

A microscopic view of numerous red blood cells, which are biconcave discs, filling the frame. The cells are densely packed and appear in various shades of red and orange, with some showing a distinct central pallor. The background is dark, making the individual cells stand out.

*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.

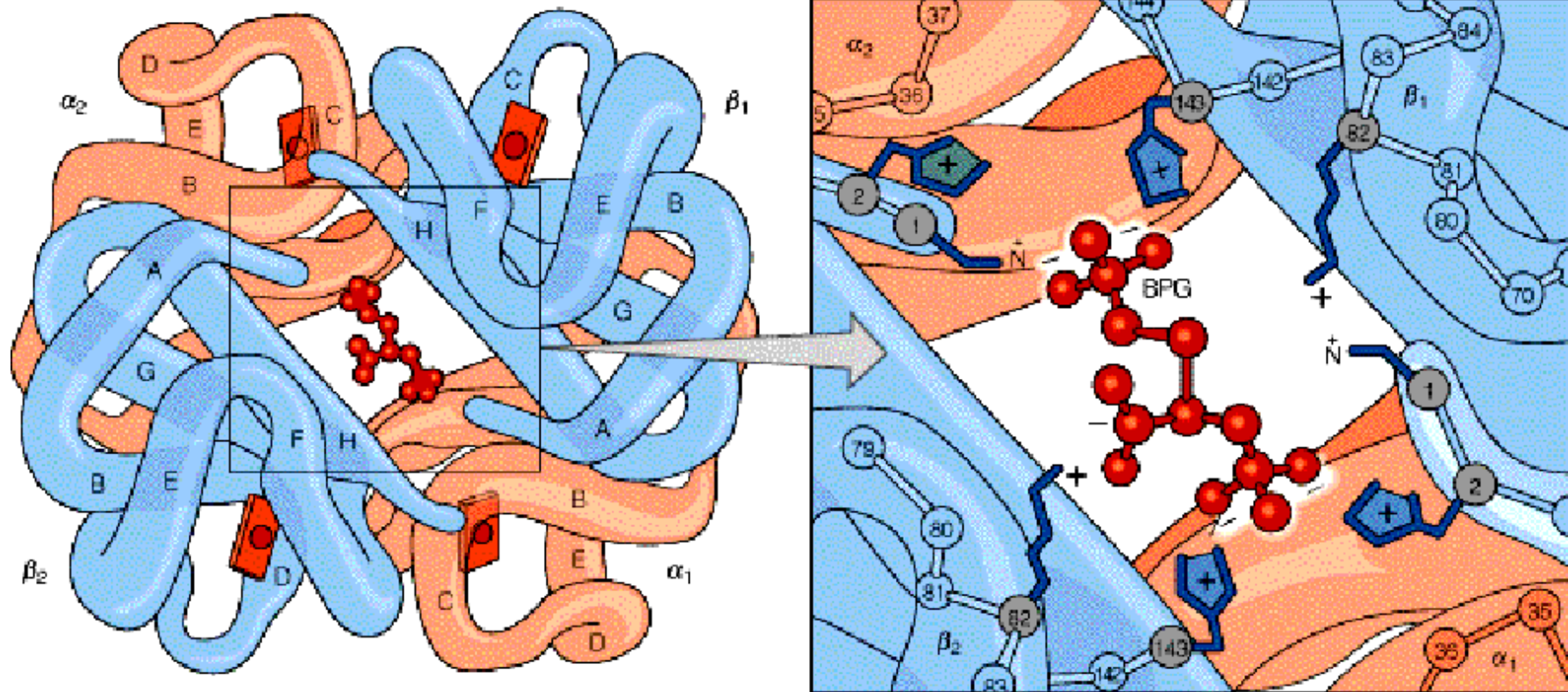
2,3 Bisphosphoglycerate (BPG)

- 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

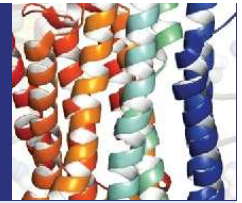
2,3 Bisphosphoglycerate (BPG)



Bisphosphoglycerate (BPG)

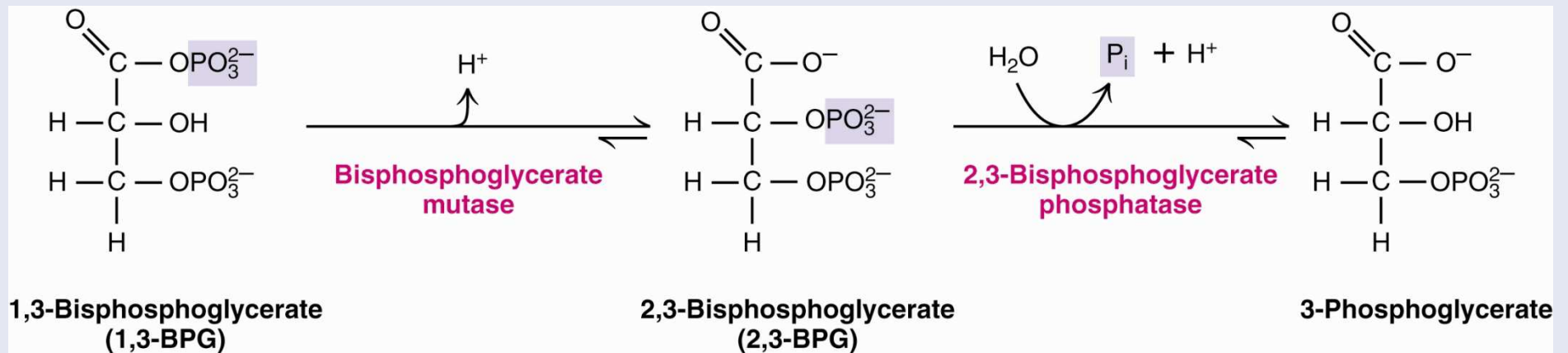
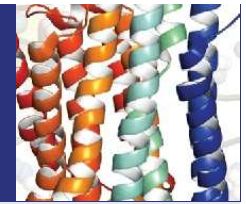
- 2,3-BPG is a glycolytic intermediate in RBCs

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



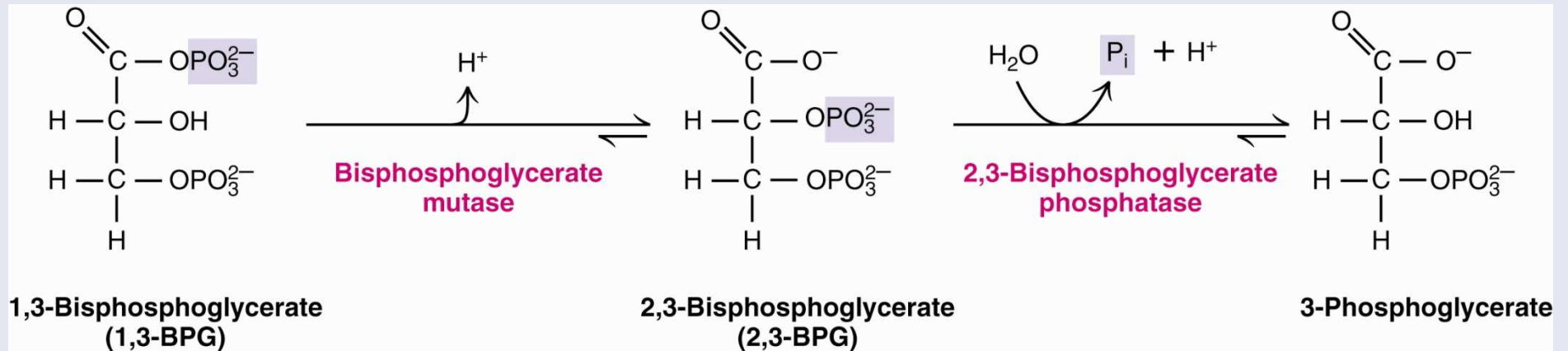
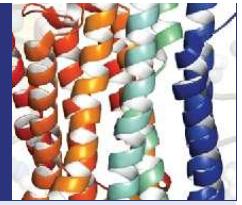
- Most cells contain only a trace of 2,3-BPG, but erythrocytes typically contain 4-5 mM 2,3-BPG
- 2,3-bisphosphoglycerate is an important **regulator of hemoglobin**
- 2,3-BPG (for hemoglobin) is made by circumventing the PGK reaction
- 2,3-BPG is formed from 1,3-BPG by bisphosphoglycerate mutase.
-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

