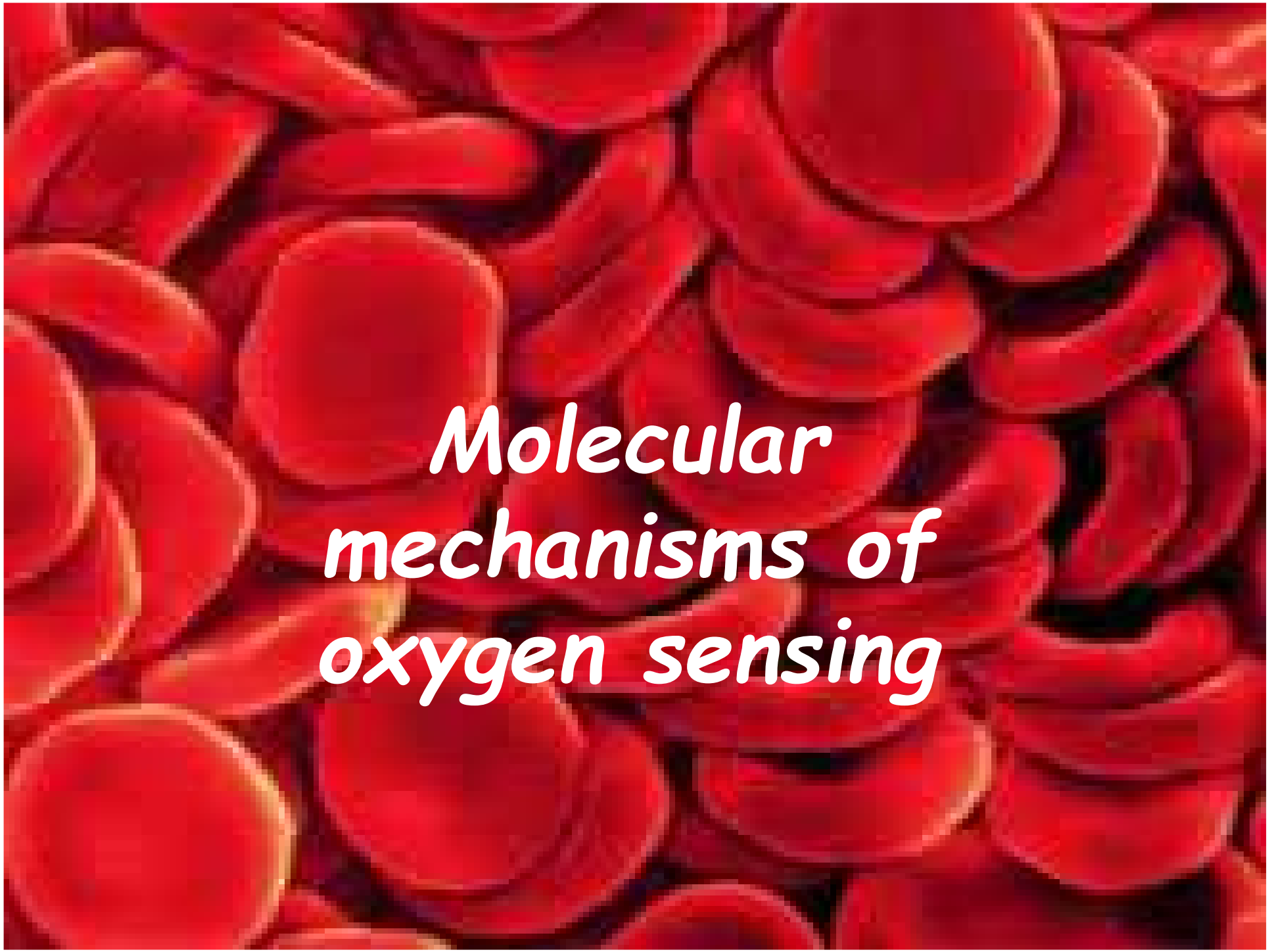

Molecular
mechanisms of
oxygen sensing

In mammals, O2 sensing occurs at many levels, leading to both acute and chronic adaptation.

Acute seconds…..
The carotid body, which is located at the bifurcation of the internal and external carotid arteries, contains highly specialized chemosensory cells. These glomus cells depolarize in response to reduction in arterial blood PO2 (hypoxemia) resulting in stimulation of the brain stem centers that control the respiratory and cardiovascular systems, which leads to rapid changes in ventilation, heart rate, and blood pressure that serve to increase O2 uptake in the lungs and O2 delivery to the tissues.

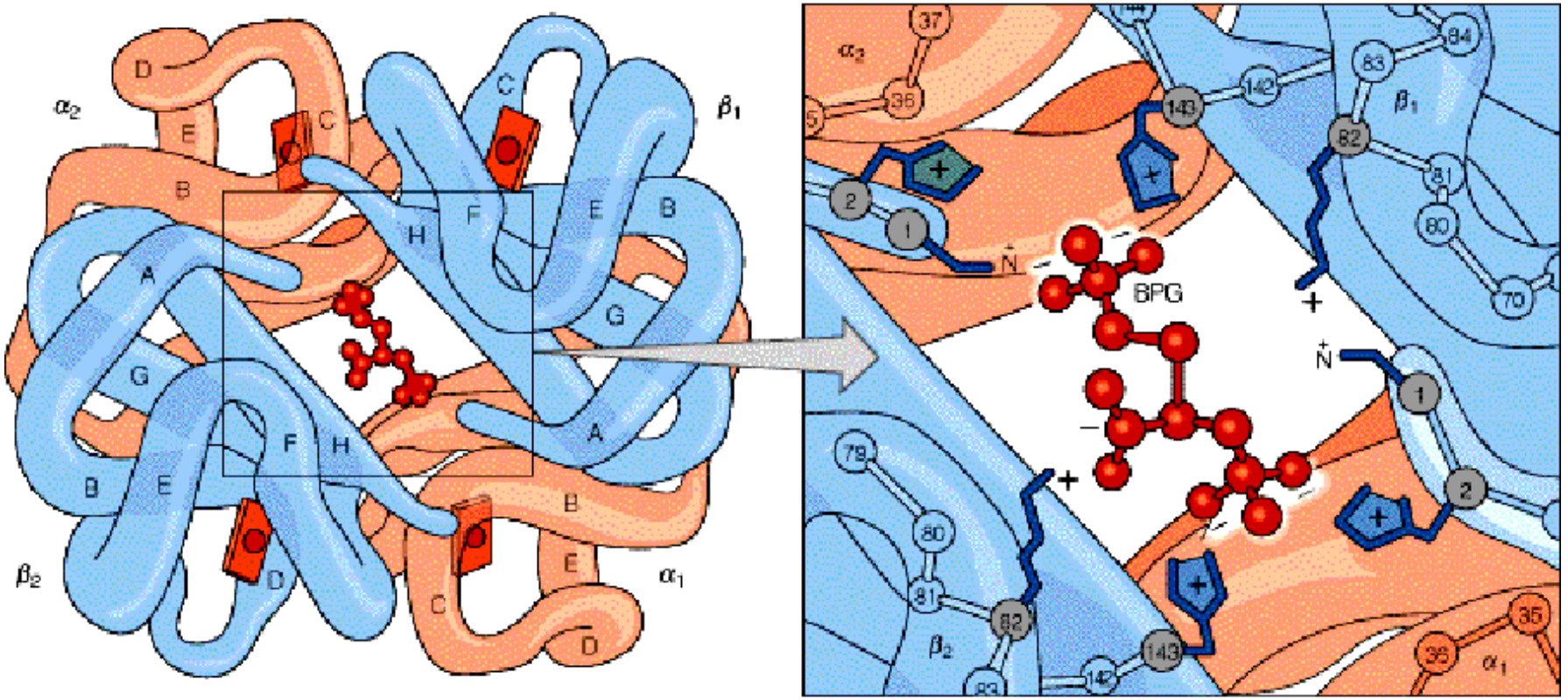
2,3 Bisphosphoglycerate (BPG)

- 2,3,BPG is involved in acclimatization to hypoxia as in high altitude

Bisphosphoglycerate (BPG)

- BPG binds in the cavity between β-Hb subunits and Stabilizes T-conformation

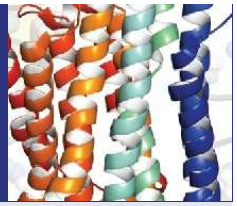
2,3 Bisphosphoglycerate (BPG)



Bisphosphoglycerate (BPG)

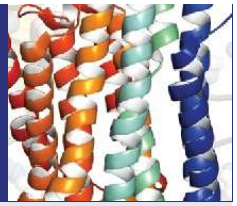
- 2,3-BPG is a glycolytic intermediate in RBCs

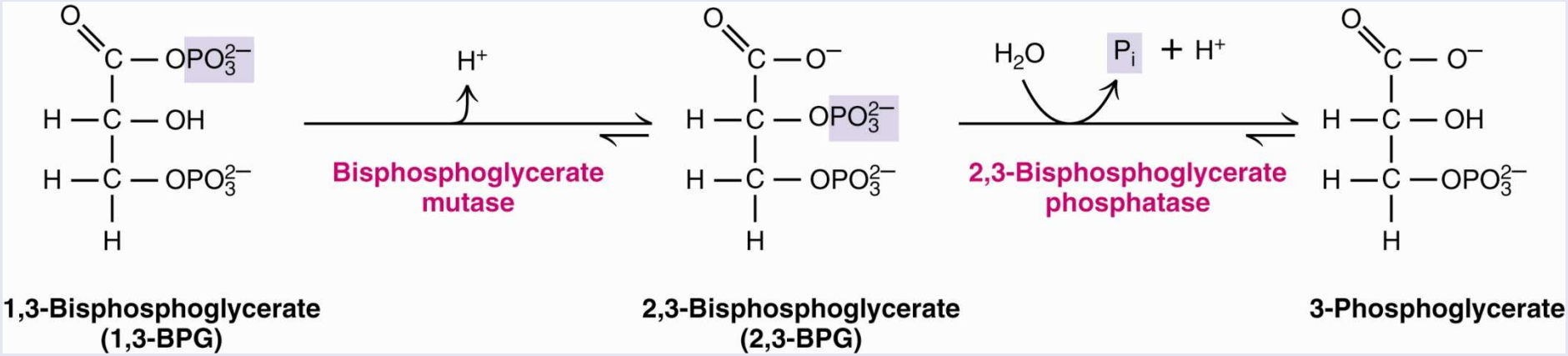
2,3-BPG synthesis in erythrocytes detours around the phosphoglycerate kinase rxn



- Most cells contain only a trace of 2,3-BPG, but erythrocytes typically contain 4-5 mM 2,3-BPG
- 2,3-bisphosphoglycerate is an important regulator of hemoglobin
- 2,3-BPG (for hemoglobin) is made by circumventing the PGK reaction
- 2,3-BPG is formed from 1,3-BPG by bisphosphoglycerate mutase.
- 3-phosphoglycerate is then formed by 2,3-bisphosphoglycerate phosphatase.

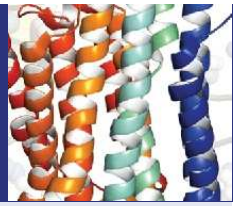
Erythrocyte synthesis of 2,3-BPG

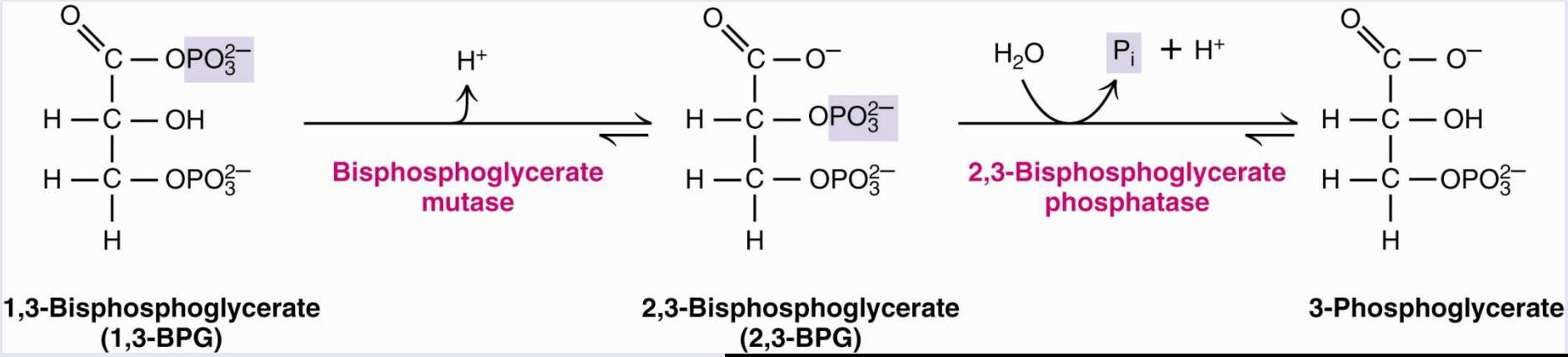




Formation and decomposition of 2,3-bisphosphglycerate in erythrocytes

Erythrocyte synthesis of 2,3-BPG



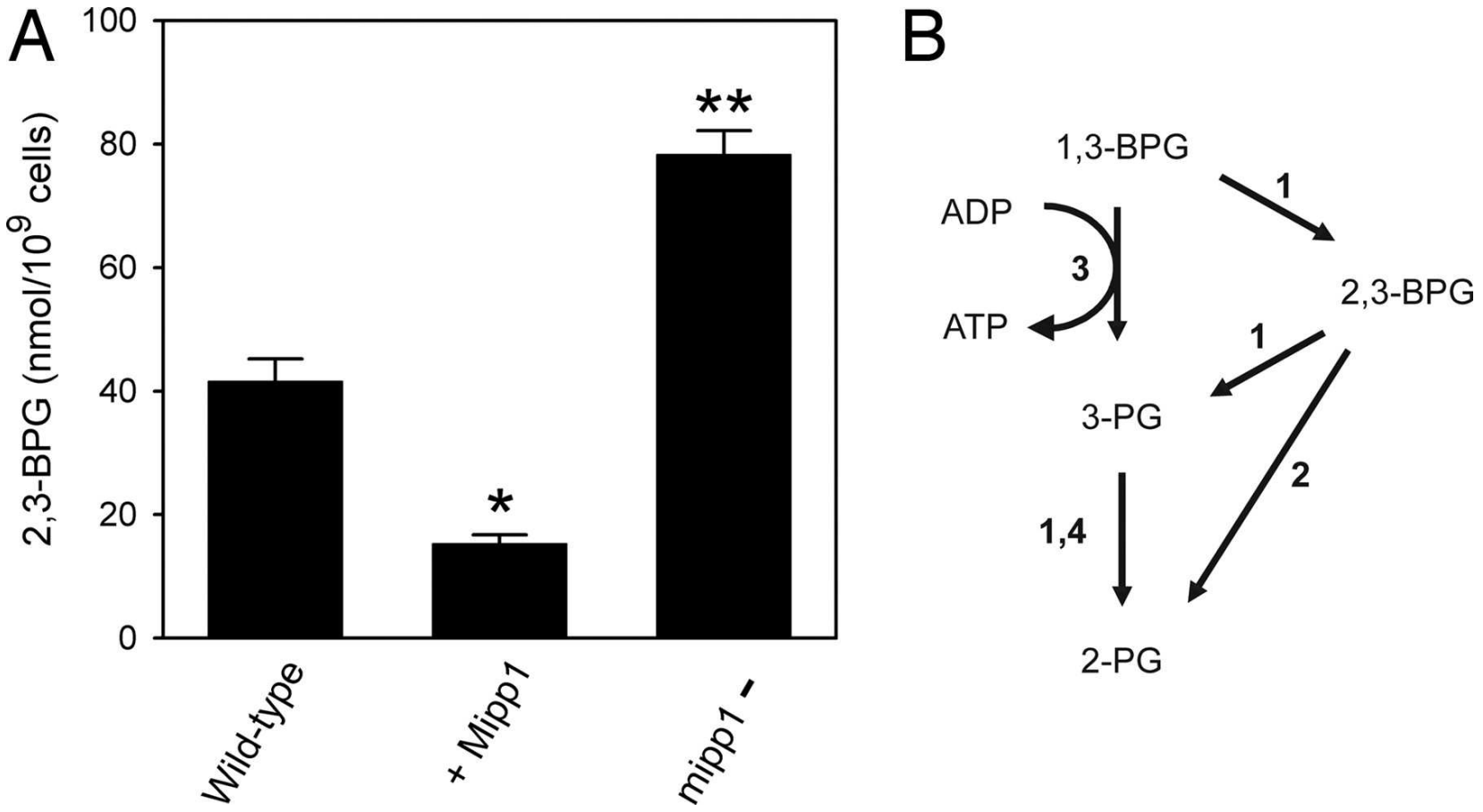


multiple inositol polyphosphate phosphatase (MIPP1)

Hydrolysis of 2,3-BPG by human MIPP1 is sensitive to physiologic alkalosis;

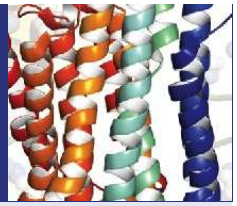
This phenomenon provides a homeostatic mechanism for elevating 2,3-BPG levels, thereby enhancing oxygen release to tissues.

