Molecular Biology of the Cell
Vol. 6, 945–951, August 1995

Essay

$InsP_3$-induced Ca^{2+} Excitability of the Endoplasmic Reticulum

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Monitoring Editor: Henry R. Bourne

Oscillations in intracellular Ca^{2+} can be induced by a variety of cellular signalling processes (Woods *et al.*, 1986; Berridge 1988; Jacob *et al.*, 1988) and appear to play a role in secretion (Stojilković *et al.*, 1994), fertilization (Miyazaki *et al.*, 1993), and smooth muscle contraction (Iino and Tsukioka, 1994). Recently, great progress has been made in understanding the mechanisms involved in a particular class of Ca^{2+} oscillation, associated with the second messenger inositol 1,4,5-trisphosphate ($InsP_3$) (Berridge, 1993). Working in concert with intracellular Ca^{2+}, $InsP_3$ controls Ca^{2+} release via the $InsP_3$ receptor in the endoplasmic reticulum (ER) (Berridge and Irvine, 1989). The IP_3 receptor is regulated by its coagonists $InsP_3$ and Ca^{2+}, which both activate and inhibit Ca^{2+} release (Finch *et al.*, 1991; Bezprozvanny *et al.*, 1991; De Young and Keizer, 1992). These processes, together with the periodic activation of Ca^{2+} uptake into the ER, have been identified as key features in the mechanism of $InsP_3$-induced Ca^{2+} oscillations in pituitary gonadotrophs (Li *et al.*, 1994), *Xenopus laevis* oocytes (Lechleiter and Clapham, 1992; Atri *et al.*, 1993), and other cell types (Keizer and De Young, 1993). Earlier discussions and models of $InsP_3$-induced Ca^{2+} oscillations focused on the nature and number of internal releasable pools of Ca^{2+} (Goldbeter *et al.*, 1990; Swillens and Mercan, 1990; Somogyi and Stucki, 1991), the importance of oscillations in $InsP_3$ (Meyer and Stryer, 1988), and other issues not based on detailed experimental findings in specific cells types. In this review we briefly summarize recent experimental findings dealing with $InsP_3$-induced Ca^{2+} excitability of the ER and describe a detailed, quantitative model that explains how intracellular Ca^{2+} oscillations occur (De Young and Keizer, 1992; Li *et al.*, 1995b).

INTRACELLULAR Ca^{2+} SIGNALS

The divalent cation Ca^{2+} is well suited to transmit signals intracellularly (Rubin, 1982). Because it is relatively large, Ca^{2+} is less well hydrated than smaller divalent cations and thus, capable of binding tightly to proteins. This permits free intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) to be buffered down to submicromolar levels (Allbritton *et al.*, 1992; Neher and Augustine, 1992; Wagner and Keizer, 1994), while the high affinity of Ca^{2+} for proteins like calmodulin makes it suitable for regulating both protein function (Hanson *et al.*, 1994) and protein synthesis (Gilchrist *et al.*, 1994). Extracellularly, however, Ca^{2+} exists at millimolar concentrations, and cells must constantly remove Ca^{2+} from the cytoplasm to keep $[Ca^{2+}]_i$ at levels where it can function effectively in signal transduction. This is carried out by Ca^{2+} pumps and Ca^{2+} exchange proteins in the plasma membrane (Carafoli, 1994) and by a variety of uptake mechanisms into intracellular organelles.

Research over the past decade has established that the endoplasmic reticulum (or sarcoplasmic reticulum in muscle) is the primary storage organelle for Ca^{2+} in most cell types (Berridge and Irvine, 1989). The endoplasmic reticulum (ER) is now thought to be a single, continuous compartment (Bird *et al.*, 1992; Terasaki *et al.*, 1994) that includes the rough ER at the nuclear

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membrane and the cortical ER near the plasma membrane. Uptake of Ca^{2+} into the ER occurs via specialized Ca^{2+}-ATPase pumps, dubbed SERCA pumps because they are found also in the sacroplasmic reticulum (Lytton *et al.*, 1992). Several tissue-specific isoforms of SERCA are known, and their high affinity for Ca^{2+} allows them to help maintain resting $[Ca^{2+}]_i$ in the range of 50–250 nM. Significantly higher concentrations of free Ca^{2+} have recently been estimated in the lumen of the ER using the fluorescent-free Ca^{2+} indicators mag-fura-2 (Hofer and Machen, 1993) and mag-indo-1 (Tse *et al.*, 1994b). Thus it now appears that the total Ca^{2+} concentration in the ER may be 10–30 mM and that the free Ca^{2+} concentration is in the range of 10–200 μM.

