CELL CALCIUM

Cell Calcium (1993) 14, 736–745

Classes and mechanisms of calcium waves

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Abstract — The best known calcium waves move at about 5–30 μm/s (at 20°C) and will be called fast waves to distinguish them from slow (contractile) ones which move at 0.1–1 μm/s as well as electrically propagated, ultrafast ones. Fast waves move deep within cells and seem to underlie most calcium signals. Their velocity and hence mechanism has been remarkably conserved among all or almost all eukaryotic cells. In fully active (but not overstimulated) cells of all sorts, their mean speeds lie between about 15–30 μm/s at 20°C. Their amplitudes usually lie between 3–30 μM and their frequencies from one per 10–300 s. They are propagated by a reaction diffusion mechanism governed by the Luther equation in which Ca^{2+} ions are the only diffusing propagators, and calcium induced calcium release, or CICR, the only reaction; although this reaction traverses various channels which are generally modulated by IP_3 or cADPR. However, they may be generally initiated by a second, lumenal mode of CICR which occurs within the ER. Moreover, they are propagated between cells by a variety of mechanisms. Slow intracellular waves, on the other hand, may be mechanically propagated via stretch sensitive calcium channels.

As shown in Table 1, there seem to be three main classes of intracellular calcium waves. The best known class of waves generally travel at about 10–30 μm/s (at 20°C) and will be called *fast* waves in this paper. They have also been called cytocal waves [1]. Such waves were first visualized in fertilizing medaka fish eggs about 15 years ago [2], move deep into cells, seem to underlie most calcium signals [3] and will probably prove to be comparable to action potentials as organizers of living systems. On the basis of earlier work on muscle cells [4–6] the medaka waves were immediately interpreted as reaction-diffusion waves propagated by regenerative or calcium-induced calcium release or CICR.

Table 1 Classes of Ca^{2+} waves.

Class	*Speed (μm/s at 20°C)*	*Locus*	*Propagator*
Slow	0.1–1	Cortex?	Mechanical tension?
Fast	5–30	Whole cytosol	Diffusing Ca^{2+}
Ultrafast	10^4–10^8	Cortex	Electrical field

A second class seems to travel at about 0.1–1 μm/s (at 20°C) and will be called *slow* calcium waves here. Such waves were first visualized in cleaving medaka eggs only a couple of years ago and may prove to drive a variety of slow contractile events such as furrow formation [7]. A third class of calcium waves are simply the subsurface or cortical increases in Ca^{2+} which accompany action

potentials in activating eggs, many neurons and muscles and certain other systems [8]. They will be called ultrafast waves here. They are briefly discussed both for logical completeness and because they sometimes start or help start the 'fast' ones that are our main topic. Moreover, a possible new class of medium speed waves, propagated by calcium-induced calcium entry or 'CICE' through the plasma membrane is proposed to explain the anomalous waves through certain cultured vascular myocytes.

Fast calcium waves

Conservation of velocity

The more reliable measurements of fast wave speeds as a function of temperature are plotted in the Figure. It updates a similar diagram published two years ago [3] with the new studies detailed in Table 2. These data bring the number of accumulated cases up to 52 and prove to sharpen the pattern first seen in 1991.

The accumulated data fall into two main classes: waves through activating eggs and waves through fully active cells (which go about twice as fast). When the latter are induced by agonists, they go a bit faster at higher agonist concentrations; so to minimize variability, I only plotted the speeds of waves induced by minimal stimuli. Except for two anomalous cases discussed below, the chief source of variability is a rise in speed with temperature; in two cases – that of the fertilizing medaka egg (case 18) and that of ventriculocytes (case 31) – this rise was measured on the same system in the same study; in three others – that of *Lytechinus* eggs (case 8), pancreatic acinar cells (case 39) and of vascular myocytes (case 44) – a comparable rise can be seen in data from different studies on the same system; overall a comparable rise can be seen in the accumulated data for all sorts of activating eggs and in that for all sorts of fully active cells. After one corrects for temperature, the residual variation in mean wave velocity is only about 2-fold for active cells and 3-fold for eggs. Moreover, there has been a clear fall in the variability in the data obtained from active cells as better methods have been applied; so much of the residual variability may lie in experimental error.

