Toxicological Consequences of Oxidative Stress

Mechanistic Toxicology (M.Sc. /Pharmacology and toxicology)

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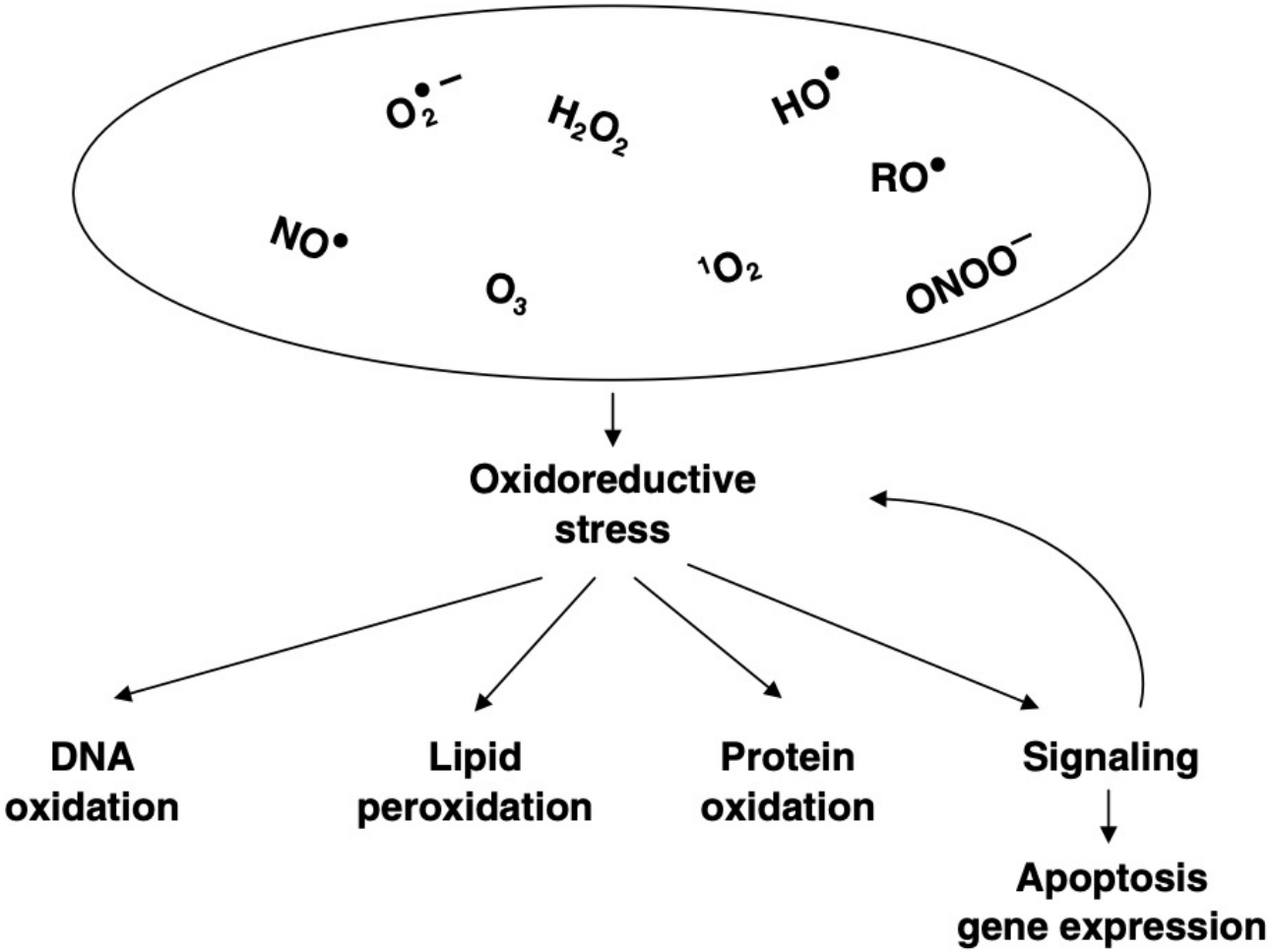
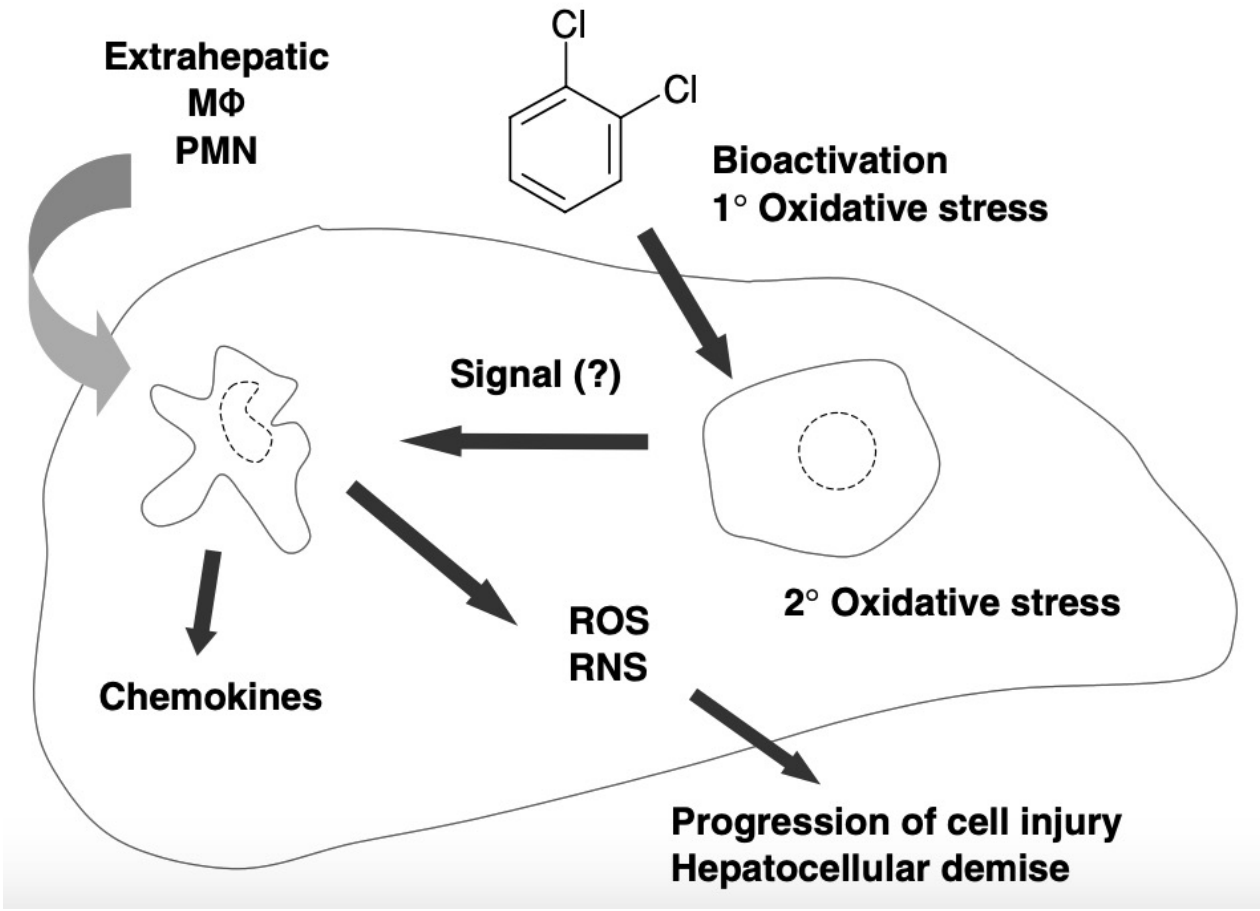
Oxidoreductive stress can have multiple consequences:

- If not compensated by antioxidant defense systems, Oxidative stress can damage cellular targets either via **direct** oxidative damage:
  1) nucleic acids oxidation
  2) Proteins oxidation
  3) Lipids peroxidation
  4) Or **indirect** by activation Redox sensitive pathway (act as a second messenger that leads to the transactivation of genes or signal transduction



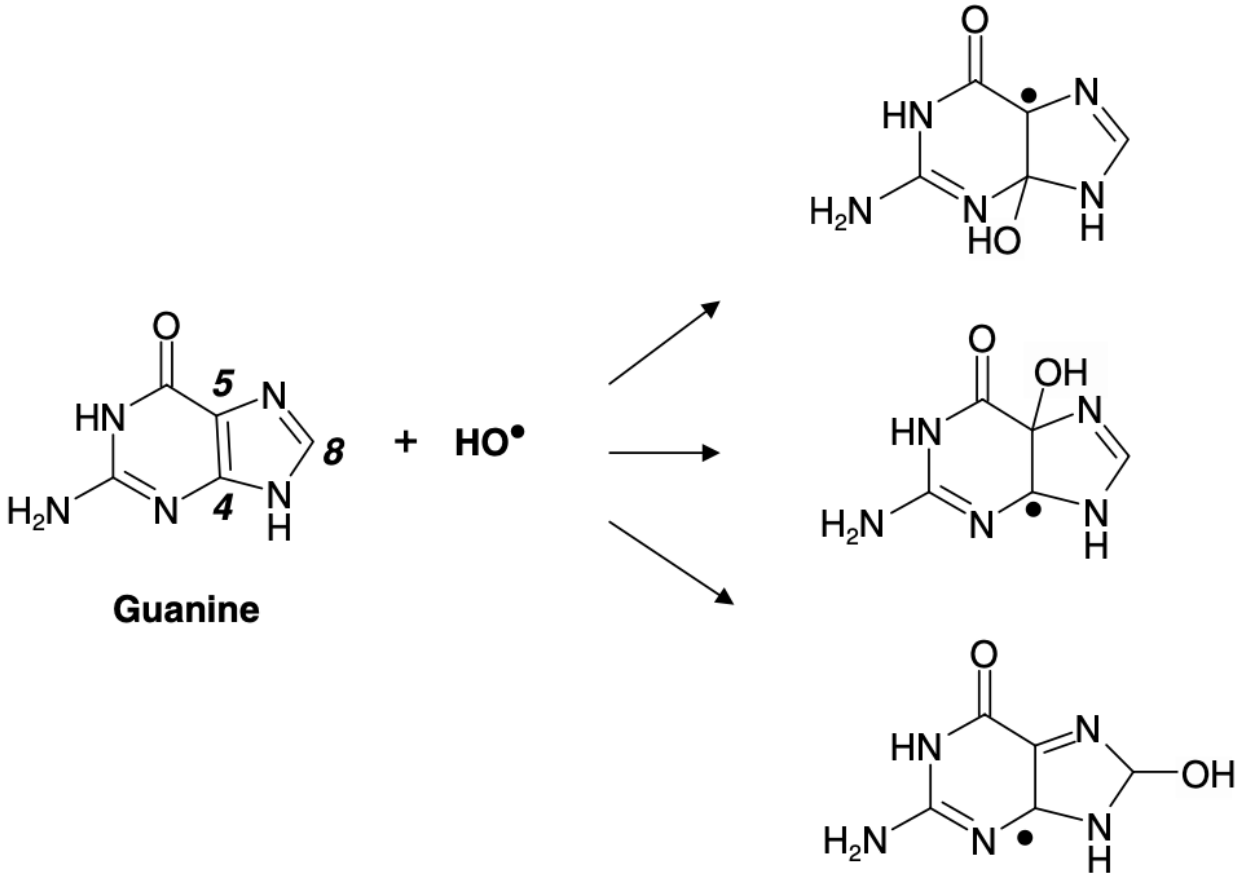
- For example, in the liver exposed to 1,2-dichlorobenzene, ROS are generated which by itself are probably insufficient to damage liver cells severely (because their effect would be antagonized by the powerful antioxidant defense system in the liver). Instead, the signal activates chemokines and other activating pathways, which results in the infiltration of extrahepatic macrophages and other immune cells, which flood the liver and produce a second and much stronger wave of oxidative stress.



Signals released from hepatocytes subjected to a primary oxidative stress, caused by activated 1,2-dichlorobenzene, recruit macrophages and neutrophils, thus amplifying the extent of oxidoreductive stress.

1. Oxidative DNA damage

- One of the consequences of oxidative stress in cells is oxidation of nucleic acids by ROS. Because ROS are physiologically generated all the time, this is a **normal process** that is counterbalanced by antioxidants and repair mechanisms.
- One of the markers to detect oxidative damage to DNA is the measurement of oxidized bases; for example, the presence of 8-hydroxy-deoxyguanosine(8-OH-dG) in urine.
- Indeed, in nuclear DNA, 1 out of 130,000 guanine residues is 8-OH-G.



