Computational Systems Biology

Lecture 8: Signal Transduction

Images from:
D. L. Nelson, Lehninger Principles of Biochemistry, IV Edition – Chapter 12
E. Klipp, Systems Biology in Practice, Wiley-VCH, 2005 – Chapter 6

Summary:

- Introduction on cell signalling
  - Metabolism vs. Signal transduction
- The signalling paradigm and some typical components:
  - The receptor
  - G protein
  - MAP kinase cascade

Cell Signalling

Signal Transduction

- The cell senses extra cellular signals:
  - Hormones, pheromones, heat, cold, light, osmotic pressure, concentration change of glucose, K^+, Ca^{2+} or cAMP.
- and commutes them in intracellular signals:
  - Signalling involves the same type of molecular modification as metabolism: production and degradation of substances, phosphorylation, activation of inhibition of reactions
- What's the difference then?

Metabolism vs. Signal Transduction

Metabolism

- Provides mass transfer
- Quantity of converted material: μM or mM
- A metabolic network is determined by the present set of enzymes
- The catalyst to substrate ratio is *low* (quasi-steady-state assumption in Michaelis-Menten kinetics)

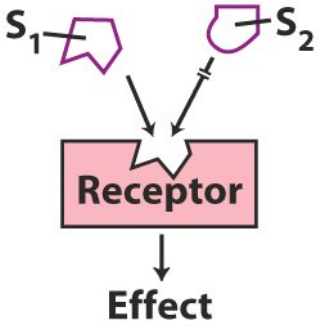
Signal Transduction

- Provides information transfer
- Quantities: 10 to 10^4 *molecules* per cell
- A signal pathway may assemble dynamically
- Amount of catalyst and substrate in the same order of magnitude

Characteristics of Signal Transduction

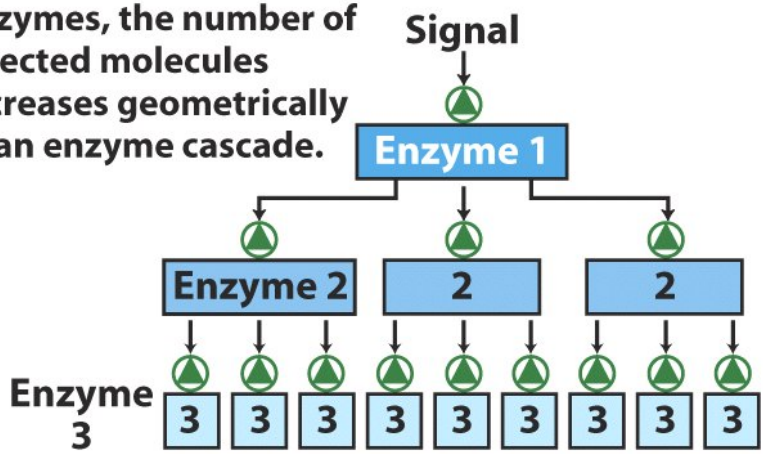
(a) Specificity

Signal molecule fits binding site on its complementary receptor; other signals do not fit.



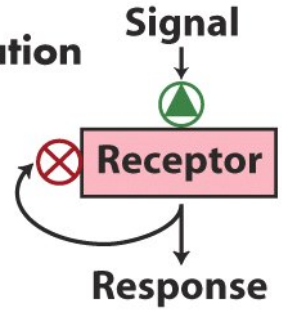
(b) Amplification

When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.



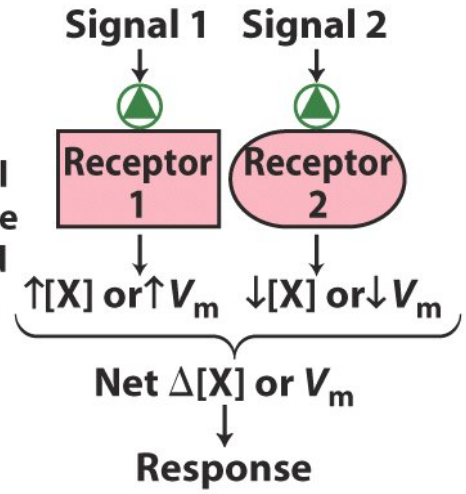
(c) Desensitization/Adaptation

Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



(d) Integration

When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger X, or the membrane potential V_m, the regulatory outcome results from the integrated input from both receptors.



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Signalling Paradigm: the Receptor

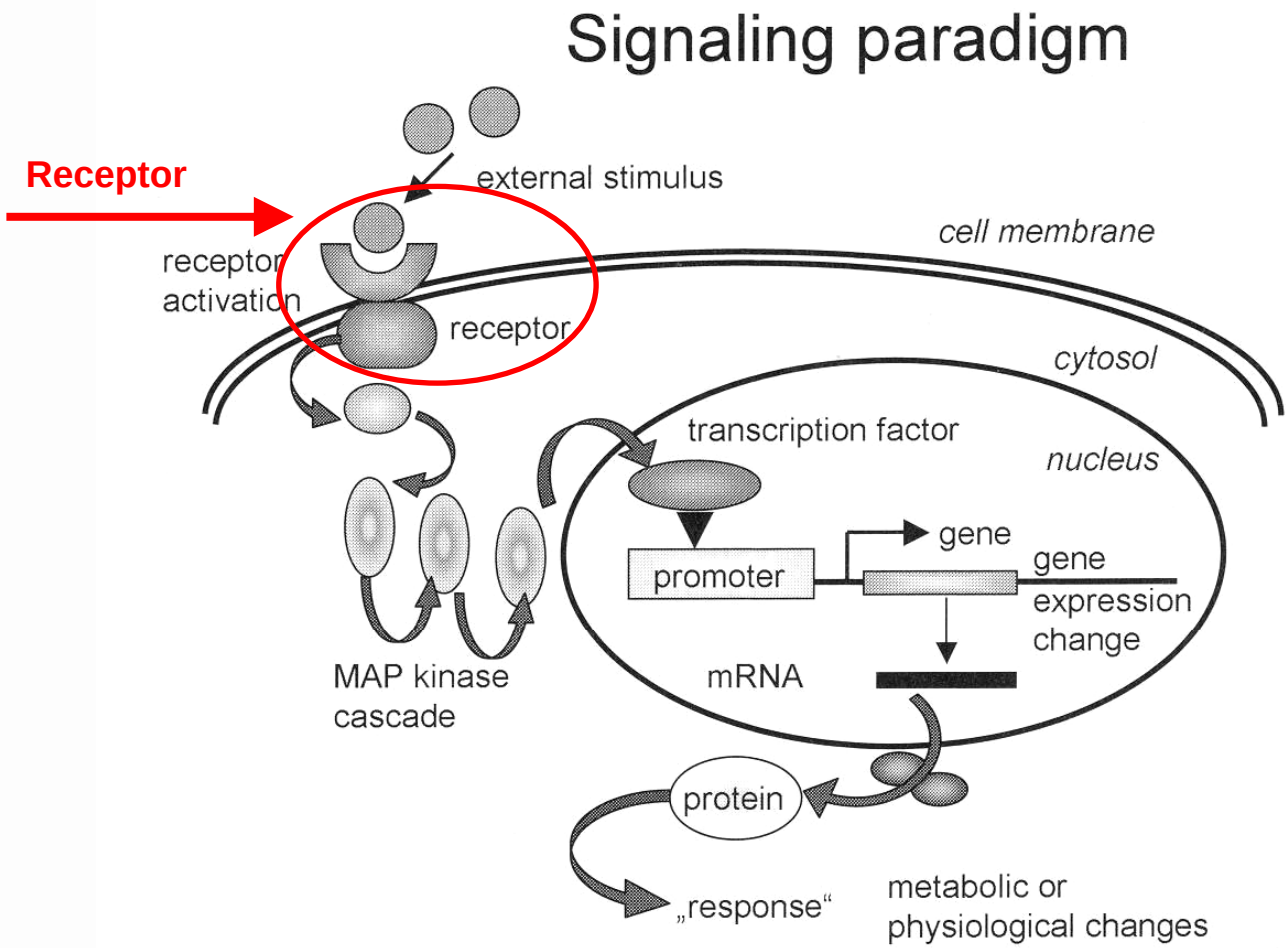

Fig. 6.1 Visualization of the signaling paradigm (for description, see text). The receptor is stimulated by a ligand or another kind of signal, and it changes its own state from susceptible to active. The active receptor initiates the internal signaling cascade, including a series of protein phosphorylation state changes. Subsequently, transcription factors are activated or deactivated. The transcription factors regulate the transcription rate of a set of genes. The absolute amount or the relative changes in protein concentrations alter the state of the cell and trigger the actual response to the signal.

