Chapter 10
New Insights in the IP_3 Receptor and Its Regulation

Jan B. Parys and Tim Vervliet

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

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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
TKO	triple-knockout

10.1 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.

10.2 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 (Fig. 10.1a): 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) (Fig. 10.1b). 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.

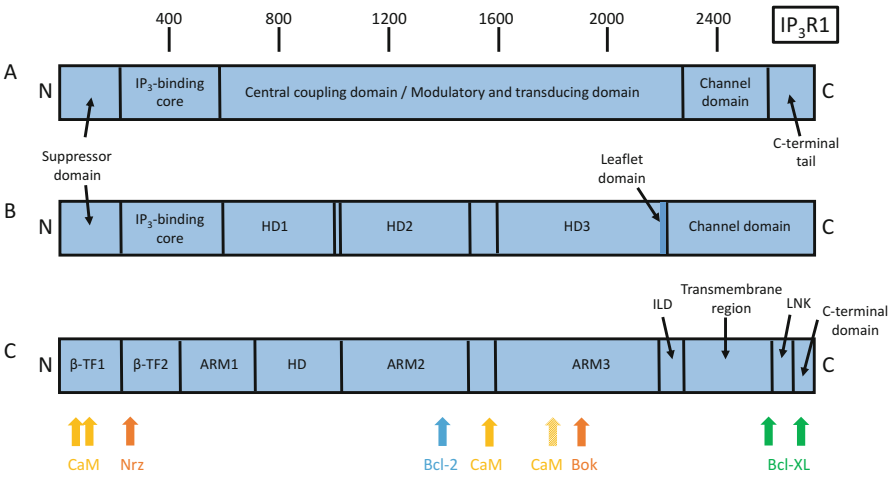

Fig. 10.1 Alignment of proposed IP_3R1 structures. (**a**) Linear representation of IP_3R1 [33]. (**b**) Linear representation of the IP_3R1 domains identified by X-ray crystallography [39]. (**c**) Linear representation of the IP_3R1 domains identified by cryo-EM [41]. For the various domains, the original 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

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 (Fig. 10.2). 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) (Fig. 10.1c). 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

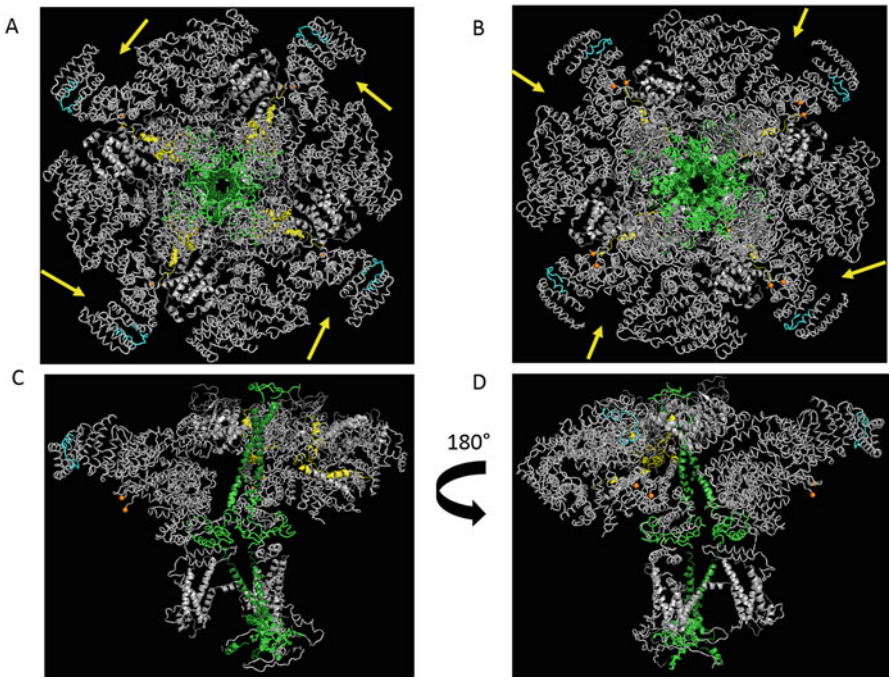

Fig. 10.2 Cryo-EM structure of IP_3R1. Structure of IP_3R1 fitted to the cryo-EM map (PDB 3JAV, [41]) showing (**a**) a cytosolic and (**b**) a luminal view of an IP_3R1 tetramer. (**c**, **d**) Side views of two neighboring IP_3R1 subunits as seen from the (**c**) inside or the (**d**) outside of the 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 to 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

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 inter-subunit 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 Sect. 10.1). 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.

