DOCUMENT SUMMARY

This document is a comprehensive review of the mechanisms of cell signaling through **Receptor Tyrosine Kinases (RTKs)**. It details the paradigms of receptor activation, the role of modular protein domains and docking proteins, and the various intracellular signaling pathways activated by **RTKs**, such as the **Ras/MAP kinase** cascade. The paper also explores the factors that determine signal specificity, attenuation, and termination, providing a foundational overview of **RTK** function in cellular processes.

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A large group of genes in all eukaryotes encode for proteins that function as membrane spanning cell surface receptors. Membrane receptors can be classified into distinct families based upon the ligands they recognize, the biological responses they induce and, more recently, according to their primary structures. A great variety of ligands bind to and regulate the activity of cell surface receptors, including small organic molecules, lipids, carbohydrates, peptides, and proteins. One large family of cell surface receptors is endowed with intrinsic protein tyrosine kinase activity. These **receptor tyrosine kinases (RTKs)** catalyze transfer of the y phosphate of ATP to hydroxyl groups of tyrosines on target proteins (Hunter, 1998).

> **RTKs** play an important role in the control of most fundamental cellular processes including the cell cycle, cell migration, cell metabolism and survival, as well as cell proliferation and differentiation.

All **receptor tyrosine kinases** contain an extracellular ligand binding domain that is usually glycosylated. The ligand binding domain is connected to the cytoplasmic domain by a single transmembrane helix. The cytoplasmic domain contains a conserved **protein tyrosine kinase (PTK)** core and additional regulatory sequences that are subjected to autophosphorylation and phosphorylation by heterologous protein kinases (Hunter, 1998; Hubbard et al., 1998). Lymphokines such as erythropoietin and interferon also mediate their responses by tyrosine phosphorylation. However, rather than containing an intrinsic protein tyrosine kinase activity, the relatively short cytoplasmic domains of these receptors interact through noncovalent interactions with members of the **Jak family** of nonreceptor tyrosine kinases (Darnell et al., 1994; Ihle, 1995). Apart from the lack of covalent linkage to a kinase, the mechanism of activation of these binary receptors largely resembles that of receptor tyrosine kinases (Lemmon and Schlessinger, 1994; Heldin, 1995; Jiang and Hunter, 1999).

> The purpose of this review is to describe general concepts underlying the mechanism of action of **RTKs** and the signaling pathways that they regulate, while attempting to shed light on the question of how specificity is defined by the action of **RTKs**, and how a specific biological response can be generated by the diverse array of signaling pathways activated by all **RTKs**.

Paradigms for Receptor Activation

With the exception of the **insulin receptor (IR)** family of **RTKs**, all known **RTKs** (e.g., **EGF receptor**, **PDGF receptor**) are monomers in the cell membrane. Ligand binding induces dimerization of these receptors resulting in autophosphorylation of their cytoplasmic domains (Schlessinger, 1988; Lemmon and Schlessinger, 1994; Jiang and Hunter, 1999). Members of the **IR family** are disulfide linked dimers of two polypeptide chains forming an α2β2 heterodimer (Van-Obberghen, 1994). Insulin binding to the extracellular domain of the **IR** induces a rearrangement in the quaternary heterotetrameric structure that leads to increased autophosphorylation of the cytoplasmic domain. As the active forms of insulin receptor and monomeric **RTKs** are both dimeric, the signaling mechanisms of the two types of receptor are likely to be very similar (Hubbard et al., 1998).

Activation by Dimerization

Although all **RTKs** are activated by dimerization, different ligands employ different strategies for inducing the active dimeric state. Structural studies of **growth hormone (GH)** in complex with **GH receptor (GHR)** and **erythropoietin (EPO)** in complex with **EPO receptor (EPOR)** show that these cytokines are bivalent, and one ligand binds simultaneously to two receptor molecules to form a 1:2 (ligand:receptor) complex (Kossiakoff and De Vos, 1998; Jiang and Hunter, 1999). Receptor dimerization is further stabilized by additional receptor:receptor interactions.

Several growth factors are homodimers (e.g., **VEGF**, **PDGF**) providing the simplest mechanism for ligand-induced receptor dimerization. The **VEGF receptors (VEGFR)** contain seven immunoglobulin (Ig)-like domains in their extracellular domain, of which

only Ig-domains 2 and 3 are required for ligand binding. The crystal structure of **VEGF** in complex with Ig-like domain 2 of the **flt-1 VEGFR** provides a view of ligand-induced receptor dimerization (Wiesmann et al., 1997). The structure shows that one receptor molecule binds at each of the two junctions between **VEGF** protomers to yield a complex that is close to 2-fold symmetric, and contains two **VEGF** protomers plus the two Ig-like domains.

The **fibroblast growth factor (FGF)** family consists of at least 21 related growth factors (Maski and Ornitz, 1998). **FGFs** are unable to activate **FGF receptors (FGFR)** without cooperation of the accessory molecule **heparin sulfate proteoglycan (HSPG)** (Yayon et al., 1991). The crystal structures of **FGF** in complex with the ligand binding domain of **FGFR** (consisting of Ig-like domains-2 [D2] and -3 [D3]) provide a molecular view of **FGFR** dimerization (Plotnikov et al., 1999; Schlessinger et al., 2000) and activation and illustrate the determinants that govern **FGF:FGFR** specificity (Plotnikov et al., 2000). Each structure shows a 2:2 **FGF:FGFR** complex, in which **FGF** interacts extensively with D2, D3, and with the linker that connects these two domains within one receptor (the primary binding site). The dimer is stabilized by a secondary binding site involving interactions between **FGF** and D2 of the second receptor in the complex, as well as by receptor:receptor interactions. In contrast to the disulfide linked **VEGF** homodimer, the two **FGF** molecules in the 2:2 **FGF:FGFR** complex do not make any contact. Indeed, interactions between **FGF** and **FGFR** alone are not sufficient for stabilizing **FGFR** dimers at the cell surface under normal physiological conditions. Heparin or heparan sulfate proteoglycans are essential for stable dimerization of **FGF:FGFR** complexes (Spivak-Kroizman et al., 1994). It has been shown that heparin binds to a positively charged canyon formed by a cluster of exposed Lys and Arg residues that extends across the D2 domains of the two receptors in the dimer and the adjoining bound **FGF** molecules (Schlessinger et al., 2000). The full-length **FGFR** contains an additional Ig-like domain (D1) and a stretch of acidic residues or "acid box" in the linker between D1 and D2. Neither D1 nor the acid box is required for **FGF** binding to the **FGFR**. In fact, deletion of D1 and the acid box enhances binding of the receptor to **FGF** and heparin (Wang et al., 1995). Recent studies we have carried out lead us to propose that D1 and the acid box in full-length **FGFR** have an autoinhibitory function (Plotnikov et al., 1999). It is thought that the acid box can bind intramolecularly to the heparin binding site in D2, competing with heparin for binding to this site. Similarly, D1 may interact intramolecularly with the ligand binding domain in D2 and D3 and thus interfere with **FGF** binding to **FGFR**. This autoinhibition would prevent accidental FGF-independent activation of **FGFR** by **HSPGs** that are abundant in the extracellular matrix and on cell surfaces. According to this view, the extracellular domain of **FGFR** has an autoregulatory function in addition to its roles in ligand recognition and receptor dimerization. A similar mechanism of autoinhibition may apply for other **RTKs** that contain multiple Ig-like domains in their extracellular domains (e.g., **PDGFR**, **VEGFR**). As only 2 out of the 5 Ig-like domains of **PDGFR**, and just 2 of the 7 Ig-like domains of **VEGFR** are essential for ligand binding, it is possible that the extra Ig-like domains not involved in ligand binding could play an autoregulatory role in these receptors.

