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Inositol trisphosphate receptor Ca^{2+} release channels in neurological diseases

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Abstract

The modulation of cytoplasmic Ca^{2+} concentration by release from internal stores through the inositol trisphosphate receptor ($InsP_3R$) Ca^{2+} release channel is a ubiquitous signaling system involved in the regulation of numerous processes. Because of its ubiquitous expression and roles in regulating diverse cell physiological processes, it is not surprising that the $InsP_3R$ has been implicated in a number of disease states. However, relatively few mutations in $InsP_3R$ genes have been identified to date. Here, I will discuss mutations in the type 1 $InsP_3R$ that have been discovered by analyses of human patients and mice with neurological disorders. In addition, I will highlight diseases caused by mutations in other genes, including Huntington's and Alzheimer's diseases and some spinocerebellar ataxias, where the mutant proteins have been found to exert strong influences on $InsP_3R$ function that may link $InsP_3R$ to disease pathogenesis.

Keywords

IP_3; Disease; Neurodegeneration; Calcium; Ion channel

Introduction

The modulation of $[Ca^{2+}]_i$ is a ubiquitous signaling system involved in the regulation of numerous processes, including transepithelial transport, learning and memory, muscle contraction, synaptic transmission, secretion, motility, membrane trafficking, excitability, gene expression, and cell division. Activation of phospholipases Cβ and Cγ by ligand interaction with G-protein- or tyrosine kinase-linked receptors, respectively, results in the hydrolysis of phosphatidlyinositol 4,5 bisphosphate, generating inositol 1,4,5-trisphosphate ($InsP_3$). $InsP_3$ binds to its receptor ($InsP_3R$), a ligand-gated Ca^{2+} release channel in the endoplasmic reticulum (ER). Analyses of $InsP_3$-mediated $[Ca^{2+}]_i$ signals in single cells have revealed them to be unexpectedly complex. In the temporal domain, this complexity is manifested as repetitive spikes or oscillations, with frequencies often tuned to levels of stimulation, suggesting that $[Ca^{2+}]_i$ signals may be transduced by amplitude as well as frequency encoding. In the spatial domain, $[Ca^{2+}]_i$ signals may initiate at specific locations and remain highly localized or propagate as waves [2,11]. Thus, $InsP_3$-mediated $[Ca^{2+}]_i$ signals are often organized to provide different signals to discrete parts of the cell.

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Three genes and alternatively spliced isoforms have identified a family of $InsP_3Rs$ in mammalian cells, including humans [15]. The three full-length sequences are 60–80% homologous. The $InsP_3R$ is ubiquitously expressed, perhaps in all cell types [15,68]. The isoforms have distinct and overlapping patterns of expression with most cells expressing more than one, and expression levels can be modified during differentiation and by use-dependent degradation [15]. This impressive diversity of expression suggests that cells require distinct $InsP_3Rs$ to regulate specific functions. Nevertheless, the functional implications of this diversity, at the single channel, cellular, and organ level, remain largely unappreciated.

The $InsP_3Rs$ are ~2,700–2,800 amino acid intracellular membrane proteins that exist as homo- or hetero-tetramers [15,32]. Structurally, the proteins contain a cytoplasmic N-terminus comprising ~85% of the protein, a hydrophobic region predicted to contain six membrane-spanning helices, and a relatively short cytoplasmic C-terminus (Fig. 1a). Functionally, the N-terminal domain can be divided into one comprising an N-terminal $InsP_3$ binding domain and a more distal 'regulatory'/'coupling' domain (Fig. 1b). $InsP_3$ binding to the $InsP_3R$ is stoichiometric and localized by mutagenesis and an X-ray structure to a region within residues 226–578 [5,80,81]. The $InsP_3R$ is itself a ligand-gated ion channel. The basic six-transmembrane (TM)-domain topology of $InsP_3Rs$ is shared with other cation channels (Fig. 2). By analogy, putative TM helices 5 and 6 and the intervening intra-luminal loop likely constitute the permeation pathway [4,53]. Binding of $InsP_3$ gates the channel open, modulated by the linker region that contains consensus sequences for phosphorylation, proteolytic cleavage, and binding by proteins and ATP that integrate other signaling pathways or metabolic states with the function of the $InsP_3R$.

Because of its ubiquitous expression and roles in regulating diverse cell physiological processes in so many cell types, it is perhaps not surprising that the $InsP_3R$ has been implicated in a number of disease states, including polycystic kidney disease [41], cholestasis [55], cardiac arrythmias [36], and inflammation [51] among others. It is surprising, however, that relatively few mutations in $InsP_3R$ genes have been identified to date. Many disease-causing mutations have been discovered in the other major and related family of intracellular Ca^{2+} release. It seems likely that the relative dearth of identified disease-causing $InsP_3R$ mutations reflects a widespread functional redundancy of multiple $InsP_3R$ isoforms expressed in most cells. In this review, I will discuss mutations in the type 1 $InsP_3R$that have been discovered by analyses of human patients and mice with neurological disorders. In addition, I will highlight diseases caused by mutations in other genes, including Huntington's and Alzheimer's diseases (ADs) and some spinocerebellar ataxias (SCAs), where the mutant proteins have been found to exert strong influences on $InsP_3R$ function that may link $InsP_3R$ to disease pathogenesis.

Role of $InsP_3R$ in spinocerebellar ataxias 15 and 16

To date, the only known diseases that have been definitively linked to a mutation(s) in an $InsP_3R$ gene are spinocerebellar ataxias 15 and 16. The SCAs are a heterogeneous group of genetic disorders with clinically distinct features. Both SCA15 and SCA16 are uncommon, autosomal, dominant, pure cerebellar ataxias with adult onset, slow progression, and pronounced cerebellar atrophy. Heterozygous deletions of the 5′ part of the type 1 *$InsP_3R$* gene, encompassing exons 1–10, 1–40, and 1–44 (of 59 exons), were identified in three human SCA15 families [73] and a heterozygous complete deletion of the gene was found in another [21]. Heterozygous deletion of exons 1–48 was identified in an individual affected by SCA16 [30]. That the diseases were associated with mutations in the type 1 channel isoform is consistent with the high level of $InsP_3R$-1 protein expression in the cerebellum, particularly in the Purkinje neurons [16,44,47]. SCA15 was linked to a complete deletion of the *$InsP_3R$-1* [21], and SCA16 revealed deletions in the *$InsP_3R$-1* gene only, without involvement of the adjacent *SUMF1* gene [30], demonstrating that the diseases are caused by $InsP_3R$-1 loss of

function. Western blot analysis of immortalized lymphoblasts from SCA15-affected family members with exons 1–10 deleted had strongly reduced $InsP_3R$-1 expression [73]. Haplo-insufficiency of $InsP_3R$-1 may account for the delayed onset and slow progression of the disease. In contrast, knockout of the mouse $InsP_3R$-1 results in ataxia and seizures within a couple of weeks of birth, with death by 4 weeks of age [46]. The absence of epilepsy or reduced life span in the SCA15 and SCA16 patients may suggest that the $InsP_3R$-1 expression from one allele provides a protection against a more severe phenotype. In agreement, heterozygote $InsP_3R$-1 knockout mice suffer only from some defects in motor coordination [50].

Two heterozygous missense mutations in $InsP_3R$-1 have also been identified in SCA15-affected individuals. In one family, patients had valine at position 494 in the amino acid sequence replaced by isoleucine (V494I) [17]. This residue is located within the $InsP_3$ binding domain (Fig. 1b). The core $InsP_3$ binding domain consists of a proximal β-trefoil domain linked to an α-helical-rich armadillo repeat domain. $InsP_3$ binding is coordinated by residues contributed by both domains [5,15]. Val494 is located in the armadillo repeat domain at the end of a loop that connects the first two alpha helices. It is located far from the $InsP_3R$ binding pocket, and it is not particularly conserved among species. In the *Xenopus* $InsP_3R$-1, the residue is an isoleucine. Thus, an association of the disease with the V494I mutation in SCA15 patients is perhaps surprising. Based on the gene deletion phenotypes, if the mutation is indeed responsible for the SCA15 disease, it must represent a loss of function. This region of the protein also binds proteins, including CaBP1 [77,79], CIB1 [77], and IRBIT [1] (Fig. 1b), although the role of this particular residue in their interactions with the $InsP_3R$ has not been studied.

