

# Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external $\text{Ca}^{2+}$

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Micro-injection of submicromolar concentrations of inositol 1,3,4,5-tetrakisphosphate caused a raising of the fertilization envelope in eggs of the sea urchin *Lytechinus variegatus*. This effect was dependent both on the presence of extracellular  $\text{Ca}^{2+}$  and on co-injection with a  $\text{Ca}^{2+}$ -mobilizing compound, inositol 2,4,5-trisphosphate. Inositol 1,3,4,5-tetrakisphosphate was the most potent compound tested in this assay; removal of the 3- or 5-phosphates or randomization of the phosphates in the inositol ring decreased its potency. These results show that inositol 1,3,4,5-tetrakisphosphate is an intracellular second messenger, and suggest that its function is to control cellular  $\text{Ca}^{2+}$  homeostasis at the plasma membrane.

## INTRODUCTION

Recent advances in our understanding of the role of inositides in cell signalling has led to the generally accepted hypothesis that stimulated phosphodiesteratic hydrolysis of phosphatidylinositol 4,5-bisphosphate occurs after receptor activation, giving rise to two intracellular messengers, diacylglycerol (Nishizuka, 1984) and  $\text{Ins}(1,4,5)\text{P}_3$  (Berridge & Irvine, 1984). The function of the latter compound is to mobilize  $\text{Ca}^{2+}$  from intracellular stores, probably in the endoplasmic reticulum. However,  $\text{Ca}^{2+}$  stores inside the cell are limited, so  $\text{Ca}^{2+}$  mobilization is short-lived. When cells are stimulated by agonists that are coupled to inositide turnover, after the initial pulse of  $\text{Ca}^{2+}$  mobilization a prolonged raised level of  $\text{Ca}^{2+}$  can be seen which is entirely dependent on extracellular  $\text{Ca}^{2+}$  (e.g. Putney, 1978; Joseph *et al.*, 1985). This shows that a change in plasma membrane  $\text{Ca}^{2+}$  transport must also occur coincident with (and perhaps controlled by) inositide metabolism, as was first suggested by Michell (1975).

The mediator of this stimulated  $\text{Ca}^{2+}$  entry (which was directly demonstrated by, for example, Mauger *et al.*, 1984) is not known, although Putney (1986) has hypothesized that it might be  $\text{Ins}(1,4,5)\text{P}_3$  acting on an intracellular  $\text{Ca}^{2+}$  pool with direct access to the extracellular space. Alternative candidates for controlling  $\text{Ca}^{2+}$  homeostasis at the plasma membrane have emerged with the discovery of two novel inositol phosphates which are formed on cell stimulation,  $\text{Ins}(1,3,4)\text{P}_3$  (Irvine *et al.*, 1984a, 1985; Burgess *et al.*, 1985) and its precursor  $\text{Ins}(1,3,4,5)\text{P}_4$  (Batty *et al.*, 1985). The latter compound in particular, with its rapid rise after cell stimulation (Batty *et al.*, 1985; Heslop *et al.*, 1985; Hawkins *et al.*, 1986), and equally rapid decrease after receptor-blocking (Hawkins *et al.*, 1986), is a prime candidate for a role in controlling  $\text{Ca}^{2+}$  homeostasis (Batty *et al.*, 1985; Irvine, 1986).  $\text{Ins}(1,3,4,5)\text{P}_4$  has been shown to be completely inactive in mobilizing  $\text{Ca}^{2+}$  from intracellular stores in Swiss mouse 3T3 cells (Irvine *et al.*, 1986a), RINm5F insulinoma cells (Wollheim & Biden, 1986), *Limulus* photoreceptors (J. E. Brown & R. F.

Irvine, unpublished work) and *Xenopus* oocytes (W. Busa, R. Nucitelli, M. J. Berridge & R. F. Irvine, unpublished work).