Attack of a hydroxyl radical on purine bases.
The C8 position of purines is a very sensitive site for oxidation by ROS

1. Oxidative DNA damage

- In contrast to nuclear DNA, **mitochondrial DNA (mtDNA)** is much more prone to be hit by an oxidative event and to be permanently damaged. In mtDNA, it is **1 out of approximately 8000 guanine residues that is 8-OH-G**.
- The biological reasons behind this increased susceptibility include the:
  a) absence of histones (which are protective) in mtDNA.
  b) the close proximity to the generation of ROS in mitochondria
  c) inefficient repair mechanisms, leading to accumulation of oxidatively damaged bases.
  d) mtDNA lacks noncoding sequences, which makes an oxidative event potentially more relevant.
- On the other hand, there are several copies of mtDNA in each mitochondrion, and there are hundreds or thousands of mitochondria in a single cell. Therefore, an oxidative event is outweighed by the rest of the intact mtDNA

1. Consequences of Oxidative DNA damage

- DNA base oxidation has severe consequences on base pairing: the oxidized form, 8-oxo- guanine (the keto form) binds now to adenine via two hydrogen bonds, this mismatch will lead to a base-pair transversion in the next cycle of DNA synthesis.

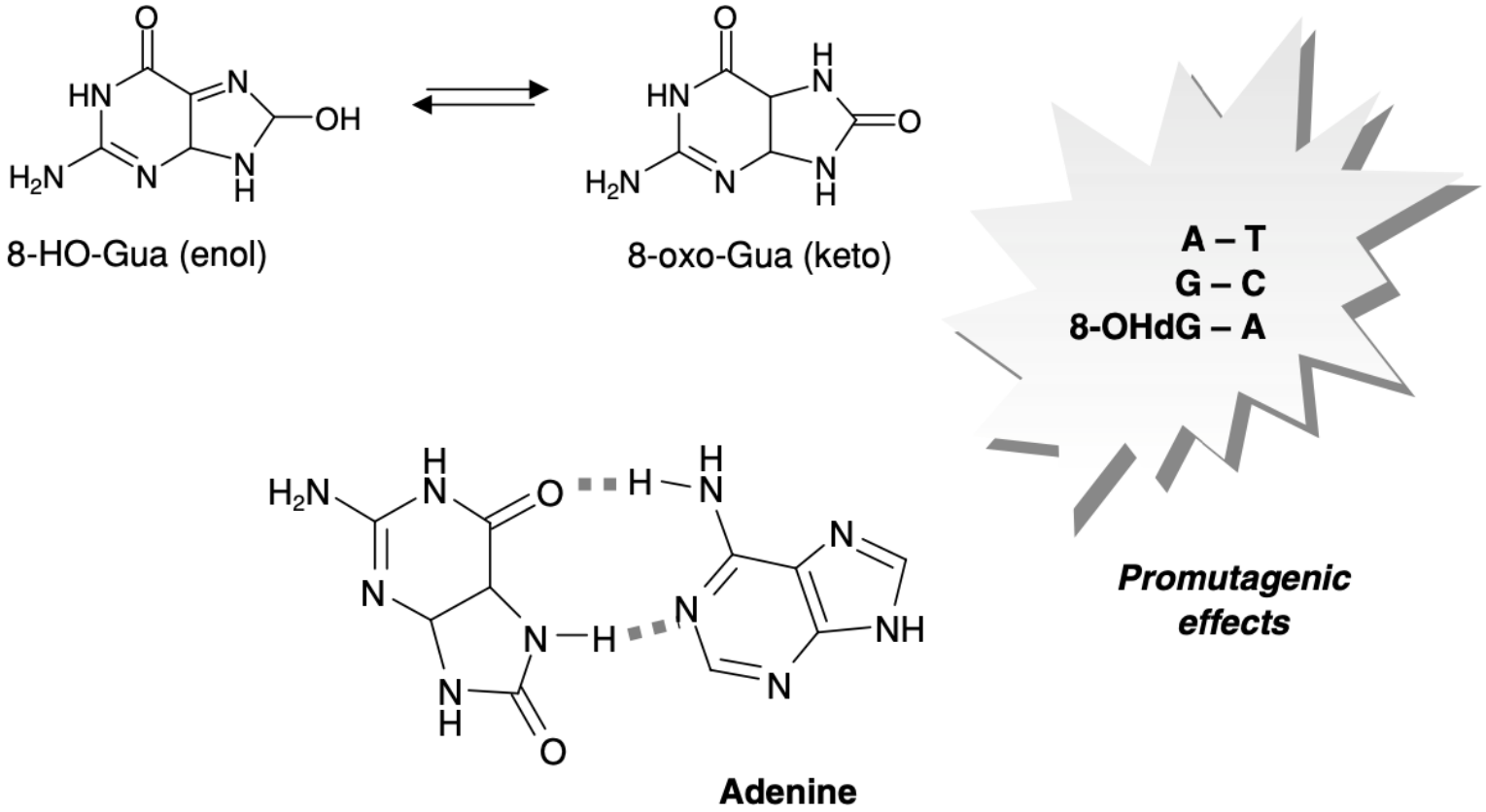
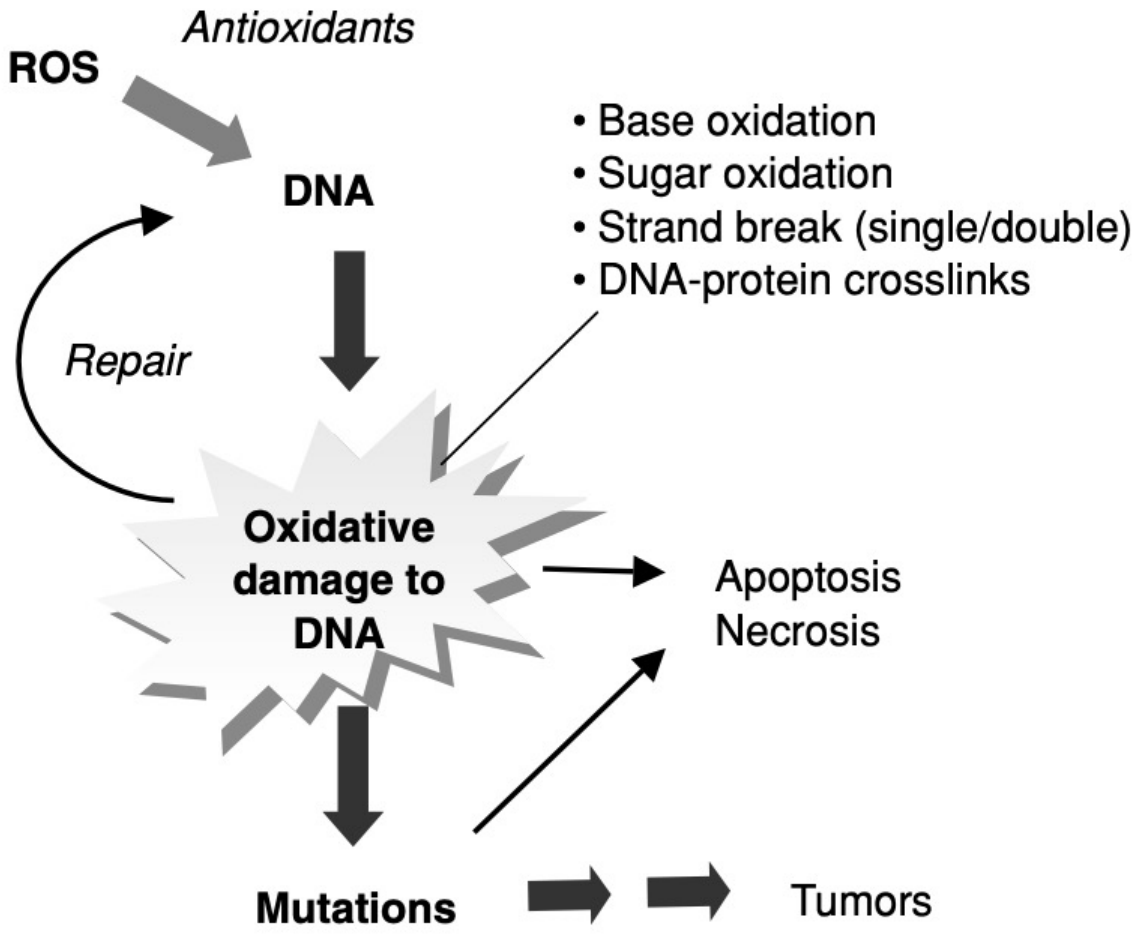


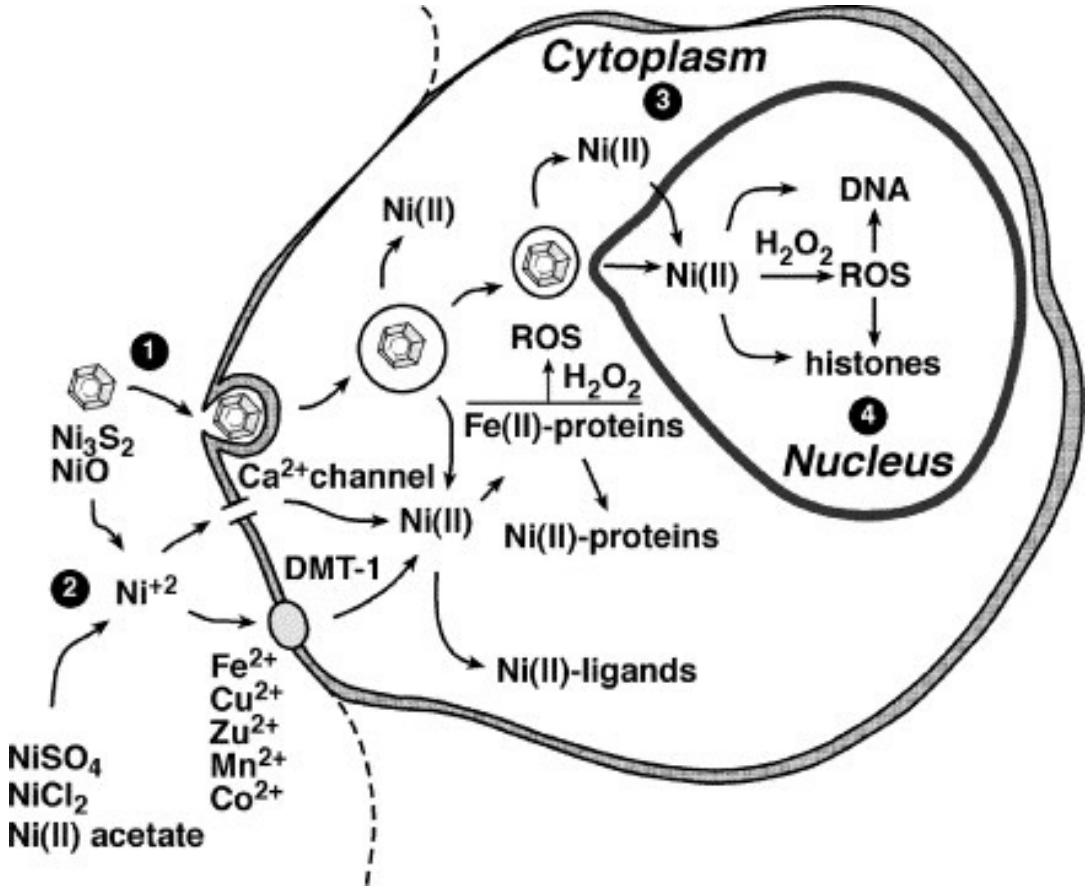
Figure: DNA base oxidation (at the guanine residue) leads to the insertion of a wrong base (adenine) due to the inability to form hydrogen bonds with cytidine.

- If these changes persist during the next replication cycle, a point mutation is generated. In most cases, such damage will be **repaired**, or mutated cells will undergo **cell death by apoptosis or necrosis**. However, DNA oxidation can also be the basis for tumor formation.



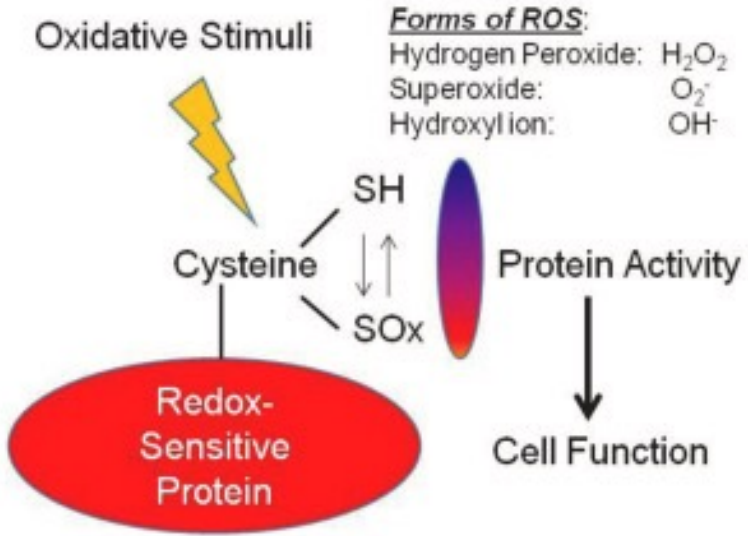
Oxidative DNA damage can ultimately lead to mutations and eventually tumors.