Erythrocyte synthesis of 2,3-BPG



Formation and decomposition of 2,3-bisphosphoglycerate in erythrocytes

Erythrocyte synthesis of 2,3-BPG

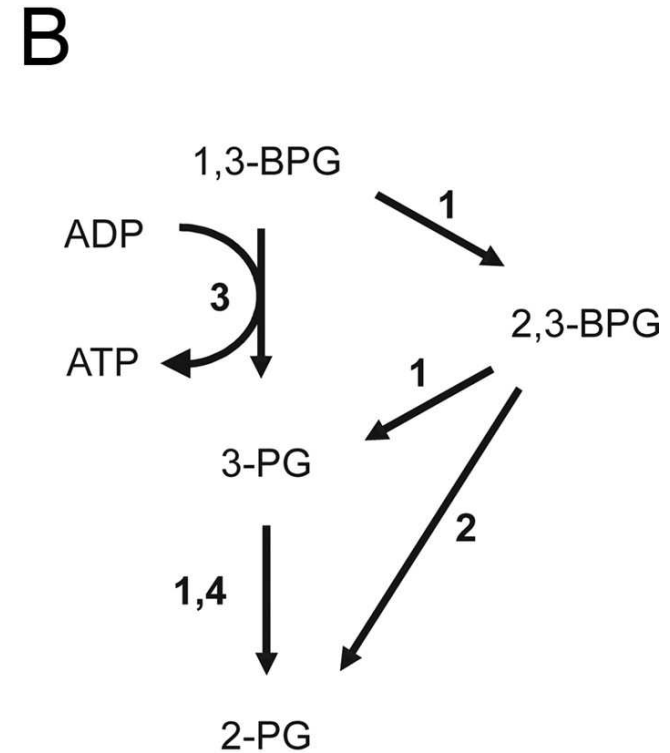
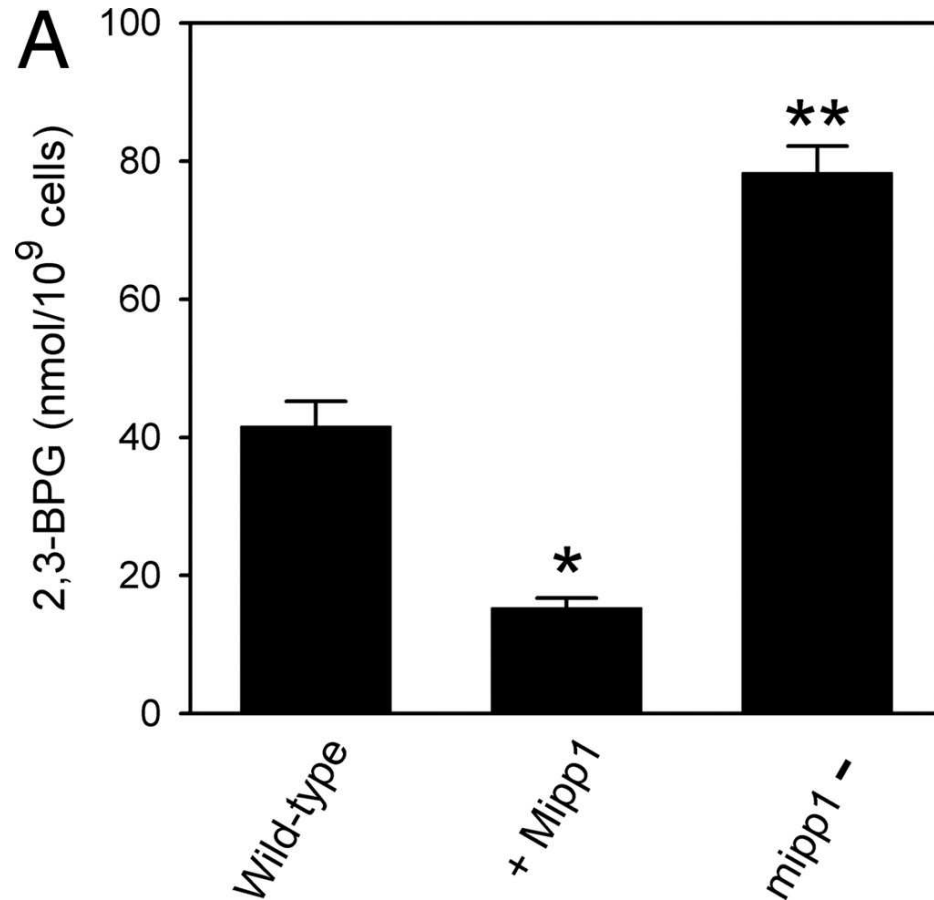


multiple inositol polyphosphate phosphatase (MIPP1)

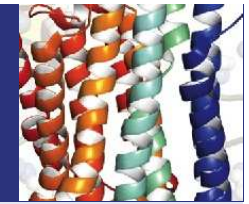
Hydrolysis of 2,3-BPG by human MIPP1 is sensitive to physiologic alkalosis;

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

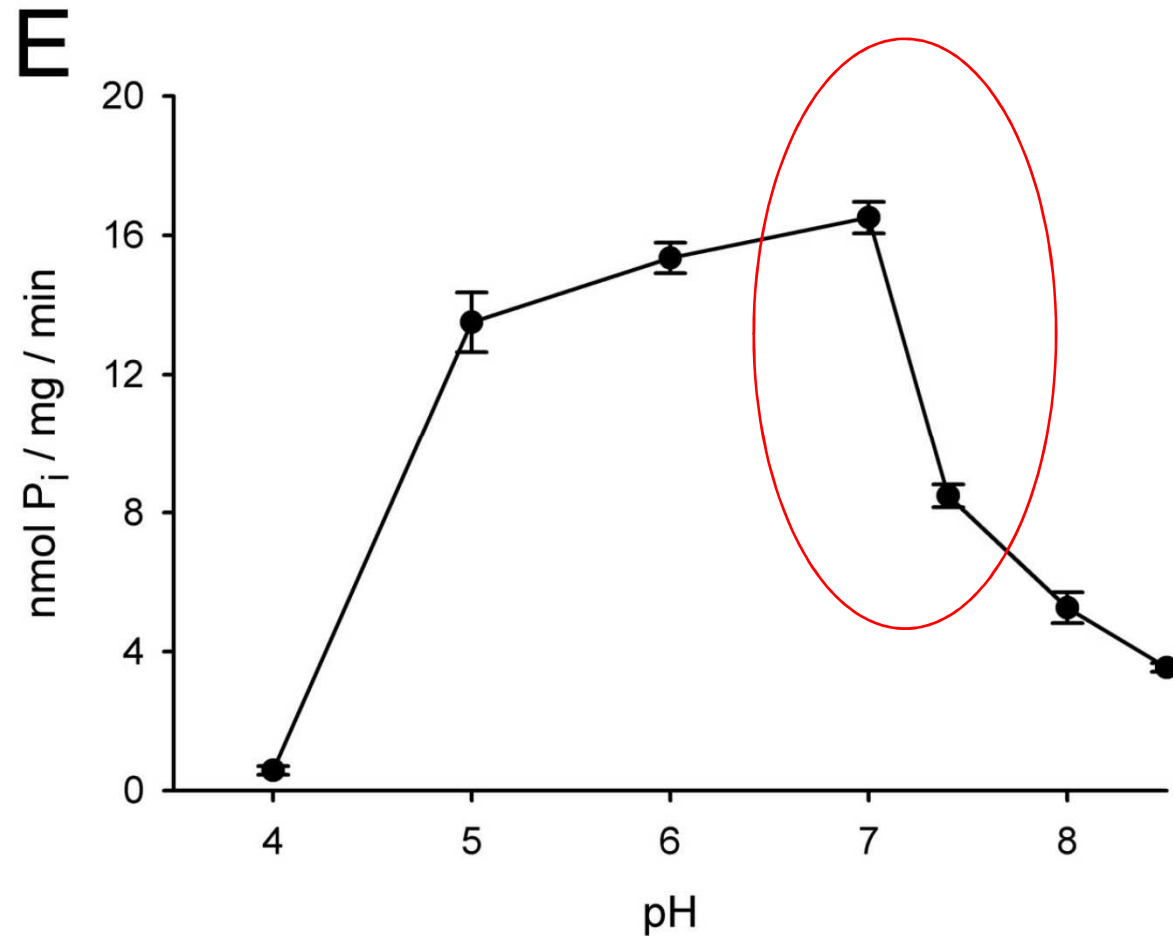
Cellular levels of 2,3-BPG in Dictyostelium respond to genetic manipulations of expression.



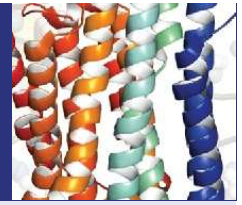
Levels of 2,3-BPG in wild-type Dictyostelium, in cells in which the mipp1 gene was disrupted - , and in cells in which DdMipp1 was overexpressed +.



The effect of pH on enzyme activity of MIPP1



2,3-BPG as substrate



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 .

