New insights in the IP_3 receptor and its regulation

Jan B. PARYS* & Tim VERVLIET

KU Leuven, Laboratory for Molecular and Cellular Signaling,
Department of Cellular and Molecular Medicine & Leuven Kanker Instituut,
Campus Gasthuisberg O/N-1 B-802, Herestraat 49,
BE-3000 Leuven, Belgium

*Corresponding author:
J.B. Parys (jan.parys@kuleuven.be), Tel. +32-16-330660

Table of content

ABSTRACT

The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) is a Ca^{2+}-release channel mainly located in the endoplasmic reticulum (ER). Three IP_3R isoforms are responsible for the generation of intracellular Ca^{2+} signals that may spread across the entire cell or occur locally in so-called microdomains. Because of their ubiquitous expression, these channels are involved in the regulation of a plethora of cellular processes, including cell survival and cell death. To exert their proper function a fine regulation of their activity is of paramount importance. In this review, we will highlight the recent advances in the structural analysis of the IP_3R and try to link these data with the newest information concerning IP_3R activation and regulation. A special focus of this review will be directed towards the regulation of the IP_3R by protein-protein interaction. Especially the protein family formed by calmodulin and related Ca^{2+}-binding proteins and the pro- and anti-apoptotic/autophagic Bcl-2-family members will be highlighted. Finally, recently identified and novel IP_3R regulatory proteins will be discussed. A number of these interactions are involved in cancer development, illustrating the potential importance of modulating IP_3R-mediated Ca^{2+} signaling in cancer treatment.

KEYWORDS

IP_3R

Ca^{2+} signaling

IP_3-induced Ca^{2+} release

Calmodulin

Bcl-2

IRBIT

TESPA1

PKM2

BAP1

Cancer

ABBREVIATIONS

a.a.	amino acids
BAP1	BRCA-associated protein 1
Bcl	B-cell lymphoma
BH	Bcl-2 homology
CaBP	neuronal Ca^{2+}-binding protein
CaM	calmodulin
CaM1234	calmodulin fully deficient in Ca^{2+} binding
cryo-EM	cryo-electron microscopy
DARPP-32	dopamine- and cAMP-regulated phosphoprotein of 32 kDa
ER	endoplasmic reticulum
IBC	IP_3-binding core
IICR	IP_3-induced Ca^{2+} release
IP_3	inositol 1,4,5-trisphosphate
IP_3R	IP_3 receptor
IRBIT	IP_3R-binding protein released by IP_3
MLCK	myosin light chain kinase
NCS-1	neuronal Ca^{2+} sensor-1
PK	pyruvate kinase
PKA	cAMP-dependent protein kinase
PKB	protein kinase B/Akt
PLC	phospholipase C
PTEN	phosphatase and tensin homolog
RyR	ryanodine receptor
TCR	T-cell receptor
TESPA1	thymocyte-expressed, positive selection-associated 1
TIRF	total internal reflection fluorescence

103	TKO	triple-knockout
104		

1 Introduction

The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) is a ubiquitously expressed Ca^{2+}-release channel mainly located in the endoplasmic reticulum (ER) (1). The IP_3R is activated by IP_3, produced by phospholipase C (PLC), following cell stimulation by for instance extracellular agonists, hormones, growth factors or neurotransmitters. The IP_3R is responsible for the initiation and propagation of complex spatio-temporal Ca^{2+} signals that control a multitude of cellular processes (2, 3). Moreover, dysfunction of the IP_3R and deregulation of the subsequent Ca^{2+} signals is involved in many pathological situations (4-10).

There are at least three main reasons for the central role of the IP_3R in cellular signaling. First, IP_3R signaling is not only dependent on the production of IP_3, but is also heavily modulated by its local cellular environment, integrating multiple signaling pathways. Indeed, IP_3R activity is controlled by the cytosolic and the intraluminal Ca^{2+} concentrations, pH, ATP, Mg^{2+} and redox state, as well as by its phosphorylation state at multiple sites. Furthermore, a plethora of associated proteins can modulate localization and activity of the IP_3R (11-15). Second, in higher organisms, three genes (ITPR1, ITPR2 and ITPR3) encode three isoforms (IP_3R1, IP_3R2, and IP_3R3). These isoforms have a homology of about 75% at the a.a. level, allowing for differences in sensitivity towards IP_3 (IP_3R2 > IP_3R1 > IP_3R3) as well as towards the various regulatory factors and proteins (12, 16-19). Splice isoforms and the possibility to form both homo- and heterotetramers further increase IP_3R diversity. Third, the intracellular localization of the IP_3Rs determines their local effect (1). Recently, an increased appreciation for the existence and functional importance of intracellular Ca^{2+} microdomains was obtained, e.g. between ER and mitochondria, lysosomes or plasma membrane where IP_3-induced Ca^{2+} release (IICR) occurs, allowing Ca^{2+} to control very local processes (20-24).

As a number of excellent reviews on various aspects of IP_3R structure and function have recently appeared (25-32), we will in present review highlight the most recent advances concerning the understanding of IP_3R structure and regulation, with special focus on recent insights obtained in relation to IP_3R modulation by associated proteins.

2 New structural information on the IP_3R

The IP_3Rs form large Ca^{2+}-release channels consisting of 4 subunits, each about 2700 a.a. long, that assemble to functional tetramers with a molecular mass of about 1.2 MDa. Each subunit consists of five distinct domains (**Figure 1 A**): the N-terminal coupling domain or suppressor domain (for IP_3R1: a.a. 1–225), the IP_3-binding core (IBC, a.a. 226–578), the central coupling domain or modulatory and transducing domain (a.a. 579–2275), the channel domain with 6 trans-membrane helices (a.a. 2276–2589) and the C-terminal tail or gatekeeper domain (a.a. 2590–2749) (33).

The crystal structure of the two N-terminal domains of the IP_3R1 were first resolved separately at a resolution of 2.2 Å (IBC with bound IP_3, (34)) and 1.8 Å (suppressor domain, (35)). Subsequent studies analyzed the crystal structure of the full ligand-binding domain, i.e. the suppressor domain and the IBC together, resolved in the presence and absence of bound IP_3 at a resolution between 3.0 and 3.8 Å (36, 37). These studies indicated that the N-terminus of IP_3R1 consisted of two successive β-trefoil domains (β-TF) followed by an α-helical armadillo repeat domain. IP_3 binds in a cleft between the second β-trefoil domain and the α-helical armadillo repeat leading to a closure of the IP_3-binding pocket and a conformational change of the domains involved (36-38). Recently, Mikoshiba and co-workers succeeded to perform X-ray crystallography on the complete cytosolic part of the IP_3R (39). This study was performed using truncated IP_3R1 proteins (IP_3R^{2217} and IP_3R^{1585}) in which additional point mutations (resp. $R^{937}G$ and $R^{922}G$) were incorporated in order to increase the quality of the obtained crystals. In addition to the three domains mentioned above (the two β-trefoil domains and the α-helical armadillo repeat domain), three large α-helical domains were described, i.e. HD1 (a.a. 605-1009), HD2 (a.a. 1026-1493) and HD3 (1593-2217) (**Figure 1 B**). Binding of IP_3 induces a conformation change that is transmitted from the IBC through HD1 and HD3, whereby a short, 21 a.a.-long domain (a.a. 2195-2215) called the leaflet domain is essential for IP_3R function.

In parallel with the analysis of the IP_3R by X-ray crystallography, the structure of full-size IP_3R1 was investigated by several groups by cryo-electron microscopy (cryo-EM), obtaining increasingly better resolution (40). The structure of the IP_3R1 at the highest resolution obtained by this method until now (4.7 Å) was published by Serysheva and co-workers and allowed modelling of the backbone topology of 2327 of the 2750 a.a. (41). As IP_3R1 was purified in the absence of IP_3 and as Ca^{2+} was depleted before vitrification, the obtained structure corresponds to the closed state of the channel (**Figure 2**). In total, ten domains were identified: two contiguous β-trefoil domains (a.a. 1–436), followed by three armadillo solenoid folds (ARM1–ARM3, a.a. 437-2192) with an α-helical domain between ARM1 and 2, an intervening lateral domain (ILD, a.a. 2193–2272), the transmembrane region with six trans-membrane α-helices (TM1-6) (a.a. 2273-2600), a linker domain (LNK, a.a. 2601–2680) and the C-terminal domain containing an ~80 Å α-helix (a.a. 2681–2731) (**Figure 1 C**). The latter domains of the four subunits form together with the four TM6 helices (~55 Å) a central core structure that is not found in other types of Ca^{2+} channels. The four transmembrane TM6 helices thereby line the Ca^{2+} conduction pathway and connect via their respective LNK domains with the cytosolic helices.

How binding of IP_3 is coupled to channel opening is still under investigation. An interesting aspect of the IP_3R structure thereby is the fact that either after mild trypsinisation of IP_3R1 (42) or after heterologous expression of the various IP_3R1 fragments corresponding to the domains obtained by trypsinisation (43), the resulting structure appeared both tetrameric and functional. This indicates that continuity of the polypeptide chain is not per se needed for signal transmission to the channel domain, although the resulting Ca^{2+} signals can differ, depending on the exact cleavage site and the IP_3R isoform under consideration (44, 45). Meanwhile, various models for the transmission of the IP_3 signal to the channel region were proposed for IP_3R1, including a direct coupling between the N-terminus and the C-terminus (41, 46-48) and a long-range coupling mediated by the central coupling domain (48), via intra and/or inter subunit interactions (41). Mechanisms for the latter can involve β-TF1 → ARM3 → ILD (41) or IBC → HD1 → HD3 → leaflet (39).

In addition to the structural studies on IP_3R1 described above, the structure of human IP_3R3 was recently analyzed at high resolution (between 3.3 and 4.3 Å) under various conditions. Its apo state was compared to the structures obtained at saturating IP_3 and/or Ca^{2+} concentrations (49). In the presence of IP_3, five different conformational states were resolved, suggesting a dynamic transition between intermediate states eventually leading to channel opening. Ca^{2+} binding appeared to eliminate the intersubunit interactions present in the apo and the IP_3-bound states and provoke channel inhibition. Two Ca^{2+}-binding sites were identified, one just upstream of ARM2 and one just upstream of ARM3, though their relative function cannot be inferred from structural data alone.

Although IP_3R1 and IP_3R3 are structurally quite similar, they are differentially activated and regulated (see 1.). Additional work, including performing a high-resolution cryo-EM analysis of IP_3-bound IP_3R1 and the further investigation of the effect of Ca^{2+} and other IP_3R modulators, including associated proteins, on IP_3R structure will therefore be needed to fully unravel the underlying mechanism of activation and to understand the functional differences between the various IP_3R isoforms.

3 Complexity of IP_3R activation and regulation

Concerning the mechanisms of activation and regulation of the IP_3R, progress has been made on several points recently.

3.1 IP_3 binding stoichiometry

First, a long-standing question in the field concerned the number of IP_3 molecules needed to evoke the opening of the IP_3R/Ca^{2+}-release channel. Some studies demonstrated a high cooperativity of IP_3 binding to its receptor, and suggested that minimally 3 IP_3 molecules should be bound to the IP_3R to evoke Ca^{2+} release (50, 51). In contrast herewith, co-expression of an IP_3R apparently defective in IP_3 binding ($R^{265}Q$) and of a channel-dead IP_3R mutant ($D^{2550}A$) resulted in a partial IP_3-induced Ca^{2+} release, suggesting that one IP_3R subunit can gate another and that therefore not all subunits need to bind IP_3 to form an active

channel (52). Moreover, these results fit with the most recent cryo-EM data discussed above (see 2.; (41)).

Recently, a comprehensive study by Yule and co-workers demonstrated in triple-knockout (TKO) cells, devoid of endogenous IP_3R expression (DT-40 TKO and HEK TKO), that the activity of recomplemented IP_3Rs depends on the occupation of the 4 IP_3-binding sites by their ligand (53). The strongest evidence for this was obtained by the expression of a concatenated IP_3R1 containing 3 wild-type subunits and 1 mutant subunit. The mutant subunit contained a triple mutation ($R^{265}Q/K^{508}Q/R^{511}Q$) in the ligand-binding domain precluding any IP_3 binding, as previously demonstrated (54), while the $R^{265}Q$ single mutant still retained ~10% binding activity. Interestingly, the tetrameric IP_3R containing only 1 defective IP_3-binding site and expressed in cells fully devoid of endogenous IP_3Rs was completely inactive in Ca^{2+} imaging experiments, unidirectional Ca^{2+} flux experiments and in patch-clamp electrophysiological experiments (53). Similar experiments were performed for IP_3R2, making use of its existing short splice isoform that lacks 33 a.a. in the suppressor domain rendering it non-functional (55). These data strongly suggest that no opening of the IP_3R can occur, unless each subunit has bound IP_3. This characteristic would strongly limit the number of active IP_3Rs and protect the cell against unwanted Ca^{2+} release in conditions in which the IP_3 concentration is only slightly increased (53, 56). However, in the case of IP_3R mutations affecting IP_3 binding / IP_3R activity it may explain why they are detrimental, even in heterozygous conditions (10).

