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Tamas Balla
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Phosphoinositides II: The Diverse Biological Functions

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Phosphoinositides II: The Diverse Biological Functions

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Phosphoinositides II: The Diverse Biological Functions

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Preface

When I was approached to shape a book about phosphoinositide signaling, I first felt honored and humbled. On second thought, this appeared to be an impossible task. Phosphoinositides have grown from being just a curious lipid fraction isolated from bovine brain, showing increased radioactive metabolic labeling during intense stimulation protocols, to become the focus of immense interest as key regulatory molecules that penetrate every aspect of eukaryotic biology. The expansion of this field in the last three decades has been enormous: it turned from a basic science exercise of a devoted few to highly translatable science relevant to a large number of human diseases (isn't this the nature of good basic science?). These include cancer, metabolic-, immuno- and neurodegenerative disorders, to name just a few. Reviewing the large number of enzymes that convert phosphoinositides would fill a book—let alone the diverse biological processes in which phosphoinositides play key regulatory roles. Given the interest, a collection of up-to-date reviews compiled in a book is clearly warranted, which was enough to sway me to accept this assignment. As one editor is unable to handle this enormous task, I was delighted when Matthias Wymann and John York were kind enough to join me in this ambitious effort.

When thinking about potential authors, the obvious choice would have been to approach the people whose contributions have been crucial to push and elevate this field to the level it is today. Bob Michell, prophetically placed phosphoinositides in the center of signal transduction in a 1975 Biochem. Biophys. Acta review (Michell 1975), Michael Berridge had a key role in linking phosphoinositides and Ca^{2+} signaling and whose fascinating reviews have inspired many of us (Berridge and Irvine 1984). Robin Irvine, whose group found that InsP_3 was a mixture of two isomers, the active Ins(1,4,5)P_3 and an inactive Ins(1,3,4)P_3, and who described the tetrakisphosphate pathway (Irvine et al. 1986), and who always challenges us with most provocative ideas. Philip Majerus, who has insisted on the importance of inositide phosphatases (Majerus et al. 1999) very early on. The group of Lewis Cantley, with the discovery of PI 3-kinase activities and the mapping of downstream effectors (Whitman et al. 1988; Franke et al. 1997), or the Waterfield lab where the first PI 3-kinase catalytic subunit was isolated and cloned (Otsu et al. 1991; Hiles et al. 1992). Peter Downes, who recognized the translational value of phosphoinositide research. Jeremy Thorner and Scott Emr, whose work in baker's yeast still forms the

foundation of our understanding of the role of inositol lipids in trafficking (Strahl and Thorner 2007) or Pietro De Camilli, whose group documented the central role of inositides in brain and synaptic biology (Cremona et al. 1999). There are many others who made valuable or even greater contributions to phosphoinositide research. The above list reflects my bias, as these researchers had the largest impact on my thinking and the directions of my work. Research is, however, a constantly evolving process and we (now Matthias and John being involved) wanted to involve contributions of scientists who represent a second or third wave of researchers infected with the interest in phosphoinositides. We made an effort to recruit authors who have been trainees of these founding laboratories. With this selection our goal was to sample the view of the current and future generation. By selecting their trainees, we feel that we pay tribute to the "Founding Fathers", and show that the research they put in motion is alive and continues with fresh ideas, new ambitions and a translational and therapeutic value.

Phosphoinositide research in the 1980s went hand in hand with research on Ca^{2+}signaling pursued in "non-excitable" cells and was also marked with the discovery of the family of protein kinase C enzymes, regulated by diacylglycerol, one of the products of phosphoinositide-specific phospholipase C enzymes. These areas of research developed and expanded to form their own fields, and could not be discussed here in detail—even though they are linked historically to the development of phosphoinositide signaling. The enormous work of the groups of Yasutomi Nishizuka on protein kinase C, and Katsuhiko Mikoshiba on cloning and characterizing the Ins(1,4,5)P_3 receptors are prime examples of these achievements. Although we could not cover all these areas, we included a chapter on Ca^{2+} signaling via the Ins(1,4,5)P_3 receptor by Colin Taylor, a trainee of the Michael Berridge's lab, where important links between Ca^{2+} release and Ins(1,4,5)P_3 receptor signaling were discovered. We also decided to allocate some space to inositol phosphates, the soluble counterparts of some of the phosphoinositides. These molecules for long had been viewed only as the metabolic products of the second messenger Ins(1,4,5)P_3 but recently gained significant prominence as regulators of important physiological processes. With the discovery of the highly phosphorylated and pyrophosphorylated inositols and the enzymes that produce them, it became clear that this system represents a whole new regulatory paradigm with exciting new developments.

Finally, it was a difficult dilemma whether to include a Chapter on the early history of phosphoinositides. We decided against it for a number of reasons. First, the really interesting history is traced back to studies that preceded the landmark 1975 Bob Michell review and included the work of the Hokins (1987), Bernard Agranoff (2009) and other pioneers of phosphoinositide research. Nobody could tell these early developments better than Bob Michell in his several recollections (Michell 1995) or Robin Irvine who commemorated the 20 years of Ins(1,4,5)P_3 and the period leading to its discovery (Irvine 2003). We encourage the young readers to go back and read these recollections, as they show several examples of how seemingly uninspiring observations formed the beginning of something that became huge as it unfolded. What came after these landmark discoveries is so overwhelming that each one of us has own views and subjective memories and stories to tell on some aspects of

it. As Editors we felt that our views should not be elevated above others on these historical aspects, and leave it to the authors of the individual Chapters to elucidate the diversity in this respect. The only exception is a Chapter on the history of PI 3-kinases by Alex Toker that we felt deserves special emphasis as it had the most transforming impact on the field since the late 1980s.

One needs to understand that selection of authors is a subjective process and does not always reflect on who contributed the most in a selected field. However, we are confident that proper credit is given in the individual Chapters to each groups and individuals whose work has moved this field forward. It should also be understood that a field that generates over 10,000 entries in PubMed with each keyword that relates to phosphoinositides cannot be covered without missing some aspects that could be important. However, we trust that this collection will be found useful for both the experts and the novices.

References

Agranoff BW (2009) Turtles all the way: Reflections on myo-Inositol. *J Biol Chem* 284(32):21121–21126

Berridge MJ, Irvine RF (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312:315–321

Cremona O, et al (1999) Essential role of phosphoinositide metabolism in synaptic vesicle recycling. *Cell* 99:179–188

Franke TF, Kaplan DR, Cantley LC, Toker A (1997) Direct regulation of the Akt protooncogene product by PI3,4P2. *Science* 275:665–668

Hiles ID et al (1992) Phosphatidylinositol 3-kinase: structure and expression of the 110 kDa catalytic subunit. *Cell* 70:419–429

Hokin LE (1987) The road to the phosphoinositide-generated second messengers. *Trends Pharmacol Sci* 8:53–56

Irvine RF (2003) 20 years of Ins(1,4,5)P3, and 40 years before. *Nat Rev Mol Cell Biol* 4(7):586–590

Irvine RF, Letcher AJ, Heslop JP, Berridge MJ (1986) The inositol tris/tetrakis phosphate pathway—demonstration of inositol (1,4,5)trisphosphate-3-kinase activity in mammalian tissues. *Nature* 320:631–634

Majerus PW, Kisseleva MV, Norris FA (1999) The role of phosphatases in inositol signaling reactions. *J. Biol Chem* 274:10669–10672

Michell B (1995) Early steps along the road to inositol-lipid-based signalling. *Trends Biochem Sci* 20(8):326–329

Michell RH (1975) Inositol phospholipids and cell surface receptor function. *Biochim Biophys Acta* 415:81–147

Otsu M et al (1991) Characterization of two 85 kDa proteins that associate with receptor tyrosine kinases, middle-T/pp60c-src complexes, and PI3-kinase. *Cell* 65:91–104

Strahl T, Thorner J (2007) Synthesis and function of membrane phosphoinositides in budding yeast, Saccharomyces cerevisiae. *Biochim Biophys Acta* 1771(3):353–404

Whitman M, Downes CP, Keeler M, Keller T, Cantley L (1988) Type-I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* 332:644–646

