Signaling Through enzyme-coupled receptors

Activation of mTOR by the PI-3-kinase–Akt signaling pathway

- The **control of cell growth** by the PI-3-kinase–Akt pathway depends in part on a large protein kinase called **TOR** (named as the target of rapamycin, a bacterial toxin that inactivates the kinase and is used clinically as both an **immunosuppressant and anticancer drug**).

- TOR was originally identified in yeasts in genetic screens for rapamycin resistance; in mammalian cells, it is called mTOR, which exists in cells in two functionally distinct multiprotein complexes:

-mTOR complex 1 contains the protein **raptor**; this complex is sensitive to rapamycin, and it stimulates cell growth—both by **promoting ribosome production** and protein synthesis and by **inhibiting protein degradation**. Complex 1 also promotes both cell growth and cell survival by stimulating **nutrient uptake** and **metabolism**.

-mTOR complex 2 contains the protein **rictor** and is insensitive to rapamycin; it helps to **activate Akt**, and it regulates the **actin cytoskeleton via Rho family GTPases**.

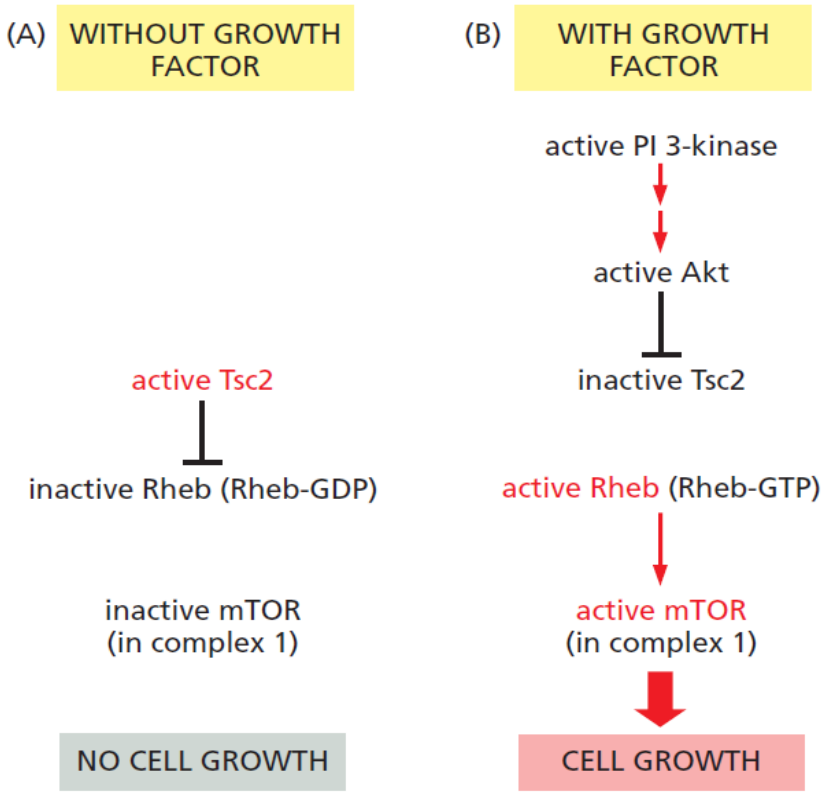
- The mTOR in complex 1 integrates inputs from various sources, including extracellular signal proteins referred to as growth factors and nutrients such as amino acids, both of which help activate mTOR and promote cell growth.

Activation of mTOR by the PI-3-kinase–Akt signaling pathway

The growth factors activate mTOR mainly via the PI-3-kinase–Akt pathway:
-Akt activates mTOR in complex 1 indirectly by **phosphorylating**, and thereby **inhibiting**, a GAP called **Tsc2**.
-Tsc2 acts on a monomeric Ras-related GTPase called **Rheb**.
-Rheb in its active form (Rheb-GTP) activates **mTOR in complex 1**.

The net result is that Akt activates mTOR and thereby promotes cell growth.

