Dimerization of Cell Surface Receptors in Signal Transduction

Review

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Introduction

Cell growth, differentiation, migration, and apoptosis are in part regulated by polypeptide growth factors or cytokines. As these factors are unable to pass the hydrophobic cell membrane, a fundamental question is how they transduce their signals into the cell. Growth factors and cytokines exert their effects via binding to cell surface receptors; results obtained during recent years have given ample evidence that such receptors often are activated by ligand-induced dimerization or oligomerization. Moreover, the elucidation of intracellular signal transduction pathways have revealed that the activity of several components in these pathways are also regulated by dimerization. For instance, certain of the cytoplasmic signal transduction molecules dimerize after activation, and the active form of transcription factors are often dimers. It thus appears that dimerization is a mechanism of general applicability for the regulation of signal transduction.

This review focuses on the role of dimerization of cell surface receptors in signal transduction. Dimerization or oligomerization have been shown to occur after binding of several polypeptide hormones, cytokines, growth factors, or growth inhibitors to their receptors. Examples include protein-tyrosine kinase receptors, cytokine receptors, antigen receptors, receptors for tumor necrosis factor (TNF) and related factors, and serine/threonine kinase receptors (Figure 1; Table 1). There are, however, many variations on the theme, as will be discussed below.

Protein-Tyrosine Kinase Receptors

Many traditional growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF), bind to receptors with tyrosine kinase activity (Table 1). Protein-tyrosine kinase receptors consist of single transmembrane domains separating the intracellular kinase domains from extracellular domains, which typically contain one or several copies of immunoglobulin-like domains, fibronectin type III–like domains, EGF-like domains, cysteine-rich domains, or other domains (reviewed by Fantl et al., 1993). Based on their structural characteristics, the tyrosine kinase receptors can be classified into families; the largest families are listed in Table 1.

Several of the ligands for protein-tyrosine kinase receptors are dimeric molecules, which thus contain two identical receptor-binding epitopes. Examples include PDGF and colony-stimulating factor 1 (CSF-1), which are disulfide-bonded dimers, and stem cell factor (SCF), which is a dimer held together by noncovalent forces. These ligands form stable receptor dimers by simultaneously binding two

receptors. In addition to the bridging of the ligand between two receptors, it is possible that direct interactions between the receptors, involving epitopes located outside the ligand-binding domains, are important for stabilization of the receptor dimer. In the case of the SCF receptor, evidence has been presented that epitopes in the fourth immunoglobulin domain are involved in such receptor–receptor interactions (Blechman et al., 1995). It is possible that such direct receptor–receptor interactions are promoted by conformational changes in the receptors induced by ligand binding. Other ligands, like EGF, have apparent monomeric configurations; interestingly, however, recent calorimetric studies have shown that a single EGF molecule also can bind simultaneously to two receptor molecules (Lemmon and Schlessinger, 1994). Another variation on the theme is exemplified by ligands for Eph-related tyrosine kinase receptors. These ligands are cell surface attached and do not activate receptors in soluble form. The possibility that receptor dimerization or clustering is involved in receptor activation, presumably facilatated by membrane attachment of ligands, is supported by the finding that antibody-mediated clustering of soluble receptors led to activation of receptors (Davis et al., 1994).

Receptor Autophosphorylation

Dimerization of protein-tyrosine kinase receptors is followed by receptor "autophosphorylation," which mainly occurs by one receptor molecule phosphorylating the other in the dimer (Ullrich and Schlessinger, 1990). The autophosphorylation occurs on two principally different classes of tyrosine residues. On one hand, autophosphorylation is commonly seen on a conserved tyrosine residue within the kinase domains (Tyr-857 in the PDGF β receptor; Figure 2). In the cases of the receptors for insulin and hepatocyte growth factor (HGF), phosphorylation of the tyrosine residue at this and neighboring sites leads to an increase in the kinase activity and precedes phosphorylation of other sites in the receptor or substrates (Naldini et al., 1991; White et al., 1988). This thus appears to be an allosteric site that regulates the V_{max} of the receptor kinase. It is still not known how the autophosphorylation is initiated; one possibility is that the monomeric receptor has a low basal kinase activity, which is sufficient to phosphorylate and activate the companion receptor after dimerization. This would then rapidly be followed by reciprocal phosphorylation. Alternatively, the interaction between the intracellular domains of the receptors in the dimer may induce a conformational change that leads to an increased kinase activity. Not all receptors are regulated by phosphorylation inside the kinase domain, e.g., in the EGF receptor, the conserved tyrosine residue in the kinase domain appears not to be autophosphorylated.

