CH1131 Biomolecular Engineering

Syllabus

Signaling

CELL SIGNALING

LECTURE OUTLINE

Cell Signaling: Introduction

I. To survive, cells must communicate with their neighbors, monitor environmental conditions & respond appropriately to a host of different stimuli that impinge on their surface
   A. Most cells in a complex multicellular organism are specialized to carry out ≥1 specific functions
   B. Many biological processes require various cells to work together & to coordinate their activities – to do this, cells must communicate, which is accomplished by a process called **cell signaling**

II. Cell signaling makes it possible for cells to talk to each other & for an organism to function as a coherent system; it affects virtually every aspect of cell structure & function
   A. An understanding of cell signaling requires knowledge about other types of cellular activity
   B. Insights into cell signaling can tie together a variety of seemingly independent cellular activities
   C. Cell signaling is also intimately involved in regulation of cell growth & division, making it important in understanding the development of a malignant tumor

The Basic Elements of Cell Signaling Systems in the Body

I. Cells usually communicate with each other through extracellular messenger molecules – cell signaling starts with release of a messenger molecule by a cell engaged in sending messages to other cells in body
   A. Sometimes the messenger molecule need only diffuse across a narrow cleft or through a tiny blood vessel before the message is received by an appropriate target cell
   B. Other times, the messenger molecule may have to circulate through the entire body before reaching specific target cells

II. Cells can only respond to an extracellular message if they express **transmembrane receptors** that specifically recognize & bind that particular messenger molecule
   A. Binding of the messenger molecule (**ligand**) to the extracellular surface of the receptor relays a signal across the membrane to the receptor's cytoplasmic domain at the inner membrane surface

III. Once it has reached the inner plasma membrane surface, there are 2 major routes by which the signal is transmitted into the cell interior; the particular route depends on the type of receptor activated
   A. One type of receptor transmits signal from its cytoplasmic domain to a nearby enzyme that generates a second messenger
      1. Since it brings about (effects) the cellular response by generating a second messenger, the enzyme responsible is called an **effector**
      2. Second messengers are small substances that typically activate (or inactivate) specific proteins

3. Depending on its chemical structure, a second messenger may diffuse through the cytosol or remain embedded in the membrane lipid bilayer

B. Another type of receptor transmits a signal by transforming its cytoplasmic domain into a recruiting station for cellular signaling proteins

IV. Whether the signal is transmitted by a second messenger or by protein recruitment, the outcome is similar, a protein that is positioned at the top of an intracellular signaling pathway is activated

A. Each signaling pathway consists of a series of distinct proteins that operate in sequence

B. Each protein in the pathway typically acts by altering the conformation of the subsequent (downstream) protein in the series, an event that activates or inhibits the protein

C. Alterations in the conformations of signaling proteins are often accomplished by protein kinases & protein phosphatases that, respectively, add or remove phosphate groups from other proteins

D. Many of the protein substrates of these enzymes are enzymes themselves, like other kinases & phosphatases, but include ion channels, transcription factors & various types of regulatory molecules

E. ~1/3 of the cell's proteins are thought to be subject to phosphorylation & phosphorylation can change protein behavior in several different ways:

1. It can activate or inactivate an enzyme
2. It can increase or decrease protein-protein interactions
3. It can induce a protein to move from one subcellular compartment to another **or**
4. It can act as a signal that initiates protein degradation

F. Signals transmitted along such signaling pathways ultimately reach target proteins involved in basic cell processes; depending on cell type & message, response initiated by target protein may involve:

1. A change in gene expression
2. An alteration of the activity of metabolic enzymes
3. A reconfiguration of the cytoskeleton
4. An increase or decrease in cell mobility
5. A change in ion permeability
6. Activation of DNA synthesis **or**
7. Even the death of the cell

G. Virtually every activity in which a cell is engaged is regulated by signals originating at cell surface

1. The overall process in which information carried by extracellular messenger molecules is translated into changes that occur inside of a cell is called **signal transduction**

