Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9883–9887, November 1991
Cell Biology

The path of calcium in cytosolic calcium oscillations: A unifying hypothesis

(calcium waves/fertilization/Luther equation/endoplasmic reticulum)

LIONEL F. JAFFE

Marine Biological Laboratory, Woods Hole, MA 02543

Communicated by Daniel Mazia, August 8, 1991

ABSTRACT **Data from 42 systems have been assembled in which the overall spatial course of relatively natural, intracellular calcium pulses has been or can be determined. These include 21 cases of solitary pulses in activating eggs and 21 cases of periodic (as well as solitary) pulses in various fully active cells. In all cases, these pulses prove to be waves of elevated calcium that travel from one pole of a cell to the other or from the periphery inward. The velocities of these waves are remarkably conserved—at ≈10 μm/sec in activating eggs and ≈25 μm/sec in other cells at room temperature. Moreover, in three cases, the data suffice to show that these velocities fit the Luther equation for a reaction/diffusion wave of calcium through the cytosol. It is proposed that (*i*) natural intracellular calcium pulses quite generally take the form of cytosolic calcium waves and (*ii*) cytoplasmically controlled calcium waves are triggered and then propagated by the successive action of two distinct modes of calcium-induced calcium release. First, in the lumenal mode, a slow increase of calcium within the lumen of the endoplasmic reticulum reaches a level that triggers fast lumenal release as well as fast localized release into the cytosol. Then, the well-known cytosolic mode drives a reaction/diffusion wave across or into the cell.**

The study of cytoplasmically controlled calcium oscillations has followed two paths. One path emerged with the discovery that sperm activate medaka fish eggs through a giant calcium wave or calcium tsunami (1). This soon led to evidence that sperm activate all eggs on the vertebrate line in this way (2). The other path emerged with the discovery that increasing concentrations of certain hormones induce increasingly frequent, periodic calcium pulses in isolated hepatocytes (3). This soon led to evidence that agonists generally affect cultured cells in this way (4). Two further findings have begun to bring these paths together. First has been the discovery of periodic postfertilization pulses in hamster eggs and in ascidian eggs (5, 6). Second has been the discovery that both these postfertilization pulses (5, 7) and many periodic calcium pulses in cultured cells (8) take the form of periodic calcium waves. This paper pursues this unification.

The Spatial Character of Calcium Pulses

Table 1 summarizes the rapidly increasing information on the spatial character of relatively natural calcium pulses. In no known case do such pulses occur synchronously throughout a cell. In most cases, they spread from pole to pole; in a few cases, they spread inwardly from the cell's periphery; however, they are never synchronous. In the great majority of cases they are known to spread at the relatively constant speeds characteristic of actively propagated, reaction/diffusion waves rather than the rapidly decreasing speeds characteristic of diffusion.

Conservation of Wave Velocities

Fig. 1 plots the wave speeds listed in Table 1 versus temperature. It includes all of these data except for those from two grossly damaged systems (cases 26 and 33) as well as those from Purkinje neurons (case 35) since this (barely) seen "wave" probably arose from sequential delay in the response to depolarization rather than to true propagation.

These data fall into two well-defined groups: waves through activating eggs and waves through fully active cells. These groups are connected by data from ascidian eggs and from hamster eggs, cases in which wave speeds have been measured both during fertilization and afterwards when the eggs are derepressed and presumably come to resemble other fully active cells. At 19°C ± 4°C—where most data are available—the velocities reported through eggs vary by ≈3-fold, from 5 to 14 μm/sec, while with two exceptions (discussed below) the measured velocities through various fully active cells also vary by ≈3-fold, but over a range from about 15 to 40 μm/sec.

Considering experimental error (and correcting for temperature effects), one can conclude that the whole range of calcium wave speeds across all eggs is ≈2-fold; so is the whole range, or almost the whole range, across all fully active cells. This remarkable constancy holds for organisms from sponges to mammals, for cells with diameters from 20 to 2000 μm, and for natural milieus varying from pond water to seawater. The only data that clearly fall outside of the main range are those for recently isolated heart myocytes (case 31) and those for cells cultured from human arteries through many divisions *in vitro* (case 43). In the former, calcium waves move 2–3 times faster than usual. Perhaps this anomaly is related to the somewhat unnatural character of isolated heart myocytes since the wave velocity through naturally syncytial heart muscle (case 32) does fall within the usual restricted range. In the latter, calcium waves move 3–4 times slower than usual; this too may be related to the rather unnatural character of these laboratory cells.

