Regulation of Enzymatic Activity

Regulation of Enzymatic Activity

Enzymatic activity can be regulated by controlling concentration of the enzyme ("fake" regulation) or by controlling its function ("real" regulation)
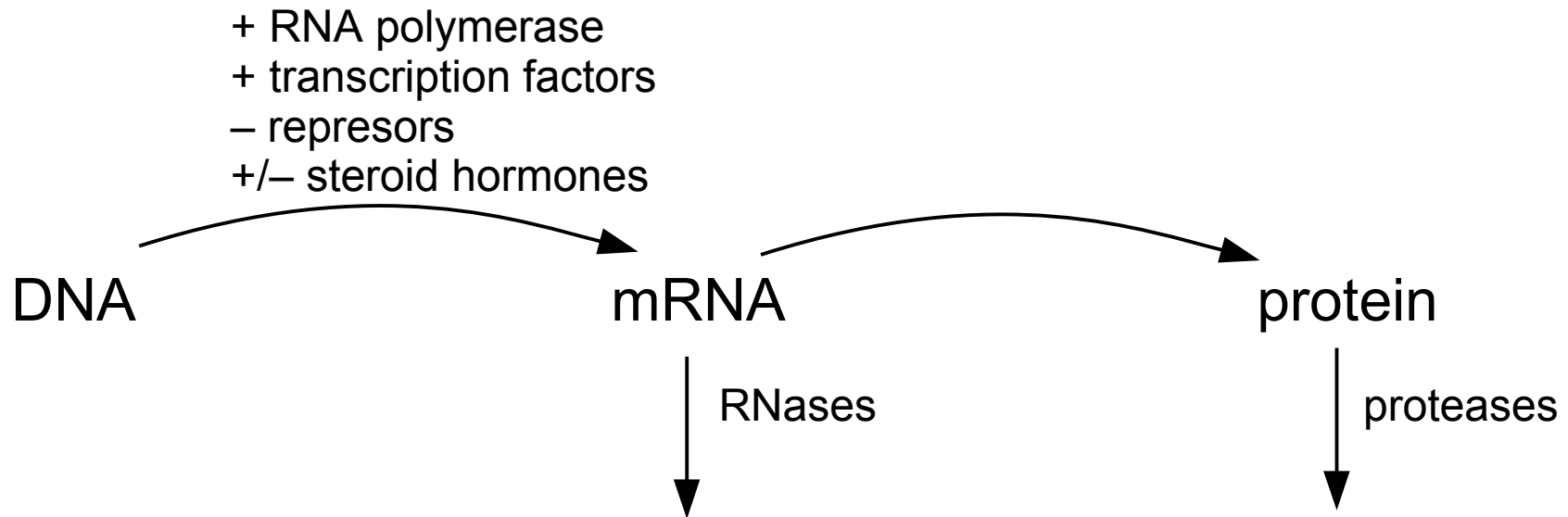
"fake" regulation:

- regulation of enzyme expression and turnover
- control of enzyme trafficking
- supply of cofactors

"real" regulation of enzymatic activity:

- activation/inhibition by small-molecule ligands
- activation/inhibition by protein binding
- regulatory domains
- activation/inhibition by covalent modifications
- activation by proteolytic cleavage
- regulation by physical conditions

Regulation of enzyme expression and turnover and control of enzyme trafficking

+ RNA polymerase
+ transcription factors
– represors
+/– steroid hormones

DNA → mRNA → protein

mRNA ↓ RNases

protein ↓ proteases

"Fake" regulations include regulations of transcription of the gene of the enzyme and regulation of its degradation or degradation of its mRNA. For most biochemist it is not natural to say that this controls the activity of the enzyme because it controls its **concentration**. However, many molecular biologists do not hesitate to say that something "activates" or "inhibits" an enzyme, despite it activates or inhibits expression of its gene.

Activation/inhibition by small-molecule ligands
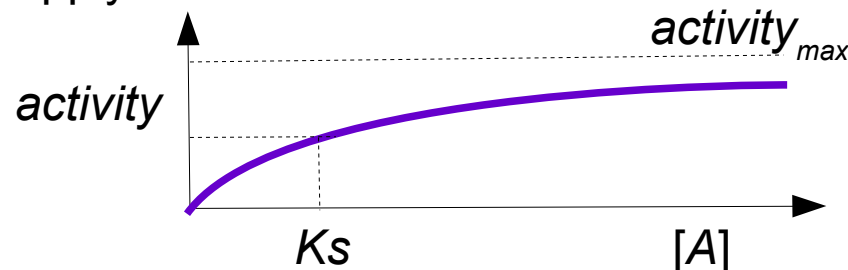
regulation: activation/inhibition

regulation: non-allosteric/allosteric

inhibition: competitive, noncompetitive, acompetitive, covalent

parameters: saturation constant, inhibition constant, IC_{50}

Many small molecule compounds such as second messengers (e.g. cAMP) bind non-covalently to an enzyme and modulate its activity. Some compounds activate the enzyme. For this it is possible to apply saturation kinetics:

$$activity = \frac{activity_{max}[A]}{K_s + [A]}$$

For inhibitors it is possible to apply standard models of enzyme inhibition (competitive, noncompetitive, acompetitive). Allosteric effect may play a role in activation as well as inhibition and may cause behavior different from standard models.
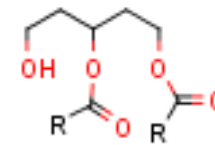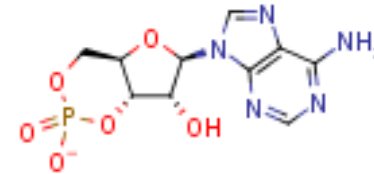
Activation/inhibition by small-molecule ligands

second messengers:
    cAMP, cGMP
    diacylglycerol
    IP_3 and other inositolphosphates
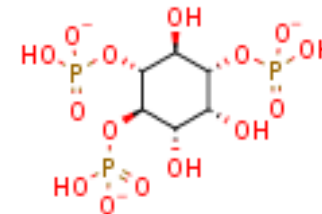 Ca^{2+}, calmodulin

other small signaling molecules:
    prostaglandins
    NO, H_2S, CO
    ethylene, ppGpp,
    etc.

Second messengers are small molecule compounds involved in signal transduction from receptor to cellular proteins. Their concentrations indicate different physiological states of the cell. Beside signaling enzymes, such as protein kinases, they may regulate activity of metabolic enzymes. Most famous secondary messengers are cAMP, diacylglycerol or inositol phosphates. They also include gaseous signaling molecules or molecules specific for plants and bacteria.

Activation/inhibition by small-molecule ligands

feedback regulation of metabolic pathways:

Pasteur effect: anaerobic glycolysis is faster than aerobic

- phosphofructokinase is activated by ADP, AMP, fructose-2,5-bisphosphate, inhibited by ATP and citrate

- pyruvate kinase is inhibited by ATP

In 1857 Pasteur discovered that oxygen increases the rate of growth of yeast. This can be explained by two types of glucose metabolism in yeast. In aerobic metabolism glucose is oxidized to carbon dioxide and water. This yields approx. 32 mols of ATP per one mol of glucose. Under anaerobic conditions yeast produce ethanol yielding approx. 2 mols of ATP per one mol of glucose. In order to sustain comparable production of ATP, yeast slow down aerobic and accelerate anaerobic glycolysis. This is done by inhibition of phosphofructokinase by ATP or citrate, which are present in high concentrations under aerobic conditions. In contrast, AMP and ADP accelerate glycolysis by activation of phosphofructokinase.