Tsc2 is short for **tuberous sclerosis protein 2**, and it is one component of a heterodimer composed of Tsc1 and Tsc2; these proteins are so called because mutations in either gene encoding them cause the genetic disease **tuberous sclerosis**, which is associated with **benign tumors** that contain abnormally large cells.



(A) In the absence of extracellular growth factors, **Tsc2** (a Rheb GAP) keeps Rheb inactive; mTOR in complex 1 is inactive, and there is no cell growth.

(B) In the presence of growth factors, activated Akt phosphorylates and inhibits Tsc2, thereby promoting the activation of Rheb.
Activated **Rheb (Rheb-GTP)** helps activate mTOR in complex 1, which in turn stimulates cell growth.

- The Erk MAP kinase can also phosphorylate and inhibit Tsc2 and thereby activate mTOR.

- Thus, both the **PI-3-kinase–Akt** and **Ras–MAP-kinase** signaling pathways converge on mTOR in complex 1 to stimulate cell growth.

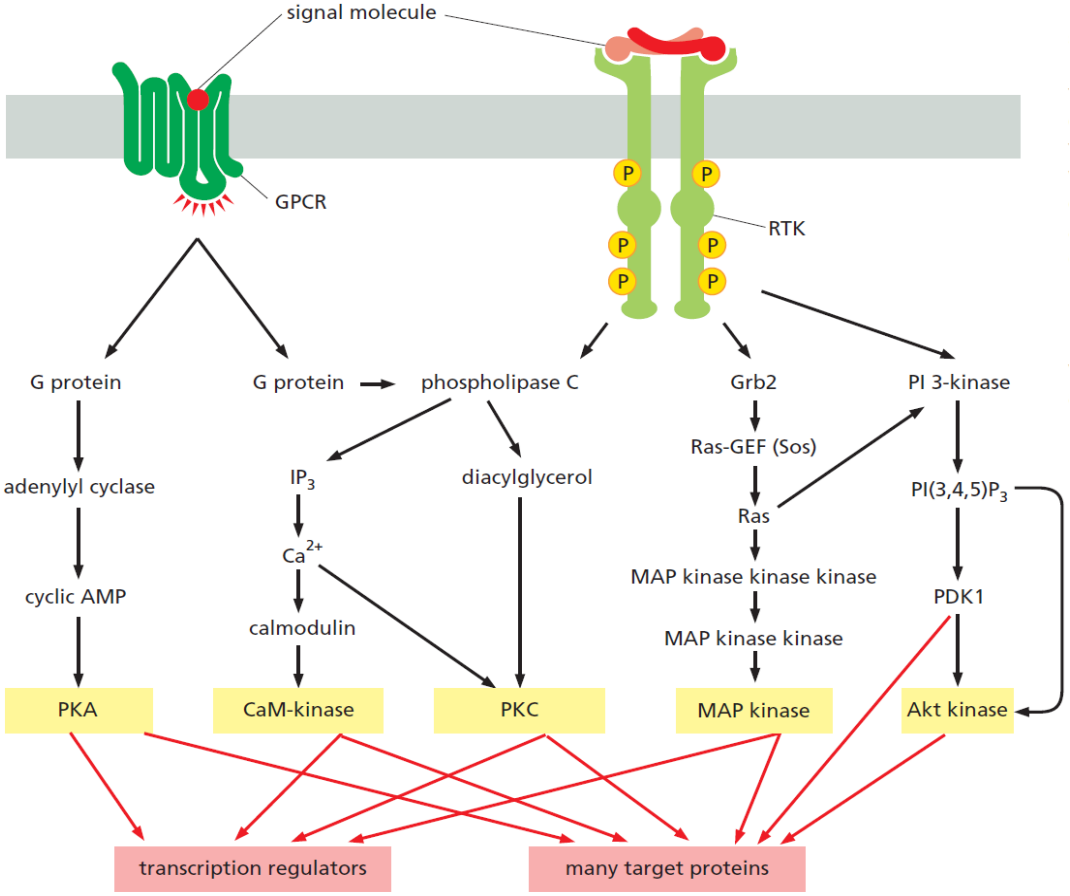
RTKs and GPCRs Activate Overlapping Signaling Pathways

RTKs and GPCRs activate some of the same intracellular signaling pathways.
Both, for example, can activate the inositol phospholipid pathway triggered by phospholipase C.

