

A microscopic view of numerous red blood cells, which are biconcave discs, filling the frame. The cells are densely packed and appear in various orientations, creating a textured, reddish background. The lighting highlights the edges and the central depression of the cells.

*Molecular mechanism
of oxygen sensing*

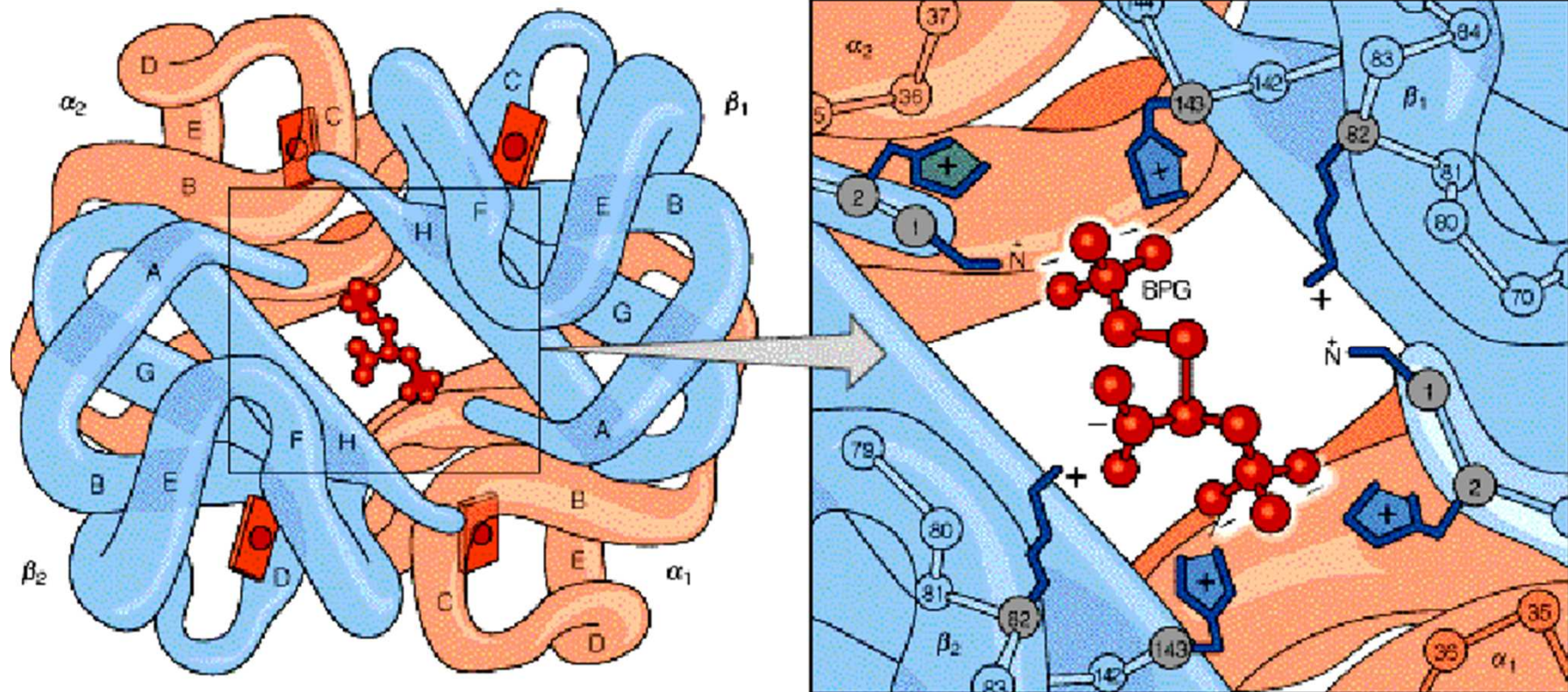
2,3 Bisphosphoglycerate (BPG)

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

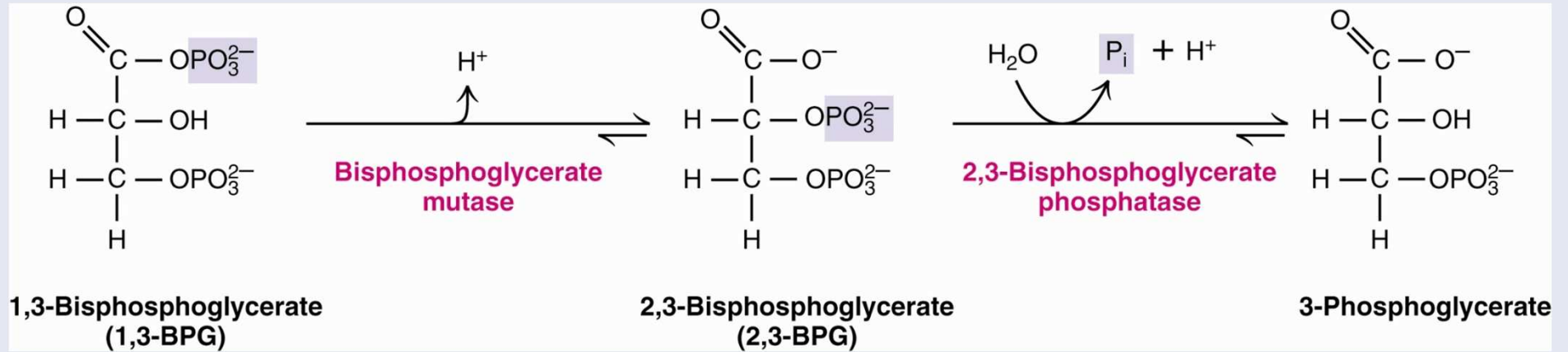
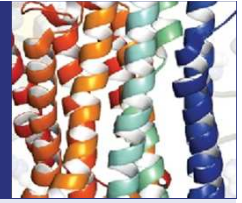
Bisphosphoglycerate (BPG)

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

2,3 Bisphosphoglycerate (BPG)

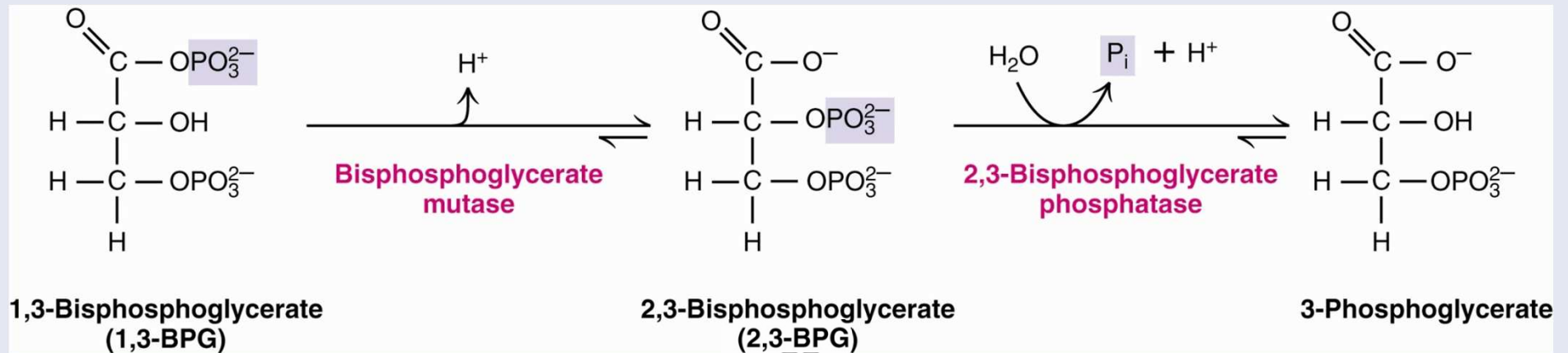
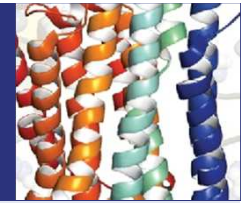


Erythrocyte synthesis of 2,3-BPG



Formation and decomposition of 2,3-bisphosphoglycerate in erythrocytes

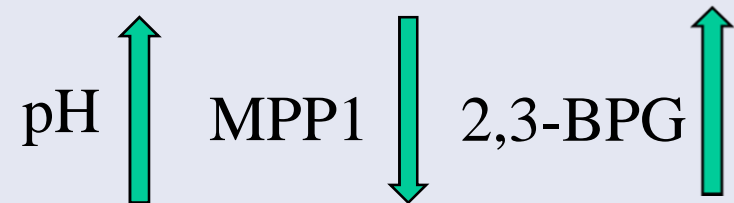
Erythrocyte synthesis of 2,3-BPG



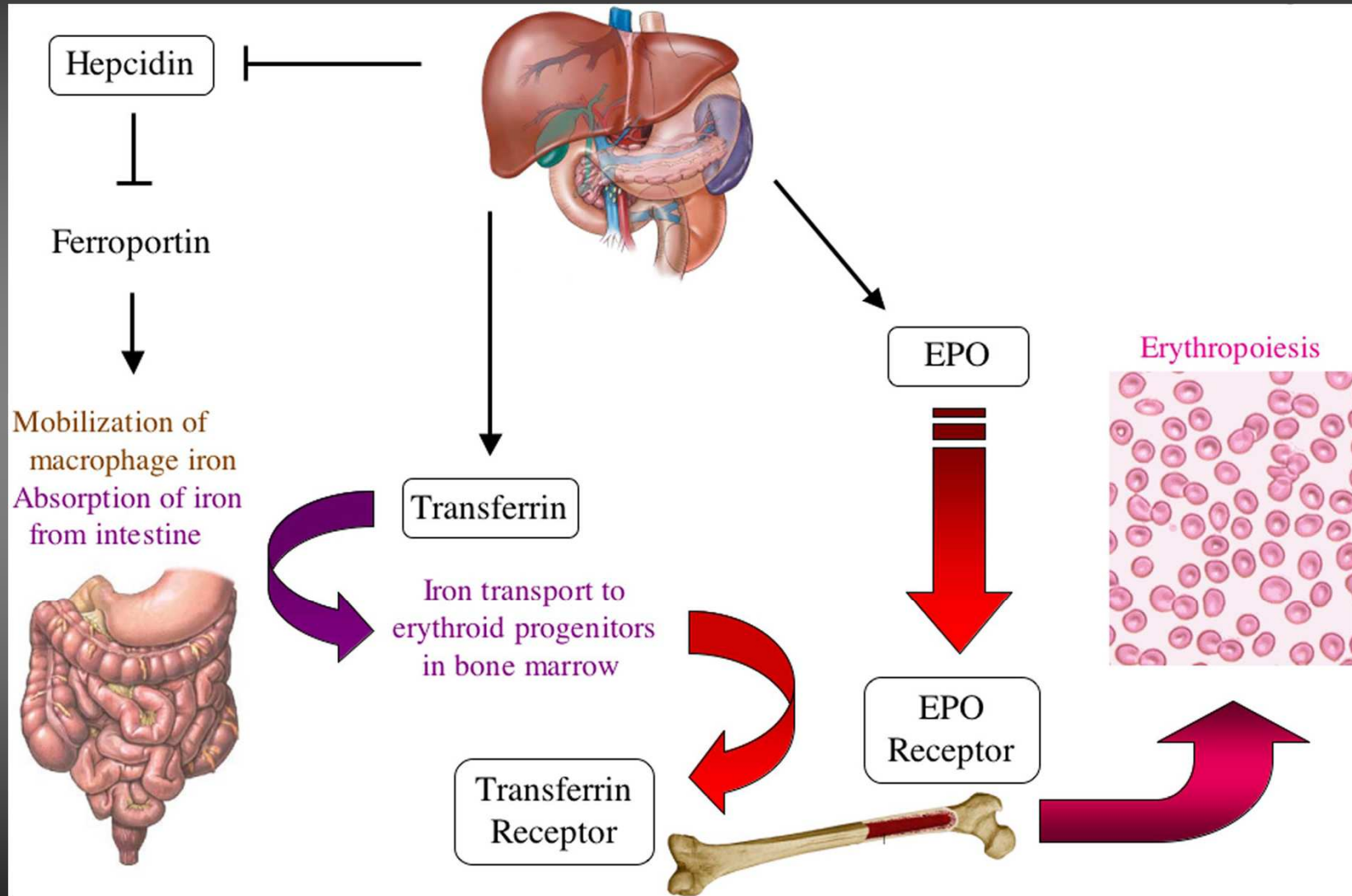
multiple inositol polyphosphate phosphatase (MIPP1)

Hydrolysis of 2,3-BPG by human MIPP1 is sensitive to physiologic alkalosis; activity decreases 50% when pH rises from 7.0 to 7.4.

