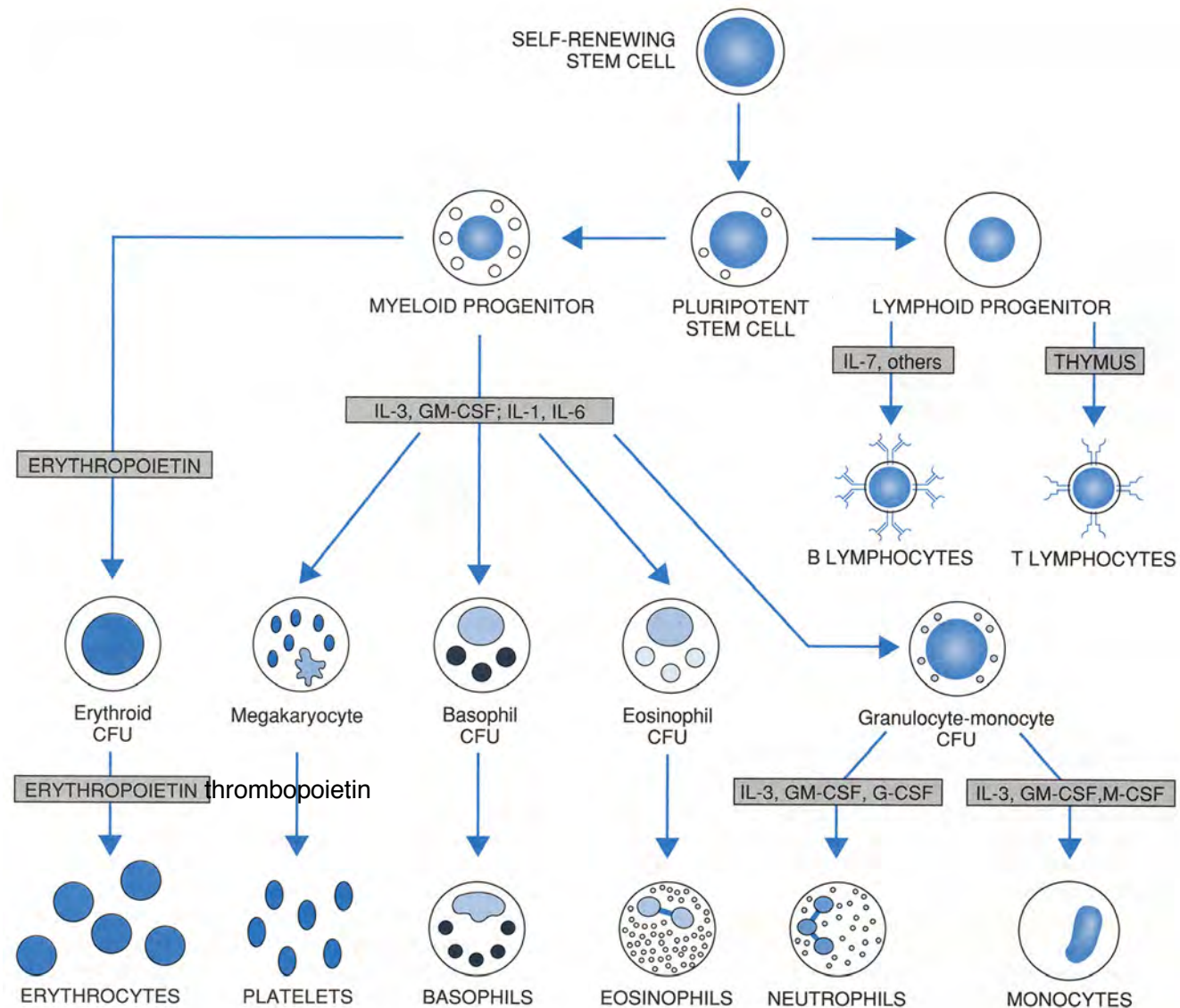


Cytokines regulate interactions between cells of the hemapoietic system

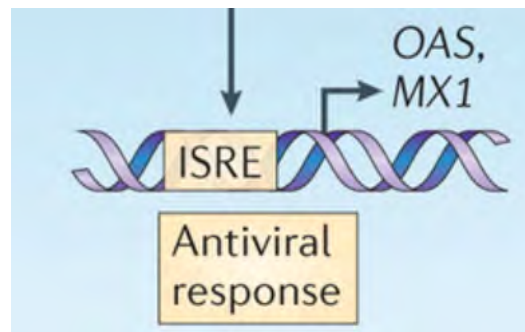
Some well-known cytokines:

- Erythropoietin (Epo)
- G-CSF
- Thrombopoietin
- IL-2
- INF



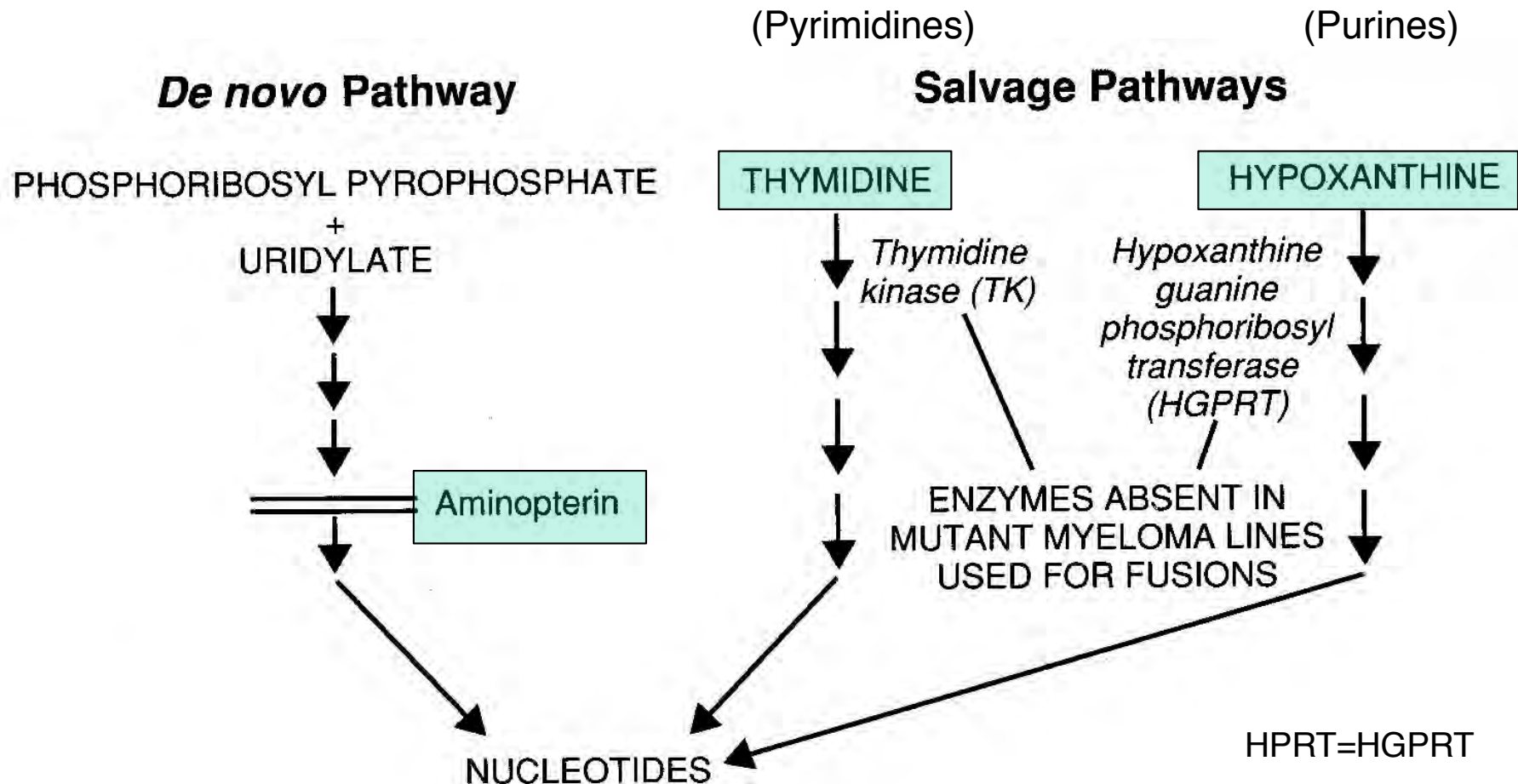
Interferons are cytokines that signal defense against pathogens

- Interferons are a group of secreted cytokines: IFN- α , IFN- β , IFN- γ , etc.
- Released by cells in response to pathogens, including viruses and bacteria.
- Activate natural killer (NK) cells, which kill host cells infected with virus.
- IFN- α induces rapid (15-30 minutes) transcription of target genes.
- Induction can be 50-fold.
- Occurs in absence of protein synthesis (cycloheximide), implying a latent, pre-existing activator.
- ISRE=interferon-stimulated response element



Ivashkiv & Donlin (2014) Nat Rev Immunol

Nucleotide metabolism and drugs



The **salvage pathway** allow nucleotides to be synthesized from degraded intermediates of DNA/RNA

HAT selection:

Aminopterin-blocks *de novo* pathway for purine/pyrimidine synthesis

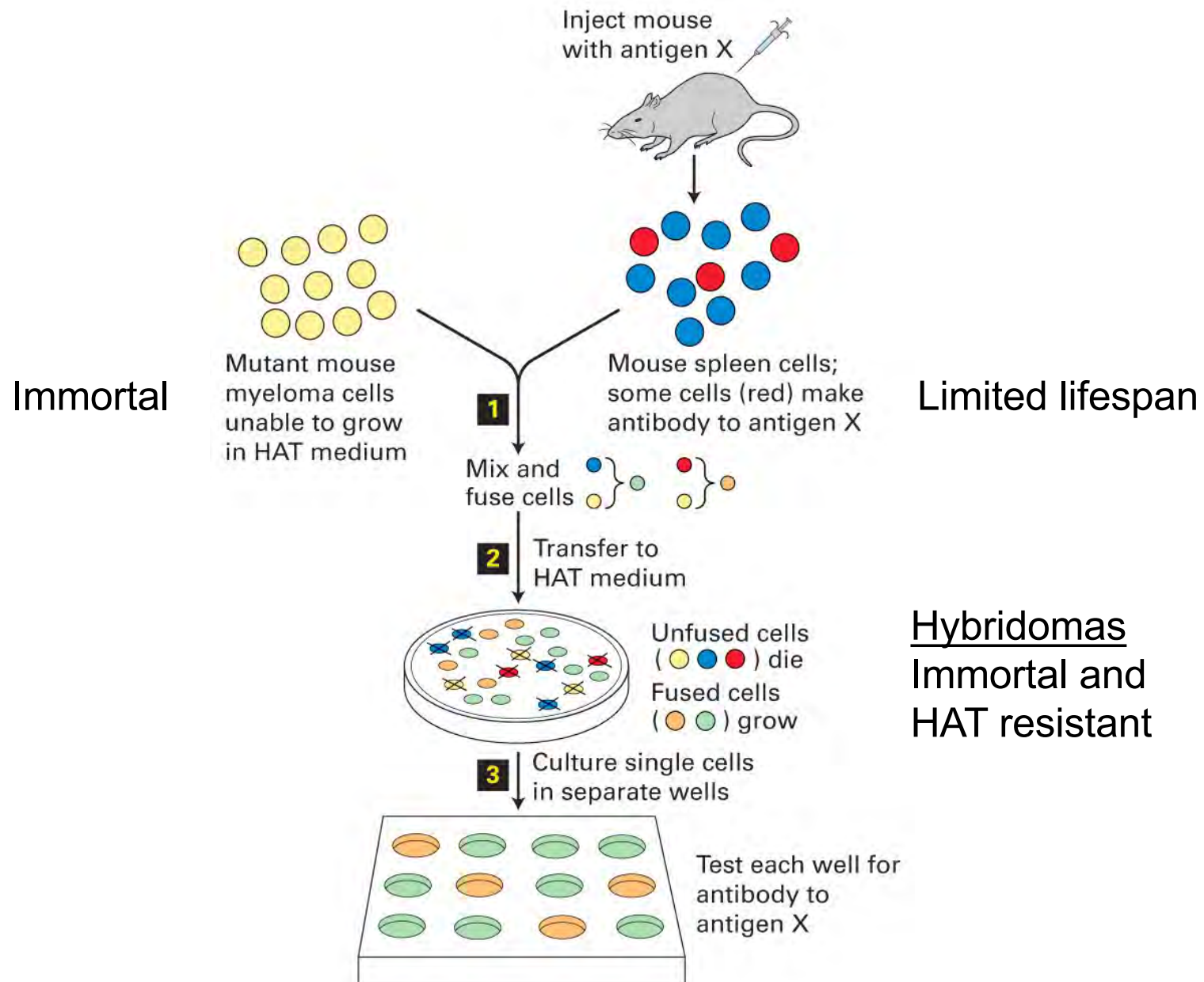
Thymidine and Hypoxanthine: substrates for salvage pathway for purine/pyrimidine synthesis

Normals cells survive in HAT; HPRT- cells die in HAT

6-TG (thioguanine) is metabolized through salvage (HPRT) pathway;

feedback inhibition of *de novo* pathway and incorporation into RNA/DNA account for toxicity

HAT selection is used to select for hybridomas



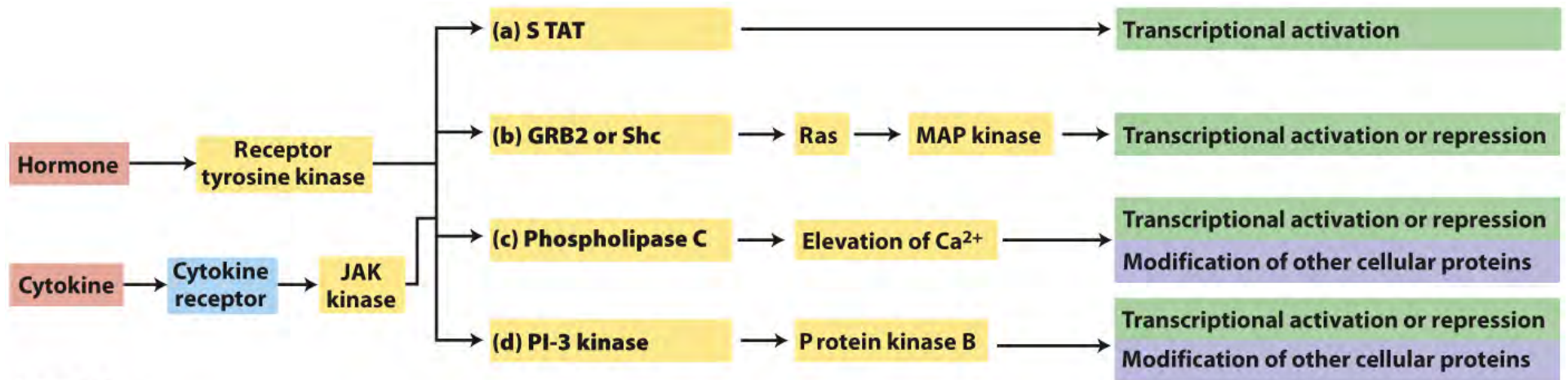
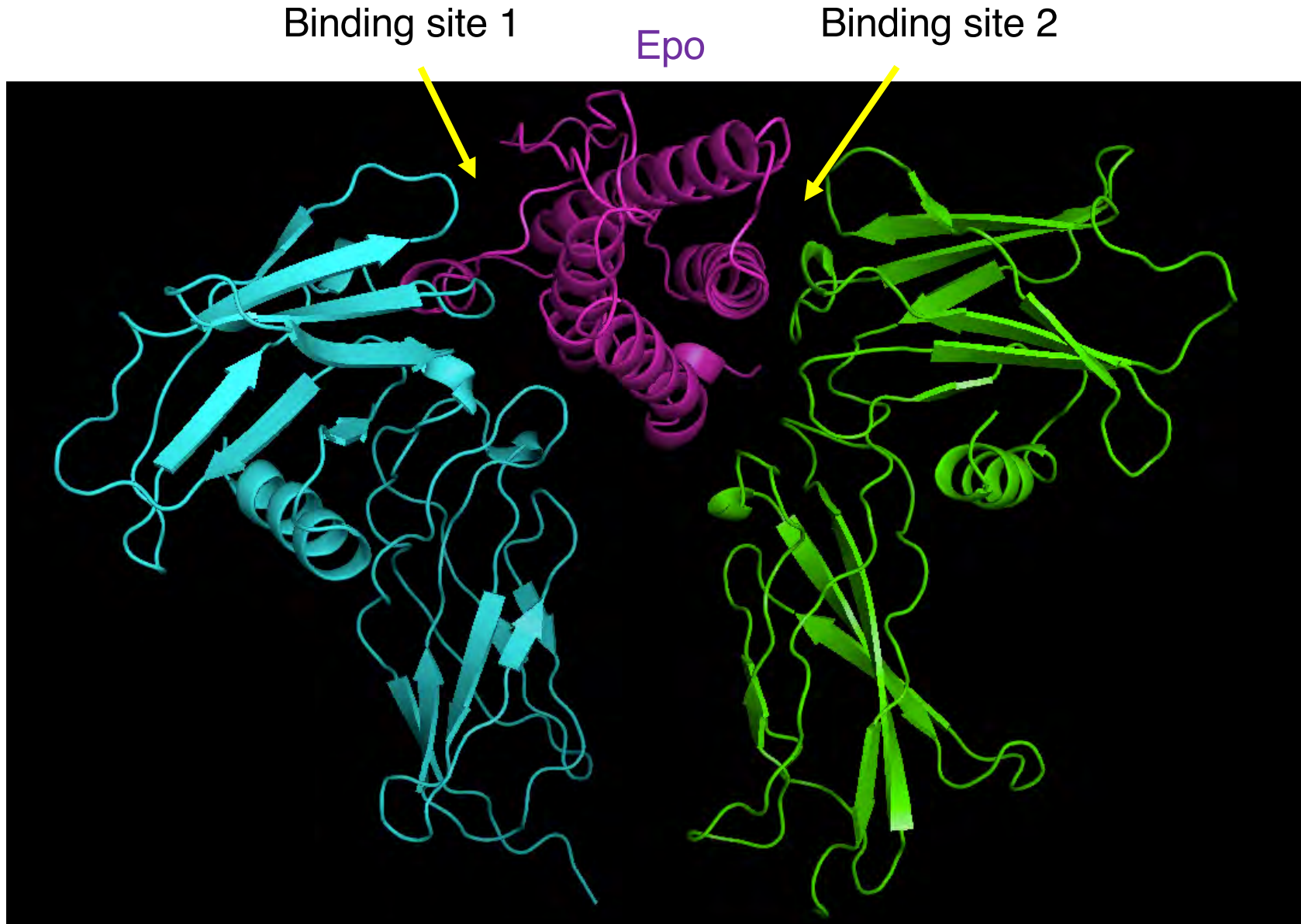


