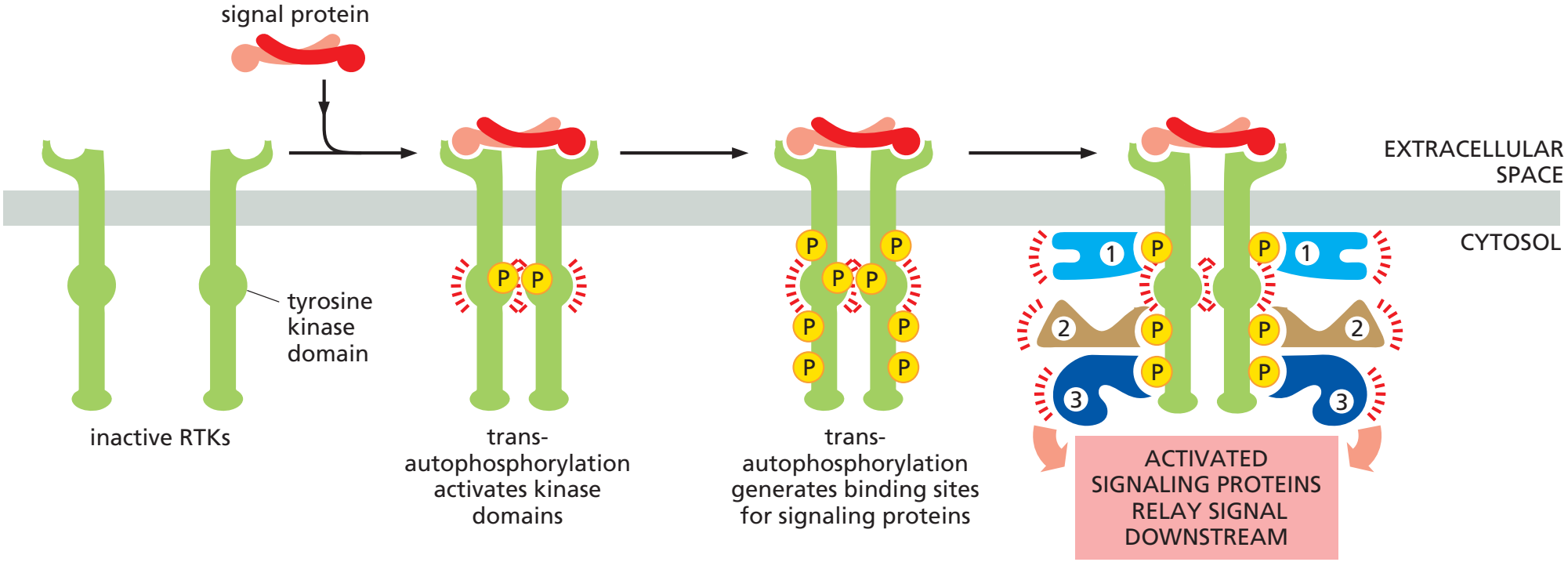
CHAPTER

15

Cell Signaling

IN THIS CHAPTER

Activation of RTKs by dimerization



Two RTK are bound by a ligand, it (Does not have to be dimerized ligand binding to two RTKs as shown in the example!)

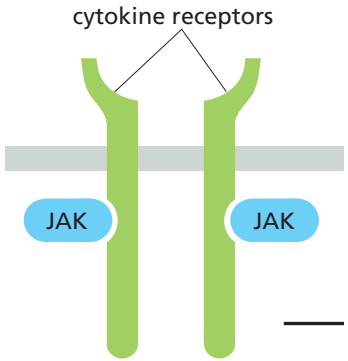
The RTK dimerize and make an initial Tyrosine autophosphorylation auto = “self”

first phosphorylation initiates trans-autophosphorylation of several tyrosines

Phospho-Tyrosine sites recruit and/or activate downstream signaling proteins

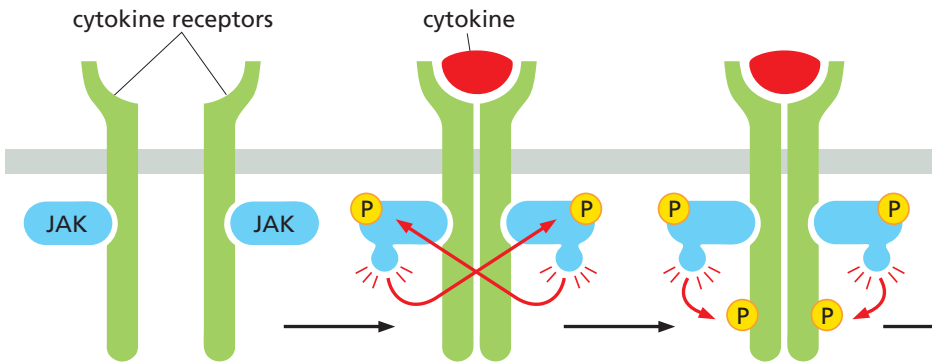
TYROSINE KINASE ASSOCIATED SIGNALING

The JAK–STAT signaling pathway activated by cytokines



Inactive JANUS Kinases are associated with receptors

The JAK–STAT signaling pathway activated by cytokines

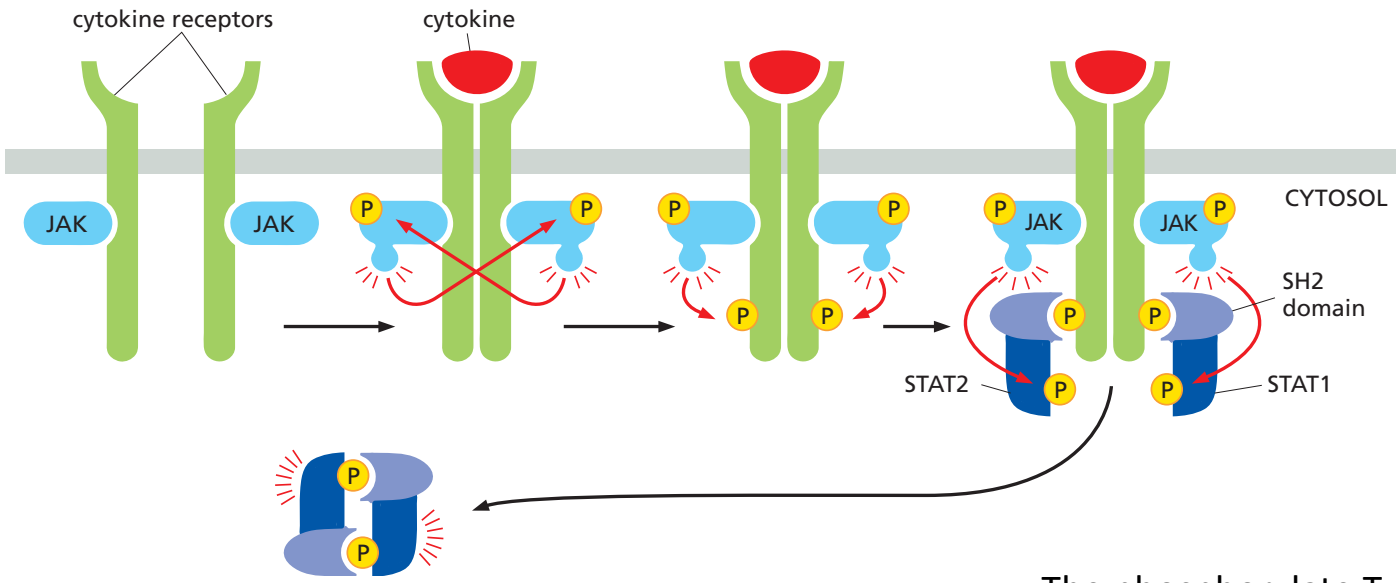


Upon binding of a ligand (Cytokine) the receptors dimerize

the JAKs cross phosphorylate each other

JAKs phosphorylate the receptors at a Tyrosine

The JAK–STAT signaling pathway activated by cytokines



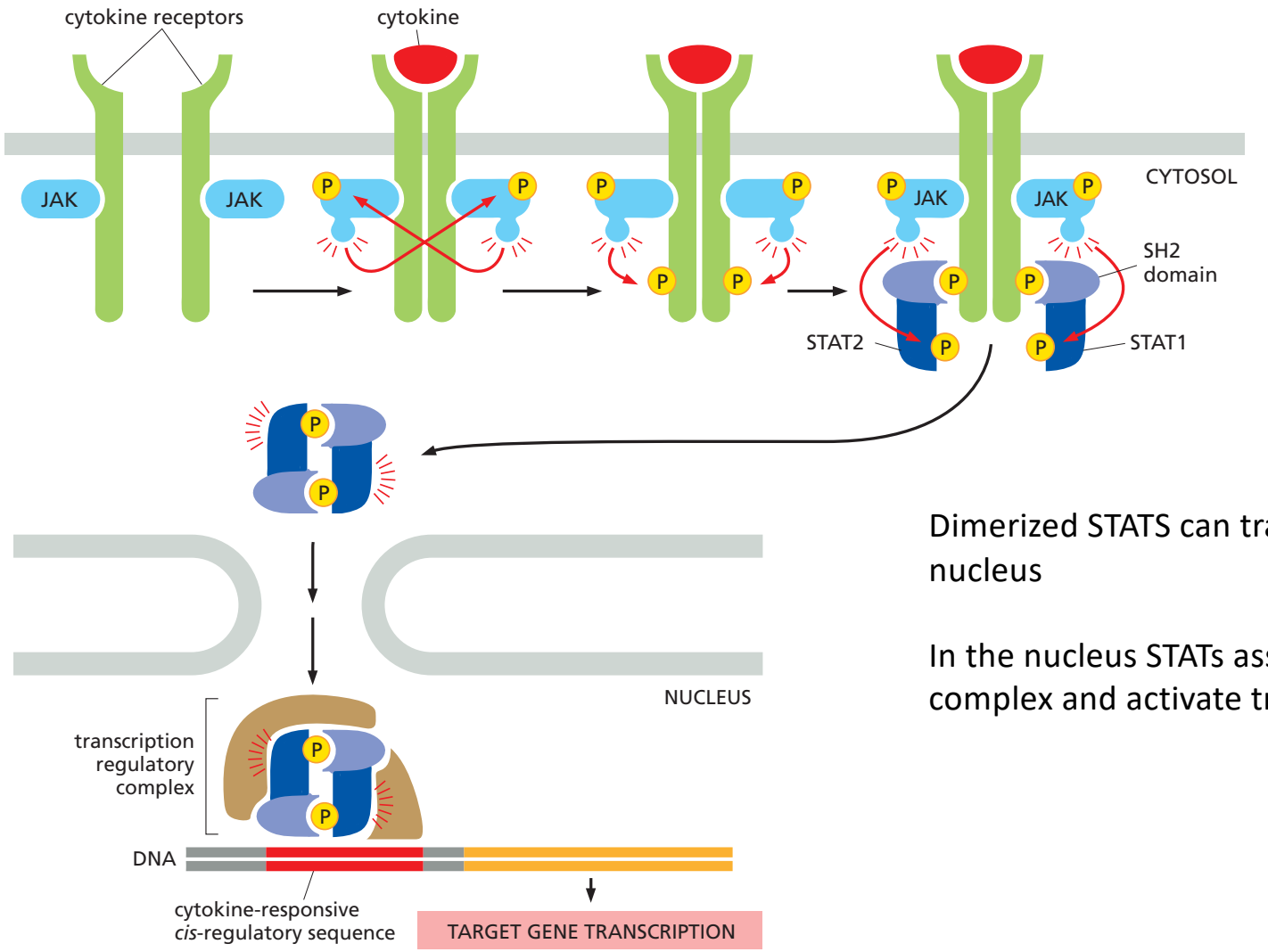
The phosphorylate Tyrosine on the receptors can recruit STAT proteins

Once docked, the STAT proteins are phosphorylated by the JAKs

This drives the release of STATs

STATs dimerize in the cytosol

The JAK–STAT signaling pathway activated by cytokines



Dimerized STATS can translocate to the nucleus

In the nucleus STATs associated with a complex and activate transcription

Some extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway

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

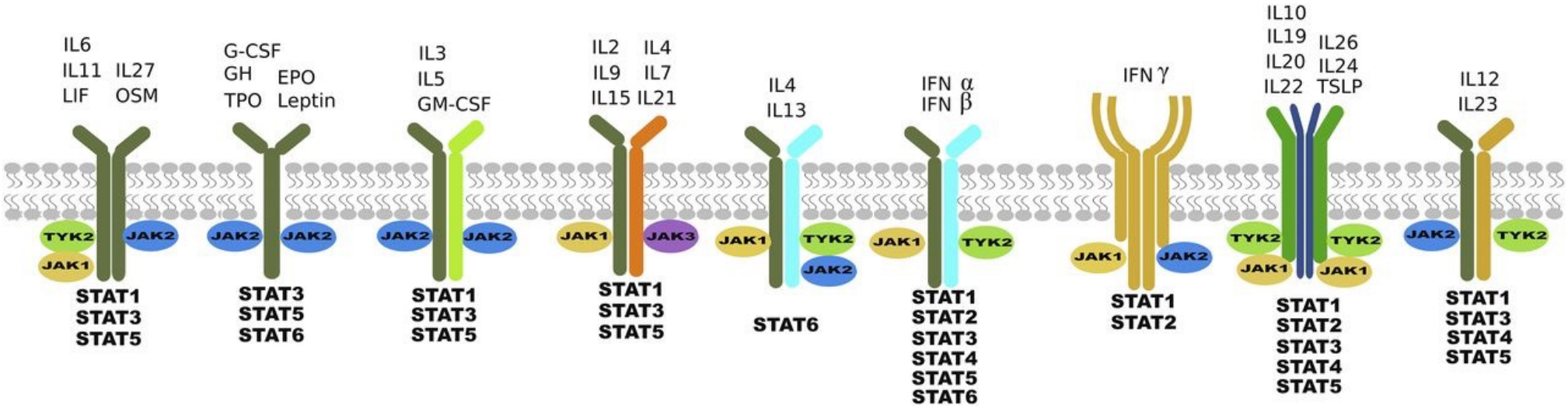
There are 4 JAKS
7 Stats

It is a promiscuous pathway!

Very important in many cell types and especially known for immune responses

And my lab worked on it recently

Some extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway



Many receptors signal through JAK-STAT!