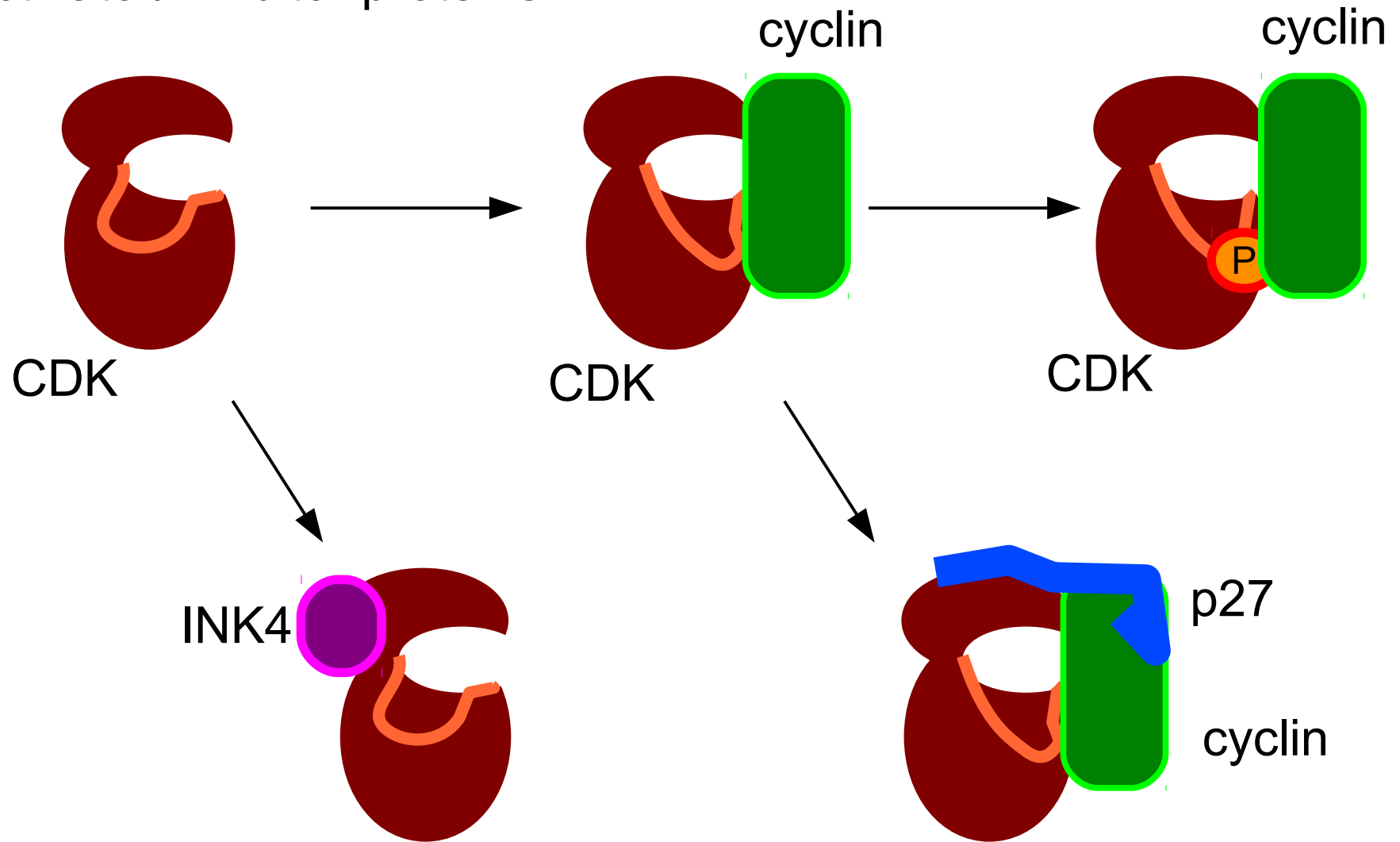# Activation/inhibition by small-molecule ligands

feedback regulation of metabolic pathways:

Pasteur effect: anaerobic glycolysis is faster than aerobic
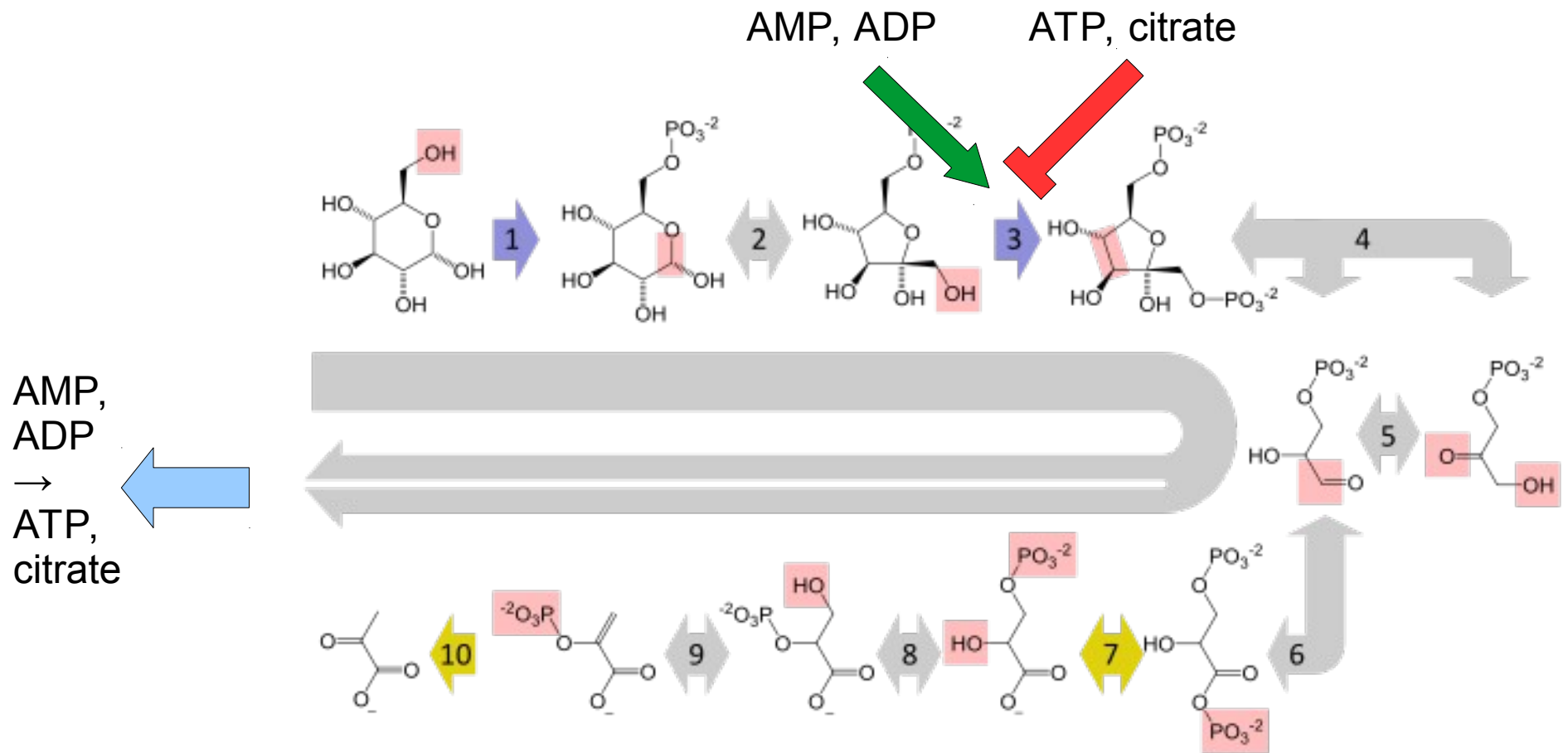
Activation/inhibition by protein binding

- activator/inhibitor proteins

Enzymes can be activated or inhibited by interactions with some other proteins.
For example, cyclins regulate activity of cyclin-dependent kinases (CDK).
Regulation of CDK is involved in the control of the cell cycle.

