ was increased is 1.31, which is identical to the 
ratio observed experimentally (Fig. 1C). In contrast to $\phi_2$, 
increasing $\phi_4$ increases the duration of Ca^{2+} puffs in eggs as 
compared to oocytes (Fig. 3), producing an oocyte/egg ratio 
of 0.82, which is distinct from the experimentally observed 
1.31 ratio (Fig. 1C). Together these data show that increasing 
IP$_3$R affinity to IP$_3$ is the only determinant that reproduces 
experimentally observed changes in Ca^{2+} signaling at both 
the elemental and global levels. The fact that our simulation 
is capable of modeling these widely different scales of Ca^{2+} 
signaling allowed us to identify increased IP$_3$R affinity and 
PMCA internalization as the most critical determinants of 
Ca^{2+} signaling differentiation during oocyte maturation.

Interestingly, when comparing puff duration in the oocyte 
and egg with an increase in $\phi_2$, the distribution differs very 
little for puffs with long durations, but quite dramatically for 
short puffs (Fig. 3). With an increased $\phi_2$, the number of 
puffs of duration 20 ms or less is much larger than in oocytes, 
causing the puffs to be shorter on average. This observa-
tion has triggered us to re-analyze the correlations between 
puff-duration and puff-amplitude, which has been considered 
small [32]. We found that although the correlation between 
puff duration and amplitude is small if averaged over puffs 
of all sizes and durations, almost all short puffs are small 
in amplitude. In the model, this is a consequence mainly 
of IP$_3$-dependent inactivation (the S-state in Fig. 2B) since 
the shut-rate $\phi_3$ is larger than $\phi_4$ for small cytosolic Ca^{2+} 
levels.

2.3. Functional coupling between the IP$_3$R and SERCA

Since our model faithfully replicates the duration of the 
Ca^{2+} plateau in eggs, we will now utilize it to explore the 
determining features underlying it. Plotting Ca^{2+} flux through 
IP$_3$Rs over time (Fig. 4A) shows that IP$_3$Rs open maximally 
immediately after the IP$_3$ pulse for a short duration, followed 
by a significantly lower steady state flux level for the duration 
of the Ca^{2+} plateau (Fig. 4A). Because SERCA flux depends 
on cytoplasmic Ca^{2+} levels, the new higher Ca^{2+} level (see 
Fig. 4B) due to potentiated IP$_3$Rs increases SERCA flux, 
balancing the increased basal Ca^{2+} flux through potentiated 
IP$_3$Rs. It is important to note that the plateau is not main-
Fig. 4. Fluxes through IP$_3$Rs (black) and SERCA (gray) (A), cytosolic Ca$^{2+}$-concentration (B), and cytosolic IP$_3$ concentration (C) as a function of time. At times $t = 20$ s and $t = 150$ s, a uniform IP$_3$ stimulus was applied to both oocyte and egg for the duration of 10 s at a rate of 1 $\mu$M/s. D) Correlation between steady-state cytosolic Ca$^{2+}$ and IP$_3$ concentrations.

3. Conclusion

Using a mathematical model that faithfully reproduces Ca$^{2+}$ signals dynamics over large time and space scales we have identified the most critical determinants of Ca$^{2+}$ signaling differentiation during oocyte maturation. This differentiation is essential to endow the egg with the competence to be fertilized and undergo the egg-to-embryo transition. Despite the fact that every aspect of Ca$^{2+}$ signals is dramatically altered during oocyte maturation, our modeling studies show that the two most critical determinants underlying these changes are PMCA internalization and increased IP$_3$R affinity. These changes effectively reproduce the sustained Ca$^{2+}$ signal observed in eggs. Furthermore, due to the increased affinity of the IP$_3$R in eggs and the dependency of both SERCA and the IP$_3$ on cytosolic Ca$^{2+}$ levels, the Ca$^{2+}$ signaling machinery shifts to a new steady state following an IP$_3$ pulse and effectively maintains elevated cytosolic Ca$^{2+}$ levels over a wide range of IP$_3$ concentrations. Therefore, our studies reveal that because of the inherent functional coupling between Ca$^{2+}$ modulators, increasing IP$_3$R affinity is sufficient to produce a sustained elevated Ca$^{2+}$ plateau in eggs, in the absence of Ca$^{2+}$ extrusion.