> 128

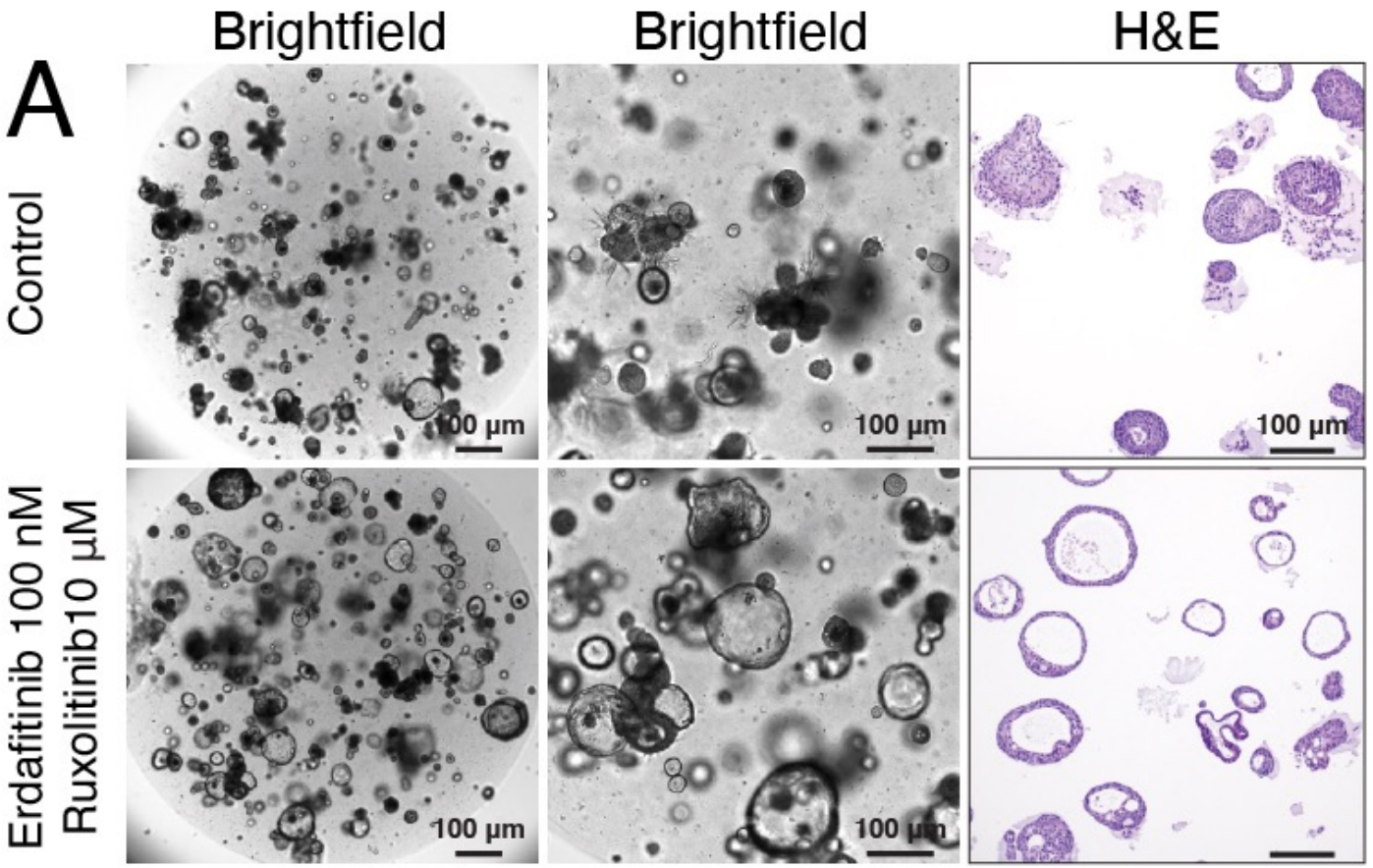
4 Kinases:

JAK1
JAK2
JAK3
TYK2

7 STATS:

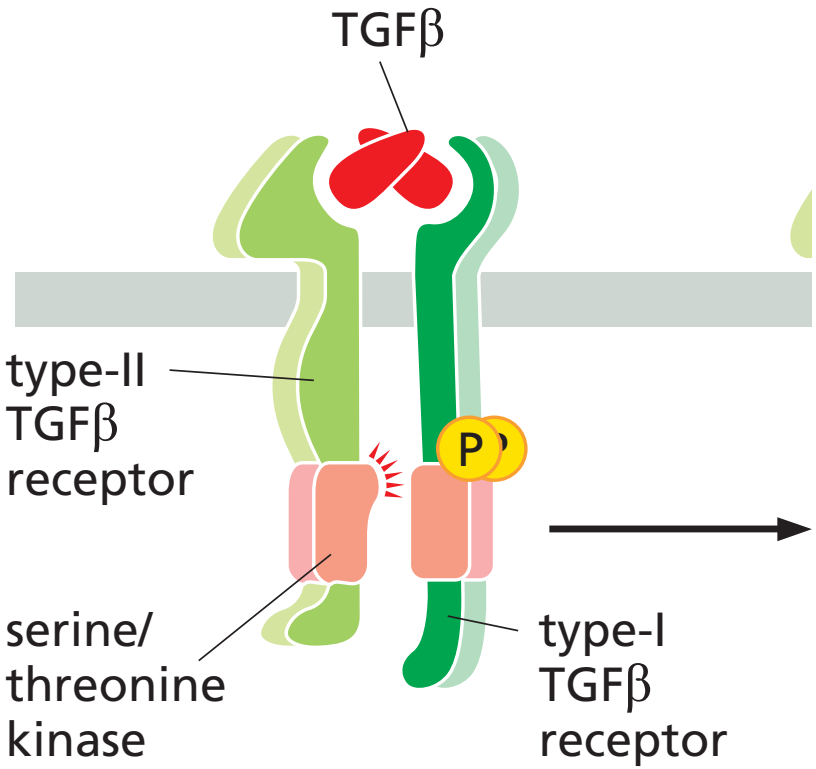
STAT1
STAT2
STAT3
STAT4
STAT5a
STAT5b
STAT6

JAK-STAT and FGFR signaling inhibition can restore sensitivity to anti-hormonal drugs in prostate cancer



TGF BETA SIGNALING

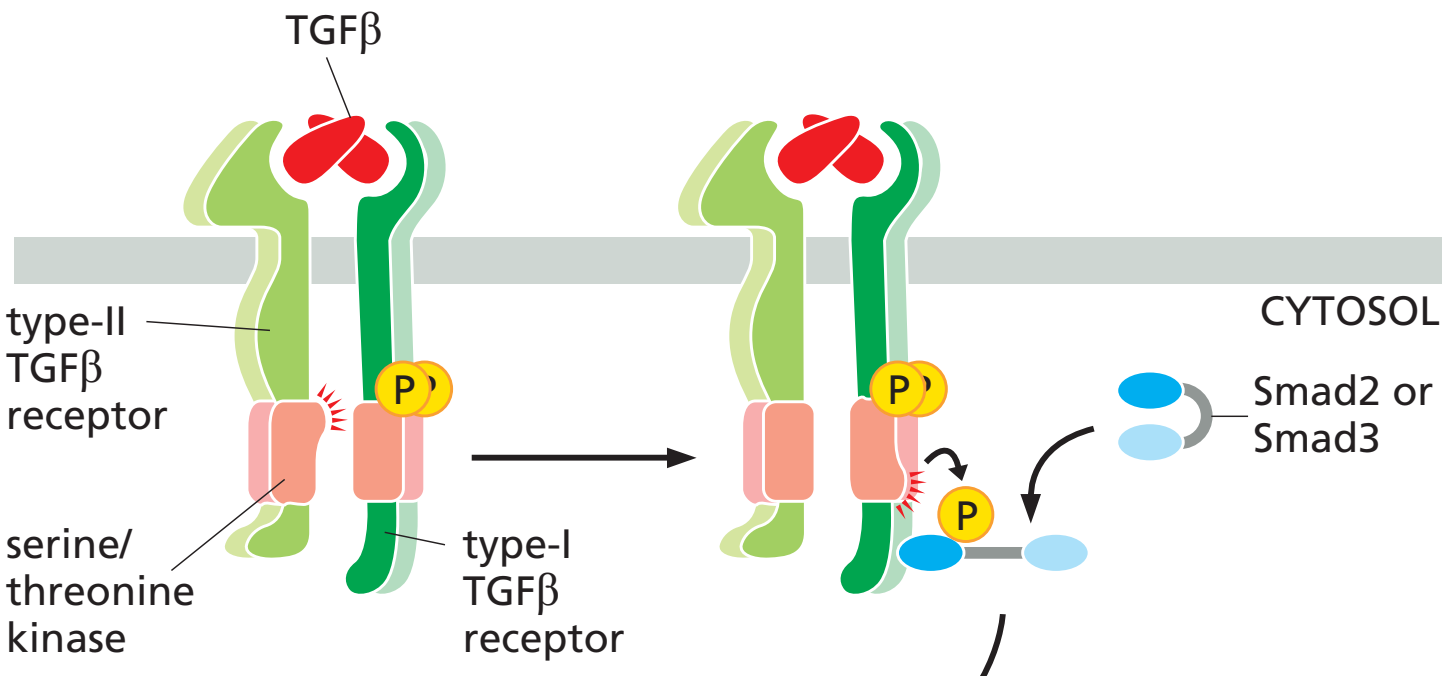
Transforming growth factor (TGF)-β



TGF-b receptors are composed of 4 individual proteins, 2x Type-I and 2x Type-II

When bound by a ligand (TGF-b) Type-II phosphorylates Type-I

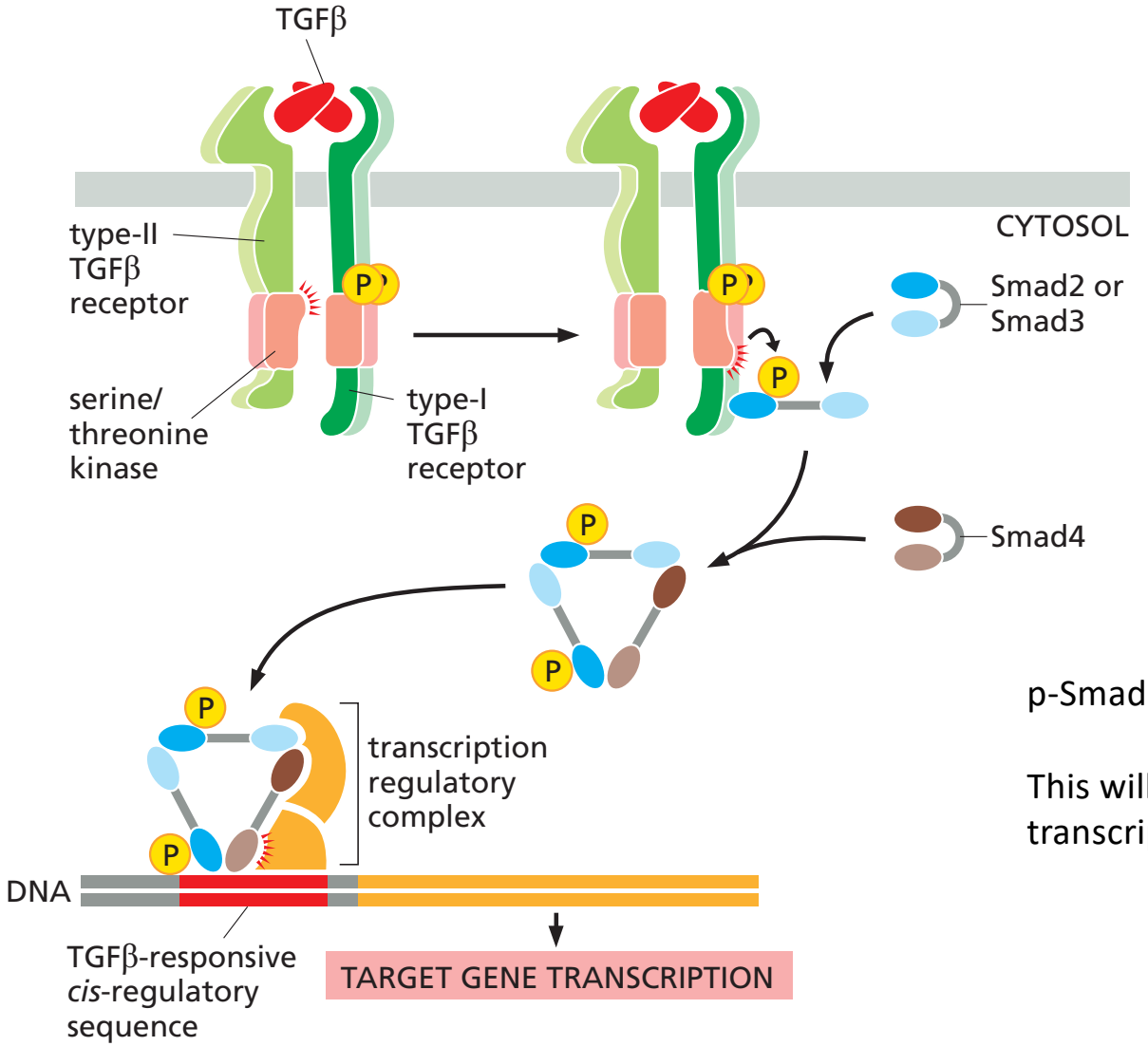
The Smad-dependent signaling pathway activated by TGFβ