Cellular levels of 2,3-BPG in Dictyostelium respond to genetic manipulations of expression.

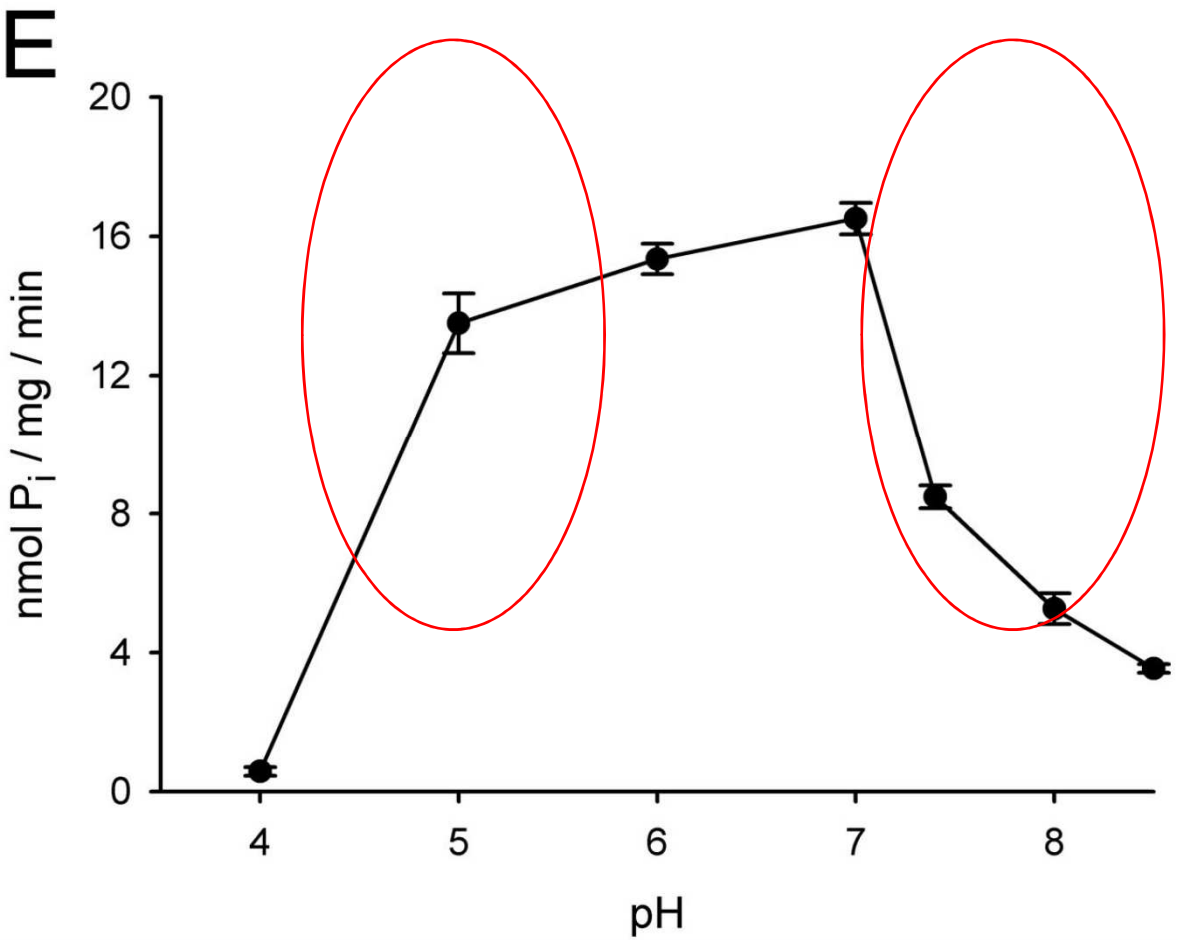


Levels of 2,3-BPG in wild-type Dictyostelium, in cells in which the mipp1 gene was disrupted - , and in cells in which DdMipp1 was overexpressed +.

Cho J et al. PNAS 2008;105:5998-6003

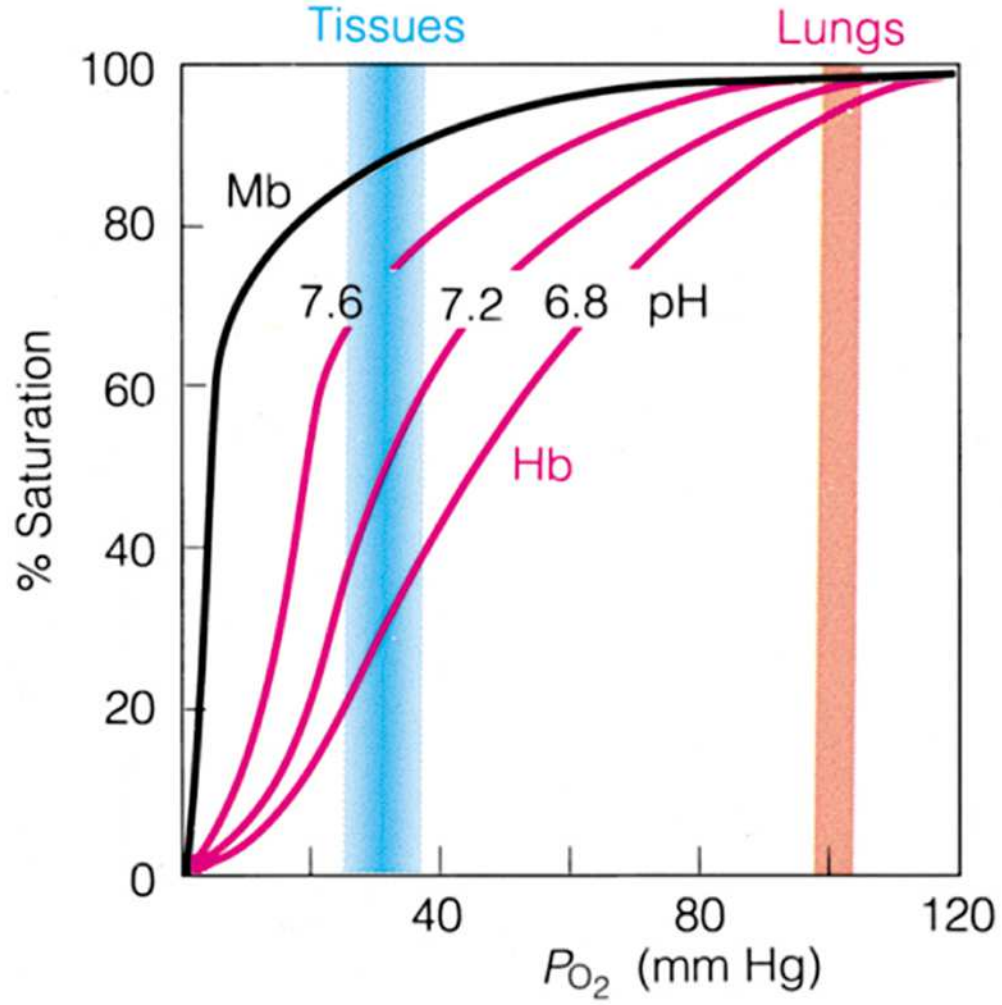


The effect of pH on enzyme activity of MIPP1



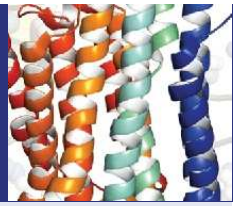
2,3-BPG as substrate

L’effetto Bohr: pH bassa ⟶ bassa affinità ⟶ rilascio di O_2



-His-H^+

Terminal-NH_3^+



The Effect of pH on MIPP1 and Its Regulatory Significance

As hemoglobin releases oxygen, its affinity for H+ increases, causing intracellular alkalinization.

This elevated intracellular pH drives a positive feedback loop, increasing levels of 2,3-BPG, thereby facilitating more oxygen release .

