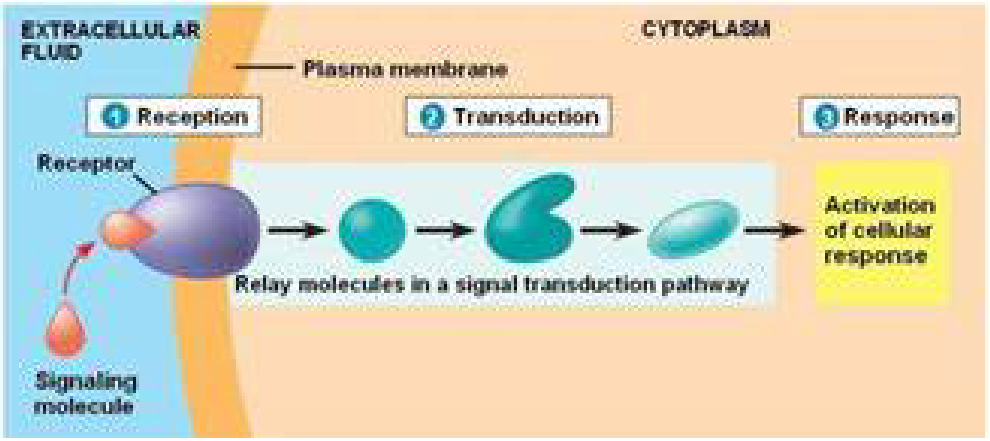
Quinases

Características das vias de sinalização

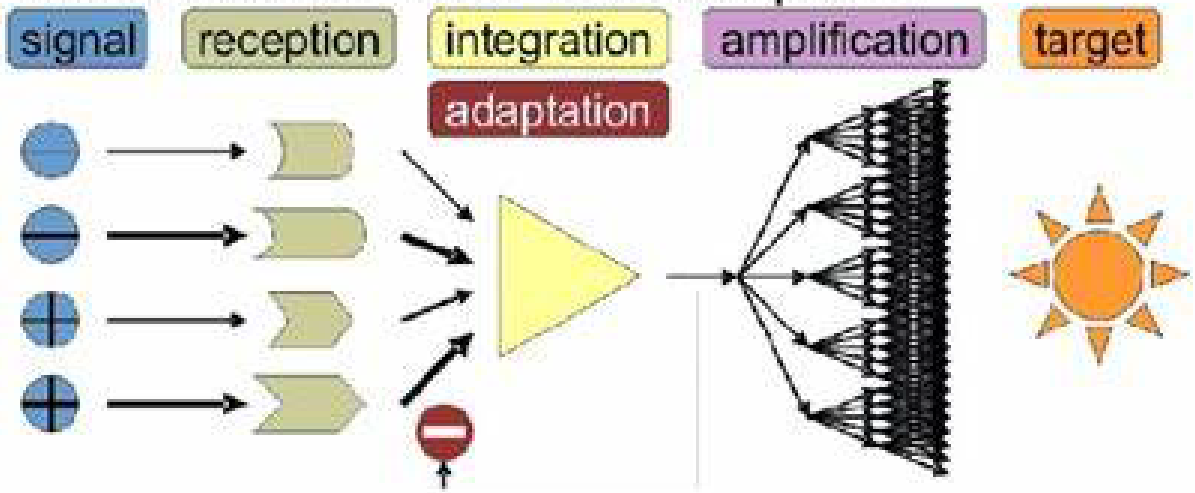
3 Phases of Signal Transduction

1. **Reception** – The target cell's detection of a signal molecule coming from outside the cell.
2. **Transduction** – The conversion of the signal to a form that can bring about a specific response.
3. **Response** – The specific cellular response to the signal molecule.

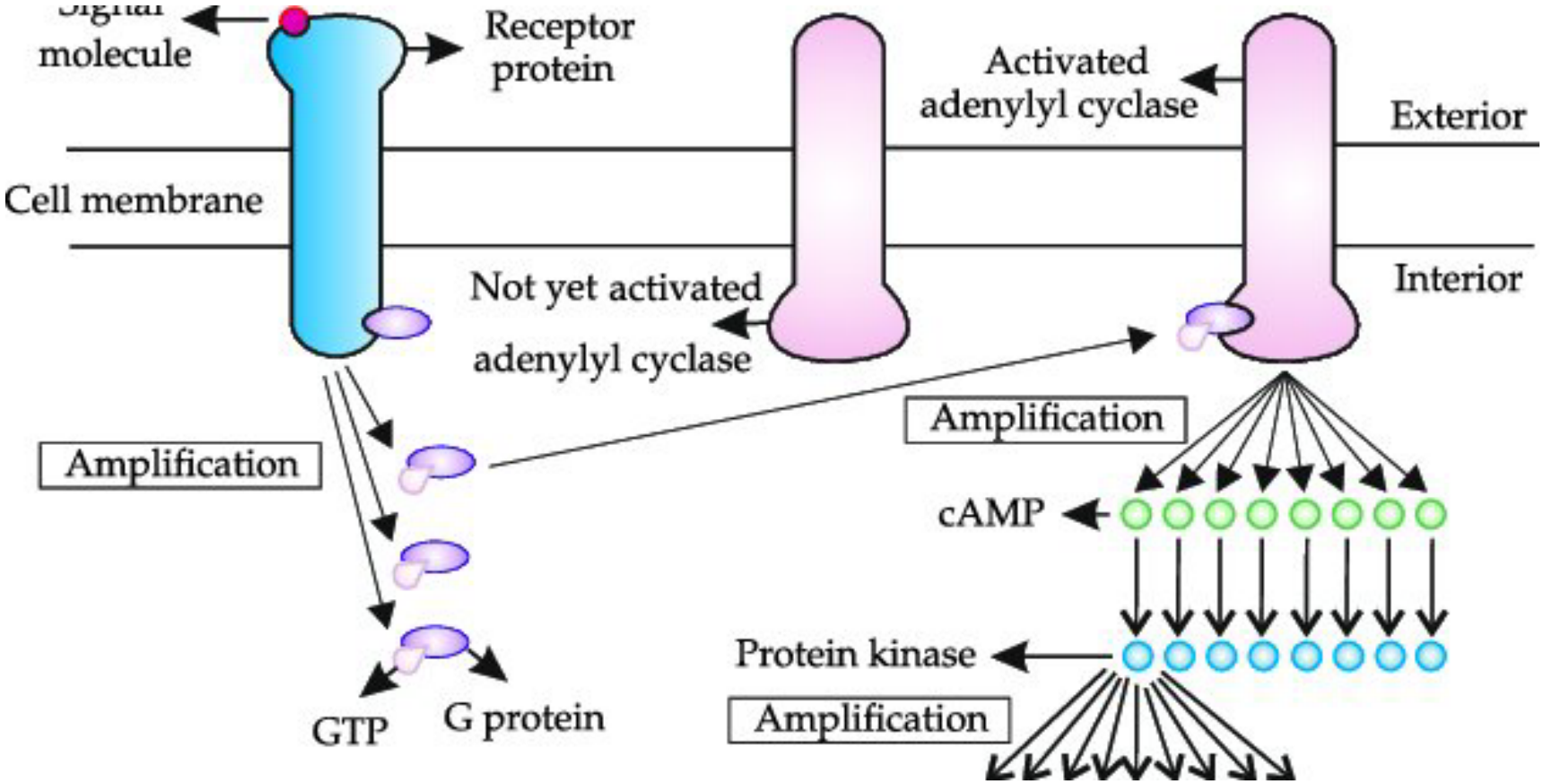


Signal Amplification

- Amplification refers to the activation of increasing numbers of molecules downstream from the receptors



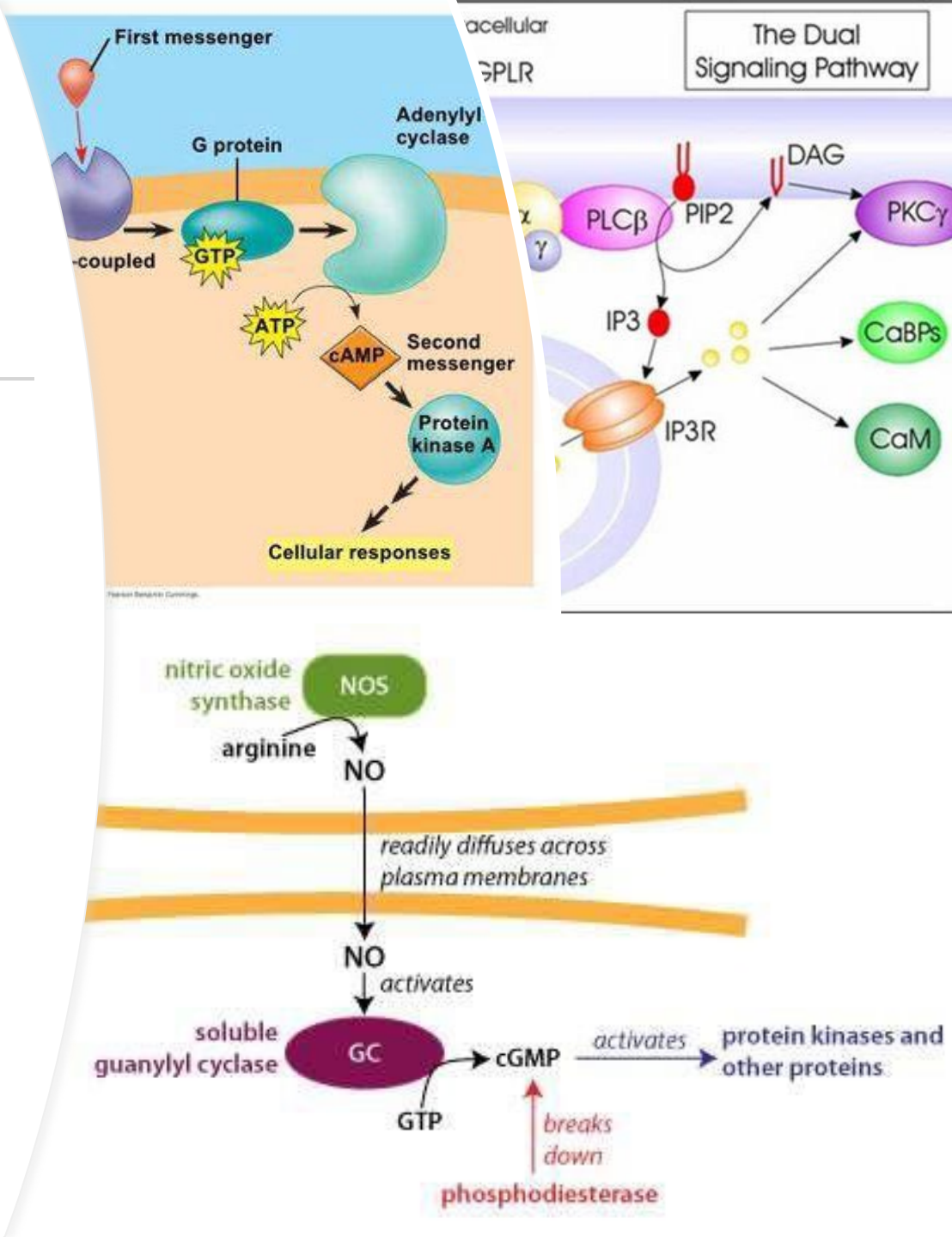
Signal Amplification



ttps://www.researchgate.net/publication/304415983_Amplify-and-Forward_Relaying_in_Two-Hop_Diffusion-Based_Molecular_Communication_Networks/figures?lo=1

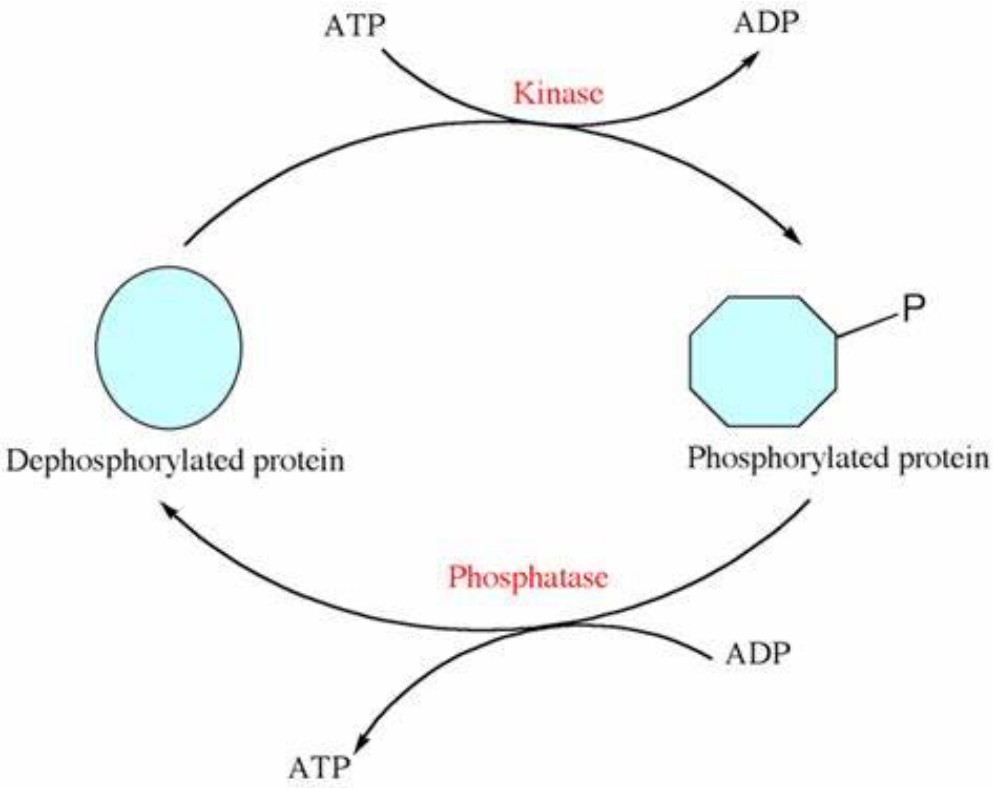
Signal Amplification

- Second messengers



Signal Amplification

- Phosphorylation and dephosphorylation



Fosforilação e remoção de um fosfato são mecanismos de “ligar” e “desligar” proteínas.

- A Fosforilação pode determinar:
- A localização sub cellular
- Interãções proteina/ proteina
- Atividade
- Mudanças conformacionais
- Degradação
- Auxiliam na propagação do sinal

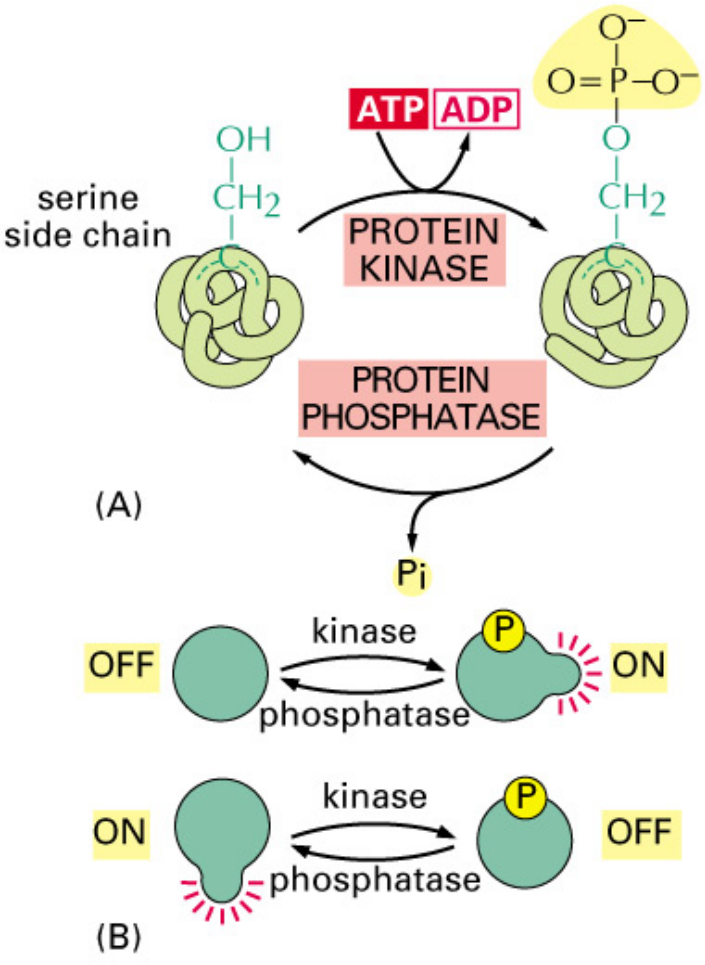
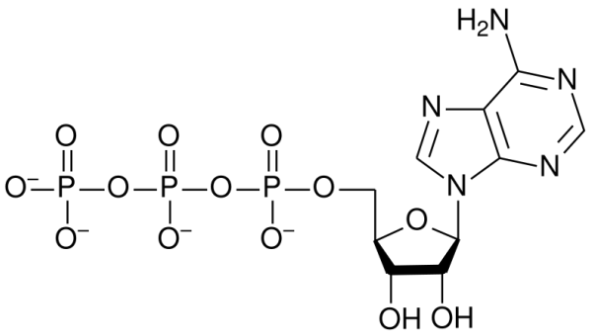
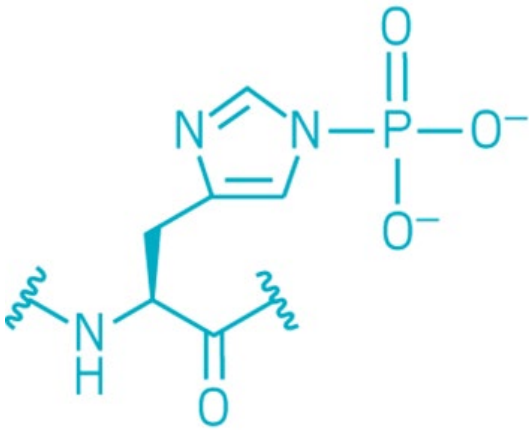


Figure 3–63. Molecular Biology of the Cell, 4th Edition.

