Oxygen, ROS/RNS, Hypoxia and Hypoxia Inducible Factors

D. Limanan and F. Ferdinal

Departemen Biokimia dan Biologi Molekular, Fakultas Kedokteran Universitas Tarumanagara Jakarta

Korespondensi: Email: frafrdl@fk.untar.ac.id

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ABSTRACT

About 2.5 billion years ago, cyanobacteria evolved to gain the ability to produce oxygen (O2) as a by-product of photosynthesis. CO2 + H20 --> Glucose + O2. Remain the Oparin-Haldane hypothesis (primordial soup), suggests that life arose gradually from inorganic molecules, with "building blocks" like amino acids forming first and then combining to make complex polymers.

All life on earth is based on redox reactions. Oxygen is both essential to human life and toxic. We are dependent on O2 for oxidation reactions in the pathways of adenosine triphosphate (ATP). The oxygen molecule itself is a radical, because at ground-state, oxygen has 2-unpaired electrons with parallel spin states. It is paramagnetic, predisposes it to reduction by a univalent pathway.and lead to excessive production of ROS.

At higher ROS can damage all macromolecules such as DNA, RNA, proteins, carbohydrates and lipids. that can contribute to the pathogenesis of various diseases including: cancer,diabetes, neurodegenerative, CVD and aging (Oxidative Damage)

Hypoxia is defined as a deficiency in either the delivery or the utilization of oxygen at the tissue level, which can lead to changes in function, metabolism and even structure of the body. Hypoxia plays an important role in both physiological and pathological.

Hypoxia inducible factors (HIFs) mediate the transcription almost 300 of genes that allow cells to adapt to hypoxic environments. Could be very important target for drug development and treatment for diseases, such as Atherosclerosis, Cancer and Stroke, in which hypoxia is a central aspect

Key Words: Oxygen, ROS/RNS, hypoxia, Hypoxia Inducible Factors

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INTRODUCTION

History

- Oxygen, discovered by: Joseph Priestley (1974), Carl Scheele (1973) and Antoine Lavoisier (1977) at: England, Sweden and France.
- The name is derived from the Latin words *oxy* and *genes*, meaning "acid forming".

Joseph Priestley

Carl W. Scheele

Antoine L. Lavoisier

Property

- About 2.5 billion years ago, cyanobacteria evolved to gain the ability to produce oxygen (O2) as a by-product of photosynthesis. CO2 + H20 ----> Glucose + O2

  1920 - Hypothesis Oparin – Haldane : Primordial Soup

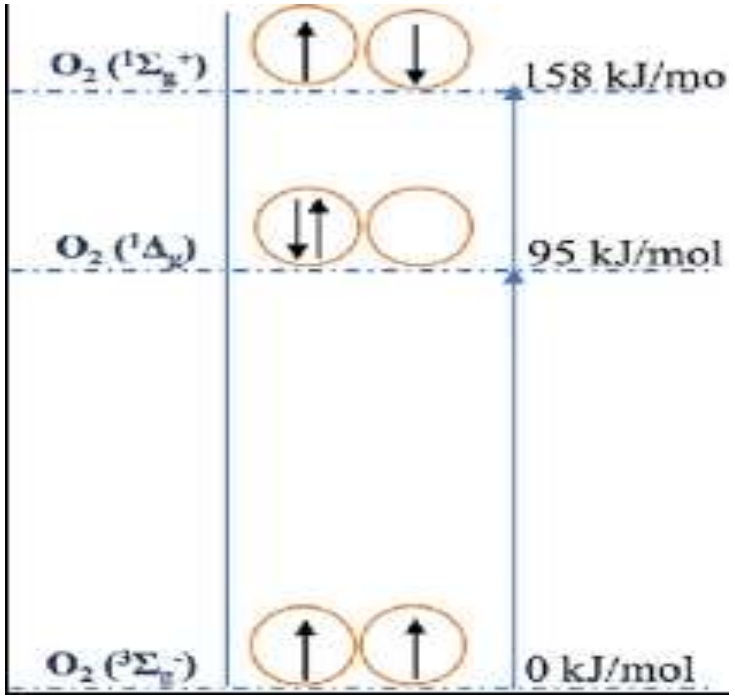
  1953 - Miller & Urey

- Oxygen soluble in water , colorless, odorless, tasteless, extremely reactive gas; in its elemental form, oxygen is found as the diatomic molecule O_2; in the liquid, solid phase, it is pale blue.
- Oxygen is the most abundant element in human body (65%) and the Earth's crust (47%), the second most abundant element in the atmosphere (21%), and the third most abundant element in the universe.
- Chemical bonds with almost all other element and three isotopes: ^{16}O, ^{17}O, and ^{18}O.
- Oxygen has numerous uses in steelmaking and other metals refining and fabrication processes, in chemicals, pharmaceuticals, petroleum processing, glass, ceramic manufacture, and pulp and paper manufacture, rocket fuel.

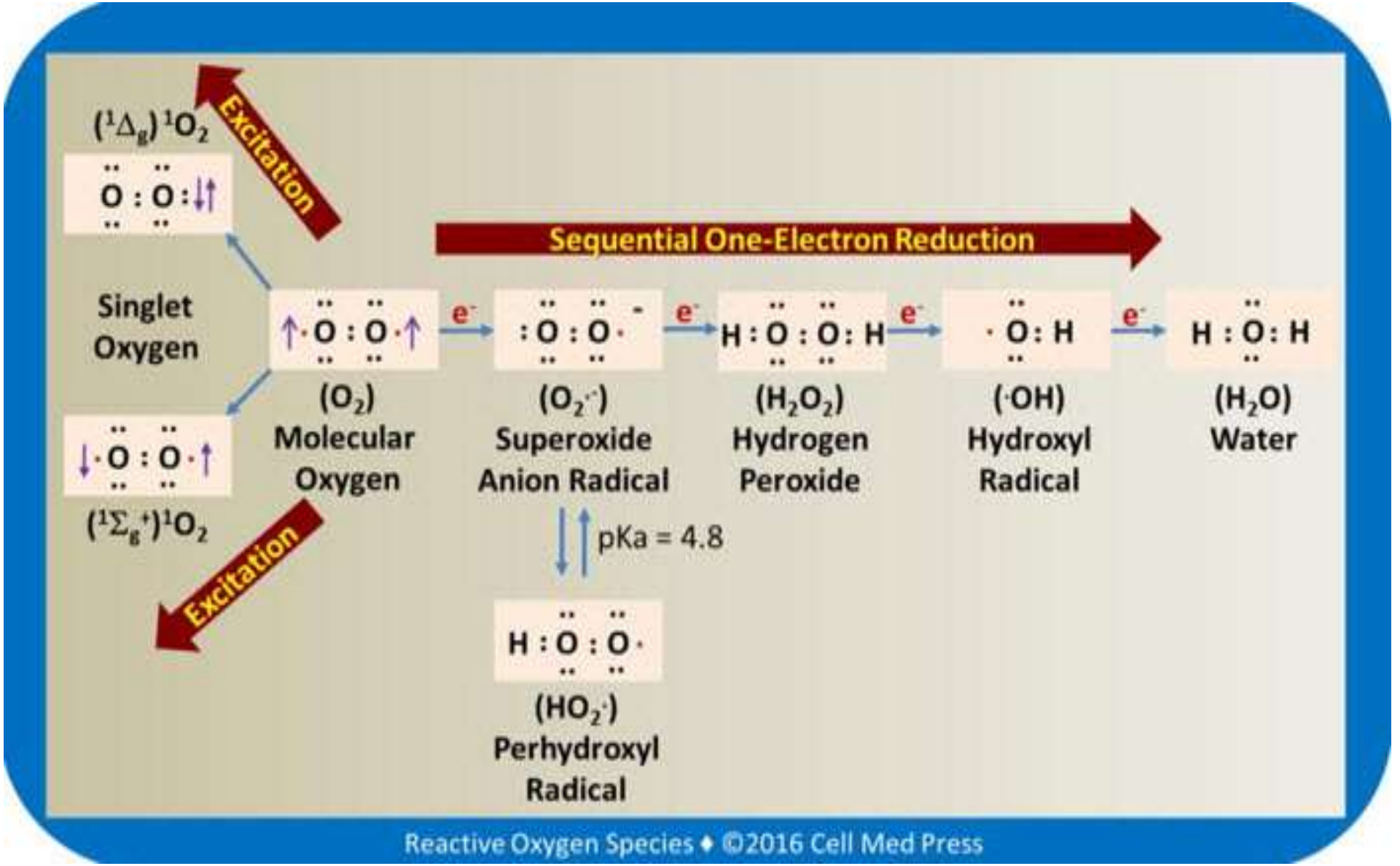
The Paradox of Aerobic Life

- All life on earth is based on redox reactions
- **Oxygen is essential, but toxic**
- **Oxygen** BIRADICAL, Ground-state oxygen has 2-unpaired electrons with parallel spin states
- It is paramagnetic, predisposes it to reduction by a univalent pathway
- Oxygen molecule is minimally reactive due to spin restrictions

