Cell signalling

Suggested reading:
Reese et al. (2013) Campbell Biology.
Sadava & Hills (2012) Life: The Science of Biology.
Chapters on cell signalling

http://www.biocenter.sk/lt.html
Link „Teaching“

25.10.2016; Ľ. Tomáška, Department of Genetics

Useful www links:

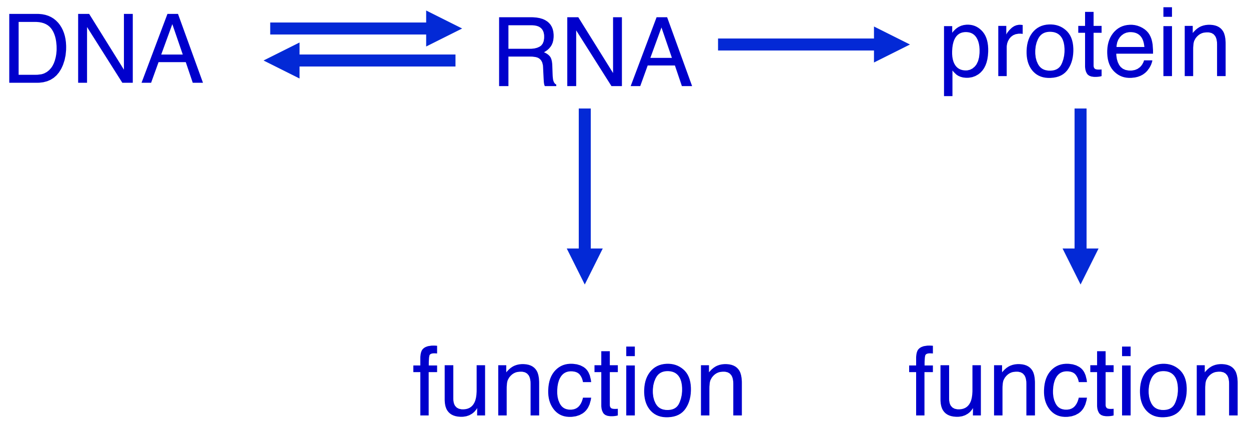
MIT Open courses: http://ocw.mit.edu/OcwWeb/Biology/index.htm

Nobel prize lectures:
http://nobelprize.org/

TED lectures:
http://www.ted.com/

iBiology:
www.ibiology.org

The **Central dogma of molecular biology** describes the direction of flow of genetic information

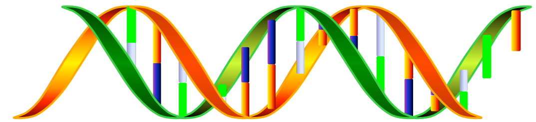


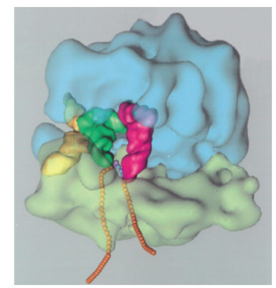
A metaphoric representation of the Central dogma is decoding of a digital message

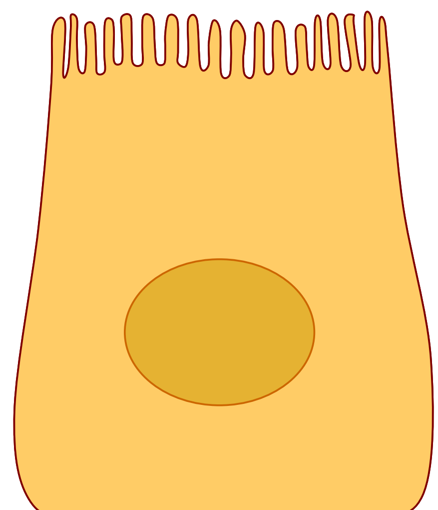
Coded instructions

Decoder

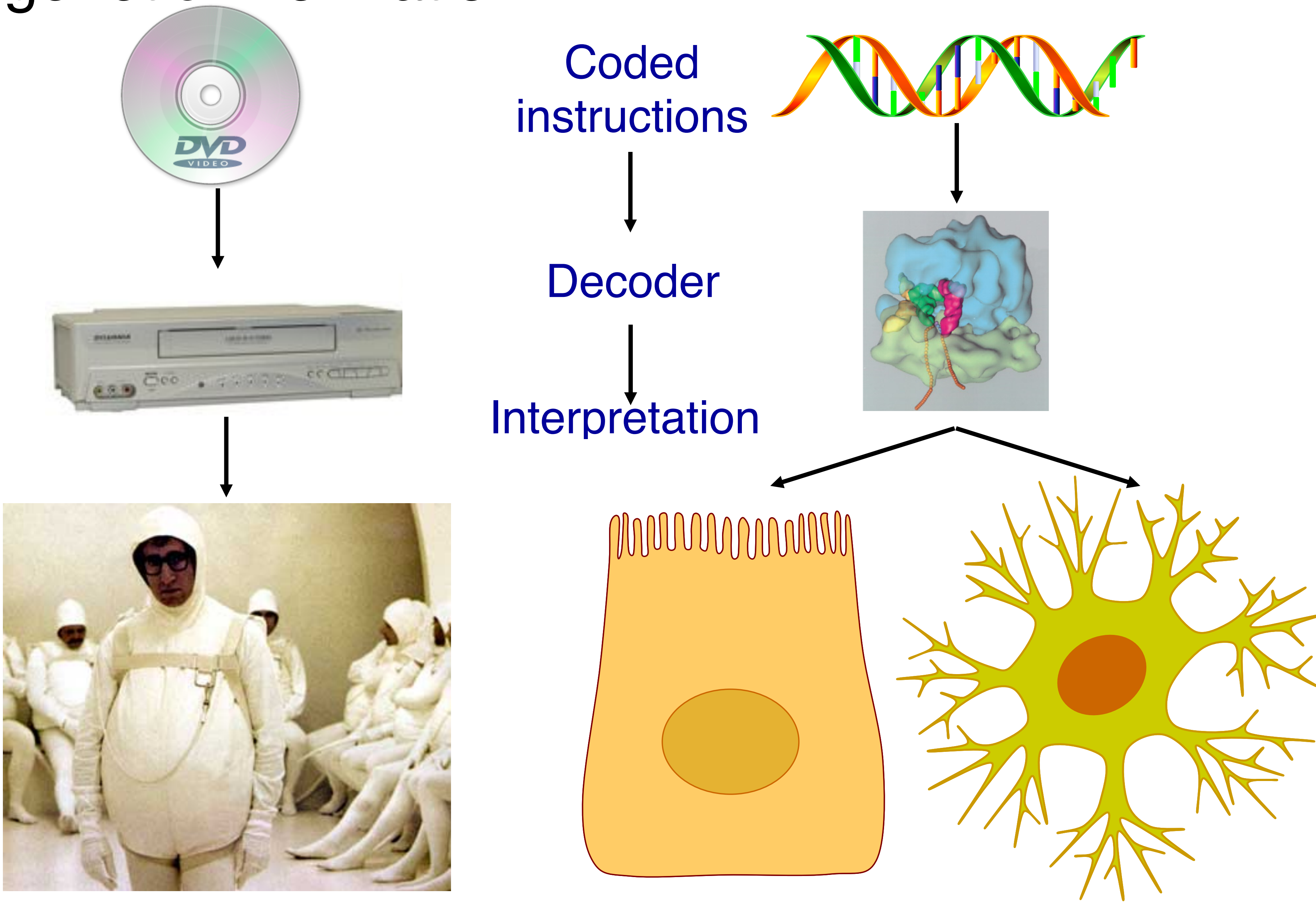
Interpretation





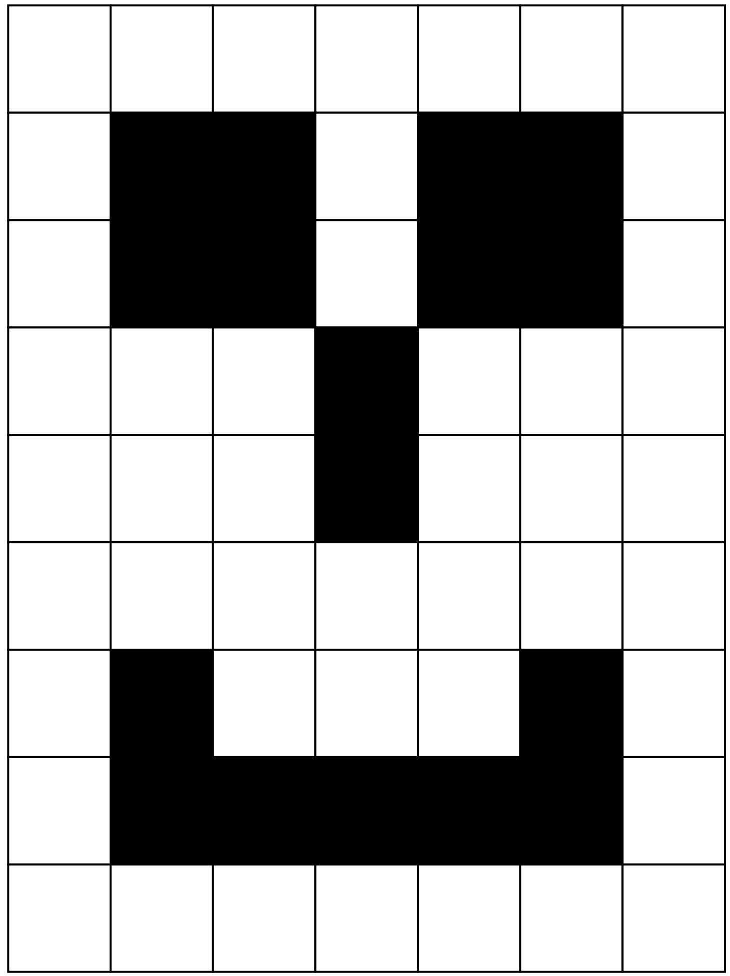


However, this metaphor cannot explain ambiguity of genetic information



Interpretation of the encoded information is context-dependent

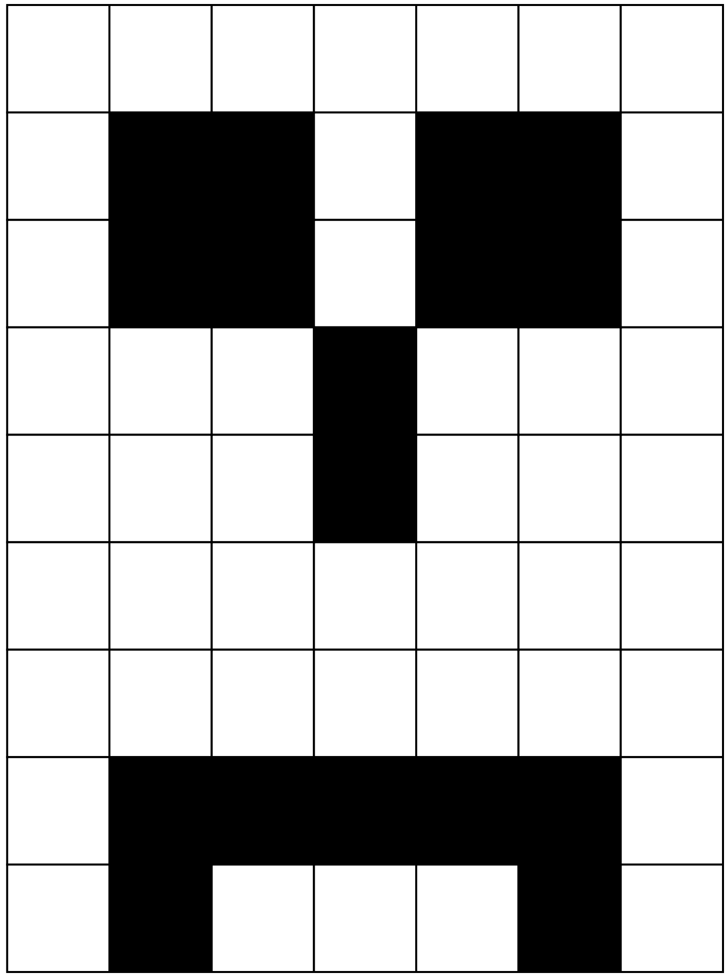
000000001101100110110000100000010000000000010001001111100000000



7x9

Interpretation of the encoded information is context-dependent

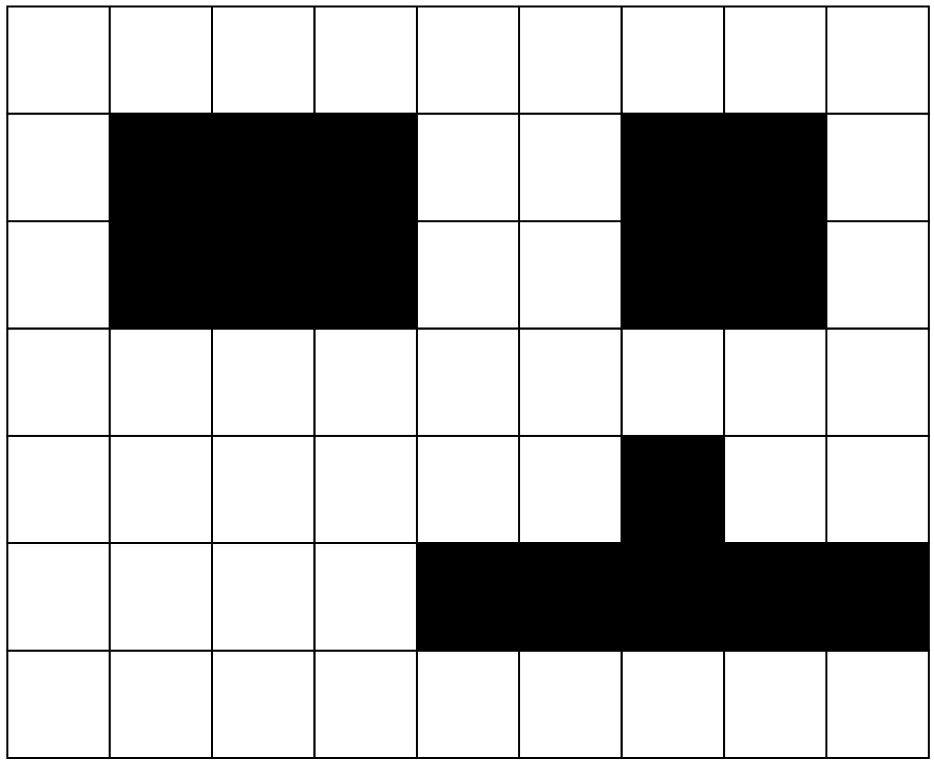
000000001101100110110000100000010000000000000000000000111110010001 0



7x9

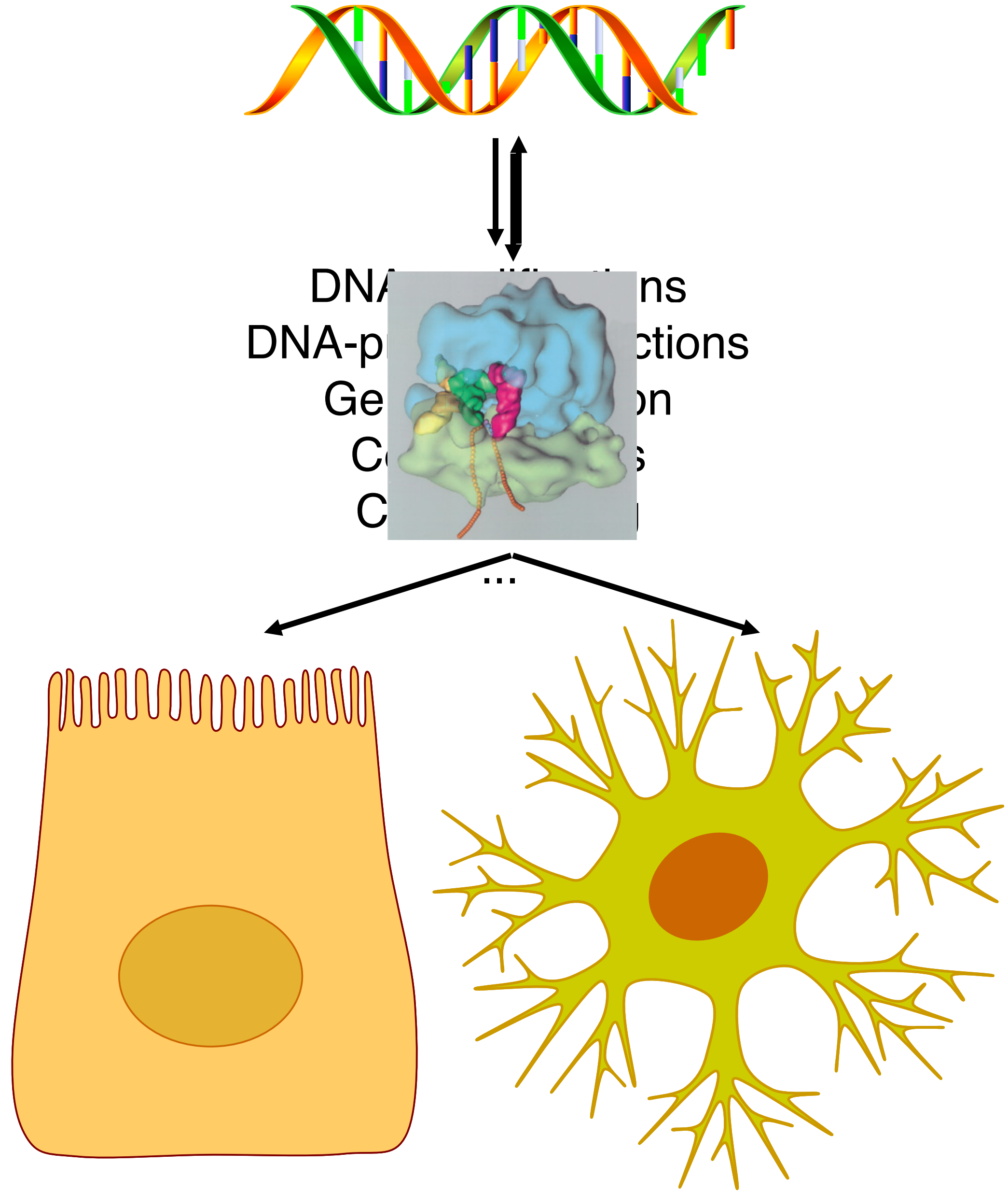
Interpretation of the encoded information is context-dependent