3.2 Physiological relevance of IP_3R heterotetramer formation

As already indicated above (see 1.), the high level of homology between the various IP_3R isoforms allows not only for the formation of homotetramers but also for that of heterotetramers (57-59). The frequency of heterotetramer occurrence is however not completely clear. A study in COS-7 cells indicated that kinetic constrains affect the formation of heterotetramers and that therefore the level of heterotetramers composed of overexpressed IP_3R1 and of either endogenously expressed or overexpressed IP_3R3 was

lower than what could be expected from a purely binomial distribution (60). In contrast herewith, by using isoform-specific IP_3R antibodies for sequential depletion of the IP_3Rs, it was shown that in pancreas, over 90% of IP_3R3 is present in heterotetrameric complexes, generally with IP_3R2 (61). This is significant as pancreas is a tissue in which IP_3R2 and IP_3R3 together constitute over 80% of the total amount of IP_3R (62, 63). It is therefore meaningful to investigate whether the presence of IP_3R heterotetramers will contribute in increasing the diversity of the IP_3R Ca^{2+}-release channels, as is generally assumed. However, due to the fact that most cells express or can express various types of homo- and heterotetrameric IP_3Rs in unknown proportions, addressing this question is in most cell types not straightforward.

Overexpressing mutated IP_3R1 and IP_3R3 in COS-7 cells at least indicated that heterotetramers are functional (52). The expression of concatenated dimeric IP_3R1-IP_3R2 (and IP_3R2-IP_3R1) in DT-40 TKO cells led to the formation of IP_3R heterotetramers with a defined composition (2:2) that could be compared with homotetrameric IP_3R1 or homotetrameric IP_3R2 that were similarly expressed (61). Investigation of their electrophysiological properties via nuclear patch-clamp recordings indicated that in the IP_3R1-IP_3R2 2:2 heterotetramers the properties of the IP_3R2 dominated with respect to the induction of Ca^{2+} oscillations and their regulation by ATP (61). A more recent study based on the same approach but now including combinations of all three IP_3R isoforms, demonstrated that 2:2 heterotetrameric IP_3Rs display an IP_3 sensitivity that is intermediate to that of their respective homotetramers (64) indicating that heterotetramerization successfully increases IP_3R diversity. In addition, the obtained results also demonstrate that IP_3R2 properties with respect to both the induction of Ca^{2+} oscillations and the regulation by ATP also dominated in IP_3R2-IP_3R3 2:2 heterotetramers. In contrast, when a tetrameric IP_3R containing 3 IP_3R1 and 1 IP_3R2 subunit was expressed, its properties were similar to that of a homotetrameric IP_3R1 (64). Taken together, these experiments indicate that IP_3R heterotetramers increase the diversity of the IP_3Rs with respect to Ca^{2+} release and that further studies are needed to fully

understand how IP_3R heterotetramers are regulated by other factors, including associated proteins.

3.3 Novel crosstalk mechanism between cAMP and IICR

cAMP and Ca^{2+}, the two most important intracellular messengers, have numerous crosstalks between them (65). At the level of the IP_3R, the most evident crosstalk is the sensitization of IP_3R1 by cAMP-dependent protein kinase (PKA) (66), while a similar regulatory role is highly probable for IP_3R2 but less likely for IP_3R3 (15, 65).

A novel line of regulation was discovered some time ago when it was shown that cAMP can, independently from PKA or cAMP-activated exchange proteins, potentiate the IP_3R (67-69). In particular, it was shown in HEK cells that adenylate cyclase 6, which in those cells accounts for only a minor portion of the adenylate cyclase isoforms, is responsible for providing cAMP to a microdomain surrounding IP_3R2, increasing its activity (69). Such mechanism would form a specific signaling complex in which locally a very high concentration of cAMP could be reached, without affecting its global concentration (65).

Recent work provided further evidence concerning the importance of cAMP for IP_3R functioning, showing that the presence of cAMP can uncover IP_3Rs that were insensitive to IP_3 alone (56). Indeed, in HEK cells heterologously expressing the parathyroid hormone (PTH) receptor, it appears that PTH, via production of cAMP, can evoke Ca^{2+} release after full depletion of the carbachol-sensitive Ca^{2+} stores. Although the identity of the Ca^{2+} stores could not yet be established, the obtained results are indicative that cAMP unmasks IP_3Rs with a high affinity for IP_3. This fits with the previous observation that IP_3R2, the IP_3R with the highest affinity for IP_3 (reviewed in (19)), is regulated by cAMP (69). The molecular mechanism on how cAMP interacts with the IP_3R remains to be determined. At this moment no discrimination can be made between a low-affinity cAMP-binding site on the IP_3R itself or a similar binding site on an associated protein (65). The possibility that the IP_3R-binding protein released by IP_3 (IRBIT)-related protein S-adenosylhomocysteine-hydrolase, known to

298 bind cAMP, is involved was however already excluded by knockdown and overexpression
299 experiments (56).
300
301 **4 Complexity of protein-protein interactions affecting the IP_3R**
302 In a comprehensive review published a few years ago, over 100 proteins that interact with
303 the IP_3R have been listed (14). For that reason, we will limit ourselves in the present review
304 to either newly discovered interacting proteins or proteins for which new information about
305 their interaction recently became available.
306
307 **4.1 Calmodulin (CaM) and related Ca^{2+}-binding proteins**
308 CaM is the most ubiquitously expressed intracellular Ca^{2+} sensor. It is a relatively small
309 protein (148 a.a.) with a typical dumbbell structure. A central, flexible linker region connects
310 the globular N-terminal and C-terminal domains, each containing two Ca^{2+}-binding EF-hand
311 motifs with a classical helix-loop-helix structure. The K_d of CaM for Ca^{2+} ranges between
312 $5x10^{-7}$ and $5x10^{-6}$ M, with the C-terminal Ca^{2+}-binding sites having a 3- to 5-fold higher affinity
313 than the N-terminal ones (70). CaM therefore displays the correct Ca^{2+} affinity to sense
314 changes in intracellular Ca^{2+} concentrations and serve as Ca^{2+} sensor. While apo-CaM has a
315 rather compact structure, Ca^{2+}-CaM exposes in each domain a hydrophobic groove with
316 acidic residues at its extremities that will allow interaction with their target (71). A plethora of
317 target proteins that are modulated by CaM exists, including various Ca^{2+}-transporting
318 proteins (72). These various proteins contain CaM-binding sites that can be categorized into
319 various types of motifs (73).
320 Although the interaction of CaM with the IP_3R was already observed soon after the
321 identification of the IP_3R as IP_3-sensitive Ca^{2+}-release channel (74) its exact mechanism of
322 action is still not completely elucidated. Moreover, there are a number of interesting features
323 related to the binding of CaM to the IP_3R: (i) the existence of multiple binding sites, (ii) the
324 possibility for both Ca^{2+}-CaM and apo-CaM to affect IP_3R function and (iii) the use of some of
325 the CaM-binding sites by other Ca^{2+}-binding proteins. The aim of this paragraph therefore is

326 to present a comprehensive view on the relation between CaM (and some related Ca^{2+}-
327 binding proteins) and the IP_3R.
328 On IP_3R1, three CaM-binding sites were described (**Figure 1**). A high-affinity CaM-binding
329 site (a.a. 1564-1585; **Figure 2 A-B**, indicated by the yellow arrows) was described in the
330 central coupling domain (75), while a low-affinity one was found in the suppressor domain
331 (76). The latter site is discontinuous (a.a. 49-81 and a.a. 106–128; **Figure 2,** indicated in
332 yellow) and can bind both apo-CaM and Ca^{2+}-CaM (77). Finally, a third site was described on
333 the S2(-) IP_3R1 splice isoform in which a.a. 1693-1732 are removed (78, 79). CaM binding to
334 this newly formed site is inhibited by PKA-mediated phosphorylation, probably on Ser1589
335 (79).
336 CaM interaction with the two other IP_3R isoforms was studied in less detail, but an IP_3R2
337 construct overlapping with the CaM-binding site in the central coupling domain interacted
338 with CaM, supporting the conservation of this site (75). However, no direct interaction
339 between CaM and IP_3R3 could be measured (75, 80) though CaM can bind to IP_3R1-IP_3R3
340 heterotetramers (79).
341 Functional effects on the IP_3R have been described for both apo-CaM and Ca^{2+}-CaM. In fact,
342 apo-CaM is equally potent in inhibiting IP_3 binding to full-length IP_3R1 as Ca^{2+}-CaM (81). In
343 agreement with the absence of CaM binding to IP_3R3, full-length IP_3R3 remained insensitive
344 to regulation by CaM (80). In contrast, a Ca^{2+}-independent inhibition of IP_3 binding was
345 observed for the isolated ligand-binding domain of IP_3R1 (82) as well as for that of IP_3R2 and
346 IP_3R3 (83).
347 Concerning IP_3-induced Ca^{2+} release, the situation is somewhat more complex. Ca^{2+} release
348 by IP_3R1 is inhibited by CaM in a Ca^{2+}-dependent way (84, 85) while similar results were
349 subsequently found for IP_3R2 and IP_3R3 (76, 86). However, linking these functional effects
350 molecularly to a CaM-binding site appeared more difficult, not only because of the apparent
351 absence of a Ca^{2+}-dependent CaM-binding site on IP_3R3 but also because the mutation
352 $W^{1577}A$ that abolishes CaM binding to IP_3R1 (75), does not abolish the CaM-mediated
353 inhibition of IICR (87).

354 Furthermore, other results suggested that the relation between CaM and the IP_3R was more
355 complex than originally thought. A detailed analysis of the CaM-binding site located in the
356 central coupling domain of IP_3R1 provided evidence that it consisted of a high-affinity Ca^{2+}-
357 CaM and a lower affinity apo-CaM site (88). Moreover, in the same study it was
358 demonstrated that a CaM mutant deficient in Ca^{2+} binding (CaM1234) could inhibit IICR in a
359 Ca^{2+}-dependent way with the same potency as CaM. In a separate study, it was
360 demonstrated that a myosin light chain kinase (MLCK)-derived peptide, which binds to CaM
361 with high affinity, fully inhibited the IP_3R (89). This inhibition could be reversed by the addition
362 of CaM but not of CaM1234 and the results were interpreted as evidence that endogenously
363 bound CaM is needed for IP_3R activity. A follow-up study by another group (90) however
364 proposed that the MLCK peptide is not removing endogenous CaM but is interacting with an
365 endogenous CaM-like domain on IP_3R, thereby disrupting its interaction with a so-called 1-8-
366 14 CaM-binding motif (a.a. 51-66) essential for IP_3R activity (91).
367 Meanwhile, the interaction of apo-CaM with the suppressor domain was studied via NMR
368 analysis (92). This study brought forward two main pieces of evidence. First, it was shown
369 that the binding of apo-CaM to the suppressor domain induced an important, general
370 conformational change to the latter. These changes further increased in the presence of
371 Ca^{2+}. Secondly, analysis of the conformational change of CaM indicated that apo-CaM
372 already binds with its C-lobe to the IP_3R1 suppressor domain, and that only after addition of
373 Ca^{2+} also the N-lobe interacts with the suppressor domain. These results can therefore
374 explain the importance of the CaM-binding sites in the suppressor domain in spite of their
375 difficult accessibility ((92); **Figure 2**).
376 Finally, some Ca^{2+}-binding proteins related to CaM (e.g. neuronal Ca^{2+}-binding protein
377 (CaBP) 1, calmyrin, also known as CIB1, and neuronal Ca^{2+} sensor-1 (NCS-1)) also regulate
378 the IP_3R. Similarly to CaM, these proteins contain 4 EF-hand motifs but in contrast with CaM,
379 not all of them bind Ca^{2+}. In CaBP1 and NCS-1 only 3 EF hands are functional (EF1, EF3,
380 EF4 and EF2, EF3, EF4 resp.) and in calmyrin only 2 (EF3 and EF4). Moreover, some of the
381 EF hands bind Mg^{2+} rather than Ca^{2+}. Furthermore, those proteins are myristoylated.