Our rationale for these studies was to use a mathematical model that faithfully replicates Ca$^{2+}$ signaling dynamics during oocyte maturation at both the elementary and global scales. Given these constraints and the experimental findings regarding Ca$^{2+}$ signaling differentiation during oocyte maturation (summarized in Fig. 1A), our modeling studies define increased IP$_3$ affinity and PMCA internalization as the most critical determinants of the sustained Ca$^{2+}$ plateau in eggs. Although other factors such as increasing Ca$^{2+}$-dependent gating of the IP$_3$ receptor could also produce a sustained plateau in eggs, it does not replicate experimentally observed puff kinetics and thus was ruled out as a phys-
ologically relevant mechanism underlying Ca\textsuperscript{2+} signaling and differentiation.

4. Mathematical methods

In oocytes and eggs intracellular Ca\textsuperscript{2+} dynamics are determined by Ca\textsuperscript{2+} fluxes from and into intracellular stores and the extracellular matrix, and by diffusion of Ca\textsuperscript{2+} in the cytosol and the ER. To model these processes we assume that the ER is distributed homogeneously over the section of the cell we are modeling, i.e. each point in the intracellular space has a cytosolic and an endoplasmic domain. These two domains interact through various fluxes: The Ca\textsuperscript{2+}-flux from the ER into the cytosol, $J_{\text{IP3}}$; ATP driven Ca\textsuperscript{2+} fluxes from the cytosol to the ER and to the extracellular space, $J_{\text{SERCA}}$, $J_{\text{PMCA}}$; leak flux from the ER to the cytosol and store-operated Ca\textsuperscript{2+} flux from the extracellular space into the cytosol $J_{\text{SOC}}$. Binding of cytosolic and luminal Ca\textsuperscript{2+} by buffers is taken into account by using the fast-buffer approximation where we replace the diffusion coefficients by effective diffusion coefficients. Such a procedure has been proven valid recently [33] even if the buffer binding and dissociation is not as fast as originally required for this approximation making it a viable first approach even for slower buffers.

Denoting the cytoplasmic and luminal Ca\textsuperscript{2+} concentrations by $c$ and $c_{\text{ER}}$, respectively, and the IP\textsubscript{3} concentration by $p$, the three coupled reaction-diffusion equations governing the system read:

$$\frac{dc}{dt} = D_{\text{Ca}} V^2 c + J_{\text{IP3}} + J_{\text{leak}} - J_{\text{SERCA}} + \delta (J_{\text{SOC}} - J_{\text{PMCA}}) \quad (1a)$$

$$\frac{dc_{\text{ER}}}{dt} = D_{\text{ER}} V^2 c_{\text{ER}} + \gamma (J_{\text{SERCA}} - J_{\text{IP3}} - J_{\text{leak}}) \quad (1b)$$

$$\frac{dp}{dt} = D_{\text{IP3}} V^2 p + \beta (p^\beta - p) + J_{\text{stim}}, \quad (1c)$$

where $\delta = 1$ for oocytes and $\delta = 0$ for eggs. The first terms of Eqs. (1a) and (1b) describe diffusion of Ca\textsuperscript{2+} in the cytosol and ER, respectively. The effective diffusion coefficients $D_{\text{Ca}}$ and $D_{\text{ER}}$ incorporate the effect of fast buffers and we use values obtained from confocal line scans [34].

Calcium is removed from the cytoplasm by the ATP-dependent SERCA pump. We assume that these pumps are distributed uniformly on the ER-membrane and use the frequently used quasi-Hill-form [35] for the corresponding flux:

$$J_{\text{SERCA}} = V_3 \frac{c}{K_p + c} \frac{1}{c_{\text{ER}}}, \quad (2)$$

where the factor $1/c_{\text{ER}}$ avoids overloading of the ER with Ca\textsuperscript{2+}. Calcium is released from the ER through non-specific channels modeled as a passive leak flux:

$$J_{\text{leak}} = V_2 (c_{\text{ER}} - c), \quad (3)$$

with a maximum leak-conductance $V_2$. In oocytes, plasma membrane pumps (PMCas) are in the membrane and functional. The extrusion currents through PMCas are modelled by a Hill-form:

$$J_{\text{PMCA}} = \frac{c^2}{K_0^2 + c^2}. \quad (4)$$

Store-operated Ca\textsuperscript{2+}-entry is also functional only in oocytes and is described by the combination of a constant flux and an IP\textsubscript{3}-dependent flux:

$$J_{\text{SOC}} = \alpha_1 + \alpha_2 p \quad (5)$$

Although these fluxes are not well-established, similar expression have been used in previous modeling studies [35,36]. To account for different volumes of the ER and the cytosol, conservation of Ca\textsuperscript{2+} requires the prefactor $\gamma$ in Eq. (1b) representing the ratio of cytoplasmic to ER volume.