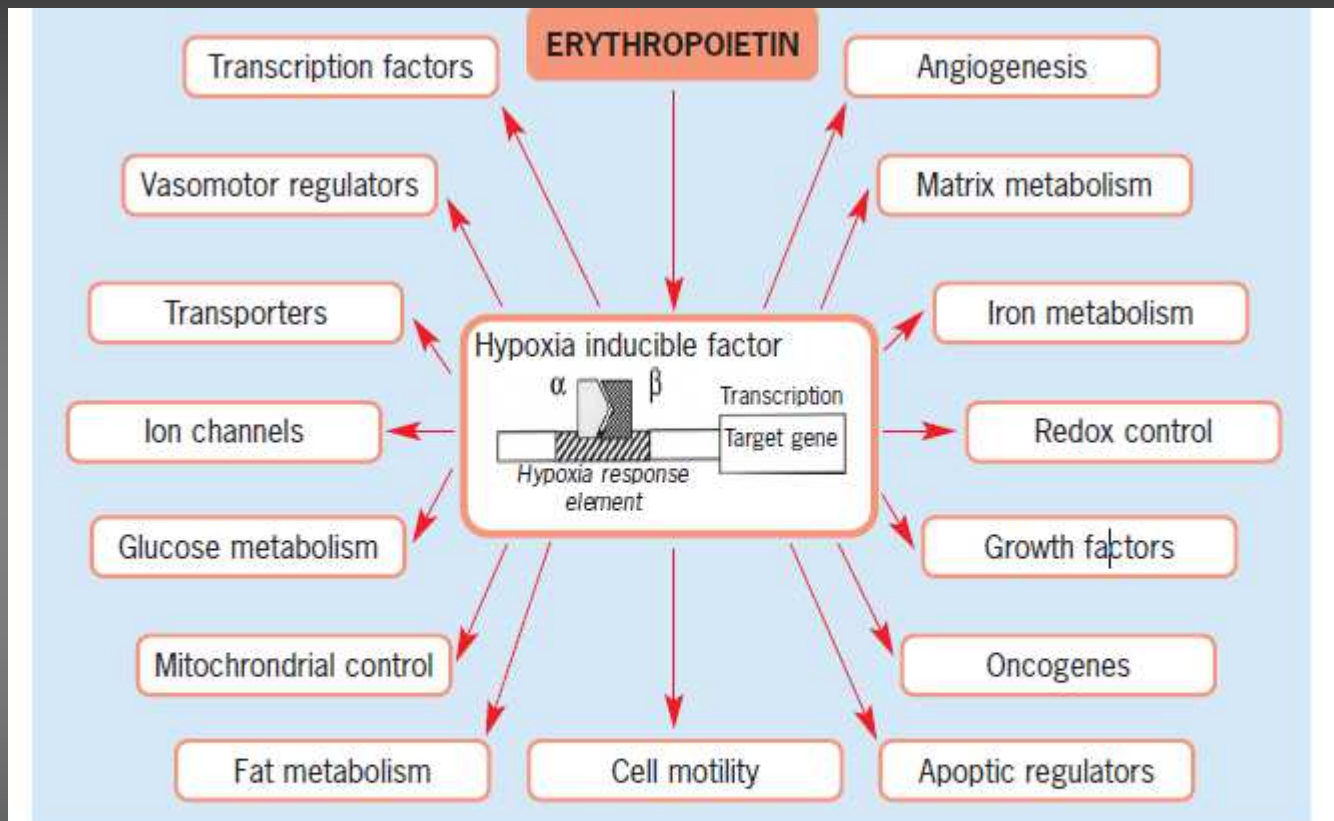
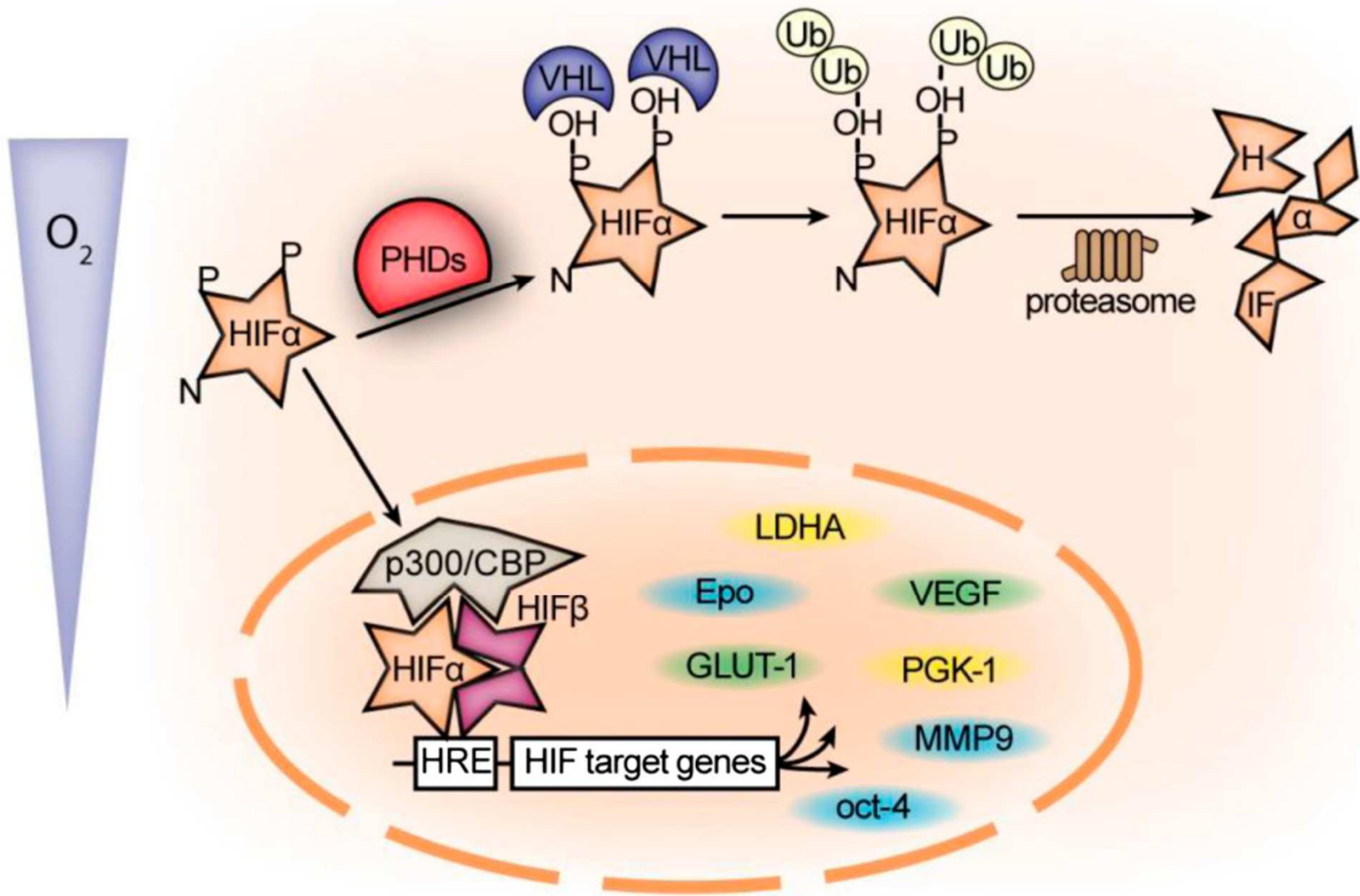
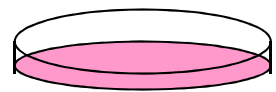


Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.



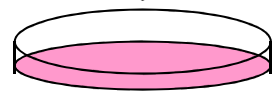
Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

Eric Huang et al. - JBC 1996



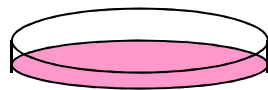
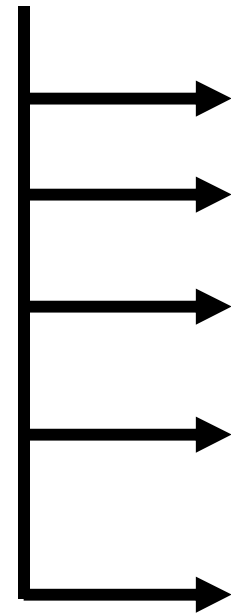
HeLa cells

20% O₂

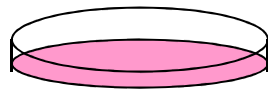


HeLa cells

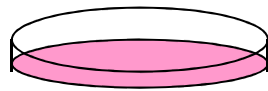
1% O₂ per 4h



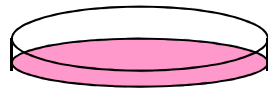
20% O₂ per 0 min



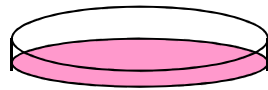
20% O₂ per 5 min



20% O₂ per 10 min



20% O₂ per 30 min



20% O₂ per 60 min

Lisi delle cellule

-Quantificazione mRNA di HIF1

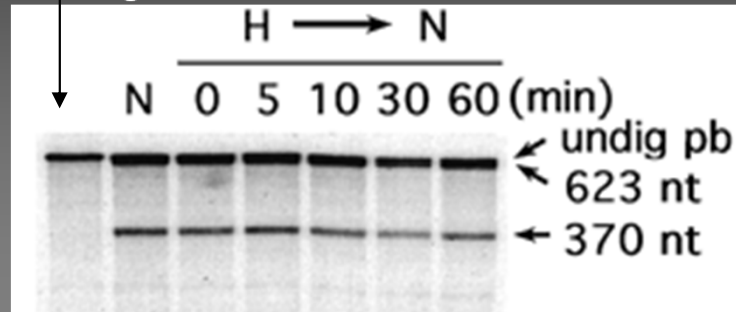
-Quantificazione proteine: HIF1 α e HIF1 β

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

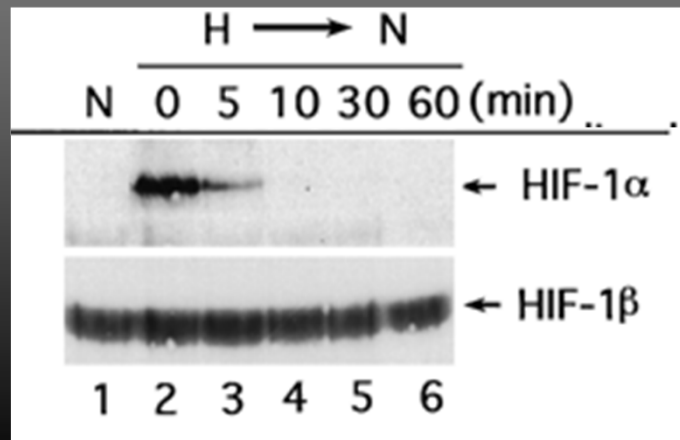
Sonda indigerita



HIF1 α è espresso a livello di mRNA.