Two anomalous cases remain. Waves through mammalian ventriculocytes (case 31) are 2–3 times faster than those through nearly 50 other systems; while those through three lines of mammalian vascular smooth myocytes (cases 43, 43a and 45) are 5–20 times slower. However, a better understanding of the much studied mammalian ventriculocyte may have emerged. Alexandre Fabiato has pointed out to me that such cells have a comparably anomalous structure. They are so penetrated and riddled by tubular invaginations of the plasmalemma that most of their cytosol is within a single micron of the medium [28] despite their superficial dimensions of about 14 x 20 x 100 μm. Calcium ions diffusing at 200 μm/s would only take about 5 ms to diffuse 1 μm, far too short a time for ER uptake to fully control it. Moreover, Ishide et al. have noted and discussed cases where 'a focal increase in $[Ca^{2+}]$ may be followed by an increase occurring almost simultaneously throughout the myocyte ... this rapid spread of the calcium wave must be mediated by a propagation of electrical excitation of the sarcolemmal membrane' (p. H949 of [11]). Put together, these facts suggest that calcium waves within ventricular myocytes are so fast because they travel so close to the plasmalemma as to encounter large increases in cytosolic $[Ca^{2+}]$ which were produced by an electrically triggered influx of Ca^{2+} from the medium. In a sense, these fast waves may be accelerated by interaction with ultrafast ones.

On the other hand, the slowness of waves through three short term lines of vascular myocytes remain puzzling. They are particularly puzzling since the speeds reported for a long term line of vascular myocytes (A7r5 cells, cases 44 and 44a) are not at all anomalous. However an interesting clue is provided by applying the Luther Equation 1a to the periodic waves which can be induced in aortic myocytes with ATP. Applying it to data in Figures 1 and 3 of [20] yields values for the propagator's diffusion constant, D_p of 130, 230 and 120 $\mu m^2/s$ for the second, third and fifth waves after ATP addition. This result is remarkably consistent with those obtained by applying the same analysis to medaka egg waves and to hepatocyte waves. Hence it argues against Ca^{2+} buffering as the anomalous factor. It also seems to argue against mechanical propagation as a cause of slowness. Indeed, it suggests a