The physical structure of the ER membrane, with its sponge-like twists and folds, is ideal for controlling not only the uptake of Ca^{2+} but its release as well. Two types of Ca^{2+} release channels have been identified in the ER (Mignery *et al.*, 1989), both referred to by their receptor properties: the so-called inositol 1,4,5-trisphosphate receptor (IP_3R) (Berridge, 1993; Putney and Bird, 1993) and the ryanodine receptor (RyR) (Wagenknecht *et al.*, 1989). Although $InsP_3$ is an endogenous agonist of the IP_3R, ryanodine is a plant alkaloid used as a marker for RyR activity. Both receptors are composed of four subunits arranged in a clover-leaf structure that is large enough to be visualized with an electron microscope (Chadwick *et al.*, 1990; Danoff *et al.*, 1991; Marshall and Taylor, 1993). The RyR is the predominant Ca^{2+} release channel in the sacroplasmic reticulum, whereas in cell types as diverse as rat pituitary gonadotrophs (Stojilković *et al.*, 1994a) and mouse oocytes (Miyazaki *et al.*, 1993), the IP_3R dominates in the ER membrane. Because the opening of the RyR channel is stimulated by increased $[Ca^{2+}]_i$ (Fabiato, 1985), it releases Ca^{2+} in a self-regenerative fashion, referred to as calcium-induced calcium release or CICR. Regulation of Ca^{2+} release via the IP_3R, referred to as $InsP_3$-induced calcium release or IICR, is more complicated. IICR involves regulation of the IP_3R by $[Ca^{2+}]_i$, $InsP_3$ and, possibly, luminal Ca^{2+} working together as coagonists and coinhibitors (Bezprozvanny *et al.*, 1991; Finch *et al.*, 1991; Missiaen *et al.*, 1992; Hajnoczky and Thomas, 1994).

Intracellular $InsP_3$ is generated by the phosphotidylinositol cascade, which can be initiated by a variety of signaling mechanisms based in the plasma membrane and elsewhere (Berridge and Irvine, 1989; Berridge, 1993). One of the best-documented examples comes from pituitary gonadotrophs (Iida *et al.*, 1991; Stojilković *et al.*, 1994b), in which gonadotropin-releasing hormone (GnRH) binds to a specific plasma membrane receptor that triggers the production of $InsP_3$ via a G_q-protein linked mechanism. This initiates a sequence of events leading to the exocytosis of intracellular vesicles containing lutenizing hormone and follicle-stimulating hormone as well as gene expression and protein synthesis. The first event in this sequence is IICR (Stojilković *et al.*, 1994a; Tse *et al.*, 1994a), which upsets the resting balance of Ca^{2+} within the cell, producing the complex Ca^{2+} signals shown in Figure 1A. These responses occur even when the cells are placed in Ca^{2+}-deficient medium, and range from Ca^{2+} oscillations with a GnRH dose-dependent frequency to large biphasic responses recorded at high doses of GnRH (Stojilković *et al.*, 1992). Such complex signals are typical of Ca^{2+} responses that occur during intracellular signalling (Tsien and Tsien, 1990; Tsunoda, 1991) and represent a form of Ca^{2+}-dependent excitability of the ER membrane.

