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InsP3R

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Gene Symbols: ITPR1, ITPR2, ITPR3
Other Names: IP_3R1, IP_3R2, IP_3R3

1. General: The role of InsP3R in pancreatic acinar and other cells

The classical pathway linking stimulation by neurotransmitters and hormones to changes in phosphoinositide metabolism and the subsequent $InsP_3$ induced Ca^{2+} release was in large part documented by work carried out in exocrine cells, in particular, acinar cells isolated from the exocrine pancreas (49). In seminal work using permeabilized rat pancreatic acini, Streb and colleagues demonstrated that the addition of $InsP_3$ resulted in Ca^{2+} release from a non-mitochondrial Ca^{2+} store (43). This store was later identified to be the endoplasmic reticulum (ER) through experiments where sub-cellular fractions from pancreatic cells were isolated and exposed to $InsP_3$ (4, 42). Ca^{2+} release is rapid, occurring as quickly as 1s following secretagogue stimulation of acinar cells. Ca^{2+} release is also isomer specific, with other structurally related inositol phosphates shown to be far less efficacious in releasing Ca^{2+}(43). The target protein for $InsP_3$ binding was later identified as the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) (45). Strikingly, the secretion of digestive enzymes from pancreatic acinar cells has been shown to be entirely dependent on the activation of $InsP_3Rs$ and the resulting elevation in intracellular [Ca^{2+}] (15). The receptor is encoded by 3 distinct genes in mammalian cells (ITPR1, ITPR2, ITPR3) that generate 3 monomeric isoforms (R1, R2, R3 respectively) that share 60-70% sequence homology (36).

Originally purified and cloned from rat cerebellum, the full length $InsP_3R$ forms a tetrameric cation selective channel *in vivo* (28). The three isoforms of $InsP_3R$ exhibit overlapping patterns of expression, with most cells and tissues expressing more than one isoform (50). Western blotting and quantitative PCR has revealed that there is relatively equal expression of the R2 and R3 isoforms in pancreas, with R1 only constituting ~3% of the total $InsP_3R$ (50). The importance of R2 and R3 isoforms to pancreatic Ca^{2+} signaling is most evident in studies employing $InsP_3R$ knockout (KO) mice. Although single R2 or R3 KO mice were found to have no observable ill effects or phenotypic alterations, double R2/R3 KO mice typically gained less body weight post-natally compared to single KO or WT mice. Furthermore, double KO mice tended to lose weight rapidly post weaning and typically died 4 weeks after birth. These observations were attributed to the key role the R2 and R3 isoforms play in the secretion of saliva and exocytosis of digestive enzymes from exocrine glands and the resulting inability of double KO mice to swallow or properly digest dry adult food. Indeed, double KO mice were rescued and reached body weights comparable to WT when a fed pre-digested wet mash diet or an elemental diet. Pancreatic acinar cells from R2/R3 double KO mice were also found to accumulate zymogen granules that failed to secrete zymogen granules on stimulation with secretagogues. The study also highlights the inability of R1 to rescue double R2/R3 KO mice (15).