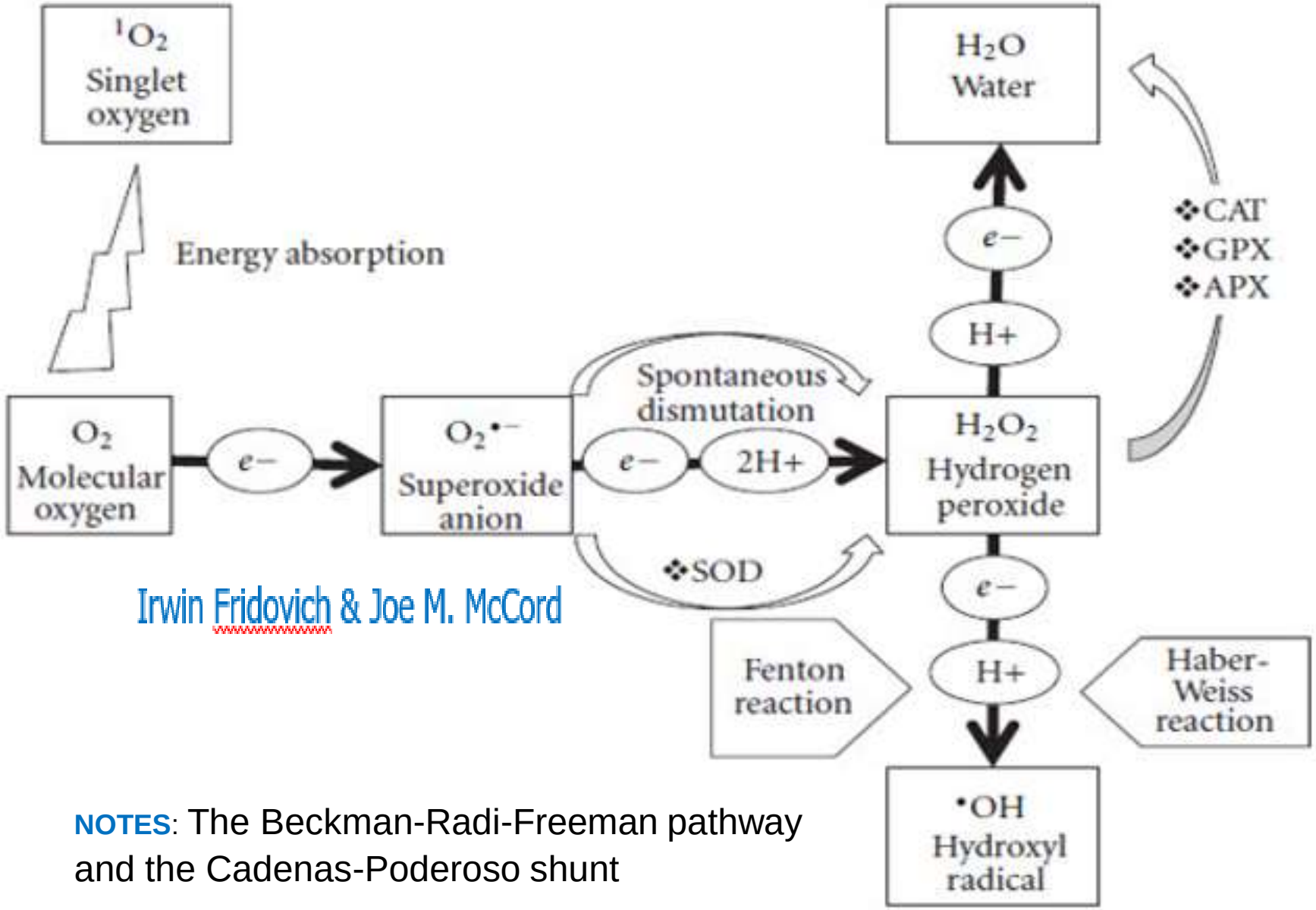
Lewis Diagram



Possible electron configuration for antibonding π orbitals of ground and excited states of molecular oxygen
Pauli Exclusion principle

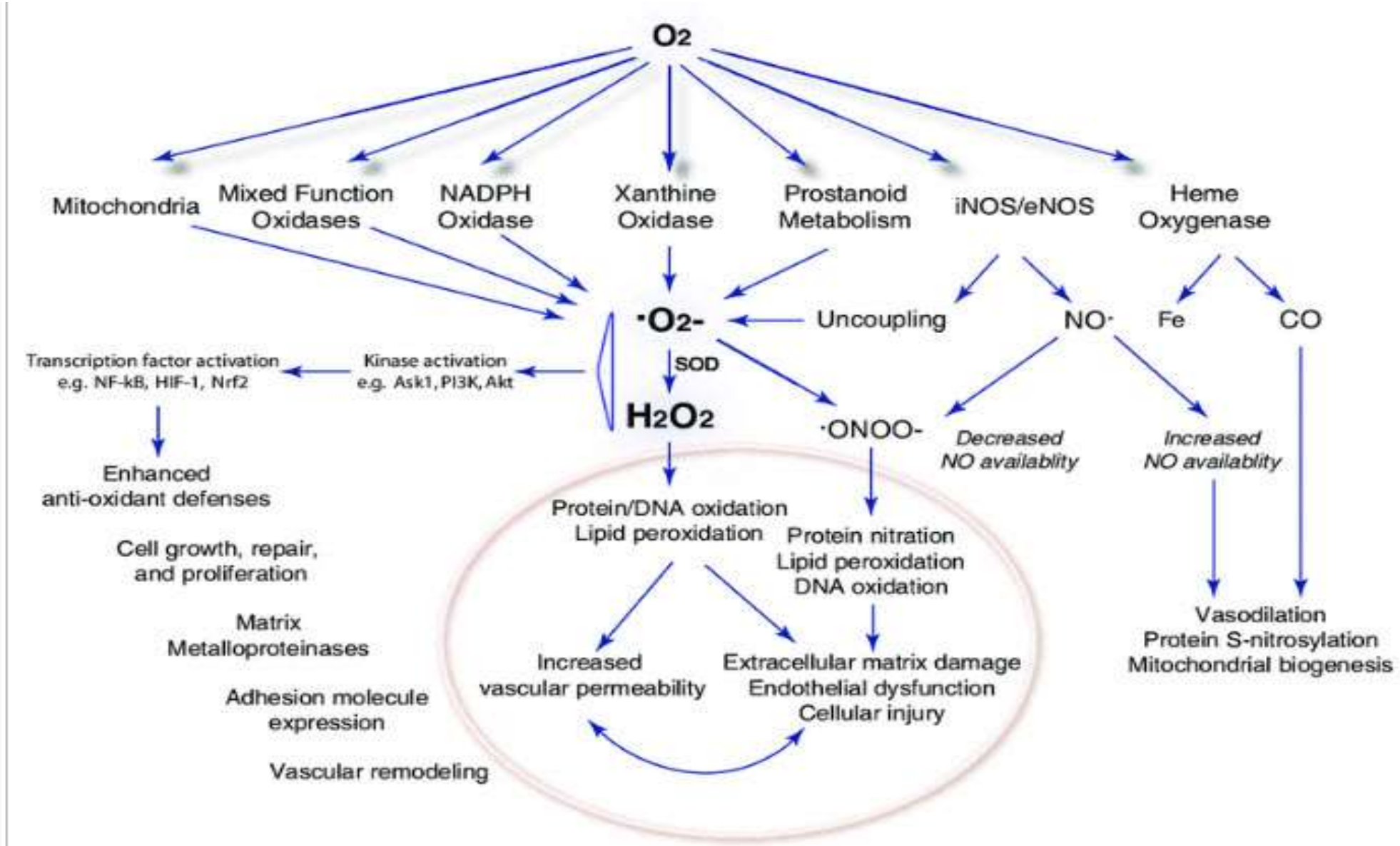


Complete and incomplete reduction of molecular oxygen. The production of specific reactive species by single electron addition (e^-)



NOTES: The Beckman-Radi-Freeman pathway and the Cadenas-Poderoso shunt

Schematic representation of generation of reactive oxygen species (ROS) in plants. Activation of O_2 occurs by: Stepwise monovalent reduction of O_2 or Energy transfer to O_2 leads to formation of $^1O_2$.



Molecular oxygen use by enzyme systems leading to reactive oxygen species production and downstream consequences. Oxygen (O 2) not only leads to superoxide anion (. O 2

ROS/RNS

- Both classes are referred to as RONS, $R_3C\cdot$ Carbon-centered, R-S· Sulfur-centered, RCS Chlorine-centered radicals.
- Definition: Reactive oxygen species (ROS) is a collective term for species derived from O2 that are more reactive than O2 it self.
- Hence all oxygen radicals are ROS, but not all ROS are radical species (the latter being defined as a species with one or more unpaired electrons).
- Reactive' is a relative term; O•2 and H2O2 are selective in their reactions with biological molecules, leaving most of them unscathed, whereas •OH attacks everything

Reactive Oxygen Species (ROS)

Radicals:

$O_2^{\cdot -}$	Superoxide
$\cdot OH$	Hydroxyl
$RO_2\cdot$	Peroxyl
$RO\cdot$	Alkoxyl
$HO_2\cdot$	Hydroperoxyl

Non-Radicals:

H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite

Reactive Nitrogen Species (RNS)

Radicals:

$NO\cdot$	Nitric Oxide
$NO_2\cdot$	Nitrogen dioxide

Non-Radicals:

$ONOO^-$	Peroxynitrite
$ROONO$	Alkyl peroxynitrites
N_2O_3	Dinitrogen trioxide
N_2O_4	Dinitrogen tetroxide
HNO_2	Nitrous acid
NO_2^+	Nitronium anion
NO^-	Nitroxyl anion
NO^+	Nitrosyl cation
NO_2Cl	Nitryl chloride

ROS/RNS: SOURCES and IMPLICATION

- Endogenous : mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells etc.) and Exogenous sources (air-pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs like paracetamol, and radiation

- Hormesis or biphasic dose response, dual effects:

  At lower concentrations, ROS play significant roles in various physiological functions including: gene activation, cell growth, proliferation, survival, apoptosis, blood pressure control, prostaglandin biosynthesis, embryonic development, cognitive function, and immune response (**REDOX SIGNALING**)

- However, at higher concentrations, ROS can damage all macromolecules such as DNA, RNA, proteins, carbohydrates and lipids. that can contribute to the pathogenesis of various diseases including: Cancer,Diabetes, neurodegenerative, CVD , aging, respiratory diseases, cataract and rheumatoid arthritis (**OXIDATIVE DAMAGE**)

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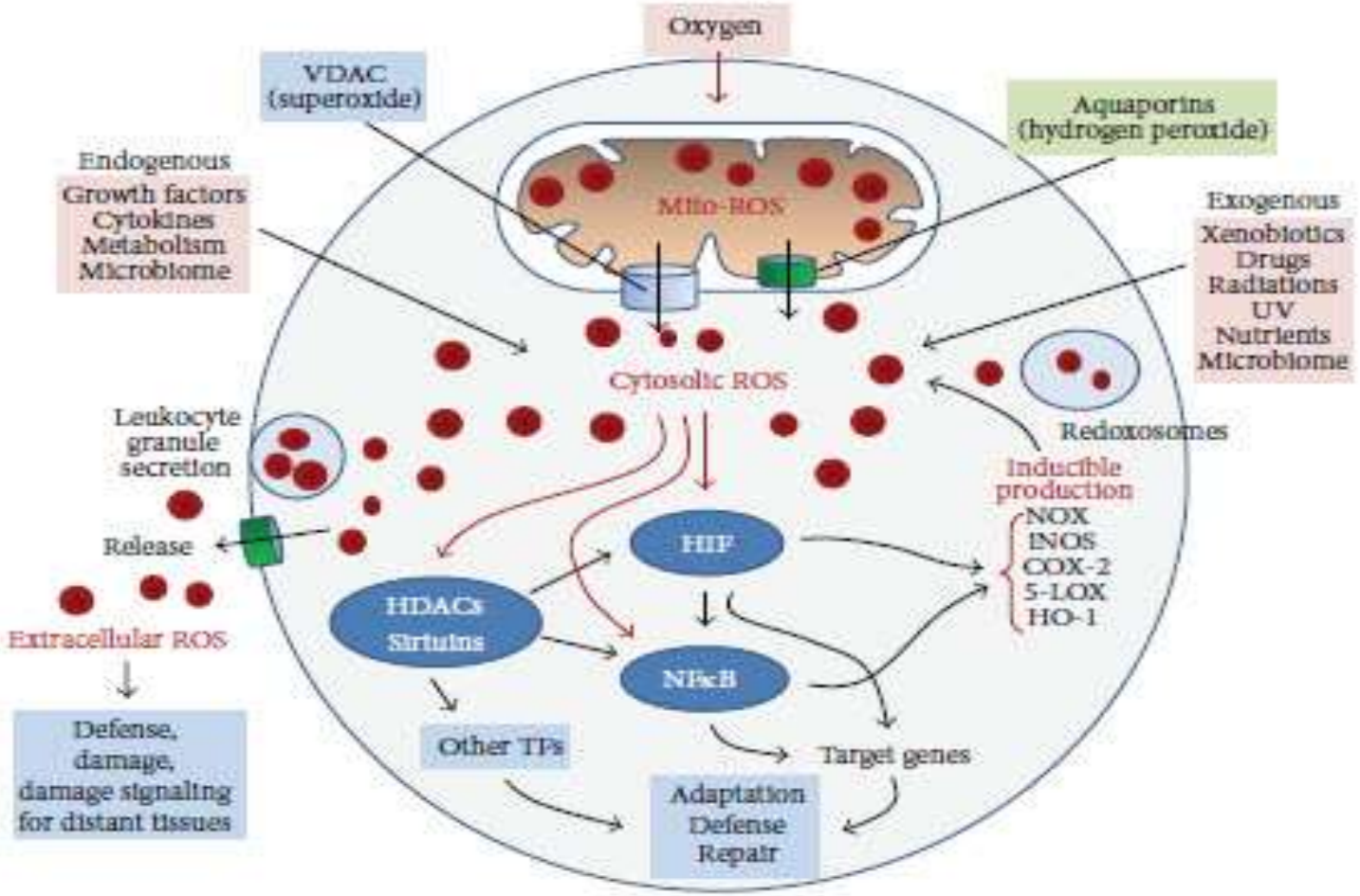


