

Elucidation of oxygen sensing mechanisms in human and animal cells
Peter J. Ratcliffe

Nobel Lecture - December 2019

Oxford-Yale expedition to Pike's Peak, Colorado
J. S. Haldane and colleagues - July 1911



Haldane, Fitzgerald, Schneider, Henderson and Douglas at top of Pike's peak, 1911

VIII. *The Changes in the Breathing and the Blood at Various High Altitudes.*

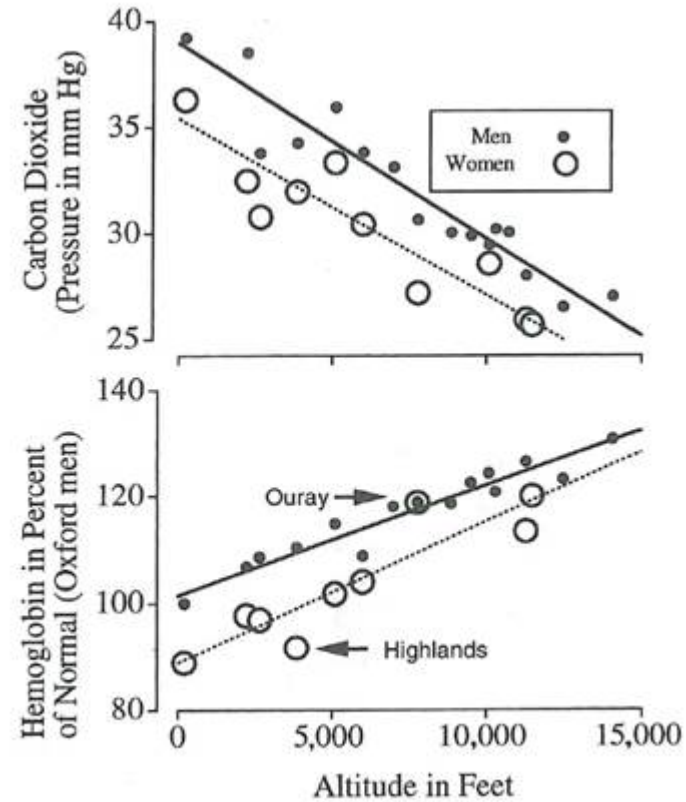
By MABEL PUREFOY FITZGERALD.

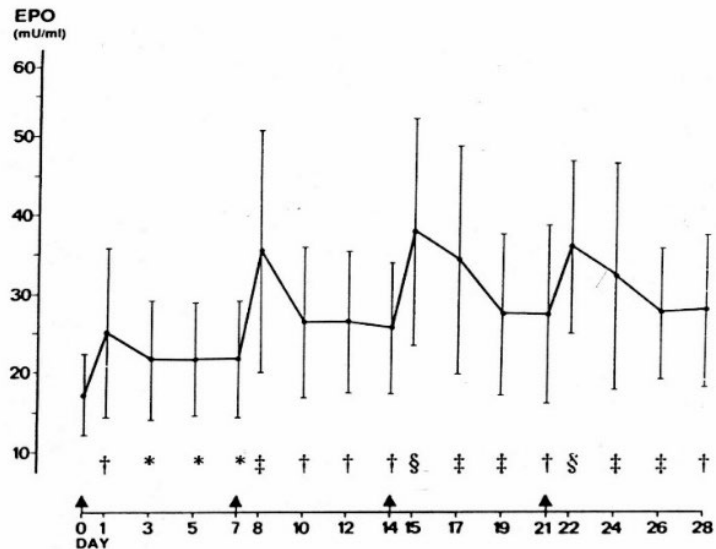
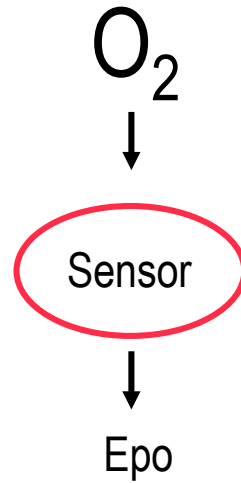
Communicated by Dr. J. S. HALDANE, F.R.S.

(Received January 16,—Read February 20, 1913.)



Fig. 3.3. Mabel FitzGerald, measuring the hemoglobin in the blood by diluting a sample of blood in one of two tubes until it matches the color of the standard in the other tube. Reprinted from Colorado Springs Herald Telegraph, July 8, 1911.





Extra-ordinary sensitivity of the erythropoietin response to changes in blood oxygen availability – responses to donation of blood

Lorentz et al.
 Transfusion 31
 650-654 (1991)

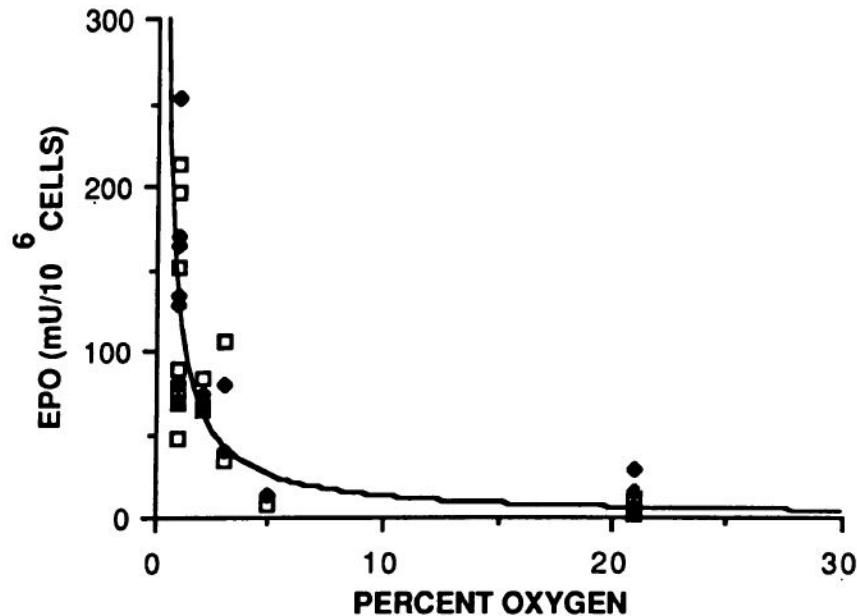
Development of a cellular model for study of Epo regulation by Oxygen