The Mechanism of Calcium Oscillations

Luther equation test. The calcium-induced calcium release (CICR) model of calcium wave propagation involves essentially planar waves propagated by a single autocatalytic reaction together with the diffusion of Ca^{2+} ions. The velocity of such a wave is given by the Luther equation, a basic equation for reaction/diffusion waves (47, 48)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CICR, calcium-induced calcium release (irrespective of the exact mechanism); ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate.

Table 1. Velocities of intracellular calcium waves

No.	Group	Genus	Cell and wave type	Calcium indicator	Cell width, μm	Pulse duration, t, °C	Pulse duration, sec	Speed, μm/sec	Year	Ref(s).
				Activating eggs*						
1	Sponges	*Tetilla*		Secretion	175	19	35	5	1952	2
2	Echinoderms	*Psammechinus*		Secretion	100	18	13	8	1955	2
3		*S. drobechiansis*		Secretion	160	8	53	3	1964	2
		S. purpuratus		Secretion	75	16	13	6	1971	2
5		*Asterias*		Aequorin	110	18	18	6	1984	9
6		*Arbacia*		Aequorin	73	19	5	14	1984	10
7		*Scaphechinus*		Aequorin	105	23	11	10	1986	11
8		*Lytechinus*		Aequorin	111	16	14	8	1986	12
8a				Fura-2	111	18	10	11	1988	13
9		*Clypeaster*		Fluo-3	123	25	8	16†	1990	14
10	Tunicates	*Phallusia*		Aequorin	140	20	11	13	1990	7
11	Hemichordates	*Saccoglossus*		Secretion	400	23	50	8	1954	2
12	Lampreys	*Lampetra*		Secretion	1600	18	270	6	1947	2
13	Bony fishes	*Perca*		Secretion	800	17	110	7	1953	2
14		*Gasterosteus*		Secretion	960	20	120	8	1953	2
15		*Carassius*		Secretion	1100	21	180	6	1954	2
16		*Hypomedusa*		Secretion	730	21	91	8	1954	2
17		*Pungitius*		Secretion	1000	18	110	9	1956	2
18		*Oryzias*		Secretion and aequorin	1100	10–30		8–18	1978	1, 2
19	Amphibians	*Rana*		Secretion	2000	15	150	13	1971	2
20		*Xenopus*		Aequorin	1300	22	160	8	1987	15
				Electrodes		22	130	10	1985	16
21	Mammals	Hamster		Aequorin	80	31	4	22	1986	5
				Fully active cells‡						
22	Characeae	*Nitella*	Internode *s in d*	Streaming	100	Room	3	20	1979	17
23	Crustaceae	Crayfish	Muscle *s in d*	Contraction	200 × 3000	Room	4	23	1974	18
24	Molluscs	*Helisoma*	Neuron *s*	Fura-2	—	Room	?	18 ± 4	—	§
25	Tunicates	*Phallusia*	Zygote	Aequorin	140	20	6	25	1990	19
26	Amphibians	*Bufo*	Skinned muscle	Contraction	70 × 1500	17	17	90	1975	20
27		*Rana*	Neuron *s in d*	Fura-2	25	22	1	≈15¶	1988	21
28		*Bufo*	Neuron *s*	Fluo-3	40	25	1–2	45 ± 10‖	1990	22
29	Birds	Chicken	Embryo	Contraction	—	37	?	33	1977	23
30	Fish	*Oryzias*	Stage 14 stellate	Contraction	40	Room	—	33 ± 14	1987	24, 25
31a	Mammals	Rabbit	Heart myocyte	Contraction	13 × 130	22	?	60–160	1979	26
31b		Rat	Heart myocyte	Contraction	25 × 90	37	1	90	1984	27
31c			Heart myocyte	Contraction	35 × 125	23–31	1	72–118	1985	28
31d			Heart myocyte	Contraction	?	Room	?	85	1985	29
31e			Heart myocyte	Fura-2	14 × 82	35	0.8	100	1987	30
31f		Cavy	Heart myocyte	Indo-1	30 × 125	22	1.3	100	1990	31
32		Rat	Heart muscle	Contraction	100–400	Room	?	33	1985	28
33			Damaged muscle *d*		100 × 3000	20	—	10^2–10^4	1989	32
34		Hamster	Zygote	Aequorin	78	31	2	50	1986	5
35		Cavy	Purkinje neuron *d*	Fura-2	—	32	2	30	1988	33**
36		Bovine	Chromaffin *s in d*	Fura-2	10	37	≈1	?	1989	34
37		Rat	Astrocyte	Fluo-3	50 × 200	Room	11	19 ± 9	1990	35
38			Mixed glia	Fura-2	20–50	37	?	34	1991	36
39			Pancreatic *d* acinar	Fura-2	12	25	?	16 ± 10	1990	37, 38
40		Human	Endothelial	Fura-2	30	37	0.6	50	1990	39
41		Rat	Hepatocyte	Fura-2	50–80	37	2	23 ± 4††	1990	40
42a		Rabbit	Airway	Ciliary speed	20	25–37	—	20–30	1988	41
42b				Fura-2	25	Room	1	28	1990	42
43		Human	Smooth muscle line *s*	Fura-2	20 × 100	37	100	10 ± 2	1990	43
44		Rat	Muscle A7r5 cell line†† *s in*	Fura-2	40 × 100	37	0.4	54	1991	44