Activation/inhibition by regulatory domains

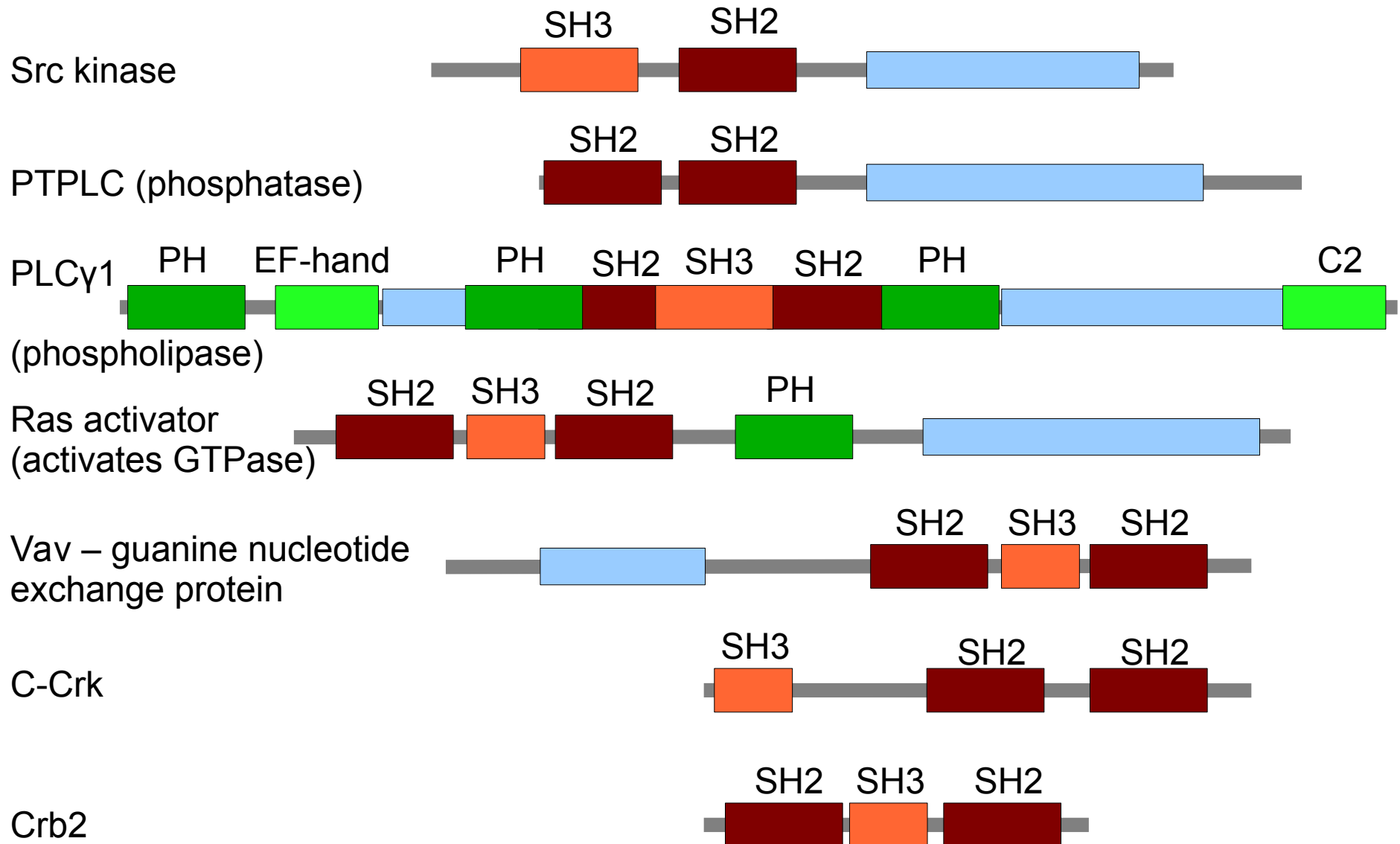## regulatory (adaptor, interaction) domains

Cyclins are examples of proteins regulating enzymatic activities by inter-molecular interactions with enzymes. Production of cyclins and cyclin-dependent kinases (CDKs) is controlled at the level of transcription. Transcription controls cyclin concentrations, they control activities of CDKs and they in turn control transcription. This forms a complicated cell cycle regulation system.

However, activities of some enzymes can be controlled by intra-molecular interaction with its regulatory domains. We will use the term domain as a smaller part of larger protein (as a one polypeptide chain) with some function. It was found that many eukaryotic proteins are composed from such regulatory domains as a kind of "molecular Lego". For example SH2 domain was found in 111 human proteins (with minor differences in amino acid sequences). It competes for binding either to the enzyme to which it is attached or to a concurrent protein containing phosphotyrosine motif.
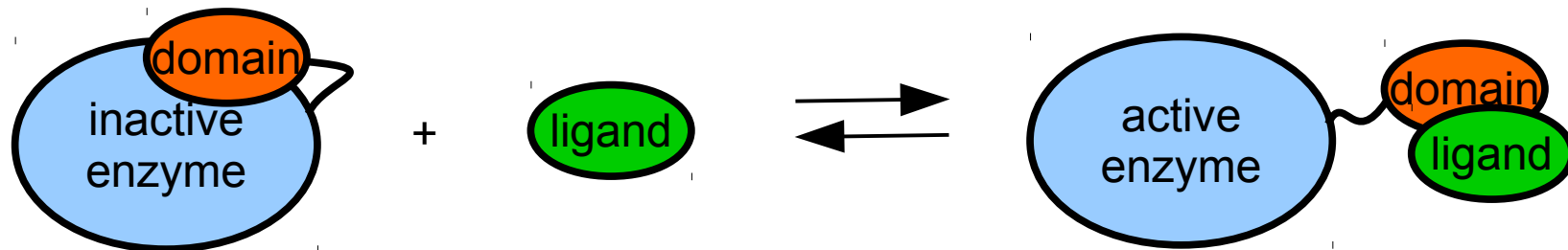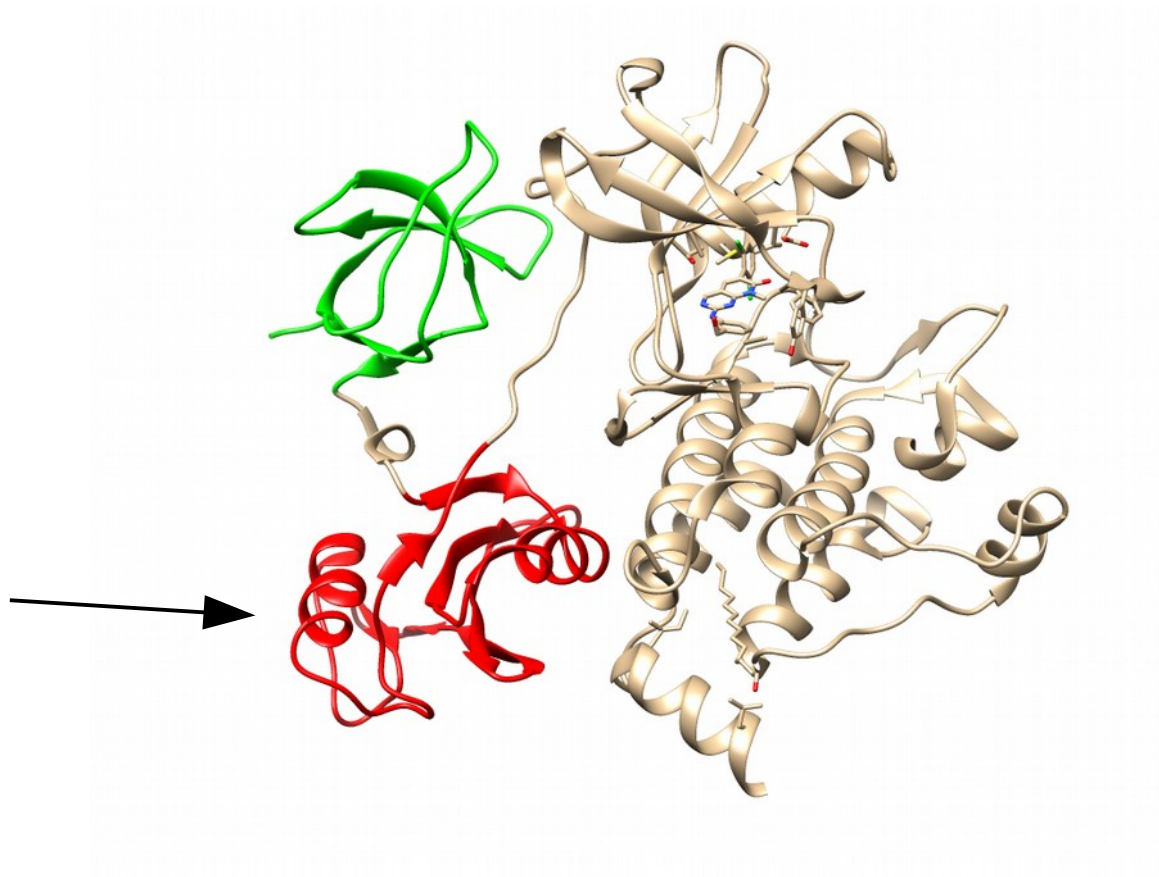
Activation/inhibition by regulatory domains

regulatory (adaptor, interaction) domains

Activation/inhibition by regulatory domains
regulatory (adaptor, interaction) domains

