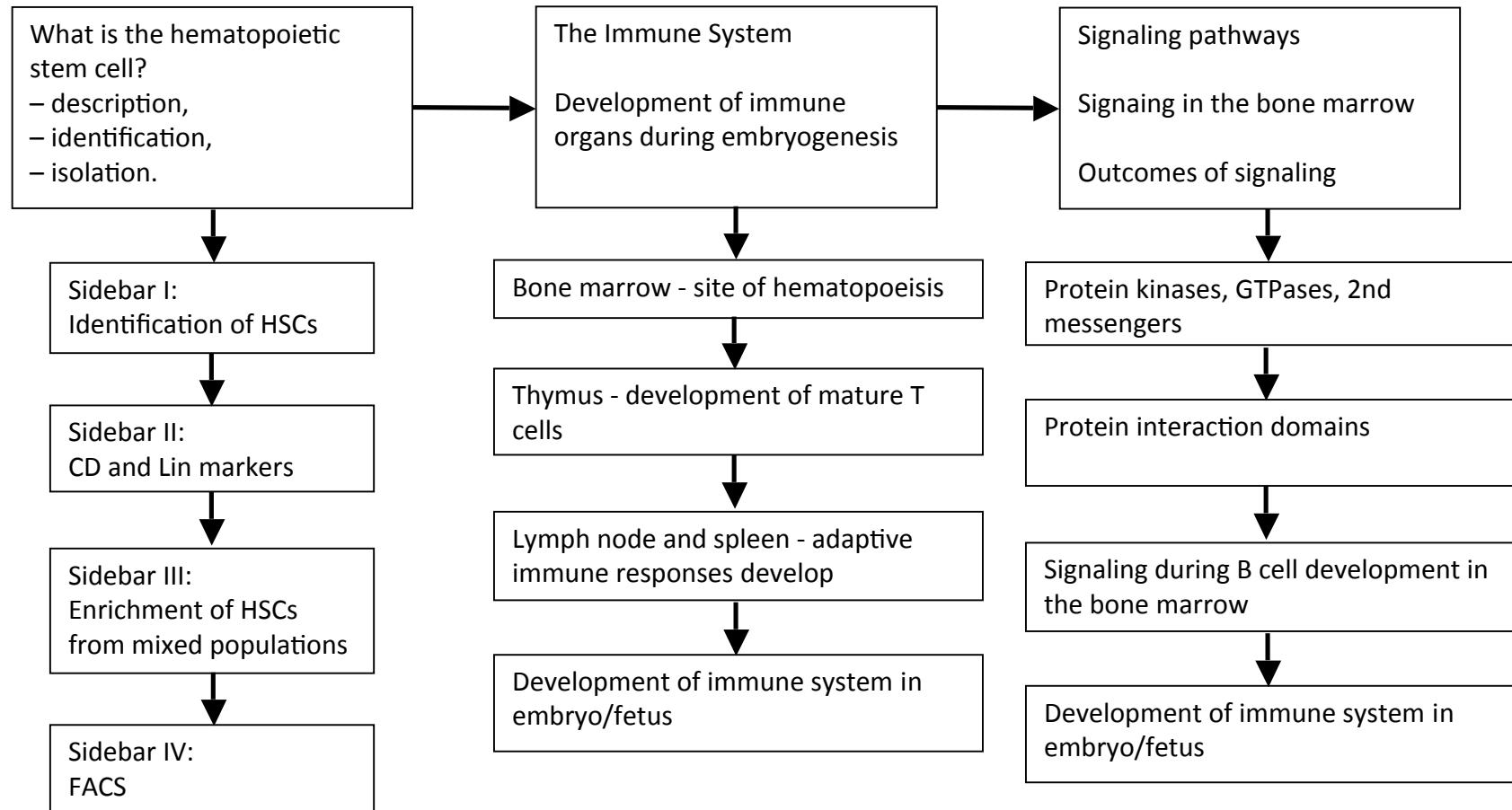


Module 1: Stem cells and the development of the immune system



Module 1: Stem cells and the development of the immune system

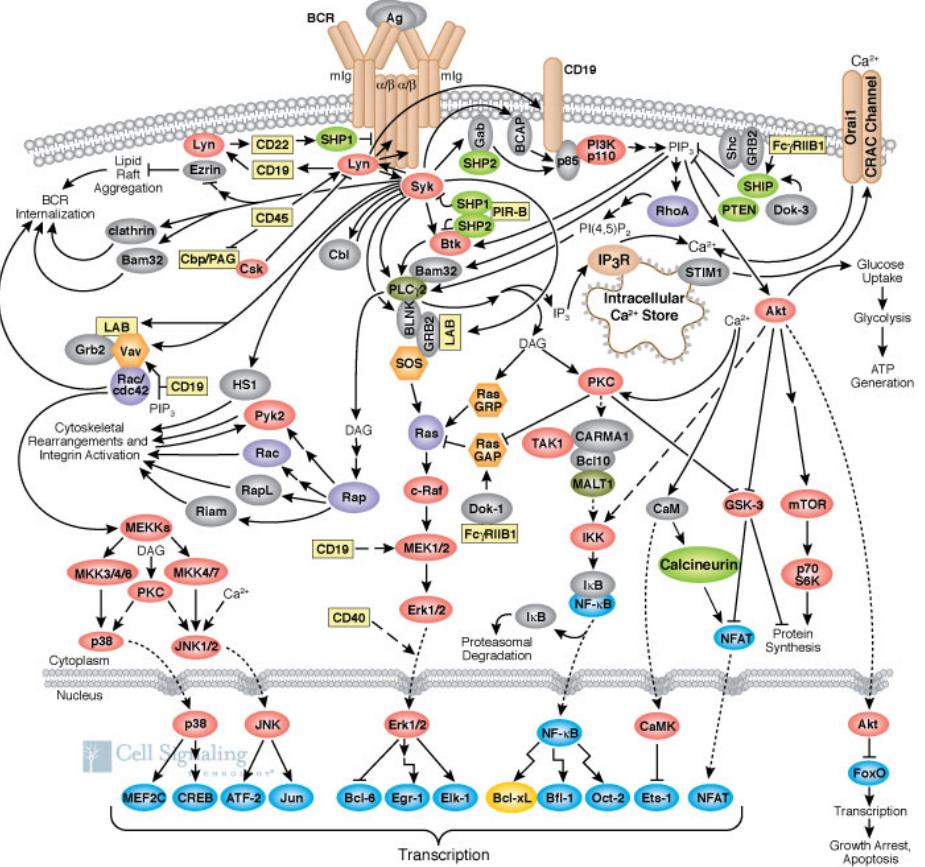
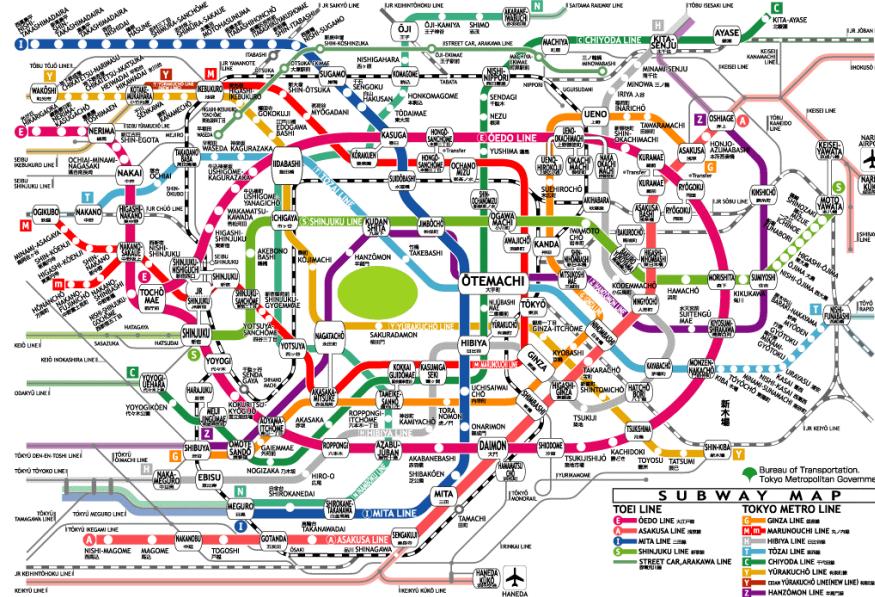
Learning Objectives

After completing this part of Module 1, students should be able to:

- explain how receptor signaling involves converting of extracellular signals into intracellular signals after binding a ligand,
- explain the activity of protein kinases, GTPases, and second messengers,
- explain how the components of the signaling cascade assemble through interaction domains,
- explain how signaling fits into the development of B cells in the bone marrow,
- explain how ligand binding of a receptor can result in two different outcomes for the cells.

Tokyo subway map vs. signaling pathways in B cells

Can you spot the difference?



http://www.wa-pedia.com/japan-guide/transportation_tokyo.shtml
http://www.cellsignal.com/reference/pathway/B_Cell_Antigen.html

Introduction to intracellular signal transduction pathways

Signal transduction refers to how extracellular stimuli (the “signal”) initiate the generation of intracellular signals (“biochemical events”) that cause changes in cell function.

The extracellular signals are transmitted across the plasma membrane by transmembrane receptor proteins, which work to convert the information outside of the cell into an intracellular biochemical event.

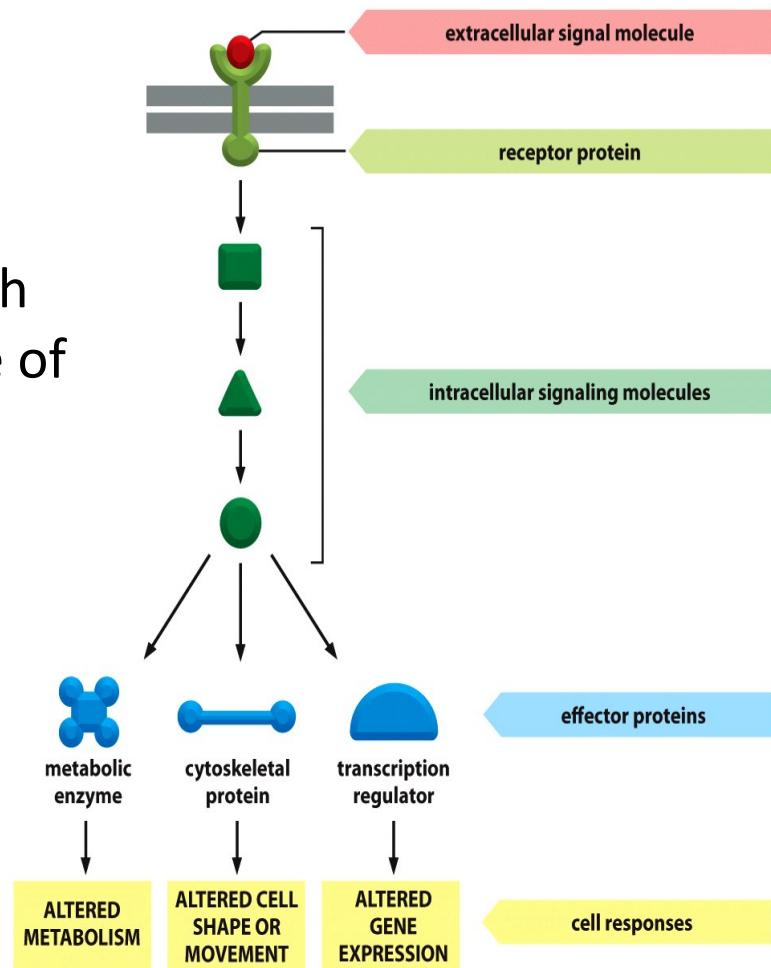


Figure 16-12 *Essential Cell Biology* (© Garland Science 2010)

Introduction to intracellular signal transduction pathways

Once inside the cell, the signal is transmitted along intracellular signaling pathways and distributed to different sites in the cell and sustained and regulated as they proceed toward their destination.

The final destinations of many of the signaling pathways are the:

- nucleus (alter gene expression – synthesis of new proteins such as cytokines or cell-adhesion molecules, cell division and differentiation, apoptosis),
- cytoskeleton (to alter cell shape, size and motility).

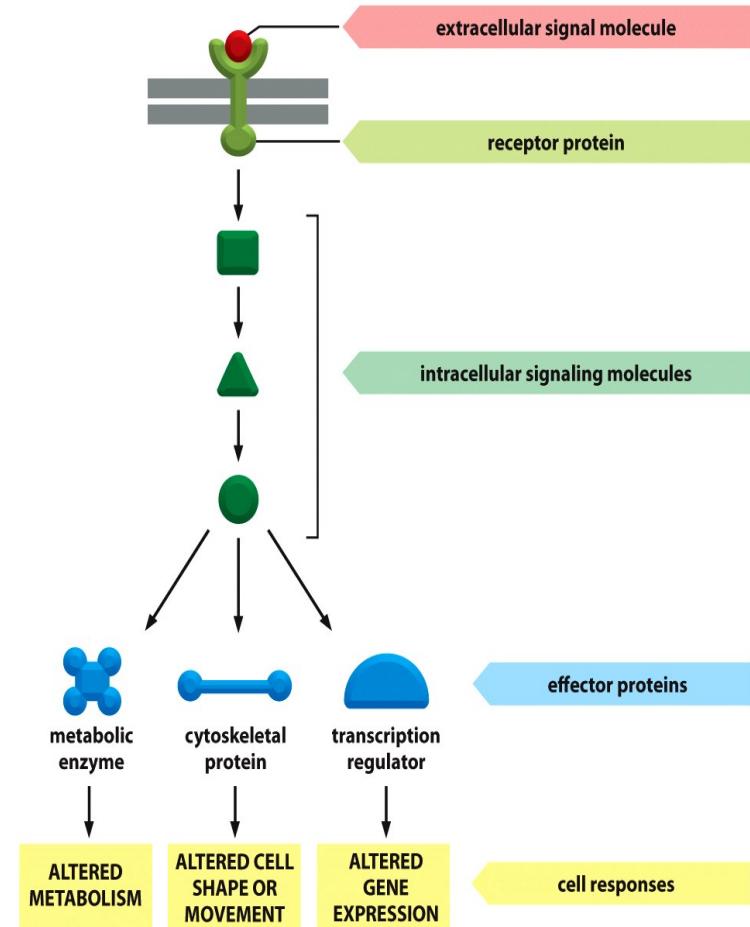


Figure 16-12 *Essential Cell Biology* (© Garland Science 2010)

Introduction to intracellular signal transduction pathways

In the immune system, signaling pathways are important in:

- hematopoiesis (e.g., B cell, T cell development),
- recognition of a pathogen during the inflammatory response,
- activation of adaptive immune responses by B cells and T cells, etc.