The equation for IP\textsubscript{3} (Eq. (1c)) includes a diffusion term, a linear decay term taking into account the degradation of IP\textsubscript{3} towards a steady-state value $p^*$, and a term $J_{\text{stim}}$ that mimics photo-release of IP\textsubscript{3}. For the decay rate we use 0.01/s based on experimental data on Xenopus oocyte [37,38] while the steady-state IP\textsubscript{3} concentration $p^*$ is 50nM.

Although IP\textsubscript{3} receptors have been modeled extensively in the past [39], not all models mimic IP\textsubscript{3} receptor behaviour properly at the elemental level (Ca\textsuperscript{2+}-puffs). The model of choice is the stochastic version of the recent model by Sneyd and Dufour [40] which has been shown [28] to generate puffs [28] with durations and amplitudes consistent with experiments.

The model is similar to older models in that it assumes 4 independent subunits, with one IP\textsubscript{3} binding site and two Ca\textsuperscript{2+} binding sites (one for activation, the other for inactivation) for each subunit. It differs, however, in three important details: a) non-mass-action law kinetics for IP\textsubscript{3} and Ca\textsuperscript{2+} binding, b) IP\textsubscript{3} induced inactivation prevalent for low cytosolic Ca\textsuperscript{2+} levels and c) sequential binding of IP\textsubscript{3} and Ca\textsuperscript{2+}. All these features are motivated by recent experimental insights into the structure [41] and kinetics [42] of the IP\textsubscript{3} receptor. A simplified scheme of the model for a subunit is shown in Fig. 2B. State R represents the state where neither IP\textsubscript{3} nor Ca\textsuperscript{2+} is bound. Binding of IP\textsubscript{3} with rate $\phi_2$ leads to state O. The fraction of IP\textsubscript{3}-bound subunits is determined by the IP\textsubscript{3} binding affinity $\phi_2/\phi_{-2}$. Only if IP\textsubscript{3} is bound can Ca\textsuperscript{2+} bind to the activating binding site and make the activated state A accessible. At low Ca\textsuperscript{2+} concentrations state S (shut-state) competes with A, modeling IP\textsubscript{3} dependent inactivation. The subunit can inactivate either from state A or directly from state R. An IP\textsubscript{3}R is open and allows for the flux of Ca\textsuperscript{2+} if all subunits are activated, i.e. bound by IP\textsubscript{3} and Ca\textsuperscript{2+}. For more details we refer the reader to Ref. [28]. All parameters in the equations above are listed in Table 1.

To model the large-scale Ca\textsuperscript{2+} signal we solve the set of partial differential equations for $c$, $c_{\text{ER}}$ and $p$ on a planar 100 $\mu$m x 100 $\mu$m patch of membrane. The patch
Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_1$</td>
<td>0.4/s</td>
</tr>
<tr>
<td>$V_2$</td>
<td>0.002/s</td>
</tr>
<tr>
<td>$V_3$</td>
<td>10 μM/s</td>
</tr>
<tr>
<td>$K_F$</td>
<td>0.18 μM</td>
</tr>
<tr>
<td>$K_0$</td>
<td>0.42 μM</td>
</tr>
<tr>
<td>$V_4$</td>
<td>14 μM/s</td>
</tr>
<tr>
<td>$a_1$</td>
<td>0.6 nM/s</td>
</tr>
<tr>
<td>$e_1$</td>
<td>0.004/s</td>
</tr>
<tr>
<td>$r^*$</td>
<td>5.405</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.015/s</td>
</tr>
<tr>
<td>$p^*$</td>
<td>50 nM</td>
</tr>
<tr>
<td>$D_x$</td>
<td>20–30 μm²/s</td>
</tr>
<tr>
<td>$D_{xy}$</td>
<td>10–20 μm²/s</td>
</tr>
<tr>
<td>$D_y$</td>
<td>280 μm²/s</td>
</tr>
<tr>
<td>$V_{\text{cluster}}$</td>
<td>600/s</td>
</tr>
</tbody>
</table>

is discretized and represented by a two-dimensional lattice with grid distance $\Delta x = 1 \mu m$. No-flux boundary conditions are applied at the edge of the integrating area. A time step of 0.001 s is used during all the simulations.