SH2 domain (Src-homology type 2)
    protein kinases, protein phosphatases, phospholipases,
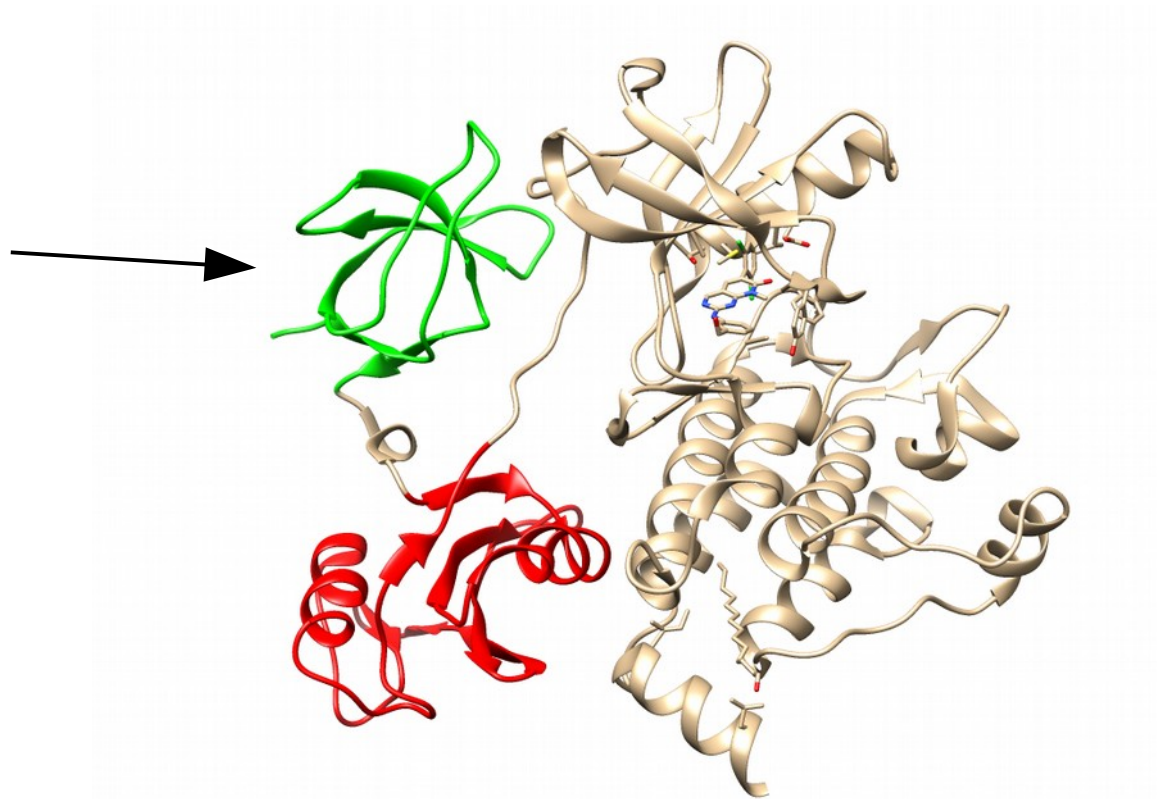   regulatory proteins and others

-pY–x–x–Φ-

Activation/inhibition by regulatory domains
regulatory (adaptor, interaction) domains

SH3 domain (Src-homology type 3)
protein kinases, protein phosphatases, phospholipases,
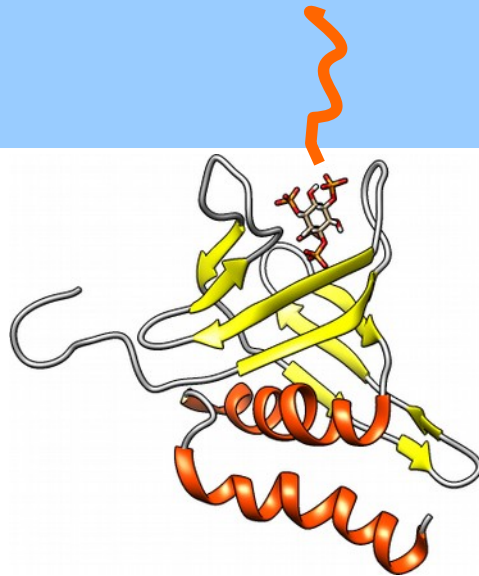regulatory proteins, myosin, spectrin and others

-R/K–x–x–P–x–x–P-
-x–P–x–x–P–x–R/K-

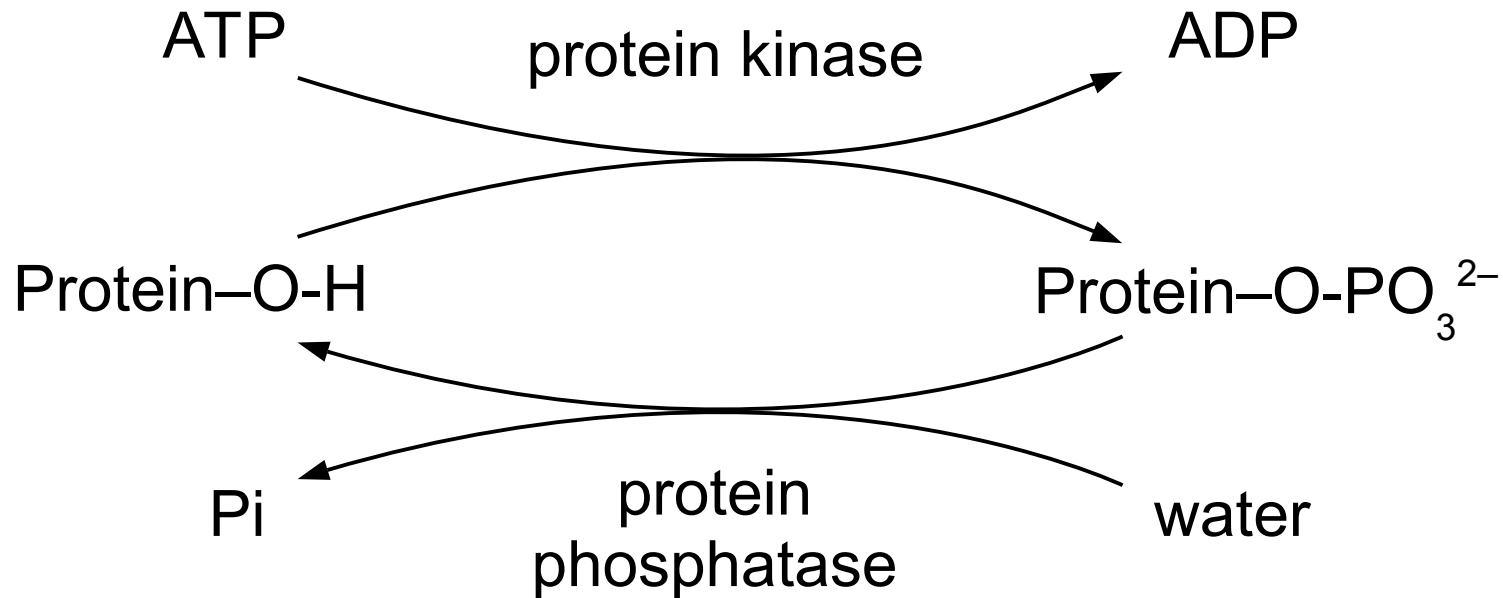**Activation/inhibition by regulatory domains**
regulatory (adaptor, interaction) domains

