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REVIEW —*Current Perspective*—

Molecular Basis of Spatio-temporal Dynamics in Inositol 1,4,5-Trisphosphate-Mediated Ca^{2+} Signalling

Masamitsu Iino

Department of Pharmacology, Graduate School of Medicine, The University of Tokyo; and CREST, Japan Science and Technology Corporation, Tokyo 113-0033, Japan

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ABSTRACT—Inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} signalling regulates many important cell functions, and the spatio-temporal dynamics of the Ca^{2+} signalling is a crucial factor for its versatility. The molecular mechanisms that control Ca^{2+} signalling are now being investigated, and I here describe the subtypes of IP_3 receptors that have distinct functional properties and contribute to the diversity of Ca^{2+} signalling patterns. I also discuss the spatio-temporal dynamics of intracellular IP_3 concentration, describing recent methodological advances in monitoring intracellular IP_3 concentration. These findings highlight the potential importance of the spatio-temporal information of any signalling molecule.

Keywords: Ca^{2+} wave, Ca^{2+} oscillation, Inositol 1,4,5-trisphosphate receptor, Phospholipase C

Introduction

Inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} signalling controls important cell functions such as smooth muscle contraction, secretion, fertilization, immune responses, gene expression and synaptic plasticity. It is remarkable that a molecule as simple as Ca^{2+} can control such a vast array of important cell functions. One of the unique features of Ca^{2+} signalling is the variety of spatio-temporal patterns of Ca^{2+} signals (1), which provides versatility of Ca^{2+} signalling in the regulation of many different cell functions. For example, an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) may start from a limited area within a cell and propagate toward the other parts of the cell, which is referred to as a Ca^{2+} wave. Ca^{2+} waves have been found in many types of cells and provide an efficient way to transmit a Ca^{2+} signal from one part of the cell to another. The velocity of Ca^{2+} waves is rather constant (20 – 100 μm/s) under different conditions (2), and Ca^{2+} waves may also play a role in producing a delay in the timing of an increase in $[Ca^{2+}]_i$ at different parts of the cell, particularly in polarized cells when the direction of the Ca^{2+} wave is parallel to the cell polarity (3). Upon activation of cells by agonists, $[Ca^{2+}]_i$ may show a steady increase, but may also undergo repetitive increases, called Ca^{2+} oscillations. The frequency of Ca^{2+} oscillations depends on the agonist concentration, and thus regulates the intensity of cell functions. For example, vascular smooth muscle contraction can be regulated by the frequency of agonist-induced Ca^{2+} oscillations (4, 5). The frequency of Ca^{2+} oscillation may also regulate the gene expression patterns and protein kinase activities (6 – 8). Thus, it is important to understand the molecular mechanisms that underlie the spatio-temporal pattern formation of Ca^{2+} signals.

The versatility of Ca^{2+} signals is based on the fact that Ca^{2+} is essentially a short-range intracellular signalling molecule due to the presence of Ca^{2+} binding proteins and Ca^{2+} sequestration mechanisms within the cytoplasm (9). Thus, an increase in $[Ca^{2+}]_i$ can be local and transient as found in a Ca^{2+} puff, which is the elementary Ca^{2+} release event via a cluster of a small number of Ca^{2+} release channels (10). The elementary events may be organized spatially to generate a Ca^{2+} wave, which is based on a regenerative mechanism in the Ca^{2+} release channels such that a local Ca^{2+} release further activates the Ca^{2+} release at the adjacent Ca^{2+} release site. To generate Ca^{2+} oscillations, the molecules involved in Ca^{2+} signalling are required to have Ca^{2+}- and time-dependent mechanisms to account for the repetitive activation and inactivation of Ca^{2+} release. Therefore, differential expression of the signal-generating molecules in different tissues or in different stages of development may control the pattern of Ca^{2+} signals that are tissue- or development-specific. As far as the IP_3-mediated Ca^{2+} signalling is concerned, such molecular mechanisms include: i) the agonist receptor-phospholipase C (PLC) sys-

tem; ii) IP_3 receptors; iii) Ca^{2+} sequestration mechanisms including sarco(endo)plasmic reticulum Ca^{2+} ATPase, mitochondria, plasma membrane Ca^{2+} ATPase, and Na^+/Ca^{2+} exchanger; iv) IP_3 phosphatases and kinases; and v) Ca^{2+} influx pathways via the plasma membrane. Since a thorough review of all theses aspects would exceed the scope of this article, I shall concentrate on the recent progress in the field with which our laboratory has been involved. These topics include subtype specificity of IP_3 receptor functions and intracellular IP_3 dynamics.

