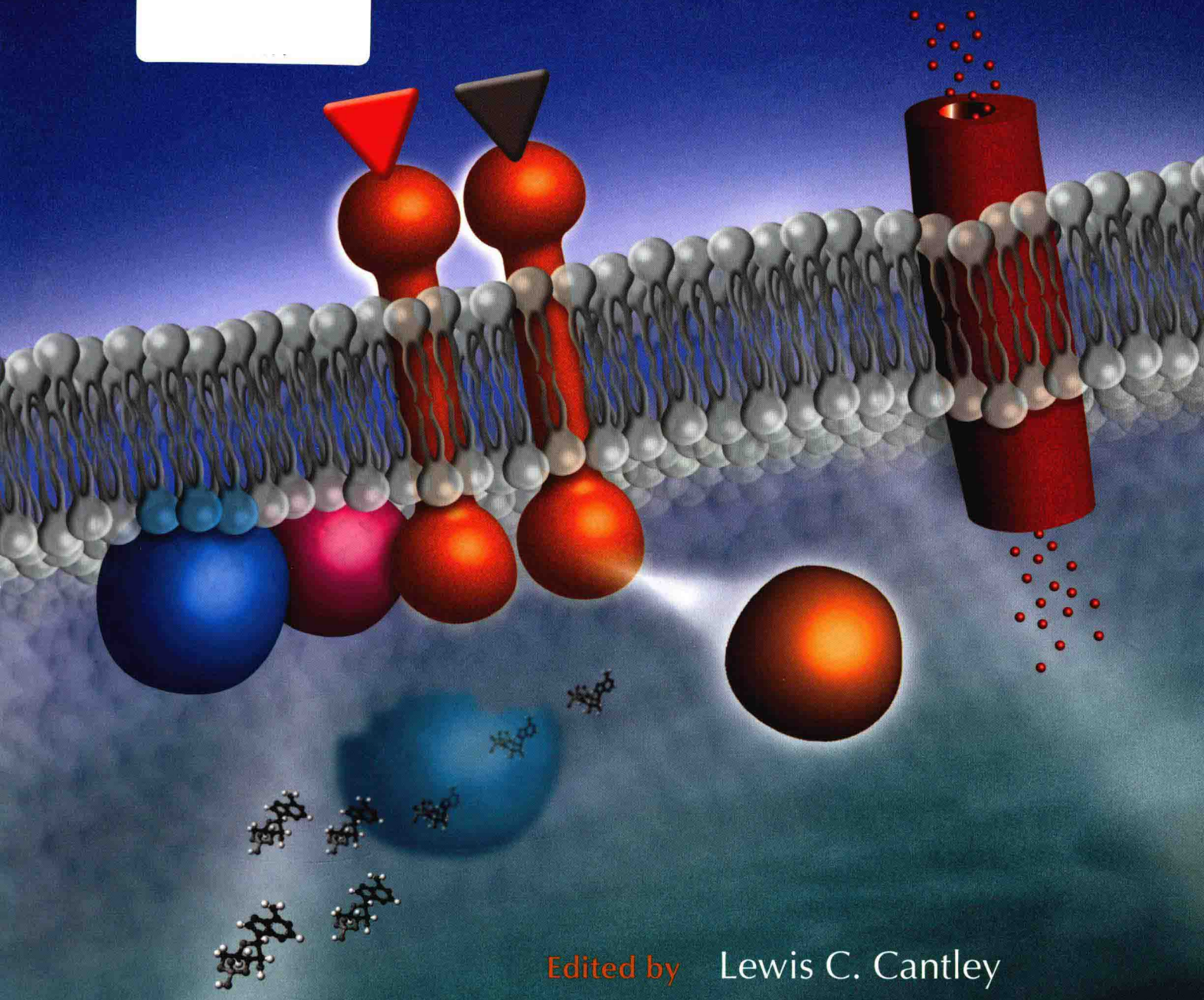
Signal Transduction

Principles, Pathways, and Processes



Edited by Lewis C. Cantley
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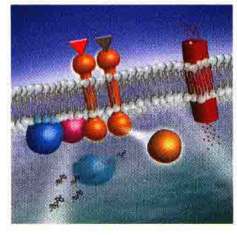

