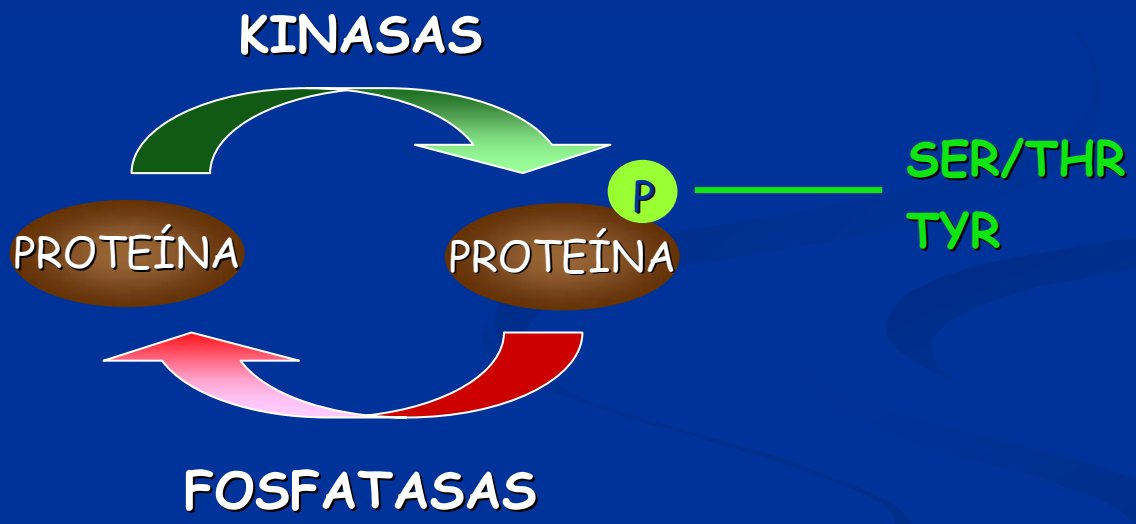
Señalización por fosforilación en tirosinas

LA FOSFORILACIÓN ES UN MECANISMO REVERSIBLE DE REGULACIÓN DE PROTEÍNAS

1959 Fischer y Krebs	La fosforilación como mecanismo reversible de regulación de la actividad de proteínas: Fosforilasa kinasa (Ser/Thr kinasa)
1980 Hunter y Sefton	Descubrimiento de la fosforilación en tirosinas



1955	Fosforilasa fosfatasa, PP1
1988 Tonks, Diltz y Fischer	Identificación de la primera fosfatasa de tirosinas

32.000 HUMAN GENES

- 20 % SIGNAL TRANSDUCTION
- 518 PROTEIN KINASES (2%)
  - 90 PTK GENES (0.3 %)
    - 58 RTK-20 subfamilies
    - 32 NON-RTK (CYTOPLASMIC)-10 subfamilies
- 130 PHOSPHATASES
  - 107 TYROSINE PHOSPHATASES

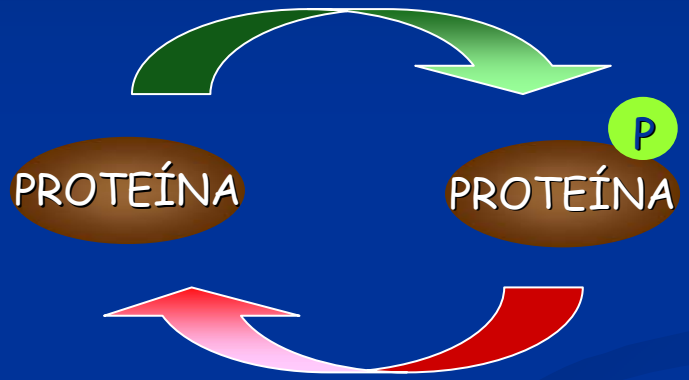
PHOSPHORYLATION

- 30 % HUMAN PROTEINS CONTAIN PHOSPHATE BOUND COVALENTLY
  - 99.9 % SER/THR
  - 0.1 % TYR

Why is so important tyrosine phosphorylation?

1. Growth factor signaling
2. Cell adhesion, spreading, migration and shape
3. Cell differentiation in development
4. Cell cycle control
5. Gene regulation and transcription
6. Endocytosis and exocytosis
7. Insulin stimulation of glucose uptake
8. Angiogenesis (formation of new blood vessels)
9. Regulation of ion channels in nerve transmission

- PROTEIN TYROSINE KINASES



- PROTEIN TYROSINE PHOSPHATASES

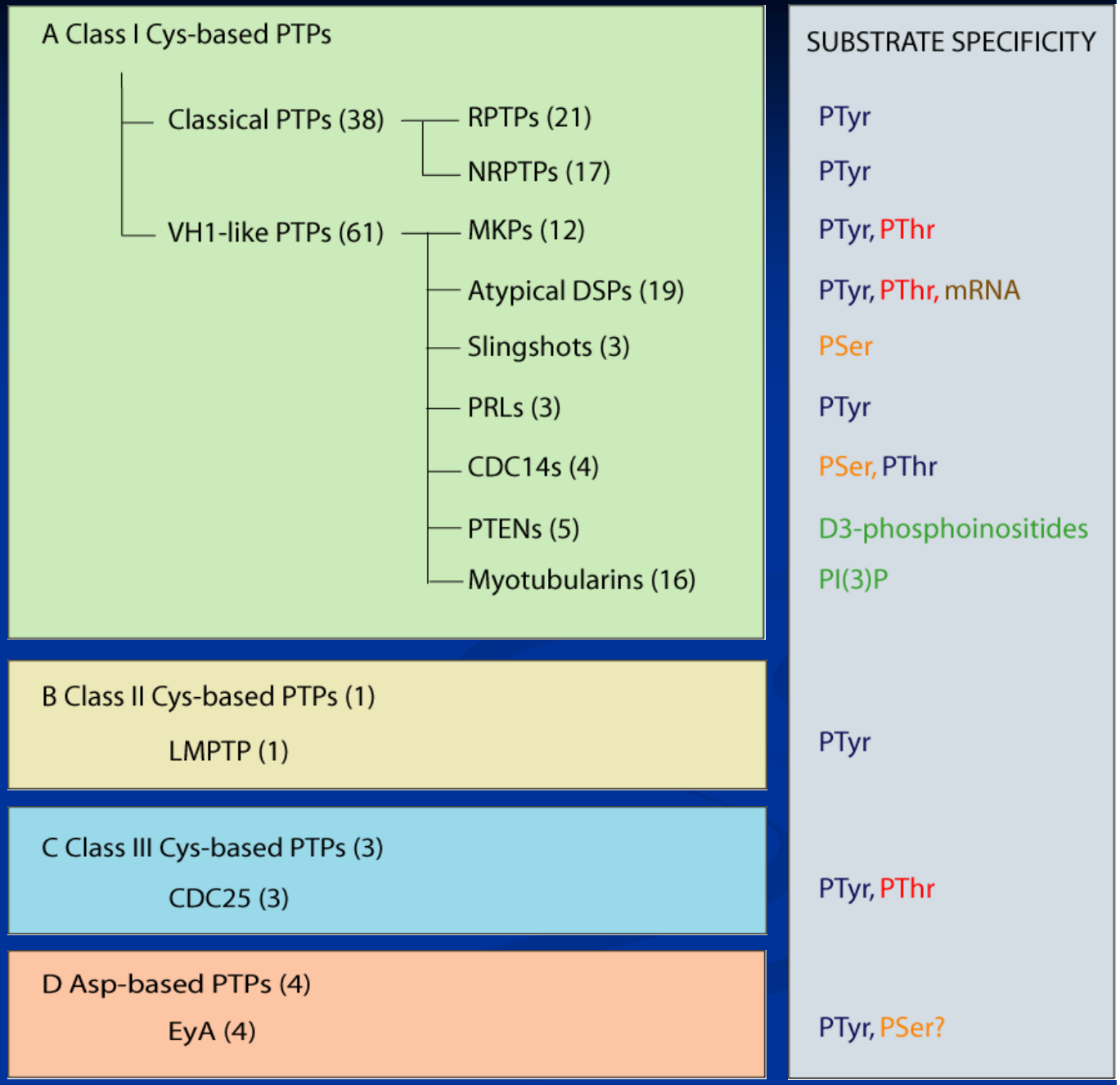
FOSFATASAS DE TIROSINAS

FOSFATASAS

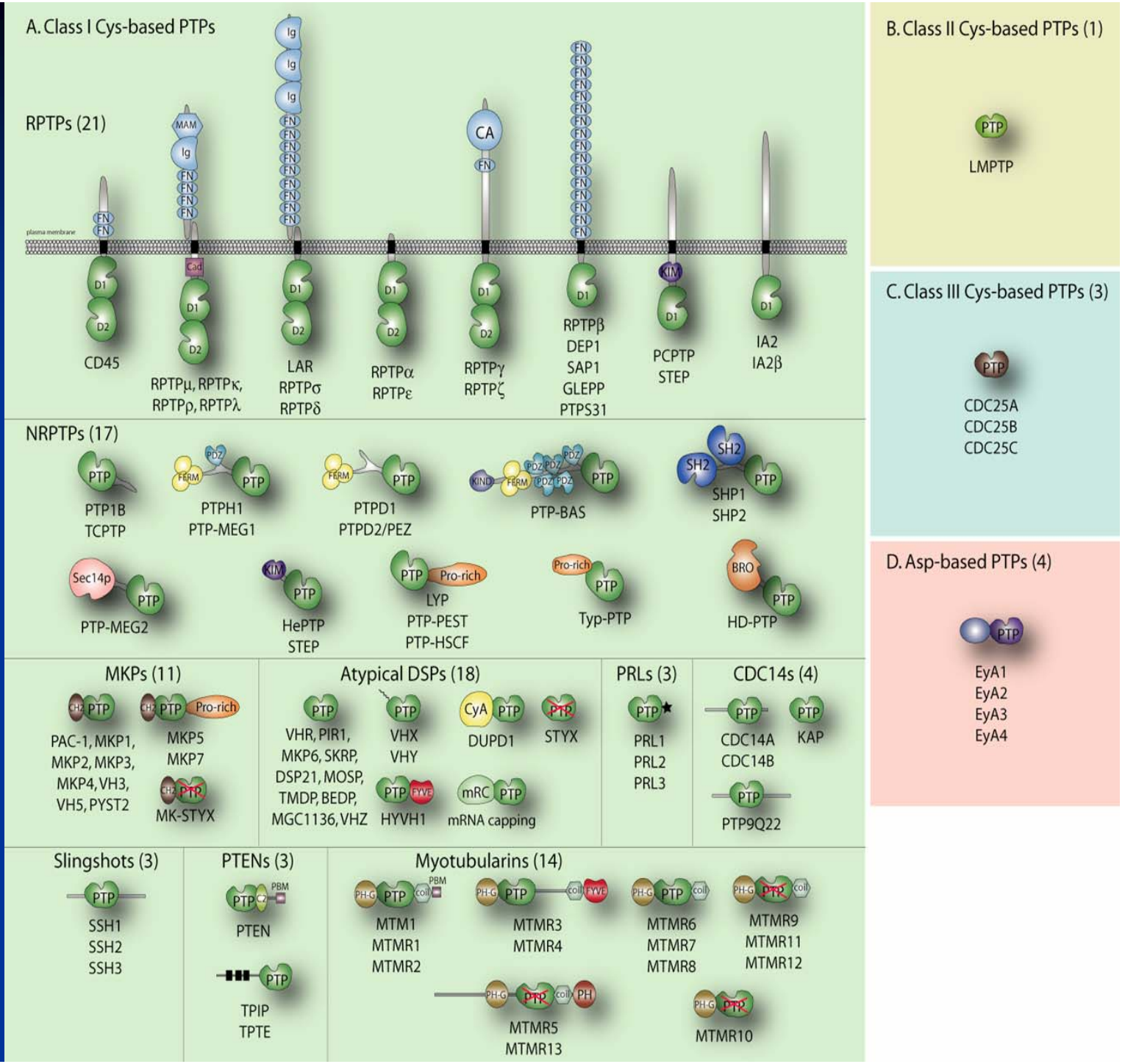
- DE SERINA/TREONINA
  - PPP: subfamilias PP1, PP2A, PP2B y PP5
  - PPM: PP2C
- DE TIROSINA
  - CISTEINA DEPENDIENTES
  - ASPÁRTICO DEPENDIENTES

FOSFATASAS DE TIROSINAS: 107 genes en el genoma humano

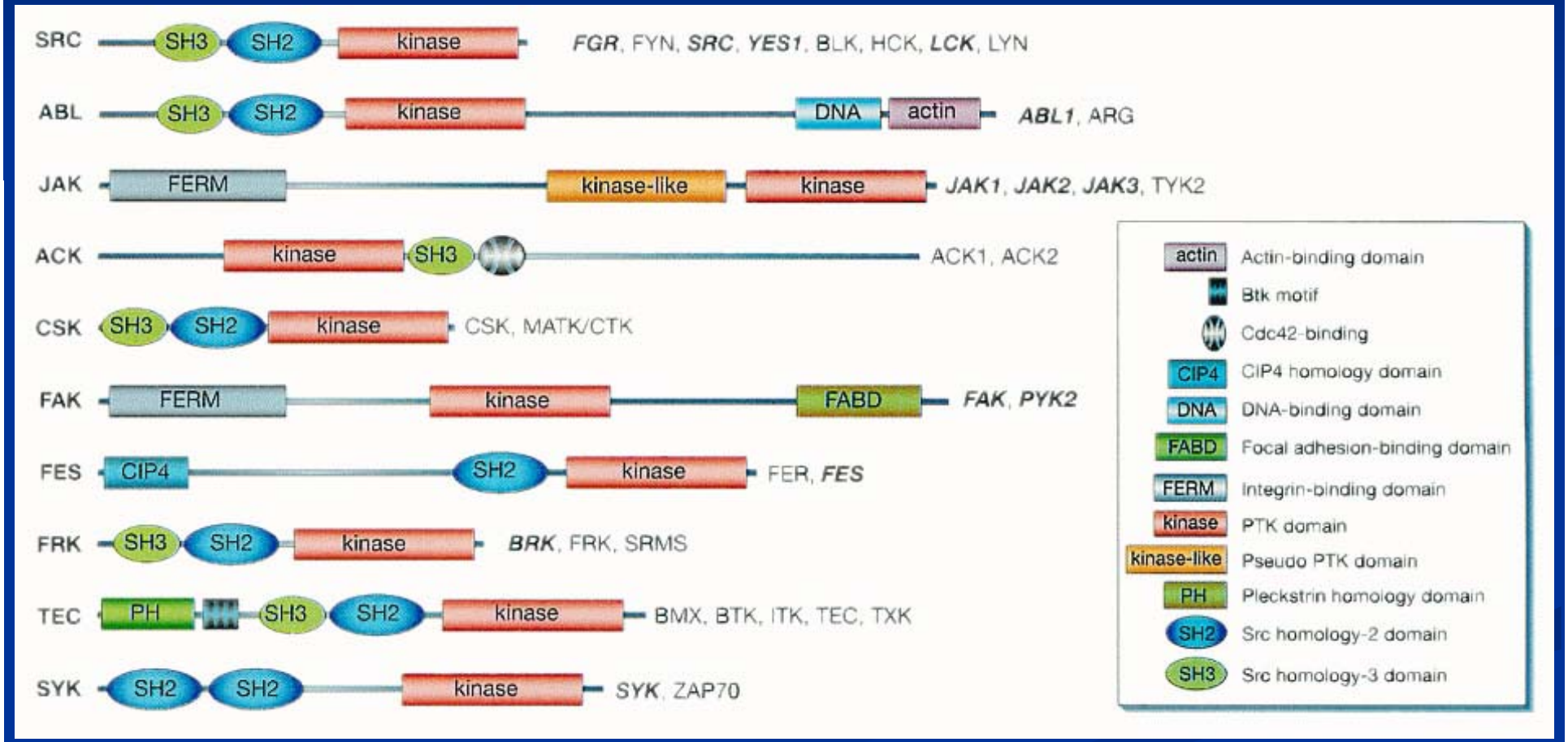
81 PTPs ACTIVAS



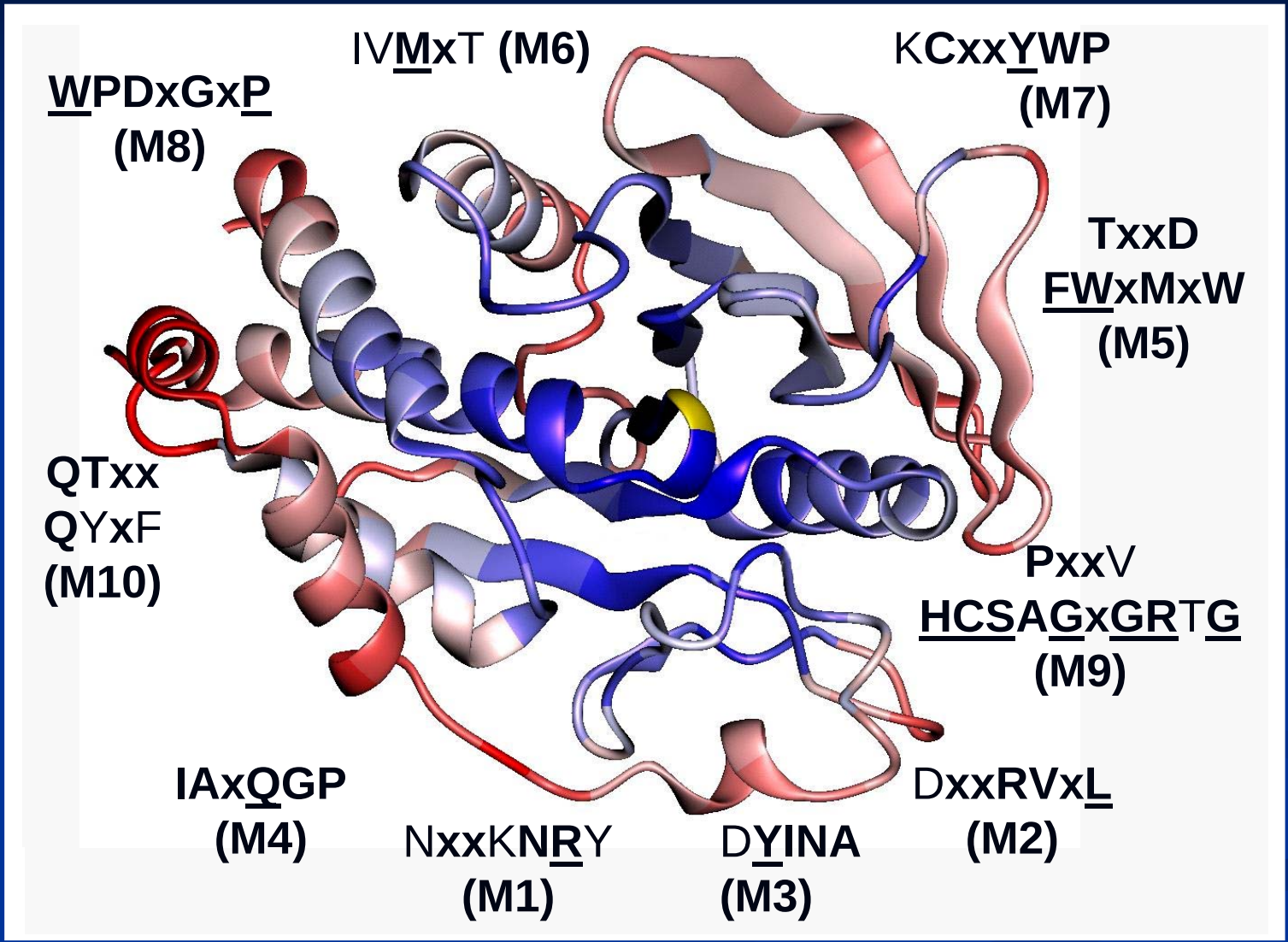
DOMINIOS PRESENTES EN LA FAMILIA DE FOSFATASAS DE TIROSINAS



Cytoplasmic protein-tyrosine kinases
32 kinases in 10 subfamilies



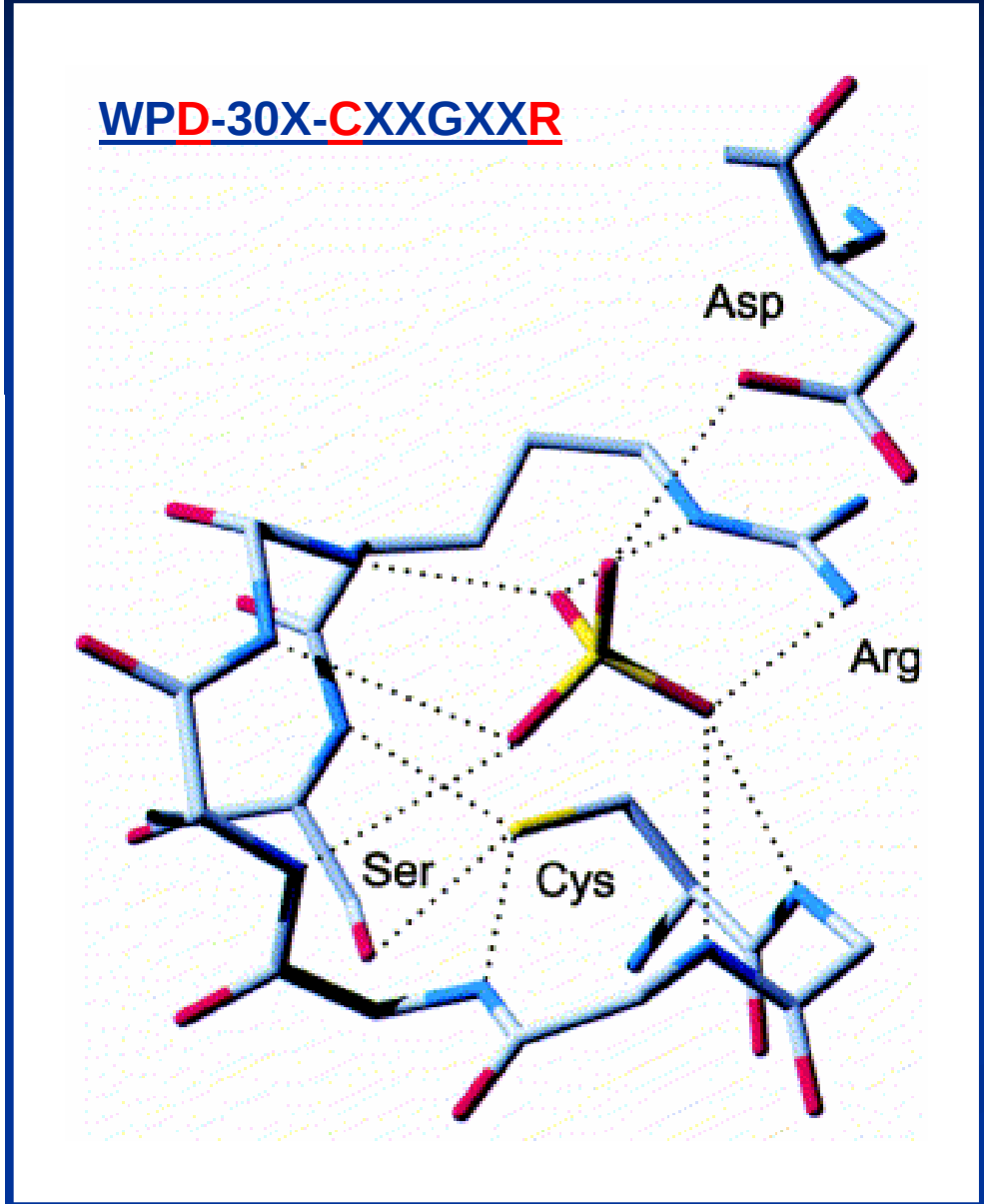
Core structures within the PTP domain are highly conserved and surface loops between secondary structure elements are least conserved

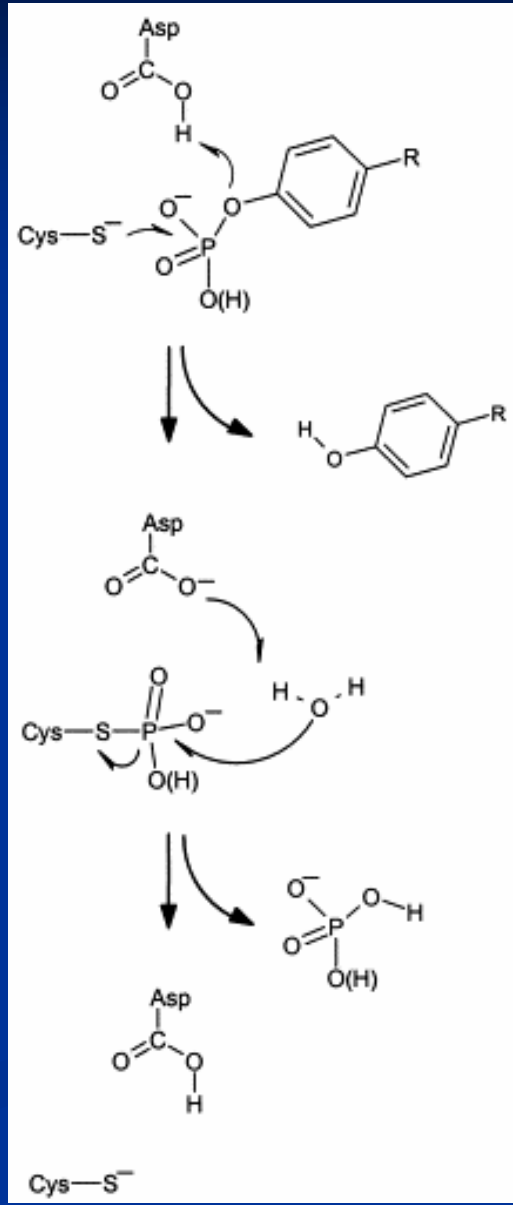


Ribbon diagram indicating the position of conserved motifs (M1-M10) within the tertiary structure of PTP1B (blue - most conserved; red - least conserved).

Andersen et al Mol. Cell. Biol. 2001

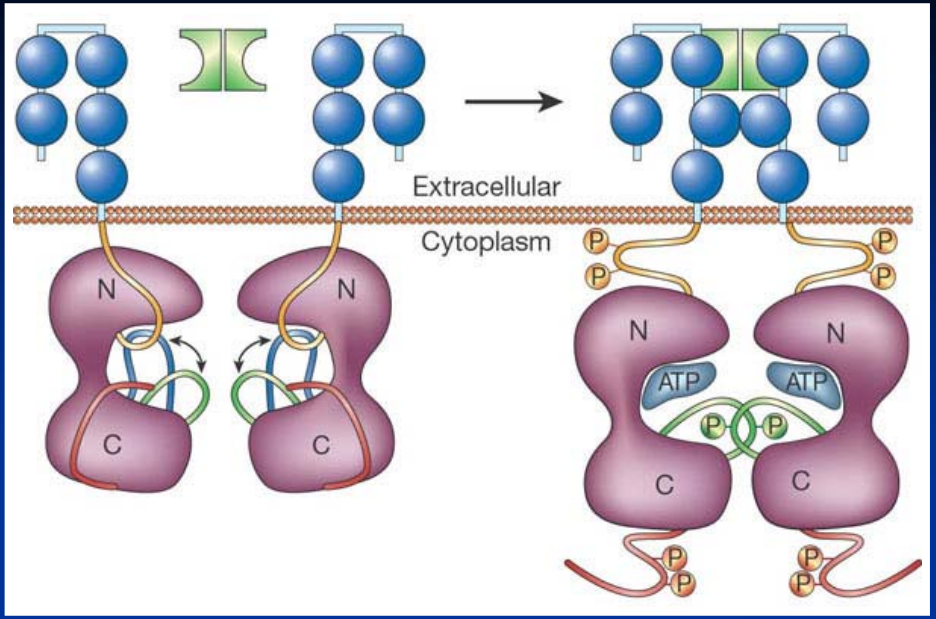
PTPs: MECANISMO CATALÍTICO



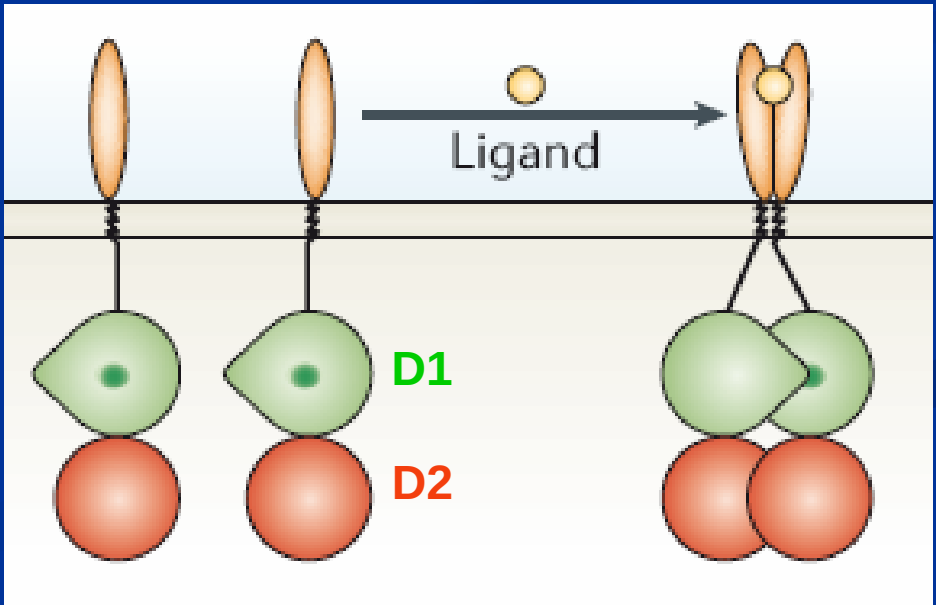


Reacción en 2 pasos iniciada por un ataque nucleofílico

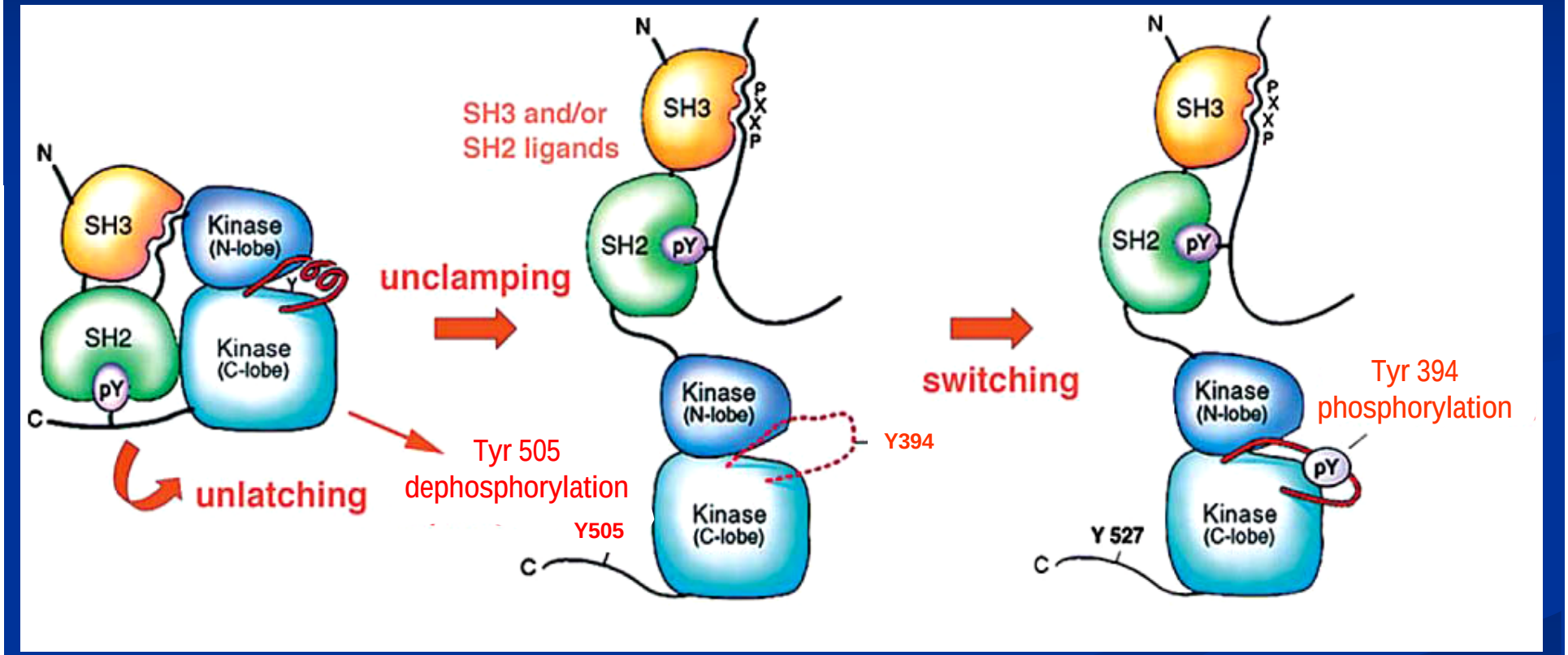
Activation of receptor-PTK by dimerization



