Alternative Signaling Routes in Genes Regulation

Hedgehog Proteins Bind to Patched, Relieving Its Inhibition of Smoothened

✓ Hedgehog proteins and Wnt proteins act in similar ways:

-Both are secreted signal molecules, which act as **local mediators** and **morphogens** in many developing invertebrate and vertebrate tissues.
-Both proteins are modified by covalently attached lipids, depend on secreted or cell-surface-bound heparan sulfate proteoglycans for their action, and activate latent transcription regulators by inhibiting their degradation.
-They both trigger a switch from transcriptional repression to transcriptional activation, and excessive signaling along either pathway in adult cells can lead to **cancer**.
-They even use some of the same intracellular signaling proteins and sometimes collaborate to mediate a response.

- The Hedgehog proteins were discovered in Drosophila, where this protein family has only one member.
- Mutation of the Hedgehog gene produces a larva covered with **spiky processes (denticles)**, like a **hedgehog**.
- At least three genes encode Hedgehog proteins in vertebrates—Sonic, Desert, and Indian hedgehog.
- The active forms of all Hedgehog proteins are covalently coupled to cholesterol, as well as to a fatty acid chain.
- The cholesterol is added during an unusual processing step, in which a precursor protein cleaves itself to produce a smaller, cholesterol-containing signal protein.
- Most of what we know about the Hedgehog signaling pathway came initially from genetic studies in flies, and it is the fly pathway that we summarize here.
- The effects of Hedgehog are mediated by a latent transcription regulator called **Cubitus interruptus (Ci)**, the regulation of which is reminiscent of the regulation of β-catenin by Wnts.

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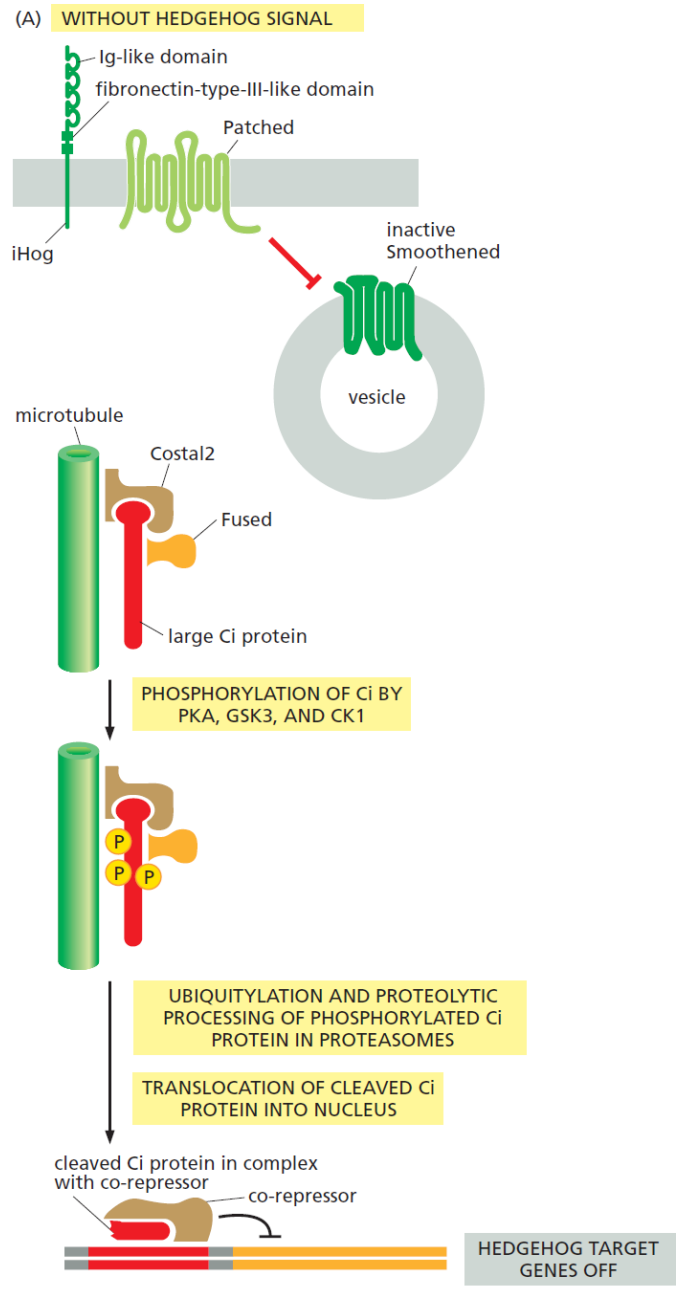
In the absence of a Hedgehog signal, Ci is ubiquitylated and proteolytically cleaved in proteasomes.
Instead of being completely degraded, however, Ci is processed to form a smaller fragment, which accumulates in the nucleus, where it acts as a **transcriptional repressor**, helping to keep Hedgehog-responsive genes silent.