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



Response to hypoxia - chronic adaptation



Erythropoiesis control

HIF-1 directly regulates the expression of 5 gene

EPO, EPOR

hepcidin, transferrin, and transferrin receptor

in 5 different organs

kidney, liver, intestine, blood, and bone marrow

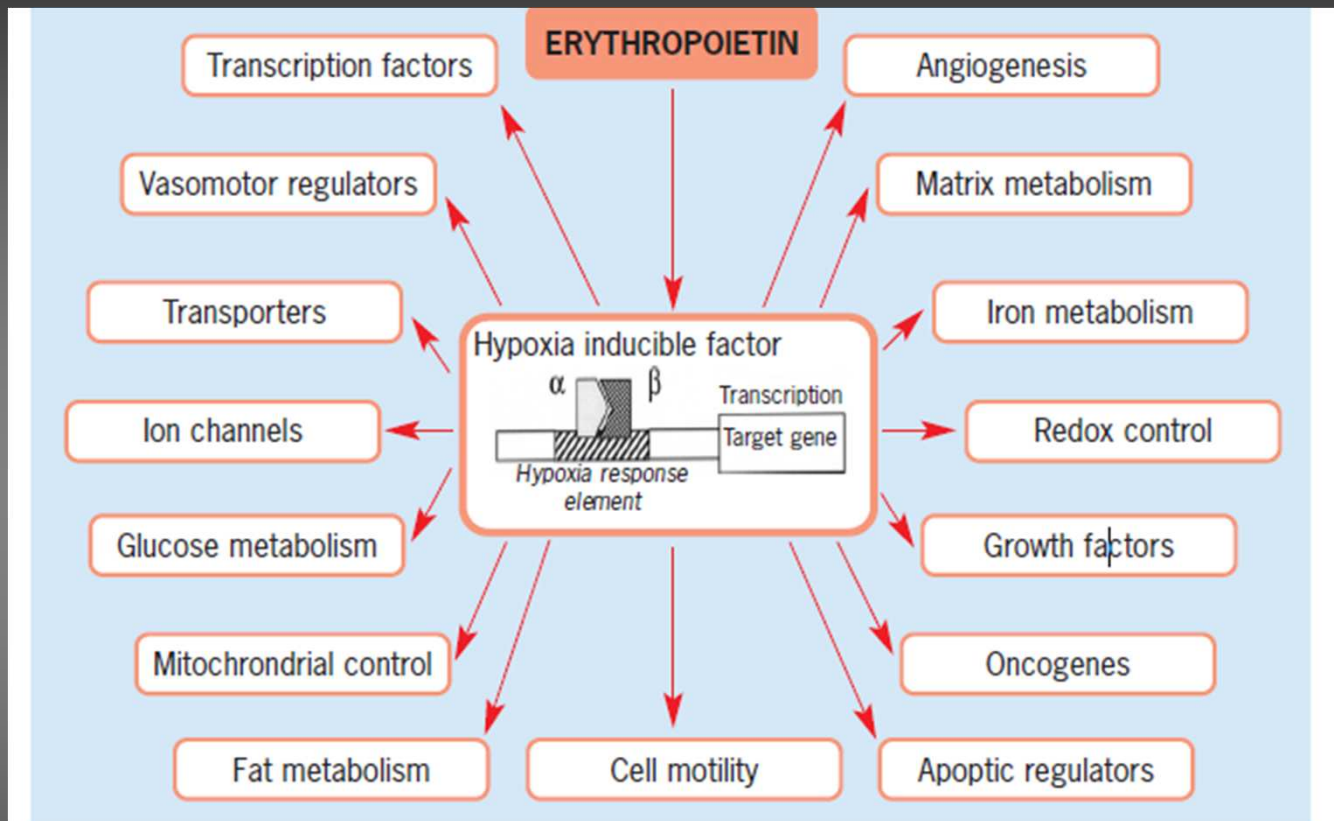


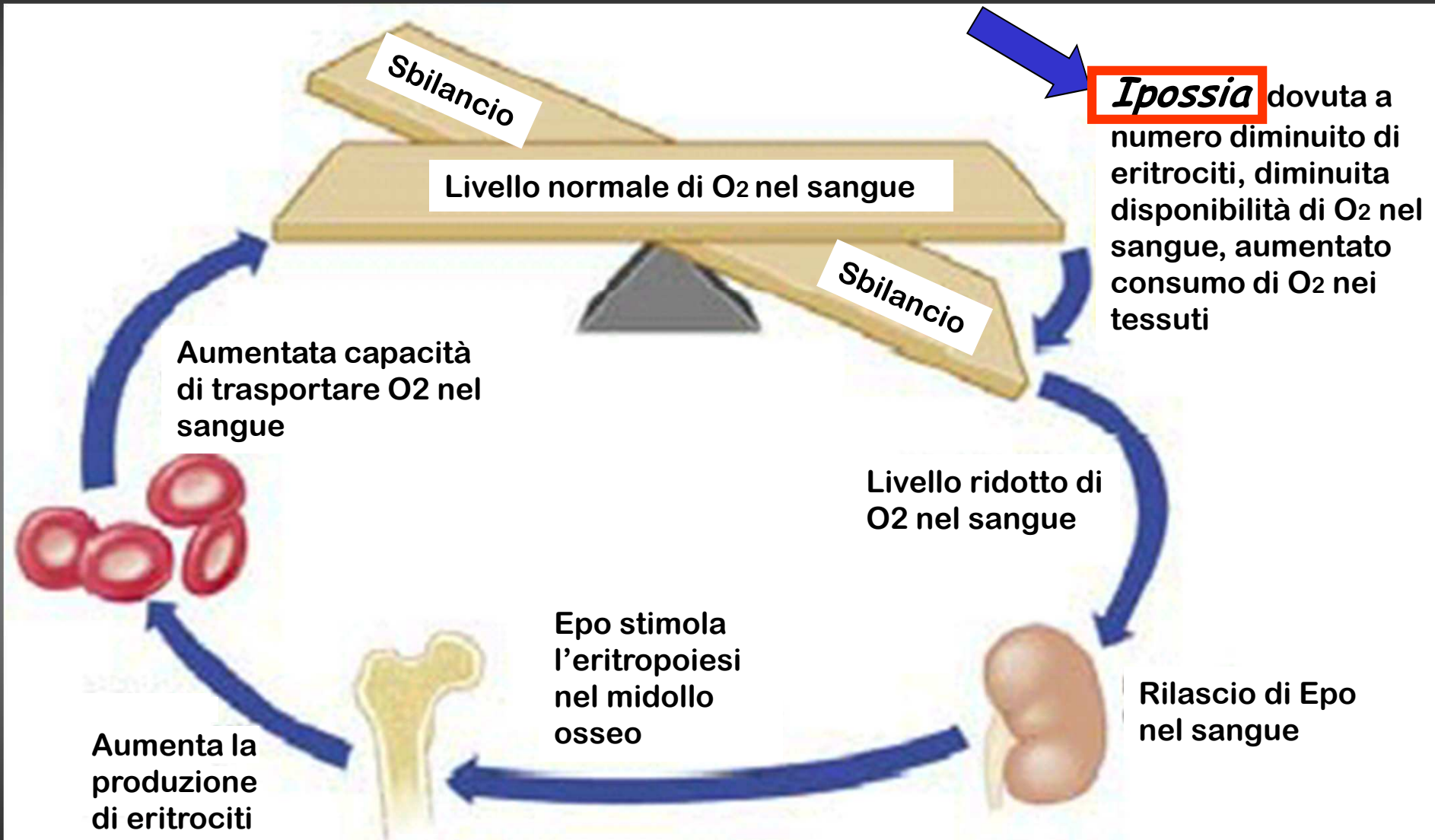
Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.

*REGOLAZIONE
DELL'ESPRESSIONE
DELL'ERITROPOIETINA
DA PARTE
DELL'IPOSSIA*

REGOLAZIONE DELL'Epo



REGOLAZIONE DELL'Epo

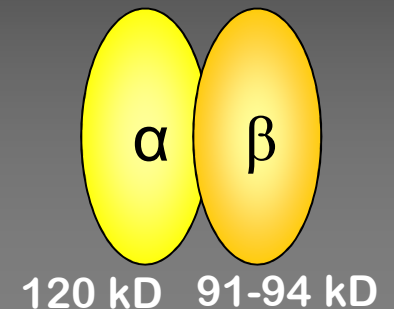


La regolazione dell'espressione del gene
Epo avviene sostanzialmente a
livello trascrizionale



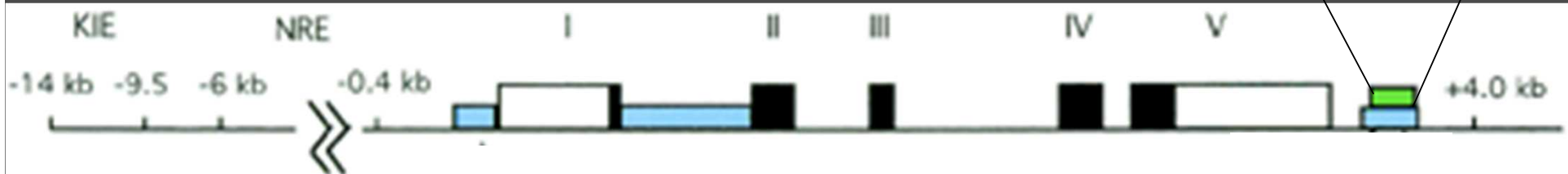
HIF-1 Hypoxia-inducible factor 1

- Fattore di trascrizione formato da due subunità (α e β), attivo SOLO come dimero
- Stimola la sintesi di Epo *in condizioni di ipossia*



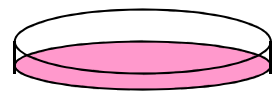
TACGTGCT

Gene Epo



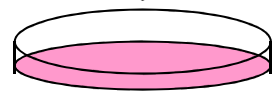
Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

Eric Huang et al. - JBC 1996



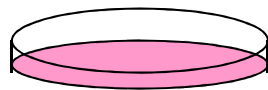
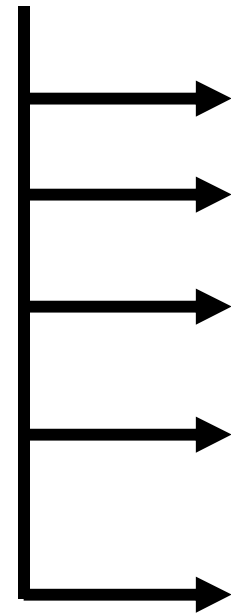
HeLa cells

20% O₂

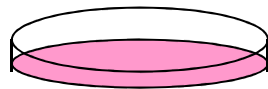


HeLa cells

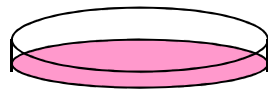
1% O₂ per 4h



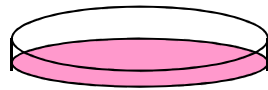
20% O₂ per 0 min



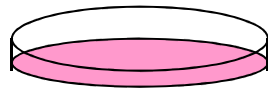
20% O₂ per 5 min



20% O₂ per 10 min



20% O₂ per 30 min



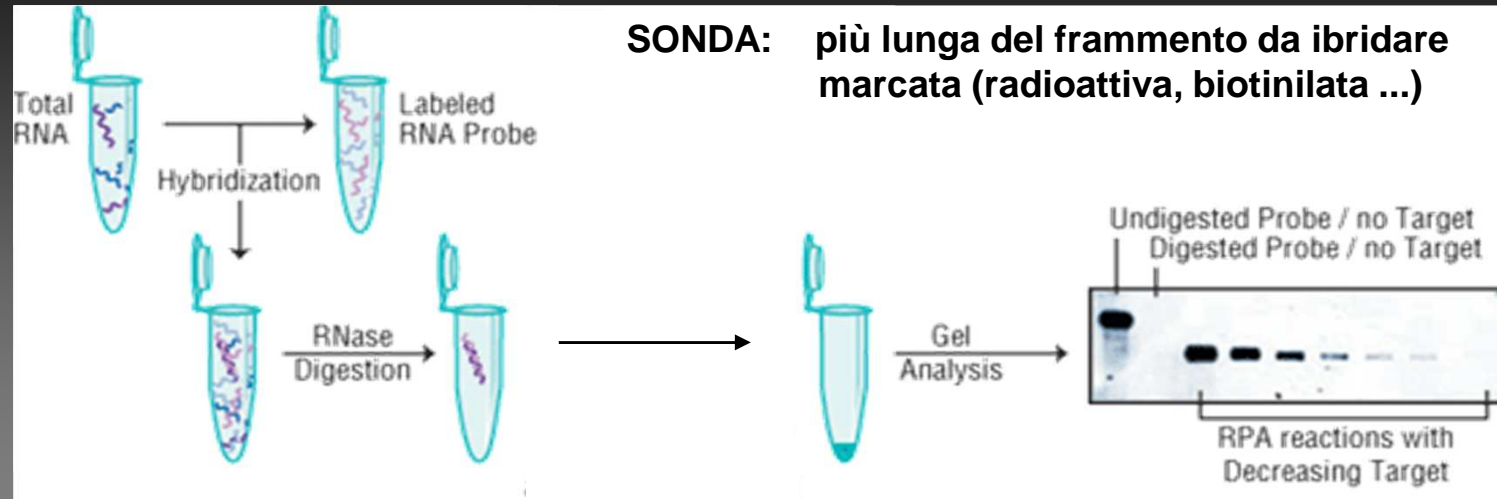
20% O₂ per 60 min

Lisi delle cellule

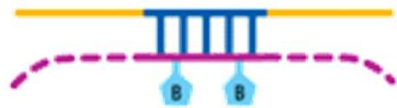
-Quantificazione mRNA di HIF1

-Quantificazione proteine: HIF1 α e HIF1 β

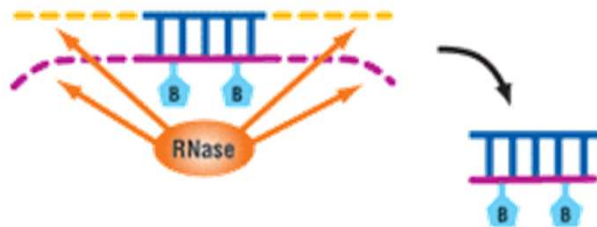
Quantificazione mRNA → RNase protection assay (RPA)



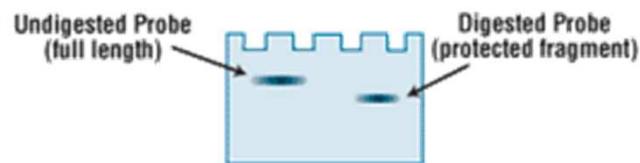
Step 1. Hybridize probe to mRNA.



Step 2. RNase digests single-stranded RNA - only the "protected" fragment remains.



Step 3. Separate by gel electrophoresis.



