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REVIEW

Inositol trisphosphate 3-kinases: focus on immune and neuronal signaling

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Abstract The localized control of second messenger levels sculpts dynamic and persistent changes in cell physiology and structure. Inositol trisphosphate [Ins(1,4,5)P_3] 3-kinases (ITPKs) phosphorylate the intracellular second messenger Ins(1,4,5)P_3. These enzymes terminate the signal to release Ca^{2+} from the endoplasmic reticulum and produce the messenger inositol tetrakisphosphate [Ins(1,3,4,5)P_4]. Independent of their enzymatic activity, ITPKs regulate the microstructure of the actin cytoskeleton. The immune phenotypes of ITPK knockout mice raise new questions about how ITPKs control inositol phosphate lifetimes within spatial and temporal domains during lymphocyte maturation. The intense concentration of ITPK on actin inside the dendritic spines of pyramidal neurons suggests a role in signal integration and structural plasticity in the dendrite, and mice lacking neuronal ITPK exhibit memory deficits. Thus, the molecular and anatomical features of ITPKs allow them to regulate the spatiotemporal properties of intracellular signals, leading to the formation of persistent molecular memories.

Keywords Inositol phosphates · Intracellular Ca^{2+} · Lymphocyte · Neutrophil · Pyramidal neuron · F-actin · Rho GTPase · Dendritic spines · Integration

Abbreviations

ACPD	1-Aminocyclopentane-trans-1, 3-dicarboxylic acid
AMPA	Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BCR	B cell receptor
CaM	Calmodulin
CamKII	Ca^{2+}/calmodulin-dependent kinase II
ERK	Extracellular signal-regulated kinase
ER	Endoplasmic reticulum
IP	Inositol phosphate
InsP_3	Inositol trisphosphate [Ins(1,4,5)P_3]
InsP_4	Inositol tetrakisphosphate [Ins(1,3,4,5)P_4]
IPK	Inositol polyphosphate kinase
F-actin	Filamentous actin
INPP5A	Inositol polyphosphate 5-kinase type 1
IPMK	Inositol polyphosphate multikinase
ITK	Interleukin-2 inducible T cell tyrosine kinase
ITPK	Inositol trisphosphate 3-kinase
ITPR	Inositol trisphosphate receptor
LTP	Long term potentiation
NMDA	*N*-methyl-D-aspartate
PH	Pleckstrin homology
PI3K	Phosphatidylinositol lipid 3-kinase
SOC	Stores operated channel
TCR	T cell receptor

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Introduction

How cells translate brief signals from their environment into persistent modifications in physiology, shape, and function during development and behavior is a fundamental problem in cell biology. In the immune system and in the central nervous system, the cellular changes triggered by experience may persist for as long as the organism, creating an integrated memory, encoded by modifications in both cell structure and gene expression. This review first summarizes our current understanding of

the structure, biochemistry, and regulation of the inositol trisphosphate 3-kinase (ITPK) family of enzymes, and then it focuses on recent breakthroughs in our understanding of ITPKs function at immune synapses [1] and in the dendritic spines of neurons [2], two prominent sites of spatiotemporally regulated, integrative signaling that produce cellular memory in higher animals.

Metabolism of inositol trisphosphate

Extracellular stimuli generate intracellular second messengers, which transmit and amplify biochemical information in the temporal and spatial domains [3]. Messengers have short lifetimes inside cells, and highly regulated attenuation systems—such as cyclic nucleotide phosphodieserases, protein phosphatases, and Ca^{2+} sequestration/buffering/extrusion systems—help sculpt intracellular signals and create signaling microdomains [4–7]. In cells enriched in the inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] signaling cascade, inositol trisphosphate 3-kinases (ITPKs) catalyze a selective phosphorylation of the second messenger Ins(1,4,5)P_3 at the 3-OH position on the inositol ring, producing Ins(1,3,4,5)P_4 (Fig. 1) [8]. While Ins(1,4,5)P_3 triggers intracellular Ca^{2+} release from the endoplasmic reticulum (ER) by gating the inositol trisphosphate receptor channel (ITPR), Ins(1,3,4,5)P_4 is not a potent ITPR agonist [9]. Thus, ITPKs terminate Ins(1,4,5)P_3 signals by shortening the Ins(1,4,5)P_3 lifetime. The Ins(1,3,4,5)P_4 produced by ITPKs may itself act as

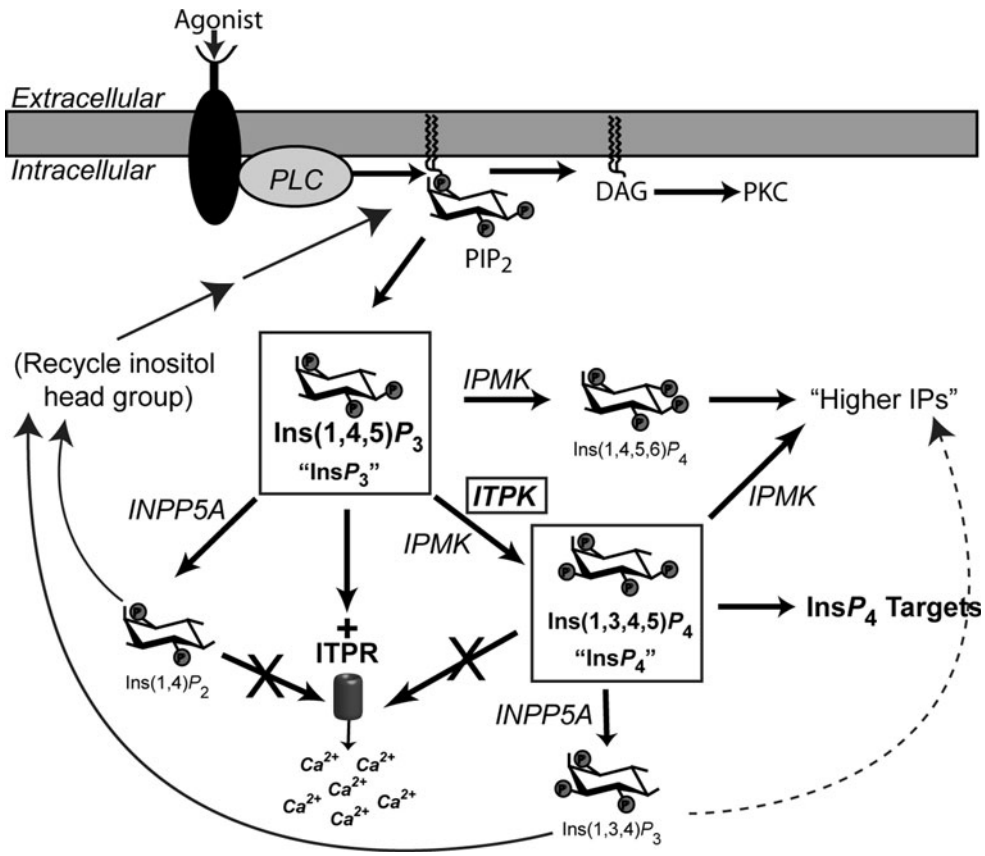

Fig. 1 Ins(1,4,5)P_3 signaling and metabolism. Activation of cell surface receptors triggers the canonical bifurcating pathway in which PLC hydrolyzes the lipid phosphatidylinositol 4,5 bisphosphate (PIP_2). The soluble Ins(1,4,5)P_3 head group diffuses to the ER, where it gates the inositol trisphosphate receptor (ITPR), triggering Ca^{2+} release from intracellular stores. The diacylglycerol (DAG) remains in the lipid bilayer, where it activates protein kinase C (PKC). Ins(1,4,5)P_3 is subject to metabolism by cascades of phosphatases and kinases [10]. Inositol trisphosphate 3-kinases (ITPKs) phosphorylate Ins(1,4,5)P_3 at the 3-OH position to produce Ins(1,3,4,5)P_4, which does not gate ITPR channels but can bind various other protein targets in cells. Inositol polyphosphate multikinase (IPMK) phosphorylates Ins(1,4,5)P_3 twice, at the 6- and 3-positions, and these reactions govern the production of higher inositol phosphates (IPs) [34]. Both Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 are substrates for the type 1 inositol 5-phosphatase (INPP5A) [14]. If Ins(1,4,5)P_3 is the substrate, Ins(1,4,)P_2 is produced, which is dephosphorylated further and ultimately re-incorporated into new inositol lipids [17]. If Ins(1,3,4,5)P_4 is the substrate, Ins(1,3,4)P_3 is produced; the fate of Ins(1,3,4)P_3 varies among cells, and involves both kinase and phosphatase pathways [211]

third messenger by binding to protein targets in cells, and the identification of physiological targets for Ins(1,3,4,5)P_4 is an active area of current research [10–12].

Ins(1,4,5)P_3 signaling to ITPR probably occurs only in metazoans [8]. Multi-cellular animals have evolved complex spatial and temporal components to their intracellular Ca^{2+} response. Since ITPKs evolved soon after the appearance of ITPR, the signaling roles for Ins(1,3,4,5)P_4 are likely to be related to messenger pathways that involve Ins(1,4,5)P_3-triggered Ca^{2+} release. The elusive physiological Ins(1,3,4,5)P_4 receptors will occur in higher organisms only, as components of spatiotemporal elaborations on Ca^{2+} signaling and homeostasis. Some of the most dynamic intracellular Ca^{2+} signaling occurs in the immune and nervous systems, where Ins(1,4,5)P_3 signaling machinery is notably enriched. Extracts from immune or neuronal tissues exhibit 3-tenfold more ITPK activity than other tissues [13]. ITPKs are positioned at points in signaling cascades where they function as translators and integrators of intracellular stimuli, leading to persistent changes in development, physiology and structure of complex metazoan cells.

Both kinases and phosphatases control the lifetime of Ins(1,4,5)P_3 (Fig. 1) [10]. The type 1 Ins(1,4,5)P_3 5-phosphatase, called INPP5A [14], works in parallel to ITPK by catalyzing the hydrolysis of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 at the 5 position. Depending on the substrate, INPP5A produces either Ins(1,4)P_2 or Ins(1,3,4)P_3, and neither of these Ins(1,4,5)P_3 metabolites has known signaling properties. In cells stimulated chronically with Ins(1,4,5)P_3-generating agonists, Ins(1,4)P_2 and Ins(1,3,4)P_3 are the major inositol phosphates that accumulate at steady-state in most [15] but not all [16] cells and tissues. The bulk of these inositol head groups are ultimately re-incorporated into lipid [17]. Thus, the rapid metabolism of receptor-generated Ins(1,4,5)P_3 is a metabolic duet [18] between the 3-kinase and 5-phosphatase, and complex co-metabolism can occur. For example, in some cases the Ins(1,3,4,5)P_4 generated by ITPKs can saturate the activity of INPP5A, prolonging the lifetime of Ins(1,4,5)P_3 [19, 20].

ITPKs resemble other kinases but phosphorylate Ins(1,4,5)P_3 only

Based on the solved crystal structures of the ITPKA catalytic domain [21, 22] (Fig. 2a), ITPKs have been assigned to the lipid kinase-like branch of the group 1 protein kinase superfamily [23]. Although ITPKs are sometimes confused with the more famous phosphatidylinositol lipid 3-kinases (PI3Ks), they are not lipid kinases at all, because their structure sterically prevents them from binding substrates in the lipid membrane [21, 22]. ITPKs share structural homology with an ATP-binding pocket found in the lipid kinase phosphatidylinositol 4-phosphate 5-kinase beta (PIPKIIβ; Fig. 2a). The most conserved part of the ITPK structure is that which binds and holds the ATP substrate in an orientation such that it can donate its gamma phosphate. ITPKs belong to the inositol polyphosphate kinase (IPK) family [11, 24]. This gene family includes not only ITPKs, but also the inositol polyphosphate multikinase (IPMK, also known as IPK2 in yeast and Arabidopsis), and the InsP_6 kinases [25, 26]. For each of these enzymes, the various inositol phosphate substrates are held in orientations that facilitate phosphate transfer [27]. The structural domains that surround this binding site in three-dimensional space determine what the kinase can phosphorylate.

