


Structure and Function in
Cell Signalling

John Nelson



 WILEY

Contents

Acknowledgments	xvii
Preface	xix
1 The components and foundations of signalling	1
1.1 Definition of terms used	2
1.1.1 First messengers	2
1.1.2 Glands and types of secretion	2
1.1.3 Ligands	4
1.1.4 Agonists	4
1.1.5 Antagonists	5
1.1.6 Receptors for first messengers	6
1.1.7 Second messengers	8
1.1.8 Soluble second messengers	9
1.1.9 Membrane-bound second messengers	9
1.2 Historical foundations	12
1.2.1 When did the discipline of cell signalling begin?	12
1.2.2 The discovery of 'hormones' – Bayliss and Starling, 1902	13
1.2.3 The discovery of insulin and the beginning of endocrine therapy – Banting and Best, 1921	14
1.2.4 Peptide sequencing – Fred Sanger, 1951	14
1.2.5 Discrimination of beta- and alpha-adrenergic responses – Ahlquist, 1948	15
1.2.6 'Acrasin' = cAMP – the ancient hunger signal	15
1.3 Early milestones in signal transduction research	16
1.3.1 Cell-free experiments and the discovery of cAMP – Sutherland, Rall and Berthet, 1957	16
1.3.2 Fluoride – a stimulator of G proteins	16
1.3.3 ATP and subcellular fractionation	17
1.3.4 Heat-stable factor – cAMP	17
1.3.5 The problem with rats	18
1.3.6 The discovery of hormonally regulated protein kinases – phosphorylase kinase, serine phosphorylation and Ca^{2+} – Krebs and Fischer, 1958–1968	18
1.3.7 Discovery of calcium as activator of phosphorylase kinase	19
1.3.8 cAMP-dependent protein kinase	19
1.4 The discovery of receptors and G proteins	20
1.4.1 Radioligand receptor assays prove receptors are discrete entities	20

1.4.2	Oestrogen receptor directly detected by radioligand binding assays – Jensen and Gorski, 1962	20
1.4.3	Purification of the β -adrenergic receptor – Caron and Lefkowitz, 1976	21
1.4.4	The discovery of G proteins. Guanine nucleotides, fluoride and aluminium – Gilman and Rodbell, 1971–1983	21
1.4.5	Magnesium	21
1.4.6	High and low glucagon affinities	22
1.4.7	GTP (contaminant of ATP) lowers 7-pass receptor affinity	22
1.4.8	GTP analogues and adenylyl cyclase activation	23
1.4.9	cAMP toxicity and clonal mutants of S49 cells	24
1.4.10	Aluminium is needed for fluoride activation of G proteins	25
1.4.11	Use of bacterial toxins	25
1.4.12	The calcium signal	26
1.5	cAMP pathways	26
1.5.1	A simple mammalian signalling pathway – F-2,6-bisP as a second messenger	26
1.5.2	PFK-1 and FBP-1	28
1.5.3	PFK-2/FBP-2 – a ‘tandem’ enzyme	29
1.5.4	Control of PFK-2/FBP-2 by phosphorylation – liver	29
1.5.5	Control of PFK-2/FBP-2 by phosphorylation – heart	30
1.5.6	F-2,6-bisP in tumours	32
1.6	cAMP: ancient hunger signal – primitive signalling in amoebazoans and prokaryotes	32
1.6.1	Slime moulds	32
1.6.2	cAMP and <i>E. Coli</i>	34
	References	35
2	Enzymes and receptors – quantitative aspects	39
2.1	Enzyme steady state assays – Michaelian enzymes	39
2.1.1	How are enzymes assayed?	40
2.1.2	Steady state	40
2.1.3	K_M – the Michaelis-Menten constant	42
2.1.4	V_{max} is reached when the enzyme becomes saturated	42
2.1.5	What does the K_M mean?	43
2.1.6	Non-Michaelian enzymes – G proteins and Ras	45
2.1.7	Non-Michaelian enzymes – cooperativity and allostery	45
2.2	Receptor equilibrium binding assays	45
2.2.1	Equilibrium	45
2.2.2	K_D – the dissociation constant	47
2.2.3	B_{max} – the maximum binding capacity is a ‘count’ of the receptors in a sample	47
2.2.4	The meaning of K_D	49
2.2.5	Displacement assays	50

