Protein kinases in signal transduction- lessons from the WNK subfamily

Mestrado em Bioquímica FCUL
Cadeira ‘Regulação dos sistemas bioquímicos’
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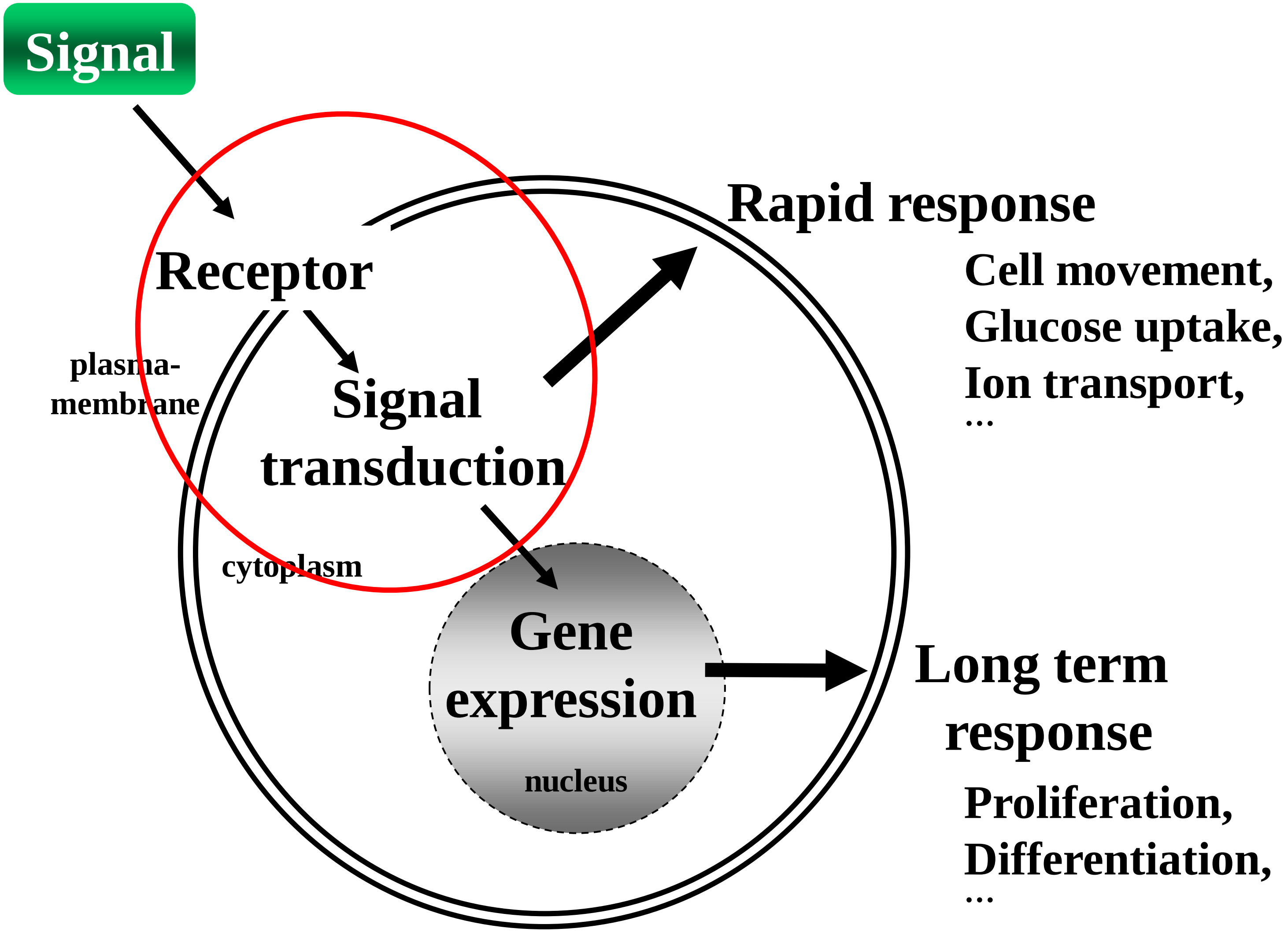
Extracellular signals and cellular response



