Regenerative release of calcium from functionally discrete subcellular stores by inositol trisphosphate

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[Plate 1]

SUMMARY

Fluorescence imaging was used to determine the spatial and temporal patterns of subcellular calcium (Ca^{2+}) liberation induced in *Xenopus* oocytes by photorelease of inositol 1,4,5-trisphosphate ($InsP_3$) from a caged precursor. Increasing levels of $InsP_3$ evoked Ca^{2+} release that began in a graded manner but, at varying threshold levels of $InsP_3$, localized sites then showed transient and asynchronous 'puffs' of Ca^{2+} release. With higher levels of $InsP_3$, Ca^{2+} from adjacent sites formed a focus for initiation of a propagating Ca^{2+} wave. The results show that $InsP_3$-sensitive Ca^{2+} stores are arranged as distinct and functionally independent units, and that Ca^{2+} is released in both graded and regenerative fashions.

1. INTRODUCTION

Inositol 1,4,5-trisphosphate ($InsP_3$) is an intracellular messenger that mediates responses to many neurotransmitters and hormones by liberating Ca^{2+} ions stored within the cell (Berridge & Irvine 1989). The spatial organization of Ca^{2+} liberation is complex, and may encode or determine the way in which information is passed along the signal transduction pathway (Berridge *et al.* 1988; Cheek 1989; O'Sullivan *et al.* 1989; Lechleiter *et al.* 1991; Delisle 1991; Meyer 1991). The result that Ca^{2+} release from permeabilized cell preparations varies in a stepwise manner with successive increments of $InsP_3$ concentration (Muallem *et al.* 1989; Taylor & Potter 1990; Meyer *et al.* 1990; Meyer & Stryer 1990) has led to the proposal that $InsP_3$-sensitive Ca^{2+} stores are organized as multiple subcellular quanta, displaying different sensitivities to $InsP_3$ (Parker & Ivorra 1990; Muallem *et al.* 1989). However, alternative interpretations are possible, including heterogeneity between cells (Taylor & Potter 1990), or modulation of $InsP_3$ receptors by intraluminal Ca^{2+} within a homogeneous store (Irvine 1990; Tregear *et al.* 1991). We have tested the 'quantal' hypothesis by using video imaging of oocytes loaded with a fluorescent indicator to visualize subcellular Ca^{2+} release with micrometre resolution, and to determine the dependence of Ca^{2+} release upon $InsP_3$ concentration.

2. MATERIALS AND METHODS

Experiments were done on oocytes of *Xenopus laevis*. These were obtained from albino frogs to avoid interference during optical recordings by the pigment that is normally present in the animal hemisphere of the cells. Oocytes were each injected with about 50 pmol of the Ca^{2+} indicator fluo-3 (Minta *et al.* 1989) and about 5 pmol caged $InsP_3$ (*myo*-inositol 1,4,5-trisphosphate, $P^{4(5)}$-1-(2-nitrophenyl) ethyl ester) (McCray & Trentham 1989), resulting in respective final intracellular concentrations of approximately 50 μM and 5 μM. Injections were made by pneumatic pressure pulses applied to a glass micropipette filled with 5 mM fluo-3 and 0.5 mM caged $InsP_3$ (Parker & Miledi 1989), and an interval of 30–90 min was allowed before recording to allow even distribution of the compounds throughout the cell.

The optical system for simultaneous flash photolysis of caged $InsP_3$ and recording of Ca^{2+}-dependent changes in fluo-3 fluorescence is shown in figure 1. This was based on an upright Zeiss microscope fitted with two stacked epifluorescence units and a 40 × water immersion objective (numerical aperture 0.75). The lower epifluorescence unit provided flashes of near UV light (wavelengths about 340–420 nm) for photolysis, by means of a continuous Xenon arc lamp and an electronically controlled shutter. A variable rectangular slit diaphragm allowed the photolysis light to be focused on the oocyte as a square or rectangle of desired size. The upper epifluorescence unit was fitted with a 100 W quartz halogen lamp, and provided blue excitation light for the fluo-3. The excitation light was restricted to circle of about 100 μm diameter on the oocyte surface, as the curvature of the cell did not allow larger regions to be viewed in sharp focus. Because of the long excitation and emission wavelengths of fluo-3, the fluorescence excitation light caused no detectable photorelease of $InsP_3$, and photolysis light flashes were almost completely blocked by the barrier filter in the recording path.