PH domain (Pleckstrin-homology)
  phospholipases, protein kinases and others

inositole phosphates (PIP_2, PIP_3), free or in phospholipids

Activation/inhibition by covalent modification

phosphorylation/dephosphorylation

$$\text{ATP} \quad \xrightarrow{\text{protein kinase}} \quad \text{ADP}$$

Protein–O-H $\qquad\qquad$ Protein–O-PO$_3^{2-}$

Pi $\qquad\qquad$ water

protein
phosphatase

Protein phosphorylation is one of most important system for regulation of enzymatic activities. Protein kinases phosphorylate either Tyr, Ser or Thr residues (other PKs exists but are rare). Phosphorylation of a residue changes the activity of the enzyme. Phosphorylation can be reverted by protein phosphatases. Protein kinases have been challenging targets for drug discovery. However, for a long time it was believed that the development of specific inhibitor is impossible due to existence of many ATP-recognizing proteins. Discovery of Imatinib (Gleevec) has shown that it is possible. This has sparked further interest in protein kinases.

Activation/inhibition by covalent modification

phosphorylation/dephosphorylation

| Enzyme	Activator
Ser/Thr	
Protein kinase A (PKA)	cAMP
Protein kinase B (PKB)	PtdInsP3
Protein kinase C (PKC)	DAG
Ca^{2+}/calmodulin-PK	Ca^{2+} - calmodulin
AMPK	AMP
MAPK	phosphorylation
Tyr	
Insulin receptor	insulin
Src, Abl, Jak ...	phosphorylation and other

There are more than 500 PKs in the human genome. PKs can be divided into two evolutionary related families – Ser/Thr and Tyr PKs. There are also receptor PKs such as insulin receptor.

Activation/inhibition by covalent modification

phosphorylation/dephosphorylation
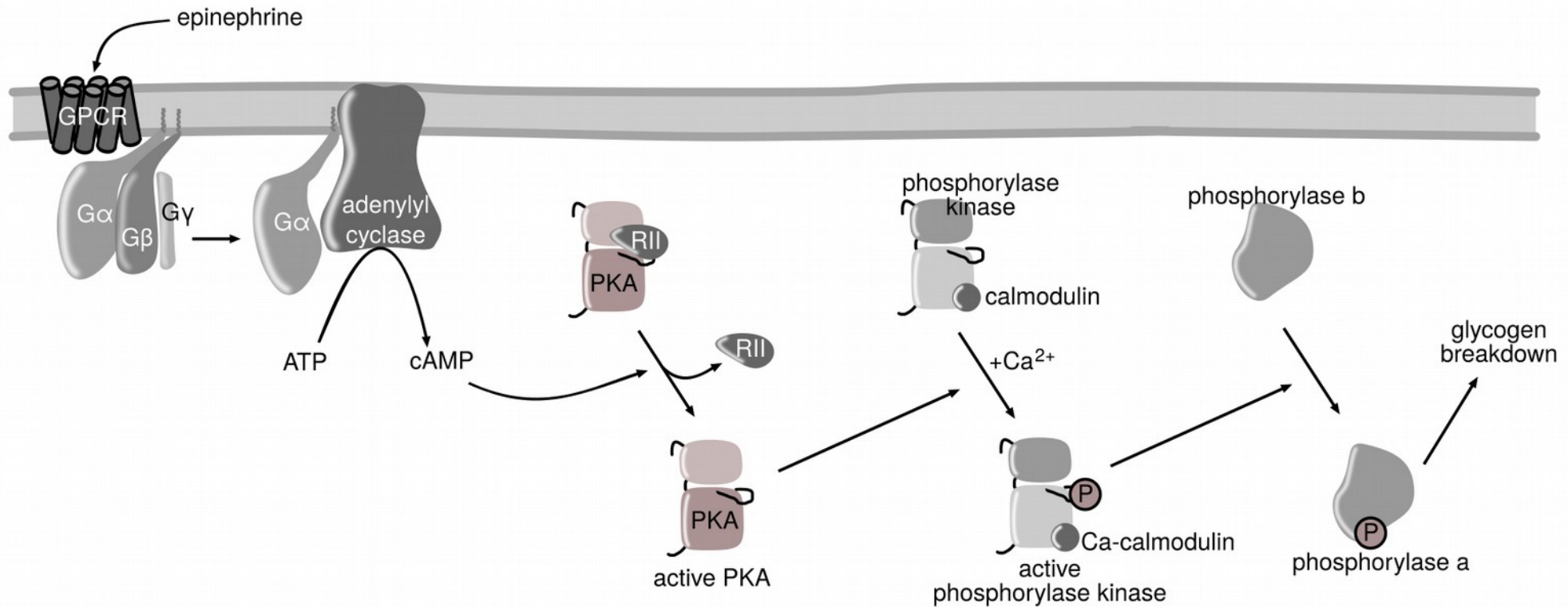
The function of PKs is shown on the example of glycogen breakdown regulation. A small molecule epinephrine binds to its receptor from GPCR family (to be explained in the next lecture). This activates G proteins. Gα protein activates adenylyl cyclase. This enzyme produces cAMP from ATP. cAMP activates PKA, PKA activates phosphorylase kinase and phosphorylase kinase activates phosphorylase. This enzyme degrades glycogen by phosphorolysis. Important feature of this pathway is that each activated molecule of enzyme may activate large number of other molecules, so the signal is amplified along the pathway. A similar pathway exists for insulin, which counteracts this pathway.

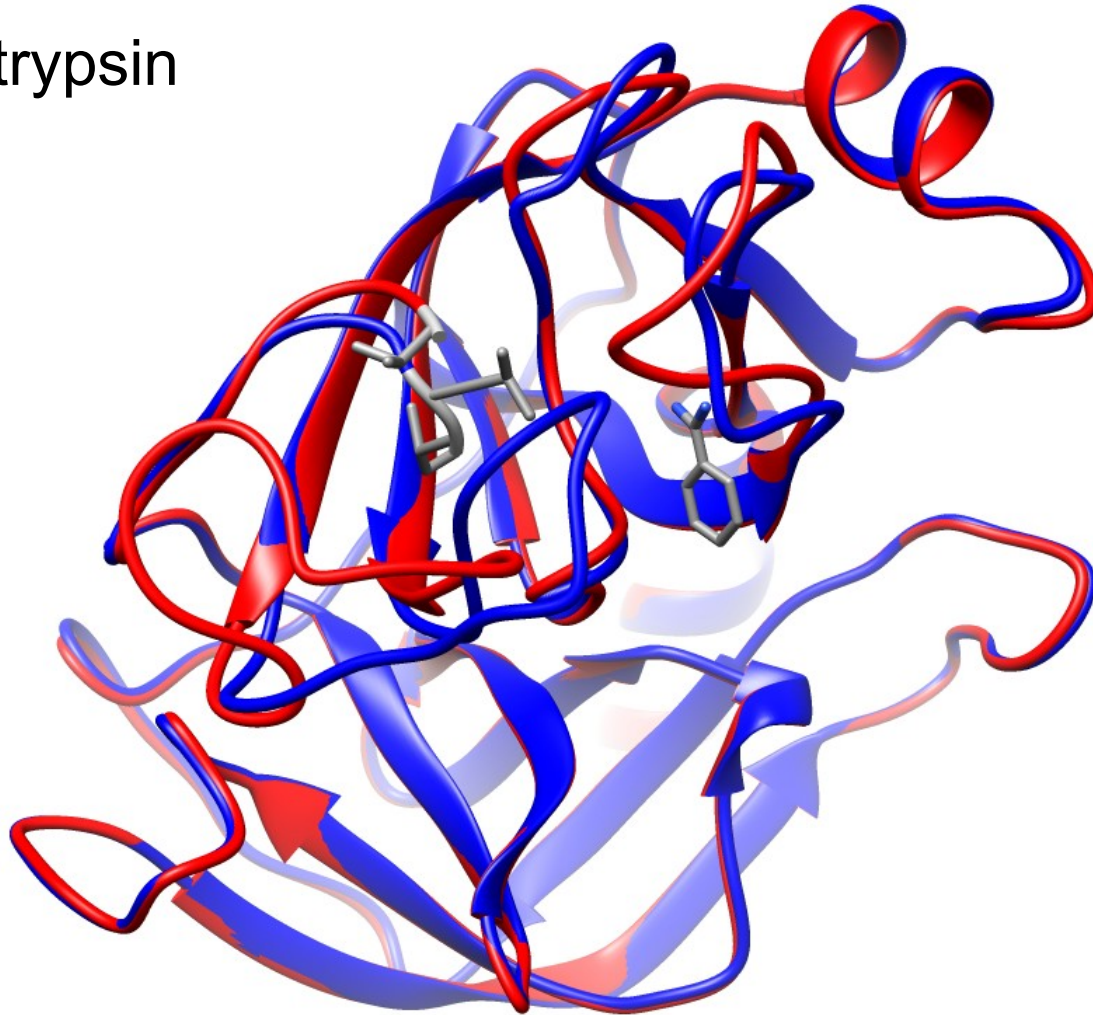**Activation/inhibition by covalent modification**