The control of **FGFR** stimulation by two ligands, **FGF** and heparin, may provide a mechanism for localized activation of **FGFR** and vectorial stimulation of cell proliferation

or differentiation. The biosynthesis of **HSPGs** in restricted areas of the extracellular matrix of different tissues may provide a scaffold to which cells expressing **FGFR** will migrate, and on which these cells will survive, proliferate, or undergo differentiation when supplied with a specific **FGF** molecule. Indeed, it was demonstrated that **FGF8** and **FGFR1** are essential for cell migration and mesodermal patterning during gastrulation (Yamaguchi et al., 1994; Sun et al., 1999).

Recent biochemical and structural studies and earlier experiments using monoclonal anti-receptor antibodies have demonstrated that only certain forms of receptor dimers with unique configurations of the extracellular and cytoplasmic domains of both **RTKs** and cytokine receptors lead to trans-autophosphorylation and **PTK** stimulation (Lemmon and Schlessinger, 1994; Jiang and Hunter, 1999). It is thought that receptor monomers are in equilibrium with receptor dimers. A limited population of receptor dimers exist with quaternary structures of their extracellular and cytoplasmic domains in configurations that are compatible with trans-autophosphorylation and stimulation of **PTK** activity (active dimer). Ligand binding to the extracellular domain stabilizes the formation of active dimers and consequently **PTK** stimulation. We propose that active dimers exist even in the absence of ligand binding since autophosphorylation of **RTKs** can be enhanced by inhibitors of protein tyrosine phosphatases or by receptor overexpression even in the absence of ligand binding.

The Role of Receptor Hetero-Oligomerization

The **EGFR family** consists of four **RTKs**, **EGFR (ErbB1)**, **ErbB2**, **ErbB3**, and **ErbB4**. While **EGFR** has numerous ligands (e.g., **EGF**, **TGF**, **HB-EGF**), a ligand for **ErbB2** has not been identified. The ligands for **ErbB3** and **ErbB4**, the two other members of this **RTK** family, are the various isoforms of the **neuregulins (NRG)**. It was demonstrated over a decade ago that EGF-induced stimulation of **EGFR** leads to activation of **ErbB2** by transduction through hetero-oligomerization (King et al., 1988; Stern and Kamps, 1988; Wada et al., 1990). Subsequently, numerous studies have demonstrated that stimulation with **EGF** or **NRG** induces a combinatorial hetero-oligomerization of different pairs of members of the **EGFR family** (Carraway and Cantley, 1994; Lemmon and Schlessinger, 1994; Olayioye et al., 2000). In the absence of a specific ligand for **ErbB2**, it was proposed that this **RTK** may function as a heterodimeric partner of the other members of the family, and could provide an additional platform for recruitment of intracellular signaling pathways in response to **EGF** or **NRG** stimulation. Moreover, since the sequence of the **ErbB3** catalytic domain suggests that this receptor does not have **PTK** activity, it is thought that **ErbB3** may function as a platform to expand the repertoire of intracellular signaling proteins recruited following its trans-phosphorylation by other members of the **EGFR family** (Carraway and Cantley, 1994).

In the absence of structural information about **EGFR**, it is difficult to present a clear molecular picture concerning the mechanism of receptor dimerization and hetero-oligomerization. Biophysical studies have suggested that **EGF** is bivalent toward **EGFR** and shown that **EGF** can drive dimerization of the **EGFR** extracellular domain ending with a stoichiometry of 2:2 **EGF:EGFR** (Lemmon et al., 1997; Ferguson et al., 2000). It has been proposed that the bivalency of **EGF** or **NRG** is the driving force for

heterodimerization of **ErbB2** with other members of the **EGFR family** (Tzahar et al., 1997). However, presently there is no evidence for binding of **EGF** or **NRG** to the extracellular domain of **ErbB2** (Feurguson et al., 2000). The exact mechanism of ligand-dependent dimerization of members of the **EGFR family** must await the determination of the three-dimensional structures of these complexes. An alternative mechanism is that two receptor homodimers form a heterodimer. A potential mechanism for EGF-induced heterotetramer formation between **EGFR** and **ErbB2** is that EGF-induced homodimers form a tetrameric complex with unoccupied homodimers of **ErbB2** by receptor:receptor interactions. The interactions between the two homodimers within the context of a heterotetramer could serve to stabilize the formation of one dimer indirectly by growth factor binding. For example, binding of two monomeric **ErbB2** proteins to an EGF-induced homodimer of **EGFR** may cause homodimerization of the **ErbB2** molecules followed by their trans-autophosphorylation and consequent activation (Honegger et al., 1990; Qian et al., 1994; Gamett et al., 1997; Huang et al., 1998). The mechanism of heterotetramer formation between **ErbB3** and **ErbB4** may be different, since both receptors bind **NRG** and may undergo NRG-dependent homodimerization. In this case, **ErbB3** homodimers may interact with NRG-induced homodimers of **ErbB4**, which in turn will phosphorylate the cytoplasmic domains of **ErbB3** proteins by trans-phosphorylation. In other words, homodimers of **ErbB3** may in fact be preferable substrates of **ErbB4** within the context of a heterotetrameric complex.