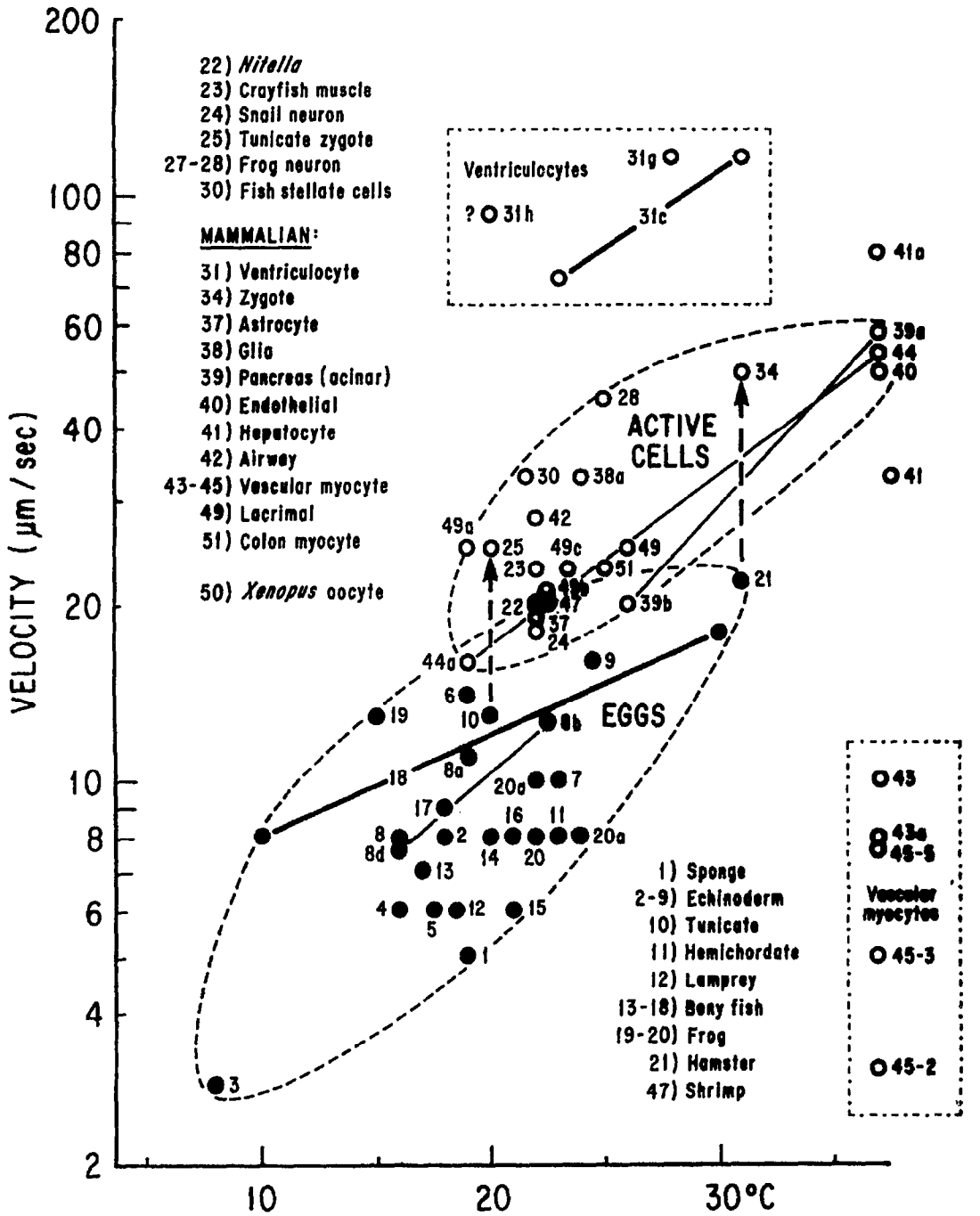

Fig. Velocities of intracellular calcium waves in fertilizing eggs (●) and in fully active cells (○) vs. temperature. Vertical dashed arrows connect data for fertilizing eggs with data for post-fertilization waves through these same eggs. Oblique solid lines connect data from the same system at different temperatures. (Heavier ones were reported in the same paper; lighter ones, separately.) Updated from Figure 1 in [3] and detailed in Table 2. Relatively uncertain data (cases 8c, 27, 31h, 33, 37a, 45 & 47) as well as speeds through large masses of cells (cases 29, 32, 42a & 52) have generally not been plotted.

new model of wave propagation in this case.

It suggests that these peculiar medium speed (?) waves are propagated by calcium-induced calcium entry through the plasma membrane or '*CICE*' rather than CICR through the ER membrane. This CICE model is consistent with the fact that the periodic oscillations – unlike the initial transient – do not occur at all if external Ca^{2+} is removed with EGTA before ATP is added and disappear rapidly if it is removed afterwards [29]. It also seems to be consistent with the insensitivity of the periodic oscillations to nifedipine and to high K^+ since there is no reason to believe that CICE channels would have such sensitivities. It would explain the remarkable observations of two independent and supposedly colocalized waves within a single cell [20] as actually arising from waves along the top and bottom membranes of the same thin cell. Other cogent arguments for the periodic oscillations being independent of intracellular Ca^{2+} release can be found in [30].

Moreover, it would attribute the clearly different character of the initial transient to its being a fast CICR wave rather than a medium speed CICE one. This last point is supported by application of the Luther equation. Figure 1B of [20] yields a value of 1.0 s for the rise time, t, and use of Equation 1 with D_P taken as 300 $\mu m^2/s$ then yields a speed of 35 $\mu m/s$ which is clearly in the fast wave range.