2.3	The receptor's environment	51
2.3.1	Heterogeneity of binding sites – positive cooperativity	52
2.3.2	Binding site heterogeneity – two site models versus negative cooperativity	53
2.3.3	Negative cooperativity of the insulin receptor or two site model	55
2.3.4	Site heterogeneity of the EGF receptor – independent two site model?	57
2.4	Guanine nucleotides and the agonist 'affinity-shift' of 7-pass receptors	59
2.4.1	The ternary complex 'equilibrium' model	60
2.4.2	The 'empty pocket' form of $G\alpha$	62
2.4.3	The thermodynamic 'catalytic' or 'kinetic' model	62
2.4.4	Constitutive signalling in the absence of ligand	64
2.4.5	The effect of limiting concentrations of G protein on agonist binding	65
2.4.6	Agonist binding in membrane preparations where the cognate G protein is in unlimited supply	65
2.4.7	<i>In vivo</i> GTP versus GDP concentrations	66
	References	67
3	Modules and motifs in transduction	71
3.1	Src homology domains	72
3.1.1	Src-homology-1 (SH1) region represents the tyrosine kinase domain	72
3.1.2	Src-homology-2 (SH2) modules are phosphotyrosine-binding domains	73
3.1.3	Src-homology-3 (SH3) modules are polyproline-binding domains	75
3.1.4	Src-homology-4 (SH4) motif and Src 'unique domain'	78
3.1.5	The C-terminal Src regulatory motif and Src family autoinhibition	82
3.2	PH superfold modules: PH-, PTB- and PDZ-domains	85
3.2.1	PH domains – phosphoinositide lipid-binding modules, or $G\beta/\gamma$ -interacting modules	86
3.2.2	PTB domains – phosphotyrosine binding modules	86
3.2.3	PDZ domains – C-terminal (and C-terminal-like) peptide binding modules	88
3.3	Bcr-homology (BcrH) domains	88
3.4	Dbl homology (DH) domains – partners of PH domains	89
3.5	Bcl-2 homology (BH) domains	90
3.6	Ras binding domains	90
3.7	Phosphoserine/phosphothreonine-binding domains	92
3.7.1	14-3-3 proteins	93

3.7.2	Forkhead-associated domains	95
3.8	EF-hands – calcium-sensing modules	95
3.9	C1 and C2 domains – a Ca^{2+} -activated, lipid-binding, module	96
	References	97
4	Protein kinase enzymes – activation and auto-inhibition	101
4.1	The protein kinase fold	102
4.1.1	Invariant residues	102
4.1.2	The phosphate-binding loop or ‘p-loop’	104
4.1.3	Critical differences between serine/threonine kinases and tyrosine kinases	107
4.1.4	Closed and open conformations	108
4.1.5	The catalytic loop or ‘C-loop’	109
4.1.6	The activation segment/loop or ‘A-loop’	112
4.2	Protein kinases activated by A-loop phosphorylation	113
4.2.1	Phosphorylation of A-loop residues and assembly of active site	114
4.2.2	The A-loop and catalysis – transition state and site closure	115
4.2.3	ATP binding	115
4.2.4	A-loop and autoinhibition	116
4.3	The insulin receptor kinase (IRK) – a ‘gated’ kinase	116
4.4	Cyclin dependent kinases	119
4.4.1	Monomeric Cdk2 structures	120
4.4.2	Cyclin-bound unphosphorylated Cdk	123
4.4.3	Cyclin-bound phosphorylated Cdk	123
4.5	Secondary inhibition mechanisms – PKA	124
4.5.1	Substrate or pseudosubstrate binding to the catalytic cleft of PKA	126
4.5.2	The extended binding surface of R_{SUB}	126
4.5.3	Effects on cAMP binding at CBD-A	129
	References	131
5	7-pass receptors and the catabolic response	133
5.1	7-pass receptor phylogeny	134
5.2	Functional mechanisms of 7-pass receptors	134
5.2.1	$G_{\alpha s}$ -coupling receptors – glucagon- and β -adrenergic receptors – stimulation of cAMP production	135
5.2.2	$G_{\alpha q}$ -coupling receptors – bombesin- and $\alpha 1$ -adrenergic receptors – stimulation of calcium release from the endoplasmic reticulum	135
5.2.3	$G_{\alpha i}$ -coupling receptors – somatostatin and $\alpha 2$ -adrenergic receptors – inhibition of adenylyl cyclase, activation of K^+ ion channels, inhibition of Ca^{2+} channels, and activation of phospholipase $\text{C}\beta 2$	136