0000000011011001101100001000000010000000000010001001111100000000

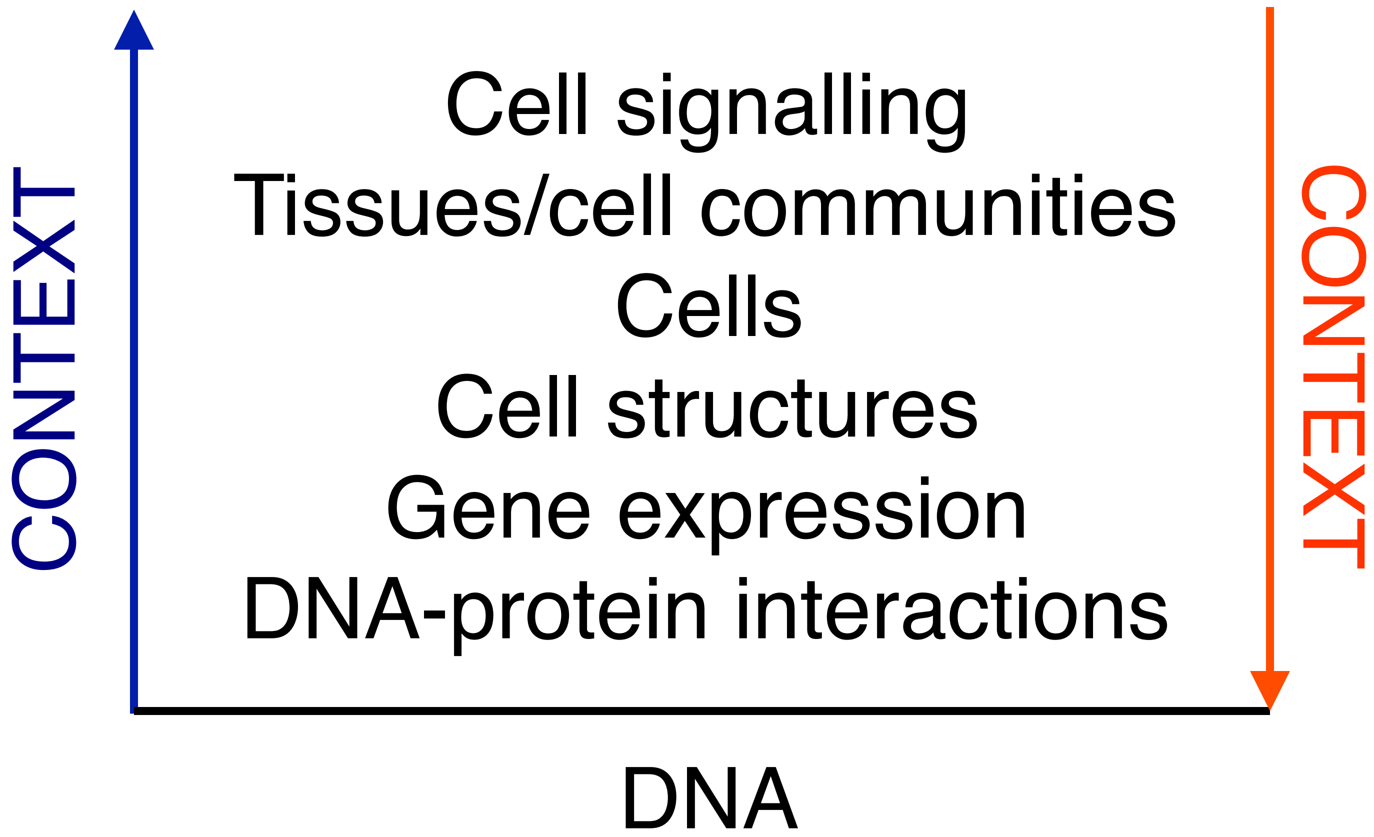


9x7

Interpretation of the encoded information is context-dependent



Cell signalling is an important tool for expression of genetic information



Cells are constantly exposed to a wide variety of signals

Sadava & Hills (2012). Life: The Science of Biology.

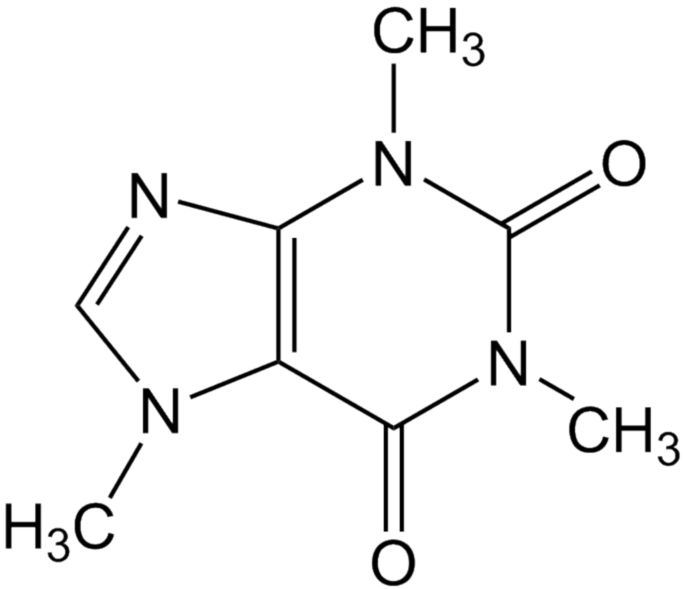
These signals come from both external and internal envirobments

Sadava & Hills (2012). Life: The Science of Biology.

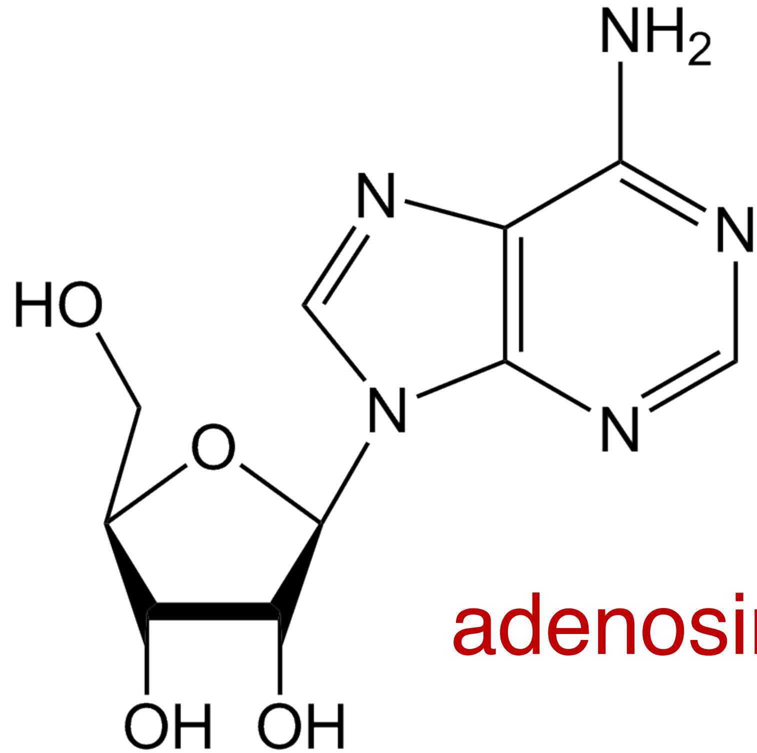
Caffeine is one of the environmental signals affecting our cells

Coffee	180 mg / cup
Tea	90 mg / cup
Coca-Cola	50 mg / cup
Chocolade	20 mg / cup

Signals from the environment affect our cells by imitating natural chemicals produced by the body



caffeine

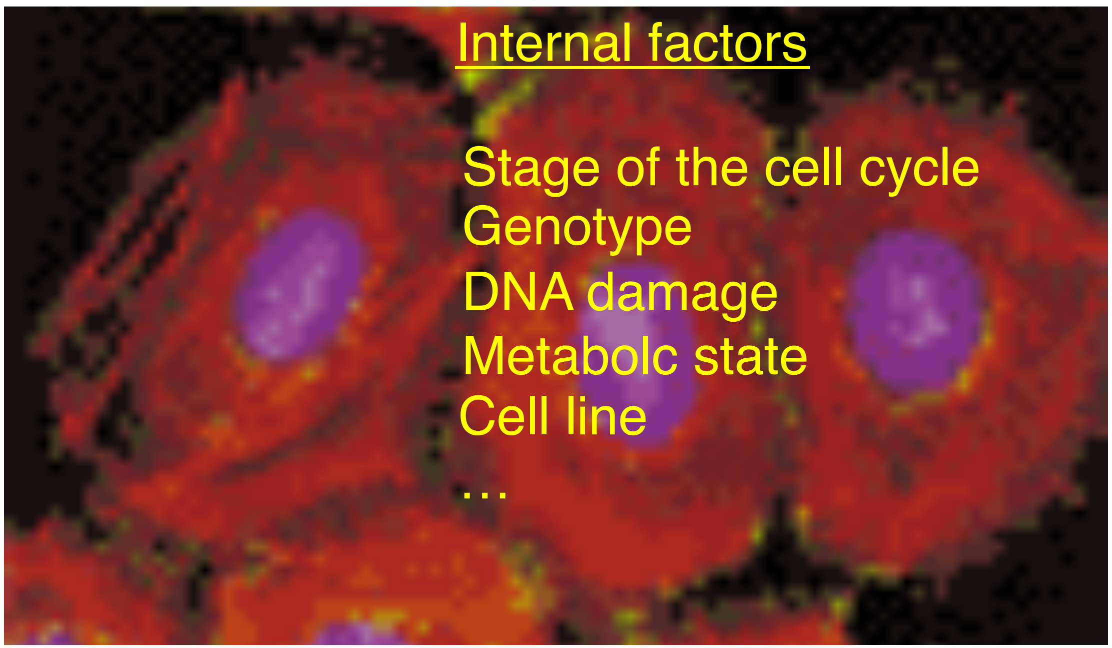


adenosine

Correct interpretation of the signals is essential for appropriate cellular decisions

External factors

Nutrients
Growth factors
Temperature
…



Cell division

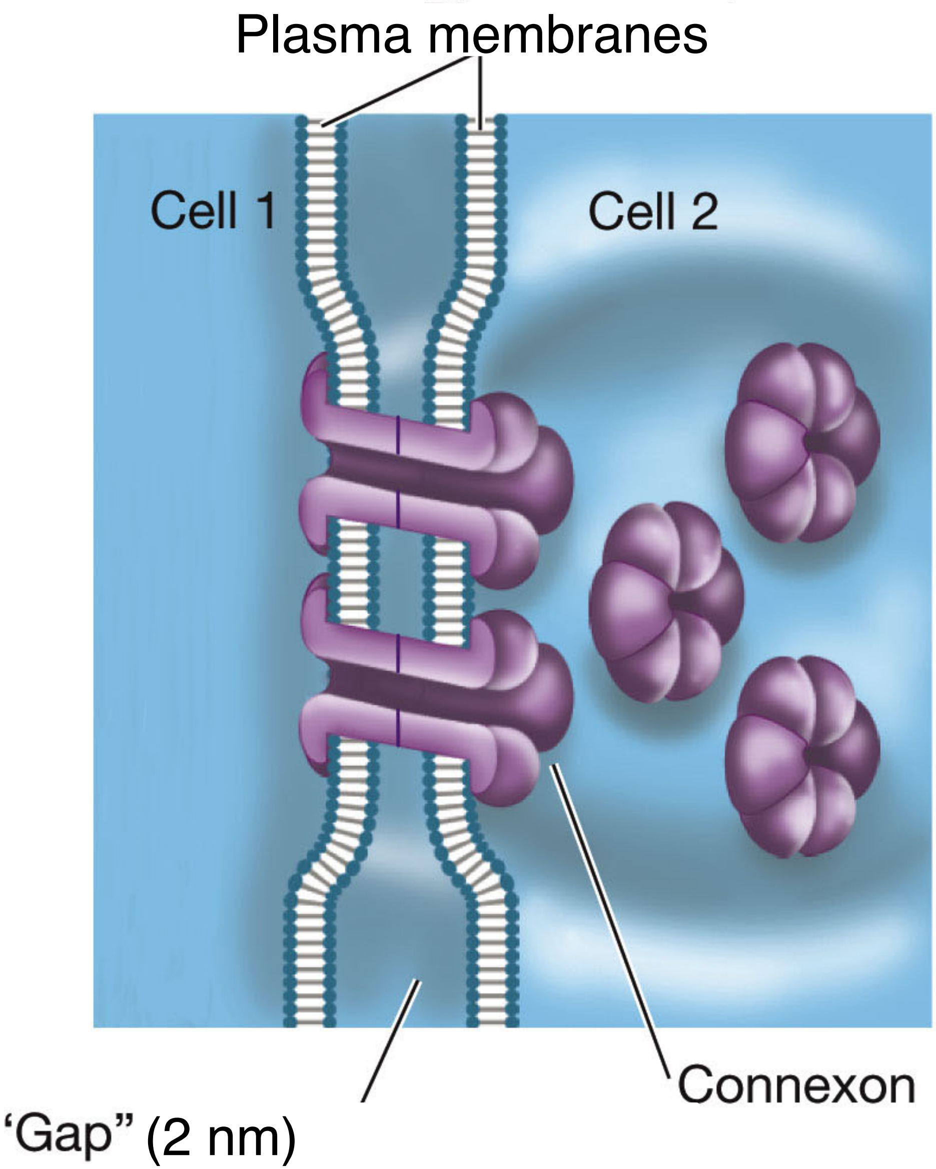
Quiscience

Differentiation

Senescence

Apoptosis

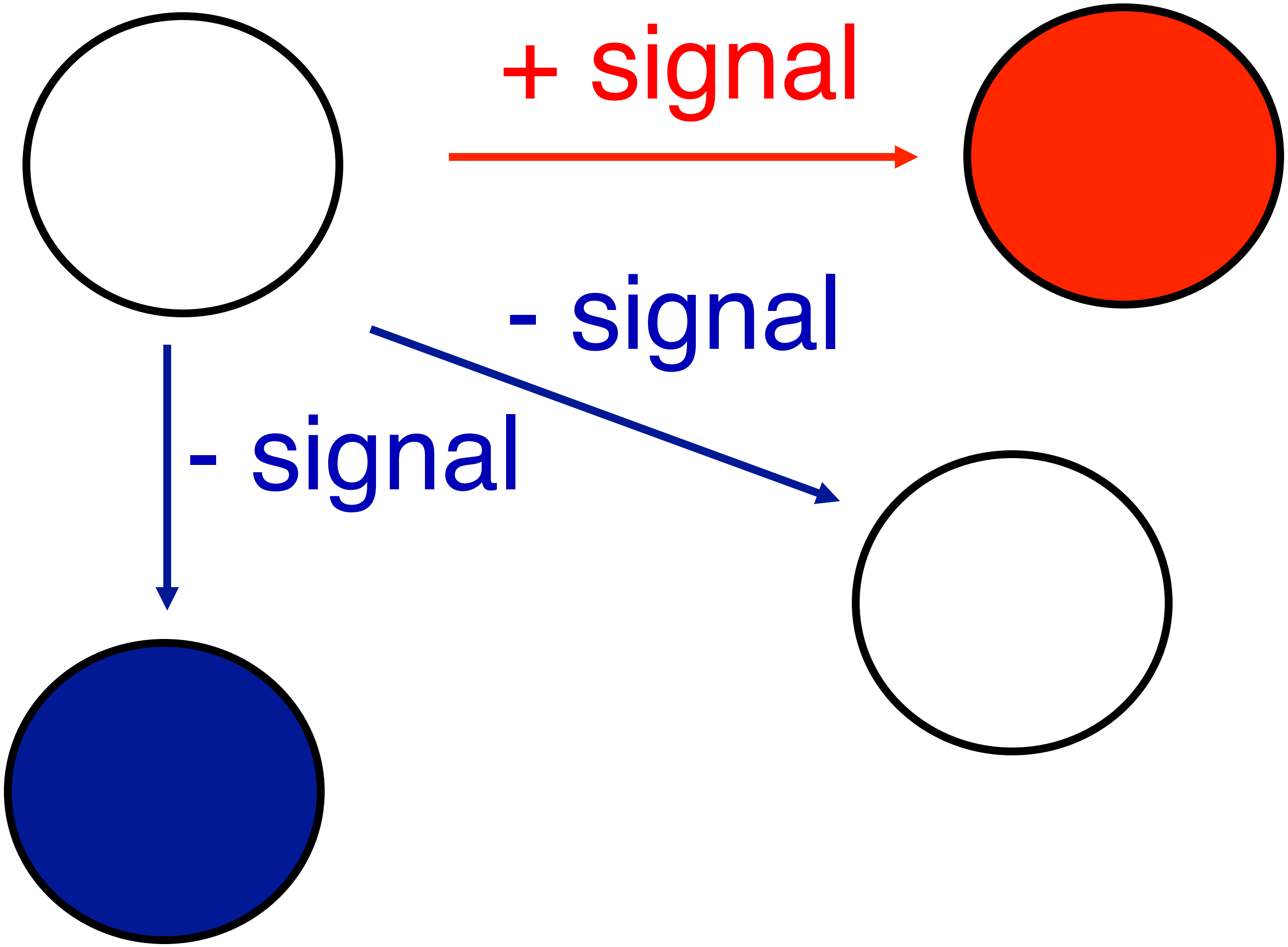
Gap junctions mediate direct cell-to-cell communications



Sadava & Hills (2012). Life: The Science of Biology.