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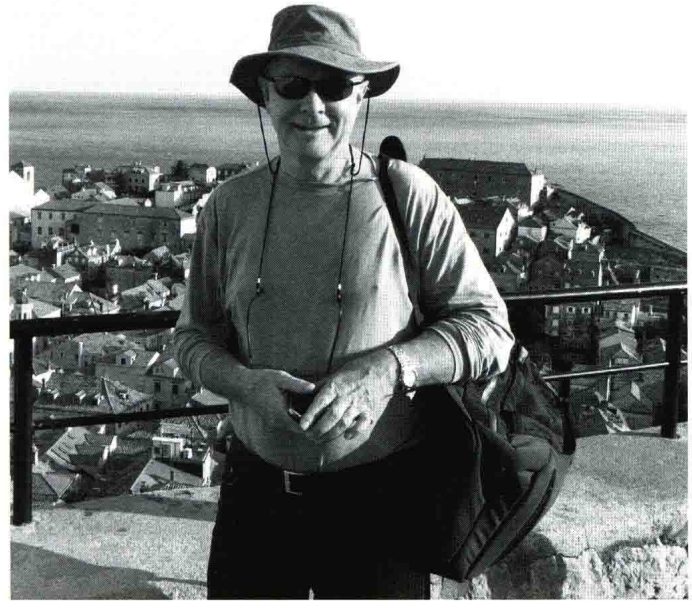

This book is dedicated to the memory of Tony Pawson (1952–2013). Tony was a giant in the field of signal transduction, who established principles of protein–protein interactions that have profoundly influenced our understanding of signal transduction. His enduring legacy will be the discovery that the Src homology 2 (SH2) domain of one protein can selectively interact with a tyrosine residue in a second protein, once it is phosphorylated in response to an upstream signal. This type of inducible protein–protein interaction can link intracellular signals generated in response to various upstream stimuli to downstream signaling events. This insight was the basis for his enormously influential idea that eukaryotic signaling systems involve modular and combinatorial interaction domains that propagate signals throughout the cell.

Preface

SIGNAL TRANSDUCTION PROCESSES CAN BE VIEWED as the higher command functions executed by cells on metabolic pathways (both catabolic and biosynthetic), macromolecular machinery and organellar compartments that allow an organism to maintain homeostasis and adjust cell number, cell behavior, and organismal physiology appropriately in response to internal cues and external stimuli. This book was conceived and organized as an instructional resource to introduce advanced students, investigators new to the field, and even researchers actively working in this general area to the underlying foundations and basic mechanisms of signal transduction in animal cells. Such a volume is needed because signaling impinges on every aspect of molecular and cellular biology—from biochemistry and structural biology to development and differentiation, endocrinology and systems biology, pharmacology and neuroscience, and immunology and cancer biology. Our objective is to explicate and illustrate the fundamental concepts, principles, and processes involved in signaling quite comprehensively, without necessarily being completely encyclopedic. We have taken a novel approach to conveying this large body of information and making it accessible, dividing the book up into distinct sections that describe principles, pathways, and processes.

The first four *principle* chapters set the stage, presenting molecular mechanisms and paradigms that are pertinent to all that follows. In Chapter 1, Carl-Henrik Heldin, Benson Lu, Ronald Evans, and Silvio Gutkind discuss signaling molecules and their receptors and downstream signaling events. In Chapter 2, Michael Lee and Michael Yaffe introduce the central role of proteins as transducers in signaling, describing the many ways by which signaling can control protein level, function, activity, and location. In Chapter 3, Alexandra Newton, Martin Bootman, and John Scott discuss the nature, generation, and action of intracellularly generated mediators ("second messengers"). In Chapter 4, Evren Azeloglu and Ravi Iyengar consider the circuit-like characteristics of signaling networks and systems, their emergent properties, and mathematical models we can use to describe them.

There follows a series of 14 *process* chapters that cover the roles of signaling in distinct biological processes and discuss how the general principles described in the four *principle* chapters apply in a specific context. Thus, the focus in these more specialized chapters is on the molecular basis of a particular aspect of signaling, its logic and its physiological consequences in biology, rather than a mere enumeration of pathway components and their interactions. Nonetheless, familiarity with signaling pathways used by cells is essential, and so separating the *principles* and *process* chapters are a series of *pathway* diagrams with short accompanying synopses written by other leaders in the field.

