Hypothesis

Possible roles of inositol 1,4,5-trisphosphate 3-kinase B in calcium homeostasis

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Abstract Some aspects of the roles of inositol trisphosphate (Insp3) and inositol tetrakisphosphate (Insp4) in Ca^{2+} homeostasis in terms of inositol trisphosphate 3-kinase B (IP3K-B) localization and activity are discussed. The model that we propose is also compatible with IP3K-B participating in the widely reported phenomenon of quantal release of Ca^{2+} from internal stores, at least in some biological systems.

Key words: Calcium homeostasis; Inositol trisphosphate; Inositol trisphosphate 3-kinase B

1. Introduction

Inositol 1,4,5-trisphosphate (Insp3) is a second messenger that mediates the release of Ca^{2+} from intracellular stores. Binding of ligands to specific plasma membrane receptors triggers the hydrolysis of membrane-associated phosphatidyl inositol bisphosphate (PIP2) by phospholipase C (PLC) into soluble Insp3 and membrane-bound diacylglycerol [1].

Although the role of Insp3 in Ca^{2+} homeostasis is well understood, much less is known about the role of its metabolites. Of particular interest is the product of inositol 1,4,5-trisphosphate 3-kinase (IP3K), inositol 1,3,4,5-tetrakisphosphate (Insp4), which has been postulated to regulate Ca^{2+} influx through the plasma membrane [2]. The recent purification and cloning of an Insp4-binding protein with both ras- and rap-GAP activity ($GAP1^{IP4BP}$) [3] may support a role for Insp4 in extracellular Ca^{2+} influx. In short, Insp4 binds and activates $GAP1^{IP4BP}$ in platelets, the final effect probably being inactivation of a small G protein, possibly rap1B [4,5]. Since binding of Insp4 to $GAP1^{IP4BP}$ would finally inactivate the G protein, it can follow that Insp4 may regulate Ca^{2+} influx from the plasma membrane in a GTP-dependent manner, at least in platelets. Thus, Insp4 is of clear physiological importance and potentially links the Insp3 signal transduction pathway with GTP-regulated signalling mechanisms.

2. Experimental

2.1. Cell culture and immunofluorescence microscopy

Normal rat kidney (NRK) cells were grown in RPMI-1640 medium (GIBCO, Life Technologies, Ltd., Paisley, Scotland), 2 mM glutamine (GIBCO), 100 μg/ml streptomycin (Evans Medical, Ltd., Langhurst, Horsham, UK) and 60 μg/ml penicillin (Glaxo Laboratories, Ltd., Greenford, UK) at 37°C in 5% CO_2. Immunofluorescence was performed as previously described [31], using polyclonal antibody 52 (against the N-terminal 12 amino acids of rat IP3K-B) and a polyclonal antibody versus Insp3 receptor type I (a kind gift from Dr. J.-P. Mauger, Institute Pasteur, Paris) [32].

3. Inositol 1,4,5-trisphosphate 3-kinase activities

As mentioned above, the conversion of Insp3 into Insp4 is catalysed by inositol 1,4,5-trisphosphate 3-kinases (IP3Ks) [6]. This activity has been shown to be associated with a variety of low molecular weight, mainly cytosolic, proteins [7–12] which are activated by Ca^{2+} and calmodulin [8,12–16]. Do these proteins represent multiple isoforms of IP3Ks, or are they proteolytic fragments of a more limited number of IP3K isoforms? The sequences of A and B isoforms of rat and human IP3K have been published [17–19], so different isoforms of the enzyme do exist. However, cloning of the B isoform of rat IP3K (IP3K-B) [20] showed that the initiating Met is in fact 205 amino acids upstream of that initially reported for the human orthologue of this enzyme [19] and that whilst the A and B isoforms share a catalytic region (the C-terminus of the B isoform and the entirety of the A isoform), the N-terminal 320 amino acids are unique to the B isoform [19]. In addition, the full-length IP3K-B is not only cytosolic. It has also been shown to bind to the cytosolic face of a well-defined area of the ER membrane system, via high-affinity, conformation-dependent, protein–protein interactions, and to have an apparent molecular weight of 90 kDa (Soriano et al., submitted). Moreover, we show here that the membrane localization of IP3K-B overlaps with that of Insp3 receptor type I, as seen by immunofluorescence (Fig. 1), implying that the membranes involved are those of the internal Ca^{2+} stores.