Modeling the elemental Ca$^{2+}$ release events requires a realistic spatial distribution of IP$_3$Rs. IP$_3$Rs in *Xenopus* oocyte are organized in clusters with a cluster size of 10–30 channels. To model elementary Ca$^{2+}$ release events we consider a single cluster of 20 IP$_3$Rs channels placed at the centre of an integrating area of 5 μm × 5 μm. The small number of release channels per cluster requires stochastic modeling of the channels and subunits [43–46] which undergo stochastic transitions due to agonist binding. The selection between competing transitions is carried through as described by Gillespie [47] while we use a fixed time-step. Hence each cluster is characterized at any time by a number of open IP$_3$Rs.

We solved the stochastic partial differential equations by forward a difference method [48] with spatial discretization lengths of $\Delta x = \Delta y = 50$ nm. In a previous study [28], we showed that a finer discretization with $\Delta x = \Delta y = 20$ nm, resulting in larger Ca$^{2+}$ concentrations at the cluster-site, does not change the amplitudes and lifetimes of puffs (for a detailed discussion on local Ca$^{2+}$ concentrations at cluster sites, see Refs. [49,50]). Each cluster of the IP$_3$R is treated as a point source placed on a single grid point, i.e. (for a justification, see Ref. [28]).

$$J_{IP3} = \xi(x, y) \times V_{\text{cluster}} \frac{N_{\text{open}}}{N} (c_{\text{ER}}(x, y) - c(x, y)),$$ (6a)

where $V_{\text{cluster}}$ denotes the maximum Ca$^{2+}$-flux through the cluster, $n$ the total number of channels per cluster, $N_{\text{open}}$ the number of open channels (a stochastic function of time) and

$$\xi(x, y) = \begin{cases} 1 & \text{at cluster} \\ 0 & \text{otherwise} \end{cases}$$ (6b)

where $V_{\text{cluster}}$ denotes the maximum flux of Ca$^{2+}$ generated by the channel. Ca$^{2+}$ puffs are spatially and temporally limited release events when a number of channels open, release Ca$^{2+}$ into the cytosol and subsequently close again. The durations and amplitudes vary stochastically as the number of channels recruited by the events varies. The puff-amplitudes are defined by averaging over a circular area with a radius of 1 μm centred around the channel cluster. The duration is characterized by the full width at half maximum amplitude (FWHM). A full characterization of puff durations is given in terms of the frequency distribution of puff durations (Fig. 3).

Conflict of interest

The authors (Ghanim Ullah, Peter Jung and Khaled Machaca) declare no conflict of interest.

Acknowledgments

This work was funded by grant GM-61829 from the NIH (KM) and grant IBN-0078055 from NSF for PJ.

References


N. Callamaras, J.S. Marchant, X.P. Sun, I. Parker, Activation and coordination of InsP3-mediated elementary Ca2+ events during global Ca2+ signals in Xenopus oocytes, J. Physiol. 509 (Pt 1) (1998) 81–91.

I. Parker, Y. Yao, Calcium puffs in Xenopus oocytes, Ciba Found Symp. 188 (1995) 50–65.


H.C. Hartzell, Activation of different Cl currents in Xenopus oocytes by Ca liberated from stores and by capacitative Ca influx, J. Gen. Phys. 108 (1996) 157–175.


A. Bugrim, R. Fontanilla, B.B. Eutenier, J. Keizer, R. Nuccitelli, Sperm initiate a Ca2+ wave in frog eggs that is more similar to calcium waves initiated by IP3 than by Ca2+, Biophys. J. 84 (2003) 1580–1590.

G. Ullah et al. / Cell Calcium 42 (2007) 556–564

initiate a Ca(2+) wave in frog eggs that is more similar to Ca(2+) waves 2088–2097.


Y. Yao, J. Choi, I. Parker, Quantal puffs of intracellular Ca2+ evoked by inositol triphosphate in Xenopus oocytes, J. Physiol. 482 (Pt 3) (1995) 553–553.