Different cell types possess a variety of mechanisms to sense and respond to diverse stimuli. Dedicated receptor cells, for example, respond to physical inputs from their surroundings, such as light, heat, and sound, as considered in the chapter by David Julius and Jeremy Nathans. The information is relayed via inorganic-ion-based electrical currents and release of and response to amino acids (glutamate and glycine), amino-acid-derived compounds, and other classes of substances that serve as neurotransmitters, as discussed in the chapters by Mary Kennedy and by Ivana Kuo and Barbara Ehrlich.

Cells respond to a plethora of other kinds of chemical signals, as disparate as inorganic substances (including gases) and a host of other organic molecules (from volatile substances to lipidic compounds to peptide hormones, growth factors, and morphogens), as presented in Chapter 1 and in the chapter by Norbert Perrimon, Chrysoula Pitsouli, and Ben-Zion Shilo. As discussed in Chapter 3, in many cases, the encounter with such extracellular ligands activates the production of second messengers, from phosphoinositides to cyclic nucleotides to less familiar, newly discovered metabolites. This allows amplification and spreading of the response by affecting the level, localization, and activity of numerous proteins and other cellular targets by mechanisms described in detail in Chapter 2. In addition to responses to native extracellular signals and normal internal cues, the specialized cells of our immune system must respond to attack by or internalization of potentially dangerous prokaryotic, viral and fungal pathogens, as reviewed in the chapters by Kim Newton and Vishva Dixit and by Doreen Cantrell. Microbes, in turn, have evolved an armamentarium of virulence factors and other effectors that they inject to specifically interdict signaling by lymphocytes and other cells, which also provide useful tools for experimentally interrogating signaling processes, as discussed in the chapter by Neal Alto and Kim Orth.

It is especially important that cells and tissues stay acutely attuned to their nutrient supply and adjust their metabolism accordingly. This aspect of signaling is described in the chapters by Patrick Ward and Craig Thompson and by Grahame Hardie. Cells also need to gauge their position in space and time and alter their morphology and adjust their movements in response to signals arising from cell–cell and cell–extracellular-matrix contacts, as presented in the chapters by Luke McCaffrey and Ian Macara and by Peter Devreotes and Rick Horowitz.

One reason for a cell to constantly gauge and integrate information about its nutrient supply, its developmental state, its neighboring cells, and demands of other tissues is to decide whether it should remain quiescent, grow and divide, or enter a developmental pathway leading to production of a highly specialized postmitotic cell type. The issue of how entry into the cell division cycle is controlled by signaling pathways is discussed in detail in the chapter by Robert Duronio and Yue Xiong. The internal, fail-safe signaling mechanisms (checkpoints) that ensure the proper spatial and temporal order of events in cell cycle progression, and act as delay timers to allow an adequate hiatus for any necessary repairs, are considered in the chapter by Nicholas Rhind and Paul Russell. When the normal signals that control the decision of cells to divide are subverted, and the negative controls on cell division are broken, malignant growth can occur. How defects in signaling lie at the heart of the molecular basis of cancers is discussed in the chapter by Richard Sever and Joan Brugge.

Concomitant with what may occur under optimal conditions, cells also have to cope with decisions about how to manage their resources and responses under more challenging and stressful conditions. Maybe the cell can overcome the problems, but, if it suffers irreversible harm to the integrity of its chromosomes, or to the functioning of a vital organelle, then alarm signals are in place to try to prevent any rogue or damaged cell from lingering. The signaling responses elicited by stressful conditions, and how those responses promote cell survival, are examined in the chapter by Gökhan Hotamisligil and Roger Davis. Conversely, how cells evoke and respond to the signals that lead to their own demise is described in the chapter by Douglas Green and Fabien Llambi.

Of course, most eukaryotes develop from multiplication of the single-celled zygote formed by the union of two germ cells, and how signaling is involved in gametogenesis and sexual reproduction is presented in the chapter by Sally Kornbluth and Rafael Fissore.

At the end of the book, we present an Outlook that provides some additional information and perspectives on recent developments (both methodological and conceptual) that further set the stage for future advances in the field of signal transduction. In it we discuss challenges and open questions that we hope will help point the way forward.