The cell's response to the signals may include:

- changes in gene expression,
- induce of apoptosis,
- induce proliferation, differentiation,
- affect cytoskeleton changes (shape, motility), etc.

Introduction to intracellular signal transduction pathways

All cell-surface receptors that have a signaling function are either transmembrane proteins themselves, or form parts of protein complexes that link the exterior and interior of the cell.

Different receptors transduce extracellular signals in a variety of ways.

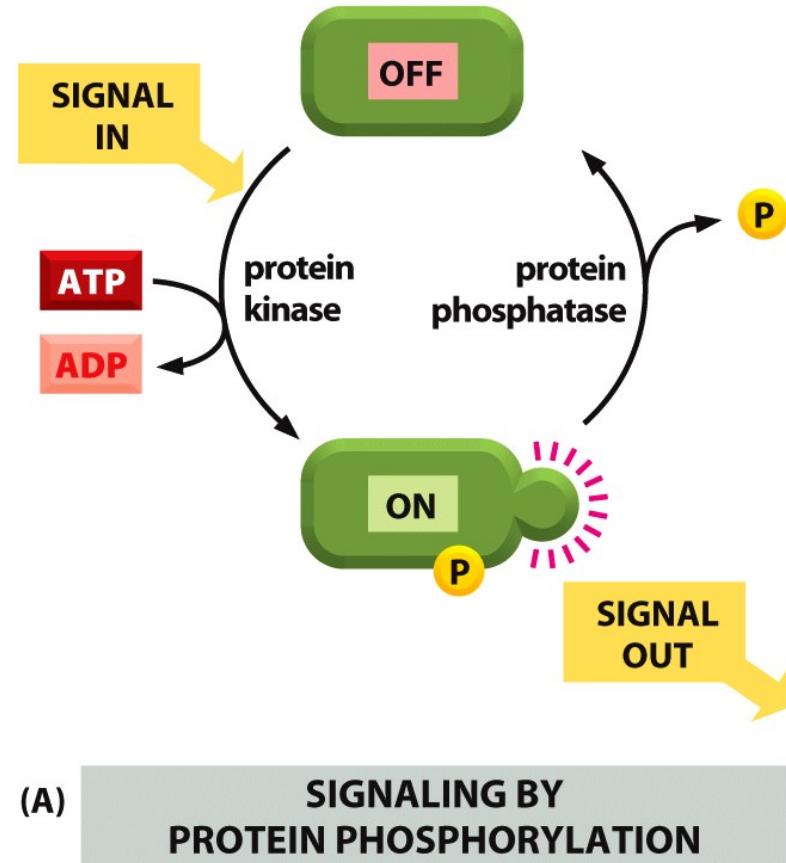
A common example is with receptors is the activation of intracellular enzyme activity after the binding of a ligand.

Protein kinases

The enzymes most commonly associated with receptor activation are the protein kinases.

Kinases are enzymes that use ATP and catalyze the addition of a phosphate group to protein.

In animals, protein kinases phosphorylate proteins one of three amino acids – tyrosine (Y), serine (S), and threonine (T).



Receptor Protein Kinases

The binding of the ligand to the extracellular domain causes dimerization of individual receptors, resulting in the allows the cytoplasmic kinase domain to become “active” and phosphorylate the cytoplasmic tails of adjacent receptors,

Most of the enzyme-linked receptors are tyrosine protein kinases.

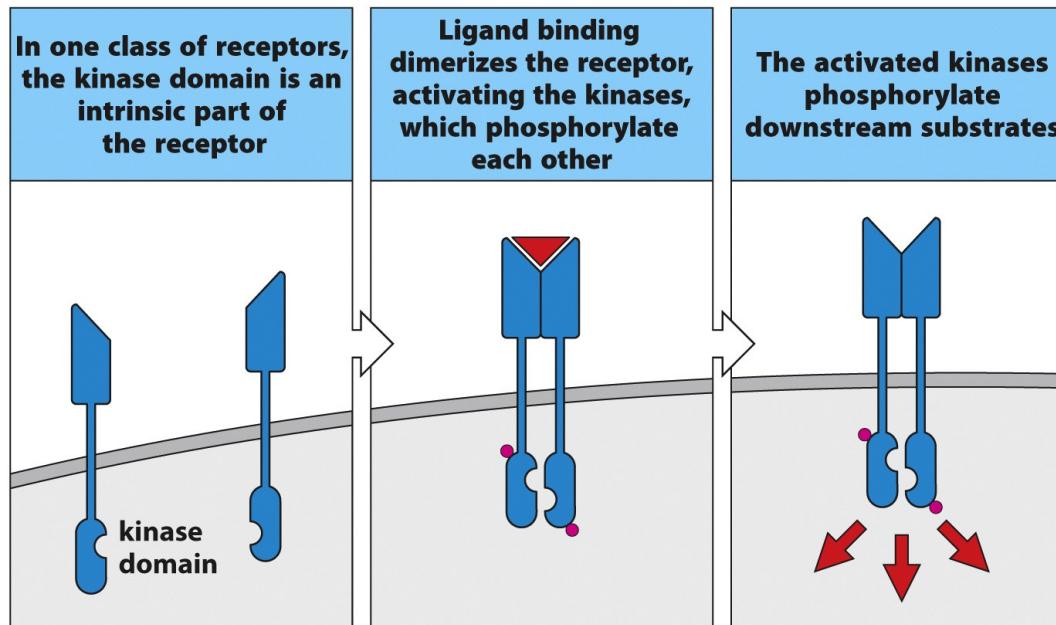


Figure 7.1 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 7.1.1 Janeway's Immunobiology, 8th edition.

Non-receptor Protein Kinases

Not all receptors have intrinsic kinase activity but associate with intracellular tyrosine kinases.

These non-receptor kinases can either be constitutively associated with the cytoplasmic domains of the receptor proteins or recruited to the receptor after it binds a ligand.

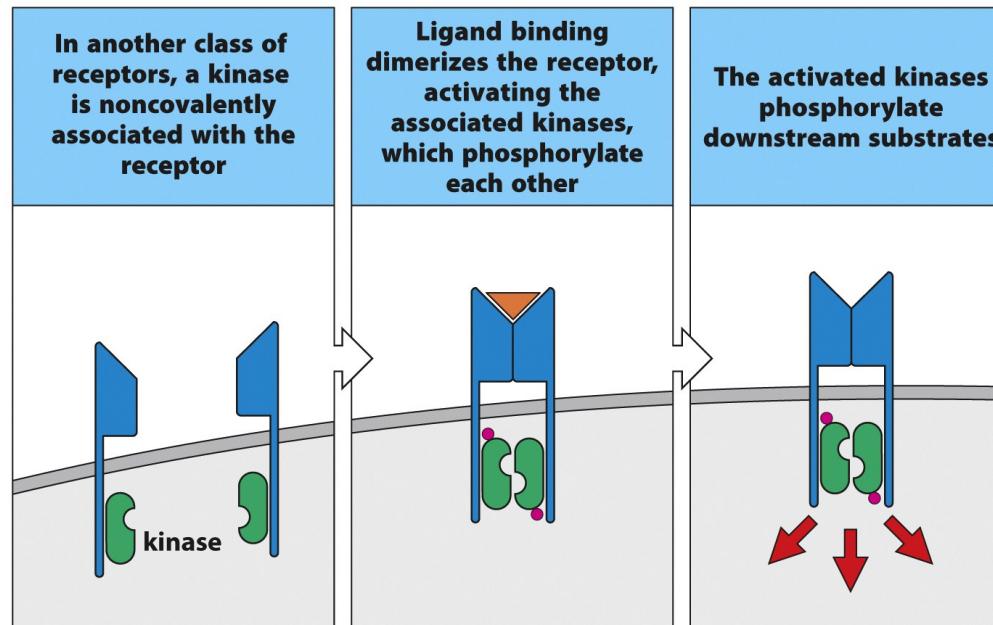


Figure 7.1 part 2 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

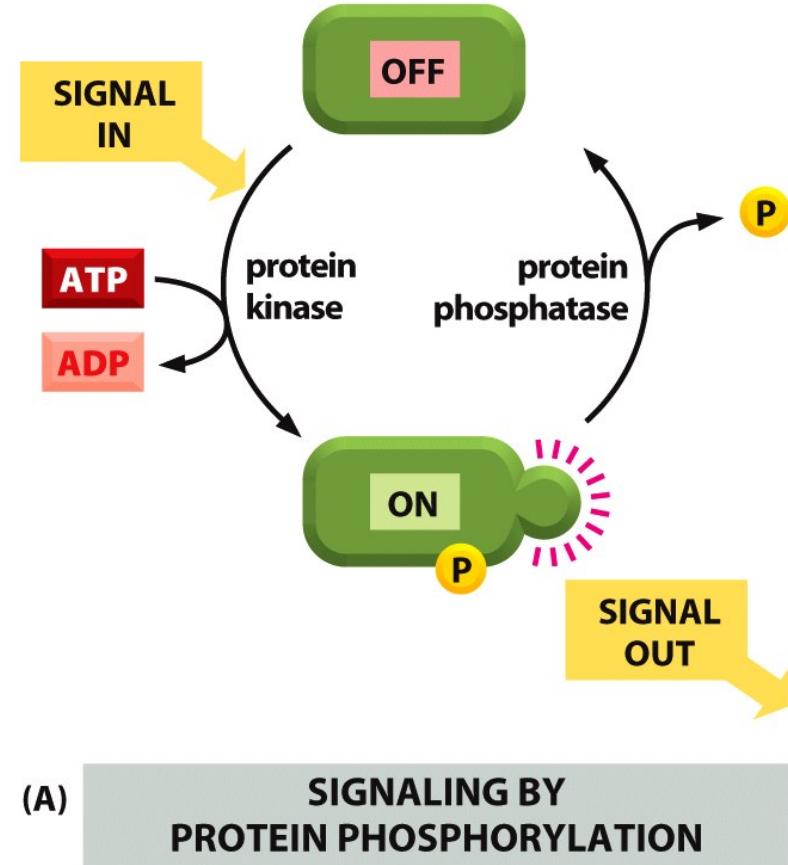
Figure 7.1.2 Janeway's Immunobiology, 8th edition.

Protein kinases

Phosphate groups can be removed by phosphatases – different classes of these enzymes too.

These regulate signaling proteins by “resetting” a protein to its original state and switching signaling off.

Protein kinases figure largely in cell signaling because phosphorylation/dephosphorylation are the means of regulating the activity of many enzymes, transcription factors and proteins.



Protein kinases

Once inside the cell, the signal is transmitted along intracellular signaling pathways.

Intracellular signaling pathways are composed of proteins that interact with each other in a variety of ways.

The role of kinases are not confined to receptor activation and they can act at many different stages of intracellular signaling pathways.

Phosphorylation can generate sites on proteins to which other signalling proteins can bind.

