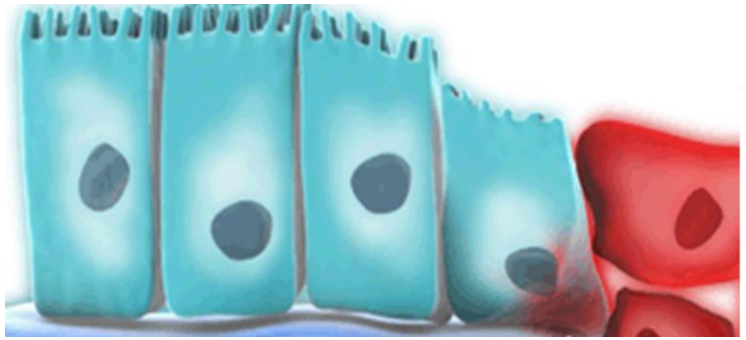
Ciências ULisboa

REPÚBLICA PORTUGUESA
SAÚDE

Instituto_Nacional de Saúde Doutor Ricardo Jorge

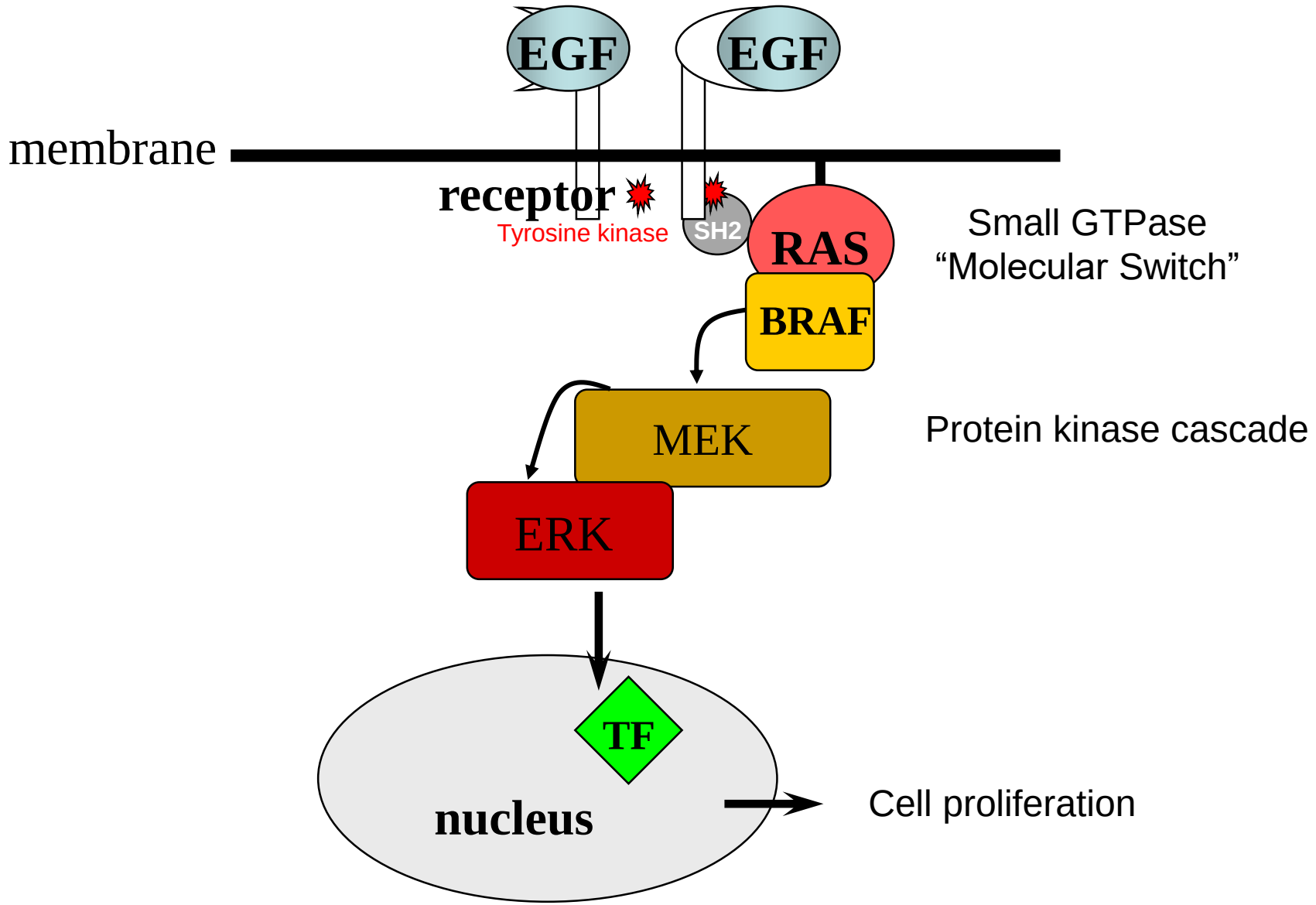


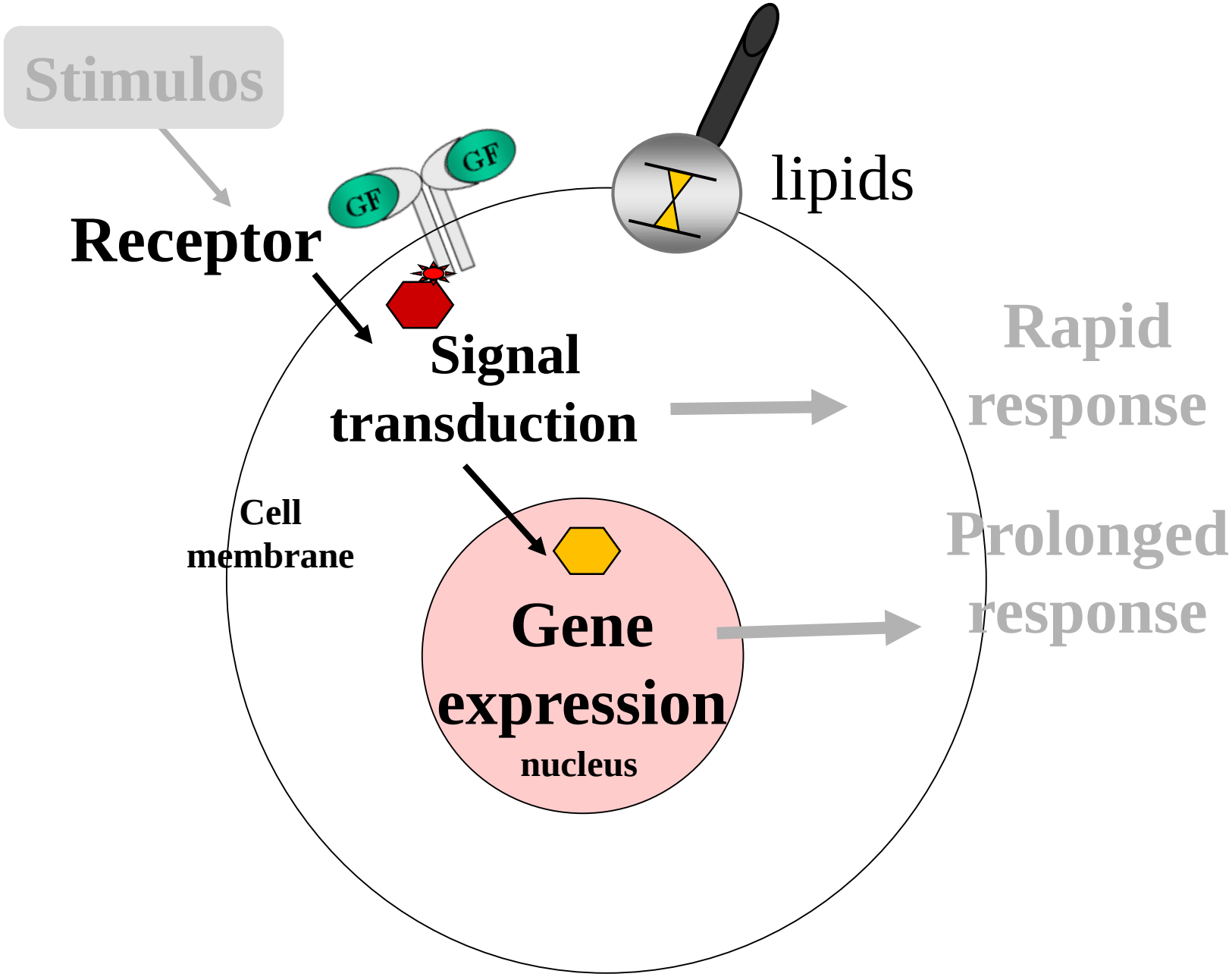
Oncobiology

Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

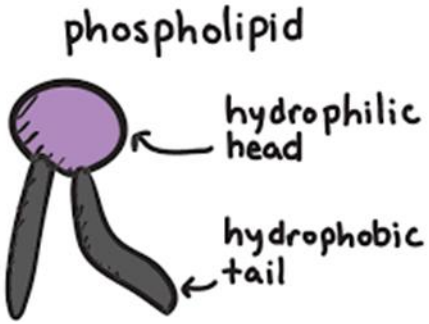
Cell Signalling – part 2

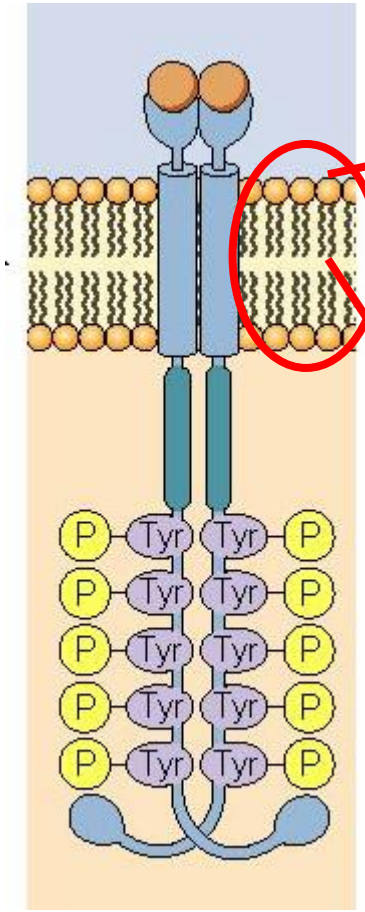
repetition: The RAS-MAP kinase pathway



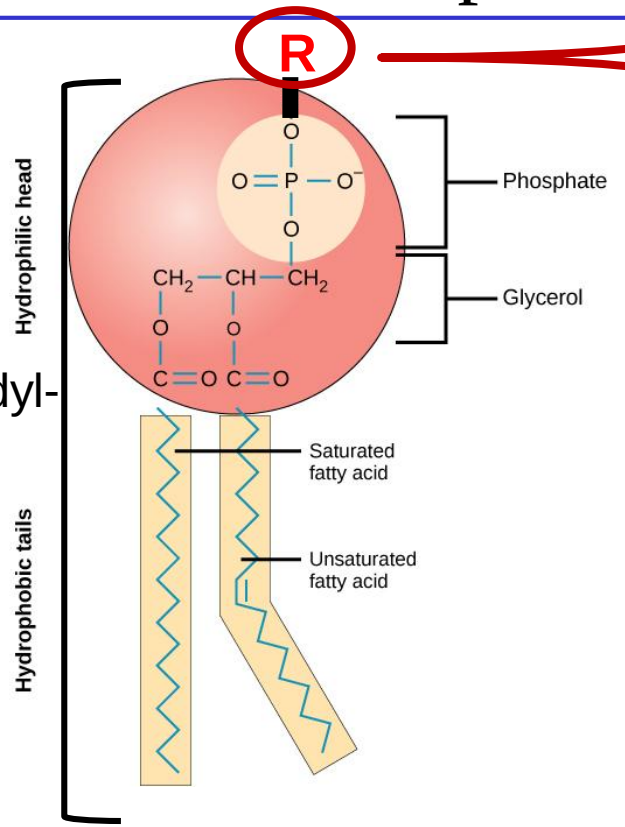

Stimulos
Receptor
GF
GF
lipids
Signal
transduction
Cell
membrane
Gene
expression
nucleus
Rapid
response
Prolonged
response

Plasma membrane lipids

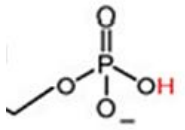




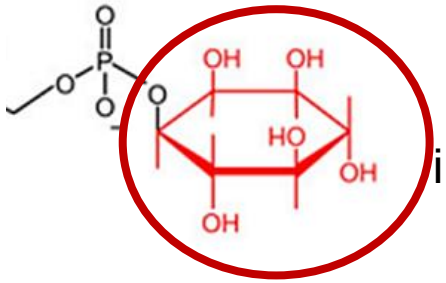
Phosphatidyl-



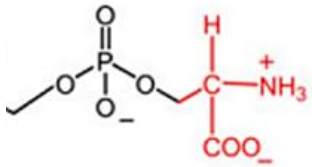
Phosphatidyl-



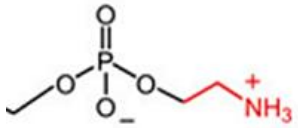
PA
acid



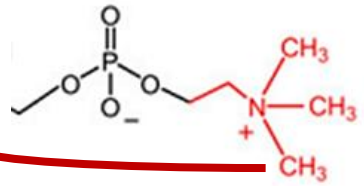
PI
inositol



PS
serine

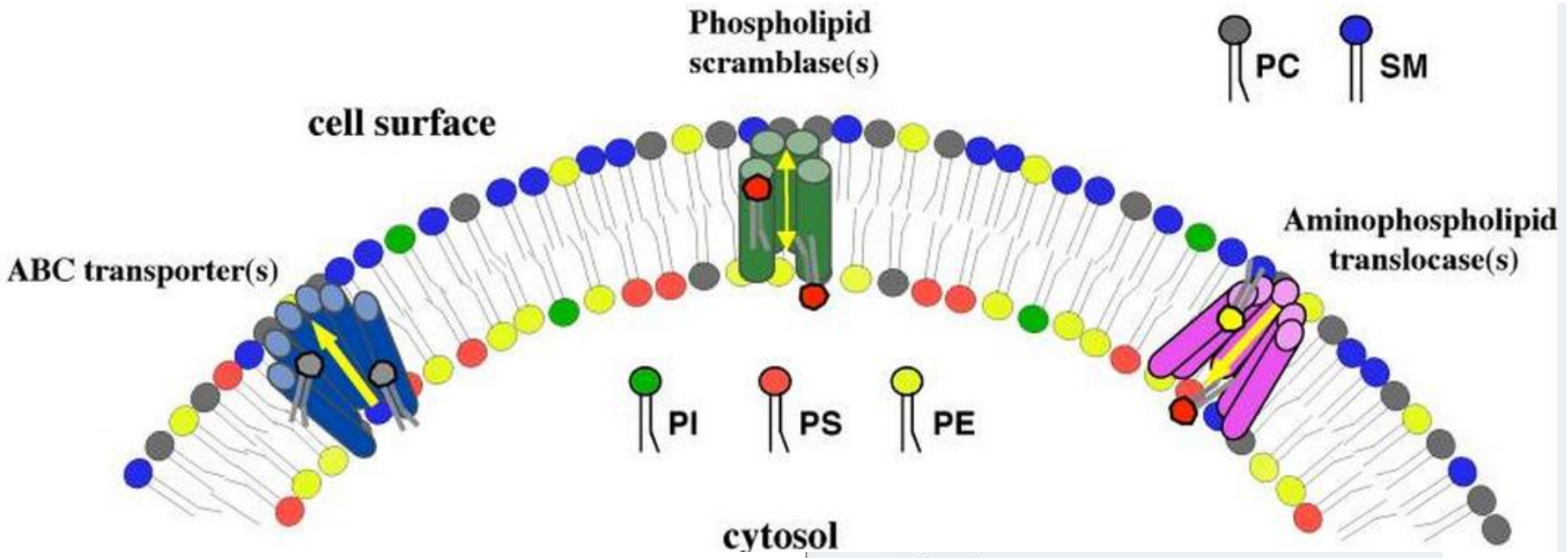


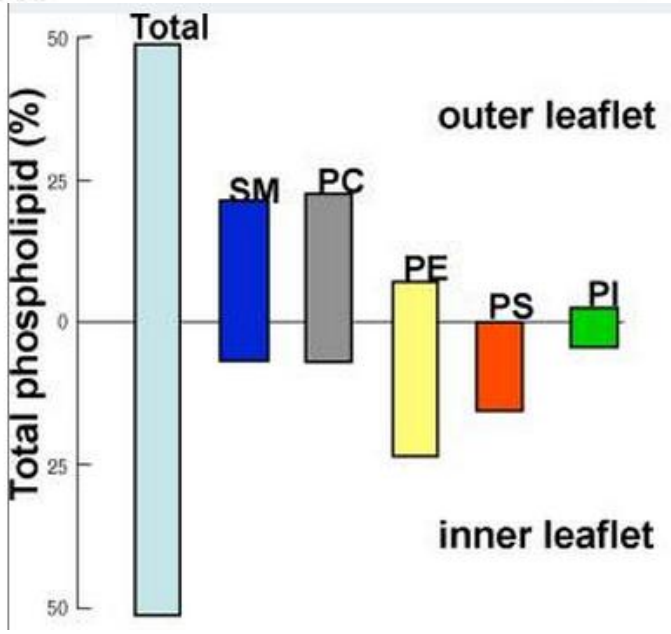
PE
ethanolamine



PC
choline

Plasma membrane lipids show an assymetric distribution





Why assymetric?