*All of these waves are solitary ones that travel pole-to-pole. This updates a 1983 table (2) but speeds are now all calculated for a path parallel to the cell surface.

†An anomalous value of 3.3 μm/sec at 25°C has also been reported for *Clypeaster*, perhaps due to excessive compression of the eggs (45).

‡These waves are periodic unless marked *s* for solitary, pole-to-pole unless marked *in*, and are not begun by membrane depolarization unless marked *d*.

§Begun by prodding (R. W. Davenport, L. R. Mills, and S. B. Kater, personal communication).

¶From figure 1 *C* and *D* in ref. 21.

‖From figure 3*H*, traces *b–e*, in ref. 22 of a caffeine-stimulated, voltage-clamped cell.

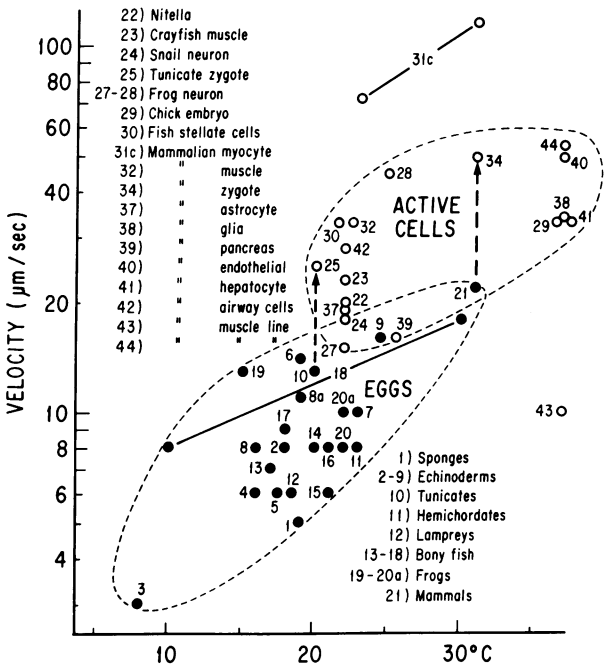

FIG. 1. Velocities of intracellular calcium waves in fertilizing eggs (●) and in fully active cells (○) vs. temperature. Arrows connect data for fertilizing eggs with data for postfertilization waves through these same eggs. See Table 1 for details.

$$v = \alpha\sqrt{D/t_r}, \qquad [1]$$

where v is wave velocity, D is the diffusion constant of the autocatalytic species—here that of free cytosolic Ca^{2+} ions, which is known to be $\approx$600 μm^2/sec (ref. 49, see p. 6610 of ref. 50)—t_r is the reaction time or time taken for Ca^{2+} to rise e-fold, and α is a factor that largely depends on the exact (and in this case unknown) kinetics of the autocatalytic reaction. However, α generally lies between 0.5 and 2, so we can test the Luther equation by somewhat arbitrarily taking α to be 1. Let us then take α to be 1 and D to be 600 μm^2/sec and apply these figures to a recent study of periodic calcium oscillations in guinea pig heart myocytes (Table 2). Then Eq. 1 predicts a speed of $\approx$112 μm/sec, a value in good agreement with the speed of 110 μm/sec found for calcium waves through these same cells. It also accounts quite well for the slower velocities of the solitary fertilization wave through medaka fish eggs as well as the periodic waves through phenylephrine-treated hepatocytes. The success of the Luther equation provides substantial quantitative support for a CICR mechanism of wave propagation.