The substrate-binding pockets of most IPK family members are promiscuous. Thus, IPMK, which probably occurs in all eukaryotes, functions as a primitive ITPK by catalyzing either the 3-phosphorlyation of Ins(1,4,5)P_3 to produce Ins(1,3,4,5)P_4 (the same reaction catalyzed by ITPKs) or else the 6-phosphorlylation to produce Ins(1,4,5,6)P_4. Both inositol tetrakisphosphate isomers are themselves substrates for the IPMK, with both reactions producing Ins(1,3,4,5,6)P_5 (Fig 1). The human IPMK is also an Ins(1,3,4,6)P_4 5-kinase [28], as is the IPK2 of Arabidopsis [29]. If substrate concentrations are high, IPMK can catalyze the phosphorylation of phosphate groups to make inositol pyrophosphates, at least in vitro [30]. Lastly, unlike ITPK, IPMK can also act as an inositol lipid 3-kinase [31], which is distinct from the more conventional PI3Ks involved in growth-factor signaling and membrane trafficking. The physiological relevance of this reaction in cells remains uncertain. The closely related InsP_6 kinases catalyze the pyrophosphorylation of various higher inositol phosphates [32]. Again, the substrate-binding pocket is flexible enough to accommodate different inositol phosphate orientations [33], and more than one reaction can be catalyzed. The IPMK pathway to InsP_6 is the major means for producing higher inositol phosphates in eukaryotes [26, 34–36]. The physiological roles of higher inositol phosphates, especially in the nucleus, are under intense study, and this topic has been reviewed elsewhere [10, 24, 37].

By contrast, ITPKs possess exquisite specificity for the 3-phosphorylation of Ins(1,4,5)P_3 only. All available evidence suggests that ITPKs, unlike other IPKs, evolved explicitly for removing the second messenger pool of Ins(1,4,5)P_3. All ITPKs have a similar domain structure, consisting of a conserved catalytic C-terminal end and a variable N-terminal end, which is involved in the targeting and regulation of the enzyme [10] (Fig 2b). The structural explanation for the exquisite ITPK enzymatic specificity

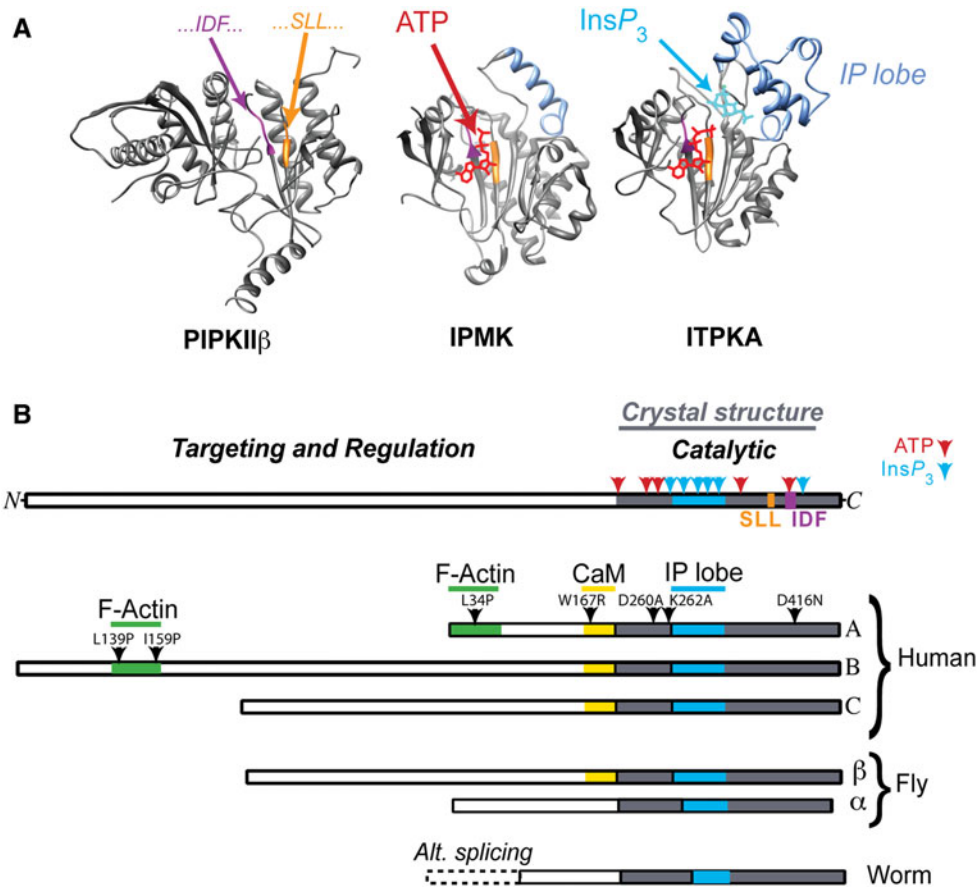

Fig. 2 Structure of ITPKs. **a** Comparison of crystal structures of kinase superfamily members. Crystal structures of human phosphatidylinositol 4-phosphate 5-kinase beta (*PIPKIIβ*, *left*) [212], yeast inositol polyphosphate multikinase (IPMK, *middle*) [27], and human ITPKA (*right*) [21]. Structures are oriented such that one is looking into the active sites, which bind ATP (*red*) through a conserved motif (SLL…IDF…, colored *purple*/*orange*). Inositol phosphates (IPs), such Ins(1,4,5)P_3 (InsP_3, *blue*) are oriented through their interaction with the IP lobe (*blue*), which holds the IP so that phosphotransfer from ATP is facilitated. IPMK (*middle*) has a smaller IP lobe than ITPK, allowing it to accommodate multiple IP substrates; by contrast, the more elaborated IP lobe found in ITPKs (right) holds Ins(1,4,5)P_3 in an orientation that allows phosphorylation at the 3-position only. **b** Domain structure of the three human ITPK isoforms compared to fly and worm varieties. The catalytic domains of ITPKs are highly conserved and located in the C-terminal parts of the protein (*top domain diagram*). By contrast, the amino terminal domains are divergent, and govern selective targeting and regulation in different cells and tissues. All ITPK crystal structures solved thus far have been obtained from catalytic fragments (*gray*), which lack the N-terminal regulatory regions; the catalytic region contains all of the substrate binding sites for ATP (*red*) and InsP_3 (*blue*). In the presence of Ca^{2+}, all three human isoforms bind calmodulin (CaM) in a Ca^{2+}-dependent manner, via a conserved domain [45]. The three human isoforms of ITPK show diversity in their N-termini [8]. Isoforms A [177] and B [77] bind filamentous actin (F-actin), while isoform C shows a mixed cytosolic/nuclear localization [213]. Arrowheads are labeled with point mutations that can selectively destroy domain functions in ITPKA or ITPKB. The L34P mutation destroys F-actin binding in ITPKA [133], while L139P and L159P reduce F-actin binding in ITPKB [77]. The W167R mutation destroys CaM binding in ITPKA; analogous mutations behave similarly for ITPKB and C [45]. *Arrowheads* in the *blue* and *gray* areas point to two of the many mutations in the active site that destroy catalytic activity [21]. The fruit fly *Drosophila melanogaster* possesses two isoforms [42], one of which is positively regulated by calmodulin [45]. The nematode *Caenorhabditis elegans* posesses one ITPK gene, which is not regulated by CaM and shows diversity generated by alternative splicing at the 5′ end [39]

lies in an elaboration of the Ins(1,4,5)P_3 binding site called the IP lobe (Fig 2a) [21]. This stretch of approximately 35 amino acids holds Ins(1,4,5)P_3 in an orientation that constrains phosphorylation to the 3-OH position only and prevents the phosphorylation of lipids. The presence of an elaborated IP lobe distinguishes ITPKs from other IPK family members (Fig 2a).

ITPKs are expressed heterogeneously in metazoan tissues

Table 1 lists some ITPK isoforms and the phenotypes associated with their over- or under-expression. The simplest biochemically validated ITPK, called LFE-2, occurs in the roundworm *C. elegans* [38]. These organisms

Table 1 Comparison of the effects of too much or too little ITPKs in metazoans

ITPK isoform	Expression pattern	Knockout/inactivation phenotype	Overexpression phenotype
Worm (C46H11.4; lfe-2)	Vulval precursor cells of the spermatheca	Suppresses IGF-1 receptor-dependent (let-23) sterility mutations [38]	Sterility due to attenuation of LET-23-triggered $InsP_3$ generation [40]
Fly α, IP3K1 (CG4026; SCIM15)	Tubule, hindgut, brain	Decreased miniochromosome inheritance [43]	Resistance to oxidative stress [44] Attenuation of $InsP_3$ Ca^{2+} response [45]
Fly β, IP3K2 (CG1630)	Tubule	None reported	Attenuation of $InsP_3$ Ca^{2+} response [45]
ITPKA (human chromosome 15q15.1)	Principal neurons in forebrain, plus Purkinje neurons. Testis; expressed late in postnatal brain development	Enhanced LTP in CA1 [48]; decreased LTP in CA3, memory deficits [132]	Attenuation of $InsP_3$ Ca^{2+} response [45, 76, 207–209]
ITPKB (human chromosome 1q42.13)	Immune system: lymphocytes and neutrophils; widespread expression in brain; detectable expression in most other tissues	Failure of T lymphoblasts to mature [51, 52]; defects in B cell selection [110, 117, 121]; hyperactive neutrophil activation [53]; enhanced proliferation of monocyte progenitors [54]	Attenuation of $InsP_3$ Ca^{2+} response [45, 75, 76, 210]
ITPKC (human chromosome 19q13.1)	Expressed in most tissues; expression differences reported between rat [57] and human [58]	Possibly Kawasaki disease [59]	Attenuation of $InsP_3$ Ca^{2+} response [45, 76]

possess one ITPK gene, which exhibits alternative splicing at the N-terminus [39] (Fig 2b, bottom). Besides splicing, no regulatory mechanisms are known for ITPK in *C. elegans*. Ca^{2+}/calmodulin (CaM) does not regulate worm ITPK as it does in mammals [38]. Genetic analysis indicates that the worm version of ITPK regulates signal transduction triggered by insulin-like growth-factor 1 as part of an ITPR and phospholipase C (PLC)-dependent pathway involved in fertility [38]. The worm homolog of INPP5A also regulates Ins(1,4,5)P_3 removal in the same pathway [40]. Thus, from the earliest point in evolution that an ITPK can be identified with certainty, it participates in the regulation of ITPR-dependent cell signaling and works as a team with INPP5A. Although both enzymes regulate Ins(1,4,5)P_3 removal downstream of insulin-like growth factor, the phenotypes produced by under- or over-expression of ITPK or INPP5A are different [40, 41]. Even in this simple organism, fine-tuning Ins(1,4,5)P_3 metabolism produces selective phenotypes.

