CHAPTER

19

Cell Growth Factors

I. GROWTH FACTOR SIGNAL TRANSDUCTION

In recent years there has been an explosion of information on the continuation of the signal generated by the interaction of growth factor with cell membrane growth factor receptor. Thus, the hormone–growth factor interacts with the receptor, and this interaction often produces a conformational rearrangement in receptor and receptor-associated proteins so that the message is carried by some initial system. This system can be a part of the receptor itself, which may contain a protein kinase catalytic center that catalyzes the phosphorylation of specific amino acid residues, such as tyrosine residues if it is a tyrosine protein kinase, resulting in autophosphorylation of the receptor on the cytoplasmic side of the receptor molecule. This could result in an activation leading to other interactions. Likewise, the receptor may dimerize upon interacting with ligand, and the dimerized receptor could undergo further interactions in the cell membrane and in the cell. The receptor may interact in specific ways with G proteins or enzymes of the phosphatidylinositol cycle to produce other changes, or the receptor could be interacting with an ion channel that could become activated following ligand–receptor interaction. These general comments set the stage for this section.

A number of growth factor receptors are members of a protein tyrosine kinase receptor family, and others have specific characteristics concerning their structure. These topics are summarized in Table 19-1.

Similarities between some groups of ligands indicate that they may have derived from the same or similar ancestors. Examples are insulin, IGF-I, and IGF-II. This group shares homology to the extent that there can be crossover binding to their receptors. For example, both insulin and IGF-II can bind to the IGF-I receptor, although much greater amounts of IGF-II and insulin are needed to activate the receptor. However, it appears that blockage of IGF-I by competition with a similar but inactive peptide, while greatly inhibiting the growth of certain cells in culture, has a much smaller effect on intact animals. Perhaps this is indicative of the growth factors (e.g., insulin or IGF-II) that are present in great enough quantity to overcome the effect of the inhibitor. Another group of hormones sharing a common ancestor is LH, FSH, hCG, ACTH, and MSH, and this has been discussed in Chapter 5. There are also similarities between receptors. The receptors for IGF-I and insulin are very similar, and receptors for IGF-I, insulin, epidermal growth factor

TABLE 19–1 Single-Transmembrane-Segment Receptors[a]

Protein Tyrosine Kinase Receptor Family
Epidermal growth factor
Neu-differentiation factor (human EGF receptor 2)
Insulin
Insulin-like growth factor-I
Fibroblast growth factors
Platelet-derived growth factors A and B
Colony-stimulating factor-1 (macrophage colony-stimulating factor)
Nerve growth factors (neurotrophins)
Hepatocyte growth factor (scatter factor)
Guanylate Cylase Receptor Family
Atrial natriuretic peptide types A, B, and C
Serine–Threonine Kinase Receptor Family
Transforming growth factor β, types I and II
Activin
Inhibin
Multisubunit Receptor Family
Growth hormone
Prolactin
Placental lactogen
Erythropoietin
Interleukin-2, -3, -4, -5, -6, -7
Granulocyte macrophage colony-stimulating factor
Granulocyte colony-stimulating factor
Interferon-α, -β, and -γ
Tumor necrosis factor p75
Leukemia inhibitory factor
Oncostatin
Ciliary neurotrophic factor
Tumor Necrosis Factor–Nerve Growth Factor Receptor Family
Tumor necrosis factor p55
Nerve growth factor p75
Phosphotyrosine Phosphatase Receptor Family
Ligands unknown
Plasma Protein Receptor Family
Low-density lipoprotein
Transferrin
Asialoglycoproteins
Polymeric immunoglobulin A–immunoglobulin M
Mannose 6-phosphate–insulin-like growth factor-II
Urokinase plasminogen activator
α_2-Macroglobulin

[a] Reproduced with permission from Gammeltoft, S., and Kahn, C. R. (1995). Hormone signaling via membrane receptors. *In* "Endocrinology" (L. J. DeGroot, ed.), 3rd ed., Vol. 1, pp. 17–65. W. B. Saunders Co., Philadelphia, PA.

(EGF), and platelet-derived growth factor (PDGF) all have tyrosine kinase domains on the cytoplasmic portion of the receptors. Receptors for PRL, GH hematopoietic growth factors, and cytokines have similar domains and interact with the Janus (JAK) cytoplasmic tyrosine kinases. Receptors that interact with G proteins often contain seven transmembrane domains, and the activation of enzymes by G-protein subunits is often similar.

II. GROWTH HORMONE (GH) AND SOMATOMEDINS (IGFs)

Although this subject overlaps that of Chapter 5 on anterior pituitary hormones, it is a reasonable starting point since IGF-I is a major growth factor. The relationship between growth hormone and IGF-I is shown in Figure 19-1.

Here it is shown that GH is released through the action of hypothalamic growth hormone-releasing hormone (GRH), as described earlier in Chapter 3. The GH released into circulation has two effects: One effect is to directly act on lipid and fat metabolism, analogously to cortisol action, in a manner to oppose the actions of insulin and IGF-I, as shown by its ability to directly increase blood sugar (Figure 19-1). The other action of GH is on liver and other organs to generate IGF-I, which, like insulin, produces skeletal growth as well as tissue growth. IGF-I is active as a negative feedback agent on the hypothalamus and causes the production of somatostatin (GIH), which inhibits the release of GH from the anterior pituitary, and it also acts at the level of the pituitary to inhibit expression of the GH gene in response to GRH. When GH causes IGF-I to be produced, IGF-I-binding proteins are also synthesized in the liver and other tissues, and both IGF-I and binding proteins are secreted into the bloodstream. Consequently, IGF-I (and other somatomedins) is complexed with binding proteins in the circulation. There are six IGF-binding proteins (IGFBPs) in humans and each is produced by a separate gene. These binding proteins contain cysteine-rich domains that are involved in the interaction with IGFs. IGFBPs I and II have R-G-D sequences that may bind to cell surface integrin receptors. Consequently, IGFs circulate in the bound form, a condition that allows for relatively high concentration and stability. In some cases the binding proteins interfere with the action of IGF at its cell membrane receptor, and in other cases, certain binding proteins enhance the growth effects of IGFs upon cells. Apparently, the extent to which a specific binding protein is phosphorylated may determine its activity with regard to the binding of IGF to its receptor. IGF-I can be dissociated from an IGF-I-BP by the action of serum endoproteases and also by heparin-like substances. Properties of human IGFBPs are given in Table 19-2.

III. INTERACTION OF IGFs WITH RECEPTORS

Not only do IGFs and insulin have high homologies, but the receptors for IGF-I and insulin are also homologous. Thus, IGF-I receptor binds IGF-I and, at higher levels, insulin, and it also carries binding sites for IGF-

