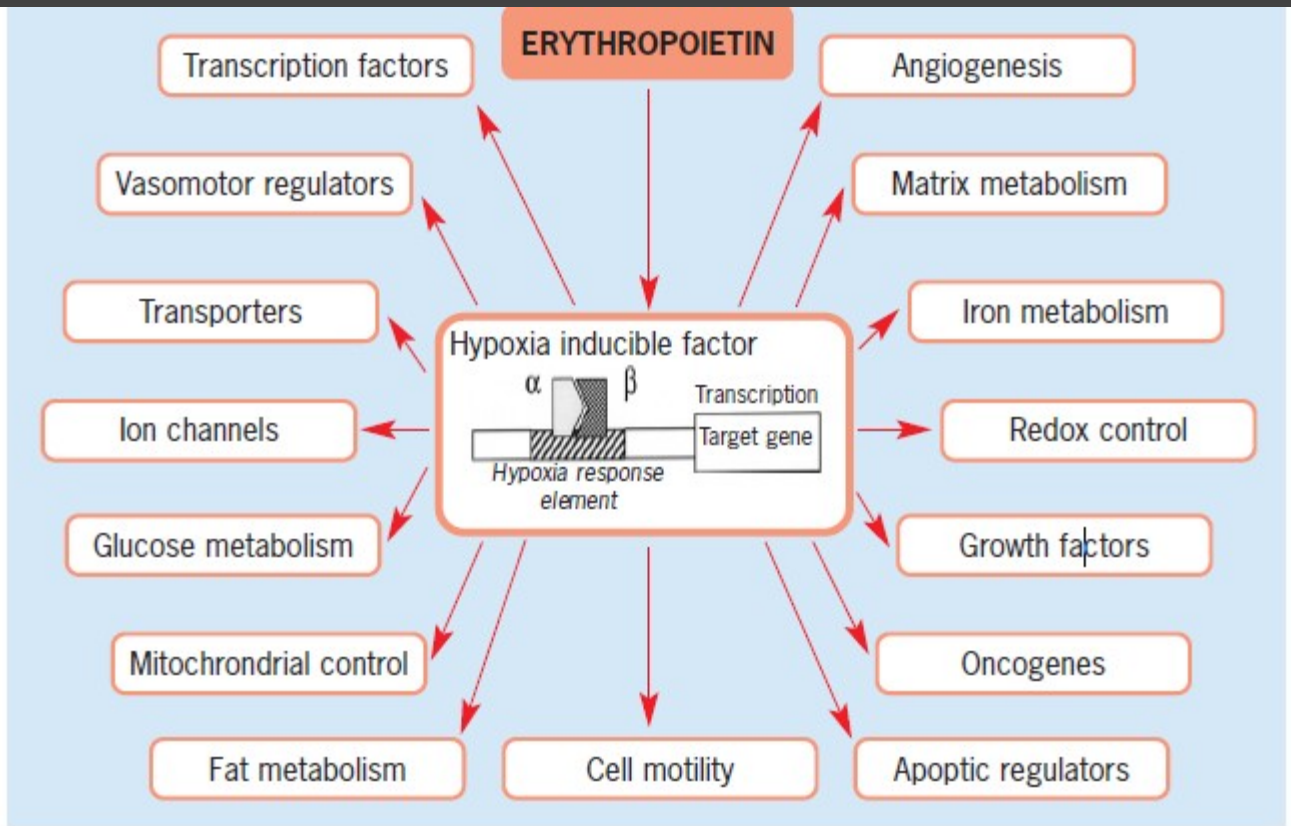
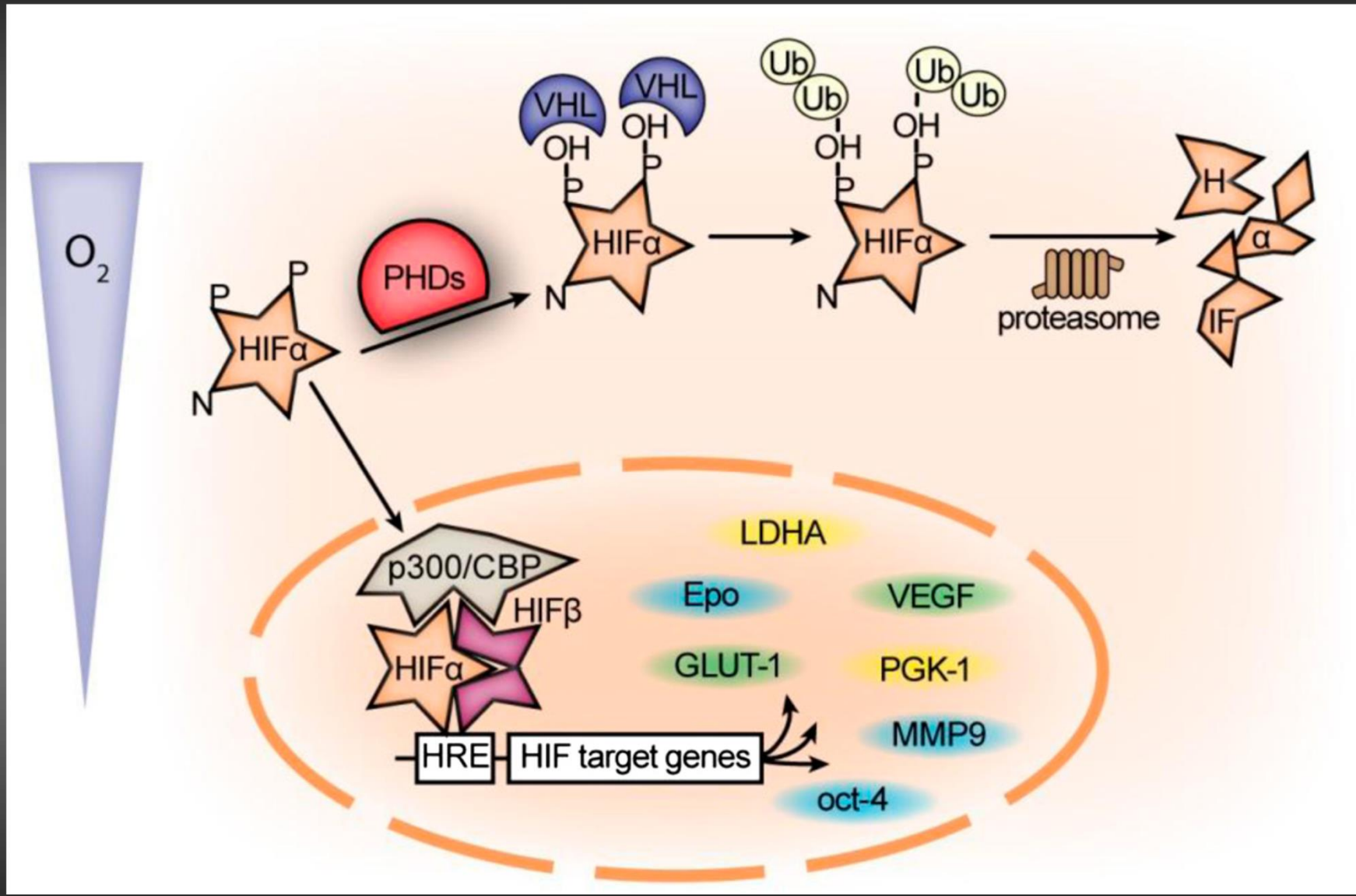
