BIOMEMBRANES

Editor: A. G. LEE

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TRANSMEMBRANE RECEPTORS
AND CHANNELS

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A Multi-Volume Treatise

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Editor: A. G. LEE
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PREFACE

The quantity of information available about membrane proteins is now too large for any one person to be familiar with anything but a very small part of the primary literature. A series of volumes concentrating on molecular aspects of biological membranes therefore seems timely. The hope is that, when complete, these volumes will provide a convenient introduction to the study of a wide range of membrane functions.

Volume 6 of *Biomembranes* covers transmembrane receptors and channels. A particularly important role for the membrane is that of passing messages between a cell and its environment. Part I of this volume covers receptors for hormones and growth factors. Here, as in so many other areas of cell biology, the application of the methods of molecular biology have led to the recognition of a number of families of receptors. Typically, such receptors contain an extracellular ligand binding domain, a transmembrane domain, and an intracellular catalytic domain whose activation, as a result of ligand binding, leads to generation of second messengers within the cell and stimulation of a range of cytosolic enzymes. An alternative signaling strategy, exploited in particular in the nervous system, is to use ion channels to allow controlled movement of monovalent (Na^+, K^+) or divalent (Ca^{2+}) cations in or out of the cell, resulting in changes in membrane potential or alterations in the intracellular concentration of Ca^{2+}. Part II of this volume is concerned with these ion channels and with other, often simpler, ion channel systems whose study can throw light on channel mechanism.

As editor, I wish to thank all the contributors for their efforts and the staff of JAI Press for their professionalism in seeing everything through to final publication.

A. G. Lee
Editor

PART I

RECEPTORS FOR HORMONES AND GROWTH FACTORS

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INSULIN RECEPTOR SIGNALING

Chin K. Sung and Ira D. Goldfine

I. INSULIN RECEPTOR (IR)

Insulin regulates the general metabolism of most differentiated cells (Goldfine, 1981; Jacobs and Cuatrecasas, 1981; Kahn, 1985; Reaven, 1988). In the major target cells—myocytes, hepatocytes, and adipocytes—insulin has specific effects on the metabolism of carbohydrates, lipids, and proteins. In other cells, insulin is a general anabolic hormone (Goldfine, 1981). The initial interaction of insulin is with the insulin receptor (IR) protein that is located on the plasma membrane. After insulin binds, the IR initiates biological responses. Accordingly, the nature of the

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IR has been intensively studied, and significant progress has been made in understanding this protein (Goldfine, 1987; Ebina et al., 1985; Ullrich et al., 1985; Seino et al., 1989).

The IR gene is located on the short arm of chromosome 19 (Goldfine, 1987; Seino et al., 1989). The IR gene is greater than 120 kilobases in length and is comprised of 22 exons ranging from 36 to >2,500 base pairs (Seino et al., 1989). The mature receptor on the plasma membrane is an $\alpha_2 \beta_2$ tetramer composed of two extracellular α-subunits that bind the hormone, and two transmembrane β-subunits that have intracellular tyrosine kinase activity (Goldfine, 1987). One α-subunit (130 kDa) and one β-subunit (95 kDa) are derived from a common precursor of 1382 amino acids. After translation and N-glycosylation, the receptor precursor is transferred to the Golgi apparatus where it is split into separate subunits, the sugar residues modified, and the $\alpha_2 \beta_2$ tetramer formed; the mature receptor is then transported to the cell surface where it initiates the actions of insulin (Goldfine, 1987).

II. ADULT ONSET DIABETES MELLITUS AND IR SIGNALING

Diabetes mellitus exists in two major forms (Harris et al., 1987). One form is insulin dependent diabetes mellitus (IDDM; also known as juvenile onset diabetes mellitus). This disease has a prevalence of 0.3% to 0.5% in the general population, and is due to an autoimmune destruction of pancreatic β cells. The other form is non-insulin dependent diabetes mellitus (NIDDM; also known as adult onset diabetes mellitus). This disease is 10-fold more common than IDDM, and has a prevalence in the general population of 3% to 5%. It is estimated that 10 to 15 million individuals in the United States have NIDDM (Harris et al., 1987). The prevalence rate of NIDDM is higher in certain populations such as Hispanic Americans and Native Americans (Zimmet, 1992). One group, Pima Indians, has a prevalence of NIDDM approaching 50% (Bogardus and Lillioja, 1992; Howard, 1993). In NIDDM patients, there is a decreased insulin secretory response of β cells to blood glucose (Halter and Porte, 1981). In addition, there is resistance to insulin in key target tissues including muscle (Olefsky, 1980; Reaven, 1988, Bogardus and Lillioja, 1992; Myers and White, 1993). Studies have suggested that the insulin resistance is genetically determined, and in most instances this resistance precedes the abnormalities in insulin secretion (Bogardus and Lillioja, 1992). In many NIDDM patients, the IR is normal and the defect in insulin action is at the post-receptor level. However, patients with defects in IR expression and function have also been described, and approximately up to 5% of NIDDM patients have been estimated to have defects in IR function and/or expression (Taylor, 1992).