other modifications

ADP-ribosylation on Arg

adenylation on Tyr
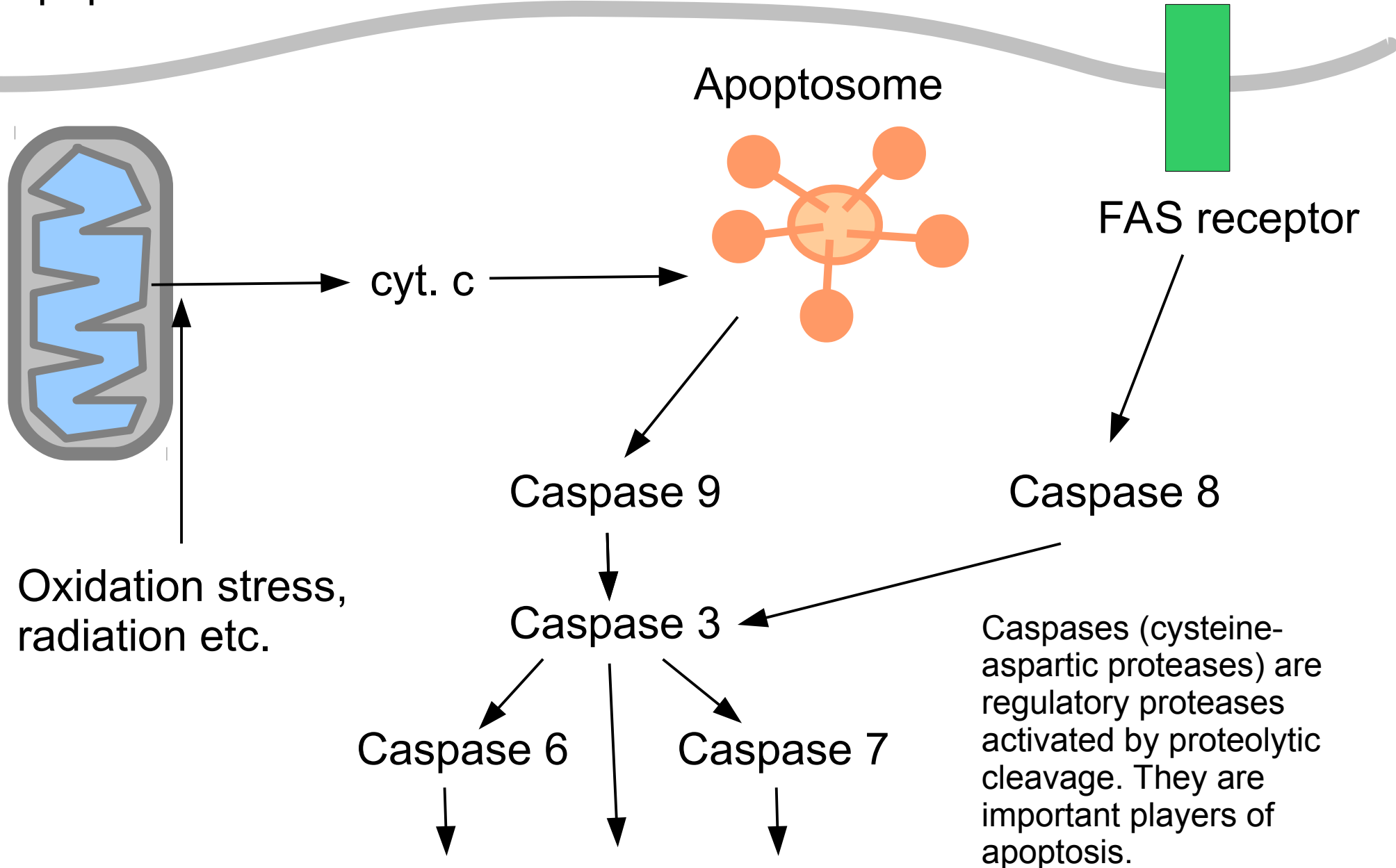
others

Activation by limited proteolysis

trypsinogen → trypsin

Some enzymes are activated by partial proteolysis. Trypsin is synthesized in pancreas as an inactive precursor called trypsinogen (blue). It is then activated by removal of N-terminal sequence (15 AA residues, gray) to obtain active trypsin (red). Other pathways regulated by partial proteolysis include apoptosis (with Caspases) and blood coagulation pathway.
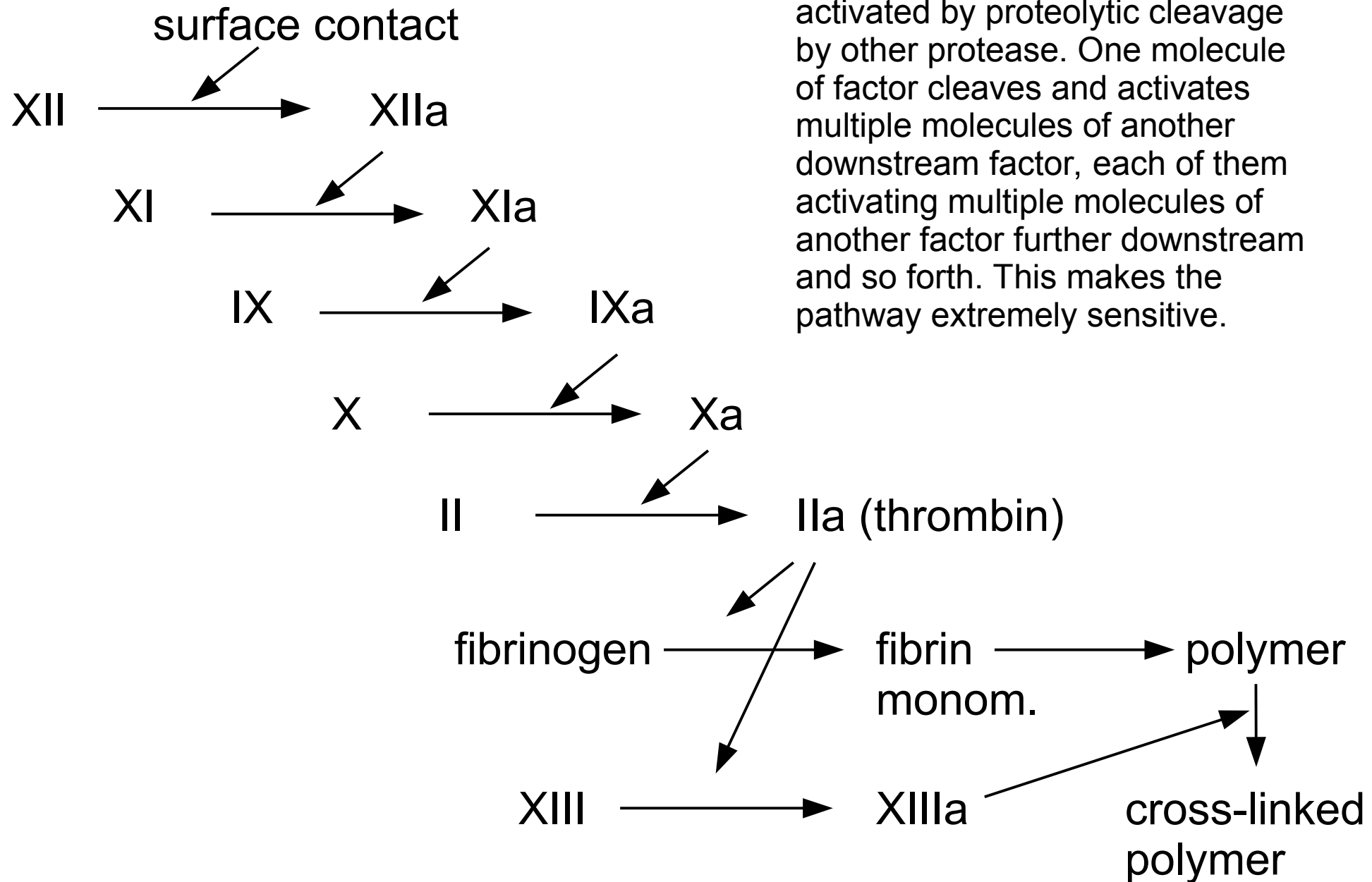
Activation by limited proteolysis