- **Oxidative DNA damage has been implicated in the carcinogenic activity of many metals** such as Cr(VII) and Ni(II) as well as indium, all of which induce tumors in lung and nose.
- **Ni_3S_2** is a highly carcinogenic form of the metal and causes severe DNA lesions and increases in 8-OH-dG levels in the lungs. It also causes an inflammatory response in the lung and greatly enhances NO production by macrophages. Thus, the combined mechanism of ROS release by macrophages during the inflammation and the reaction between Ni and H2O2 produces a Ni-ROS complex which can release OH radicals and hence can damage DNA.



2. Oxidative protein damage

- Oxidative stress can lead to the oxidation of cellular proteins, in particular:
  a) oxidation of side chains of amino acid residues
  b) formation of protein-protein cross-links
  c) protein fragmentation due to the oxidation of the peptide backbone.
- The sulfur-containing amino acids cysteine and methionine are particularly susceptible to oxidation, leading to disulfide bonds or sulfoxide formation, respectively. Furthermore, aromatic amino acids (tyrosine, tryptophan) are also prone to being attacked by ROS.



- cells harbor a host of **antioxidant** defense mechanisms to limit the oxidative stress of proteins, and there are repair mechanisms to reverse the damage. For example, **heat-shock proteins** (hsp) are able to renature damaged proteins or to resolubilize aggregates of damaged proteins (repairment).
- Also, oxidatively damaged proteins are recognized and readily degraded by the major cytosolic protease system, the **proteasome** (Proteasomes recognize, unfold, and digest protein substrates that have been marked for degradation by the attachment of ubiquitin moieties. The damaged proteins are then degraded into peptides and amino acids. Undamaged amino acids are recycled for protein biosynthesis (digestion and recycling)

Consequences of Oxidative protein damage

- If oxidation cannot be compensated or the damage repaired, the major consequences are:

1. loss of catalytic function for enzymes
2. impairment of function for structural proteins (example Actin).
3. increased surface hydrophobicity due to partial unfolding of the protein and result in formation of large protein aggregates, which are often toxic when they accumulate in a cell (hemoglobin)

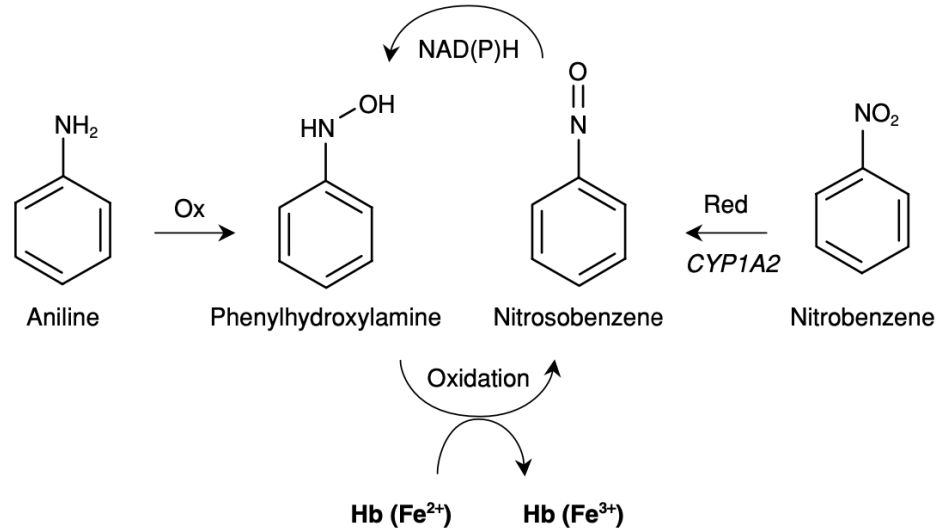
One special example of protein oxidation arising from xenobiotic-induced oxidative damage is the red cell and its major protein, hemoglobin.

- As the bulk (>90%) of the erythrocyte's protein content consists of hemoglobin, this protein is a frequent target for oxidative damage by xenobiotics by two ways:

1. oxidation of the a- and b globin chains of hemoglobin in erythrocytes can lead to disulfide bond formation. Such cross-links can also be formed with spectrin, a component of the erythrocyte's cytoskeleton. The resulting aggregates of dark bodies attached to the cell membrane and were named "Heinz bodies."
2. Oxidation of heme moiety, containing Fe(II) (ferrous) into Fe(III)-(ferric) hemoglobin, which is termed methemoglobin (MetHb), is no longer able to carry oxygen

Mechanisms of metHb formation and hemotoxicity:

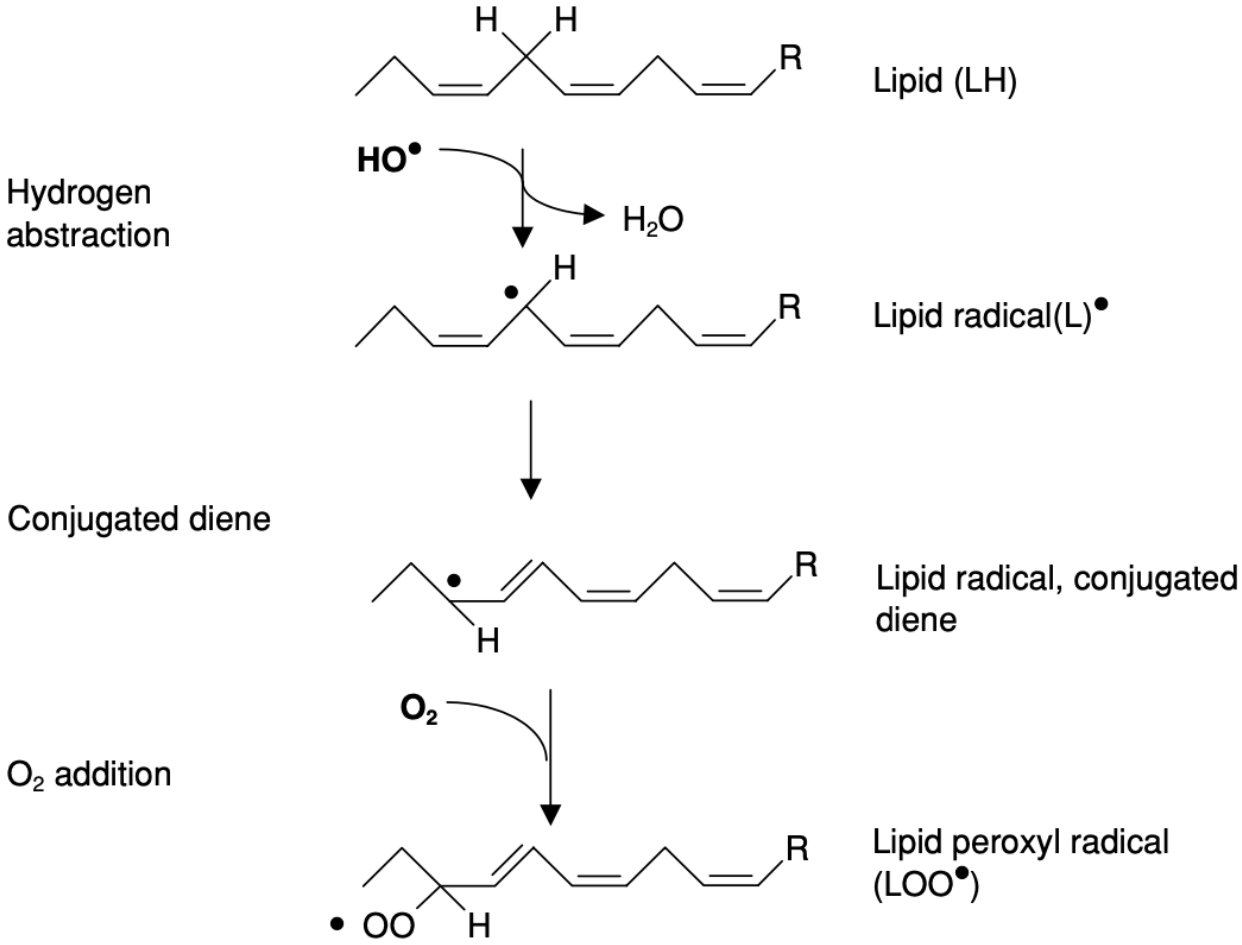
- MetHb can be formed directly from reduced aromatic amines (e.g., aniline) or indirectly from oxidized aromatic amines (e.g., nitrobenzene). After bioactivation, the resulting intermediates drive the formation of hemoglobin oxidation in a cycle until the reducing equivalents (NADPH) are exhausted.
- Therefore, even relatively small amounts of strong MetHb-forming compounds can be potentially dangerous, in particular in patients with compromised erythrocytic antioxidant defense systems (G6PDH deficiency)



The formation of methemoglobin by aromatic amines

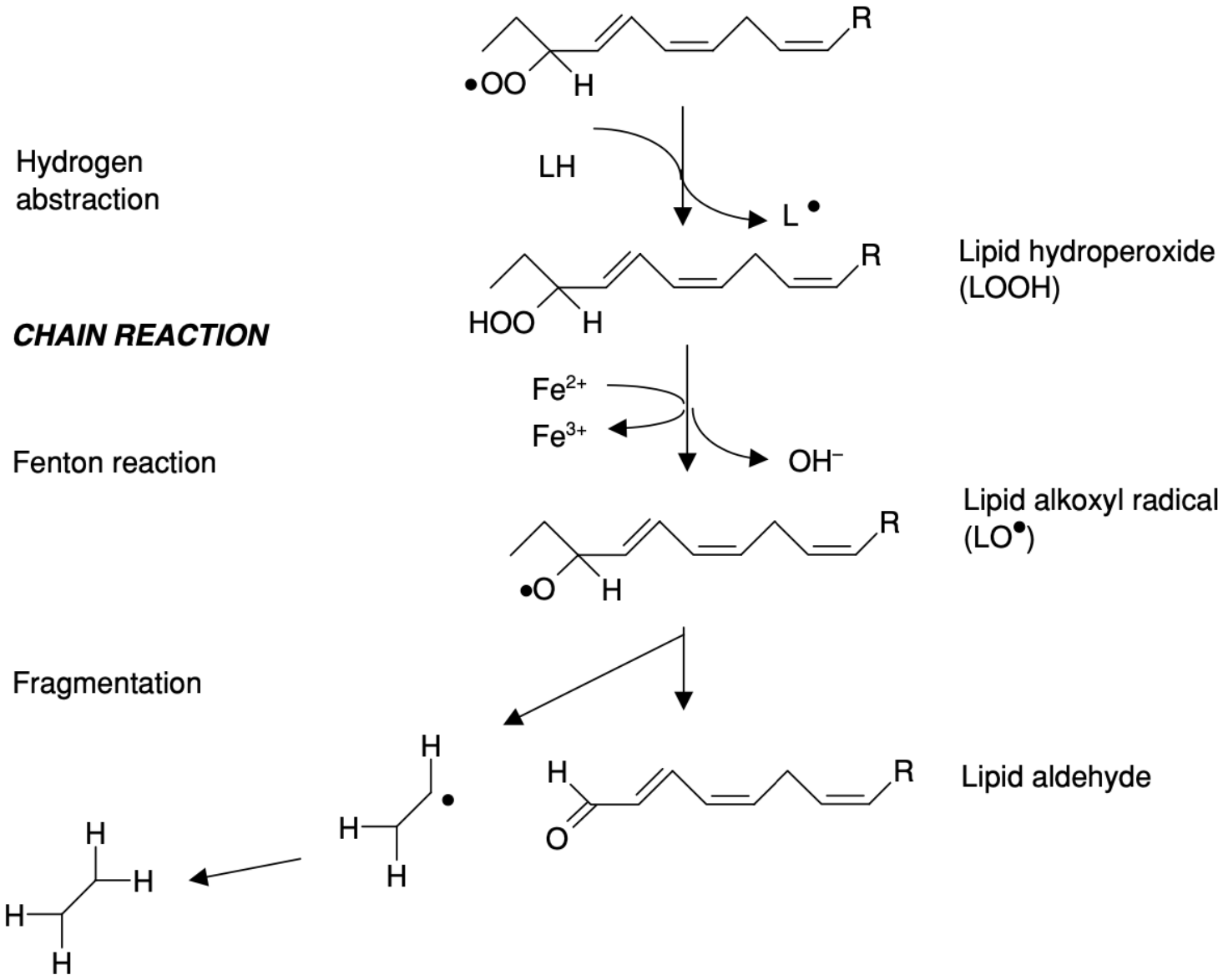
3. Oxidative lipid damage

If the microenvironmental conditions favor the generation of ROS in the hydrophobic compartments of a cell, then lipids can be oxidized. As a result, biomembranes may be peroxidized and damaged.



3. Oxidative lipid damage

- Two important features characterize lipid peroxidation and distinguish it from others:
  1. **Propagation of the original radical-induced damage across the biomembrane.**
  2. The products arising from lipid peroxidation(e.g. **alkoxy radicals or toxic aldehydes) can be equally reactive as the original ROS themselves** and damage cells by additional mechanisms.