IP_3Rs AND SERCA PUMPS MAKE THE ER EXCITABLE

The concentration level of $InsP_3$ in many cells is set by the membrane-associated enzyme, phospholipase C (PLC), which phosphorylates and cleaves phosphotidylinositol 4,5-bisphosphate to $InsP_3$ and diacylglycerol. The β isoform of PLC is activated by specific G-proteins, including the α and the $\beta\gamma$ subunits of G_q. PLC_γ, on the other hand, is activated directly by phosphorylation (Cockcroft and Thomas, 1992). At basal levels of $InsP_3$, uptake and release of Ca^{2+} from the ER are in a stable balance with influx and efflux through the plasma membrane (see Figure 2). The nature of this balance at higher concentrations of $InsP_3$ is determined by the kinetic properties of the IP_3R and the SERCA pumps. The major events involved in IICR are summarized in Figure 2. These key features of the kinetics of IICR have been established through a variety of experiments, including Ca^{2+} imaging of paired photo-released pulses of caged $InsP_3$ (Payne *et al.*, 1988; Parker and Ivorra, 1990), kinetic measurements of Ca^{2+} release from synaptosomes (Finch *et al.*, 1991), and single channel recordings of the IP_3R in phospholipid bilayers (Bezprozvanny *et al.*, 1991; Watras *et al.*, 1991; Bezprozvanny and Ehrlich, 1994). Both $InsP_3$ and Ca^{2+} are thought to bind to the cytoplasmic face of the channels (Marshall and Taylor, 1993a; 1994), with each subunit accomodating a single $InsP_3$ molecule and two Ca^{2+} ions. The regulatory role of $InsP_3$ appears to be permissive, i.e., it "potentiates" opening of the channel by Ca^{2+}, which is without effect in the absence of $InsP_3$. The action of Ca^{2+} to activate the channel and the binding of $InsP_3$ are relatively rapid processes that have been estimated to occur in less than 50 ms (De Young and Keizer, 1992). Furthermore, fast activation by $[Ca^{2+}]_i$ occurs only above a threshold of about 0.2–0.4 μM (Bezprozvanny *et al.*, 1991) and is counteracted at higher concentrations by a second,

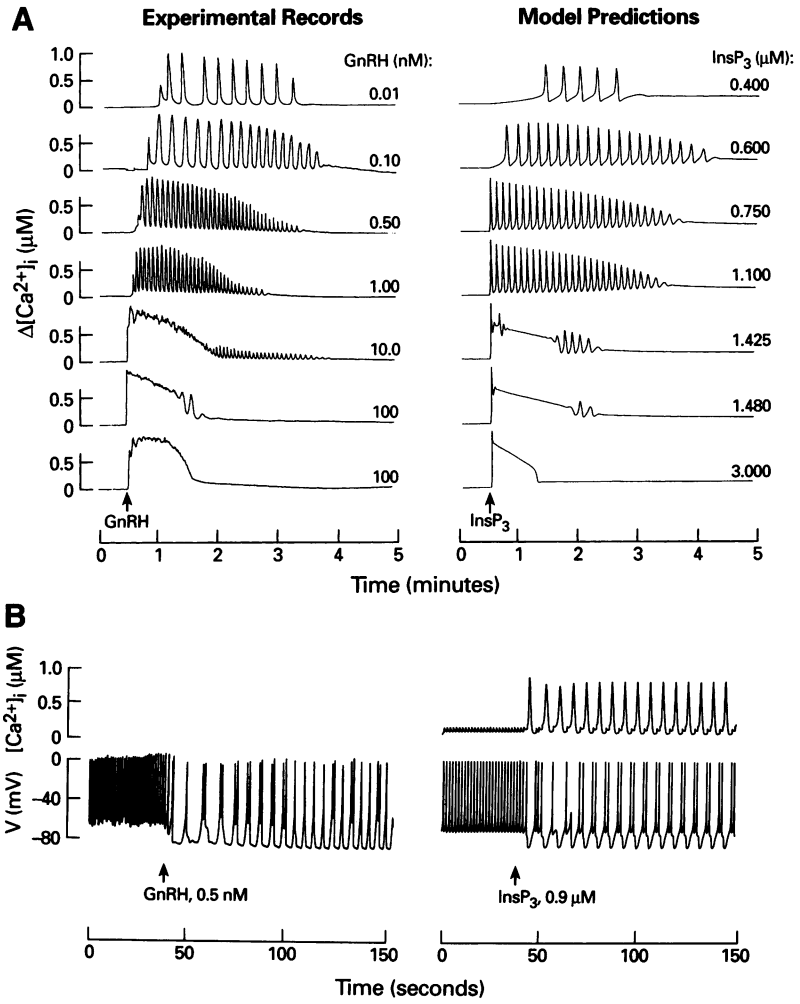

Figure 1. Excitable calcium reponses in gonadotrophs. (A, left panel) (Li *et al.*, 1994). Measured $[Ca^{2+}]_i$ responses in Ca^{2+}-deficient medium for isolated rat pituitary gonadotrophs at the indicated doses of GnRH. (A, right panel). Comparable $[Ca^{2+}]_i$ responses simulated using a model of the IP_3R-mediated Ca^{2+} excitability at the indicated concentrations of $InsP_3$. (B, left panel). Plasma membrane potential measured for a single rat pituitary gonadotroph in 1.8 mM external Ca^{2+} with GnRH added at the arrow; methods described in Li *et al.*, 1995b. (B, right panel). Comparable simulations of $[Ca^{2+}]_i$ and membrane potential for a model of the rat pituitary gonadotroph cell with voltage-gated Ca^{2+} and K^+ channels and Ca^{2+}-activated K^+ channels included in the plasma membrane. Concentration of $InsP_3$ increased from 0.01 to 0.9 μM at the arrow.