A general model of cytosolically controlled calcium oscillations. Fig. 2 diagrams the proposed calcium pulse cycle during an internally controlled, periodic oscillation. Between pulses (Fig. 2*a*), calcium is slowly pumped into the lumen of the endoplasmic reticulum (ER). As a pulse starts in a pacemaker region (Fig. 2*b*), high lumenal calcium triggers calcium release, which was bound to calsequestrin, into the lumen and then into the nearby cytosol. The release process then spreads (Fig. 2*c*) via a reaction/diffusion wave driven by cytosolic calcium. Behind the wave front, further release is blocked by the slow effect of an increase in cytosolic calcium as well as the decrease in lumenal calcium. Then pumping continues the cycle.

When pulses are induced by a (deuterostome) sperm (Fig. 2*a'*), calcium flows from the medium through the fused sperm into the local cytosol from which it is slowly pumped into the ER. The lag before a pulse starts corresponds to the interpulse process shown in Fig. 2*a*, and subsequent events are endogenous. When pulses are induced by an agonist, it acts via inositol 1,4,5-trisphosphate (IP_3) to speed calcium flow into the cytosol; this cytosolic calcium is again pumped into the ER, as shown in Fig. 2*a*, and again subsequent events are endogenous. However, when pulses are induced by action potentials (52), each pulse is still a wave, but one triggered by a rapid influx of calcium from the medium rather than the ER.

The idea that cells slowly pump calcium into the ER between calcium pulses is both old and widely supported (53–55). The most convincing evidence for this conclusion is that both in skinned cardiac cells (54) and in various inexcitable cells *in vitro* (3, 53, 56) the frequency of such oscillations is far more variable than their amplitude. Direct evidence for a slow increase of lumenal Ca^{2+} between pulses can be found in Fabiato's work (57, 58). Moreover, leakage from the filling lumen should account for the slow increase in cytosolic Ca^{2+} that is sometimes seen between pulses (59). Arguments for the idea that IP_3 initially releases Ca^{2+} from a second pool have been presented elsewhere (p. 3078 in ref. 4; see also ref. 60); so have arguments for the idea, shown in Fig. 2*a'*, that the sperm is a calcium conduit (61). Cogent qualitative arguments for the idea shown in Fig. 2*c* of CICR propagation goes back to the first visualized calcium wave (1). Quantitative support for this idea was then provided by Gilkey's buffer injection experiments (62) and now by application of the Luther equation. Arguments against more complex, alternative views—involving IP_3 oscillations—are discussed below. What needs detailed consideration in this section is the idea, shown in Fig. 2*b*, of a second, lumenal mode of CICR.

The most compelling argument for a luminal trigger is the fact that the release channels in cardiac cells can only be opened by increases in cytosolic calcium that are far faster than actually occur between pulses in various cells. To trigger luminal Ca^{2+} release in canine cardiac cells, cytosolic calcium concentrations must double in the order of a few milliseconds or less (figure 12 in ref. 63). Yet, in histamine-stimulated human endothelial cells, for example, it takes $\approx$30 sec for cytosolic calcium concentrations to double between pulses (59). Thus, cytosolic calcium increases on the order of 10,000 times more slowly than the minimal rate needed to induce its own release in cardiac cells. Another cogent argument against a cytosolic trigger in cardiac cells is the remarkable observation that under certain circumstances the amplitude of a pulse can be enhanced (if not initiated) by lowering cytosolic calcium (29). Also, the ineffectiveness of slow increases in cytosolic calcium and the paradoxical enhancement by a decrease can both be understood as reflecting a slowly acting but powerful inhibition of calcium release by cytosolic calcium (64, 65). Moreover, ER vesicles isolated from brain synaptosomes exhibit very similar responses to calcium changes (66). These reflect a dual action of cytosolic calcium on these neuronal structures that is remarkably similar to its action on cardiac ER.

Several observations support the large, initial brief increase in lumenal calcium suggested by Fig. 2 and thus

Table 1. (*Continued*)

**From figure 2 *B* and *C* in ref. 33.

††This speed is for cells stimulated with 2 nM vasopressin, but it scarcely differs in cells stimulated with 2–10 μM phenylephrine or even by 50 mM NaF plus 10 μM $AlCl_3$ (40) or 200 μM *t*-butylhydroperoxide (46).