Altogether, the evidence for a remarkable conservation of fast calcium wave velocities is now compelling. However, this is certainly not to assert that these velocities can not be substantially modulated by changes in the cytosolic concentrations of second messengers or modulators like IP_3, cADP-ribose, cAMP, ATP, pH etc. One clear indicator that they can is the approximate doubling of such velocities after eggs are fertilized [31,32]. Another is the sensitivity of such velocities to the concentration of an initiating agonist. Waves through isolated pancreatic acini, for example, travel 40% more slowly when initiated by 0.1 mM ACh than by 1 or 10 mM ACh [15]. What is being asserted is that at a given temperature and modulator background, there is an astonishing conservation of velocities over an immense range of organisms and cell types. Compare the 5-fold range of velocities at 20°C which is shown in the Figure with the 10 000-fold range of conduction velocities for action potentials in various cells ([33], p. 81).

This conservation of velocity implies a conservation of mechanism. That in turn plainly indicates the value of incorporating data from a variety of systems in studying fast wave mechanisms. Moreover, it also indicates that these conserved mechanisms involve a rather complex machine. For it is very difficult to believe that wave velocities were conserved by direct environmental pressures. What on earth would drive all cells to propagate calcium waves over a roughly 2-fold range of speeds at a given temperature etc.? Rather, one is driven to imagine that the wave machine is so complex that having once evolved, the shift to a different working system with a different inherent velocity would require more simultaneous genetic changes than chance would ever allow.

Amplitudes and frequencies of fast calcium waves

Wave speeds can and usually are reliably measured with fluorescent dyes like Fura–2 and Fluo–3. However, the peak Ca^{2+} levels or amplitudes of calcium waves can only be reliably measured with the luminescent photoprotein aequorin and its variants. The main reason for aequorin's reliability is its wide dynamic range: its luminescence rises with nearly the third power of $[Ca^{2+}]$ up to about 300–600 μM $[Ca^{2+}]$; while the fluorescent dyes like Fura–2 do not report cytosolic Ca^{2+} levels above about 1–2 μM [34,35].

In all cases where wave amplitudes in eggs have been measured with aequorin, they generally prove to lie in the 3–30 μM range. These include: the fertilization wave through the medaka egg where this peak is about 30 μM [36,37]; the fertilization waves through ascidian eggs where they are about 7–10 μM; and the postfertilization waves through these eggs where they are usually about 2–4 μM [38] as well as the fertilization and postfertilization waves through hamster eggs where they can be deduced to lie in the 5–10 μM range from Figure 3 of [31]. The few available aequorin studies of $[Ca^{2+}]$ oscillations in smaller cells have not usually given such clear results. Nevertheless, such a study of agonist-induced oscillations in rat hepatocytes indicated peak levels of 0.6–0.8 μM Ca^{2+} assuming that peak

Ca^{2+} is evenly distributed [39]. However a correction for the unevenness later seen in rat hepatocytes [40] suggests true peak levels of a few μM.

It is well known that the pattern of periodic calcium spikes seen in temporal records of various cultured mammalian cells can be driven to confluency by sufficient stimuli. In most cases, it is hard to assess the natural range of spiking frequencies. However, in their recent review of cultured hepatocyte behaviour, Cobbold et al. state that in the range between threshold agonist concentrations and doses sufficient to just activate glycogenolysis, that periods vary by about 10-fold, from up to 240 s to ≈20–30 s [41]; while among the periodic postfertilization pulses in ascidian eggs, these intervals showed a comparable variation from about 30–120 s [38].