The other class of autophosphorylation sites are normally localized outside the kinase domains and serve the important function of creating docking sites for downstream signal transduction molecules containing Src-homology 2 (SH2) domains. The SH2 domains consists of about 100 amino acid residues folded in such a way

EGF receptor GH receptor T cell receptor TNF receptor TGF-β receptor

box1
box2

α β
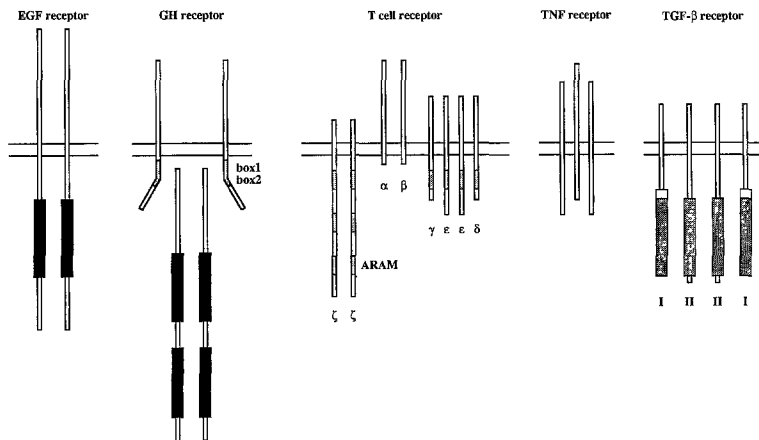
γ ε ε δ

ARAM

ζ ζ

I II II I

Figure 1. Examples of Receptors Activated by Dimerization or Oligomerization

Schematic representations of the complexes formed after ligand binding to receptors from the families discussed in the text, i.e., protein-tyrosine kinase receptors (the EGF receptor shown as an example), cytokine receptors (GH receptor bound to JAK kinases), antigen receptors (TCR), trimeric receptors (TNF receptor), and serine/threonine kinase receptors (TGFβ receptor). Tyrosine kinase domains are closed and serine/threonine kinase domains dark stippled. Box1, box2 (light stippled), refers to a region in cytokine receptors to which JAK kinases bind. ARAM (light stippled) refers to antigen recognition activation motifs in different subunits of antigen receptors that become phosphorylated by tyrosine kinases of the Src family and thereafter bind tyrosine kinases of the ZAP-70/Syk family.

Table 1. Families of Receptors Activated by Dimerization or Oligomerization

Receptor Type	Family	Examples	Characteristics
Protein-tyrosine kinase receptors	PDGF receptor family	PDGFR-α, PDGFR-β, SCFR (Kit), CSF-R (Fms), Flk-2	Five immunoglobulin-like domains extracellularly
	EGF receptor family	EGFR (ErbB), ErbB2 (Neu), ErbB3, ErbB4	Two cysteine-rich domains extracellularly
	FGF receptor family	FGFR-1, FGFR-2, FGFR-3, FGFR-4	Two to three immunoglobulin-like domains extracellularly
	IGF receptor family	insulin R, IGF-1R	Disulphide-bound heterotetramer of α and β chains
	HGF receptor family	HGFR (Met), MSPR (Ron)	Extracellular domain cleaved into an α and β chain
	VEGF receptor family	Flt-1, Flk-1 (KDR)	Seven immunoglobulin-like domains extracellularly
	Neurotrophin receptor family	Trk, TrkB, TrkC	
	Eph receptor family	Eph, Elk, Eck, Cck5, Sek, Eck, Erk	Two FNIII-like domains and a cysteine-rich domain extracellularly
Cytokine receptors	Class I cytokine receptor family		
	GH receptor subfamily	GHR, EPOR, PRLR, G-CSFR	Form homodimers
	IL-3 receptor subfamily	IL-3R, GM-CSFR, IL-5R	Form complexes with the β$_c$ subunit
	IL-6 receptor subfamily	IL-6R, LIFR, CNTFR, IL-11R	Form complexes with gp130
	IL-2 receptor subfamily	IL-2Rα, IL-2rβ, IL-4R, IL-7R	Form complexes with IL-2Rγ
	Class II cytokine receptor family	IFN-α/βR, IFN-γRα, IFN-γRβ, IL-10R	
TNF receptor family		TNFR-1, TNFR-II, LNGFR, CD40, OX-40, Fas, CD27, CD30	Form trimers
Antigen receptors		TCR	Complex of α, β, γ, δ, ε, ζ an η subunits
		BCR	Complex of IgM and heterodimers of α/β subunits
Serine/threonine kinase receptor family	Type II receptor family	TGFβR-II, ActR-II, ActR-IIB	Form hetero-oligomers with type I receptors, i.e., TGFβR-I, ActR-1, ActR-1B, BMPR-IA, BMPR-IB, ALK-1