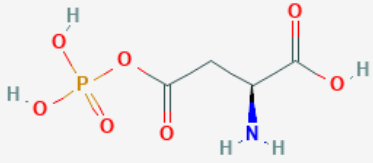
Em eucariontes os resíduos mais comumente fosforilados são serinas, treoninas and tirosinas

Nos procariontes histidina e aspartato

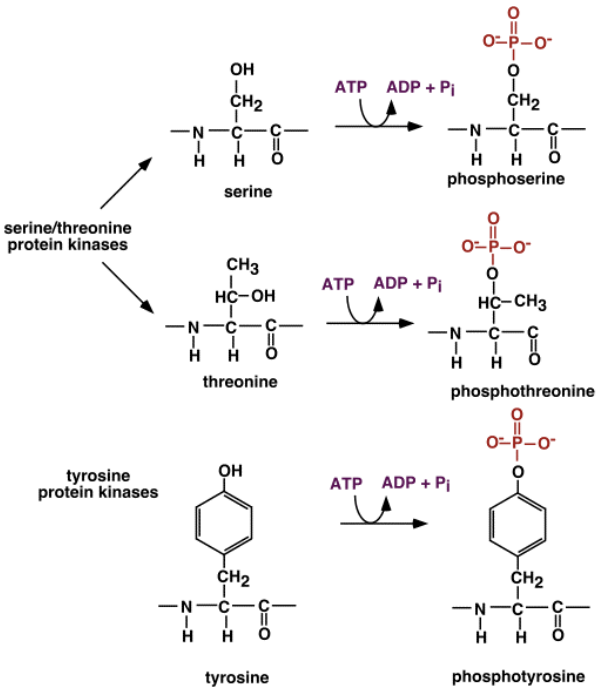




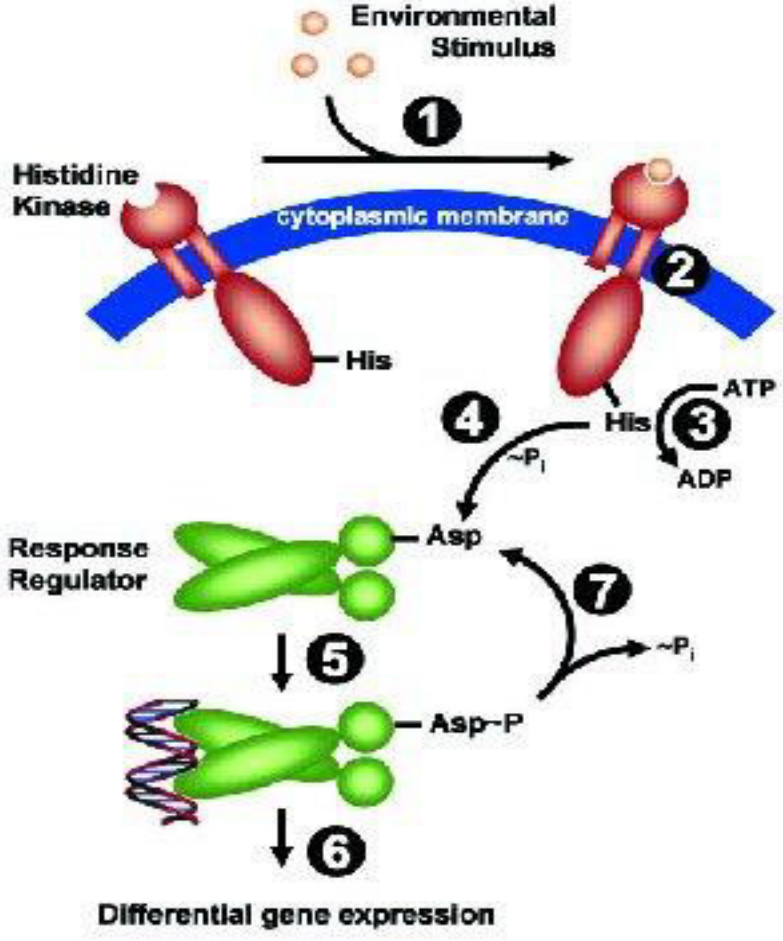
Phosphohistidine

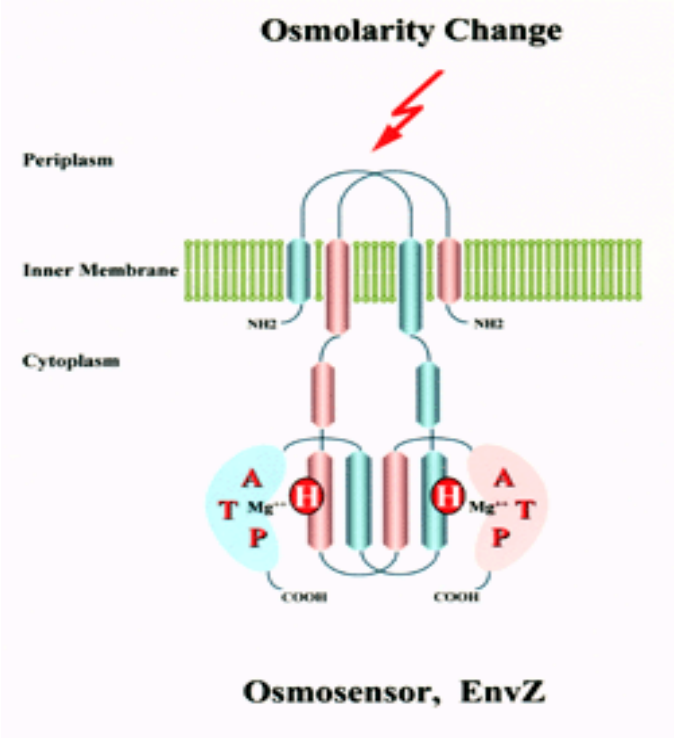


Aspartilfosfato

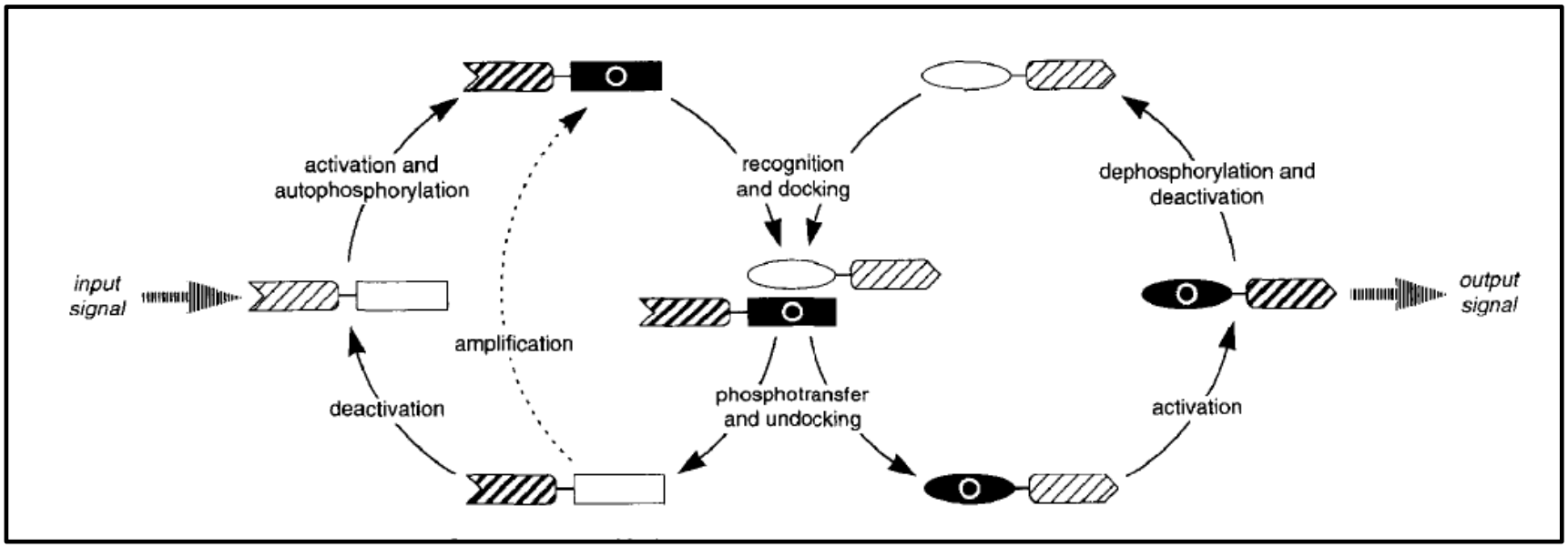


Sistema dois componentes (eucariontes)

