3rd semester **Zoo-18301-CR**

Signal Transduction Pathways: JAK/STAT and MAP Kinase pathways

Most cell surface receptors stimulate intracellular target enzymes, which may be either directly linked or indirectly coupled to receptors by G proteins. These intracellular enzymes serve as downstream signaling elements that propagate and amplify the signal initiated by ligand binding. In most cases, a chain of reactions transmits signals from the cell surface to a variety of intracellular targets—a process called intracellular signal transduction. The targets of such signaling pathways frequently include transcription factors that function to regulate gene expression. Intracellular signaling pathways thus connect the cell surface to the nucleus, leading to changes in gene expression in response to extracellular stimuli.

I. JAK/STAT Pathway:

The MAP kinase pathway provides an indirect connection between the cell surface and the nucleus, in which a cascade of protein kinases ultimately leads to transcription factor phosphorylation. An alternative pathway, known as the JAK/STAT pathway, provides a much more immediate connection between protein-tyrosine kinases and transcription factors. In this pathway protein-tyrosine phosphorylation directly affects transcription factor localization and function.

The key elements in this pathway are the STAT proteins (signal transducers and activators of transcription), which were originally identified in studies of cytokine receptor signaling. The STAT proteins are a family of transcription factors that contain SH2 domains. They are inactive in unstimulated cells, where they are localized to the cytoplasm. Stimulation of cytokine receptors leads to recruitment of STAT proteins, which bind via their SH2 domains to phosphotyrosine-containing sequences in the cytoplasmic domains of receptor polypeptides. Following their association with activated receptors, the STAT proteins are phosphorylated by members of the JAK family of nonreceptor protein-tyrosine kinases, which are associated with cytokine receptors. Tyrosine phosphorylation promotes the dimerization of STAT proteins, which then translocate to the nucleus, where they stimulate transcription of their target genes.

Further studies have shown that STAT proteins are also activated downstream of receptor protein-tyrosine kinases, where their phosphorylation may be catalyzed either by the receptors themselves or by associated nonreceptor kinases. The STAT transcription factors thus serve as direct links between both cytokine and growth factor receptors on the cell surface and regulation of gene expression in the nucleus.

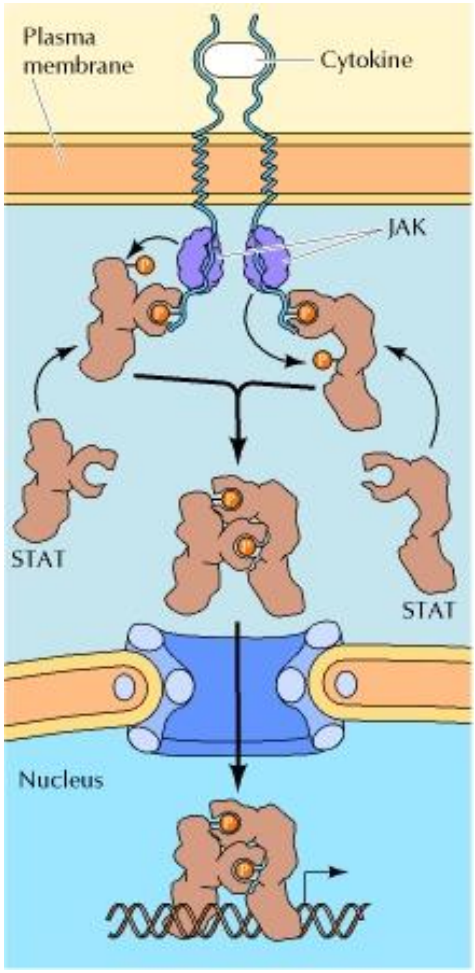

Fig. The JAK/STAT pathway [The STAT proteins are transcription factors that contain SH2 domains that mediate their binding to phosphotyrosine-containing sequences. In unstimulated cells, STAT proteins are inactive in the cytosol. Stimulation of cytokine receptors leads to the binding of STAT proteins, where they are phosphorylated by the receptor-associated JAK protein-tyrosine kinases. The phosphorylated STAT proteins then dimerize and translocate to the nucleus, where they activate the transcription of target genes.]

II. MAP Kinase Pathway:

The MAP kinase pathway refers to a cascade of protein kinases that are highly conserved in evolution and play central roles in signal transduction in all eukaryotic cells, ranging from yeasts to humans. The central elements in the pathway are a family of protein-serine/threonine kinases called the MAP kinases (for mitogen-activated protein kinases) that are activated in response to a variety of growth factors and other signaling molecules. In yeasts, MAP kinase pathways control a variety of cellular responses, including mating, cell shape, and sporulation. In higher eukaryotes (including *C. elegans*, *Drosophila*, frogs, and mammals), MAP kinases are ubiquitous regulators of cell growth and differentiation.