382 Although early results suggested that CaBP1 and calmyrin could, in the absence of IP_3,
383 activate the IP_3R under some circumstances (93, 94), there is presently a large consensus
384 that they, similarly to CaM, generally inhibit the IP_3R (93, 95, 96).
385 CaBP1 was proposed to interact with the IP_3R1 with a higher affinity than CaM itself (94, 96),
386 while in contrast to CaM it does not affect the ryanodine receptor (RyR), another family of
387 intracellular Ca^{2+}-release channels. Additionally, the interaction with the IP_3R would be
388 subject to regulation by caseine kinase 2, an enzyme that can phosphorylate CaBP1 on S^{120}
389 (96). Similarly to CaM, CaBP1 binds in a Ca^{2+}-independent way to the IP_3R1 suppressor
390 domain, but in contrast to CaM, only to the first of the two non-contiguous binding sites
391 described for CaM (**Figure 1**). However, CaM and CaBP1 similarly antagonized the
392 thimerosal-stimulated interaction between the suppressor domain and the IBC of IP_3R1,
393 suggesting a common mechanism of action whereby they disrupt intramolecular interactions
394 needed for channel activation (97). More recent work confirmed the inhibitory effect of
395 CaBP1 on IP_3R1, while expanding the knowledge concerning the CaBP1 binding site. In
396 particular, NMR analysis indicated that CaBP1 interacts with its C lobe with the suppressor
397 domain of the IP_3R and that even at saturating Ca^{2+} concentrations EF1 is bound to Mg^{2+},
398 precluding a conformational change of the N lobe (98). The same study demonstrated that
399 Ca^{2+}-bound CaBP1 bound with an ~10-fold higher affinity than Mg^{2+}-bound CaBP1 and an at
400 least 100-fold higher affinity than CaM itself. Functional analysis performed in DT-40 cells
401 solely expressing IP_3R1 demonstrated that CaBP1 stabilized the closed conformation of the
402 channel, probably by clamping inter-subunit interactions (99). The interaction of specific
403 hydrophobic a.a. in the C lobe of CaBP1 (V^{101}, L^{104}, V^{162}) that become more exposed in the
404 presence of Ca^{2+} with hydrophobic a.a. in the IBC (L^{302}, I^{364}, L^{393}) appeared hereby essential.
405 The action of NCS-1 on the IP_3R forms a slightly different story. It co-immunoprecipitates with
406 IP_3R1 and IP_3R2 in neuronal cells and in heart thereby stimulating IICR in a Ca^{2+}-dependent
407 way (100, 101). Interestingly, paclitaxel by binding to NCS-1 increases its interaction with
408 IP_3R1 and so induces Ca^{2+} oscillations in various cell types (102, 103). This Ca^{2+} signaling
409 pathway was proposed to lead to calpain activation and to underlie the origin of paclitaxel-

410 induced peripheral neuropathy (104). However, the interaction site of NCS-1 on the IP_3R,
411 either direct or indirect, has not yet been identified.
412 Taken together these results confirm that Ca^{2+}-binding proteins interact in a complex way
413 with the IP_3R and that the various Ca^{2+}-binding proteins have distinct, though sometimes
414 overlapping, roles. The functional effect or CaM has been studied in detail and it appeared to
415 inhibit the IP_3R. The results described above support a view that the main action of CaM on
416 the IP_3R is at the level of the suppressor domain. Indeed, apo-CaM can via its C lobe bind to
417 the suppressor domain of all three IP_3R isoforms while a subsequent binding of the N lobe
418 will depend on the Ca^{2+} concentration. The binding of CaM in that domain can disturb an
419 intra-IP_3R interaction needed for IP_3R function and therefore inhibits IICR. This behavior can
420 be particularly important in cells having high CaM expression levels, as for example Purkinje
421 neurons that also demonstrate high levels of IP_3R1. In that case, CaM was proposed to be
422 responsible for suppressing basal IP_3R activity (81). Moreover, as the intracellular distribution
423 of CaM can depend on intracellular Ca^{2+} dynamics, it was also hypothesized that it allows
424 IP_3R regulation is a non-uniform way (84). Additionally, it should be emphasized that CaM
425 can act on other Ca^{2+}-transporting proteins in the cell, like the RyR (105), the plasma
426 membrane Ca^{2+} ATPase (106) and various plasma membrane Ca^{2+} channels including
427 voltage-operated Ca^{2+} channels and transient receptor potential channels (107, 108). In all
428 these cases CaM tends to inhibit Ca^{2+} influx into the cytosol (inhibition of IP_3Rs, RyRs and
429 plasma membrane Ca^{2+} channels) while promoting Ca^{2+} efflux out of the cell (stimulation of
430 plasma membrane Ca^{2+} ATPase).
431 An IP_3R-inhibiting behavior can similarly be expected for CaM-related Ca^{2+}-binding proteins,
432 though their interaction sites are not strictly identical to that of CaM. The binding site for
433 NCS-1, which rather stimulates the IP_3R, is even still unknown. In comparison to CaM,
434 CaBP1 demonstrates a much higher affinity for the IP_3R (99) and a higher specificity, as it
435 does not affect the RyR (96). In cells expressing CaBP1, the major control of IICR will
436 therefore depend on the interaction of the IP_3R with CaBP1, while RyR activity will depend on
437 the presence and activation of CaM. Further work will be needed to completely unravel the

exact role of these various proteins in the control of intracellular Ca^{2+} signaling. From the present results, it can already be expected that the relative role of the various Ca^{2+}-binding proteins in the control of IICR will strongly depend on the exact cell type in consideration.

4.2 The Bcl-2-protein family

The B-cell lymphoma (Bcl)-2 protein family has been extensively studied as critical regulator of apoptosis (109). This family consists of both anti- and pro-apoptotic members. The anti-apoptotic family members inhibit apoptosis in at least two different manners. First, at the mitochondria anti-apoptotic Bcl-2 proteins such as Bcl-2, Bcl-XL and Mcl-1, bind to the pro-apoptotic Bcl-2-family members thereby inhibiting the permeabilization of the outer mitochondrial membrane by Bax and Bak and subsequent release of cytochrome C (110, 111). Second, the anti-apoptotic Bcl-2-family members also affect intracellular Ca^{2+} signaling. On the one hand they promote pro-survival Ca^{2+} oscillations while on the other hand they inhibit pro-apoptotic Ca^{2+} release from the ER that otherwise could lead to mitochondrial Ca^{2+} overload (112). These combined actions mean that anti apoptotic Bcl-2 proteins can, by modulating several protein families involved in intracellular Ca^{2+} signaling, both fine tune mitochondrial bio-energetics and inhibit Ca^{2+}-mediated mitochondrial outer membrane permeabilization (113-116). Both the interaction between Bcl-2-family members and their ability to regulate intracellular Ca^{2+} signaling is critically dependent on the presence of so-called Bcl-2 homology (BH) domains. Anti-apoptotic Bcl-2 proteins contain four of these domains (BH1, 2, 3 and 4) (111). The BH1-3 domains together form a hydrophobic cleft that inactivates the pro-apoptotic Bcl-2-family members via interaction with their BH3 domain. For regulating intracellular Ca^{2+} signaling events, anti-apoptotic Bcl-2 proteins rely to a great extent, however not exclusively, on their BH4 domain. In this review we will focus on how IP_3Rs are regulated by Bcl-2 proteins. For a more extensive revision of how Bcl-2-family members regulate the various members of the intracellular Ca^{2+} signaling machinery we would like to refer to our recent review on the subject (112).

The various IP_3R isoforms are important targets for several anti-apoptotic Bcl-2-family members (112). To complicate matters, multiple binding sites on the IP_3R have been described for anti-apoptotic Bcl-2 proteins (117). First, Bcl-2, Bcl-XL and Mcl-1 were shown to target the C-terminal part (a.a. 2512-2749) of IP_3R1 (**Figure 2**, indicated in green) thereby stimulating pro-survival Ca^{2+} oscillations (114, 115, 118). Additionally, Bcl-2, and with lesser affinity also Bcl-XL, also target the central coupling domain (a.a. 1389-1408 of IP_3R1; **Figures 1 and 2**, indicated in blue) of the IP_3R where binding of these proteins inhibits pro-apoptotic Ca^{2+}-release events (116, 118-120). Finally, the zebrafish protein Nrz (121) and its mammalian homolog Bcl-2-like 10 (122) were shown to interact with the IBC and to inhibit IICR.

The group of Kevin Foskett performed a more in-depth study into how the IP_3R is regulated by Bcl-XL and proposed a mechanism unifying the regulation at the C-terminal and at the central coupling domain of the IP_3R (123). Two domains containing BH3-like structures (a.a. 2571-2606 and a.a. 2690-2732; **Figures 1 and 2,** indicated in dark green) were identified in the C-terminal part of the IP_3R. When Bcl-XL is, via its hydrophobic cleft, bound to both BH3-like domains it sensitizes the IP_3R to low concentrations of IP_3, thereby stimulating Ca^{2+} oscillations. If Bcl-XL binds to only one of these BH3 like domains while also binding to the central coupling domain, it will inhibit IICR. Whether Bcl-XL occupies one or the two BH3-like domains at the C-terminus of the IP_3R was proposed to be dependent on Bcl-XL levels and on the intensity of IP_3R stimulation. Whether Bcl-2 operates in a similar manner is still unclear. As there is evidence that Bcl-2 shows a greater affinity than Bcl-XL for the inhibitory binding site in the central coupling domain it is likely that this site is the preferential target for Bcl-2 (118). In addition, for Bcl-2 not its hydrophobic cleft but rather its transmembrane domain seems to play an important role for targeting and regulating the IP_3R via both its C-terminus and the site located in the central coupling domain (124). Based on the recent cryo-EM structure of IP_3R1 (29, 41), this central site in the coupling domain resides in a relatively easily accessible area of IP_3R1 (**Figure 2**, indicated in blue). The C-terminal transmembrane domain of Bcl-2 may thus serve to concentrate the protein at the ER near the IP_3R from where its N-terminal BH4

492 domain can more easily bind to the central coupling domain. In addition, sequestering Bcl-2
493 proteins at the ER membrane via their transmembrane domain may increase their ability to
494 interact with the C-terminus of the IP_3R (**Figure 2**, indicated in green). As this C-terminal
495 binding site seems to be located more at the inside of the IP_3R1 tetramer one can expect a
496 local high concentration of Bcl-2 proteins to be necessary for this interaction. Besides directly
497 modulating IICR, Bcl-2 can serve as an anchor for targeting additional regulatory proteins to
498 the IP_3R. It was shown that Bcl-2 attracts dopamine- and cAMP-regulated phosphoprotein of
499 32 kDa (DARPP-32) and calcineurin to the IP_3R thereby regulating the phosphorylation state
500 of the latter and consequently its Ca^{2+}-release properties (125). Finally, recent data indicate
501 also for Bcl-2 an additional interaction site in the ligand-binding domain (126) highlighting the
502 complexity of the interaction of the anti-apoptotic Bcl-2 family members with the IP_3R. Further
503 research will be needed to unravel the precise function of each of these sites.

504 Another Bcl-2-family member that regulates the IP_3R is the zebrafish protein Nrz. The latter
505 was shown to bind via its BH4 domain to the IBC of zebrafish IP_3R1, whereby E^{255} appeared
506 essential for interaction (**Figure 1**). Nrz prevents IP_3 binding to the IP_3R thereby inhibiting IICR
507 (121). Interestingly, although the Nrz BH4 domain is sufficient for interaction with the IP_3R,
508 inhibition of IICR required the BH4-BH3-BH1 domains. Furthermore, phosphorylation of Nrz
509 abolished its interaction with the IP_3R. Recently, Bcl-2-like 10, the human orthologue of Nrz,
510 was shown that just like Nrz in zebrafish, it interacts with the IBC, indicating a conserved
511 function for this protein (122).

512 Besides anti-apoptotic Bcl-2-family members, also pro-apoptotic Bcl-2 proteins and other BH3
513 domain-containing proteins are known to target and regulate IP_3Rs. For instance, Bok, a pro-
514 apoptotic Bcl-2-family member, interacts with the IP_3R (a.a. 1895–1903 of IP_3R1; **Figures 1**
515 **and 2**) (127). This interaction protects IP_3R1 and IP_3R2 from proteolytic cleavage by caspase
516 3 that results in a Ca^{2+} leak that may contribute to mitochondrial Ca^{2+} overload and thus
517 apoptosis (128, 129). Subsequent work demonstrated that the majority of all cellular Bok is
518 bound to the IP_3R thereby stabilizing the Bok protein (130). Unbound, newly synthesized Bok

519 is rapidly turned over by the proteasome pathway. Both the association of mature Bok with the
520 IP_3R and the rapid degradation of newly synthesized Bok by the proteasome restrict the pro-
521 apoptotic functions of Bok thus preventing cell death induction.