Fluorescence images were detected by an intensified CCD camera (Quantex Corp., Sunnyvale, California, U.S.A.) and stored on a domestic super VHS videocassette recorder at video frame rate (30 s^{-1}). The intensifier and camera were operated at their minimum gain settings, and the intensity of

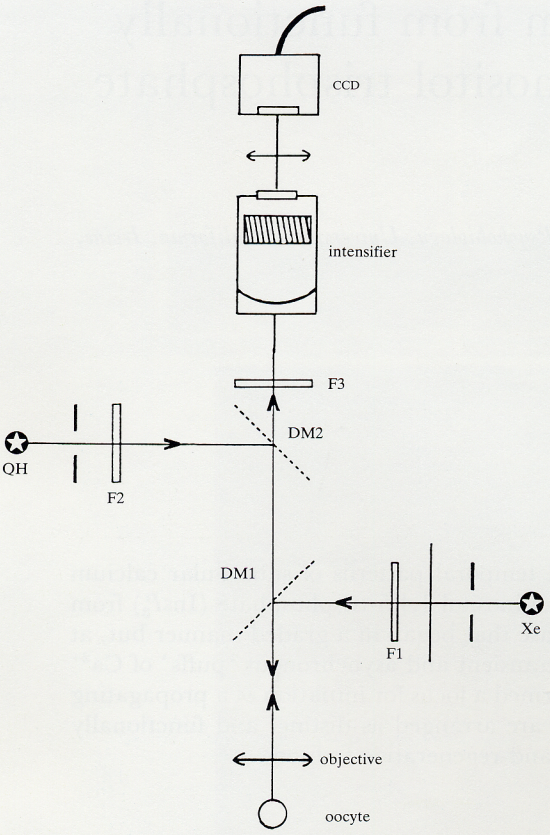

Figure 1. Schematic diagram of the optical system. Xe = 75 W xenon arc lamp; QH = 100 W quartz-halogen lamp; DM1, DM2 = dichroic mirrors with respective cut-off wavelengths of 420 nm and 500 nm; Fl = Schott UG5 ultraviolet filter; F2 = 470 nm narrow bandpass filter; F3 = 540 nm long pass filter. See text for further details.

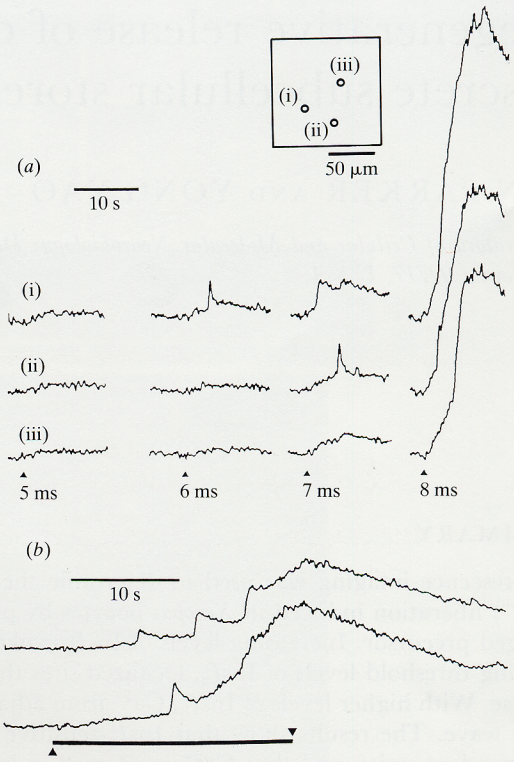

Figure 3. Timecourse of Ca^{2+} signals recorded from localized areas (2 μm diameter). Records were obtained using a photodiode and lens to measure intensities from small spots on a monochrome display screen, and were low pass filtered at 3 Hz. The vertical scale is arbitrary, but is consistent within each section of the figure. (*a*) Measurements from the same experiment as figure 1*a*, showing simultaneous records of Ca^{2+} at the three sites marked on the inset. Responses were evoked by photolysis light flashes of the durations indicated, applied at the arrowheads. (*b*) Asynchronous and repetitive puffs of Ca^{2+} recorded simultaneously at two sites 30 μm apart in a different oocyte. $InsP_3$ was gradually liberated by continued illumination with UV light attenuated to 0.4% of the normal intensity for the time indicated by the bar.

the fluorescence excitation light was adjusted so that the camera operated within its linear range. Video frames were captured with 8 bit (256 level) resolution and processed using the JAVA image processing system (Jandel Scientific, Corte Madre, California, U.S.A.). The images in figure 2 show regions of about 200 × 200 pixels (100 μm × 100 μm) in the centre of the recording field. Control frames of resting fluorescence were subtracted and, to reduce noise, the lowest four intensity levels were set to zero and the images were low-pass filtered through a 3 × 3 convolution mask. Timecourses of Ca^{2+} signals were monitored by using a photodiode and collector lens to measure intensities from small spots on a monochrome display screen. Because fluo-3 does not permit the use of ratio measurements to determine absolute free Ca^{2+} levels, data are presented in arbitrary units. However, comparison of fluorescence signals within each experiment provides a relative indication of free Ca^{2+} levels.