When bound by a ligand (TGF-b) Type-II phosphorylates Type-I

This in turn allows Type-I to phosphorylate Smad2 or Smad3

The Smad-dependent signaling pathway activated by TGFβ



p-Smad2/3 will form a complex with Smad4

This will go to the nucleus activate transcription

The Smad-dependent signaling pathway activated by TGFβ

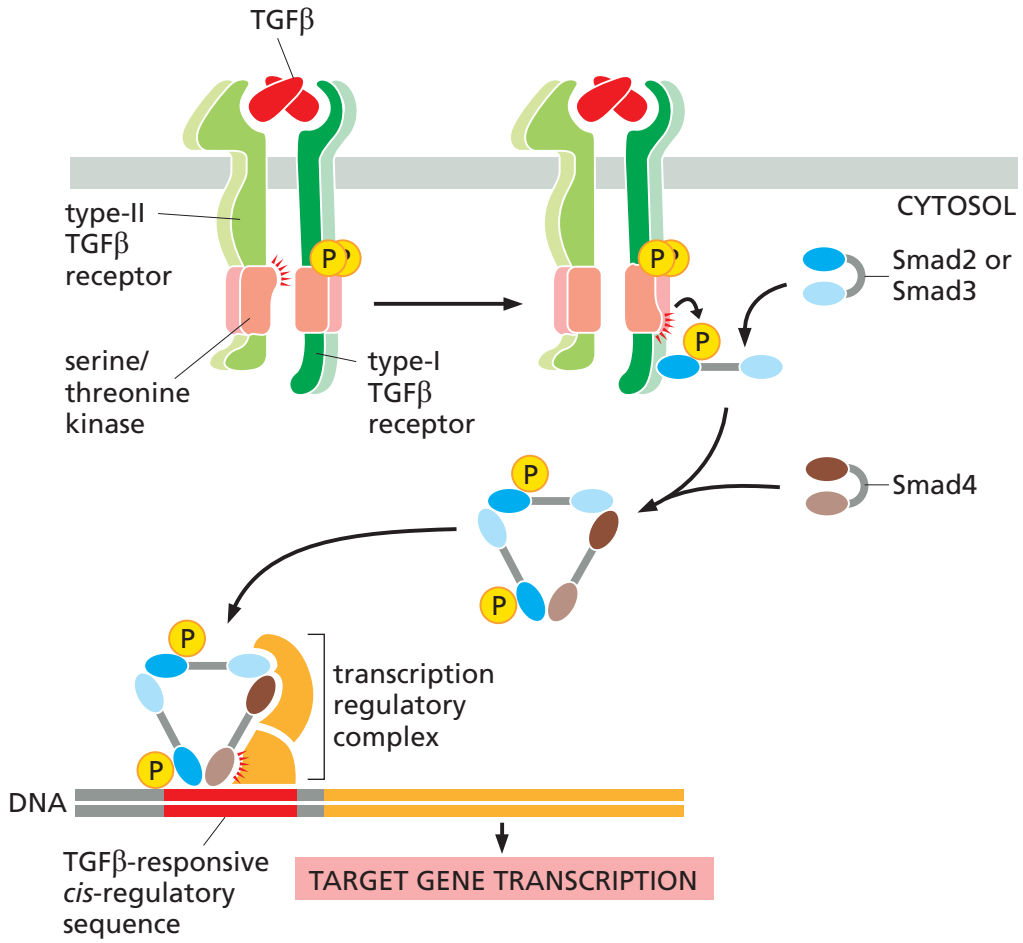


Figure 15–57 The Smad-dependent signaling pathway activated by TGFβ. The TGFβ dimer promotes the assembly of a tetrameric receptor complex containing two copies each of the type-I and type-II receptors. The type-II receptors phosphorylate specific sites on the type-I receptors, thereby activating their kinase domains and leading to phosphorylation of R-Smads such as Smad2 and Smad3. Smads open up to expose a dimerization surface when they are phosphorylated, leading to the formation of a trimeric Smad complex containing two R-Smads and the co-Smad, Smad4. The phosphorylated Smad complex enters the nucleus and collaborates with other transcription regulators to control the transcription of specific target genes.

SIGNALING WITH REGULATED PROTEOLYSIS
NOTCH
WNT
HEDGEHOG

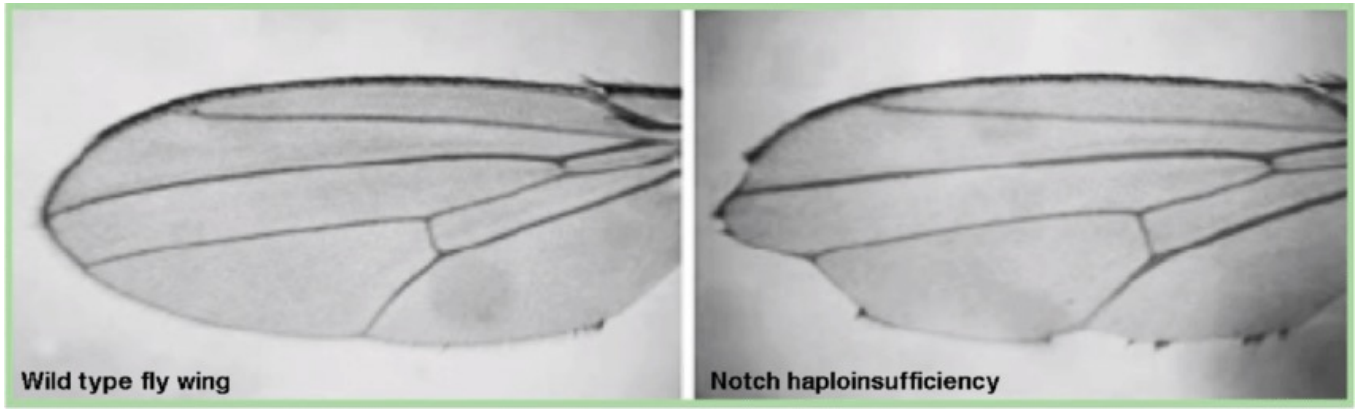
Why the funny names?

Named after phenotypes I The mighty fruit fly (*Drosophila Melanogaster*)

NOTCH = V-shaped indentation in the wing

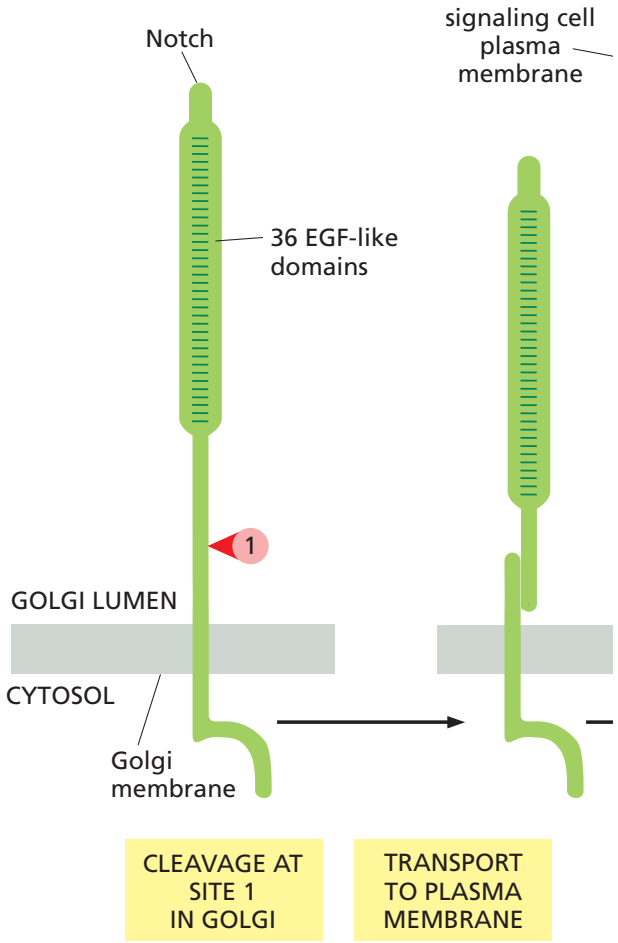
WNT = Wingless (Wg) + Integration-1 (Int)

Hedgehog = Larva looking like a hedgehog (!)



NOTCH SIGNALING

The processing and activation of Notch by proteolytic cleavage

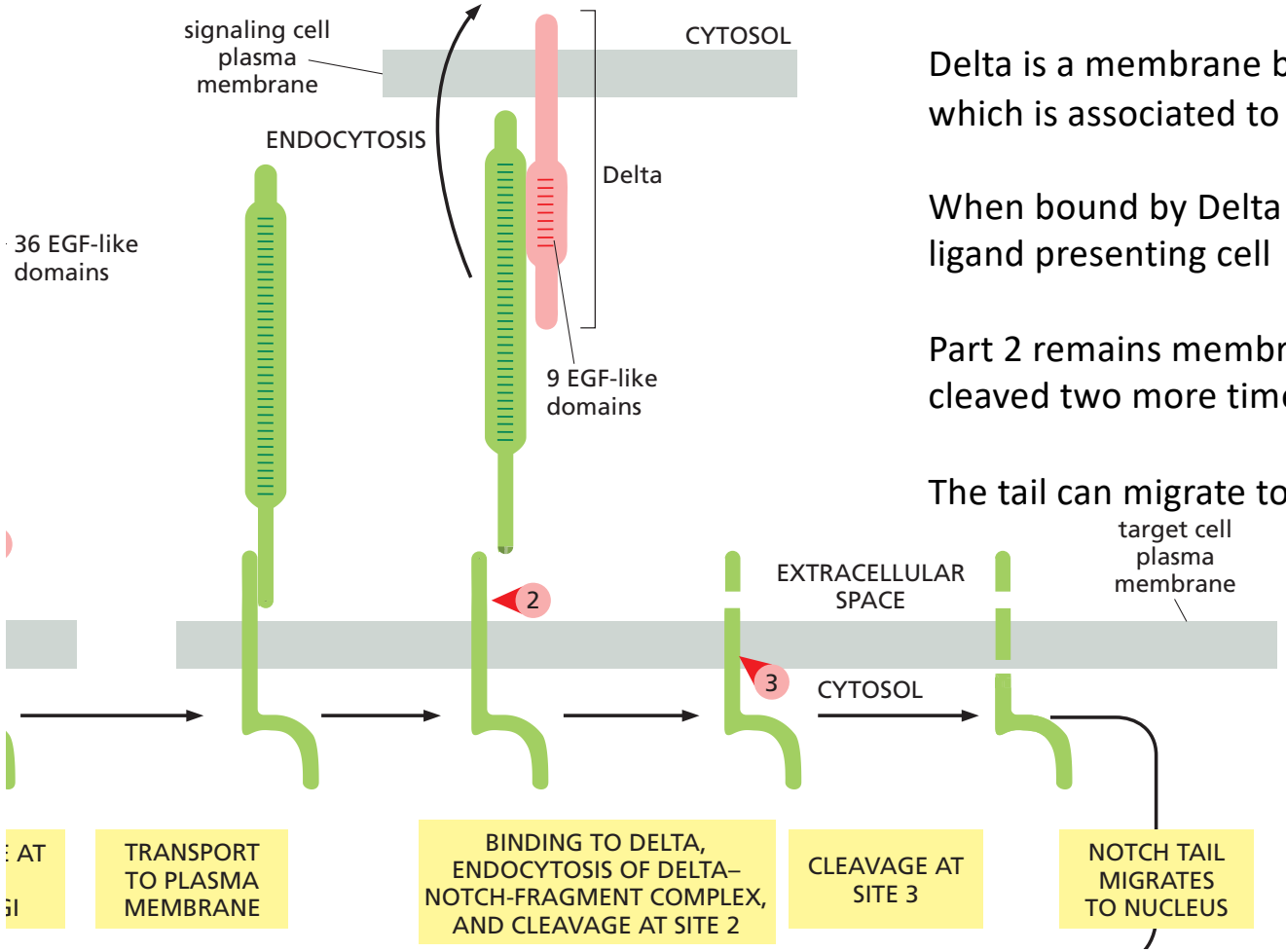


The Notch receptor is first cleaved in the Golgi

The two cleaved parts will still associate with each other

This receptor is transported to the plasma membrane

The processing and activation of Notch by proteolytic cleavage



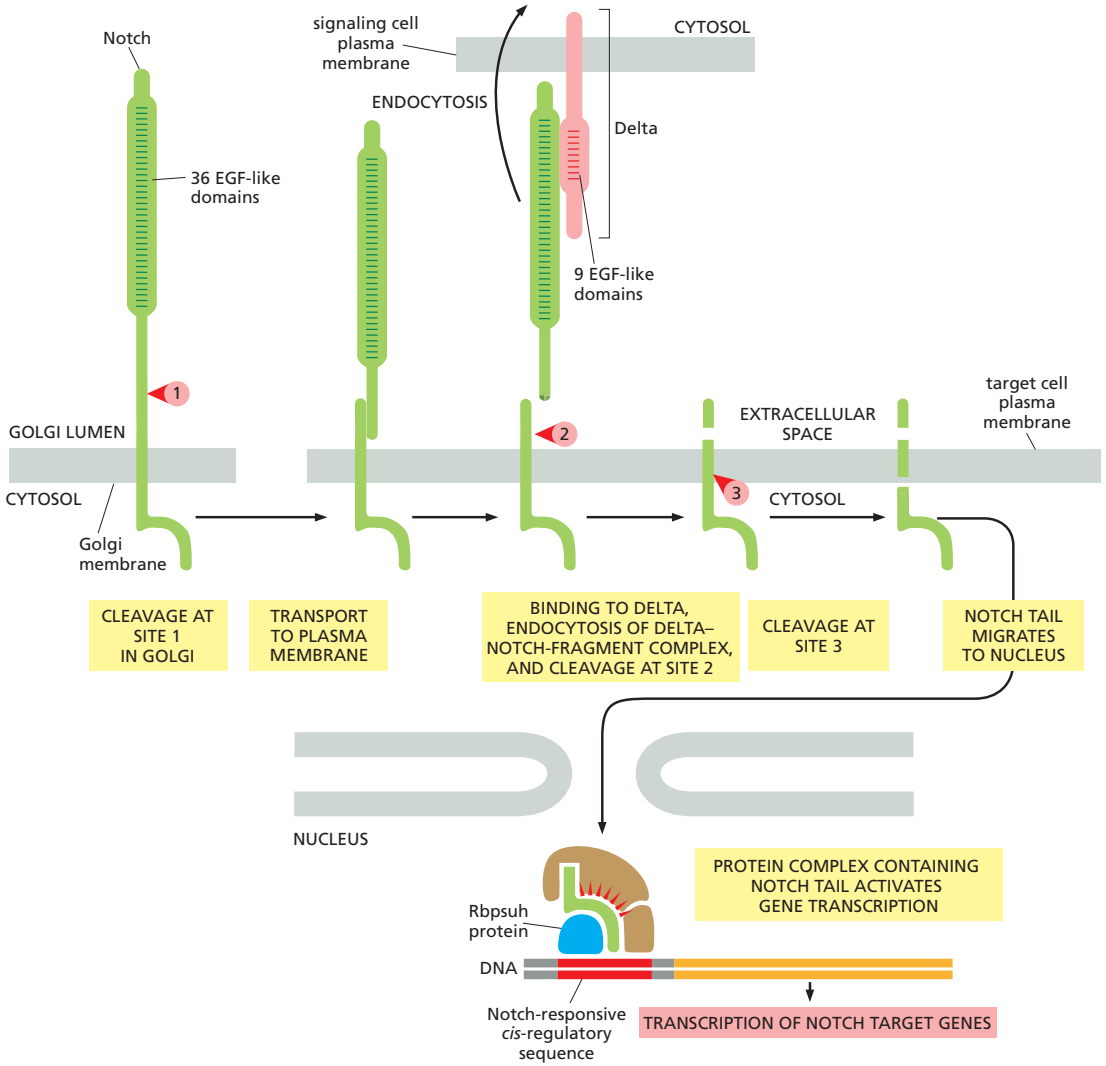
Delta is a membrane bound ligand for notch, which is associated to the membrane.