Structural studies of the catalytic core of several **RTKs**, together with biochemical and kinetic studies of receptor phosphorylation and activation have provided insights into the mechanism by which **RTK** dimerization activates enzymatic activity (Hubbard et al., 1994; Mohammadi et al., 1996; Hubbard, 1997). The emerging picture is that receptor oligomerization increases the local concentration of the **PTK**, leading to more efficient transphosphorylation of tyrosine residues in the activation loop of the catalytic domain (Hubbard et al., 1998). Structural studies have shown that, upon tyrosine phosphorylation, the activation loop adopts an "open" configuration that permits access to ATP and substrates, and enables phosphotransfer from MgATP to tyrosines on the receptor itself and on cellular proteins involved in signal transmission.

Mechanism of Activation of Signaling Proteins

In addition to its central role in the control of protein tyrosine kinase activity, tyrosine autophosphorylation of **RTKs** is crucial for recruitment and activation of a variety of signaling proteins. Most tyrosine autophosphorylation sites are located in noncatalytic regions of the receptor molecule. These sites function as binding sites for **SH2 (Src homology 2)** or **PTB (phosphotyrosine binding)** domains of a variety of signaling proteins. **SH2** domain-mediated binding of signaling proteins to tyrosine autophosphorylation sites provides a mechanism for assembly and recruitment of signaling complexes by activated receptor tyrosine kinases.

> According to this view, every **RTK** should be considered not only as a receptor with tyrosine kinase activity but also as a platform for the recognition and recruitment of a specific complement of signaling proteins (Pawson and Schlessinger, 1993).

Signaling proteins containing **SH2** and **PTB** domains are modular in nature (Kuriyan and Cowburn, 1997; Pawson and Scott, 1997; Margolis, 1999). Many of these proteins contain intrinsic enzymatic activities and protein modules that bring about interactions with other proteins, with phospholipids, or with nucleic acids.

Modular Domains of Signaling Proteins

SH2 domains bind specifically to distinct amino acid sequences defined by 1 to 6 residues C-terminal to the pTyr moiety (Songyang et al., 1993), while **PTB** domains bind to pTyr within context of specific sequences 3 to 5 residues to its N terminus (Margolis, 1999). Certain **PTB** domains bind to nonphosphorylated peptide sequences, while still others recognize both phosphotyrosine-containing and nonphosphorylated sequences equally well (Margolis, 1999). **SH3** domains bind specifically to the proline-rich sequence motif PXXP, while **WW** domains bind preferentially to another proline-rich motif PXPX (Kuriyan and Cowburn, 1997). **Pleckstrin homology (PH)** domains comprise a large family of more than a hundred domains. While certain **PH** domains bind specifically to Ptdins (4,5)P2, another subset of **PH** domains binds preferentially to the products of agonist-induced **phosphoinositide-3-kinases (PI-3 kinase)** (Ferguson et al., 1995; Lemmon et al., 1995, 1996; Czech, 2000). As only a small subset of **PH** domains bind specifically to phosphoinositides or to their soluble head groups, the physiological ligands of the majority of **PH** domains remain to be identified. However, the weak and nonspecific binding of most **PH** domains to phosphoinositides may be compensated for by the oligomeric nature of certain **PH** domain-containing proteins leading to strong membrane association (Lemmon and Ferguson, 2000). Finally, **FYVE** domains comprise another family of small protein modules that specifically recognize PtdIns-3-P (Fruman et al., 1999), and **PDZ** domains belong to another large family of independent protein modules that bind specifically to hydrophobic residues at the C termini of their target proteins (Gomperts, 1996).

A large family of **SH2** domain-containing proteins possess intrinsic enzymatic activities such as **PTK** activity (**Src kinases**), **protein tyrosine phosphatase (PTP)** activity (**Shp2**), **phospholipase C activity (PLC)**, or **Ras-GAP** activity among other activities. Another family of proteins contains only **SH2** or **SH3** domains. These **adaptor proteins** (e.g., **Grb2**, **Nck**, **Crk**, **Shc**) utilize their **SH2** and **SH3** domains to mediate interactions that link different proteins involved in signal transduction. For example, the adaptor protein **Grb2** links a variety of surface receptors to the **Ras/MAP kinase** signaling cascade. **Grb2** interacts with activated **RTKs** via its **SH2** domain and recruits the guanine nucleotide releasing factor **Sos** close to its target protein **Ras** at the cell membrane (Schlessinger, 1994; Pawson, 1995).

Docking Proteins

Agonist-induced membrane recruitment of signaling proteins stimulated by tyrosine phosphorylation is also mediated by a family of **docking proteins**. All **docking proteins** contain in their N termini a membrane targeting signal and in their C termini a large region that contains multiple binding sites for the **SH2** domains of signaling proteins (Sun et al., 1993; Kouhara et al., 1997). Some **docking proteins** are associated with the cell membrane by a myristyl anchor (e.g., **FRS2**), while others have

their own transmembrane domain (e.g., **LAT**) (Zhang et al., 1998a). However, most **docking proteins** contain a **PH** domain at their N terminus. **Docking proteins** such as **Gab1** become associated with the cell membrane by binding of its **PH** domain to Ptdins (3,4,5)P3 in response to agonist-induced stimulation of **PI-3 kinase** (Rodrigues et al., 2000). In addition to the membrane targeting signal, most **docking proteins** contain specific domains such as **PTB** domains that are responsible for complex formation with a particular set of cell surface receptors. The **PTB** domains of **IRS1** and **IRS2**, for example, bind specifically to **IR**, **IGF1-R** or **IL4-R**. The **PTB** domains of **FRS2α** and **FRS2β** on the other hand, bind preferentially to **FGFR** or **NGFR**. It has been shown that **docking proteins** function as platforms for the recruitment of signaling proteins in response to receptor stimulation. In fact, most of the signaling proteins that are activated in response to insulin or **FGF** stimulation are recruited via the **IRS** or **FRS** families of **docking proteins** and not by their direct binding to **IR** or **FGFR**. It appears that the total amount of signaling proteins that are recruited by a given activated **RTK** is the sum of the proteins recruited by the receptor directly, and those recruited by **docking proteins** that are tyrosine phosphorylated by the same receptor. Although many proteins serve as substrates of, and are activated by, **RTKs**, there appear to be three different general mechanisms for how signaling proteins are activated in response to **RTK** stimulation.

Paradigms for Activation of Effector Proteins

Activation by Membrane Translocation.

