Computational Systems Biology

Lecture 5:
Metabolic Pathways and beyond

Tuesday 22 January 2010

Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, Freeman ed.
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Suggested reading

- *Enzymes*
  - Lehninger Chapter 14 – Glycolisis (as general overview of a pathway, the biochemical details will not be examinable)

***Book**:*
David L. Nelson, Lehninger - Principles of Biochemistry, 4th Edition (or 3rd Edition), W. H. Freeman ed.
http://bcs.whfreeman.com/lehninger/

Summary

- Metabolic Pathways
- An example: Glycolysis
- Databases: KEGG and MetaCyc
- Beyond metabolism: proteins regulating other proteins
  - Phosphorylation
  - Transcriptional regulation
  - Signal transduction

Metabolic pathways: glycolysis

What is a pathway in biochemistry

- A metabolic pathway is a chain of enzymatic reactions.

- The pathway is a collection of step by step modifications: the initial substance used as substrate by the first enzyme is transformed into a product. This product will then be the substrate for the next reaction, until the exact chemical structure necessary for the cell is reached

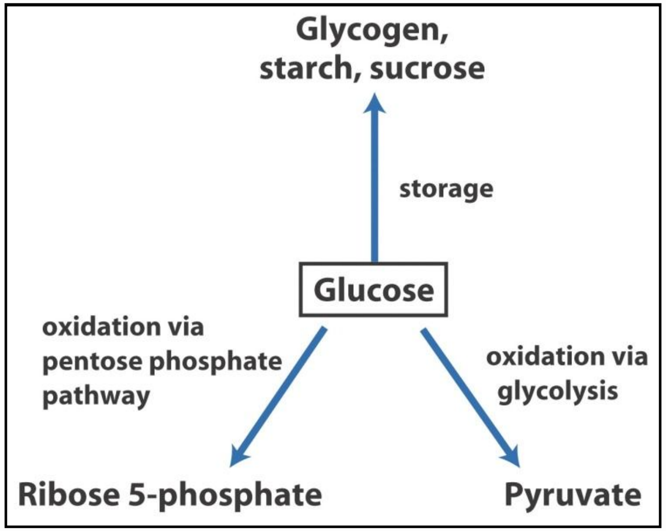
  http://en.wikipedia.org/wiki/Metabolic_pathway

- Click here for pathway animation (flash) *http://www.maxanim.com/biochemistry/Metabolic%20Pathway/Metabolic%20Pathway.htm*

A central pathway: glycolysis

- Glycolysis (from the Greek *glykys* meaning “sweet” and *lysis* meaning splitting)

- Glucose is the major fuel for most organism > it’s rich in potential energy: its complete oxidation to CO2 and water has a standard free-energy change of -2840 kJ/mol



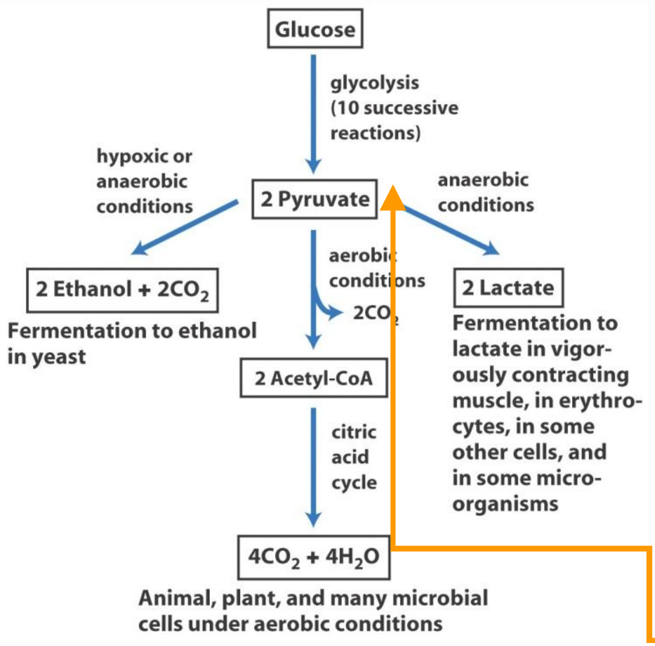
- Glucose is stored in big polymers like starch or glycogen
- When energy demand increases, it can be released quickly and used to produce ATP either aerobically (using oxigen) or anaerobically (fermentation)

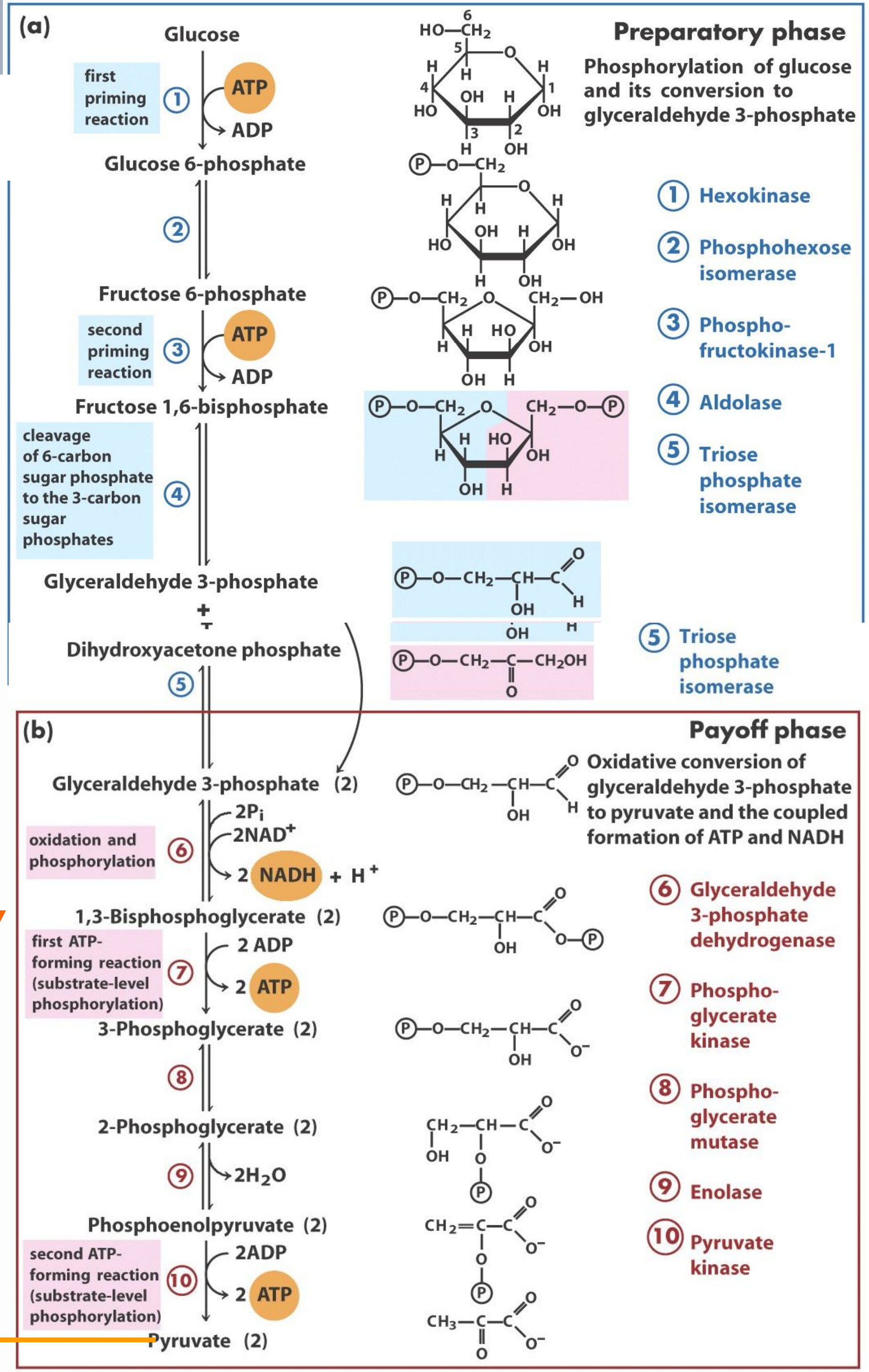
- Glucose is also a versatile molecule for building the basic carbon skeleton of other molecules like fatty acids, amino acids etc.