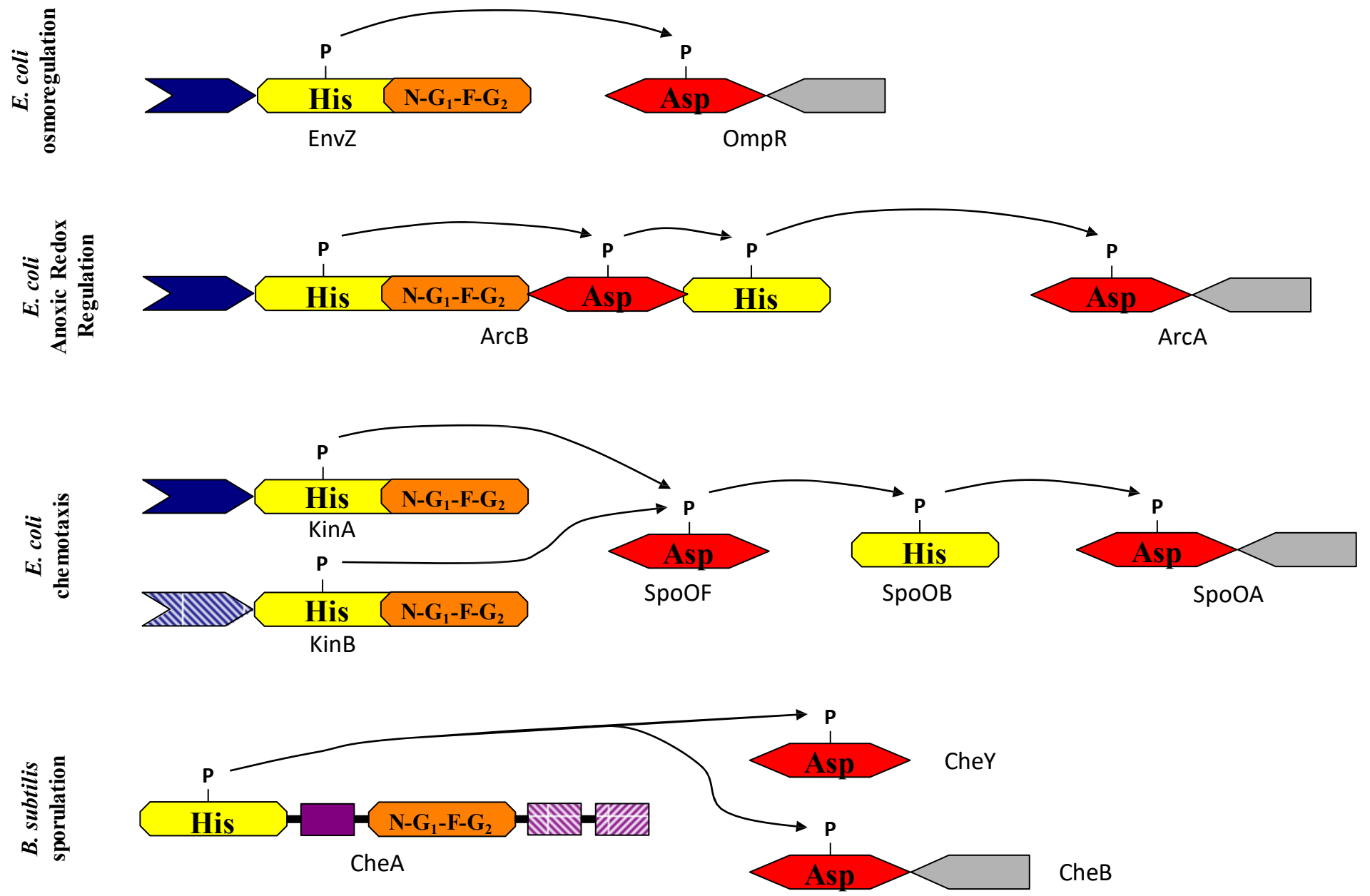




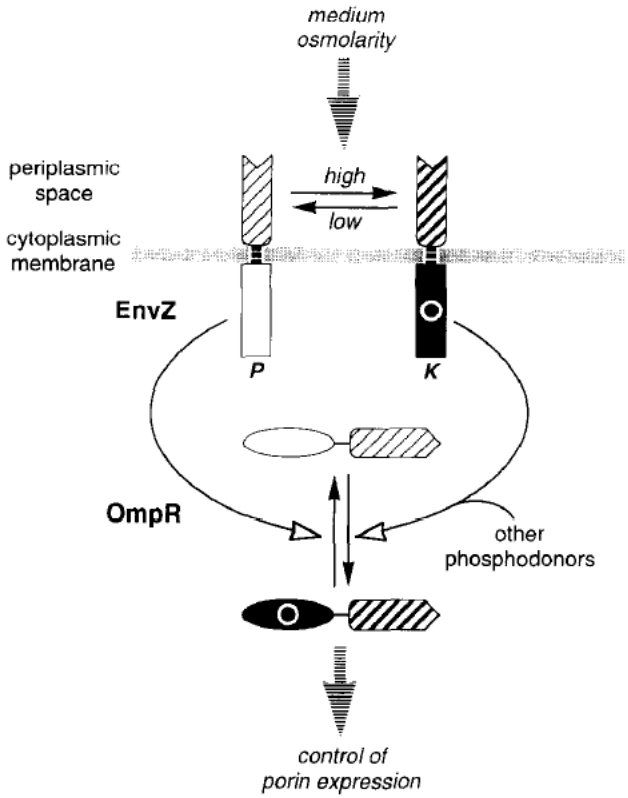
Two component systems

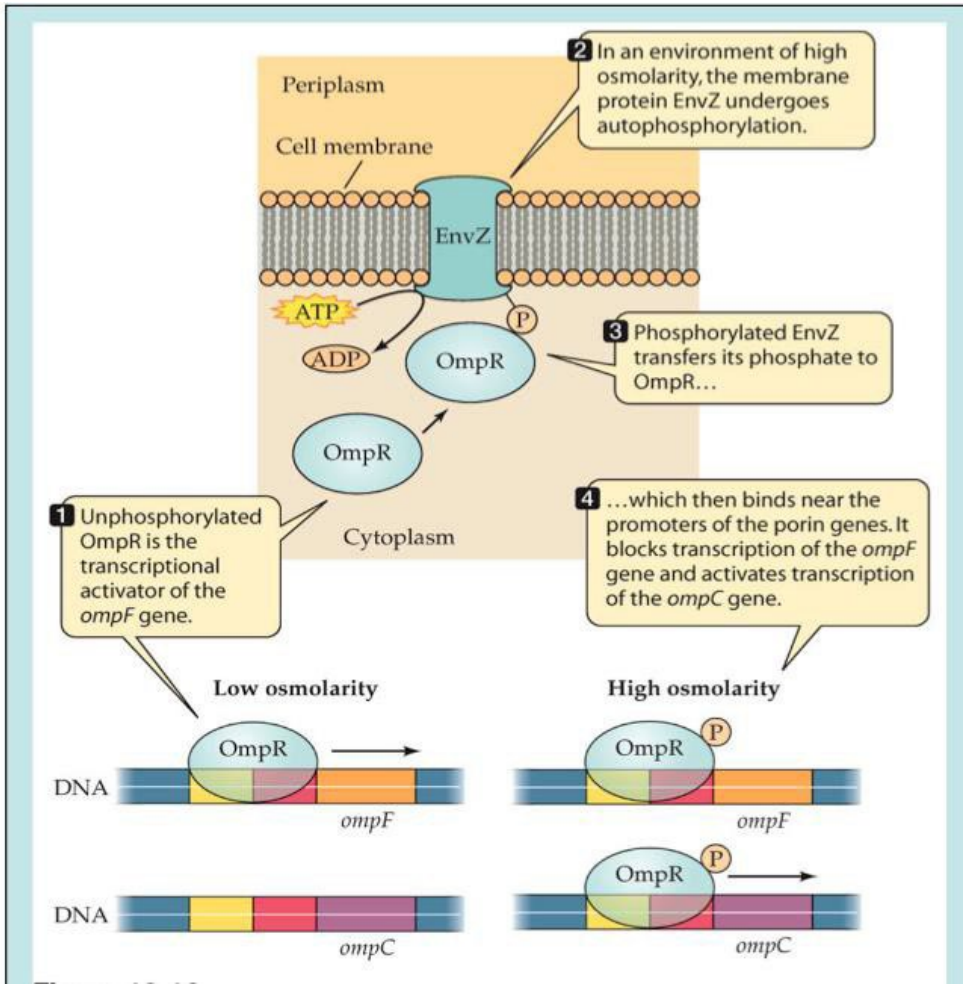


Modular Organization of two component systems

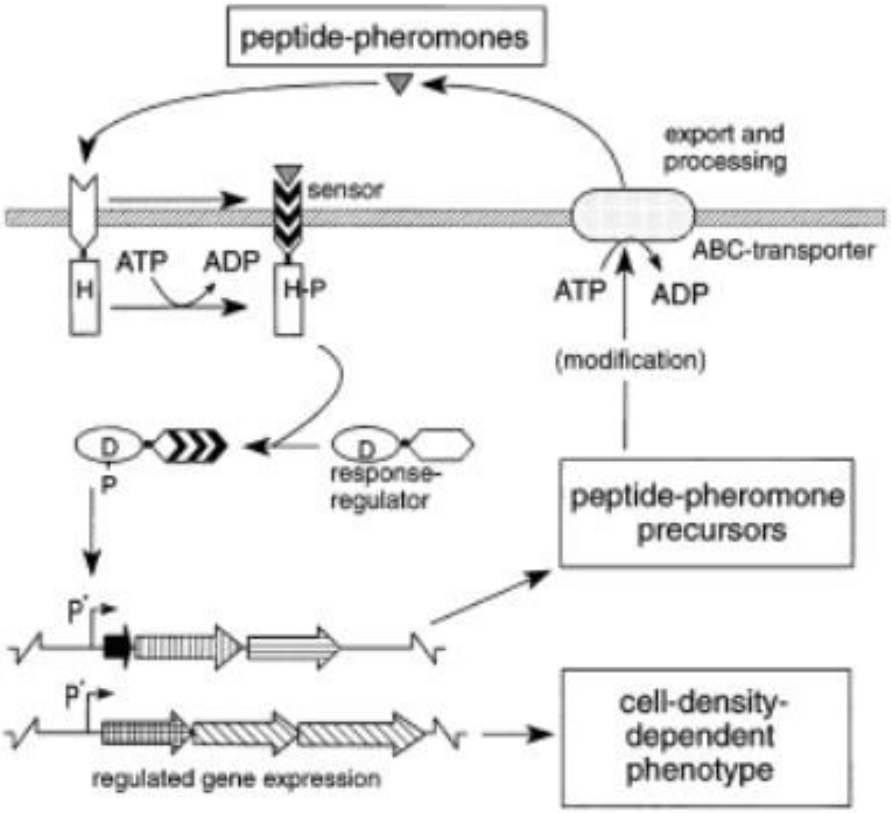


Examples of two component systems

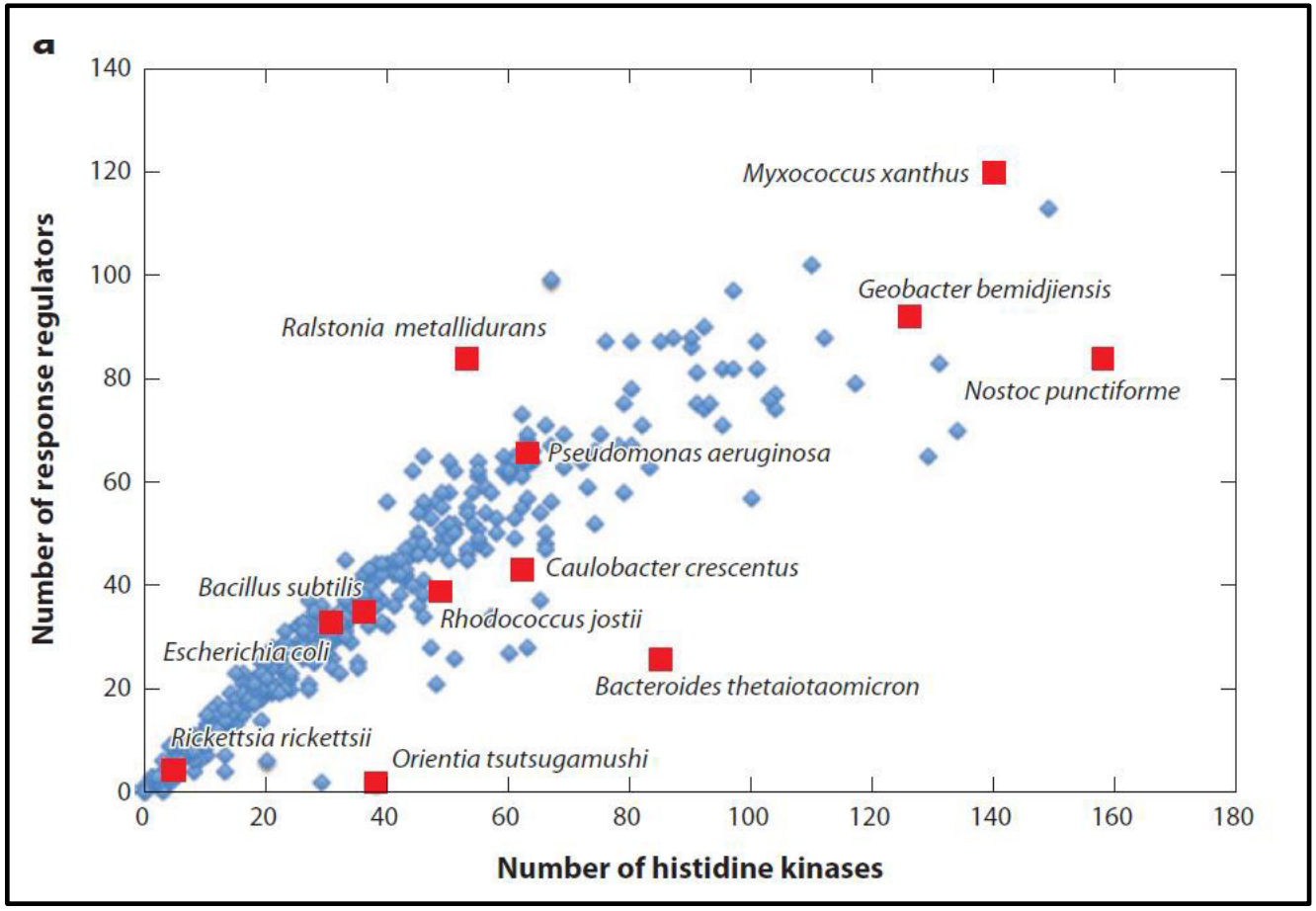




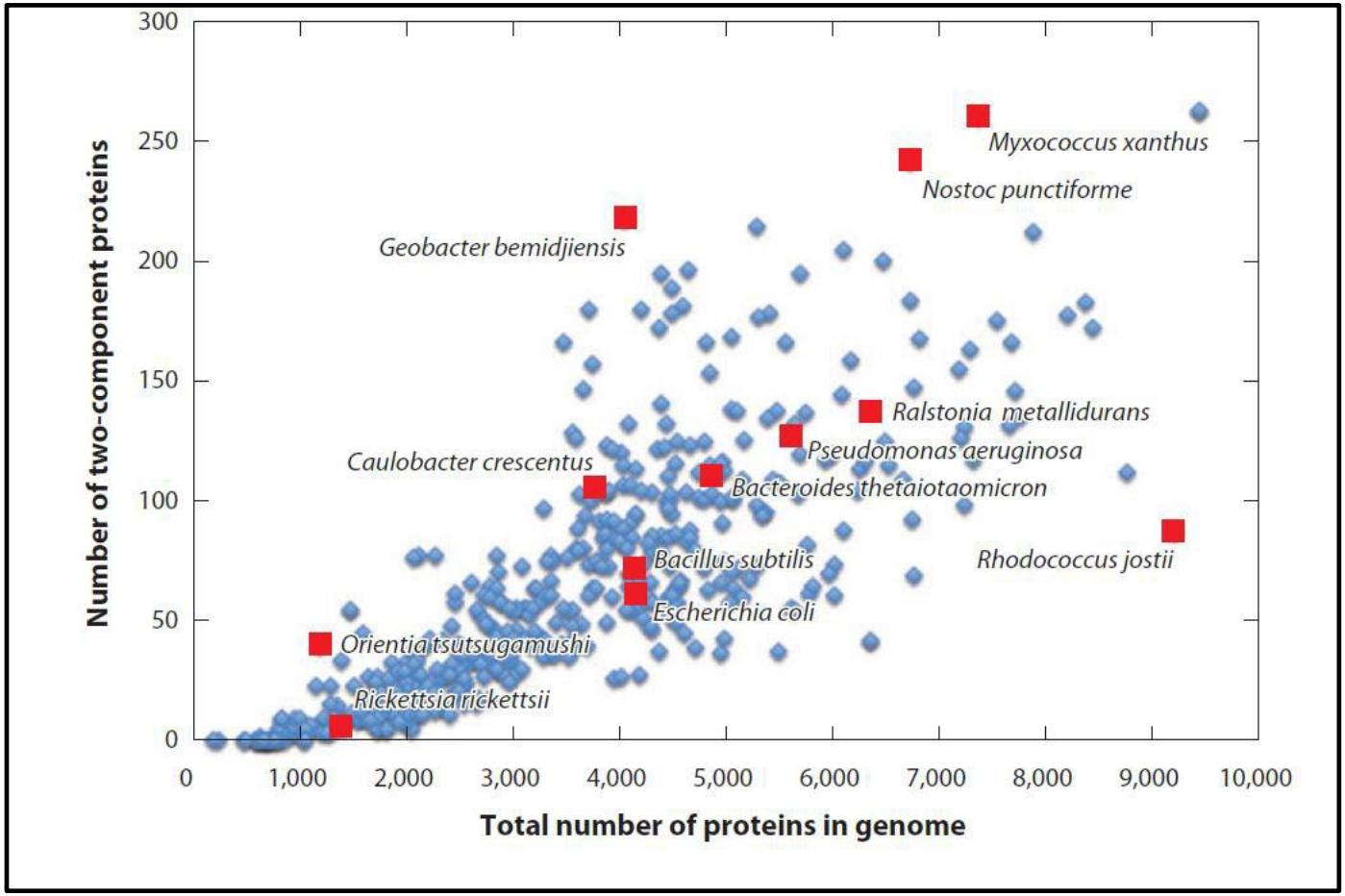
Examples of two component systems
Quorum Sensing



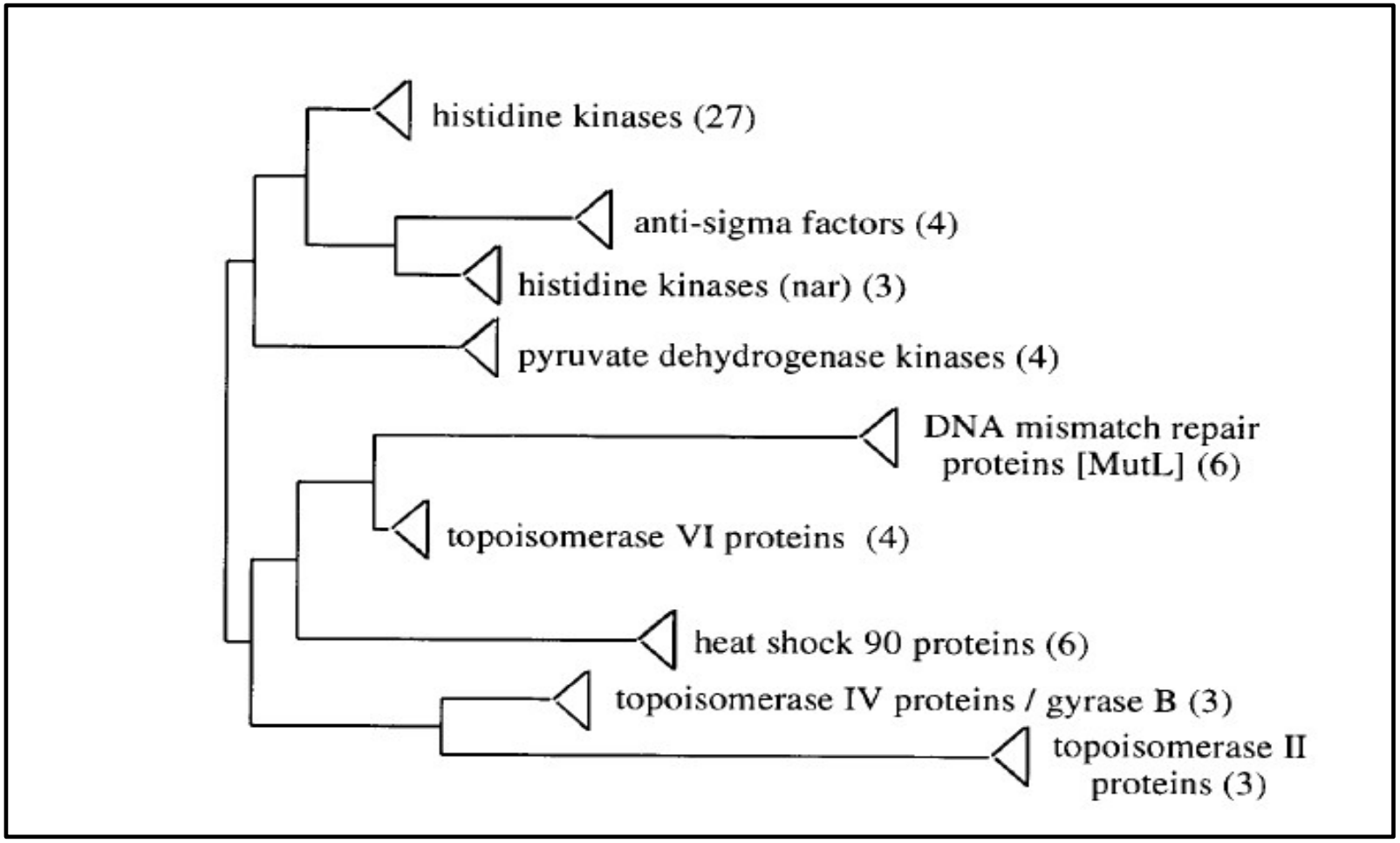
Kinase and response regulators identified by sequence homology in the same operon



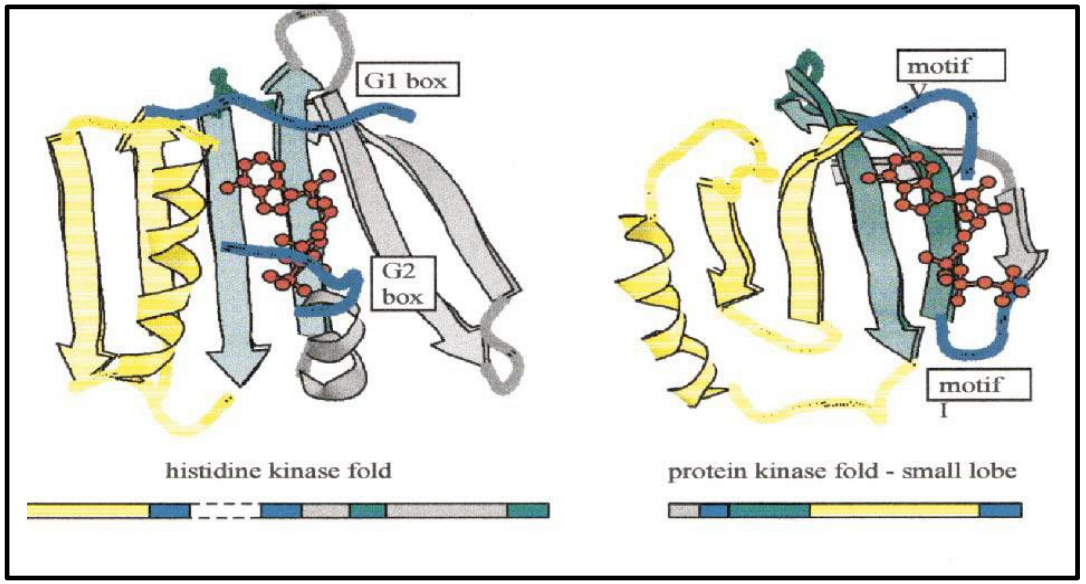
Number of two component proteins

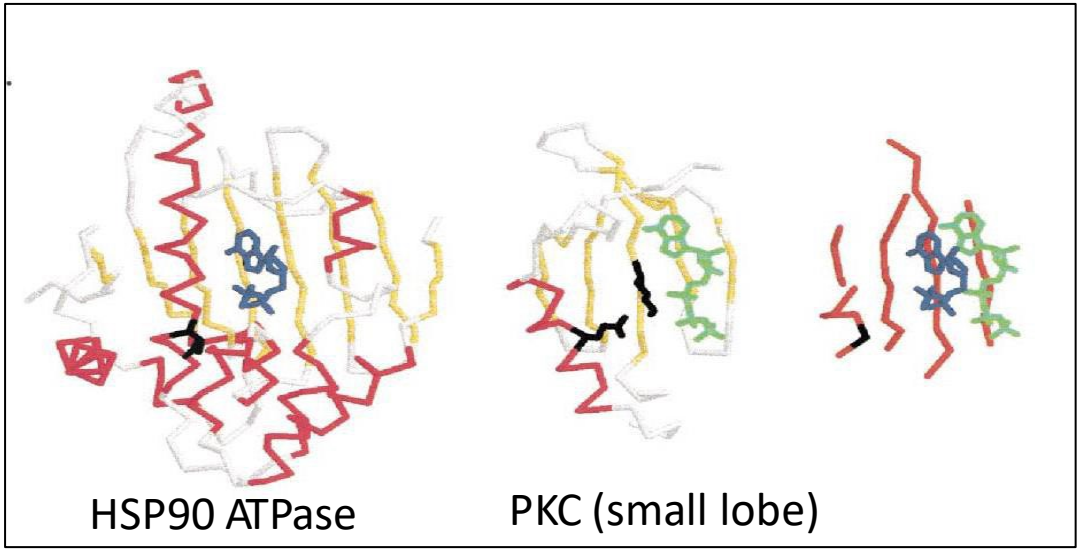


Evolution of the histidine kinase domain



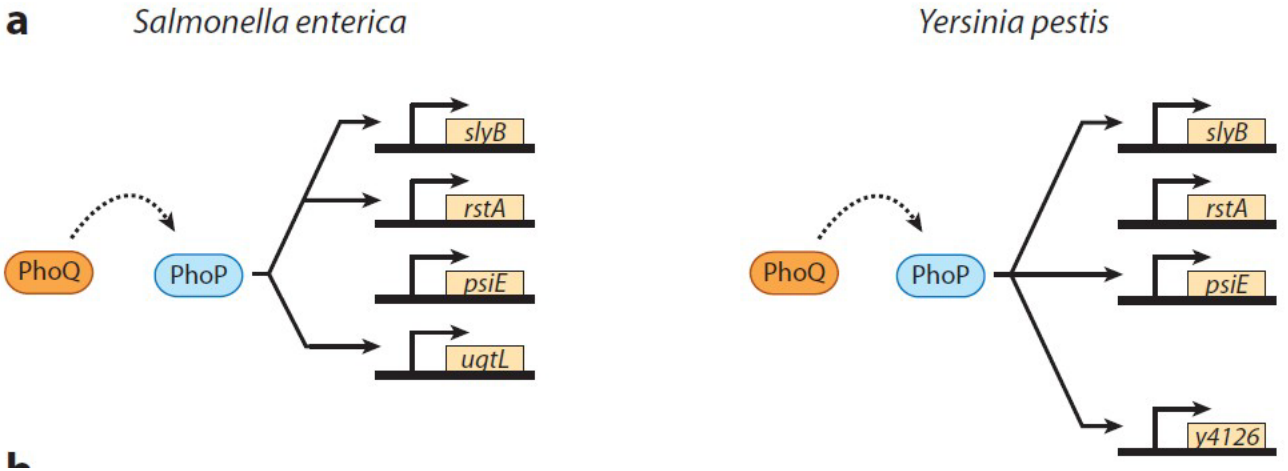
Evolution of the histidine kinase domain