V. Finally, signaling has to be terminated so that cells can be responsive to additional messages that they may receive

A. First, they must eliminate the extracellular messenger molecule – certain cells produce extracellular enzymes that destroy specific extracellular messengers

B. In other cases, activated receptors are internalized; once internalized, the receptor may be degraded together with its ligand, leaving the cell with decreased sensitivity to subsequent stimuli
   1. Alternatively, receptor & ligand may be separated within an endosome, after which the ligand is degraded & the receptor is returned to the cell surface

C. Cells also contain enzymes that are necessary to return cellular signaling proteins back to the resting state
   1. Sometimes, activated signaling proteins are targeted for degradation & new signaling proteins are synthesized to maintain signaling capabilities

A Survey of Extracellular Messengers and Their Receptors

I. A large variety of molecules can function as extracellular carriers of information, including:
   A. Small molecules like amino acids and amino acid derivatives – glutamate, glycine, acetylcholine, epinephrine, dopamine & thyroid hormone; these molecules act as neurotransmitters & hormones

   B. Steroids, which are derived from cholesterol – steroid hormones regulate sexual differentiation, pregnancy, carbohydrate metabolism, & excretion of sodium & potassium ions
   C. A wide variety of polypeptides & proteins
      1. Some are present as transmembrane proteins on the surface of an interacting cell
      2. Others are part of, or associate, with the extracellular matrix
   3. Finally, many proteins are excreted in the extracellular environment where they are involved in regulating processes like cell division, differentiation, immune response or cell death & cell survival

II. Extracellular signaling molecules are usually, but not always, recognized by specific receptors that are present on the surface of the responding cell
   A. Receptors bind their signaling molecules with high affinity
   B. They then translate this interaction at cell outer surface into changes that take place on inside of cell

III. The receptors that have evolved to mediate signal transduction are:
   A. G-protein coupled receptors (GPCRs) – huge family of receptors that contain 7 transmembrane α-helices
      1. They translate binding of extracellular signaling molecules into activation of GTP-binding proteins
      2. GTP-binding proteins (G proteins) are involved in vesicle budding & fusion, MT dynamics, protein synthesis & nucleocytoplasmic transport & transmitting messages along cell information circuits
   B. Receptor protein-tyrosine kinases (RTKs) – a second class of receptors that have evolved to translate the presence of extracellular messenger molecules into changes inside the cell

1. Binding of specific extracellular ligand to RTK usually results in receptor dimerization followed by activation of receptor's protein-kinase activity, which is associated with its cytoplasmic domain
2. Upon activation, these protein kinases phosphorylate cytoplasmic substrate proteins, thereby altering their activity, their localization or their ability to interact with other proteins within the cell
3. Most protein kinases transfer phosphate groups to serine or threonine residues of their protein substrates, but RTKs phosphorylate tyrosine residues
4. RTKs are typically involved in the regulation of cell division & differentiation

G Protein-Coupled Receptors: Background Information on Receptors

I. G-protein coupled receptors (GPCRs) – are so-named because they interact with G proteins; also referred to as seven-transmembrane (7TM) receptors because they contain 7 transmembrane helices
   A. Hundreds of different GPCRs have been identified in organisms ranging from yeast to flowering plants & mammals; they regulate an extraordinary spectrum of cellular processes
   B. Included among the natural ligands that bind to GPCRs are a diverse array of hormones, chemoattractants, neurotransmitters, opium derivatives, odorants, tastants & photons

II. G protein-coupled receptors normally have the following topology:
   A. Their amino-terminus is present on the outside of the cell
   B. The 7 α-helices that traverse the plasma membrane are connected by loops of varying length
   C. Carboxy-terminus is present on the inside of the cell
   D. There are 3 loops present on the outside of the cell that, together, form the ligand-binding site
   E. There are also 3 loops present on the cytoplasmic side of the plasma membrane that provide binding sites for intracellular signaling proteins
      1. G proteins bind to the third intracellular loop
      2. Arrestins also bind to the third intracellular loop & compete with G proteins for binding to receptor