‡‡This is a noncontractile cell line from vascular smooth muscle. The speed listed is a longitudinal one from figure 6 of ref. 44.

further support a lumenal CICR trigger. First, early studies of skinned cardiac cells with both chlorotetracycline and several potential-sensitive dyes indicated such an increase (57, 58). Second, recent studies of intact ER vesicles isolated from striated muscle and observed with tetramethylmurexide directly show such an increase under some conditions (figure 5 in ref. 67; see also ref. 68). Third, observations of such vesicles, which are broken open with detergent (the so-called junctional face membrane), show a massive release of bound calcium in response to caffeine (figures 7–9 in ref. 67). Since this release requires a binding of calsequestrin to the inner faces of the opened vesicles, it presumably comes from the calsequestrin as this lumenal protein undergoes a sharp conformational change. One can easily imagine that overloading the calsequestrin–membrane complex with lumenal calcium could trigger this release.

One interesting question raised by this line of thought is whether calcium waves are propagated by calcium diffusion within the ER network instead of (or in addition to) calcium diffusion within the cytosol. In systems with ER networks, which are pulled out along one axis, lumenal propagation should be indicated by higher wave speeds along this axis. There may even be a hint of such anisotropy in figure 6 of ref. 44.

Alternative oscillation models. Several alternative models require IP_3 oscillations (reviewed in refs. 4 and 8). Such models do not easily account for waves that move inward away from the plasma membrane in *Nitella*, crayfish muscle, etc., or for oscillations and waves in skinned muscle cells (20, 69), since IP_3 is thought to be exclusively generated by the plasma membrane. Nor do they easily account for recent findings on oscillations in pancreatic acinar cells (38), chromaffin cells (70), and hepatocytes (71). In pancreatic cells, one difficulty is that calcium oscillations persist despite the presence of up to 50 μM inositol trisphosphorothioate, an effective but stable analog of IP_3 (38); another difficulty is that intracellular infusion of heparin, an inhibitor of IP_3 receptors, fails to inhibit calcium oscillations caused by Ca^{2+} infusion but blocks oscillations evoked by acetylcholine or IP_3 itself (72). In chromaffin cells, the problem is that pretreatment with neomycin, a drug that blocks IP_3 generation, fails to inhibit spontaneous or caffeine-evoked oscillations although it blocks agonist-evoked ones (70). In hepatocytes, the difficulty is that pretreatment with *t*-butylhydroperoxide induces typical, periodic 25-μm/sec calcium waves without inducing formation of any inositol polyphosphate (71).

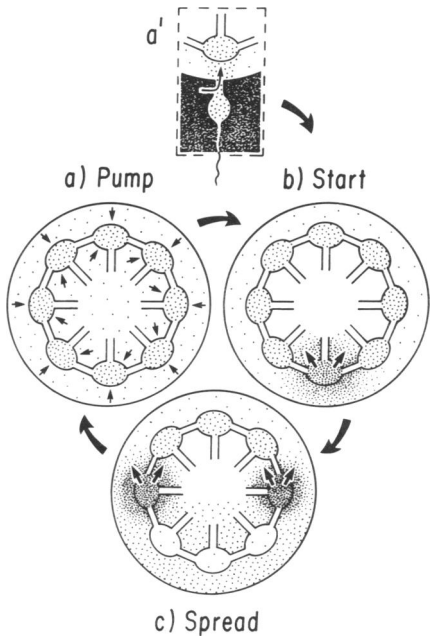

FIG. 2. A proposed mechanism for endogenous calcium oscillations in cells. (*a*) Between (or before) pulses, calcium is slowly pumped into the lumen of the ER. (*a'*) In fertilization, this calcium comes from the medium via the fused sperm. (*b*) Somewhere in the lumen of the ER, calcium that is bound to calsequestrin reaches a level at which it triggers its own sudden release into the lumen and into the cytosol. (*c*) This cytosolic calcium then diffuses to a nearby region where further calcium release is induced. This chain reaction spreads to give a calcium wave and an overall calcium pulse. This pump/release cycle is then repeated. Note that this model involves the alternation of two modes of regenerative calcium release. The mode triggered by cytosolic calcium (shown in *c*) was invoked earlier to explain propagation (1). The lumenal mode (shown in *b*) is now invoked to explain pulse initiation.

