Preface

The Annual Symposium of the Biochemical Society *Lipids, Rafts and Traffic* took place over 2 days at the BioScience2004 meeting in Glasgow, in July 2004. This book brings together many of the presentations from that meeting and provides a unique description of the endocytosis and exocytosis of proteins and lipids, focusing on the roles and mechanisms of action of lipid rafts and clathrin-coated vesicles in these processes. The roles of lipids and rafts in cell signalling and in disease states are also described.

The book begins with a discussion from David Stephens and colleagues of our current understanding of the mechanisms that link membrane traffic in the early secretory pathway to the microtubule cytoskeleton. In addition to a description of the roles of the different proteins involved in this process, the relative merits of the hypotheses to describe how directionality of vesicle movement is generated are contrasted, namely the 'tug-of war' and 'co-ordinated motor' theories. This is followed by a chapter from Christian Preisinger and Francis Barr describing the role of kinases, such as Cdk1–cyclin and the MAP kinases, in the regulation of Golgi function and re-organization during the cell cycle. In addition, the role of the mammalian Ste protein kinase family and protein kinase D in the establishment and maintenance of cell polarity and migration is examined.

In Chapter 3, Martin Lowe and colleagues turn our attention to phosphoinositide regulation of the formation of cargo vesicles at the *trans*-Golgi network with particular emphasis on PtdIns(4)*P*, which is enriched in the Golgi apparatus. They describe how low levels of another phosphoinositide, PtdIns(4,5)P_2, and its associated phosphatase OCLR1 are found on Golgi membranes and suggest mechanisms by which PtdIns(4,5)P_2 might play a role in membrane traffic. The next two chapters deal with the trafficking of proteins in polarized epithelial cells. Paula Urquhart and colleagues (Chapter 4) describe how N-glycans, rather than the glycosylphosphatidylinositol (GPI) anchor, are responsible for the apical targeting of membrane dipeptidase, while Ashley Toye (Chapter 5) shows that although some mutations in the kidney anion exchanger cause the protein to remain in the endoplasmic reticulum, other mutations misdirect the protein from the basolateral to the apical membrane due to the removal of a basolateral targeting motif.

Liz Smythe focuses our attention in Chapter 6 on clathrin-mediated endocytosis, and the roles that dynamin I has in constricting the neck of the vesicle and the adaptor protein AP-2 has in stimulating cargo sequestration into coated pits.

In Chapter 7, our view of clathrin-mediated endocytosis is challenged by Tom Kirchhausen and colleagues, who summarize their data obtained from live

imaging of fluorescently tagged clathrin or its adaptor protein AP-2. Analysis of the results suggests that there are no preferred nucleation sites for endocytosis and that assembly of coated pits aborts unless cargo is captured. They propose a new model of endocytosis, termed the 'exploratory model'. Continuing the theme of vesicle fusion or formation, in the next chapter Paul Luzio and colleagues describe how, using live imaging of cells pre-loaded with Rhodamine–dextran, and then exposed to Oregon Green–dextran, both 'kissing and running' and 'explosive' fusion events contribute to the maturation of endosomes to lysosomes, but not vesicle trafficking between the organelles. Using a cell-free assay system, they describe how lysosomes fuse with endosomes to form a hybrid organelle and that different SNAREs, VAMP 7 (vesicle-associated membrane protein 7) and VAMP 8, are required for heterotypic endosome–lysosome fusion and homotypic endosome fusion respectively.

The next two chapters deal with endocytosis, with reviews by Karen Smillie and Michael Cousin on the role of dynamin I phosphorylation in the control of synaptic vesicle endocytosis, and by Jeremy Simpson and Arwyn Jones on the role of the Rab proteins in endocytosis. The former describes the identification of cyclin-dependent kinase and calcineurin as the proteins important in regulating the phosphorylation of dynamin, and discusses the possible consequences of these processes, with respect to changing the interaction of dynamin I with other proteins and lipids. The latter concentrates on Rab5, Rab21 and Rab22, which localize to the early endocytic pathway, and examines their roles in this process, and highlights the difficulties in dissecting the functions of these widely expressed proteins.

The evidence that the reggie proteins 1 and 2 (otherwise known as the flotillins) form intracellular non-caveolar lipid rafts, contained within the inner leaflet of the lipid bilayer, is presented by Claudia Stuermer and Helmut Plattner in Chapter 11. These rafts are thought to function as scaffolds for the assembly of signalling complexes containing kinases and other membrane proteins.