III. When a hormone or neurotransmitter binds to a GPCR, it induces a change in concentration in the extracellular ligand-binding site
   A. The change in conformation is transferred across the plasma membrane & causes a change in conformation in the cytoplasmic loops of the receptor
   B. This, in turn, leads to an increase in the receptor's affinity for a G protein that is present on the cytoplasmic surface —> the ligand-bound receptor forms a receptor-G protein complex
   C. The interaction with the receptor induces a conformational shift in the α subunit of the G protein, causing the release of GDP, which is followed by the binding of GTP —> G protein is activated

G Protein-Coupled Receptors: G Proteins – General Structure and Function

I. Heterotrimeric G proteins are called G proteins because they bind guanine nucleotides, either GDP or GTP
   A. They are described as heterotrimeric because all of them consist of 3 different polypeptide subunits (α, β and γ), distinguishing them from small, monomeric G proteins, like Ras
   B. Heterotrimeric G proteins are held at the plasma membrane by lipid chains that are covalently attached to the α & γ subunits
   C. The guanine-nucleotide-binding site is present on the G_{α} subunit

II. Replacement of GDP by GTP after an interaction with an activated GPCR causes a conformational change in the G_{α} subunit
   A. In its GTP-bound conformation, the G_{α} subunit has a low affinity for $G_{\beta\gamma}$, leading to its dissociation from the complex
      1. Each dissociated G_{α} subunit with GTP attached is free to activate an effector protein like adenylyl cyclase, which in this case leads to the production of the second messenger, cAMP
   B. Other effectors include phospholipase C-β and cyclic GMP phosphodiesterase
   C. Second messengers, in turn, activate one or more cellular signaling proteins

III. After its interaction with an effector, G_{α} hydrolyzes its bound GTP to GDP and P_I
   A. GTP hydrolysis induces a shape change causing G_{α} subunit to dissociate from effector & to reassociate with βγ dimer forming an inactive heterotrimeric G protein, waiting for next round of activation
   B. In a sense, heterotrimeric G proteins function as molecular timers
      1. They are turned on by the interaction with an activated receptor & turn themselves off by hydrolysis of bound GTP after a certain amount of time has passed
      2. While they are active, G_{α} subunits can turn on downstream effectors

G Protein-Coupled Receptors: G Proteins - Response Termination

I. To prevent overstimulation, receptors must be blocked from continuing to activate G proteins & to regain sensitivity to future stimuli, receptor, G protein & effector must all return to inactive state

II. Desensitization – the process that blocks active receptors from turning on additional G proteins; it takes place in 2 steps
   A. Step 1 of desensitization – the cytoplasmic domain of the activated receptor GPCR is phosphorylated by a specific type of kinase, G protein-coupled receptor kinase (GRK)
   B. Step 2 – GPCR phosphorylation sets the stage for second step, which is the binding of proteins (**arrestins**)

1. Arrestins form a small group of proteins that bind GPCRs & compete for binding with heterotrimeric G proteins —> thus, arrestin binding prevents further activation of additional G proteins
2. This is termed desensitization because the cell stops responding to the stimulus, while that stimulus is still acting on the outer surface of the cell
3. Desensitization is one of the mechanisms that allows a cell to respond to a change in its environment, rather than continuing to fire endlessly in the presence of an unchanging environment
4. Importance of desensitization - mutations interfering with rhodopsin phosphorylation by a GRK lead to retinal photoreceptor cell death (thought to be one cause of blindness due to retinitis pigmentosa)