Assembling intracellular signaling complexes

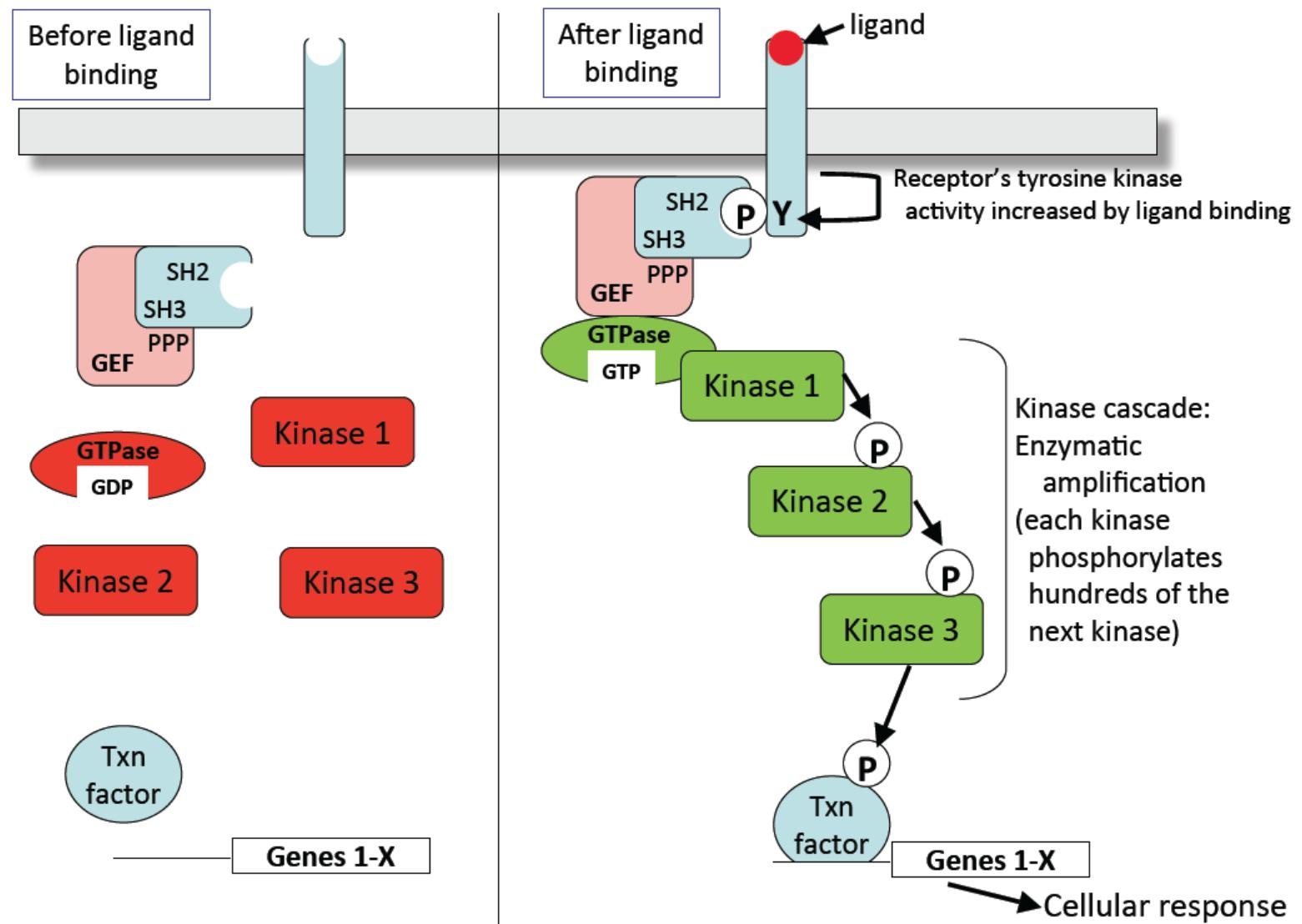
The binding of a ligand to a receptor initiates a cascade of events involving intracellular signaling proteins.

The unique enzymes and other components assembled into a particular multi-protein complex will determine the character of the signal it generates.

These components may be shared by many pathways or exclusive to one receptor pathway, thus allowing distinct signaling pathways to be built from a relatively limited number of components.

These signaling proteins are assembled into complexes that work together to generate the intracellular signal.

Assembling intracellular signaling complexes



Assembling intracellular signaling complexes

Signaling cascades are made up of many components interacting with each other via domains (like 2 jigsaw pieces fitting together) and in sequence of interactions (like electrons that are passed from one ETC component to the next).

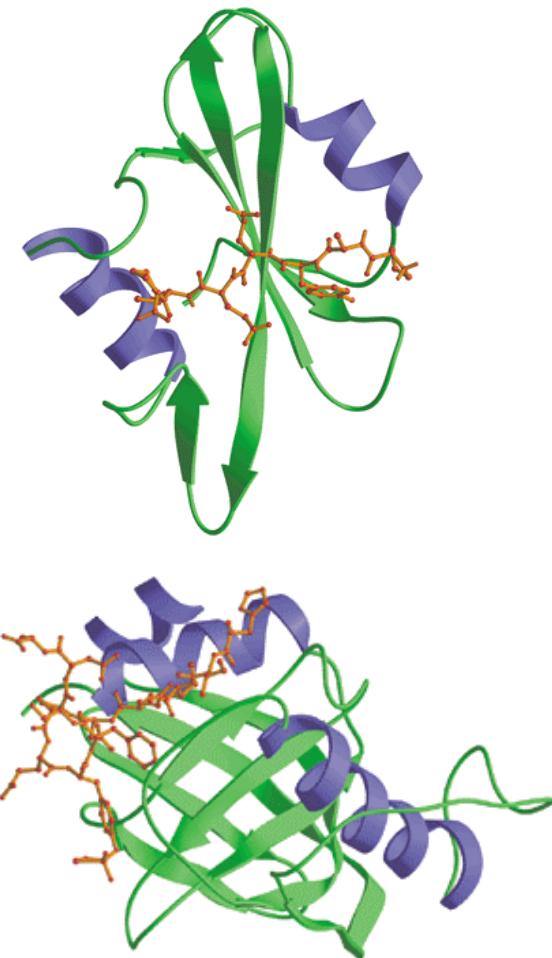
The receptor activates enzymes that create molecular determinants that are recognized by “interaction domains” that are found in signaling proteins.

These allow the next protein to assemble to start the formation of a signaling complex.

Assembling intracellular signaling complexes

There are ~ 50 different types of protein interaction domains.

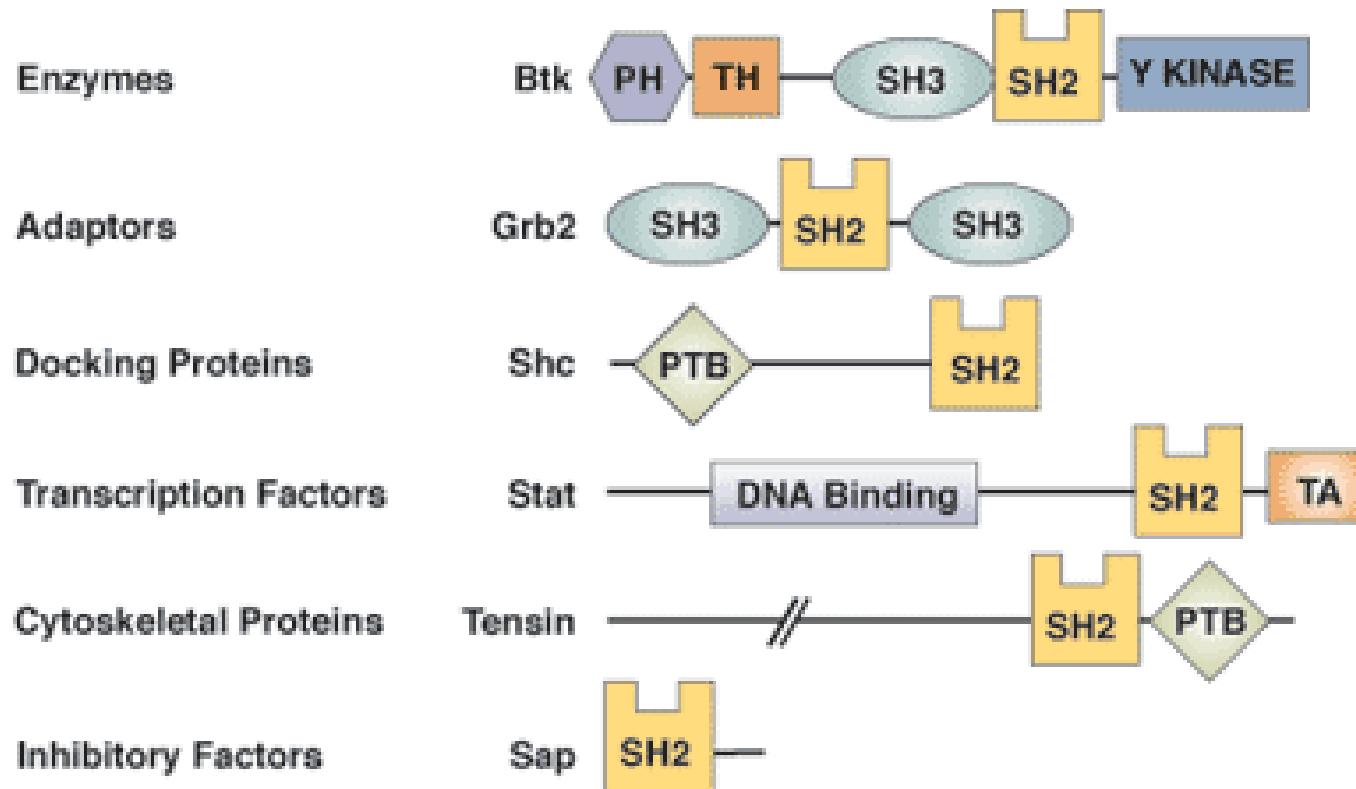
Each type of interaction domain has a “pocket” (i.e., a 3D structure) that binds to a specific amino acid sequence (often containing a phosphorylated S/T or Y) in other proteins or to a specific membrane lipid.



SH2 and PTB domains bind phospho-tyrosine-containing sequences

Assembling intracellular signaling complexes

A protein that is part of a signal cascade contains at least one domain and may have several different domains to interact with many other proteins.



SH2 domains

An important aspect in the formation of the signaling complexes is the activity of tyrosine kinases.

Phosphylated tyrosines (pY) are binding sites for a number of protein-interaction domains (e.g., SH2 domains – Src homology 2).

This module (about 100 aa) is present in many intracellular signaling proteins.

SH2 domains

SH2 domains bind in a sequence specific fashion, recognizing the pY and typically the amino acid three positions away (pYXXXZ)*.

Different SH2 domains prefer different combinations of amino acids.

The SH2 domain of a protein can act as a key that allows inducible and specific association with another protein that contains the appropriate pY-containing amino acid sequence.

* X is any amino acid and Z is a specific amino acid

Examples of protein interaction domains

Examples of protein domains and what they recognize.

Domain	What it recognizes
SH2	pY in a peptide motif of about 3 – 6 amino acids
SH3	proline rich motifs, e.g., X–P–(P)–X–P
PH (PHD)	phosphatidylinositol membrane lipids (PIP3)
PTB	pY in a peptide motif different than a SH2 domain
TH	Proline rich motif, different than a SH3 domain

A protein that is part of a signal cascade may have several different domains to interact with many other proteins.

Assembling intracellular signaling complexes

Y kinase associated receptors can also assemble multi-protein signaling complexes using proteins called scaffolds and adaptors.

These proteins lack enzymatic activity themselves and can function by recruiting other proteins into a signaling complex so that these proteins can interact with each other.

Assembling intracellular signaling complexes

Scaffolds are relatively large proteins and they can have several pY.

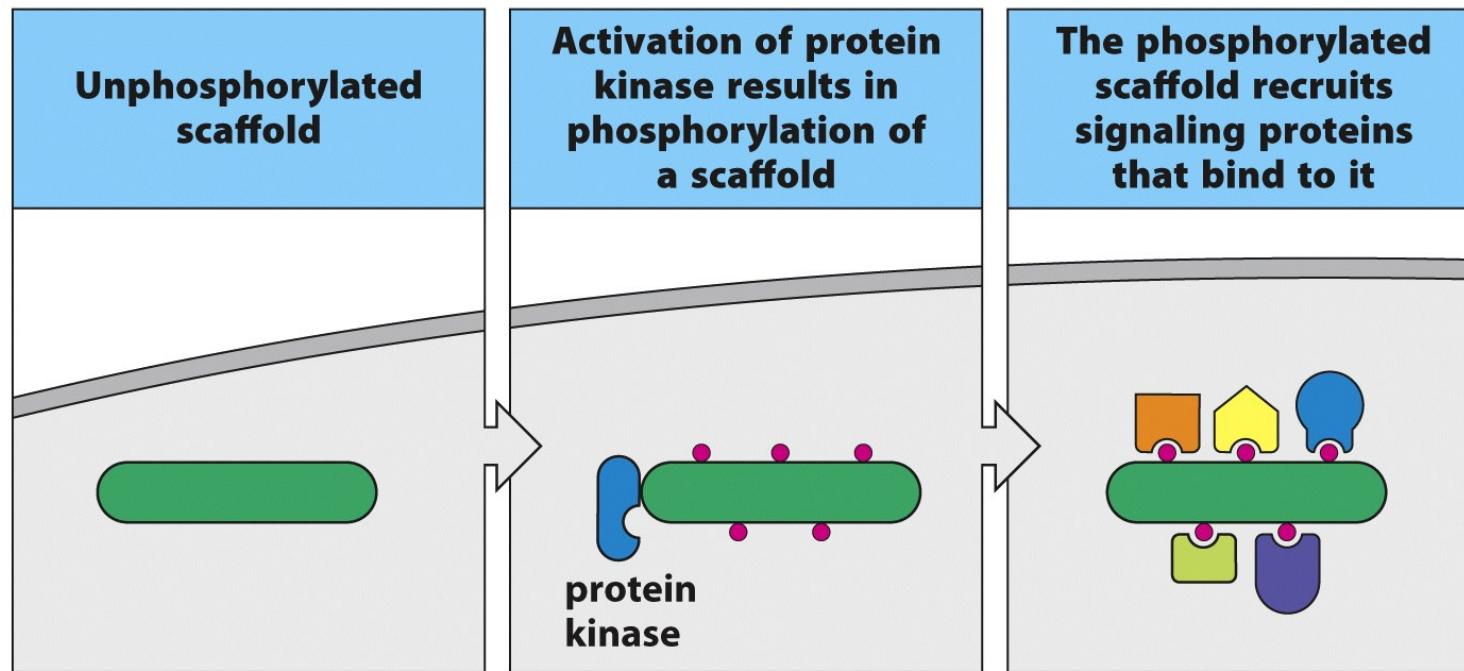


Figure 7.3 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 7.3.1 Janeway's Immunobiology, 8th edition.

