PtdIns-3,4,5-P3: A Regulatory Nexus between Tyrosine Kinases and Sustained Calcium Signals

Minireview

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Introduction

Elevation of the cytoplasmic calcium concentration is a common and crucial event which follows the activation of many types of cell surface receptors. It is produced by the second messenger inositol-1,4,5-trisphosphate (IP3), itself the product of the action of phosphoinositide-specific phospholipase C (PI-PLC) enzymes on plasma membrane phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P2). PI-PLC enzymes fall into three distinct subtypes (reviewed in Rhee and Bae, 1997): (1) PLCβ, which are controlled by various Gα and Gβγ subunits; (2) PLCδ, whose regulation may involve a novel class of GTP-binding proteins; and (3) PLCγ, which are the enzymes utilized by tyrosine kinases to produce IP3, and which have been thought to be controlled primarily via tyrosine phosphorylation. However, as detailed below, a series of reports from the field of immunoreceptor signaling together show that specifically blocking phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P3) accumulation inhibits phospholipase Cγ-dependent IP3 production and completely blocks sustained calcium influx. Based on these and related reports from other systems, PtdIns-3,4,5-P3 appears to function as a general component of phospholipase Cγ (PLCγ)-dependent calcium signaling pathways.

Model for PLCγ Activation by Tyrosine Kinases and PtdIns-3,4,5-P3

A model for receptor-mediated regulation of PLCγ function that includes a role for PtdIns-3,4,5-P3 is depicted in Figure 1. During a tyrosine kinase-mediated calcium signal, PLCγ is recruited to an upstream tyrosine kinase via its SH2 domains, producing at least part of its tyrosine phosphorylation along with proximity to the plasma membrane where its substrate PtdIns-4,5-P2 resides. Phosphoinositide 3-kinase (PI3K) is activated on a similar time course with PLCγ tyrosine phosphorylation, resulting in conversion of a small fraction of presumably the same pool of PtdIns-4,5-P2 to PtdIns-3,4,5-P3 (top panel). The PtdIns-3,4,5-P3 then feeds back positively both directly and indirectly to enhance the PLCγ-catalyzed breakdown of PtdIns-4,5-P2 to IP3 and diacylglycerol (bottom panel). Its direct actions are mediated through interactions with the PLCγ amino-terminal PH domain and tandem SH2 domains (Bae et al., 1998; Falasca et al., 1998). These interactions may be required for an intramolecular PLCγ function or, since PtdIns-3,4,5-P3 can only be made where PtdIns-4,5-P2 exists, they may help target PLCγ to PtdIns-4,5-P2-rich areas of the plasma membrane. The indirect actions of PtdIns-3,4,5-P3 are mediated through its ability to interact with the PH domains of TEC family tyrosine kinases, thereby promoting their membrane targeting and activation. TEC family kinases are then thought to influence PLCγ activation at least in part by participating in PLCγ tyrosine phosphorylation (Takata and Kurosaki, 1996; Fluckiger et al., 1998; Liu et al., 1998; Scharenberg et al., 1998).

The combined effects of the various upstream pathways on PLCγ function typically result in a large accumulation of IP3 over the first 1–2 min after receptor engagement, followed by a low level of sustained IP3 accumulation. The effect that modulation of PI3K-dependent signals has on IP3 production suggests that PI3K is involved in the regulatory processes that produce these IP3 accumulation kinetics. Blockade of PtdIns-3,4,5-P3 accumulation either pharmacologically or through engagement of specific inhibitory receptors
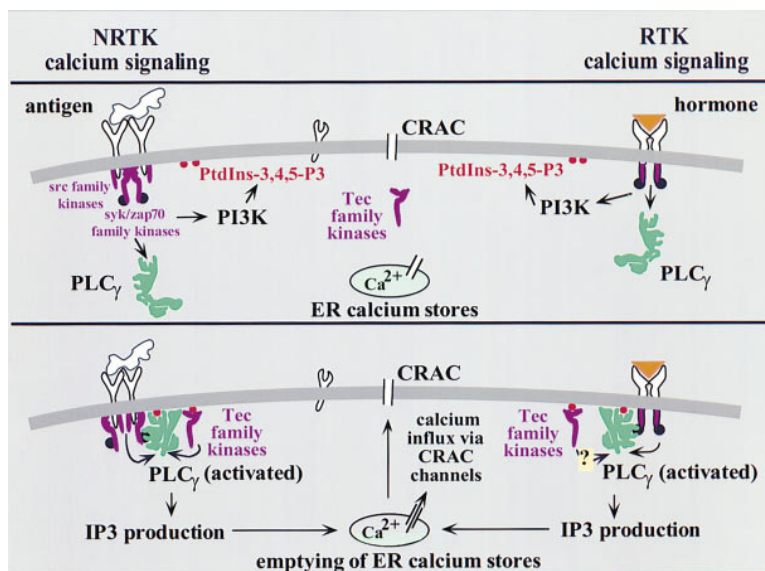