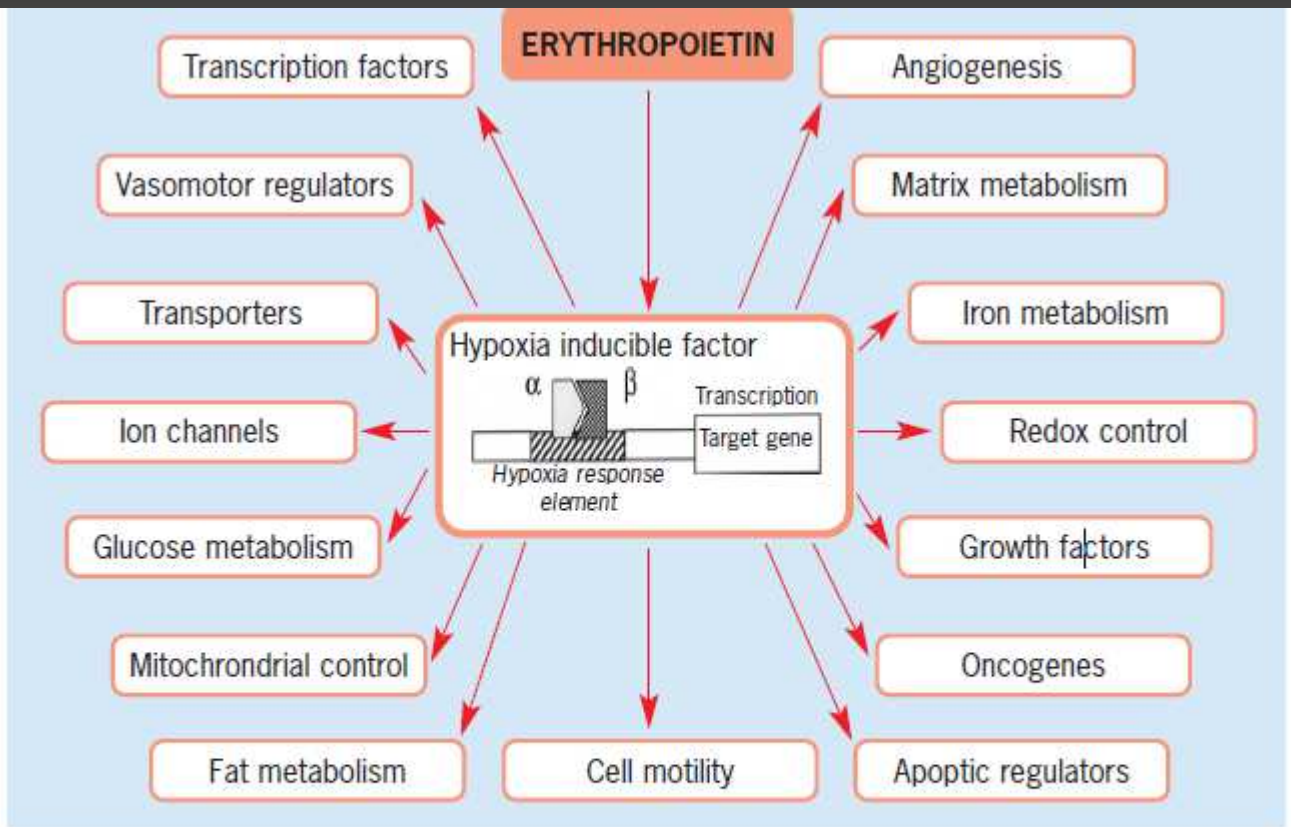
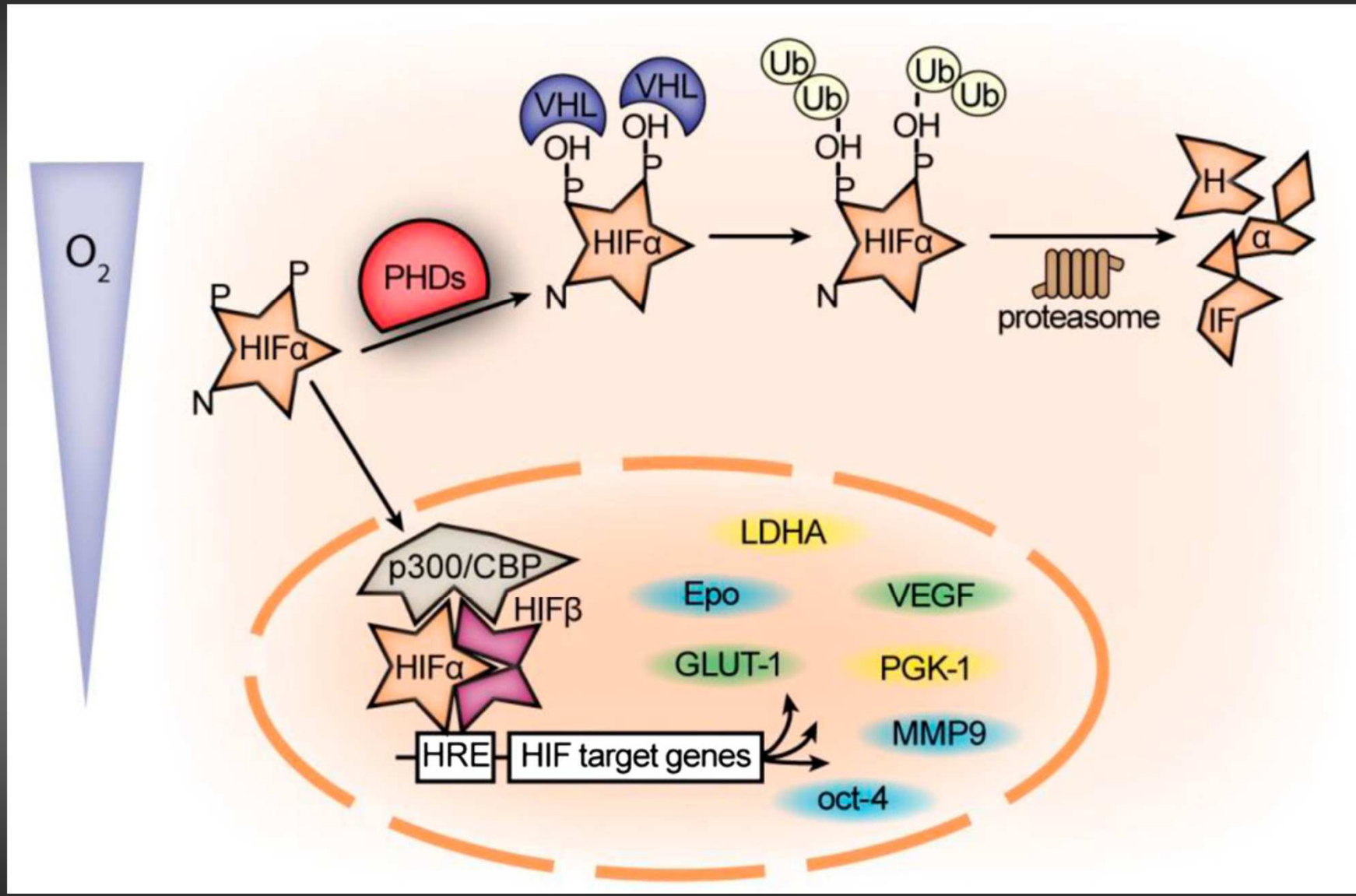
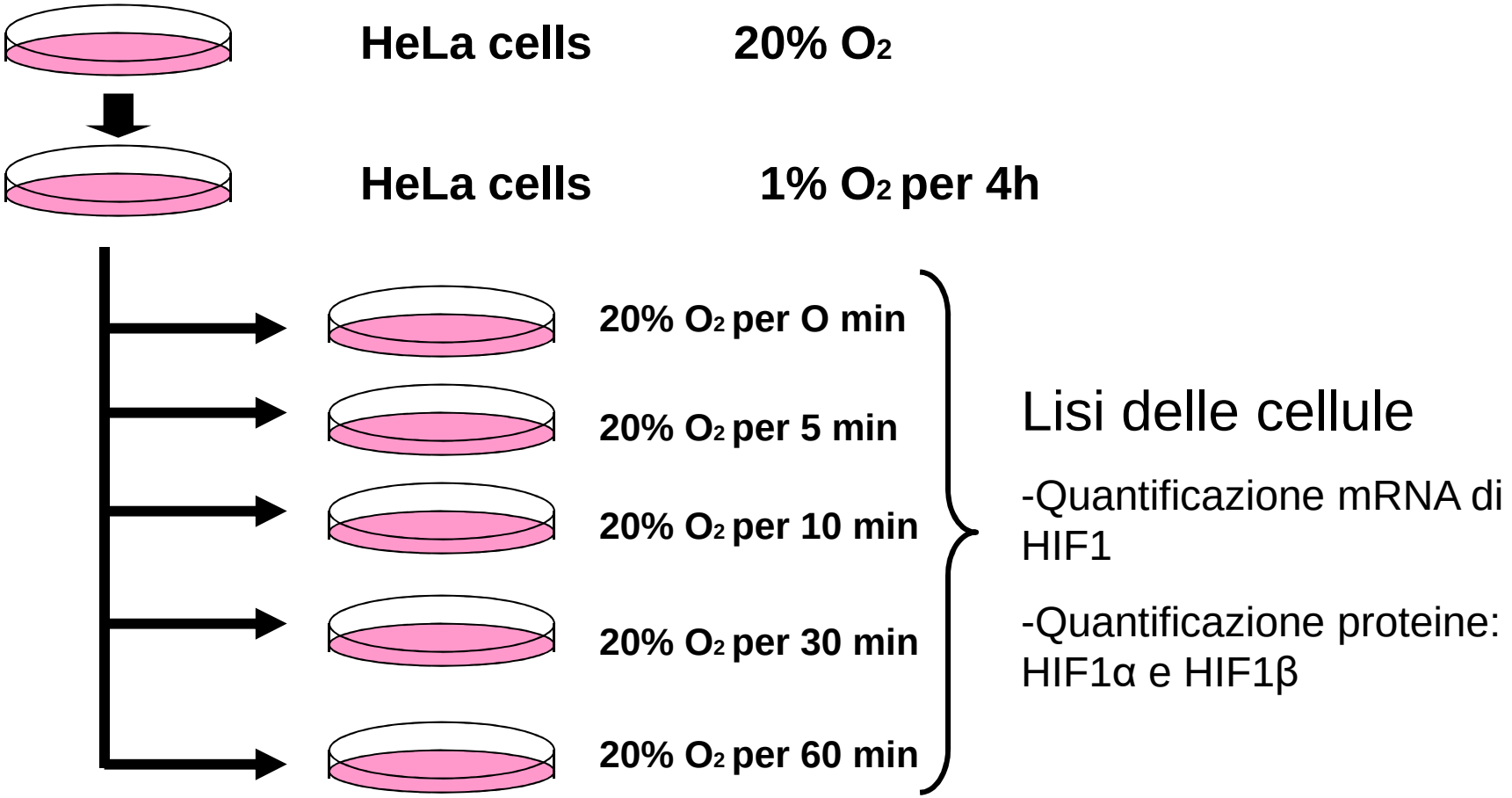


Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.


O2
HIFα
P
P
N
PHDs
VHL
VHL
OH
OH
P
P
HIFα
N
Ub
Ub
Ub
Ub
OH
OH
P
P
HIFα
N
proteasome
H
α
IF
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

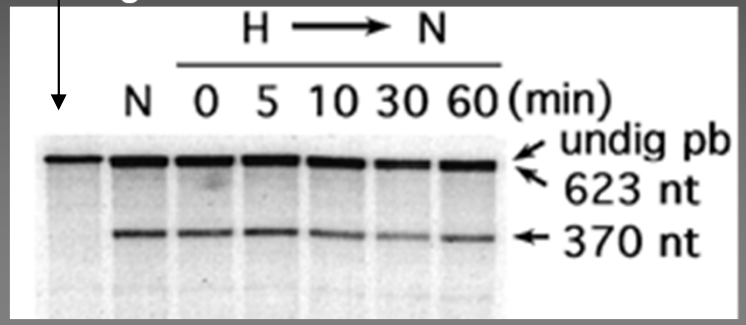
Eric Huang et al. - JBC 1996



Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit
Huang et al. - JBC 1996

H=hypoxia; N=normoxia

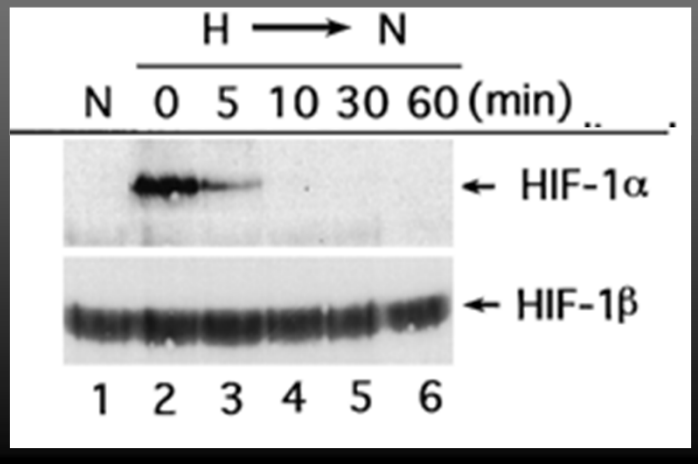
Sonda indigerita



HIF1α è espresso a livello di mRNA.

Quantificazione proteine → **Western blot**

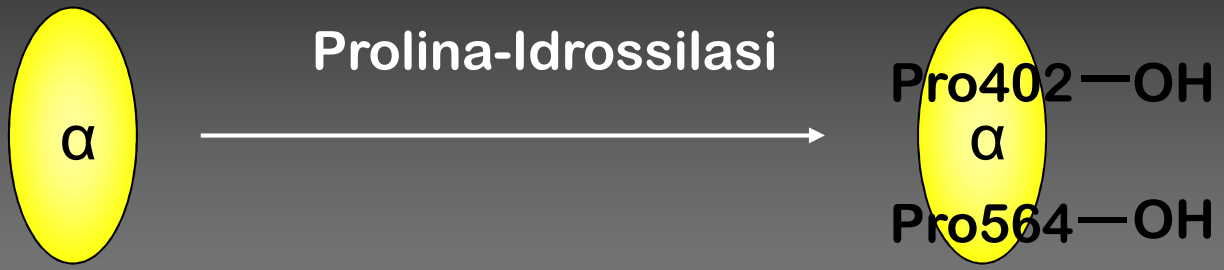
Ibridazione con anticorpi anti HIF1α e HIF1β



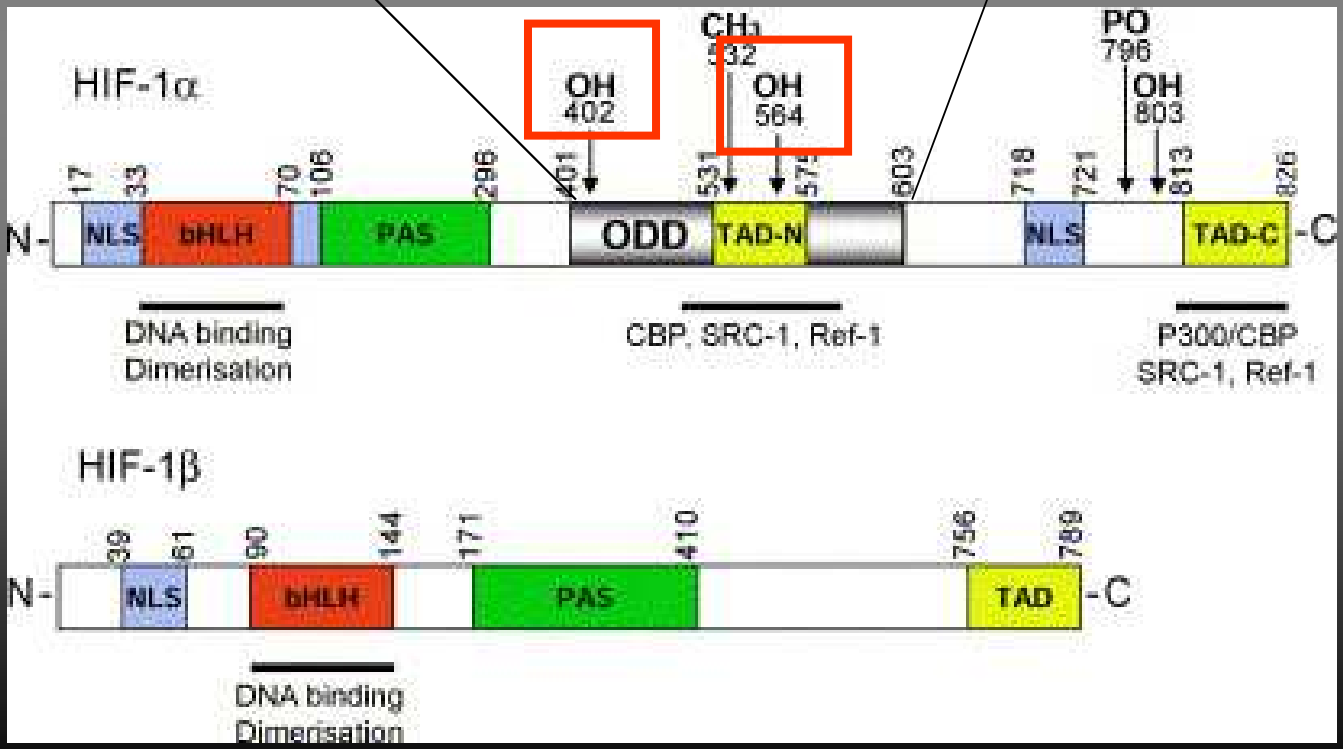
HIF1α è presente solo in condizioni di ipossia