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Abbreviations

AD	Alzheimer's disease
AMPK	5′-AMP-activated protein kinase
ALL	acute lymphocytic leukemia
ALS	amyotrophic lateral sclerosis
AML	acute myeloblastic leukemia
ARNO	Arf nucleotide binding site opener
ASK1	apoptosis signal-regulating kinase 1
ATM	ataxia telangiectasia mutated
ATX	arabidopsis trithorax 1
Bad	Bcl-XL/Bcl-2-associated death promoter
BAFF	B cell activation factor of the TNF family
BCR	B cell receptor
Bcr/Abl	break point cluster region/Abelson kinase fusion protein
Btk	Bruton's tyrosine kinase
c-Kit	stem cell growth factor receptor
CAD	caspase activated DNase
CCR(L)	C-C chemokine receptor (ligand) type
CDK	cyclin-dependent kinase
CDKN2A	cyclin-dependent kinase inhibitor 2A
CERT	ceramide transfer protein
CIN85	Cbl-interactin protein of 85kD (also Ruk (regulator of ubiquitous kinase), SETA (SH3 domain-containing gene expressed in tumorigenic astrocytes))
CML	chronic myeloid leukemia
CMT	Charcot-Marie-Tooth
COPI/II	coatomer protein complex I/II
CXCR(L)	C-X-C chemokine receptor (ligand) type
DAAX	death domain-associated protein
DAG	diacylglycerol
DGK	diacylglycerol kinase
DH	dbl-homology
DMSO	dimethyl sulfoxide

DNA-PK_{cs}	DNA-dependent protein kinase, catalytic subunit
DOCK2	dedicator of cytokinesis 2
Dpm1	dolichol phosphate mannosyltransferase
EGF(R)	epidermal growth factor (receptor)
eEF1A	eukaryotic elongation factor 1A
eIF4E	elongation initiation factor 4E
EMT	epithelial-to-mesenchymal transition
EnaC	epithelial sodium channel
ER	estrogen receptor, or endoplasmic reticulum
ErbB1	epidermal growth factor receptor
ERM	ezrin/radexin/moesin
FAK	focal adhesion kinase
FAPP1	phosphoinositol 4-phosphate adaptor protein 1
FAPP2	phosphoinositol 4-phosphate adaptor protein 2
FcεRI	high affinity receptor for Fc fragment of IgE
FOXO	forkhead transcription factor, class O
FYVE	Fab1, YOTB, Vac1, EEA-1 homology
G6P	glucose-6-phosphatase
Gab	Grb2-associated binder
GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
GIST	gastrointestinal stromal tumors
GK	glucokinase
GLUT4	glucose transporter type 4
GM-CSF	granulocyte and macrophage colony stimulating factor
GPCR	G protein-coupled receptors
GRK2	G protein-coupled receptor kinase 2 (also βARK1 (adrenergic receptor kinase 1)
Grp1	general receptor for phosphoinositides
GSK-3	glycogen synthase kinase-3
GST-2xFYVE	glutathione S-transferase-tagged to tandem FYVE domains
HAUSP	herpesvirus-associated ubiquitin-specific protease
Hdac2	histone deacetylase 2
HSCs	hematopoietic stem cells
IκBK	IκB kinase
ING2	inhibitor of growth protein 2
Inpp5e/INPP5E	72 kDa inositol polyphosphate 5-phosphatase
Ins	*myo*-inositol
IGF1(R)	insulin-like growth factor (receptor)
ILK	integrin-linked kinase
Ins(1,4)P_2	inositol 1,4-bisphosphate
Ins(1,4,5)P_3	inositol 1,4,5-trisphosphate; also used $InsP_3$
IPMK	inositol polyphosphate multikinase
IRS	insulin receptor substrate

ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibitory motif
JAK	Janus-activated kinase
JNK	Jun N-terminal Kinase
Kv1.3	Voltage-gated K^+ channel
LAT	linker for activation of T cells
LOH	loss of heterozygosity
LSCs	leukemic stem cells
LTP	long term potentiation
MAPK	mitogen-activated protein kinase
MAPKAP-2	mitogen-activated protein kinase-activated kinase 2
M-CSF	macrophage colony-stimulating factor
MDM2	murine double minute 2
MDS	myelodysplastic syndrome
MEFs	mouse embryonic fibroblasts
miRNA	microRNA
MPP(+)	1-methyl-4-phenylpyridinium iodide
MSN	medium sized spiny projection neurons
MTM	myotubularin
MTMR	myotubularin related
mTOR	mammalian target of rapamycin, see also TOR
MVB	multivesicular body
MVP	major vault protein
Nedd4	neural-precursor-cell-expressed developmentally down-regulated 4
NFκB	nuclear factor κB
NLS	nuclear localization signal
NMDA(R)	N-methyl-D-aspartate (receptor)
NOS3/eNOS	NO-synthase 3
NTAL	non-T cell activation linker, also named LAB (Linker of activation for B cells) or LAT2
OSBP	oxysterol binding protein
OCRL	oculocerebrorenal syndrome of Lowe
OGD	oxygen–glucose deprivation
PAO	phenyalrsine oxide
PCAF	p300/CBP-associated factor
PDE	phosphodiesterase
PDGF(R)	platelet-derived growth factor (receptor)
PDZ	post synaptic density protein, Drosophila disc large tumor suppressor, zonula occludens-1 protein
PDK1	phosphoinositide-dependent kinase 1
PEPCK	phosphoenolpyruvate carboxy kinase
PEST	proline, glutamic acid, serine, threonine
PH	plecktrin-homology
PHD	plant homeodomain
PH-GRAM	pleckstrin homology glucosyltransferase Rab-like GTPase activator

PHTS	PTEN hamartoma tumor syndrome
PI3K	phosphoinositide 3-kinase; catalytic subunits of class I PI3K are referred to as p110α, p110β, p110γ and p110δ
PI3Kc	PI3K catalytic domain
PI3Kr	PI3K regulatory subunit
PI4K	phosphatidylinositol 4-kinase
PI4KII	type II phosphatidylinositol 4-kinase
PI4KIII	type III phosphatidylinositol 4-kinase
PICS	Pten-loss–induced cellular senescence
PID	phosphoinositide interacting domain
PIKE	PI-3-kinase enhancer
PIKK	phosphoinositide 3-kinase-related kinase
PIP4K	phosphatidylinositol 5-phosphate 4-kinase (also called type II PIP kinase)
PIP5K	phosphatidylinositol 4-phosphate 5-kinase (also called type I PIP kinase)
PIPP	proline-rich inositol polyphosphate 5-phosphatase
PIX	PAK-associated guanine nucleotide exchange factor
PKA	protein kinase A
PKB/Akt	protein kinase B, also called Akt after the transforming kinase encoded by the AKT8 retrovirus
PKC	protein kinase C
PLC	phospholipase C
PLD	phospholipase D
PM	plasma membrane
PML	promyelocytic leukemia protein
PPI	polyphosphoinositide
pRB	retinoblastoma protein
PRD	proline-rich domain
P-Rex	PtdIns(3,4,5)P_3-dependent Rac exchanger
PSD95	post synaptic density protein 95
PtdIns	phosphatidylinositol
PtdIns4P	phosphatidylinositol 4-phosphate; short PIP
PtdIns3P	phosphatidylinositol 3-phosphate; PIP should not be used here
PtdIns5P	phosphatidylinositol 5-phosphate; PIP should not be used here
PtdIns(4,5)P_2	phosphatidylinositol 4,5-bisphosphate; short PIP_2
PtdIns(3,4)P_2	phosphatidylinositol 3,4-bisphosphate; the abbreviation PIP_2 should not be used here.
PtdIns(3,5)P_2	phosphatidylinositol 3,5-bisphosphate; the abbreviation PIP_2 should not be used here.
PtdIns(3,4,5)P_3	phosphatidylinositol 3,4,5-trisphosphate; short PIP_3
PtdOH	phosphatidic acid (also used PA)
PTEN	Phosphatase and Tensin homolog deleted on chromosome Ten, [also MMAC (mutated in multiple advanced cancers), TEP1 (TGF-β-regulated and epithelial cell enriched phosphatase 1)]

PX	Phox-homology
RAN	Ras-related nuclear protein
RID	Rac-induced recruitment domain
RNAi	ribonucleic acid interference
ROS	reactive oxygen species
RSK	ribosomal S6 kinase
R-SMAD	receptor regulated SMAD
RTK	receptor tyrosine kinase
Rb2	retinoblastoma-related gene $p130^{Rb2}$
RYR1	type 1 ryanodine receptor
Sac	suppressor of actin
SCIP	Sac domain-containing inositol phosphatases
SCV	*Salmonella*-containing vacuole
SGK	serum- and glucocorticoid-induced protein kinase
SH2	Src homology 2
SHIP	SH2 domain-containing inositol 5'-phosphatase
SID	Set interacting domain
siRNA	short-interfering RNA
SKICH	SKIP carboxy homology
SKIP	skeletal muscle and kidney enriched inositol phosphatase
SNP	single nucleotide polymorphism
SSC	squamos cell carcinoma
Star-PAP	poly(A) polymerase
Syk	spleen tyrosine kinase, member of the Src tyrosine kinase family
TAC	transverse aortic constriction
TDLU	terminal ductal lobuloalveolar units
TGFβ	transforming growth factor β
Tiam	T-lymphoma invasion and metastasis inducing protein
TNF	tumour necrosis factor
TopoIIα	topoisomerase IIα
TOR	target of rapamycin (also called FRAP or mTOR)
TPIP	TPTE and PTEN homologous inositol lipid phosphatase
TPTE	trans-membrane phosphatase with tensin homology
TRAPs	transmembrane adapter proteins, link immune-receptors to downstream signaling cascades. Examples: LAT, NTAL/LAB
TSC	tuberous sclerosis complex
UTR	untranslated region
Vps34p	vacuolar protein sorting mutant 34 protein
WASP	Wiskott Aldrich Syndrome protein
Wm	wortmannin
WT	wild type