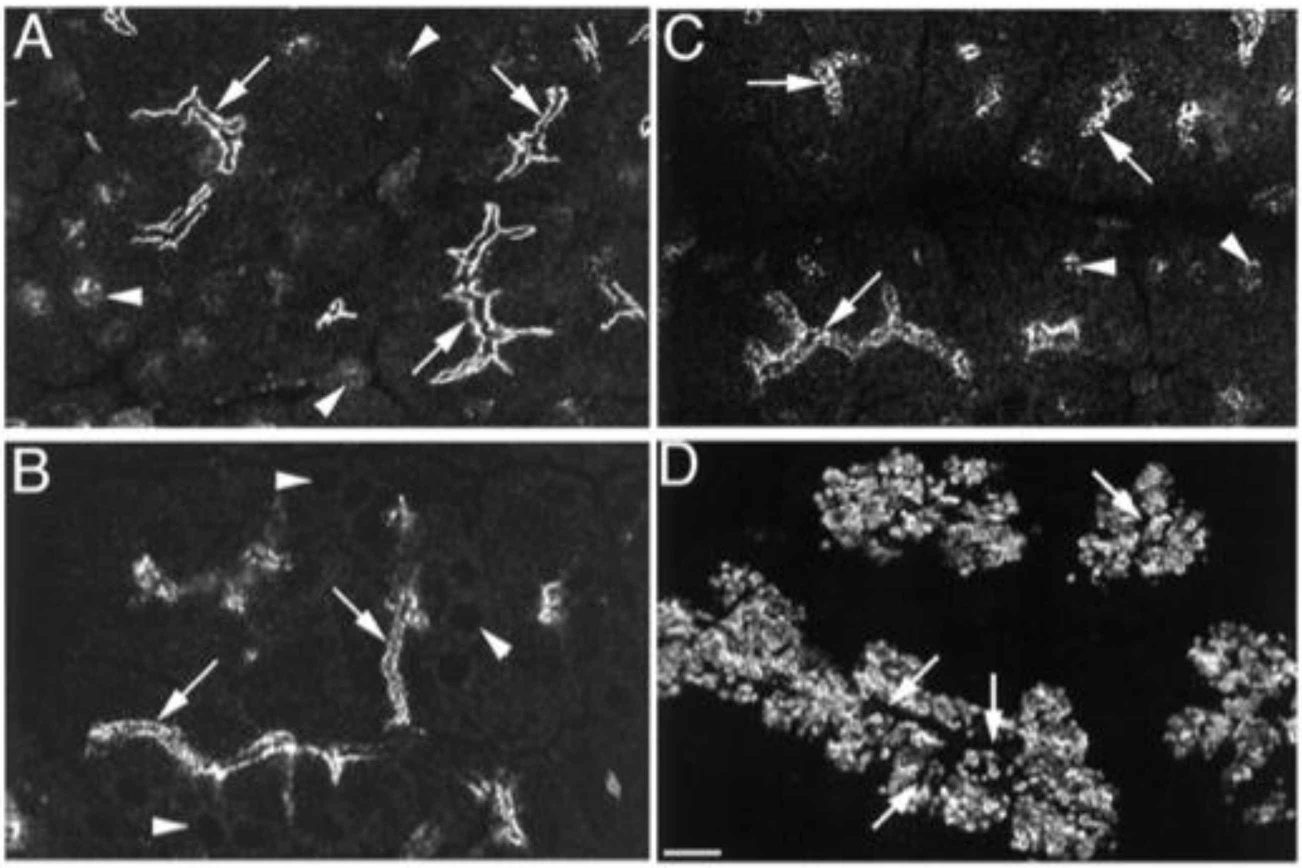

Figure 1. Localization of $InsP_3Rs$ in pancreatic acinar cells. Immunofluorescence localizations in pancreatic lobules of $InsP_3Rs$ by confocal microscopy demonstrate the localization of $InsP_3R1$, R2, R3 and amylase (A,B,C,D respectively) respectively. All 3 isoforms are predominantly localized to the apical pole of acinar cells directly abutting the plasma membrane (arrows in panels A-C). R1 and R3 also appeared to localize to perinuclear structures. (Scale bar = 10 μm). From (57).

The fidelity and specificity of the Ca^{2+} signal required for exocytosis is thought to be largely determined by the differential expression, localization and modulation exhibited by the 3 $InsP_3R$ isoforms (14). For the most part, $InsP_3Rs$ are predominantly localized to the ER, although the golgi, nucleus, plasma membrane, peroxisomes and endolysosomal vesicles have also been reported to express small levels of $InsP_3Rs$. Pancreatic acinar cells are highly polarized, both functionally and morphologically and $InsP_3Rs$ are predominantly expressed in ER extensions in the apical regions juxtaposed to the acinar lumen (22, 24, 32, 57). Using fluorescence imaging techniques, studies have shown that stimulating $InsP_3$ generation in these cells results in the initiation of Ca^{2+} signals in the apical regions, followed by the propogation of a Ca^{2+} wave that is facilitated by peripheral $InsP_3Rs$. Stimulation of basal secretagogue receptors as well as uncaging $InsP_3$ in various regions of the cell aso confirm that Ca^{2+} signals initiate in apical regions of the pancreatic acinar cell (3, 13, 21, 47, 53).