III. While bound to phosphorylated GPCRs, arrestin molecules are also capable of binding to clathrin molecules that are situated in clathrin-coated pits
   A. The interaction between bound arrestin & clathrin promotes the uptake of phosphorylated GPCRs into the cell by endocytosis
      1. Depending upon circumstances, receptors that have been removed from the surface by endocytosis may be dephosphorylated & returned to the plasma membrane
      2. Alternatively, internalized receptors are degraded in lysosomes
   B. If the receptors are degraded, the cells lose, at least temporarily, sensitivity for the ligand in question; if receptors are returned to the cell surface, the cells remain sensitive to the ligand

IV. Signaling by the activated G_{α} subunit is terminated by a very different mechanism: the bound GTP molecule is simply hydrolyzed to GDP
   A. Thus, strength & duration of the signal are determined partly by the G_{α} subunit GTP hydrolysis rate
      1. G_a subunits have weak GTPase activity, allowing them to slowly hydrolyze bound GTP, inactivating themselves
   B. Termination of the response is accelerated by **r**egulators of **G** protein **s**ignaling (RGSs)
      1. The interaction with an RGS protein increases the rate of GTPase hydrolysis by the G_{α} subunit
      2. Once the GTP is hydrolyzed, the G_{α}-GDP reassociates with the $G_{\beta\gamma}$ subunits to reform the inactive trimeric complex —> returns system to the resting state

The Specificity of G Protein-Coupled Responses

I. Wide variety of agents (hormones, neurotransmitters, sensory stimuli) act by way of GPCRs & heterotrimeric G proteins to transmit information across plasma membrane; triggers a wide variety of cell responses

II. The various parts of the signal transduction machinery are not identical in every cell type

A. Receptors for a given ligand can exist in several different versions (**isoforms**)
B. The heterotrimeric G proteins that transmit signals from receptor to effector can also exist in multiple forms, as can many of the effectors
1. At least 20 different G_{α} subunits, 5 different G_{β} subunits & 11 different G_{γ} subunits identified
2. 9 isoforms of the effector adenylyl cyclase have also been identified
C. Different combinations of specific subunits construct G proteins having different capabilities of reacting with specific isoforms of both receptors & effectors
D. Some G proteins act by inhibiting their effectors; the same stimulus can activate a stimulatory G protein (with a $G_{\alpha s}$ subunit) in one cell & an inhibitory G protein (with a $G_{\alpha i}$ subunit) in a different cell

III. Thus, the same extracellular messenger can activate a variety of pathways in different cells

Regulation of Blood Glucose Levels

I. Glucose can be utilized as a source of energy by all cell types present in the body
A. Glucose is oxidized to CO_2 & H_2O by glycolysis
B. The ATP produced provides cells with ATP that can be used to drive energy-requiring reactions

II. Animal cells store glucose in glycogen (a large, insoluble, branched glucose polymer linked by glycosidic bonds); glucose oxidation gives cell its primary energy source; its regulation has been studied for years
A. In vertebrates, hormones control glycogen breakdown into its energy-rich building blocks to capture its energy, most notably:
1. Glucagon - secreted by α-cells in the pancreas in response to low blood glucose levels
B. Increased blood glucose levels due to the action of these hormones provide the body with energy resources needed to deal with the situation at hand
C. Each hormone binds to its specific receptor protruding from membrane of target cell & the binding starts a series of reactions that leads to the activation of the enzyme glycogen phosphorylase
1. Catalyzes the breakdown of glycogen to glucose 1-phosphate (first step in glucose catabolism)
2. Both hormones also inhibit glycogen synthase; catalyzes glycogen formation (addition of glucose to glycogen), the opposite reaction
D. After activation by their respective ligands, both receptors activate the same type of heterotrimeric G proteins that cause an increase in the levels of cAMP

III. Glucose mobilization: An example of a response induced by cAMP

A. Integral membrane protein adenylyl cyclase (catalytic domain resides at inner membrane surface) is effector (brings about cell response); makes cAMP, which starts reaction chain that mobilizes glucose