IP_3 receptors

IP_3 receptors (IP_3R) are Ca^{2+} release channels that are located on the endoplasmic reticulum membrane and formed as a tetramer of subunits with approximately 2,700 amino acids. Thus, the total molecular mass of a single channel molecule exceeds 1 MDa. Phylogenetic analyses suggest that the IP_3Rs share a common origin with the ryanodine receptors, other members of the family of intracellular Ca^{2+} release channels (11). The N-terminal end of the IP_3R forms the IP_3-binding domain (12), while the C-terminal end traverses the ER membrane to form the Ca^{2+} channel domain (13).

The activity of IP_3R depends not only on the IP_3 concentration but also on other cytoplasmic ion concentrations; i.e., Ca^{2+} concentration (14), ATP concentration (15, 16) and pH (17). The Ca^{2+} and ATP dependence will be discussed further in conjunction with the IP_3R subtypes. The molecular mechanism of such dependence is not fully understood, but there are regions along the IP_3R that show high-affinity Ca^{2+} or ATP binding. One of the major effects of pH is on the affinity between IP_3 and IP_3R, which increases at higher pH. pH also has a modulatory effect on the channel gating.

IP_3R subtypes

There are three subtypes of IP_3 receptors (IP_3R-1, -2 and -3) encoded by different genes. Although all three subtypes are coexpressed in most tissues, the relative content varies in a tissue- and development-specific manner (18–20). For example, IP_3R-1 is the major subtype expressed in the central nervous system. In particular, Purkinje cells in the cerebellum express a very high density of IP_3R-1 (11). Hepatocytes and cardiac myocytes, on the other hand, predominantly express IP_3R-2. Furthermore, IP_3R subtypes form heterotetrameric channels. These complex expression patterns of IP_3R subtypes may be involved in the generation of cell-type-specific Ca^{2+} signalling (see below).

Single channel properties of the individual IP_3R subtypes were estimated using cells that predominantly express a single subtype: subtype 1, either Purkinje cells or HEK293 cells transformed to express IP_3R-1 (21, 22); subtype 2, cardiac myocytes (23); and subtype 3, RIN-5F cells (24). However, the properties of individual subtypes were not compared under similar conditions. To examine the properties of each subtype under the same cellular context,