Figures illustrate results from individual oocytes but, with the exception of figure 4, are representative of findings in at least five oocytes.

3. RESULTS

(*a*) *Punctate release of Ca^{2+}*

The spatial distribution of subcellular Ca^{2+} liberation induced by $InsP_3$ was monitored by recording images of fluo-3 fluorescence from small (*ca.* 100 μm diameter) regions of the oocyte after photorelease of $InsP_3$. Despite the considerable thickness of the oocyte (1 mm or more in diameter), the fluorescence signals reflect changes in free Ca^{2+} occurring only in a thin layer close to the plasma membrane. This is because the turbidity of the cytoplasm severely restricts light transmission, and measurements with a confocal microscope showed that the recorded fluorescence declined to one-half at a distance of about 15 μm into the cell. Photolysis flashes of varying duration were used to release different amounts of $InsP_3$. A roughly linear relation is expected because the photolysis reaction involves single-photon absorption, and even the longest flashes used would have photolysed only a small fraction of the available caged $InsP_3$ (Parker 1991). The timecourses of the evoked Ca^{2+} transients were slow (several hundred ms) as compared both to the flash durations used and the kinetics of photorelease of $InsP_3$ (< 10 ms; McCray & Trentham 1989).

Figure 2*a* illustrates typical patterns of Ca^{2+} liberation evoked by stimulus flashes of various durations. In this oocyte, a flash of 6 ms duration evoked a single, localized 'puff' of Ca^{2+}, beginning after a latency of 3.2 s and lasting less than 1 s. The same site responded