Thus, whilst some of the reported IP3K activity associated with low molecular weight, cytosolic proteins may correspond to different isoforms of IP3K, some are likely to derive from proteolytic release of C-terminal, catalytically active fragments from IP3K-B (and other isoforms). This hypothesis is supported by the observation that IP3K is highly sensitive to proteases, in particular calpain, and it is common to find several degradation products of IP3K even in fresh preparations [10,18].

Here we discuss some aspects of Insp3 and Insp4 roles in Ca^{2+} homeostasis in terms of IP3K-B localization and activity.

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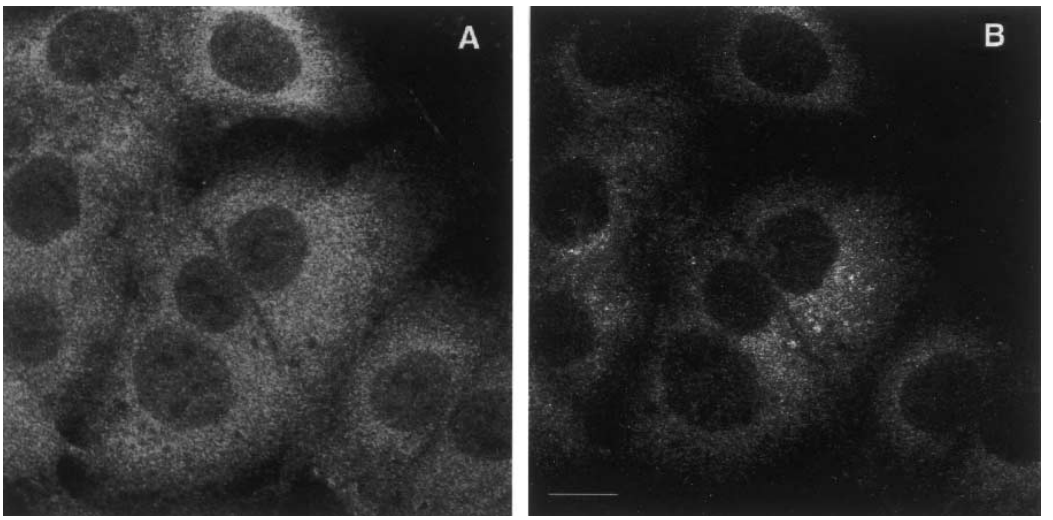

Fig. 1. Colocalization of Insp3 receptor type I (A) and IP3K-B (B) in NRK cells. Cells were processed for immunofluorescence as described in Section 2. Bar: 10 μm.

4. Possible roles of inositol 1,4,5-trisphosphate 3-kinase B in Ca^{2+} homeostasis

There is extensive evidence showing that, at least in several biological systems, Insp3 and Insp4 may regulate Ca^{2+} mobilization synergistically [33–35]. And more recently, evidence from Dawson's laboratory [36] strongly suggests that the synergistic effect of Insp4 is unlikely to be due to artefacts such as conversion into Insp3 or by contamination of Insp4 preparations with Insp3 traces. Moreover, it has also been shown that there is a mechanism requiring GTP which links Insp3-sensitive and insensitive pools [25,27–29], and Irvine [37] has suggested that Insp4 could regulate such GTP-dependent linking.

Based on the fact that IP3K-B is membrane associated and localised to specific regions of the ER membrane system involved in Ca^{2+} homeostasis (Fig. 1) (Soriano et al., submitted), we take these ideas further and postulate that the mechanism for Ca^{2+} influx from the plasma membrane, dependent on small G proteins, and activated by Insp4, also exists in intracellular membranes; such a system would be analogous to different Rab proteins functioning in membrane traffic events involving different intracellular membranes. In our model, based on the localization and activity of IP3K-B, Insp3 would release Ca^{2+} from the ER during cell activation, which in turn would stimulate Insp4 production and subsequent Insp4-regulated Ca^{2+} release in a GTP-dependent manner, in a similar way to that proposed for a Rap-like protein and Insp4 at the plasma membrane of platelets [4,5]. Thus, production of Insp4 on ER membranes would cause Ca^{2+} release into the cytosol, as it causes Ca^{2+} influx from the plasma membrane.

Although Insp3 receptors may be regulated in several ways, including covalent modifications [21], the partial co-localization with IP3K-B, the enzyme distribution being more restricted, offers an additional regulatory mechanism, since only the receptors proximal to the enzyme would be further activated by the G protein-dependent pathway.