Signal

Receptor

plasma-
membrane

Signal
transduction

cytoplasm

Gene
expression

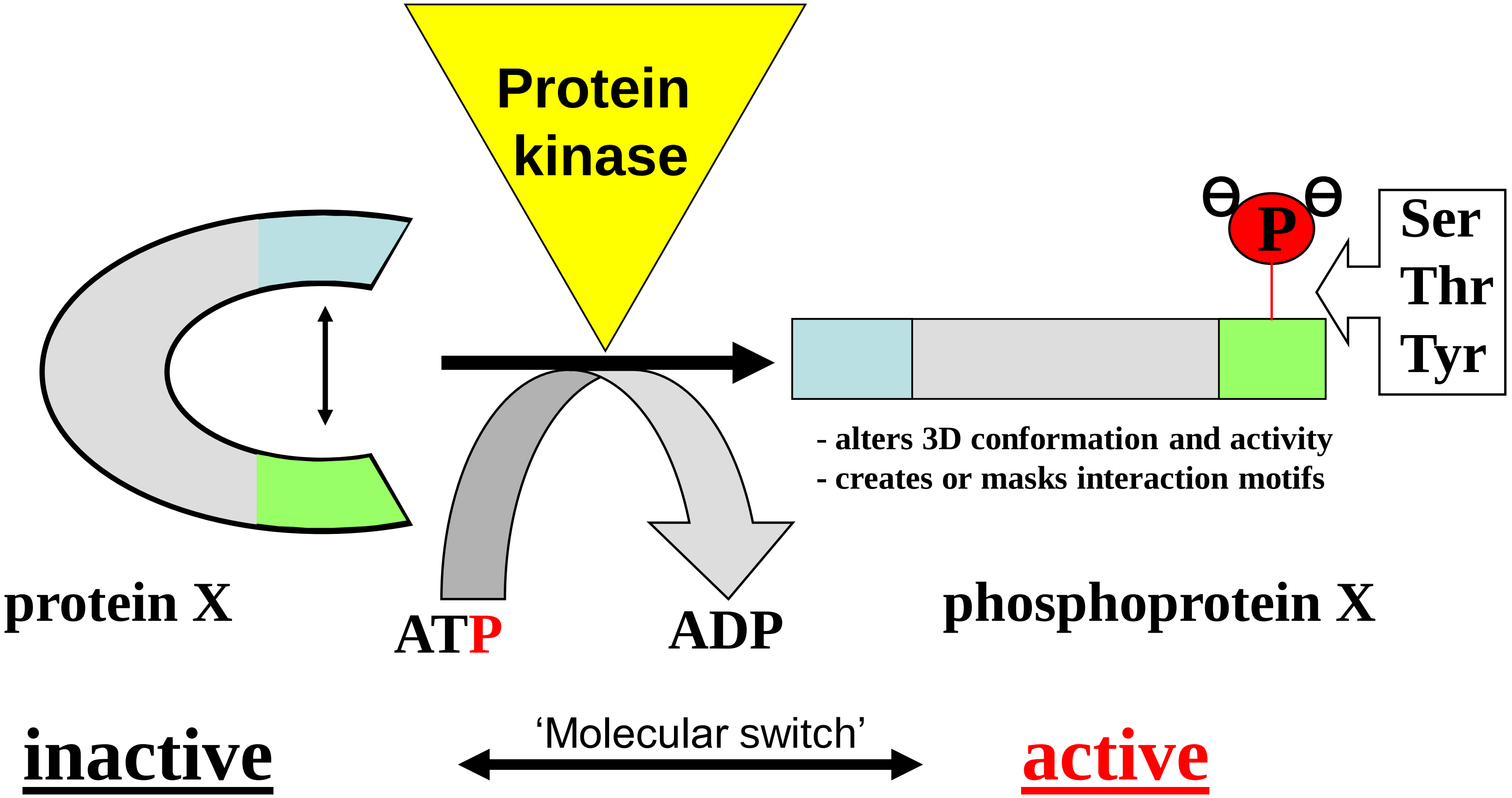
nucleus

Rapid response

Cell movement,
Glucose uptake,
Ion transport,
…

Long term
response

Proliferation,
Differentiation,
…

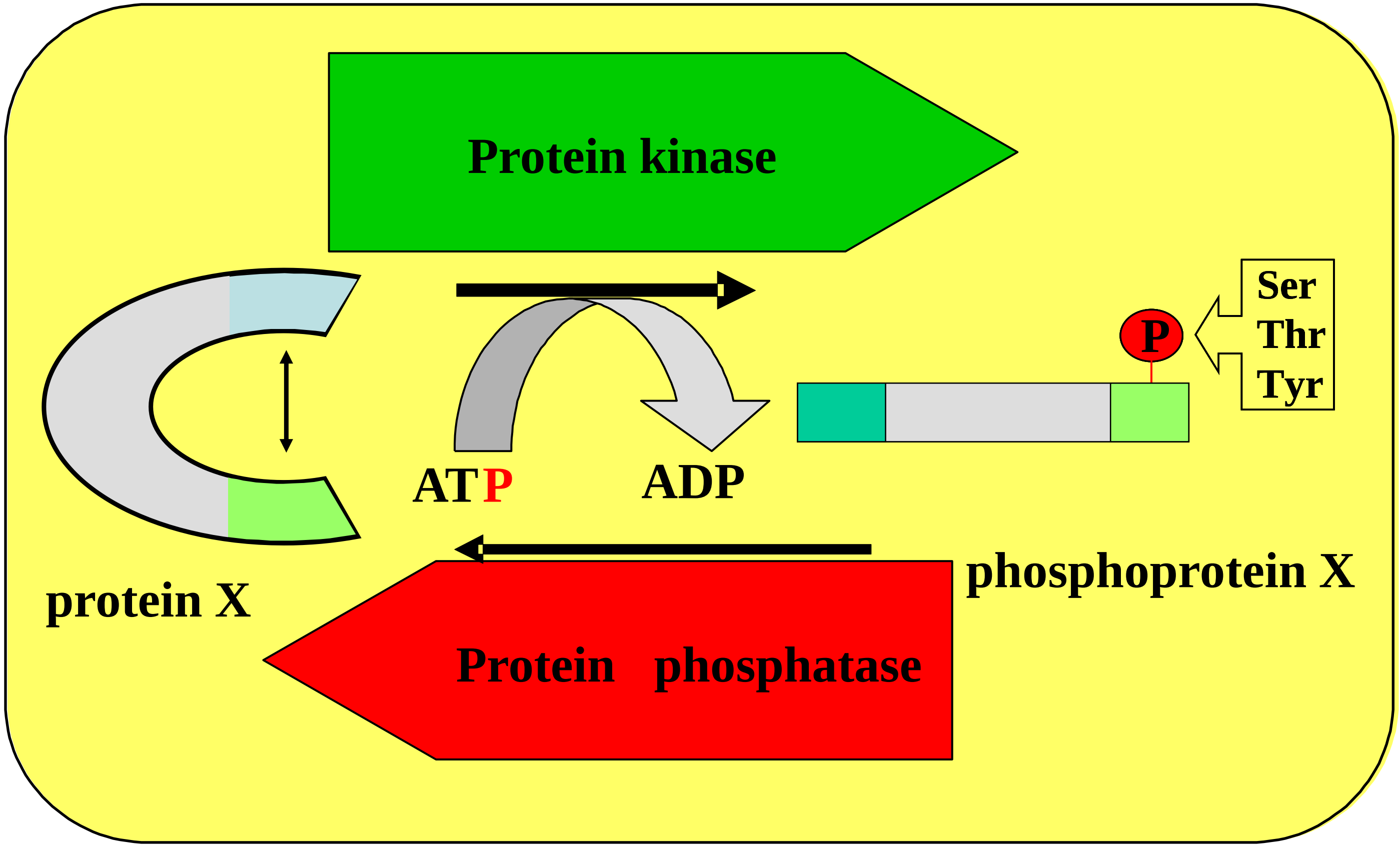


- alters 3D conformation and activity
- creates or masks interaction motifs

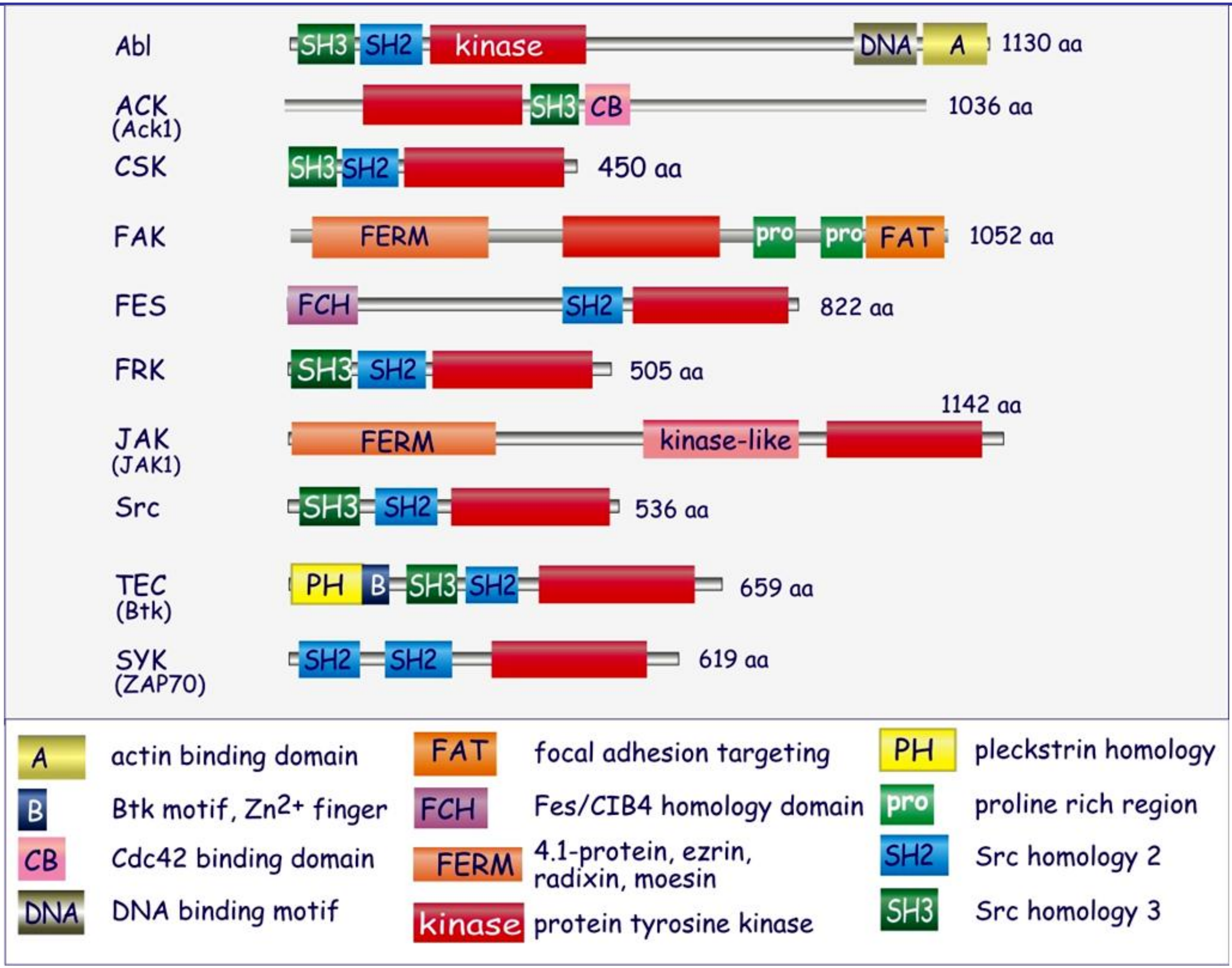
inactive ‘Molecular switch’ **active**

	-	EGF	+
pSer	89%		85%
pThr	10%		12%
pTyr	0.05%		2%

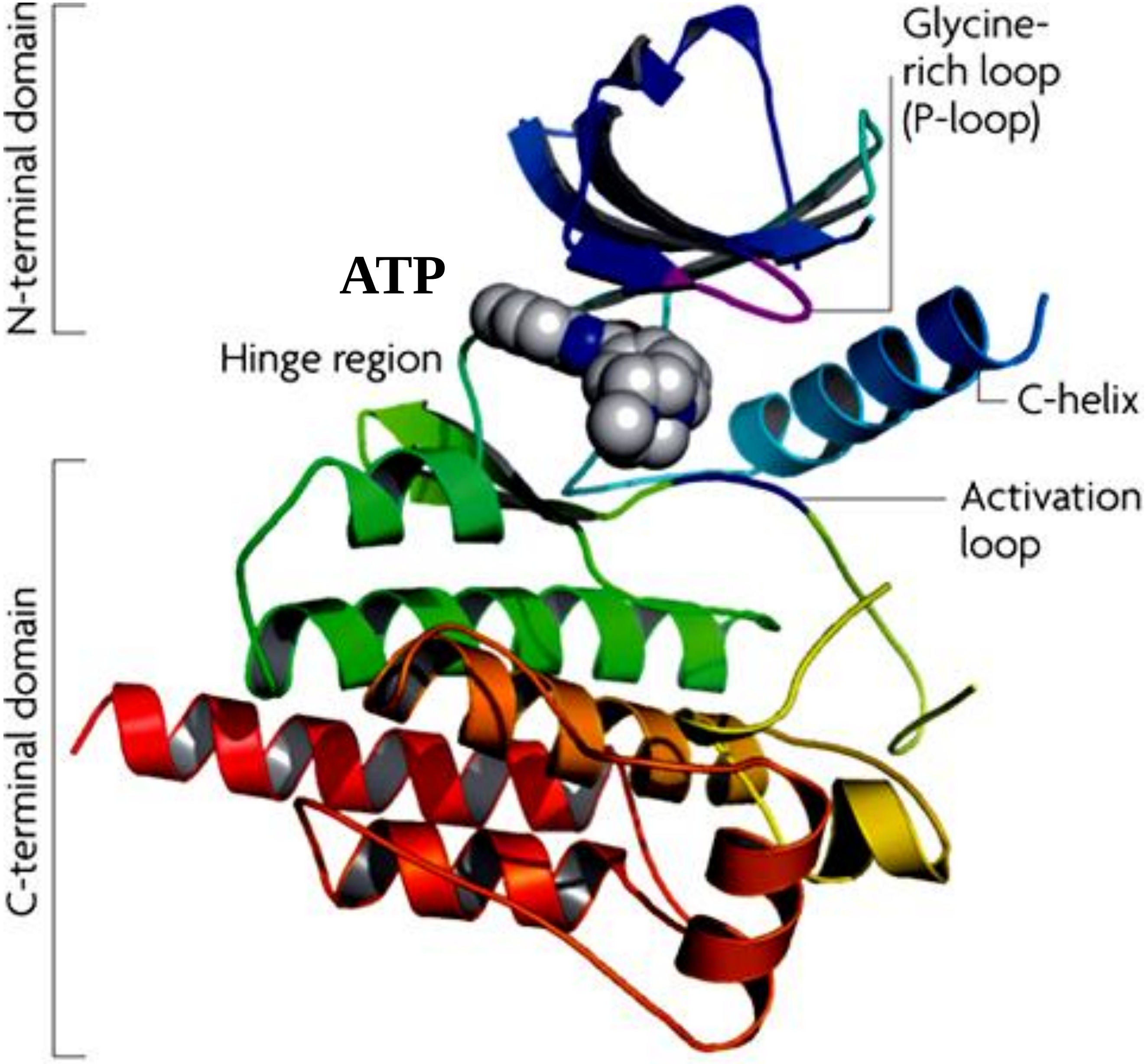
Reversible protein phosphorylation



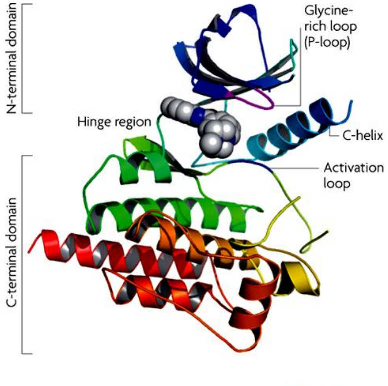
Protein kinase= catalytic domain + remaining protein



Structure of the catalytic kinase domain

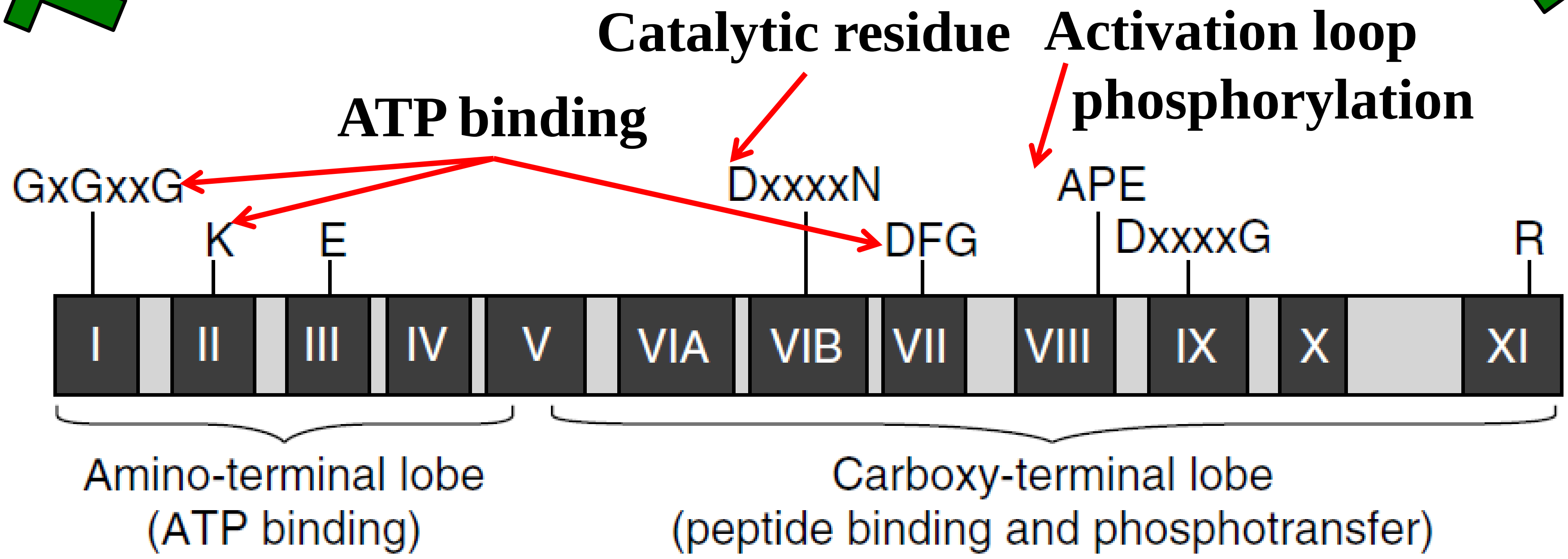


Nature Reviews | Drug Discovery



Nature Reviews | Drug Discovery

The catalytic domain


Catalytic residue

Activation loop phosphorylation

ATP binding

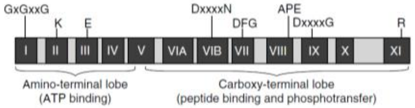
Amino-terminal lobe
(ATP binding)

Carboxy-terminal lobe
(peptide binding and phosphotransfer)

Hanks SK (2003) Genome Biology 4 (5), Article 111

The human kinome

518
protein kinase genes



478 ePKs
conserved eucaryotic domain

40 aPKs
atypical catalytic domain

50 Pseudo-ePK kinases lacking conserved residues

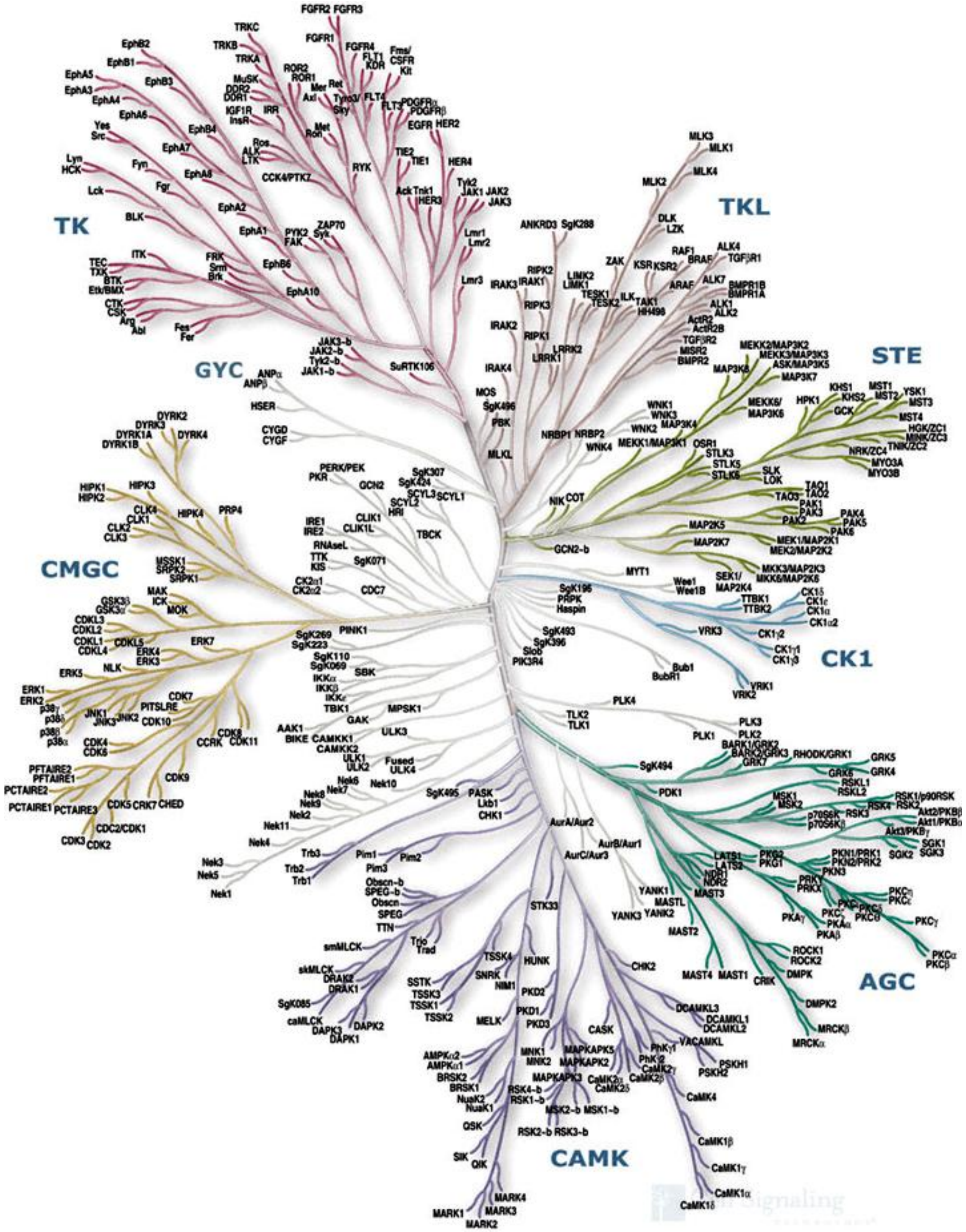
428 ePKs with known or likely kinase activty

8 subgroups:
TK- 84; CAMK- 66; AGC- 61; CMGC- 61;
STE- 45; TKL- 37; CK1- 11; **Other- 63**