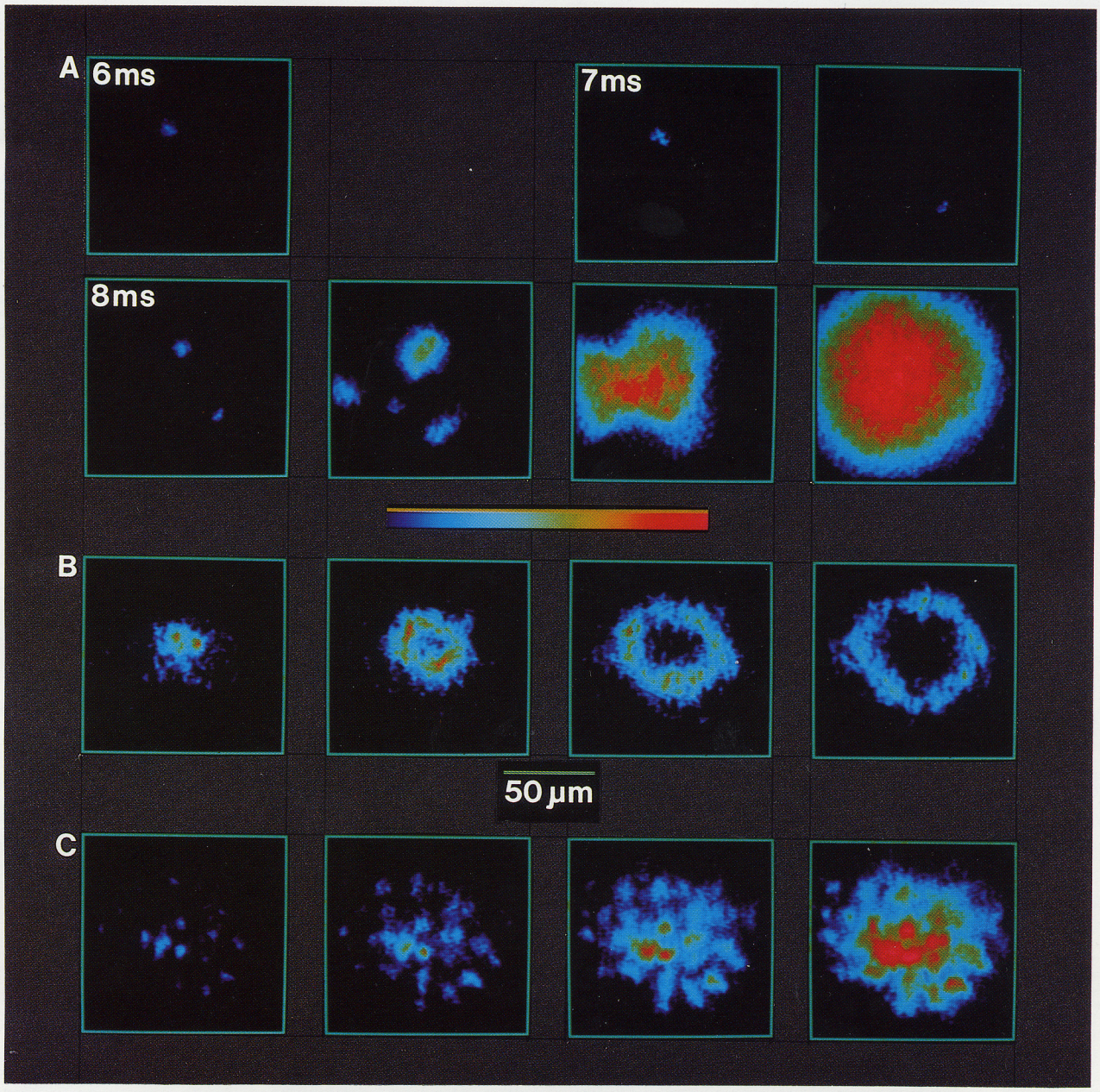

Figure 2. Spatial patterns of subcellular Ca^{2+} liberation induced by photoreleased $InsP_3$. Increasing free Ca^{2+} levels are depicted on a pseudocolour scale from blue to red, after subtraction of resting images captured before stimulation. Fluorescence images were recorded from a circular field, just encompassed within the frame borders. The photolysis light was arranged as a square slightly larger than the frames, to ensure uniform liberation of $InsP_3$ throughout the recording area. A. Ca^{2+} release occurs at discrete sites, showing different thresholds for $InsP_3$. All frames show Ca^{2+} release within the same recording area, evoked by photolysis flashes of various durations. The images were captured at intervals of: 3.2 s following a 6 ms flash; 1.6 s and 2.4 s following a 7 ms flash; and 1.7 s, 2.3 s, 3.3 s and 6.3 s following an 8 ms flash. B. Active wavefront of propagating Ca^{2+} release. Images were formed by sequential subtraction of video frames captured at ten-frame intervals, and thus show increases in Ca^{2+} occurring during the preceding 333 ms. A 20 ms photolysis flash given 1.6 s before the first frame evoked a focus of Ca^{2+} release near the center of the recording area. C. Punctate release of Ca^{2+} following a strong (duration about 3-times threshold) light flash. Images show successive video frames (33 ms intervals) beginning two frames after the end of a 20 ms flash.

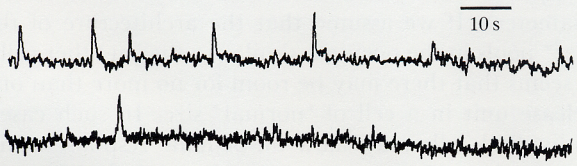

Figure 4. Spontaneous puffs of Ca^{2+} in the absence of stimulation. Traces show simultaneous records from two localized (2 μm diameter) sites situated about 20 μm apart.

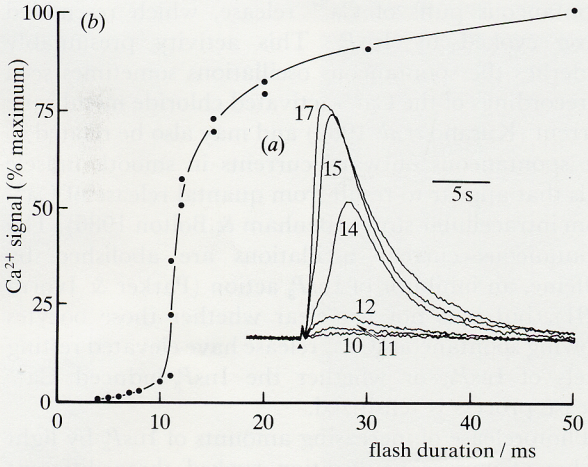

Figure 5. Dose-dependence of $InsP_3$-evoked Ca^{2+} release. (*a*) Inset shows superimposed traces of fluorescence Ca^{2+} signals evoked by photolysis flashes of the indicated durations (in ms). Flashes were applied when marked by the arrowhead, and the initial transient deflections are flash artefacts. Fluorescence measurements were integrated over a 20 μm diameter recording spot. (*b*) Graph shows normalized peak size of the fluorescent Ca^{2+} signals evoked in a different oocyte as a function of flash duration. Points are single measurements, obtained by using the image processing software to average the fluorescence increase occurring within a 50 μm × 20 μm rectangular area. The curve was drawn by eye. The inflexion at about 11 ms corresponded to the initiation of a spreading Ca^{2+} wave.