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Simple receptor activation

- The simplest mechanism is the reversible binding of the ligand L to the receptor R to form the complex RL

$$R + L \rightleftarrows RL$$

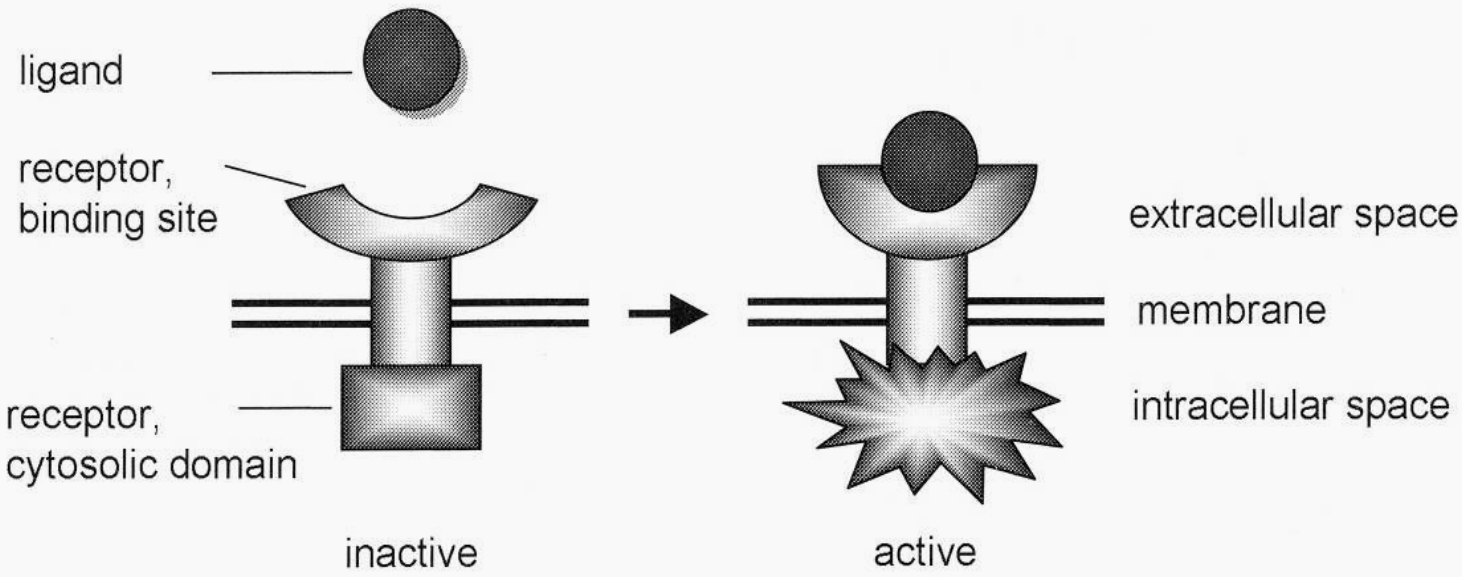

Fig. 6.3 Schematic representation of receptor activation.

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Scatchard Analysis

- The receptor-ligand binding, like the enzyme-substrate binding, depends on the concentration of the interacting components
- The resulting equilibrium can be described in term of K_a (the association constant) or K_d (the dissociation constant)

$$R + L \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} RL$$

$$K_a = \frac{[RL]}{[R][L]} = \frac{k_{+1}}{k_{-1}} = \frac{1}{K_d}$$

A more realistic Receptor activation

- Cells can regulate the activity of their receptors, e.g. to weaken the signal transmission during long term stimulation

- A classic mechanism is the phosphorylation of the receptor (on a serine, threonine or tyrosine amino acid) on the cytosolic domain

- Hence, a more realistic depiction should include not only the active and bound receptor, but:
  - R_i = the inactivated receptor (cannot be activated)
  - R_s = the susceptible receptor (can be activated)
  - R_a = the activated receptor (bound to the ligand)

A more realistic Receptor activation (1): the model

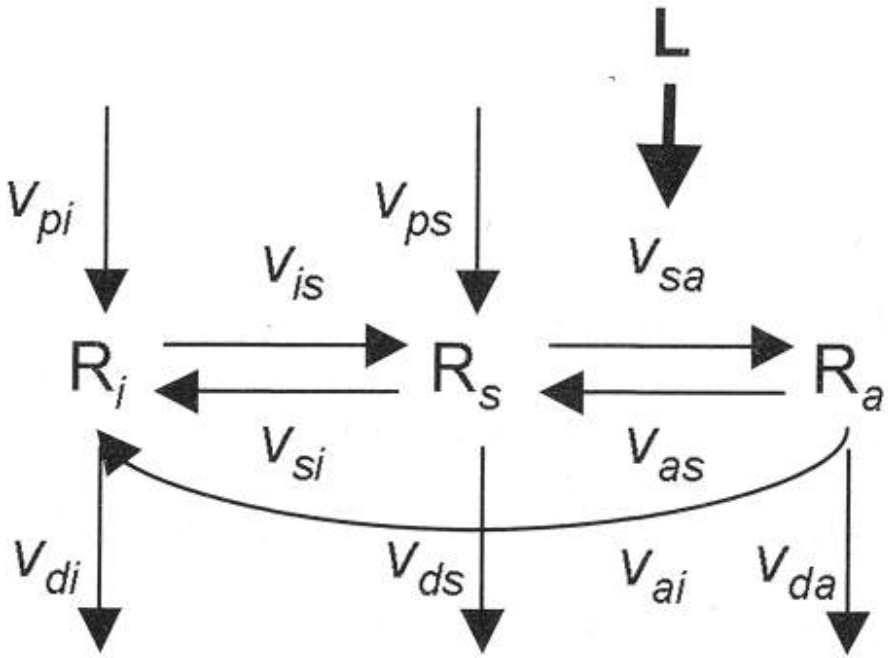

Fig. 6.4 Schematic representation of processes involved in receptor activation by a ligand. L = ligand, R_i = inactive receptor, R_s = susceptible receptor, R_a = active receptor. v_{p*} = production steps, v_{d*} = degradation steps, other steps = transition between inactive, susceptible, and active state of receptor.