The proteolytic processing of the Ci protein depends on **its phosphorylation** by three protein kinases—**PKA** and two kinases also used in the Wnt pathway, namely **GSK3** and **CK1**.
As in the Wnt pathway, the proteolytic processing occurs in a multiprotein complex.

The complex includes the protein kinase **Fused** and a **scaffold protein Costal2**, which stably associates with Ci, recruits the three other kinases, and binds the complex **to microtubules**, thereby keeping unprocessed Ci out of the nucleus.



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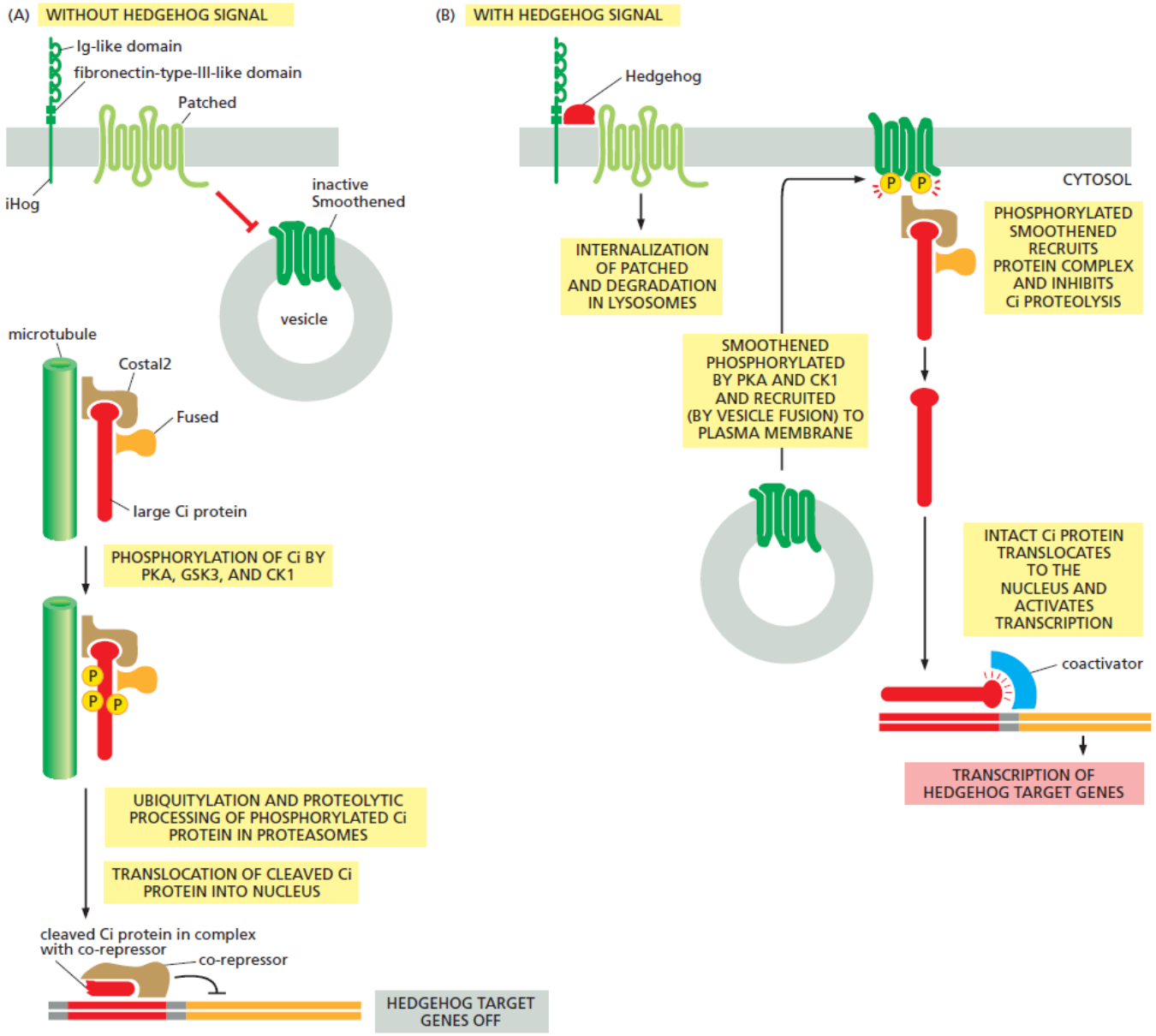
- Hedgehog functions by blocking the proteolytic processing of Ci, thereby changing it into a **transcriptional activator**.
- It does this by a convoluted signaling process that depends on three transmembrane proteins: **Patched**, **iHog**, and **Smoothened**.
- Patched is predicted to cross the plasma membrane 12 times, and, although much of it is in intracellular vesicles, some is on the cell surface where it can bind the Hedgehog protein.
- iHog is also on the cell surface and is thought to serve as a **co-receptor** for Hedgehog.
- Smoothened is a seven-pass transmembrane protein with a structure very similar to a GPCR, but it does not seem to act as a Hedgehog receptor or even as an activator of G proteins; **it is controlled by Patched and iHog**.
- In the absence of a Hedgehog signal, Patched employs an unknown mechanism to keep Smoothened sequestered and inactive in intracellular vesicles.
- The binding of Hedgehog to iHog and Patched **inhibits the activity of Patched and induces its endocytosis and degradation**.
- The result is that Smoothened is liberated from inhibition and translocates to the plasma membrane, where it recruits the protein complex containing Ci, Fused, and Costal2.