again with a Ca^{2+} puff after a 7 ms flash, but the latency was shorter (1.6 s) and a second puff subsequently appeared at a different location. Increasing further the flash duration to 8 ms evoked nearly simultaneous increases in Ca^{2+} at the two sites, followed by responses at additional locations. However, unlike the transient puffs seen with shorter flashes, Ca^{2+} then remained elevated for several seconds, and Ca^{2+} from adjacent release spots fused to form a focus from which a regenerative Ca^{2+} wave propagated throughout the recording area. This propagating wave is shown more clearly in figure 2*b*, recorded in a different oocyte. The images in this case were processed by sequential subtraction of frames captures at $\frac{1}{3}$ s intervals, and thus display the advancing wavefront where the free Ca^{2+} level had increased during the preceding interval. The velocity of propagation of the Ca^{2+} wave was approximately 18 μm s^{-1} (mean value from three oocytes).

Release of Ca^{2+} as transient puffs was seen in all oocytes ($n = 11$) examined from one donor, but in six out of 13 oocytes from another donor frog, Ca^{2+} waves were triggered as soon as one or a group of adjacent release sites were activated, so that it was not possible in these cells to observe transient puffs of Ca^{2+} release.

Following stimulation by longer light flashes, Ca^{2+} release occurred nearly simultaneously at multiple discrete loci, spaced about 10–20 μm apart (figure 2*c*). 'Spots' of Ca^{2+} first became apparent within one or two video frames following the flash, and subsequently became brighter and more diffuse over the next several frames. Even at early times, when diffusion of Ca^{2+} would be minimal, the Ca^{2+} spots did not appear as point sources. Instead, they were often shaped as elongated blobs or as rings, with dimensions of approximately 5 μm. No Ca^{2+} waves were apparent, probably because Ca^{2+} was released simultaneously from all of the $InsP_3$-sensitive stores within the recording area.

(*b*) *Timecourse of Ca^{2+} puffs*

Figure 3*a* shows the timecourses of cytoplasmic free Ca^{2+} monitored at three points within the same recording field as figure 2*a*. Site i was the most sensitive and showed a Ca^{2+} transient in response to a 6 ms flash, whereas site ii began to respond only when the flash was lengthened to 7 ms. Site iii did not show Ca^{2+} puffs, but was invaded by the wave of Ca^{2+} initiated following an 8 ms flash. Free Ca^{2+} levels during the wave rose higher than during the puffs, and decayed more slowly.

An important result was that different subcellular sites operated independently, and generated asynchronous puffs of Ca^{2+}. Another example of this is shown in figure 3*b*, obtained using prolonged exposure to UV light of low intensity to produce a gradually rising level of $InsP_3$ within the cell. Two sites about 30 μm apart gave repetitive puffs of Ca^{2+}, superimposed on a gradual rise in baseline Ca^{2+}.

(*c*) *Spontaneous Ca^{2+} puffs*

Oocytes did not usually show any changes of Ca^{2+} in the absence of stimulation. However, by chance, we observed one oocyte which displayed spontaneous puffs of Ca^{2+}. These occurred apparently at random, at multiple sites within the recording field. Figure 4 shows traces of Ca^{2+} transients recorded at two sites. One site (upper trace) gave frequent puffs, whereas other sites (e.g. lower trace) were less active.

(*d*) *Pacemaker and regenerative Ca^{2+} release*

In addition to the release of Ca^{2+} as puffs or waves, brief flashes evoked also a smaller, gradual elevation of Ca^{2+}. This 'pacemaker' Ca^{2+} was not resolved in images such as figure 2*a*, but is evident in several traces in figure 3*a*, where it can be seen to precede the appearance of puffs, as well as occurring at sites that failed to show puffs. Because the fluorescence signals from the pacemaker Ca^{2+} were faint, we integrated measurements over wider (several hundred μm^2) areas

of the cell to obtain better resolution. For example, the oocyte in figure 5*a* first showed a detectable response to a 10 ms flash, and the Ca^{2+} signal then increased progressively as the flash was lengthened to 12 ms. However, the response to a 14 ms flash showed an inflexion after about 2 s, after which the Ca^{2+} level rose abruptly as a Ca^{2+} wave propagated through the recording area. Increasing the flash duration to 15 ms increased the size of the response and shortened the latency to the inflexion but, although a further increase to 17 ms gave a still shorter latency, the response size increased little. Measurements from a different oocyte of peak sizes of Ca^{2+} signals evoked by flashes of various durations are plotted in figure 5*b*, and fit a curve with three distinct components: (i) an initial 'foot' increasing in a graded manner with flash duration; (ii) an abrupt rise; and (iii) a more gradual rise over which the signal grew only 30 % for a fivefold increase in flash duration.