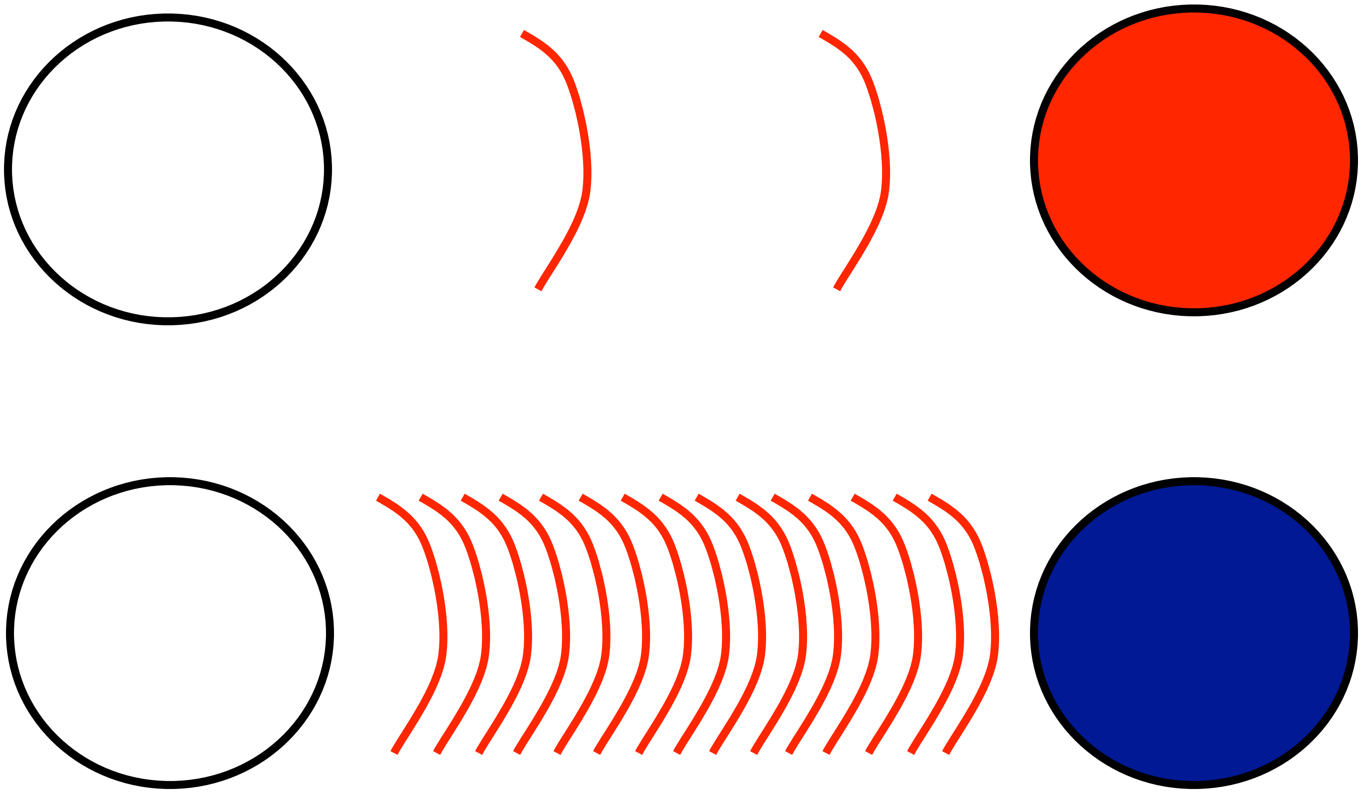
In most cases the cell-cell communication takes place over longer distances

1. Binary choice



In most cases the cell-cell communication takes place over longer distances

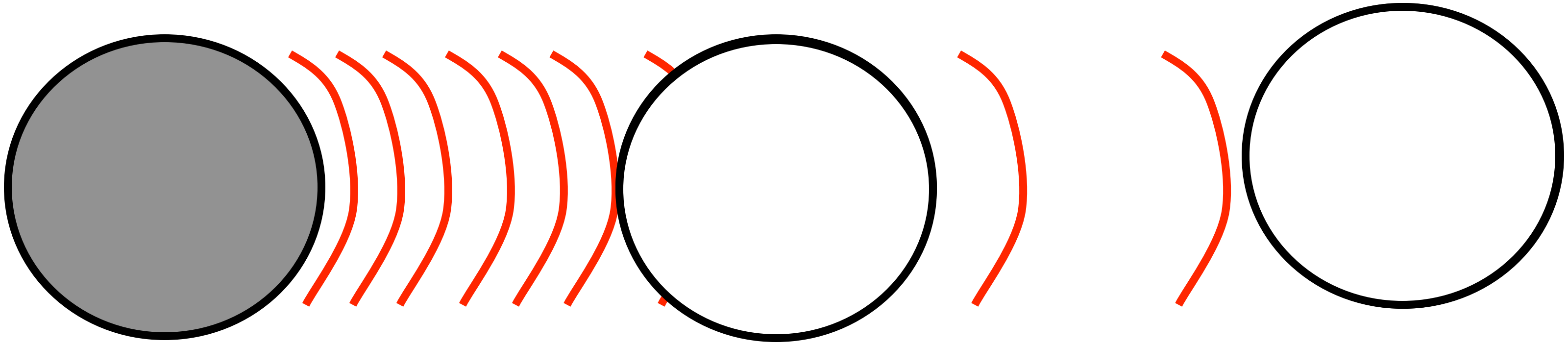
2. Cell fate can depend on the concentration of the signal



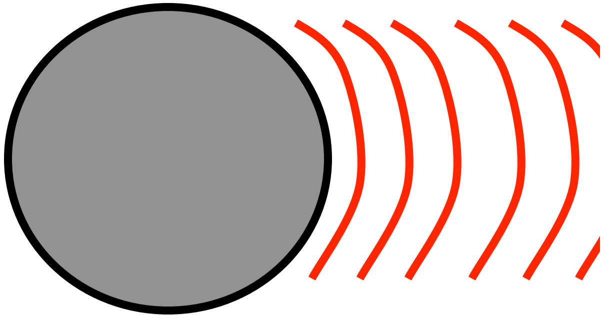
In most cases the cell-cell communication takes place over longer distances

Cell signalling can mediate formation of patterns during ontogenesis

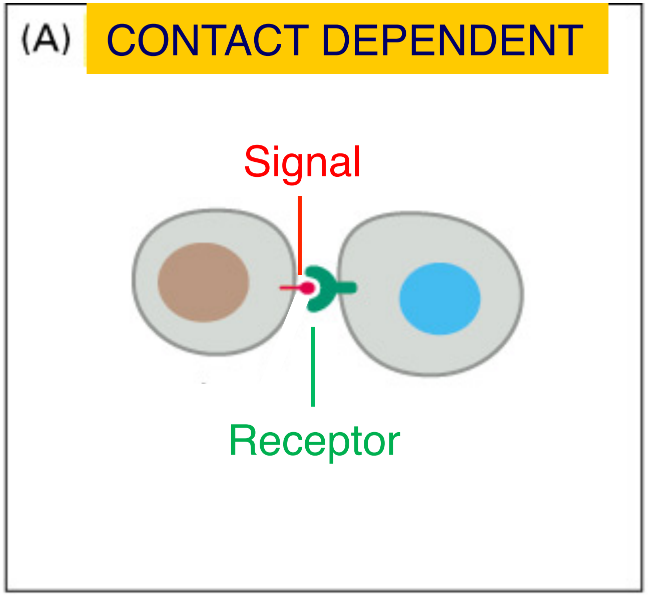
Gradient model



„Relay“ model



There are various forms of cell signalling



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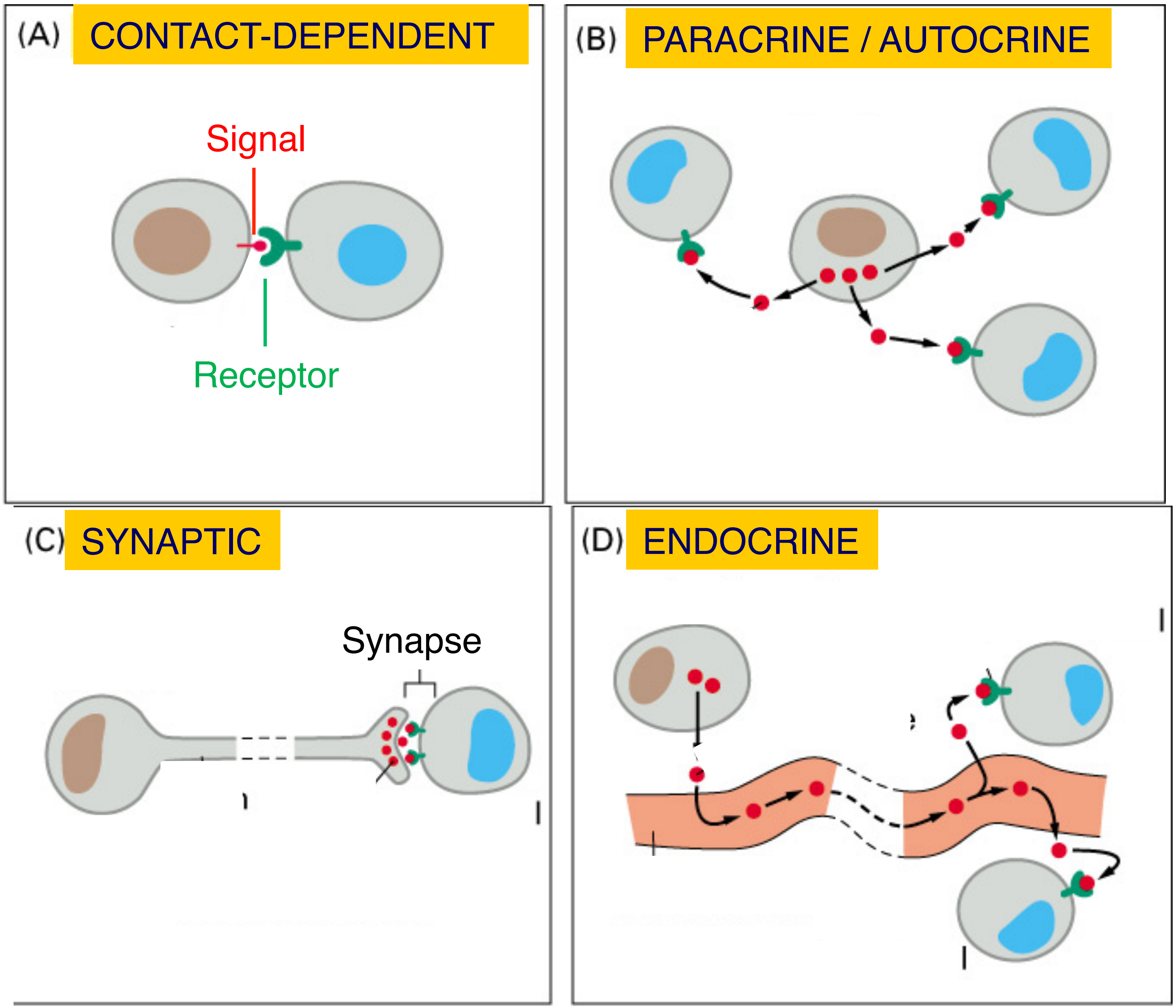
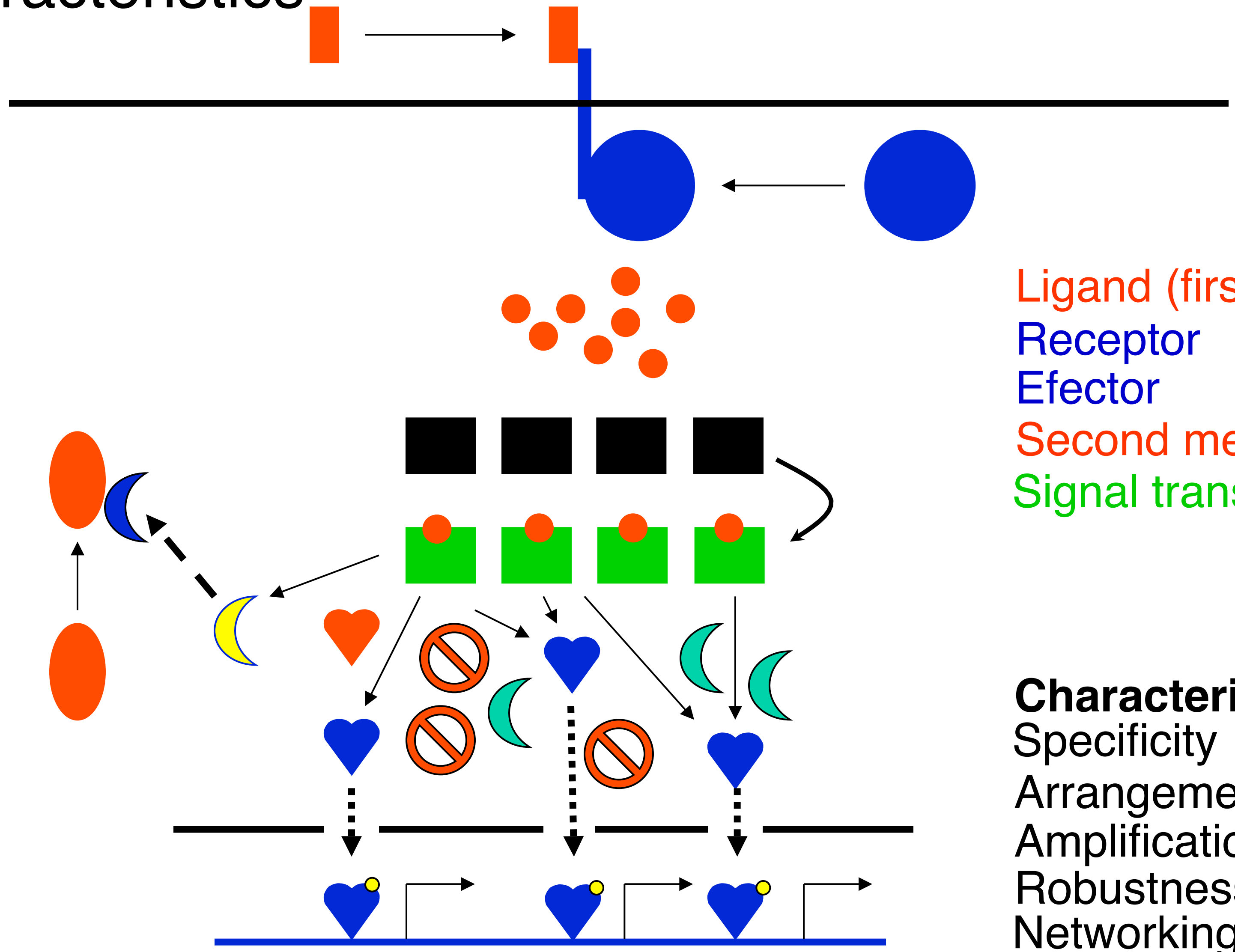


The forms of communications between cells can be compared with the following means of inter-personal communications:

• Phone conversation	**SYNAPTIC**
• Communication with people at the hotel reception	**PARACRINE**
• Statement in the radio	**ENDOCRINE**
• Talking to oneself	**AUTOCRINE**

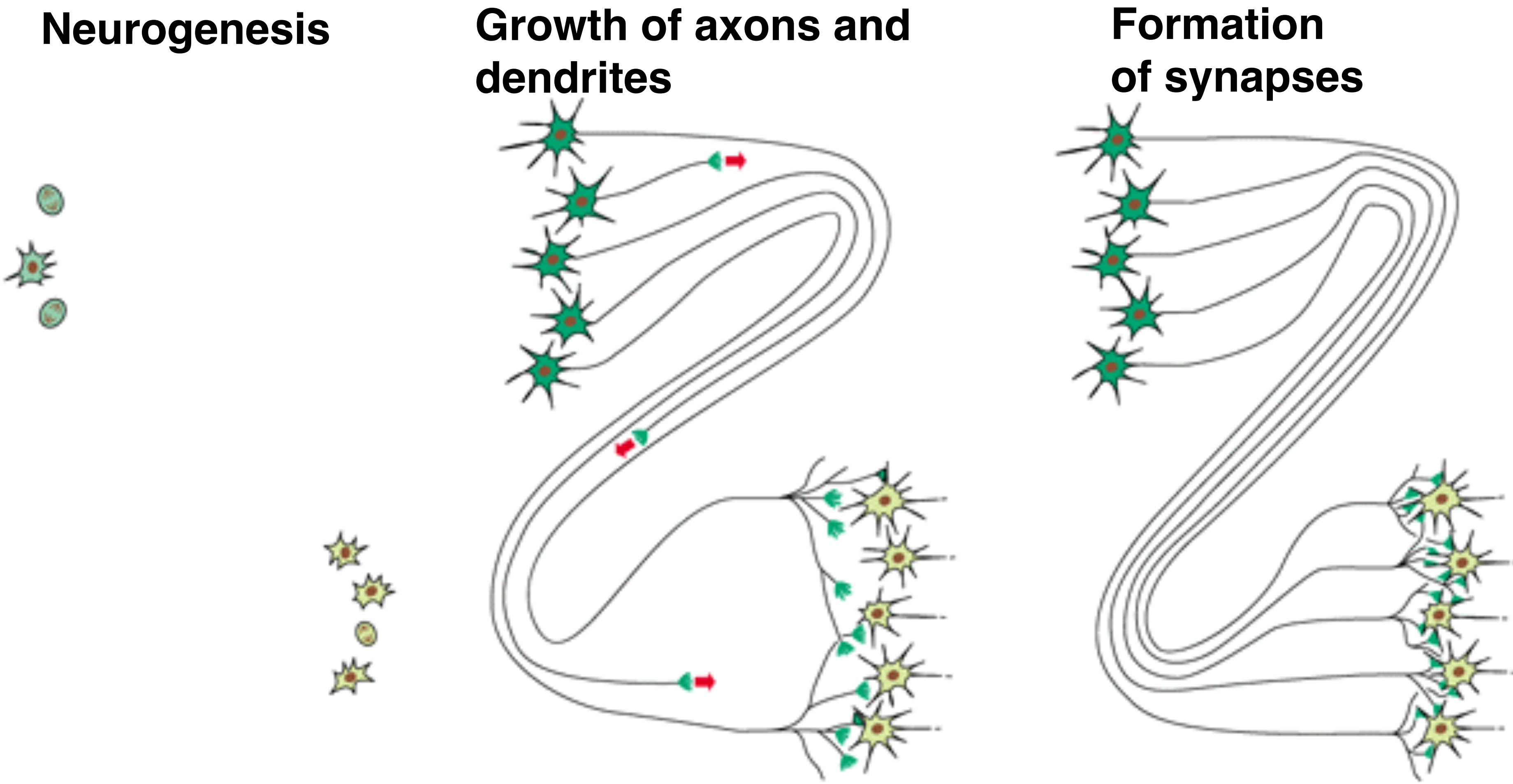
Cell signalling pathways exhibit several universal characteristics