Glycolysis: spend a little get a lot

- For each molecule of glucose:
- 2 ATP molecules are spent in the activation/preparatory phase, but 4 ATP molecules are produced in the payoff phase
- The final product, pyruvate is then further metabolised

- *In the next slides we will concentrate on the cleavage (lysis, that gives this pathway its name) of Fructose 1,6-Biphosphate into two smaller molecules: Glyceraldehyde and Dihydroxyacetone (the enzyme doing this is the fructose 1,6-biphosphate* ***aldolase****)*





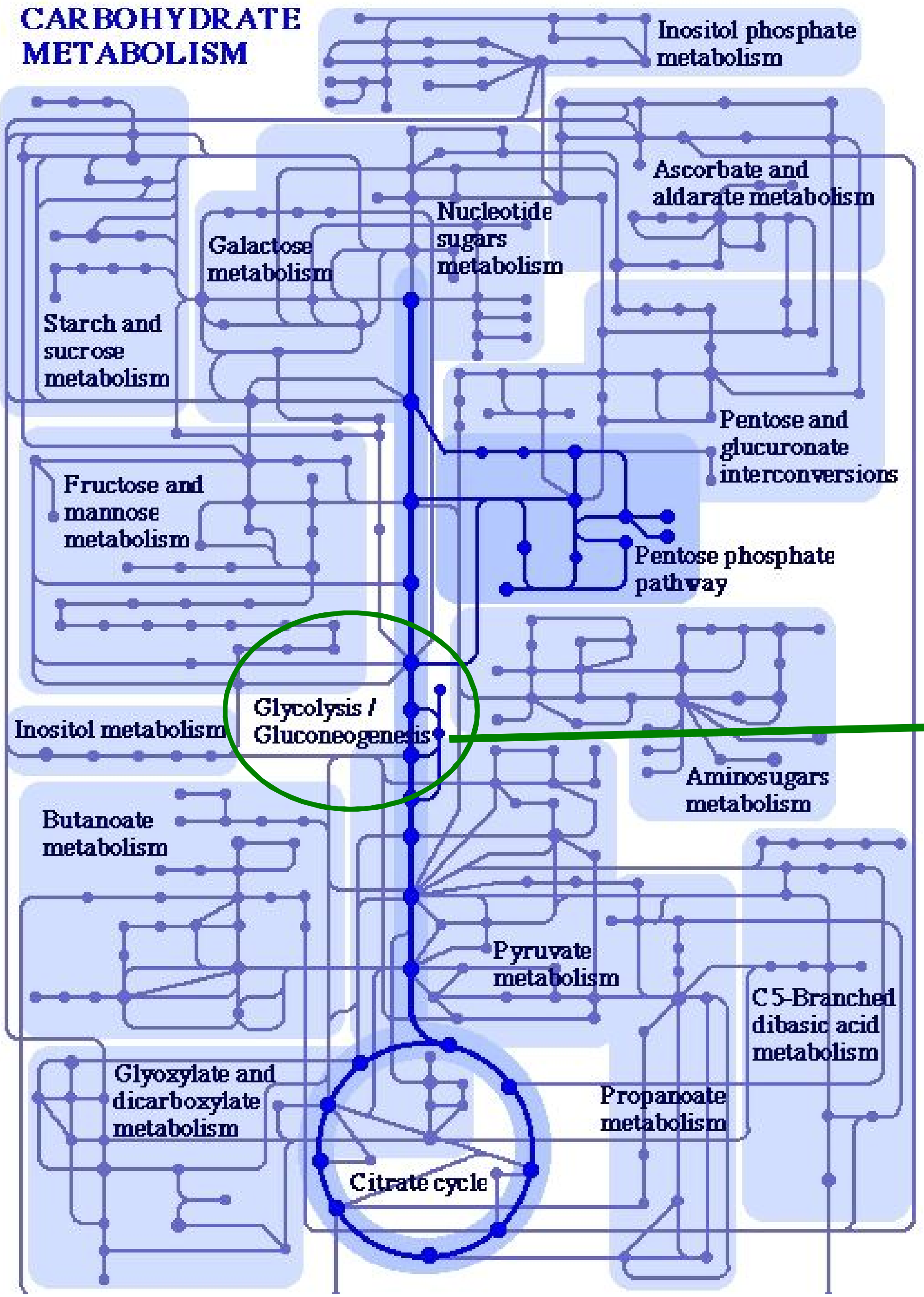
Pathway databases: KEGG and MetaCyc

The KEGG Pathway Database

- The KEGG resource (http://www.genome.jp/kegg/) is a knowledge base of building blocks in the genomic space (KEGG GENES), chemical space (KEGG LIGAND), and reaction space (KEGG PATHWAY)

- KEGG (Kyoto Encyclopedia of Genes and Genomes) can be queried via web sevice http://www.genome.jp/kegg/soap/ or on the web at: http://www.genome.ad.jp/kegg/pathway.html

Kegg metabolic maps: glycolysis (1)

