

Advanced Cell Biology. Lecture 37

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Outline

Questions and answers

Enzyme-coupled receptors

What is the main function of calmodulin?

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- ▶ Bind Ca^{2+} , then Ca^{2+} /calmodulin-dependent protein kinases (CaM-kinases)

- ▶ Point mutation in target amino acids are used
- ▶ Typically, amino acid of interest is replaced with non-polar Ala (alanine)
- ▶ Co-immunoprecipitation will the help to obtain protein complexes

Mutants and binding sites

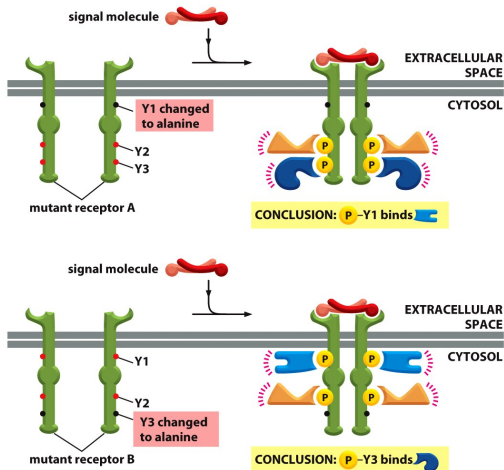


Figure 16-36 Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ In many cancers, constantly active form of Ras is involved
- ▶ There is also dominant-negative form of Ras which blocks pathway
- ▶ siRNAs will also help to block the synthesis of particular protein

Constatntly active Ras

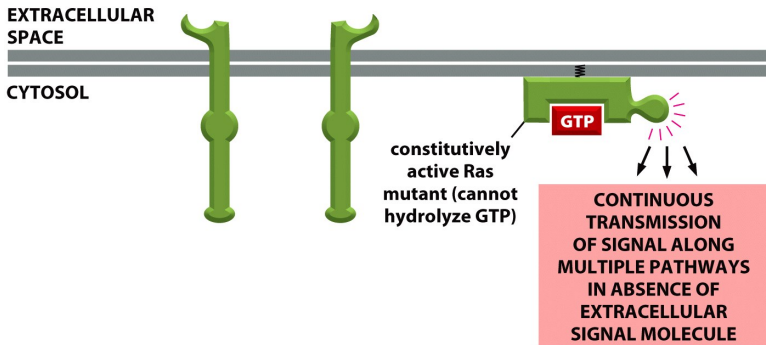


Figure 16-37 Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ Mutant forms of each pathway protein are needed
- ▶ With combination of constantly active Ras, effects will be different if protein positioned upstream or downstream of Ras

Mutation in upstream protein

MUTATION IN PROTEIN X BLOCKS SIGNALING UPSTREAM OF RAS

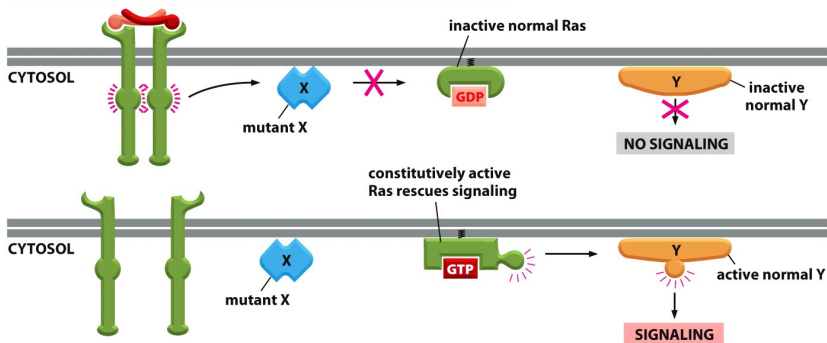


Figure 16-38b Essential Cell Biology 3/e (© Garland Science 2010)