The fruit fly *Drosophila melanoganster* has two ITPK genes, IP3Kα and IP3Kβ (Table 1) [42]. P-element-based disruption of a chromosomal region that contains the gene for ITPKα (SCIM23) produces a diminution in mini-chromosome inheritance [43], while stable over-expression of ITPKα increases resistance to oxidative stress in a way genetically related to ITPR expression [44]. No phenotypes have yet been reported associated with changes in ITPKβ expression. The two ITPK isoforms in fruit flies can be distinguished by their differential regulation by Ca^{2+}/CaM [45]. Like the worm ITPK, ITPKα is not regulated by CaM (and lacks a CaM-binding domain). A head-to-head comparison of various ITPKs in a mammalian expression system indicated that drosophila ITPKα was the most efficient ITPK tested for its ability to attenuate an agonist-generated Ins(1,4,5)P_3-dependent Ca^{2+} signal, despite its lack of stimulation by Ca^{2+} [45]. By contrast, ITPKβ in drosophila binds CaM in a Ca^{2+}-dependent manner, increasing its activity. Thus, CaM-regulated ITPKs seem to have a lower basal activity in the absence of Ca^{2+}/CaM than do the non-CaM-regulated varieties. This property may allow for better control of the dynamic range of the Ins(1,4,5)P_3 signal. Interestingly, honeybees don't express the isoform that lacks CaM regulation but rather possess different alternatively spliced versions of a CaM-dependent isoform [46]. Ca^{2+}/CaM positively regulates all known ITPKA isoforms in higher animals, creating a negative feedback loop following Ca^{2+} release.

Most mammals have three ITPK genes (Fig. 2b). The isoforms exhibit variable expression patterns [13], and reducing or enhancing their expression produces different phenotypes in different cells and tissues (Table 1). One consistent finding is that over-expression of ITPKs in cells produces a reduction in the Ins(1,4,5)P_3-triggered Ca^{2+} signal in response to agonist. Isoform A (ITPKA) is found mainly in the principal neurons of the forebrain and in the cerebellar Purkinje neurons [47]. Mice with targeted disruption of ITPKA show large changes in the degree of long-term potentiation (LTP) in the hippocampus [48, 49]

(see later section). Isoform B (ITPKB) is widely expressed [50], with notably high levels in immune tissues. Mice lacking ITPKB exhibit major deficits in lymphocyte maturation [51, 52], neutrophil activation [53], and myelopoiesis [54]. The proposed molecular mechanisms underlying these deficits will be discussed in more detail in the sections below. Abundant ITPKB expression also occurs in brain, probably in glial cells [55, 56]. It should be noted, however, that microarrays from human tissue (rather than rodents) indicate substantial ITPKB expression in gray matter regions that lack ITPKA expression [52]. This suggests that a subset of neurons expresses ITPKB, at least in humans. Isoform C (ITPKC) is also widely expressed [57, 58]. Recently, genetic mutations in the human ITPKC gene have been linked to Kawasaki disease, an autoimmune disease [59]. The available evidence suggests that an overactive Ins(1,4,5)P_3 signaling pathway leading to the transcription factor NFAT may underlie the immune hyperactivity (see later section).

ITPKs are regulated by phosphorylation

In addition to direct stimulation by Ca^{2+}/CaM, at least three other mechanisms regulate ITPKs: phosphorylation, proteolysis, and intracellular targeting (Fig. 3). No new studies addressing the regulation of ITPKs by protein phosphorylation have been published in almost a decade, yet a surprising amount remains unclear. In cells or tissues expressing ITPKA or ITPKB, pretreatment with the protein phosphatase inhibitor okadaic acid prior to addition of agonist is the most effective means of prolonging Ins(1,3,4,5)P_4 generation [56, 60]. This may be because Ca^{2+}/CaM-dependent protein kinase II (CaMKII) stimulates ITPKA and ITPKB and simultaneously inhibits INPP5A [61]. CamKII increases enzyme activity by increasing V_{max} but no change in K_m. Protein phosphorylation increases the affinity of ITPKA (but not ITPKB) for Ca^{2+}/CaM. By contrast, phosphorylation causes ITPKB (but not ITPKA) to translocate from the cytosolic to the membrane fraction [56]. ITPKC lacks the CamKII phosphorylation site altogether, and its regulation by phosphorylation has been examined in much less detail than the other isoforms.

In vitro, ITPKA is also a substrate for both cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC). The effects of these protein kinases on ITPKs are difficult to generalize and may be different among the isoforms. Some studies found that PKA increased the V_{max} of ITPKA and ITPKB about twofold [62, 63], but another study found no effect of the adenylate cyclase activator forskolin on either isoform [56]. Likewise, the effects of PKC differ among the reports in the literature, with some studies suggesting inhibition [62, 63], and others a modest stimulation [56]. The discrepancies appear to be due to the particular enzyme preparation assayed, its state of

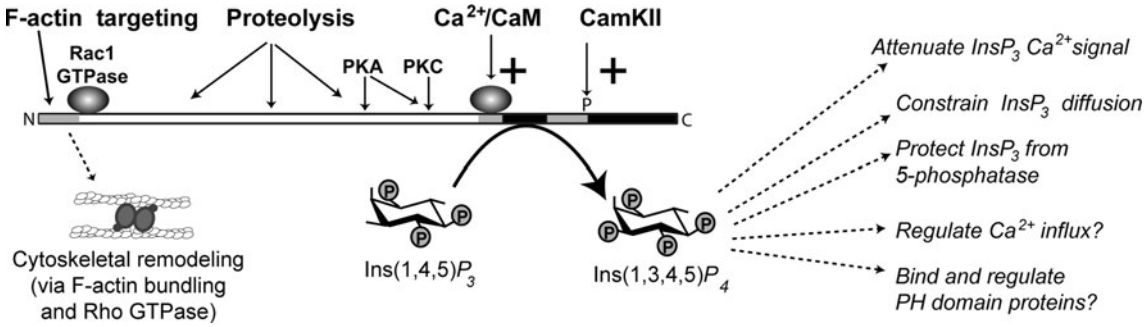

Fig. 3 Regulation of ITPKs and its consequences. ITPK action is regulated by at least four mechanisms. All mammalian ITPKs are positively regulated by Ca^{2+}/calmodulin; this creates a negative feedback loop on the Ins(1,4,5)P_3 signal to release Ca^{2+} via ITPR [45]. ITPKs are also regulated by various protein kinases, such as protein kinase C (PKC), protein kinase A (PKA), and Ca^{2+}/CaM-activated protein kinase II (CamKII). Phosphorylation at some sites activates the enzyme, and at others inhibits it (see text). The amino terminal regions of ITPKA and ITPKB bind F-actin, which targets the enzymes near sites of Ins(1,4,5)P_3 generation. The ITPKA amino terminal-binding region [177] affects F-actin structure through a mechanism independent of ITPKA catalytic activity [179]. This mechanism has been linked to the Rho family GTPase Rac1 [132]. The F-actin remodeling involves cross-linking or bundling of the actin filaments, and controls the selective targeting of ITPKA to dendritic spines [133]. ITPKs are subject to the actions of proteases, which usually cleave between the N-terminal targeting region and the catalytic region. Protease cleavage of ITPKs changes enzymatic localization [74, 75], and can also affect the sensitivity to or substrate sites for protein kinases [70]. ITPK catalysis has many suggested functions in cells [10]. These can be subdivided into functions involving the attenuation of Ins(1,4,5)P_3 signals via restriction of those signals to spatial and temporal domains through the control of Ins(1,4,5)P_3 lifetime, and those which depend on the specific actions of the enzymatic product Ins(1,3,4,5)P_4. Cells possess a variety of Ins(1,3,4,5)P_4-binding proteins. Some of these bind Ins(1,3,4,5)P_4 selectively via their pleckstrin homology (PH) domains; others are channel proteins whose gating or conductance properties change upon Ins(1,3,4,5)P_4 binding (see text)

proteolysis, the time of incubation with the kinase, the isoform being studied, and/or the presence of CaM. Furthermore, combinations of protein kinases may produce effects different from each kinase alone [56, 64]. The available data suggest that multiple kinases, perhaps in conjunction with proteolysis and CaM, differentially regulate different ITPK isoforms. Further studies will be required to resolve the specifics.

Regulation by proteolysis and intracellular targeting

Early biochemical studies showed that ITPKA is highly susceptible to proteolysis during purification [65, 66]. ITPKA purified from brain runs on gels as a ladder of three to five catalytically active bands between 53 kD and about 30 kD. Inclusion of calpain inhibitors in the buffers during purification reduces the laddering [66]. When ITPKA was molecularly cloned [67], it was reported that the protein contained a PEST sequence, which is a stretch of roughly 12–20 amino acids hypothesized to cause a protein to be highly susceptible to proteolysis through Ca^{2+}-regulated proteases (calpains), followed by rapid (1–2 h) degradation [68]. PEST stands for a proline (P), glutamine (E), serine (S), and threonine (T)-rich stretch of amino acids, and originally the PEST sequence was said to occur between residues 99 and 136 of the full-length rat sequence [67].

PEST sequences occur with high frequency in CaM-binding proteins [69]. More refined versions of the original prediction program (PESTfind) identify one very weak PEST sequence in ITPKA, now re-assigned to the middle of the IP lobe, where proteolysis would almost certainly destroy enzyme activity [70]. Since all proteolytic products isolated biochemically retain enzymatic activity [65, 66], the PEST sequence in ITPKA, if it exists, is not directing the proteolysis by Ca^{2+}-activated proteases. Indeed, the PEST hypothesis has now been modified to exclude calpain-mediated mechanisms [68, 71]. Immunolocalization of ITPKA in brain suggests very low levels of ITPKA in the neuronal cell soma, indicating that most or all of the protein remains anchored to the synaptic cytoskeleton in vivo [72]. Consistent with this, we have tagged ITPKA with enhanced green fluorescent protein (EGFP) at the C-terminus and then expressed the fusion protein in hippocampal neurons for up to 2 weeks, yet we do not observe a significant cytosolic GFP fluorescence. Therefore, despite the obvious susceptibility of ITPKA to proteolysis during purification, the idea that ITPKA contains a PEST sequence that directs its proteolysis inside cells should be retired.

By contrast, many studies indicate the importance of proteolysis in regulating ITPKB and ITPKC. PESTfind predicts a number of strong PEST sequences in the amino terminal halves of ITPKB and ITPKC, as has been discussed in more detail elsewhere [70, 73]. No data have yet emerged to indicate that these sequences play a significant role in the regulation of ITPK proteolysis. Nevertheless, recent experimental evidence supports the general idea that ITPKB is regulated by proteolysis, with the functional consequence being that proteolysis changes the enzyme's subcellular localization [74, 75]. When full-length ITPKB is expressed in cells, it exhibits a heterogeneous localization and occurs with different molecular weights [50, 74–76]. Depending on the cell, ITPKB is associated with the actin cytoskeleton, on intracellular membranes, or in the cytosol. The F-actin-binding domain of rat ITPKB was identified between amino acids 108 and 170 [77]. Proteolytic cleavage separates the catalytic region from its cytoskeletal-targeting domain, thereby changing the cellular location of ITPK activity (Fig. 3). A shift in the cellular location of Ins(1,4,5)P_3 phosphorylation could potentially affect the spatiotemporal dynamics of Ins(1,4,5)P_3 Ca^{2+} signals. In fact, we have demonstrated that cytoskeletal anchoring of ITPKA [45] and ITPKB [75] enhances the ability of the enzyme to attenuate a Ca^{2+} signal. Earlier studies of ITPKB that used a truncated version of the protein that lacked the F-actin-binding region localized ITPKB to the ER membrane, and it would be interesting to investigate how placing an ITPK near the ITPR instead of close to the site of Ins(1,4,5)P_3 generation would influence the spatiotemporal dynamics of the ITPR-induced Ca^{2+} signal.