FIGURE 2: ROS are produced mainly in the mitochondria. Superoxides are rapidly detoxified by mitochondrial MnSOD as hydrogen peroxide or can cross mitochondrial membranes through the VDAC. Hydroperoxides travel easily to cytosol through membrane aquaporin. In addition to ROS coming from mitochondria, cytosolic ROS can originate from many endogenous or exogenous sources, including nutrients, radiation, microbiome, growth factors, cytokines, and other metabolisms. Proinflammatory inducible enzymes such as NADPH-oxidases (NOX), inducible nitric oxide synthase (iNOS), inducible cyclooxygenase (COX2), 5-lipoxigenase, and inducible heme-oxigenase-1 (HO-1) may produce an additional burst of ROS. HIF1α, NFκB, and HDACs, especially Sirtuins, are activated by ROS in synergy with the specific signaling from receptors and metabolism. Target genes of activated TFs are aimed at adaptation to hypoxia, proinflammatory harmful agents' inactivation, and damage repair. ROS are also released in the extracellular space by secretion of granules of activated leukocytes or crossing plasma membrane through anionic channels (superoxides) or aquaporins (hydroperoxides). Extracellular ROS are important for defense (as in case of ROS released by eosinophils against macroparasite) and produce collateral damage not only in adjacent healthy tissues but also in distant tissues and organs, signaling the local damage and activating improper mechanisms of adaptation, remodeling, and chronic damage.

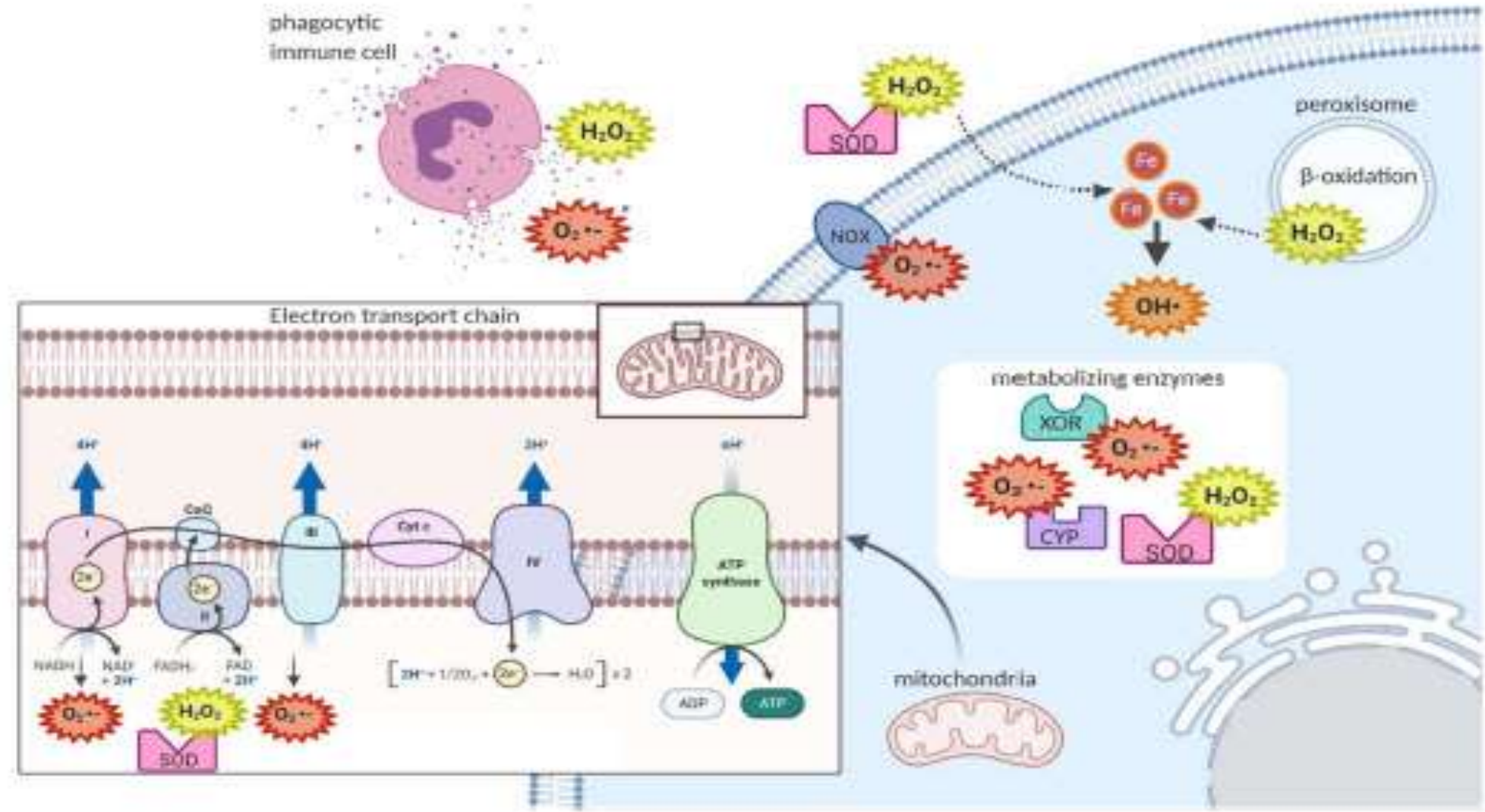
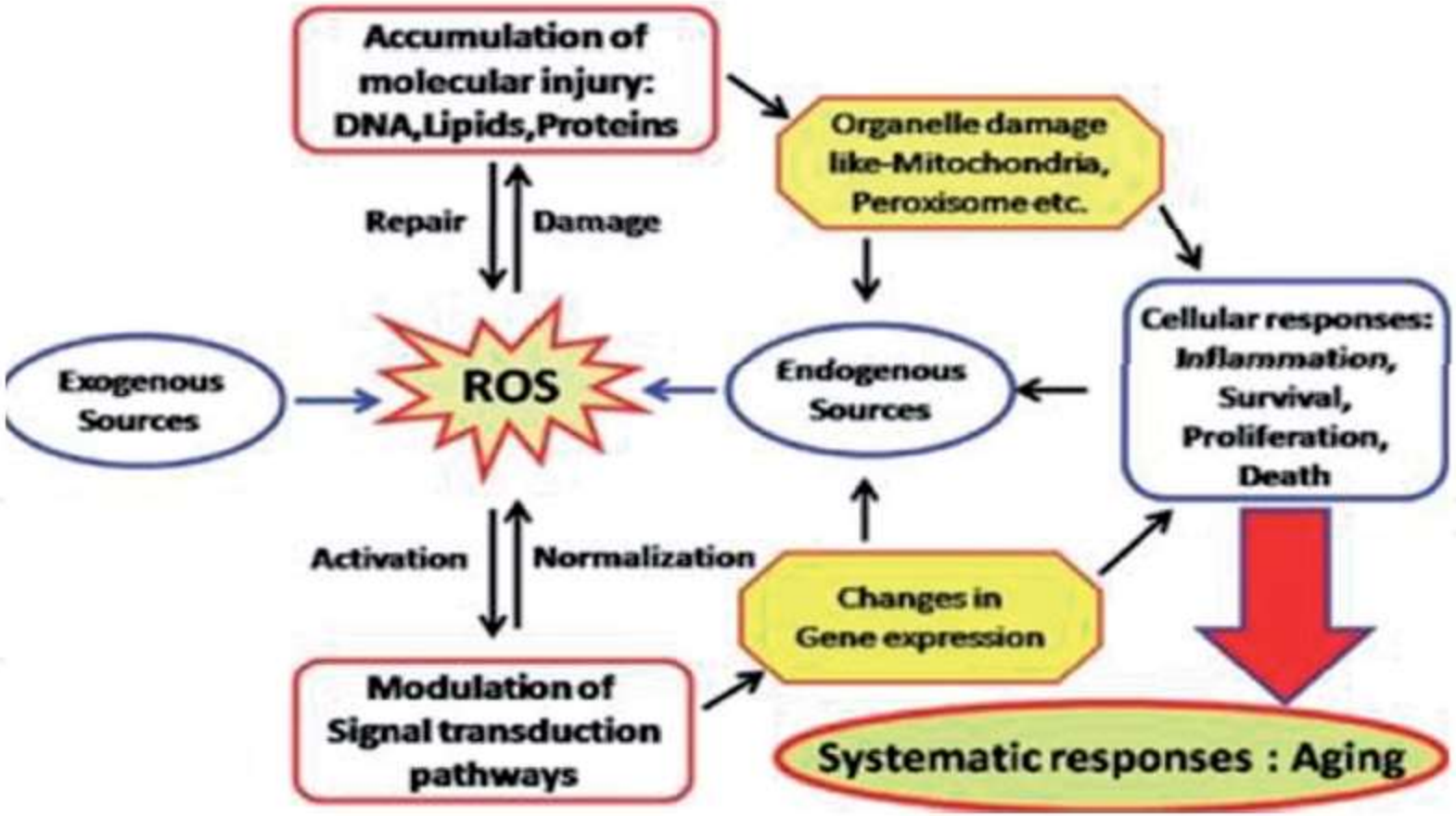
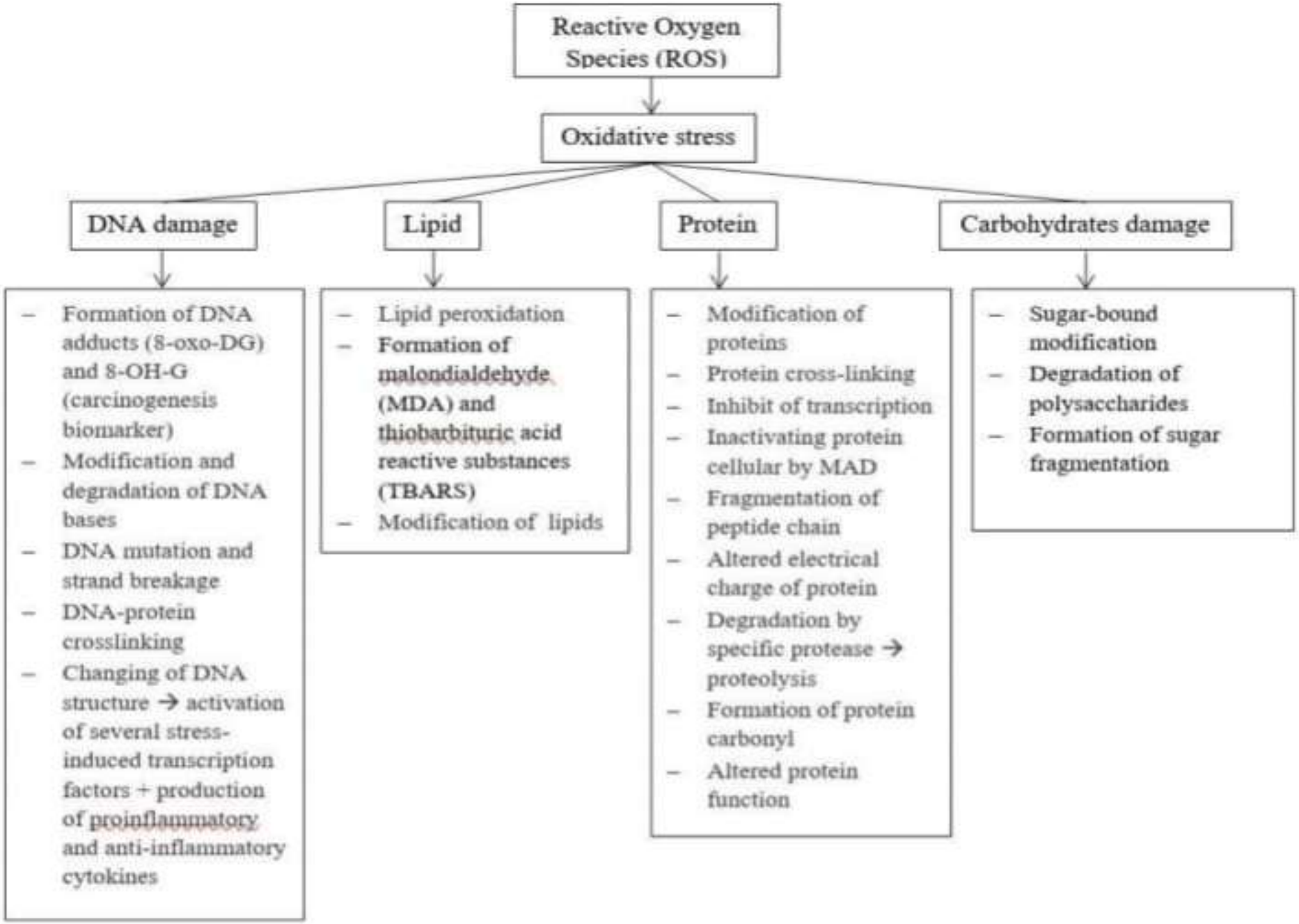
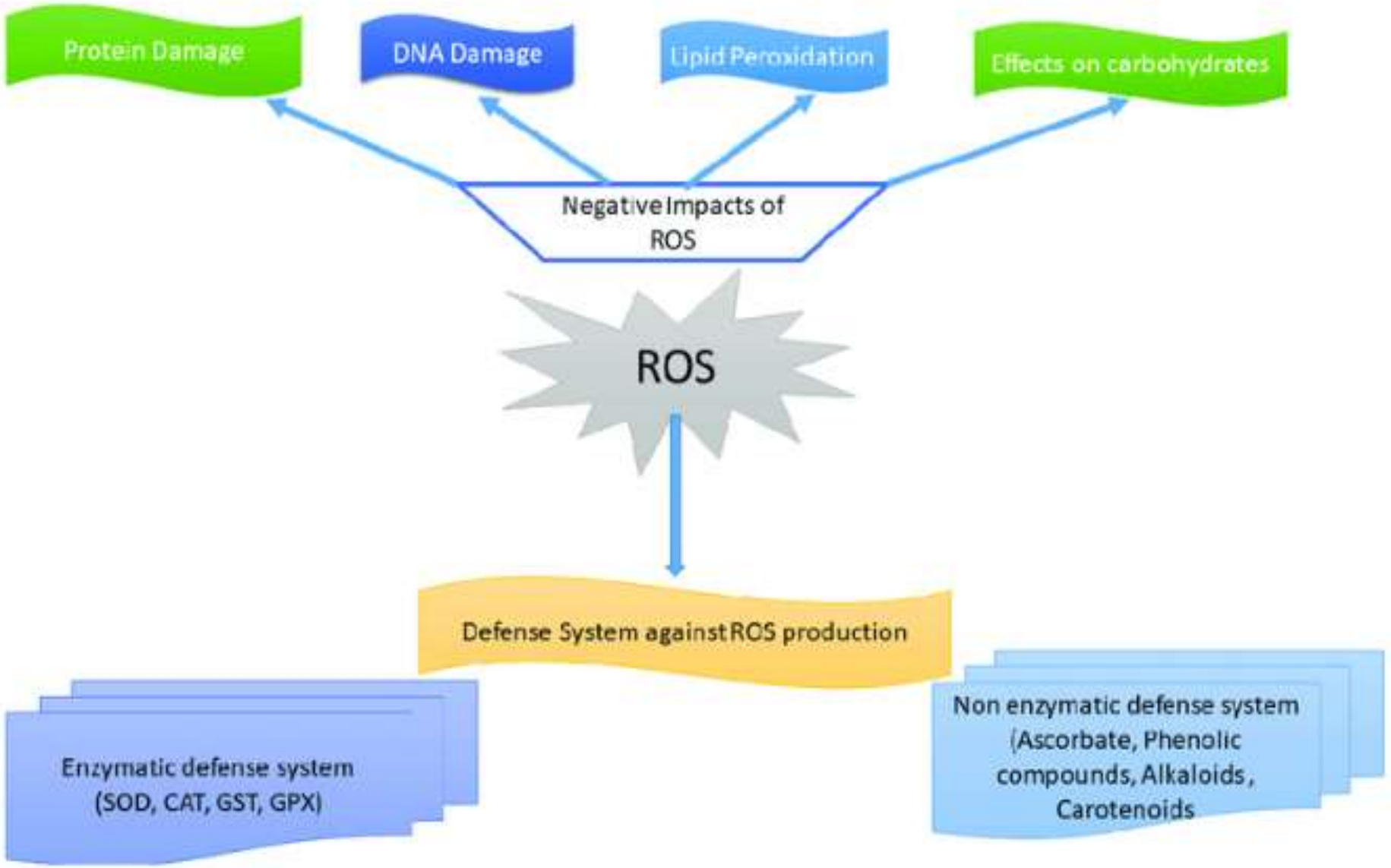