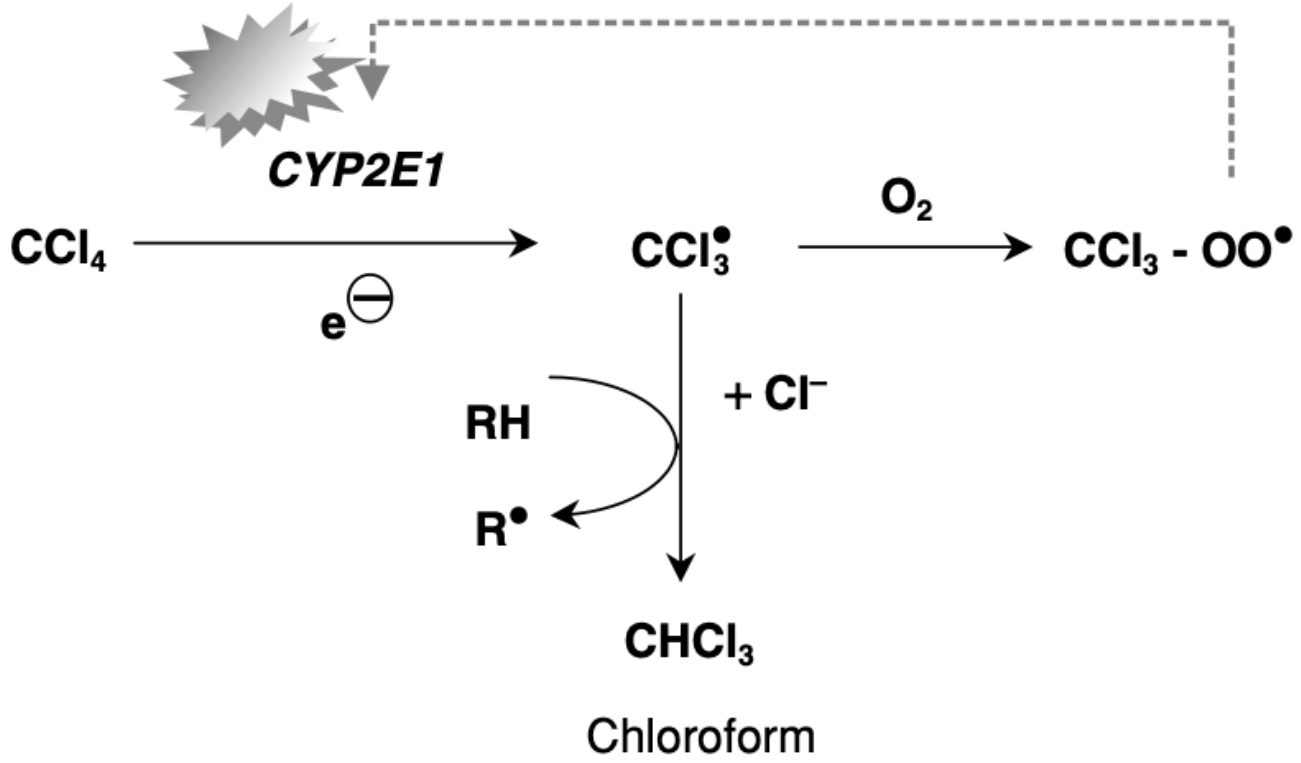


Mechanism of lipid peroxidation: propagation of reaction and formation of aldehyde degradation products.

Mechanism of carbon tetrachloride-induced hepatotoxicity:

- under conditions of **low pO2** : CCl4 undergoes bioreductive metabolism in the liver by CYP2E1 to the CCl3 radical which react with fatty acyl residue and initiate lipid peroxidation.
- In contrast, in the presence of **high pO2** , the trichloromethyl peroxy radical is formed. This is an extremely reactive species that reacts and inactivates CYP2E1 itself (suicide inactivation of CYP).

How the acute intoxication with CCl4 can be treated??

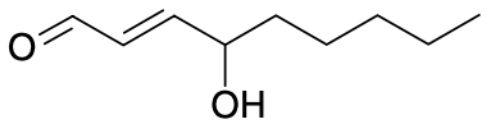


Consequences of Oxidative lipid damage

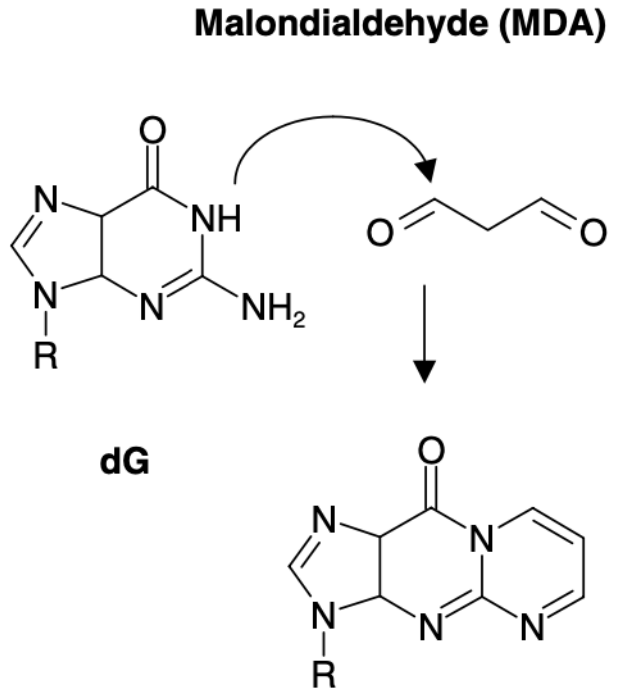
A general and important consequence of membrane lipid peroxidation is the production of two toxic aldehydes.

1. malondialdehyde (MDA)
2. 4-hydroxynonenal (4-HNE).

- Both aldehydes are not only **biomarkers** to prove that lipid peroxidation has occurred, but they have more recently been recognized to be **reactive electrophilic molecules** themselves that can covalently bind to proteins or DNA.

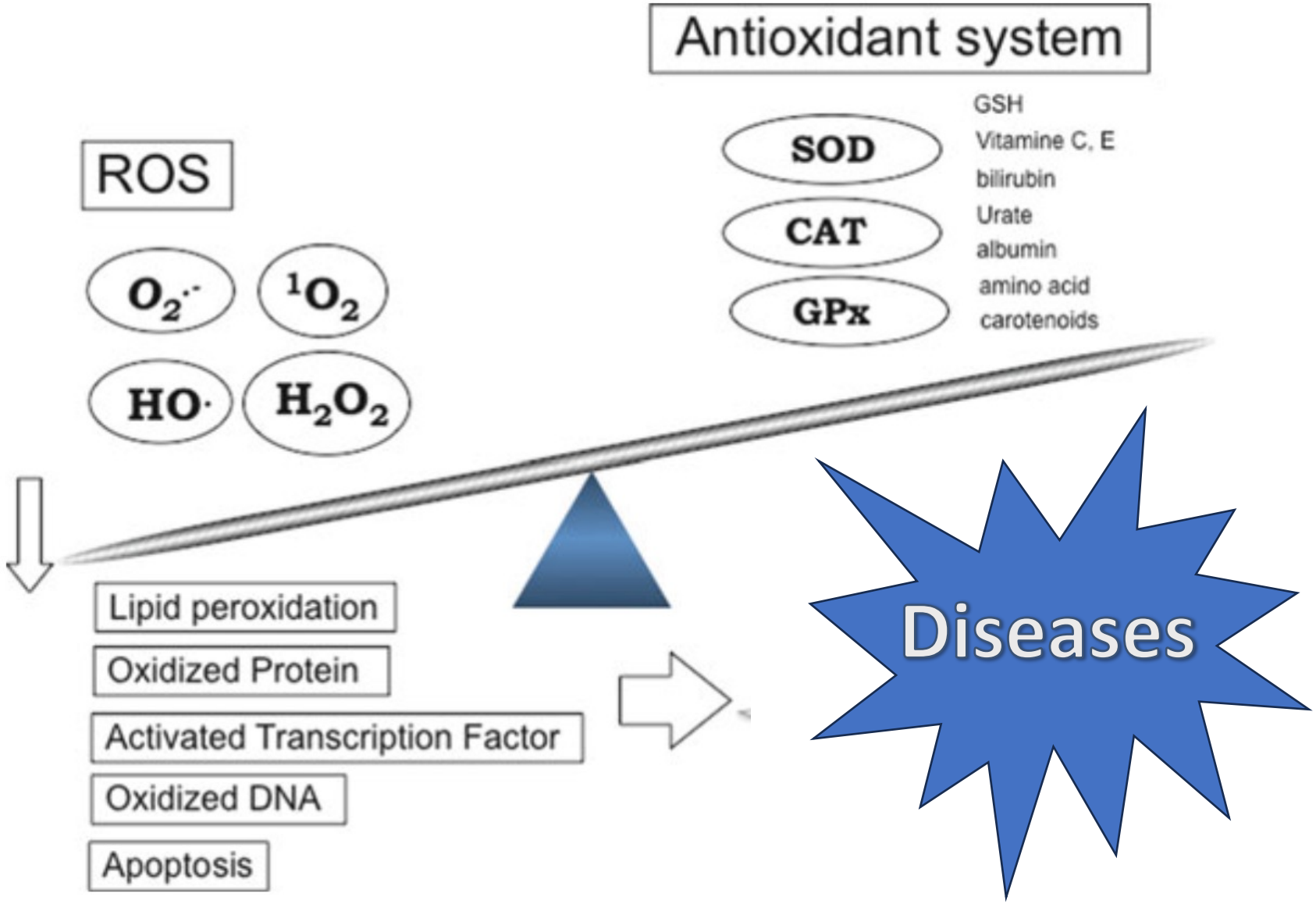


4-OH-Nonenal (4HNE)



Aldehydes from lipid peroxidation are protein- and DNA-reactive species. MDA reacts with deoxyguanosine (dG) to form a covalent adduct.

The disruption of redox balance between ROS and antioxidant system may lead to ROS-induced disease



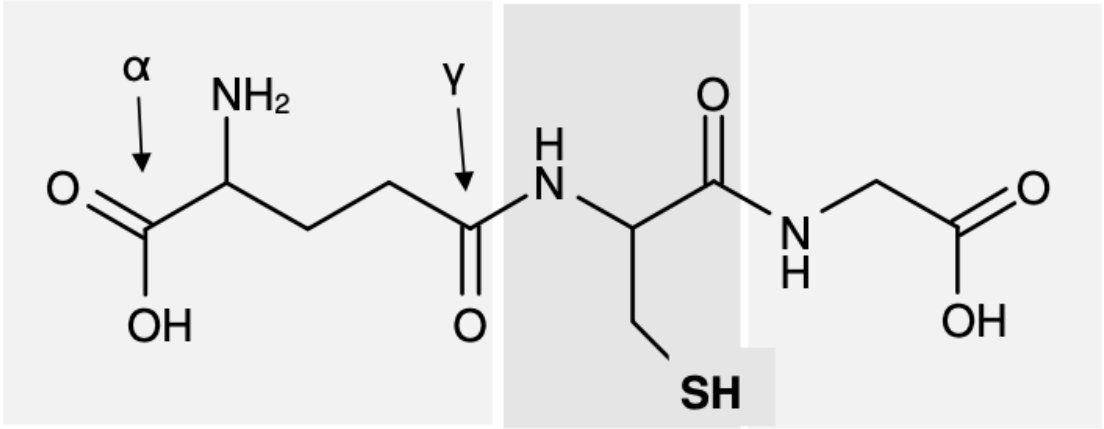
Interference with antioxidant defense mechanisms

- Organisms have evolved a biological antioxidant systems to prevent the maintenance of mild oxidative stress. The major functions of these antioxidants are to:

1. Provide scavengers for ROS and RNS
2. Keep the cellular thiol redox status in the reduced form
3. Prevent or repair the oxidation of lipids
4. Sequester redox-active metals and prevent Fenton-type reactions

1. Glutathione

- Glutathione (GSH), together with its coupled enzyme systems, is one of the most important antioxidant defense lines in the body. Upon need, the levels of GSH can even become upregulated.
- There are several cellular pools of GSH: the cytosolic pool, the mitochondrial pool, and, importantly, a large nuclear pool of GSH. GSH is produced and released from many other cells, too (except erythrocytes).
- **Glutathione is a ubiquitous tripeptide consisting of glutamate, cysteine, and glycine**.