Wave Function

The advantages of a so-called digital calcium control system, which acts to change pulse frequency rather than pulse size, have been discussed elsewhere (p. 3080 in ref. 4). Here those functions of calcium pulses that derive from their being waves are briefly discussed. The most obvious function of calcium waves is to carry calcium signals deep into individual cells, into the whole cytosol, into the lumen of the ER network, and into the nucleus (22). Moreover, there is growing evidence that typical calcium waves (and thus calcium signals) often spread across large groups of similar contiguous cells (cases 29, 30, 37, and 42). Thus, in the remarkable case of cultured hippocampal astrocytes, calcium waves are directly seen to travel across many confluent cells with about the same 19-μm/sec speed that they cross each astrocyte (case 37; see ref. 35); moreover, similar waves can even be seen in 400-μm-thick hippocampal slices (73) and probably underlie Leão's spreading depression (74–76). Similarly, calcium waves are directly seen to cross large groups of cultured confluent airway cells, crossing each cell at ≈28 μm/sec with only a small delay between cells (case 42b; see ref. 42). These airway waves apparently serve to spread and coordinate increases in ciliary beat frequency from points of local mechanical disturbance (41).

This paper argues that the mechanism of calcium oscillations has been highly conserved; however, evolution is an

Table 2. Applying the Luther equation (Eq. 1) (with $\alpha = 1$) to observed calcium wave velocities

Cell	Cell width, μm	*t*, °C	Rise time, sec (ref.)	Eq. 1, μm/sec	Wave velocity observed (ref.)
Medaka egg	1100	20	2.5–5 (51)*	11–16	12 (1)
Rat hepatocyte	50–80	37	1.2–2.6 (40)†	17–23	23 ± 4 (40)‡
Cavy heart myocyte	30 × 125	22	0.54 ± 0.016 (31)§	112 ± 18	110 (31)¶

*Obtained by multiplying the published 1–2 sec time for aequorin luminescence to rise *e*-fold by 2.5, since this luminescence rises with about the 2.5 power of calcium concentration.
†From figure 1 *B–D* in ref. 40.
‡From table 1 in ref. 40.
§Waves 1, 2, 4, 7, and 8 in ref. 31.
¶From figure 2 in ref. 31.

opportunist and their functions should go beyond communication and prove to be highly diverse. Thus, the somewhat attenuated postfertilization waves in *Phallusia* eggs are equivalent to an oscillating gradient of calcium with calcium periodically increased at the vegetal pole (19). Perhaps they serve the purpose of pattern development. Yet another possibility is one of transport. Kasai and Augustine (37) have put forward an attractive "push–pull" model whereby calcium waves through the exocrine cells of the pancreas act to pump out chloride and then fluid.

Note Added in Proof. For case 31, see ref. 77.

I thank the National Science Foundation for financial support and Anthony Galione for his helpful discussion.