When bound by Delta Part 1 is taken up by the ligand presenting cell

Part 2 remains membrane bound and is cleaved two more times

The tail can migrate to the nucleus

The processing and activation of Notch by proteolytic cleavage



In the nucleus the tail end or notch intracellular domain (NICD) can associate with new proteins (Rbpsuh) and form a transcriptional complex

In the absence of NICD Rbpsuh is a transcriptional repressor

In the presence an activator!

The processing and activation of Notch by proteolytic cleavage

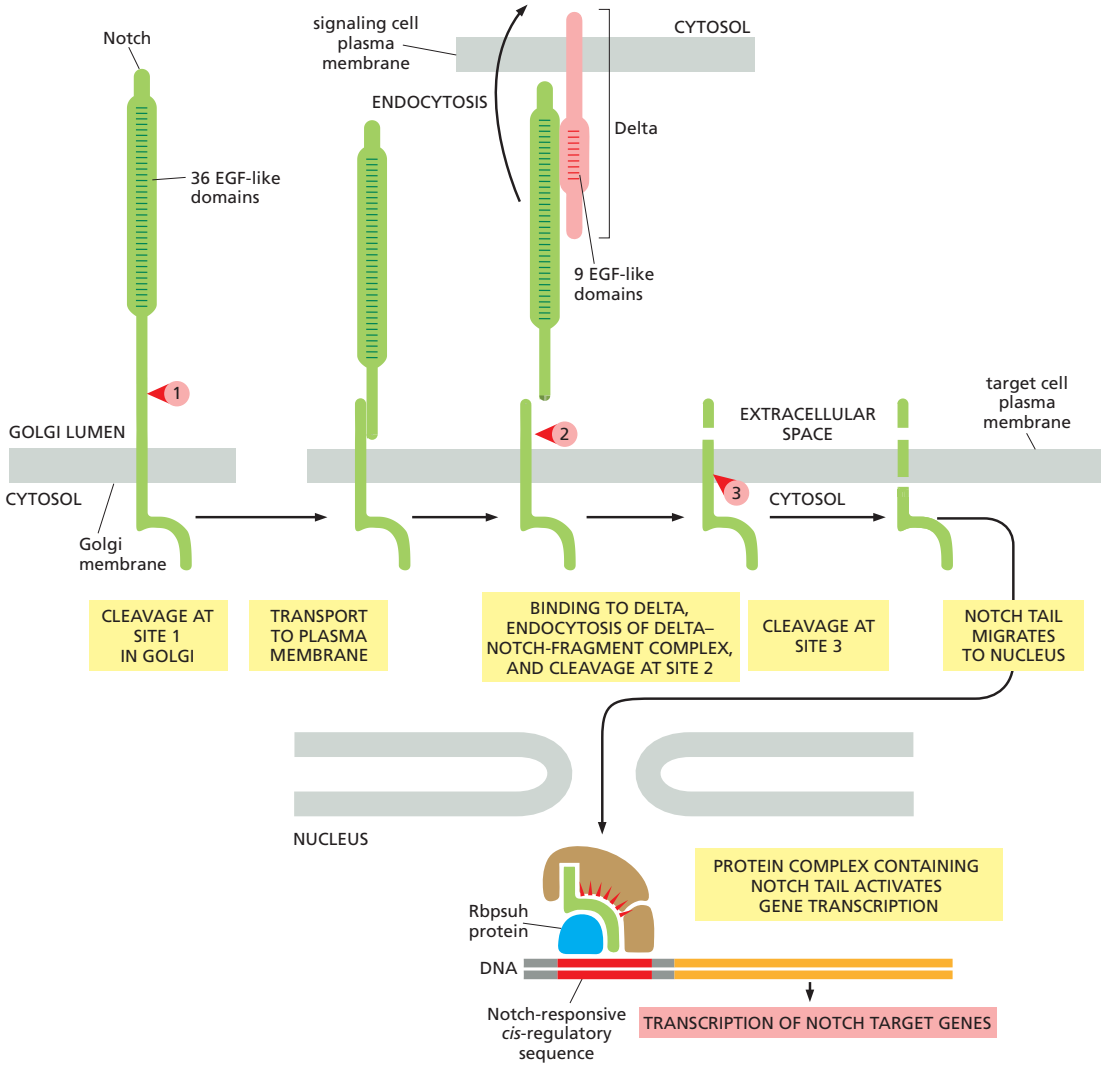


Figure 15–59 The processing and activation of Notch by proteolytic cleavage. The *numbered red arrowheads* indicate the sites of proteolytic cleavage. The first proteolytic processing step occurs within the *trans* Golgi network to generate the mature heterodimeric Notch receptor that is then displayed on the cell surface. The binding to Delta on a neighboring cell triggers the next two proteolytic steps: the complex of Delta and the Notch fragment to which it is bound is endocytosed by the Delta-expressing cell, exposing the extracellular cleavage site in the transmembrane Notch subunit. Note that Notch and Delta interact through their repeated EGF-like domains. The released Notch tail migrates into the nucleus, where it binds to the Rbpsuh protein, which it converts from a transcriptional repressor to a transcriptional activator.

Lateral inhibition mediated by Notch and Delta during neural cell development in *Drosophila*

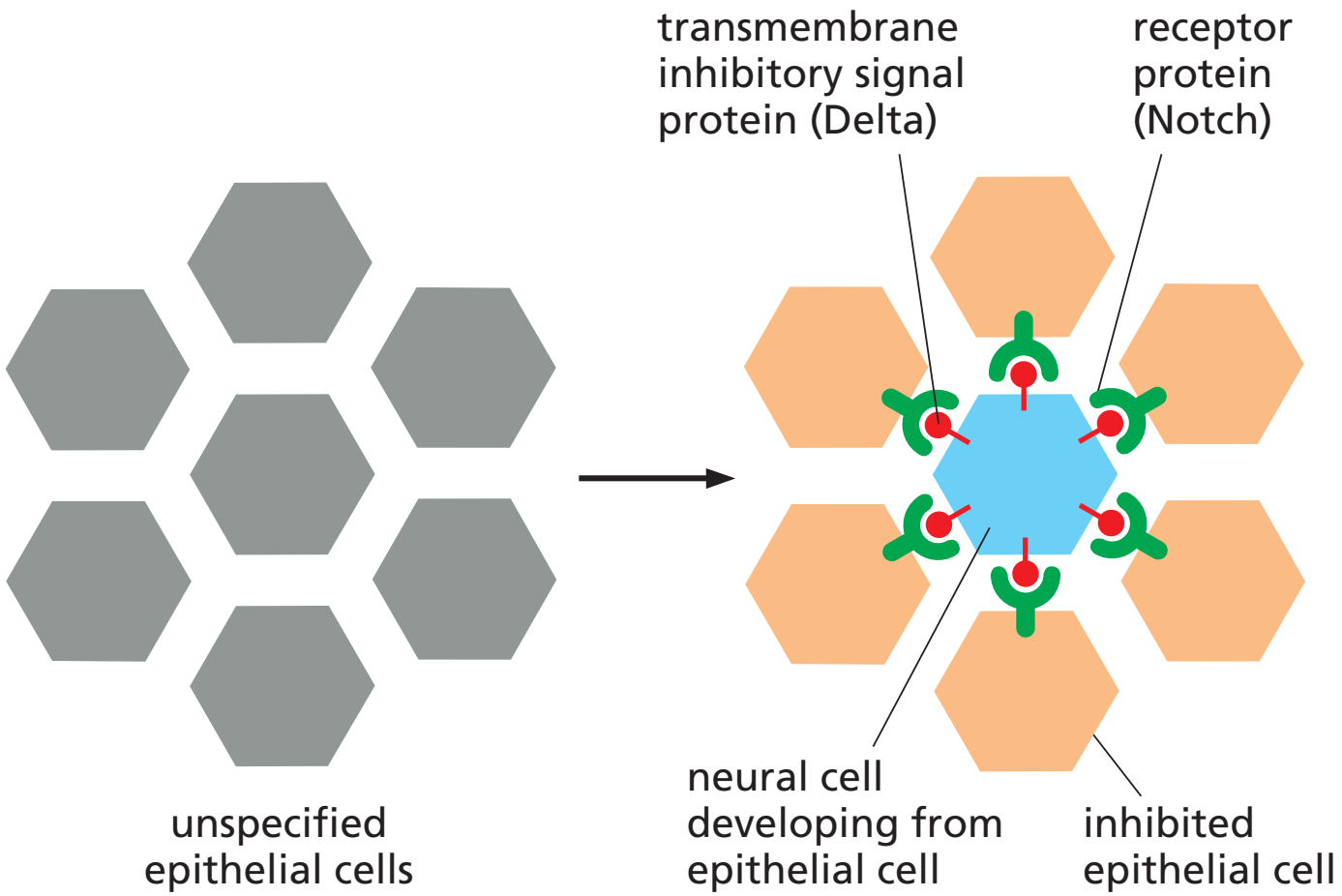
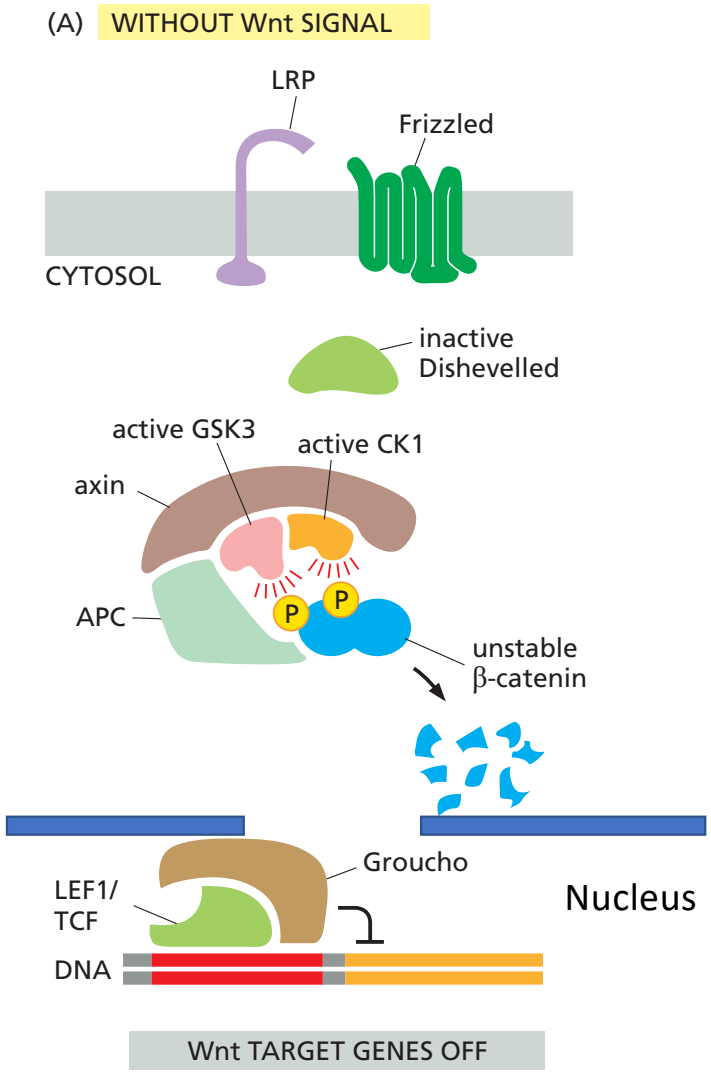


Figure 15–58 Lateral inhibition mediated by Notch and Delta during neural cell development in *Drosophila*. When individual cells in the epithelium begin to develop as neural cells, they signal to their neighbors not to do the same. This inhibitory, contact-dependent signaling is mediated by the ligand Delta, which appears on the surface of the future neural cell and binds to Notch receptor proteins on the neighboring cells. In many tissues, all the cells in a cluster initially express both Delta and Notch, and a competition occurs, with one cell emerging as winner, expressing Delta strongly and inhibiting its neighbors from doing likewise. In other cases, additional factors interact with Delta or Notch to make some cells susceptible to the lateral inhibition signal and others unresponsive to it.

WNT SIGNALING

The Wnt/β-catenin signaling pathway

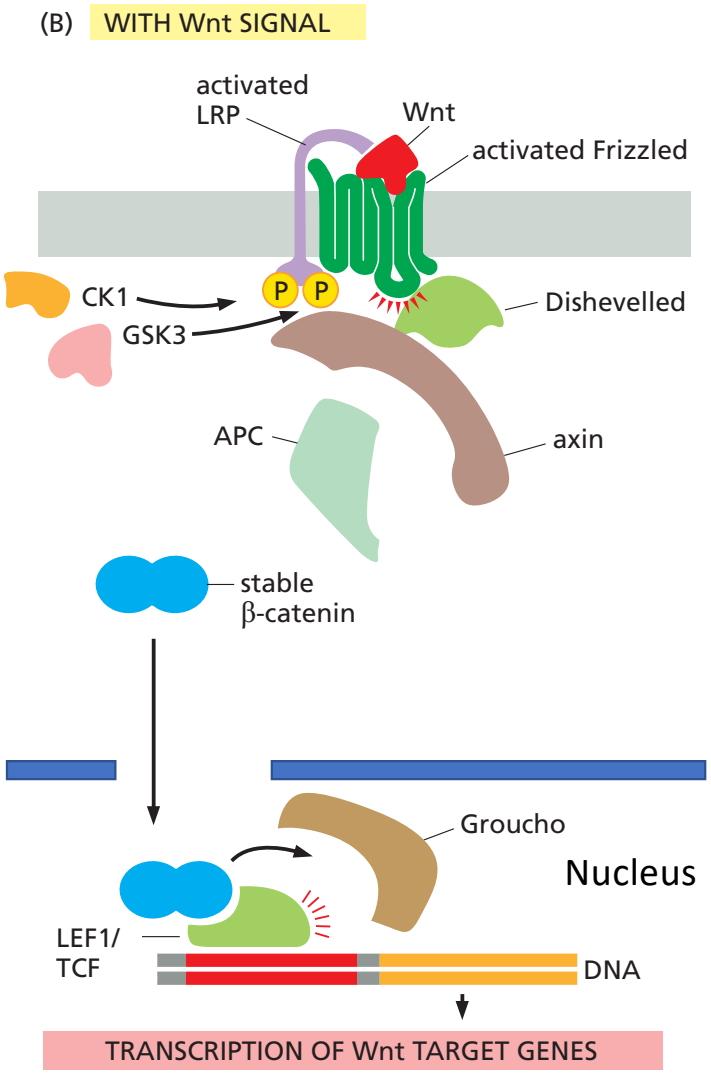


WNT off

B-Catenin is degraded

TCF or LEF1 forms a transcriptional repressor with Groucho

The Wnt/β-catenin signaling pathway



WNT on

B-Catenin is no longer degraded

B-Catenin goes to the nucleus

B-Catenin displaced Groucho and forms a transcriptional activator with TCF or LEF1

