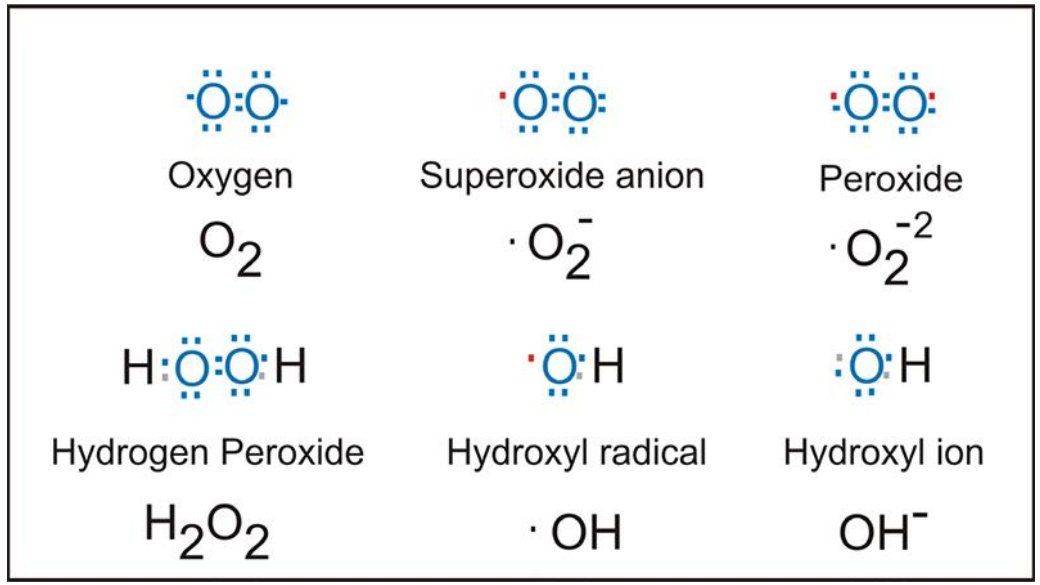
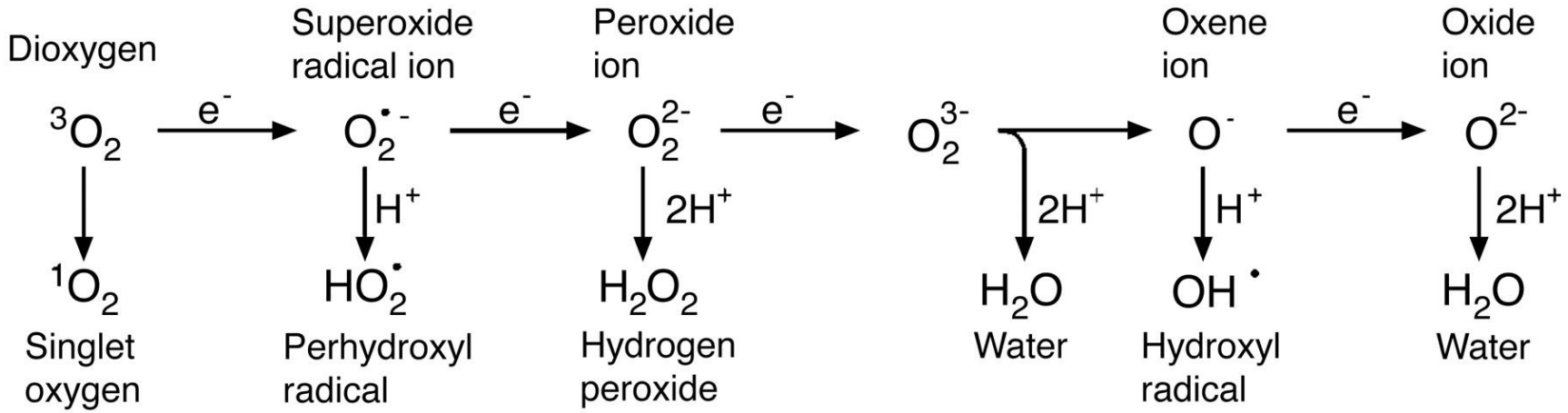
Role of reactive oxygen species in physiological processes

Petr Babula

ROS = reactive oxygen species





Radical	Structure	Reactivity	Half-life	Production/localization	Diffusion	Targets	Biological effect	Pathological effect
Hydroxyl radical	$OH^{\bullet}$	High	10^{-9} sec	Mitochondria Phagosome Endoplasmic reticulum (ER)	Highly localized where is produced	Any cell component	Unknown	Toxicity
Superoxide	O_2^-	Low	1–15 minutes	Mitochondria cytosol ER Peroxisome	Localized, it can diffuse through an anion channel	Fe-S centers Nitric oxide	Protein modification (activation or inhibition)	Protein damage
Hydrogen peroxide	H_2O_2	Moderate Reversible	Hours to days	Mitochondria cytosol ER Peroxisome	Diffuse, it can travel through aquaporins	Iron-sulphur Cysteine residues	Activation of signaling	Mutation, accumulation, and genomic instability

Reactive Oxygen Species (ROS) include:

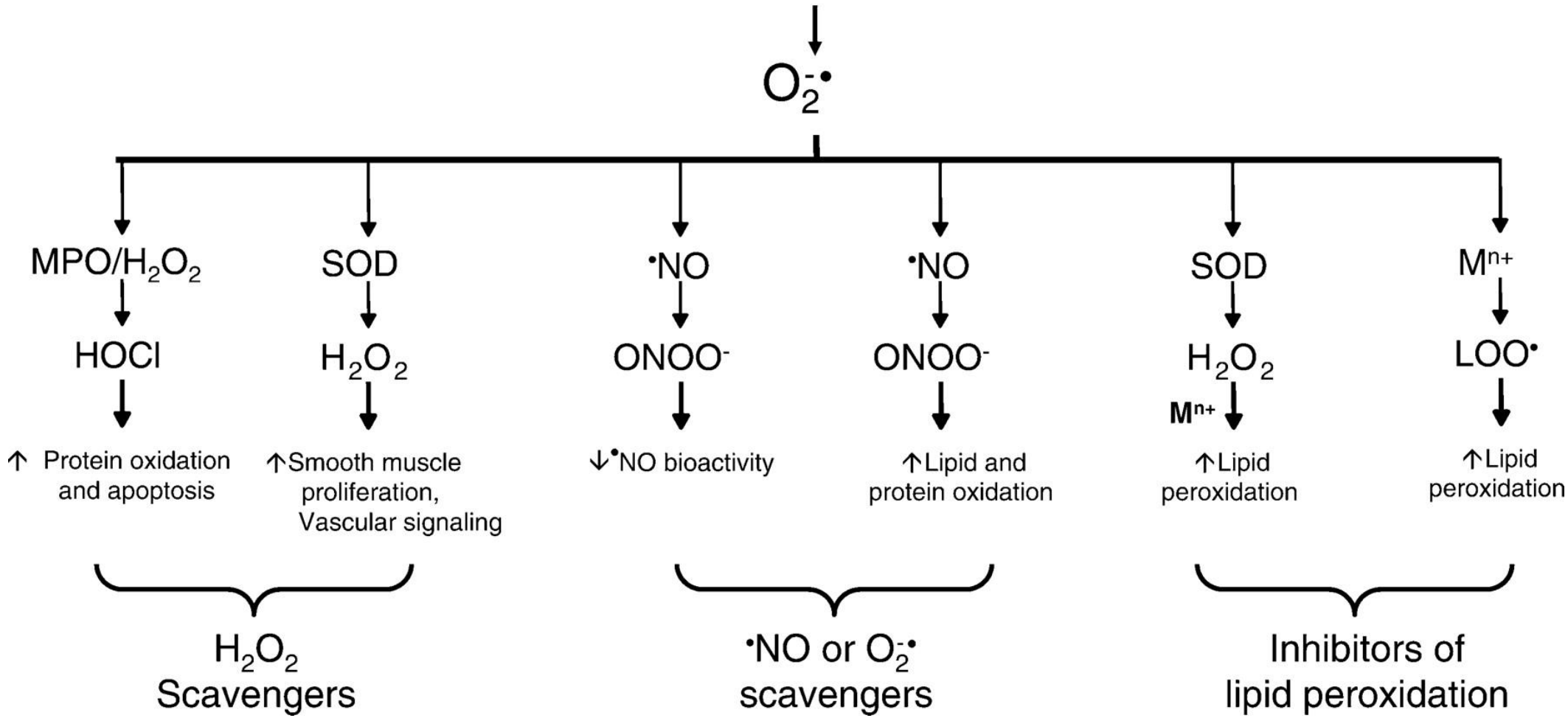
Radicals		*Non-Radicals*	
Hydroxyl	$OH^{\cdot}$	Peroxynitrite	$ONOO^{-}$
Superoxide	$O_2^{\cdot -}$	Hypochloric acid	HOCl
Nitric Oxide	$NO^{\cdot}$	Hydrogen Peroxide	H_2O_2
Thyl	$RS^{\cdot}$	Singlet Oxygen	$^1?_g$ ($^{-1}O_2$)
Peroxyl	$RO_2^{\cdot}$	Ozone	O_3
Lipid peroxyl	$LOO^{\cdot}$	Lipid peroxide	LOOH

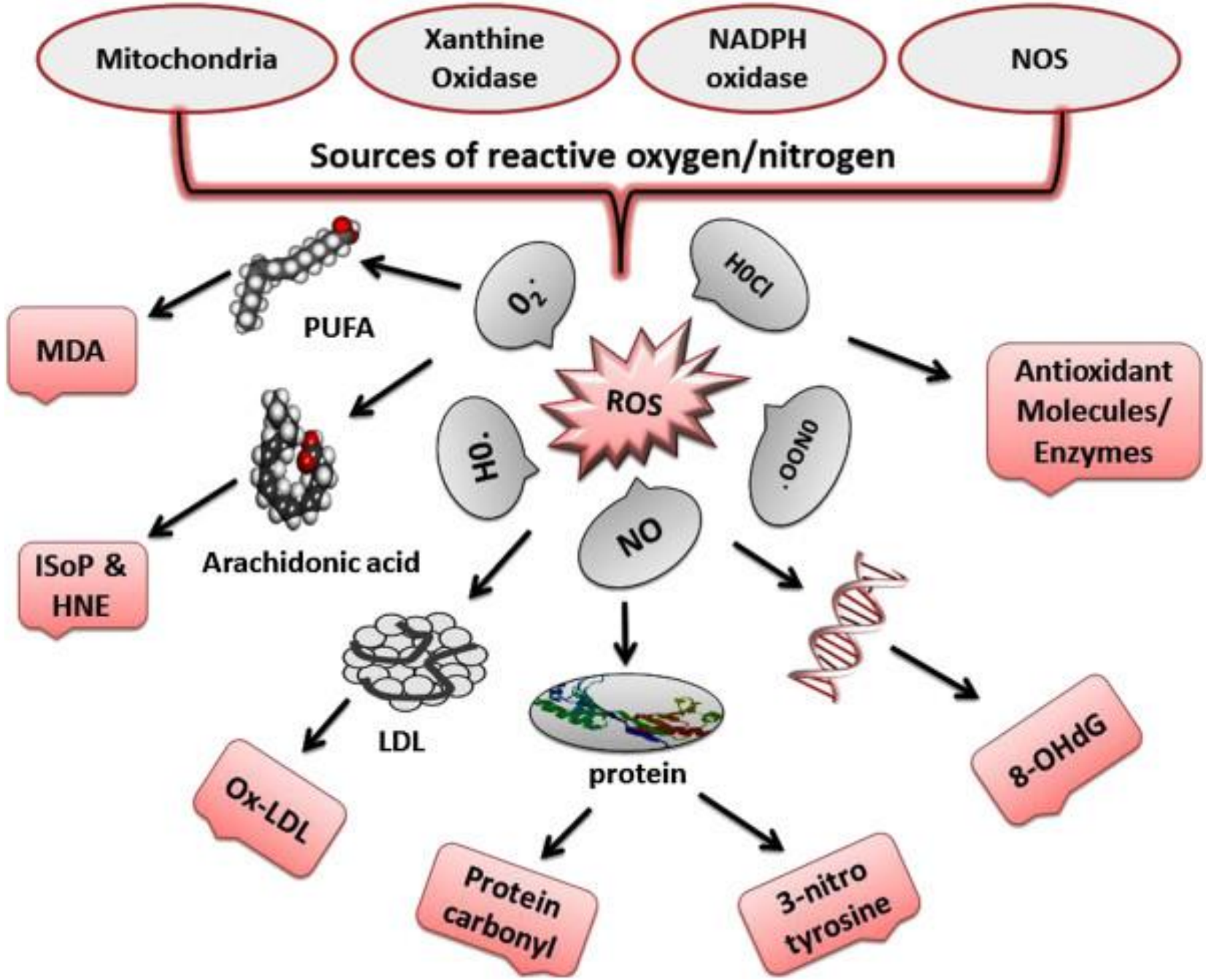
Reactive Nitrogen Species (RNS) include:

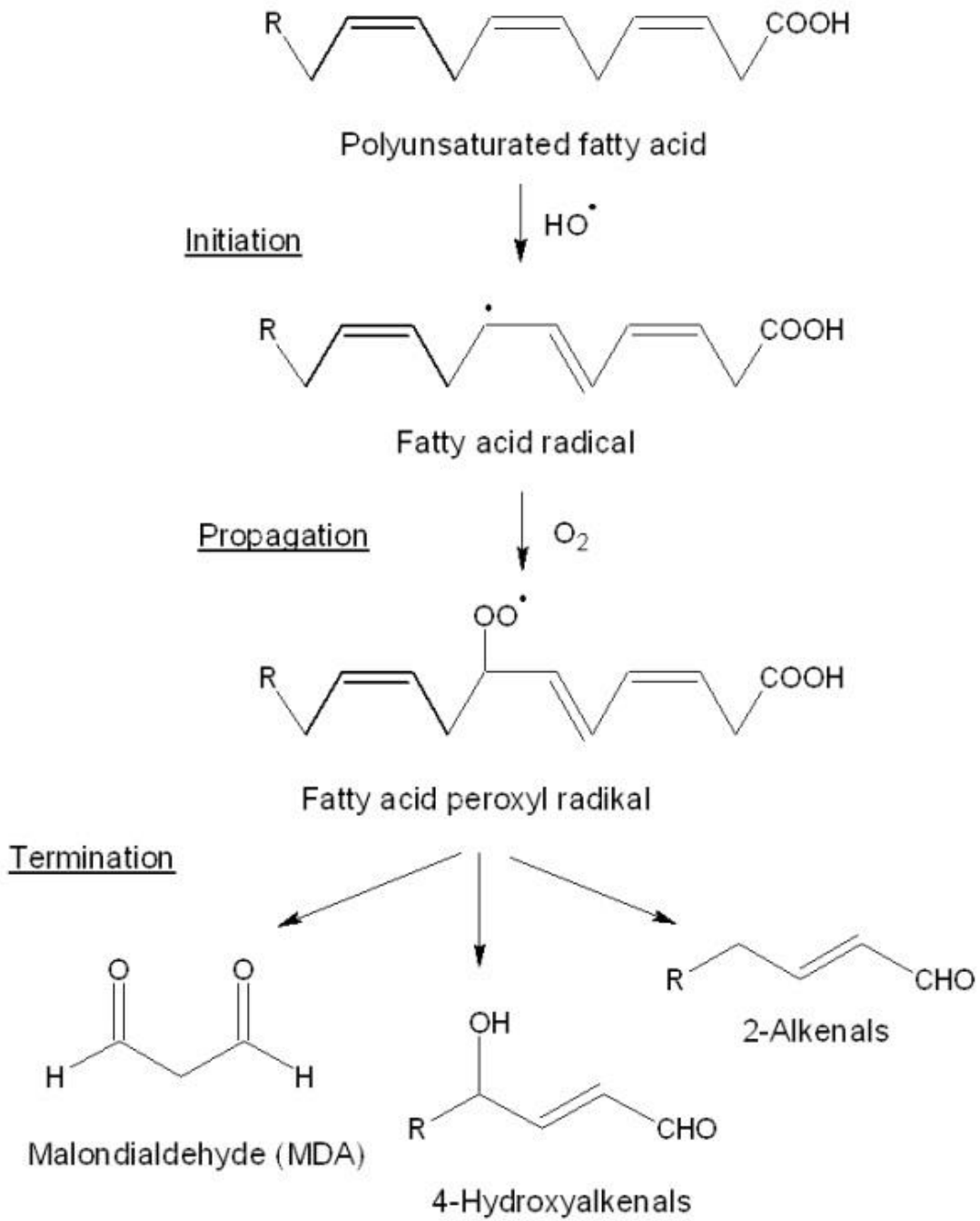
Nitrous oxide	$NO^{\cdot}$	Nitrosyl cation	NO^{+}
Peroxynitrite	$OONO^{-}$	Nitrogen dioxide	$NO_2^{\cdot}$
Peroxynitrous acid	ONOOH	Dinitrogen trioxide	N_2O_3
Nitroxyl anion	NO^{-}	Nitrous acid	HNO_2
Nitryl chloride	NO_2Cl		

Sources of oxidants

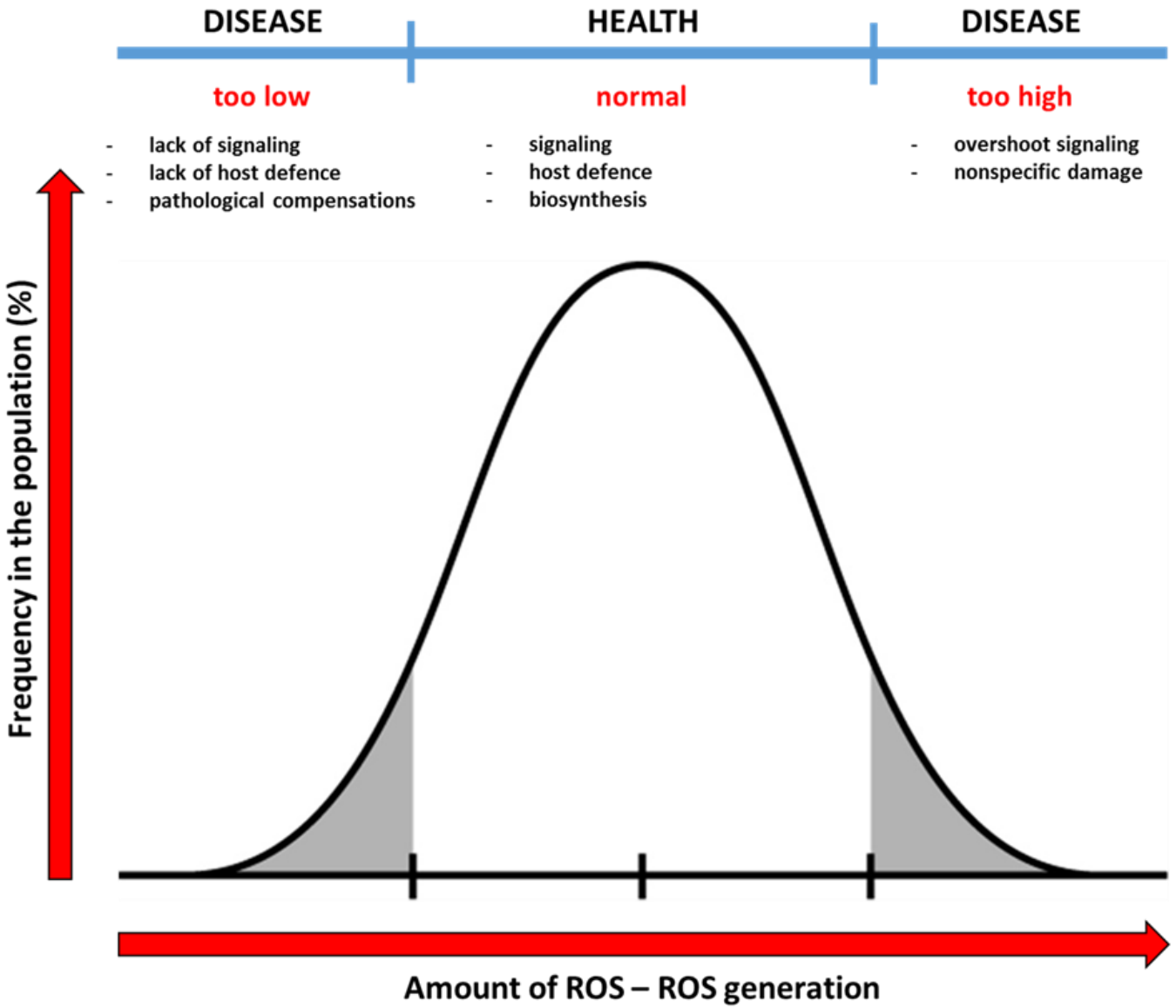
(oxidases, lipoxygenases, mitochondria, cytochrome P-450)




Mitochondria
Xanthine Oxidase
NADPH oxidase
NOS
Sources of reactive oxygen/nitrogen
PUFA
MDA
$O_2^{\cdot}$
HOCl
ROS
Antioxidant Molecules/ Enzymes
HO·
·OONO
Arachidonic acid
ISoP & HNE
NO
LDL
Ox-LDL
protein
8-OHdG
Protein carbonyl
3-nitro tyrosine


R
COOH
Polyunsaturated fatty acid
Initiation
HO•
R
COOH
Fatty acid radical
Propagation
O2
OO•
R
COOH
Fatty acid peroxyl radikal
Termination
O
O
H
H
Malondialdehyde (MDA)
OH
R
CHO
4-Hydroxyalkenals
R
CHO
2-Alkenals

Variation in ROS production



Amount of ROS as a result of variations in ROS production. Both, genetically (epigenetically) and environmentally based factors are involved in ROS production. In healthy people (= moderate level of ROS), ROS are involved in physiological processes including cell signalling, host defence and biosynthetic processes. On the other hand, reduced amounts of ROS participate in decreased antimicrobial defence, hypothyroidism, or changes in blood pressure. ROS play a crucial role in all above-mentioned physiological processes. When ROS levels are too high, overshoot signalling and nonspecific damage of biomolecules (cells, tissues) occur. ROS overproduction is involved in many pathological processes, including chronic inflammation and autoimmune diseases, cancer, sensory impairment, cardiovascular diseases, such as atherosclerosis, hypertension, re-stenosis, ischaemia/reperfusion injury, neurological disorders, fibrotic disease, other age-associated diseases, or infectious diseases. Adapted and modified from (Brieger et al., 2012).

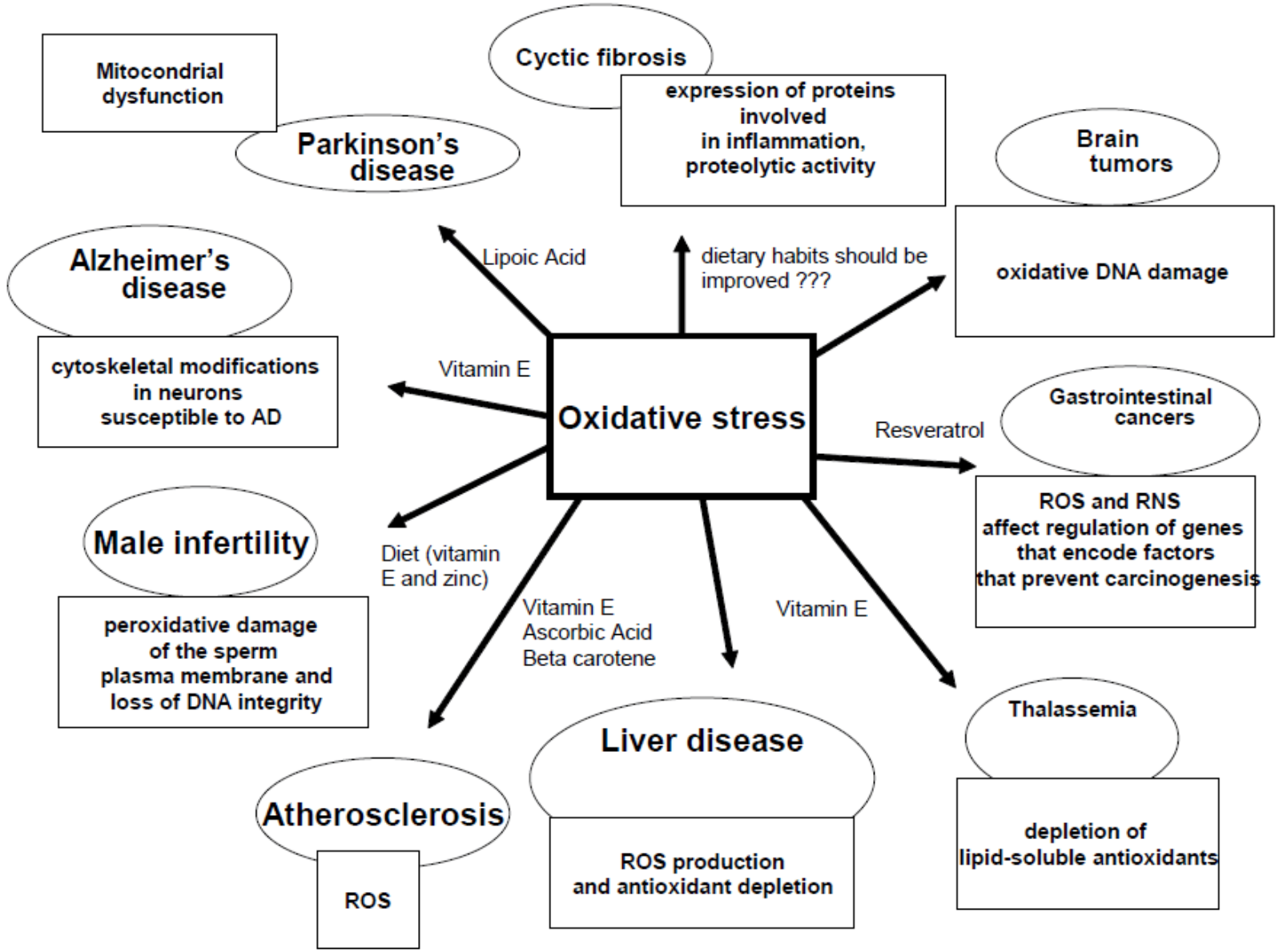


Figure 1: The pro-oxidant/antioxidant balance is shifted in favour of the pro-oxidants in oxidative stres. Here is a diagram showing some of the disease processes driven by oxidative stres, related mechanisms involved and the role of antioxidants discussed. ROS: Reactive oxygen species. RNS: Reactive nitrogen species. AD: Alzheimer's Disease.

