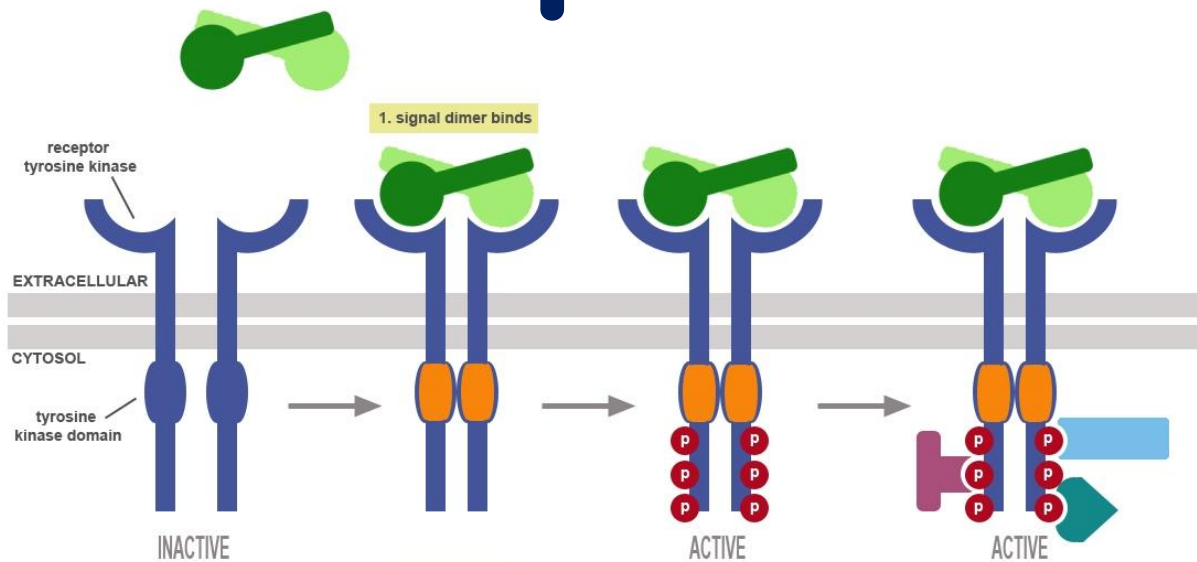


Enzyme-linked receptors



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BT 16-02

Content

1. Introduction
2. Classification
3. Structure
4. Mechanism of action
 - 4.1 Activation and regulation



Introduction

Enzyme-linked receptors (catalytic receptors) are a second major type of cell-surface receptor. They were recognized initially through their role in responses to extracellular signal proteins that promote the growth, proliferation, differentiation, or survival of cells in animal tissues.

The responses to them are typically slow (on the order of hours) and usually require many intracellular signaling steps that eventually lead to changes in gene expression. in gene expression.

Classification

1. *Receptor tyrosine kinases*

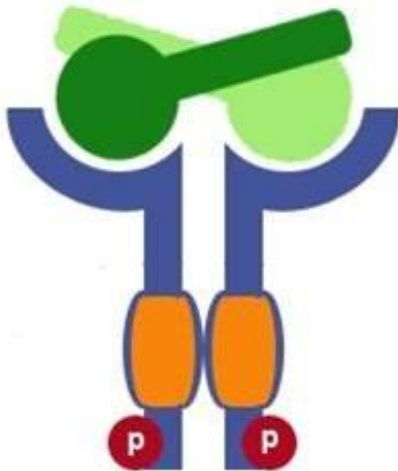
2. *Tyrosine-kinase-associated receptors*

3. *Receptorlike tyrosine phosphatases*

4. *Receptor serine/threonine kinases*

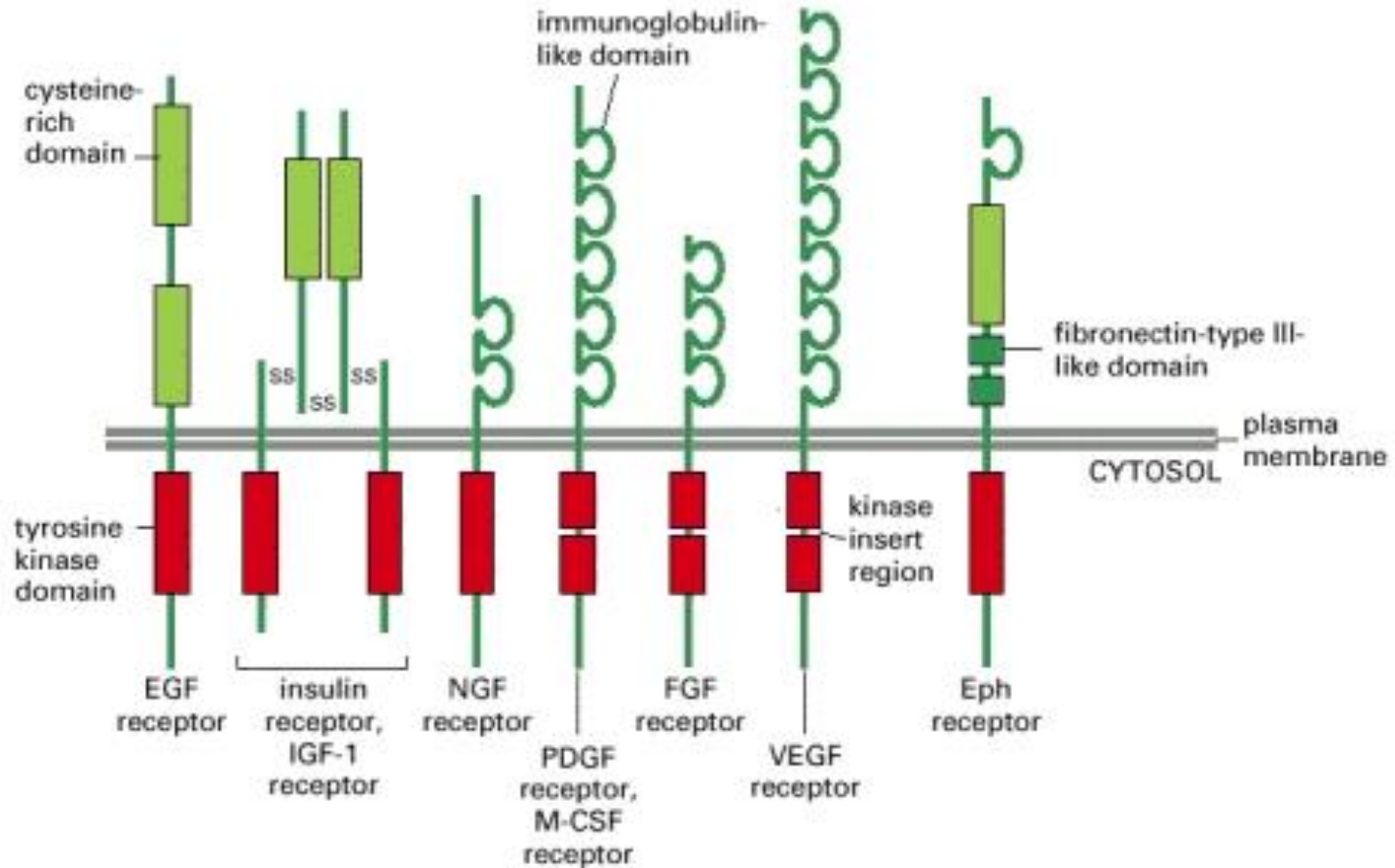
5. *Receptor guanylyl cyclases*

6. *Histidine-kinase-associated receptors*



1. Receptor tyrosine kinases

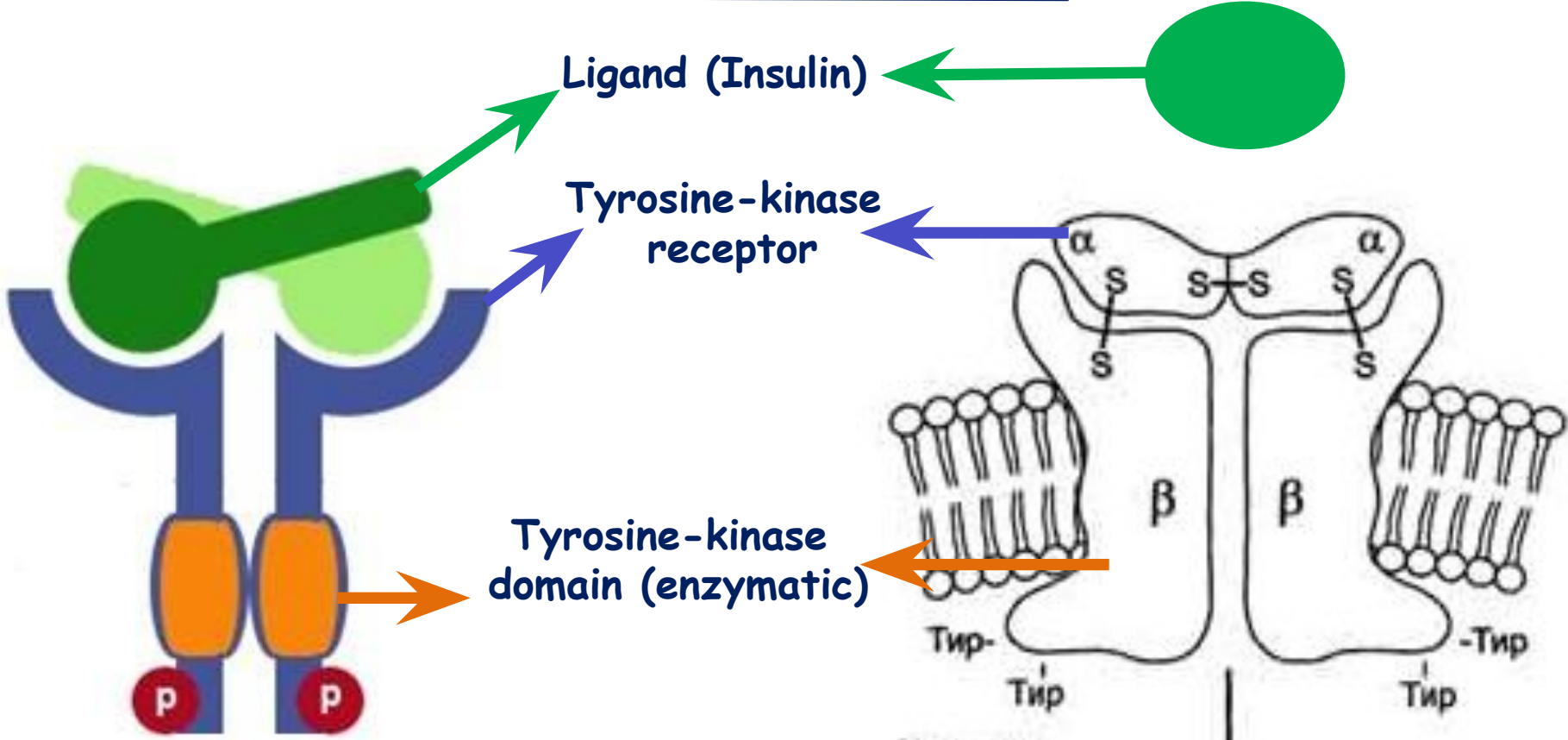
Phosphorylate specific tyrosines on a small set of intracellular signaling proteins.