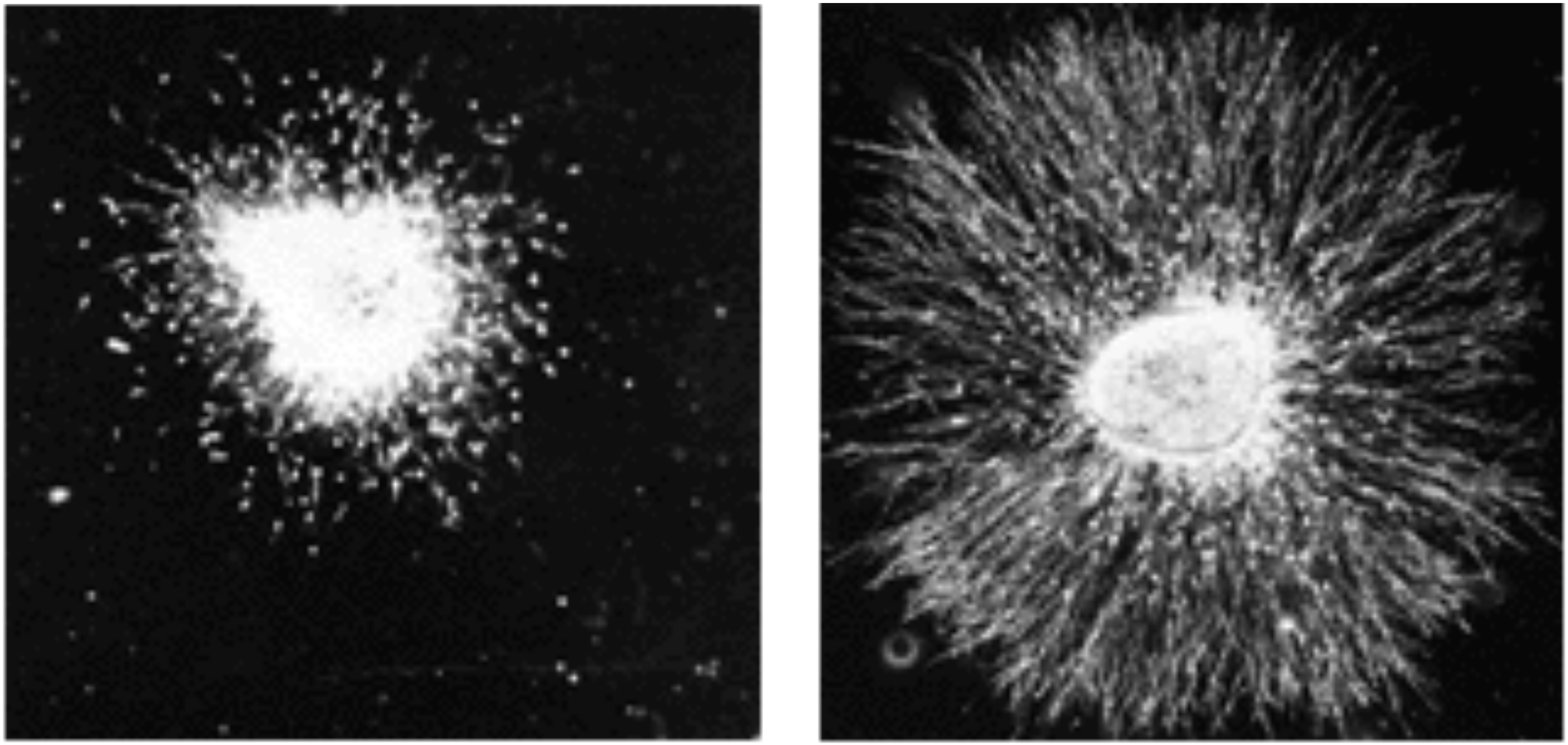
Ligand (first messenger)
Receptor
Efector
Second messenger
Signal transducer

Characteristics:
Specificity
Arrangement in a cascade
Amplification(1→10^8)
Robustness
Networking

Multicellular bodies produce a large number of ligands affecting the target cells via their binding to specific receptors



Growth of neurons is under the control of a chemical signal



Control +signal

What is the nature of the signal?

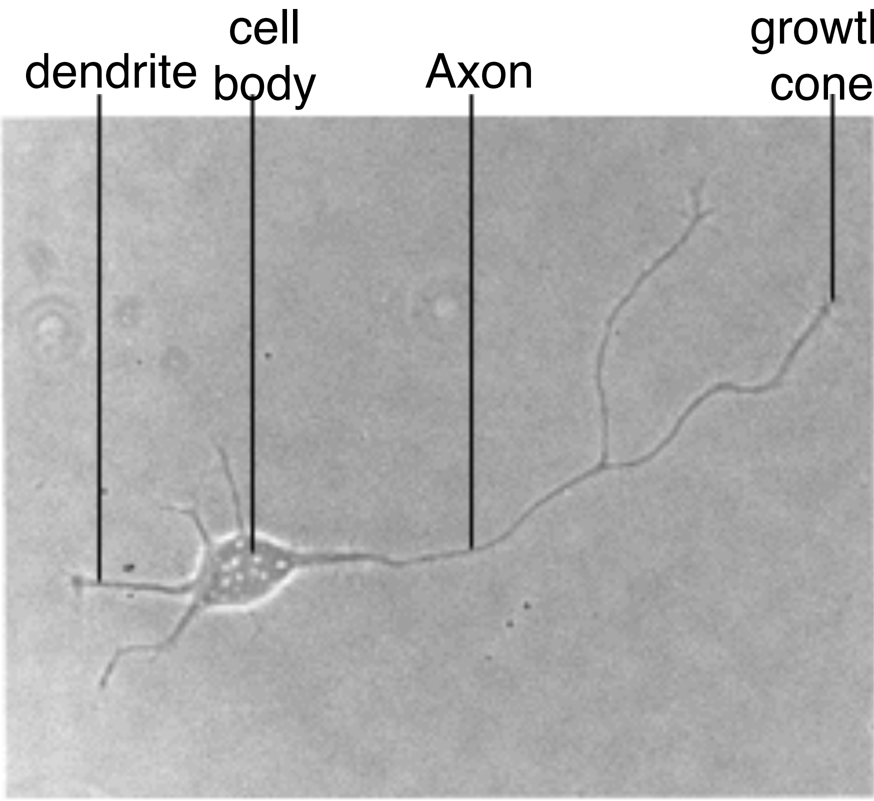
Rita Levi-Montalcini (1909-2012)

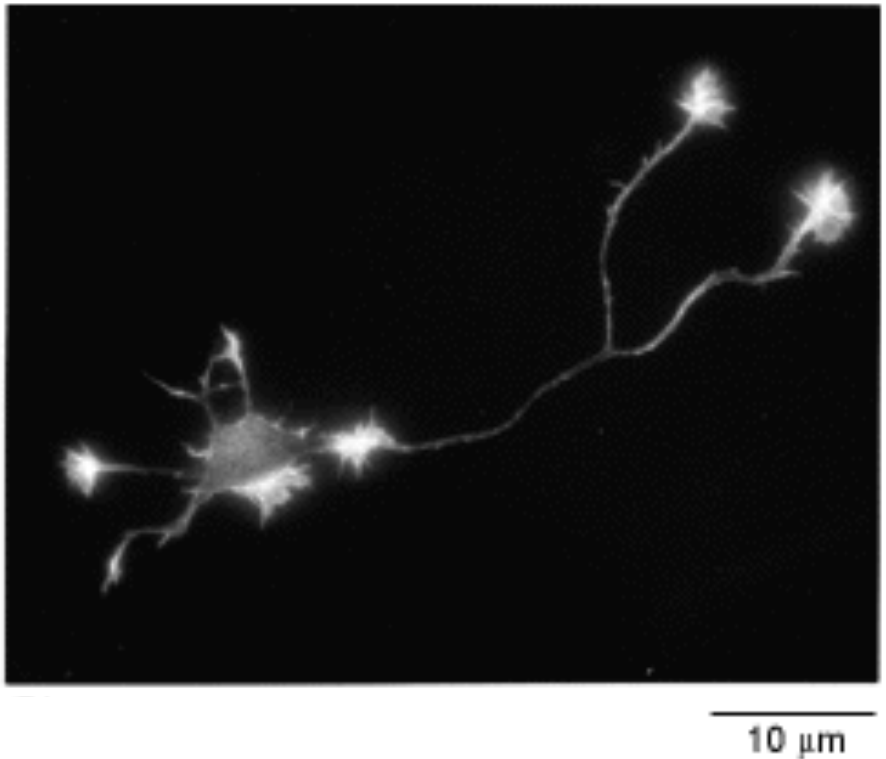
Paola Levi-Montalcini (1909-2000)

The active substance is a polypeptide called (neuronal growth factor, NGF)

Stanley Cohen (1922-)

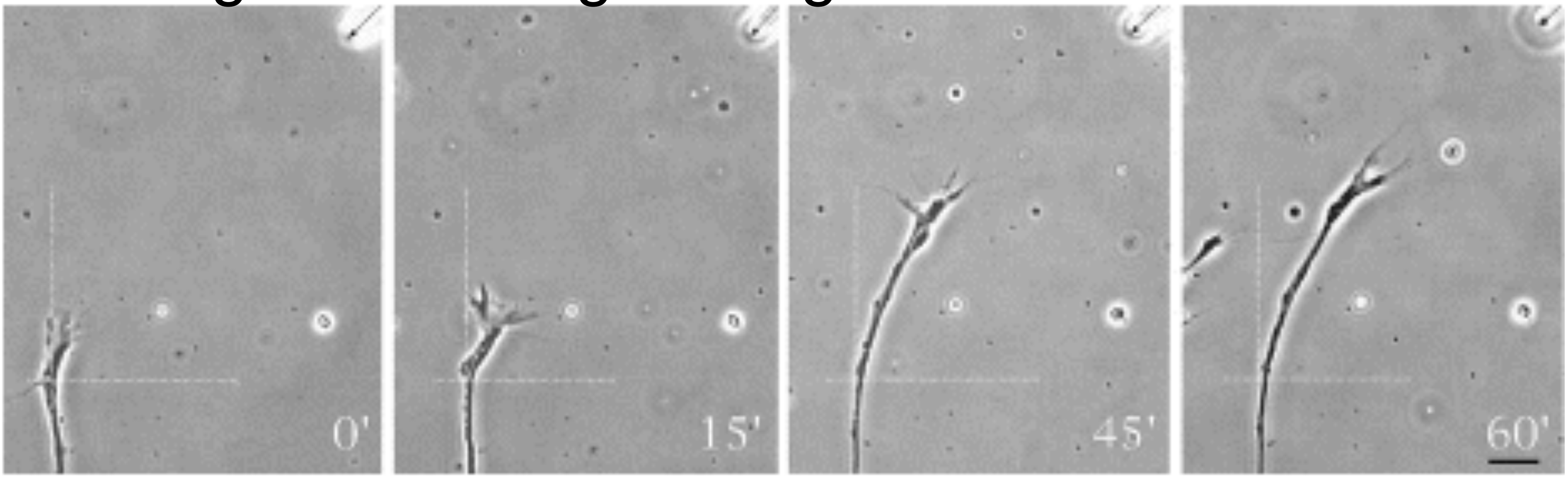
Growth factors are also involved in navigation of axonal growth during neurogenesis





Fluorescently stained actin

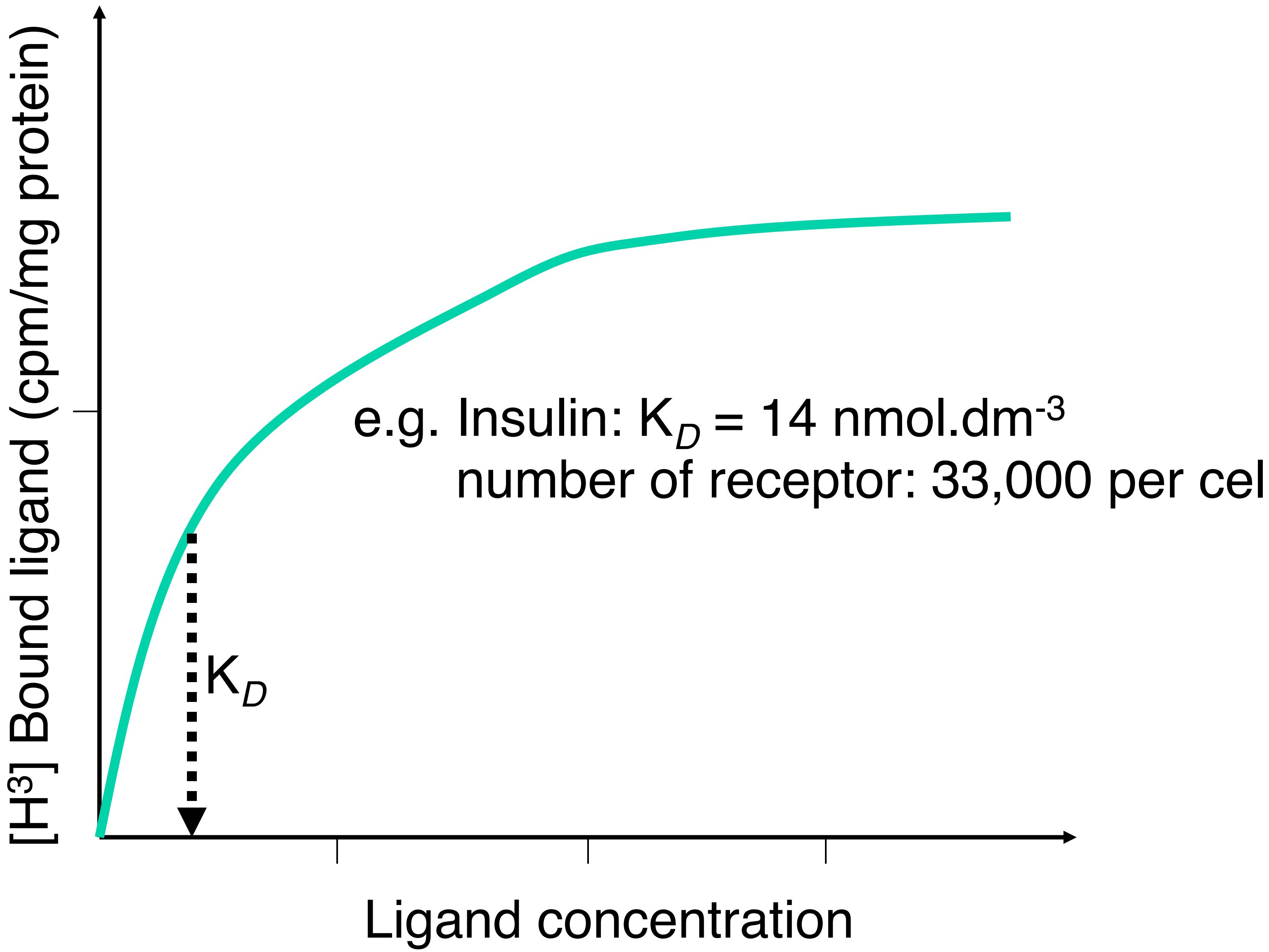
Growth factors are also involved in navigation of axonal growth during neurogenesis



CHEMOATTRACTION

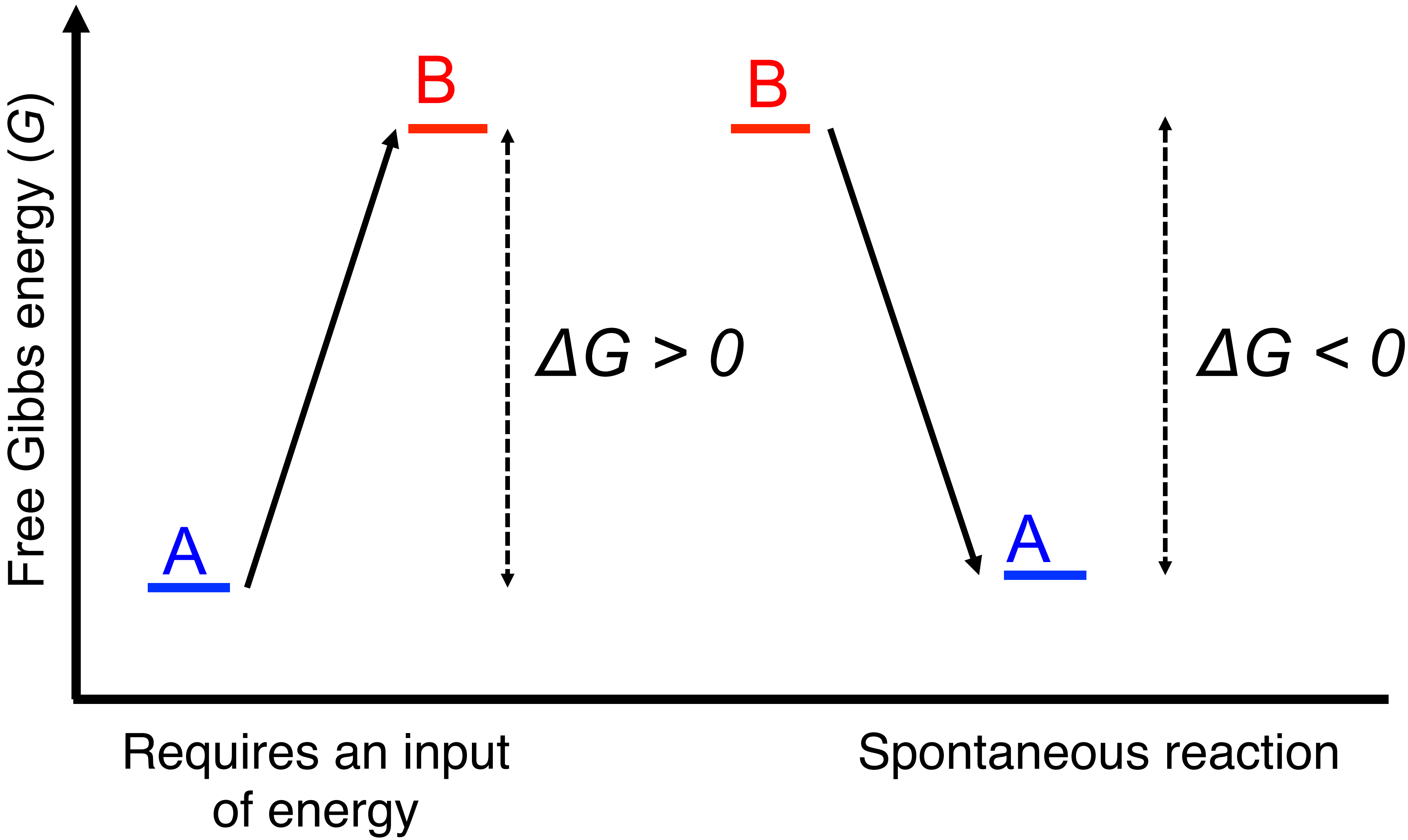
How is it possible to quantitatively express the specificity of the receptor-ligand binding?