We would like to express our gratitude to all the authors who took time out of their busy schedules to contribute the fantastic chapters that make up this book. We also want to express our deep gratitude to the many investigators, too numerous to name individually here, who served as anonymous referees to evaluate the accuracy and effectiveness of the contents of this book. We would also like to thank Cell Signaling Technology, Inc., for financial support and for making available figures from which the pathway diagrams shown in the book were derived and adapted. Finally, we are indebted to Inez Sialiano, Diane Schubach, and Kathleen Bubbeo at Cold Spring Harbor Laboratory Press for all their hard work helping to get the book into print and online.

Jeremy Thorner
Richard Sever
Tony Hunter
Lewis C. Cantley

Foreword

THIS TEXTBOOK ON *Signal Transduction*, edited by some of the foremost experts in this area, presents an encyclopedic view of a field that essentially did not exist 60 years ago. In those days, almost nothing was known about the mechanism by which enzymes and physiological processes were regulated, and terms such as "signaling" or "signal transduction" that are so commonly used today would not have been understood.

First, although endocrinology was already well established as a discipline, it remained purely at the phenomenological, mostly intact animal, level. The action of hormones stopped at the cell membrane and what happened next was totally unknown until Earl Sutherland and Ted Rall came along with their stunning discovery of cAMP, which served as a second, intracellular messenger for the action of epinephrine. Second, there was a fundamental difference in the way science was conducted. At that time and, in fact, since the days of Claude Bernard in the second half of the 19th century, one first observed a physiological phenomenon and then tried to identify the factors or enzymes involved. Whereas today, by and large, it is the other way around: new proteins are first identified mostly through genome sequencing projects and then, by overexpressing them or by knocking them in or out, one tries to define their function. Finally, essentially nothing was known about enzyme regulation. The prevailing idea was that they were regulated simply by the rate at which they were synthesized and degraded. But in the late 1940s/early 1950s, people began to realize that this could not be the case, that this would not work because protein synthesis and degradation are far too slow. Cells had to have ways of modulating the activity of their enzymes once they had been produced and liberated within the cells. They had to have the capability of adapting to their environment, of satisfying their metabolic needs, almost instantaneously in response to whatever internal or external demands are placed upon them. And this is where cell signaling and signal transduction came into play.

These fields did not originate from a single, explosive breakthrough or discovery. They grew step-by-step through successive small advances in the second half of the last century, originating perhaps with the finding that the control of glycogen phosphorylase, an enzyme shown by the Coris to catalyze the first step in the degradation of glycogen, occurs through a phosphorylation–dephosphorylation reaction. Since then, reversible protein phosphorylation has been found to be one of the most prevalent and versatile means by which cellular processes are regulated, being involved in the control of metabolism, gene expression, the immune response, cell development and differentiation, and what not. In fact, it would be difficult to find a physiological process that would not be, directly or indirectly, regulated by this kind of mechanism. It is implicated in innumerable hereditary diseases and pathological conditions, such as diabetes, Alzheimer's and Parkinson's diseases, and myelogenous leukemia, in viral diseases such as smallpox, and bacterial diseases such as cholera and plague.

Quantitatively, better than 99.9% of all these phosphorylation reactions occur on serine and threonine. But one of the most exciting developments in this field was the discovery, more than 30 years ago, that phosphorylation of proteins on tyrosyl residues was intimately implicated in cell transformation and oncogenesis, bringing into play a multitude of tyrosine kinases of cellular or viral origin, or linked to growth factor receptors.

Although reversible protein phosphorylation seemed to be for many years the main form of cellular regulation, a just as prevalent and far more complex regulatory mechanism has since been uncovered—namely, ubiquitylation. And it is very likely that other general regulatory systems might come to light, such as reversible protein acetylation, methylation, and oxidoreduction or the interaction of enzymes with their specific binding modules, anchors, and chaperones.

These advances could not have been possible without the development of sophisticated methodologies such as X-ray crystallography, nuclear magnetic resonance, mass spectrometry, and cryo-electron microscopy for protein structure determination and nanochemistry and the use of nanoparticles, monoclonal antibodies, and genetically encoded fluorescent marker proteins allowing one to monitor molecular processes without disrupting cell function.