In another family, patients had proline at position 1059 in the amino acid sequence replaced by leucine (P1059L; (21)). The proline is conserved in type 1 $InsP_3R$ isoforms among species, although it is alanine in the human types 2 and 3 isoforms. Nothing is known regarding the role of this residue in the ion channel function of the $InsP_3R$-1. This residue is localized in the coupling domain (Fig. 1b), with the surrounding region not known to be involved in protein interactions. It is possible that the missense mutation does not cause disease, although this was felt to be unlikely [21]. Again, if the mutation is indeed responsible for the SCA15 disease, it must represent a loss of function. In a preliminary report, expression of recombinant rat $InsP_3R$-1 containing the corresponding mutation formed a functional channel [49]. As discussed below, an $InsP_3R$-1 mutation in the *opistotonus* (*opt*) mouse is phenotypically equivalent to that of the knockout [57], whereas the recombinant channel is functional [70]. Thus, the mechanisms that account for the loss-of-function phenotype remain to be determined.

$InsP_3R$ mutant mice

The *opt* mouse

The *opistotonus* mouse was identified as having a naturally occurring deletion of exons 43 and 44 in the type 1 *$InsP_3R$* channel that results in an in-frame deletion of residues 1732–1839 in the regulatory domain immediately after the SII splice region [57] (Fig. 1b). The phenotype of the homozygous *opt* mouse is similar to that of the type 1 *$InsP_3R$* homozygous knock-out mouse. Both mice are smaller than their normal littermates at birth, lack normal locomotor behaviors, display seizures at about 2 weeks of life, and then die by 3–4 weeks of age [46, 57]. Metabotropic glutamate receptor-mediated Ca^{2+} release was only moderately diminished in Purkinje cell soma in P4 cerebellar slices from homozygous *opt* mice [57]. A reconstituted recombinant *opt* $InsP_3R$-1 was functional, although it had apparent diminished ATP sensitivity compared with wild-type (WT) channels [70]. The *opt*-deleted region of the $InsP_3R$ contains a putative ATP binding site (Fig. 1b) that may account for reduced ATP responsiveness, and it also contains a PKA phosphorylation site. The mutant protein is expressed at lower levels than the wild-type protein [57]. We have confirmed that $InsP_3R$-1 protein level is reduced by

~50% in the heterozygote *opt* brain and is nearly undetectable in brain lysates from homozygous *opt* mice (our unpublished results). It seems most likely that the major deficit in *opt* mice is reduced $InsP_3R$-1 protein expression, consistent with the nearly identical phenotypes of *opt* and $InsP_3R$-1 knock-out mice. However, the Ca^{2+} imaging results may not be easily reconciled with this conclusion. The mechanisms that account for the reduced channel expression are unknown. The fact that the recombinant channel behaves relatively normally suggests that the 108-residue deletion does not prevent normal oligomerization, ligand binding, permeation, or gating. Persistent activation of Gαq induces down-regulation of $InsP_3R$ protein levels in some cells [78]. However, the published single channel studies do not indicate that the *opt* channel is hyper-active, suggesting that this mechanism is not responsible. It is possible that cellular quality control mechanisms recognize the channel as defective and quickly degrade it.

The Δ18 mouse

The Δ18 mouse was identified as having an in-frame deletion of 18 base pairs within exon 36 of *$InsP_3R$-1* that results in the deletion of six residues (residues 1533–1538; Glu-Ser-Cys-Ile-Arg-Val) in the regulatory domain [73] (Fig. 1b). It was observed as a severe autosomal recessive progressive movement disorder, with a survival time of approximately 4 weeks, phenotypes reminiscent of both *$InsP_3R$-1* knock-out and *opt* mice. The six residues are not particularly conserved across the three channel isoforms and species, although the Cys and a basic amino acid 2 residue downstream appear to be. The functional significance of these residues is unknown. In a preliminary study, it was reported that the recombinant rat channel with the six-residue deletion formed a functional channel [49]. Nevertheless, as in the *opt* mouse, the deletion appears to be associated with a pronounced reduction of $InsP_3R$-1 protein levels, measured by immunostaining in cerebellar Purkinje neurons and Western blotting of whole brain lysates [73]. It is likely therefore that the disease phenotype is caused by a lack of $InsP_3R$-1 protein expression as a consequence of the deletion.

Hints of defective $InsP_3R$ roles in other ataxias

CA8

A homozygous mutation in the *CA8* gene was discovered in a consanguineous family as the cause of a syndrome of ataxia and mild mental retardation and ambulation on all four extremities (quadrupedal gait) [72]. *CA8* encodes for the carbonic anhydrase-related protein VIII, a catalytically inactive carbonic anhydrase with strong expression in cerebellar Purkinje neurons. In affected humans, serine at position 298 was replaced by proline. In cell culture, the mutation resulted in severely reduced CA8 protein expression, which was in part restored by inhibition of the proteasome [72]. It was therefore speculated that the mutation destabilizes the protein and causes disease as a result of a loss of CA8 function. Loss of function of CA8 was previously identified as the autosomal recessive deficit in the waddles (*wdl*) mouse [31], a spontaneous model that has a 19-base-pair deletion in *CA8* that results in a lack of mRNA and protein in the homozygote. Thus, it, too, is a CA8 loss of function. The mouse has ataxia and appendicular dystonia that produces nearly straight limbs and a “waddling” side-to-side gait during ambulation [31]. Homozygous mice have normal gross cerebellar morphology with abnormalities of parallel fiber–Purkinje cell synapses and defects in excitatory transmission [24].

The only reported function of CA8 is to inhibit $InsP_3$ binding to the $InsP_3R$-1 [26]. CA8 was discovered in a yeast two-hybrid screen as an interactor with the regulatory domain of the channel within residues 1387–1647 (Fig. 1b). CA8 and $InsP_3R$-1 co-localized extensively in isolated cerebellar Purkinje cells. Binding of CA8 reduced the apparent affinity of the channel for [^{3}H]$InsP_3$ binding [26]. Since the binding region is distinct from the $InsP_3$ binding domain,

this reflects an allosteric effect, suggesting that CA8 binding induces conformational changes in the protein. However, nothing else is known regarding the functional consequences of CA8 binding to the $InsP_3$Ror whether the binding is related to the phenotypes described above. Lack of interaction of CA8 with the $InsP_3$R in the *CA8* patients or *wdl* mice would not be expected, a priori, to result in loss of $InsP_3$R function. As discussed below, polyglutamine-expanded huntingtin and ataxins 1 and 2, the latter that result in cerebellar ataxias, all bind to the $InsP_3$R and enhance its sensitivity to $InsP_3$. Furthermore, mutant presenilins that cause Alzheimer's disease bind to the $InsP_3$R and increase its activity (below). It is conceivable therefore that $InsP_3$R-1 is more sensitive to $InsP_3$ in *CA8* patients and *wdl* mice, and that the disease is a result of gain of function of the channel. Further studies are necessary to establish a role of the $InsP_3$Rin *CA8* patients and *wdl* mice.