It might be argued that, because of its rapid rate of difusion, there would be no need for the in situ generation of Insp4. However, the key step in the above scheme is not so much the downstream effects of Insp4 production but rather regulation of that production, i.e. the activation of IP3K-B, an enzyme which is activated by Ca^{2+}. Proximity of IP3K-B to the Ca^{2+} channel of the Insp3 receptor provides the ideal microenvironment for such modulation of enzyme activity.

5. Quantal release from intracellular Ca^{2+} stores

The preceding model also provides one explanation for the widely reported phenomenon of quantal release of Ca^{2+} from internal stores, at least in some biological systems.

It has been proposed that there are both Insp3-sensitive and Insp3-insensitive Ca^{2+} stores in the ER, and this idea has been used to explain the phenomenon of quantal release, by which the release of Ca^{2+} from intracellular stores can be graded [22,23]. However, it has also been argued that, in a large number of cases, experimental procedures for cell permeabilization can account for the apparent quantal release [26]. It has been reported that, in intact cells, all Ca^{2+} stores are available for Insp3 mobilization, whereas disruption of the cytoskeleton and/or inhibition of GTP-dependent interactions results in fragmentation into Insp3-sensitive and insensitive Ca^{2+} stores [24,25]. Treatments disrupting the cytoskeleton result in ER vesicularization [26], effectively causing luminal discontinuity, whereas inhibition of GTP-dependent interactions disrupts membrane trafficking by interfering with both Rab and ARF G proteins. Also, and more relevantly, such inhibition would be predicted to inhibit Ca^{2+} influx from the plasma membrane by interfering with the action of G proteins [3–5]. We suggest that IP3K-B would participate in the 'bridging' of IP3-sensitive and insensitive Ca^{2+} stores in the systems where quantal Ca^{2+} release is observed. In the model that we suggest, outlined in Fig. 2, Ca^{2+} stores would be continuous in normal conditions, and Insp3 and Insp4 would work in conjunction to release Ca^{2+}, the latter by activating a pathway analogous to the one present for Insp4 and a G protein on the plasma membranes of human platelets (Fig. 2A). In this case, graded release of Ca^{2+} would be achieved by graded activation of IP3K-B, according to the initial, Insp3-induced, Ca^{2+} release, which would in turn activate a subset of the IP3K-B population. In short, IP3K-B would be responsible for the Ca^{2+}-induced Ca^{2+} release, by producing Insp4 and thereby

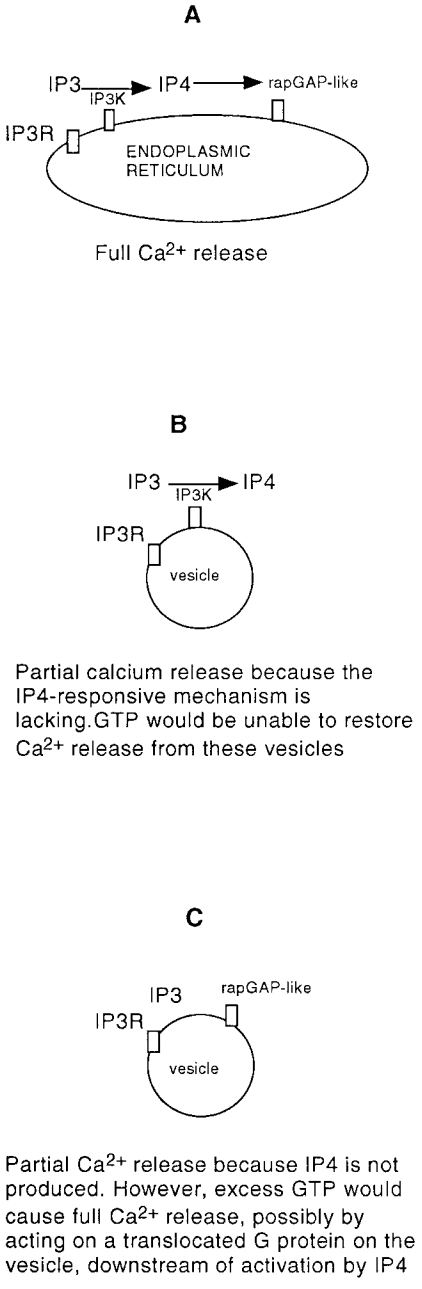

Fig. 2. Possible role of IP3K-B in calcium homeostasis in intact (A) or disrupted (B,C) Ca^{2+} stores. See text for full explanation.

activating the GTP-dependent further Ca^{2+} release. Therefore, quantal Ca^{2+} release, at least in some systems, would be dependent on the relative amounts, and degree of association, of IP3K-B and Insp3 receptor. The more IP3K-B present in proximity to Insp3 receptors, the more likely it is that all Ca^{2+} is released by a single dose of Insp3, since more IP3K-B would be activated by the release of Ca^{2+} via Insp3 receptors, leading to further release of Ca^{2+} (potentially from Insp3-insensitive stores) in an Insp4-regulated, GTP-dependent manner.