Of course, the most spectacular advance occurred in genetic engineering with the cloning, manipulation, expression, and sequencing of genes, without which we would know essentially nothing about our genetic makeup or about a variety of hereditary and viral diseases. With the pervasive presence of the computer that allows one to

display and analyze data and store and retrieve them at the touch of a button, today's investigators have at their disposal an array of technologies absolutely undreamed of just a few years ago.

Finally, what are some of the main problems that remain to be solved in signal transduction? Most of the major signaling pathways have probably been elucidated, and the structure, properties, regulation, and physiological function of the molecules involved have been well characterized. But these molecules are only the words the cells use to perform their daily chores. We know many of these words; we recognize probably bits and pieces of some of the sentences they spell out to elicit a particular response. But we are only just starting to understand the language the cell has to use to allow different receptors or pathways to speak with one another to coordinate all the reactions that take place. This communication often occurs through the formation of large macromolecular complexes comprising anchoring and scaffolding proteins and modules that link them to the cytoskeleton, providing those systems with the specificity and selectivity they require; however, how cells maintain and preserve the fidelity of signaling processes remains poorly understood.

The problem is further complicated by the fact that during the several billion years over which cells have evolved, they have had all the opportunities in the world to put in place the vast array of secondary or parallel pathways, shunts, compensatory mechanisms, feedback loops, and fail-safe systems they need to regulate their growth and development, to protect themselves against all sorts of adversity, and to program their own death when the time comes. And we do not know the myriads of signals that must exist to sort out all the reactions that take place.

Perhaps even more importantly, we do not understand the cross talk—the interactivity that must exist among cells and how they communicate with one another to synchronize their behavior in response to internal or external signals. This cross talk, this sharing of information, is crucial for the establishment of such sophisticated networks of communication as seen, for instance, during embryonic development and organogenesis, in the immune system, or in the infinitely more complex central nervous system, where a thousand billion cells speak with one another through more than a million billion synapses, leading ultimately to the generation of memory and thought and consciousness. Solving these problems will be one of the major challenges that will confront biologists in the years to come.

This textbook on signal transduction addresses most of these problems. It is directed toward future practitioners of biology and medicine: advanced graduate students, postdoctoral fellows, or researchers working in an academic, biotechnological, or pharmaceutical environment. It will be of enormous help to all those who would want to remain abreast of the field.

EDMOND FISCHER
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Contents

SECTION I

GENERAL PRINCIPLES AND MECHANISMS

CHAPTER 1

Signals and Receptors

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SUMMARY

Communication between cells in a multicellular organism occurs by the production of ligands (proteins, peptides, fatty acids, steroids, gases, and other low-molecular-weight compounds) that are either secreted by cells or presented on their surface, and act on receptors on, or in, other target cells. Such signals control cell growth, migration, survival, and differentiation. Signaling receptors can be single-span plasma membrane receptors associated with tyrosine or serine/threonine kinase activities, proteins with seven transmembrane domains, or intracellular receptors. Ligand-activated receptors convey signals into the cell by activating signaling pathways that ultimately affect cytosolic machineries or nuclear transcriptional programs or by directly translocating to the nucleus to regulate transcription.

Outline

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1 INTRODUCTION

Cells within multicellular organisms need to communicate with each other to coordinate their growth, migration, survival, and differentiation. They do so by direct cell–cell contact and secretion or release of molecules that bind to and activate receptors on the surface of or inside target cells. Such factors can stimulate the producer cell itself (autocrine stimulation), cells in the immediate vicinity (paracrine stimulation), or cells in distant organs (endocrine stimulation). The signaling induced within target cells is important during embryonic development, as well as in the adult, where it controls cell proliferation, differentiation, the response to infection, and numerous organismal homeostatic mechanisms.

Many cell-surface receptors contain an extracellular ligand-binding region, a single transmembrane segment, and an intracellular effector region, which may or may not have an associated enzyme activity. Some receptors contain multiple subunits that together form the ligand-binding site. Others, including those encoded by the largest gene family in the human genome, consist of a polypeptide that spans the cell membrane seven times. Finally, there are receptors that are located intracellularly and are activated by ligands that cross the cell membrane, such as steroid hormones. Below, we describe the major families of ligands and receptors and the signal transduction mechanisms they activate.