Chapter 1
Ca^{2+} Signalling by IP_3 Receptors

Colin W. Taylor and David L. Prole

Abstract The Ca^{2+} signals evoked by inositol 1,4,5-trisphosphate (IP_3) are built from elementary Ca^{2+} release events involving progressive recruitment of IP_3 receptors (IP_3R), intracellular Ca^{2+} channels that are expressed in almost all animal cells. The smallest events ('blips') result from opening of single IP_3R. Larger events ('puffs') reflect the near-synchronous opening of a small cluster of IP_3R. These puffs become more frequent as the stimulus intensity increases and they eventually trigger regenerative Ca^{2+} waves that propagate across the cell. This hierarchical recruitment of IP_3R is important in allowing Ca^{2+} signals to be delivered locally to specific target proteins or more globally to the entire cell. Co-regulation of IP_3R by Ca^{2+} and IP_3, the ability of a single IP_3R rapidly to mediate a large efflux of Ca^{2+} from the endoplasmic reticulum, and the assembly of IP_3R into clusters are key features that allow IP_3R to propagate Ca^{2+} signals regeneratively. We review these properties of IP_3R and the structural basis of IP_3R behavior.

Keywords Ca^{2+} signalling · Ca^{2+} channel · Endoplasmic reticulum · IP_3 receptor

1.1 Introduction

As the breadth of these volumes confirms, phosphoinositides fulfill many and varied roles in the activities of all eukaryotic cells, whether from unicellular or multicellular organisms (Balla et al. 2011). Pathways for synthesis or uptake of *myo*-inositol, and for synthesis of phosphatidylinositol (PtdIns) appear to have evolved early in a common ancestor of archaea and eukaryotes. Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) and many other polyphosphoinositides, which are ubiquitous in eukaryotes, made their appearance soon after the divergence of eukaryotes and archaea (Michell 2008). The phosphoinositide-specific phospholipases C (PLC),

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which provide the only route to inositol 1,4,5-trisphosphate (IP_3) in animal cells (Irvine and Schell 2001), are also ancient, although the subtypes known to be regulated by extracellular stimuli appear to be a later invention and are restricted to multicellular animals (Michell 2008). This distribution is broadly similar to that of IP_3 3-kinases (Irvine and Schell 2001), a major route for inactivation of IP_3, and of IP_3 receptors (IP_3R). Genes encoding related IP_3R are probably found in all animals, including all vertebrates, many invertebrates, including arthropods, nematodes, coelenterates and even *Paramecium*, a unicellular ciliate; and in the unicellular green algae, *Chlamydomonas* (Wheeler and Brownlee 2008), but not in fungi or land plants (Krinke et al. 2007). The latter despite evidence that IP_3 can release Ca^{2+} from the intracellular stores of plants, within either the ER or vacuole (Krinke et al. 2007). The identity of the protein(s) through which IP_3 evokes Ca^{2+} signals in plants remains unknown. One possibility is that land plants lost genes encoding IP_3R (and ryanodine receptors) and re-invented intracellular IP_3-gated Ca^{2+} channels after their divergence from animals (Wheeler and Brownlee 2008). We can speculate that the vacuole of plants, with its membrane potential and steep electrochemical gradient for H^+, may have demanded evolution of IP_3R with greater selectivity for Ca^{2+} than is required for IP_3R within the ER of animal cells (Sect. 1.5).

Our focus here is on IP_3 and the receptors (IP_3R) through which it releases Ca^{2+} in animal cells, but there are many additional interactions between phosphoinositides and Ca^{2+} signalling. Examples of the web of interactions that entangle phosphoinositides and Ca^{2+} signalling include regulation of membrane trafficking and cytoskeletal organization by phosphoinositides (Di Paolo and De Camilli 2006); stimulation of ion channels and transporters, including Ca^{2+}-permeable channels and Na^+-Ca^{2+} exchangers, by PtdIns(4,5)P_2 (Suh and Hille 2008); the possibility that PtdIns(4,5)P_2 may be an antagonist of IP_3R (Lupu et al. 1998); and PtdIns(3,4,5)P_3-mediated activation of protein kinase B and thereby phosphorylation of IP_3R (Khan et al. 2006; Szado et al. 2008).

Use of phosphoinositides to control membrane trafficking by defining the identity of different membranes, and signalling via 3-phosphorylated phosphoinositides (Di Paolo and De Camilli 2006) probably evolved before the appearance of IP_3-mediated Ca^{2+} signalling (Michell 2008), but it was the latter that was first experimentally elucidated. A relationship between phospholipid turnover and secretion was first noted in 1953 (Hokin and Hokin 1953), but it was an influential review that first proposed a causal link between PLC-mediated hydrolysis of phosphoinosides and Ca^{2+} signalling (Michell 1975). The proposal initially envisaged a direct link between PLC activity and Ca^{2+} entry across the plasma membrane (Michell 1975), but it was later extended to Ca^{2+} release from intracellular stores. Indeed it is only very recently that the relationship between PLC, intracellular Ca^{2+} stores and Ca^{2+} entry has been resolved with the elucidation of the mechanisms linking empty Ca^{2+} stores to activation of store-operated Ca^{2+} channels in the plasma membrane (Park et al. 2009).

In the years following Michell's provocative review, and amidst some skepticism about the role of PLC in triggering Ca^{2+} signals, evidence progressively accumulated to support his insightful hypothesis (Michell 2009). Key breakthroughs included the

demonstration that PLC-mediated catalysis of PtdIns(4,5)P_2 (rather than PtdIns) was the first step in the signalling sequence (Berridge 1983; Michell et al. 1981); evidence from blowfly salivary gland that depletion of phosphoinositides reversibly abrogated 5-HT-evoked Ca^{2+} signals (Fain and Berridge 1979); a demonstration that the ER, rather than mitochondria, was the likely source of the Ca^{2+} released by PLC-linked receptors (Burgess et al. 1983); and finally, in 1983, proof that IP_3 stimulated Ca^{2+} release from a non-mitochondrial store in permeabilized pancreatic acinar cells (Streb et al. 1983). The latter paper—one of the most cited in biology—moved the field into new territory with countless groups rapidly confirming the findings in numerous cell types (Berridge 1987, 1993; Berridge and Irvine 1984). This was followed by purification and functional reconstitution of IP_3R (Ferris et al. 1989), cloning of three vertebrate IP_3R genes (Blondel et al. 1993; Furuichi et al. 1989; Mignery et al. 1989), and structural and functional analyses of IP_3R behavior. We focus on the latter in this review. Several of the key players have published personal perspectives of the historical development of our current understanding of the roles of IP_3R in Ca^{2+} signalling (Berridge 2005; Irvine 2003; Michell 2009; Mikoshiba 2007; Putney 1997), and there are recent reviews on many aspects of IP_3R (Choe and Ehrlich 2006; Foskett et al. 2007; Patterson et al. 2004; Serysheva 2010; Taylor and Dellis 2006; Taylor et al. 1999; Yule et al. 2010).

1.2 Ca^{2+} Is a Versatile and Ubiquitous Intracellular Messenger

At least three features of Ca^{2+} are likely to have contributed to the evolutionary steps leading to it becoming a ubiquitous intracellular messenger. First, it seems likely that as soon as early prokaryotes adopted a phosphate-based energy economy, there was a strong selective pressure to exclude Ca^{2+} from the cytosol to avoid precipitation of calcium phosphate (Kretsinger 1977). The result is that all eukaryotic cells now maintain a low free cytosolic Ca^{2+} concentration, typically about 100 nM. This they achieve by actively extruding Ca^{2+} from the cytosol across both the plasma membrane and the membranes of intracellular organelles. Among the latter, the endoplasmic reticulum (ER) is the most important and, at least in most cells, the major intracellular Ca^{2+} reservoir from which extracellular stimuli can release Ca^{2+}. The important point is that in all eukaryotic cells, the plasma membrane and the membranes of the ER separate the cytosol from much higher concentrations of Ca^{2+}. Both gradients are used to evoke rapid, and usually transient, increases in cytosolic Ca^{2+} concentration. Interactions between these sources of Ca^{2+} are also important. The second key feature of Ca^{2+} is its ability, in the presence of much higher concentrations of other cations, notably Mg^{2+}, to be recognized selectively and with appropriate affinity by simple protein folds—EF-hands are the most common (Celio et al. 1996). These Ca^{2+}-binding proteins provide the means of decoding cytosolic Ca^{2+} signals. Third, Ca^{2+} binds also with relatively low-affinity to countless cellular components—proteins, lipids, small organic anions, etc—causing it to diffuse in cytosol up to 100-times more slowly than expected for a small freely diffusing cation

(Allbritton et al. 1992). The result is that the increases in cytosolic Ca^{2+} concentration that result from opening of Ca^{2+}-permeable channels are, at least initially, restricted to volumes within tens of nm of the channel pore, where the free Ca^{2+} concentration (at least tens of μM) might be some 1000-times higher than that of the bulk cytosol (Shuai et al. 2006). The significant point is that Ca^{2+} signalling can be local, such that Ca^{2+} entering the cytosol via one channel may cause a local increase that is spatially distinct from that evoked by another channel. Different Ca^{2+} channels can thereby direct their Ca^{2+} to different intracellular targets (Berridge et al. 2003; Di Capite et al. 2009; Dick et al. 2008; Neher 1998; Willoughby and Cooper 2007).