PDGF-induced activation of **PI-3 kinase** leads to generation of the second messengers Ptdins (3,4)P2 and PtdIns(3,4,5)P3. The generation of these second messengers plays a crucial role in the activation of **PDK1** and **PKB** (also known as **AKT**), two highly conserved protein kinases that play an important role in stimulation of cell survival, protein synthesis, and metabolic processes. **PDK1** has a **PH** domain at the C terminus of the protein through which it binds to Ptdlns(3,4,5)P3 leading to membrane translocation (Alessi et al., 1997; Anderson et al., 1998). **PKB**, which is also recruited to the membrane via its N-terminal **PH** domain binding to **PI-3 kinase** products (Franke et al., 1995; Frech et al., 1997), is phosphorylated by **PDK1** on Thr308 in its activation loop. It has been proposed that an as yet unidentified protein kinase (hypothetical **PDK2**) is responsible for **PKB** phosphorylation on Ser473 leading to complete stimulation of **PKB** activity. However, it was recently reported that phosphorylation of Ser473 is mediated by **PKB** trans-autophosphorylation (Toker and Newton, 2000a, 2000b).

Activation by a Conformational Change.

There is good evidence that **SH2** domain-mediated binding of certain signaling proteins to phosphotyrosines on activated receptors induces a conformational change that releases an autoinhibition resulting in stimulation of enzymatic activity. For example, the protein tyrosine kinase activity of **Src** is activated when its **SH2** domain binds to tyrosine autophosphorylation sites on **PDGFR** (Thomas and Brugge, 1997; Xu et al., 1999). Similarly, binding of **p85**, the regulatory subunit of **PI-3 kinase**, to phosphotyrosines in the **PDGFR** or **IRS1** causes conformational changes in **p85** that are transmitted to the

catalytic subunit **p110** leading to enhancement of **PI-3 kinase** activity. In addition, by binding to tyrosine phosphorylated **PDGFR** or **IRS1**, **PI-3 kinase** is translocated to the cell membrane where its substrate Ptdlns(4,5)P2 is found.

Activation by Tyrosine Phosphorylation.

It has been shown that tyrosine phosphorylation of certain target proteins is required for ligand stimulation of their enzymatic activity. In response to **EGF**, **PDGF**, or **FGF** receptor activation, the **SH2** domains of **PLCγ** bind to specific phosphotyrosines in the C-terminal tails of these receptors. Binding of **PLCγ** to the activated receptor facilitates its efficient tyrosine phosphorylation by the **RTK**. PDGF-induced activation of phospholipase C activity is abrogated in cells expressing **PLCγ** mutated in the tyrosine phosphorylation sites (Kim et al., 1991). Activation of **PLCγ** is also dependent upon agonist-induced generation of **PI-3 kinase** products. Both tyrosine phosphorylation and membrane translocation of **PLCγ** through binding of its **PH** domain to Ptdlns(3,4,5)P3 are essential for complete activation of phospholipase-C activity leading to the generation of the two second messengers diacylglycerol and Ins(1,4,5)P3 (Falasca et al., 1998).

As many of the targets of **RTKs** are membrane linked, membrane translocation of key signaling components is critical in the process of signal transduction. At least two molecular events must take place before agonist-induced activation of each of the effector proteins described can occur. **PKB** activation, for example, requires translocation to the plasma membrane and phosphorylation by **PDK1** on a key Thr residue. Furthermore, it was proposed that translocation of **PKB** to the cell membrane is accompanied by release of an autoinhibition suggesting that a conformational change in **PKB** may also take place and be required for phosphorylation by **PDK1** and for kinase activation. PDGF-induced activation of **PI-3 kinase** is mediated by a conformational change in **PI-3 kinase** induced by **p85** binding to pTyr sites on activated **PDGFRS**. Stimulation of **PLCγ**, on the other hand, is dependent on both tyrosine phosphorylation and **PI-3 kinase** activation. Membrane translocation is essential for **PI-3 kinase** and **PLCγ** activation, as Ptdlns(4,5)P2, the substrate of these two enzymes is located in the cell membrane.

Intracellular Signaling Pathways

The rapid progress in understanding intracellular signaling pathways that took place during the 1990s was largely due to the convergence of information generated by multiple scientific disciplines. Similar proteins were repeatedly identified by applying totally different methodologies. Key components of signaling pathways have been discovered in biochemical studies in which cellular proteins were isolated, cloned, and analyzed. The invertebrate *C. elegans* and *Drosophila* homologs of the same proteins have been found in genetic screens. Moreover, in many cases the same proteins have been identified as products of genes that are mutated in different human diseases such as cancer, severe skeletal disorders, immunodeficiencies, and neurological diseases. A picture is starting to emerge with regard to the different components of several signal transduction pathways and signaling networks that are activated by cell surface receptors. General principles that govern the spatiotemporal information flow from the

cell surface to the nucleus, and the modes of communication between the different signaling pathways are becoming unveiled.

The Ras/MAP Kinase Signaling Cascade

All **RTKs** and many other cell surface receptors stimulate the exchange of GTP for GDP on the small G protein **Ras**. Both biochemical and genetic studies have demonstrated that **Ras** is activated by the guanine nucleotide exchange factor, **Sos**. The adaptor protein **Grb2** plays an important role in this process by forming a complex with **Sos** via its **SH3** domains. The **Grb2/Sos** complex is recruited to an activated **RTK** through binding of the **Grb2 SH2** domain to specific pTyr sites of the receptor, thus translocating **Sos** to the plasma membrane where it is close to **Ras** and can stimulate exchange of GTP for GDP (Schlessinger, 1994; Pawson, 1995; Bar-Sagi and Hall, 2000). Membrane recruitment of **Sos** can be also accomplished by binding of **Grb2/Sos** to **Shc**, another adaptor protein that forms a complex with many receptors through its **PTB** domain (Margolis, 1999). Alternatively, **Grb2/Sos** complexes can be recruited to the cell membrane by binding to membrane-linked **docking proteins** such as **IRS1** or **FRS2α** which become tyrosine phosphorylated in response to activation of certain **RTKs** (Sun et al., 1993; Kouhara et al., 1997). There is also evidence that the **PH** domain of **Sos** is essential for membrane translocation and for complete activation of **Ras**. Once in the active GTP-bound state, **Ras** interacts with several effector proteins such as **Raf** and **PI-3 kinase** to stimulate numerous intracellular processes. Activated **Raf** stimulates **MAP-kinase-kinase (MAPKK, MEK)** by phosphorylating a key Ser residue in the activation loop. **MAPKK** then phosphorylates **MAPK (ERK)** on Thr and Tyr residues at the activation-loop leading to its activation. Activated **MAPK** phosphorylates a variety of cytoplasmic and membrane linked substrates (e.g., **EGFR**, **Sos**). In addition, **MAPK** is rapidly translocated into the nucleus where it phosphorylates and activates transcription factors (Karin and Hunter, 1995; Hunter, 2000). The signaling cassette composed of **MAPKKK**, **MAPKK**, and **MAPK** is highly conserved in evolution and several **MAPK** cascades exist in yeast, in invertebrates and vertebrates (Waskiewicz and Cooper, 1995; Madhani and Fink, 1998; Garrington and Johnson, 1999). These highly conserved signaling cascades play an important role in the control of metabolic processes, cell cycle, cell migration, and cell shape as well as in cell proliferation and differentiation (Davis, 2000).