Receptor families and subfamilies discussed in the text are presented. Abbreviations used: R, receptor; PDGF, platelet-derived growth factor; SCF, stem cell factor; CSF, colony-stimulating factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; HGF, hepatocyte growth factor; MSP, macrophage-stimulating protein; VEGF, vascular endothelial growth factor; FN, fibronectin; GH, growth hormone; EPO, erythropoietin; PRL, prolactin; IL, interleukin; LIF, leukemia inhibitory factor; CNTF, ciliary neurotrophic factor; IFN, interferon; TNF, tumor necrosis factor; LNGFR, low affinity nerve growth factor receptor; TCR, T cell receptor; BCR, B cell receptor; TGFβ, transforming growth factor β; Act, activin; BMP, bone morphogenic protein. Alternative designations are given within parentheses.
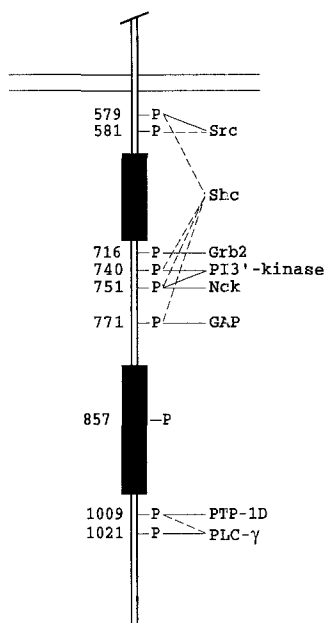
Figure 2. Interaction of SH2 Domain–Containing Signal Transduction Molecules with Different Autophosphorylation Sites in the PDGF β Receptor

Schematic illustration of the intracellular portion of a PDGF β receptor after activation. The kinase domain (closed boxes) in the receptor is divided into two parts by an inserted sequence. The tyrosine residues in the receptor known to be autophosphorylated are indicated by numbers. The interaction of individual autophosphorylated tyrosine residues with different SH2 domain–containing proteins are also indicated. Shc, Grb2, and Nck are adaptor molecules; Src denotes different members of the Src family of tyrosine kinases; PI3-kinase, phosphatidylinositol 3'-kinase; GAP, GTPase-activating protein; PTP1D, protein tyrosine phosphatase 1D; PLC-γ, phospholipase C-γ.

that a binding pocket for a phosphorylated tyrosine and the immediately surrounding amino acid residues is formed (Pawson and Schlessinger, 1993; Cohen et al., 1995 [this issue of *Cell*]). Of particular importance are the three to six amino acid residues C-terminal of the phosphorylated tyrosine (Eck et al., 1993; Pascal et al., 1994; Waksman et al., 1993); since different SH2 domains have different preferences for this region, there is specificity in the interaction. As an example, the PDGF β receptor has been shown to contain at least nine autophosphorylated tyrosine residues; Tyr-857 in the second part of the kinase domain is of importance for the catalytic activity of the kinase, whereas the others interact in a specific manner with at least eight different signal transduction molecules (reviewed by Claesson-Welsh, 1994; Figure 2).

Homodimerization or Heterodimerization

Protein-tyrosine kinase receptors are activated after homodimerization or after heterodimerization. In the case of the PDGF receptor subfamily, the different isoforms of PDGF induce different dimeric forms of the receptors. Since the A chain of PDGF binds only α receptors while the B chain binds both α and β receptors with high affinity, PDGF-AA induces αα receptor homodimers only, PDGF-AB induces αα receptor homodimers and αβ receptor heterodimers, and PDGF-BB induces all three combinations

of receptors (Heldin et al., 1989; Kanakaraj et al., 1991; Seifert et al., 1989). There are certain differences in the signals transduced via αα receptor homodimers and ββ receptor homodimers, e.g., regarding the stimulation of chemotaxis and actin reorganization. Moreover, PDGF-AB, which preferentially induces αβ receptor dimers, induces a stronger mitogenic response than the other PDGF isoforms. A possible explanation for the unique properties of the αβ receptor heterodimer is the presence of unique autophosphorylation sites, not seen in the homodimeric receptors, and that may mediate interactions with additional signal transduction molecules (Rupp et al., 1994). Thus, the response to PDGF depends both on the particular isoform of PDGF and on the number of α and β receptors expressed on the target cells.

The EGF receptor was the first protein-tyrosine kinase receptor to be shown to dimerize after ligand binding (Yarden and Schlessinger, 1987). However, within the same subfamily of tyrosine kinase receptors, heterodimerization of receptors has also been observed. A candidate ligand for ErbB2 (Neu differentiation factor [NDF], also called heregulin, glial growth factor, or acetylcholin-receptor-inducing activity), which is structurally related to EGF, was found to induce heterodimeric complexes between ErbB2 and ErbB3 or ErbB4 (Peles et al., 1993; Plowman et al., 1993; Sliwkowski et al., 1994). Moreover, the presence of ErbB3 or ErbB4 was necessary for high affinity binding of NDF and signal transduction through ErbB2 to occur. Interestingly, ErbB3 lacks certain highly conserved amino acid residues in its kinase domain; consistent with this finding, ErbB3 was found to have low or no kinase activity (Prigent and Gullick, 1994). It is thus possible that the major function of ErbB3 in the heterodimer is to act as a substrate for the ErbB2 kinase and thus provide docking sites for downstream SH2 domain–containing signal transduction molecules (Carraway and Cantley, 1994); for example, binding motifs for the SH2 domains of the phosphatidylinositol 3'-kinase (PI3-kinase) are lacking in the EGF receptor and in ErbB2, but occur in several copies in ErbB3 (Fedi et al., 1994; Soltoff et al., 1994). Also, EGF itself can induce heterodimerization of EGF receptors and ErbB2 (Soltoff et al., 1994; Wada et al., 1990). In fact, heterodimerization is preferred in cells expressing both EGF receptors and ErbB2. Although heterodimerization occurred also with a kinase-inactivated ErbB2 receptor mutant, this complex was inactive, showing that in this case signaling can not occur via ErbB2 serving as a EGF receptor substrate (Qian et al., 1994).