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7972-7976, November 1987
Cell Biology

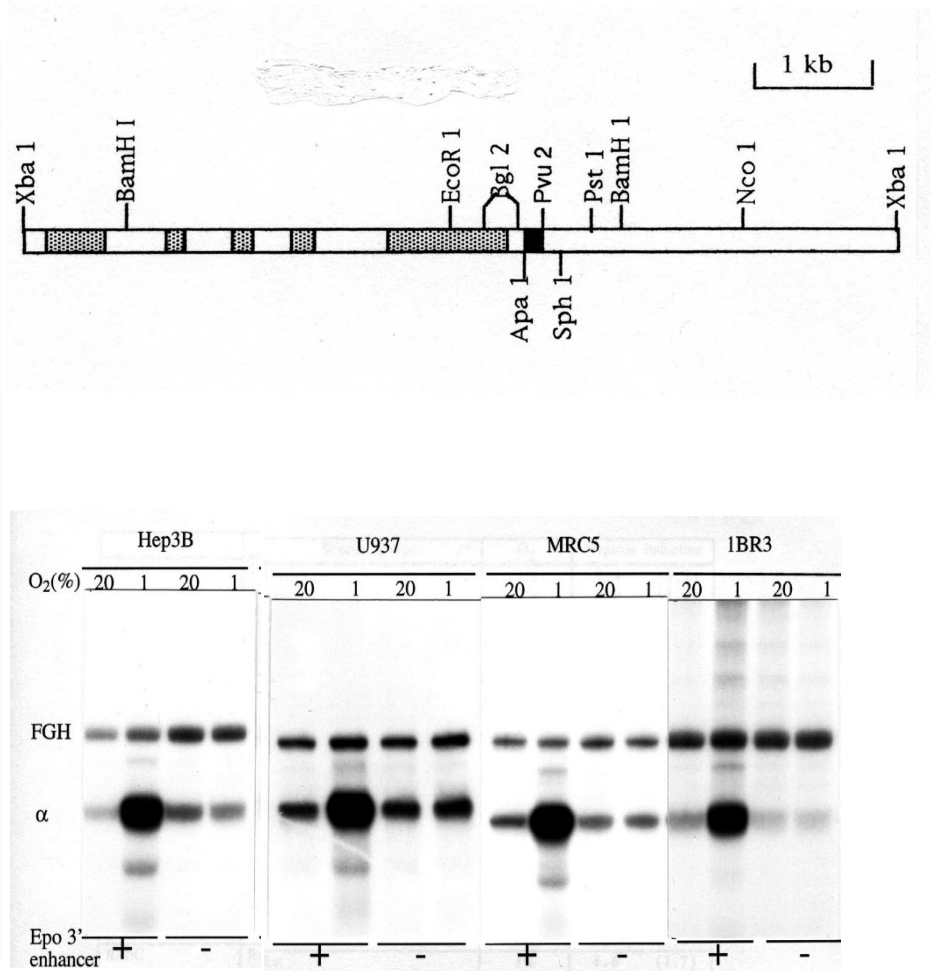
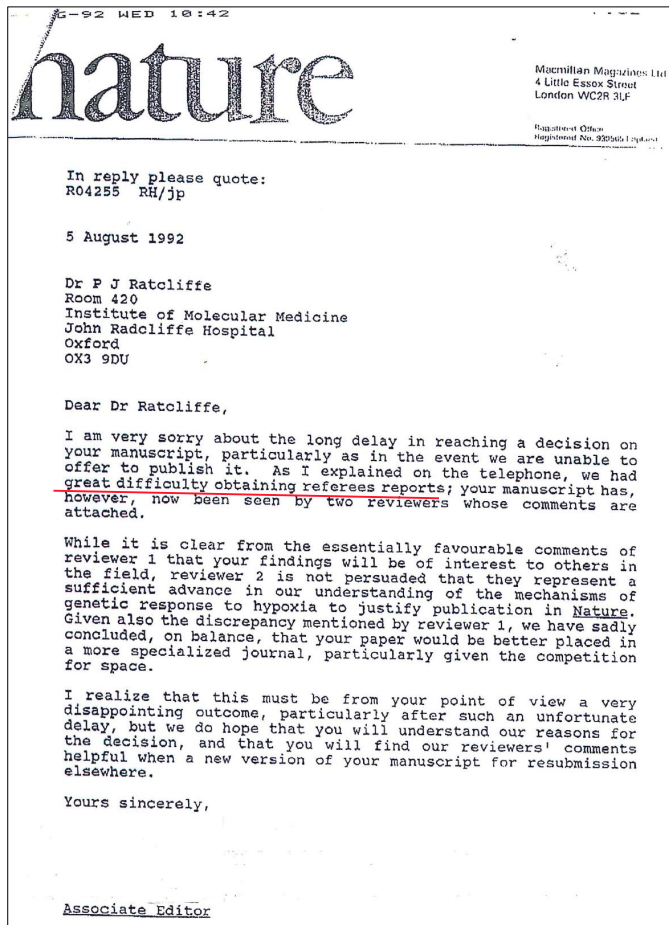
The regulated expression of erythropoietin by two human hepatoma cell lines