At present we do not know whether the pacemaker Ca^{2+} is liberated diffusely, or in a punctate manner like the regenerative Ca^{2+} release. The faint Ca^{2+} fluorescence signals did not allow good spatial resolution and, in any case, Ca^{2+} ions would become diffusely distributed during the slow rising phase of the pacemaker Ca^{2+}.

4. DISCUSSION

Our results show that InsP_3 caused punctate release of sequestered Ca^{2+} in *Xenopus* oocytes. Individual Ca^{2+} release units functioned independently and showed varying sensitivities to InsP_3, as predicted by a 'quantal' model of Ca^{2+} release (Parker & Ivorra 1990; Muallem *et al.* 1989). The morphological correlate of the release units is presently unclear; they may, for example, be discrete organelles, or represent 'hot spots' within an extensive reticulum (Rossier & Putney 1991). In the latter case, possible explanations for heterogeneity in sensitivity to InsP_3 include clustering of InsP_3 receptors, or variations in affinity of InsP_3 receptors resulting from differences in either cytoplasmic (Bezprozvanny *et al.* 1991) or intraluminal (Irvine 1990) free Ca^{2+} level. Fine structure was evident in patterns of Ca^{2+} from single units, suggesting they have dimensions of a few μm, rather than being point sources. High speed confocal imaging should provide better resolution, and it will be interesting to correlate the foci of Ca^{2+} release with the distributions of InsP_3 receptors and other endoplasmic reticulum marker proteins.

From experiments like figure 2*c* we estimate that the oocyte may contain a few thousand Ca^{2+} release units if these are arranged as a single layer under the cell membrane, and this number will obviously be greater if additional units are present deeper in the cytoplasm where they are not visible in our recordings. The incremental contribution made by each unit is, therefore, only a small fraction of the total, so that the whole cell may show an apparently smoothly graded response as increasing numbers of units are recruited by increasing concentrations of InsP_3. However, the *Xenopus* oocyte is an extraordinarily large cell (> 1 mm diameter). If we assume that the architecture of the Ca^{2+} pools in the oocyte is similar to that in other cells, it seems that there may be room for no more than one release unit in a cell of 'normal' size. In such cases, individual cells may show all-or-none responses, and observations of quantal or incremental Ca^{2+} release in cell populations (Muallem *et al.* 1989; Taylor & Potter 1990; Meter *et al.* 1990; Meyer & Stryer 1990) could result from inter- rather than intra-cellular heterogeneity.

In one oocyte we were fortunate to observe spontaneous puffs of Ca^{2+} release, which resembled those evoked by InsP_3. This activity presumably underlies the spontaneous oscillations sometimes seen in recordings of the Ca^{2+}-activated chloride membrane current (Kusano *et al.* 1982) and may also be related to the spontaneous outward currents in smooth muscle cells that appear to result from quantal release of Ca^{2+} from intracellular stores (Benham & Bolton 1986). The spontaneous current oscillations are abolished by caffeine, an inhibitor of InsP_3 action (Parker & Ivorra 1991), but it is not yet clear whether those oocytes showing spontaneous Ca^{2+} release have elevated resting levels of InsP_3, or whether the InsP_3-induced Ca^{2+} release process is sensitized.

Photorelease of increasing amounts of InsP_3 by light flashes of increasing duration evoked three different modes of Ca^{2+} liberation. We refer to these, respectively, as pacemaker Ca^{2+}, Ca^{2+} puffs and Ca^{2+} waves. After brief flashes, the level of Ca^{2+} rose and fell gradually over a few seconds, with an amplitude that was graded with flash duration. However, when the flash was lengthened beyond a certain threshold an abrupt regenerative release of Ca^{2+} was triggered, which appeared either as transient puffs restricted to particular release sites, or as a wave of Ca^{2+} that propagated throughout the stimulated area. The all-or-none dose–response relation we had previously observed (Parker & Ivorra 1990) using confocal optics to monitor Ca^{2+} at a point source probably reflected the initiation of a regenerative response, whereas the pacemaker Ca^{2+} may have been below the limit of resolution in those experiments. Regenerative Ca^{2+} release has also been observed in mammalian oocytes (Miyazaki 1988; Peres 1990).