522 From the above it is clear that the IP_3R is heavily regulated by both pro- and anti-apoptotic Bcl-
523 2-family members. The occurrence of multiple binding sites for the same Bcl-2-family member
524 further increases the complexity (112). Furthermore, it should be stressed that the regulation
525 of the IP_3R by Bcl-2 proteins is conserved during evolution. This is illustrated by the ability of
526 the zebrafish Nrz protein to regulate IICR via its BH4 domain (121) and is further validated by
527 the observation that the BH4 domains of Bcl-2 derived from different vertebrates are able to
528 inhibit IICR with similar efficiency (131). The large number of both pro-and anti-apoptotic Bcl-
529 2 proteins that regulate the IP_3R, targeting it at multiple sites, suggests that throughout
530 evolution regulating IICR became an important functional aspect of the Bcl-2 protein family.

531 Mcl-1, Bcl-2 and Bcl-XL all target the C-terminal region of the IP_3R stimulating the occurrence
532 of pro-survival Ca^{2+} oscillations and thus Ca^{2+} transfer to the mitochondria (114, 115, 118).
533 These Ca^{2+} transfers into the mitochondria are important for normal cell functioning (113) but
534 are also involved in cancer development and could potentially form a novel therapeutic target
535 (132). Mitochondrial Ca^{2+} contributes to maintaining proper ATP production. When Ca^{2+}
536 transfer into the mitochondria is inhibited, ATP levels decrease, activating autophagy. At the
537 same time the cell cycle progression is halted (113, 133). In cancer cells, decreased Ca^{2+}
538 transfer into the mitochondria, consecutive loss of ATP and the start of autophagy is not
539 accompanied by a stop in the cell cycle. Continuing the cell cycle without sufficient building
540 blocks and ATP results in necrotic cell death (132). Cancer cells are therefore reliant on proper
541 Ca^{2+} transfer to the mitochondria to maintain mitochondrial function, including the production
542 of ATP and metabolites necessary for completing the cell cycle. It is therefore common for
543 cancer cells to upregulate one or several anti-apoptotic Bcl-2 proteins. By interacting with the
544 C-terminus of the IP_3R the Bcl-2 proteins may stimulate Ca^{2+} oscillations assuring proper
545 mitochondrial Ca^{2+} uptake and an adequate mitochondrial metabolism. On the other hand,

upregulation of Bcl-2 and/or Bcl-XL also protects the cells from excessive IP_3R-mediated Ca^{2+} release by binding to the central regulatory site (116, 118-120) and prevents apoptosis, even in the presence of cell death inducers (109, 134). In healthy cells a similar regulation of IICR by Bcl-2 proteins occurs. However, when cell death is induced in the latter, the amount of anti-apoptotic Bcl-2 proteins declines (134) potentially decreasing the level of their association with the IP_3R. This alleviates the inhibitory actions on IICR allowing pro-death Ca^{2+} signals while also reducing the opportunities for the occurrence of pro-survival Ca^{2+} oscillations.

4.3 Beclin 1

Beclin 1 is a pro-autophagic BH3 domain-containing protein (135). It interacts with various proteins involved in the regulation of autophagy, including Bcl-2 (136, 137). The latter protein, by sequestering Beclin 1, prevents its pro-autophagic action. A first study presenting evidence that Beclin 1 also interacted with the IP_3R showed an interaction between Beclin 1 and the IP_3R that depended on Bcl-2 and which was disrupted by the IP_3R inhibitor xestospongin B (138). The release of Beclin 1 from the Bcl-2/IP_3R complex resulted in the stimulation of autophagy which could be counteracted by overexpressing the IBC. This suggested that the IBC was able to sequester the xestospongin B-released Beclin 1 thus halting its pro-autophagic function. From subsequent work, it appeared that the role of Beclin 1 with respect to the IP_3R was more complex (139). Indeed, the binding of Beclin 1 to the ligand-binding domain was confirmed, though it appeared that in IP_3R1 and to a lesser degree in IP_3R3 the suppressor domain (a.a. 1-225) played a more prominent role in the interaction than the IBC. Interestingly, during starvation-induced autophagy Beclin 1 binding to the IP_3R sensitized IICR that was shown to be essential for the autophagy process (139). Using the $F^{123}A$ Beclin 1 mutant that does not interact with Bcl-2, it was shown that the sensitization of the IP_3R by Beclin 1 was not due to counteracting the inhibitory effect of Bcl-2, although, in agreement with the previous study (138) it appeared that Beclin 1 binding to Bcl-2 may be needed to target the protein in proximity of the IP_3R.

4.4 IRBIT

IRBIT regulates IICR by targeting the IP_3R ligand-binding domain thereby competing with IP_3. Moreover, this interaction is promoted by IRBIT phosphorylation (140). Besides the IP_3R, IRBIT binds to several other targets regulating a wide range of cellular processes (141). How IRBIT determines which target to interact with and modulate was recently described (142). First, various forms of IRBIT exist, IRBIT, the long-IRBIT homologue and its splice variants, which were shown to have distinct expression patterns. Besides this, the N-terminal region of the various members of the IRBIT-protein family showed distinct differences. These differences, obtained by N-terminal splicing, are important in maintaining protein stability and in determining which target to interact with.

Recently, it was shown that Bcl-2-like 10, which binds to a distinct site in the ligand-binding domain (see 4.2), functionally and structurally interacts with the action of IRBIT on the IP_3R (122). When both proteins are present, Bcl-2-like 10, via its BH4 domain, interacts with IRBIT, thereby mutually strengthening their interaction with the IP_3R and decreasing IICR in an additive way. Upon dephosphorylation of IRBIT, both IRBIT and Bcl-2-like 10 are released from the IP_3R, increasing pro-apoptotic Ca^{2+} transfer from the ER to the mitochondria. Interestingly, this study also showed that IRBIT is involved in regulating ER-mitochondrial contact sites as IRBIT knockout reduced the number of these contact sites (122).

4.5 Thymocyte-expressed, positive selection-associated 1 (TESPA1)

T-cell receptor (TCR) stimulation triggers a signaling cascade ultimately leading to the activation of PLC, production of IP_3 and IICR important for T-cell maturation (143). TESPA1, a protein involved in the development/selection of T cells (144), has been shown to regulate these Ca^{2+} signals. TESPA1 has a significant homology with KRAS-induced actin-interacting protein (147), a protein that was already shown to interact and control the IP_3R (145, 146). TESPA1 similarly interacts with the various IP_3R isoforms and it appeared that the full ligand-binding domain was needed for this interaction. However, at first no functional effect was described for this interaction (147). Recently this topic was revisited and it was shown that

TESPA1 recruits IP_3R1 to the TCR where PLC signaling is initiated and IP_3 produced (143). In this way, TESPA1 promotes IP_3R1 phosphorylation on Y^{353} by the tyrosine kinase Fyn, increasing the affinity of the IP_3R for IP_3. The combination of both these effects increases the efficiency by which Ca^{2+} signaling occurs after TCR stimulation, which is beneficial for T-cell selection and maturation (148). Furthermore, in Jurkat cells TESPA1 interacts at the ER-mitochondria contact sites with GRP75 (149), a linker protein coupling IP_3R with the mitochondrial VDAC1 channel favoring Ca^{2+} transfer from ER to mitochondria (150). Consequently, TESPA1 knockout diminished the TCR-evoked Ca^{2+} transfers to both mitochondria and cytosol and confirm the important role for TESPA1 in these processes.

4.6 Pyruvate kinase (PK) M2

PKs catalyze the last step of glycolysis and convert phosphoenolpyruvate to pyruvate resulting in the production of ATP. Many cancer cells preferentially upregulate glycolysis over oxidative phosphorylation suggesting a potential role for the PK family in cancer development. Four distinct PK isoforms exists, having each a distinct tissue expression pattern but PKM2 has the peculiarity to be expressed at an elevated level in most tumoral cells where it has a growth-promoting function. Moreover, although PKM1 and PKM2 are nearly identical, differing in only 22 a.a., they are regulated differently and have non-redundant functions (151). Besides its metabolic functions, PKM2 is also involved in several non-metabolic functions. The latter encompass a nuclear role in transcriptional regulation, protein kinase activity towards various proteins in different cellular organelles, and even an extracellular function as PKM2 is also present in exosomes (152, 153). It is therefore interesting that also a role for PKM2 at the ER was described since a direct interaction was found between PKM2 and the central coupling domain of the IP_3R, inhibiting IICR in various cell types (154, 155). Moreover, a recent study links the switch from oxidative phosphorylation to glycolysis in breast cancer cells with PKM2 methylation (156). Methylated PKM2 promoted proliferation, migration and growth of various breast cancer cell lines. Strikingly, PKM2 methylation did not seem to alter its enzymatic activity but did however alter

mitochondrial Ca^{2+} homeostasis by decreasing IP_3R levels. Finally, co-immunoprecipitation experiments showed an interaction between methylated PKM2 and IP_3R1 and IP_3R3, though in this study it was not investigated whether the interaction was direct or indirect (156). As PKM2 is in a variety of cancers considered as a good prognostic marker with a strong potential as therapeutic target (152) these new data, linking directly a metabolic enzyme with an intracellular Ca^{2+}-release channel and ER mitochondria Ca^{2+} transfer, provide new possibilities for therapeutic intervention.

4.7 BRCA-associated protein 1 (BAP1) and the F-box protein FBXL2

Prolonged stimulation of the IP_3Rs leads to a downregulation of the IP_3R levels (157-159). This downregulation is mainly due to IP_3R ubiquitination followed by their degradation via the proteasomal pathway (31, 160). Ubiquitination is therefore an important IP_3R modification that may severely impact IICR signaling to for instance the mitochondria, thereby greatly affecting cell death and cell survival decisions. Recently a number of proto-oncogenes and tumor suppressors have been identified that critically control IP_3R3 ubiquitination.

BAP1 is a tumor suppressor with deubiquitinase activity that is known to have important roles in regulating gene expression, DNA stability, replication, and repair and in maintaining chromosome stability (161-164). Besides this, BAP1 was also shown to influence cellular metabolism, suggesting potential roles for BAP1 outside the nucleus (165, 166).

Heterozygous loss of BAP1 results in decreased mitochondrial respiration while increasing glycolysis (167, 168). These cells produced a distinct metabolite signature, indicative for the occurrence of the Warburg effect that is supporting cells towards malignant transformation.

Heterozygous loss of BAP1 leads to a decreased ER-mitochondria Ca^{2+} transfer and altered mitochondrial metabolism (167). BAP1 regulates this Ca^{2+} transfer by interacting with the N-terminal part (a.a. 1-800) of IP_3R3, a region which contains the complete ligand-binding domain and a small part of the central coupling domain. The deubiquitinase activity of BAP1 prevents degradation of IP_3R3 by the proteasome. Loss of BAP1 consequently results in excessive reduction of IP_3R3 levels thereby lowering mitochondrial Ca^{2+} uptake. This not only

reduces the cell its responsiveness to Ca^{2+}-induced cell death but also promotes glycolysis over oxidative phosphorylation, both important aspects of malignant cell transformation. The nuclear function of BAP1 with respect to maintaining DNA integrity (161-164) together with its extra-nuclear role in regulating cell metabolism and sensitivity to Ca^{2+}-induced cell death (165-168) suggests that this protein may be an excellent target for cancer drug development.

F-box protein FBXL2 that forms a subunit of a ubiquitin ligase complex has the opposite effect of BAP1 on IP_3R3. FBXL2 interacts with a.a. 545-566 of IP_3R3, promoting its ubiquitination and its subsequent degradation. Reduced IP_3R3 leads to a decreased transfer of Ca^{2+} to the mitochondria and a reduced sensitivity towards apoptosis, thus promoting tumor growth (169). The phosphatase and tensin homolog (PTEN) tumor suppressor could inhibit this pro-tumorigenic effect of FBXL2. PTEN not only promotes apoptosis by inhibiting protein kinase B/Akt (PKB) (170-172) thereby counteracting PKB-mediated IP_3R3 phosphorylation (173, 174) but also by directly binding to IP_3R3 (169). Binding of PTEN to IP_3R3 displaces FBXL2 from its binding site, reducing IP_3R3 ubiquitination, stabilizing IP_3R3 levels, and thus increasing pro-apoptotic Ca^{2+} signaling to the mitochondria (169). In accordance with the fact that the FBXL2-binding site is only partially conserved in IP_3R1 and IP_3R2, the stability of the two latter isoforms appeared to be affected neither by FBXL2 nor by PTEN.

In several tumors, PTEN function is impaired which results in accelerated IP_3R3 degradation and impaired apoptosis induction. Treatment with drugs that stabilize IP_3R levels may therefore also be of interest for cancer therapy in cases where PTEN is affected.