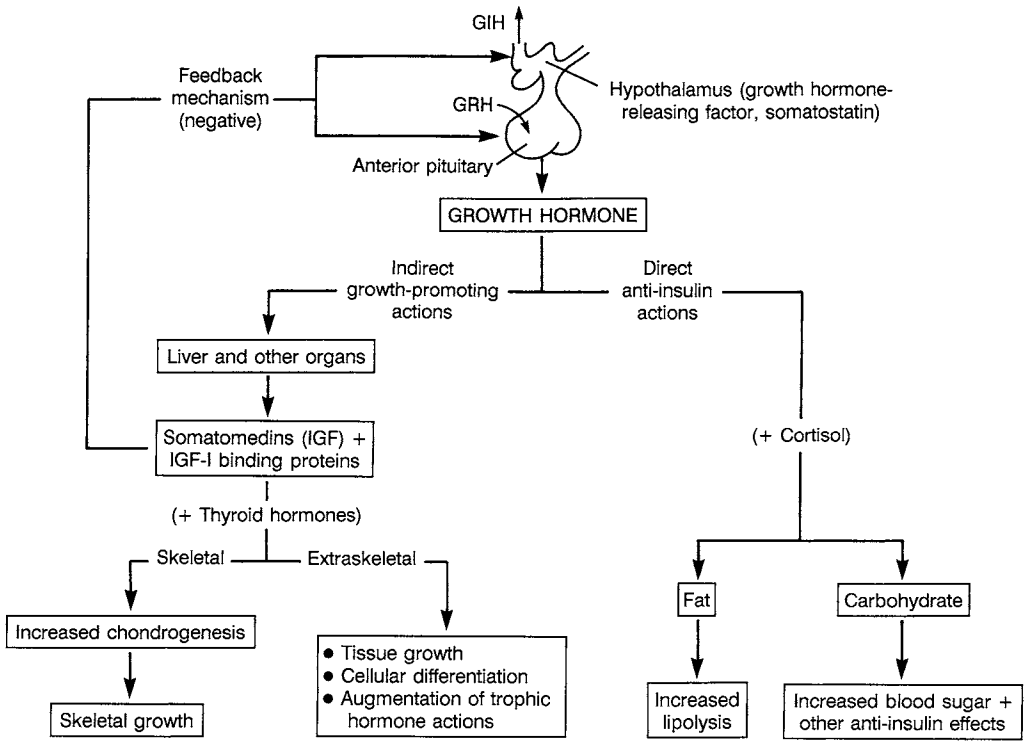

FIGURE 19-1 Somatomedin hypothesis of growth hormone (GH) action. The direct actions of GH include diabetogenic and lipolytic actions and stimulatory action on several hepatic enzymes. These direct actions are antagonistic to insulin and often synergistic with cortisol. The anabolic and growth-promoting actions of GH are mediated through the somatomedins. IGF-I participates in the negative feedback on GH secretion at the hypothalamic level by stimulating somatostatin production and at the pituitary level by directly blocking the effect of GH-releasing hormone on the expression of the GH gene. Reproduced with permission from Russell, W. E., and Van Wyk, J. J. (1995). Peptide growth factors. *In* "Endocrinology" (L. J. DeGroot, ed.), 3rd ed., Vol. 1, pp. 2590–3623. W.B. Saunders Co., Philadelphia, PA.

II, which are apparently different from the sites to which IGF-I binds. The β-subunits of both receptors contain a tyrosine kinase and ligand binding triggers the activity of the tyrosine kinase, resulting in autophosphorylation on tyrosine residues. Other proteins may be phosphorylated on tyrosine residues by the receptor kinase. IGF-I receptors are present in most tissues: the hormone exerts its effect in concert with other hormones produced in specialized tissues, and the IGF-I receptor is down-regulated after ligand binding in a typical process characteristic of many membrane receptors. IGF-II receptors bind IGF-II nearly exclusively and interact poorly with IGF-I. On the other hand, as has already been pointed out, IGF-II can bind to the IGF-I receptor and this interaction is important in humans, who, in contrast to some rodents, produce IGF-II. The IGF-II receptor is identical to the mannose 6-phosphate receptor and contains separate domains for binding IGF-II and mannose 6-phosphate. Interestingly, the mannose 6-phosphate-binding domain activates the TGF-β precursor by removing mannose, and this domain has other activities as well. The interaction of IGF-II with the IGF-II receptor may lead to the uptake of extracellular calcium possibly mediated by a G protein.

IV. SIGNAL TRANSDUCTION OF RECEPTORS FOR GH, IGFs, AND INSULIN

The growth hormone receptor and its immediate signaling partners are shown in Figure 19-2.

The human GH receptor binds human GH exclusively and does not interact with other species of GH. The structure of the extracellular domain of the GH receptor has been determined by X-ray crystallography and when studied in the presence of ligand it was shown that the extracellular domain forms a dimer that interacts with one GH molecule. The structure of the hormone-binding domain of the GH receptor is shown in Figure 19-3.

Dimerization is required for binding of GH and cellular responses.

Figure 19-4 shows a signal transduction scheme for cytokine receptor, growth factor receptor, and insulin

TABLE 19-2 Properties of IGF-Binding Proteins (IGFBPs)[a]

IGF-binding protein	Major tissues producing	Increases concentration	Decreases concentration	Other comments
IGFBP-1	Liver, kidney reproductive tissues: prostate, uterus, decidua	Decreased insulin or insulin sensitivity, fasting, diabetes, insulin resistance, pregnancy, hypopituitarism	Hyperinsulinism: feeding, glucose infusion, GH, glucocorticoids, partial hepatectomy increases liver level	Independent of GH; may transport IGFs out of vascular space
IGFBP-2	Fetal tissues, but declines after birth; CNS and cerebral spinal fluid, higher in lymph than serum	Hypophysectomy Diabetes	GH Glucocorticoids Insulin	May transport IGFs out of vascular space
IGFBP-3	Serum	GH treatment Acromegaly Adolescence Infusion of IGF-1 Various growth factors, *in vitro* feeding after nutrient restriction	GH deficiency Stimulators of cAMP Nutrient restriction	IGF-I or IGF-II is linked to other subunits, one of which is IGFBP-3; this complex prevents loss of IGFs from intravascular compartment; serum levels dependent on GH and nutritional status; IGF-I may mediate GH induction of IGFBP-3'; helpful in diagnosing GH deficiency
IGFBP-4	Liver Plasma Many other organs	cAMP	Reduction of cAMP	Binds to IGF-I and IGF-II equally; blocks effects of IGF-I in cell culture; may modulate effects of IGFs in CNS
IGFBP-5	Kidney Bone Endocrine tissues			Binds strongly to extracellular matrix and may serve as storage compartment for IGF-I when bound to extracellular matrix
IGFBP-6	All tissues		Acromegaly	10-fold higher affinity for IGF-II than for IGF-I; regulation may resemble that of IGFBP-1 and -2

[a] Information for this table was derived from Russell, W. E., and Van Wyk, J. J. (1995). Peptide growth factors. *In* "Endocrinology" (L. J. DeGroot, ed.), 3rd ed., Vol. 1, pp. 2590–3623. W. B. Saunders Co., Philadelphia, PA.

receptor. While this scheme may describe signal transduction for EGF, PDGF, FGF, NGF, and insulin, it is not clear whether this pathway utilizes IGF-I.

It would be surprising if IGF-I signal transduction operated by a different pathway, given that IGF-I can produce insulin effects by binding to the insulin receptor and insulin can produce IGF-I-like effects by binding to the IGF-I receptor. Figure 19-4 shows one pathway for receptor-linked tyrosine kinases and starts with autophosphorylation on tyrosine residues after ligand binding. Other proteins may also be phosphorylated, as in the case of the insulin receptor phosphorylating the insulin receptor substrate-1 (IRS-1). GRB2 associates with phosphorylated tyrosine residues in the receptor kinase. This is followed by the action of SOS complexed to GRB2 on membrane-bound *ras* by GDP–GTP exchange and *ras* activates raf kinase, which starts a phosphorylation cascade involving the four kinases shown in the figure. The end of this process is the effect on growth and metabolism.

V. EPIDERMAL GROWTH FACTOR (EGF) FAMILY

This growth factor is part of a family of growth factors that includes, besides EGF, TGF-α, amphiregulin, heparin-binding EGF, and cripto. The linear

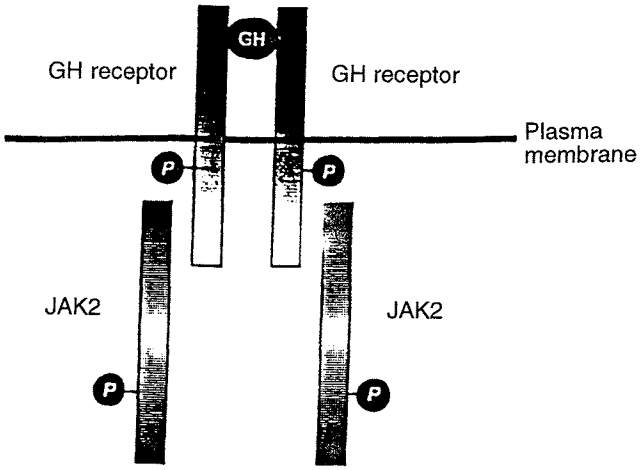

FIGURE 19-2 Structure of single-transmembrane-segment receptors that form homologous dimers; the growth hormone receptor. One GH molecule binds to identical binding sites on the extracellular domains of two GH receptors that form a homodimer. The intracellular domains associate with a tyrosine kinase of the Janus family of kinases. An encircled P denotes a potential tyrosine phosphorylation site on the intrecellular portion of GH receptor. Reproduced with permission from Gammeltoft, S. and Kahn, C. R. (1995). Hormone signaling via membrane receptors. *In* "Endocrinology" (L. J. DeGroot, ed.), 3rd ed., Vol. 1, pp. 17–65. W.B. Saunders Co., Philadelphia, PA.

sequences of these hormones are shown in Figure 19-5. The signal transduction process of the EGF receptor can be formulated as shown in Figure 19-6. While EGF promotes mitosis in cells of mesodermal and ectodermal origin, TFG-α confers the transformed phenotype on normal cells. TGF-α has substantial homology to EGF and both growth factors operate through the EGF receptors. TGF-α may be the fetal ligand for EGF receptor. Amphiregulin stimulates the growth of normal fibroblasts and keratinocytes while it inhibits the growth of several breast carcinoma lines. It has a 43-amino-acid extension at its terminus compared to EGF, and this extension may contain a nuclear translocation signal. Heparin-binding EGF also has an N-terminal extension and acts through EGF-R. While the gene for cripto has been cloned, little is known about its signal transduction.