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A more realistic Receptor activation (2): kinetic equations

- The receptor is present either in the inactive form (R_i) or susceptible form (R_s)
- R_s can interact with the ligand L to give the active form R_a
- The inactive or susceptible form is produced from precursors with rates v_{pi} or v_{ps}
- All three forms can be degraded with rates v_{di}, v_{ds}, v_{da}

$$\frac{d}{dt}R_i = v_{pi} - v_{di} - v_{is} + v_{si} + v_{ai}$$

$$\frac{d}{dt}R_s = v_{ps} - v_{ds} + v_{is} - v_{si} - v_{sa} + v_{as}$$

$$\frac{d}{dt}R_a = -v_{da} + v_{sa} - v_{as} - v_{ai}$$

A more realistic Receptor activation (3):

rate approximation

- The degradation terms are assumed to be linearly dependent on the concentration of substrates (in this case the receptor R) (here the asterisks means any species, either i, s or a):

$$v_{d*} = k_{d*} \cdot R_*$$

- Also the state changes can be approximated linearly as first guess:

$$v_{is} = k_{is} \cdot R_i$$

- The receptor activation is dependent on ligand concentration. A linear approximation is:

$$v_{sa} = k_{sa} \cdot R_s \cdot L$$

A practical example…

Example 6-1

An experimentally confirmed example for the activation of receptor and G protein of the pheromone pathway has been presented by Yi and colleagues (2003) for the binding of the pheromone α-factor to the receptor Ste2 in yeast. Concerning the receptor activation dynamics, they report a susceptible and an active form of the receptor, but no inactive form ($R_i = 0$, $v_{*i} = v_{i*} = 0$). The remaining rates are determined as follows:

$$
\begin{aligned}
v_{ps} &= k_{ps} \\
v_{ds} &= k_{ds} \cdot R_s \\
v_{da} &= k_{da} \cdot R_a \\
v_{sa} &= k_{sa} \cdot R_s \cdot L \\
v_{as} &= k_{as} \cdot R_a \,, \qquad (6\text{-}4)
\end{aligned}
$$

with the following values for the rate constants: $k_{ps} = 4$ (molecules per cell) s^{-1}, $k_{ds} = 4 \cdot 10^{-4}\, s^{-1}$, $k_{da} = 4 \cdot 10^{-3}\, s^{-1}$, $k_{sa} = 2 \cdot 10^{6}\, M^{-1}\, s^{-1}$, and $k_{as} = 1 \cdot 10^{-2}\, s^{-1}$. The time course of receptor activation is depicted in Fig. 6.5.

…and its time course

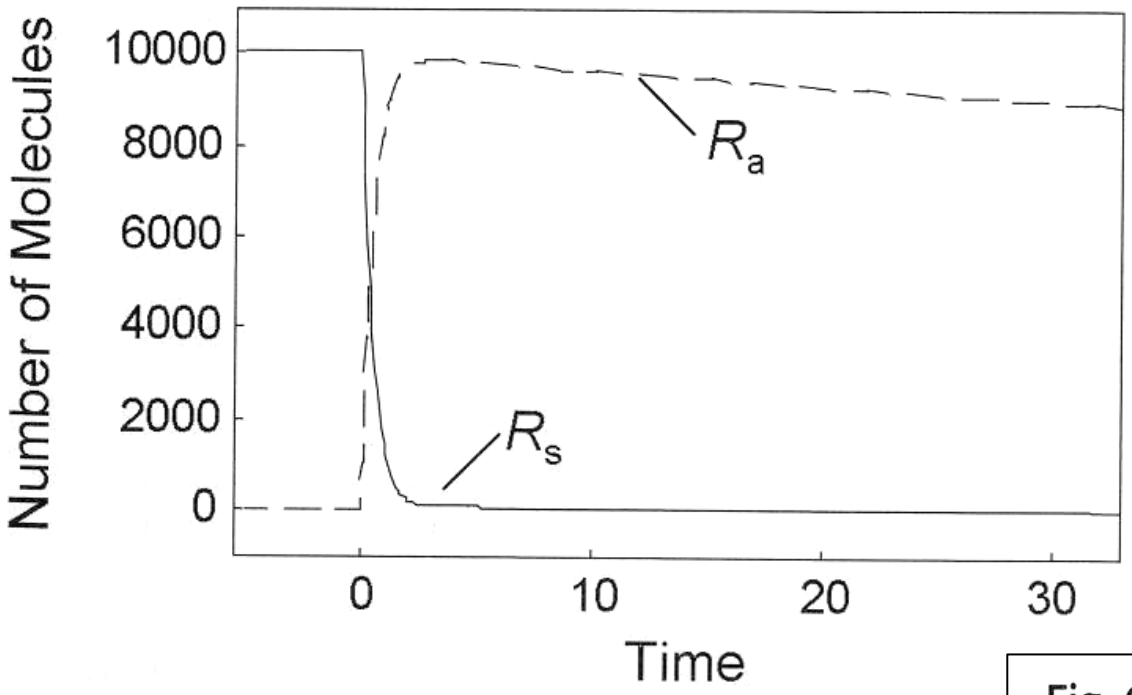

Fig. 6.5 Time course of active (solid line) and susceptible (dashed line) receptor after stimulation with 1 μM α-factor at $t = 0$. The total number of receptors is 10,000. The concentration of the active receptor increases immediately and then declines slowly, while the susceptible receptor is effectively reduced to zero.

Signalling Paradigm: the structural components

- **G protein cycle**
- MAP kinase cascade

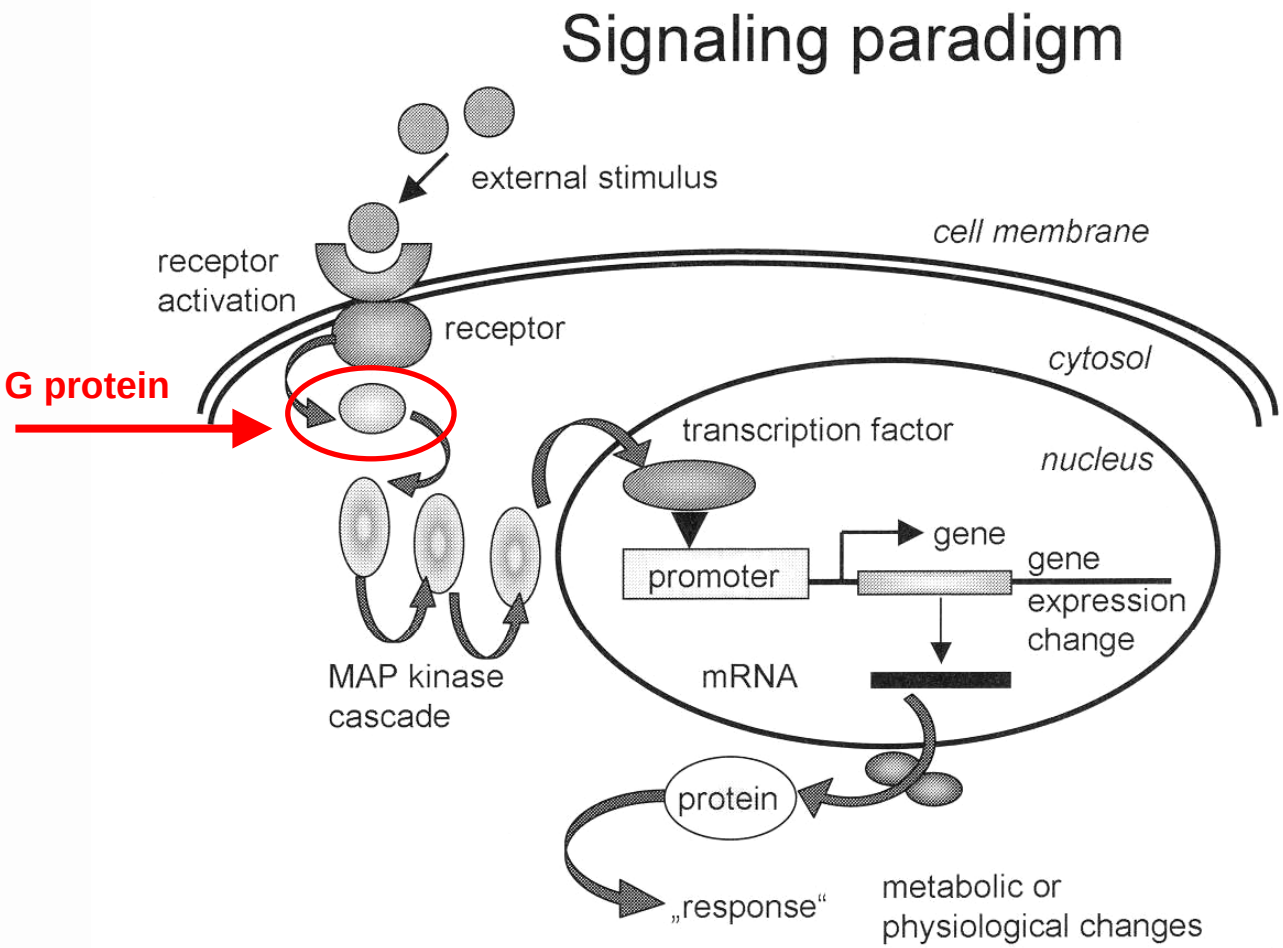

Fig. 6.1 Visualization of the signaling paradigm (for description, see text). The receptor is stimulated by a ligand or another kind of signal, and it changes its own state from susceptible to active. The active receptor initiates the internal signaling cascade, including a series of protein phosphorylation state changes. Subsequently, transcription factors are activated or deactivated. The transcription factors regulate the transcription rate of a set of genes. The absolute amount or the relative changes in protein concentrations alter the state of the cell and trigger the actual response to the signal.