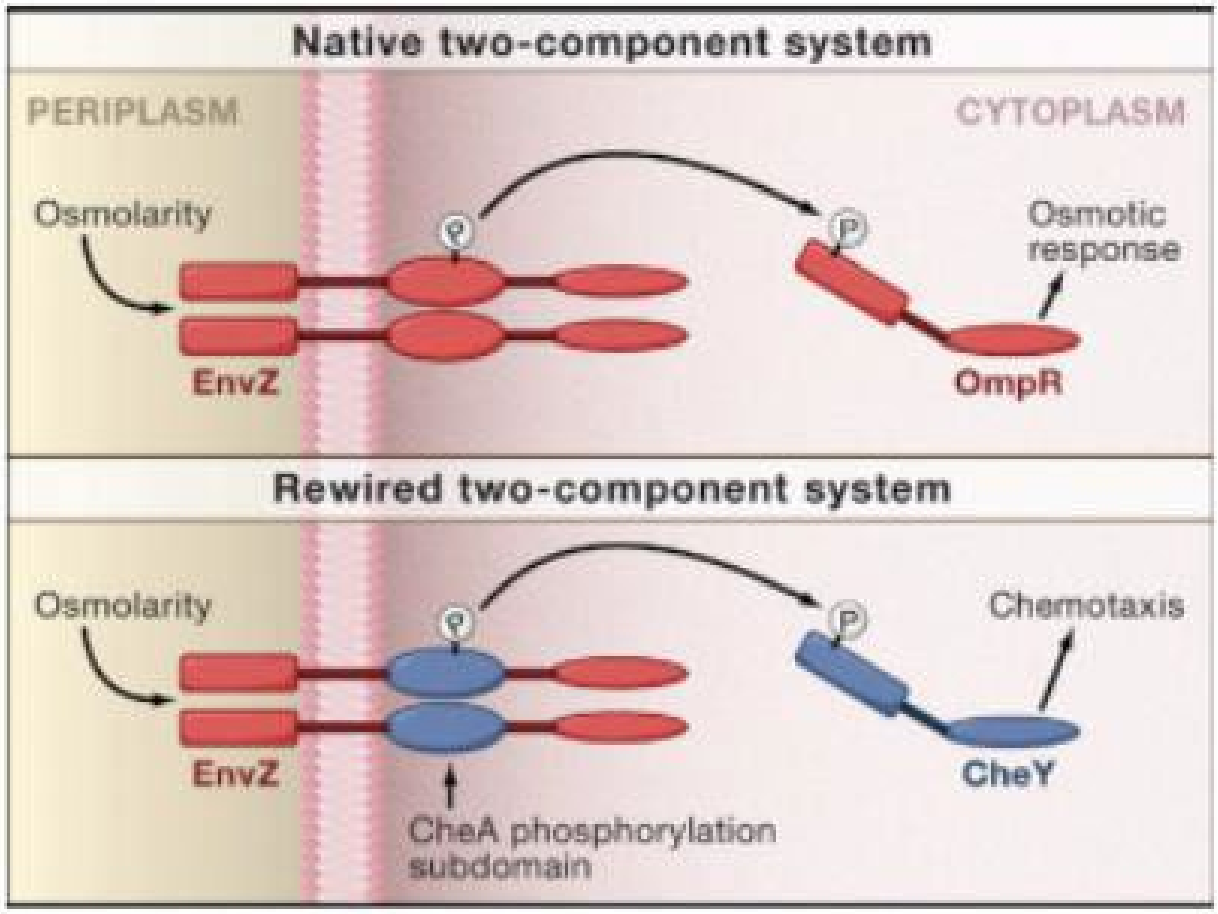
Evolution of pathway outputs

PhoQ-PhoP system response to extracellular Mg^{2+} concentration



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4097194/pdf/nihms589840.pdf/?tool=EBI

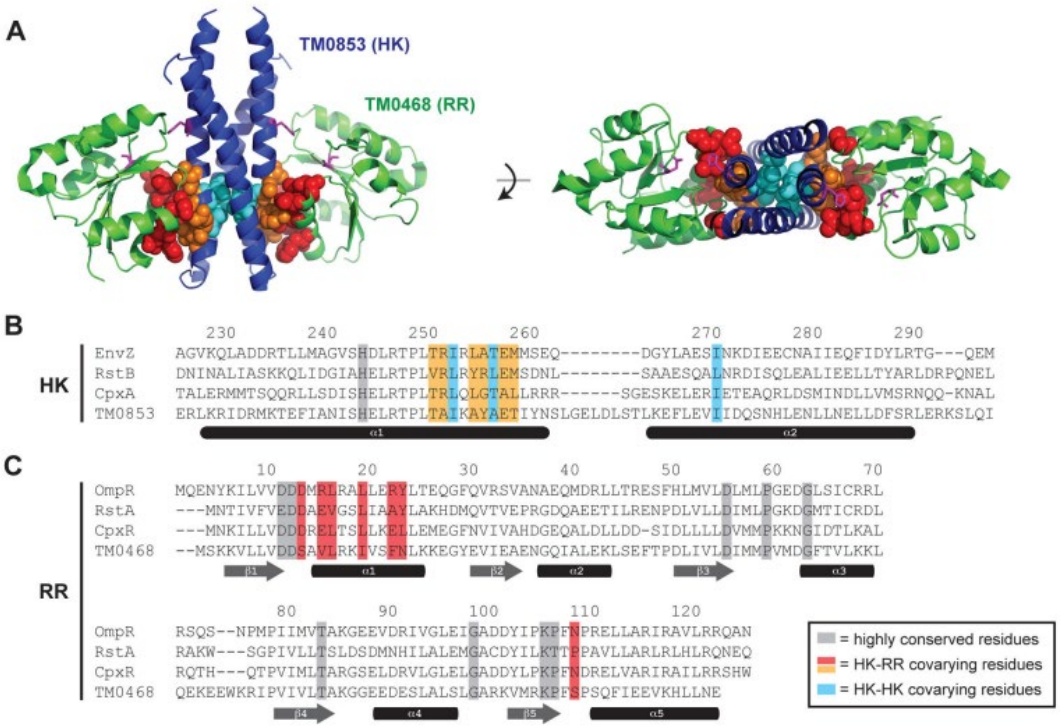
Specificity based on molecular recognition not scaffolds



https://www.cell.com/fulltext/S0092-8674(08)00697-1

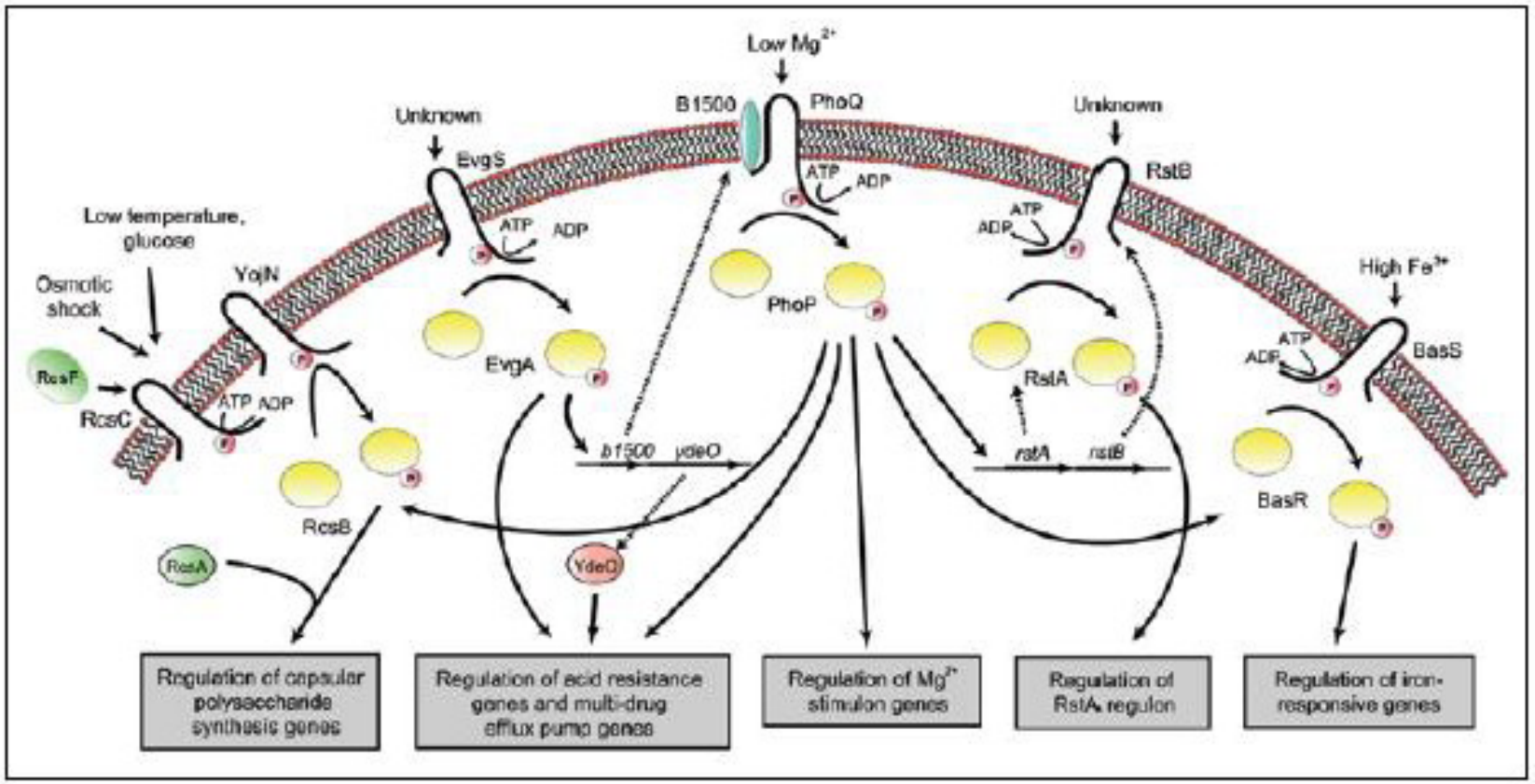
Specificity based on molecular recognition not scaffolds

Disadvantages mutations can disrupt interactions they must co-evolve



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4097194/pdf/nihms589840.pdf/?tool=EBI

Integration of Signals



Sinalização em eucariontes

Quinases classificadas de acordo com a sua atividade catalítica

- Tirosina quinases

Acoplada a receptores ex: NGFR

Não acopladas: Src

- Serina/ Treonina quinases

PKC, PKA

- Quinases de dupla especificidade (serina/ threonina e tirosina)

MEK1, MEK2

Fosforilação e Quinases

- ~ 500 proteina quinases, 2% do genoma humano e 2% do genoma de diversos organismos.

- ~700,000 resíduos potencialmente fosforilados no genoma humano.

-~10.000 proteins, 30% fosforiladas tamanho médio de ~400 aminoácidos, 17% resíduos fosforilados : serinas (8,5%) threonine (5.7) and tyrosine (3%).

.

Ubersax and Ferrell, 2007

Classificação das proteína quinases de eucariontes

- O genoma humano tem ~ 518 proteina quinases
- 478 são proteína quinases típicas com o domínio catalítico conservado.
- 40 com estruturas atípica

Manning et al. Science 298, 1912-1934 (2002)

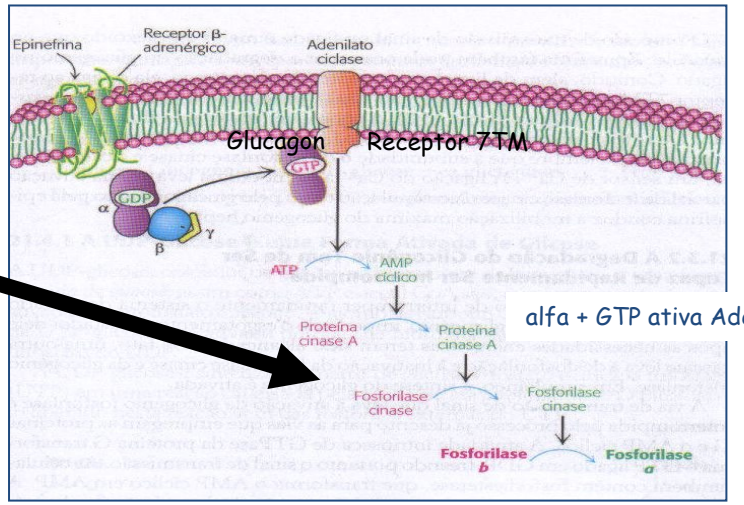
Grupos de quinases em diferentes espécies

Table 1. Kinase distribution by major groups in human and model systems. A detailed classification is available in tables S1 and S6.

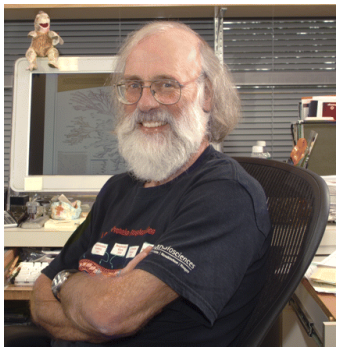
Group	Families	Subfamilies	Yeast kinases	Worm kinases	Fly kinases	Human kinases	Human pseudogenes	Novel human kinases
AGC	14	21	17	30	30	63	6	7
CAMK	17	33	21	46	32	74	39	10
CK1	3	5	4	85	10	12	5	2
CMGC	8	24	21	49	33	61	12	3
Other	37	39	38	67	45	83	21	23
STE	3	13	14	25	18	47	6	4
Tyrosine kinase	30	30	0	90	32	90	5	5
Tyrosine kinase–like	7	13	0	15	17	43	6	5
RGC	1	1	0	27	6	5	3	0
Atypical-PDHK	1	1	2	1	1	5	0	0
Atypical-Alpha	1	2	0	4	1	6	0	0
Atypical-RIO	1	3	2	3	3	3	1	2
Atypical-A6	1	1	1	2	1	2	2	0
Atypical-Other	7	7	2	1	2	9	0	4
Atypical-ABC1	1	1	3	3	3	5	0	5
Atypical-BRD	1	1	0	1	1	4	0	1
Atypical-PIKK	1	6	5	5	5	6	0	0
Total	134	201	130	454	240	518	106	71

Descoberta das quinases

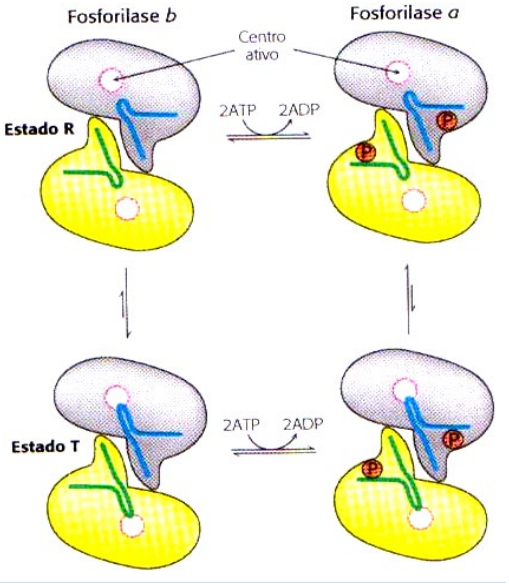
Krebs e Fischer
(1964) Prêmio nobel (1992)



alfa + GTP ativa Adenilato ciclase



Tony Hunter descoberta das tirosina quinases(1979)



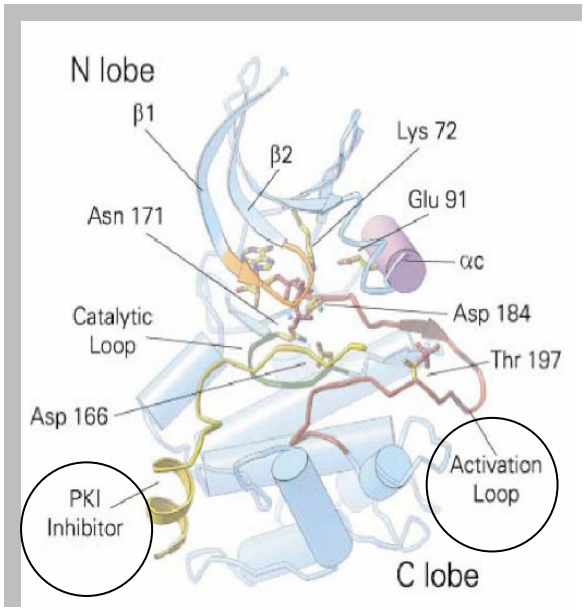
https://www.youtube.com/watch?v=8XKRAduG2Hk

Domínio catalítico das quinases

A maioria das proteínas quinases tem um domínio catalítico conservado conhecido como ePK.

ePKs contém o sítio de ligação do ATP e o sítio de ligação de substratos, sendo composto por ~260 residuos.