The studies on dimerization of receptors in the PDGF receptor and EGF receptor families thus provide examples of different types of dimeric complexes induced after ligand binding, i.e., homodimeric (Figure 3A) or heterodimeric (Figure 3B) complexes between two catalytically active subunits, or a heterodimeric complex between one active and one inactive or less active subunit (Figure 3C). Given that tyrosine kinase receptors and ligands occur in families of structurally related molecules, it is not unlikely that homodimerization and heterodimerization of receptors occur in parallel also in other families, thus increasing
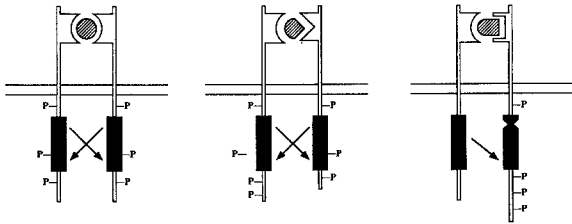
Figure 3. Different Dimeric Complexes of Protein-Tyrosine Kinase Receptors

Schematic representation of different forms of dimeric complexes of tyrosine kinase receptors formed after ligand binding. (A) a homodimeric complex; (B) a heterodimeric complex of two kinase-active subunits; (C) a heterodimeric complex of one active and one inactive or less active subunit.

the range of responses possible from a given number of receptor molecules.

One special case is the insulin and insulin-like growth factor 1 (IGF-1) receptor family. These receptors exist in the cell membrane as disulfide-bonded homo- or heterodimers of receptor subunits (each subunit is further cleaved into α and β chains by proteolysis) (Soos and Siddle, 1989). Thus, ligand binding does not induce receptor dimerization, but presumably causes a conformational alteration in the preformed dimeric receptor, which leads to receptor activation. Moreover, autophosphorylated tyrosine residues in the receptor molecules are not so important for the binding of downstream components in the signal transduction pathways; rather, the insulin receptor kinase phosphorylates insulin receptor substrate 1 (IRS-1), which mediates the interactions with SH2 domain proteins (White, 1994).

Cytokine Receptors

The cytokine receptor classes include receptors for many interleukins, colony-stimulating factors, interferons, and certain other factors and hormones (reviewed by Kishimoto et al., 1994; Mui and Miyajima, 1994; see Table 1). Class I cytokine receptors are characterized by the presence in their extracellular domains of one or two copies of a conserved domain of about 200 amino acids, which contains two modules of fibronectin type III–like motifs, four conserved cysteine residues, and the conserved motif Trp–Ser–Xaa–Trp–Ser (Bazan, 1990). Class II cysteine receptors, including receptors for interferons and interleukin-10 (IL-10), contain another conserved motif of four cysteine residues and lack the Trp–Ser–Xaa–Trp–Ser motif. The intracellular domains of cytokine receptors lack intrinsic enzymatic activities. However, despite the structural difference between cytokine receptors and tyrosine kinase receptors, their mechanism of activation appears to be similar. Ligand binding induces dimerization or oligomerization of cytokine receptors, and this allows interaction and activation of cytoplasmic protein-tyrosine kinases that are associated with the intracellular domain of the receptors.

Activation of Class I Cytokine Receptors through Formation of Hetero-Oligomeric Complexes

Most of the class I cytokine receptors undergo heterodi-

merization or hetero-oligomerization after ligand binding (Table 1). In many cases, the ligand-binding subunit(s) form signaling complexes with signal-transducing molecules that are structurally related to cytokine receptors, but that are themselves unable to bind ligands. For instance IL-3, granulocyte/macrophage colony-stimulating factor (GM-CSF), and IL-5 bind to specific α subunit receptors; the α subunits all interact with a common β subunit that is required for high affinity ligand binding and signal transduction (Mui and Miyajima, 1994).

Similarly, IL-6, leukemia inhibitory factor (LIF), oncostatin M, IL-11, and ciliary neurotrophic factor (CNTF) share a common signal transducer, gp130 (Taga et al., 1989); signaling is triggered by the formation of homo- or heterodimers of gp130. IL-6 binds to the IL-6 receptor and induces a complex containing a homodimer of gp130 (Murakami et al., 1993). Interestingly, signaling occurs also with a truncated IL-6 receptor lacking the cytoplasmic domain, which indicates that the IL-6 receptor is needed only to increase the binding affinty for IL-6. The CNTF receptor acts similarly, lacking a cytoplasmic domain in its natural form and being anchored in the membrane through a phosphatidylinositol group. The CNTF receptor–CNTF complex signals via formation of a heteromeric complex of gp130 and the LIF receptor (Davis et al., 1993). LIF and oncostatin M signal via binding directly to a heteromeric complex of gp130 and the LIF receptor (Gearing et al., 1992). IL-11 is dependent on gp130 but not the LIF receptor for signaling (Hilton et al., 1994).