The Wnt/β-catenin signaling pathway

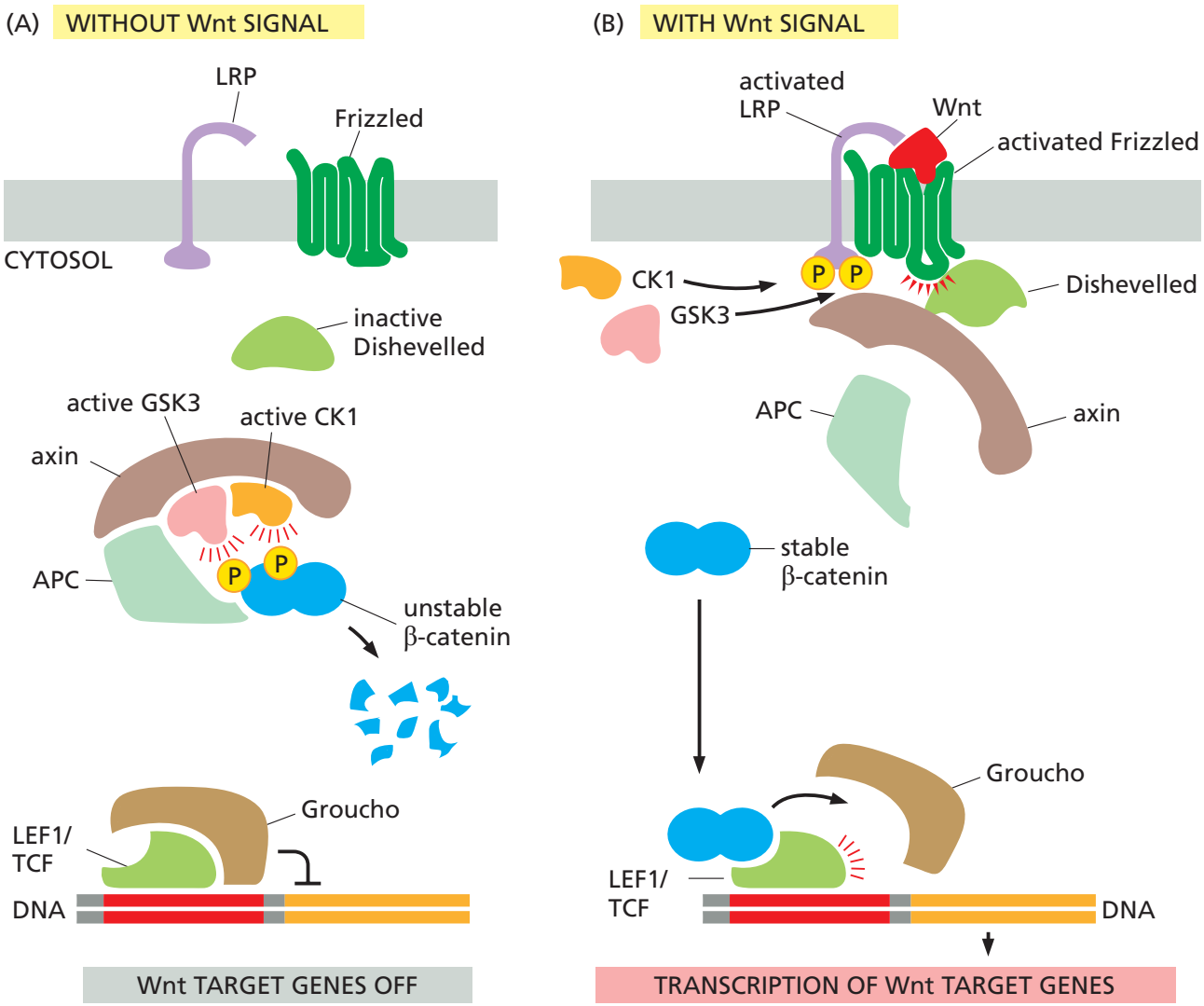
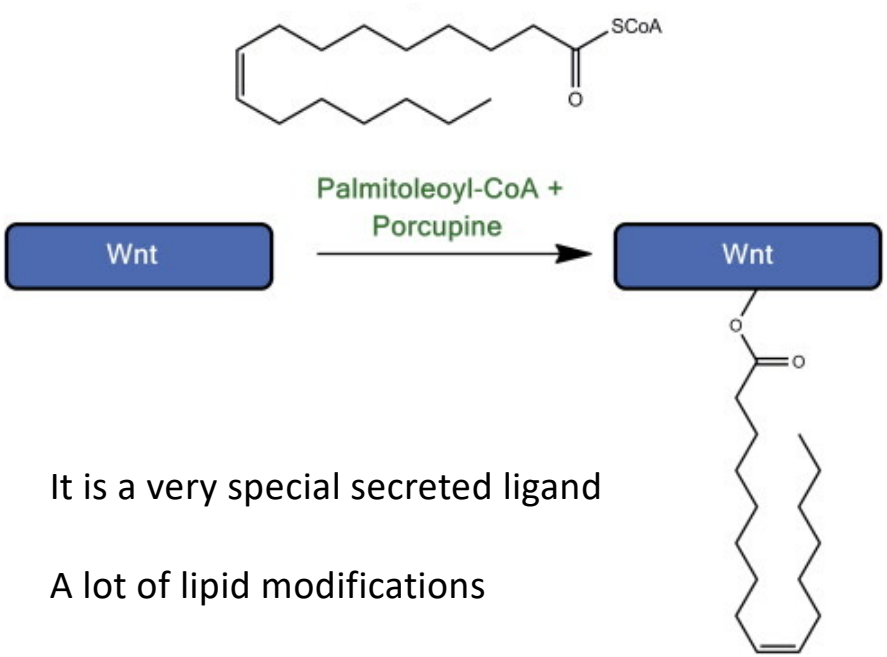


Figure 15–60 The Wnt/β-catenin signaling pathway. (A) In the absence of a Wnt signal, β-catenin that is not bound to cell–cell adherens junctions (not shown) interacts with a degradation complex containing APC, axin, GSK3, and CK1. In this complex, β-catenin is phosphorylated by CK1 and then by GSK3, triggering its ubiquitylation and degradation in proteasomes. Wnt-responsive genes are kept inactive by the Groucho co-repressor protein bound to the transcription regulator LEF1/TCF. (B) Wnt binding to Frizzled and LRP clusters the two co-receptors together, and the cytosolic tail of LRP is phosphorylated by GSK3 and then by CK1. Axin binds to the phosphorylated LRP and is inactivated and/or degraded, resulting in disassembly of the degradation complex. The phosphorylation of β-catenin is thereby prevented, and unphosphorylated β-catenin accumulates and translocates to the nucleus, where it binds to LEF1/TCF, displaces the co-repressor Groucho, and acts as a coactivator to stimulate the transcription of Wnt target genes. The scaffold protein Dishevelled is required for the signaling pathway to operate; it binds to Frizzled and becomes phosphorylated (not shown), but its precise role is unknown.

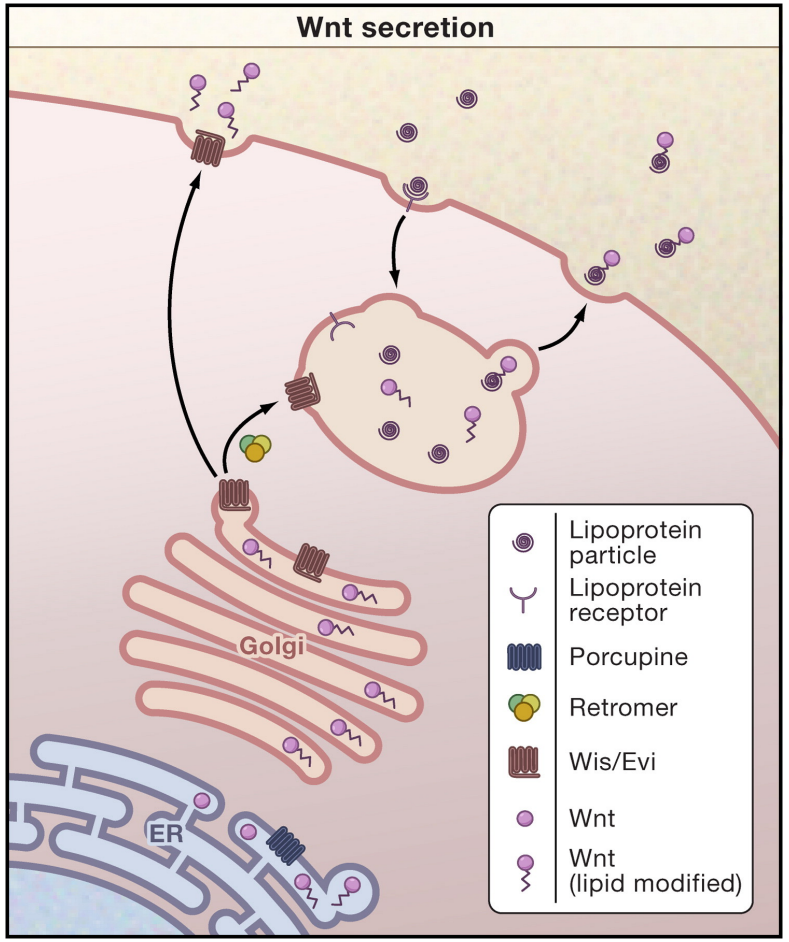
The Wnt ligand



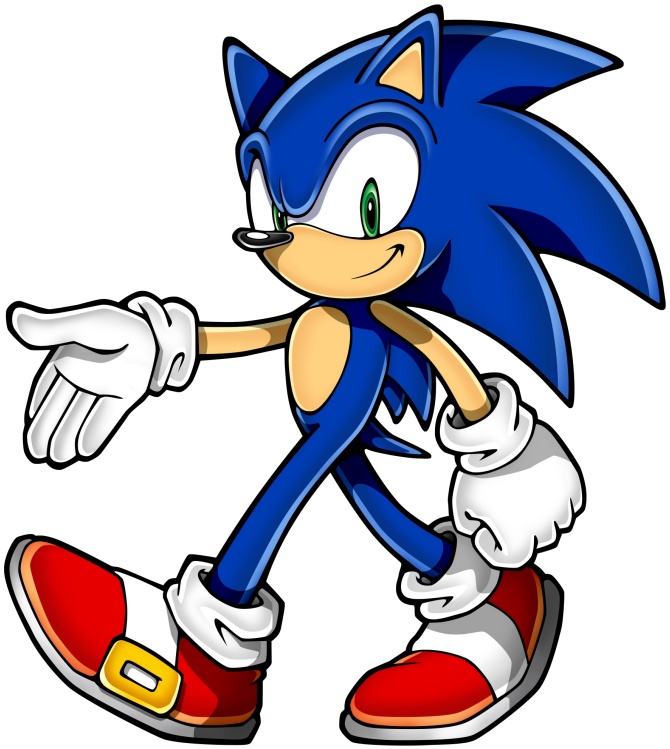
It is a very special secreted ligand

A lot of lipid modifications

It associates to the plasma membrane and does not travel far!

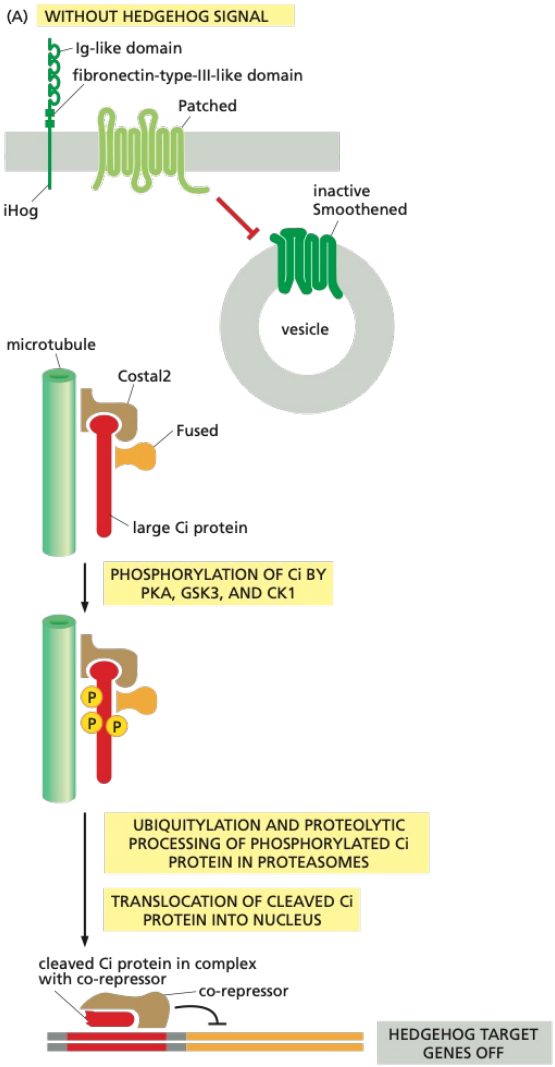


HEDGEHOG SIGNALING



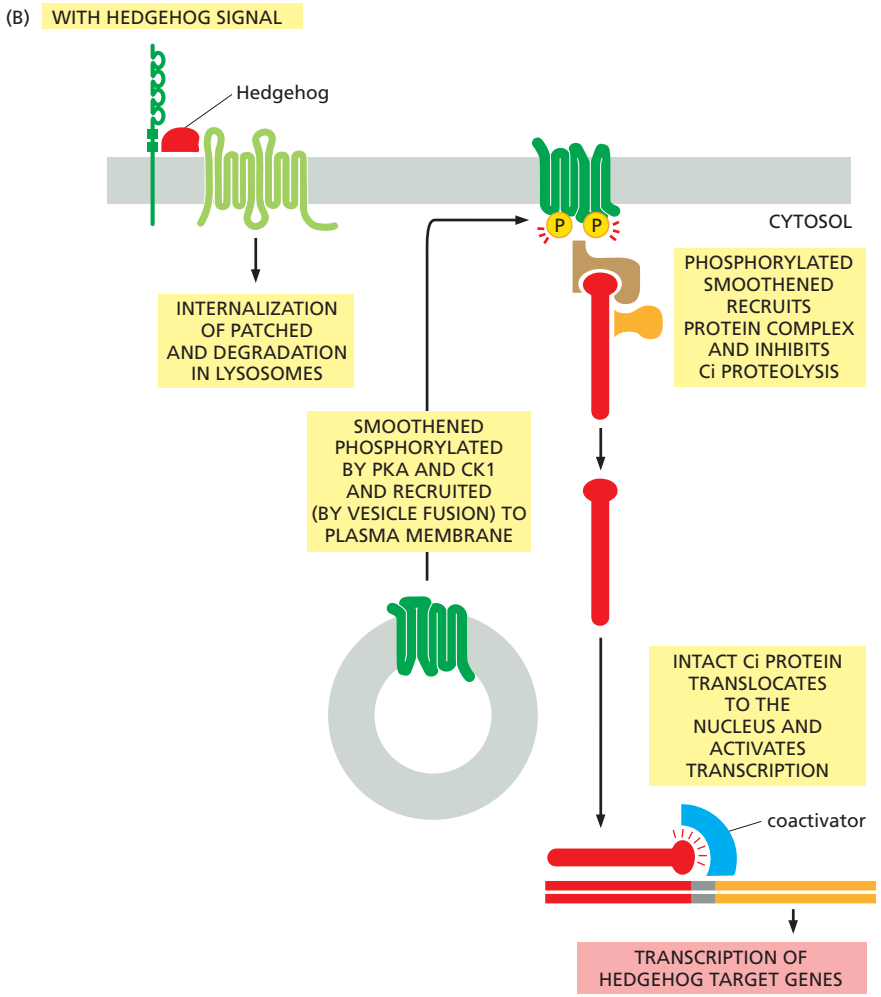
Indian HH
Desert HH
Sonic HH

Hedgehog signaling in *Drosophila*



In absence of a ligand Ci protein is cleaved and a truncated version represses Hedgehog target genes