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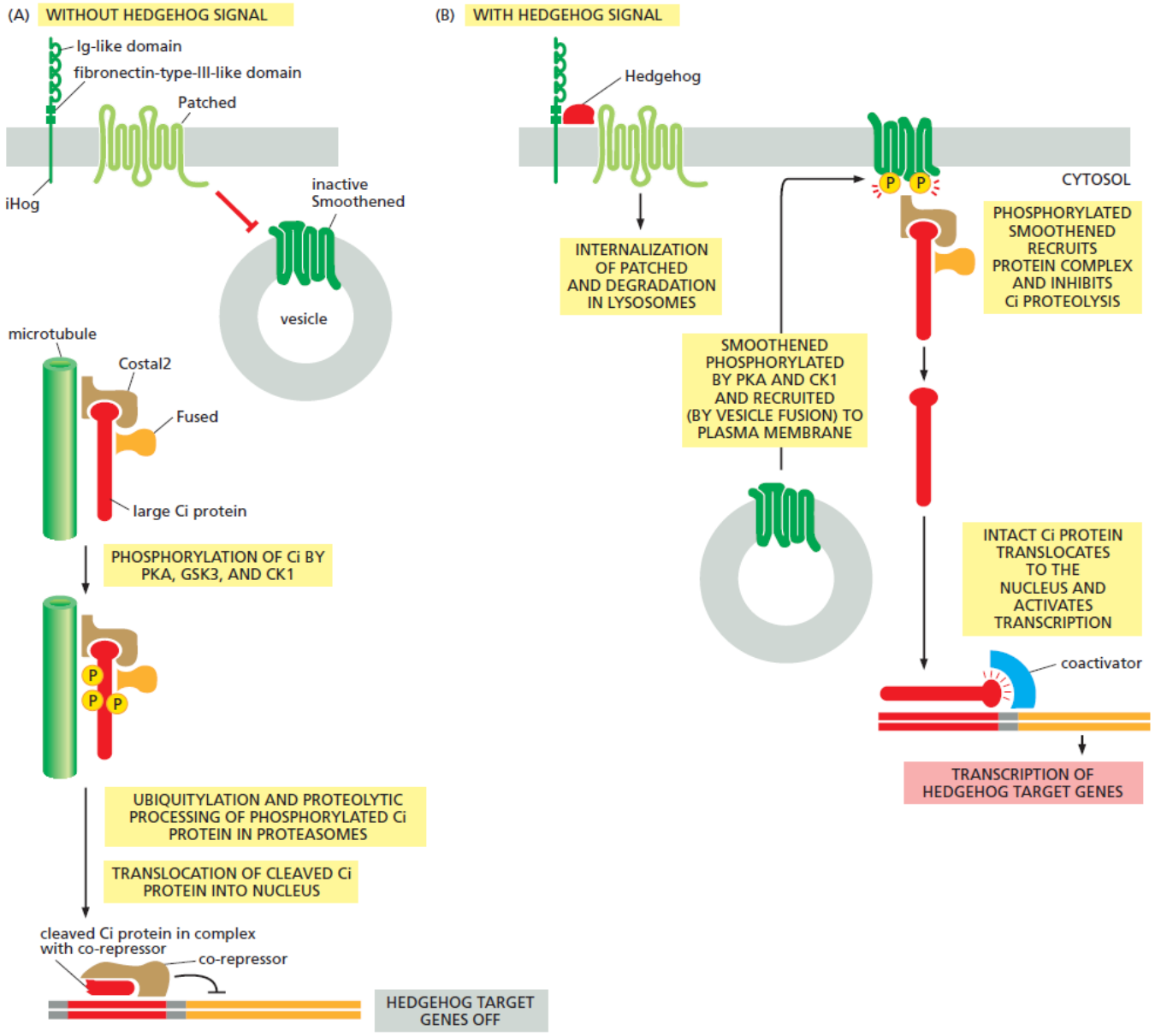
- Costal2 is no longer able to bind the other three kinases, and so Ci is no longer cleaved and can now enter the nucleus and **activate the transcription of Hedgehog target genes**.
- Among the genes activated by Ci is Patched itself; **the resulting increase in Patched protein on the cell surface inhibits further Hedgehog signaling—providing another example of negative feedback.**
- Many gaps remain in our understanding of the Hedgehog signaling pathway.
- It is not known, for example, how Patched keeps Smoothened inactive and intracellular.
- As the structure of Patched resembles a transmembrane transporter protein, it has been proposed that it may transport a small molecule into the cell that keeps Smoothened sequestered in vesicles.