ITPKs regulate Ins(1,4,5)P_3 lifetime

The ER Ca^{2+} stores are contiguous [78, 79]. Cytosolic Ca^{2+} sequestered in one part of the cell's ER can be released at remote sites at the other end of the cell [80]. Signals controlling the intracellular release of Ca^{2+} depend not only on the site(s) of Ins(1,4,5)P_3 generation, but also the spatiotemporal relationships between Ca^{2+} dynamics, Ins(1,4,5)P_3 diffusion/metabolism, and ITPR. In this context, where and when Ins(1,4,5)P_3 is removed may determine the local or global properties of the Ca^{2+} signal. ITPK-regulated signals can be subdivided into those that involve modulating of Ins(1,4,5)P_3 lifetime and those that require the rapid, selective generation of a messenger pool of Ins(1,3,4,5)P_4 [8]. Figure 3 depicts a summary of ITPK regulatory mechanisms and also indicates the major consequences of ITPK activity suggested from a variety of studies.

How ITPKs control Ins(1,4,5)P_3 lifetime and influence the spatiotemporal properties of the Ca^{2+} signal remains largely unknown. Ca^{2+} itself does not diffuse very far within a cell, owing to extrusion, sequestration, and

buffering [6, 81–83]. Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 can diffuse much longer distances and may persist in cells for seconds or even minutes [83–86]. The lifetime of an Ins(1,4,5)P_3 molecule is determined chiefly by its rate of metabolism, and possibly by the presence of Ins(1,4,5)P_3 buffering proteins. The functional lifetime of an Ins(1,4,5)P_3 molecule is also subject to passive diffusion [87]. As locally generated Ins(1,4,5)P_3 equilibrates in a cell, its concentration may fall below its affinity for binding ITPR, silencing the signal. ITPKs, INPP5A, and IPMK probably all participate in Ins(1,4,5)P_3 metabolism (Fig. 1). Their relative influence in shaping the lifetime of the receptor-generated pool of Ins(1,4,5)P_3 is poorly understood, and varies among cells types and within subdomains of single cells [10, 24, 37, 45].

In at least eight published reports, the over-expression of an ITPK isoform causes a dramatic attenuation in the ability of cells to produce a Ca^{2+} signal via Ins(1,4,5)P_3-generating agonists (see Table 1). In these studies, it is assumed that ITPKs shorten the lifetime of Ins(1,4,5)P_3 in the cell. ITPKs have a higher affinity for Ins(1,4,5)P_3 than does INPP5A, so they have privileged access to pools of Ins(1,4,5)P_3 produced rapidly upon receptor stimulation. By contrast, INPP5A has a much greater V_{max}, so its influence is more on the steady-state concentration of Ins(1,4,5)P_3 in the cell. These studies are borne out by knockout and knockdown studies. ITPK knockout mice show no change in their basal Ins(1,4,5)P_3 levels [48, 51], while INPP5A knockdown cells show increased steady-state Ins(1,4,5)P_3 levels, and a lower threshold for triggering a Ca^{2+} response [88].

On the other hand, Ins(1,3,4,5)P_4 can also prolong the lifetime of Ins(1,4,5)P_3 by competitively inhibiting the phosphatase INPP5A [19]. In this scenario, a cell stimulus generates Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4. The Ins(1,3,4,5)P_4 that lingers following the stimulus functions as a "memory trace" of recent cellular activity. The counterintuitive prolonging of the Ins(1,4,5)P_3 lifetime by ITPK occurs because the affinity of Ins(1,3,4,5)P_4 for INPP5A is greater than for Ins(1,4,5)P_3 [89]. Thus, if a second Ins(1,4,5)P_3-generating stimulus arrives before Ins(1,3,4,5)P_4 returns to basal levels, Ins(1,3,4,5)P_4 will "protect" the Ins(1,4,5)P_3 by competitively inhibiting INPP5A. This mechanism may be further reinforced if the Ca^{2+}/CaM-dependent kinase II (CamKII) is present in the cell, since this protein kinase enhances the activity of ITPKA [60] and ITPKB [56], but inhibits INPP5A [61]. In any cell where ITPK and INPP5A collaborate to control the Ins(1,4,5)P_3 lifetime, a prior Ins(1,3,4,5)P_4 signal will likely prime the system towards enhanced Ins(1,3,4,5)P_4 production and possibly increased Ins(1,4,5)P_3 lifetime if a second Ins(1,4,5)P_3 signal arrives while Ins(1,3,4,5)P_4 levels remain elevated. This model of cellular coincidence detection has been called the "InsP_4 protection racket" [20].

There is no a priori reason to assume that Ins(1,4,5)P_3 metabolism is involved at all in the generation of frequency-encoded calcium signals in cells. Ca^{2+} oscillations can be triggered in cells by introducing metabolically inert Ins(1,4,5)P_3 analogs [90]. A number of ITPK-independent mechanisms can account for physiological oscillations, such as Ca^{2+} or protein kinase feedback on surface receptors, ITPRs, or on phospholipase C [91, 92]. Nevertheless, mathematical modeling suggests that physiological conditions occur inside cells when ITPK activity affects the threshold, waveform, or frequency of Ca^{2+} oscillations [93–96]. Such models take into account enzyme and receptor affinities, enzyme velocities, and positive and negative feedbacks by Ca^{2+}. The aim of these models is to predict the conditions under which cells exhibit global calcium spiking and oscillation, thereby adding frequency encoding to the Ca^{2+} signal. A related question is whether or not cellular levels of inositol phosphates themselves exhibit frequency encoding. The development of fluorescent biosensors has led to evidence that Ins(1,4,5)P_3 levels in cells do indeed exhibit periodic behaviors [97–99].

ITPKs as generators of Ins(1,3,4,5)P_4 signals to downstream targets

The rapid and highly selective generation of Ins(1,3,4,5)P_4 in response to intracellular stimuli implies that the attenuation of a second messenger may lead to the creation of a third one. Despite more than 20 years of research, the identities of Ins(1,3,4,5)P_4-based signaling systems remain unclear [10, 100]. Following the discovery of the receptor-generated Ins(1,3,4,5)P_4 pathway in cells, receptor binding studies spearheaded the search for Ins(1,3,4,5)P_4 receptors [101–103]. The vast majority of Ins(1,3,4,5)P_4-binding proteins isolated in these studies contain pleckstrin homology (PH) domains that also bind to inositol lipids (Table 2). These include the Centaurins/p42IP4 [104, 105] and the GAP1 family of Ras GTPases, especially RASA3 [106, 107]. All have been suggested to be involved in Ins(1,3,4,5)P_4 signaling, but how is far from clear.

In the majority of cases, the downstream consequence of Ins(1,3,4,5)P_4 action is modulation of the Ras/extracellular signal-regulated protein kinase (ERK) signaling system [51, 52, 107, 108]. Among the various models suggested, Ins(1,3,4,5)P_4 has been proposed to function either as an activator of RASA3 GAP activity [107, 109], or as an inhibitor RASA3 GAP activity by removing RASA3 from the plasma membrane through competition with the lipid phosphatidylinositol (3,4,5)P_3 [110] (see Fig. 4). Differences in results may be partly explained by which isoform of Ras (or Rap; [111]) is activated. Moreover, the cellular location of Ras activity (plasma membrane versus Golgi

Table 2 Candidate Ins(1,3,4,5)P_4 targets. Ins(1,3,4,5)P_4 targets are usually PH domains or channels. The immune system is rich in a variety of PH-domain containing proteins, and putative Ins(1,3,4,5)P_4-modulated channels

Cell type	Molecular target	Suggested effect of $InsP_4$
Thymocyte/T cell	ITK	Enhanced binding of ITK to plasma membrane lipid [123]
B lymphoblast	$GAP1^{IP4BP}$ (Rasa3), a GAP for Ras or Rap [111]	Reduced GAP1 activity at plasma membrane [110]
Many cells	Centaurin/p42IP4, an Arf6 GAP	Compete with PIP_3 binding at plasma membrane [105]
B lymphoblast	Store-operated Ca^{2+} channel	Inhibition of Ca^{2+} influx [117, 121]
Granulocyte/monocyte progenitors	PH domain of AKT	Reduced AKT translocation to plasma membrane [54]
Neutrophil	PH domain of AKT	Reduced AKT translocation to plasma membrane [53]
RBL mast cell	INPP5A	Saturation of INPP5A, leading to increased IP_3 lifetime [19]
RBL mast cell	RASA3 ($GAP1^{IP4BP}$)	Enhanced Ras GAP activity [109]
Ras-transformed NIH/3T3 fibroblast	Voltage-regulated influx channel in response to bradykinin	$InsP_4$-enhanced channels, especially if cell is hyperpolarized [116]
Pyramidal neuron	Voltage-regulated Ca^{2+} channel(s)	Increased channel open time [118, 119]

apparatus) has emerged as a major mechanism of Ras regulation relevant to these different signaling systems, adding an additional layer of complexity to Ras activation relevant to Ins(1,3,4,5)P_4 [112, 113].

The second major class of Ins(1,3,4,5)P_4 receptors are channel proteins located in the plasma membrane. Channels were one of earliest suggested identities for Ins(1,3,4,5)P_4 effectors [100, 114]. For example, one series of studies has demonstrated Ins(1,3,4,5)P_4 enhancement of channels in a tyrosine kinase-triggered PLCγ signaling pathway that used ras-transformed NIH/3T3 fibroblasts [115]. This pathway required tyrosine kinase activation [116] and was hypothesized to require the GAP1 protein RASA3 [115]. This channel system resembles one characterized in immune cells [117]. Other channels implicated as Ins(1,3,4,5)P_4 targets include a Ca^{2+}-dependent Cl-channel [114]. In the nervous system, the available data indicate that Ins(1,3,4,5)P_4 can enhance the activity of voltage-gated Ca^{2+} channels in pyramidal neurons located in the CA1 layer of the hippocampus [118, 119].

Widespread roles for ITPKs in the immune response

Some of the most compelling recent data supporting a signaling role for Ins(1,3,4,5)P_4 have been obtained from studies in the immune system. ITPKB knockout mice exhibit deficits throughout both the acquired and innate branches of the system (Fig. 4). Deficits in innate immunity include overactive neutrophils [53, 54] and mast cells [109]. The suggested molecular mechanisms involve competition of Ins(1,3,4,5)P_4 with the lipid PIP_3, to bind to and regulate the function of proteins with PH domains, notably the serine/threonine kinase AKT [54] and RASA3 [109]. Some of the roles of ITPKs in innate immunity are depicted in Fig. 4d, e, and this topic was recently reviewed [120].

ITPKB plays a critical role in T cell development [51, 52]. Mice with targeted disruption in ITPKB exhibit massive defects in T cell maturation in the thymus. Specifically, T cells undergo negative selection normally but not positive selection. Lack of ITPKB during T cell selection causes a 90% reduction in the number of T cells circulating in peripheral lymphoid organs [51]. ITPKB also plays a crucial role in B cell survival and selection [110, 117, 121]. Mice with ITPKB knockout have tolerant B lymphocytes, which undergo inappropriate apoptosis. Recent data suggest that the B cell anergy observed in ITPKB knockout mouse lymphocytes is linked to enhancement of Ca^{2+} entry [121]. Whether the effect of ITPKB involves a direct Ins(1,3,4,5)P_4 inhibition of Ca^{2+} stores-operated channels (SOCs), or rather an indirect effect via enhanced Ca^{2+} stores depletion (or both) remains unclear.

