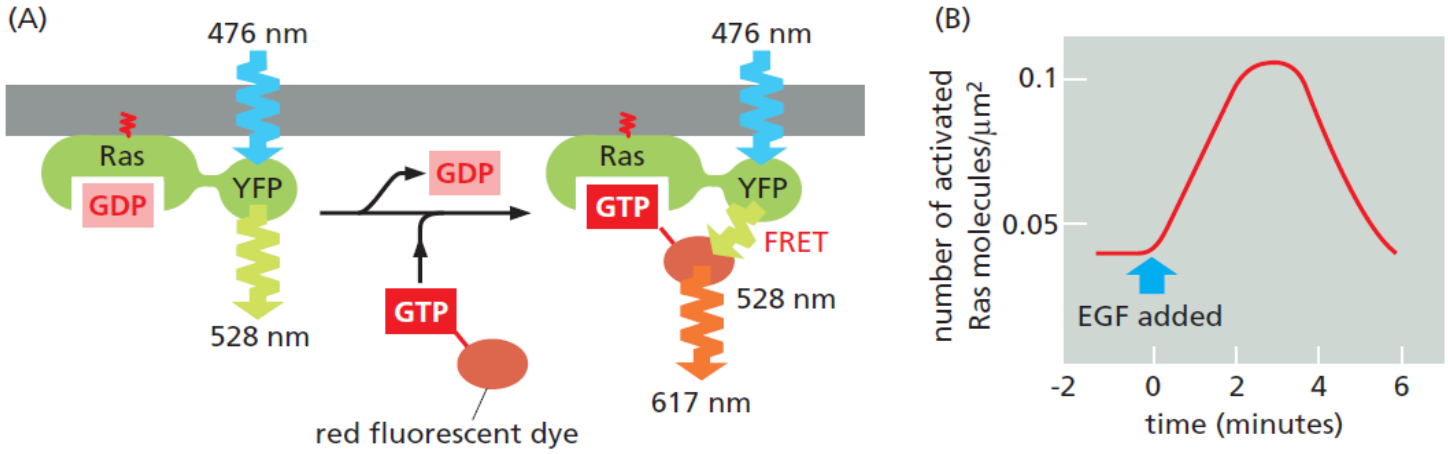
Signaling Through enzyme-coupled receptors

Ras Activates a MAP Kinase Signaling Module

Both the tyrosine phosphorylations and the activation of Ras triggered by activated RTKs are usually short-lived.



(A) Schematic drawing of the experimental strategy. Cells of a human cancer cell line are genetically engineered to express a Ras protein that is covalently linked to yellow fluorescent protein (**YFP**). GTP that is labeled with a **red** fluorescent dye is microinjected into some of the cells.

The cells are then stimulated with the extracellular signal protein EGF, and single fluorescent molecules of Ras-YFP at the inner surface of the plasma membrane are followed by video fluorescence microscopy in individual cells:

-When a fluorescent Ras-YFP molecule becomes activated, it exchanges unlabeled GDP for fluorescently labeled GTP; the energy emitted by the YFP now activates the fluorescent GTP to emit red light (called **fluorescence resonance energy transfer**, or **FRET**).

-Thus, the activation of single Ras molecules can be followed by the emission of red fluorescence from a previously yellow-green fluorescent spot at the plasma membrane.

(B) Activated Ras molecules can be detected after about 30 seconds of EGF stimulation. The red signal peaks at about 3 minutes and then decreases to baseline by 6 minutes.

As **Ras-GAP** is found to be recruited to the same spots at the plasma membrane as Ras, it presumably plays a major part in rapidly shutting off the Ras signal.