5 Conclusions

Intracellular Ca^{2+} signaling is involved in a plethora of cellular processes. The ubiquitously expressed IP_3R Ca^{2+}-release channels play an important role in the generation of these signals and serve as signaling hubs for several regulatory factors and proteins/protein complexes. Since the first identification of the IP_3R (175), IP_3R-interacting proteins and their modulating roles on Ca^{2+} signaling and (patho)physiological processes have been the subject of many studies and well over 100 interaction partners were reported (14), though for many of them it is unclear how they exactly interact with the IP_3R and how they affect IP_3R function. Moreover, for many regulatory proteins, multiple binding sites were described of which the importance is not directly apparent. The recent (and future) advances in the elucidation of the IP_3R structure will pave the way for a better understanding how IP_3R gating exactly occurs and how different cellular factors and regulatory proteins influence IICR. As several of these proteins affect life and death decisions and/or play important roles in tumor development, the exact knowledge of their interaction site and their action of the IP_3R may lead to the development of new therapies for e.g. cancer treatment.

ACKNOWLEDGEMENTS

TV is recipient of a postdoctoral fellowship of the Research Fund—Flanders (FWO). Work performed in the laboratory of the authors was supported by research grants of the FWO, the Research Council of the KU Leuven and the Interuniversity Attraction Poles Programme (Belgian Science Policy).

LEGENDS TO THE FIGURES

Figure 1. Alignment of proposed IP_3R1 structures. (a) Linear representation of IP_3R1 (33). (b) Linear representation of the IP_3R1 domains identified by X-ray crystallography (39). (c) Linear representation of the IP_3R1 domains identified by cryo-EM (41). For the various domains, the original nomenclature was used. Additionally, the interaction sites for calmodulin (CaM) and for the various Bcl-2 family members (Bcl-2, Bcl-XL, Nrz and Bok) are indicated with colored arrows at the bottom of the figure. Please note that the name of the interacting protein indicated at each arrow represents the protein for which binding was initially described. As discussed in the text, related proteins share in some cases common binding sites. The striped arrow indicates that this binding site is only present in a specific IP_3R1splice isoform. For further explanations, please see text.

Figure 2. Cryo-EM structure of IP_3R1. Structure of IP_3R1 fitted to the cryo-EM map (PDB 3JAV, (41)) showing (A) a cytosolic and (B) a luminal view of an IP_3R1 tetramer. (C, D) Side views of two neighboring IP_3R1 subunits as seen from the (C) inside or the (D) outside of the tetramer. The discontinuous CaM-binding site in the suppressor domain is indicated in yellow (a.a. 49-81 and a.a. 106–128). The yellow arrows in panels A and B indicate where the CaM-binding site in the central coupling domain should be located (a.a. 1564-1585). This could not be indicated on the structure itself because the part between a.a. 1488 and 1588 of the IP_3R is not resolved. The binding site for Bcl-2 and, to a lesser extent, Bcl-XL located in the central coupling domain is indicated in blue (a.a. 1389-1408). The C-terminal binding site for Bcl-2, Bcl-XL and Mcl-1 is shown in green (a.a. 2512-2749). The domains indicated in dark green (a.a. 2571-2606 and a.a. 2690-2732) thereby represent the BH3-like structures that were identified to bind Bcl-XL. The region where Bok interacts with IP_3R1 (a.a. 1895-1903) was not resolved in this cryo-EM structure. The two orange spheres (a.a. 1883 and 1945) however

show the boundaries of this non-characterized IP_3R1 region to which Bok binds. These images were obtained using PyMOL. For further explanations, please see text.

REFERENCES

1. Vermassen E, Parys JB, Mauger JP (2004) Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants. Biol Cell 96:3-17.
2. Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: Dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 4:517-529.
3. Berridge MJ, Lipp P, Bootman MD (2000). The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1:11-21.
4. Berridge MJ (2016) The inositol trisphosphate/calcium signaling pathway in health and disease. Physiol Rev 96:1261-1296.
5. Tada M, Nishizawa M, Onodera O (2016) Roles of inositol 1,4,5-trisphosphate receptors in spinocerebellar ataxias. Neurochem Int 94:1-8.
6. Egorova PA, Bezprozvanny IB (2017) Inositol 1,4,5-trisphosphate receptors and neurodegenerative disorders. FEBS J, in press.
7. Hisatsune C, Mikoshiba K (2017) IP_3 receptor mutations and brain diseases in human and rodents. J Neurochem 141:790-807.
8. Hisatsune C, Hamada K, Mikoshiba K (2018) Ca^{2+} signaling and spinocerebellar ataxia. Biochim Biophys Acta, in press.
9. Kerkhofs M, Seitaj B, Ivanova H, Monaco G, Bultynck G, Parys JB (2018) Pathophysiological consequences of isoform-specific IP_3 receptor mutations. Biochim Biophys Acta, in press.
10. Terry LE, Alzayady KJ, Furati E, Yule DI (2018) Inositol 1,4,5-trisphosphate receptor mutations associated with human disease. Messenger, in press.
11. Fedorenko OA, Popugaeva E, Enomoto M, Stathopulos PB, Ikura M, Bezprozvanny I (2014) Intracellular calcium channels: Inositol-1,4,5-trisphosphate receptors. Eur J Pharmacol 739:39-48.
12. Foskett JK, White C, Cheung KH, Mak DO (2007) Inositol trisphosphate receptor Ca^{2+} release channels. Physiol Rev 87:593-658.
13. Parys JB, De Smedt H (2012) Inositol 1,4,5-trisphosphate and its receptors. Adv Exp Med Biol 740:255-279.
14. Prole DL, Taylor CW (2016) Inositol 1,4,5-trisphosphate receptors and their protein partners as signalling hubs. J Physiol 594:2849-2866.
15. Vanderheyden V, Devogelaere B, Missiaen L, De Smedt H, Bultynck G, Parys JB (2009) Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release by reversible phosphorylation and dephosphorylation. Biochim Biophys Acta 1793:959-970.
16. Ivanova H, Vervliet T, Missiaen L, Parys JB, De Smedt H, Bultynck G (2014) Inositol 1,4,5-trisphosphate receptor-isoform diversity in cell death and survival. Biochim Biophys Acta 1843:2164-83.
17. Patel S, Joseph SK, Thomas AP (1999) Molecular properties of inositol 1,4,5-trisphosphate receptors. Cell Calcium 25:247-264.
18. Taylor CW, Genazzani AA, Morris SA (1999) Expression of inositol trisphosphate receptors. Cell Calcium 26:237-251.
19. Vervloessem T, Yule DI, Bultynck G, Parys JB (2015) The type 2 inositol 1,4,5-trisphosphate receptor, emerging functions for an intriguing Ca^{2+}-release channel. Biochim Biophys Acta 1853:1992-2005.
20. Gutierrez T, Simmen T (2018) Endoplasmic reticulum chaperones tweak the mitochondrial calcium rheostat to control metabolism and cell death. Cell Calcium 70:64-75.
21. La Rovere RM, Roest G, Bultynck G, Parys JB (2016) Intracellular Ca^{2+} signaling and Ca^{2+} microdomains in the control of cell survival, apoptosis and autophagy. Cell Calcium 60:74-87.
22. Marchi S, Bittremieux M, Missiroli S, Morganti C, Patergnani S, Sbano L, et al. (2017) Endoplasmic reticulum-mitochondria communication through Ca^{2+} signaling: The importance of mitochondria-associated membranes (MAMs). Adv Exp Med Biol 997:49-67.
23. Marchi S, Patergnani S, Missiroli S, Morciano G, Rimessi A, Wieckowski MR, et al. (2018) Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. Cell Calcium 69:62-72.

24. Raffaello A, Mammucari C, Gherardi G, Rizzuto R. (2016) Calcium at the center of cell signaling: Interplay between endoplasmic reticulum, mitochondria, and lysosomes. Trends Biochem Sci 41:1035-1049.
25. Ando H, Kawaai K, Bonneau B, Mikoshiba K. (2018) Remodeling of Ca^{2+} signaling in cancer: Regulation of inositol 1,4,5-trisphosphate receptors through oncogenes and tumor suppressors. Adv Biol Regul 68:64-76.
26. Garcia MI, Boehning D (2017) Cardiac inositol 1,4,5-trisphosphate receptors. Biochim Biophys Acta 1864:907-914.
27. Kania E, Roest G, Vervliet T, Parys JB, Bultynck G (2017) IP_3 receptor-mediated calcium signaling and its role in autophagy in cancer. Front Oncol 7:140.
28. Roest G, La Rovere RM, Bultynck G, Parys JB (2017) IP_3 receptor properties and function at membrane contact sites. Adv Exp Med Biol 981:149-178.
29. Serysheva, II, Baker MR, Fan G (2017) Structural insights into IP_3R function. Adv Exp Med Biol 981:121-147.
30. Wang L, Alzayady KJ, Yule DI (2016) Proteolytic fragmentation of inositol 1,4,5-trisphosphate receptors: a novel mechanism regulating channel activity? J Physiol 594:2867-2876.
31. Wright FA, Wojcikiewicz RJ (2016) Chapter 4 - Inositol 1,4,5-trisphosphate receptor ubiquitination. Prog Mol Biol Transl Sci 141:141-159.
32. Eid AH, El-Yazbi AF, Zouein F, Arredouani A, Ouhtit A, Rahman MM, et al. (2018) Inositol 1,4,5-trisphosphate receptors in hypertension. Front Physiol 9:1018.
33. Uchida K, Miyauchi H, Furuichi T, Michikawa T, Mikoshiba K (2003) Critical regions for activation gating of the inositol 1,4,5-trisphosphate receptor. J Biol Chem 278:16551-16560.
34. Bosanac I, Alattia JR, Mal TK, Chan J, Talarico S, Tong FK, et al. (2002) Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand. Nature 420:696-700.
35. Bosanac I, Yamazaki H, Matsu-Ura T, Michikawa T, Mikoshiba K, Ikura M (2005) Crystal structure of the ligand binding suppressor domain of type 1 inositol 1,4,5-trisphosphate receptor. Mol Cell 17:193-203.
36. Lin CC, Baek K, Lu Z (2011) Apo and $InsP_3$-bound crystal structures of the ligand-binding domain of an $InsP_3$ receptor. Nat Struct Mol Biol 18:1172-1174.
37. Seo MD, Velamakanni S, Ishiyama N, Stathopulos PB, Rossi AM, Khan SA, et al. (2012) Structural and functional conservation of key domains in $InsP_3$ and ryanodine receptors. Nature 483:108-112.
38. Bosanac I, Michikawa T, Mikoshiba K, Ikura M (2004) Structural insights into the regulatory mechanism of IP_3 receptor. Biochim Biophys Acta 1742:89-102.
39. Hamada K, Miyatake H, Terauchi A, Mikoshiba K (2017) IP_3-mediated gating mechanism of the IP_3 receptor revealed by mutagenesis and X-ray crystallography. Proc Natl Acad Sci USA 114:4661-4666.
40. Taylor CW, da Fonseca PC, Morris EP (2004) IP_3 receptors: the search for structure. Trends Biochem Sci 29:210-219.
41. Fan G, Baker ML, Wang Z, Baker MR, Sinyagovskiy PA, Chiu W, et al. (2015) Gating machinery of $InsP_3R$ channels revealed by electron cryomicroscopy. Nature 527:336-341.
42. Yoshikawa F, Iwasaki H, Michikawa T, Furuichi T, Mikoshiba K (1999) Trypsinized cerebellar inositol 1,4,5-trisphosphate receptor. Structural and functional coupling of cleaved ligand binding and channel domains. J Biol Chem 274:316-327.
43. Wang L, Wagner LE, 2nd, Alzayady KJ, Yule DI (2017) Region-specific proteolysis differentially regulates type 1 inositol 1,4,5-trisphosphate receptor activity. J Biol Chem 292:11714-11726.
44. Wang L, Yule DI (2018) Differential regulation of ion channels function by proteolysis. Biochim Biophys Acta, in press.
45. Wang L, Wagner LE, 2^{nd}, Alzayady KJ, Yule DI (2018) Region-specific proteolysis differentially modulates type 2 and type 3 inositol 1,4,5-trisphosphate receptor activity in models of acute pancreatitis. J Biol Chem, in press.