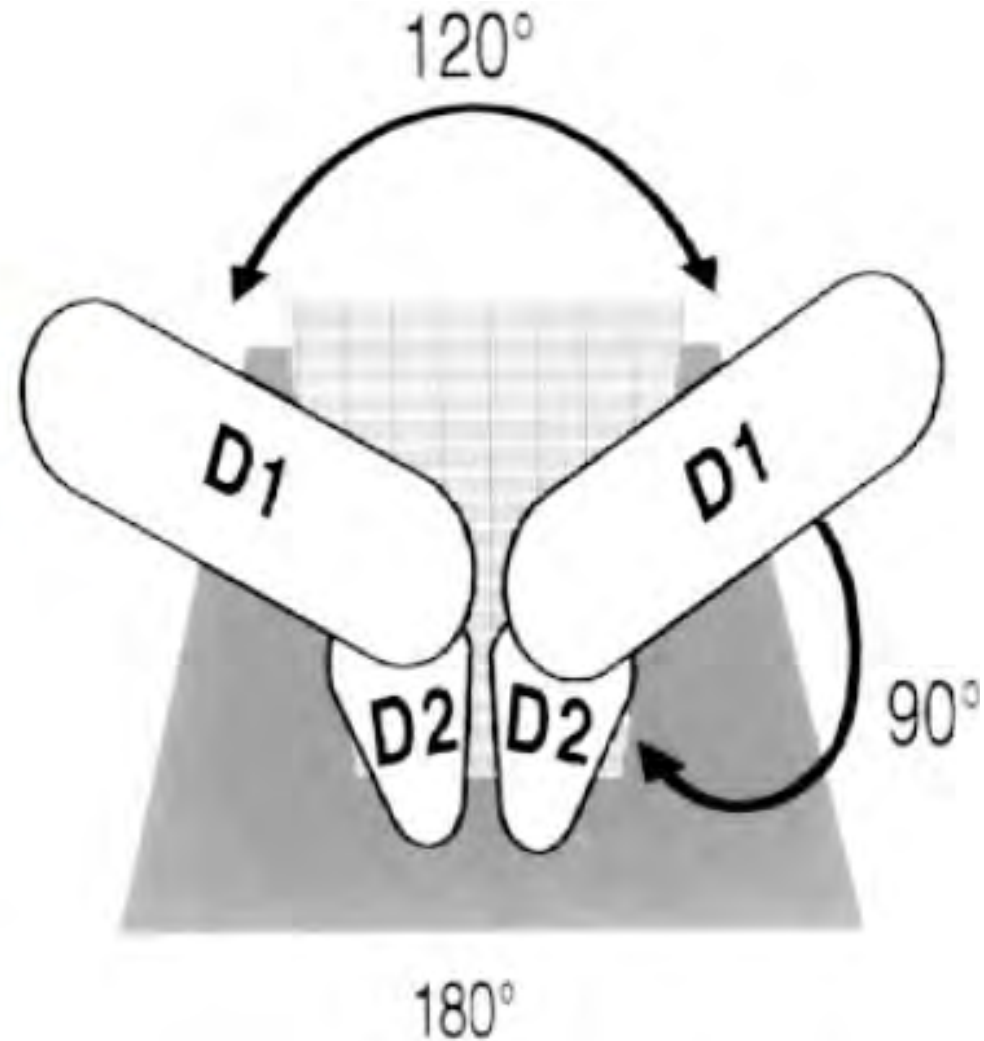
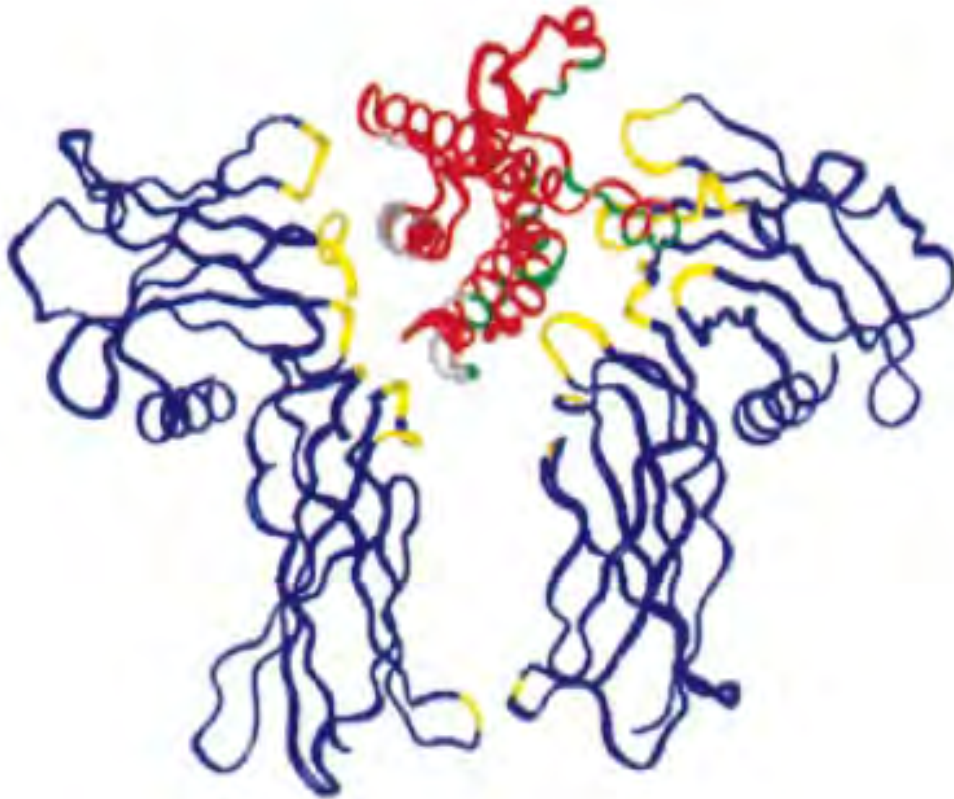
Figure 16-6
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Homodimerization of EpoR by ligand

- Each EpoR binds a distinct site on Epo
- Epo is not a symmetric molecule
- This mode of binding orients the EpoR dimer for optimal activation

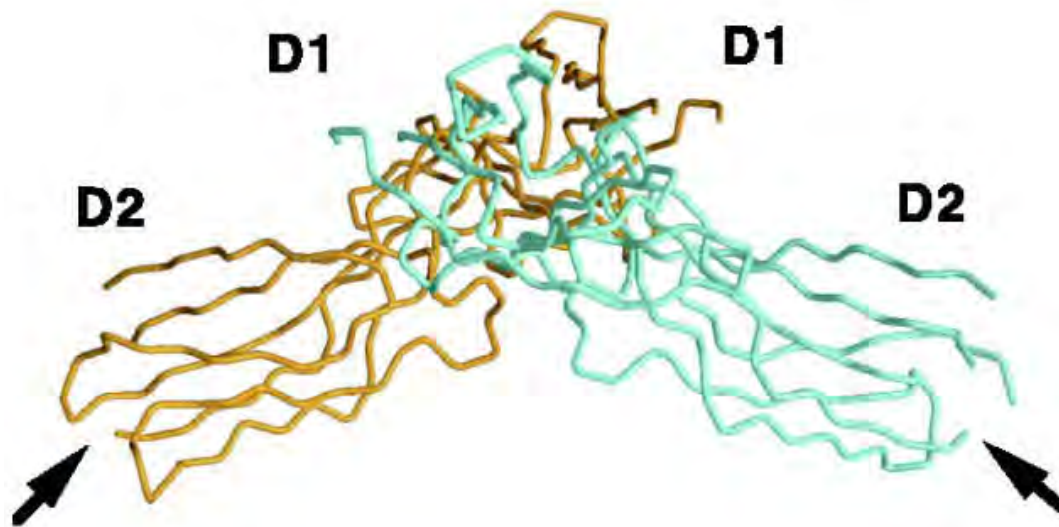


Homodimerization of EpoR by ligand

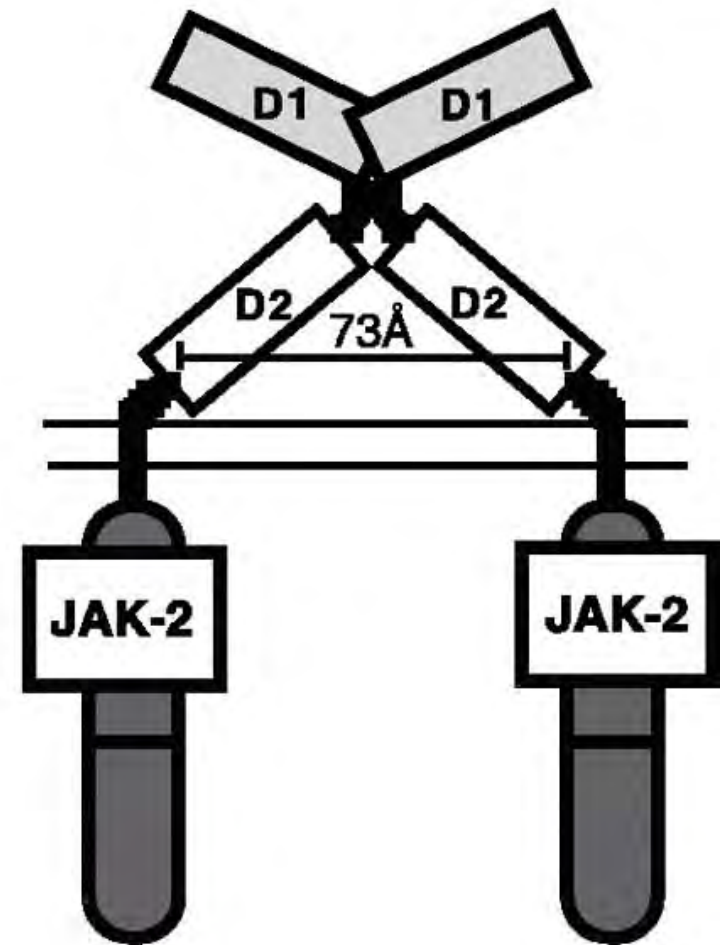


- The natural ligand, Epo, orients the receptors in an orientation that many peptide mimics do not.

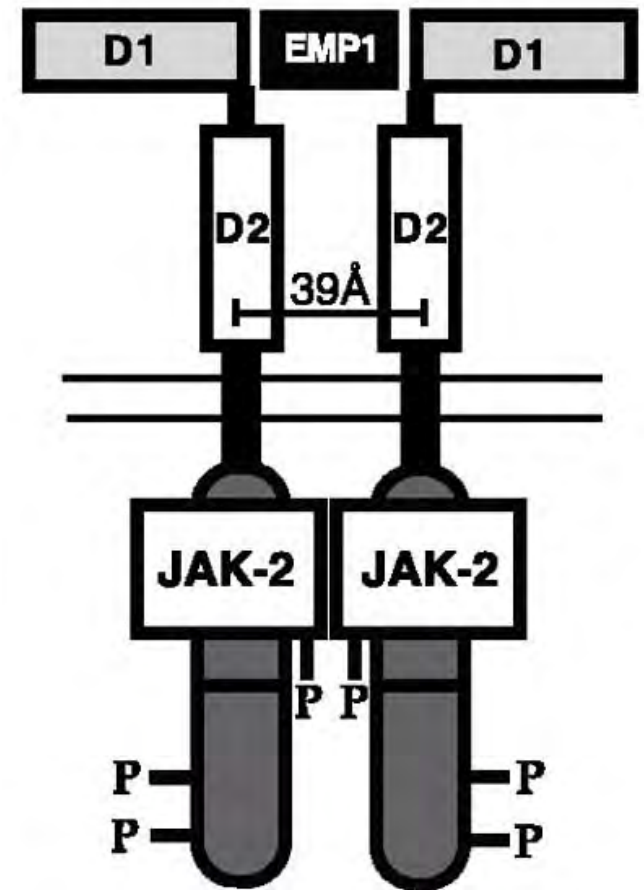
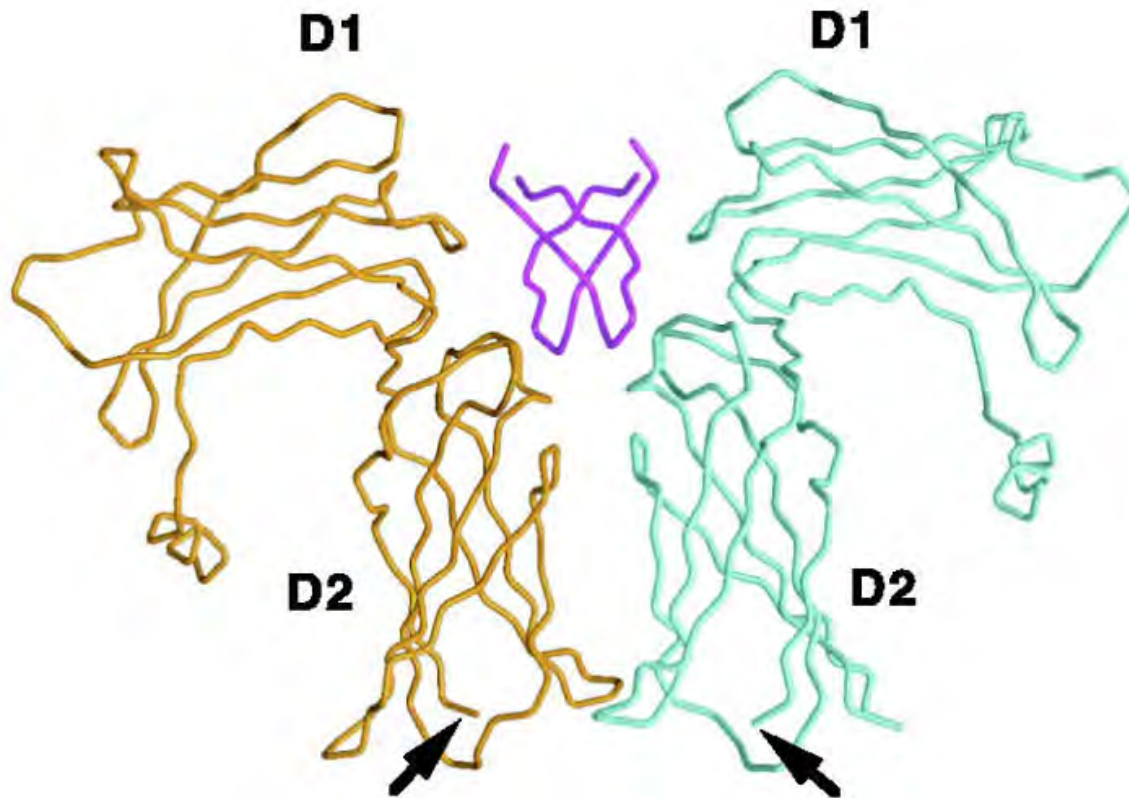
Unliganded EpoR is a preformed, cross shaped-dimer