The affinity of a receptor to a ligand is expresses as a dissociation constant K_D

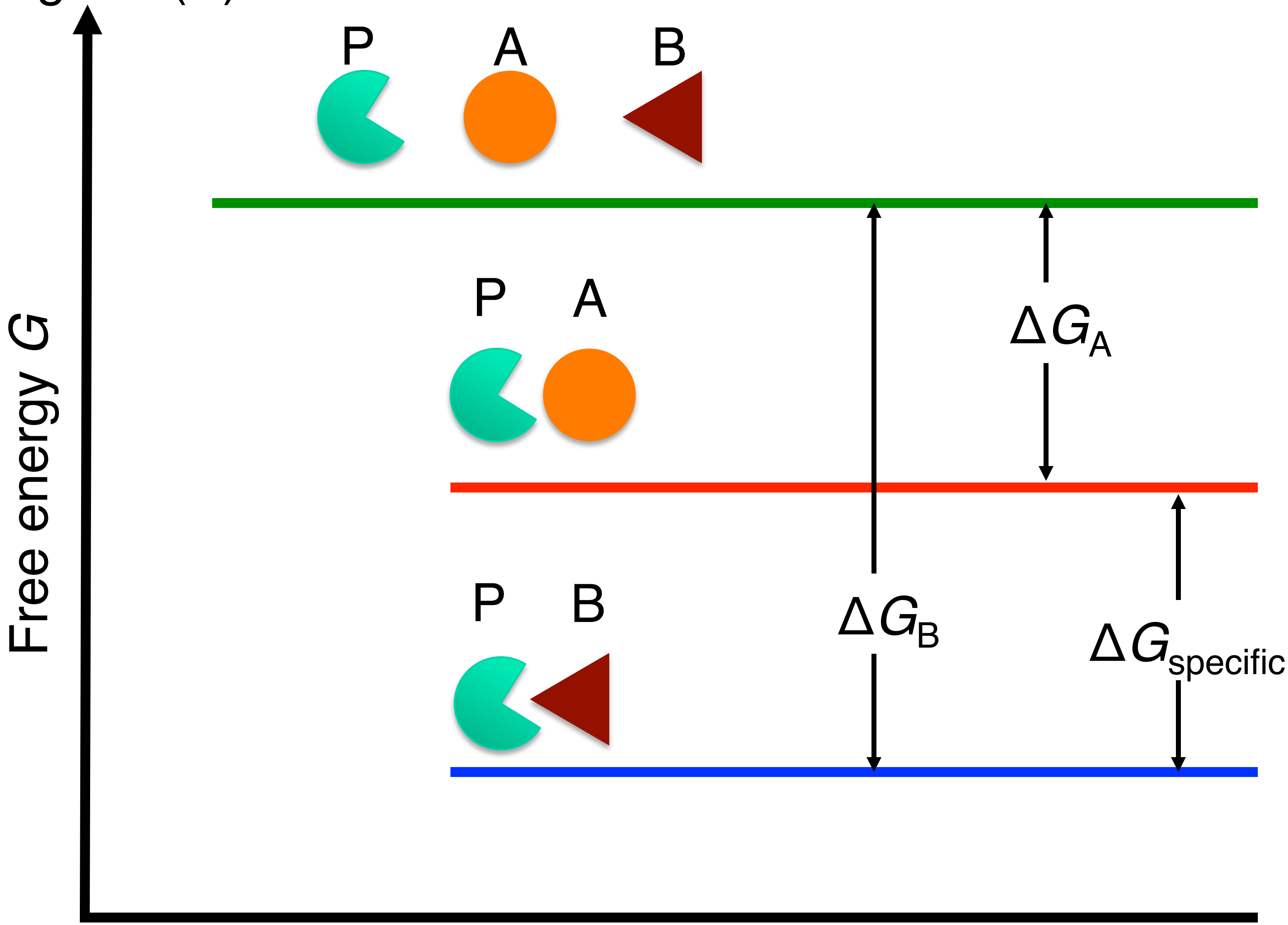


The binding of ligands to their receptors occurs spontaneously, if it is associated with a release of free Gibbs energy (i.e. $\Delta G < 0$)

$$\Delta G = G_B - G_A$$



Specific binding of a protein (P) to a ligand (B) is mediated by a greater net free energy change compared to a nonspecific binding to a ligand (A)



Specificity of binding can be quantified in a form of a dissociation constant (K_D)

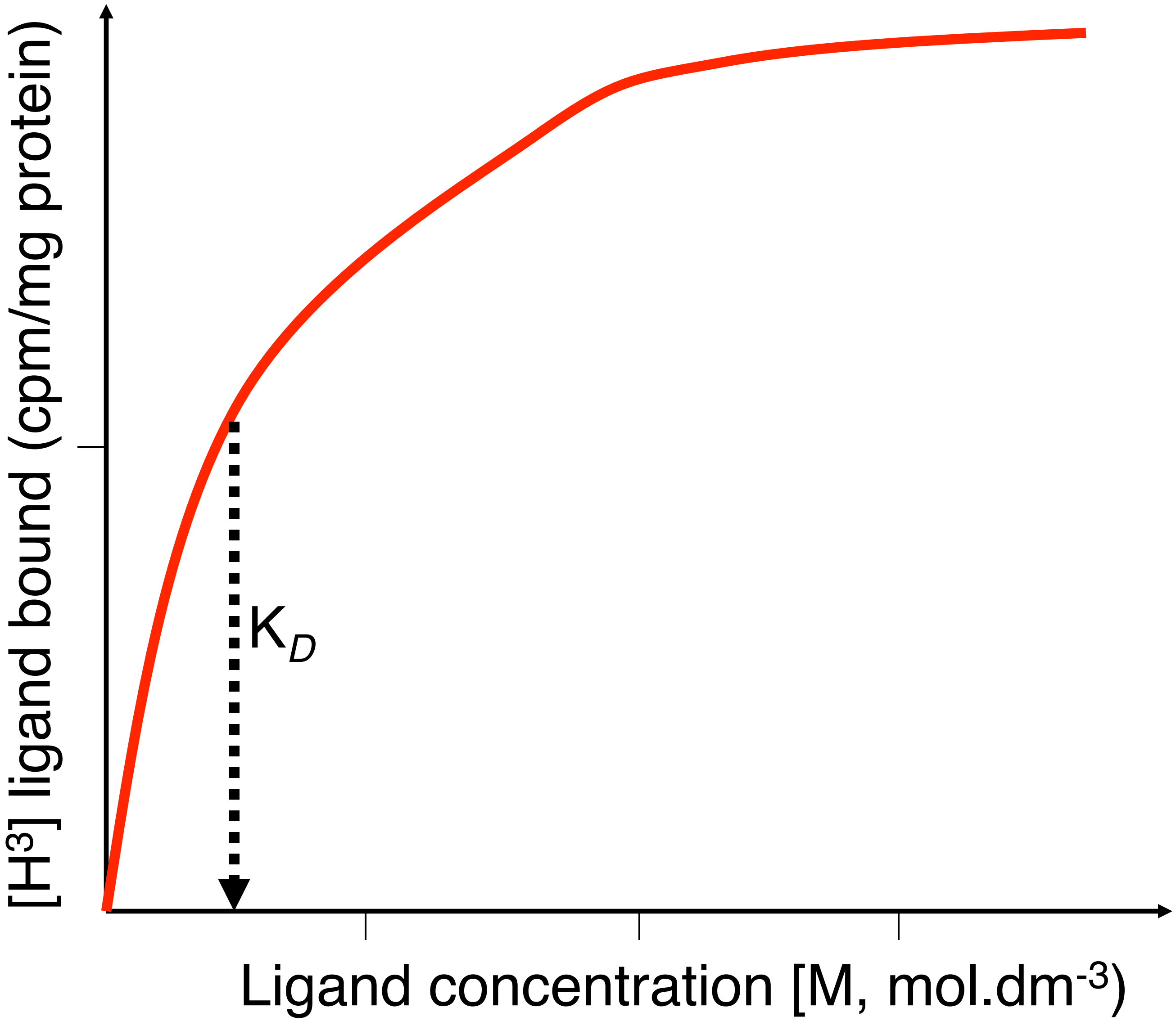
$$[R] + [H] \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [RH]$$

At equilibrium:

$$K_D = \frac{k_{off}}{k_{on}}$$

$$K_D = \frac{[R][H]}{[RH]}$$

Specificity of binding can be quantified in a form of a dissociation constant (K_D) that can be calculated from a binding curve



There is a relationship between dissociation constant (K_D) and free energy of association ($\Delta G_{association}$)

$$\Delta G_{association} = RT \ln K_D$$

K_D [M]	$\Delta G_{association}$ (kJ/mol)
10^{3}	17.1
1	0
10^{-3}	-17.1
10^{-6}	-34.2
10^{-9}	-51.4
10^{-12}	-68.5
10^{-15}	-85.6

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10^{-9}	**-51.4**
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10^{-15}	-85.6

Small differences in two molecules can have a dramatic consequences on their binding properties

Example: a single hydrogen bond can have a free energy value of 4.2-13 kJ/mol, so addition or subtraction of a single hydrogen bond can affect the K_D by several orders of magnituted

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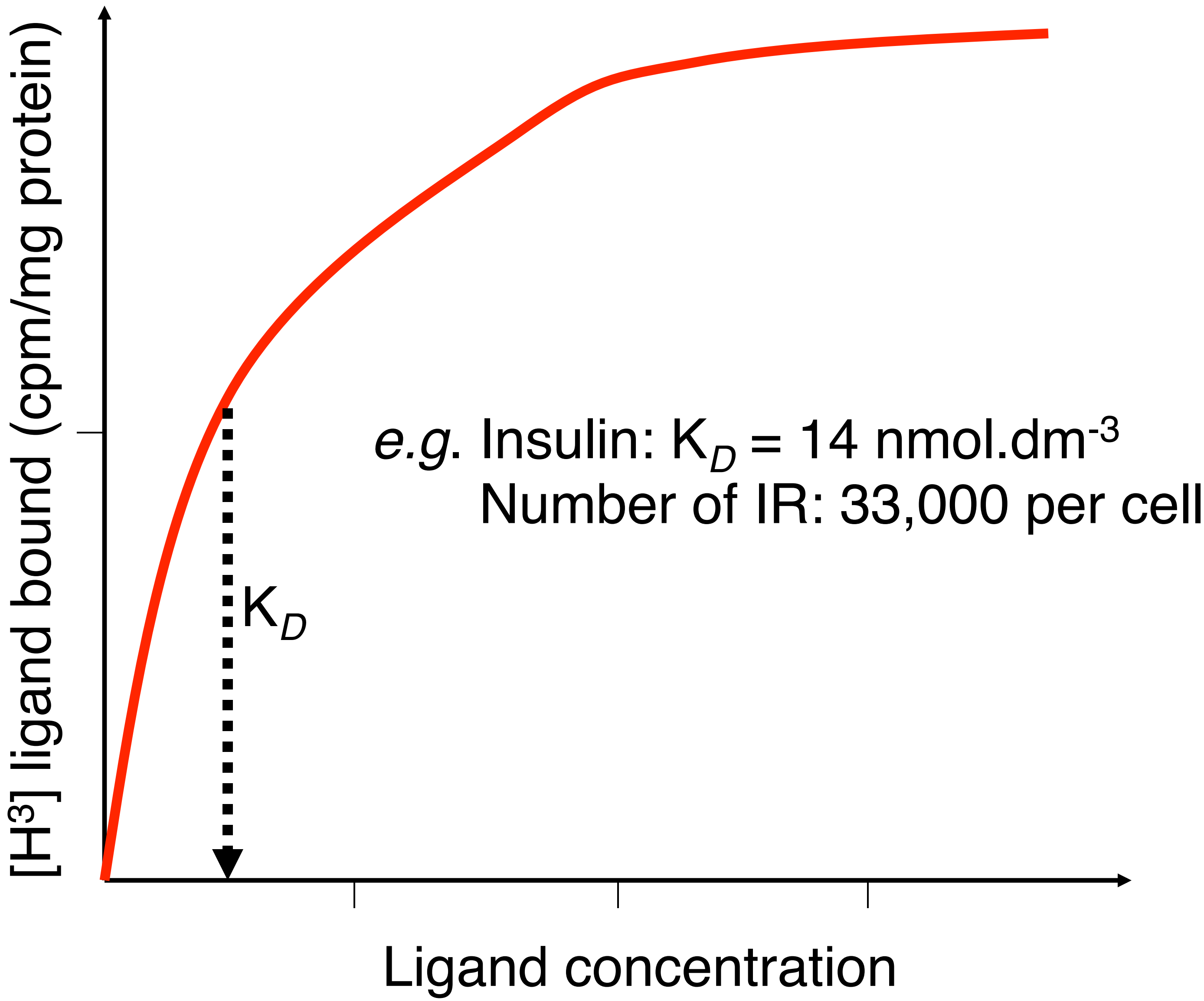
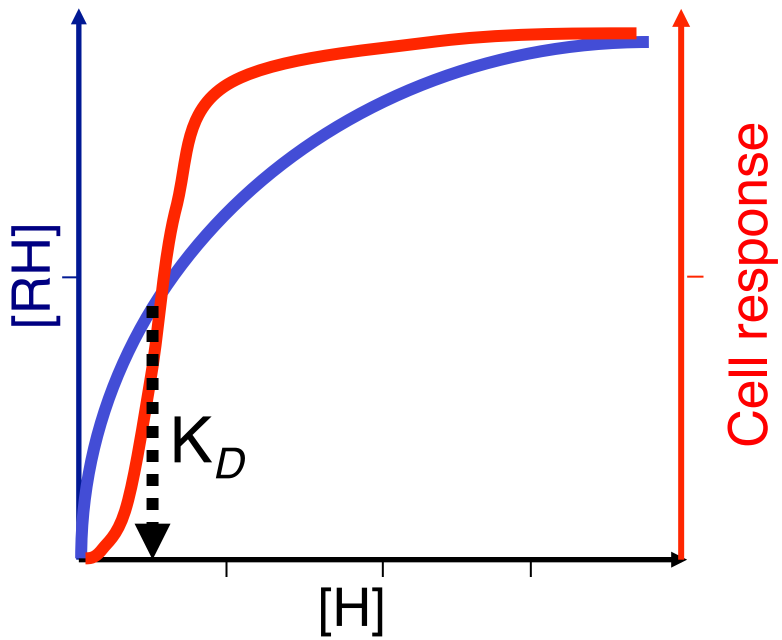
The binding curve is helpful in determination of both K_D and the number of binding sites (e.g. receptors)



Kinetic parameters of the ligand-receptor interaction are finely tuned to mediate appropriate cell response



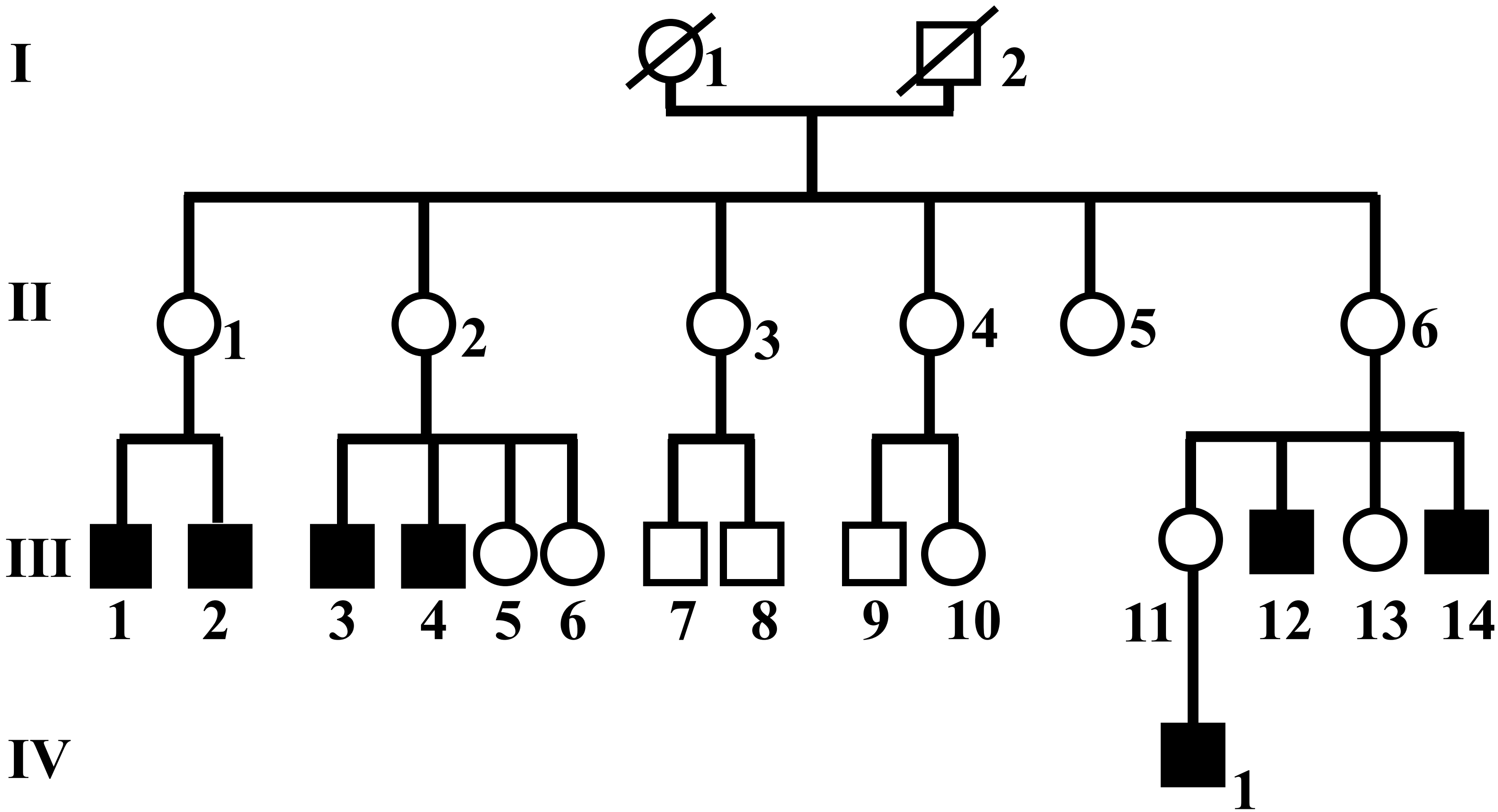
Adrenaline/Epinephrine:

$K_D = 5\ \mu M$

[Adrenaline] <<$0.5\ \mu M$

Changes in kinetic parameters of the ligand-receptor interactions can result in pathological states

Kennedy disease (spinal and bulbar myotonic atrophy)



Kennedy *et al*. (1968). *Neurology*. 18: 671-680.