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Even less is known about the more complex Hedgehog pathway in vertebrate cells.

In addition to there being at least three types of vertebrate Hedgehog proteins, there are three **Ci-like transcription regulator proteins (Gli1, Gli2, and Gli3)** downstream of Smoothened.

Gli2 and Gli3 are most similar to Ci in structure and function, and Gli3 has been shown to undergo proteolytic processing like Ci and to act as either a transcriptional repressor or a transcriptional activator.

Moreover, in vertebrates, **Smoothened**, upon activation, becomes localized to the surface of the **primary cilium**, where the Gli proteins are also concentrated, thereby increasing the **speed and efficiency of signaling**.

Hedgehog signaling can promote cell proliferation, and excessive Hedgehog signaling can lead to cancer.

Inactivating mutations in one of the two **human Patched genes**, for example, which lead to excessive Hedgehog signaling, occur frequently in **basal cell carcinoma of the skin**, the most common form of cancer in Caucasians.

A small molecule called **cyclopamine**, made by a meadow lily, is being used to treat cancers associated with excessive Hedgehog signaling:

-It blocks Hedgehog signaling by binding tightly to Smoothened and inhibiting its activity.

-It was originally identified because it causes severe developmental defects in the progeny of sheep grazing on such lilies; these include the presence of a single central eye (a condition called **cyclopia**), which is also seen in mice that are deficient in Hedgehog signaling.

Many Stressful and Inflammatory Stimuli Act Through an NF κB-Dependent Signaling Pathway

The **NFκB** proteins are latent transcription regulators that are present in most animal cells and are central to many **stressful**, **inflammatory**, and **innate immune responses**.
These responses occur as a reaction to infection or injury and help protect stressed multicellular organisms and their cells.

An excessive or inappropriate inflammatory response in animals can also damage tissue and cause severe pain, and chronic inflammation can lead to cancer; as in the case of Wnt and Hedgehog signaling, excessive NFκB signaling is found in a number of human cancers.

NFκB proteins also have important roles during normal animal development: the Drosophila NFκB family member Dorsal, for example, has a crucial role in specifying the dorsal–ventral axis of the developing fly embryo.

Various cell-surface receptors activate the NFκB signaling pathway in animal cells:
-Toll receptors in Drosophila and **Toll-like receptors** in vertebrates, for example, recognize pathogens and activate this pathway in triggering innate immune responses.

The receptors for **tumor necrosis factor α (TNFα)** and **interleukin-1 (IL1),** which are vertebrate **cytokines** especially important in inducing inflammatory responses, also activate this signaling pathway.

The Toll, Toll-like, and IL1 receptors belong to the same family of proteins, whereas TNF receptors belong to a different family; all of them, however, act in similar ways to activate NFκB.