10.3 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.

10.3.1 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 Sect. 10.2.; [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 [50, 53]. 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].

10.3.2 Physiological Relevance of IP_3R Heterotetramer Formation

As already indicated above (see Sect. 10.1.), 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.

10.3.3 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, evokes 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 to protein S-adenosylhomocysteine-hydrolase, known to bind cAMP, is involved was however already excluded by knockdown and overexpression experiments [56].

10.4 Complexity of Protein-Protein Interactions Affecting the IP_3R

In a comprehensive review published a few years ago, over 100 proteins that interact with the IP_3R have been listed [14]. For that reason, we will limit ourselves in the present review to either newly discovered interacting proteins or proteins for which new information about their interaction recently became available.

10.4.1 Calmodulin (CaM) and Related Ca^{2+}-Binding Proteins

CaM is the most ubiquitously expressed intracellular Ca^{2+} sensor. It is a relatively small protein (148 a.a.) with a typical dumbbell structure. A central, flexible linker region connects the globular N-terminal and C-terminal domains, each containing two Ca^{2+}-binding EF-hand motifs with a classical helix-loop-helix structure. The K_d of CaM for Ca^{2+} ranges between 5×10^{-7} and 5×10^{-6} M, with the C-terminal Ca^{2+}-binding sites having a three to five-fold higher affinity than the N-terminal ones [70]. CaM therefore displays the correct Ca^{2+} affinity to sense changes in intracellular Ca^{2+} concentrations and serve as Ca^{2+} sensor. While apo-CaM has a rather compact structure, Ca^{2+}-CaM exposes in each domain a hydrophobic groove with acidic residues at its extremities that will allow interaction with their target [71]. A plethora of target proteins that are modulated by CaM exists, including various Ca^{2+}-transporting proteins [72]. These various proteins contain CaM-binding sites that can be categorized into various types of motifs [73].

Although the interaction of CaM with the IP_3R was already observed soon after the identification of the IP_3R as IP_3-sensitive Ca^{2+}-release channel [74] its exact mechanism of action is still not completely elucidated. Moreover, there are a number of interesting features related to the binding of CaM to the IP_3R: (i) the existence of multiple binding sites, (ii) the possibility for both Ca^{2+}-CaM and apo-CaM to affect IP_3R function and (iii) the use of some of the CaM-binding sites by other Ca^{2+}-binding proteins. The aim of this paragraph therefore is to present a comprehensive view on the relation between CaM (and some related Ca^{2+}-binding proteins) and the IP_3R.

On IP_3R1, three CaM-binding sites were described (Fig. 10.1). A high-affinity CaM-binding site (a.a. 1564–1585; Fig. 10.2a–b, indicated by the yellow arrows) was described in the central coupling domain [75], while a low-affinity one was found in the suppressor domain [76]. The latter site is discontinuous (a.a. 49–81

and a.a. 106–128; Fig. 10.2**,** indicated in yellow) and can bind to both apo-CaM and Ca^{2+}-CaM [77]. Finally, a third site was described on the S2(−) IP_3R1 splice isoform in which a.a. 1693–1732 are removed [78, 79]. CaM binding to this newly formed site is inhibited by PKA-mediated phosphorylation, probably on Ser^{1589} [79].

CaM interaction with the two other IP_3R isoforms was studied in less detail, but an IP_3R2 construct overlapping with the CaM-binding site in the central coupling domain interacted with CaM, supporting the conservation of this site [75]. However, no direct interaction between CaM and IP_3R3 could be measured [75, 80] though CaM can bind to IP_3R1-IP_3R3 heterotetramers [79].