Figure 1. Model of Receptor and Non–Receptor Tyrosine Kinase–Mediated Activation of Phospholipase Cγ

(Top panel) Model of the signaling events that are upstream from the activation of PLCγ in receptor tyrosine kinase (RTK) and non–receptor tyrosine kinase (NRTK) signaling systems.

(Bottom panel) Model of the subsequent events involved in the ongoing production of IP3, and its ensuing effects on cellular calcium fluxes.

Red filled circles represent PtdIns-3,4,5-P3; black filled circles represent tyrosine phosphorylation. CRAC, *C*alcium *R*elease *A*ctivated *C*hannel; PI3K, phosphoinositide 3-kinase; PLCγ, phospholipase Cγ; ER, endoplasmic reticulum.
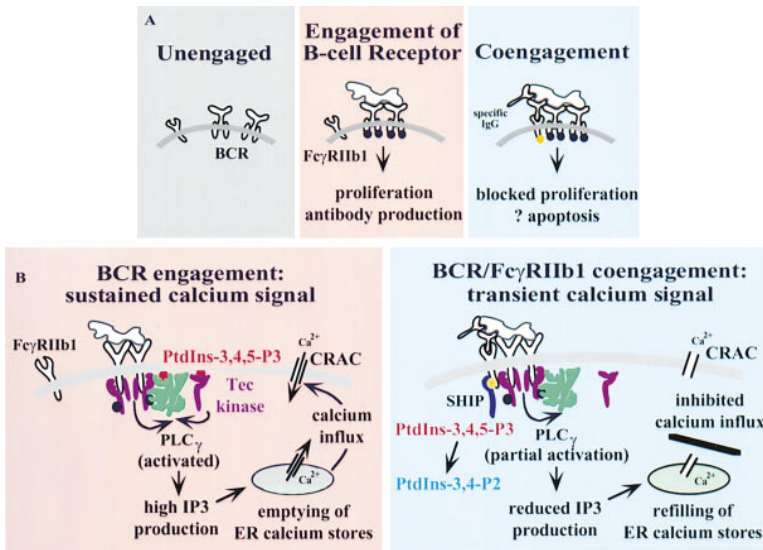
Figure 2. Mechanism of Inhibitory Signaling by FcγRIIb1 in B Cells

(Top panels) FcγRIIb1, a receptor for the Fc portion of immunoglobulin G (IgG), provides a negative feedback signal for terminating humoral immune responses. Black filled circles represent tyrosine phosphorylation of the cytoplasmic portions of the activating B-cell receptor (BCR). Yellow filled circle represents tyrosine phosphorylation of the cytoplasmic portion of the inhibitory FcγRIIb1 receptor. (Bottom panels) Model of biochemical events involved in the inhibition of B-cell receptor calcium signaling by FcγRIIb1. Red filled circles represent PtdIns-3,4,5-P3; black filled circles represent tyrosine phosphorylation of the respective tyrosine kinases. SHIP, *SH*2-containing *I*nositol *P*hosphatase.

partially blocks IP3 production in all systems examined (typically 30%–50%), indicating that PtdIns-3,4,5-P3 is not absolutely required for PLCγ function (Hippen et al., 1997; Bae et al., 1998; Falasca et al., 1998). In addition, in the B-cell system, blockade of PtdIns-3,4,5-P3 accumulation most notably affects the low levels of IP3 accumulation present at later time points after receptor engagement (Hippen et al., 1997). Finally, overexpression of TEC kinases markedly enhances sustained IP3 accumulation and total inositol phosphate turnover (Takata and Kurosaki, 1996; Fluckiger et al., 1998). Therefore, PtdIns-3,4,5-P3 seems to play an adjunctive role in PLCγ activation that is particularly important in sustaining IP3 production.