Glutamate Cysteine Glycine

1. Glutathione

- A number of characteristics must be recalled for a better understanding of its biological function

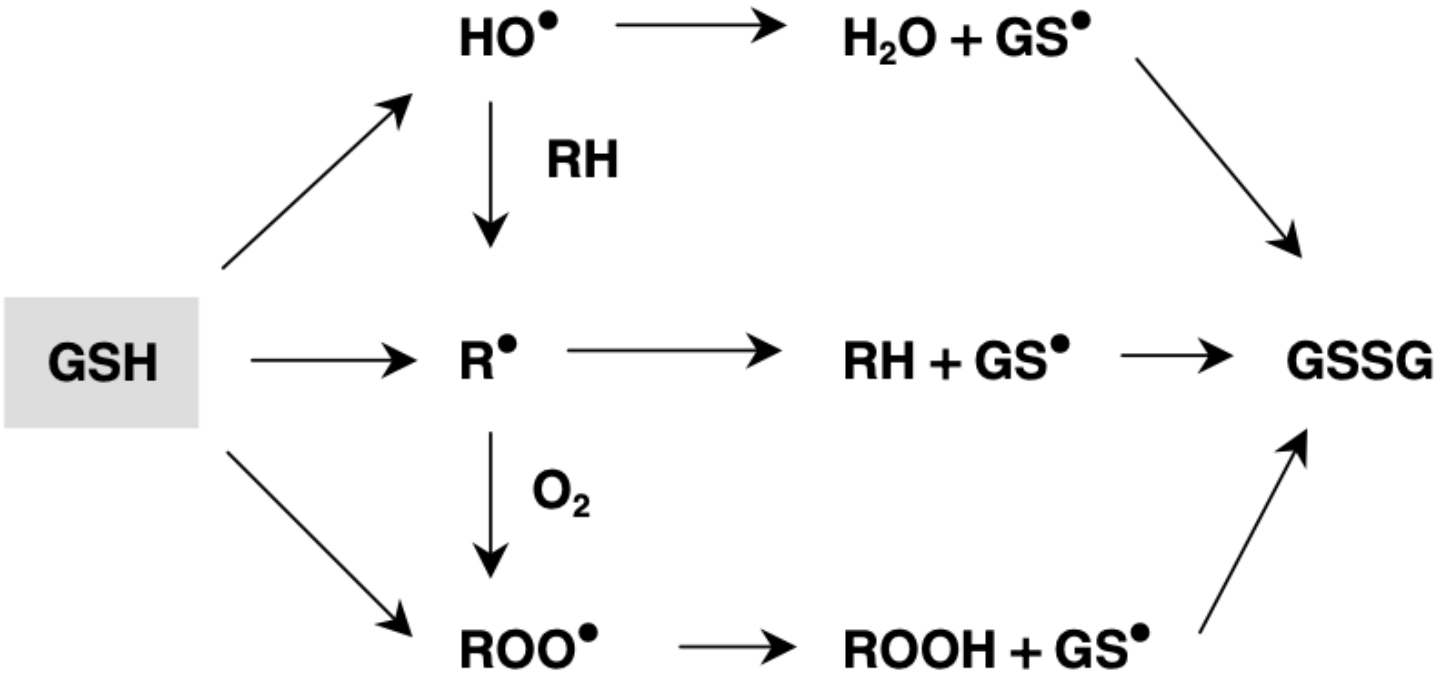
1. GSH has a reactive sulfhydryl group (cysteinyl), which is responsible for its antioxidant activities.
2. Glutamate and cysteine are not coupled via a peptide bond at the a-carbon (as in regular peptide), but the glutamyl moiety is attached to cysteine via its y-carboxyl group. This feature protects it from protease digestion.
3. In many tissues, GSH reaches high (i.e., millimolar) steady-state concentrations, and these high levels are maintained.

Mechanisms of GSH-mediated antioxidant activities

- As an antioxidant, GSH fulfills at least three pivotal tasks:

1. GSH is a radical scavenger (nonenzymatically).
2. GSH is a co substrate for the enzymatic (GS peroxidase-catalyzed) degradation of H2O2.
3. GSH keeps the cells in a reduced state and is involved in the regeneration of oxidized proteins.

Mechanisms of GSH-mediated antioxidant activities

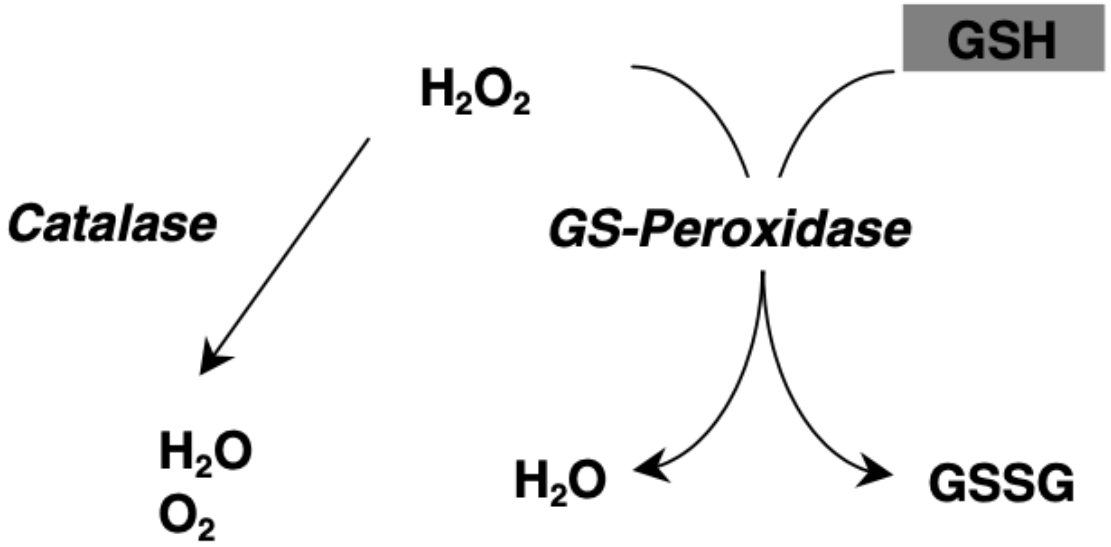


1. GSH is a radical scavenger and becomes oxidized to GSSG.

Mechanisms of GSH-mediated antioxidant activities

2. Enzymatic degradation of hydrogen peroxide by glutathione peroxidase.

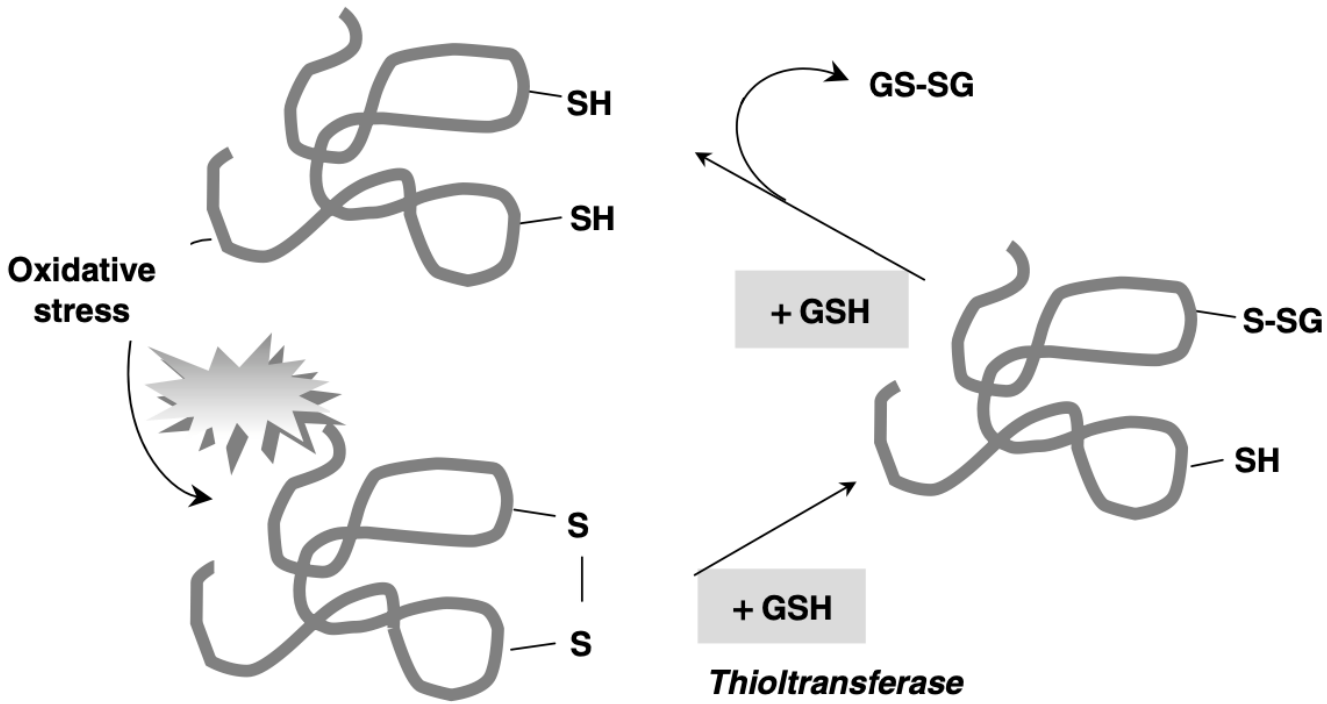
GSH is a substrate for glutathione peroxidase (GS-Px), and is involved in the degradation of hydrogen peroxide.



Mechanisms of GSH-mediated antioxidant activities

3. Reduction of oxidized sulfhydryl groups in proteins by GSH.

- GSH can regenerate oxidized proteins by reducing disulfide bonds back to the SH by thiol reductase.
- The GSH/GSSG ratio in the ER is therefore kept at a much lower level than in the cytosol) **Why?**



Situations where GSH can become depleted.

1. extensive conjugation to reactive electrophilic metabolites during exposure to xenobiotic-induced increased oxidant stress.
2. nutritional status (GHS levels are low after fasting)
3. diurnal changes (GHS levels are low at night)
4. genetic cases due to a deficiency of glucose-6-phosphate dehydrogenase.

GSH levels in the liver and the GSH de novo synthesis are lowest at the end of the day. This chronotoxicological characteristic can explain the quite impressive differential toxicity induced by a single dose of APAP given either in the morning or early in the night

TABLE 6.4
Hepatic Levels and Cytoprotective Effects of GSH against Acetaminophen Toxicity Are Dependent on the Time of Administration

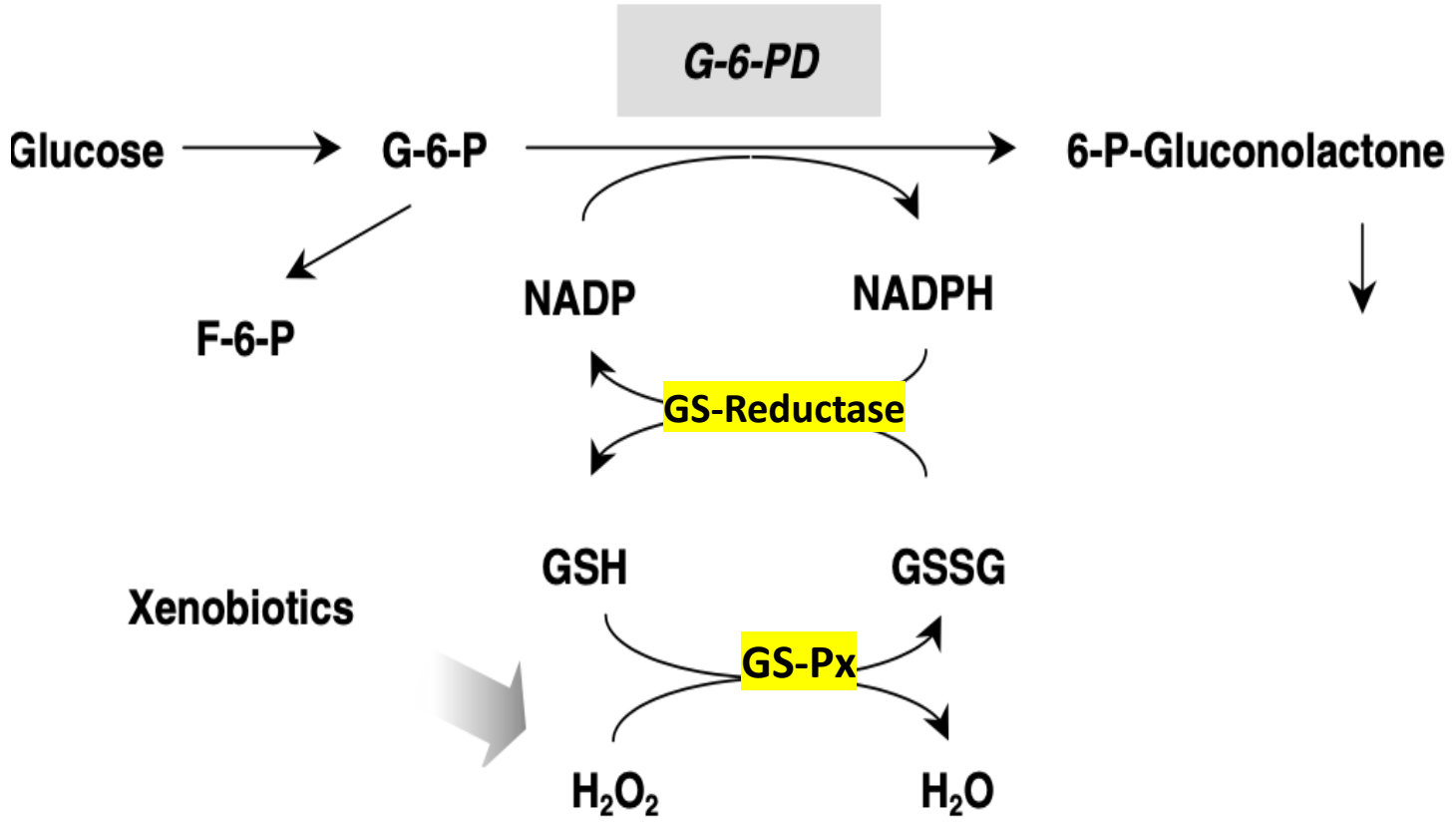
Time of APAP Administration	Plasma ALT Activity (U/l)	Hepatic GSH Content (μmol/g liver)	
		Before APAP	After APAP (3 h)
8 A.M.	27 ± 3	8.7 ± 0.2	4.2 ± 0.6
2 P.M.	70 ± 28	6.8 ± 0.4	2.4 ± 0.5
8 P.M.	3451 ± 1036	6.1 ± 0.4	1.5 ± 0.2

Note: Mice were treated with a single dose of APAP (400 mg/kg, i.p.). Plasma ALT activities were determined 24 h post administration. Data are mean + SEM ($n = 6$ to 13). There were no differences in plasma concentrations of APAP or its metabolites in the different treatment groups.