HIF1β è sempre presente

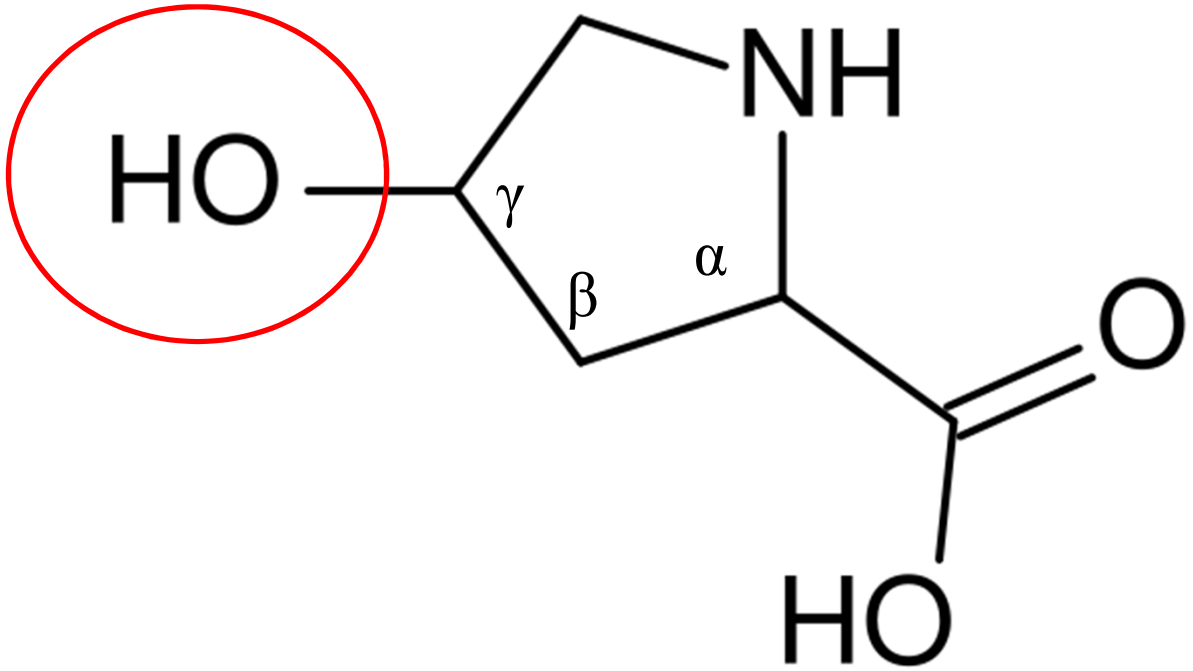
Struttura di HIF1



Sequenza di idrossilazione



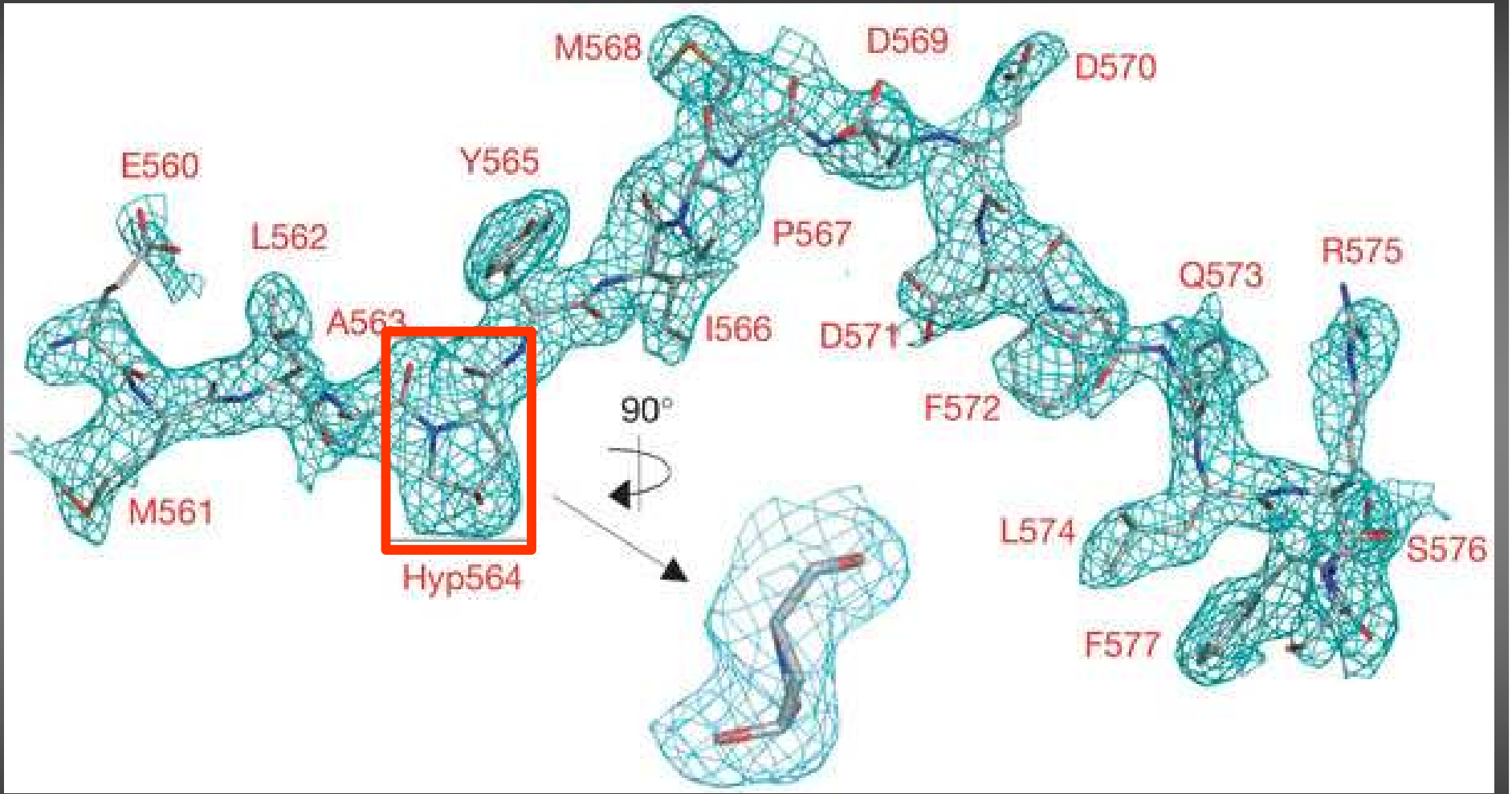
Idrossiprolina



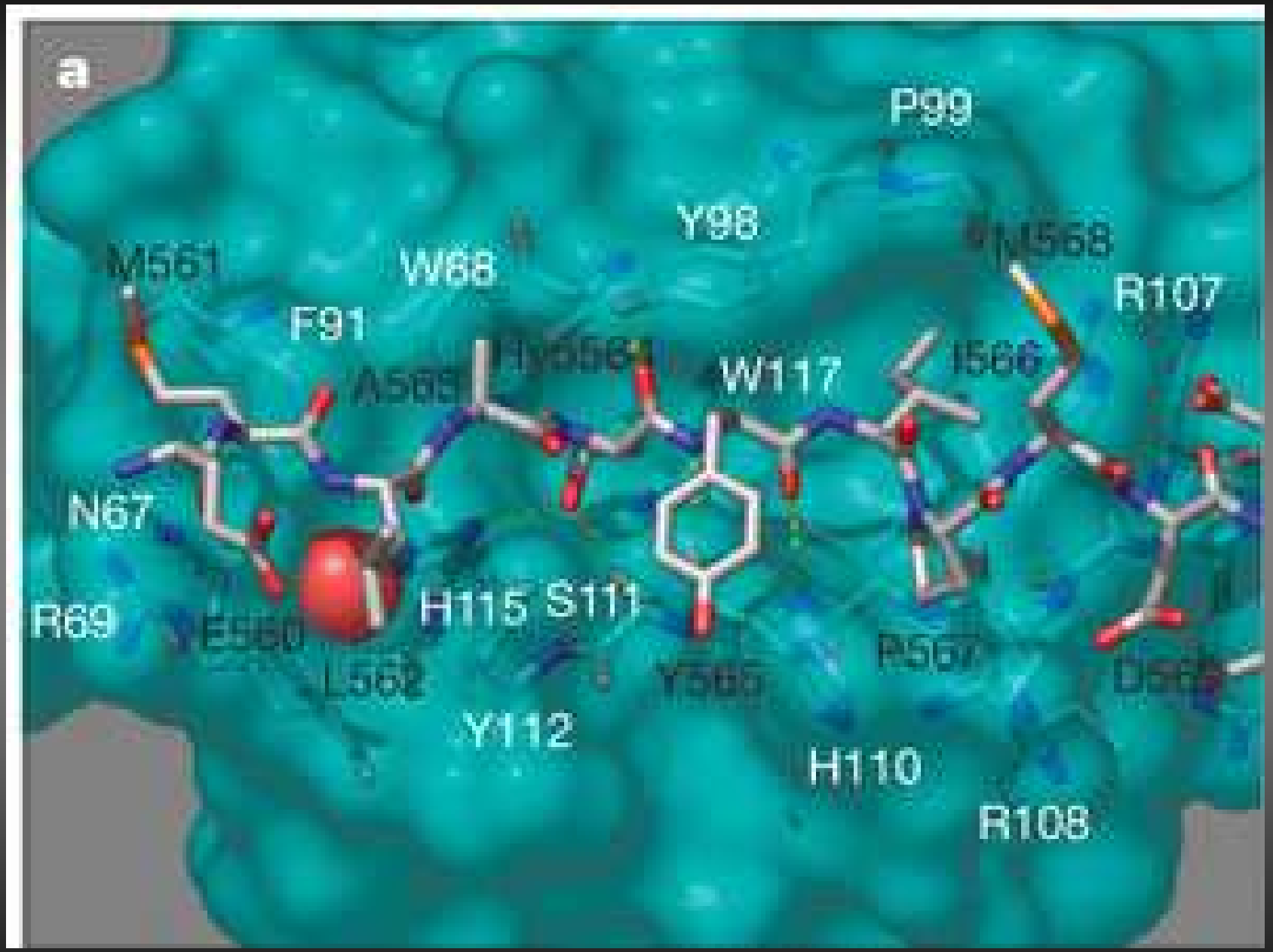
4-hydroxypyrrolidine-2-carboxylic acid

Riconoscimento specifico dell'idrossiprolina da parte del complesso di VHL

The boomerang-shaped CODD peptide (Hif)

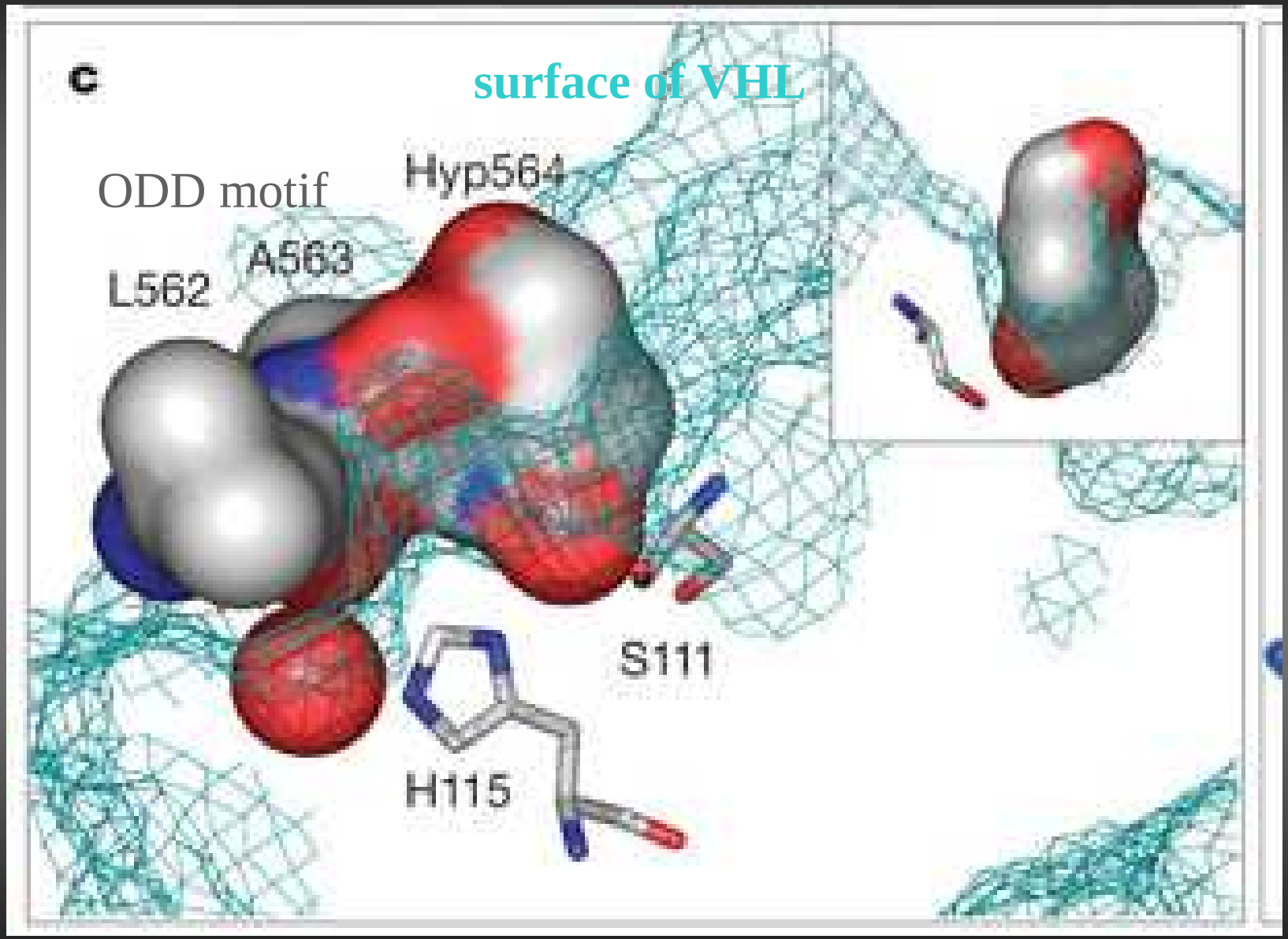


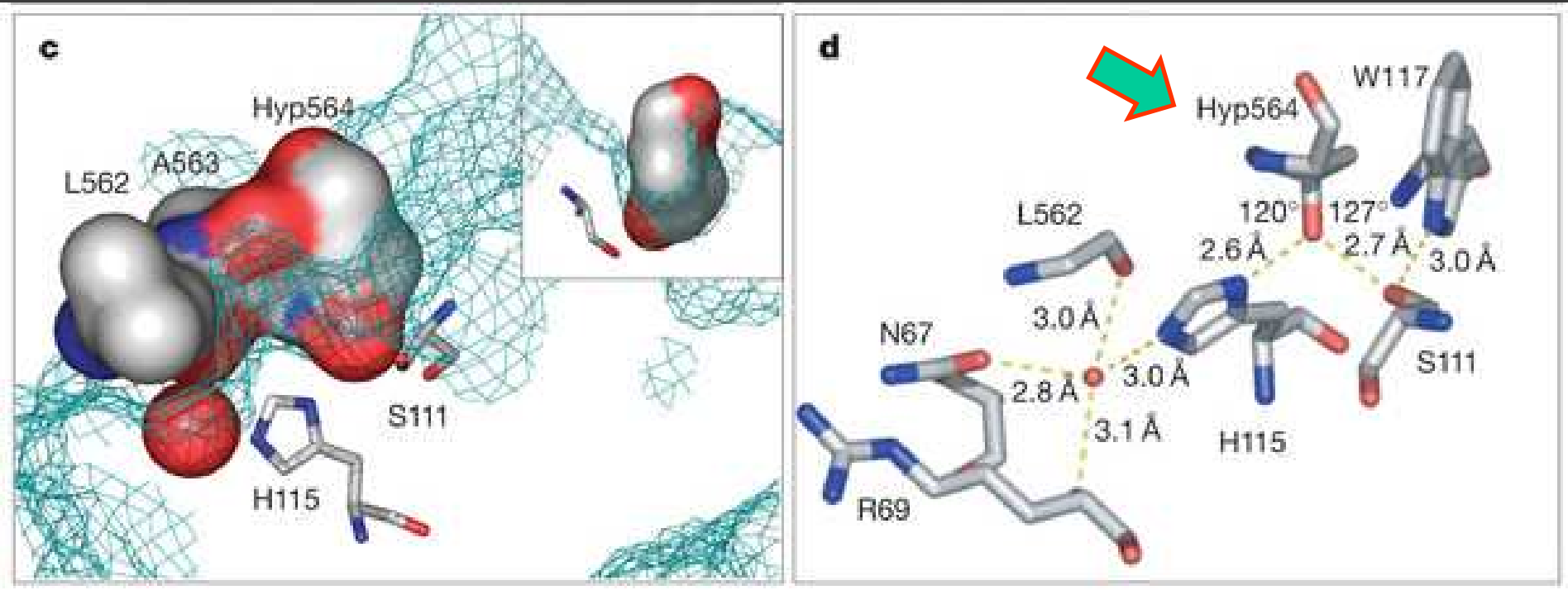
CODD: Carboxyl oxygen dependent domain



CODD-contacting residues of VHL (stick models)

Hyp-binding pocket (VHL)