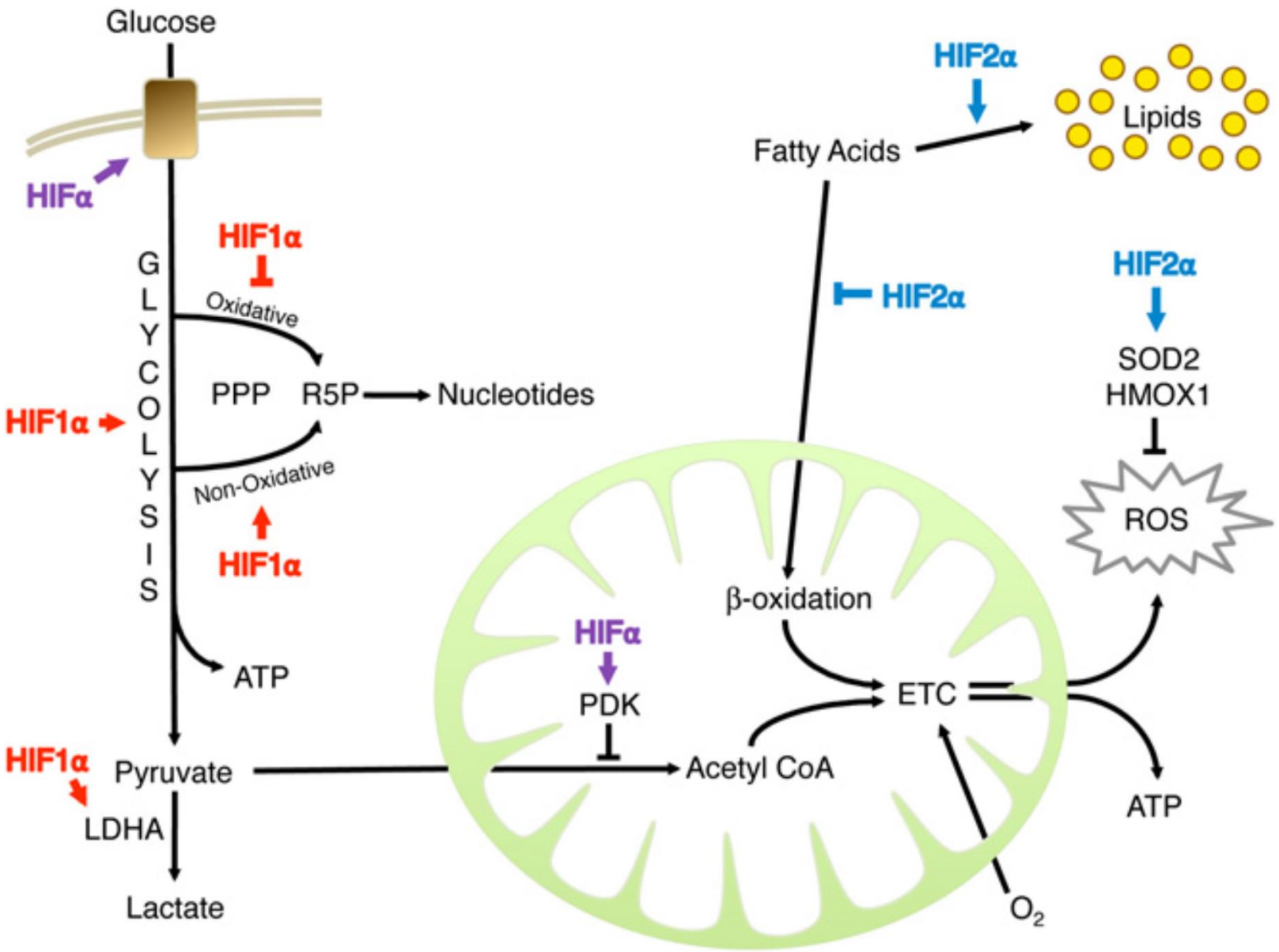


Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.


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

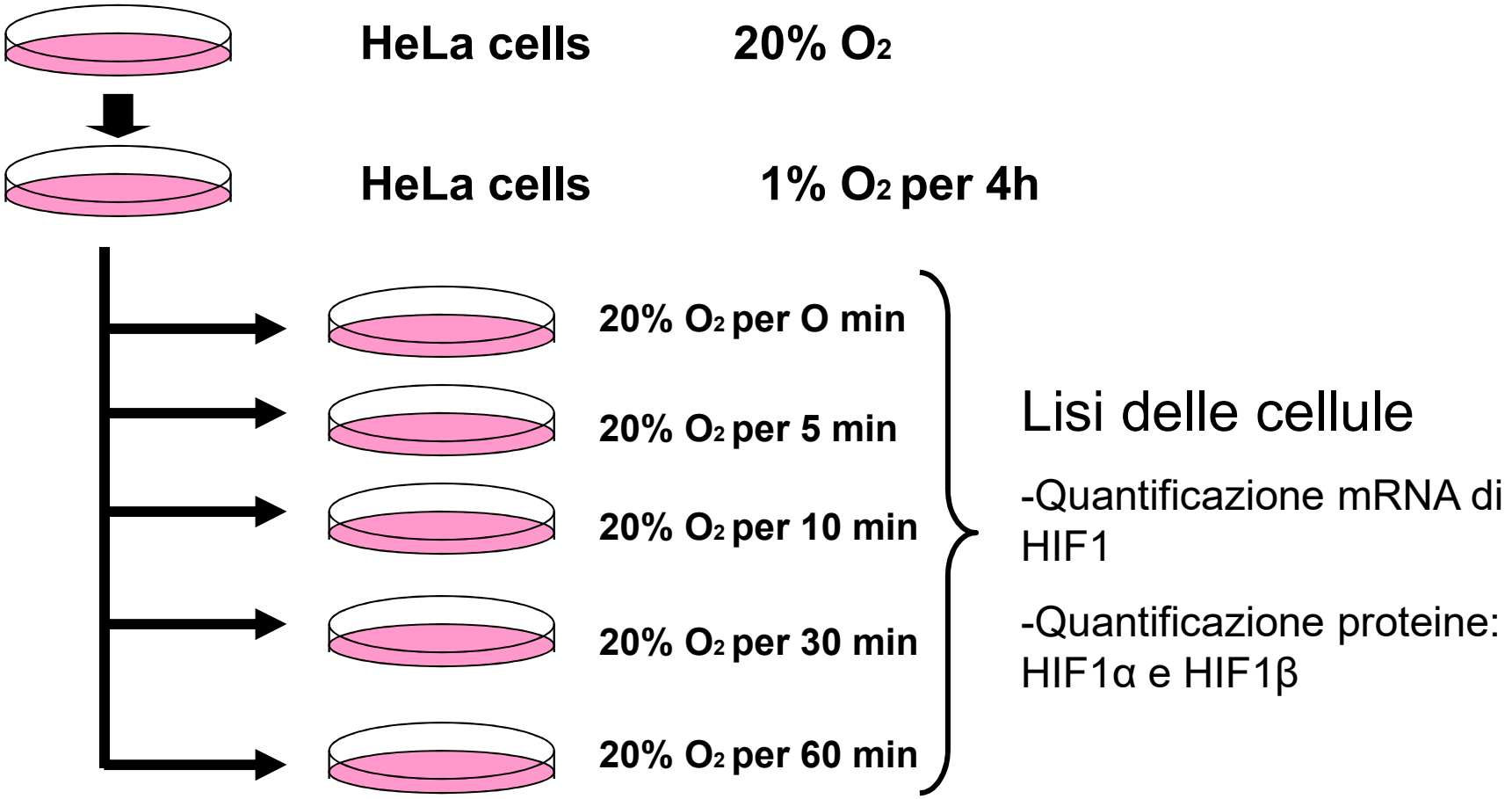
HIF Metabolismo e Mitocondrio

HIFa Control of Cell Metabolism



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

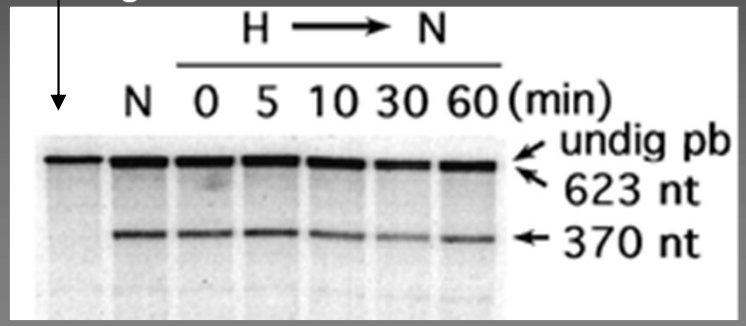
Eric Huang et al. - JBC 1996



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

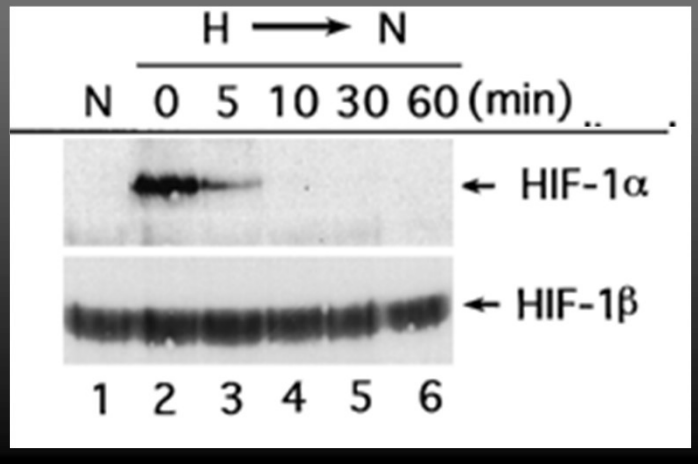
Sonda indigerita



HIF1α è espresso a livello di mRNA.

Quantificazione proteine → **Western blot**

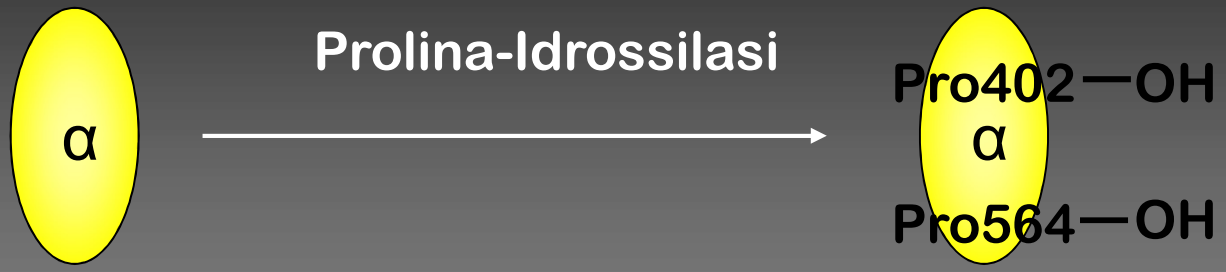
Ibridazione con anticorpi anti HIF1α e HIF1β



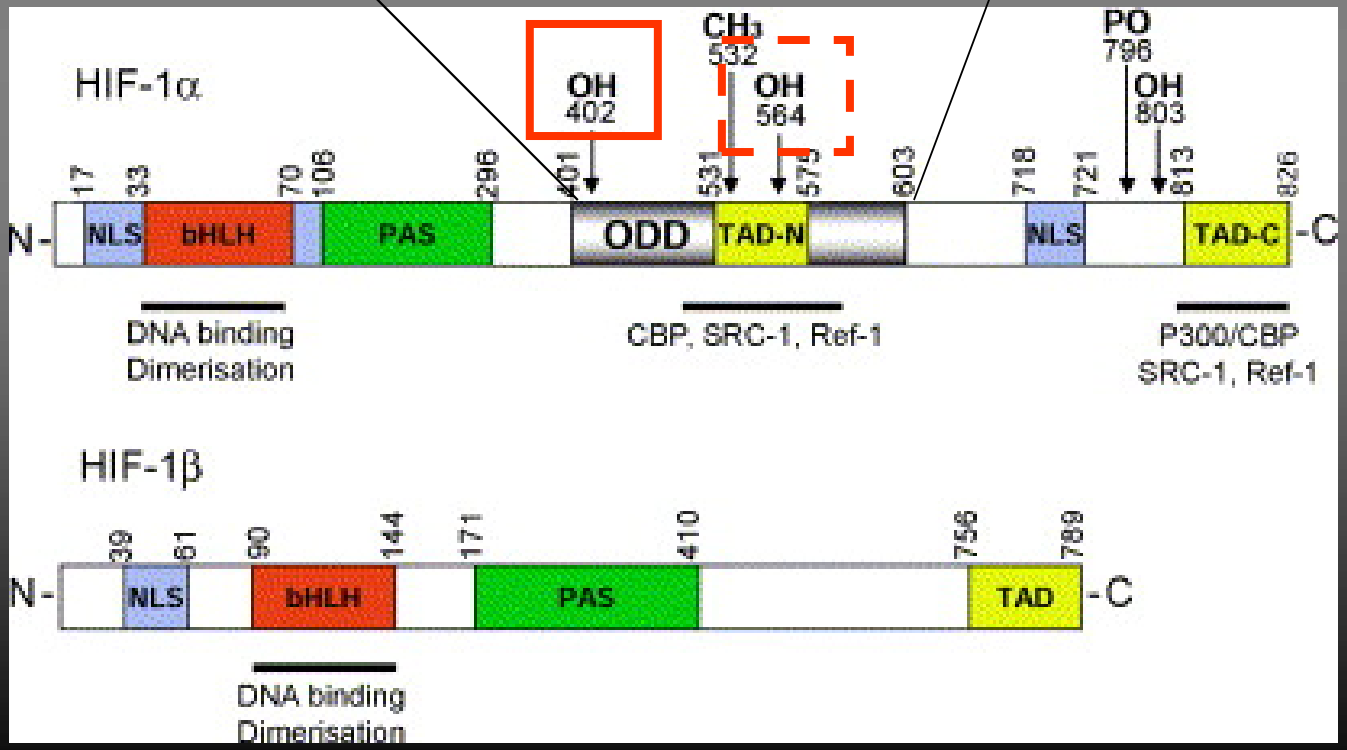
HIF1α è presente solo in condizioni di ipossia