FIGURE 3 | Sites of reactive oxygen species generation in the cell. As electrons are being passed from Complex I or Complex II to Complex III via ubiquinone in the mitochondrial electron transport chain, some of these electrons can escape and react with oxygen to form superoxide. The enzymes superoxide dismutase can convert the superoxide to hydrogen peroxide, which can then exit the mitochondria. In the cytoplasm, metabolism reactions such as those of the cytochrome p family of enzymes (CYP) produce ROS. In the peroxisome, fatty acid beta-oxidation produces hydrogen peroxide. At the plasma membrane, NADPH Oxidase produces superoxide. Extracellularly, ROS can be released in processes such as the respiratory burst, where phagocytic immune cells release ROS to attack pathogens. Extracellular superoxide dismutase can then convert extracellular superoxide to hydrogen peroxide. Hydrogen peroxide, which can cross membranes, can be converted to the potent hydroxyl radical when in contact with cellular ferrous iron.

Oxidative Damage




Reactive Oxygen Species (ROS)
Oxidative stress
DNA damage
Lipid
Protein
Carbohydrates damage
– Formation of DNA adducts (8-oxo-DG) and 8-OH-G (carcinogenesis biomarker)
– Modification and degradation of DNA bases
– DNA mutation and strand breakage
– DNA-protein crosslinking
– Changing of DNA structure → activation of several stress-induced transcription factors + production of proinflammatory and anti-inflammatory cytokines
– Lipid peroxidation
– Formation of malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS)
– Modification of lipids
– Modification of proteins
– Protein cross-linking
– Inhibit of transcription
– Inactivating protein cellular by MAD
– Fragmentation of peptide chain
– Altered electrical charge of protein
– Degradation by specific protease → proteolysis
– Formation of protein carbonyl
– Altered protein function
– Sugar-bound modification
– Degradation of polysaccharides
– Formation of sugar fragmentation



Effects of oxidative stresses on plants parts and different defence mechanism.In plants, ROS cause serious damage to the cells by inhibiting proteins, DNA and others metabolic pathways. Conversely, the defense system is activated in the plants against ROS to regulate its functional activity by activating different enzymatic and non-enzymatic antioxidant agents

Marker or Type of Damage	Sample Type				
	Cells	Tissues	Blood	Urine	
DNA / RNA Damage					
8-hydroxyguanosine (8-OHG)	X	X	X	X	
*8-hydroxydeoxyguanosine (8-OHdG)	X	X	X	X	
Abasic (AP) sites	X	X			
BPDE DNA Adduct	X	X			
Double-strand DNA breaks	X				
Comet Assay (general DNA damage)	X				
UV DNA Damage (CPD, 6-4PP)	X				
Lipid Peroxidation					
4-Hydroxynonenal (4-HNE)	X	X	X		
8-iso-Prostaglandin F2alpha (8-isoprostane)	X	X	X	X	
Malondialdehyde (MDA)	X	X	X	X	
TBARS	X	X	X	X	
Protein Oxidation / Nitration					
*Protein Carbonyl Content (PCC)	X	X	X		
3-Nitrotyrosine	X	X	X		
Advanced Glycation End Products (AGE)	X	X	X		
Advanced Oxidation Protein Products (AOPP)	X	X	X		
BPDE Protein Adduct	X	X	X		
Reactive Oxygen Species					
Universal ROS / RNS	X	X	X	X	
Hydrogen Peroxide	X	X	X	X	
Nitric Oxide	X	X	X	X	
Antioxidants					
Catalase	X	X	X		
Glutathione	X	X	X	X	
Superoxide Dismutase	X	X	X		
Oxygen Radical Antioxidant Capacity (ORAC)	X	X	X	X	
Hydroxyl Radical Antioxidant Capacity (HORAC)	X	X	X	X	
Total Antioxidant Capacity (TAC)	X	X	X	X	
Cell-Based Exogenous Antioxidant Assay					Fo

Free Radical Detection:

- Single photon counting
- Chemiluminescence
- Fluorescent probe
- Electro Paramagnetic Resonance EPR/ESR

Hypoxia

Hypoxia is defined as a deficiency in either the delivery or the utilization of oxygen at the tissue level, which can lead to changes in function, metabolism and even structure of the body.