Moreover, even when they activate different pathways, the different pathways can converge on the same target proteins.

Figure below illustrates both of these types of signaling overlaps: it summarizes five parallel intracellular signaling pathways that we have discussed so far—one triggered by GPCRs, two triggered by RTKs, and two triggered by both kinds of receptors.

Interactions among these pathways allow different extracellular signal molecules to modulate and coordinate each other's effects.



Some Enzyme-Coupled Receptors Associate with Cytoplasmic Tyrosine Kinases

- Many cell-surface receptors depend on tyrosine phosphorylation for their activity and yet **lack a tyrosine kinase domain.**
- These receptors act through **cytoplasmic tyrosine kinases**, which are associated with the receptors and phosphorylate **various target proteins**, often including the **receptors themselves**, when the receptors bind their ligand.
- These tyrosine-kinase-associated receptors thus function in much the same way as RTKs, except that their kinase domain is encoded by a separate gene and is noncovalently associated with the receptor polypeptide chain.
- A variety of receptor classes belong in this category, including the receptors for **antigen and interleukins on lymphocytes, integrins, and receptors for various cytokines and some hormones.**

- As with RTKs, many of these receptors are either preformed dimers or are cross-linked into dimers by ligand binding.

Some of these receptors depend on members of the largest family of mammalian cytoplasmic tyrosine kinases, the **Src family**, which includes Src, Yes, Fgr, Fyn, Lck, Lyn, Hck, and Blk:
-These protein kinases all contain **SH2** and **SH3** domains and are located on the cytoplasmic side of the plasma membrane, held there partly by their interaction with transmembrane receptor proteins and partly by covalently attached lipid chains.
-Different family members are associated with **different receptors** and phosphorylate overlapping but distinct sets of target proteins.
-Lyn, Fyn, and Lck, for example, are each associated with different sets of receptors on **lymphocytes**.
-In each case, the kinase is activated when an extracellular ligand binds to the appropriate receptor protein.
-Src itself, as well as several other family members, can also bind to activated RTKs; in these cases, the receptor and cytoplasmic kinases **mutually** stimulate each other's catalytic activity, thereby **strengthening** and **prolonging** the signal.
-There are even some G proteins (Gs and Gi) that can activate Src, which is one way that the activation of GPCRs can lead to tyrosine phosphorylation of intracellular signaling proteins and effector proteins.

Some Enzyme-Coupled Receptors Associate with Cytoplasmic Tyrosine Kinases

Another type of cytoplasmic tyrosine kinase associates with **integrins**, the main receptors that cells use to bind **to the extracellular matrix**.

The binding of matrix components to integrins activates intracellular signaling pathways that influence the behavior of the cell.

When integrins cluster at sites of matrix contact, they help trigger the assembly of cell–matrix junctions called **focal adhesions**.

Among the many proteins recruited into these junctions is the cytoplasmic tyrosine kinase called **focal adhesion kinase (FAK)**, which binds to the cytosolic tail of one of the integrin subunits with the assistance of other proteins.

The clustered FAK molecules **phosphorylate each other**, creating **phosphotyrosine docking sites** where the Src kinase can bind.

Src and FAK then phosphorylate each other and other proteins that assemble in the junction, including many of the signaling proteins used by RTKs.

In this way, the two tyrosine kinases signal to the cell that it has adhered to a suitable substratum, where the cell can now survive, grow, divide, migrate, and so on.

Cytokine Receptors Activate the JAK–STAT Signaling Pathway

- The largest and most diverse class of receptors that rely on cytoplasmic tyrosine kinases to relay signals into the cell is the class of **cytokine receptors**.