Functional effects on the IP_3R have been described for both apo-CaM and Ca^{2+}-CaM. In fact, apo-CaM is equally potent in inhibiting IP_3 binding to full-length IP_3R1 as Ca^{2+}-CaM [81]. In agreement with the absence of CaM binding to IP_3R3, full-length IP_3R3 remained insensitive to regulation by CaM [80]. In contrast, a Ca^{2+}-independent inhibition of IP_3 binding was observed for the isolated ligand-binding domain of IP_3R1 [82] as well as for that of IP_3R2 and IP_3R3 [83].

Concerning IP_3-induced Ca^{2+} release, the situation is somewhat more complex. Ca^{2+} release by IP_3R1 is inhibited by CaM in a Ca^{2+}-dependent way [84, 85] while similar results were subsequently found for IP_3R2 and IP_3R3 [76, 86]. However, linking these functional effects molecularly to a CaM-binding site appeared more difficult, not only because of the apparent absence of a Ca^{2+}-dependent CaM-binding site on IP_3R3 but also because the mutation $W^{1577}A$ that abolishes CaM binding to IP_3R1 [75], does not abolish the CaM-mediated inhibition of IICR [87].

Furthermore, other results suggested that the relation between CaM and the IP_3R was more complex than originally thought. A detailed analysis of the CaM-binding site located in the central coupling domain of IP_3R1 provided evidence that it consisted of a high-affinity Ca^{2+}-CaM and a lower affinity apo-CaM site [88]. Moreover, in the same study it was demonstrated that a CaM mutant deficient in Ca^{2+} binding (CaM1234) could inhibit IICR in a Ca^{2+}-dependent way with the same potency as CaM. In a separate study, it was demonstrated that a myosin light chain kinase (MLCK)-derived peptide, which binds to CaM with high affinity, fully inhibited the IP_3R [89]. This inhibition could be reversed by the addition of CaM but not of CaM1234 and the results were interpreted as evidence that endogenously bound CaM is needed for IP_3R activity. A follow-up study by another group [90] however proposed that the MLCK peptide is not removing endogenous CaM but is interacting with an endogenous CaM-like domain on IP_3R, thereby disrupting its interaction with a so-called 1–8-14 CaM-binding motif (a.a. 51–66) essential for IP_3R activity [91].

Meanwhile, the interaction of apo-CaM with the suppressor domain was studied via NMR analysis [92]. This study brought forward two main pieces of evidence. First, it was shown that the binding of apo-CaM to the suppressor domain induced an important, general conformational change to the latter. These changes further increased in the presence of Ca^{2+}. Secondly, analysis of the conformational change of CaM indicated that apo-CaM already binds with its C-lobe to the IP_3R1 suppressor domain, and that only after addition of Ca^{2+} also the N-lobe interacts

with the suppressor domain. These results can therefore explain the importance of the CaM-binding sites in the suppressor domain in spite of their difficult accessibility ([92]; Fig. 10.2).

Finally, some Ca^{2+}-binding proteins related to CaM (e.g. neuronal Ca^{2+}-binding protein (CaBP) 1, calmyrin, also known as CIB1, and neuronal Ca^{2+} sensor-1 (NCS-1)) also regulate the IP_3R. Similarly to CaM, these proteins contain 4 EF-hand motifs but in contrast with CaM, not all of them bind Ca^{2+}. In CaBP1 and NCS-1 only 3 EF hands are functional (EF1, EF3, EF4 and EF2, EF3, EF4 resp.) and in calmyrin only 2 (EF3 and EF4). Moreover, some of the EF hands bind Mg^{2+} rather than Ca^{2+}. Furthermore, those proteins are myristoylated. Although early results suggested that CaBP1 and calmyrin could, in the absence of IP_3, activate the IP_3R under some circumstances [93, 94], there is presently a large consensus that they, similarly to CaM, generally inhibit the IP_3R [93, 95, 96].

