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GROWTH FACTORS AND CYTOKINE SIGNALS THAT CONTROL GENE EXPRESSION

REVIEW THE CONCEPTS

1. GRB2 serves as an adapter molecule that contains both SH2 and SH3 domains. GRB2 binds to phosphotyrosine residues on activated receptor tyrosine kinases via its SH2 domain and binds to the guanine nucleotide exchange factor Sos via two SH3 domains. Sos then binds to and activates the Ras protein. Although all SH2 domains bind to phosphotyrosine residues, specificity is determined by the conformation of the binding pocket, which interacts with amino acids on the carboxy-terminal side of the phosphotyrosine.
2. Negative feedback occurs when a signaling pathway induces expression or activation of its own inhibitor. (a) When erythropoietin binds to its receptor, EpoR, an SH2 domain on the phosphatase SHP1 binds to a phosphotyrosine on the receptor. Binding induces a conformation change that activates SHP1, which is in close proximity to JAK (associated with EpoR). SHP1 dephosphorylates and inactivates JAK, thus inhibiting signal transduction. The erythropoietin signaling pathway also possesses negative feedback that triggers long-term downregulation in which STAT proteins induce the expression of SOCS proteins. SOCS proteins contain SH2 domains that bind EpoR, preventing the binding of signaling molecules. One SOCS protein also binds the activation lip of JAK2 and inhibits its kinase activity. SOCS proteins also recruit E3 ubiquitin ligases, which ubiquitinate JAKs and target them for degradation by the proteasome. (b) TGF β signaling induces the expression of SnoN and Ski, two proteins that bind to Smads and inhibit their ability to regulate transcription. TGF β signaling also induces the expression of I-Smads, which prevent the phosphorylation of R-Smads by the TGF β receptor.

3. Constitutive activation is the alteration of a protein or signaling pathway such that it is functional or engaged even in the absence of an upstream activating event. For example, Ras^D is constitutively active because it cannot bind GAP and therefore remains in the GTP-bound, active state even when cells are not stimulated by growth factor to activate a receptor tyrosine kinase. Constitutively active Ras is cancer promoting because cells will proliferate in the absence of growth factors, and thus normal regulatory mechanisms for cell proliferation are bypassed.
 - a. A mutation that resulted in Smad3 binding Smad4, entering the nucleus, and activating transcription independent of phosphorylation by the TGF β receptor would render Smad3 constitutively active.
 - b. A mutation that made MAPK active as a kinase and able to enter the nucleus without being phosphorylated by MEK would render MAPK constitutively active.
 - c. A mutation that prevented NF- κ B from binding to I κ -B or that allowed NF- κ B to enter the nucleus and regulate transcription even when bound to I κ -B would render NF- κ B constitutively active.
4. In the mating factor signaling pathway Ste7, another serine/threonine kinase is the substrate for Ste11. When the mating factor signaling pathway is activated, Ste7, Ste11, and the other relevant kinases in the cascade form a complex with the scaffold protein Ste5. Binding to Ste5 ensures that Ste7 is the only substrate to which Ste11 has access.
5. Maximal activation of protein kinase B requires 1) release of inhibition by its own PH domain, which is achieved when PI 3-phosphate binds the PH domain; 2) phosphorylation of a serine in the activation lip of protein kinase B by PDK1, which occurs when both protein kinase B and PDK1 are recruited to the cytosolic surface of the plasma membrane by binding PI 3-phosphates; and 3) phosphorylation of an additional serine residue located outside the activation lip.
6. PTEN phosphatase removes the 3-phosphate from PI 3,4,5-triphosphate, thus reversing the reaction catalyzed by PI-3 kinase and rendering the PI phosphate unable to bind protein kinase B and PDK1. Loss-of-function mutations are cancer promoting because constitutive activation of protein kinase B results in constitutive phosphorylation and inactivation of proapoptotic proteins such as Bad and Forkhead-1. Cancers are typically characterized by cells that are resistant to apoptosis. A gain-of-function mutation in PTEN phosphatase would promote cell death by causing the apoptotic pathway to be active even in the presence of survival factors that signal through protein kinase B.