Ras Activates a MAP Kinase Signaling Module

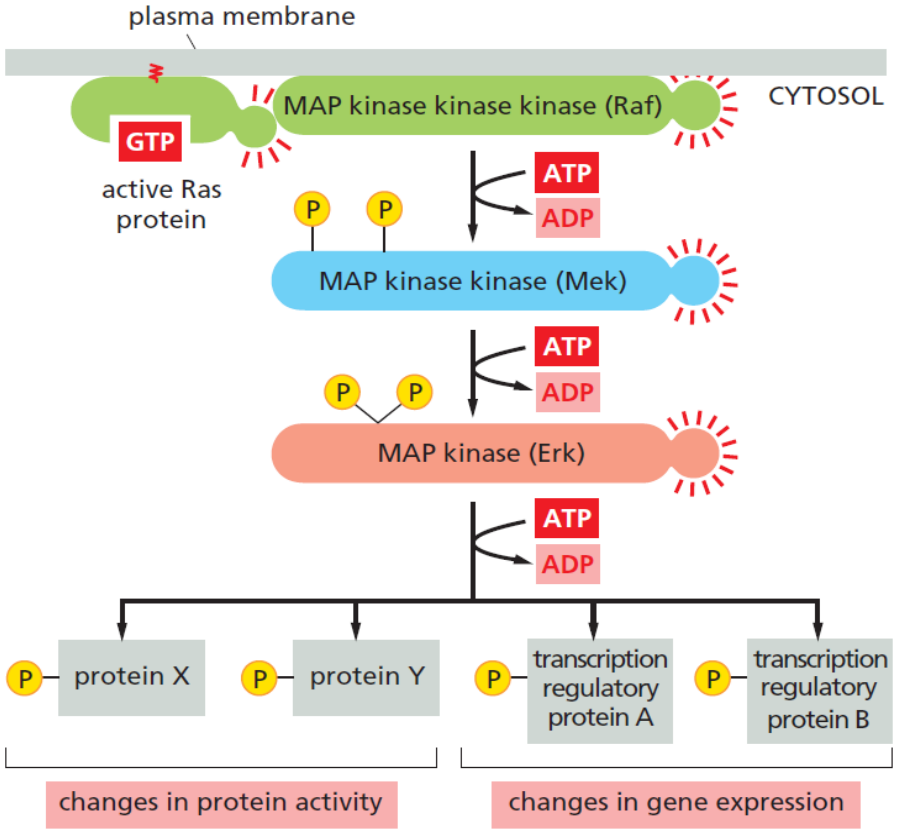
- **Tyrosine-specific protein phosphatases** quickly reverse the phosphorylations, and **Ras-GAPs** induce activated Ras to inactivate itself by hydrolyzing its bound GTP to GDP.

- To stimulate cells to proliferate or differentiate, these short-lived signaling events must be converted into **longer-lasting** ones that can sustain the signal and relay it downstream to the nucleus to alter the pattern of gene expression.

- One of the key mechanisms used for this purpose is a system of proteins called the **mitogen-activated protein kinase module (MAP kinase module)**.

The three components of this system form a functional signaling module that has been remarkably well **conserved** during evolution and is used, with variations, in many different signaling contexts.
The three components are all protein kinases:
-The final kinase in the series is called simply **MAP kinase (MAPK)**.
-The next one upstream from this is **MAP kinase kinase (MAPKK)**: it phosphorylates and thereby activates MAP kinase.
-Next above that, receiving an activating signal directly from Ras, is **MAP kinase kinase kinase (MAPKKK)**: it phosphorylates and thereby activates MAPKK.

In the mammalian Ras–MAP-kinase signaling pathway, these three kinases are known by shorter names: **Raf** (=MAPKKK), **Mek** (=MAPKK), and **Erk** (=MAPK).



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- Once activated, the MAP kinase relays the signal downstream by phosphorylating various proteins in the cell, including transcription regulators and other protein kinases.

- Erk, for example, enters the nucleus and phosphorylates one or more components of a transcription regulatory complex.

- This activates the transcription of a set of **immediate early genes**, so named because they turn on within minutes after an RTK receives an extracellular signal, even if protein synthesis is experimentally blocked with drugs.

- Some of these genes encode other **transcription regulators** that turn on other genes, a process that requires both protein synthesis and more time.

- In this way, the Ras–MAP-kinase signaling pathway conveys signals from the cell surface to the nucleus and alters the pattern of gene expression.