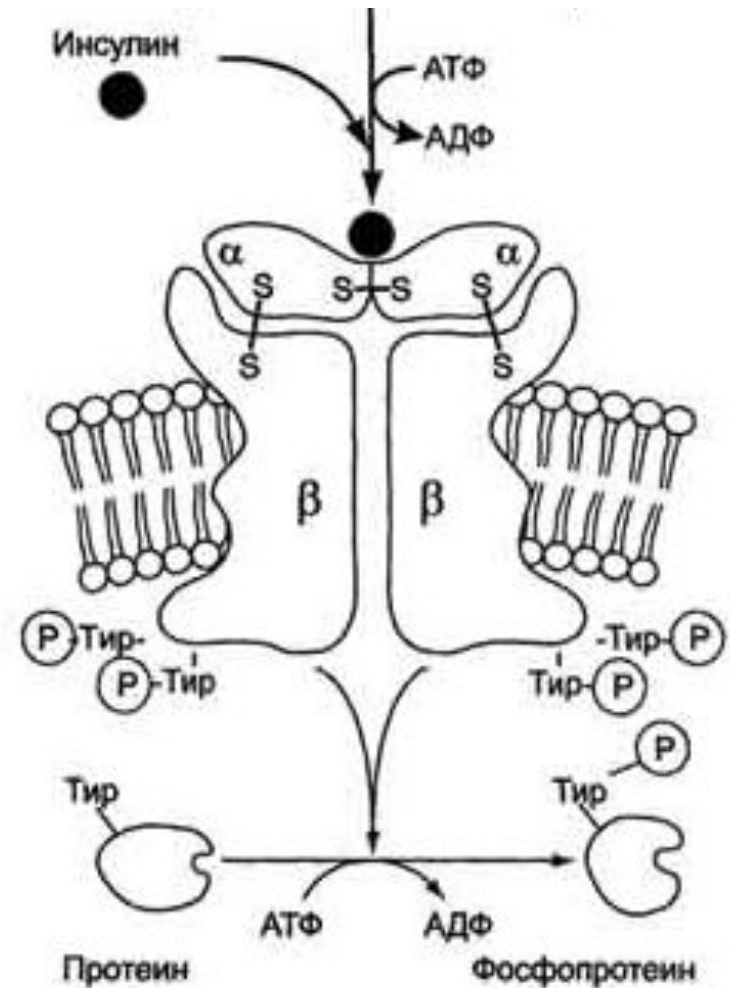
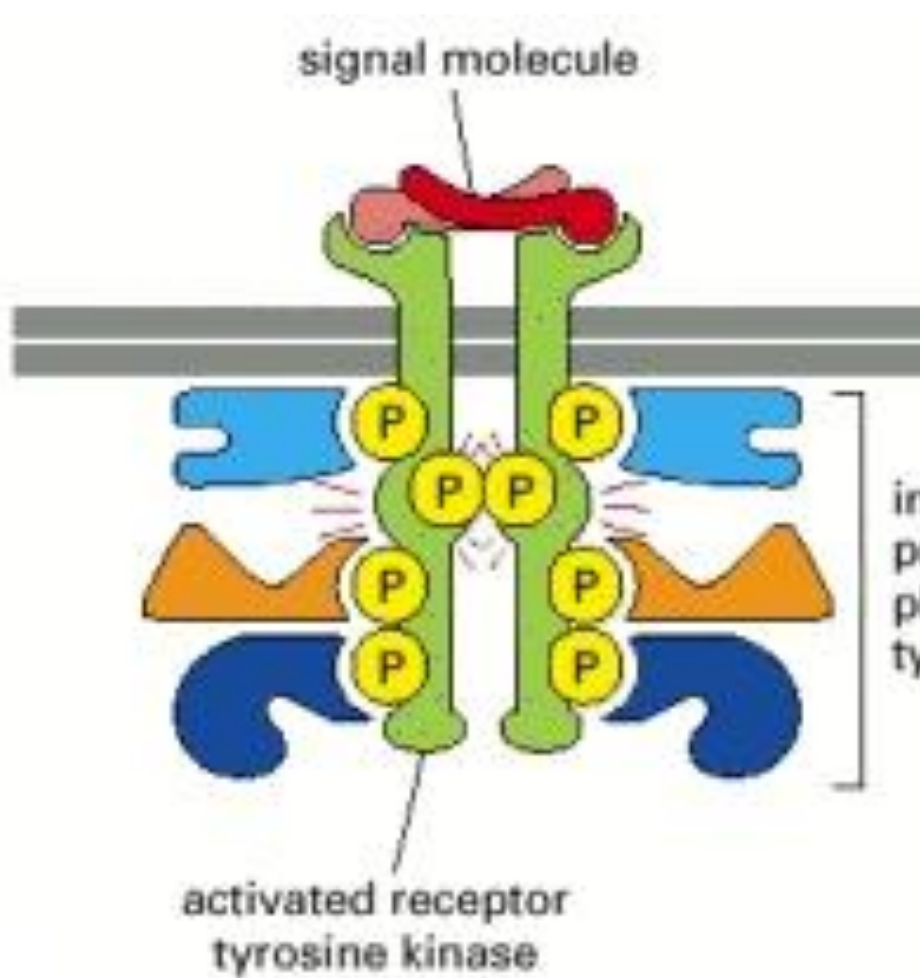
Seven subfamilies of receptor tyrosine kinases: Note that the tyrosine kinase domain is interrupted by a "kinase insert region" in some of the subfamilies. The functional roles of most of the cysteine-rich, immunoglobulin-like, and fibronectin-type III-like domains are not known.

SIGNALING LIGAND	RECEPTORS	SOME RESPONSES
Epidermal growth factor (EGF)	EGF receptor	stimulates proliferation of various cell types
Insulin	insulin receptor	stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factors (IGF-1 and IGF-2)	IGF receptor-1	stimulate cell growth and survival
Nerve growth factor (NGF)	Trk A	stimulates survival and growth of some neurons
Platelet-derived growth factors (PDGF AA, BB, AB)	PDGF receptors (α and β)	stimulate survival, growth, and proliferation of various cell types
Macrophage-colony-stimulating (M-CSF)	M-CSF receptor factor	stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factors (FGF-1 to FGF-24)	FGF receptors (FGF-R1-FGF-R4, plus multiple isoforms of each)	stimulate proliferation of various cell types; inhibit differentiation of some precursor cells; inductive signals in development
Vascular endothelial growth factor (VEGF)	VEGF receptor	stimulates angiogenesis
Ephrins (A and B types)	Eph receptors (A and B types)	stimulate angiogenesis; guide cell and axon migration

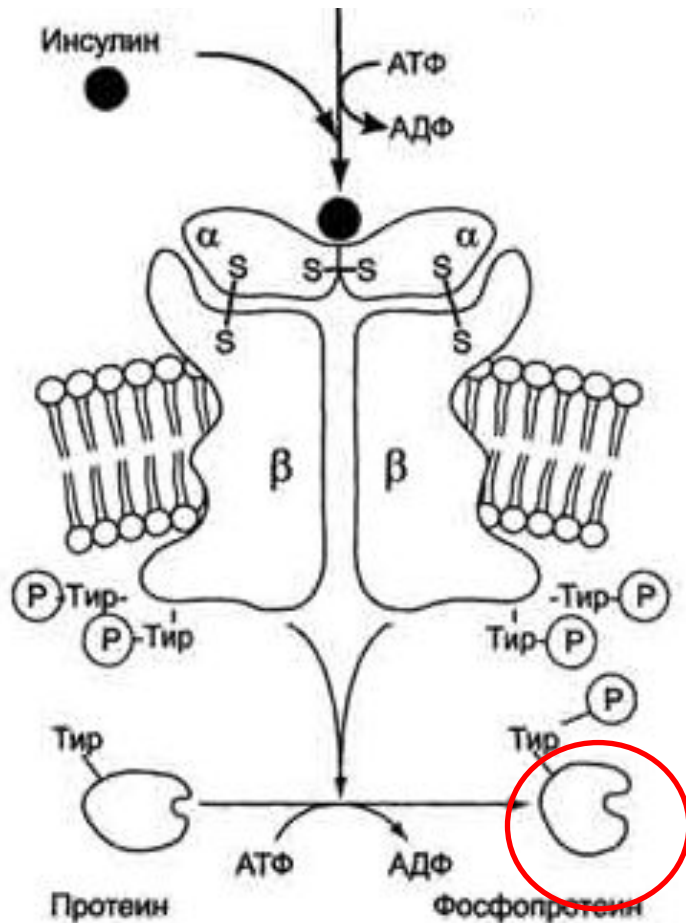
Structure



Mechanism of action: activation



Mechanism of action: activation

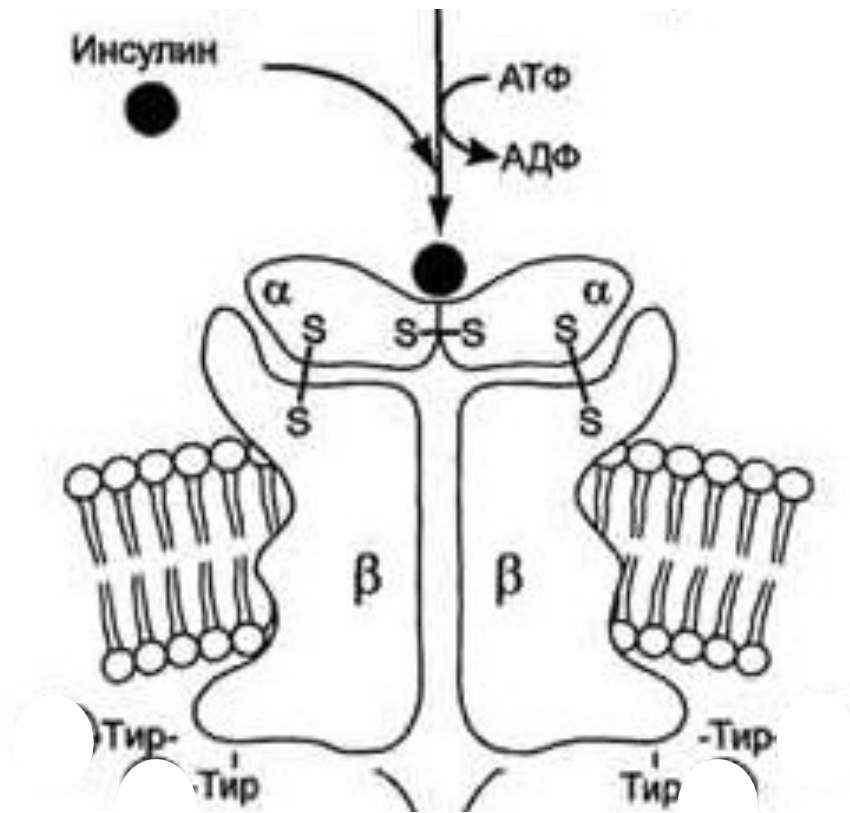


IRS-1 stimulates the cascade of intercellular protein activation like:

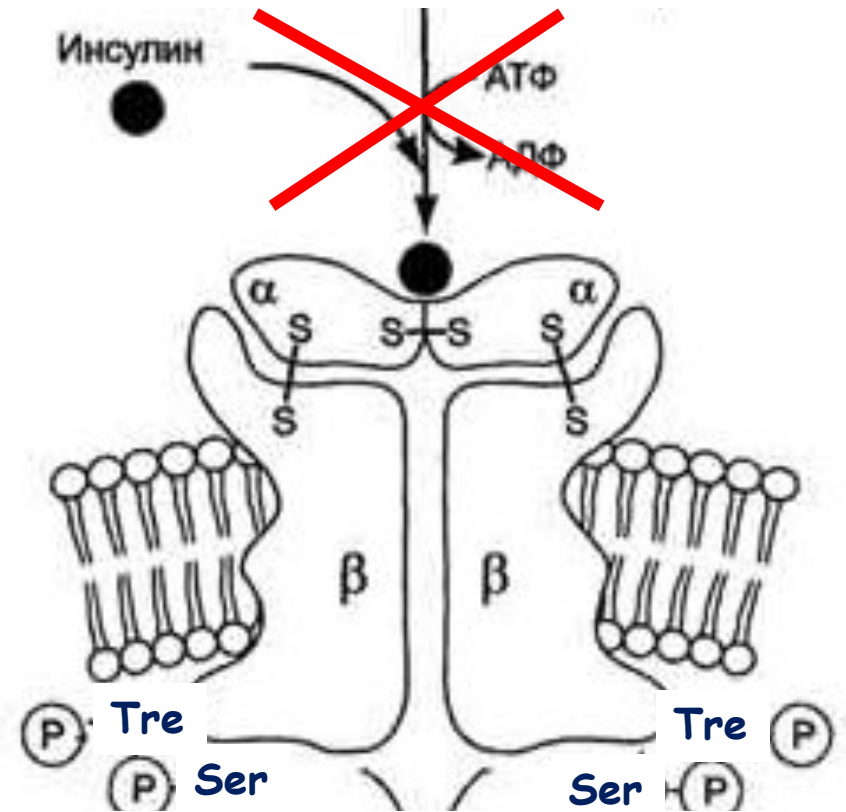
1. Translocation of **GLUT4** transporter to plasma membrane (pass of glucose molecules into the cell);
2. Expression of insulin regulating genes ();
3. Glycogen synthesis;
4. Glycolysis;
5. Synthesis of fatty acids.

IRS-1 (insulin receptor substrate)

Mechanism of action: regulation



IRS-1 activates tyrosine phosphoproteinphosphotase



Phosphorylation of serine/threonine residues decrease the affinity of insulin with receptor

2. Tyrosine-kinase-associated receptors

Associate with intracellular proteins that have tyrosine kinase activity and also called "Cytokine receptors".

Example-**The Jak-STAT signaling pathway**, initially discovered in the studies on the effects of interferon.

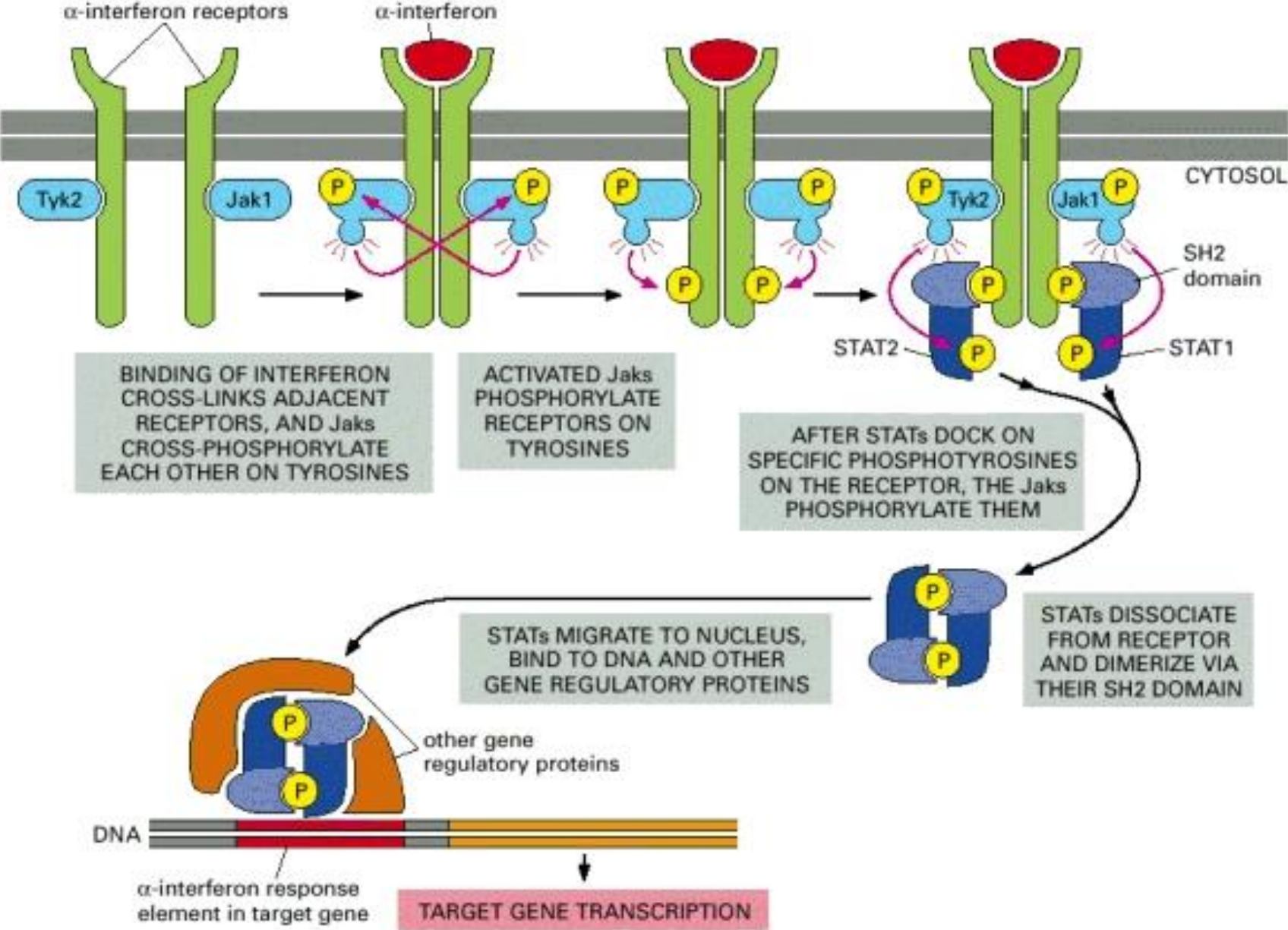
***Jak** - cytoplasmic tyrosine kinases called Janus kinases (Jaks) (after the two-faced Roman god)