The smallest form of active TGF-α contains 50 amino acids and is homologous to EGF. As shown in Figure 19-5, a presentation of six cysteine residues occurs in this family and provides the similar three-loop structures of EGF and TGF-α that explain the ability of both structures to bind to the EGF receptor. There is no evidence for a specific TGF-α receptor or for the activities of TGF-α on epithelial and mesenchymal cell proliferation; migration and differentiation appear to be due to the mediation of the TGF-α–EGFR interaction.

The 50-amino acid form of TGF-α is released from a membrane precursor, as shown in Figure 19-7. Two forms of this 159- or 160-amino-acid pro-TGF-α are coexpressed due to the microheterogeneity of mRNA splicing. The cysteine-rich cytoplasmic domain is highly conserved. The 50-amino-acid TGF-α is released from the precursor by proteases that cleave AV bonds at both termini (black arrows). Sometimes the processing is incomplete and larger forms of TGF-α are released that are still biologically active. This type of processing to a mature hormone from a membrane precursor is common throughout the EGF family. EGF is matured in a similar fashion, although the membrane precursor is larger than pro-TGF-α. Presumably the release of mature soluble growth factor from the precursor membrane form is a regulated event.

VI. TRANSFORMING GROWTH FACTOR-β (TGF-β) FAMILY

The crystal structure of TGF-β has been determined, and a computer model is shown in Figure 19-8. TGF-β either stimulates or inhibits cell growth or affects cellular functions distinct from mitosis. Among the activities of TGF-β are inhibition of proliferation of mesenchymal, epithelial, endothelial, and transformed cells, stimulation of proliferation of these cell types, control of extracellular matrix interactions, suppression of immune function, regulation of embryogenesis and cellular differentiation, fibroblast transformation, inhibition of adrenal steroidogenesis, and regulation of the biosynthesis and action of FSH. These represent many diverse activities, including several that are distinct from the effects on cellular proliferation. There are three isoforms in the mammal (TGF-β1–3), a chicken form (TGF-β4), and a frog form (TGF-β5). There are other more distant members of this family. The members of this family are related by sequence homology but, for the most part, act through separate receptors, although some of these receptors, as expected, may share some homology. TGF-β is found in the α-granules of platelets, and some species, such as pig, chicken, and mouse, contain isoforms. Aspects of the structure of TGF-β are described in Figure 19-8.

Members of the TGF-β family are pictured in Figure 19-9. Among the family members are inhibins, activins, Müllerian inhibitory substance (MIS), bone morphogenetic protein, Vg1 and Vgr-1 from xenopus, and DPP-C in *Drosophila* development. Inhibins, activins, and Müllerian inhibitory substance are discussed in Chapters 12 and 13.

TGF-β is generated from an inactive precursor protein. The precursors are glycosylated and substituted

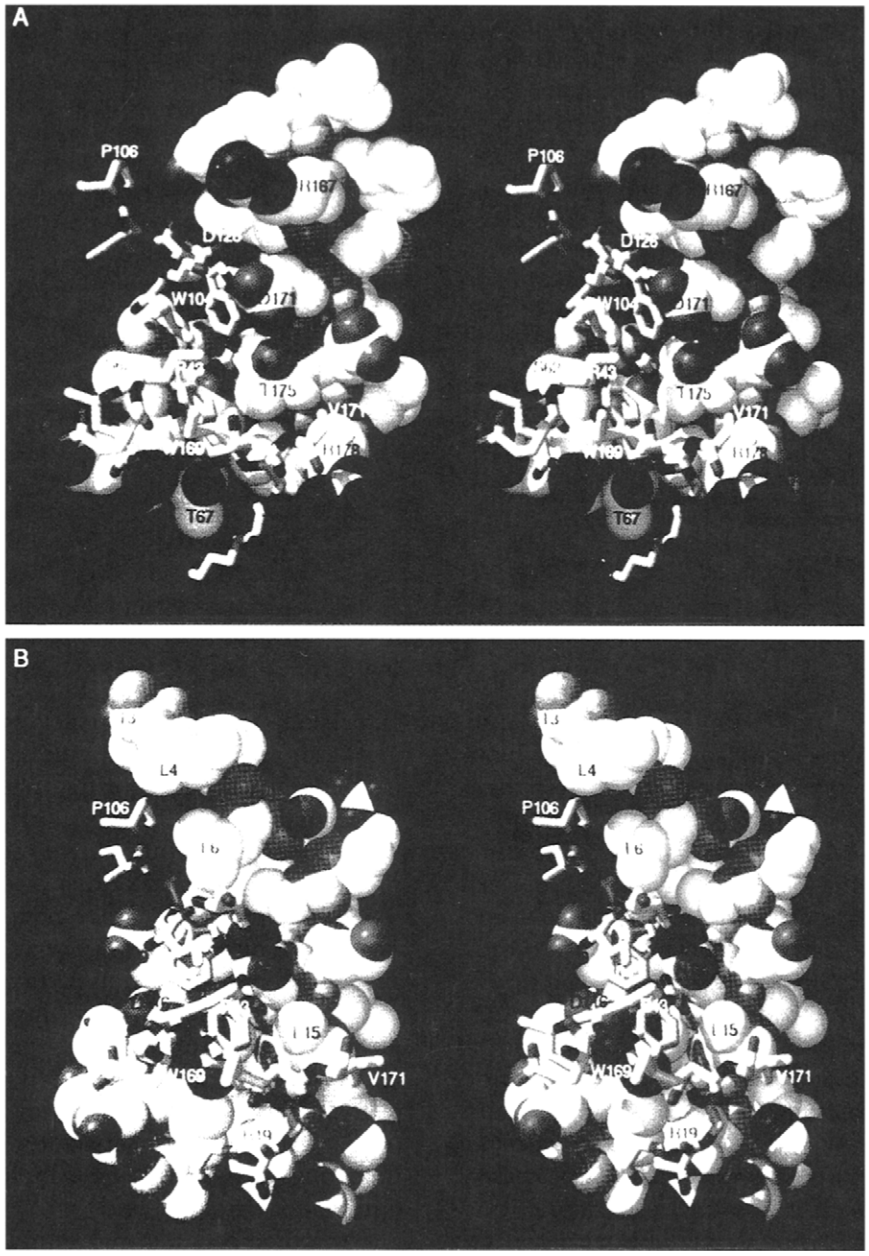

FIGURE 19-3 Close-up of interfaces between hormone and receptors: (A) binding site I; (B) binding site II. The hGH is represented by a space-filling model and the receptors by a stick model. The hGH backbone atoms are cyan, side chain carbons are white, and side chain oxygens and nitrogens are red and blue, respectively. The receptor carbon atoms are in yellow, with red oxygens and blue nitrogens. Selected residues are labeled. This figure is reproduced in black and white from a colored illustration with permission from de Vos, A. M., Ultsch, M., and Mossiakoff, A. (1992). Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. *Science* **255,** 306–212.