Genetic deficiency in erythrocyte G6PDH

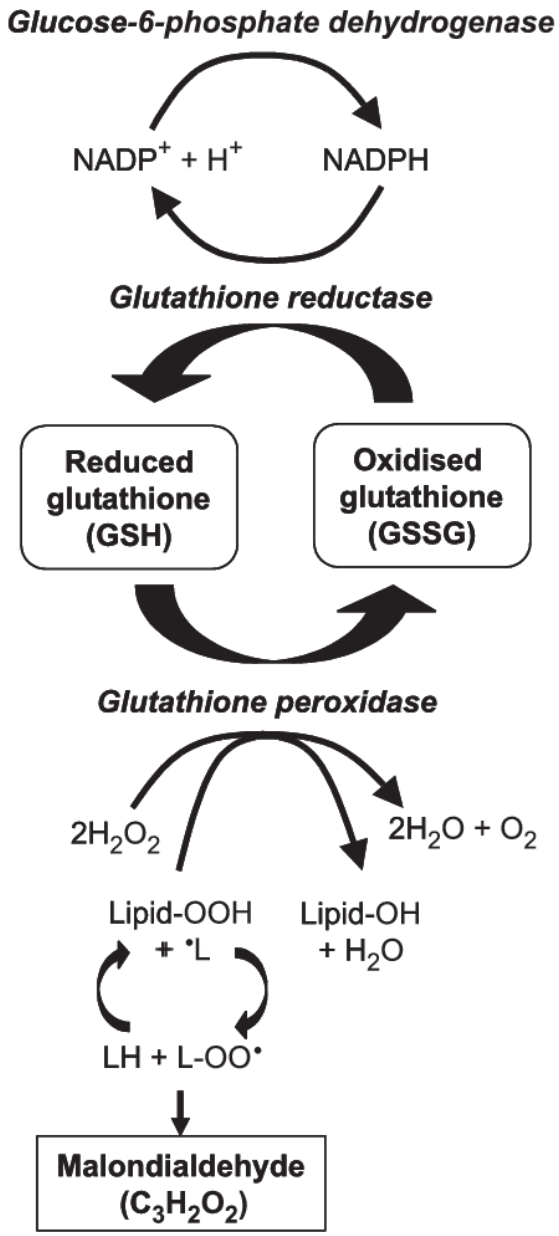
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is apparently the most frequent pharmacogenetic polymorphism in human populations.

G6PD catalyzes the production of NADPH and thus indirectly drives the reduction of GSSG to GSH.



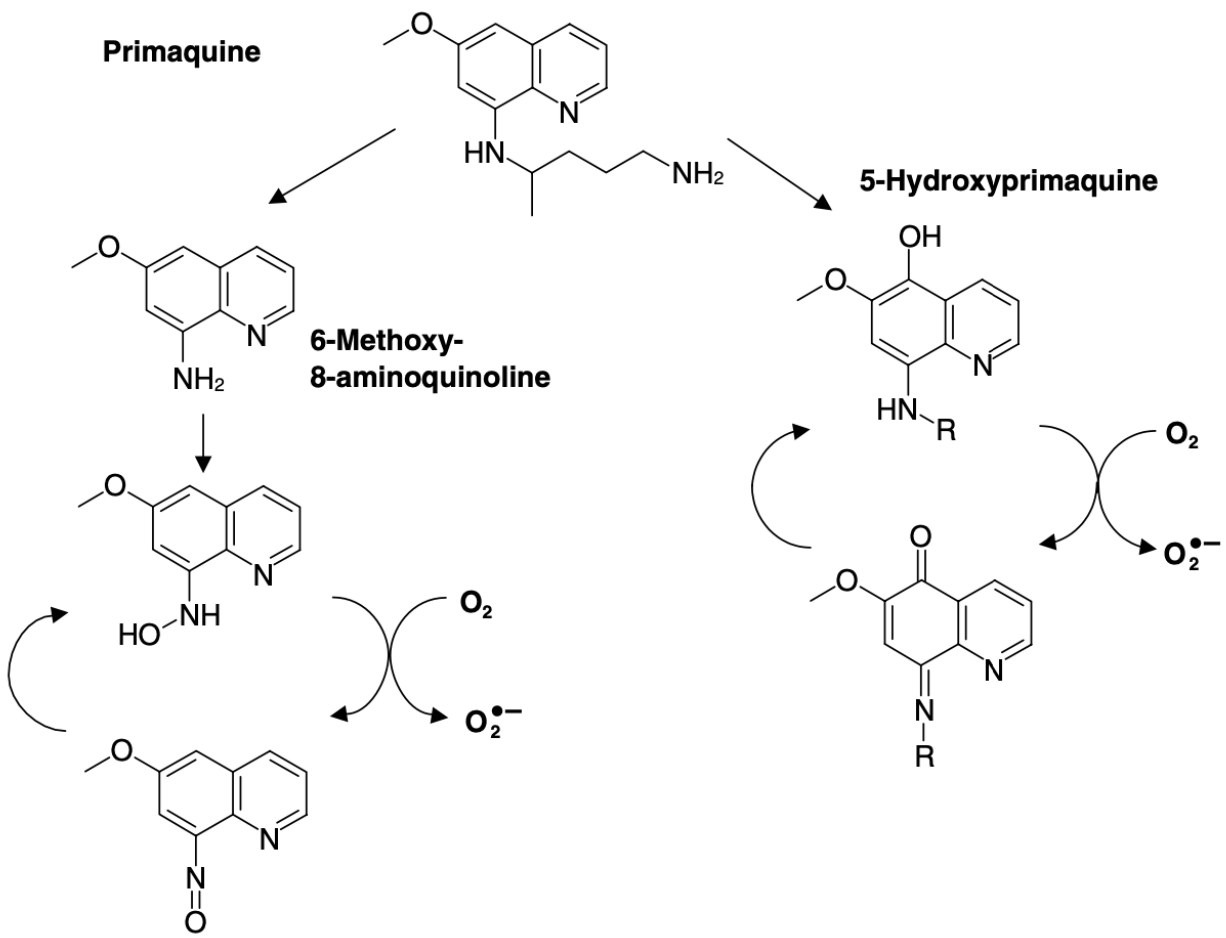
Genetic deficiency in erythrocyte G6PDH

- GS-Px is dependent on GSH, and catalase requires NADPH to remain in a functional state. Consequently, hydrogen peroxide can only be incompletely degraded under G6PDH deficiency
- under xenobiotic-enhanced production of large amounts of ROS, the residual antioxidant system is no longer capable of coping with the oxidoreductive stress, and erythrocyte injury ensues (**such as hemotoxicity following ingestion of the antimalarial drug primaquine)**



Mechanisms of primaquine hemotoxicity:

- primaquine is extensively metabolized to form: an 8- amino metabolite. This arylamine is further oxidized to the N-hydroxy derivative. It is possible that the redox cycling between the N-hydroxy and the nitroso form poses an oxidative stress in red cells that could damage the cell if it is uncompensated.
- Alternatively, the 5-hydroxylation of primaquine also forms a potential redox-cycling active pair.

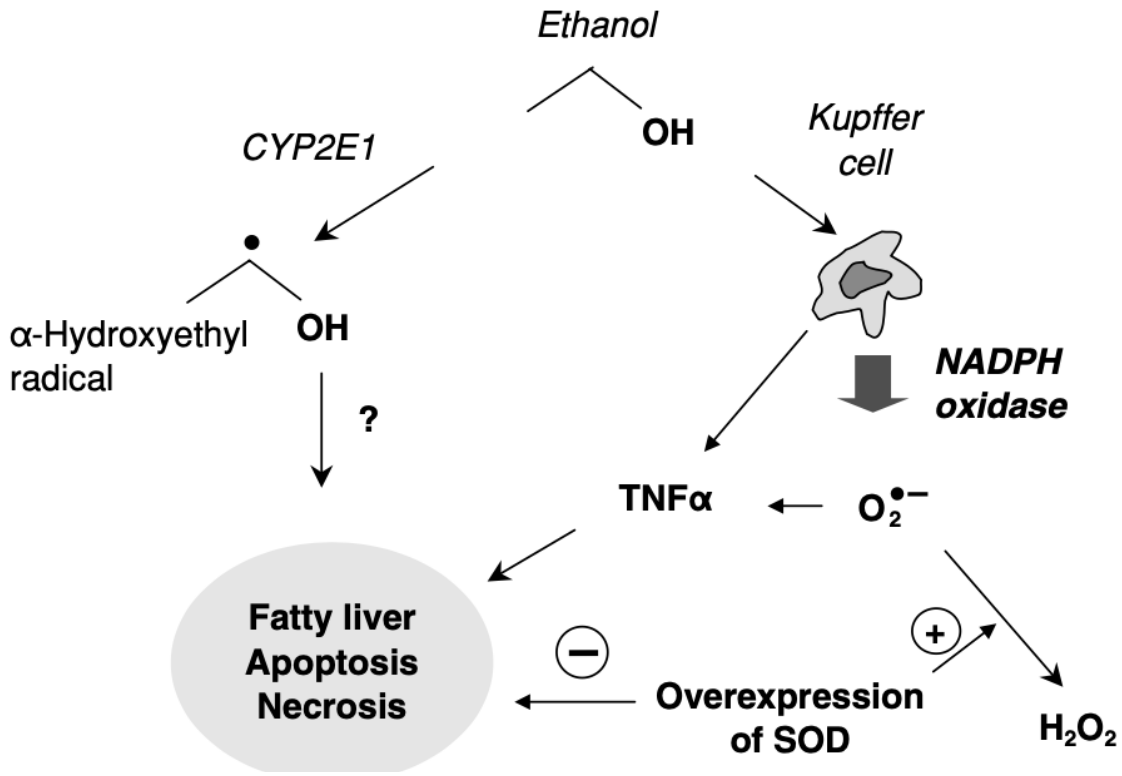


In cells with compromised NADPH-regenerating capabilities, this leads to hemolysis.

2. Superoxide dismutase

An example that illustrates that SOD plays a paramount and direct role as an antioxidant is ethanol-induced liver injury.

- Ethanol increases the permeability of the intestine for G- ve bacterial endotoxin (LPS).
- In the blood, LPS can activate Kupffer cells in the liver to release both ROS and proinflammatory cytokines, including TNFa.
- The produced superoxide anion radical dismutates to H2O2 (which is rapidly inactivated by both catalase and GS-Px).
- In contrast, superoxide anion, if not eliminated by dismutation, can activate a number of signaling pathways that aggravate the oxidative stress.