Assembling intracellular signaling complexes

Adaptors are smaller proteins with usually no more than 2 or 3 interaction domains that serve to link two proteins together.

For example, it may contain an SH2 domain and 1 or 2 SH3 domains.

The arrangement of these domains can be used to link Y phosphorylation of a receptor to molecules acting in the next stage of signaling.

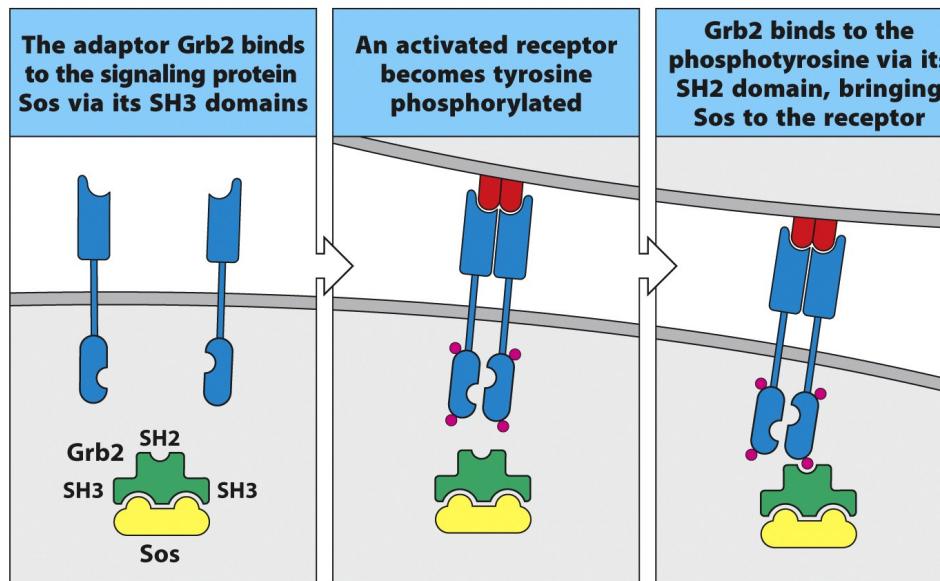


Figure 7.3 part 2 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 7.3.2 Janeway's Immunobiology, 8th edition.

Hypothetical signaling pathway

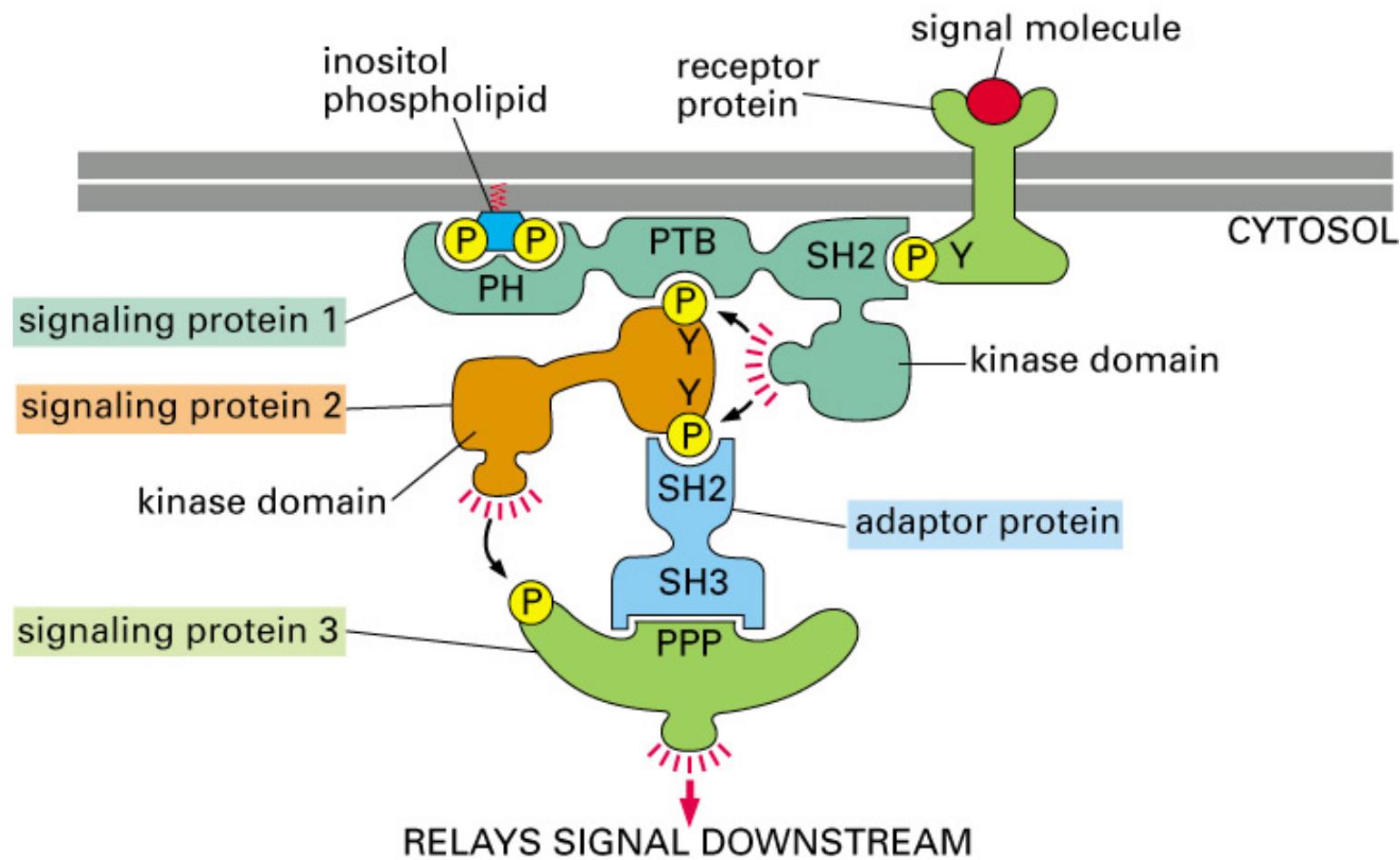


Figure 15–20 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Small G proteins (GTPases)

GTPases act as “molecular switches” in many signaling pathways.

These are localized to the inner surface of the plasma membrane via fatty acids that are attached to the protein post-translationally.

There are more than 100 proteins in the small GTPase family.

Some of the GTPases are involved in signaling pathways that result in proliferation or pathways that lead to changes in the cell’s cytoskeleton.

GTPases exist in two states depending on whether they are bound to GTP or GDP.

Each GTPase has its own GEFs and GAPs which help to confer specificity on the pathway.

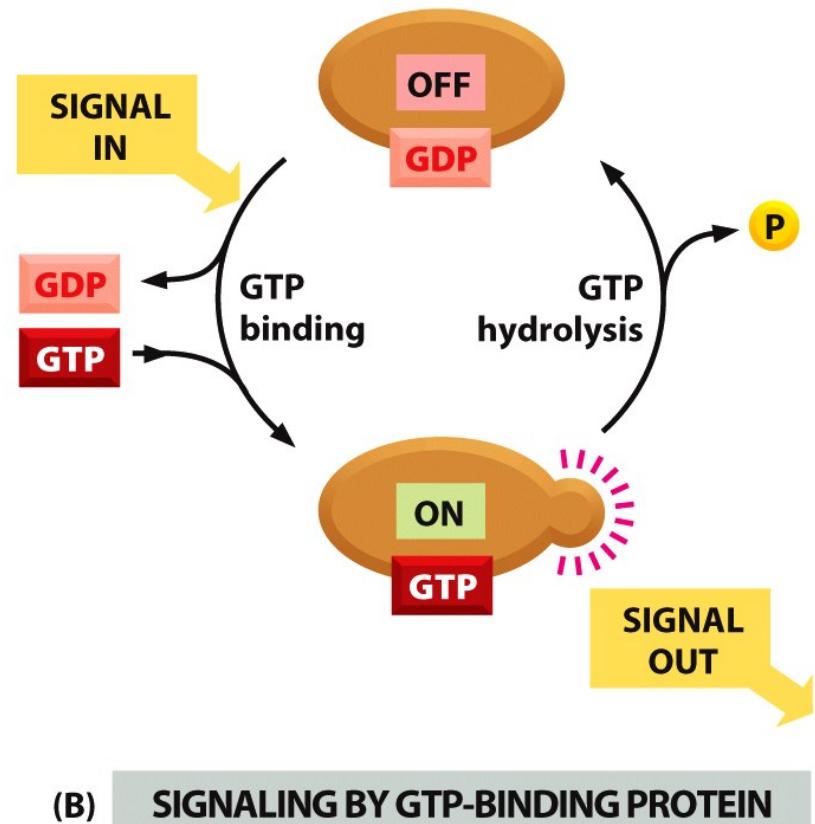
Small G proteins (GTPases)

GDP-bound form is inactive.

Guanine exchange factors (GEFs) convert the GTPase to an active form by causing it to release GDP and then bind the more abundant GTP.

GEFs are recruited to signaling pathways by adaptor proteins through the protein-interaction domains.

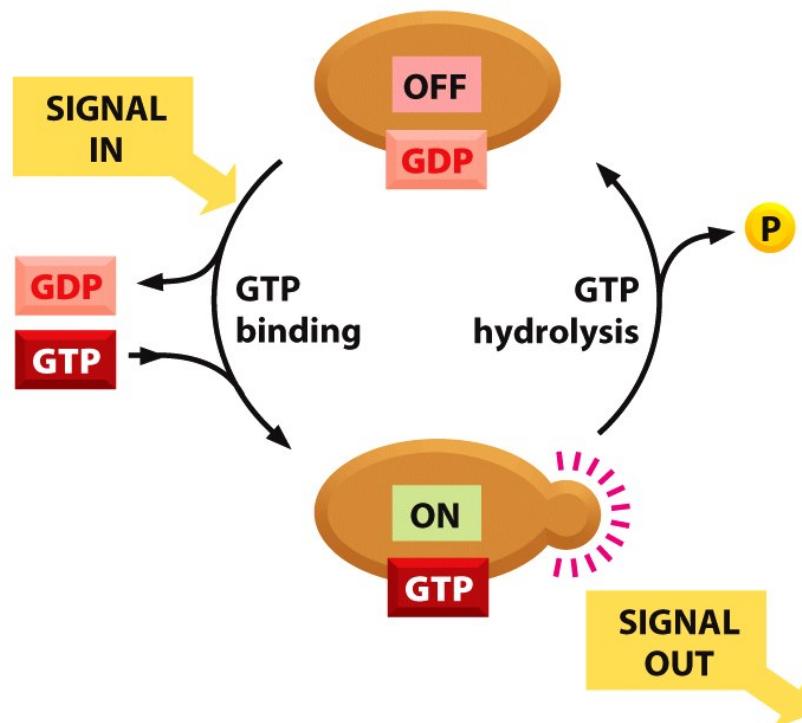
The active GTP-bound form can bind to and activate “effector” proteins that relay the signal further.



Small G proteins (GTPases)

The GTP-bound form is eventually converted back to the GDP-bound form by its intrinsic GTPase activity by the removal of a phosphate group from the GTP.

GTPase-activating proteins (GAPs) accelerate this conversion to GDP.



(B) SIGNALING BY GTP-BINDING PROTEIN

Assembling intracellular signaling complexes

Signaling proteins can be recruited to the membrane in a variety of ways.

The first method discussed involved the phosphorylation of Y in the receptor or scaffold proteins, creating molecular determinants for the recruitment of SH2-domain-containing signaling proteins.

Another mechanism is the activation of membrane associated GTPases which can then recruit signaling molecules to the membrane.

A third method is the local production of modified membrane lipids as a result of receptor activation.