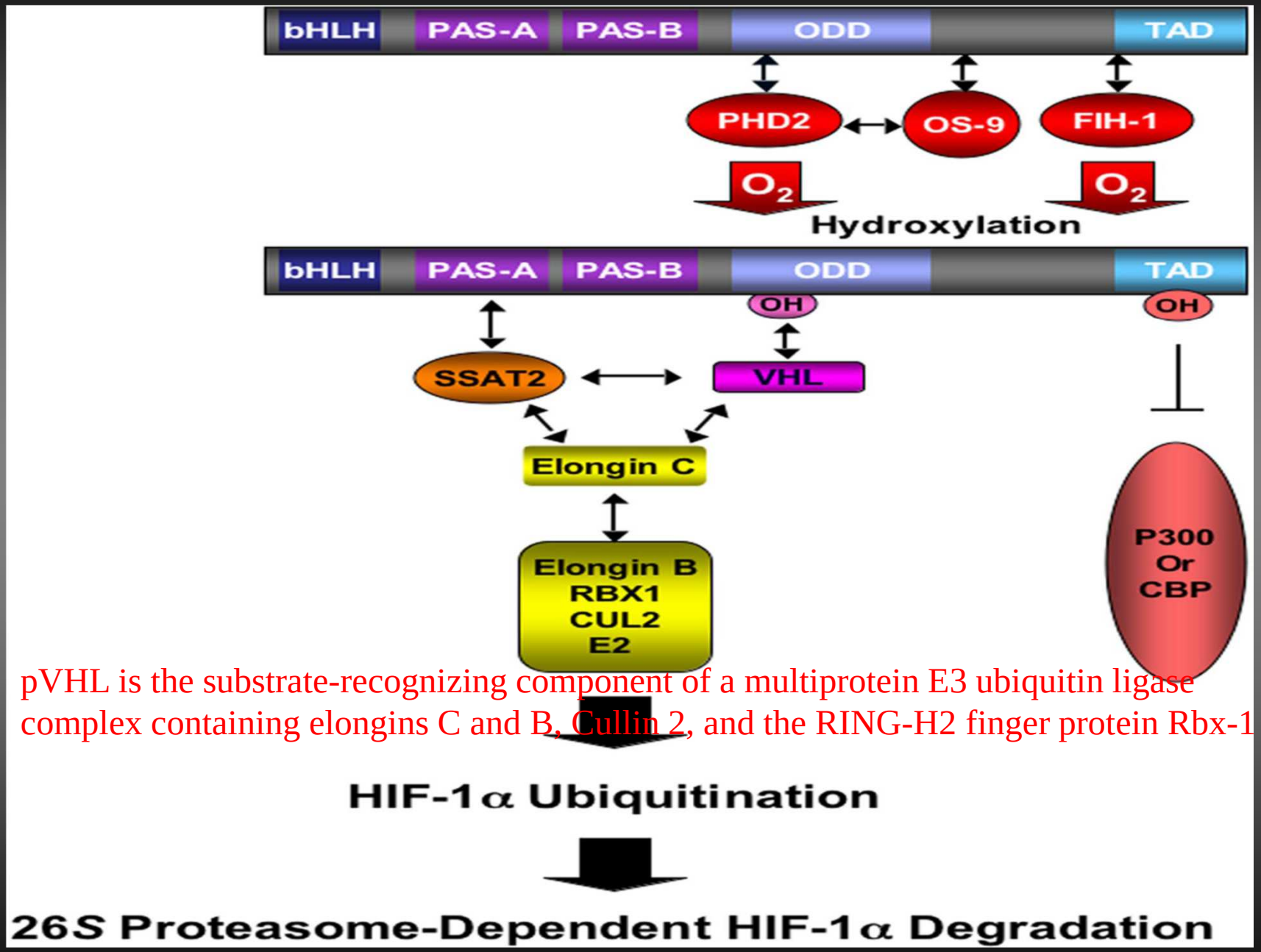




The hydrogen-bonding network (VHL) involved in binding of the Hyp564 hydroxyl group (Hif)

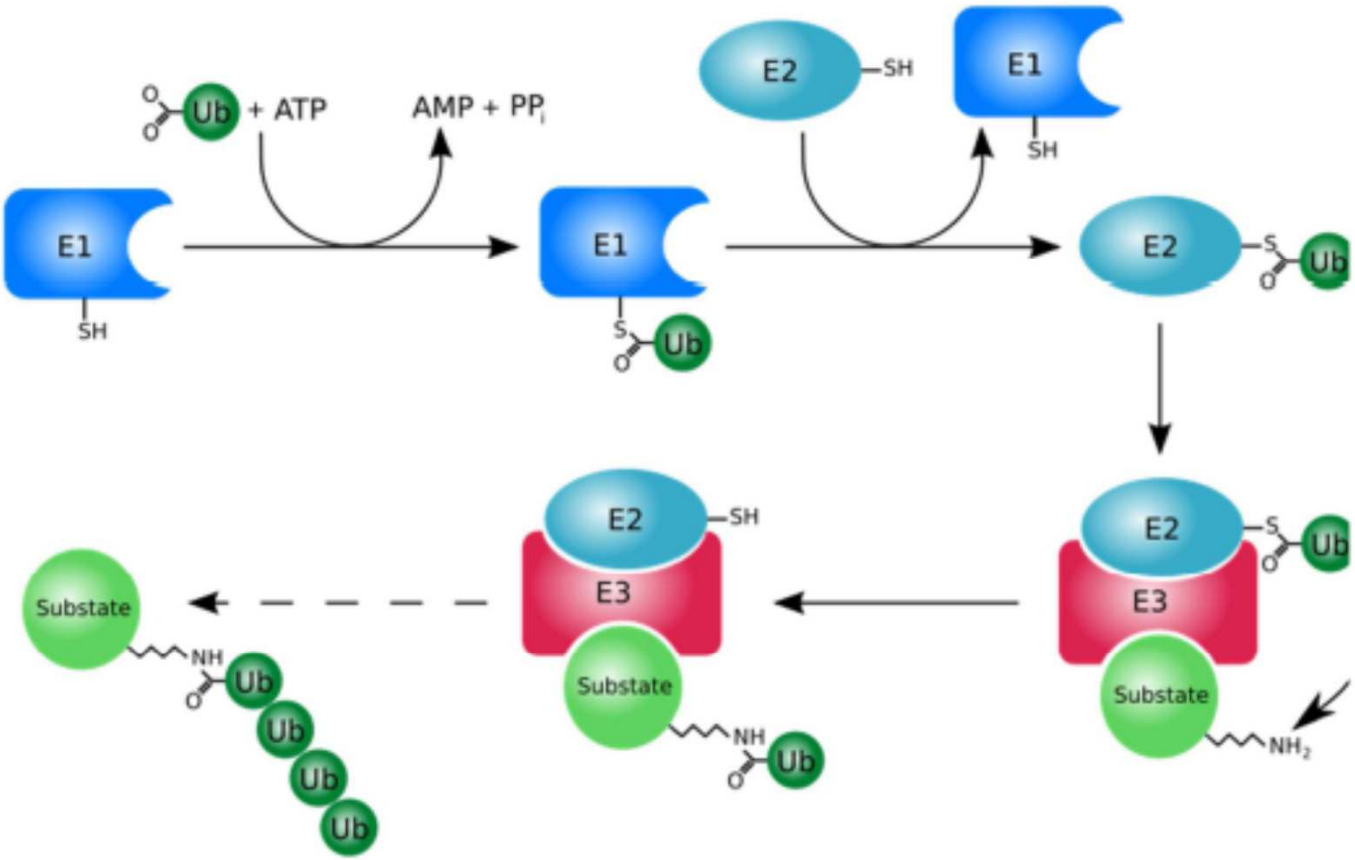
red sphere = key water molecule

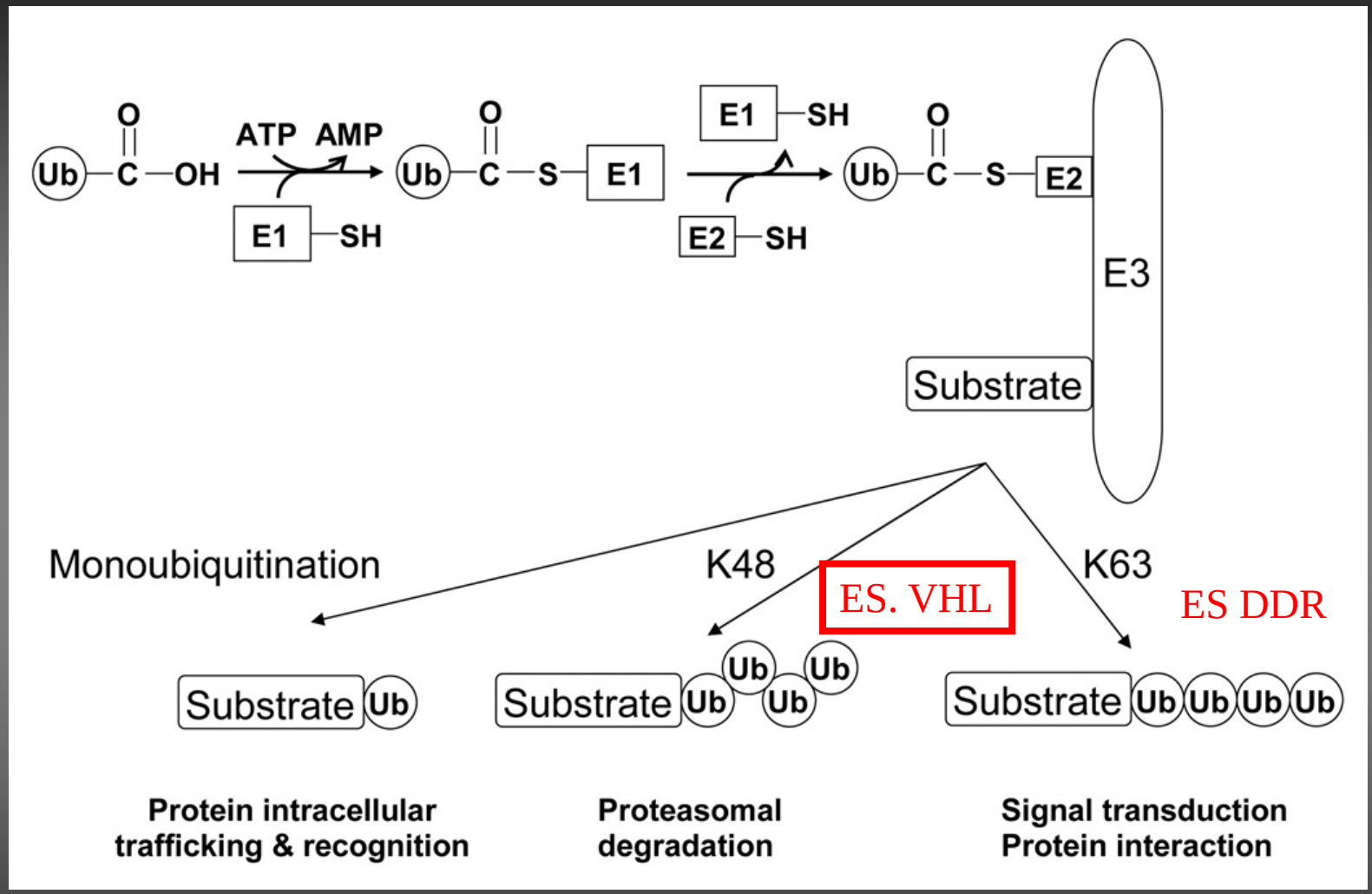
HIF e VHL fanno parte di complessi molecolari con molte componenti



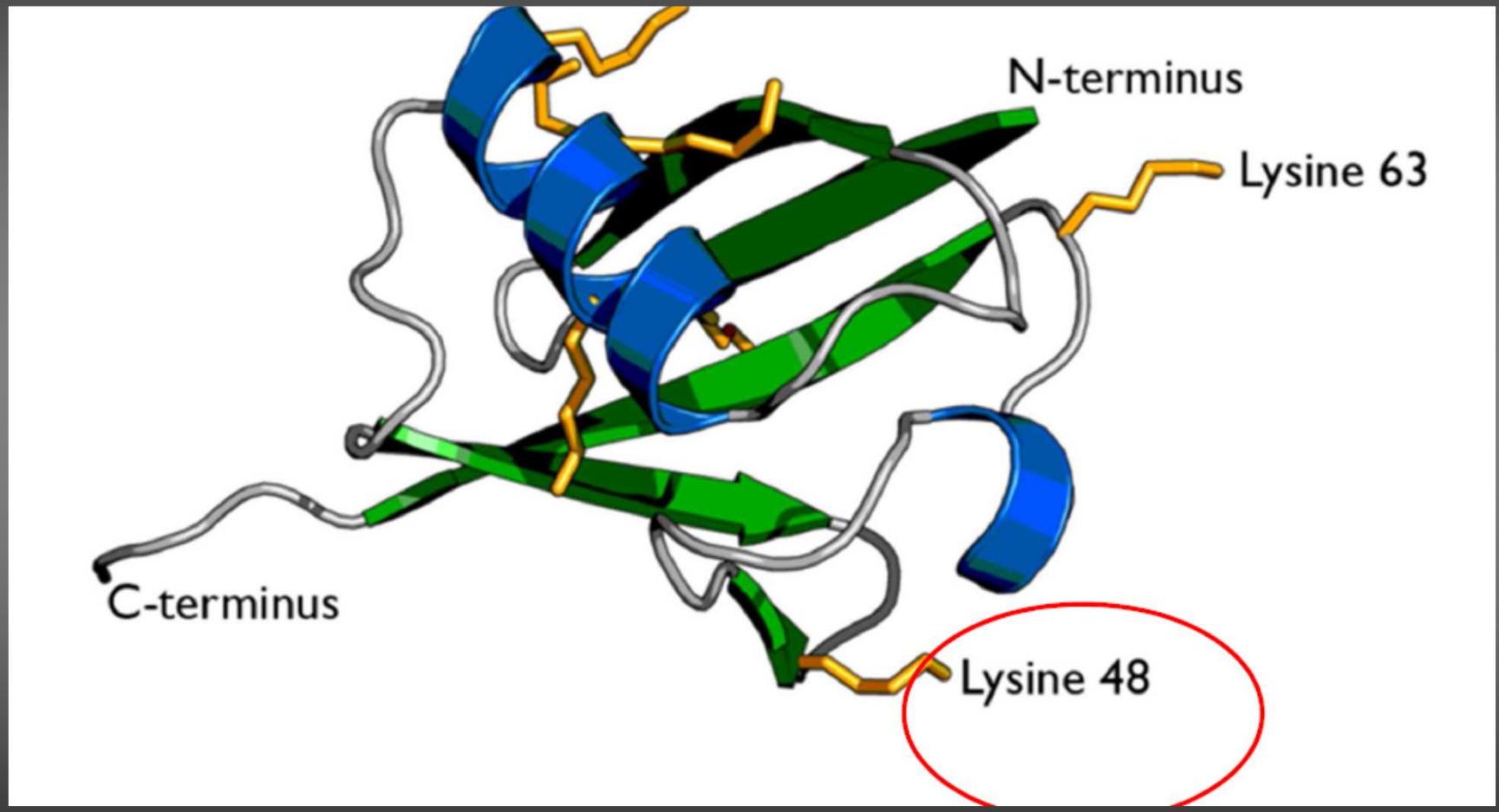
pVHL is the substrate-recognizing component of a multiprotein E3 ubiquitin ligase complex containing elongins C and B, Cullin 2, and the RING-H2 finger protein Rbx-1