Table 1 The good, bad and ugly of ROS

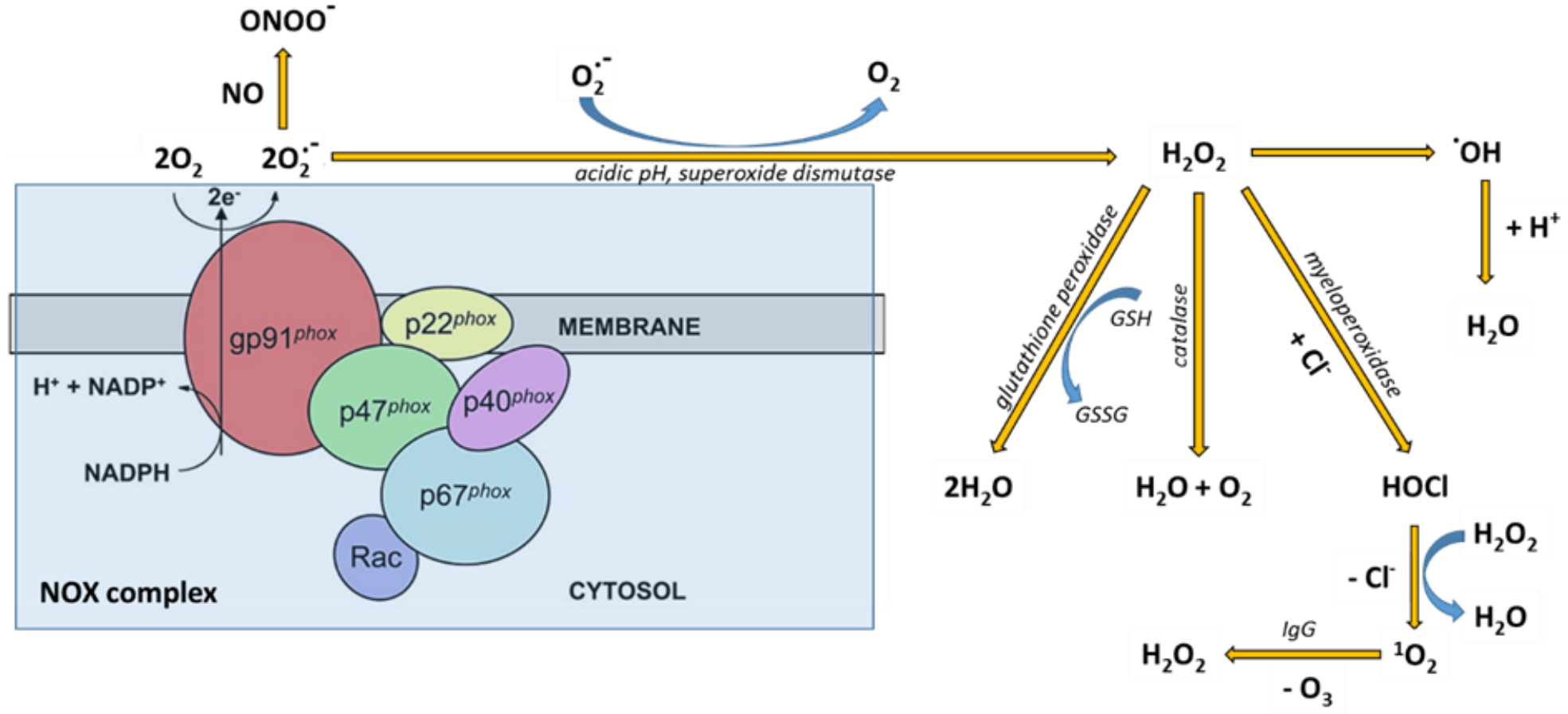
Nature of ROS	Biology/Physiology	Effects of ROS	Citations
Good	Cellular activities	Involved in cellular response to stressors Regulates mitochondrial function, expression of certain stress proteins and antioxidant levels	Allen & Tresini (2000), Banerjee Mustafi *et al.* (2009), Bushell *et al.* (2002), Chandel *et al.* (1996), Yamashita *et al.* (1997)
	Immune system	Activates NLRP3 inflammasomes or other immune-related receptors Helps combat invading pathogens	Bedard & Krause (2007), Bonini & Malik (2014), Tschopp (2011)
	Synaptic plasticity	Involved in the formation of LTP	Massaad & Klann (2011)
Bad	Protein degradation	Leads to protein modification Influences protein translation Increases the susceptibility of proteins to proteolysis	Aiken *et al.* (2011), Breusing & Grune (2008), Cheeseman & Slater (1993), Stadtman (1991), Valko *et al.* (2007, 2006), Winrow *et al.* (1993)
	DNA damage	Induces mutagenesis Oxidizes nucleotides (guanine is particularly susceptible)	Sassa *et al.* (2013), Sheng *et al.* (2012)
	Muscle fatigue	Increases fatigue thus reducing muscle function Promotes oxidative damage to muscle protein	Khassaf *et al.* (2001), Mangner *et al.* (2013), McArdle *et al.* (2001), Pattwell *et al.* (2004), Reid *et al.* (1992)
Ugly	Cancer	Induces DNA mutation Upregulates HIF-1α, which is involved in tumor angiogenesis	Liao *et al.* (2007), Waris & Ahsan (2006)
	Pulmonary diseases	Enhances inflammation response and damages diaphragm function Contributes to pulmonary diseases such as COPD or asthma	Barreiro *et al.* (2005), Zuo *et al.* (2012, 2013a)
	Cardiovascular diseases	Involved in IR damage Causes hypertension via mechanisms such as lipid peroxidation	Zuo *et al.* (2013b, 2014c)
	Neurodegenerative disorders	Correlated with neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and ALS	Brieger *et al.* (2012), Mitsumoto *et al.* (2014), Saccon *et al.* (2013), Sorce & Krause (2009)

ALS, amyotrophic lateral sclerosis; COPD, chronic obstructive pulmonary disease; HIF-1α, hypoxic-inducible transcription factor; IR, ischaemia–reperfusion; LTP, long-term potentiation; NLRP3, cryopyrin; ROS, reactive oxygen species.

Table 1: Selected ROS-mediated diseases.

Disease family	Example disease	Potential mechanisms of ROS involvement
Cancer	Renal cell carcinoma	HIF-1α expression induces vascular endothelial growth factor (VEGF), a mediator of angiogenesis, tumour growth and metastasis; NOX4 activity is required for HIF-1α expression. Fumarate hydratase deficiency may induce hypoxia-inducible transcription factor stabilisation by glucose-dependent generation of ROS. Obligate glycolytic switch is critical to HIF stabilisation via ROS generation. Decrease in mitochondrial energy metabolism, upregulation of glycolysis. von Hippel-Lindau tumour suppressor-deficient. Cells exhibit upregulation of p22phox, NOX4, and NOX-mediated ROS generation. Tumour cell growth is suppressed by DPI.
Cardiovascular	Hypertension	Superoxide reacts with $^{\cdot}NO$, forming peroxynitrite ($ONOO^{-}$), causing a reduction in $^{\cdot}NO$ bioavailability and endothelium-dependent vasodilation. NOX4 is strongly expressed in media of small pulmonary arteries and is causally involved in development of pulmonary hypertension. NOX-derived ROS are a hypertensive signalling element. Decreased systolic BP response to angiotensin II and to bone morphogenetic protein-4 in p47phox-deficient mice. Decreases in BP in NOX1-deficient mice.
Neurological	Schizophrenia	NOX2 involved in neurotransmitter release. NOX2 contributes to changes in interneurons, including the loss of parvalbumin expression and the capacity to secrete GABA. Oxidative stress may change the set of active transcription factors within GABAergic interneurons.

ROS signalling depends on ability of cells to detoxify ROS



NOX (= NADPH oxidase) complex as a source of ROS and degradation cascade of ROS that contributes to ROS balance. Major enzymes involved in ROS conversions are shown – superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and myeloperoxidase (MPO). Scheme also shows a way leading to production of singlet oxygen and ozone. Superoxide anion radical is rapidly converted into hydrogen peroxide by superoxide dismutases with specific cellular localization. On the other hand, hydrogen peroxide is able to oxidize cysteine residues on proteins to initiate redox biology.

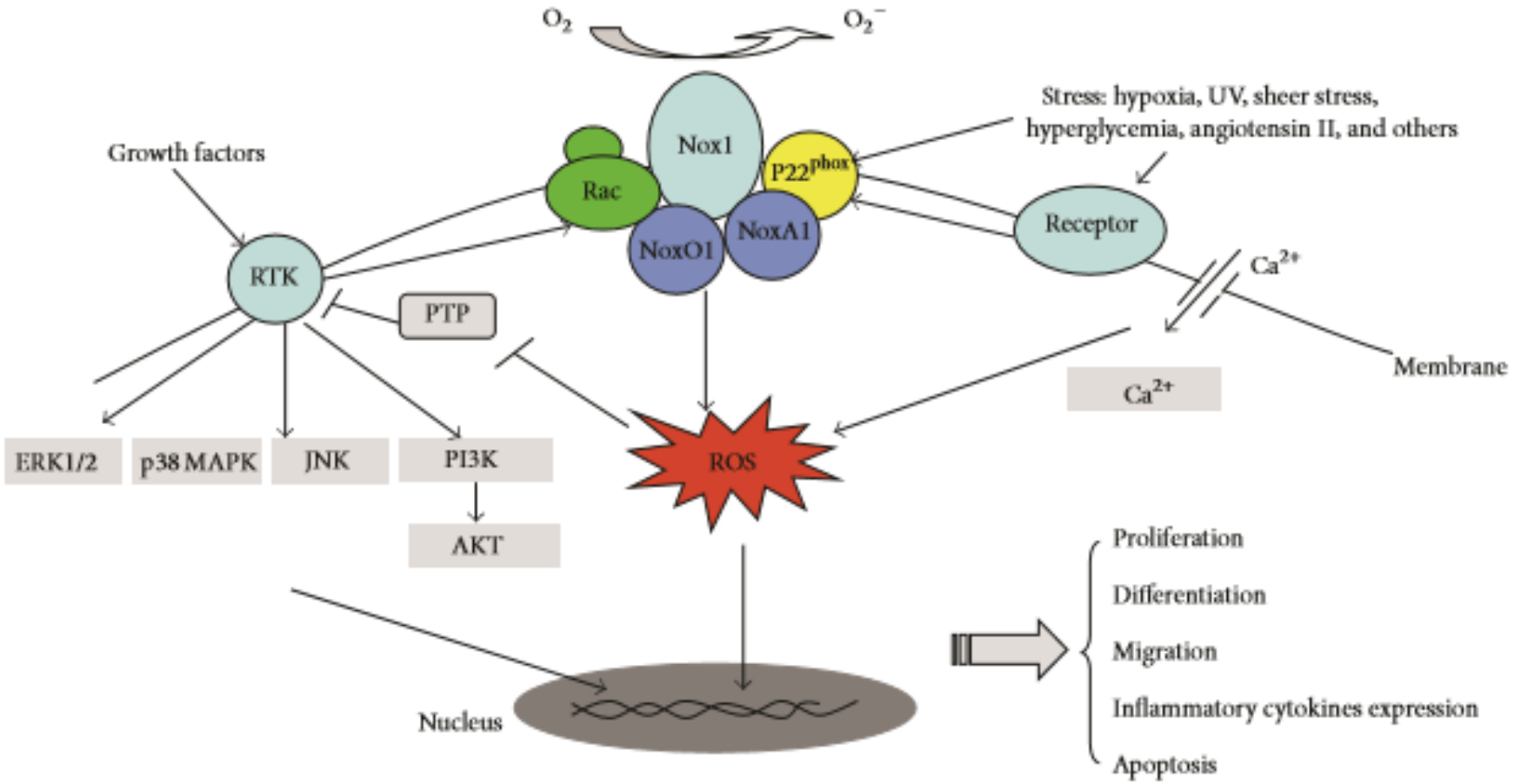
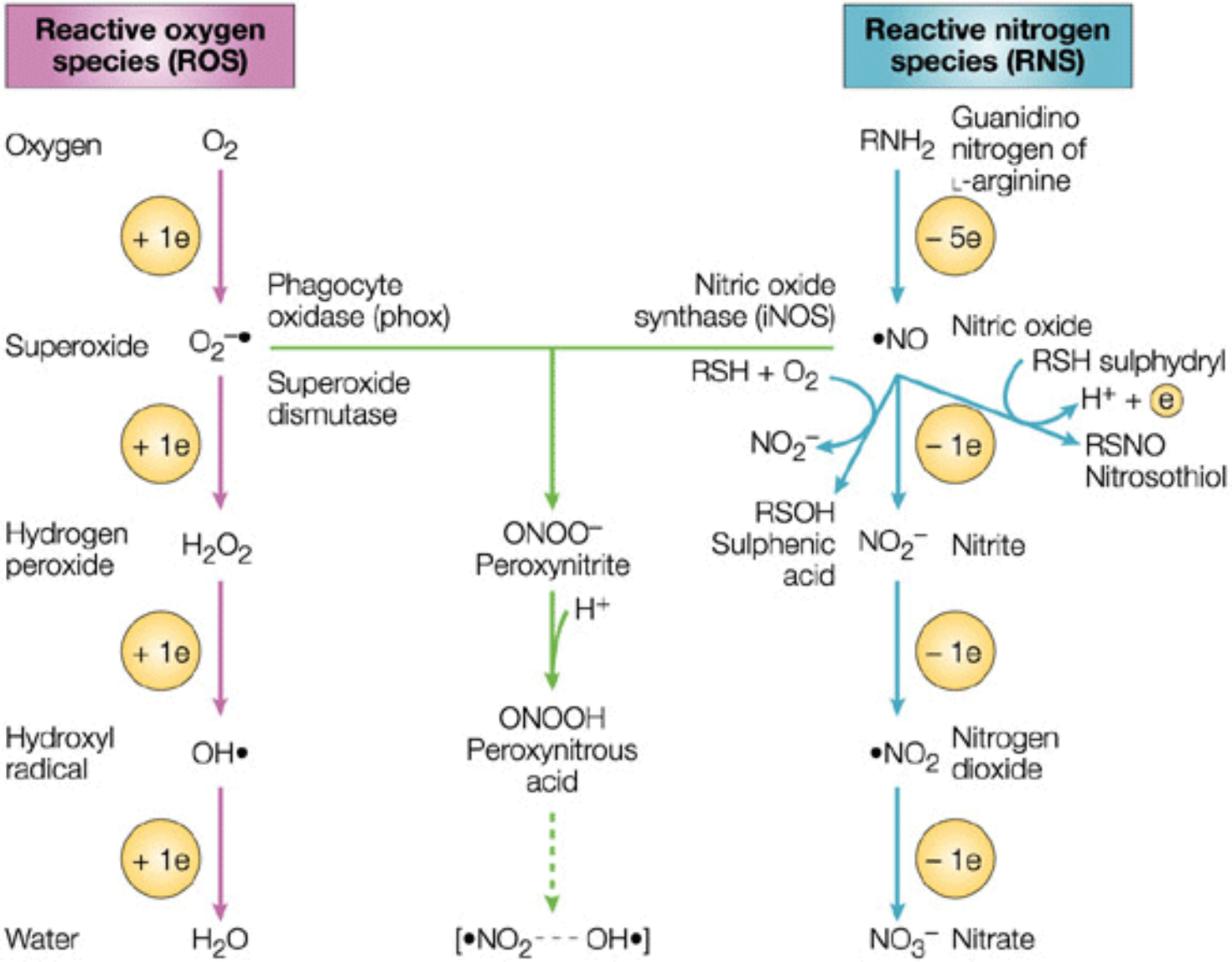
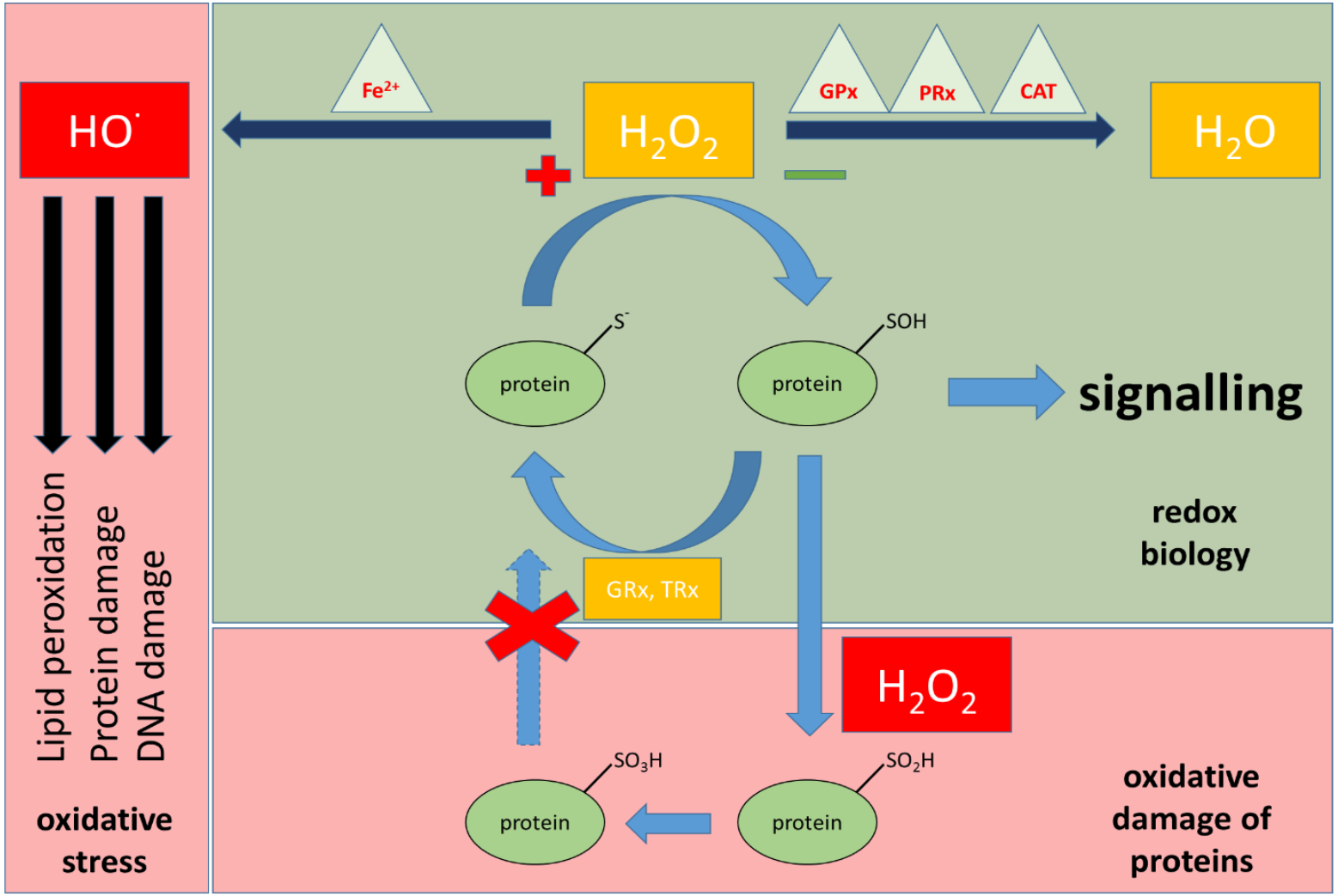


FIGURE 2: Nox1 modulates intracellular signaling. Nox1 can be activated by a diverse array of stimuli, such as the binding of growth factors to their receptor tyrosine kinases (RTK) and the stimulation by agonists such as angiotensin II. ROS produced by activated Nox1 oxidize the cysteine residue of protein tyrosine phosphatases (PTP), inactivate these enzymes, and lead to enhanced activation of MAPK system and PI3 K. ROS may also interact with intracellular Ca^{2+} by enhancing the entry of Ca^{2+} through cell membrane. The activated intracellular signals may further activate Nox1 or other Nox homologues, causing additional increasing of ROS. All these mechanisms may be involved in regulating cell proliferation, differentiation, apoptosis, and migration, and angiogenesis, which are crucial components of tissue injury and repair.



Nitroxyl anion (NO), a one-electron reduction product of nitric oxide (NO), is unlikely to arise from NO under physiological conditions. The reaction of reactive nitrogen species with cysteine sulphydryls can result in either S-nitrosylation or oxidation to the sulphenic acid, as well as disulphide-bond formation, all of which are potentially reversible. The peroxynitrite anion (ONOO-) and peroxynitrous acid (ONOOH) have distinct patterns of reactivity. ONOOH spontaneously decomposes through a series of species that resemble the reactive radicals hydroxyl (OH) and/or nitrogen dioxide (NO2). When the concentration of L-arginine is limiting, nitric oxide synthase (NOS) can produce superoxide (O2-) along with NO, which favours the formation of peroxynitrite. Reproduced with permission from Ref. 13 © (2000) National Academy of Sciences, USA.

Physiological and pathophysiological role of ROS



Overview of physiological and pathological (excessive) role of ROS in the cell. Under physiological conditions, superoxide anion radical is rapidly converted by SOD enzymes to hydrogen peroxide. Cellular level of hydrogen peroxide is strictly converted to water by glutathione peroxidase (GPx), peroxiredoxins (PRx), and catalase (CAT). Low concentration of hydrogen peroxide is involved in the first step of cysteine oxidation in proteins moieties from thiolate anion ($Cys\text{-}S^-$) to the sulfenic form (Cys-SOH). This reaction is reversible and glutaredoxin (GRx) and thioredoxin (TRx) play crucial role in it. Sulfenic form of cysteine moieties in proteins causes allosteric changes in proteins, which means change in the protein function. On the other hand, excess of hydrogen peroxide causes next oxidation steps to sulfinic (SO_2H) or sulfonic (SO_3H) species; these steps are irreversible and these species cannot be converted back to initial form of protein. Increased level of hydrogen peroxide leads to creation of hydroxyl radicals ($HO^{\cdot}$); the reaction is catalysed by Fe^{2+} ions. Hydroxyl radicals irreversibly damage cellular biomolecules including lipids, proteins, and nucleic acids.

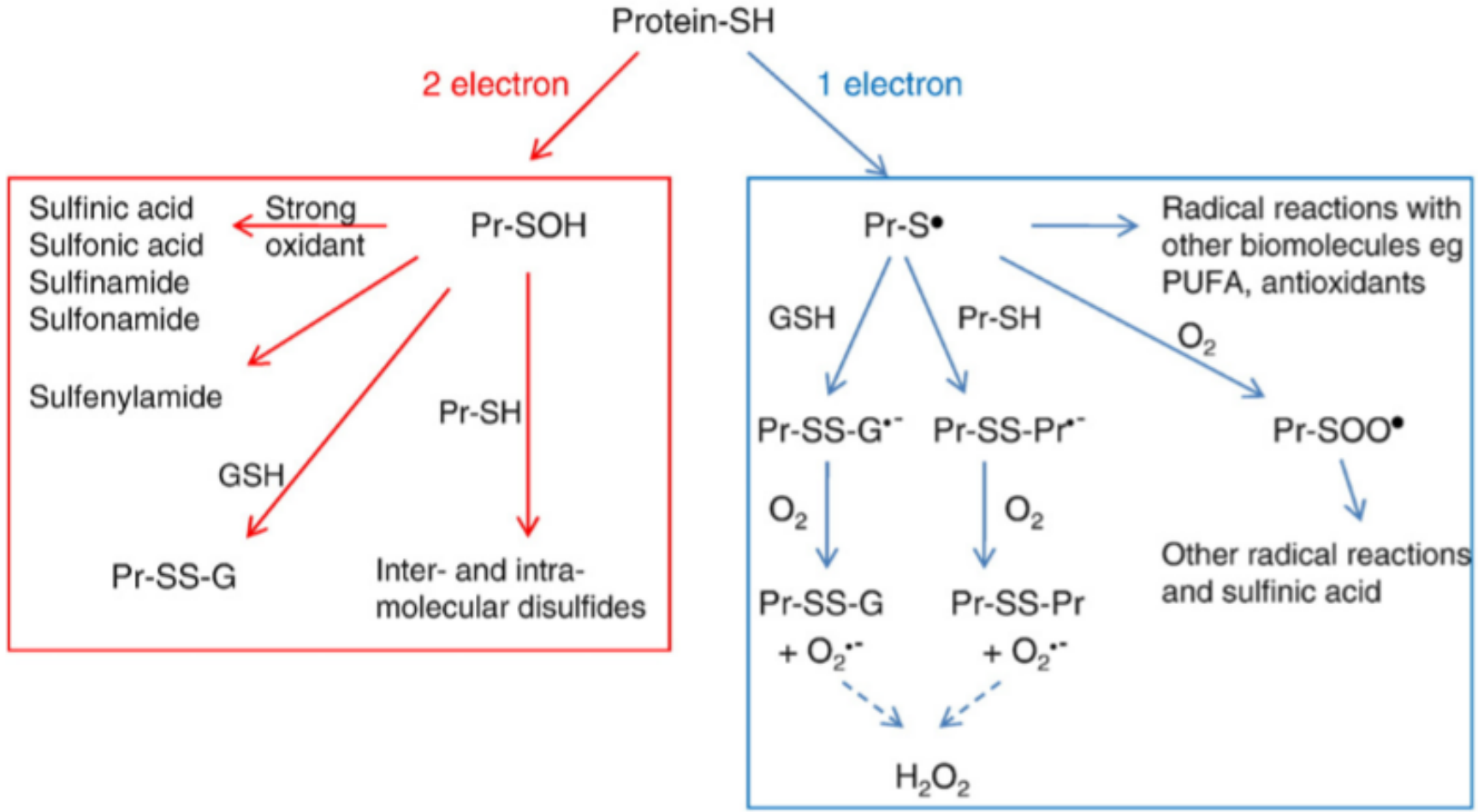
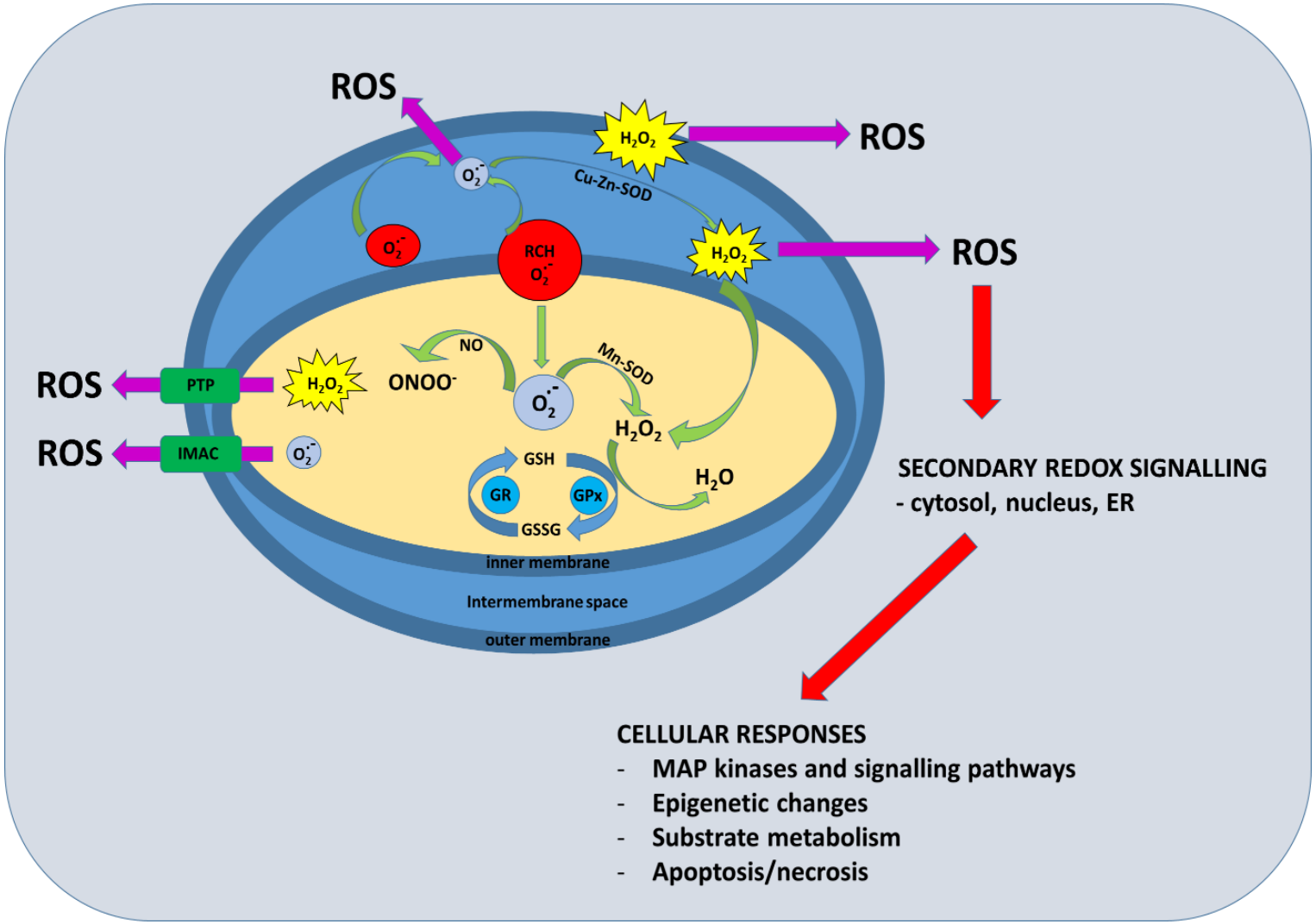


Fig. 2. Pathways for one electron and two electron oxidation of protein thiols. Two electron oxidation of a protein thiol (Pr-SH), shown in the left-hand pathway, initially gives the sulfenic acid (Pr-SOH) that, unless in a shielded environment, is a transient intermediate that undergoes a range of secondary reactions. It can form mixed disulfides with GSH (Pr-SS-G); vicinal thiols favor formation of intramolecular disulfides; intermolecular disulfides can form between proteins; or as in PTP1B, reversible condensation with an adjacent amide gives a sulfenylamide. Hypohalous acids and chloramines initially form sulfenyl halides, which hydrolyze or undergo similar reactions to the sulfenic acid. Hypohalous acids are more likely to generate the higher oxidation products. As shown on the right-hand side, one electron oxidants (radicals and transition metal ions) give rise to the relevant thiyl radical (Pr-S•). Under aerobic conditions its most favored reaction is with a thiolate anion (protein or GSH) to give the disulfide anion radical ($GSSG^{\bullet-}$ for GSH). Reaction with oxygen drives this reaction forward generating superoxide and thus amplifying oxidative reactions. Alternatively, the thiyl radical can propagate radical reactions or be quenched by scavengers. Dimerization of two thiyl radicals is usually a minor pathway. Most reactions of thiyl radicals are reversible, so to be efficient may require rapid removal of products. Many of the reactions involve the thiolate anion but for simplicity are shown for the thiol. As the degree of oxygenation of the sulfur increases, pK_a decreases, so if a thiol is present as the thiolate, its oxidation products will also be ionized. More extensive discussion of these reactions can be found in Refs. [48,51,52,85,86,209–211].