Phosphoinositol Metabolism and Cell Signaling

Activation of **RTKs** leads to rapid stimulation of phosphoinositol metabolism and generation of multiple second messengers (Rameh and Cantley, 1999; Czech, 2000). **PLCγ** is rapidly recruited by an activated **RTK** through the binding of its **SH2** domains to pTyr sites in the receptor molecules. Upon activation **PLCγ** hydrolyzes its substrate Ptdlns(4,5)P2 to form two second messengers, diacylglycerol and Ins(1,4,5)P3. By binding to specific intracellular receptors, Ins(1,4,5)P3 stimulates the release of Ca2+ from intracellular stores. Ca2+ then binds to calmodulin, which in turn activates a family of Ca2+/calmodulin-dependent protein kinases. In addition, both diacylglycerol and Ca2+ activate members of the **PKC family** of protein kinases. The second messengers generated by Ptdlns(4,5)P2 hydrolysis stimulate a variety of intracellular responses in

addition to phosphorylation and activation of transcriptional factors (Karin and Hunter, 1995; Hunter, 2000).

The phospholipid kinase **PI-3 kinase** is activated by virtually all **RTKs**. One group of **PI-3 kinases** are heterodimers composed of a regulatory subunit **p85**, which contains two **SH2** and one **SH3** domain and a catalytic subunit designated **p110**. Like other **SH2** domain-containing proteins, **PI-3 kinase** forms a complex with pTyr sites on activated receptors or with tyrosine phosphorylated **docking proteins** such as **IRS1** and **Gab1**. Activated **PI-3 kinase** phosphorylates PtdIns(4)P and Ptdlns(4,5)P2 to generate the second messengers Ptdlns(3,4)P2 and Ptdlns(3,4,5)P3. Ptdlns(3,4,5)P3 mediates membrane translocation of a variety of signaling proteins, such as the non-receptor protein tyrosine kinases **Btk** and **Itk**, the Ser/Thr kinases **PDK1** and **PKB**, the Arf exchange factor **Grp1**, the docking protein **Gab1**, and **PLCγ1**, among many others (Rameh and Cantley, 1999; Czech, 2000). Membrane translocation is mediated through binding of their **PH** domains to agonist-induced **PI-3 kinase** products leading to their activation and subsequent stimulation of a variety of cellular responses. One important response is stimulation of cell survival. It has been shown that **PI-3 kinase**-dependent activation of **PKB** leads to phosphorylation and inactivation of **BAD**. Phosphorylation of **BAD** prevents apoptotic cell death by blocking its complex formation with the apoptotic protein **Bcl-2** and **Bcl-xl** (Datta et al., 1999). Another mechanism for inhibition of apoptosis is via **PKB**-induced phosphorylation of the transcription factor **FKHR1** (Brunet et al., 1999), which in turn suppresses proapoptotic gene expression. Insulin-induced activation of **PDK1** leads to phosphorylation and activation of **S-6 kinase**. Furthermore, **glycogen synthase kinase-3 (GSK-3)** and phosphofruckokinase, two enzymes that are regulated in response to insulin stimulation, are phosphorylated by **PKB**. **PDK1** and **PKB** may play a role in the control of protein synthesis, gluconeogenesis, and glycolysis in response to insulin stimulation (Toker and Newton, 2000a).

PI-3 kinase also plays an important role in growth factor-induced hydrogen peroxide generation. It has been recently shown that PDGF-induced H2O2 generation is dependent upon activation of **PI-3 kinase** and the small G protein **Rac** (Bae et al., 2000). Earlier studies demonstrated that activation of **NADPH synthase**, the enzyme complex that catalyzes the production of hydrogen peroxide, is an effector of **Rac**. Interestingly, EGF-induced generation of H2O2 is essential for sustained tyrosine autophosphorylation and activation of **EGFR** (Bae et al., 1997). Hydrogen peroxide that is generated in response to **EGF** stimulation oxidizes and inactivates a **protein tyrosine phosphatase (PTP)** that dephosphorylates activated **EGFR** (Lee et al., 1998; Bae et al., 2000). Regulation of **EGFR** kinase activity is not the only role of hydrogen peroxide in response to growth factor stimulation. There is good evidence that H2O2 plays an active role in the control of multiple cellular processes.

The activity of effector proteins that are dependent on **PI-3 kinase** activation can be negatively regulated by **PTEN** and **SHIP**, two phosphoinositide-specific phosphatases that dephosphorylate the 3' and 5' positions of the inositol ring of phosphoinositides, respectively (Bolland et al., 1998; Maehama and Dixon, 1998). **PTEN** is a tumor suppressor protein that is mutated in a variety of human cancers leading to aberrant stimulation of cell survival pathway (Maehama and Dixon, 1998).

Nuclear Translocation of STATS

All lymphokines induce gene transcription by activating the **JAK/STAT signaling pathway** (Darnell et al., 1994; Ihle, 1995). The binding of lymphokines to their binary receptor complexes leads to the activation of **JAK** and subsequent tyrosine phosphorylation of **STATs**. This is followed by binding of the **SH2** domain of **STAT** to pTyr sites on homotypic or heterotypic **STATs** enabling formation of **STAT** homodimers or heterodimers. The dimeric **STATs** migrate to the nucleus to activate transcription in a target DNA sequence designated the GAS element. In addition to their central role in signaling via lymphokine receptors, there is good evidence that **STATs** play a role in signaling via **RTKs**. **PDGF**, **EGF**, or **FGF** stimulation leads to rapid tyrosine phosphorylation and migration of **STATs** to the nucleus and transcription of target DNA genes. The transcriptional program initiated by **STATs** is an integral component of the genetic program induced by growth factor stimulation. Moreover, there is good evidence that **STAT3** plays a role in PTK-induced oncogenic transformation, as constitutively dimeric forms of **STAT3** promote tumor formation.