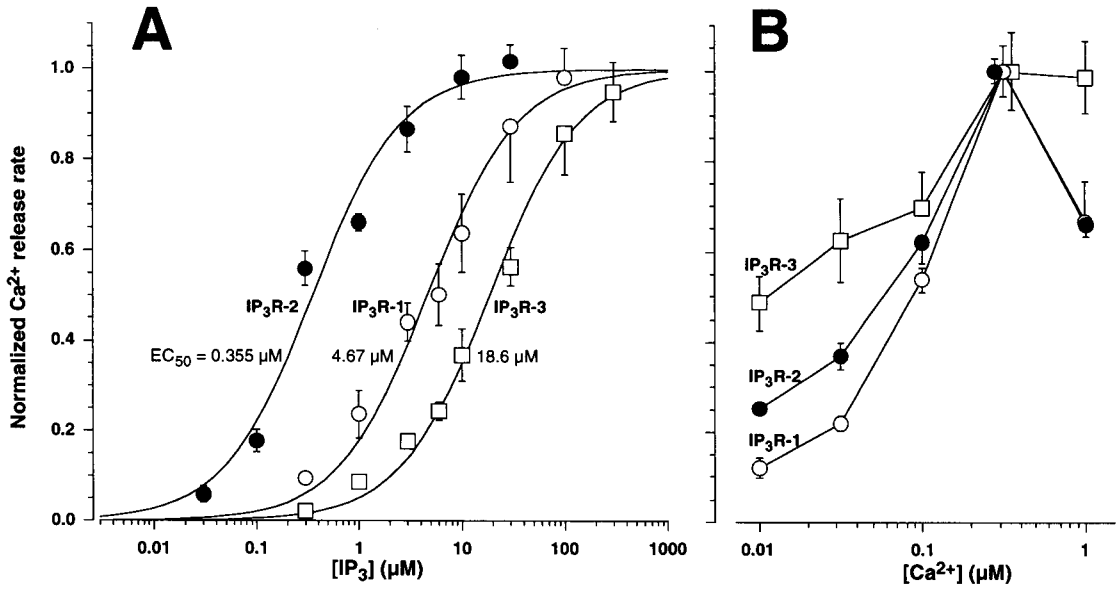

Fig. 1. IP_3- and Ca^{2+}-dependence of IP_3-induced Ca^{2+} release in different IP_3R subtypes. A: IP_3 concentration-Ca^{2+} release rate relationship in three avian IP_3R subtypes. Michaelis-Menten curve was fitted to the data and the data points were normalized by the fitted maximal rate of Ca^{2+} release. EC_{50} values are given in the figure. B: Ca^{2+} concentration-Ca^{2+} release rate relationship. Data points were normalized by the Ca^{2+} release rate at 300 nM Ca^{2+} in each IP_3R subtype.
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we used genetically engineered B cells that express either a single or a combination of IP_3R subtypes (25).

The results of our study show that IP_3 receptor subtypes differ markedly in their response to intracellular agonists; i.e., IP_3, Ca^{2+} and ATP. Figure 1A shows the IP_3 concentration-response relationship. IP_3R-2 is the most sensitive to IP_3, while IP_3R-3 is the least sensitive subtype. The order of IP_3 sensitivity among the three IP_3R subtypes is in general agreement with the IP_3 binding affinity of the recombinant IP_3-binding domains of each mammalian IP_3R subtype. The Ca^{2+} sensitivity is also subtype-dependent (Fig. 1B). The activity of IP_3R-1 is steeply dependent on Ca^{2+} concentrations below 300 nM; i.e., Ca^{2+} has an agonistic action on the IP_3R, while at higher Ca^{2+} concentrations (>300 nM), there is an inhibitory effect. Such biphasic Ca^{2+} dependence has been reported in both smooth muscle cells (14, 26) and Purkinje cells (21), in which the dominant IP_3R subtype is IP_3R-1. IP_3R-2 has a moderate Ca^{2+} sensitivity. IP_3R-3 has a rather flat Ca^{2+} dependence, in other words, IP_3R-3 can open at a very low cytoplasmic Ca^{2+} concentration and is not inhibited at high Ca^{2+} concentrations. IP_3R-1 requires ATP for its full activity, while IP_3R-3 has a weaker ATP requirement than IP_3R-1. IP_3R-2 has no ATP requirement for channel activity. The effect of ATP is kinase-independent, and a non-hydrolyzable analog of ATP is also effective in inducing the potentiatory effects. The ATP dependence has also been noted in the IP_3R studied in smooth muscle cells and cerebellar Purkinje cells (16, 27).

Interaction between IP_3R subtypes

It has been shown that different IP_3R subtypes can form heterotetrameric channels (28, 29), and such heteromerization is thought to result in inter-subunit functional interaction. However, there has been no experimental proof that there are such interactions among the IP_3R subtypes. We studied the properties of IP_3Rs in cells expressing two IP_3R subtypes (25). Were it not for the interaction between the subunits, we would expect to find the properties of IP_3-induced Ca^{2+} release in cells expressing two IP_3R subtypes to be the summation of the properties of individual subtypes. Our results show that as far as the IP_3 sensitivity is concerned, cells expressing two IP_3R subtypes have an IP_3 sensitivity intermediate between those of single subtypes. However, regarding ATP sensitivity, the property of one of the subtypes predominated (25). These results clearly indicate the presence of functional interactions among the coexpressed IP_3R subtypes, and such interactions would generate a higher degree of complexity in the IP_3R functions.