III. TYROSINE KINASE FAMILY

The IR is a member of the tyrosine kinase family (Yarden and Ullrich, 1988). A number of growth factors stimulate cellular mitogenesis by interacting with a family of cell-surface receptors that possess an intrinsic ligand-sensitive protein tyrosine kinase activity. Tyrosine phosphorylation of key cellular proteins initiates changes in cell growth. Tyrosine kinase receptors are typically composed of an extracellular ligand binding domain that is linked to a cytoplasmic catalytic domain, which not only transduces the growth factor or hormonal signal, but also generates mitogenic second messengers. There are four subclasses of receptor tyrosine kinases (I, II, III, IV); and non-receptor tyrosine kinases (V) (Figure 1; Yarden and Ullrich, 1988).

Subclass I includes the epidermal growth factor-receptor (EGF-R), which is activated by the ligands EGF, TGF-α, and the closely related HER-2/*neu* receptor. Subclass II receptors include the IR and the closely related insulin like growth factor-1-receptor (IGF-1-R), which are activated by insulin and IGF-1, respectively; and the insulin receptor-related receptor (IRR), whose ligand is unknown. Subclass III receptors include the platelet-derived growth factor-receptor (PDGF-R), the colony stimulating factor-1-receptor (CSF-1-R), and the protooncogene, c-kit. Subclass IV receptors include the fibroblast growth factor-receptor (FGF-R) and its

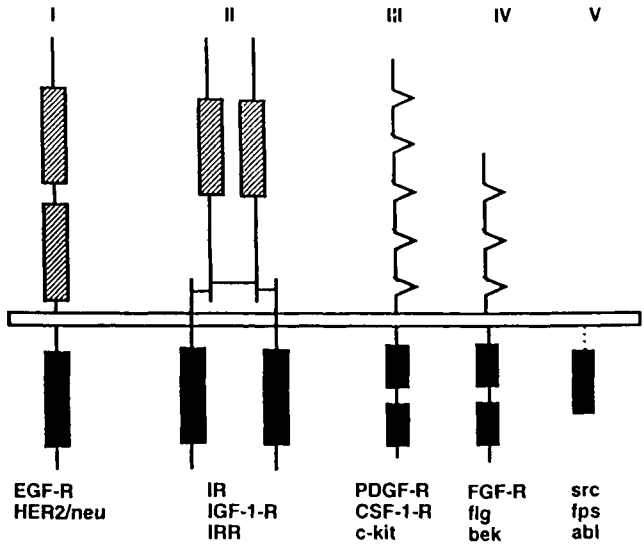

Figure 1. Schematic diagram of receptor and non-receptor tyrosine kinases. Receptor tyrosine kinases are classified into 4 subclasses (I, II, III, IV). Non-receptor tyrosine kinase subclass (V) includes viral oncogene tyrosine kinases. Hatched areas = cysteine rich regions; solid areas = tyrosine kinase domain; jagged lines = amino acid sequence repeats.

relatives, including flg and bek (Lee et al., 1989). In addition to these protooncogene encoded tyrosine kinase receptors, there are also receptor-derived viral oncogene products. v-*erb* B is derived from the EGF-R, and v-*fms* from the CSF-1-R. In general, these viral oncogene products differ from their normal receptor counterparts in that they have either amino acid deletions or substitutions that enable them to possess ligand-independent (and thus constitutively activated) tyrosine kinase activity. Subclass V includes non-receptor tyrosine kinases such as src, fps and abl.

IV. RECEPTOR TYROSINE KINASES AND SH2 PROTEINS

Several major clues in IR signaling have come from studies of other related receptor tyrosine kinases, such as the EGF-receptor (Skolnik et al., 1991) and the PDGF-receptor (Kaplan et al., 1990). In the case of these receptors, it has been demonstrated that intracellular adaptor and effector molecules attach to specific phosphorylated tyrosines of the receptors via Src homology 2 (SH2) domains (Table 1; Moran et al., 1990; Songyang et al., 1993). The SH2 domain is a 100 amino acid consensus sequence that was originally described in the oncogene product src and has the ability to bind to phosphotyrosines in specific motifs (Koch et al., 1991). At least five molecules containing SH2 domains bind to either the EGF or the PDGF receptors via phosphotyrosines, and become activated. These molecules include: phospholipase C, an enzyme that hydrolyzes phosphatidylinositol-4,5-bisphosphate to generate inositol-1,4,5-trisphosphate and 1,2-diacylglycerol; the p85 regulatory subunit of phosphatidylinositol-3-kinase (PIK) whose p110 catalytic subunit phosphorylates inositol at the 3 position; Ras GTPase activating protein (GAP) that