Cellular sources of ROS – mitochondria



Sources of ROS in mitochondria and regulation of their production – a schematic overview. Mitochondria have several sources of ROS. Respiratory chain (RCH) produces superoxide anion radical; production of both superoxide and hydrogen peroxide in the intermembrane space is usually not considered, major source of them is oxidation of dihydroorotate to orotate with reduction of CoQ. Intermembrane space contains Cu-Zn-SOD, which dismutates superoxide anion radical to hydrogen peroxide. In mitochondrial matrix, superoxide very quickly reacts with nitric oxide to form peroxynitrite. Mitochondrial matrix contains several systems that consume hydrogen peroxide formed by an action of Mn-SOD: peroxiredoxins 3 and 5 (PRx3/5), catalase (CAT), and glutathione peroxidases 1 and 4 (GPx1/4), which represent together with peroxiredoxins the most important ROS-detoxifying systems. Glutathione reductase (GR) regenerates reduced glutathione (GSH) from its oxidized (GSSG) form. Both superoxide anion radical and hydrogen peroxide are transported out of mitochondria into the cytosol. Permeability transition pore (PTP - mPTP) is involved in the efflux of hydrogen peroxide, inner membrane anion channel (IMAC) as the ion channel opens transiently in response to an elevation of superoxide anion radical. Hydrogen peroxide alters structure of many different proteins (cytosolic or nuclear enzymes, carriers or transcription factors), which leads to changes in activities. ROS are involved in secondary redox signalling, which finally causes cellular responses based on changes in MAPK signalling pathway, epigenetic changes, changes in metabolism of substrates, or eventually may result in cell death (apoptosis, necrosis).

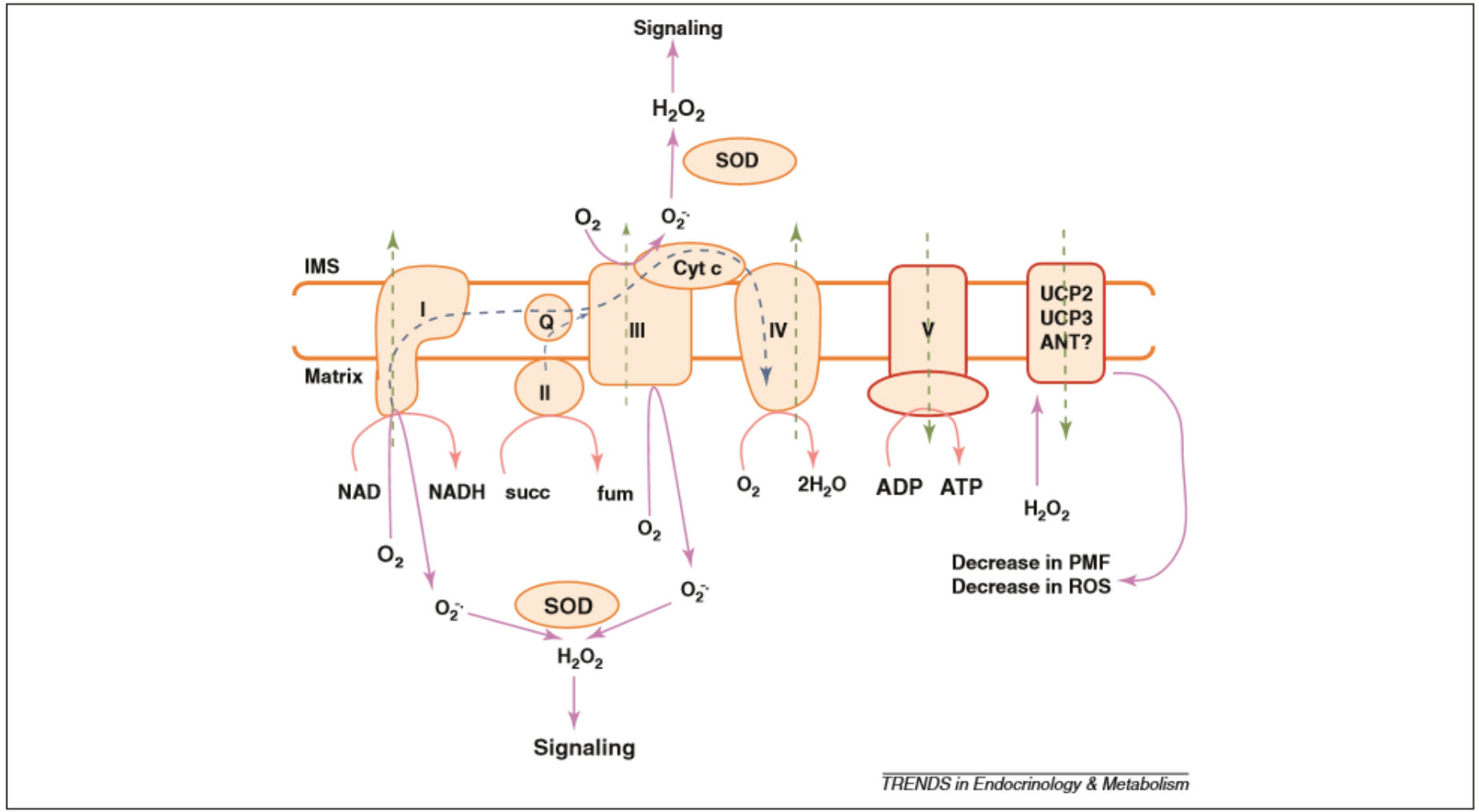
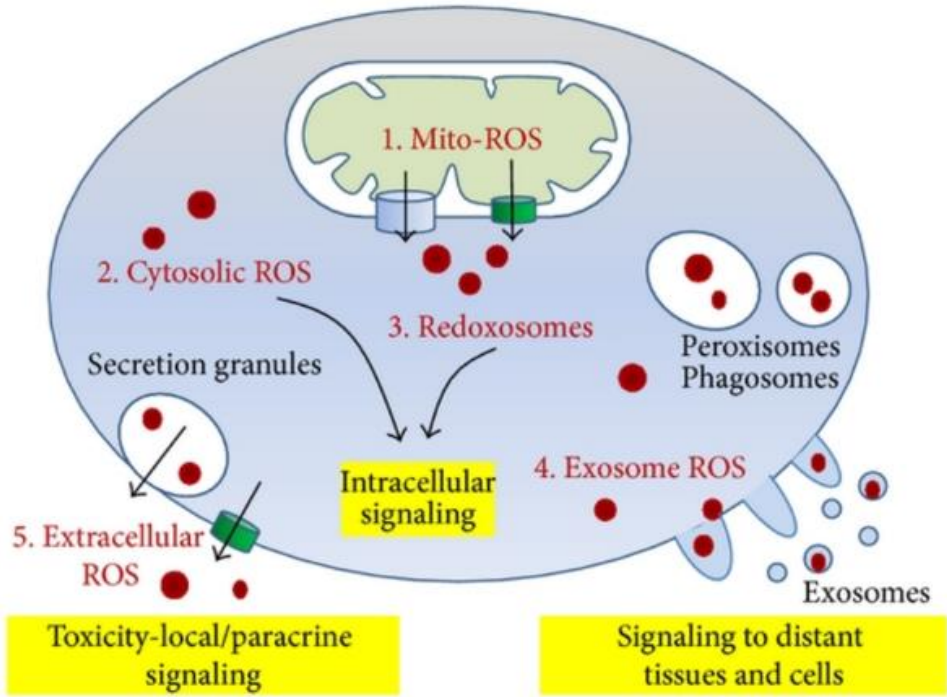
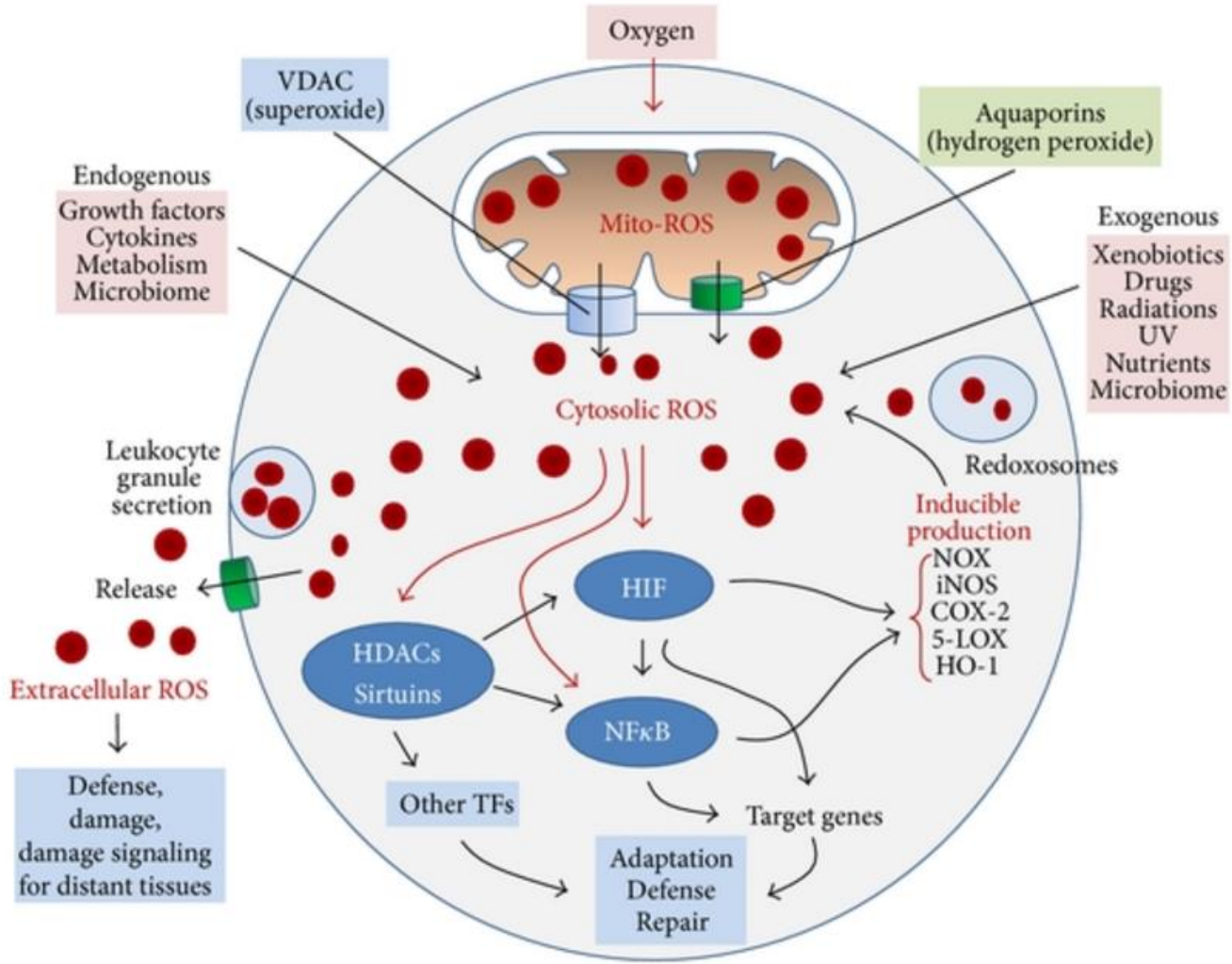
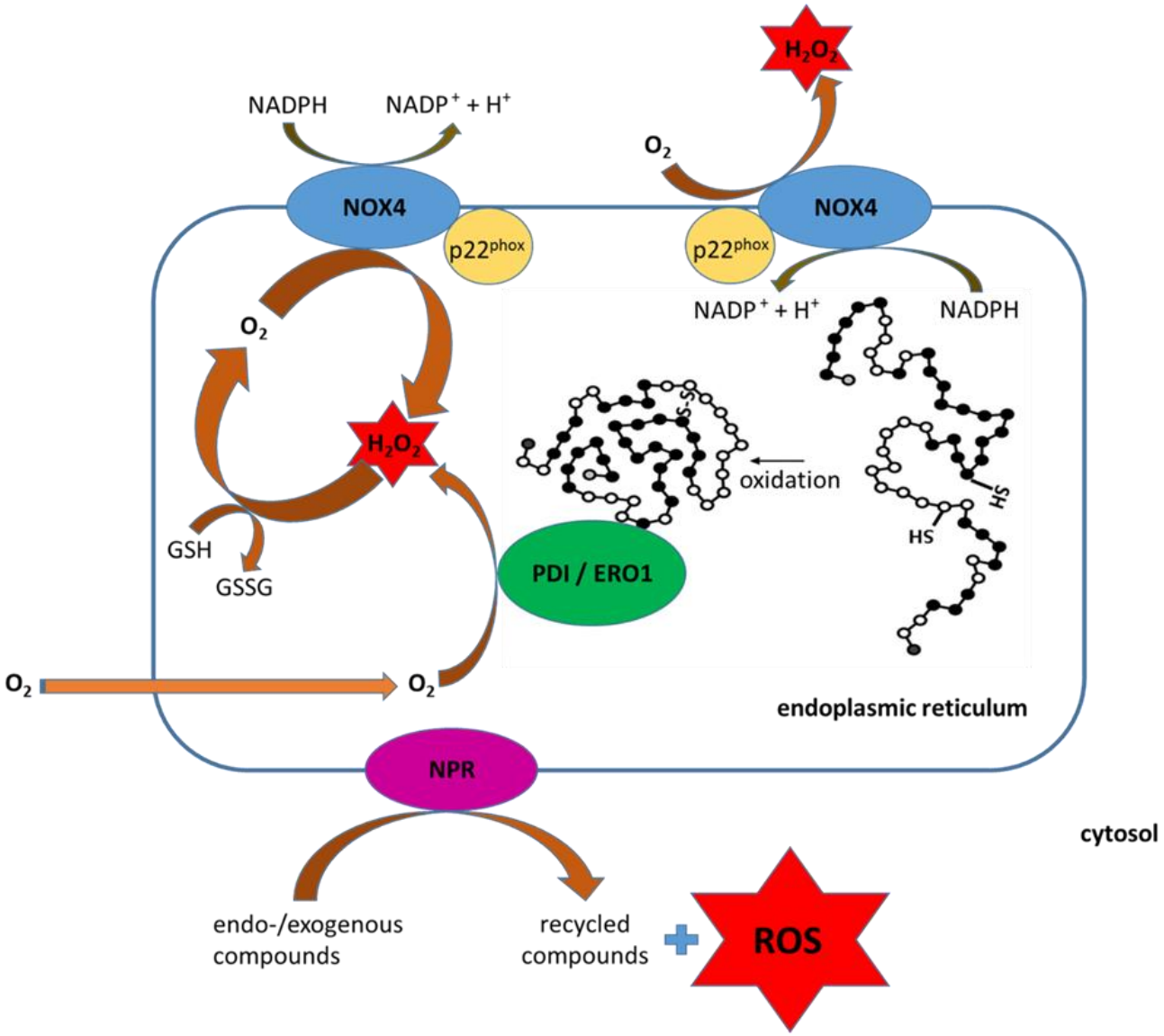


Figure 1. Aerobic respiration and the self-regulation of mitochondrial ROS production. Following NADH (complex I), succinate (complex II), and other fuel oxidations (fatty acid or glyceraldehyde-3-phosphate oxidation that donate electrons directly to Q), electrons systematically pass through the respiratory complexes (complexes III and IV) to the terminal electron acceptor, O_2. The difference in redox potential between components of the respiratory chain allows proton extrusion from the matrix at complexes I, III and IV, which establishes protonmotive force (PMF). The latter is then used to drive ATP synthesis through complex V (ATP synthetase). When PMF is high and the components of the respiratory chain are mainly in reduced states, liberated electrons from complexes I and III can univalently reduce O_2 to superoxide ($O_2^{-\bullet}$). In contrast to complex I, complex III generates ROS on both the intermembrane space (IMS) and matrix side of the membrane. The production of $O_2^{-\bullet}$ by mitochondria is self-regulated through the activation of uncoupling proteins (UCPs) 2 and 3, which decrease the PMF and thus decrease ROS production. Although adenine nucleotide translocase (ANT) is known to induce basal proton leak, its role in using proton leak to control mitochondrial ROS production remains controversial. Direction of electron flow is indicated by the blue-dotted line. Proton extrusion or import is indicated by green-dotted arrows.

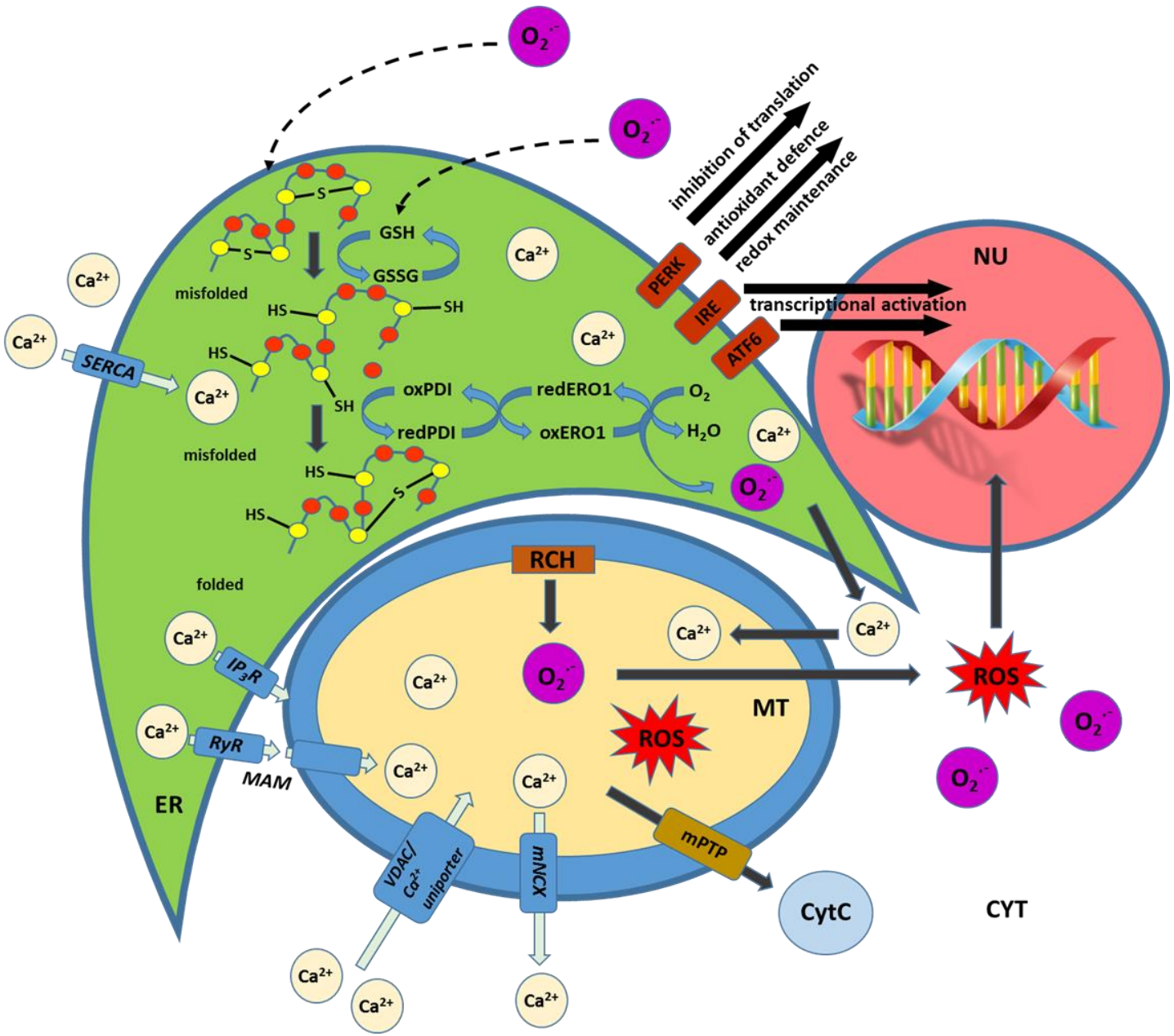

1. Mito-ROS
2. Cytosolic ROS
3. Redoxosomes
Secretion granules
Peroxisomes
Phagosomes
Intracellular signaling
4. Exosome ROS
5. Extracellular ROS
Exosomes
Toxicity-local/paracrine signaling
Signaling to distant tissues and cells


Oxygen
VDAC (superoxide)
Aquaporins (hydrogen peroxide)
Endogenous
Growth factors
Cytokines
Metabolism
Microbiome
Mito-ROS
Exogenous
Xenobiotics
Drugs
Radiations
UV
Nutrients
Microbiome
Cytosolic ROS
Redoxosomes
Leukocyte granule secretion
Release
Extracellular ROS
Inducible production
NOX
iNOS
COX-2
5-LOX
HO-1
HIF
HDACs
Sirtuins
NFκB
Other TFs
Target genes
Adaptation
Defense
Repair
Defense, damage, damage signaling for distant tissues

Cellular sources of ROS – endoplasmic reticulum

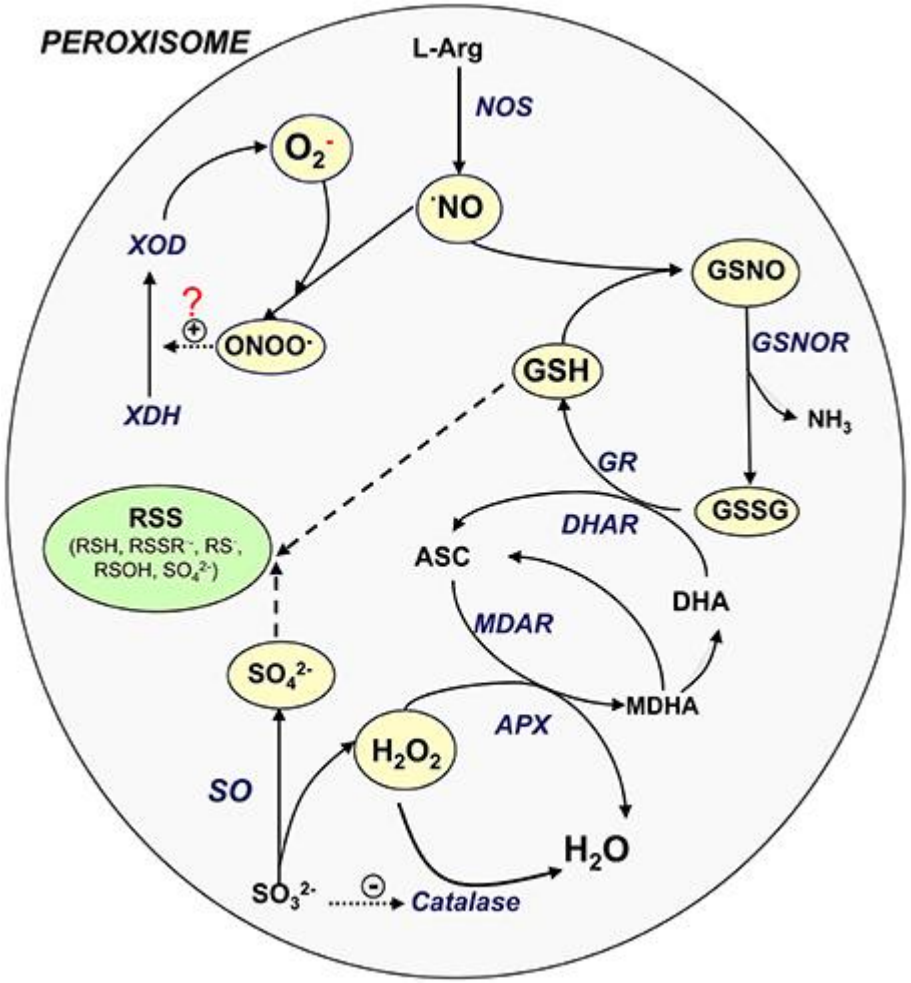


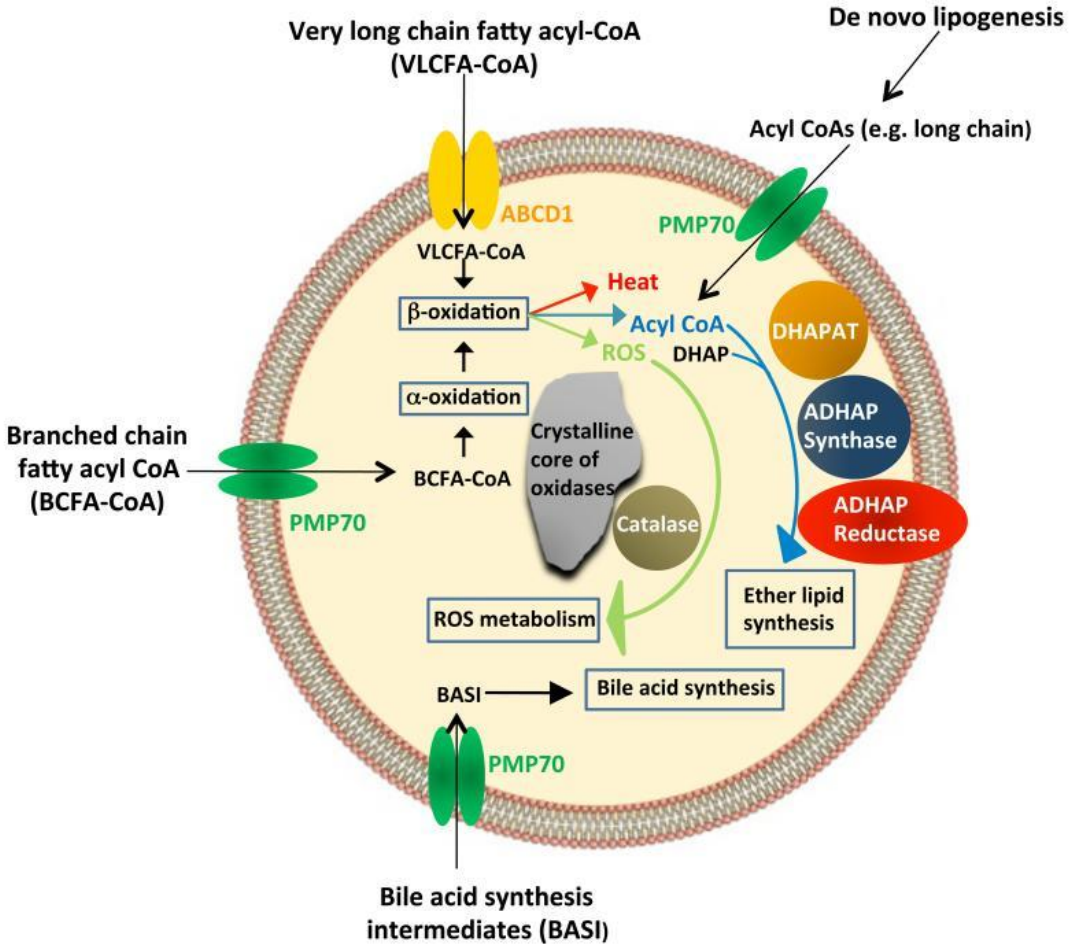
Scheme of the ER ROS sources and their mutual communication. Production of ROS occurs during a protein folding. In this process, protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductin-1 (ERO1) play a crucial role. Another source of ROS is NADPH oxidase 4/p22phox (NOX4) and also NADPH-P450 reductase (NPR), which is involved in the recycling of both endogenous and exogenous compounds, but also amount of reduced glutathione (GSH) itself.



An interplay between mitochondria, endoplasmic reticulum and cytoplasm in handling ROS and calcium ions. Briefly, endoplasmic reticulum is the crucial and major site for calcium storage in cell. Sarco-/endoplasmic reticulum Ca^{2+}-ATPase represents the most important transport mechanism for influx of calcium ions. On the other hand, mitochondria represent the second most important calcium store in the cell. However, these two organelles are closely connected in calcium handling, mainly in response to ROS. Increase in ROS levels in the mitochondria, where the respiratory chain (RCH) represents the major site for creation of ROS, triggers the ER to release calcium and sensitizes a calcium-releasing channel in the ER membrane, sending a feedback signal. On the other hand, process of folding proteins contributes significantly to creation ROS directly in ER. When incorrect disulfide bonds form, they need to be reduced by GSH, resulting in a further decrease of GSH/GSSG ratio, altering the redox state within the ER. Alternatively, misfolded proteins can be directed to degradation through ER-associated degradation machinery. Accumulation of misfolded proteins in the ER initiates the unfolded protein response, which includes involvement of protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme (IRE), and activating transcription factor 6 (ATF6). All these proteins influence cellular responses at different levels (transcription, translation, antioxidant defence). Calcium ions released from ER during these processes (inositol 1,4,5-trisphosphate receptors (IP3R) and ryanodine receptors (RyR) – accumulated in membranes with close connection with mitochondria - in mitochondrial associated membranes (MAMs) trigger mitochondrial ROS stimulation via stimulation of the tricarboxylic acid cycle. Mitochondria release calcium ions *via* mitochondrial sodium/calcium exchanger (mNCX), influx of calcium ions from cytoplasm in provided by voltage-dependent anion channel (VDAC) and calcium uniporter. Increased load of mitochondria with calcium ions stimulate release of cytochrome c and pro-apoptic factors *via* mitochondrial permeability transition pore (mPTP).