The main types of hypoxia include:

- Hypoxic hypoxia (hypoxemic hypoxia)**:** There is a lack of oxygen in the blood flowing to the tissues.
- Anemic hypoxia**:** Blood isn't able to carry oxygen as well as it should because of an insufficient amount of healthy red blood cells. This leads to a lower supply of oxygen in the tissues.
- Stagnant/circulatory hypoxia**:** Poor blood flow leads to less oxygen available to the tissues. This may occur in one specific area or throughout the whole body.
- Histiotoxic hypoxia**:** Enough oxygen is taken in through the lungs and delivered to the tissues, but the body has difficulty using it.
- Cytopathic hypoxia**:** Oxygen is able to be used properly by the tissues, but there is a higher demand for oxygen than usual.

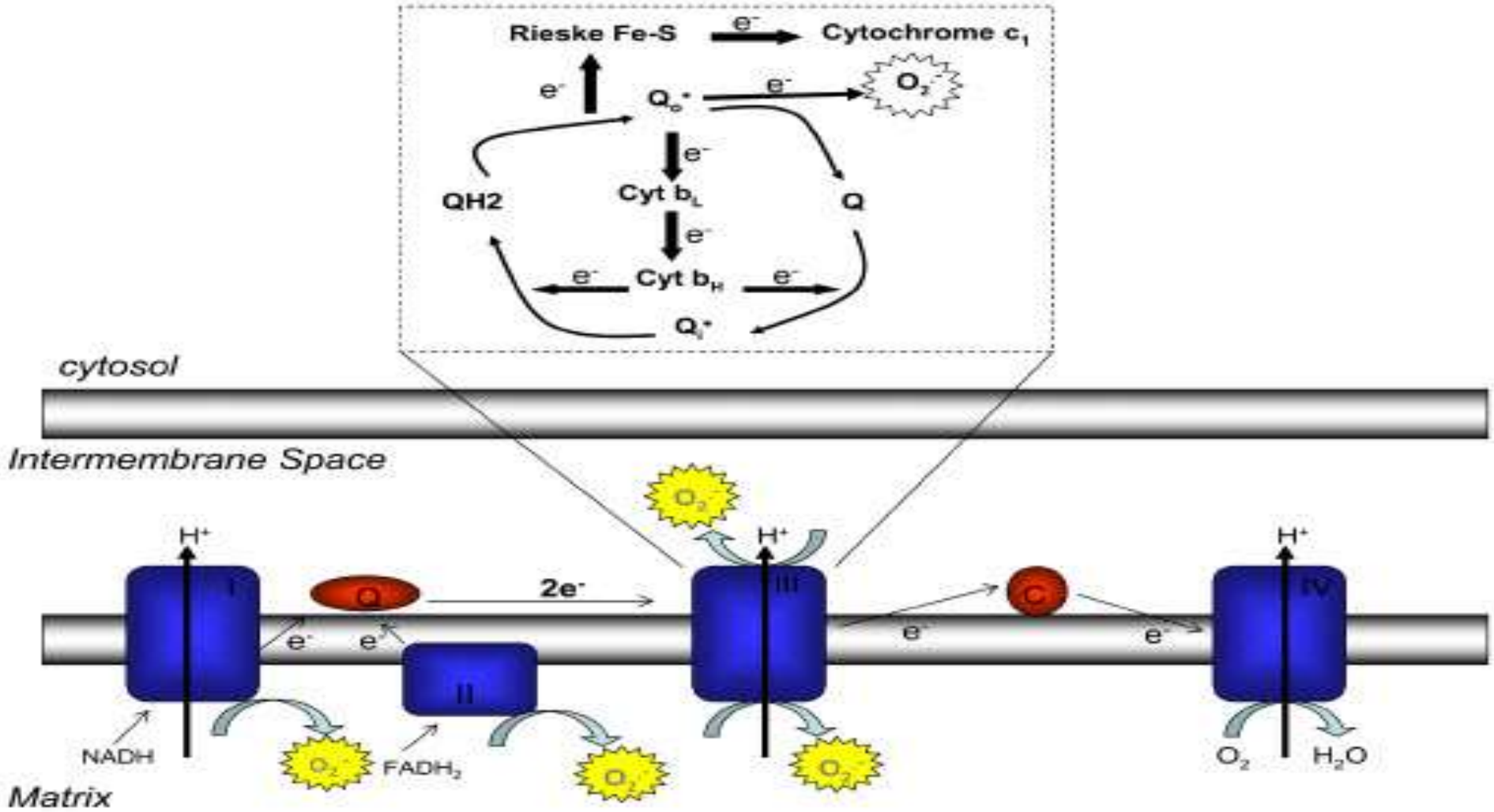


Figure 1. Mitochondrial generation of reactive oxygen species
Mitochondrial complexes I, II, and III produce superoxide. While complexes I and II only produce superoxide into the mitochondrial matrix, complex III can produce superoxide on both sides of the mitochondrial inner membrane in a process termed the Q-cycle. Complexes I and II donate two electrons to coenzyme Q, forming ubiquinol. At complex III, the first of these electrons is transferred by the Rieske iron-sulfur protein (RISP) to cytochrome *c*1, leaving the radical ubisemiquinone. Subsequently, ubisemiquinone transfers the second electron to cytochrome *b*. Ubisemiquinone formed at the Q_o site can donate its electron directly to oxygen, producing superoxide.

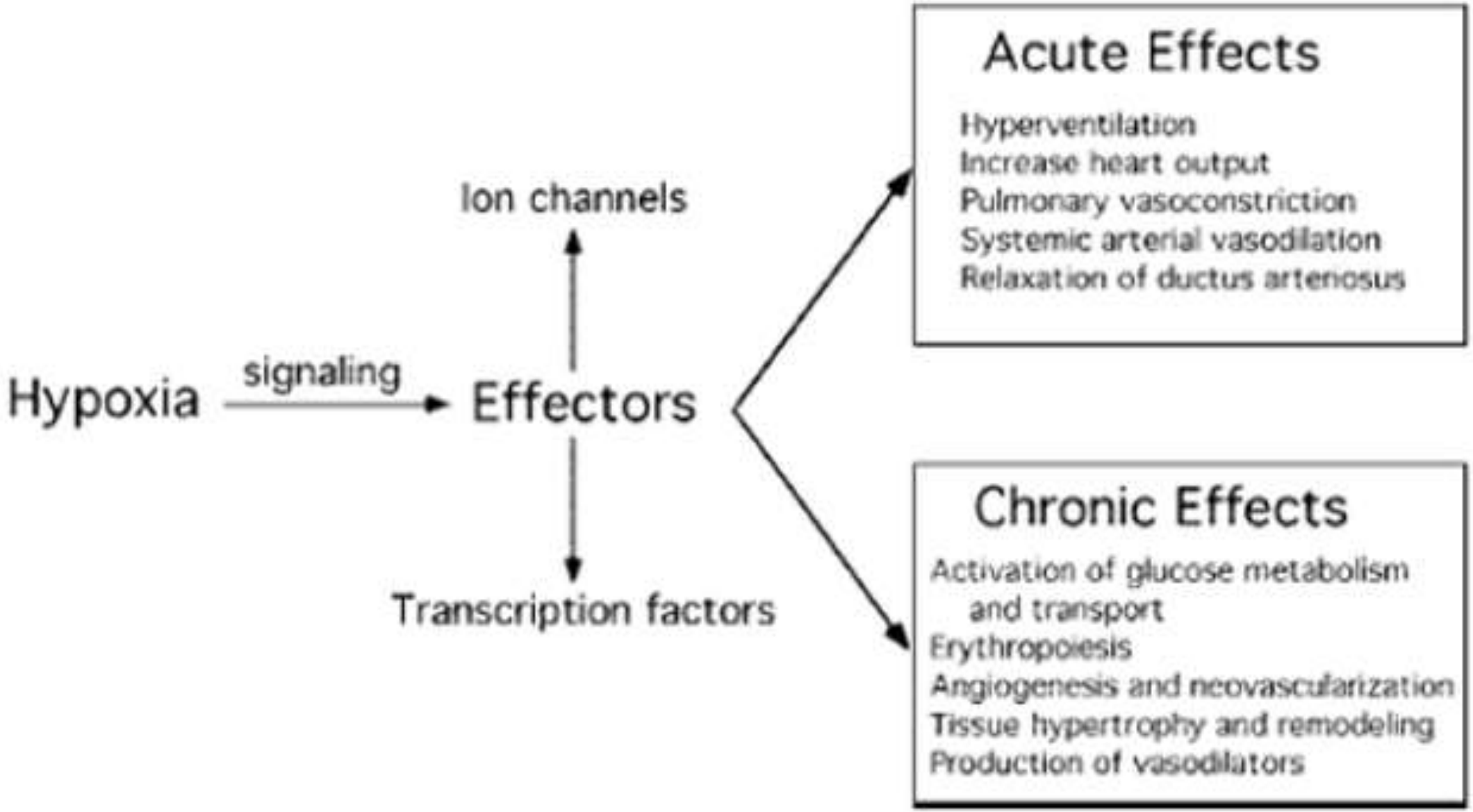
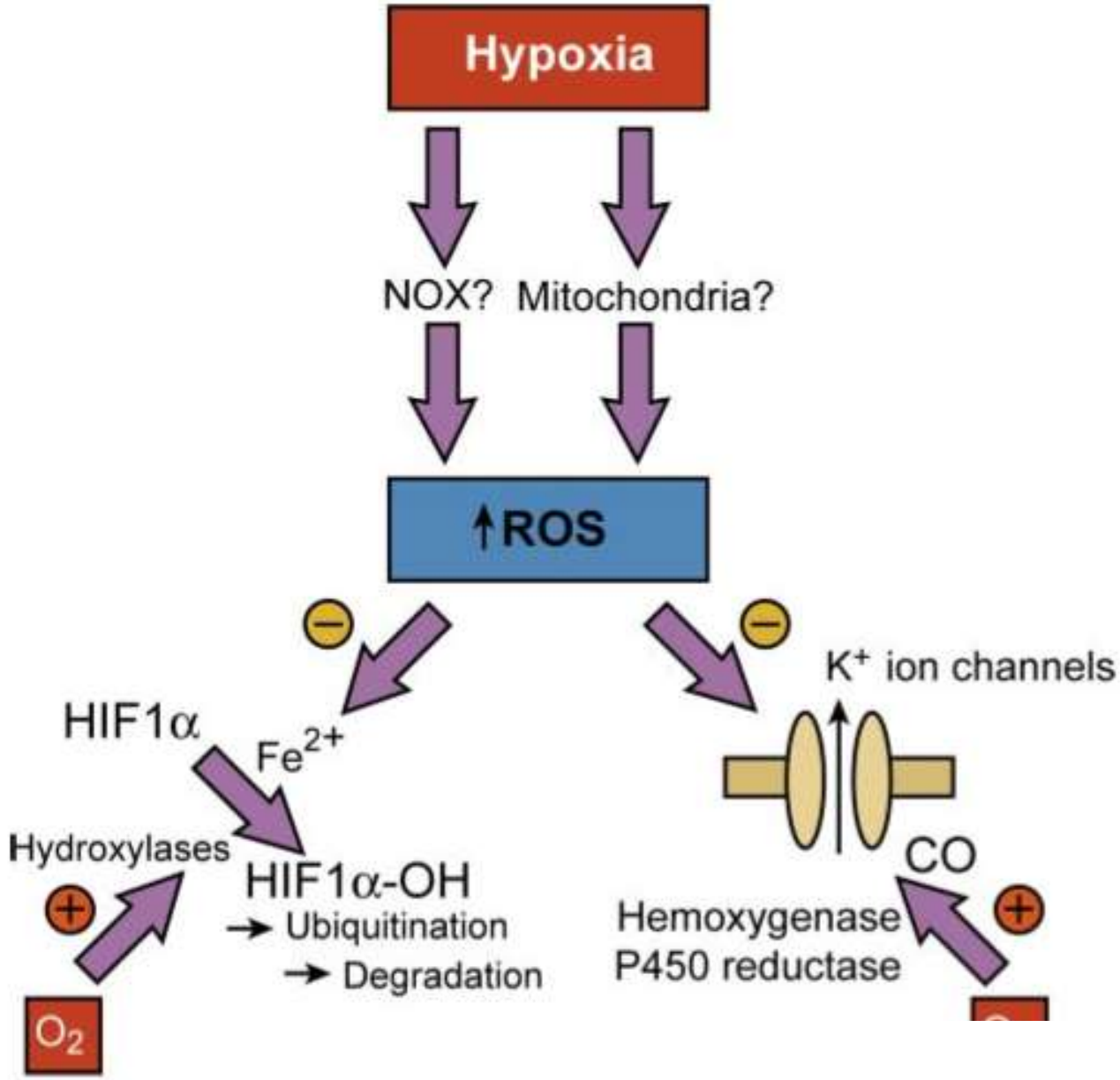