Quantificazione proteine → **Western blot**

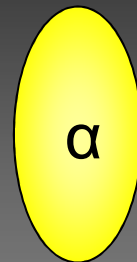
Ibridazione con anticorpi anti HIF1 α e HIF1 β



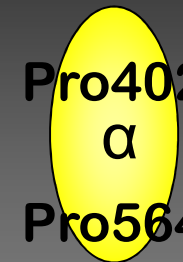
HIF1 α è presente solo in condizioni di ipossia

HIF1 β è sempre presente

Struttura di HIF1



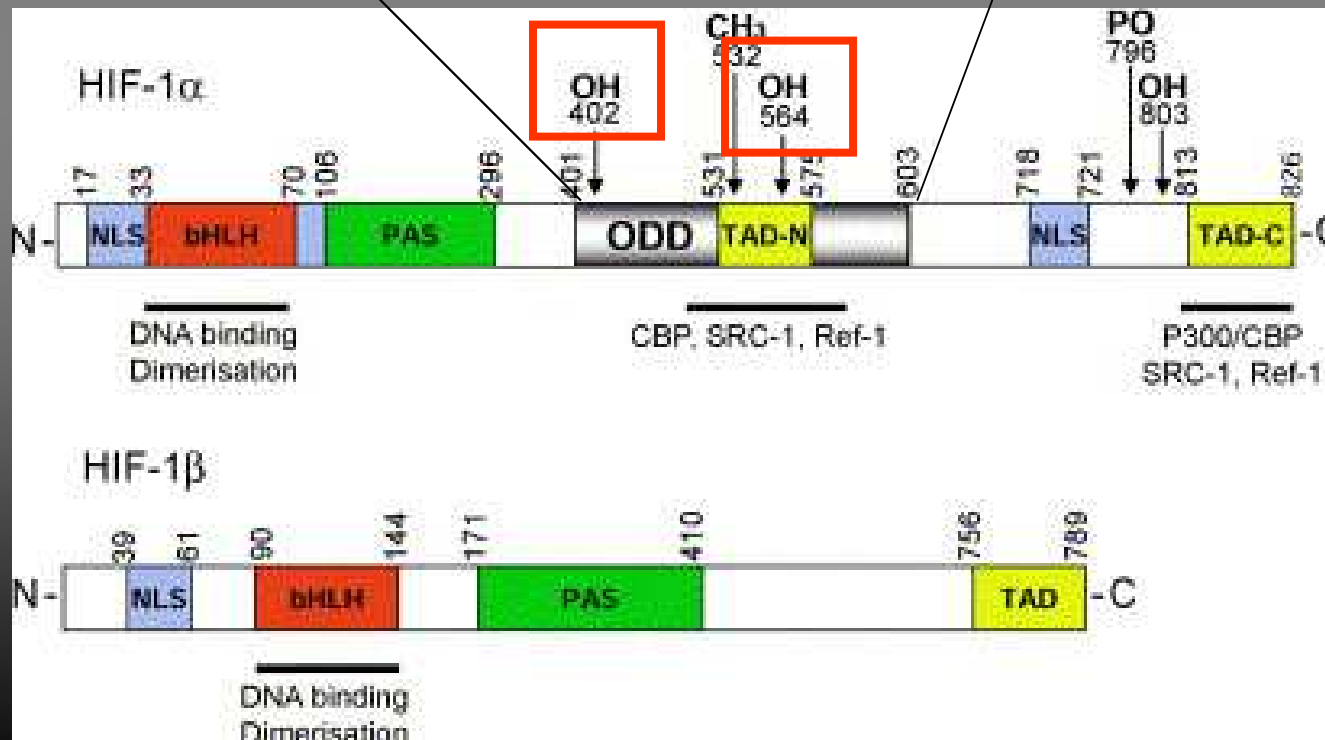