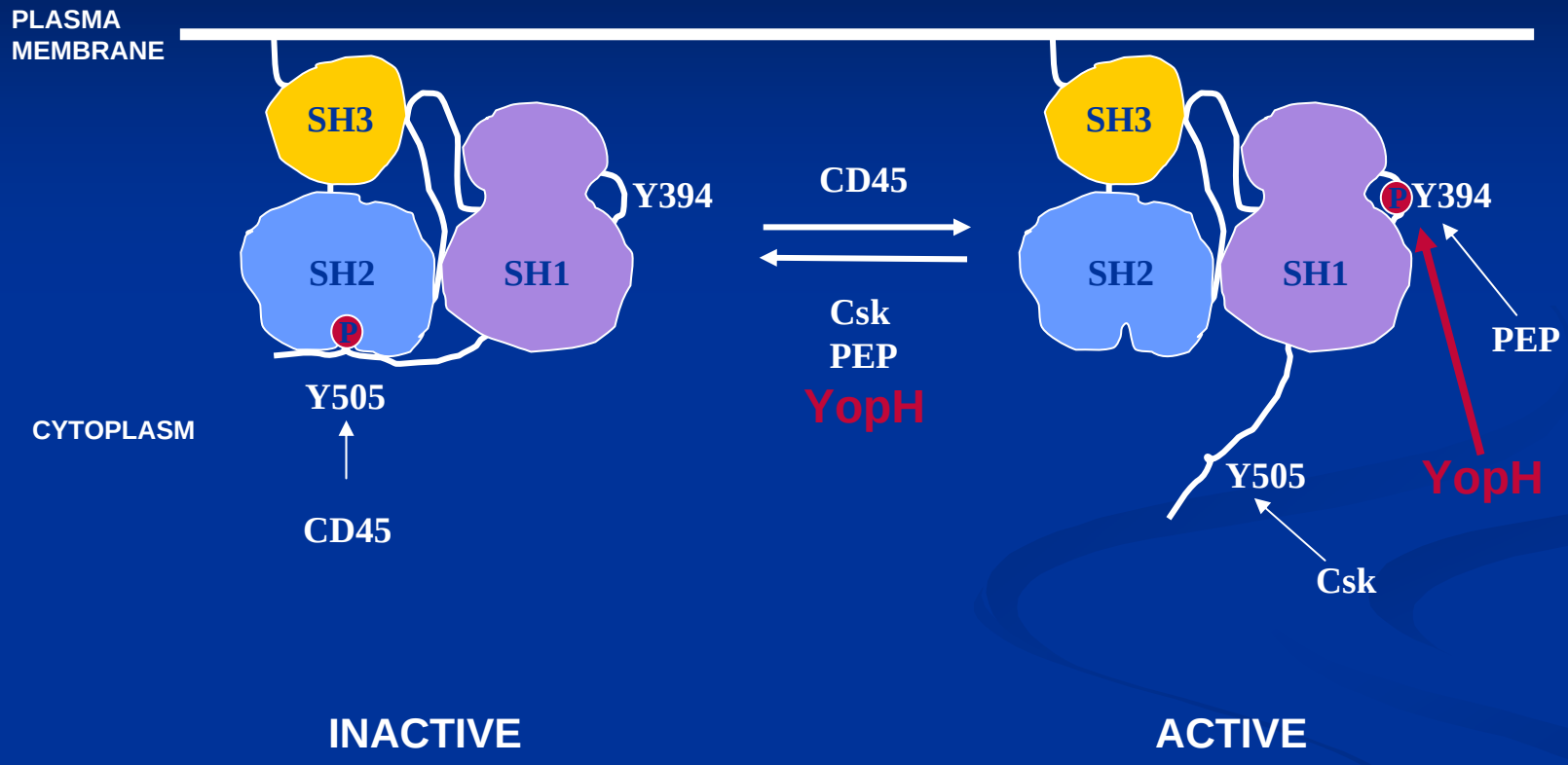
Inactivation of receptor-PTP by dimerization



Activación de Lck



LCK REGULATION BY TYROSINE PHOSPHORYLATION



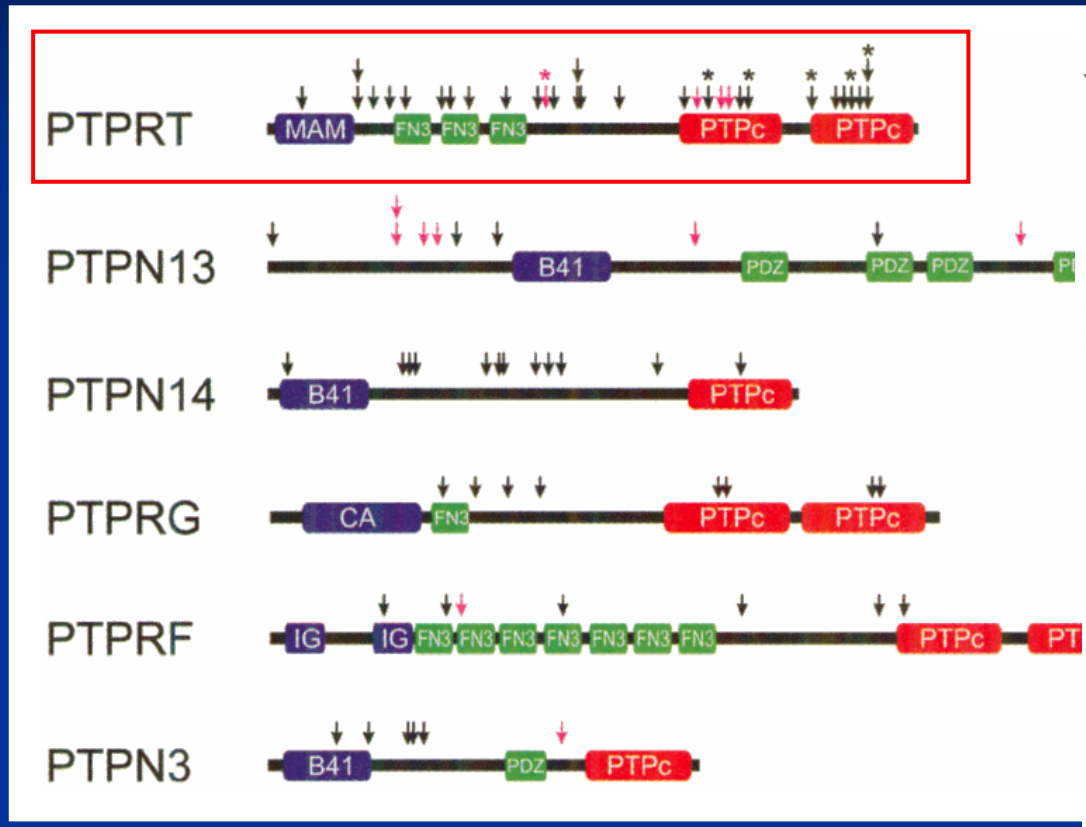
ALTERACIONES EN LAS FOSFATASAS DE TIROSINAS CAUSAN ENFERMEDADES

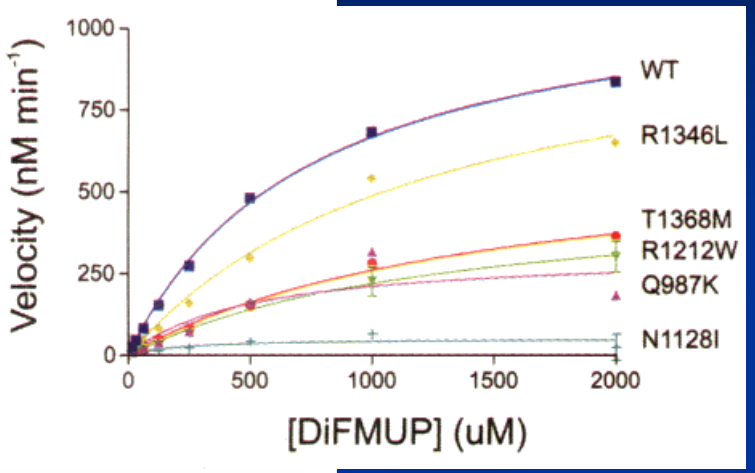
PTPN1 (PTP1B)	Insulin resistance, obesity
PTPN6 (SHP1)	Sezary syndrome
PTPN9 (PTP-MEG2)	Autism
PTPN11 (SHP2)	Noonan syndrome
PTPN22 (LYP)	SNP polymorphism in type I diabetes
PTEN (PTEN)	Bannayan-Zonana , Cowden syndrome and Lhermitte-Duclos disease
MTM1 (myotubularin)	X-linked myotubular myopathy
MTMR2 (MTMR2)	Charcot-Marie-Tooth syndrome type 4B
MTMR13 (MTMR13)	Charcot-Marie-Tooth syndrome type 4B
EPM2A (laforin)	Progressive myoclonus epilepsy (Lafora's disease)

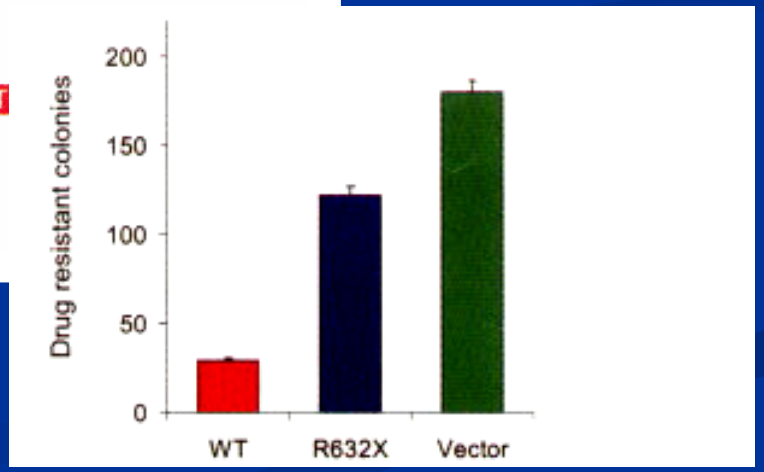
PTPs and CANCER

PTP (encoding gene)	Tumour suppressing functions
PTEN (*MMAC1*)	Tumour suppressor mutated in various human cancers. Cowden disease
DEP1 (*PTPRJ*)	Colon cancer susceptibility locus SCC1. Deletions and mutations in human colon, lung and breast cancer
PTPκ (*PTPRK*)	Potential tumour suppressor in primary central nervous system lymphomas
PTPρ (*PTPRT*) LAR (*PTPRF*) PTPγ (*PTPRG*) PTPH1 (*PTPN3*) PTPBAS (*PTPN13*) PTPD2 (*PTPN14*)	Potential tumour suppressor in colorectal cancers
GLEPP1 (*PTPRO*)	Promoter methylation in lung tumours and hepatocellular carcinoma — potential tumour suppressor
SHP1 (*PTPN6*)	Promoter methylation in leukaemia and/or lymphoma — potential tumour suppressor
FAP1 (*PTPN13*)	Promoter methylation in hepatocellular carcinoma — potential tumour suppressor
SHP2 (*PTPN11*)	Oncogene in leukaemia. Target of *Helicobacter pylori* CagA protein in gastric carcinoma
MKP3 (*DUSP6*)	Candidate pancreatic tumour suppressor at locus 12q22. Promoter methylation
cdc25	Cell-cycle control. Target of Myc and overexpressed in primary breast cancer
PRL3 (*PTP4A3*)	Upregulated in metastases of colon cancer
(*PTPRR*)	*TEL* and *PTPRR* chimeric gene. It fuses exon 4 of the *TEL* gene with exon 7 of the *PTPRR* gene in acute myelogenous leukaemia

Distribution of mutations in PTPRT, PTPN13, PTPN14, PTPRG, PTPRF, and PTPN3, in colorectal cancer



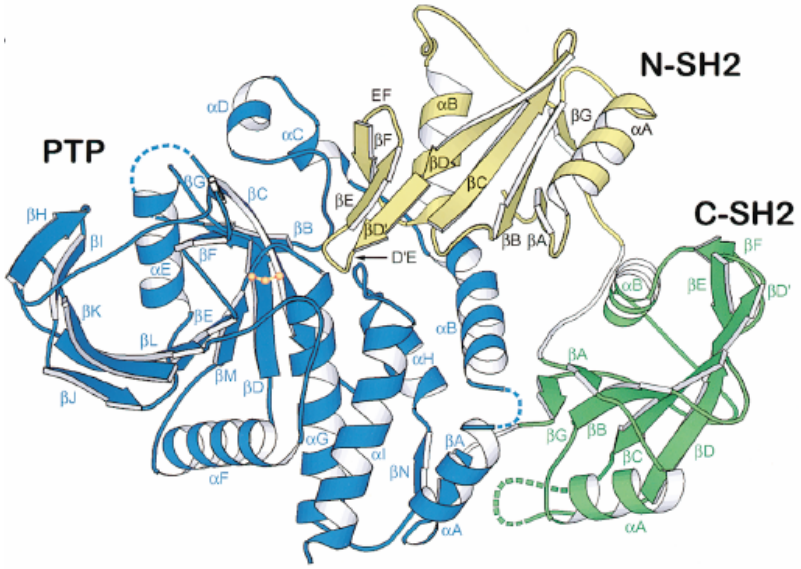


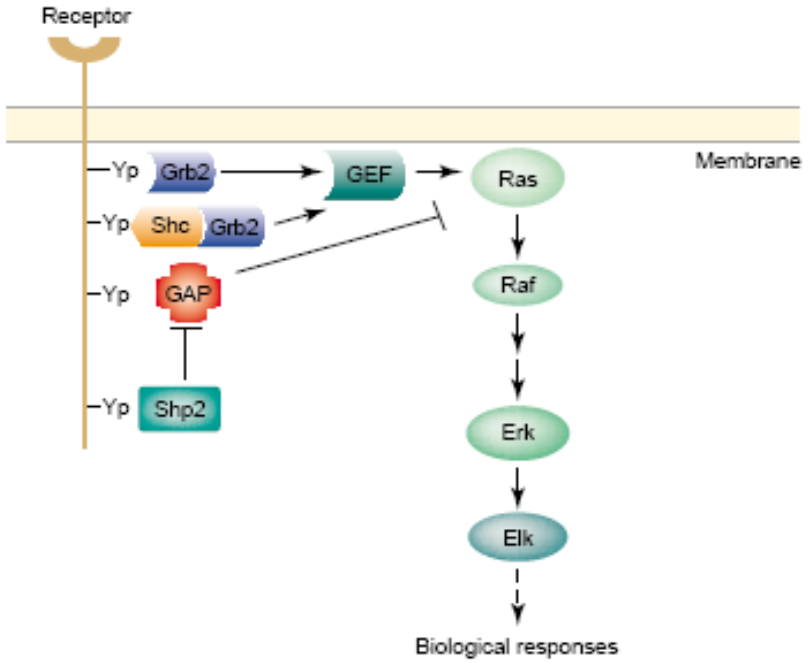


Wang Z, et al. Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science* (2004) 304:1164-6.

Shp2

- Ubiquitous
- Substrates are unknown
- Shp2 has a positive effect on RPTK signalling
- Activating mutations make Shp2 a proto-oncogene

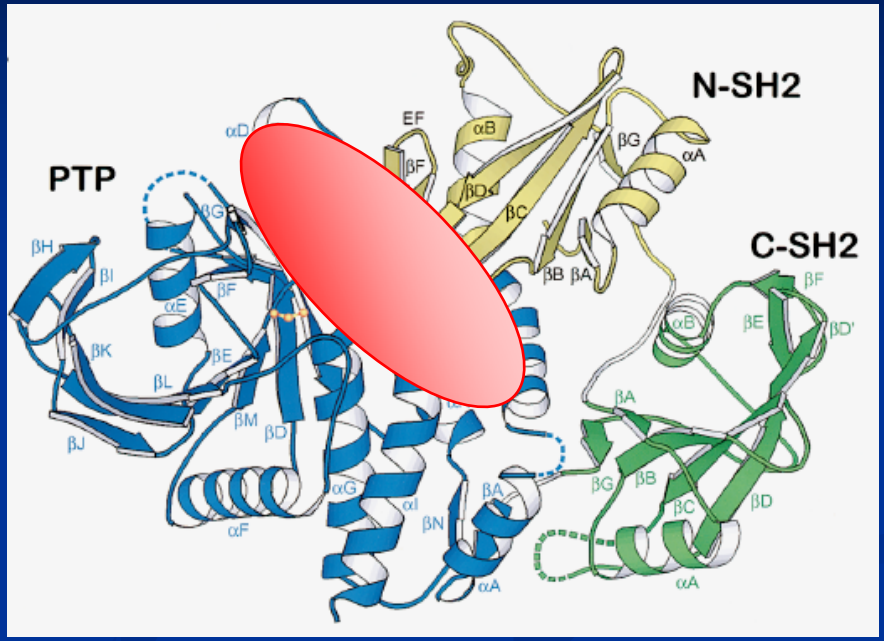




PTPN11 (SHP2) VARIANTS

Table 2 | **SHP2 (*PTPN11*) variants in Noonan syndrome, and malignancies**

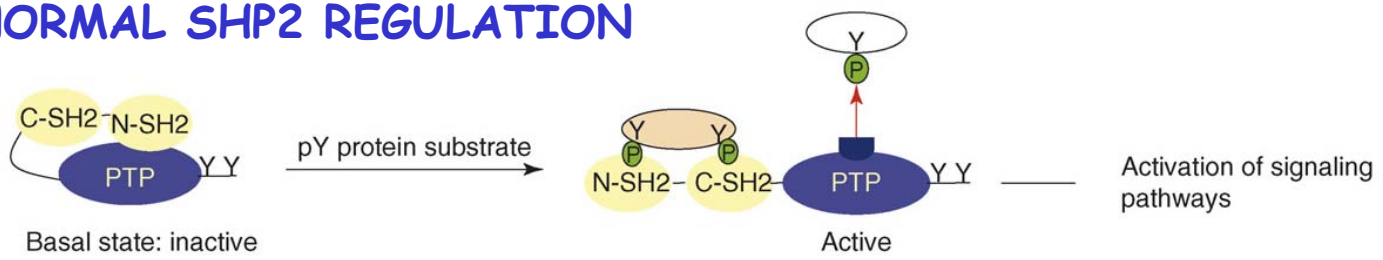
Mutations	Domain of SHP2	Occurrence
T42A	N-terminal SH2	NS
V46L, N58S	N-terminal SH2	Lung carcinoma
D61G	N-terminal SH2	NS
D61H, Y, V	N-terminal SH2	JMML, ALL, AML
E69K	N-terminal SH2	JMML, ALL, AML, neuroblastoma
A72G, S	N-terminal SH2	NS
A72D, T, V	N-terminal SH2	JMML, ALL, AML
T73I	N-terminal SH2	NS, JMML, AML
E76A, K, V, Q	N-terminal SH2	JMML, ALL, AML, lung carcinoma
Q79P, R	N-terminal SH2	NS
D106A	Inter-SH2 region	NS
R138Q	C-terminal SH2	Melanoma
R289G	PTP	AML
N208D, S	PTP	NS
G503V	PTP	JMML, AML
Q506P	PTP	NS, JMML
T507K	PTP	Neuroblastoma



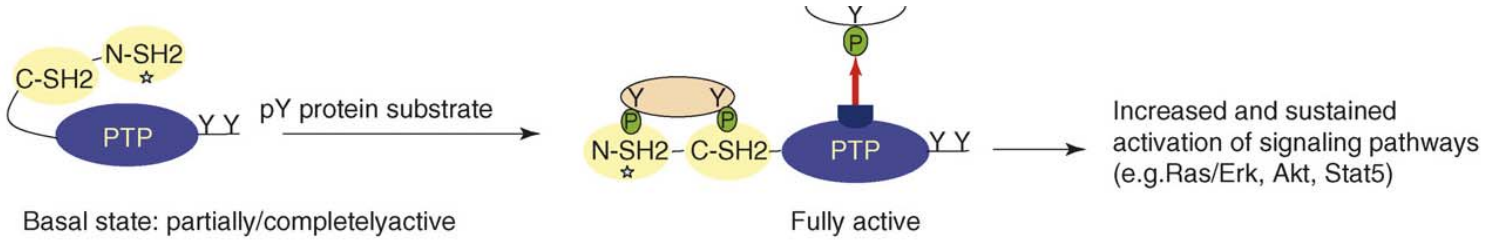
ALL: acute lymphoblastic leukaemia.
AML: acute myeloid leukaemia; JMML: juvenile myelomonocytic leukaemia.
NS: Noonan syndrome

Normal Shp2 regulation and its disruption in disease

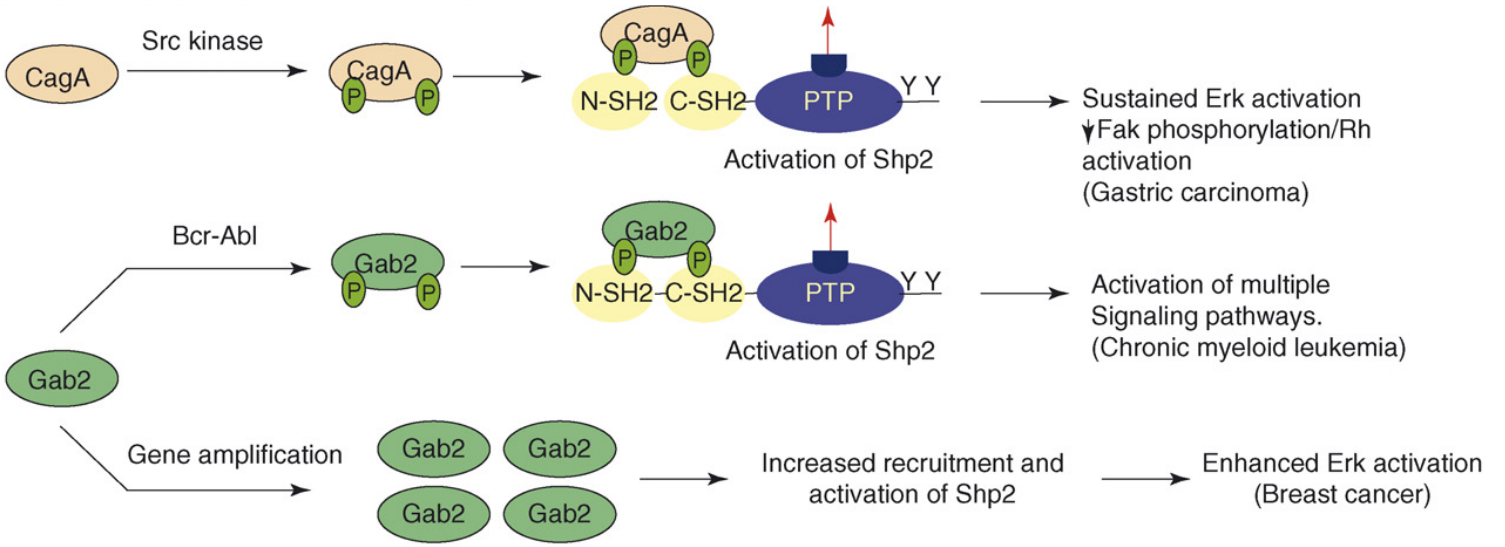
NORMAL SHP2 REGULATION



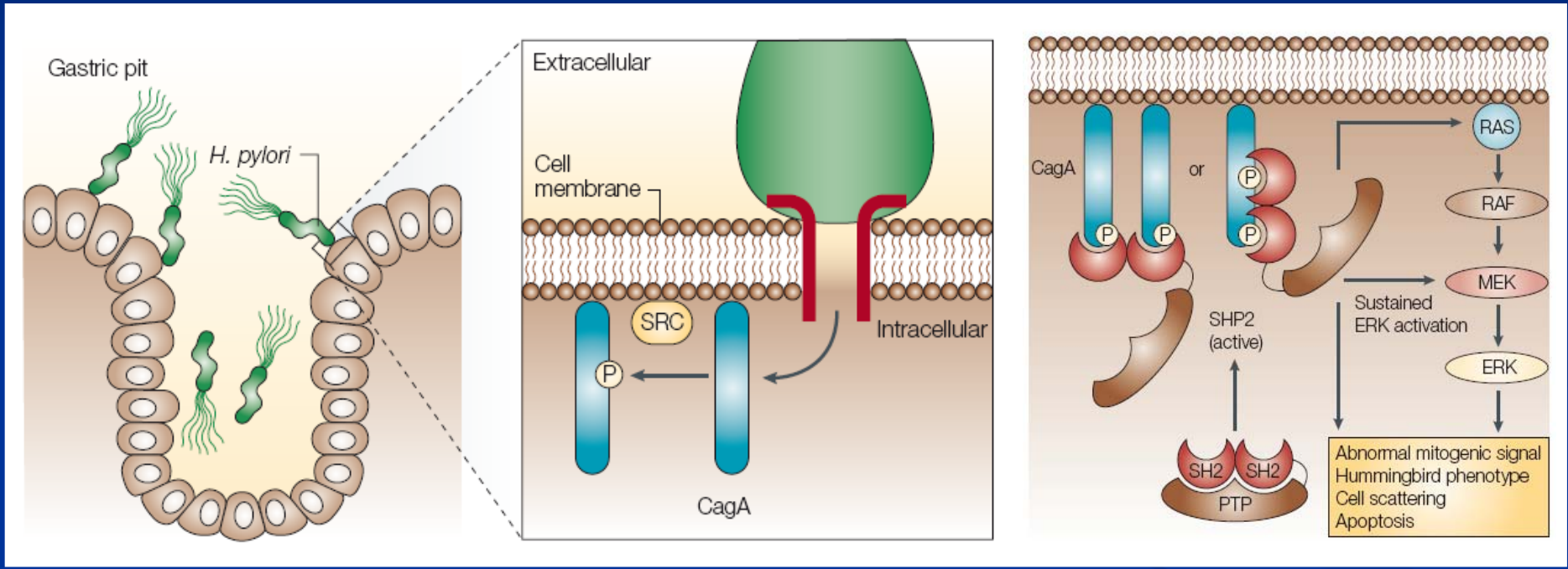
ENHANCED ACTIVATION BY SHP2 MUTATION



ENHANCED ACTIVATION BY SHP2 BINDING PROTEIN

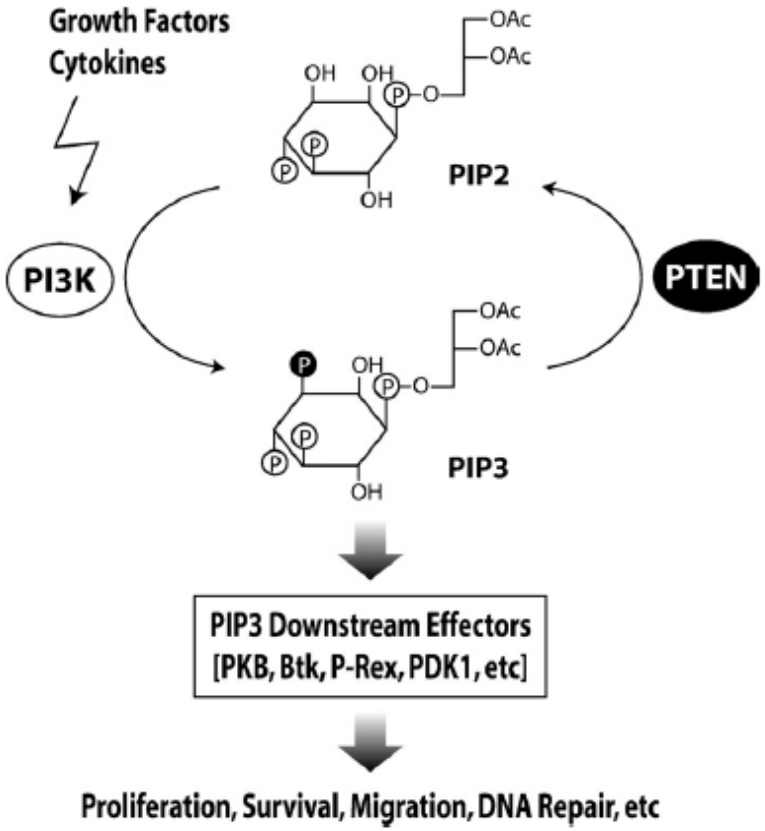


SHP-2 DEREGULATION BY CagA (cytotoxin-associated antigen A) a protein of *Helicobacter pylori*



PTEN (Phosphatase and tensin homolog)

- PTEN regulates signaling pathways activated by PI3K.
- PTEN dephosphorylates PtdIns(3,4,5)P3, at position 3.
- PTEN dephosphorylates FAK.
- *PTEN/MMAC1* is a common event in diverse tumours.
- PTEN is a tumor suppressor

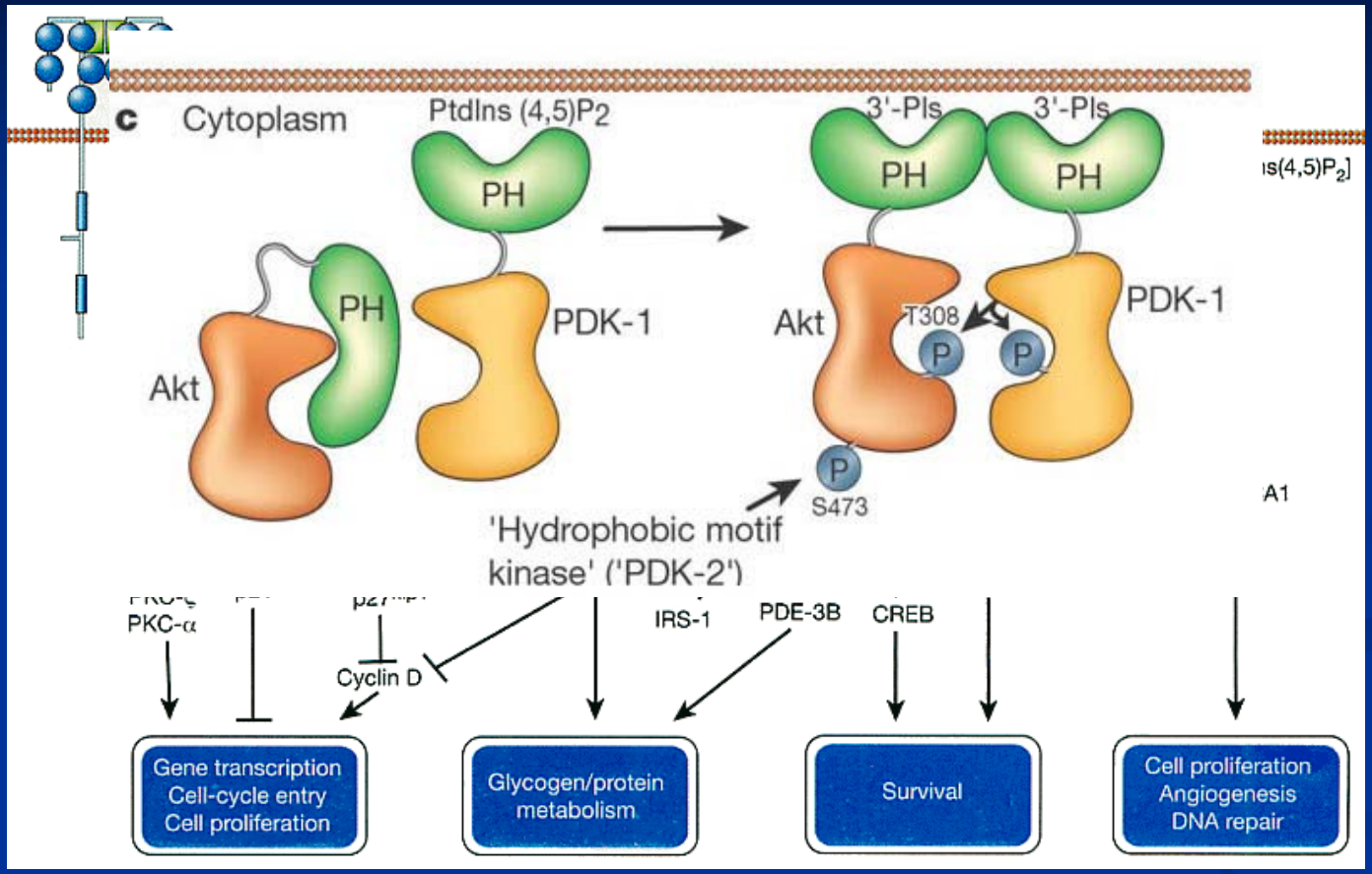


PTEN

Table 1 | **Evidence of PI3K-signalling deregulation in human malignancies**

Cancer type	Type of alteration	References
Glioblastoma	*PTEN* mutation	133
Ovarian	Allelic imbalance and mutations of *PTEN* gene	134
	Elevated AKT1 kinase activity	135
	AKT2 amplification and overexpression	71
	PI3K *p110α* amplification	70
	PI3K *p85α* mutation	74
Breast	Elevated AKT1 kinase activity	135
	AKT2 amplification and overexpression	71
	RSK amplification and overexpression	78,79
	Loss of heterozygosity at *PTEN* locus	136
	PI3K and AKT2 overactivation	137
Endometrial	*PTEN* mutation	138
	PTEN silencing	139
Hepatocellular carcinoma	*PTEN* mutation	140
Melanoma	*PTEN* mutation	141
	PTEN silencing	142
Digestive tract	Aberrant *PTEN* transcripts	143
	PI3K *p85α* mutation	74
Lung	PTEN inactivation	144
Renal-cell carcinoma	*PTEN* mutations	145
Thyroid	*PTEN* mutations	146–148
	AKT overexpression and overactivation	149
Lymphoid	*PTEN* mutations	150,151
	p85–EPH fusion (only one case reported)	75

THE PI3K-PTEN SIGNALING NETWORK



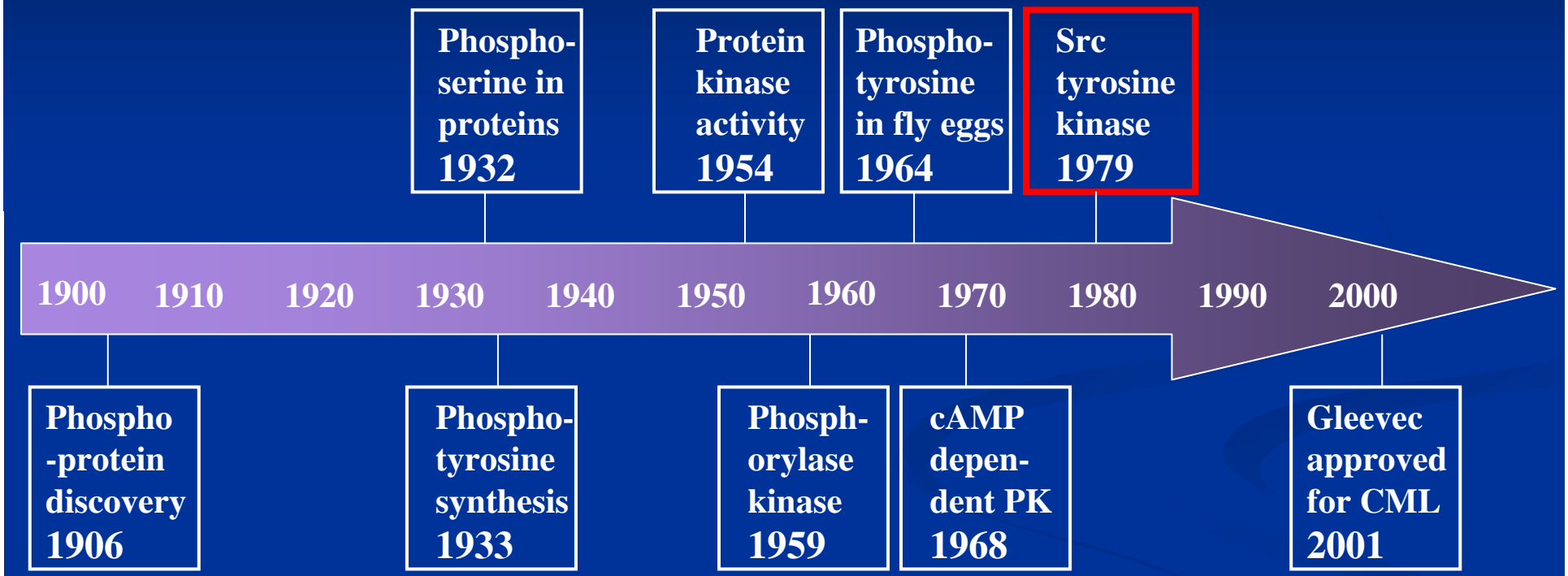
TUMOR GROWTH AND ANGIOGENESIS

PROTEIN TYROSINE KINASES (PTKs)