MARK A. GOLDBERG, G. ALLISON GLASS, JAMES M. CUNNINGHAM, AND H. FRANKLIN BUNN

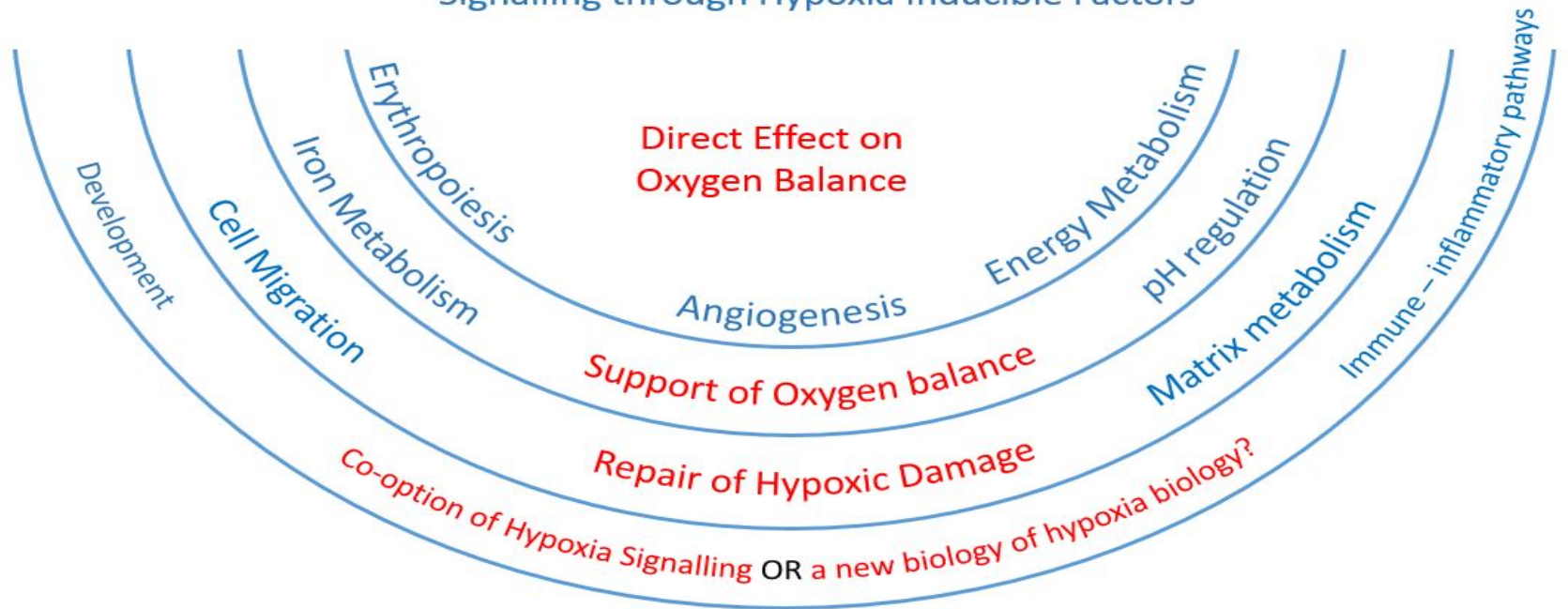
Howard Hughes Medical Institute, Division of Hematology, Brigham and Women's Hospital, and the Department of Medicine, Harvard Medical School, Boston, MA 02115