Cellular sources of ROS – peroxisomes





Other cellular sources of ROS

- Autooxidation of small molecules (dopamine, epinephrine, flavins, and hydroquinones)
- XO, COXs, cytochrome P450 enzymes (cytochrome P450 monooxygense), LOXs, flavin-dependent demethylase, oxidases for polyamines and amino acids, and NOSs that produce oxidants as part of their normal enzymatic function

Nitric oxide

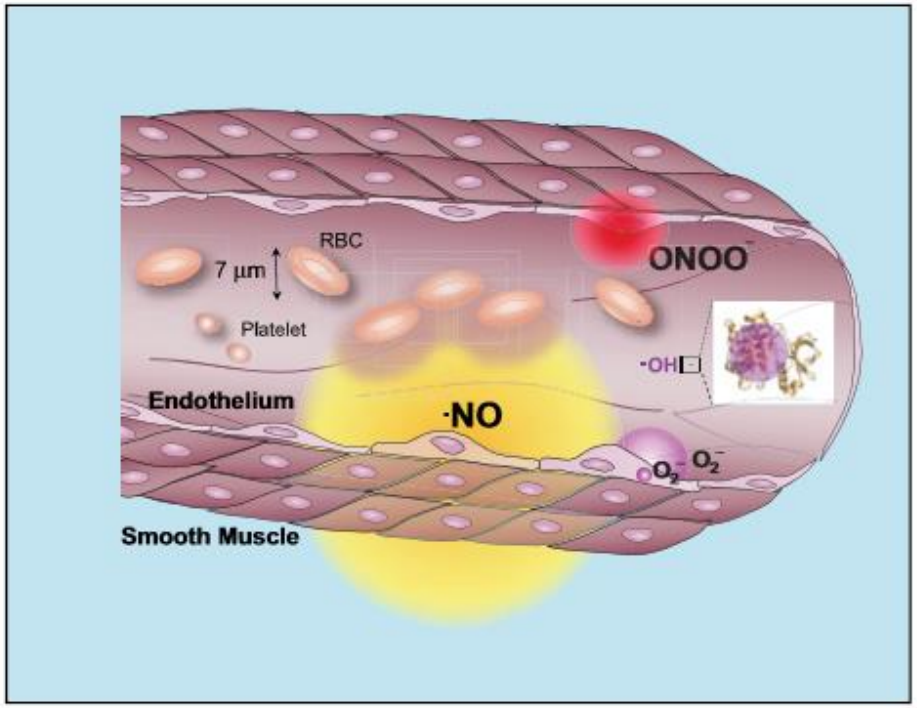


FIG. 1. Cellular diffusion of superoxide, peroxynitrite, and hydroxyl radical within their estimated first half-lives. These circles indicate the extent to where the concentration of each species from a point source would decrease by 50%. The diffusion of peroxynitrite accounts for its rapid reaction with carbon dioxide and with intracellular thiols. The diffusion distance for nitric oxide is calculated based on its half-life of 1 s in vivo, which results mostly from its diffusion into red blood cells. The diffusion distance for hydroxyl radical is about the same diameter as a small protein, or 10,000 times smaller than peroxynitrite. All of these estimates involve many approximations, but varying the estimated half-lives by 10-fold would only alter the diameters by the square root of 10 or by 3.2-fold.

Press Release

NOBELFÖRSAMLINGEN KAROLINSKA INSTITUTET
THE NOBEL ASSEMBLY AT KAROLINSKA INSTITUTET

October 12, 1998

The Nobel Assembly at Karolinska Institutet has today decided to award the Nobel Prize in Physiology or Medicine for 1998 jointly to

Robert F. Furchgott, Louis J. Ignarro and **Ferid Murad**

for their discoveries concerning "nitric oxide as a signalling molecule in the cardiovascular system".

Summary

Nitric oxide (NO) is a gas that transmits signals in the organism. Signal transmission by a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell represents an entirely new principle for signalling in biological systems. The discoverers of NO as a signal molecule are awarded this year's Nobel Prize.

Robert F Furchgott, pharmacologist in New York, studied the effect of drugs on blood vessels but often achieved contradictory results. The same drug sometimes caused a contraction and at other occasions a dilatation. Furchgott wondered if the variation could depend on whether the surface cells (the endothelium) inside the blood vessels were intact or damaged. In 1980, he demonstrated in an ingenious experiment that acetylcholine dilated blood vessels only if the endothelium was intact. He concluded that blood vessels are dilated because the endothelial cells produce an unknown signal molecule that makes vascular smooth muscle cells relax. He called this signal molecule EDRF, the endothelium-derived relaxing factor, and his findings led to a quest to identify the factor.

Ferid Murad, MD and pharmacologist now in Houston, analyzed how nitroglycerin and related vasodilating compounds act and discovered in 1977 that they release nitric oxide, which relaxes smooth muscle cells. He was fascinated by the concept that a gas could regulate important cellular functions and speculated that endogenous factors such as hormones might also act through NO. However, there was no experimental evidence to support this idea at the time.

a

着舌以通心氣。治中悪、急心痛、手足逆冷者、頃刻可殺人。
看其人指、爪青者是。
硝石 五銭匕　雄黄一銭匕
右二味、共爲極細末。啓病者舌、着散一匕於舌下、若有涎出、令病者随涎咽下、必愈。

Putting under the tongue to cause heart *qi* to flow freely

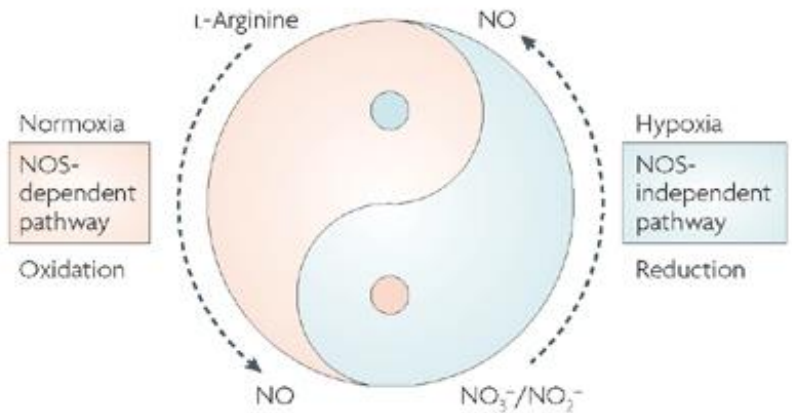
For treating symptoms such as struck by evil, acute heart pains and cold in the hands and feet which can kill a patient in an instant.

Look at the patient's fingers and those with greenish-black nails are such cases.

Take potassium nitrate (5 measures of a bi spoon) and arsenic sulphide (1 measure of a bi spoon) and combine the two into a fine powder. Lift the patient's tongue and sprinkle 1 measure of a bi spoon under the tongue. If saliva is produced have the patient swallow it.

This is a sure cure.

b

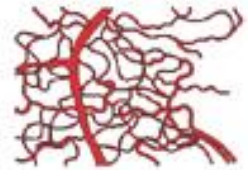


Nitrate and some nitrite from food

An active uptake of nitrate from the blood occurs in the salivary glands

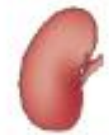
Bacteria in the oral cavity reduce nitrate to nitrite

Nitrate and nitrite in blood originate from the food and from systemic NO production

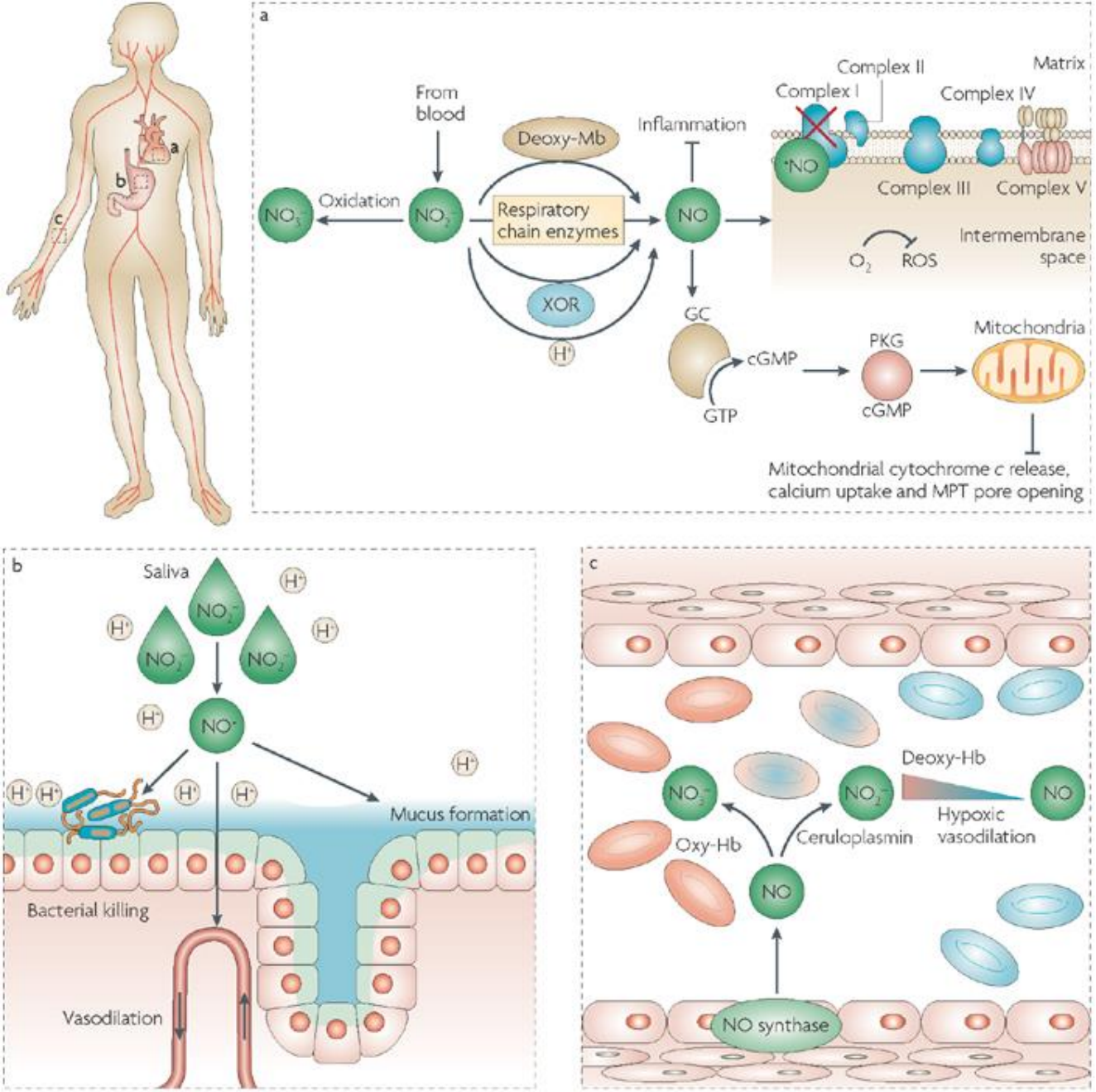


In the gastric acidic milieu, a non-enzymatic reduction of nitrite to NO occurs

Nitrate and remaining nitrite is absorbed in the intestine

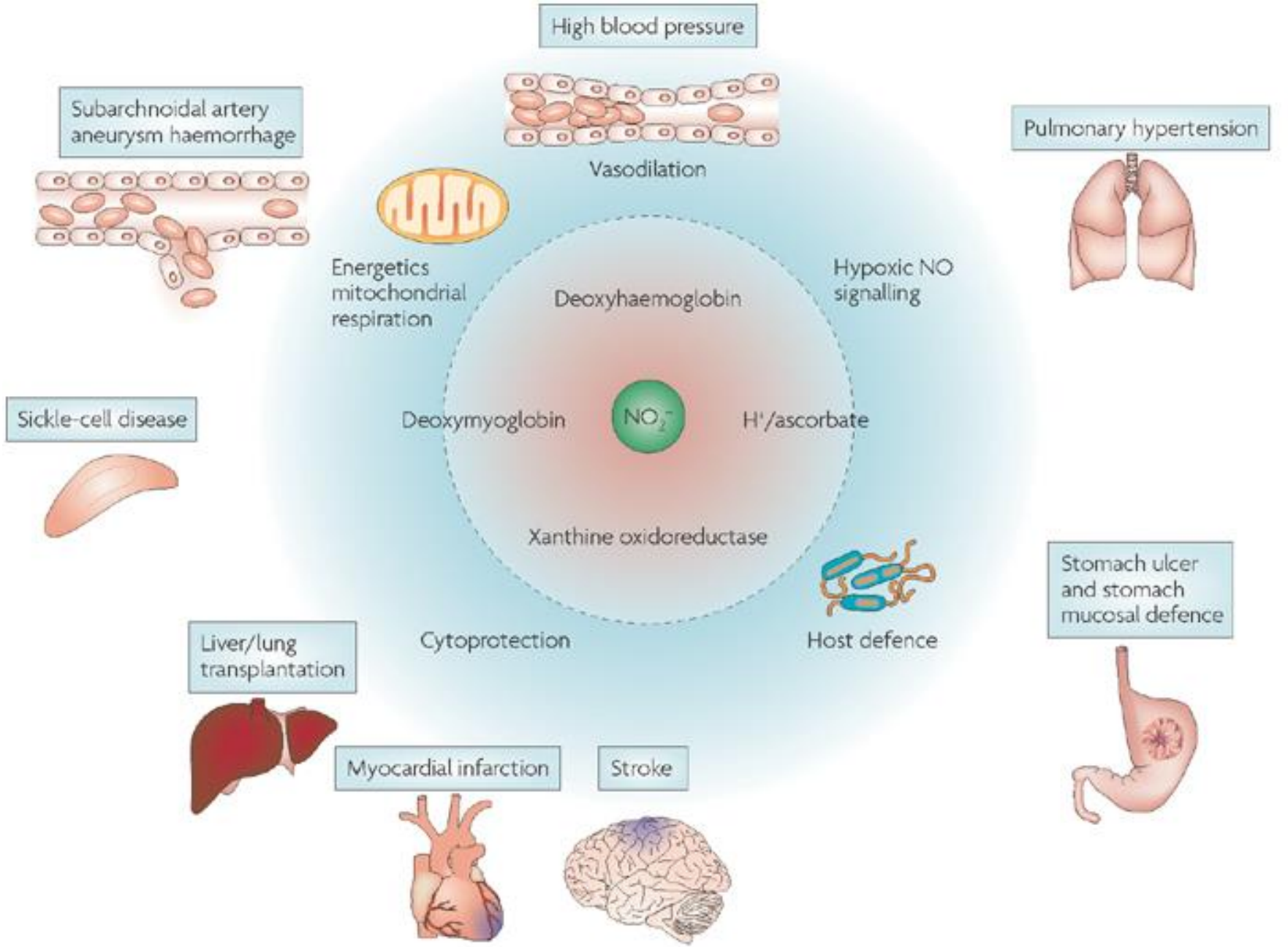


Nitrate is excreted by the kidneys



Nature Reviews | Drug Discovery

In the tissues, such as the heart, there are numerous pathways for the generation of NO from nitrite, all greatly potentiated during hypoxia, including xanthine oxidoreductase (XOR), deoxygenated myoglobin (deoxy-Mb), enzymes of the mitochondrial chain and protons. Nitrite-dependent NO formation and S-nitrosothiol formation can modulate inflammation, inhibit mitochondrial respiration and mitochondrial derived reactive oxygen species formation, and drive cyclic GMP-dependent signalling under anoxia. NO-dependent cytochrome c oxidase (complex IV) inhibition can also drive reactive oxygen species (ROS)-dependent signalling. b | The formation of bioactive nitric oxide (NO) from the inorganic anion nitrite is generally enhanced under acidic and reducing conditions. In the acidic gastric lumen, NO is generated non-enzymatically from nitrite in saliva after formation of nitrous acid (HNO_2) and then decomposition into NO and other reactive nitrogen oxides. This NO helps to kill pathogenic bacteria and it also stimulates mucosal blood flow and mucus generation, thereby enhancing gastric protection. Detrimental effects have also been suggested, including nitrite-dependent generation of nitrosamines with potentially carcinogenic effects. c | In the blood vessels, nitrite forms vasodilatory NO after a proposed reaction with deoxygenated haemoglobin (deoxy-Hb) and contributes to physiological hypoxic blood flow regulation. GC, guanylate cyclase; MPT, mitochondrial permeability transition; Oxy-Hb, oxygenated haemoglobin; PKG, protein kinase G.


High blood pressure
Vasodilation
Subarchnoidal artery aneurysm haemorrhage
Pulmonary hypertension
Energetics mitochondrial respiration
Hypoxic NO signalling
Deoxyhaemoglobin
Sickle-cell disease
Deoxymyoglobin
NO_2^-
H+/ascorbate
Xanthine oxidoreductase
Stomach ulcer and stomach mucosal defence
Liver/lung transplantation
Cytoprotection
Host defence
Myocardial infarction
Stroke

Cellular antioxidant system

- Non-enzymatic antioxidants - both lipophilic (e.g. tocopherols) and hydrophilic (e.g. ascorbate, GSH) compounds
- a system of antioxidant enzymes, which convert superoxide anion radical to hydrogen peroxide and water
  - SOD
  - CAT
  - Peroxidases + TRx + glutathione systems

SOD = superoxide dismutase

SOD isoform	Metal ion(s)	Molecular mass (kDa)	Assembly of subunits	Cellular localization
Cu-Zn-SOD (SOD1)	Cu^{2+} (catalytic activity) Zn^{2+} (enzyme stability)	88	homodimer	cytoplasm, nucleus, mitochondrial intermembrane space, lysosomes
Mn-SOD (SOD2)	Mn^{2+} (catalytic activity)	32	homotetramer	mitochondrial matrix
Cu-Zn-SOD (SOD3, ECSOD)	Cu^{2+} (catalytic activity) Zn^{2+} (enzyme stability)	132	homotetramer, resp. homotetrameric glycoprotein	plasma membrane, extracellular fluid

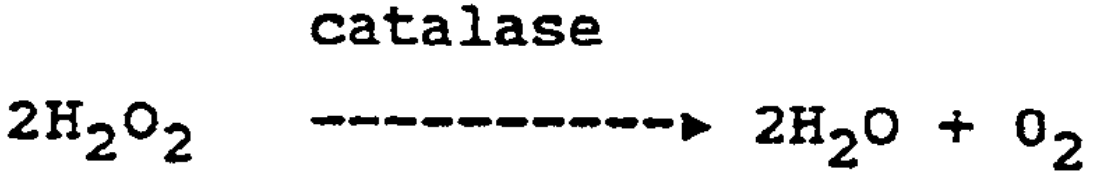
Expression of SOD regulated by several transcription factors = nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein 1 (AP-1), activator protein 2 (AP-2), specificity protein 1 (Sp1), or CCAAT-enhancer-binding protein (C/EBP).

Table 1. Common Intra- and Extracellular Stimuli Affecting Expression of SOD1, SOD2, and SOD3

	SOD1	SOD2	SOD3
Proximal promoter			
TATA or CAAT box	Yes	No	No
GC-rich region	Yes	Yes	Yes
Proinflammatory cytokines			
TNF-α	↔ Rat lung [146] ↔ Human vascular smooth muscle [147]	↑ Rat smooth muscle [148] ↑ Human pulmonary adenocarcinoma cells [149] ↑ Human vascular smooth muscle [147]	↓ Human fibroblast [122] ↓ Human vascular smooth muscle [147]
IL-1β	↔ Rat lung [150]	↑ Rat smooth muscle [148] ↑ Rat glial and neuronal cells [151]	
IL-1α	↔ Human vascular smooth muscle [147]	↑ Human vascular smooth muscle [147]	↑ Human fibroblast [122] ↑ Rat sertoli cells [123]
IFN-γ	↔ Human vascular smooth muscle [147]	↑ Human vascular smooth muscle [147] ↑ Human lung adenocarcinoma [107] ↑ Rat glial and neuronal cells [151]	↑ Human fibroblasts [122] ↑ Human vascular smooth muscle [147] ↔ Rat sertoli cells [123]
TNF-α + IFN-γ		↑ Murine fibrosarcoma [152] ↑ Human lung adenocarcinoma [107]	↑ Rat pneumocytes [124]
Growth factors			
TGF-β		↓ Murine fibrosarcoma [152]	↔ Human fibroblast [122]
Fibroblast and epidermal growth factors	↑ Rat fibroblasts [153]		↓ Human vascular smooth muscle [125] ↔ Rat sertoli cells [123]
Platelet-derived growth factor		↑ NIH 3T3 [113]	↓ Human vascular smooth muscle [125]
GM-CSF and GH			↔ Human fibroblast [122]
Nitric oxide	↑ Human keratinocytes [87]	↑ Rat vascular smooth muscle [154]	↑ Human aortic smooth muscle [126]
Ozone	↑ Rat lung [86]	↑ Rat lung [86]	
Lipopolysaccharide	↔ Pulmonary epithelial cells [106] ↓ Rat astrocytes [155]	↑ Rat smooth muscle [148] ↑ Pulmonary epithelial cells [106] ↑ Rat glial and neuronal cells [151]	↔ Human fibroblasts [122]
cAMP			↑ Rat glioma [128]
TPA	↑ HeLa cells [23]	↑ Human lung adenocarcinoma [110] ↑ HeLa cells [156]	
Angiotensin II	↔ Mouse aorta [127]		↑ Human vascular smooth muscle [125] ↑ Mouse aorta [127]

GM-CSF = granulocyte-macrophage colony-stimulating factor; GH = growth hormone.

CAT = catalase



- enzymes that catalyse decomposition of hydrogen peroxide to water and oxygen, therefore, it can prevent formation of hydroxyl radicals via the Fenton reaction
- CAT plays an important role in removing higher intracellular concentrations of hydrogen peroxide
- CAT contributes to ethanol metabolism in the body after ingestion of alcohol, but it only breaks down a small fraction of the alcohol
- CAT seems to have a specific red cell membrane location that may facilitate both catalatic and peroxidatic roles in erythrocytes that do not contain classical dehydrogenase systems
- Three subgroups of CAT have been classified:
  - Two of them are heme-containing enzymes: monofunctional heme CAT (typical CAT) and catalase-peroxidase (only fungi among eukaryotic organisms, activity 2 or 3 orders of magnitude less than that of a typical CAT)
  - the third one is (nonheme) manganese CAT (only several species with manganese CAT have been identified)

PRxs = Peroxiredoxins

- = cysteine-dependent peroxide reductases
- family of enzymes that use thioredoxin (TRx) to recharge after reducing hydrogen peroxide to water
- one of the most important mechanisms of hydrogen peroxide detoxification !
- E.g. human erythrocytes:
  - PRx2, is one of the most abundant protein and plays a preponderant role in maintaining low endogenous levels of hydrogen peroxide produced by haemoglobin autoxidation

GPx = glutathione peroxidase

- family of enzymes (8 isoforms) that catalyse conversion of lipid hydroperoxides to corresponding alcohols and hydrogen peroxide to water with the use of GSH as a typical co-substrate in the reaction

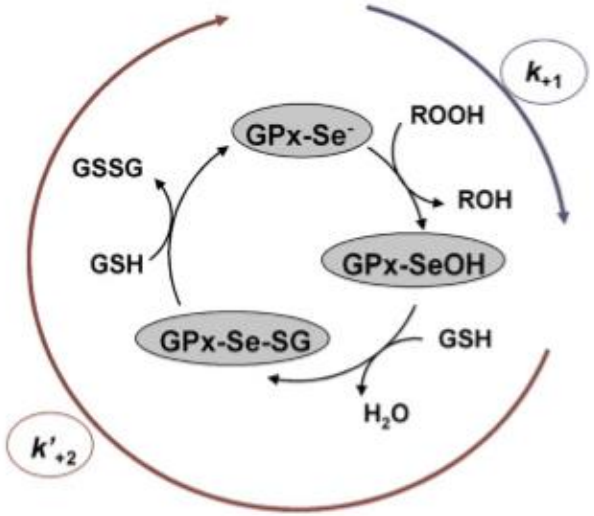
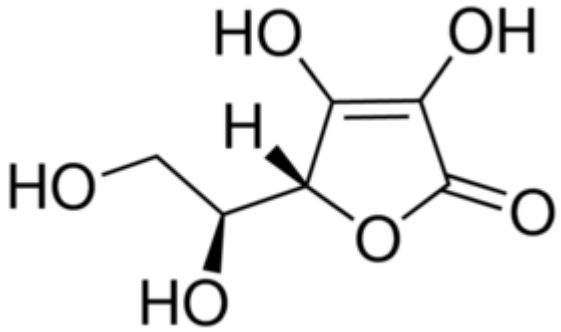


Fig. 1. Catalytic cycle of glutathione peroxidases. This basic catalytical scheme has been evaluated for GPx1 and is valid also for GPx3 and 4. In the peroxidatic part, selenol in GPx becomes oxidized to selenenic acid by a hydroperoxide (ROOH) (Eq. (1)). The respective rate constant is k_{+1}. The entire reductive part is characterized by k'_{+2}. Here the first GSH forms a selenadisulfide with the selenenic acid, the oxygen is removed as H_2O. The second GSH reduces the selenadisulfide by a thiol–disulfide exchange. Thereby GSSG is released and the enzyme regenerated to the selenol form which is now ready for the next cycle. For more details see text and references therein.

Characterization of human isoforms of GPxs – structural features, localization on chromosomes, reducing and oxidizing substrates, and their tissue localizations. ChOOH, cholesterol hydroperoxide; CEOOH cholesterol ester hydroperoxide; DTT, dithiotreitol; GRx, glutaredoxin; LOOH, lipid (fatty acid) hydroperoxide; PLOOH, phospholipid hydroperoxide of different classes; ROOH, small synthetic hydroperoxides (e.g. cumene hydroperoxide and *tert*-butyl-hydroperoxide); TRx, thioredoxin; PDI, protein disulfide isomerase; n.d. not determined so far. Adapted from (Brigelius-Flohe and Maiorino, 2013) and modified.