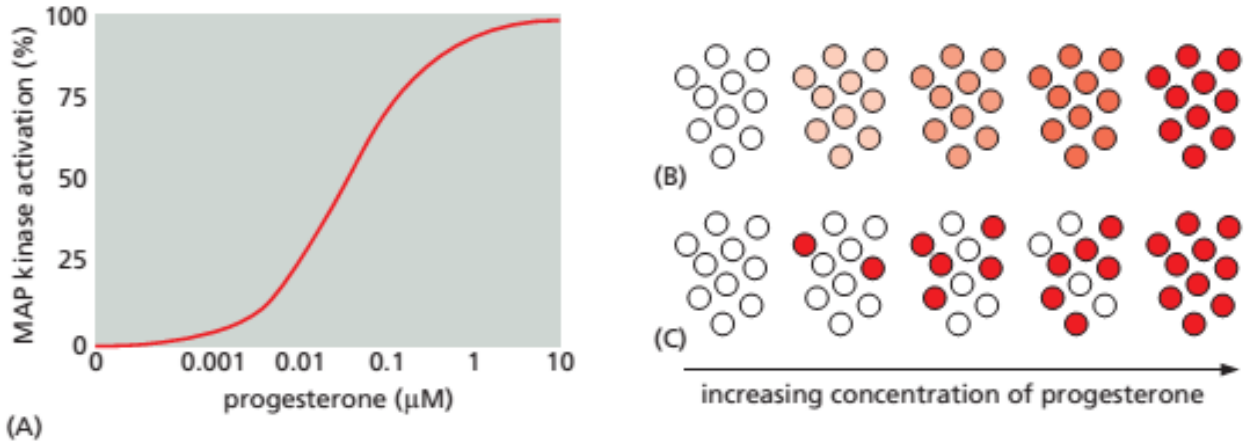
- Among the genes activated by this pathway are some that stimulate **cell proliferation**, such as the genes encoding G1 cyclins.

-When **EGF** activates its receptors in a neural precursor cell line, for example, Erk MAP kinase activity peaks at 5 minutes and rapidly declines, and the cells later go on to divide.
-By contrast, when **NGF** activates its receptors on the same cells, Erk activity remains high for many hours, and the cells stop proliferating and differentiate into neurons.

Ras Activates a MAP Kinase Signaling Module

Many factors influence the duration and other features of the signaling response, including **positive and negative feedback loops**, which can combine to give responses that are either graded or switchlike and either brief or long lasting.

MAP kinase activates a complex positive feedback loop to produce an **all-or-none**, irreversible response when frog oocytes are stimulated to mature by a brief exposure to the extracellular signal molecule progesterone.



In many cells, MAP kinases activate a **negative feedback loop:**

1.By **increasing the concentration of a protein phosphatase** that removes the phosphate from MAP kinase. (The increase in the phosphatase results from both an increase in the transcription of the phosphatase gene and the stabilization of the enzyme against degradation).

2.In the Ras–MAP-kinase pathway, **Erk also phosphorylates and inactivates Raf**, providing another negative feedback loop that helps shut off the MAP kinase module.

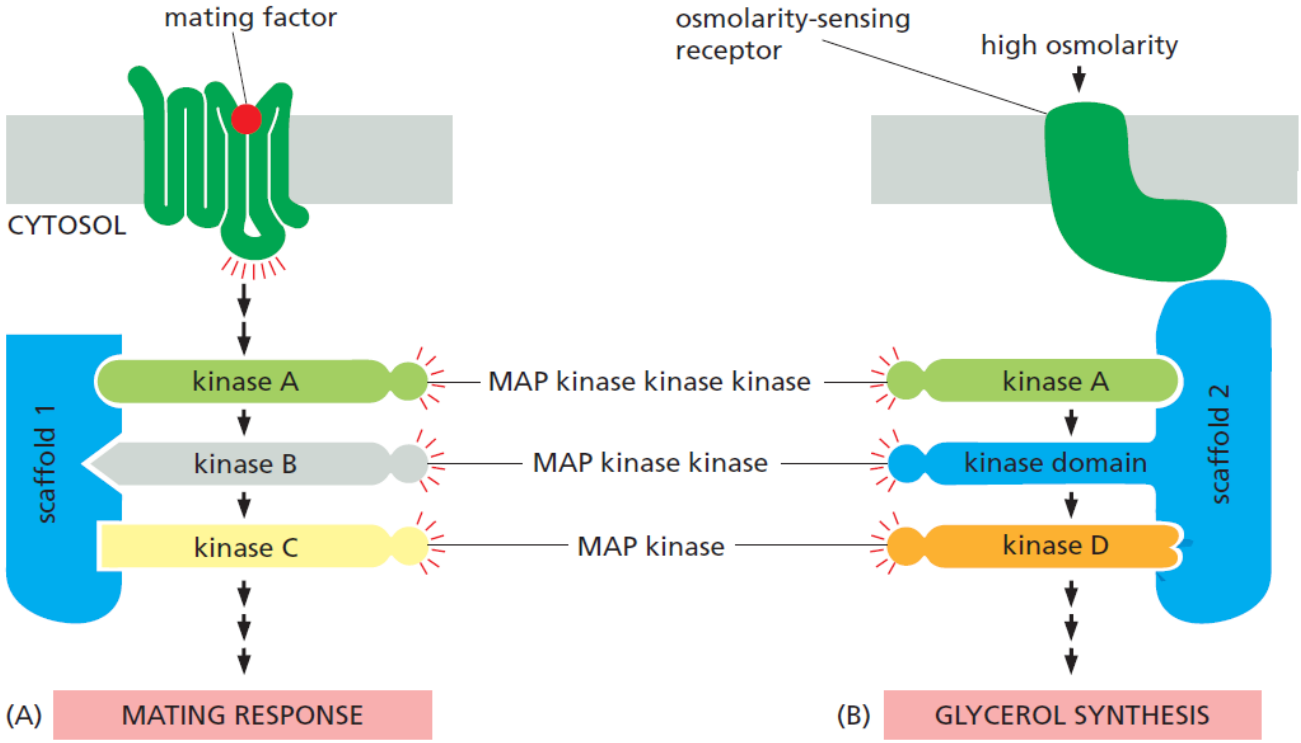
Scaffold Proteins Help Prevent Cross-talk Between Parallel MAP Kinase Modules