Mutation in downstream protein

MUTATION IN PROTEIN Y BLOCKS SIGNALING DOWNSTREAM OF RAS

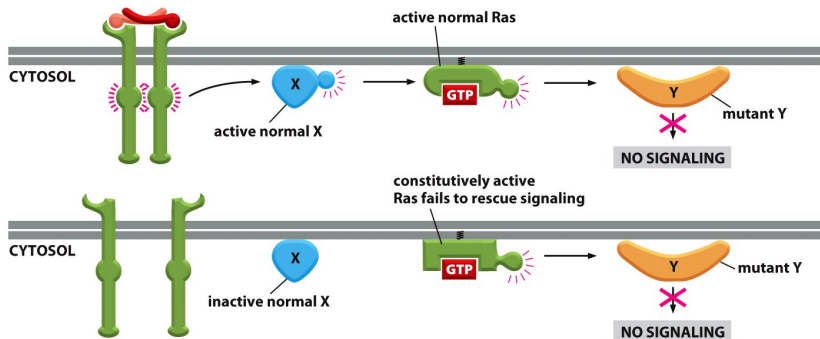


Figure 16-38c Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ RTK activated from IGF (insulin-like growth factor) activate phosphoinositide 3-kinase (PI 3-kinase)
- ▶ PI 3-kinase phosphorylates inositol phospholipid which in turn (through other kinases) activated protein kinase B (Akt, or PKB)
- ▶ In short, PI 3-kinase creates membrane docking site where different proteins (including Akt) will be phosphorylated

PI 3-kinase pathway

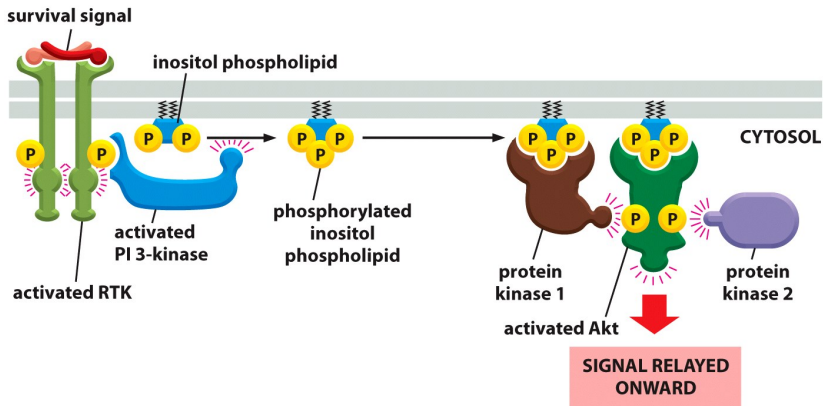


Figure 16-33 Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ Activated Akt inactivates apoptosis through dissolving of Bad-Bcl2 complex
- ▶ Activated Akt also indirectly activates Tor protein kinase which increase level of protein synthesis and inhibit protein degradation
- ▶ Suppressing Akt may stop different cancers

Activated Akt, Bad, Bcl2 and apoptosis

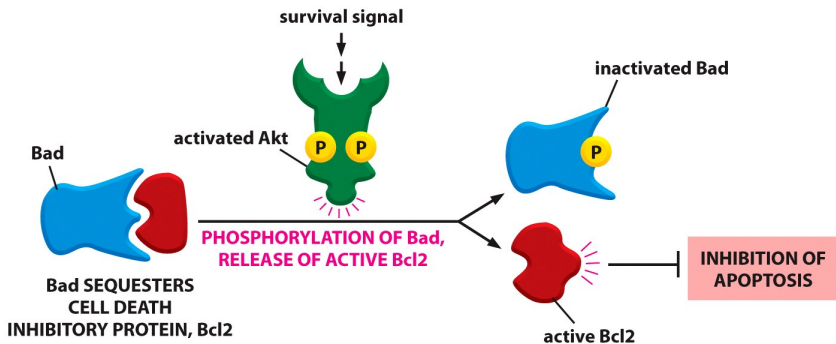


Figure 16-34 Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ Cytokines are local mediators which activate transcription regulators (e.g., interferons activate synthesis of viral-resistant proteins)
- ▶ These transcription regulators (STATs) may come straight to nucleus

- ▶ Unlike RTKs, cytokine receptors have no enzymatic activity: instead, they associate with tyrosine kinases JAKs
- ▶ JAKs may reciprocally phosphorylate themselves and then phosphorylate STATs
- ▶ STATs dissociated from complex, dimerize and migrate to nucleus