A possible way of testing directly the hypothesis that  $\text{Ins}(1,3,4,5)\text{P}_4$  can control  $\text{Ca}^{2+}$  entry through the plasma membrane is by micro-injection into eggs.  $\text{Ins}(1,4,5)\text{P}_3$  can activate sea urchin eggs (Whittaker & Irvine, 1984; Turner *et al.*, 1986; Slack *et al.*, 1986), and this  $\text{Ins}(1,4,5)\text{P}_3$ -induced activation has recently been shown to depend on extracellular  $\text{Ca}^{2+}$  (Slack *et al.*, 1986). Slack *et al.* (1986) demonstrated two distinct responses in membrane potential generated by micro-injection of  $\text{Ins}(1,4,5)\text{P}_3$ : an external  $\text{Ca}^{2+}$ -independent depolarization, and an external  $\text{Ca}^{2+}$ -dependent prolonged hyperpolarization which is presumably associated with the raising of the fertilization envelope.  $\text{Ins}(1,3,4,5)\text{P}_4$  is now known to be formed from  $\text{Ins}(1,4,5)\text{P}_3$  by a specific  $\text{Ins}(1,4,5)\text{P}_3$  3-kinase (Irvine *et al.*, 1986b; Hawkins *et al.*, 1986; Stewart *et al.*, 1986; Hansen *et al.*, 1986; Biden & Wollheim, 1986) and this discovery opens up the possibility that  $\text{Ins}(1,4,5)\text{P}_3$  may not activate sea urchin eggs directly by  $\text{Ca}^{2+}$  mobilization, but at least in part indirectly by being phosphorylated to  $\text{Ins}(1,3,4,5)\text{P}_4$ , which in turn stimulates  $\text{Ca}^{2+}$  entry. We report here experimental evidence consistent with this suggestion.

## MATERIALS AND METHODS

### Inositol phosphates

$\text{Ins}(2,4,5)\text{P}_3$  was prepared as in Irvine *et al.* (1984b).  $\text{Ins}(1,4,5)\text{P}_3$ ,  $\text{Ins}(1,3,4)\text{P}_3$  and  $\text{Ins}(1,3,4,5)\text{P}_4$  were prepared as in Irvine *et al.* (1986a) and the latter two compounds were further purified by h.p.l.c. so that their  $\text{Ins}(1,4,5)\text{P}_3$  content was less than 0.1%.

A *myo*-inositol tetrakisphosphate preparation was also made by partial acid hydrolysis of phytic acid and isolation of the  $\text{InsP}_4$  fraction by ionophoresis (Seiffert & Agranoff, 1965; Batty *et al.*, 1985). The isomer content of this  $\text{InsP}_4$  is unknown. From a purely random hydrolysis one would expect about 6% to be  $\text{Ins}(1,3,4,5)\text{P}_4$ ; however, in view of the predominance of

Abbreviations used:  $\text{InsP}_3$  and  $\text{InsP}_4$ , inositol trisphosphate and tetrakisphosphate respectively; locants are given in parentheses, e.g.  $\text{Ins}(1,2\text{cyc}4,5)\text{P}_3$  is inositol 1,2-cyclic 4,5-trisphosphate.

inositol phosphates with a 2-phosphate resulting from acid hydrolysis of phytic acid (e.g. Pizer & Ballou, 1959), the actual  $\text{Ins}(1,3,4,5)P_4$  content is probably less than that. This  $\text{Ins}P_4$  is designated  $\text{Ins}(w,x,y,z)P_4$ .