Spinocerebellar ataxias 2 and 3

Abnormal Ca^{2+} release through the $InsP_3$R has been implicated in two spinocerebellar ataxias in addition to SCAs 15 and 16. Following their observations of the interaction of mutant huntingtin protein with the carboxyl terminus of the $InsP_3$R-1 (below), the Bezprozvanny group explored whether other disease-causing polyglutamine repeat proteins similarly bind there and affect the channel activity [8,42]. SCA2 and SCA3 are caused by polyglutamine expansions in ataxin2 and ataxin3, respectively [35,38]. It was discovered in pull-down and co-immunoprecipitation assays that the polyglutamine expanded forms, but not the normal proteins, and each interacted with the same region of the $InsP_3$R-1 carboxyl terminus (residues 2627–2749) [8,42] (Fig. 1b). To explore the functional consequences of these interactions, either the wild-type or mutant ataxin proteins were co-expressed with rat $InsP_3$R-1 in Sf9 cells, and microsomes purified from the infected cells were reconstituted into lipid planar bilayers. In each case, reconstituted channels from the cells expressing the mutant ataxins were more sensitive to activation by lower concentrations of $InsP_3$. In cells in culture, medium spiny neurons in the case of SCA2 and Purkinje neurons in the case of SCA3, metabotropic glutamate receptor stimulation caused higher-peak cytoplasmic Ca^{2+} responses in cells from the mutant mice. It was speculated that these Ca^{2+} responses may underlie cellular toxicity because enhanced cell death induced by prolonged glutamate exposure in the disease neurons was diminished by treatment with dantrolene, a RyR Ca^{2+} release channel inhibitor. It was suggested that dantrolene provided this protection by inhibiting Ca^{2+} signals that emanate from $InsP_3$-induced Ca^{2+} release that is amplified by CICR by the RyR. Dantrolene feeding provided protection against age-dependent disease-associated morphological and behavioral deficits. The authors concluded that abnormal neuronal Ca^{2+} signaling through the $InsP_3$R-1 may play a role in the pathogenesis of many polyglutamine expansion disorders [8,42].

The role of $InsP_3$R in Huntington's disease

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by amino-terminal polyglutamine expansions in huntingtin (Htt), a large ubiquitously expressed protein [20,75,82]. The disease is associated with movement disorders, cognitive decline, and psychiatric symptoms that progress over 15–20 years before death. Brain neurons in HD have characteristic cytoplasmic and nuclear aggregates containing htt and other proteins [82]. HD pathogenesis is due to a toxic gain of function of mutant htt that results in neuronal loss in the cortex and striatum, although the molecular mechanisms that underlie pathogenesis and the selective vulnerability of particular neuronal populations are still debated on [69,75,82]. The physiological function of wild-type htt is unknown. Its sequence indicates that it possesses many HEAT repeats, protein interaction domains that suggest that it may serve as a molecular scaffold [43].

The type 1 $InsP_3$R was identified in a comprehensive screen of protein interactors with htt [33]. Importantly, it behaved as a genetic modifier of HD-associated neurodegeneration. In a

Drosophila model, in which a polyglutamine-expanded human *Htt* transgene expression in the eye caused retinal degeneration, $InsP_3R$ expression modified the eye phenotype: reduced expression of the channel suppressed it, whereas enhanced expression made it more severe [33]. In a separate screen, huntingtin-associated protein 1 (HAP1), a protein that also interacts with Htt, particularly the polyglutamine expanded forms (Htt^{exp}) [13], was also identified to interact with $InsP_3R$-1. The interaction was with the carboxyl terminus of the channel within residues 2627–2736 [65] (Fig. 1b). This channel construct also directly bound to Htt^{exp} and to a lesser extent to Htt. Both interactions were strengthened by the presence of HAP1, suggesting that the three proteins might exist in a complex [65]. Whereas the addition of recombinant HAP1 to the cytoplasmic aspect of reconstituted $InsP_3R$-1 was without effect on channel P_o evoked by a sub-saturating [$InsP_3$], subsequent additions of amino-terminal fragments of Htt or Htt^{exp}, or application of premixed HAP1–Htt/Htt^{exp}, increased channel activity [65,66]. Full-length Htt^{exp} but not Htt increased P_o in response to sub-saturating [$InsP_3$] without HAP1 pre-exposure [65]. Consistent with these effects, over-expression of full-length Htt^{exp}, but not Htt, increased $InsP_3$-dependent Ca^{2+} release in cultured medium spiny neurons in response to the threshold levels of the agonist. Enhanced Ca^{2+} release caused by Htt^{exp} was partially dependent on HAP1, as the effect was less robust in $HAP1^{-/-}$ cells [66]. It was suggested that the enhanced $InsP_3$ sensitivity of the Htt/HAP-bound $InsP_3R$-1 may contribute to the progression of HD by exaggerated Ca^{2+} release-dependent neuronal apoptosis [64].

Expression in vitro of a GFP-fused carboxyl-terminal interacting region in medium spiny neurons from an HD transgenic mouse normalized exaggerated cytoplasmic Ca^{2+} responses to glutaminergic stimulation and provided protection from glutamate excitotoxicity [63]. Long-term expression of the construct in the striatum of virally injected mice reduced the biochemical interaction of $InsP_3R$-1 and htt^{exp} and reduced motor coordination deficits and loss of medium spiny neurons. Remarkably, the fusion protein also reduced the load of aggregated Htt^{exp} [63]. Although many questions remain, these studies indicate a strong involvement of the $InsP_3R$ in HD pathogenesis and suggest novel therapeutic targets and strategies.

The role of $InsP_3R$ in Alzheimer's disease

Alzheimer's disease is a common form of dementia involving slowly developing and ultimately fatal neurodegeneration. The etiology of AD is debated on, with age being the main risk factor but with major molecular mechanisms remaining unclear. A hallmark feature of AD is accumulation of extracellular β amyloid (Aβ) plaques, intracellular neurofibrillary tangles, and neuronal loss [14]. Mutations in presenilins (PS1 and PS2) and amyloid precursor protein (APP) cause most early-onset, autosomal dominant familial cases of the disease (FAD) [67]. Presenilins are components of a protein complex that proteolytically processes APP into Aβ peptides [54]. In the “amyloid hypothesis” of AD, an accumulation of Aβ due to defective processing and clearance leads to pathological sequelae associated with the disease [22]. The identification of three components in FAD—PS1, PS2, and APP—that are linked in a biochemical pathway that impinges on Aβ production has strongly influenced the acceptance of the amyloid hypothesis [22]. Nevertheless, much evidence suggests that altered Ca^{2+} signaling is associated with expression of FAD mutant PS in symptomatic or pre-symptomatic patient cells and in brain neurons in AD mouse models long before the appearances of plaques or tangles and in a variety of heterologous expression systems [3,19,37,61]. Before the molecular identification of PS, it was shown that fibroblast lines from AD patients (later shown to harbor a FAD mutation in PS1) generated exaggerated $[Ca^{2+}]_i$ responses to sub-maximal concentrations of two G-protein coupled receptor agonists that activate PLC [29]. Subsequent studies have confirmed that FAD PS expression is associated with an exaggerated ER Ca^{2+} release in several cell systems. However, these studies have not led to consensus regarding the molecular mechanisms involved (reviewed in 37,58]. Exaggerated ER Ca^{2+} release has been ascribed to the enhanced loading of the ER lumen [58] due either to enhanced SERCA Ca^{2+}

pump activity [18] or to disruption of a putative Ca^{2+} channel function of wild-type PS [48, 71]. Alternately, exaggerated Ca^{2+} release has been accounted for by enhanced Ca^{2+} liberation from normal stores through $InsP_3R$[40,58] or RyR [6,56,60]Ca^{2+} release channels, both in vivo [6,56,59,60] and in vitro [12,25,29,39], either as a consequence of enhanced channel expression [6,7,34,61] or, in the case of the $InsP_3R$, of enhanced activity in response to its ligand $InsP_3$ [10,29].