When activated, they trigger a **multiprotein ubiquitylation and phosphorylation cascade** that releases NFκB from an inhibitory protein complex, so that it can translocate to the nucleus and turn on the transcription of hundreds of genes that participate in inflammatory and innate immune responses.

There are five NFκB proteins in mammals (**RelA, RelB, c-Rel, NFκB1, and NFκB2**), and they form a variety of homodimers and heterodimers, each of which activates its own characteristic set of genes.

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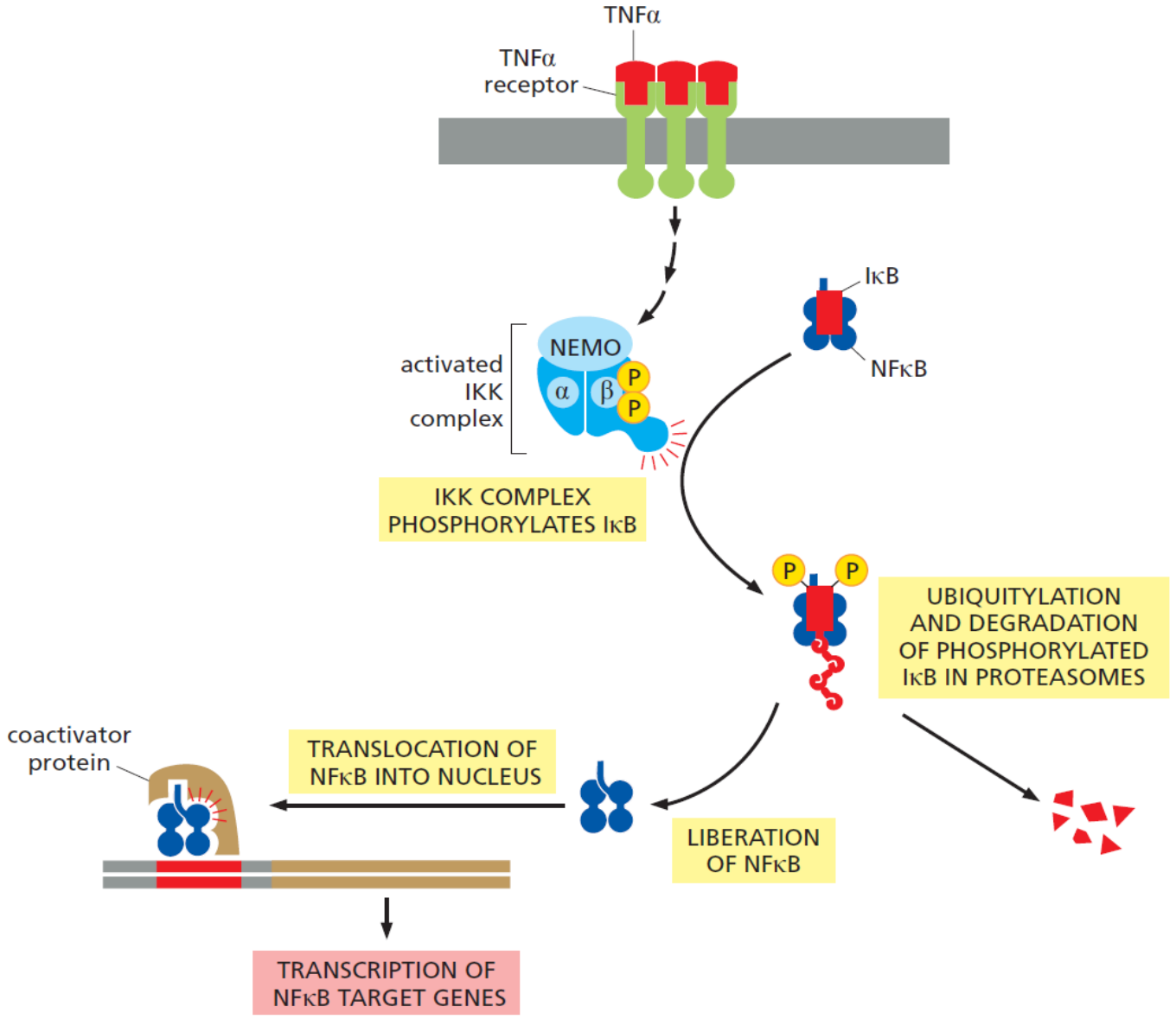
Inhibitory proteins called **IκB** bind tightly to the dimers and hold them in an inactive state within the cytoplasm of unstimulated cells.