The molecular mechanisms underlying the defects in thymocyte maturation are intriguing because they provide the strongest evidence for ITPK-generated, Ins(1,3,4,5)P_4-selective signal transduction [12, 122]. The intracellular Ca^{2+} responses measured in thymocytes lacking ITPKB appear to be normal following signaling through the T cell receptor [51]. However, the thymocytes exhibited reduced activation of the Ras–ERK pathway following TCR stimulation [51, 52]. The molecular mechanisms explaining these phenotypes remain incompletely defined. In one study [123], Ins(1,3,4,5)P_4 is suggested to function in a feed-forward mechanism in which it binds to the interleukin-2 inducible T cell tyrosine kinase (ITK) and enhances its phosphorylation of downstream targets (Fig. 4a). However, other studies in lymphocytes suggest a different molecular mechanism that involves Ins(1,3,4,5)P_4 modulation of the Ras pathway, possibly via its action on a GTPase activating protein of the GAP1 family (Fig 4a, b) [111]. The situation in B cells is also hypothesized to

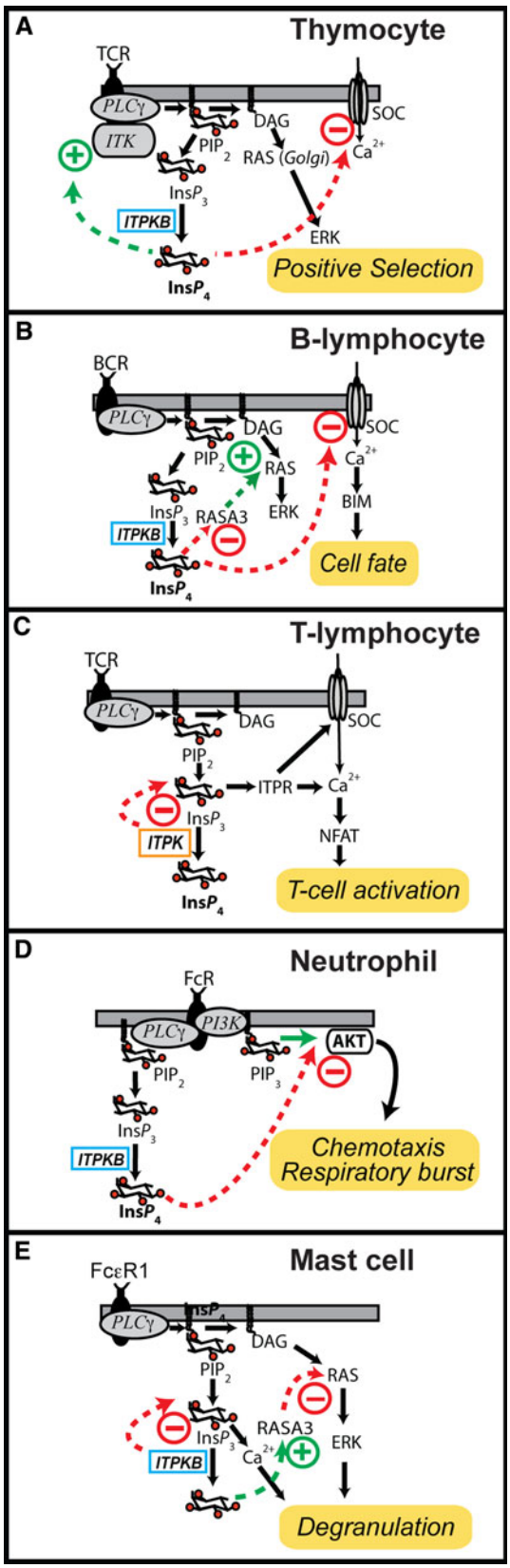

Fig. 4 Diversity of ITPK signaling in immune cells. **a** Mice with ▶ targeted disruption in the gene for ITPKB exhibit major deficits in thymocyte maturation, with very few thymocytes undergoing positive selection [51, 52]. Activation of the T cell receptor in thymocytes derived from these mice results in less MAP kinase activation than in controls. At least two molecular mechanisms have been proposed to explain the phenotype. In one mechanism, Ins(1,3,4,5)P_4 triggers a feed-forward mechanism, whereby it binds and activates the interleukin-2 inducible T cell tyrosine kinase (ITK) [123] (*green arrow*). Downstream targets of ITK, such as phospholipase C gamma (PLCγ1), are thus hypoactive in the knockouts. A second mechanism proposes that store-operated Ca^{2+} channels (SOCs) are negatively regulated by Ins(1,3,4,5)P_4 (*red arrow*). In this model, lack of Ins(1,3,4,5)P_4 causes excessive Ca^{2+} influx, and this leads to deficits in Ca^{2+} homeostasis and its associated signaling [117]. **b** B lymphocytes from ITPKB knockout are anergic and undergo excessive apoptosis. Knockout B cells exhibit enhanced Ca^{2+} influx through SOCs, and this is attributed to a loss of normal inhibition on SOC by Ins(1,3,4,5)P_4 [117, 121] (*red arrow*). A second model suggests that Ins(1,3,4,5)P_4 is a negative regulator RASA3, a Ras GTPase activating protein that binds Ins(1,3,4,5)P_4 with high affinity [110] (*red arrow*). In this model, lack of Ins(1,3,4,5)P_4 leads to excessive GTPase activity, thus attenuating Ras and its downstream targets [110]. **c** Proposed role for ITPK signaling in mature, circulating T lymphocytes based on polymorphisms in the ITPKC gene linked to Kawasaki disease, an autoimmune disorder [59]. This model posits a hyperactive Ins(1,4,5)P_3-triggered Ca^{2+} response following activation of the T cell receptor (TCR), leading to excessive activation of the phosphatase calcineurin, causing dephosphorylation and over activation of the transcription factor NFAT. The hyperactive NFAT would result in an inappropriately large immune response in the vascular and mucosal systems, accounting for the Kawasaki disease phenotype. In contrast to the other immune models, no gain-of-function role for Ins(1,3,4,5)P_4 is invoked. **d** ITPKB also plays widespread roles in the innate immune system [120]. In neutrophils, Ins(1,3,4,5)P_4 generated downstream of Fc receptor (FcR) activation is proposed to compete with the head group of the lipid phosphatidylinositol (3,4,5)P_3 (PIP_3) for binding to the tyrosine kinase AKT. Thus, neutrophils derived from ITPKB knockouts exhibit enhanced PIP_3-regulated responses, such as chemotaxis and the respiratory burst [53]. **e** Yet another mechanism is suggested to explain ITPKB signaling in mast cells [109]. Here, Fc receptor epsilon 1 (FcεR1) regulates degranulation via two ITPKB-dependent processes. The first is a straightforward reduction of Ca^{2+} release via ITPKB attenuation of Ins(1,4,5)P_3 signals (red arrow). The second, in contrast to the mechanism depicted in panel B, involves a positive regulation of RASA3 and concomitant negative effect on Ras activation status. In either mechanism shown in panel **e**, lack of ITPKB would produce hyperactive degranulation

involve GAP1 modulation of the Ras pathway, leading defects in B cell selection to the control of the pro-apoptotic gene Bim following BCR stimulation (Fig 4b) [110]. In addition, ITPKB knockout B cells exhibit increased capacitative Ca^{2+} influx via SOCs following activation the BCR [121]. The proposed mechanism is that Ins(1,3,4,5)P_4 negatively regulates SOC (Fig. 4b), but it is not clear if Ins(1,3,4,5)P_4 binds directly to the channel or requires a transducer.

A summary of the immune data obtained from knockout studies (Fig. 4) suggests that Ins(1,3,4,5)P_4 is capable of acting on different or multiple targets in different cells, and surprisingly, most of the ITPKB-dependent mechanisms do not seem to involve the attenuation of Ins(1,4,5)P_3 Ca^{2+} signals. As further data emerge, the situation is likely to turn out to be less absolute because the interplay among Ca^{2+} release, Ca^{2+} entry, and Ras regulation is coordinated both spatially and temporally [124]. The spatiotemporal control of Ca^{2+} responses is critical for proper lymphocyte maturation and activation [125, 126]. The Ins(1,4,5)P_3-triggered Ca^{2+} response and subsequent sustained Ca^{2+} influx through stores operated channels (SOCs) regulate a range of T cell responses on different spatiotemporal scales [125]. The proper coordination of local and global Ca^{2+} signals is essential for eliciting appropriate immune signals emanating from the immunological synapse and determines both transcriptional profile and developmental fate [1].

Many immune responses require that the signal produced by a surface receptor be tuned to "just right," meaning that either too much or too little activation can lead to disease [125, 127–129]. Ca^{2+} spikes and oscillations occur following TCR or BCR activation over time scales from seconds to hours. For example, the intracellular polarity and frequency of the Ca^{2+} signals are crucial to developing T cells in the thymus [130]. Here, they regulate positive selection by prolonging thymocyte interactions with the stroma [131]. Furthermore, the degree of activation of the Ras/ERK pathway depends both on the frequency coding of Ca^{2+} signal [124] and on the intracellular membrane locus of Ras [112, 113]. The localized, Ca^{2+}-dependent generation of Ins(1,3,4,5)P_4 from Ins(1,4,5)P_3 can be imagined to participate in many of these processes, and investigations into ITPKB functions in immune development, tolerance, selection, and memory will remain fruitful areas of research for the foreseeable future [12, 120, 122].

Independent of ITPKB control of Ca^{2+} signals and Ins(1,3,4,5)P_4 levels, the phenotypes of ITPKB knockout mice require reconsideration in the context of the recent discovery that the amino terminus of the brain isoform ITPKA functions as a scaffold for Rho GTPases and associated F-actin machinery [132]. It is sobering to consider that the amino terminal regulatory region of ITPKB is about four times as long as that of ITPKA. It interacts with F-actin, shows structural homology to the actin-binding domain of ITPKA [77, 133], and remains largely uncharacterized. Since Rho GTPases are crucial regulators of lymphocyte development, polarity, and function [134], it is interesting to speculate that some of the phenotypes observed in the immune system of ITPKB knockouts are explained by the loss of a non-enzymatic function of ITPKB, such as control of the cytoskeleton.

ITPKC polymorphisms linked with Kawasaki disease

Because thymocytes in ITPKB knockout mice fail to mature normally, the knockout studies tell us little about the roles for ITPKs in mature, circulating T cells during activation of TCR. One recent hint of ITPK function in T cells comes from a suggested link between ITPKC and the auto-immune disorder Kawasaki disease—an acute, systemic vasculitis, which affects mainly infants and children [59]. In Kawasaki disease, an unidentified infectious agent triggers hyper-activation and inflammation of the vascular and mucosal systems in genetically susceptible children [135]. Kawasaki disease can cause coronary aneurisms, and this is the most common cause of acquired heart disease in children in Japan and the US [136].

Single-nucleotide polymorphisms in ITPKC suggest that reduced ITPC activity produces a hyper-activated T cell responsible for inappropriate immune activation [59]. In this model, increased Ins(1,4,5)P_3 lifetimes in ITPKC-hypoactive patients increases the release of intracellular Ca^{2+}. The Ca^{2+}/CaM-activated protein phosphatase calcineurin becomes hyper-activated, and the transcription factor NFAT becomes dephosphorylated, activating inappropriate transcription in the T cell [128]. This model is depicted in Fig. 4c, and it is distinguished from other cell signaling systems depicted in the figure because there is no explicit requirement for Ins(1,3,4,5)P_4.

Cellular and developmental co-expression of ITPR1 and ITPKA in brain

The sequestration and release of intracellular Ca^{2+} contributes substantially to the broad palette of spatiotemporal signaling in neurons, and this topic is comprehensively reviewed elsewhere [7, 137, 138]. The goal here is to highlight those systems most likely to be regulated by ITPKA. Brain is more enriched in the intracellular Ca^{2+} signaling "toolkit" than other tissues [3, 9, 139]. ITPR1, which is the main neuronal isoform of the intracellular Ca^{2+} release channel, is notably concentrated within large spiny neurons, such as pyramidal neurons in the hippocampus and cerebral cortex, and cerebellar Purkinje cells [140]. ITPKA is concentrated in most of these same cells, but the levels of co-expression with ITPR1 are not absolutely correlated [141, 142]. For example, the expression of ITPKA in CA1 pyramidal neurons is greater than it is in Purkinje cells, despite the much higher expression of ITPR1 in Purkinje neurons. By contrast, Purkinje neurons have much higher levels of the Ins(1,4,5)P_3 phosphatase INPP5A than do pyramidal neurons [143]. Thalamic areas have moderate ITPR1 expression, but virtually no ITPKA [142].