JAK-STAT pathway

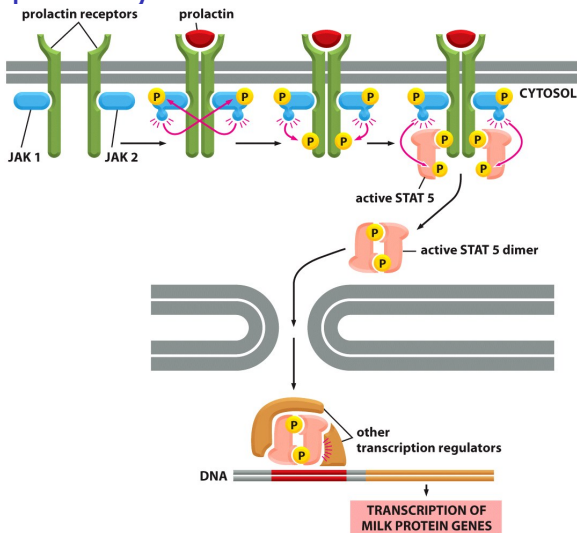


Figure 16-39 Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ In fly neural cells, membrane Delta signal proteins activate Notch receptors
- ▶ By activation, Notch cleaved and its tail migrates to nucleus where activates responsible genes

Notch pathway

developing nerve cell

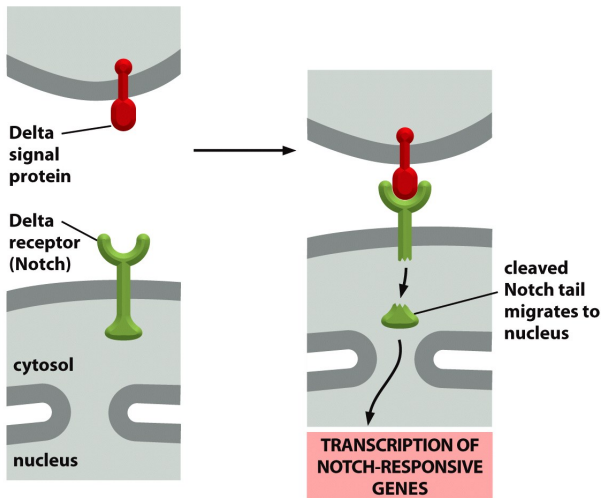


Figure 16-40 Essential Cell Biology 3/e (© Garland Science 2010)

- ▶ Plant cells are not using RTKs, steroid hormones, cyclic AMPs
- ▶ GPCRs are known for plants, but there are few of them
- ▶ In contrast, plants have specific receptors for specific plant molecules—plant hormones

- ▶ Ethylene controls ripening of fruits
- ▶ Ethylene receptor is somewhat similar to enzyme-coupled receptors of animals but it is dimeric and active with an absence of signal (!)
- ▶ Ethylene presence will make receptor kinase inactive and transcription regulators will not degrade but start to activate transcription

Ethylene signaling pathway

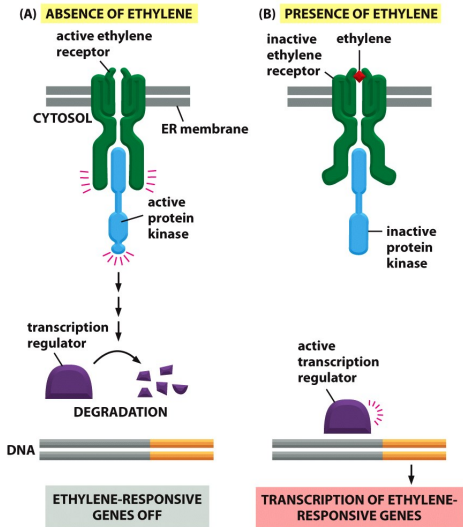
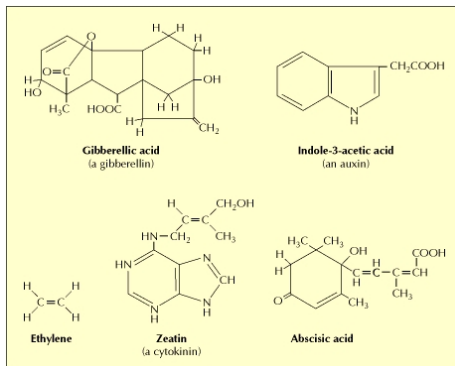