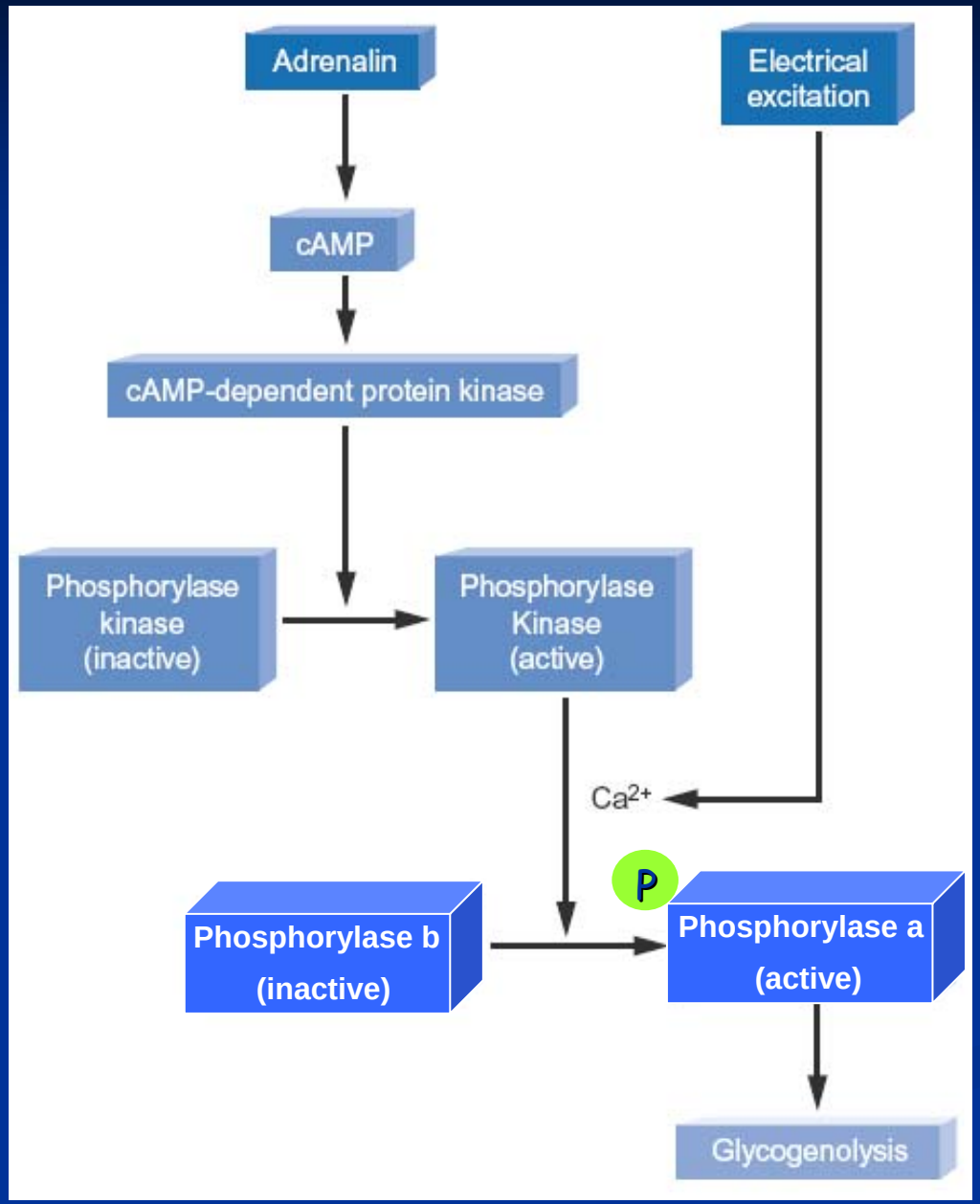
PROTEÍN KINASAS

- Perspectiva Histórica
- Clasificación
- Evolución
- Estructura
- Tyr Kinasas

The History of Protein Phosphorylation



GLUCOGENOLISIS



1968 Krebs: PKA

1950 Sutherland: hormone
NP 1971

1955/59 Fisher & Krebs
NP 1992

Años 30 C. & G. Cori
NP 1947

Finales de los 70 principios de los 80

- Nuevos ejemplos de fosforilación
  (L. Reed 1969 Piruvato deshidrogenasa)
- Nuevos sustratos para la PKA
- Proteínas fosforiladas en más de un sitio por más de una kinasa
- Fosfatasas específicas de Ser/Thr
- v-Src
- Fosforilación en Tyr

Descubrimiento de la fosforilación de tirosinas

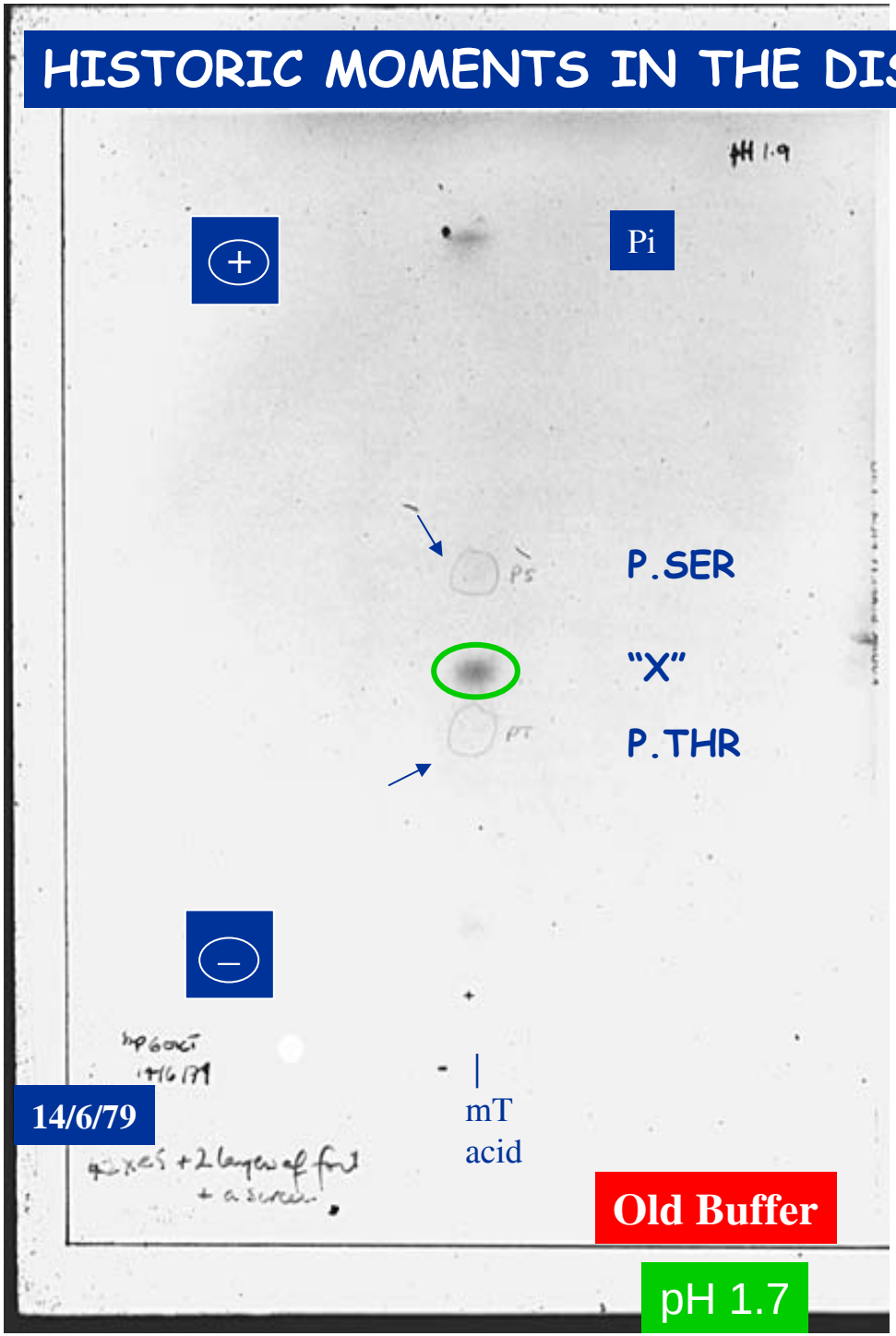
Cell, Vol. 18, 925–933, December, 1979, Copyright ©1979 by Cell Press

An Activity Phosphorylating Tyrosine in Polyoma T Antigen Immunoprecipitates

Walter Eckhart, Mary Anne Hutchinson and Tony Hunter
Tumor Virology Laboratory
The Salk Institute
Post Office Box 85800
San Diego, California 92138

large T antigen between 74 and 79 and 86 and 24 map units (Smart and Ito, 1978; Hutchinson et al., 1978; G. Carmichael and T. Benjamin, unpublished results). The medium and large T antigens are translated in different reading frames from the viral DNA between 86 and 99 map units (Hunter et al., 1979).

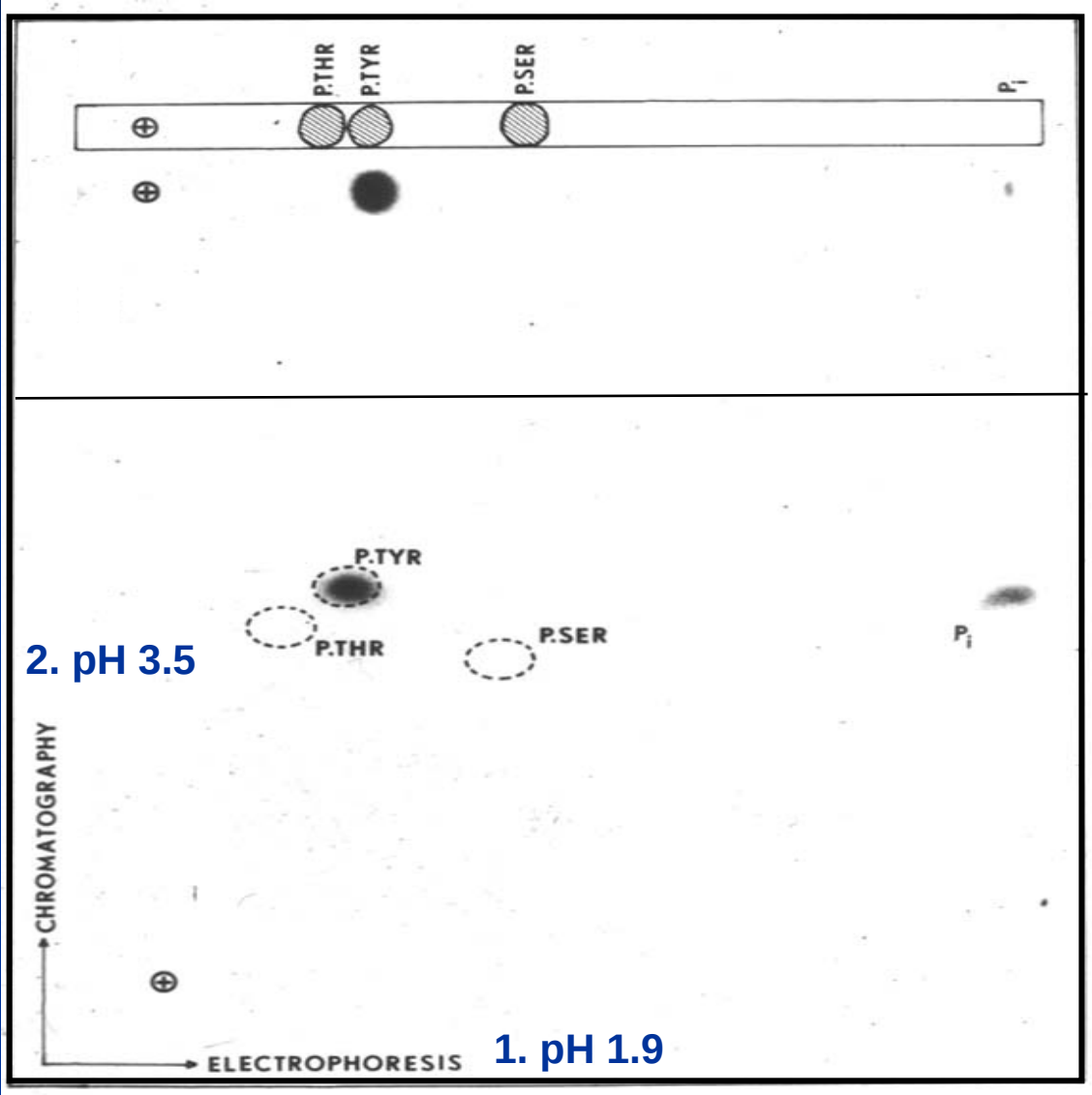
HISTORIC MOMENTS IN THE DISCOVERY OF PHOSPHOTYROSINE



Comparison of 1-D and 2-D phosphoamino acid analysis of phosphorylated Polyoma virus middle T antigen

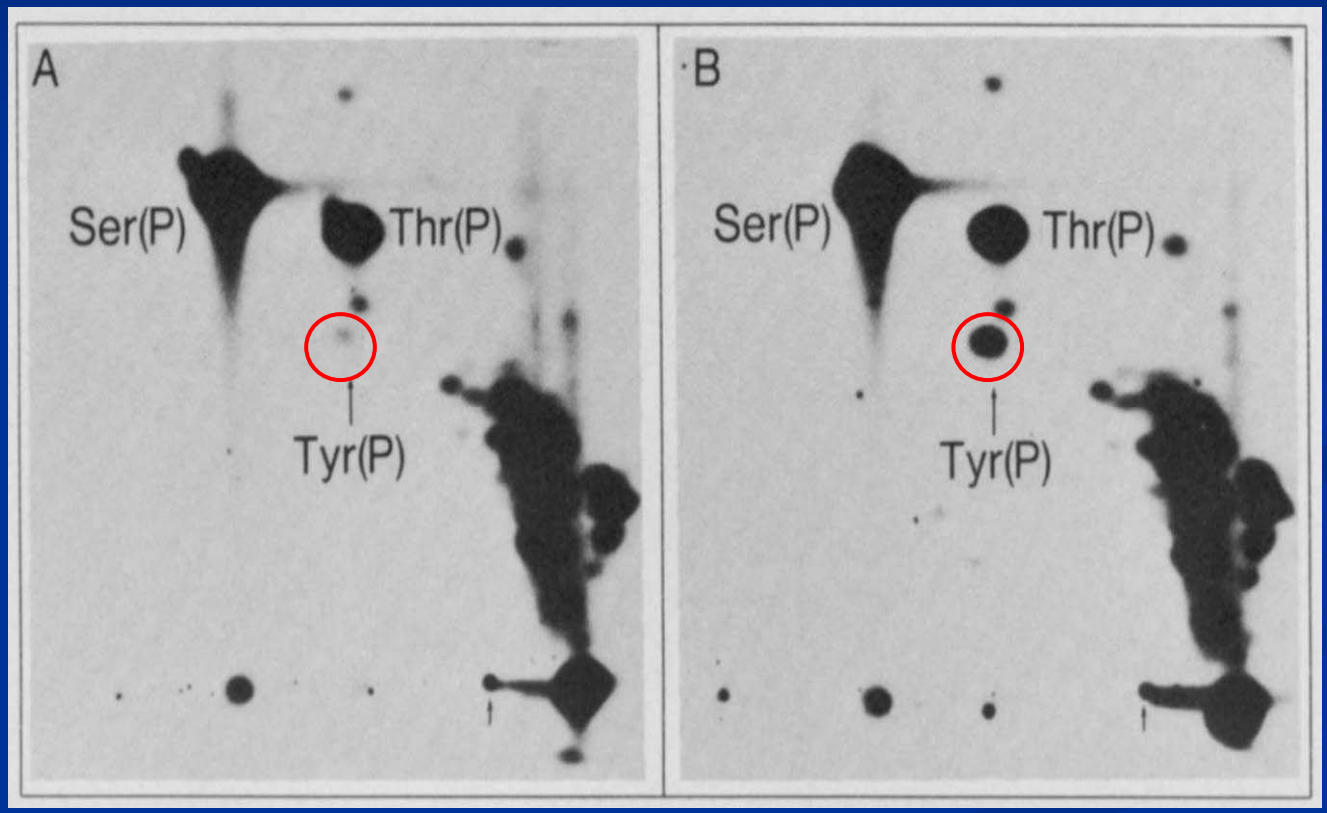
1-D PAA analysis

2-D PAA analysis



Eckhart, Hutchinson and Hunter, Cell 18:925 (1979)

RSV (v-src) transformed cells have increased levels in phosphotyrosine



uninfected **RSV-transformed**

Hunter and Sefton, PNAS 77:1311 (1980)

How many tyrosine kinases are there?

- The finding that v-Src and c-Src was a kinase provided the first evidence for tyrosine kinase in 1979

- By the end of 1980 four tyrosine kinases were known (Src, Abl, EGF receptor, Fps/Fes)

- By the end of 1990 over 50 tyrosine kinases had been identified in vertebrates and equal numbers of tyrosine kinases and serine kinases were known, leading to the prediction that there might be several 100 tyrosine kinases in a vertebrate genome and a total of over a 1000 protein kinases.

- The complete human genome sequence reported in 2001 reveals that there are 90 tyrosine kinases, out of a total of 518 protein kinases

What is tyrosine phosphorylation used for?

1. Growth factor signaling (and oncogenesis)
2. Cell adhesion, spreading, migration and shape
3. Cell differentiation in development
4. Cell cycle control
5. Gene regulation and transcription
6. Endocytosis and exocytosis
7. Insulin stimulation of glucose uptake
8. Angiogenesis (formation of new blood vessels)
9. Regulation of ion channels in nerve transmission

PROTEÍN KINASAS

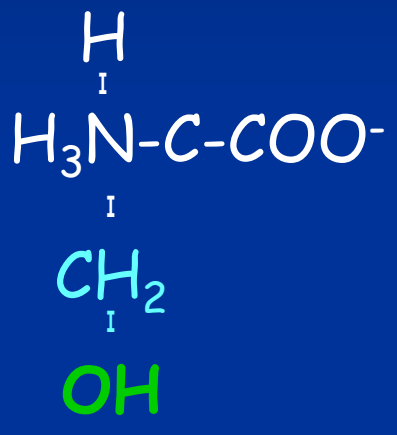
- Perspectiva Histórica
- Clasificación
- Evolución
- Estructura
- Tyr Kinasas

PROTEÍN KINASAS

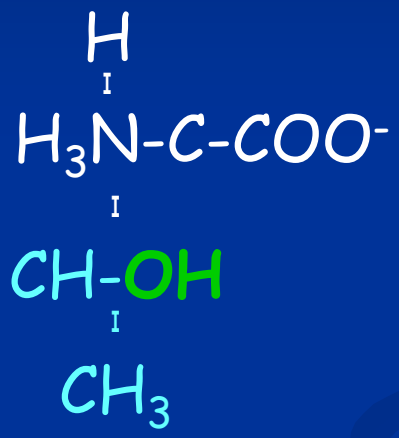
- Fosfotransferasas que transfieren el fosfato γ del ATP al –OH libre de la cadena lateral de los amino ácidos
- Se clasifican por la especificidad del amino ácido que fosforilan y por la secuencia de amino ácidos del domino catalítico
- La fosforilación de proteínas es ubicua y esta intimamente ligada a la regulación del metabolismo, del crecimiento y de la diferenciación.

Protein Kinases Classified by Specificity

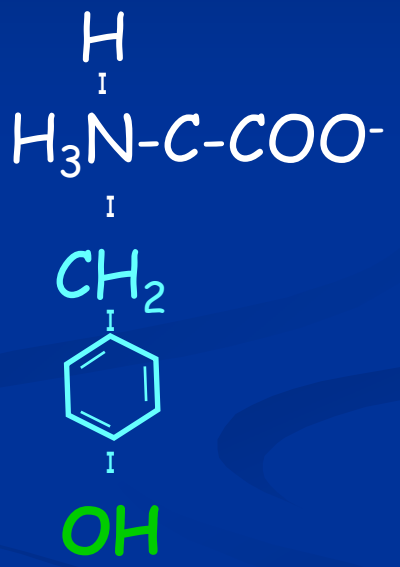
serine



threonine



tyrosine

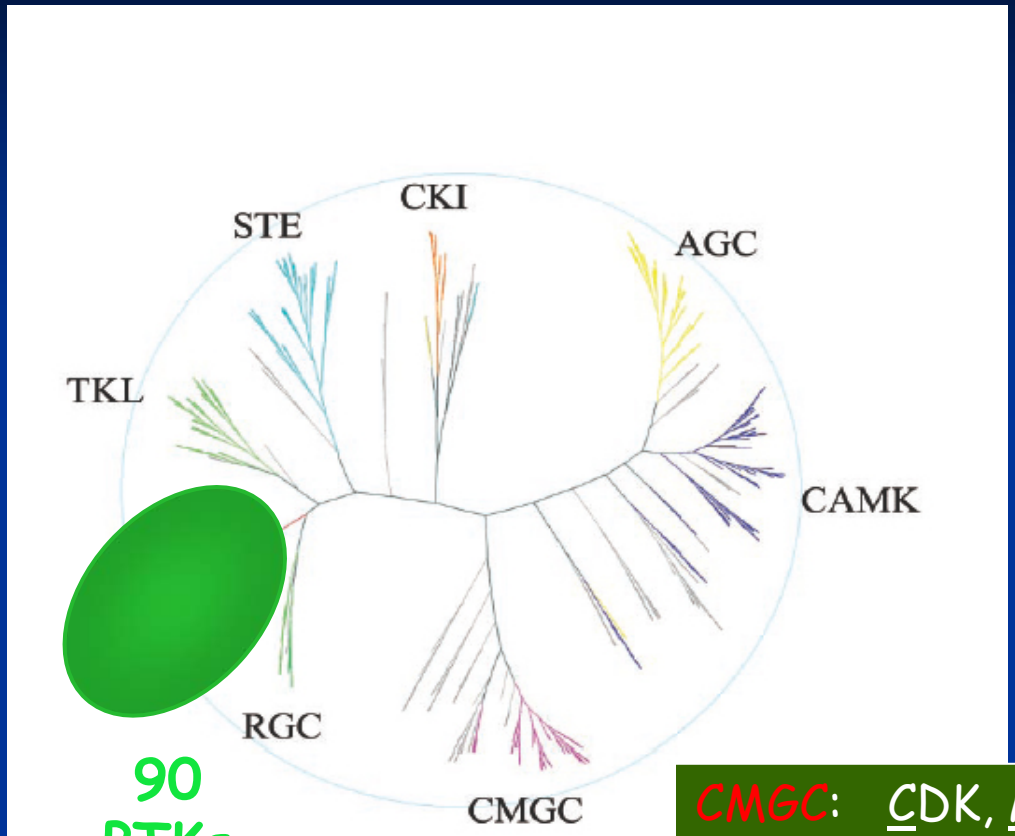


Serine/Threonine Kinases

Tyrosine kinases

Dual Specificity Kinases

KINOMA HUMANO



90
PTKs
85 activas

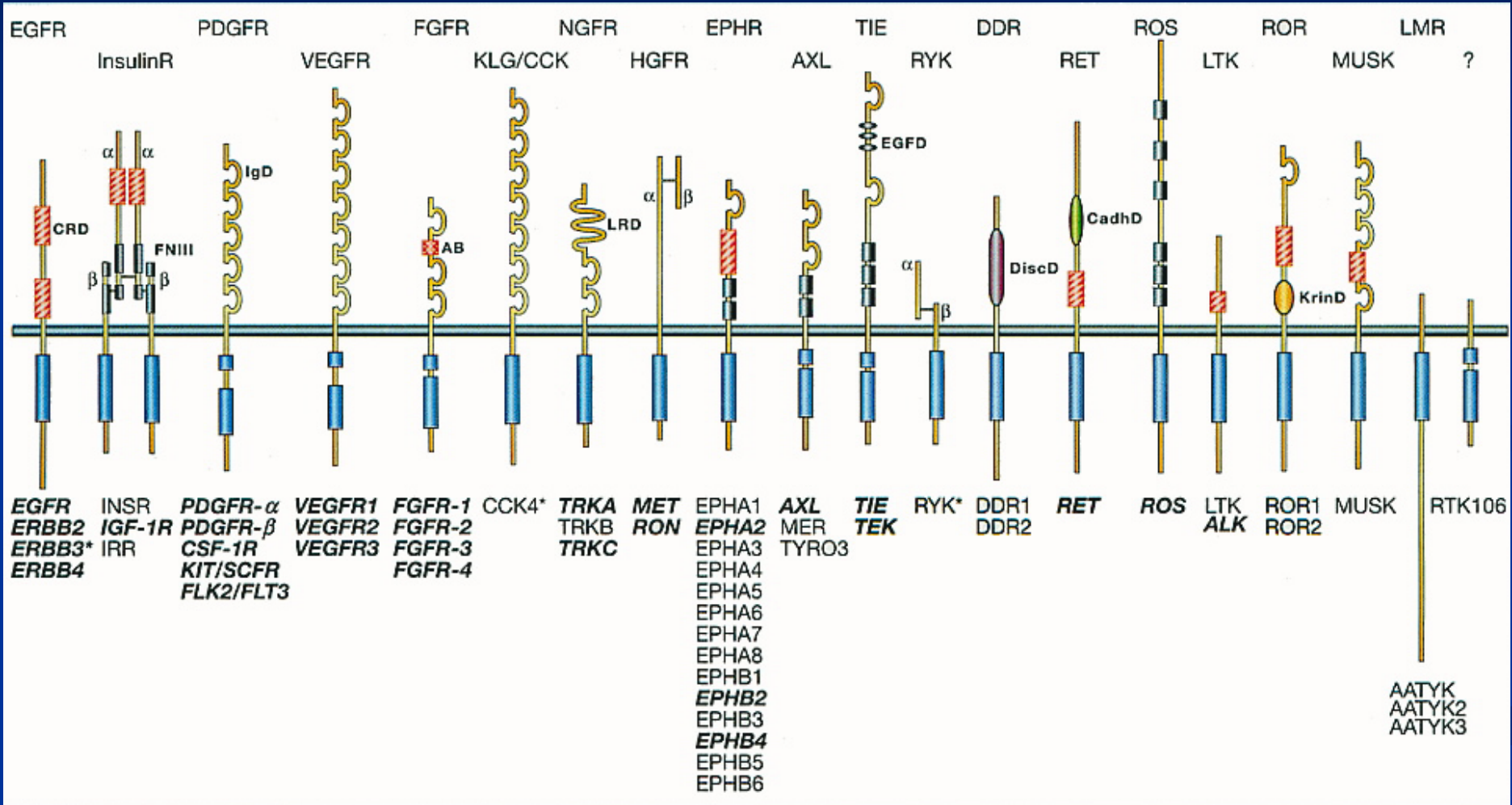
518 genes (2% del total)

* 478 ePK
* 50 aPK

CMGC:	CDK, MAPK, GSK3 y CLK
CAMK:	Calcium/calmodulin dependent kinases
AGC:	PKA, PKG y PKC
CK1:	Caseína Kinasa 1
STE:	Homólogos de STERILE (kinasa de levaduras)
TKL:	Tyrosin Kinase Like
TK :	Tyrosin Kinase
RGC:	Receptor Guanylato Cyclase

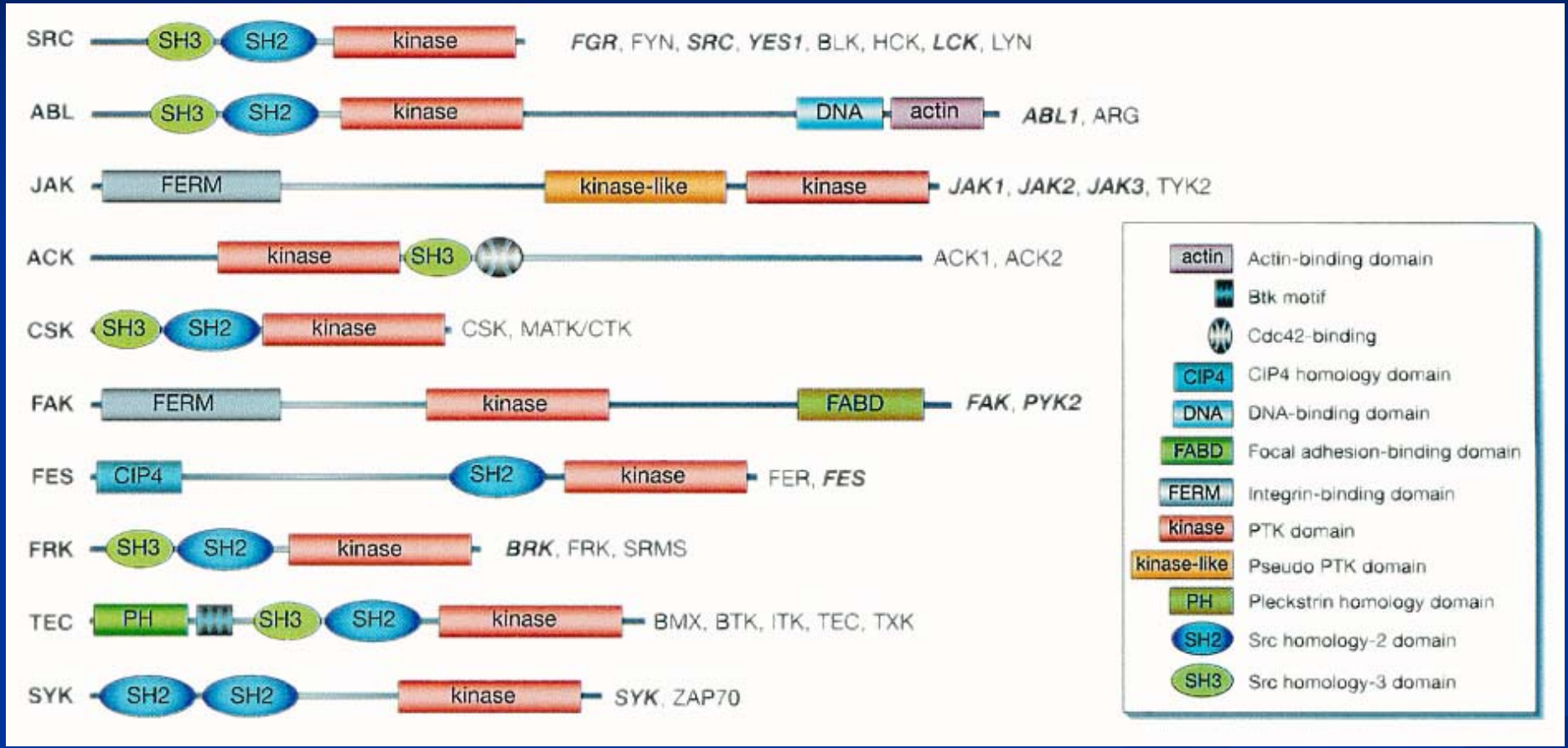
The Protein Kinase Complement of the Human Genome. Manning, DB Whyte, R Martinez, T Hunter, S Sudarsanam (2002). Science **298**:1912-1934

RECEPTOR TYROSINE KINASES
59 kinases in 20 subfamilies



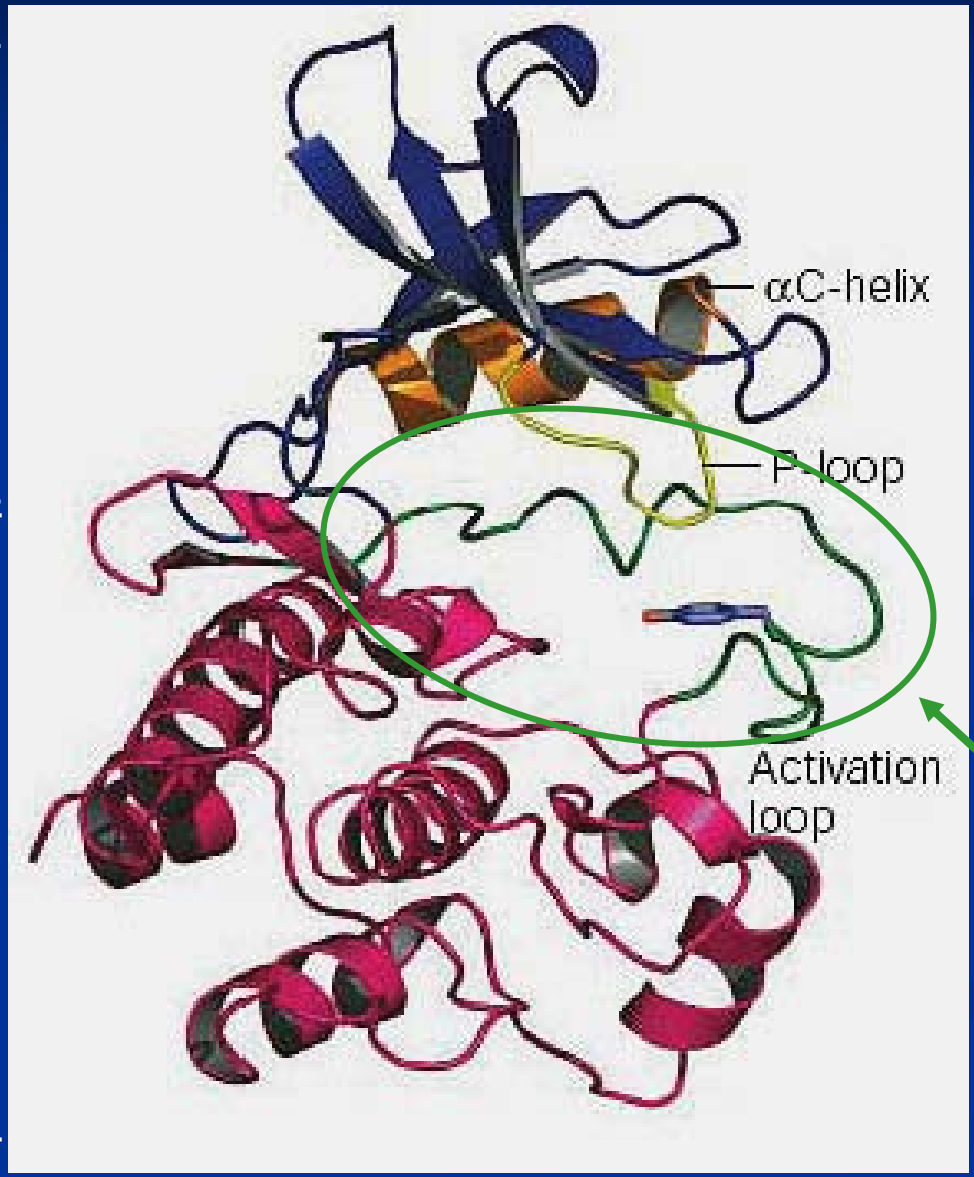
31 have been repeteadly found mutated or overexpressed in human cancers