The best-characterized forms of MAP kinase in mammalian cells belong to the ERK (extracellular signal-regulated kinase) family. ERK activation plays a central role in signaling cell proliferation induced by growth factors that act through either protein-tyrosine kinase or G protein-coupled receptors. Activation of ERK is mediated by two upstream protein kinases, which are coupled to growth factor receptors by a GTP-binding protein called Ras. Activation of Ras leads to activation of the Raf protein-serine/threonine kinase, which

phosphorylates and activates a second protein kinase called MEK (for MAP kinase/ERK kinase). MEK is a dual-specificity protein kinase that activates members of the ERK family by phosphorylation of both threonine and tyrosine residues separated by one amino acid (e.g., threonine-183 and tyrosine-185 of ERK2). Once activated, ERK phosphorylates a variety of targets, including other protein kinases and transcription factors.

The central role of the ERK pathway in mammalian cells emerged from studies of the Ras proteins, which were first identified as the oncogenic proteins of tumor viruses that cause sarcomas in rats (hence the name Ras, from rat sarcoma virus). Interest in Ras intensified considerably in 1982, when mutations in ras genes were first implicated in the development of human cancers. The importance of Ras in intracellular signaling was then indicated by experiments showing that microinjection of active Ras protein directly induces proliferation of normal mammalian cells. Conversely, interference with Ras function by either microinjection of anti-Ras antibody or expression of a dominant negative Ras mutant blocks growth factor-induced cell proliferation. Thus, Ras is not only capable of inducing the abnormal growth characteristic of cancer cells, but also appears to be required for the response of normal cells to growth factor stimulation.

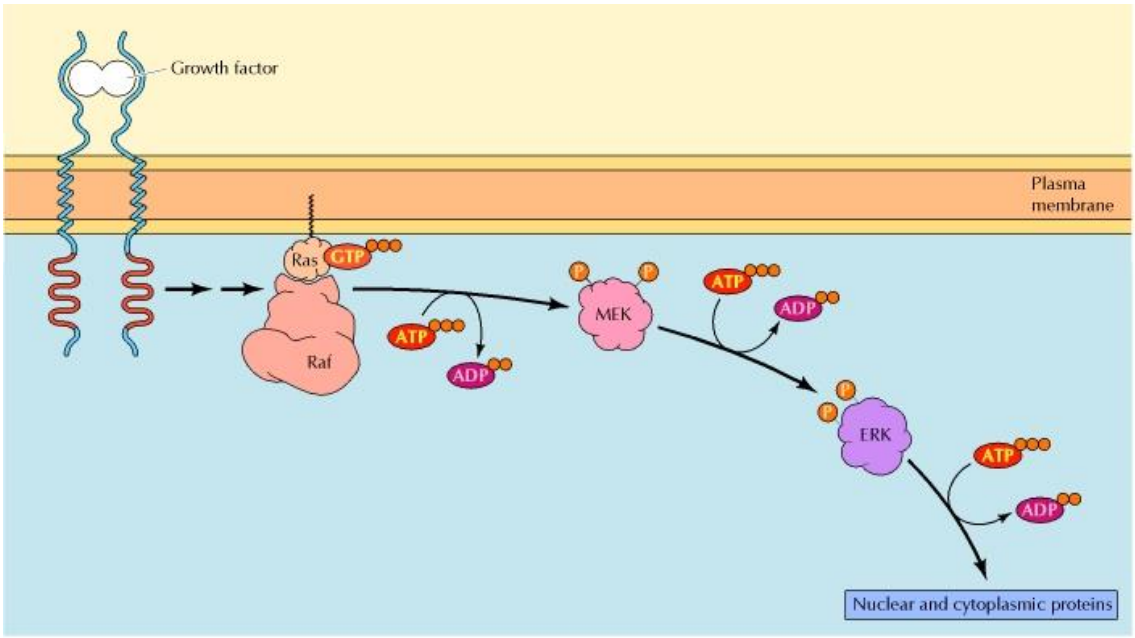

Fig. Activation of the ERK MAP kinases [Stimulation of growth factor receptors leads to activation of the small GTP-binding protein Ras, which interacts with the Raf protein kinase. Raf phosphorylates and activates MEK, a dual-specificity protein kinase that activates ERK by phosphorylation on both threonine and tyrosine residues (Thr-183 and Tyr-185). ERK then phosphorylates a variety of nuclear and cytoplasmic target proteins.]