2. Structural features of the $InsP_3R$

Structurally, the $InsP_3R$ monomer is conventionally divided into 3 functional domains: an N-terminal ligand binding domain (LBD), a coupling/modulatory domain and a C-terminal transmembrane domain (TMD) that contains the channel (14) (Figure 2). $InsP_3$ binding is mediated by the 'core' ligand binding domain, which constitutes amino acids (AA) 224-578 of the LBD. This region contains 10 conserved positively charged arginine and lysine residues (3 critical, R265, K508, R511) that are thought to allosterically coordinate the negatively charged PO_4^{3-} groups of $InsP_3$ in a binding pocket (55). The three isoforms have differing $InsP_3$ binding affinities that are regulated by the first 223 AA of the LBD, termed the suppressor domain (SD). Specifically, competitive $InsP_3$ binding assays using GST fusion constructs encoding AA 1-604 demonstrate that R2 has an 11-fold greater affinity (Kd) for $InsP_3$ compared to R3 (14nM vs. 163nM), with R1 having an intermediate affinity (49.5nM) (19). Similar studies in pancreatic acini revealed a Kd of 6nM (17), comparable to that of R2, while permeabilized Ca^{2+} release assays in acinar cells demonstrated an EC_{50} for $InsP_3$ of 0.8μM (34) (Figure 3).