O sítio de ligação do ATP é altamente conservado de forma que os inibidores de quinases atuando neste sítio são menos específicos.



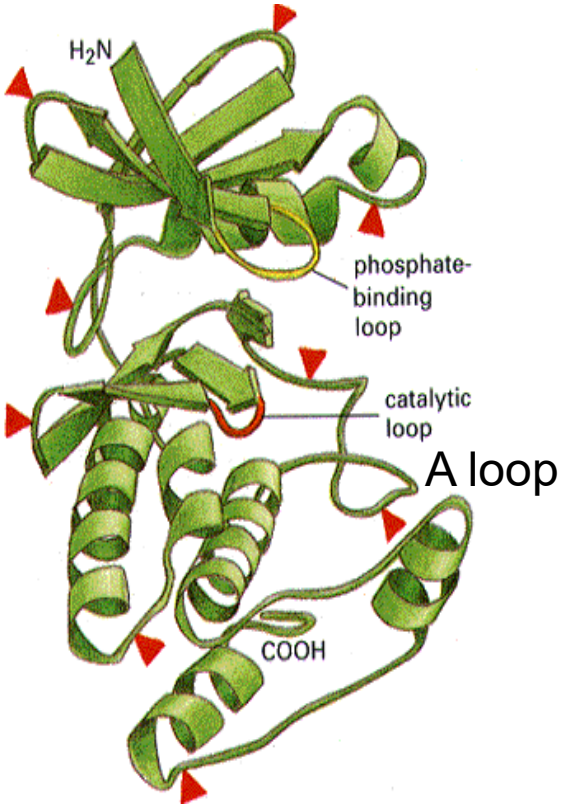
Estrutura geral das quinases

Often N and C-terminal lobes.

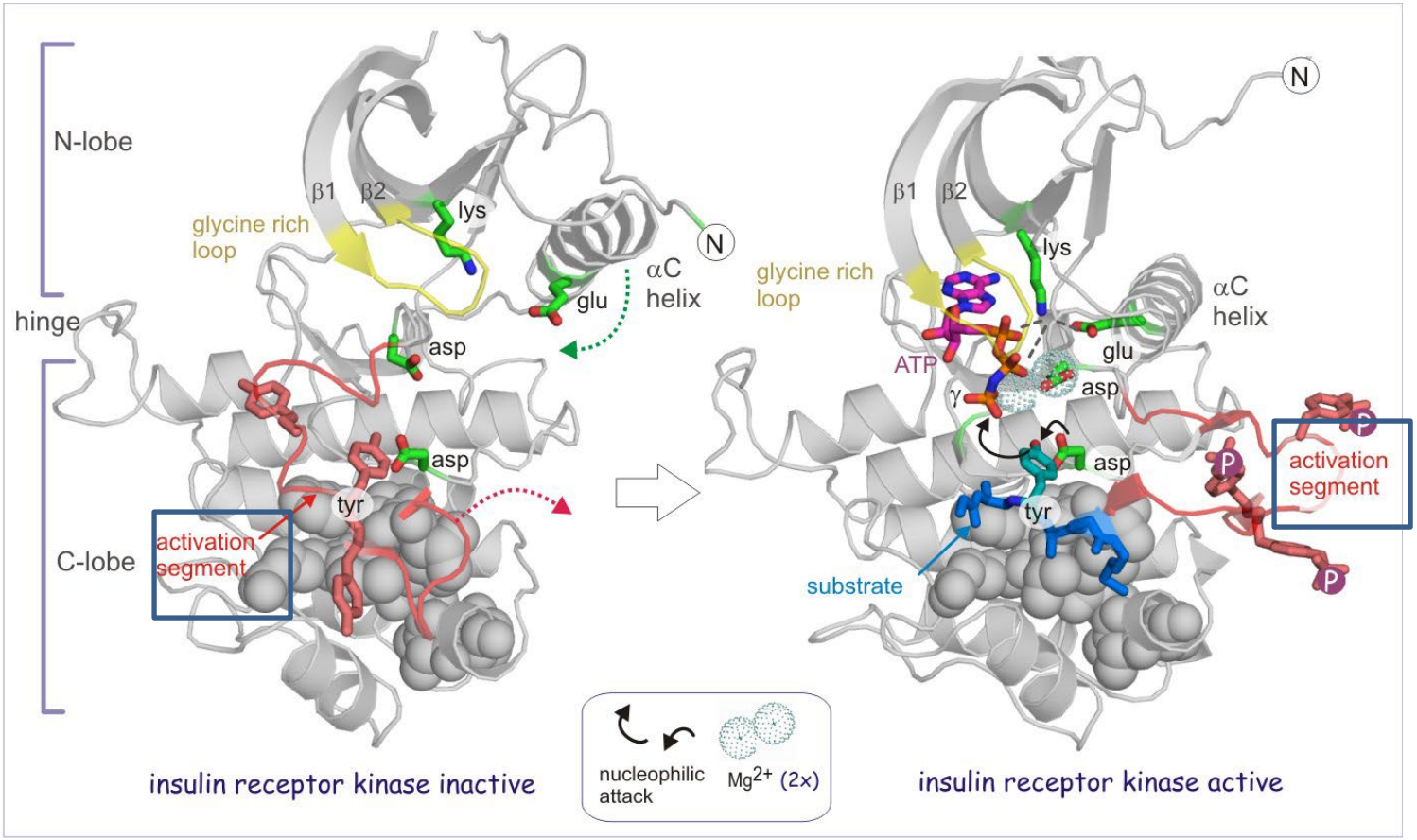
N-terminal ATP binding site.

Activating / catalytic loop (phosphorylated)

Short sequences (red arrowheads) a unique to specific kinase and may determine substrate specificity.

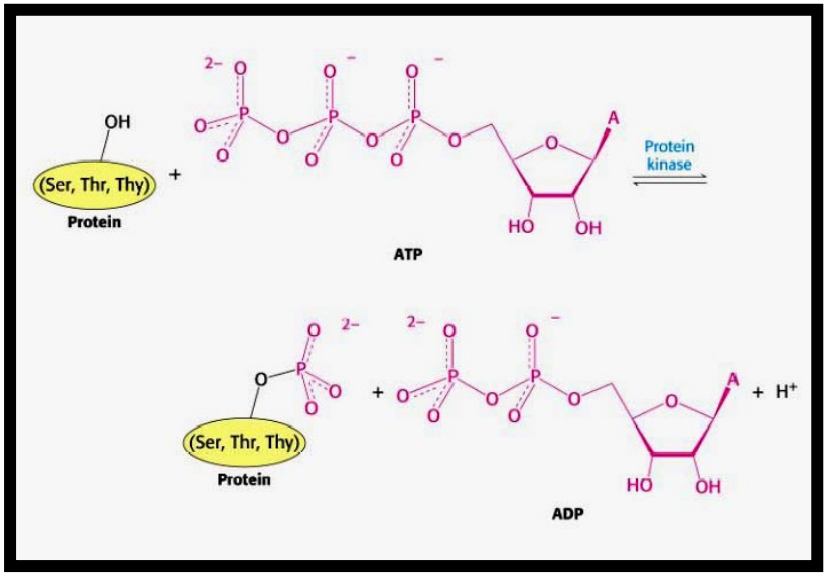


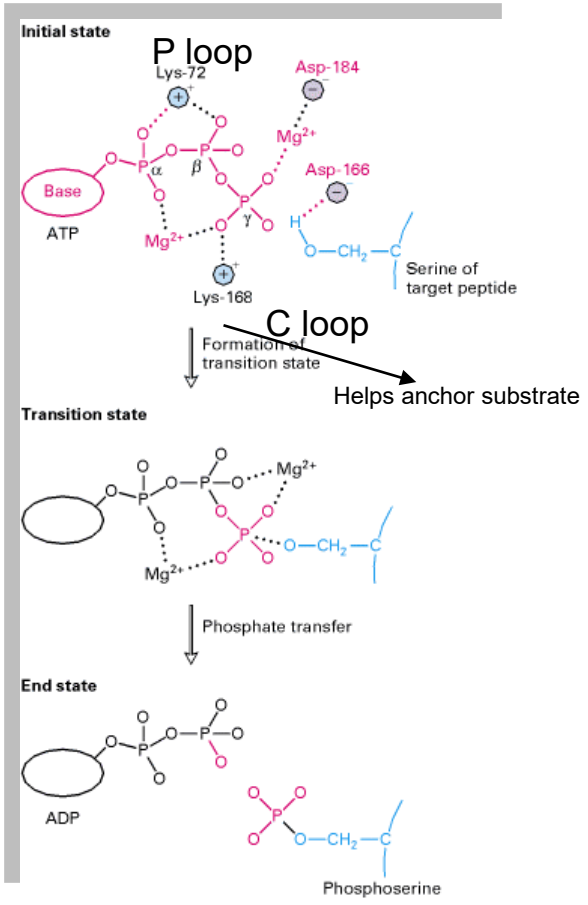
As quinases sofrem mudanças conformacionais que levam à ativação das mesmas



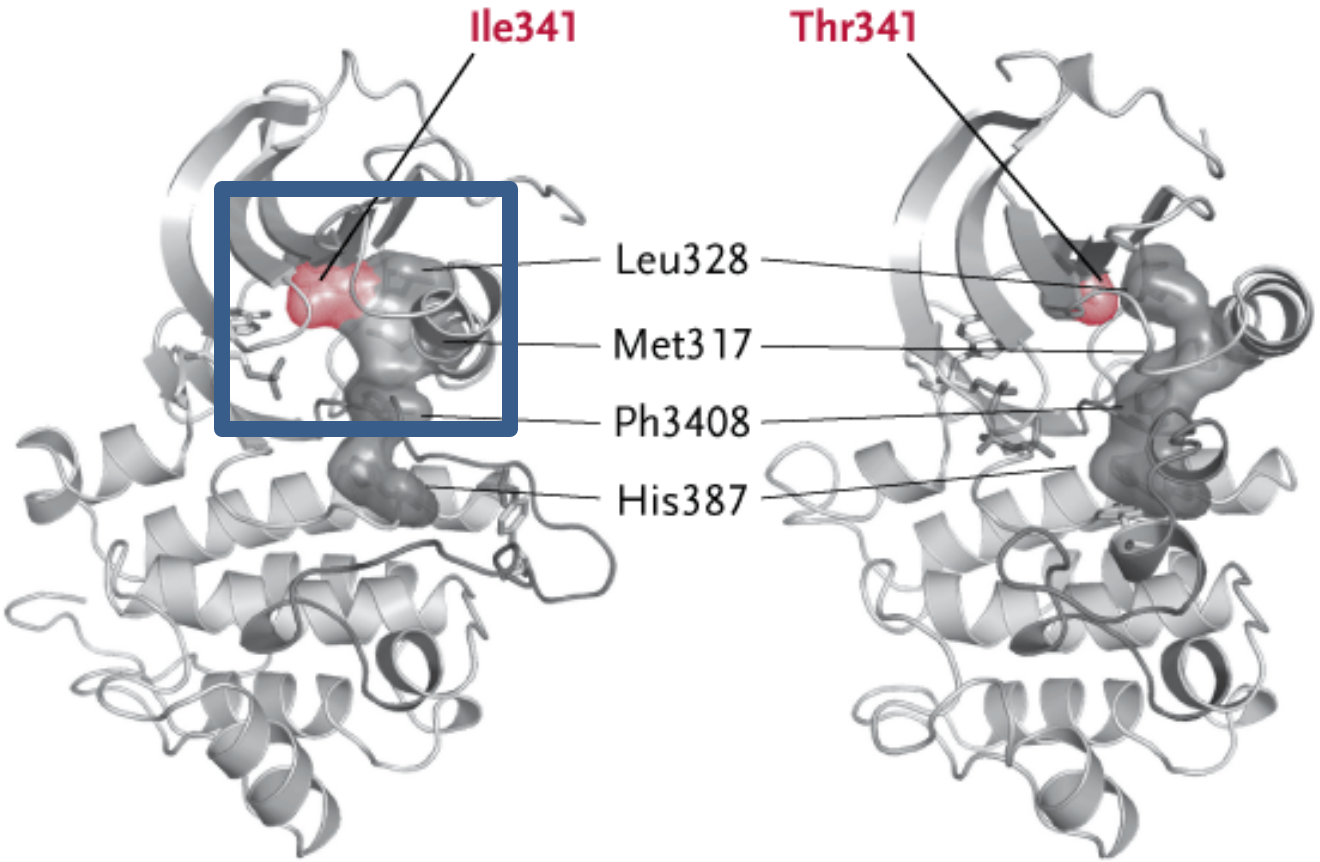
Changbong Hyeona, Patricia A. Jenningsb, Joseph A. Adamsc and José N. Onuchicd PNAS 2008

Phosphate transfer





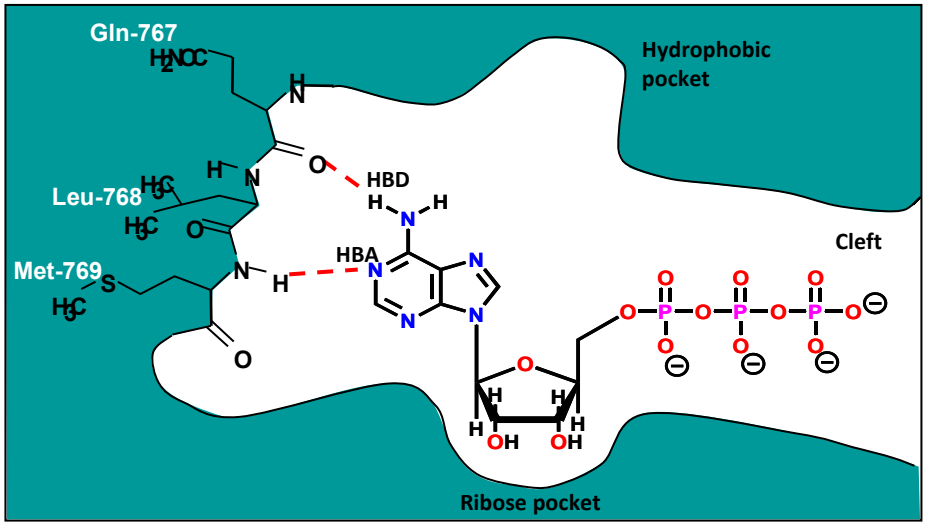
Mutations in c-Src T341I stabilizes the H spine increasing catalytic activity



George Daley (left), Mohammad Azam

Proteína Quinase

Sítio de ligação do ATP



- ***Hydrophobic pocket*** em frente ao **pocket** de ligação da ribose.

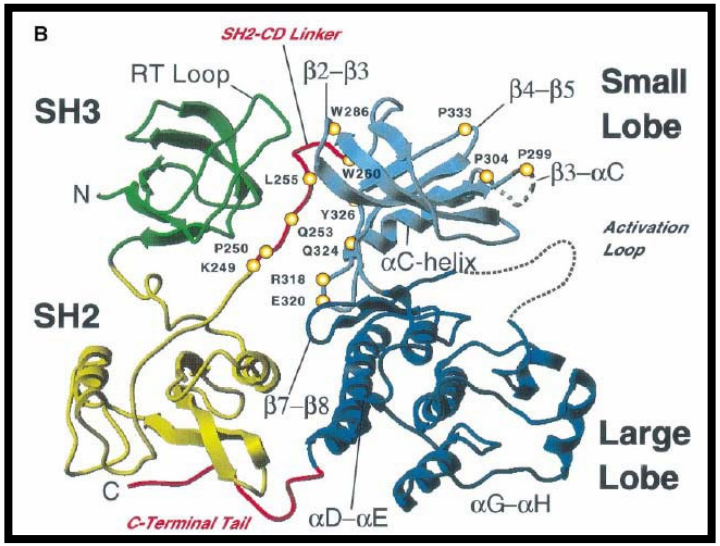
- ***O gatekeeper residue*** é um aminoácido na entrada do *Hydrophobic pocket* .

- O tamanho desse resíduo é importante para o *desenho* de drogas.

Domínios encontrados em proteínas quinases

Table 3. Most common Pfam domains in protein kinases. See table S7 for a fuller listing.