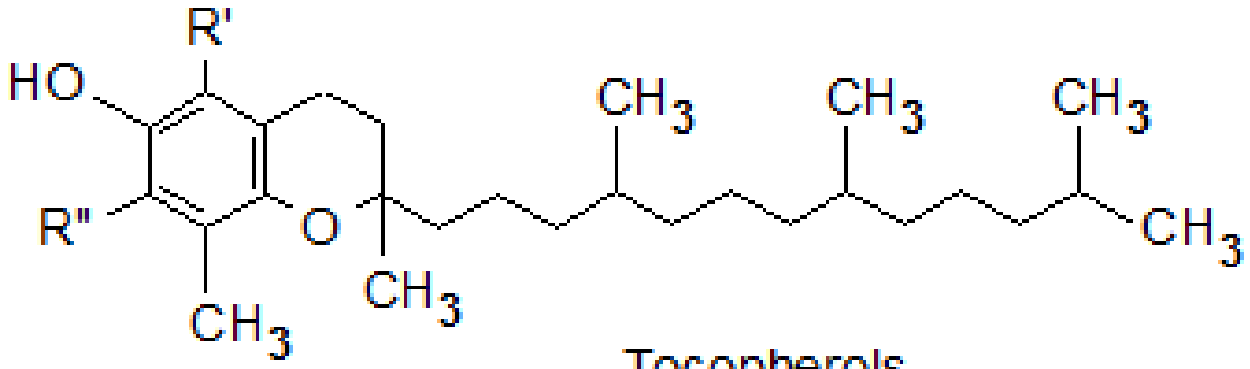
Ascorbic acid



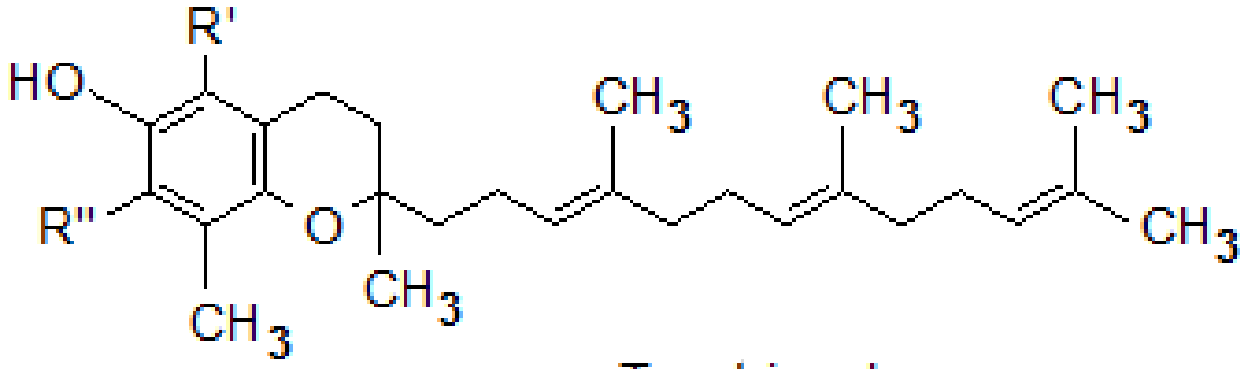
- intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging oxygen free radicals
- it converts vitamin E free radicals back to vitamin E
- vitamin C possesses proactive role against some types of cancer and also demonstrated the immunomodulatory effects enhancing host defence; generally vitamin C is considered supportive for immune responsiveness
- vitamin C is transported in the form of dehydroxyascorbic acid (DHA) into various cells through glucose transporters
- it is maintained in its reduced form by reaction with GSH, which can be catalysed by protein disulfide isomerase and glutaredoxins

Tocopherols and tocotrienols – vitamin E

- Vitamin E is formed by a set of eight related tocopherols and tocotrienols – α-, β-, γ-, δ-forms of tocopherols and tocotrienols that vary only in the number and position of methyl substituents attached to the chromanol ring
- it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction
- Antioxidant activity of tocopherols and tocotrienols strongly depends on the degree of methylation in the aromatic ring (α > β = γ > δ), size of the heterocyclic ring, stereochemistry at position 2, and finally length of the prenyl chain (optimum between 11 and 13 carbons)



Tocopherols



Tocotrienols

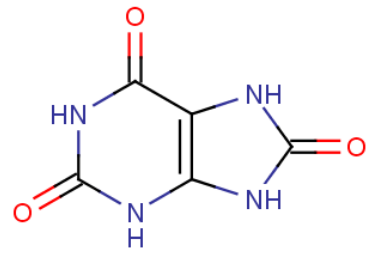
α: R' = CH_3, R" = CH_3

β: R' = CH_3, R" = H

γ: R' = H, R" = CH_3

δ: R' = H, R" = H

Uric acid



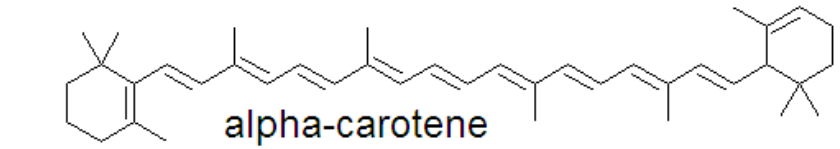
- final product of purine metabolism
- accounts for roughly half antioxidant ability of plasma
- uric acid is similarly produced in the liver, adipose tissue and muscle and is primarily excreted through the urinary tract
- high uric acid levels regulate the oxidative stress, inflammation and enzymes associated with glucose and lipid metabolism, suggesting a mechanism for the impairment of metabolic homeostasis
- possible neuroprotective properties based on mechanisms involving chelating Fenton reaction transitional metals, antioxidant quenching of superoxide and hydroxyl free radicals, and as an electron donor that increases antioxidant enzyme activity, e.g. SOD

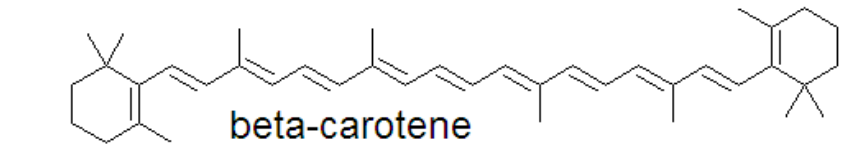
Carotenoids

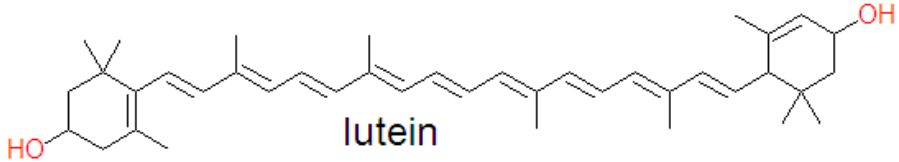
- Carotenoids are pigments found in plants
- Primarily, β-carotene has been found to react with peroxyl, hydroxyl, and superoxide anion radicals
- Carotenoids exhibit their antioxidant effects in low oxygen partial pressure, but may have pro-oxidant effects at higher oxygen concentrations
- genetic polymorphisms in the beta-carotene oxygenase 1 (*BCO1*) = *BCO1* encodes an enzyme that is expressed in the intestine and converts provitamin A to vitamin A-aldehyde
- carotenoids and retinoic acids are capable of regulating transcription factors - β-carotene inhibits the oxidant-induced NF-κB activation via thiol groups of both IkB kinase and p65 and interleukin IL-6 and tumour necrosis factor-α (TNF-α) production
  - inhibition the production of inflammatory cytokines, such as IL-8 or prostaglandin E2
  - blocking oxidative stress by interacting with the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway, enhancing its translocation into the nucleus, and activating phase II enzymes and antioxidants, such as GST

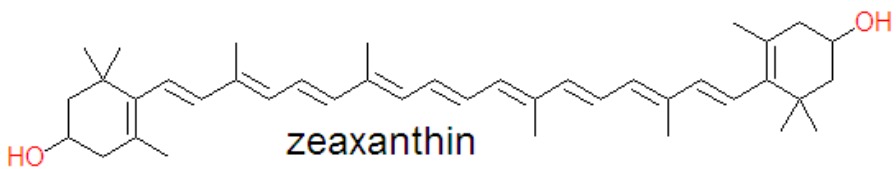
NATURALLY OCCURING CAROTENOIDS

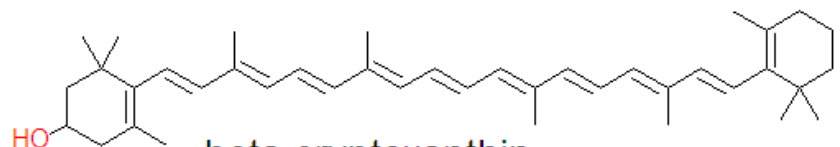
- **Hydro-Carbons**
  - Lycopersene, Phytofluene, Healhydro-lycopene, Torulene, α-zeacarotene
- **Alcohols**
  - Lutein, Zeaxanthin, Alloxanthin, Gazania-xanthin, Lycoxanthin, Loroxanthin, Saproxanthin
- **Glycoside, Ethers And Aldehydes**
  - Oscillaxanthin, Phlei-xanthophyll, Rhodovibrin, Speroidin & Rhodopinal, Warmingone, Torularhodin-aldehyde
- **Epoxides**
  - Mutato-xanthin, Citroxanthin, Luteoxanthin, Foliachrome, Trollichrome, Vaucheria-xanthin
- **Ketones**
  - Astaxanthin, Canthaxanthi, Capsanthin, Capsorubin, Cryptocapsin, Flexixanthin
- **Esters Of Alcohol**
  - Fucoxanthin, Isofuco-xanthin, Physalien, Astacein, Siphonein
- **Higher Carote-noids**
  - Nonapreno-xanthin, Decapreno-xanthin, Bacterio-ruberin
- **Apo-car otenoids**
  - Apo-2-lycopenal, Bixin, Citrana-xanthin, Sintaxanthin

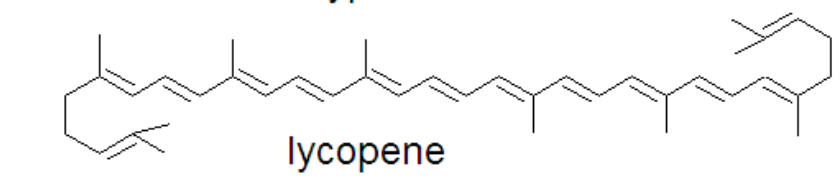








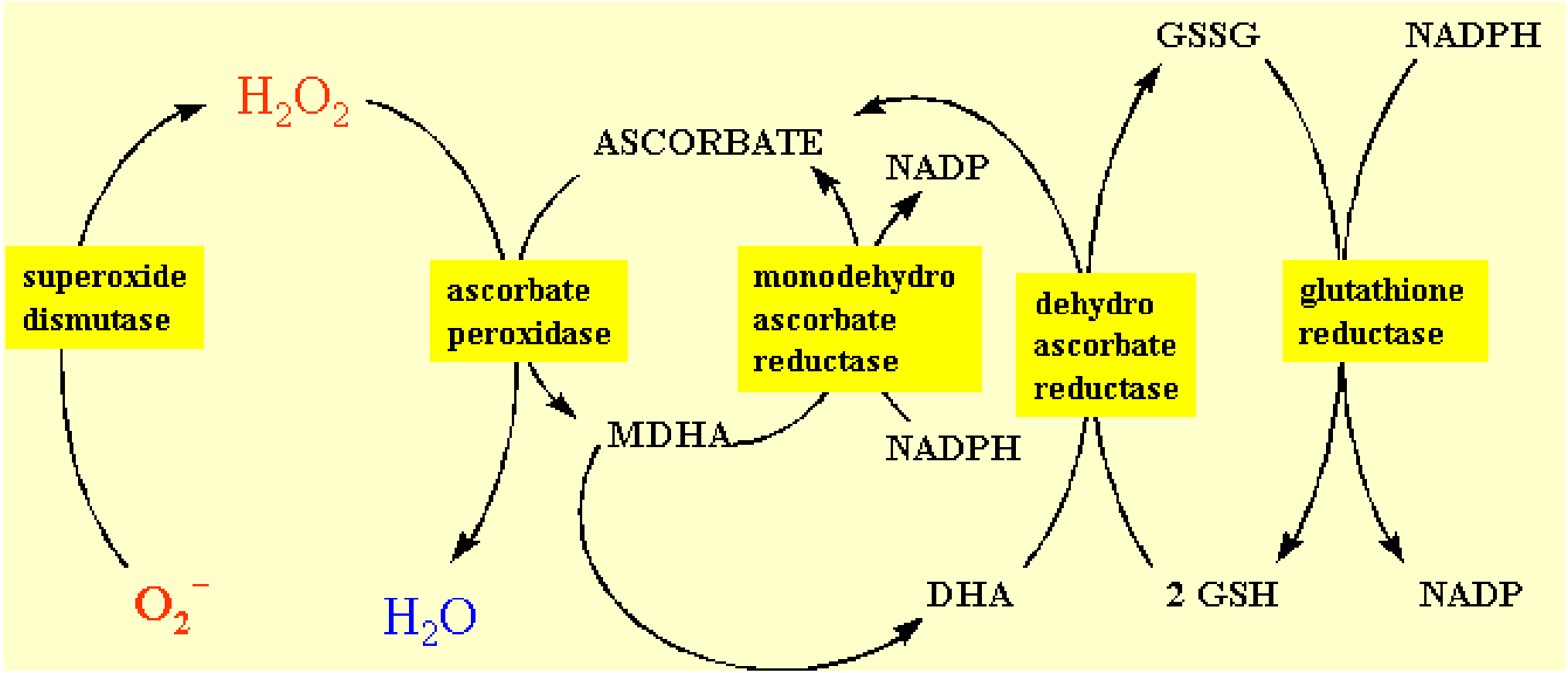




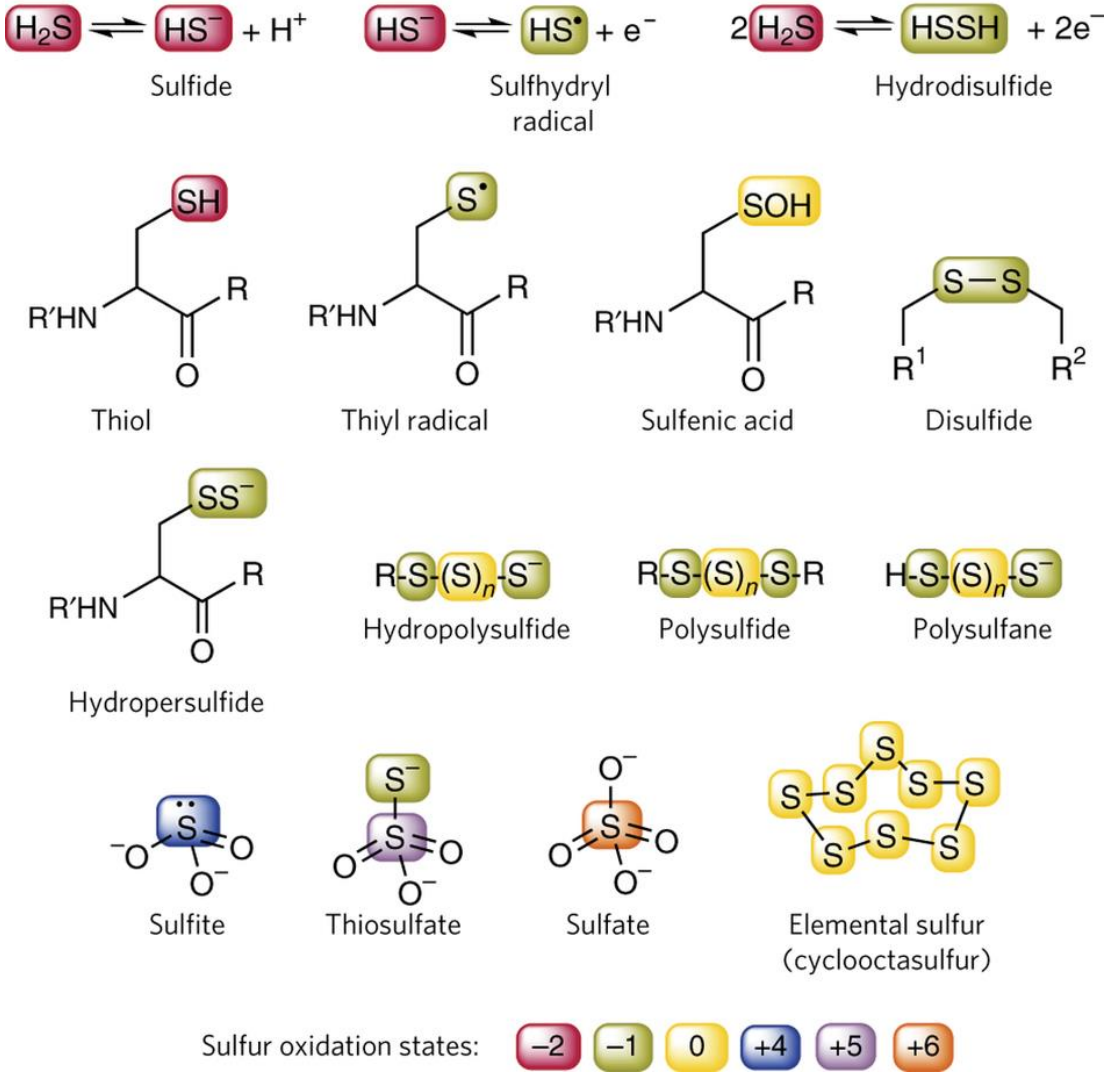
Glutathione - GSH

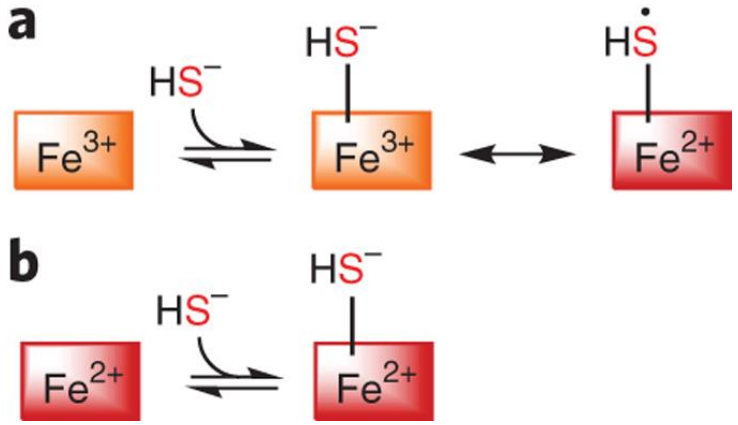
- a cysteine-containing peptide found in most forms of aerobic life and is highly abundant in all cell compartments and is the major soluble antioxidant
  - a part of glutathione system includes GSH, GR, GPxs, and GST
- thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced - GSH/GSSG ratio is a major determinant of oxidative stress
- it detoxifies H_2O_2 and lipid peroxides via action of GPxs
- GSH donates protons to membrane lipids and protects them from oxidant attacks
- GSH protects cells against apoptosis by interacting with pro-apoptotic and anti-apoptotic signalling pathways
- It also regulates and activates several transcription factors, such as AP-1, NF-κB, and Sp-1

Glutathione – ascorbate cycle

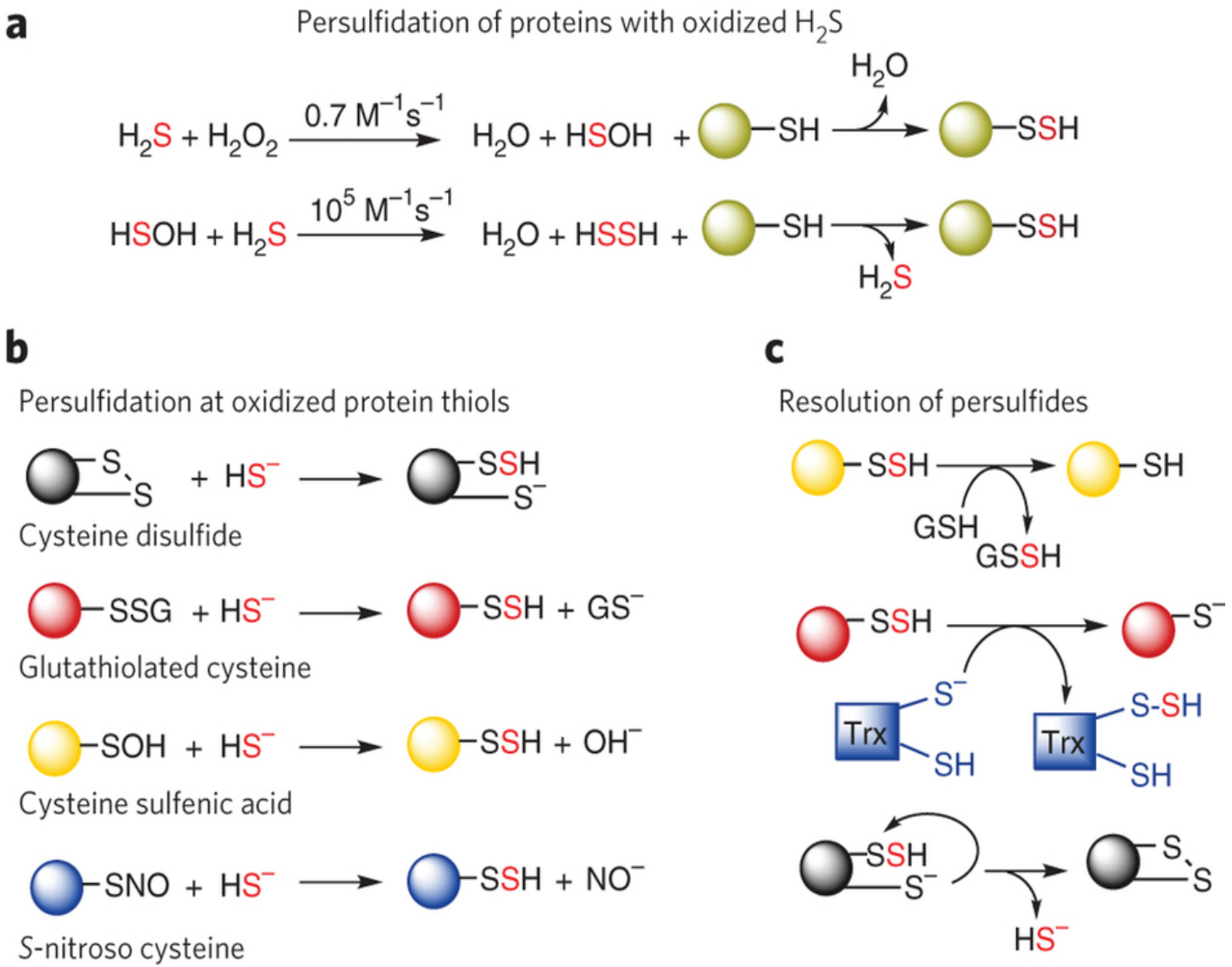


Reactive sulphur species

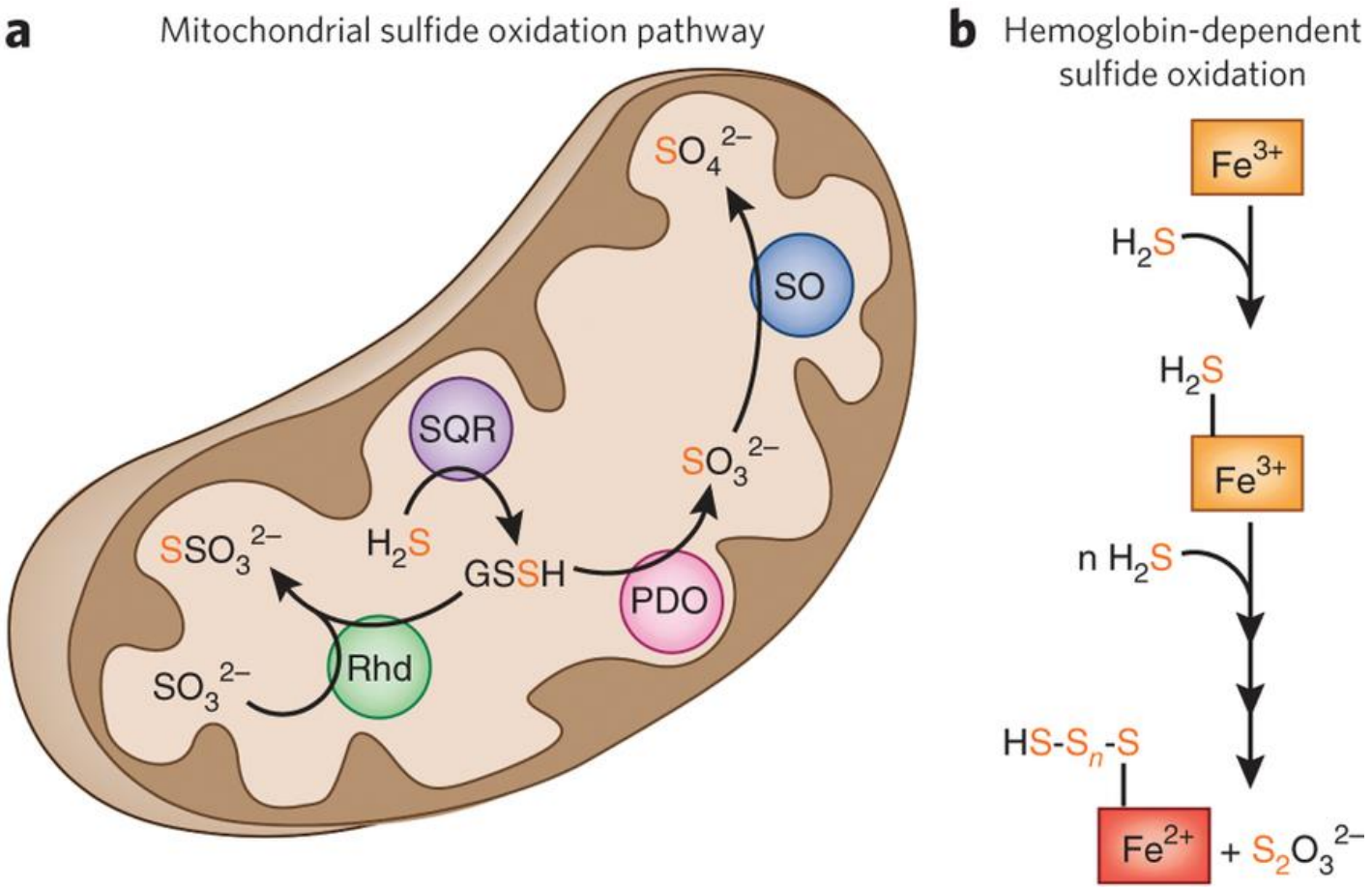




Binding of sulfide anion to ferric heme results in the formation of ferric sulfide complex, which, depending on the polarity of the distal heme pocket, could lead to heme iron reduction and formation of the sulfur radical. Depending on the heme protein, either H_2S or HS^- can bind ferric heme. (**b**) In principle, H_2S can also bind ferrous hemes, as seen with model porphyrinate complexes.



H2S is relatively stable and is oxidized slowly by H2O2 to give HSOH. Either HSOH or HSSH formed from HSOH in the presence of a second equivalent of H2S can be attacked by a reactive cysteine on a protein to generate the persulfide modification. The bimolecular rate constants for HSOH and HSSH formation in solution at pH 7.4 and 37 °C are noted. (b) Persulfidation can result from the nucleophilic attack of a sulfide anion on an oxidized protein thiol (such as disulfide, mixed disulfide, cysteine sulfenic acid or S-nitrosylated cysteine). (c) Persulfide modifications on proteins are reversible and, unless sequestered, labile. They can be removed via persulfide interchange reactions involving glutathione (GSH), thioredoxin (Trx) or a cysteine on the same or a different protein. In all cases, the product is ultimately H2S, formed upon reduction of the persulfide moiety by either a second mole of GSH or the NADPH–thioredoxin reductase system.



The canonical sulfide oxidation pathway found in most tissues resides in the mitochondrion and involves four enzymes. In the first step of the pathway, sulfide quinone oxidoreductase (SQR) oxidizes sulfide to persulfide, which is transferred from the active site of SQR to a small molecule acceptor, such as glutathione (GSH). The glutathione persulfide (GSSH) product can be oxidized by persulfide dioxygenase (PDO) to sulfite or can be used in a sulfurtransferase reaction catalyzed by rhodanese (Rhd) in the presence of sulfite, to form thiosulfate. In the final step, sulfite is oxidized to sulfate by sulfite oxidase (SO). (b) Heme-dependent sulfide oxidation pathway. An alternative pathway for sulfide oxidation involves ferric heme–dependent conversion of H2S to a mixture of thiosulfate and polysulfides. This newly discovered mechanism has been established for human hemoglobin and could be an activity of other heme proteins as well. For clarity, the fate of the H2S sulfur atom is highlighted in red and other reactants and reaction stoichiometries are omitted.

H_2S in cellular signalling

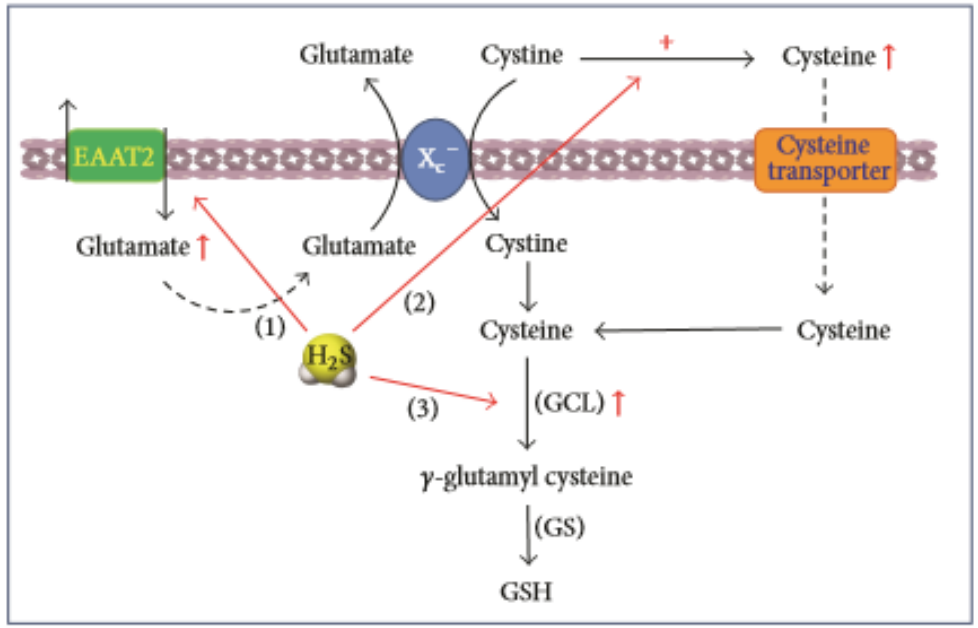


FIGURE 3: H_2S increases intracellular GSH synthesis. Cellular GSH is mainly synthesized from cysteine. (1) H_2S increases EAAT2-mediated glutamate uptake, which thereby increases cystine transportation through cystine/glutamate antiport system (X_c^-). (2) Intracellular H_2S is released into extracellular space and reduces cystine into cysteine, which would be efficiently imported into cells through a cysteine transporter distinct from system X_c^-. These two pathways provide more substrate to produce GSH. (3) H_2S increases glutamate cysteine ligase (GCL) expression and promotes GSH synthesis.