Fig. Hypoxia signaling pathway with indication of the major adaptive responses to acute and chronic hypoxia


Hypoxia
NOX?
Mitochondria?
↑ROS
HIF1α
Fe2+
Hydroxylases
HIF1α-OH
Ubiquitination
Degradation
O2
K+ ion channels
CO
Hemoxygenase
P450 reductase

HYPOXIA INDUCIBLE FACTORS

- Hypoxia-inducible factors (HIFs) are master regulators of oxygen homeostasis that match O2 supply and demand for each of the 50 trillion cells in the adult human body

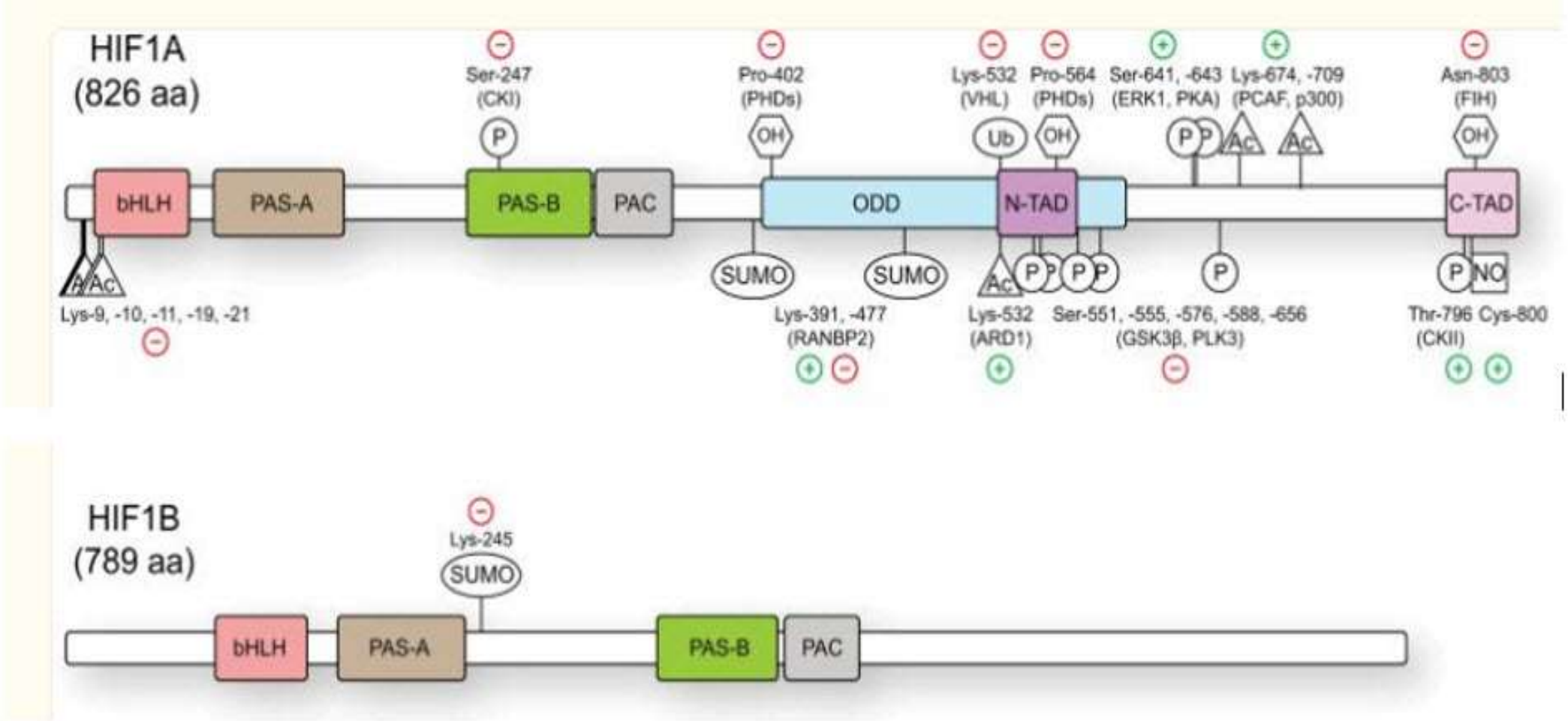
- The HIF-1 transcription factor was first identified based on its ability to activate the erythropoetin gene in response to hypoxia (Wang and Semenza, 1993).

- HIFs activate the transcription of thousands of genes that mediate angiogenesis, cancer stem cell specification, cell motility, epithelial-mesenchymal transition, extracellular matrix remodeling, glucose and lipid metabolism, immune evasion, invasion, and metastasis

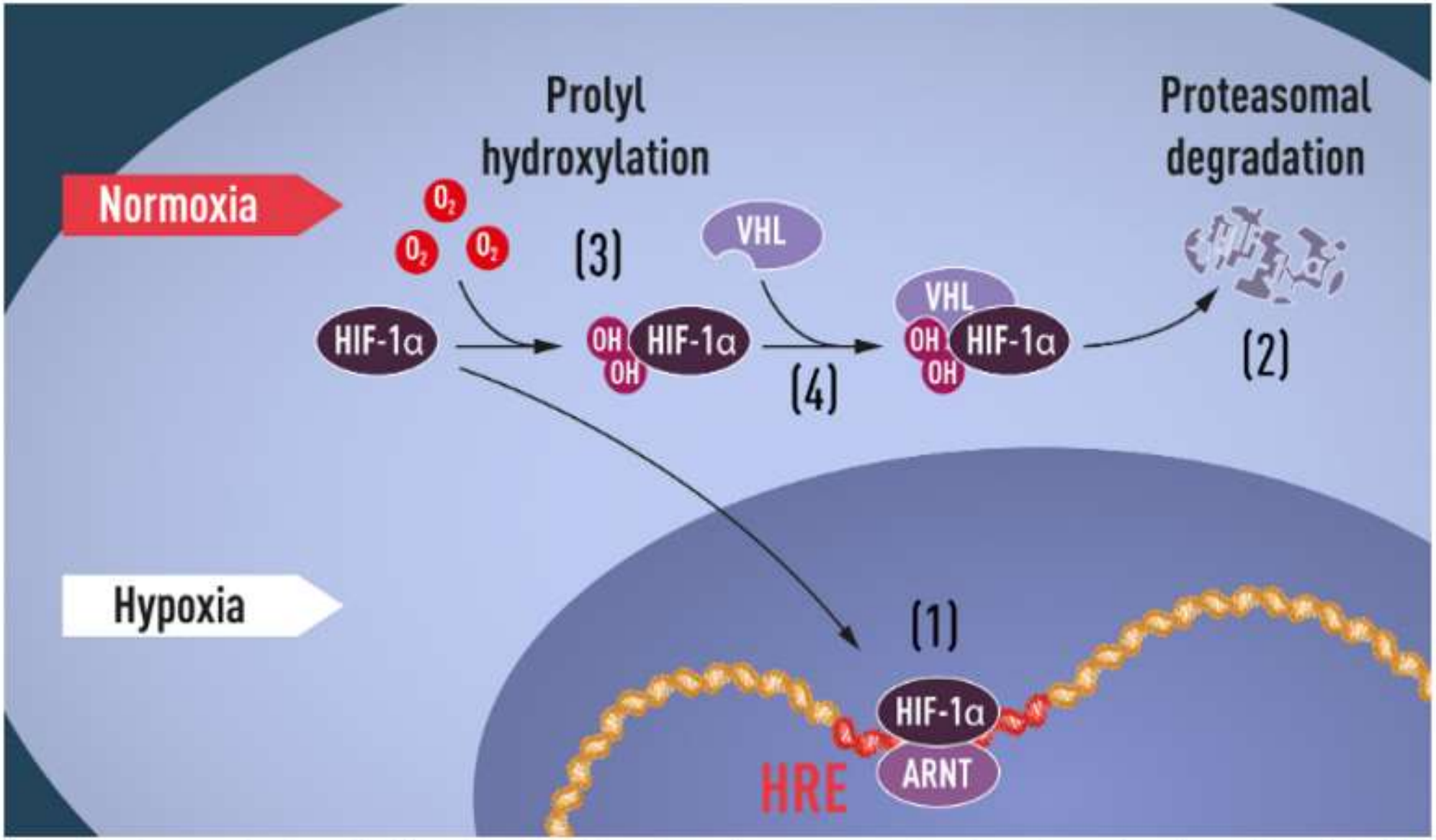
- HIFs are heterodimeric proteins consisting of an O2-sensitive HIF-1α, HIF-2α, or HIF-3α subunit and a constitutively expressed HIF-1β subunit (also known as ARNT)

- Cancer cells co-opt this homeostatic system to drive cancer progression

HYPOXIA INDUCIBLE FACTORS (HIFs)



HIF protein domains and post-translational modifications. The HIF proteins are comprised of several conserved domains that are involved in DNA binding (basic Helix-Loop-Helix, bHLH), protein-protein interactions and dimerization (PER-ARNT-SIM, PAS-A, PAS-B, and PAS-associated C-terminal domain), oxygen-dependent degradation (ODD) and transcriptional activation (N-TAD, C-TAD). Numerous HIF3A isoforms exist, with several longer forms possessing transactivation and leucine zipper (LZIP) domains (HIF3A-1) while others lack any known transactivation domains and act as negative regulators (HIF3A-4). Multiple post-translational modifications are known to modulate HIF protein stability and transcriptional activity. Selected modifications are shown here along with the enzyme responsible and the overall positive (+) or negative (−) effects on HIF transcriptional function.