5.2.4	Glucagon- and β -adrenergic-receptors – the catabolic cAMP-dependent protein kinase (PKA) pathway leading to glycogenolysis	137
5.3	Amplification	137
5.3.1	Stimulated changes in cytosolic cAMP concentration	139
5.3.2	'Spare receptors' – maximum signal transduction from partial occupancy of receptors	139
5.3.3	Collision coupling <i>versus</i> pre-coupling	139
5.3.4	How much cAMP is needed?	139
5.3.5	The PKA 'cascade'	140
5.4	Adenylyl cyclase – signal limitation	140
5.4.1	Adenylyl cyclase – signal termination and PDE isoforms	140
5.4.2	Crosstalk and negative feed back	141
5.5	Adenylyl cyclase isoforms	141
5.5.1	Transmembrane isoforms of adenylyl cyclase	141
5.6	G proteins and the adenylyl cyclase effector isoforms	143
5.6.1	G _s -coupling catabolic receptors	148
5.6.2	β -Adrenergic/glucagon-receptor stimulation of glycogenolysis	148
5.6.3	β -Adrenergic/glucagon-receptor inhibition of glycogen synthesis	148
5.6.4	α 1-adrenergic receptor stimulation of glycogenolysis	148
5.6.5	Diffusible cascade or scaffolded pathway	149
5.7	Regulatory subunits of PKA and A-Kinase Anchoring Proteins	149
5.7.1	RII regulatory subunits – reversible phosphorylation and scaffolding	150
5.7.2	Segregation of pathways	153
5.7.3	PKA and its inhibitors	154
5.8	Phosphorylase kinase	155
5.8.1	PhK structure	155
5.8.2	The catalytic γ -subunit of PhK	156
5.8.3	Regulatory subunits	156
5.8.4	PhK substrates and autophosphorylation sites	157
5.8.5	Possible mechanisms of activation and holoenzyme conformation	157
5.9	Glycogen phosphorylase	160
5.9.1	Glycogen phosphorylase isoforms	160
5.9.2	Glycogen phosphorylase allosteric sites	161
5.9.3	Control by hormones or metabolite effectors – functional differences between muscle and liver isoforms	162
5.9.4	How do these properties of GP isoforms fit with metabolic necessities?	163
5.9.5	Structural changes induced by GP activating signals	165
5.9.6	T-state to R-state transition	165

5.9.7	Activation by phosphorylation	165
5.9.8	Activation by 5'-AMP	168
5.9.9	Inhibition by glucose	169
5.10	Glycogen synthase	169
5.10.1	GSK-3 – a multi-tasking enzyme	170
5.11	Remaining questions – scaffolds and alternate second messenger 'receptors'	171
5.11.1	Protein kinase C	171
5.11.2	Lipid activation of PKC – DAG-binding isoforms are also activated by phorbol esters	172
5.11.3	Alternative DAG/phorbol ester receptors	172
5.11.4	PKC scaffolds	173
5.11.5	What does PKC actually do?	173
5.11.6	Alternate cAMP receptors	174
5.12	G protein coupled receptor kinases – downregulators, signal integrators	174
	References	175
6	Single pass growth factor receptors	179
6.1	Receptor tyrosine kinases – ligands and signal transduction	179
6.1.1	RTK ligands and receptors	180
6.2	The PDGFR family – signal transduction	181
6.2.1	PDGFR signal transduction particle	182
6.2.2	MAP kinases and MAPK kinases	184
6.2.3	PDGFR kinase insert tyrosines – PI-3-kinase, and Ras <i>versus</i> Rac	186
6.2.4	PDGFR, PI-3-kinase, Ras and mitosis	187
6.2.5	PDGFR, PI-3-kinase, Rac and motility	187
6.2.6	PDGFR insert phosphotyrosines and Ras regulators	188
6.2.7	Sos-1 – a bi-functional guanine nucleotide exchange factor (GEF)	189
6.2.8	Sos – the switch from RasGEF to RacGEF	190
6.2.9	PDGFR C-terminal tail tyrosines	192
6.2.10	Alternative Grb-2 docking sites: SHP-2 and Shc	192
6.2.11	Shc	192
6.2.12	PLC γ	193
6.2.13	PDGFR Juxtamembrane tyrosines	193
6.3	PDGFR family autoinhibition: juxtamembrane and A-loop tyrosines	194
6.3.1	PDGFR juxtamembrane and A-loop tyrosines \rightarrow phenylalanines	194
6.3.2	PDGFR juxtamembrane (Y-Y \rightarrow A-A) mutant unresponsive to ligand	195
6.3.3	PDGFR Y579/581F is stuck in an autoinhibited state	195