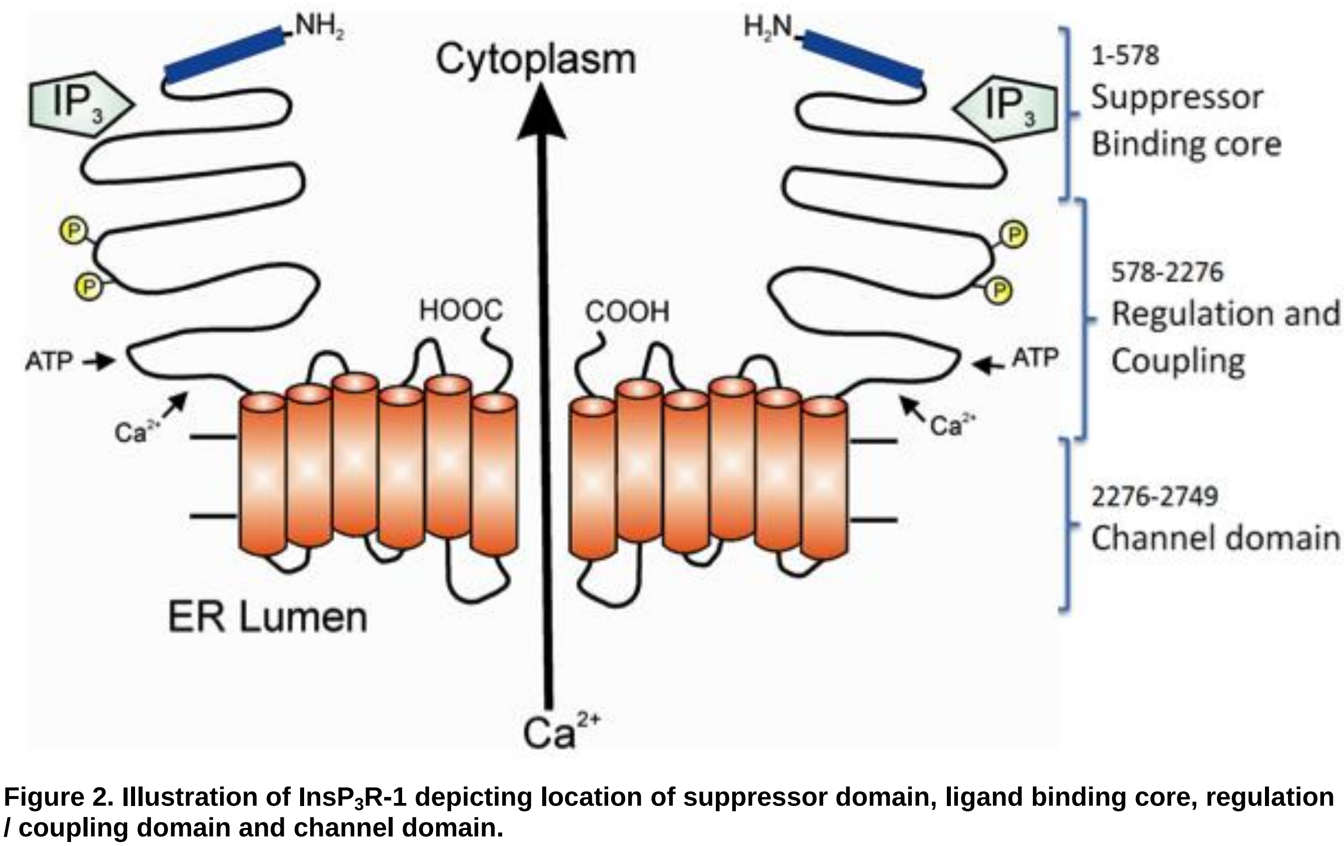

Figure 2. Illustration of $InsP_3R$-1 depicting location of suppressor domain, ligand binding core, regulation / coupling domain and channel domain.

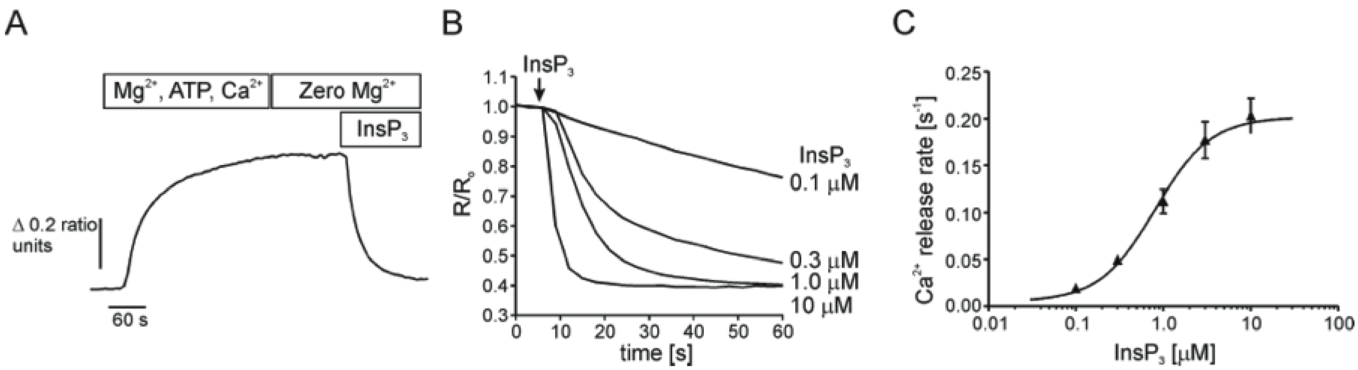

Figure 3. (A) Permeabilized cell Ca^{2+} release assay in acinar cells. Isolated pancreatic acini were loaded with furaptra prior to permeabilization with β-escin. ER is loaded through SERCA upon application of Ca^{2+} containing buffer containing $MgCl_2$, and ATP, as indicated by the increase in fluorescence ratio. Removal of $MgCl_2$ deactivates SERCA and **(B)** addition of varying [$InsP_3$] releases from stores as indicated by the decrease in fluorescence ratio. **(C)** Concentration response analysis for $InsP_3$. EC_{50} for InP_3 is 0.8µM. From (8).

Deletion of the suppressor domain (SD) results in the loss of distinct $InsP_3$ affinities between the isoforms and a 10-100 fold increase in $InsP_3$ affinity (19). Despite an increased $InsP_3$ binding affinity, deletion of the SD also results in the loss of channel activity, indicating that the SD is required for inducing $InsP_3R$ activation and Ca^{2+} release. Based on hydropathy plots, the TMD is similar in structure to that of RyRs and voltage gated K^+, Na^+ and Ca^{2+} channels and constitutes 6 putative transmembrane regions (TM1-6) (12). The TMD is responsible for the ER targeting (33, 35) and for the oligomerization of the $InsP_3R$ into tetramers, which occurs co-translationally (20). Lastly, TM5 and 6 forms the pore through which Ca^{2+} is conducted (37). The loop between TM5 and 6 contains a selectivity filter (GVGD; similar to the super family of cation selective channels) that provides some degree of cation selectivity to the $InsP_3R$ (14). However, it is poorly Ca^{2+} selective and allows conduction of monovalent cations (Ca^{2+}:K^+ = 6:1). In fact, it is believed that "functional" Ca^{2+} selectivity of $InsP_3R$ is primarily determined by virtue of SERCA being a highly selective Ca^{2+} pump and Ca^{2+} being by the far the most abundant cation in the ER. To "gate" and open the channel, evidence suggest that the SD interacts with the cytosolic loop between TM4-5, and that $InsP_3$ binding results in a conformational change that moves TM5 away from TM6 and opens the channel (38). The CT tail (last 160 AA) and the large (1700 AA) but less conserved modulatory domain contains putative binding sites for the numerous modulators of $InsP_3R$ activity (56). These modulators, which include Ca^{2+}, ATP and PKA, all contribute in distinct ways to the differential Ca^{2+} release profiles encoded by the 3 isoforms.