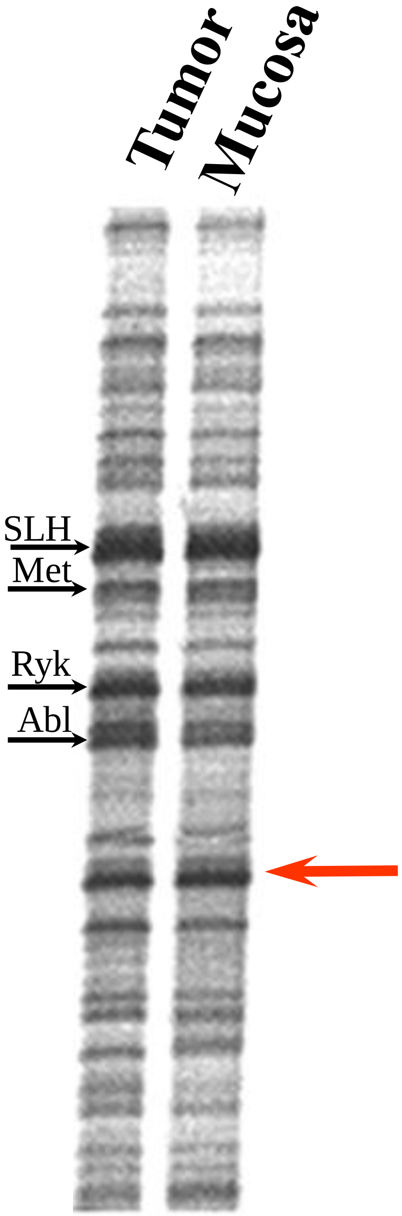
Hanks SK (2003) Genome Biology 4 (5), Article 111

The human kinome tree:

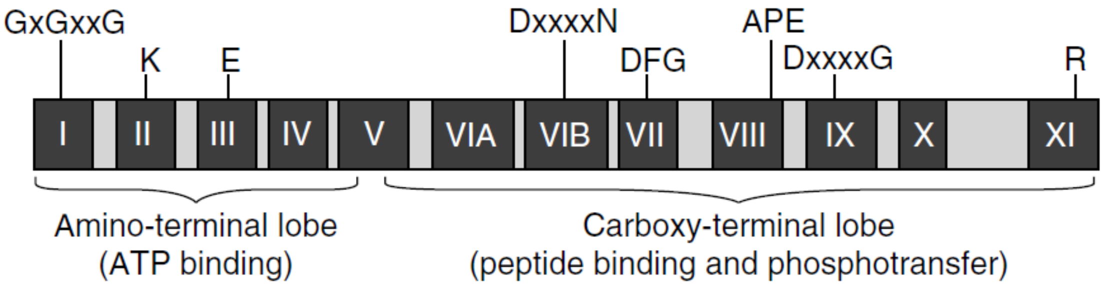
clustering by sequence similarity in the kinase domain led to identification of different subfamilies (incl. 60 receptor kinases)



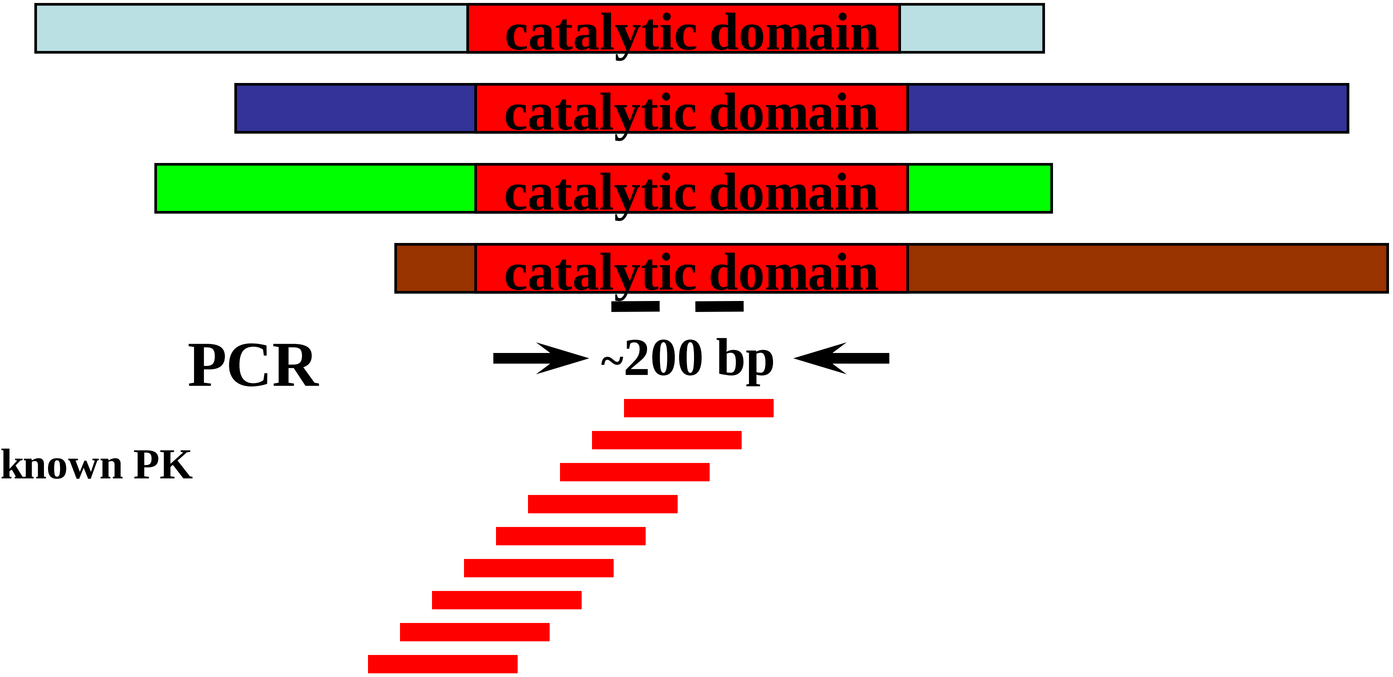
Manning et al. (2002) Science 298, 1912-1934; Hanks (2003) Genome Biol 4: 111

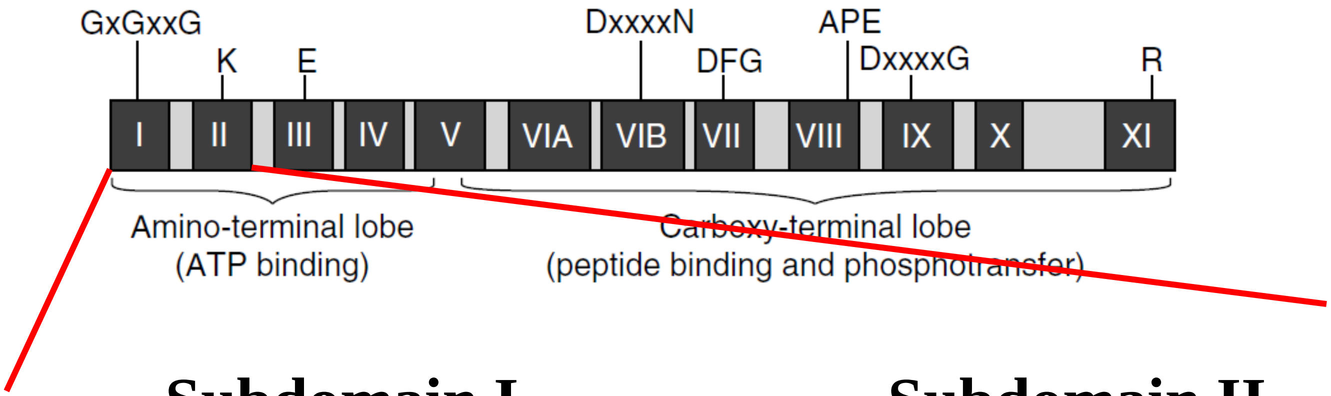


unknown PK



Superfamily of protein kinases





Subdomain I **Subdomain II**

typical catalytic lysine (Subdomain II)

glycine flap

ePK motif: **GXGXXGXV**xxxxxxxxxx**VAIK**xxx

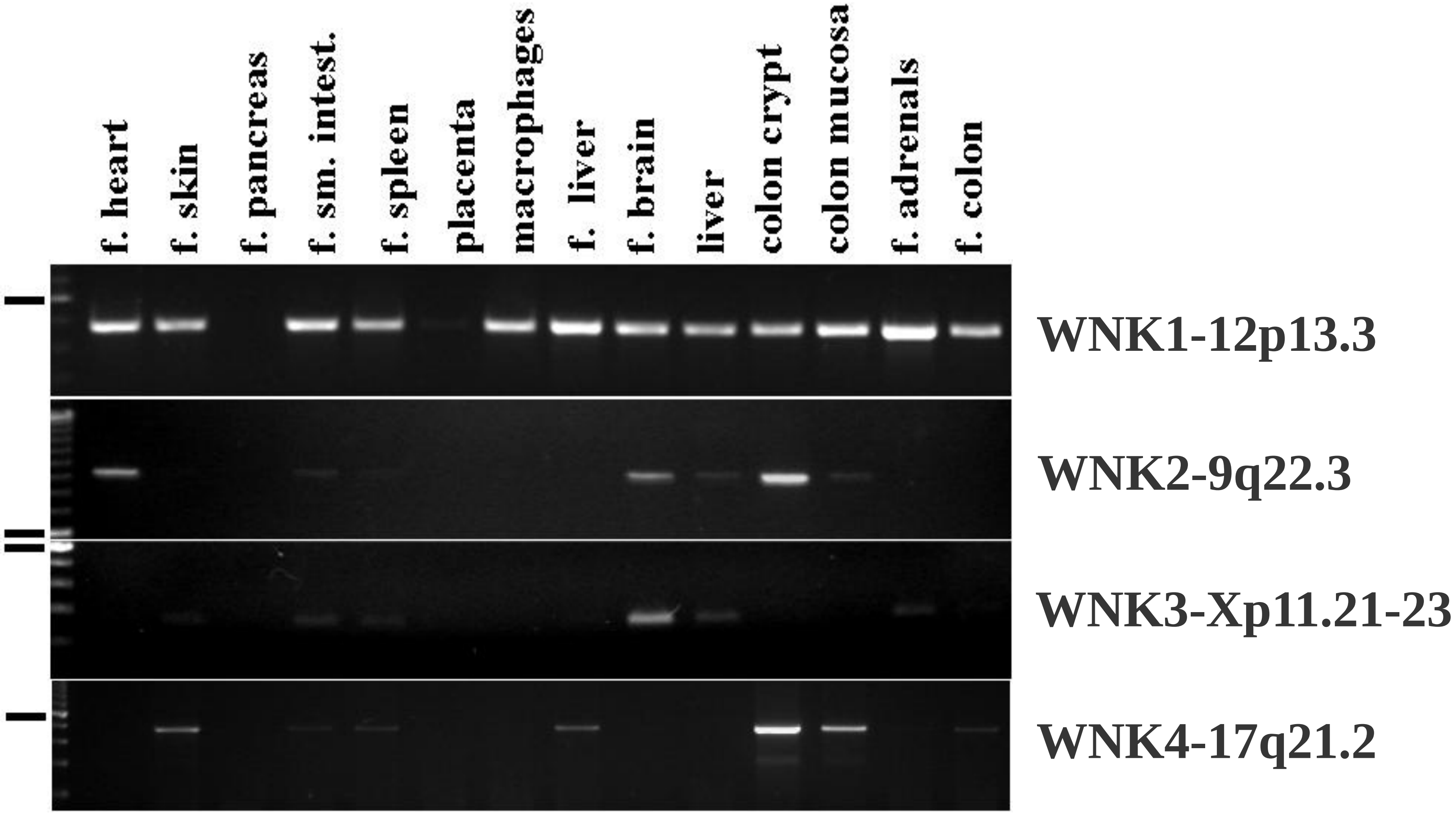
WNK motif: **GXGXFKXV**xxxxxxxxxx**VAWC**xxx

Distinct catalytic lysine in WNKs (Subdomain I)