- PI generates second messengers (PLC: IP3 + DAG; PI3K: PIP3)

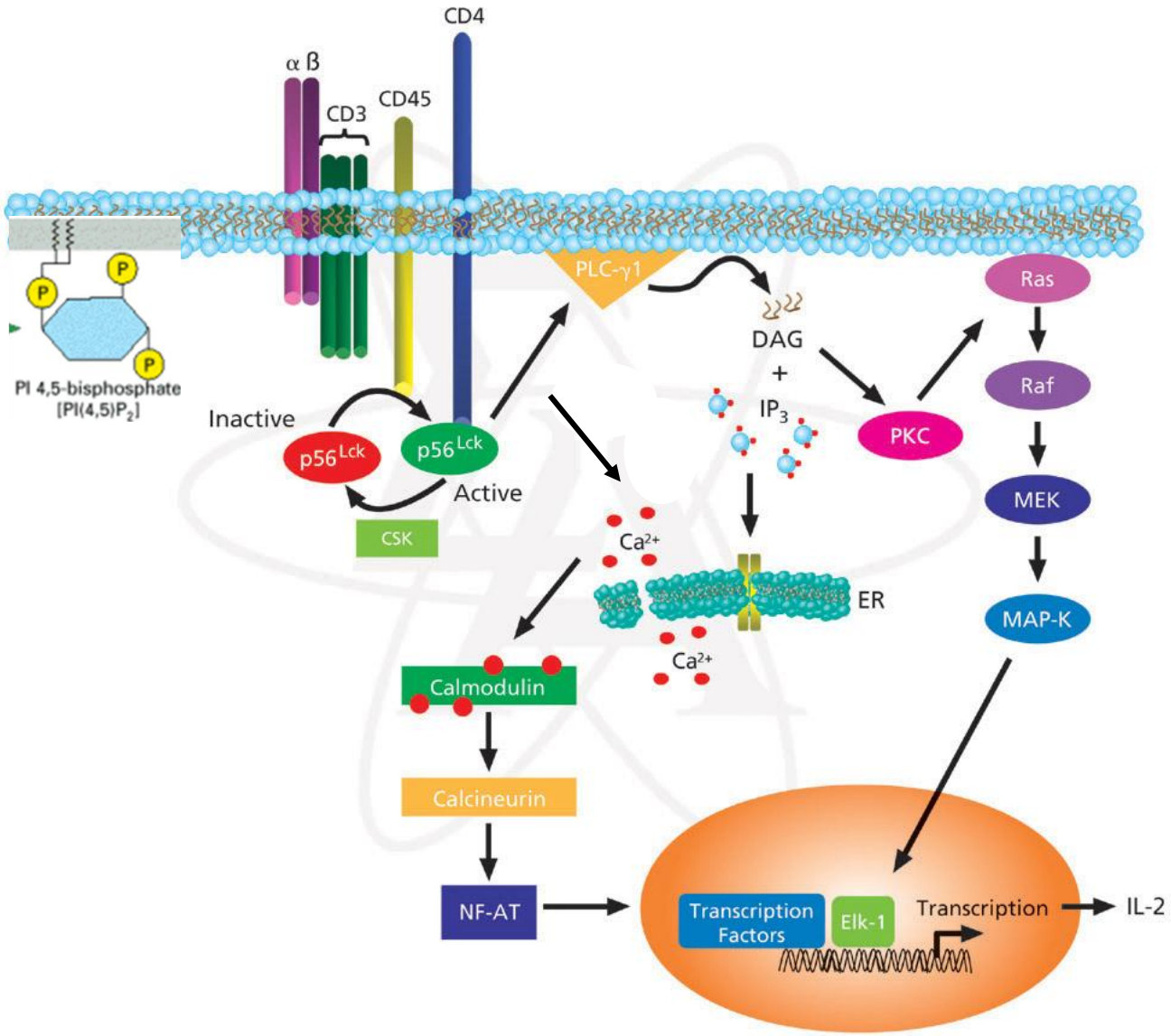
- PS at outer leaflet marks apoptotic cells

Phosphatidyl-inositol-derived second messengers

group 1: IP3 and DAG

e. g: T Cell Receptor (TCR) Signaling

via Phospholipase C



Receptor

↓

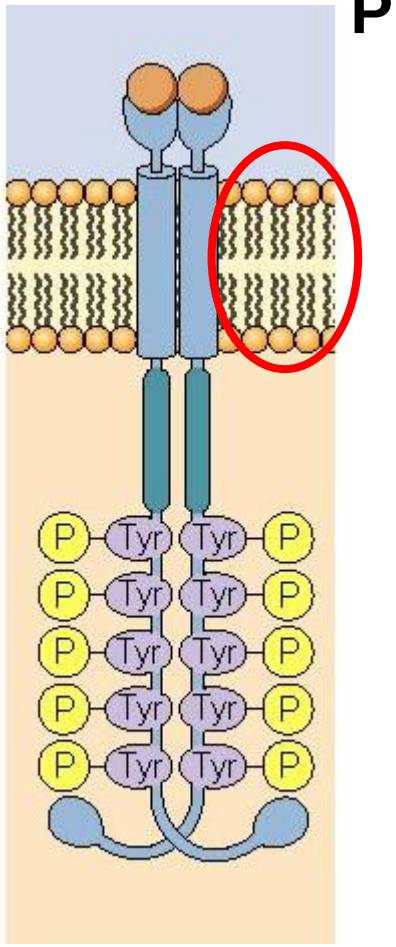
Protein kinase

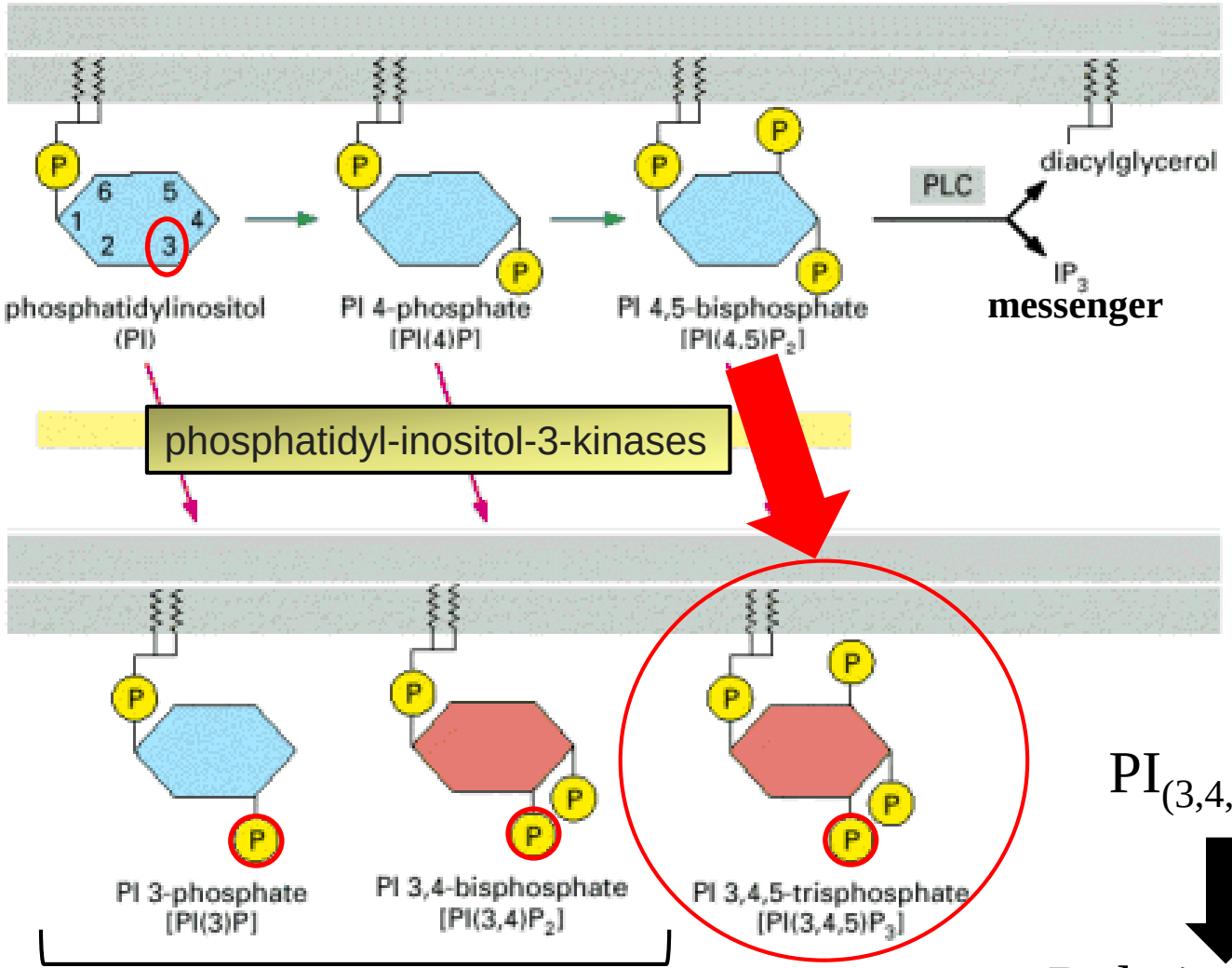
↓

Second messenger

Phosphatidyl-inositol-derived second messenger:

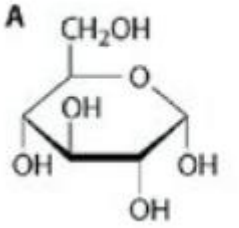
group 2: phosphorylation at position 3



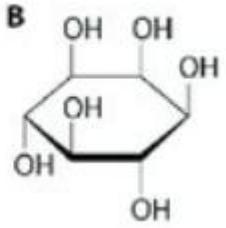


$PI_{(3,4,5)}P3$

Role in cancer



Glucose

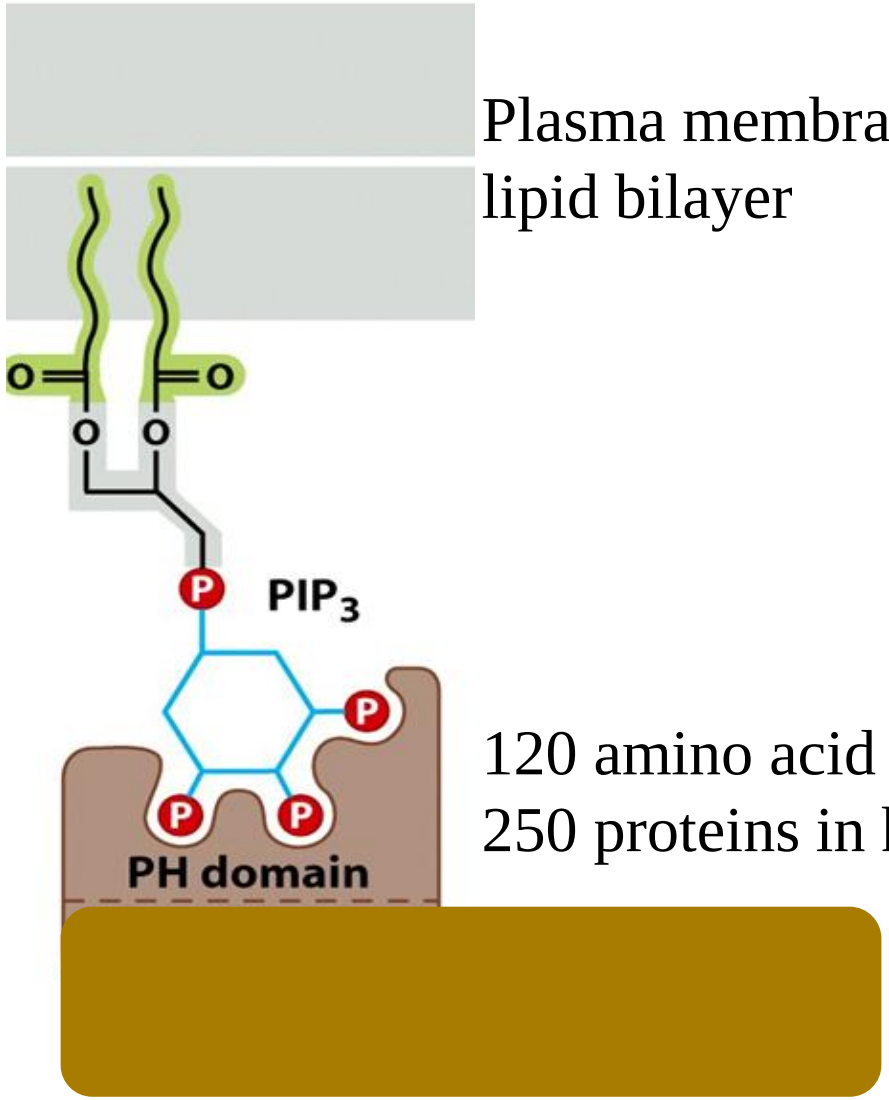


Inositol

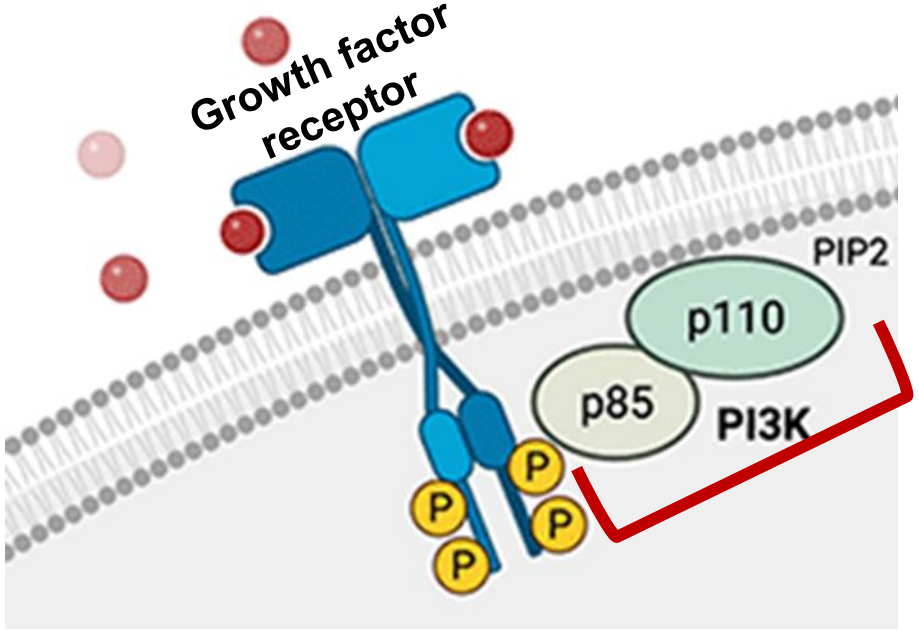
The PI3 kinase pathway

$PI_{(3,4,5)}P3$
=

anchor for recruitment of cytoplasmic PH-domain containing protein kinases to the membrane for their activation