3. Modulation of the $InsP_3R$

Cytosolic Ca^{2+} is the most important regulator of $InsP_3Rs$, modulating activity in a biphasic manner (11). Numerous putative Ca^{2+} binding sites have been identified, and Ca^{2+} has been shown to induce dramatic conformational changes in R1 (1, 2, 18, 39, 40). For the most part, studies have shown that in the presence of $InsP_3$, low to optimal [Ca^{2+}] (300nM) stimulates channel activity while higher cytosolic [Ca^{2+}] inhibits it (11). To explain this biphasic regulation, Foskett and collaborators have proposed a model in which $InsP_3R$ activity is regulated by two distinct Ca^{2+} binding sites: a stimulatory and an inhibitory site. Under resting conditions, the inhibitory site has a higher affinity for Ca^{2+} than the stimulatory site. Accordingly, this site is occupied at resting conditions and inhibits $InsP_3R$ activity. The binding of $InsP_3$ decreases the inhibitory sites' affinity for Ca^{2+}, thereby allowing Ca^{2+} to bind to the stimulatory site and positively regulating channel activity. The downstroke of the biphasic curve is the result of Ca^{2+} binding to the inhibitory sites due to cytosolic [Ca^{2+}] being elevated beyond 300nM (46). In effect, they suggest that Ca^{2+} is only an essentially $InsP_3R$ co-agonist, with $InsP_3$s sole role being simply to modulate Ca^{2+} sensitivities (25).

Cytosolic ATP has also been shown to differentially modulate the activity of all 3 isoforms in an allosteric manner. This mode of regulation is thought to link the metabolic status of the cell to the Ca^{2+} release. Specifically, studies show that R2 is only modulated by ATP at sub-maximal [$InsP_3$] while R1 and R3 activity is affected irrespective of [$InsP_3$], implying that ATP is required for maximal activity of R1 and R3 (6, 7). Each isoform also differs in its affinity for ATP, with R2 having 3 fold and 10 fold higher affinities than R1 and R3, respectively (6). ATP binding was originally believed to occur at Walker A like motifs (G-X-G-X-X-G) that exist in each isoform. One such motif, called ATPB, is conserved across all 3 isoforms. Additionally, R1 contains 2 other sites: ATPA and ATPC, the latter only being found in the S2- variant which is expressed in peripheral tissues (48). Recently, mutagenesis studies have demonstrated that only the ATPB site in R2 is important in mediating the modulatory effects of ATP (34) and this regulation is important for defining the sensitivity of Ca^{2+} release in pancreatic acinar cells (17). In contrast, the ATPB sites in R1 and R3, in addition to the ATPA and ATPC sites in R1, play no role in modulating $InsP_3R$ activity through ATP.

Lastly, protein kinase A (PKA) mediated phosphorylation has been shown to directly increase $InsP_3R$ Ca^{2+} flux and single channel activity of R1 and R2 albeit by phosphorylation at different residues. Specifically, studies show that PKA mediated phosphorylation at S1598 and S1755 on R1 and S937 on R2 significantly enhance Ca^{2+} release and single channel activity (5, 9, 56). Conversely, no effects on Ca^{2+} release through R3 have been observed after PKA stimulation, despite evidence that R3 is phosphorylated by PKA at 3 sites in vivo (41, 56). Thus far, no single channel studies have been performed on R3 to rule out any PKA mediated effects on channel activity.