-La sonda radioattiva si lega al mRNA di HIF1 α

-RNase digerisce i frammenti a singola elica

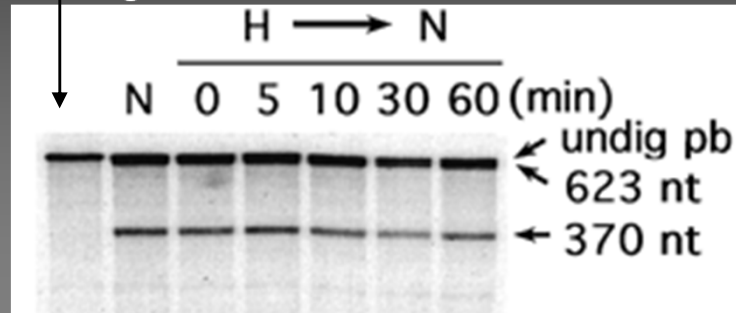
-Corsa elettroforetica ed esposizione su lastra → visualizzazione delle bande corrispondenti all'mRNA di HIF1

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

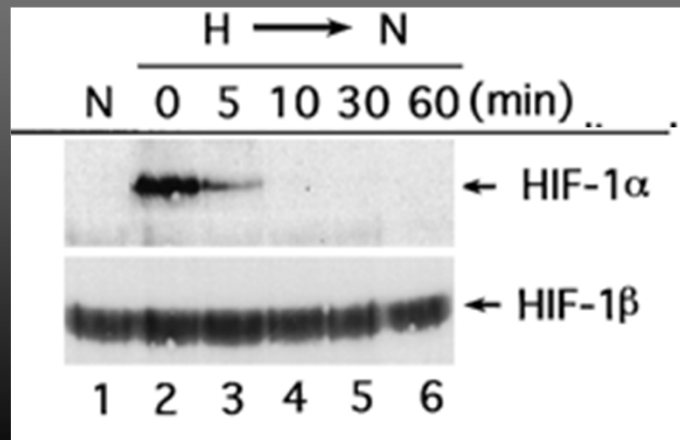
Sonda indigerita



HIF1 α è espresso a livello di mRNA.

Quantificazione proteine \rightarrow **Western blot**

Ibridazione con anticorpi anti HIF1 α e HIF1 β



HIF1 α è presente solo in condizioni di ipossia

HIF1 β è sempre presente

Regulation of hypoxia-inducible factor 1 is mediated by an O₂-dependent degradation domain via the ubiquitin-proteasome pathway

Huang et al. - JBC 1998

- Cellule epatiche incubate (20% O₂) per 16 h con inibitori per:

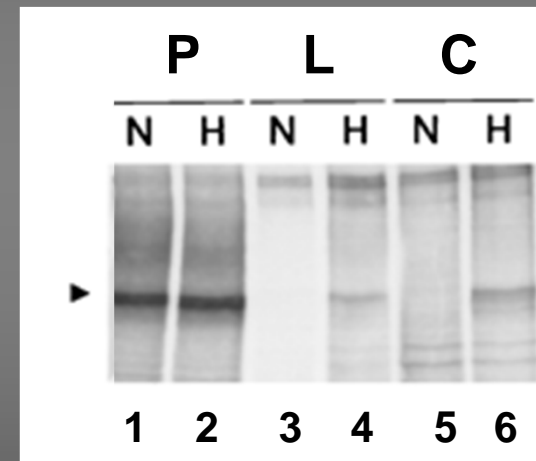
Proteosoma → P

Proteasi lisosomiali → L

Calpaine → C

→ 20% O₂ → N=normoxia

→ 1% O₂ per 4 h → H=hypoxia

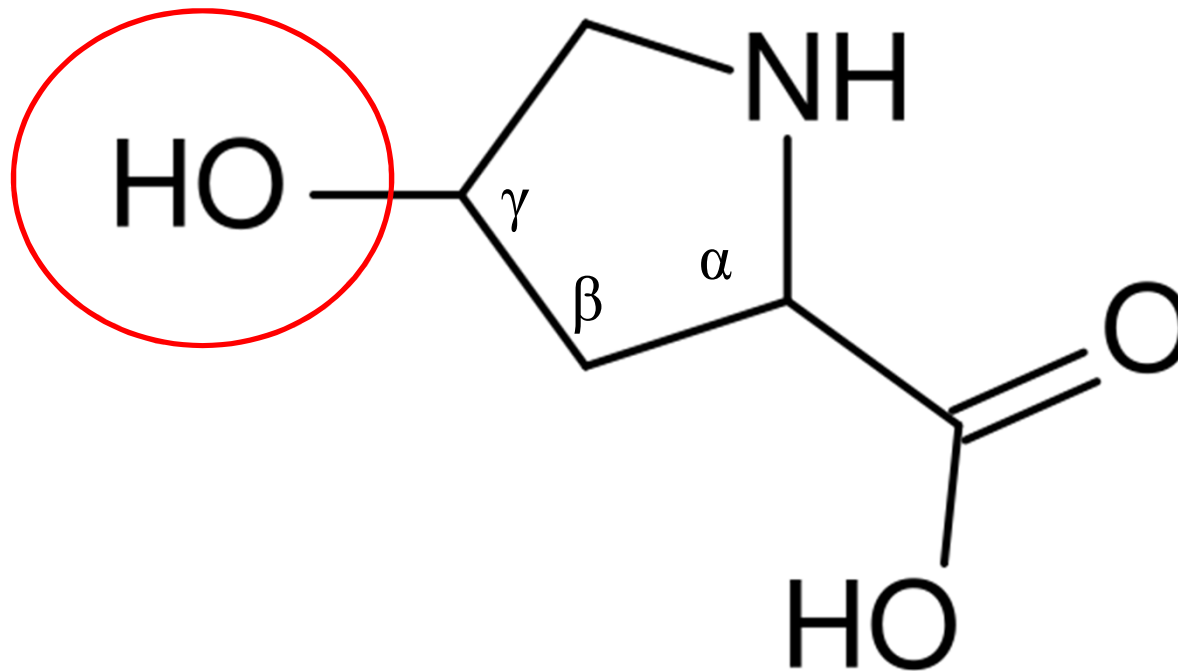


- Lisi cellulare

- Western blot: Ibridazione con anticorpi anti HIF1α

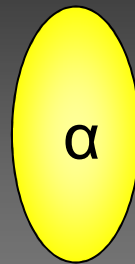
HIF1α è degradato dal sistema proteosomico

Idrossiprolina

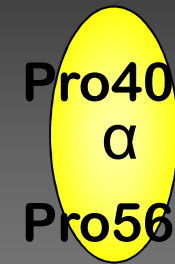


4-hydroxypyrrolidine-2-carboxylic acid

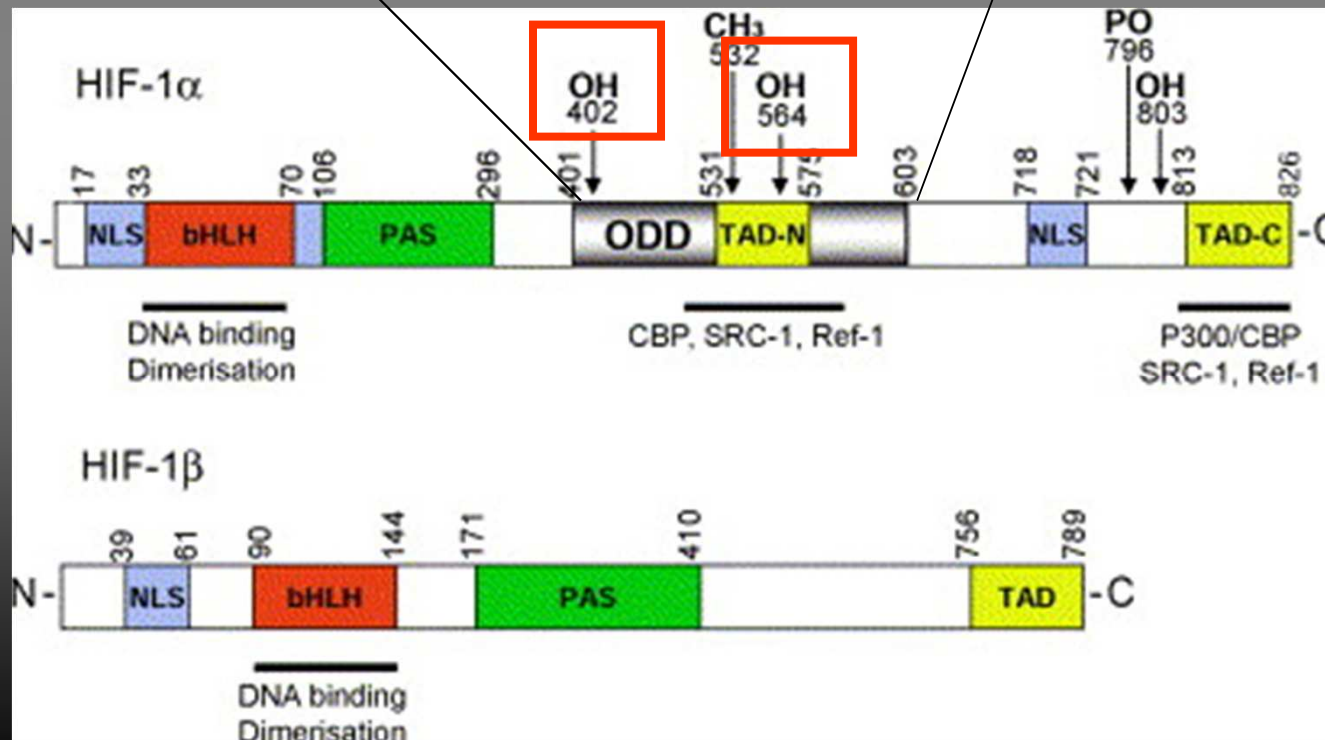
Struttura di HIF1

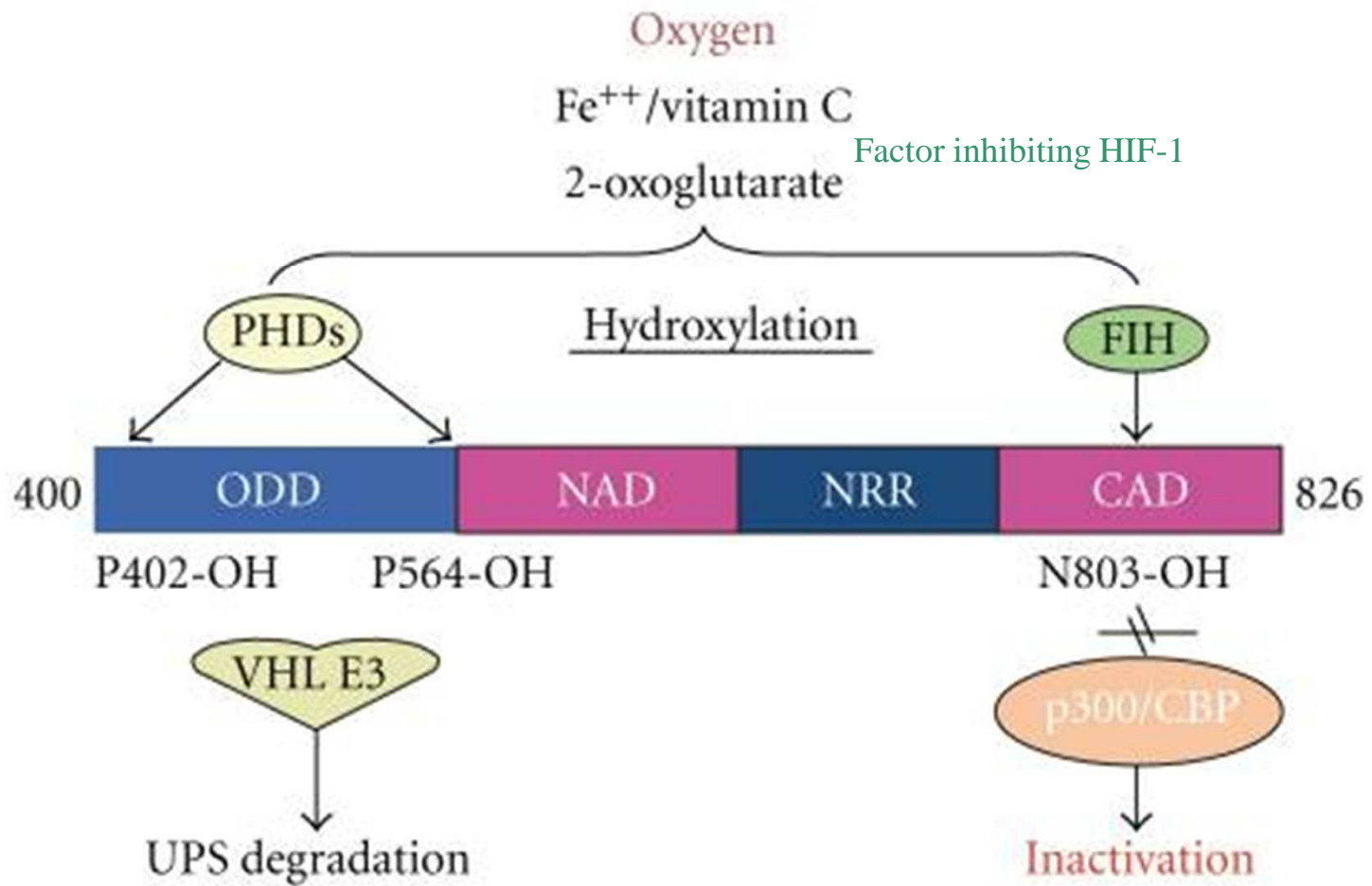


Prolina-Idrossilasi

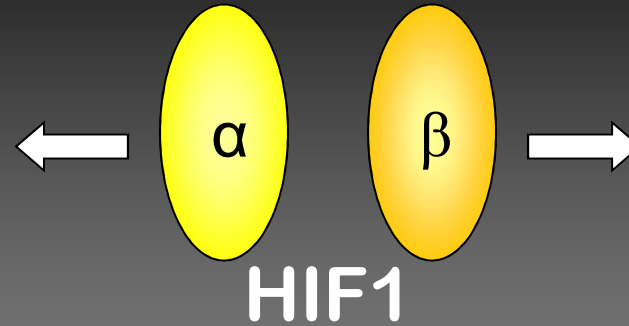
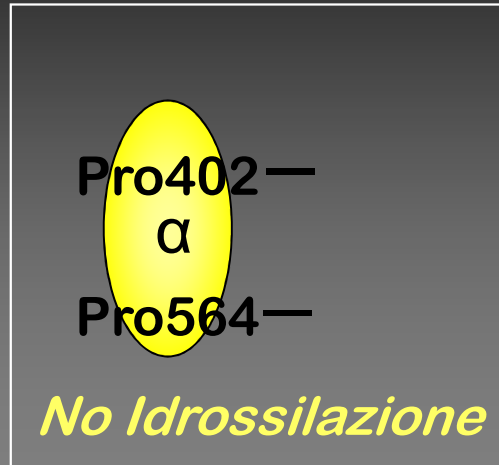