EpoR once thought to be dimerized
by ligand binding



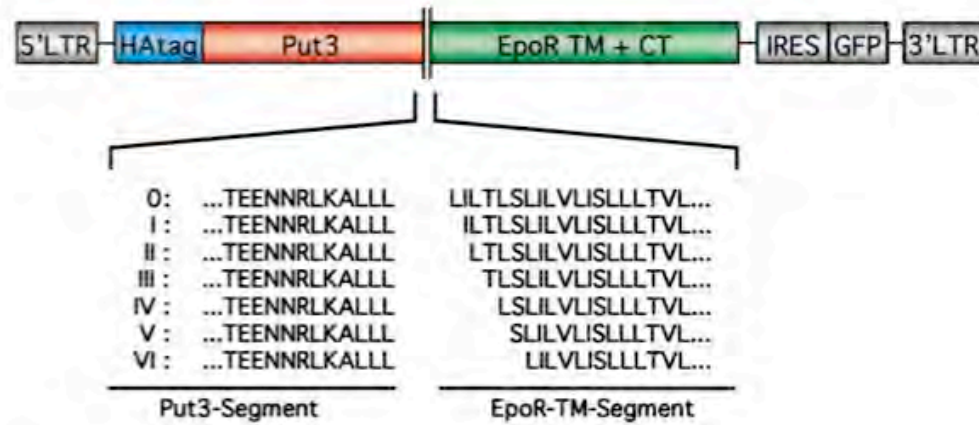
Crystal structure of EpoR bound to peptide agonist



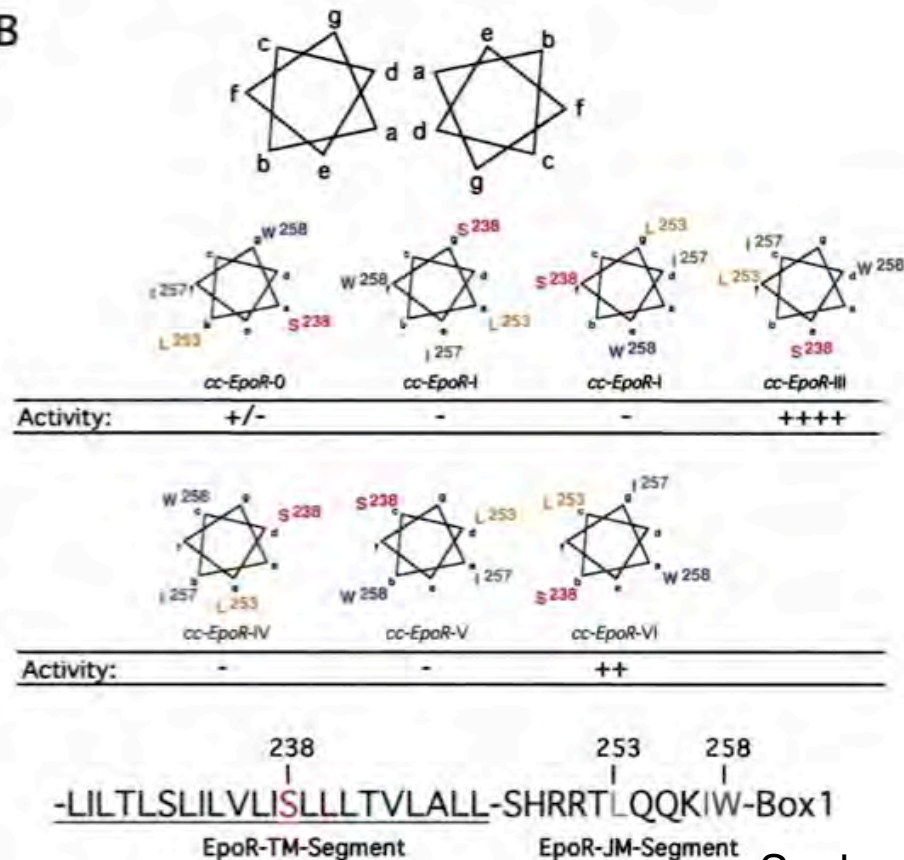
Livnah et al. (1999) Science 283:987

Probing orientations of EpoR

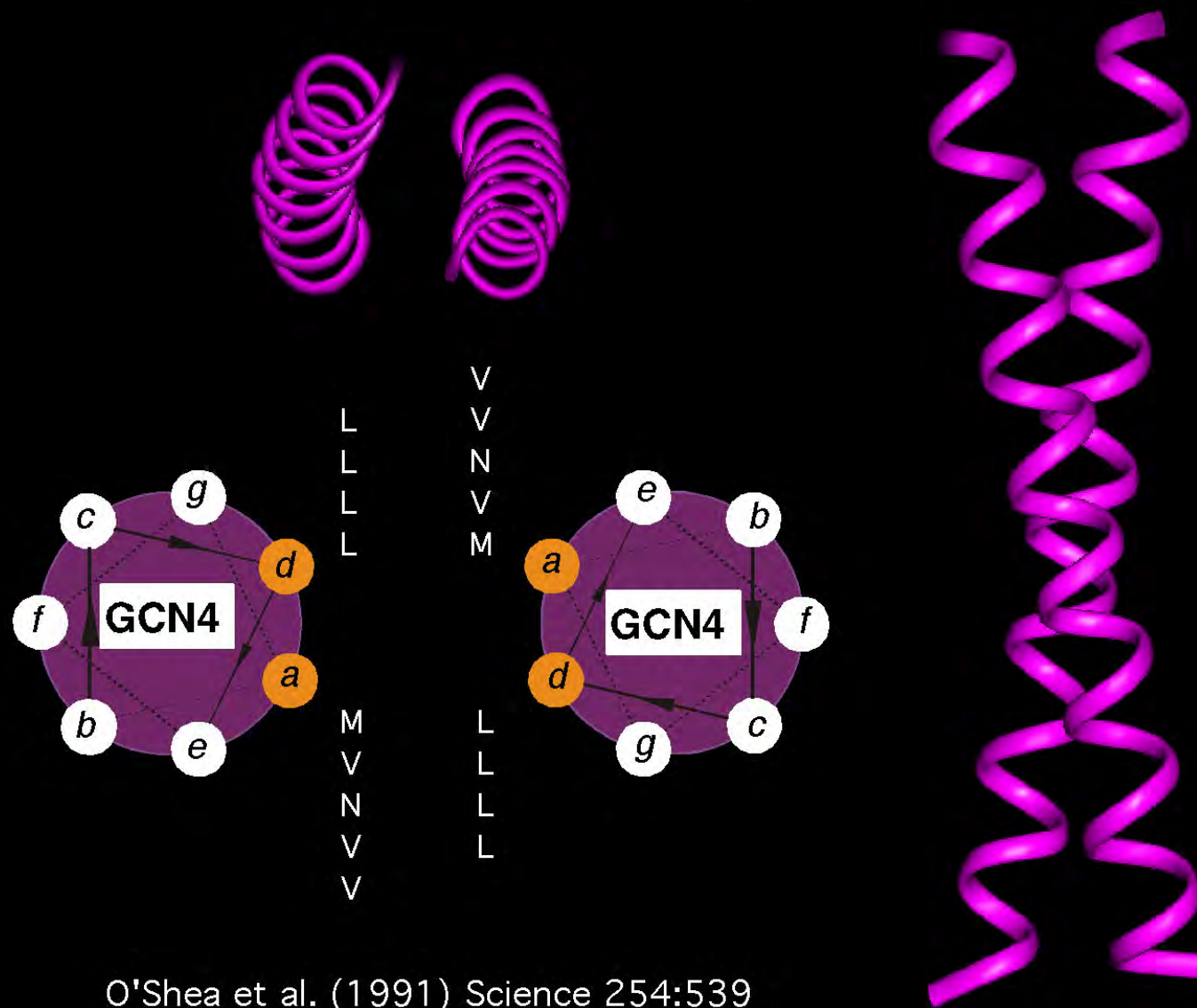
A



B



Coiled coiled are stabilized by hydrophobic packing
in the core *a* and *d* positions



O'Shea et al. (1991) Science 254:539

Activation of cytokine receptors by ligand

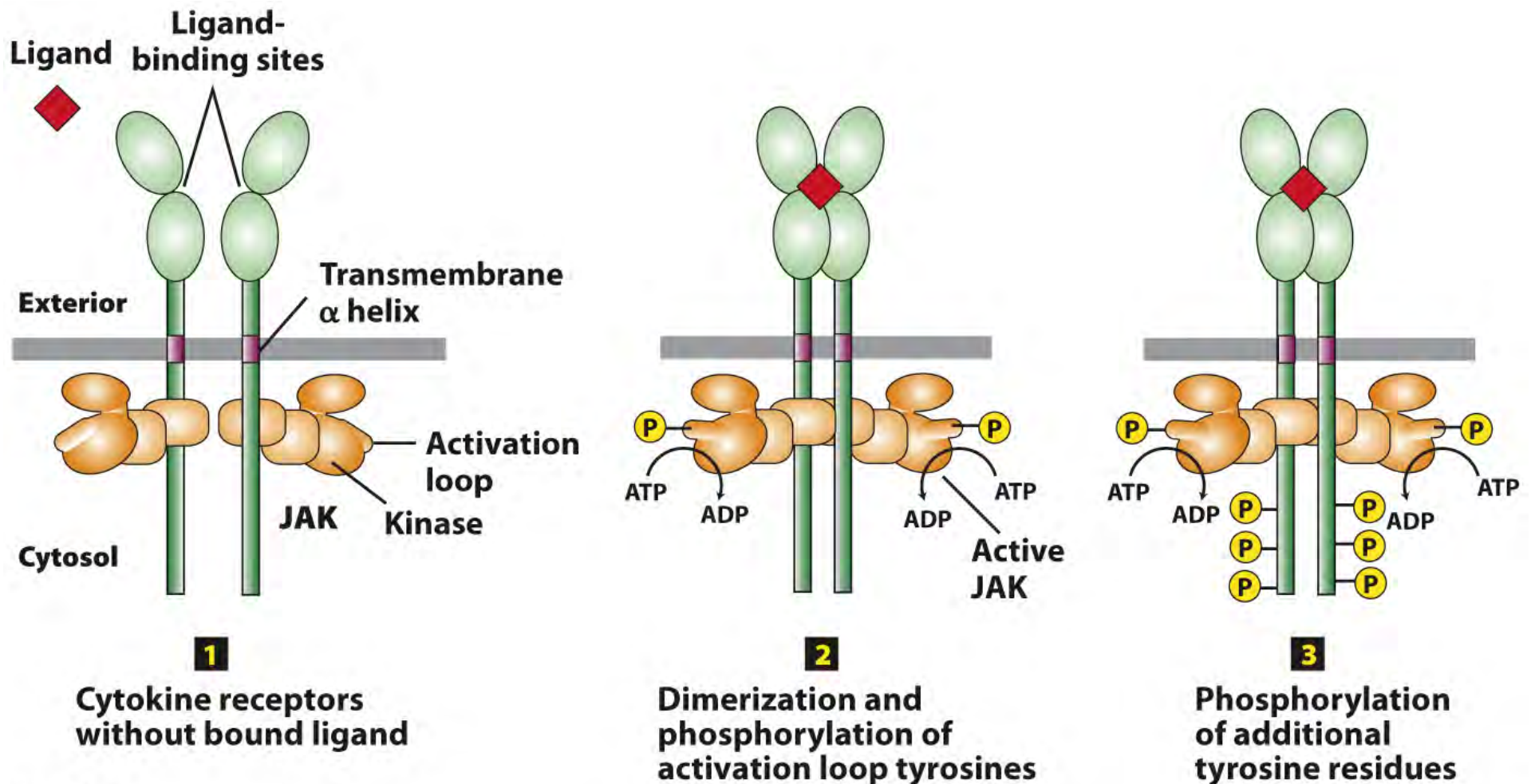
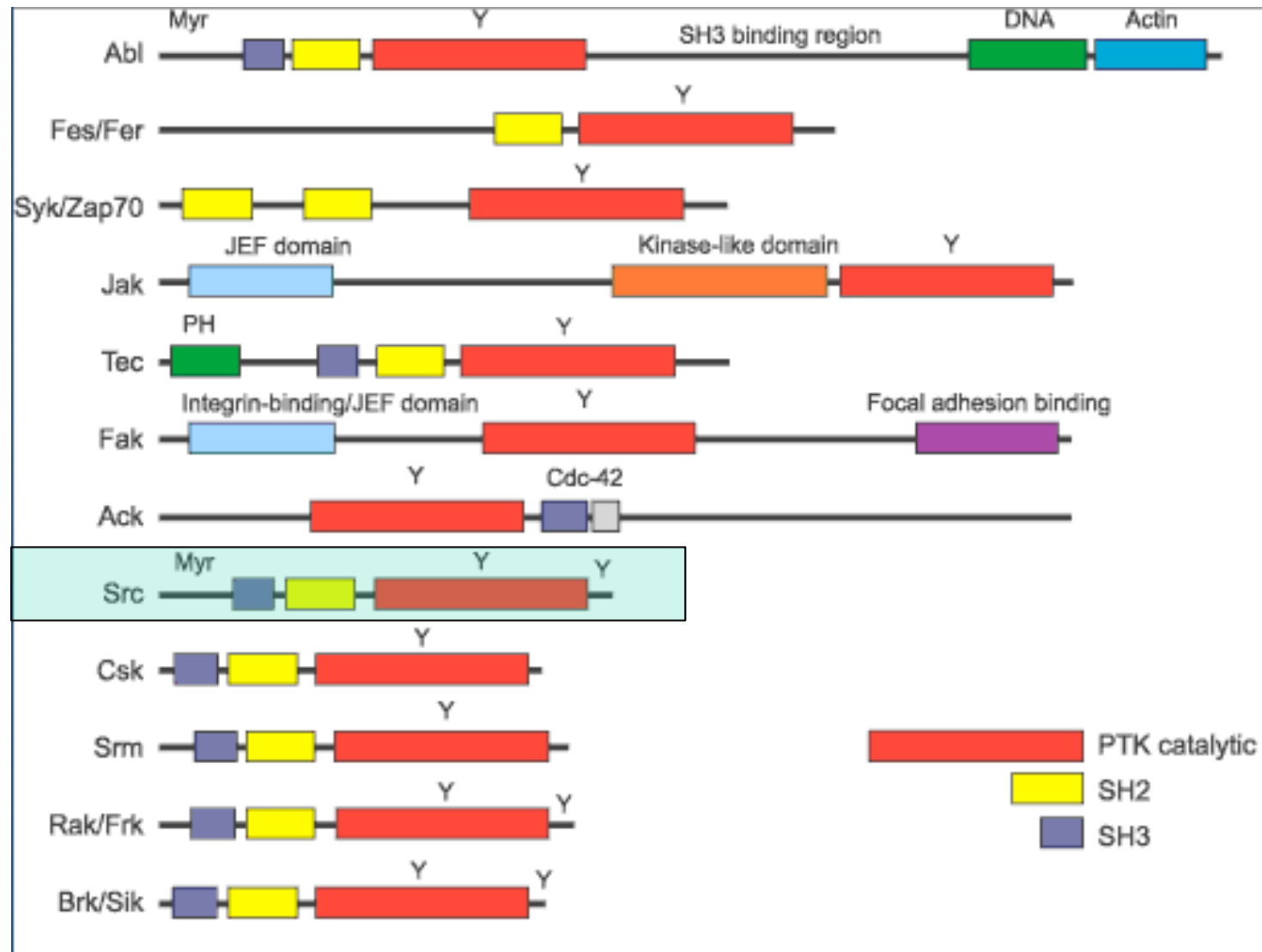


