Inositol 1,4,5-trisphosphate 3-kinases: functions and regulations

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ABSTRACT

Inositol 1,4,5-trisphosphate 3-kinase (IP_3 3-kinase/IP3K) plays an important role in signal transduction in animal cells by phosphorylating inositol 1,4,5-trisphosphate (IP_3) to inositol 1,3,4,5-tetrakisphosphate (IP_4). Both IP_3 and IP_4 are critical second messengers which regulate calcium (Ca^{2+}) homeostasis. Mammalian IP3Ks are involved in many biological processes, including brain development, memory, learning and so on. It is widely reported that Ca^{2+} is a canonical second messenger in higher plants. Therefore, plant IP3K should also play a crucial role in plant development. Recently, we reported the identification of plant IP3K gene (*AtIpk2β/AtIP3K*) from *Arabidopsis thaliana* and its characterization. Here, we summarize the molecular cloning, biochemical properties and biological functions of IP3Ks from animal, yeast and plant. This review also discusses potential functions of IP3Ks in signaling crosstalk, inositol phosphate metabolism, gene transcriptional control and so on.

Keywords: inositol 1,4,5-trisphosphate 3-kinase (IP_3 3-kinase/IP3K), inositol polyphosphate kinase (Ipk), inositol phosphate multikinase (Ipmk), calcium (Ca^{2+}), signal transduction

INTRODUCTION

Inositol 1,4,5-trisphosphate (IP_3) is an important second messenger in animal cells that mediates calcium (Ca^{2+}) release from the endoplasmic reticulum (ER) to the cytosol [1-3]. Inositol 1,3,4,5-tetrakisphopsphate (IP_4) is another messenger responsible for mediating Ca^{2+} entry through plasma membrane and mobilize intracellular Ca^{2+} by acting synergistically with IP_3 [4]. Inositol 1,4,5-trisphosphate 3-kinase (IP_3 3-kinase/IP3K) phosphorylates IP_3 to IP_4 [1, 5]. Thus, IP3K plays a key role in maintaining Ca^{2+} homeostasis by regulating the concentrations of IP_3 and IP_4.

IP_3 also serves as a precursor for the synthesis of other higher inositol phosphate (IP) isomers in IP metabolism [6, 7]. These water-soluble IP isomers are involved in multiple cellular events such as modulating Ras GTPase-activating protein [8], blocking tumor cell growth [9], regulating mRNA export [10] and so on. In addition, inositol 1,2,3,4,5,6-hexakisphosphate (IP_6) is related to human neutrophil function [11] and plant seed germination [12, 13]. Yeast and *Arabidopsis* IP3Ks, also referred to as inositol polyphosphate kinase (Ipk) and inositol phosphate multikinase (Ipmk), recognize IP_3 as substrate and add a phosphate to position 6 on the inositol ring to generate inositol 1,4,5,6-tetrakisphosphate ($I(1,4,5,6)P_4$) [10, 14, 15]. It is further phosphorylated by yeast and *Arabidopsis* IP3Ks to produce inositol 1,3,4,5,6-pentakisphosphate (IP_5) [10, 14, 15]. Therefore, the physiological function of IP3K is not only regulating intracellular Ca^{2+} homeostasis, but also controlling IP metabolism (Fig. 1).

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MOLECULAR CLONING OF IP3K

Inostiol 1,4,5-trisphosphate 3-kinase (IP_3 3-kinase)

The first IP_3 3-kinase cDNA ($RnIP_3K$-A) was isolated from rat brain in 1990 [16-18]. Afterwards, several cDNAs encoding IP_3 3-kinase were consequently cloned from human ($HsIP_3K$-A, $HsIP_3K$-B, $HsIP_3K$-C) and rat ($RnIP_3K$-B, $RnIP_3K$-C) [19-23]. Rat $RnIP_3K$-B is 204 amino acids longer than that of the human $HsIP_3K$-B, but remaining similar to its human homologue with 93% identity in amino acids [21]. The recently identified human $HsIP_3K$-C shares a highly conserved catalytic domain with human isoforms A and B [22, 23]. It is about 75% identical to rat $RnIP_3K$-C [22, 23]. IP_3 3-kinase from chicken [24], nematode [25] and fruit fly [26] has also been identified.