slower $[Ca^{2+}]_i$-dependent inactivation of the channel (Iino, 1990; Keizerand and DeYoung, 1992). The opposing processes of fast activation and slow inactivation are reflected in the equilibrium open probability of the channel, which exhibits a characteristic bell-shaped dependence on $[Ca^{2+}]_i$ with a maximum between 0.2–0.5 μM (Bezprozvanny *et al.*, 1991; Iino and Tsukioka, 1994).

Physiologically, the fast activation of the IP_3R by $[Ca^{2+}]_i$ permits rapid transduction of the $InsP_3$ signal into an increased level of $[Ca^{2+}]_i$. Because of the high luminal Ca^{2+} concentration, if left unchecked this self-regenerating signal would soon swamp the cytoplasmic Ca^{2+} buffers, and $[Ca^{2+}]_i$ would rise to toxic levels. Well before that can happen, however, elevated $[Ca^{2+}]_i$ turns off release by inactivating the channel. Inactivation occurs within 1–10 s and acts as a regulatory "brake" on the CICR caused by Ca^{2+} activation of the IP_3R. Another brake on CICR is provided by the SERCA ATPases, which pump $[Ca^{2+}]_i$ back into the ER lumen. Although the rates of the various SERCA isoforms saturate as $[Ca^{2+}]_i$ increases (with a Hill coefficient of 2), their affinity for $[Ca^{2+}]_i$ (0.1–0.4 μM) is in the physiological range (Lytton *et al.*, 1992). Thus pumping by SERCA into the ER is also activated by $[Ca^{2+}]_i$.

Using these experimental findings, De Young and Keizer (1992) constructed a kinetic model of the coagonist regulation of the IP_3R by $InsP_3$ and $[Ca^{2+}]_i$ that mimicked many of the observed properties of IICR. When the sigmoidal activation of SERCA activity by $[Ca^{2+}]_i$ was added to the model, simulations produced Ca^{2+} oscillations for a physiological range of $InsP_3$ concentrations. This type of model represents an idealized "closed" cell in which Ca^{2+} influx and efflux through the plasma membrane are ignored. This model provides an explanation for Ca^{2+} oscillations induced by the SERCA pump inhibitor thapsigargin, and by Ca^{2+} leak enhancement using ionomycin (Li *et al.*, 1994). By inhibiting Ca^{2+} uptake or increasing the leak, $[Ca^{2+}]_i$ rises to levels that trigger Ca^{2+} activation of release from the IP_3R,

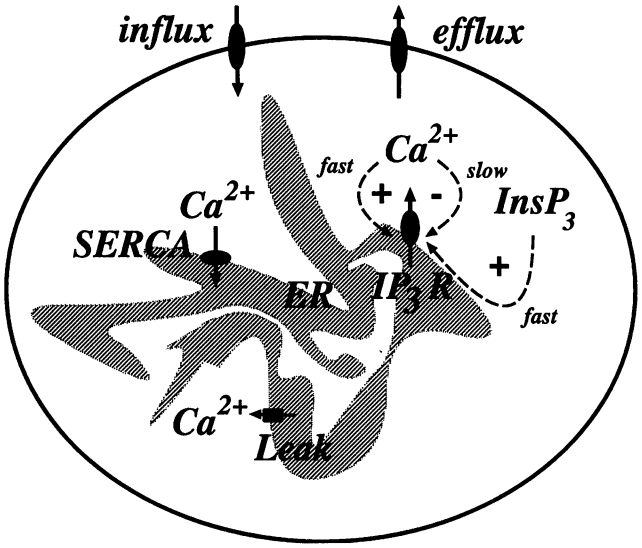

Figure 2. Schematic diagram of Ca^{2+} transport mechanisms affecting IICR. In the ER, Ca^{2+} release occurs via a passive leak and the IP_3R Ca^{2+} channel. IP_3R activity is potentiated by $InsP_3$, activated rapidly by $[Ca^{2+}]_i$ above a threshold, and then slowly inactivated by $[Ca^{2+}]_i$. Uptake into the ER occurs via SERCA ATPase Ca^{2+} pumps. IICR is influenced by influx and efflux of Ca^{2+} through the plasma membrane.

initiating repetitive cycles of release and uptake of Ca^{2+}.