46. Chan J, Yamazaki H, Ishiyama N, Seo MD, Mal TK, Michikawa T, et al. (2010) Structural studies of inositol 1,4,5-trisphosphate receptor: coupling ligand binding to channel gating. J Biol Chem 285:36092-36099.
47. Schug ZT, Joseph SK (2006) The role of the S4-S5 linker and C-terminal tail in inositol 1,4,5-trisphosphate receptor function. J Biol Chem 281:24431-24440.
48. Yamazaki H, Chan J, Ikura M, Michikawa T, Mikoshiba K (2010) Tyr-167/Trp-168 in type 1/3 inositol 1,4,5-trisphosphate receptor mediates functional coupling between ligand binding and channel opening. J Biol Chem 285:36081-36091.
49. Paknejad N, Hite RK (2018) Structural basis for the regulation of inositol trisphosphate receptors by Ca^{2+} and IP_3. Nat Struct Mol Biol 25:660-668.
50. Marchant JS, Taylor CW (1997) Cooperative activation of IP_3 receptors by sequential binding of IP_3 and Ca^{2+} safeguards against spontaneous activity. Curr Biol 7:510-518.
51. Meyer T, Holowka D, Stryer L (1988) Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. Science 240:653-656.
52. Boehning D, Joseph SK (2000) Direct association of ligand-binding and pore domains in homo- and heterotetrameric inositol 1,4,5-trisphosphate receptors. EMBO J 19:5450-5459.
53. Alzayady KJ, Wang L, Chandrasekhar R, Wagner LE, 2nd, Van Petegem F, Yule DI (2016) Defining the stoichiometry of inositol 1,4,5-trisphosphate binding required to initiate Ca^{2+} release. Sci Signal 9:ra35.
54. Yoshikawa F, Morita M, Monkawa T, Michikawa T, Furuichi T, Mikoshiba K (1996) Mutational analysis of the ligand binding site of the inositol 1,4,5-trisphosphate receptor. J Biol Chem 271:18277-18284.
55. Iwai M, Tateishi Y, Hattori M, Mizutani A, Nakamura T, Futatsugi A, et al. (2005) Molecular cloning of mouse type 2 and type 3 inositol 1,4,5-trisphosphate receptors and identification of a novel type 2 receptor splice variant. J Biol Chem 280:10305-10317.
56. Konieczny V, Tovey SC, Mataragka S, Prole DL, Taylor CW (2017) Cyclic AMP recruits a discrete intracellular Ca^{2+} store by unmasking hypersensitive IP_3 receptors. Cell Rep 18:711-722.
57. Joseph SK, Lin C, Pierson S, Thomas AP, Maranto AR (1995) Heteroligomers of type-I and type-III inositol trisphosphate receptors in WB rat liver epithelial cells. J Biol Chem 270:23310-23316.
58. Monkawa T, Miyawaki A, Sugiyama T, Yoneshima H, Yamamoto-Hino M, Furuichi T, et al. (1995) Heterotetrameric complex formation of inositol 1,4,5-trisphosphate receptor subunits. J Biol Chem 270:14700-14704.
59. Wojcikiewicz RJ, He Y (1995) Type I, II and III inositol 1,4,5-trisphosphate receptor co-immunoprecipitation as evidence for the existence of heterotetrameric receptor complexes. Biochem Biophys Res Commun 213:334-341.
60. Joseph SK, Bokkala S, Boehning D, Zeigler S (2000) Factors determining the composition of inositol trisphosphate receptor hetero-oligomers expressed in COS cells. J Biol Chem 275:16084-16090.
61. Alzayady KJ, Wagner LE, 2nd, Chandrasekhar R, Monteagudo A, Godiska R, Tall GG, et al. (2013) Functional inositol 1,4,5-trisphosphate receptors assembled from concatenated homo- and heteromeric subunits. J Biol Chem 288:29772-29784.
62. De Smedt H, Missiaen L, Parys JB, Henning RH, Sienaert I, Vanlingen S, et al. (1997) Isoform diversity of the inositol trisphosphate receptor in cell types of mouse origin. Biochem J 322:575-583.
63. Wojcikiewicz RJ (1995) Type I, II, and III inositol 1,4,5-trisphosphate receptors are unequally susceptible to down-regulation and are expressed in markedly different proportions in different cell types. J Biol Chem 270:11678-11683.
64. Chandrasekhar R, Alzayady KJ, Wagner LE, 2nd, Yule DI (2016) Unique regulatory properties of heterotetrameric inositol 1,4,5-trisphosphate receptors revealed by studying concatenated receptor constructs. J Biol Chem 291:4846-4860.
65. Taylor CW (2017) Regulation of IP_3 receptors by cyclic AMP. Cell Calcium 63:48-52.
66. Wagner LE, 2nd, Joseph SK, Yule DI (2008) Regulation of single inositol 1,4,5-trisphosphate receptor channel activity by protein kinase A phosphorylation. J Physiol 586:3577-3596.

67. Meena A, Tovey SC, Taylor CW (2015) Sustained signalling by PTH modulates IP_3 accumulation and IP_3 receptors through cyclic AMP junctions. J Cell Sci 128:408-420.
68. Tovey SC, Dedos SG, Rahman T, Taylor EJ, Pantazaka E, Taylor CW (2010) Regulation of inositol 1,4,5-trisphosphate receptors by cAMP independent of cAMP-dependent protein kinase. J Biol Chem 285:12979-12989.
69. Tovey SC, Dedos SG, Taylor EJ, Church JE, Taylor CW (2008) Selective coupling of type 6 adenylyl cyclase with type 2 IP_3 receptors mediates direct sensitization of IP_3 receptors by cAMP. J Cell Biol 183:297-311.
70. Chin D, Means AR (2000) Calmodulin: a prototypical calcium sensor. Trends Cell Biol 10:322-328.
71. Villarroel A, Taglialatela M, Bernardo-Seisdedos G, Alaimo A, Agirre J, Alberdi A, et al. (2014) The ever changing moods of calmodulin: how structural plasticity entails transductional adaptability. J Mol Biol 426:2717-2735.
72. Tidow H, Nissen P (2013) Structural diversity of calmodulin binding to its target sites. FEBS J 280:5551-5565.
73. Yap KL, Kim J, Truong K, Sherman M, Yuan T, Ikura M (2000) Calmodulin target database. J Struct Funct Genomics 1:8-14.
74. Maeda N, Kawasaki T, Nakade S, Yokota N, Taguchi T, Kasai M, et al. (1991) Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum. J Biol Chem 266:1109-1116.
75. Yamada M, Miyawaki A, Saito K, Nakajima T, Yamamoto-Hino M, Ryo Y, et al. (1995) The calmodulin-binding domain in the mouse type 1 inositol 1,4,5-trisphosphate receptor. Biochem J 308:83-88.
76. Adkins CE, Morris SA, De Smedt H, Sienaert I, Török K, Taylor CW (2000) Ca^{2+}-calmodulin inhibits Ca^{2+} release mediated by type-1, -2 and -3 inositol trisphosphate receptors. Biochem J 345:357-363.
77. Sienaert I, Nadif Kasri N, Vanlingen S, Parys JB, Callewaert G, Missiaen L, et al. (2002) Localization and function of a calmodulin-apocalmodulin-binding domain in the N-terminal part of the type 1 inositol 1,4,5-trisphosphate receptor. Biochem J 365:269-277.
78. Islam MO, Yoshida Y, Koga T, Kojima M, Kangawa K, Imai S (1996) Isolation and characterization of vascular smooth muscle inositol 1,4,5-trisphosphate receptor. Biochem J 316:295-302.
79. Lin C, Widjaja J, Joseph SK (2000) The interaction of calmodulin with alternatively spliced isoforms of the type-I inositol trisphosphate receptor. J Biol Chem 275:2305-2311.
80. Cardy TJ, Taylor CW (1998) A novel role for calmodulin: Ca^{2+}-independent inhibition of type-1 inositol trisphosphate receptors. Biochem J 334:447-455.
81. Patel S, Morris SA, Adkins CE, O'Beirne G, Taylor CW (1997) Ca^{2+}-independent inhibition of inositol trisphosphate receptors by calmodulin: redistribution of calmodulin as a possible means of regulating Ca^{2+} mobilization. Proc Natl Acad Sci USA 94:11627-11632.
82. Sipma H, De Smet P, Sienaert I, Vanlingen S, Missiaen L, Parys JB, et al. (1999) Modulation of inositol 1,4,5-trisphosphate binding to the recombinant ligand-binding site of the type-1 inositol 1,4,5-trisphosphate receptor by Ca^{2+} and calmodulin. J Biol Chem 274:12157-12162.
83. Vanlingen S, Sipma H, De Smet P, Callewaert G, Missiaen L, De Smedt H, et al. (2000) Ca^{2+} and calmodulin differentially modulate myo-inositol 1,4, 5-trisphosphate (IP_3)-binding to the recombinant ligand-binding domains of the various IP_3 receptor isoforms. Biochem J 346:275-280.
84. Michikawa T, Hirota J, Kawano S, Hiraoka M, Yamada M, Furuichi T, et al. (1999) Calmodulin mediates calcium-dependent inactivation of the cerebellar type 1 inositol 1,4,5-trisphosphate receptor. Neuron 23:799-808.
85. Missiaen L, Parys JB, Weidema AF, Sipma H, Vanlingen S, De Smet P, et al. (1999) The bell-shaped Ca^{2+} dependence of the inositol 1,4, 5-trisphosphate-induced Ca^{2+} release is modulated by Ca^{2+}/calmodulin. J Biol Chem 274:13748-13751.