1. Gilkey, J. C., Jaffe, L. F., Ridgway, E. B. & Reynolds, G. T. (1978) *J. Cell Biol.* **76,** 448–466.
2. Jaffe, L. F. (1983) *Dev. Biol.* **99,** 265–276.
3. Woods, N. M., Cuthbertson, K. S. R. & Cobbold, P. H. (1986) *Nature (London)* **139,** 600–602.
4. Berridge, M. J. & Galione, A. (1988) *FASEB J.* **2,** 3074–3082.
5. Miyazaki, S.-I., Hashimoto, N., Yoshimoto, Y., Kishimoto, T., Igusa, Y. & Hiramoto, Y. (1986) *Dev. Biol.* **118,** 259–267.
6. Speksnijder, J. E., Corson, D. W., Sardet, C. & Jaffe, L. F. (1989) *Dev. Biol.* **135,** 182–190.
7. Speksnijder, J. E., Sardet, C. & Jaffe, L. F. (1990) *J. Cell Biol.* **110,** 1589–1598.
8. Rooney, T. A. & Thomas, A. P. (1991) *Pharmacol. Ther.* **49,** 223–237.
9. Eisen, A. & Reynolds, G. T. (1984) *J. Cell Biol.* **99,** 1878–1882.
10. Eisen, A., Kiehart, D. P., Wieland, S. J. & Reynolds, G. T. (1984) *J. Cell Biol.* **99,** 1647–1654.
11. Yoshimoto, Y., Iwamatsu, T., Hirano, K.-I. & Hiramoto, Y. (1986) *Dev. Growth Differ.* **28,** 583–596.
12. Swann, K. & Whitaker, M. (1986) *J. Cell Biol.* **103,** 2333–2342.
13. Hafner, M., Petzelt, C., Nobiling, R., Pawley, J. B., Kramp, D. & Schatten, G. (1988) *Cell Motil. Cytoskel.* **9,** 271–277.
14. Hamaguchi, Y. & Hamaguchi, M. S. (1990) *Cell Struct. Funct.* **15,** 159–162.
15. Kubota, H. Y., Yoshimoto, Y., Yoneda, M. & Hiramoto, Y. (1987) *Dev. Biol.* **119,** 129–136.
16. Busa, W. & Nuccitelli, R. (1985) *J. Cell Biol.* **100,** 1325–1329.
17. Hayama, T., Shimmen, T. & Tazawa, M. (1979) *Protoplasma* **99,** 305–321.
18. Reuben, J. P., Brandt, P. W. & Grundfest, H. (1974) *J. Mechanochem. Cell Motil.* **2,** 269–285.
19. Speksnijder, J. E., Sardet, C. & Jaffe, L. F. (1990) *Dev. Biol.* **142,** 246–249.
20. Natori, R. (1975) *Jpn. J. Physiol.* **25,** 51–63.
21. Lipscombe, D., Madison, D. V., Poenie, M., Reuter, H., Tsien, R. W. & Tsien, R. Y. (1988) *Neuron* **1,** 355–365.
22. Hernandez-Cruz, A., Sala, F. & Adams, P. R. (1990) *Science* **247,** 858–862.
23. Stern, C. & Goodwin, B. (1977) *J. Embryol. Exp. Morphol.* **41,** 15–22.
24. Barber, B., da Cruz, M. J. B., de Leon, J., Fluck, R. A., Hasenfeld, M. P. & Unis, L. A. (1987) *J. Exp. Zool.* **242,** 35–42.
25. Cope, J., Fluck, R., Nicklas, L., Plumhoff, L. A. & Sincock, S. (1990) *J. Exp. Zool.* **254,** 270–275.
26. Rieser, G., Sabbadini, R., Paolini, P., Fry, M. & Inesi, G. (1979) *Am. J. Physiol.* **236,** C70–C77.
27. Cobbold, P. H. & Bourne, P. K. (1984) *Nature (London)* **312,** 444–446.
28. Kort, A. A., Capogrossi, M. C. & Lakatta, E. G. (1985) *Circ. Res.* **57,** 844–855.
29. Fabiato, A. (1985) *Basic Res. Cardiol.* **80** (Suppl. 2), 83–88.
30. Wier, W. G., Cannell, M. B., Berlin, J. R., Marban, E. & Lederer, W. J. (1987) *Science* **235,** 325–328.
31. Takamatsu, T. & Wier, W. G. (1990) *FASEB J.* **4,** 1519–1525.
32. Mulder, B. J. M., de Tombe, P. P. & ter Keurs, H. E. D. J. (1989) *J. Gen. Physiol.* **93,** 943–961.
33. Tank, J. W., Sugimori, M., Connor, J. A. & Llinas, R. R. (1988) *Science* **242,** 773–777.
34. O'Sullivan, A. J., Cheek, T. R., Moreton, R. B., Berridge, M. J. & Burgoyne, R. D. (1989) *EMBO J.* **8,** 401–411.
35. Cornell-Bell, A. H., Finkbeiner, S. M., Cooper, M. S. & Smith, S. J. (1990) *Science* **247,** 470–473.
36. Charles, A. C., Merrill, J. E., Dirksen, E. R. & Sanderson, M. J. (1991) *Neuron* **6,** 983–992.
37. Kasai, H. & Augustine, G. G. (1990) *Nature (London)* **348,** 735–738.