Figure 16-10
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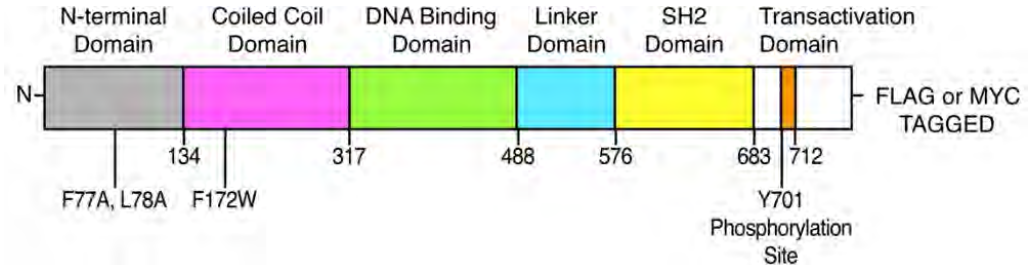
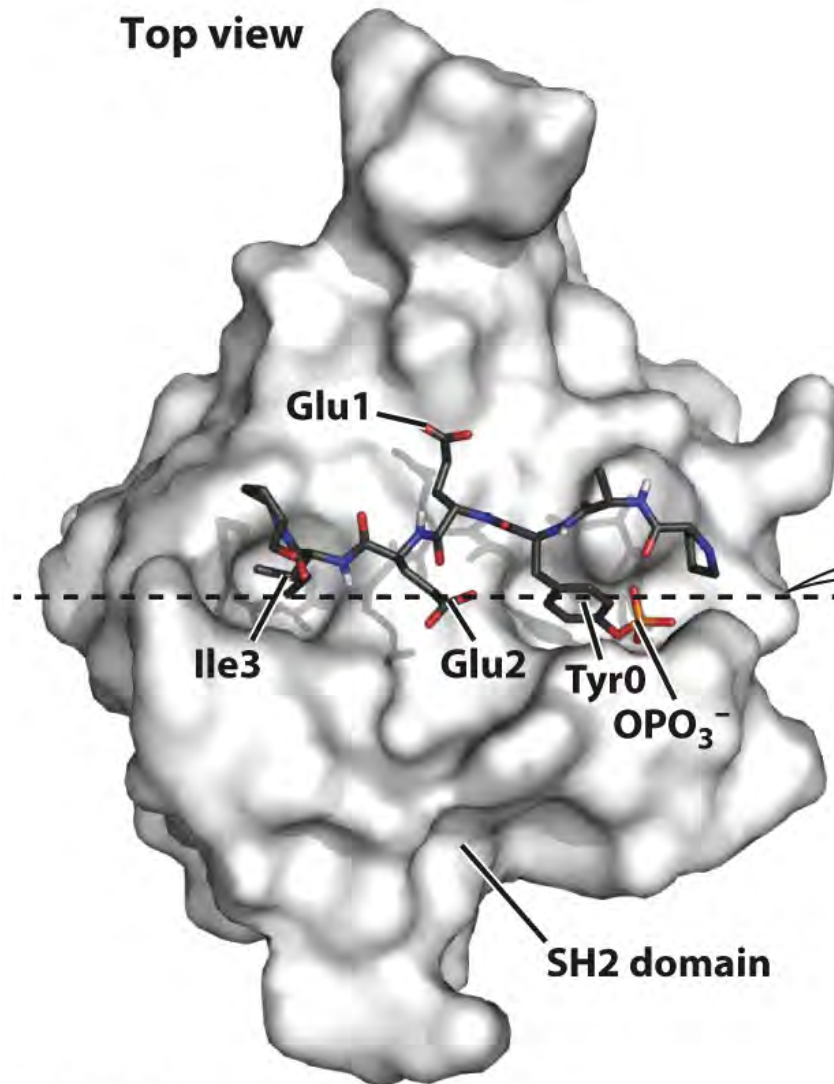
1. Receptor dimers in absence of ligand have poor kinase activity
2. Ligand binding brings JAK kinases together for cross-phosphorylation of activation loop.
3. The activated JAK kinase phosphorylates the C tail of the receptor.

Src homology domains originally found in c-Src



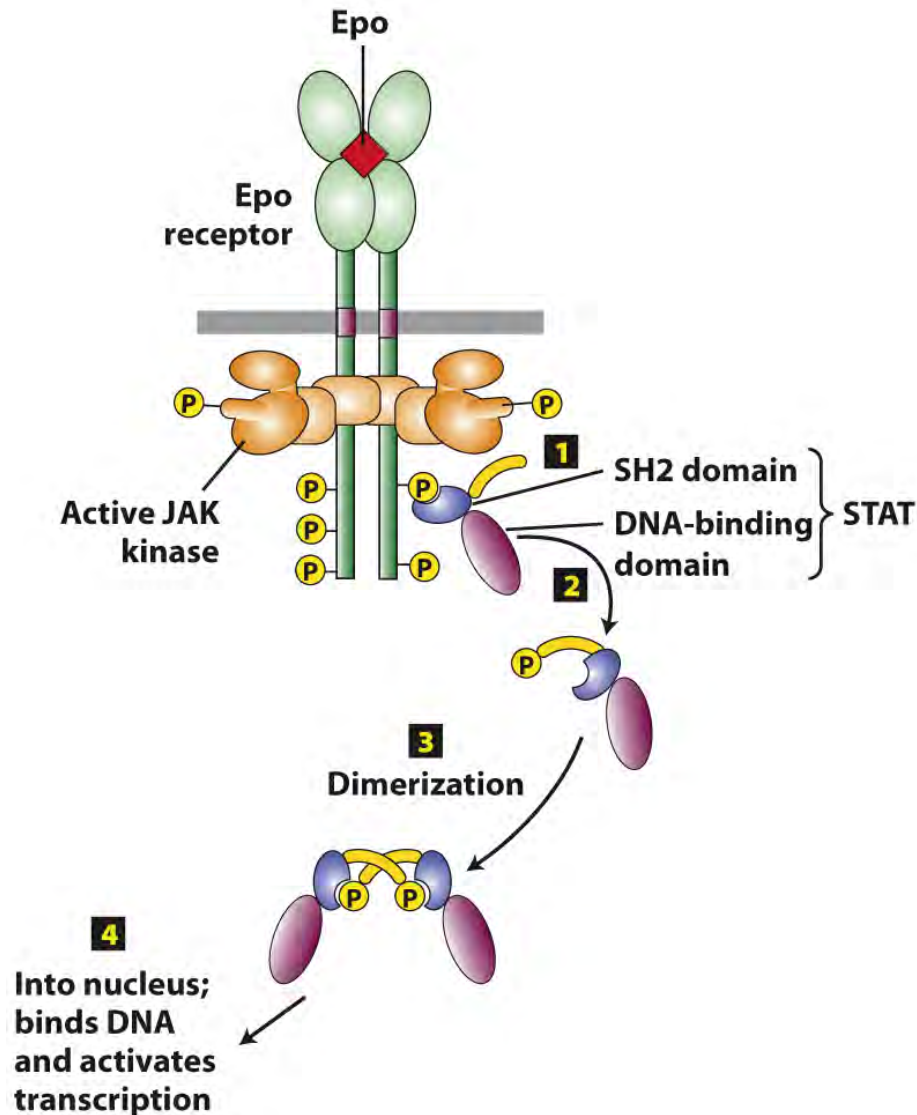
SH2 domains bind phosphotyrosine peptides

- SH2 is a small, phosphotyrosine-binding domain
- SH2= Src homology domain 2
- STAT transcription factors contain N-terminal DNA binding domain, an SH2 domain, and a C-terminal region with a tyrosine.



Zhong et al. (2005) PNAS

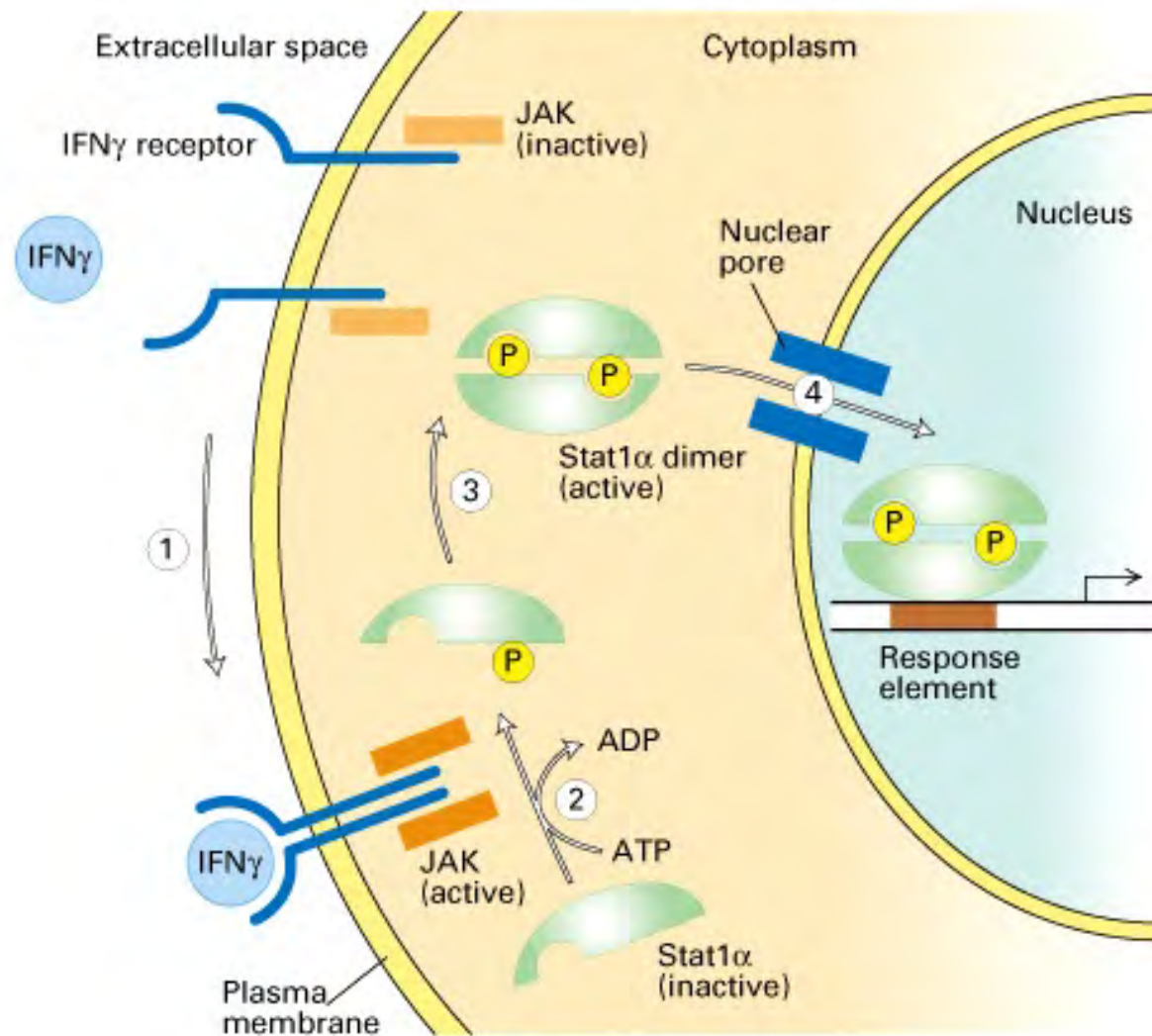
Activated receptors recruit and activate STAT



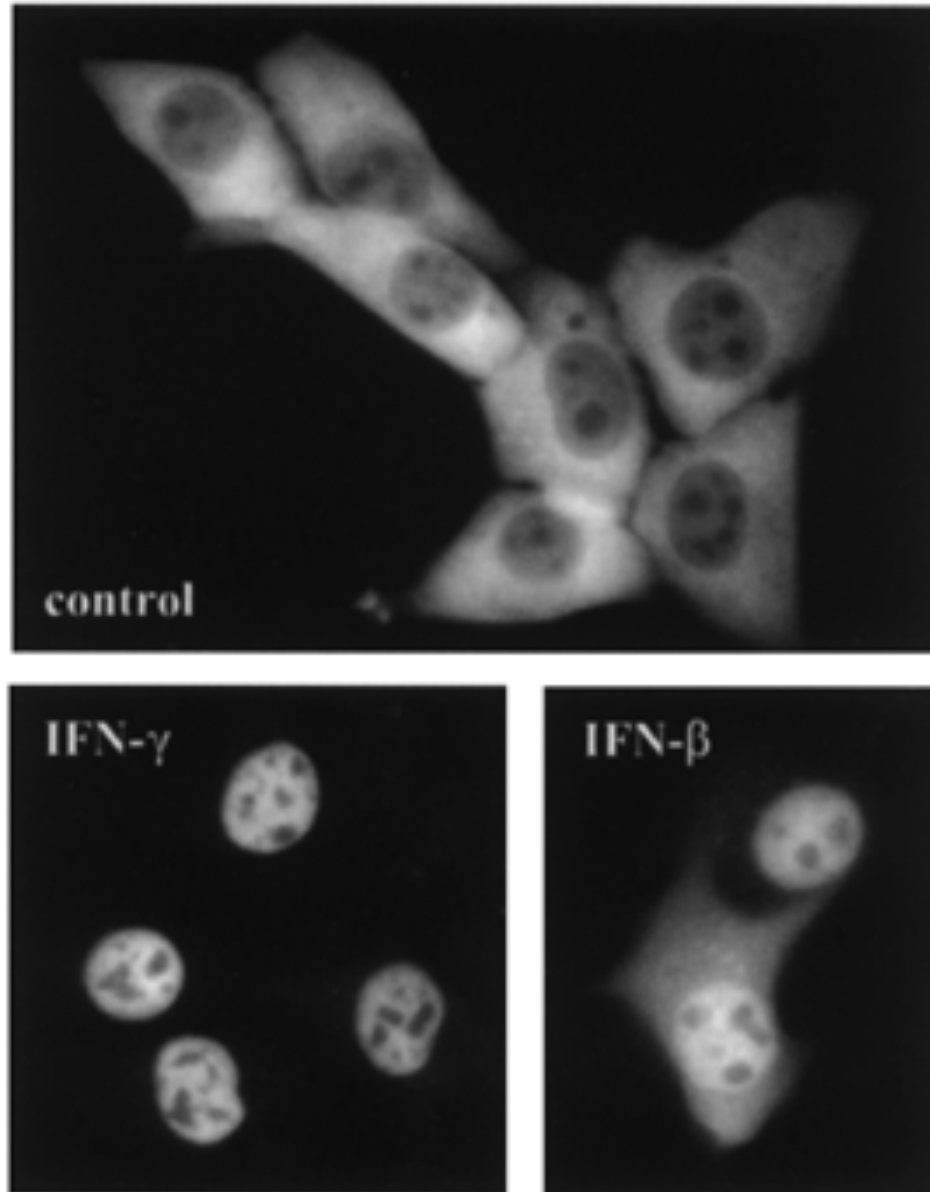
- Monomeric STAT is recruited to receptor due to SH2 binding to phosphotyrosine.
- C-terminal tyrosine of STAT is phosphorylated.
- Phosphorylated STAT dissociates, homodimerizes via SH2-phosphotyrosine interactions.
- Dimerization exposes NLS, and STAT is imported into the nucleus.
- STATs activate transcriptional program.