Ca^{2+} signalling patterns in cell expressing different IP_3R subtypes

What would be the impact of the different IP_3R subtypes, with different Ca^{2+} release activities, on the Ca^{2+} signalling in intact cells? We studied the temporal patterns

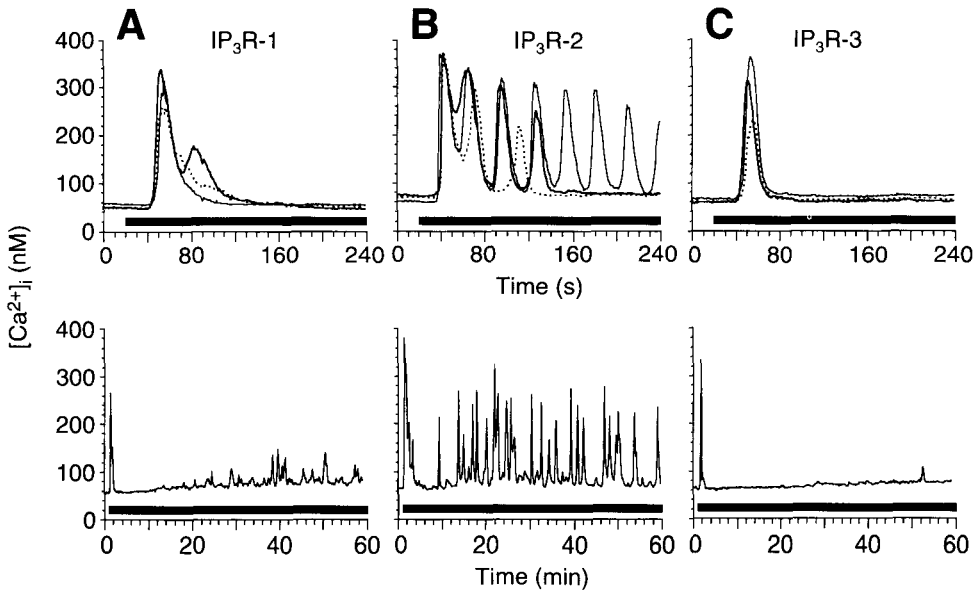

Fig. 2. Ca^{2+} signalling patterns in DT40 cells expressing different IP_3R subtypes upon B-cell receptor stimulation. Traces obtained from cells expressing either IP_3R-1 (A), IP_3R-2 (B) or IP_3R-3 (C). Early time course is shown for three representative cells (thick, thin, and dotted traces; upper panels) and a 60-min time course is shown for one of the cells (lower panels). BCR stimulation was applied as indicated by the horizontal bars below the traces.

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of Ca^{2+} signals in genetically engineered DT40 cells expressing a single IP_3R subtype and found that the Ca^{2+} signalling pattern depended critically on the expressed IP_3R subtypes (Fig. 2) (25). In IP_3R-2 expressing cells, robust Ca^{2+} oscillations were observed, while in IP_3R-1- or IP_3R-3-expressing cells, only a single Ca^{2+} transient or rapidly attenuating Ca^{2+} oscillations were observed. Thus, although Ca^{2+} oscillation involves complex molecular mechanisms (see below), the differential expression of IP_3R subtypes is definitely one of the major factors that determine the Ca^{2+} signalling patterns.

Mechanism of Ca^{2+} wave and oscillation

As shown above, cells may respond to a constant level of agonist stimulation with oscillatory changes in $[Ca^{2+}]_i$. There are two major theories with regard to the mechanism of Ca^{2+} oscillations (30). One theory states that Ca^{2+} oscillations are generated by intermittent activation of IP_3R at a constant level of IP_3 concentration (31). Such mechanism is based on the Ca^{2+}-induced activation of the IP_3R, and progressive activation of the IP_3R by a gradual increase in the ambient Ca^{2+} concentration is thought to underlie the intermittent Ca^{2+} release (Ca^{2+}-induced Ca^{2+} release model) (30). Indeed, we found a pacemaker-like Ca^{2+} rise ("foot") preceding a rapid rise in $[Ca^{2+}]_i$ in single smooth muscle cells (32). Recent Ca^{2+} imaging studies indicate that the gradual increase in ambient Ca^{2+} concentration is brought about by increased frequency and peak size of Ca^{2+} puffs (33). Since the activity of the IP_3R is Ca^{2+}-sensitive (see above), increased generation of Ca^{2+} puffs would result in gradual accumulation of the ambient Ca^{2+} concentration, which would further activate the IP_3R even at a constant IP_3 concentration. At a certain activation level of Ca^{2+} release at a rate that overcomes the rate of Ca^{2+} sequestration, a catalytic Ca^{2+} release due to the positive feedback loop will start. Such regenerative Ca^{2+} release would be the basis of the generation of a Ca^{2+} wave (Fig. 3A). Ca^{2+} release then subsides due to depletion of the stores or high Ca^{2+} concentration-induced inhibition of IP_3R because of the biphasic Ca^{2+} dependence of the IP_3R. Gradual replenishment of the stores is required before the next Ca^{2+} oscillation. It has also been postulated that IP_3R may enter into an inactivated state in a Ca^{2+}- and IP_3-dependent manner and the time-dependent recovery from the putative inactivated state is required before the cell is ready for the next Ca^{2+} oscillation. Although the inactivation mechanism of IP_3R has been proposed in hepatocytes (34), this requires confirmation in other cell systems.