(see figure next slide)

Assembling intracellular signaling complexes

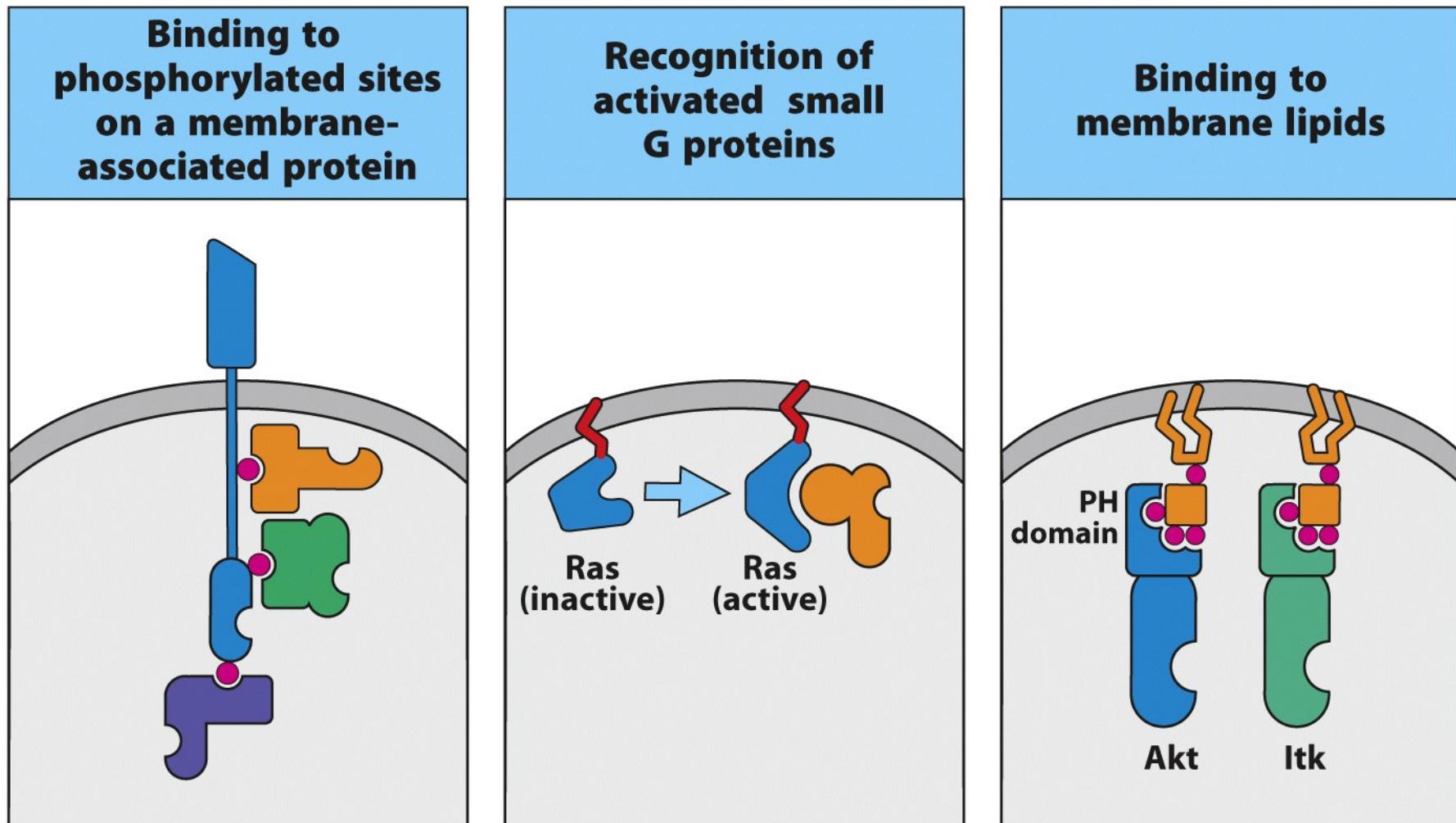


Figure 7.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 7.5 Janeway's Immunobiology, 8th edition.

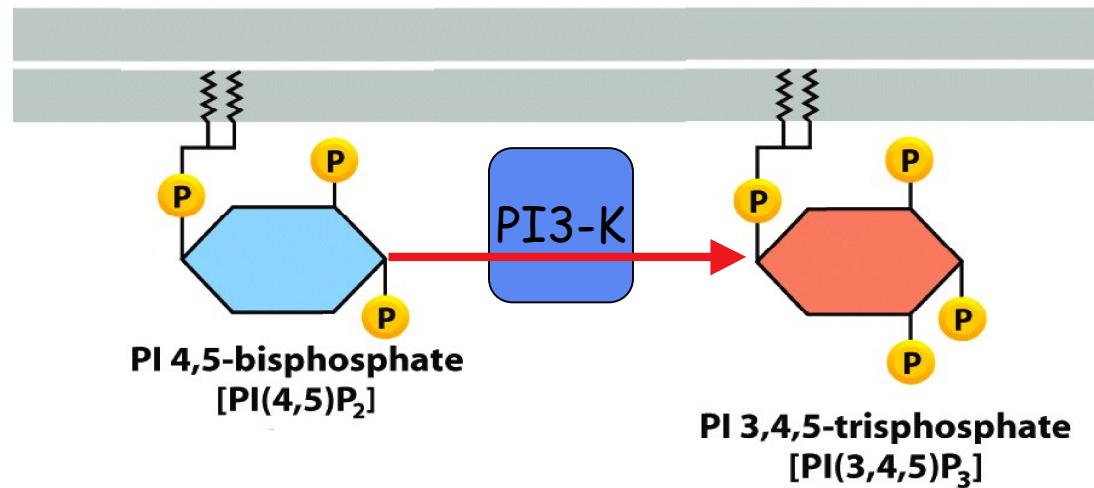
Assembling intracellular signaling complexes

These modified lipids are produced by the phosphorylation a sugar in the inositol head groups in phosphatidylinositol (a type of membrane lipid).

The important ones are:

- phosphatidylinositol 3,4-biphosphate (PIP_2),
- phosphatidylinositol 3,4,5-triphosphate (PIP_3).

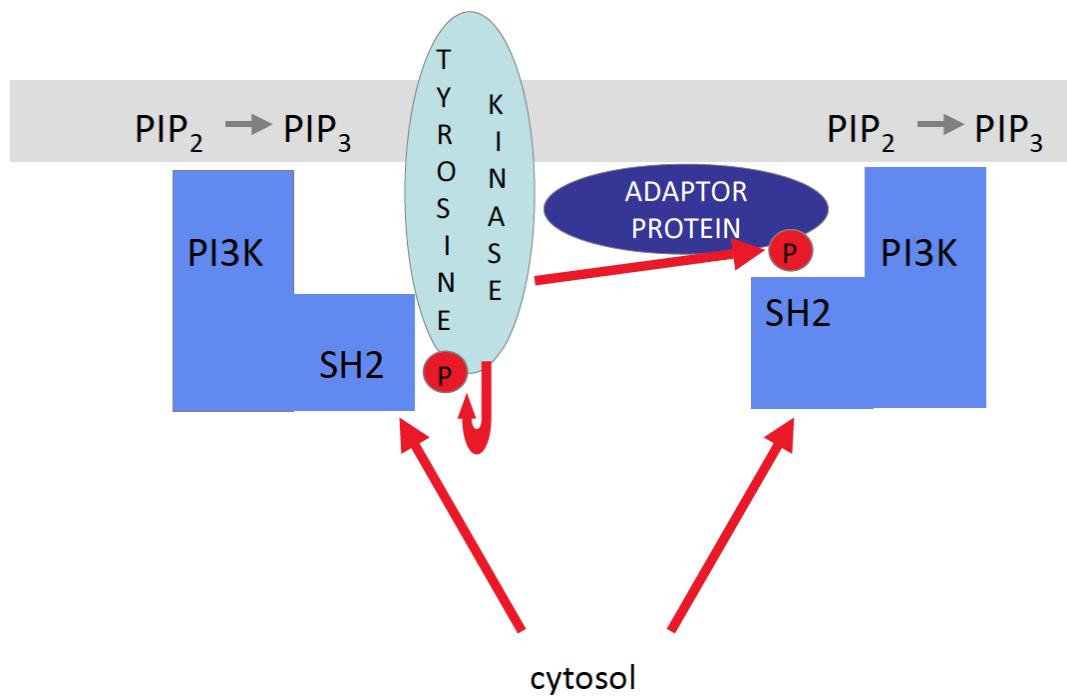
PIP_3 is generated from PIP_2 by the enzyme PI 3-kinase.



Adapted from Figure 15-63 *Molecular Biology of the Cell* (© Garland Science 2008)

Assembling intracellular signaling complexes

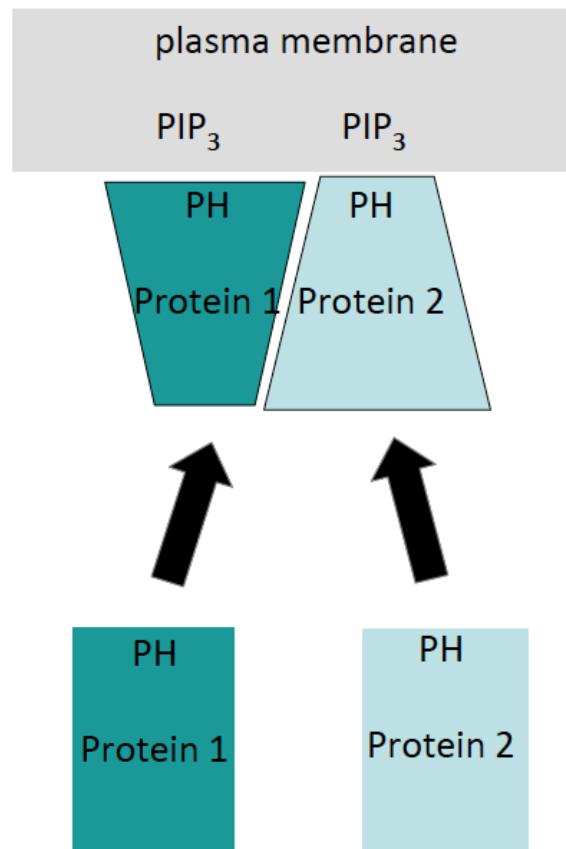
PI 3-kinase (PI 3-K) is recruited to the plasma membrane by the interaction of its SH2 domain with a pY receptor tail.



Assembling intracellular signaling complexes

PIP₃ is recognized by proteins that contain a PH or PX domain.

The membrane phosphoinositides are produced rapidly after activation and are short-lived which are ideal for signaling cascades.



Second Messengers

The activation of some receptors generates small-molecule second messengers (e.g., release of store Ca^{+2}).

These mediators can diffuse throughout the cell, enabling the signal to activate a variety of target proteins.

They also amplify the initial signal, as one activated enzyme can produce hundreds of second messengers.

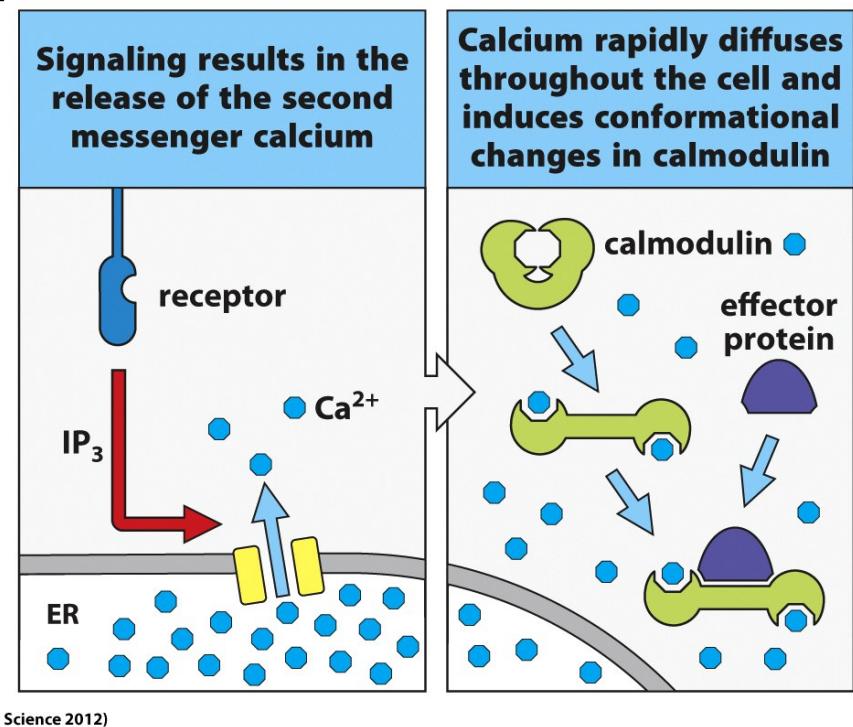


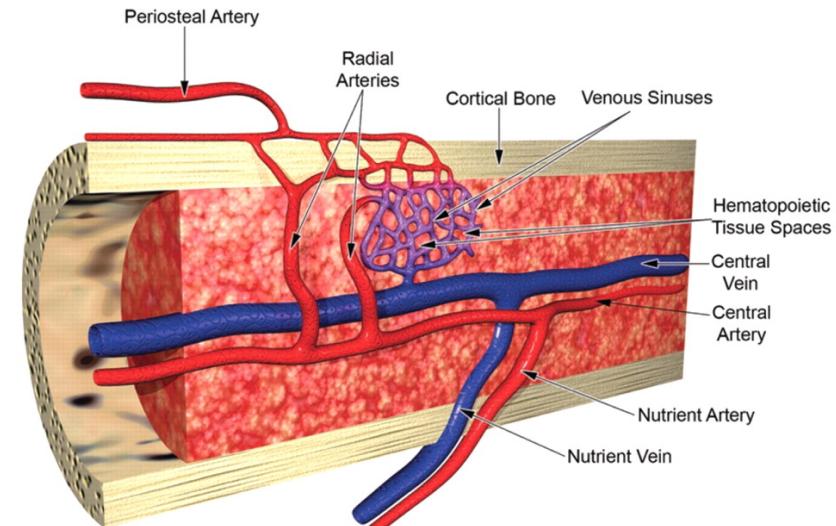
Figure 7.7 Janeway's Immunobiology, 8th edition.

Signaling in the bone marrow

The specialized microenvironment of the bone marrow provides signals both for the development of lymphoid progenitors from HSCs and for the subsequent differentiation of B cells.

Such signals act on the developing lymphocytes to switch on key genes that direct the developmental program.

External signals are provided by the bone marrow stromal cells that interact with the developing lymphocytes.



Adapted from: G.S. Travlos, *Toxicol Pathol* 2006 34: 548.

Signaling in the bone marrow

The stromal cells for specific adhesive contacts with the developing lymphocytes by interactions of cell-adhesion molecules and their ligands.

They also provide soluble and membrane-bound cytokines and chemokines that control lymphocyte proliferation and differentiation.

HSCs first differentiate into MPPs, which can then produce CLPs and CMPs.