Three-component MAP kinase signaling modules operate in **all eukaryotic cells**, with different modules mediating different responses in the same cell.

In budding yeast, for example, one such module mediates the response to mating pheromone, another the response to starvation, and yet another the response to osmotic shock.

Some of these MAP kinase modules use one or more of the **same kinases** and yet **manage** to activate different effector proteins and hence different responses.

One way in which cells avoid **cross-talk** between the different parallel signaling pathways and ensure that each response is specific is to use **scaffold proteins**. In budding yeast cells, such scaffolds bind all or some of the kinases in each MAP kinase module to form a complex and thereby help to ensure response specificity.



Scaffold Proteins Help Prevent Cross-talk Between Parallel MAP Kinase Modules

Mammalian cells also use this **scaffold strategy** to prevent cross-talk between different MAP kinase modules.

At least five parallel MAP kinase modules can operate in a mammalian cell.

These modules make use of at least 12 MAP kinases, 7 MAPKKs, and 7 MAPKKKs.

Two of these modules (terminating in MAP kinases called **JNK** and **p38**) are activated by different kinds of cell stresses, such as ultraviolet (UV) irradiation, heat shock, and osmotic stress, as well as by inflammatory cytokines;
others mainly mediate responses to signals from other cells.

Although the scaffold strategy provides **precision** and avoids **cross-talk**, it reduces the opportunities for amplification and spreading of the signal to different parts of the cell, which require at least some of the components to be diffusible.

It is unclear to what extent the individual components of MAP kinase modules can dissociate from the scaffold during the activation process to permit amplification.

Rho Family GTPases Functionally Couple Cell-Surface Receptors to the Cytoskeleton

- Besides the Ras proteins, the other class of Ras superfamily GTPases that relays signals from cell-surface receptors is the **large Rho family**:

Rho family monomeric GTPases regulate both the **actin** and **microtubule** cytoskeletons,
-controlling cell shape, polarity, motility, and adhesion; they also regulate cell-cycle progression, gene transcription, and membrane transport.
-They play a key part in the guidance of cell migration and nerve axon outgrowth, mediating cytoskeletal responses to the activation of a special class of **guidance receptors**.

- The three best-characterized family members are **Rho** itself, **Rac**, and **Cdc42**, each of which affects multiple downstream target proteins.

- In the same way as for Ras, GEFs activate and GAPs inactivate the Rho family GTPases; there are more than 80 Rho-GEFs and more than 70 Rho-GAPs in humans.

- Some of the GEFs and GAPs are specific for one particular family member, whereas others are less specific.

- Unlike Ras, which is membrane-associated even when inactive (with GDP bound), inactive Rho family GTPases are often bound to **guanine nucleotide dissociation inhibitors (GDIs)** in the cytosol, which prevent the GTPases from interacting with their Rho-GEFs at the plasma membrane.