In Chapter 12, Tamara Golub and Pico Caroni describe the role of plasmalemmal rafts and actin regulation in neurons, with particular focus on PtdIns(4,5)P_2-rich rafts that accumulate at the leading edge of motile lamellipoda and promote actin assembly. In Chapter 13, Maria Dolores Ledesma and Carlos Dotti describe work showing that the low level of plasmin observed in brains from Alzheimer's disease patients is due to decreased binding of its precursor plasminogen to rafts, resulting in reduced metabolism of amyloid-β peptide. They also challenge the currently accepted stance concerning cholesterol and Alzheimer's disease by claiming that increasing cholesterol levels actually blocks amyloid-β production. Endocytosis and retrograde transport in neurons are the subject of the next chapter by Katrin Deinhardt and Giampietro Schiavo. They describe how tetanus and botulinum toxins bind to different arrays of gangliosides, GPI-anchored and transmembrane proteins in order to enter cells, and that tetanus toxin is internalized via clathrin-coated pits. Once internalized, the endocytic vesicles are transported retrogradely along the axon in a microtubule -dependent manner. Anja Becher and Jeff McIlhinney discuss the consequences of lipid raft association on G-protein-coupled receptor function in Chapter 15, and Eva Janas and colleagues describe that the B-cell antigen CD20, which is involved

in store-operated calcium entry, does not normally reside in rafts, but that on cross-linking with therapeutic antibodies moves into rafts and that this affects its activity (Chapter 16).

The biophysics (and sociology!) of ceramides and how short- and long-chain ceramides differently affect the curvature of the membrane and flip-flop transverse motion across the bilayer are the subject of Chapter 17 by Felix Goñi and colleagues. The theme of proteins binding to the surface of membranes is the focus of Chapter 18 by Stuart McLaughlin and colleagues, who describe how proteins with clusters of basic residues interact with acidic phospholipid head-groups in a reversible manner mediated through calmodulin, thereby affording a mechanism of regulation. In Chapter 19, Paula Booth and colleagues describe the translocation of the cell-penetrating Tat peptide across artificial bilayers and into living cells. Jurate Kazlauskaite and Teresa Pinheiro then describe experiments using recombinant prion protein and lipid vesicles to investigate the role of lipids in the conversion and pathogenesis of the prion protein. In the final chapter, Jennifer Gallop and Harvey McMahon describe how endophilin 1 and other proteins containing BAR domains, through binding selectively to curved membranes, may be involved in mediating clathrin-coated pit formation.

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Abbreviations

A568	Alexa Fluor 568
Aβ	β-amyloid peptide
AAK1	adaptor-associated kinase 1
AD	Alzheimer's disease
ADCC	antibody-dependent cell cytoxicity
AE1	anion-exchanger 1
AKAP	A-kinase-anchoring protein
ANTS	8-aminonaphthalene-1,3,6-trisulphonic acid
AP	adaptor protein
AP180	assembly protein 180
ApoE4	apolipoprotein E4
APP	amyloid precursor protein
APR	array of pre-synaptic receptors
AQP-2	aquaporin-2
ARAP1	ARF-GAP, Rho-GAP, ankyrin repeat and PH domain-containing protein 1
ARF	ADP-ribosylation factor
ARH	autosomal recessive hypercholesterolaemia
Arp	actin-related protein
AT_1	angiotensin II type 1 receptor
AT_2	angiotensin II type 2 receptor
BAPTA	1,2-bis[2-aminophenoxy]ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid
BAR	bin, amphiphysin and Rvs161/167
BCR	B-cell receptor
BDNF	brain-derived neurotrophic factor
BicD	Bicaudal D
BIG2	brefeldin-A-inhibited guanine nucleotide-exchange factor 2
BoNT	botulinum neurotoxin
BRAP	breast-cancer-associated protein
BSE	bovine spongiform encephalopathy
CaM	calmodulin
CAP	c-Cbl-associated protein
CAP23	cortical cytoskeleton-associated protein of 23 kDa
CCPA	2-chloro-N^6-cyclopentyladenosine
CCR5	chemokine receptor 5
Cdc	cell-division control
Cdk	cyclin-dependent kinase