- The large family of cytokine receptors includes receptors for many kinds of **local mediators** (collectively called **cytokines**), as well as receptors for some **hormones**, such as **growth hormone** and **prolactin**.

- These receptors are stably associated with cytoplasmic tyrosine kinases called **Janus kinases (JAKs)** (after the two-faced Roman god), which phosphorylate and activate transcription regulators called **STATs (signal transducers and activators of transcription)**.

- STAT proteins are located in the cytosol and are referred to as **latent transcription regulators** because they migrate into the nucleus and regulate gene transcription only after they are activated.

- Although many intracellular signaling pathways lead from cell-surface receptors to the nucleus, where they alter gene transcription, the JAK–STAT signaling pathway provides one of the more direct routes.

- Cytokine receptors are dimers or trimers and are stably associated with one or two of the four known JAKs (JAK1, JAK2, JAK3, and Tyk2).

Cytokine Receptors Activate the JAK–STAT Signaling Pathway

Cytokine binding alters the arrangement so as to bring two JAKs into close proximity so that they **phosphorylate** each other, thereby increasing the activity of their tyrosine kinase domains.

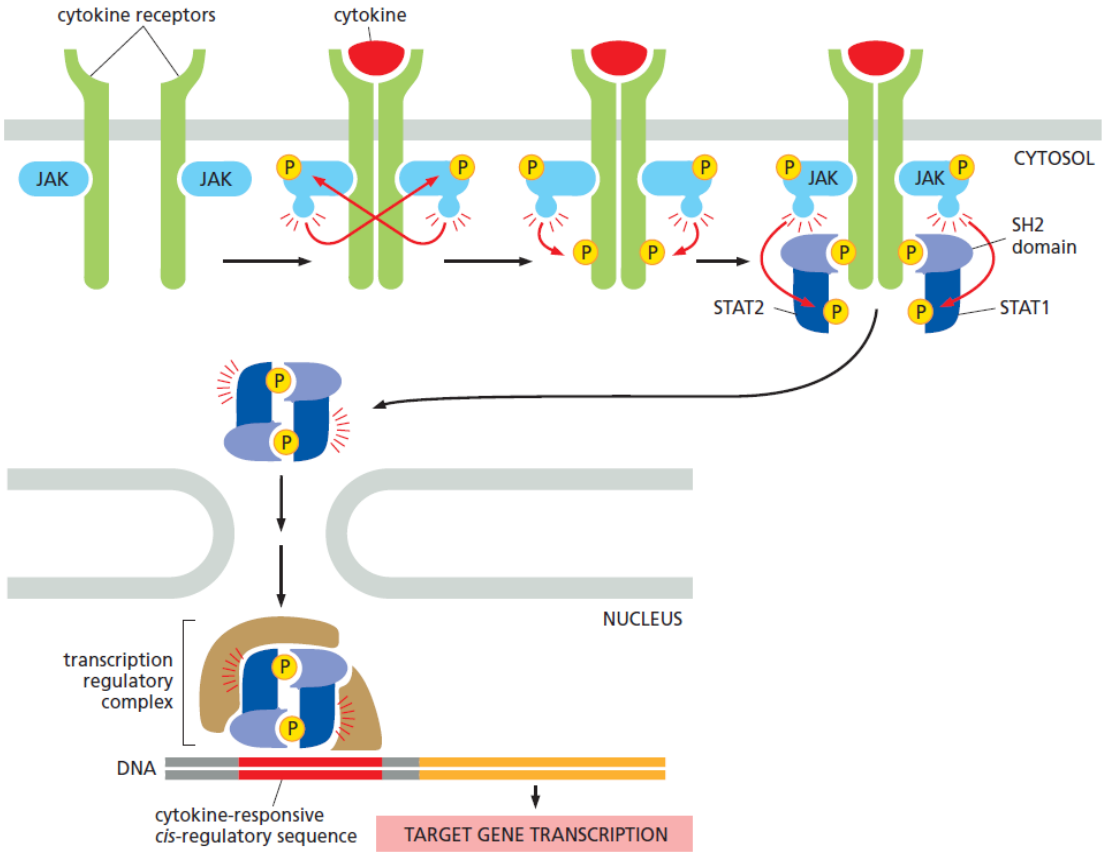
The JAKs then phosphorylate tyrosines on the cytoplasmic tails of cytokine receptors, creating **phosphotyrosine docking sites for STATs**.