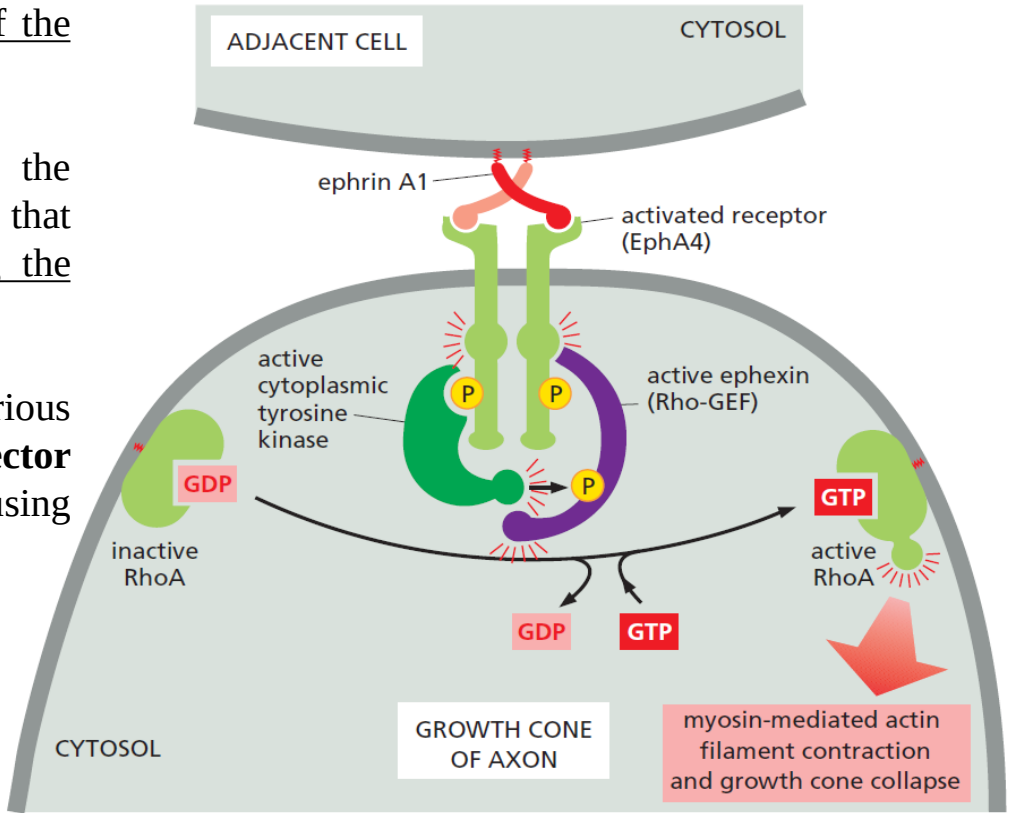
Rho Family GTPases Functionally Couple Cell-Surface Receptors to the Cytoskeleton

- Signaling by extracellular signaling proteins of the **ephrin family** provides an example of how RTKs can activate a Rho GTPase.
- **Ephrins** bind and thereby activate members of the Eph family of RTKs.
- One member of the Eph family is found on the surface of motor neurons and helps guide the migrating tip of the axon (called a **growth cone**) to its muscle target.
- The binding of a cell-surface ephrin protein activates the Eph receptor, causing the growth cones to **collapse**, thereby repelling them from inappropriate regions and keeping them on track.

The response depends on a Rho-GEF called **ephexin**, which is stably associated with the cytosolic tail of the Eph receptor.