CEACAM1-L	long isoform of carcinoembryonic antigen-related cell-adhesion molecule 1
CFTR	cystic fibrosis transmembrane conductance regulator
CGN	*cis*-Golgi network
CHO	Chinese-hamster ovary
CIN	Cbl-interacting protein
CJD	Creutzfeldt–Jakob disease
CK	casein kinase
CLEM	correlative live cell and electron microscopy
CMV	cytomegalovirus
CNT	clostridial neurotoxin family
COPI	coatamer protein I
COPII	coatamer protein II
CPP	cell-penetrating peptide
cryo-EM	cryo-electron microscopy
CSF	cerebrospinal fluid
CWD	chronic wasting disease
DAG	diacylglycerol
DAPI	4,6-diamidino-2-phenylindole
DEPE	dielaidoyl PE
DHC	dynein heavy chain
7-DHC	7-dehydrocholesterol
DIC	dynein intermediate chain
DIV	days *in vitro*
DLC	dynein light chain
DLIC	dynein light intermediate chain
DMPC	dimyristoyl phosphatidylcholine
dn	dominant-negative
DOPC	1,2-dioleoyl-*sn*-3-glycerophosphocholine
DPPC	dipalmitoyl phosphatidylcholine
DPX	p-xylenebispyridinium bromide
DRM	detergent-resistant membrane
dRTA	distal renal tubular acidosis
DSC	differential scanning calorimetry
dynactin	dynein activator
EEA1	early endosome antigen 1
EGF	epidermal growth factor
EGFP	enhanced green fluorescent protein
EH	eps1S homology
EHNA	erythro-9-(2-hydroxy-3-nonyl) adenine
EM	electron microscopy
eps15	EGF receptor pathway substrate 15
ER	endoplasmic reticulum
ERAD	ER-associated degradation
ERK	extracellular-signal-regulated kinase
ERM	ezrin, radixin and moesin
ESCRT	endosomal sorting complexes required for transport

F-actin	filamentous actin
FAK	focal adhesion kinase
FAPP	four-phosphate-adapter protein
FcεRIβ	β-subunit of the high-affinity IgE receptor
FCS	fluorescence correlation spectroscopy
FGF	fibroblast growth factor
FM 4-64	*N*-(3-triethylammoniumpropyl)-4-{6-[4-(diethylamino)phenyl]hexatrienyl} pyridinium dibromide
Fmoc	fluoren-9-ylmethoxycarbonyl
FRET	fluorescence resonance energy transfer
FSH	follicle-stimulating hormone
GAP	GTPase-activating protein
GAP-43	growth-associated protein 43
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDI	GDP-dissociation inhibitor
GDNF	glial-derived neurotrophic factor
GED	GTPase effector domain
GEF	guanine nucleotide-exchange factor
GFP	green fluorescent protein
GFRα1	GDNF-family receptor α1
GGA	Golgi-localized, γ-ear-containing, ARF-binding protein
GluT	glucose transporter
GM130	Golgi matrix protein of 130 kDa
GnRH	gonadotropin-releasing hormone
GPI	glycosylphosphatidylinositol
GPBP	Goodpasture antigen-binding protein
GPCR	G-protein-coupled receptor
GRASP	Golgi reassembly stacking protein
GRK5	G-protein-coupled receptor kinase 5
GST	glutathione S-transferase
HA	haemagglutinin
H_C	non-toxic fragment of tetanus neurotoxin
H_{CC}	C-terminal domain of H_C
H_{CN}	N-terminal domain of H_C
HEK-293	human embryonic kidney
Hsc70	heat-shock cognate 70
HTm4	haematopoietic transmembrane-4 protein
IAF	5-iodoacetamidofluorescein
IDE	insulin-degrading enzyme
IgCAMs	Ig superfamily cell-adhesion molecules
IPTG	isopropyl β-D-thiogalactoside
IrreC-rst	irregular chiasm C roughest
JNK	c-Jun N-terminal kinase
KAP	kinesin-associated protein
KDELR	KDEL (Lys-Asp-Glu-Leu) receptor
kAE1	kidney isoform of AE1
LAT	linker for activation of T-cells