ITPR and ITPKA are also co-expressed ontologically. The intracellular Ca^{2+} signaling system arises late in development, appearing during the first postnatal week and reaching its full expression only after 4 weeks in rodents [18, 143–145]. In hippocampus, ITPR-related systems increase during and following periods of robust synaptogenesis (postnatal weeks 2 and 3 in rodents). ITPR and ITPKA expression in neurons both appear to be controlled by an endogenous developmental clock, because the increase in expression between postnatal weeks 1 and 3 is retained when neonatal cells are grown in dissociated culture, separated from anatomical constraints of their normal synaptic inputs [146, 147].

There are probably no ITPKA-positive/ITPR1-negative neurons, yet certain higher brain regions are notable for their extreme ITPKA levels relative to ITPR1 [141, 142]. The pyramidal neurons in the CA1 region of hippocampus express more ITPKA than anywhere else in the body (Fig. 5a, left). Within the cerebral cortex, ITPKA protein levels are especially high in the synapse-rich layers 1 and 3 [72], while IPTR1 localizes in soma-rich layers 2 and 5 [140]. The frontal cortex, amygdala, nucleus accumbens, septum, and striatum are all rich in ITPKA. It is in these brain regions where ITPKA and Ins(1,3,4,5)P_4-dependent signaling systems are most likely to occur. ITPKA gene expression in hippocampus increases and remains elevated for days following the training of rodents in a spatial memory task, suggesting a role for ITPKA in long-term consolidation and storage of recent synaptic activity [49].

Seminal early studies using cerebral cortical slices showed that stimulation of muscarinic acetylcholine receptors with the agonist carbachol triggered a rapid and transient production of Ins(1,3,4,5)P_4 [148]. Subsequent studies demonstrated that depolarization of brain slices by increasing [K+] from 5 to 20 mM is also an effective means of producing Ins(1,3,4,5)P_4 in cortical slices [149]. Moreover, a modest elevation in [K+] synergizes with muscarinic activation to enhance production of Ins(1,3,4,5)P_4 [150]. By far the best glutamatergic agonist for stimulating Ins(1,3,4,5)P_4 production in cortical slices is quisqualate, which possesses dual agonist activity at AMPA (amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and class 1 metabotropic glutamate receptors, but has little direct effect on NMDA receptors. Quisqualate-stimulated Ins(1,3,4,5)P_4 effects were mimicked by the combination of the class 1 metabotropic glutamate agonist ACPD (1-aminocyclopentane-trans-1,3-dicarboxylic acid) and AMPA, but not by carbachol and AMPA under similar conditions [151]. These experiments suggest that Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 pools mobilized by muscarinic versus metabotropic glutamatergic agonists are not identical.

Exposure of cortical brain slices to concentrations of *N*-methyl-D-aspartate (NMDA) between 1 and 10 μM enhanced Ins(1,3,4,5)P_4 generation up to fourfold, but higher concentrations were inhibitory; no Ins(1,3,4,5)P_4 was produced at 100 μM NMDA or higher [152]. In numerous studies, kainic acid has been shown to block inositol phosphate generation triggered by carbachol [153]. In summary, a modest Ca^{2+} influx is necessary to elicit significant Ins(1,3,4,5)P_4 production, but larger Ca^{2+} influxes are inhibitory to ITPKA. The stimulation by Ca^{2+} is likely explained by ITPKA being positively regulated by Ca^{2+}/CaM, CaMKII, and also by the stimulation of phospholipase C via Ca^{2+}. The inhibition by Ca^{2+} may occur because, in biochemical experiments, the Ca^{2+} effect on ITPKA shows an inverted U shape, with inhibition of enzyme activity occurring at Ca^{2+} concentrations above 1 mM [154]. A second contributing factor for the Ca^{2+} inhibition is that large Ca^{2+} influxes drive the ITPKA-decorated F-actin out of dendritic spines, away from sites of Ins(1,4,5)P_3 generation [147].

ITPKA may participate in ITPR-dependent signal integration

Neurons rich in Ins(1,4,5)P_3-dependent signaling machinery specialize in synaptic integration—the spatiotemporal reception of hundreds or thousands of synaptic inputs to compute a binary output [155, 156]. For example, the cerebellar Purkinje neurons, which perform astounding feats of integration, express more ITPR than any other cell type [144]. Compelling experimental evidence supports the idea that Ins(1,4,5)P_3 signaling participates in synaptic integration in Purkinje neurons [157–159] and in the CA1 pyramidal neurons of hippocampus [160, 161]. ITPRs themselves are subject to numerous molecular inputs, which impart integrative properties at the level of individual channels [162].

The ER Ca^{2+} stores of neurons, as in other cells, are contiguous [78]. This property positions the ER to perform integrative functions at electrical, biochemical, and transcriptional levels [163, 164]. The neuronal ER is excitable because it can produce regenerative intracellular Ca^{2+} waves (Fig. 5c) [137, 165–169]. The fill state of the Ca^{2+} stores is regulated by the extent of recent synaptic activity, which imparts the ER with a "memory" of recent action potentials [170–173]. The disposition of Ca^{2+} in the ER may also regulate biochemical changes relevant to neuronal plasticity—such as protein translation, and especially transcription in the nucleus (since the nuclear envelope is contiguous with the ER). Indeed, the rise in nuclear Ca^{2+} required to activate transcription is triggered preferentially through the Ins(1,4,5)P_3-sensitive intracellular stores, compared to other Ca^{2+} sources [163, 166–168, 173].

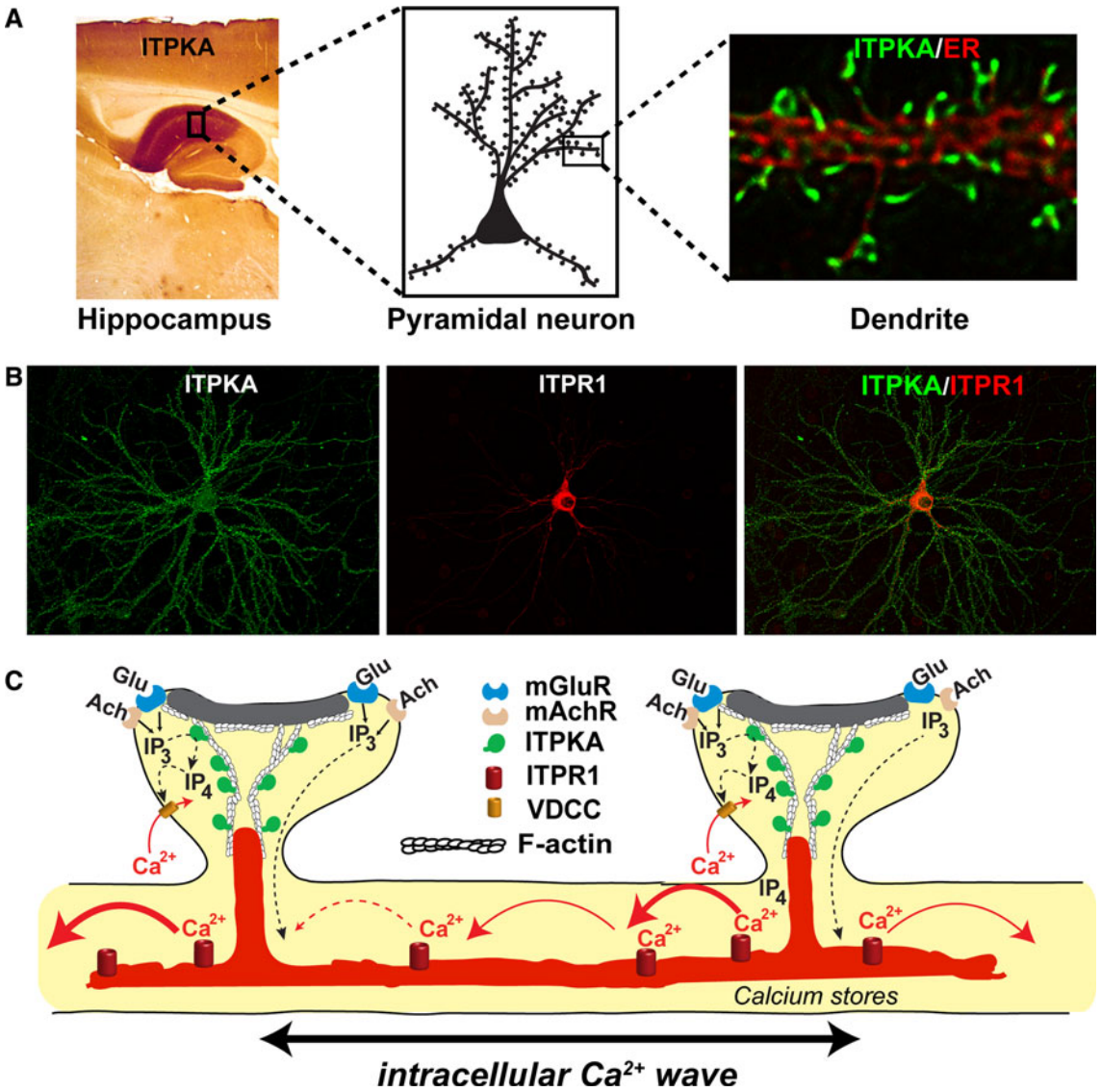

Fig. 5 ITPKA-regulated signaling in dendritic spines. **a** Regional and cellular localization of ITPKA in brain. Antibody staining against ITPKA (*left*) shows that the highest levels of ITPKA protein occur in the synapse-rich neuropil of the CA1 region of hippocampus (*left*, *dark brown stain*), while much lower expression occurs in the adjacent CA3 region. ITPKA is enriched in large pyramidal neurons, in dendritic spines (*cartoon*, *middle*). Deconvolved microscopic images (*right*) of a dendrite from a hipocampal neuron, co-labeled for ITPKA (*green*) and the endoplasmic reticulum (ER, *red*). Note how ITPKA is localized to Y-shaped structures, which lie inside dendritic spines and are situated between the synapse and the ER [147]. **b** Comparison of the localization of ITPKA and ITPR1 in a cultured hippocampal neuron. The image on the left (*green*) depicts a neuron transfected with ITPKA, which is localized toward the distal dendritic processes, in postsynaptic zones. The middle image (*red*) depicts transfected ITPR1, which is localized in the proximal ER and envelops the nucleus. The overlay (*right*) illustrates the inverse gradients of ITPK, located at distal (synaptic) sites, and ITPR1, located more proximally. **c** Cartoon depicting the spatial distribution of Ca^{2+} signaling systems regulated by ITPKA. Ins(1,4,5)P_3 is generated in spines chiefly through the activation of class 1 metabotropic glutamate (Glu) receptors (mGluR), which couple to phospholipase C beta (PLCβ). In the ITPKA-rich CA1 region of hippocampus, the mGluR subtype is mGluR5. Muscarinic acetylcholine (Ach) receptor (mAchR) subtypes M1 and M3 also occur in pyramidal neurons, and they constitute a second prominent Ins(1,4,5)P_3-generating system. Ins(1,4,5)P_3 generated inside dendritic spines diffuses to reach ITPR1, located in ER in or near the spine. ITPKA, which is intensely concentrated on bundles of filamentous actin (F-actin) inside spines [133], lies between sites of Ins(1,4,5)P_3 generation and action. Thus, the enzymatic activity of ITPKA is positioned as a molecular gatekeeper for synaptic Ins(1,4,5)P_3 signals. The Ca^{2+} released from intracellular stores can trigger an intracellular Ca^{2+} wave, which is driven by Ca^{2+}-triggered Ca^{2+} release and also by Ins(1,4,5)P_3 produced in adjacent spines and propagates bi-directionally inside the main dendrite [137, 165]. Ins(1,3,4,5)P_4, the enzymatic product, has a direct effect on Ca^{2+} influx across the spine plasma membrane through its ability to enhance the activity of voltage-dependent Ca^{2+} channels (VDCCs) [118, 119]

The role of ITPKA in neuronal integrative systems remains largely unexplored. In this context, however, the spatial relationship between ITPR and IPKA within neurons appears relevant. Although ITPR1-labeled ER can extend to distal dendritic processes, it is most concentrated proximally, within the somatodendritic regions of neurons (Fig. 5b) [174, 175]. By contrast, ITPKA is concentrated in distal regions of the dendrite, in dendritic spines, near sites of Ins(1,4,5)P_3 generation at synapses. Figure 5b depicts a hippocampal pyramidal neuron grown in culture, which is co-expressing a red-tagged ITPR1 and a green-tagged ITPKA. They form an inverse gradient, where ITPK predominates distally and ITPR1 predominates in the large apical dendrites at summation zones [176] and ITPR1 "hotspots" [169] located at dendritic branch conversion points near the cell soma.