6.3.4	PDGFR A-loop (Y → F) mutant cannot bind exogenous substrate polypeptides	195
6.3.5	PDGFR juxtamembrane and A-loop tyrosines → alanines	196
6.3.6	PDGFR Y579/581A is constitutively active	196
6.4	Crystal structure of kinase domain of PDGFR family-A member: Flt-3	197
6.4.1	Flt-3 juxtamembrane interactions and autoinhibition/activation	197
6.5	The ErbB family	200
6.5.1	EGFR family members and ligands	200
6.6	ErbB-type receptor signal transduction particles	202
6.6.1	The epidermal growth factor receptor kinase – a pre-assembled active site	204
6.7	Autoinhibition of EGFR and activation	205
6.7.1	Ligand binding, dimerisation and activation	206
6.7.2	The EGFR juxtamembrane domain – a nexus for crosstalk	207
6.7.3	EGFR activation and calcium	209
	References	211
7	G proteins (I) – monomeric G proteins	215
7.1	Classification	216
7.2	ON and OFF states of Ras-like proteins	217
7.3	Raf – a multi-domain serine/threonine kinase family of Ras effectors	218
7.3.1	Raf-Ras binding – translocation of Raf from cytosol to membrane	219
7.3.2	cAMP inhibition of cell division <i>via</i> sequestration of Raf	221
7.3.3	Raf activation by translocation	221
7.3.4	B-Raf is less stringently inhibited than C-Raf	222
7.3.5	Homologous or heterologous trans-autophosphorylation	223
7.3.6	Erk-1/2-type MAPK pathway activation	223
7.3.7	MAPK scaffolds	223
7.3.8	Signal termination	224
7.3.9	Other activating signals for Raf	225
7.4	Ras protein structure and function	225
7.4.1	The GTPase site of Ras: G-boxes and switch regions	226
7.4.2	The P-loop (G-1)	226
7.4.3	Switch I (G-2)	228
7.4.4	Switch II (G-3)	230
7.5	The switch mechanism: hydrolysis-driven conformational change in Ras	231
7.6	GTP hydrolysis	232
7.6.1	Structural effects of loss of γ -phosphate	234
7.7	Effector and regulator binding surfaces of Ras	234
7.7.1	RasGAP	234