PI3-kinase activation

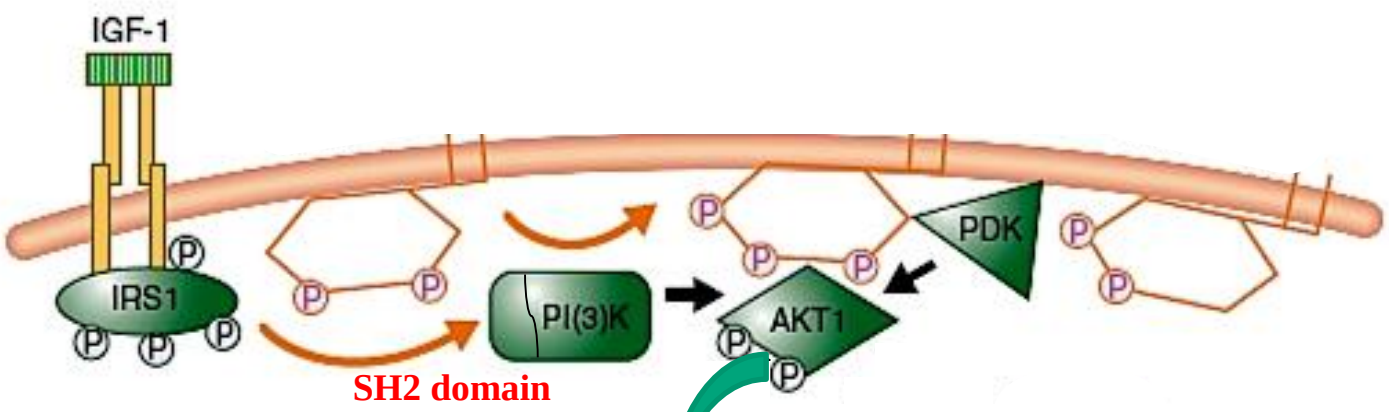


p110α,
PIK3CA gene

Class IA PI3K molecules are **heterodimers** composed of
1. a regulatory, SH2 domain-containing subunit (p85) and
2. a catalytic (p110) subunit (*PIK3CA* gene).

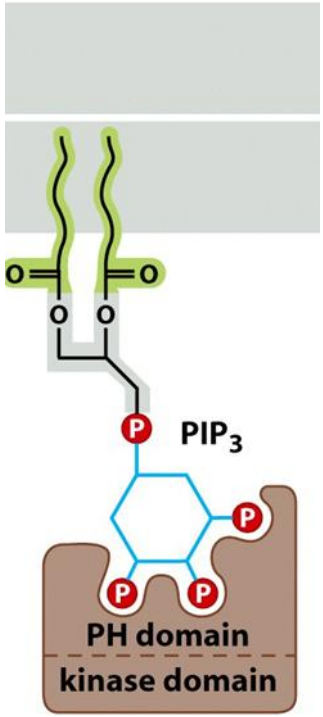
The PI3 kinase pathway

$PI_{(3,4,5)}P3$ = anchor for recruitment of PH-domain proteins



1- Ligand-receptor complex leads to PI3K activation via SH2 domains

2- Recruitment of cytoplasmic protein kinases to the membrane for their activation

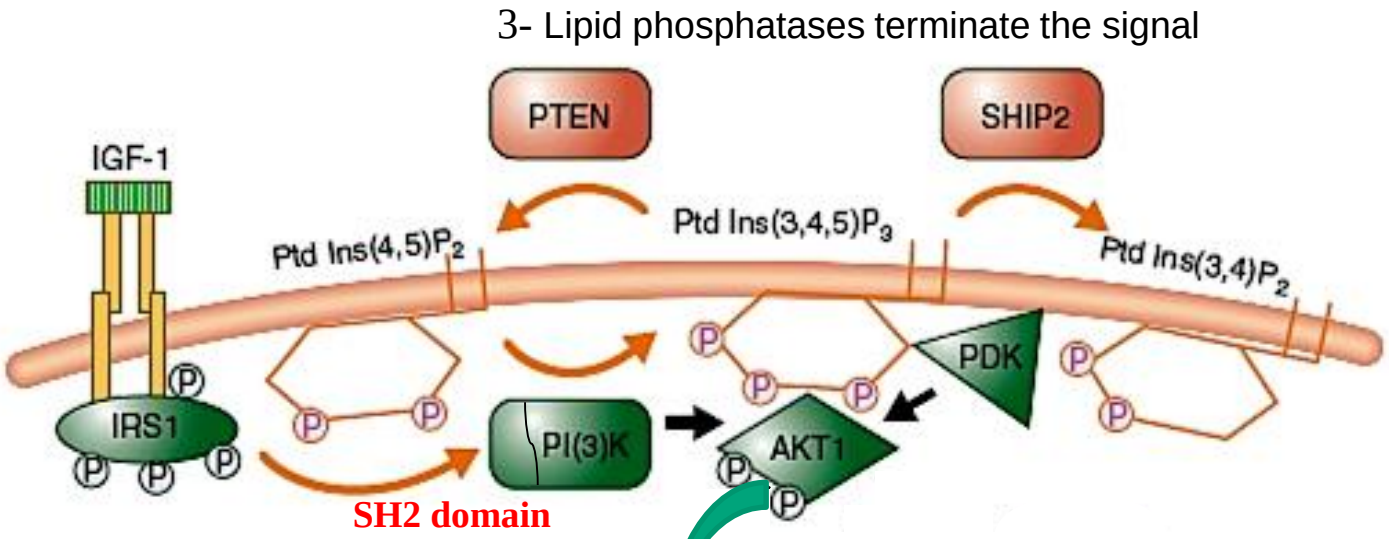


AKT (PKB)
PDK1

PI3K-AKT pathway

The PI3 kinase pathway

$PI_{(3,4,5)}P3$ = anchor for recruitment of PH-domain proteins



PI3K-AKT pathway

Genetic alterations that activate the pathway in tumours

PTEN

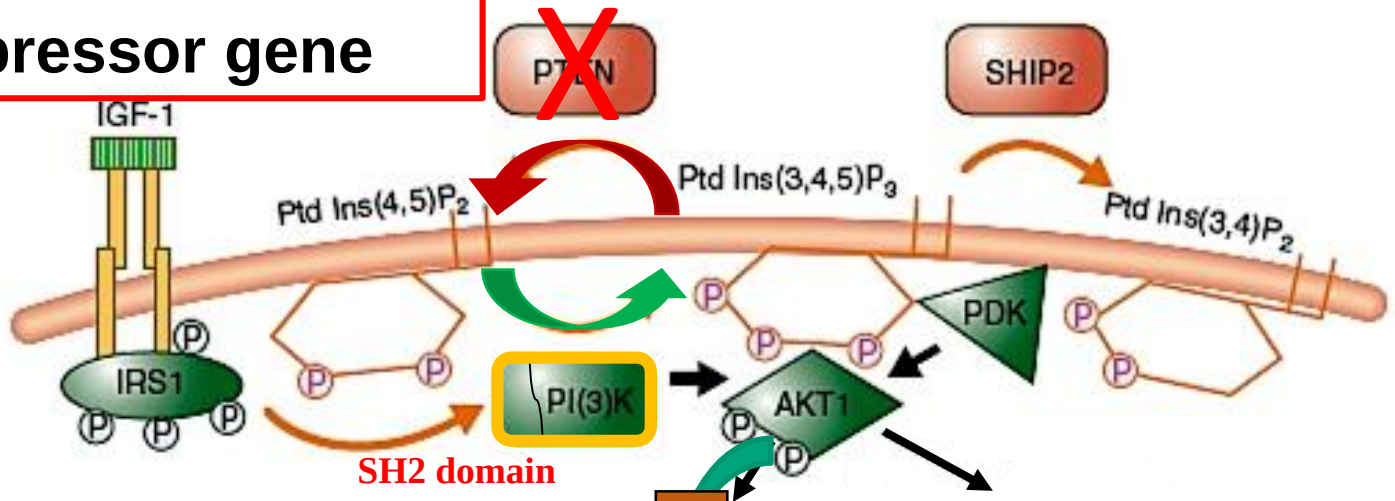
Gene deletion or gene silencing

↓

Brain and breast tumours

PTEN tumour suppressor gene

Lipid phosphatase required to terminate the signal



PIK3CA oncogene

Gain-of-function missense mutations lead to receptor-independent activation

PI3K-AKT pathway

Oncogenic PIK3CA mutations

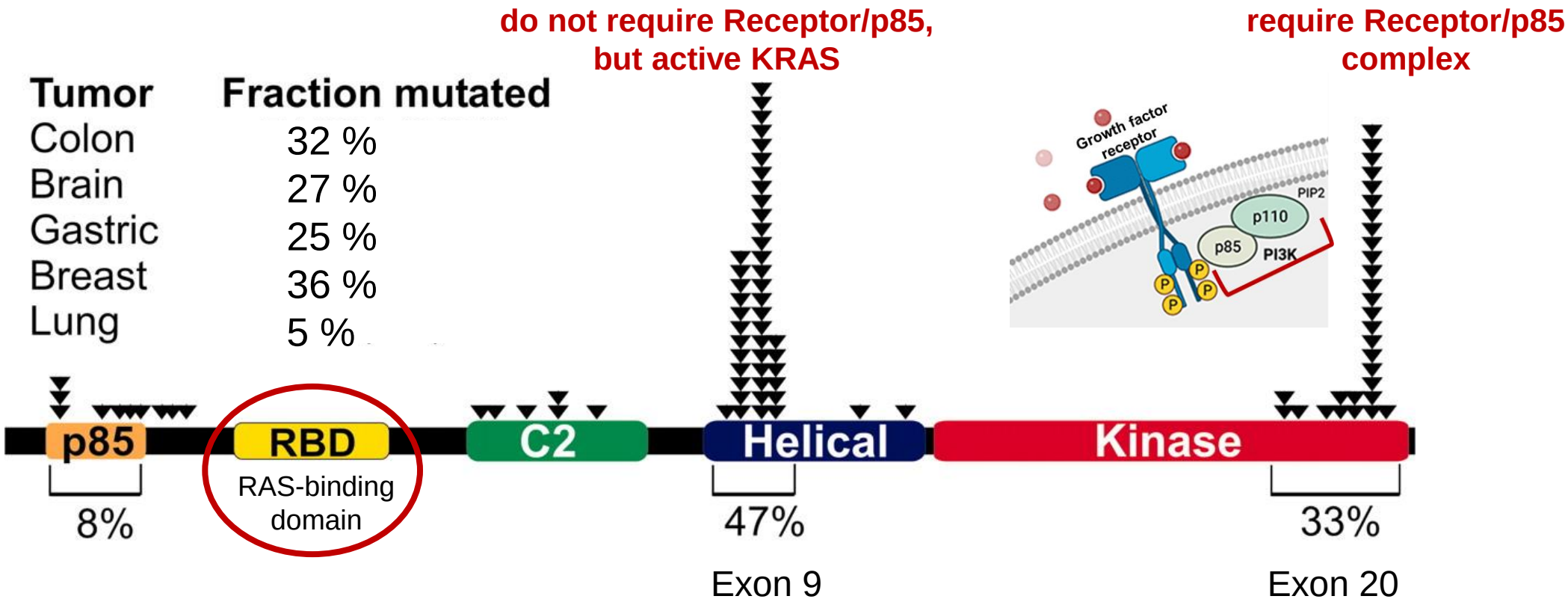
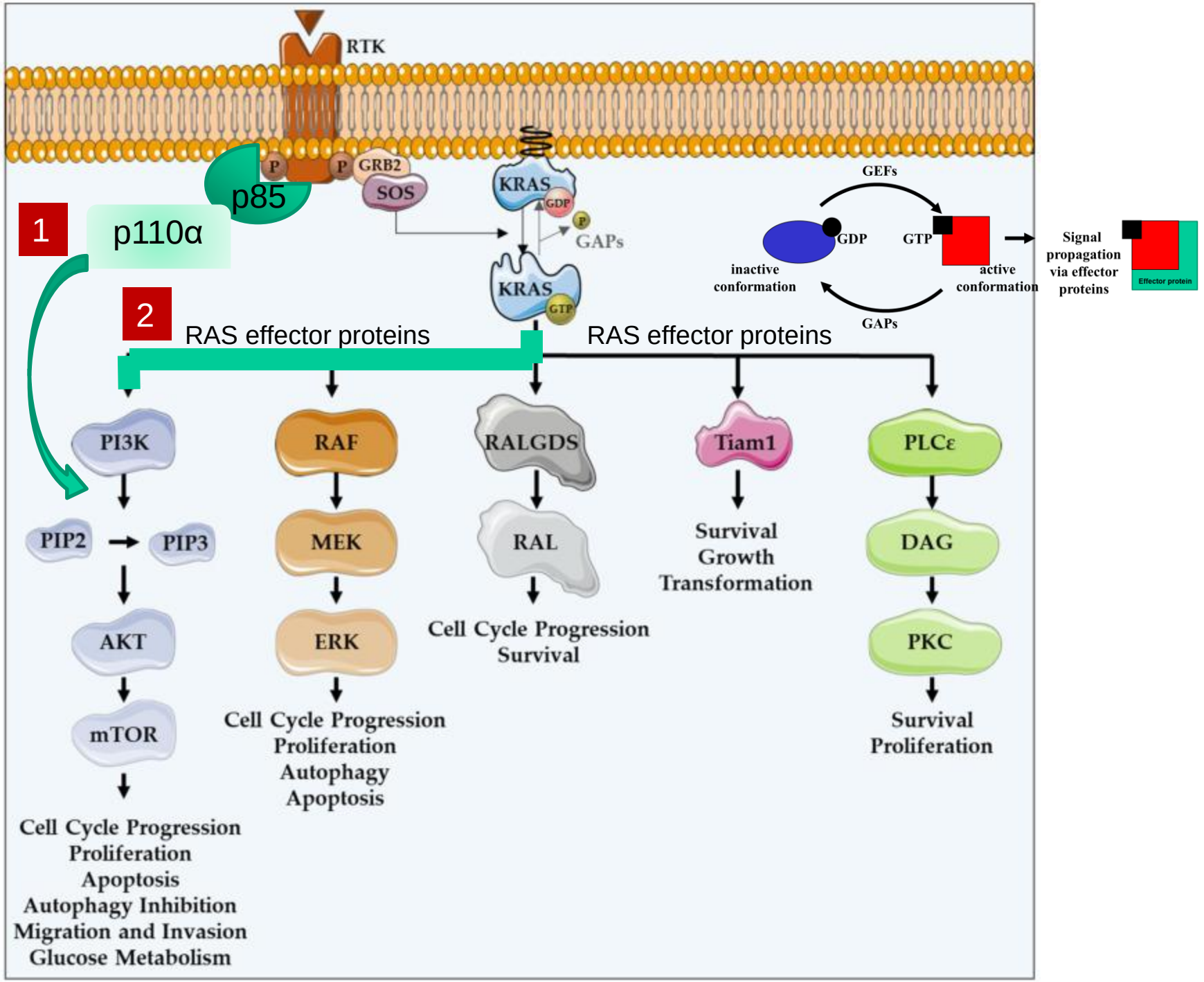