That Ca^{2+} can function as either a local messenger, signalling only to proteins in close proximity to specific Ca^{2+} channels, or as a global messenger invading the cell adds enormously to its versatility as an intracellular messenger (Berridge et al. 2000). For intracellular Ca^{2+} channels, notably IP_3R and ryanodine receptors (RyR), the growth of Ca^{2+} signals from local events to larger ones depends on their ability to propagate Ca^{2+} signals regeneratively via Ca^{2+}-induced Ca^{2+} release. This regenerative capacity arises from three key attributes of both major families of intracellular Ca^{2+} channels, namely their permeability to Ca^{2+}, their stimulation by increases in cytosolic Ca^{2+} concentration and their organization into groups of channels spaced to allow effective Ca^{2+}-mediated communication between them. Other Ca^{2+} channels, such as the NAADP-regulated two-pore channels (TPC) in endosomes or lysosomes (Patel et al. 2010; Zhu et al. 2010), or voltage-gated Ca^{2+} channels in the plasma membrane (Berridge et al. 2003) can also provide the Ca^{2+} that recruits the activity of IP_3R or RyR. Here we concentrate on the interactions *between* IP_3R (Fig. 1.1).

Confocal microscopy, and more recently total internal reflection fluorescence microscopy (TIRFM) (Parker and Smith 2010), have shown that in intact cells IP_3-evoked Ca^{2+} signals are built from 'elementary events' with characteristics (durations, amplitudes and spatial spreads) that are relatively unaffected by IP_3 concentration (Rose et al. 2006). The smallest events, 'blips', are the Ca^{2+} signals that arise from opening of single IP_3R; they typically last ~ 10 ms and spread about ~ 500 nm from the source. Larger events, 'Ca^{2+} puffs', which are often preceded by a triggering Ca^{2+} blip (Rose et al. 2006), reflect the near-synchronous opening of several IP_3R within a cluster, as Ca^{2+} released by an active IP_3R very rapidly (< 20 ms) ignites the activity of its neighbors (Smith and Parker 2009) (Fig. 1.1). Although there is considerable variability, reflecting the stochastic recruitment of IP_3R, a typical puff might last ~ 100 ms and spread ~ 1 μm (Marchant et al. 1999; Thomas et al. 1998). The number of IP_3R contributing to a puff is unresolved, but on average it is probably no more than a handful; indeed with high-resolution TIRFM the falling phase of a puff often suggests the stepwise closure of about 5–6 IP_3R (Smith and Parker 2009). The shapes of individual blips and puffs are relatively insensitive to IP_3 concentration, but IP_3 increases the number of IP_3R ready to respond to Ca^{2+} (Sect. 1.4), thereby allowing Ca^{2+} signals to grow with increasing stimulus intensity. Hence, as the IP_3 concentration increases, Ca^{2+} signals grow from blips to puffs and then, as Ca^{2+} diffuses between puff sites, into regenerative Ca^{2+} waves that

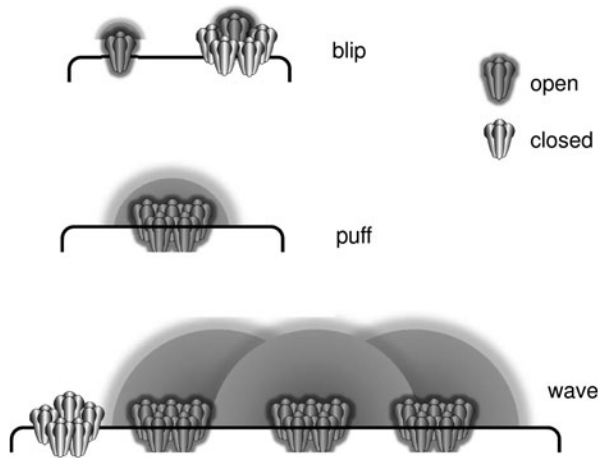

Fig. 1.1 Elementary Ca^{2+} release events mediated by IP_3 receptors. Ca^{2+} blips reflect the opening of single IP_3R, either lone IP_3R (*left*) or a single IP_3R opening within a cluster (*right*). IP_3 causes IP_3R to cluster (Sect. 1.7), and at higher IP_3 concentrations more IP_3R within a cluster are primed to respond to Ca^{2+}, allowing the near simultaneous opening of several IP_3R to give a Ca^{2+} puff. At still higher IP_3 concentrations, Ca^{2+} diffusing from one puff site can ignite the activity of a neighboring cluster of IP_3R to give a regenerative Ca^{2+} wave

can invade the entire cytosol (Bootman and Berridge 1995; Marchant et al. 1999). These Ca^{2+} waves viewed from the perspective of the entire cell appear as repetitive Ca^{2+} spikes, the frequency of which also increases with stimulus intensity (Berridge 1997; Woods et al. 1986).

Many studies, each with their strengths and limitations, suggest that most IP_3R are mobile within ER membranes (Chalmers et al. 2006; Cruttwell et al. 2005; Ferreri-Jacobia et al. 2005; Iwai et al. 2005; Tateishi et al. 2005; Tojyo et al. 2008; Wilson et al. 1998). Our fluorescence recovery after photobleaching (FRAP) analyses of IP_3R1 and IP_3R3, for example, suggest that each IP_3R moves freely within the ER membrane (Pantazaka and Taylor 2011), and our patch-clamp analyses of the same IP_3R in the nuclear envelope likewise suggests they are mobile (Rahman et al. 2009) (Sect. 1.7). But optical measurements of IP_3-evoked Ca^{2+} blips (Smith et al. 2009) and Ca^{2+} puffs (Smith and Parker 2009; Thomas et al. 2000; Tovey et al. 2001) suggest that the events repeatedly initiate at a limited number of fixed sites. How might these apparently conflicting observations be reconciled? A second issue relates to the scarcity of elementary events in cells. A typical mammalian cell, like the SHSY5Y neuroblastoma cells most used for TIRFM analyses of elementary Ca^{2+} release events (Parker and Smith 2010; Smith and Parker 2009; Smith et al. 2009), probably expresses about 10^4–10^5 IP_3R (Tovey et al. 2008). Yet there typically appear to be only 4–5 puff sites in each cell (Smith and Parker 2009), a scarcity that appears not to result from imaging only in the TIRF field (Parker and Smith 2010). Why should less than 1% of a cell's IP_3R contribute to puffs? We return briefly to these issues in Sect. 1.7.