### Injection of eggs and assessment of egg activation

Eggs of *Lytechinus variegatus* in artificial sea water [(430 mM-NaCl, 10 mM-KCl, 25 mM-MgCl<sub>2</sub>, 25 mM-MgSO<sub>4</sub>, 2 mM-NaHCO<sub>3</sub>, 10 mM-CaCl<sub>2</sub>, 10 mM-Hepes/NaOH (pH 8.0)] were held in an apparatus very similar to that described by Turner *et al.* (1986), and were injected with inositol phosphate solutions made up in 0.5 M-KCl/0.1 mM-EGTA (Whittaker & Irvine, 1984). Quantification of the amount of solution injected was by measurement of the diameter of an oil droplet injected at the same time (see Turner *et al.*, 1986). Egg activation was defined as the raising of a distinct fertilization envelope (e.g. Whittaker & Irvine, 1984; Turner *et al.*, 1986). Ten to twenty eggs were injected with the solution under trial, and the number of eggs showing a full fertilization envelope was counted; partially-activated eggs were excluded. Each batch of eggs was routinely tested for its ability to respond to  $10^{-16}$  mol of  $\text{Ins}(1,4,5)P_3$  or  $10^{-17}$  mol of  $\text{Ins}(1,3,4,5)P_4$  [co-injected with  $\text{Ins}(2,4,5)P_3$ ; see below]. If a batch of eggs did not show a 100% response it was disregarded. All data are representative of a number of trials with different batches of eggs, and wherever two compounds are described in the text as being compared, the comparison was done at the same time on a single batch of eggs. Over many experiments we found some variation in the sensitivity of eggs to inositol phosphates, depending on the animal from which they were obtained and the time which had elapsed since they were taken out of the animals. Although these differences were to some extent corrected for by standardization with  $\text{Ins}(1,4,5)P_3$  or  $\text{Ins}(1,3,4,5)P_4$  (see above), because of them the assays for biological potency can only be regarded as semi-quantitative; in most instances the differences in potencies of inositol phosphates were of at least an order of magnitude, and so any relative activities recorded below are quantifiable, and were reproducible between batches of eggs of differing sensitivity.

## RESULTS AND DISCUSSION

### Effect of $\text{Ins}(1,4,5)P_3$

Preliminary trials injecting  $\text{Ins}(1,4,5)P_3$  into eggs of *Lytechinus variegatus* showed that they behaved in our hands just as in others' experiments with the same species (Turner *et al.*, 1986) or other species (Whittaker & Irvine, 1984; Slack *et al.*, 1986), i.e. microinjection of about  $10^{-17}$  mol of  $\text{Ins}(1,4,5)P_3$  (e.g. 10 pl of 1  $\mu\text{M}$ ) would fully activate 100% of the eggs. We also confirmed the results of Slack *et al.* (1986) in that if EGTA (2 mM) was substituted for  $\text{Ca}^{2+}$  in the artificial sea water, no (or only partial) activation occurred, even with  $10^{-16}$  mol of  $\text{Ins}(1,4,5)P_3$ . This effect was readily reversible by restoring the  $\text{Ca}^{2+}$  to the eggs. The practicalities of egg micro-injection prevented us from investigating in detail the time course of this EGTA effect, but less than 5 min without  $\text{Ca}^{2+}$  would abolish activation by  $\text{Ins}(1,4,5)P_3$  in 90% of eggs, and a similar time of re-incubation in  $\text{Ca}^{2+}$  (after 30 min in EGTA) would restore it. This effect of EGTA was not instantaneous, and that is probably

because the EGTA has to decrease  $\text{Ca}^{2+}$  in the perivitelline space before  $\text{Ca}^{2+}$  entry through the plasma membrane is affected. Slack *et al.* (1986) observed that long after EGTA had abolished the raising of fertilization envelopes by  $\text{Ins}(1,4,5)P_3$ , the earliest responses in membrane potential to  $\text{Ins}(1,4,5)P_3$  (presumably caused by intracellular  $\text{Ca}^{2+}$  mobilization) were entirely unaffected. Thus in their hands even prolonged incubation with EGTA was not depleting intracellular stores, but was only preventing  $\text{Ca}^{2+}$  entry through the plasma membrane; therefore, as  $\text{Ca}^{2+}$  mediates the whole fertilization membrane response (Steinhardt & Epel, 1974; Steinhardt *et al.*, 1977), there is a very strong implication that stimulated  $\text{Ca}^{2+}$  entry through the plasma membrane is caused directly or indirectly by  $\text{Ins}(1,4,5)P_3$ , and is a prerequisite of the activation mechanism (see also Putney, 1986). We assume, though we cannot prove in these experiments, that the same applies here.