A biochemical interaction of WT and FAD mutant PS1 and 2 with the $InsP_3R$ was identified. Functionally, FAD PS specifically had gain-of-function consequences for the activity of the channel, which in turn was associated with exaggerated Ca^{2+} signaling in intact cells [9,10]. An example is shown in (Fig. 3). FAD and WT-PS1 were expressed in insect Sf9 cells, and patch clamp experiments were performed on isolated nuclei to record $InsP_3R$ ion channels in their native membrane [45]. The $InsP_3$ and Ca^{2+} regulation of the Sf9 channel, the type 1 isoform, is similar to the dominant neuronal type in the mammalian brain [27]. In conditions optimal for channel activity, $InsP_3R$ channels in control nuclei had a high P_o (Fig. 3b). In nuclei from either M146L-PS1 or WT-PS1-expressing cells, no novel ion channels were detected (Fig. 3a), nor were channels observed in the absence of $InsP_3$ (Fig. 3a) or in the presence of $InsP_3$ and its competitive inhibitor heparin. Activated $InsP_3R$ channels in WT-PS1-infected cells had P_o similar to control cells, whereas P_o was elevated significantly in FAD M146L-PS1-infected cells (Fig. 3b, d). With sub-saturating [$InsP_3$], P_o was elevated approximately threefold in nuclei from FAD PS1-expressing cells compared with control and WT-PS1 expressing cells, to a degree comparable to that observed in saturating [$InsP_3$] (Fig. 3c, d). Similar results were obtained with an FAD PS2 mutant (N141I) [9,10].

Enhanced $InsP_3R$ channel activity appears to be a conserved feature of FAD PS-expressing cells, since similar results were observed in cells expressing other FAD mutant PS [9]. γ-Secretase-dead mutants also significantly enhanced the $InsP_3R$ channel activity, although to a lesser extent than the FAD mutants, indicating that the secretase activity of PS is not required for its effects on $InsP_3R$ gating [9]. Interestingly, the P_o of channels recorded from cells infected with frontotemporal dementia-associated mutant PS1 was not different from controls [9]. Thus, several FAD-mutant PS have similar effects on $InsP_3R$ gating, and these effects appear not to be recapitulated in PS mutants associated with a different neurological disease.

These effects of FAD PS on $InsP_3R$ gating appear to be remarkably robust, since they have now been observed in five different cellular systems: Sf9 cells, chicken DT40 B cells, human FAD patient B cell-derived lymphoblasts and fibroblasts, and neurons from FAD-PS AD mice, in the absence of and preceding disease pathology [9,10]. The strikingly similar effects of mutant PS expression of $InsP_3R$ channels in such diverse cellular systems from different species suggest that the effects of mutant PS on channel activity is a robust one in all cell types and $InsP_3R$ isoforms. The results at the single-channel level are consistent with the observations of exaggerated $InsP_3$-mediated $[Ca^{2+}]_i$ signals in FAD patient fibroblasts [29] and other cells with mutant PS expressed. An example in DT40 B cells, where the electrophysiological effects of FAD PS have been demonstrated [10], is shown in Fig. 4. $[Ca^{2+}]_i$ signals mediated by $InsP_3R$ were elicited by cross-linking the B cell receptor (BCR). Weak stimulation triggered repetitive Ca^{2+} oscillations and spiking in ~50% of control cells (Fig. 4c, d), due to periodic release from ER through $InsP_3R$, because they are absent in DT40-KO cells in which all three isoforms of the $InsP_3R$ were genetically deleted [62][76]. In cells expressing FAD PS1, oscillation frequency and number of cells responding was increased, and the latency between application of agonist and first response decreased (Fig. 4e, f). In a subset (30%) of cells expressing M146L-PS1, the latency was nearly abolished (Fig. 4c,d,f), a response that was reminiscent of that of normal cells to strong stimulation [76]. These effects demonstrate that FAD PS1 generates exaggerated $InsP_3R$-mediated $[Ca^{2+}]_i$ responses, as observed in other cell

types, and they suggest, consistent with the single channel studies, that FAD PS expression enhances $InsP_3R$ sensitivity to $InsP_3$.

The electrophysiological studies suggest that FAD PS stimulate $InsP_3R$ gating by a mechanism that involves PS-mediated effective sensitization of the channel to $InsP_3$, most likely through an allosteric mechanism. Nevertheless, this conclusion is somewhat tentative, as are those regarding of the effects of polyglutamine-expanded protein interaction effects on $InsP_3R$ gating discussed earlier, since the effects of PS have in each case only been examined at a single $[Ca^{2+}]_i$. $InsP_3$ and Ca^{2+} regulate the channel in a complicated manner, with $InsP_3$ affecting gating through modulation of Ca^{2+} inhibition. The relationship between channel P_o and $[InsP_3]$ and $[Ca^{2+}]_i$ cannot be adequately characterized by determining P_o at different $[InsP_3]$ at just one $[Ca^{2+}]_i$. Depending on the $[Ca^{2+}]_i$ used, different apparent functional affinities for $InsP_3$ can be observed [15]. Thus, it will be important to extend these studies to examine the effects over physiologically relevant ranges on both $[InsP_3]$ and $[Ca^{2+}]_i$. With this caveat in mind, modal gating analysis suggested that the FAD mutant PS regulates channel activity by impinging upon the normal ligand activation mechanisms. Ligand regulation of $InsP_3R$ gating is largely mediated by altering the propensity of the channel to gate in particular modes [28]. Strongly activated channels gate in a high P_o H mode characterized by long bursting activities: an intermediate P_o I mode is characterized by fast channel openings and closings and a low P_o L mode is characterized by long closings with brief openings. In nuclei isolated from control cells expressing an irrelevant protein or from Sf9 cells infected with WT-PS1 or PS2, similar modal distributions were observed (Fig. 5). In contrast, the H mode was the dominant gating mode of $InsP_3R$ recorded from FAD PS-expressing cells (Fig. 5). FAD PS therefore enhances $InsP_3R$ gating by mode switching, causing the channel to spend more time in the H mode. Modal gating regulation may have important functional consequences. The channel open time in the L mode (~10 ms) is short enough that it may not increase local $[Ca^{2+}]$ sufficiently to recruit additional $InsP_3R$- or RyR-mediated CICR. In contrast, the much longer activity bursts in the H mode (>200 ms) can provide a sufficiently large Ca^{2+} flux to enable a normally local Ca^{2+} signaltobeamplified andpropagatedbyCICR [15]. It was suggested [9] that, because $InsP_3$ R and RyR are clustered and spatially organized to provide local $[Ca^{2+}]_i$ signals as a critical element of physiological specificity, mode-shifting by FAD PS may result not only in exaggerated local Ca^{2+} signaling but also in the disruption of spatial specificity by enabling CICR to transmit signals more globally [15,28]. Mode switching by FAD PS of $InsP_3R$ gating may account for observations $InsP_3$-dependent, exaggerated RyR-mediated Ca^{2+} signals in neurons (e.g., 60,61).

$InsP_3$-mediated $[Ca^{2+}]_i$ signals regulate many cell physiological processes. A major question is whether the effects of mutant PS on $InsP_3R$-mediated Ca^{2+} signaling impinge on disease pathogenesis. It is possible that exaggerated Ca^{2+} signals in AD may influence reactive oxygen species generation, mitochondrial function, gene transcription, and Aβ production [52], features associated with AD. The possible role of $InsP_3R$inAβ production was examined in DT40 cells that were engineered to stably express APP harboring Swedish mutations (APP_{SWE}) that enhance Aβ production, together with either PS1-WT or PS1-M146L (Fig. 6, top). PS1-M146L specifically enhanced $Aβ_{40}$ and $Aβ_{42}$ secretion by approximately two and threefold, respectively, compared with control cells. Of note, the $Aβ_{42}/Aβ_{40}$ ratio was enhanced in mutant PS1-expressing cells (Fig. 6), as observed in AD patients. To determine the role of the $InsP_3R$ in PS1-dependent APP processing, APP and PS1-expressing cells were generated in the DT40 cells that lacked expression of $InsP_3R$ ($InsP_3R$-KO). Notably, mutant PS1 enhancement of Aβ secretion observed in $InsP_3R$-expressing cells was abolished. Furthermore, the absolute levels of Aβ peptides detected were strongly reduced in all control and PS1-expressing $InsP_3R$-KO lines (Fig. 6, bottom). These results suggest that APP processing by FAD PS1 has a strong dependence on $InsP_3R$ activity (Fig. 7).