2 CELL-SURFACE RECEPTORS

2.1 Receptors with Associated Protein Kinase Activity

Several types of cell-surface receptors contain or are associated with kinase activities that respond to the binding of a ligand. Perhaps best understood are receptors with intrinsic protein tyrosine kinase domains. This receptor tyrosine kinase (RTK) family has more than 50 human members (Lemmon and Schlessinger 2010). RTKs have important roles in the regulation of embryonic development, as well as in the regulation of tissue homeostasis in the adult. Each has an extracellular, ligand-binding region, which consists of different combinations of various domains, such as Ig-like, fibronectin type III, and cysteine-rich domains. This is linked to a single transmembrane segment and an intracellular region that includes a tyrosine kinase domain. Based on their structural features, RTKs can be divided into 20 subfamilies (Fig. 1), a well-studied example being the epidermal growth factor (EGF) receptors (EGFRs).

Members of the cytokine receptor family in contrast lack intrinsic kinase activity but associate with intracellular kinases. They have important roles in the regulation of the immune system and also promote cell differentiation. Cytokine receptors can be divided into two classes. The extracellular domains of class I cytokine receptors contain cytokine receptor homology domains (CHDs) consisting of two tandem fibronectin type III domains with a characteristic WSXWS motif in the second (Liongue and Ward 2007). Based on the number of CHDs and the presence of other types of domains, the class I cytokine receptors can be divided into five groups (Fig. 2), the growth hormone (GH) receptor being typical of the first group. Interferon receptors are typical of the 12-member class II cytokine receptor family, which also have extracellular regions based on tandem fibronectin domains but differ from those of class I receptors (Fig. 2) (Renauld 2003). Both classes of cytokine receptors have conserved box 1 and box 2 regions in their intracellular regions, which bind to JAK family tyrosine kinases that are activated upon ligand binding. The multisubunit antigen receptors on B cells and T cells (Zikherman and Weiss 2009) and the Fc receptors present on macrophages, mast cells, basophils, and other immune cells (Nimmerjahn and Ravetch 2008) are also associated with intracellular tyrosine kinases; activation of these receptors involves tyrosine phosphorylation by members of the Src family, followed by docking and activation of SH2-domain-containing Syk/Zap70 tyrosine kinases (see p. 125 [Samelson 2011]; Ch. 16 [Cantrell 2014]). Although receptors that have intrinsic tyrosine kinase activity and those associated with tyrosine kinases are structurally different and bind ligands of different kinds, the principles underlying their activation and the intracellular signals they initiate are similar (see below).

There is also a family of receptors that have intrinsic serine/threonine kinase domains, and these respond to members of the transforming growth factor β (TGFβ) family (see Moustakas and Heldin 2009; p. 113 [Wrana 2013]). The human genome has only 12 genes encoding receptors of this type (Fig. 3). These receptors have rather small cysteine-rich extracellular domains; their intracellular domains are most often also small and consist mainly of the kinase domains. TGFβ receptors mediate signaling events during embryonic development. Because they often inhibit cell growth, they also exert a controlling function on the immune system and other tissues.

2.2 Ligands

Each of these different receptor types responds to a subfamily of structurally similar ligands. The ligands are normally small monomeric, dimeric, or trimeric proteins, often derived by proteolytic processing from larger precursors, some of which are transmembrane proteins. There is not a strict specificity in ligand–receptor interactions with-