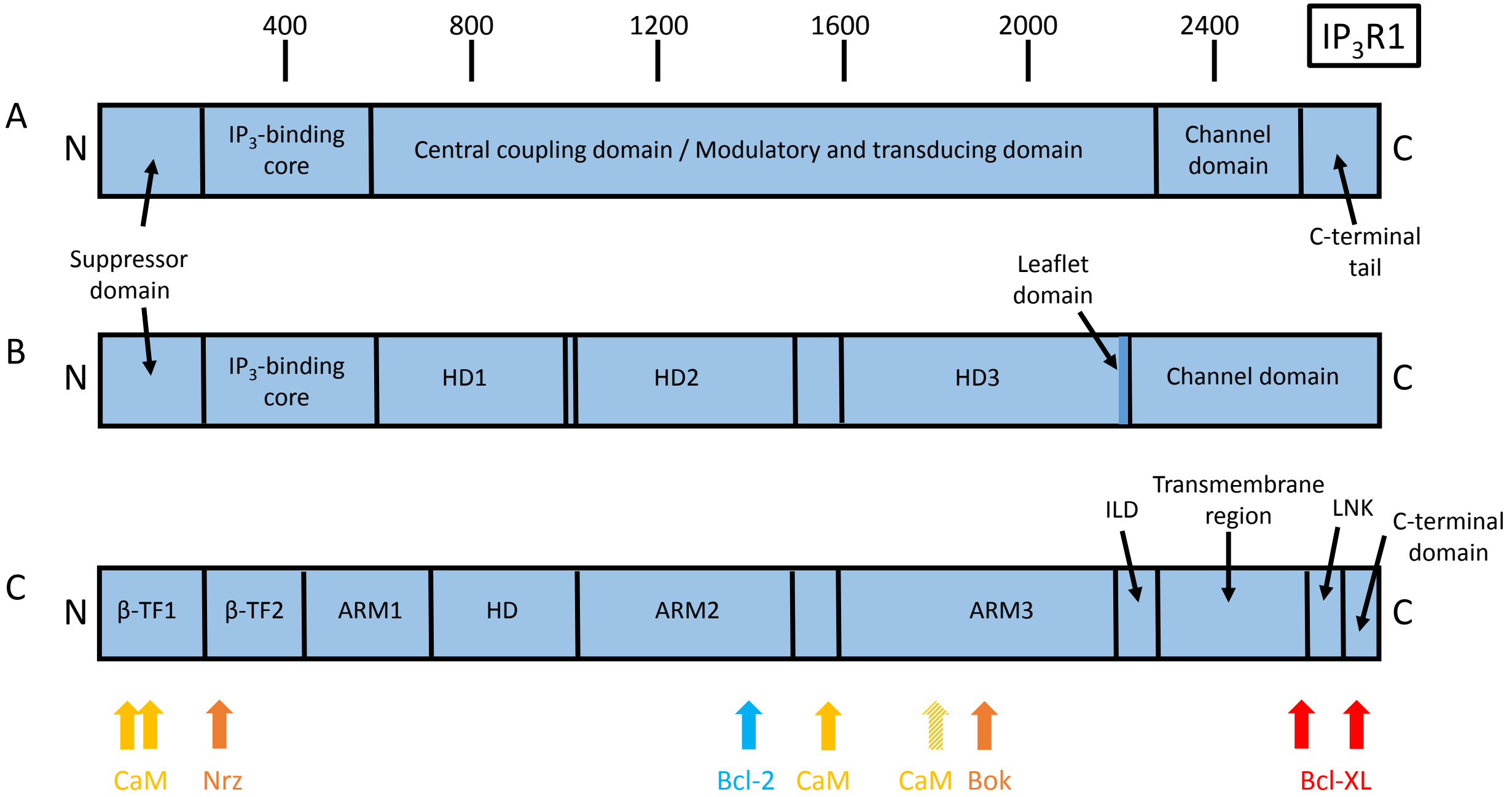
86. Missiaen L, DeSmedt H, Bultynck G, Vanlingen S, Desmet P, Callewaert G, et al. (2000) Calmodulin increases the sensitivity of type 3 inositol-1,4, 5-trisphosphate receptors to Ca^{2+} inhibition in human bronchial mucosal cells. Mol Pharmacol 57:564-567.
87. Nosyreva E, Miyakawa T, Wang Z, Glouchankova L, Mizushima A, Iino M, et al. (2002) The high-affinity calcium-calmodulin-binding site does not play a role in the modulation of type 1 inositol 1,4,5-trisphosphate receptor function by calcium and calmodulin. Biochem J 365:659-367.
88. Kasri NN, Bultynck G, Smyth J, Szlufcik K, Parys JB, Callewaert G, et al. (2004) The N-terminal Ca^{2+}-independent calmodulin-binding site on the inositol 1,4,5-trisphosphate receptor is responsible for calmodulin inhibition, even though this inhibition requires Ca^{2+}. Mol Pharmacol 66:276-284.
89. Kasri NN, Török K, Galione A, Garnham C, Callewaert G, Missiaen L, et al. (2006) Endogenously bound calmodulin is essential for the function of the inositol 1,4,5-trisphosphate receptor. J Biol Chem 281:8332-8338.
90. Sun Y, Taylor CW (2008) A calmodulin antagonist reveals a calmodulin-independent interdomain interaction essential for activation of inositol 1,4,5-trisphosphate receptors. Biochem J 416:243-253.
91. Sun Y, Rossi AM, Rahman T, Taylor CW (2013) Activation of IP_3 receptors requires an endogenous 1-8-14 calmodulin-binding motif. Biochem J 449:39-49.
92. Kang S, Kwon H, Wen H, Song Y, Frueh D, Ahn HC, et al. (2011) Global dynamic conformational changes in the suppressor domain of IP_3 receptor by stepwise binding of the two lobes of calmodulin. FASEB J 25:840-850.
93. White C, Yang J, Monteiro MJ, Foskett JK (2006) CIB1, a ubiquitously expressed Ca^{2+}-binding protein ligand of the $InsP_3$ receptor Ca^{2+} release channel. J Biol Chem 281:20825-20833.
94. Yang J, McBride S, Mak DO, Vardi N, Palczewski K, Haeseleer F, et al. (2002) Identification of a family of calcium sensors as protein ligands of inositol trisphosphate receptor Ca^{2+} release channels. Proc Natl Acad Sci USA 99:7711-7716.
95. Haynes LP, Tepikin AV, Burgoyne RD (2004) Calcium-binding protein 1 is an inhibitor of agonist-evoked, inositol 1,4,5-trisphosphate-mediated calcium signaling. J Biol Chem 279:547-555.
96. Kasri NN, Holmes AM, Bultynck G, Parys JB, Bootman MD, Rietdorf K, et al. (2004) Regulation of $InsP_3$ receptor activity by neuronal Ca^{2+}-binding proteins. EMBO J 23:312-321.
97. Bultynck G, Szlufcik K, Kasri NN, Assefa Z, Callewaert G, Missiaen L, et al. (2004) Thimerosal stimulates Ca^{2+} flux through inositol 1,4,5-trisphosphate receptor type 1, but not type 3, via modulation of an isoform-specific Ca^{2+}-dependent intramolecular interaction. Biochem J 381:87-96.
98. Li C, Chan J, Haeseleer F, Mikoshiba K, Palczewski K, Ikura M, et al. (2009) Structural insights into Ca^{2+}-dependent regulation of inositol 1,4,5-trisphosphate receptors by CaBP1. J Biol Chem 284:2472-2481.
99. Li C, Enomoto M, Rossi AM, Seo MD, Rahman T, Stathopulos PB, et al. (2013) CaBP1, a neuronal Ca^{2+} sensor protein, inhibits inositol trisphosphate receptors by clamping intersubunit interactions. Proc Natl Acad Sci USA 110:8507-8512.
100. Nakamura TY, Jeromin A, Mikoshiba K, Wakabayashi S (2011) Neuronal calcium sensor-1 promotes immature heart function and hypertrophy by enhancing Ca^{2+} signals. Circ Res 109:512-523.
101. Schlecker C, Boehmerle W, Jeromin A, DeGray B, Varshney A, Sharma Y, et al. (2006) Neuronal calcium sensor-1 enhancement of $InsP_3$ receptor activity is inhibited by therapeutic levels of lithium. J Clin Invest 116:1668-1674.
102. Zhang K, Heidrich FM, DeGray B, Boehmerle W, Ehrlich BE (2010) Paclitaxel accelerates spontaneous calcium oscillations in cardiomyocytes by interacting with NCS-1 and the $InsP_3R$. J Mol Cell Cardiol 49:829-835.
103. Boehmerle W, Splittgerber U, Lazarus MB, McKenzie KM, Johnston DG, Austin DJ, et al. (2006) Paclitaxel induces calcium oscillations via an inositol 1,4,5-trisphosphate receptor and neuronal calcium sensor 1-dependent mechanism. Proc Natl Acad Sci USA 103:18356-18361.
104. Boeckel GR, Ehrlich BE (2018) NCS-1 is a regulator of calcium signaling in health and disease. Biochim Biophys Acta, in press.

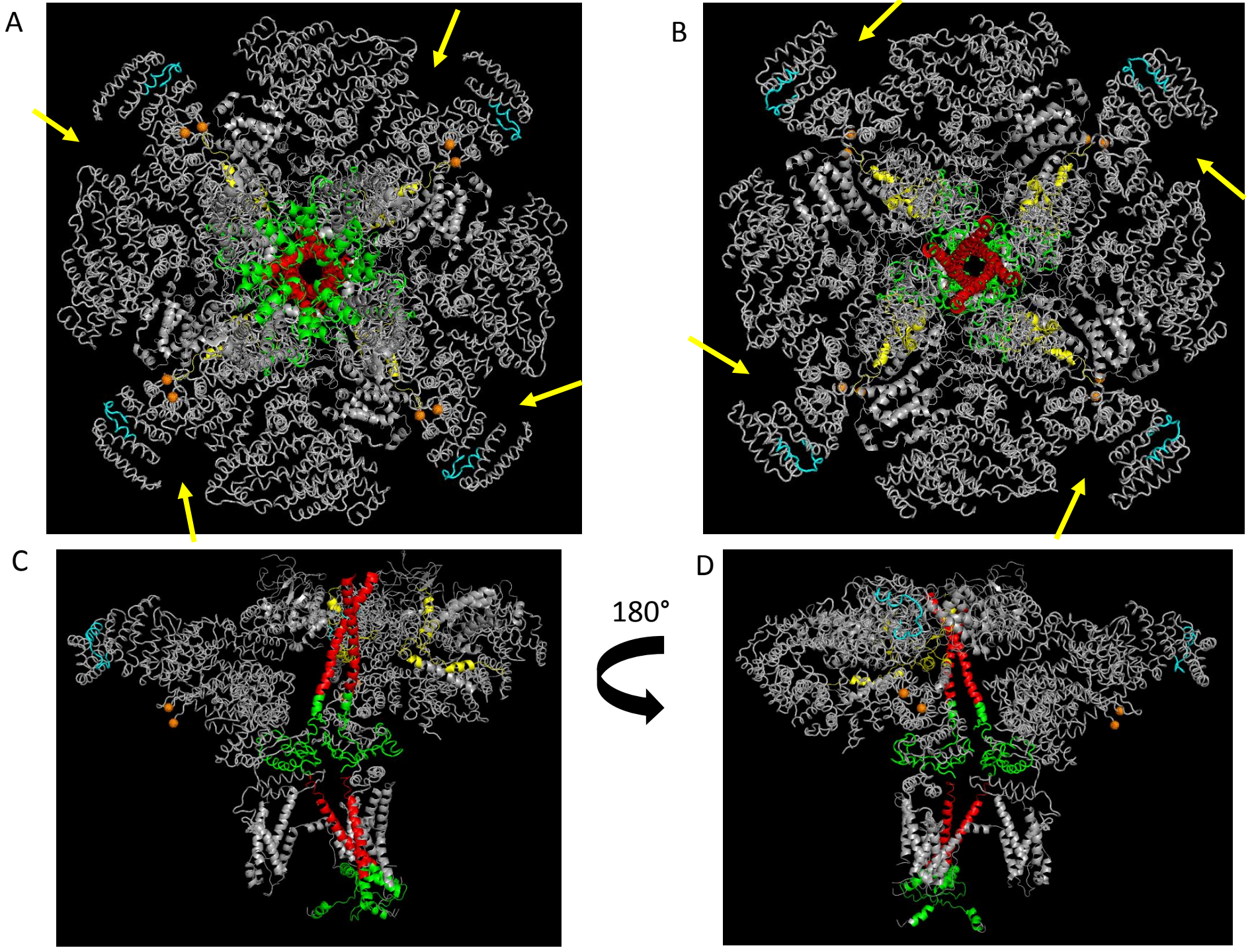
990 105. Meissner G (2017) The structural basis of ryanodine receptor ion channel function. J Gen
991 Physiol 149:1065-1089.
992 106. Brini M, Cali T, Ottolini D, Carafoli E (2013) The plasma membrane calcium pump in health
993 and disease. FEBS J 280:5385-5397.
994 107. Hasan R, Zhang X (2018) Ca^{2+} regulation of TRP ion channels. Int J Mol Sci 19:1256.
995 108. Saimi Y, Kung C (2002) Calmodulin as an ion channel subunit. Annu Rev Physiol 64:289-311.
996 109. Letai AG (2008) Diagnosing and exploiting cancer's addiction to blocks in apoptosis. Nat Rev
997 Cancer 8:121-32.
998 110. Brunelle JK, Letai A (2009) Control of mitochondrial apoptosis by the Bcl-2 family. J Cell Sci
999 122:437-441.
1000 111. Davids MS, Letai A (2012) Targeting the B-cell lymphoma/leukemia 2 family in cancer. J Clin
1001 Oncol 30:3127-3135.
1002 112. Vervliet T, Parys JB, Bultynck G (2016) Bcl-2 proteins and calcium signaling: complexity
1003 beneath the surface. Oncogene 35:5079-5092.
1004 113. Cárdenas C, Miller RA, Smith I, Bui T, Molgó J, Müller M, et al. (2010) Essential regulation of
1005 cell bioenergetics by constitutive $InsP_3$ receptor Ca^{2+} transfer to mitochondria. Cell 142:270-283.
1006 114. Eckenrode EF, Yang J, Velmurugan GV, Foskett JK, White C (2010) Apoptosis protection by
1007 Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling. J Biol
1008 Chem 285:13678-13684.
1009 115. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, et al. (2005) The endoplasmic
1010 reticulum gateway to apoptosis by Bcl-X_L modulation of the $InsP_3R$. Nat Cell Biol 7:1021-1028.
1011 116. Rong YP, Bultynck G, Aromolaran AS, Zhong F, Parys JB, De Smedt H, et al. (2009) The BH4
1012 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling
1013 domain of the IP_3 receptor. Proc Natl Acad Sci USA 106:14397-14402.
1014 117. Parys JB (2014) The IP_3 receptor as a hub for Bcl-2 family proteins in cell death control and
1015 beyond. Sci Signal 7:pe4.
1016 118. Monaco G, Beckers M, Ivanova H, Missiaen L, Parys JB, De Smedt H, et al. (2012) Profiling of
1017 the Bcl-2/Bcl-X_L-binding sites on type 1 IP_3 receptor. Biochem Biophys Res Commun 428:31-35.
1018 119. Monaco G, Decrock E, Akl H, Ponsaerts R, Vervliet T, Luyten T, et al. (2012) Selective
1019 regulation of IP_3-receptor-mediated Ca^{2+} signaling and apoptosis by the BH4 domain of Bcl-2 versus
1020 Bcl-Xl. Cell Death Differ 19:295-309.
1021 120. Rong YP, Aromolaran AS, Bultynck G, Zhong F, Li X, McColl K, et al. (2008) Targeting Bcl-2-IP_3
1022 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals. Mol Cell 31:255-265.
1023 121. Bonneau B, Nougarede A, Prudent J, Popgeorgiev N, Peyrieras N, Rimokh R, et al. (2014) The
1024 Bcl-2 homolog Nrz inhibits binding of IP_3 to its receptor to control calcium signaling during zebrafish
1025 epiboly. Sci Signal 7:ra14.
1026 122. Bonneau B, Ando H, Kawaai K, Hirose M, Takahashi-Iwanaga H, Mikoshiba K. (2016) IRBIT
1027 controls apoptosis by interacting with the Bcl-2 homolog, Bcl2l10, and by promoting ER-mitochondria
1028 contact. Elife 5:e19896.
1029 123. Yang J, Vais H, Gu W, Foskett JK (2016) Biphasic regulation of $InsP_3$ receptor gating by dual
1030 Ca^{2+} release channel BH3-like domains mediates Bcl-xL control of cell viability. Proc Natl Acad Sci USA
1031 113:E1953-62.
1032 124. Ivanova H, Ritaine A, Wagner L, Luyten T, Shapovalov G, Welkenhuyzen K, et al. (2016) The
1033 trans-membrane domain of Bcl-2α, but not its hydrophobic cleft, is a critical determinant for efficient
1034 IP_3 receptor inhibition. Oncotarget 7:55704-55720.
1035 125. Chang MJ, Zhong F, Lavik AR, Parys JB, Berridge MJ, Distelhorst CW (2014) Feedback
1036 regulation mediated by Bcl-2 and DARPP-32 regulates inositol 1,4,5-trisphosphate receptor
1037 phosphorylation and promotes cell survival. Proc Natl Acad Sci USA 111:1186-1191.
1038 126. Ivanova H, Wagner LE, 2nd, Tanimura A, Vandermarliere E, Luyten T, Welkenhuyzen K, et al.
1039 (2018) Mutual antagonism between IP_3 and anti-apoptotic Bcl-2 modulates IP_3R activity by
1040 competing for the ligand-binding domain. Abstract, 15th International Meeting of the European
1041 Calcium Society (Hamburg, Germany).