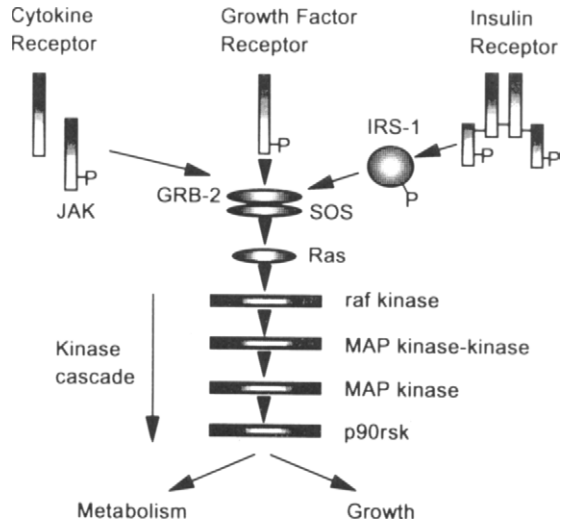

FIGURE 19-4 Signal pathway of receptor-activated tyrosine kinase receptors. Activation of receptor-linked tyrosine kinases by cytokines, growth factors, and insulin induces receptor autophosphorylation. The complex of GRB2 and the son of sevenless (SOS) guanine nucleotide-releasing protein is recruited to the plasma membrane by the receptor via SH2 domain–phosphotyrosine interaction. SOS activates raf kinase, which initiates a phosphorylation cascade, mitogen-activated protein (MAP) kinase-kinase, MAP-kinase, and $p90^{rsk}$, leading to cellular effects on metabolism and cell growth. Reproduced with permission from Gammeltoft, S., and Kahn, C. R. (1995). Hormone signaling via membrane receptors. *In* "Endocrinology" (L. J., DeGroot, ed.), 3rd ed., Vol. 1, pp. 17–65. W.B. Saunders Co., Philadelphia, PA.

with mannose phosphate. Dimerization follows to a 110-kDa inactive precursor. The precursor has to be activated in a process that is not well understood, but one that may involve proteases. TGF-β in serum is bound to α_2-macroglobulin, which is cleared by the liver; however, the latent complex of TGF-β has an extended half-life and is probably activated by proteases and binding to the IGF-II receptor through substituent mannose 6-phosphate residues. This complex between latent TGF-β and IGF-II receptor may be the best conformational form to allow the activating proteases to attack the latent TGF-β. Latency is apparently a function of the content of sialyl substituents. Binding of latent TGF-β to IGF-II receptor could also facilitate endocytosis of the complex to avail the latent TGF-β to the lysosomes and an activating environment. The active TGF-β is a 12.5-kDa peptide in the form of a 25-kDa homodimer linked by disulfide bonds.

There is a membrane-binding protein that forms a 300-kDa complex with TGF-β. The function of the binding protein is unknown except that it contains large amounts of heparin sulfate and chondroitin sulfate. This complex may represent a storage form of TGF-β. The signal-transducing form of TGF-β appears to involve the growth factor in an heteromeric complex of type I and type II TGF-β receptors, which form ligand–receptor complexes of about 65 and 130 kDa, respectively, generating a heterodimer of about 200 kDa. The direct effects of TGF-β through its heterodimeric receptor complex appear to be reversible forms of inhibition of cell growth directed at the G_1 phase of the cell cycle. TGF-β may also interfere with the expression of c-*myc*, which is a growth factor for many cells. Also, TGF-β may affect c-*myc* expression together with pRB, the protein product of the retinoblastoma gene. The stimulatory effects of this growth factor are more indirect than the negative effects and may be accounted for by the ability of TGF-β to induce other growth factors, for example, the β-chain of PDGF. In some cases, TGF-β has been shown to be a signal for inducing apoptosis. Although this mechanism is not well understood, it is becoming clear that cascades of cysteine proteases are involved in apoptosis, and these proteases, once activated, catalyze the cleavage of important proteins in the cell and may also account for the activation of other destructive enzymes, such as nucleases. Which particular proteases are activated by TGF-β will prove to be of interest.

VII. PLATELET-DERIVED GROWTH FACTOR (PDGF)

PDGF consists of two chains, A and B, which are separate gene products joined by a disulfide bond. Consequently, three forms of PDGF exist: PDGF-AA, PDGF-AB, and PDGF-BB. The molecular weight of the dimer is about 30 kDa, the A chain being 12 kDa and the B chain 18 kDa. Human platelets contain all three isoforms. PDGF-AA has been found in the context of the osteosarcoma cell-derived growth factor (ODGF), and PDGF-BB has been found in the context of $p28^{sis}$ secreted by simian sarcoma virus-infected cells. PDGF-AA is a secreted form of the growth factor, whereas PDGF-BB is associated with intracellular compartments.

There are two PDGF receptors, α and β. Both receptors have five extracellular Ig-like domains and intracellular tyrosine kinase domains (Figure 19-10). When the dimeric ligand binds to a receptor, a receptor dimer is formed that is capable of activating the signal transduction process, including autophosphorylation of intracellular tyrosine residues as an initial step. It is of interest that both receptors α and β induce signal transduction, but only the β-receptor is involved in chemotaxis. The α-receptor binds both the A and B chains of PDGF, but the β-receptor binds only the B chain of the growth factor. Thus, receptor dimer $\alpha\alpha$ binds PDGF-

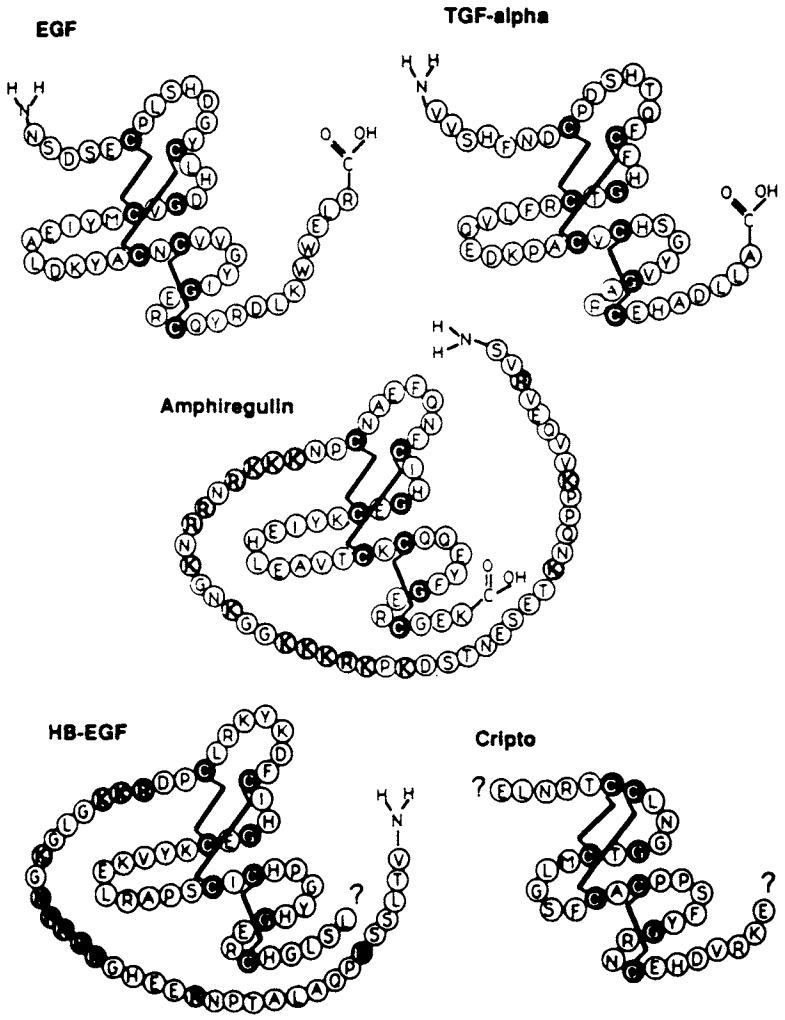

FIGURE 19-5 Members of the EGF superfamily. Cysteine bridges are indicated by solid black lines. Conserved amino acids are shown in black, and positively charged residues in the N-terminal extensions of HB-EGF and amphiregulin are indicated by shaded circles. All members of the EGF family of mitogens contain a highly conserved 36- to 40-amino acid motif, containing six cysteines that link internally to produce three peptide loops. In addition, all members of the family are synthesized embedded within larger precursor proteins. All three forms of TGF-α are capable of activating EGF–TGF-α receptors. Reproduced with permission from Russell, W. E., and Van Wyk, J. J. (1995). Peptide growth factors. *In* "Endocrinology" (L. J., DeGroot, ed.), 3rd ed., Vol. 1, pp. 2590–3623. W. B. Saunders Co., Philadelphia, PA.