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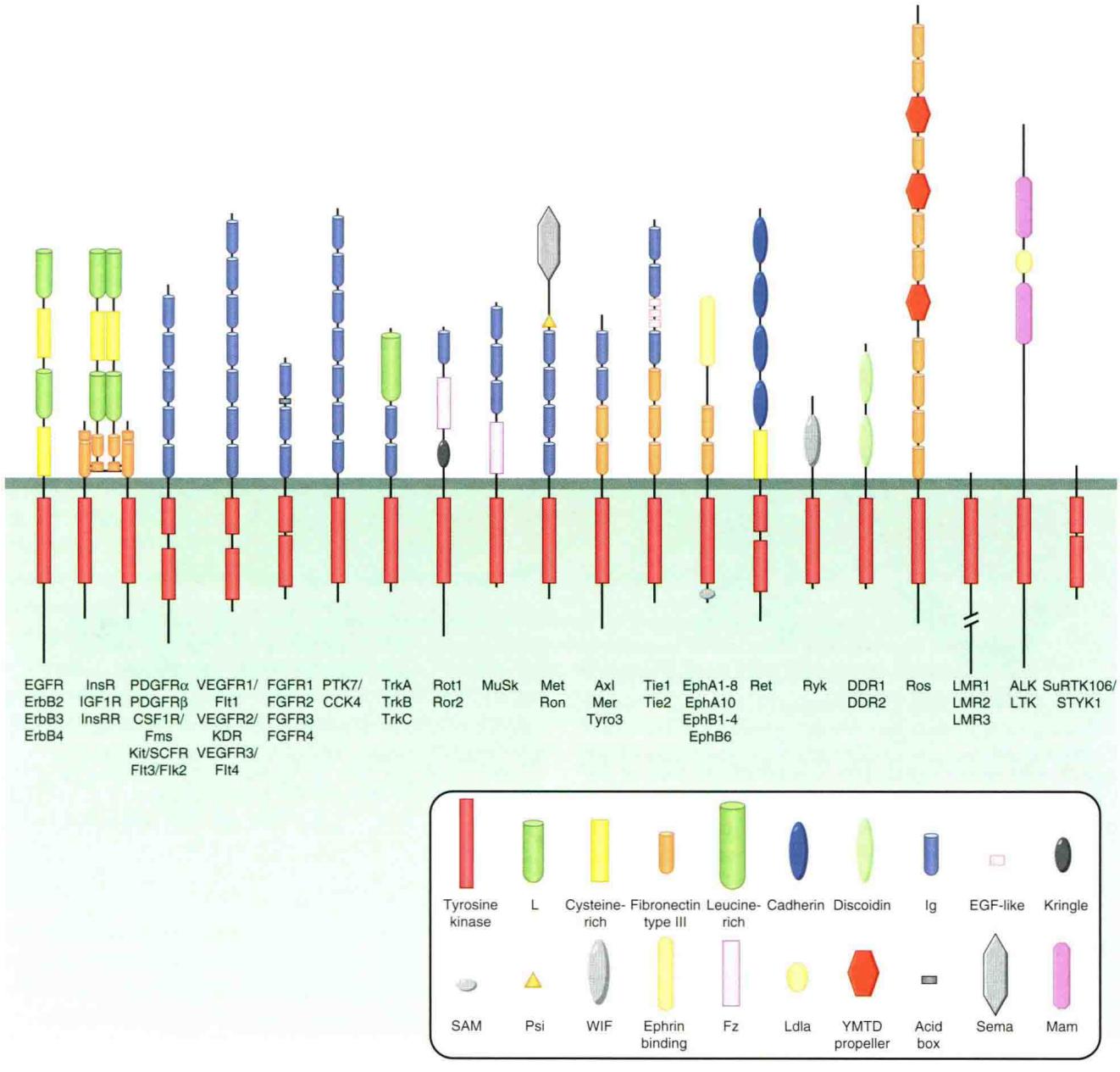

Figure 1. Receptor tyrosine kinase (RTK) families. The 20 subfamilies of human RTKs and their characteristic structural domains are shown. The individual members of each family are listed below. (From Lemmon and Schlessinger 2010; adapted, with permission.)

in the families; normally each ligand binds to more than one receptor, and each receptor binds more than one ligand. Although it is rare that ligands for completely different types of receptors bind to kinase-associated receptors, examples do exist.[4]

2.3 Activation by Dimerization

A common theme for activation of kinase-associated receptors is ligand-induced receptor dimerization or oligomerization (Heldin 1995). The juxtaposition of the intracellular kinase domains that occurs as a consequence allows autophosphorylation in *trans* within the complex. For RTKs, the autophosphorylation has two important consequences: it changes the conformation of the kinase domains, leading to an increase in their kinase activities; and it produces docking sites (phosphorylated sequences) for intracellular signaling molecules containing SH2 or PTB domains (see

[4]The Ryk and Ror families of RTK, for example, bind members of the Wnt family. Ryk has a Wnt inhibitory factor-1 (WIF1) domain, and the two Ror receptors have cysteine-rich domains related to a domain in the Frizzled family of serpentine receptors to which ligands of the Wnt family bind (van Amerongen et al. 2008).