Prolina-Idrossilasi



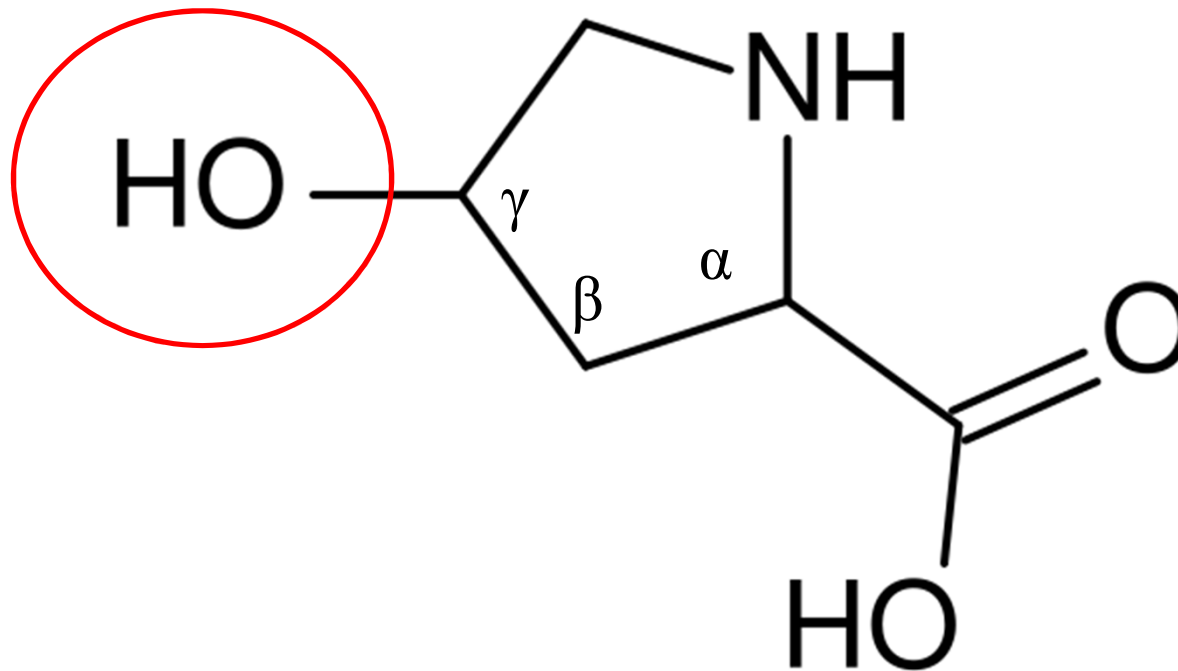
Pro402—OH

Pro564—OH

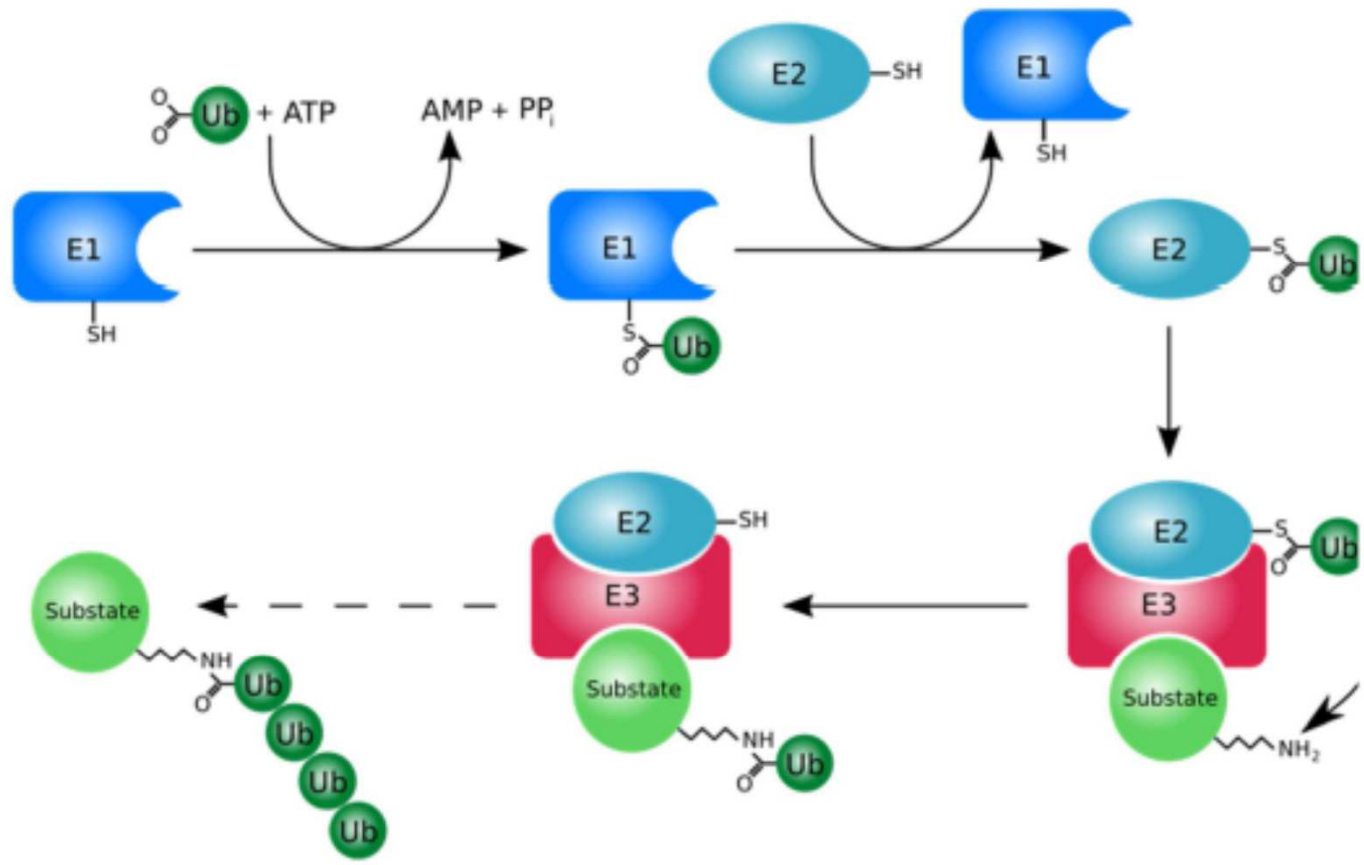
Sequenza di idrossilazione

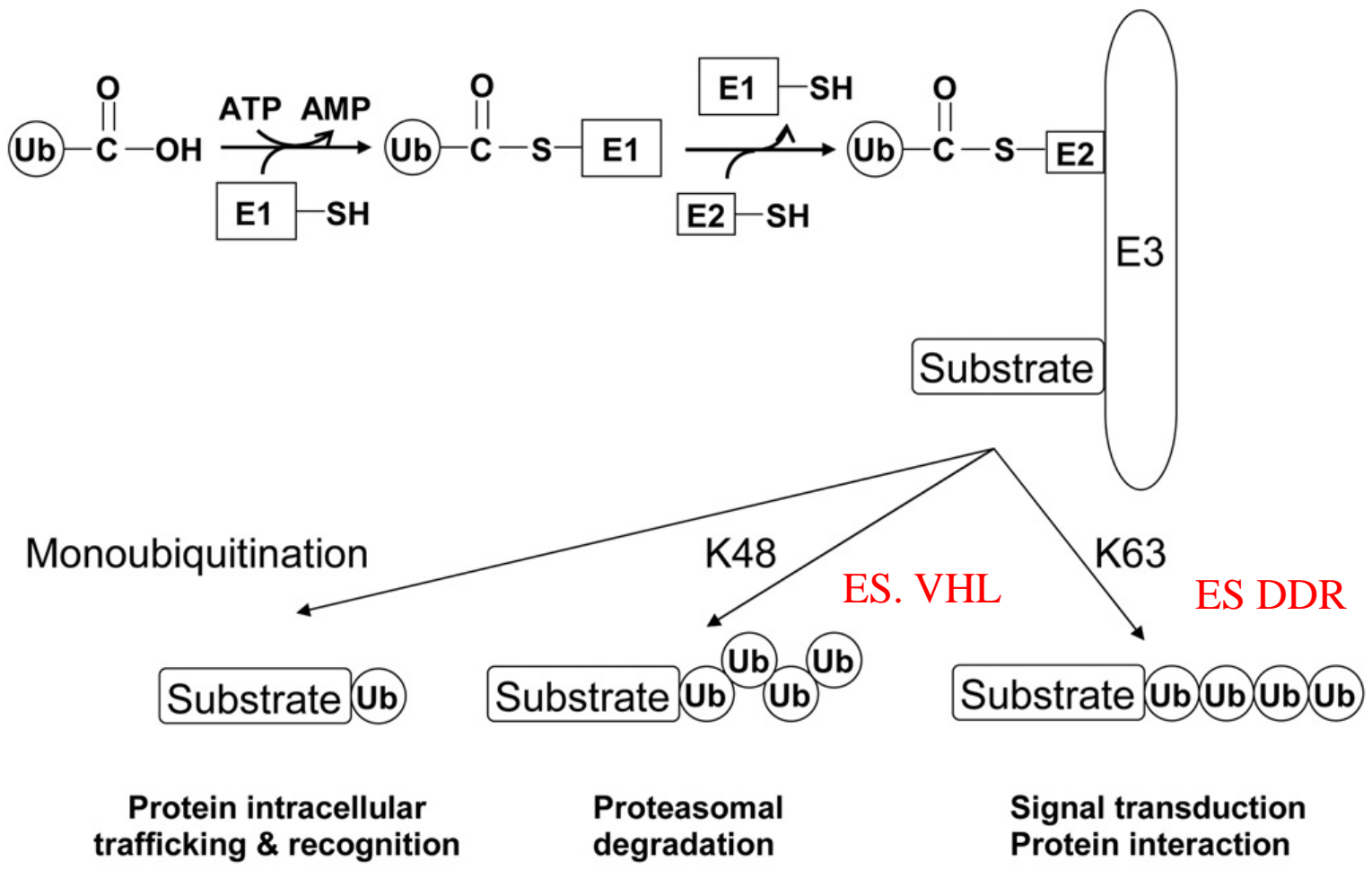


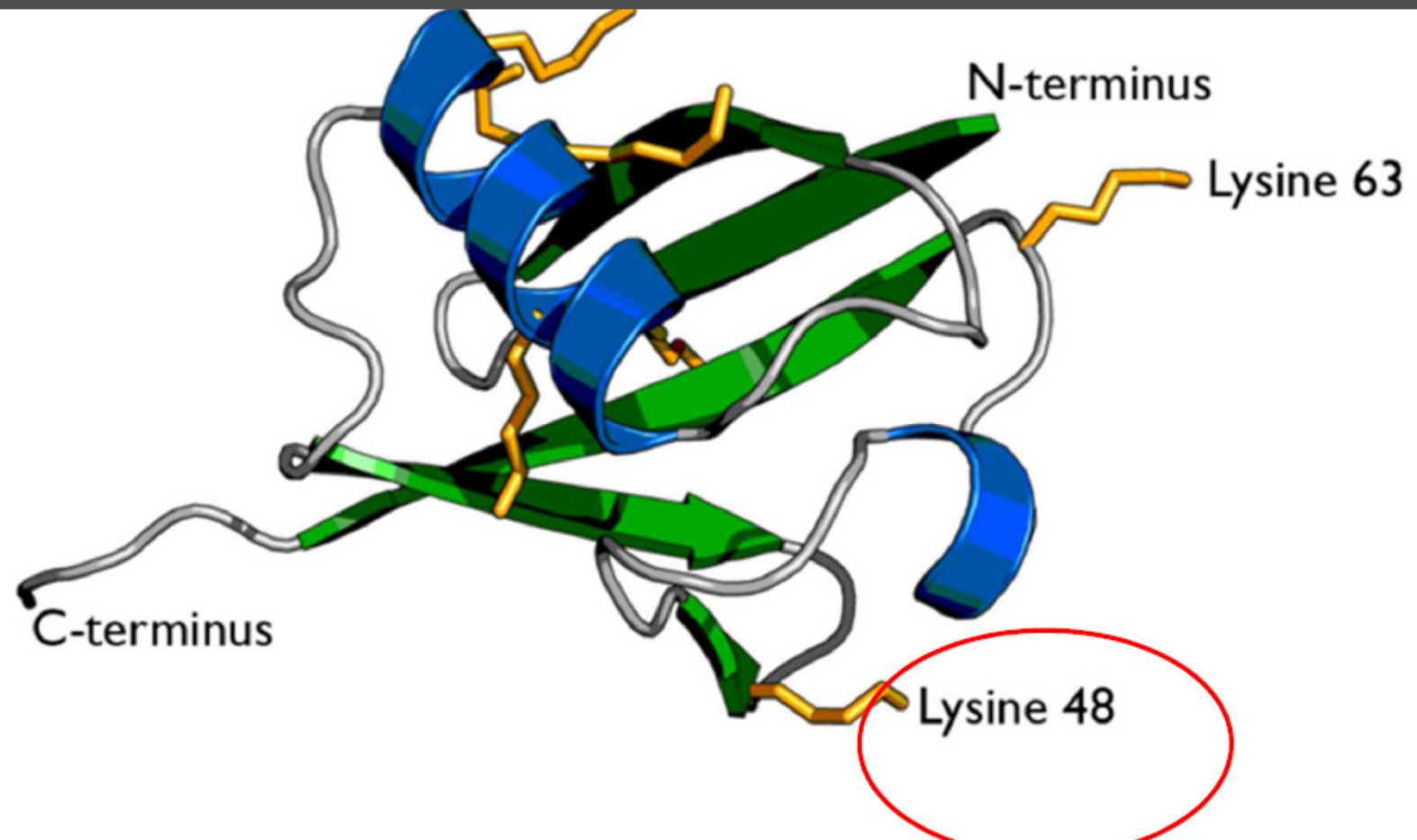
Idrossiprolina



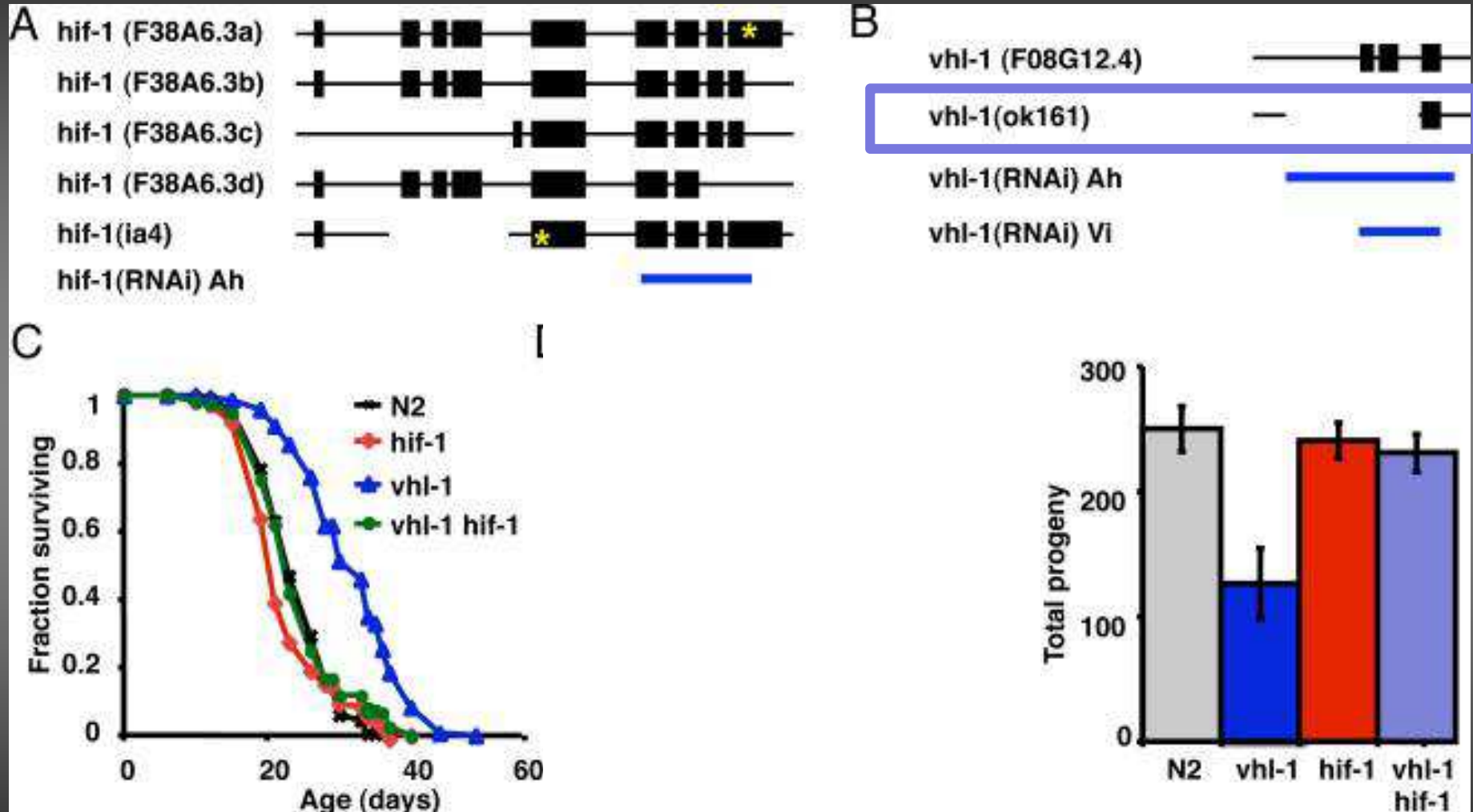