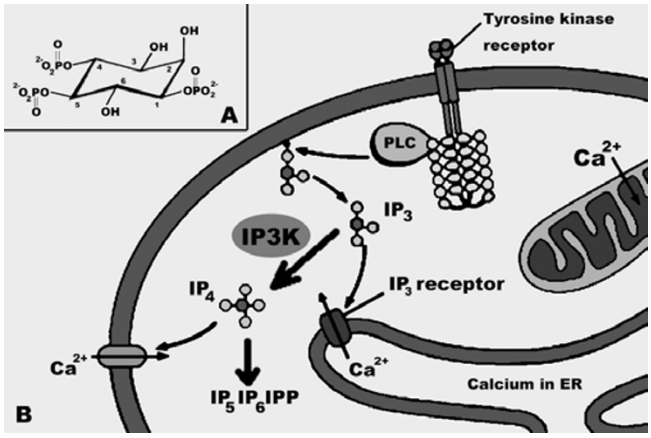

Fig. 1 IP3K function in inositol metabolism and calcium homeostasis. Panel A shows the structure of inositol 1,4,5-trisphosphate (IP_3). Panel B shows IP3K's role in phosphorylating IP_3 to IP_4 and IP_5. IP3K regulates intracellular calcium homeostasis by controlling the balance of IP_3 and IP_4.

There are at least three distinct IP_3 3-kinase isoforms (A, B, and C). They are different in their molecular masses, Ca^{2+}/calmodulin (Ca^{2+}/CaM) sensitivity, intracellular distribution and tissue expression [23, 27] (Tab. 1). Mammalian IP_3 3-kinases are usually activated by Ca^{2+}/ CaM [28]. Nematode IP_3 3-kinase instead lacks a consensus CaM-binding site and thus is insensitive to Ca^{2+}/CaM [25]. There are evidences suggesting that the N-terminal sequence of IP_3 3-kinases is involved in intracellular localization [27, 29]. For example, the N-terminal 320 amino acid sequence of rat $RnIP_3K$-B is unique and is necessary for the binding of rat $RnIP_3K$-B to the cytosolic face of the ER membrane [30]. IP_3 3-kinase isoforms show tissue specificity, such as rat $RnIP_3K$-A is specifically expressed in brain and testes, whereas rat $RnIP_3K$-B is predominantly expressed in lung and also in thymus, heart, testes and brain [29]. Such specified distribution and expression pattern of IP_3 3-kinases may contribute to their various physiological functions. However, all these IP_3 3-kinases seem to have strict biochemical activity in phosphorylating IP_3 to IP_4 [25-27].

Inositol phosphate multikinase (Ipmk)/inositol polyphosphate kinase (Ipk)

Inositol phosphate multikinase (Ipmk) is widely distributed in the kingdoms of animal, plant and yeast [31]. The first identified Ipmk cDNA (also called Ipk2) was from yeast [10, 14]. Yeast Ipk2/Ipmk/IP3K is a dual-specificity IP_3/IP_4 6/3-kinase and identical to Arg82/ArgRIII which is an indispensable component of ArgR-Mcm1 transcriptional complex [10]. The ArgR-Mcm1 complex functions in transcriptional control of genes involved in arginine metabolism [32, 33]. However, the inositol phosphorylation activity Arg82 is not required for the transcriptional regulation [34]. We previously reported the molecular cloning and characterization of a plant IP3K gene (*AtIpk2β*/*AtIP3K*) from *Arabidopsis* [15]. The amino acid sequence of AtIpk2β shares 73% identity and 84% similarity to that of a second *Arabidopsis* IP3K, AtIpk2α. [15, 35]. Similar to yeast IP3K, *Arabidopsis* IP3K is also a dual-specificity 6/3-kinase [15, 35]. York and his colleagues reported that

Tab. 1 Biochemical and molecular characteristics of IP3K isoforms from human, rat and *Arabidopsis*.