Thus, ITPKA may be involved in spatially based integration by establishing an inositol phosphate gradient, with high Ins(1,3,4,5)P_4 levels predominating near distal synapses, in contrast to much higher densities of ITPR near the soma and nucleus. This may in turn affect the location and intracellular Ca^{2+} wave trigger zones, and the directionality or geometry of wave propagation, and this arrangement could in turn influence the Ca^{2+} signal in and near the nucleus. At the level of individual synapses, ITPKA is positioned in the neuron as a "metabolic gatekeeper" or "firewall" situated between sites of synaptic Ins(1,4,5)P_3 generation in dendritic spines, and sites of Ins(1,4,5)P_3 action at ITPRs, located more proximally on the ER in the dendritic shaft (Fig. 5c).

ITPKA possesses a number of properties that make it attractive as a molecular coincidence detector. ITPKA is directly activated by Ca^{2+}/CaM as part of a classical negative feedback loop on Ins(1,4,5)P_3 levels following intracellular Ca^{2+} release. ITPKA is also positively regulated by the "memory kinase" CamKII [60], which is highly enriched at synapses [2] near ITPKA [177]. By contrast, the Ins(1,4,5)P_3 phosphatase INPP5A becomes inhibited by 90% upon phosphorylation by CamKII [61]. Thus, synaptic activity will produce a Ca^{2+}/CaM-dependent "memory trace" that affects the routes of Ins(1,4,5)P_3 metabolism [10]. Recent synaptic activity will drive metabolic pathways towards more Ins(1,3,4,5)P_4 production in a dendrite, and lead to longer Ins(1,3,4,5)P_4 lifetimes in and near dendritic spines. The consequence of this remains unclear. The simplest consequence of positive regulation of ITPKA by Ca^{2+}/CaM is that Ins(1,4,5)P_3 signals will be more rapidly attenuated, allowing activation of protein kinase C without triggering Ca^{2+} release. However, the ability of Ins(1,3,4,5)P_4 to protect Ins(1,4,5)P_3 from destruction by INPP5A [19] (which itself may have also become inactivated by CaMKII) implies that Ins(1,3,4,5)P_4 levels may remain elevated for seconds (or longer) following synaptic activity. If Ins(1,4,5)P_3 escapes ITPKA metabolism in the spine, it may diffuse to the dendritic shaft or cell soma, where its lifetime and steady-state concentration will be determined more by INPP5A. If INPP5A has been inhibited by a prior burst of Ins(1,3,4,5)P_4, the concentration of Ins(1,4,5)P_3 may rise along with Ins(1,3,4,5)P_4. The effects of elevated Ins(1,3,4,5)P_4 on its putative molecular targets in neurons are largely unexplored, but the currently available data suggest an enhancement of Ca^{2+} channel openings in the plasma membrane and/or a modulation of signaling that involves small G-proteins such as Ras (see below).

Regulation of F-actin by ITPKA in dendritic spines

The microstructure of the actin cytoskeleton in neurons is the focus of intense study because it participates in most kinds of structural plasticity [178]. Independent of its enzymatic activity, ITPKA interacts with actin filaments and may function as a persistent regulator of activity-dependent changes in postsynaptic F-actin microstructure [132, 133, 147, 179]. Early electron microscope studies localized ITPKA in brain and observed that it was concentrated in the dendritic spines of pyramidal neurons in cerebral cortex and hippocampus, and in cerebellar Purkinje neurons [47, 72, 180, 181]. These studies also noted that adjacent spines from the same cortical pyramidal neuron sometimes showed very different levels of ITPKA content, ranging from very high to not detected [47]. The disposition of ITPKA as a synaptic enzyme seemed at odds with many biochemical studies showing that ITPK activity occurred predominantly in the cytosolic fraction from brain [65, 66].

The morphological and biochemical data were reconciled when we demonstrated that ITPKA is enriched in dendritic spines because its 66 most amino terminal amino acids bind F-actin [177]. ITPKA binds a labile pool of synaptic actin [147] with micromolar affinity [133]. When ITPKA is isolated from brain homogenates (usually done in sucrose buffers containing no salt), the F-actin becomes depolymerized, and ITPKA dissociates from its cytoskeletal anchoring. We recently demonstrated that the F-actin binding is the sole means by which ITPKA is targeted to spines. A single point mutation at residue L34, located in a putative alpha helical region in the F-actin-binding N-terminus of rat ITPKA (Fig. 2), was sufficient to render the enzyme cytosolic [133].

Owing to the delicate and labile state of F-actin located beneath the posynaptic density in dendritic spines, much remains unclear about the structure and function of postsynaptic actin. If brain tissue is processed using standard electron microscopy techniques, ITPKA appears localized

on "cytosolic matrix" [47] and "fluffy material" [72] associated with the plasma membrane and with the spine ER. Better methods have been developed, which prevent the destruction of F-actin during processing for EM [182]. However, ITPKA has not yet been localized ultrastructurally under these conditions. In recent studies where F-actin microstructure in spines has been properly preserved [182], actin filaments occur as bundles that, in part, span between the spine ER lamellae and the plasma membrane (Fig. 5c) [183]. Our studies using deconvolution light microscopy localized ITPKA to bundles of actin that formed Y-shaped projections between the ER and the PM [147], consistent with the observations of Campani et al. [183] (Fig. 5).

Recently, we demonstrated that the N-terminus of rat ITPKA bundles F-actin via a mechanism that involves multimerization of the predicted alpha helix centered near amino acid L34 [133]. The bundling of filaments contributes to the targeting of ITPKA to dendrites and away from axons, and it may also affect spine actin microstructure by increasing the length of the spine neck. Since we previously demonstrated that IPKA-decorated F-actin in dendritic spines is dynamic, and that it moves in and out of dendritic spines in a Ca^{2+} regulated manner [147], these observations suggest a complex interplay between the targeting of ITPKA to synaptic actin and the regulation of F-actin microstructure during and after synaptic activity. This may in turn affect the ability of ITPKA to modulate and compartmentalize synaptic Ins(1,4,5)P_3. The idea that ITPK regulates F-actin structure in spines recently gained further support with the discovery that it functions as a scaffold for the Rho family GTPase Rac1 [132]. During synaptic activity, ITPKA recruits Rac1 and associated signaling machinery to dendritic spine actin, and this regulates the shape of the spine. These studies open up a new and exciting line of research investigating how synaptic signals in spines lead to a rapid yet persistent change in spine shape and internal microstructure. It is worth noting that the ITPKA interaction with F-actin is unique to mammals (including marsupials), and some birds. Inspection of the recently published genome of the platypus [184] indicates that the N-terminal region of ITPKA from this "pre-mammal" is unlikely to interact with F-actin (MJS, unpublished). This implies that the targeting of ITPKA to synaptic actin is a very recent elaboration in animal evolution, which may contribute to a selective advantage for big-brained animals.

Does ITPKA regulate Ins(1,4,5)P_3 lifetime in dendritic spines?

The positioning of ITPKA on the F-actin inside dendritic spines—between sites of Ins(1,4,5)P_3 generation and sites of Ins(1,4,5)P_3 action—implies that it regulates Ins(1,4,5)P_3 concentrations in the spatial and/or temporal domains during synaptic activity. However, very few studies have addressed this question experimentally.

Although ITPKA is incredibly enriched in the dendritic spines in the CA1 region, where it would be expected to have privileged access to Ins(1,4,5)P_3 generated following activation of metabotropic glutamate receptors and muscarinic acetylcholine receptors, clear evidence that ITPKA regulates Ins(1,4,5)P_3 lifetime at synapses is lacking. One recent study examined Ins(1,4,5)P_3 lifetime in CA1 pyramidal neurons using hippocampal slices and Ins(1,4,5)P_3 released by photolysis [87]. They concluded that the major determinant of the timing window for Ins(1,4,5)P_3-regulated coincidence detection with action potentials was simply Ins(1,4,5)P_3 diffusion, rather than metabolism. Even if localized metabolism of Ins(1,4,5)P_3 in spines does occur, the functional consequence of ITPKA activity, and Ins(1,3,4,5)P_4 synthesis, remains unclear.

The physiological mechanism for Ins(1,4,5)P_3 production in neurons has been delineated best in the cerebellum. In Purkinje spines, mGluR1 and Ca^{2+} influx through AMPA receptors and/or voltage-gated receptors synergize to activate Ca^{2+}sensitive phospholipase C, producing Ins(1,4,5)P_3 as part of a Ca^{2+}-dependent feed-forward mechanism that controls long-term depression, a physiological system of coincidence detection between different synaptic inputs onto Purkinje neurons [157, 185, 186]. In this system, Ca^{2+} release from intracellular stores in and near dendritic spines reports the coincidence of synaptic inputs originating from climbing and parallel fibers [158]. The role, if any, for ITPKA in this system is not known. One recent study that examined Ins(1,4,5)P_3 diffusion in Purkinje cell dendrites concluded that both trapping of Ins(1,4,5)P_3 in spines and Ins(1,4,5)P_3 metabolism contributed to anomalous diffusion of Ins(1,4,5)P_3 [83]. Another recent study released Ins(1,4,5)P_3 via photolysis in and near Purkinje cell spines from cerebellar slices and estimated that about 20% of the Ins(1,4,5)P_3 generated in a spine gets degraded in the spine before passing through the spine neck [86]. The authors then pursued the spatiotemporal relationship between climbing fiber activation and Ins(1,4,5)P_3 release via flash photolysis, and suggested that the predominant integrative role for Ins(1,4,5)P_3 metabolism in Purkinje cells is spatial, not temporal [159]. Since Purkinje spines contain substantial levels of both ITPKA and INSP5A, the metabolic pathway(s) operating are unclear.

The hippocampal CA1 region is another prominent site of ITPKA expression, but surprisingly little is known about how ITPKA regulates Ins(1,4,5)P_3 lifetime in a CA1 neuron. The mechanisms of Ca^{2+} release operating in Purkinje

cells may not be the same as those in CA1 pyramidal neurons. Compared to Purkinje neurons, CA1 pyramidal neurons have more ITPKA, less ITPR, and less INPP5A, and they express NMDA receptors abundantly (while adult Purkinje cells have none). All dendritic spines of Purkinje neurons contain ITPR-rich ER, but only the largest spines in CA1 neurons contain ER called the spine apparatus [187, 188]. Whether the spine apparatus of CA1 neurons even contains ITPR1 is controversial [174].