→ **WNK: With no K (=lysine)**

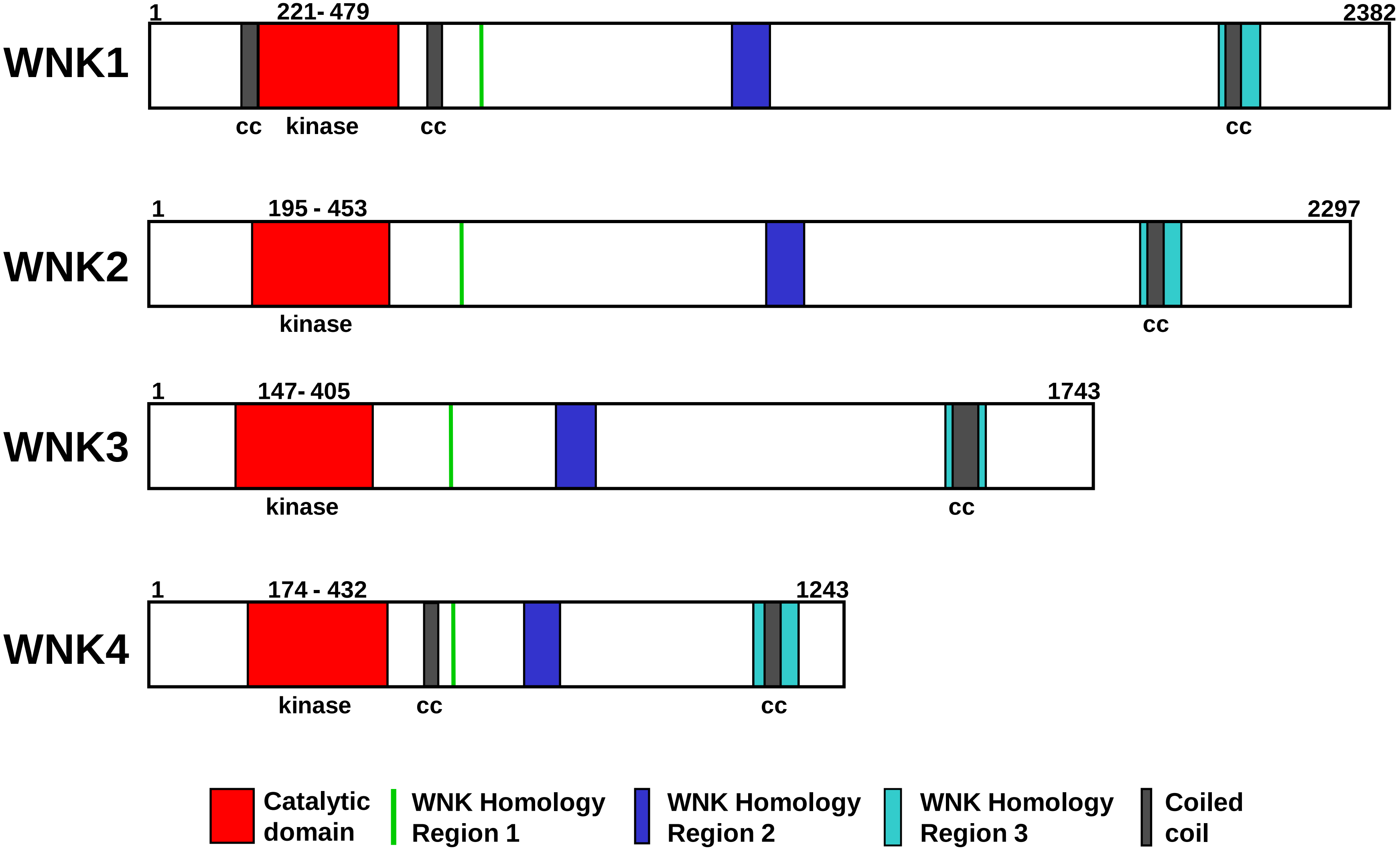
Min et al (2004) Structure 12: 1303-11

Four human *WNK* genes exist and differ in expression



Veríssimo and Jordan (2001) Oncogene 20

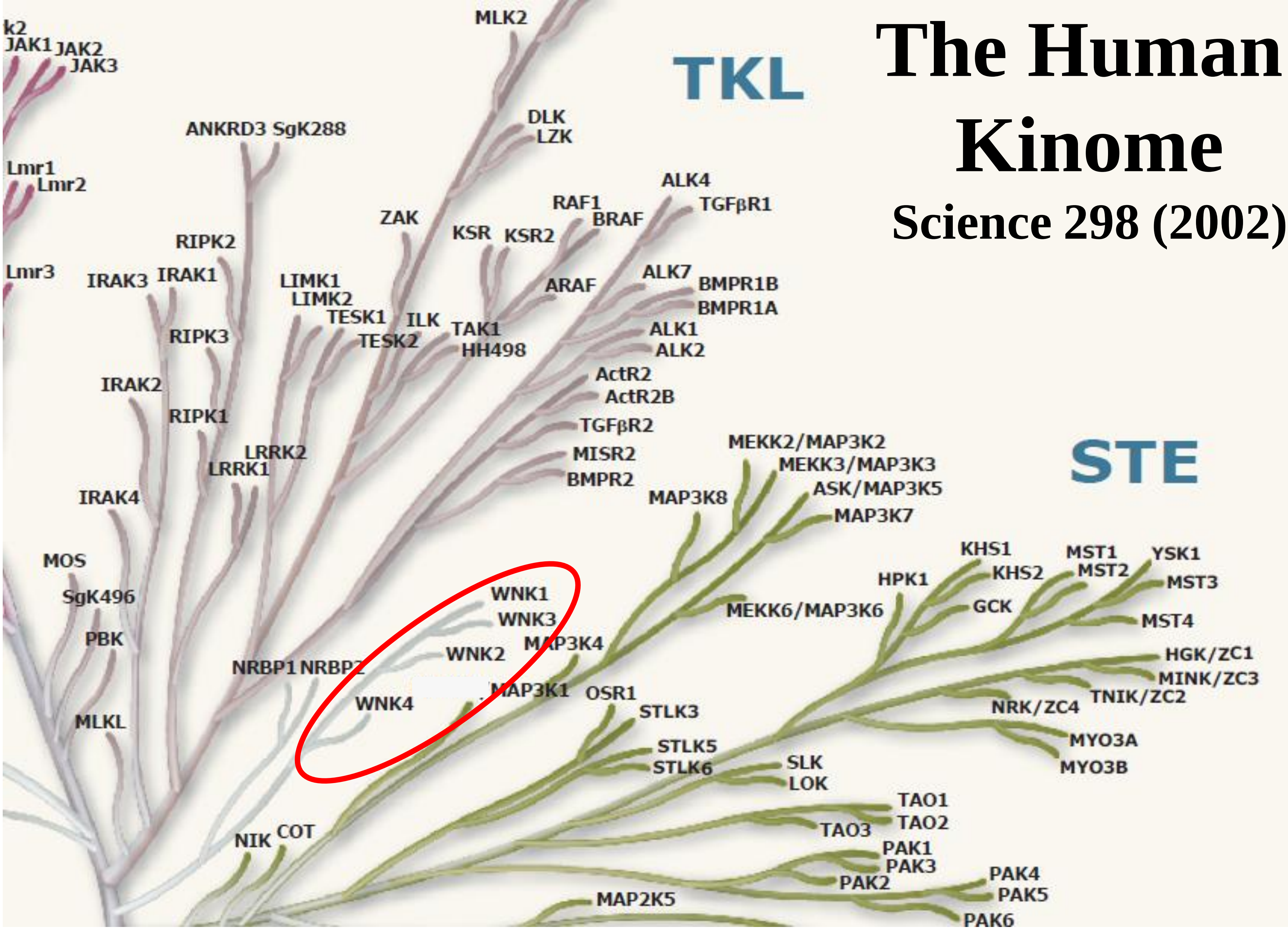
The mammalian WNK subfamily



Moniz and Jordan (2010) Cell. Mol. Life Sci. 67

The Human Kinome

Science 298 (2002)



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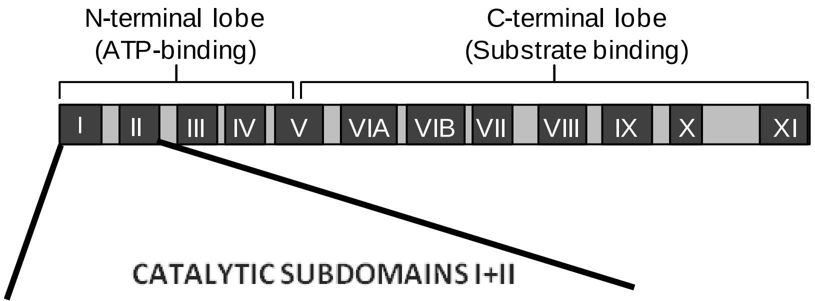
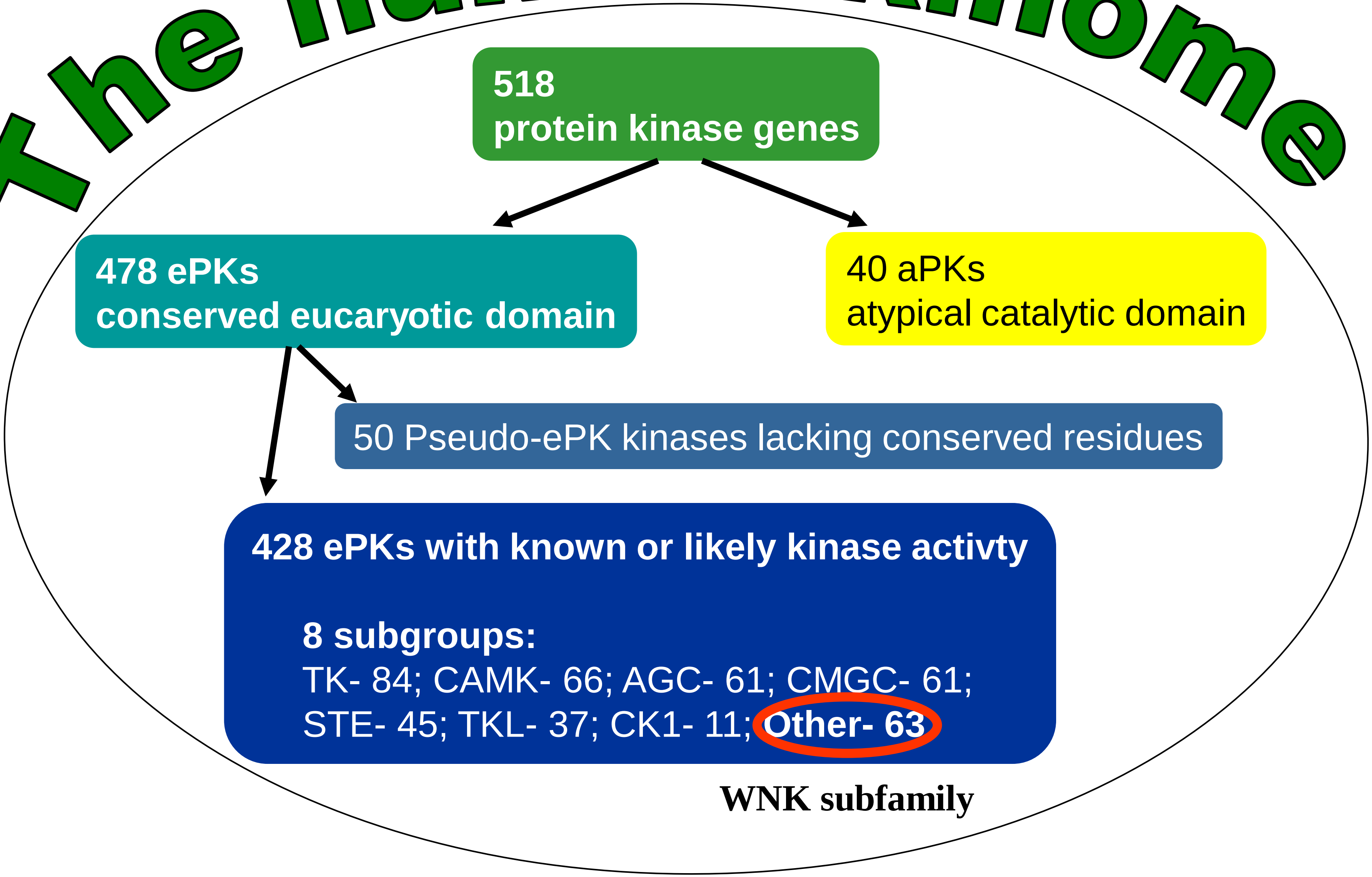
(2001) Oncogene 20



GENE	CATALYTIC SUBDOMAINS I+II	GENE ID
hs WNK1	LKFDIEIGRGSFKTVYKGLDTETTVEVAWCELQ	65125
hs WNK2	LKFDIELGRGSFKTVYKGLDTETWVEVAWCELQ	65268
hs WNK3	LKFDIELGRGAFKTVYKGLDTETWVEVAWCELQ	65267
hs WNK4	LKFDIEIGRGSFKTVYRGLDTDTTVEVAWCELQ	65266
mm WNK1	LKFDIEIGRGSFKTVYKGLDTETTVEVAWCELQ	232341
mm WNK2	LKFDIELGRGSFKTVYKGLDTETWVEVAWCELQ	75607
mm WNK3	LKFDIELGRGAFKTVYKGLDTETWVEVAWCELQ	279561
mm WNK4	LKFDIEIGRGSFKTVYRGLDTDTTVEVAWCELQ	69847
at WNK1	GRYNEVLGKGASKTVYRAFDEYEGIEVAWNQVK	819651
at WNK2	GRYDEILGKGASKTVYRAFDEYEGIEVAWNQVK	821810
at WNK3	GRYKEVLGKGAFKEVYRAFDQLEGIEVAWNQVK	823984
at WNK4	GRFAEILGRGAMKTVYKAIDEKLGIEVAWSQVK	835947
at WNK5	GRFREVLGKGAMKTVYKAFDQVLGMEVAWNQVK	824326
at WNK6	IRYKEVIGKGAFKTVYKAFDEVDGIEVAWNQVR	821406
at WNK7	IRYKEVIGKGASKTVFKGFDEVDGIEVAWNQVR	841339
at WNK8	IRYDDVLGRGAFKTVYKAFDEVDGIEVAWNLVS	834204
at WNK9	GRYNEVLGKGSSKTVYRGFDEYQGIEVAWNQVK	832881
rn WNK1	LKFDIEIGRGSFKTVYKGLDTETTVEVAWCELQ	116477
pt WNK1	LKFDIEIGRGSFKTVYKGLDTETTVEVAWCELQ	451739
clf WNK1	LKFDIEIGRGSFKTVYRGLDTDTTVEVAWCELQ	477728
gg WNK1	LKFDIEIGRGSFKTVYKGLDTDTTVEVAWCELQ	427925
dm CG7177	FKYDKEVGRGSFKTVYRGLDTLTGVPVAWCELL	40391
ce C46C2.1	LKFDEELGRGSFKTVFRGLDTETGVAVAWCELQ	177743
Invariant	G G K V D VAW	**WNK signature sequence**

The human kinome



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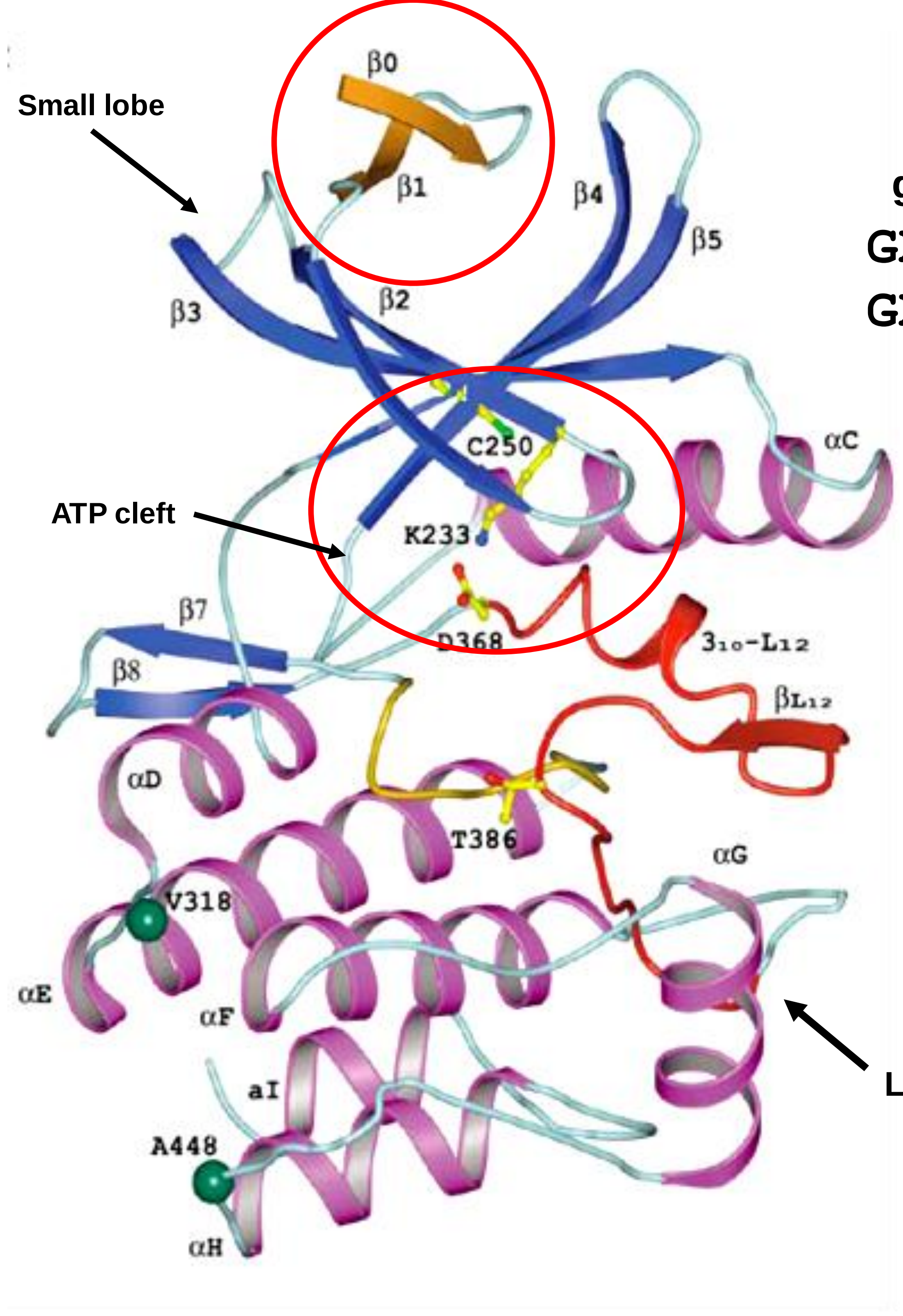
WNK subfamily