Organism	Isoforms	Molecular mass (kD)	Amino acids (aa)	Ca^{2+}/CaM sensitivity	Intracellular distribution	Tissue expression
Human	$HsIP_3K$-A	50.0	461	2~3 fold	Cytoskeleton	
	$HsIP_3K$-B	53.5	472	7~8 fold	Plasma membrane, cytoskeleton and ER	
	$HsIP_3K$-C	75.2	684	Ca^{2+} decrease CaM reverse	Cytoplasmic	
Rat	$RnIP_3K$-A	50.9	459	3~6 fold		Brain and Testes
	$RnIP_3K$-B	74.0	673			Lung, thymus, heart, testes, and brain
	$RnIP_3K$-C	74.5	678			Heart, brain, testes, tongue epithelium
Arabidopsis	AtIpk2α	31.9	286	Not affected		Leaf, stem, root, flower, silique
	AtIpk2β	33.5	300	Not affected		Pollen, flower, root, mesophyll cells

Arabidopsis IP3K has a novel 5-kinase activity to phosphate I(1,3,4,6)P_4 to generate I(1,2,3,4,6)P_5 [35]. Identified Ipmks include those from human [36] and rat [37].

Human Ipmk is very similar to rat Ipmk with 84% amino acid sequence identity [31]. *Arabidopsis* IP3K and yeast IP3K are less conserved in the Ipmk superfamily with 25% and 16% amino acid sequence identity to human Ipmk respectively [31]. Within the catalytic domain of Ipmks family, mammalian homologues share 25-33% and 42-54% identity to yeast and *Arabidopsis* IP3Ks respectively. Ipmks have conserved IP-binding consensus sequence and ATP-binding site in their catalytic domain [31]. Fig. 2 depicts a schematic alignment of IP3Ks from rat, human, yeast and *Arabidopsis*. The expression patterns of *Ipmk*s are different: rat *Ipmk* is highly expressed in kidney and brain [37], whereas human *Ipmk* is ubiquitously expressed [36]. Although *Arabidopsis IP3K* has similar transcript level in flower, root, stem, and leave [15], its activity is detected only in mature pollens, but not in immature pollen grains [15].

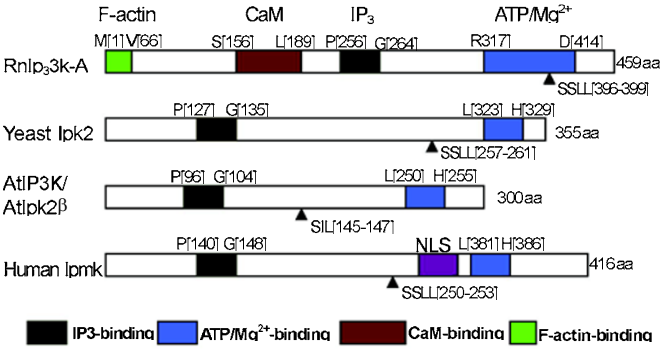

Fig. 2 The structure of IP3K family. The various conserved domains are marked by colored boxes. The dark green box and the blue box show IP_3-binding domain and ATP/Mg^{2+}-binding domain, respectively. The red box represents CaM-binding domain. The light green box shows F-actin-binding domain. The SSLL-like motif is also conserved in IP3K. A nucleus localization signal (NLS) is represented by the purple box.

IP3K STRUCTURE

There are two major functional domains in mammalian IP3Ks: a highly conserved C-terminal catalytic domain and a divergent N-terminal regulatory domain. The structure of mammalian IP3Ks catalytic core (residues 185-459 of rat Rn$IP_3$3K-A) consists of two domains: a large α/β-class structure and a small α-helical structure [38-40]. The α/β-class structure has two lobes that are necessary for ATP/Mg^{2+}-binding with critical residues Lys-197, Lys-262, Arg-317 and Asp-414, whereas the small α-helical structure is responsible for IP_3-binding in dependence of a 35 amino acid sequence of Arg-276 to Lys-303 [39, 40-43]. Many hydrophobic residues of the large domain also participate in ATP binding [39]. The IP_3-binding core is inserted between two lobes of the large domain acting together during ATP binding and phosphate transfer [43, 44]. Sequence alignment of IP3Ks shows that consensus sequence PxxxDxKxG is a highly conserved motif for substrate binding [31]. However, the small helix domain is absent in mammalian Ipmks, yeast and *Arabidopsis* IP3Ks [39]. This may explain the substrate specificity of IP_3 3-kinase and Ipmk from the structure level. Motif [L/M][I/V]D[F/L][A/G][H/K] is also considered as a putative ATP/Mg^{2+}-binding sequence in Ipmks [37]. Furthermore Saiardi *et al* identified a new domain designated "SSLL" in rat Ipmk [37]. The SSLL-like motif is also conserved within other IP3Ks [31, 37]. Mutational analysis shows that loss of this motif may impair catalysis activity of IP3Ks [37].