Conclusions

Human and mouse genetics have provided the strongest evidence that $InsP_3R$ mutations can result in disease. However, the number of mutations and diseases identified are limited. To date, only one channel isoform, the type 1, has been implicated in these studies. Furthermore, the diseases have all been neurological and predominantly cerebellar. Cerebellar Purkinje neurons, with the highest $InsP_3R$-1 protein expression levels in the body, are the final integrators of sensory inputs and provide cerebellar cortex outputs that control motor coordination. The genetics has suggested that the lack of two functional *$InsP_3R$-1* alleles disrupts cerebellar function. It is interesting that the recombinant expression of several of the $InsP_3R$-1 mutant proteins produces functional ion channels, including *opt*, *Δ18*, and P1059L, but appear to cause a disease due to diminished expression, perhaps due to rapid degradation by cellular quality control mechanisms. It will be interesting, in future studies, to examine the effects of these mutations not only on ion channel properties but also on the kinetics of channel biogenesis and turnover. *RyR1* and *RyR2* are the predominant RyR isoforms in skeletal and cardiac muscle, respectively. Many mutations in these isoforms have been identified because of the pronounced muscle phenotypes they cause. The reliance in the cerebellum on one particular $InsP_3R$ isoform seems to be the exception in the human body. It seems likely that most cell types and tissues have functional $InsP_3R$ redundancy that renders $InsP_3R$ mutations phenotypically non-penetrant. Nevertheless, it seems likely that other diseases will be discovered that have mutations in $InsP_3R$ genes as their basis. A recent genome-wide association study linked an intronic single nucleotide polymorphism in the *$InsP_3R$-2* gene to risk of amyotrophic lateral sclerosis, a motor neuron disease [74].

Acknowledgments

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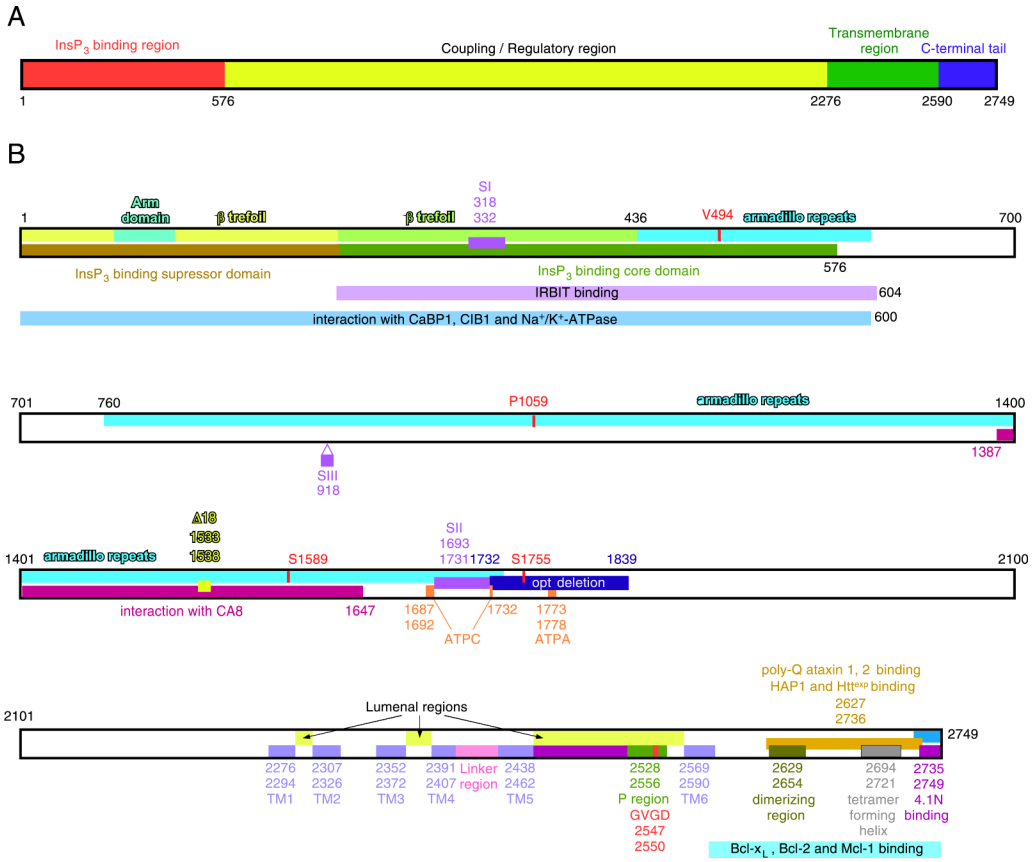

Fig. 1.

Structural determinants of the $InsP_3R$. **a** Overall domain structure. The $InsP_3R$ molecule depicted as a linear amino acid sequence, with the amino terminal $InsP_3$ binding region (*red*), coupling region (*yellow*), transmembrane region (*green*), and carboxyl tail (*blue*) depicted. **b** Linear amino acid sequence. Residues are numbered according to the rat type 1 SI+, SII+, SIII– sequence (protein accession number 121838). The structural features shown are: Arm sub-domain and β-trefoil in the $InsP_3$-binding suppressor domain; β-trefoil and armadillo repeats in $InsP_3$ binding core-domain; armadillo repeats in the coupling/regulatory domain; alternative splicing regions SI, SII, and SIII for type 1 $InsP_3R$; *opt* deletion in type 1 $InsP_3R$mutant; Δ18 deletion in type 1 $InsP_3R$; ATP-binding site ATPA; transmembrane helices TM1–6, and pore-forming P region with selectivity filter; dimerizing region; tetramer forming region; S1589 and S1755 are PKA/PKG phosphorylation sites; mutation of Pro1059 to Leu in type 1 $InsP_3R$ associated with SCA15; mutation of Val494 to Ile in type 1 $InsP_3R$ associated with SCA15. The sequences involved in the interaction of $InsP_3R$ channel with the following proteins are also depicted: CaBP1; IRBIT; CIB1; Na^+/K^+-ATPase; HAP1 and Htt^{exp}; poly-glutamine (poly-Q)-expanded ataxins 1 and 2; protein 4.1N; CA8; Bcl-2, Bcl-x_L, and Mcl-1. Modified from [15] with permission from the American Physiological Society