A third subfamily is constituted by IL-2, IL-4, IL-7, and IL-9. In this family, signaling involves the formation of heterodimeric receptor complexes between specific β subunits and a common γ subunit (Kawahara et al., 1994). In the case of IL-2, the ligand binding affinity is increased by the presence also of an α subunit, which has a structure unrelated to that of cytokine receptors. Whereas the α subunit is not needed for signal transduction, both the β and the γ subunits are needed, presumably in a heterodimeric configuration (Nakamura et al., 1994; Nelson et al., 1994).

Activation by Homodimerization

Although activation by heterodimerization appears to be most common among cytokine receptors, there are examples of cytokine receptors that are activated by homodimerization, e.g., the receptors for growth hormone (GH), erythropoietin (EPO), prolactin, and granulocyte colony-stimulating factor (G-CSF) (Table 1). A well-characterized example is the GH receptor. Analysis of crystals of GH and the extracellular part of the receptor revealed that each ligand binds two receptor molecules simultaneously (Cunningham et al., 1991; de Vos et al., 1992; Ultsch et al., 1991). This finding was surprising since GH is a monomeric molecule without apparent symmetry. The two receptor-binding sites in GH are therefore different, although they bind to similar epitopes in the receptors. Site 1 is larger and is supposed to bind receptor first; the smaller site 2 thereafter binds a second receptor, and the dimeric receptor complex is further stabilized by direct interaction between the two receptors. The importance of the latter

epitope in stabilizing a GH receptor dimer is illustrated by the finding that a mutation in this region abolishes receptor homodimerization and is responsible for a form of familial GH resistance (Laron's syndrome; Duquesnoy et al., 1994). The results from the three-dimensional structure studies are supported by titration calorimetry in solution; the heat of binding was found to be saturated at a 1:2 ratio of ligand and receptor (Ultsch et al., 1991).

Signal Transduction

Much information regarding the signal transduction pathways from cytokine receptors to the nucleus has come from a genetic approach in which mutant cell lines defective in the response to interferons were isolated and characterized (Darnell et al., 1994). This approach led to the identification of three categories of proteins, a DNA-binding protein (p48), STATs (signal transducers and activators of transcription), and cytoplasmic protein-tyrosine kinases of the JAK family.

The JAK kinases are characterized by the presence of two kinase domains in each molecule, which is the basis for their name (Janus kinases, after the Roman god with two faces) (reviewed by Ziemiecki et al., 1994). Several members of this family are currently known (JAK1, JAK2, Tyk2, JAK3 [Ziemiecki et al., 1994; Takahashi and Shirasawa, 1994]); they associate in a specific manner with different cytokine receptors and are activated upon receptor dimerization. An important class of substrates for JAK kinases is members of the STAT family (STAT1α, STAT1β, STAT2, STAT3, STAT4, STAT5, and IL-4 STAT) (Darnell et al., 1994; Zhong et al., 1994; Gouilleux et al., 1994; Hou et al., 1994). After phosphorylation on tyrosine residues, the STAT molecules form homo- or heterodimers.

In the case of signaling from the interferon-α receptor, heterodimers of STAT1α (p91) or STAT1β (p84) and STAT2 (p113) are created, which move into the nucleus and form a complex with a DNA-binding protein (p48), allowing them to bind and stimulate transcription from elements in the promoters of interferon-α-induced genes (Schindler et al., 1992). Interestingly, another combination of STATs is formed after stimulation by interferon-γ, either a homodimer of STAT1α (p91) or a homodimer of STAT1β (p84) (Shuai et al., 1994; Shuai et al., 1992). These dimers do not associate with p48, but may form complexes with other related proteins. Both homodimers bind to interferon-γ-activated sites (GAS), which are present in interferon-γ-inducible genes, although only the STAT1α homodimer activates transcription (Shuai et al., 1993).

It thus appears that the activities of STATs are regulated by specific assembly into homo- or heterodimers. The dimerization is triggered by phosphorylation. A single phosphorylated tyrosine residue has been identified in STAT1 after stimulation with interferon-α as well as after stimulation with interferon-γ; mutation of this tyrosine residue to a phenylalanine residue prevents dimerization (Shuai et al., 1993). Since STATs contain SH2 domains, it is likely that the dimerization involves reciprocal interactions between the SH2 domains and the tyrosine-phosphorylated regions in the STAT molecules (Shuai et al., 1994). How is the specificity regulated? One possibility is that STATs

may associate in a differential manner with different receptors (Fu and Zhang, 1993; Greenlund et al., 1994).