It has been suggested that wave amplitudes are much less variable than wave frequencies [42]. In assessing this proposition, one really must restrict oneself to aequorin data since fluorescent amplitudes are truncated above about 1 μM Ca^{2+}. Using this criterion, one finds that in cultured hepatocytes, that a 10-fold range in frequency is accompanied by a less than 2-fold one in amplitude [39,41]. However, among the postfertilization pulses in ascidian eggs, amplitudes and frequencies seem to be equally variable. Thus in *Phallusia*, both vary by about 4-fold; while in *Ciona*, both vary by about 2.5-fold. [38]. So 'frequency modulation' remains an attractive but uncertain proposition.

Ca^{2+} ions are the only propagators of fast calcium waves

Few would disagree that fast waves are propagated within cells by a reaction-diffusion mechanism in which Ca^{2+} is one essential, diffusing propagator. However, some still argue that IP_3 is another essential, diffusing propagator [43,44]. In the most plausible version of that model – the so-called ICC model – diffusing Ca^{2+} induces IP_3 formation which in turn diffuses to induce Ca^{2+} release.

Let me outline the compelling arguments against the ICC model:

1. The oldest and simplest one is that fast waves travel deep inside of cells, a point seen in the first visualized calcium wave [2] and well confirmed by the confocal images listed in Table 2. Hence the ICC model requires Ca^{2+} to rapidly induce IP_3 formation from inner membranes; yet no substantial evidence for such induction seems to exist. The argument for Ca^{2+}-induced IP_3 formation relies entirely upon studies of preparations which contain plasma membranes (p. 160 of [43]).

2. For a wave of IP_3-induced Ca^{2+} release to cross a cell, a wave of high IP_3 would also have to cross it. Yet pancreatic acinar cells injected with high concentrations of a stable and effective IP_3 analogue, $InsPS_3$, were found to support typical Ca^{2+} oscillations and hence waves [45]. The same has been recently found to be true for *Xenopus* oocytes [23,25]. Moreover, 10-fold increases in the concentration of IP_3 injected into these cells – while they much increase wave frequency – have no effect upon its velocity [24].

3. Three drugs that should block IP_3 waves sometimes fail to block fast Ca^{2+} waves. Neomycin, at concentrations that should block IP_3 formation, nevertheless does not affect spontaneous or caffeine-induced Ca^{2+} oscillations (hence waves) in chromaffin cells [46]. Thapsigargin can induce typical Ca^{2+} oscillations (and hence waves) in salivary cells, under conditions in which the IP_3 sensitive Ca^{2+} pools remain continuously depleted [47]. While concentrations of heparin that should block IP_3 induced Ca^{2+} release allow a wave of undiminished speed to cross smooth muscle cells [19] and a typical calcium pulse (and hence wave) to occur during the fertilization of sea urchin eggs [10,48,49].

4. Moreover, despite a recent claim to the contrary [50], the evidence indicates that Ca^{2+} ions diffuse fast enough through the cytosol to support fast calcium waves. In three different systems kept at the 3–30 μM levels of $[Ca^{2+}]$ – which are the levels found in fast calcium waves – Ca^{2+} has been found to diffuse at about 100–500 $\mu m^2/s$. In the somas of live *Aplysia* neurons, Ca^{2+} diffuses at 70–120 $\mu m^2/s$ [51]; in *Myxicola* axoplasm, at about 530 $\mu m^2/s$ [52]; and in *Xenopus* ooplasm, at 100–200 $\mu m^2/s$ [50]. Moreover, D_p, the unknown propagator's diffusion constant can be inferred to lie in this same 100–500 $\mu m^2/s$ range.

This inference can be made for the medaka egg and the rat hepatocyte by applying the Luther equation to fast calcium waves [3].

$$v = 2\left(\frac{Dp}{t}\right)^{\frac{1}{2}} \quad \text{Eq. 1}$$

Hence

$$D_p = \frac{tv^2}{4} \quad \text{Eq. 1a}$$

Where v is the wave's speed; t, the time for the autocatalytic reaction to raise the propagator's concentration e-fold. In my 1991 paper [3], the constant, 2 in Equation 1 was casually taken to be 1. However, the exponential rise of Ca^{2+} in the fertilizing medaka egg indicates that the autocatalytic reaction involves only one Ca^{2+} ion and in waves driven by such reactions, this term, α, is known to be 2 rather than 1 (equation 26b in [53]). If one therefore takes α to be 2, then the data in Table 2 of [3] yield values for D_p of 90–180 and 160–340 $\mu m^2/s$ through medaka eggs and hepatocytes, respectively. Values of D_p are also obtainable from measurements of the rise in v with a wave's radius of curvature. In *Xenopus* oocytes, this gives a value of 150–300 $\mu m^2/s$ [54].