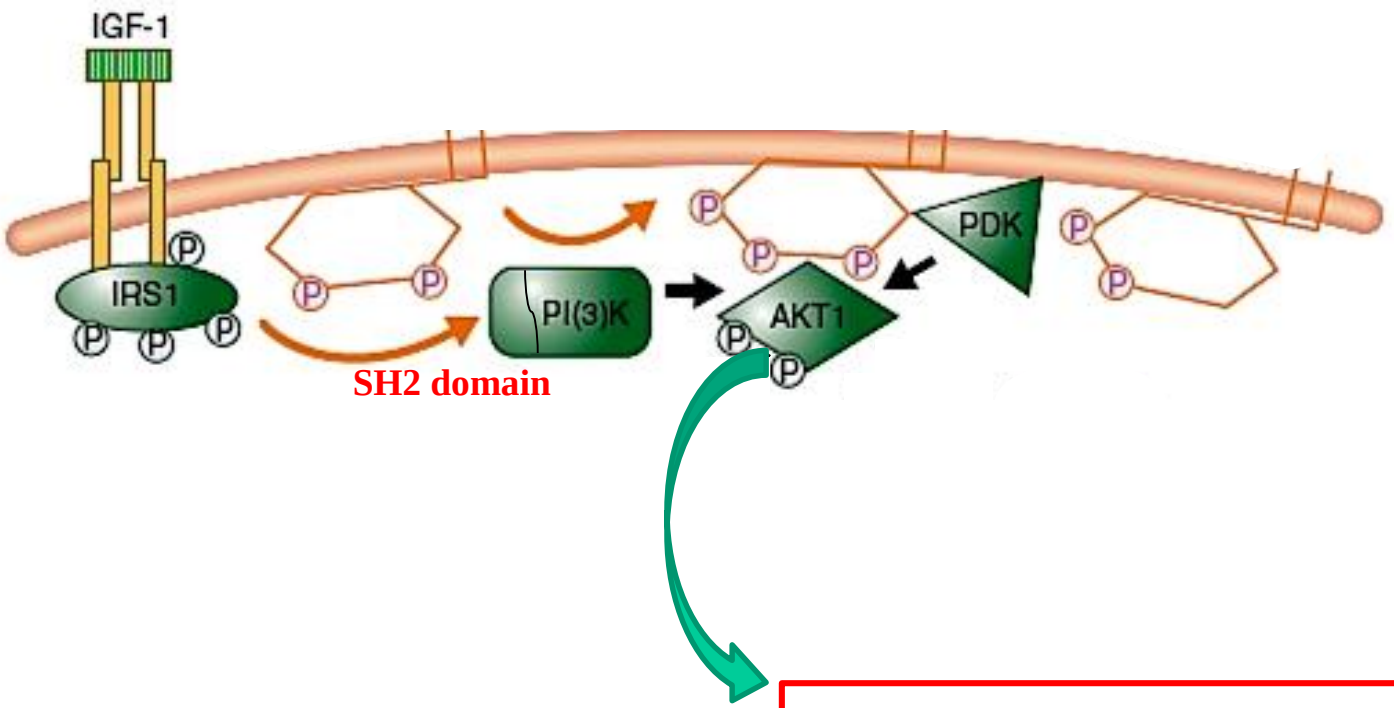
Fig. 1. Mutations in *PIK3CA*. Arrowheads indicate the location of missense mutations

Samuels et al., (2004) Science. 2004 304,554. (*updated*)

2 modes of PI3K activation

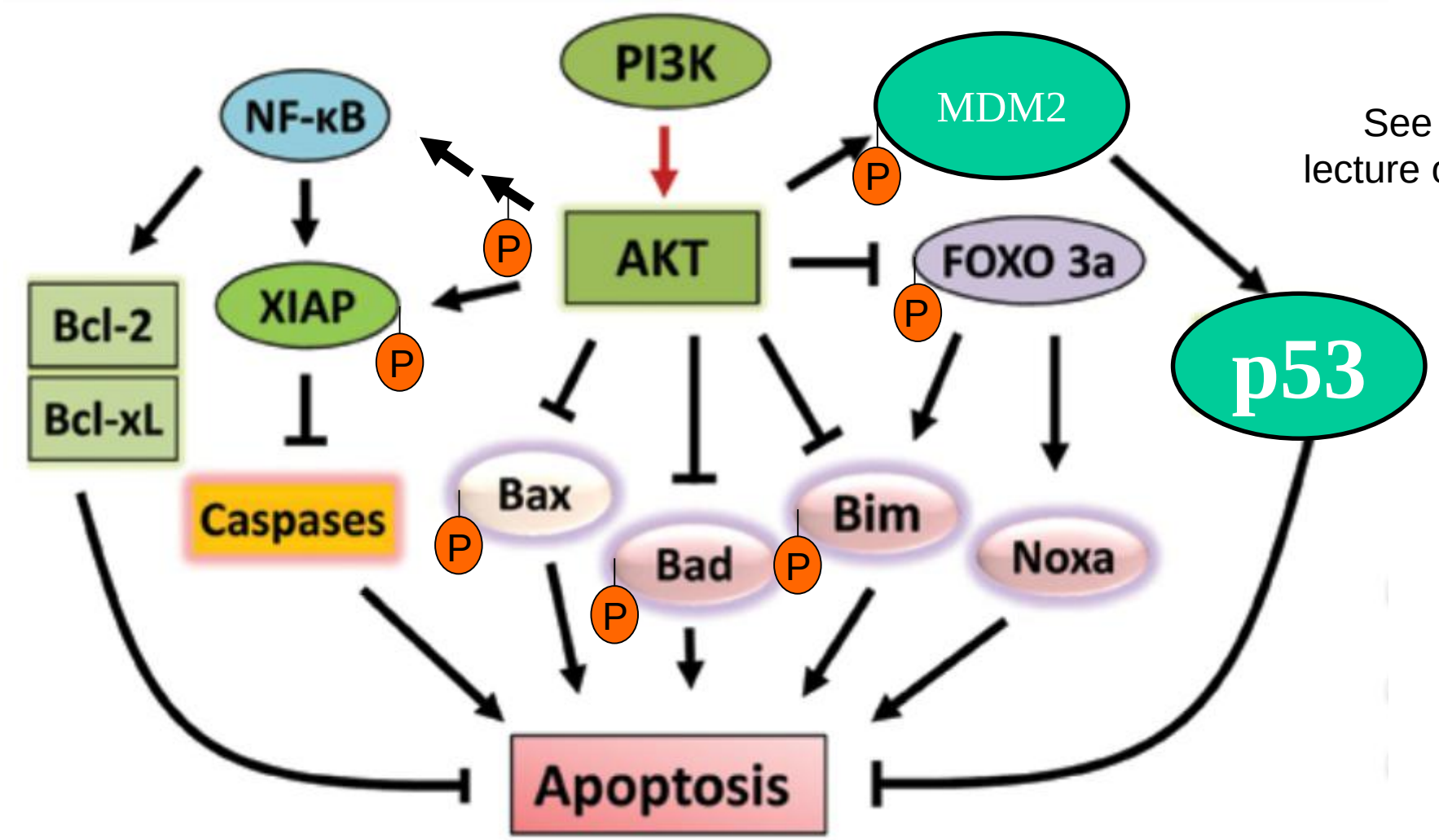


What is AKT doing?



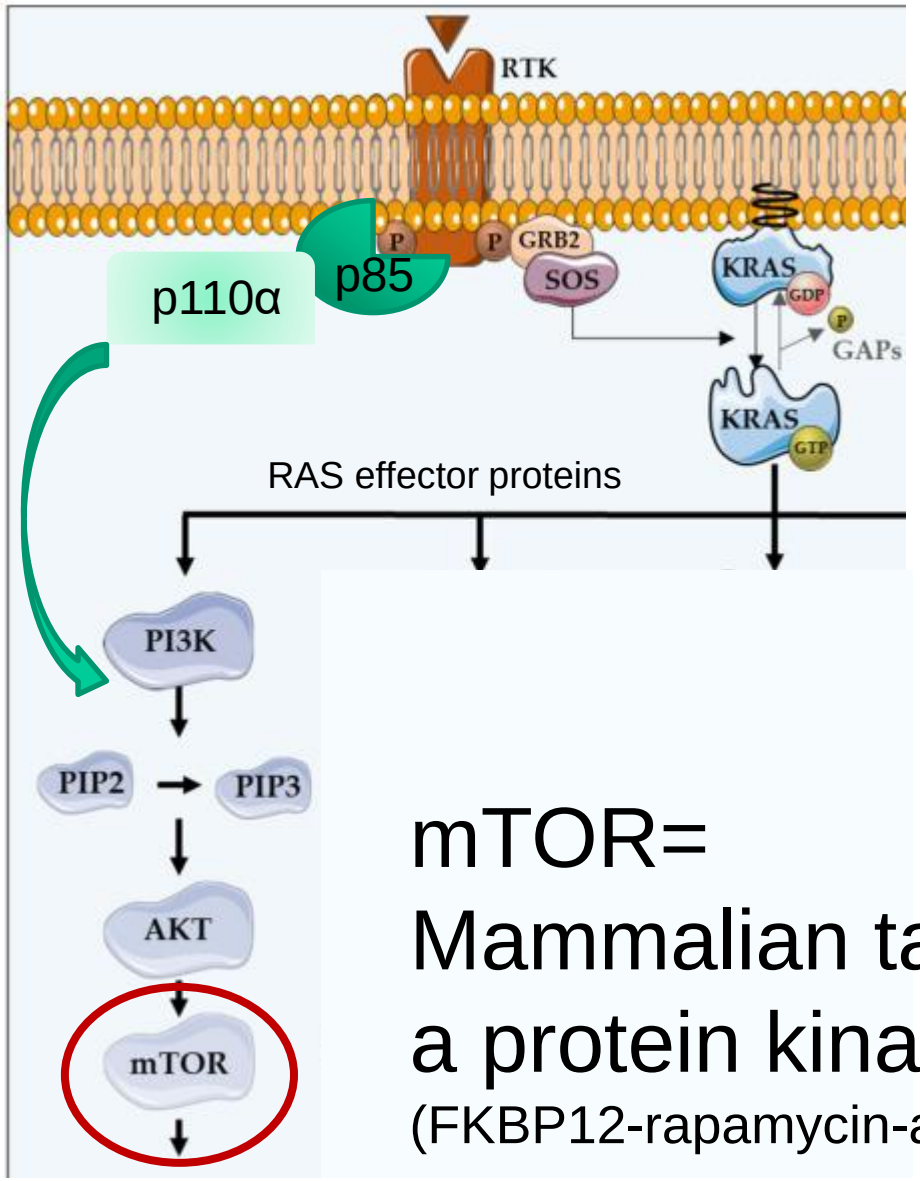
PI3K-AKT pathway

PI3K-AKT signalling:
1) Anti-apoptotic signalling



See separate lecture on Apoptosis

PI3K-AKT signalling:
2) Cell growth stimulation via mTOR



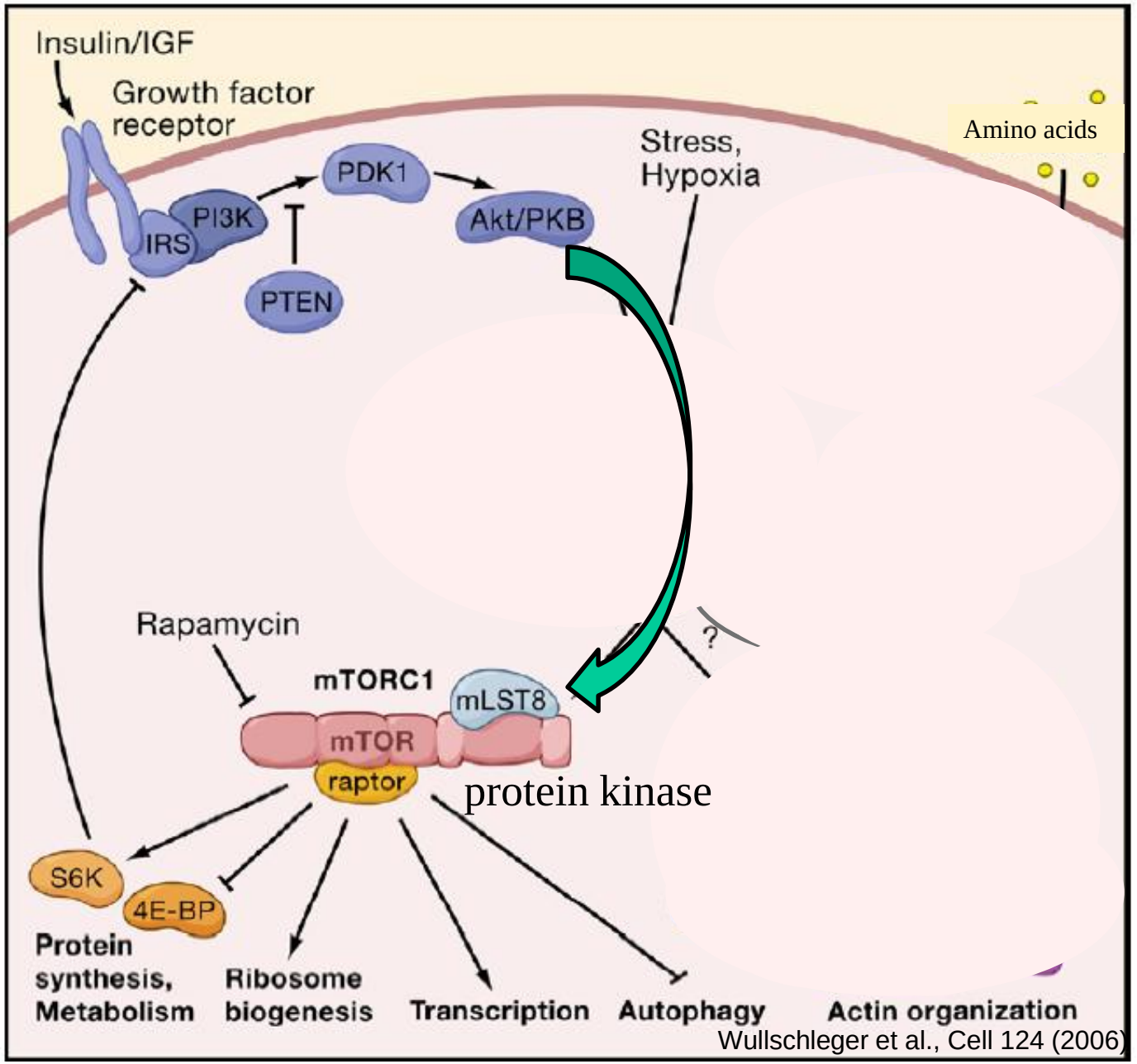
Cell growth
Protein synthesis
Metabolism

Rapamycin-
Antifungal compound isolated from Streptomyces hygroscopicus in 1972;
(FDA approved in 1999 as Sirolimus)

mTOR=
Mammalian target of rapamycin, a protein kinase
(FKBP12-rapamycin-associated protein (FRAP))