Figure 16-12a
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Model for cytokine receptor activation: ligand dimerizes receptor



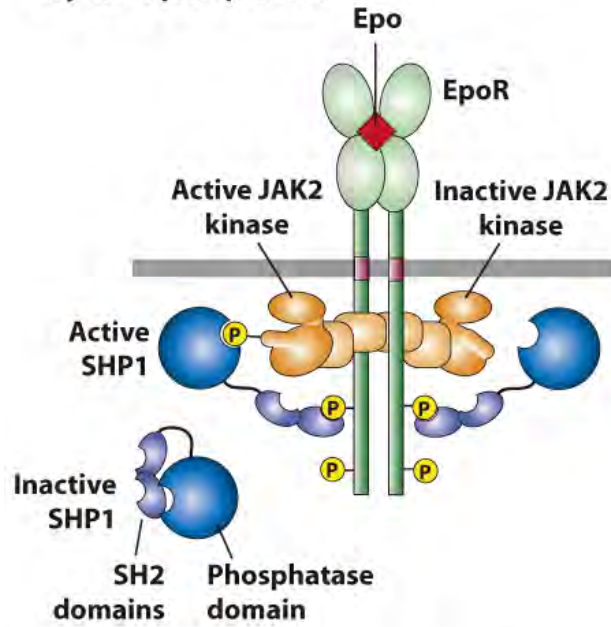
Interferon treatment induces nuclear translocation of STAT



Koster & Hauser (1999). E. J. Biochem 260:137.

Downregulation of receptor activity

(a) Short-term regulation: JAK2 deactivation by SHP1 phosphatase



(b) Long-term regulation: signal blocking and protein degradation by SOCS proteins

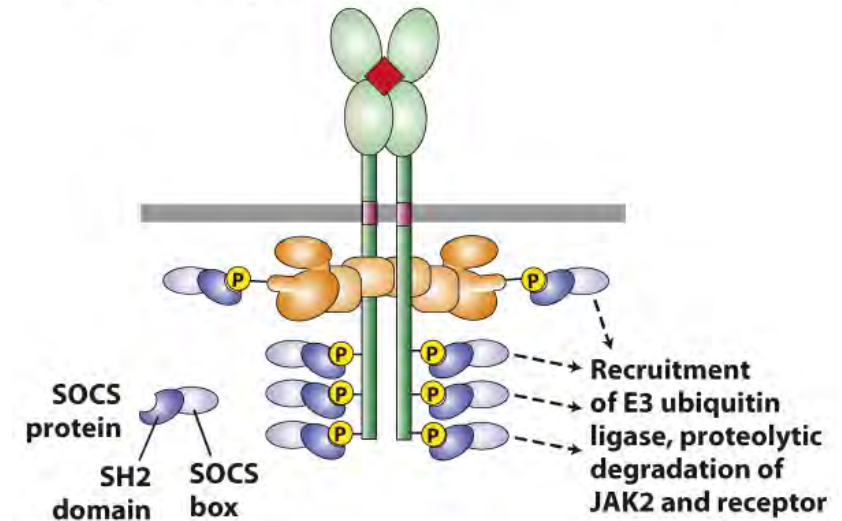


Figure 16-13

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- Binding of SH2 domain of SHP1 to the activated receptor:
 - Activates its phosphatase catalytic activity
 - Allow it to dephosphorylate the JAK2 activation loop, inactivating the JAK kinase activity
- STAT5 induces SOCS protein expression in erythropoietin-stimulated erythroid progenitor cells.
- binds to specific phosphotyrosine residues on EpoR or JAK2, blocking binding of other signaling proteins.
- targets the receptor and JAK2 for degradation by the ubiquitin proteasome pathway.

Receptor tyrosine kinases

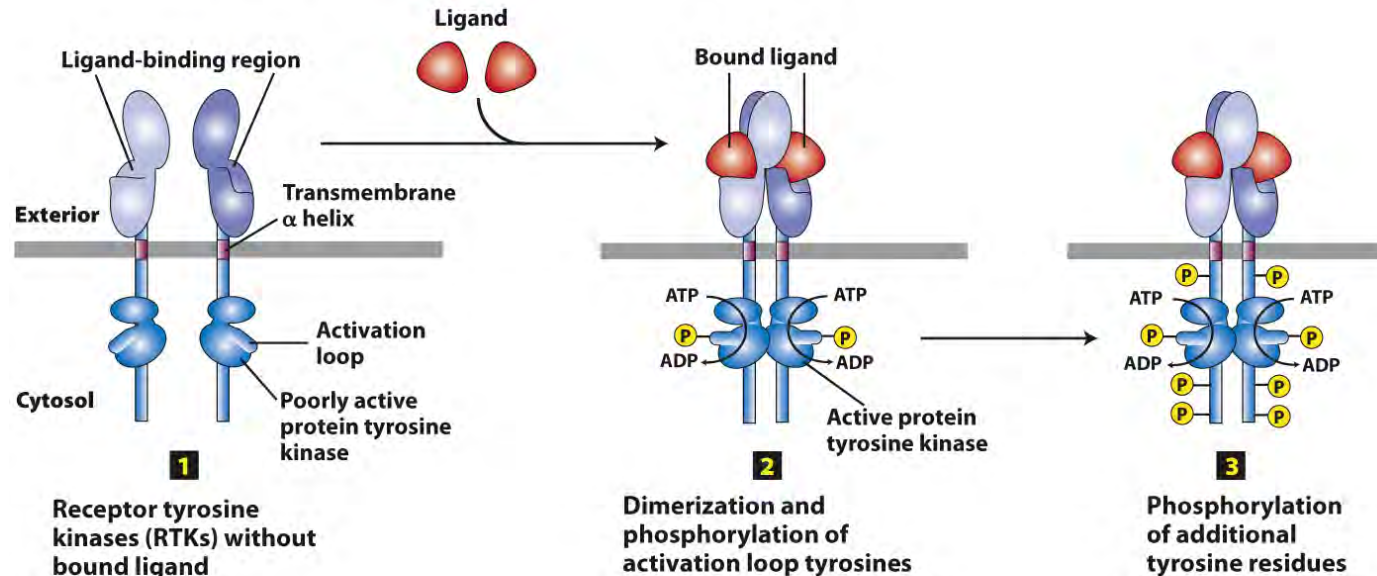
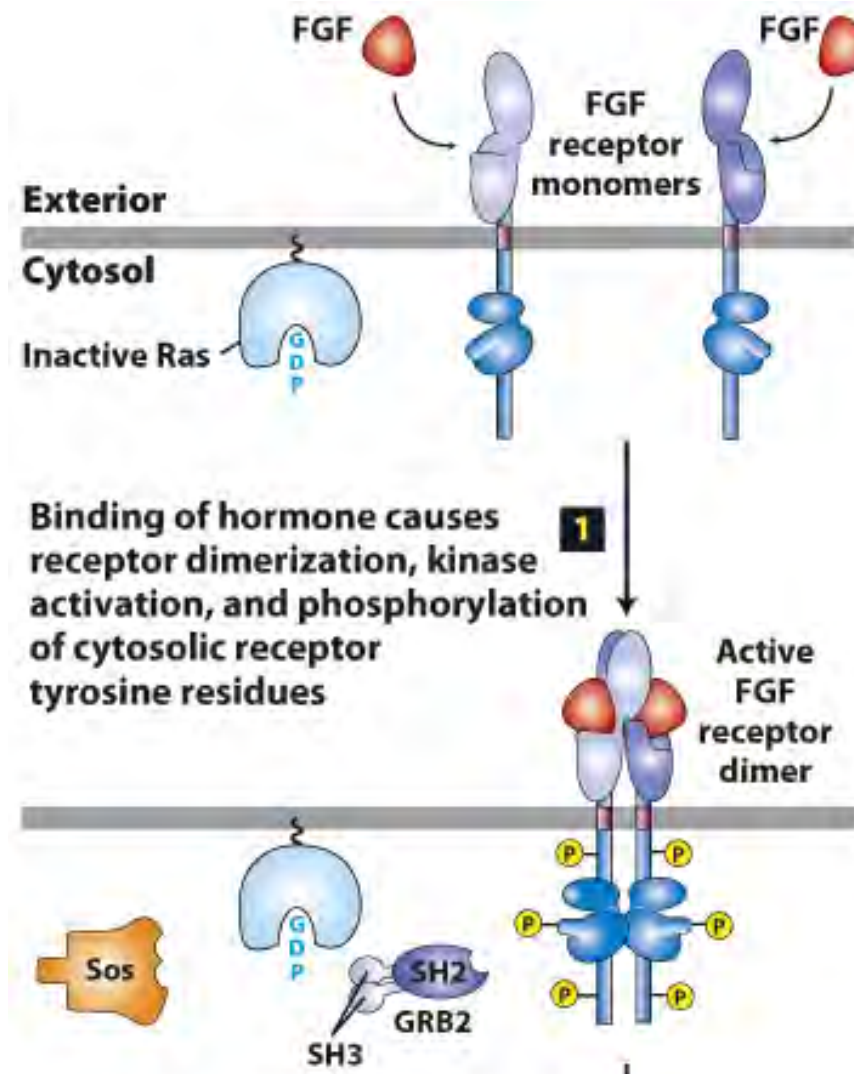


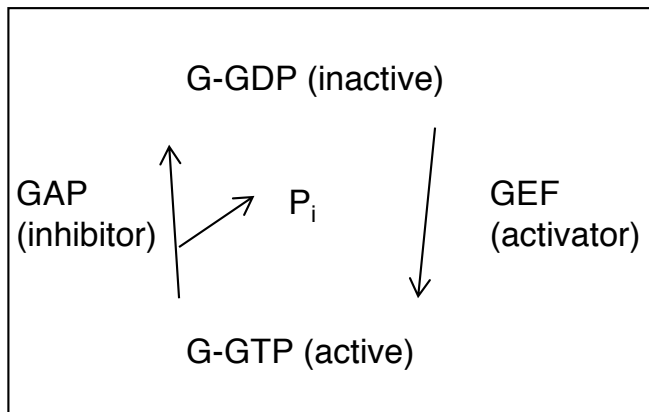
Figure 16-14
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- Some well-known receptor tyrosine kinases: receptors for NGF, PDGF, FGF, EGF
- RTK's have intrinsic kinase activity that is activated by ligand-induced dimerization.
- Cross-phosphorylation of kinase domains at activation lip leads to conformational change and activation.
- Cytosolic tail of receptor is phosphorylated on tyrosines and these sites then act as docking sites for signaling proteins. (Analogous to JAK kinases and cytokine receptors)

An adapter protein and GEF link activated RTKs to Ras



An adapter protein and GEF link activated RTKs to Ras



Grb2 is an adaptor protein, linking sos to RTK.

Sos activates Ras by nucleotide exchange.

What is downstream of Ras?

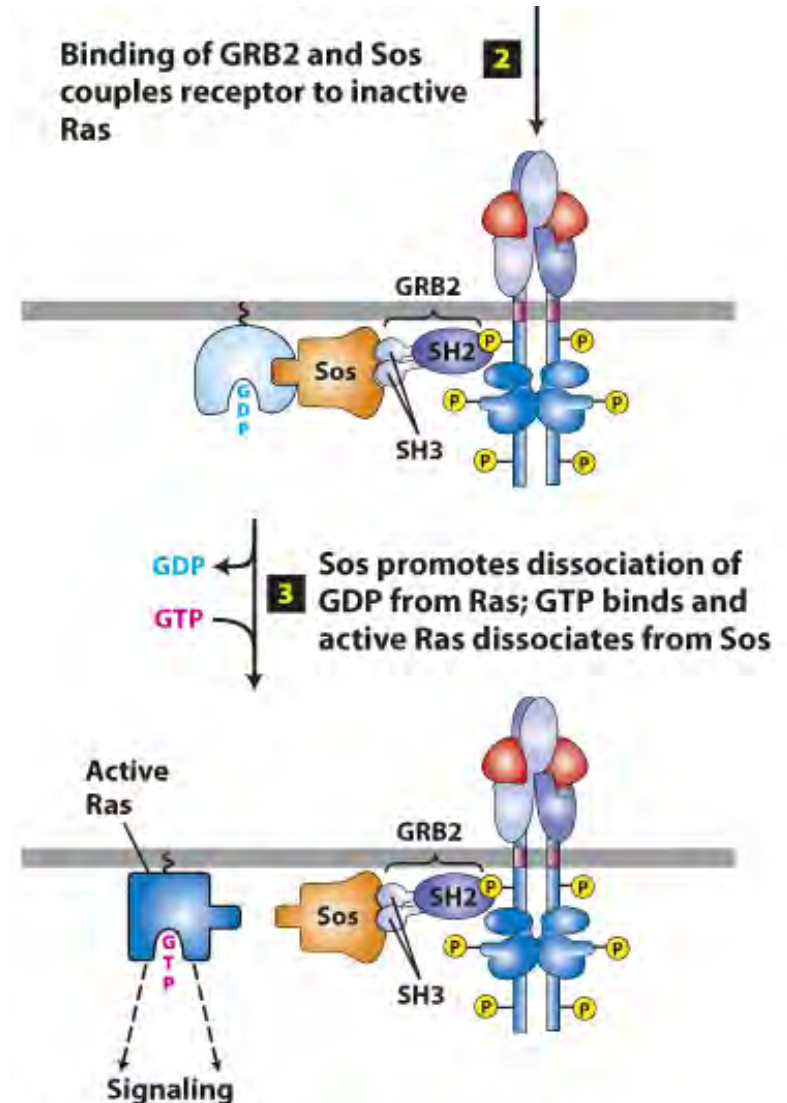
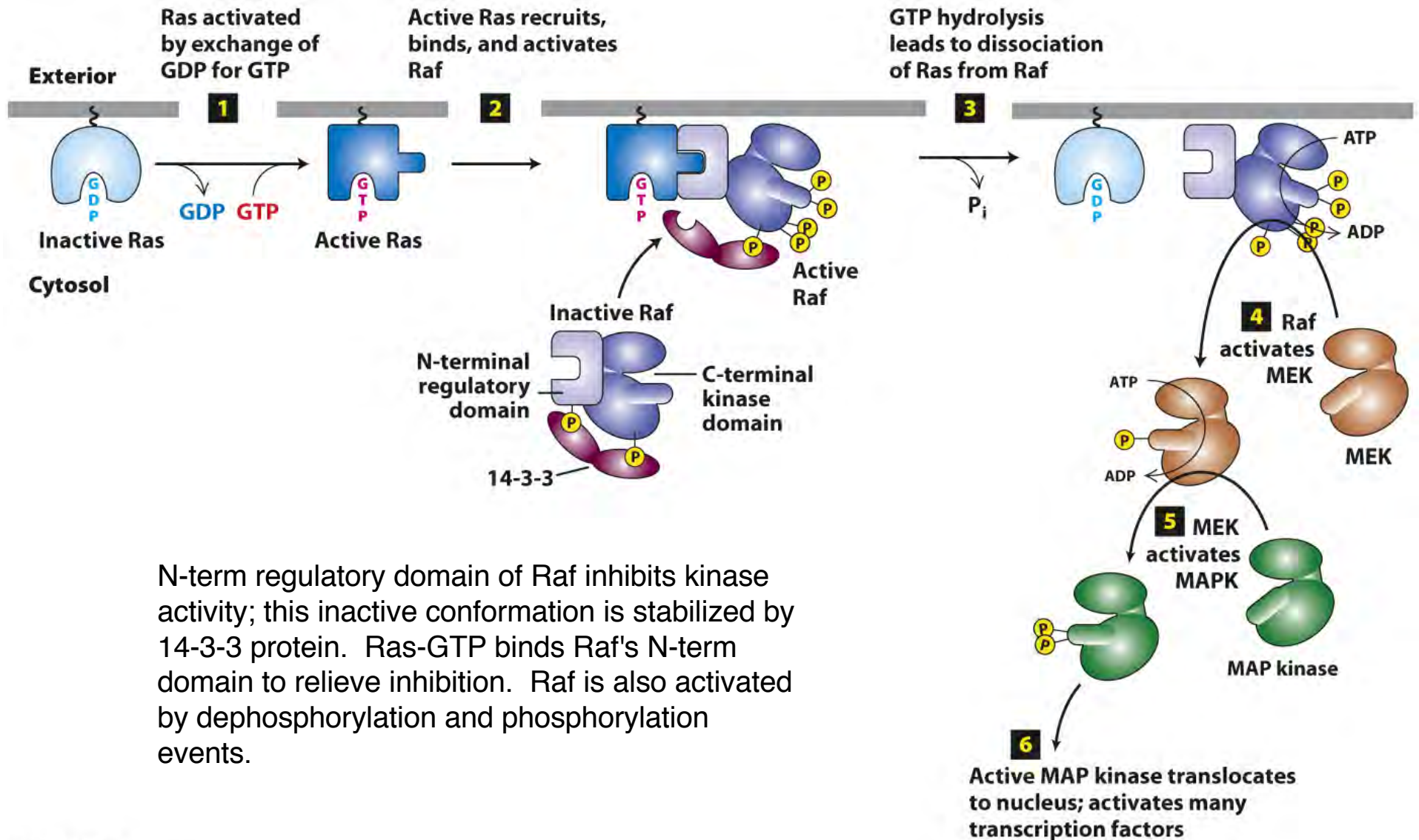


Figure 16-21

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Ras activates the MAP kinase cascade



N-term regulatory domain of Raf inhibits kinase activity; this inactive conformation is stabilized by 14-3-3 protein. Ras-GTP binds Raf's N-term domain to relieve inhibition. Raf is also activated by dephosphorylation and phosphorylation events.