Cytoplasmic protein-tyrosine kinases
32 kinases in 10 subfamilies



15 of these kinases are altered in human cancers

Protein Kinase Domain Structure



300 aminoacids

N-lobe (small)

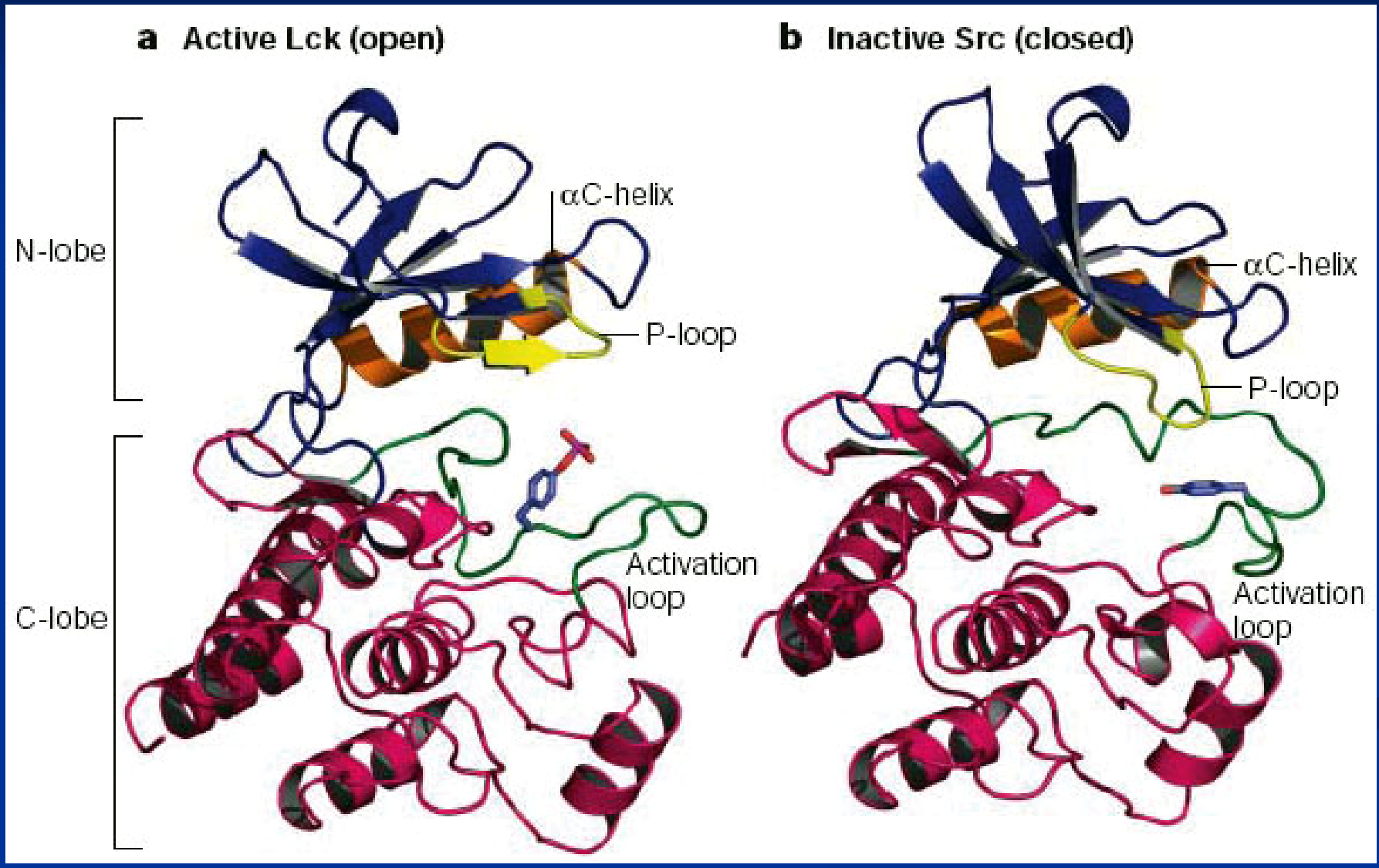
5 stranded β-sheet
Alpha-C helix
P-loop: roof of the active site, coordinates ATP γ phosphate

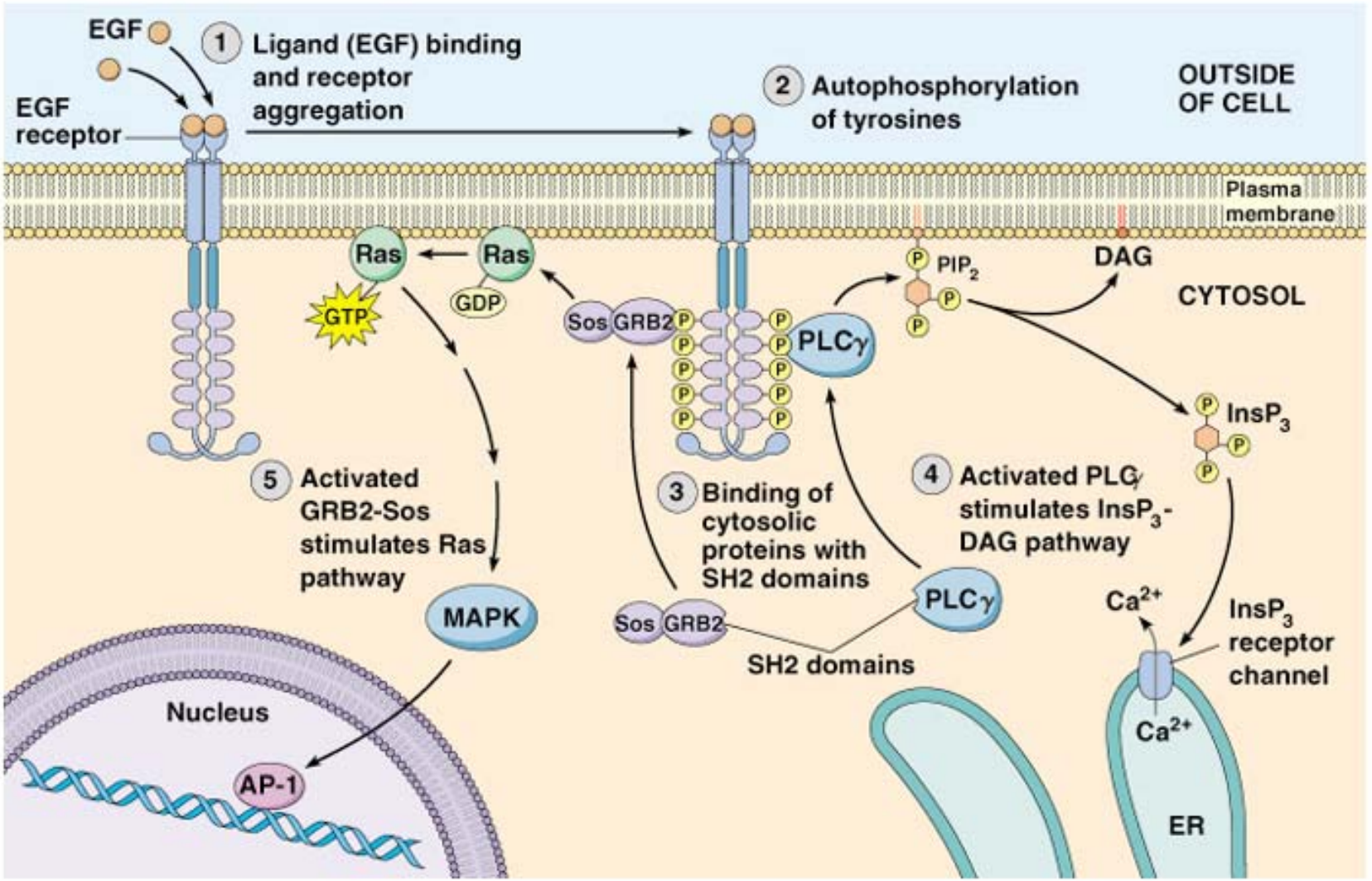
C-lobe (large)

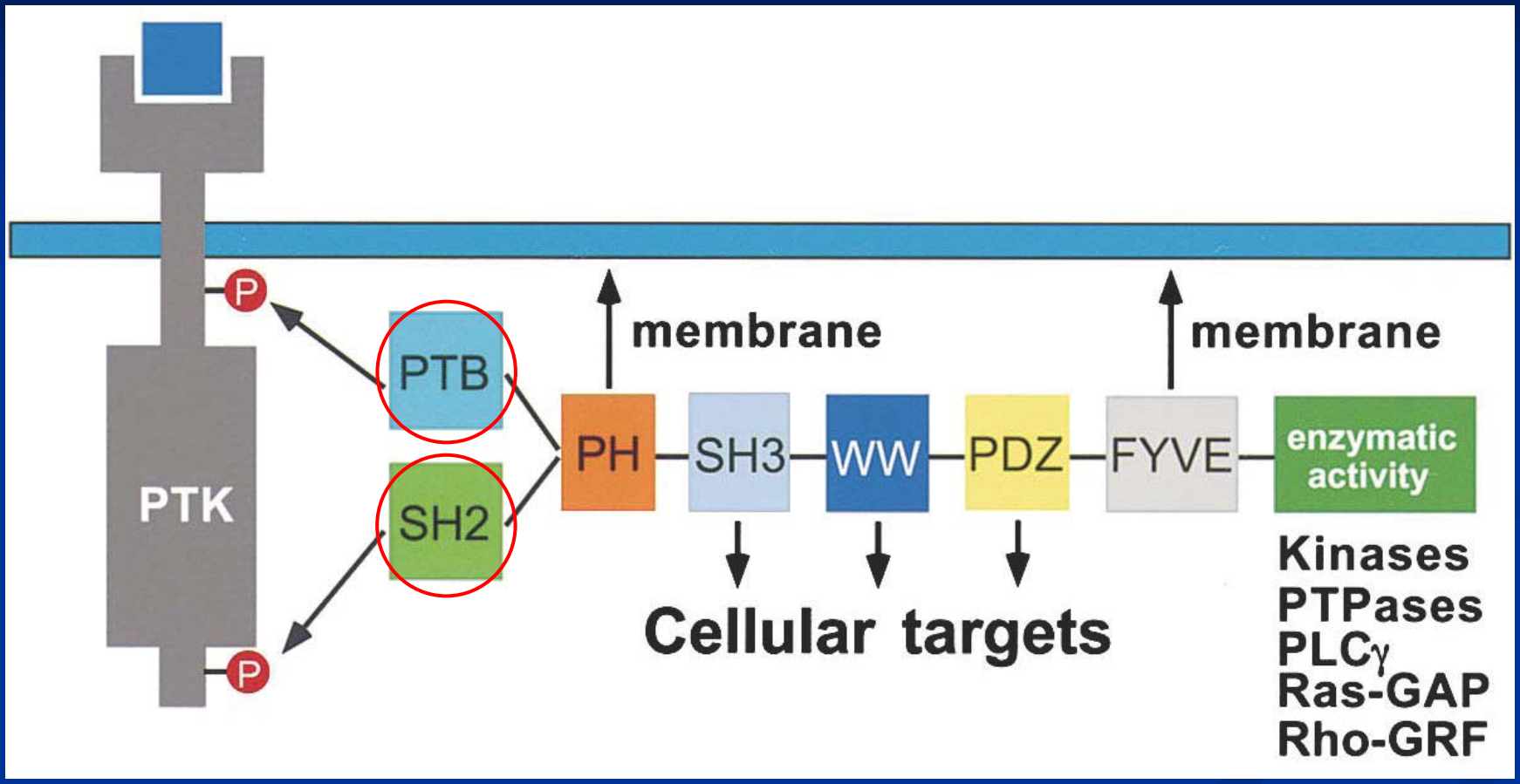
Substrate binding
Activation loop

ACTIVE SITE

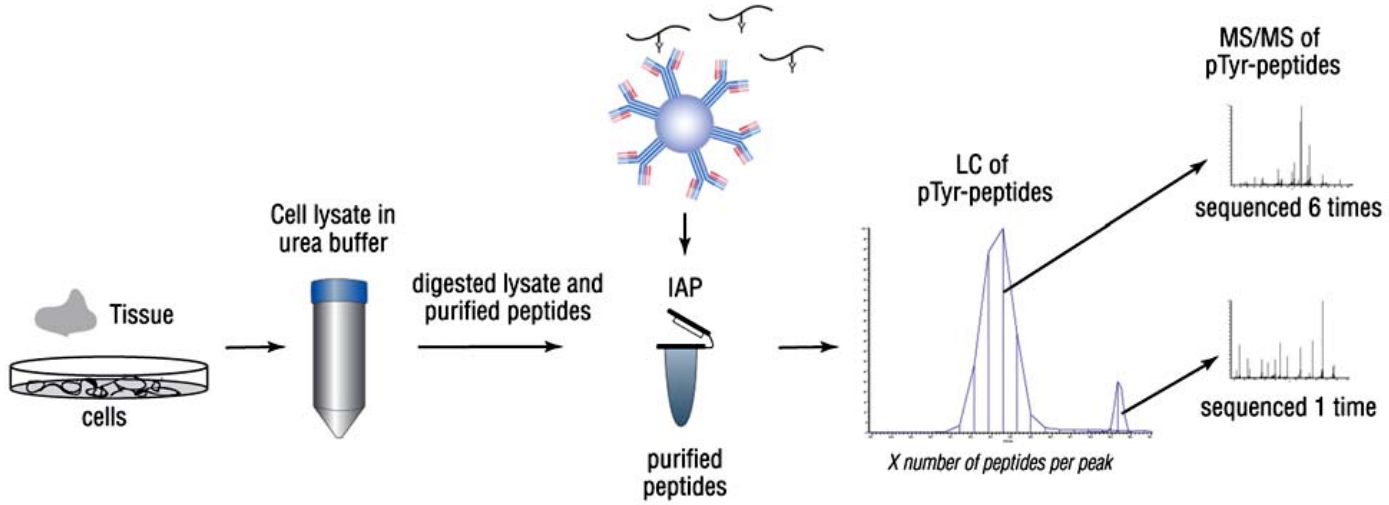
Activation conformational change

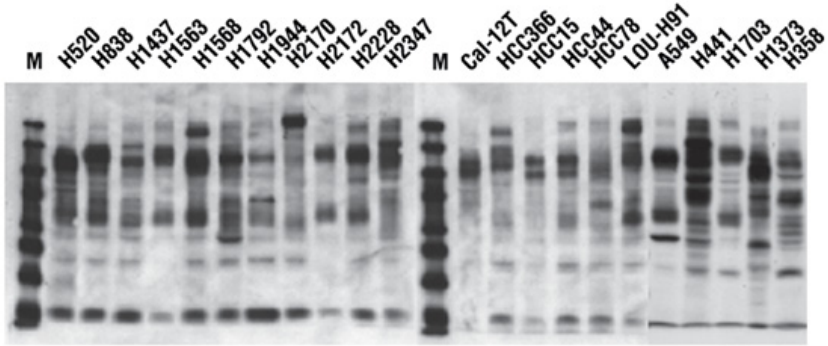


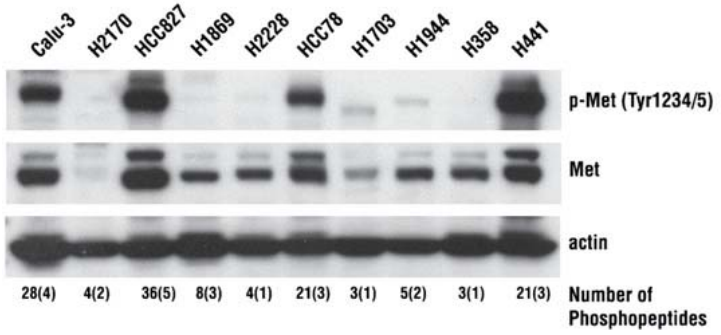



P
PTB
SH2
PTK
P
membrane
membrane
PH
SH3
WW
PDZ
FYVE
enzymatic activity
Cellular targets
Kinases
PTPases
PLCγ
Ras-GAP
Rho-GRF

Global Survey of Phosphotyrosine Signaling Identifies Oncogenic Kinases in non-small cell lung cancer (NSCLC)





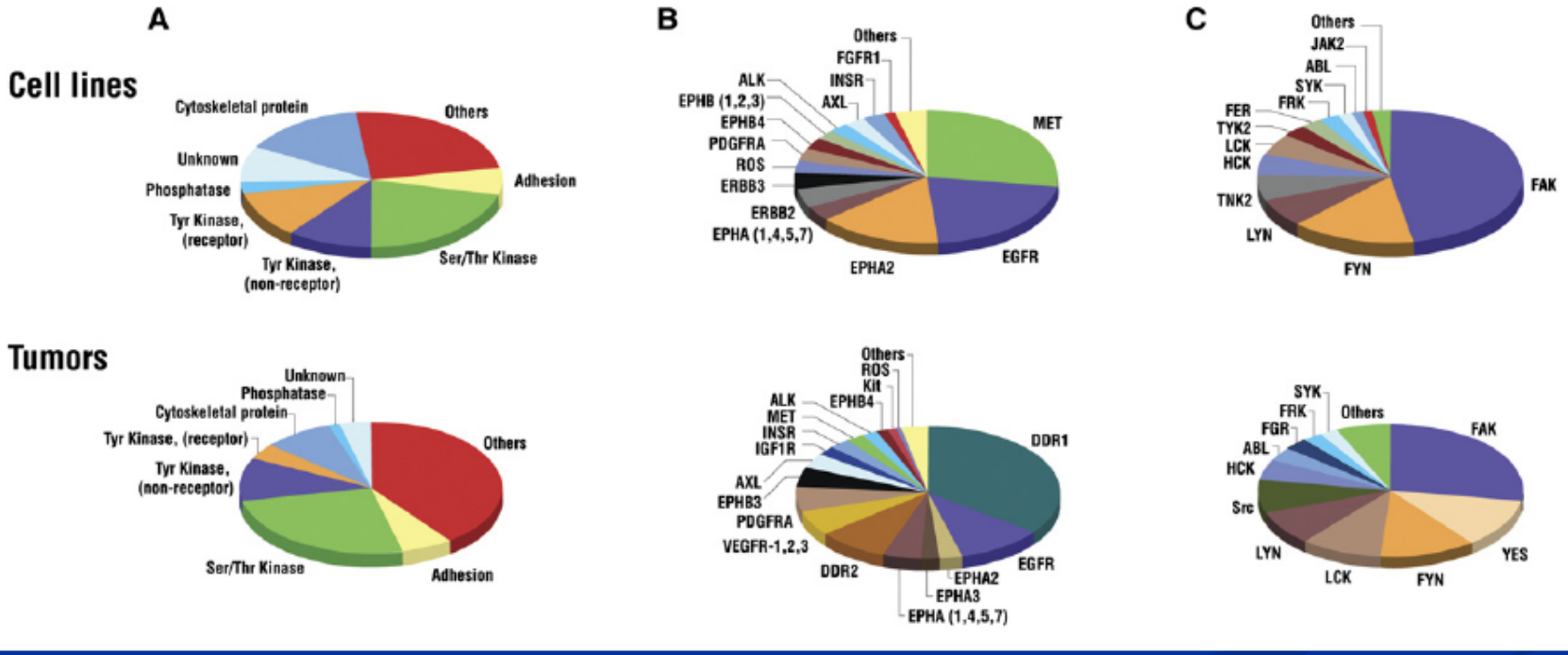


Cell, Vol 131, 1190-1203, 14 December 2007

PROTEINS

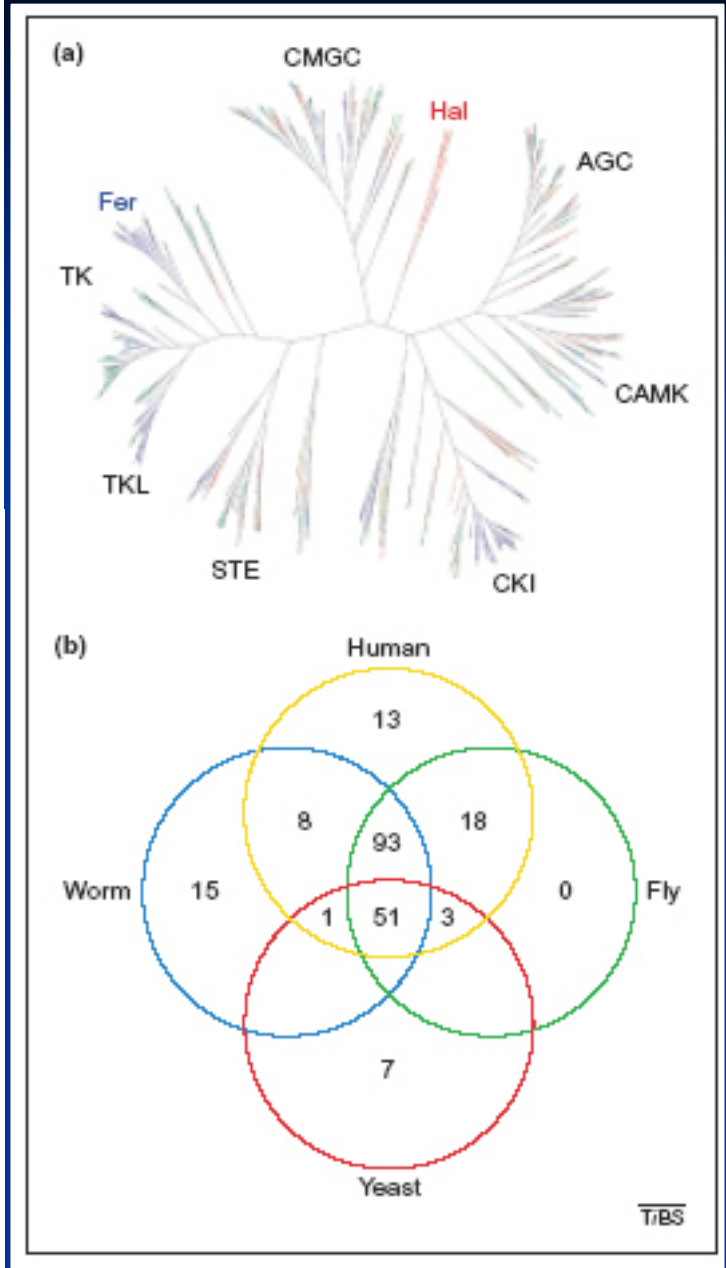
RPTKs

NON-PTKs



PROTEÍN KINASAS

- Perspectiva Histórica
- Clasificación
- Evolución
- Estructura
- Tyr Kinasas



LEVADURA, GUSANO, MOSCA, HUMANO :
51 familias (median funciones relacionadas con la célula eucariota)

LEVADURA:
7 familias propias (median funciones específicas de organismos unicelulares)
55 familias compartidas (median funciones relacionadas con la célula eucariota)

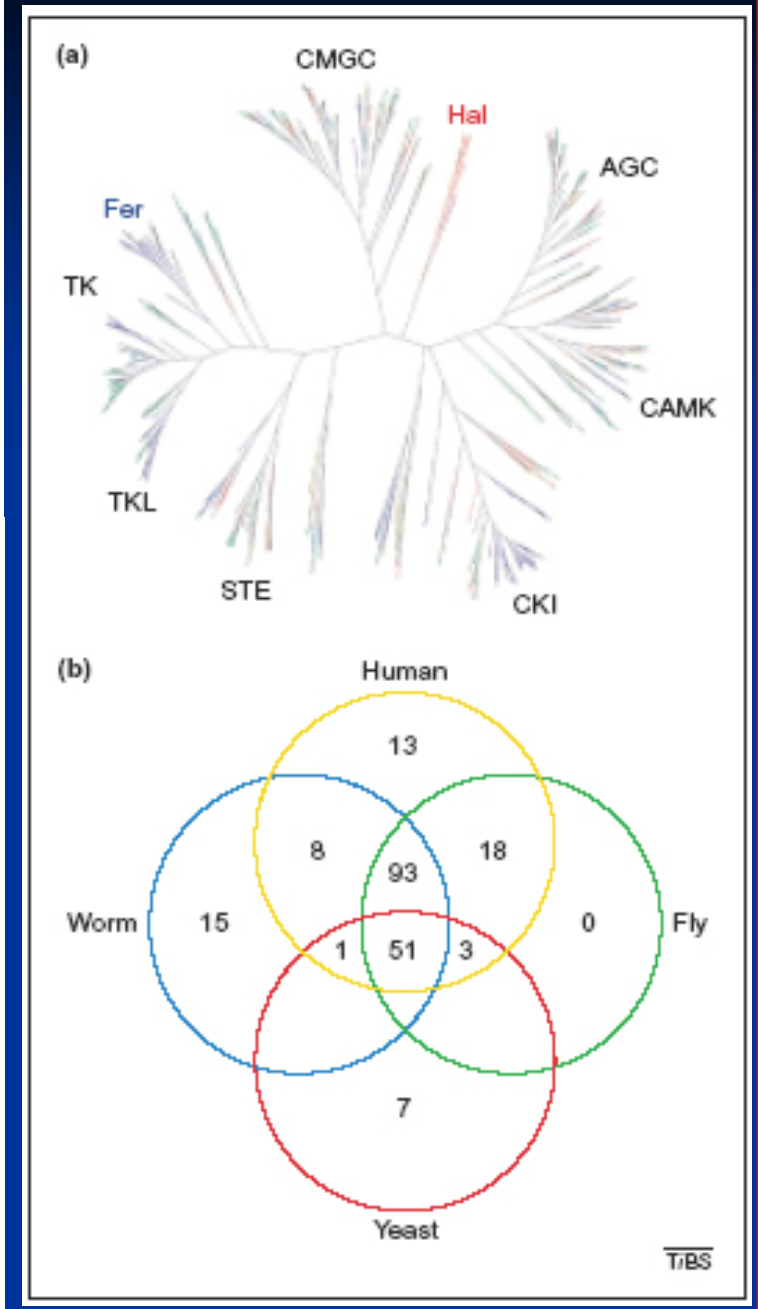
GUSANO, MOSCA, HUMANO (Y NO EN LEVADURA):
94 familias, incluyendo dos grupos TK y TKL (señalizacion y regulación de funciones propias de organismos pluricelulares)

HUMANO:
13 familias exclusivas: la mayor expansión génica tuvo lugar en el ancestro común del gusano, la mosca y el hombre

EXPANSIONES DE FAMILIAS DE KINASAS EN HUMANO

Table 2. Kinase families expanded in human relative to those in fly and worm. See table S6 for more details.

Function	Family	Human	Fly	Worm	Notes
Immunology, hemopoiesis, angiogenesis	JAK	4	1	0	Couple cytokine receptors to transcription
	PDGFR/VEGFR	8	2	0	Angiogenesis, vascular growth factor receptors
	Tec	5	1	0	Nonreceptor tyrosine kinase
	Src	11	2	3	Nonreceptor tyrosine kinase
	IRAK	4	1	1	IL-1 receptor–associated kinase
	Tie	2	0	0	Tie and Tek RTKs
	IKK	4	2	0	IκB kinase, NF-κB signaling
	RIPK	5	0	0	Receptor-interacting protein kinase, NF-κB signaling
	Axl	3	0	0	Immune system homeostasis
Neurobiology	Eph	14	1	1	Ephrin receptors
	Trk	3	0	0–1	Neurotrophin receptors
MAPK cascades	Ste11	9	2	2	(MAP3K)
	Ste20	31	13	12	(MAP4K)
	Ste7	8	4	10	(MAP2K) Has distinct worm-specific expansion
Apoptosis	DAPK	5	1	1	Death-associated protein kinase family
	RIPK	5	0	0	Transduces death signal from TNF-α receptor
	Lmr	3	0	0	Lmr1, aka apoptosis-associated tyrosine kinase (AATYK)
Calcium signaling	CaMK1	5	1	1	Calmodulin (CaM)–regulated kinases
	CaMK2	4	1	1	Calmodulin (CaM)–regulated kinases
EGF signaling	EGFR	4	1	1	Epidermal growth factor receptor family
	RSK/RSK	4	1	1	Ribosomal protein S6 kinases; RSK1-3 activated by MAPK in response to EGF
	Tao	3	1	1	Tao3 activated by EGFR
	Src	11	2	3	Src implicated in EGF signaling
	HUNK	1	0	0	Hormonally up-regulated Neu-associated kinase
Other	Trio	3	0	0	Fly and worm orthologs lack the kinase domain
	Trbl	3	1	0	Unpublished homologs of *Drosophila* trbl
	PDK	5	1	1	Mitochondrial pyruvate dehydrogenase kinases
	HIPK	4	1	1	Homeodomain-interacting protein kinases
	STKR	12	5	3	TGF-β, Activin receptors
	BRD	4	1	1	Bromodomain-containing atypical kinases
	Wnk	4	1	1	Implicated in hypertension
	NKF3	2	0	0	Uncharacterized (new kinase family 3)
	NKF4	2	0	0	Uncharacterized (new kinase family 4)
	NKF5	2	0	0	Uncharacterized (new kinase family 5)
	CDKL	5	1	1	Cyclin-dependent kinase-like



MOSCA, HUMANO :

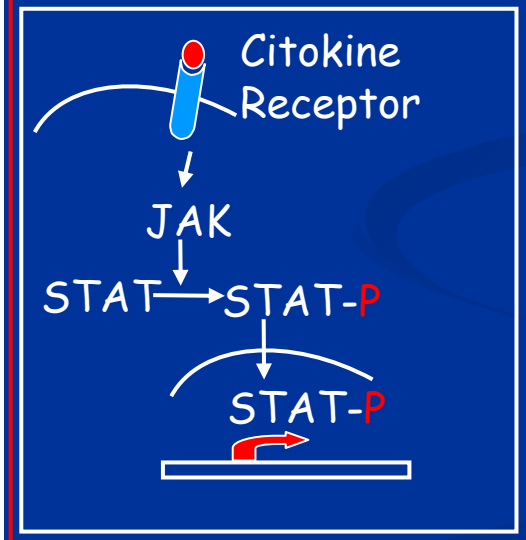
18 familias

*funciones recientes desarrolladas con posterioridad a la divergencia con el nemátodo

*algunos podrían haber estado en metazoos más primitivos y haberse perdido

*se relacionan con funciones de inmunidad, neurobiología, ciclo celular y morfogénesis.