In another theory, Ca^{2+} oscillation is postulated to be accompanied by an oscillatory change in the IP_3 concentration due to Ca^{2+}-dependent activation of PLC and the positive feedback regulation of the PLC activity (IP_3-Ca^{2+} cross-coupling model) (30). In this theory a Ca^{2+} wave would accompany an IP_3 wave (Fig. 3B). Although it has been difficult to test this hypothesis, the cross-coupling model received support from a study using a population of cells whose Ca^{2+} oscillation was artificially synchronized by re-addition of extracellular Ca^{2+} after suppression of Ca^{2+} oscillation by extracellular Ca^{2+} deprivation (35). An IP_3 concentration increase was observed simultaneously with the initial Ca^{2+} spike. Since the subsequent Ca^{2+} oscillations were asynchronous among different cells, IP_3 concentration changes corresponding to the subsequent Ca^{2+} oscillations could not be studied. Therefore, to study the IP_3 dynamics during physiological Ca^{2+} oscillations, monitoring of the real-time changes in the intracellular IP_3 concentration ($[IP_3]_i$) at the single cell level is required.

Intracellular IP_3 dynamics

To monitor the $[IP_3]_i$, we developed a new method in which the pleckstrin homology domain (PHD) of PLC $\delta 1$ was used as the IP_3 probe (36). In vitro studies of the recombinant PHD show specific binding of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] as compared to Ins(1,3,5)P_3 and Ins(1,3,4,5)P_4. PHD also binds phosphatidylinositol bisphosphate (PIP_2) but at a 20-fold lower affinity than the binding between PHD and Ins(1,4,5)P_3. PHD was fused with the GFP (GFP-PHD) and was expressed in Madin-Darby canine kidney epithelial cells (MDCK cells). Due to

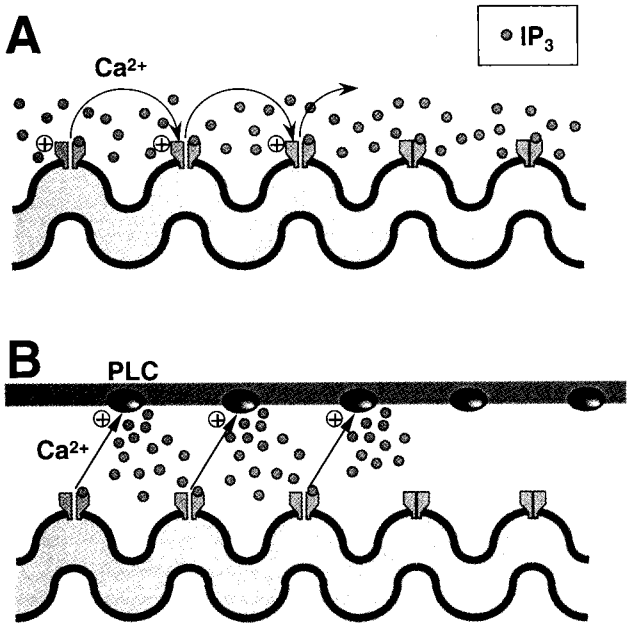

Fig. 3. Two alternative models of Ca^{2+} waves. A: CICR model, in which Ca^{2+} released from one site successively activates the adjacent sites at a constant IP_3 concentration. B: IP_3-Ca^{2+} cross-coupling model, in which Ca^{2+} released from one site will activate the local PLC, thus producing IP_3 to induce Ca^{2+} release from the neighbouring site. Small circles represent IP_3 molecules, and the arrows indicate the flow of Ca^{2+}.

its affinity for PIP_2, a membrane phospholipid, GFP-PHD was bound to the cell membrane during the resting state. Upon activation of the receptor and PLC, GFP-PHD was translocated to the cytoplasm. This translocation was caused by an increase in $[IP_3]_i$; microinjection of IP_3 into these cells induced dose-dependent translocation of GFP-PHD, and overexpression of IP_3-5-phosphatase, which would induce immediate consumption of IP_3, abolished the agonist-induced translocation.