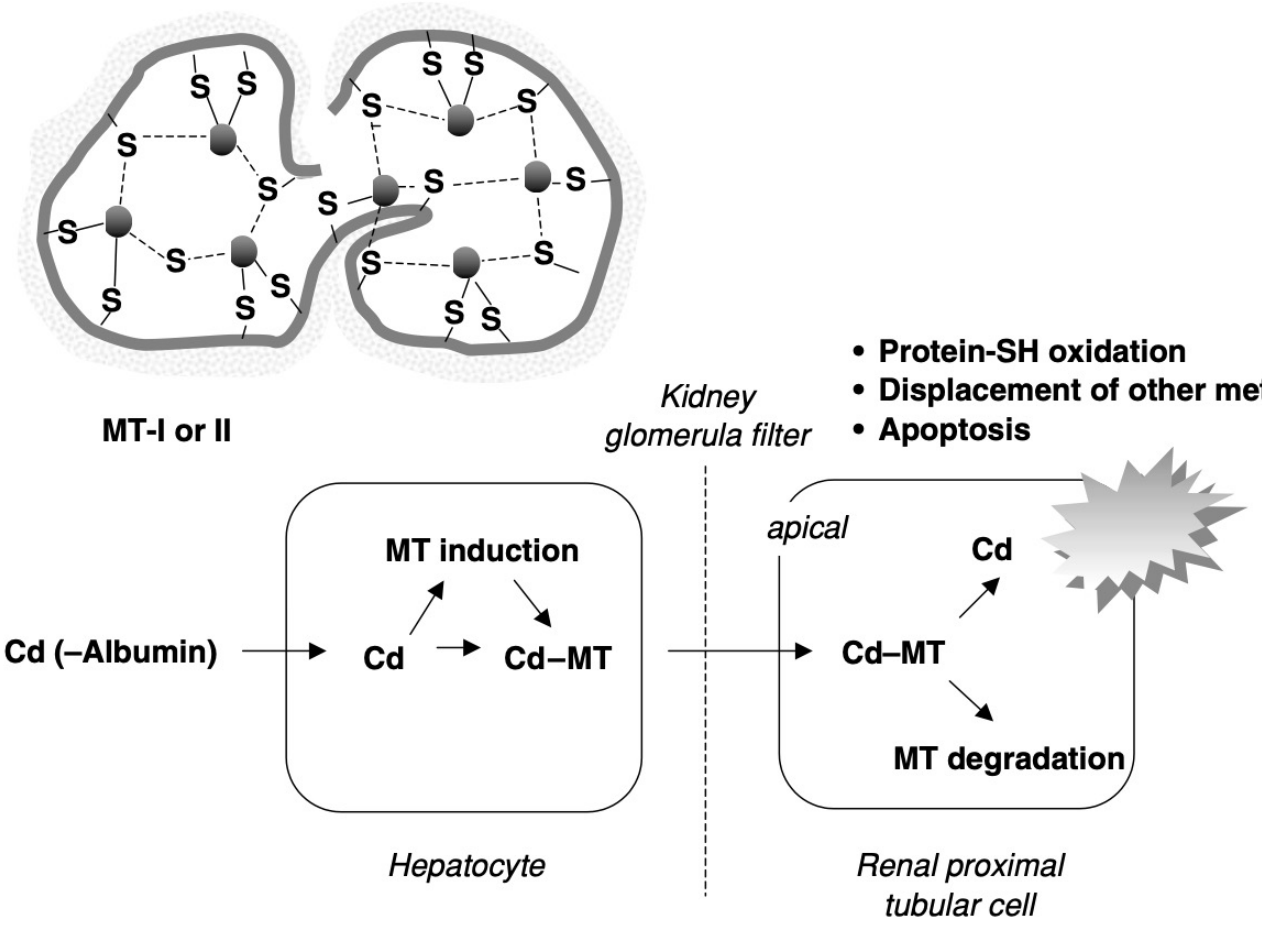
Kupffer cell-mediated production of superoxide anion by ethanol. The resulting OS can be prevented by overexpression of SOD.

3 Metallothionein

- MT is a ubiquitous low-molecular-weight (6.5 kDa) protein.
- Its most unique feature is that 30% of the amino acid residues of MT are cysteines. Therefore, the protein has a **large number of sulfhydryl groups that can be coordinated by heavy metal ions.** In fact, one molecule of MT can bind seven Cd atoms.
- Thus, **MT can sequester heavy metals and prevent oxidation of critical protein or nonprotein (GSH) thiol groups**.

Mechanisms of metallothionein induction and Cd-induced nephrotoxicity:

- The constitutive levels of MT are low, but MT is readily induced upon a variety of cellular stresses, including exposure to metals. How?.
- The Cd-MT complex is excreted from the liver and reaches the systemic circulation. Due to the small size of MT, the complex can cross the glomerular filter and accumulate over time in the tubular cells.

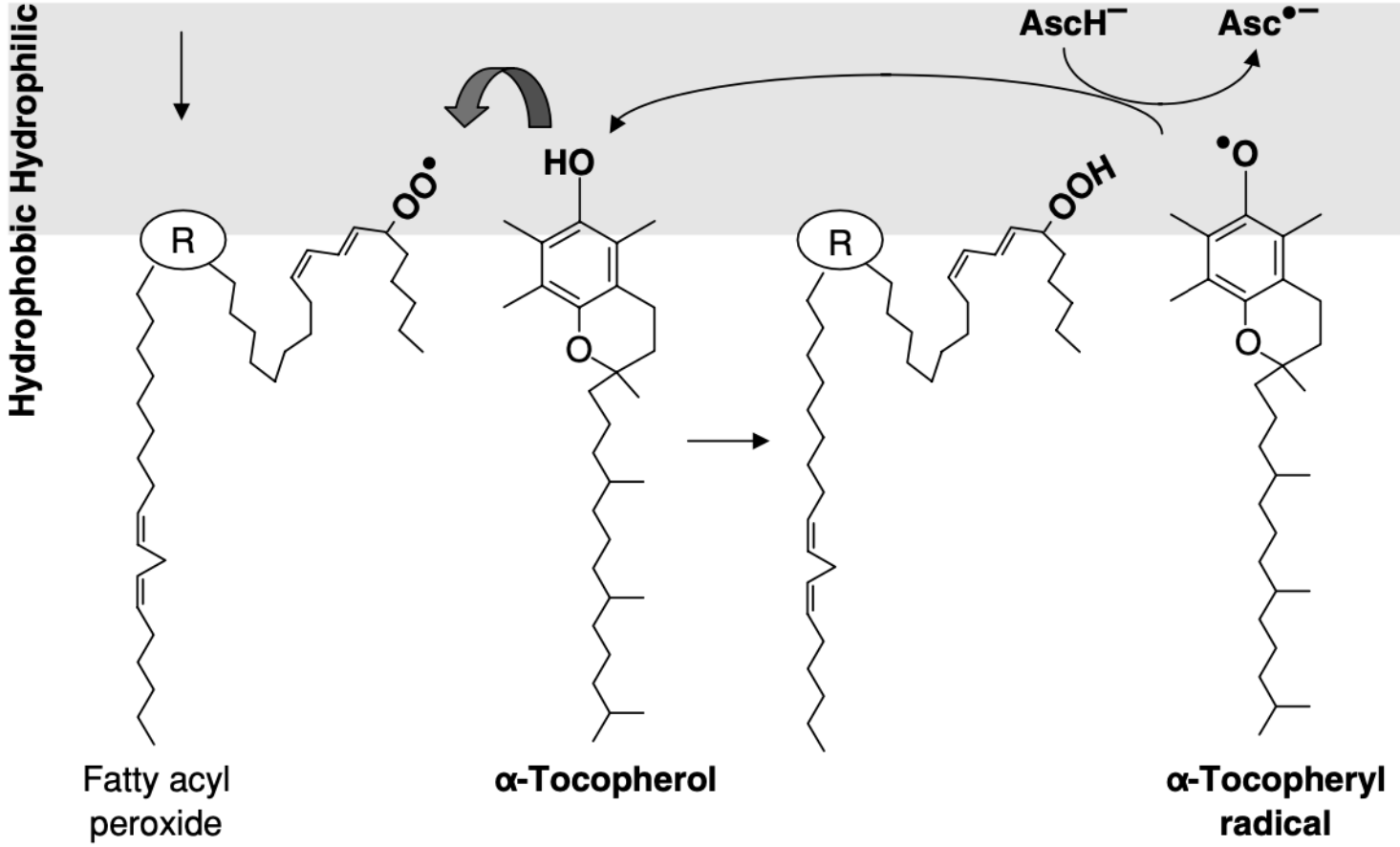


- Upon lysosomal degradation of the protein, the Cd is liberated and, exert its nephrotoxic effects. These are mediated by oxidation of critical sulfhydryl groups and by a displacement of other ions from MT.

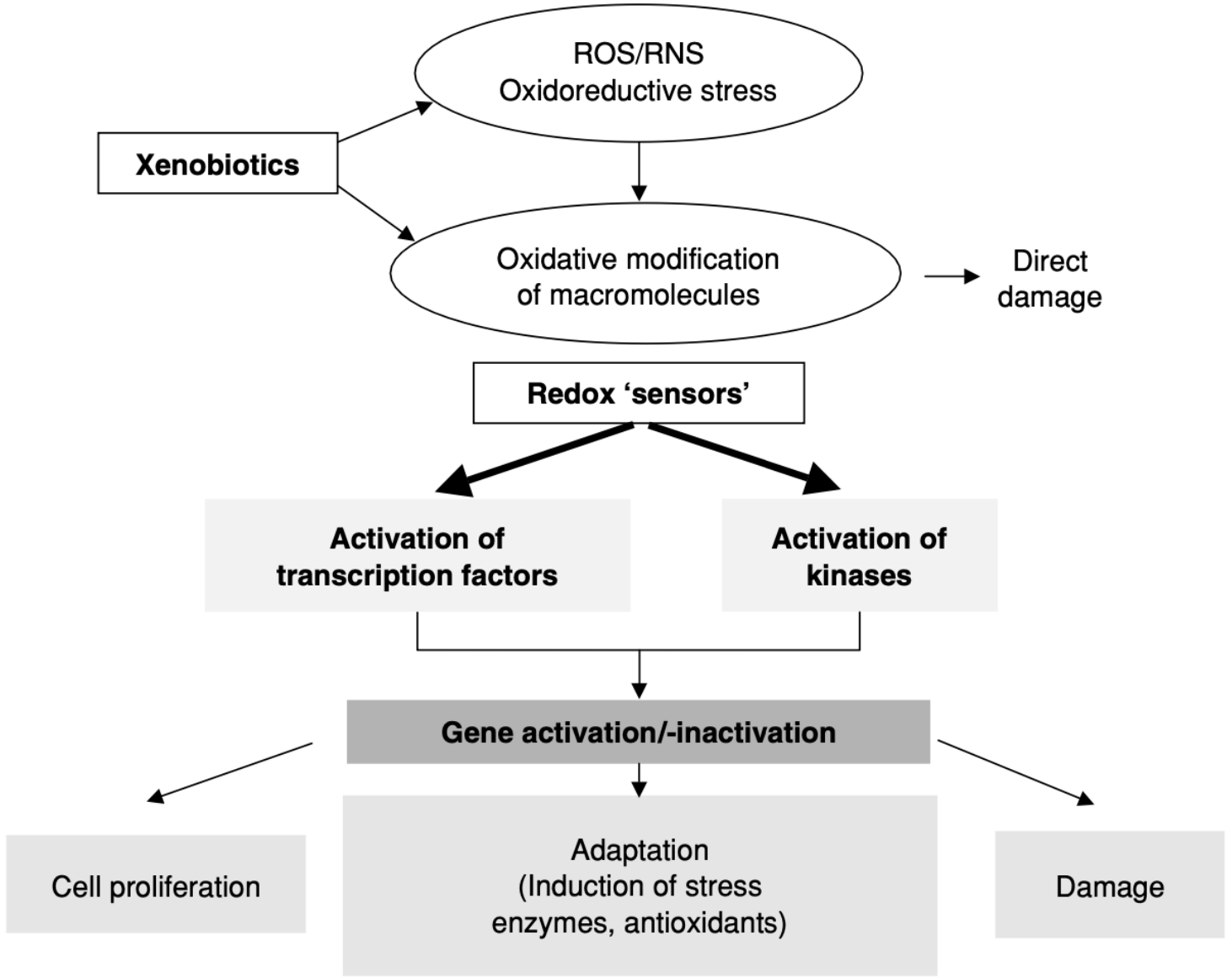
4. α-TOCOPHEROL

- Specific antioxidant defense systems in the cell's hydrophobic environment exist to prevent the propagation of lipid peroxidation. The major one is "vitamin E," which is actually a mixture of several tocopherols. Among these, a- tocopherol is the most important. a-Tocopherol consists of a lipophilic and a hydrophilic phenolic group.