$InsP_3Rs$ are also regulated and bound by numerous other kinases and accessory proteins, including cGMP protein kinase (PKG), Akt kinase, FK506 binding protein, calmodulin, CaBP, IRBIT, Bcl-2/XL, cytochrome C, RACK and Erp44 (14).

Lastly, due to the differences in modulation and Ca^{2+} signals generated by each isoform, it is entirely plausible that the formation of heterotetrameric $InsP_3Rs$ would add another layer to the diversity of generated Ca^{2+} signals. To date, co-immunoprecipitation (IP) studies that utilize isoform specific antibodies have primarily provided evidence for the formation of heterotetramers (31, 52). Such experiments have been performed on the AR42J rat pancreatoma cell line (52) and pancreatic acinar cells (51) and have shown that such cells are capable of forming heterotetramers.

4. Tools available to study the $InsP_3R$

a. Antibodies

A multitude of commercially available isoform specific antibodies can be utilized to probe for each isoform of the $InsP_3R$. Additionally, there are antibodies that detect all 3 isoforms.

- $InsP_3R1$ Rabbit polyclonal (H-80) from Santa Cruz (sc-28614); epitope corresponding to AA 1894-1973 in the cytosolic modulatory domain.

- $InsP_3R1$, UC Davis/NIH NeuroMab Facility, clone L24/18; epitope against AA 2680-2749 in C-terminus.
- $InsP_3R3$ Goat polyclonal (C-20) from Santa Cruz (sc-7277); epitope corresponding to C-terminus.
- $InsP_3R3$ purified mouse monoclonal from BD Transduction Laboratories (Cat: 610313).
- $InsP_3R1/2/3$ Rabbit polyclonal (H-300) from Santa Cruz (sc-28613); epitope corresponding to AA 2402-2701 in C-terminus of $InsP_3R$-2 (human).

b. cDNA

Accession number:

$InsP_3R1$: rat, 55925609.

$InsP_3R2$: mouse, 60593031.

$InsP_3R3$: rat, 6981109.

c. Cell Lines

SHSY5Y Neuroblastoma: 99% $InsP_3R1$ (50).

AR42J Rat pancreatoma: 86% $InsP_3R2$; 12% $InsP_3R1$; 2% $InsP_3R3$ (50).

RINM5F Mouse insulinoma: 96% $InsP_3R3$; 4% $InsP_3R1$(50).

DT40 triple knockout cell lines: Kurosaki and colleagues have generated a DT40 chicken B lymphocyte cell line with the endogenous $InsP_3Rs$ stably knocked out (44). These triple knockout cells can subsequently be stably transfected with constructs encoding individual mammalian $InsP_3R$ isoforms, allowing the study of activity and regulation of each isoform in isolation. These cell lines are available on request from our laboratory.

d. Mouse Lines

$InsP_3R$-1 knockout mice (27).

$InsP_3R$-2 (23) and R-3 single and double knockout mice (15).

e. Agonists/Antagonists

Commercially available $InsP_3$: D-myo-inositol 1,4,5-trisphoshate hexapotassium salt; Enzo Life Sciences (cat: ALX-307-00). Adenophostin is a high affinity analogue available from SIGMA (cat: A5094). Various chemically caged forms of $InsP_3$ are available which have no activity until a masking group is liberated by UV light exposure; e.g NPE-$InsP_3$ is available from Invitrogen (cat: I-23580). A cell permeable version of caged $InsP_3$ is available from Sichem (cat: cag-iso-145).

Cells are often stimulated with Gq coupled GPCR agonists to stimulate $InsP_3$ production. Agonists include acetylcholine, trypsin and cholecystokinin.

Antagonists

Only poorly selective/specific antagonists are currently available, these include Heparin (54); Caffeine (10); 2-Aminoethoxydiphenyl borate (2-APB) (26, 30); Xestospongin (16).

f. Techniques used to study InsP3R function

See reviews by Betzenhauser MJ., Wagner LE., Won JW and Yule DI (8) and Betzenhauser, Matthew J., Won, Jong Hak, Park, Hyungseo and Yule, David I. (2011). Measurement of Intracellular Calcium Concentration in Pancreatic Acini. The Pancreapedia: Exocrine Pancreas Knowledge Base, DOI:10.3998/panc.2011.34

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