Intracellular localization of IP3Ks is contributed by special domains. A novel N-terminal 66 amino acid sequence in rat Rn$IP_3$3K-A is involved in F-actin binding [45]. A similar actin-binding domain was also identified in rat Rn$IP_3$3K-B [46]. Rat Rn$IP_3$3K-B can bind to ER membrane with high-affinity, depending upon conformation, and protein-protein interaction [30]. Soriano and Banting hypothesized that the N-terminus of Rn$IP_3$3K-B was only required for the binding of the enzyme to the ER in proximity of the IP_3 receptor [30]. This N-terminal 320 amino acids are unique for the rat IP3K isoform B, which contributes to its subcellular localization to the ER [30]. Rat Rn$IP_3$3K-C is exclusively cytoplasmic but shuttles between cytoplasm and the nucleus [23]. A nuclear export signal (NES) has been identified at its N-terminus [23]. A similar nuclear localization signal (NLS) has also been discovered in human Ipmk [47]. Both yeast and *Arabidopsis* IP3Ks are nucleus localized [10, 15]. However, no obvious NSL can be found through sequence alignment [15]. Different domains are presented in Fig. 2.

IP3K REGULATORS

Ca^{2+}/CaM

Mammalian IP3Ks can be activated by CaM in a Ca^{2+}-dependent manner to different degrees. CaM recognizes sequences which contain amphiphilic α-helices with clusters of positively charged and hydrophobic amino acids [38]. Sequence from Ser-156 to Leu-189 together with site Trp-165 in rat $IP_3$3K-A is required for CaM binding and the enzyme activation [38, 48, 49]. The level of stimulation appears to be cell-, tissue- and isoform-specific [27, 50] (Tab. 1). Up to 20-fold of increase in IP3Ks enzymatic activities by Ca^{2+}/CaM can be observed in a *in vitro* assay using purified IP3Ks from rat [17, 51], pig

and human [29, 52, 53]. However, IP3Ks from nematode [25], *Arabidopsis* [15] and yeast [10] lack the consensus CaM-binding sites and thus are insensitive to Ca^{2+}/CaM.

PKA, PKC and CaMKII

Mammalian IP3Ks are substrates of camp-dependent kinase (PKA), protein kinase C (PKC) and Ca^{2+}/CaM-denpendent kinase II (CaMKII). PKA can stimulate IP3K activity. In contrast, PKC is a negative regulator of IP3K [54]. Ser-175 on $RnIP_3$3K-A is the phosphorylation site for PKC, and Ser-109 for both PKC and PKA [28]. Simultaneous phosphorylation of Ser-109 and Ser-175 leads to inactivation of the enzyme, whereas a single phosphorylation at Ser-109 activates it, suggesting that Ser-175 is probably the inhibitory phosphorylation site [28]. CaMKII is also a positive regulator of IP3K [55]. Thr-311 of human $HsIP_3$3K-A is a CaMKII phosphorylation site. CaMKII can stimulate enzyme activity by 8~10-fold [54, 55]. The phosphorylation level of IP3K varies depending upon Ca^{2+}/CaM-sensitivity and different isoforms [55]. To date, it is not clear whether Ipmk is sensitive to PKC, PKA, CaMKII. But *Arabidopsis* IP3K can be phosphorylated by PKC *in vitro* [15]. Further experiments are needed to elucidate how the activity of *Arabidopsis* IP3K is regulated.

Other regulators

Mammalian IP3Ks activity can be stimulated by 12-O-tetradecanoylphorbol-13-acetate (TPA) in the presence of cAMP [56-58]. Protein stability is also involved such as mammalian IP3Ks are very sensitive to calpains [59]. Pp60v-src kinase can also increase IP3K activity, although the src-phosphorylation site in IP3K has not been identified yet [60].