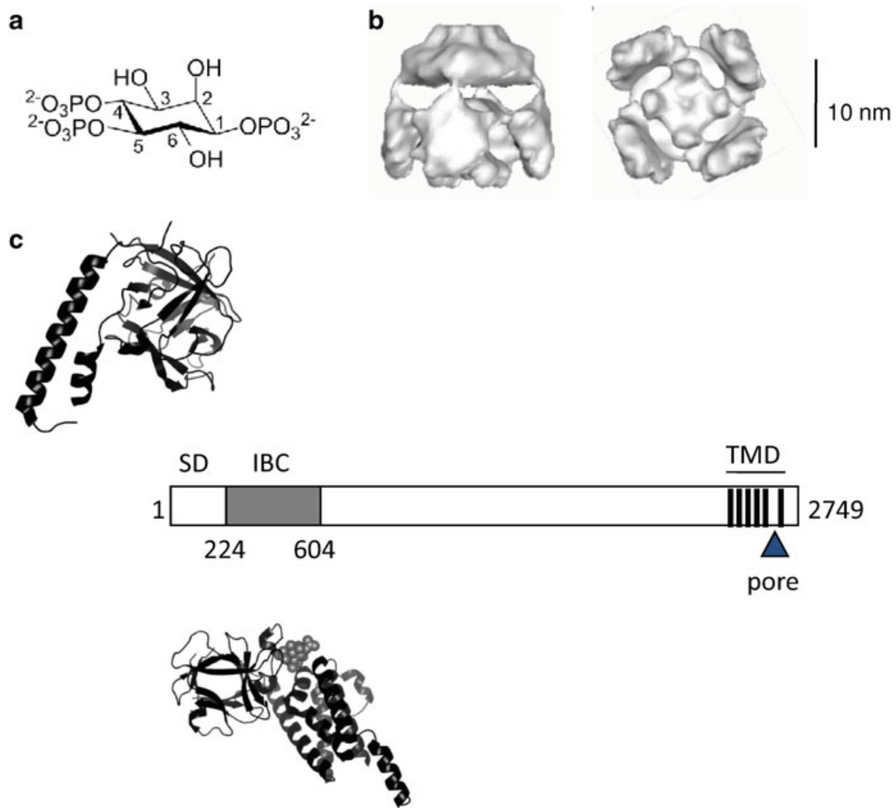

Fig. 1.2 Key structural features of IP_3 receptors. **a** Stucture of IP_3. **b** Low-resolution ($\sim$30 Å) 3D reconstruction of IP_3R1 derived from single particle reconstruction (da Fonseca et al. 2003). **c** Key regions of a single IP_3R subunit showing the structures of the IBC with IP_3 bound (Protein Data Base, 1N4K) (Bosanac et al. 2002) and of the SD of IP_3R1 (Protein Data Base, 1ZXX) (Bosanac et al. 2005)

1.3 IP_3 Receptors: An Overview

IP_3R are unusually large proteins, each assembled from four homologous subunits arranged around a central pore (Fig. 1.2b) (Taylor et al. 2004). Invertebrate IP_3R are homomeric, but for vertebrates, where there are three genes and multiple splice variants, functional IP_3R may be either homo- or hetero-tetrameric (Taylor et al. 1999). Each subunit comprises about 2700 residues. Although there are differences in the distribution and regulation of IP_3R subtypes, the similarities are generally more striking than the differences. We concentrate on the properties that are either known, or seem very likely, to be shared by all IP_3R.

The IP_3-binding core (IBC), which is entirely responsible for IP_3 recognition, lies towards the N-terminal of the sequence, preceded by the so-called ‘suppressor

domain' (SD). Both the IBC and SD are essential for the initial steps in IP_3R activation (Sect. 1.6). High-resolution structures of the IBC with IP_3 bound (Bosanac et al. 2002) and of the SD from IP_3R1 (Bosanac et al. 2005) and IP_3R3 (Chan et al. 2010) have been published (Fig. 1.2c). Towards the C-terminal, there are six predicted transmembrane domains (TMD) in each subunit (Fig. 1.2c). The last pair of TMD (TMD5-6) together with the intervening luminal loop from each of the four subunits assemble to form a central pore (Sect. 1.6). The TMD are also responsible for co-translational targeting of IP_3R to the ER (Joseph 1994; Pantazaka and Taylor 2010; Parker et al. 2004), they play a major role in oligomerization of IP_3R (Galvan et al. 1999), and they retain IP_3R within the ER (Parker et al. 2004). It is, however, noteworthy that although most IP_3R in most cells reside in ER membranes, IP_3 also stimulates Ca^{2+} release from the Golgi apparatus (Pinton et al. 1998), from within the nucleus (Echevarria et al. 2003; Gerasimenko et al. 1995; Marchenko et al. 2005) and perhaps also from secretory vesicles (Gerasimenko et al. 1996). In some cells, small numbers of IP_3R are also selectively targeted to the plasma membrane, where they contribute to the Ca^{2+} entry evoked by physiological stimuli (Dellis et al. 2006; Taylor et al. 2009).

As described in Sect. 1.2, the versatility of Ca^{2+} as an intracellular messenger derives largely from regulating the growth of Ca^{2+} signals from local to global events. For IP_3-evoked Ca^{2+} signals, this depends on the distribution of IP_3R, their Ca^{2+}-permeability and their co-regulation by IP_3 and Ca^{2+}. We therefore concentrate on these features in this short review. It is, however, important to note that there are many other facets to IP_3R behavior. They are, for example, modulated by many additional intracellular signals, like ATP (Betzenhauser et al. 2008), cAMP (Tovey et al. 2008, 2010) and redox state (Higo et al. 2005; Vais et al. 2010); they are substrates for a variety of proteins kinase, which also modulate their activity (deSouza et al. 2007; Fritsch et al. 2004; Szado et al. 2008; Wagner et al. 2008); and many proteins, including calmodulin (Taylor and Laude 2002), Bcl-2 (Li et al. 2007; Rong et al. 2008), presenilins (Cheung et al. 2010), $\beta\gamma$ subunits of G proteins (Zeng et al. 2003) and various luminal proteins (Choe and Ehrlich 2006; Higo et al. 2005) associate with and regulate IP_3R. These modulatory factors, which endow IP_3R with an ability to integrate diverse intracellular signals, effect their influence by tuning the sensitivity of IP_3R to IP_3 or Ca^{2+}, or the interactions between them. It is clear that IP_3R have considerable computational ability: detecting and processing diverse inputs before deciding whether to return them to the cytosol as a Ca^{2+} signal.

Opening of the pore, giving rise to a cytosolic Ca^{2+} signal, is the most important output from IP_3R, but they can also relay information directly to other proteins. IRBIT (IP_3R-binding protein released by IP_3), for example, is a small phosphoprotein that associates with IP_3R1 and appears then to dissociate after IP_3 binding (Ando et al. 2006), freeing IRBIT to then regulate other cellular activities, like Cl^- channels and Na^+-HCO_3^- exchangers in ion-transporting epithelia (Yang et al. 2009). In aortic smooth muscle, IP_3R1 interacts directly with Ca^{2+}-sensitive K^+ channels (BK_{Ca}) and, independent of a Ca^{2+} flux through the IP_3R, promotes their opening and consequent hyperpolarization of the plasma membrane (Zhao et al. 2010). IP_3R

have also been implicated in regulating Ca^{2+} entry via interactions with other Ca^{2+}-permeable channels in the plasma membrane (van Rossum et al. 2004) including some members of the transient receptor potential (trp) channel family (Kiselyov et al. 1999; Tang et al. 2001; Xi et al. 2008; Zhang et al. 2001). These suggestions remain contentious (DeHaven et al. 2009; Taylor et al. 2009).

1.4 Activation of IP_3 Receptors by Ca^{2+} and IP_3

The conformational changes in the IP_3R that lead to opening of its pore are initiated by IP_3 binding to the IP_3-binding core (IBC) (Fig. 1.2a, c). There have been reports of IP_3-independent activation of IP_3R by CaBP1 (Yang et al. 2002), a member of the neuronal Ca^{2+}-sensor family, and by Gβγ subunits of G proteins (Zeng et al. 2003), but the physiological relevance is unclear (Haynes et al. 2004; Nadif Kasri et al. 2004). The current consensus is that binding of IP_3 is essential to initiate activation of all IP_3R, but whether all four IP_3-binding sites of the tetrameric IP_3R must be occupied is unresolved. Positively cooperative responses to IP_3 in some (Dufour et al. 1997; Marchant and Taylor 1997; Tu et al. 2005a; Marchenko et al. 2005), but not all (Finch et al. 1991; Laude et al. 2005; Watras et al. 1991), studies and delays before the first response to IP_3 that decrease with increasing IP_3 concentration (Mak and Foskett 1994; Marchant and Taylor 1997) indicate that channel opening probably requires occupancy of several IP_3-binding sites. However, even heteromeric IP_3R in which at least one subunit has been mutated to prevent IP_3 binding can open in response to IP_3, suggesting that occupancy of fewer than four IP_3-binding sites may be sufficient to cause some gating (Boehning and Joseph 2000).

IP_3R subtypes differ in their affinities for IP_3; the consensus is that IP_3R2 is more sensitive than IP_3R1, and both are more sensitive than IP_3R3 (Iwai et al. 2007; Tu et al. 2005b). Within intact cells, however, differences in expression (Dellis et al. 2006; Tovey et al. 2010), distribution (Petersen et al. 1999), post-transcriptional and post-translational modifications, and association of IP_3R with accessory proteins (Patterson et al. 2004) are likely to be more important determinants of IP_3 sensitivity.