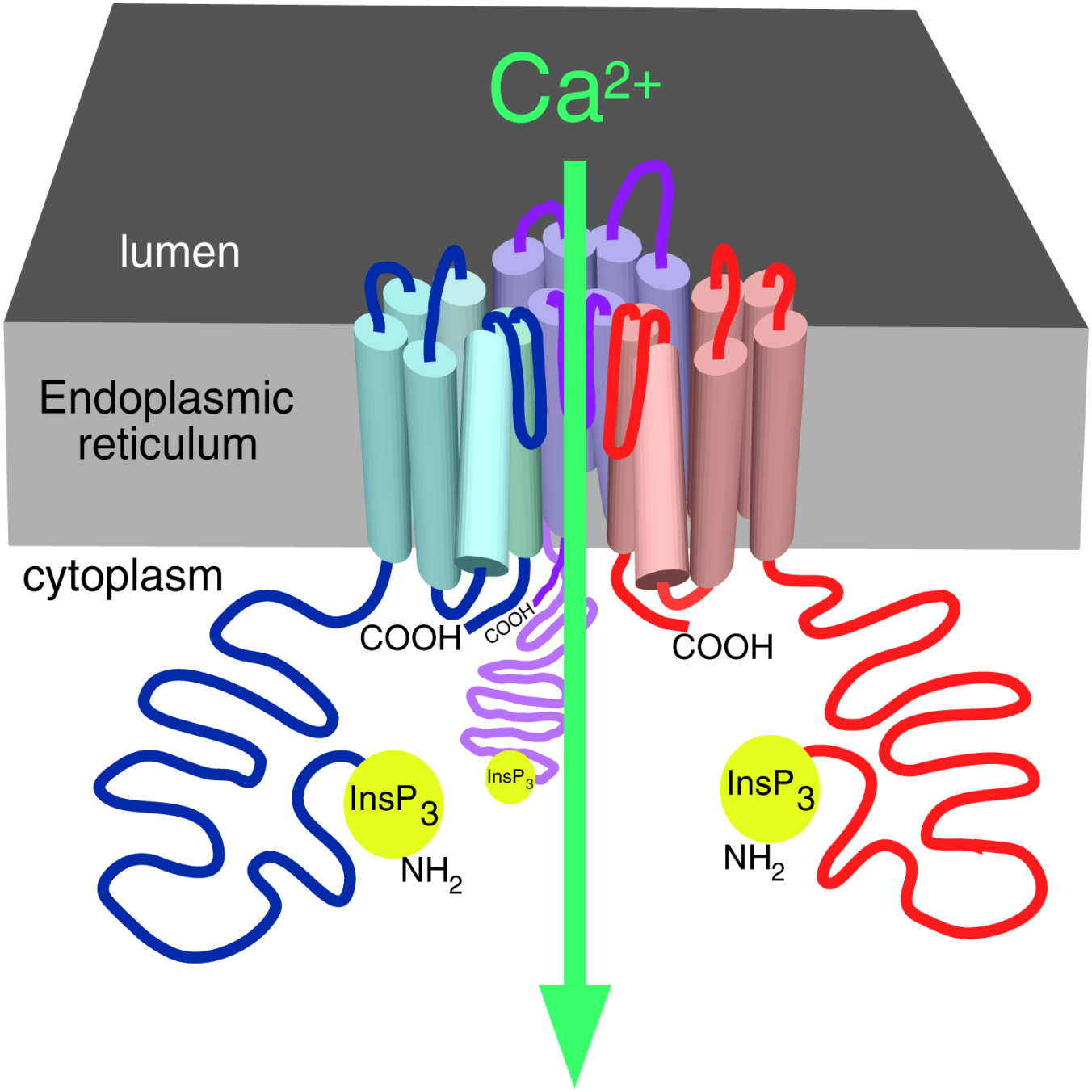

Fig. 2.
The $InsP_3R$ Ca^{2+} release channel. Cartoon depicting three of four $InsP_3R$ molecules (in different colors) in a single tetrameric channel structure. Part of the lumenal loop connecting transmembrane helices 5 and 6 of each monomer dips into the fourfold symmetrical axis, creating the permeation pathway for Ca^{2+} efflux from the lumen of the endoplasmic reticulum. Reproduced from [15] with permission from the American Physiological Society

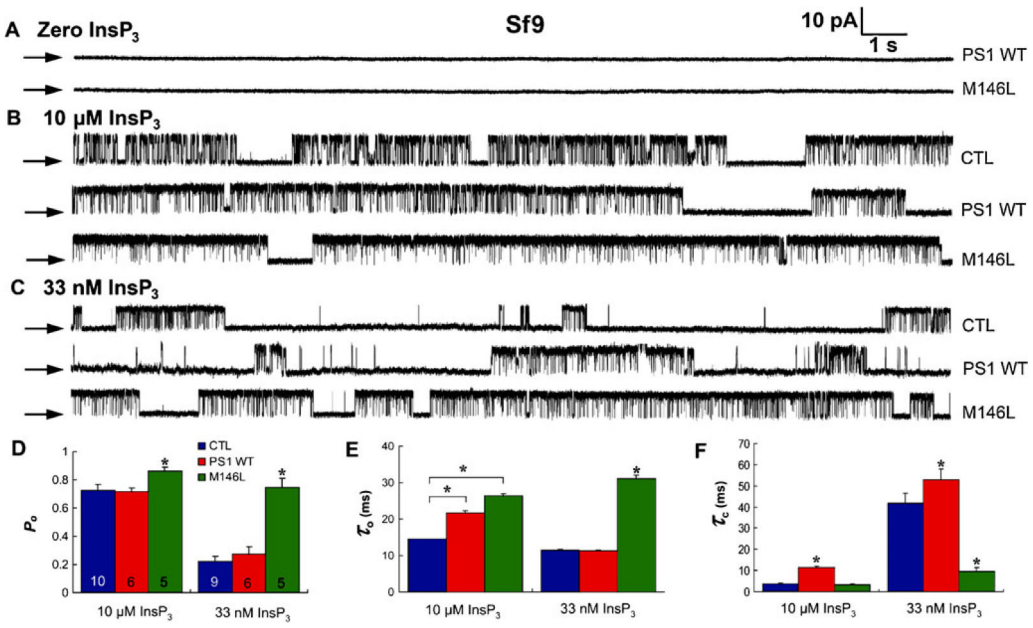

Fig. 3.

Effects of PS1 expression on $InsP_3R$ single channel activity in Sf9 cells. **a–c** Representative current recordings in isolated nuclei from Sf9 cells infected with PS1 WT or M146L baculoviruses in the absence (**a**) or presence of saturating (10 μM; **b**) or sub-saturating (33 nM; **c**) $InsP_3$ in pipette solution. Channel activity was not evoked by PS1 alone in the absence of $InsP_3$ (**a**), whereas $InsP_3R$ channels were activated in the presence of $InsP_3$ (**b, c**). Pipette $[Ca^{2+}]$ was 1 μM; *arrows*, zero current level. Summary of effects of PS1 expression on $InsP_3R$ channel open probability P_o (**d**) mean open time (τ_o) (**e**), and mean closed time (τ_c) (**f**). *Asterisks*, $p<0.01$, unpaired *t*-test. From [10] with permission from Elsevier

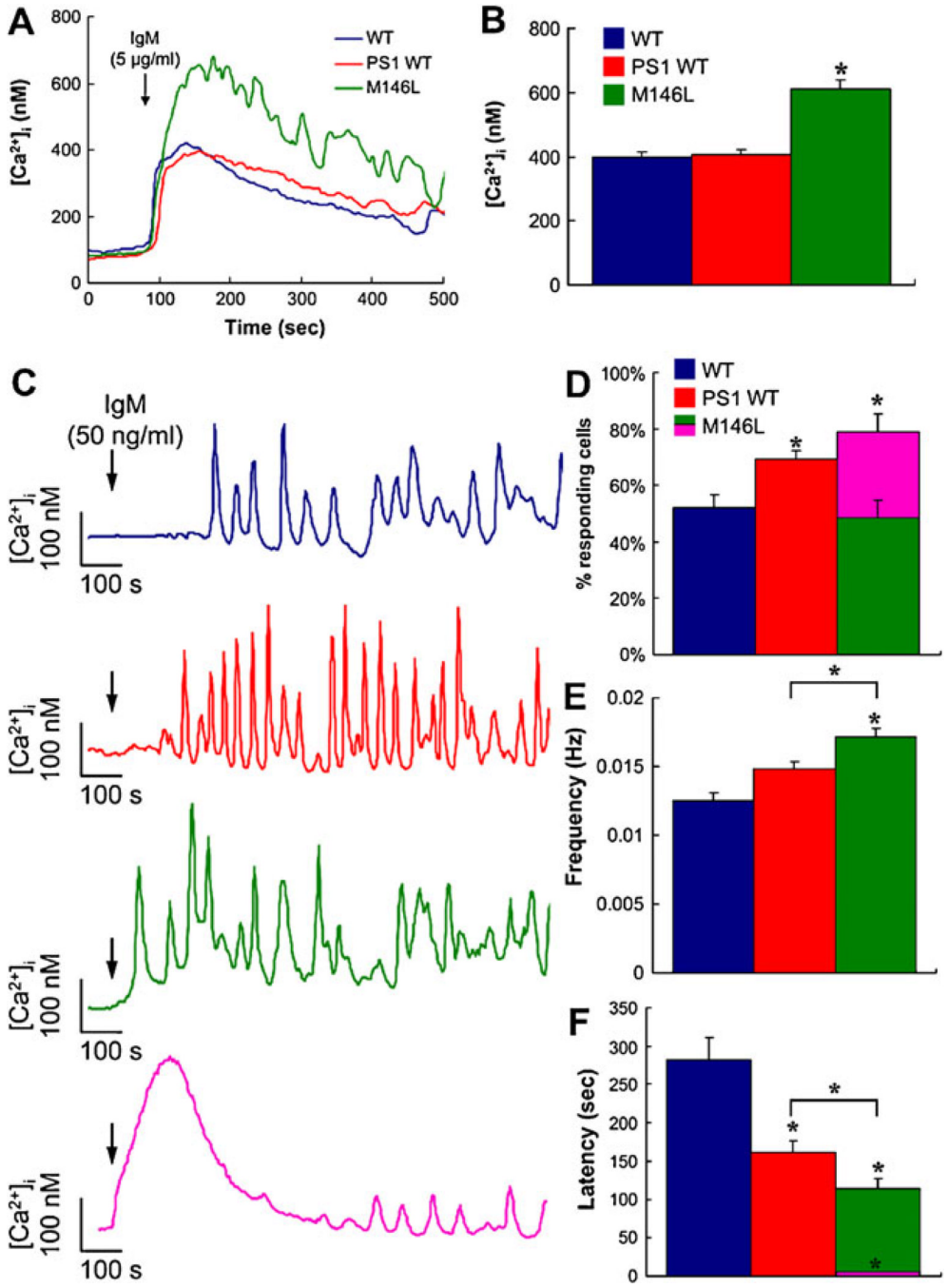

Fig. 4.