IP3K FUNCTIONS

IP3Ks are involved in inositol signaling pathway, calcium signal transduction, brain development, stress responses and gene transcription (Fig. 3).

Inositol signaling pathway

Mammalian IP3Ks mainly phosphorylate IP_3 to IP_4 to provide precursors for synthesis of higher IPs [5, 31]. Yeast and *Arabidopsis* IP3Ks participate additional pathway in IP metabolism [10, 15]. In yeast, there is a subdivision of lipid-dependent pathway for IP_6 synthesis [10]. IP3K phosphorylates IP_3 stepwise at the D-6 and D-3 positions to generate IP_5 or as a minor pathway to phosphorylate IP_3 to bring about IP_4 [10, 14]. There is evidence showing that expansion of an IP_3 pool could lead to increases of IP_4, IP_5 and IP_6 levels via Ipmk [61]. Thus, in higher eukaryotes Ipmk, but not IP_3 3-kinase, may be the main contributor for IP_5 and IP_6 syntheses [61]. Plant react in a similar way. Maize IP3K (ZmIpk) is responsible for IP_6 biosynthesis in developing maize seed [62]. *Arabidopsis* IP3K has 6-/3-kinase activity and can phosphorylate IP_3 to give rise to IP_5 [15, 35]. Besides *Arabidopsis* IP3K exhibits a novel 5-kinase activity to produce IP_5 from $I(1,3,4,6)P_4$ [35]. The 5-kinase activity has also been detected in human and Drosophila Ipmks [36, 61], which

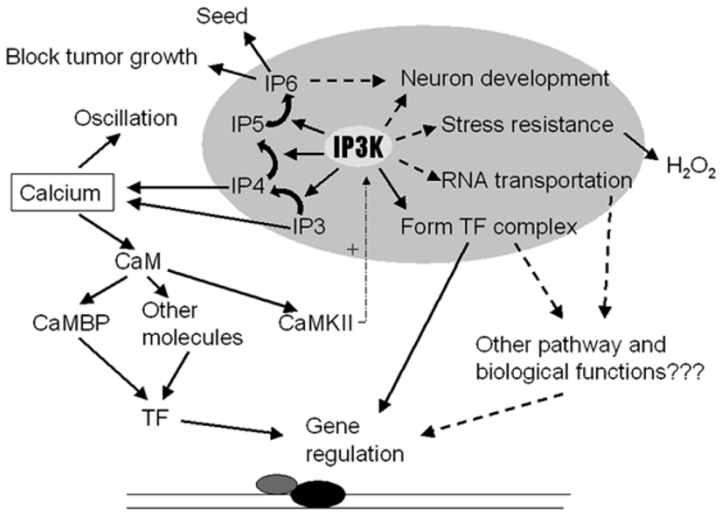

Fig. 3 The network of IP3K functions.

is especially important for fruit flies since no IP_3 5-/6-kinase can be found in this animal. Human Ipmk can also phosphorylate inositol 4,5-biphosphate (IP_2) to generate to IP_3 and can make pyrophosphate disphosphoinositolP tetrakisphosphate (PP-IP_4) from IP_5 [36].

Calcium signal transduction

IP_3 and IP_4 regulate Ca^{2+} mobilization synergistically [2, 4]. Increase of IP3K activity may reduce cellular IP_3 concentration and correspondingly terminate IP_3 action. The function of IP_4 is implicated in promoting Ca^{2+} entry from extracellular space [4]. Evidence shows that IP_4 can activate a protein with ras- and rap-GAP activity and finally inactivate the G protein [30]. This indicates that IP_4 regulates Ca^{2+} influx in a GTP-dependent way, which potentially links the IP_3 signaling pathway to GTP-regulated signaling mechanisms [30]. IP_4 is demonstrated to be a common regulator in Ca^{2+} homeostasis [63]. A complete inhibition of IP3K activity in Hela cells by adriamycin or by IP3K-specific antibody blocked Ca^{2+} oscillations, whereas a partial inhibition caused a significant reduction in oscillations frequency [63]. Taken together, IP3K activity is related to the levels of IP_3 and IP_4 and subsequently to Ca^{2+} oscillations (Fig. 3). However, it remains unknown whether yeast and *Arabidopsis* IP3Ks are involved in regulation of Ca^{2+} oscillations. Recombinant yeast IP3K mainly phosphorylates IP_3 to give rise I(1,4,5,6)P_4 [10, 35]. However, I(1, 4,5,6)P_4 is not as efficient as IP_4 in Ca^{2+} influx. Yeast IP3K thus may not be relevant to Ca^{2+} oscillations *in vivo*.