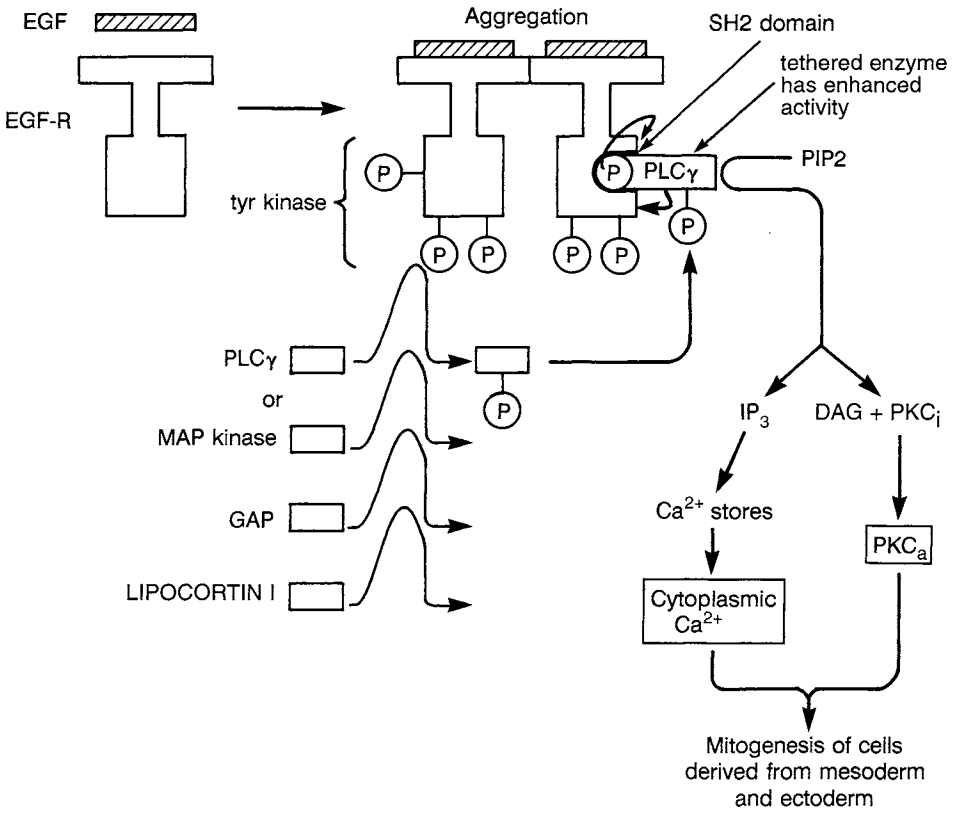

FIGURE 19-6 Signal transduction of EGF-R.

AA while receptor dimers $\alpha\alpha$ and $\alpha\beta$ bind PDGF-AB, and all of the receptor dimers, including $\beta\beta$, bind PDGF-BB. The PDGF–receptor complex is internalized and degraded, and the signal transduction process set off by the growth factor–receptor complex has already been indicated (Figure 19-4).

PDGF promotes cell division and does this by inducing a state of competence to undergo DNA synthesis. Other growth factors in the PDGF-stimulated cells are involved in progression through the G_1 phase of the cell cycle on the way to the S phase. As mentioned earlier, PDGF also functions in smooth muscle cells and fibroblasts as a chemoattractant, although other growth factors involved in progression are not required for PDGF to exert chemoattractant activity. PDGF is also involved in wound healing, and it is responsible for the appearance of macrophages in wounds where fibroblasts and endothelial cells replicate as part of the wound-healing process under the influence of PDGF. PDGF may also be involved in atherosclerosis, where initial injury to the intima can lead to platelet aggregation and degranulation. In this process, PDGF is released and smooth muscle cells migrate to the site and cause the sequestering of lipids. PDGF may have other important activities.

VIII. FIBROBLAST GROWTH FACTOR (FGF)

There are two separate forms of FGF, the basic FGF (bFGF) and the acidic FGF (aFGF), and these factors stimulate cell division in cell lines derived from neuroectoderm and mesoderm. The angiogenesis factor appears to be FGF, and FGF causes the proliferation of vascular endothelial cells. *int*-2, present in tumors, is related to FGF. *hst*-1 and kFGR are FGF-4. FGF-4 and -5 are related proteins, both oncogenes, and FGF-6 is related to *hst*.

bFGF is a 16-kDa, acid-labile protein, and aFGF is 15 kDa and has homology to bFGF. FGFs have affinity for heparin, which enhances the biological activity of FGF and increases its stability. FGFs are extracellular and may be secreted from the cell by Ca^{2+}-dependent exocytosis. Outside the cell, bFGF associates with negatively charged proteoglycans. Thus, FGFs seem to be stored in the extracellular matrix. Receptors for FGF are *flg* and *bek*. They contain three IgG-like domains in the extracellular region; they have single transmembrane domains and tyrosine kinase in the cytoplasmic domains. Either receptor binds both forms of FGF. There are a large number of FGF receptor isoforms

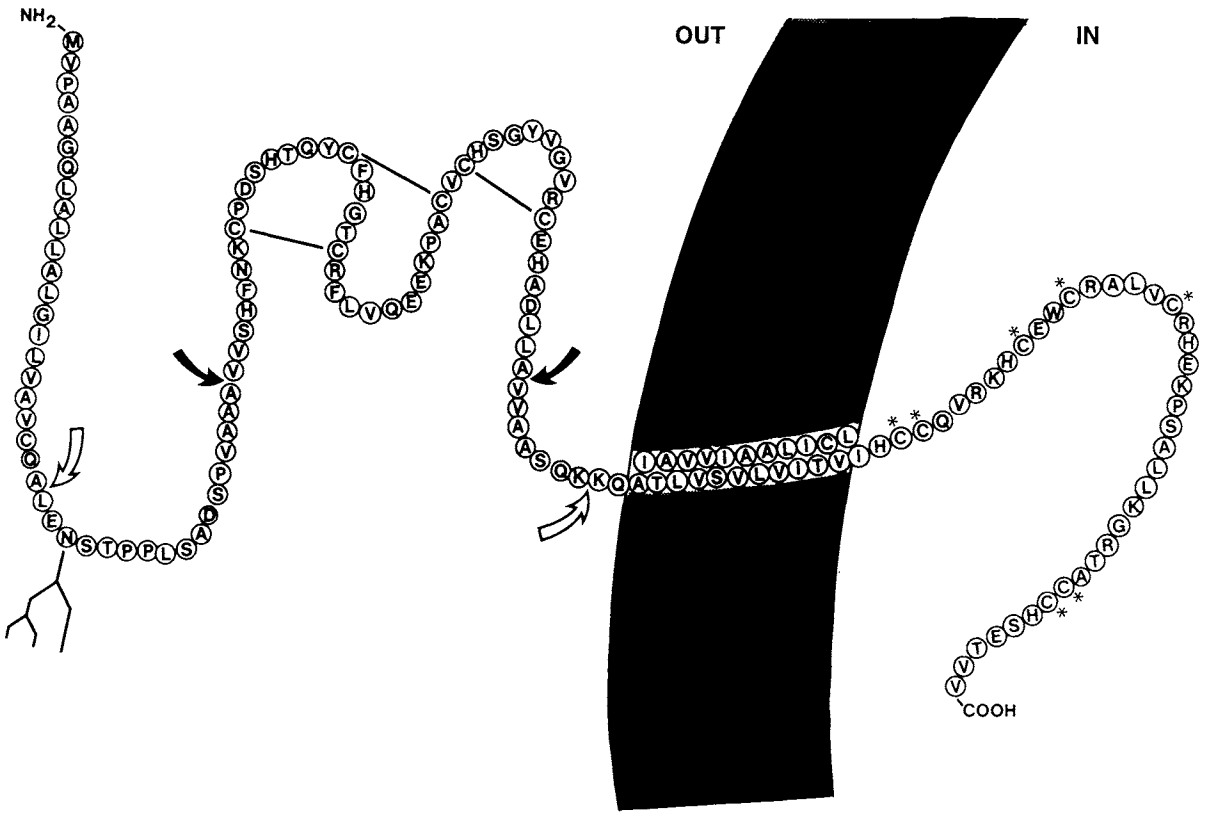

FIGURE 19-7 Complete amino acid sequence of the 159-amino acid pro-TGF-α is shown as embedded in the plasma membrane. A consensus site for N-linked glycosylation is indicated, and cysteine residues within the cytoplasmic domain are marked with asterisks. Black arrows indicate sites of proteolytic cleavage that release the mature, 50-amino acid form. The open arrow near the amino terminus marks the apparent signal peptide cleavage site between amino acids 22 and 23. The open arrow immediately external to the plasma membrane marks a lysine–lysine bond that could serve as a cleavage site for a trypsin-like protease. Reproduced with permission from Lee, D. C., Luetteke, N. C., Qiu, T. H., Chen, X., and Berkowitz, E. A. (1993). Transforming growth factor-alpha. *In* "Growth Factors in Perinatal Development" (R. C. Tsang, J. A. Lemons, and W. F. Balistreri, eds.), pp. 21–38. Raven Press, NY.

deriving from splice variants of four different genes. FGF resembles PDGF in its biological activity, and its signal transduction process is similar to that of other growth factor receptors, including PDGF (Figure 19-4).