The pacemaker Ca^{2+} signal appeared to increase as a steeper than linear function of the amount of photoreleased InsP_3. This might reflect cooperative binding of InsP_3 to its tetrameric receptor (Meyer *et al.* 1988; Meyer & Stryer 1990), or could arise through a subthreshold regenerative effect. However, it is clear that cooperativity cannot explain the abrupt appearances of Ca^{2+} puffs and waves. Instead, the striking resemblance of these regenerative responses to a nerve action potential points to the existence of positive feedback in the release process. Ca^{2+} ions released into the cytosol have been proposed to exert positive feedback at various stages in the messenger pathway, including: (i) stimulation of phospholipase C resulting in increased formation of InsP_3 (Swann & Whitaker, 1986; Harootunian *et al.* 1991); (ii) Ca^{2+}-induced Ca^{2+} release from InsP_3-insensitive stores (Berridge 1991;

Goldbeter *et al.* 1990); and (iii) Ca^{2+} acting as a coagonist to potentiate $InsP_3$-mediated Ca^{2+} liberation (Iino 1990; Finch *et al.* 1991; Missiaen *et al.* 1991; Bezprozvanny *et al.* 1991). Of these models we favour the latter as a possible explanation for our results, as intracellular injections of Ca^{2+} ions into *Xenopus* oocytes evoke smoothly graded membrane current responses (Miledi & Parker 1984) and do not evoke propagating Ca^{2+} waves (unpublished data). Thus puffs of Ca^{2+} may result from regenerative positive feedback localized to individual release units, and a wave will be initiated if regenerative release is triggered from surrounding units. The organization of $InsP_3$-sensitive Ca^{2+} release units is, therefore, probably important not only in determining spatial and temporal aspects of $InsP_3$ signalling, but also its dose-dependence.

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REFERENCES

Benham, C. D. & Bolton, T. B. 1986 Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. *J. Physiol., Lond.* **381**, 385–406.

Berridge, M. J. 1988 Inositol trisphosphate-induced membrane potential oscillations in *Xenopus* oocytes. *J. Physiol., Lond.* **403**, 589–599.

Berridge, M. J. 1991 Caffeine inhibits inositol trisphosphate-induced membrane potential oscillations in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **244**, 57–62.

Berridge, M. J. & Irvine, R. F. 1989 Inositol phosphates and cell signalling *Nature, Lond.* **341**, 197–205.

Berridge, M. J., Cobbold, P. E. & Cuthbertson, K. S. R. 1988 Spatial and temporal aspects of cell signalling. *Phil. Trans. R. Soc. Lond.* B **320**, 325–343.

Bezprozvanny, I., Watras, J. & Erlich, B. E. 1991 Bell shaped calcium-response curves of $Ins(1,4,5)P_3$- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature, Lond.* **351**, 751–754.

Brooker, G., Seki, T., Croll, D. & Wahlestedt, C. 1990 Calcium wave evoked by activation of endogenous or exogenously expressed receptors in *Xenopus* oocytes. *Proc. natn. Acad. Sci. U.S.A.* **87**, 2183–2187.

Busa, W. B. & Nucitelli, R. 1985 An elevated free cytosolic Ca^{2+} wave follows fertilization in eggs of the frog *Xenopus laevis*. *Devl Biol.* **100**, 1325–1329.

Cheek, T. R. 1989 Spatial aspects of calcium signalling. *J. Cell Sci.* **93**, 211–216.

Delisle, S. 1991 The four dimensions of calcium signalling in *Xenopus* oocytes. *Cell Calcium* **12**, 217–227.

Finch, E. A., Turner, T. J. & Goldin, S. M. 1991 Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science, Wash.* **252**, 443–446.

Goldbeter, A., Dupont, G. & Berridge, M. J. 1990 Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1461–1465.

Harootunian, A. T., Kao, J. P. Y., Paranjape, S. & Tsien, R. Y. 1991 Generation of calcium oscillations in fibroblasts by positive feedback between calcium and IP_3. *Science, Wash.* **251**, 75–78.

Iino, M. 1991 Biphasic Ca^{2+}-dependence of inositol 1,4,5-trisphosphate-induced Ca release in smooth muscle cells of the guinea pig *Taenia caeci. J. gen. Physiol.* **95**, 1103–1122.

Irvine, R. F. 1990 'Quantal' Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates – a possible mechanism. *FEBS Lett.* **263**, 5–9.

Kubota, H. Y., Yoshimoto, Y., Yoneda, M. & Hiramoto, Y. 1987 Free calcium wave upon activation in *Xenopus* eggs. *Devl Biol.* **119**, 129–136.