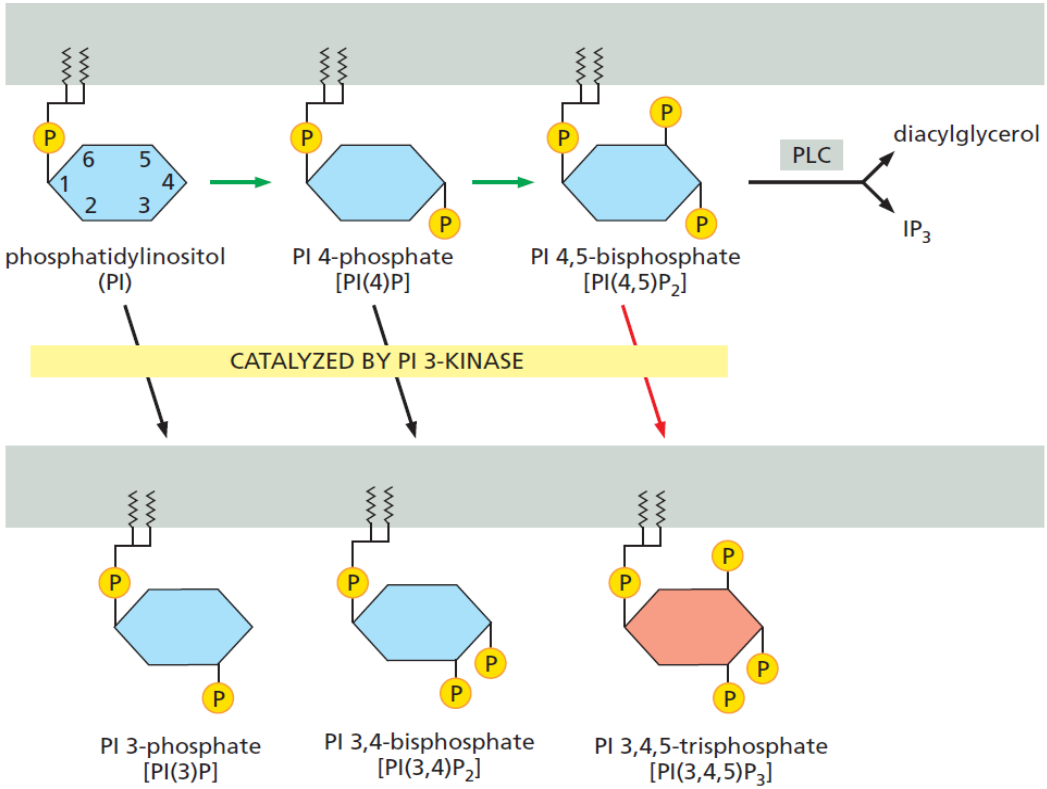
When ephrin binding activates the Eph receptor, the receptor activates a cytoplasmic tyrosine kinase that **phosphorylates** ephexin on a tyrosine, enhancing the ability of ephexin to activate the Rho protein RhoA.

The activated RhoA (RhoA-GTP) then regulates various downstream target proteins, including some **effector proteins that control the actin cytoskeleton**, causing the growth cone to collapse.



PI 3-Kinase Produces Lipid Docking Sites in the Plasma Membrane

- One of the proteins that binds to the intracellular tail of RTK molecules is the plasma-membrane-bound enzyme **phosphoinositide 3-kinase (PI 3-kinase).**
- This kinase principally phosphorylates inositol phospholipids rather than proteins, and both RTKs and GPCRs can activate it.
- It plays a central part in **promoting cell survival and growth**.
- Phosphatidylinositol (PI) is unique among membrane lipids because it can undergo reversible phosphorylation at multiple sites on its inositol head group to generate a variety of phosphorylated PI lipids called phosphoinositides.
- When activated, PI 3-kinase catalyzes phosphorylation at the 3 position of the inositol ring to generate several phosphoinositides.



The most important phosphorylation (indicated in red) is of PI(4,5)P2 to **PI(3,4,5)P3**, which can serve as a docking site for signaling proteins with **PI(3,4,5)P3-binding PH domains.**

PI 3-Kinase Produces Lipid Docking Sites in the Plasma Membrane

- The production of PI(3,4,5)P3 matters most because it can serve as a docking site for various intracellular signaling proteins, which assemble into signaling complexes that relay the signal into the cell from the cytosolic face of the plasma membrane.

- Notice the difference between this use of phosphoinositides and their use described earlier, in which PI(4,5)P2 is **cleaved** by PLCβ (in the case of GPCRs) or PLCγ (in the case of RTKs) to generate soluble IP3 and membrane-bound diacylglycerol.

- By contrast, **PI(3,4,5)P3** is not cleaved by either PLC. It is made from PI(4,5)P2 and then remains in the plasma membrane until specific phosphoinositide phosphatases dephosphorylate it.

- Prominent among these is the **PTEN phosphatase**, which dephosphorylates the 3 position of the inositol ring.
- Mutations in PTEN are found in many cancers: by prolonging signaling by PI 3-kinase, they promote uncontrolled cell growth.

-There are various types of PI 3-kinases.
-Those activated by RTKs and GPCRs belong to class I.
-These are **heterodimers** composed of a common catalytic subunit and different regulatory subunits.
-**RTKs** activate class Ia PI 3-kinases, in which the regulatory subunit is an adaptor protein that binds to two phosphotyrosines on activated RTKs through its two SH2 domains.
-**GPCRs** activate class Ib PI 3-kinases, which have a regulatory subunit that binds to the **βγ complex** of an activated trimeric G protein (**Gq**) when GPCRs are activated by their extracellular ligand.
-The direct binding of activated **Ras** can also activate the common class I catalytic subunit.