Hanks SK (2003) Genome Biology 4 (5), Article 111



typical catalytic lysine
(Subdomain II)

glycine flap

GXGXXGXVxxxxxxxxxx**VAIK**xxx

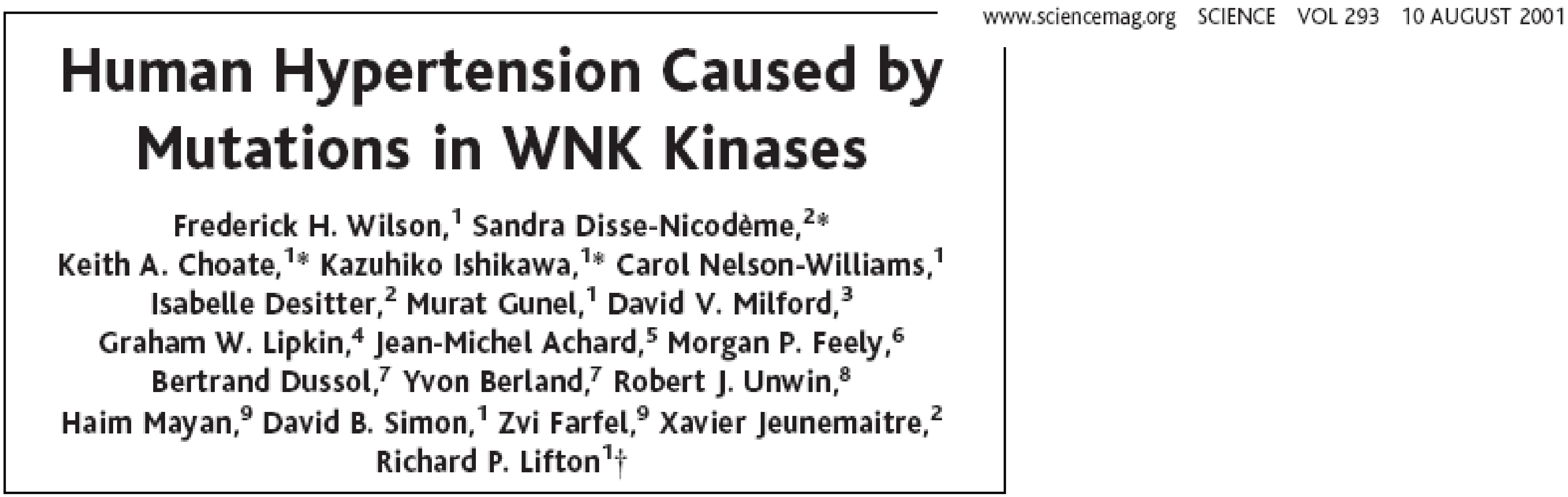
GXGXFKXVxxxxxxxxxx**VAWC**xxx

Distinct catalytic lysine in WNKs
(Subdomain I)

WNK1 crystal structure

(Min et al., 2004, Structure 12)

Large lobe



www.sciencemag.org SCIENCE VOL 293 10 AUGUST 2001

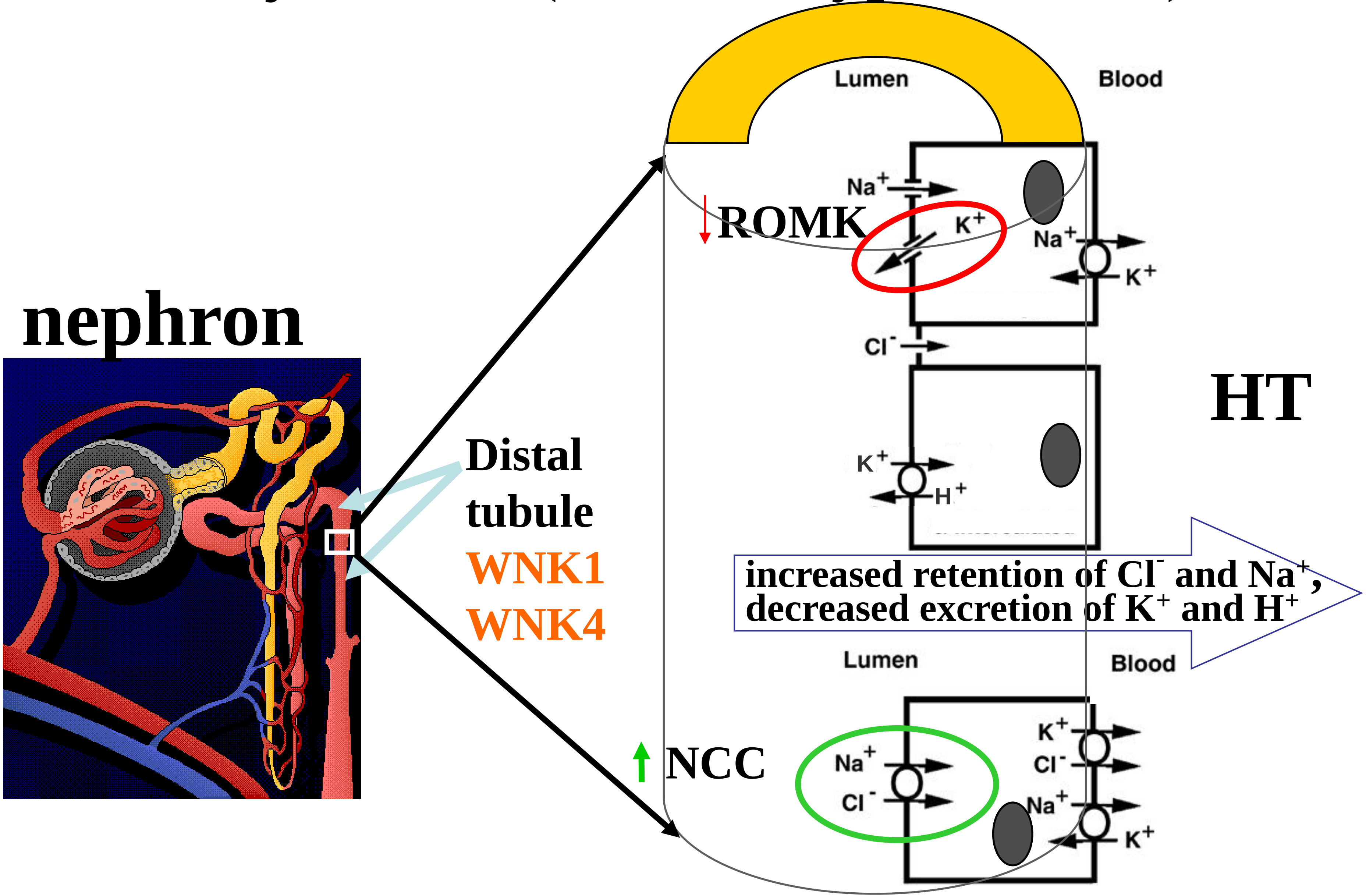
Human Hypertension Caused by Mutations in WNK Kinases

Frederick H. Wilson,[1] Sandra Disse-Nicodème,[2]*
Keith A. Choate,[1]* Kazuhiko Ishikawa,[1]* Carol Nelson-Williams,[1]
Isabelle Desitter,[2] Murat Gunel,[1] David V. Milford,[3]
Graham W. Lipkin,[4] Jean-Michel Achard,[5] Morgan P. Feely,[6]
Bertrand Dussol,[7] Yvon Berland,[7] Robert J. Unwin,[8]
Haim Mayan,[9] David B. Simon,[1] Zvi Farfel,[9] Xavier Jeunemaitre,[2]
Richard P. Lifton[1]†

The Gordon syndrome

- **or pseudo-hypo-aldosteronism type II (PHA II)**
- **hypertension↑, salt retention↑, potassium secretion↓**
- **familial disease, autosomal dominant**
- **germline mutations in the *WNK1* or *WNK4* genes**

Mutations in the *WNK1* and *WNK4* genes cause Gordon syndrome (familial hypertension)