Therefore, the translocation of GFP-PHD can be used as the measure of $[IP_3]_i$. Hirose et al. (36) found IP_3 waves and oscillations concomitant with Ca^{2+} waves and oscillations in ATP-stimulated MDCK cells (Fig. 4). Thus, these results support the IP_3-Ca^{2+} cross-coupling model. However, the presence of spatio-temporal IP_3 dynamics does not rule out the CICR model, and it is likely that both CICR and IP_3-Ca^{2+} cross-coupling mechanisms are involved in generating complex Ca^{2+} signalling patterns, and that the relative importance of these mechanisms might vary in different tissues.

Molecules underlying IP_3 dynamics

The presence of intracellular IP_3 dynamics suggests the presence of the Ca^{2+}- and time-dependent metabolism of IP_3. The Ca^{2+}-dependent activation of PLC has been well known and was demonstrated in MDCK cells using the GFP-PHD method (36). Ca^{2+} and time-dependent inactivation of IP_3 production was also shown in MDCK cells (36). These results suggest the presence of Ca^{2+}- and time-dependent regulation of PLC or IP_3 metabolizing enzymes such as IP_3-5-phosphatase and IP_3-3-kinase. The relative contribution of these enzymes in the generation of IP_3 dynamics requires further elucidation.

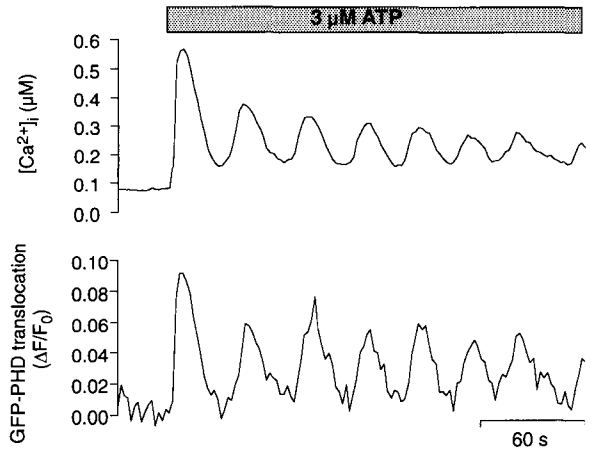

Fig. 4. IP_3 oscillation concomitant with Ca^{2+} oscillation during purinergic activation. IP_3 concentration in MDCK cells was measured using the GFP-PHD method, and $[Ca^{2+}]_i$ was simultaneously measured using fura-2.
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Perspectives

The dynamic spatio-temporal patterns of Ca^{2+} signalling have intrigued many investigators, and the molecular mechanisms involved have been recently elucidated. Such studies will help us to understand the molecular basis of the tissue-specific aspects of Ca^{2+} signalling. Although Ca^{2+} signalling regulates many cell functions, only very few drugs that specifically intervene in Ca^{2+} signalling are currently available except for the Ca^{2+} channel antagonists. When the tissue-specific Ca^{2+} signalling mechanism is better understood, we will expect to have a better view with regard to the development of new drugs.

Another important point here is that the spatio-temporal dynamics is not unique to Ca^{2+} signalling as initially thought. We have recently shown that the $[IP_3]_i$ also displays spatio-temporal dynamics (36). Furthermore, dynamic translocation of protein kinases, such as C-kinases (37, 38) and cytosolic phospholipase A_2 (39), within the cell in response to stimulation has been demonstrated. The translocation of these enzymes may increase the substrate specificity. The distribution of ion channels (40) or calmodulin-dependent protein kinase II (41) changes dramatically near the synapse in response to stimuli that cause long-term changes in the synaptic transmission. Thus, it will be increasingly important to examine the spatio-temporal patterns of intracellular signals in general.

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