Sequenza di idrossilazione

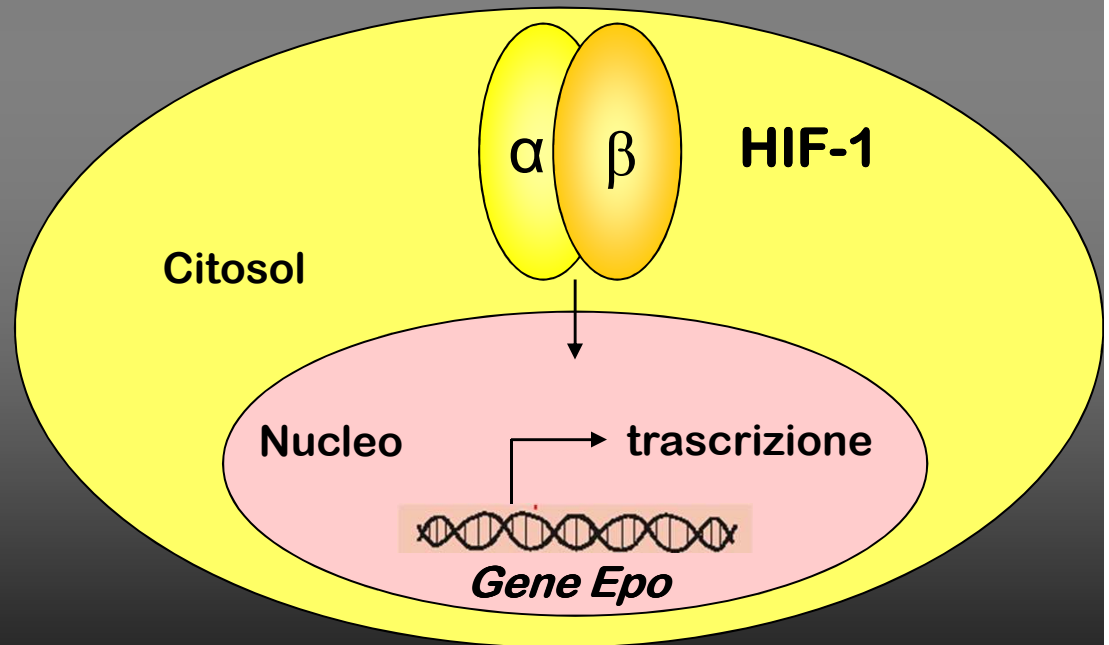




Condizione di Ipossia



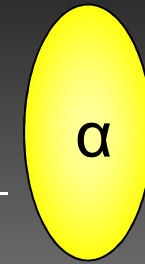
Espressa costitutivamente



Dimerizzazione

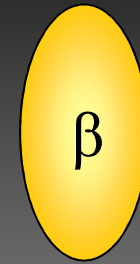
HIF-1 Hypoxia-inducible factor 1

Eterodimero



α

120 kD



β

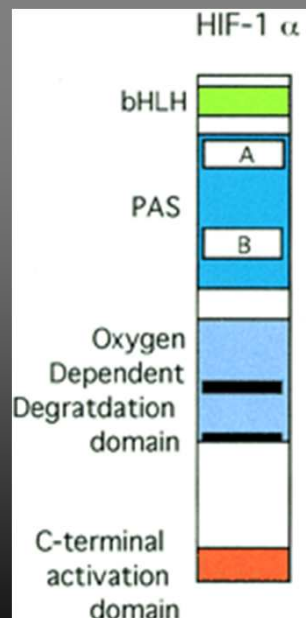
91-94 kD

ARNT (aryl hydrocarbon nuclear receptor translocator)

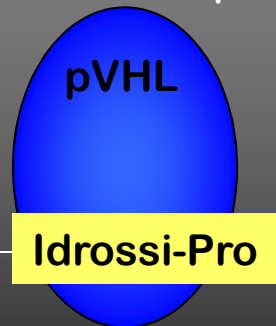
Normali livelli di O₂:

Ubiquitinizzazione pVHL-dipendente e degradazione proteosomica

Abbondante nel citosol



pVHL: von Hippel-Lindau protein

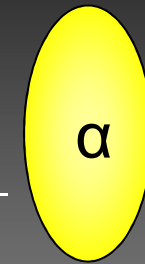


← idrossilazione

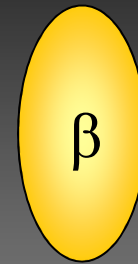
Protil-idrossilasi:
attiva ad elevate concentrazioni di O₂

HIF-1 Hypoxia-inducible factor 1

Eterodimero



120 kD



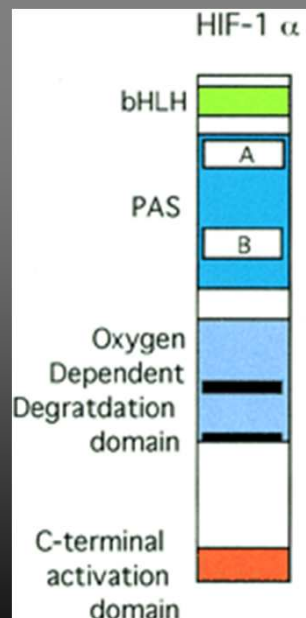
91-94 kD

ARNT (aryl hydrocarbon nuclear receptor translocator)

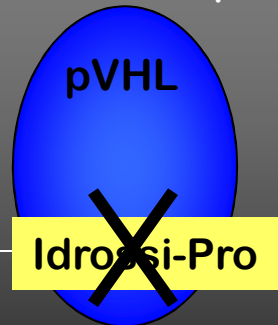
Ipossia:

~~Ubiquitinizzazione pVHL-dipendente e degradazione proteasomica~~

Abbondante nel citosol



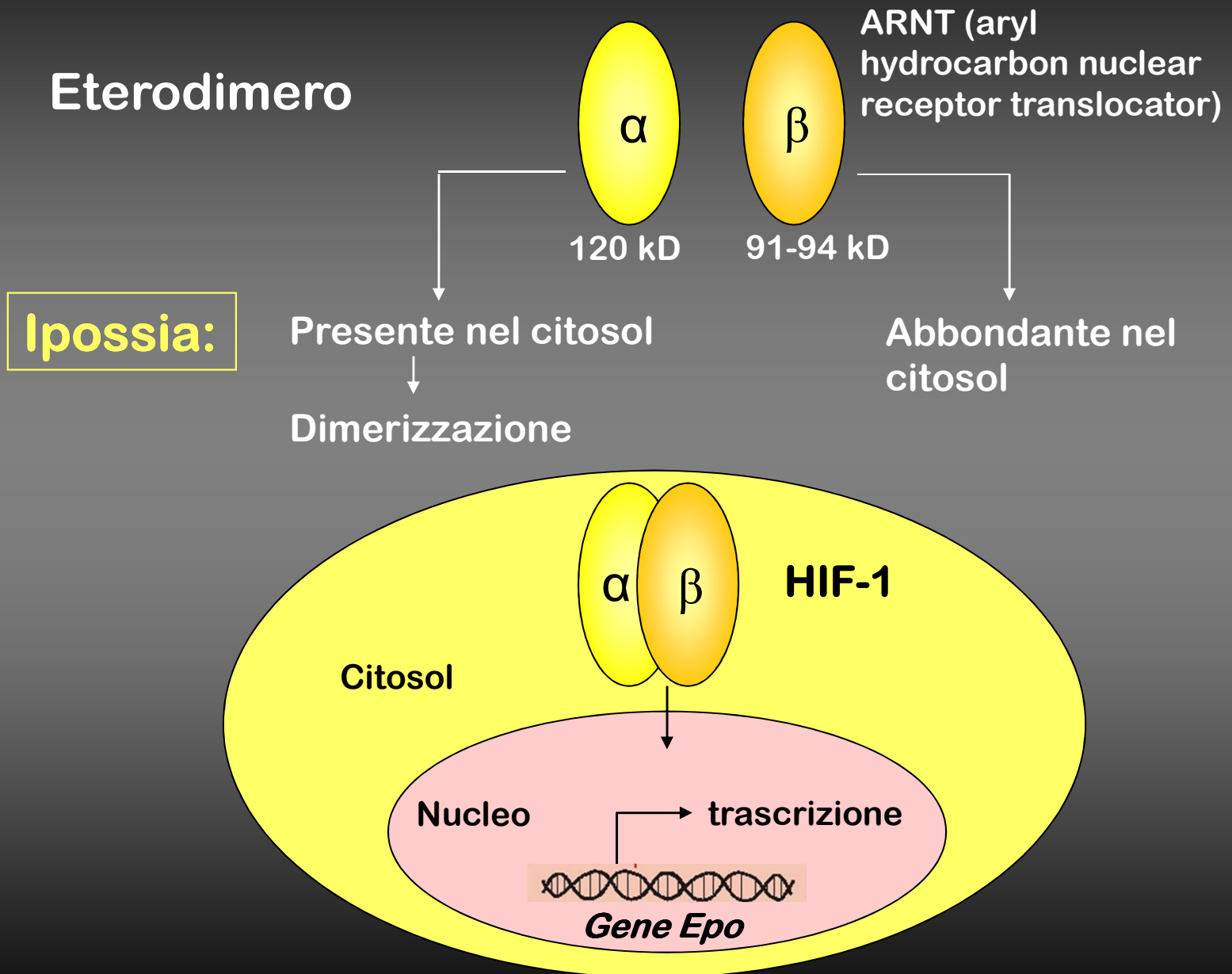
pVHL: von Hippel-Lindau protein



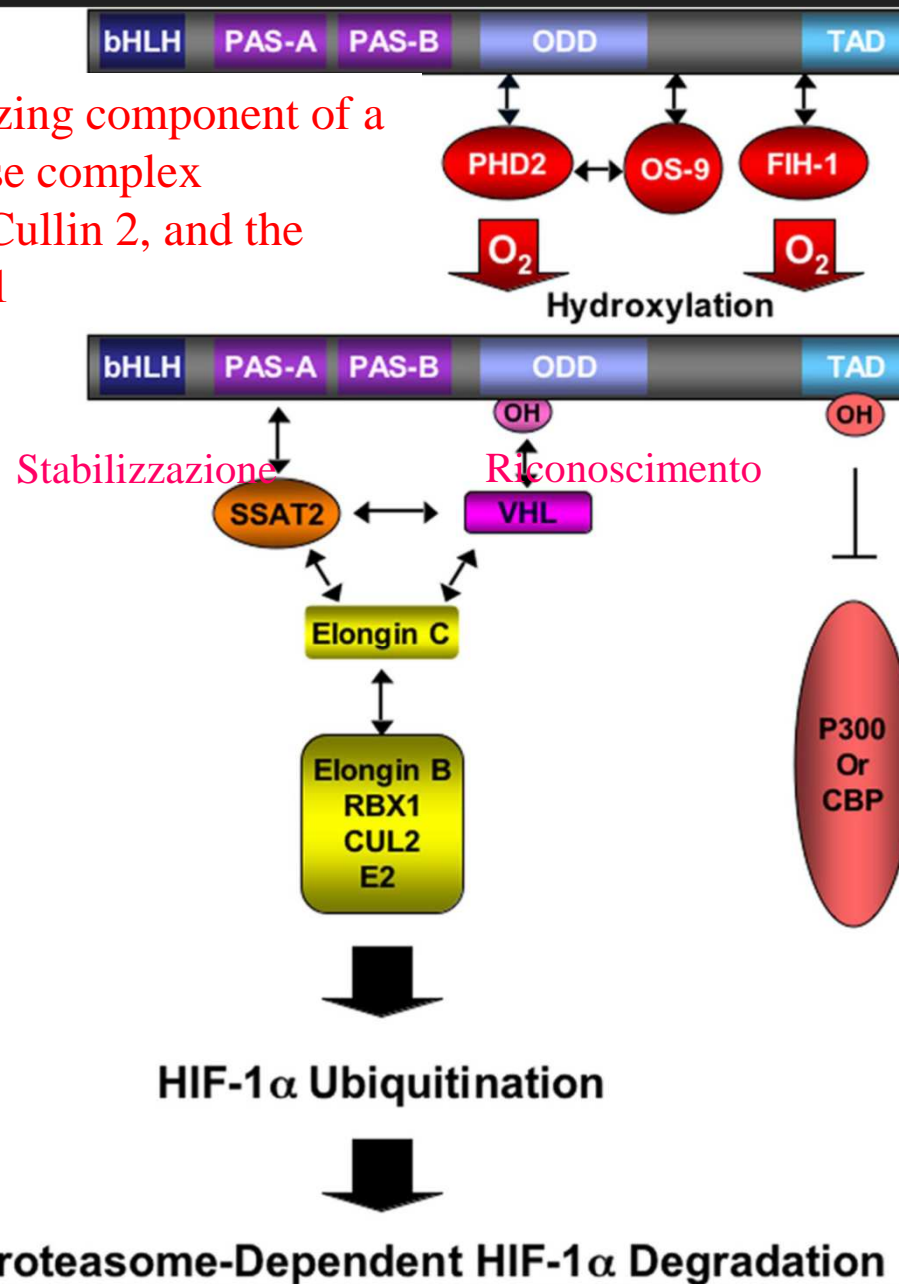
~~idrossilazione~~

Protil-idrossilasi:
attiva ad elevate concentrazioni di O₂

HIF-1 Hypoxia-inducible factor 1

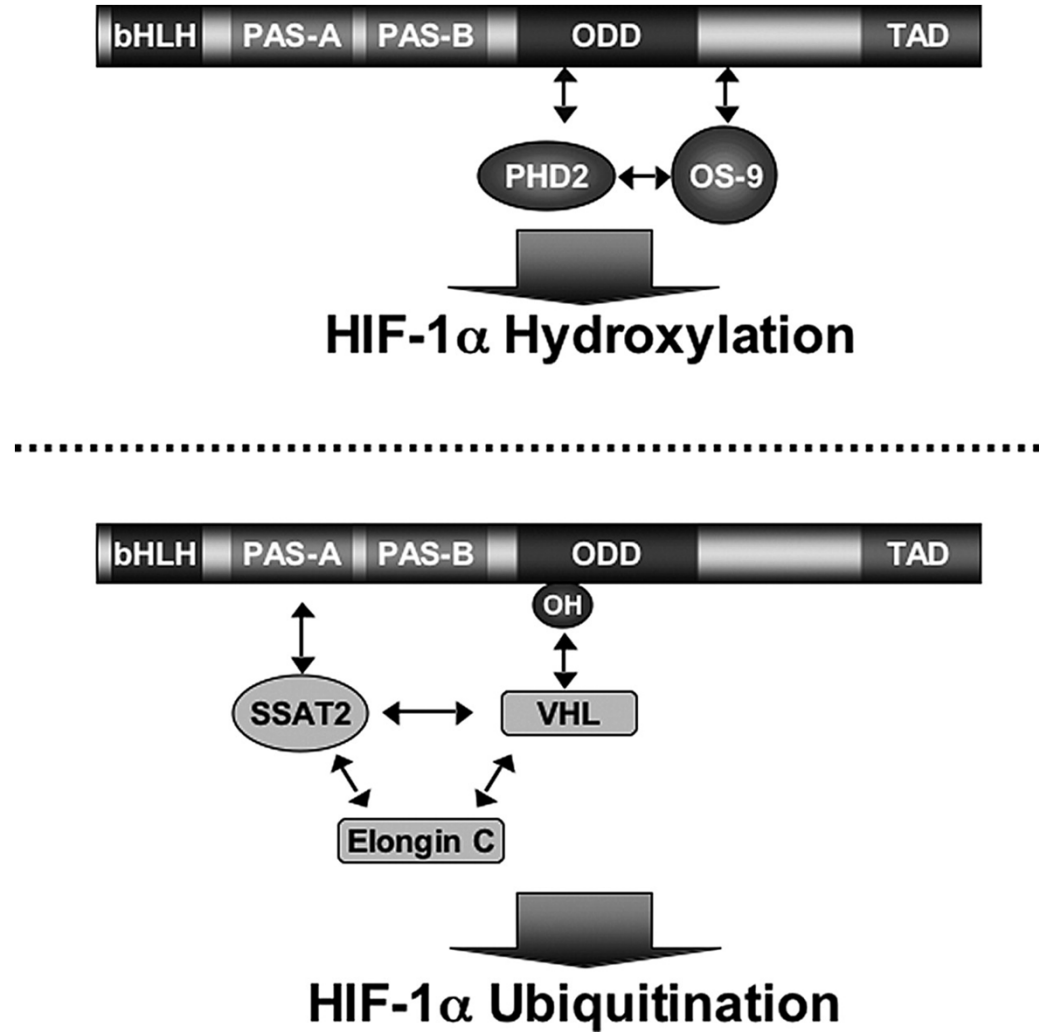


pVHL is the substrate-recognizing component of a multiprotein E3 ubiquitin ligase complex containing elongins C and B, Cullin 2, and the RING-H2 finger protein Rbx-1



26S Proteasome-Dependent HIF-1 α Degradation

Multivalent protein complexes regulate hydroxylation and ubiquitination of HIF-1 α .



Baek J H et al. J. Biol. Chem. 2007;282:23572-23580

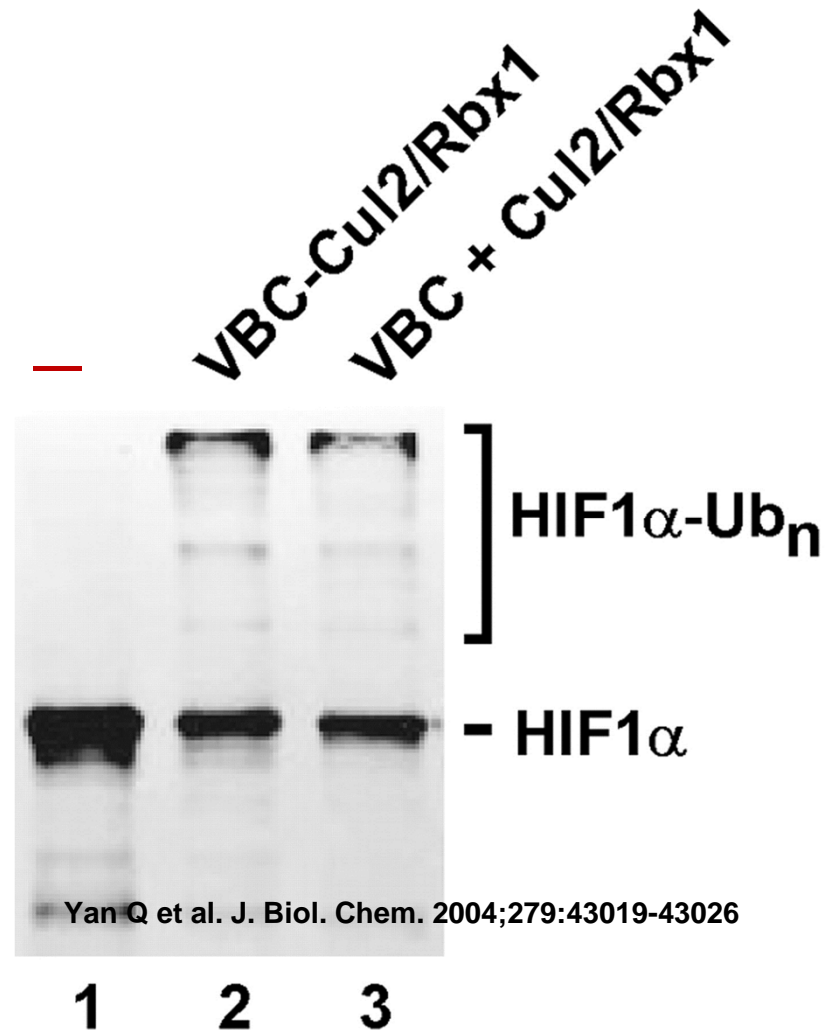
The multiprotein von Hippel-Lindau (VHL) tumor suppressor and Skp1-Cul1-F-box protein (SCF) complexes belong to families of structurally related E3 ubiquitin ligases.

In the VHL ubiquitin ligase, the VHL protein serves as the substrate recognition subunit, which is linked by the adaptor protein Elongin C to a heterodimeric Cul2/Rbx1 module that activates ubiquitylation of target proteins by the E2 ubiquitin-conjugating enzyme Ubc5.

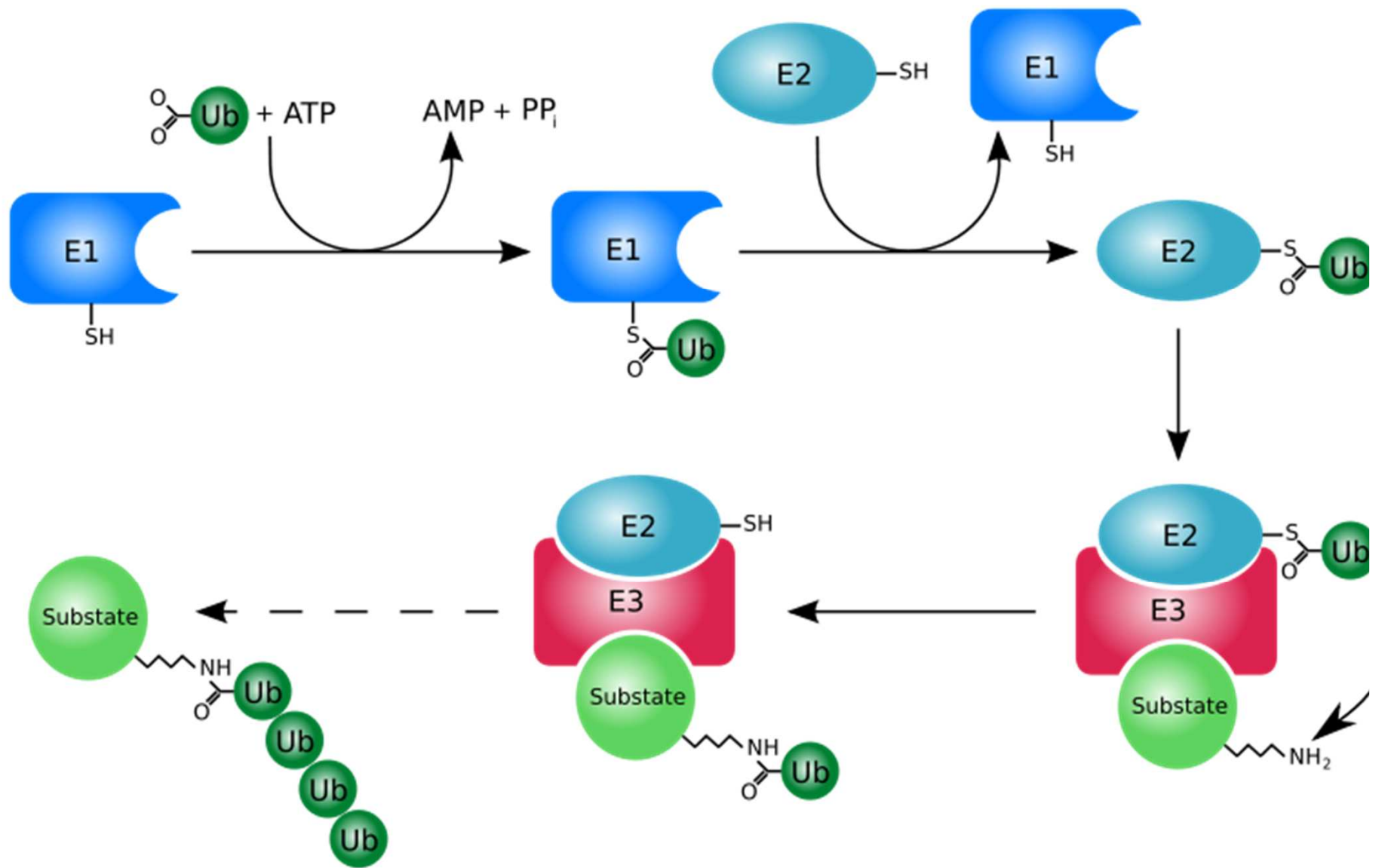
In SCF ubiquitin ligases, F-box proteins serve as substrate recognition subunits, which are linked by the Elongin C-like adaptor protein Skp1 to a Cul1/Rbx1 module that activates ubiquitylation of target proteins, in most cases by the E2 Cdc34.

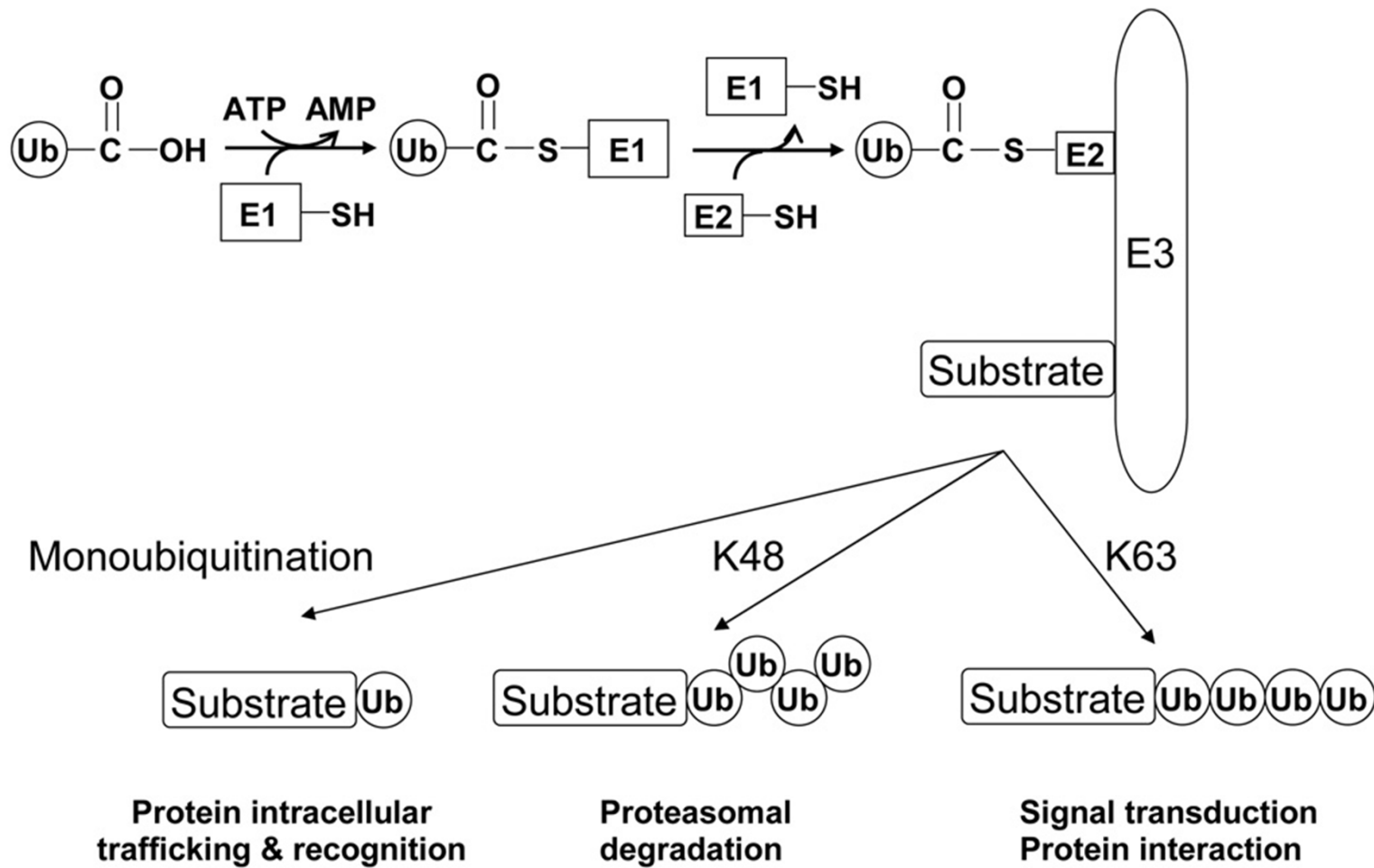
Reconstitution of VHL ubiquitin ligase with isolated VBC and Cul2/Rbx1 complexes.