HIF1β è sempre presente

Struttura di HIF1

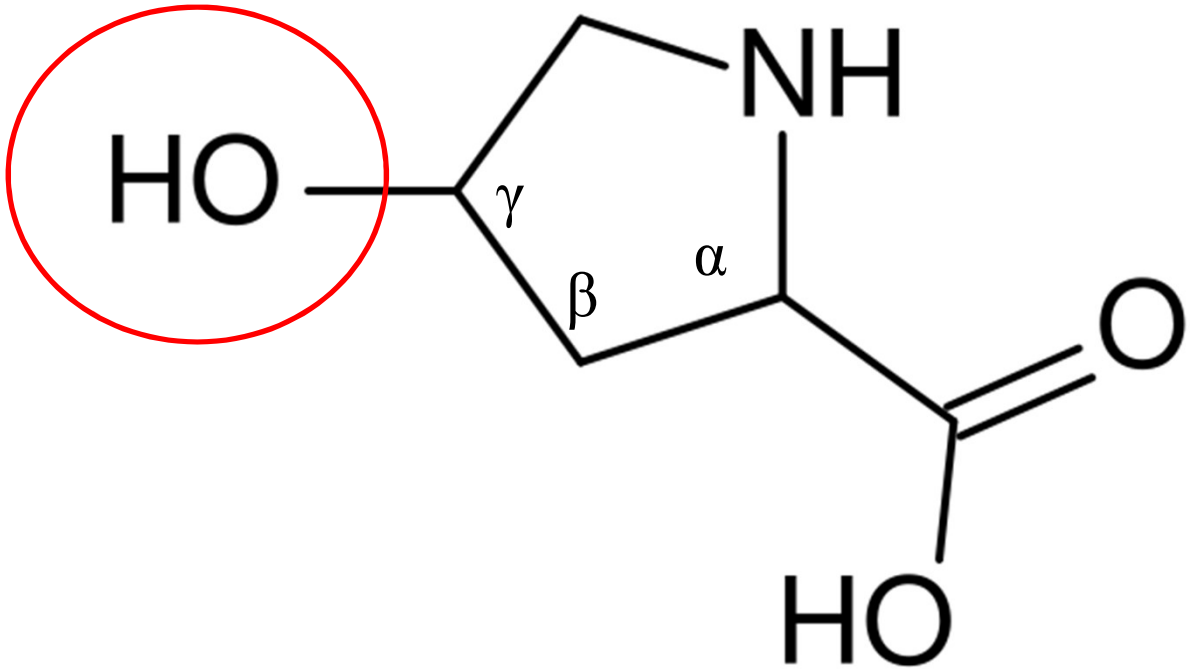


Sequenza di idrossilazione



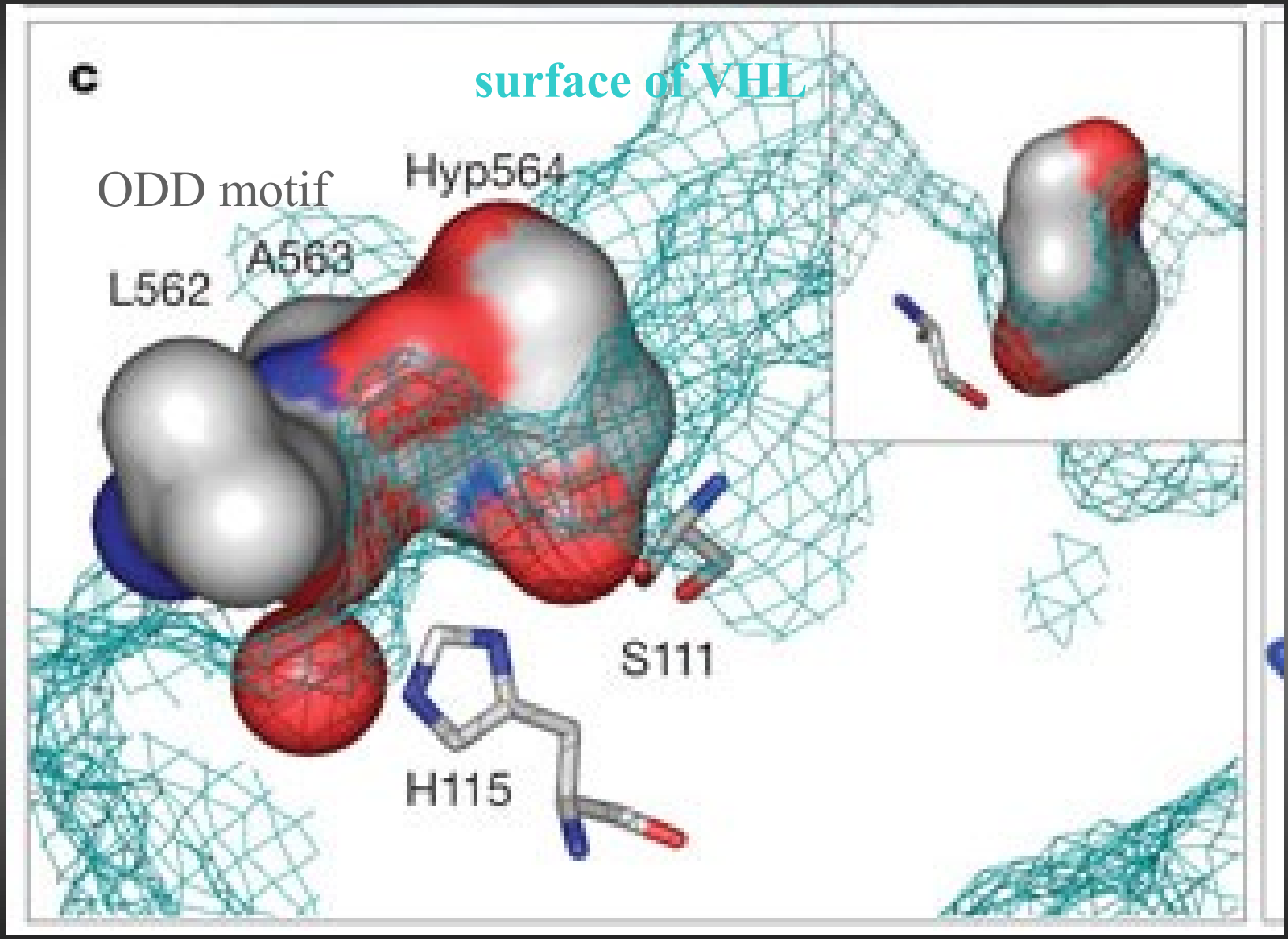
Riconoscimento specifico dell'idrossiprolina da parte del complesso di VHL