nephron

Distal tubule

WNK1

WNK4

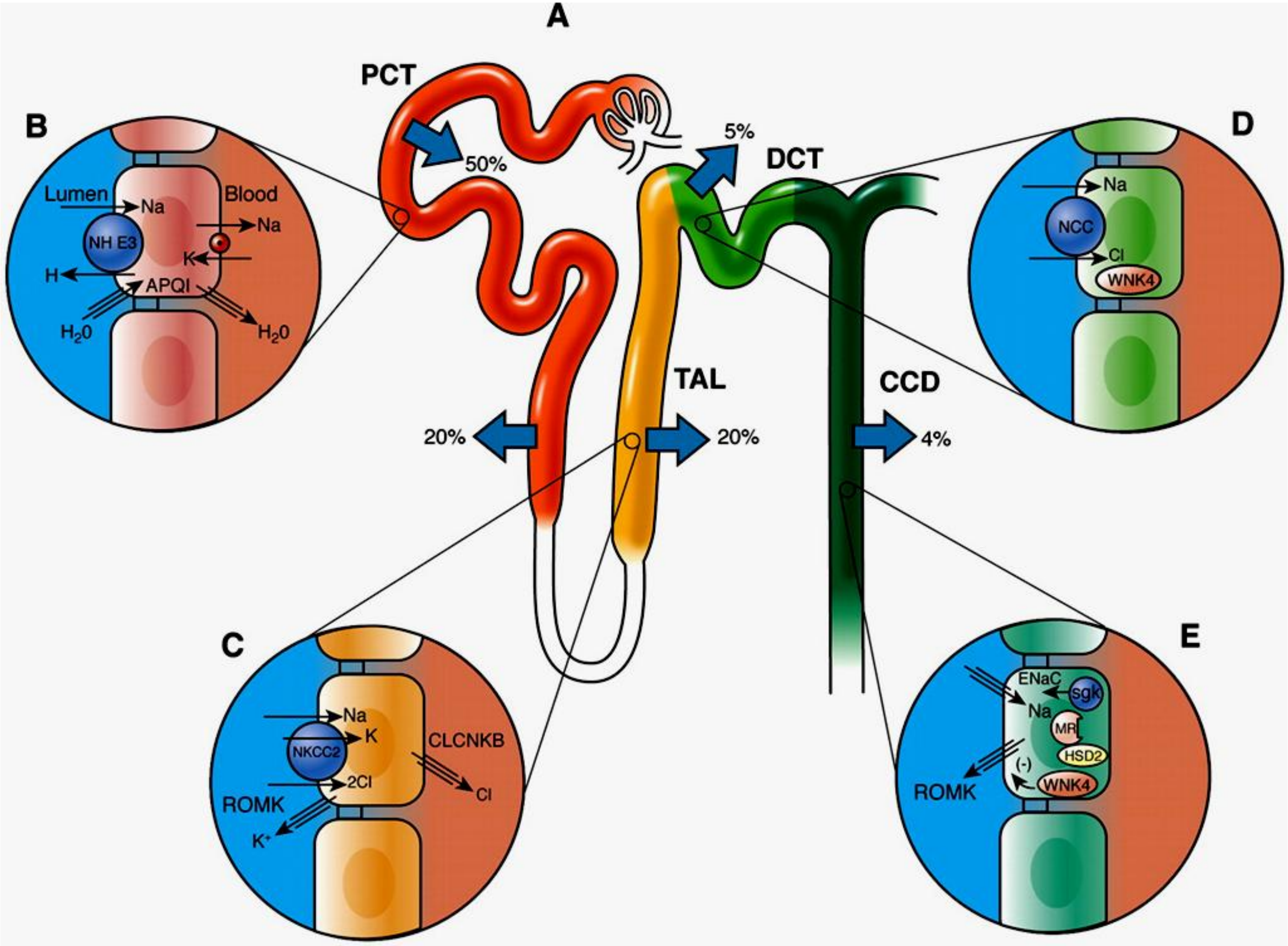
↓ROMK

↑NCC

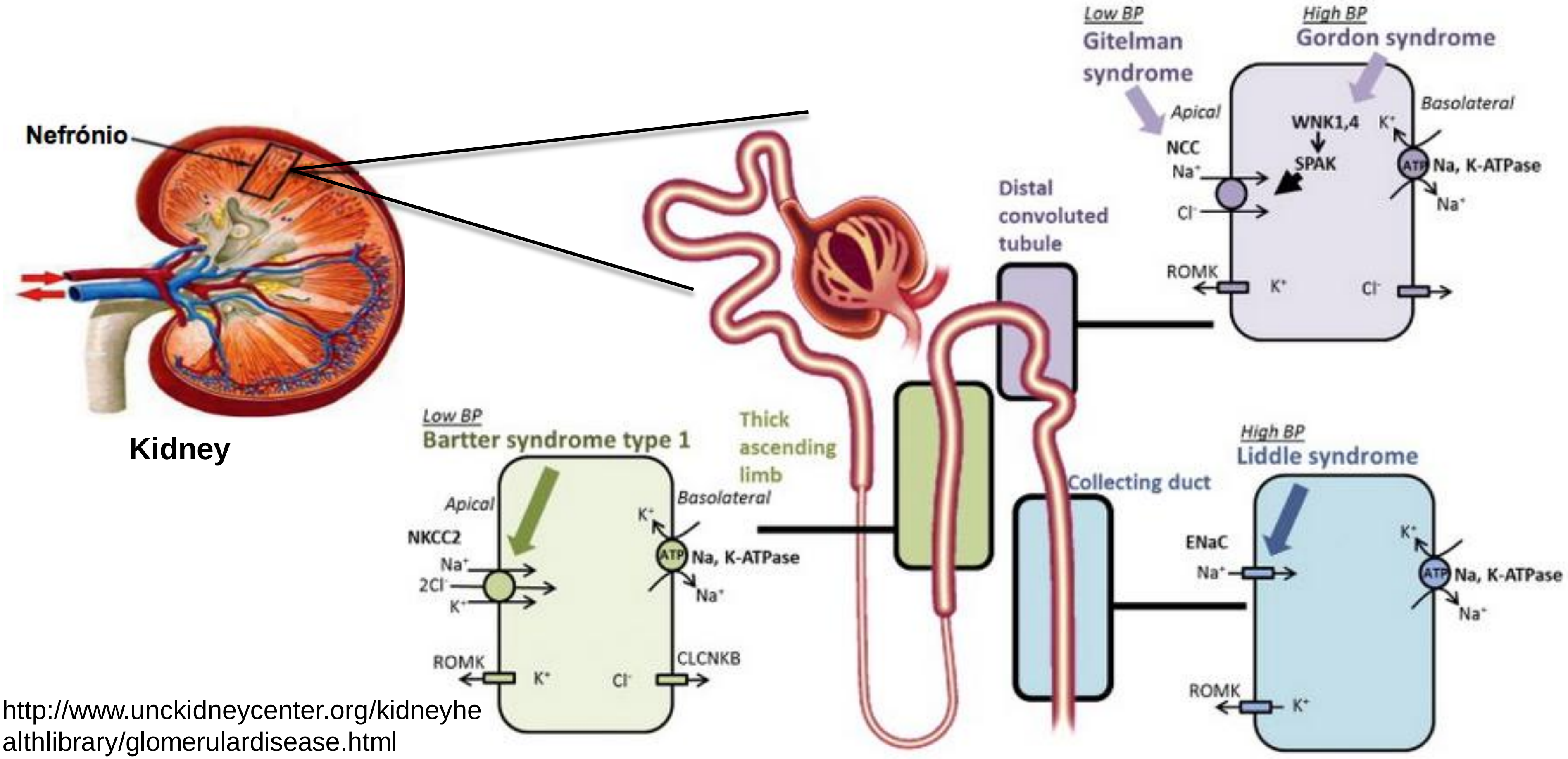
HT

increased retention of Cl^- and Na^+, decreased excretion of K^+ and H^+

The role of nephron segments in sodium retention



Rare genetic diseases affecting the renal regulation of blood pressure

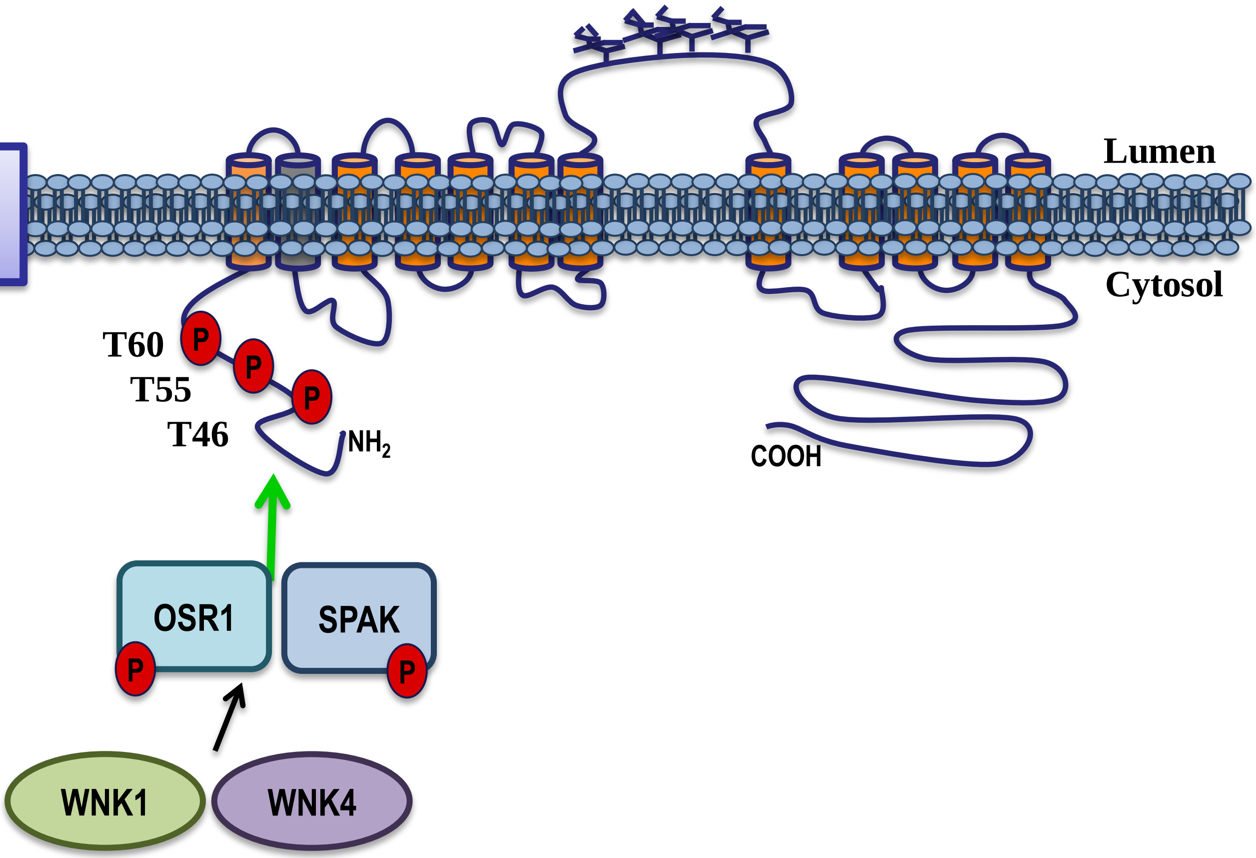


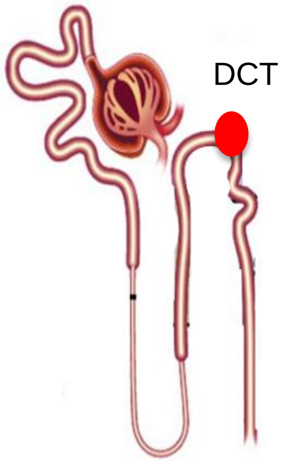
http://www.unckidneycenter.org/kidneyhealthlibrary/glomerulardisease.html

Chen & Coffman (2012) Canadian Journal of Cardiology

Regulation of renal NCC sodium/chloride co-transport by phosphorylation

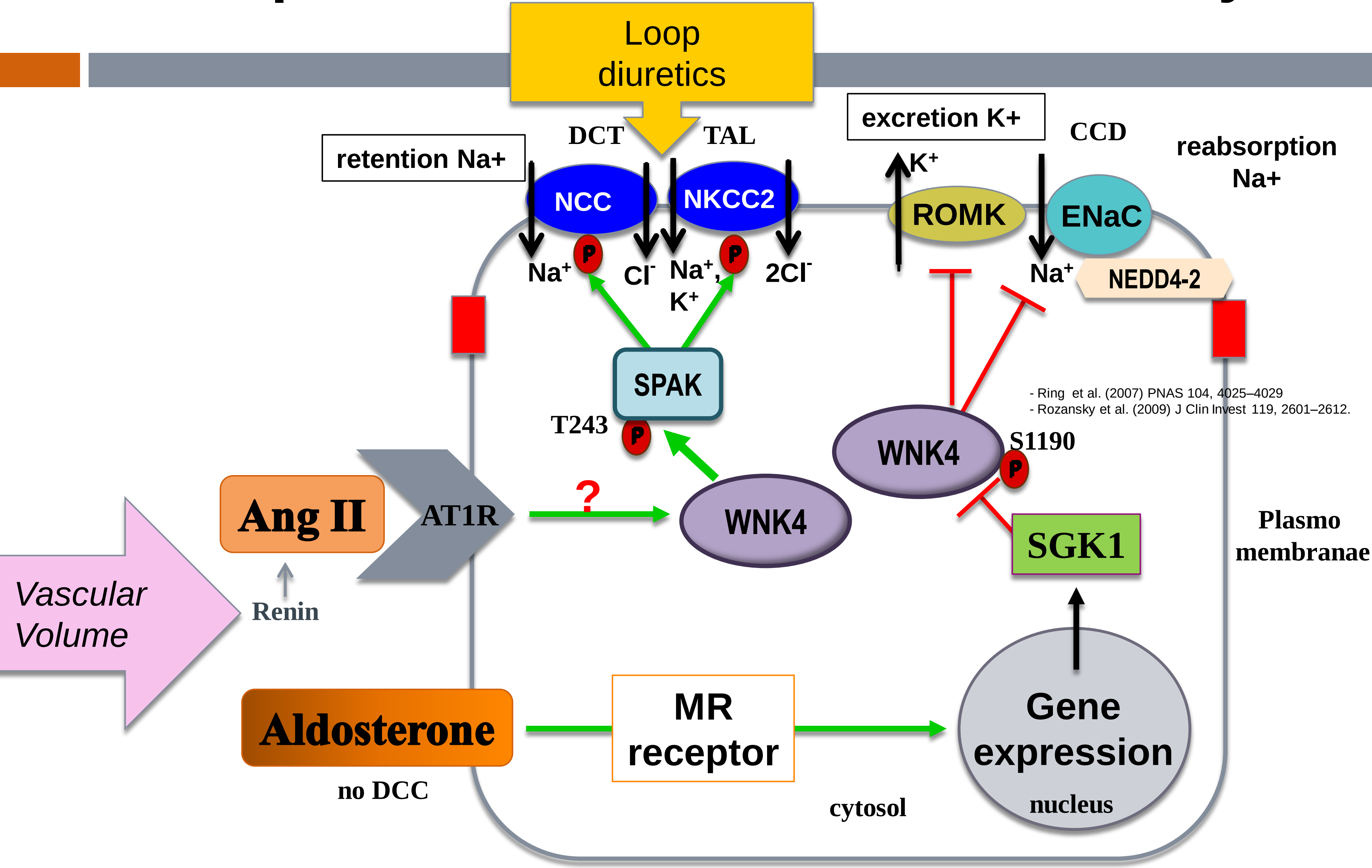
Renal cotransporter
NCC





DCT= distal cortical tubule;
regulates ~5% of total sodium resorption

Molecular regulation of sodium retention and potassium excretion in the kidney



Loop diuretics

retention Na+

DCT

TAL

NCC

NKCC2

Na^+

Cl^-

Na^+, K^+

$2Cl^-$

excretion K+

CCD

reabsorption Na+

K^+

ROMK

ENaC

Na^+

NEDD4-2

SPAK

T243

WNK4

S1190

SGK1

- Ring et al. (2007) PNAS 104, 4025–4029
- Rozansky et al. (2009) J Clin Invest 119, 2601–2612.

Ang II

AT1R

?