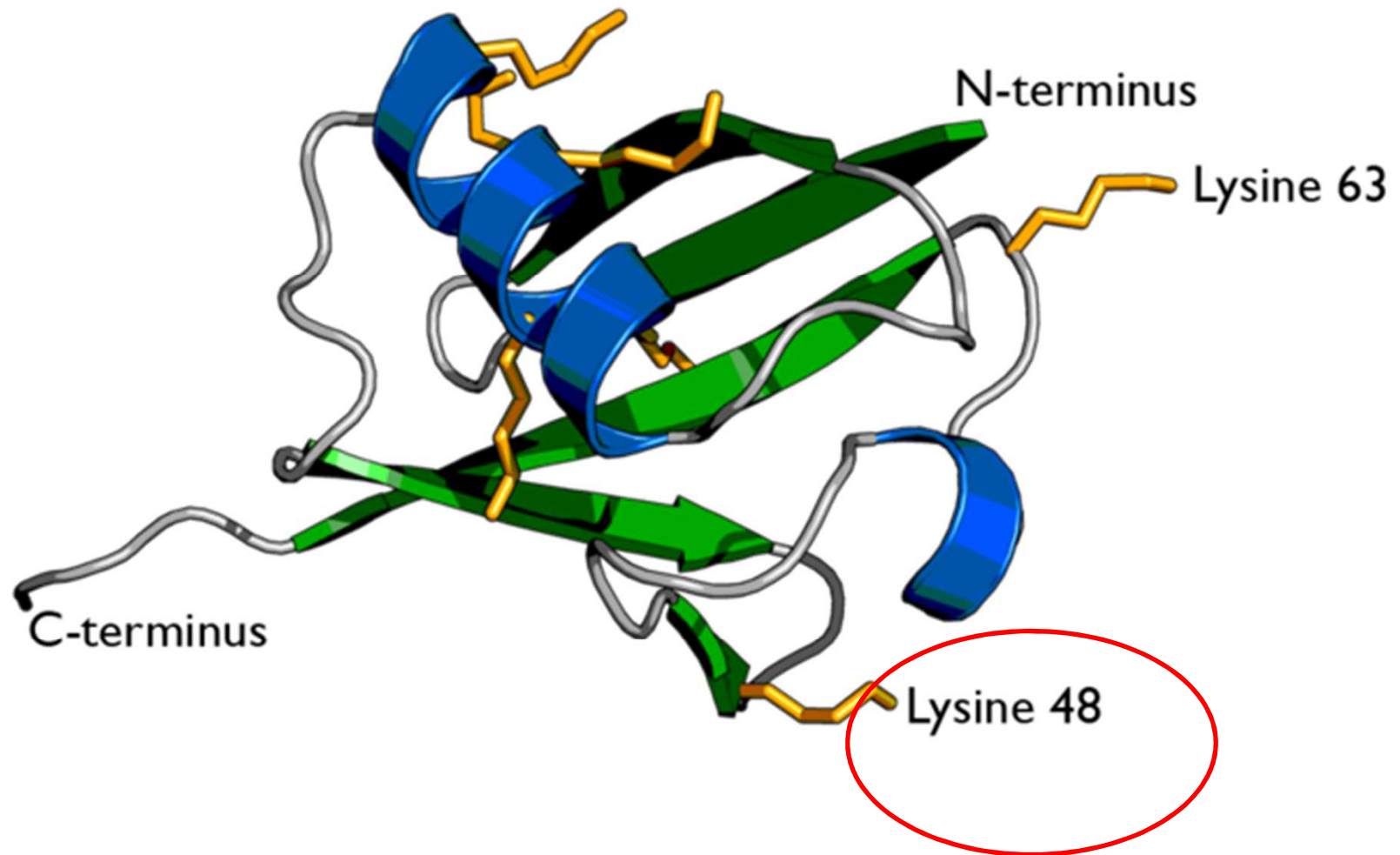
VBC- VHL-Elongin BC complex;



Yan Q et al. J. Biol. Chem. 2004;279:43019-43026

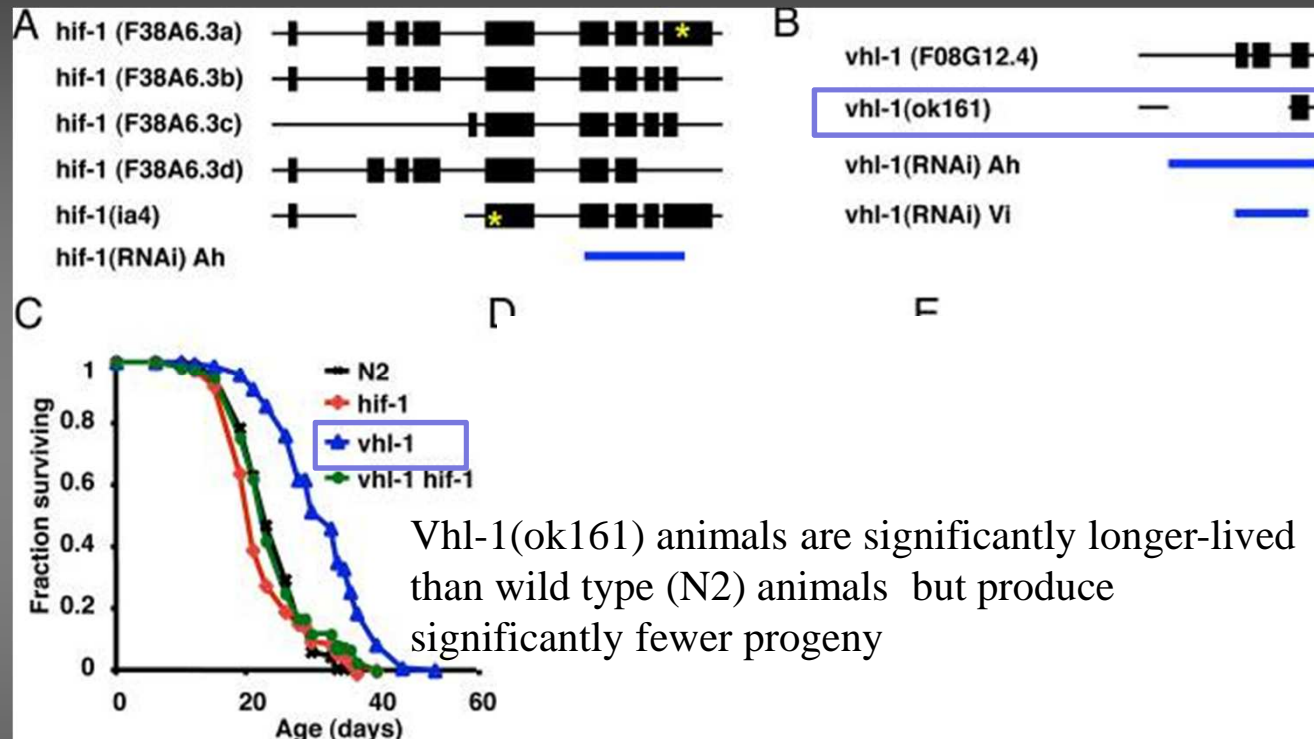






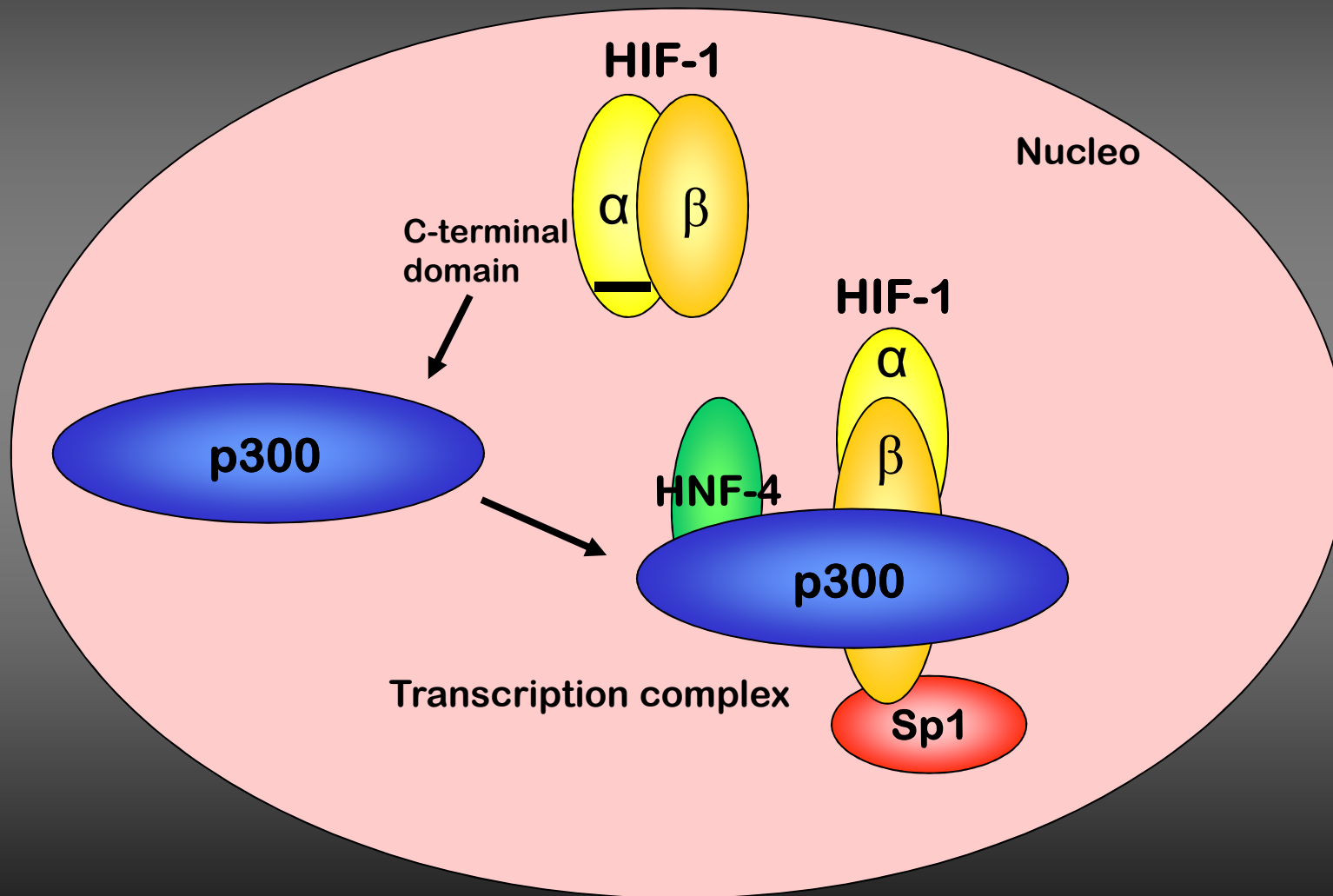
K48-linked polyubiquitin chains on substrate proteins result in their degradation in proteasomes

VHL-1 modulates longevity

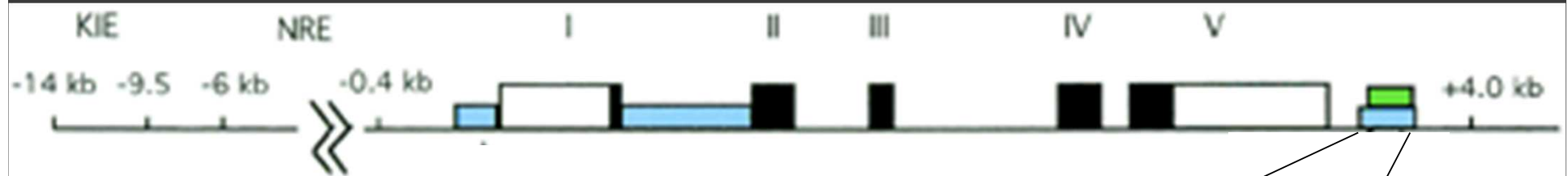


Vhl-1(ok161) animals (C.E) are significantly longer-lived than wild type (N2) animals