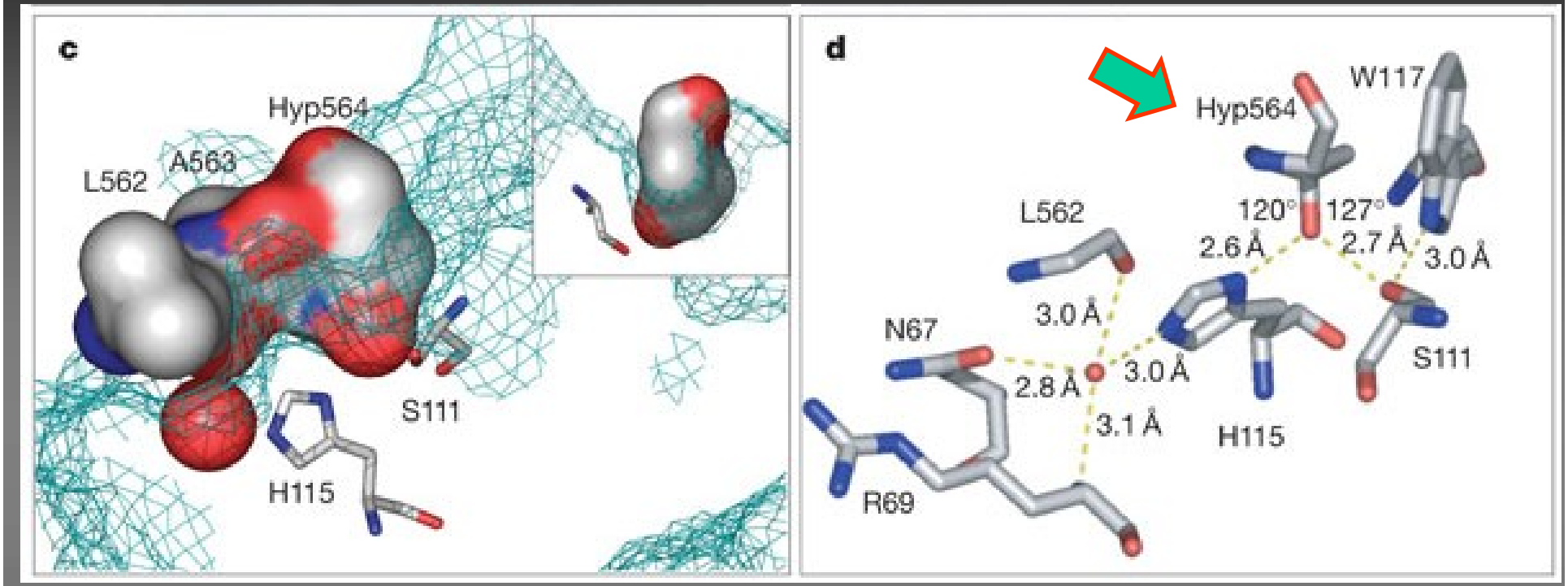
Idrossiprolina - Hyp



4-hydroxypyrrolidine-2-carboxylic acid

Hyp-binding pocket (VHL)



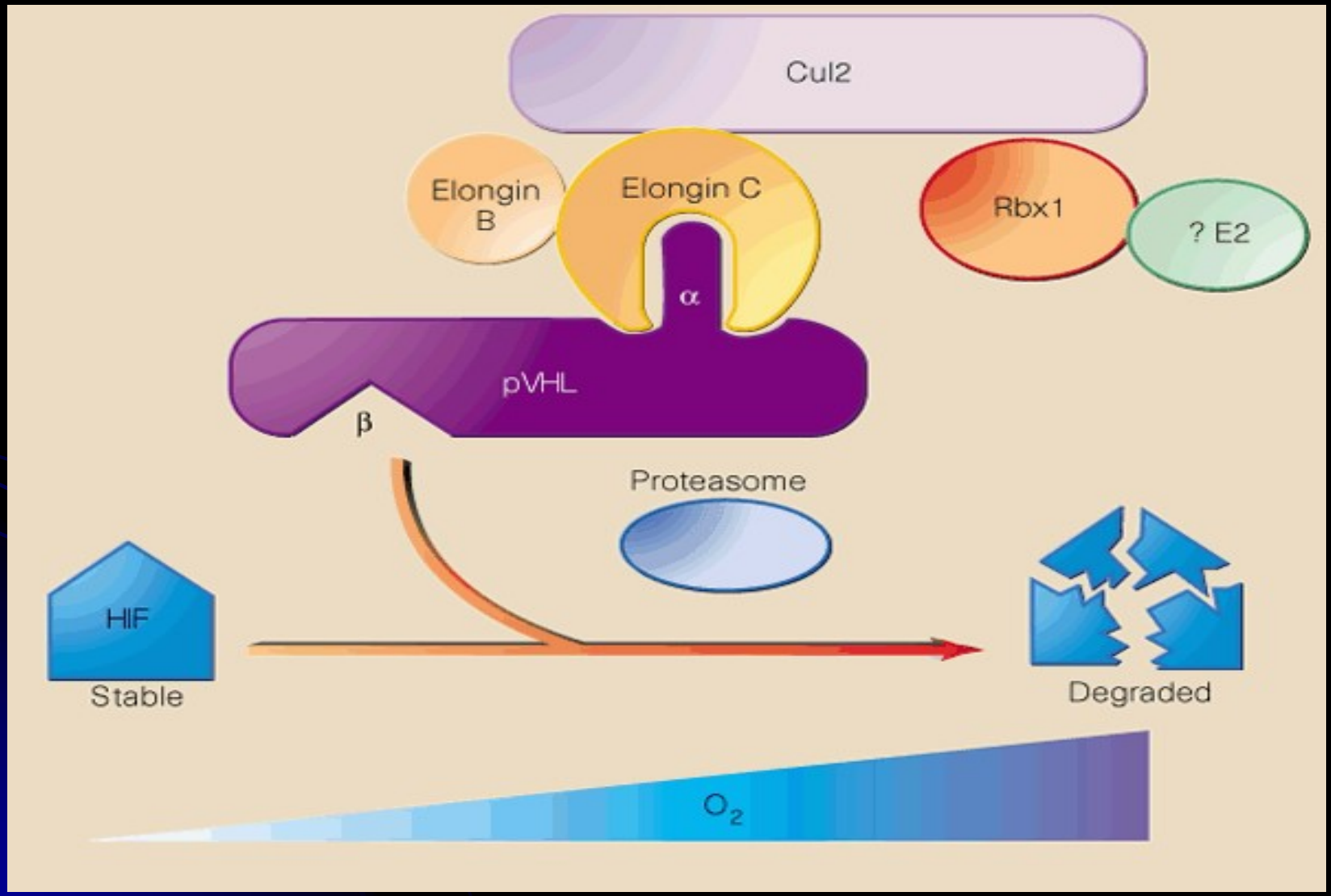


The hydrogen-bonding network (VHL) involved in binding of the Hyp564 hydroxyl group (Hif)