Figure 16-24

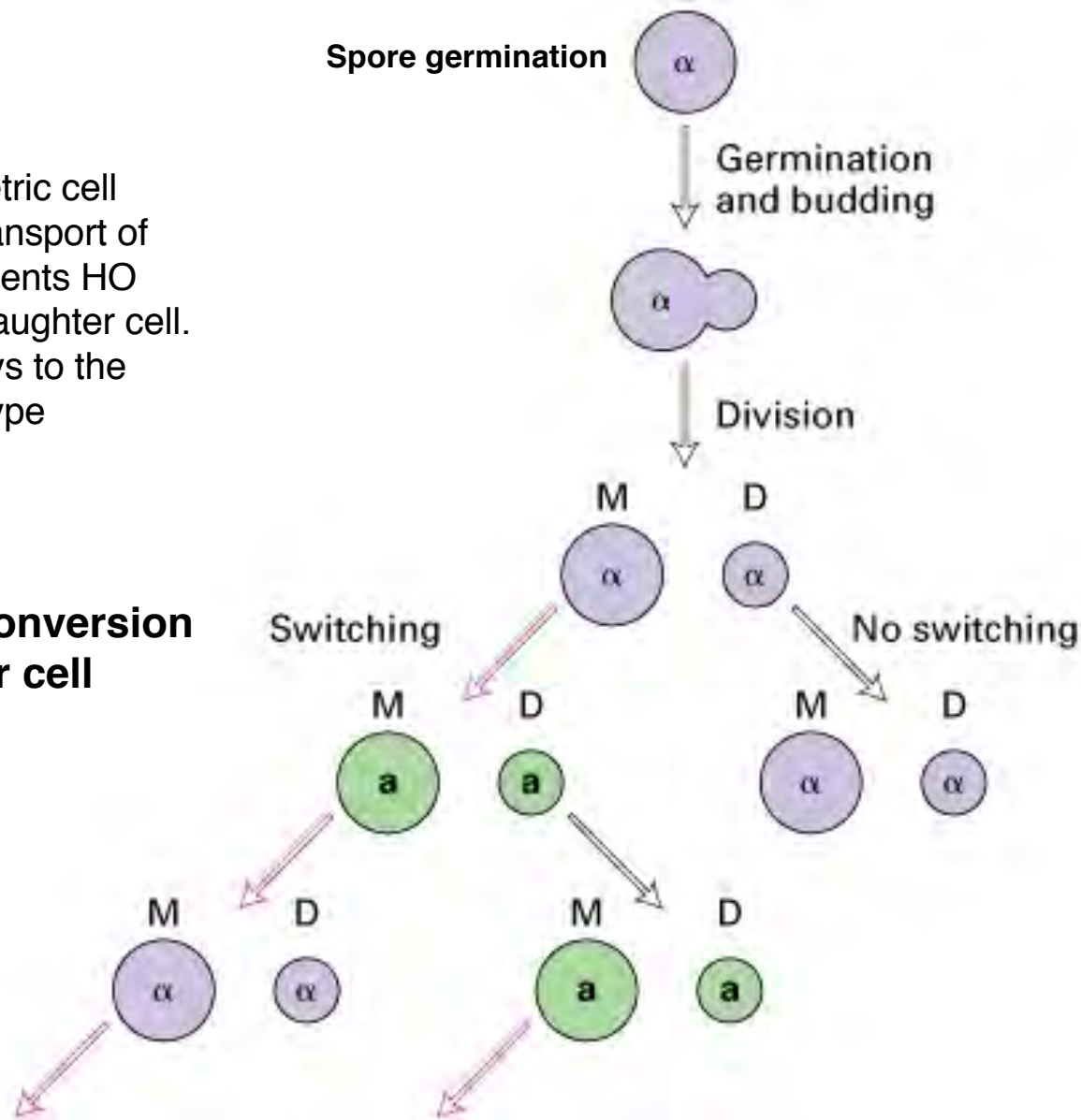
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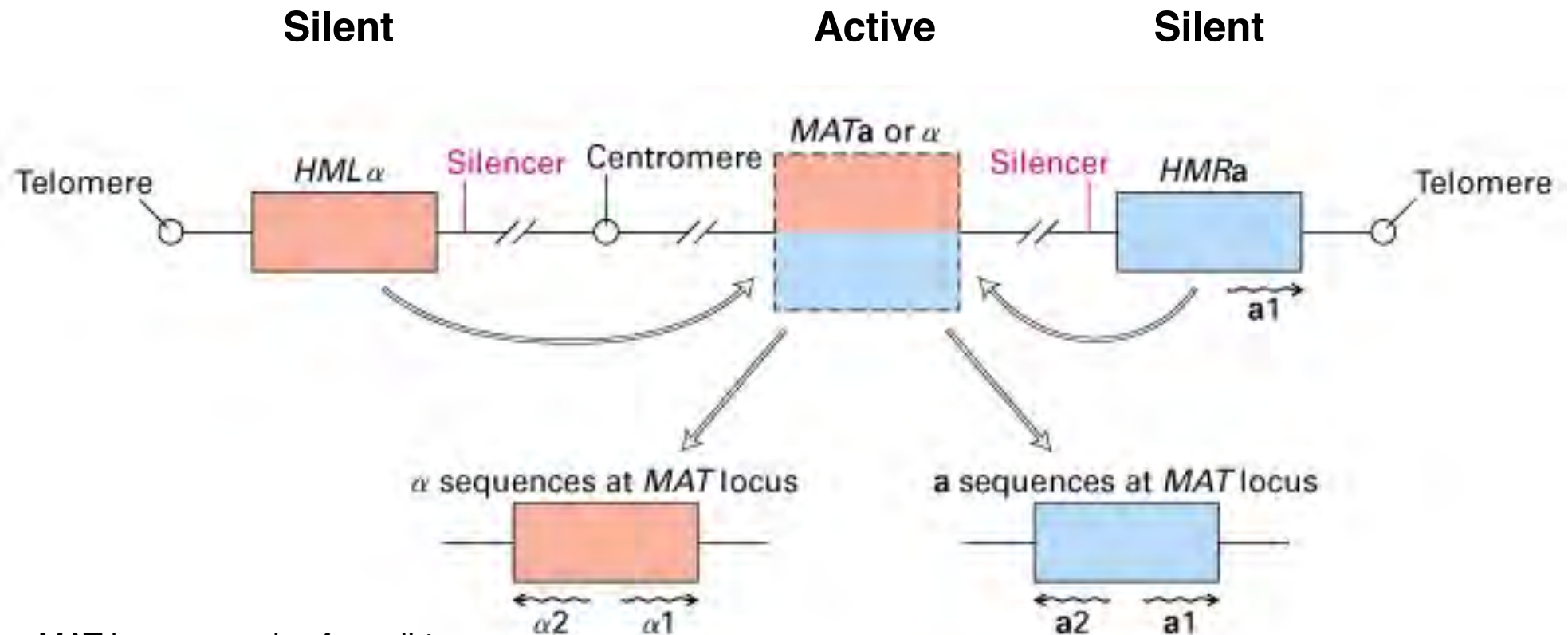
Haploid yeast cells can be “a” or “α” mating type

A form of asymmetric cell division, due to transport of Ash1 mRNA (prevents HO transcription) to daughter cell. Switching is always to the opposite mating type

Mating-type conversion in mother cell



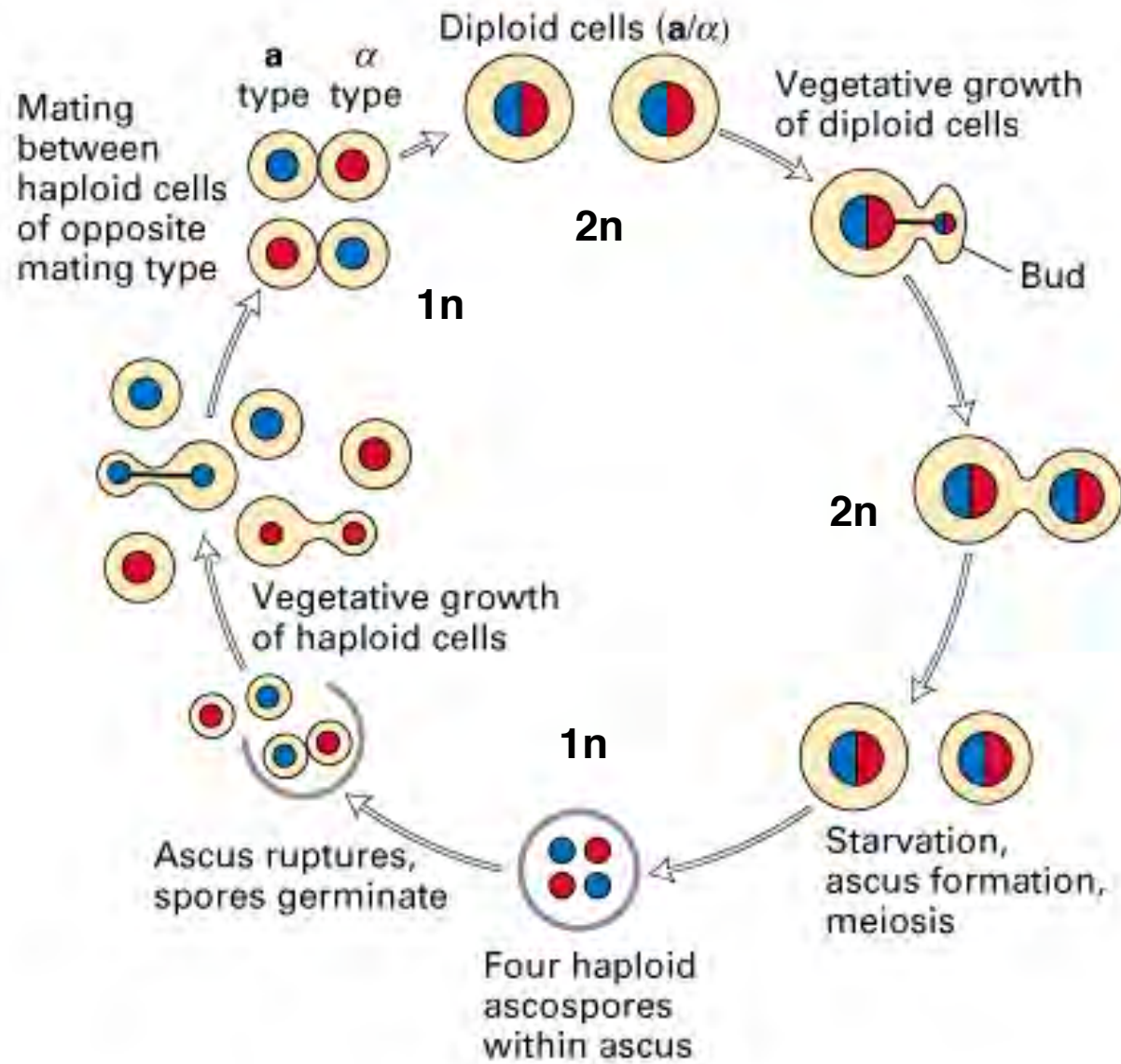
The MAT locus determines mating type



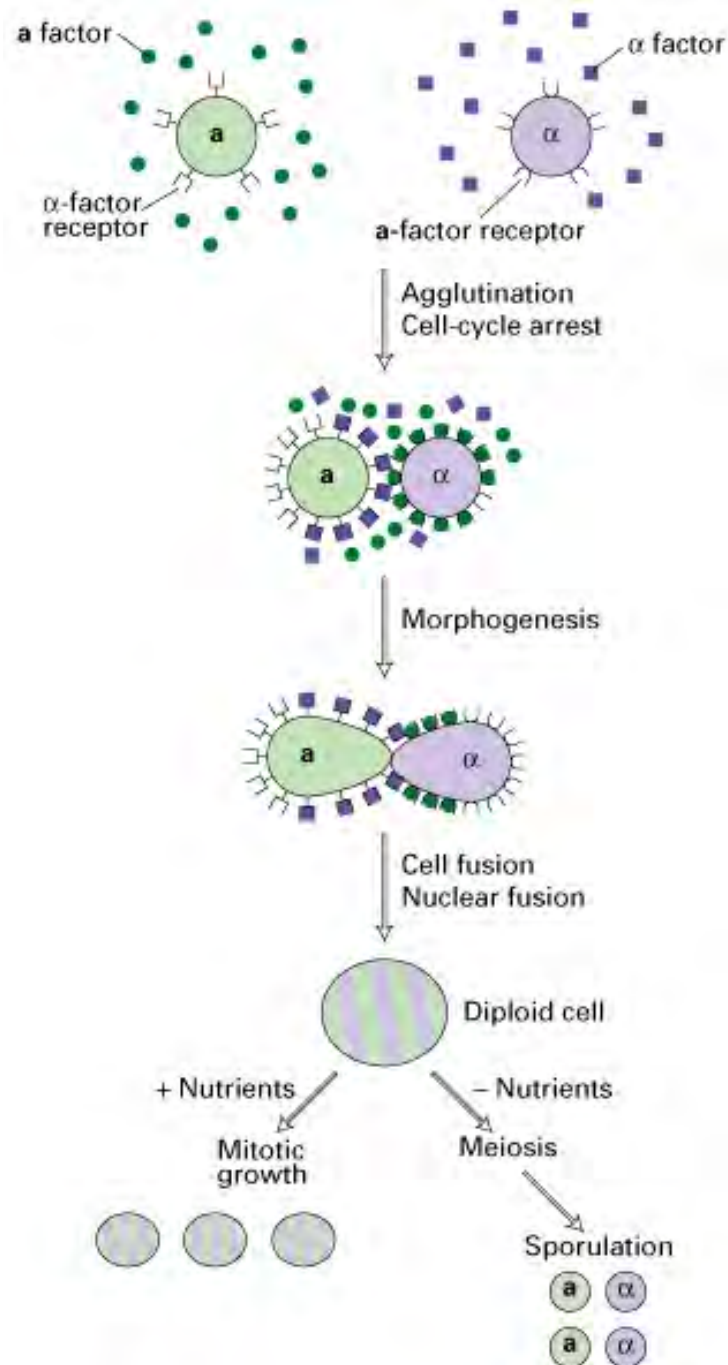
MAT locus encodes for cell-type specific transcription factors.

As a result, *a* haploids, α haploids, and *a*/ α diploids each have their own transcriptional profile.