7. Both cytokine receptors and receptor tyrosine kinases (RTKs) form functional dimers upon binding their ligands. For both cytokine receptors and RTKs, upon dimerization, one of the poorly active cytosolic kinases phosphorylates the other. This phosphorylation activates the kinase, which phosphorylates the second kinase in the dimer as well as other tyrosine residues in the receptor. Both cytokine receptors and RTKs then serve as docking sites for signaling molecules that bind to these phosphotyrosine sites. A major distinction between cytokine receptors and RTKs is that the RTK is itself the tyrosine kinase, whereas the cytokine receptor has no catalytic activity but rather is associated with a JAK kinase.
8. When GRB2 binds the Epo receptor, the Ras/MAP kinase signaling pathway is activated, resulting in the translocation of MAP kinase to the nucleus, where it phosphorylates and regulates the activity of transcription factors. When JAK phosphorylates and activates STAT5, STAT5 itself translocates to the nucleus as a homodimer and functions as a transcription factor. Epo signaling induces the production and survival of erythrocytes, or red blood cells (RBCs), which increases the oxygen carrying (aerobic) capacity of the blood. In most normal people and even elite athletes, RBCs comprise 40–50% in men and 35–45% in women of the volume of the blood (hematocrit). Epo doping can raise that level to 75%, which makes the blood very viscous and can cause heart failure and death.
9. A dominant-negative mutant of JAK cannot be phosphorylated and thus is inactive, but it binds normally to the Epo receptor, blocking the binding of wild-type JAK.
10. In multiple cell types, TGF β activates a conserved signaling pathway that results in translocation of Smad2 or Smad3 to the nucleus in complexes with co-Smad4. Once in the nucleus, the Smads interact with other transcription factors to regulate the expression of target genes. The complement of these other transcription factors is cell-type specific, and thus the TGF β signaling pathway will induce the transcription of different genes in different cell types.
11. TGF β binds to its type II receptor either directly or when presented by the type III receptor. The type II receptor is a constitutively active serine/threonine kinase. When the type II receptor binds TGF β , it forms a tetrameric complex consisting of two molecules of the type II receptor and two molecules of the type I receptor. The type II receptor then phosphorylates the type I receptor, activating the type I receptor as a serine/threonine kinase. The type I receptor then phosphorylates R-Smad2 or R-Smad3, inducing a conformational change that exposes a nuclear localization signal on the R-Smad. The R-Smad then forms a complex consisting of two molecules of R-Smad and one molecule each of co-Smad4 and importin- β . This complex translocates to the nucleus, where it interacts with other transcription factors to elicit changes in gene expression. A nuclear phosphatase continuously dephosphorylates and inactivates Smads in the nucleus. Maintenance of Smad activity in the nucleus thus requires continued activation of Smads by TGF β -activated receptors.

12. Delta is a single-pass plasma membrane protein with an extracellular Notch-binding signaling domain. Unlike EGF precursors, which can be cleaved by proteases to release diffusible EGF signaling molecules, Delta remains intact for signaling and therefore can only activate Notch receptors on neighboring cells with which it is in direct contact.
13. γ -secretase is an intramembrane protease.
14. Hedgehog is covalently linked to cholesterol and also has a palmitoyl group added to the N-terminus. Cholesterol addition occurs during autoproteolytic cleavage of the protein into two fragments. Together, these modifications make the Hh signaling domain hydrophobic so that it remains tethered to the cell membrane. Tethering of Hh to cell membranes may limit its range of action in tissues, allowing spatial restriction of its action.
15. Hedgehog (Hh) protein binding to the Patched receptor relieves its inhibition of Smoothed delivery to the cell surface, thus initiating the Hh signaling pathway. Nonfunctional versions of either Hedgehog or Smoothed protein would block the Hh signaling pathway, yielding the same phenotype. In contrast, nonfunctional Patched would allow Smoothed delivery to the plasma membrane even in the absence of Hh protein, permanently activating the Hh signaling pathway.
16. The signaling pathway that activates NF- κ B is considered irreversible because I- κ B, the inhibitor of NF- κ B, is degraded when the pathway is activated. Thus, the signal cannot be switched off rapidly as with a kinase-induced signal that can be reversed by the action of an opposing phosphatase. The NF- κ B pathway is eventually disengaged by negative feedback in which NF- κ B stimulates the transcription of I- κ B. However, synthesis of the I- κ B inhibitor de novo is a relatively slow process, and thus, the NF- κ B pathway can remain active for some time after the original stimulus, such as TNF- α , is removed.
17. Stimulation of a cell by an infectious agent or inflammatory cytokine activates β kinase to phosphorylate I- κ B α . This stimulates polyubiquitination of I- κ B α lysine 48 (K48) by an E3 ubiquitin ligase, which targets the I- κ B α for degradation by a proteasome. The E3 ligase TRAF6 polyubiquitinates I- κ B α lysine 63 (K63). Rather than targeting the protein for degradation, the K63-linked polyubiquitin chain acts as a scaffold for poly K63 ubiquitin-binding domain proteins to bring together the kinase TAK1 with its substrate I κ B α .