7.7.2	RasGEFs	236
7.7.3	The Ras effector region and Raf binding	239
7.7.4	Rap1 and cAMP effects	240
	References	241
8	G proteins (II) – heterotrimeric G proteins	245
8.1	Classification and structural relationship with Ras	246
8.2	G α -subunits: the Ras-like core, G-boxes and switch regions	249
8.2.1	The P-loop	250
8.2.2	Switch I/G-2	250
8.2.3	Switch I/insert-1: a tethered GTPase-activating protein (GAP)	253
8.2.4	Switch II/G-3	253
8.2.5	Switch III	254
8.3	GTP exchange, hydrolysis and switch movements	254
8.3.1	GTP conformations	255
8.3.2	The transition state	256
8.4	β/γ - and receptor-binding surfaces of α -subunits	256
8.4.1	The β/γ binding site of GDP-occupied α	256
8.4.2	The receptor-binding interface of GDP-occupied G proteins	257
8.4.3	Receptor-induced GDP dissociation and nucleotide exchange	259
8.4.4	Switch II helix rotation	259
8.4.5	Switch II – the primary effector-binding surface of α -subunits	262
8.5	Modulators of G protein activity – the ‘RGS’ protein family	263
8.5.1	RGS proteins and GTPase activation	263
8.5.2	RGS proteins: inhibition of nucleotide exchange; crosstalk with other pathways	264
8.5.3	G α i/o/q GEF proteins – unrelated to RGS	265
8.5.4	GRKs – RGS domain-containing S/T-kinases	265
8.6	Signal transduction by β/γ subunits	266
	References	268
9	The insulin receptor and the anabolic response	271
9.1	The insulin receptor – a pre-dimerised RTK with a unique substrate	271
9.1.1	Insulin receptor residues numbering	273
9.1.2	Three clusters of autophosphorylated tyrosines in the InsR intracellular region	273
9.2	InsR and IGF-IR: differentiation leads differential tissue effects	275
9.3	Features of metabolic control in key tissues	276
9.4	InsR downstream signalling pathways	277
9.4.1	MAPK/p90Rsk pathway only mediates growth effects	277
9.4.2	PI-3-kinase is the prime anabolic effector – is there a second (non-MAPK) anabolic pathway: (CAP-Cbl-Crk)?	278

9.5	The insulin receptor substrate – a surrogate signal transduction particle	278
9.5.1	IRS protein targetting	279
9.5.2	IRS-interacting proteins – Class 1A PI-3-kinases	280
9.6	IRS-1/2 phosphorylation and PI-3-kinase activation	280
9.7	Protein phosphatase-1 (PP-1)	281
9.7.1	Glycogen granule targetting of PP-1	281
9.7.2	p70Rsk – inducer of GS dephosphorylation?	282
9.8	Insulin reverses effects of adrenaline and/or glucagon	283
9.8.1	Insulin's reversal of adrenaline-induced glycogenolysis in muscle	283
9.8.2	Insulin's reversal of adrenaline- and glucagon-induced glycogenolysis in liver	283
9.8.3	Insulin's reversal of adrenalin/glucagon-induced lipolysis in adipose tissue	285
9.9	PIP3 downstream effects – glycogen synthesis	285
9.9.1	PKB and GSK-3 inactivation	286
9.9.2	PKC- ζ – negative feedback control	287
9.9.3	PIP3 downstream effects – GLUT4 mobilisation	287
9.10	Many questions remain	289
9.10.1	Insulin activates the Erk1/2 MAPK pathway – why, then, is the insulin receptor not as mitogenic as the PDGF receptor	289
9.10.2	PDGR- β activates PI-3-kinase but does not exert anabolic effects like the insulin receptor Why?	290
9.10.3	The insulin receptor and the IGF-I receptor are homologues – why is one anabolic and the other mitogenic?	290
9.10.4	Do differing C-terminal tails cause differing regulation of growth responses in InsR <i>versus</i> IGF-IR?	291
9.10.5	IFG-II, insulin receptor-A and 'half receptors'	292
	References	293
10	Mitogens and cell cycle progression	297
10.1	The mitogenic response and the cell division cycle	298
10.1.1	Large scale biophysical events in the cell division cycle	298
10.1.2	The cyclin model	298
10.1.3	Summary of the budding yeast cell cycle	301
10.1.4	Mammalian cyclin cycle model	301
10.1.5	Embryonic cell cycle has no 'gaps'	302
10.2	G ₀ , competency, and the point of no return in G ₁ – the 'R-point'	303
10.2.1	What is G ₀ ?	303
10.2.2	The commitment point and competency factors	304
10.2.3	Growth factors and the fibroblast cell cycle	304