Hedgehog signaling in *Drosophila*



In presence of a ligand Ci protein is no longer cleaved and the full version activates Hedgehog target genes

Hedgehog signaling in *Drosophila*

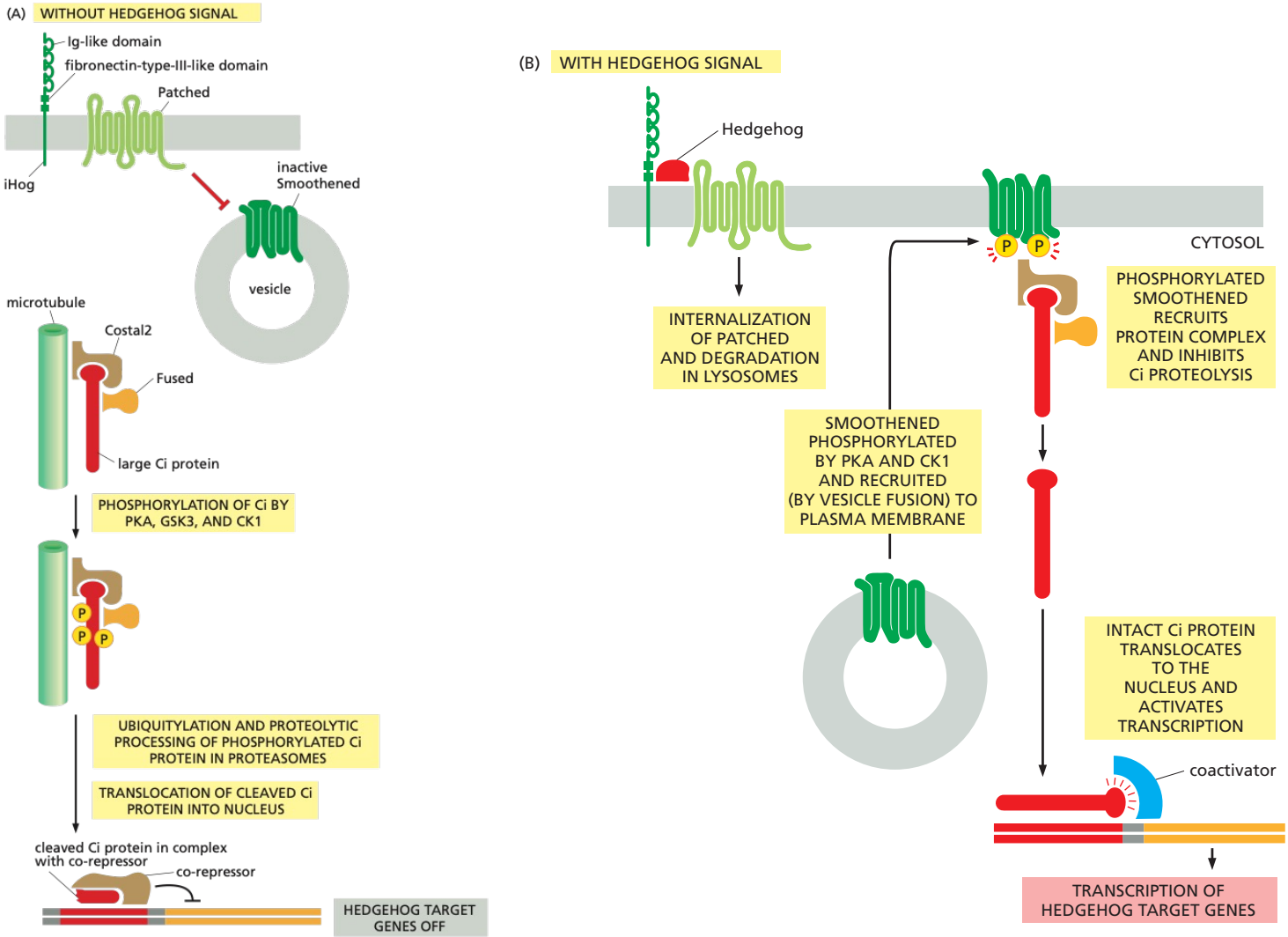


Figure 15–61 Hedgehog signaling in *Drosophila*. (A) In the absence of Hedgehog, most Patched is in intracellular vesicles (not shown), where it keeps Smoothened inactive and sequestered. The Ci protein is bound in a cytosolic protein degradation complex, which includes the protein kinase Fused and the scaffold protein Costal2. Costal2 recruits three other protein kinases (PKA, GSK3, and CK1; not shown), which phosphorylate Ci. Phosphorylated Ci is ubiquitylated and then cleaved in proteasomes (not shown) to form a transcriptional repressor, which accumulates in the nucleus to help keep Hedgehog target genes inactive. (B) Hedgehog binding to iHog and Patched removes the inhibition of Smoothened by Patched. Smoothened is phosphorylated by PKA and CK1 and translocates to the plasma membrane, where it recruits the complex containing Fused, Costal2, and Ci. Costal2 releases unprocessed Ci, which accumulates in the nucleus and activates the transcription of Hedgehog target genes. Many details in the pathway are poorly understood, including the role of Fused.

NFKB SIGNALING

The activation of the NFκb pathway

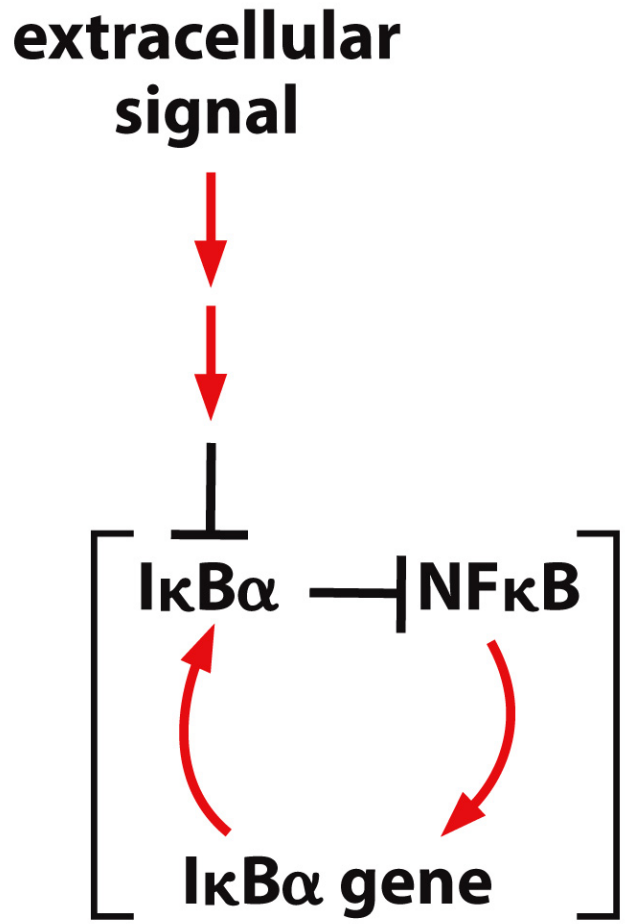


Figure 15-63a Molecular Biology of the Cell 6e (© Garland Science 2015)

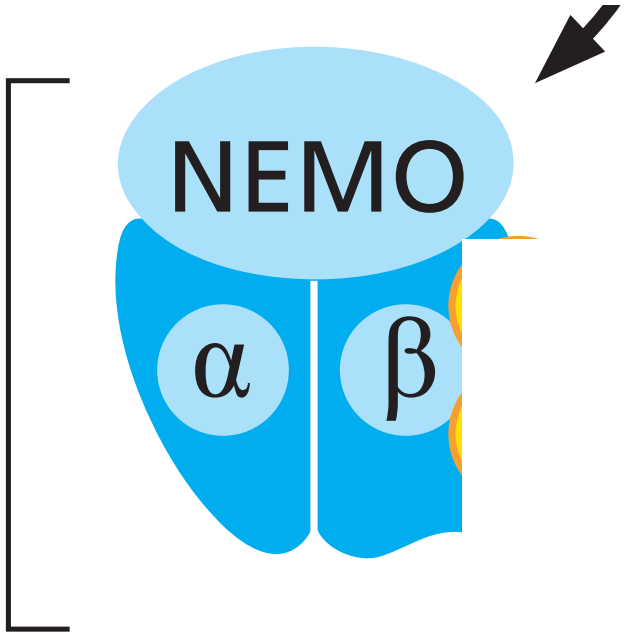
negative feedback loop

NF-kB can be activated by many signals

A lot of them are inflammatory signals (Virus infection, Bacteria, and immune cells secreting ligands)

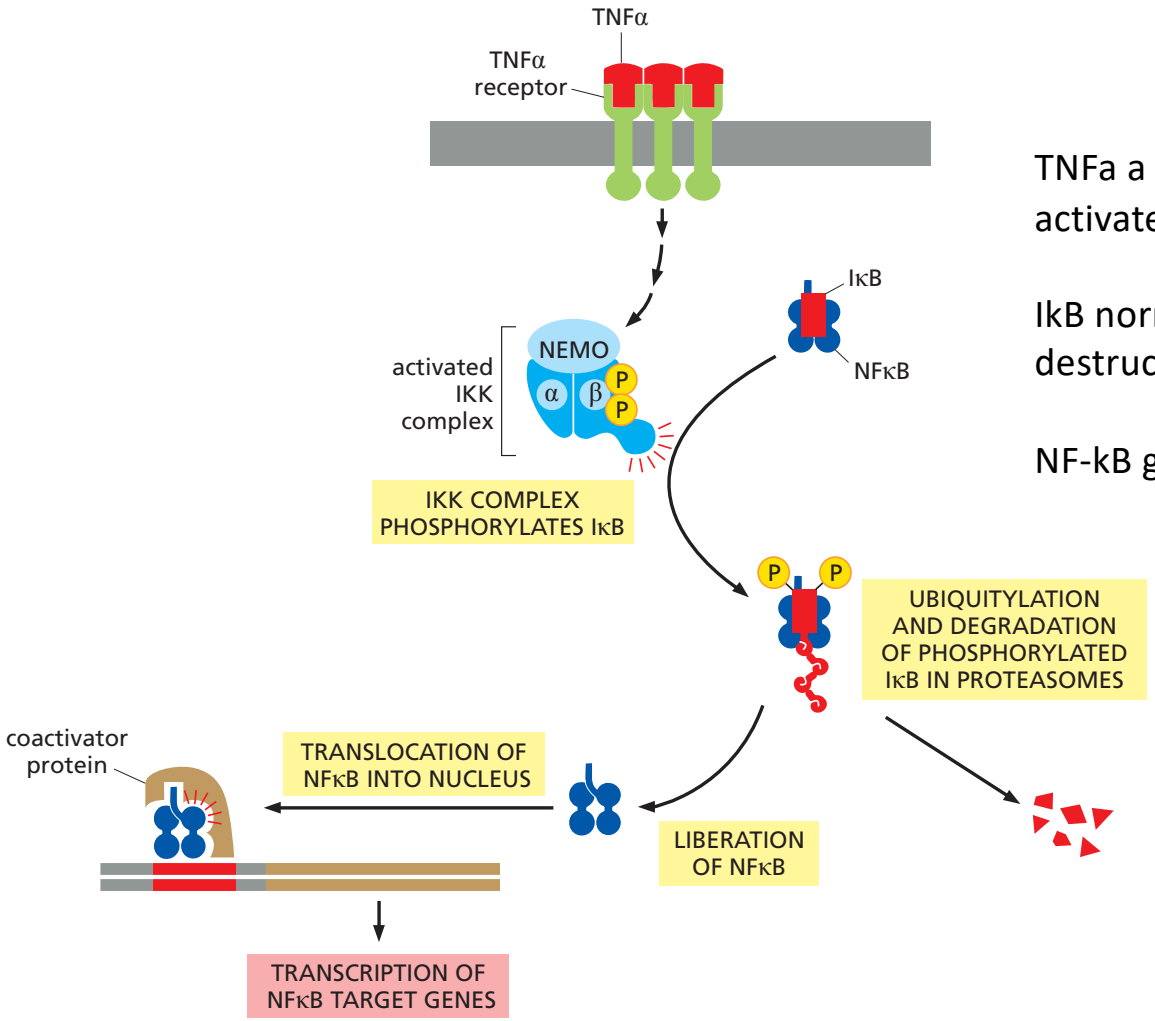
The activation of the NFκb pathway by TNFα