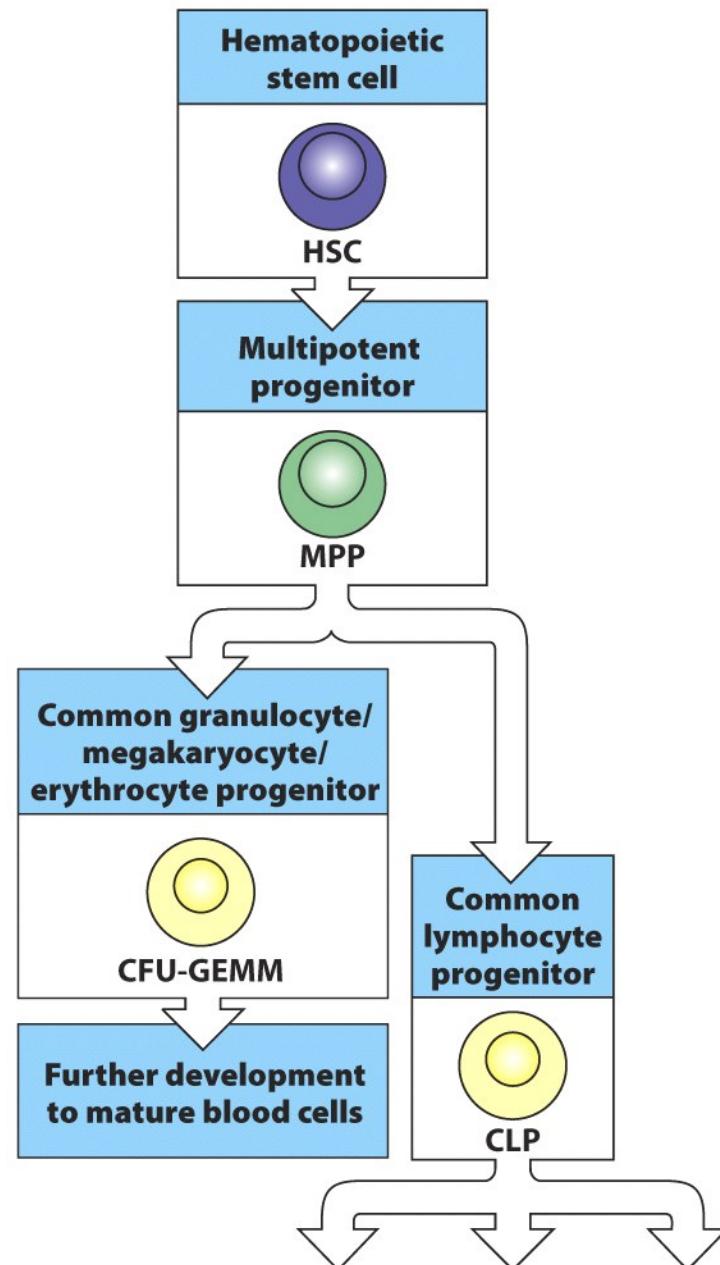


Figure 8.2.1 Janeway's Immunobiology, 8th edition.

Figure 8.2 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Signaling in the bone marrow

MPPs express a cell-surface receptor Y kinase known as FLT3 that binds the membrane-bound FLT3-ligand on the stromal cells.

Signaling through FTL3 is needed for differentiation to the next stage, CLP.

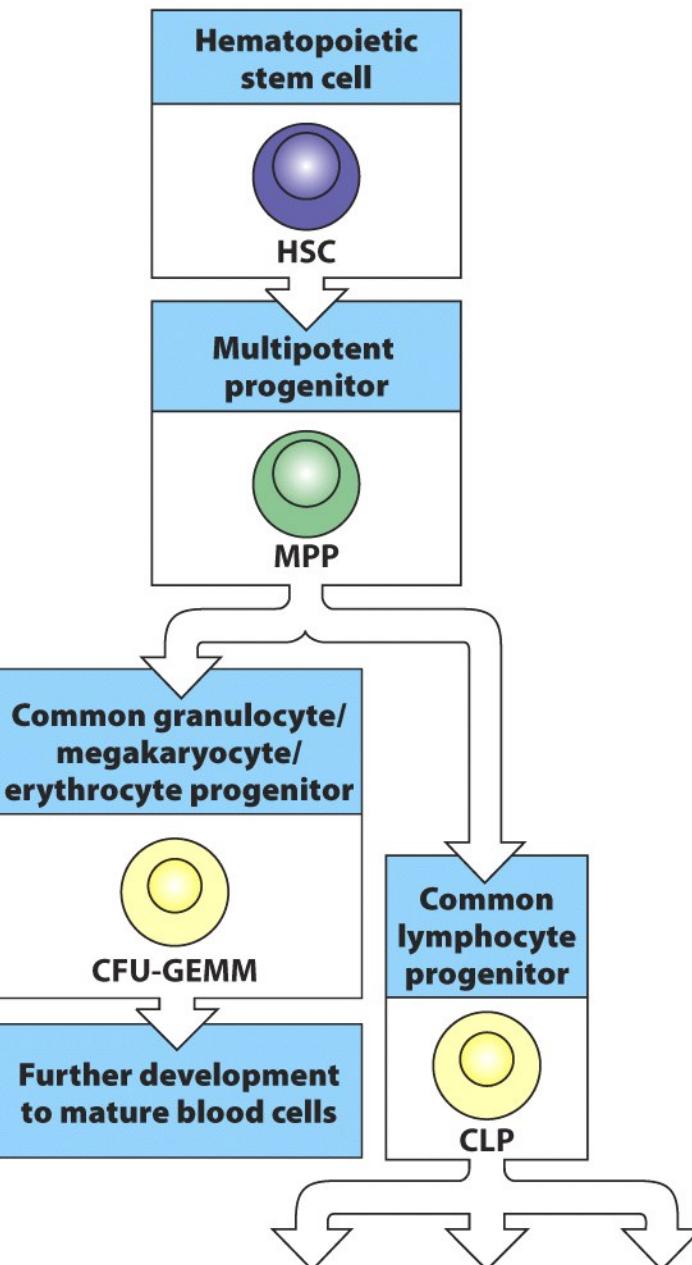


Figure 8.2.1 Janeway's Immunobiology, 8th edition.

Figure 8.2 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Signaling in the bone marrow

Lymphocyte differentiation is accompanied by the expression of the IL-7 receptor (which was induced by FTL3 signaling together with the activity of the transcription factor PU.1.).

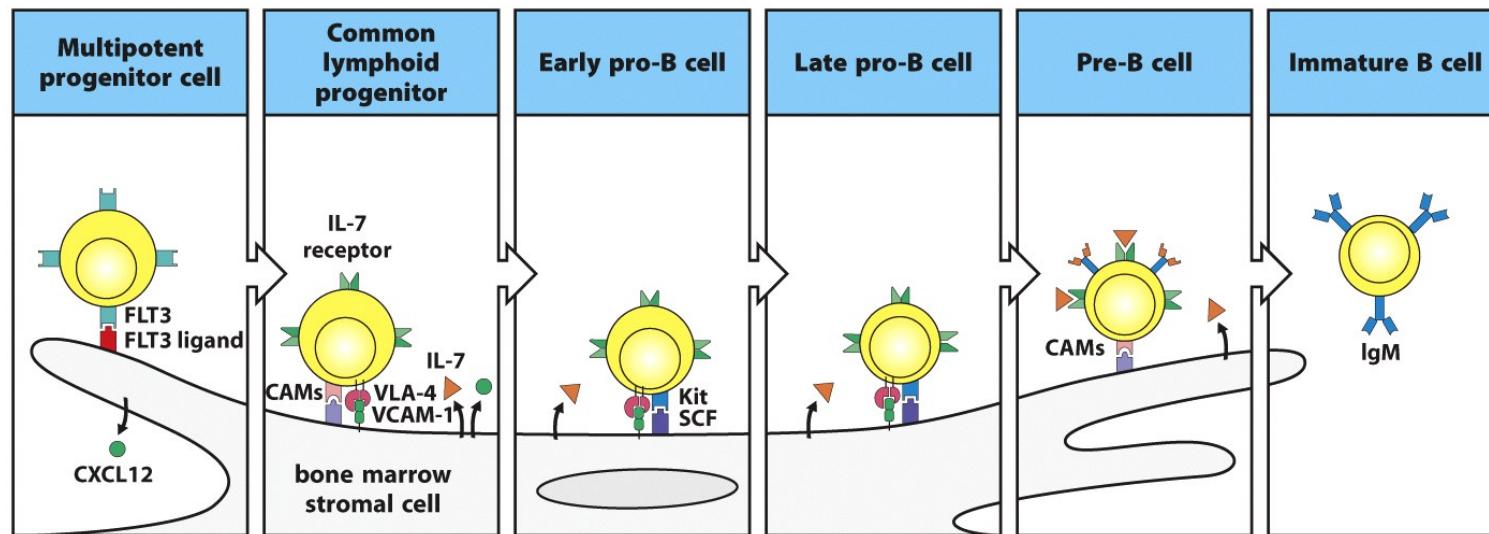


Figure 8.3 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

IL-7 is a cytokine and is secreted by the stromal cells, is essential for growth and survival of the developing B cells (in mice, maybe humans) and developing T cells (in mice and humans).

Figure 8.3 Janeway's Immunobiology, 8th edition.

Signaling in the bone marrow

Stem-cell factor (SCF) a membrane-bound cytokine that is also present on the stromal cells. It stimulates growth of HSCs and the earliest B-lineage progenitors.

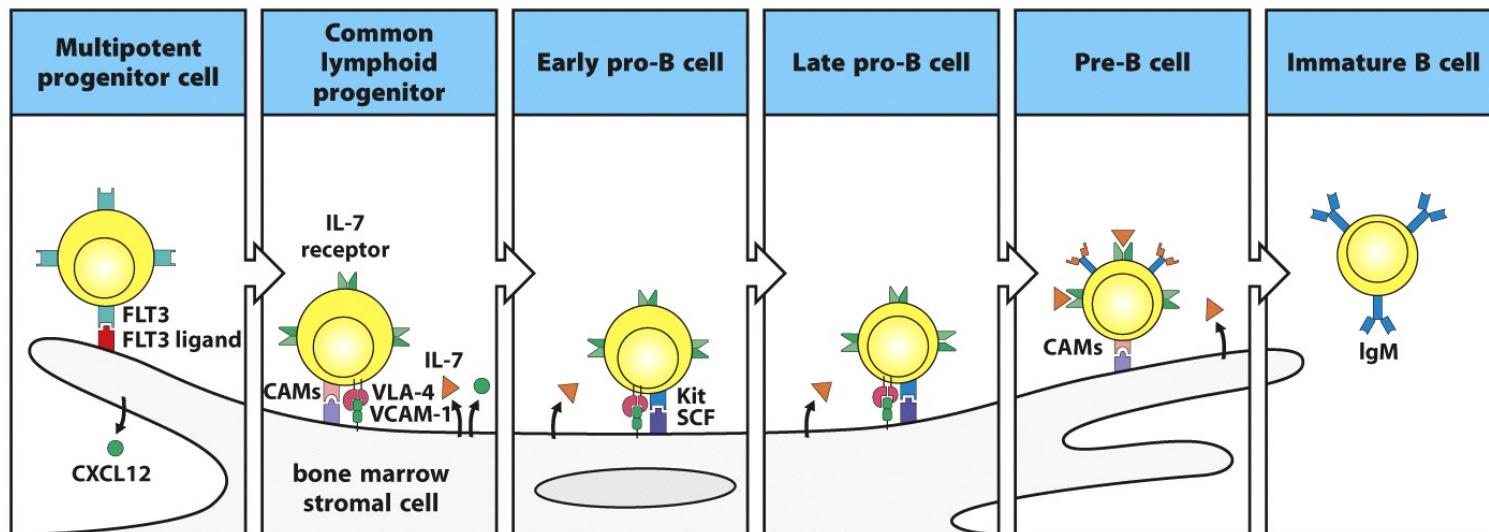


Figure 8.3 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

SCF interacts with a receptor tyrosine kinase Kit on the precursor cells.

The stromal cells also produce chemokine CXCL12 to retain developing B cells precursors in the bone marrow environment.

Figure 8.3 Janeway's Immunobiology, 8th edition.

Signaling in the bone marrow

The CLP gives rise to the earliest B-lineage cell, the pro-B cell.

The definitive B cell fate is specified by the induction of the B-lineage specific transcription factors E2A and the early B cell factor (EBF).