WNK4

Renin

Vascular Volume

Plasmo membranae

Aldosterone

MR receptor

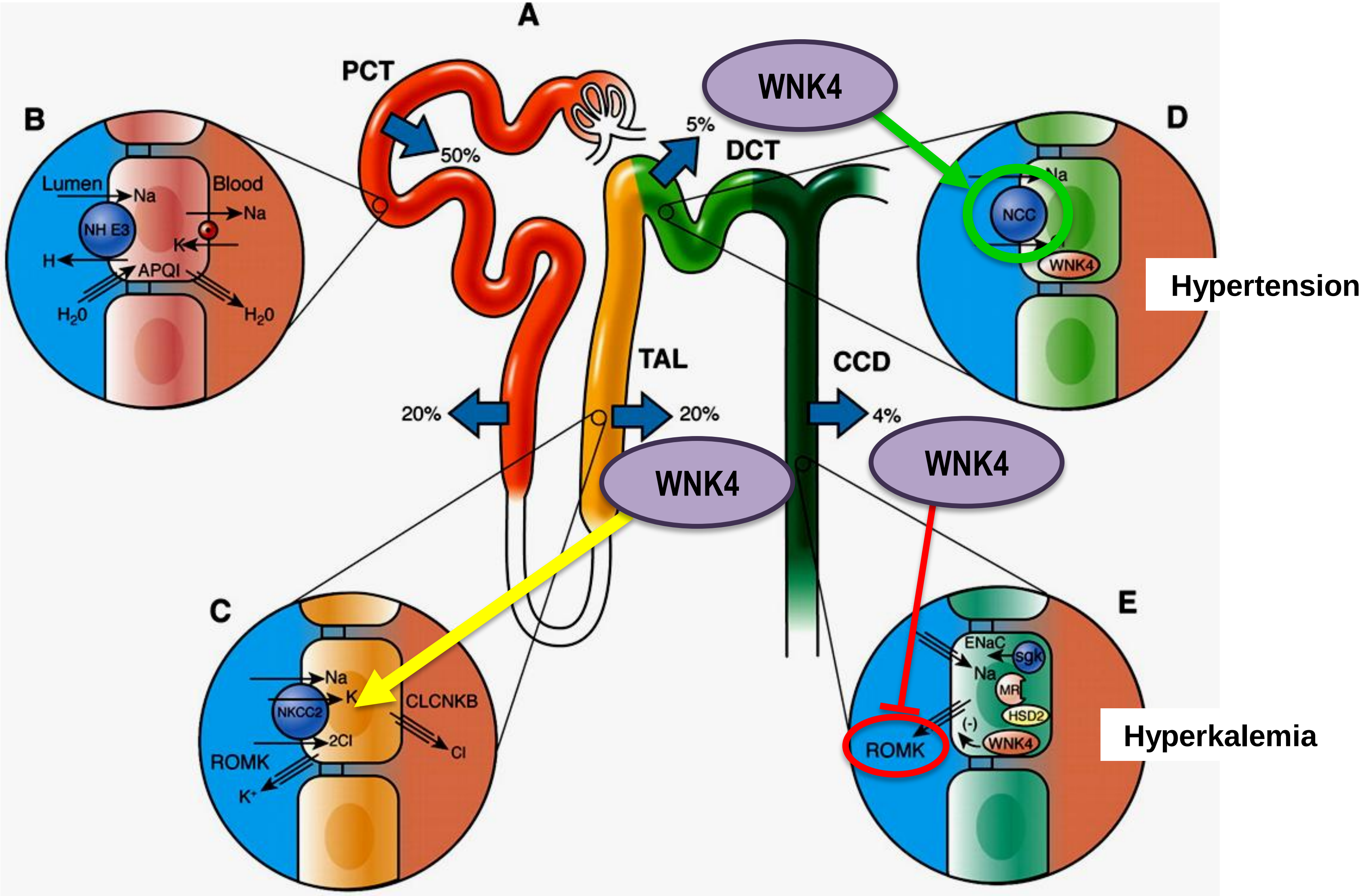
Gene expression

nucleus

no DCC

cytosol

The role of nephron segments in sodium retention



Experimental evidence for WNK-regulated ion transport

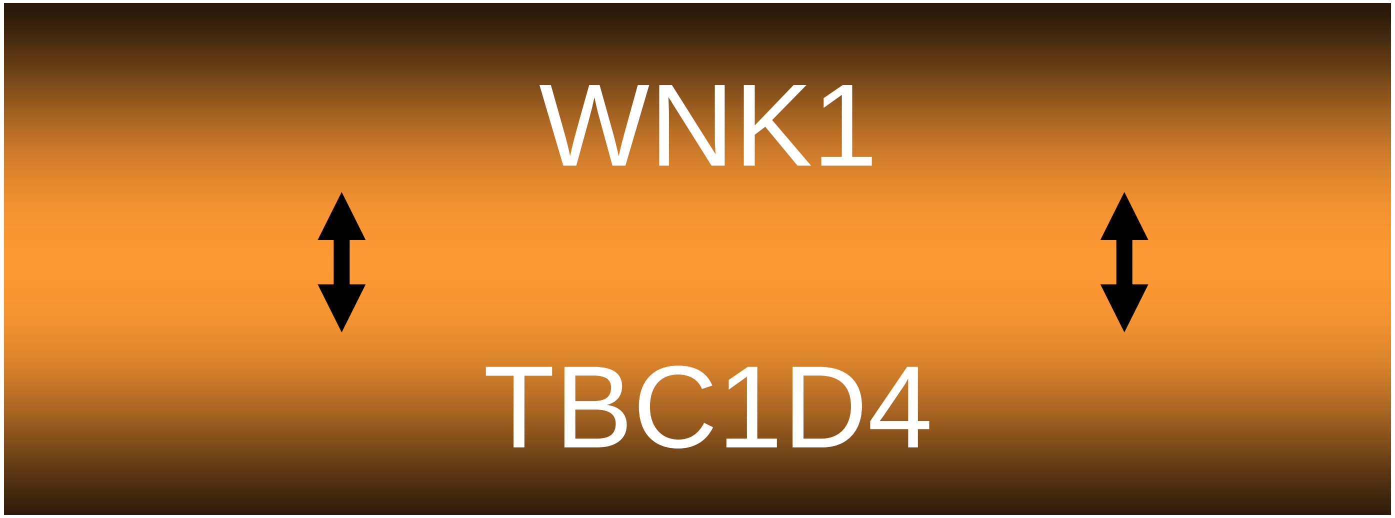
Channel	**Activity**	**Effect**	**WNK activity**
NCC	Na^+ Cl^- cotransporter	surface	dependent
NKCC	$Na^+/K^+/2Cl^-$ cotransporters	activity	dependent
KCC	K^+/Cl^- cotransporters	activity	dependent
CFTR	Cl^- channel	surface	independent
SLC26A9	$Cl^-/HCO3^-$ exchanger	surface	independent
ENaC	epithelial Na^+ channel	surface	independent
ROMK	K^+ channel	surface	independent
TRPV	Ca^+ channels	surface	dependent

Xenopus oocyte assays

How do WNK kinases affect expression at the cell surface??

→ identify interacting proteins

(co-immunoprecipitation/MassSpec)

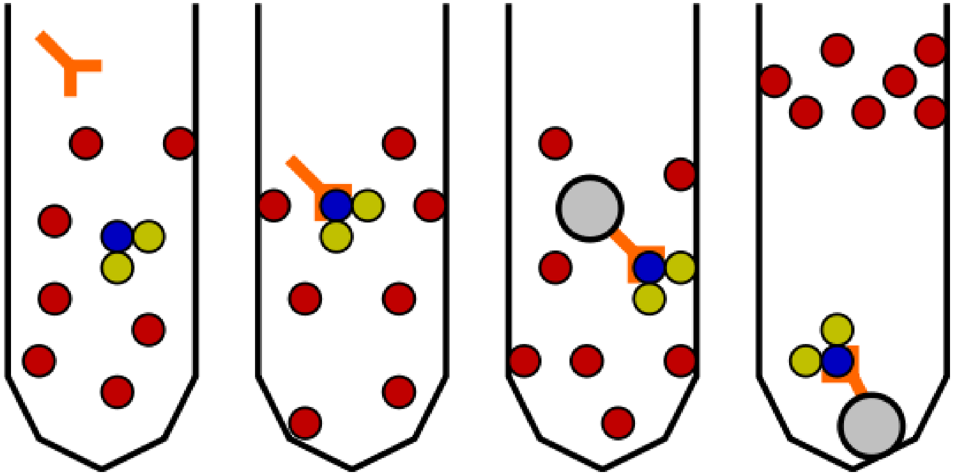


WNK1

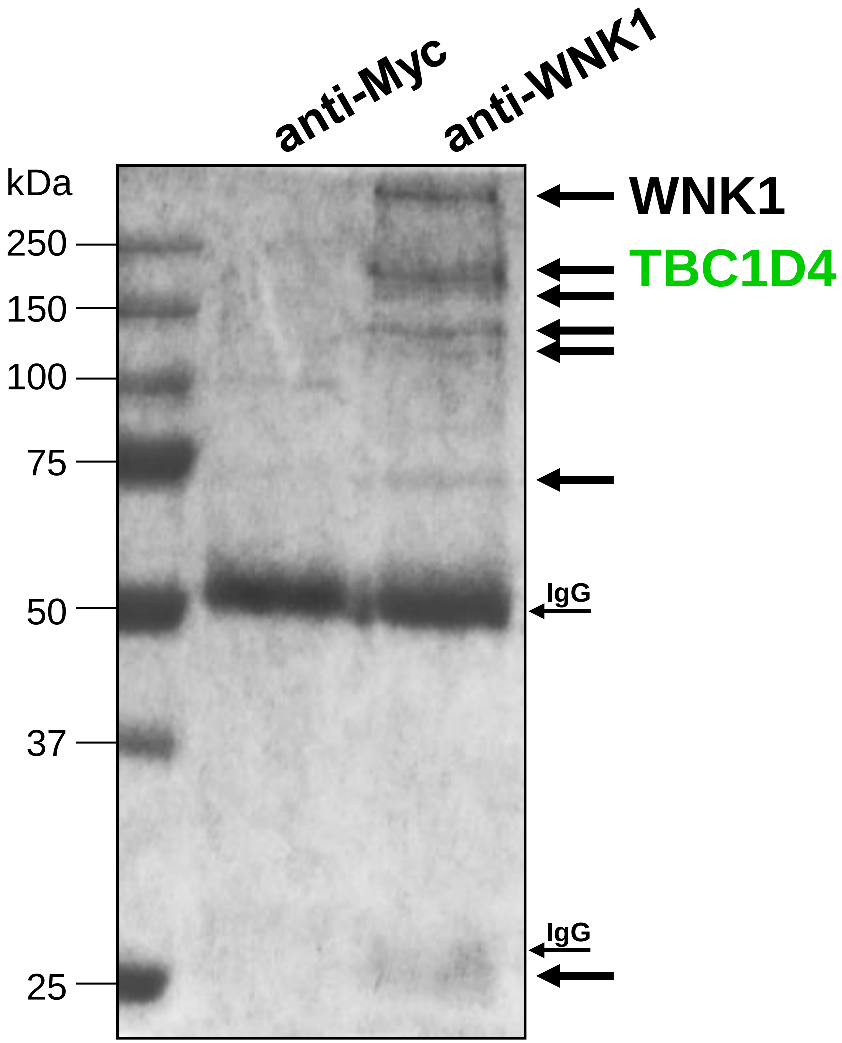
↕ ↕

TBC1D4

Co-immunoprecipitation



Endogenous WNK1 co-immunoprecipitates with TBC1D4



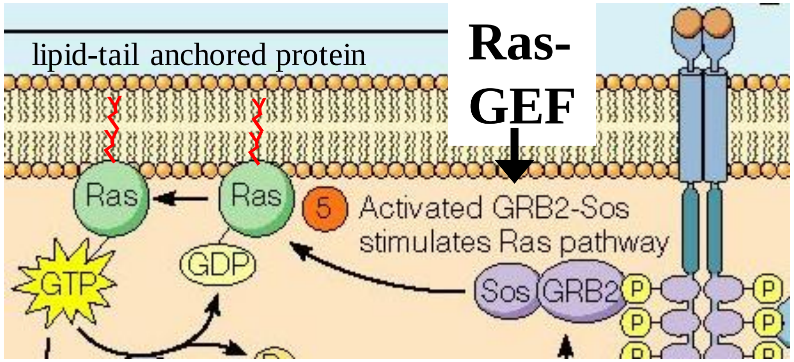
WNK1
↕
TBC1D4

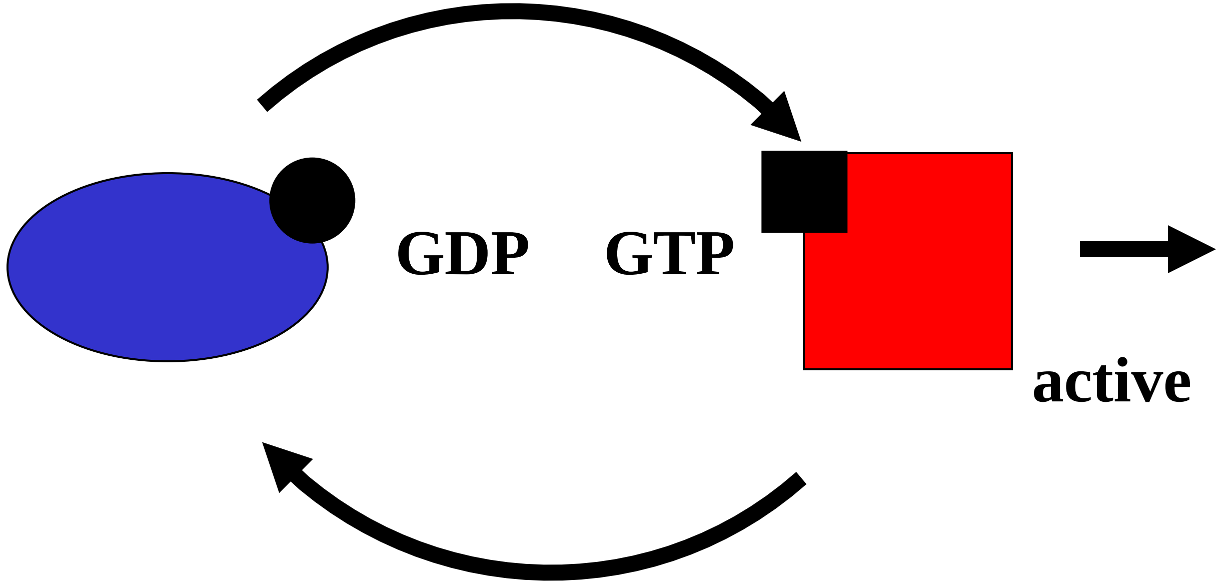
TBC domain* 1 family member 4/AS160 (Akt substrate of 160 kDa)