PI3K-AKT signalling: 2) Cell growth stimulation via mTOR

4 min-video recommendation:
https://www.youtube.com/watch?v=ewgLd9N3s-4

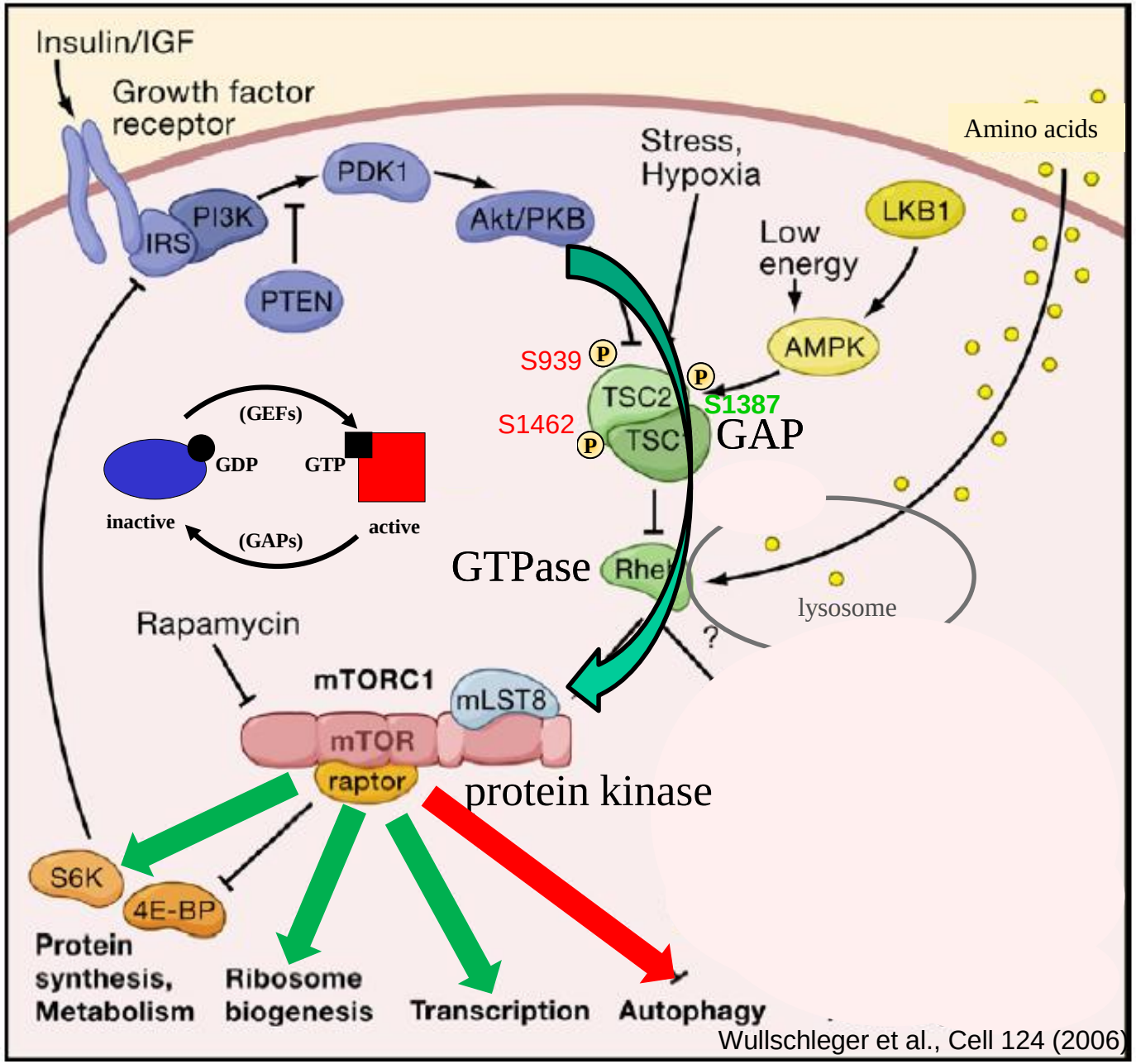


Wullschleger et al., Cell 124 (2006)

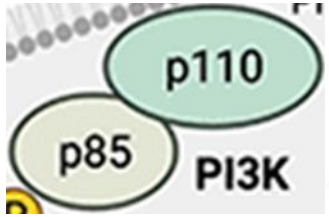
PI3K-AKT signalling: 2) Cell growth stimulation via mTOR

4 min-video recommendation:
https://www.youtube.com/watch?v=ewgLd9N3s-4

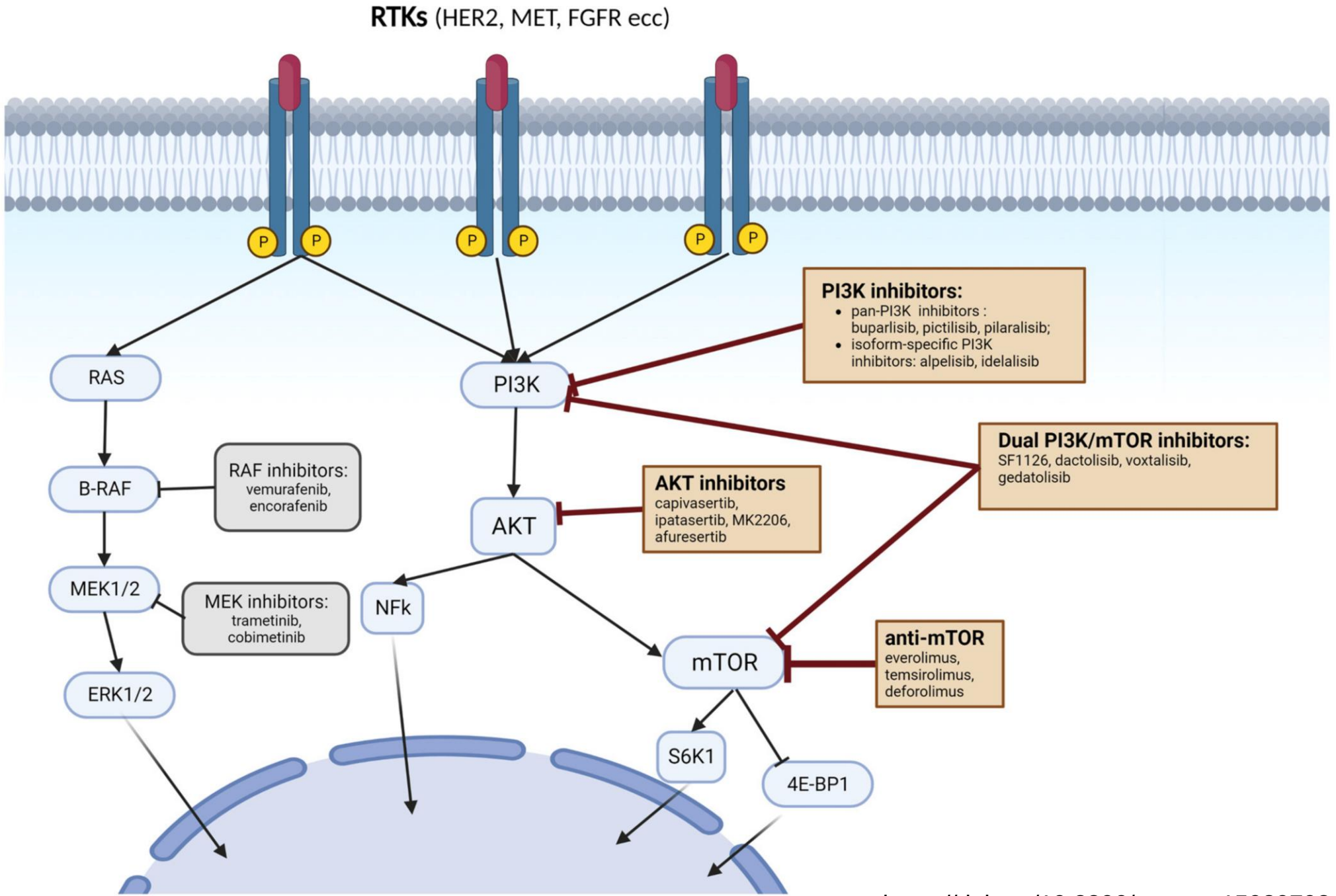
mTOR as a signalling hub that integrates growth signals and nutritional conditions with cell growth



Genetic changes found in the PI3K-AKT pathway

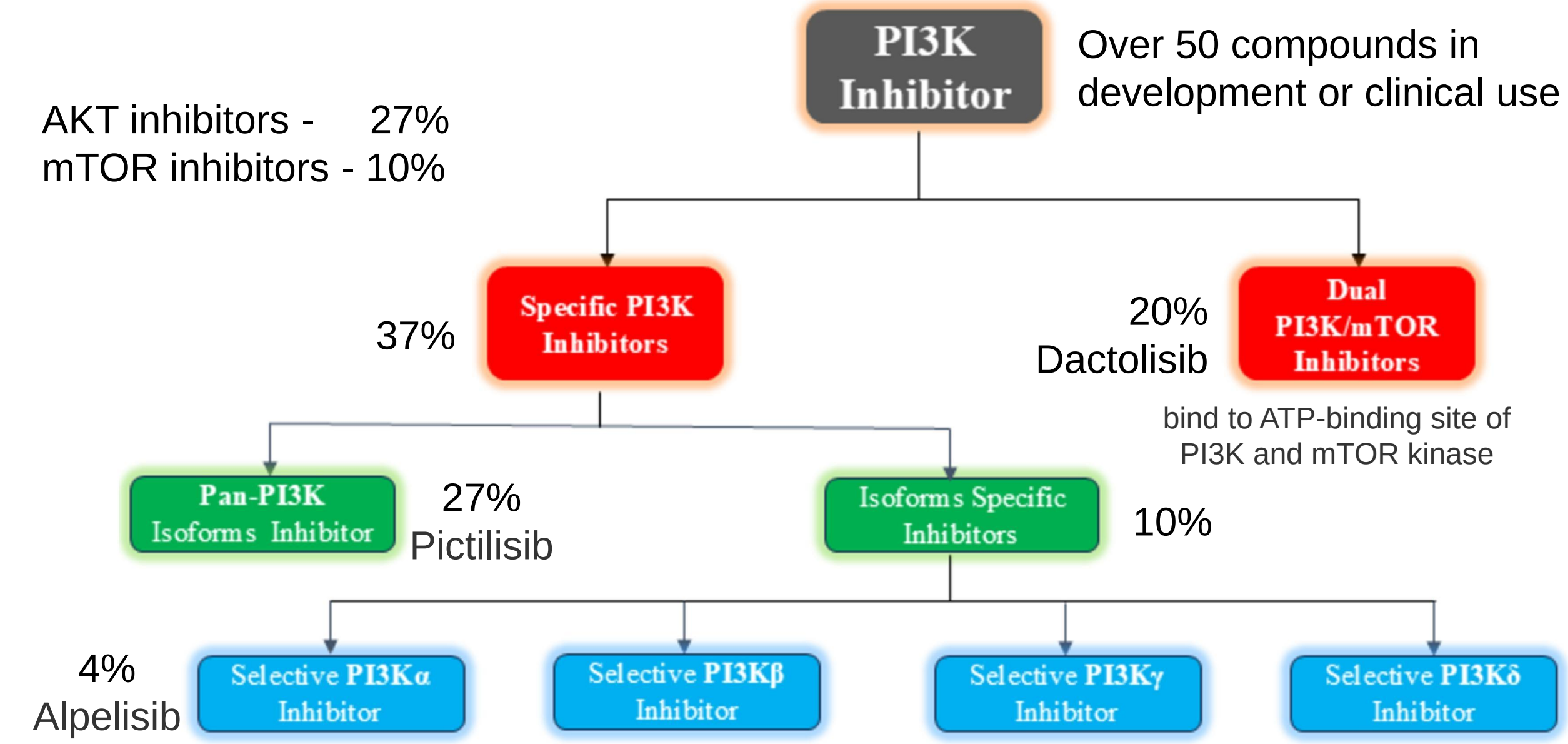


	CRC	Esoph	Panc	Gastric	HCC
• Mutation					
– PIK3CA	20%	6%	2%	22%	<1%
– PIK3R1	4%	4%	2%	4%	1%
– AKT	3%	<1%	4%	4%	<1%
– PTEN	4%	3%	2%	6%	<1%
– MTOR	7%	2%	2%	8%	3%
• Amplification					
– PIK3CA	19%	?	4%	6%	<1%
• Epigenetic					
– PTEN loss	15%	14%			12%