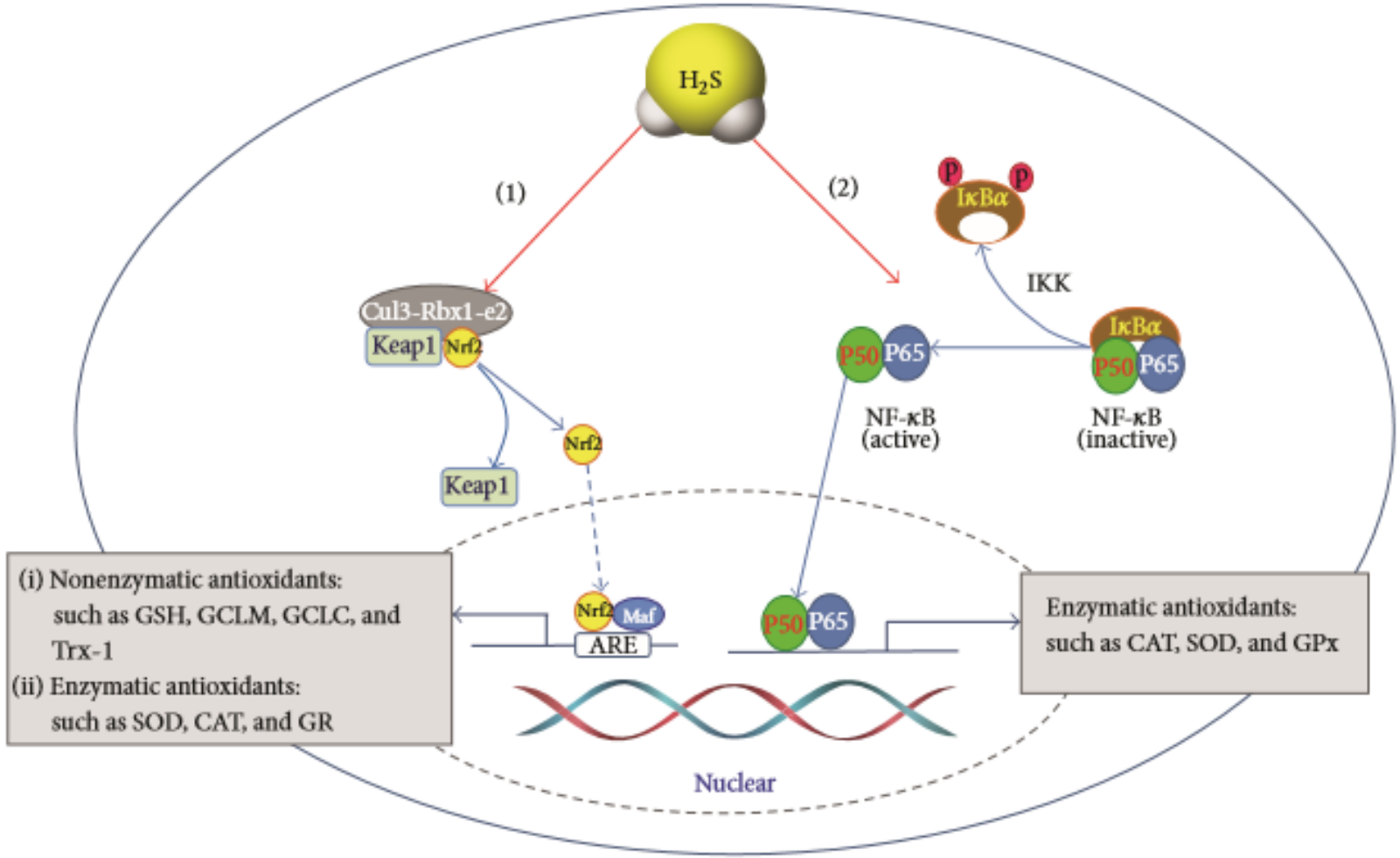


FIGURE 4: Effect of H_2S on intracellular enzymatic and nonenzymatic antioxidant production. (1) H_2S activates Nrf2, which translocates to nuclear, binds to ARE, and upregulates enzymatic and nonenzymatic antioxidant production. (2) H_2S stimulates NF-κB signaling, which further upregulates the expression of numerous genes including SOD, CAT, and GPx.

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Sodium/calcium exchanger is involved in apoptosis induced by H_2S in tumor cells through decreased levels of intracellular pH

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ABSTRACT

We explored possibility that sodium/calcium exchanger 1 (NCX1) is involved in pH modulation and apoptosis induction in GYY4137 treated cells. We have shown that although 10 days treatment with GYY4137 did not significantly decreased volume of tumors induced by colorectal cancer DLD1 cells in nude mice, it already induced apoptosis in these tumors. Treatment of DLD1 and ovarian cancer A2780 cells with GYY4137 resulted in intracellular acidification in a concentration-dependent manner. We observed increased mRNA and protein expression of both, NCX1 and sodium/hydrogen exchanger 1 (NHE1) in DLD1-induced tumors from GYY4137-treated mice. NCX1 was coupled with NHE1 in A2780 and DLD1 cells and this complex partially disintegrated after GYY4137 treatment. We proposed that intracellular acidification is due to uncoupling of NCX1/NHE1 complex rather than blocking of the reverse mode of NCX1, probably due to internalization of NHE1. Results might contribute to understanding molecular mechanism of H_2S–induced apoptosis in tumor cells.

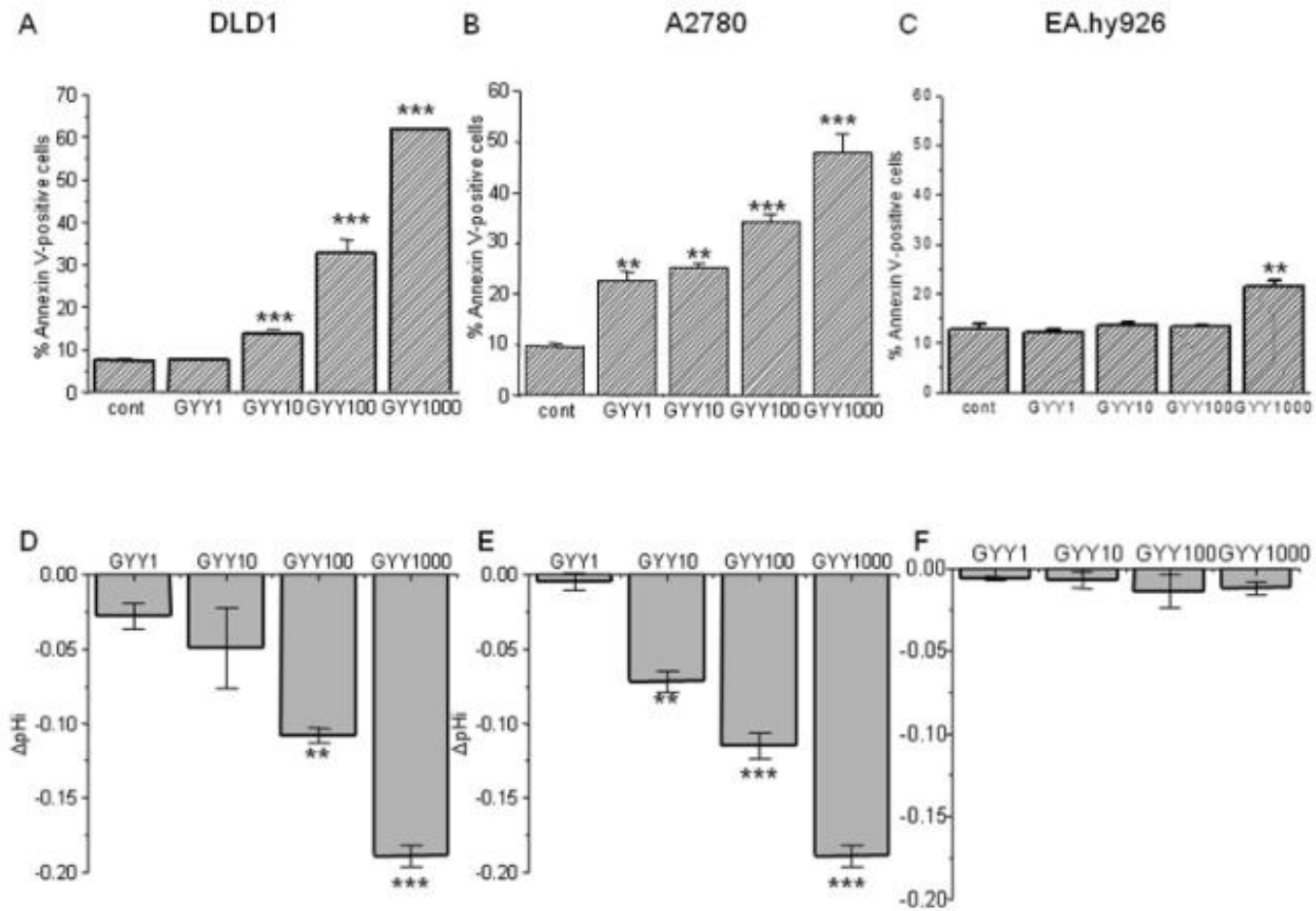


Fig. 1. Concentration-dependent effect of the GYY4137 on apoptosis induction and intracellular pH in DLD1 (A, D), A2780 (B, E) and EA.Hy926 (C, F) cells. Apoptosis was detected by Annexin V-FLUOS in cells treated with 1 μmol/L, 10 μmol/L, 100 μmol/L and 1000 μmol/L GYY4137 for 24 h (A, B, C). As expected, concentration-dependent increase in apoptosis due to GYY4137 treatment was observed in DLD1 and A2780 tumor cells (A, B), but not in non-tumor endothelial cell line EA.hy926 (C). Also, intracellular pH decreased when DLD1 and A2780 cells were treated with 1 μmol/L, 10 μmol/L, 100 μmol/L and 1000 μmol/L GYY4137 for 24 h (D, E). In EA.hy926 cells, no changes in the intracellular pH were observed after GYY4137 treatment (F). Each column is displayed as mean ± S.E.M and represents an average of three independent cultivations, each performed in triplicates. Statistical significance ** compared to corresponding control represents $p < 0.01$ and ***$p < 0.001$.

ROS in cellular signaling

- MAPK signalling pathway
  - regulates many physiological processes:
    - bone development and homeostasis of bone tissue
    - regeneration of connective tissue
    - epidermal homeostasis
    - haematopoiesis
    - circadian rhythms (in this case, MAPK pathways can function as inputs allowing the endogenous clock to entrain to 24 h environmental cycles)
  - It also modulates effect of some hormones, e.g. glucocorticoids, on target tissues and participates in regulation of homeostasis of glucose

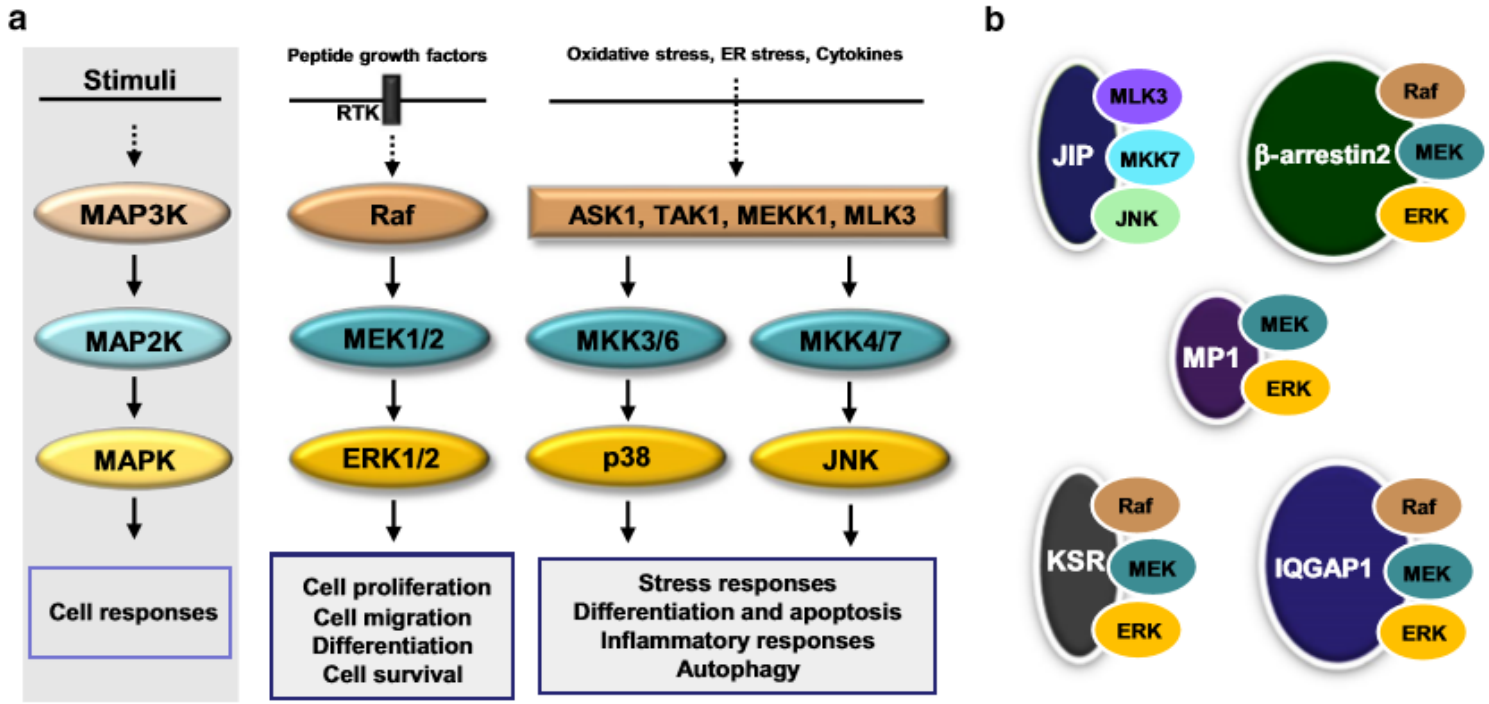
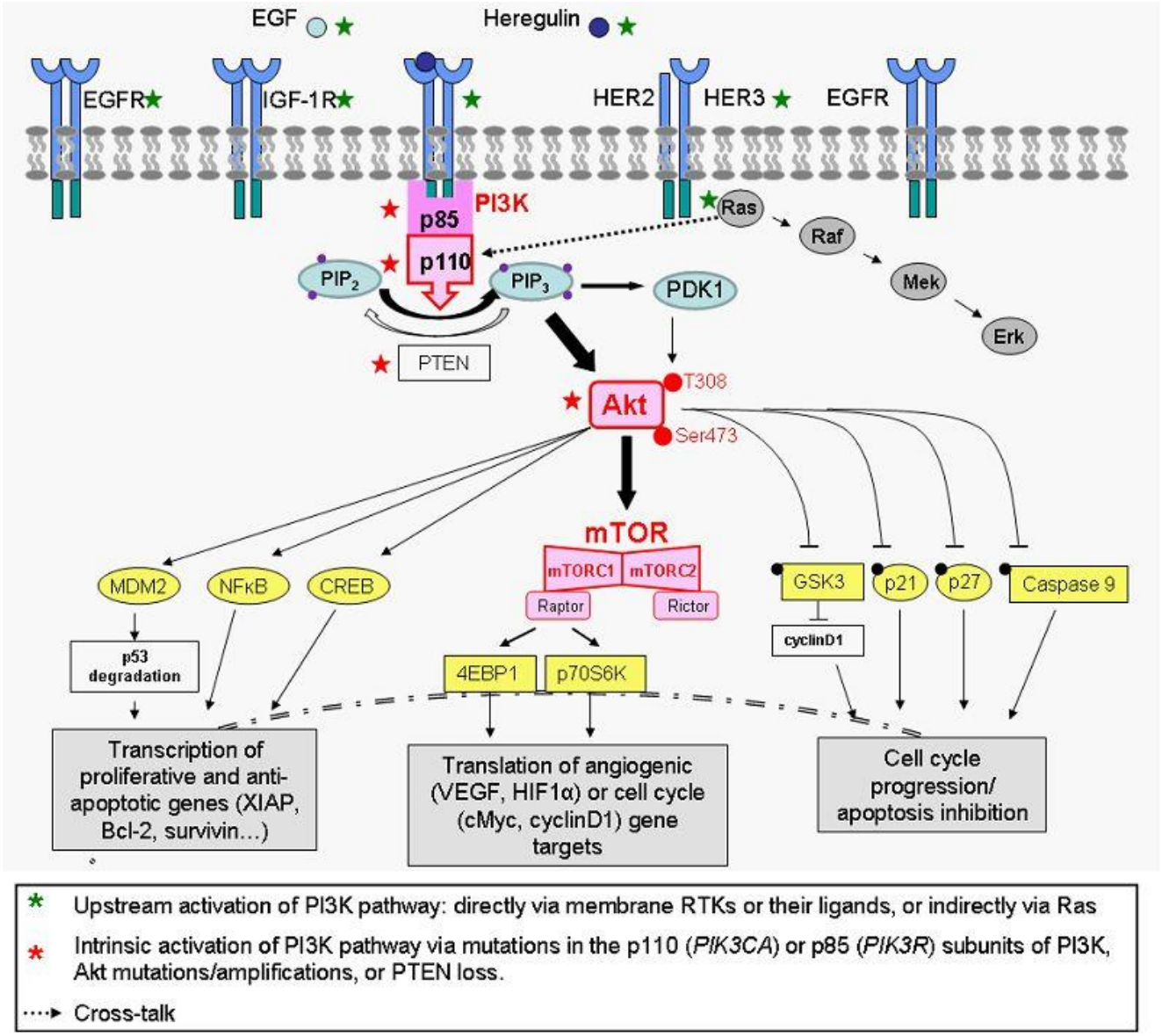
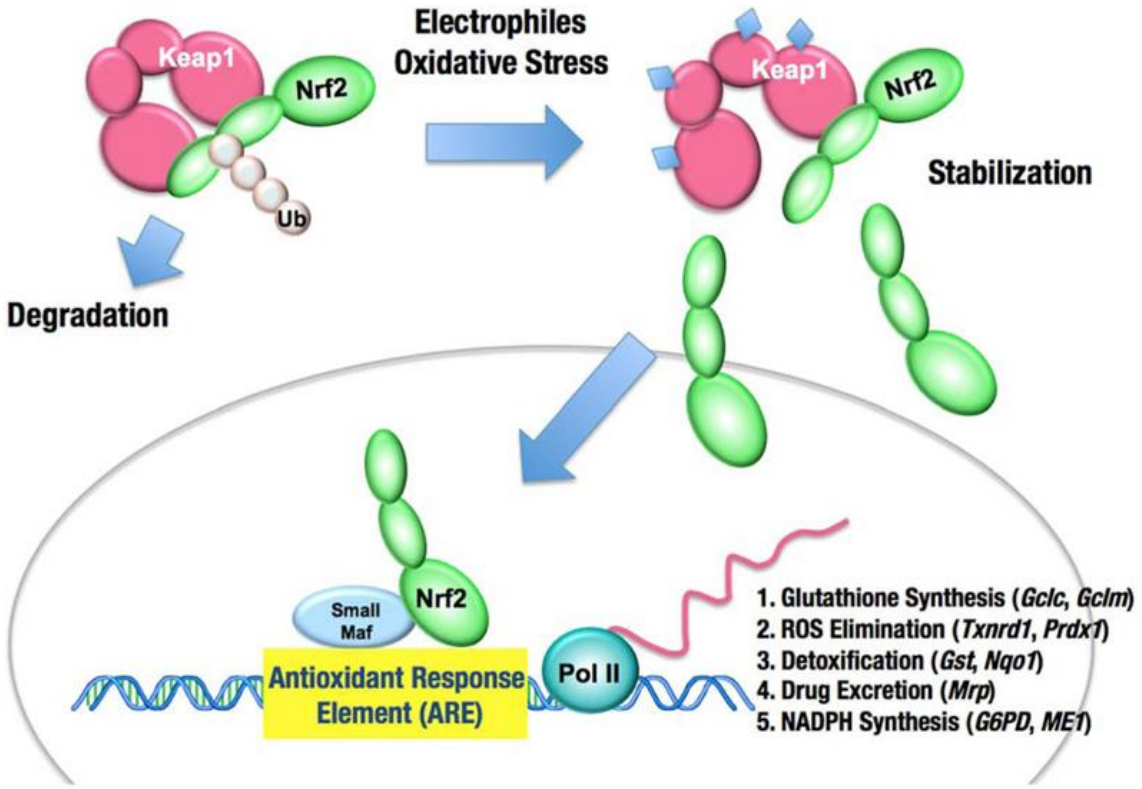


Fig. 1 Mitogen-activated protein kinase (MAPK) signaling pathways. **a** The MAPK pathways mediate intracellular signaling triggered by extracellular stimuli such as growth factors and cytokines as well as by intracellular stimuli such as oxidative and endoplasmic reticulum (ER) stress. The MAPK signaling cascades are hierarchies of three kinase components: a MAPK kinase kinase (MAP3K), a MAPK kinase (MAP2K), and a MAPK. MAP3Ks phosphorylate and activate MAP2Ks, which then phosphorylate and activate MAPKs. Activated MAPKs phosphorylate diverse molecules including transcription factors such as c-Jun, c-Myc, and ATF2 and thereby give rise to various cellular responses including proliferation, migration, differentiation, survival or apoptosis, autophagy, and inflammatory reactions. The MAPKs in mammals consist of c-Jun NH_2-terminal kinase (JNK), p38 MAPK, and extracellular signal-regulated kinase (ERK). In the ERK signaling pathway ERK1/2 is activated by MEK1/2, which is activated by Raf. Raf is activated by Ras, which is recruited to the plasma membrane as a result of receptor tyrosine kinase (RTK) activation. In the p38 MAPK pathway, MKK3 and MKK6 act as MAP2Ks and are activated by MAP3Ks such as ASK1, TAK1, MEKK1, and MLK3. These MAP3Ks also function in the JNK pathway, in which they target the MAP2Ks MKK4 (SEK1) or MKK7. **b** The MAPK signaling pathways are activated as a result either of direct interaction between the kinase components or of the formation of a signaling complex by multiple kinases centered on a scaffold protein. Such scaffold proteins include JIP for the JNK signaling pathway as well as KSR, MP1, β-arrestin, and IQGAP1 for the ERK signaling pathway

- PI3K signalling pathway
  - PI3K/Akt/mTOR pathway regulates cell cycle in response to growth factors, hormone, and cytokine stimulations and primarily is involved in the regulation of cellular quiescence, proliferation, cancer, and longevity
  - PI3K pathway regulates several physiological processes:
    - immune functions, mainly anti-viral and anti-tumour responses mediated by natural killer cells
    - activity of ion channels, which influences neuronal excitability and synaptic plasticity
    - regeneration of neural tissue
    - insulin-mediated cardioprotection
    - regulation of ovarian function including quiescence, activation, and survival of primordial follicles, granulosa cell proliferation and differentiation, and meiotic maturation of oocytes



- Nrf2 and Ref1-mediated redox cellular signaling
  - Nrf2 and Ref1-mediated redox cellular signalling is important in antioxidant signalling in order to prevent oxidative stress
  - induction of many cytoprotective enzymes in response to oxidation stress is regulated primarily at the transcriptional level
  - this transcriptional response is mediated by a *cis*-acting element termed antioxidant response element (ARE) initially found in the promoters of genes encoding the detoxification enzymes, such as GST and NADPH quinone oxidoreductase-1



Signalling pathway	Signal molecules	Molecules modified by ROS and subsequent processes	Cell responses
MAPK - ERK1/2 - JNK/p38 - ERK5	Cytokines Growth factors Hormones Genotoxic stress Oxidative stress Abiotic stress stimuli	ASK1 (homo-oligomerization): 1. 2 Cys residues and creation disufhide bond (Cys32-Cys35) 2. oligomerization and autophosphorylation 3. TRAF binding ASK1 (hetero-oligomerization) ASK1/ASK2	Non-apoptic: - Cell differentiation - Immune signalling
		PKG* 1. Oxidation of Cys42 by H_2O_2 in PKG1α (redox sensor) 2. Creation of homodimer via disulfide bonds	Regulation of MAPK cascade?
		PKC* 1. Creation of intramolecular disulfide bonds	
		PKA* 1. Activation via redox mechanism	
		JKN-inactivating phosphatases Inactivation of phosphatases involved in p38 pathway 1. Reversible oxidation of catalytic Cys to sulfenic acid 2. Possible role of thioredoxin or glutathione in reducing sulfenic acid residues and reversing the oxidative inactivation	Transducing and sustaining growth factor signals - Apoptosis - Autophagy - Inflammation? - Cell cycle control - Cell differentiation

The cellular signalling pathways in which ROS play key role. The table summarizes individual signalling pathways, signal molecules involved in these pathways, molecules that are modified by ROS and processes that are subsequently initiated, and cell responses. ARE - antioxidant response element, ATM - ataxia–telangiectasia mutated, EGF - epidermal growth factor, IRP-1/2 - iron regulatory protein-1 and -2, IRE - iron-responsive element, MAPK - mitogen-activated protein kinase cascade. NGF - nerve growth factor, Nrf2 – nuclear factor-like 2, PI3K - phosphatidylinositol-4,5-bisphosphate 3-kinase, PDGF - platelet-derived growth factor, PKA – protein kinase A, PKC – protein kinase C, PKG – protein kinase G, PTEN - phosphatase and tensin homolog, Ref1 – redox factor 1, TfR1 - transferrin receptor 1, VEGF - vascular endothelial growth factor. G. * - activation of MAPK signalling. According to citations in the chapter.