10.3	Oncogene products derived from growth factor pathway components	305
10.4	Transcription and cyclins	306
10.5	Cyclin dependent kinases	307
10.5.1	Activating and inactivating phosphorylations	307
10.5.2	Inactivating phosphorylations of Cdks	307
10.5.3	Activating dephosphorylations of Cdks	309
10.5.4	DNA damage prevents dephosphorylation of Cdks	309
10.6	Deactivation by cyclin destruction	309
10.6.1	APC/cyclosome (APC/C) and SCF – E3 ubiquitin ligase complexes	309
10.6.2	APC/C	310
10.6.3	SCF	310
10.7	Cyclin dependent kinases – activation through cyclin synthesis	311
10.7.1	Two sets of early genes – immediate and delayed	311
10.8	Mitogenic pathway downstream of single pass tyrosine kinase receptors	311
10.8.1	Transcription factor families involved in triggering the mitogenic response	311
10.8.2	Myc	311
10.8.3	Induction of Fos by ‘serum response element’ binding	312
10.8.4	The Ets family of ‘ternary complex transcription factors’ – Elk-1, Sap-1/2	312
10.8.5	The ‘serum response factor’ – MADS box-containing transcription factors	312
10.8.6	Signalling sequence of single-pass tyrosine kinase receptors leading to cyclin D induction – serum response element	313
10.8.7	Activation of Jun (and Myc) by inactivation of glycogen synthase kinase-3 (GSK-3)	313
10.8.8	AP-1 complexes – bZip transcription factors	315
10.8.9	AP-1 response elements on DNA	318
10.8.10	AP-1 and cyclin D1 induction	319
10.9	CyclinD/Cdk-4/6 – only important substrate is R _B	319
10.10	Retinoblastoma-related ‘pocket proteins’ – negative modulators of E2F	319
10.10.1	Phosphorylation/inactivation mechanism	321
10.10.2	The E2F family of transcription factors – the targets of the ‘pocket proteins’	323
10.10.3	R _B – a DNA-binding, E2F protein-binding tumour suppressor	324
10.10.4	E2F targets – genes for DNA replication and licensing, delayed early response genes (cyclin E and A), and NPAT	324

10.11	De-repression of the cyclin E gene by cyclin D/Cdk-4/6	325
10.11.1	Cyclin E/Cdk-2 substrates – R _B , NPAT, nucleophosmin	325
10.11.2	Cyclin E – licensing and loading of helicase	326
10.12	Cyclin A/Cdk-2 – S-phase progression and termination	327
10.12.1	Cyclin A/Cdk-2 – prevention of origin re-firing	327
10.12.2	Terminating S-phase – cyclin A effects	327
10.13	The controlled process of mammalian DNA replication	328
10.13.1	How does a cell know when to divide?	328
10.13.2	DNA replication	328
10.13.3	Pre-replicative complex formation begins in G ₁	328
10.13.4	Helicase loading	330
10.13.5	Geminin control of helicase loading and licensing	331
10.13.6	Origin firing – Ddk and Cdc45	331
10.14	Cyclin B translocations and M-phase	332
10.14.1	What triggers mitosis?	332
10.14.2	POLO – the ultimate mitotic trigger?	333
10.15	Cdk inhibitors	334
10.15.1	The INK proteins	334
10.15.2	The Cip/WAF family	335
10.16	p53 cell cycle arrest and apoptosis	336
10.16.1	p53 and Cip/WAF	336
10.16.2	Mdm2 and p19 ^{Arf} – control of p53	337
10.16.3	Apoptosis	338
10.16.4	Apoptosis or cell cycle arrest – majority verdict by a jury of Cdk inhibitors, survival factors, and pro-apoptotic factors	338
10.16.5	BH domain proteins and mitochondrial outer membrane permeabilisation	339
10.16.6	p53 and apoptosis	339
10.16.7	Survival factors opposing induction of apoptosis	340
10.17	7-pass receptors and mitosis	340
10.17.1	The Gsp oncogene	340
10.17.2	Wnt/ β -catenin	341
10.18	Concluding remarks and caveats	345
	References	347
Appendix 1: Worked examples		355
A.1	Enzyme and receptor assays worked out from raw data examples	355
A.1.1	An alkaline phosphatase assay	355
Appendix 2: RasMol: installation and use		365
Index		377