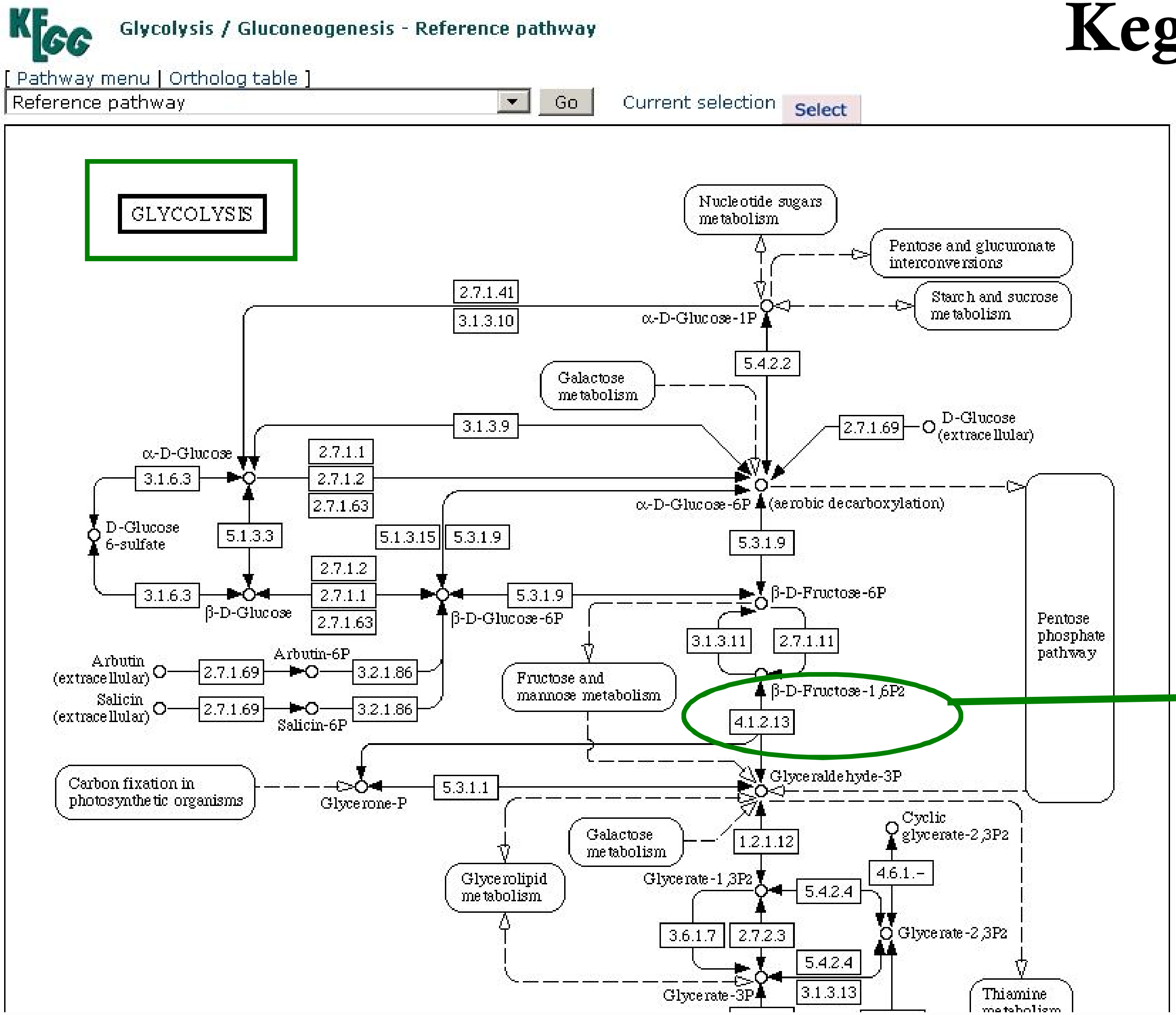


- Clicking on the web interface you can drill down into the single reactions

Next slide

http://www.genome.jp/dbget-bin/www_bget?pathway+map01110

Kegg metabolic maps: glycolysis



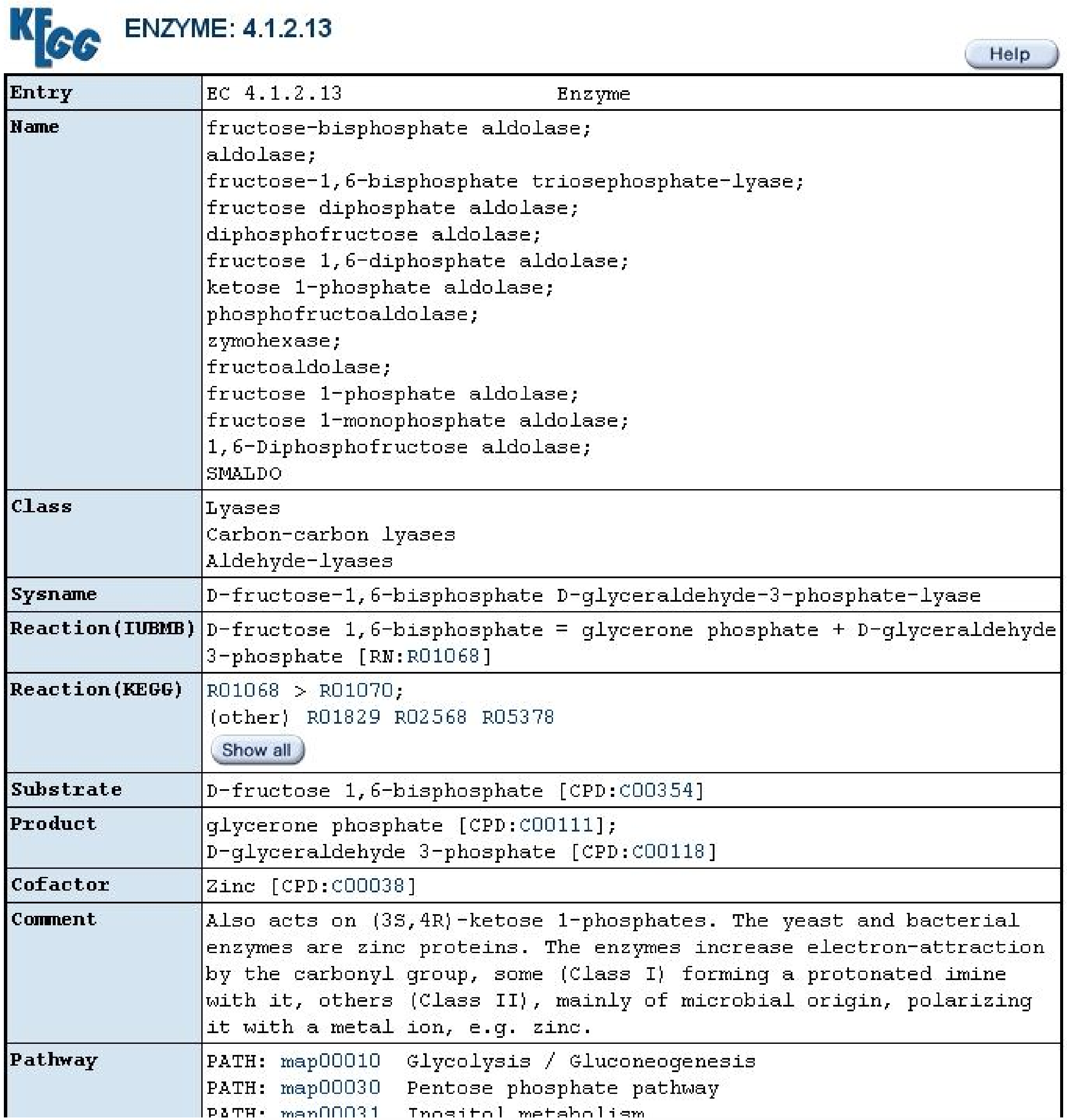
- Different databases use different notations to represent metabolic pathways