The Ras proteins are guanine nucleotide-binding proteins that function analogously to the α subunits of G proteins, alternating between inactive GDP-bound and active GTP-bound forms. In contrast to the G protein α subunits, however, Ras functions as a monomer rather than in association with βγ subunits. Ras activation is mediated by guanine nucleotide exchange factors that stimulate the release of bound GDP and its exchange for GTP. Activity of the Ras-GTP complex is then terminated by GTP hydrolysis, which is stimulated by the interaction of Ras-GTP with GTPase-activating proteins. It is interesting to note that the mutations of ras genes in human cancers have the effect of inhibiting GTP hydrolysis by the Ras proteins. These mutated Ras proteins

therefore remain continuously in the active GTP-bound form, driving the unregulated proliferation of cancer cells even in the absence of growth factor stimulation.

The Ras proteins are prototypes of a large family of approximately 50 related proteins, frequently called small GTP-binding proteins because Ras and its relatives are about half the size of G protein α subunits. While the Ras proteins regulate cell growth and differentiation, other subfamilies of small GTP-binding proteins control distinct cellular activities. For example, the largest subfamily of small GTP-binding proteins (the Rab proteins) function to regulate vesicle trafficking. Other small GTP-binding proteins are involved in nuclear protein import (the Ran protein) and organization of the cytoskeleton (the Rho subfamily).

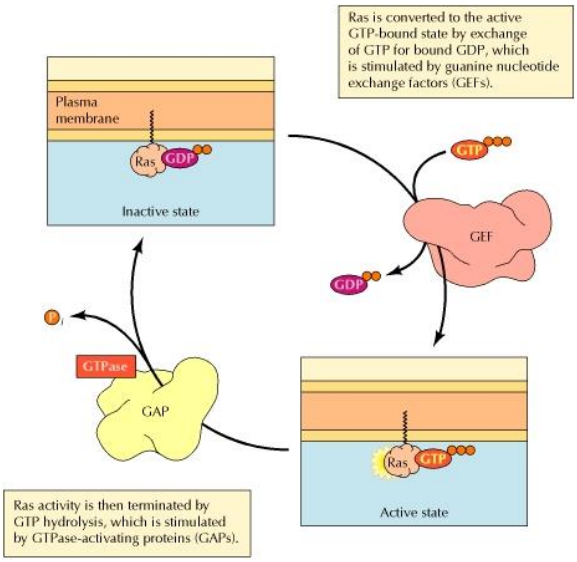

Fig. Regulation of Ras proteins [Ras proteins alternate between inactive GDP-bound and active GTP-bound states.]

The best understood mode of Ras activation is that mediated by receptor protein-tyrosine kinases. Autophosphorylation of these receptors results in their association with Ras guanine nucleotide exchange factors as a result of SH2-mediated protein interactions. One well-characterized example is provided by the guanine nucleotide exchange factor Sos, which is bound to the SH2-containing protein Grb2 in the cytosol of unstimulated cells. Tyrosine phosphorylation of receptors (or of other receptor-associated proteins) creates a binding site for the Grb2 SH2 domains. Association of Grb2 with activated receptors localizes Sos to the plasma membrane, where it is able to interact with Ras proteins, which are anchored to the inner leaflet of the plasma membrane by lipids attached to the Ras C terminus. Sos then stimulates guanine nucleotide exchange, resulting in formation of the active Ras-GTP complex. In its active GTP-bound form, Ras interacts with a number of effector proteins, including the Raf protein-serine/threonine kinase. This interaction with Ras recruits Raf from the cytosol to the plasma membrane, where it is activated as a result of phosphorylation by both protein-tyrosine and protein-serine/threonine kinases.

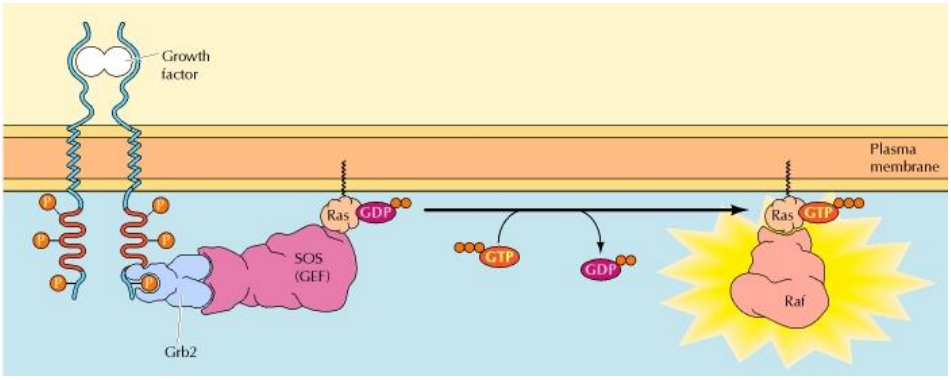

Fig. Ras activation downstream of receptor protein-tyrosine kinases [A complex of Grb2 and the guanine nucleotide exchange factor Sos binds to a phosphotyrosine-containing sequence in the activated receptor via the Grb2 SH2 domain. This interaction recruits Sos to the plasma membrane, where it can stimulate Ras GDP/GTP exchange. The activated Ras-GTP complex then binds to the Raf protein kinase.]