Regolazione del gene Epo da parte dell'ipossia

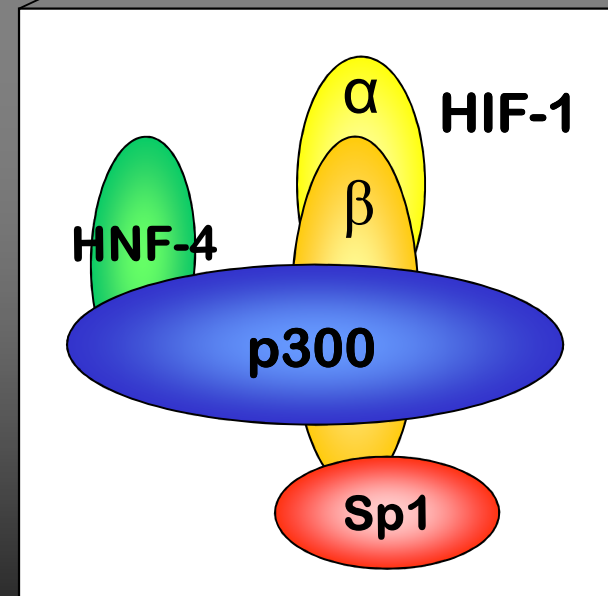


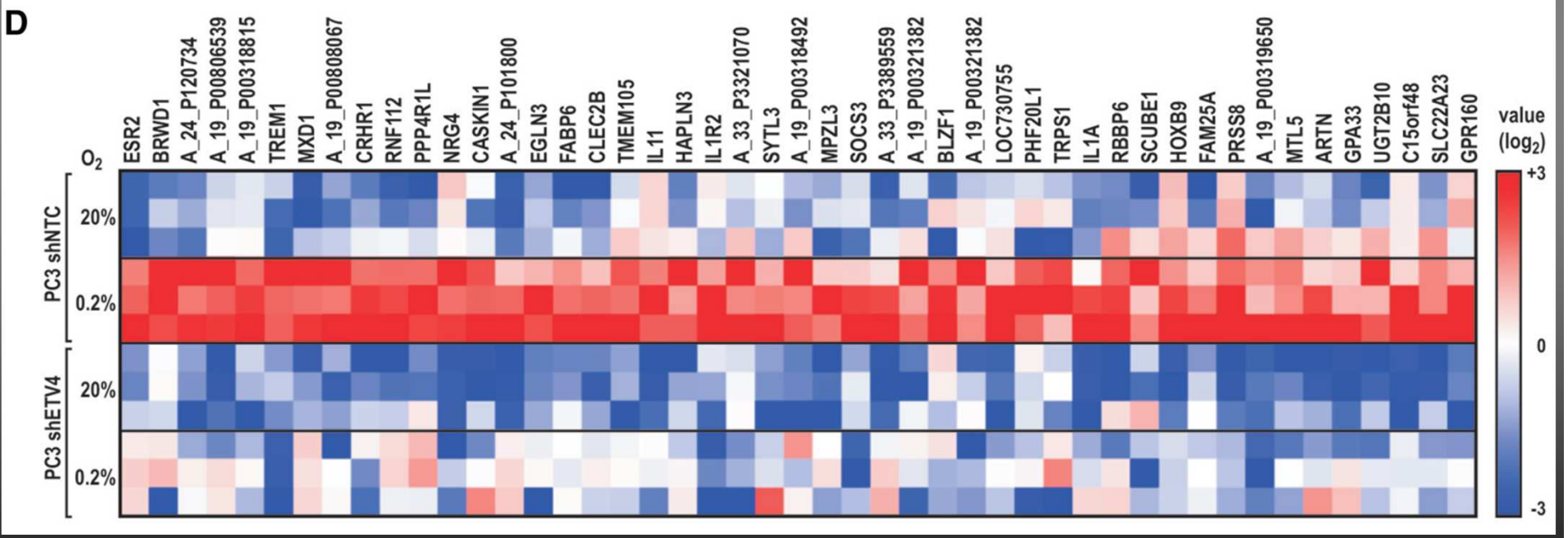
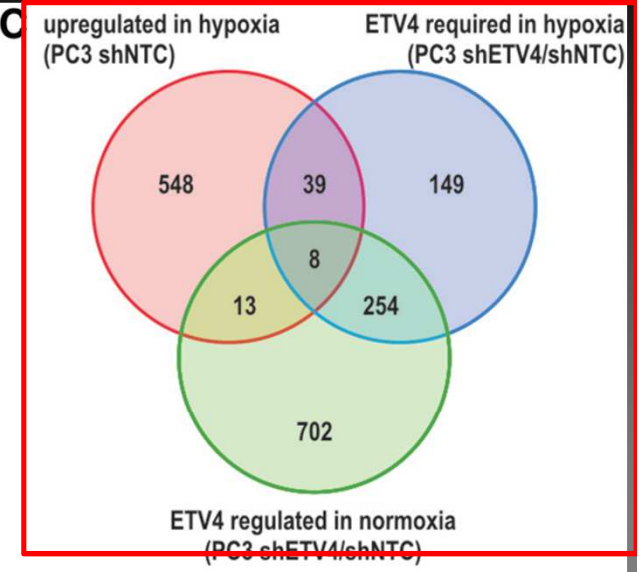
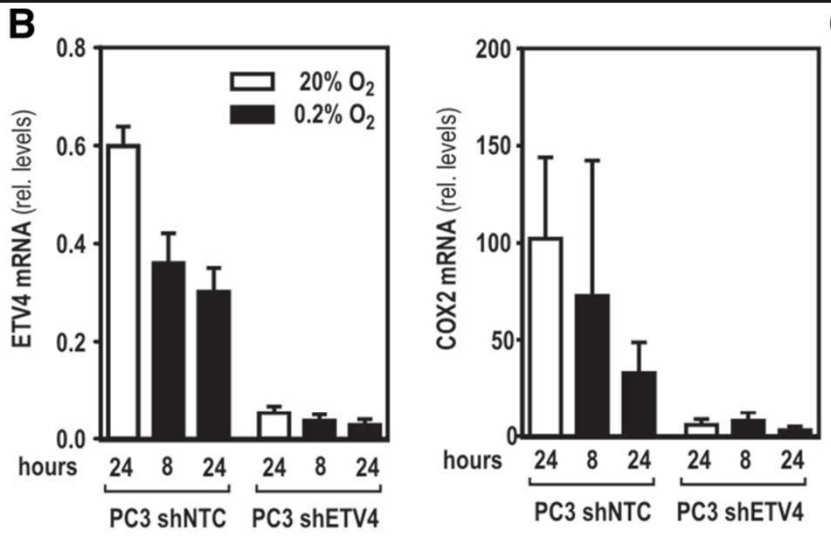
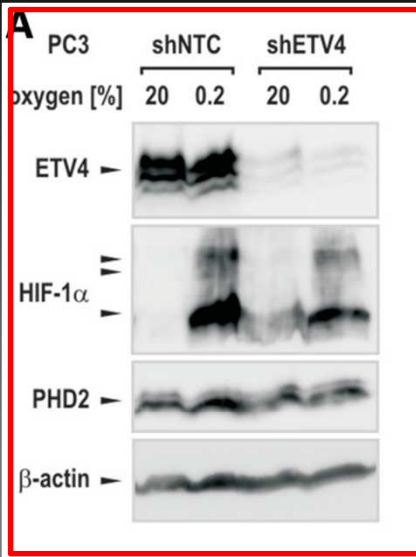
Regolazione del gene Epo da parte dell'ipossia

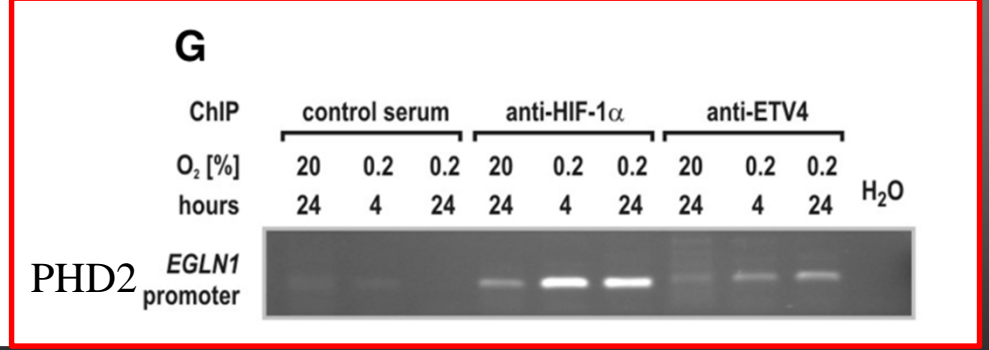
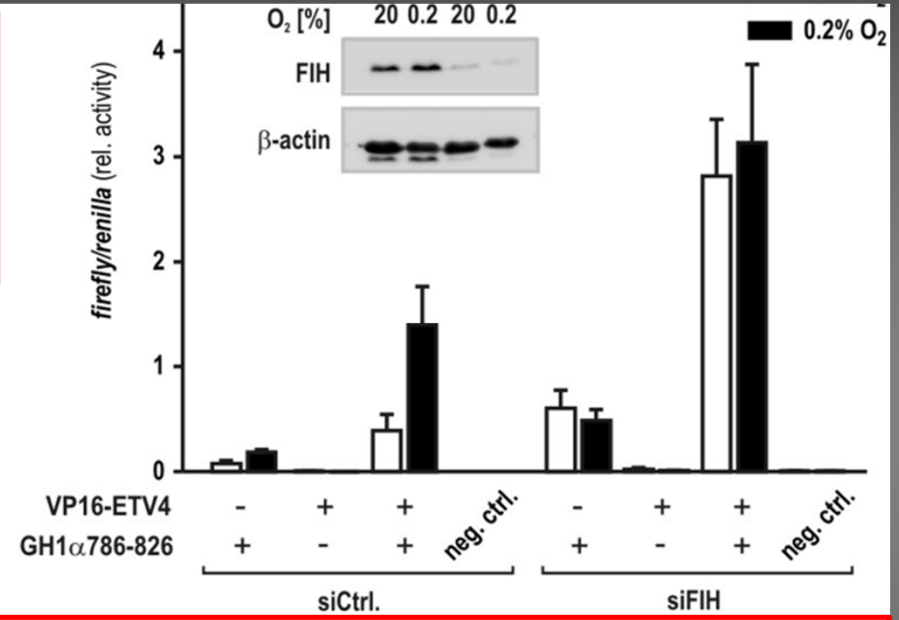
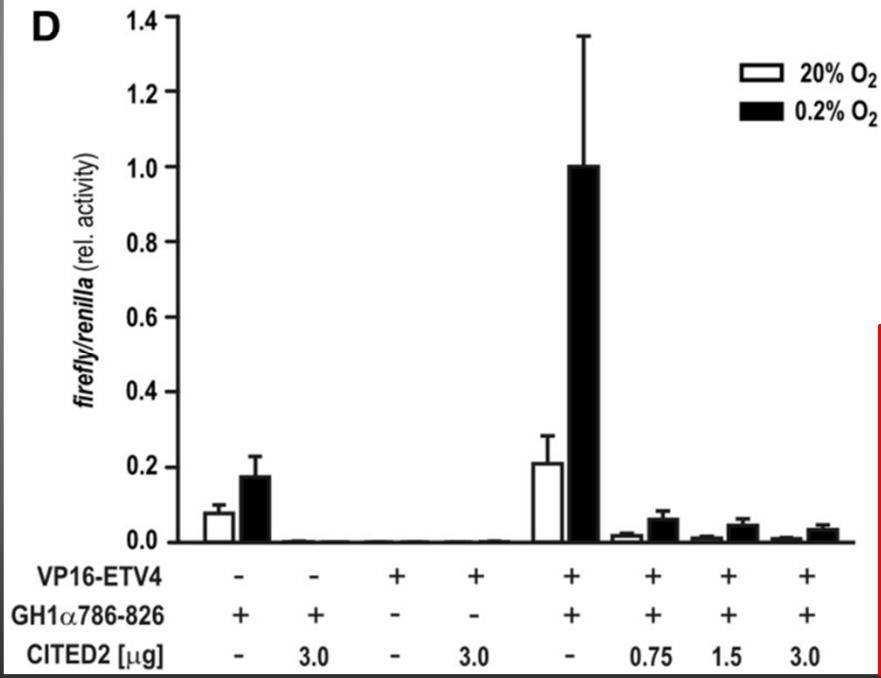
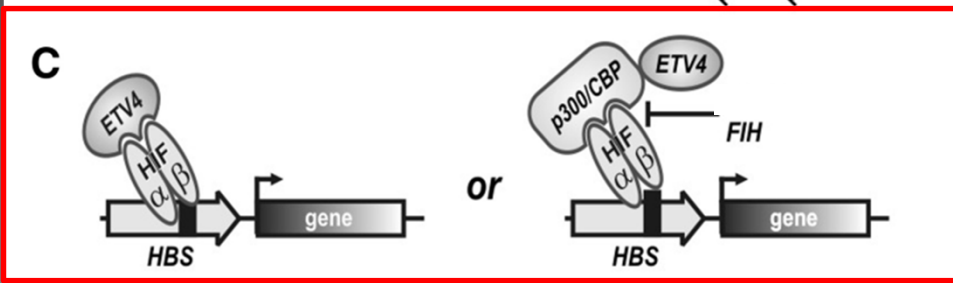