Domain name	Number of genes	Number of domains	Function class
Protein kinase C terminal domain	44	44	Accessory domain
Immunoglobulin domain (Ig)	30	254	Extracellular, protein interactions
Fibronectin type III domain (FnIII)	28	194	Extracellular, protein interactions
SH2 domain	25	27	Adaptor: Binds phosphotyrosine
SH3 domain	27	28	Adaptor: Binds proline-rich motifs
PH domain	23	22	Signaling; phospholipid binding
Diacylglycerol binding (C1, DAG_PE)	23	33	Phospholipid binding
Calmodulin binding motif	23	25	Not in Pfam. From literature and sequence alignment
SAM domain (Sterile alpha motif)	15	16	Dimerization domain
Ephrin receptor ligand binding domain	14	14	Ligand binding
CNH domain	12	12	Cytoskeletal?
HEAT, armadillo/β-catenin repeats	10	27	Protein interaction
Activin receptor	11	11	Ligand binding
Ankyrin repeat (ANK)	9	59	Protein interaction
Regulator of G protein signaling (RGS)	7	7	GTPase interaction
PDZ/DHR/GLGF domain	7	7	Membrane targeting
Ubiquitin-associated domain A (UBA)	7	8	Protein degradation
Receptor L domain	7	14	Ligand binding
Furin-like cysteine rich region	7	21	Receptor dimerization?
p21-Rho-binding domain (PBD, CRIB)	9	9	GTPase interaction
Phosphatidylinositol 3′-kinase (PI3K)	6	6	Catalytic: Protein kinase
FAT	6	6	Accessory domain for PI3K
FATC	6	6	Accessory domain for PI3K
Alpha kinase	6	6	Catalytic: Atypical kinase
C2 domain	6	6	Ca^{2+}, phospholipid binding
Guanylate cyclase catalytic domain	5	5	Catalytic: cGMP production
HSP90-like ATPase	5	5	Catalytic: Atypical kinase
ANF receptor	5	5	Ligand binding
Kinase-associated domain 1 (KA1)	5	5	Unknown
Bromodomain	8	13	Acetyl-lysine (chromatin) binding domain
HR1 repeat	5	13	GTPase interaction
Leucine-rich repeat	5	30	Ligand binding, protein interaction
ABC1 family	5	5	Catalytic: Atypical kinase
Death domain	6	6	Dimerization domain
BTK motif	4	4	Signaling
RhoGEF domain	4	5	GTPase interaction (guanine exchange factor)



A atividade das quinases pode ser regulada por mecanismos específicos

- Fosforilação reversível do *loop* de ativação.

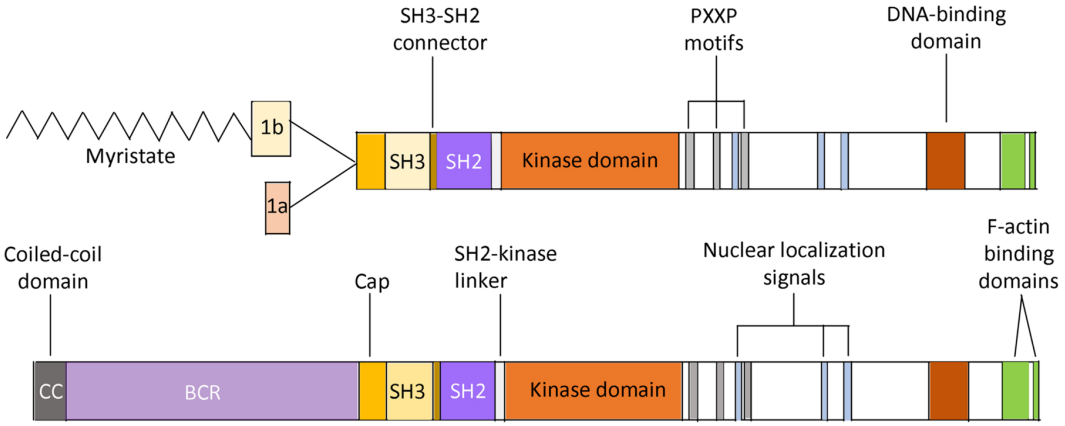
- Interação com outras proteínas e quinases.

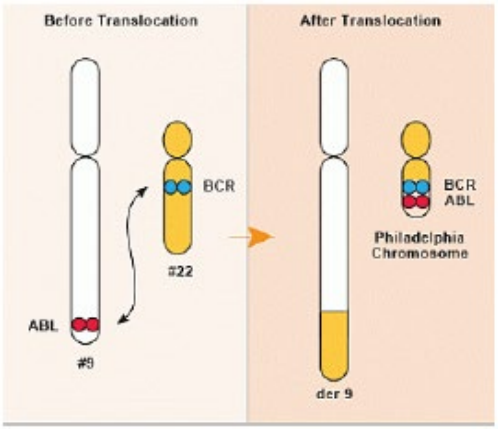
- Interação com co-fatores.

-Translocação para localizações sub-celulares específicas.

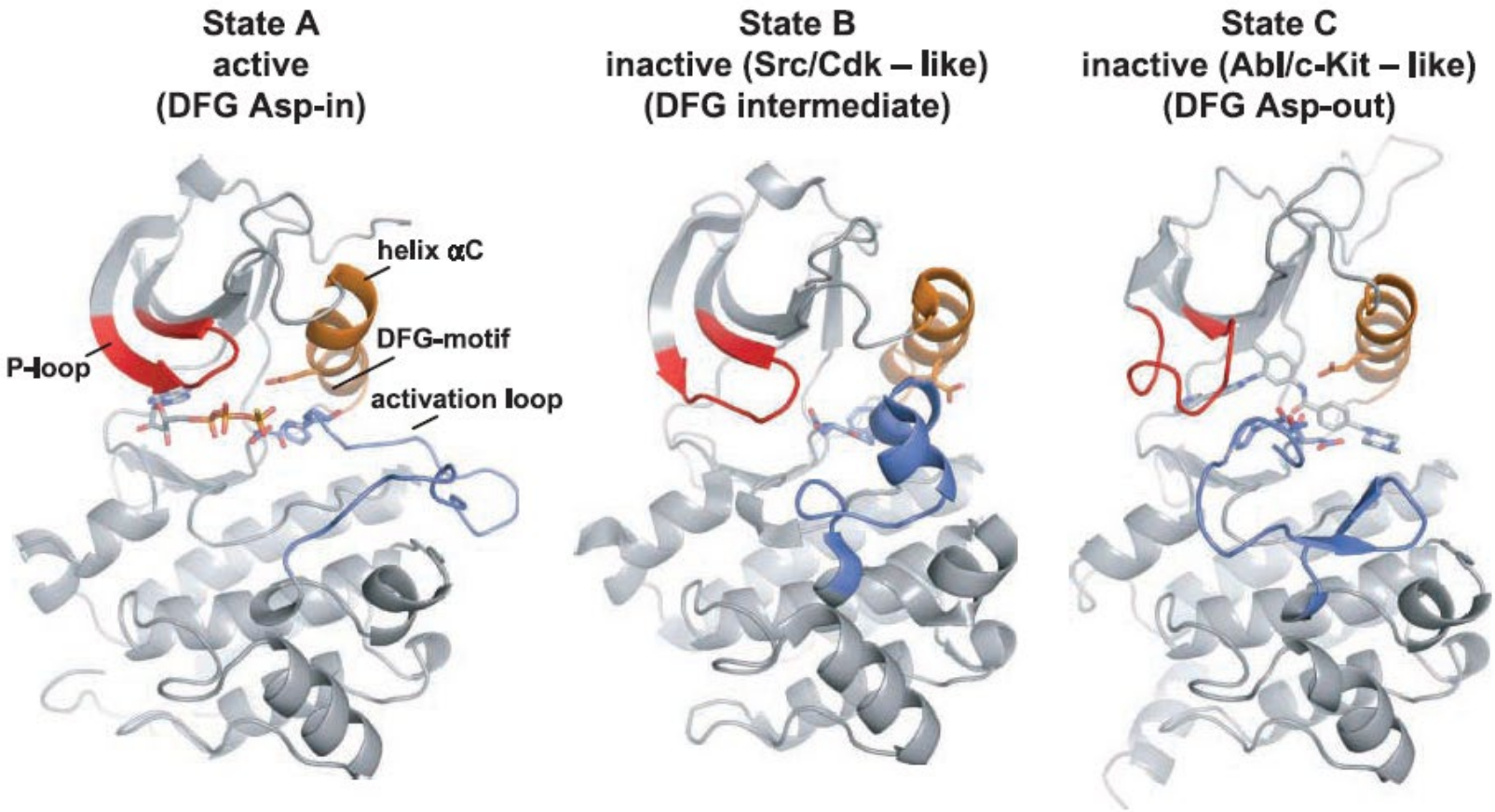
Esses processos em conjunto auxiliam na determinação da especificidade das quinases.

Leucemia mieloide crônica





https://www.sciencedirect.com/science/article/pii/S1043661822001013?via%3Dihub


State A
active
(DFG Asp-in)
State B
inactive (Src/Cdk – like)
(DFG intermediate)
State C
inactive (Abl/c-Kit – like)
(DFG Asp-out)
helix αC
DFG-motif
P-loop
activation loop

Inibidores de Proteína Quinase

- Tipo I atuam sobre a conformação ativa da enzima.

- Tipo I ligam o sítio de ligação do ATP bloqueando o acesso ao ATP

- Tipo II atuam sobre a conformação inativa da enzima.

- Tipo II estabilizam a conformação inativa.

- Tipo II são mais seletivos

Type I inhibitors

Gefitinib, erlotinib, SU11248 and seliciclib

Type II inhibitors

Imatinib, lapatinib, sorafenib and vatalanib

BIOCHEMISTRY

Revisiting protein kinase–substrate interactions: Toward therapeutic development

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REVIEW

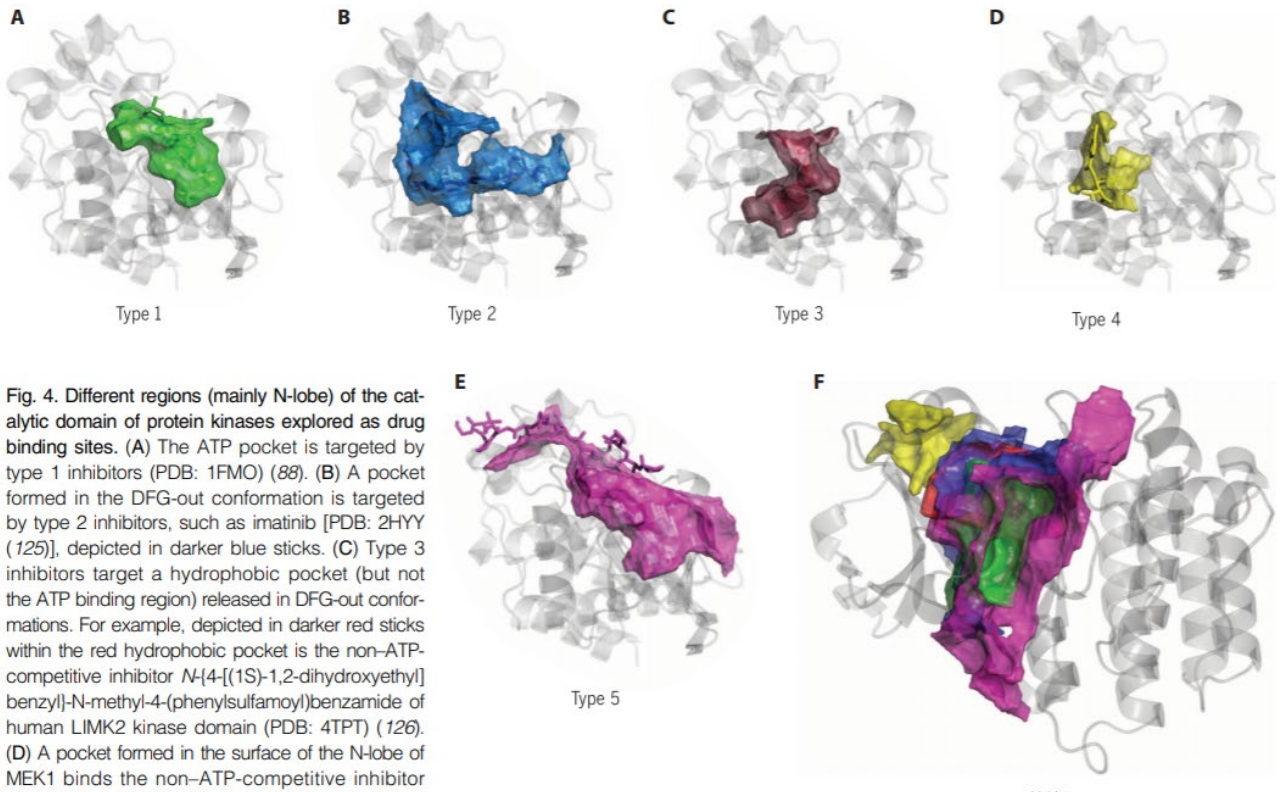


Fig. 4. Different regions (mainly N-lobe) of the catalytic domain of protein kinases explored as drug binding sites. (**A**) The ATP pocket is targeted by type 1 inhibitors (PDB: 1FMO) (*88*). (**B**) A pocket formed in the DFG-out conformation is targeted by type 2 inhibitors, such as imatinib [PDB: 2HYY (*125*)], depicted in darker blue sticks. (**C**) Type 3 inhibitors target a hydrophobic pocket (but not the ATP binding region) released in DFG-out conformations. For example, depicted in darker red sticks within the red hydrophobic pocket is the non–ATP-competitive inhibitor *N*-[4-[(1S)-1,2-dihydroxyethyl] benzyl]-N-methyl-4-(phenylsulfamoyl)benzamide of human LIMK2 kinase domain (PDB: 4TPT) (*126*). (**D**) A pocket formed in the surface of the N-lobe of MEK1 binds the non–ATP-competitive inhibitor 2-([3R-3,4-dihydroxybutyl]oxy)-4-fluoro-6-[(2-fluoro-4-iodophenyl)amino]benzamide (PDB: 4ARK) (*127*). (**E**) A shallow crevice and ATP binding pocket are occupied by an inhibitor formed by a synthetic peptide linked to thiophosphoric acid *o*-((adenosyl-phospho)phospho)-*s*-acetamidyl-diester, a typical type 4 inhibitor (magenta sticks; PDB: 1GAG) (*117*). (**F**) General view of all surfaces of pockets used by different inhibitor types. The reference structure (gray) is PDB: 2HYY (*125*).

Sítio de ligação do Substrato

Proteína Quinase

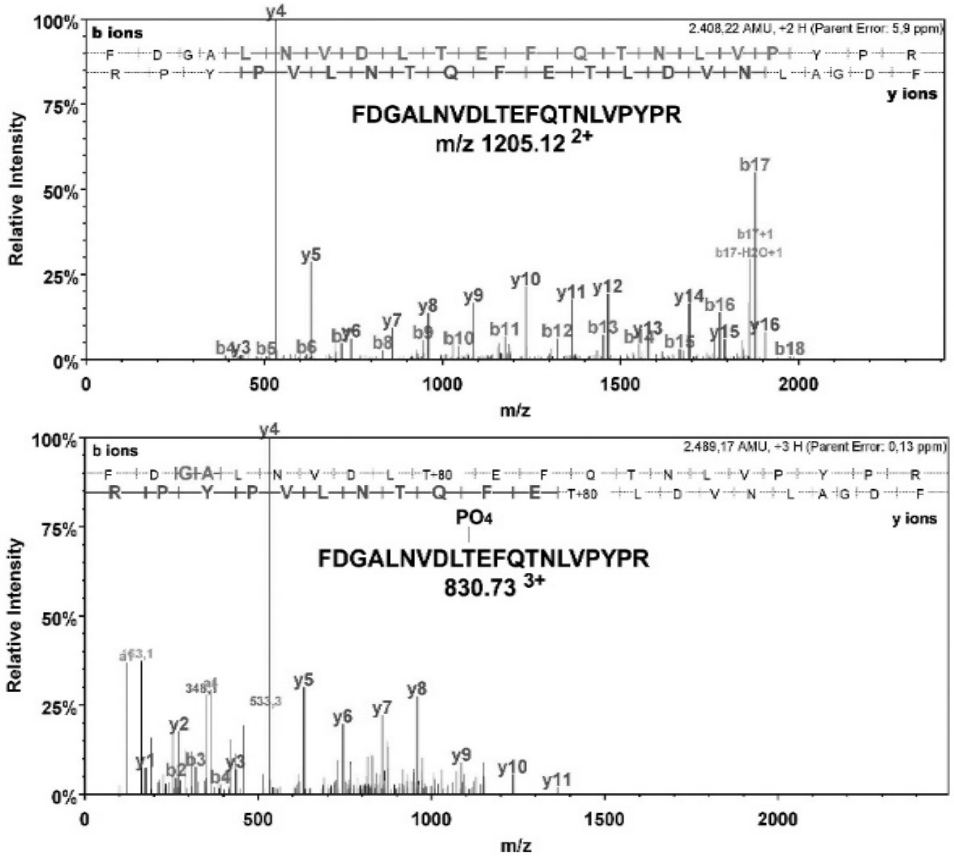
- A especificidade é dada pelo *canal de ligação do substrato* que reconhece o aminoácido que será fosforilado e aminoácidos perto dele (geralmente no N-terminal do aminoácido fosforilado).