EJEMPLO: Familia JAK

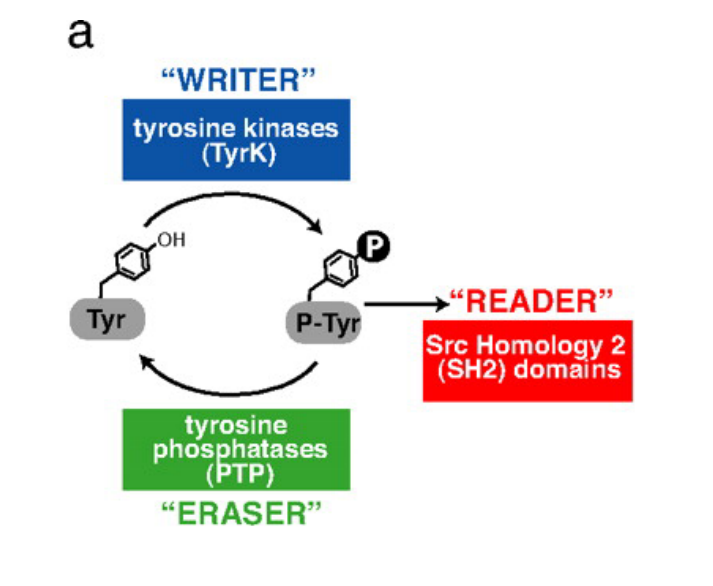


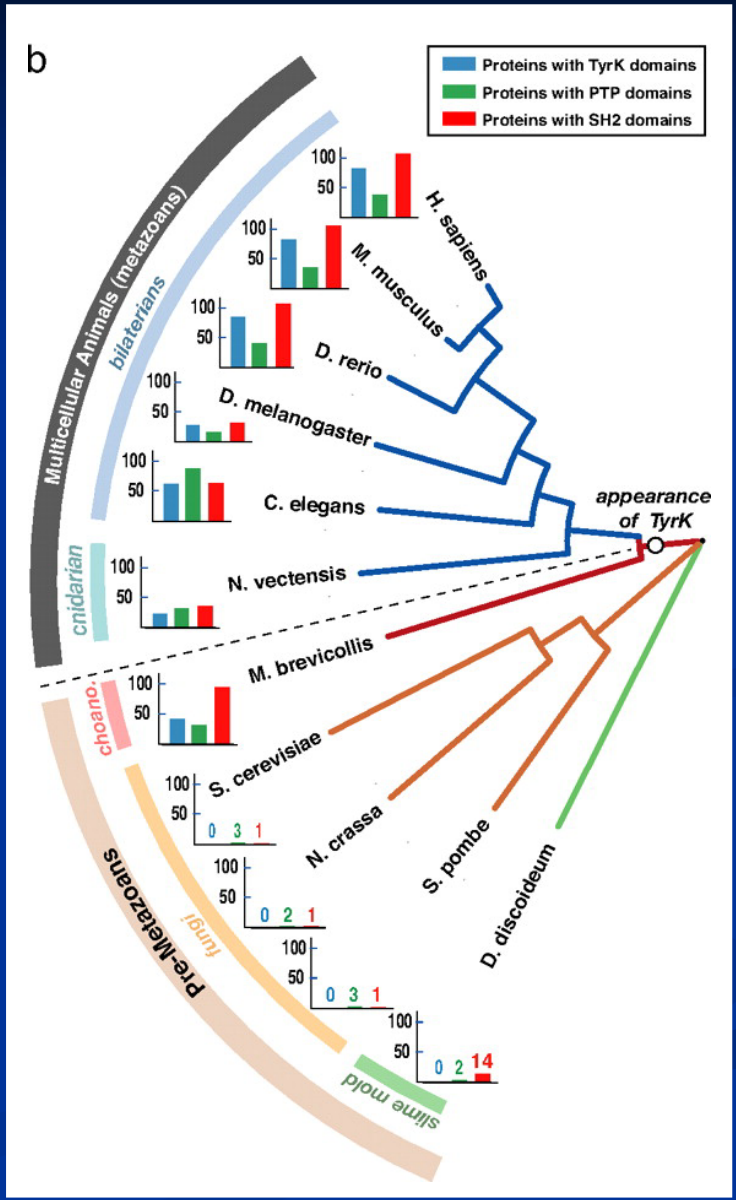
HUMANO: 8 JAK

MOSCA: 1 JAK, 1 STAT Y 1 CLR

GUSANO: 0 JAK, varios STAT activados por RTK

Phospho-tyrosine signaling machinery in different eukaryotic lineages

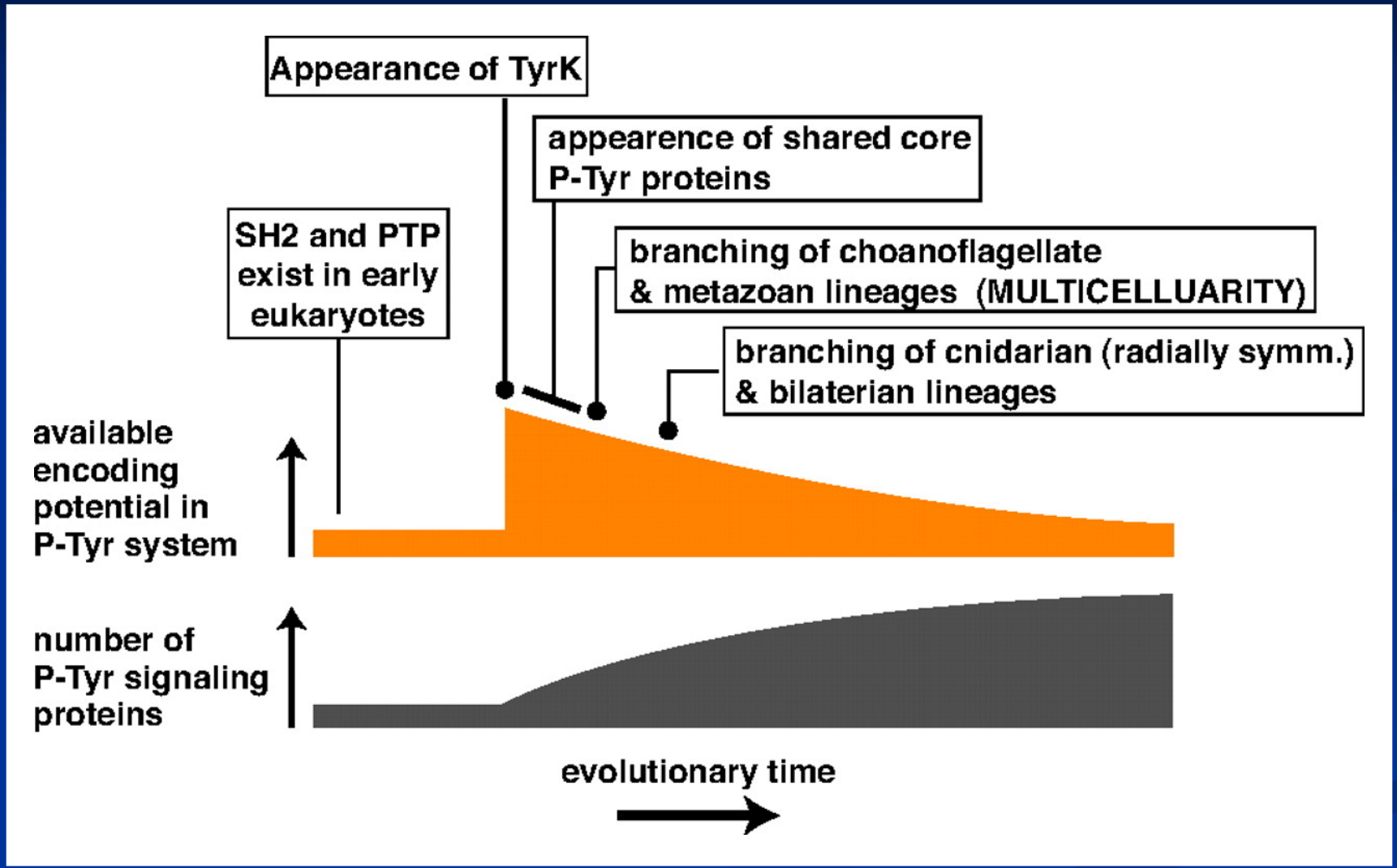




Pincus D. et.al. PNAS 2008;105:9680-9684

PNAS

Model: Timeline for the evolution of the P-Tyr signaling system



Pincus D. et.al. PNAS 2008;105:9680-9684

PNAS

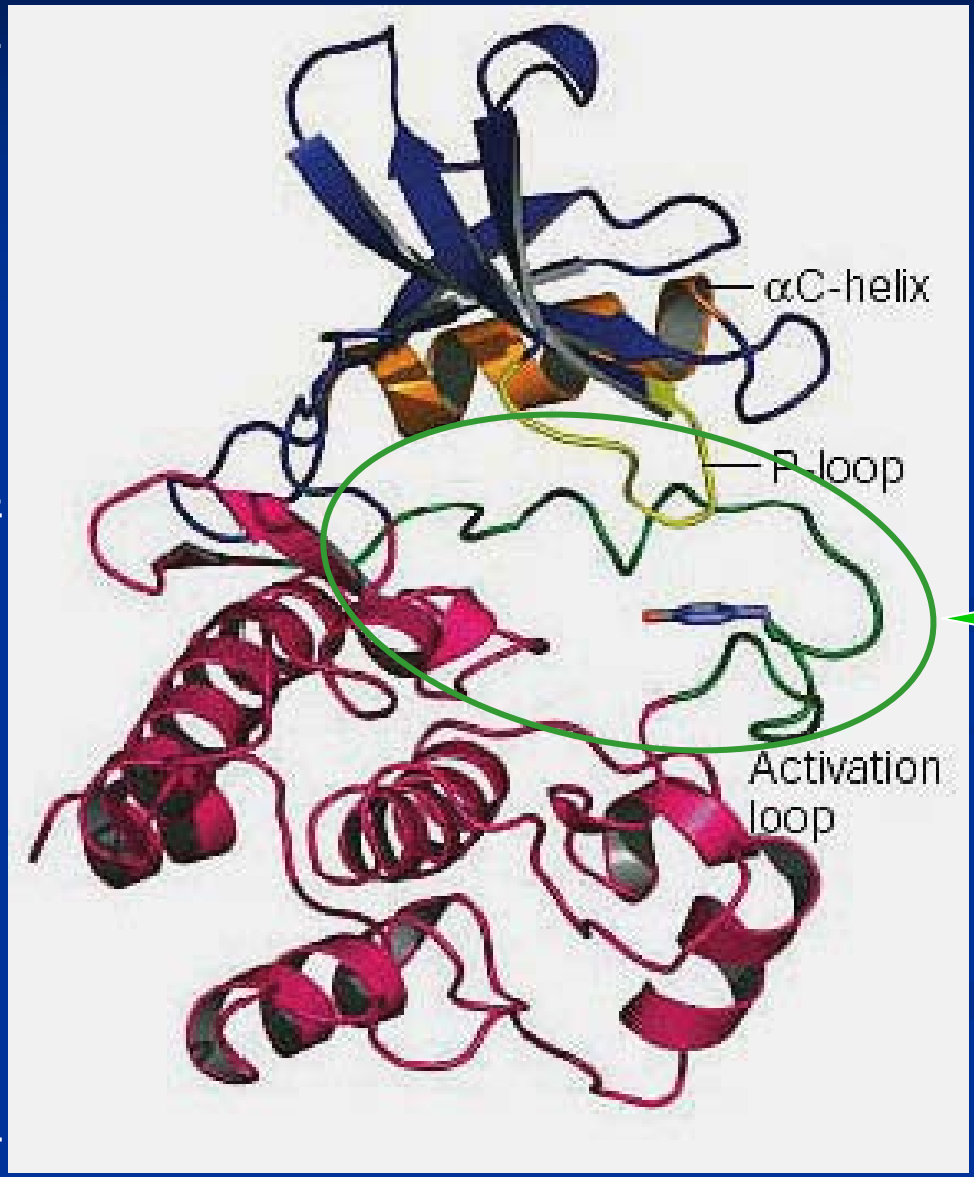
PROTEÍN KINASAS

- Perspectiva Histórica
- Clasificación
- Evolución
- Estructura
- Tyr Kinasas

Protein Kinase Structure

N-LOBE

C-LOBE



300 aminoacids

N-lobe (small)

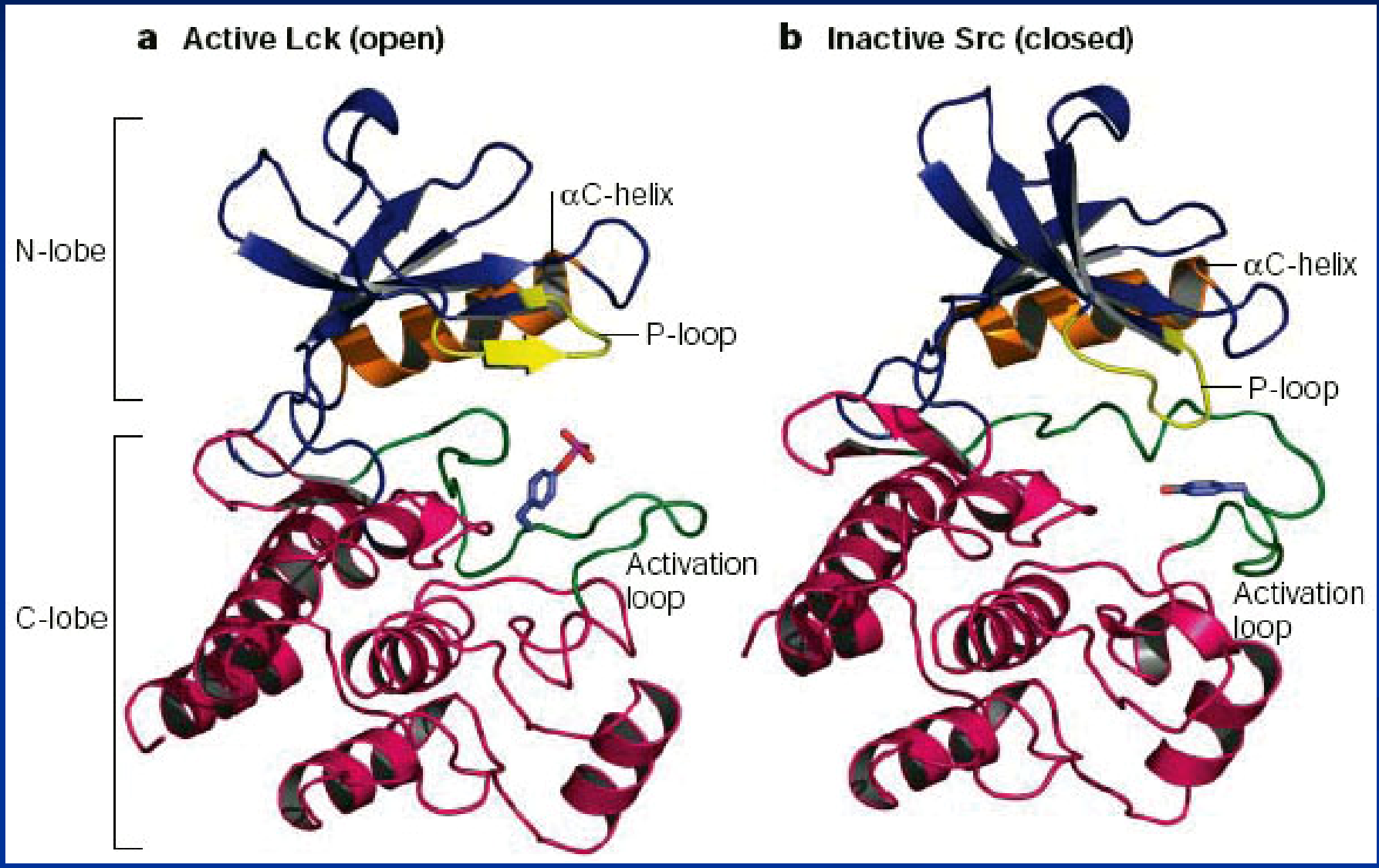
5 stranded β-sheet
Alpha-C helix
P-loop: roof of the active site, coordinates ATP γ phosphate

ACTIVE SITE

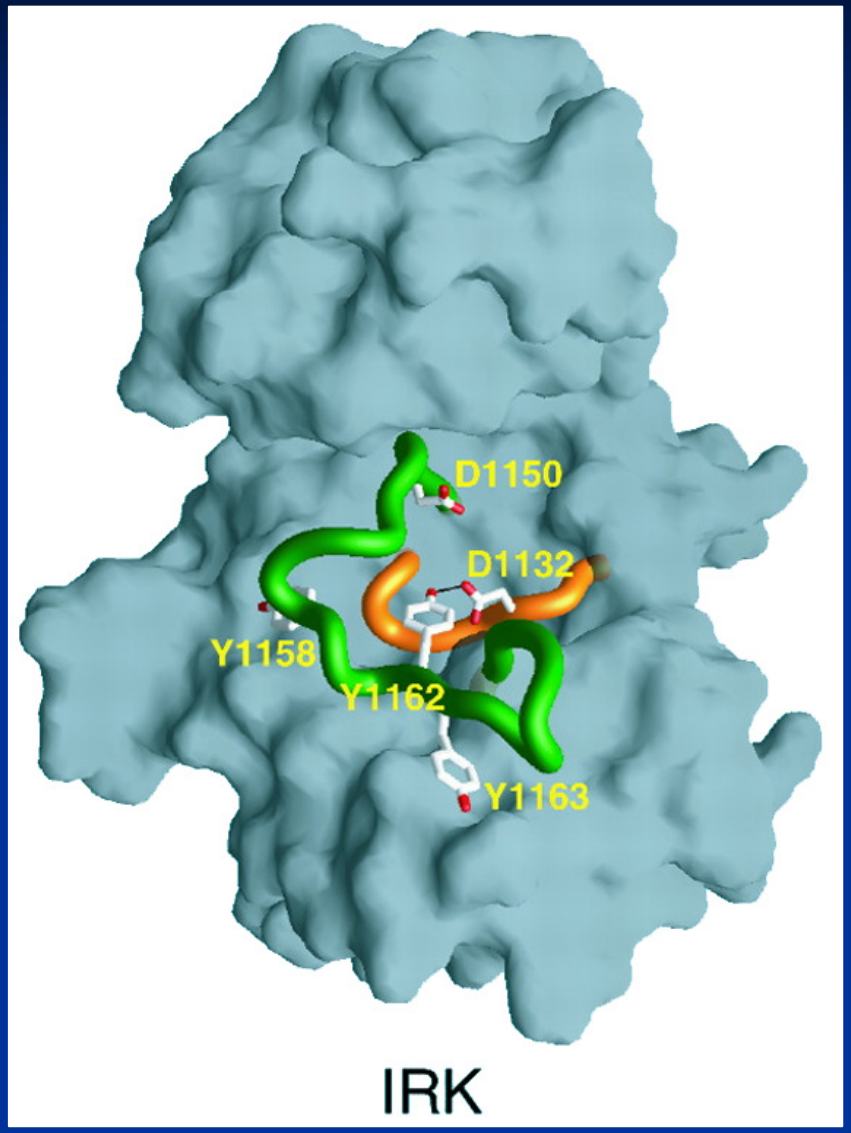
C-lobe (large)

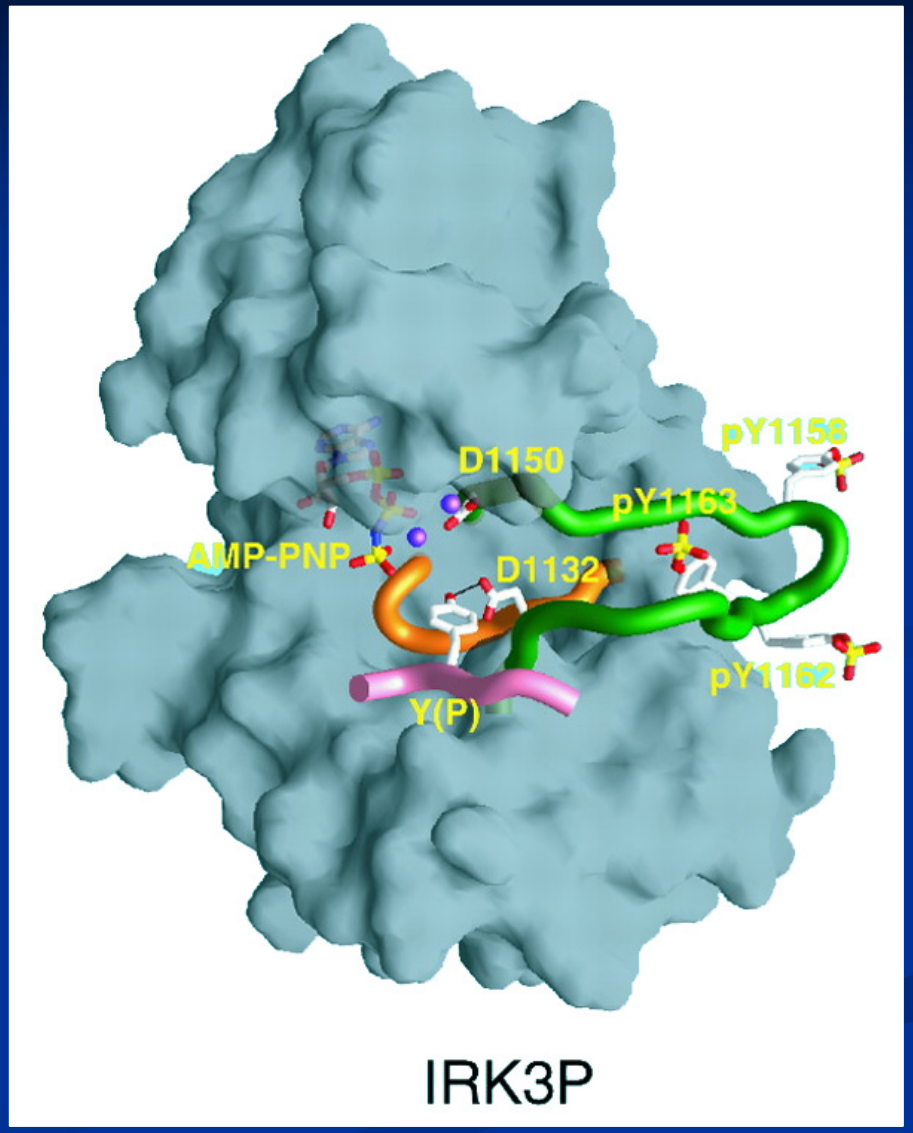
Substrate binding
Activation loop

Activation conformational change



Change in Activation loop conformations in IRK (Insulin receptor kinase)





The activation loop is green, the catalytic loop is orange, and the peptide substrate is pink.

Hubbard & Till, ARB 69, 373 (2000)

PROTEÍN KINASAS

- Perspectiva Histórica
- Clasificación
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- Estructura
- Tyr Kinasas

FOSFORILACIÓN EN Tyr

- Está ausente en levaduras: surgió al mismo tiempo que los metazoos, lo que sugiere que es necesaria para una comunicación celular coordinada.

- Surge en coordinación con otros componentes del sistema que usa la fosforilacion en Tyr para propagar la señal (p.ej. Dominios SH2, PTPs)

KINASAS DE TIROSINA

- CLASIFICACIÓN
- ACTIVACIÓN
- DOMINIOS ASOCIADOS CON LAS KINASAS DE TIROSINAS
- LAS KINASAS DE TIROSINAS COMO ONCOGENES

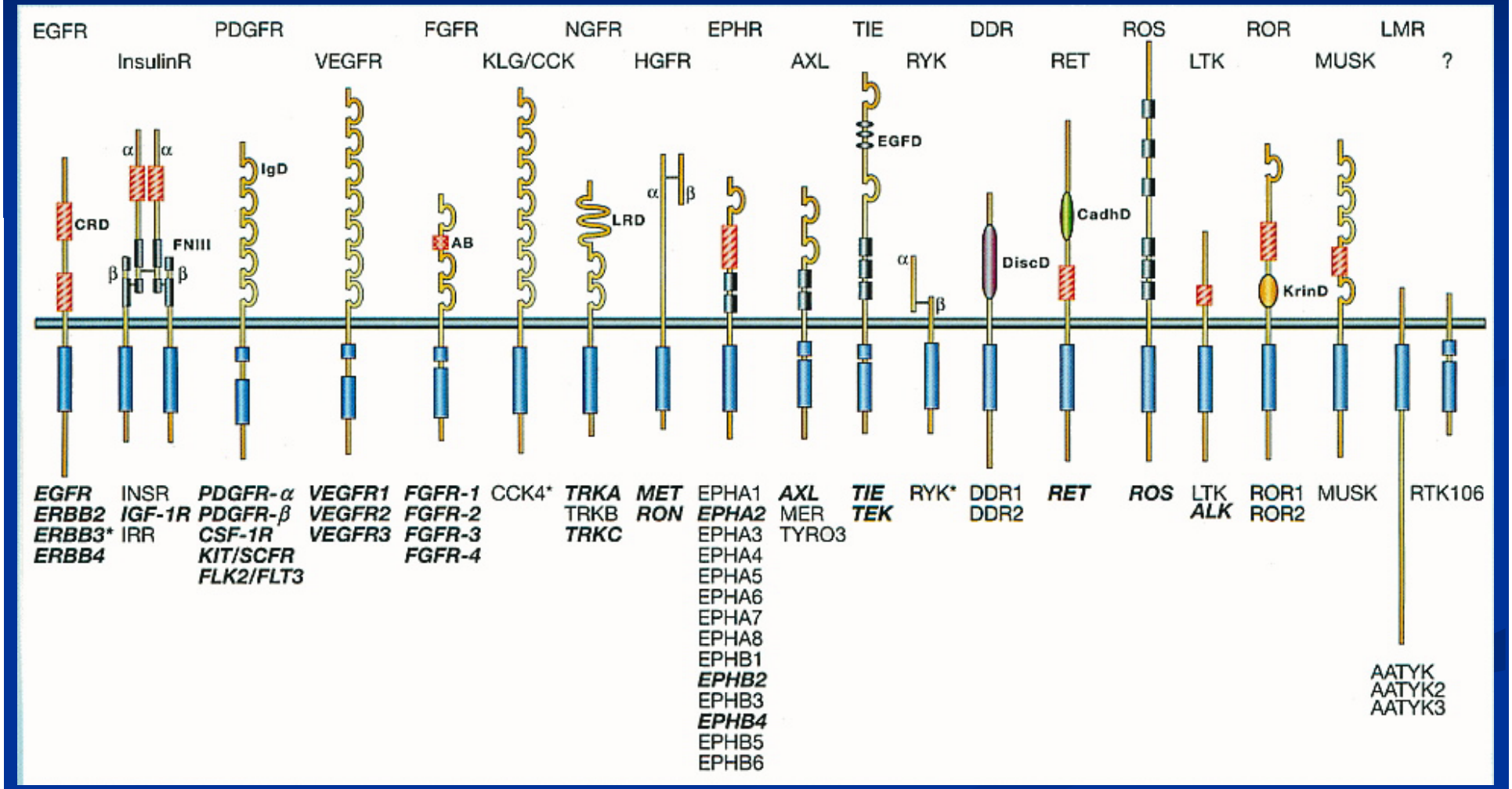
CLASIFICACIÓN DE LAS Tyr KINASAS HUMANAS

- Receptores de proteín-tirosina kinasa (RPTK):20 Familias (59 kinasas)

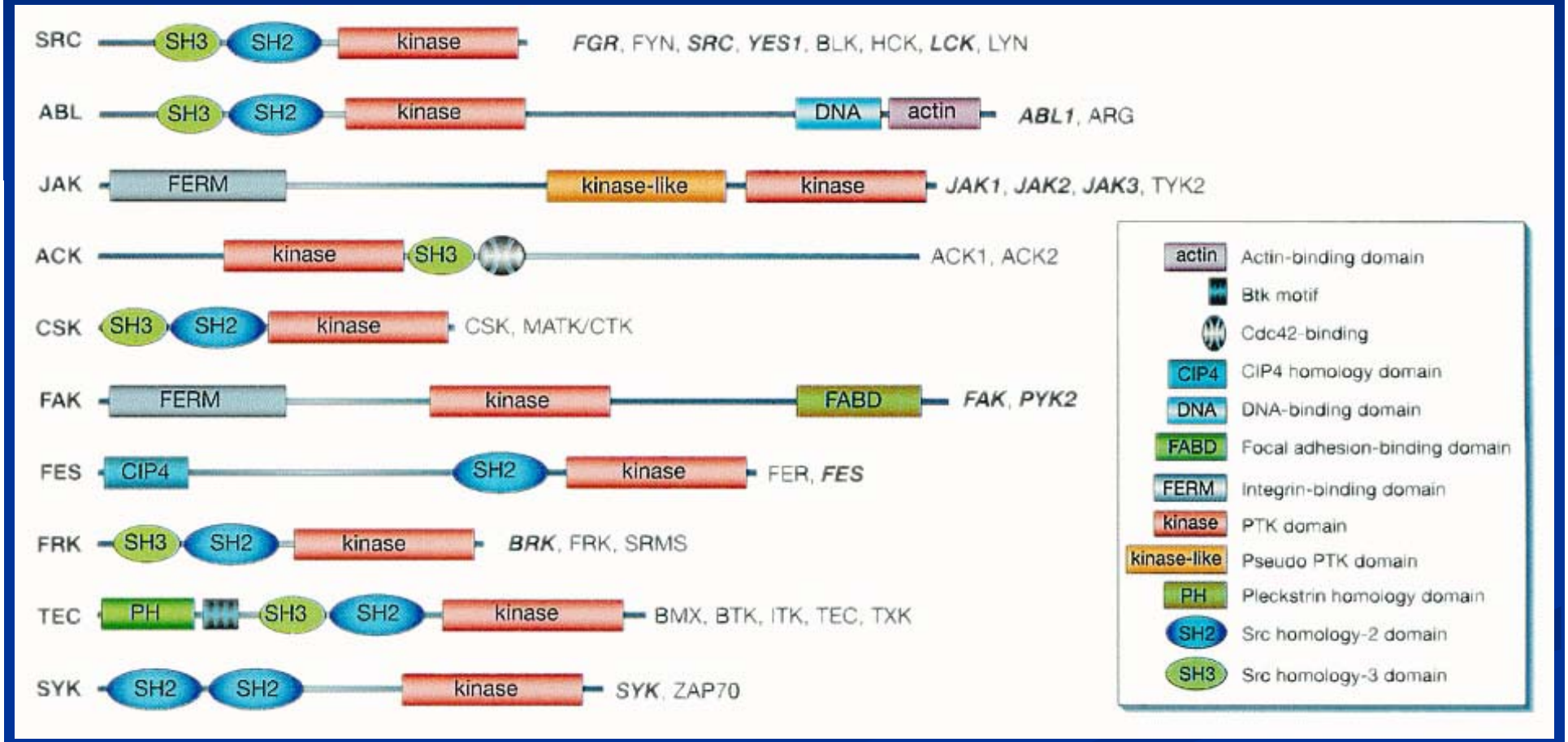
- Proteín-tirosina kinasas citoplásmicas: 10 familias (32 kinasas)

RECEPTOR TYROSINE KINASES

59 kinases in 20 subfamilies



Cytoplasmic protein-tyrosine kinases
32 kinases in 10 subfamilies



KINASAS DE TIROSINA

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- ACTIVACIÓN
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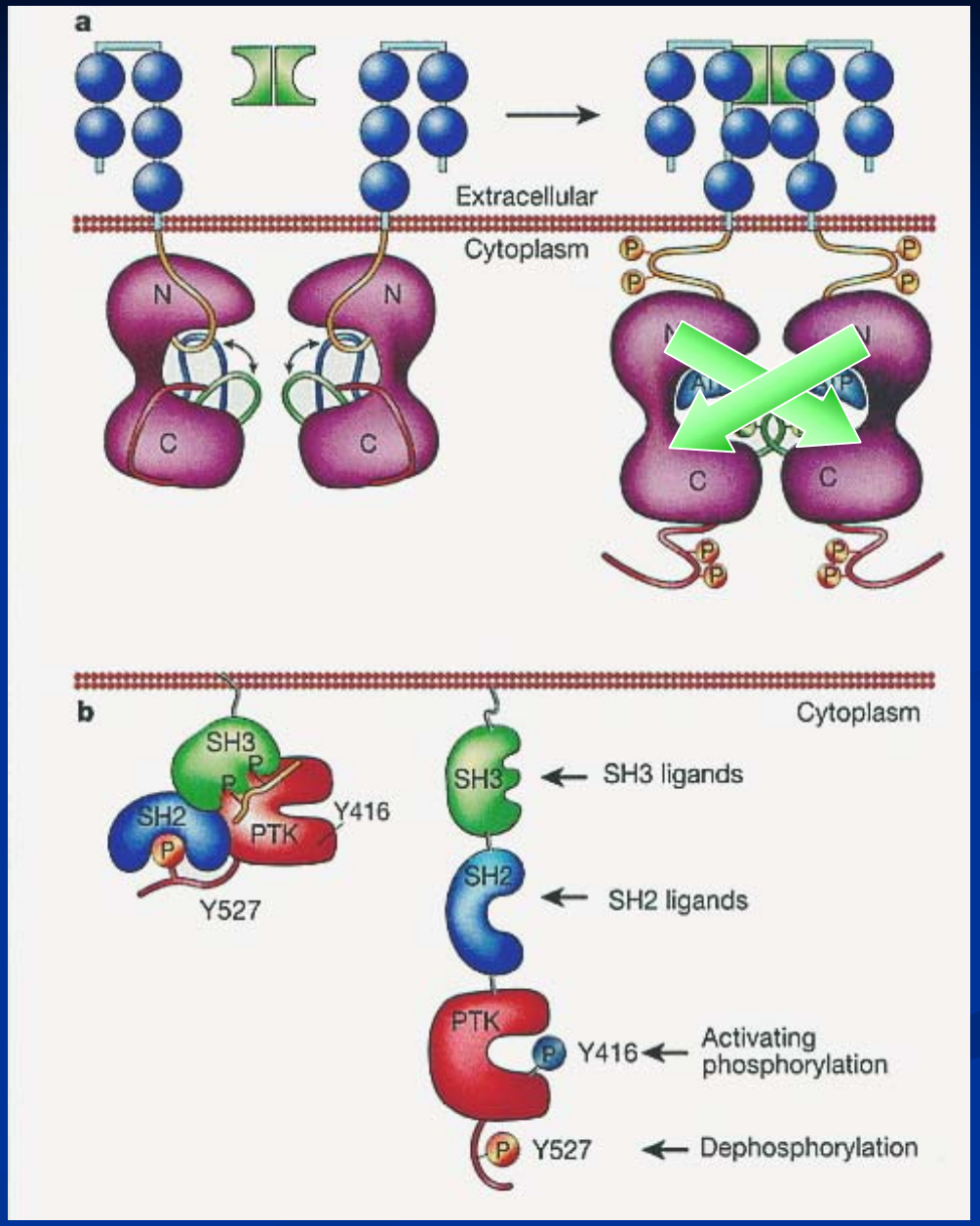
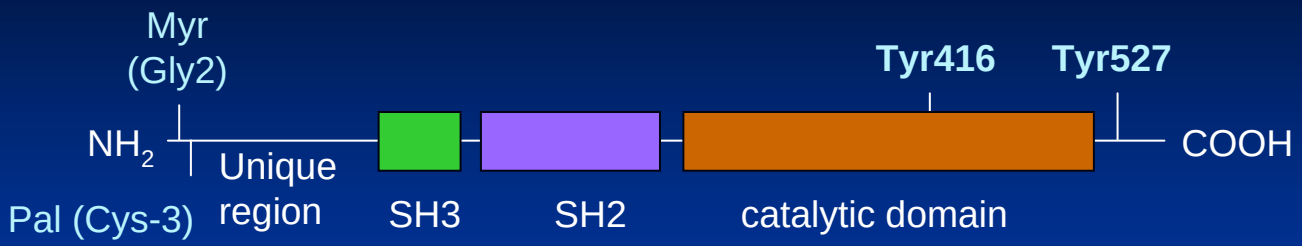
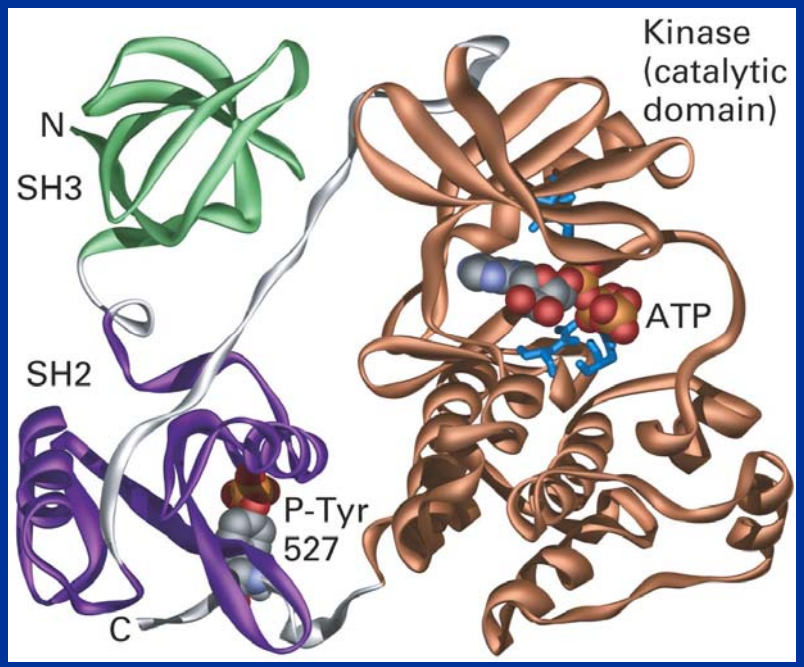
Protein tyrosine kinase activation mechanisms