As already noted, activation of Raf initiates a protein kinase cascade leading to ERK activation. ERK then phosphorylates a variety of target proteins, including other protein kinases. Importantly, a fraction of activated ERK translocates to the nucleus, where it regulates transcription factors by phosphorylation. In this regard, it is notable that a primary response to growth factor stimulation is the rapid transcriptional induction of a family of approximately 100 genes called immediate-early genes. The induction of a number of immediate-early genes is mediated by a regulatory sequence called the serum response element (SRE), which is recognized by a complex of transcription factors including the serum response factor (SRF) and Elk-1. ERK phosphorylates and activates Elk-1, providing a direct link between the ERK family of MAP kinases and immediate-early gene induction. Many immediate-early genes themselves encode transcription factors, so their induction in response to growth factor stimulation leads to altered expression of a battery of other downstream genes, thereby establishing new programs of gene expression

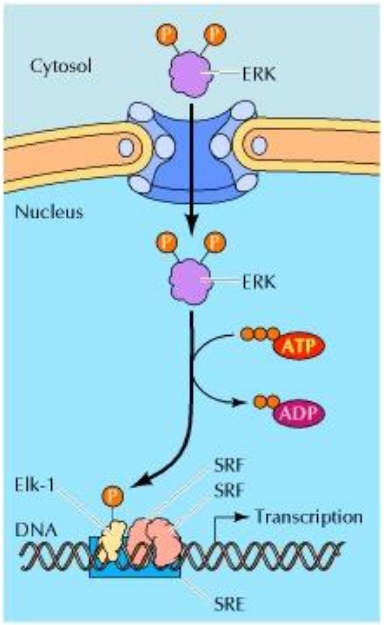

Fig. Induction of immediate-early genes by ERK [Activated ERK translocates to the nucleus, where it phosphorylates the transcription factor Elk-1. Elk-1 binds to the serum response element (SRE) in a complex with serum response factor (SRF). Phosphorylation stimulates the activity of Elk-1 as a transcriptional activator, leading to immediate-early gene induction.]

Both yeasts and mammalian cells have multiple MAP kinase pathways that control distinct cellular responses. Each cascade consists of three protein kinases: a terminal MAP kinase and two upstream kinases (analogous to Raf and MEK) that regulate its activity. In the yeast S. cerevisiae, five different MAP kinase cascades regulate mating, sporulation, filamentation, cell wall remodeling, and response to high osmolarity. In mammalian cells, at least five MAP kinases have been identified. In addition to members of the ERK family, these include the JNK and p38 MAP kinases, which are preferentially activated in response to inflammatory cytokines and cellular stress (e.g., ultraviolet irradiation). Whereas ERK signaling principally leads to cell proliferation, survival, and differentiation, the JNK and p38 MAP kinase pathways often lead to inflammation and cell death. Like ERK, the JNK and p38 MAP kinases can translocate to the nucleus and phosphorylate transcription factors that regulate gene expression. Multiple MAP kinase pathways thus function in all types of eukaryotic cells to control cellular responses to diverse environmental signals.

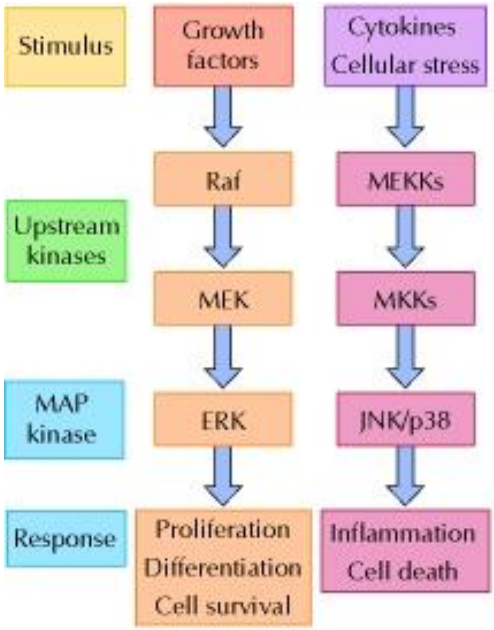

Fig. Pathways of MAP kinase activation in mammalian cells [In addition to ERK, mammalian cells contain JNK and p38 MAP kinases. Activation of JNK and p38 is mediated by protein kinase cascades parallel to that responsible for ERK activation. The protein kinase cascades leading to JNK and p38 activation appear to be preferentially activated by cytokines or cellular stress and generally lead to inflammation and cell death.]

Courtesy: The Cell: A Molecular Approach (Geoffrey M Cooper.)