Another possibility is that the JAK family members differ in their substrate specificities and thus phosphorylate different STAT molecules. Interestingly, the genetic approach led to the identification of different JAK kinases in the signaling pathways of interferon-α (JAK1 and Tyk2) and interferon-γ (JAK1 and JAK2) (Müller et al., 1993; Velazques et al., 1992; Watling et al., 1993). Thus, in each case, there was a need for two different JAK kinases. It is unlikely that the two kinases are needed in a sequential activation mechanism, since in cells deficient in JAK1 no activation of JAK2 was seen after stimulation with interferon-γ, or vice versa (Müller et al., 1993). Thus, it is possible that the active forms of the JAK kinases involved in the signal pathways of interferons are activated by heteromeric interactions, possibly involving cross-phosphorylations.

A possible mechanism to achieve such heterodimerization is via ligand-dependent formation of heteromeric receptor complexes. The receptor for interferon-γ consists of at least two different chains (Aguet et al., 1988; Hemmi et al., 1994; Soh et al., 1994), and it has been suggested that JAK1 and JAK2 interact with these chains in a differential manner (Greenlund et al., 1994). An interferon-α/β receptor that binds JAK1 has been identified (Novick et al., 1994); whether another receptor subunit with affinity for Tyk2 exists remains to be elucidated. An analogous situation appears to prevail for the IL-2 receptors; the β and γ subunits have been shown to bind JAK1 and JAK3, respectively (Miyazaki et al., 1994; Russell et al., 1994).

Common and Unique Signals

In addition to the receptors for interferon-α and interferon-γ, many other receptors, including GH, EPO, prolactin, G-CSF, LIF, gp130, the common β subunit for the IL-3 subfamily of receptors, and the common γ subunit for the IL-2 subfamily, have been shown to bind different members of the JAK family (Ihle et al., 1994). JAKs bind in a specific manner to conserved regions called box 1 and box 2 regions in the juxtamembrane parts of cytokine receptors (Murakami et al., 1991).

Other signal transduction pathways are also initiated at the activated cytokine receptor complexes; these pathways are dependent on more C-terminal regions in the receptors. For instance, members of the Src family of kinases bind to the C-terminal tail of the IL-2 β receptor (Hatakeyama et al., 1991) and to gp130 (Ernst et al., 1994). Moreover, whereas JAK kinases bind to the juxtamembrane part of the common β subunit of IL-3, IL-5, and GM-CSF, deletion of the C-terminus abrogates Shc phosphorylation, Ras activation, and induction of c-fos and c-jun (Sato et al., 1993). Likewise, a region C-terminal of the JAK kinase–binding site of the G-CSF receptor mediates induction of granulocyte-specific genes (Fukunaga et al., 1993).

The fact that certain receptor subunits/signal transducers are shared by several cytokines, as well as the fact that different receptors may bind and activate the same JAK kinases and possibly also share other signal transduc-

tion molecules, provides an explanation for the functional redundancy and pleiotropy of different cytokines. Conversely, the presence of unique epitopes in receptors or receptor combinations may allow the transduction of specific signals that mediate unique properties of the different cytokines.

Sharing of Signal Transduction Pathways between Tyrosine Kinase Receptors and Cytokine Receptors

There is no sharp division in the modes of signaling between tyrosine kinase receptors and cytokine receptors. EGF and PDGF, acting via tyrosine kinase receptors, induce the phosphorylation of STAT1α, perhaps directly or via activation of JAK kinases (Fu and Zhang, 1993; Ruff-Jamison et al., 1993; Sadowski et al., 1993; Silvennoinen et al., 1993). Moreover, after activation of cytokine receptors, JAK kinases or possibly other kinases phosphorylate the cytokine receptors themselves. This gives SH2 domain–containing signal transduction molecules the possibility to interact with the cytokine receptors and initiate pathways initially identified for tyrosine kinase receptors, e.g., leading to activation of Ras and PI3-kinase (Boulton et al., 1994; Mui and Miyajima, 1994).

Antigen Receptors

The T cell receptor is composed of transmembrane proteins with very short cytoplasmic sequences, which are associated with a large number of invariant subunits also lacking intrinsic enzymatic activities, but capable of interacting with cytoplasmic tyrosine kinases (Figure 1) (for reviews see Cambier and Jensen, 1994; Weiss and Littman, 1994). The invariant subunits (γ, δ, ϵ, ζ, and η chains) contain one to three copies of a conserved 26 amino acid motif of pairs of tyrosine and leucine residues (Reth, 1989), called the antigen recognition activation motif (ARAM; also called tyrosine-based activation motif, or antigen receptor homology 1). Tyrosine kinases of the Src family bind to the T cell receptor even in the resting state. In conjunction with receptor activation, the tyrosine residues in the ARAMs are phosphorylated, presumably by Src family kinases (in T cells primarily Lck). This gives another tyrosine kinase, ZAP-70, which has two SH2 domains, the opportunity to bind to the phosphorylated sites, after which it becomes activated by phosphorylation on tyrosine residues, most likely also by Src family tyrosine kinases (Iwashima et al., 1994; Letourneur and Klausner, 1992). The precise mechanism that triggers antigen receptor activation and phosphorylation of the ARAM sequences is not known, although a possible scenario is that antigen binding causes receptor aggregation that makes possible interactions and cross-phosphorylation of tyrosine kinases in the Src family. Consistent with this possibility are the observations that chimeric molecules consisting of cytoplasmic parts of ARAM–containing T cell receptor subunits and extracellular domains of other molecules mediate activation of T cells after cross-linking (Letourneur and Klausner, 1992; Irving and Weiss, 1991; Romeo and Seed, 1991).