***STAT**- signal transducers and activators of transcription.

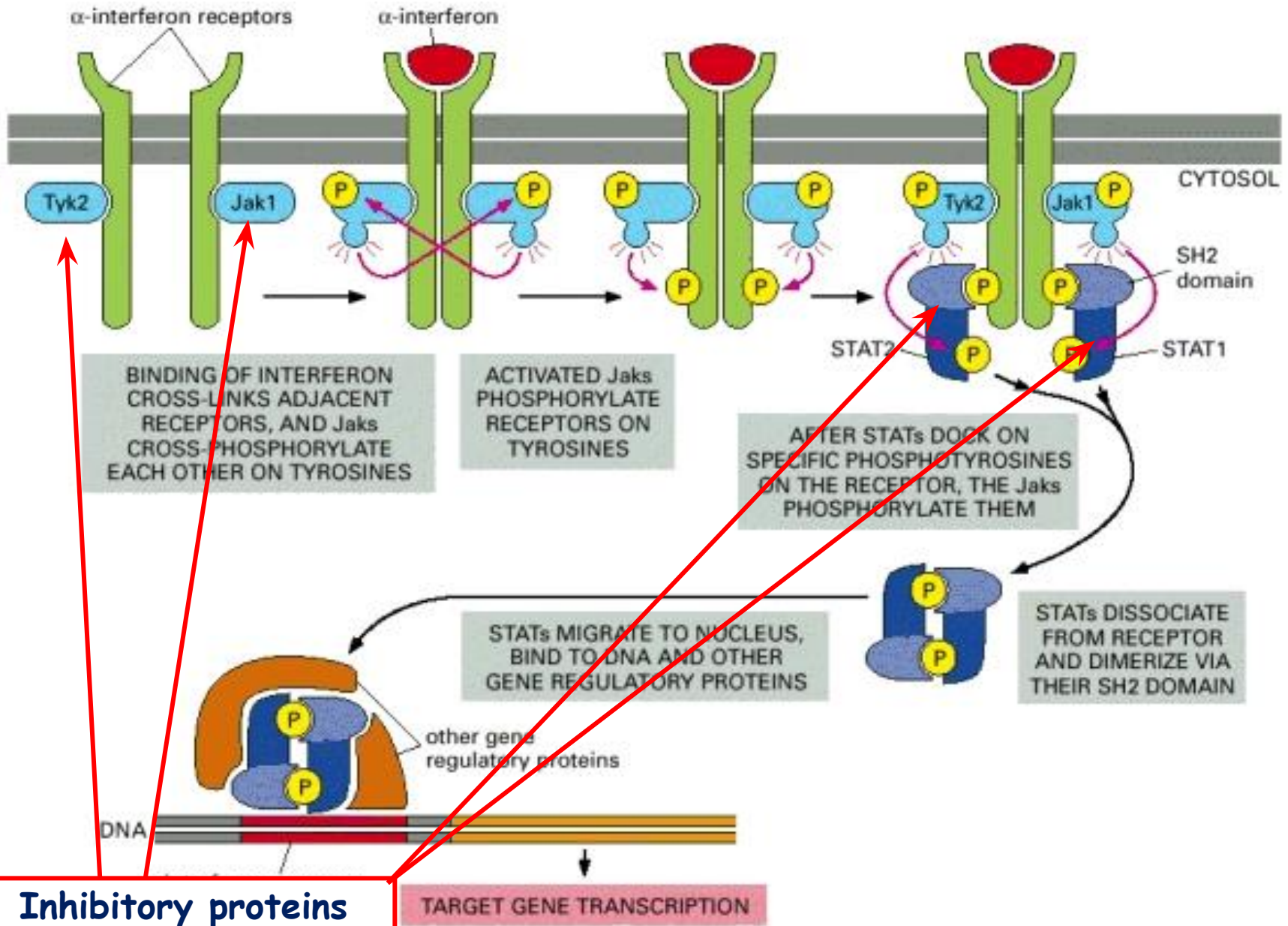
2. Tyrosine-kinase-associated receptors

SIGNALING LIGAND	RECEPTOR-ASSOCIATED JAKS	STATS ACTIVATED	SOME RESPONSES
γ -interferon	Jak1 and Jak2	STAT1	activates macrophages; increases MHC protein expression
α -interferon	Tyk2 and Jak2	STAT1 STAT2	increases cell resistance to viral infection
Erythropoietin	Jak2	STAT5	stimulates production of erythrocytes
Prolactin	Jak1 and Jak2	STAT5	stimulates milk production
Growth hormone	Jak2	STAT1 STAT5	stimulates growth by inducing IGF-1 production
GM-CSF	Jak2	STAT5	stimulates production of granulocytes and macrophages
IL-3	Jak2	STAT5	stimulates early blood cell production

Mechanism of action: activation



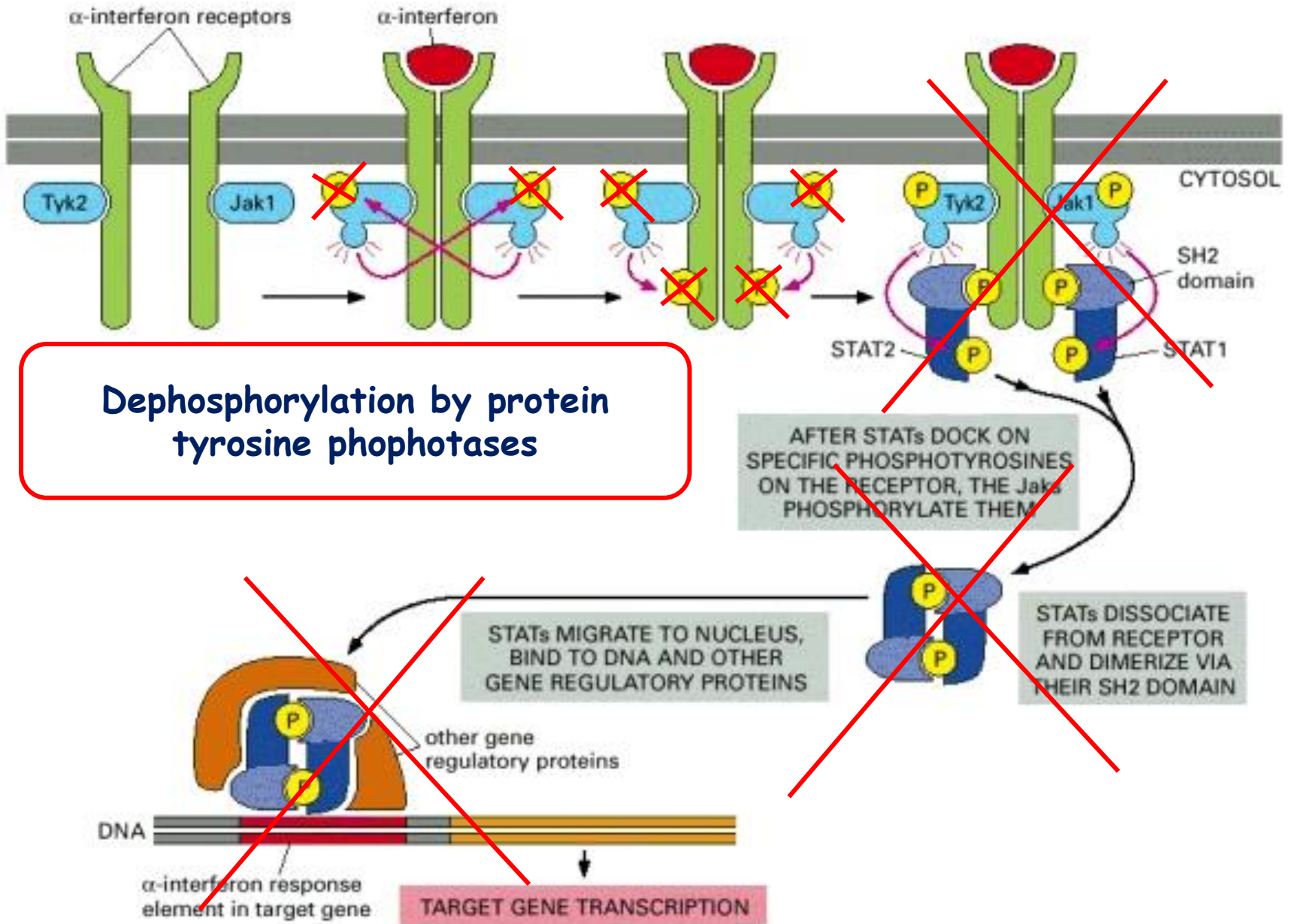
Mechanism of action: regulation

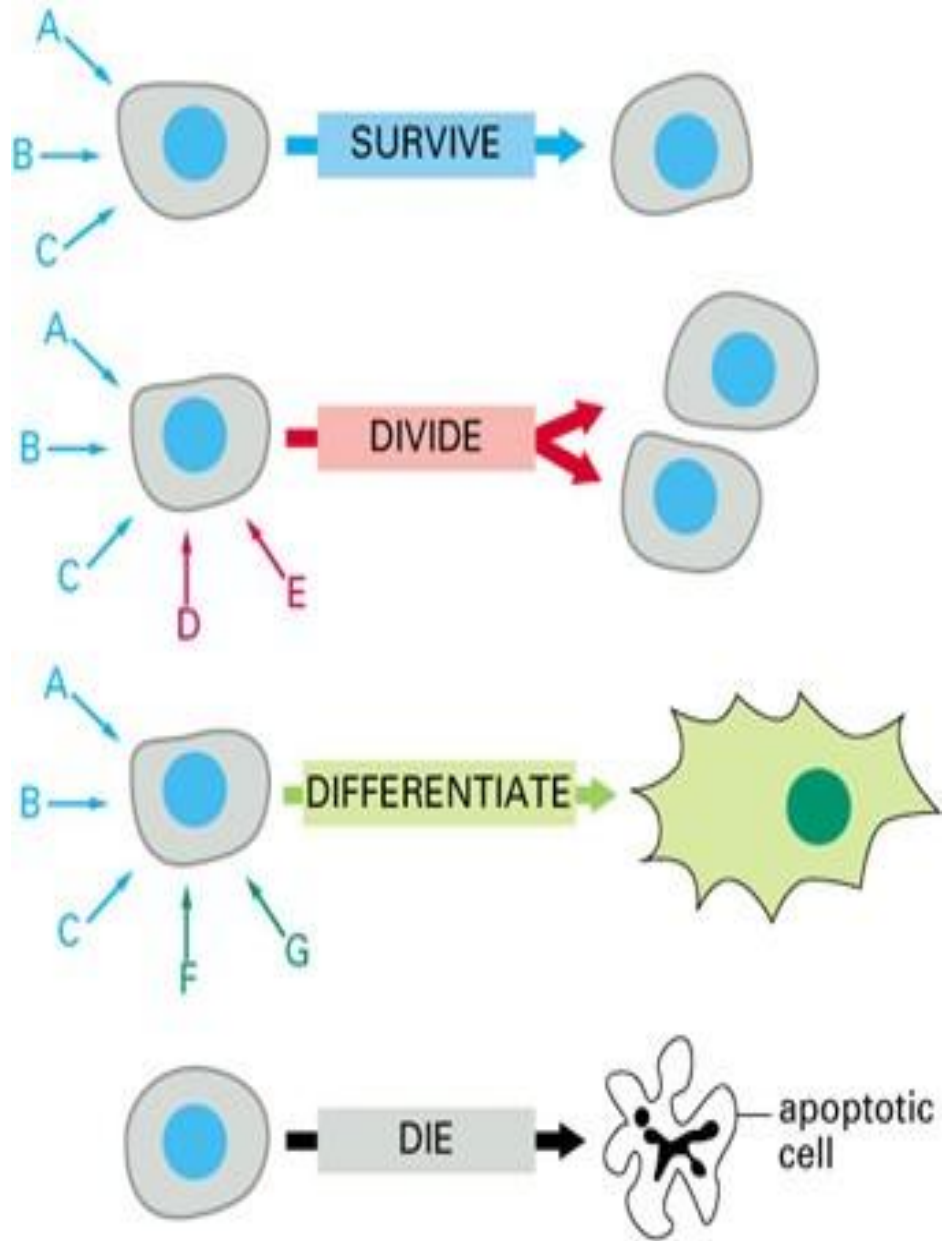


Inhibitory proteins

TARGET GENE TRANSCRIPTION

Mechanism of action: regulation





- They respond to extracellular signaling proteins called growth factors that promote growth, proliferation, differentiation or cell survival

Figure 15-8. Molecular Biology of the Cell, 4th Edition.