1. Receptor binds glucagon —> conformational shift; 1st step in reaction cascade
2. Change transmitted across membrane as the receptor activates a $G_{\alpha s}$ subunit, which activates an adenylyl cyclase effector on the inner membrane surface
3. Activated adenylyl cyclase converts ATP to cAMP that rapidly diffuses to cytoplasm

B. Once formed, cAMP diffuses into cytoplasm, where it binds to allosteric site on regulatory subunit of a cAMP-dependent protein kinase (protein kinase A; PKA)

1. PKA in its inactive form is a heterotetramer made of 2 regulatory (R) & 2 catalytic (C) subunits
2. The regulatory subunits normally inhibit the catalytic activity of the enzyme
3. cAMP binding causes the dissociation of the inhibitory subunits, thereby releasing the catalytic subunits of PKA in their active form

C. The target substrates of PKA in a liver cell include 2 enzymes that play a pivotal role in glucose metabolism: glycogen synthase & phosphorylase kinase

1. Phosphorylation of glycogen synthase inhibits its catalytic activity & thus prevents the conversion of glucose to glycogen
2. Phosphorylation of phosphorylase kinase activates the enzyme to catalyze the transfer of phosphate groups to phosphorylase molecules

G Protein-Coupled Receptors: Other Aspects of cAMP Signal Transduction Pathways

I. A few PKAs also translocate into the nucleus where they phosphorylate key nuclear proteins; one such nuclear protein is a transcription factor called CREB (**c**AMP **r**esponse **e**lement-**b**inding protein)

A. Phosphorylated CREB binds as a dimer to sites on DNA containing a particular nucleotide sequence (TGACGTCA), known as the **c**AMP-**r**esponse **e**lement (CRE)

1. Response elements are sites in the DNA where transcription factors bind & increase the rate of transcription initiation
2. CREs are found in the regulatory regions of genes that play a role in the response to cAMP

II. A mechanism must exist to reverse the hormone's effect or the cell would remain in the activated state indefinitely - liver cells contain phosphatases that remove phosphate groups added by kinases

A. cAMP phosphodiesterase helps to terminate the response to cAMP through the destruction of cAMP molecules present in the cell

III. cAMP is made in response to a wide variety of different ligands (1st messengers) in many different cells & mediates varied responses; operates in mammals & invertebrates; each cell has different response

Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

I. Protein-tyrosine kinases are enzymes that phosphorylate specific tyrosine residues on protein substrates

A. Protein-tyrosine phosphorylation is mechanism for signal transduction that appeared with the evolution of multicellular organisms; >90 different protein-tyrosine kinases are encoded by the human genome

B. These kinases are involved in the regulation of cell growth, cell division, cell differentiation, cell survival, attachment to the extracellular matrix & migration

II. Protein-tyrosine kinases can be divided into 2 groups: receptor protein-tyrosine kinases (RTKs) & non-receptor or cytoplasmic protein-tyrosine kinases

A. RTKs – integral membrane proteins containing an extracellular ligand-binding domain

1. Activated directly by extracellular growth & differentiation factors – epidermal growth factor (EGF) & platelet derived growth factor (PDGF) or by metabolic regulators like insulin

B. Cytoplasmic protein-tyrosine kinases – regulated indirectly by extracellular signals

III. Receptor dimerization – it is widely accepted that ligand binding to protein-tyrosine kinases causes the dimerization of the extracellular ligand-binding domains of a pair of receptors – 2 mechanisms recognized

A. Ligand-mediated dimerization – early work suggested that ligands of RTKs have 2 receptor-binding sites, making it possible for one growth or differentiation factor to bind to 2 receptors at the same time

1. Thus, the ligand connects two receptors & causes ligand-mediated dimerization
2. Each of the subunits of the growth or differentiation factor contains a receptor-binding site

B. Receptor-mediated dimerization – more recently, it was established that some growth factors contain only a single receptor-binding site

1. Structural work supports a second mechanism in which ligand binding induces a conformational change in the extracellular domain of a receptor
2. Conformational change leads to the formation or exposure of a receptor dimerization interface
3. The proposal is that the ligands act as allosteric regulators that turn on the ability of their receptors to form dimers