UBIQUITINIZZAZIONE

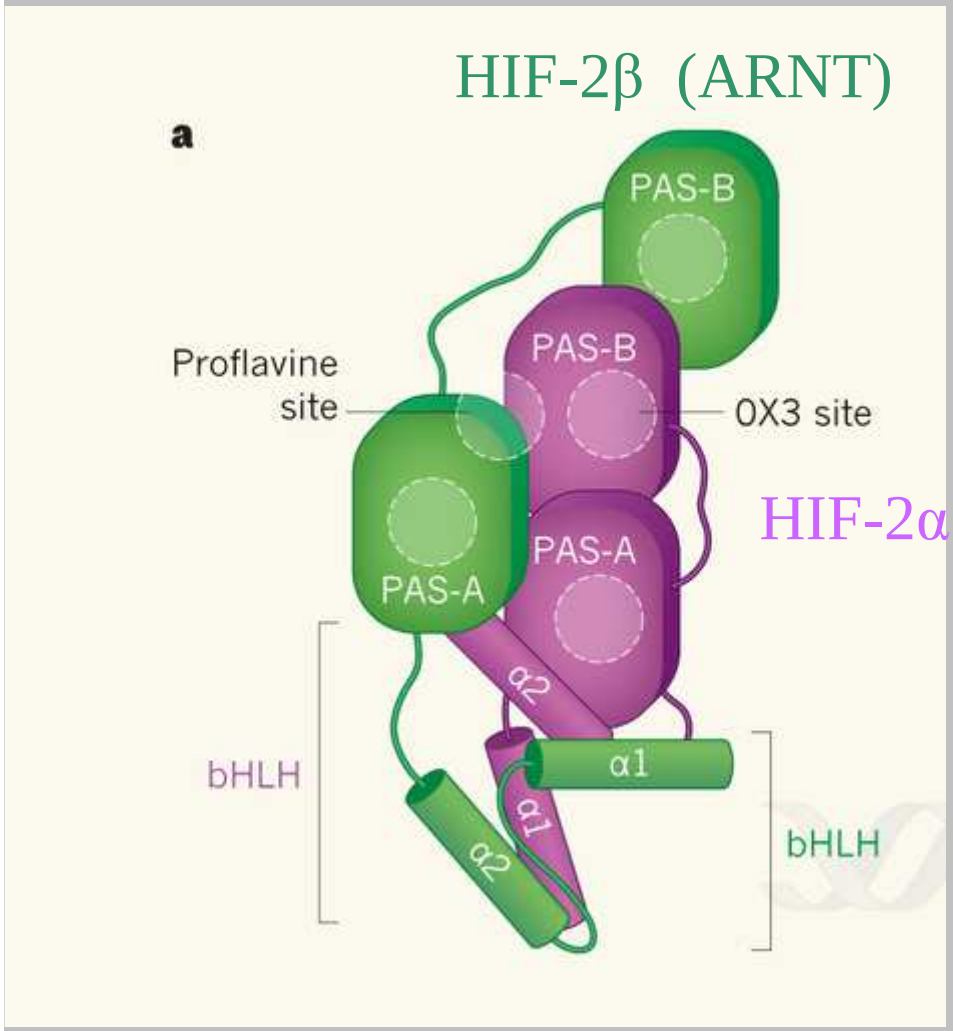

Ub
+ ATP
AMP + PPi
E1
SH
E1
E2
SH
E1
SH
E2
S
Ub
E2
S
Ub
E3
Substate
NH
Ub
Ub
Ub
Ub
E2
SH
E3
Substate
NH
Ub
E2
S
Ub
E3
Substate
NH2


Ub
C
O
OH
ATP
AMP
E1
SH
S
E2
E3
Substrate
Monoubiquitination
K48
K63
ES. VHL
ES DDR
Protein intracellular trafficking & recognition
Proteasomal degradation
Signal transduction Protein interaction

UBIQUITINA



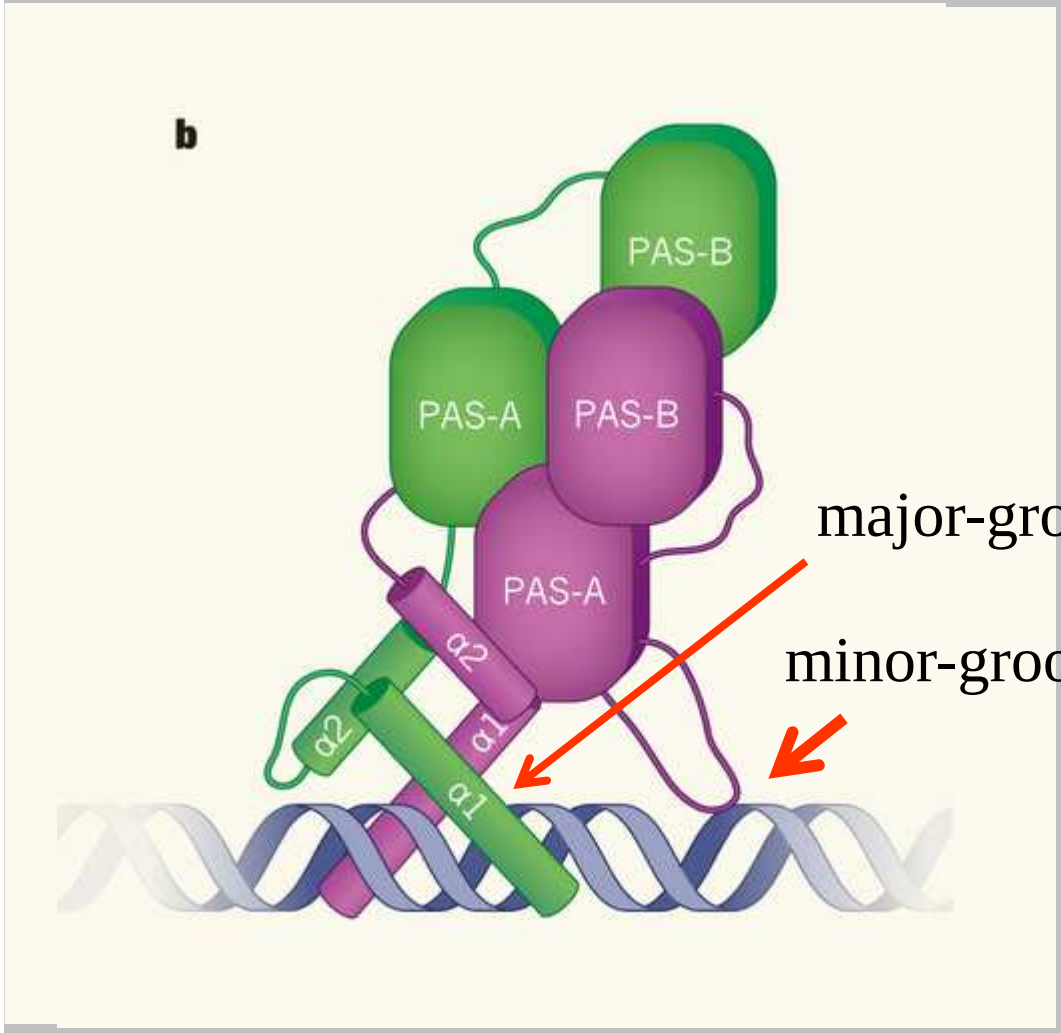
Struttura HIF



PAS,PAS-B (interaction domains)

bHLH (DNA Binding domain)

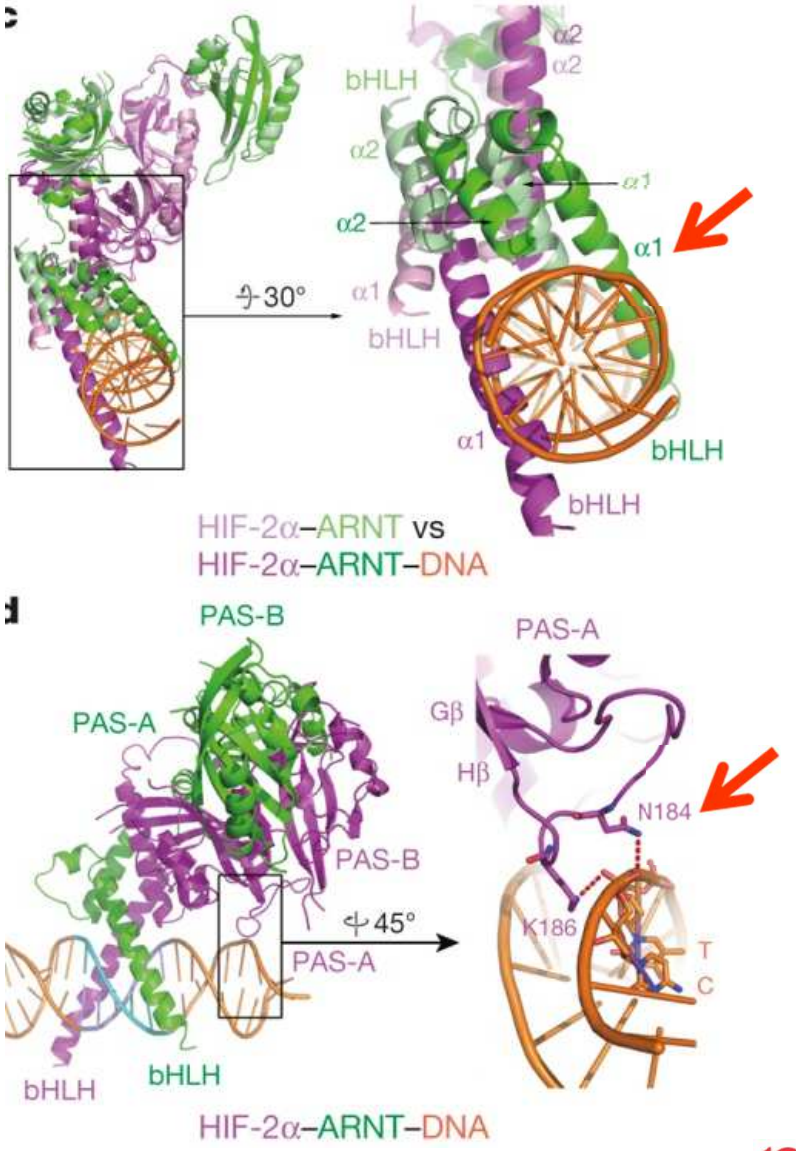
bHLH (DNA Binding domain
PAS,PAS-B (interaction domains



major-groove DNA interactions

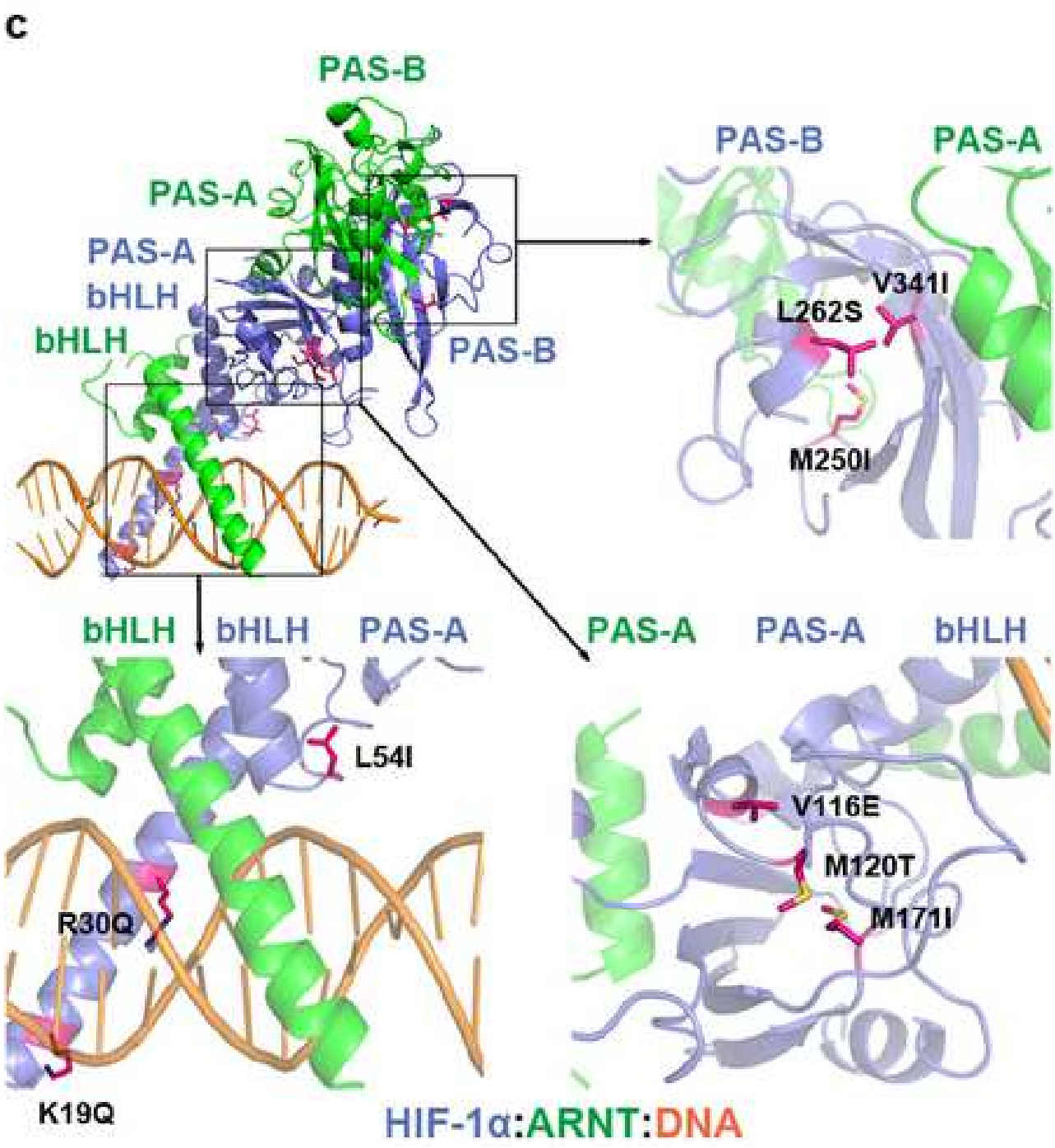
minor-groove DNA interactions

DNA-bound HIF-α–ARNT structures.