- also known as a **catalytic receptor**
 - **transmembrane receptor**, where the binding of an extracellular ligand causes enzymatic activity on the intracellular side
- • **integral membrane protein** possessing both enzymatic catalytic and receptor functions
 - Upon ligand binding a **conformational change** is transmitted which activates the enzyme, initiating signaling cascades

Physiology and diseases

- involved in growth, proliferation, differentiation, or survival
- Because of this, their ligands are collectively called growth factors. •
- The effects of enzyme-linked receptors typically are slow requiring the expression of new genes
- Mutations in receptor tyrosine kinases are responsible for a wide array of diseases, including cancers, neurodegeneration, achondroplasia and atherosclerosis.

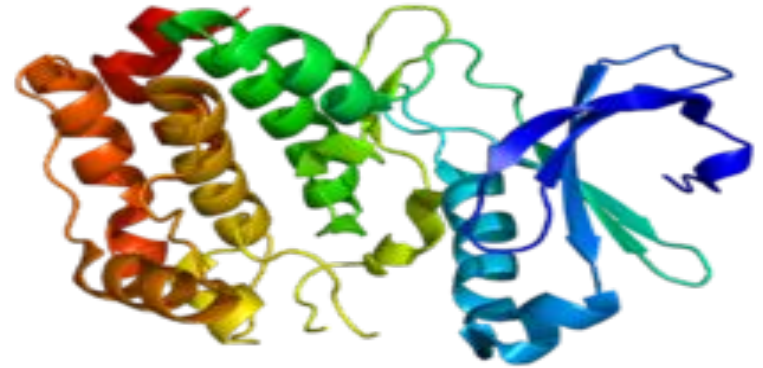
Six classes of enzyme-linked receptors have thus far been identified:

- 1. *Receptor tyrosine kinases* phosphorylate specific tyrosines on a small set of intracellular signaling proteins.
- 2. *Tyrosine-kinase-associated receptors* associate with intracellular proteins that have tyrosine kinase activity.
- 3. *Receptorlike tyrosine phosphatases* remove phosphate groups from tyrosines of specific intracellular signaling proteins. (They are called “receptorlike” because the presumptive ligands have not yet been identified, and so their receptor function has not been directly demonstrated.)
- 4. *Receptor serine/threonine kinases* phosphorylate specific serines or threonines on associated latent gene regulatory proteins.
- 5. *Receptor guanylyl cyclases* directly catalyze the production of cyclic GMP in the cytosol.
- 6. *Histidine-kinase-associated receptors* activate a “two-component” signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

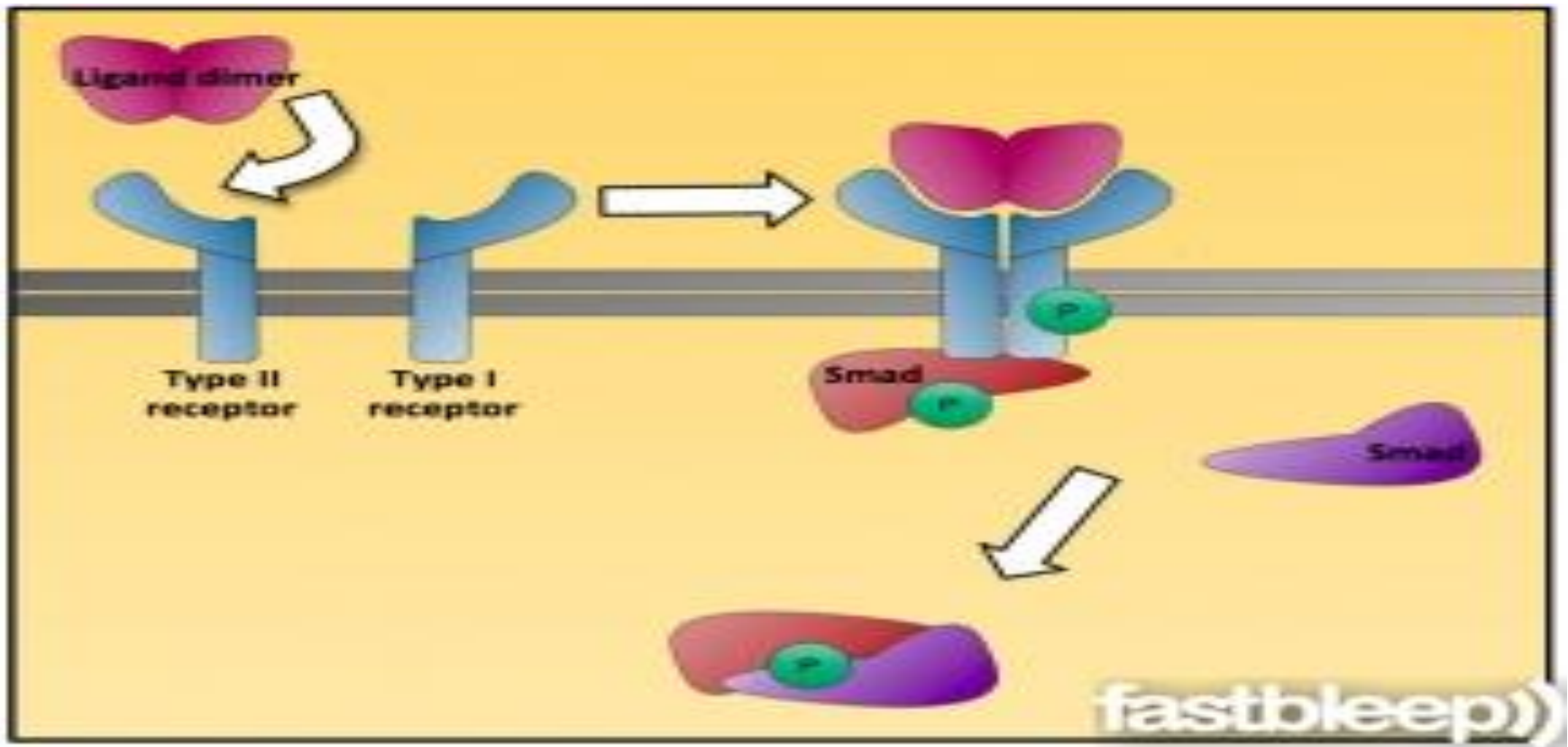
receptor protein serine/threonine kinase

- In enzymology, a **receptor protein serine/threonine kinase** (EC 2.7.11.30) is an enzyme that catalyzes the chemical reaction
- $\text{ATP} + [\text{receptor-protein}] \rightleftharpoons \text{ADP} + [\text{receptor-protein}] \text{ phosphate}$
- Thus, the two substrates of this enzyme are ATP and receptor protein, whereas its two products are ADP and receptor protein phosphate.
- This enzyme belongs to the family of transferases, to be specific those transferring phosphorus-containing groups protein-serine/threonine kinases.
- The systematic name of this enzyme class is **ATP:[receptor-protein] phosphotransferase**. Other names in common use include **activin receptor kinase**, **receptor type I serine/threonine protein kinase**, **receptor type II serine/threonine protein kinase**, **STK13**, **TGF-beta kinase**, and **receptor serine/threonine protein kinase**.
- This enzyme participates in 7 metabolic pathways: MAPK signaling pathway, cytokine-cytokine receptor interaction, TGF beta signaling pathway, adherens junction, colorectal cancer, pancreatic cancer, and chronic myeloid leukemia.

Receptor serine/threonine kinases



- phosphorylate specific Serine/ Threonine
 - There are two types of serine/threonine kinase receptors, both of which contain an intracellular kinase domain. They are each dimeric proteins, so an active receptor complex is made up of four receptors.
1. Type I receptors
 - Inactive unless in complex with type II receptors.
 - Do not interact with ligand dimers.
 - Contain conserved sequences of serine and threonine residues near to their kinase domains.
 2. Type II receptors
 - Constitutively active kinase domains (even in the absence of the bound ligand).
 - Able to phosphorylate and activate the type I receptor.



Type I receptors are kept inactive by a portion of its cytosolic domain that blocks its kinase activity.

TGFB/bone morphogenetic protein (BMP)/activin ligands bind as dimers to Type II receptors.

Type II receptors then bind to, and phosphorylate, Type I receptors. This removes the inhibition of Type I kinase activity.

Type I receptors then phosphorylate Smad transcription factors, allowing them to dimerise and enter the nucleus to repress or activate target gene expression.

- Serine/Threonine Kinase receptors play a role in the regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation, and embryonic development.

Selectivity

While serine/threonine kinases all phosphorylate serine or threonine residues in their substrates, they select specific residues to phosphorylate on the basis of residues that flank the phosphoacceptor site, which together comprise the *consensus sequence*. Since the consensus sequence residues of a target substrate only make contact with several key amino acids within the catalytic cleft of the kinase (usually through hydrophobic forces and ionic bonds), a kinase is usually not specific to a single substrate, but instead can phosphorylate a whole "substrate family" which share common recognition sequences. While the catalytic domain of these kinases is highly conserved, the sequence variation that is observed in the kinome (the subset of genes in the genome that encode kinases) provides for recognition of distinct substrates. Most kinases are inhibited by a pseudosubstrate that binds to the kinase like a real substrate but lacks the amino acid to be phosphorylated. When the pseudosubstrate is removed, the kinase can perform its normal function.

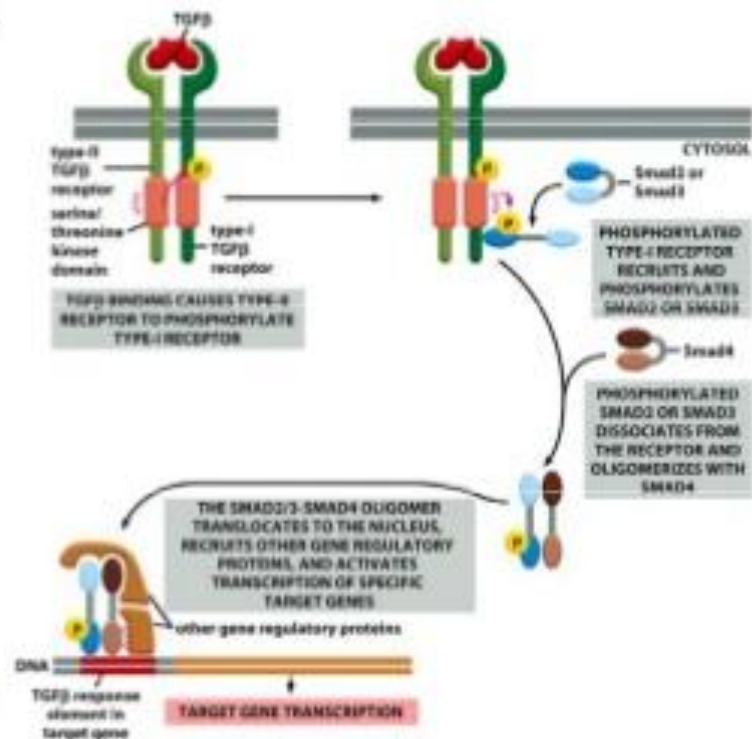
- Many serine/threonine protein kinases do not have their own individual EC numbers and use "2.7.11.1". These were formerly included in EC number "2.7.1.37", which was a general EC number for any enzyme that phosphorylates proteins while converting ATP to ADP (i.e., ATP:protein phosphotransferases.)
- Types include those acting directly as receptors (Receptor protein serine/threonine kinase) and Intracellular signaling peptides and proteins. Of the latter, types include:

EC number	Name	Description
EC2.7.11.1	CK2, also known by the misnomer casein kinase 2	was discovered in 1954 by Burnett and Kennedy.
EC2.7.11.1	Protein kinase A	consists of two domains, a small domain with several β sheet structures and a larger domain containing several α helices. The binding sites for substrate and ATP are located in the catalytic cleft between the domains (or lobes). When ATP and substrate bind, the two lobes rotate so that the terminal phosphate group of the ATP and the target amino acid of the substrate move into the correct positions for the catalytic reaction to take place.
EC2.7.11.1	Protein kinase C('PKC')	is actually a family of protein kinases consisting of ~10 isozymes. They are divided into three subfamilies: conventional (or classical), novel, and atypical based on their second messenger requirements.
EC2.7.11.1	Mos/Raf kinases	form part of the MAPKK Kinase family and are activated by growth factors. The enzyme functions to stimulate growth of cells. Raf inhibition has become the target for new anti-metastatic cancer drugs as they inhibit the MAPK cascade and reduce cell proliferation.