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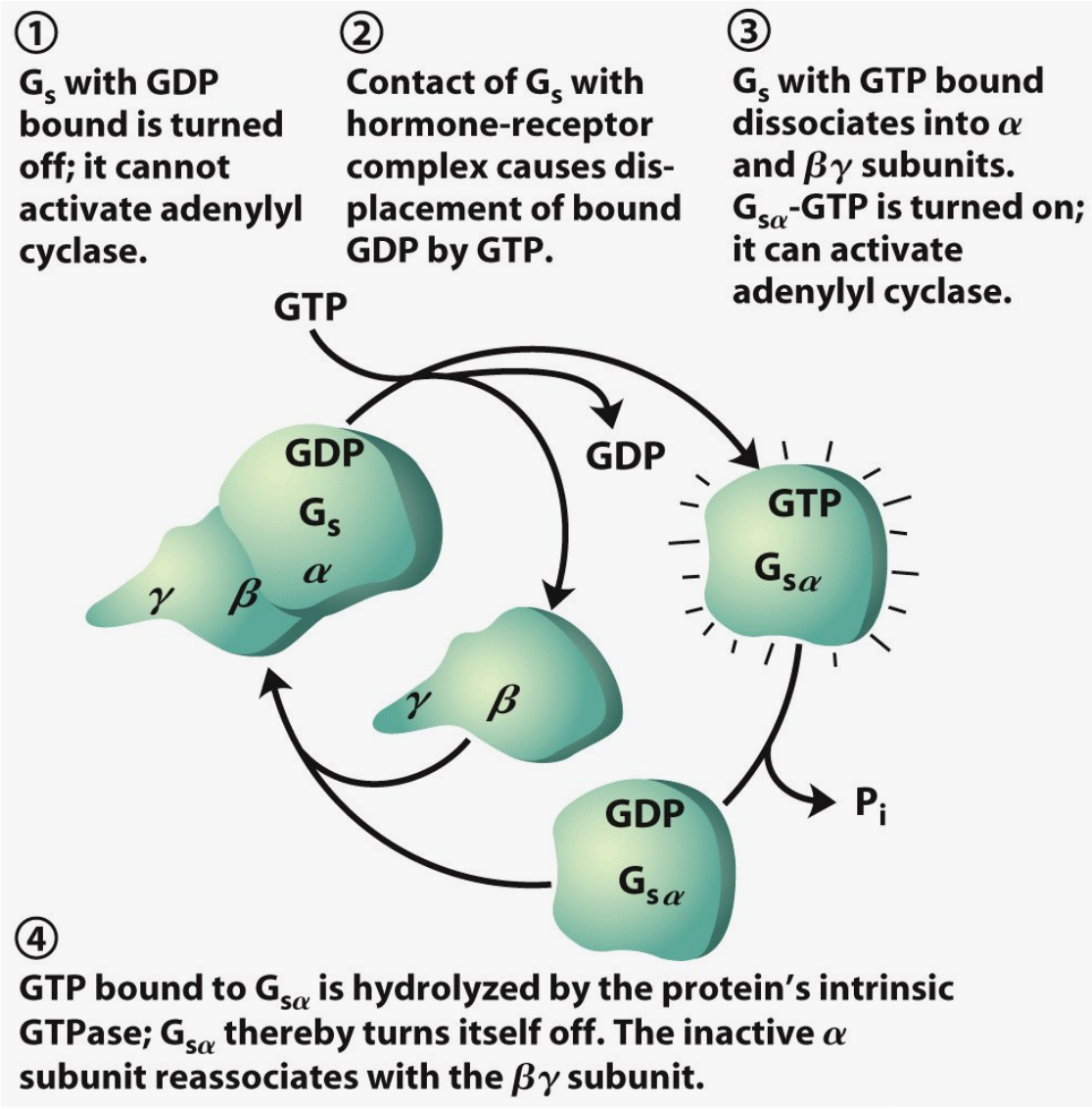
Guanosine nucleotide-binding protein (G protein)

- The **G protein** is an heterotrimer, consisting of 3 different subunits (alpha, beta and gamma)
- The alpha subunit bound to GDP (guanosine di-[two] phosphate) has high affinity for the beta and gamma subunits
- When it is stimulated by the activated receptor, the alpha subunit exchanges bound GDP for GTP (guanosine tri-[three] phosphate)
- The GTP-bound alpha subunit dissociates from the beta/gamma components, and it binds to a nearby enzyme, altering its activity
- GTP is then hydrolysed to GDP (looses a phosphate group) by the intrinsic GTPase activity of the alpha subunit, that regains its affinity for the beta/gamma subunits

The G protein cycle



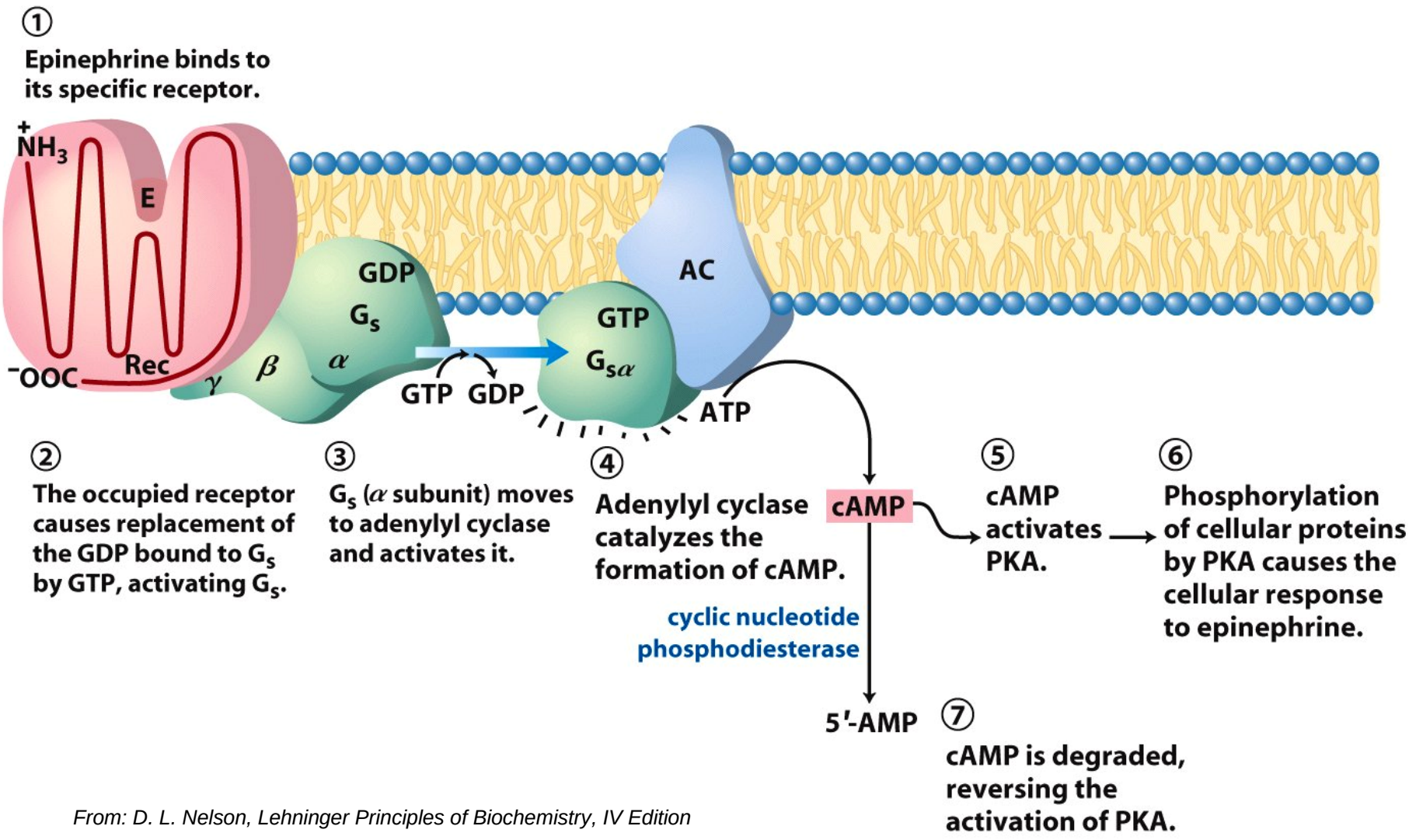
From: D. L. Nelson, Lehninger Principles of Biochemistry, IV Edition