Widespread operation of hypoxia signalling pathways



Signalling through Hypoxia Inducible Factors



Regulation of HIF by oxygen

Oxygen availability

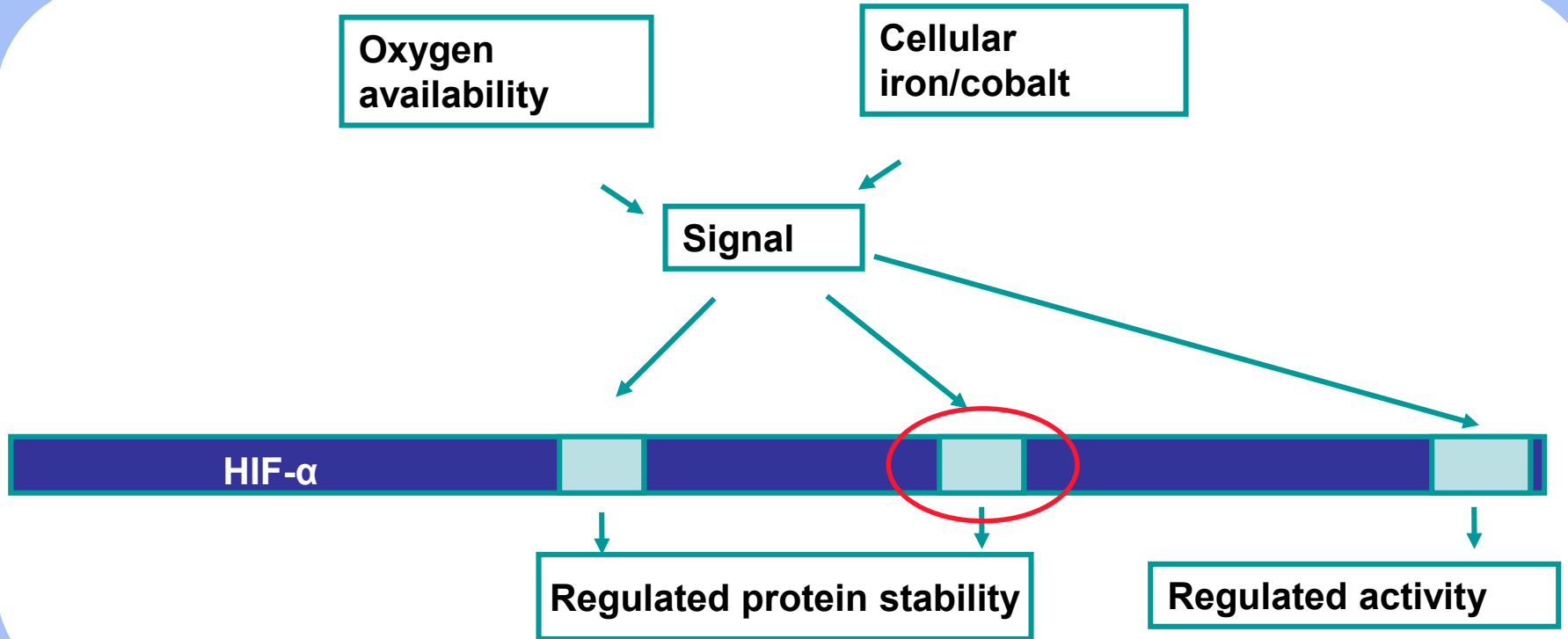
Cellular iron/cobalt

Signal

HIF- α

Regulated protein stability

Regulated activity

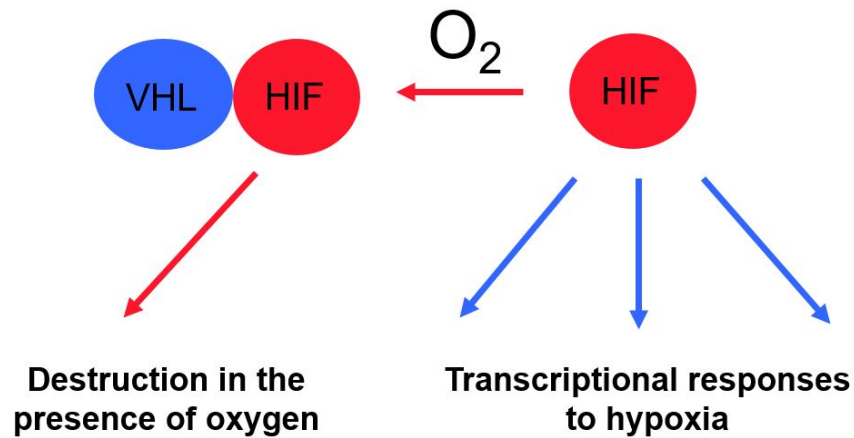


Sequencing the human genome suggests existence of HIF paralogues

Multiple hypoxia inducible transcripts identified

Making anti-EPAS antibodies (PM9) proves regulation by oxygen

Transcripts constitutively upregulated in VHL defective cells



Distortion from HIF-1 to HIF-2 during RCC development

Oxygen sensing pathway

Role in Cancer Treatment by HIF-2 antagonists

Signalling modification is prolyl hydroxylation

Biochemical analysis

Heat labile extract

~~Non-enzymatic oxidation~~

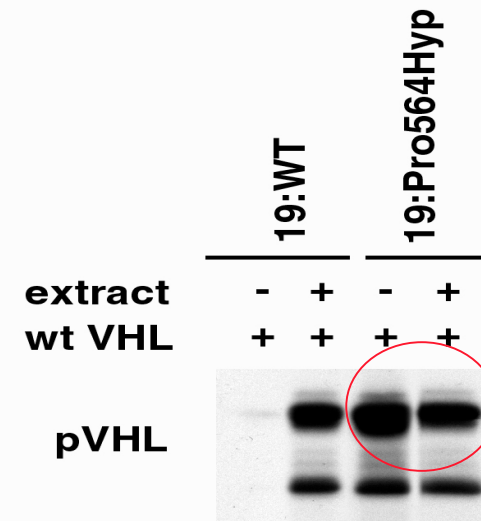
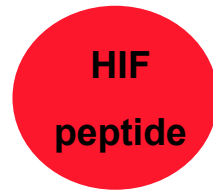
~~NADH/NADH oxidase~~