IP_3 is the only known endogenous small ligand of the IBC, but there are many synthetic agonists, all with structures equivalent to the equatorial 6-hydroxyl and the 4- and 5-phosphate groups of IP_3 (Rossi et al. 2010) (Fig. 1.2a). Because neither of the immediate products of IP_3 metabolism, IP_2 and IP_4, binds to the IBC, both metabolic pathways are effective means of inactivating IP_3. The structure of the IBC with IP_3 bound (Bosanac et al. 2002) shows IP_3 held in a clam-like structure with the phosphate groups of IP_3 coordinated by basic residues (Fig. 1.2b). The two sides of the clam, the α- and β-domains, form a network of interactions with the essential groups of IP_3. The 4-phosphate is hydrogen-bonded with residues in the β-domain, the 5-phosphate forms hydrogen bonds with residues that lie predominantly in the α-domain, and the 6-hydroxyl interacts with the backbone of a residue in the α-domain. It is easy to imagine how these interactions might allow IP_3 to pull the α- and β-domains together, causing the clam to close in a manner similar to glutamate

binding to ionotropic glutamate receptors (Mayer 2006). In the absence of a structure of the IBC without IP_3 bound, two lines of evidence lend circumstantial support to this proposal. First, the IBC adopts a more constrained structure when it binds IP_3 (Chan et al. 2007). Second, adenophostins, which are high-affinity agonists of IP_3R (Rossi et al. 2010), retain some activity after loss of the 3"-phosphate (analogous to the 5-phosphate of IP_3), probably because their adenine moiety interacts strongly with a residue in the α-domain and thereby partially mimics the clam-closure that would otherwise require binding of the 5-phosphate to the α-domain (Sureshan et al. 2009). We envisage, therefore, that when IP_3 binds to the IBC, the essential vicinal phosphate groups through their contacts with the α- and β-domains cross-bridge the two sides of the clam-like structure causing it to close and thereby initiate the processes that will culminate in opening of the pore (Sect. 1.6).

There are no *specific* antagonists of IP_3R, although with caution some antagonists can be useful (Michelangeli et al. 1995). Heparin is a competitive antagonist of IP_3 (Worley et al. 1987), but it is not membrane-permeant and it has many additional effects (Dasso and Taylor 1991; Ehrlich et al. 1994). 2-aminoethyl diphenylboronate (2-APB) is membrane-permeant and inhibits IP_3-evoked Ca^{2+} release without affecting IP_3 binding (Maruyama et al. 1997); its mechanism of action is unresolved. But 2-APB also inhibits Ca^{2+} uptake and many other Ca^{2+} channels and it is also a modulator of STIM (stromal interaction molecule) and so store-operated Ca^{2+} entry (Goto et al. 2010). Xestospongins are high-affinity membrane-permeant inhibitors of IP_3-evoked Ca^{2+} release that do not affect IP_3 binding (Gafni et al. 1997), but they too have side-effects (Solovyova et al. 2002). High concentrations of caffeine inhibit IP_3-evoked Ca^{2+} release (Parker and Ivorra 1991) without affecting IP_3 binding (Worley et al. 1987), but caffeine also stimulates RyR, inhibits cyclic nucleotide phosphodiesterases and interferes with many Ca^{2+} indicators. Membrane-permeant peptide antagonists of IP_3R may yet provide another source of selective antagonists (Sun and Taylor 2008).

Despite some initially conflicting evidence, it is now accepted that gating of all IP_3R is biphasically regulated by cytosolic Ca^{2+}. Modest increases in Ca^{2+} concentration rapidly potentiate responses to IP_3, while higher concentrations cause a slower inhibition (Finch et al. 1991; Iino 1987, 1990; Marshall and Taylor 1993; Parys et al. 1992; Foskett et al. 2007). Both elements of this Ca^{2+} regulation are widely invoked to explain both the recruitment of elementary Ca^{2+} release events by Ca^{2+}-induced Ca^{2+} release (Sect. 1.2 and 1.7) and to curtail the potentially explosive release of Ca^{2+} resulting from such positive feedback (Smith and Parker 2009).

IP_3 and Ca^{2+} are essential co-agonists of all IP_3R (Adkins and Taylor 1999; Finch et al. 1991; Foskett et al. 2007; Marchant and Taylor 1997; Taylor and Laude 2002), but the interplay between them that leads to channel opening remains incompletely understood. Foskett and colleagues argue from analyses of patch-clamp recordings of nuclear IP_3R that IP_3 decreases the sensitivity of the IP_3R to inhibition by cytosolic Ca^{2+} and that this alone is how IP_3 stimulates channel opening (Ionescu et al. 2006; Mak et al. 1998; Mak et al. 2001). This appealingly simple explanation, where IP_3 serves only to relieve tonic inhibition by resting Ca^{2+} concentrations, is difficult to reconcile with the observation that treatments that abolish Ca^{2+} inhibition do not

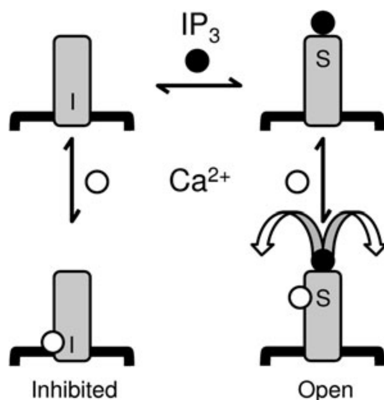

Fig. 1.3 Ca^{2+} regulation of IP_3 receptors. A simplified scheme for the interplay between IP_3 and Ca^{2+} binding is shown. IP_3 binding is proposed to cause both exposure of a stimulatory Ca^{2+}-binding site (*S*) (or massively to increase its affinity), while simultaneously causing an inhibitory Ca^{2+}-binding site (*I*) to be occluded (or massively to reduce its affinity). Opening of the IP_3R requires Ca^{2+} to bind to the stimulatory site

prevent IP_3 from activating IP_3R (Mak et al. 2003). Nor is the scheme compatible with evidence that nuclear IP_3R from cerebellar Purkinje cells show exactly the same biphasic Ca^{2+} concentration-dependence whether activated by low or saturating concentrations of IP_3 (Marchenko et al. 2005). The simple model was later elaborated to include at least three different Ca^{2+} sensors (Mak et al. 2003), but at the core of this revised scheme is a single Ca^{2+}-binding site that switches from being inhibitory in the absence of IP_3 to stimulatory in its presence (Mak et al. 2003). The core of this scheme is consistent with our initial model, derived from rapid superfusion analysis, which suggests that IP_3 both relieves Ca^{2+} inhibition and promotes binding of Ca^{2+} to a stimulatory site (Adkins and Taylor 1999; Marchant and Taylor 1997). The latter is essential for the channel to open. We, however, argue that the stimulatory and inhibitory Ca^{2+}-binding sites are distinct (Marshall and Taylor 1994). We suggest, therefore, that the essential role of IP_3 is to promote Ca^{2+} binding to a stimulatory Ca^{2+}-binding site. IP_3, by priming this site, allows Ca^{2+} to provide instantaneous control over whether the channel opens (Fig. 1.3).

Patch-clamp recordings from excised nuclear patches of insect cells in which the cytosolic surface is accessible to rapid changes in cytosolic IP_3 and Ca^{2+} concentration, have allowed further high-resolution analyses of the interplay between IP_3 and Ca^{2+} (Mak et al. 2007). The results reveal that activation of IP_3R is, as previously suggested (Adkins and Taylor 1999; Marchant and Taylor 1997), much slower than for other ligand-gated ion channels. The reasons for such slow conformational changes in the IP_3R as it passes though different closed states before opening, are unknown. The results also indicate that the interactions of Ca^{2+} with the IP_3R are faster than those with IP_3, consistent with rapid recruitment of IP_3-bound IP_3R by Ca^{2+} within elementary Ca^{2+} release events (Sect. 1.2). The authors claim, from their analyses of the latencies between rapid changes in IP_3 and/or Ca^{2+} concentration and

channel opening, that sequential binding of IP_3 and then Ca^{2+} (Marchant and Taylor 1997) is an unlikely explanation for IP_3R activation (Mak et al. 2007). However, the results from these challenging analyses include unexpected findings that are difficult to reconcile with any simple interactions between IP_3 and Ca^{2+} (Mak et al. 2007). We conclude that while IP_3 and Ca^{2+} are clearly both required for activation of IP_3R, no single scheme wherein IP_3 gates IP_3R solely by regulating Ca^{2+} binding to either an inhibitory or stimulatory site is entirely consistent with all published data.