Table 1. SH2 Containing Proteins

I. Effectors	*II. Adaptors*
src, abl, syk[a]	p85 of PIK
PTPIC	c-crk
PLCγ	shc
	nck
GAP	Sem-5/Grb2
vav	

Note: [a]src,abl, syk are non-receptor tyrosine kinases. PTPIC is a phosphotyrosine phosphatase. PLCγ is a phospholipase. GAP accelerates Ras GTPase activity. vav has possible guanine nucleotide exchanger activity. p85 is a regulatory subunit of PIK. Sem-5/Grb 2 is an adaptor molecule involved in the Ras pathway. c-crk, shrc, and nck are adaptor molecules involved in cellular signaling.

promotes hydrolysis of Ras-GTP to Ras-GDP; phosphotyrosine phosphatase; and growth factor receptor bound protein 2 (Grb2), an intracellular adaptor molecule (Ullrich and Schlessinger, 1990, Cantley et al., 1991; Skolnik et al., 1991; Feng et al., 1993).

Although association of these molecules with the IR has been observed *in vitro* (Pronk et al., 1992; Yonezawa et al., 1992), none of the intracellular molecules mentioned previously have been documented to bind to phosphotyrosines of the IR in intact cells (Hadari et al., 1992; Songyang et al., 1993). However, insulin stimulation of the IR has been demonstrated to regulate PIK and Ras activity (*vida infra*), raising the possibility that the IR may indirectly interact with one or more of these regulatory proteins containing SH2 domains.

V. IRS-1 AND PHOSPHATIDYLINOSITOL-3-KINASE (PIK)

A major breakthrough in the studies of IR signaling has been the cloning and sequencing of insulin receptor substrate-1 (IRS-1; Sun et al., 1991). IRS-1 is a cytoplasmic protein with MW 160–190 kD and is a major cellular substrate for both the IR and the IGF-1 receptors (Myers and White, 1993). IRS-1 contains 20 tyrosine phosphorylation consensus sequences, six of which appear in YMXM (Tyr–Met–Xaa–Met) motifs (Sun et al., 1991). These motifs interact with SH2 domains of the p85 regulatory subunit of PIK (Myers and White, 1993; Songyang et al., 1993). PIK has a cytoplasmic location and phosphorylates inositol at the 3 position to yield potential intracellular signaling compounds such as PI-3,4-bisphosphate and PI-3,4,5-trisphosphate (Cantley et al., 1991). PIK activity is stimulated by oncogenes such as v-src and is believed to play a role in mitogenesis (Cantley et al., 1991). PIK consists of two subunits: a p110 catalytic subunit (Hiles et al., 1992) and a p85 regulatory subunit that contains two SH2 domains and one SH3 domain (Escobedo et al., 1991; Otsu et al., 1991; Skolnik et al., 1991). When tyrosine phosphorylated IRS-1 interacts with p85 of PIK, p110 is activated. This activation can be demonstrated *in vitro* (Myers and White, 1993; Carpenter et al., 1993). While the SH2 domain of p85 links PIK to tyrosine phosphorylated IRS-1, the role of the SH3 domain of the p85 remains to be defined.

VI. THE RAS SIGNALING PATHWAY AND RECEPTOR TYROSINE KINASES

Recent studies have strongly suggest that Ras, a monomeric membrane-bound GTP-binding protein, may be an intermediate in the IR signaling pathway and regulate certain biological functions of insulin. Evidence for this concept includes: (1) microinjection of anti-Ras antibodies inhibits insulin-induced maturation of *Xenopus* oocytes (Korn et al., 1987); (2) over-expression of a dominant inhibitory Ras mutant blocks insulin action on both gene expression and differentiation of 3T3-L1 cells to adipocytes (McCormick, 1993; Skolnik et al., 1991); (3) insulin

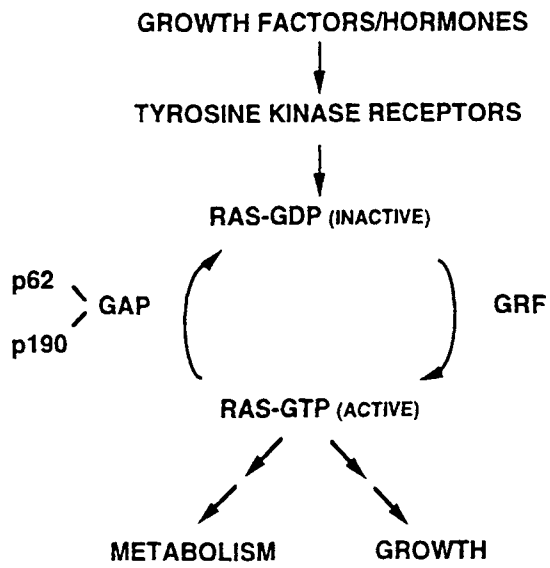

Figure 2. Regulation of Ras activity. In response to growth factors and hormones, receptor tyrosine kinases are activated. Activation of these kinases lead to activation of Ras by converting inactive Ras-GDP to active Ras-GTP. Two regulatory proteins, GRF and GAP, are identified. The activated Ras results in stimulation of metabolism and growth. GRF = guanine nucleotide releasing factors; GAP = GTPase activating protein; p62 and p190 = GAP-associated proteins.