EC2.7.11.1	Mitogen-activated protein kinases (MAPKs)	respond to extracellular stimuli (mitogens) and regulate various cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis.
EC2.7.11.17	Ca ²⁺ /calmodulin-dependent protein kinases or CaM kinases	are primarily regulated by the Ca ²⁺ /calmodulin complex.
EC2.7.11.19	Phosphorylase kinase	was in fact, the first Ser/Thr protein kinase to be discovered (in 1959 by Krebs <i>et al.</i>).
EC2.7.1.37	Protein Kinase B, also known as AKT kinase	The v-akt gene was identified as the oncogene of retrovirus AKT8. The gene codes for a protein kinase. Human homologs of the AKT8 oncogenic protein were identified in 1987. By 1995 it had been found that Akt kinases function as mitogen-activated kinases downstream from cell surface receptors that activate phosphoinositide 3-kinase. Three human akt genes exist. All three Akt kinases regulate cell proliferation and Akt2 is particularly important for insulin actions in cells. A major

Receptor Serine/Threonine Kinases

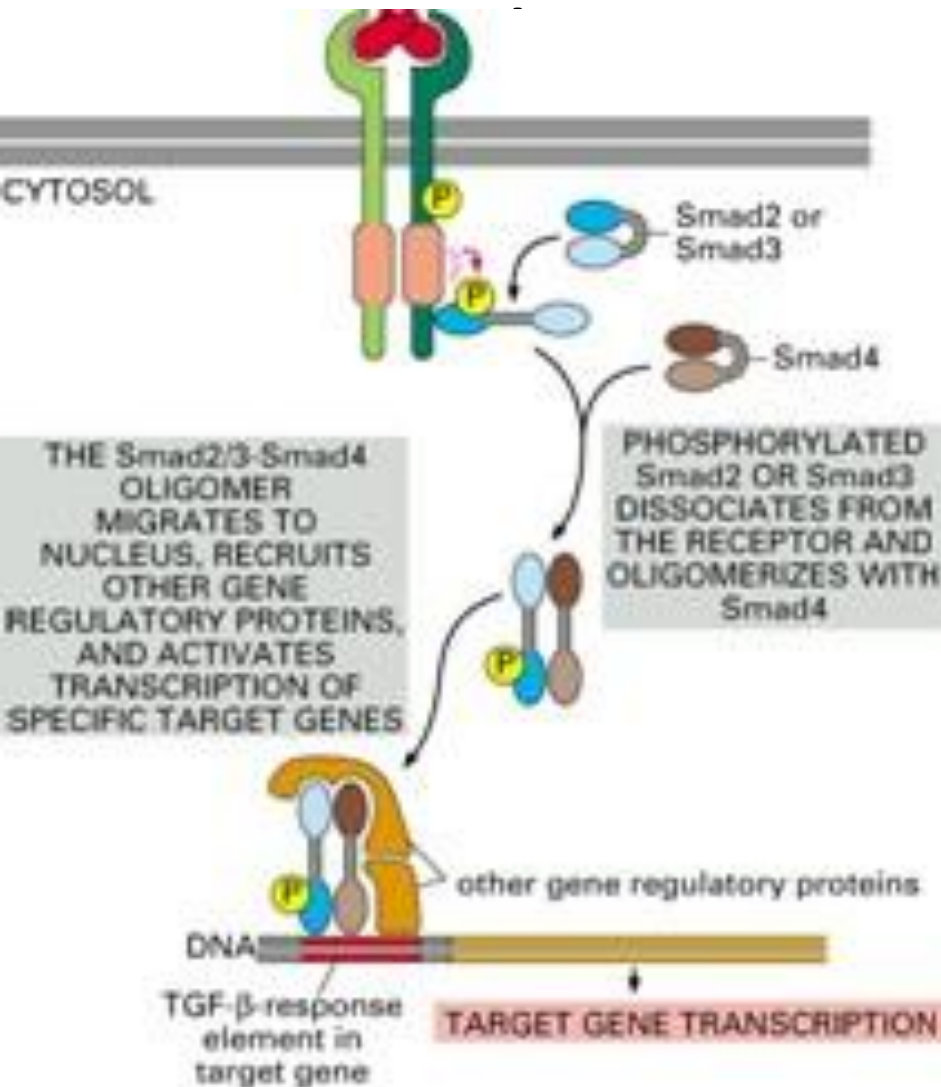
- Binds to about 40 human proteins, e.g. TGF- β and bone morphogenetic protein.
- TGF- β acts through receptor serine/threonine kinase and Smads.
- TGF- β
 - Embryonic development signaling.
 - Inhibits proliferation of most adult cells.
 - Stimulate extracellular matrix production
 - Regulate cell death in development.
 - Regulate tissue repair and immune response in adults.



Smad: Sma in *C. elegans* & Mad in *Drosophila*

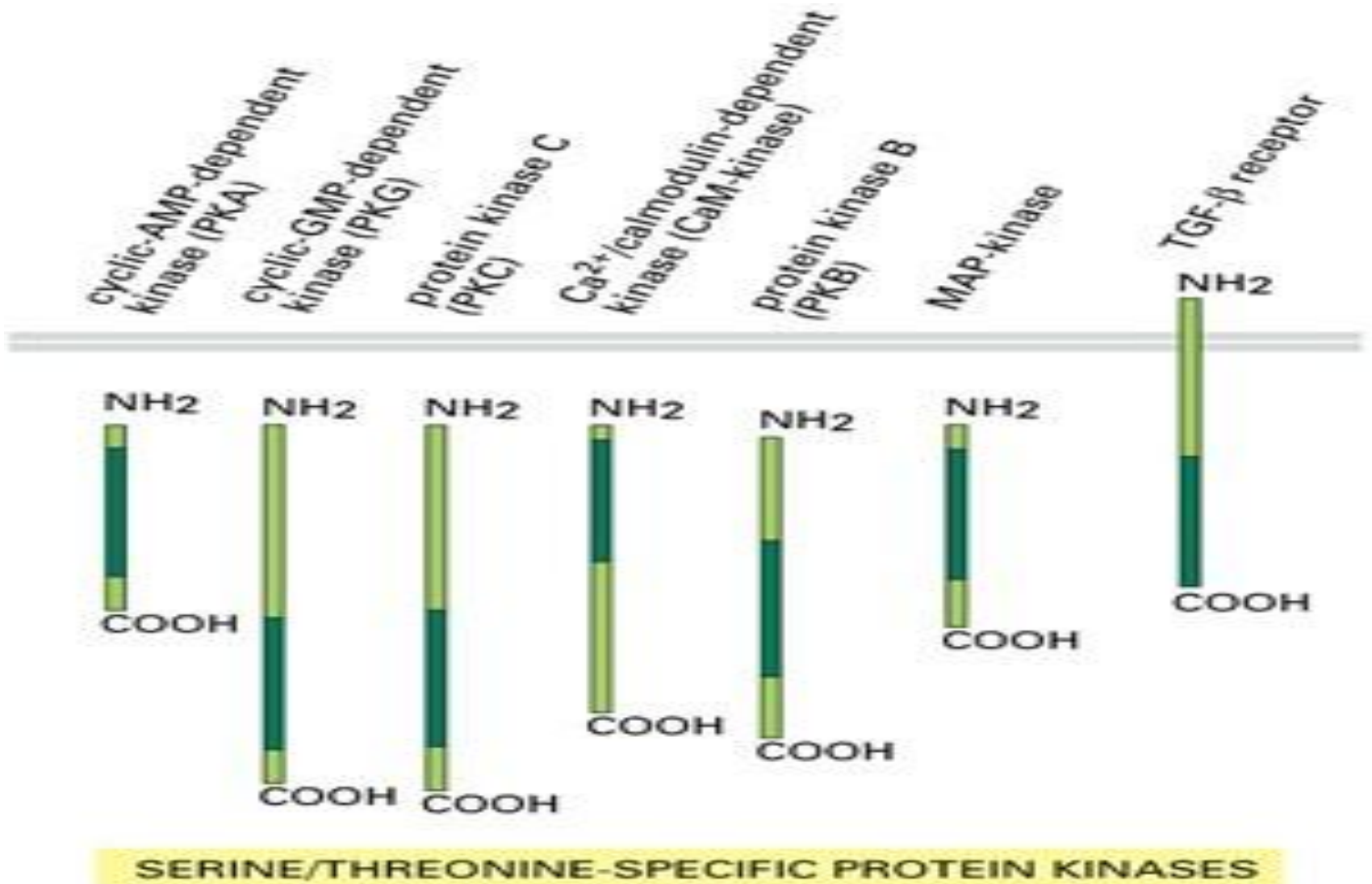
Receptor Serine/Threonine Protein Kinase Smad-dependent signaling

activated by TGF- β



- Transforming growth factor (TGF- β) consists of a large number of structurally related, secreted, dimeric proteins. They mediate a wide range of biological functions in animals: proliferation, differentiation, ECM production, cell death, tissue repair and immune regulation.
- They act through receptor serine/threonine kinases type I and type II
- Smad family members are directly phosphorylated by the type I receptor, and moves to the nucleus to direct gene transcription

The size and location of protein kinases

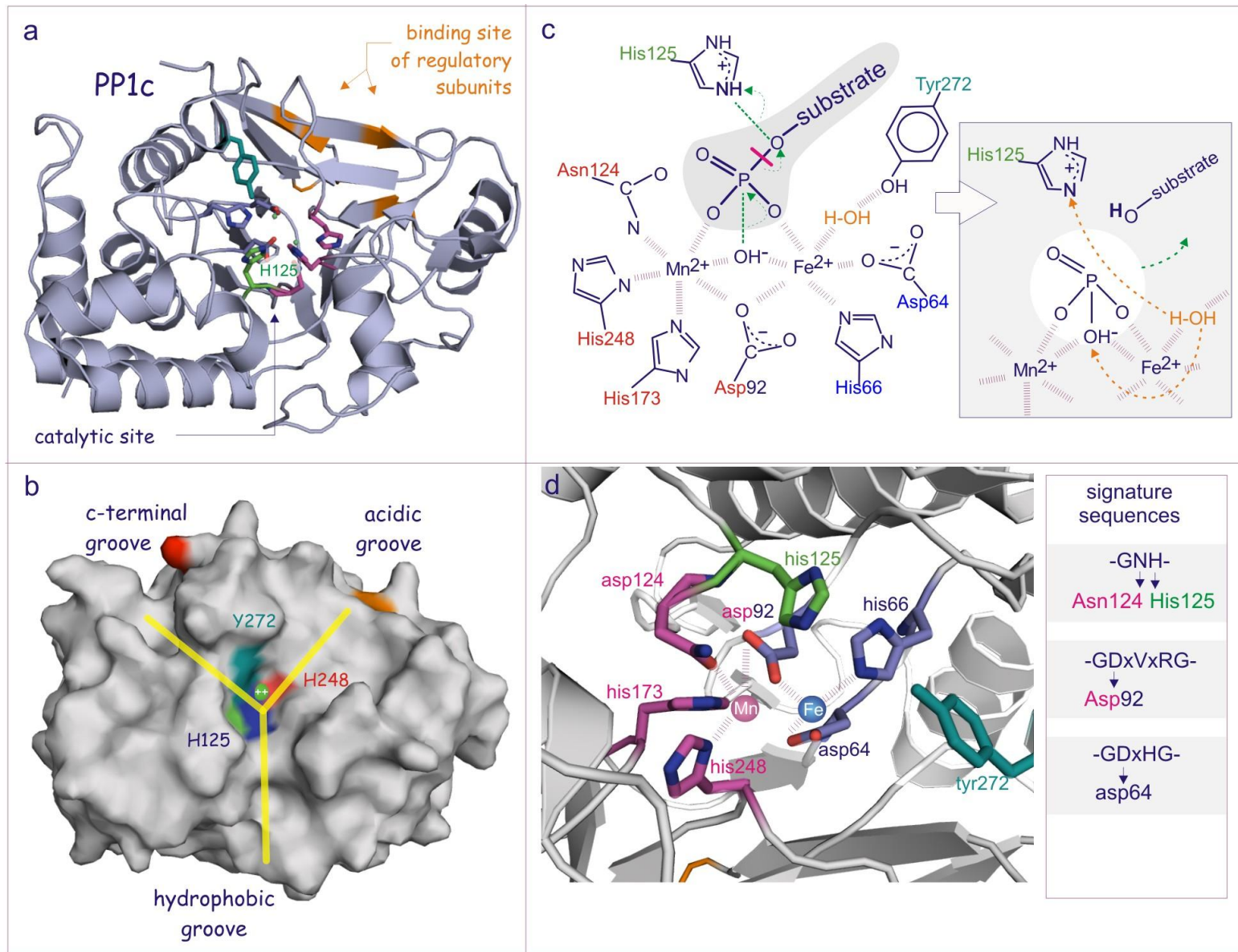


Receptor like tyrosine phosphatases

- *Receptor like tyrosine phosphatases* remove phosphate groups from tyrosines of specific intracellular signaling proteins. (They are called “receptorlike” because the presumptive ligands have not yet been identified, and so their receptor function has not been directly demonstrated.)
- Together with tyrosine kinases, PTPs regulate the phosphorylation state of many important signalling molecules, such as the MAP kinase family. PTPs are increasingly viewed as integral components of signal transduction cascades, despite less study and understanding compared to tyrosine kinases
- PTPs have been implicated in regulation of many cellular processes, including, but not limited to:
 - Cell growth
 - Cellular differentiation
 - Mitotic cycles
 - Oncogenic transformation
 - Receptor endocytosis

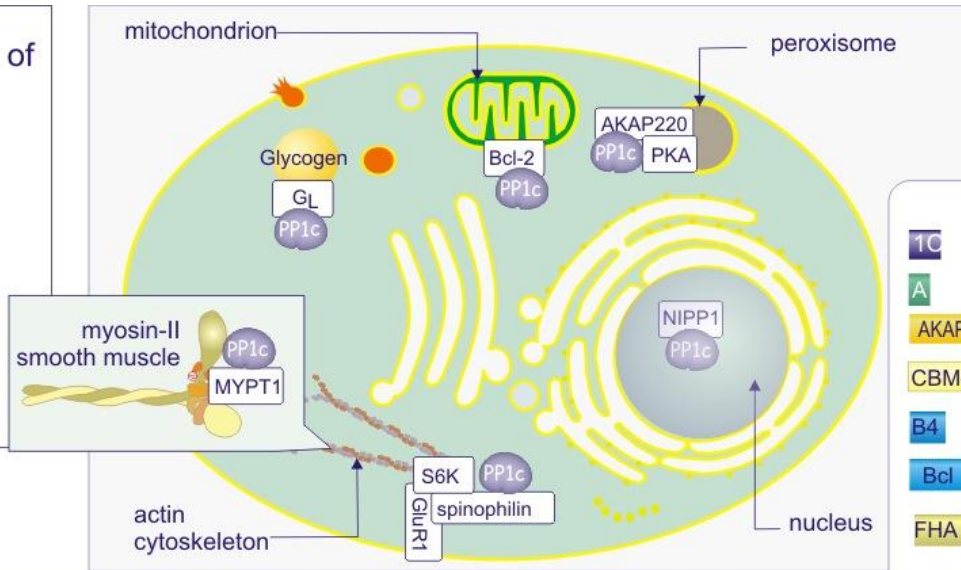
- Based on their cellular localization, PTPases are also classified as:
- Receptor-like, which are transmembrane receptors that contain PTPase domains. In terms of structure, all known receptor PTPases are made up of a variable-length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains, or carbonic anhydrase-like domains in their extracellular region. In general, the cytoplasmic region contains two copies of the PTPase domain. The first seems to have enzymatic activity, whereas the second is inactive.
- Non-receptor (intracellular) PTPases