G protein Coupled Receptors

- The human genome encodes more than 1000 G-protein Coupled Receptors (GPCR), that transduce messages as diverse as light, smells, taste, and hormones

- An example is the beta-adrenergic receptor, that mediates the effects of epinephrine on many tissues:…

An example of G protein Coupled Receptor…



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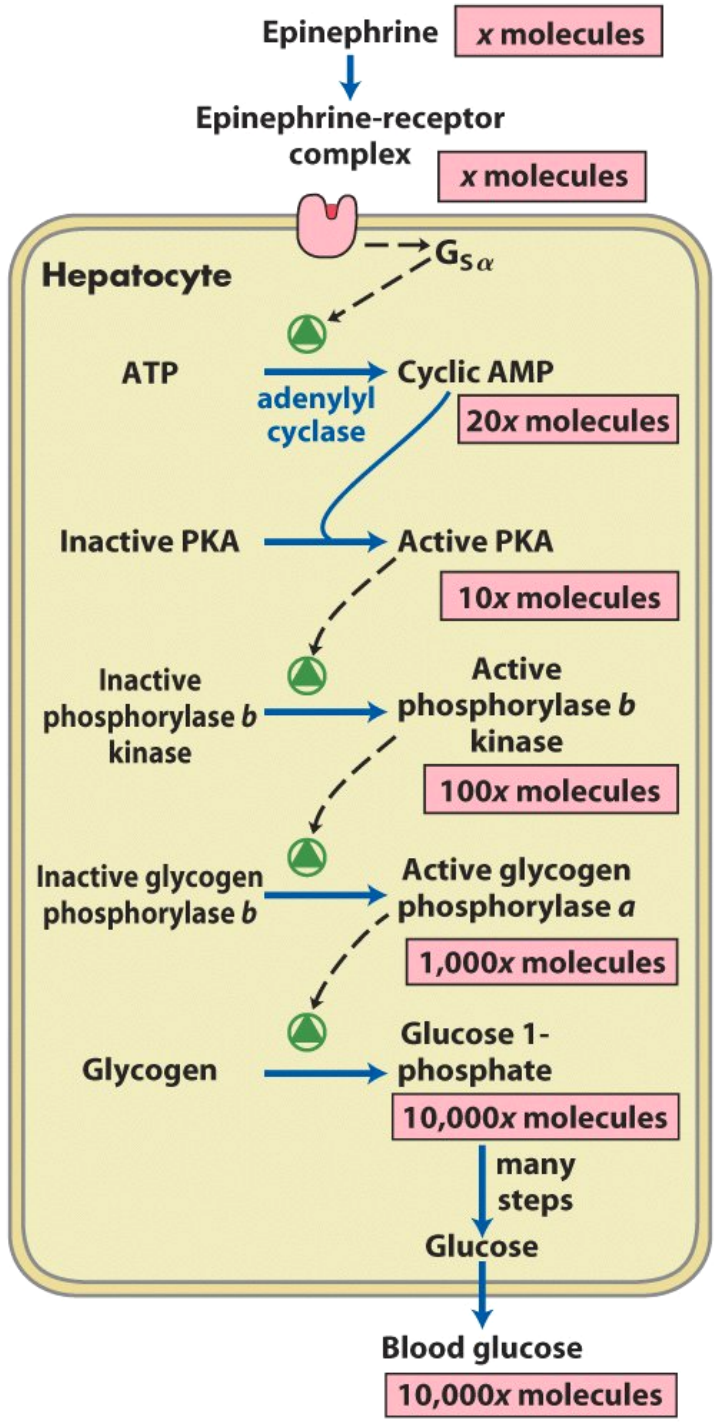
… and the following signalling cascade

- An example of signal transduction <u>amplification</u>



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A more realistic G protein cycle

- Note: in this model in addition to the intrinsic GTPase activity of the G protein (slow rate v_{h0}), there is a faster hydrolysis (fast rate v_{h1}) supported by RGS (Regulator of G protein Signalling)

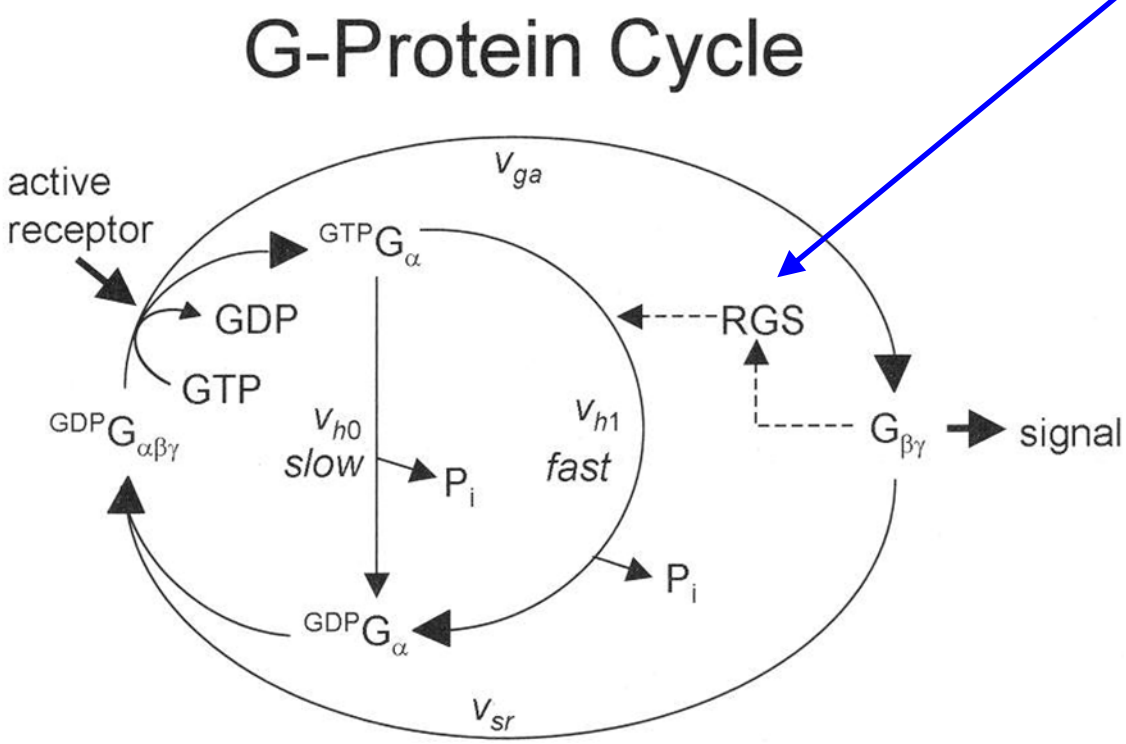

Fig. 6.6 Activation cycle of G protein. Without activation, the heterotrimeric G protein is bound to GPD. Upon activation by the activated receptor, an exchange of GDP with GTP occurs and the G protein is divided into GTP-bound Gα and the heterodimer Gβγ. Gα-bound GTP is hydrolyzed, either slowly in reaction v_{h0} or fast in reaction v_{h1}, supported by the RGS protein. GDP-bound Gα can re-associate with Gβγ (reaction v_{sr}).