Kusano, K., Miledi, R. & Stinnakre, J. 1982 Cholinergic and catecholaminergic receptors in the oocyte membrane. *J. Physiol., Lond.* **328**, 143–170.

Lechleiter, J., Girard, S., Peralta, E. & Clapham, D. 1991 Spiral calcium wave propagation and annihilation in *Xenopus laevis* oocytes. *Science, Wash.* **252**, 123–126.

McCray, J. A. & Trentham, D. R. 1989 Properties and uses of photoreactive caged compounds. *A. Rev. Biophys. biophys. Chem.* **18**, 239–270.

Meyer, T. 1991 Cell signalling by second messenger waves. *Cell* **64**, 675–678.

Meyer, T., Holowka, D. & Stryer, L. 1988 Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. *Science, Wash.* **240**, 653–656.

Meyer, T. & Stryer, L. 1990 Transient calcium release induced by successive increments of inositol 1,4,5-trisphosphate. *Proc. natn. Acad. Sci. U.S.A.* **87**, 3841–3845.

Meyer, T., Wensel, T. & Stryer, L. 1990 Kinetics of calcium channel opening by inositol 1,4,5-trisphosphate. *Biochemistry* **29**, 32–37.

Miledi, R. & Parker, I. 1984 Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol., Lond.* **357**, 173–183.

Minta, A., Kao, J. P. Y. & Tsien, R. Y. 1989 Fluorescent indicators for cytosolic calcium based on rhodamine and fluoresceine chromophores. *J. biol. Chem.* **264**, 8171–8178.

Missiaen, L., Taylor, C. W. & Berridge, M. J. 1991 Spontaneous calcium release from inositol trisphosphate-sensitive calcium stores. *Nature, Lond.* **352**, 241–244.

Miyazaki, S.-i. 1988 Inositol 1,4,5-trisphosphate-induced calcium release and guanine nucleotide-binding protein-mediated periodic calcium rises in golden hamster eggs. *J. Cell Biol.* **106**, 345–353.

Muallem, S., Pandol, S. J. & Beeker, T. G. 1989 Hormone-evoked calcium release from intracellular stores is a quantal process. *J. biol. Chem.* **264**, 205–212.

O'Sullivan, A. J., Cheek, T. R., Moreton, R. B., Berridge, M. J. & Burgoyne, R. D. 1989 Localization and heterogeneity of agonist-induced changes in cytosolic calcium concentration in single bovine adrenal chromaffin cells from video imaging of fura-2. *EMBO J.* **8**, 401.

Parker, I. & Ivorra, I. 1990 Localized all-or-nothing calcium liberation by inositol trisphosphate. *Science, Wash.* **250**, 977–979.

Parker, I. & Ivorra, I. 1991 Caffeine inhibits inositol trisphosphate-mediated liberation of intracellular calcium in *Xenopus* oocytes. *J. Physiol., Lond.* **433**, 229–240.

Parker, I. & Miledi, R. 1989 Non-linearity and facilitation in phosphoinositide signalling studied by the use of caged inositol trisphosphate in *Xenopus* oocytes. *J. Neurosci.* **9**, 4068–4077.

Parker, I. & Yao, Y. 1991 Imaging of Ca^{2+} liberated in *Xenopus* oocytes by agonists and photo-released inositol trisphosphate. *Soc. Neurosci. Abstr.* **17**, 17.

Peres, A. 1990 $InsP_3$- and Ca^{2+}-induced Ca^{2+} release in single mouse oocytes. *FEBS Lett.* **275**, 213–216.

Rossier, M. F. & Putney, J. W. 1991 The identity of the calcium-storing, inositol 1,4,5-trisphosphate-sensitive organelle in non-muscle cells: calciosome, endoplasmic reticulum... or both? *Trends Neurosci.* **14**, 310–314.

Swann, K. & Whitaker, M. 1986 The part played by

inositol trisphosphate and calcium in the propagation of the fertilization wave in sea urchin eggs. *J. Cell. Biol.* **103**, 2333–2342.

Taylor, C. & Potter, V. B. 1990 The size of inositol 1,4,5-trisphosphate-sensitive Ca^{2+} stores depends on inositol 1,4,5-trisphosphate concentration. *Biochem. J.* **266**, 189–194.

Tregear, R. T., Dawson, A. P. & Irvine, R. F. 1991 Quantal release of Ca^{2+} from intracellular stores by $InsP_3$: test of the concept of control of Ca^{2+} release by intraluminal Ca^{2+}. *Proc. R. Soc. Lond.* B **243**, 263–268.

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