Patients with Kennedy´s disease exhibit changes in K_D of androgen receptor

K_D (normal) = 0.19 +/-0.06 $nmol.dm^{-3}$
K_D (patient) = 0.34 +/-0.17 $nmol.dm^{-3}$

MacLean *et al*. (1995). *J. Clin. Endocrinol. Metabol*. 80: 508-516.

Information about kinetic parametrs of ligand-receptor interaction is important for a preparation of clinically important drugs

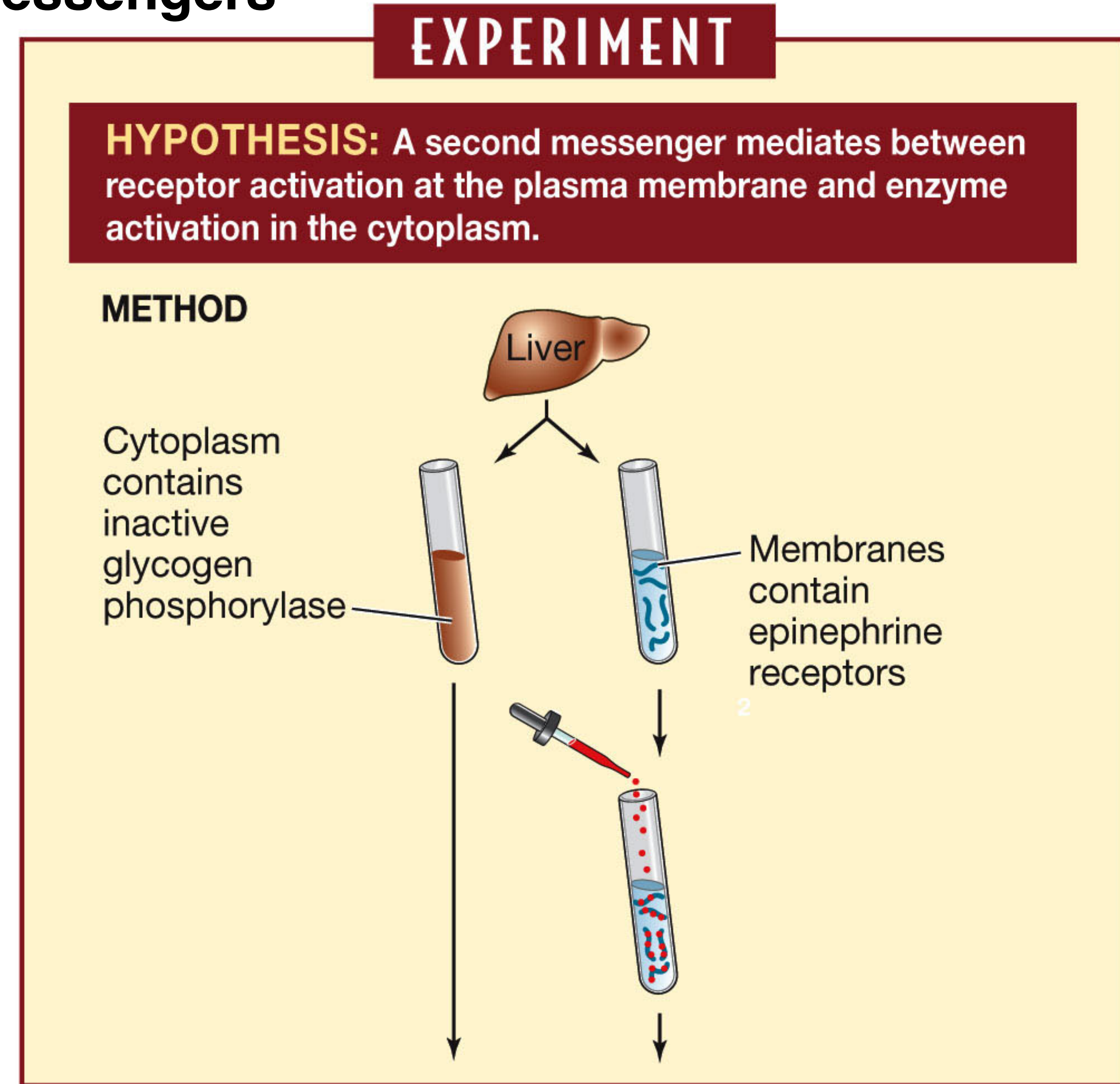
Ligand – agonist- antagonist
adrenaline – isoproterenol - alprenolol

K_D / response:

Adrenaline: $5x10^{-6}$ M /↑cAMP
Isoproterenol: $0.4x10^{-6}$ M /↑cAMP
Alprenolol: $0.0034x10^{-6}$ M /---cAMP

Binding of a ligand to a receptor induces a series of biochemical reactions

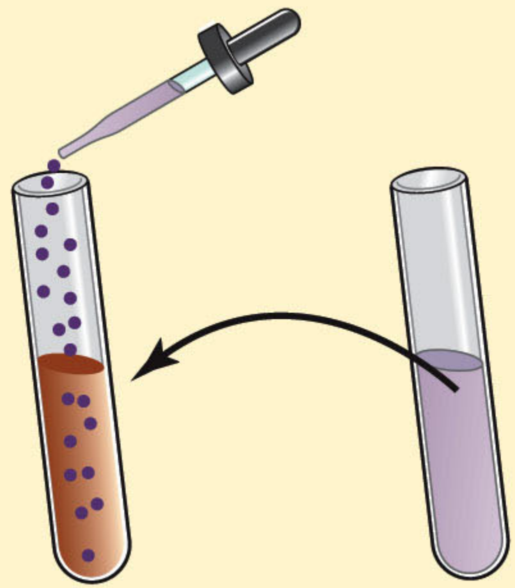
One of these reactions is production of small molecules called **second messengers**



Sadava *a kol.* (2007). Life: The Science of Biology. Sinnauer Ass.

One of these reactions is production of small molecules called **second messengers**

EXPERIMENT



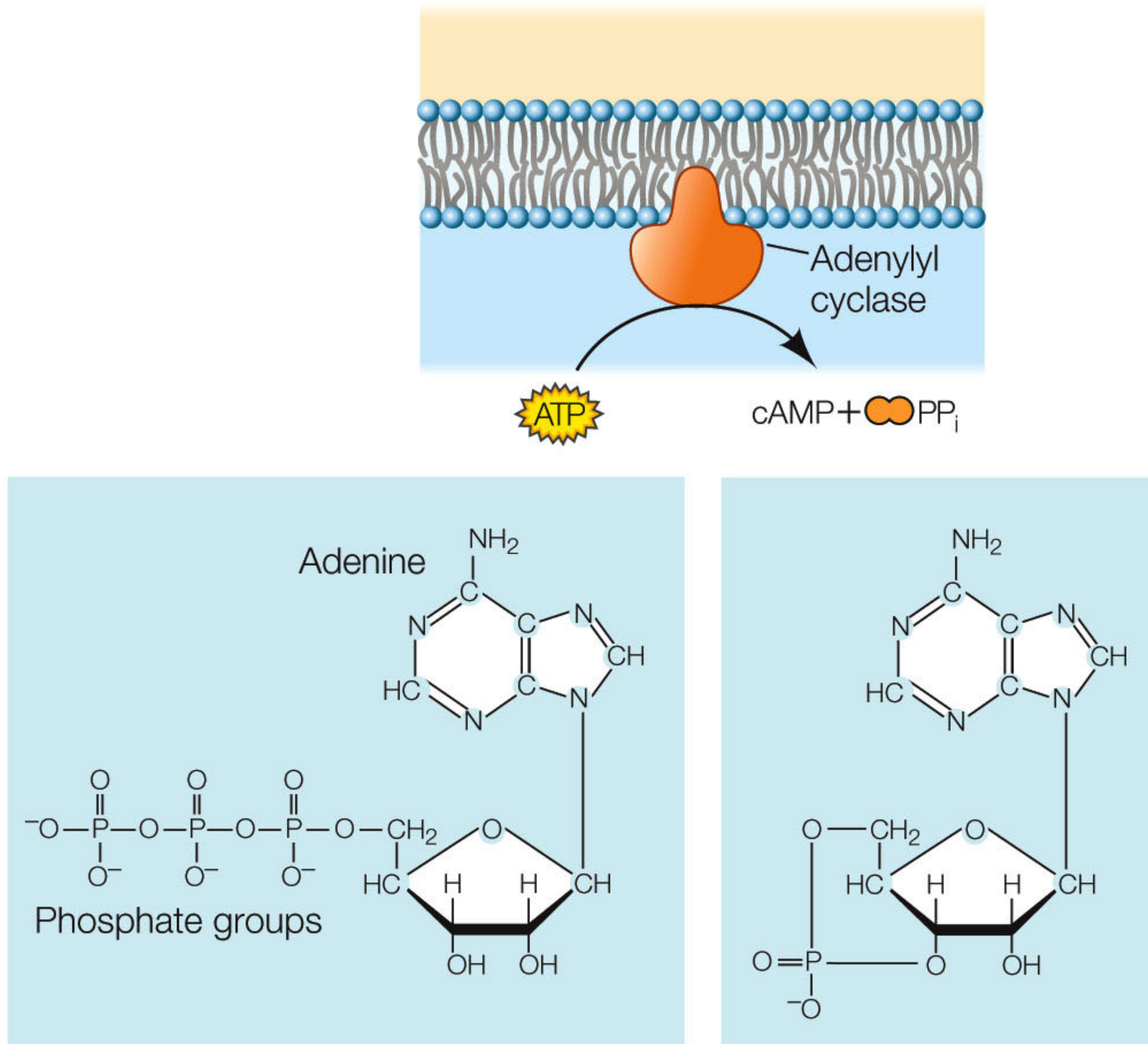
RESULT

Active glycogen phosphorylase is present in the cytoplasm.

CONCLUSION: A soluble second messenger, produced by hormone-activated membranes, is present in the solution and activates enzymes in the cytoplasm.

Sadava *a kol.* (2007). Life: The Science of Biology. Sinnauer Ass.

Cyclic adenosine monophosphate (cAMP) is a typical second messenger



ATP **Cyclic AMP (cAMP)**

Sadava *a kol.* (2007). Life: The Science of Biology. Sinnauer Ass.

Another types of reactions triggered by activated receptors are various **reversible** post-translational modifications of proteins

Phosphorylation ($-PO_4^{-3}$)
Methylation ($-CH_3$)
Acetylation ($-COO^-$)
...

http://selene.princeton.edu/PTMCuration/

Phosphorylation is catalyzed by special family of enzymes called **kinases**

$$ATP + X \xrightarrow{\text{kinase}} X\text{-}P + ADP$$

$$X\text{-}P \xrightarrow{\text{phosphatase}} X + P_i$$

X:

