

# CYTOKINES, GROWTH FACTORS, AND HORMONES

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Growth factors and cytokines, secreted by a variety of cells, act through cell surface receptors and can elicit similar as well as distinct biochemical responses in their target cells. Growth factors are proteins that bind to receptors on cell membranes and elicit biological responses leading to cell proliferation and differentiation. Some growth factors are versatile and promote growth in a variety of cell, however others may be specific for a particular cell type.

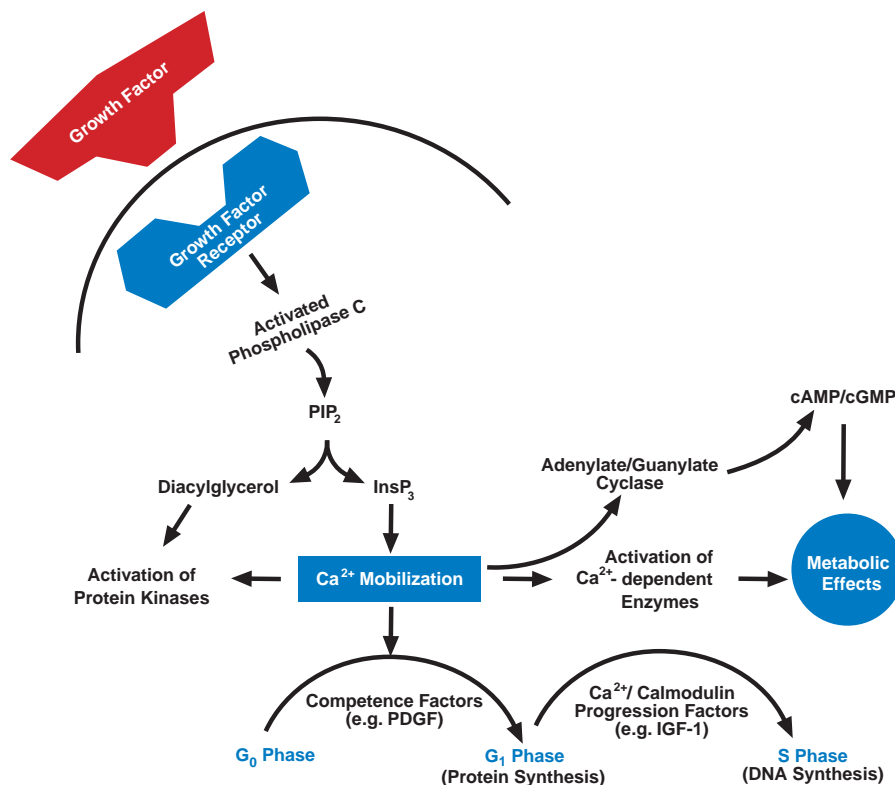
Major families of growth factor receptors are the tyrosine kinases, the small G-protein-associated receptors, and the serine/threonine kinases. Tyrosine phosphorylation is considered one of the most characteristic features of several growth factors. Receptors for EGF, TGF- $\beta$ , PDGF, and FGF contain a polypeptide domain with tyrosine kinase activity. Binding of growth factors to the receptors on quiescent cells leads to the activation of intrinsic receptor-associated tyrosine kinase activity and concomitant phosphorylation of tyrosine residues of cellular proteins.

The EGF-receptor, in general terms, typifies the tyrosine kinase family of growth factor receptors. Nearly all tyrosine kinase receptors described thus far are composed of an extracellular ligand-binding domain, a single transmembrane domain, a region containing the tyrosine kinase activity, and a carboxy terminus extending into the cytoplasm. Within the tyrosine kinase domain of

the EGF-receptor are amino acid residues that are conserved in all protein kinases and are thought to be involved in the binding of the ATP substrate.

The figure below provides an overview of some of the major pathways stimulated by growth factors. Here, the interaction of a growth factor with its receptor leads to the activation of membrane-associated phospholipase C that hydrolyzes phosphatidylinositol 4,5-diphosphate (PIP<sub>2</sub>) leading to the formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP<sub>3</sub>). The IP<sub>3</sub> causes the release of endogenous Ca<sup>2+</sup> that binds to the cytosolic PKC and exposes the phospholipid-binding site. The binding of Ca<sup>2+</sup> translocates PKC to the membrane where it interacts with DAG to transform PKC into a fully active enzyme.