Oxygen

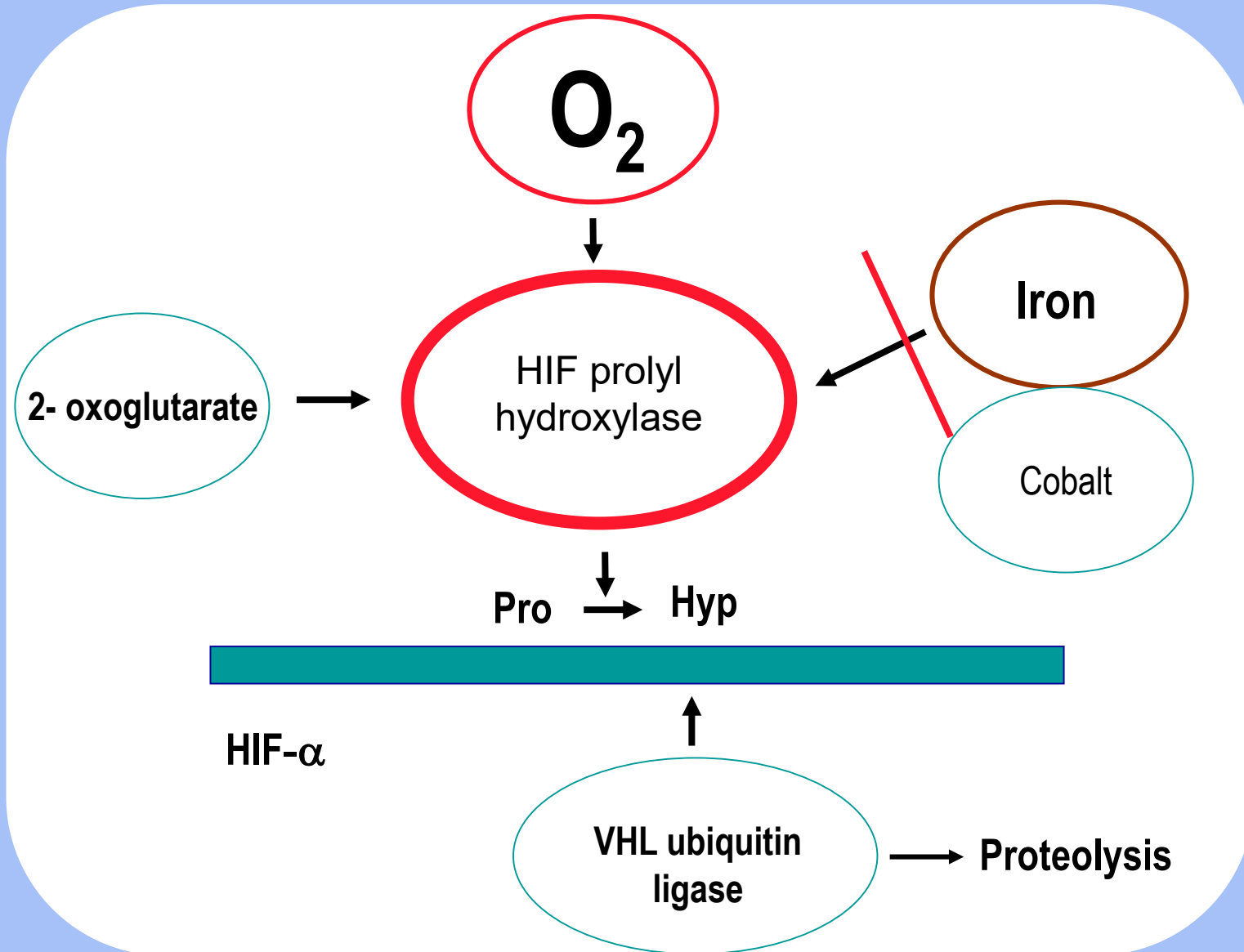
Iron

~~ATP~~

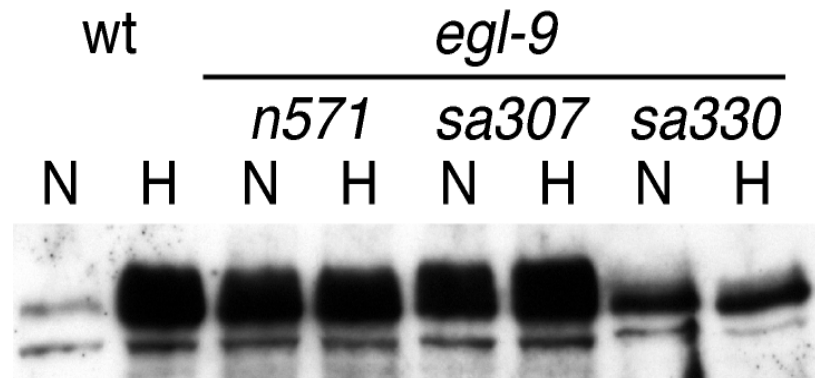
Treatment
with cell
extract

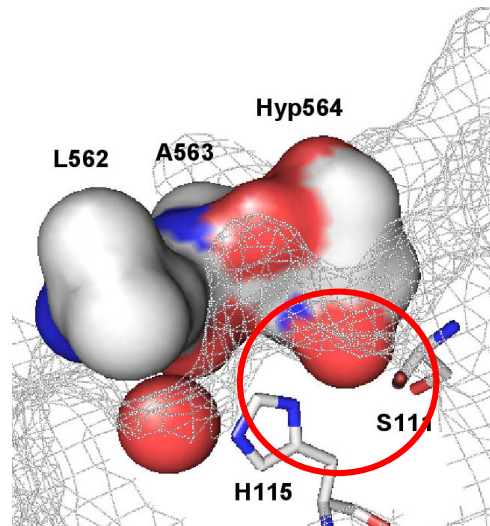
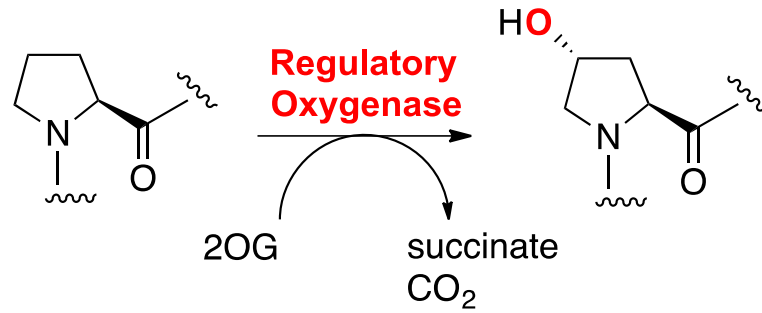


HIF prolyl hydroxylation implies a mechanism of oxygen sensing



HIF prolyl hydroxylases - a set of Fe(II) and 2-oxoglutarate dioxygenases that are conserved throughout the animal kingdom