Mechanism of Signal Attenuation and Termination

The activity of **RTKs** must be tightly regulated and properly balanced in order to mediate their normal cellular tasks and their many physiological responses. Indeed, aberrant expression or dysfunction of **RTKs** is responsible for several diseases and developmental disorders. It is to be expected, therefore, that several mechanisms exist for the attenuation and termination of **RTK** activity induced by stimulatory ligands.

Antagonistic Ligands

In *Drosophila*, activation of the **EGFR** homolog by an EGF-like factor (e.g., **Spitz**) leads to the expression of a secreted EGF-like protein designated **Argos**. Genetic and biochemical experiments suggest that **Argos** binds to **EGFR**, competes with **Spitz** for receptor binding, and inhibits **EGFR** activity. It has been proposed that the regulated expression of an **EGFR** agonist (**Spitz**), and **EGFR** antagonist (**Argos**) is essential for the control of various regulatory networks in which **EGFR** plays an important role in *Drosophila* development (Casci and Freeman, 1999). No vertebrate homolog of **Argos** has been identified and the mechanism of its antagonistic activity is not yet understood (Jin et al., 2000).

Another example of an **RTK** antagonist comes from the family of **angiopoietins**. **Angiopoietins** belong to a family of multimeric proteins that regulate mammalian vascularization and angiogenesis. **Angiopoietins** bind specifically to and activate **Tie2**, an **RTK** expressed on the surface of endothelial cells that is implicated in the control of vascularization and angiogenesis. Interestingly, one group of **angiopoietins** inhibits the biological responses mediated by the **Tie2** receptor (Maisonpierre et al., 1997). It is thought that the spatiotemporal expression of the stimulatory and inhibitory **angiopoietins** is critical for shaping and remodeling the vascular system during development. Moreover, the degree of receptor oligomerization induced by the inhibitory or stimulatory **angiopoietins** may determine biological outcome.

Hetero-Oligomerization with Receptor Mutants

In addition to transcripts encoding for full-length **RTKs**, certain tissues express naturally occurring soluble or membrane-linked receptor variants that are deficient in **RTK** activity. Expression of an inactive deletion mutant in the same cell may result in dominant negative inhibition of full-length receptor through generation of inactive heterodimers or hetero-oligomers (Jaye et al., 1992). It is thought that one biological role of mutant receptor variants coexpressed in the same cell with full-length receptors is to provide a mechanism for attenuating of the signal generated by ligand stimulation of the full-length receptor.

Inhibition of RTK Activity

Activation of **protein kinase-C (PKC)** by G protein-coupled receptors or by **PDGF** or phorbol-esters (PMA) results in **EGFR** phosphorylation on multiple Ser and Thr residues, including Thr654 in the juxtamembrane domain of **EGFR**. PKC-induced phosphorylation of **EGFR** results in an inhibition of its **PTK** activity and in strong inhibition of **EGF** binding to the extracellular ligand binding domain (Cochet et al., 1984; Davis and Czech, 1985). PKC-mediated phosphorylation of the juxtamembrane domain of **EGFR** thus appears to provide a negative feedback mechanism for control of receptor activity.

SOCS (suppressor of cytokine signaling) belongs to a family of proteins that function as negative regulators for feedback inhibition in response to cytokine stimulation (Hilton et al., 1998). It has been shown that **SOCS** proteins inhibit signaling in response to cytokine stimulation by direct binding to the **PTK** domain of **JAK** via their **SH2** domains. There is now evidence that insulin stimulation induces the expression of **SOCS-3** and that **SOCS-3** binds directly to the **IR** suggesting that a similar negative feedback mechanism may take place in signaling via **RTKs** (Emmanuelli et al., 2000).

Inhibition by Tyrosine Phosphatases

Protein tyrosine phosphatases (PTP) play an important role in the control of **RTK** activity and the signaling pathways that they regulate. Virtually all **RTKs** can be activated, even in the absence of ligand binding, by treatment of cells with **PTP** inhibitors. This experiment demonstrates that the activity of **RTKs** is continuously being monitored and checked by inhibitory **PTPs**. The protein tyrosine kinase activity of most **RTKs** is positively regulated by one or several phosphotyrosine sites in the activation loop. **Protein tyrosine phosphatases** that dephosphorylate these regulatory p-Tyr residues will inhibit **RTK** activity and the biological responses mediated by downstream effectors that depend on **PTK** activity. It was recently demonstrated that targeted gene disruption of **PTP1B** in mice leads to hyperphosphorylation of **IR** and **IRS1** and sensitization of signaling via the **IR** in vitro and in the mutant mice. These data argue that **PTP1B** is an important negative regulator of **IR** (EIchebly et al., 1999).

Receptor Endocytosis and Degradation

Growth factor stimulation results in rapid endocytosis and degradation of both the receptor and the ligand. Ligand binding induces receptor clustering in coated pits on the

cell surface, followed by endocytosis, migration to multivesicular bodies and eventual degradation by lysosomal enzymes. It has been shown that degradation of **EGFR** is dependent on protein tyrosine kinase activity and that a kinase-negative receptor mutant recycles to the cell surface for reutilization (Ullrich and Schlessinger, 1990). The rapid endocytosis and degradation of activated **EGFR** and other **RTKs** attenuates the signal generated at the cell surface in response to growth factor stimulation. Recent studies suggest that the oncogenic protein **Cbl** plays a role in regulating **EGFR** and **PDGFR** degradation. **Cbl** contains several subdomains, including an SH2-like domain that is responsible for binding to activated **RTKs**, and a **RING finger domain** that functions as a ubiquitin ligase. Binding of **EGFR** or **PDGFR** to **Cbl** leads to ubiquitination of the receptor and subsequent degradation by the proteosome (Joazeiro et al., 1999). On the other hand, complex formation with activated receptors results in tyrosine phosphorylation of **Cbl** followed by recruitment to it of signaling proteins such as **PI-3 kinase**, arguing that **Cbl** may also function as a docking protein for recruitment of effector proteins.