The B cell receptor and Fc receptors also occur in complexes containing signal transducing molecules with ARAMs, suggesting similar mechanisms of signal trans-

duction (Clark et al., 1994; Law et al., 1993; Ravetch, 1994). Interestingly, a sequential activation of Src family members and ZAP-70/Syk family members may also be involved in cytokine signaling. The G-CSF receptor has been shown to be associated with Lyn, a member of the Src family; after stimulation, an ARAM-like motif in the C-terminus of the G-CSF receptor is phosphorylated, which binds Syk leading to its activation (Corey et al., 1994).

TNF Receptor Family

An interesting variation on the "activation by oligomerization" theme is provided by members of the TNF receptor family, which are involved in regulation of cytotoxicity, apoptosis, and proliferation (for reviews see Bazan, 1993; Smith et al., 1994). TNF occurs as two forms, TNFα and TNFβ, which both binds to two different receptors, TNF receptor 1 and 2 (p55 and p75, respectively). The TNFs are nondisulfide-bonded trimers, and elucidation of the X-ray structure of TNFβ and TNF receptor 1 (Banner et al., 1993) revealed that ligand binding induces trimerization of the receptor. Each TNF subunit makes contact with two adjacent receptor molecules, thus stabilizing the receptor trimer. It is likely that the activating event is receptor aggregation, but it is not clear whether there is a need for receptor trimerization, or whether receptor dimerization would be sufficient for activation. In support of the possibility that trimerization of TNF receptor 1 is, in fact, necessary for signal transduction, monoclonal antibodies against this receptor, which are expected to dimerize the receptor, do not lead to activation, whereas activation occurs after cross-linking of the monoclonals with a second antibody, or after stimulation by two monoclonals directed against different epitopes (Engelmann et al., 1990).

A novel family of molecules that associate with the cytoplasmic part of TNF receptor 2 and that may serve as signal transducers was recently identified (Rothe et al., 1994); TNF receptor associated factors, TRAF1 and TRAF2, contain a novel region of homology and form homo- or heterodimers. This finding represents an important step in the understanding of signaling from the TNF receptor 2, but the mode of activation of TRAFs, their downstream effectors, and whether related molecules are involved in signaling from other members in the TNF receptor family remain to be elucidated.

Protein-Serine/Threonine Kinase Receptors

Transforming growth factor β (TGFβ) is a prototype for a large family of structurally related factors that regulate cell growth and differentiation, including in addition to TGFβs, e.g., activins and inhibins, bone morphogenic proteins, and Müllerian inhibition substance. As far as has been characterized, these molecules exert their cellular effects by binding to heteromeric complexes of serine/threonine kinase receptors (reviewed by Massagué et al., 1994; Miyazono et al., 1994).

Both type I and type II receptors have rather small cysteine-rich extracellular domains; the type I receptors, which are more similar to each other than to the type II receptors, all have a characteristic region rich in glycine and serine
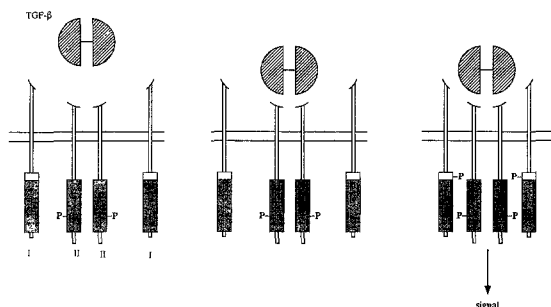
Figure 4. Signaling via TGFβ Receptors

Schematic illustration of the mechanism of signaling via TGFβ receptors as suggested by Wrana et al. (1994). TGFβ binds first to type II receptors that have a constitutively active serine/threonine kinase. The type I receptor is then incorporated in the complex and activated by phosphorylation in the GS box. Serine/threonine kinase domains are stippled, and GS boxes are open.

residues (GS domain) in their cytoplasmic juxtamembrane domains. Both receptors are needed for signaling (Wrana et al., 1992), and the cytoplasmic parts of the receptors are not interchangeable (Okadome et al., 1994). Ligand binding induces a hetero-oligomeric complex of type I and type II receptors, most likely a heterotetramer containing two receptors of each type (Yamashita et al., 1994). Studies on TGFβ-induced phosphorylation of the receptor molecules have led to an interesting model for activation of the receptors (Wrana et al., 1994; Figure 4). The type II receptor, which occurs in a dimer also in the absence of ligand (Henis et al., 1994; Chen and Derynck, 1994) and has a constitutively active kinase, first binds TGFβ. This complex then recruits the type I receptor, which can not bind ligand in the absence of type II receptor, resulting in the phosphorylation of the type I receptor on serine residues in the GS domain. The phosphorylation presumably activates the type I receptor kinase that now can act on downstream components in the signal transduction pathway. Other members in the TGFβ family also form heteromeric complexes containing different members of the type I and type II receptor subfamilies. Thus, sequential phosphorylation between the type II and type I receptors may be a general mechanism of receptor activation of members of the serine/threonine kinase receptor family.