LCa	light chain A
LDL	low-density lipoprotein
LH	luteinizing hormone
LIM	Lin-11, Isl-1 and Mec-3
Lis1	lissencephaly 1
LM	light microscopy
LUV	large unilamellar vesicle
MacMARCKS	macrophage myristoylated alanine-rich C kinase substrate
MAL	myelin and lymphocyte protein
MAPK	mitogen-activated protein kinase
MARCKS	myristoylated alanine-rich C kinase substrate
MDCK	Madin–Darby canine kidney
MDP	membrane dipeptidase
MEK	MAPK/ERK kinase
MHCI	major histocompatibility complex class I
MLK	mixed-lineage kinase
MMP9	matrix metalloproteinase 9
MN	motor neuron
MPR	mannose 6-phosphate receptor
MS4A	membrane-spanning 4A
MST4	mammalian Ste20 4
MT	microtubule
MVB	multivesicular body
N-BAR	N-terminal domain of BAR
NCAM	neural cell-adhesion molecule
NGF	nerve growth factor
NLS	nuclear localization signal
NMDA	*N*-methyl-D-aspartate
NMJ	neuromuscular junction
NPC	Niemann–Pick type C
NRK	normal rat kidney
NSF	*N*-ethylmaleimide-sensitive factor
Nudel	NudE-like
N-WASP	neural Wiskott–Aldrich syndrome protein
OCRL	oculocerebrorenal syndrome of Lowe
OSBP	oxysterol-binding protein
$p75^{NTR}$	p75 low-affinity NGF receptor
PA	phosphatidic acid
PAI	plasminogen-activator inhibitor
PAK	p21-activated kinase
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEG	poly(ethylene glycol)
PH	pleckstrin homology
PI3K	phosphoinositide 3-kinase
PI4K	phosphoinositide 4-kinase
PI5K	phosphoinositide 5-kinase

PI4P5K	phosphoinositide 4-phosphate 5-kinase
PICK-1	protein interacting with protein kinase C
PIPK1γ	phosphoinositide phosphate kinase 1γ
PIPLC	phosphatidylinositol-specific phospholipase C
PITP	phosphatidylinositol-transfer protein
PKA	protein kinase A (or cAMP-dependent protein kinase)
PKC	protein kinase C
PKD	protein kinase D
PLC	phospholipase C
PLD	phospholipase D
Plk	polo-like kinase
POPC	1-palmitoyl-2-oleoyl phosphatidylcholine
POPG	1-palmitoyl-2-oleoyl phosphatidylglycerol
PP	protein phosphatase
PRD	proline-rich domain
PRK	PKC-related kinase
PrP	prion protein
α-PrP	PrP with high α-helix content
β-PrP	PrP with high β-sheet content
PrP*	PrP with altered conformation
PrP^{c}	cellular PrP
PrP^{Sc}	disease-specific PrP
PS	phosphatidylserine
PSKH1	protein serine kinase H1
PTB	phosphotyrosine-binding domain
PTD	protein transduction domain
PX	phox homology
RabF	Rab-specific motif
RabSF	Rab-specificity-determining region
REP	Rab-escort protein
Rh	rhodamine
RNAi	RNA interference
ROCKII	Rho-associated, coiled-coil-containing protein kinase 2
R-SNARE	arginine-containing SNARE
SAO	Southeast Asian ovalocytosis
SAV	sucrase-isomaltase-carrying vesicle
SERCA	sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase
SH3	Src homology 3
SHP-2	Src-homology-2-domain-containing tyrosine phosphatase
siRNA	small intefering RNA
SNAP	soluble NSF-attachment protein
SNARE	soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor
SnS	sticks and stones
Snx1	sorting nexin 1
SOCC	store-operated calcium channel
SV	synaptic vesicle

SV40	simian virus 40
SVE	SV endocytosis
SYBL1	synaptobrevin-like 1
Tat	*trans*-activating transcription factor
TCR	T-cell receptor
TeNT	tetanus neurotoxin
tER	transitional ER
TGN	*trans*-Golgi network
TI-VAMP	toxin-insensitive VAMP
TM	transmembrane
$T_{1/2}$	midpoint transition temperature
$\Delta T_{1/2}$	endotherm width at mid-height
T_m	melting temperature
tPA	tissue-type plasminogen activator
TRPC1	transient receptor potential canonical 1
TSE	transmissible spongiform encephalopathy
t-SNARE	target SNARE
uPA	urokinase-type plasminogen activator
VAMP	vesicle-associated membrane protein
VIP36	vesicular integral protein 36
Vps	vacuolar protein sorting
VSV-G	G glycoprotein of the vesicular stomatitis virus
VTC	vesicular tubular cluster
WASP	Wiskott–Aldrich syndrome protein
WIPI49	WD40 repeat protein interacting with phosphoinositides of 49 kDa
YFP	yellow fluorescent protein
Ypt	yeast protein transport
YSK1	yeast Sps1/Ste20-related kinase 1
ZO-1	zona occludens 1 protein