apoptosis

Apoptosome

FAS receptor

cyt. c

Oxidation stress, radiation etc.

Caspase 9

Caspase 8

Caspase 3

Caspase 6

Caspase 7

Caspases (cysteine-aspartic proteases) are regulatory proteases activated by proteolytic cleavage. They are important players of apoptosis.

Activation by limited proteolysis

blood coagulation

Blood coagulation pathway is an example of an activation cascade. Each member is a protease activated by proteolytic cleavage by other protease. One molecule of factor cleaves and activates multiple molecules of another downstream factor, each of them activating multiple molecules of another factor further downstream and so forth. This makes the pathway extremely sensitive.

surface contact

XII ⟶ XIIa

XI ⟶ XIa

IX ⟶ IXa

X ⟶ Xa

II ⟶ IIa (thrombin)

fibrinogen ⟶ fibrin monom. ⟶ polymer

XIII ⟶ XIIIa

cross-linked polymer

Allosteric effect

homo/heterotropic
homotropic – ligand influences protein's affinity towards itself,
substrate influences enzyme's activity towards itself
positive/negative
positive – strengthening affinity, activation
negative – weakening affinity, inhibition

An important role in regulation of enzymatic activities is played by allosteric effect. The word allosteric means "different place". It means that something happens at one side of the protein, and that affect the other side of the protein. For example, binding of a ligand into the binding site A changes the shape of a distant active site B of the enzyme, thus it changes its activity. It can be homotropic (the same molecule is ligand of A and ligand or substrate of B) or heterotropic (different molecules bind to A and B). It can be also positive (binding to A increases affinity or activity in B) or negative.

Allosteric effect is usually accompanied by a change in the 3D structure of the protein. It was not possible to demonstrate the allosteric effect directly in the times when determination of protein structures was not possible or its possibilities were limited. It was demonstrated indirectly. In this sense, homotropic allosteric effect and hemoglobin play the key role in the demonstration of the allostery phenomenon.

Allosteric effect

Hemoglobin

Hemoglobin ("honorary enzyme") binds oxygen with low affinity at low concentrations (or low partial pressure) and with high affinity at high concentrations. Therefore it can be almost fully loaded in lungs and it releases almost all oxygen in tissues. This makes oxygen transport highly efficient. Different affinity is achieved by following mechanism. Hemoglobin is homotetramer. Binding of oxygen to the first subunit supports binding of the second oxygen to the second subunit and so forth. The saturation curve is therefore not hyperbolic but rather sigmoid:
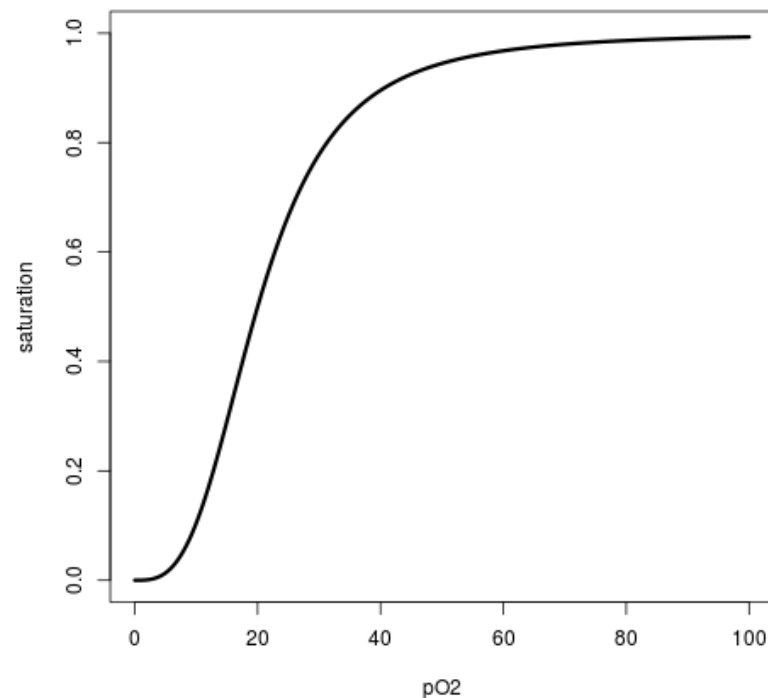
Allosteric effect

Hemoglobin

Several models were proposed to explain allosteric effect in hemoglobin. Hill's model assumes that hemoglobin can bind either no or certain number (κ) of molecules. The problem of Hill's model is that elementary reactions with more than two reactants are kinetically unfavorable, it cannot explain non-integer values of κ and it cannot explain negative allosteric effect.

Aldair's model simply uses different dissociation constants for binding of the first, second, third and fourth oxygen. This model was further developed by Koshland in order to provide a structural explanation. The disadvantage of Aldair's model is the high number of parameters.

