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

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

Direct Effect on Oxygen Balance

Support of Oxygen balance

Repair of Hypoxic Damage

Co-option of Hypoxia Signalling OR a new biology of hypoxia biology?
Regulation of HIF by oxygen

Oxygen availability
Cellular iron/cobalt

Signal

HIF-\(\alpha\)
Regulated protein stability
Regulated activity
Sequencing the human genome suggests existence of HIF paralogues.

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

Multiple hypoxia inducible transcripts identified.

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 peptide → VHL

<table>
<thead>
<tr>
<th>extract</th>
<th>19:WT</th>
<th>19:Pro564Hyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt VHL</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pVHL</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
HIF prolyl hydroxylation implies a mechanism of oxygen sensing

- $O_2$
  - HIF prolyl hydroxylase
  - Pro $\rightarrow$ Hyp
  - 2-oxoglutarate
  - Iron
    - Cobalt
  - HIF-α
    - VHL ubiquitin ligase $\rightarrow$ Proteolysis
HIF prolyl hydroxylases - a set of Fe(II) and 2-oxoglutarate dioxygenases that are conserved throughout the animal kingdom
Fig. 3.2, Mabel FazGerald, measuring the hemoglobin in the blood by diluting a sample of blood in one of two tubes and it matches the color of the standard in the other tube. Reprinted from Colorado Springs Herald Telegraph, July 8, 1911.
All eukaryotic kingdoms use protein oxidation and proteolysis to signal oxygen levels.

<table>
<thead>
<tr>
<th>Funghi</th>
<th>Animals</th>
<th>Protists</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>Homo sapiens</td>
<td>Dictyostelium discoideum</td>
<td>Arabidopsis thaliana</td>
</tr>
<tr>
<td>Ofd1 - Prolyl 3 hydroxylase</td>
<td>Prolyl 4 hydroxylase</td>
<td>Prolyl 4 hydroxylase</td>
<td>Cysteine oxidases</td>
</tr>
<tr>
<td>SRE proteolysis</td>
<td>Hypoxia inducible factors</td>
<td>Skp1 ubiquitin ligase</td>
<td>MCxxx N-end rule</td>
</tr>
<tr>
<td>Sterol response</td>
<td>VHL ubiquitin ligase</td>
<td>Culmination factors</td>
<td>Ethylene response factors</td>
</tr>
</tbody>
</table>
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’