Fast calcium waves may be initiated by lumenal CICR

It has been suggested that fast calcium waves are generally started by a second form of regenerative calcium release – one which occurs within the lumen of the ER rather than the cytosol [3]. This dual CICR model implies three subsidiary propositions:

1. Calcium is generally pumped into the ER and rises within it in the intervals between periodic waves or in the delays between an external stimulus and wave initiation. Few calcium physiologists seem to doubt that.

Table 2 Velocities of intracellular calcium waves. This updates Table 1 of [3][a].

No.	*Genus*	*Cell(s)*	*Method*	*°C*	*μm/s*	*Ref*
8b	*Lytechinus*	Activating egg	Fluo–3	22.4	13	b
8c	*Lytechinus*	Activating egg	Fluo–3*	18	5–16	[9]
8d	*Lytechinus*	Activating egg	calc green*	16	8	[10]
20a	*Xenopus*	Activating egg	calc green*	24	8	[10]
31g	Rat	Heart myocyte	Fura–2/AM	26–30	116 ± 31	[11]
31h	Cavy	Heart myocyte	Fluo–3*	20	≈93	[12]
37a	Rat	Astrocyte	Fluo–3/AM	21	≈15	[13]
38a	Rat	Brain glial	Fura–2/AM	23–25	33 ± 6	[14]
39a	Rat	Pancreatic acini: whole	Fluo-3/AM*	37	58 ± 10[c]	[15]
39b	Mouse	Pancreatic acinar	Fura–2 AM	25–27	20[d]	[16]
41a	Rat	Hepatocyte couplets	Fluo–3/AM*	37	80 ± 34	[17]
44a	Rat embryo	Vascular myocyte line	Fura–2 or Fura–2/AM	19	16	[18]
43a	Human	Vascular myocyte	Fura–2/AM	37	≈8	[19]
45	Pig	Vascular myocyte	Fura dextran	37	3,5,8[e]	[20]
46	Cavy	Gastric parietal	Fura–2/AM	37	≈30	[21]
47	Shrimp	Activating egg	Fluo–3*	21–23	20 ± 5	[22]
48	Murine	Neuroblastoma	Fluo–3*	28	≈15	f
49	Mouse	Lacrimal	Fluo–3*	25–27	25 ± 3[g]	[16]
50a	*Xenopus*	Oocyte: stage 5	Fluo–3*	19	25 ± 0.8	[23]
50b	*Xenopus*	Oocyte: stage 5	calc green*	21–24	21 ± 1.4[h]	[24]
50c	*Xenopus*	Oocyte: stage 5	calc green dextran*	22–25	23 ± 0.5[h]	[25]
51	Rabbit	Colon myocyte	Fura–2/AM	25	23 ± 3	[26]
52	?	Mast cells	Fura–2/AM	25	5 to 10[i]	[27]

Footnotes : [a]As in that table, all speeds are calculated for a path parallel to the cell surface. Confocal studies are marked with an asterisk. [b]Chambers EL., unpublished. [c]With minimal stimuli of 0.1 μM Ach or 0.5 nM CCK. [d]Stimulated by 0.5 mM Ach (Fig. 2C) or 200 pM CCK (Fig. 2D). [e]3, 5 & 8 μm/s are speeds of the second, third & fifth waves after applying 50 μM ATP. [f]Morielli AD. Thompson S., unpublished. [g]Stimulated by 0.5 mM Ach (Fig. 3B) or 1 mM ATP (Fig 3C). [h]After minimal stimulation. [i]Across a sheet of cells.