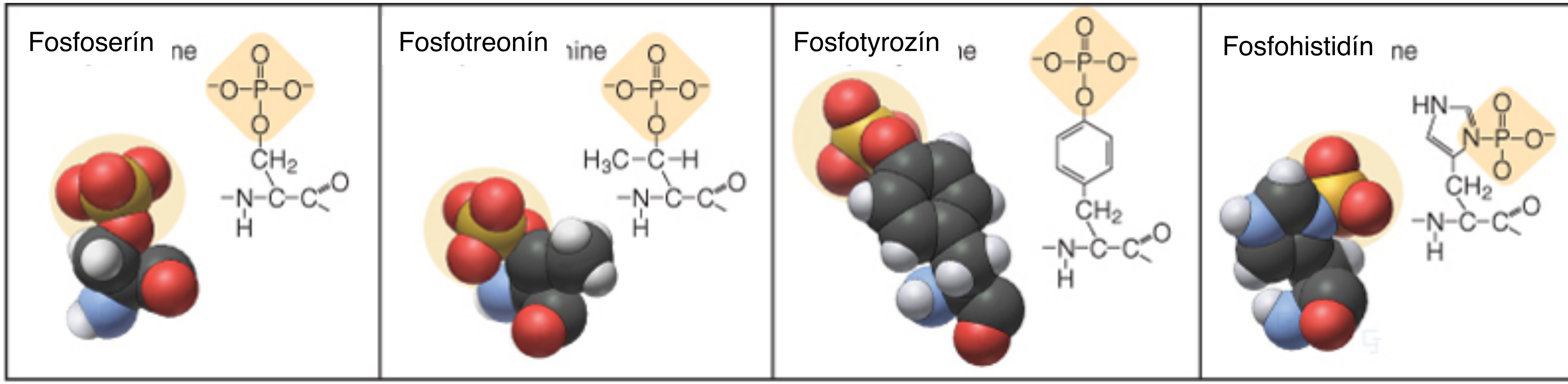
sugars

polynucleotides

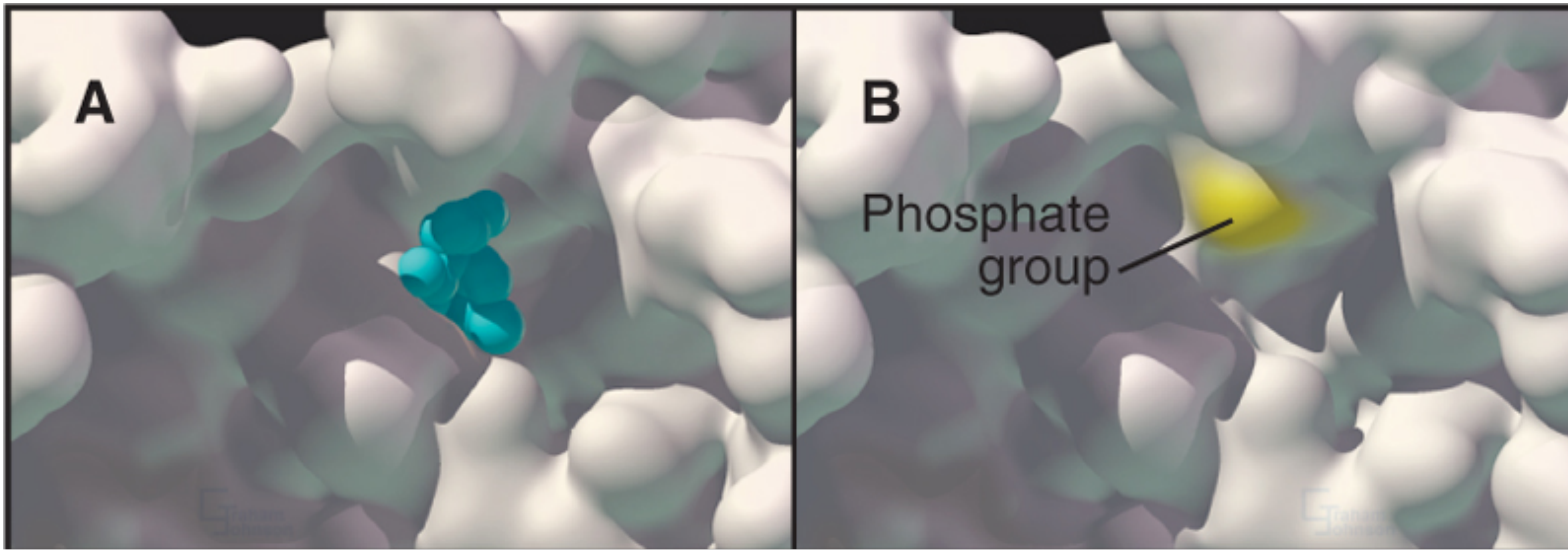
Inositol ring in phospholipids

Ser, Thr, Tyr, His, Arg residues in proteins

The most frequent **phosphoamino acids**



Phosphorylation may affect biochemical properties of a protein

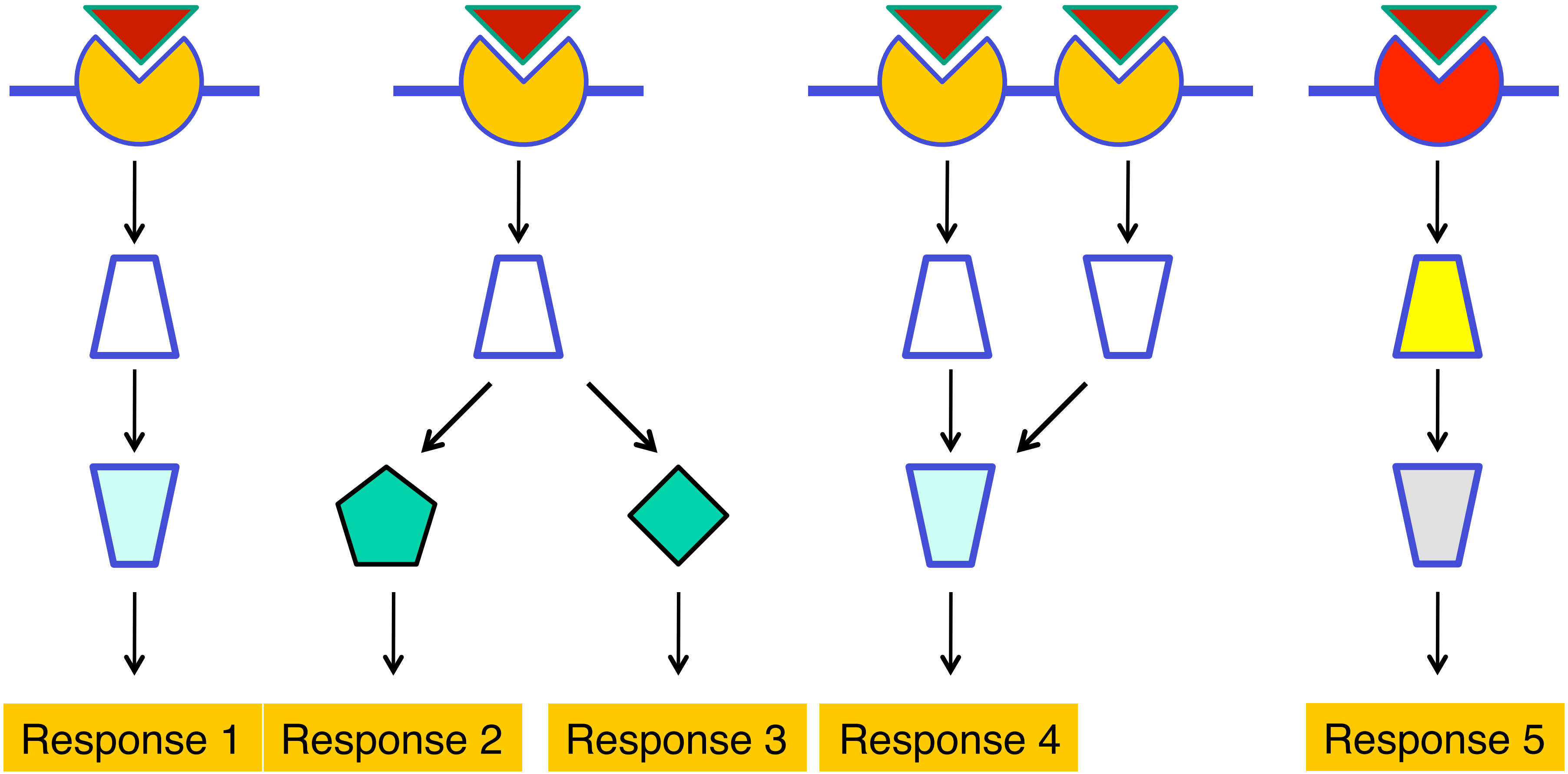


isocitrate dehydrogenase

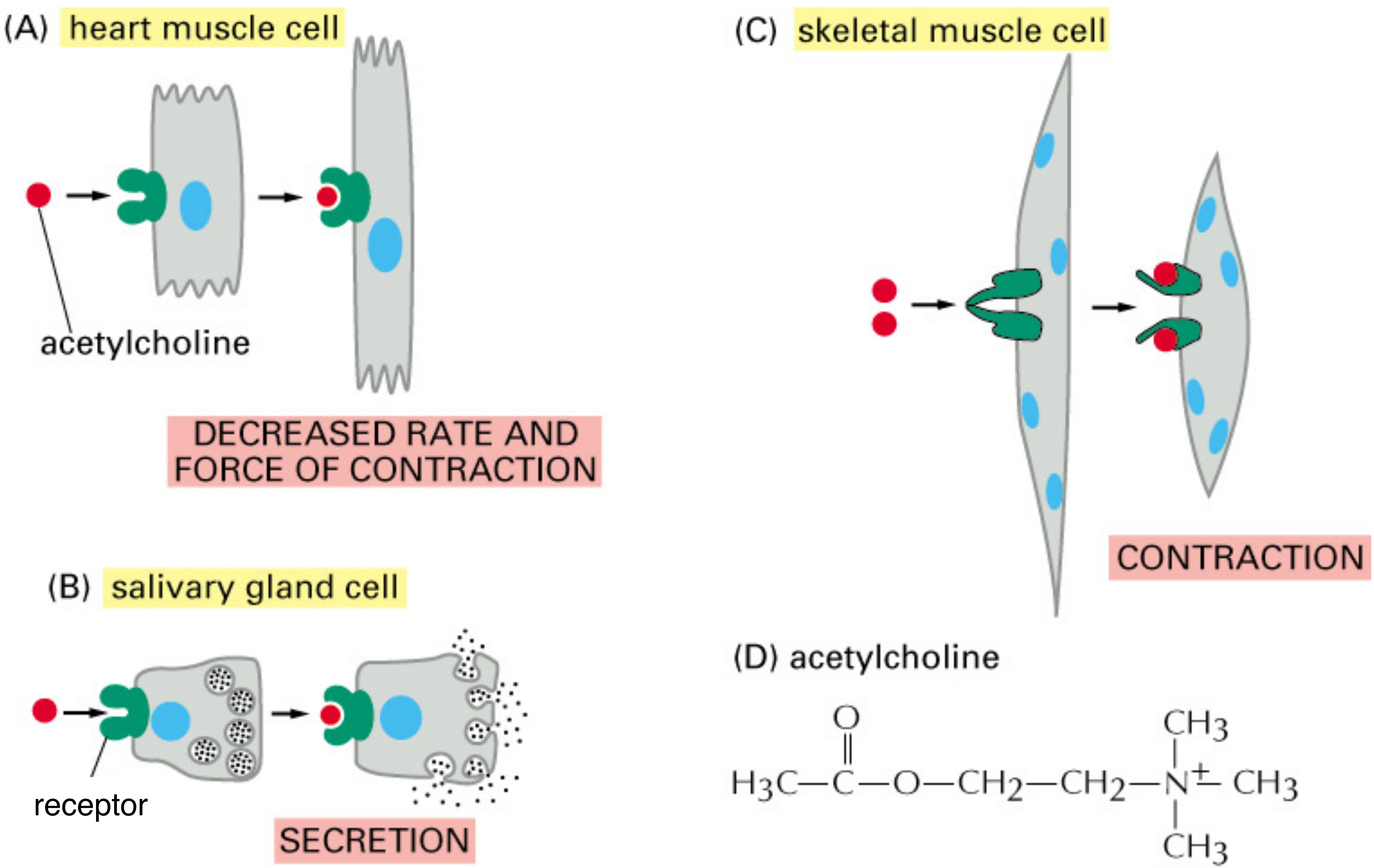
Binding of insulin released from pancreatic β-cells to its receptor on the host cells induces release of glucose

http://vcell.ndsu.nodak.edu/animations/insulinsignaling/movie-flash.htm

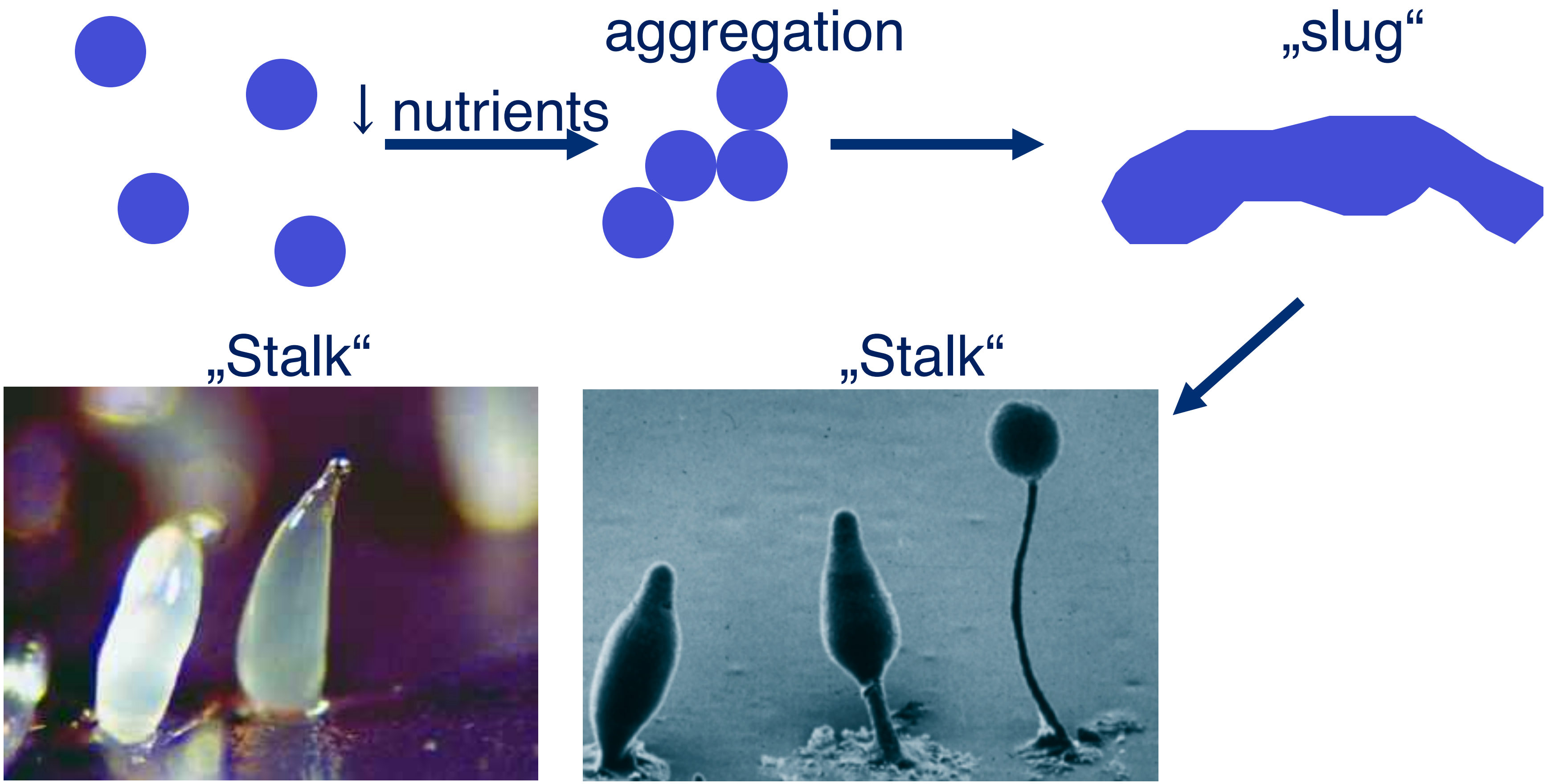
One ligand can induce several cellular responses



One ligand (such as acetylcholine) can induce several cellular responses



Dictyostelium discoideum –
A simple model of cellular differentiation



Dictyostelium discoideum –
A simple model of cellular differentiation

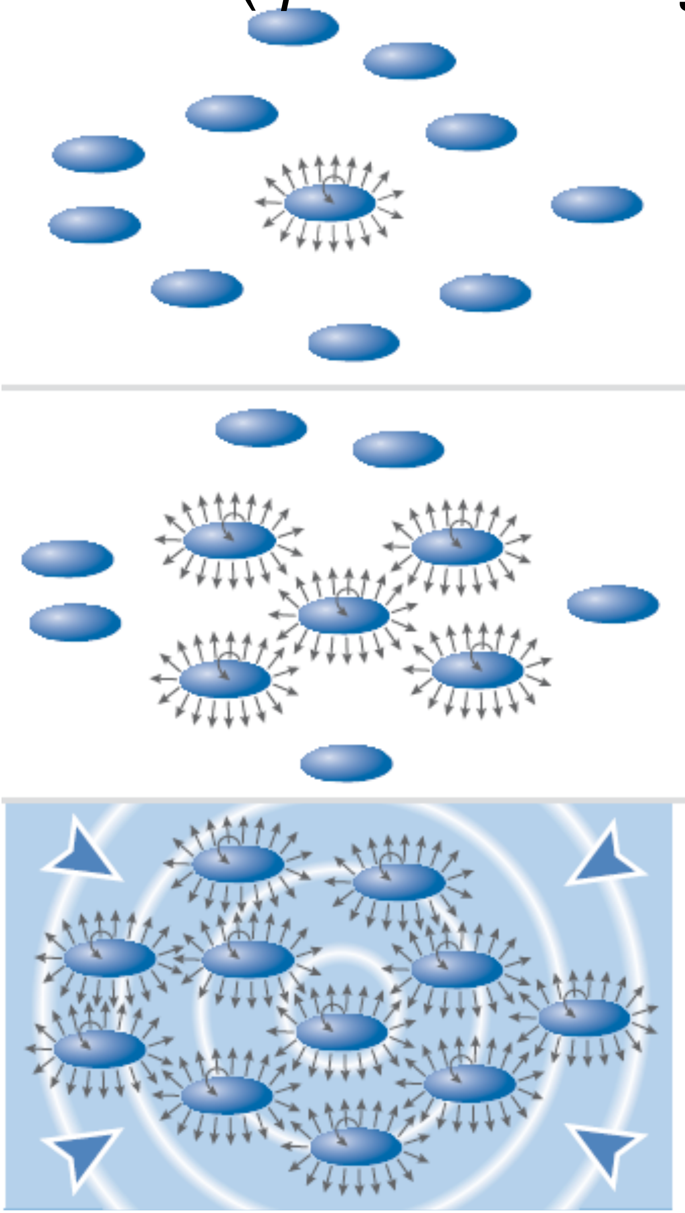
https://www.youtube.com/watch?v=5h8WOWEqP6o

Coordinated behaviour of *D. discoideum* is possible due to their ability to measure cell concentrations (*quorum sensing*)

Production of „autoinducer“

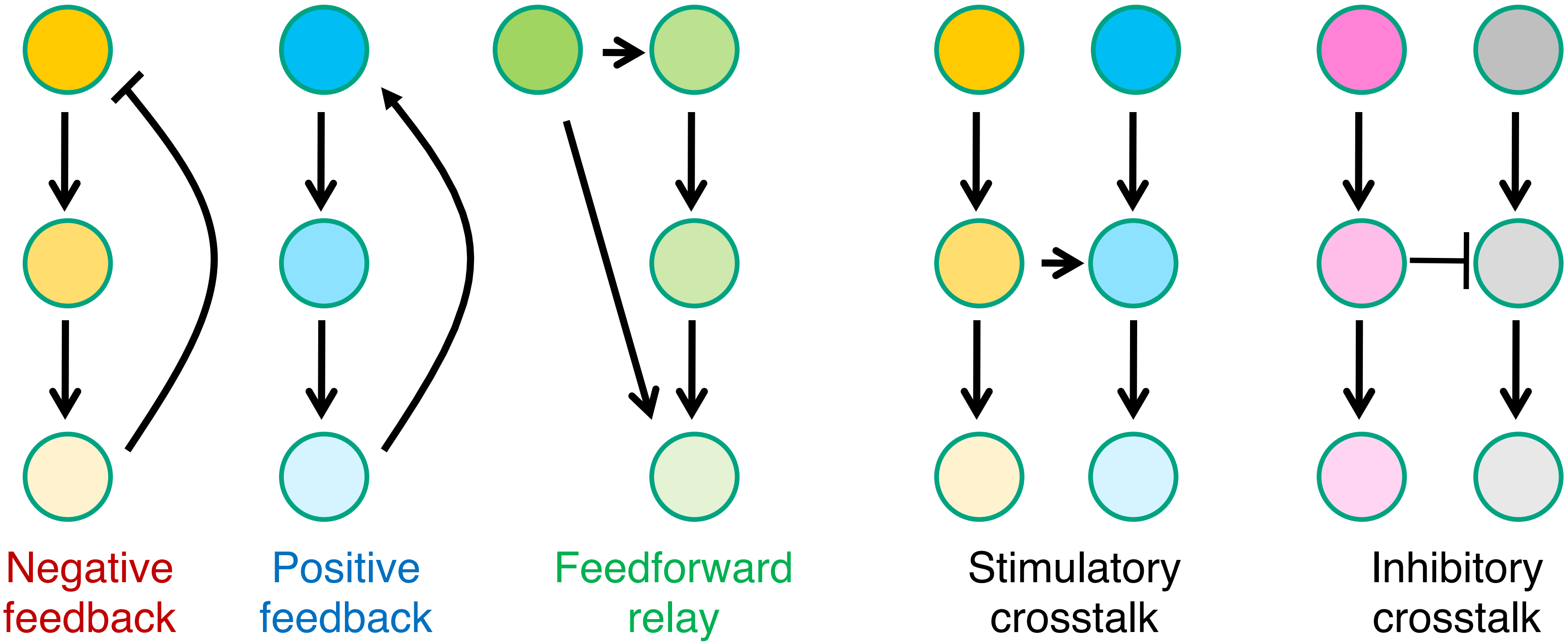
Induction of cellular response in the neighboring cells