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The G protein model: kinetic equations

Example 6-2

The model of the heterotrimeric G protein cycle of the yeast pheromone pathway was already mentioned in Example 6-1 and is linked to the receptor activation model via the concentration of the active receptor. The G protein cycle model comprises two ODEs and two algebraic equations for the mass conservation of the subunits Gα and Gβγ. This gives

$$\frac{d}{dt} G_{\alpha\beta\gamma} = -v_{ga} + v_{sr}$$

$$\frac{d}{dt} G_{\alpha} GTP = v_{ga} - v_{h0} - v_{h1}$$

$$G_t = G_{\alpha\beta\gamma} + G_{\alpha} GTP + G_{\alpha} GDP$$

$$G_{total} = G_{\alpha\beta\gamma} + G_{\beta\gamma} \,. \qquad (6\text{-}5)$$

The rate equations for the G protein activation, v_{ga}, the hydrolysis of $G_{\alpha}GTP$, v_{h0} and v_{h1}, and the subunit re-association, v_{sr}, follow simple mass action kinetics:

$$v_{ga} = k_{ga} \cdot R_a \cdot G_{\alpha\beta\gamma}$$

$$v_{hi} = k_{hi} \cdot G_{\alpha} GTP, \; i = 0,1$$

$$v_{sr} = k_{sr} \cdot G_{\beta\gamma} \cdot G_{\alpha} GDP \,. \qquad (6\text{-}6)$$

The parameters are $k_{ga} = 1 \cdot 10^{-5}$ (molecules per cell)$^{-1}$ s^{-1}, $k_{h0} = 0.004$ s^{-1}, $k_{h1} = 0.11$ s^{-1}, and $k_{sr} = 1$ (molecules per cell)$^{-1}$ s^{-1}. Fig. 6.7 shows the respective simulation. Note that in the original work two different yeast strains have been considered. For the strains with a constantly active RGS ($SST2^{+}$) or with a deletion of RGS ($sst2\Delta$), the rate constants k_{h1} and k_{h0} have been set to zero, respectively.