D Wu *et al. Nature* **000**, 1-6 (2015) doi:10.1038/nature14883

cancer-related mutations in HIF-1α



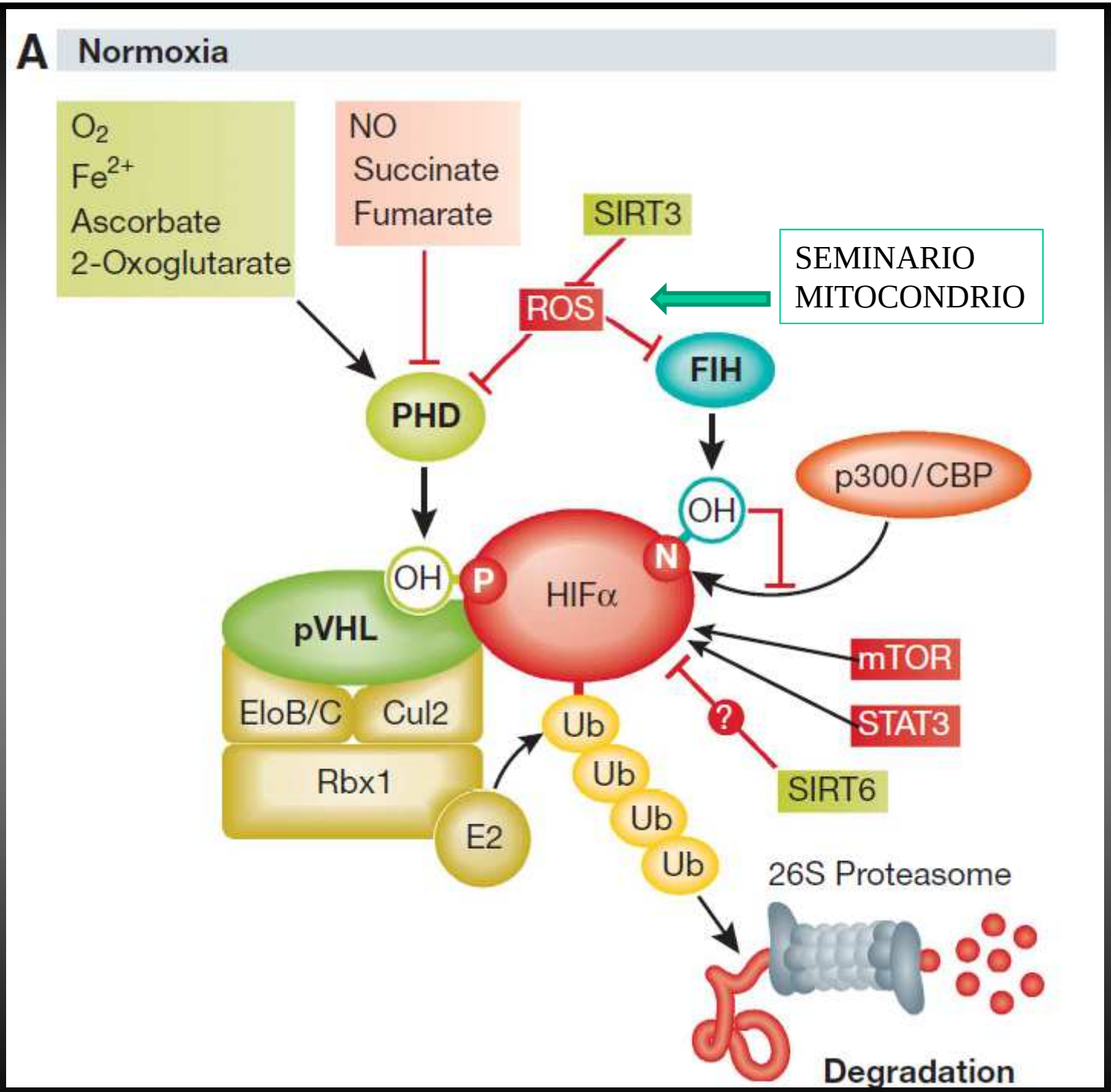
a

	Location	Possible Role	Primary Tissue (Subtype)	Associated Histology
HIF-2α				
K18E	bHLH α1	DNA interaction	Stomach	Adenocarcinoma
A23V	bHLH α1	DNA interaction	Endometrium	Endometrioid carcinoma
V47M	bHLH α1-α2 loop	Interface 6 (bHLH/PAS-A)	Central nervous system (brain)	Glioma
F98L	PAS-A Aβ	Internal stability	Large intestine (colon)	Adenocarcinoma
R166L	PAS-A Gβ	Internal stability	Kidney	Clear cell renal cell carcinoma
I223M	PAS-A Iβ	Interface 2 (PAS-A/PAS-A)	Lung	Adenocarcinoma
H248N	PAS-B Aβ	Internal stability	Large intestine (colon)	Adenocarcinoma
R275H	PAS-B Dα-Eα loop	Internal stability	Cervix	Squamous cell carcinoma
A277P	PAS-B Eα	Internal stability	Lung	Squamous cell carcinoma
E279V	PAS-B Eα	Internal stability	Liver	Hepatocellular carcinoma
HIF-1α				
K19Q	bHLH α1	DNA interaction	Endometrium	Endometrioid carcinoma
R30Q	bHLH α1	DNA interaction	Skin	Malignant melanoma
L54I	bHLH α1-α2 loop	Interface 6 (bHLH/PAS-A)	Kidney	Clear cell renal cell carcinoma
V116E	PAS-A Cα	Internal stability	Kidney	Clear cell renal cell carcinoma
M120T	PAS-A Cα	Internal stability	Large intestine (colon)	Adenocarcinoma
M171I	PAS-A Gβ	Internal stability	Kidney	Clear cell renal cell carcinoma
M250I	PAS-B Aβ-Bβ loop	Internal stability	Lung	Adenocarcinoma
L262S	PAS-B Cα	Internal stability	Skin	Malignant melanoma
V341I	PAS-B Iβ	Internal stability	Endometrium	Endometrioid carcinoma

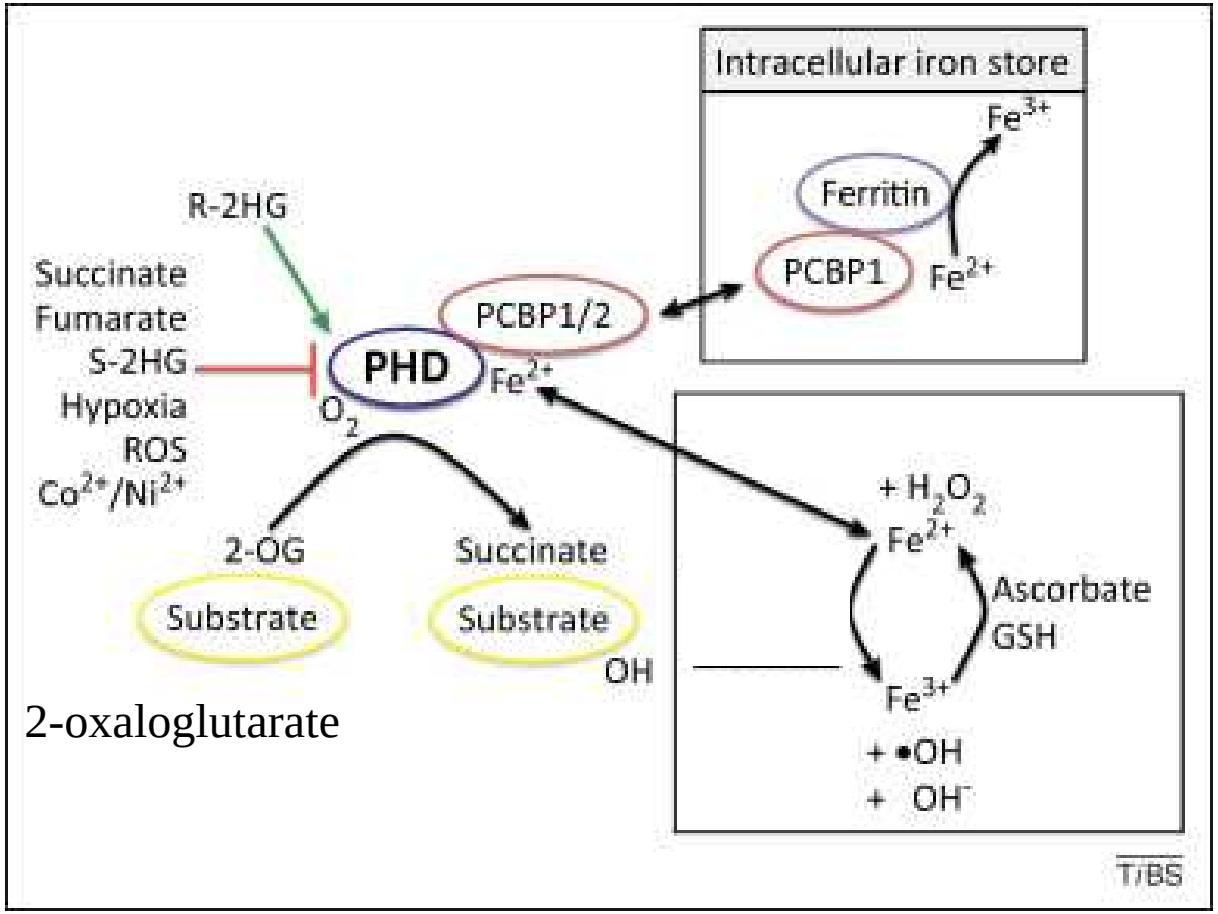
cancer-related mutations in HIF-2α and HIF-1α

Genere: carcinoma endometrio

Le prolil idrossilasi (PHD) hanno ruolo c
e sono finemente regolate


A Normoxia
O2
Fe2+
Ascorbate
2-Oxoglutarate
NO
Succinate
Fumarate
SIRT3
ROS
SEMINARIO
MITOCONDRIO
FIH
PHD
p300/CBP
OH
N
OH
P
HIFα
pVHL
EloB/C
Cul2
Rbx1
E2
Ub
Ub
Ub
Ub
mTOR
STAT3
?
SIRT6
26S Proteasome
Degradation

Regulation of prolyl hydroxylase domain enzyme (PHD) activity



PHDs require 2-oxaloglutarate and the cofactors oxygen and iron to hydroxylate substrates, such as hypoxia inducible factor (HIF)-1α. Inhibitory factors of PHD function include the metabolic intermediates succinate and fumarate, or 2-hydroxyglutarate (2-HG), which compete with 2-oxaloglutarate (2-OG); divalent metal ions such as Co2+ or Ni2+, which compete with Fe2+ binding to PHDs; and reactive oxygen species (ROS), which can disrupt oxygen interaction with PHDs.

PCBP1 not only delivers iron to ferritin for intracellular iron storage, but also delivers Fe2+ to PHDs, which is necessary for their activation and function.

Fe2+, which binds the proline substrate and the oxygen molecule, undergoes oxidation in the Fenton reaction. Ascorbate /glutathione maintains iron in the active site of PHDs in the reduced (ferrous) state.

g **Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes** Brian W. Won

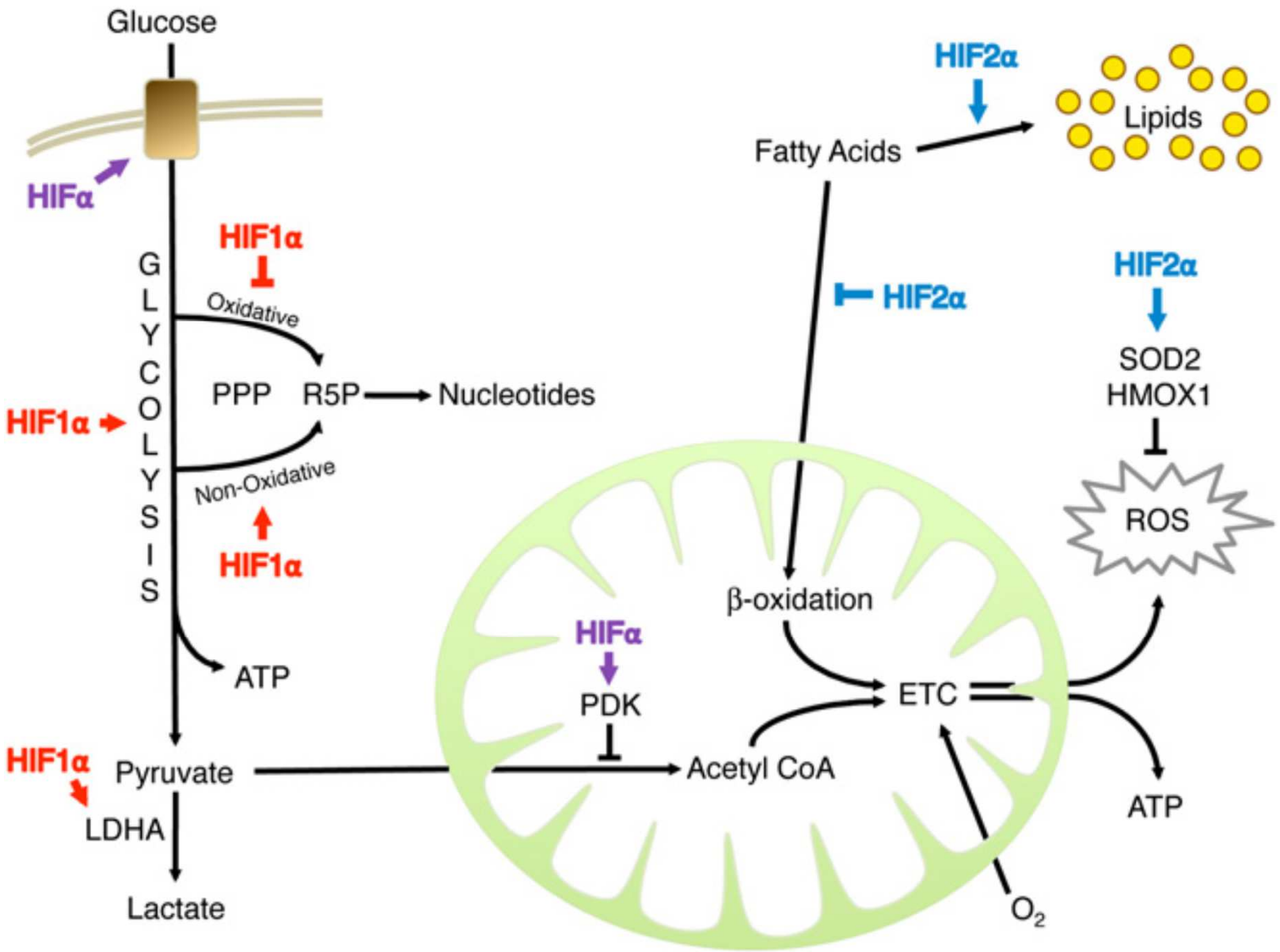
Concentrations of oxygen in tissues
- range 10–30 μM-

below the Km for oxygen of the hydroxylases

Concentrations of oxygen is limiting for enzyme activity over the entire physiological range.