Coordinated action of all members of the cell population

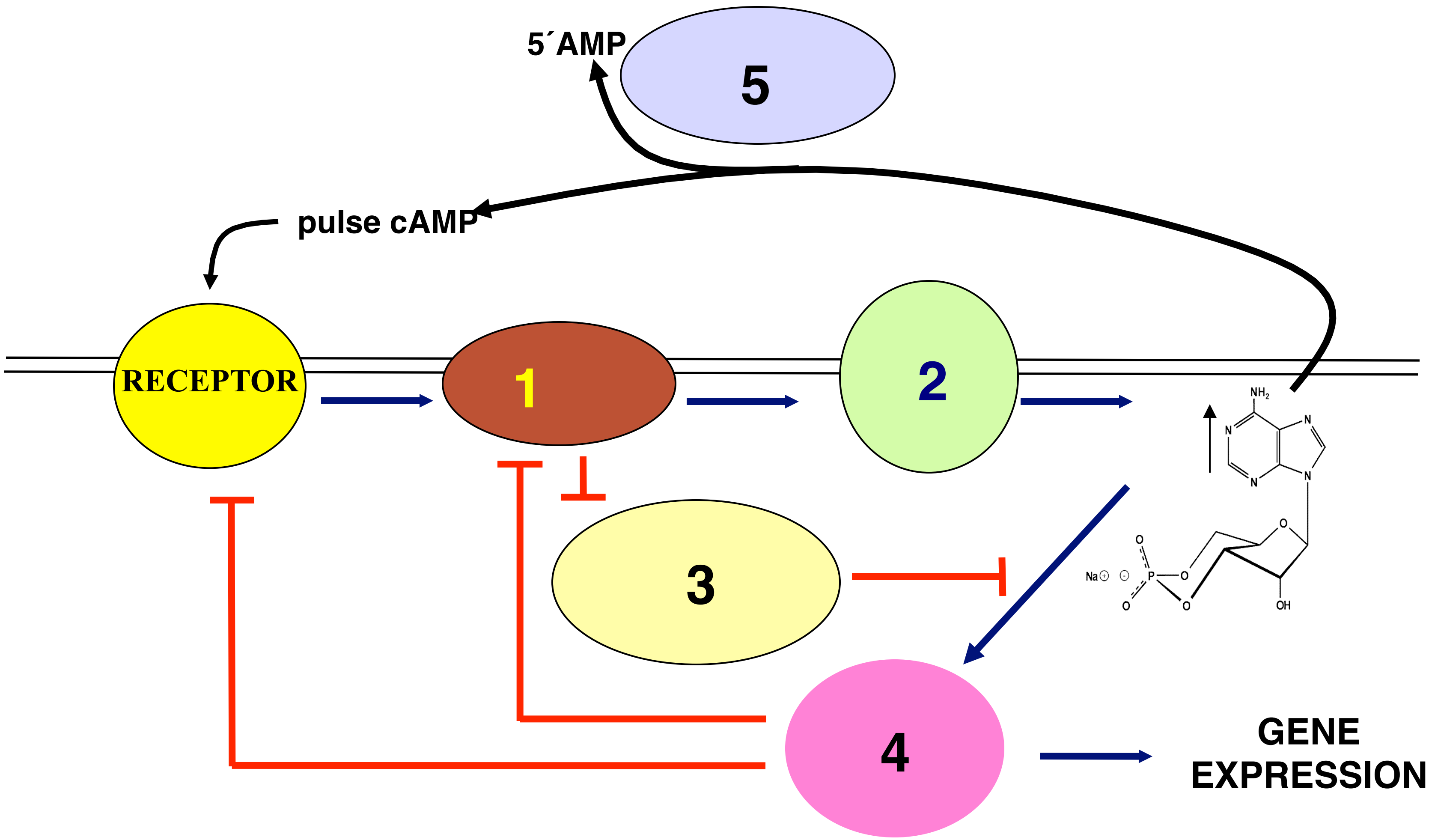


AGGREGATION

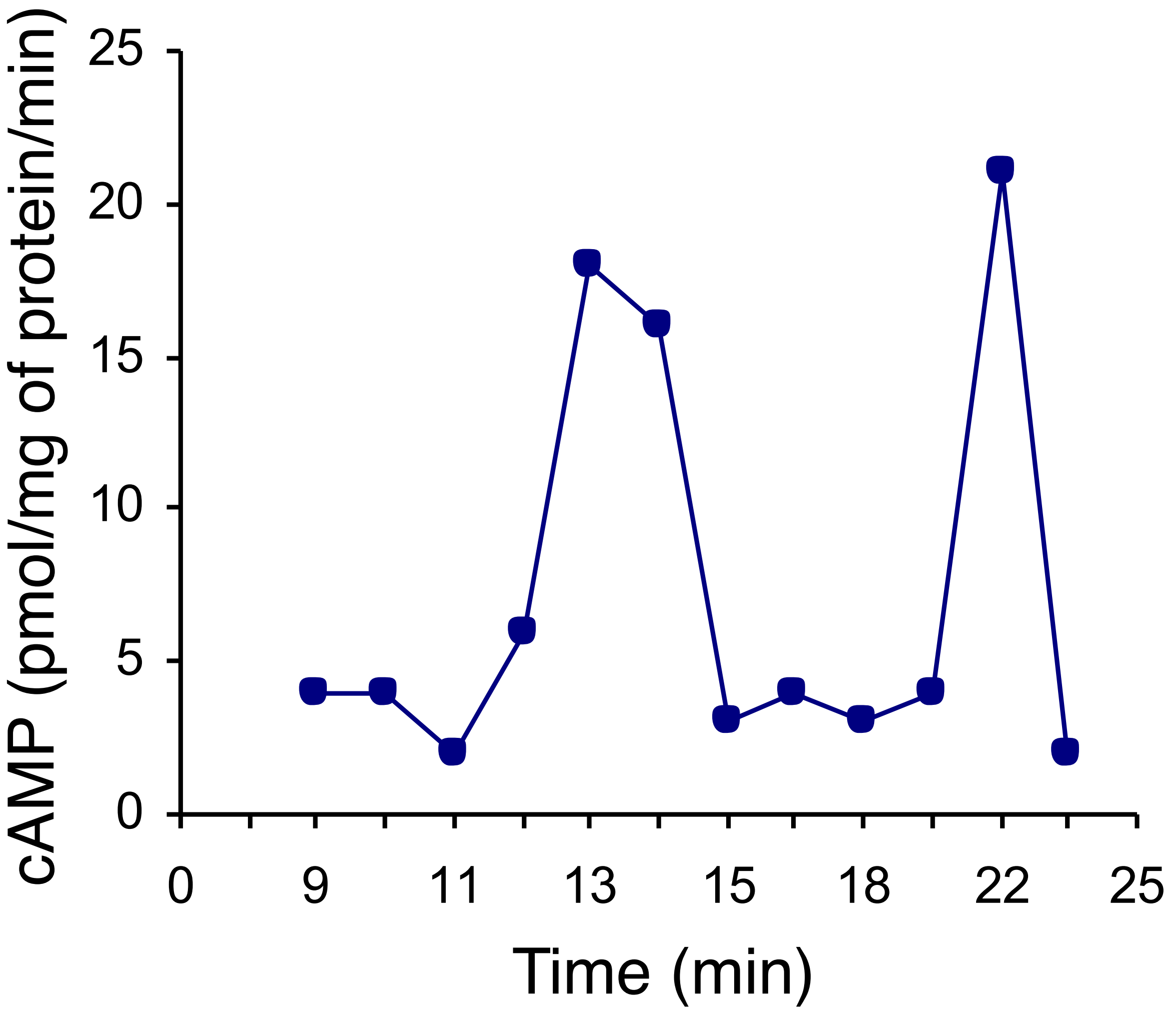
Signalling pathways are arranged in networks containing simple elements



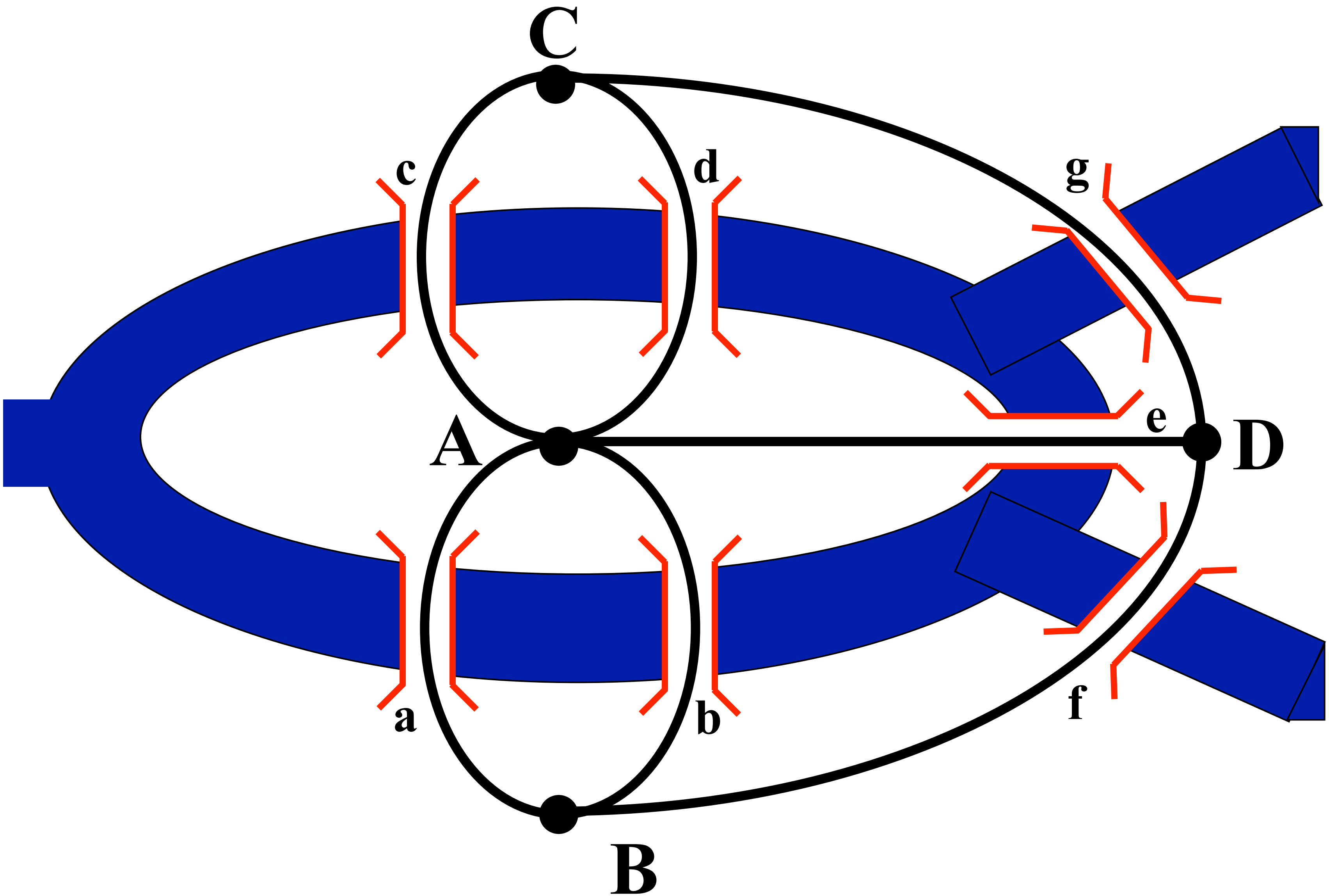
Signalling pathway mediating aggregation of *Dictyostelium* contains several feedback loops



Signaling networks exhibit non-intuitive cell bebaviour



Analysis of networks is an important approach to understand complex systems



Barabási, A.L. (2002). *Linked*. Perseus Publishing.

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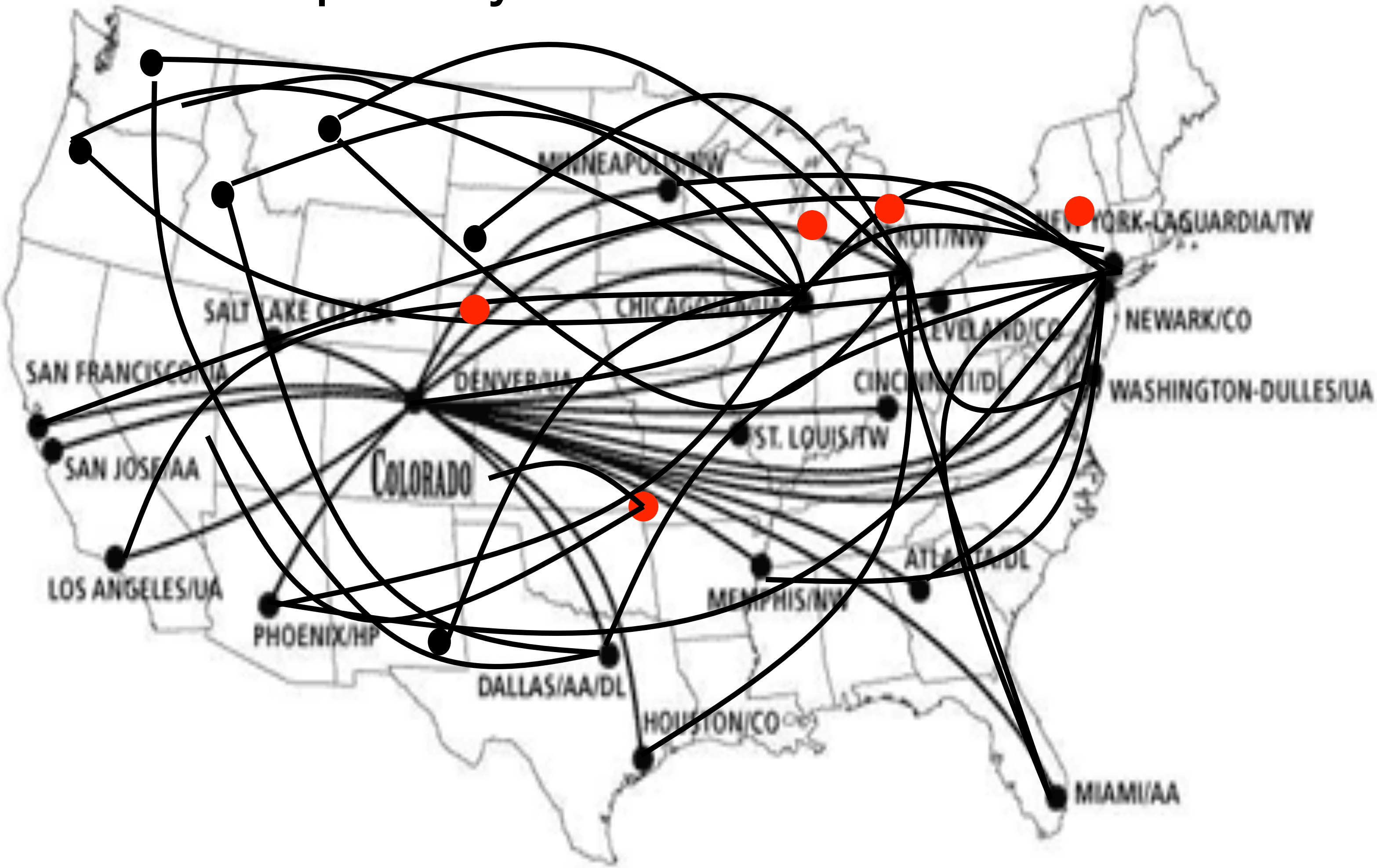
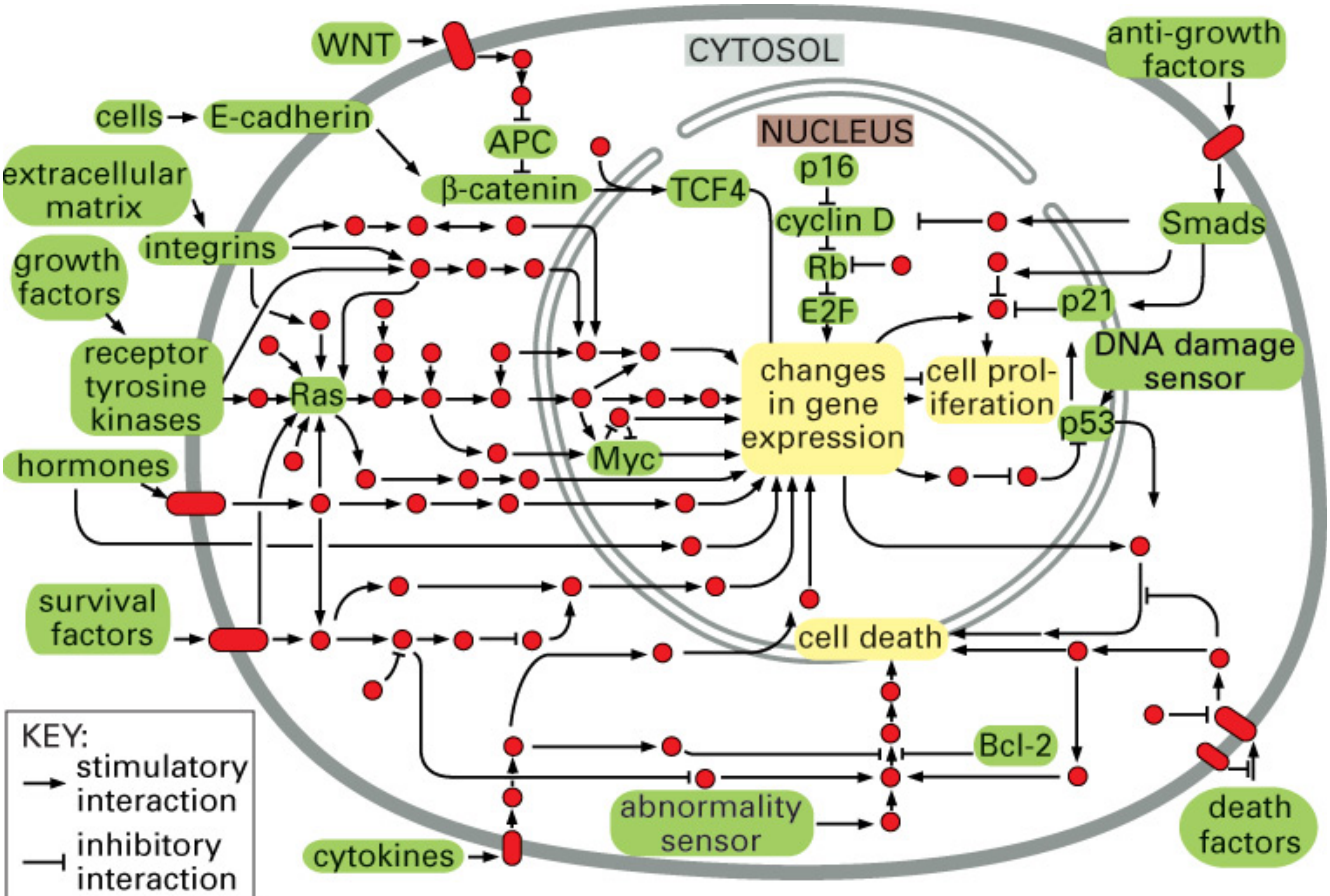


A detailed description of cell signalling pathways/networks willbe instrumental in a construction of a vrtual cell



Biology+
+chemistry+mathematics+informatics

Systems biology

http://www.ibiology.org/ibioeducation/exploring-biology/cell-bio/signaling/ten-craziest-things-cells-do.html

http://www.cellsignal.com/contents/resources-applications-western-blotting-amp-immunoprecipitation/western-blotting-protocol-video/wb-protocol-video/
http://www.jove.com/video/2359/western-blotting-sample-preparation-to-detection
http://www.jove.com/video/5071/western-blotting-troubleshooting-guide-cell-signaling-technology

http://www.jove.com/video/3495/assaying-the-kinase-activity-of-lrrk2-in-vitro

http://www.jove.com/video/3203/imaging-estrogen-receptor-rat-pial-arterioles-using-digital

http://www.jove.com/video/51285/profiling-of-estrogen-regulated-micrornas-in-breast-cancer-cells

http://www.jove.com/video/51809/determination-protein-ligand-interactions-using-differential-scanning