4-hydroxypyrrolidine-2-carboxylic acid





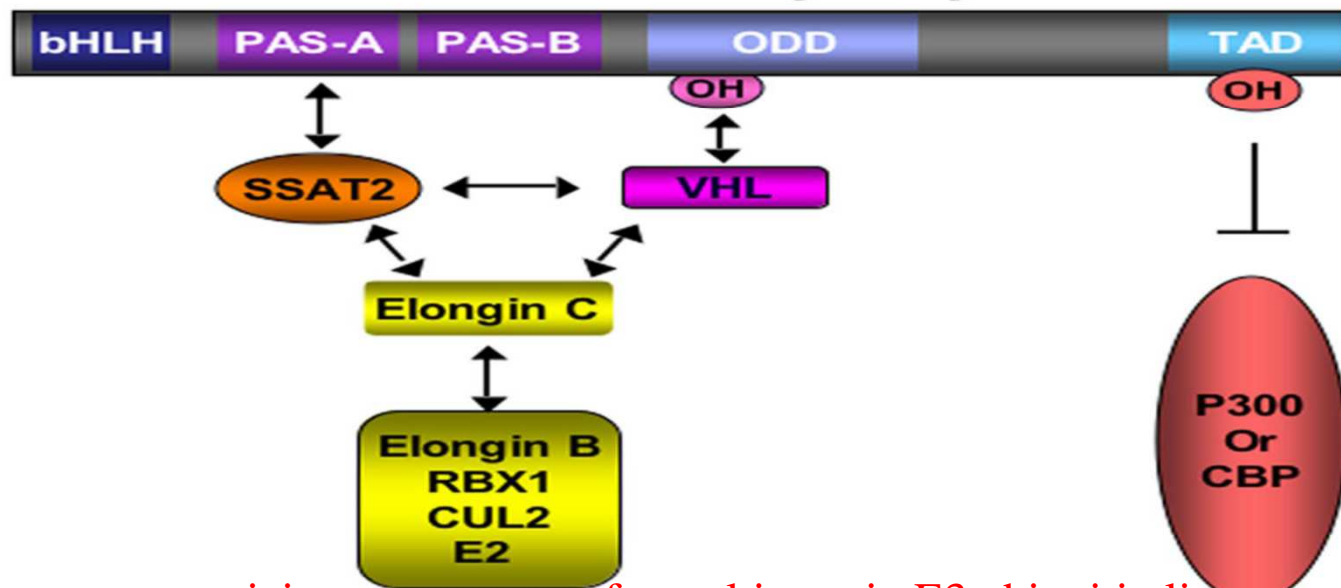
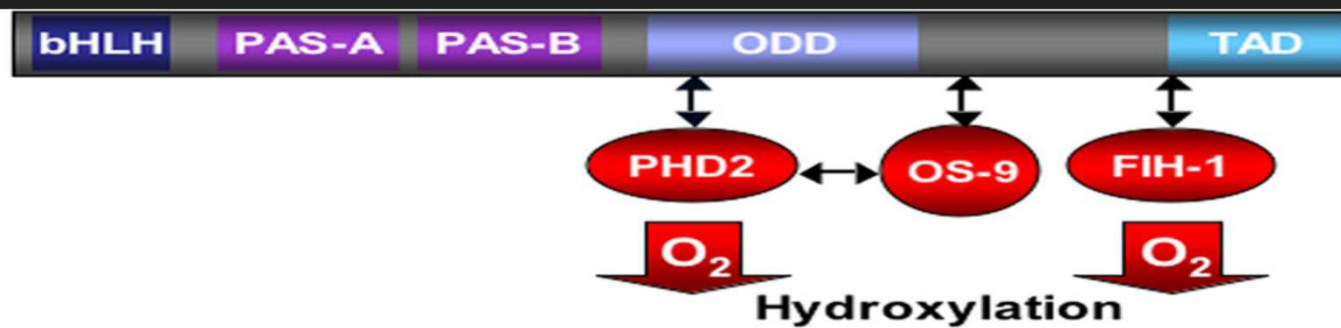


VHL-1 modulates longevity and reproduction



Vhl-1 deletion (ok161) animals are significantly longer-lived than wild type (N2) animals but the total progeny is decreased