### Effect of $\text{Ins}(2,4,5)P_3$ and $\text{Ins}(1,3,4)P_3$

When we injected eggs of *Lytechinus variegatus* [which respond rapidly and fully to  $5 \times 10^{-17}$  mol of  $\text{Ins}(1,4,5)P_3$ ] with various doses of  $\text{Ins}(2,4,5)P_3$  or  $\text{Ins}(1,3,4)P_3$ , there was very little response. At extremely high doses ( $10^{-14}$  mol; 20 pl of 0.5 mM) 20% of eggs raised envelopes in response to  $\text{Ins}(2,4,5)P_3$ , and at a similar high dose of  $\text{Ins}(1,3,4)P_3$  a morphologically different (and very small) envelope was raised around some eggs, but no full envelopes were observed. The overall potency of  $\text{Ins}(2,4,5)P_3$  was at least three orders of magnitude less than  $\text{Ins}(1,4,5)P_3$ , and  $\text{Ins}(1,3,4)P_3$  was therefore even less effective.

These results were very surprising, as  $\text{Ins}(2,4,5)P_3$  is known to be an efficient  $\text{Ca}^{2+}$  mobilizer with a potency about 6-fold less than  $\text{Ins}(1,4,5)P_3$  in many vertebrate systems, e.g. permeabilized Swiss mouse 3T3 cells (Irvine *et al.*, 1984b), guinea-pig hepatocytes (Burgess *et al.*, 1984) or mouse pancreatic acinar cells (H. Streb, R. F. Irvine, M. J. Berridge & I. Schulz, unpublished work), and also on washed rat liver microsomes (Dawson & Irvine, 1984). Also,  $\text{Ins}(2,4,5)P_3$  has been shown to compete with  $\text{Ins}(1,4,5)P_3$  binding in permeabilized mammalian cells (Spät *et al.*, 1986). Furthermore, in invertebrate photoreceptors it is virtually indistinguishable from  $\text{Ins}(1,4,5)P_3$  in inducing depolarization and adaptation (Brown *et al.*, 1984; Fein *et al.*, 1984), both of which are known to be mediated by  $\text{Ca}^{2+}$  mobilization (Rubin & Brown, 1985; Payne *et al.*, 1986).  $\text{Ins}(1,3,4)P_3$  will also mobilize  $\text{Ca}^{2+}$  in Swiss mouse 3T3 cells (Irvine *et al.*, 1986a), RIN-insulinoma cells (Wollheim & Biden, 1986) or *Xenopus* oocytes (W. Busa, R. Nucitelli, M. J. Berridge & R. F. Irvine, unpublished work) albeit at a 20-fold higher dose than  $\text{Ins}(1,4,5)P_3$ . Pressure injection of  $\text{Ins}(1,3,4)P_3$  mobilizes  $\text{Ca}^{2+}$  in invertebrate photoreceptors with a potency similar to that of  $\text{Ins}(1,4,5)P_3$  (J. E. Brown & R. F. Irvine, unpublished work). Sea urchin eggs have also been reported to be unresponsive to a third inositol trisphosphate,  $\text{Ins}(1,2\text{cyc}4,5)P_3$  (M. Whittaker & K. A. Swann, personal communication) which has calcium-mobilizing properties very similar to those of  $\text{Ins}(1,4,5)P_3$  in both vertebrate and invertebrate tissues (Wilson *et al.*, 1985; Irvine *et al.*, 1986a).