IX. NERVE GROWTH FACTOR (NGF)

NGF was discovered in the mouse submaxillary gland. It has a molecular weight of 13,259 and exists in a complex of 140 kDa. The complex contains two molecules of NGF (β-subunits) together with two α- and two γ-subunits (the γ-subunit is arginine esterase). Apparently the identity of the α-subunits remains unclear. Although NGF is not a mitogen, it prevents apoptosis of neurons. A now classical demonstration of the induction of apoptosis is the withdrawal of NGF, usually in chick embryos, that triggers programmed cell death. This is the case during development, but dependence on NGF for neuronal survival does not occur in the adult. NGF causes a rat pheochromocytoma, PC-12, to differentiate into sympathetic neurons. Experimentally, NGF can also act as a chemoattractant for neutrophilic leukocytes. The structure of the complex containing NGF is shown in Figure 19-11.

Other growth factors with homology to NGF do exist. Examples of these are neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF). Two other factors fall into this class of NGF homologues, but they have not been well described. Ciliary neurotrophic factor (CNTF) is a survival factor for chick ciliary ganglia parasympathetic neurons; however, it is different from neurotrophins NT-3 and BDNF. CNTF is expressed throughout the central nervous system and the peripheral nervous system and is a survival factor for neurons in these regions at various developmental stages. It is

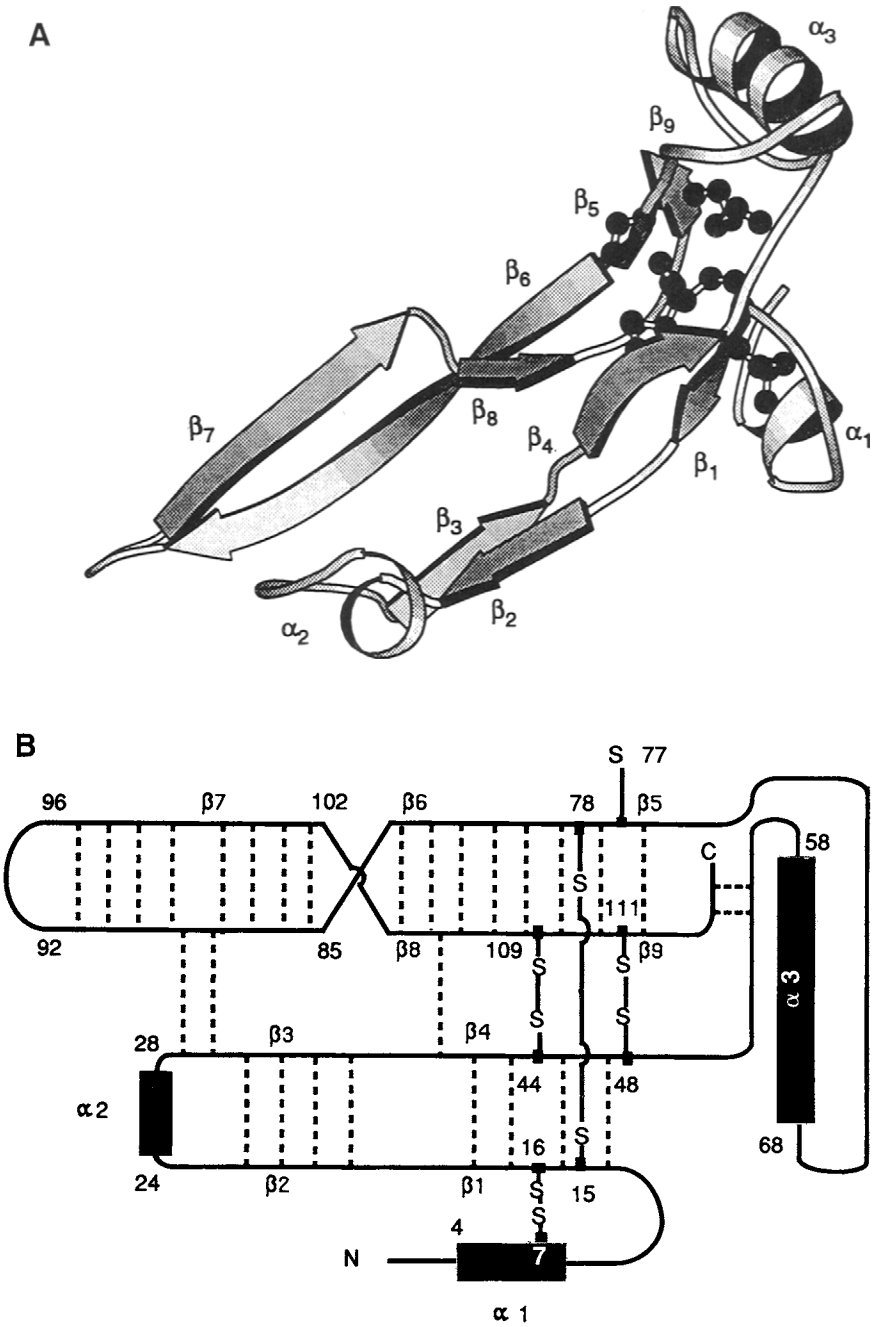

FIGURE 19-8 Crystal structure of TGF-β. (A) Topology diagram of a TGF-β2 subunit. The α-helices are labeled as α_1, α_2, and α_3, and peptide strands in β-sheets are labeled from β_1 through β_9. The residues involved in the regular secondary structure are as follows: α_1, residues 4–8; α_2, 24–28; α_3, 58–68; β_1, 15–18; β_2, 20–23; β_3, 37–40; β_4, 42–46; β_5, 77–80; β_6, 82–91; β_7, 96–102; β_8, 104–106; and β_9, 109–112. (B) Schematic drawing of the primary and secondary structure of a TGF-β2 subunit. Hydrogen bonds in the β-strands and loops are indicated by dashed lines. The analogy to a left hand can be seen. The heel (helix α3) is to the right and the fingers (β-strands) are to the left with the third and fourth fingers twisted. Reproduced with permission from Daopin, A., Piez, K. A., Ogawa, Y., and Davies, D. R. (1992). Crystal structure of transforming growth factor-β-2: An unusual fold for the superfamily. *Science* **257**, 369–373.