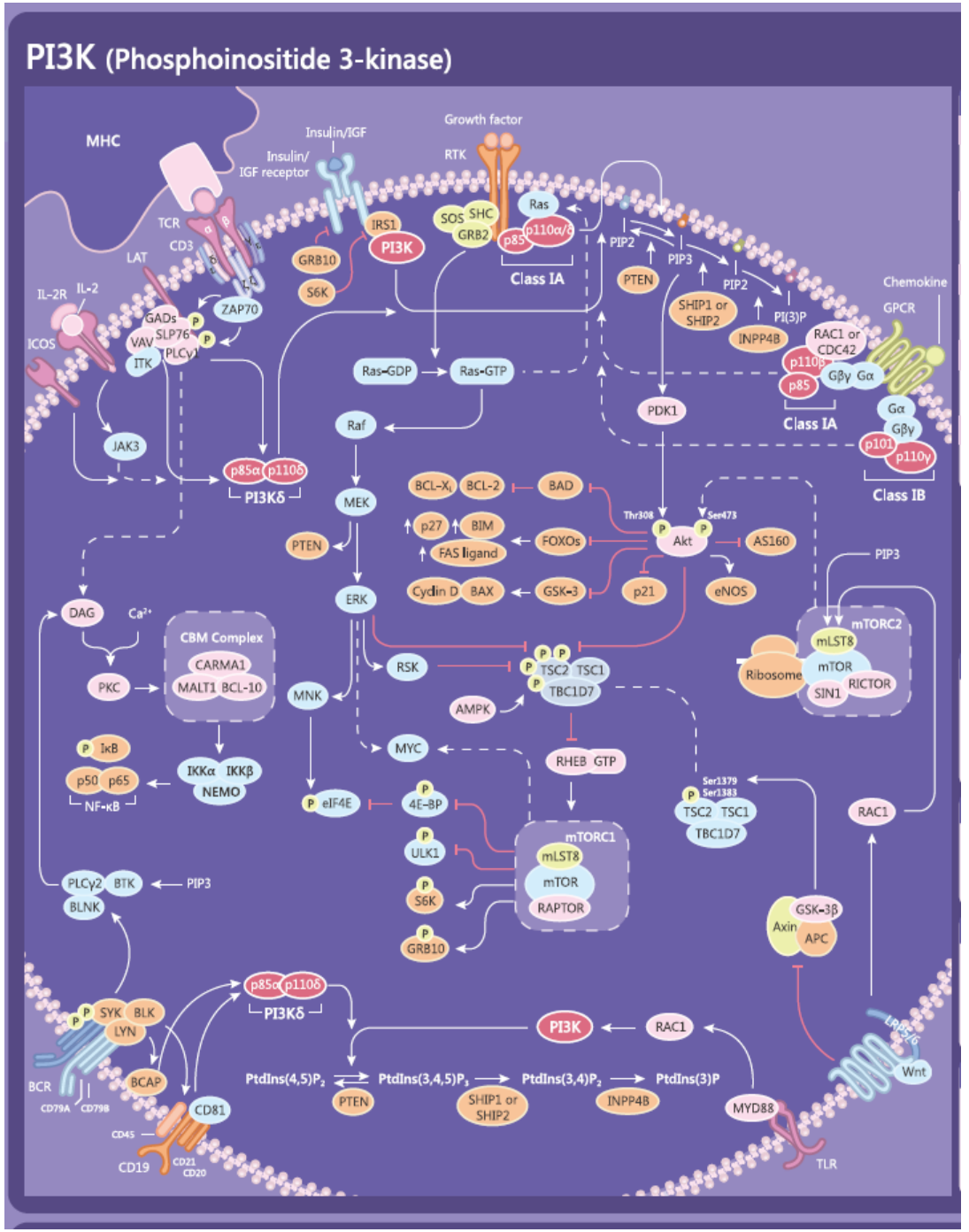


https://doi.org/10.3390/cancers15030703

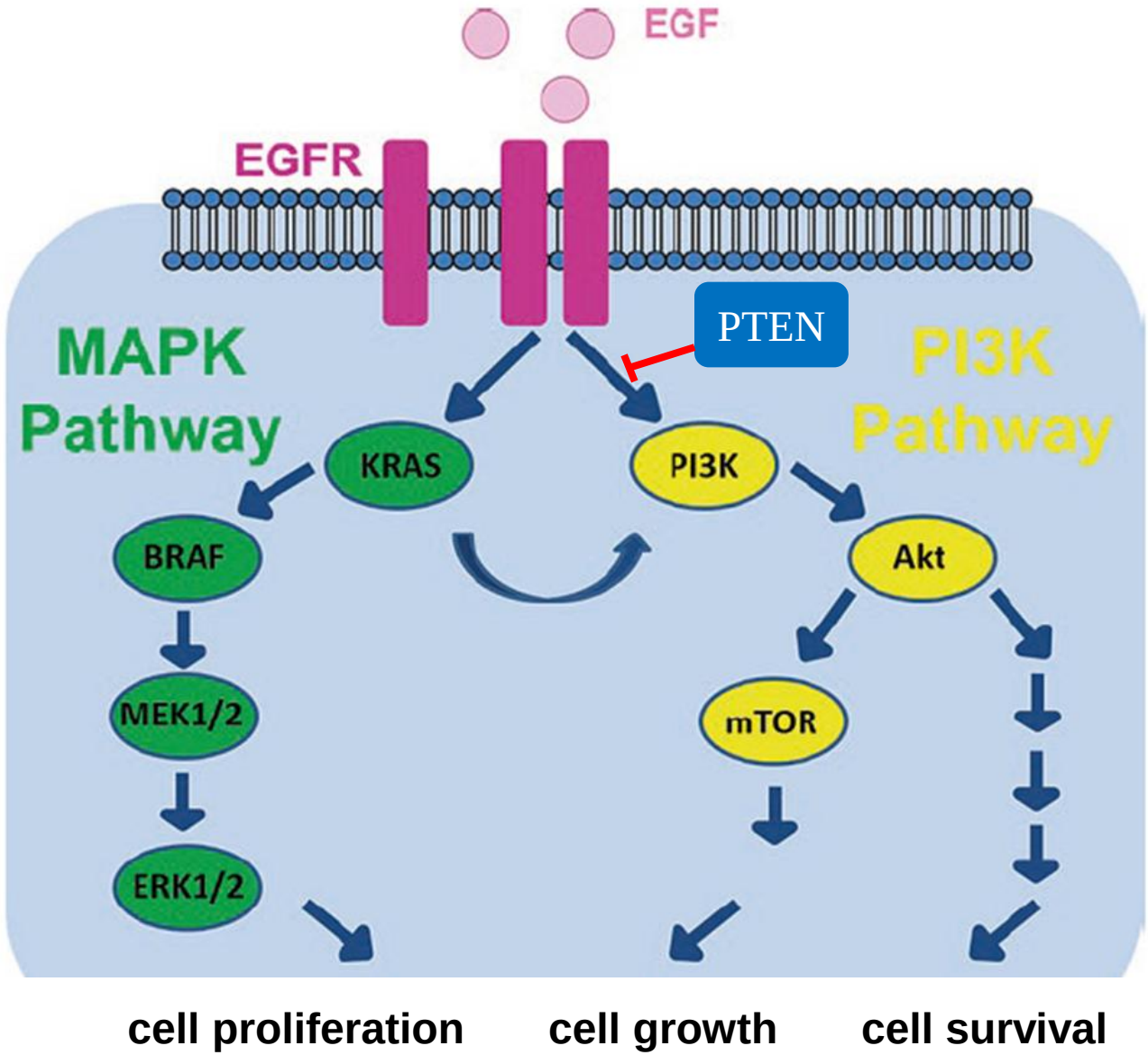
PI3K pathway inhibitors



Take 5 minutes: a PI3K-AKT pathway poster



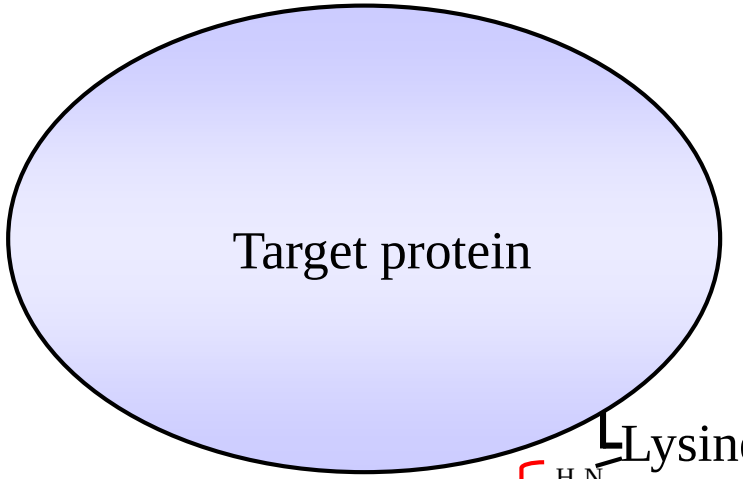
Graphical abstract of
key tumour-promoting
cancer cell signalling pathways

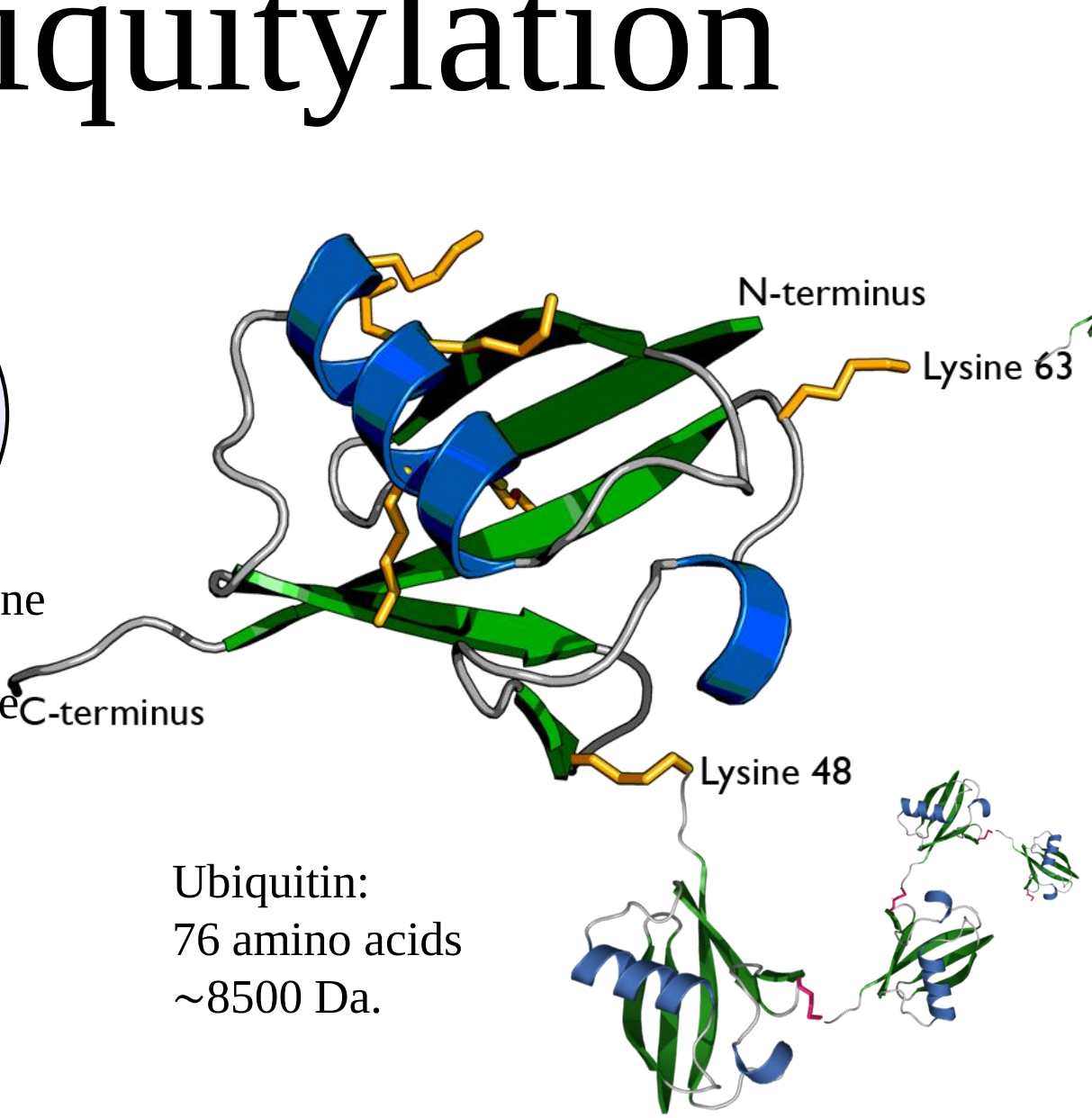


Activation and inactivation of signalling proteins by reversible post-translational modifications

- Phosphorylation	- Ser, Thr, Tyr
- O-GalNAcetylation	- Ser, Thr
- N-GlcNAcetylation	- Asn
- Sulfation	- Tyr
- Ubiquitylation	- Lys
- Acetylation	- Lys
- Methylation	- Lys, Arg
- Oxidation	- Cys
- Nitrosylation	- Cys
- (…)	

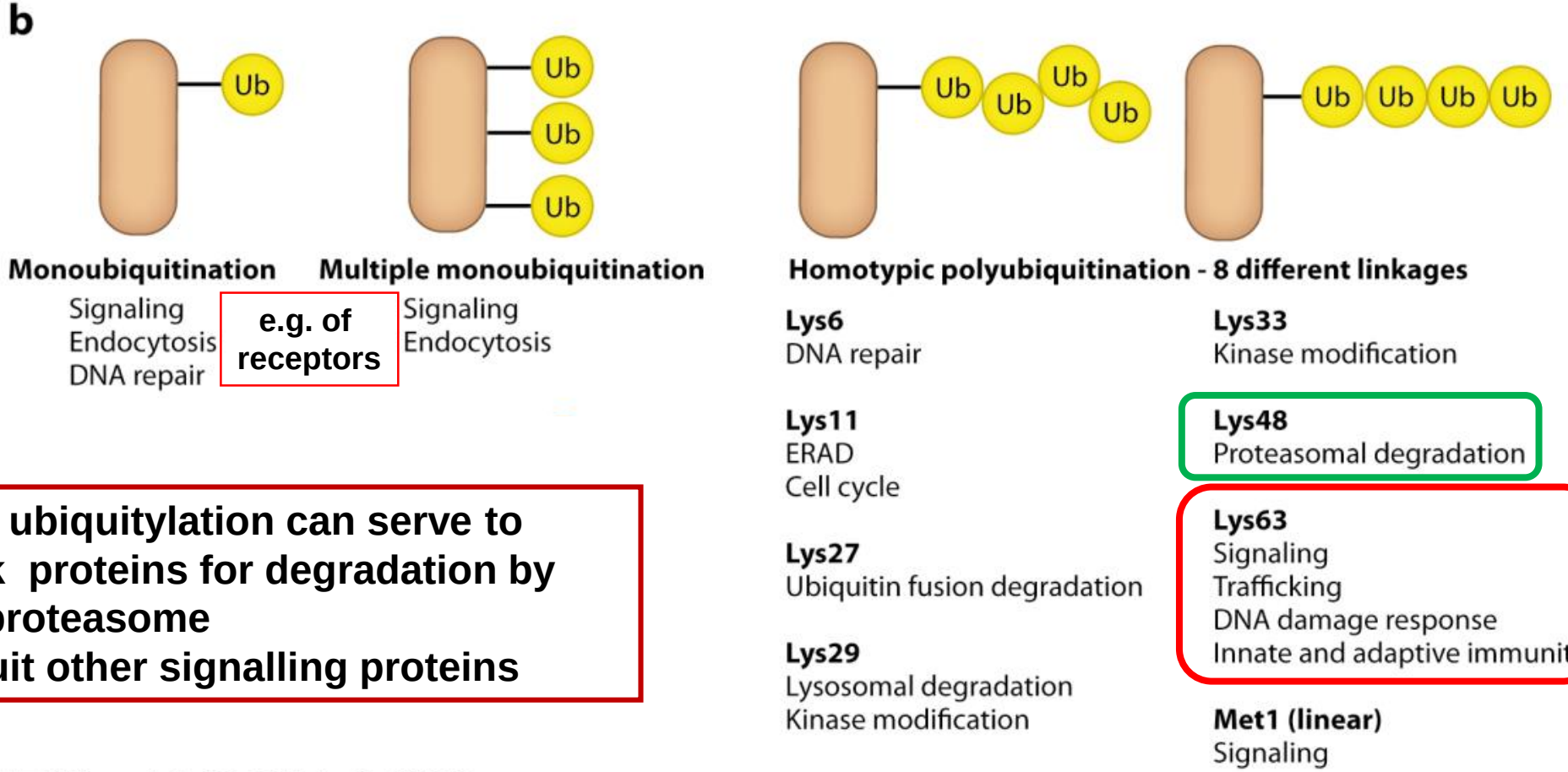
ubiquitylation





Ubiquitin:
76 amino acids
~8500 Da.

Different types of ubiquitylation

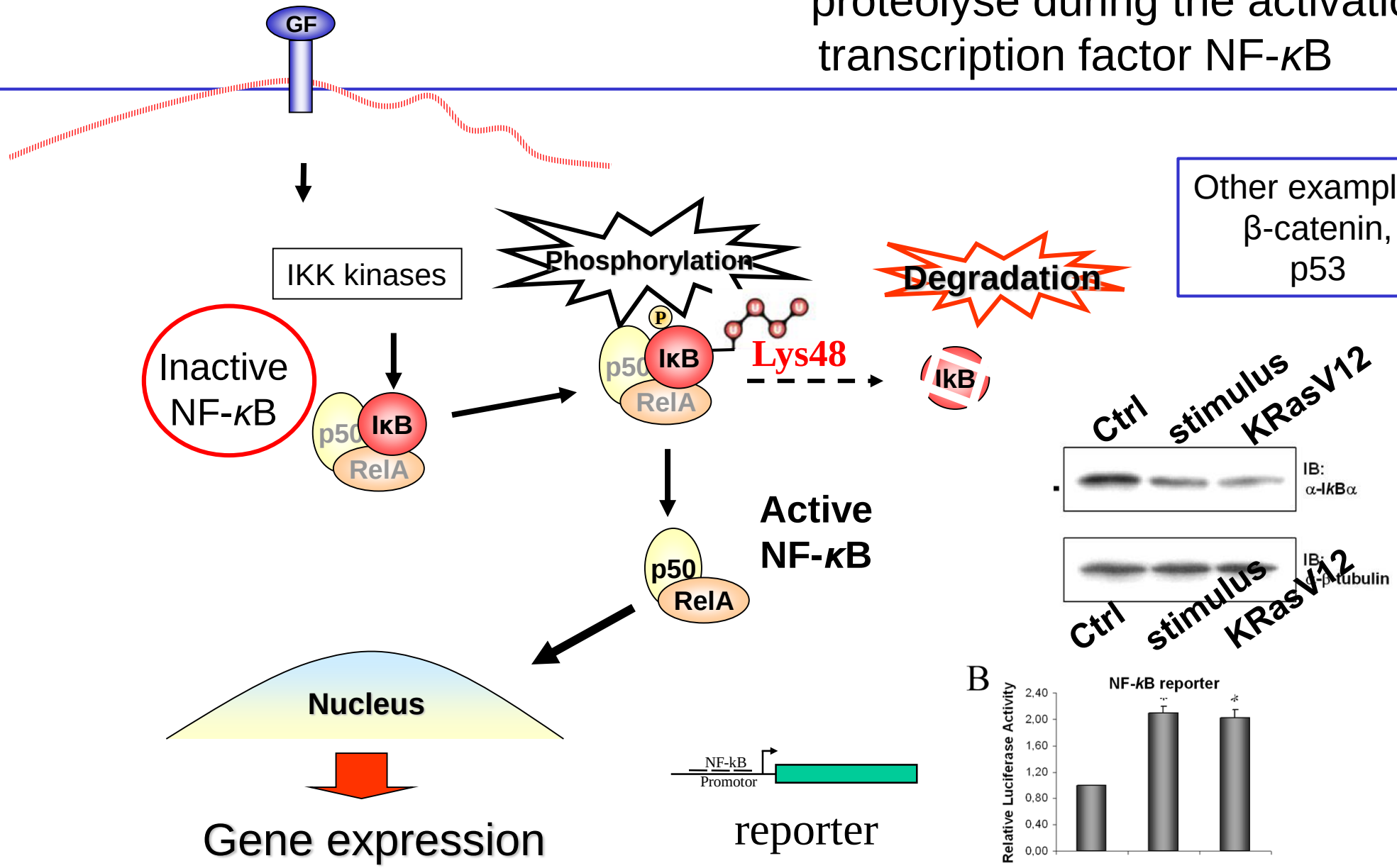


Protein ubiquitylation can serve to
- **mark proteins for degradation by the proteasome**
- **recruit other signalling proteins**