The structural basis for Ca^{2+} regulation of IP_3R is unresolved. It may be direct, via Ca^{2+} binding to a site intrinsic to the IP_3R, or via an accessory Ca^{2+}-binding protein (Taylor et al. 2004). Stimulation of IP_3R by cytosolic Ca^{2+} is universally observed even with purified IP_3R reconstituted into lipid bilayers (Ferris et al. 1989; Hirota et al. 1995; Michikawa et al. 1999), suggesting that this essential Ca^{2+}-binding site probably resides within the primary sequence of the IP_3R. At least seven cytosolic Ca^{2+}-binding sites have been identified within IP_3R1 (Sienaert et al. 1996, 1997), but their physiological relevance is unresolved. Two sites (residues 304–381 and 378–450) are within the IBC, in which there are two surface-exposed clusters of acidic residues that overlap with residues in the second of these Ca^{2+}-binding regions. However, mutation of several of these acidic residues had no effect on Ca^{2+} regulation of IP_3R (Joseph et al. 2005). The remaining Ca^{2+}-binding sites fall within the central region of the IP_3R (Sienaert et al. 1996, 1997), but there is no evidence to link any of them directly to Ca^{2+} regulation of IP_3R. The only tangible link between specific residues and Ca^{2+} regulation comes from mutagenesis of a glutamate residue that is conserved in all IP_3R and RyR. Mutation of this residue (E2100 in IP_3R1) to another acidic residue (D) caused a $\sim$5–10-fold decrease in the Ca^{2+}-sensitivity of the IP_3R to both stimulation and inhibition, abolished oscillatory Ca^{2+} transients in response to agonist stimulation, and reduced the Ca^{2+}-binding affinity of a large fragment of the IP_3R that includes the residue (Miyakawa et al. 2001; Tu et al. 2003). A puzzling aspect of these results is the observation that mutation of a single residue similarly attenuated both stimulation and inhibition by Ca^{2+}, when other evidence suggests that the two effects are mediated by distinct sites (Hajnóczky and Thomas 1997; Marshall and Taylor 1994). This, together with the lack of direct evidence that Ca^{2+} is coordinated by the conserved glutamate, leaves open the possibility that rather than itself contributing to an essential Ca^{2+}-binding site, this residue may allosterically couple to the site.

It remains unclear whether inhibition of IP_3R by Ca^{2+} is mediated by Ca^{2+} binding directly to IP_3R or to an associated protein (Taylor and Laude 2002). The effects of Ca^{2+} on IP_3 binding differ between subtypes (Taylor and Laude 2002), purified IP_3R1 is not inhibited by Ca^{2+} (Benevolensky et al. 1994; Danoff et al. 1988; Lin et al. 2000; Richardson and Taylor 1993) and in some bilayer recordings of reconstituted IP_3R there is no Ca^{2+}-inhibition (Hagar et al. 1998; Michikawa et al. 1999; Ramos-Franco et al. 2000). These observations lend support to the idea that Ca^{2+} inhibition may be mediated by an accessory protein. However, deletion of the suppressor domain (SD, residues 1–223) of IP_3R1, which appears not to include a Ca^{2+}-binding site, abolishes inhibition of IP_3 binding by Ca^{2+} (Sienaert et al. 2002). This suggests that regulation by an accessory protein might require the SD. This is significant because

the conformational changes initiated by IP_3 binding to the IBC are proposed to both pass entirely via the SD (Rossi et al. 2009) (Sect. 1.6) and to regulate the behavior of an inhibitory Ca^{2+}-binding site (Adkins and Taylor 1999; Mak et al. 1998) (Fig. 1.3).

Calmodulin (CaM) was considered a candidate for the accessory protein through which Ca^{2+} inhibition is exercised, but that now seems improbable (Nadif Kasri et al. 2002; Taylor and Laude 2002). All IP_3R subtypes are inhibited by Ca^{2+}-CaM (Adkins et al. 2000; Hirota et al. 1999; Michikawa et al. 1999; Missiaen et al. 1999, 2000) and CaM has been shown to restore Ca^{2+} inhibition to purified IP_3R (Hirota et al. 1999; Michikawa et al. 1999; Nosyreva et al. 2002). But it has proven difficult to relate these functional effects of CaM to either its effects on IP_3 binding or to identified CaM-binding sites within IP_3R. CaM inhibits IP_3 binding to IP_3R1 in a Ca^{2+}-independent manner (Cardy and Taylor 1998; Patel et al. 1997), through a site that probably lies within the SD (Adkins et al. 2000; Sienaert et al. 2002), but the properties of this site are inconsistent with the ability of CaM to inhibit IP_3R function only in the presence of Ca^{2+}. There is a high-affinity Ca^{2+}-CaM-binding site within the central region of IP_3R1 and IP_3R2, but it is not present in IP_3R3 (Lin et al. 2000; Yamada et al. 1995). However, mutations that prevent Ca^{2+}-CaM binding to this site have no effect on Ca^{2+}-dependent inhibition of IP_3R (Nosyreva et al. 2002; Zhang and Joseph 2001). This evidence and the absence of the site in IP_3R3 suggest that the central Ca^{2+}-CaM-binding site cannot be responsible for Ca^{2+} inhibition of IP_3R. An additional high-affinity Ca^{2+}-CaM-binding site is created in IP_3R1 after removal of the S2 splice region. This may increase the Ca^{2+}-CaM sensitivity of peripheral $S2^-$ IP_3R1, but it is not a universal candidate for mediating Ca^{2+} inhibition of IP_3R (Islam et al. 1996; Lin et al. 2000).

Recently it was suggested that bound CaM is essential for IP_3R function because a peptide antagonist of CaM inhibited IP_3-evoked Ca^{2+} release (Nadif Kasri et al. 2006). It is now clear that this peptide acts directly on IP_3R with no requirement for CaM (Sun and Taylor 2008). While this eliminates an *essential* role for tethered CaM in activating IP_3R, it raises the intriguing possibility that an endogenous CaM-like structure might be essential for IP_3R activation (Sun and Taylor 2008). In summary, all IP_3R subtypes are inhibited by Ca^{2+}-CaM, but the molecular basis of this inhibition has not been established. The site through which Ca^{2+} inhibits IP_3R remains unresolved, but it is unlikely to be CaM.

Whether Ca^{2+} also regulates IP_3R from the luminal surface is another unresolved issue. Ca^{2+} release by RyR terminates before Ca^{2+} stores are entirely depleted because luminal Ca^{2+} is required to maintain RyR activity (Györke and Györke 1998; Jiang et al. 2008; Launikonis et al. 2006), possibly via its interaction with calsequestrin, a luminal high-capacity Ca^{2+}-binding protein (Launikonis et al. 2006; Terentyev et al. 2006). A similar scheme has been proposed to account for the initiation of Ca^{2+} release after the quiescent interval between repetitive Ca^{2+} spikes (Berridge 2007) and for 'quantal Ca^{2+} release' via IP_3R (Muallem et al. 1989). The latter describes the situation wherein after stimulation with sub-maximally effective concentrations of IP_3, unidirectional Ca^{2+} efflux from intracellular stores terminates before they have fully emptied (Bootman et al. 1992; Brown et al. 1992; Combettes et al. 1992; Ferris et al. 1992; Hirota et al. 1995; Meyer and Stryer 1990; Muallem

et al. 1989; Oldershaw et al. 1991; Taylor and Potter 1990). The proposal is that luminal Ca^{2+} sets the gain on the regulation by cytosolic IP_3 and Ca^{2+}, so that as the luminal free Ca^{2+} concentration falls it causes the sensitivity of the IP_3R to IP_3 to fall until, as Ca^{2+} leaks from the ER, the IP_3R closes despite the continued presence of cytosolic IP_3 and residual Ca^{2+} within the ER (Irvine 1990). Conversely, as stores refill between Ca^{2+} spikes in an intact cell, the model predicts that the sensitivity of the IP_3R increases until it exceeds the threshold at which prevailing cytosolic IP_3 and Ca^{2+} concentrations become sufficient to trigger opening. Despite the appeal of the model, evidence that luminal Ca^{2+} directly regulates IP_3R is not yet wholly convincing.

Stores have been shown to become more sensitive to IP_3 as they load with Ca^{2+} in some studies (Combettes et al. 1996; Horne and Meyer 1995; Missiaen et al. 1992, 1994; Nunn and Taylor 1992; Oldershaw and Taylor 1993; Parys et al. 1993; Tanimura and Turner 1996; Yamasaki-Mann et al. 2010), but not in others (Combettes et al. 1992, 1993; Shuttleworth 1992; van de Put et al. 1994). But even the supportive results do not generally eliminate the possibility that the increased sensitivity to IP_3 comes from having Ca^{2+} pass through active IP_3R and increase their sensitivity from the cytosolic surface (Laver 2009; Marchenko et al. 2005). An exception that provides direct evidence for regulation of IP_3R by luminal Ca^{2+}*per se* is provided by work where buffering of luminal Ca^{2+} attenuated IP_3-evoked Ca^{2+} release under conditions where feed-forward regulation via a cytosolic Ca^{2+}-binding site was unlikely (Caroppo et al. 2003). In bilayer recordings of IP_3R1, where essential accessory proteins may be lost, luminal Ca^{2+} either failed to potentiate responses to IP_3 (Bezprozvanny and Ehrlich 1994) or inhibited them (Thrower et al. 2000). Despite the caveats, regulation of IP_3R by luminal Ca^{2+} deserves serious consideration. A high-affinity Ca^{2+}-binding site within the luminal loop linking TMD5 and 6 (Sienaert et al. 1996) contains conserved acidic residues that could mediate luminal Ca^{2+} regulation, although the sub-μM affinity of this site for Ca^{2+} would be poorly suited to detecting likely changes in luminal Ca^{2+} concentration. Luminal accessory proteins, akin to those that regulate RyR, are another possibility, with ERp44 being one candidate. ERp44 belongs to the thioredoxin protein family and regulates IP_3R1 in a pH- and luminal Ca^{2+}-dependent manner (Higo et al. 2005). Binding of ERp44 to the TMD5-6 loop of IP_3R1 inhibits channel activity, and the interaction is disrupted by high concentrations of Ca^{2+}, consistent with the suggestion that luminal Ca^{2+} might enhance IP_3R activity.