Kinase name	Consensus sequence	# phosph.
PKACa (PRKACA)	xxrxRRl**S**lxxxxxx	734
PKCa (PRKCA)	xxxxRRx**S**fKrkkxx	523
CK2a1 (CSNK2A1)	xxxeeed**S**DdEeeee	483
ERK2 (MAPK1)	xxpxpPl**S**Ptppxxx	410
CDK1 (CDC2)	xxxxlpx**S**Pxkkxxx	393
SRC	xxxeedv**Y**gxvxxxx	385
ERK1	xxpppPl**S**Ptptxxx	292
CDK2	xxxxxpx**S**PgKkxlx	201
PDK1 (PDPK1)	xxgxttx**T**FCGTpeY	43

T^{253} da α-tubulina não está contida numa sequência *consenus* reconhecida pela PKC

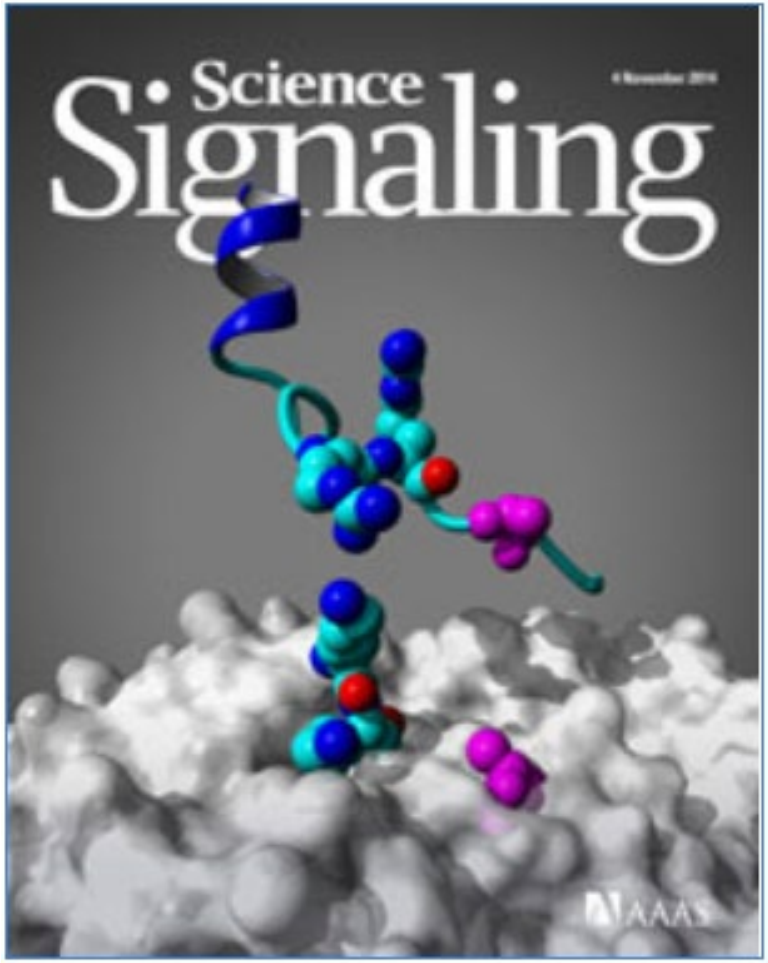
α-tubulina DL[T]EF

"PKC consensus sequence" RRX[S/T]



Lemos Duarte, et. al., Science Signaling 2014

Sequências *consensus* podem ser estruturalmente formadas



Lemos Duarte, et. al., Science Signaling 2014

Kinase resources
cAPK structural walkthrough
http://pkr.sdsc.edu/html/3D/xray/2cpk/2cpkwalk.html

Protein kinase databases
http://www.kinasenet.org/pkr/Welcome.do
http://kinase.ucsf.edu/ksd/
http://bioinf.uta.fi/KinMutBase/main_frame.html
http://www.kinase.com/

Phosphorylation sites
http://phospho.elm.eu.org/
http://scansite.mit.edu/
http://www.phosphosite.org/Login.jsp

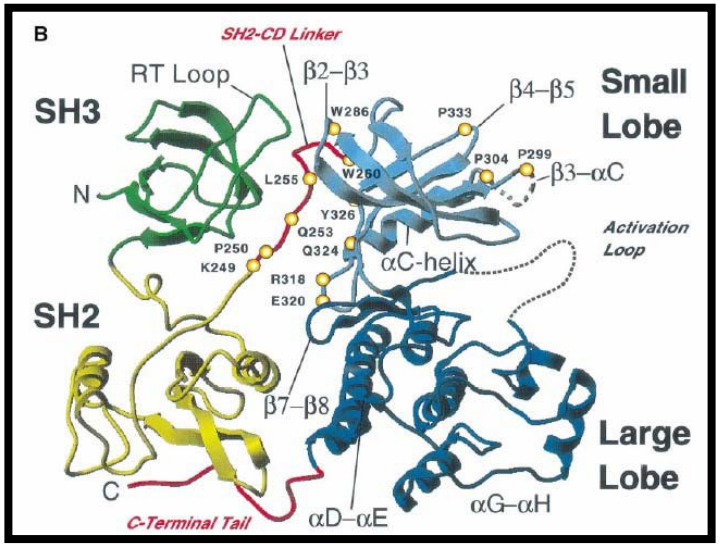
Signal transduction illustrated
http://www.bio.davidson.edu/courses/Immunology/Flash/MAPK.html

http://www.youtube.com/watch?v=TQ2C-5P-I5E

Domínios encontrados em proteínas quinases

Table 3. Most common Pfam domains in protein kinases. See table S7 for a fuller listing.

Domain name	Number of genes	Number of domains	Function class
Protein kinase C terminal domain	44	44	Accessory domain
Immunoglobulin domain (Ig)	30	254	Extracellular, protein interactions
Fibronectin type III domain (FnIII)	28	194	Extracellular, protein interactions
SH2 domain	25	27	Adaptor: Binds phosphotyrosine
SH3 domain	27	28	Adaptor: Binds proline-rich motifs
PH domain	23	22	Signaling; phospholipid binding
Diacylglycerol binding (C1, DAG_PE)	23	33	Phospholipid binding
Calmodulin binding motif	23	25	Not in Pfam. From literature and sequence alignment
SAM domain (Sterile alpha motif)	15	16	Dimerization domain
Ephrin receptor ligand binding domain	14	14	Ligand binding
CNH domain	12	12	Cytoskeletal?
HEAT, armadillo/β-catenin repeats	10	27	Protein interaction
Activin receptor	11	11	Ligand binding
Ankyrin repeat (ANK)	9	59	Protein interaction
Regulator of G protein signaling (RGS)	7	7	GTPase interaction
PDZ/DHR/GLGF domain	7	7	Membrane targeting
Ubiquitin-associated domain A (UBA)	7	8	Protein degradation
Receptor L domain	7	14	Ligand binding
Furin-like cysteine rich region	7	21	Receptor dimerization?
p21-Rho-binding domain (PBD, CRIB)	9	9	GTPase interaction
Phosphatidylinositol 3′-kinase (PI3K)	6	6	Catalytic: Protein kinase
FAT	6	6	Accessory domain for PI3K
FATC	6	6	Accessory domain for PI3K
Alpha kinase	6	6	Catalytic: Atypical kinase
C2 domain	6	6	Ca^{2+}, phospholipid binding
Guanylate cyclase catalytic domain	5	5	Catalytic: cGMP production
HSP90-like ATPase	5	5	Catalytic: Atypical kinase
ANF receptor	5	5	Ligand binding
Kinase-associated domain 1 (KA1)	5	5	Unknown
Bromodomain	8	13	Acetyl-lysine (chromatin) binding domain
HR1 repeat	5	13	GTPase interaction
Leucine-rich repeat	5	30	Ligand binding, protein interaction
ABC1 family	5	5	Catalytic: Atypical kinase
Death domain	6	6	Dimerization domain
BTK motif	4	4	Signaling
RhoGEF domain	4	5	GTPase interaction (guanine exchange factor)



Interações proteína-proteína e proteínas adaptadoras auxiliam na propagação do sinal.

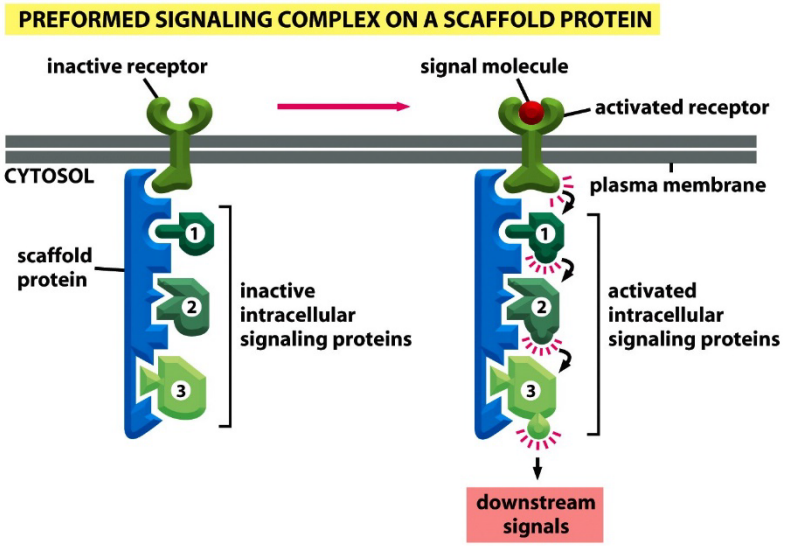


Figure 15-21a Molecular Biology of the Cell 5/e (© Garland Science 2008)

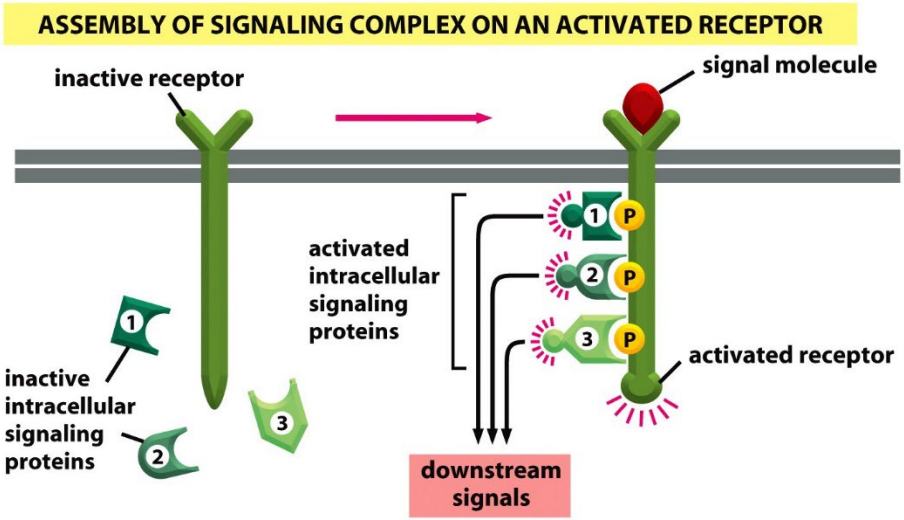
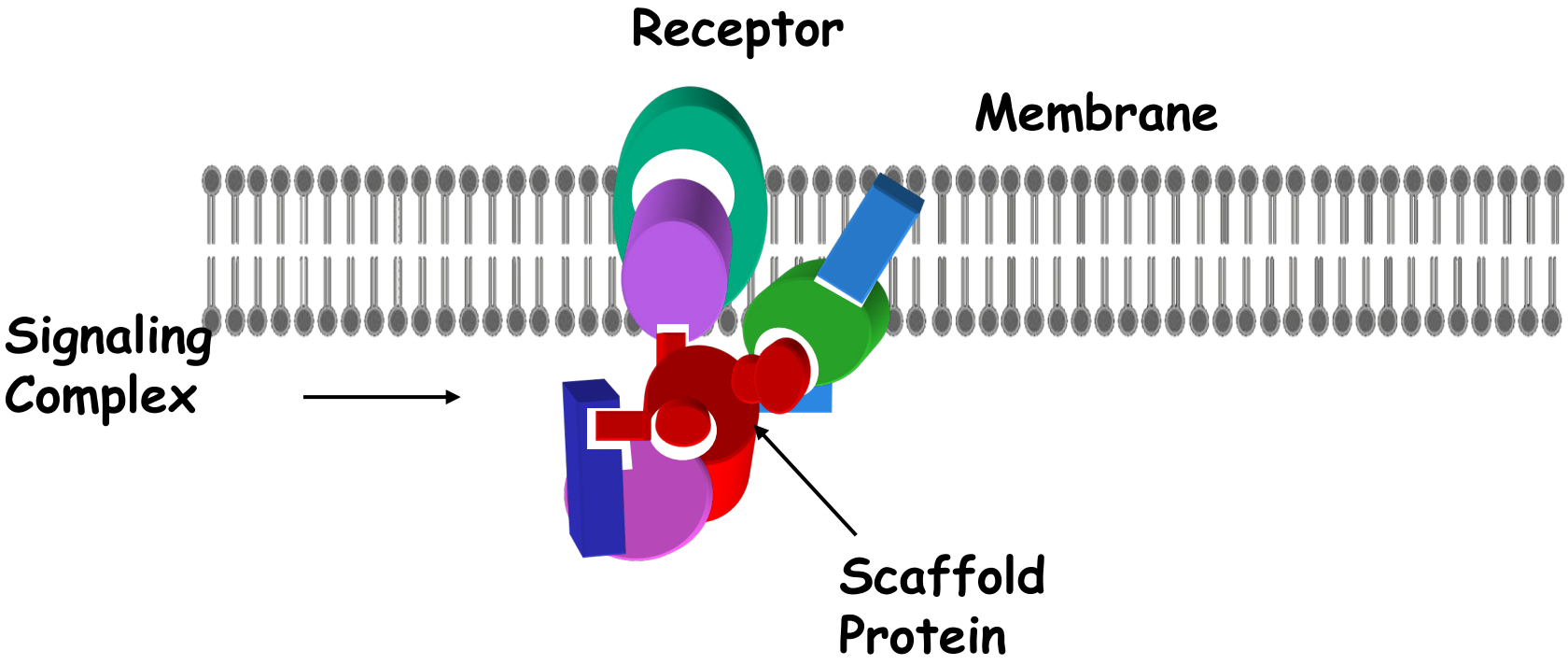
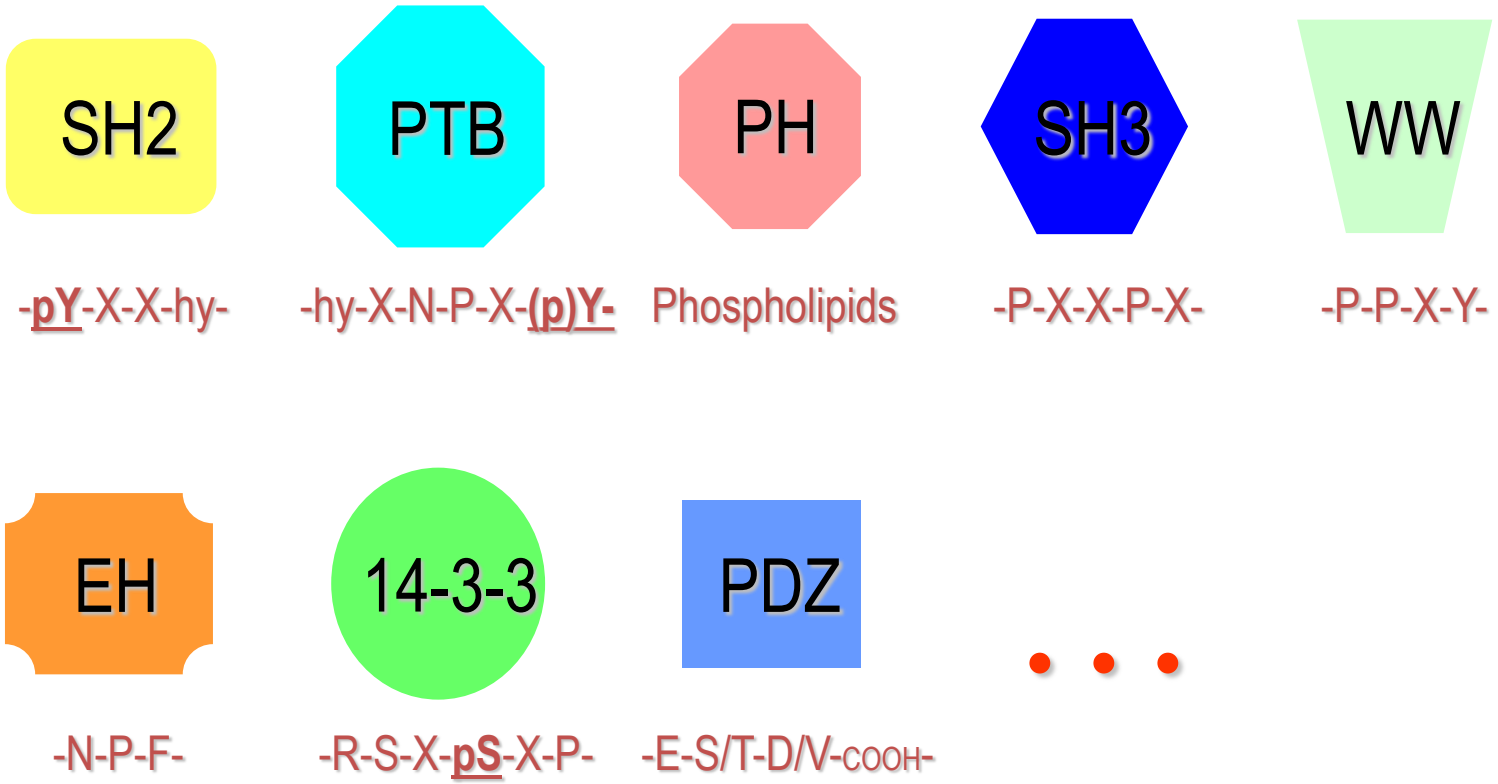


Figure 15-21b Molecular Biology of the Cell 5/e (© Garland Science 2008)