Figure 16-41 Essential Cell Biology 3/e (© Garland Science 2010)

Plant hormones



- ▶ Some pathway components like Ca^{2+} or phospholipase C could participate in both GPCR and RTK pathways
- ▶ Pathway may also integrate signal via double phosphorylation or activating complementary proteins

Interconnections of pathways

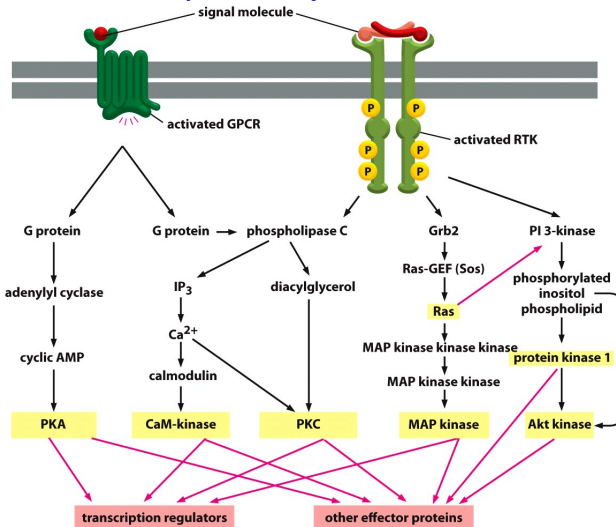


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Integration of signal

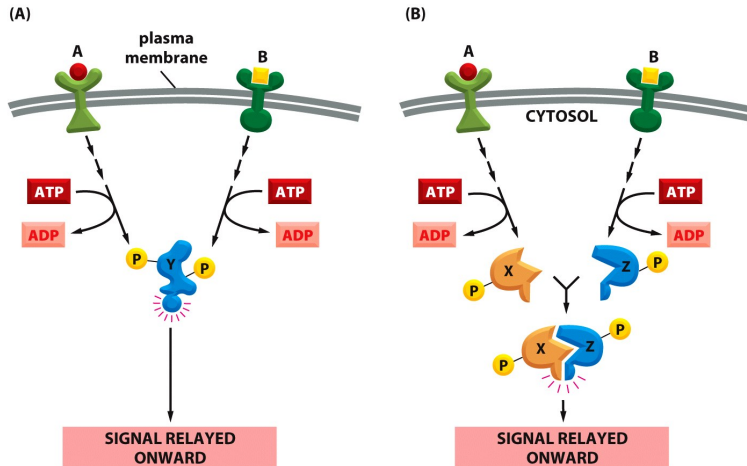


Figure 16-43 Essential Cell Biology 3/e (© Garland Science 2010)

Chemotaxis movie

Neutrophil movie

Lymphocyte movie

How do researchers use constantly active Ras protein?

Short anonymous absolutely voluntary survey

1. What do you **like** most in advanced cell course?
2. What do you **dislike** most in advanced cell course?
3. **Which lab** do you remember most of all?
4. Please grade (1—bad, 5—excellent):
 - 4.1 Lectures
 - 4.2 Labs
 - 4.3 Final questions
 - 4.4 Exams

- ▶ PI 3-kinases will activate cell growth (similarly to MAP kinases) via creation of membrane docking sites
- ▶ Notch and cytokine receptors activate a direct pathway into nucleus
- ▶ Plant signaling systems are different from animal

For Further Reading



A. Shipunov.

Advanced Cell Biology [Electronic resource].

2011—onwards.

Mode of access: [http:](http://)

[//ashipunov.info/shipunov/school/biol_250](http://ashipunov.info/shipunov/school/biol_250).



B. Alberts et al.

Essential Cell Biology. 3rd edition.

Garland Science, 2009.

Chapter 16.