127. Schulman JJ, Wright FA, Kaufmann T, Wojcikiewicz RJ (2013) The Bcl-2 protein family member Bok binds to the coupling domain of inositol 1,4,5-trisphosphate receptors and protects them from proteolytic cleavage. J Biol Chem 288:25340-25349.
128. Assefa Z, Bultynck G, Szlufcik K, Nadif Kasri N, Vermassen E, Goris J, et al. (2004) Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis. J Biol Chem 279:43227-43236.
129. Hirota J, Furuichi T, Mikoshiba K (1999) Inositol 1,4,5-trisphosphate receptor type 1 is a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent manner. J Biol Chem 274:34433-34437.
130. Schulman JJ, Wright FA, Han X, Zluhan EJ, Szczesniak LM, Wojcikiewicz RJ (2016) The stability and expression level of Bok are governed by binding to inositol 1,4,5-trisphosphate receptors. J Biol Chem 291:11820-11828.
131. Ivanova H, Luyten T, Decrock E, Vervliet T, Leybaert L, Parys JB, et al. (2017) The BH4 domain of Bcl-2 orthologues from different classes of vertebrates can act as an evolutionary conserved inhibitor of IP_3 receptor channels. Cell Calcium 62:41-66.
132. Cárdenas C, Müller M, McNeal A, Lovy A, Jana F, Bustos G, et al. (2016) Selective vulnerability of cancer cells by inhibition of Ca^{2+} transfer from endoplasmic reticulum to mitochondria. Cell Rep 14:2313-2324.
133. Finkel T, Hwang PM (2009) The Krebs cycle meets the cell cycle: mitochondria and the G_1-S transition. Proc Natl Acad Sci USA 106:11825-11826.
134. Distelhorst CW (2018) Targeting Bcl-2-IP_3 receptor interaction to treat cancer: A novel approach inspired by nearly a century treating cancer with adrenal corticosteroid hormones. Biochim Biophys Acta, in press.
135. He C, Levine B (2010). The Beclin 1 interactome. Curr Opin Cell Biol 22:140-149.
136. Decuypere JP, Parys JB, Bultynck G (2012) Regulation of the autophagic Bcl-2/Beclin 1 interaction. Cells 1:284-312.
137. Erlich S, Mizrachy L, Segev O, Lindenboim L, Zmira O, Adi-Harel S, et al. (2007) Differential interactions between Beclin 1 and Bcl-2 family members. Autophagy 3:561-568.
138. Vicencio JM, Ortiz C, Criollo A, Jones AW, Kepp O, Galluzzi L, et al. (2009) The inositol 1,4,5-trisphosphate receptor regulates autophagy through its interaction with Beclin 1. Cell Death Differ 16:1006-1017.
139. Decuypere JP, Welkenhuyzen K, Luyten T, Ponsaerts R, Dewaele M, Molgo J, et al. (2011) $Ins(1,4,5)P_3$ receptor-mediated Ca^{2+} signaling and autophagy induction are interrelated. Autophagy 7:1472-1489.
140. Ando H, Mizutani A, Matsu-ura T, Mikoshiba K (2003) IRBIT, a novel inositol 1,4,5-trisphosphate (IP_3) receptor-binding protein, is released from the IP_3 receptor upon IP_3 binding to the receptor. J Biol Chem 278:10602-10612.
141. Ando H, Kawaai K, Mikoshiba K (2014) IRBIT: a regulator of ion channels and ion transporters. Biochim Biophys Acta 1843:2195-2204.
142. Kawaai K, Ando H, Satoh N, Yamada H, Ogawa N, Hirose M, et al. Splicing variation of long-IRBIT determines the target selectivity of IRBIT family proteins. Proc Natl Acad Sci USA 114:3921-3926.
143. Liang J, Lyu J, Zhao M, Li D, Zheng M, Fang Y, et al. (2017) Tespa1 regulates T cell receptor-induced calcium signals by recruiting inositol 1,4,5-trisphosphate receptors. Nat Commun 8:15732.
144. Wang D, Zheng M, Lei L, Ji J, Yao Y, Qiu Y, et al. (2012) Tespa1 is involved in late thymocyte development through the regulation of TCR-mediated signaling. Nat Immunol 13:560-568.
145. Dingli F, Parys JB, Loew D, Saule S, Mery L (2012) Vimentin and the K-Ras-induced actin-binding protein control inositol-(1,4,5)-trisphosphate receptor redistribution during MDCK cell differentiation. J Cell Sci 125:5428-5440.

146. Fujimoto T, Machida T, Tanaka Y, Tsunoda T, Doi K, Ota T, et al. (2011) KRAS-induced actin-interacting protein is required for the proper localization of inositol 1,4,5-trisphosphate receptor in the epithelial cells. Biochem Biophys Res Commun 407:438-443.
147. Matsuzaki H, Fujimoto T, Ota T, Ogawa M, Tsunoda T, Doi K, et al. (2012) Tespa1 is a novel inositol 1,4,5-trisphosphate receptor binding protein in T and B lymphocytes. FEBS Open Bio 2:255-259.
148. Malissen B, Gregoire C, Malissen M, Roncagalli R. (2014) Integrative biology of T cell activation. Nat Immunol 15:790-797.
149. Matsuzaki H, Fujimoto T, Tanaka M, Shirasawa S. (2013) Tespa1 is a novel component of mitochondria-associated endoplasmic reticulum membranes and affects mitochondrial calcium flux. Biochem Biophys Res Commun 433:322-326.
150. Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, et al. (2006) Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. J Cell Biol 175:901-911.
151. Dayton TL, Jacks T, Vander Heiden MG (2016) PKM2, cancer metabolism, and the road ahead. EMBO Rep 17:1721-1730.
152. Hsu MC, Hung WC (2018) Pyruvate kinase M2 fuels multiple aspects of cancer cells: from cellular metabolism, transcriptional regulation to extracellular signaling. Mol Cancer 17:35.
153. Dong G, Mao Q, Xia W, Xu Y, Wang J, Xu L, et al. (2016) PKM2 and cancer: The function of PKM2 beyond glycolysis. Oncol Lett 11:1980-1986.
154. Lavik AR (2016) The role of inositol 1,4,5-trisphosphate receptor-interacting proteins in regulating inositol 1,4,5-trisphosphate receptor-dependent calcium signals and cell survival. PhD thesis, Case Western Reserve University, USA; https://etdohiolinkedu/pg_10?0::NO:10:P10_ACCESSION_NUM:case1448532307.
155. Lavik A, Harr M, Kerkhofs M, Parys JB, Bultynck G, Bird G, et al. (2018) IP_3Rs recruit the glycolytic enzyme PKM2 to the ER, promoting Ca^{2+} homeostasis and survival in hematologic malignancies. Abstract, 15th International Meeting of the European Calcium Society (Hamburg, Germany).
156. Liu F, Ma F, Wang Y, Hao L, Zeng H, Jia C, et al. (2017) PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis. Nat Cell Biol 19:1358-1370.
157. Sipma H, Deelman L, Smedt HD, Missiaen L, Parys JB, Vanlingen S, et al. (1998) Agonist-induced down-regulation of type 1 and type 3 inositol 1,4,5-trisphosphate receptors in A7r5 and DDT1 MF-2 smooth muscle cells. Cell Calcium 23:11-21.
158. Wojcikiewicz RJ, Furuichi T, Nakade S, Mikoshiba K, Nahorski SR (1994) Muscarinic receptor activation down-regulates the type I inositol 1,4,5-trisphosphate receptor by accelerating its degradation. J Biol Chem 269:7963-7969.
159. Wojcikiewicz RJ, Nakade S, Mikoshiba K, Nahorski SR (1992) Inositol 1,4,5-trisphosphate receptor immunoreactivity in SH-SY5Y human neuroblastoma cells is reduced by chronic muscarinic receptor activation. J Neurochem 59:383-386.
160. Oberdorf J, Webster JM, Zhu CC, Luo SG, Wojcikiewicz RJ (1999). Down-regulation of types I, II and III inositol 1,4,5-trisphosphate receptors is mediated by the ubiquitin/proteasome pathway. Biochem J 339:453-461.
161. Lee HS, Lee SA, Hur SK, Seo JW, Kwon J (2014) Stabilization and targeting of INO80 to replication forks by BAP1 during normal DNA synthesis. Nat Commun 5:5128.
162. Zarrizi R, Menard JA, Belting M, Massoumi R (2014) Deubiquitination of γ-tubulin by BAP1 prevents chromosome instability in breast cancer cells. Cancer Res 74:6499-6508.
163. Yu H, Pak H, Hammond-Martel I, Ghram M, Rodrigue A, Daou S, et al. (2014) Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. Proc Natl Acad Sci USA 111:285-290.
164. Yu H, Mashtalir N, Daou S, Hammond-Martel I, Ross J, Sui G, et al. (2010) The ubiquitin carboxyl hydrolase BAP1 forms a ternary complex with YY1 and HCF-1 and is a critical regulator of gene expression. Mol Cell Biol 30:5071-5085.

165. Baughman JM, Rose CM, Kolumam G, Webster JD, Wilkerson EM, Merrill AE, et al. (2016) NeuCode proteomics reveals Bap1 regulation of metabolism. Cell Rep 16:583-595.
166. Ruan HB, Han X, Li MD, Singh JP, Qian K, Azarhoush S, et al. (2012) O-GlcNAc transferase/host cell factor C1 complex regulates gluconeogenesis by modulating PGC-1α stability. Cell Metab 16:226-237.
167. Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, Tanji M, et al. (2017) BAP1 regulates IP_3R3-mediated Ca^{2+} flux to mitochondria suppressing cell transformation. Nature 546:549-553.
168. Bononi A, Yang H, Giorgi C, Patergnani S, Pellegrini L, Su M, et al. (2017) Germline BAP1 mutations induce a Warburg effect. Cell Death Differ 24:1694-1704.
169. Kuchay S, Giorgi C, Simoneschi D, Pagan J, Missiroli S, Saraf A, et al. (2017) PTEN counteracts FBXL2 to promote IP_3R3- and Ca^{2+}-mediated apoptosis limiting tumour growth. Nature 546:554-558.
170. Worby CA, Dixon JE (2014). PTEN. Annu Rev Biochem 83:641-669.
171. Carnero A, Paramio JM (2014) The PTEN/PI3K/AKT pathway in vivo, cancer mouse models. Front Oncol 4:252.
172. Milella M, Falcone I, Conciatori F, Cesta Incani U, Del Curatolo A, Inzerilli N, et al. (2015) PTEN: Multiple functions in human malignant tumors. Front Oncol 5:24.
173. Bittremieux M, Parys JB, Pinton P, Bultynck G (2016) ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca^{2+} signaling. Biochim Biophys Acta 1863:1364-1378.
174. Bononi A, Bonora M, Marchi S, Missiroli S, Poletti F, Giorgi C, et al. (2013) Identification of PTEN at the ER and MAMs and its regulation of Ca^{2+} signaling and apoptosis in a protein phosphatase-dependent manner. Cell Death Differ 20:1631-1643.
175. Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K (1989). Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P_{400}. Nature 342:32-38.


400
800
1200
1600
2000
2400
IP3R1
A
N
IP3-binding core
Central coupling domain / Modulatory and transducing domain
Channel domain
C
Suppressor domain
C-terminal tail
B
N
IP3-binding core
HD1
HD2
HD3
Leaflet domain
Channel domain
C
C
N
β-TF1
β-TF2
ARM1
HD
ARM2
ARM3
ILD
Transmembrane region
LNK
C-terminal domain
C
CaM
Nrz
Bcl-2
CaM
CaM
Bok
Bcl-XL


A
B
C
180°
D