Mechanism of action and highly conserved (signature) sequences of serine / threonine protein phosphatases. The catalytic subunits have broad specificity



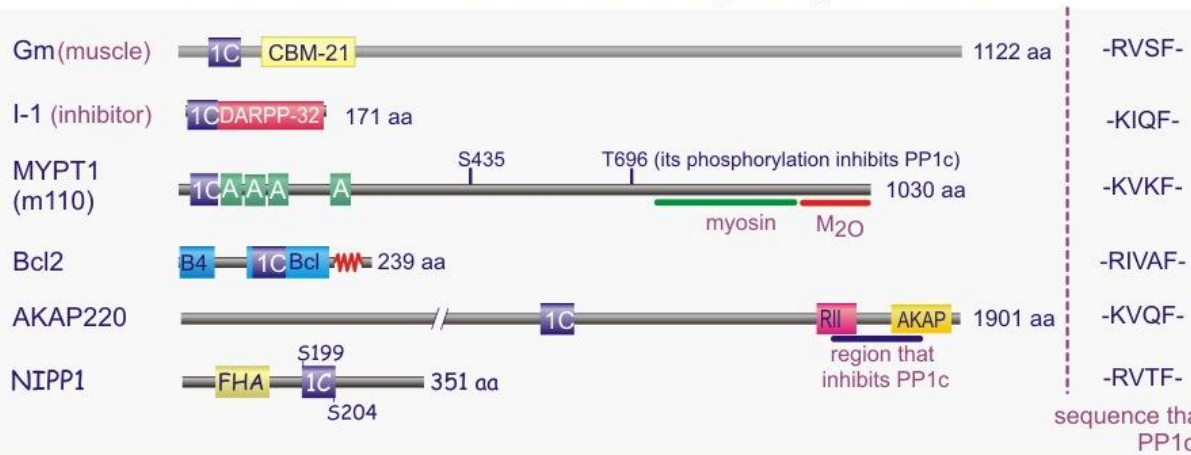
Specificity of serine/threonine phosphatases is largely determined by association of regulatory subunits that affect subcellular localisation as well as substrate specificity

subcellular localisation of ser/thr protein phosphatases is determined by the regulatory subunit they are associated with. These subunits affect the catalytic activity as well the choice of substrate



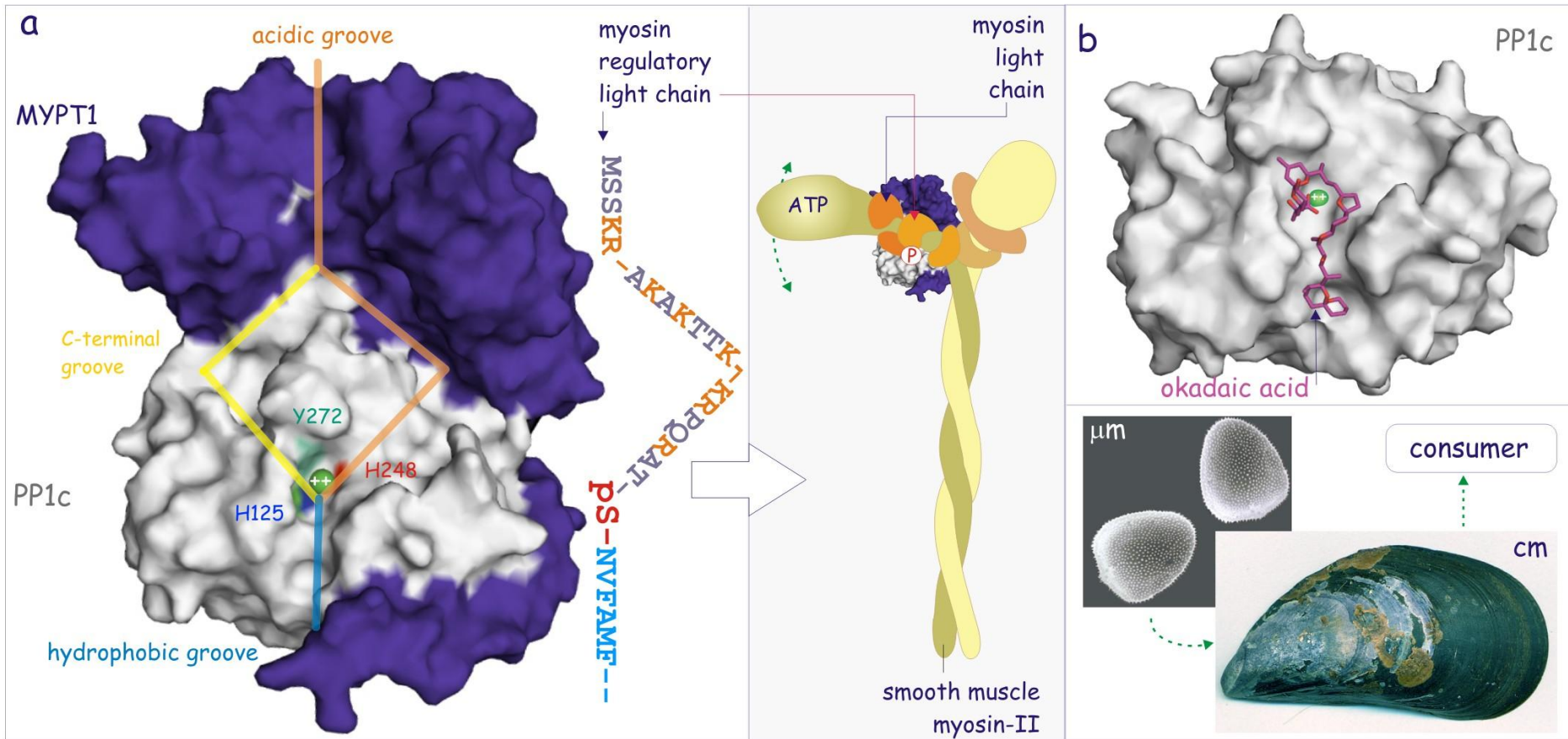
- 1C** consensus PP1c binding site
- A** ankyrin repeats
- AKAP** AKAP consensus domain
- CBM-21** carbohydrate binding type-21
- B4** Bcl-homology region 4
- Bcl** B-cell lymphoma domain
- FHA** Forhead associated phosphopeptide binding domain
- M20** interaction site of PP1c regulatory subunit, unknown function
- PDZ** PDS95, DlgA, ZO-1 homology domain
- PL** phosphorylase binding site
- RII** PKA-RII binding site
- DARPP-32** PPI/dopamine cAMP regulated neuronal phosphoprotein-32

domain architecture of some of the regulatory subunits of PP1c



sequence that binds PP1c

The regulatory subunit MYPT1 turns the broad-specificity PP1c (catalytic subunit) into a myosin regulatory-light chain-specific phosphatase



- R= arginine & K= lysine are positively charged residues that fit the negatively charged acidic groove
- Dephosphorylation of myosin regulatory light chain causes smooth muscle cell relaxation (no hydrolysis of ATP)

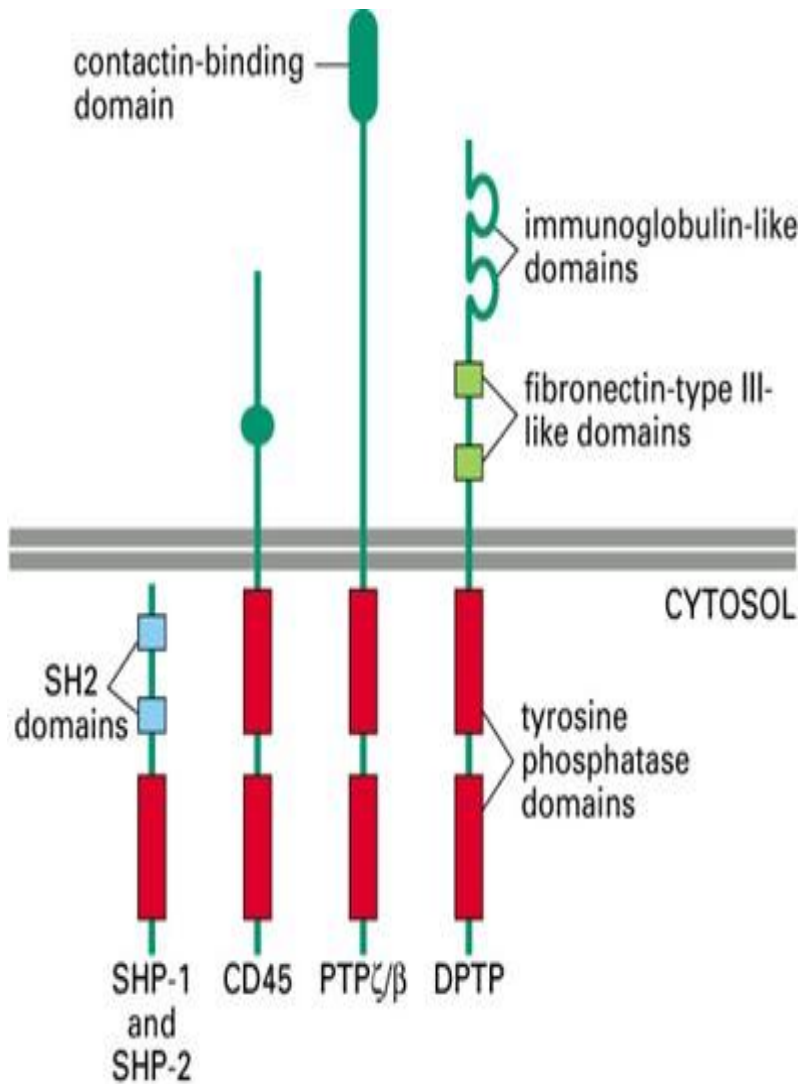
Clinical significance

Serine/threonine kinase (STK) expression is altered in many types of cancer

Serine/threonine protein kinase p90-kDa ribosomal S6 kinase (RSK) is involved in development of some prostate cancers.

Raf inhibition has become the target for new anti-metastatic cancer drugs as they inhibit the MAPK cascade and reduce cell proliferation.

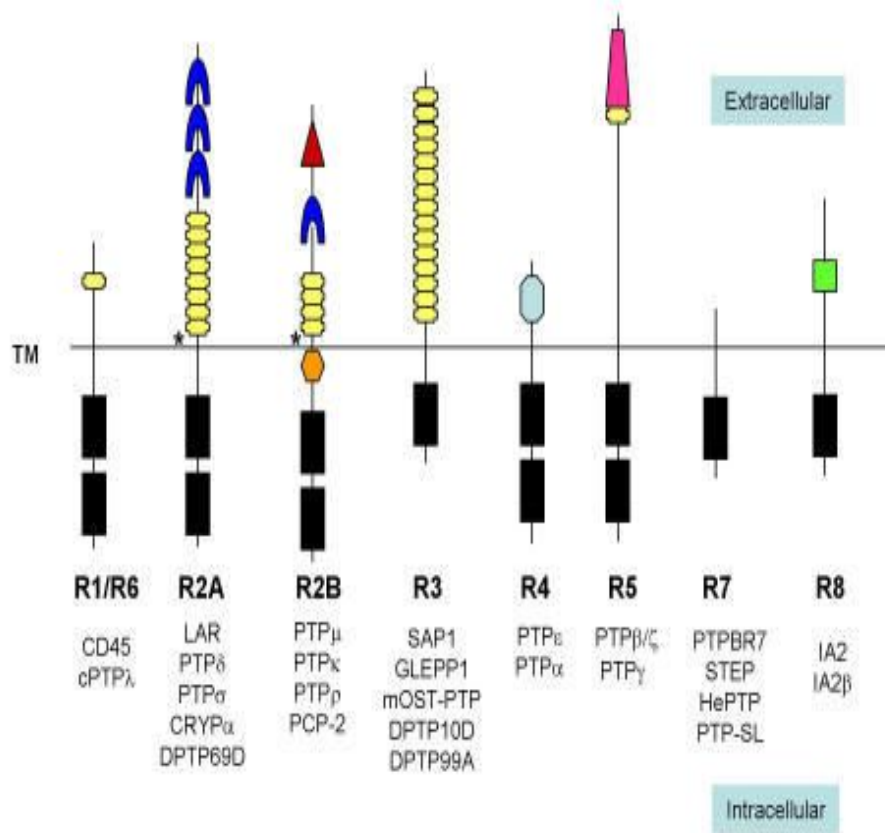
Act as Cell-Surface Receptors Protein tyrosine phosphatases (PTPs) remove selected phosphotyrosines on a subset of tyrosine-phosphorylated proteins. Exhibit high degree of substrate selectivity. These enzymes ensure that the tyrosine phosphorylations are short-lived and are responsible for regulating the intensity of the signal. There are about 30 known PTPs and occur as both transmembrane and cytoplasmic forms.