Coupling with Heterologous Signaling Pathways

In recent years it has become apparent that **RTKs** and the signaling pathways they activate are part of a large signaling network that can be regulated by multiple extracellular cues such as cell adhesion, agonists of G protein-coupled receptors, lymphokines or stress signals (Carpenter, 1999). It has also been shown that cell adhesion via integrin receptors leads to activation of several **RTKs** including the receptors for insulin, **EGF**, **PDGF**, and **FGF** resulting in tyrosine phosphorylation of target proteins and activation of signaling pathways that are normally activated by these receptors. It has been proposed that receptor activation induced by cell adhesion is mediated by coclustering of integrins with **RTKs**, although the precise mechanism of complex formation between integrins and **RTKs** is not understood.

RTKs have also been shown to be activated by membrane depolarization, by various stress responses including hyperosmotic conditions and ultraviolet radiation, as well as by G protein-coupled receptors (Carpenter, 1999). Agonists of several G protein-coupled receptors (e.g., endothelin, lysophosphatidic acid, angiotensin, and thrombin) have been shown to stimulate the tyrosine phosphorylation of **EGFR** or **PDGFR**. It has also been proposed that **EGFR** and **PDGFR**, as well as the nonreceptor **PTKs**, **Src** and **PYK2**, are crucial for coupling G protein-coupled receptors stimulation with the **Ras/MAP kinase** signaling cascade (Luttrell et al., 1999; Hackel et al., 1999). However, it is not yet clear how Gi- and Gq-dependent pathways activate these protein tyrosine kinases. Moreover, **MAP kinase** stimulation induced by G protein-coupled receptors is normal in fibroblasts deficient in **EGFR** or in **Src kinases**.

There is also good evidence for coupling between **EGFR** signaling and the signaling pathway activated by **transforming growth factor-β (TGFβ)** receptors. The **TGFβ** family of cytokines mediate their biological responses by binding to and activating a hetero-tetrameric complex composed of receptors with Ser/Thr activity designated **TGFβ receptor-I** and **-II** (Massague et al., 2000). Stimulation of **TGFβ** receptors results in the phosphorylation of **Smad proteins**, followed by their translocation to the cell

nucleus and consequent enhancement of transcriptional activity of target genes. **EGF** exerts an inhibitory response on **TGFβ** signaling, by inducing phosphorylation of **Smad proteins** at specific sites that prevent nuclear translocation and cause an inhibition of transcriptional activity (Kretzschmar et al., 1997; de Caestecker et al., 1998; Zhang et al., 1998b).

> It is already apparent that signaling pathways do not function in isolation, and cannot be presented or considered in a simple linear fashion as would be proposed by genetic analyses. A more realistic picture is that signaling pathways are linked together in a large protein network that is subjected to multiple stimulatory and inhibitory inputs, as well as complex feedback mechanisms. Such complexity is essential for mediating the pleiotropic responses of growth factors in development and in the adult animal.

Factors that Determine the Specificity of Signaling Pathways

A major unanswered question in the field of signal transduction concerns the origin of signal specificity. How are the myriad of extracellular cues transmitted to induce specific biological responses? It is not at all clear how activation of a given **RTK** at the cell membrane by a specific ligand could utilize the currently known repertoire of intracellular signaling pathways to transduce a unique biological response. Insulin and **NGF**, for instance, stimulate unique biological responses in their target tissues. Yet, the intracellular signaling pathways that are activated by insulin, **NGF**, or other growth factors are very similar indeed. In other instances, activation of the same signaling molecules in different cells leads to distinct responses. Why, for example, does stimulation of **PI-3 kinase** by insulin in muscle cells result in enhancement of metabolic processes, while stimulation of **PI-3 kinase** by **NGF** in neuronal cells leads to an antiapoptotic signal? Moreover, what are the factors that determine the biological outcome of a signal generated by a given receptor tyrosine kinase in different cellular context? Why does stimulation of an **RTK** (e.g., **TrkA**, **FGFR**, **Ret**) in fibroblasts result in cell proliferation whereas stimulation of the same **RTK** in neuronal cells results in cell differentiation? Several mechanisms have been proposed for the control of specificity in cell signaling.

Combinatorial Control

Signal specificity may be defined in part by a combinatorial control. Every **RTK** recruits and activates a unique set of signaling proteins via its own tyrosine autophosphorylation sites and by means of the tyrosine phosphorylation sites on closely associated **docking proteins** (e.g., **Gab1**, **FRS2**). The combinatorial recruitment of a particular complement of signaling proteins from a common preexisting pool of signaling cassettes is one mechanism for control of signal specificity. This process is further regulated by differential recruitment of stimulatory and inhibitory proteins by the different receptors and downstream effector proteins leading to fine tuning of cellular responses.

The Role of Scaffold Proteins

It has been shown that **scaffolding proteins** that bind simultaneously to several proteins are able to insulate key components of signaling pathways from closely related signaling cascades (Whitmarsh and Davis, 1998). In yeast, the scaffolding protein **Ste5** has been shown to interact with a pheromone-activated G protein and with components of **MAP kinase** cascade. **Ste5** forms a complex with **Ste11**, **Ste7**, and **Fus3P** leading to insulation of pheromone-induced **MAP kinase** cascade from closely related signaling pathways. Another example is **JIB**, a protein that functions as a scaffolding protein in the **JNK** signaling cascade in mammalian cells (Davis, 2000). There is also evidence that particular members of the **MAPK** cascade form a complex with a specific upstream activating kinase and downstream effector-kinase to provide insulation from other **MAP kinase** cascades (Kallunki et al., 1994). It remains to be determined whether **RTKs** induce specific biological responses by utilizing specific scaffold proteins.

Cellular Compartmentalization

In recent years it has become apparent that the cellular localization of proteins involved in cell signaling has a profound impact on their biological activity. As many of the targets of **RTKs** are located at the cell membrane, membrane translocation is required for activation of many cellular processes. Binding of **SH2**, **PTB**, or **SH3** domains to activated receptors or to membrane-linked **docking proteins** leads to membrane translocation. In addition, membrane translocation is regulated in part by **PH** or **FYVE** domains, two protein modules that bind to different phosphoinositides. It has been shown that binding of proteins containing **PDZ** domains to their canonical target sequences at the C termini of signaling proteins will induce the assembly of specific sets of signaling proteins in specific regions at the inner face of the cell membrane. Protein assembly at the cell membrane, mediated by multi-**PDZ** domain containing proteins, may facilitate the phosphorylation of specific substrates by a kinase that is part of the same complex or activation of a GTPase by an exchange factor that is located at the same assembly.