PI3K	EGF PDGF NGF Insulin VEGF	PTEN phosphatase 1. reversible redox regulation (inactivation) by ROS generated by growth factor stimulation 2. PTEN inactivation by H_2O_2 3. disulfide bond formation between Cys124 and Cys71 in catalytic domain 4. Possible role of peroxiredoxin 3 in reversible reaction 5. Note: PTEN knockdown enhances transcription of ARE-regulated antioxidant genes	Regulation of: - Cell cycle - Cell quiescence - Cell proliferation - Cancer

Nrf2 and Ref-1-mediated redox cellular signalling	Genotoxic agents Oxidants Various stimuli	Ref-1 redox activity on several transcription factors: - AP-1 - p53 - NFκB - HIF-1a 1. increased DNA binding and transcriptional activation of target genes Ref-1-mediated transcriptional activation of Nrf2-target genes under oxidative stress 1. reversible oxidation of cysteine to sulfenic or sulfinic acids 2. Regulatory role of thioredoxin 3. Translocation of cytoplasmic Ref-1 to the nucleus under oxidative stress	Protection against DNA damage and oxidative stress

p53-p66Shc signalling	Genotoxic stress - UVC Oxidative stress - H_2O_2	Phosphorylation of p66Shc 1. p66Shc = redox protein 2. Ser-54 and Thr-386 in a p38 dependent manner 3. phosphorylation of p66Shc at Ser-36 by PKC-β 4. interaction of the prolyl isomerase Pin1 with p66Shc 5. isomerization of a p66shc phospho-Ser36-Pro37 bond 6. translocation of p66Shc into mitochondria Alternatively: 1. redox-dependent reversible tetramerization 2. Cu-dependent ROS generation 3. initiation of apoptosis 4. Regulatory role of glutathione or thioredoxin	Regulation of mitochondrial ROS metabolism Oxidative stress responses and control of cell redox status Regulation of rate of DNA oxidative damage Regulation of steady-state levels of intracellular ROS Regulation of p53-induced ROS up-regulation and cytochrome c release selective regulation of p53-dependent apoptosis

IRE-IRP regulatory network	Oxidative stress NO Hypoxia	Interaction of IRP-1/2 with IRE 1. Regulation of expression of different genes (also oxidases) 2. 4Fe–4S iron–sulfur cluster 3. Destabilisation of 4Fe–4S iron–sulfur cluster with H_2O_2 via posttranslational modifications of IRP1 4. Phosphorylation of IRP1 by PKC at Ser-138 5. Regulation of iron storage and export proteins – Fenton reaction 6. Role of ARE transcriptional activation of the ferritin gene	Regulation of ferritin and TfR1 mRNA expression – iron homeostasis

ATM-mediated DNA damage responses	Genotoxic stress Oxidation stress (H_2O_2)	Activation of ATM by H_2O_2 1. formation of active ATM dimers via intermolecular disulfide bond 2. Cys2991 oxidation	Suppression of protein synthesis and induction of autophagy under oxidative stress

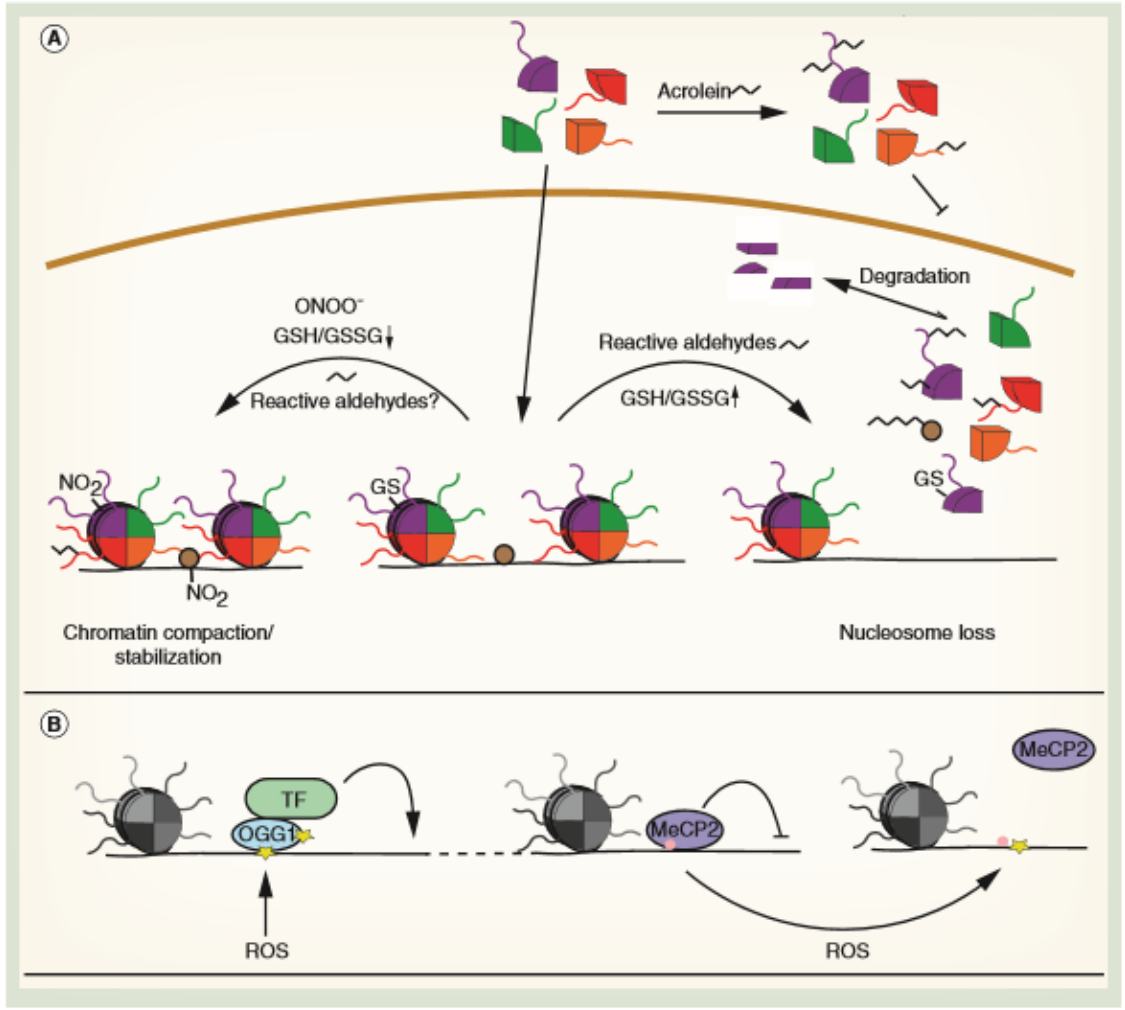


Figure 2. Oxidative stress induces direct modifications of chromatin. (A) Different oxidative histone modifications lead to alterations of chromatin structure. **(B)** Oxidation of deoxyguanine directly influences gene expression. GS: Reduced glutathione; GSH: Glutathione; GSSG: Glutathione disulfide; NO: Nitric oxide; ROS: Reactive oxygen species; TF: Transcription factor.

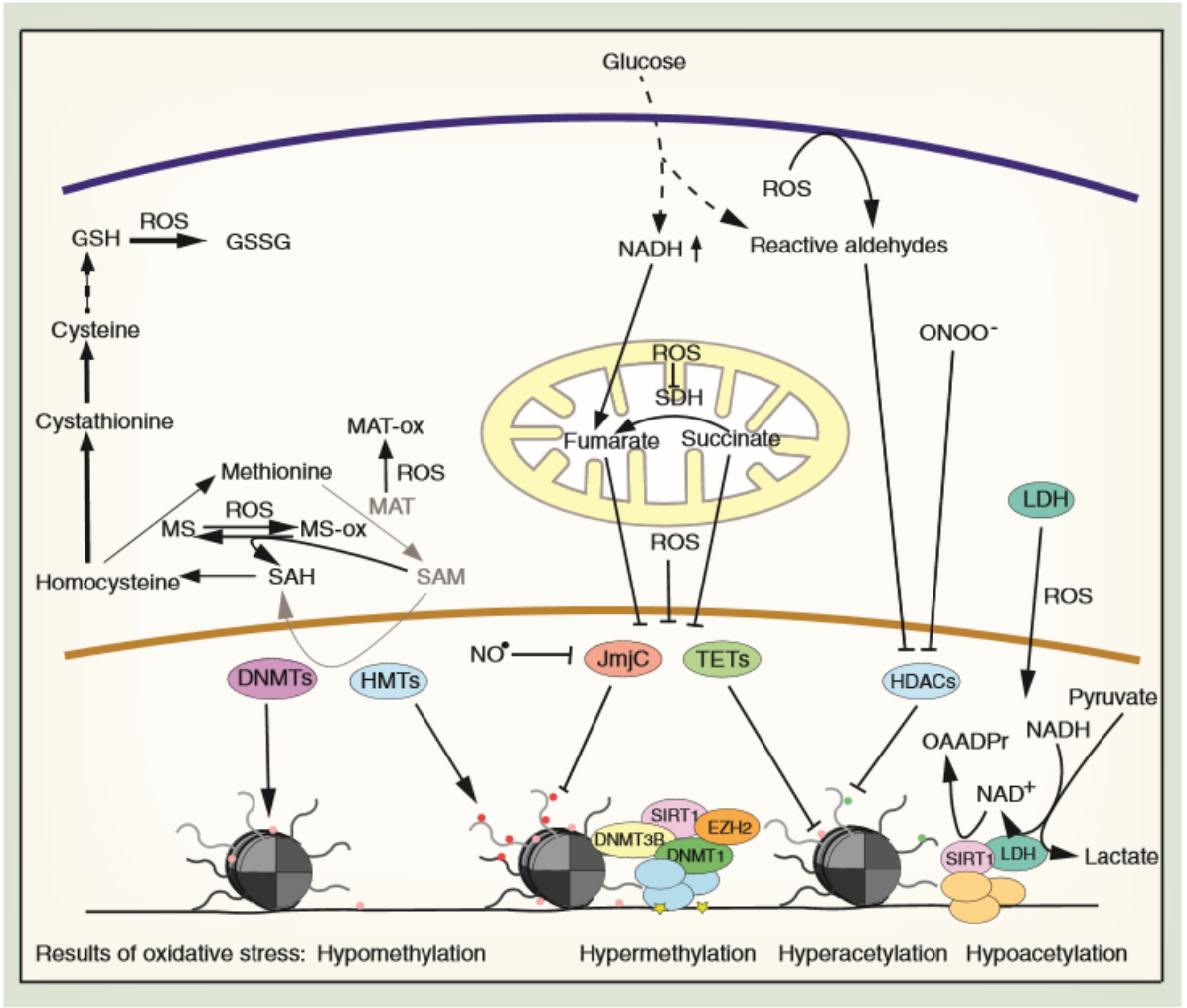


Figure 3. Oxidative stress influences chromatin modifiers. Chromatin modifying enzymes can be inhibited under oxidative stress conditions by different mechanisms, including SAM depletion, oxidation, carbonylation or nitration. Activation of DNA methylation is mediated by oxidative DNA damage and the SIRT1 HDAC can be stimulated by LDH.
DNMT: DNA methyltransferase; GSH: Glutathione; HDAC: Histone deacetylase; HMT: Histone methyltransferase; LDH: Lactate dehydrogenase; MAT: Methionine adenosyltransferase; MS: Methionine synthase; NAD+: Nicotinamide adenine dinucleotide; NO: Nitric oxide; ROS: Reactive oxygen species; SAM: S-adenosylmethionine; TET: Ten-Eleven-Translocation DNA demethylase.

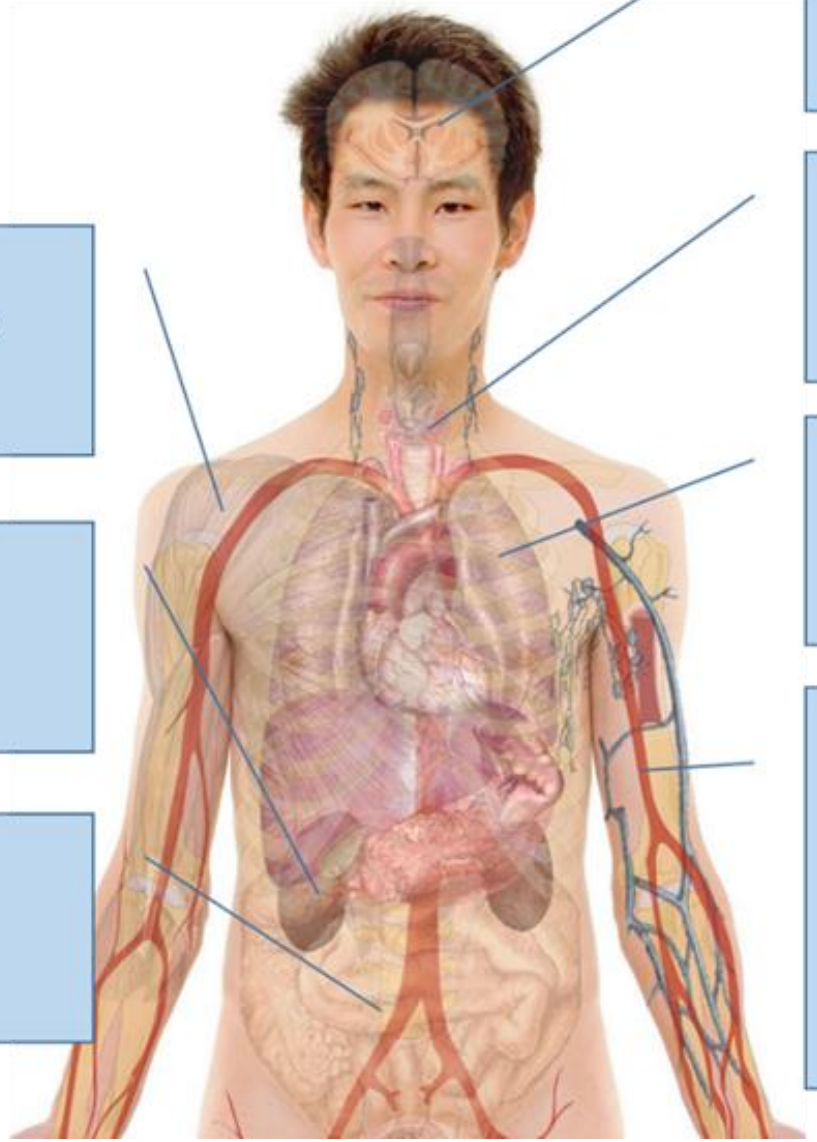
ROS in physiological processes

- synaptic processes
- synaptic plasticity
- memory formation

- production of hormones
- release of hormones
- mediation of hormone action

- sensing of partial pressure of O_2
- pulmonary perfusion

- contractility of smooth muscle
- vascular endothelial cell proliferation
- platelet activation and haemostasis
- immune response



- glucose transport/uptake
- glycogen biogenesis

- medullary blood flow
- sodium homeostasis
- blood pressure control

- haematopoiesis
- bone metabolism
- bone remodelling

- Cardiovascular physiology
  - differentiation and contractility of vascular smooth muscle cells
    - hydrogen peroxide, as signalling moiety, induces increase in intracellular calcium level and contraction in pulmonary artery smooth muscle cells
    - responses induced by different vasoconstrictor stimuli, including hypoxia (pulmonary arteries constrict in response to hypoxia)
    - involvement of ROS in the development of pulmonary hypertension
  - control of vascular endothelial cell proliferation and migration
  - platelet activation and haemostasis
  - ROS, in turn, activate membrane transporters, as sodium/hydrogen exchanger (NHE-1) and sodium/bicarbonate cotransporter (NBC) via stimulation of the ROS-sensitive MARK cascade and finally stimulates of such effectors leads to an increase in cardiac contractility

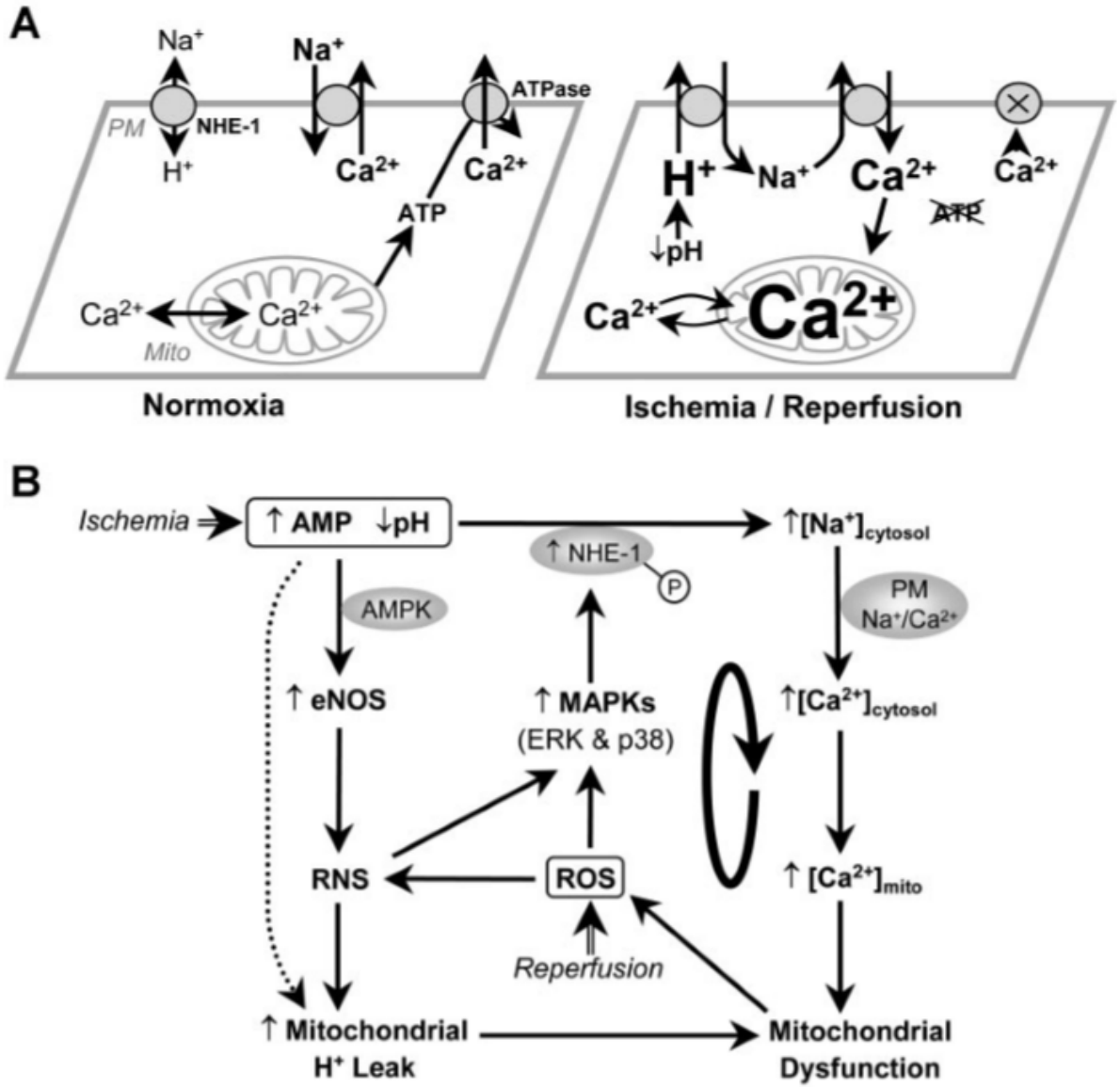


Fig. 7. Mitochondria, Ca^{2+}, and ROS in myocardial ischemia-reperfusion (I/R) injury. *A*: the events leading up to $[Ca^{2+}]_m$ overload during I/R. Briefly, a drop in pH triggers Na^+/H^+ exchanger (NHE)-1-mediated Na^+ influx, resulting in Na^+ overload. Reverse-mode Na^+/Ca^{2+} exchange across the plasma membrane (PM) then results in Ca^{2+} overload and subsequent $[Ca^{2+}]_m$ overload. Ca^{2+} cycling across the mitochondrial membrane can lead to propagation and amplification of $[Ca^{2+}]_m$ overload. In addition, ATP deficiency prevents adequate Ca^{2+} export by Ca^{2+}-ATPases. *B*: cross-talk between the pathways of mitochondrial dysfunction, ROS, and Ca^{2+} overload in I/R injury. The precipitating events (boxes) are acidosis, elevated AMP, and ROS generation. A feed-forward loop is hypothesized, encompassing mitochondrial ROS generation, MAPKs, and $[Ca^{2+}]_m$ overload. For further explanation, see text. RNS, reactive nitrogen species; AMPK, AMP-dependent protein kinase.

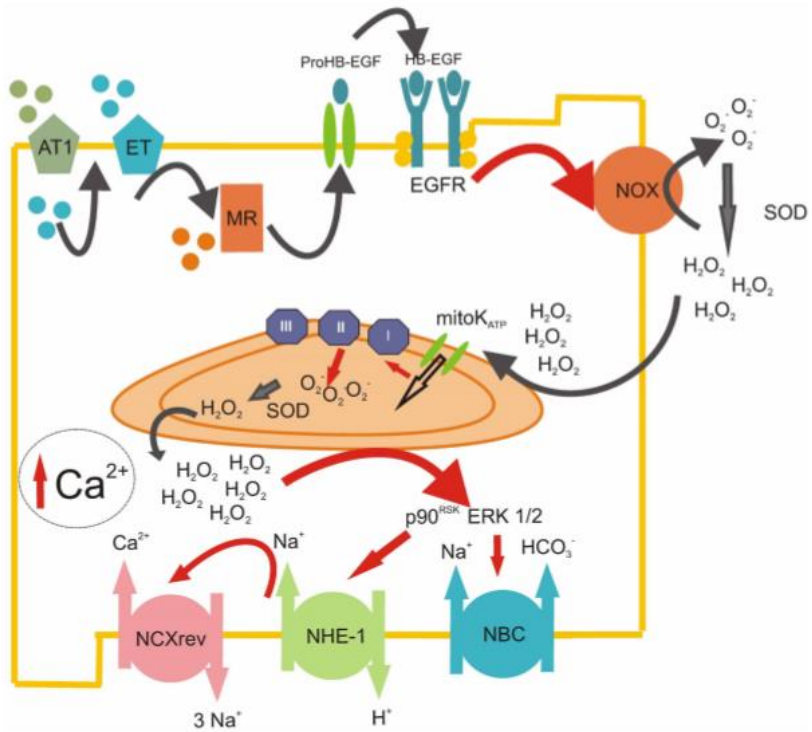


FIGURE 1 | ROS-induced ROS-release mechanism triggered by RAAS. Scheme representing the sequential steps involved in the production of mitochondrial ROS after the initial Ang II stimulation. Ang II acting on AT_1 receptors induces the release of intracellular ET-1, which, in turn, acts in an autocrine manner on ET_A receptors. This autocrine action leads to the activation of the mineralocorticoid receptor (MR), which induces the transactivation of the EGFR, possibly via the release of membrane heparin-bound EGF (HB-EGF). The stimulation of the EGFR leads to the activation of the NADPH oxidase (NOX), which produces superoxide anion (O_2^-) and quickly dismutate by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). This permanent and stable oxidant molecule produces the opening of mitochondrial ATP-dependant potassium channels (mitoK$_{ATP}$) with the subsequent enhanced production of mitochondrial O_2^- by the electron transport chain (mainly by complex II). These mitochondrial ROS are released to the cytosol (ROS-induced ROS-release mechanism), where they stimulate redox sensitive MAPkinases ERK 1/2 and p90^{RSK}, which, in turn, activate NHE-1 and NBC, pH regulation transporters that induce the increase in intracellular Na^+. Finally, this cytosolic Na^+ increase favors the operation of the reverse mode of NCX, promoting the influx of Ca^{2+} into the cell. The enhancement of intracellular Ca^{2+} in the cardiomyocyte could lead to a positive inotropic effect in the short term and/or the development of cardiac hypertrophy in a time-prolonged scenario.

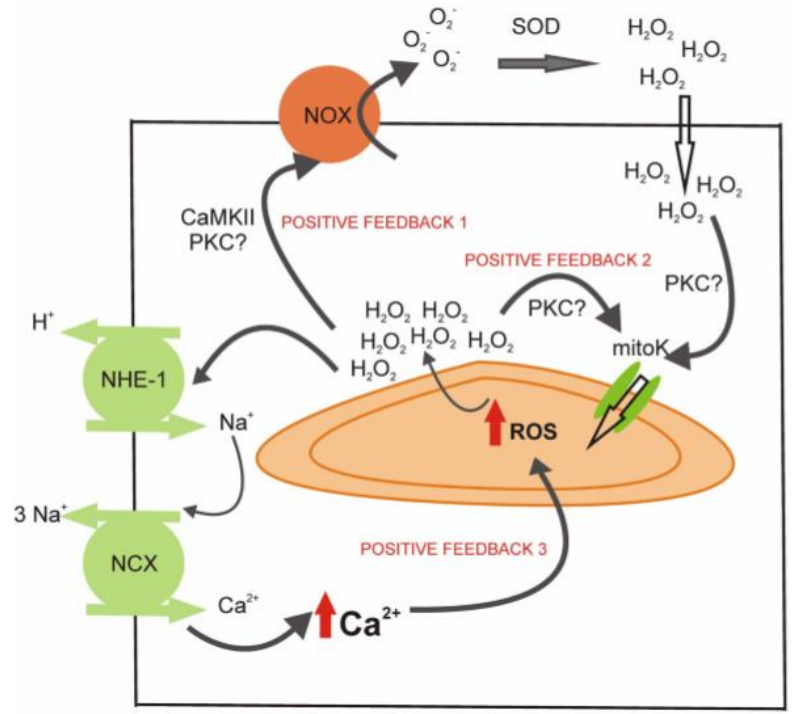
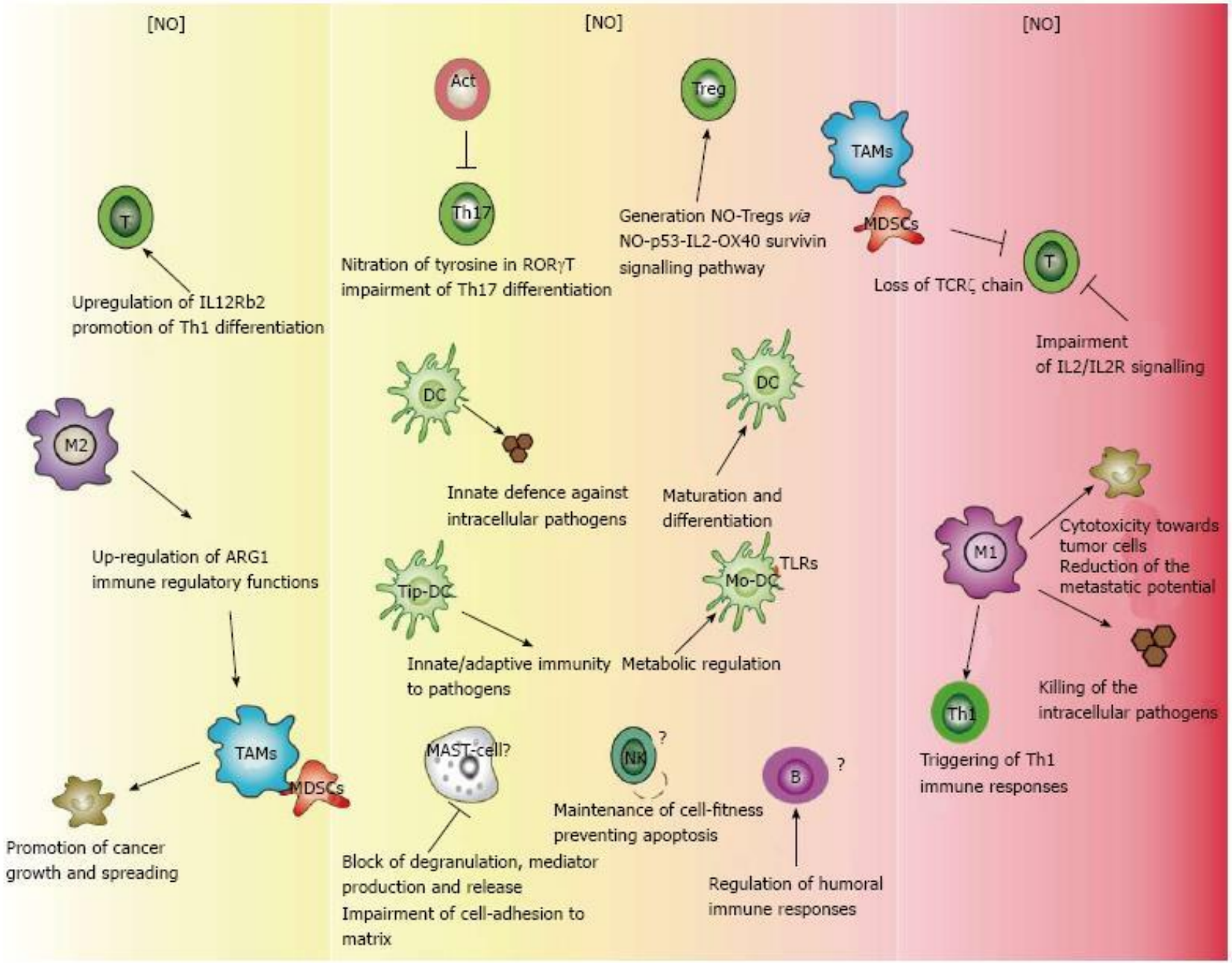


FIGURE 2 | Potential sites of positive feedback mechanisms involved in the mitochondrial ROS production during the activation of RAAS. The H_2O_2 released by the mitochondria during the ROS-induced ROS-release mechanism could activate NOX (possibly via CaMKII or PKC activation), cycling the mitochondrial ROS production (positive feedback 1). The mitochondrial ROS could also help to maintain the opening of mitoK$_{ATP}$ (positive feedback 2), perhaps through the activation of PKC. Finally, the intracellular Ca^{2+} augmentation after NHE-1 and NCX reverse mode stimulation could induce mitochondrial Ca^{2+} load and further ROS production (positive feedback 3).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Act
Treg
TAMs
Th17
MDSCs
T
T
Upregulation of IL12Rb2
promotion of Th1 differentiation
Nitration of tyrosine in RORγT
impairment of Th17 differentiation
Generation NO-Tregs *via*
NO-p53-IL2-OX40 survivin
signalling pathway
Loss of TCRζ chain
Impairment
of IL2/IL2R signalling
DC
DC
M2
Innate defence against
intracellular pathogens
Maturation and
differentiation
Up-regulation of ARG1
immune regulatory functions
Tip-DC
Mo-DC
TLRs
M1
Cytotoxicity towards
tumor cells
Reduction of the
metastatic potential
Innate/adaptive immunity
to pathogens
Metabolic regulation
Killing of the
intracellular pathogens
Th1
TAMs
MDSCs
MAST-cell?
NK
?
B
?
Triggering of Th1
immune responses
Promotion of cancer
growth and spreading
Maintenance of cell-fitness
preventing apoptosis
Block of degranulation, mediator
production and release
Impairment of cell-adhesion to
matrix
Regulation of humoral
immune responses

LETTER

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Hypoxia induces heart regeneration in adult mice

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The adult mammalian heart is incapable of regeneration following cardiomyocyte loss, which underpins the lasting and severe effects of cardiomyopathy. Recently, it has become clear that the mammalian heart is not a post-mitotic organ. For example, the neonatal heart is capable of regenerating lost myocardium[1], and the adult heart is capable of modest self-renewal[2,3]. In both of these scenarios, cardiomyocyte renewal occurs via the proliferation of pre-existing cardiomyocytes, and is regulated by aerobic-respiration-mediated oxidative DNA damage[4,5]. Therefore, we reasoned that inhibiting aerobic respiration by inducing systemic hypoxaemia would alleviate oxidative DNA damage, thereby inducing cardiomyocyte proliferation in adult mammals. Here we report that, in mice, gradual exposure to severe systemic hypoxaemia, in which inspired oxygen is gradually decreased by 1% and maintained at 7% for 2 weeks, results in inhibition of oxidative metabolism, decreased reactive oxygen species production and oxidative DNA damage, and reactivation of cardiomyocyte mitosis. Notably, we find that exposure to hypoxaemia 1 week after induction of myocardial infarction induces a robust regenerative response with decreased myocardial fibrosis and improvement of left ventricular systolic function. Genetic fate-mapping analysis confirms that the newly formed myocardium is derived from pre-existing cardiomyocytes. These results demonstrate that the endogenous regenerative properties of the adult mammalian heart can be reactivated by exposure to gradual systemic hypoxaemia, and highlight the potential therapeutic role of hypoxia in regenerative medicine.