Inactive IKK complex



This complex is in the cytosol and waiting to be activated

The activation of the NFκb pathway by TNFα



TNFa a pro-inflammatory ligand binds and the IKK complex is activated

IkB normally keeps NF-kB inactive, but is now targeted for destruction

NF-kB goes to the nucleus and activates transcription

The activation of the NFκb pathway by TNFα

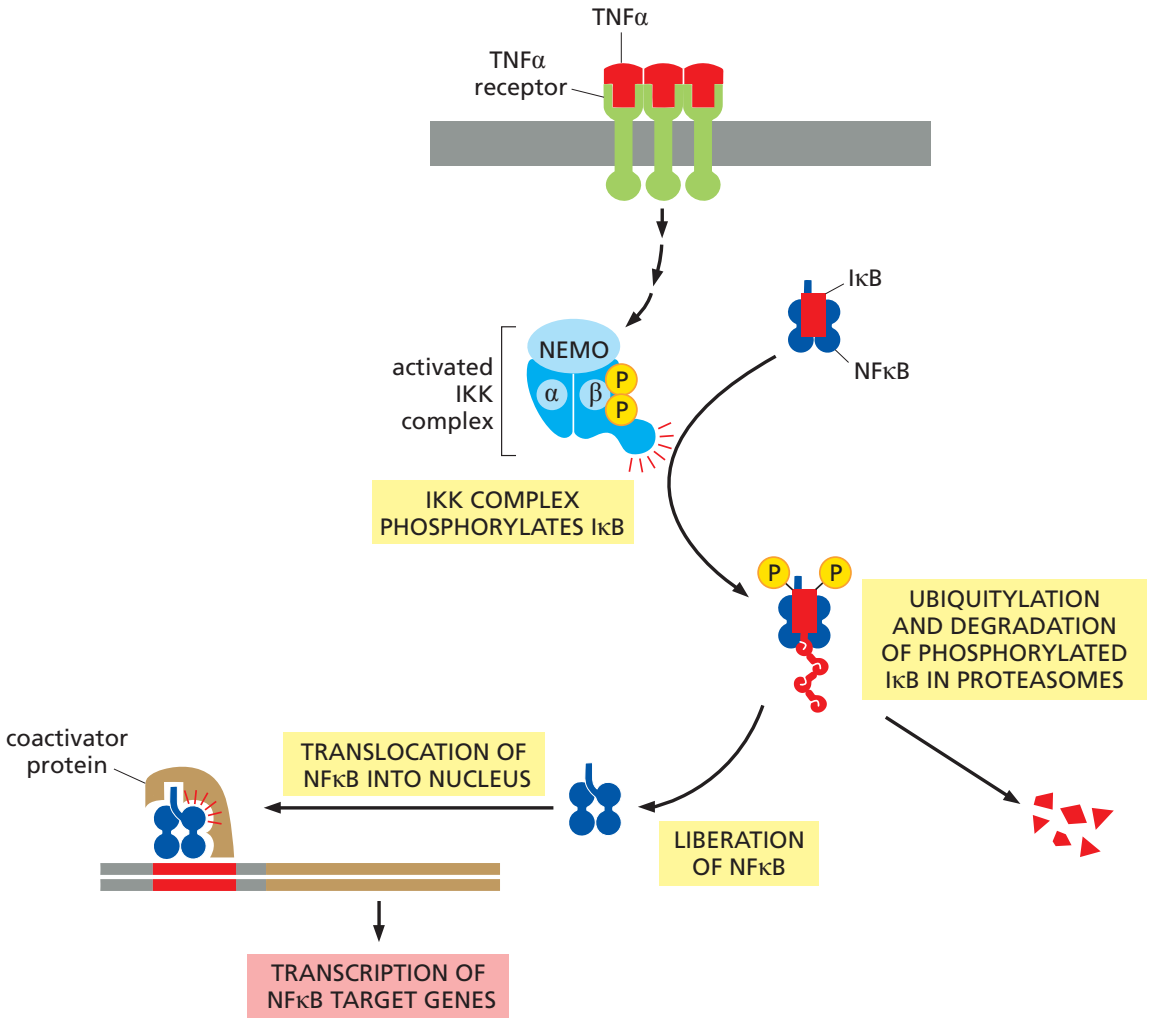
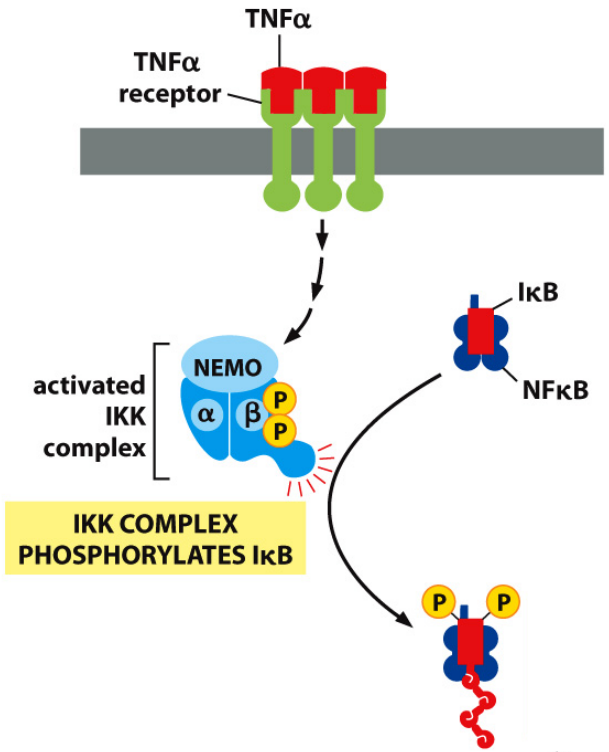
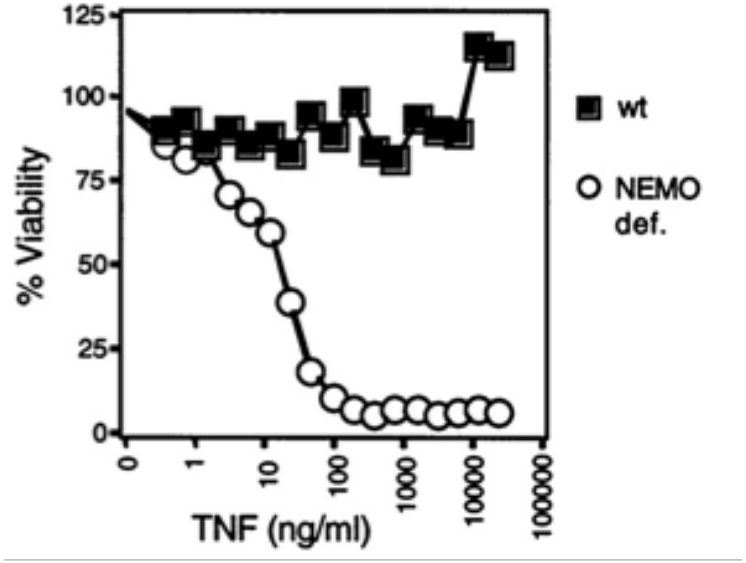


Figure 15–62 The activation of the NFκB pathway by TNFα. Both TNFα and its receptors are trimers. The binding of TNFα causes a rearrangement of the clustered cytosolic tails of the receptors, which now recruit various signaling proteins, resulting in the activation of a protein kinase that phosphorylates and activates IκB kinase kinase (IKK). IKK is a heterotrimer composed of two kinase subunits (IKKα and IKKβ) and a regulatory subunit called NEMO. IKKβ then phosphorylates IκB on two serines, which marks the protein for ubiquitylation and degradation in proteasomes. The released NFκB translocates into the nucleus, where, in collaboration with coactivator proteins, it stimulates the transcription of its target genes.

In NEMO-deficient cells, TNF triggers cell death





Micheau O et al. Mol. Cell. Biol. 2001; doi:10.1128/MCB.21.16.5299-5305.2001

Negative feedback in the NFκb signaling pathway induces oscillations in NFκb activation

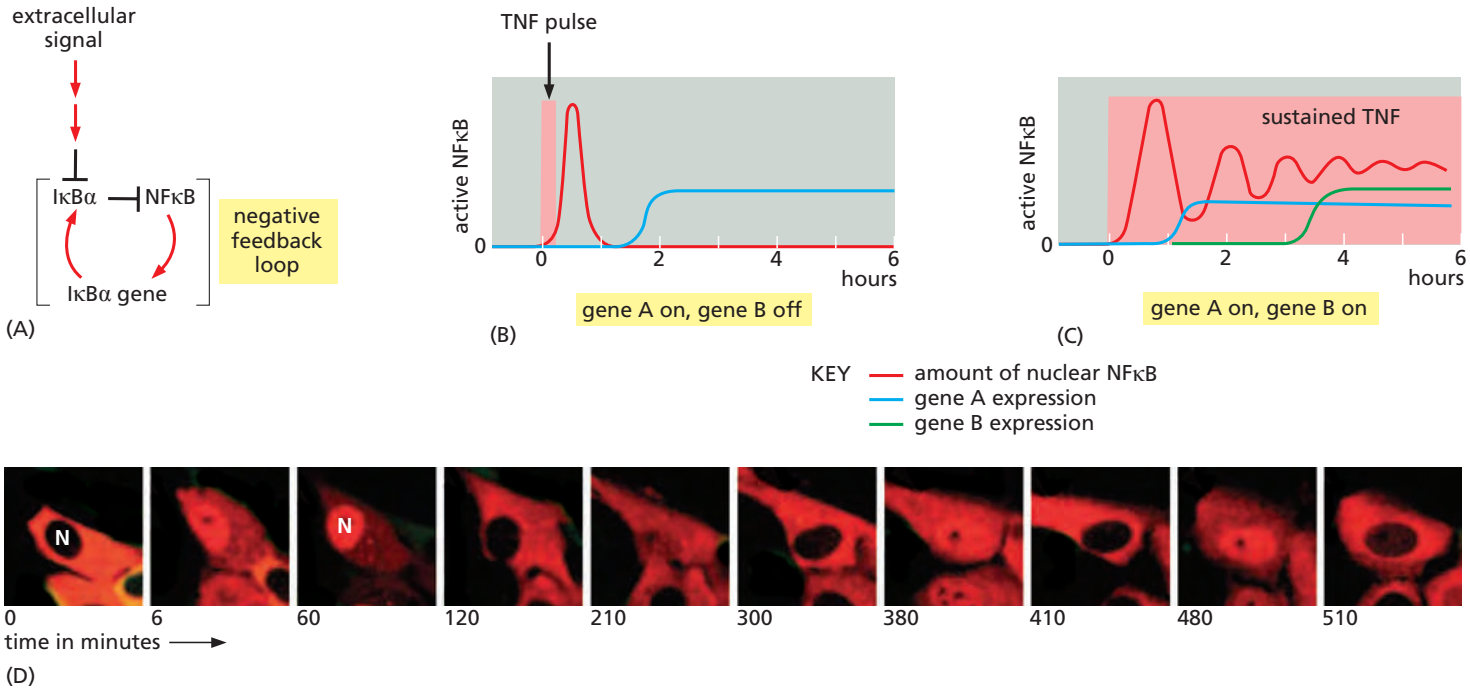


Figure 15–63 Negative feedback in the NFκB signaling pathway induces oscillations in NFκB activation. (A) Drawing showing how activated NFκB stimulates the transcription of the IκBα gene, the protein product of which acts back in the cytoplasm to sequester and inhibit NFκB there; if the stimulus is persistent, the newly made IκBα protein will then be ubiquitylated and degraded, liberating active NFκB again so that it can return to the nucleus and activate transcription (see Figure 15–62). (B) A short exposure to TNFα produces a single, short pulse of NFκB activation, beginning within minutes and ending by 1 hour. This response turns on the transcription of gene A but not gene B. (C) A sustained exposure to TNFα for the entire 6 hours of the experiment produces oscillations in NFκB activation that damp down over time. This response turns on the transcription of both genes; gene B turns on only after several hours, indicating that gene B transcription requires prolonged activation of NFκB, for reasons that are not understood. (D) These time-lapse confocal fluorescence micrographs from a different study of TNFα stimulation show the oscillations of NFκB in a cultured cell, as indicated by its periodic movement into the nucleus (N) of a fusion protein composed of NFκB fused to a red fluorescent protein. In the cell at the center of the micrographs, NFκB is active and in the nucleus at 6, 60, 210, 380, and 480 minutes, but it is exclusively in the cytoplasm at 0, 120, 300, 410, and 510 minutes. (A–C, based on data from A. Hoffmann et al., *Science* 298:1241–1245, 2002, and adapted from A.Y. Ting and D. Endy, *Science* 298:1189–1190, 2002; D, from D.E. Nelson et al., *Science* 306:704–708, 2004. All with permission from AAAS.)

NUCLEAR RECEPTOR SIGNALING

Some signal molecules that bind to intracellular receptors

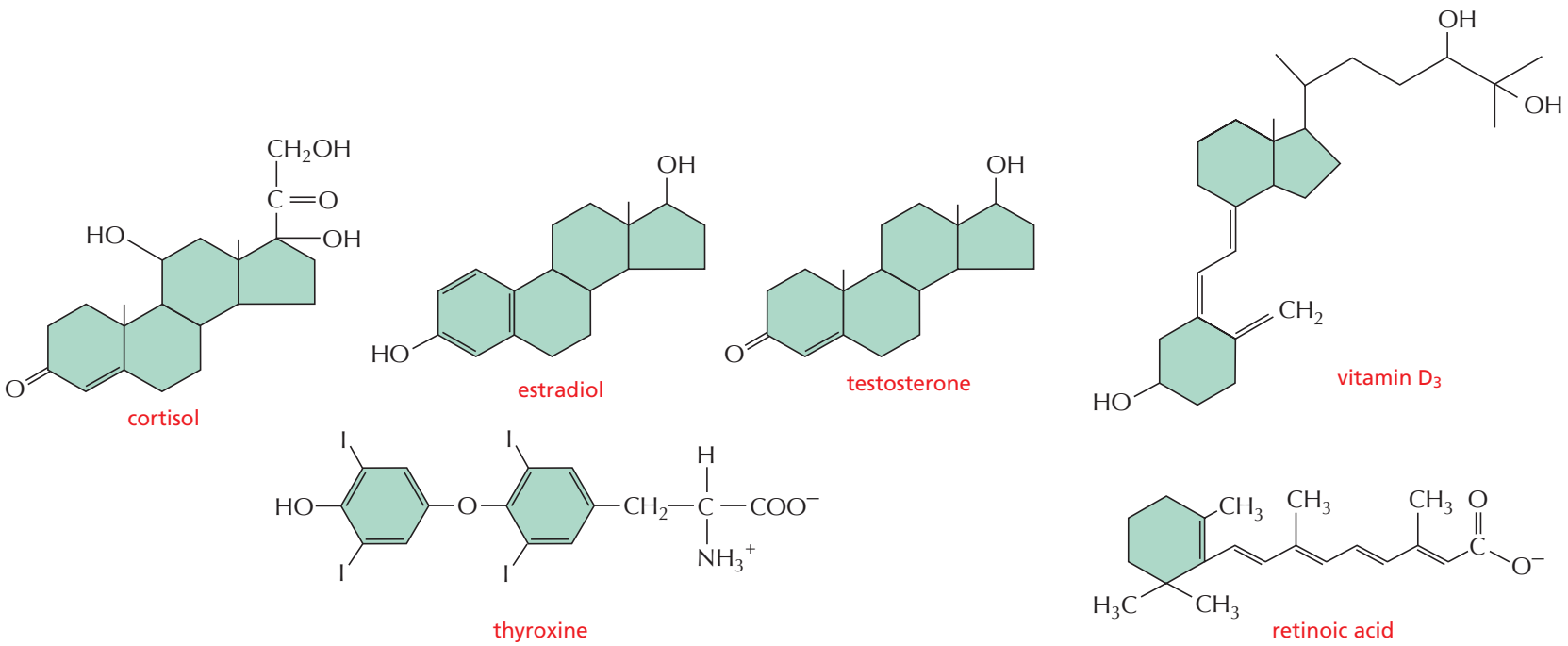


Figure 15–64 Some signal molecules that bind to intracellular receptors. Note that all of them are small and hydrophobic. The active, hydroxylated form of vitamin D_3 is shown. Estradiol and testosterone are steroid sex hormones.