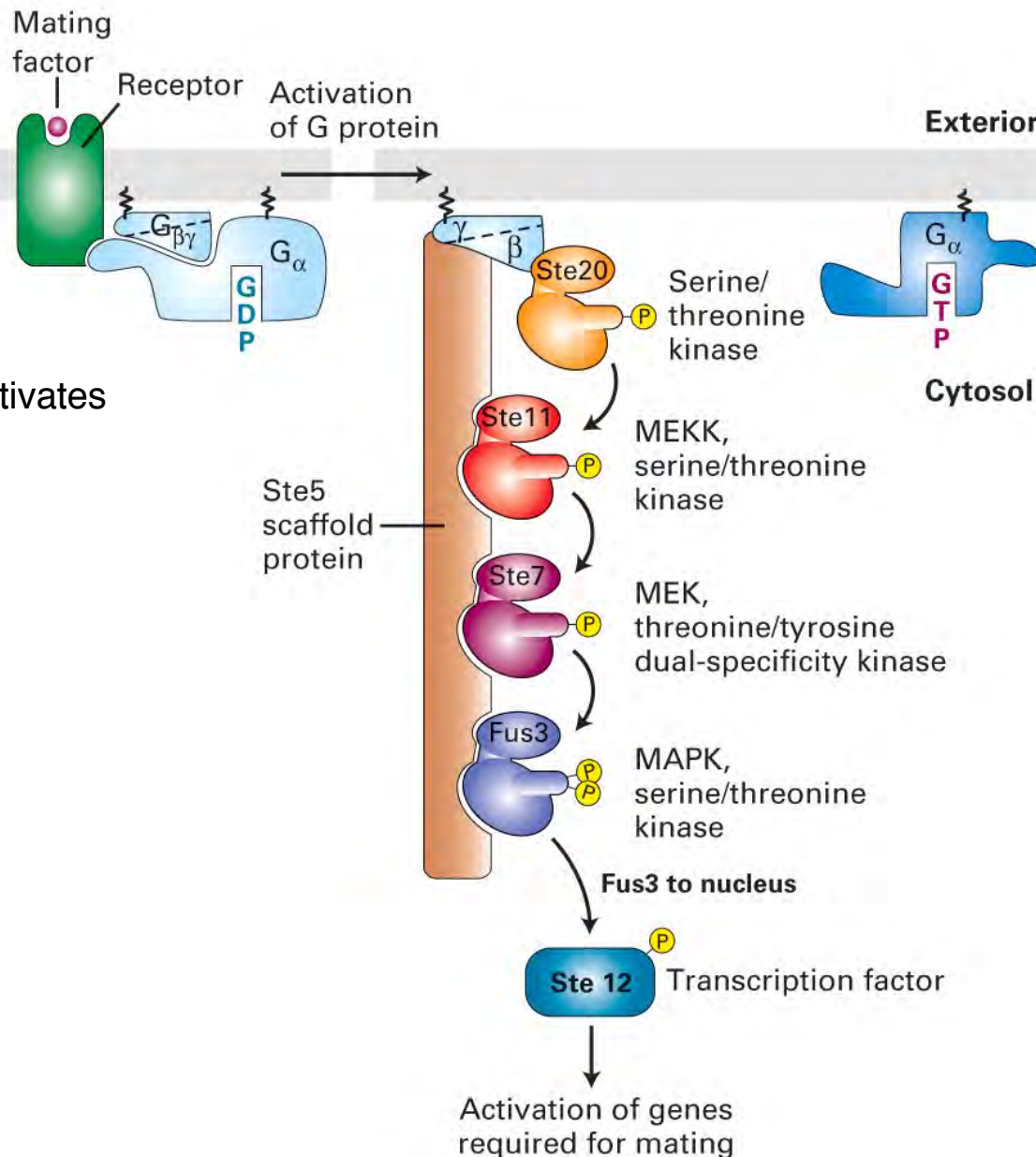
Life cycle of the budding yeast, *S. cerevisiae*



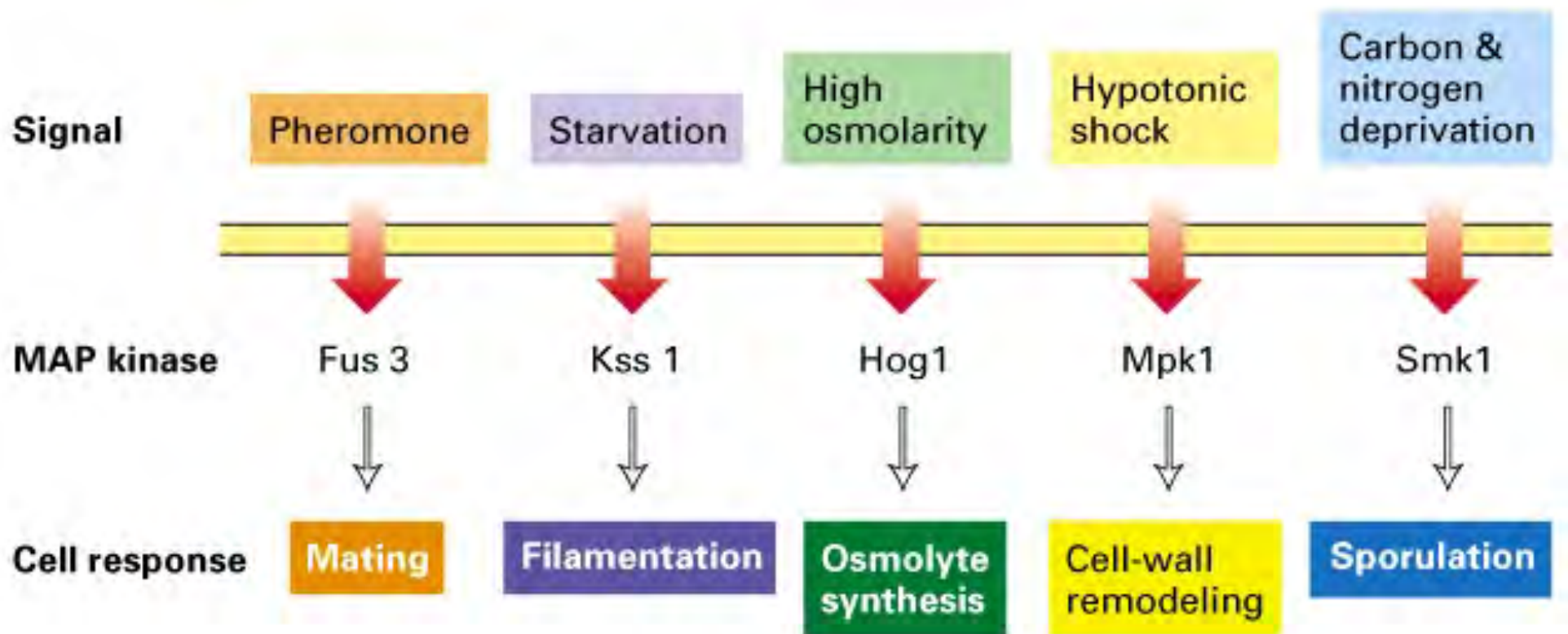
Mating factors induce yeast mating



A MAP kinase cascade mediates signaling in the yeast mating pathway

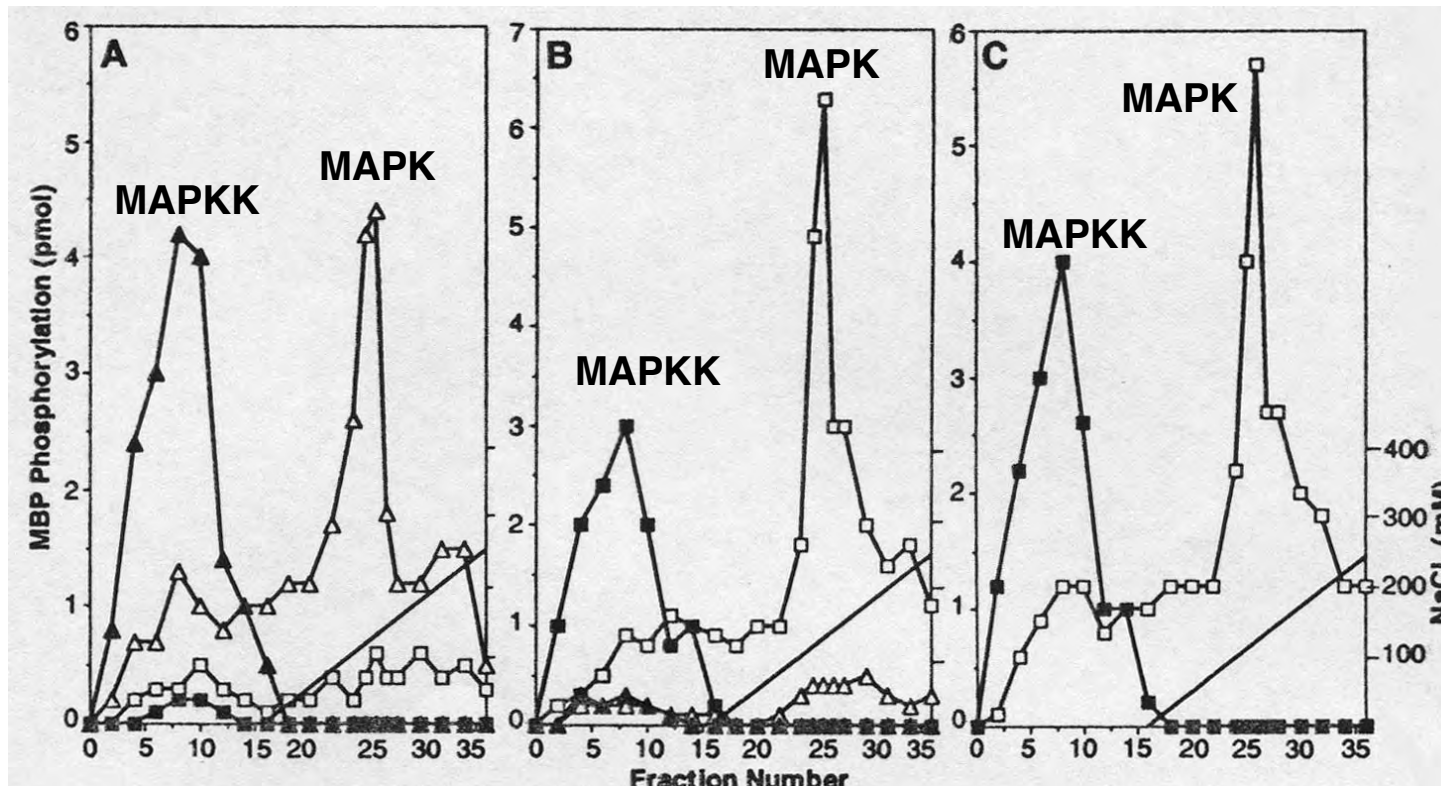


Multiple MAP kinase pathways are involved in response of yeast cells to stimuli



Scaffold protein may help to keep specificity of pathways

Infection of cells with v-Raf virus leads to activation of MAPK and MAPKK



Control virus

No serum: □

Serum: Δ

No serum

No virus: Δ

EC12 virus: □

No serum

22W virus: □

EC12, 22W = Moloney mouse sarcoma viruses containing activated v-Raf (N-term truncations)

The three cellular Raf genes are ser/thr kinases with N-term regulatory domain.

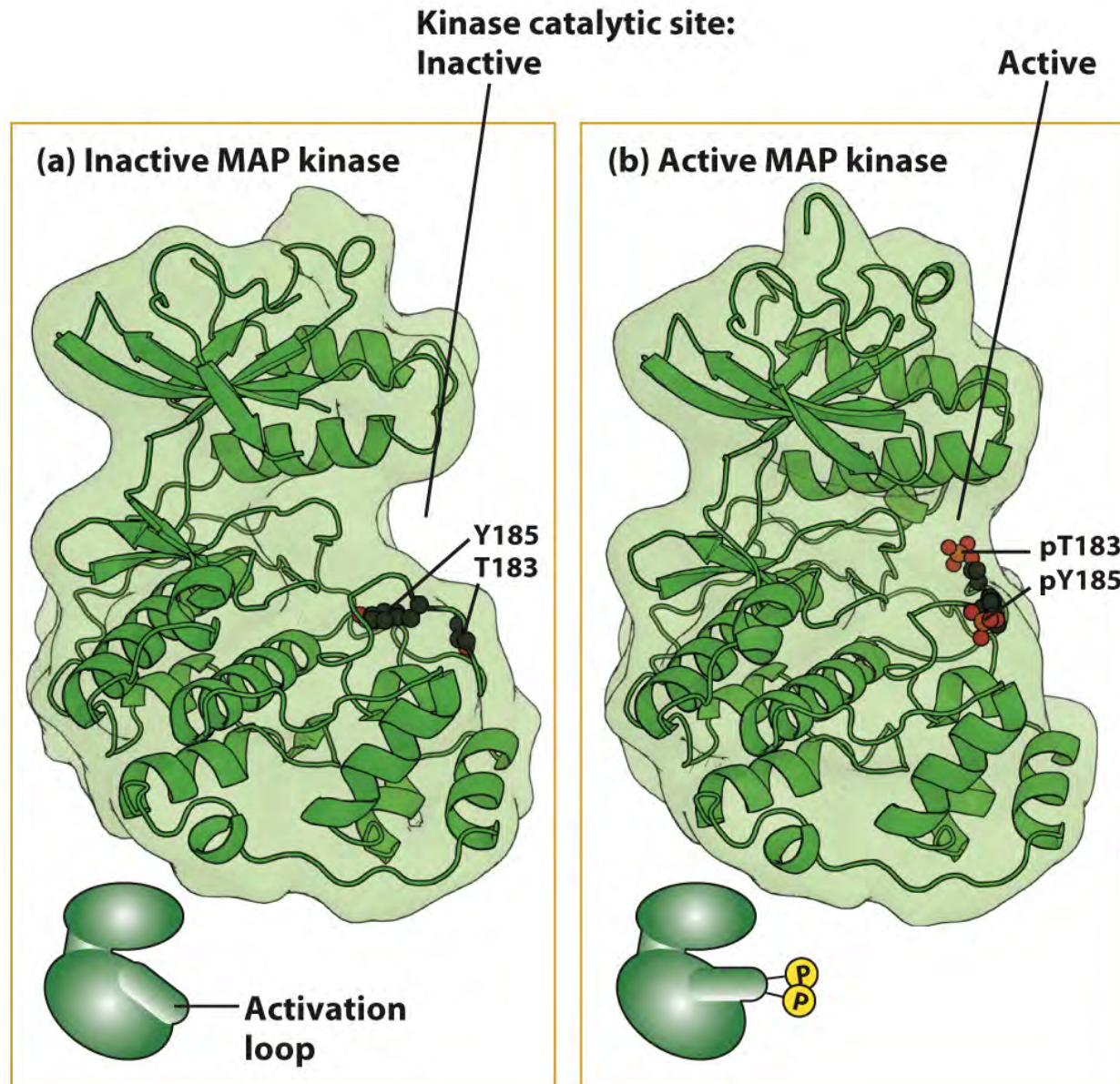
Cell lysates fractionated by ion exchange, and fractions assayed for MAPK and MAPKK activity

MAPK activity: assay phosphorylation of MBP

MAPKK activity: assay phosphorylation of MBP through recombinant inactive MAPK

Control virus expresses a more severely truncated v-Raf that is inactive.
 Serum or v-Raf is able to induce MAPKK and MAPK activity in serum-starved cells.