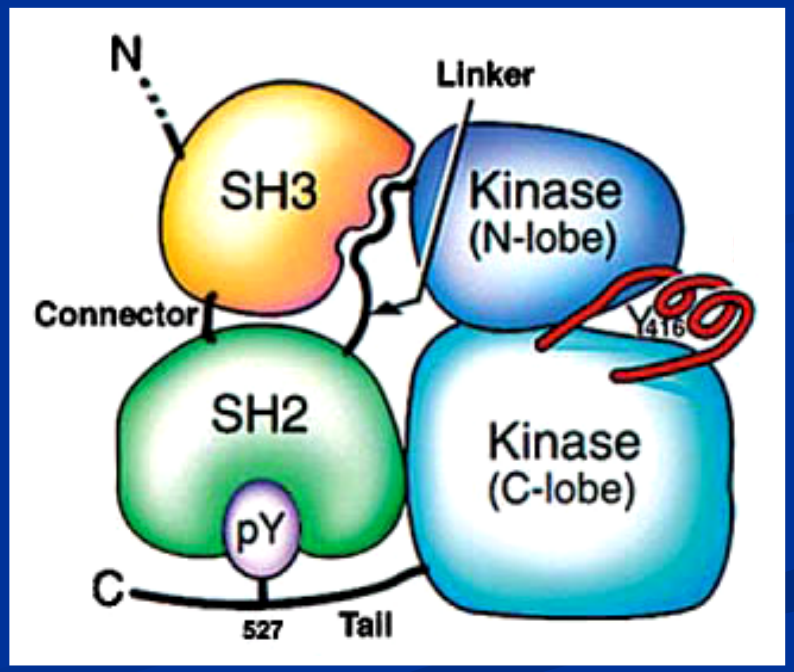


ACTIVACIÓN DE LAS SRC KINASAS

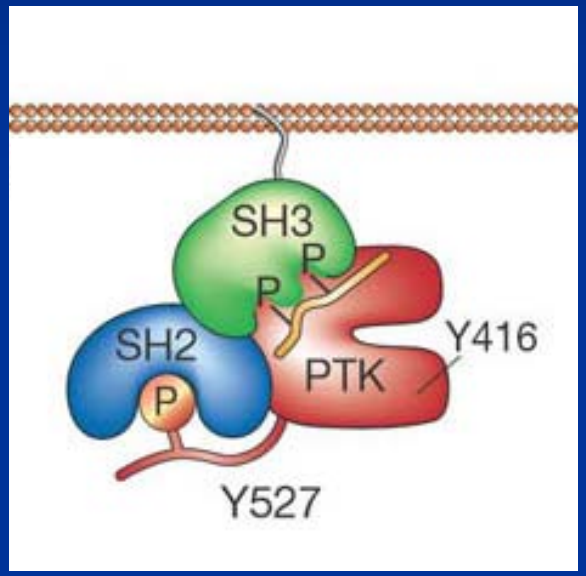
Structure of the Src tyrosine kinase



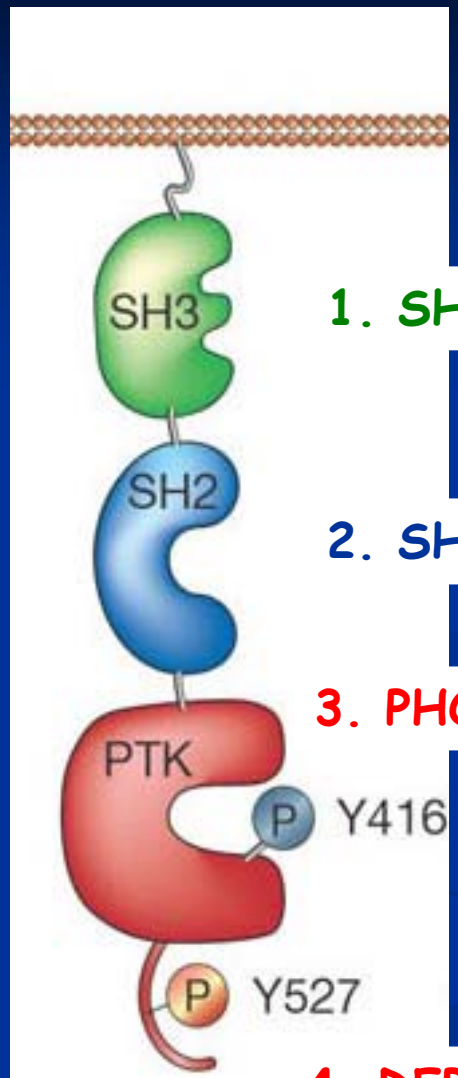




Activation of Src tyrosine kinases



INACTIVE



1. SH3 LIGANDS

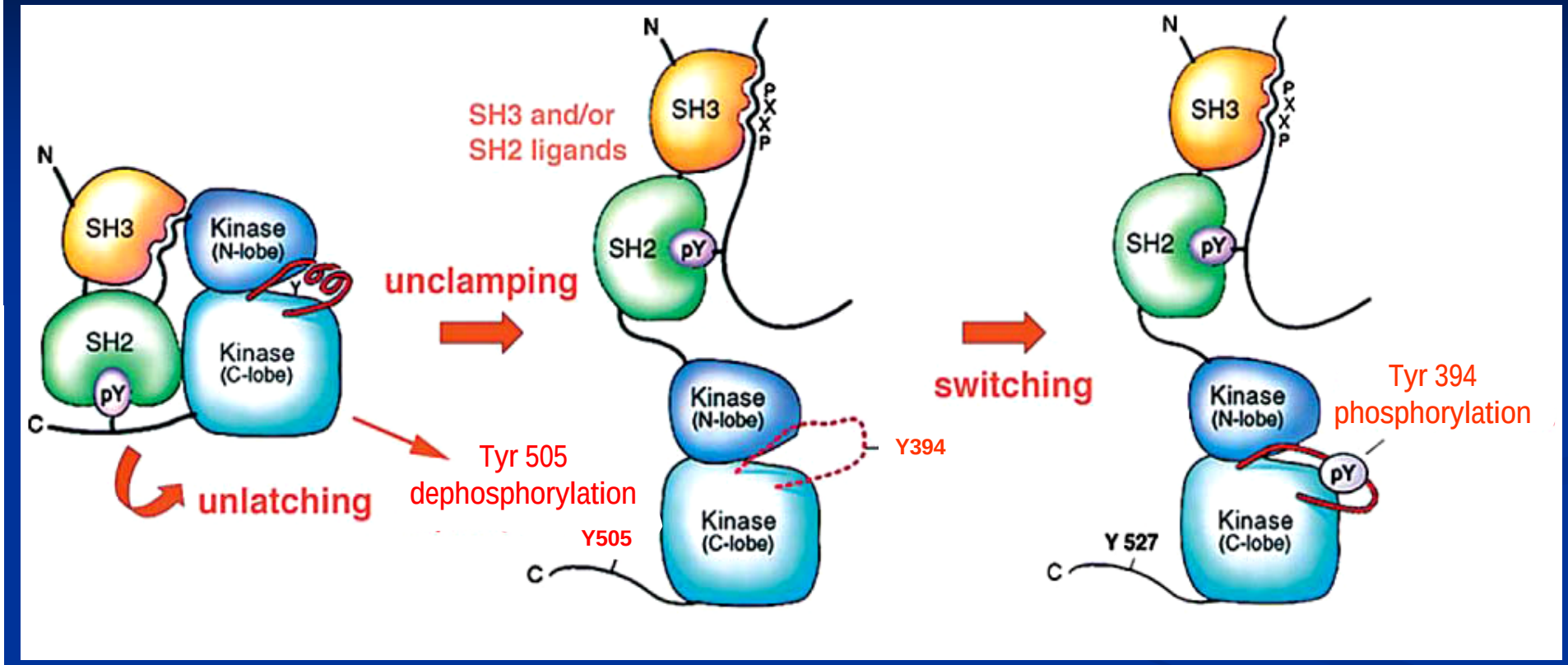
2. SH2 LIGANDS

3. PHOSPHORYLATION

4. DEPHOSPHORYLATION

ACTIVE

Activación de Lck

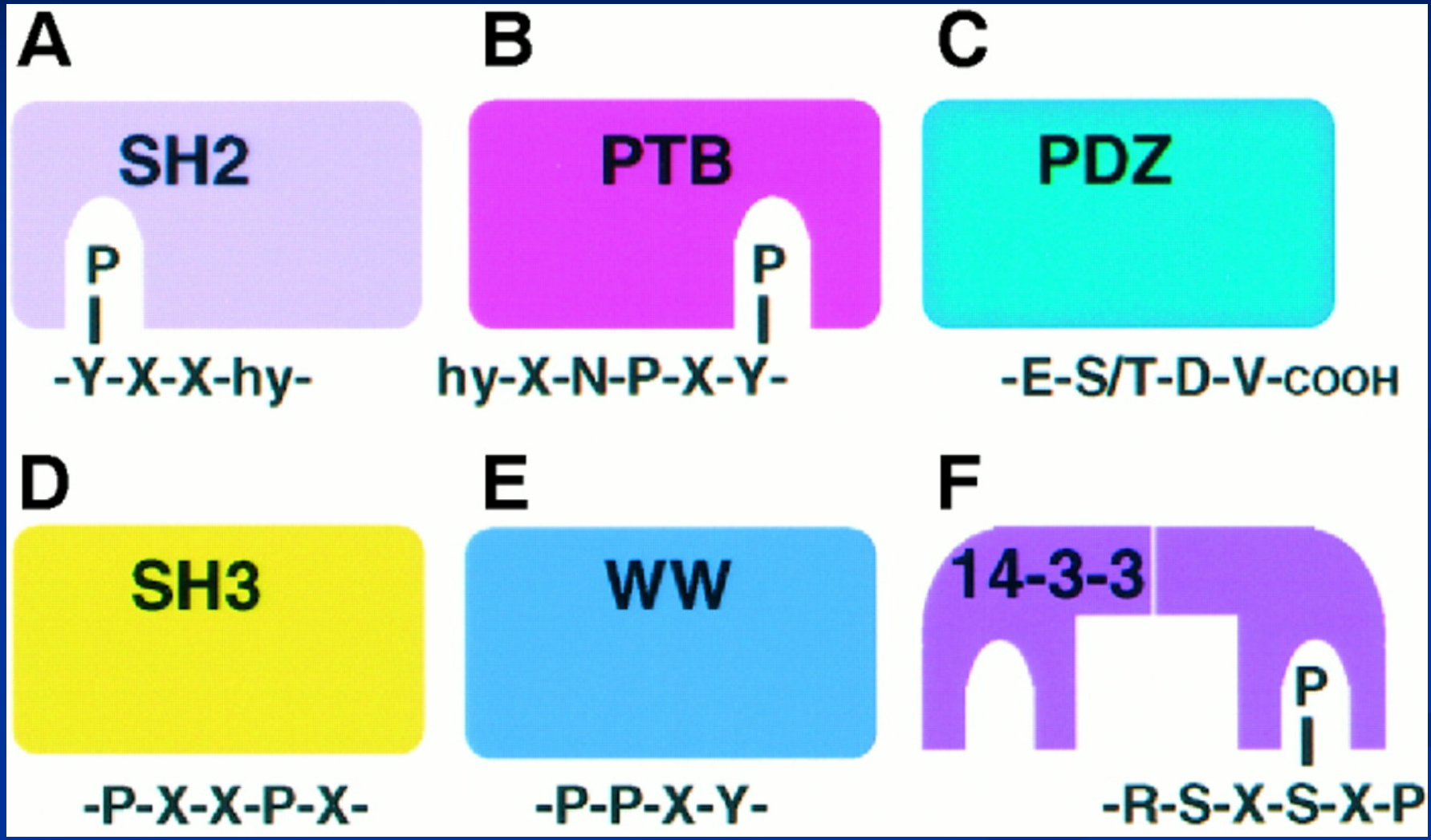


SH2 and SH3 domains assist tyrosine kinases in recognizing cellular substrates. Many of the best substrates for Src kinases contain ligands for the SH3 and/or SH2 domains. Binding promotes phosphorylation by the catalytic domain; in this way, kinase activation is coupled to substrate recognition.

KINASAS DE TIROSINA

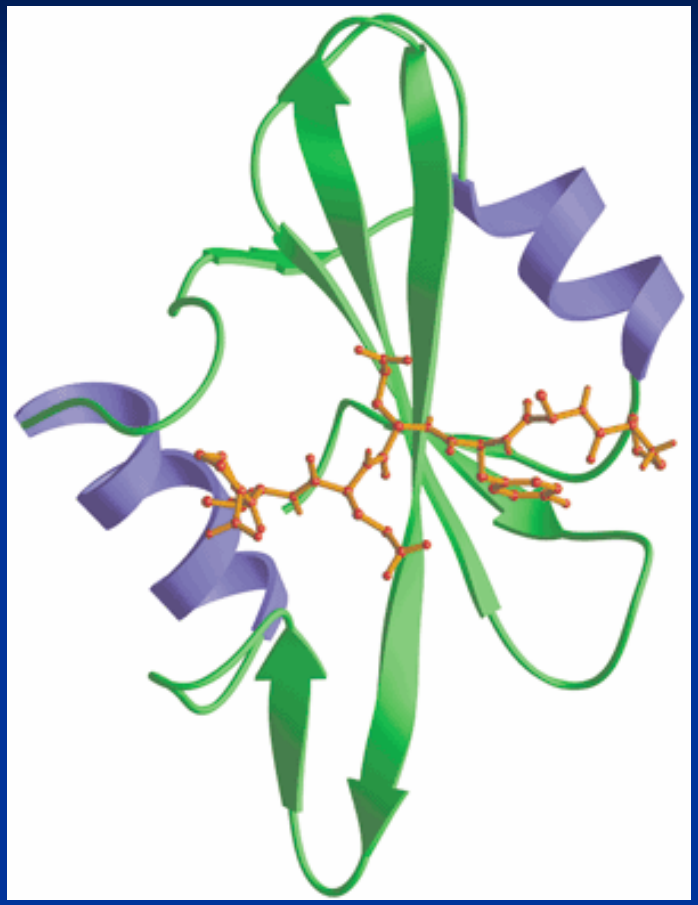
- CLASIFICACIÓN
- ACTIVACIÓN
- DOMINIOS ASOCIADOS CON LAS KINASAS DE TIROSINAS
- LAS KINASAS DE TIROSINAS COMO ONCOGENES

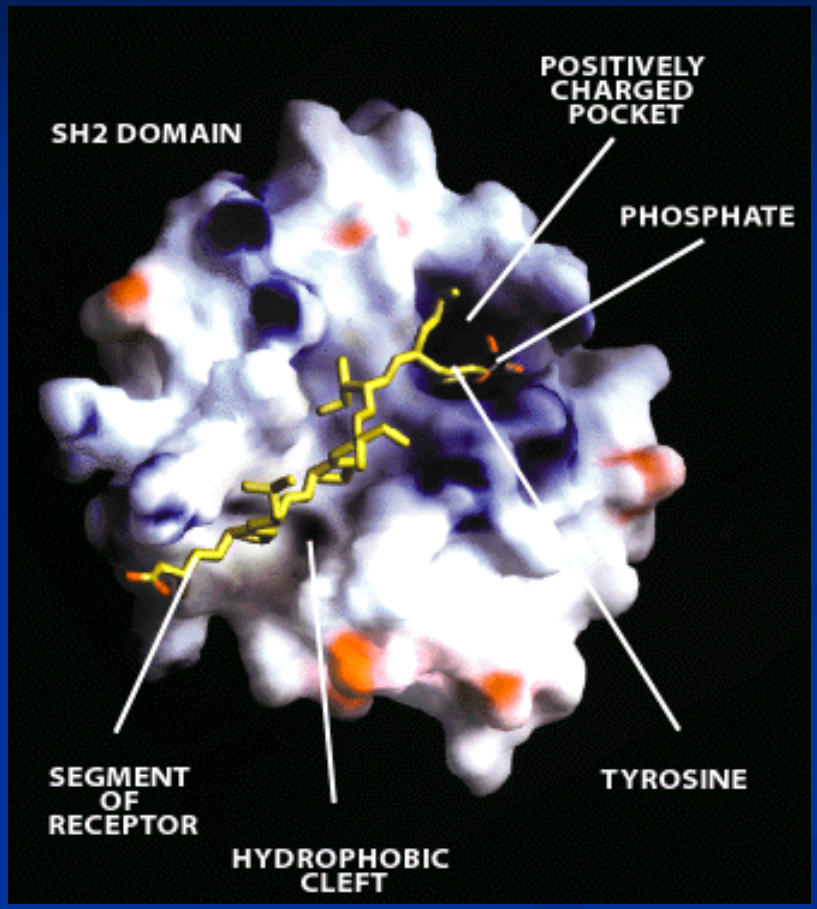
Protein modules for the assembly of signaling complexes



From Pawson & Scott, 1997. Science 278:2075-2080.

SH2 domains (Src-homlogy 2)

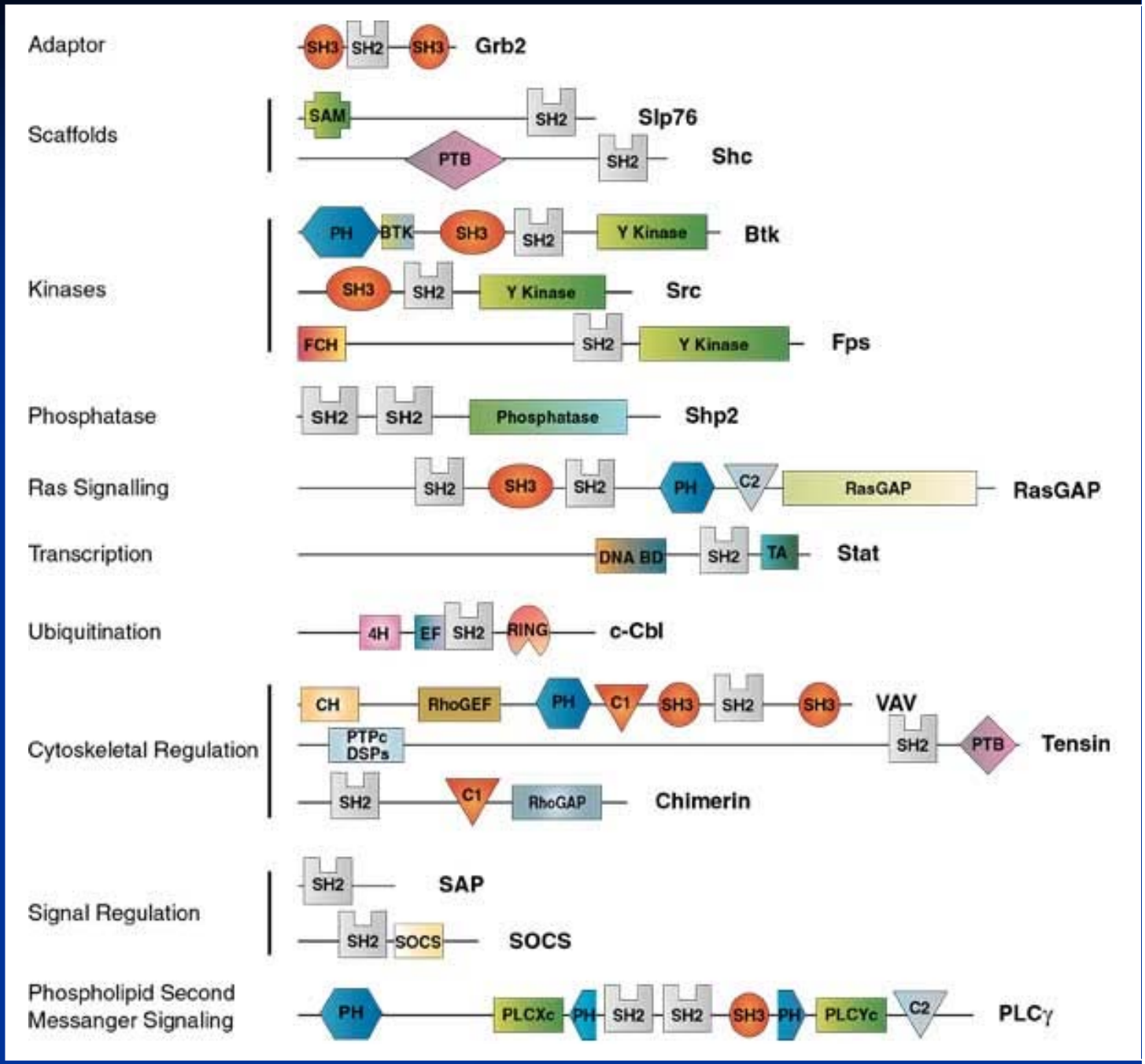




SH2 domains are modules of ~100 amino acids that bind to specific phospho (pY)-containing peptide motifs

The Pawson Lab http://www.mshri.on.ca/pawson/domains.html

The SH2 domain is found in a wide variety of metazoan proteins that regulate functionally diverse processes.



Pawson, T. et al., Trends in Cell Biology Vol.11 No.12 December 2001

KINASAS DE TIROSINA

- CLASIFICACIÓN
- ACTIVACIÓN
- DOMINIOS ASOCIADOS CON LAS KINASAS DE TIROSINAS
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MECANISMOS DE TRANSFORMACIÓN ONCOGÉNICA POR KINASAS DE TIROSINAS

1. TRANSDUCCIÓN RETROVIRAL DEL PROTO-ONCOGEN DE UNA PTK (ROEDORES Y AVES)
2. REORDENACIONES GENÓMICAS (TRANSLOCACIONES CROMOSÓMICAS)
3. MUTACIONES DE GANANCIA DE FUNCIÓN (GOF)
4. AUMENTO DE LA EXPRESIÓN POR AMPLIFICACIÓN GÉNICA

AUMENTO DE LA ACTIVIDAD KINASA

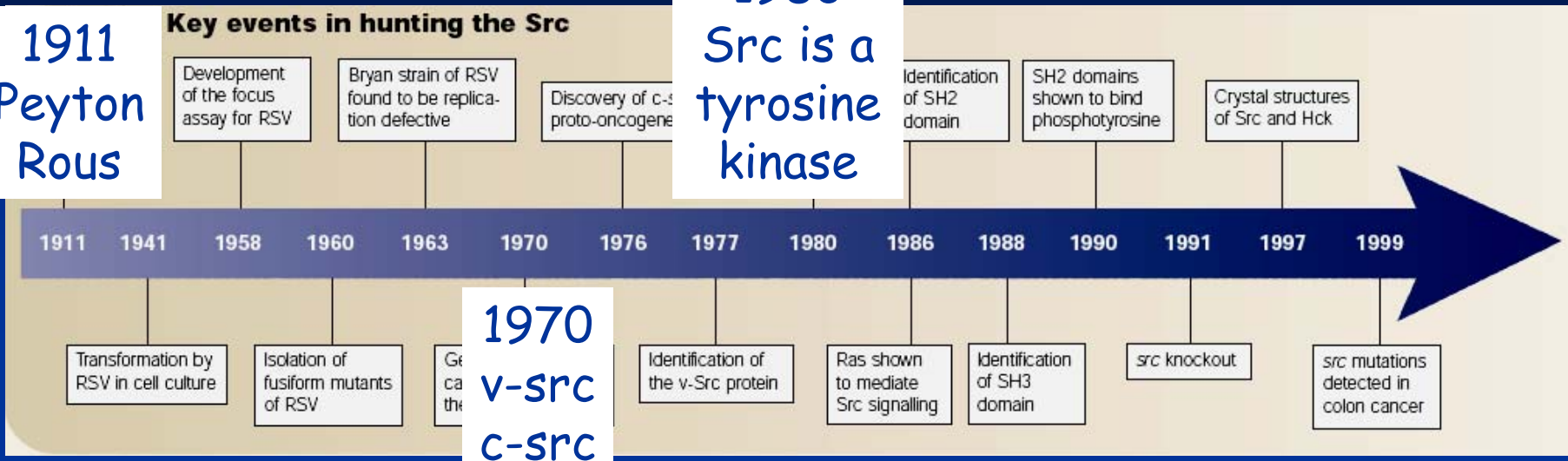
THE HUNTING OF THE SRC

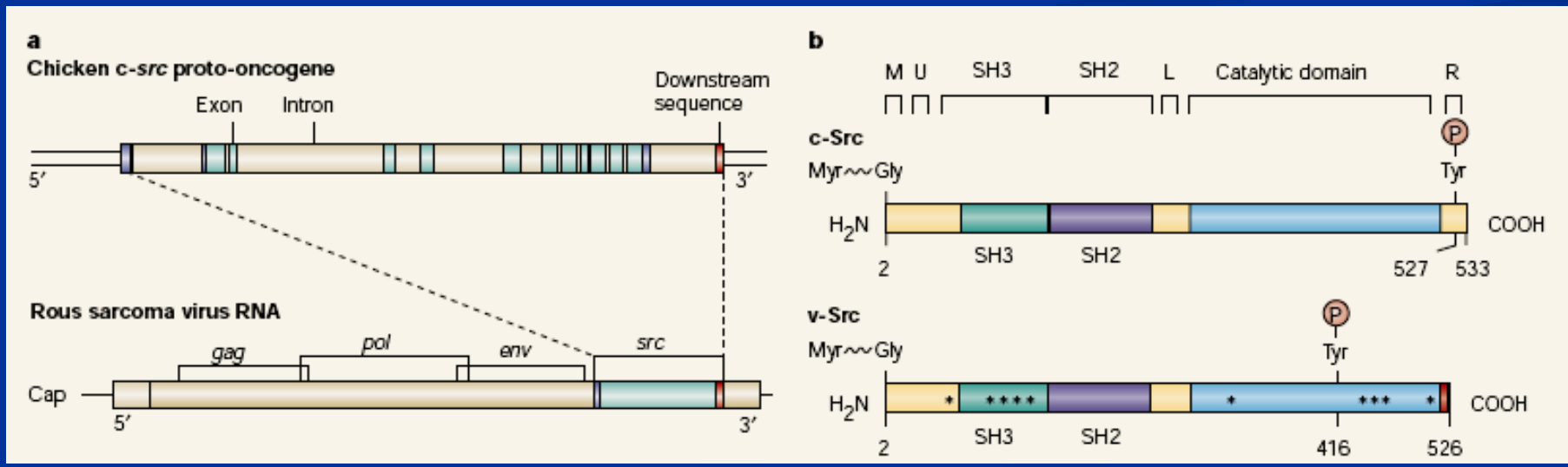
TRANSDUCCIÓN RETROVIRAL

1911
Peyton
Rous

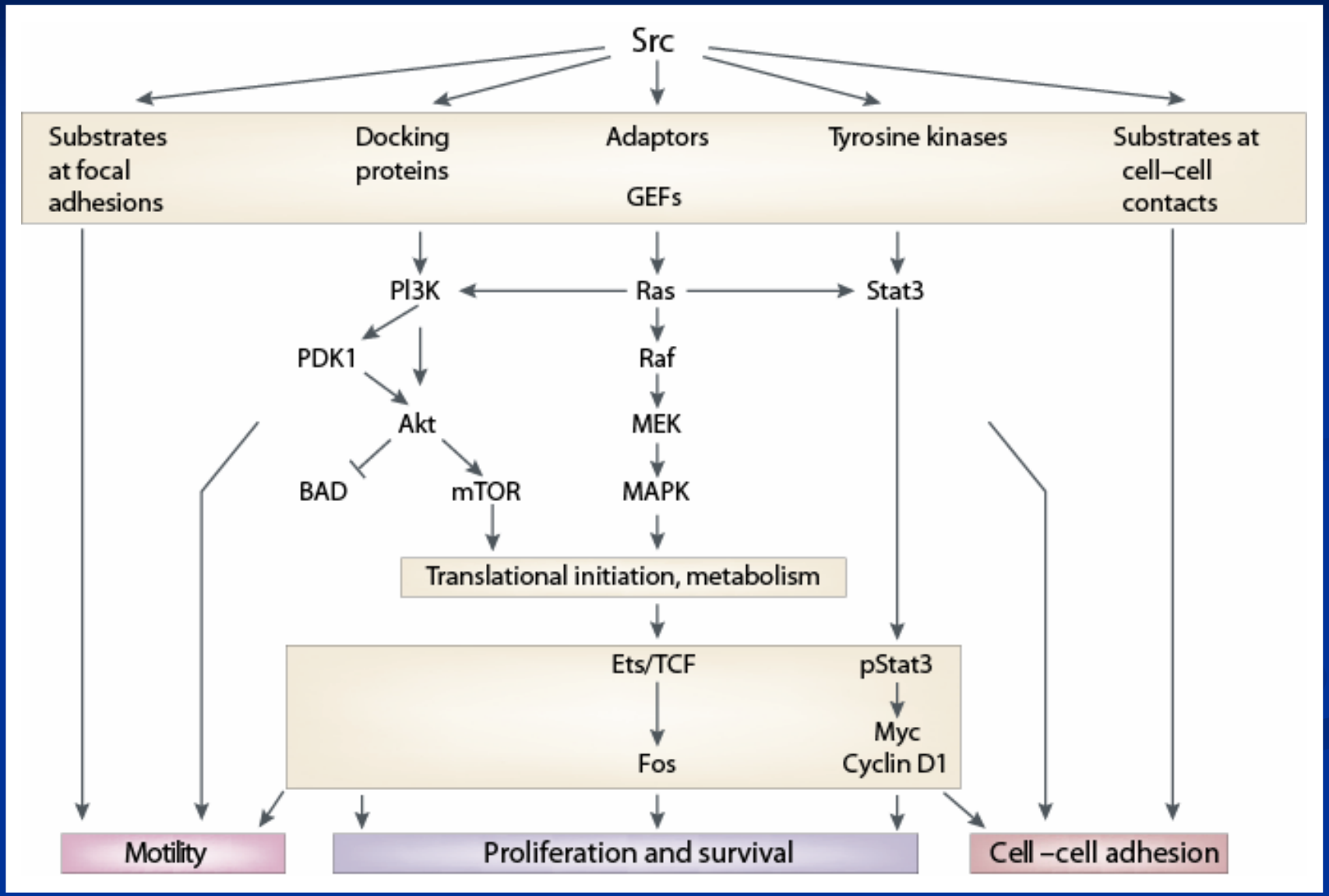
1980
Src is a
tyrosine
kinase

1970
v-src
c-src

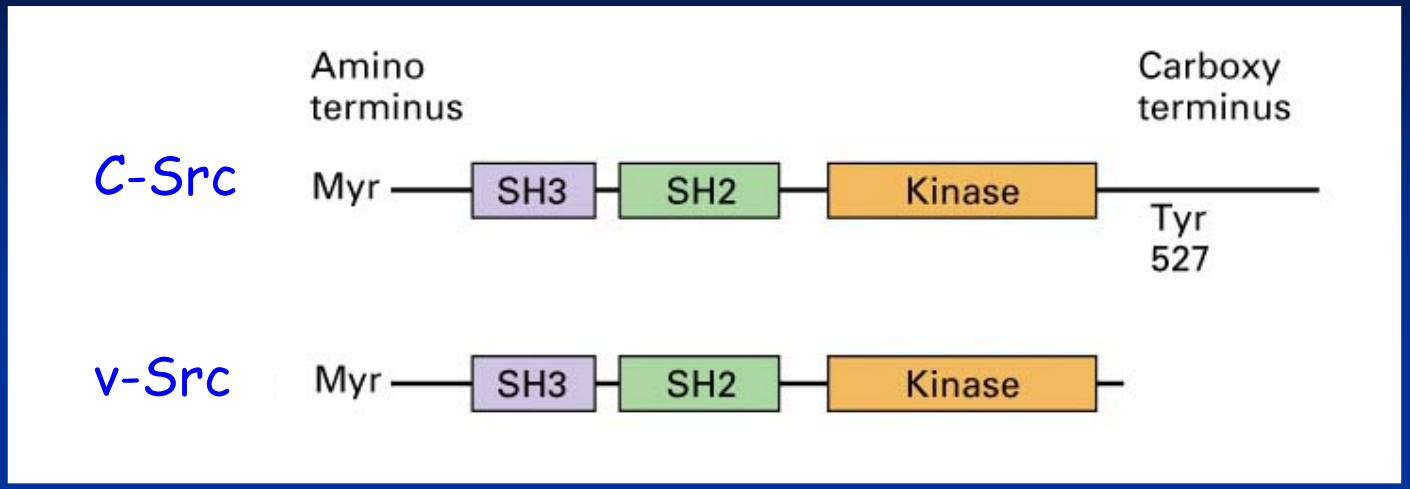


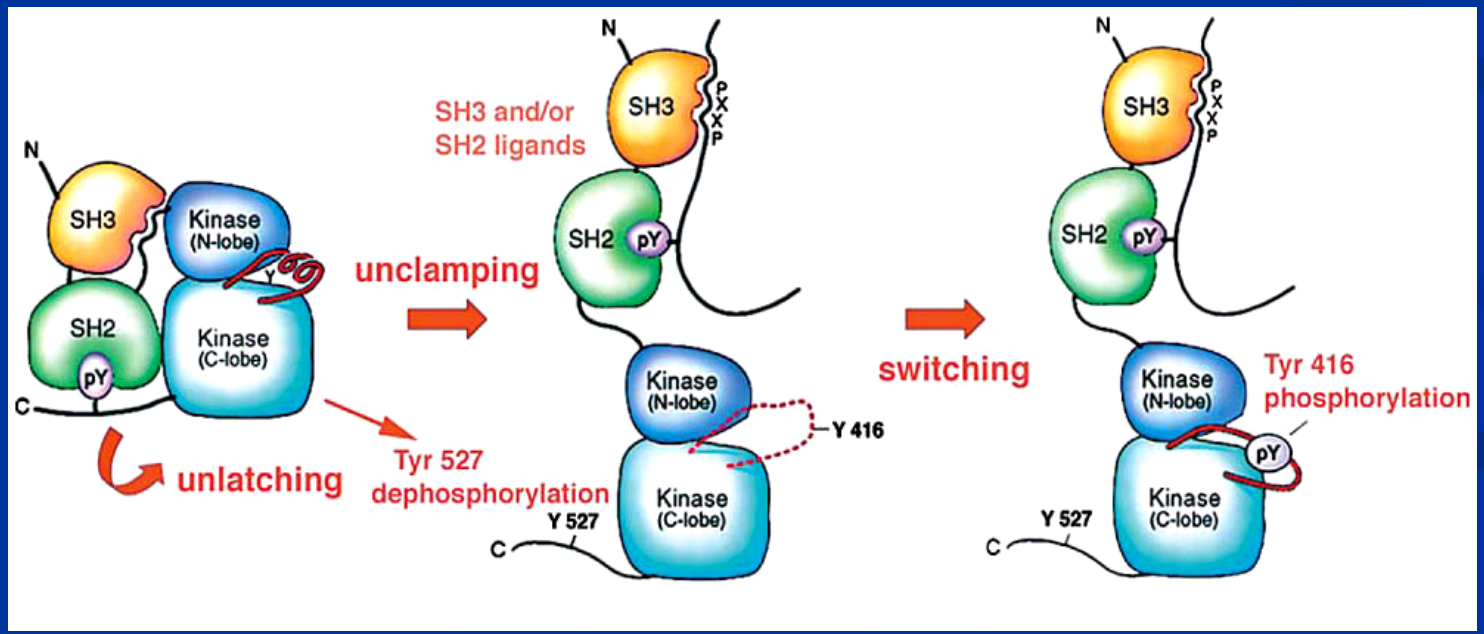


SIGNALING BY SRC



Activación de Src





GENOMIC REARRENGEMENTS, CHROMOSOMAL TRANSLOCATIONS: BCR-ABL

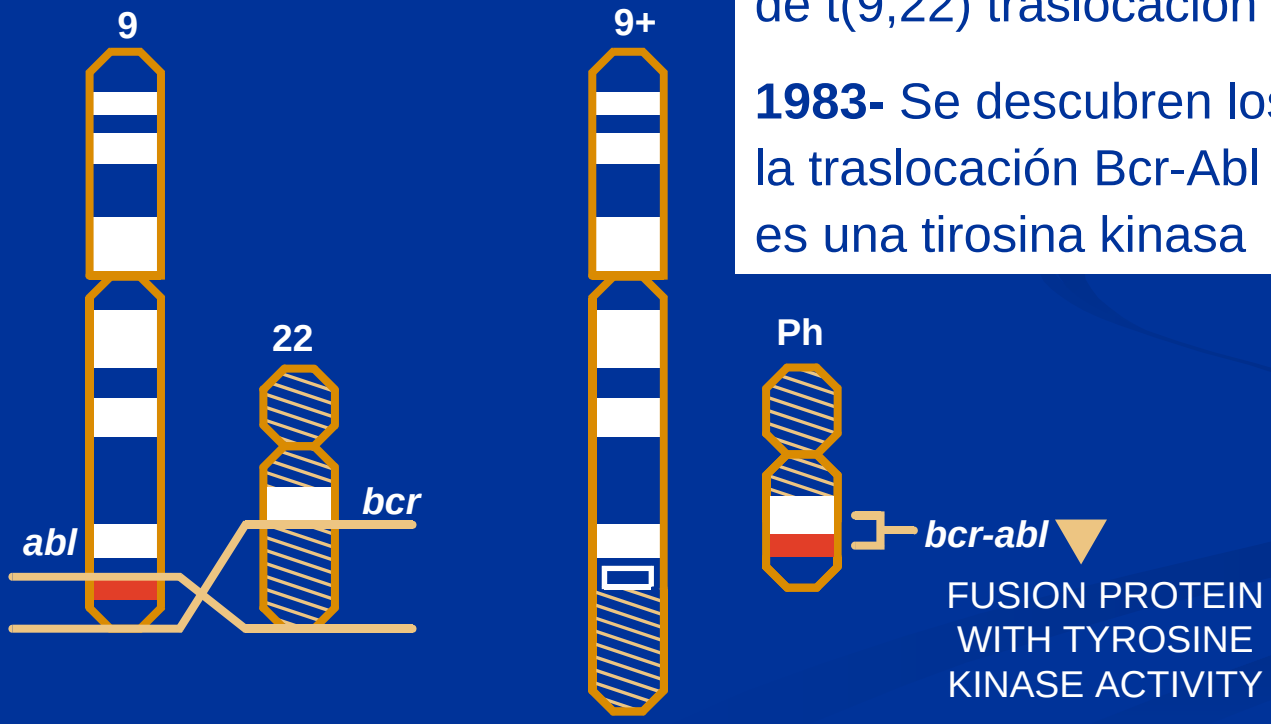
The Philadelphia Chromosome: t(9;22) Translocation