C. Regardless of the mechanism, receptor dimerization results in the juxtapositioning of 2 protein-tyrosine kinase domains on the cytoplasmic side of the plasma membrane

1. This brings 2 kinase domains into close contact allowing for trans-autophosphorylation
2. Thus, the protein kinase activity of one receptor of the dimer phosphorylates the tyrosine residues in the cytoplasmic domain of the other receptor of the dimer, and vice versa

IV. Autophosphorylation sites have a dual function: they regulate kinase activity & they function as binding sites for cytoplasmic signaling molecules

A. Kinase activity is usually regulated by autophosphorylation on tyrosine residues that are present in the activation loop of the kinase domain

1. The activation loop, when unphosphorylated, obstructs the substrate-binding site, thereby preventing ATP from entering
2. After its phosphorylation, the activation loop is stabilized in a position away from the substrate-binding site, resulting in activation of the kinase domain
3. Once their kinase domain has been activated, the receptor subunits proceed to phosphorylate each other on tyrosine residues that are present in regions adjacent to the kinase domain

B. It is these autophosphorylation sites that act as binding sites for cellular signaling proteins

V. Phosphotyrosine-dependent protein-protein interactions – signaling pathways consist of a chain of signaling proteins that interact with one another in a sequential manner

A. Signaling proteins can associate with activated protein-tyrosine kinase receptors, since they contain domains that bind specifically to phosphorylated tyrosines – 2 such domains have been identified

1. The Src-homology 2 (**SH2**) domain

B. SH2 domains were initially identified as part of protein-tyrosine kinases encoded by the genome of tumor-causing (oncogenic) viruses

1. They are composed of ~100 amino acids & contain a conserved binding-pocket that accommodates a phosphorylated tyrosine residue
2. SH2 domains mediate a large number of phosphorylation-dependent protein-protein interactions
3. These interactions occur after phosphorylation of specific tyrosine residues
4. The specificity of the interactions is determined by the amino acid sequence immediately adjacent to the phosphorylated tyrosine residues
5. Interestingly, the budding-yeast genome encodes only one SH2-domain-containing protein; this correlates with the overall lack of tyrosine kinase signaling activity in these lower eukaryotes

VI. Downstream signaling pathway activation – receptor activation —> signaling complex formation with SH2- or PTB-containing signaling proteins (several groups) binding to specific receptor autophosphorylation sites

A. Adaptor proteins – function as linkers enabling ≥2 signaling proteins to be joined together as part of a signaling complex; they contain an SH2 domain & ≥1 additional protein-protein interaction domains

1. Ex.: – adaptor protein Grb2 contains 1 SH2 & 2 SH3 (Src-homology 3) domains; Grb SH3 domains bind constitutively to other proteins (like Sos & Gab)
2. The SH2 domain binds to phosphorylated tyrosine residues within a Tyr-X-Asn motif
3. Thus, tyrosine phosphorylation of Sos or Gab Tyr-X-Asn motif results in translocation of Grb2-Sos or Grb2-Gab from the cytoplasm to the receptor, which is present at the plasma membrane

VII. Ending the response – signal transduction by RTKs is usually terminated by receptor internalization; what causes this to happen is still an area of active research; example – receptor-binding protein named Cbl

A. When RTKs are activated by ligands, they autophosphorylate tyrosine residues, which can act as a binding site for Cbl

B. Cbl then associates with the receptor & brings along an enzyme capable of attaching a ubiquitin molecule to the receptor

1. Ubiquitin is a small protein that is linked covalently to other proteins, thereby marking those proteins for internalization or degradation

C. Cbl complex binding to activated receptors is followed by receptor ubiquitination & internalization

RTK-Activated Signaling Pathways: The Ras-MAP Kinase Pathway

I. Retroviruses are small viruses that carry their genetic information in the form of RNA – some have genes called oncogenes that enable them to transform normal cells into tumor cells