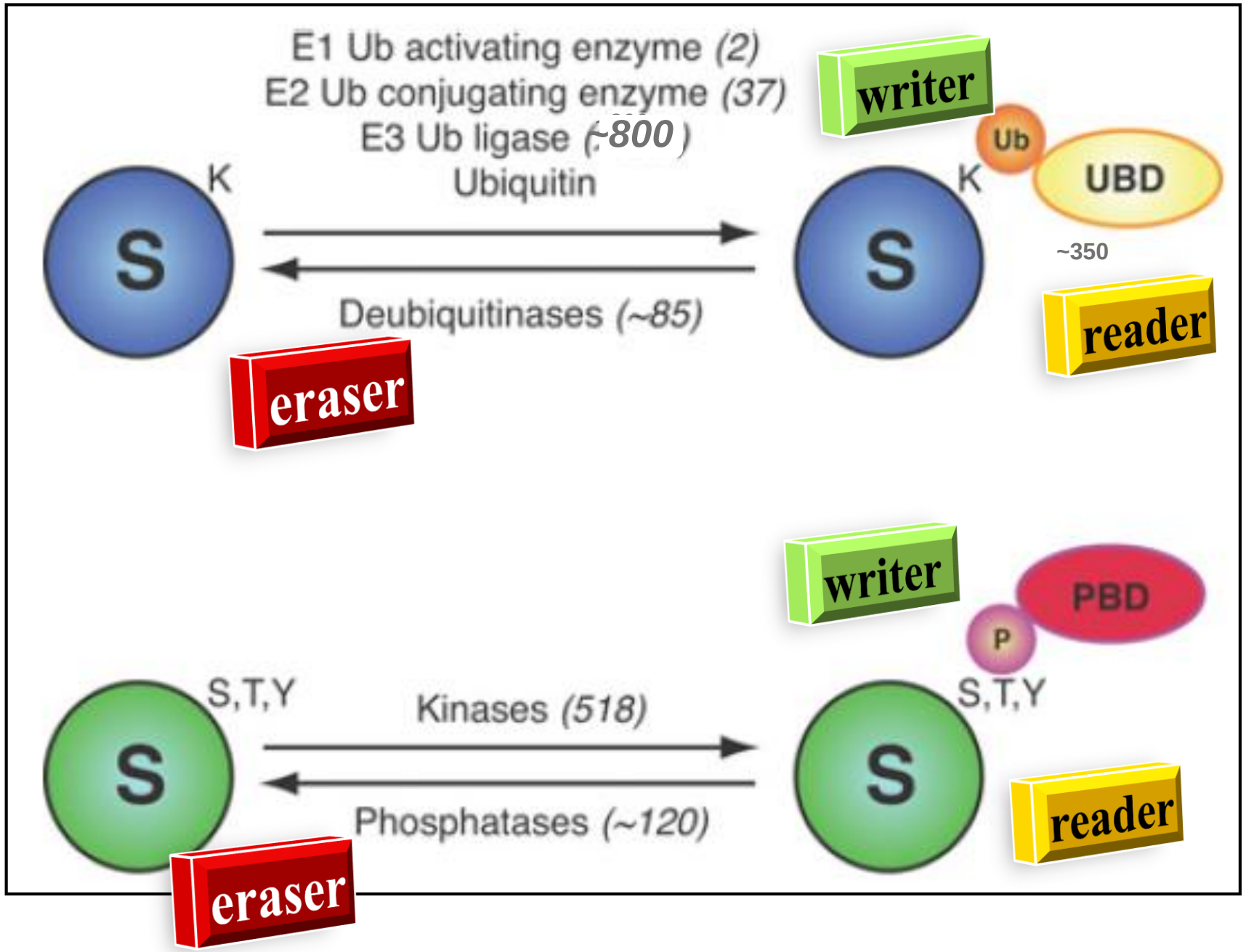
Husnjak K, Dikic I. 2012.
Annu. Rev. Biochem. 81:291–322

⟶ More than 5 000 ubiquitylated proteins exist in the human proteome (~**25%**) (Kim et al. 2011 MolCell 44)

Ubiquitin-mediated signalling, example 1: Role of Lys48-ubiquitylation for proteolyse during the activation of transcription factor NF-κB

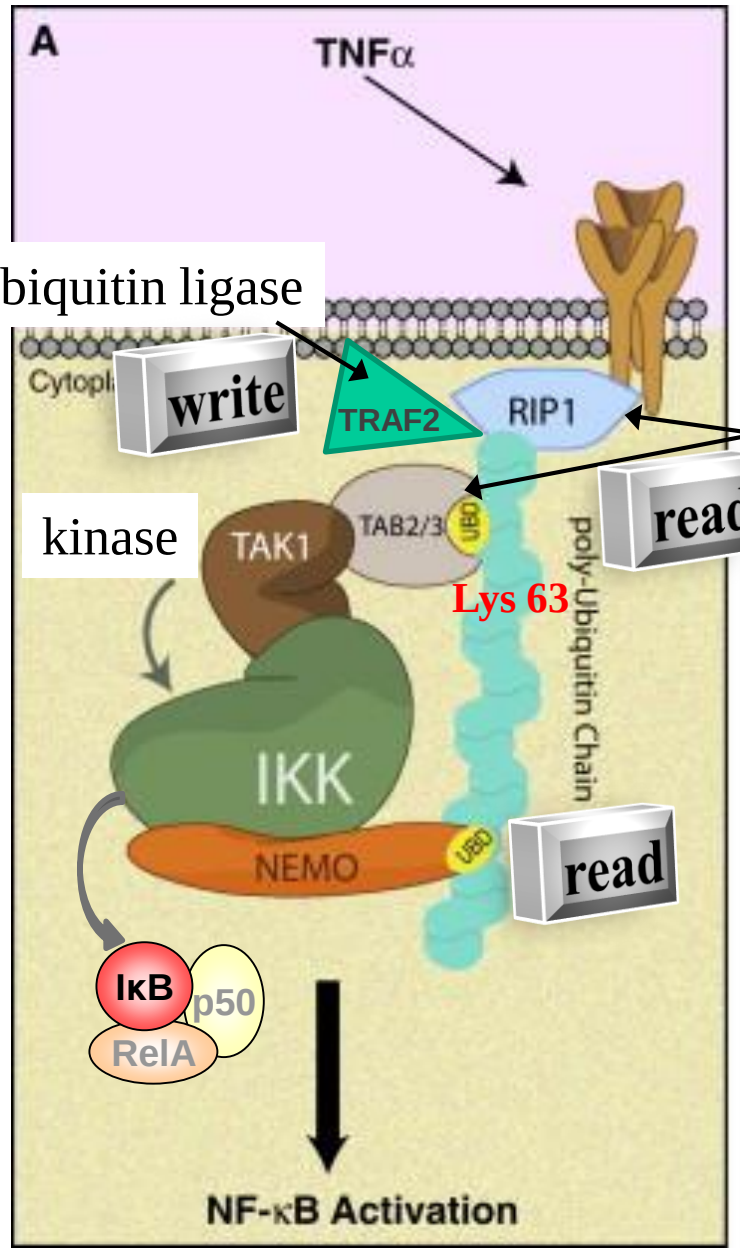


Other examples:
β-catenin,
p53


E1 Ub activating enzyme (2)
E2 Ub conjugating enzyme (37)
E3 Ub ligase (~800)
Ubiquitin
writer
Ub
UBD
~350
K
S
K
S
reader
Deubiquitinases (~85)
eraser
writer
PBD
P
S,T,Y
S,T,Y
Kinases (518)
S
S
reader
Phosphatases (~120)
eraser

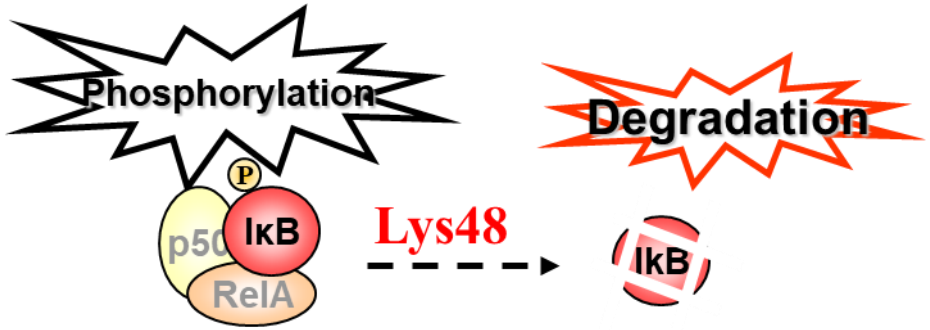
Ubiquitin-mediated signalling, example 2: Role of Lys63-ubiquitylation for NF-κB

UBD- Ubiquitin-binding domain
=
Reader:
Protein domain for recognition
to Ub-chain-modified proteins



Protein ubiquitylation can serve as a **platform for protein interaction** that promotes protein kinase activation

Regulation of signalling proteins by ubiquitin-mediated proteolyse

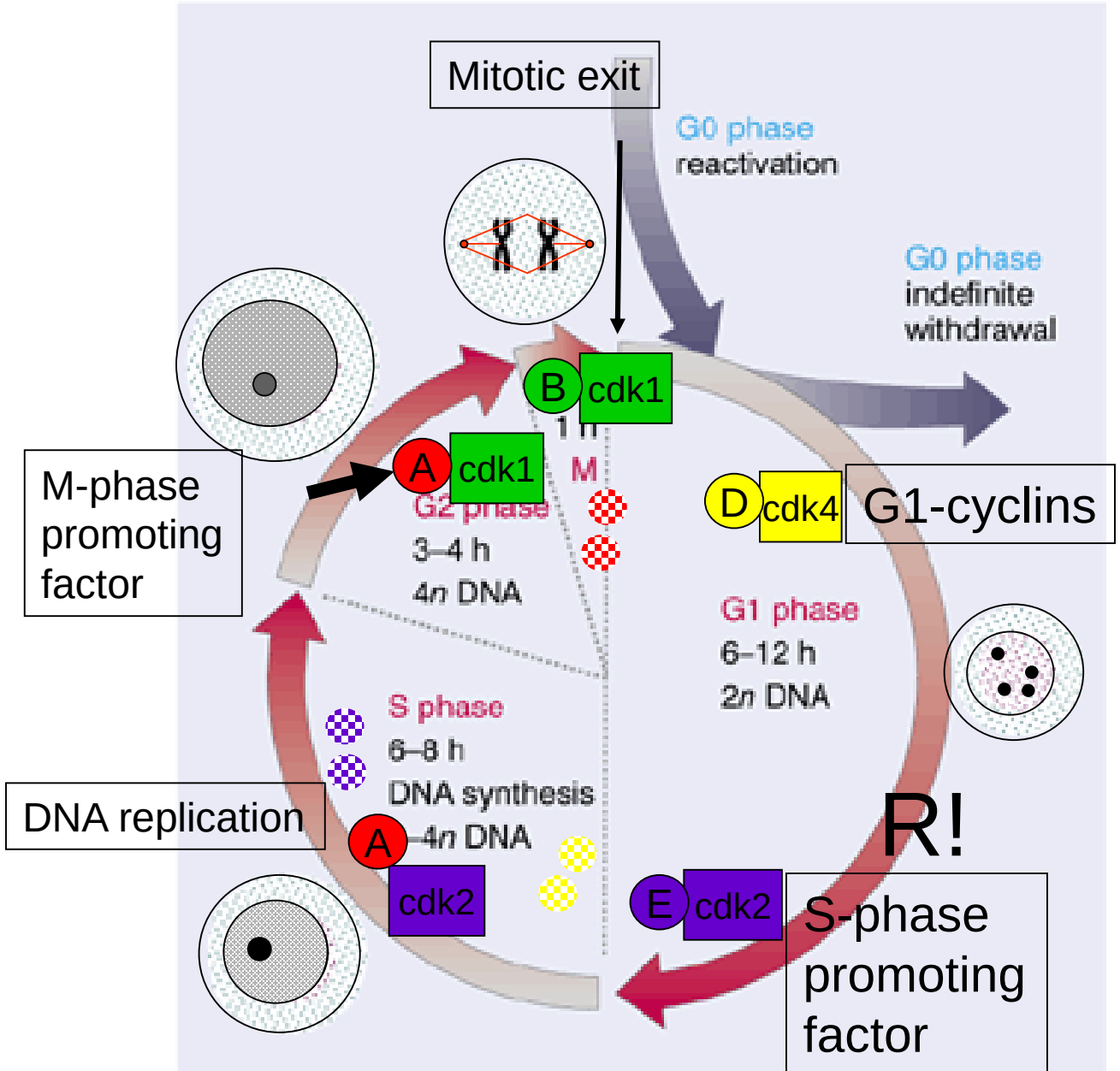


Irreversible

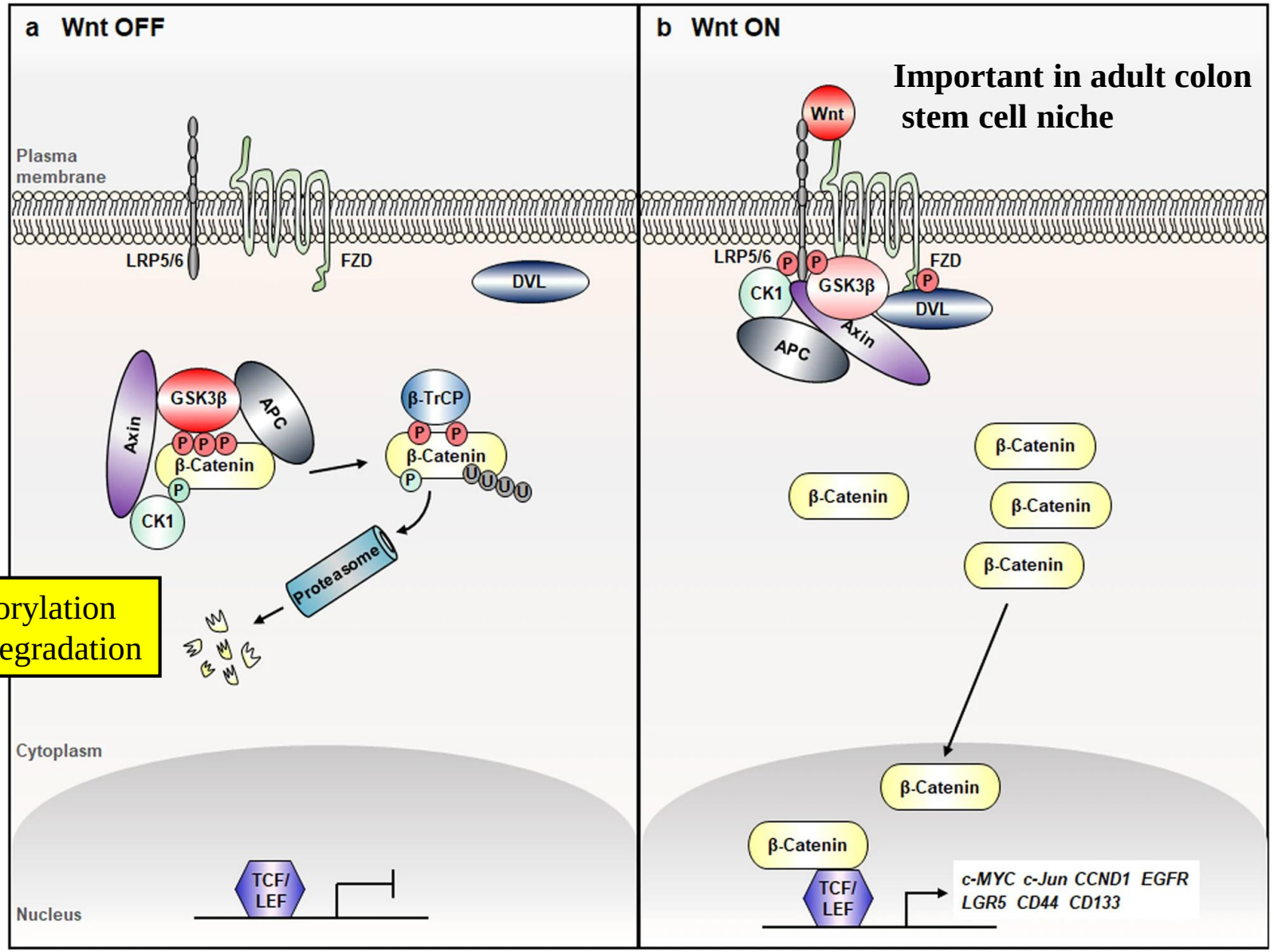
(except through *de novo* synthesis)