To explain experimental measurements on gonadotrophs, the model has been expanded to include the effect of luminal Ca^{2+}, which in gonadotrophs appears to partially inhibit the receptor (Li *et al.*, 1994). The model also explains the "puffs" of calcium (Yao *et al.*, 1994) that appear in localized confocal images recorded at low $InsP_3$ in *X. laevis* oocytes. These are comparable to calcium "sparks" seen in cardiac cells (Cheng *et al.*, 1993) and appear to be self-regenerative release sites composed of clusters of IP_3R. The De Young-Keizer model also provides an explanation for so-called "quantal" calcium release (Kindman and Meyer, 1993), i.e., the observation that repetitive increments in $[InsP_3]$ yield roughly equal increments in $[Ca^{2+}]_i$. This is a consequence of the fact that increments in $[InsP_3]$ increase the height of the bell-shaped dependence on $[Ca^{2+}]_i$ of the IP_3R open probability whereas increased $[Ca^{2+}]_i$ increases the rate of SERCA pumps back into the ER. Similar conclusions have been reached by Swillens *et al.* (1994) using a nearly identical model.

ANALOGY TO PLASMA MEMBRANE ELECTRICAL EXCITABILITY

Ca^{2+} excitability of the ER membrane is reminiscent of electrical excitability of the plasma membrane in neurons and other so-called "excitable" cells (Meyer, 1991; Sneyd, *et al.*, 1993). In fact, the detailed characteristics of the regulation of IP_3R channels and SERCA pumps by $[Ca^{2+}]_i$ can be put into a one-to-one correspondence (Li *et al.*, 1995a) with the regulation of sodium and potassium channels by membrane potential in the Hodgkin-Huxley model (Hodgkin and Huxley, 1952). In Ca^{2+} excitability, the role of the sodium channel is filled by the IP_3R, with Ca^{2+} replacing the membrane potential as the excitation variable (Li *et al.*, 1995a). Both the IP_3R and the sodium channel activate quickly above a threshold for the excitation variable and both then inactivate more slowly at higher levels. Similarly SERCA activity, in analogy to the delayed rectifier potassium channel, activates with $[Ca^{2+}]_i$, although it does so instantaneously rather than with a delay. Even the ER Ca^{2+} leak fits into the analogy, acting like the leak current in the Hodgkin-Huxley model. Given these similarities between Ca^{2+} excitability of the ER membrane and electrical excitability, it is not surprising that $InsP_3$ can promote "action potential"–like spikes of $[Ca^{2+}]_i$ when $[Ca^{2+}]_i$ is perturbed above a small threshold value (Li *et al.*, 1995a). Indeed, just as repetitive action potential spikes in the Hodgkin-Huxley model occur in the presence of a sufficiently depolarizing external current, increasing the concentration of $InsP_3$ organizes $[Ca^{2+}]_i$ action potential spikes into Ca^{2+} oscillations (Li *et al.*, 1995a).

Several groups have pursued the analogy between Ca^{2+} excitability and electrical excitability by constructing kinetic models of the open probability of the IP_3R using Ca^{2+}-dependent activation and inactivation gates analogous to those in the Hodgkin-Huxley model. In fact, Li and Rinzel (1994) have shown that the De Young-Keizer model, which is based on subunit states of the IP_3R, actually reduces to this form when Ca^{2+} activation is much faster than inactivation and when changes in Ca^{2+} are not too rapid. These Hodgkin-Huxley–like models have been used successfully to describe Ca^{2+} oscillations in gonadotrophs (Li *et al.*, 1994) and Ca^{2+} waves in *X. laevis* oocytes (Atri *et al.*, 1993).

ROLE OF PLASMA MEMBRANE Ca^{2+} FLUXES

Closed cell models of ER Ca^{2+} excitability neglect the influence of Ca^{2+} fluxes through the plasma membrane. These fluxes are often several orders of magnitudes smaller than the fluxes through the IP_3R and SERCA (Tse *et al.*, 1994a) and function in part to maintain total Ca^{2+} levels in the cell. When plausible values of these fluxes are used to simulate Ca^{2+} responses in gonadotrophs (Li *et al.*, 1994), the results are strikingly similar to those caused by GnRH (cf. Figure 1A). In Ca^{2+}-deficient medium, they predict a monotonic decrease in total free Ca^{2+} concentration.