II. Ras was originally described as a retroviral oncogene; eventually, it was found that the retroviral Ras gene was derived from its mammalian hosts; ~30% of all human cancers contain mutant versions of RAS genes

A. Ras is a small GTPase (G protein) that is held at the inner surface of the plasma membrane by a lipid group that is embedded in the inner leaflet of the bilayer

1. Ras has a function similar to that of heterotrimeric G proteins discussed above; Ras acts like a molecular timer; unlike heterotrimeric G proteins, Ras consists of only a single small subunit

2. The Ras superfamily contains numerous subfamilies of proteins (Ras, Rab, Arf, Ran & Rho subfamilies), all of which are conserved from yeast to mammals

B. Ras proteins are present in 2 different forms: an active GTP-bound form & an inactive GDP-bound form

1. Ras-GTP binds & activates downstream signaling proteins

2. Ras is turned off by hydrolysis of its bound GTP to GDP

C. Mutations in the RAS gene that lead to tumor formation prevent the protein from hydrolyzing the bound GTP back to the GDP form; the mutant version of "Ras" stays in the "on" position

1. The mutant thus sends a continuous signal downstream along the signaling pathway, keeping the cell in the proliferative mode

III. Cycling of monomeric G proteins like Ras between active & inactive states is aided by accessory proteins that bind to the G protein & regulate its activity; these accessory proteins include:

A. GTPase-activating proteins (GAPs) – most monomeric G proteins possess some capability to hydrolyze a bound GTP, but this capability is greatly accelerated by interaction with specific GAPs

1. Since they stimulate hydrolysis of the bound GTP, which inactivates the G protein, GAPs dramatically shorten the duration of a G protein-mediated response
2. Mutations in one of the Ras-GAP genes (*NF1*) cause neurofibromatosis 1, a disease in which patients develop large numbers of benign tumors (neurofibromas) along the sheaths lining the nerve trunks

B. Guanine nucleotide-exchange factors (GEFs) – an inactive G protein is converted to the active form when the bound GDP is replaced with a GTP
1. GEFs are proteins that bind to an inactive monomeric G protein & stimulate dissociation of the bound GDP
2. Once the GDP is released, the G protein rapidly binds a GTP, which is present at relatively high concentration in the cell —> the G protein is activated

C. Guanine nucleotide-dissociation inhibitors (GDIs) – GDIs are proteins that inhibit the release of a bound GDP from a monomeric G protein, thus maintaining the protein in the inactive, GDP-bound state

IV. Ras's best-studied role is as a key component of the Ras-MAP kinase (***m***itogen-***a***ctivated ***p***rotein kinase) cascade, which plays a key role in regulating vital activities like cell proliferation

A. The MAP kinase cascade is activated by mitosis-stimulating growth factors (i.e., mitogens) like EGF
1. The pathway relays extracellular signals from the plasma membrane through the cytoplasm & into the nucleus & is activated when a growth factor (EGF, PDGF) binds to its RTK's extracellular domain

B. Many activated RTKs have phosphorylated tyrosine residues that act as docking sites for the adaptor protein Grb2, which, in turn, binds to Sos, a guanine nucleotide-exchange factor (GEF) for Ras
1. Creation of a Grb2 binding-site on an activated receptor promotes the translocation of Grb2-Sos from the cytoplasm to the cytoplasmic surface of the plasma membrane, in close proximity to Ras

C. Simply bringing Sos to plasma membrane is sufficient to cause Ras activation – illustrated by experiment with a mutant version of Sos that is permanently tethered to the plasma membrane inner surface
1. Expression of this membrane-bound Sos mutant results in constitutive activation of Ras & transformation of the cell to a malignant phenotype

D. Interaction with Sos opens the Ras nucleotide-binding site —> GDP is released & replaced by GTP

E. Exchange of GDP for GTP in the Ras nucleotide-binding site results in a conformational change resulting in the creation of a binding interface for an important signaling protein called Raf