Role of TEC Kinases: Obligatory Component or Specific Adaptation?

One issue to be resolved in the above model is whether or not TEC family kinases are obligatory components of the PtdIns-3,4,5-P3/PLCγ signaling pathway. TEC kinases are thought to have a relatively restricted expression pattern, having been detected so far primarily in hematopoietic cells and specialized tissues such as heart or endothelia, suggesting that they function to enhance PtdIns-3,4,5-P3-dependent calcium signals in specific contexts. However, available data from the B-cell system indicate that they are required for activation of PLCγ in the surface immunoglobulin receptor (designated B-cell receptor or BCR) context—for example, a chicken B-cell line that is deficient in TEC family kinase expression is essentially unable to produce IP3 in response to BCR engagement (Takata and Kurosaki, 1996). This in turn raises the possibility that TEC kinases might be required cofactors for PLCγ activation in other systems as well. Since the lack of detection of TEC kinases in many tissues may reflect factors such as expression level and so capacity for detection, it is conceivable that known TEC kinases or currently undiscovered homologs are broadly expressed obligatory components of all PLCγ signaling systems, but have simply escaped detection to date.

PtdIns-3,4,5-P3-Dependent IP3 Production as a Target of SHIP-Mediated Inhibitory Signals

The importance of PtdIns-3,4,5-P3 as a regulatory target is dramatically illustrated in the B-cell system by the effect of an inhibitory receptor that blocks PtdIns-3,4,5-P3 accumulation (Figure 2A). During a humoral immune response, antigen binds to cognate (antigen-specific) BCRs, causing B cells bearing those BCRs to proliferate and secrete antigen-specific antibodies (middle panel). Antibody production is in part controlled by negative feedback through an inhibitory signal mediated by the FcγRIIb1 subtype of Fc receptor for immunoglobulin G (IgG). FcγRIIb1 is expressed on all B cells and is coengaged with the BCR once an adequate level of antigen-specific IgG is reached in the course of the response, resulting in a block in proliferation and in some cases apoptosis of the B cell (right panel). For approximately 10 years, it has been known that the inhibitory signal mediated by FcγRIIb1 involves a block in calcium influx and so early termination of the calcium signal initiated by the BCR, engendering a tremendous amount of interest in the inhibitory mechanism of FcγRIIb1. Over the past 2 years this mechanism has been mostly solved (Figure 2B), beginning with the demonstration that CD19-associated PI3K activity is modulated by the FcγRIIb1 inhibitory signal (Hippen et al., 1997; Kiener et al., 1997), and subsequently that the FcγRIIb1 inhibitory signal requires recruitment of SH2-containing inositol 5′-phosphatase (SHIP) (Chacko et al., 1996; Ono et al., 1996, 1997; Gupta et al., 1997). SHIP removes 5′-phosphates from phosphoinositides that are phosphorylated at the D3 position, hence its recruitment into the activated receptor complex blocks the accumulation of PtdIns-3,4,5-P3 (Scharenberg et al., 1998). This eliminates a membrane targeting signal for TEC kinases (Bolland et al., 1998), and both direct PtdIns-3,4,5-P3 and PtdIns-3,4,5-P3/TEC kinase-dependent activation of PLCγ (Fluckiger et al., 1998; Scharenberg et al., 1998), resulting in decreased IP3 accumulation and a transient calcium signal.
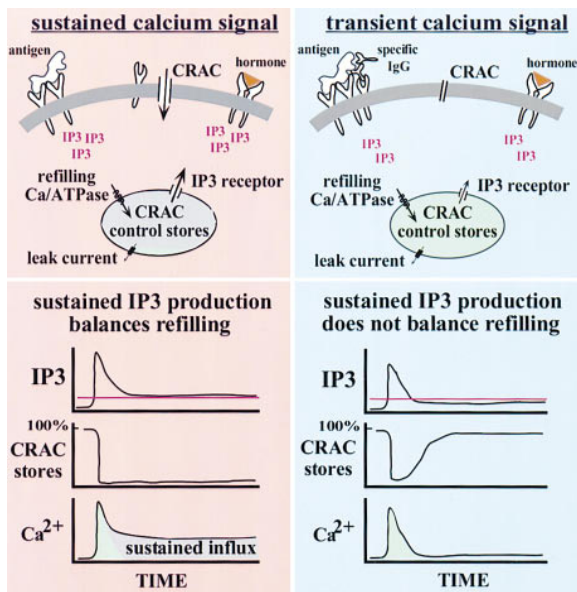
Figure 3. Model for Control of Calcium Influx by Sustained IP3 Production