- This is an example of the “KEGG” notation

EC 4.1.2.13 Aldolase

Kegg metabolic maps: glycolysis

- EC 4.1.2.13 Fructose - biphosphate **aldolase**



ENZYME: 4.1.2.13

Entry	EC 4.1.2.13 Enzyme
Name	fructose-bisphosphate aldolase; aldolase; fructose-1,6-bisphosphate triosephosphate-lyase; fructose diphosphate aldolase; diphosphofructose aldolase; fructose 1,6-diphosphate aldolase; ketose 1-phosphate aldolase; phosphofructoaldolase; zymohexase; fructoaldolase; fructose 1-phosphate aldolase; fructose 1-monophosphate aldolase; 1,6-Diphosphofructose aldolase; SMALDO
Class	Lyases Carbon-carbon lyases Aldehyde-lyases
Sysname	D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase
Reaction(IUBMB)	D-fructose 1,6-bisphosphate = glycerone phosphate + D-glyceraldehyde 3-phosphate [RN:R01068]
Reaction(KEGG)	R01068 > R01070; (other) R01829 R02568 R05378
Substrate	D-fructose 1,6-bisphosphate [CPD:C00354]
Product	glycerone phosphate [CPD:C00111]; D-glyceraldehyde 3-phosphate [CPD:C00118]
Cofactor	Zinc [CPD:C00038]
Comment	Also acts on (3S,4R)-ketose 1-phosphates. The yeast and bacterial enzymes are zinc proteins. The enzymes increase electron-attraction by the carbonyl group, some (Class I) forming a protonated imine with it, others (Class II), mainly of microbial origin, polarizing it with a metal ion, e.g. zinc.
Pathway	PATH: map00010 Glycolysis / Gluconeogenesis PATH: map00030 Pentose phosphate pathway PATH: map00031 Inositol metabolism

MetaCyc

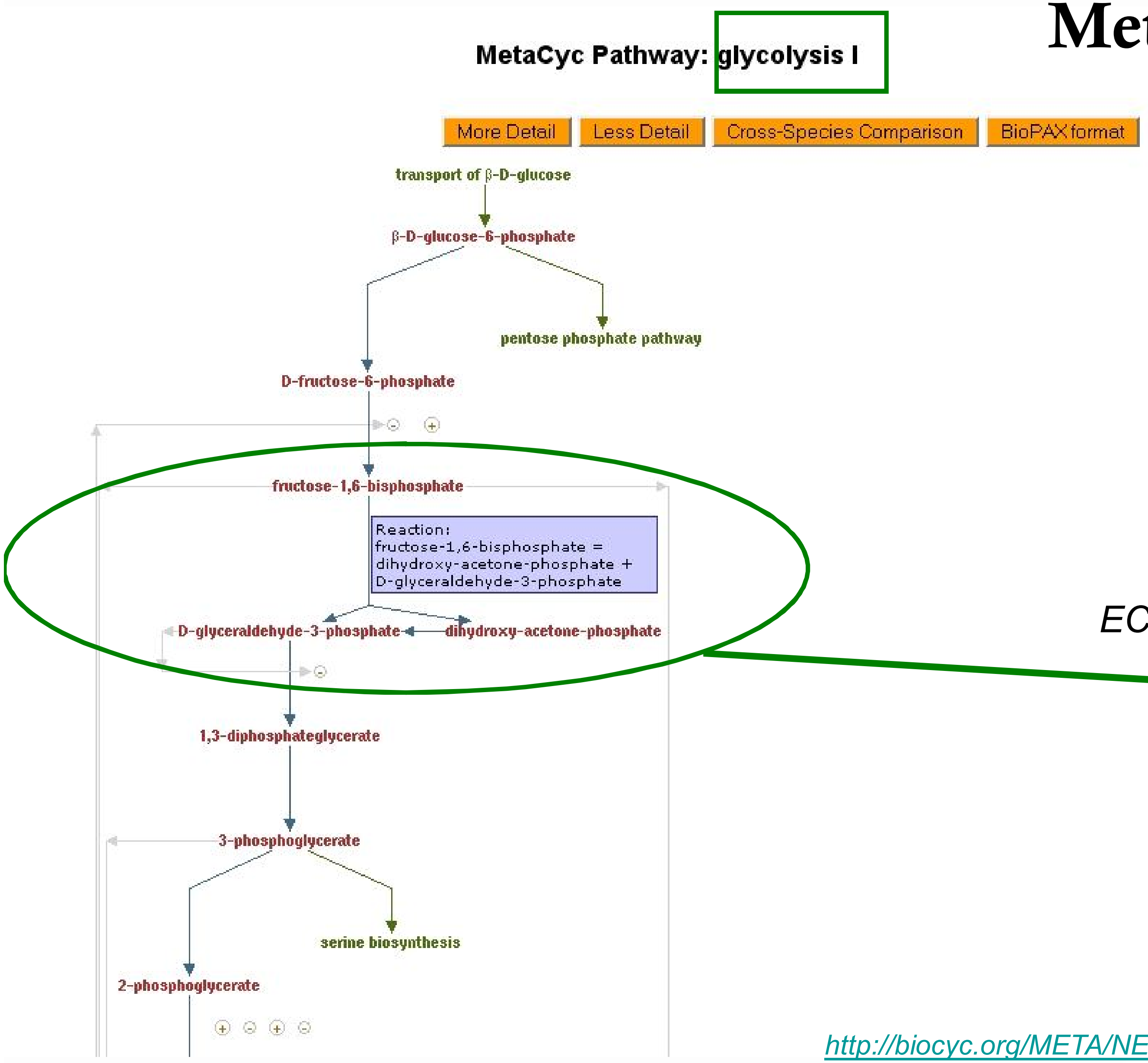
- MetaCyc is a database of nonredundant, experimentally elucidated metabolic pathways.
- MetaCyc contains over 900 pathways from more than 900 different organisms.
- It is curated from the scientific experimental literature.

- MetaCyc pathways can be browsed from the web, via ontologies or queried programmatically using Java or PERL when installed locally.