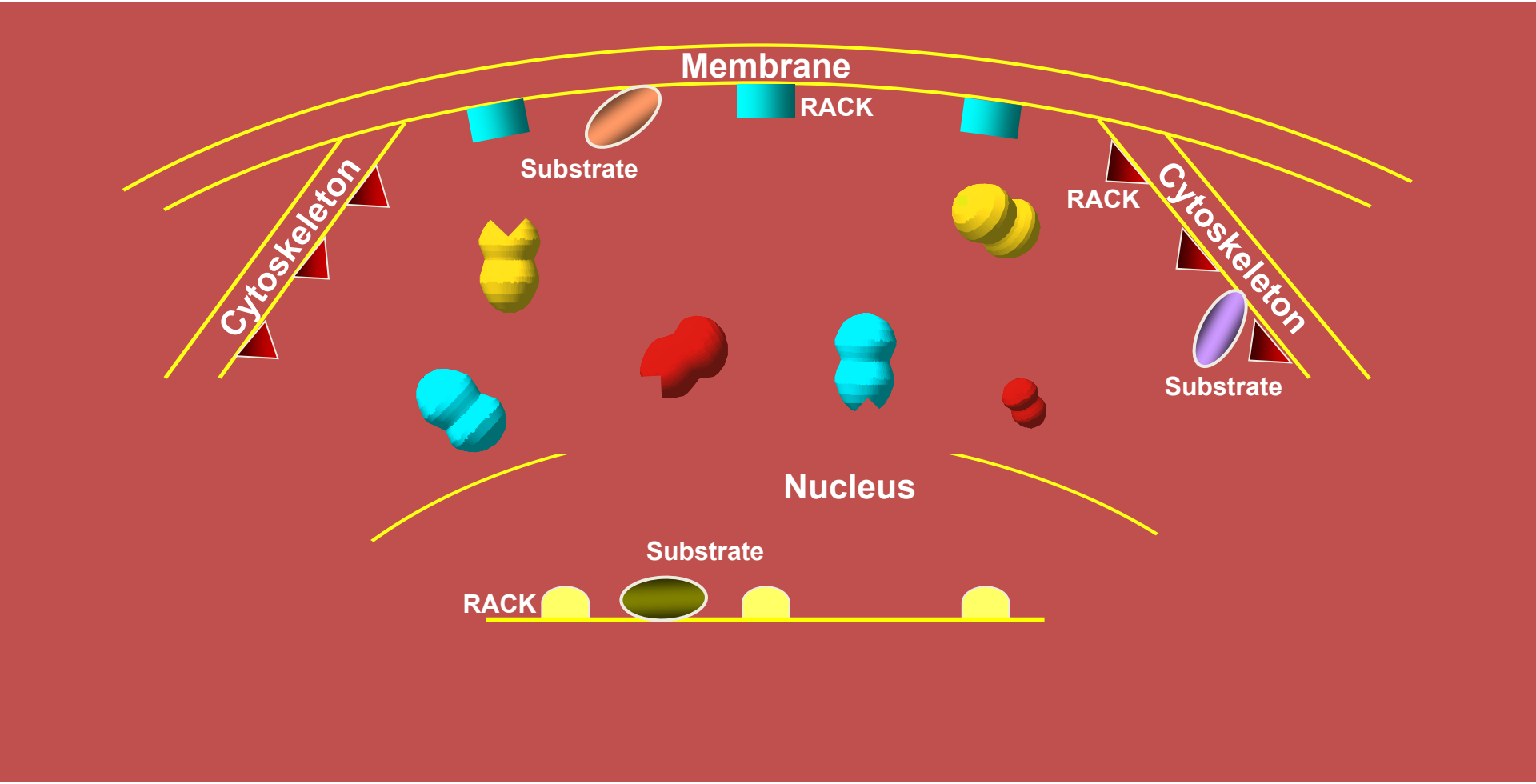
Scaffolds



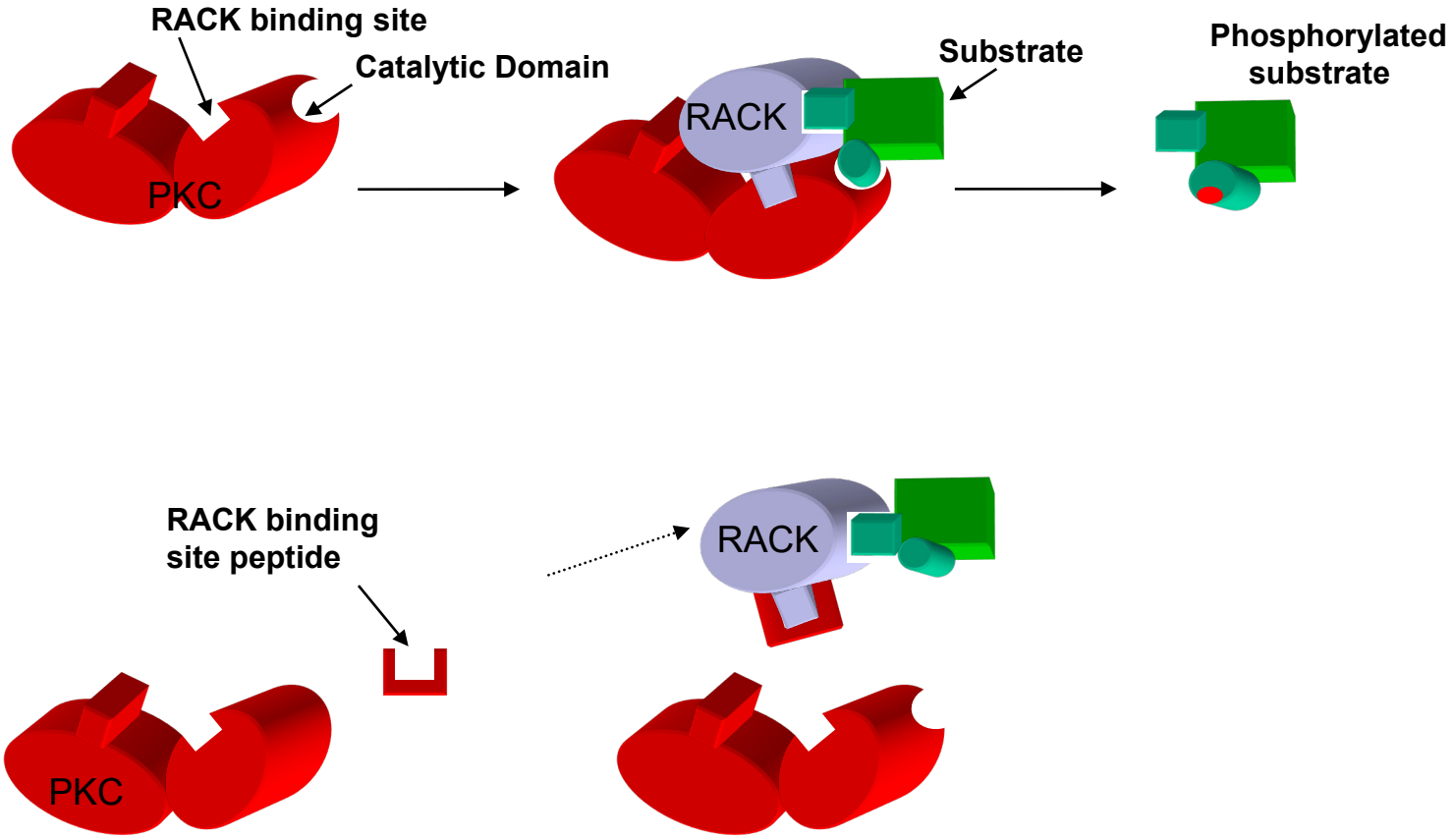
Protein Interaction Modules



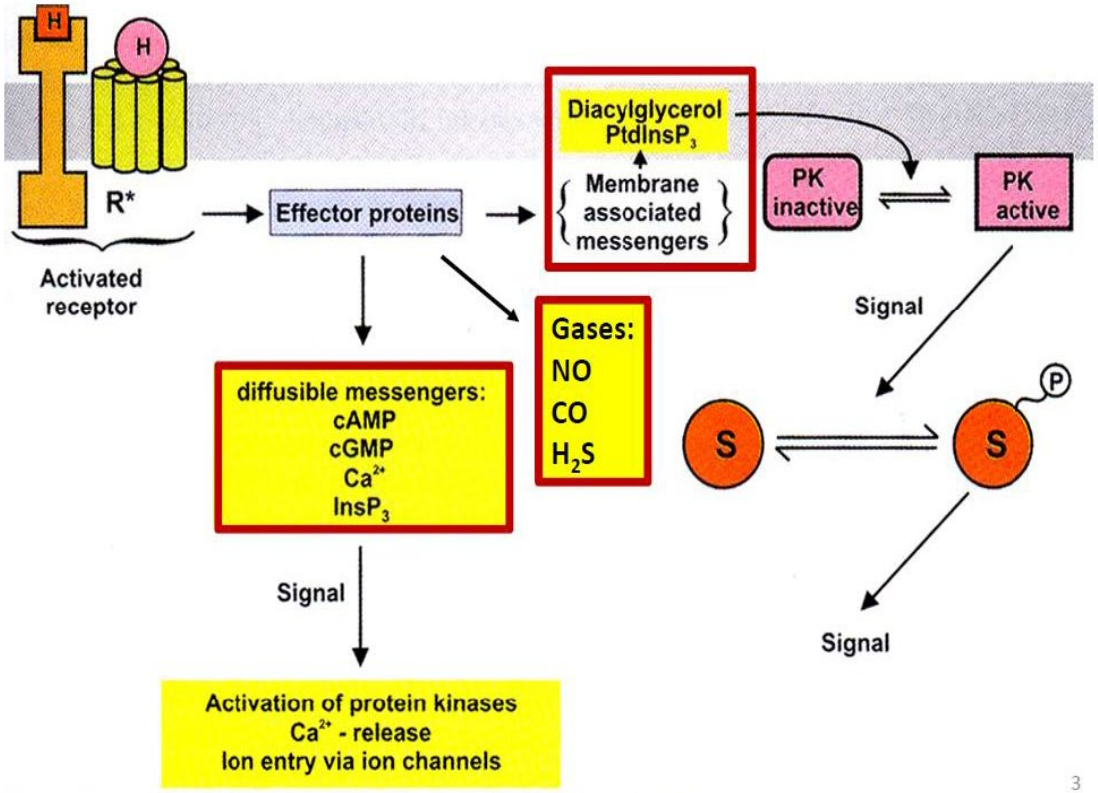
Sinalização da Proteína Quinase C



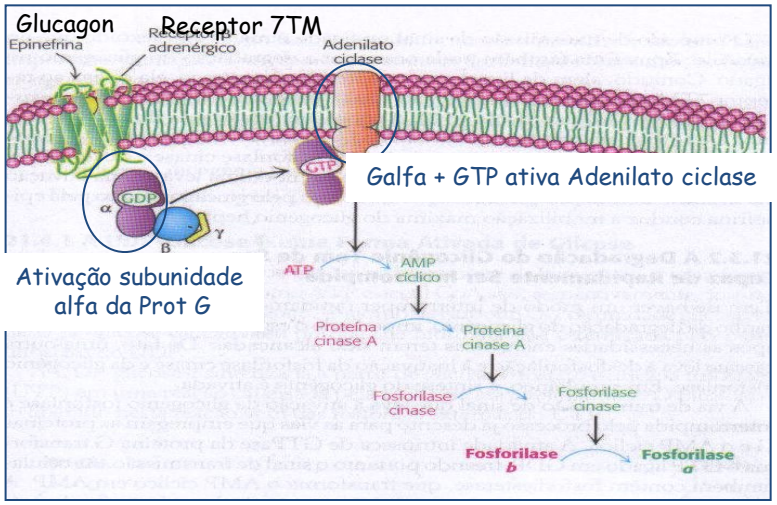
Peptide inhibitors of PKC isoenzymes



Second messengers



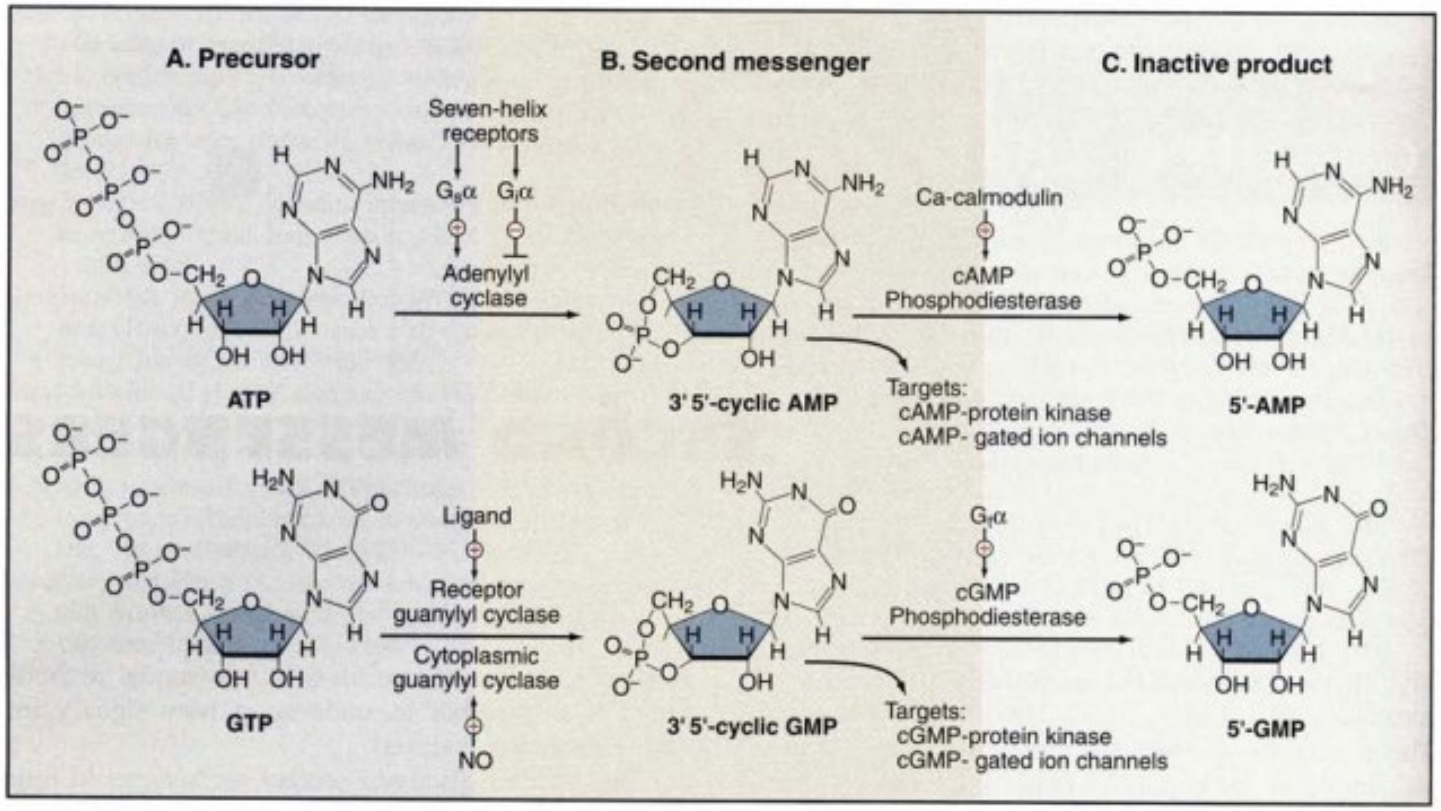
modified from Biochemistry of Signal Transduction and Regulation. 2008. 4th ed. Gerhard Kraus



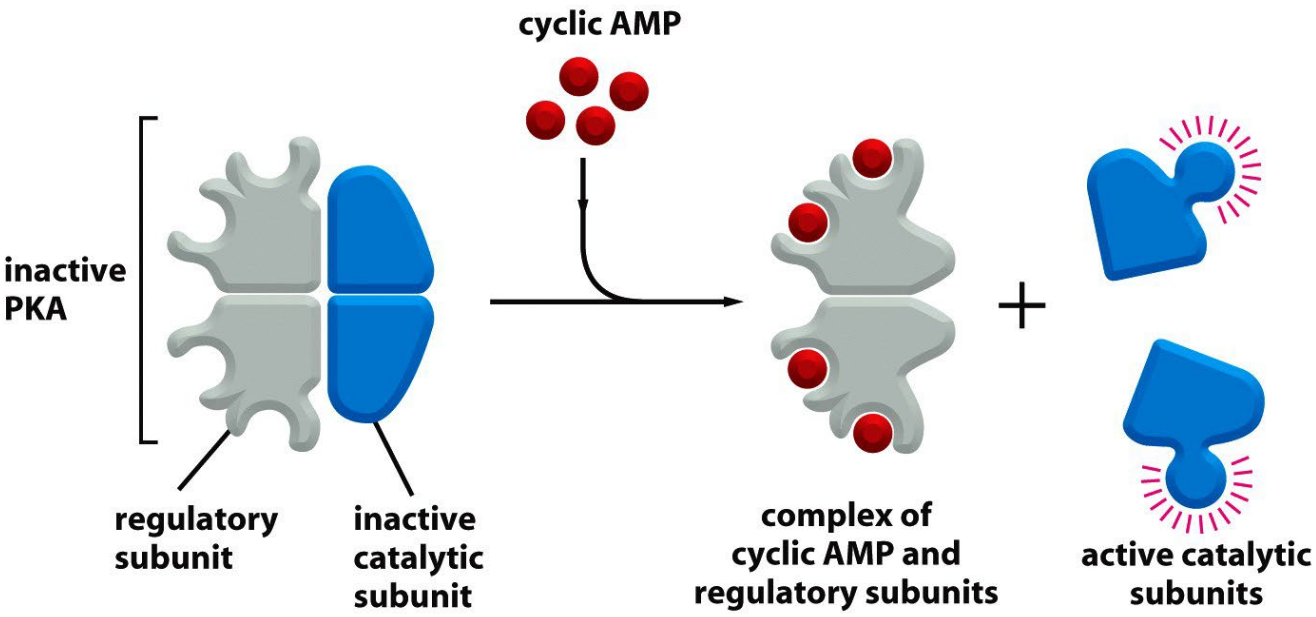
Earl Wilbur Sutherland, Jr., discovered second messengers, for which he won the 1971 Nobel Prize in Physiology or Medicine. Sutherland saw that epinephrine would stimulate the liver to convert glycogen to glucose (sugar) in liver cells, but epinephrine alone would not convert glycogen to glucose.

He found that epinephrine had to trigger a second messenger, cyclic AMP, for the liver to convert glycogen to glucose. The mechanisms were worked out in detail by Martin Rodbell and Alfred G. Gilman, who won the 1994 Nobel prize

Cyclic Nucleotides



PKA


cyclic AMP
inactive PKA
regulatory subunit
inactive catalytic subunit
complex of cyclic AMP and regulatory subunits
active catalytic subunits

PKA Signaling Scafold and second mesenger