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

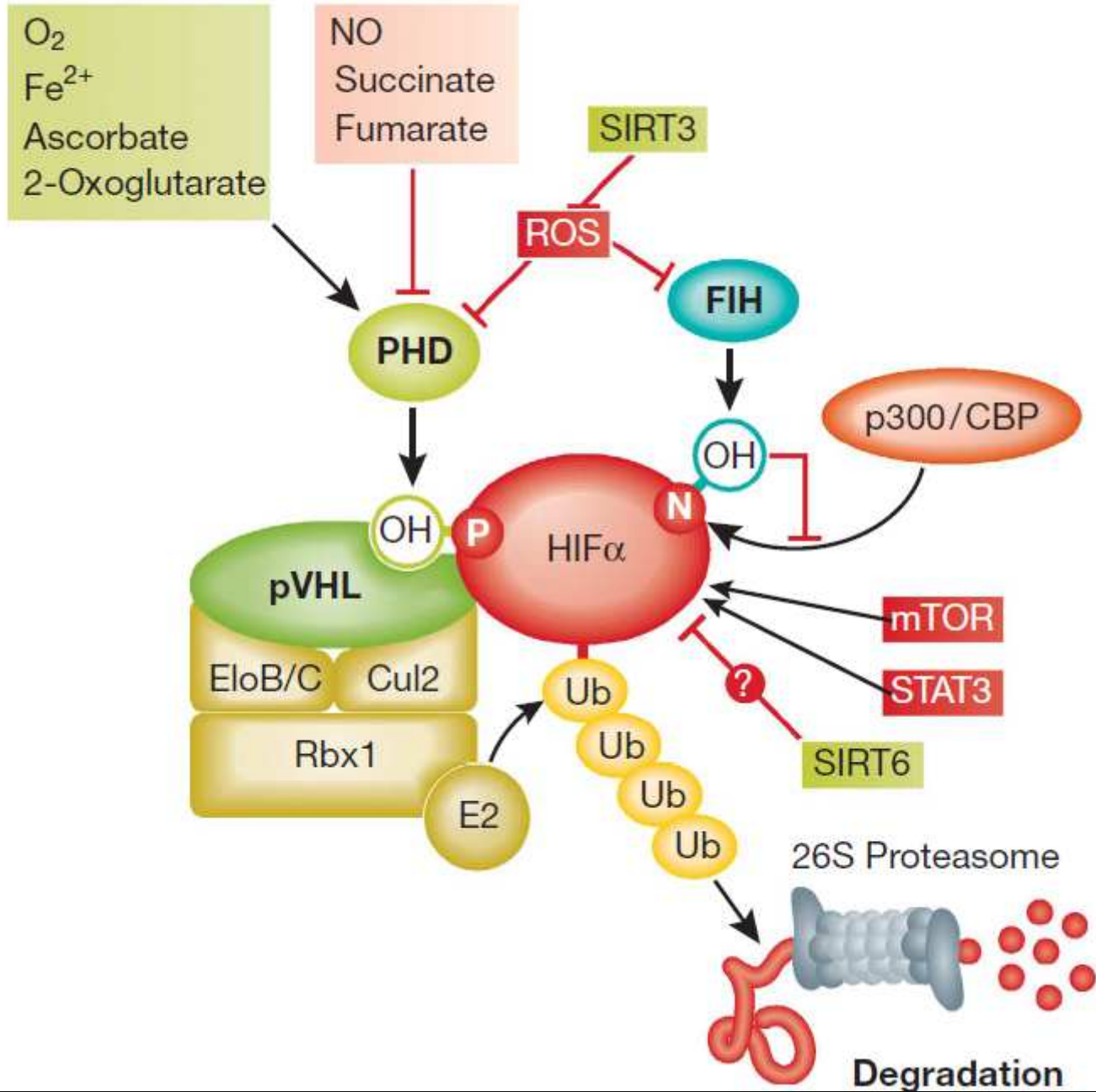
HIF-1 α Ubiquitination



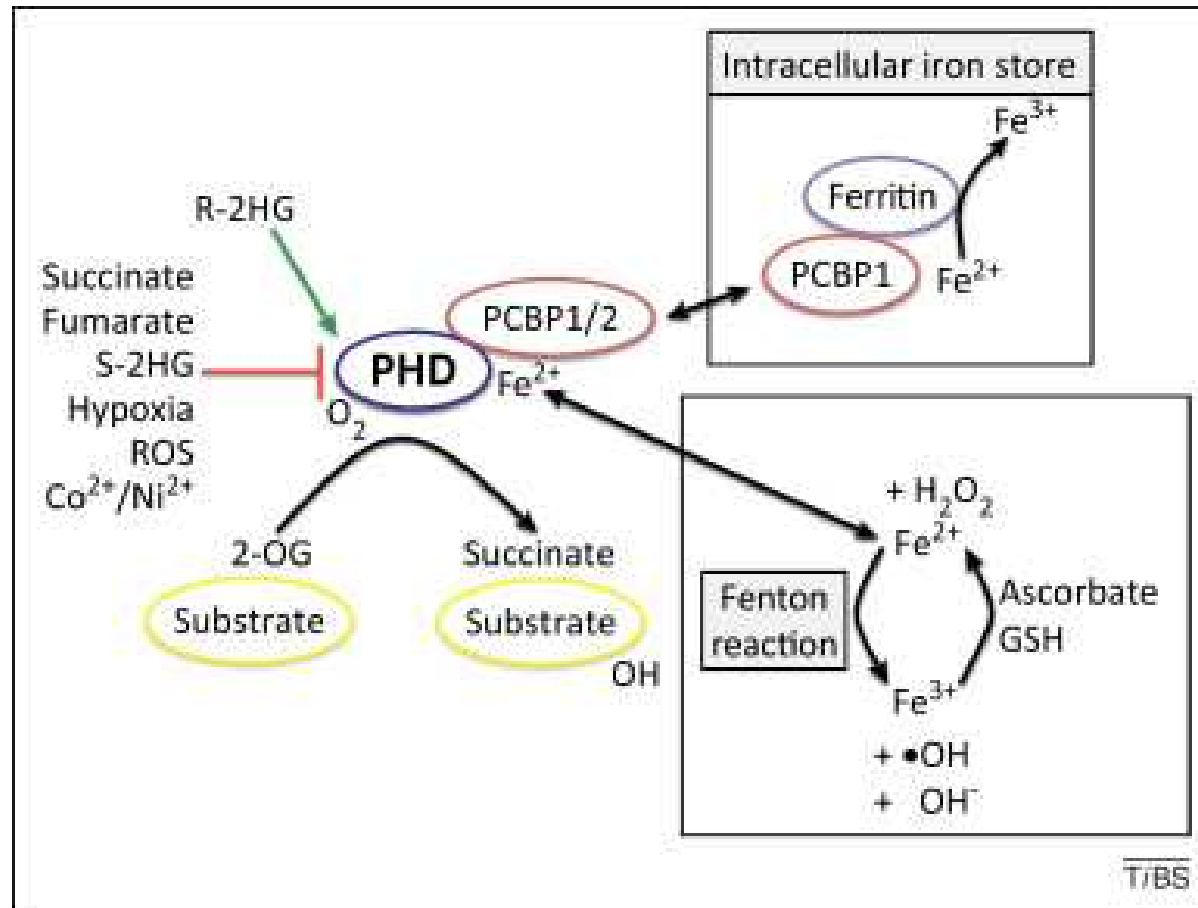
26S Proteasome-Dependent HIF-1 α Degradation

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

A Normoxia



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)-1 α . 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.

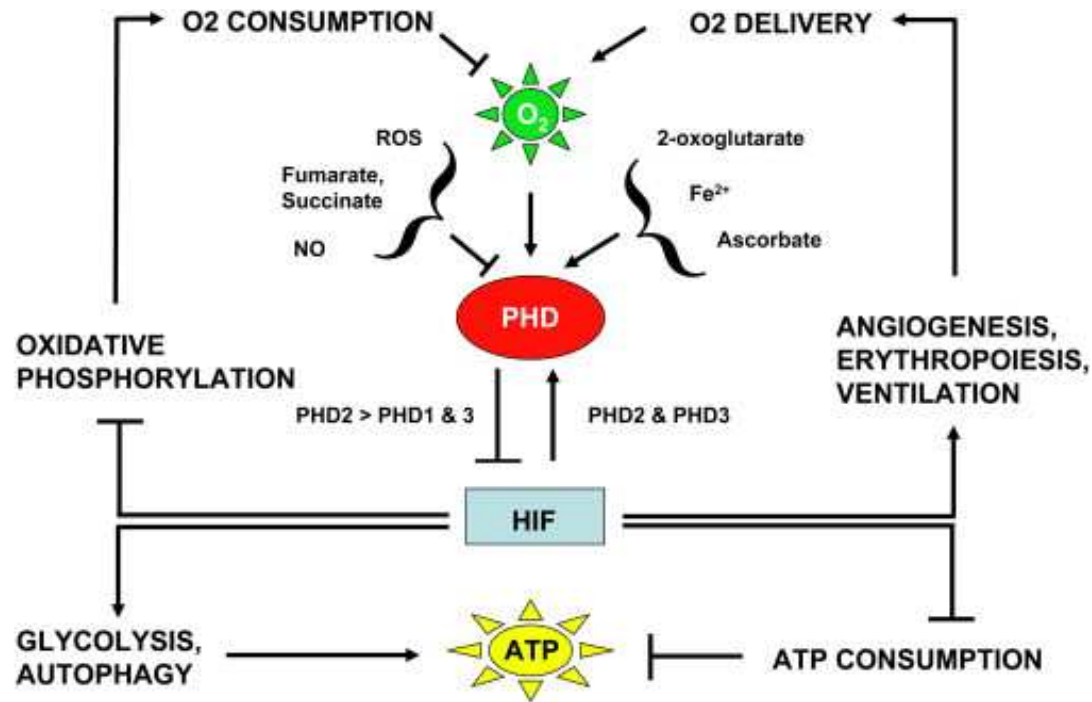


Figure 1. Central Role of PHD Prolyl Hydroxylases and the HIF Transcription Factor in Response to Hypoxia