HIF Metabolismo e Mitocondrio

HIFa Control of Cell Metabolism



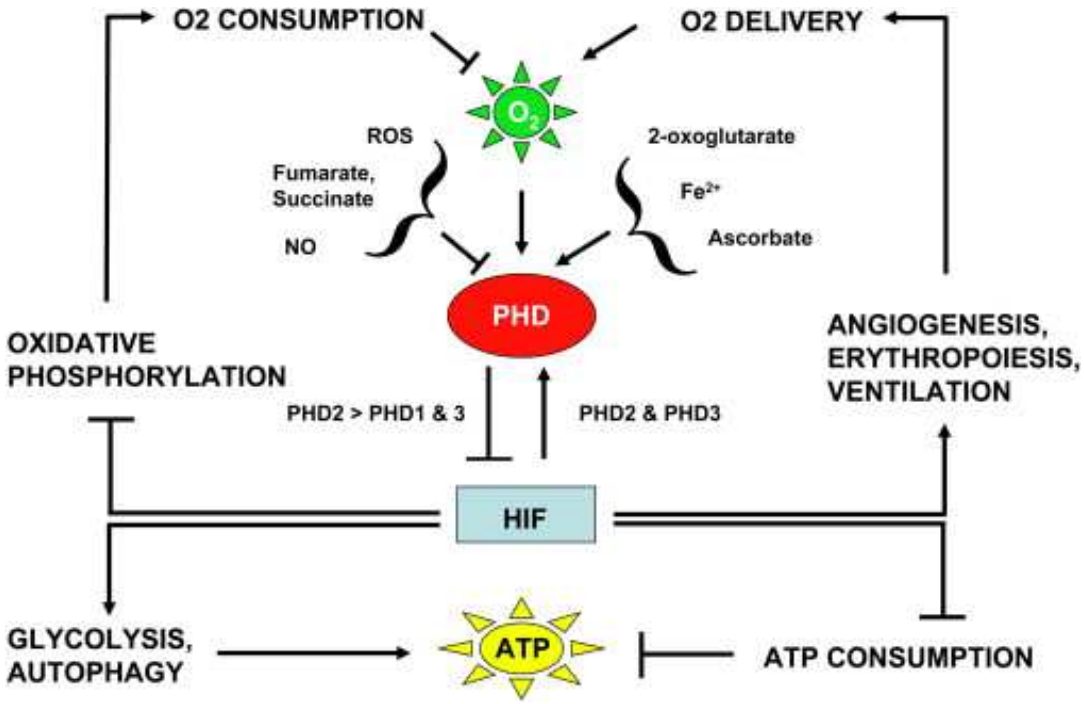
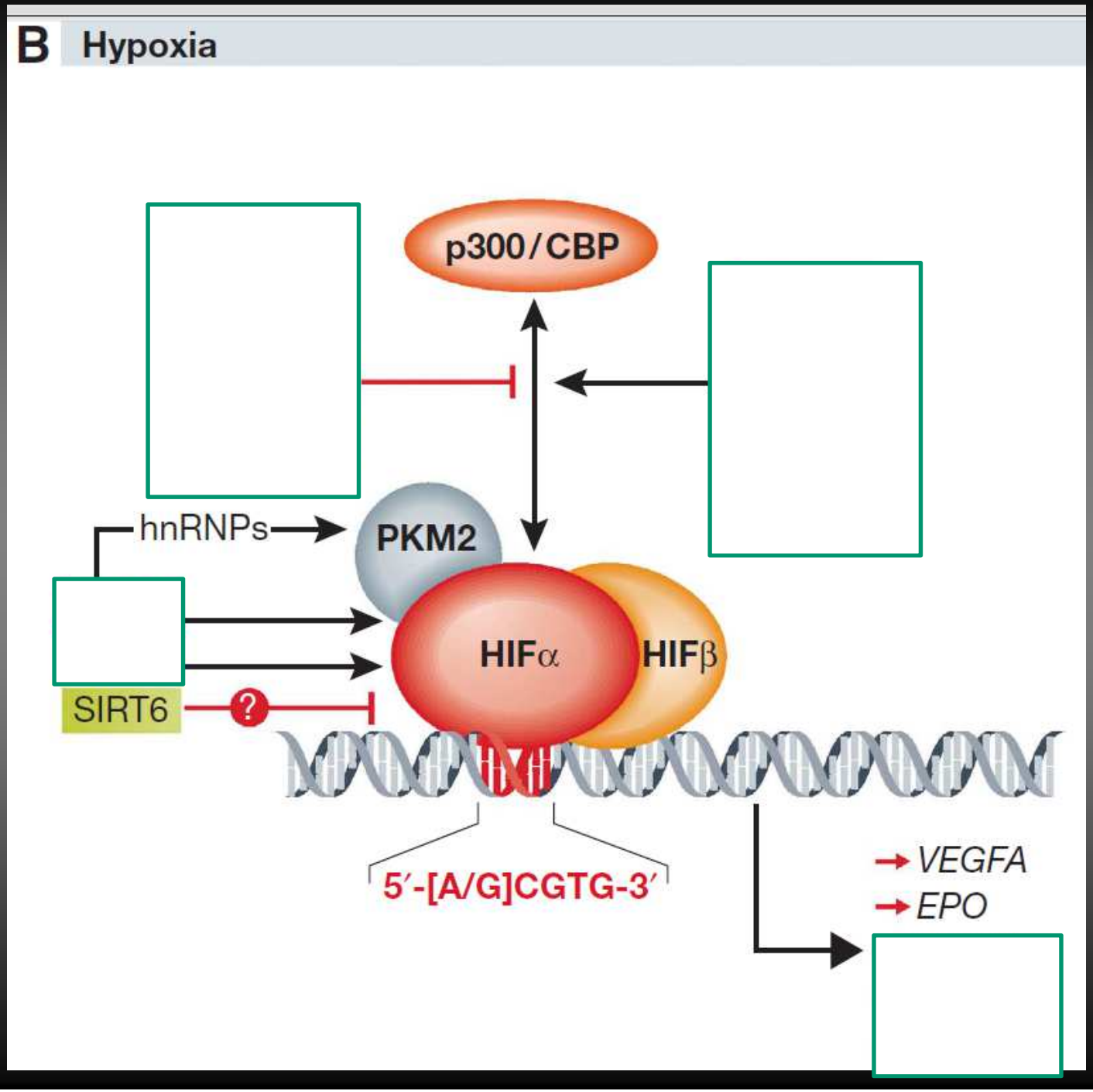


Figure 1. Central Role of PHD Prolyl Hydroxylases and the HIF Transcription Factor in Response to Hypoxia

Oxygen Sensing by Metazoans: The Central Role of the HIF Hydroxylase Pathway William G. Kaelin Jr., Peter J. Ratcliffe

null, Volume 30, Issue 4, 2008, 393–402


B Hypoxia
p300/CBP
hnRNPs
PKM2
HIFα
HIFβ
SIRT6
?
5′-[A/G]CGTG-3′
VEGFA
EPO

(B) Under low oxygen tension HIFa associates with HIFb.
The heterodimer binds to a core consensus sequence at the promoters of HIF-responsive genes, and upon binding to the coactivators p300/CBP and PKM2, initiates transcription.
The interaction between HIFa and p300 may be regulated by a variety of factors that to influence the transcriptional activity.

(PHD, prolyl-hydroxylase domain-containing enzyme; SIRT, sirtuin; FIH, factor inhibiting HIF; CBP, Creb-binding protein; OH, hydroxyl group; STAT3, signal transducer and activator of transcription 3; ub, ubiquitin moiety; EloB/C, elongins B and C; Cul2, cullin 2; pVHL, von Hippel-Lindau protein; ROS, reactive oxygen species; CITED2/4, CBP/p300 interacting transactivator; PKM2, pyruvate kinase isoform M2; hnRNPs, heterogeneous nuclear ribonucleoproteins).

Mutazioni nella Pathway oxygen sensing

Policitemia di Chuvash

Ang et al. Nature Genetics 2002

- Policitemia autosomica recessiva trovata in Russia

Table 1 • Biochemical parameters in Chuvash polycythemia

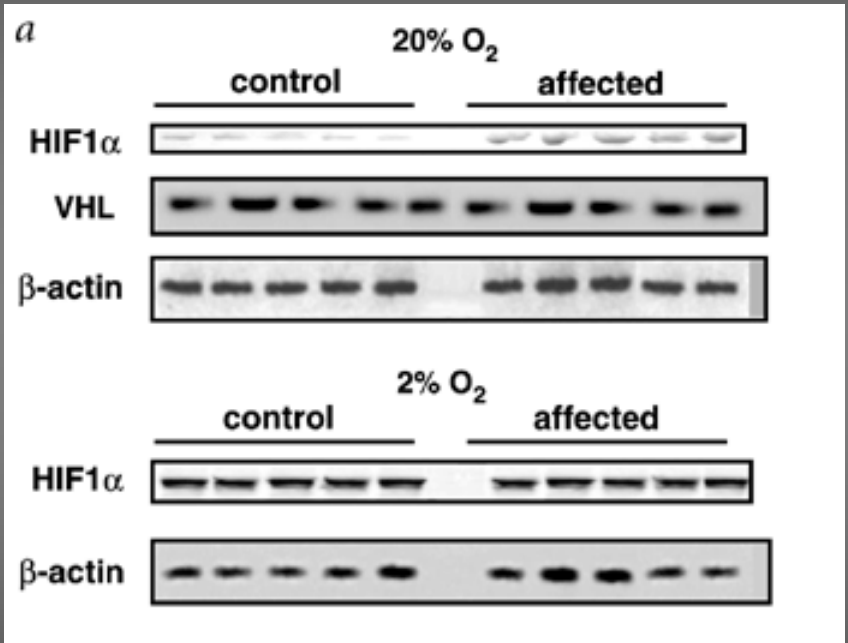
	Individuals with Chuvash polycythemia ($n = 20$)	Unaffected relatives ($n = 51$)	P
Erythropoietin (mIU ml^{-1})	61.9 ± 12.8	6.4 ± 6.9	0.001
Serum ferritin[a] (ng ml^{-1})	19 (15–24)	28 (25–32)	0.2
Serum iron (µg dL^{-1})	64 ± 15	81 ± 9	0.4
Total iron binding capacity (µg dL^{-1})	427 ± 18	346 ± 10	0.001
Transferrin saturation (%)	16 ± 4	24 ± 2	0.1

Sequenziamento gene von Hippel Lindau (VHL) C/T transition, Arg/Trp200 (Pazienti omozigoti)

Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia

Sonny O. Ang

Nature genetics 2002, volume 32 no. 4 pp 614 - 621



Western blot, 5 pazienti + 5 controlli

20% O_2:

- Livelli di proteina VHL normali in mutato e Wt
- Livelli di HIF1α maggiori nei soggetti affetti

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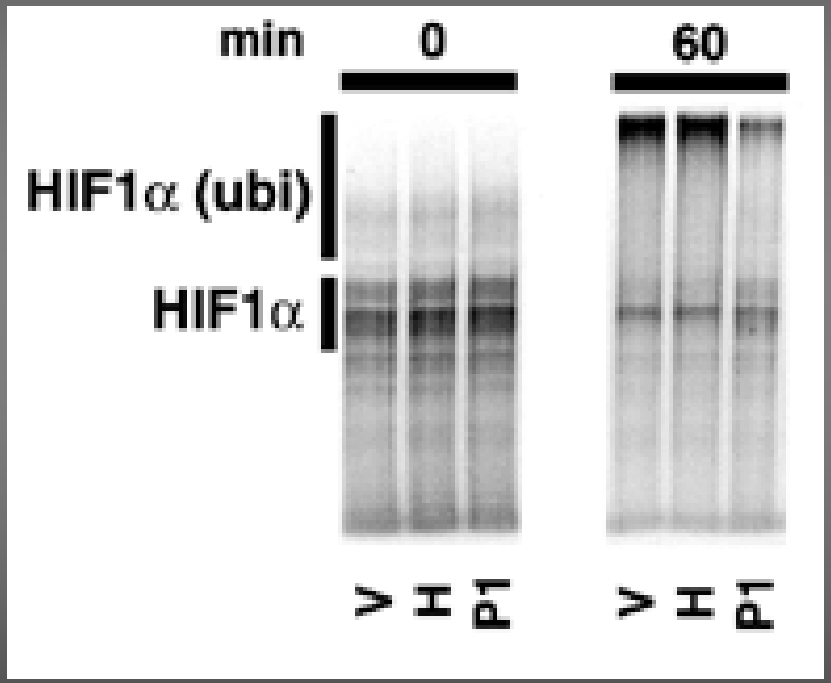


V= controllo (Wild type)

H= eterozigote

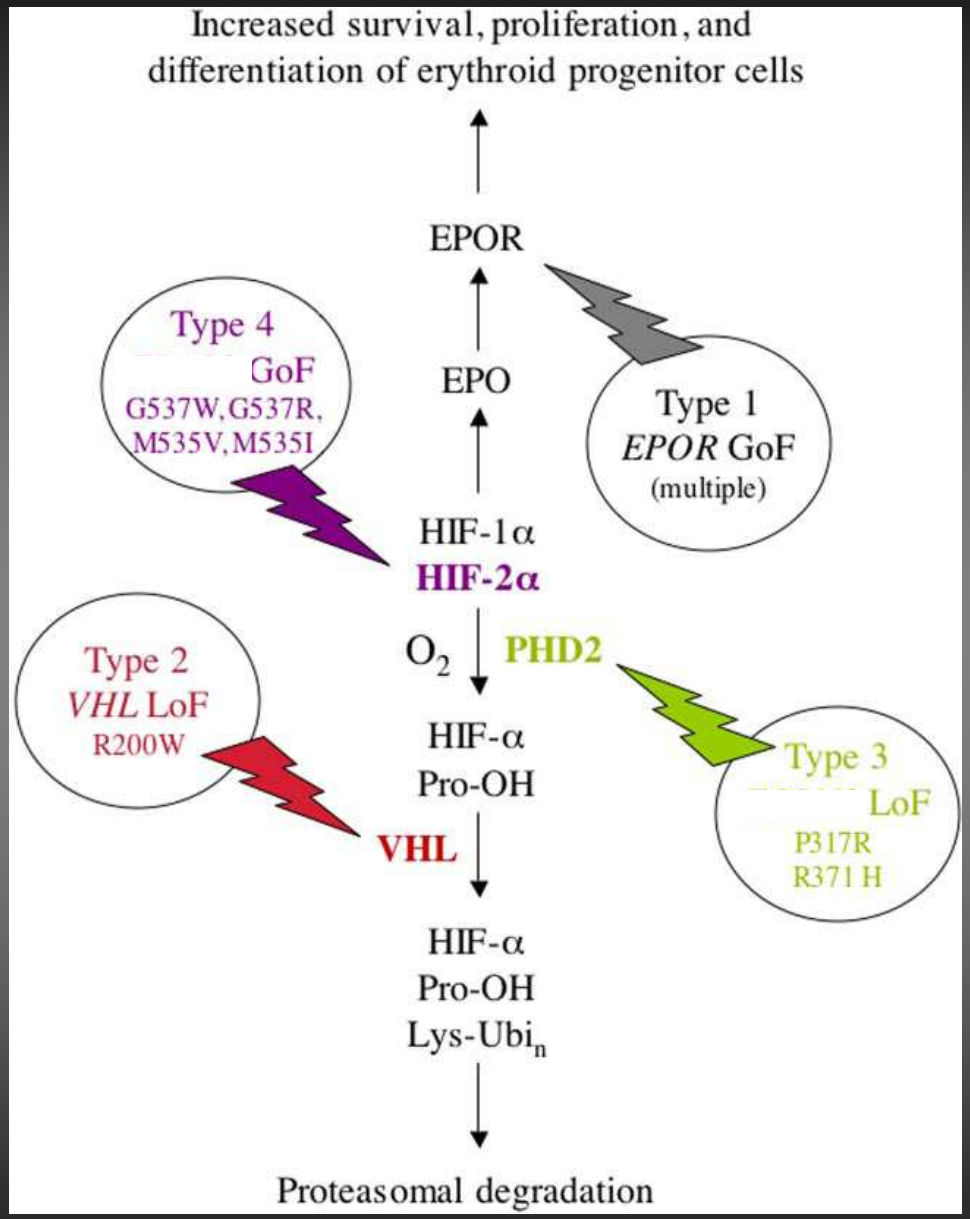
P1= paziente (omozigote)

La forma ubiquitinizzata è meno presente nelle cellule del paziente

Mutazione Arg200Trp:

- Ridotta ubiquitinizzazione di HIF1α

- Aumentata espressione del gene Epo policitemia


Increased survival, proliferation, and differentiation of erythroid progenitor cells
EPOR
EPO
HIF-1α
HIF-2α
O2
PHD2
HIF-α
Pro-OH
VHL
HIF-α
Pro-OH
Lys-Ubin
Proteasomal degradation
Type 4 GoF G537W, G537R, M535V, M535I
Type 1 EPOR GoF (multiple)
Type 2 VHL LoF R200W
Type 3 LoF P317R R371H