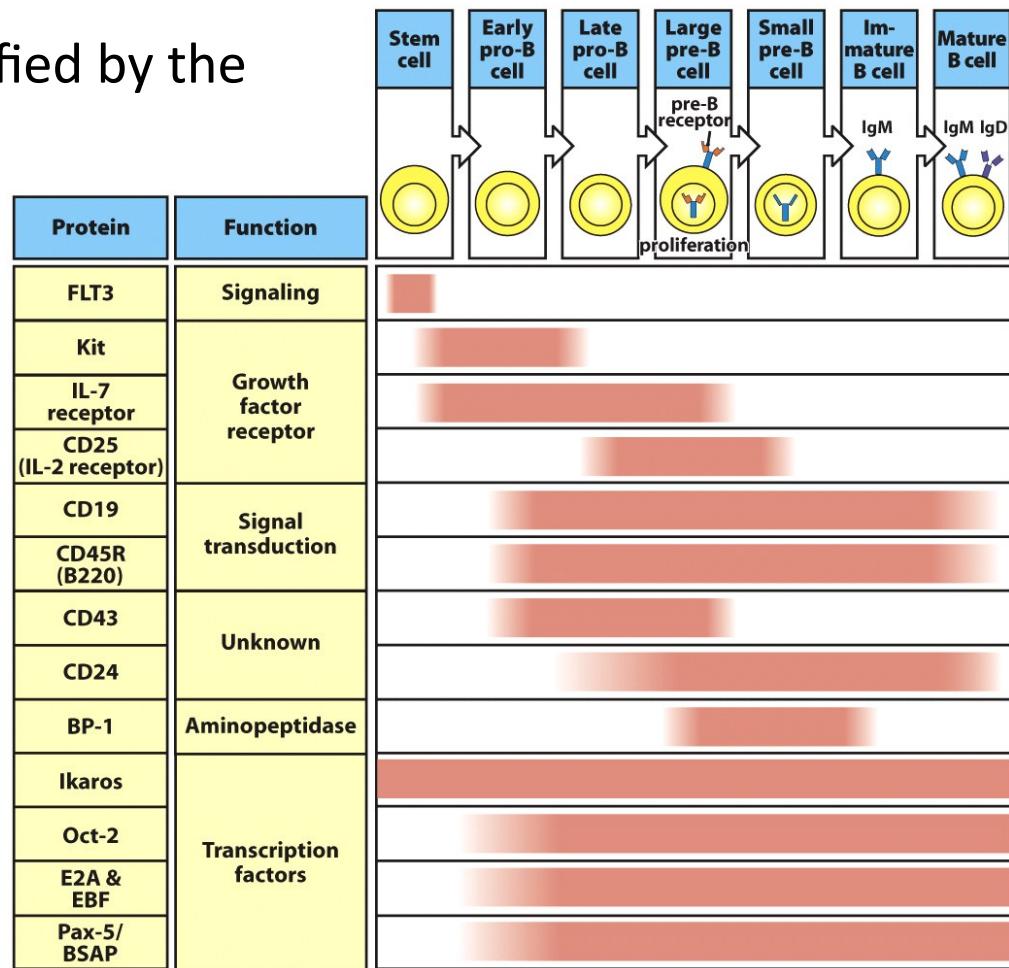


Figure 8.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 8.5 Janeway's Immunobiology, 8th edition.

Signaling in the bone marrow

IL-7 signaling is through to promote expression of E2A, which cooperates with PU.1 to induce expression of EBF.

E2A and EBF drive the expression of proteins that determine the pro-B cell state.

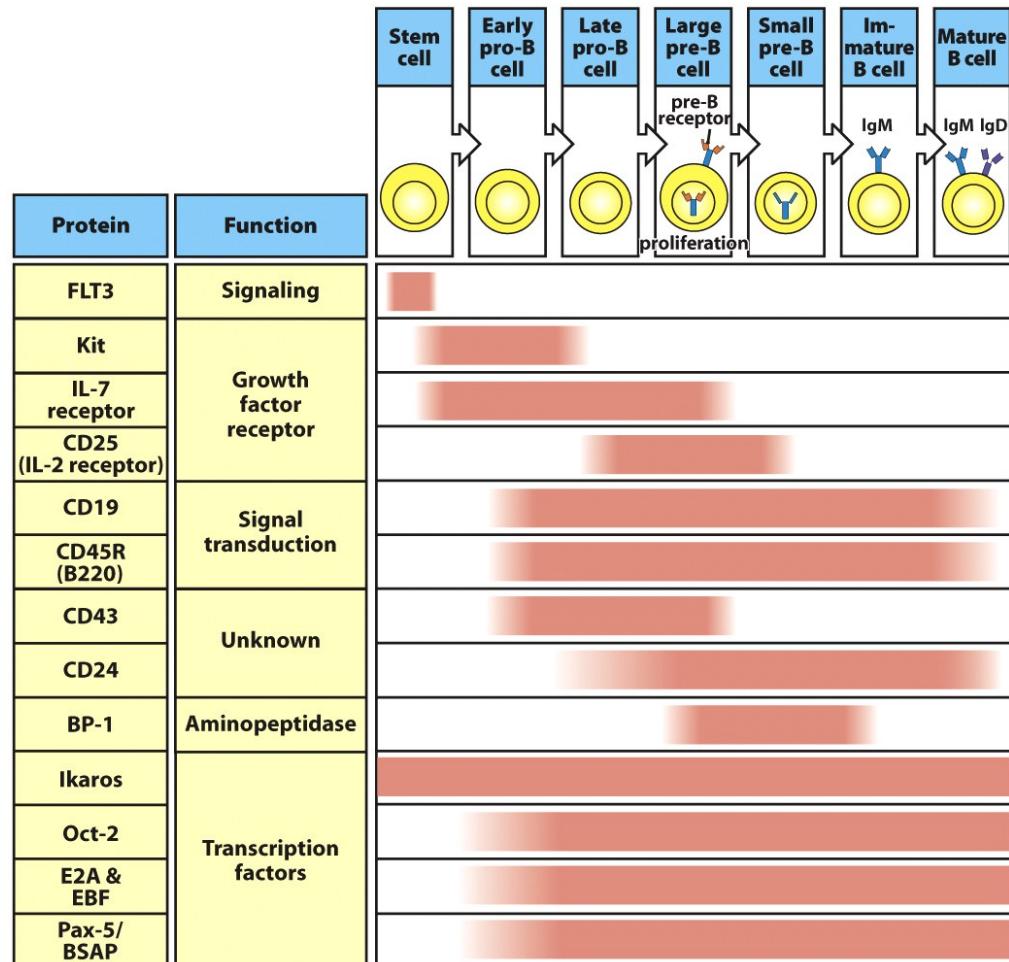


Figure 8.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 8.5 Janeway's Immunobiology, 8th edition.

Signaling in the bone marrow

As the B-lineage cells matures, they migrate within the bone marrow, remaining in contact with the stromal cells.

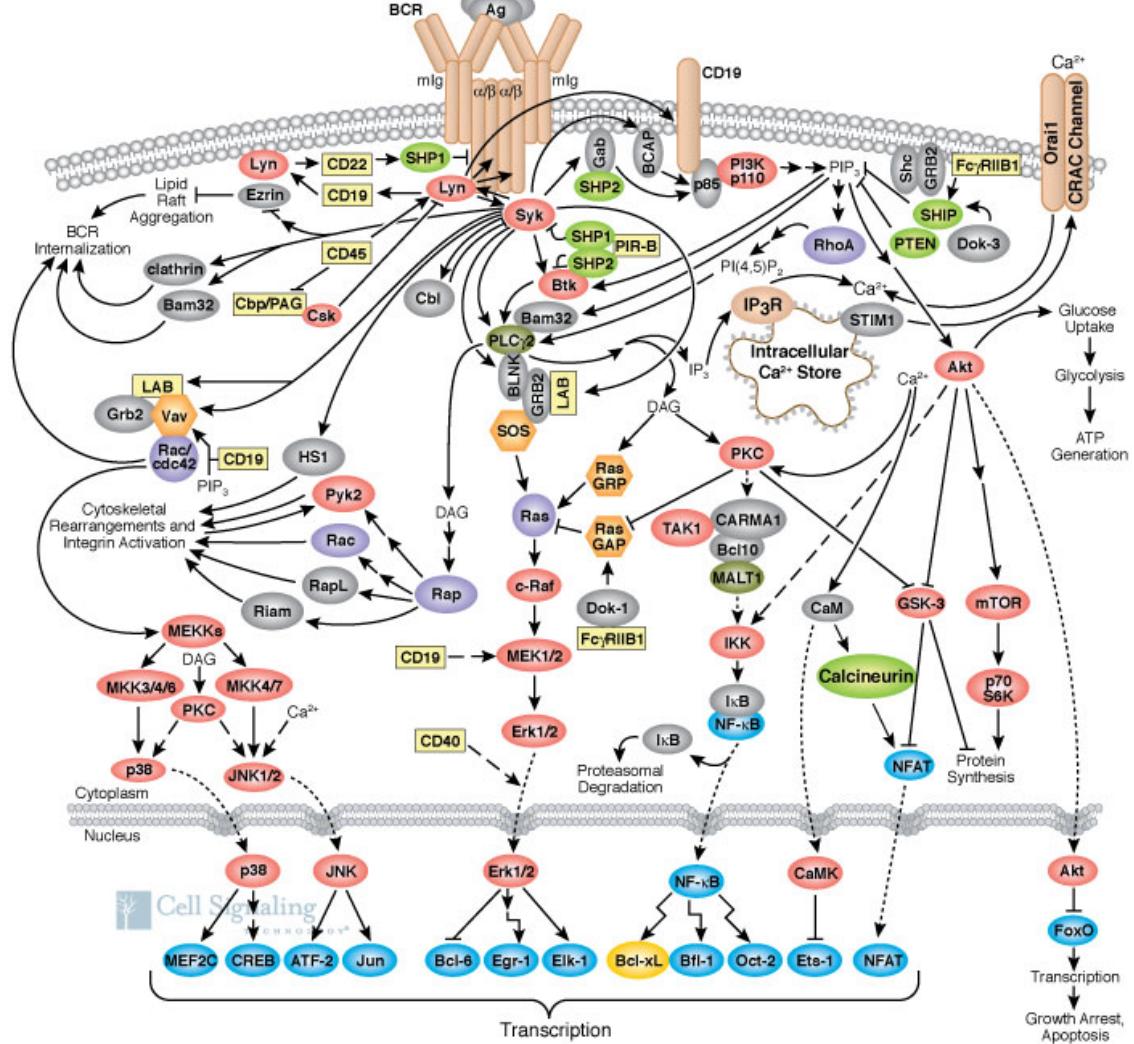
The earliest stem cells lie in a region called the endosteum, which is adjacent to the inner surface of the bone and as they develop into B cells, they move toward the central sinus of the bone marrow cavity.

During this time, the B cells will be rearranging their H and L chains to make the functional BCR.

One cell, same receptor engaged, two different outcomes

Immature B cell

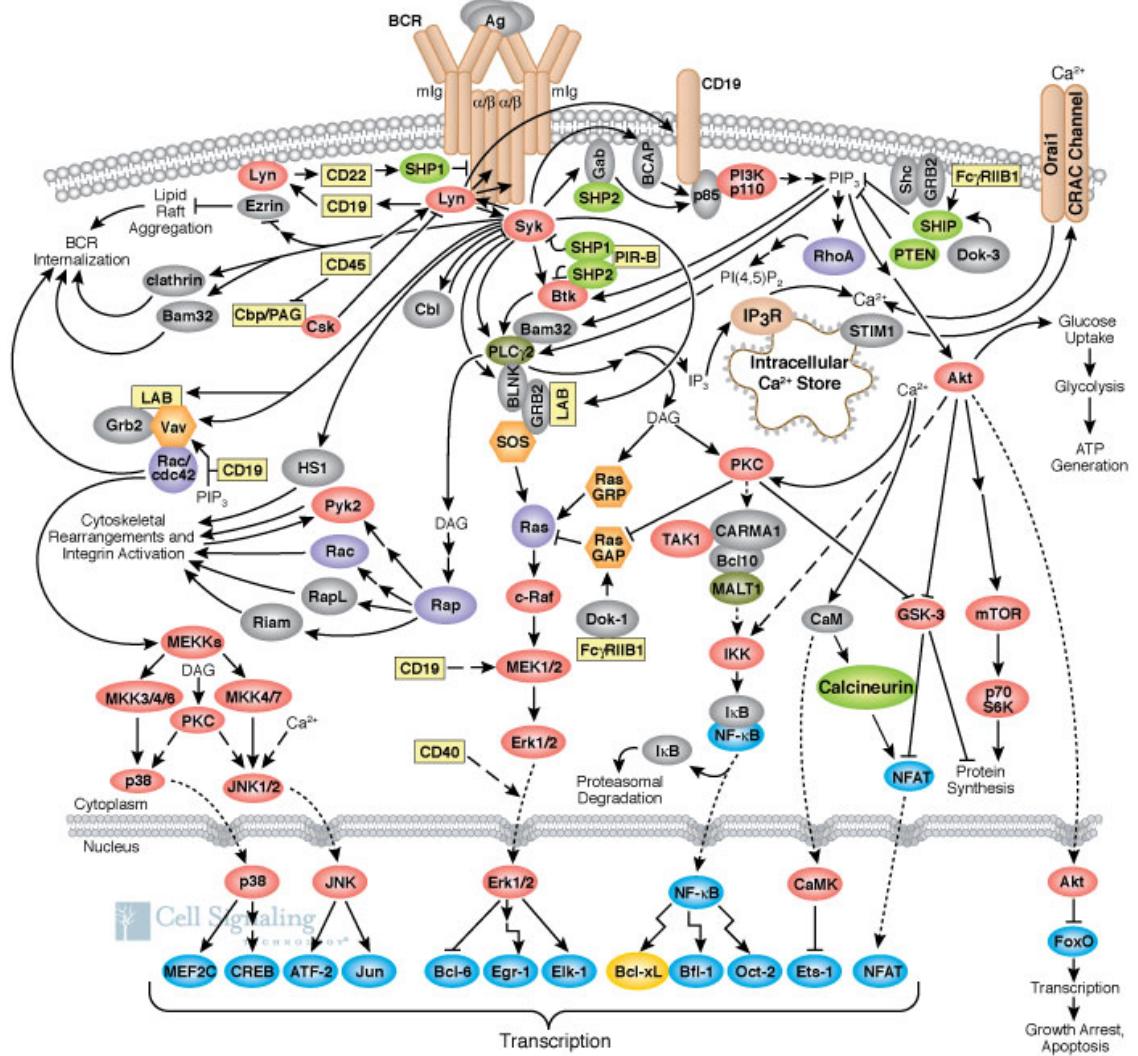
- BCR engaged while in the bone marrow environment
- most likely encounter with a self antigen
- outcome - apoptosis



One cell, same receptor engaged, two different outcomes

Mature B cell

- BCR engaged while in the spleen or lymph node
- most likely foreign antigen
- outcome - survival
- proliferation and differentiation to plasma cell - Ab production



One cell, same receptor engaged, two different outcomes

How did two very different outcomes result from the engagement of the same receptor on the B cell?