2. A rise in lumenal Ca^{2+} tends to open ER Ca^{2+} channels. This proposition has been repeatedly considered over many years [6,44,55–58]. Yet it remains uncertain. It remains so because the available techniques for measuring luminal Ca^{2+} remain so limited.

3. Wave initiation starts with an explosive, autocatalytic rise in free calcium **within the ER's lumen.** The experimental basis for this proposition lies in two remarkable reports by Ikemoto et al. [59,60]. First, they reported that when sarcoplasmic reticulum vesicles are stimulated to release calcium, that luminal calcium rises substantially for about half a second before it falls (Fig. 5 in [60]). Second – and even more remarkable – when such vesicles are broken open with detergent to yield flat, so-called junctional face membranes and then treated with caffeine, there is a massive release of calcium from the calsequestrin-membrane complex (Figs 7–9 in [60]). Since these membrane pieces are flat, the release of free Ca^{2+} cannot result from channel opening; rather it must result from a conformational change within the calsequestrin-membrane complex. This release was observed in response to caffeine or polylysine.

What is being imagined and proposed in the dual CICR model is that this luminal release occurs naturally in response to a slow rise in luminal Ca^{2+}. Whether channel opening occurs as part of the same conformational change which apparently releases luminal Ca^{2+} or whether it occurs in response to that release cannot even be guessed at yet.

One can imagine two tests of the dual CICR model. One is to test its implication that speeding the ER pump should increase the frequency of periodic Ca^{2+} pulses or waves. Since cAMP is know to speed this pump in many systems [61], yet is unknown to affect ER channels, one would expect it to raise pulse or wave frequencies in many systems.

Another is to develop effective means of measuring and imaging luminal Ca^{2+} within living cells. Recent advances in engineering an apoaequorin with reduced affinity for calcium [62] and in transfecting cells with apoaequorin [63] promise to soon make this possible. When it does become possible, one should be able to see luminal Ca^{2+} rise as cytosolic waves start. One should also be able to see whether luminal waves of rising Ca^{2+} accompany (and help propogate?) cytosolic waves. It seems possible that calcium waves are relayed by a conformational change in multimolecular complexes which can be triggered by a rise in calcium on their cytosolic **and/or** luminal ends. The complexity which seems needed to explain the conservation of wave velocity might well lie in such tandem waves.

Various Ca^{2+} release channels can relay fast calcium waves

It has been argued that fast Ca^{2+} waves are generally initiated and relayed by two modes of CICR through Ca^{2+} release channels in the ER. However, this is not to suggest that the molecular machines which are involved have the same modulating switches or 'receptors' in every cell. Indeed, there is growing evidence that some can be modulated by cytosolic IP_3 [10,64] and others by cytosolic cADPR [10] as well as the possibility that some may be modulated by neither IP_3 nor cADPR [65]. Perhaps the conserved machines which account for the conservation of velocity lie at the lumenal rather than the cytosolic ends of the calcium release channels.

Cell-to-cell propagation mechanisms are quite distinct from intracellular ones

The discovery in 1990 of fast calcium waves through **sheets** of cultured astrocytes [66] and airway epithelial cells [67] has exciting consequences for biology and medicine. Older observations of comparably fast contraction waves through normally developing chick embryos [68] as well as fish embryos [69] can now be interpreted as fast calcium waves. While the observations on cultured astrocytes have already led to the visualization of comparable waves in cultured brain slices [13]. Moreover, there is good evidence to suspect that the injury waves thought to underlie migraine attacks [1,3] as well, perhaps, as epileptic seizures (Cornell-Bell et al., unpublished) are all propagated by the same fast multicellular calcium waves which are seen in brain slices. All this makes the mechanisms of intercellular or cell-to-cell propagation of particular interest.