ODE = ordinary differential equation

The G protein model: time course

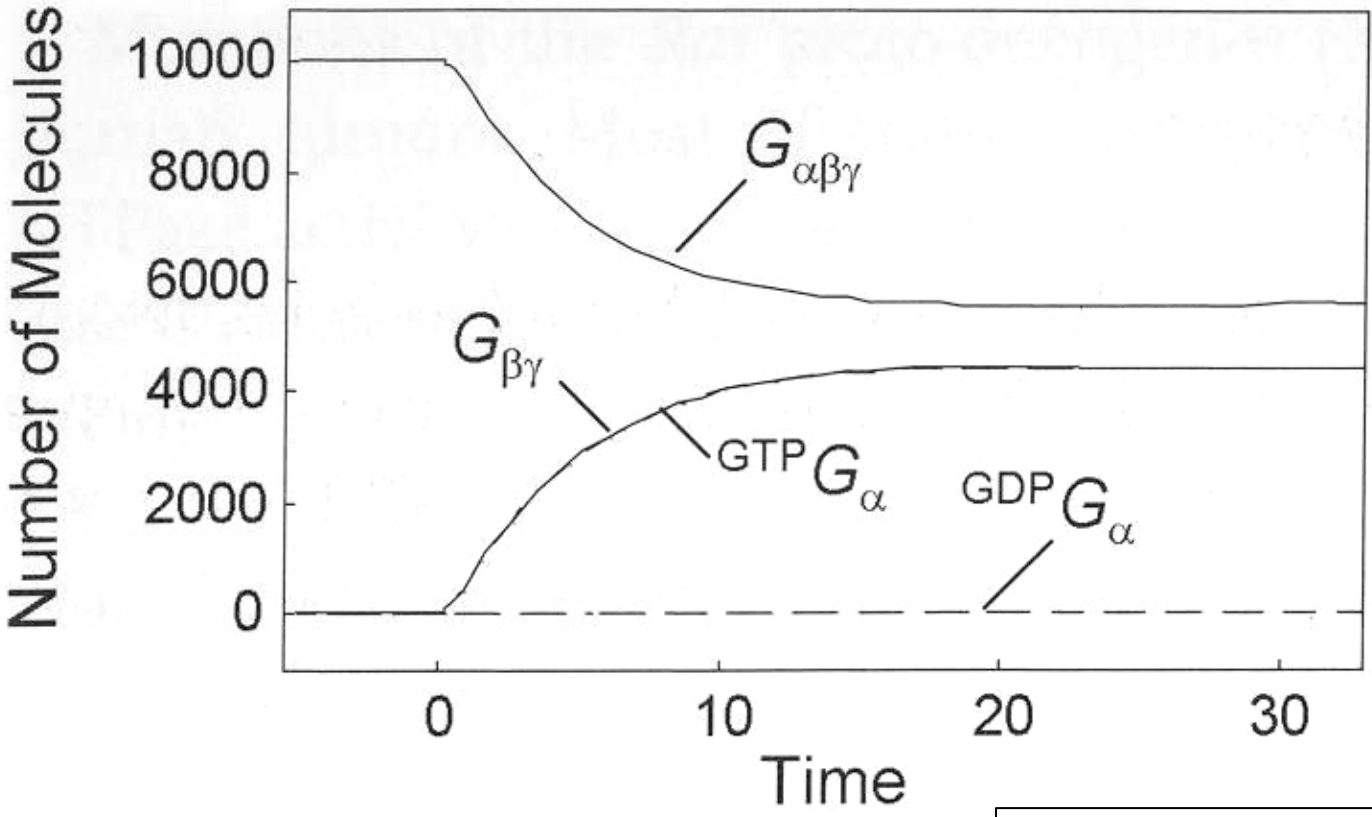

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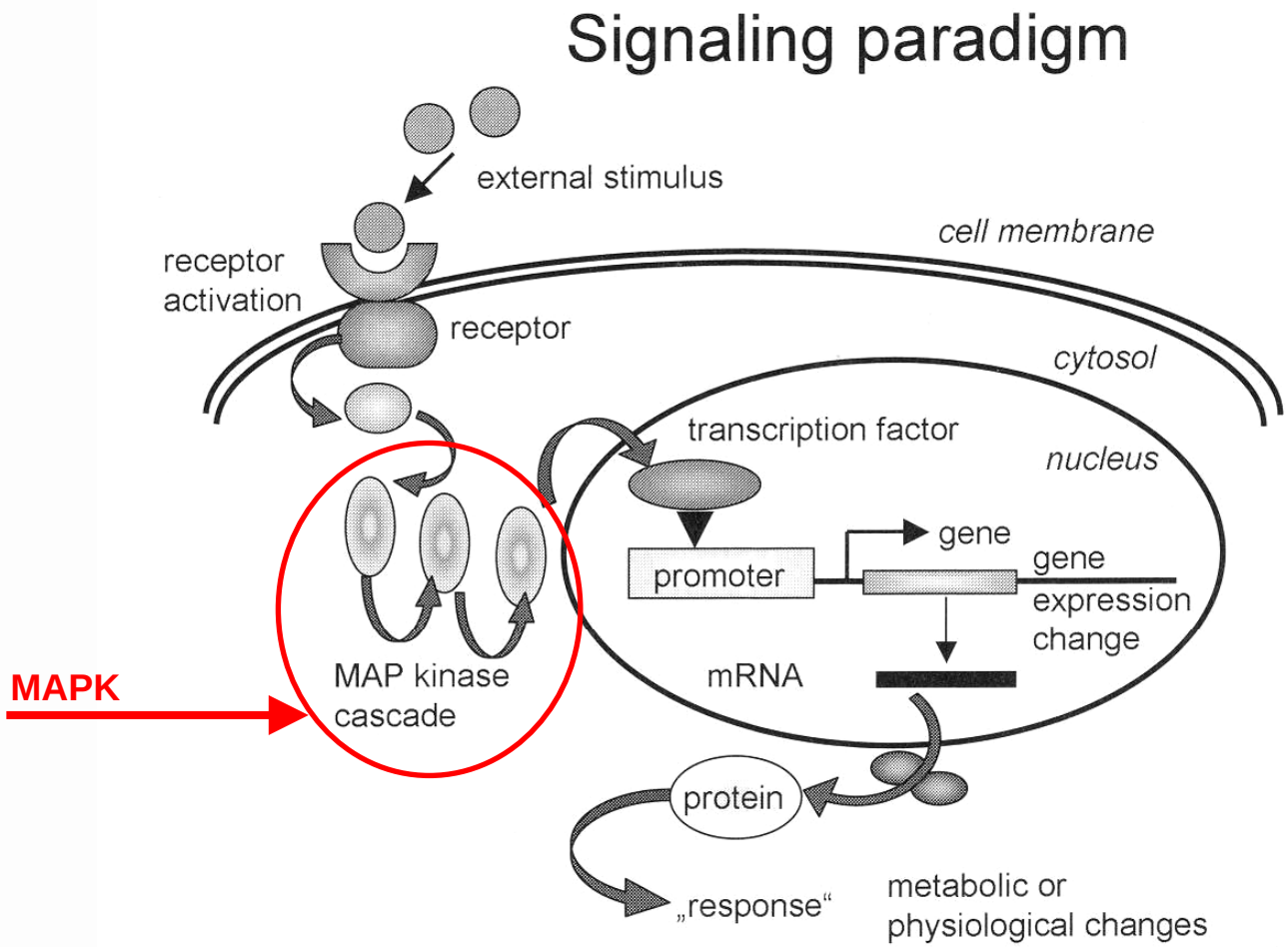
Fig. 6.7 Time course of G protein activation. The total number of molecules is 10,000. The concentration of GDP-bound Gα is low for the whole period due to fast complex formation with the heterodimer Gβγ.

Signalling Paradigm: the structural components

- G protein cycle
- **MAP kinase cascade**



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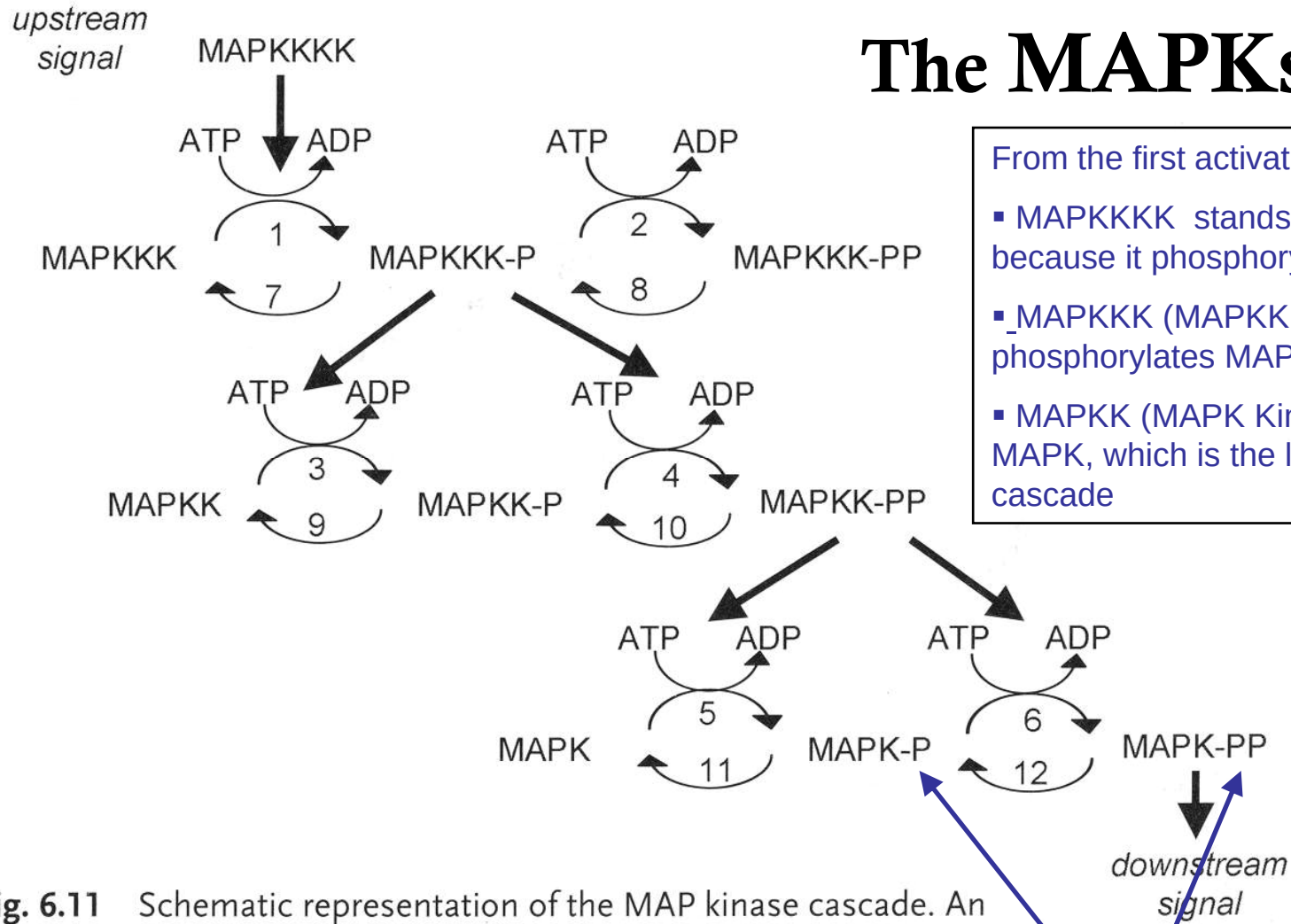
Mitogen-activated protein kinases (MAPKs)

- MAPKs belong to a family of serine/threonine kinases (kinases add phosphate groups to/phosphorylate other proteins)
- They participate in cell growth, differentiation, transformation, apoptosis (controlled cell death) and others
- They are conserved from yeast to mammal, but their names differ in different species:

Tab. 6.1 Names of the components of MAP kinase pathways in different organisms and different pathways.

Organism	*Budding yeast*		*Xensopus oocytes*	*Human, cell cycle regulation*		
	HOG pathway	Pheromone pathway			p38 pathway	JNK pathway
MAPKKK	Ssk2/Ssk22	Ste11	Mos	Rafs (c-, A- and B-),	Tak1	MEKKs
MAPKK	Pbs2	Ste7	MEK1	MEK1/2	MKK3/6	MKK4/7
MAPK	Hog1	Fus3	p42 MAPK	ERK1/2	p38	JNK1/2

The MAPKs cascade



From the first activation step:

- MAPKKKK stands for "MAPKKK Kinase" because it phosphorylates MAPKKK
- MAPKKK (MAPKK Kinase) phosphorylates MAPKK
- MAPKK (MAPK Kinase) phosphorylates MAPK, which is the last enzyme of the cascade

Fig. 6.11 Schematic representation of the MAP kinase cascade. An upstream signal (often by a further kinase called MAP kinase kinase kinase kinase) causes phosphorylation of the MAPKKK. The phosphorylated MAPKKK in turn phosphorylates the protein at the next level. Dephosphorylation is assumed to occur continuously by phosphatases or autodephosphorylation.