Exaggerated $[Ca^{2+}]_i$ signaling in mutant PS-expressing DT40 cells. **a, b** Responses to strong stimulation by BCR antibody of DT40 cell $[Ca^{2+}]_i$. **a** Representative single-cell responses to 5 μg/ml anti-IgM (added at *arrow*) in untransfected (*blue*) and PS1-WT (*red*) and PS1-M146L (*green*) stably transfected DT40 cells. **b** Summary of peak $[Ca^{2+}]_i$ responses triggered by 5 μg/ml anti-IgM (n=90). Asterisk, p<0.01 compared with WT and PS1-WT (**c–f**). Responses to weak stimulation by BCR antibody of DT40 cell $[Ca^{2+}]_i$. **c** Representative single cell $[Ca^{2+}]_i$ responses to 50 ng/ml anti-IgM (IgM; added at *arrow*) stimulation of BCR in control (*blue*), PS1-WT (*red*), and PS1-M146L (*green and pink*) stably transfected DT40 cells. **d** Summary of percentage of cells responding to 50 ng/ml anti-IgM (n=90). Of PS1-M146L-expressing

cells, ~30% (*purple*) exhibited a different, exaggerated $[Ca^{2+}]_i$ response. **e** $[Ca^{2+}]_i$ oscillation frequency triggered by anti-IgM in WT DT40, PS1-WT- and PS1-M146L-expressing cells. **f** Summary of latencies to first response in WT DT40, PS1-WT- and PS1-M146L-expressing cells. The 30% of PS1-M146L-expressing cells that exhibited the exaggerated response had nearly no latency (*purple*). *Asterisks*, $p<0.01$ compared with WT DT40 cells. *Asterisks with bars*, $p<0.01$ PS1-Wt vs PS1-M146L. From [10] with permission from Elsevier

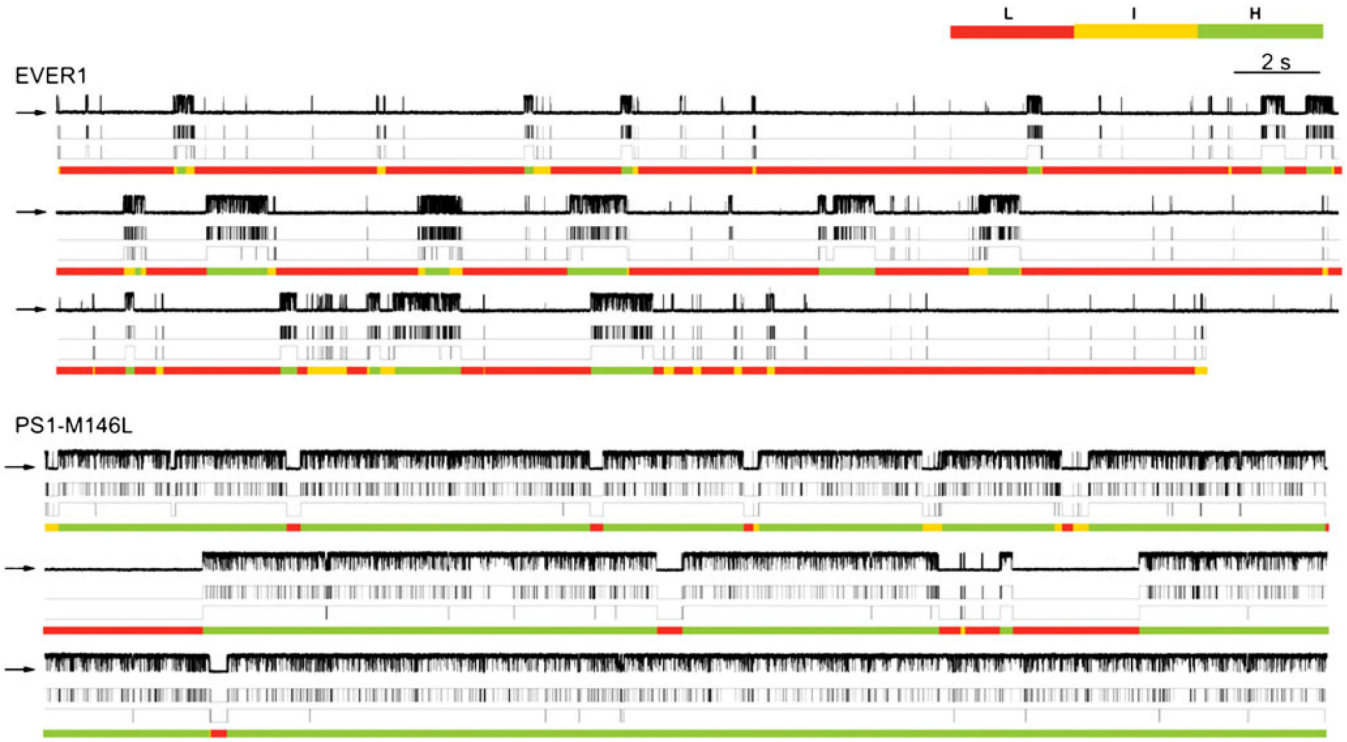

Fig. 5.

Modal gating analyses of $InsP_3R$ channels under the influence of FAD-linked mutant PS. Distinct single channel $InsP_3R$ gating behaviors from EVER1 (control) *vs* PS1-M146L-expressing Sf9 cells. Each section consists of a set of four traces of the same single channel current record: (*top*) unprocessed current trace, (*second*) idealized current trace generated using Qub software (*third*), idealized current trace after burst analysis (closing events <10 ms were filtered), and (bottom) modal assignment by analyzing channel burst (t_b) and gap (t_g) durations [28]. In EVER1-infected cells, low P_o is associated with prevalence of L gating mode. In PS1-M146L infected cells, enhanced P_o is manifested by increased t_b and decreased t_g. Channel occupancy of H mode is dominant, whereas occupancy of L gating mode is significantly decreased. From [9] with permission from the American Association for the Advancement of Science

