16 Cell Signaling II: Signaling Pathways That Control Gene Activity

Review the Concepts

1. In multiple cell types, TGFβ 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 TGFβ signaling pathway will induce the transcription of different genes in different cell types.

2. TGFβ 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 TGFβ, 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. The TGFβ signaling pathway is diagrammed in Figure 16-4 in the text.

3. Both cytokine receptors and receptor tyrosine kinases (RTKs) form functional dimers upon binding their ligands. Upon dimerization, one of the poorly active cytosolic kinases phosphorylates the other on a particular tyrosine residue in the activation lip. 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.

4a. When JAK phosphorylates and activates STAT5, STAT5 itself translocates to the nucleus as a homodimer and functions as a transcription factor.

4b. 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.

5a. TGFβ signaling induces the expression of SnoN and Ski, two proteins that bind to Smads and inhibit their ability to regulate transcription. TGFβ signaling also induces the expression of I-Smads, which prevent the phosphorylation of R-Smads by the TGFβ receptor. Negative feedback occurs when a signaling pathway induces expression or activation of its own inhibitor.

5b. 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.

6. 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.

7. 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.

7a. A mutation that resulted in Smad3 binding Smad4, entering the nucleus, and activating transcription independent of phosphorylation by the TGFβ receptor would render Smad3 constitutively active.

7b. 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.

7c. A mutation that prevented NF-κB from binding to Iκ-B or that allowed NF-κB to enter the nucleus and regulate transcription even when bound to Iκ-B would render NF-κB constitutively active.

8. 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.

9. Maximal activation of protein kinase B requires 1) release of inhibition by its own PH domain, which is achieved when PI 3-phosphates bind 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. In muscle cells, insulin-stimulated activation of protein kinase B causes the GLUT4 glucose transporter to locate to the cell surface, resulting in increased influx of glucose. Insulin-stimulated activation of protein kinase B also promotes glycogen synthesis because protein kinase B phosphorylates and inhibits glycogen synthase kinase 3 (GSK3), an inhibitor of glycogen synthase.

10. 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.

11. Activation of PKA can stimulate the expression of many genes. Gene expression leads to its long-term effects. In the liver, activated PKA induces the synthesis of several enzymes involved in converting three-carbon compounds to glucose, thus increasing blood glucose levels over the long term.

PKA acts through phosphorylating the CREB (cAMP response element binding) protein.

Phosphorylated CREB binds to CRE elements on DNA. Together with the co-activator CBP/300, CREB stimulates transcription of target genes.

12. 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.

13. Although their exact role in signal transduction is not completely known, genetic studies have implicated IFT proteins in the Hh signal pathway. In vertebrates, mutations that eliminate IFT function cause derepression of Hh pathway target genes.

14. The signaling pathway that activates NF-κB is considered irreversible because I-κB, the inhibitor of NF-κB, 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-κB pathway is eventually disengaged by negative feedback in which NF-κB stimulates the transcription of I-κB. However, synthesis of the I-κB inhibitor de novo is a relatively slow process, and thus, the NF-κB pathway can remain active for some time after the original stimulus, such as TNF-α, is removed.

15. Presenilin I is a protease that is responsible for cleaving the integral membrane Notch receptor to release a cytosolic fragment of Notch that diffuses to the nucleus to activate transcription factors. In neurons, presenilin I is a component of γ-secretase, which cleaves the integral membrane protein APP after the extracellular domain of APP is cleaved by β-secretase. The 42-amino-acid peptide that remains in the membrane has been implicated in the formation of amyloid plaques in Alzheimer's patients.

Analyze the Data

a. This experiment reveals that MEK5 and MEKK2 co-localize within a complex since immunoprecipitation with a MEK5 antibody also precipitates MEKK2. However, these data give no evidence as to whether MEKK2 activates MEK5 or MEK5 activates MEKK2.

b. MEKK2 is required for the activation of ERK. However, MEKK2 cannot activate ERK unless MEK5 is present. MEKK2 alone partially activates ERK5 because there is some endogenous MEK5, but in the presence of MEK5AA, which inhibits endogenous MEK5, MEKK2 cannot activate ERK5. These experiments clearly place ERK5 downstream of MEKK2 and MEK5 in the signaling pathway; however, they do not unambiguously order MEKK2 and MEK5. To do so would require co-expression of ERK5, MEK5AA and a constitutively active form of MEKK2. If ERK5 were phosphorylated, then MEKK2 would be downstream of MEK5. If not, then MEKK2 would be upstream of MEK5.