***** TBC (Tre-2, Bub2p, and Cdc16p) domain

Regulation
of
Rab-mediated
vesicle traffic

The GTPase cycle





GEFs

inactive

GDP

GTP

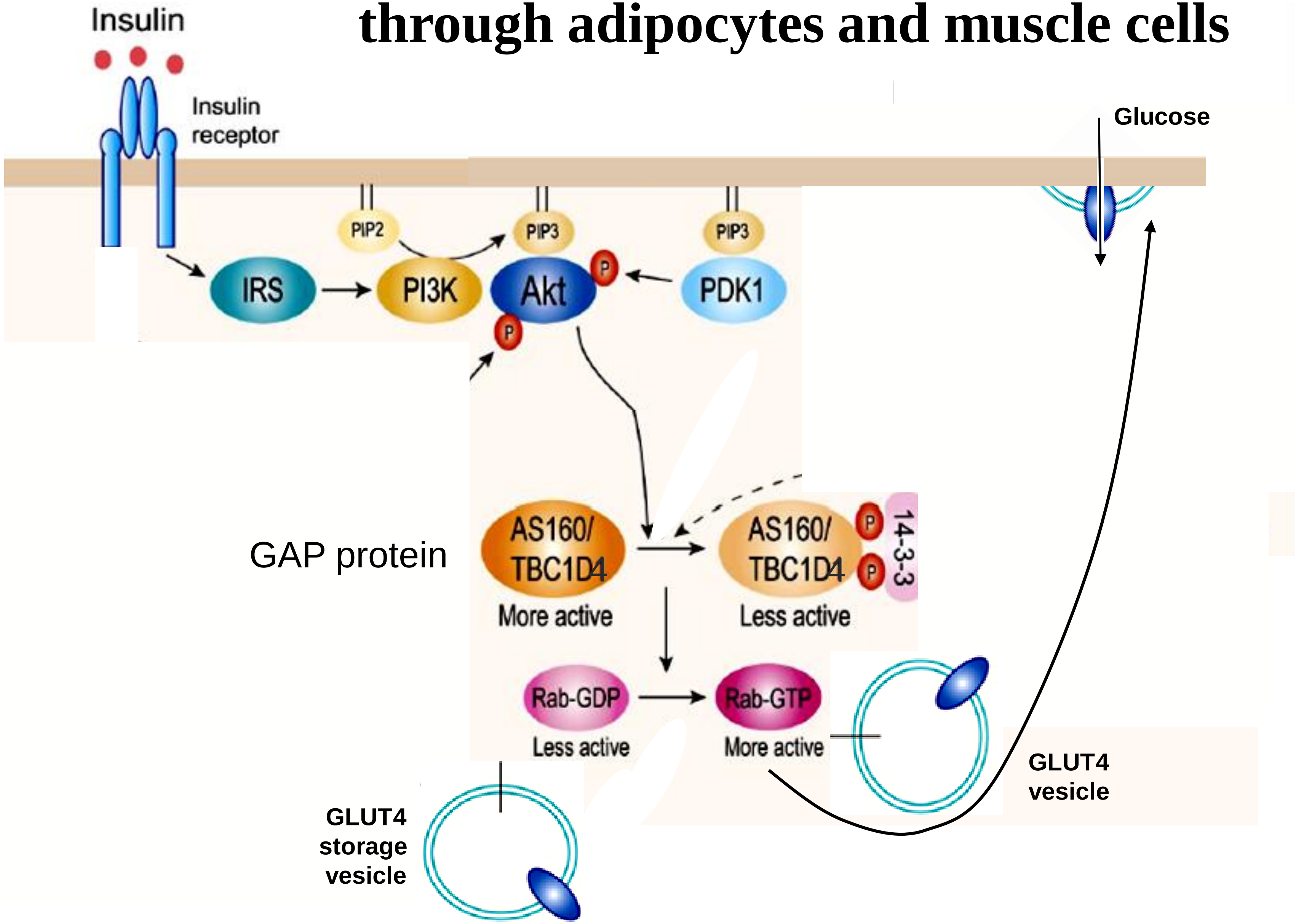
active

GAPs

Signal propagation to effector proteins

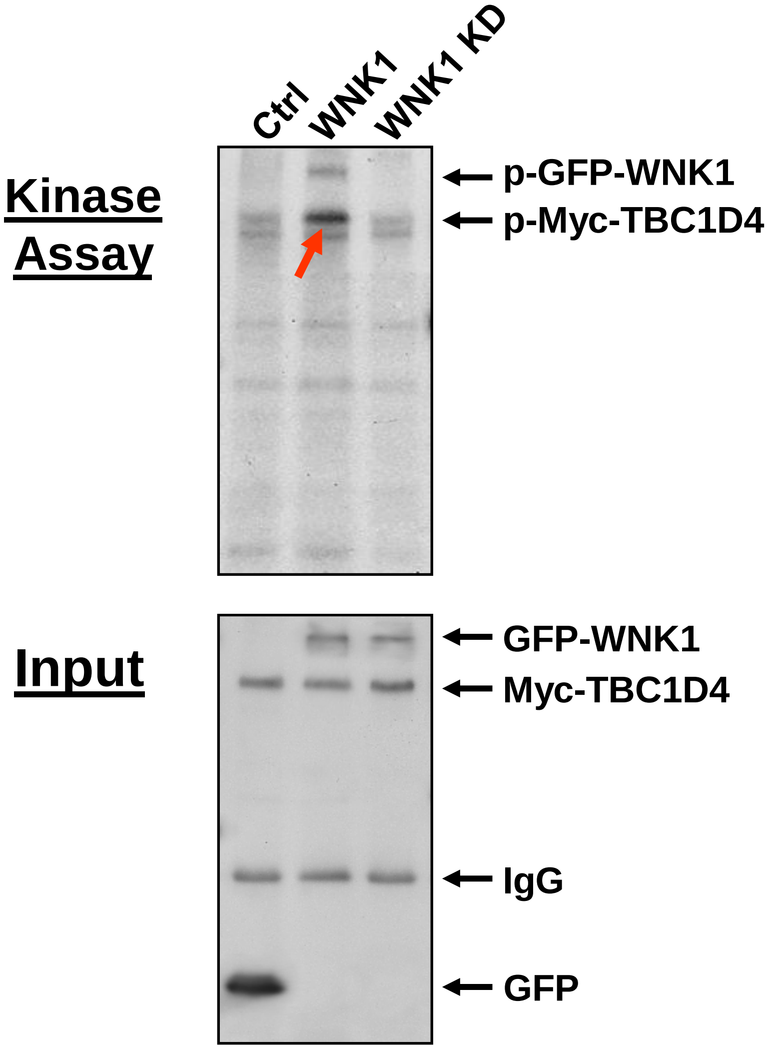
Cellular response

Insulin regulates blood glucose levels through adipocytes and muscle cells



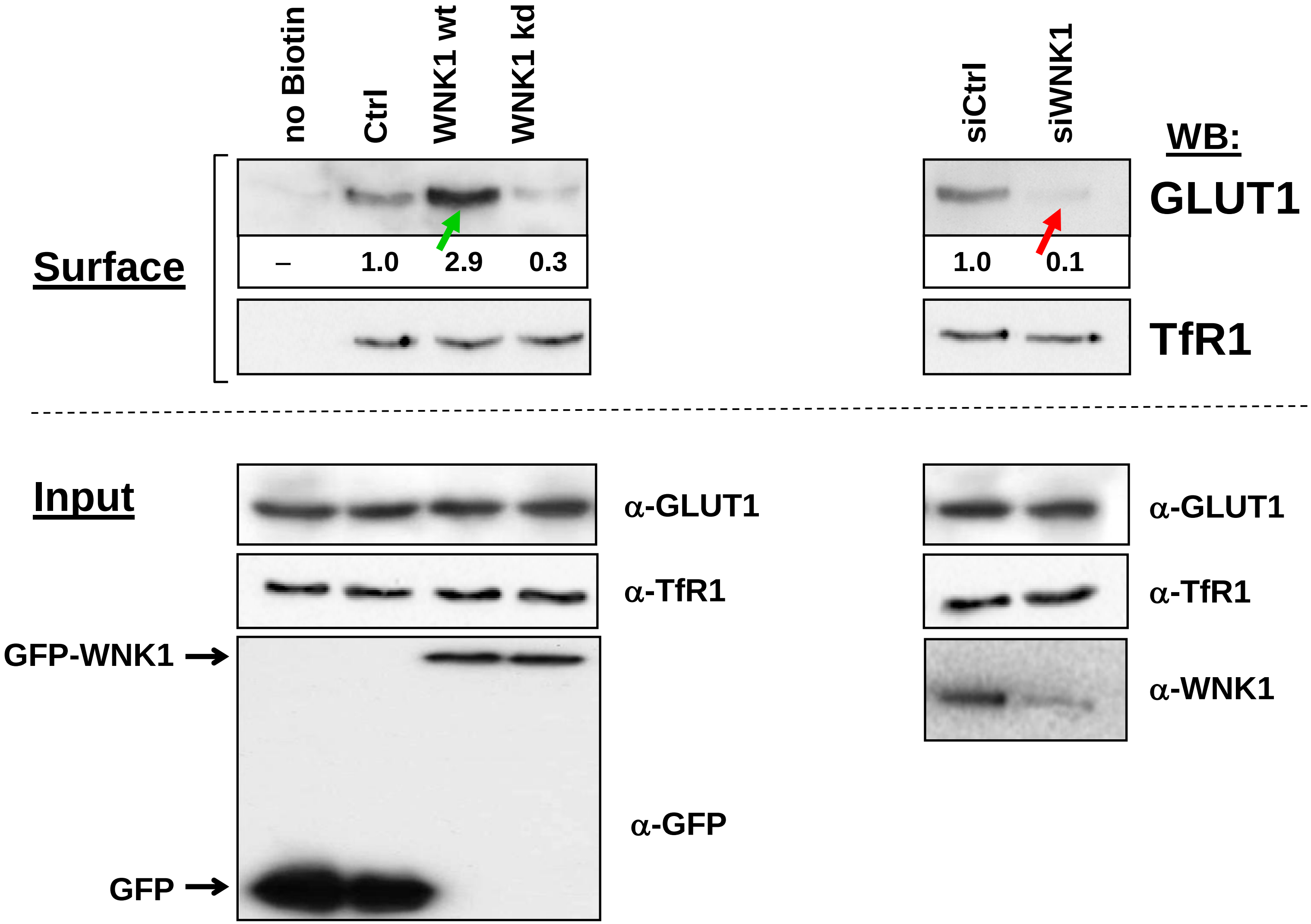
Modificado de: Sakamoto and Holman (2008) Am J Physiol Endocrinol Metab 295:29-37

WNK1 also phosphorylates TBC1D4 *in vitro*



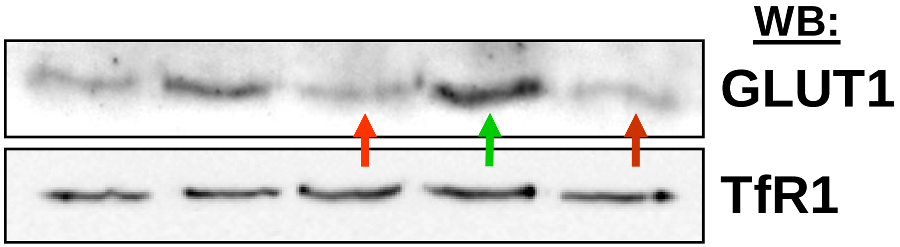
WNK1 increases surface levels of GLUT1

(the constitutive glucose transporter of most cell types)

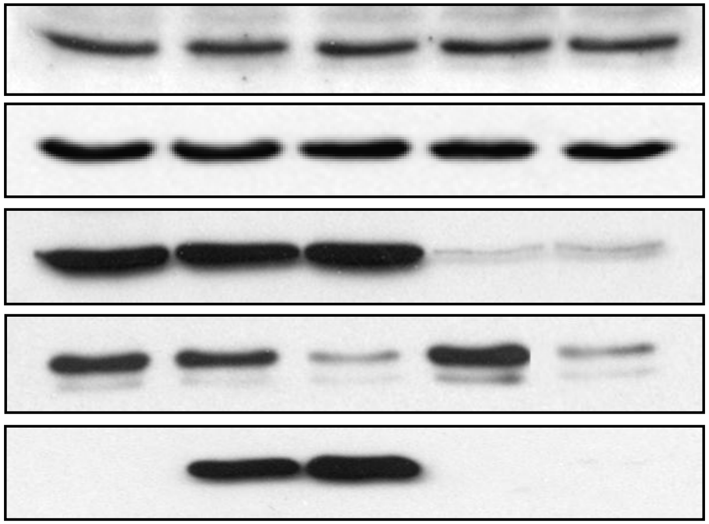


WNK1 mobilizes Rab8A

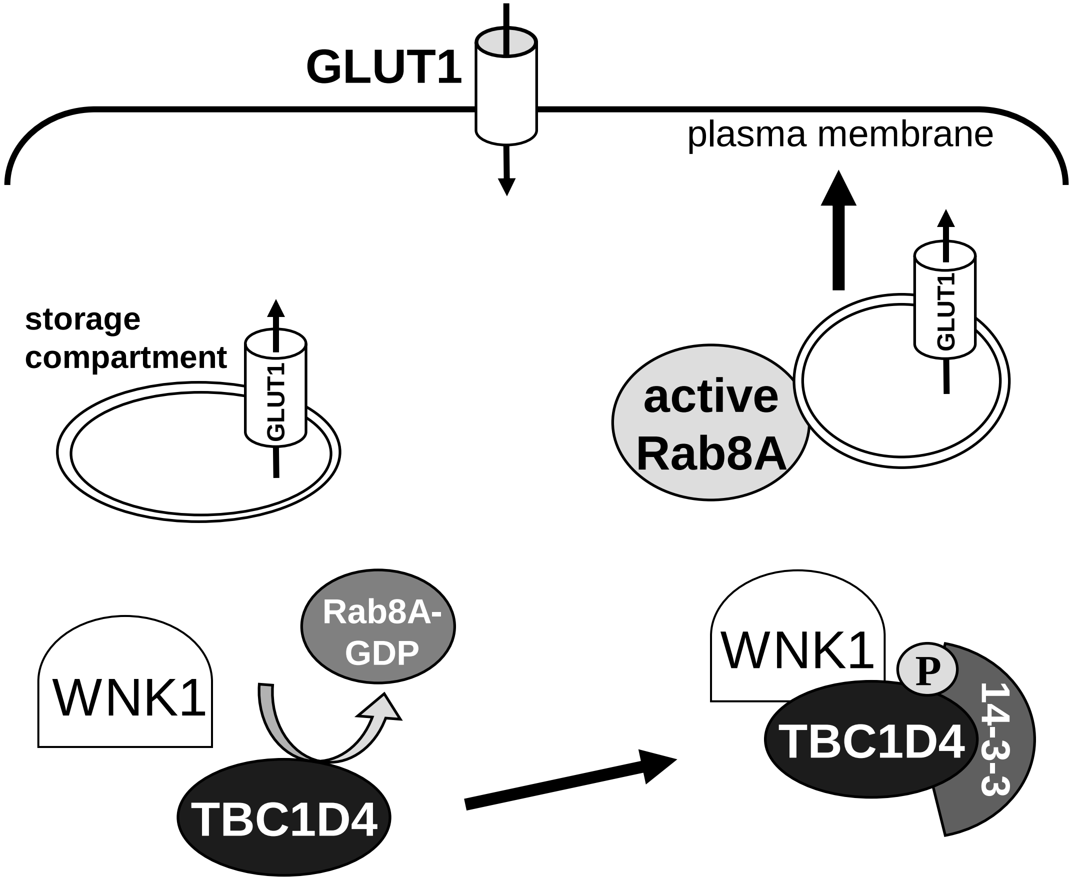
WNK1
↓
TBC1D4
↓
Rab



siCtrl	+	+	−	−	−
WNK1	−	+	+	−	−
siRab8A	−	−	+	−	+
siTBC1D4	−	−	−	+	+



Model for the regulation of GLUT1 surface expression



Implication: GLUT1 is overexpressed by many tumour cells

Mendes et al. (2010) J Biol Chem 285, 39117-39126