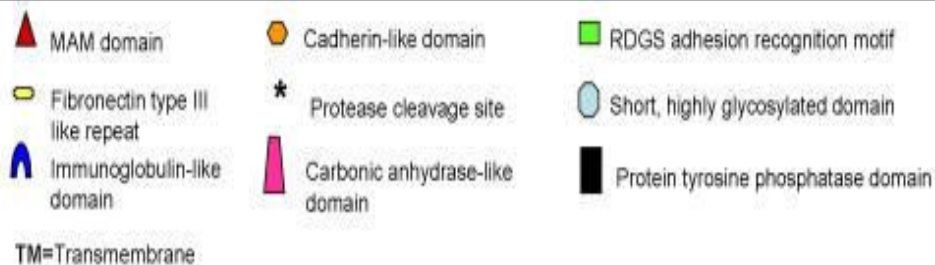
The role of receptor-like tyrosine phosphatases is not yet clearly understood. They are thought to act as receptors, but their ligands have not been identified. Some have been shown to display features of cell-adhesion. Some can activate receptors on neighboring cells.

Figure 15-64. Molecular Biology of the Cell, 4th Edition.

Classification of receptor-like protein tyrosine phosphatases (RPTPs) into eight subfamilies (R1-R8), based on sequence similarity among PTP catalytic domains. PTP μ , κ , ρ and PCP-2 are members of the R2B subfamily.



RPTPs have been divided into eight major subfamilies, based on phylogenetic analysis of the phosphatase domains. Four of these subfamilies (R2A, R2B, R3, and R4) play critical roles in CNS development. R2B is an extracellular segment containing a combination of multiple fibronectin and immunoglobulin (Ig)-like domains, and a single transmembrane region. The intracellular region contains a membrane proximal juxtamembrane domain, followed by a catalytically active tyrosine phosphatase domain and a second inactive domain. Type 2 RPTPs have been further subdivided into two distinct classes (R2A and R2B). Genes in the R2B class are differentiated from the R2A class by an additional MAM (Meprin/ A5/PTP μ) domain at the N-terminus, R2B molecules have cell adhesive properties. Because no invertebrate homologues of the four R2B molecules have been found to date, and no ESTs indicative of R2Bs have been isolated from invertebrates, the function(s) of these phosphatases is likely to be highly specific to vertebrate species.

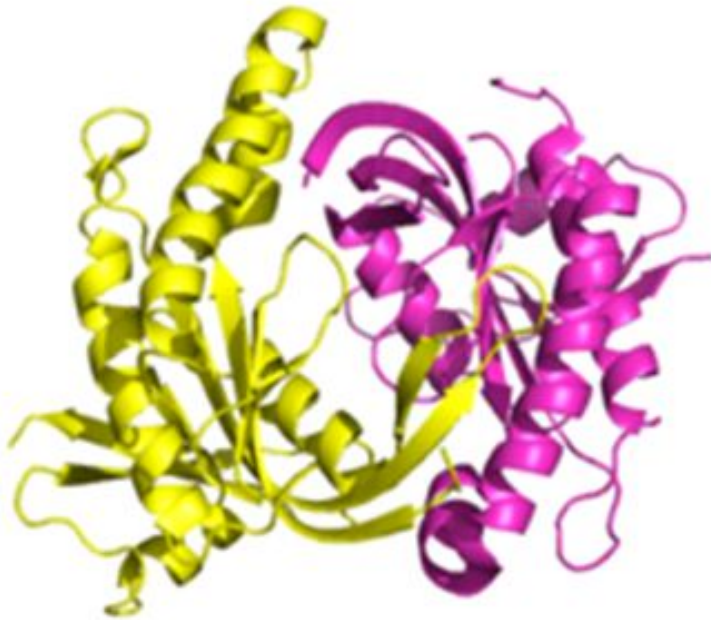


Receptor guanylyl cyclases

Receptor guanylyl cyclases

- Single-pass transmembrane proteins with an extracellular binding site for a signal molecule and an intracellular guanylyl cyclase catalytic domain. The binding of the signal molecule activates the cyclase domain to produce cyclic GMP, which in turn binds to and activates a cyclic GMP-dependent protein kinase (PKG), which phosphorylates specific proteins on serine or threonine.

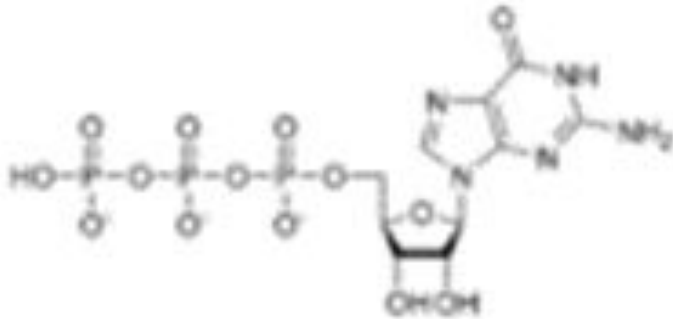
Catalytic domain of human soluble guanylate cyclase 1



EC 4.6.1.2, also known as guanyl cyclase, guanylate cyclase, or GC) is a lyase enzyme. Guanylyl cyclase is often part of the G protein signaling cascade that is activated by low intracellular calcium levels and inhibited by high intracellular calcium levels.

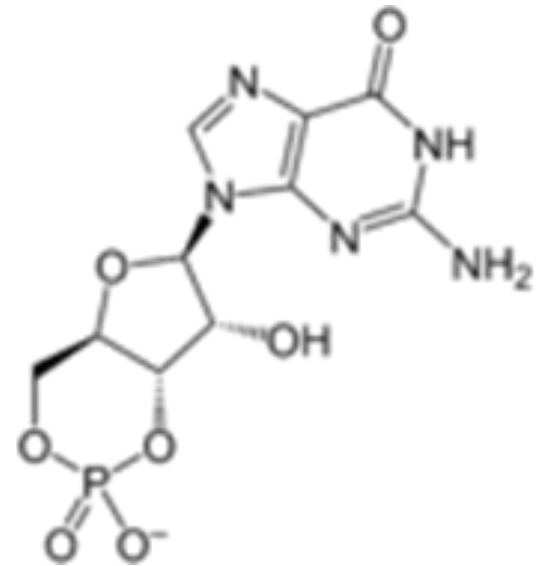
Guanylyl cyclase catalyzes the reaction

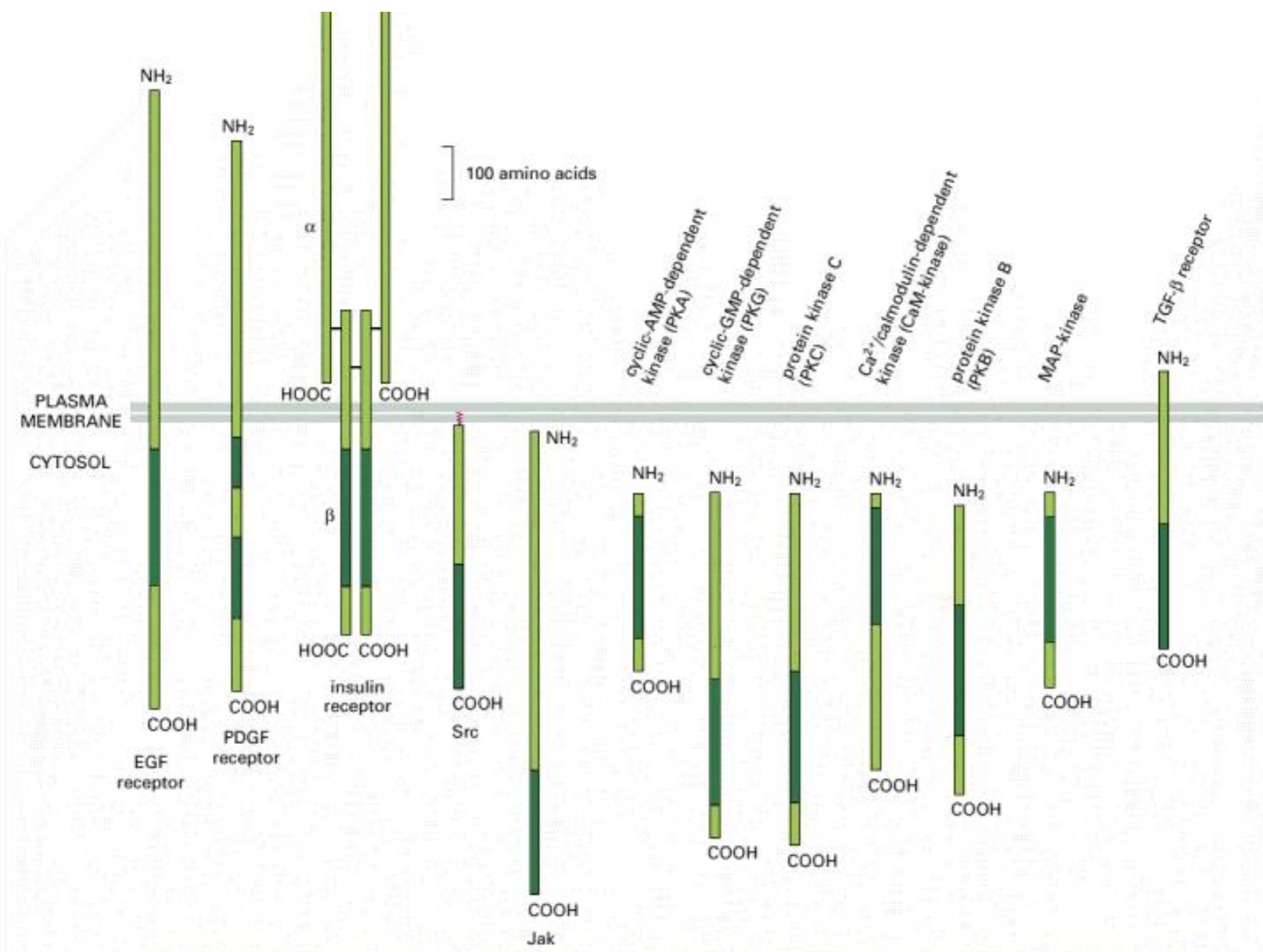
...



of guanosine triphosphate (GTP)

3',5'-cyclic guanosine
monophosphate (cGMP)





THYROSINE SPECIFIC PROTEIN KINASES SERINE/THYROSINE SPECIFIC PROTEIN KINASES

some of the protein kinases

Histidine-kinase-associated receptors

Histidine-kinase-associated receptors

- Activate a “two-component” signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

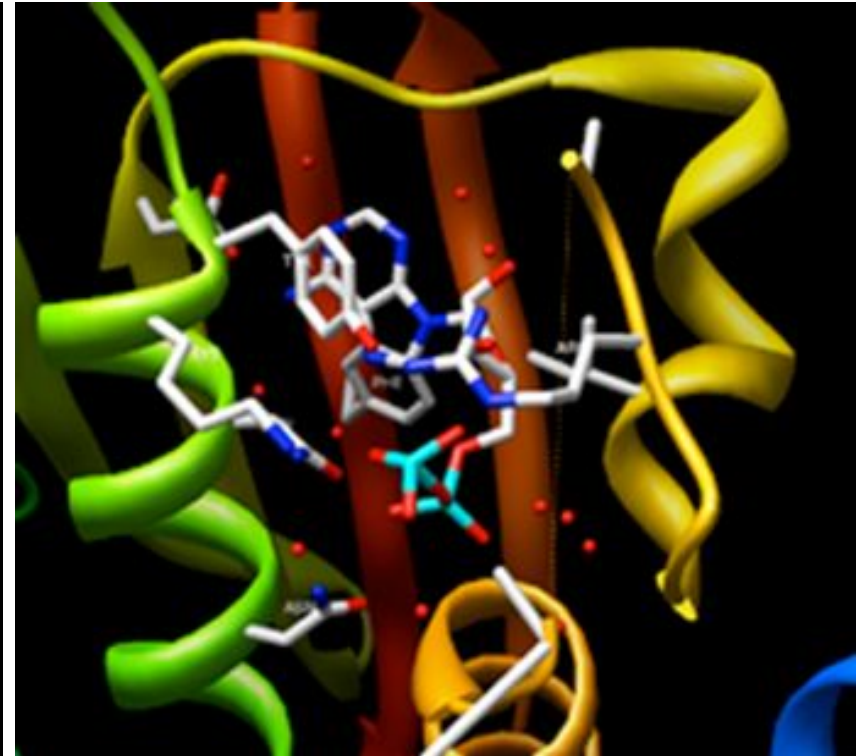
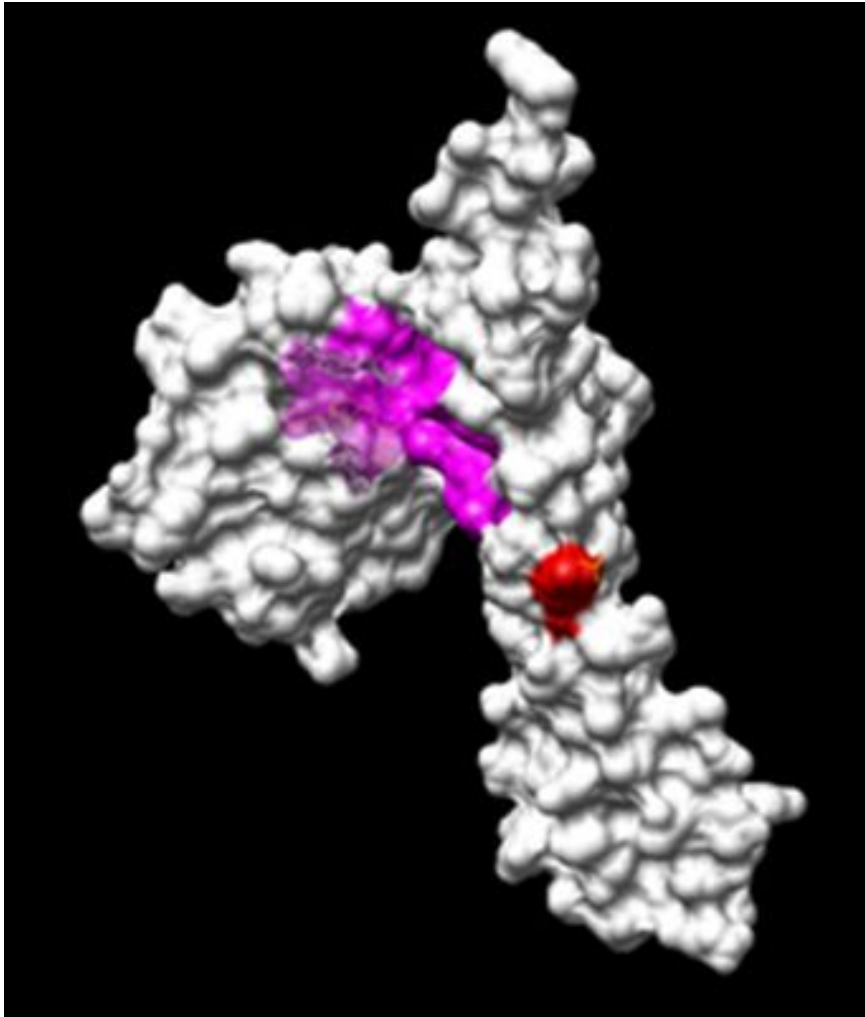
Protein histidine kinase