It has been proposed that a variety of proteins that are involved in cell signaling are concentrated in cholesterol-rich microdomains designated "**membrane rafts**" (Simons and Ikonen, 1997). It is thought that "**membrane rafts**" function as sites of assembly of proteins involved in cell signaling including cell surface receptors, GPI-liked proteins, **Src kinases**, and **Ras** proteins (Brown and London, 2000). However, it is not clear yet whether **membrane rafts** exist in the context of living cells (Edidin, 1997) or whether this phenomenon represents an artifact caused by detergent solubilization.

The translocation of **STAT** proteins from the cell membrane into the nucleus is another example for the role of protein localization in cell signaling (Darnell et al., 1994; Ihle, 1995). Initially, **STAT** proteins are bound to the cytoplasmic domains of lymphokine receptors in proximity to protein tyrosine kinases of the **JAK family**. Stimulation of lymphokine receptor or **RTKs** leads to tyrosine phosphorylation of **STAT** resulting in homotypic or heterotypic dimerization followed by nuclear translocation and regulation of transcription of target genes.

Signal Duration and Amplitude

Cellular signaling pathways could be considered as components of intracellular circuits that are generated by protein networks. According to this view, signal transmission and biological outcome should be affected by quantitative considerations such as signal duration and signal strength (Marshall, 1995). For instance, **RTKs** that induce transient stimulation of **MAPK** (e.g., **EGFR**, **IR**) stimulate PC12 cell proliferation while **RTKs** that stimulate a sustained and robust **MAPK** response (e.g., **NGFR**, **FGFR**) promote neuronal differentiation of the same cells. In fact, overexpression of **IR** or **EGFR** in PC12 cells leads to sustained **MAPK** response resulting in cell differentiation, although the same receptors give a proliferative response when expressed at lower levels.

> These experiments shows that biological outcome (proliferation versus differentiation) is determined by quantitative modulation of signal threshold (Marshall, 1995).

Signal threshold can be determined by the specific activity of a given **RTK**, and by the balanced action of the various inhibitory or stimulatory signals that are activated by the **RTK**. For example, the signal generated by an **RTK** can be prolonged by generation of hydrogen peroxide that blocks inhibitory protein tyrosine phosphatases or by phosphorylation of **docking proteins** that promote signal amplification by recruiting of multiple signaling molecules. Signaling pathways are also subjected to multiple negative feedback mechanisms at the level of the receptor itself by inhibitory protein tyrosine phosphatases and by receptor endocytosis and degradation. In addition, the specific activity of key effector proteins can be negatively regulated by inhibitory signals. For example, **MAPK** responses are inhibited by protein phosphatases that dephosphorylate and inactivate this enzyme. The two phosphoinositide phosphatases **PTEN** and **SHIP** dephosphorylate specifically the 3' or 5' phosphate of the Ptdlns(3,4,5)P3 inositol ring, respectively, leading to inhibition of cellular responses mediated by **PI-3 kinase** products. The balance between the various stimulatory and inhibitory responses will ultimately determine the strength and duration of the signals that are transmitted through the networks of signaling cascades following their initiation at the cell surface in response to **RTK** stimulation.

Cellular Context

The biological outcome of signals generated at the cell surface in response to **RTK** stimulation is strongly dependent on cellular context. The same **RTK** will induce a totally different response when expressed in different cells or at different stages of differentiation of a particular cell lineage (Sahni et al., 1999). For instance, in early development, **FGFR1** plays an important role in control of cell migration, a process crucial for mesodermal patterning and gastrulation. Stimulation of **FGFR1** in fibroblasts on the other hand, leads to cell proliferation while stimulation of **FGFR1** expressed in neuronal cells induces cell survival and differentiation. The most plausible explanation for these observations is that different cells express cell type-specific effector proteins and transcription factors that mediate the different responses. According to this view, **RTKs** and their signaling pathways are capable of feeding into multiple processes thus regulating the activity of different effector proteins and transcriptional factors in different cellular environments. A similar input can therefore generate a different output in a

different cellular context. In other words, signaling cassettes that are activated by **RTKs** have evolved in order to relay information from the cell surface to the nucleus and other cellular compartments irrespective of the biological outcome of their activation.

Finally, there is good evidence that critical signaling cascades are regulated by multiple and parallel steps leading to redundancy in signaling pathways. For example, activated **EGF** receptor recruits the adaptor protein **Grb2** directly and indirectly via **Shc** and **Gab1**. Therefore, **EGFR** mutants defective in **Grb2** binding are capable of recruiting the adaptor protein **Grb2** indirectly resulting in efficient activation of the **Ras/MAP kinase** signaling cascade. Another example is the redundancy seen in the expression and function of **Src kinases** (Klinghoffer et al., 1999). While most cells express at least three of the nine known members of the **Src family**, expression of a single **Src kinase** is sufficient for mediating an intracellular signal that requires a **Src family kinase**.

Conclusions

It has been 20 years since protein tyrosine phosphorylation was discovered (Hunter and Sefton, 1980). The last two decades have seen a rapid progress in the characterization of protein tyrosine kinases, the signaling pathways they activate, and the mechanisms underlying their action and regulation. With the complete determination of the sequences of the genomes of *C. elegans*, *Drosophila*, and *Homo sapiens*, the entire plethora of kinases, phosphatases, and signaling proteins has become accessible to biochemists and geneticists who are interested in deciphering the roles played by **RTKs** in normal biological processes and in pathological situations.

It is already clear that signaling pathways activated by **RTKs** are interconnected with other signaling pathways via protein networks that are subjected to multiple positive and negative feedback mechanisms. The frequently applied tool of targeted gene disruption used by geneticists for analyzing signaling pathways is complicated by the existence of redundant signaling pathways and because key components are sometimes shared by multiple signaling cascades. Consequently, more sophisticated tools should be developed and applied for the analysis of cellular signaling pathways. There is need for new techniques for determination of protein localization (Teruel and Meyer, 2000) and measurement of kinetics of cellular reactions in the context of living cells and even in the live animal. In addition, detailed analyses of gene expression patterns by microarray analysis of genes that are expressed in response to growth factor stimulation (Fambrough et al., 1999) of cells derived from normal or pathological tissues will reveal new links between signaling pathways. Finally, the modern biochemist and geneticists will have to adopt approaches that have been developed by engineers to describe complicated networks (e.g., system analysis) in order to obtain a coherent and realistic perspective on cell signaling (Levchenko et al., 2000; Jordan et al., 2000).

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