(Top panels) Schematic of responses of cellular calcium homeostasic mechanisms during transient or sustained calcium signals. Relevant characteristics of cellular calcium homeostasis: Cytoplasmic calcium concentration is maintained at a low level via the action of plasma membrane calcium/ATPases, which pump calcium out of the cell (not shown), and endoplasmic reticulum (ER) store calcium/ATPases (for example, SERCA-type calcium/ATPases), which pump calcium from the cytoplasm into what are thought to be a heterogeneous set of ER stores. Membrane leak currents also run from areas of high calcium (extracellular space [not shown], inside ER stores) to areas of low calcium (cytoplasm). Calcium can exit the various types of ER stores into the cytoplasm via either the action of IP3 receptors or via the leak current. In nonexcitable cells, extracellular calcium influx is thought to occur predominantly through specialized calcium channels (Calcium Release Activated Channels, or CRAC channels), which produce a highly selective calcium current (I_{CRAC}) in response to the emptying of a subset of intracellular calcium stores (CRAC control stores). Patch clamp studies have shown that release of calcium from most intracellular stores occurs in response to a relatively low threshold level of IP3 (Parekh et al., 1997), but that this release can occur without associated activation of I_{CRAC}. In contrast, a higher level of IP3 is thought to be required to saturate cellular IP3 metabolizing processes and thereby raise the IP3 concentration high enough to release the calcium from the CRAC control stores (Parekh et al., 1997). When this threshold level of IP3 is reached, the CRAC control stores empty and calcium influx via CRAC channels is initiated.

(Bottom panels) Proposed model for how cellular calcium fluxes may be dramatically altered in response to slightly different levels of sustained IP3 production. Top graph: IP3 production. Red line on the IP3 graphs indicates the IP3 level at which exit from the CRAC stores would balance refilling of the CRAC stores. Middle graph: filling status (100% = full) of stores that control calcium influx via CRAC channels (CRAC control stores). Bottom graph: resulting cytoplasmic calcium concentration.

One of the most intriguing aspects of PtdIns-3,4,5-P3 function during calcium signaling in the B-cell system is its apparent role in maintaining calcium influx. It has been proposed that PtdIns-3,4,5-P3 and TEC kinases act independently of PLCγ to control calcium influx (Ono et al., 1997; Bolland et al., 1998). While this is formally possible, we outline below a model in which the ability of PtdIns-3,4,5-P3 to support sustained IP3 production, in combination with known characteristics of store-operated calcium influx, is sufficient to explain what is observed in the B-cell system, and we suggest that this mechanism may be of general importance during PLCγ-dependent calcium signals.