The cytokine family of signaling molecules includes several interleukins, a variety of growth and colony-stimulating factors, ciliary neurotrophic factor, interferons, and several other molecules that exhibit pleiotropic effects on cell differentiation, tissue development and homeostasis. Cytokines share many properties with hormones and growth factors. Cytokines mediate communication among cells in the immune system through binding to specific receptors on target cells. Their biological actions vary widely depending upon the type of target tissue involved. They are endowed with anti-proliferative



**Mechanism of Growth Factor Action and Related Events**

properties and regulate the synthesis of acute phase proteins following tissue injury, trauma, inflammation, and sepsis. The receptors for a large number of cytokines have been cloned and shown to be membrane-spanning glycoproteins with their amino termini in the extracellular space. Unlike receptors for growth factors, cytokine receptors generally lack identifiable catalytic activity. The major diagnostic feature of the 'cytokine' receptors is the presence, in the extracellular region of the receptor, of a domain containing multiple cysteine residues and a conserved amino acid motif, WSXWS (Trp-Ser-X-Trp-Ser) that functions in the recognition and binding of the ligand.

Most cytokine receptors such as those for IL-3, GM-CSF, and the interferons lack intrinsic kinase activity. They are thought to transmit their regulatory signals primarily by the receptor-associated JAK (Janus kinase) family of tyrosine kinases. Ligand-binding to the receptor leads to JAK activation that phosphorylates cytoplasmic STAT (signal transducer and activator of transcription) proteins. Following phosphorylation on tyrosine residues, STATs are dimerized (resulting from phosphotyrosine — SH2 domain association). This dimerization is accompanied by translocation of STAT to the nucleus resulting in DNA binding to specific response elements and stimulation of gene transcription.

Chemokines belong to a family of structurally related, low molecular weight (8 to 10 kDa), glycoproteins of approximately 70 to 80 amino acid residues. Most chemokines fall into two subfamilies (a) CXC chemokines, contain a single amino acid between the first and second cysteine residues; (b) CC chemokines that have adjacent cysteine residues. Most CXC chemokines are chemoattractants for neutrophils whereas CC chemokines generally attract monocytes, lymphocytes, basophils, and eosinophils. Within the CXC subfamily, the chemokines can be further divided into (a) CXC chemokines with characteristic ELR sequence (glutamic acid-leucine-arginine) immediately preceding the first cysteine residue near the amino terminus, and (b) CXC chemokines lacking the ELR domain. The ELR containing chemokines, such as IL-8, act primarily as chemoattractants and activators of neutrophils. The ELR lacking chemokines, such as RANTES, eotaxin etc., chemoattract and activate monocytes, B and T lymphocytes, NK cells, basophils, and eosinophils. In addition to these two major groups there are two other small sub-groups. The C group contains only one member, lymphotactin, which lacks one of the cysteines in the four-cysteine motif, but shares homology at its carboxyl terminus with the C-C chemokines. The other subgroup is the C-X3-C subgroup, which has three amino acids separating the first two cysteine residues. Fractalkine/neurotactin belong to this subgroup. They are tethered directly to the cell membrane via a long mucin stalk and are involved in adhesion and migration of leukocytes.

Chemokines exert their effect through chemokine receptors, which belong to a superfamily of seven transmembrane loops and trans-

duce their signals through heterotrimeric G Proteins. The chemokine receptors that bind CXC chemokines are designated CXCRs and the receptors that bind CC chemokines are designated CCRs. The various CXCRs and CCRs are known to exhibit some overlapping ligand specificities. CXCR-1 and CXCR-2 share about 77% amino acid sequence homology. IL-8 binds to both receptors with high affinity and induces rapid elevation of cytosolic Ca<sup>2+</sup> levels. CXCR-1 exhibits much higher specificity for IL-8, however, CXCR-2 has broader specificity and binds with high-affinity to other ELR motif containing  $\alpha$  chemokines including GRO $\alpha$ ,  $\beta$  and  $\gamma$ . Those  $\alpha$  chemokines that lack the ELR motif do not exhibit any affinity for CXCR-2. CXCR-3, which is highly expressed by IL-2- activated T lymphocytes, binds IP-10 and Mig with high affinity and mediates Ca<sup>2+</sup> mobilization and chemotaxis. It does not bind ELR-containing CXC chemokines. CXCR-4, also known as fusin, is reported to be a necessary cofactor for entry of T cell-tropic HIV viruses into CD4<sup>+</sup> cells. PBSF/SDF-1 chemokines act as ligands for CXCR-4.

CCR-1, the first identified C-C chemokine receptor, is expressed on monocytes and neutrophils and binds macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), RANTES, monocyte chemoattractant protein-3 (MCP-3) with high affinity. CCR-2A and CCR-2B are expressed on monocytes and specifically bind (MCP-1) and MCP-3. CCR-3 exhibits high affinity for eotaxin. CCR-4 was originally cloned from a human immature basophilic cell line and has also been shown to be expressed in T cells and IL-5-primed basophils. It mediates the effects of RANTES, MIP-1 $\alpha$ , and MCP-1. CCR-5, the most recent entry to the group of CC receptors exhibits about 48 to 75% amino acid sequence homology with other CC receptors is expressed in primary adherent monocytes, but not in neutrophils or eosinophils. It mediates the activities of RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$ .

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