When oxygen levels are low (hypoxia), HIF-1α is protected from degradation and accumulates in the nucleus, where it associates with ARNT and binds to specific DNA sequences (HRE) in hypoxia-regulated genes (1). At normal oxygen levels, HIF-1α is rapidly degraded by the proteasome (2). Oxygen regulates the degradation process by the addition of hydroxyl groups (OH) to HIF-1α (3). The VHL protein can then recognize and form a complex with HIF-1α leading to its degradation in an oxygen-dependent manner (4).

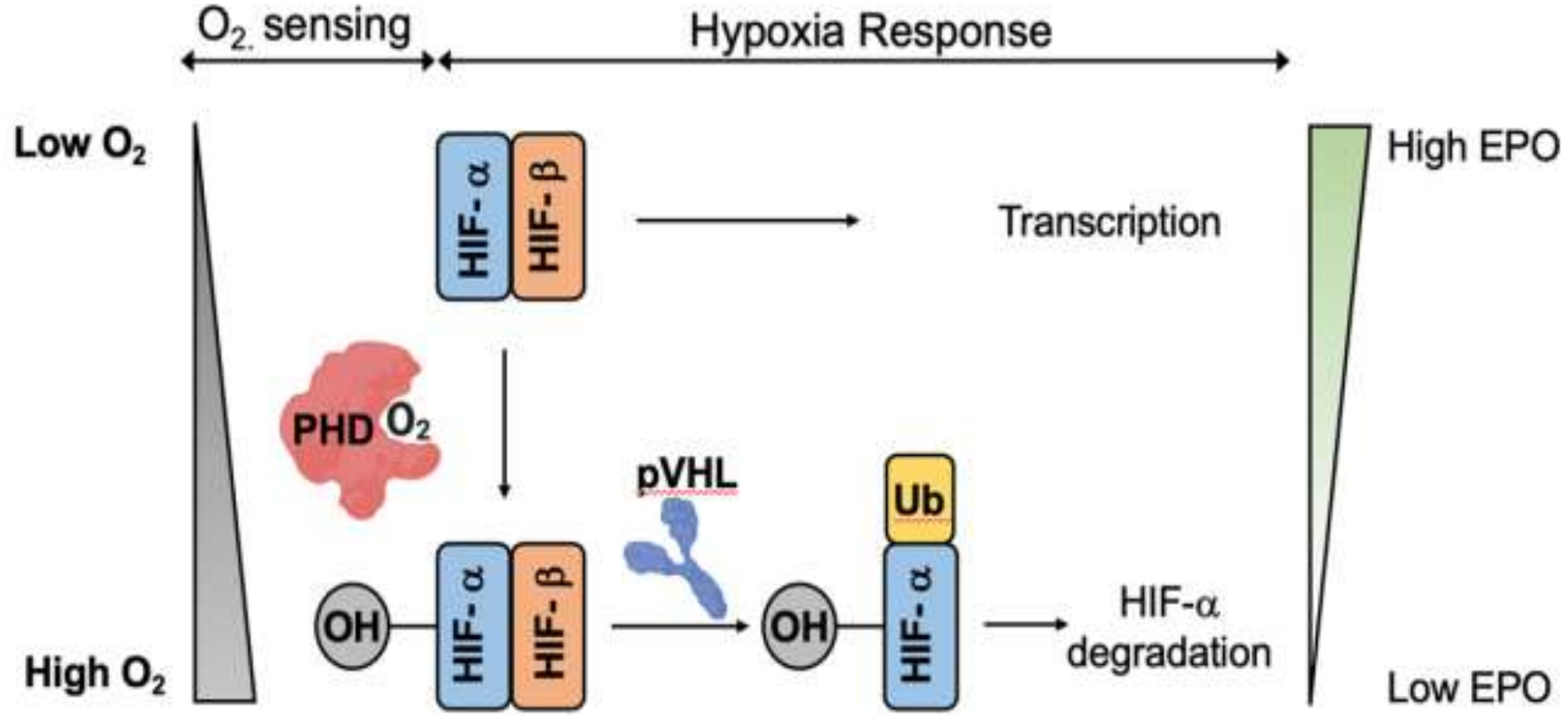
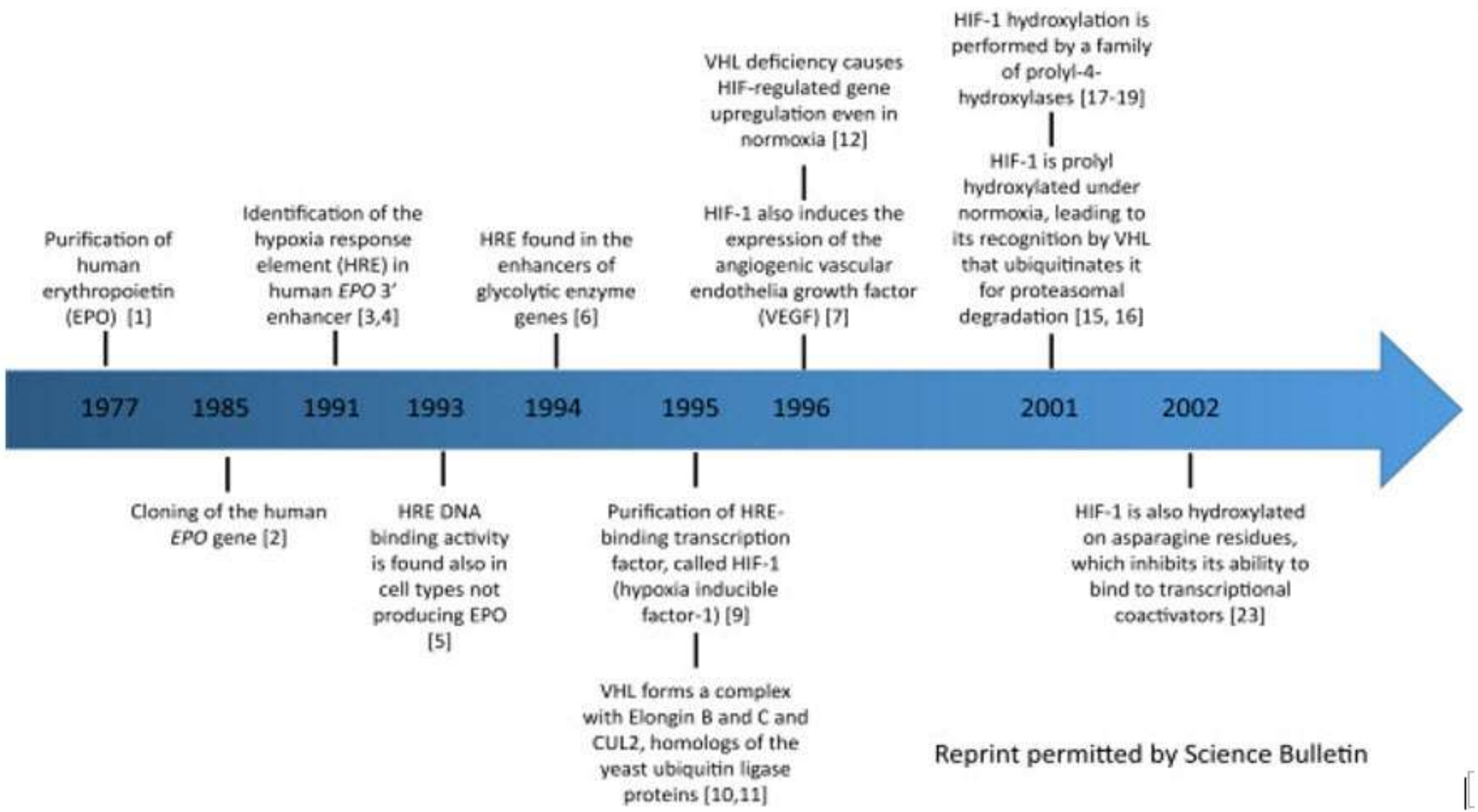


Figure 1. Essential elements of the hypoxic response in animal cells: the O_2 sensing and hypoxia response modules are depicted. Core proteins involved in the process are depicted: PHD, prolyl hydroxylase; HIF-α, hypoxia inducible factor α subunit; HIF-β, hypoxia inducible factor β subunit; pVHL, von Hippel Lindau protein; Ub, ubiquitin. Hydroxylation indicated by –OH. EPO, erythropoietin.