Given the wide range of both vertebrate and invertebrate tissues in which  $\text{Ins}(2,4,5)P_3$ ,  $\text{Ins}(1,3,4)P_3$  and  $\text{Ins}(1,2\text{cyc}4,5)P_3$  will mobilize  $\text{Ca}^{2+}$ , it therefore

seems extraordinary that they are so ineffective in activating sea urchin eggs. The most likely explanation is that, although they will indeed mobilize Ca<sup>2+</sup> [i.e. they do induce the Ca<sup>2+</sup>-independent phase recorded by Slack *et al.* (1986)], they do not induce the subsequent Ca<sup>2+</sup> entry. Ins(1,4,5)P<sub>3</sub> is clearly capable of doing both, and the possible explanation for this, which we explore here, is that only Ins(1,4,5)P<sub>3</sub> can be phosphorylated to Ins(1,3,4,5)P<sub>4</sub> which in turn mediates the Ca<sup>2+</sup> entry.

In view of the remarkable 3-hydroxyl specificity of Ins(1,4,5)P<sub>3</sub> 3-kinase (Irvine *et al.*, 1986a), Ins(1,3,4)P<sub>3</sub> will be a very poor substrate for that enzyme. Furthermore, although Ins(1,2cyc4,5)P<sub>3</sub> may serve as a substrate for Ins(1,4,5)P<sub>3</sub> 3-kinase (Irvine *et al.*, 1986b), subsequent studies by the laboratories of Downes (Hawkins *et al.*, 1987) and Majerus (Connolly *et al.*, 1987) have shown that it is also a poor substrate, with a K<sub>m</sub> at least 100 times higher than that for Ins(1,4,5)P<sub>3</sub>. Ins(2,4,5)P<sub>3</sub> is therefore likely to be an even poorer substrate for rat brain InsP<sub>3</sub> kinase, and we have confirmed this by comparing it directly with Ins(1,4,5)P<sub>3</sub> (both substrates at 100 μM) using [γ-<sup>32</sup>P]ATP, under conditions described by Irvine *et al.* (1986b), and analysing the products by h.p.l.c. Even though the concentration of substrates was 150 times the K<sub>m</sub> of the rat brain kinase for Ins(1,4,5)P<sub>3</sub> (Irvine *et al.*, 1986b), Ins(2,4,5)P<sub>3</sub> was phosphorylated at less than 10% of the rate of Ins(1,4,5)P<sub>3</sub>, this being the limit of detection under these assay conditions.

Thus we can suggest that the inability of Ins(2,4,5)P<sub>3</sub> and Ins(1,3,4)P<sub>3</sub> (the present data) or of Ins(1,2cyc4,5)P<sub>3</sub> (M. Whittaker & K. A. Swann, personal communication) to activate sea urchin eggs lies in the fact that, unlike Ins(1,4,5)P<sub>3</sub>, they are not readily phosphorylated to their corresponding InsP<sub>4</sub>.

#### Effect of Ins(1,3,4,5)P<sub>4</sub>

Our initial observations with injecting Ins(1,3,4,5)P<sub>4</sub> were disappointing with regard to this latter suggestion because microinjection of up to 10<sup>-14</sup> mol of this compound (10 pl of 1 mM, h.p.l.c. pure) failed to activate eggs. However, it is likely that the second stage of egg activation (Slack *et al.*, 1986) would depend on the first; i.e. that Ins(1,3,4,5)P<sub>4</sub> might require prior Ca<sup>2+</sup> mobilization to exert an effect on Ca<sup>2+</sup> entry. We therefore undertook to test again all the inositol phosphates that we had prepared in eggs in which the intracellular Ca<sup>2+</sup> had already been mobilized. There are two alternative ways open to us for doing this. Firstly, we could co-inject the compounds with Ca<sup>2+</sup>; this would be difficult both to do and to interpret because we know neither the local Ca<sup>2+</sup> concentrations nor the kinetics of Ca<sup>2+</sup> release induced by Ins(1,4,5)P<sub>3</sub>. A second, and more physiological, approach was to co-inject Ins(2,4,5)P<sub>3</sub>, which as discussed above is a well-established Ca<sup>2+</sup> mobilizer that does not raise fertilization envelopes in these eggs.