treatment of intact cells increases the steady-state level of active endogenous Ras–GTP (McCormick, 1993; Porras et al., 1992); (4) activation of Ras also stimulates insulin-induced glucose transport via Glut-4 translocation (Kozma et al., 1993). Recently, it has been reported that a new member of the Ras-related family, Rad, is over-expressed in muscles of patients with NIDDM and may play a role in the insulin resistance of NIDDM (Kahn, 1993).

The content of active Ras-GTP is regulated by two classes of proteins (Wigler, 1990; Marx, 1992; Feig, 1993; Figure 2). One class, guanine nucleotide releasing factors (GRF), such as *Drosophila* Sos, exchanges GDP for GTP to activate Ras. Conversely, another class of proteins, such as GAP, promotes the hydrolysis of Ras-GTP to Ras-GDP to inactivate Ras. Two proteins, p62 and p190, are associated with GAP (Ellis et al., 1990; Settleman et al., 1992; Wong et al., 1992). It has been proposed that p62 and p190 regulate GAP activity. It should be also noted that GAP itself has been proposed as an effector molecule (Moran et al., 1991; Medema et al., 1992). Once Ras is activated by either activation of GRFs or inhibition of GAPs (or both), the activated Ras can trigger cascade of serine/threonine protein kinases (Figure 3). These protein kinases include: raf-1 kinase, which binds to and is activated by Ras-GTP; microtubule associated protein (MAP) kinase kinase; MAP kinase;

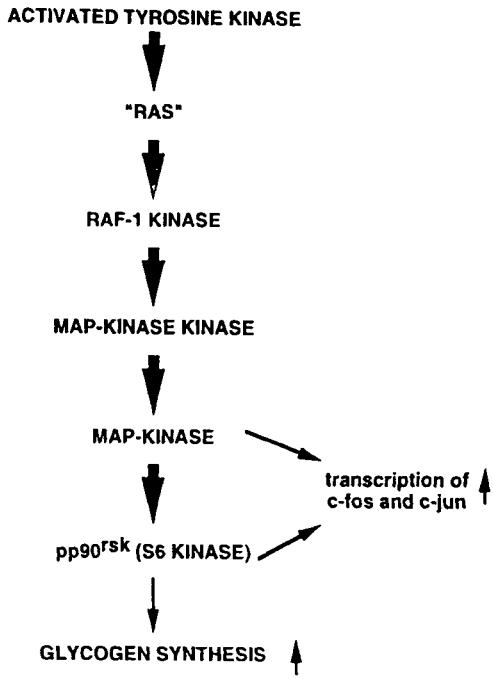

Figure 3. Tyrosine kinase induced serine/threonine kinase cascade via Ras activation. Ras activated by tyrosine kinases (either receptor or non-receptor bound) will active a series of serine/threonine kinases. Some of these kinases are reported to play a role in regulating transcription of nuclear genes and protein phosphatases leading to glycogen synthesis. MAP = microtubule associated protein.

and p90 ribosomal S6 kinase (Roberts, 1992). Activation of these protein kinases regulates various cellular functions including: c-fos and c-jun transcriptional activity, glycogen synthetase activity and cell proliferation (Roberts, 1992).

The major question arises, therefore, as to what is the link between activation of the receptor tyrosine kinases and activation of the Ras signaling pathway. In the case of the PDGF-receptor, GAP directly binds to a phosphotyrosine of the receptor via an SH2 domain, providing a direct link between the receptor and Ras (Kaplan et al., 1990). In the case of the EGF-receptor, it has been reported that the intracellular adaptor molecule, Grb2, binds to a phosphotyrosine of the EGF-receptor via its SH2 domain (Skolnik et al., 1991). Grb2 is a homolog of Sem-5 in *Caenorhabditis elegans* and drk in *Drosophila* (McCormick, 1993). Grb2 then interacts with mSos1 via its SH3 domain (a 50 amino acid consensus sequence found in src) and a proline-rich region of mSos1. mSos1 is the mammalian homolog of *Drosophila* Sos. Interaction of Grb2 with mSos1 activates Ras leading to receptor