http://metacyc.org/

METACYC™

MetaCyc metabolic maps: glycolysis

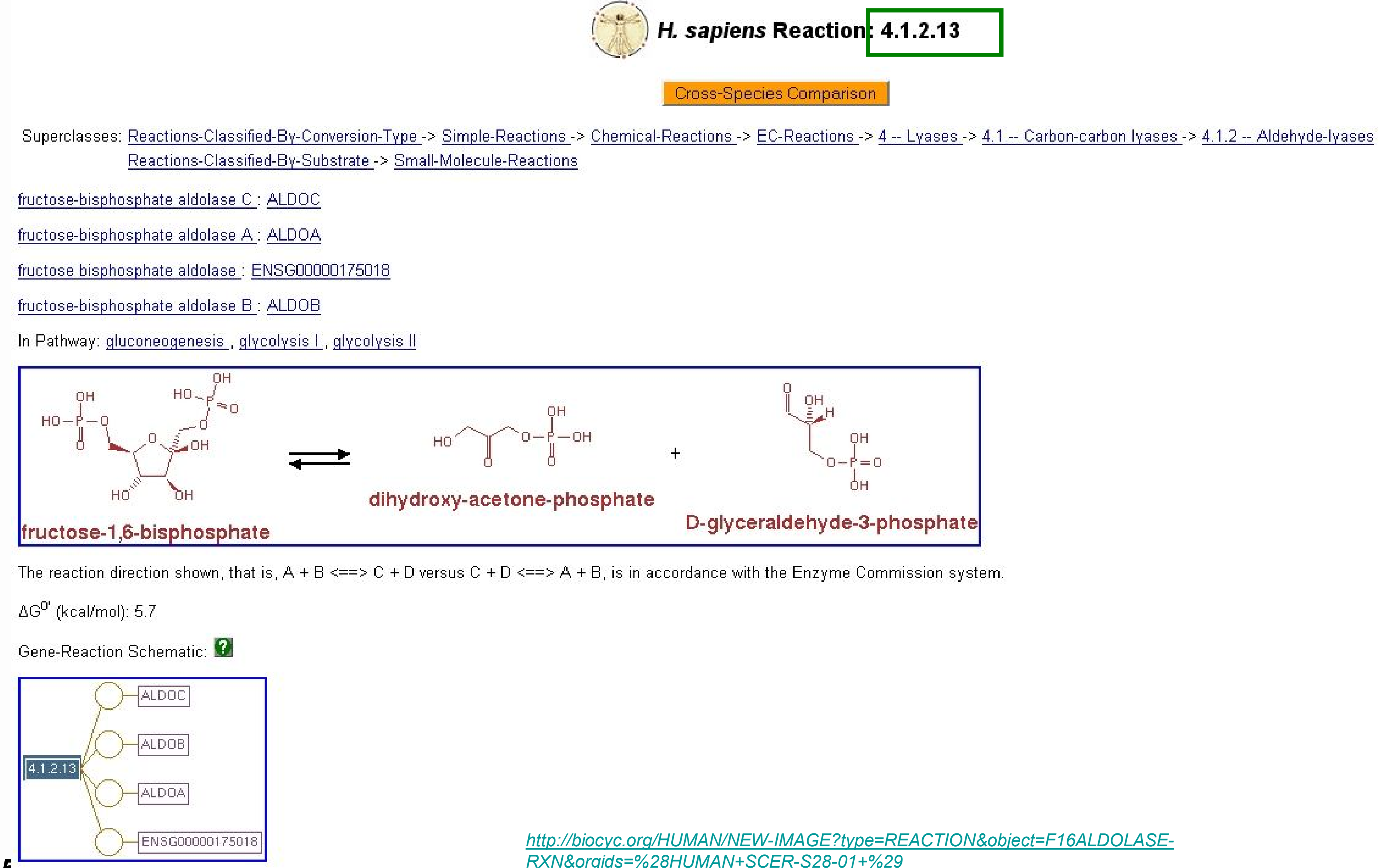


Reaction:
fructose-1,6-bisphosphate = dihydroxy-acetone-phosphate + D-glyceraldehyde-3-phosphate

EC 4.1.2.13 Aldolase

http://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=GLYCOLYSIS

MetaCyc metabolic maps: glycolysis



***H. sapiens* Reaction: 4.1.2.13**

Superclasses: Reactions-Classified-By-Conversion-Type -> Simple-Reactions -> Chemical-Reactions -> EC-Reactions -> 4 -- Lyases -> 4.1 -- Carbon-carbon lyases -> 4.1.2 -- Aldehyde-lyases
Reactions-Classified-By-Substrate -> Small-Molecule-Reactions

fructose-bisphosphate aldolase C : ALDOC

fructose-bisphosphate aldolase A : ALDOA

fructose bisphosphate aldolase : ENSG00000175018

fructose-bisphosphate aldolase B : ALDOB

In Pathway: gluconeogenesis , glycolysis I , glycolysis II

The reaction direction shown, that is, A + B <==> C + D versus C + D <==> A + B, is in accordance with the Enzyme Commission system.

$\Delta G^{0'}$ (kcal/mol): 5.7

Gene-Reaction Schematic: ?

http://biocyc.org/HUMAN/NEW-IMAGE?type=REACTION&object=F16ALDOLASE-RXN&orgids=%28HUMAN+SCER-S28-01+%29

Beyond metabolism

Cell life is regulated in a complex network of metabolic, transcriptional and signalling activities

Proteins regulating other proteins:
Phosphorylation

Phosphorylation

- Many proteins/enzymes are regulated inside the cell by the addition of a highly energetic phosphate group (PO_4) to one (or more) of the aminoacids on their surface: this process is called *phosphorylation*.

- The phoshpate addition alters the functional shape of the enzyme (or molecule), physically locking it in an activated (or inactivated) form until the phosphate group is removed

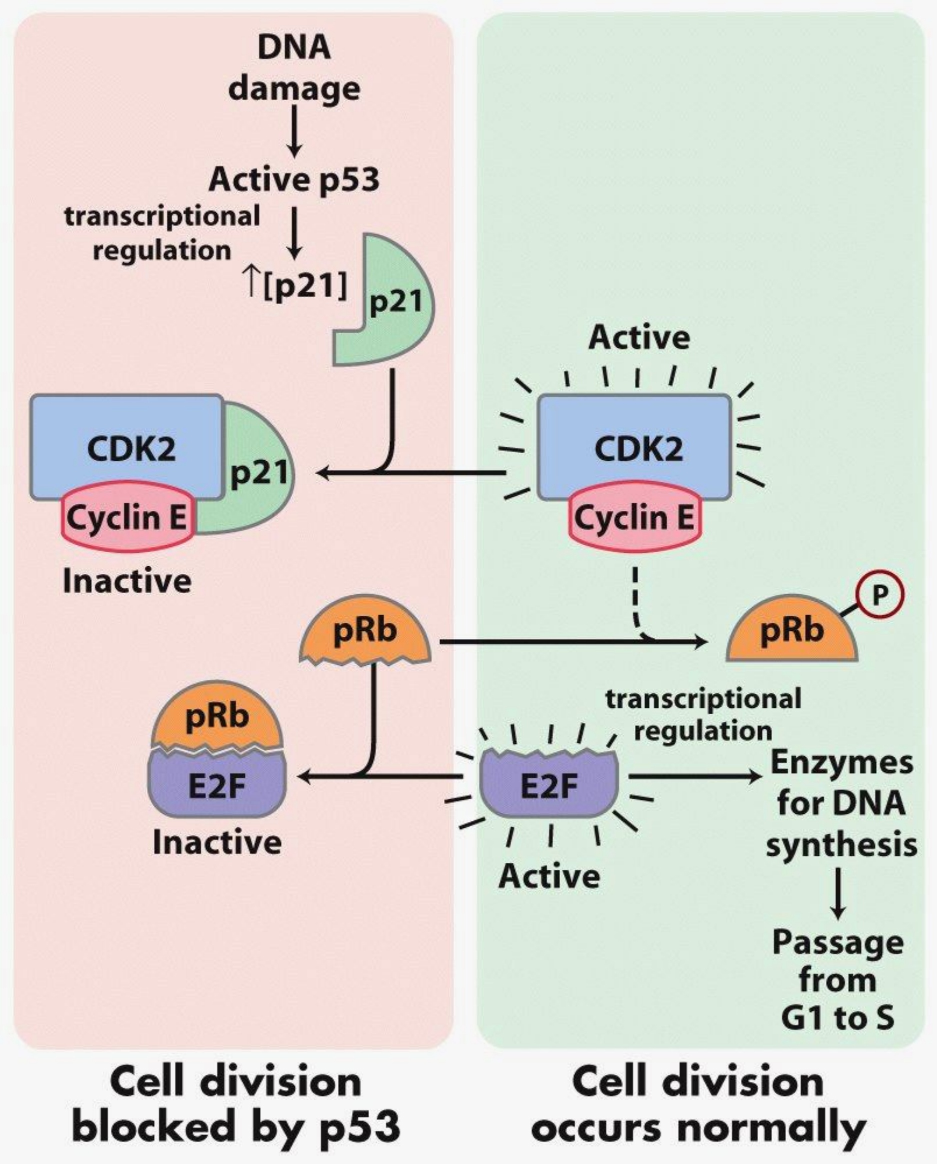
- Hence, two important classes of enzymes are:

1. Kinases → they *add* a phosphate group to another protein/enzyme
2. Phosphatases → they *remove* a phosphate group from a protein/enzyme

- Kinases and phosphatases are highly specific in their choice of substrate and often a kinase will act only on a single, particular kind of substrate protein. And the substrate protein can itself be another kinase, generating a cascade of activations/inactivations through the cell

A process affected by phosphorilation: the cell cycle

- From top left: if the DNA is damaged the cell should not divide before repairing it.
- When the DNA is repaired p53 detaches p21 from the CDK2-CyclinE complex. This complex is a kinase.
- The CDK2-CyclinE kinase phosphorylates pRb.
- The addition of a phosphate group modifies pRb's shape and affects its ability to bind E2F.
- E2F is hence released and free to carry out its role: to promote transcription of other enzymes needed for the cell division cycle



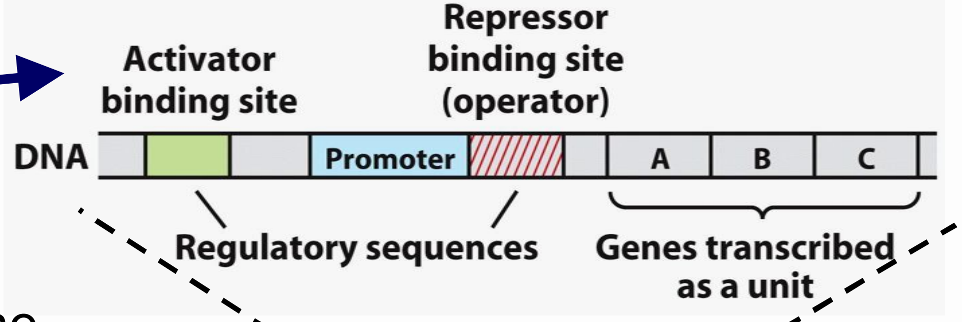
Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

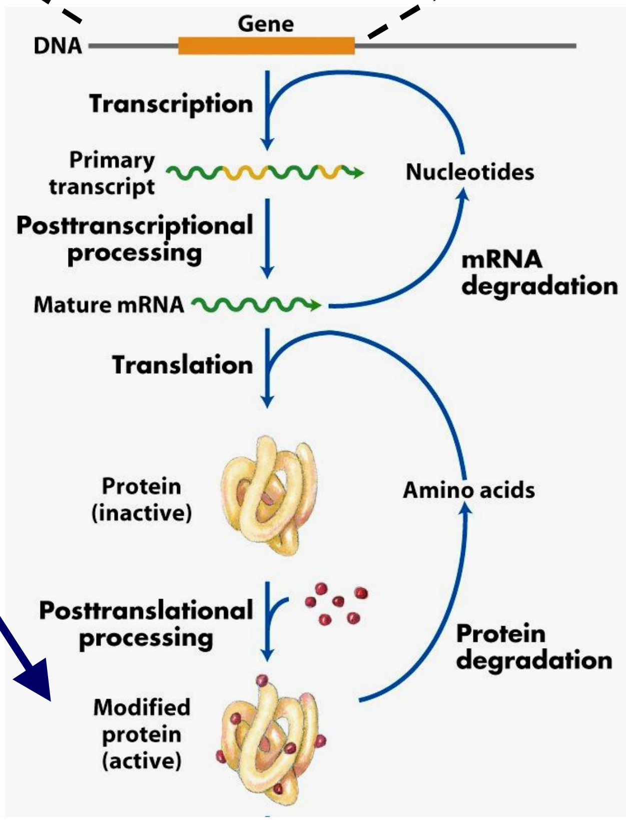
Proteins regulating other proteins:

Transcriptional regulation

Regulation at transcription level

- Some proteins - transcriptional *activators* or *repressors* - bind to regulatory sequences near the promoter region upstream of a gene (or of a group of genes - operon) to regulate the production of the protein(s) coded by that gene(s)





- Activators facilitate the binding of RNA polymerase on the promoter, while repressors impede the binding and hence the transcription

Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

Transcriptional regulation: the Lactose operon

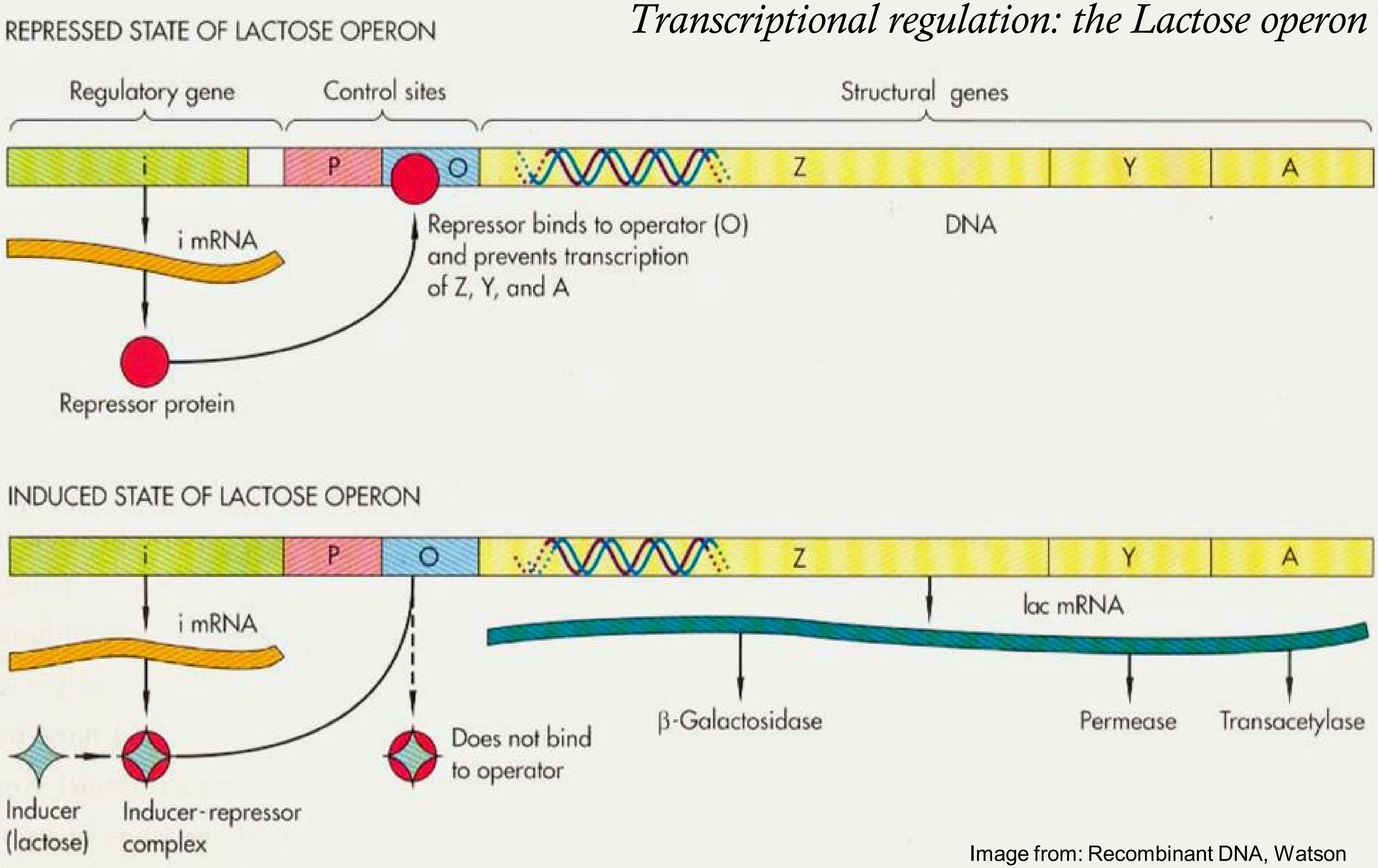


Image from: Recombinant DNA, Watson

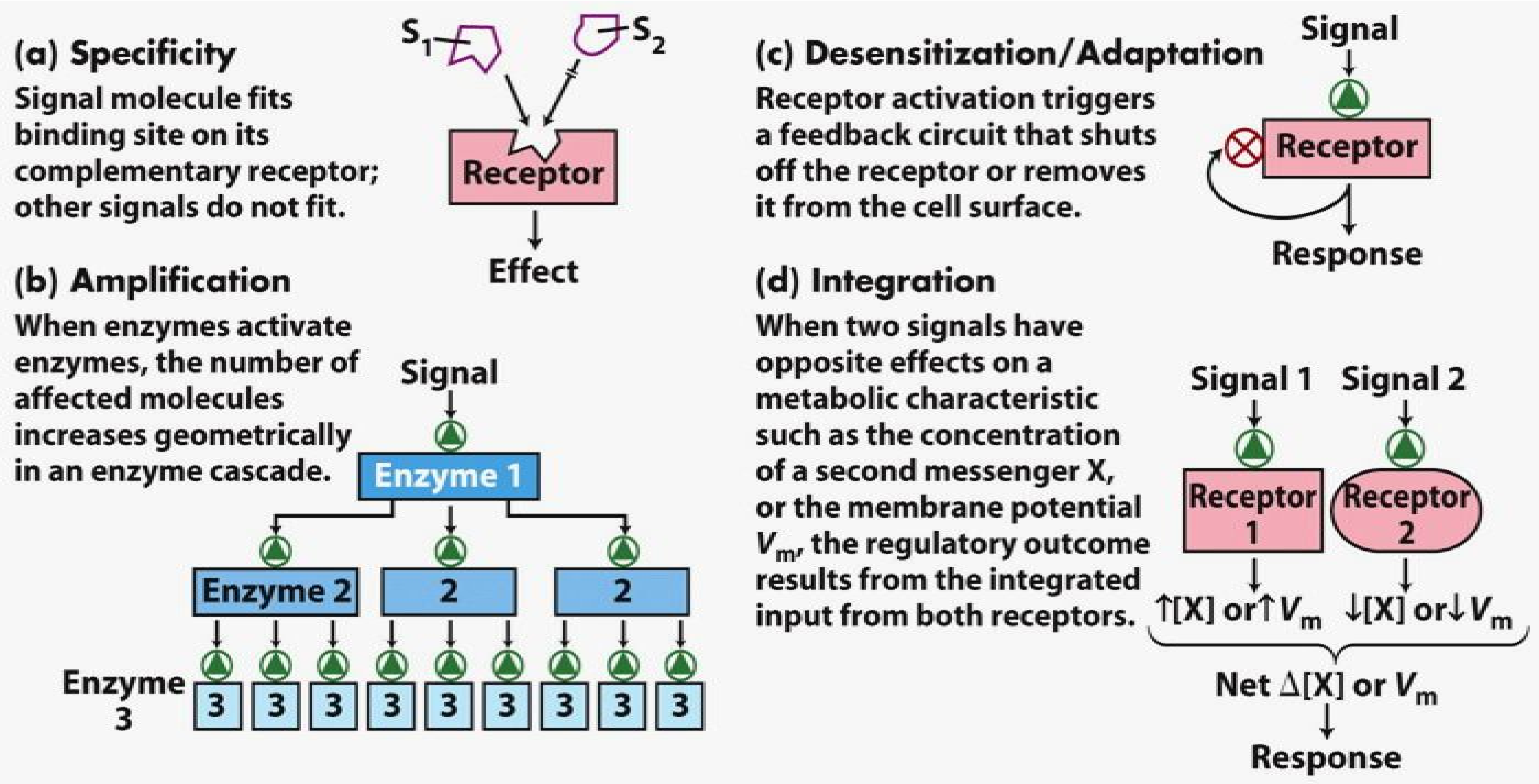
FIGURE **4-1**

Repressors and inducers control the functioning of the genes belonging to the lactose (lac) operon. The regulatory gene (i) codes for the lactose repressor. The P segment of the DNA chain is the "promoter" and is discussed in the next figure.

Proteins regulating other proteins:

Signal transduction

Characteristics of Biological 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.