#### Effect of inositol phosphates co-injected with a Ca<sup>2+</sup> mobilizer

In an extensive series of experiments we therefore injected into eggs inositol phosphate solutions all of which contained 50 μM-Ins(2,4,5)P<sub>3</sub>; from studies on other vertebrate and invertebrate tissues (see above) we can be sure that this level of Ins(2,4,5)P<sub>3</sub> will maximally mobilize Ca<sup>2+</sup> even if only 1% of cell volume [10 pl,

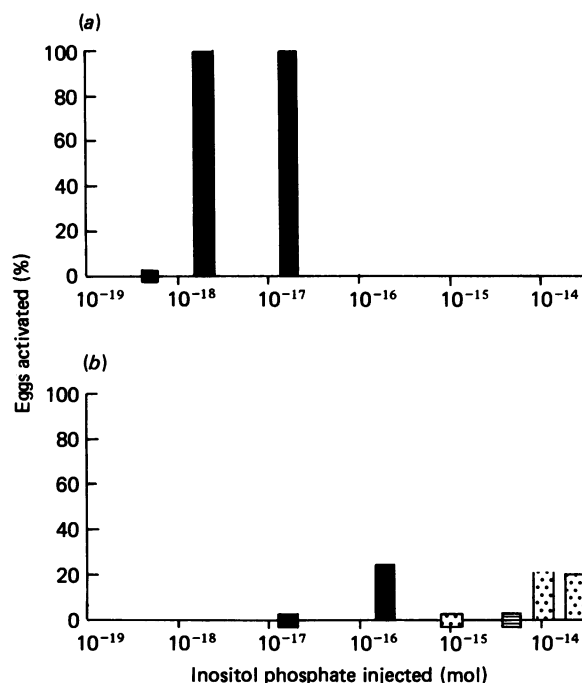


Fig. 1. Activation of sea urchin eggs by inositol phosphates co-injected with Ins(2,4,5)P<sub>3</sub>

The data are the results of a number of independent experiments. All solutions contained 50 μM-Ins(2,4,5)P<sub>3</sub>, and so in addition to the inositol phosphates shown, each egg received 5 × 10<sup>-16</sup>–1.5 × 10<sup>-15</sup> mol of Ins(2,4,5)P<sub>3</sub>. (a) ■, Ins(1,3,4,5)P<sub>4</sub>; (b) ■, Ins(w,x,y,z)P<sub>4</sub>. Doses which produced no activation are shown by the histogram straddling the axis. On (b) are also shown Ins(1,3,4)P<sub>3</sub> ▨ and Ins(2,4,5)P<sub>3</sub> ▤. For the latter two, only the highest concentrations tested that gave no activation are depicted, for simplicity.

5 × 10<sup>-16</sup> mol of Ins(2,4,5)P<sub>3</sub> is injected. Ten times this dose of Ins(2,4,5)P<sub>3</sub> does not activate eggs on its own (see above). The results of some of these experiments are recorded in Fig. 1. The difference between Ins(1,3,4,5)P<sub>4</sub> injected on its own (see above) and co-injected with Ins(2,4,5)P<sub>3</sub> (Fig. 1) can only be described as spectacular. Whereas 10<sup>-14</sup> mol of Ins(1,3,4,5)P<sub>4</sub> was ineffective on its own, now as little as 2.1 × 10<sup>-18</sup> mol [21 pl of 0.1 μM-Ins(1,3,4,5)P<sub>4</sub>] would fully activate all of a batch of eggs. Ins(1,3,4,5)P<sub>4</sub> was slightly more potent than its precursor, Ins(1,4,5)P<sub>3</sub>, though with this semiquantitative assay technique a much greater number of direct comparison experiments would be necessary for us to quantify the difference between them (results not shown). The breakdown product of Ins(1,3,4,5)P<sub>4</sub>, Ins(1,3,4)P<sub>3</sub>, was inactive up to 6 × 10<sup>-15</sup> mol (Fig. 1). It is important to demonstrate that the effect of Ins(1,3,4,5)P<sub>4</sub> is not due to an artefact of injection of a substance more highly charged than Ins(1,4,5)P<sub>3</sub>, and this we did by comparing directly Ins(1,3,4,5)P<sub>4</sub> with Ins(w,x,y,z)P<sub>4</sub> (see the Materials and methods section). Whereas Ins(1,3,4,5)P<sub>4</sub> (2.3 × 10<sup>-17</sup> mol; 23 pl of 1 μM) activated eggs fully, the same dose of Ins(w,x,y,z)P<sub>4</sub> was inactive and a higher dose (2.1 × 10<sup>-16</sup> mol; 21 pl of 10 μM) only partly effective (Fig. 1), which indicates a precise requirement for a correct distribution of the phosphates on the inositol ring. The effect of Ins(1,3,4,5)P<sub>4</sub> is dependent on