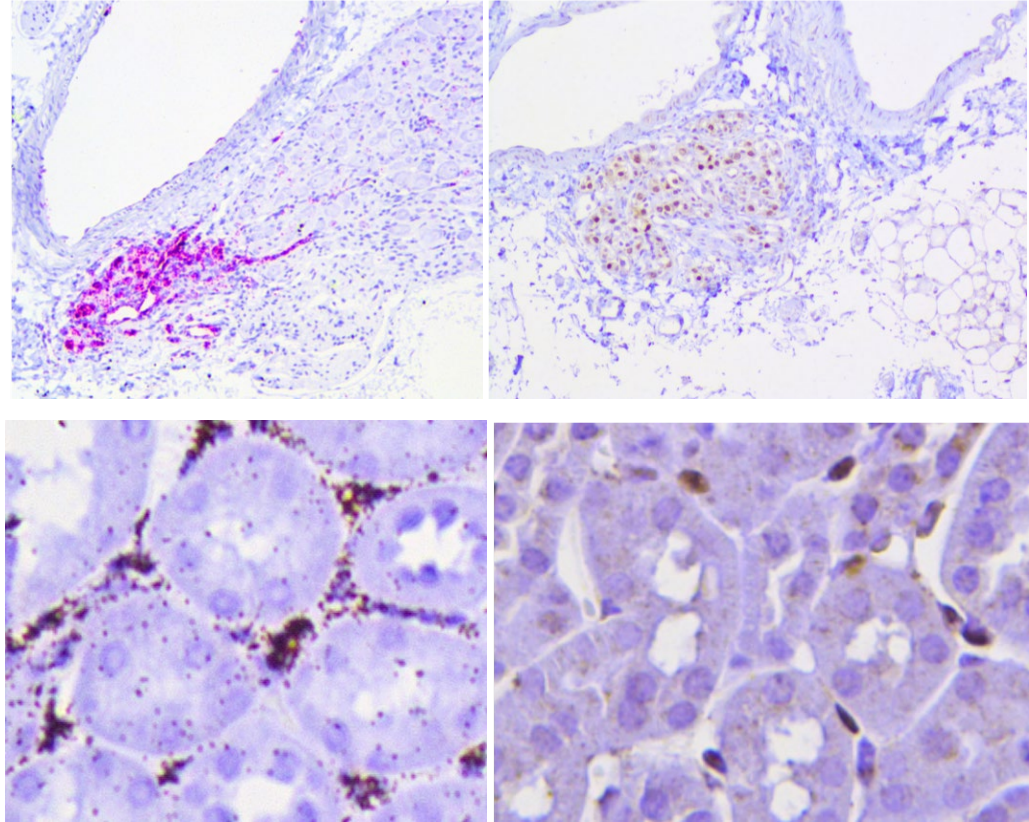


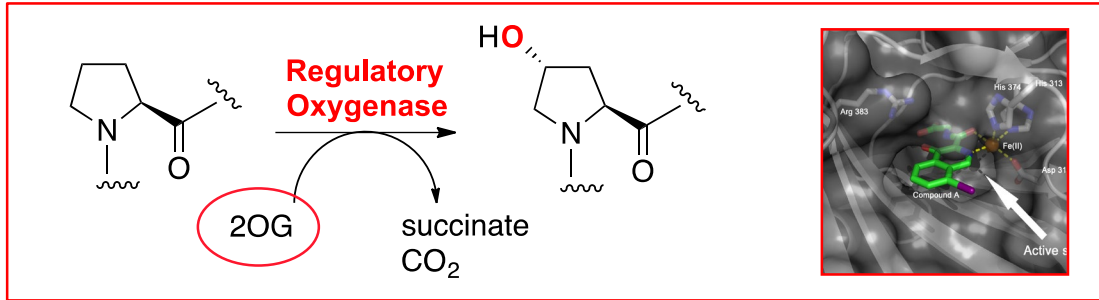
HIF-2 mRNA

HIF-2 IHC

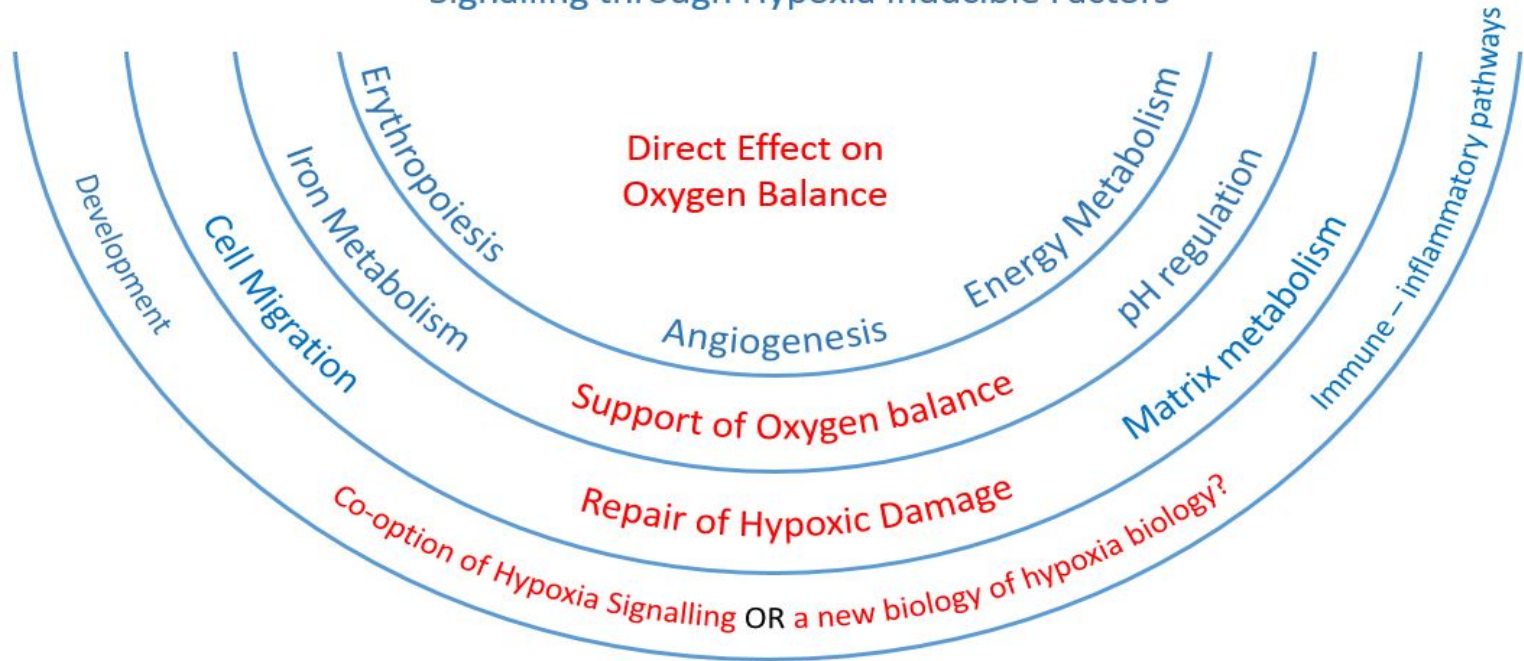


Fig. 3.3. Mabel FitzGerald, measuring the hemoglobin in the blood by diluting a sample of blood in one of two tubes until it matches the color of the standard in the other tube. Reprinted from Colorado Springs Herald Telegraph, July 8, 1911.