Timeline HIF-1 Research

The Nobel Prize in Physiology or Medicine 2019

William G. Kaelin Jr

Sir Peter J. Ratcliffe

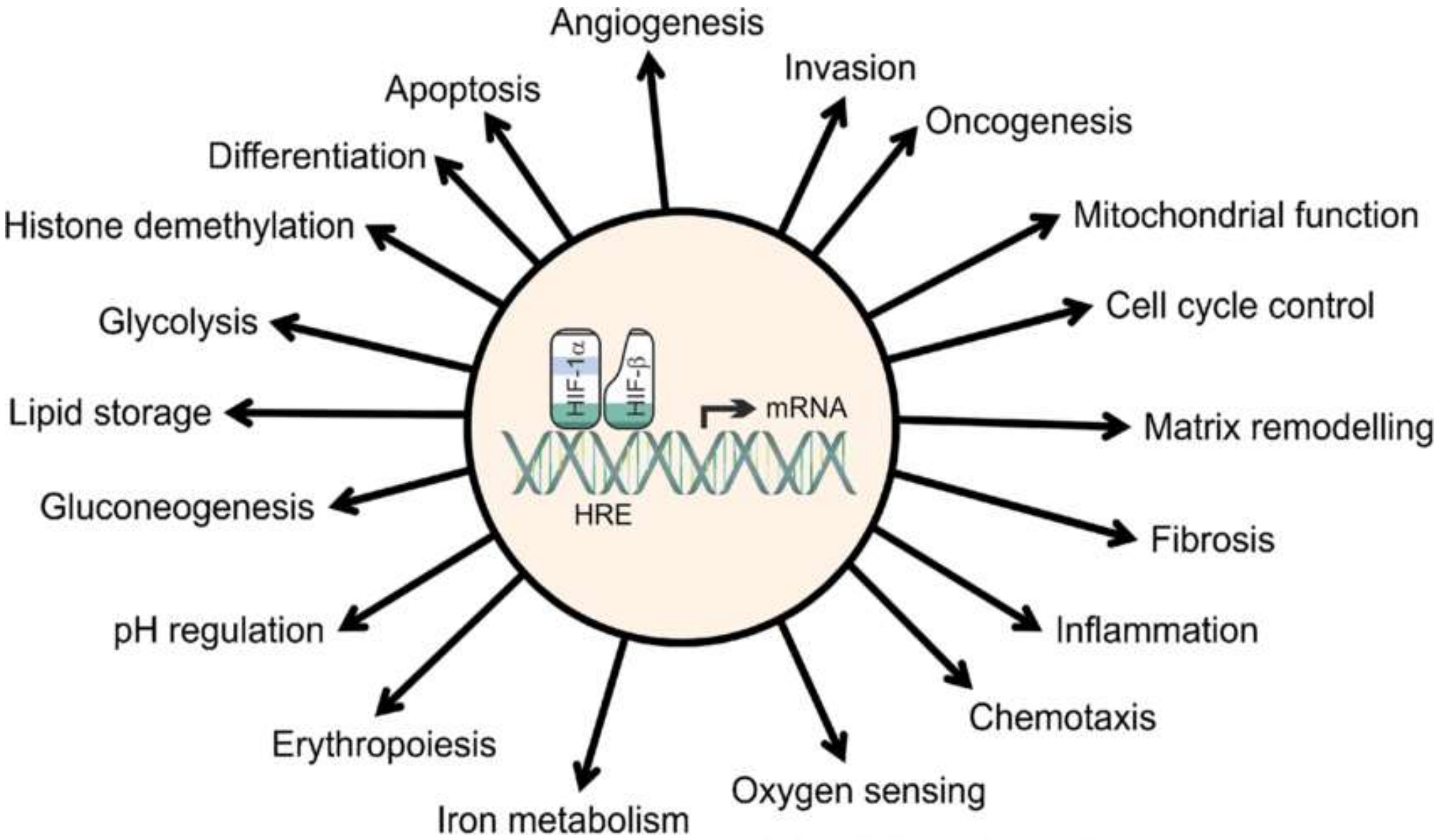
Gregg L. Semenza

"for their discoveries of how cells sense and adapt to oxygen availability"

FUTURE DIRECTION

HIF-1 as a target for drug development

- Hypoxia plays an important role in both physiological and pathological
- HIF-1 function as a Master Regulator of O2 Homeostasis by controlling both O2 delivery and utilization
- Hypoxia inducible factors (HIFs) mediate the transcription almost 300 of genes that allow cells to adapt to hypoxic environments.
- Could be very important target for drug development and treatment for diseases, such as Atherosclerosis, Cancer and Stroke, in which hypoxia is a central aspect



Pathway-targeted by the hypoxia inducible factor HIF via binding to hypoxia response element

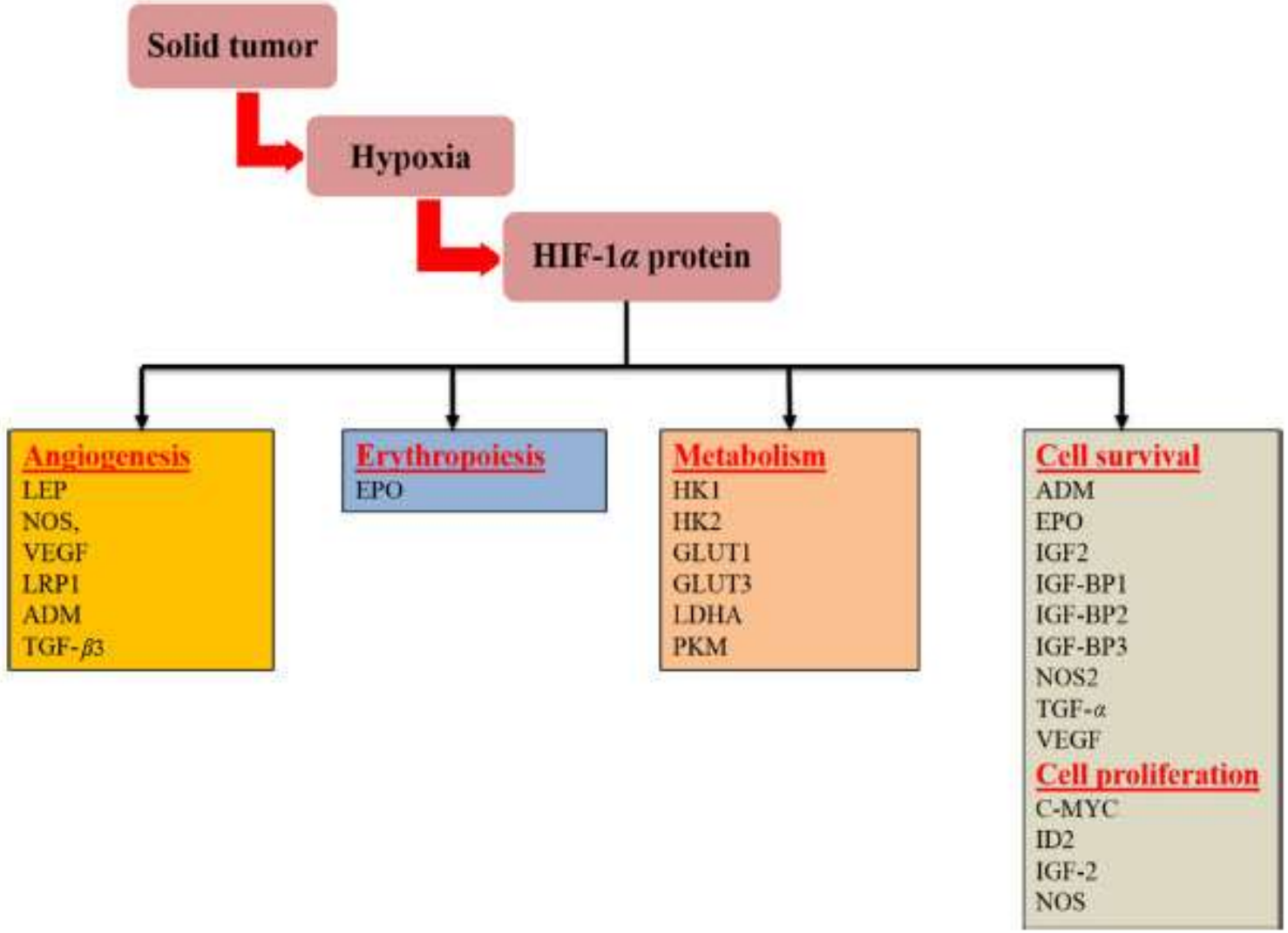
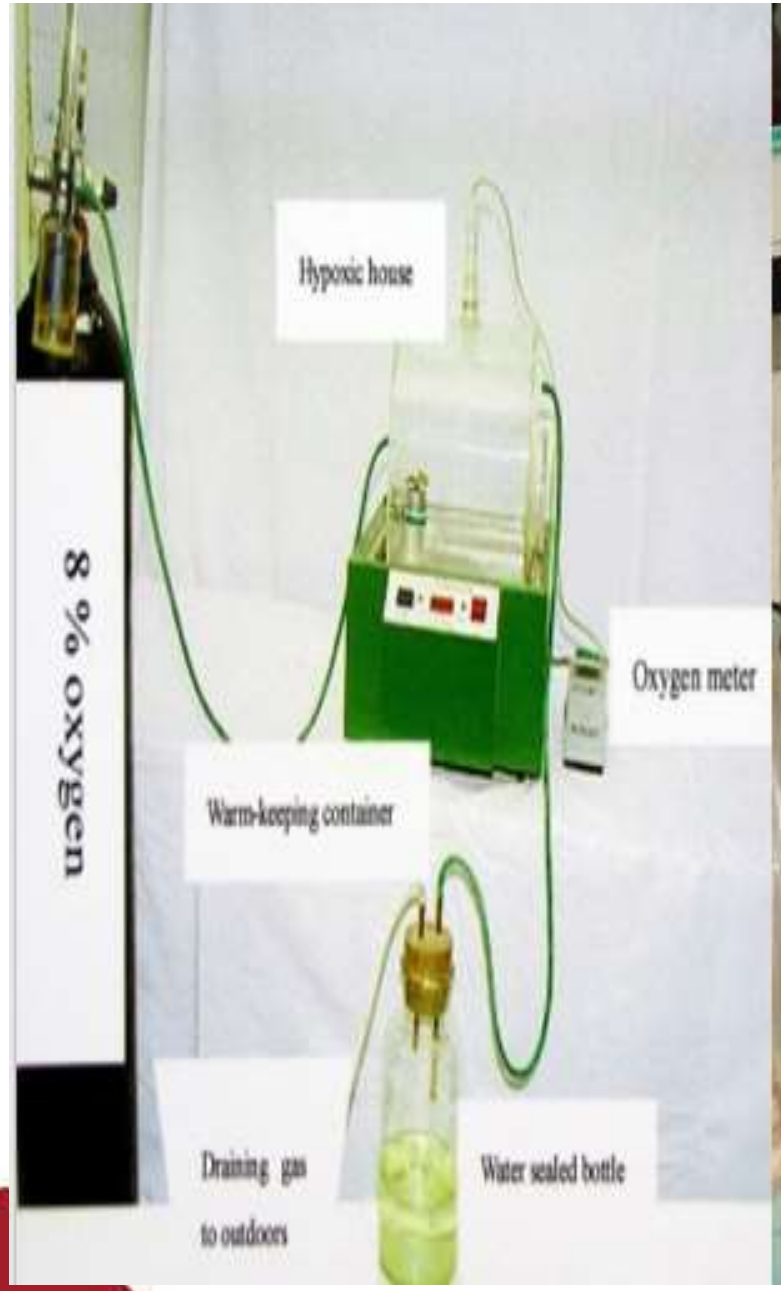


Figure 1 Representative HIF-1α regulatory genes and their effects on cancer progression. LEP, leptin; NOS, nitric oxide synthase; VEGF, vascular endothelial growth factor; LRP1, LDL-receptor-related protein 1; ADM, adrenomedullin; TGF-β3, transforming growth factor-β3; EPO, erythropoietin; HK1, hexokinase 1; HK2, hexokinase 2; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; LDHA, lactate dehydrogenase; PKM, pyruvate kinase M; IGF2, insulin-like growth factor 2; IGF-BP2, IGF-factor-binding protein 2; IGF-BP3, IGF-factor-binding protein 3; TGF-α, transforming growth factor α; C-MYC, myelocytomatosis virus oncogene cellular homolog; ID2, DNA-binding protein inhibitor[7].



Small size capacity 1-3 rats

Medium Capacity for 4-6 rats

Large size capacity 7-10 rats

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