95% LEUCEMIAS MIELOIDES CRÓNICAS

1960- Se asocia Cromosoma Filadelfia y LMC

1973- El cromosoma Filadelfia es el resultado de t(9;22) traslocación

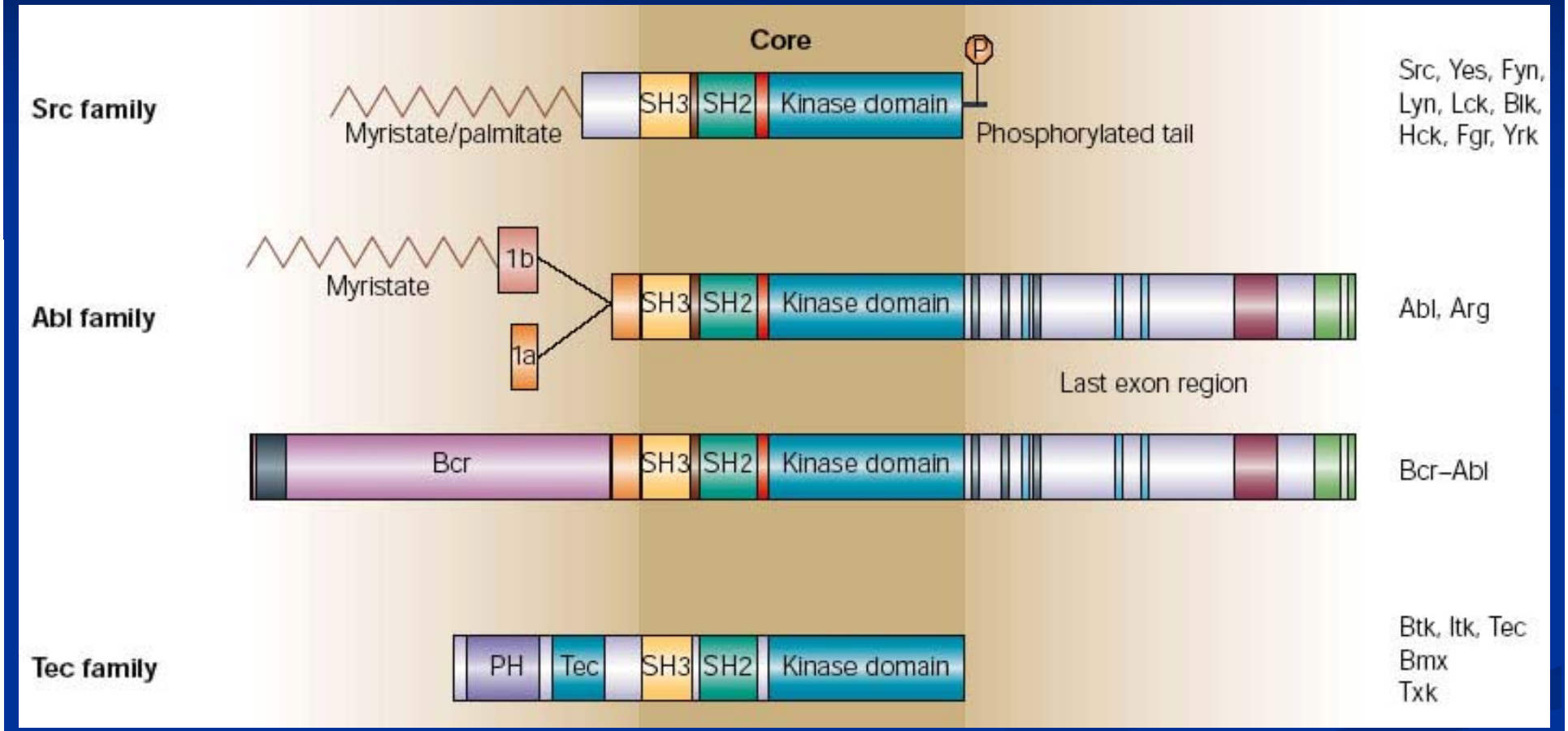
1983- Se descubren los genes involucrados en la traslocación Bcr-Abl y se determina que ABL es una tirosina kinasa



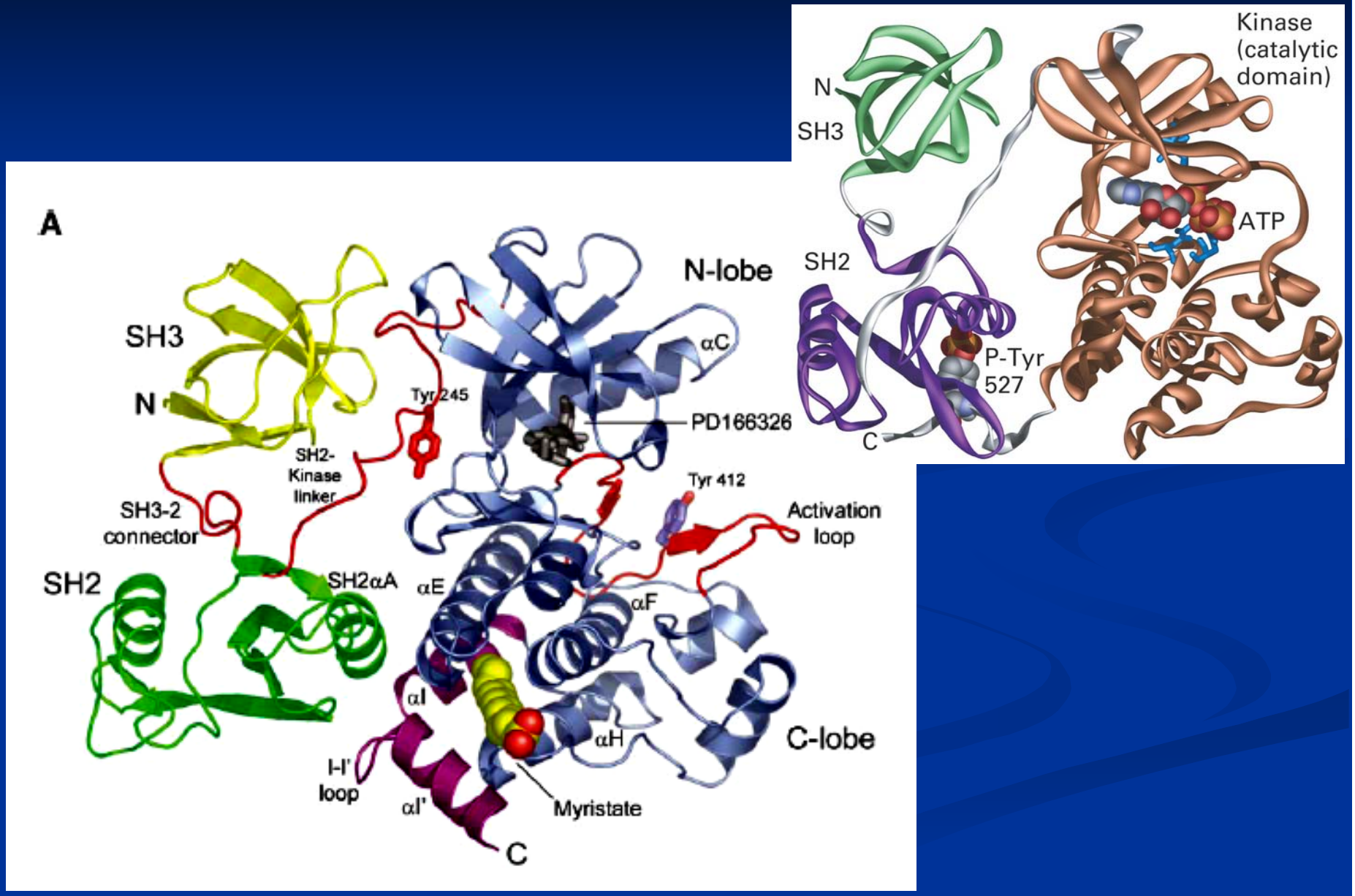
Abl physiological role in cells

- Non-receptor PTK
- Non-erytroid myelopoiesis
- Cytoskeletal rearrengement: small GTPase regulation, inhibition of cell migration and F-actin binding.
- Cell proliferation, survival and apoptosis

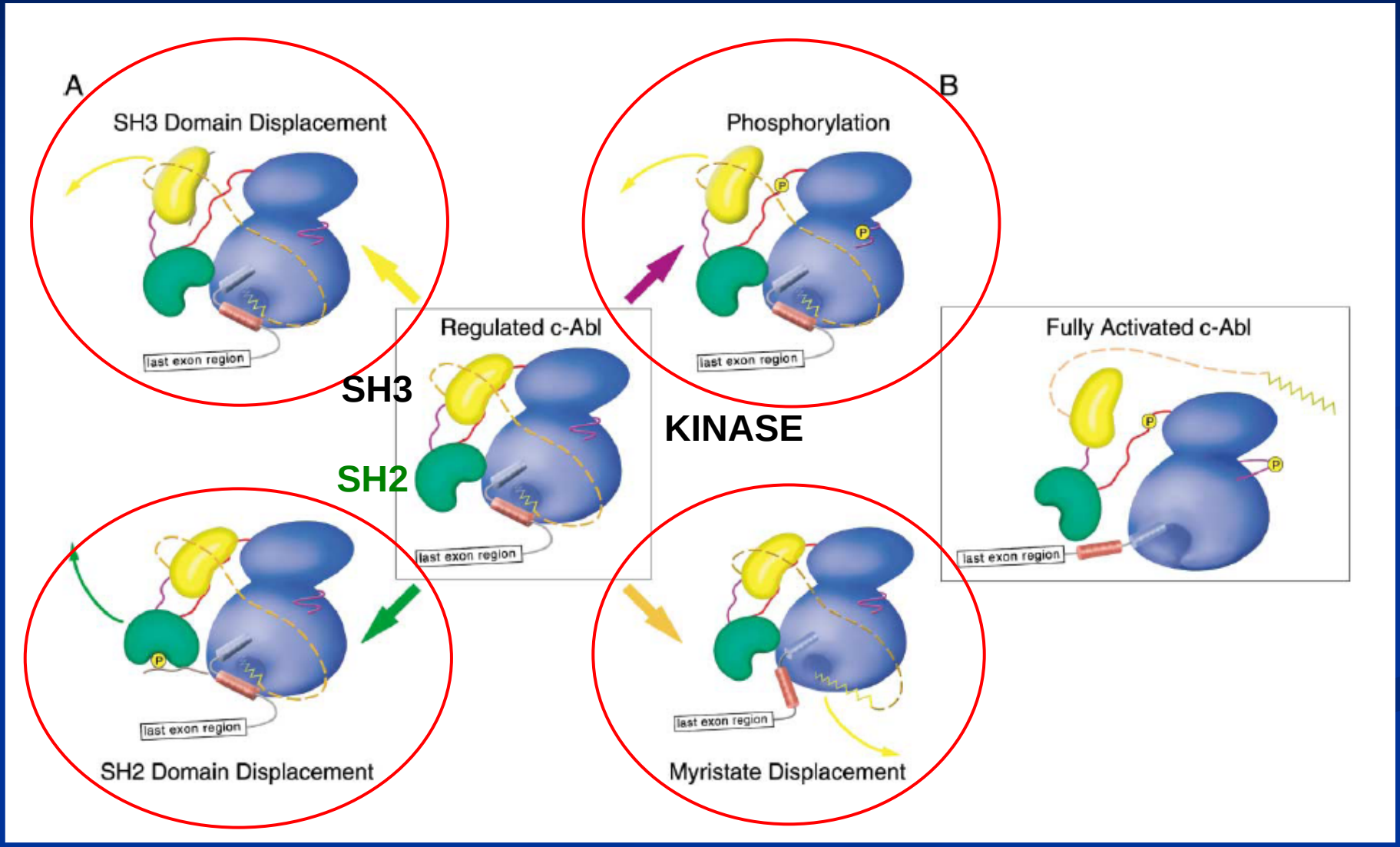
Domain structures of the SH3- and SH2-domain-containing tyrosine-kinases



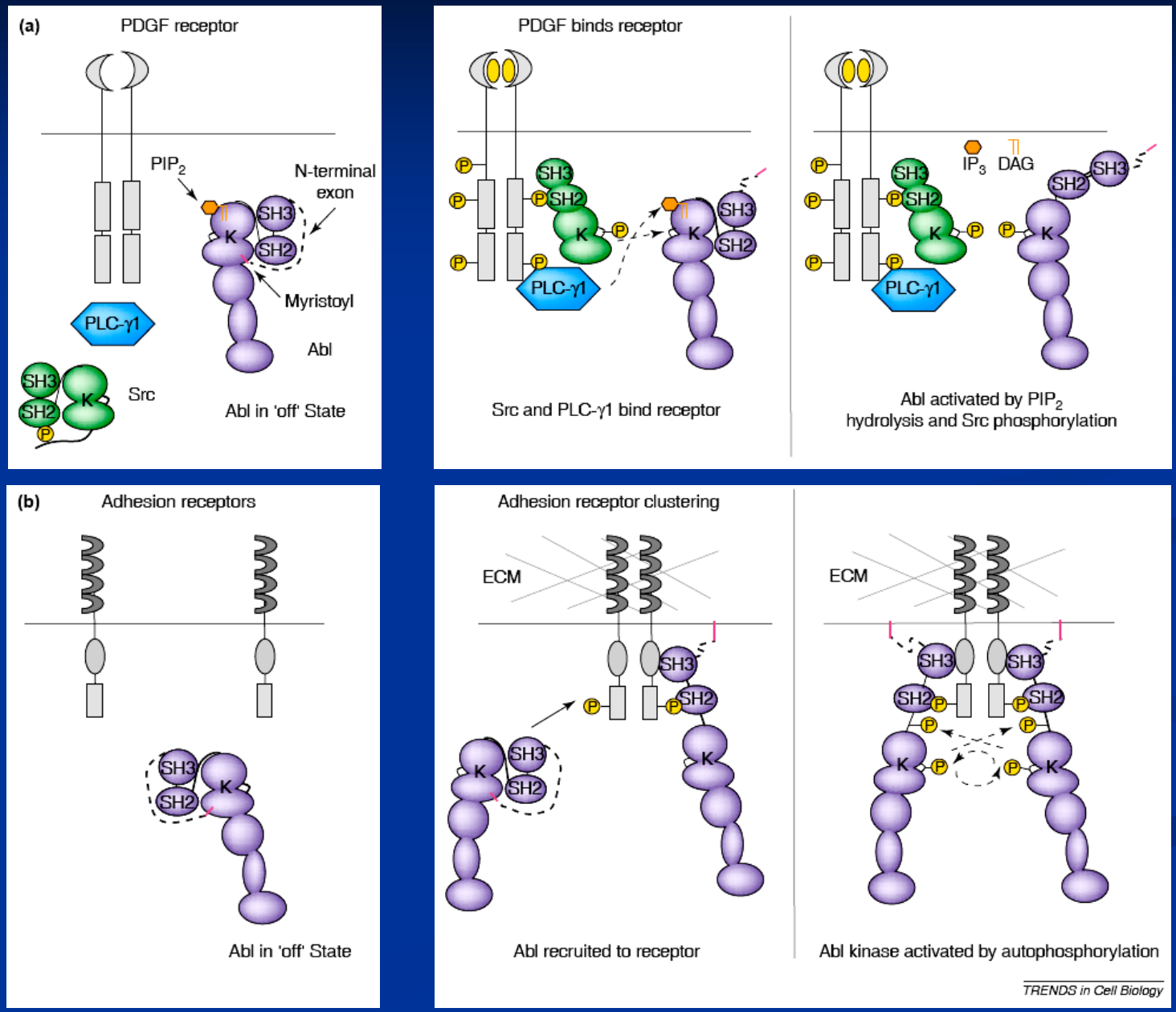
ABL 3-dimensional structure



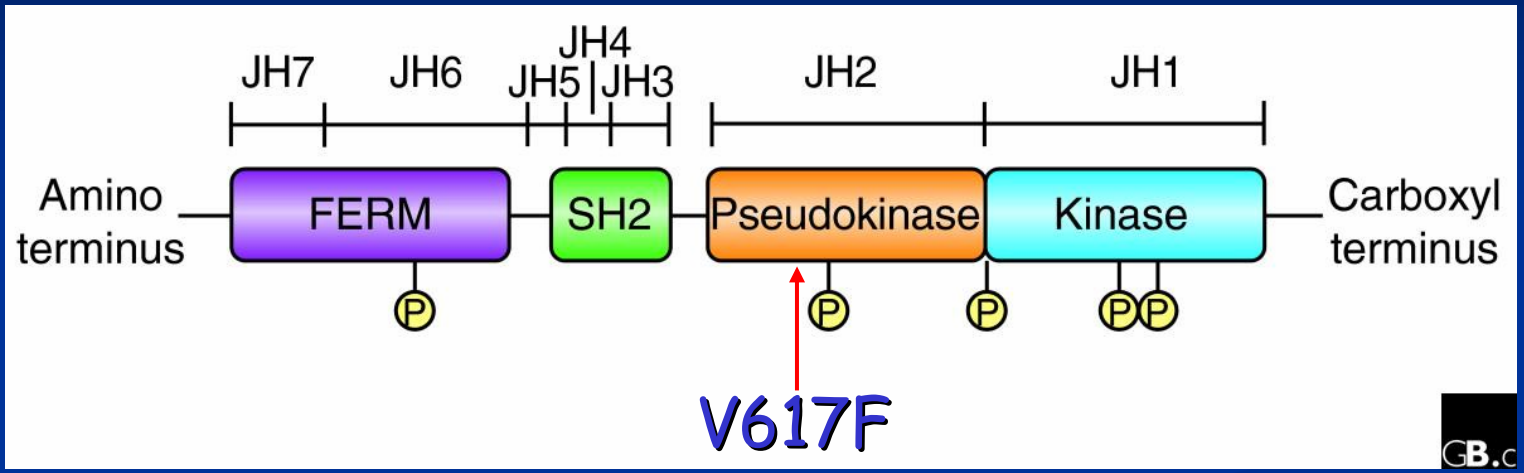
ABL activation



Possible mechanisms for the activation of Abl family kinases by cell surface receptors



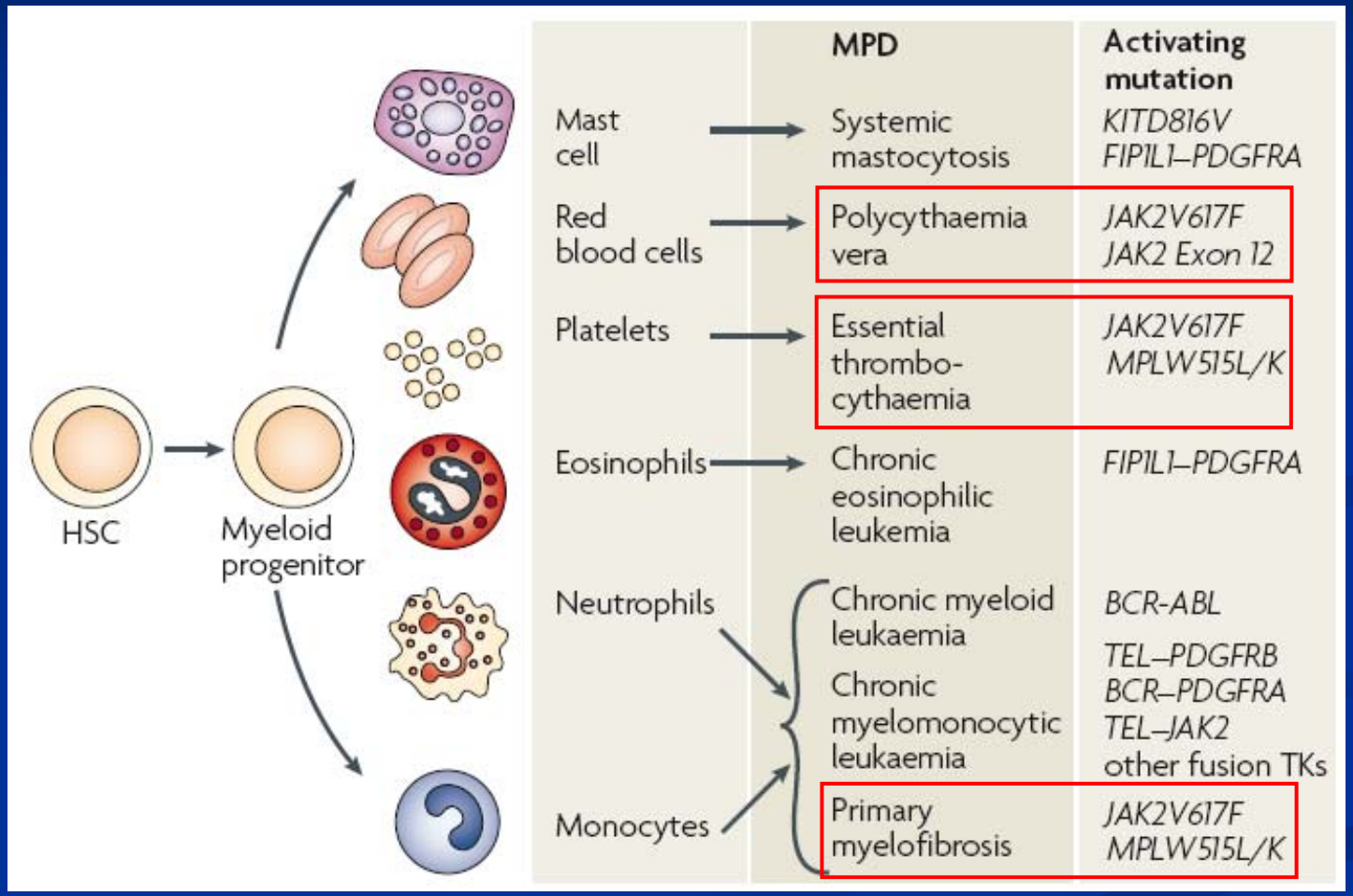
MUTACIONES DE GANANCIA DE FUNCIÓN (GOF): JAK2V617F



In 2005, several independent groups used different experimental approaches to identify a recurrent mutation in the *JAK2* tyrosine kinase in most patients with PV, ET or PMF8–11

Baxter….Green, Lancet 2005
James…Vainchencker, Nature 2005
Kralovics….Skoda, New Engl J Med, 2005
Levine…Tefferi…Gilliland, Cancer Cell 2005

Classification and molecular pathogenesis of the MPD (myeloproliferative disorders)

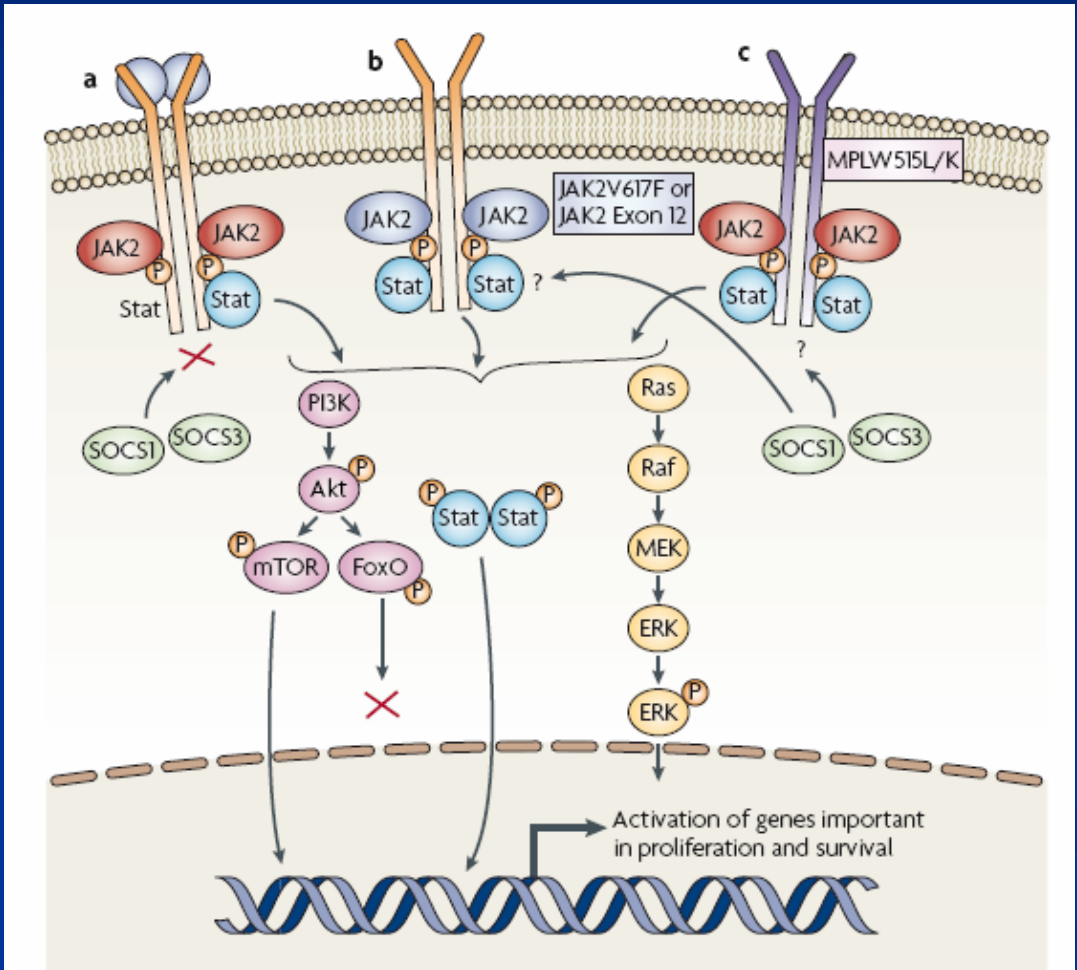


90%

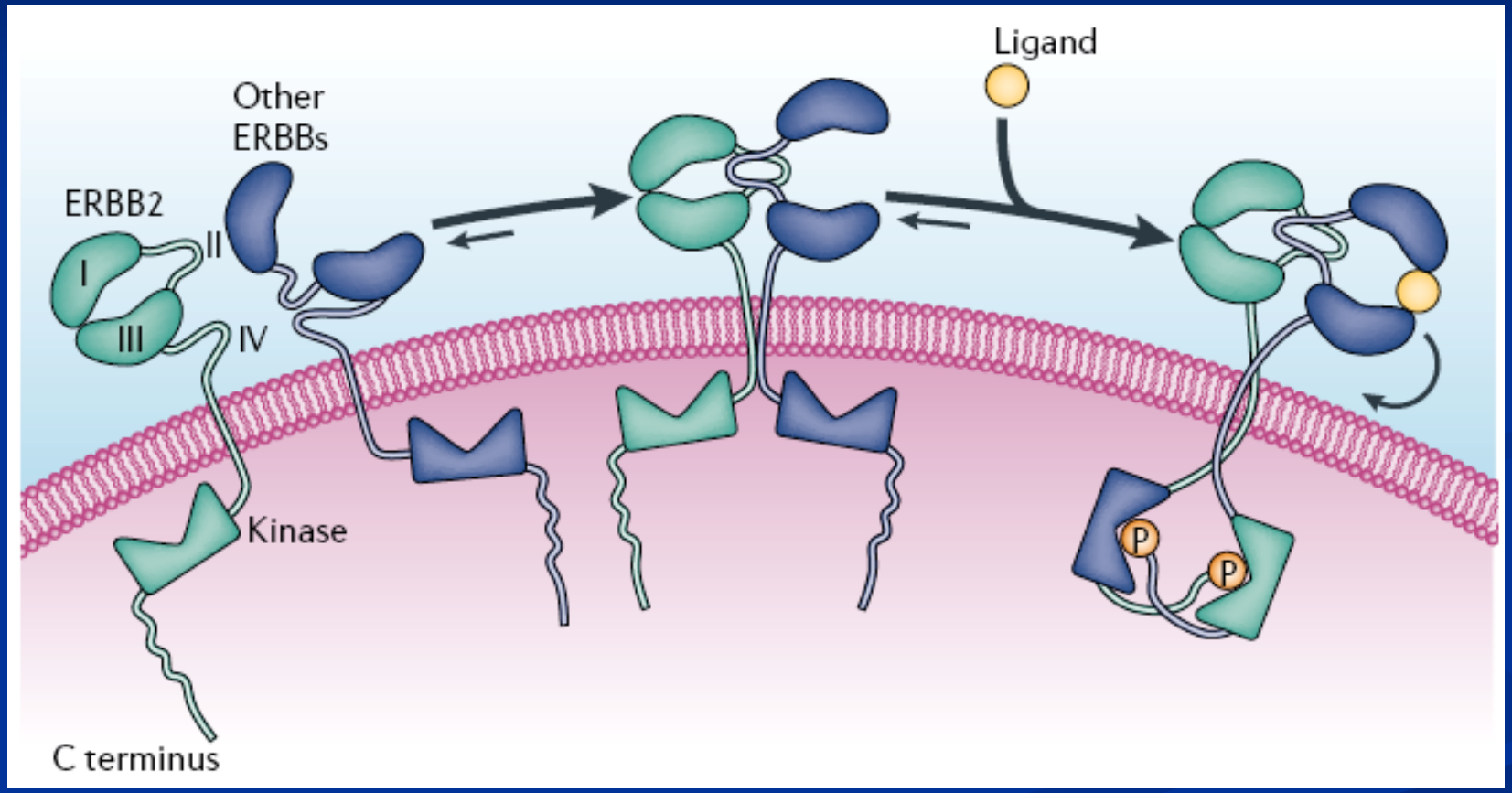
~50%

~50%

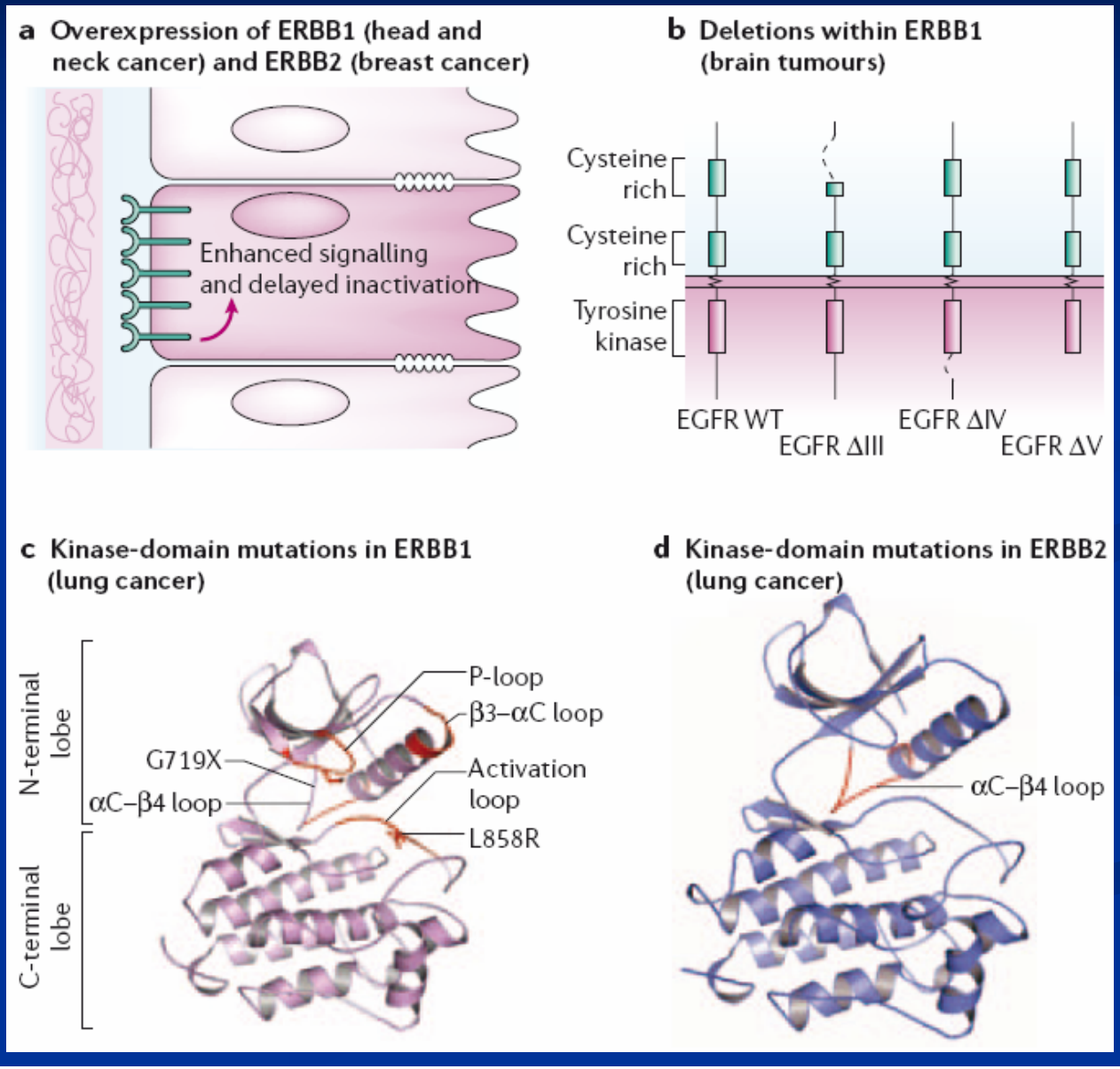
Mechanism of activation of JAK2 kinase activity by mutations in the JAK2