Brain development, memory and learning

Rat and human IP3Ks may be involved in brain development, memory and learning. Rat IP3K activity is low at birth and reaches approximately 50% of adult levels [64]. Rat IP3K activities are the highest in the hippocampal CA1 pyramidal neurons, dentate gyrus granule cells, and cerebellar purkinje cells [64, 65]. On the other hand, low activities were found in cerebellar granule cells, thalamus, hypothalamus, brainstem, spinal cord, and white matter tracts [64, 65]. The expression pattern of human *$HsIP_3$3K-A* is similar to that of rat *$RnIP_3$3K-A*. Human Hs$IP_3$3K-B is predominantly present in astrocytes [66, 67]. The distribution of IP3Ks in rat and human brain suggests that IP3K might be involved in brain development and in memory process [68]. Spatial learning training leads to the increase of rat Rn$IP_3$3K-A level [69], suggesting a possible role of rat Rn$IP_3$3K-A in spatial learning [69].

Stress responses

Interestingly, a *Drosophila IP3K* gene (*D-IP3K1*) appears to be oxidative damage resistant [26]. Ubiquitous overexpression of *D-IP3K1* confers resistance of flies to H_2O_2- but not to paraquat-induced oxidative stress [26]. Evidence suggests that the protective effect of *D-IP3K1* is mainly due to a reduced IP_3 level and thus reduced calcium release from internal stores, rather than an increased IP_4 level [26]. IP3K activity is the key player in this process [26]. In yeast, the IP3K activity has also been demonstrated to be required for resistance to salt stress, cell wall integrity and vacuolar morphogenesis [70].

Gene transcription

Yeast IP3K (Ipk2/Arg82) was identified as a regulator of arginine metabonism [10]. The complex ArgR-Mcm1 is required to ensure the coordination of gene expression in response to arginine [10, 71]. Arg80 and Mcm1 are members of the MADS-box transcription factor family, whereas Arg81, a zinc cluster protein, is the sensor of arginine [72]. Three components (Arg80, Arg81 and Mcm1) are sufficient to form a complex with DNA (arginine boxes) in the presence of arginine [72]. Yeast IP3K stabilizes Mcm1 and Arg80, and facilitates their assembly into a multimeric complex [72]. A poly-Asp domain between amino acid residues 282-303 of yeast IP3K is essential for stability of Arg80 and Mcm1 [73]. It was argued that the absence of this domain leads to the failure of forming ArgR-Mcm1 transcriptional complex [73]. *Arabidopsis IP3K* has a similar function, which complements yeast *Arg82/Ipk2* mutant lacking a functional ArgR-Mcm1 transcriptional complex [15]. However, no significant poly-Asp domain is found in *Arabidopsis IP3K* [15]. This evidence is somewhat contradicted to previous hypothesis about the role of yeast IP3K in forming transcriptional complex.

Others

IP_4 can bind with high affinity to several intracellular proteins—synaptotagmin (I and II), Gap1, Btk, and centaurin-α—and may interact with synaptotagmin to inhibit synaptic transmission [74]. IP_4 also acts as a mediator in neuronal death in the ischemic hippocampus [75]. The changes in IP_3 metabolism may be correlated to critical stages of muscle development and differentiation, which suggests a possible role for IP3K in these processes [76]. Moreover, yeast IP3K is involved in cellular mRNA export from the nucleus with Ipk1 and plays a role in determining messenger RNA export from yeast nucleus [10, 77]. Recent analysis shows that *Arabidopsis* IP3K (AtIpk2α) is also associated with pollen germination and root growth [78].