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

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

Concentrations of oxygen in tissues

- range 10–30 μM -

below the K_m for oxygen of the hydroxylases

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

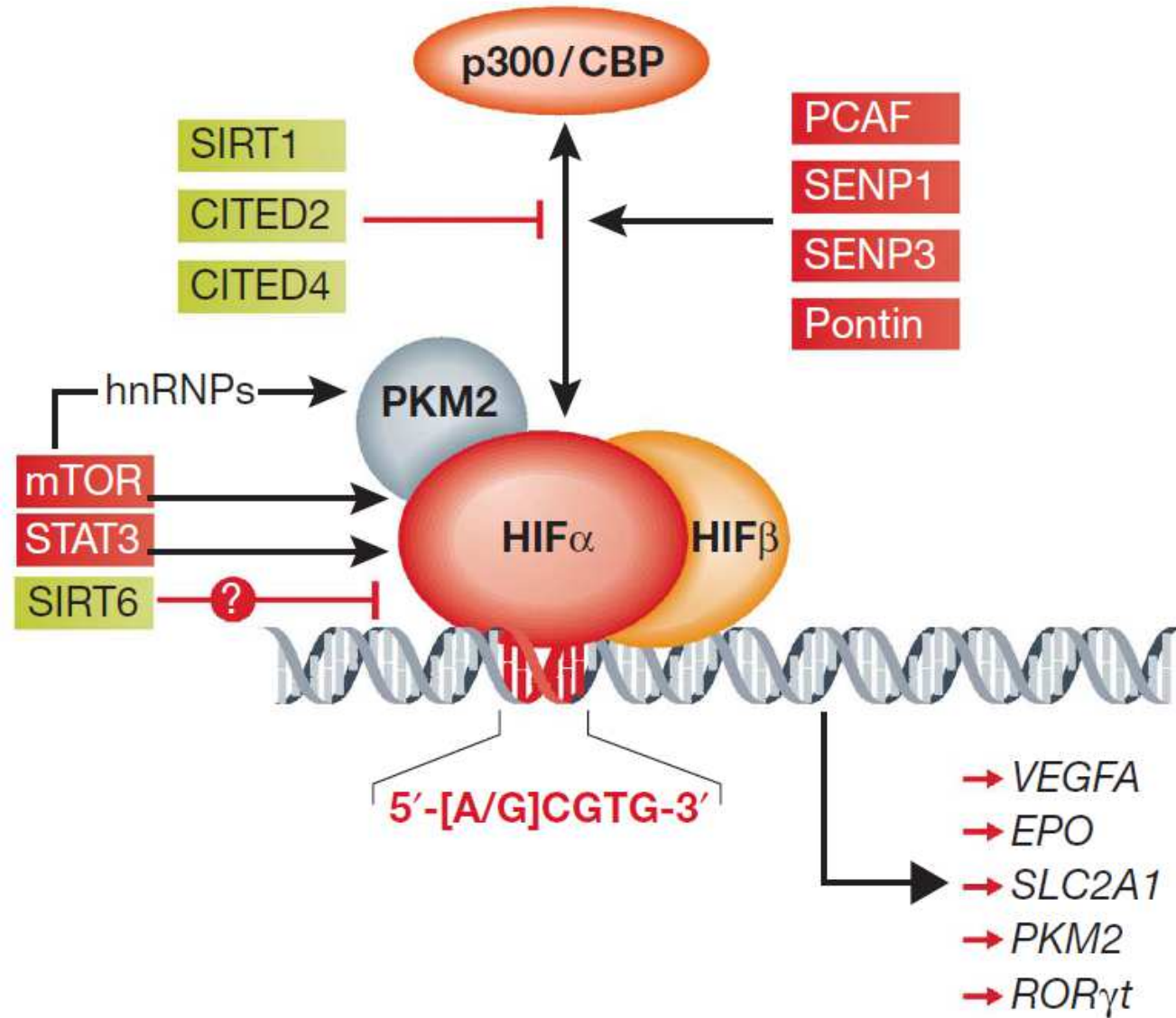
(B) Under low oxygen tension HIFa associates with HIFb.

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).

B Hypoxia



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

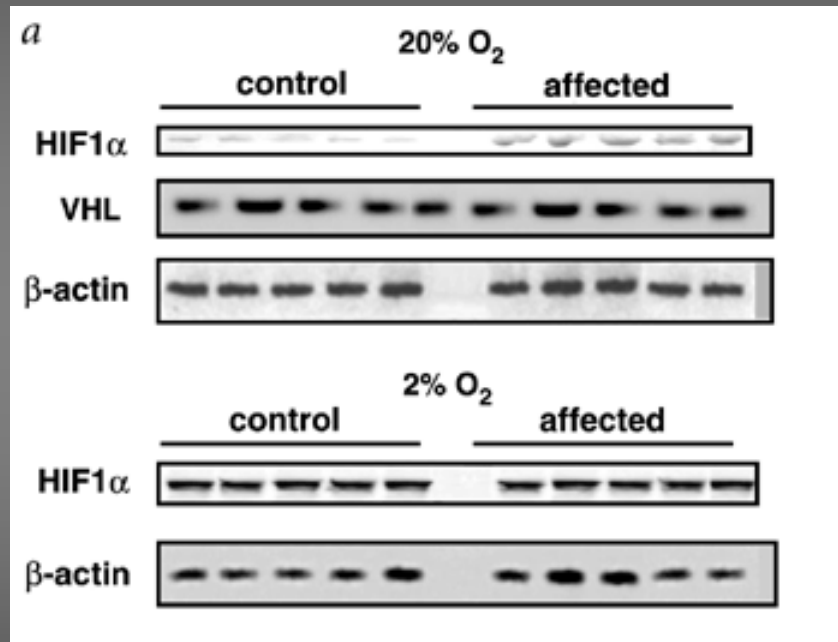
	Individuals with Chuvash polycythemia (n = 20)	Unaffected relatives (n = 51)	<i>P</i>
Erythropoietin (mIU ml ⁻¹)	61.9 ± 12.8	6.4 ± 6.9	0.001
Serum ferritin ^a (ng ml ⁻¹)	19 (15–24)	28 (25–32)	0.2
Serum iron (μg dL ⁻¹)	64 ± 15	81 ± 9	0.4
Total iron binding capacity (μg dL ⁻¹)	427 ± 18	346 ± 10	0.001
Transferrin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

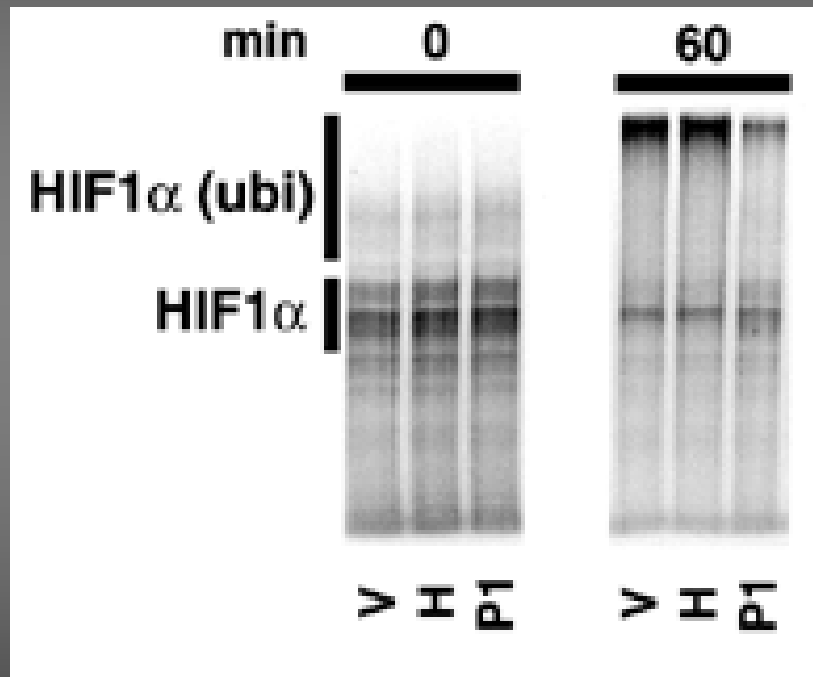
20% O₂:

- 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 HIF1 α

- Aumentata espressione del gene Epo \rightarrow policitemia

Increased survival, proliferation, and differentiation of erythroid progenitor cells