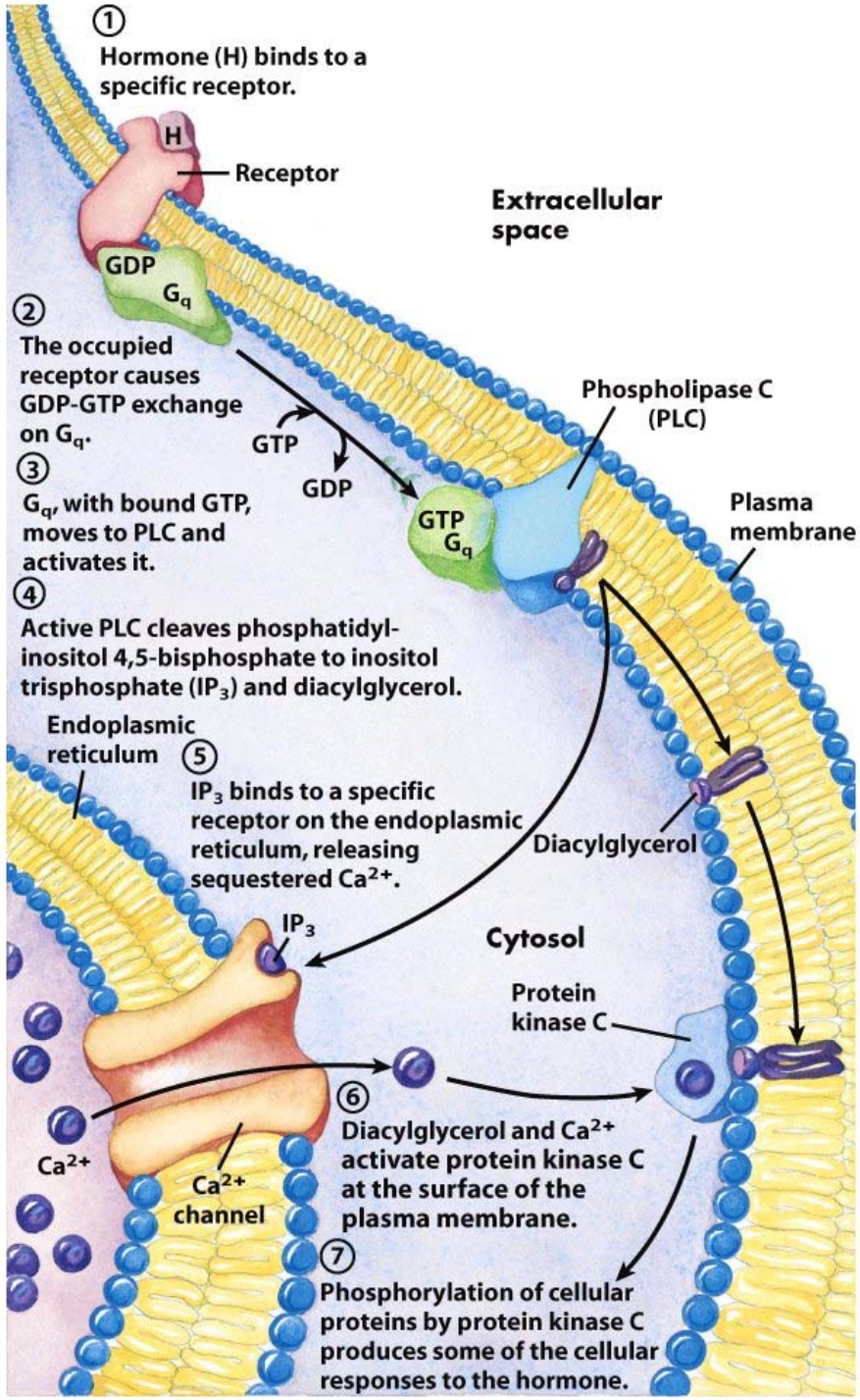
MAPKs are phosphorylated on two separate sites (amino acids)

Reading

- D. L. Nelson, Lehninger Principles of Biochemistry, IV Edition:
  - Parts of Chapter 12 on Biosignalling

- E. Klipp, Systems Biology in Practice, Wiley-VCH, 2005:
  - Parts of Chapter 6 on Signal Transduction

Appendix (1): additional example of G protein coupled receptor



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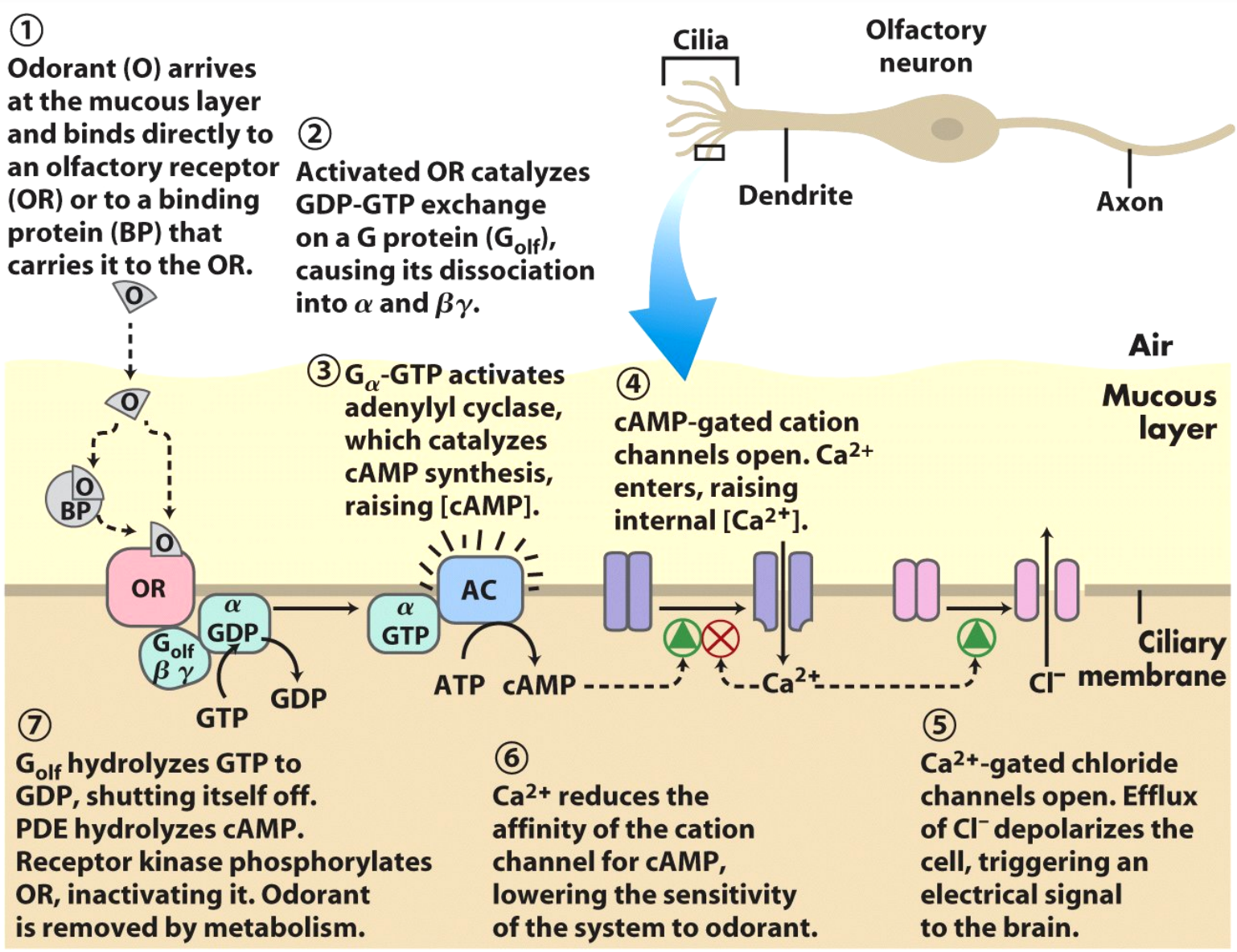
Appendix (2): additional example of G protein coupled receptor



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