The activation of nuclear receptors

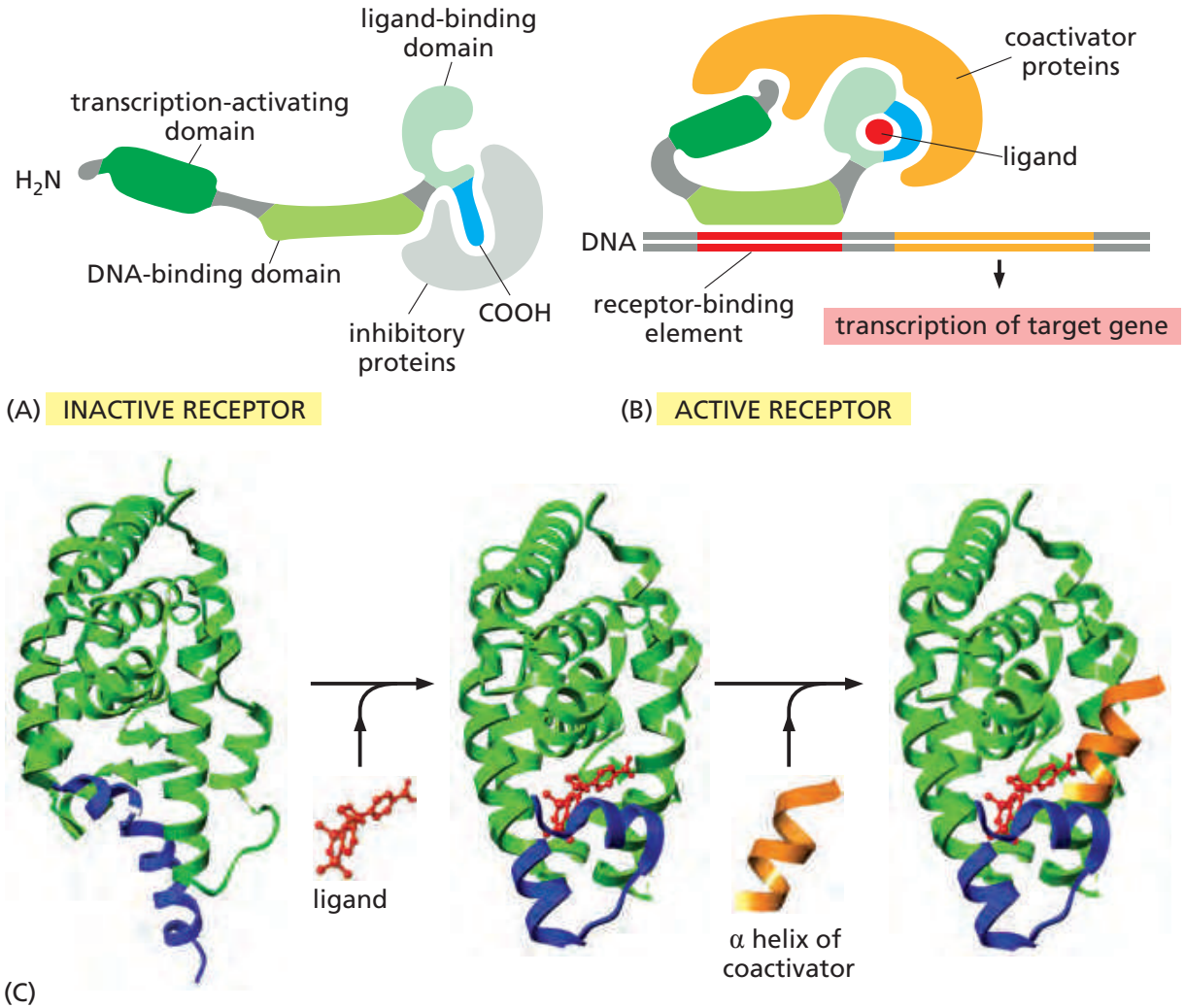
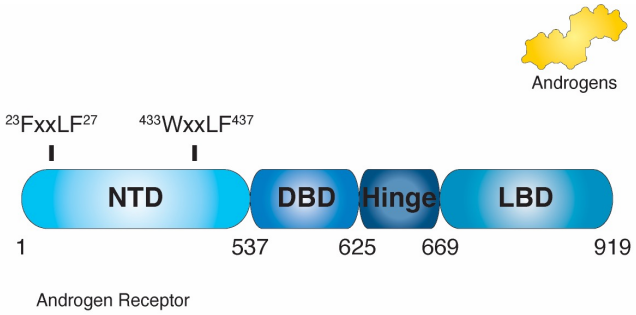
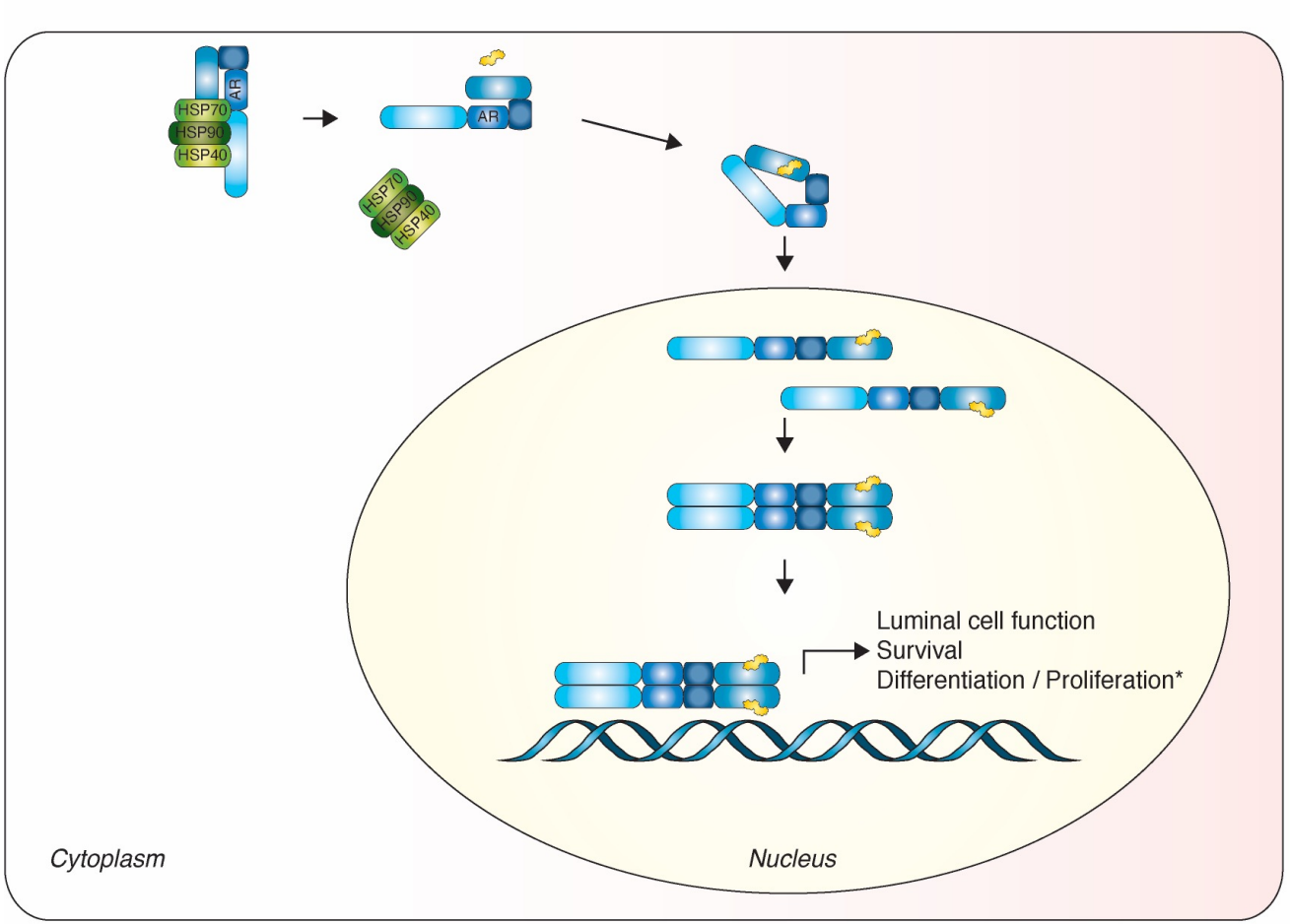


Figure 15–65 The activation of nuclear receptors. All nuclear receptors bind to DNA as either homodimers or heterodimers, but for simplicity we show them as monomers. (A) The receptors all have a related structure, which includes three major domains, as shown. An inactive receptor is bound to inhibitory proteins. (B) Typically, the binding of ligand to the receptor causes the ligand-binding domain of the receptor to clamp shut around the ligand, the inhibitory proteins to dissociate, and coactivator proteins to bind to the receptor's transcription-activating domain, thereby increasing gene transcription. In other cases, ligand binding has the opposite effect, causing co-repressor proteins to bind to the receptor, thereby decreasing transcription (not shown). (C) The structure of the ligand-binding domain of the retinoic acid receptor is shown in the absence *(left)* and presence *(middle)* of ligand (shown in *red*). When ligand binds, the *blue* α helix acts as a lid that snaps shut, trapping the ligand in place. The shift in the conformation of the receptor upon ligand binding also creates a binding site for a small α helix *(orange)* on the surface of coactivator proteins. (PDB codes: 1LBD, 2ZYO, and 2ZXZ.)

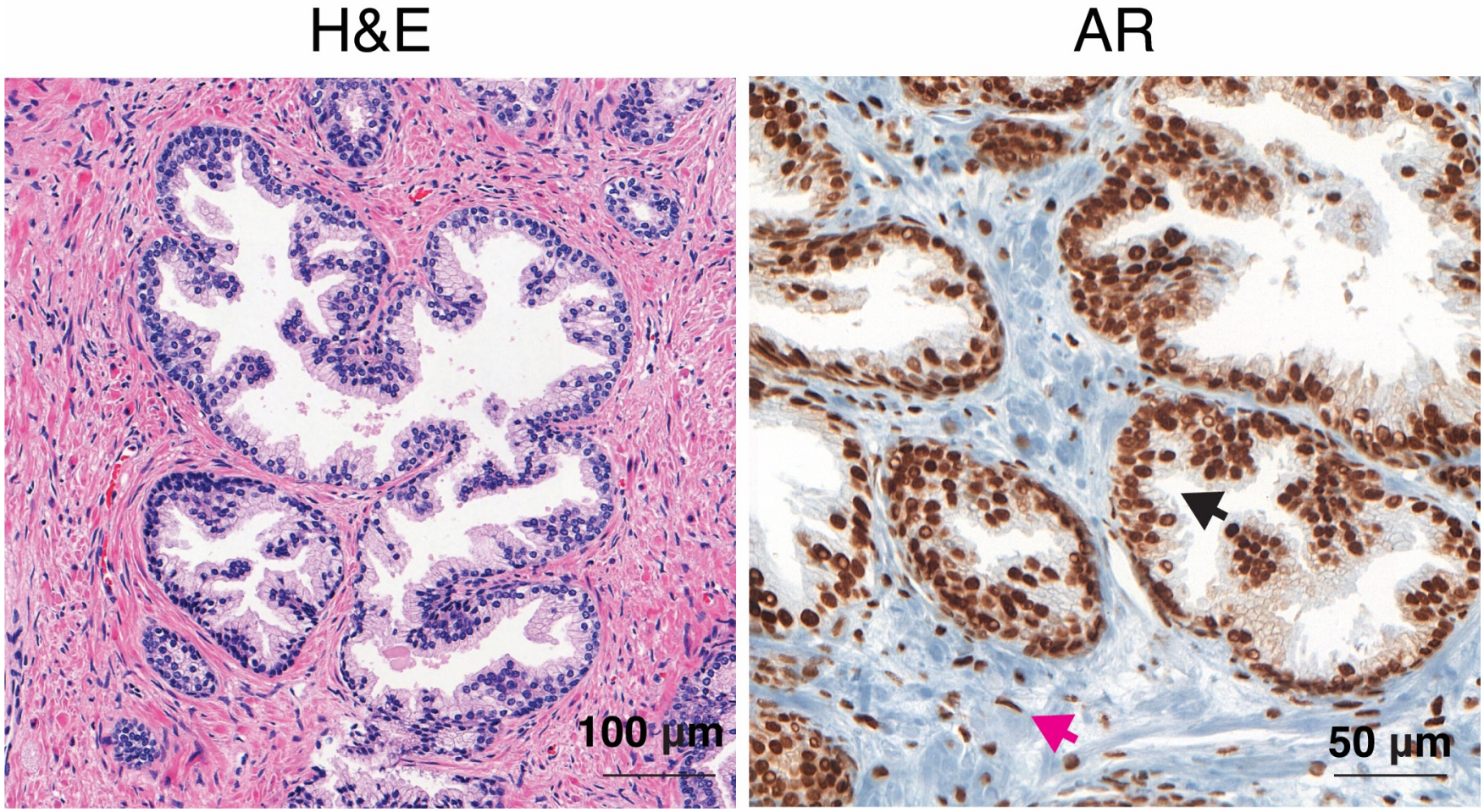
AR signaling is reprogrammed in prostate cancer





Benign Luminal cell

AR is expressed in the luminal cells and mesenchyme of the prostate



Signaling key points week 3

- Concept of short or long-distance communication
- Differences in the speed of cell response to a stimulus
- Mechanisms of signaling activation at the plasma membrane
- Comprehend the importance of protein domains in signal transduction
- Describe primary and secondary responses, and feedback mechanisms
- GPCR signaling and downstream signaling events
- RTK signaling and downstream signaling events
- RTK-Associated and downstream signaling events
- Main signaling pathways with regulated proteolysis (Notch, WNT, Hedgehog, Nf-KB)
- Understand there is a lot of overlap in downstream signaling targets
- Understand how mutations can hyperactivate signaling