There are at least six STATs in mammals.

Each has an **SH2 domain** that performs two functions:

-First, it mediates the binding of the STAT protein to a phosphotyrosine docking site on an activated cytokine receptor. Once bound, the JAKs phosphorylate the STAT on tyrosines, causing the **STAT to dissociate from the receptor**.
-Second, the **SH2 domain** on the released STAT now mediates its binding to **a phosphotyrosine on another STAT molecule**, forming either a STAT homodimer or a heterodimer.

The STAT dimer then translocates to the nucleus, where, in combination with other transcription regulatory proteins, it binds to a specific **cis-regulatory** sequence in various genes and stimulates their transcription.



Cytokine Receptors Activate the JAK–STAT Signaling Pathway

- In response to the hormone **prolactin**, for example, which stimulates breast cells to produce milk, activated STAT5 stimulates the transcription of genes that encode milk proteins.

Table 15–6 lists some of the more than 30 cytokines and hormones that activate the JAK–STAT pathway by binding to cytokine receptors.

- **Negative feedback** regulates the responses mediated by the JAK–STAT pathway.

In addition to activating genes that encode proteins mediating the cytokine-induced response, the STAT dimers can also activate genes that encode **inhibitory proteins** that help shut off the response:
-Some of these proteins bind to and inactivate **phosphorylated JAKs** and their associated phosphorylated receptors;
-others bind to **phosphorylated STAT dimers** and prevent them from binding to their DNA targets.

- Such negative feedback mechanisms, however, are not enough on their own to turn off the response.

- Inactivation of the activated JAKs and STATs requires **dephosphorylation of their phosphotyrosines**.

TABLE 15–6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway

Signal protein	Receptor-associated JAKs	STATs activated	Some responses
Interferon-γ (IFNγ)	JAK1 and JAK2	STAT1	Activates macrophages
Interferon-α (IFNα)	Tyk2 and JAK2	STAT1 and STAT2	Increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	Stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	Stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	Stimulates growth by inducing IGF1 production
Granulocyte–Macrophage-Colony-Stimulating Factor (GMCSF)	JAK2	STAT5	Stimulates production of granulocytes and macrophages

Protein Tyrosine Phosphatases Reverse Tyrosine Phosphorylations

- In all signaling pathways that use tyrosine phosphorylation, the tyrosine phosphorylations are reversed by protein **tyrosine phosphatases**.

- These phosphatases are as important in the signaling process as the protein tyrosine kinases that add the phosphates.

- Whereas only a few types of serine/threonine protein phosphatase catalytic subunits are responsible for removing phosphate groups from phosphorylated serines and threonines on proteins, there are about **100** protein tyrosine phosphatases encoded in the human genome, including some **dual-specificity** phosphatases that also dephosphorylate serines and threonines.

- Like tyrosine kinases, the tyrosine phosphatases occur in both cytoplasmic and transmembrane forms.

- Unlike serine/threonine protein phosphatases, which generally have **broad specificity**, most tyrosine phosphatases display **exquisite specificity** for their substrates, removing phosphate groups from only selected phosphotyrosines on a subset of proteins.

- Together, these phosphatases ensure that tyrosine phosphorylations are short-lived and that the level of tyrosine phosphorylation in resting cells is very low.

- They do not, however, simply continuously reverse the effects of protein tyrosine kinases; they are often regulated to act only at the appropriate time and place.