PI 3-Kinase Produces Lipid Docking Sites in the Plasma Membrane

- Intracellular signaling proteins bind to PI(3,4,5)P3 produced by activated PI 3-kinase via a specific interaction domain, such as a **pleckstrin homology (PH) domain**, first identified in the platelet protein pleckstrin.

- PH domains function mainly as protein–protein interaction domains, and it is only a small subset of them that bind to PI(3,4,5)P3; at least some of these also recognize a **specific membrane-bound protein as well as the PI(3,4,5)P3**, which greatly increases the specificity of the binding and helps to explain why the signaling proteins with PI(3,4,5) P3-binding PH domains do not all dock at all PI(3,4,5)P3 sites.

- PH domains occur in about 200 human proteins, including the Ras-GEF Sos.

- One especially important PH-domain-containing protein is the **serine/threonine protein kinase Akt**.

- The **PI-3-kinase–Akt signaling pathway** is the major pathway activated by the hormone insulin.

- It also plays a key part in promoting the **survival and growth** of many cell types in both invertebrates and vertebrates.

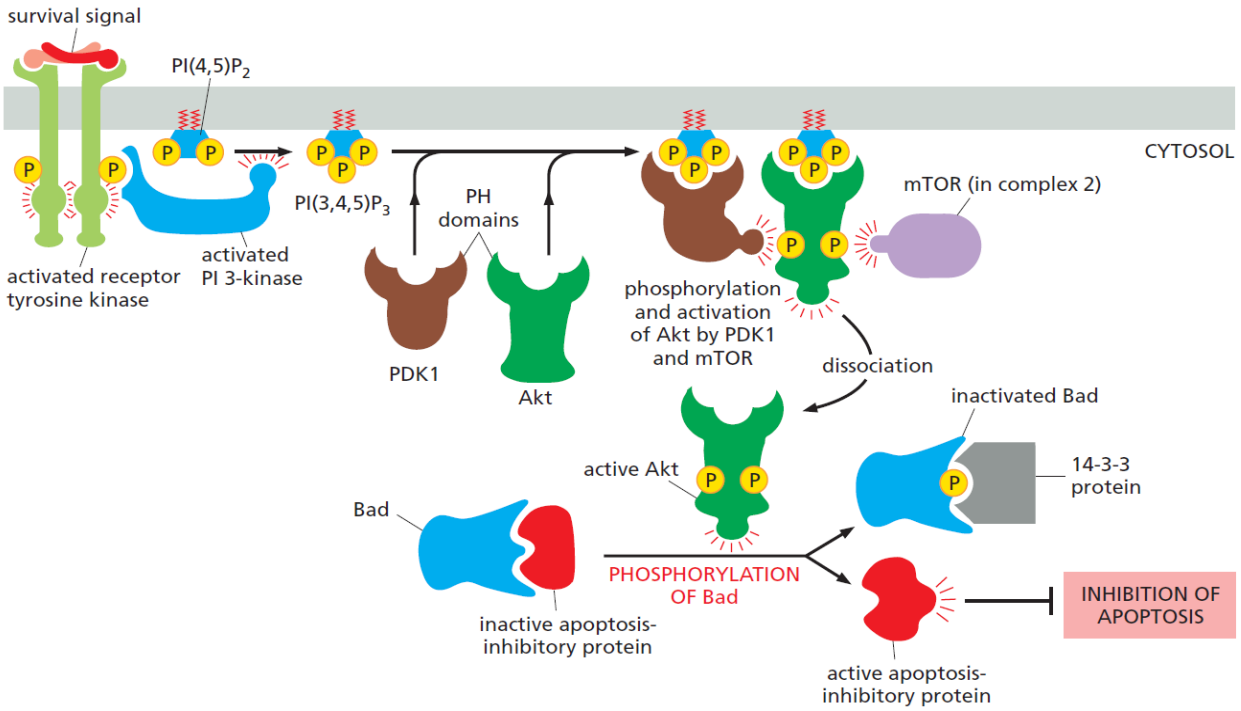
The PI-3-Kinase–Akt Signaling Pathway Stimulates Animal Cells to Survive and Grow

Extracellular signals are usually required for animal cells **to grow and divide**, as well as **to survive**.