Crystallographic
structure of
ATP:protein-L-histidine
N-phosphotransferase

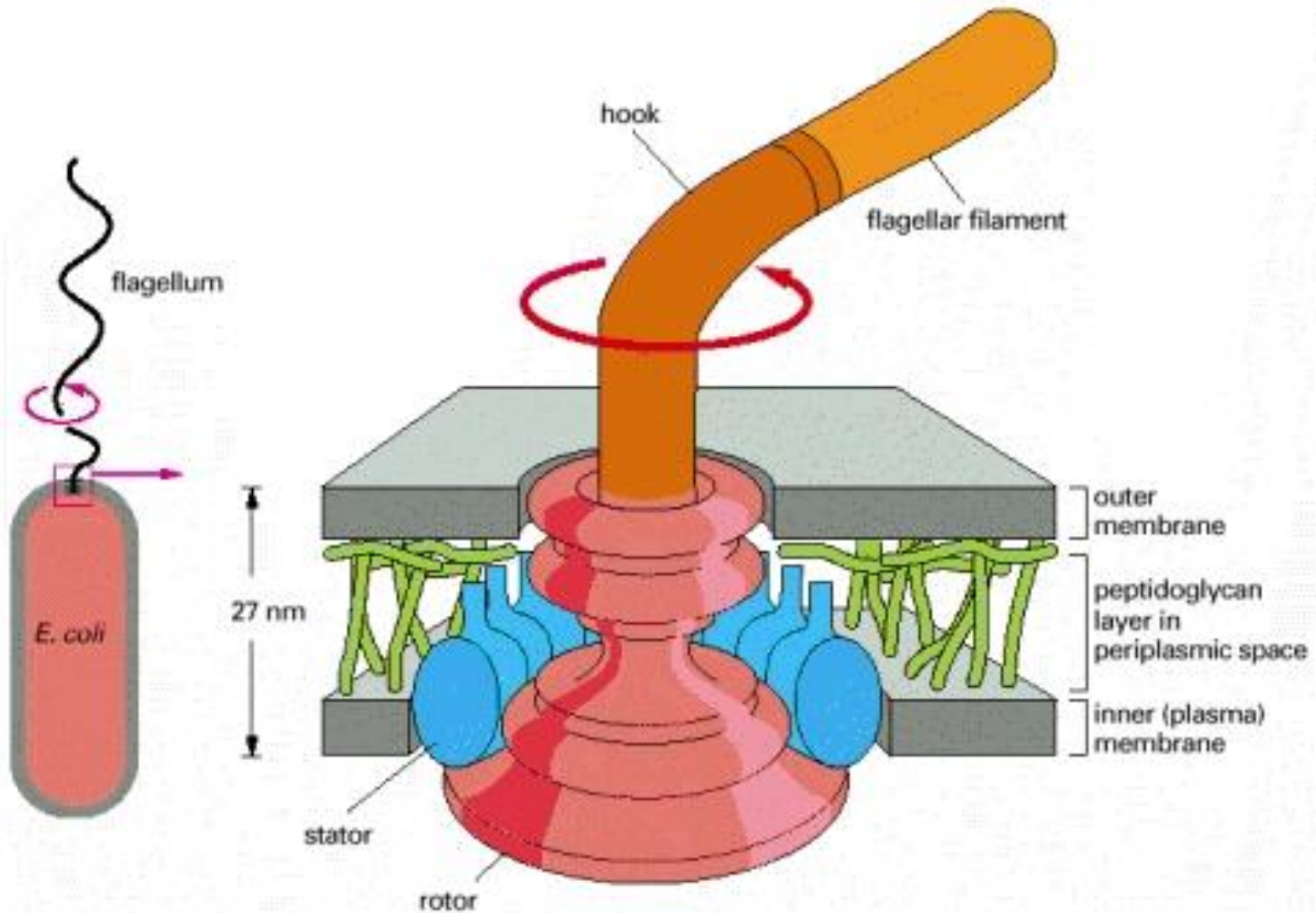
- Multifunctional, typically transmembrane, proteins of the transferase class of enzymes that play a role in signal transduction across the cellular membrane.

Single monomer. Red residue is His-260, ligand (ADP and SO_4) is yellow, ATP lid is magenta

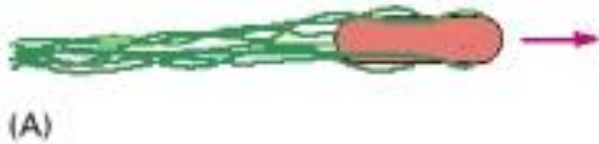


Structure and environment of HK853 ATP binding pocket. Important residues are labeled and red spheres are water molecules.

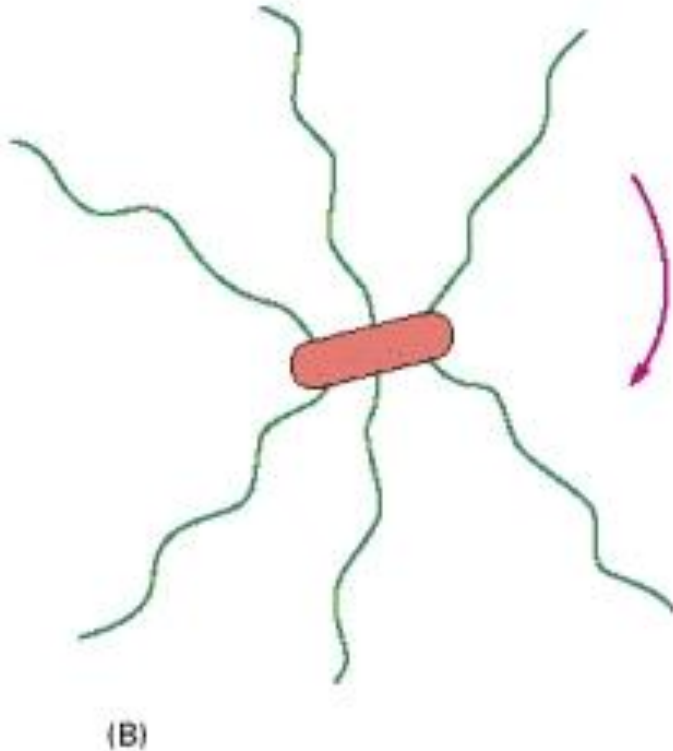
The bacterial flagellar motor



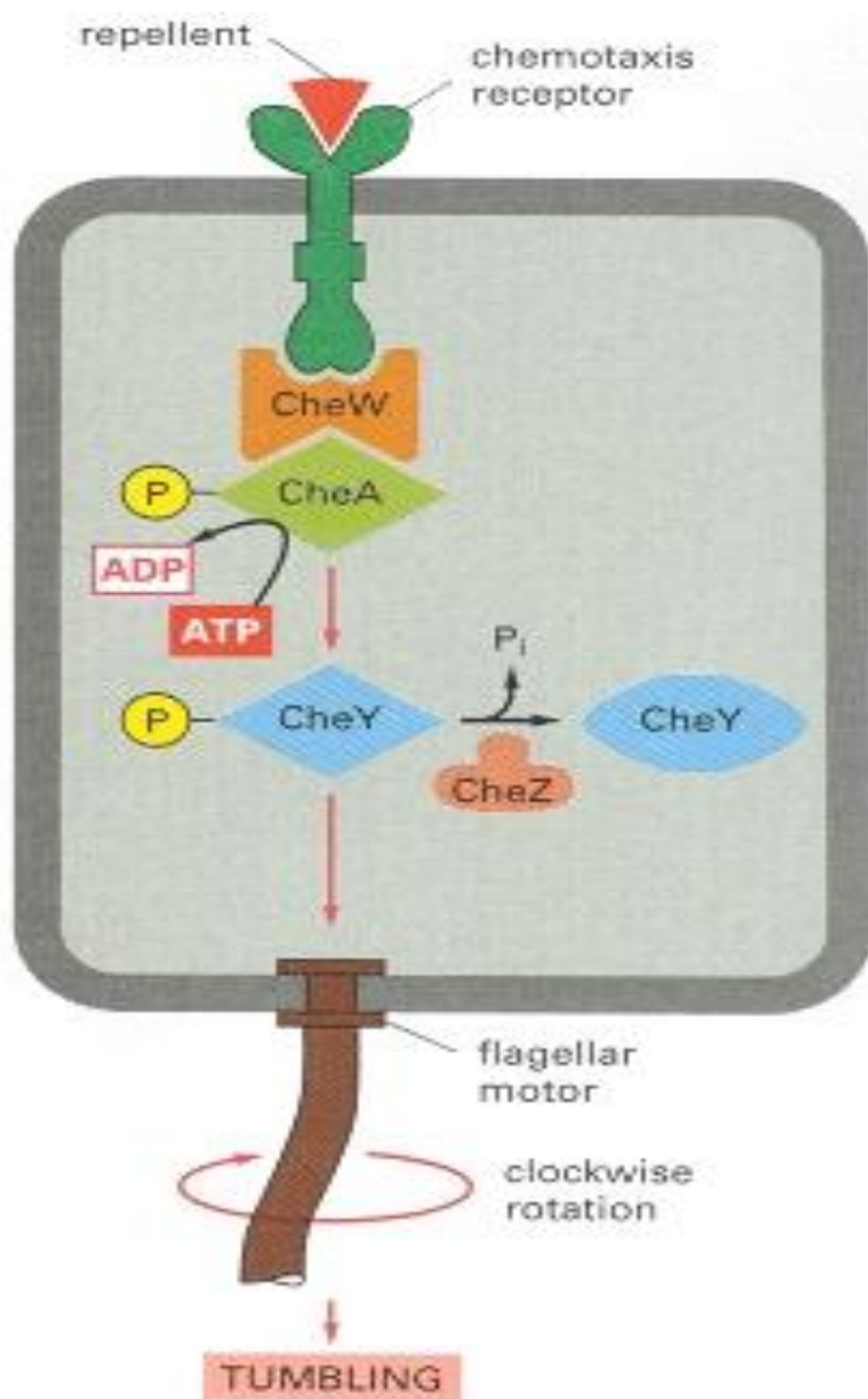
Positions of the flagella on E. coli during swimming



(A) Flagella rotate counterclockwise, they are drawn together into a single bundle, which acts as a propeller to produce smooth swimming



(B) Flagella rotate clockwise, they fly apart and produce tumbling



The two-component signaling pathway that enables chemotaxis receptors to control the flagellar motor during bacterial chemotaxis

Conclusion

- (1) receptor tyrosine kinases
- (2) tyrosine-kinase-associated receptors
- (3) receptor serine/threonine kinases
- (4) transmembrane guanylyl cyclases
- (5) histidine-kinase-associated receptors.

- Some transmembrane tyrosine phosphatases, which remove phosphate from phosphotyrosine side chains of specific proteins, are thought to function as receptors, although for the most part their ligands are unknown. The first two classes of receptors are by far the most numerous.

- Tyrosine-kinase-associated receptors depend on various cytoplasmic tyrosine kinases for their action. These kinases include members of the Src family, which associate with many kinds of receptors, and the focal adhesion kinase (FAK), which associates with integrins at focal adhesions. The cytoplasmic tyrosine kinases then phosphorylate a variety of signaling proteins to relay the signal onward. The largest family of receptors in this class is the cytokine receptors family. When stimulated by ligand binding, these receptors activate Jak cytoplasmic tyrosine kinases, which phosphorylate STATs. The STATs then dimerize, migrate to the nucleus, and activate the transcription of specific genes. Receptor serine/threonine kinases, which are activated by signaling proteins of the TGF- β superfamily, act similarly: they directly phosphorylate and activate Smads, which then oligomerize with another Smad, migrate to the nucleus, and activate gene transcription.

- Bacterial chemotaxis is mediated by histidine-kinase-associated chemotaxis receptors. When activated by the binding of a repellent, the receptors stimulate their associated protein kinase to phosphorylate itself on histidine and then transfer that phosphate to a messenger protein, which relays the signal to the flagellar motor to alter the bacterium's swimming behavior. Attractants have the opposite effect on this kinase and therefore on swimming.

Thanks
for
attention