CaBP1 was proposed to interact with the IP_3R1 with a higher affinity than CaM itself [94, 96], while in contrast to CaM it does not affect the ryanodine receptor (RyR), another family of intracellular Ca^{2+}-release channels. Additionally, the interaction with the IP_3R would be subject to regulation by caseine kinase 2, an enzyme that can phosphorylate CaBP1 on S^{120} [96]. Similarly to CaM, CaBP1 binds in a Ca^{2+}-independent way to the IP_3R1 suppressor domain, but in contrast to CaM, only to the first of the two non-contiguous binding sites described for CaM (Fig. 10.1). However, CaM and CaBP1 similarly antagonized the thimerosal-stimulated interaction between the suppressor domain and the IBC of IP_3R1, suggesting a common mechanism of action whereby they disrupt intramolecular interactions needed for channel activation [97]. More recent work confirmed the inhibitory effect of CaBP1 on IP_3R1, while expanding the knowledge concerning the CaBP1 binding site. In particular, NMR analysis indicated that CaBP1 interacts with its C lobe with the suppressor domain of the IP_3R and that even at saturating Ca^{2+} concentrations EF1 is bound to Mg^{2+}, precluding a conformational change of the N lobe [98]. The same study demonstrated that Ca^{2+}-bound CaBP1 bound with an 10-fold higher affinity than Mg^{2+}-bound CaBP1 and an at least 100-fold higher affinity than CaM itself. Functional analysis performed in DT-40 cells solely expressing IP_3R1 demonstrated that CaBP1 stabilized the closed conformation of the channel, probably by clamping inter-subunit interactions [99]. The interaction of specific hydrophobic a.a. in the C lobe of CaBP1 (V^{101}, L^{104}, V^{162}) that become more exposed in the presence of Ca^{2+} with hydrophobic a.a. in the IBC (L^{302}, I^{364}, L^{393}) appeared hereby essential.

The action of NCS-1 on the IP_3R forms a slightly different story. It co-immunoprecipitates with IP_3R1 and IP_3R2 in neuronal cells and in heart thereby stimulating IICR in a Ca^{2+}-dependent way [100, 101]. Interestingly, paclitaxel by binding to NCS-1 increases its interaction with IP_3R1 and so induces Ca^{2+} oscillations in various cell types [102, 103]. This Ca^{2+}-signaling pathway was proposed to lead to calpain activation and to underlie the origin of paclitaxel-induced peripheral neuropathy [104]. However, the interaction site of NCS-1 on the IP_3R, either direct or indirect, has not yet been identified.

Taken together these results confirm that Ca^{2+}-binding proteins interact in a complex way with the IP_3R and that the various Ca^{2+}-binding proteins have distinct, though sometimes overlapping, roles. The functional effect of CaM has been studied in detail and it appears to inhibit the IP_3R. The results described above support a view that the main action of CaM on the IP_3R is at the level of the suppressor domain. Indeed, apo-CaM can via its C lobe bind to the suppressor domain of all three IP_3R isoforms while a subsequent binding of the N lobe will depend on the Ca^{2+} concentration. The binding of CaM in that domain can disturb an intra-IP_3R interaction needed for IP_3R function and therefore inhibits IICR. This behavior can be particularly important in cells having high CaM expression levels, as for example Purkinje neurons that also demonstrate high levels of IP_3R1. In that case, CaM was proposed to be responsible for suppressing basal IP_3R activity [81]. Moreover, as the intracellular distribution of CaM can depend on intracellular Ca^{2+} dynamics, it was also hypothesized that it allows IP_3R regulation is a non-uniform way [84]. Additionally, it should be emphasized that CaM can act on other Ca^{2+}-transporting proteins in the cell, like the RyR [105], the plasma membrane Ca^{2+} ATPase [106] and various plasma membrane Ca^{2+} channels including voltage-operated Ca^{2+} channels and transient receptor potential channels [107, 108]. In all these cases CaM tends to inhibit Ca^{2+} influx into the cytosol (inhibition of IP_3Rs, RyRs and plasma membrane Ca^{2+} channels) while promoting Ca^{2+} efflux out of the cell (stimulation of plasma membrane Ca^{2+} ATPase).

An IP_3R-inhibiting behavior can similarly be expected for CaM-related Ca^{2+}-binding proteins, though their interaction sites are not strictly identical to that of CaM. The binding site for NCS-1, which rather stimulates the IP_3R, is even still unknown. In comparison to CaM, CaBP1 demonstrates a much higher affinity for the IP_3R [99] and a higher specificity, as it does not affect the RyR [96]. In cells expressing CaBP1, the major control of IICR will therefore depend on the interaction of the IP_3R with CaBP1, while RyR activity will depend on 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.