PERSPECTIVE

Signaling crosstalk

IP3K may be a key player in integrating Ca^{2+} signaling, IP metabolism and other signaling pathways. In plant, Ca^{2+} levels are modulated by IP_3 in response to various signals

including hormones, light and abiotic stresses. For example, the addition of abscisic acid (ABA) leads to increase in endogenous IP_3 levels [79]; red light elicits a rapid Ca^{2+} intracellular release which can be mimicked by microinjection of IP_3 [80]; gravity stimulates a rapid increase of IP_3 in maize [81]. However, this may not be the only way for IP3K function in many biological processes. IP_4, IP_5, and IP_6 have been demonstrated functionally important [9-13, 82]. They have recently been implicated as messengers regulating cellular processes including transcription, DNA repair and channel activity [6, 7]. IP_6 serves as a storage poll of IPs and mineral nutrients in seeds [12, 13]. Thus, IP3K may also participate in controlling plant development by regulating subsequent IP signaling pathways. A new exciting function for yeast and *Arabidopsis* IP3Ks was found in regulation of gene expression [10, 15]. A fully understand of the physiological function of IP3K needs a comprehensive consideration of IP3K network. IP3K may simultaneously regulate Ca^{2+} homeostasis, IP metabolism and gene transcription in response to external stimulus.

Inositol phosphate metabolism

Signals induced by IP_3 can be terminated by two ways; either through dephosphorylation by a 5- phosphatase to give inositol 1,4-biphosphate (IP_2) or through phosphorylation by IP3K to produce IP_4 [83]. Ipmk may replace $IP_3$3-kinase due to their similar enzymatic activities [10, 35]. Cellular IP_3 serves as a substrate for both IP_3 3-kinase and Ipmk to form IP_6 [61]. Ipmk, but not $IP_3$3-kinase, is the major enzyme in IP_6 synthesis, whereas $IP_3$3-kinase mainly function in IP_4 synthesis from IP_3 [61]. Different from animal homologues, only two IP3K isoforms (AtIpk2α and AtIpk2β) were isolated from *Arabidopsis* [15, 35]. The general pathway for IP_6 synthesis in plant as well as in yeast has been identified as follow: $IP_3 \rightarrow IP_4/I(1,4,5,6)P_4 \rightarrow IP_5 \rightarrow IP_6$ [35]. The first two steps can be phosphorylated by yeast and plant IP3Ks [10, 15, 35]. But 3-kinase activity of yeast and *Arabidopsis* IP3Ks seems less active than their *in vivo* 6-kinase activity [10, 35]. Thus, of particular interest is the mechanism of regulating Ca^{2+} release and influx in plant cells. However, *Arabidopsis* IP3K regulating pollen tube growth under different environmental conditions is Ca^{2+}-independent [78].

Gene transcriptional control

The crystal structure of Ipmk is not yet known. Information from mammalian IP3K catalytic domain suggests that yeast and *Arabidopsis* IP3Ks may interact with other molecules [39, 40]. Sun *et al* copurified COP9 signalsome/CSN from calf brain with inositol 1,3,4-trisphosphate 5/6-kinase [84]. This kinase can phosphorylate several transcription factors (NF-κB, c-Jun, p53 etc,) to avoid of degradation by the ubiquitin system [84]. Both NF-κB and c-Jun play important roles in brain development and anti-oxidative stress [85, 86]. Yeast IP3K activates ArgR-Mcm1 complex and then drives transcription [31]. We have also demonstrated that *Arabidopsis* IP3K complemented *ipk2* mutant yeast [15], indicating a potential function of *Arabidopsis* IP3K in transcription regulation.

IP3K regulation

Mammalian IP3Ks can be modulated by Ca^{2+}/CaM, PKA, PKC, CaMKII and other regulators. However, there is little information about the mechanism. Both yeast and *Arabidopsis* IP3Ks lack CaM-binding sites and are insensitive to Ca^{2+}/CaM [10, 15]. Therefore, compared to mammalian IP3Ks, they are most regulated by different mechanisms. Our preliminary experiments suggest that *Arabidopsis* IP3K can be phosphorylated by PKC *in vitro*. Whether such phosphorylation is physiologically relevant to the regulation of *Arabidopsis* IP3K activity *in vivo* is not clear. It is important to understand the function regulation of yeast and plant IP3Ks in the near future.

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