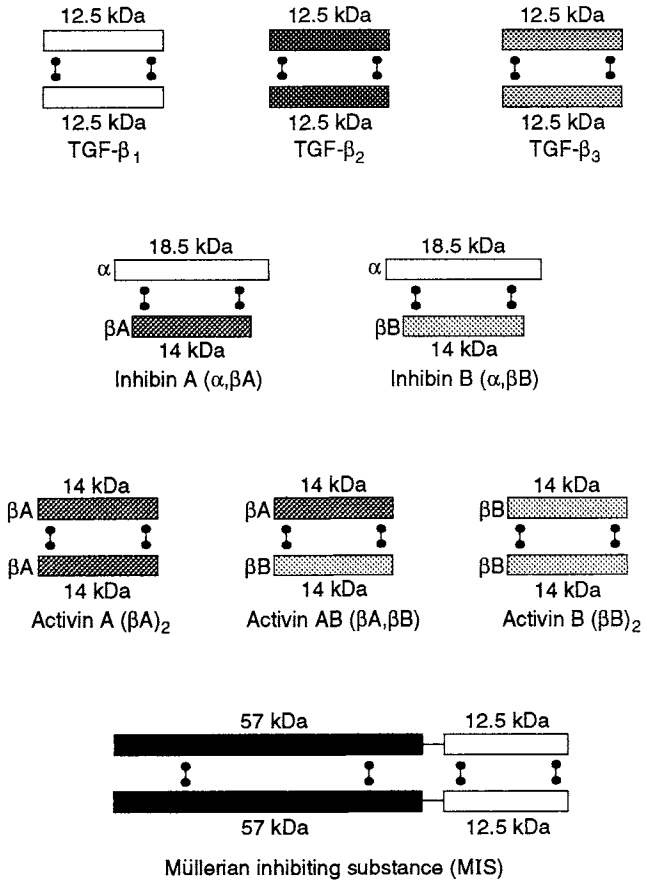

FIGURE 19-9 Structural relationships between members of the transforming growth factor-β (TGF-β) family. TGF-β_{1-3} are homodimers with different subunits. The homology between the subunits is greater than 80%. The α-subunits of inhibins and activins are unlike any other structures in the TGF-β family. The β-subunits of inhibin A and inhibin B (designated β_A and β_B) are approximately 30% homologous to TGF-β subunits. The activins consist of either homodimers or heterodimers of the β_A and/or β_B-subunits. Members of this family were isolated from intact cells and tissues as larger prohormones, which, in the case of the three patriarchal TGF-β's, remain inactive until the mature dimers shown are liberated from their latent form by exposure to acidic conditions or proteases. An exception to this is the Müllerian inhibiting substance (MIS), which was isolated as a much larger active dimer, each element of which is 70 kDa. The smaller homodimer, consisting of identical 12.5-Da subunits, is cleaved from the precursor by plasmin digestion and found to retain the same biological activity as the larger form of MIS. Reproduced with permission from Russell, W. E., and Van Wyk, J. J. (1995). Peptide growth factors. *In* "Endocrinology" (L. J., DeGroot, ed.), 3rd ed., Vol. 1, pp. 2590–3623. W.B. Saunders Co., Philadelphia, PA.

also a factor that prevents the death of motor neurons. CNTF seems to belong to a family of hematopoietic cytokines, as shown in Figure 19-12. These cytokines include granulocyte colony-stimulating factor (G-CSF), oncostatin M (OSM), leukemia inhibitory factor (LIF), and interleukin (II-6).

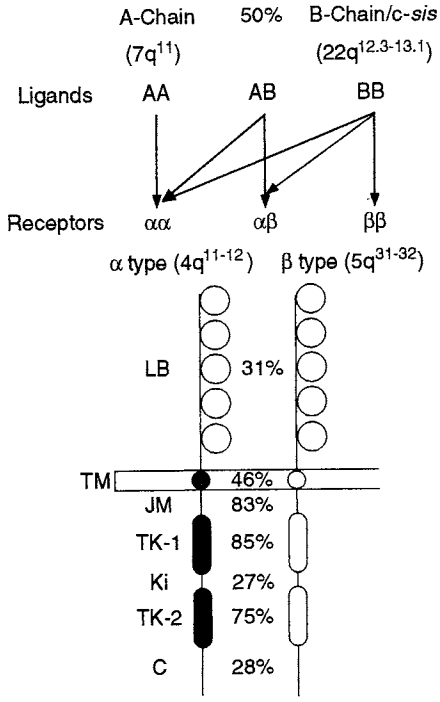

FIGURE 19-10 Biological relationship between three PDGF isoforms and two PDGF receptors. Arrows show the binding affinities for each dimerized receptor. The amino acid sequence homology (%) between α and β-PDGF receptors is shown in each domain. Abbreviations: LB, ligand binding; TM, transmembrane; JM, juxta membrane; TK, tyrosine kinase; Ki, kinase insert; C, carboxy terminal. Human chromosomal location is shown in parentheses. Reproduced with permission from Matsui, T. (1994). Platelet derived growth factor system in human oncogenesis and its signaling pathways. *In* "Growth Factors: Cell Growth, Morphogenesis, and Transformation." (T. Nakamura and K. Matsumoto, eds.), Gann Monograph on Cancer Research pp. 42, 1–12, Japan Scientific Societies Press, Tokyo, CRC Press, Boca Raton/Ann Arbor/London/Tokyo.

NGF binds to two receptor types, one of which is high affinity and the other is low affinity. The latter is found in lymphocytes and macrophages, and both forms may derive from a single gene. The tyrosine kinase receptor, *trk*, is found in neural tissues

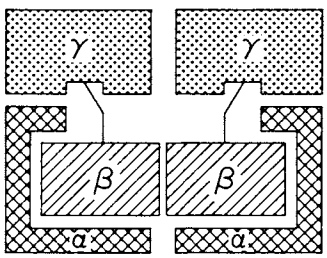

FIGURE 19-11 Mouse 7 S nerve growth factor (NGF). The β-subunit represents the monomeric NGF. Reproduced with permission from Underwood, L. E., and van Wyk, J. J. (1985). *In* "Textbook of Endocrinology" (R. H. Williams, ed.) 7th ed., p. 172. W.B. Saunders Co., Philadelphia, PA.

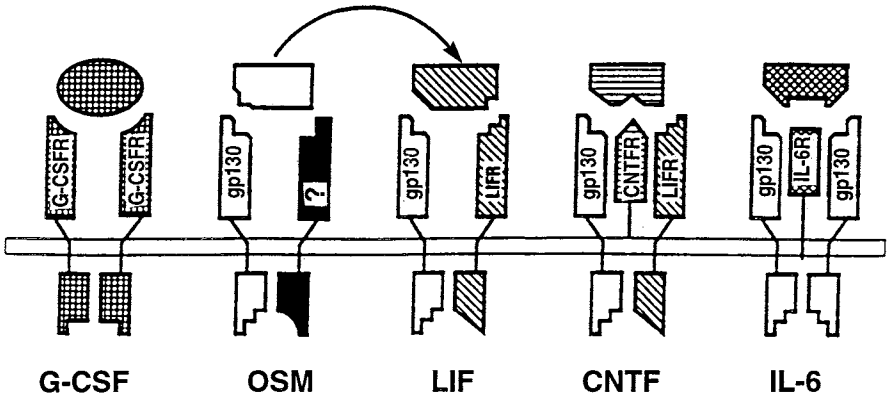

FIGURE 19-12 Diagram to illustrate similarities between the ligand–receptor complexes of the ciliary neurotrophic factor (CNTF) and related cytokines. Abbreviations: G-CSF, granulocyte colony-stimulating factor; OSM, oncostatin M; LIF, leukemia inhibitory factor; and IL-6, interleukin-6. The initial step in signal transduction is binding of the respective ligands to a low-affinity binding site, the expression of which determines cellular specificity. The low-affinity binding sites for the CNTF and IL-6 receptors are soluble, extracellular α-subunits that attach to the cell membrane through glycosophosphatidylinositol (GPI) linkages. A similar α-subunit has not been identified for LIF, OSM, and G-CSF, and the initial low-affinity binding is thought to be to one of the transmembrane subunits. Initial binding of the ligand is rapidly followed by dimerization of two transmembrane β-subunits. Dimerization creates high-affinity binding sites for their respective ligands and permits signal transduction. The binding subunit of OSM has not yet been identified. Note that the gp-130 subunit is utilized by CNTF, IL-6, LIF, and OSM, whereas the subunit initially described as the LIF receptor (LIFR) is shared with CNTF. The arrow signifies that OSM can also bind to the LIF receptor. Reproduced with permission from Russell, W. E., and Van Wyk, J. J. (1995). Peptide growth factors. *In* "Endocrinology" (L. J., DeGroot, ed.), 3rd ed., Vol. 1, pp. 2590–3623. W.B. Saunders Co., Philadelphia, PA.