Is Dimerization Sufficient for Activation?

There are several examples in which activation of receptors occurs after dimerization or oligomerization induced by means other than ligand binding. Many tyrosine kinase receptors, for instance, are activated after binding of antibodies, whereas Fab fragments generally are inactive. Insertion of an extra cysteine residue in the extracellular juxtamembrane region of the EGF receptor led to the formation of a constitutively active dimeric receptor (Sorokin et al., 1994). Moreover, mutated forms of many of the tyrosine kinase receptors have been identified as transforming oncogenes. In some cases, the activating mechanism is a gene rearrangement that leads to the production of a

fusion protein between a novel protein and the kinase domain of the receptor. The fusion partners are often domains of proteins that undergo oligomerization in their normal context. Examples include tropomyosin, which has been found fused to Trk (Martin-Zanca et al., 1986), the regulatory subunit of the cyclic AMP-dependent protein kinase, which has been found fused to Ret (Takahashi et al., 1985), and sequences from Tpr, containing a leucine zipper, which has been found fused to Met (Park et al., 1986; Rodrigues and Park, 1994) as well as to Trk (Greco et al., 1992). Another mechanism is exemplified by the Neu (ErbB2) oncogene product, which obtained transforming activity by a single amino acid exchange in the transmembrane region that promotes receptor aggregation (Weiner et al., 1989). In these cases, artificially induced receptor dimerization leads to activation of the kinase domains and autophosphorylation in a ligand-independent manner.

Also cytokine receptors can acquire transforming properties after mutation. A constitutively active EPO receptor mutant was found to have an arginine residue replaced with a cysteine residue in a region corresponding to the receptor dimer interface of the related GH receptor; this resulted in the formation of a disulfide bond that stabilized the receptor dimer in a ligand-independent manner (Watowich et al., 1992). This finding further supports the concept that dimerization is sufficient for activation of many receptor types.

Antagonists

There are many examples of tyrosine kinase receptors and cytokine receptors that after mutations in their cytoplasmic domains act in a dominant negative manner, i.e., when expressed in cells with the corresponding wild-type receptor, they attenuate the signals induced by ligands. The mechanism for the dominant negative effect is that the wild-type receptors after ligand binding are locked up in sterile heteromeric complexes with the mutated receptors. These findings provide support for the notion that dimerization of wild-type receptors is necessary for activation of many receptor types; however, alternative modes for activation have not been excluded. Another way in which the oligomerization process can be antagonized is through mutated versions of certain ligands. For example, mutation of one of the two receptor-binding sites in GH yielded a GH protein with antagonistic properties (Fuh et al., 1992). Moreover, mutation of a glutamic acid residue in GM-CSF (Glu-21) that is important for the interaction with the common β subunit (Hercus et al., 1994), mutation of Tyr-124 in IL-4, which is important for interaction with the common γ subunit (Kruse et al., 1992), or mutation of Tyr-31 and Gly-35 in IL-6, which are important for interaction with the gp130 signal transducer (Savino et al., 1994), yielded molecules with antagonistic effects in their respective systems.

It is possible that inhibition of receptor oligomerization is a generally applicable method to antagonize growth factor and cytokine action. Antagonistic ligands and antibodies may have particular clinical utility in conditions of overactivity of growth factors and cytokines, since they can act specifically.

Conclusions

It is now well established that several receptor types are activated through ligand-induced receptor dimerization or oligomerization. Dimerization combines accuracy with flexibility; there is specificity in binding of the ligand to the receptors and flexibility in the assembly of different homo- or heterodimeric receptor subunits depending on which receptors and signal transducers are expressed by a particular cell. There are also examples of receptors that do not dimerize after ligand binding, e.g., the serpentine receptor family, which transverses the cell membrane seven times and couples to G proteins, and ion channel receptors. However, for receptor molecules that are anchored in the membrane with a single transmembrane domain, dimerization or oligomerization may be a general mechanism for receptor activation.

A general feature of receptors generating growth stimulatory signals seems to be activation of tyrosine kinases in the receptor complex. Although the exact mechanisms for activation of the kinases remain to be elucidated, interactions and cross-phosphorylations between identical or related kinases induced by receptor dimerization are common. The resulting phosphorylations of tyrosine residues on receptor and signal transducing components trigger interactions with SH2-containing molecules (see Cohen et al., 1995). Growth inhibitory signals from the activated TGFβ receptor complex involve phosphorylation on serine/threonine residues in yet unknown substrates. Thus, much of intracellular signaling is regulated by phosphorylation events. To understand the regulation of signal transduction, it will therefore be important to characterize not only the kinases involved, but also the phosphatases that counteract the effects of kinases (see Hunter, 1995 [this issue of *Cell*]).

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