10.4.2 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 to 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 (Fig. 10.2, indicated in green) thereby stimulating pro-survival Ca^{2+} oscillations [114, 115, 118]. Additionally, Bcl-2, and Bcl-XL with lesser affinity, also target the central coupling domain (a.a. 1389–1408 of IP_3R1; Figs. 10.1 and 10.2, 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 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; Figs. 10.1 and 10.2**,** 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 (Fig. 10.2, 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 domain can more easily bind to the central coupling domain. In addition, sequestering Bcl-2 proteins at the ER membrane via their transmembrane domain may increase their ability to interact with the C-terminus of the IP_3R (Fig. 10.2, indicated in green). As this C-terminal binding site seems to be located more at the inside of the IP_3R1 tetramer one can expect a local high concentration of Bcl-2 proteins to be necessary for this interaction. Besides directly modulating IICR, Bcl-2 can serve as an anchor for targeting additional regulatory proteins to the IP_3R. It was shown that Bcl-2 attracts dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) and calcineurin to the IP_3R thereby regulating the phosphorylation state of the latter and consequently its Ca^{2+}-release properties [125]. Finally, recent data indicate also for Bcl-2 an additional interaction site in the ligand-binding domain [126] highlighting the complexity of the interaction of the anti-apoptotic Bcl-2-family members with the IP_3R. Further research will be needed to unravel the precise function of each of these sites.

Another Bcl-2-family member that regulates the IP_3R is the zebrafish protein Nrz. The latter was shown to bind via its BH4 domain to the IBC of zebrafish IP_3R1, whereby E^{255} appeared essential for interaction (Fig. 10.1). Nrz prevents IP_3 binding to the IP_3R thereby inhibiting IICR [121]. Interestingly, although the Nrz BH4 domain is sufficient for interaction with the IP_3R, inhibition of IICR required the BH4-BH3-BH1 domains. Furthermore, phosphorylation of Nrz abolished its interaction with the IP_3R. Recently, Bcl-2-like 10, the human orthologue of Nrz, was shown, just like Nrz in zebrafish, to interact with the IBC, indicating a conserved function for this protein [122].

Besides anti-apoptotic Bcl-2-family members, also pro-apoptotic Bcl-2 proteins and other BH3 domain-containing proteins are known to target and regulate IP_3Rs. For instance, Bok, a pro-apoptotic Bcl-2-family member, interacts with the IP_3R (a.a. 1895–1903 of IP_3R1; Figs. 10.1 and 10.2) [127]. This interaction protects IP_3R1 and IP_3R2 from proteolytic cleavage by caspase 3 that results in a Ca^{2+} leak that may contribute to mitochondrial Ca^{2+} overload and thus apoptosis [128, 129]. Subsequent work demonstrated that the majority of all cellular Bok is bound to the IP_3R thereby stabilizing the Bok protein [130]. Unbound, newly synthesized Bok is rapidly turned over by the proteasome pathway. Both the association of mature Bok with the IP_3R and the rapid degradation of newly synthesized Bok by the proteasome restrict the pro-apoptotic functions of Bok thus preventing cell death induction.

From the above it is clear that the IP_3R is heavily regulated by both pro- and anti-apoptotic Bcl-2-family members. The occurrence of multiple binding sites for the same Bcl-2-family member further increases the complexity [112]. Furthermore, it should be stressed that the regulation of the IP_3R by Bcl-2 proteins is conserved during evolution. This is illustrated by the ability of the zebrafish Nrz protein to regulate IICR via its BH4 domain [121] and is further validated by the observation

that the BH4 domains of Bcl-2 derived from different vertebrates are able to inhibit IICR with similar efficiency [131]. The large number of both pro-and anti-apoptotic Bcl-2 proteins that regulate the IP_3R, targeting it at multiple sites, suggests that throughout evolution regulating IICR became an important functional aspect of the Bcl-2-protein family.

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

10.4.3 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 interacts 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.

10.4.4 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 Sect. 10.4.2), functionally and structurally interferes 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].

10.4.5 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 with 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.

10.4.6 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.

10.4.7 BRCA-Associated Protein 1 (BAP1) and the F-Box Protein FBXL2

Prolonged stimulation of 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.

10.5 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 relative 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.

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