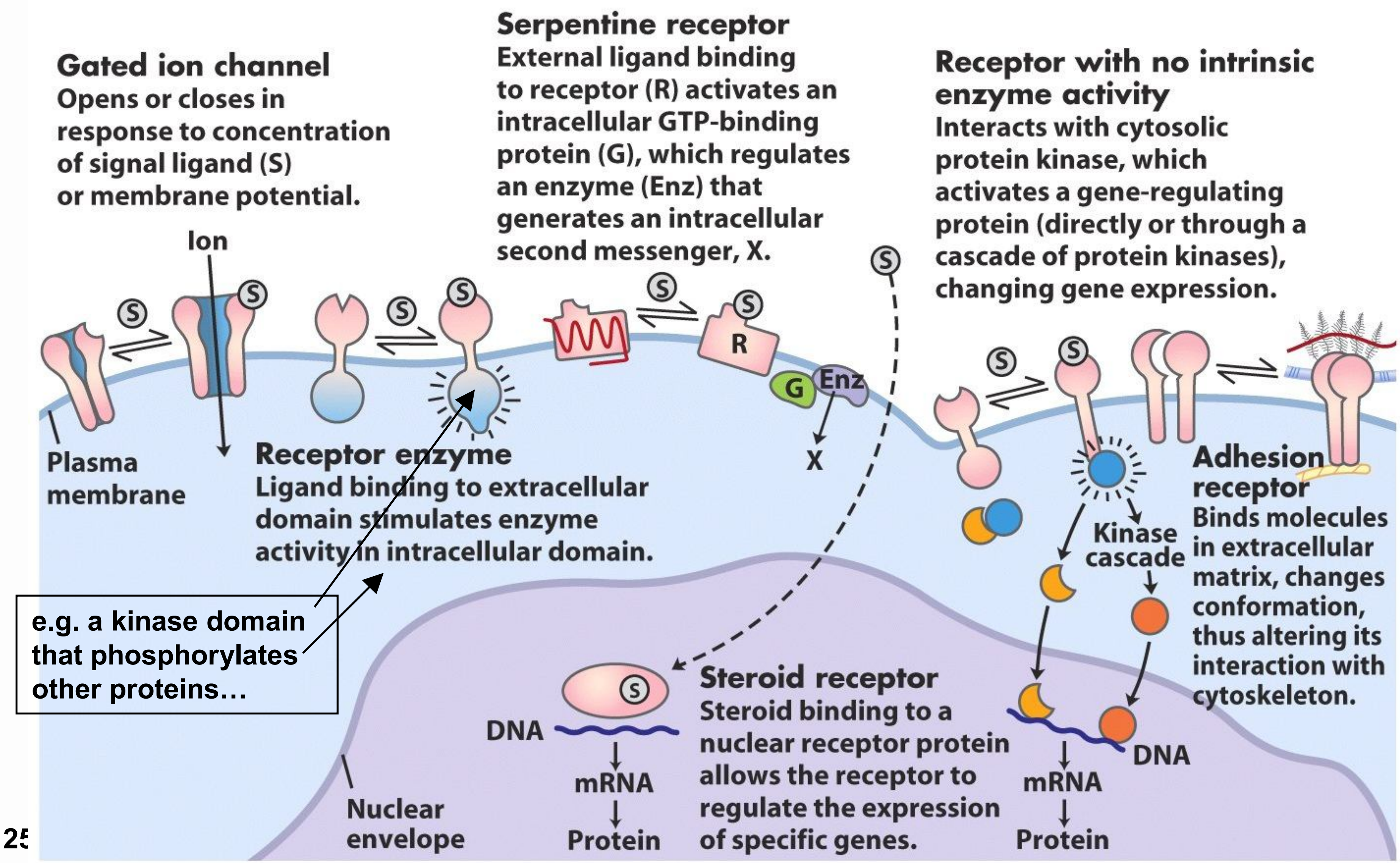
(c) Desensitization/Adaptation

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.

Biological Signal Transducers: proteins



Gated ion channel
Opens or closes in response to concentration of signal ligand (S) or membrane potential.

Serpentine receptor
External ligand binding to receptor (R) activates an intracellular GTP-binding protein (G), which regulates an enzyme (Enz) that generates an intracellular second messenger, X.

Receptor with no intrinsic enzyme activity
Interacts with cytosolic protein kinase, which activates a gene-regulating protein (directly or through a cascade of protein kinases), changing gene expression.

Receptor enzyme
Ligand binding to extracellular domain stimulates enzyme activity in intracellular domain.

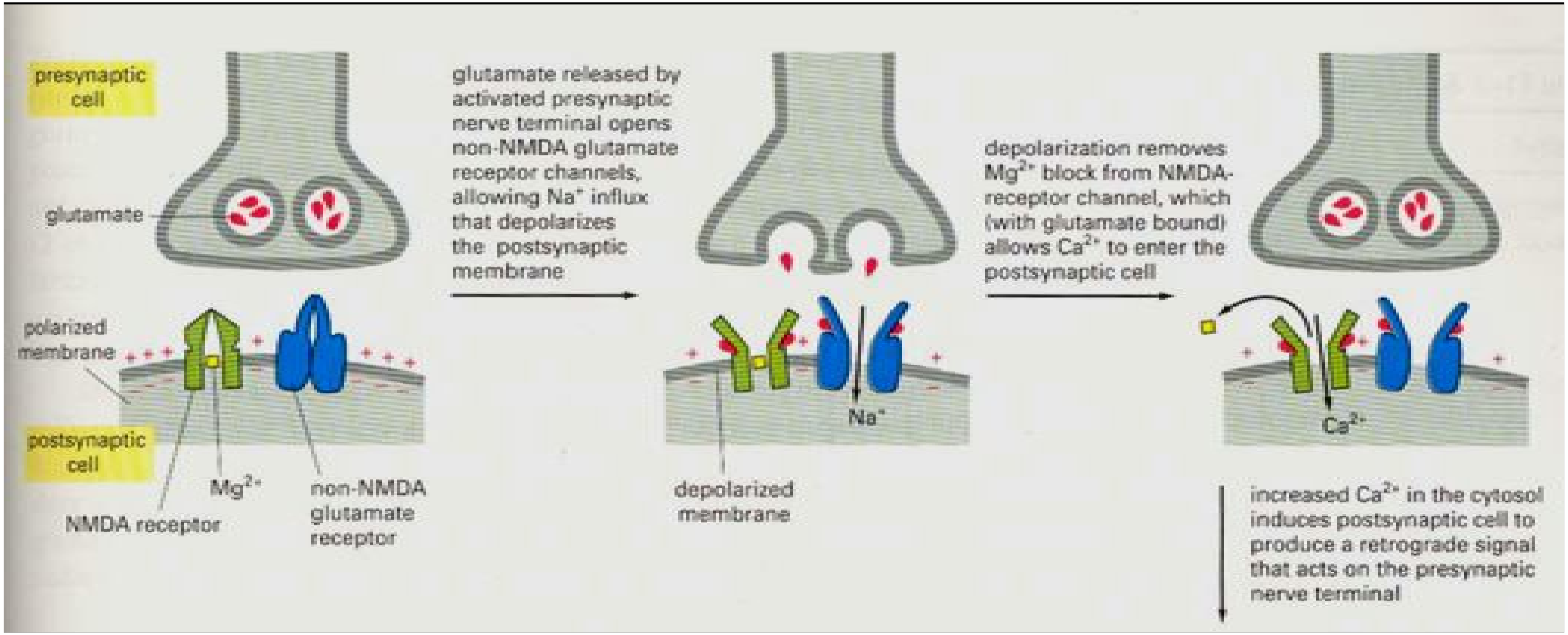
Adhesion receptor
Binds molecules in extracellular matrix, changes conformation, thus altering its interaction with cytoskeleton.

Steroid receptor
Steroid binding to a nuclear receptor protein allows the receptor to regulate the expression of specific genes.

e.g. a kinase domain that phosphorylates other proteins…

Neuronal signalling

- Some proteins exposed on the cell surface cross the entire membrane and act as gates
- The gates (or receptor channels) can "open" due to a conformation change, following phosphorylation or binding of a small chemical molecule (for example a drug)
- This is how synapses of neurons fire to help you think



glutamate released by activated presynaptic nerve terminal opens non-NMDA glutamate receptor channels, allowing Na^+ influx that depolarizes the postsynaptic membrane

depolarization removes Mg^{2+} block from NMDA-receptor channel, which (with glutamate bound) allows Ca^{2+} to enter the postsynaptic cell

increased Ca^{2+} in the cytosol induces postsynaptic cell to produce a retrograde signal that acts on the presynaptic nerve terminal