38. Wakui, M., Potter, B. V. L. & Petersen, O. H. (1989) *Nature (London)* **339,** 317–320.
39. Jacob, R. (1990) *Cell Calcium* **11,** 241–249.
40. Rooney, A. T., Sass, E. J. & Thomas, A. P. (1990) *J. Biol. Chem.* **265,** 10792–10796.
41. Sanderson, M. J., Chow, I. & Dirksen, E. R. (1988) *Am. J. Physiol.* **254,** C63–C74.
42. Sanderson, M. J., Charles, A. C. & Dirksen, E. R. (1990) *Cell Regul.* **1,** 585–596.
43. Neylon, C. B., Heyland, J., Mason, W. T. & Irvine, R. F. (1990) *Am. J. Physiol.* **259,** C675–C686.
44. Wier, W. G. & Blatter, L. A. (1991) *Cell Calcium* **12,** 241–254.
45. Mohri, T. & Hamaguchi, Y. (1990) *Cell Struct. Funct.* **15,** 309–315.
46. Rooney, A. T., Renard, D. C., Sass, E. J. & Thomas, A. P. (1991) *J. Biol. Chem.* **266,** 12272–12282.
47. Luther, R. (1906) *Z. Elektrochemie* **12,** 596.
48. Arnold, R., Showalter, K. & Tyson, J. J. (1987) *J. Chem. Educ.* **64,** 740–742.
49. Donahue, B. S. & Abercrombie, R. F. (1987) *Cell Calcium* **8,** 437–448.
50. Speksnijder, J. E., Miller, A. L., Weisenseel, M. H., Chen, T.-H. & Jaffe, L. F. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6607–6611.
51. Ridgway, E. B., Gilkey, J. C. & Jaffe, L. F. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 623–627.
52. Schlegel, W., Winiger, B. P., Mollard, P., Vacher, P., Wuarin, F., Zahnd, G. R., Wollheim, C. B. & Dufy, B. (1987) *Nature (London)* **329,** 719–721.
53. Berridge, M. J. & Irvine, R. F. (1989) *Nature (London)* **341,** 197–205.
54. Fabiato, A. & Fabiato, F. (1975) *J. Physiol. (London)* **249,** 469–495.
55. Fabiato, A. (1985) *J. Gen. Physiol.* **85,** 291–320.
56. Rooney, T. A., Sass, E. J. & Thomas, A. P. (1989) *J. Biol. Chem.* **264,** 17131–17141.
57. Fabiato, A. & Fabiato, F. (1979) *Nature (London)* **281,** 146–148.
58. Fabiato, A. (1982) *Can. J. Physiol. Pharmacol.* **60,** 556–567.
59. Jacob, R., Merritt, J. E., Hallam, T. J. & Rink, T. J. (1988) *Nature (London)* **335,** 40–45.
60. Bird, G. S. J., Rossier, M. F., Hughes, A. R., Shears, S. B., Armstrong, D. L. & Putney, J. W., Jr. (1991) *Nature (London)* **352,** 162–165.
61. Jaffe, L. F. (1990) in *Mechanisms of Fertilization*, ed. Dale, B. (Springer, Berlin), pp. 389–417.
62. Gilkey, J. C. (1983) *J. Cell Biol.* **97,** 669–678.
63. Fabiato, A. (1985) *J. Gen. Physiol.* **85,** 247–289.
64. Fabiato, A. (1983) *Am. J. Physiol.* **245,** C1–C14.
65. Parker, I. & Ivorra, I. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 260–264.
66. Finch, E. A., Turner, T. J. & Goldin, S. M. (1991) *Science* **252,** 443–446.
67. Ikemoto, N., Antonia, B., Kang, J. J., Meszaros, L. G. & Ronjat, M. (1991) *Biochemistry* **30,** 5230–5237.
68. Ikemoto, N., Ronjat, M. & Meszaros, L. G. (1989) *J. Bioenerg. Biomembr.* **21,** 247–265.
69. Fabiato, A. & Fabiato, F. (1978) *J. Gen. Physiol.* **72,** 667–699.
70. Malgaroli, A., Fesce, R. & Meldolesi, J. (1990) *J. Biol. Chem.* **265,** 3005–3008.
71. Rooney, A. T., Renard, D. C., Sass, E. J. & Thomas, A. P. (1991) *J. Biol. Chem.* **266,** 12272–12282.
72. Wakui, M., Osipchuk, Y. V. & Petersen, O. H. (1990) *Cell* **63,** 1025–1032.
73. Dani, J. W., Cherjavsky, A. & Smith, S. J. (1990) *Soc. Neurosci. Abstr.* **16,** 970.
74. Lauritzen, M. (1987) *Trends Neurosci.* **10,** 8–13.
75. Leibowitz, D. H. (1990) *Soc. Neurosci. Abstr.* **16,** 970.
76. Cornell-Bell, A. H. & Finkbeiner, S. M. (1991) *Cell Calcium* **12,** 185–204.
77. Tirri, R. & Talo, A. (1989) *J. Therm. Biol.* **14,** 63–66.