The plasma membrane of gonadotrophs, like other excitable secretory cells, possesses voltage-gated Ca^{2+}

and potassium channels that undergo spontaneous electrical activity (Stojilković *et al.*, 1992; Tse and Hille, 1993; Kukuljan *et al.*, 1994; Tse *et al.*, 1994a). The operation of this plasma membrane oscillator is crucial for regulation of Ca^{2+} in these unstimulated cells. Carefully constructed models of these plasma membrane ion channels in gonadotrophs (Li *et al.*, 1995b) have been used to assess the influence of Ca^{2+} influx on ER Ca^{2+} excitability. Typical simulations shown alongside experimental measurements of the plasma membrane potential (Figure 1B) confirm the idea that GnRH-induced electrical bursting in these cells is caused by Ca^{2+} oscillations originating in the ER, periodically hyperpolarizing the plasma membrane through Ca^{2+}-activated potassium channels (Stojilković *et al.*, 1992). Comparable calculations for pancreatic β cells (Keizer and De Young, 1993) show that voltage-gated Ca^{2+} influx can be as effective as agonist dose in modulating Ca^{2+} oscillations, which has been confirmed experimentally in gonadotophs (Stojilković *et al.*, 1993). These and other calculations that take into account ER depletion–activated channels in the plasma membrane (Bertram *et al.*, 1995; Smith *et al.*, 1995) emphasize the importance of the interaction between the plasma membrane and IICR in the ER membrane in generating stable, but highly dynamic, Ca^{2+} signals within cells.

FUTURE DIRECTIONS

Several specific predictions of the IP_3R-based model of Ca^{2+} oscillations have been successfully tested, e.g., stimulation of oscillations via thapsigargin and ionomycin (Li *et al.*, 1994) and the interaction between plasma membrane and ER Ca^{2+} handling (cf. Figure 1B). Others, including the influence of Ca^{2+} buffers on amplitude and frequency and the absence of oscillations in $InsP_3$ (Wagner and Keizer, 1994), remain to be examined experimentally. Nonetheless, the minimal Hodgkin-Huxley–like model (even with plasma membrane fluxes included) is not the correct description of Ca^{2+} oscillations for all cell types. For example, T lymphocytes can be stimulated to oscillate with a period of the order of 2–3 min with low doses of thapsigargin (Dolmetsch and Lewis, 1994). Those oscillations, however, are utterly dependent on the presence of external Ca^{2+} and involve regulation of ER depletion–activated Ca^{2+} channels. Ca^{2+} oscillations in the bullfrog sympathetic neuron also have a long period and terminate as soon as the medium is made deficient in Ca^{2+}, yet they involve a third type of mechanism involving the ryanodine receptor (Friel, 1995). Thus in looking for verification of predictions of the present model the cellular system must be chosen carefully. It appears that in addition to gonadotrophs, $InsP_3$-induced oscillations in immature *X. laevis* oocytes (but not mature oocytes) and possibly pancreatic β-cells are correctly described by this mechanism.

Much of our present understanding of $InsP_3$-induced Ca^{2+} oscillations is based on kinetic data collected from isolated receptors, vesicle preparations, and permeabilized cells. Referring to Figure 2, it is evident that further tests and refinements of the model will depend on examination of the properties of Ca^{2+} handling in situ. Although large cells, such as *X. laevis* oocytes, provide great flexibility for measuring and maniupulating Ca^{2+} handling (Parker and Ivorra, 1990; Camacho and Lechleiter, 1993), it also has been possible to measure the refractory time (ca. 1.3 s) for Ca^{2+} oscillations in gonadotrophs in situ. Other key quantities that should be accessible experimentally with current in situ techniques include the following: $[Ca^{2+}]_i$ dependence of activation and inactivation of the IP_3R; the Ca^{2+} content of the ER; rates of the ER Ca^{2+} leak, the SERCA pump, plasma membrane Ca^{2+} extrusion, and influx from the external medium; and the extent of Ca^{2+} buffering in the ER and cytoplasm. Future experimental input of this sort is essential to improving and refining the model.

ACKNOWLEGMENTS

This work was partially supported by funds from National Science Foundation grants BIR-9234381 and BIR-9300799 (to J.K.) and the Agricultural Experiment Station at University of California, Davis.

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