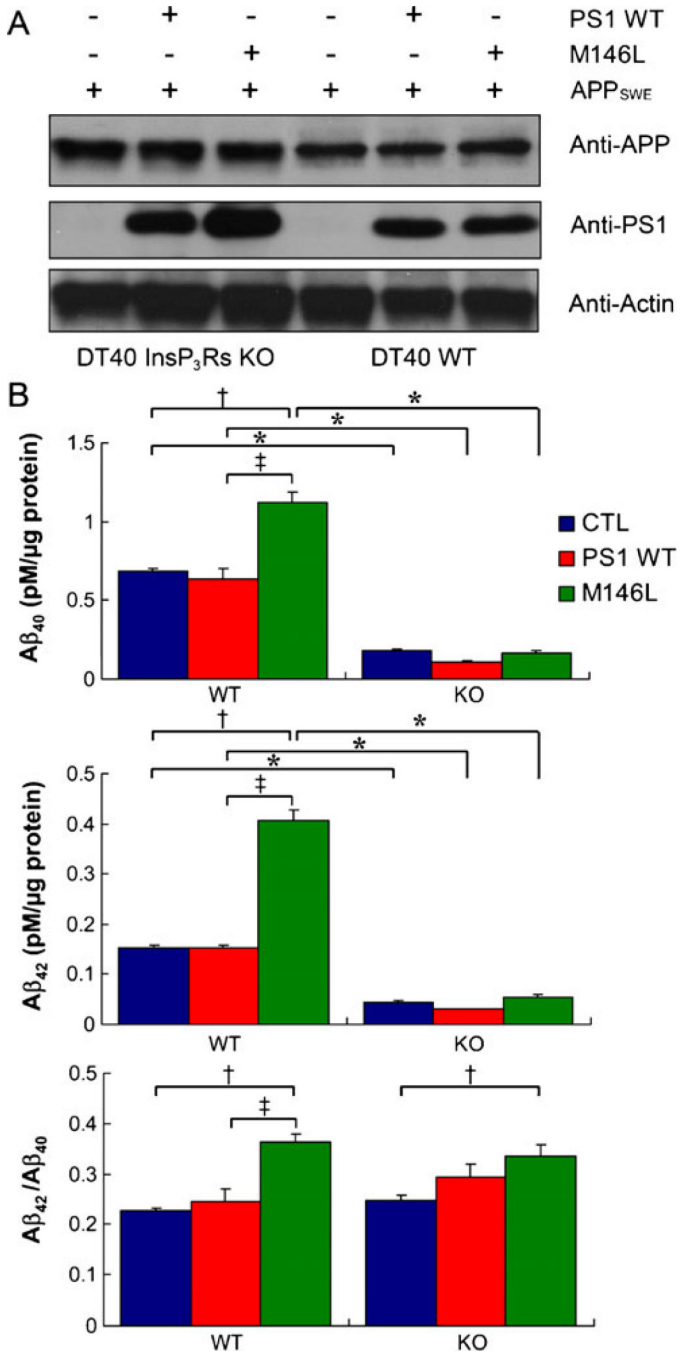

Fig. 6.

APP processing is dependent on $InsP_3R$. **a** Stable expression of PS1-WT and PS1-M146L proteins in wild-type (*WT*) and $InsP_3R$-deficient (*KO*) DT40 cell lines that stably expressed APP_{SWE}. Actin probed as loading control. *b* ELISA measurements of $A\beta_{40}$ (*top*), $A\beta_{42}$ (*middle*), and $A\beta_{42}/A\beta_{40}$ ratio (*bottom*) secreted over 48 h by $InsP_3R$-expressing wild-type (WT; *left*) or $InsP_3R$-deficient (KO DT40 cells; *right*) DT40 cells stably expressing APP_{SWE} alone (*blue*) or APP_{SWE} with PS1-WT (*red*) or PS1-M146L (*green*). Asterisks, $p < 0.01$ compared with control WT cells. *Cross*, $p<0.01$ compared with control WT cells; *double cross*, $p<0.01$ compared with PS1-WT cells. From [10] with permission from Elsevier

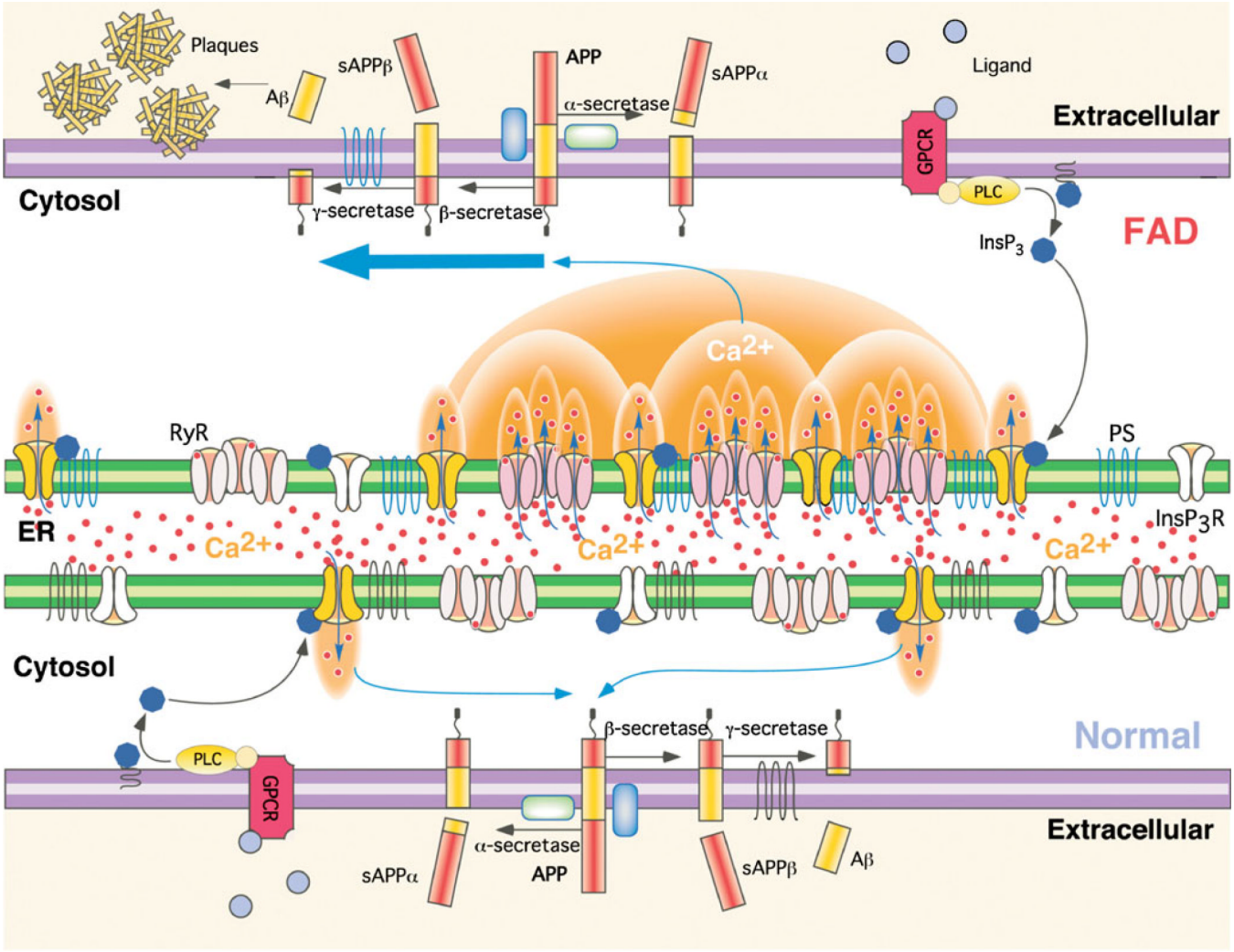

Fig. 7.

Hypothetical molecular mechanism of enhanced Aβ production due to Ca^{2+} disruption in FAD PS cells. APP is processed by either α-secretase or β-secretase, the latter leading to Aβ generation after subsequent cleavage by γ-secretase. Stimulation of G-protein coupled receptors or other cell surface receptors by extracellular ligands activates phospholipase C (*PLC*), which cleaves phosphatidylinositol bisphosphate to produce $InsP_3$. $InsP_3$ binds to and activates the $InsP_3R$ to release Ca^{2+} from ER stores, increasing cytoplasmic Ca^{2+} concentration. In normal cells, these Ca^{2+} signals are tightly regulated in time, space, and amplitude. In FAD cells, mutant PS exerts stimulatory effects on $InsP_3R$ gating by modal switching to the H mode associated with prolonged channel openings. H mode gating generates exaggerated Ca^{2+} signaling by promoting additional release channel recruitment by CICR. Increased cytoplasmic Ca^{2+} concentration promotes β-secretase activity [23] and Aβ production [19,52] which, together with mutant PS-enhanced production of amyloidogenic Aβ, results in plaque formation. From [9] with permission from the American Association for the Advancement of Science