Signal Proteins of the TGF β Superfamily Act Through Receptor Serine/Threonine Kinases and Smads

- **The transforming growth factorβ (TGF β) superfamily** consists of a large number (33 in humans) of structurally related, secreted, dimeric proteins.

- They act either as hormones or, more commonly, as local mediators to regulate a wide range of biological functions in all animals:

-During development, they regulate **pattern formation** and influence various cell behaviors, including **proliferation**, **specification** and **differentiation**, **extracellular matrix production**, and **cell death**.
-In adults, they are involved in **tissue repair** and in **immune regulation**, as well as in many other processes.

- The superfamily consists of the **TGFβ/activin family** and the larger **bone morphogenetic protein (BMP) family**.

- All of these proteins act through enzyme-coupled receptors that are single pass transmembrane proteins with a serine/threonine kinase domain on the cytosolic side of the plasma membrane.

- There are two classes of these receptor serine/threonine kinases —type I and type II—which are structurally similar homodimers.

- Each member of the TGF β superfamily binds to a characteristic combination of type-I and type-II receptor dimers, bringing the kinase domains together so that the **type-II receptor can phosphorylate and activate the type-I receptor, forming an active tetrameric receptor complex**.

Signal Proteins of the TGF β Superfamily Act Through Receptor Serine/Threonine Kinases and Smads

- Once activated, the receptor complex uses a strategy for rapidly relaying the signal to the **nucleus** that is very similar to the JAK–STAT strategy used by cytokine receptors.

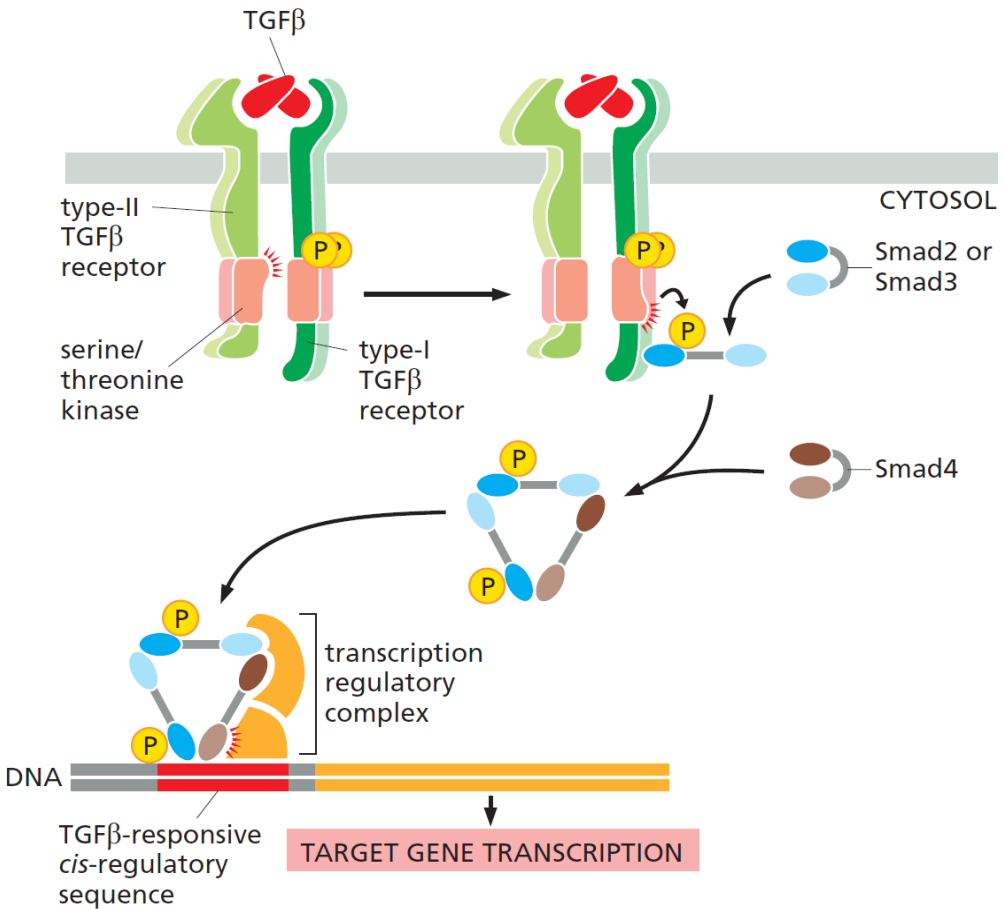
- The activated type-I receptor directly binds and phosphorylates a **latent transcription regulator** of the **Smad family** (named after the first two proteins identified, Sma in *C. elegans* and Mad in *Drosophila*).