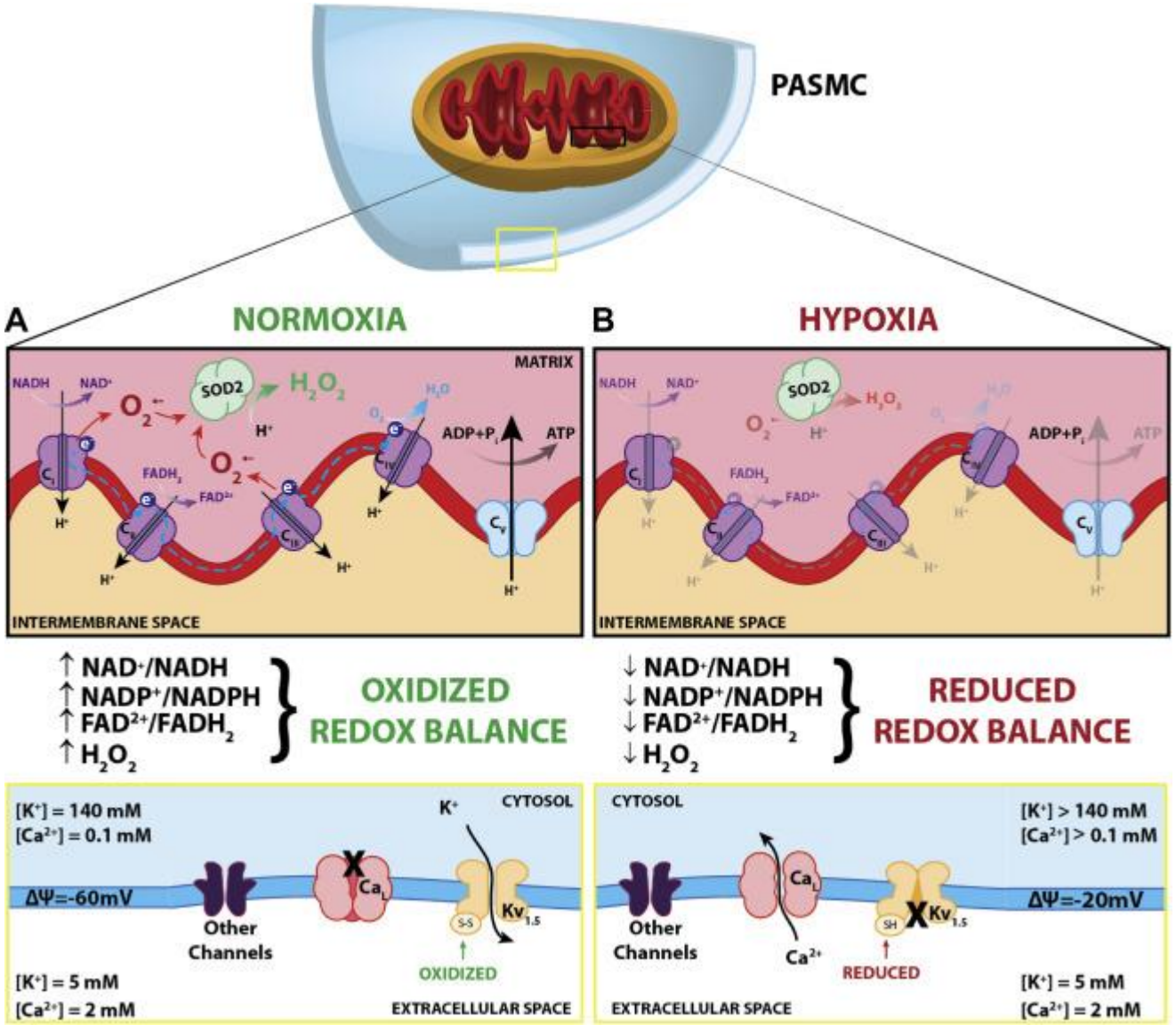
Zebrafish, urodele amphibians, and immature mammals are capable of myocardial regeneration following various types of injury, mediated primarily through the proliferation of pre-existing cardiomyocytes[1,6–8]. Given that most cardiomyocytes in mammals exit cell cycle shortly after birth, induction of cell cycle re-entry of adult cardiomyocytes has emerged as a central focus for cardiac regeneration.

Here we show that chronic severe hypoxaemia, induced by a gradual reduction in inspired oxygen, reduces ROS and oxidative DNA damage in the cardiomyocytes. Importantly, this was sufficient to induce cell cycle re-entry of adult cardiomyocytes, which resulted in significant functional recovery following myocardial infarction (MI). Although counterintuitive, these results suggest that targeting this pathway could be a viable strategy for mammalian heart regeneration.

In order to examine the effect of systemic hypoxia on mitochondrial metabolism, we exposed mice to low oxygen tension (7% O_2) for 2 weeks. To avoid hypobaropathy caused by a rapid drop in partial oxygen pressure[10], we gradually dropped the fraction of inspired oxygen (FiO_2) by 1% per day from 20.9% (room air oxygen) to 7% over the course of 2 weeks followed by exposure to 7% oxygen for an additional 2 weeks (Fig. 1a). We observed a reduction in food intake during hypoxia exposure (Extended Data Fig. 1a), therefore the normoxic mice were given an equivalent amount of food. Blood gas analysis of the normoxic mice were found to be within normal levels previously reported in anaesthetized rodents[11]. As expected, arterial pH in hypoxic mice was markedly decreased, and arterial pO_2 was decreased. Moreover, pCO_2 level was decreased in the hypoxia group, probably owing to hyperventilation (respiratory compensation) (Extended Data Fig. 1b). Two days following exposure to 7% oxygen, stabilization of hypoxia inducible factor 1 α subunit (Hif1α) in cardiomyocytes was observed as indicated by an increase in the number of fluorescent protein tdTomato$^+$ cardiomyocytes in αMHC-CreERT2-ODD;R26/tdTomato double transgenic mice[5] (Extended Data Fig. 1c). The acute increase in tdTomato$^+$ cardiomyocytes seen here was due to hypoxic stabilization of Hif1α rather than cardiomyocyte expansion. Following 2 weeks of hypoxia exposure, we observed a significant decrease in mitochondrial cristae density (Fig. 1b) and in cardiac mitochondrial DNA copy number (Fig. 1c). Mass spectrometry-based quantification of enzymes involved in mitochondrial Krebs cycle and fatty acid β-oxidation provided further support for the reduction in mitochondrial metabolism

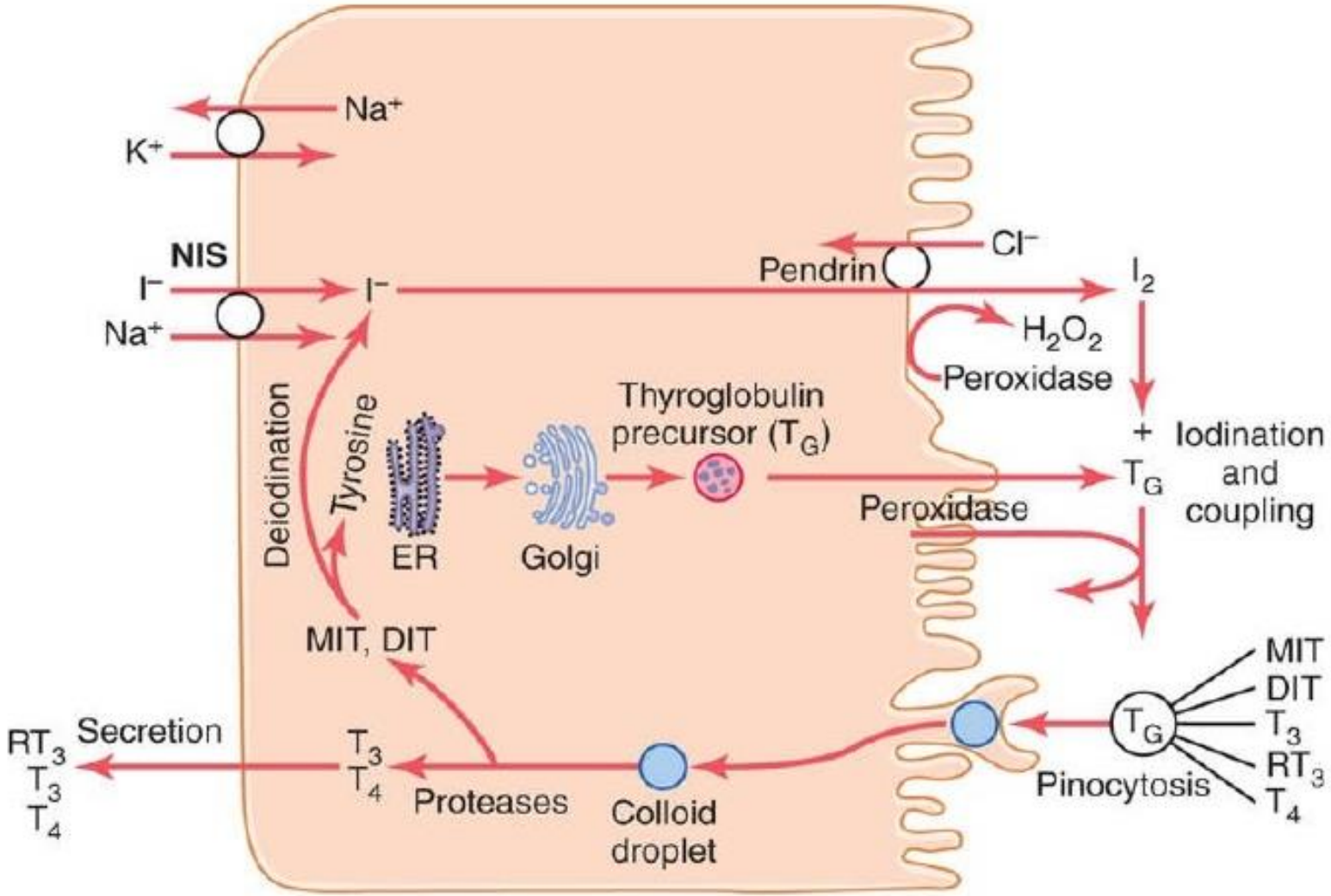
- ROS and respiration
  - sensory plasticity of the carotid body (*glomus caroticum*)
- ROS and skeletal muscles
  - circulation during exercise in humans
  - requirements of adapting to osmotic challenges, hyperthermia challenges, and loss of circulating fluid volume
  - stimulation of glucose transport in isolated skeletal muscle preparations during intense repeated contractions
- ROS and nervous system
  - ROS are generated by microglia and astrocytes
  - modulate synaptic and non-synaptic communication between neurons and glia
  - synaptic long-term potentiation, a form of activity-dependent synaptic plasticity and memory consolidation
- ROS and kidneys
  - production of superoxide, hydrogen peroxide, and nitric oxide in the renal medullary thick ascending limb of Henle regulates medullary blood flow, sodium homeostasis, and long- term control of blood pressure

hypoxic pulmonary vasoconstriction

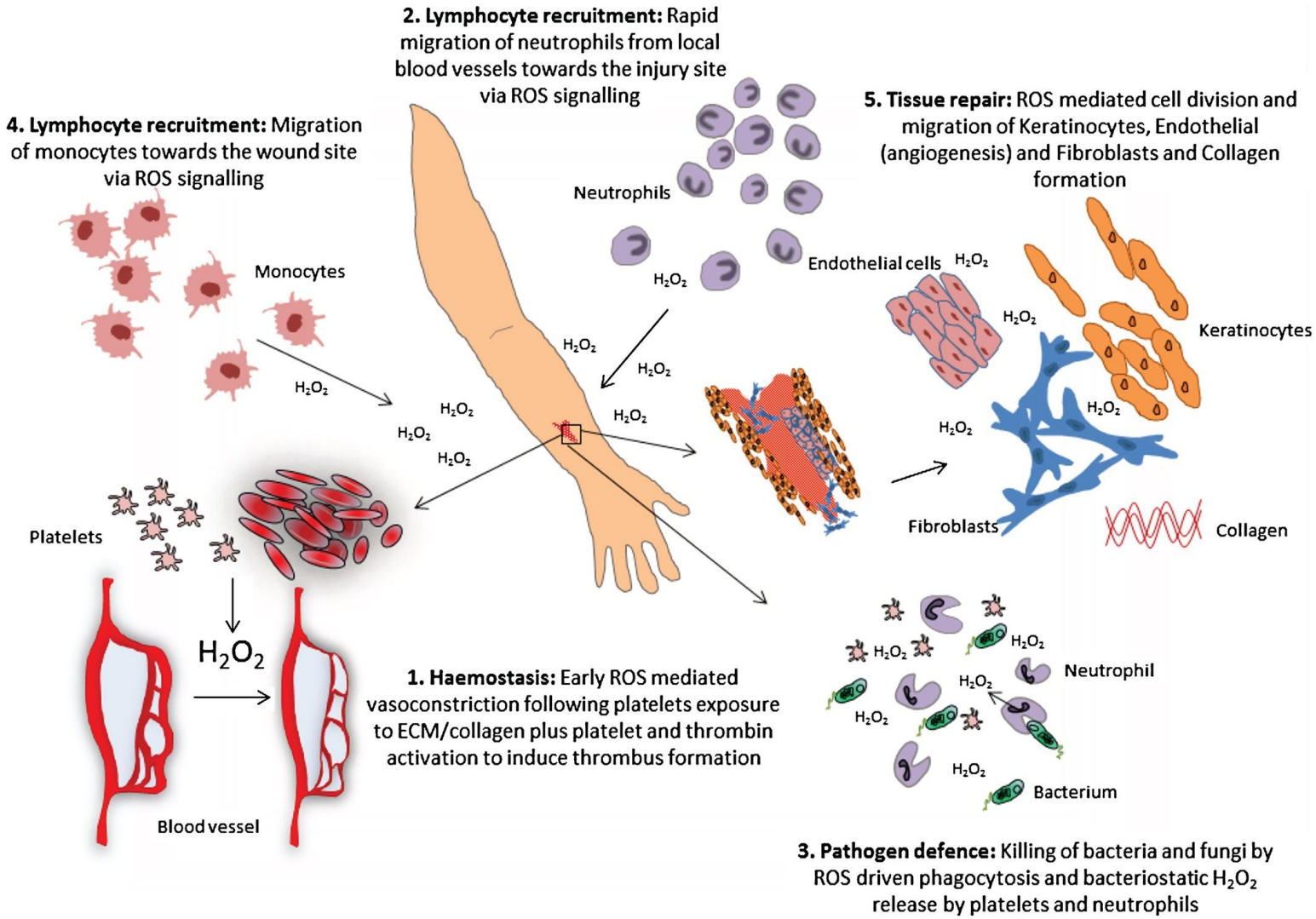


Mitochondrial redox oxygen sensing. The sensor-effector mechanism of hypoxic pulmonary vasoconstriction (HPV). (A) Under normoxic conditions, generation of reactive oxygen species (ROS) occurs at mitochondrial electron transport chain (ETC) complexes I and III, producing superoxide (O2–), which is converted to hydrogen peroxide (H2O2) by superoxide dismutase 2 (SOD2). Hydrogen peroxide, along with the oxidized redox couples (eg, nicotinamide adenine dinucleotide [NAD+], nicotinamide adenine dinucleotide phosphate [NADP+], and flavin adenine dinucleotide [FAD2+]) maintain Kv1.5 sulfhydryl group oxidation and channel open state, resulting in tonic egress of K+. This efflux of K+ sustains the resting membrane potential (ΔΨ) of the cell at –60 mV and inhibits voltage-gated calcium channel [CaL]-mediated Ca2+ influx into the cell. (B) During hypoxia, the limited presence of oxygen (1) prevents generation of hydrogen peroxide, (2) decreases the ratio of oxidized/reduced redox couples, and (3) reduces sulfhydryl groups on Kv1.5 channels, causing them to close. The subsequent buildup of K+ increases the resting membrane potential of the cell to –20 mV. This stimulates the opening of CaL, influx of Ca2+, and subsequent activation of the contractile apparatus (ie, vasoconstriction). ADP = adenosine diphosphate; FADH2 = flavin adenine dinucleotide; NADH = nicotinamide adenine dinucleotide.

ROS and endocrine system



ROS and wound healing



Reactive oxygen species (ROS) and its role in wound healing. The schematic diagram depicts the multiple roles of ROS during acute wound healing (note that this refers to homeostatic, not excessive, levels of ROS). (i) ROS are important in initial wound protection by reducing blood flow and local cell signalling for thrombus formation; (ii) local ROS release attracts blood vessel-bound local neutrophils to the wound site for bacterial protection; (iii) phagocytosis releases ROS to stunt bacterial growth and provide further signals supporting the wound response; (iv) other immunocytes, including monocytes, migrate towards the wound site to help attack invading pathogens; (v) wound edge and general release of ROS stimulates endothelial cell division and migration for blood vessel reformation, fibroblast division and migration for new ECM formation (including collagen synthesis) and promote keratinocyte proliferation and migration.

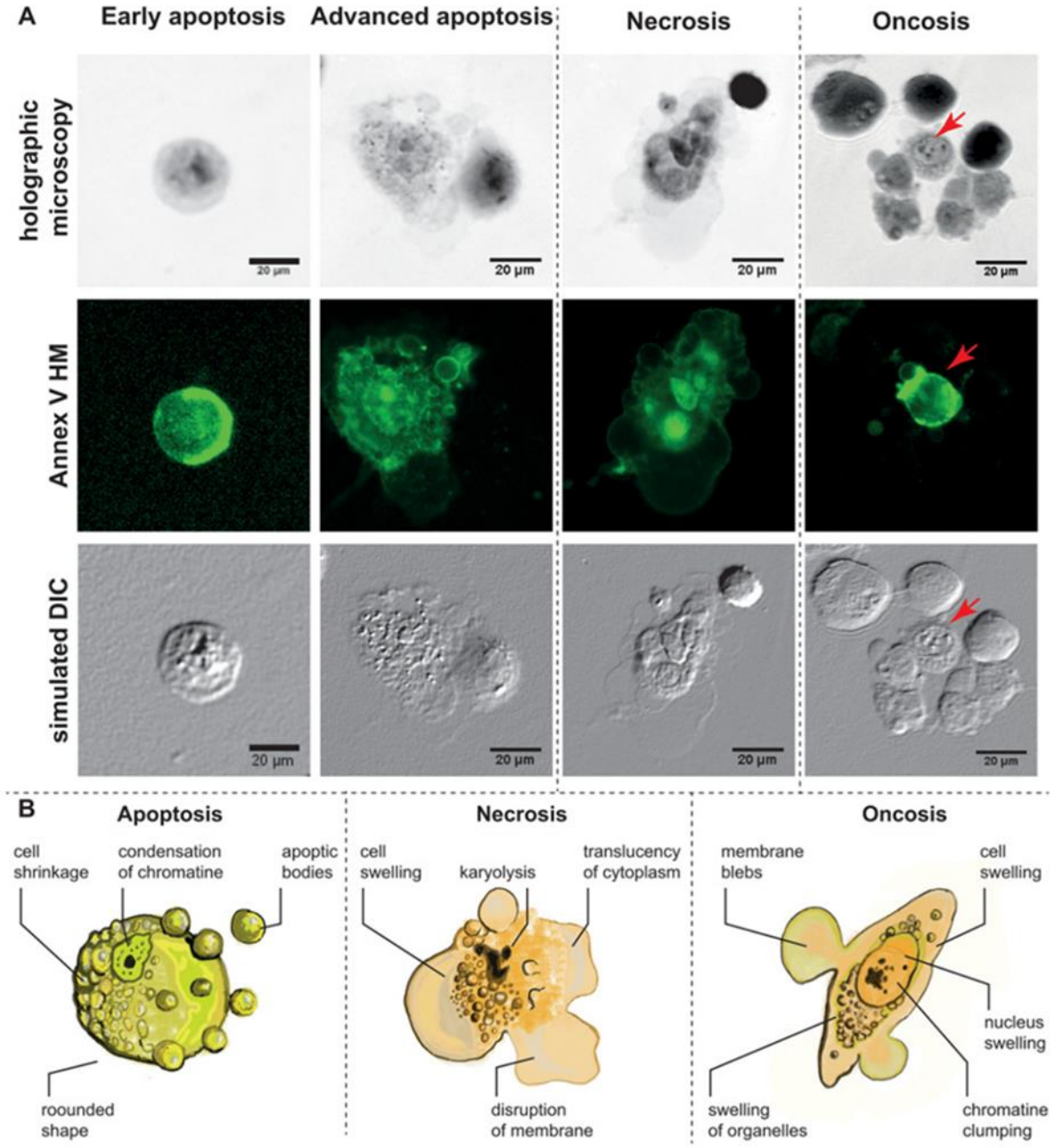
ROS and wound healing

ROS-modulating therapeutic approach	Evidence for positive physiological ROS effects on wounds
Topical H_2O_2 (or related ROS intermediates)	Anti-bacterial, promotes O_2 formation, increased angiogenesis, various immunocyte recruitments, keratinocyte proliferation and migration 35-40, 44
Recombinant glucose oxidase	Increased perfusion via NO, early facilitation of wound closure, keratinocyte differentiation and collagen formation, H_2O_2-related effects 46
Honey	Anti-bacterial, immunocyte recruitment, H_2O_2-related effects 45-50
Galvanic particles	Reduced inflammation, fibroblast migration H_2O_2-related effects 50, 52
Hyperbaric O_2 therapy	Reduced wound hypoxia thus better anabolism, efficient phagocytic respiratory bursts, H_2O_2-related effects 56, 57
Recombinant PDGF	Increased perfusion via NO, angiogenesis, neutrophil, macrophage, fibroblast, endothelial cell wound migration 59-62
Recombinant Galectin-1	Myofibroblast signalling and ROS release via NADPH oxidase, H_2O_2-related effects 63

NADPH, nicotinamide adenine dinucleotide phosphate; PDGF, platelet-derived growth factor; ROS, reactive oxygen species.

ROS and cell death

Cell features	Apoptosis	Autophagy	Necrosis	Oncosis
Cell size	reduced -shrinkage	reduced/massive vacuolization of cytoplasm (accumulation of autophagic vacuoles)	increased - swelling	increased - swelling
Plasma membrane	intact, changes in membrane symmetry, changes in orientation of lipids	intact	disrupted	intact in the early phase; increased throughput depending on the phase of oncosis
Nucleus	condensation of chromatin, changes in shape of nucleus, fragmentation of DNA (the end of the process)	no chromatin condensation;	karyolysis and caspase independent DNA fragmentation, lysis of nucleolus (the beginning of the process)	nucleus dilatation and clumping of chromatin, reticular nucleolus
Specific features	apoptotic bodies; pseudopod retraction; spherical shape of cells	presence of autophagic vacuoles	increasingly translucent cytoplasm; swelling of ER and loss of ribosomes; swollen mitochondria with amorphous densities; lysosome rupture; plasma membrane rupture; myelin figures	swelling of organelles; membrane blebs
Energy balance	retained ATP production	retained ATP production	ATP depletion	ATP depletion
Adjacent inflammation	rare	no	frequent	frequent
Involvement of ROS	yes	yes	yes	yes



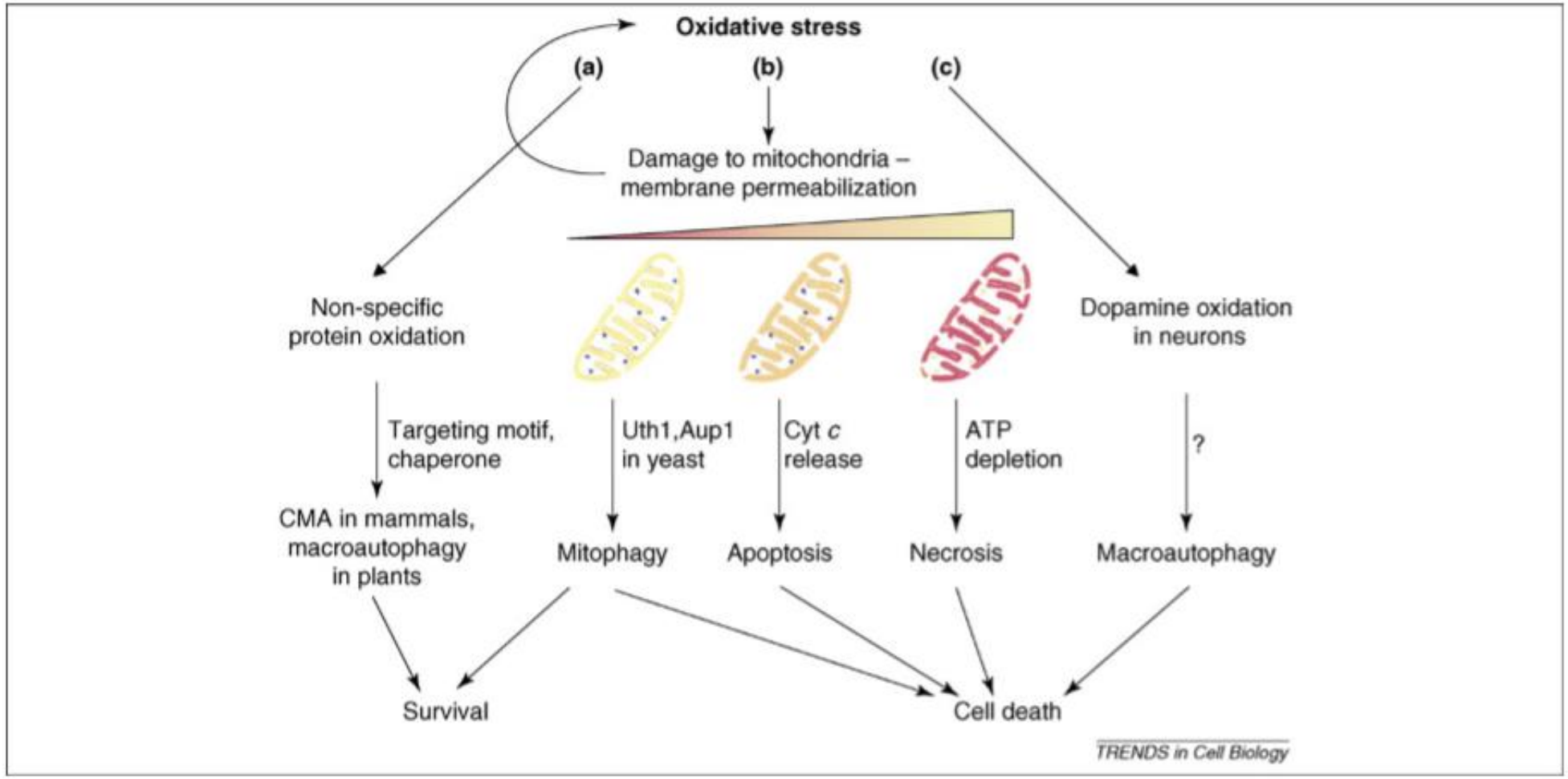


Figure 1. Different pathways of autophagy are activated in response to oxidative stress. Massive oxidative stress leads to autophagy through three different pathways. **(a)** Non-specific protein oxidation caused by oxidative stress activates CMA in mammals [18] and macroautophagy in plants [19] as a survival pathway. **(b)** Mitochondrial permeability transition induced by oxidative stress triggers one of several processes, depending on the severity of the oxidative damage: mild damage (pale yellow) induces mitophagy, as either survival or death pathway, through Uth1 and Aup1 in yeast and yet unknown factors in mammals [14,52–55]; increased damage (orange) triggers apoptosis in mammals following permeabilization and release of cytochrome *c*; and severe damage (red), both in yeast and mammals, results in necrosis owing to ATP depletion. Massive accumulation of ROS in the mitochondria can trigger the release of additional ROS from the mitochondria, which further increases the oxidative stress in the cell [50]. **(c)** In the nervous system, oxidative stress induces dopamine oxidation that, in turn, leads to autophagic cell death [23]. Where known, factors associated with the different processes are indicated.