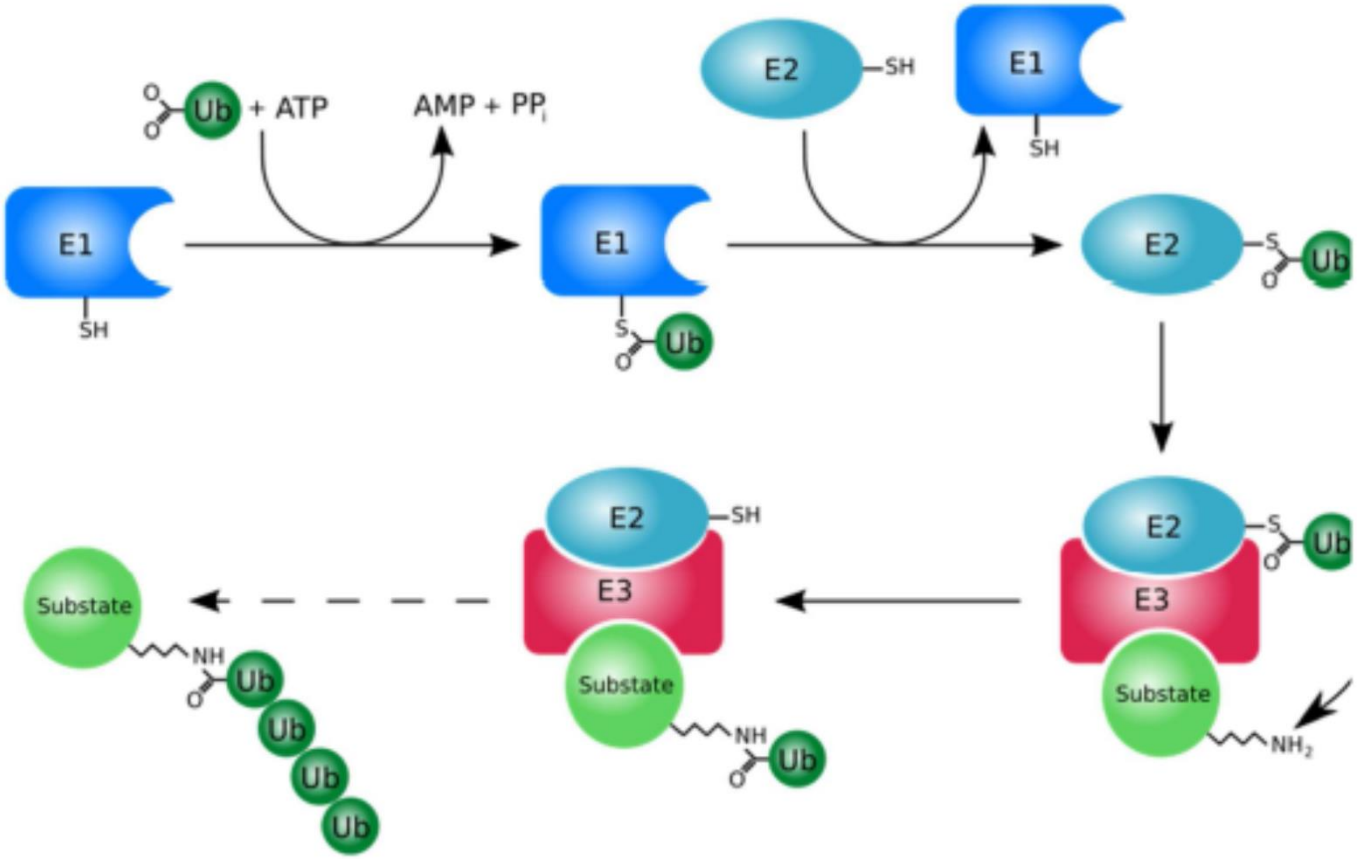
red sphere = key water molecule

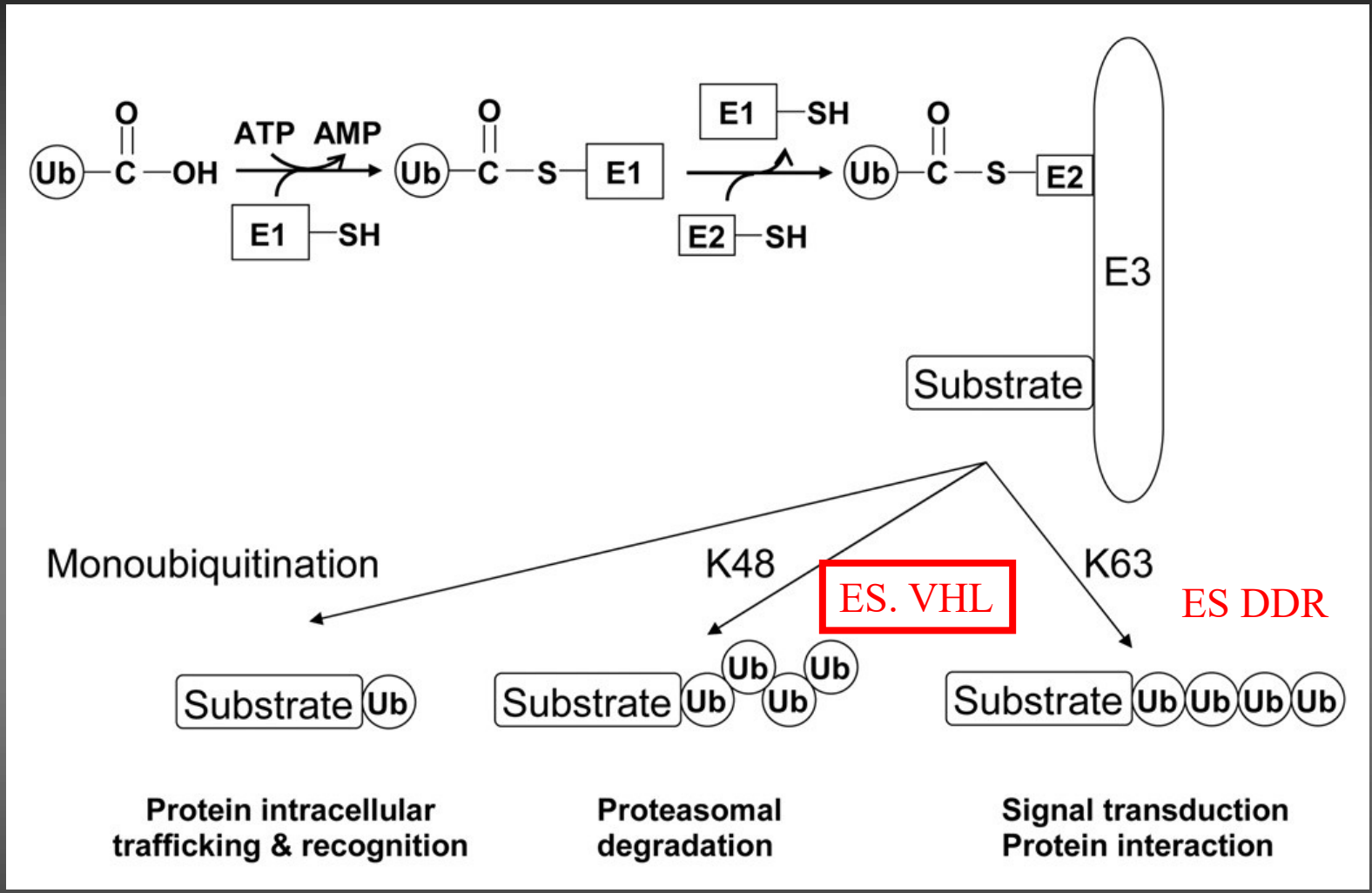
HIF e VHL fanno parte di complessi molecolari con molte componenti