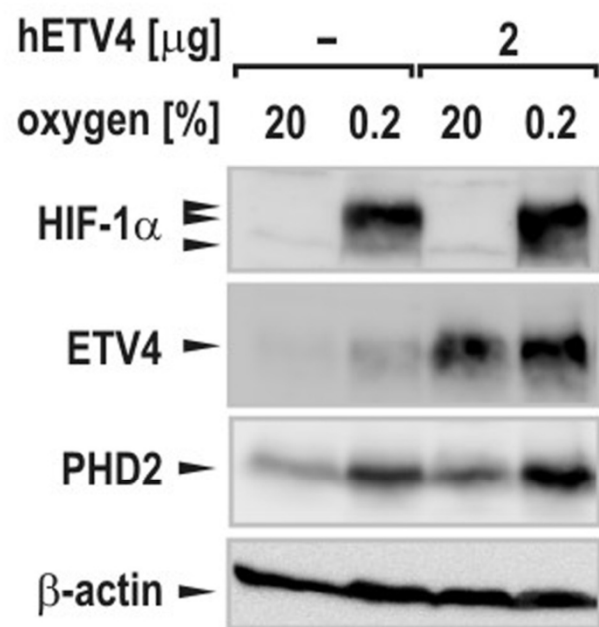
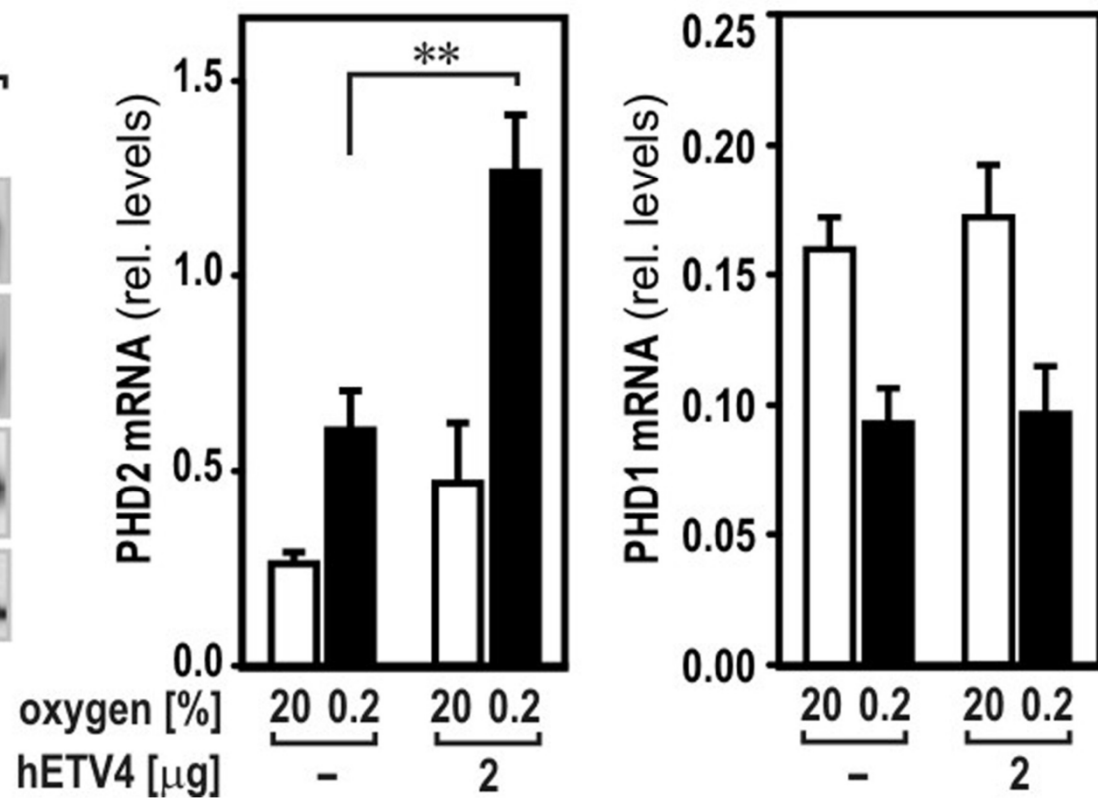
Promotore

Transcription complex







D**E**

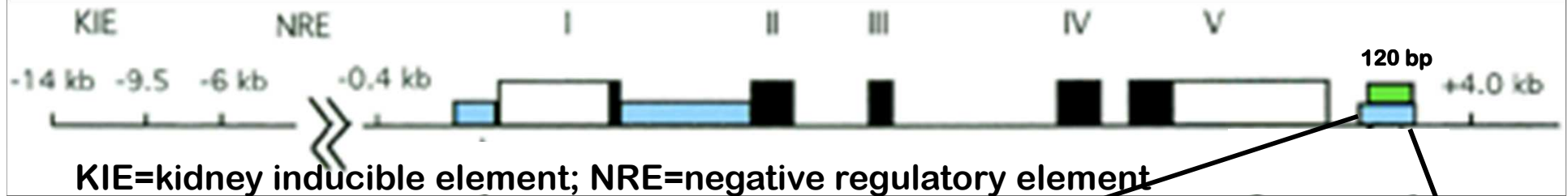
Regolazione del gene Epo da parte dell'ipossia

Sommario

Eventi che portano alla trascrizione di Epo

	O ₂ SI	O ₂ NO (Ipissia)
Prolil-idrossilasi	SI	NO
Legame di pVHL a HIF- α	SI	NO
Degradazione di HIF- α	SI	NO
Attivazione di HIF-1	NO	SI
Migrazione di HIF-1 al nucleo	NO	SI
Formazione del complesso di trascrizione	NO	SI
Trascrizione del gene Epo	NO	SI

Regolazione del gene Epo da parte dell'ipossia



50 pb nella
Regione 3'

GGCCCTACGCTGCTGTCTCACACAGCCTGTCTGACCTCTCGACCTACCG

Sequenza di
legame per
HIF-1

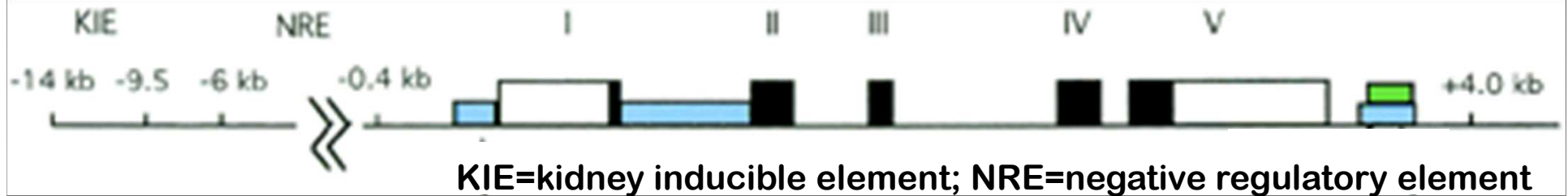
Sequenza
CACA

Sequenza di
legame per
HNF-4

Mutazioni a carico di una di queste sequenze
inibiscono l'induzione di Epo da parte
dell'ipossia

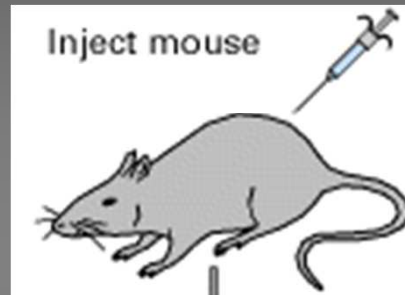
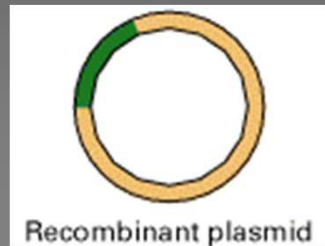
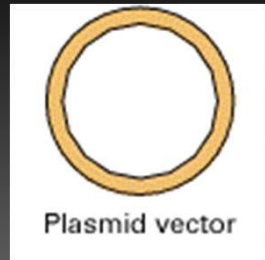
HNF-4 (Hepatocyte nuclear factor 4)
fattore di trascrizione
espresso nel cortex renale e nel fegato
come Epo →
contribuisce alla regolazione tessuto-specifica

Regolazione tessuto-specifica del gene Epo



Regione 5'

- 9.5-14 kb → Sequenza richiesta per l'espressione nel rene
- Entro le 9.5 kb → Sequenza richiesta per l'espressione nel fegato
- 0.4-6 kb → Sequenza regolatoria negativa che inibisce l'espressione di Epo nei tessuti che non producono Epo



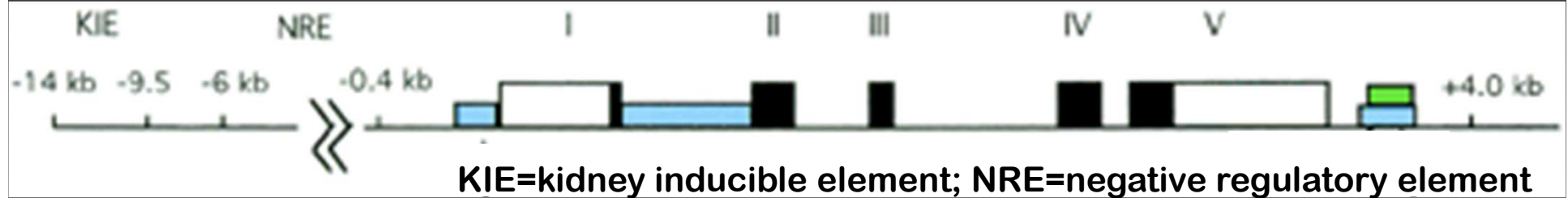
Regione 5'

Regione 3'



1. Epo espressa in fegato, rene e tessuti che normalmente non esprimono Epo
2. Epo espresso nel fegato ma non nel rene —————> 400 bp-6 kb: sequenze regolatorie negative
3. Epo espresso nel fegato ma non nel rene —————> Entro le 9.5 kb: sequenze per l'espressione nel fegato
4. Epo espresso nel rene —————> 9.5-14 kb: sequenze per l'espressione nel rene

Regolazione tessuto-specifica del gene Epo



Promotore

Sp1: fattore di trascrizione ubiquitario

- Omologia >73% con il promotore del gene Epo murino
- Regione -61 -45: contribuisce alla regolazione da parte dell'ipossia → sequenza di legame per **Sp1**
- Sito di legame per GATA: inibizione dell'espressione di Epo
- Sito CACCC: sequenza stimolatrice dell'espressione di Epo

Policitemia di Chuvash

Ang et al. Nature Genetics 2002

- Policitemia autosomica recessiva trovata in Russia

Table 1 • Biochemical parameters in Chuvash polycythemia

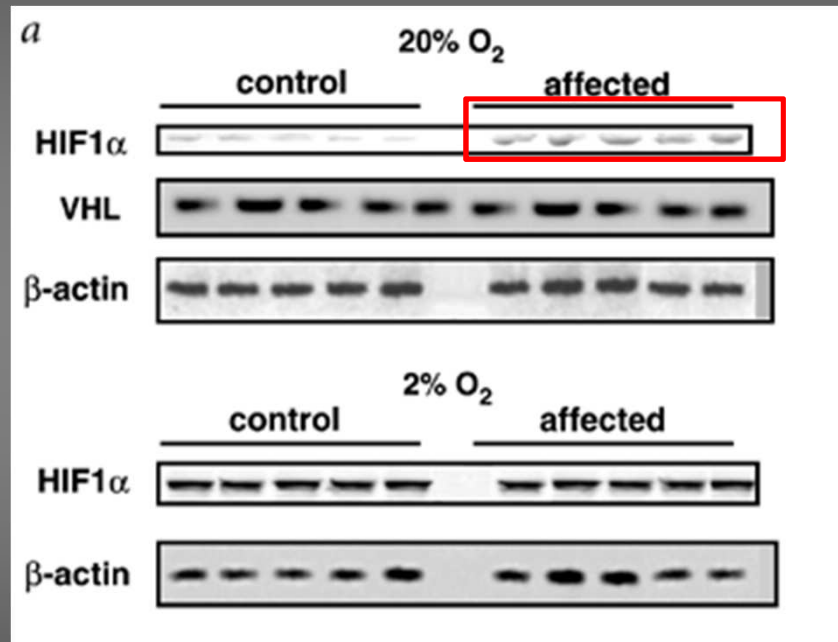
	Individuals with Chuvash polycythemia (n = 20)	Unaffected relatives (n = 51)	P
Erythropoietin (mIU ml ⁻¹)	61.9 ± 12.8	6.4 ± 6.9	0.001
Serum ferritin ^a (ng ml ⁻¹)	19 (15–24)	28 (25–32)	0.2
Serum iron (μg dL ⁻¹)	64 ± 15	81 ± 9	0.4
Total iron binding capacity (μg dL ⁻¹)	427 ± 18	346 ± 10	0.001
Transferrin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



- Livelli di HIF1α maggiori nei soggetti affetti

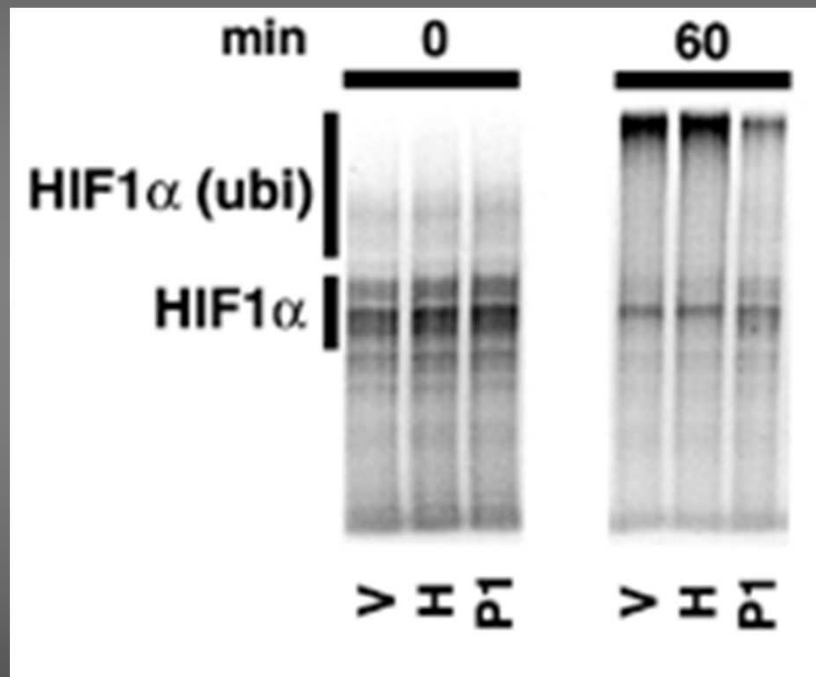
- Livelli di proteina VHL normali in mutato e Wt

Western blot, 5 pazienti + 5 controlli

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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