Studies using ITPR1 and ITPKA knockout mice have not led to clear conclusions about the roles of the Ins(1,4,5)P_3 signaling system in hippocampal synaptic plasticity. Mice with targeted disruption of ITPR1 exhibit enhanced LTP, suggesting that ITPR1 functions as a negative regulator [189]. Yet a number of studies have demonstrated that Ins(1,4,5)P_3 production following activation of metabotropic glutamate receptors "primes" neurons, lowering the threshold of stimulus required to trigger LTP [190]. Mice with targeted disruption of ITPKA exhibit a large increase in the magnitude of LTP in the CA1 region, but normal spatial learning in the Morris water maze [48]. Basal levels of Ins(1,4,5)P_3 are not different from control animals, but levels of Ins(1,3,4,5)P_4 were reduced by about 75%. The ITPK knockout study also examined global Ca^{2+} responses following perfusion of glutamate into neuronal cultures derived from knockout mice, and they found responses to be similar to controls [48]. However, it should be noted that these measurements were taken from neurons cultured for between 7 and 10 days, which was not old enough to allow significant ITPKA expression in the controls.

Recently, new physiological and behavioral phenotypes were reported in ITPKA knockout mice [132]. In contrast to the case in the CA1 region, LTP in the CA3 region is dramatically reduced. Furthermore, deficits in spatial learning were uncovered using the behavioral tests of novel object recognition and the radial arm maze [132]. This study provides considerable new evidence that ITPKA is indeed critical for some types of learning and memory storage in brain. However, it remains unclear if the phenotypes are a consequence of modified Ins(1,4,5)P_3 metabolism, modified F-actin functions in dendritic spines, or both.

Finally, Ins(1,4,5)P_3 generated by muscarinic receptors was recently shown to produce a long-lasting enhancement of synaptic transmission in CA1 pyramidal neurons [191]. The Ins(1,4,5)P_3 generated through cholinergic pathways may function differently than when it arises from stimulation of class 1 metabotropic glutamate receptors. For example, in the amygdala, the two pools of receptor-generated Ins(1,4,5)P_3 may work cooperatively to regulate levels of Ca^{2+} in the cell soma and nucleus [168].

Does Ins(1,3,4,5)P_4 bind protein targets in spines?

The most attractive candidate downstream targets for Ins(1,3,4,5)P_4 in dendritic spines are the same as those found in lymphocytes: Ca^{2+} channels and modulators of the small G-proteins Ras and Rap. The evidence is strongest for Ins(1,3,4,5)P_4 regulation of voltage-dependent Ca^{2+} channels, located postsynaptically in hippocampal neurons. These channels become more active following a stroke [118]. In one study, Ins(1,3,4,5)P_4 was applied to the cytoplasmic face of excised patches of CA1 pyramidal neurons [118]. At a holding potential of −60 mV, a small increase in channel opening was observed in the presence of 7.5 μM Ins(1,3,4,5)P_4 (see Fig. 1 of that study). However, if animals had undergone an ischemic stroke 24–36 h before the patch-clamp experiment, the open probability of the Ins(1,3,4,5)P_4-modulated channels increased dramatically. The single channel conductance of these channels was 7.5 pS, and they were permeable to cations (Ca^{2+}, Ba^{2+}, and Sr^{2+})—but not chloride. Pharmacological tests indicated that the channels operated at negative holding potentials and were blocked by ω-conotoxin GVIA, but not ω-agatoxin IVA or nifedipine.

A different study of CA1 pyramidal neurons used intracellular applications of Ins(1,3,4,5)P_4 in the whole cell configuration [119]. If 100 μM Ins(1,3,4,5)P_4 was infused into the cell prior to (but not after) tetanization to induce long-term potentiation (LTP), the degree of potentiation was enhanced. When the experiment was repeated, substituting 2,3-dideoxy Ins(1,4,5)P_3, a non-metabolizable Ins(1,4,5)P_3 analog, no potentiation was observed, showing that the potentiation was selective for Ins(1,3,4,5)P_4. Similar to the other study, the Ins(1,3,4,5)P_4 effect was blocked by ω-conotoxin GVIA. However, the Ins(1,3,4,5)P_4 effect was also dependent on release of Ca^{2+} from intracellular stores through ITPR because co-application of the ITPR-blocker heparin (but not the ryanodine receptor blocker ryanodine) during the tetanus occluded the Ins(1,3,4,5)P_4-dependent component of the potentiation. Thus, this study suggested that Ins(1,3,4,5)P_4 participates in the complex interplay between voltage-dependent Ca^{2+} entry and Ins(1,4,5)P_3-dependent Ca^{2+} release that occurs in the dendrites of CA1 pyramidal neurons during synaptic activity. These studies are difficult to reconcile with the phenotypes of ITPKA knockout mice, which exhibit enhanced LTP in CA1, decreased LTP in CA3, but apparently normal Ca^{2+} responses [48, 132].

Direct evidence for neurons using ITPK-regulated signaling systems analogous to those found lymphocytes does not yet exist, but circumstantial evidence makes the possibility worthy of further investigation. The regulation of small G-protein signaling by Ins(1,3,4,5)P_4 has not been reported in hippocampal neurons, but the analogy with

Ins(1,3,4,5)P_4 signaling in the immune system makes this an attractive possibility. The likely downstream targets for Ins(1,3,4,5)P_4 in neurons are signaling proteins that control the activation state of Ras and Rap (see Fig. 4). These participate in the biochemical integration of signals in spines (reviewed in [2]). In some cases, the regulation appears to occur via modulation of the GAP1 family GTPase activating protein RASA3, also known as GAP1^{IP4BP}. This protein was originally purified from platelet membranes and cloned based on its high affinity binding for Ins(1,3,4,5)P_4 [107], and it is widely expressed in lymphocytes and in neurons [192].

Ras and Rap signaling are prominent in CA1 pyramidal neurons, especially in and near dendritic spines, where they regulate spine morphology through Ca^{2+}-dependent mechanisms [193–197]. In lymphocytes, Ras and Rap operate downstream of tyrosine kinase pathways coupled to phospolipase C γ to control cell fate [113], and activation of the Map kinase cascade is the usual downstream effect. PLCγ is also expressed abundantly in pyramidal neurons, where its activation via EphrinB regulates dendritic spine morphology through control of the actin cytoskeleton [198]. In neurons, the generation of Ins(1,4,5)P_3 through PLCγ activation downstream of growth factors or "growth-factor like" stimuli is much less studied compared to the more classical PLCβ pathways downstream of G-proteins. The understandably intense focus on Ins(1,4,5)P_3 generation via metabotropic and muscarinic-driven PLCβ pathways for release of Ca^{2+} may have focused attentions away from PLCγ-dependent Ins(1,4,5)P_3/Ins(1,3,4,5)P_4-generating systems more similar to those operating in lymphocytes.

The potential of ITPK-based therapies

The high expression of ITPK isoforms in the immune and nervous systems suggests that small molecules targeted to ITPK isoforms have potential as drugs and as tools for research [122]. ITPK-targeted drugs would function in a way analogous to the pharmacologically useful phosphodiesterase inhibitors, which can control cellular levels of cyclic nucleotides. The pharmaceutical aim would be to produce a drug to enhance or inhibit ITPK activity selectively in the immune system or in the brain. The suggested medical aims for such therapies vary from immunosuppression or immuno-activation, cognition enhancement and/or neuroprotection. Because of the universal importance of Ins(1,4,5)P_3-based Ca^{2+} signaling in all animals and the controversial mechanisms of Ins(1,3,4,5)P_4 signaling, it remains unclear whether the desired effect would be to enhance or inhibit ITPK. The critical role for ITPKB during thymocyte maturation and the late expression of ITPKA in neurons during and after synaptogenesis suggests that the age of the organism or patient given an ITPK modulator drug would be an important consideration if such compounds are developed as therapy. While no ITPK-targeted drug is near clinical use, the example of phosphodiesterase inhibitors as therapeutic agents points to the promise of modulating second messenger systems as therapy.

All ITPK inhibitors described so far interact with either the Ins(1,4,5)P_3 or ATP recognition sites in the catalytic domain [199–201]. Early attempts to identify inhibitory lead compounds employed exhaustive screens of inositol phosphate isomers [199, 202]. The results of these screens emphasize the incredibly high selectivity of the ITPK catalytic site for Ins(1,4,5)P_3, as no other isomer is binds with similar or higher affinity. The selectivity of ITPK for Ins(1,4,5)P_3 even exceeds that of ITPR [203]. If effective drugs acting at this site are to be developed, they must be strict structural mimics of Ins(1,4,5)P_3 in the ITPK-binding pocket. Screens of purine-based inhibitors acting at the ATP site have identified possible lead compounds for ITPK inhibition, but the likely problem here is lack of selectivity over other types of kinases, especially other members of the IPK family [203, 204]. Indeed, a promising purine-based lead compound for ITPKA inhibition [200] was later shown to be a significantly more potent inhibitor of $InsP_6$ kinase [205]. The structural similarity of the ITPK ATP-binding site with an ancient kinase family emphasizes the difficulties of obtaining selectivity at the ATP site [23].

Creating isoform-selective ITPK inhibitors may prove even more difficult because of the very high structural homology among the catalytic sites of ITPKA [21, 22], ITPKB [206], ITPKC (PDB accession 2a98), and IPMK [27]. Developing isoform-selective compounds acting at these sites poses a significant challenge for medicinal chemistry. A recent compound library screen for ITPK inhibitors found the isoform selectivity to be poor among the highest affinity inhibitors identified [201]. Moreover, molecules acting at the active sites are likely to be positively charged, and highly charged drugs may prove difficult to target to cells and tissues inside organisms. One unexplored possibility is allosteric modulation, which could circumvent problems of cellular and/or tissue penetration encountered with charged molecules. The potential of allosterically modulating ITPKs is unproven. The hypothetical allosteric ITPKA modulator might interact with the calmodulin-binding site, mimic the effects on activity produced when ITPKA becomes phosphorylated, inhibit ITPKA dimerization, or affect ITPKA localization.

Conclusions

ITPKs are conserved among metazoans, where they function as "off" signals for the second messenger Ins(1,4,5)P_3.

In doing so they produce Ins(1,3,4,5)P_4, a putative "third messenger." While Ins(1,4,5)P_3 signals can be attenuated by both kinase and phosphatase pathways, ITPKs are notably expressed in immune and neuronal tissues, and their predominant roles in these tissues are borne out by the phenotypes of knockout mice. Compared to the Ins(1,4,5)P_3 phosphatase INPP5A, ITPKAs have higher affinity and selectivity for Ins(1,4,5)P_3. They are regulated by extensive cross-talk with other signaling systems, and they exhibit targeting to subcellular compartments. These properties suggest a prominent role for ITPKs in controlling Ca^{2+} signals within the spatiotemporal domains of cells via control of Ins(1,4,5)P_3 lifetimes. The status of Ins(1,3,4,5)P_4 as a messenger is bolstered by phenotypes of immune cells and neurons lacking ITPK isoforms. In some of these cases, the Ins(1,4,5)P_3-dependent Ca^{2+} signals measured appeared normal, suggesting that it was a loss of Ins(1,3,4,5)P_4 function that accounted for the cellular phenotype. The two types of likely Ins(1,3,4,5)P_4 targets are channels residing in the plasma membrane and various regulators of the small G proteins Ras and Rap. The intense concentration of ITPKA on actin filaments in association with Rho GTPases inside dendritic spines of hippocampal neurons suggests a role in signal integration in the dendrite via control of biochemical, structural, and transcriptional plasticity. Further studies are required to define this role precisely. The selective expression of ITPKA isoforms in cells of the immune and nervous systems suggests that small molecule modulators of ITPKs have potential as therapeutic agents. However, conservation among the catalytic sites of ITPK isoforms and the highly charged nature of the natural substrates imply that ITPKA modulators pose a significant challenge for drug development.

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