Proteolyse controls the unidirectional progression of the cell cycle

Cyclins are required for the activity of cyclin-dependent protein kinases



WNT pathway controls the transcriptional cofactor β-catenin



Default phosphorylation and proteolytic degradation

Receptor

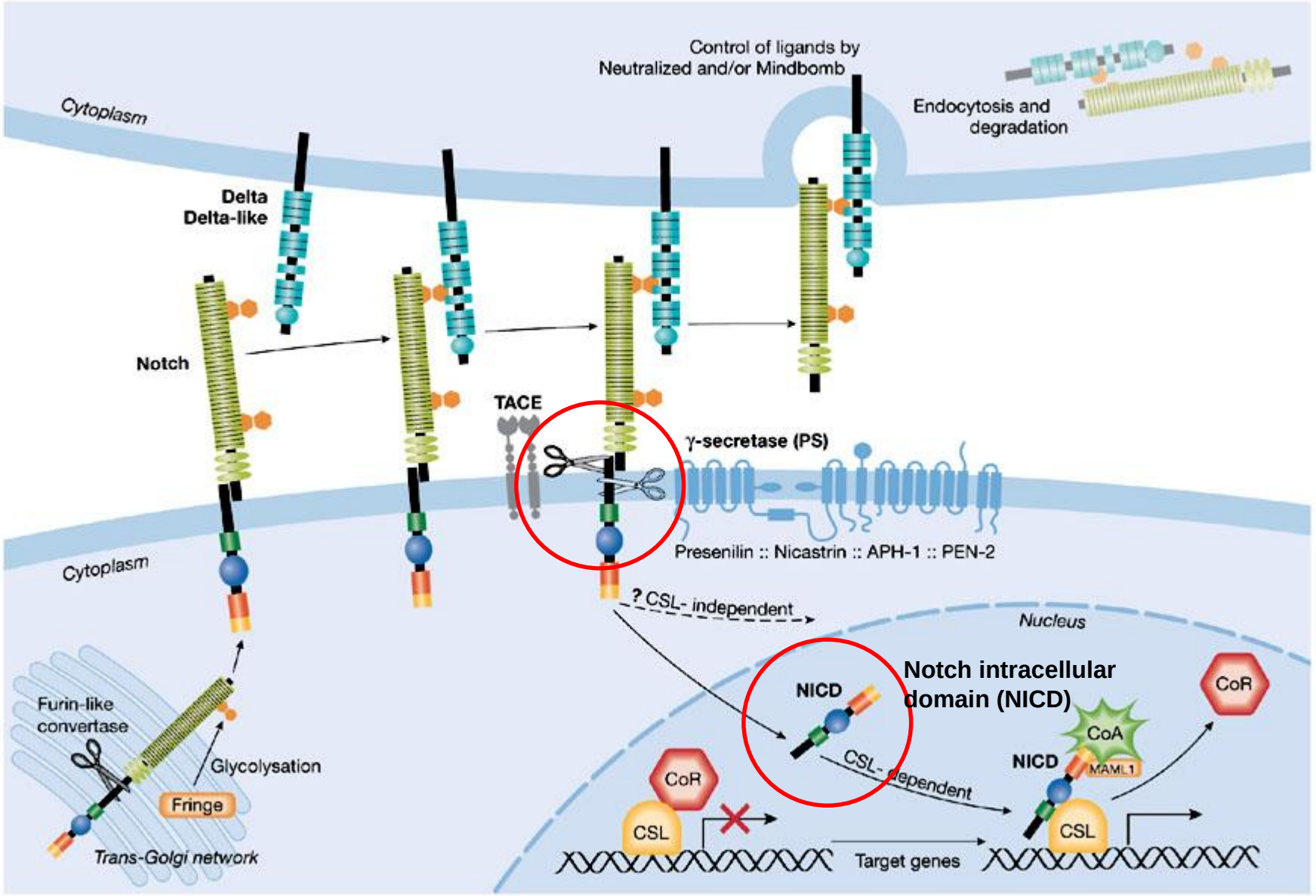
↓

Protein kinase inhibition

↓

Transcription factor stabilization

Membrane proteases in Notch signalling



EMBO reports 6, 12, 1120–1125 (2005)

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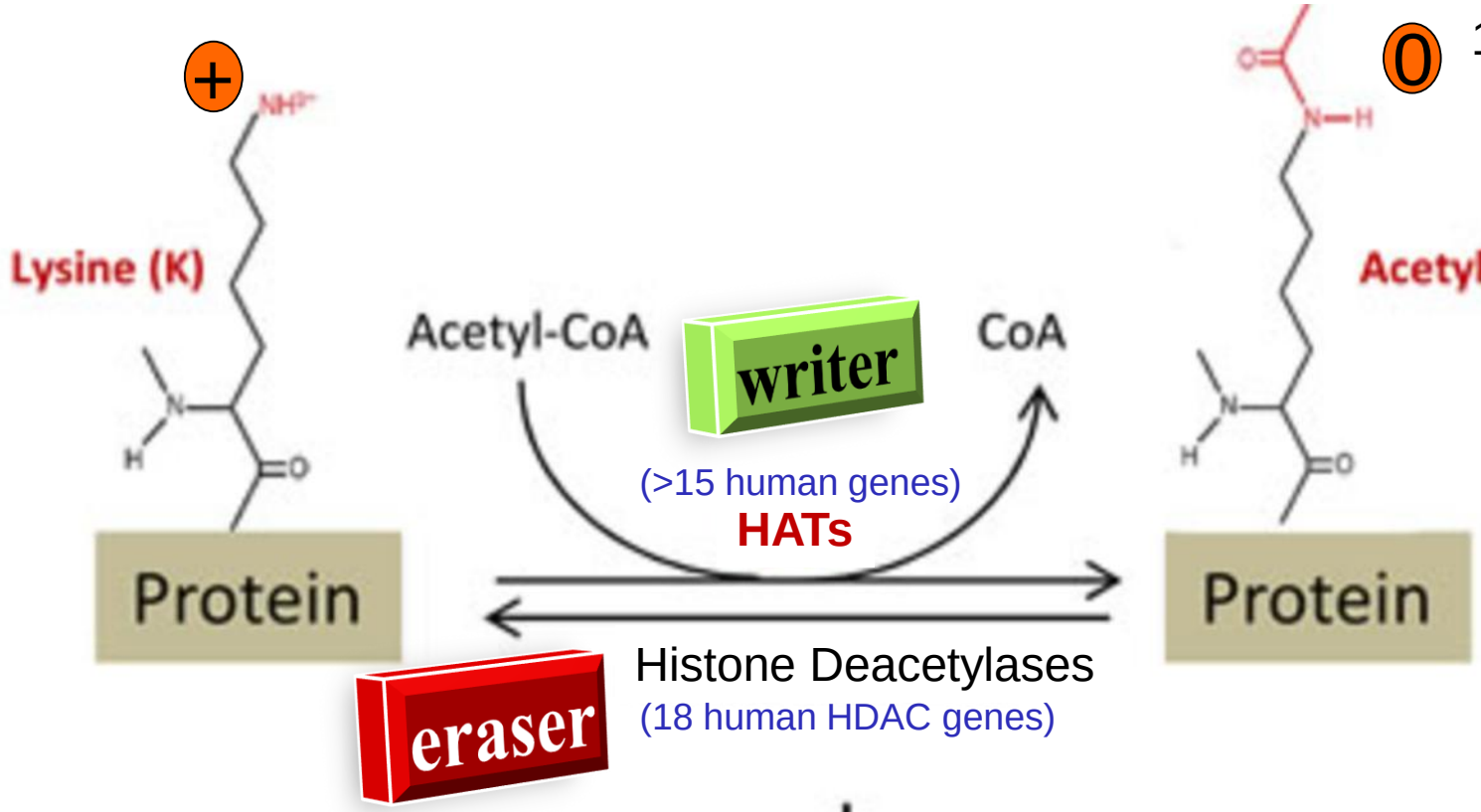
- Acetylation	- Lys
- Methylation	- Lys, Arg
- Oxidation	- Cys
- Nitrosylation	- Cys
- (…)	



1. decreases the electrostatic interaction of histones with DNA, allowing transcription

2. Recognition by bromodomain-containing proteins
(46 BRD protein genes)

reader

Epigenetic regulation of gene expression

- Regulation of chromatin higher-order structures and gene regulation
- Regulation of nucleic acid binding
- Crosstalk with other PTM
- Regulation of enzymatic activity, protein stability, protein–protein interaction, protein subcellular localization

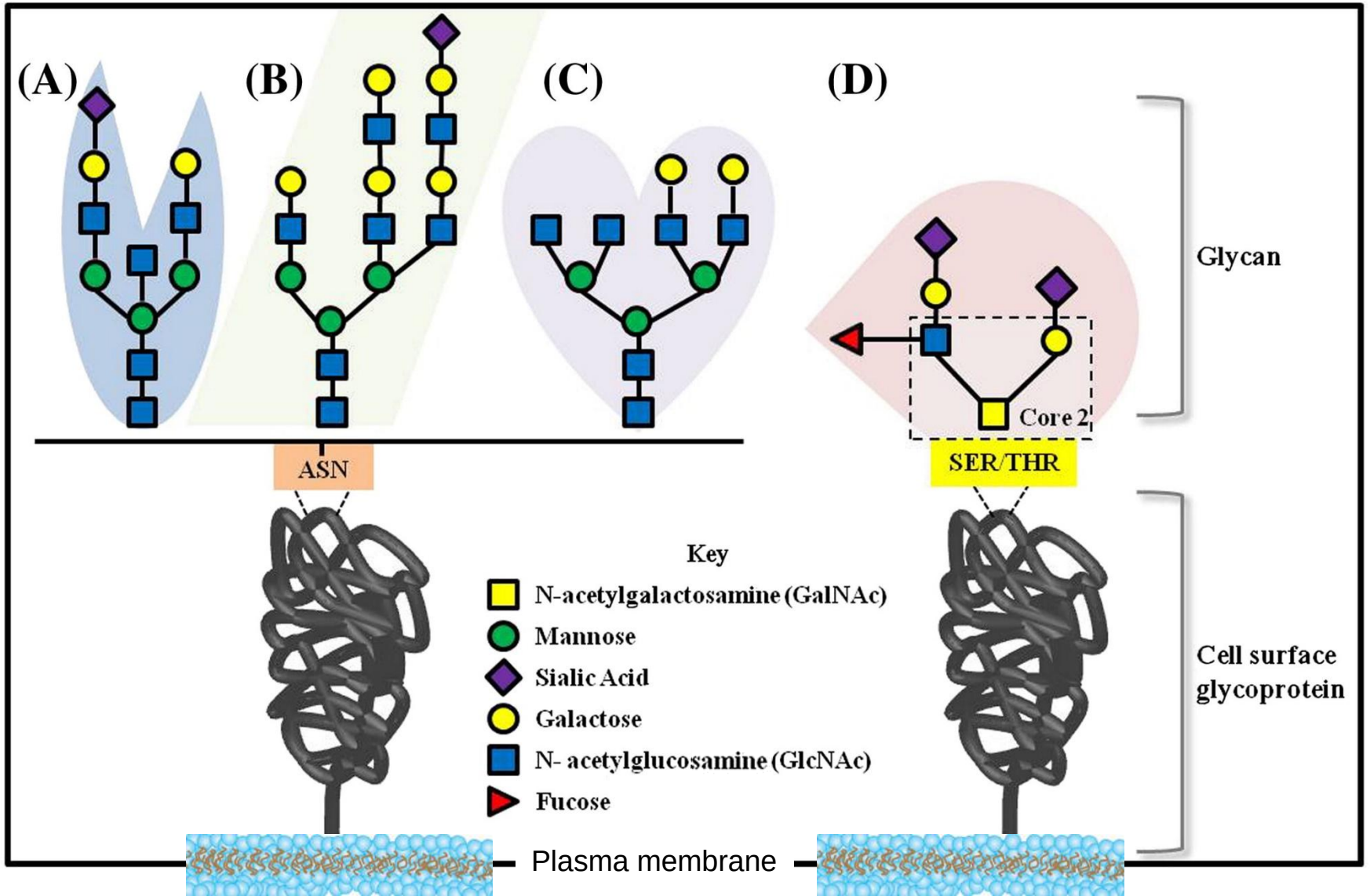
Activation and inactivation of signalling proteins by reversible post-translational modifications

- Phosphorylation	- Ser, Thr, Tyr
- O-GalNAcetylation	- Ser, Thr
- N-GlcNAcetylation	- Asn
- Sulfation	- Tyr
- Ubiquitylation	- Lys
- Acetylation	- Lys
- Methylation	- Lys, Arg
- Oxidation	- Cys
- Nitrosylation	- Cys
- (…)	

Cancer cell glycome - Changes in glycosylation of **cell surface adhesion molecules** (selectin ligands, integrins and mucins) are associated with the progression of cancers and can have prognostic implications.

- interfere with epithelial cadherin-mediated cell–cell adhesion;
- modulate tumour cell–matrix interactions;
- hide tumour-cell surface antigens from immune cells;
- regulate immune cell activation.

https://doi.org/10.1038/nrc3982- Salomé Pinho & Celso Reis



Lecture 5- Some take-home concepts

- **Besides the RAS-MAPK pathway, the PI3K/AKT pathway is another tumour-promoting pathway;**
- **Growth factor receptor-mediated activation of PI3K leads to phosphorylation of the plasma membrane lipid PI, which then is recognized by PH domain-containing proteins, including AKT;**
- **Activated AKT phosphorylates target proteins leading both, to activation of mTOR and cell growth promotion, and to inhibition of apoptosis;**
- **Oncogenic alterations leading to pathway activation include loss of PTEN and missense mutations in PIK3CA.**
- **Regulation of protein activation during cell signalling also occurs through other post-translational protein modfications, including ubiquitylation, acetylation, cysteine oxidation, or glycosylation**