- Activated TGFβ/activin receptors phosphorylate Smad2 or Smad3, while activated BMP receptors phosphorylate Smad1, Smad5, or Smad8.

- Once one of these receptor-activated Smads (**R-Smads**) has been phosphorylated, it dissociates from the receptor and binds to **Smad4 (called a co-Smad)**, which can form a complex with any of the five R-Smads.

- The Smad complex then translocates into the nucleus, where it associates with other transcription regulators and controls the transcription of specific target genes.

- Because the partner proteins in the nucleus vary depending on the **cell type** and **state** of the cell, the genes affected vary.



Signal Proteins of the TGF β Superfamily Act Through Receptor Serine/Threonine Kinases and Smads

- Activated TGFβ receptors and their bound ligand are endocytosed by two distinct routes:
  - one leading to further **activation** and
  - the other leading to **inactivation.**

- The activation route depends on clathrin-coated vesicles and leads to early endosomes, where most of the Smad activation occurs.
- **An anchoring protein called SARA (for Smad anchor for receptor activation) has an important role in this pathway; it is concentrated in early endosomes and binds to both activated TGFβ receptors and Smads, increasing the efficiency of receptor-mediated Smad phosphorylation.**

- The inactivation route depends on caveolae and leads to receptor ubiquitylation and degradation in proteasomes.

- During the signaling response, the Smads **shuttle** continuously between the cytoplasm and the nucleus: they are dephosphorylated in the nucleus and exported to the cytoplasm, where they can be rephosphorylated by activated receptors.

- In this way, the effect exerted on the target genes reflects both the **concentration of the extracellular signal** and the **time the signal continues** to act on the cell-surface receptors (often several hours).

- Cells exposed to a morphogen at high concentration, or for a long time, or both, will switch on one set of genes, whereas cells receiving a lower or more transient exposure will switch on another set.

Signal Proteins of the TGF β Superfamily Act Through Receptor Serine/Threonine Kinases and Smads

- As in other signaling systems, **negative feedback regulates** the Smad pathway:
  Among the target genes activated by Smad complexes are those that **encode inhibitory Smads**, either Smad6 or Smad7.

- Smad7 (and possibly Smad6) **binds to the cytosolic tail of the activated receptor** and inhibits its signaling ability in at least three ways:

(1) it competes with R-Smads for binding sites on the receptor, decreasing R-Smad phosphorylation;

(2) it recruits a ubiquitin ligase called **Smurf**, which ubiquitylates the receptor, leading to receptor internalization and degradation (it is because Smurfs also ubiquitylate and promote the degradation of Smads that they are called **Smad ubiquitylation regulatory factors, or Smurfs**); and

(3) it recruits a protein phosphatase that dephosphorylates and inactivates the receptor.

- In addition, the inhibitory Smads bind to the co-Smad, Smad4, and inhibit it, either by preventing its **binding to R-Smads** or by promoting its **ubiquitylation and degradation**.

- Although receptor serine/threonine kinases operate mainly through the Smad pathway just described, they can also stimulate other intracellular signaling proteins such as MAP kinases and PI 3-kinase.
- Conversely, signaling proteins in other pathways can phosphorylate Smads and thereby influence signaling along the Smad pathway.