Signalling through Hypoxia Inducible Factors



Protein Oxidation in Signalling hypoxia Evolutionary Origins?

All eukaryotic kingdoms use protein oxidation and proteolysis to signal oxygen levels

Funghi
Schizosaccharomyces pombe

Ofd1 - Prolyl 3 hydroxylase

SRE proteolysis

Sterol response

Animals
Homo sapiens

Prolyl 4 hydroxylase

Hypoxia inducible factors

VHL ubiquitin ligase

Protists
Dictyostelium discoideum

Prolyl 4 hydroxylase

Skp1 ubiquitin ligase

Culmination factors

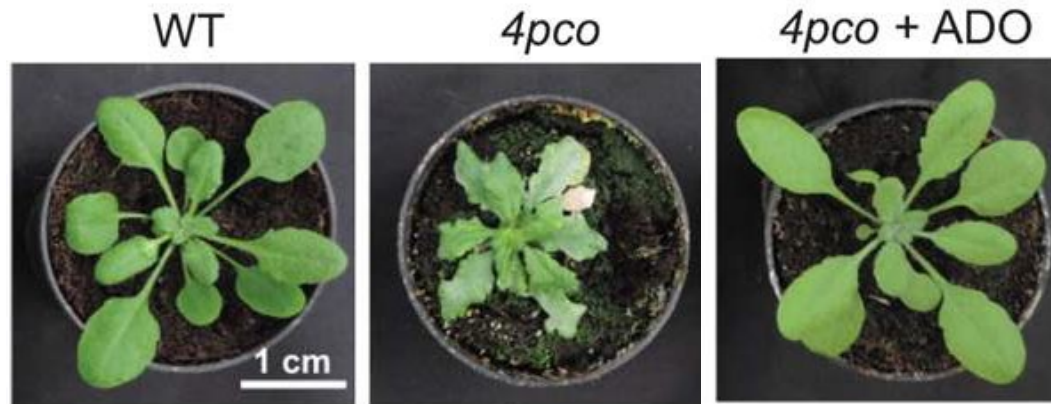
Plants
Arabidopsis thaliana

Cysteine oxidases

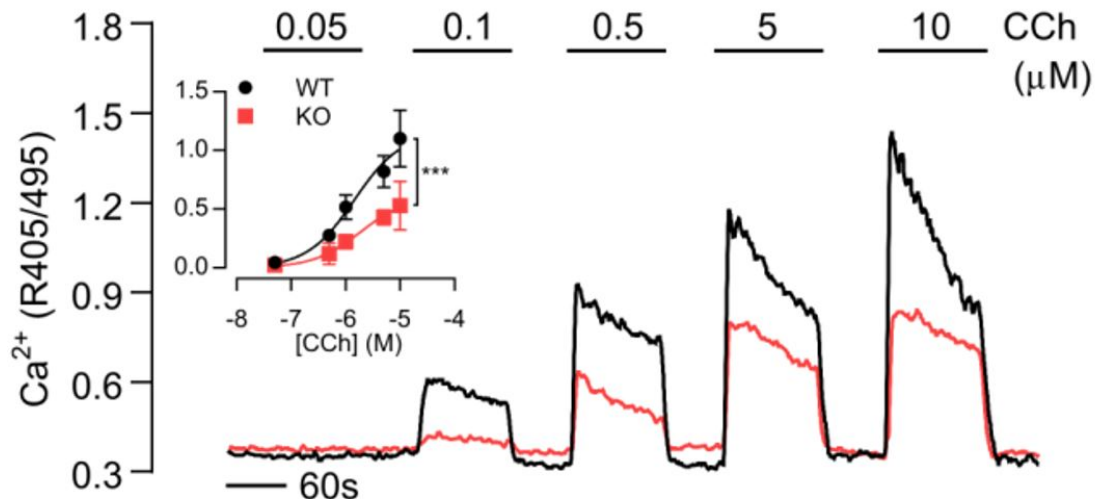
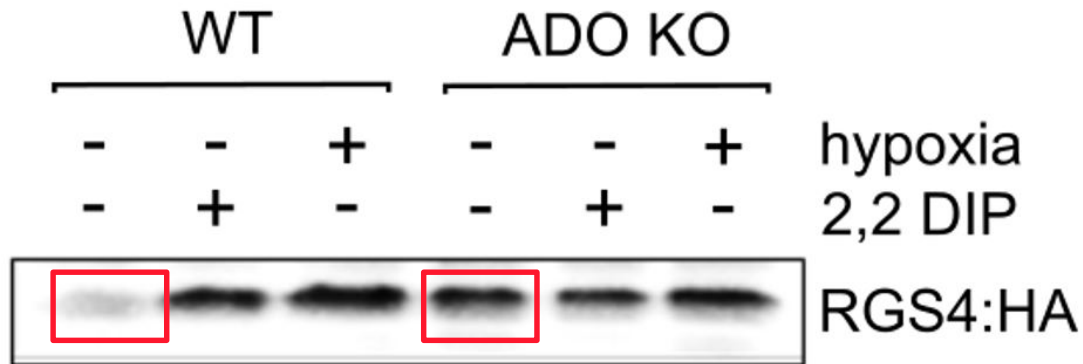
MCxxx N-end rule