There are three major IκB proteins in mammals (IκB α, β, and ε), and the signals that release NFκB dimers do so by triggering a signaling pathway that leads to the phosphorylation, ubiquitylation, and consequent degradation of the IκB proteins.

Among the genes activated by the released NFκB is the gene that encodes **IκBα**.



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.

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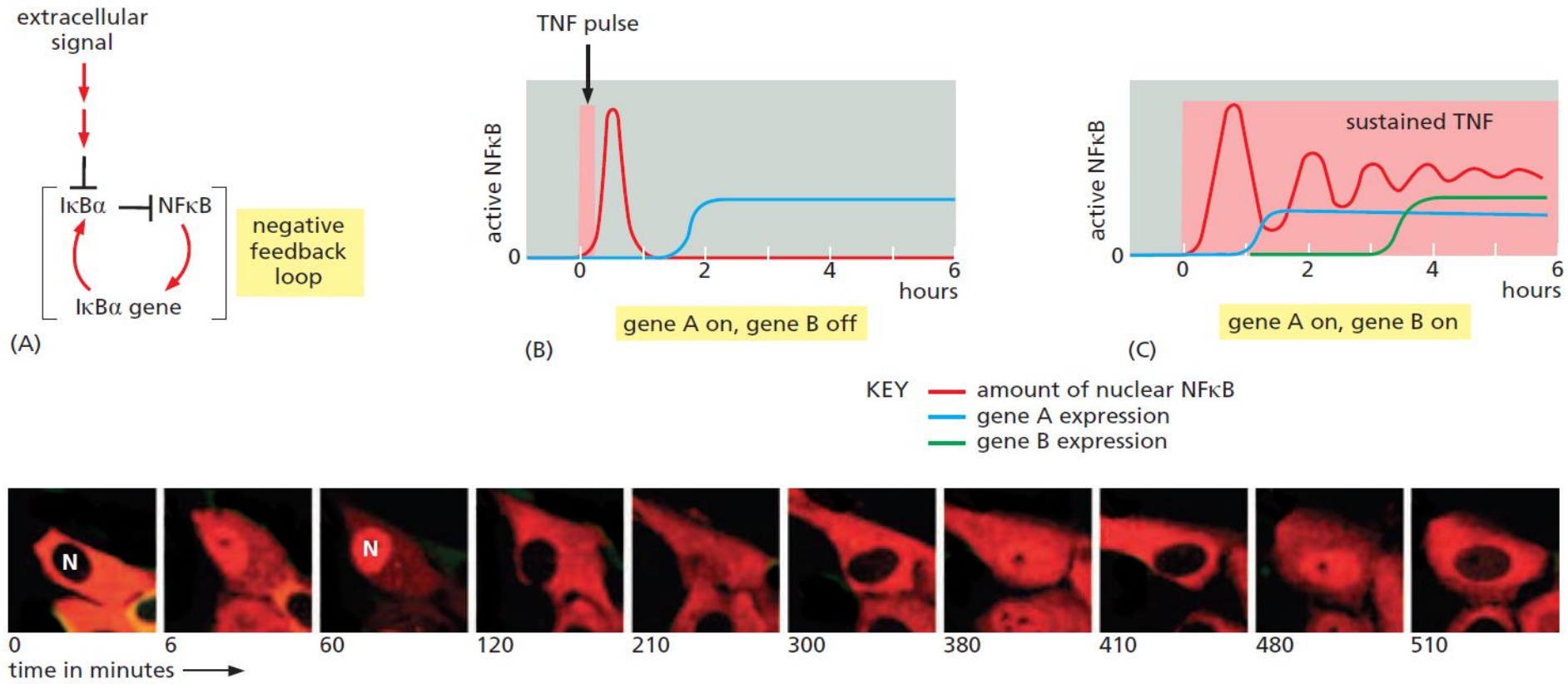
Among the genes activated by the released NFκB is the gene that encodes **IκBα**.
This activation leads to increased synthesis of IκBα protein, which binds to NFκB and inactivates it, creating a negative feedback loop.

Experiments on TNFα-induced responses, as well as computer modeling studies of the responses, indicate that the **negative feedback** produces two types of NFκB responses, **depending on the duration of the TNFα stimulus**; importantly, the two types of responses induce **different patterns of gene expression**.

The negative feedback through IκBα is required for both types of responses: in cells deficient in IκBα, even a short exposure to TNFα induces a sustained activation of NFκB, without oscillations, and all of the NFκB-responsive genes are activated.



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

-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.

-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.

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