extracellular  $\text{Ca}^{2+}$  by the same criteria as those used above, and, with the same reservations as above, we conclude that the activation by  $\text{Ins}(1,3,4,5)P_4$  is likely to be a direct or indirect result of stimulated  $\text{Ca}^{2+}$  entry.

In conclusion, we should note that we have not yet directly demonstrated that  $\text{Ins}(2,4,5)P_3$  is mobilizing  $\text{Ca}^{2+}$  in our experiments although, as discussed above, from known data it is the only probable explanation; exactly what  $\text{Ins}(2,4,5)P_3$  is doing is not, strictly speaking, relevant to the data in Fig. 1, which clearly show the specificity and potency with which  $\text{Ins}(1,3,4,5)P_4$  activates sea urchin eggs under these defined conditions. The remarkable requirement for  $\text{Ins}(2,4,5)P_3$  to be present in order for  $\text{Ins}(1,3,4,5)P_4$  to exert its effect raises the intriguing question of whether in other tissues also the promotion of  $\text{Ca}^{2+}$  entry is dependent on prior  $\text{Ca}^{2+}$  mobilization, or whether this is a unique feature of the acute response of these eggs. Experiments on parotid glands (Putney, 1977; see also Putney, 1986) do not support the idea that a raised  $\text{Ca}^{2+}$  level in the cytoplasm is a prerequisite for stimulated  $\text{Ca}^{2+}$  entry, although a priming mechanism stable for several minutes cannot be discounted. These parotid data could alternatively be interpreted to show that an empty intracellular  $\text{Ca}^{2+}$  store is the requirement for stimulated  $\text{Ca}^{2+}$  entry, and so the possibility that  $\text{Ins}(1,3,4,5)P_4$  controls  $\text{Ca}^{2+}$  entry by modulating a mechanism of the sort proposed by Putney (1986) is just one of several possible modes of action of  $\text{Ins}(1,3,4,5)P_4$  to be explored. It may be that in other tissues  $\text{Ins}(1,3,4,5)P_4$  can act on its own, and if so then clearly  $\text{Ins}(1,4,5)P_3$  kinase, by phosphorylating  $\text{Ins}(1,4,5)P_3$  at resting or stimulated levels, will play a major role in cell physiology by its long-term control of cellular  $\text{Ca}^{2+}$ .

Whatever the possible subtleties and variations, our results show that  $\text{Ins}(1,3,4,5)P_4$  joins  $\text{Ins}(1,4,5)P_3$  and diacylglycerol as a second messenger generated from inositides. That this second messenger,  $\text{Ins}(1,3,4,5)P_4$ , is derived in turn from another second messenger,  $\text{Ins}(1,4,5)P_3$ , and that it may partly depend on the latter to exert its effect, is a fascinating new facet of the role of inositides in cell signalling.

We are very grateful to A. J. Letcher and D. J. Lander for their invaluable help in preparing the inositol phosphates. We also thank Richard Sankey for his help in obtaining the sea urchins and Dr. J. W. Putney Jr. for useful discussions.

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