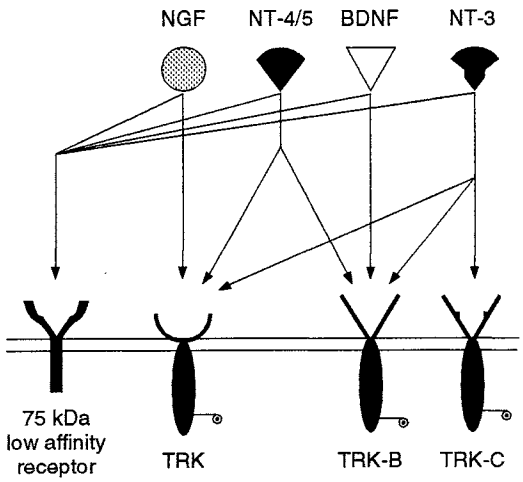

FIGURE 19-13 Neurotrophin–neurotrophin receptor interactions. Reproduced with permission from Maness, L. M., Kastin, A. J., Weber, J. T., Banks, W. A., Beckman, B. S., and Zadina, J. E. (1994). The neurotrophins and their receptors: Structure, function, and neuropathology. *Neurosci. Biobehav. Rev.*, **18**, 143–159.

and binds NGF as well as NF-3 and BDNF. Binding of neurotrophic factors to *trk* receptors is summarized in Figure 19-13. Furthermore, the signal transduction pathway following interaction with the *trk* receptor involves several components, including PI_3k, SRC, RAS, PLC-γ-1, and downstream pathways. These are summarized in Figure 19-15. Thyroid hormone positively regulates the levels of NGF in the brain during development and is required for normal development.

Hematopoietic growth factors are discussed in Chapter 15. Other growth factors relating to the gastrointestinal tract are discussed in Chapter 8.

X. HEPATOCYTE GROWTH FACTOR (HGF, SCATTER FACTOR)

HGF is a disulfide-linked heterodimer of 69-kDa α- and 34-kDa β-subunits derived by cleavage of an 85-kDa precursor protein. The precursor protein has four double-loop structures (kringle domains). The cleavage site for the release of α- and β-subunits of HGF is identical to that in plasminogen for the genera-

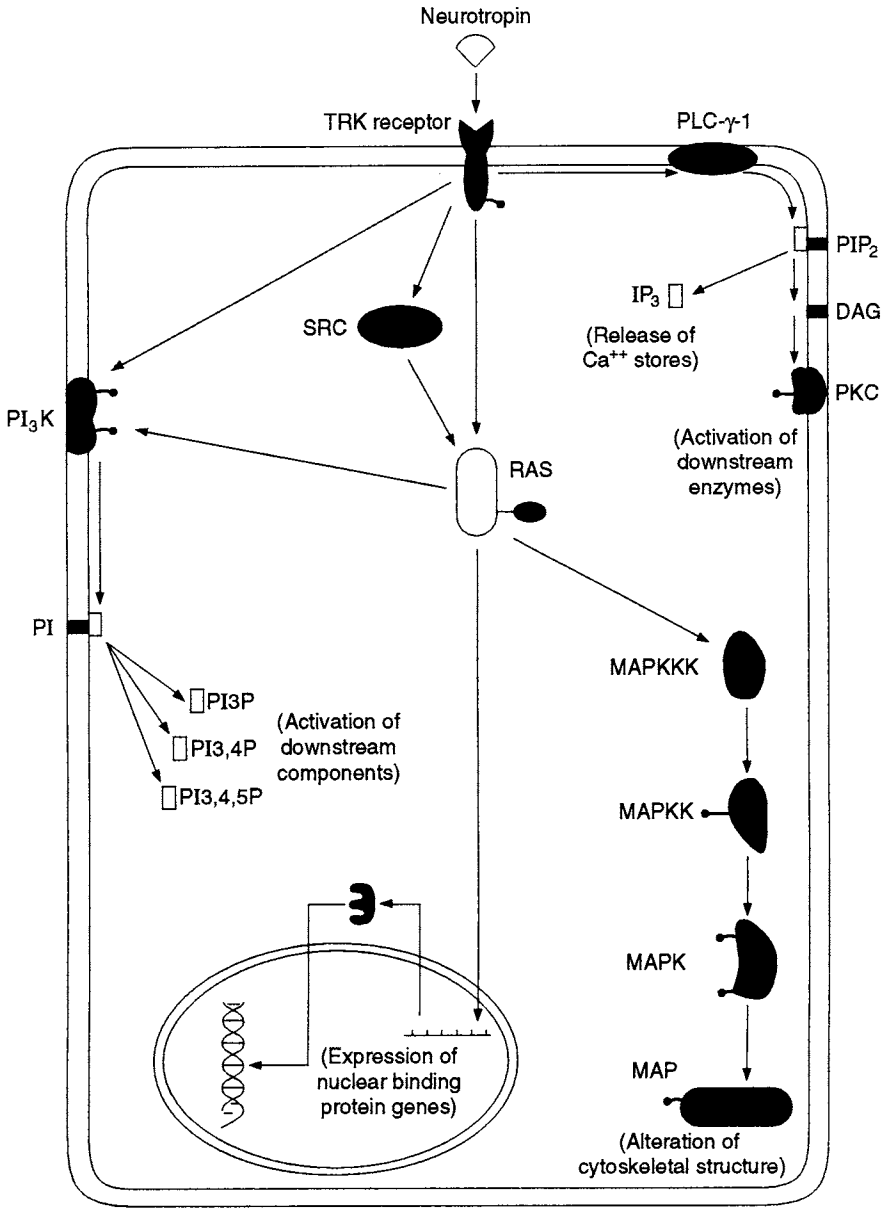

FIGURE 19-14 Putative neurotrophin signal transduction mechanisms. Reproduced with permission from Maness, L. M., Kastin, A. J., Weber, J. T., Banks, W. A., Beckman, B. S. and Zadina, J. E. (1994). The neurotrophins and their receptors: Structure, function, and neuropathology. *Neurosci. Biobehav. Rev.* **18,** 143–159.

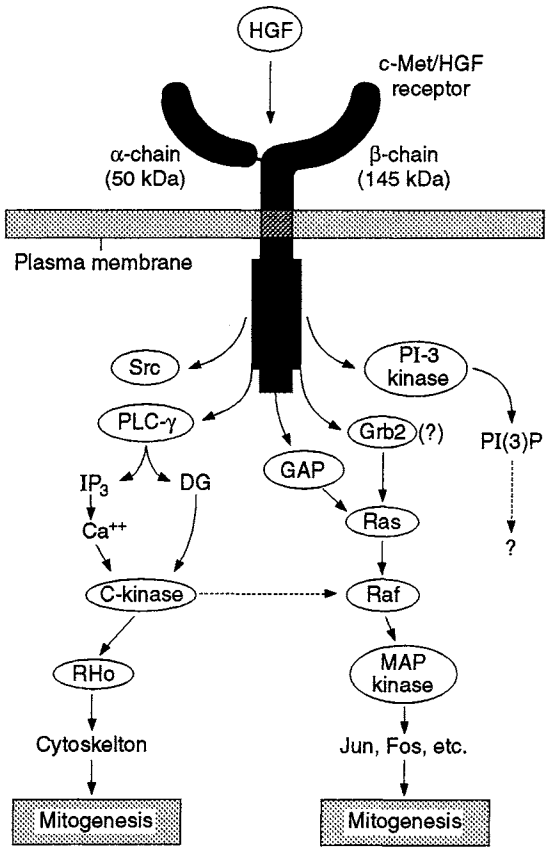

FIGURE 19-15 Schematic structure of *c-met*–HGF receptor and signal transduction pathways from the receptor. Reproduced with permission from Matsumoto, K., and Nakamura, T. (1994). Pleiotropic roles of HGF in mitogenesis, morphogenesis, and organ regeneration. *In* "Growth Factors: Cell Growth, Morphogenesis, and Transformation" (T. Nakamura and K., Matsumoto, eds.), Gann Monograph on Cancer Research, Vol. 42, pp. 91–112, Japan Scientific Societies Press, Tokyo, CRC Press, Boca Raton/Ann Arbor/London/Tokyo.

tion of plasmin through cleavage by plasminogen activator. Curiously, the precursor of HGF is as active as the heterodimer of α- and β-subunits.

The HGF receptor is the *c-met* protooncogene protein product, which is a membrane tyrosine kinase receptor. The kinase is in the β-chain of the receptor on the cytoplasmic side of the cell membrane. HGF stimulates growth in a wide variety of tissues, in spite of its name, besides hepatocytes, including melanoma cells, melanocytes, keratinocytes, renal tubule cells, and mammary epithelia. Within an hour of partial hepatectomy, there is a 15-fold increase in serum HGF levels.

The signal transduction pathways for HGF are indicated in Figure 19-15.

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