- This phenolic group can reduce radicals (lipid peroxy radicals) arising from lipid peroxidation and is thereby oxidized in turn to the tocopheryl radical (nonreactive). It is regenerated by ascorbate, which in turn is kept in its reduced state by GSH

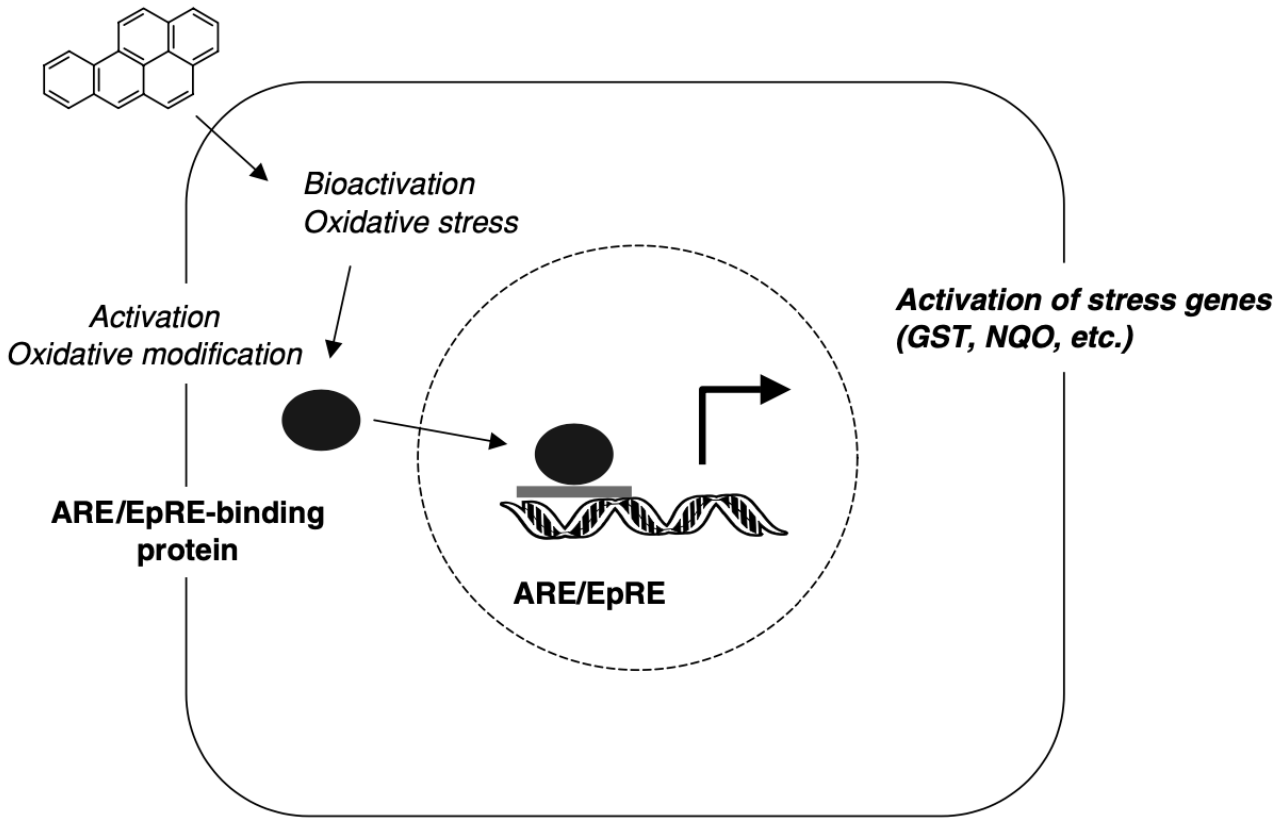


Intracellular signaling and gene regulation by oxidative stress



Mechanisms of oxidative stress-mediated gene activation

An example that illustrates how the production of oxidative stress in a cell can activate a number of genes involved in an antioxidant or stress response is polycyclic aromatic hydrocarbons (PAHs). For example, benzo[*a*]pyrene is subject to redox cycling following metabolic activation.



- Oxidative stress (and electrophilic metabolites) of benzo[*a*]pyrene can modify and thus activate **ARE/EpRE-binding protein**. This complex translocates into the nucleus where it binds to the ARE/EpRE (antioxidant/electrophilic response element), located in the promoter region of genes involved in antioxidant and stress response.

Redox sensors in the cell are modified by prooxidant xenobiotics. They can in turn activate transcription factors, or activate redox- sensitive kinase cascades, and thus activate specific sets of genes.

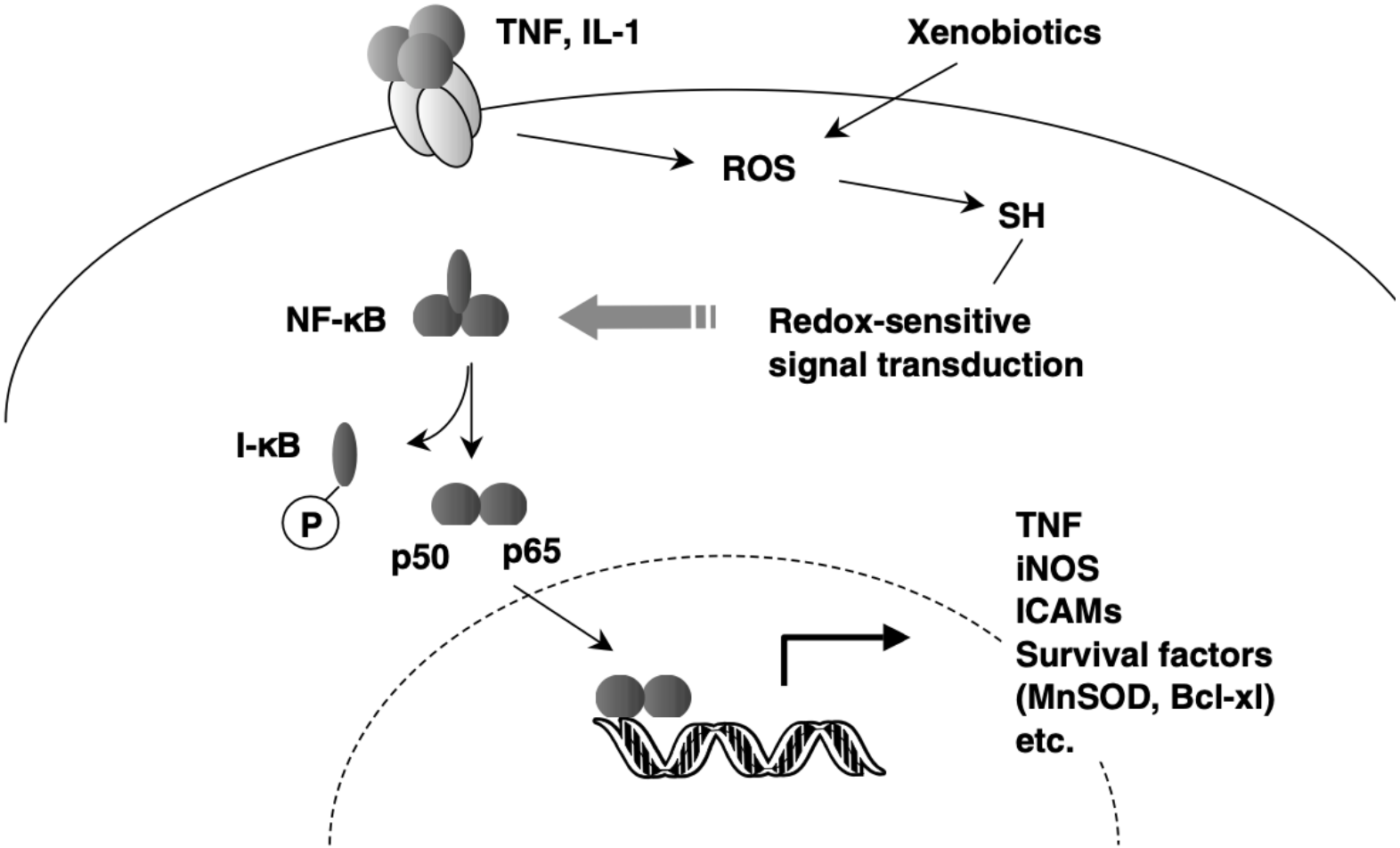
1. **HO-1 (heme oxygenase) .**
2. **NF-κB (nuclear factor-κB)**
3. **AP-1 (activator protein-1)**
4. **Nrf2**

- **Heme oxygenase-1 (HO-1)** is the rate-limiting enzyme in the degradation of heme, converting it to biliverdin. Biliverdin in turn is degraded by biliverdin reductase to bilirubin. Both biliverdin and bilirubin are potent antioxidants that can scavenge reactive oxygen species (ROS) and protect cells from oxidative damage.
- **Nrf2**: is a member of transcription factor family. It is kept in an inactive state by the protein **Keapl 1**. Upon activation by ROS, Keapl releases Nrf2, which then can transmigrate into the nucleus, bind to the ARE, and transactivate antioxidant genes.

- **NF-κB** is a transcription factor that is activated by a number of stimuli including oxidative stress. It plays a central role in the regulation of many genes involved in antioxidant defense, anti-apoptosis, immunological responses, cytokine production, and others. In the promoter region of all these NF-κB-responsive genes there is a functional NF-κB-binding site.

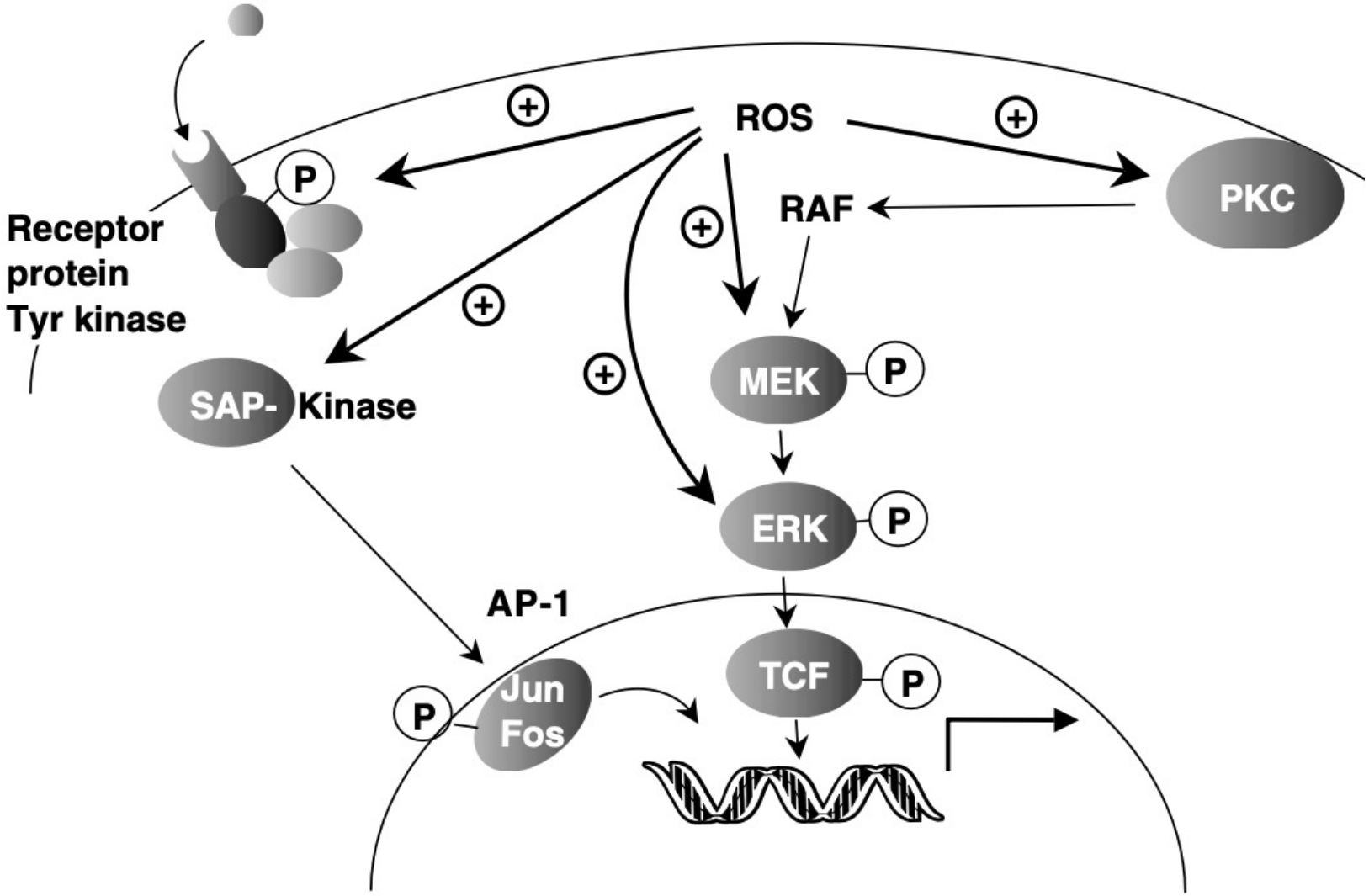
The transcription factor NF-kB is activated by oxidative stress and induces the transcription of target genes.

The inactive form of NF-κB is found in the cytoplasm and is kept in its inactive form by an inhibitor protein, I-κB. Upon stimulation, I-κB is phosphorylated, ubiquitinated and rapidly degraded. The free NF-κB then rapidly translocates into the nucleus, where it binds to the DNA.



Mechanisms of oxidative stress-mediated signal transduction:

- Xenobiotic- induced oxidative stress not only induces the transcription of many genes but also regulates the activity of preexisting proteins. In particular, prooxidant xenobiotics can be involved in many forms of signal transduction.
- Signal transduction is the transmission of a molecular signal triggered by hormones, growth factors, cytokines, etc. at a cell surface receptor and/or via second messengers all the way down to a final metabolic target or to a genetic target in the nucleus.
- These signaling pathways often proceed via self-amplifying cascades of protein kinases and phosphatases. Interestingly, ROS and increased oxidative stress can mimic some of these pathways and induce cell proliferation in many cells.
- ROS can regulate the signal transduction because many of the proteins involved in transmitting these signals exhibit critical and reactive cysteinyl residues, the redox status of which is crucial for their activity.



ROS activate the protein kinase cascade by oxidizing (and thus activating) the kinases' thiol groups. Mitogen-activated protein (MAP) kinase pathways include (ERK) and (SAP) kinases, as well as upstream and downstream kinases.