Alteraciones en RECEPTOR-PTKs



MULTIPLE PATHWAYS TO ONCOGENESIS IN EGFR (Epidermal growth factor receptor)

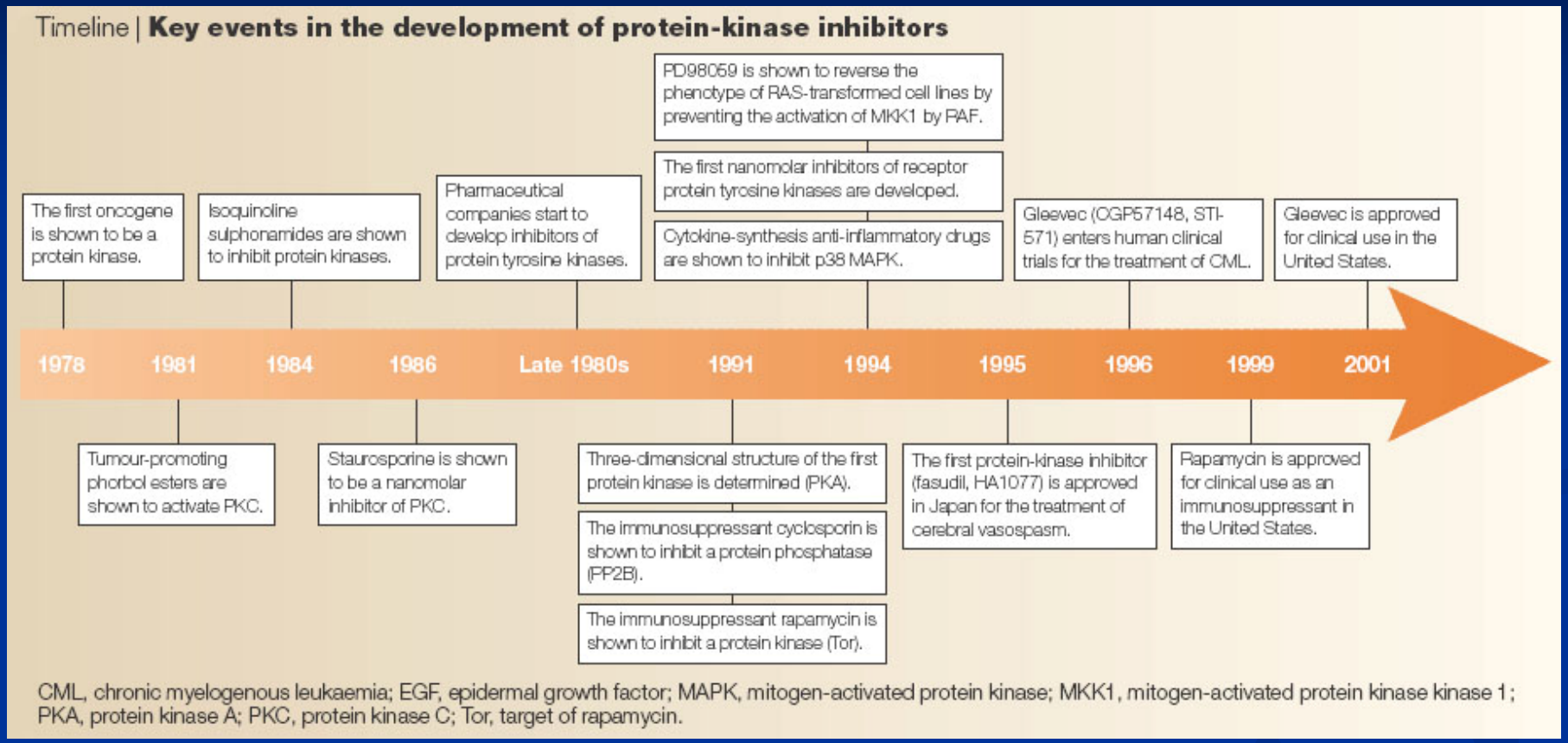


TERAPIAS DIRIGIDAS PARA KINASAS DE TIROSINA EN CÁNCER

- Pequeños compuestos químicos que inhiban la actividad kinasa

- Anticuerpos que inhiban la dimerización de los RPTK

DEVELOPMENT OF PROTEIN-KINASE INHIBITORS



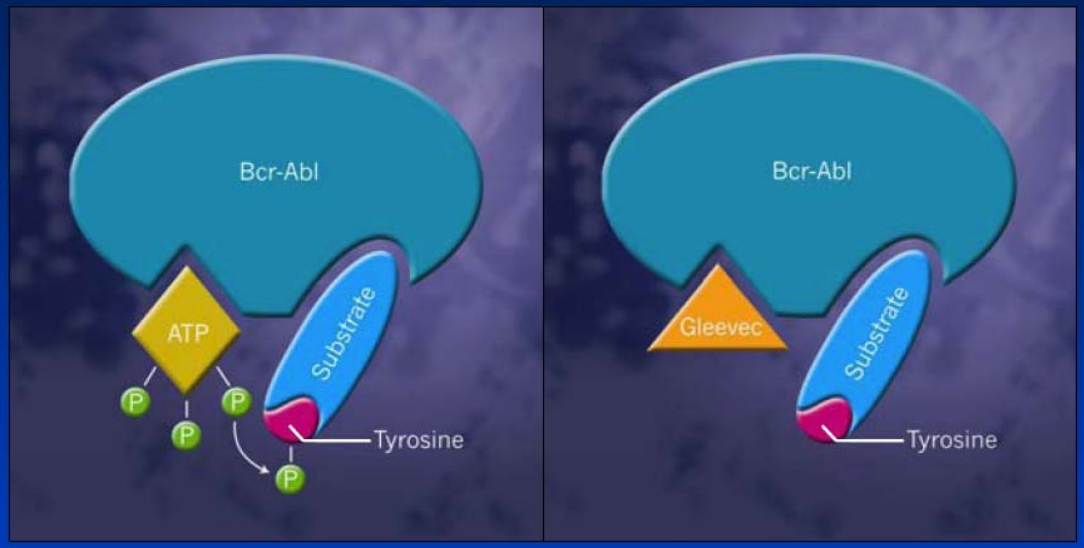
CML, chronic myelogenous leukaemia; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; MKK1, mitogen-activated protein kinase kinase 1; PKA, protein kinase A; PKC, protein kinase C; Tor, target of rapamycin.

CANCER THERAPIES TARGETED TYROSINE KINASES

Names	Targets	Status	Description	Company
Trastuzumab, Herceptin	HER2	Approved for metastatic breast cancer	Humanized anti-HER2 IgG1κ	Genentech
Imatinib, Glivec, STI571	BCR–ABL, KIT, PDGFR	Approved for CML and GIST	2-Phenylaminopyrimidine	Novartis
Gefitinib, Iressa, ZD1839	EGFR	Approved for NSCLC	Quinazoline	AstraZeneca
Cetuximab, Erbitux	EGFR	Approved for colorectal cancer	Chimeric anti-EGFR IgG1	ImClone/Merck
Bevacizumab, Avastin	VEGF	Approved for colorectal cancer	Humanized anti-VEGF (rhu mAb-VEGF)	Genentech
OSI-774, Tarceva	EGFR	Clinical development	Quinazoline	Genentech/ Roche/OSI
CI-1033	EGFR, HER2	Clinical development	4-Anilinoquinazoline, irreversible inhibitor	Pfizer
EKB-569	EGFR, HER2	Clinical development	4-Anilinoquinoline-3-carbonitrile, irreversible inhibitor	Wyeth
CDP860	PDGFR	Clinical development	Anti-PDGFβ-receptor antibody fragment	Celltech
Pertuzumab, Omnitarg, 2C4	HER2	Clinical development	Humanized anti-HER2 (heterodimerization inhibitor)	Genentech
SU6668	VEGFR2, PDGFR, FGFR	Clinical development	Indoline-2-one	Sugen/Pfizer
SU11248	VEGFR2, KIT, PDGFR, FLT3	Clinical development	Indoline-2-one	Sugen/Pfizer
ZD6474	VEGFR2	Clinical development	Quinazoline	AstraZeneca
PTK-787/ZK222584	VEGFR1/2, PDGFR	Clinical development	Anilinophthalazine	Novartis/Schering
AG013736	VEGFR2, PDGFR	Clinical development	–	Pfizer
CP549, 632	VEGFR2, FGFR1, TIE2	Clinical development	–	Pfizer
PKC-412, midostaurin	PKC, VEGFR2, PDGFR, FLT3, KIT	Clinical development	*N*-Benzoylstaurosporine	Novartis
CEP-701	FLT3, TRK kinases	Clinical development	Indolocarbazole alkaloid	Cephalon
MLN-518, CT53518	PDGFR, KIT, FLT3	Clinical development	Quinazoline	Millennium

El imatinib (Gleevec), primer antitumoral de uso en clínica descubierto por "búsqueda racional"

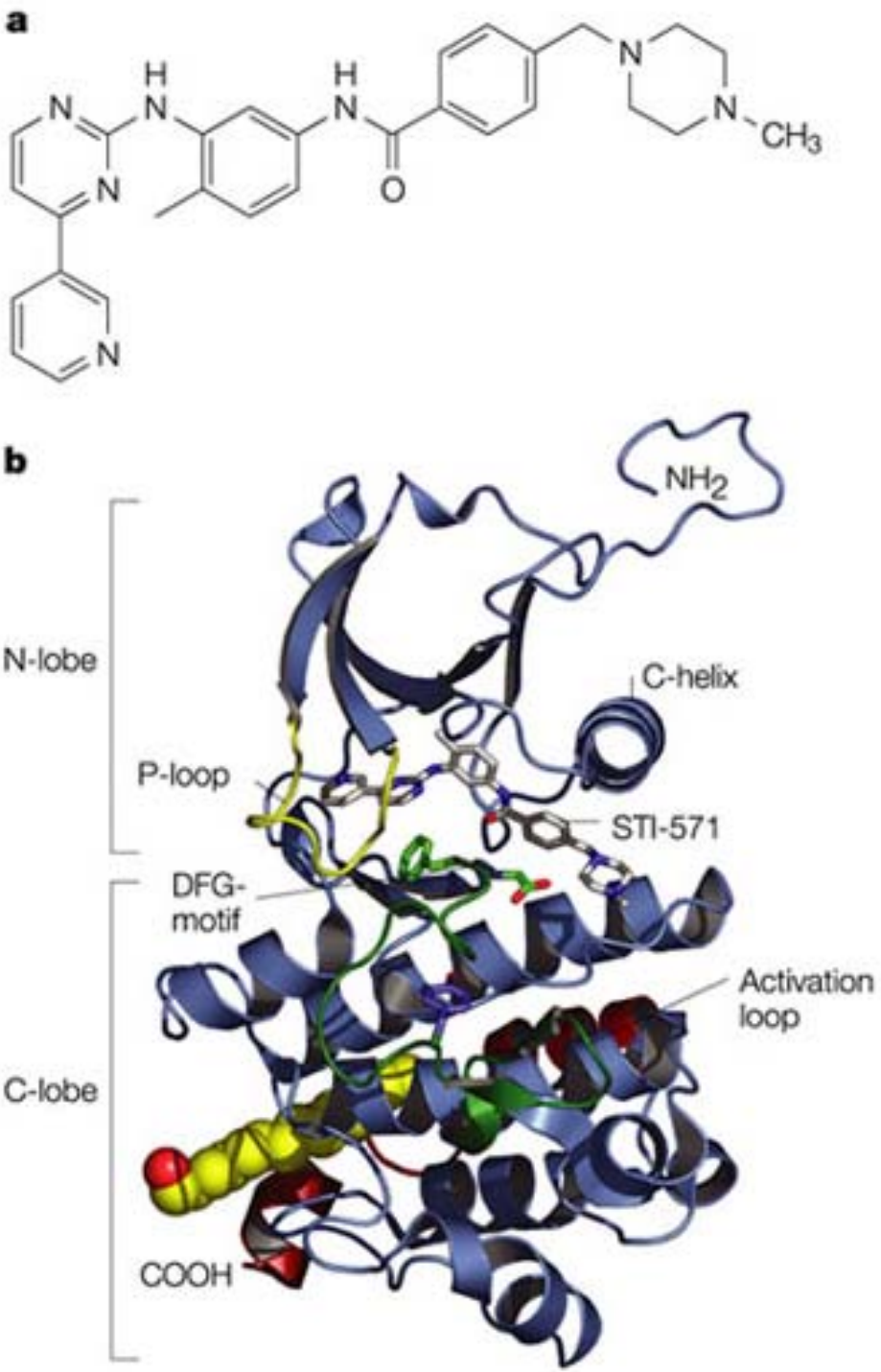
Gleevec® Targets the Cause of CML



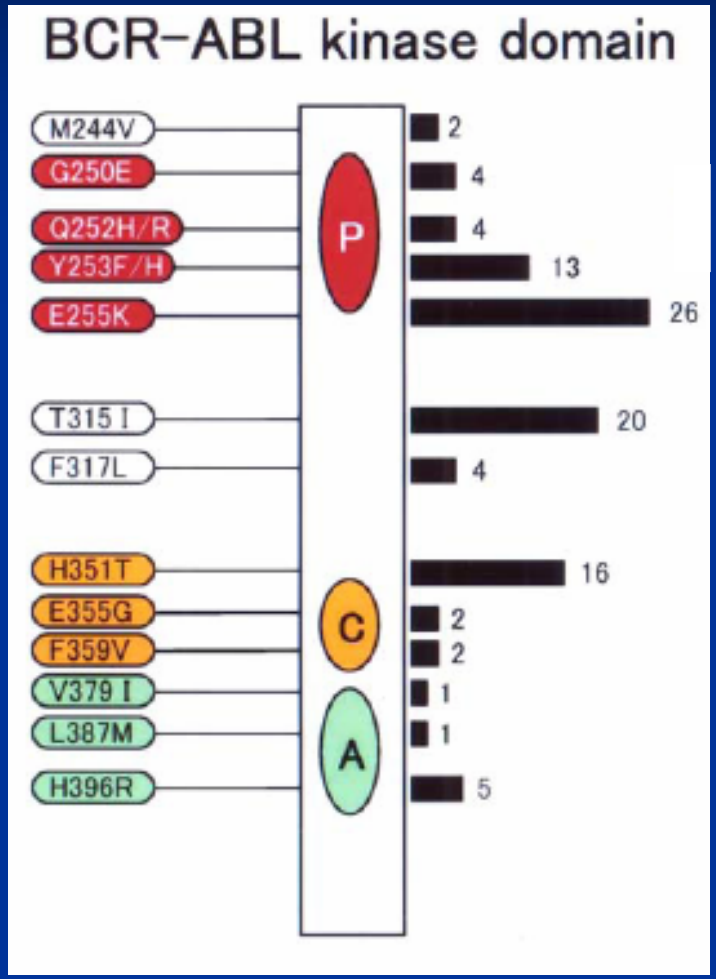
- Gleevec—a specific inhibitor of a small family of tyrosine kinases, including Bcr-Abl, Kit, and PDGF receptor

GLEEVEC (Imatinib)

- Se une al sitio de unión al ATP solo cuando el loop de activación está cerrado y así estabiliza la conformación inactiva de la proteína.
- Además distorsiona el loop de unión al grupo fosfato.
- Produce remisión en leucemias mieloides crónicas con una toxicidad mínima.



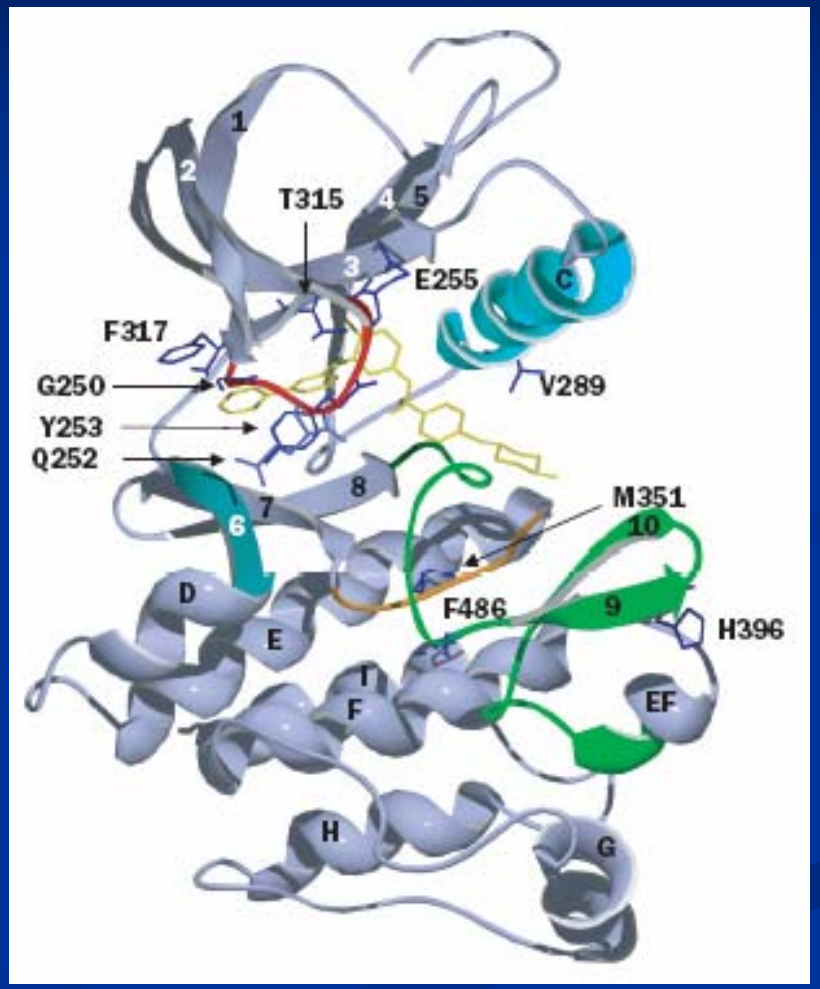
BCR-ABL Kinase Domain Mutations



P-loop

CATALYTIC DOMAIN

ACTIVATION loop

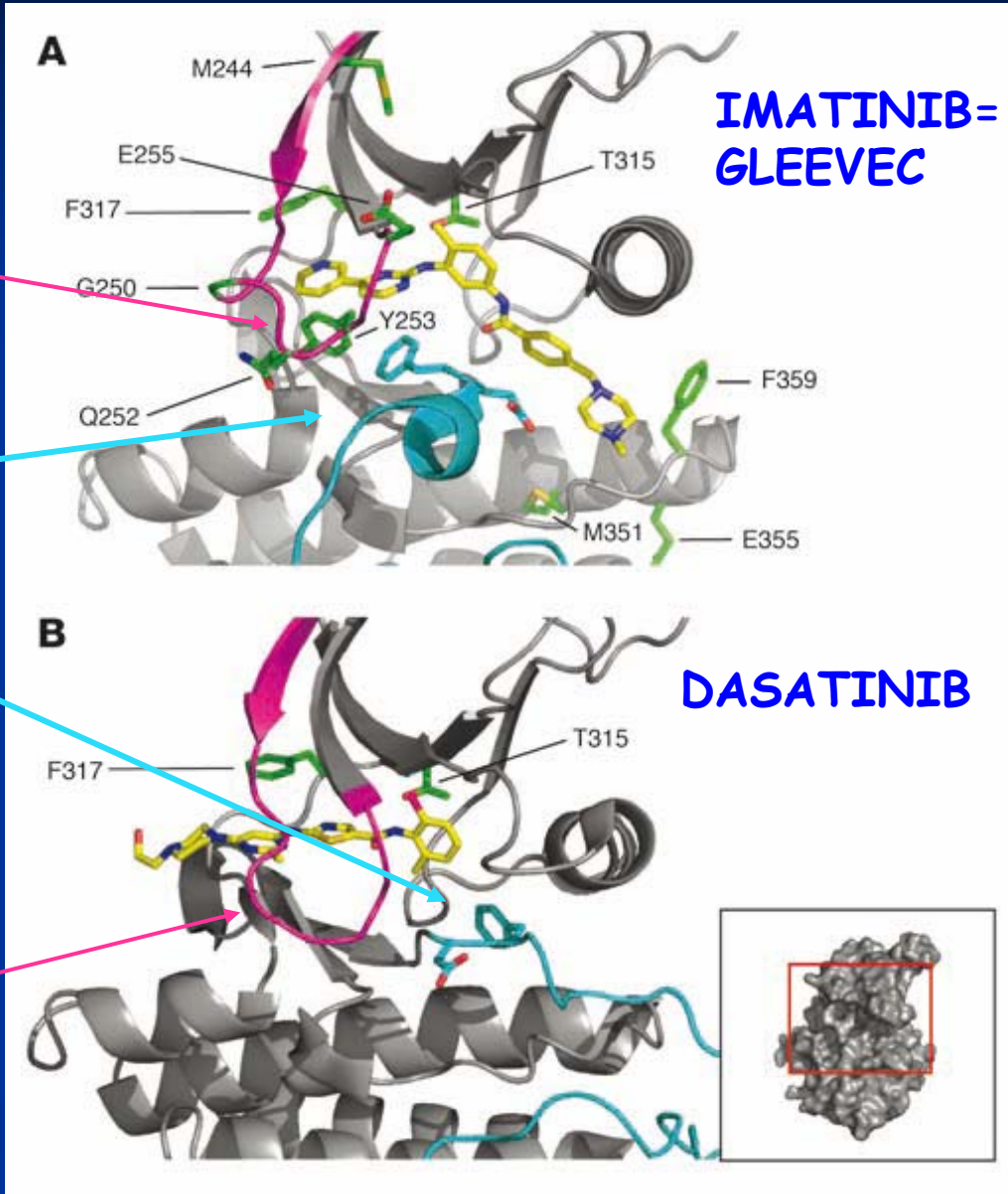


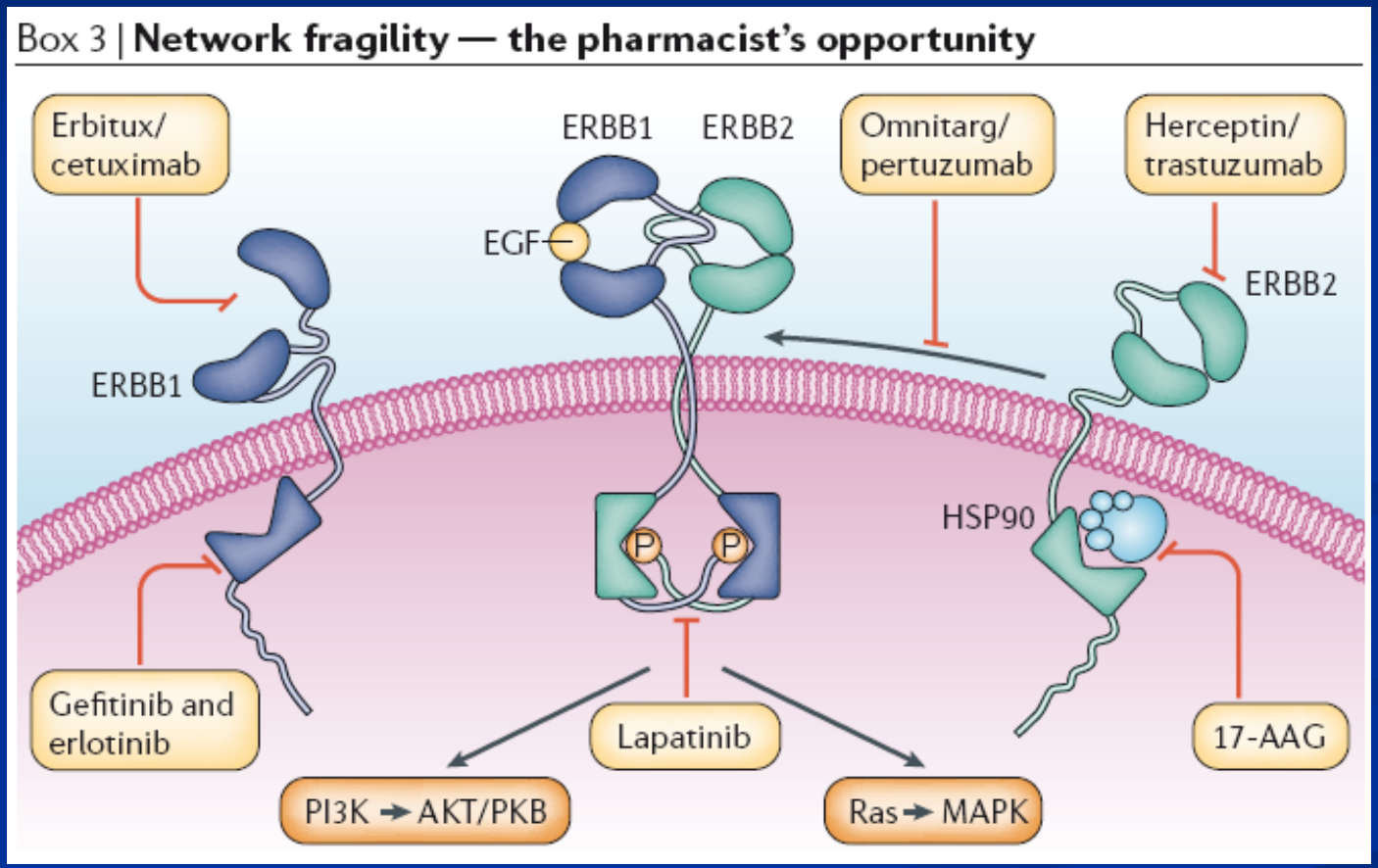
P-loop

ACTIVATION LOOP
Glu-Phe-Gly

coordinates a Mg2+ ion during catalysis. Oriented properly for catalysis, whereas in the imatinib this residue points away from the active site

P-loop



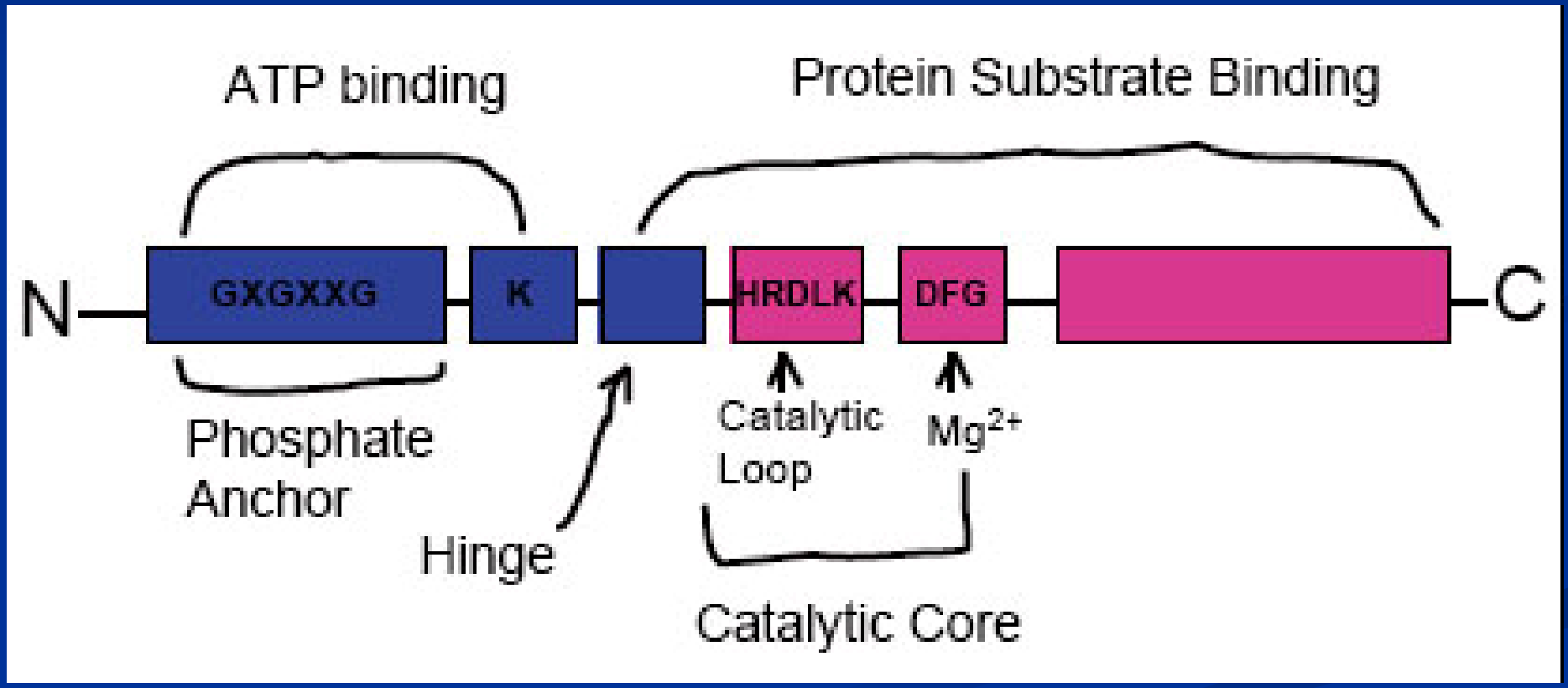

Box 3 | Network fragility — the pharmacist's opportunity
Erbitux/ cetuximab
ERBB1
ERBB2
Omnitarg/ pertuzumab
Herceptin/ trastuzumab
EGF
ERBB2
ERBB1
HSP90
P
P
Gefitinib and erlotinib
Lapatinib
17-AAG
PI3K → AKT/PKB
Ras → MAPK

CONCLUSIONES

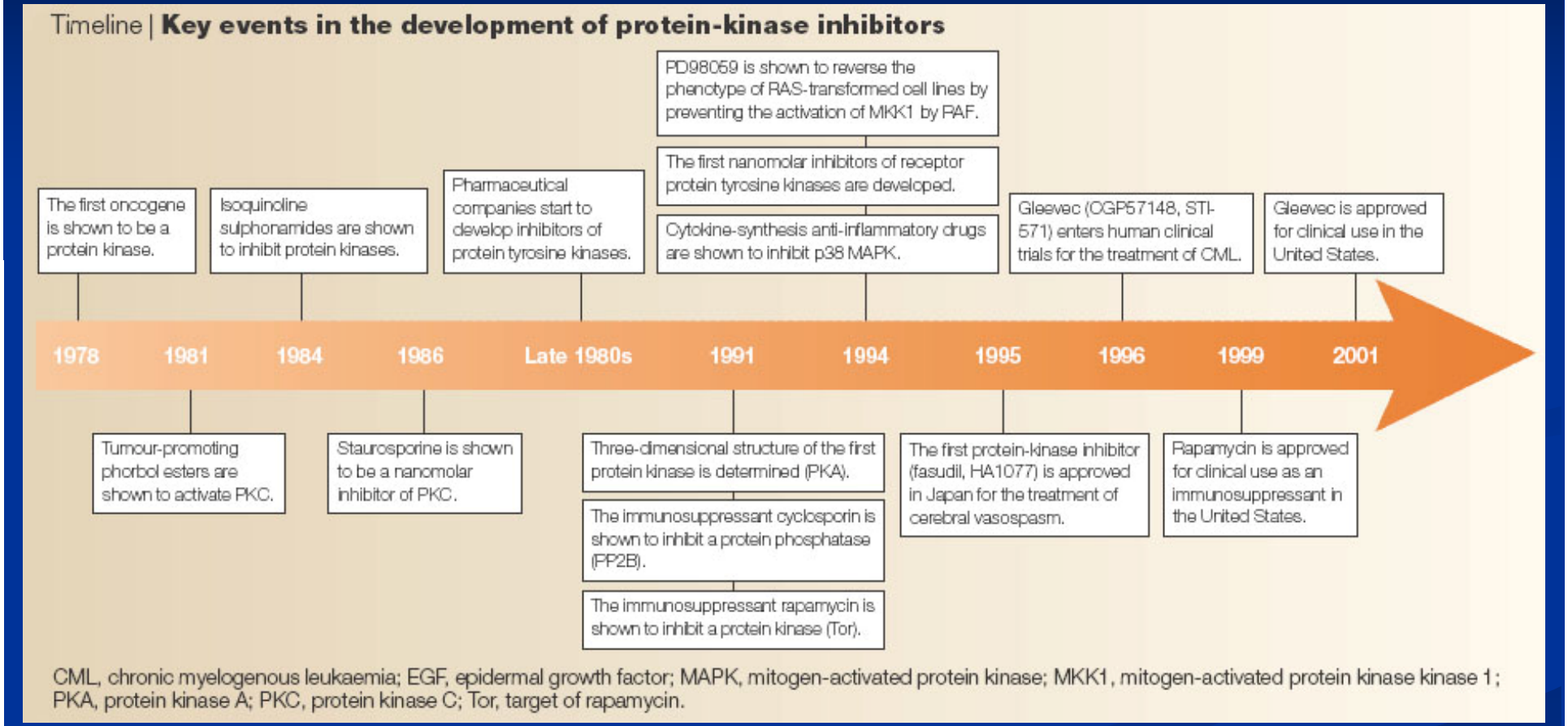
- La fosforilación de proteínas es un mecanismo postraduccional que permite regular las vías de señalización intracelular.
- Las kinasas y fosfatasas de tirosinas se sitúan en el inicio de las vías de señalización.
- La desregulación de las kinasas y fosfatasas de tirosinas conduce a la alteración de las vías de señalización y lleva a la aparición de tumores.

MUCHAS GRACIAS…

STRUCTURE OF PROTEIN KINASES AND FUNCTIONAL SEQUENCES



DEVELOPMENT OF PROTEIN-KINASE INHIBITORS



CML, chronic myelogenous leukaemia; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; MKK1, mitogen-activated protein kinase kinase 1; PKA, protein kinase A; PKC, protein kinase C; Tor, target of rapamycin.