Model for Control of Calcium Influx by PtdIns-3,4,5-P3-Dependent IP3 Production

In this model, the critical relationship between PtdIns-3,4,5-P3 and calcium influx arises from the manner in which the kinetics and PtdIns-3,4,5-P3-dependence of IP3 accumulation functionally interact with the mechanisms of calcium homeostasis (a schematic of the relevant aspects of cellular calcium stores and their homeostatic mechanisms are shown in the top panels of Figure 3, and described in the accompanying legend). As noted above, receptor-mediated activation of PLCγ typically results in a large initial peak of IP3 accumulation followed by a low level of sustained accumulation, roughly half of which seems to be dependent on PtdIns-3,4,5-P3. At the initiation of a calcium signal, IP3 causes the release of ER store calcium into the cytoplasm. Since by their very nature intracellular calcium stores are limited, their release into the cytoplasm will only be capable of producing a transient calcium signal. In fact, it is well known that extracellular calcium influx is required for sustained calcium signals. Assuming that Calcium Release–Activated calcium Channels (CRAC channels, see Figure 3 legend) are the primary route for entry of extracellular calcium in nonexcitable cells, it follows that a sustained calcium signal requires sustained opening of CRAC channels, which in turn requires sustained depletion of the subset of ER stores that control their activity (CRAC control stores). If one then considers how the kinetics of IP3 accumulation would affect the depletion of the CRAC control stores, two factors emerge that point to the importance of the PtdIns-3,4,5-P3-dependent component of IP3 production. The first is quantitative. As noted in the Figure 3 legend, it has been shown that a higher IP3 threshold is required to empty the CRAC control stores than other ER stores (Parekh et al., 1997), and the PtdIns-3,4,5-P3-dependent component of IP3 production would contribute to reaching this higher IP3 threshold (Fluckiger et al., 1998). A second factor involves the role of PtdIns-3,4,5-P3 in sustained IP3 production (Figure 3, left panels). The importance of sustained IP3 production arises because the large initial peak of IP3 accumulation would empty the CRAC control stores, thereby initiating calcium influx. Once the CRAC control stores are empty, if the sustained IP3 accumulation generates a total calcium outflow adequate to counteract the pumping effect of the refilling pumps, CRAC store depletion and so calcium influx will be maintained. On the other hand (Figure 3, right panels), just a slightly lower level of IP3 would allow the CRAC control stores to refill and eventually terminate the calcium signal. In this way, once CRAC control stores are emptied, sustained IP3 production is de facto the critical determinant of calcium influx. Consequently, if the contribution of PtdIns-3,4,5-P3 to the sustained IP3 production is necessary to keep calcium exit greater than or equal to calcium refilling, this would explain why modulation of PtdIns-3,4,5-P3 in the B-cell system produces

such a dramatic effect on calcium influx. Furthermore, most other types of nonexcitable cells are thought to have similar calcium homeostatic mechanisms (Parekh et al., 1997), and PI3K inhibition blocks PLCγ-mediated IP3 production and calcium signaling in these systems as well (Barker et al., 1995; Bae et al., 1998; Falasca et al., 1998). This suggests that modulation of PtdIns-3,4,5-P3 during PLCγ-dependent IP3 production may be an important general mechanism for regulating calcium influx and so the nature of any resulting calcium signal.

PtdIns-3,4,5-P3-Mediated Control of Calcium Influx: Downstream Implications

Calcium signals are required to initiate many types of transcriptional events (for example, those involving NFAT-type transcription factors) and proliferative responses (Berridge, 1995). The temporal characteristics of the calcium signal, including whether the signal is transient, sustained, or oscillatory, can be important determinants of the specific transcription factor that is activated, and so the type of transcriptional response that occurs (Dolmetsch et al., 1997). Similarly, sustained calcium signals are often associated with enhanced proliferative responses, as illustrated in the B-cell system discussed above. Therefore, a role for PtdIns-3,4,5-P3 in producing sustained calcium signals implies that regulation of calcium signaling may be an important pathway through which PI3K affects downstream events, and so the ultimate response of a cell.

Summary

PtdIns-3,4,5-P3 has been identified as a component of the signaling pathway utilized by tyrosine kinases for the production of IP3, and so the regulation of cytoplasmic calcium concentrations. Its role in this pathway probably involves both direct actions on PLCγ and indirect actions on PLCγ mediated by TEC family tyrosine kinases. The participation of PtdIns-3,4,5-P3 in calcium signaling implicates its enzymatic parent PI3K in the calcium signaling process, thereby placing calcium within the rapidly expanding group of PI3K effector pathways. Finally, PtdIns-3,4,5-P3-dependent IP3 production appears to be crucial for maintaining the sustained calcium influx required for certain types of calcium-dependent gene regulation and proliferative responses, suggesting that this functional link between the PI3K and PLCγ/calcium signaling pathways may be of broad importance.

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