How did the immature B cell “know” it had to commit apoptosis?

How did the mature B cell “know” it should make Ab?

One cell, different outcomes of signaling

Different combinations of signals result in the activation of different (combinations of) pathways give different outcomes.

In the case of the B cells, additional, but different signals would have come from the environment that the B cell was existing in.

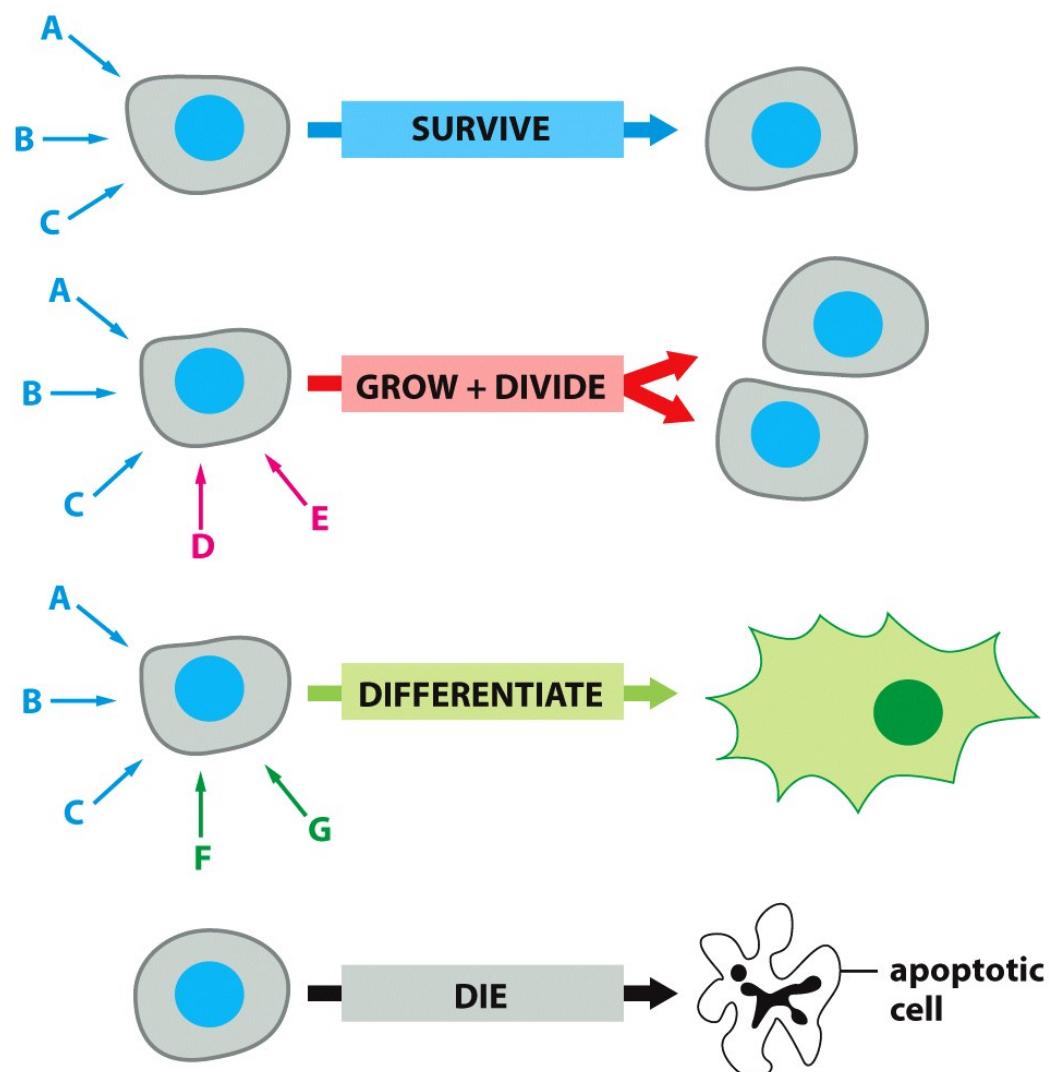
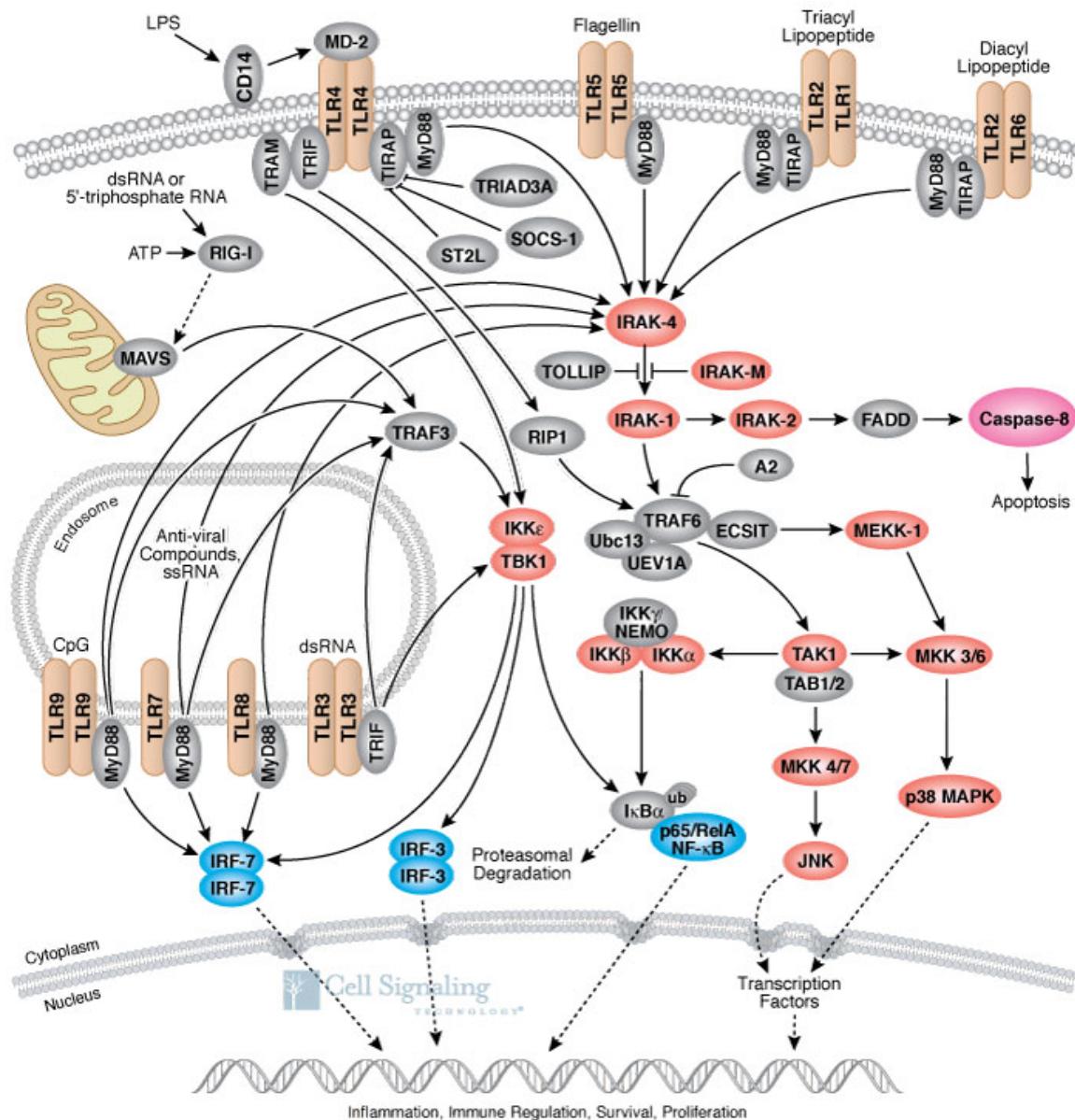


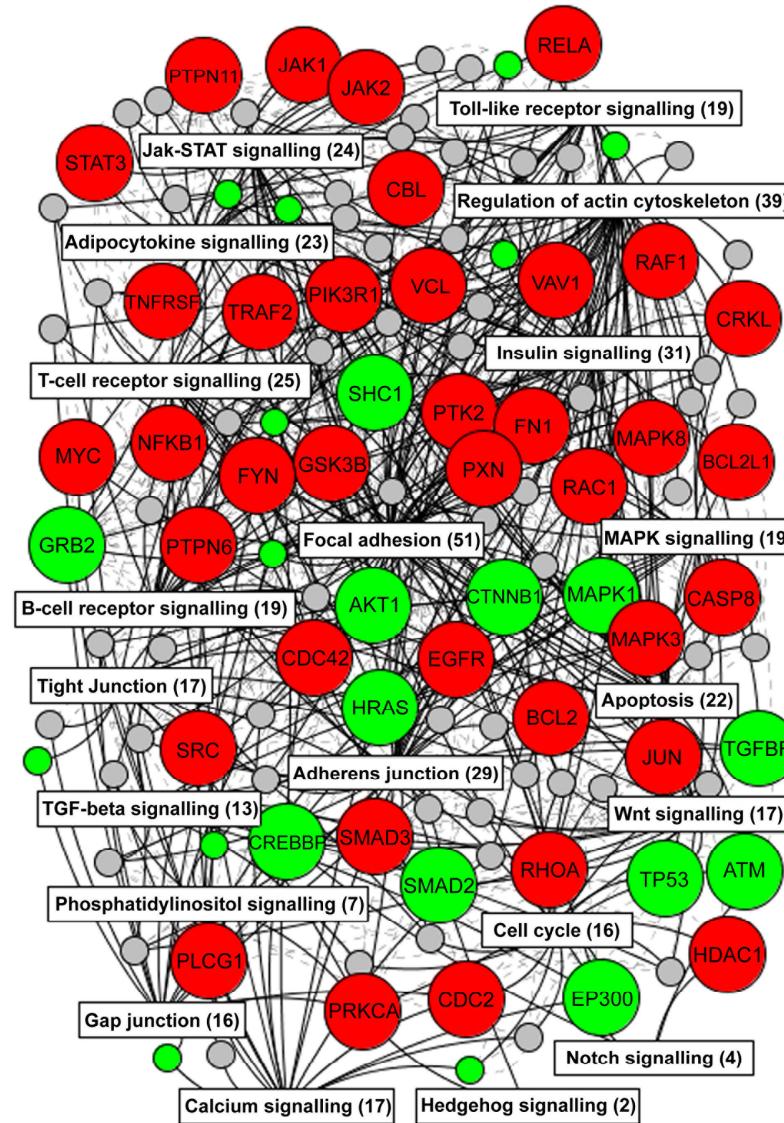
Figure 16-6 *Essential Cell Biology* (© Garland Science 2010)

Signaling pathways are connected to other signaling pathways



http://www.cellsignal.com/reference/pathway/Toll_Like.html

If one tries to connect all signaling pathways together, one can get carried away!



http://netage-project.org/?page_id=42&experiment_id=1

Two basic types of signal transduction pathways

Signal amplification: For each ligand molecule bound, receptor-associated kinases phosphorylate many substrate molecules.

Each step in the pathway results in a larger number of activated components than the previous step

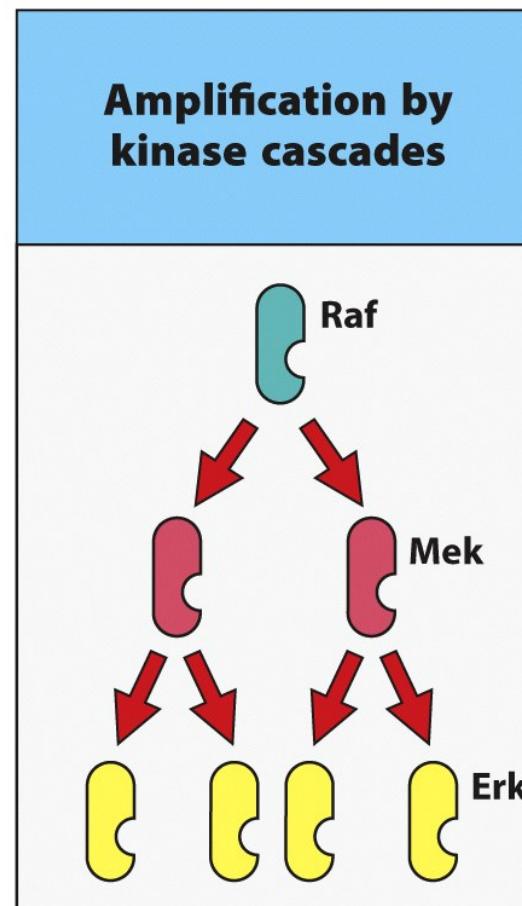


Figure 7.7 Janeway's Immunobiology, 8ed. (© Garland S

Figure 7.7 *Janeway's Immunobiology* (Garland Science 2012)