Ethylene response factors

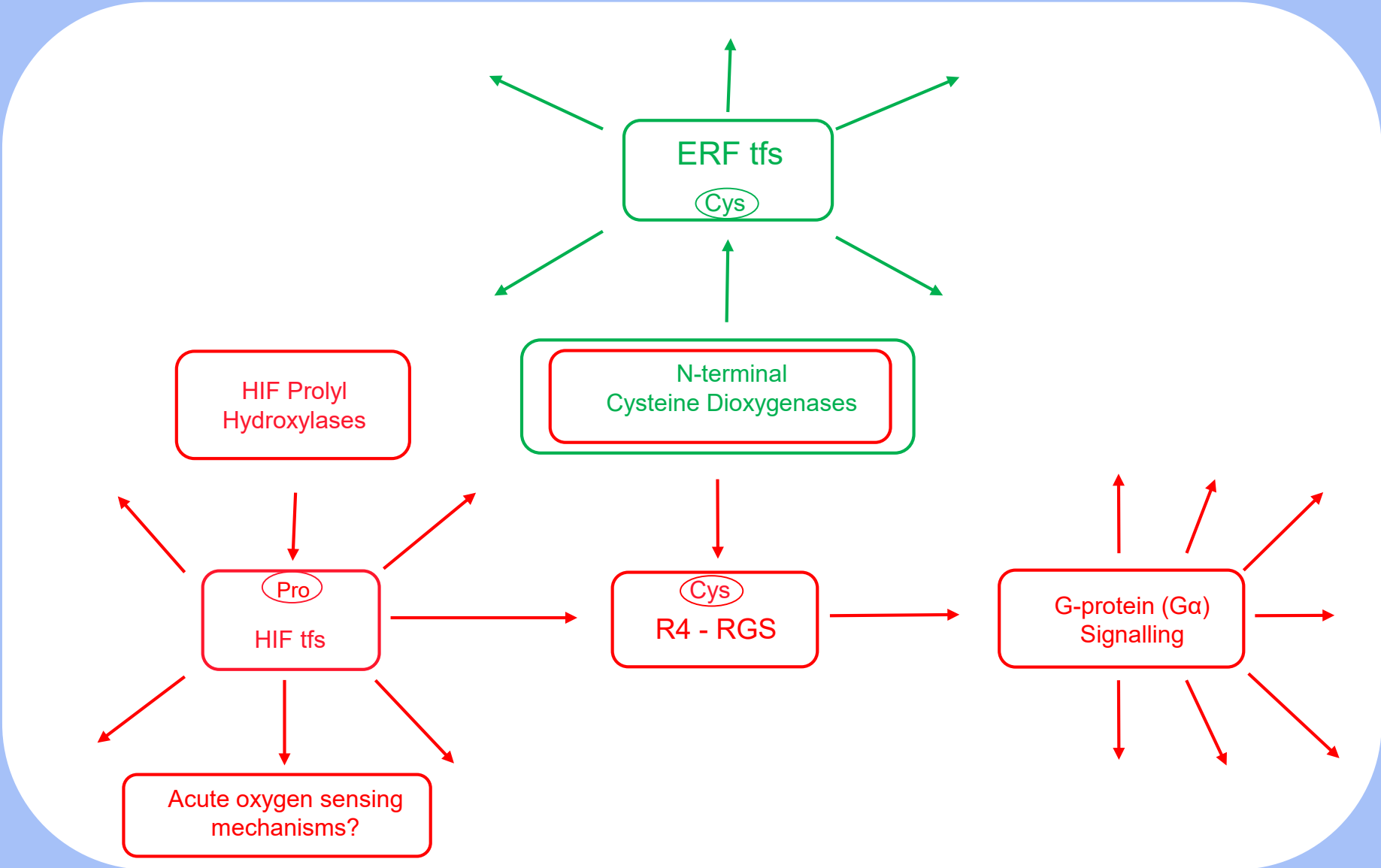
A conserved oxygen sensing mechanism in plants and animals?



Oxygen sensing by enzymatic protein oxidation
 A conserved N-terminal cysteine dioxygenase regulates
 G-protein signalling in human cells

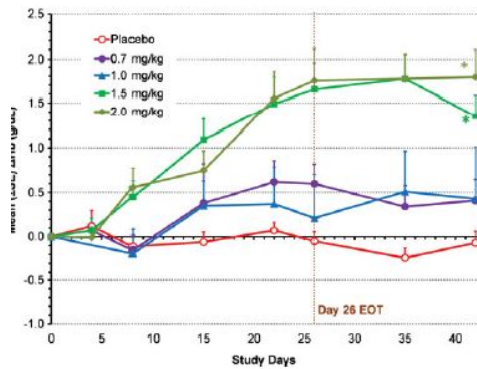


Modulation and integration of oxygen sensing systems employing enzymatic protein oxidation linked to degradation

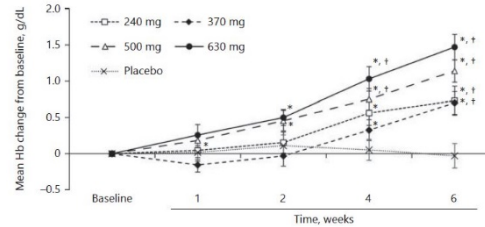


Clinical trials show efficacy of prolyl hydroxylase inhibitors in raising haemoglobin levels in pre-dialysis and dialysis patients

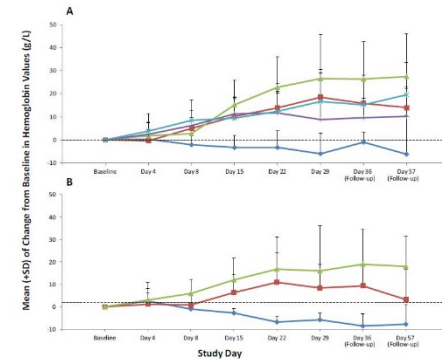
Roxadustat, Fibrogen



Vadadustat, Akebia



Daprodustat, GSK



*'You can't always get what you want
But if you try sometime you find
You get what you need'*