To summarize, IP_3 works by tuning the Ca^{2+} sensitivity of the IP_3R, although the details are not resolved. We propose that IP_3 stimulates Ca^{2+} binding to a stimulatory site and inhibits Ca^{2+} binding to an inhibitory site (Fig. 1.3). Binding to the former is the trigger for opening of the pore. Others suggest that IP_3 works solely by preventing Ca^{2+} from binding to an inhibitory site. The identity of neither Ca^{2+}-binding site is known: the stimulatory site probably resides within the IP_3R, but the inhibitory site may require an accessory protein, though this is unlikely to be CaM. Luminal Ca^{2+} may further tune the sensitivity of the IP_3R to regulation by its cytosolic ligands, but this remains unproven.

1.5 Structure and Function of the IP_3 Receptor Pore

Although commonly referred to as 'intracellular Ca^{2+} channels', IP_3R are only modestly selective for bivalent over monovalent cations (P_{Ca}/P_K or P_{Ba}/P_K ~ 6–8) (Dellis et al. 2006; Foskett et al. 2007; Mak and Foskett 1994; Marchenko et al. 2005). Within the ER, where Ca^{2+} is probably the only permeant ion with an appreciable electrochemical gradient, there is no need for IP_3R to discriminate between cations. Ion selectivity is delegated to the SR/ER Ca^{2+}-ATPase (SERCA), the Ca^{2+} pump that creates the Ca^{2+} concentration gradient across the ER membrane. Indeed within the ER, the ability to conduct K^+ may allow IP_3R to mediate both Ca^{2+} release into the cytosol and the retrograde movement of K^+ required to maintain electroneutrality and so sustain high rates of Ca^{2+} release (Gillespie and Fill 2008). The weak cation selectivity of IP_3R might therefore be viewed as an adaptation to allow rapid rates of Ca^{2+} release. Within the plasma membrane, where IP_3R are expressed in some cells (Dellis et al. 2006; Kuno and Gardner 1987; Tanimura et al. 2000), the situation is different and opening of such a relatively non-selective cation channel would be expected to cause both Ca^{2+} entry and depolarization.

Although parallel studies of each of the IP_3R subtypes suggest that each has similar ion selectivity and conductance (Tu et al. 2005b), there is considerable, and largely unexplained, variation in published values for the single-channel conductance of IP_3R. One point, however, is clear: all IP_3R have large conductance for monovalent cations (up to ~ 500 pS) (Boehning et al. 2001a, b; Dellis et al. 2006; Ionescu et al. 2006; Mak and Foskett 1998; Marchenko et al. 2005; Perez et al. 1997; Ramos-Franco et al. 2000) and smaller, though still large, conductance for bivalent cations (up to ~ 80 pS) (Watras et al. 1991). We need, therefore, to understand how IP_3R allow cations to pass selectively and rapidly through the pore.

IP_3R lacking TMD1-4 assemble to form constitutively active channels that are insensitive to IP_3, but with appropriate permeation and conduction properties (Ramos-Franco et al. 1999). This demonstrates that the single ion-conducting pore, almost certainly lying at the centre of the tetrameric IP_3R, is formed by the TMD5-6 region (Fig. 1.4a). This is consistent with mutations within this region affecting conductance and/or ion selectivity (Boehning et al. 2001b; Dellis et al. 2006, 2008; Schug et al. 2008). Because available 3D reconstructions of the IP_3R are not yet sufficient to resolve details of the pore (Taylor et al. 2004), our present understanding of its structure is inferred from comparisons with K^+ channels and RyR. Each of these tetrameric channels is thought to share a pore structure formed by two TMD from each subunit cradling a selectivity filter, but the K^+ channels for which there are high-resolution structures (eg, KcsA, KirBac1.1 and MthK) have minimal sequence similarity with IP_3R or RyR. Indeed even RyR, the closest relatives of IP_3R, while sharing some sequence similarity with IP_3R within this region, differ from them in both the length and primary sequence of the TMD5-6 region (Fig. 1.4a). High resolution maps (~ 10 Å) of RyR1 come close to revealing the likely secondary structure of the pore (Ludtke et al. 2005; Samso et al. 2005, 2009). This region appears to have six α-helices contributed by each of the four subunits (Samso et al. 2009), and along the central axis there is a luminal constriction (probably the selectivity filter) and

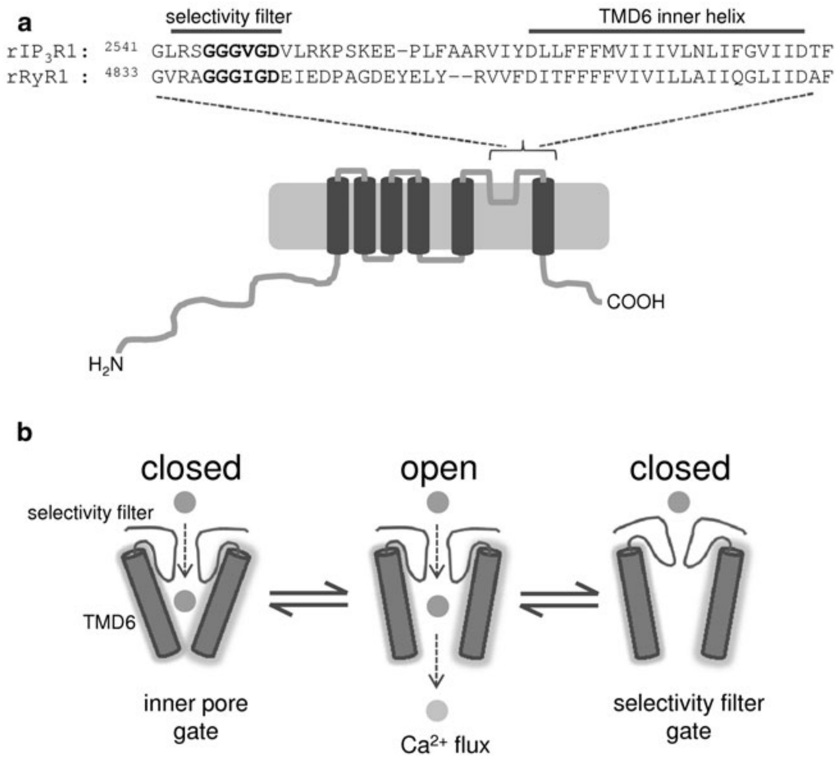

Fig. 1.4 Structure of the pore of the IP_3 receptor. **a** Alignment of the selectivity filter and TMD6 of rat IP_3R1 (ACT21453.1) and RyR1 (XP_001078539). **b** Schematic of the IP_3R pore showing TMD6 from two of the four subunits. Gates located within the inner pore (*left*) or selectivity filter (*right*) my prevent ion flow until the channel opens (*centre*)

a tepee-like assembly of four inner helices (probably TMD6) with the apex pointing into the cytoplasmic structure. In all likelihood the IP_3R forms a similar overall structure.

The only clear sequence similarity between K^+ channels, RyR and IP_3R is that known to form the selectivity filter in K^+ channels (^{2545}GGGXGD^{2550} in IP_3R1, Fig. 1.4a). Mutations within this sequence are also consistent with its role as a selectivity filter in IP_3R. Mutation of D2550 abolishes Ca^{2+} conductance without affecting K^+ conductance (Boehning et al. 2001b; Dellis et al. 2006; van Rossum et al. 2004), although in the mutant, low concentrations of Ca^{2+} block the K^+ conductance (Dellis et al. 2008). Whether this acidic residue binds directly to Ca^{2+}, as occurs for voltage-gated Ca^{2+} channels (Yang et al. 1993), or whether it facilitates coordination of Ca^{2+} by neighboring carbonyl groups, as occurs for cyclic nucleotide-gated (CNG) channels and their bacterial relatives, NaK channels, is unknown (Alam et al. 2007). Mutation of other residues within the putative selectivity filter (G2545A, G2546A, G2547A, G2549C or G2549W of IP_3R1) abolished IP_3-evoked Ca^{2+} release (Schug et al. 2008), and the G2547A mutant substantially reduced the K^+ conductance (Dellis et al. 2006). This suggests that these residues also contribute to either ion-binding