VHL Function

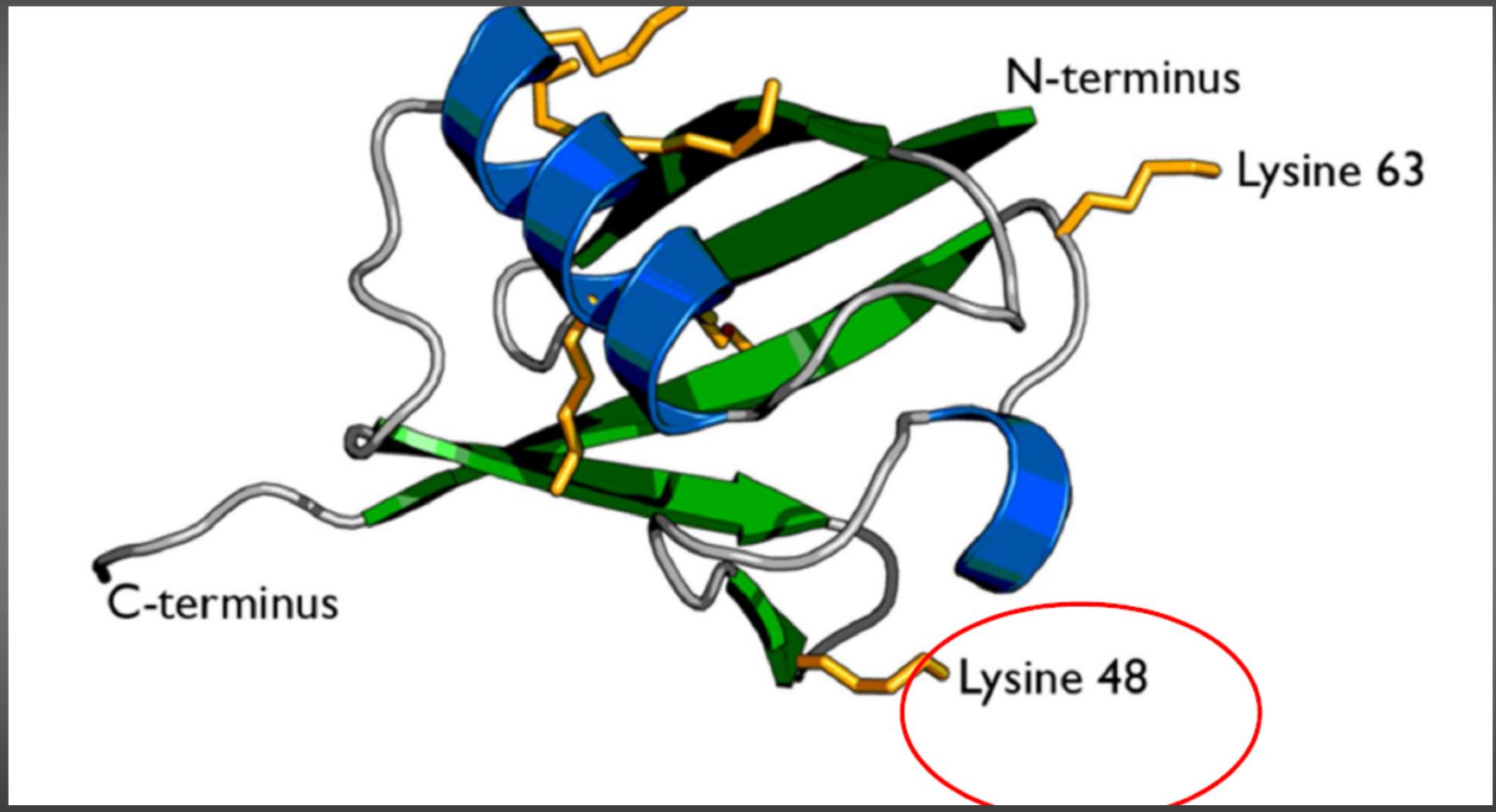


UBIQUITINIZZAZIONE

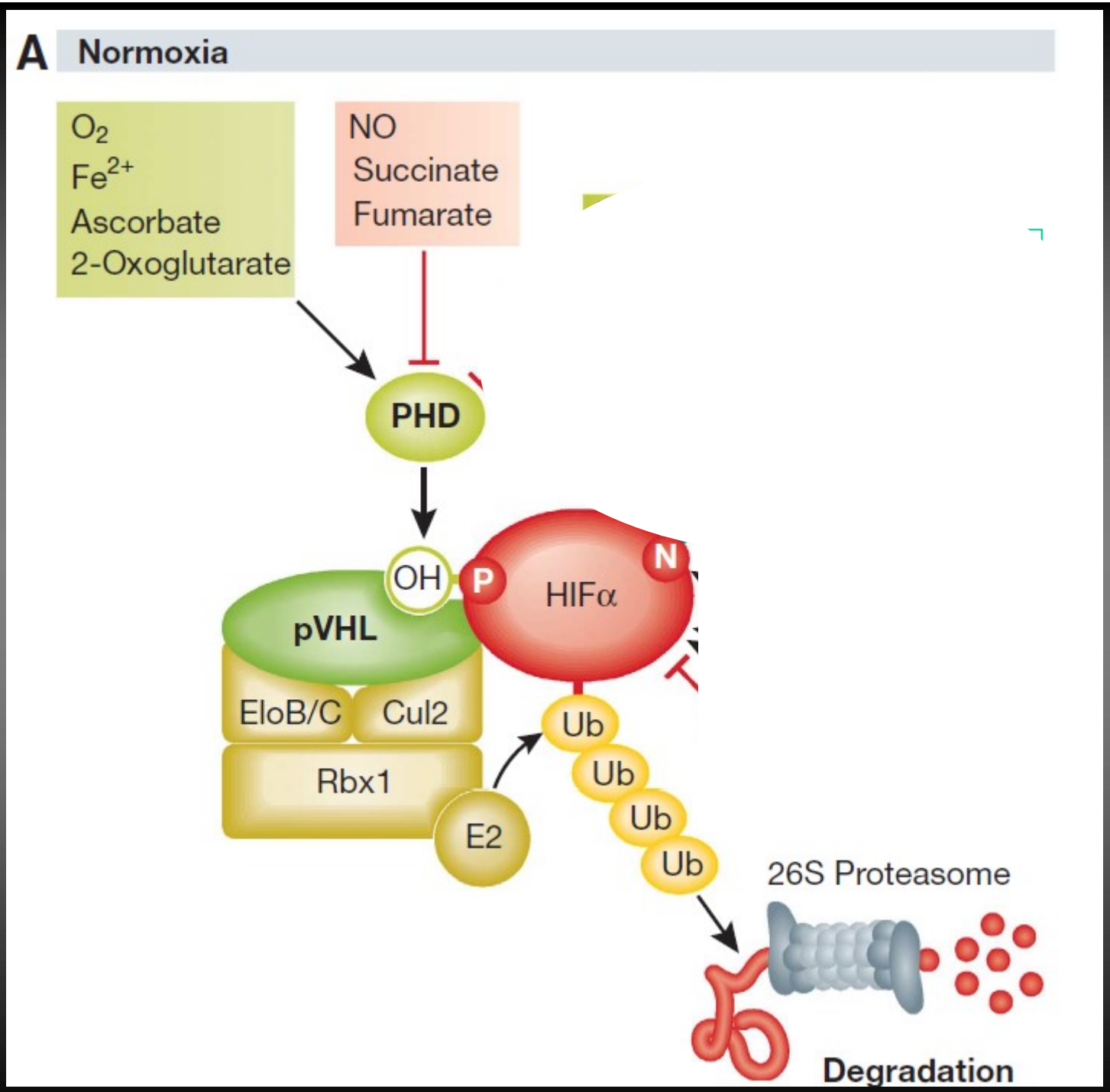

Ub
+ ATP
AMP + PPi
E1
SH
E1
S
Ub
E2
SH
E1
SH
E2
S
Ub
E2
SH
E3
Substate
NH
Ub
Ub
Ub
Ub
E2
S
Ub
E3
Substate
NH2


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



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


A Normoxia
O_2
Fe^{2+}
Ascorbate
2-Oxoglutarate
NO
Succinate
Fumarate
PHD
OH
P
N
HIFα
pVHL
EloB/C
Cul2
Rbx1
E2
Ub
Ub
Ub
Ub
26S Proteasome
Degradation

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.