F. Raf is then recruited to the inner surface of the plasma membrane where it is activated; Raf is a serine-threonine protein kinase, one of whose substrates is the protein kinase MEK

G. MEK is activated as a consequence of phosphorylation by Raf & goes on to phosphorylate & activate a MAP kinase (e.g., ERK)

H. Once activated, the MAP kinase is able to move into the nucleus where it phosphorylates & activates specific transcription factors, such as Elk-1

1. Eventually, the pathway leads to the activation of genes involved in cell proliferation, including cyclin D1, which plays a key role in driving a cell from G1 into S phase

V. Oncogenes are identified by their ability to cause cells to become cancerous; they are derived from normal cellular genes that have either become mutated or are overexpressed
   A. Many of the proteins that play a role in the Ras signaling pathway were discovered because they are encoded by cancer-causing oncogenes
      1. This includes the genes for Ras, Raf & a number of the transcriptional factors activated at the end of the pathway (e.g., Fos & Jun)
   B. Genes for several of the RTKs situated at the beginning of the pathway, including receptors for both EGF & PDGF have also been identified among the several dozen known oncogenes
   C. The fact that so many proteins in this pathway are encoded by genes that can cause cancer when mutated emphasizes the importance of the pathway to the control of cell growth & proliferation

Convergence, Divergence and Crosstalk Among Different Signaling Pathways

I. Cell signaling pathways are often much more complex than a direct linear connection leading from a cell surface receptor to an end target; for example:

A. Signals from a variety of unrelated receptors (each binding to its own ligand) can converge to activate a common effector (like Ras or Raf)

B. Signals from same ligand (EGF, insulin) can diverge to activate a variety of different effectors —> leads to diverse cellular responses

C. Signals can be passed back & forth between different pathways (a phenomenon called **crosstalk**)

II. Signaling pathways are like nervous system & provide a mechanism for routing information through a cell; cell gets information about its environment via activation of various surface receptors (they detect stimuli)

A. Cell-surface receptors can bind only to specific ligands & are unaffected by a large variety of unrelated molecules

B. A single cell may have dozens of different receptors sending signals to the cell interior simultaneously

C. Once in a cell, signals from receptors can be selectively routed along many different signaling pathways; may cause cell division, shape changes, specific metabolic pathway activation or even cell suicide

D. The cell integrates information arriving from different sources & mounts an appropriate & comprehensive response

III. Example 1: convergent signaling – there are 3 different kinds of receptors (G protein-coupled receptors, RTKs, integrins) that bind to different ligands but…..

A. All of them can lead to the formation of phosphotyrosine docking sites for SH2 domain of the Grb2 adaptor protein

B. Grb2 recruitment results in Ras activation & transmission of signals down the MAP kinase pathway

C. Thus, signals from diverse receptors can lead to the transcription & translation of a similar set of growth-promoting genes in each target cell

IV. Example 2: crosstalk between signaling pathways - cell signaling pathways highly interdependent

A. Information circuits operating in cells are more likely to resemble an interconnected web in which components produced in one pathway can participate in events occurring in other pathways

1. The more that is learned about information signaling in cells, the more crosstalk between signaling pathways is discovered – cAMP is an example

B. cAMP leads not only to glucose mobilization, but is also involved in other pathways; it inhibits growth in a variety of cells (fibroblasts, fat cells), by blocking signals transmitted through MAP kinase cascade

1. cAMP activates PKA, the cAMP-dependent kinase, which can phosphorylate & inhibit Raf, the protein that heads the MAP kinase cascade

2. These 2 pathways also intersect at another important signaling effector, the transcription factor CREB (a terminal effector of cAMP-mediated pathways)

3. It was assumed for years that CREB could only be phosphorylated by the cAMP-specific kinase, PKA; it is now known that CREB is a substrate for a much wider range of kinases