The model developed by Monod, Wyman and Changeux assumes that hemoglobin exists in two forms – relaxed (R) and tense (T). The relaxed form has higher and tense form lower affinity towards oxygen. There is an equilibrium between the relaxed and tense form and it is shifted towards the tense form in absence of oxygen. Oxygen binds strongly to the relaxed form and thus shifts the equilibrium towards the relaxed form. The terms relaxed and tense are also used in other allosteric proteins.
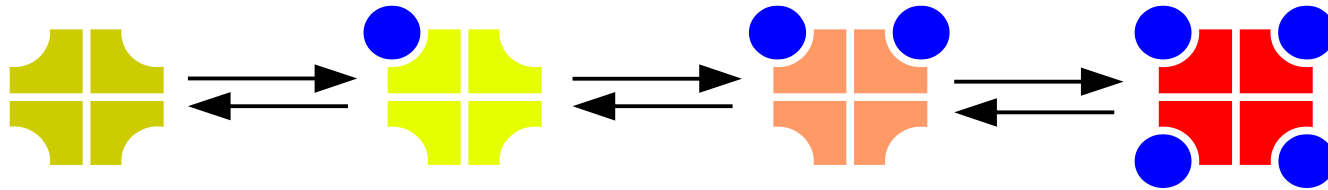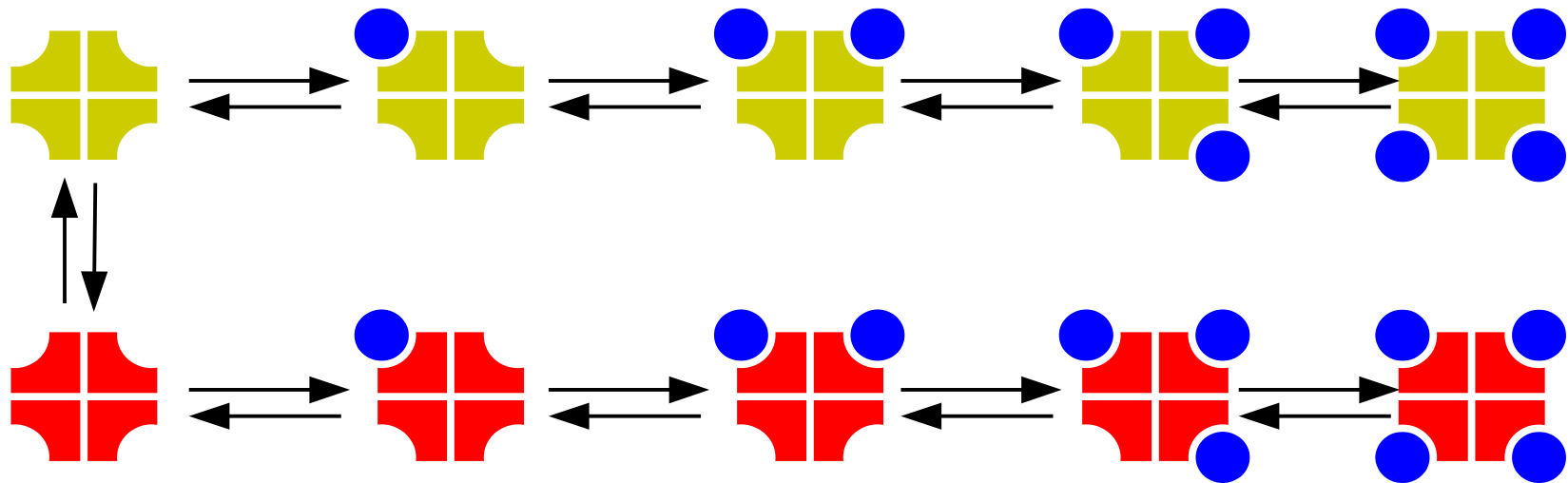
Allosteric effect

Hill's model
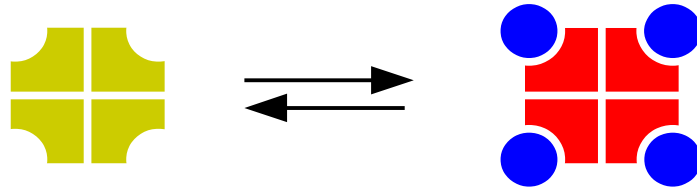
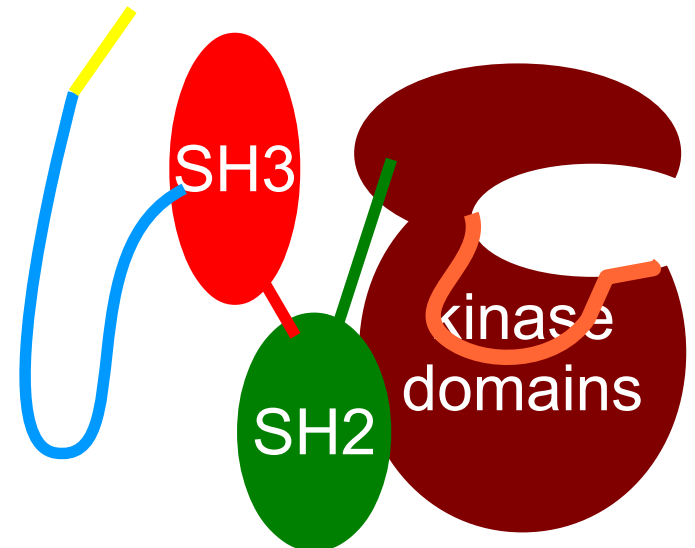$$S = \frac{[A]^{\kappa}}{[A]^{\kappa} + K_S}$$
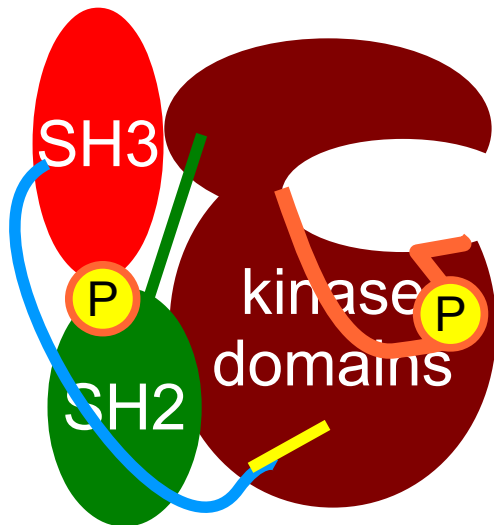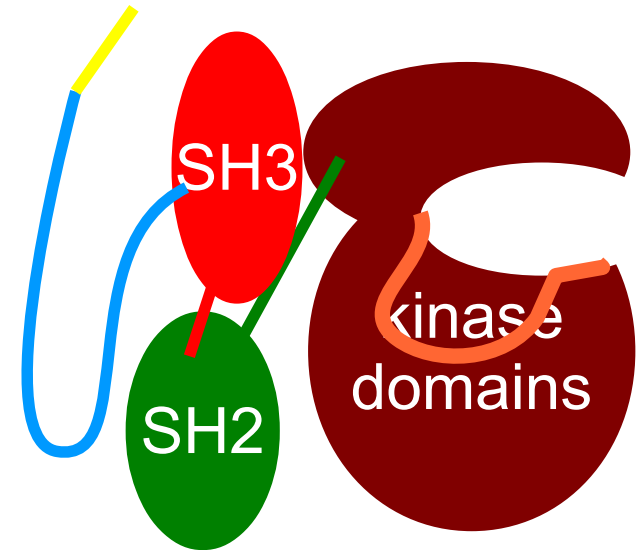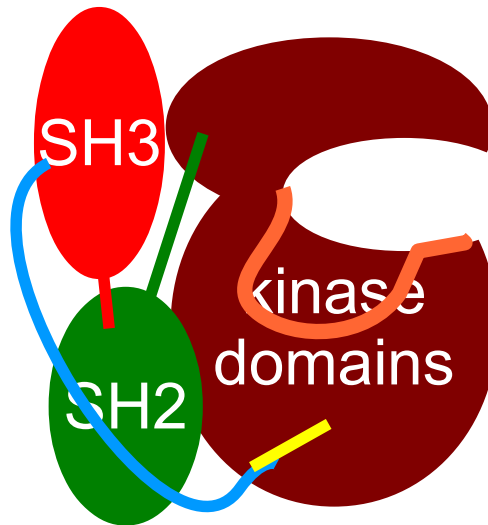
Aldair's model
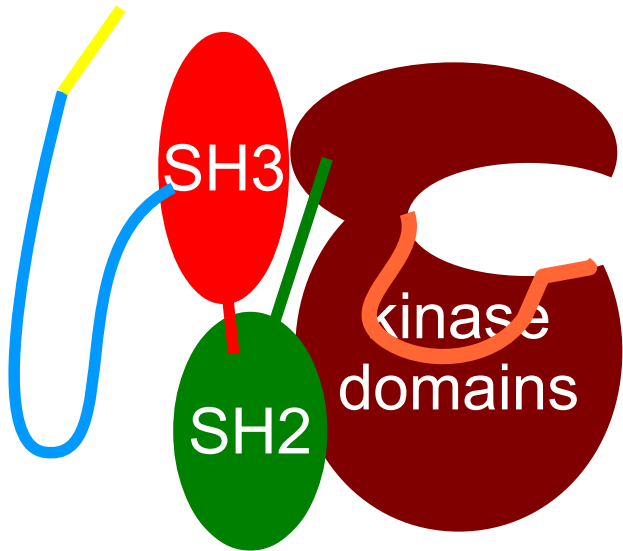

Monod-Wyman-Changeux model

Koshland's model

Abelson protein kinase

Abelson protein kinase

Chromosome
22   9   Philadelphia

BCR

SH3

SH2

kinase
domains

BCR

BCR

SH3

P

SH2

kinase
domains

P