In heart, brain and airway, intercellular propagation is believed to go through gap junctions. Such propagation obviously cannot be mediated by luminal Ca^{2+} diffusion but must be carried – at least in part – by cytosolic Ca^{2+} diffusion. In part, because the cogent arguments against IP_3 feedback deep within cells are less compelling for gap junctions: the plasma membrane source of IP_3 is obviously concentrated there and the limited data do not preclude there being enough delay at gap junctions for waves to be relayed by Ca^{2+} induced IP_3 formation followed by IP_3 induced Ca^{2+} release. Moreover, Boitano et al. have reported that when heparin is electroporated into sheets of cultured airway cells, that intercellular wave movement is blocked and therefore 'suggest that IP_3 moves through gap junctions to communicate intercellular Ca^{2+} waves' [70]. However, heparin could well have acted by blocking IP_3 receptors deep within the airway cells rather than the gap junctions and might even have acted on some other target than the IP_3 receptors (p. 804, [71]).

In certain other groups of cells, however, fast waves seem to go through individual cells and then induce waves in nearby cells which are not connected by gap junctions. Intercellular propagation can surely not occur via calcium diffusion in such cases; for the 3–30 μM levels of wave calcium would be lost in the usual 1 mM calcium moats between cells. So by analogy with chemical synapses, secretion of a variety of intercellular propagators can be expected in such cases. Indeed, in *Dictyostelium*'s aggregation waves (≈5 μm/s at 20°C), there is reason to suspect that fast calcium waves traverse each cell [72] and compelling evidence that these aggregation waves (whatever their intracellular nature) are carried between cells by secreted cAMP [73]. While in cultured mast cells, ATP seems to be the (secreted ?) propagator of 5–10 μm/s waves (at 25°C) [27]. In any case, it is clear that cell-to-cell mechanisms will prove to be far more varied than intracellular ones.

Slow calcium waves

About 8 years ago it was suggested that various slow (0.1–1 μm/s) contractile waves which occur during animal growth and development are driven by slow calcium waves [74]. With the aid of aequorin, the first such calcium waves were recently visualized in the slowly (≈0.5 μm/s) elongating cleavage furrows of the medaka egg [7]. If these furrowing waves travel within the growing, 0.1–0.2 μm thick contractile bands, then they can be estimated to reach a level of 5–8 μM free calcium. Comparable, if less studied, calcium waves accompany furrowing in *Xenopus* eggs [75] and the dramatic inhibitory effects of BAPTA-type calcium buffer injections indicate that the growing zone of high calcium in such furrows is more than a consequence of furrowing. It acts back to initiate, extend and maintain these structures [76].

A number of cogent arguments indicate that these slow waves are propagated by mechanical forces rather than the diffusion of Ca^{2+} or some other small molecule. To be specific, they indicate that the elongating and contracting furrow extends itself by tugging on cables – perhaps microtubular ones – attached to stretch-sensitive calcium channels in the ER or plasma membrane ahead. For one thing, this mechanism explains why furrows grow straight ahead. The pre-existing contractile arc contains aligned actomyosin filaments whose contraction should exert greatest pull straight ahead; while any diffusing propagator should spread out in all directions. For another, there is no reason why mechanical propagation could not be slow enough; however, there is strong reason to doubt that CICR could be: the Luther equation 1 shows that the needed autocatalytic reaction time, t, is proportional to the inverse square of the velocity attained. Since slow waves are the order of 100 times slower than fast ones, the reaction rates would have to be the order of 10 000 times slower or an hour. This is most unlikely; moreover, it is difficult to imagine how a CICR mechanism could maintain calcium at high and undiminished levels for the many minutes observed.

Pioneering studies of Zotin indicated the presence of stretch sensitive calcium channels in the plasma membrane and/or cortical ER of fish eggs [77]. However, absolutely nothing is yet known about this quantitatively. How much distortion at what rate is needed to open Ca^{2+} channels in the peripheral membranes of various cells?

Acknowledgements

I am happy to acknowledge helpful criticism by Antony Galione and financial support by NSF grant #DCB-9103569.

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Received : 28 July 1993
Accepted : 28 July 1993