Members of the ***insulin-like growth factor (IGF)*** family of signal proteins, for example, stimulate many types of animal cells to survive and grow.

They bind to specific RTKs, which activate PI 3-kinase to produce PI(3,4,5)P3.

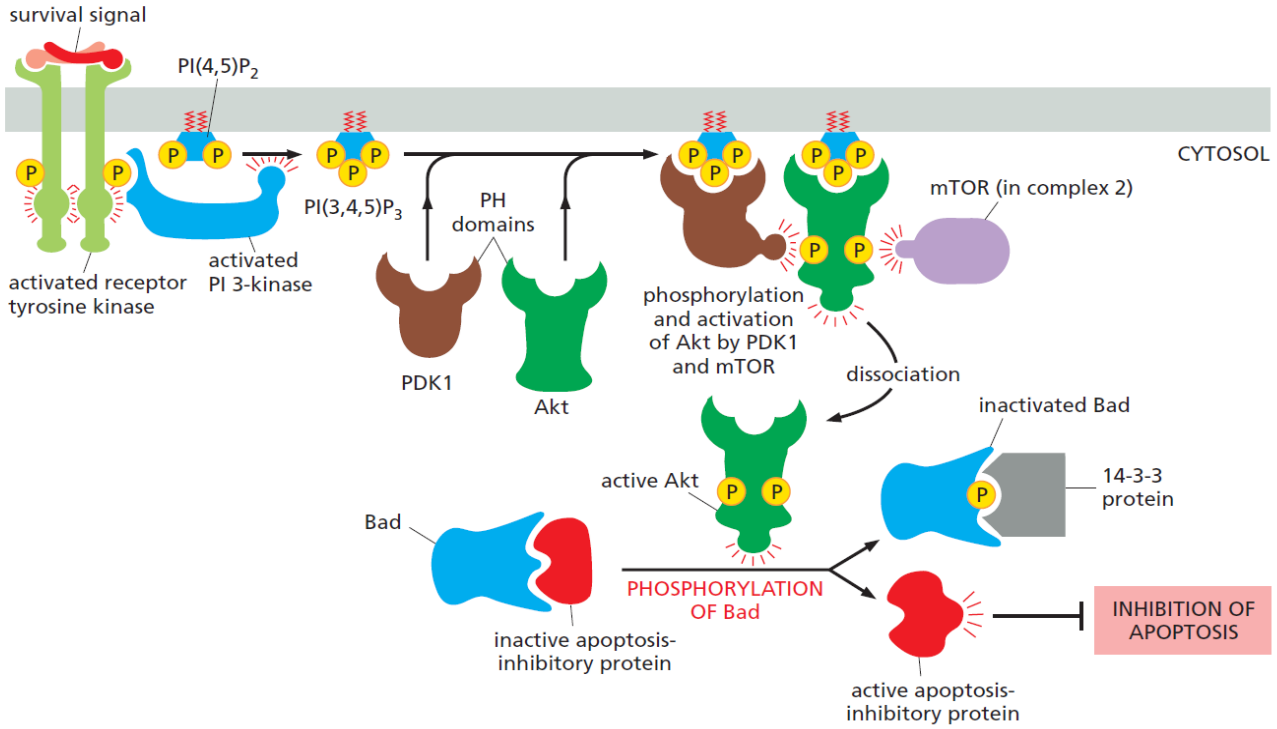
The PI(3,4,5)P3 recruits <u>two protein kinases</u> to the plasma membrane via their PH domains—**Akt** (also called protein kinase B, or PKB) and **phosphoinositide-dependent protein kinase 1 (PDK1)**, and this leads to the activation of Akt.



The PI-3-Kinase–Akt Signaling Pathway Stimulates Animal Cells to Survive and Grow

Once activated, Akt phosphorylates various target proteins at the plasma membrane, as well as in the **cytosol** and **nucleus**.

The effect on most of the known targets is to inactivate them; but the targets are such that these actions of Akt all conspire **to enhance cell survival and growth**.



The activated Akt dissociates from the plasma membrane and phosphorylates various target proteins, including the **Bad** protein.

When **unphosphorylated**, Bad holds one or more **apoptosis-inhibitory proteins** in an inactive state.

Once phosphorylated, Bad releases the inhibitory proteins, which now can block apoptosis and thereby promote cell survival.

The phosphorylated Bad binds to a ubiquitous cytosolic protein called 14-3-3, which keeps Bad out of action.