Basi molecolari della policitemia

Policitemia di Chuvash

Ang et al. Nature Genetics 2002

- Policitemia autosomica recessiva trovata in Russia

Table 1 • Biochemical parameters in Chuvash polycythemia

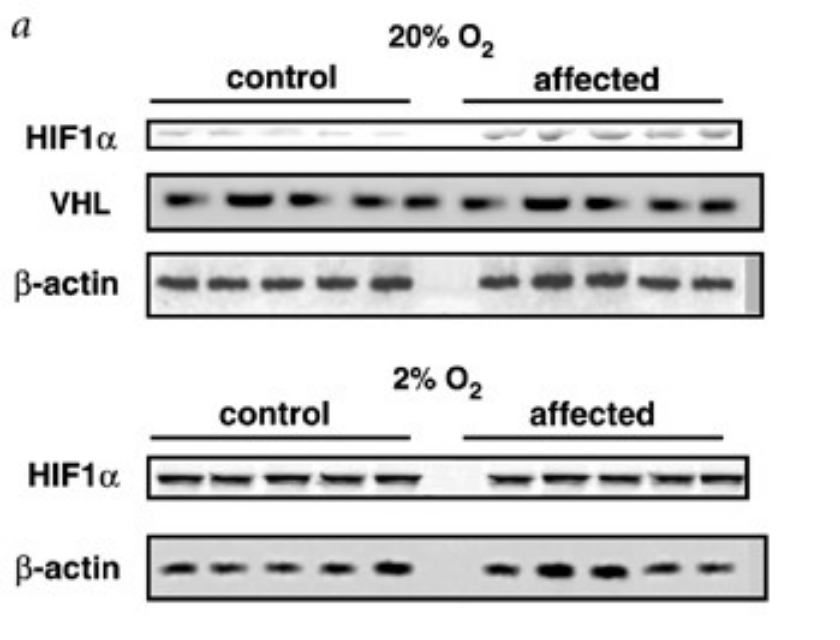
	Individuals with Chuvash polycythemia ($n = 20$)	Unaffected relatives ($n = 51$)	P
Erythropoietin (mIU ml^{-1})	61.9 ± 12.8	6.4 ± 6.9	0.001
Serum ferritin[a] (ng ml^{-1})	19 (15–24)	28 (25–32)	0.2
Serum iron (µg dL^{-1})	64 ± 15	81 ± 9	0.4
Total iron binding capacity (µg dL^{-1})	427 ± 18	346 ± 10	0.001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

Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia
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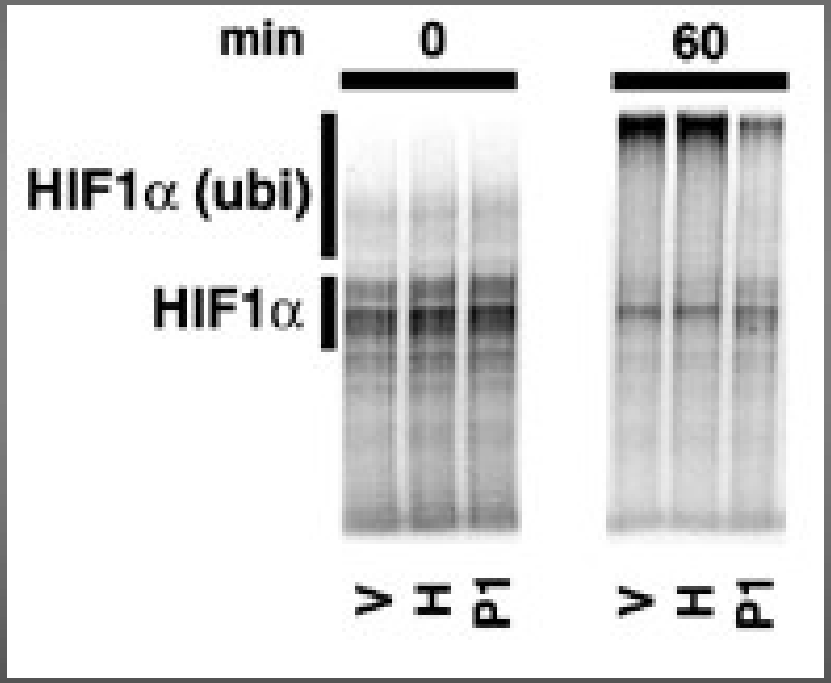


V= controllo (Wild type)

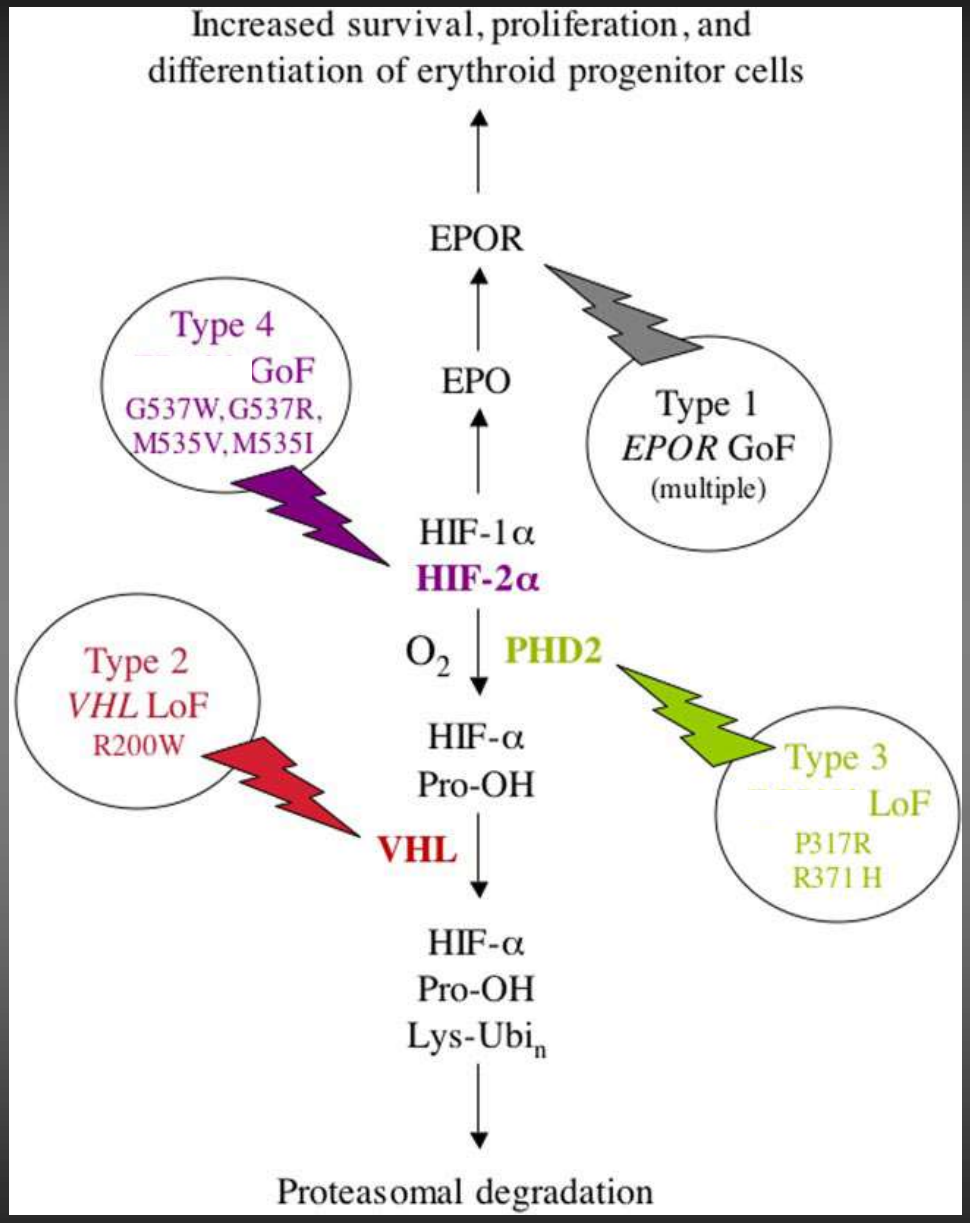
H= eterozigote

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