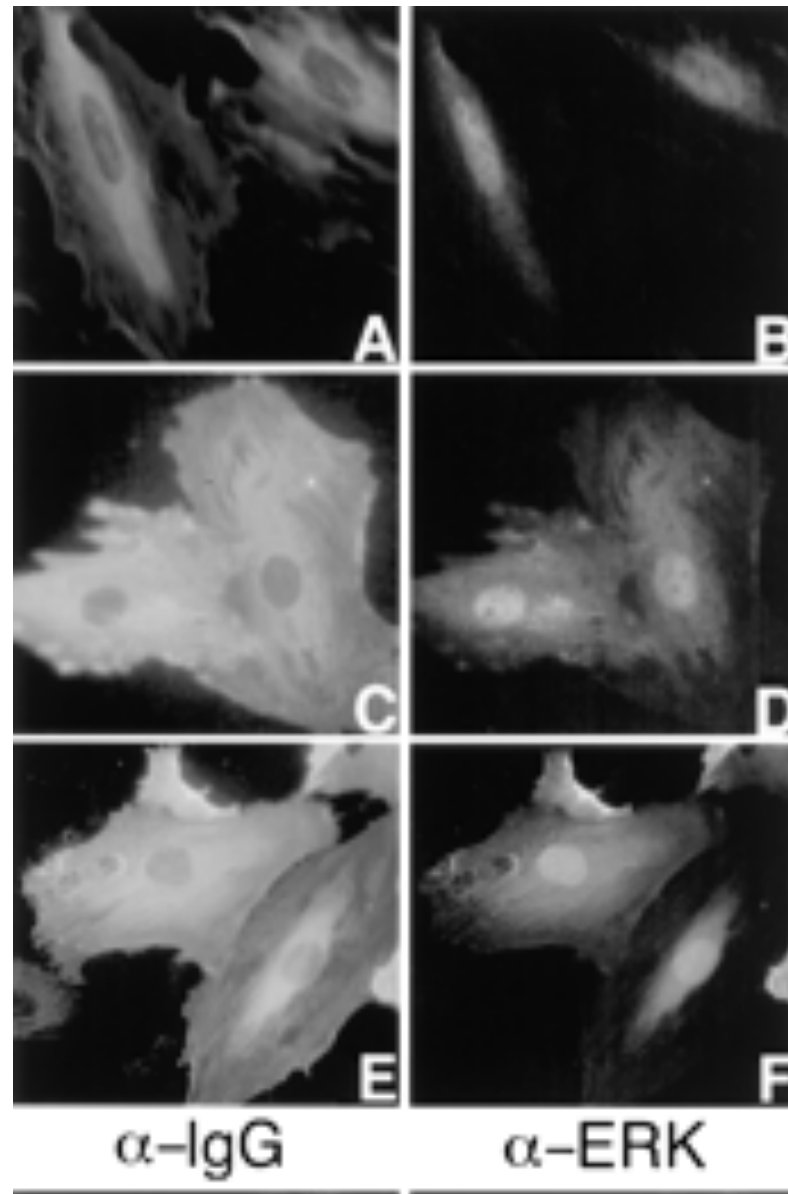
Phosphorylation of “activation lip” activates MAP kinase



In inactive state, catalytic site is blocked by lip. Phosphorylation of lip by MEK leads to binding of ATP to catalytic site and dimerization, which allow nuclear localization

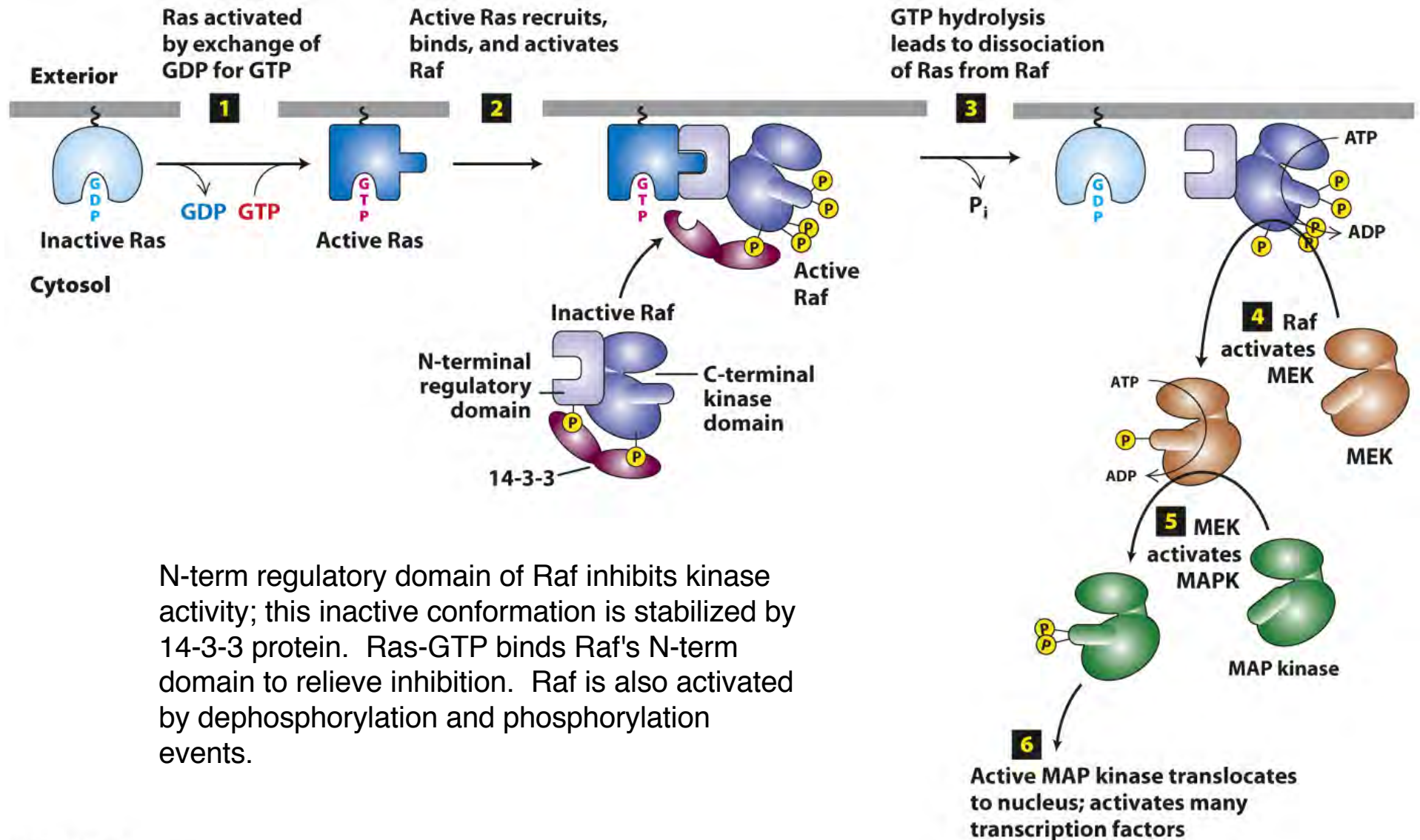
Figure 16-25
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Phosphorylation of MAPK leads to nuclear translocation



Khokhlatchev et al (1998). Cell 93:605

Summary of mammalian MAP kinase cascade



N-term regulatory domain of Raf inhibits kinase activity; this inactive conformation is stabilized by 14-3-3 protein. Ras-GTP binds Raf's N-term domain to relieve inhibition. Raf is also activated by dephosphorylation and phosphorylation events.

Figure 16-24

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Phosphorylation of MAPK leads to nuclear translocation and gene activation

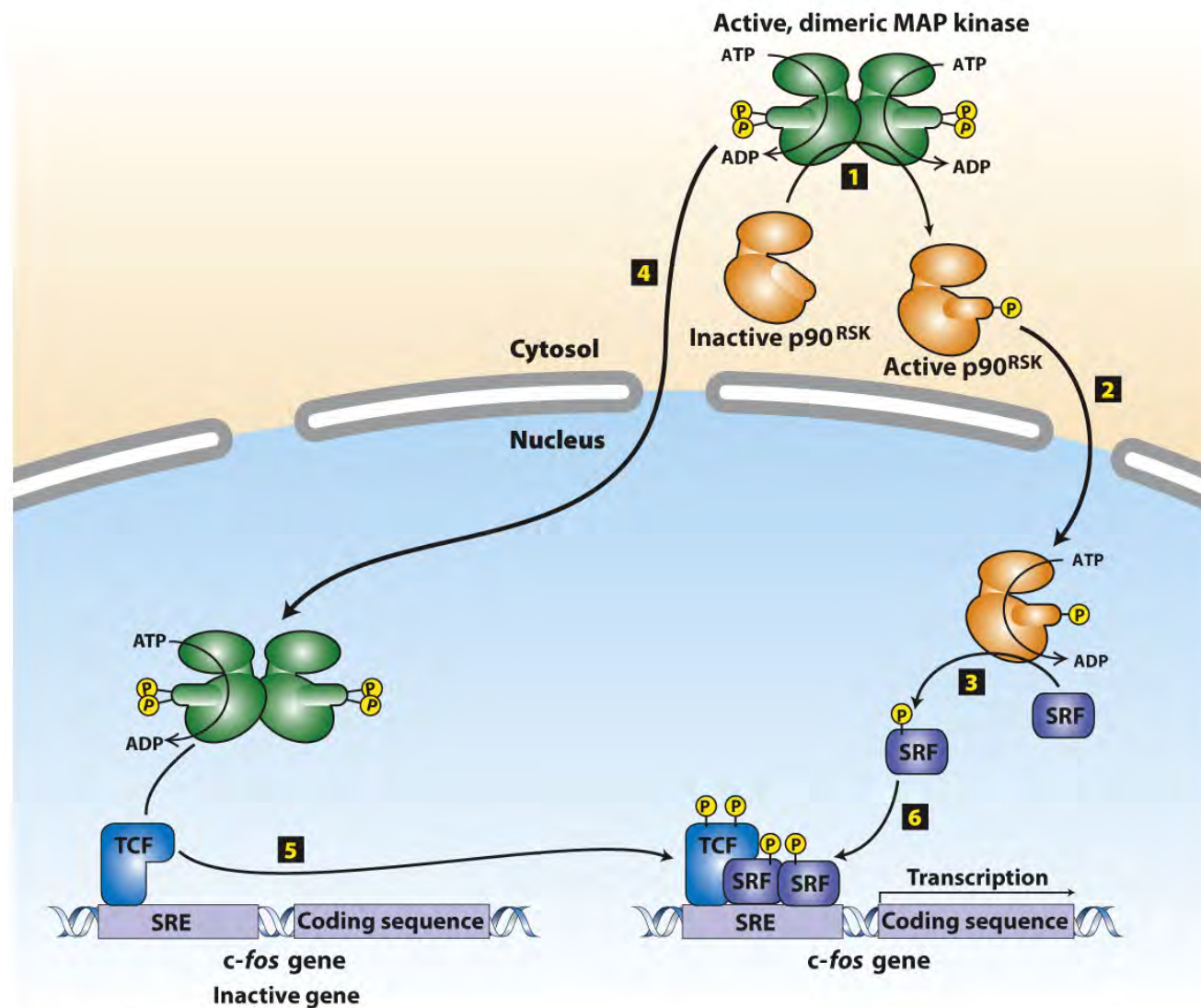
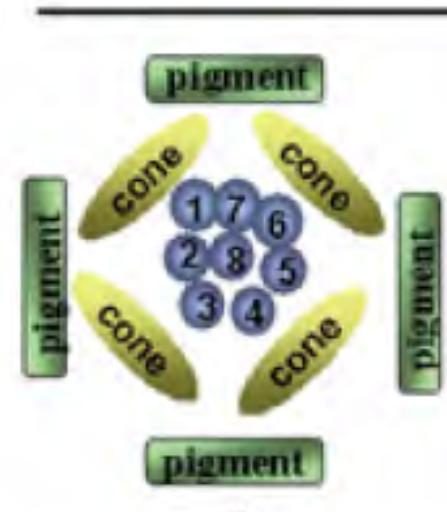
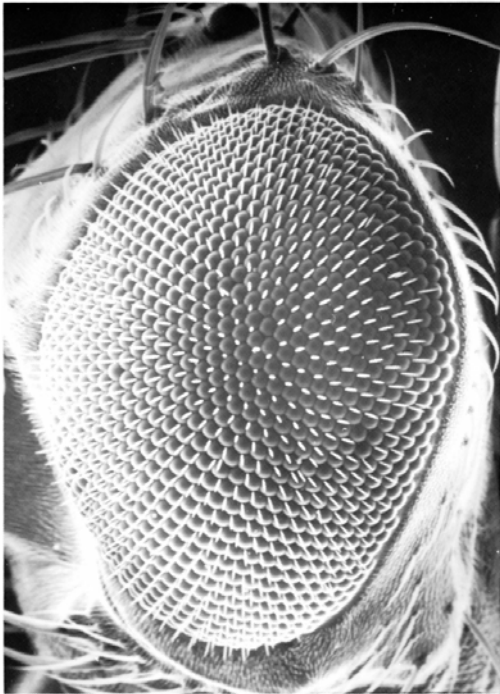


Figure 16-26
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The R7 photoreceptor in *Drosophila* is used to study RTK pathways



Nagaraj & Banerjee (2004) Int J Dev Biol
Gilbert. Developmental Biology, 8th edition

Each ommatidia is an eye that contains 20 cells (8 photoreceptors). R8 differentiates first; R7 last.

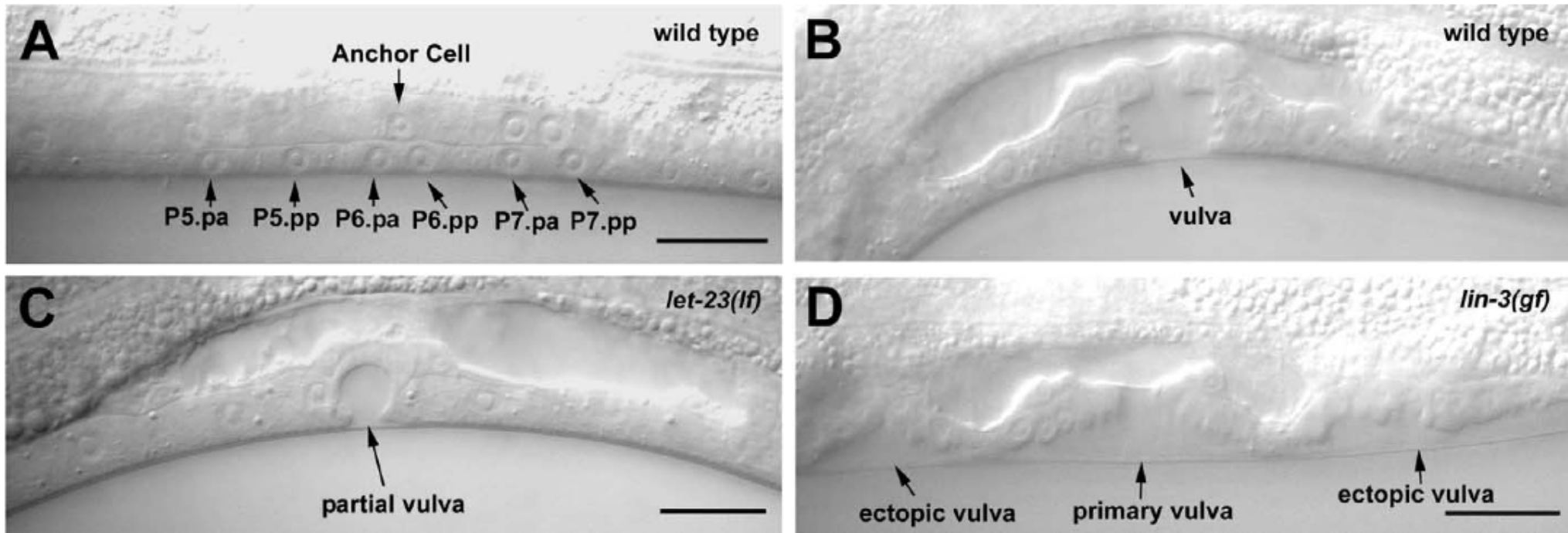
In *sevenless* mutant, R7 has non-neuronal fate, and the fly cannot sense UV light. Mosaic analysis shows *sev* is required cell autonomously in R7.

Boss (*Bride of sevenless*) mutants also lack R7, but the gene product is required in R8.

Sev is a receptor tyrosine kinase; *Boss* is its ligand.

Screens in this system identified GRB2, SOS (son of sevenless), and Ras.

Genetic dissection of vulva development in *C. elegans*



- The gonadal anchor cells send an inductive signal to ventral precursor cells that generate vulval tissue.
- The anchor cell produces Lin-3 (an EGF molecule). The precursor cells require let-23 (RTK) and Ras (let-60) for vulva development.

The MAPK cascade in vulva development