Different types of vesiculated structures ('vesicles') are formed under conditions which fragment the ER. These would be predicted to contain various combinations of IP3K-B, Insp3 receptor and the Insp4-regulated, G protein-containing system for the release of intracellular Ca^{2+} stores. The large majority of these 'vesicles' will contain the Insp3 receptor, but fewer will also contain IP3K-B and the appropriate G protein. Those containing all components (Fig. 2B) will release all Ca^{2+}. The ones containing both Insp3 receptor and IP3K-B, but lacking the G protein components will release Ca^{2+} only partially after IP3 activation (Fig. 2C). GTP would be unable to fully release Ca^{2+} from these 'vesicles'. Similarly, 'vesicles' lacking IP3K-B but containing the G components will release Ca^{2+} only partially after Insp3 activation, but full release would be achieved after GTP addition, bypassing the need for Insp4 production. This model would predict that, after GTP addition to permeabilised cells, there would be an apparent enlargement of the Insp3-sensitive intracellular Ca^{2+} store, although not to a full extent. Such an effect has been observed in permeabilised hepatocytes [25]. Also, the enhancing effect of GTP in Ca^{2+} release is well documented in liver microsomes and permeabilised hepatocytes [27–29].

The mechanism by which GTP acts could be by helping in the fusion of different types of vesicles to increase luminal Ca^{2+} available for further release or by acting on individual vesicles.

An absolute requirement for this model is that IP3K-B be either active or at least regulatable when associated to the ER membrane. As mentioned above, the enzyme has been shown to be both cytosolic and membrane-bound. Our results (S.S. and G.B. unpublished) indicate that both populations can produce Insp4. If this is the case, it is then possible that the presence of the N-terminus of the enzyme is only required to bind to the ER in the proximity of the IP3 receptor (it is intriguing to speculate that the Insp3 receptor itself could be the IP3K-B binding protein) so that it can be activated by the local Ca^{2+} concentrations, rather than by conformational changes brought about by shuttling from membrane to cytosol or vice versa.

As mentioned above, Insp3 receptors can be regulated in a variety of ways [21]. Which mechanism is chosen for receptor regulation is likely to depend on the system of study. Regulation by IP3K-B could be present in tissues like liver, where the full-length enzyme was first cloned [20], or in kidney, where the restricted localization of the enzyme was first described (Fig. 1) (Soriano et al., submitted). However, other cells may not express the B isoform of the enzyme (where Insp4 could be involved in Insp3 signal termination, or in Ca^{2+} influx). An example of this may be the finding in rat cerebellum that reconstituted vesicles containing purified Insp3 receptors release Ca^{2+} in a quantal fashion, suggesting that it is an inherent property of the receptors in this tissue [30]. Although this may well be the case, it is also true that the fact that purified Insp3 receptors show heterogeneity in their sensitivity to Insp3 does not provide information about the mechanism involved. For example, such heterogeneity may arise in the cerebellum by any known mechanism [21] and be carried along in the reconstitution of the vesicles.

6. Conclusions

In summary, some aspects of the regulation of IP3K-B have been addressed. Its localization to the cytosolic face of some ER membranes is likely to be relevant to its physiology, although its exact role in Ca^{2+} homeostasis remains unclear. We have outlined a model which is compatible with the data available from several biological systems. However, Ca^{2+} mo-

bilization in other systems may remain unaffected by Insp4, or its role could be different from that proposed here, for example removal of Insp3 or participation in Ca^{2+} influx. The latter would be particularly relevant to platelets, where a role for IP3K-B is difficult to imagine, since platelets do not have a well-defined intracellular membrane system (i.e. they contain a few granules but there is no indication of a 'proper' endoplasmic reticulum). In summary, it is clear that the effect of Insp4 on Ca^{2+} mobilization varies considerably among cell lines, suggesting that the role of IP3K isoforms will depend on the system of study.

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