

Removal of Free Radicals

Cellular antioxidant defenses and oxidative stress

Mechanisms of cell injury

Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

www.abusuraisakhri.com

Learning objectives

- Identify the chemical species that must be controlled during oxidative stress.
- Explain nonenzymatic antioxidants and sequestration of iron and copper.
- Trace superoxide disposal through SOD, catalase, and glutathione peroxidase.
- Predict cellular consequences when production exceeds antioxidant capacity.

Why cells remove free radicals

Removing oxidants preserves cellular macromolecules

Normal physiology

Reactive species arise during routine metabolism and regulated cell functions.

Injury prevention

Scavenging prevents excess oxidants from damaging membranes, proteins, and DNA.

Free radicals are not identical to ROS

Free radical

- Contains an unpaired electron
- High chemical reactivity can modify nearby molecules
- Examples: $\text{O}_2\bullet^-$ and $\bullet\text{OH}$

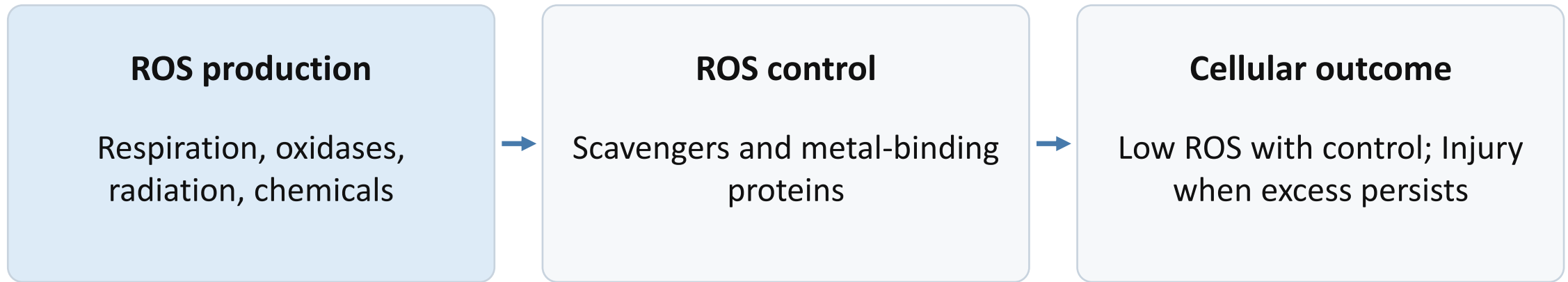
Reactive oxygen species (ROS)

- Broader oxygen-derived oxidant category
- Includes free radicals and nonradical oxidants
- H_2O_2 is an ROS but not itself a free radical

Principal reactive species in cell injury

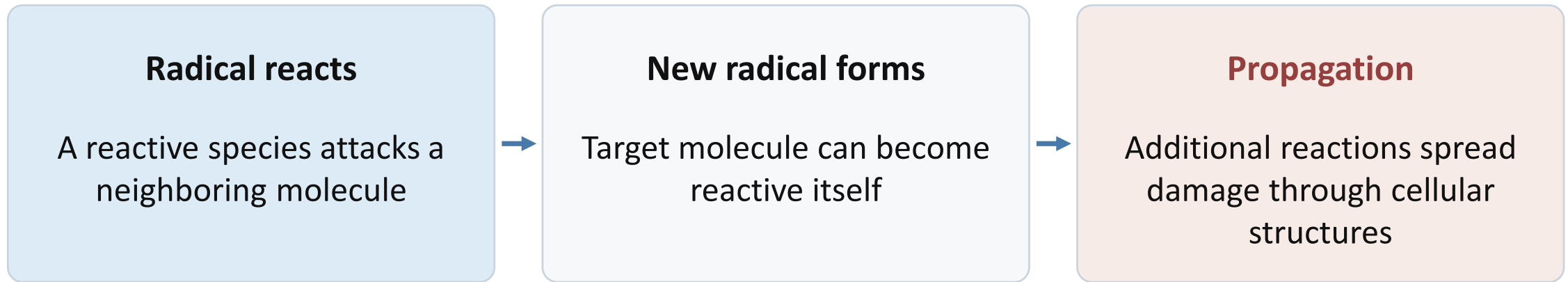
Species	Formation / identity	Why it matters
$\text{O}_2\bullet^-$ (superoxide)	Partial reduction of oxygen	SOD converts it to H_2O_2
H_2O_2 (peroxide)	SOD product; cellular oxidases	Detoxified by catalase or GPx
$\bullet\text{OH}$ (hydroxyl radical)	Radiation or Fenton reaction	Very reactive; no dedicated enzyme shown
ONOO^- (peroxynitrite)	Reaction of superoxide with NO	Reactive nitrogen/oxygen species; damages macromolecules

Oxidative stress is a balance problem



Oxidative stress occurs if oxidant generation exceeds removal capacity.

Why a single radical can start wider injury



Chain reactions make timely scavenging and prevention important.

Oxidant sources are diverse

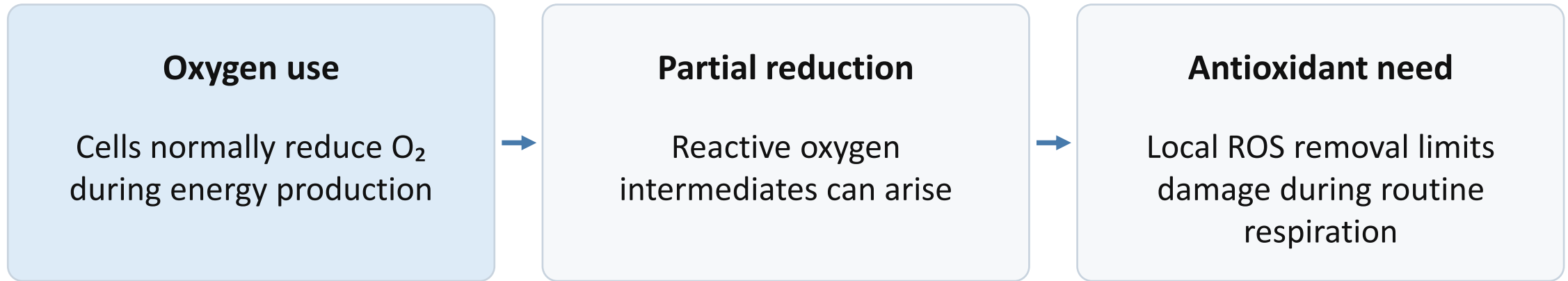
Endogenous metabolism

- Mitochondrial respiration partially reduces oxygen
- Intracellular oxidases also form superoxide
- Some ROS formation is a normal by-product

Inflammation / external insults

- Activated phagocytes deliberately produce ROS
- Ionizing radiation can generate $\bullet\text{OH}$ from water
- Chemicals and transition metals may amplify radical formation

Mitochondrial oxygen metabolism



This source explains why mitochondrial SOD is strategically localized.

Activated leukocytes generate ROS

Inflammation

Phagocytes are activated near microbes



NADPH oxidase

Regulated rapid oxidant generation



Microbial killing

ROS contribute to destruction of microbes and unwanted material

The aim is controlled antimicrobial activity, not zero ROS.

Ionizing radiation produces hydroxyl radicals

Radiant energy

X-rays can interact with cellular water



Water radiolysis

•OH and other radicals are generated



Cellular injury

Reactive species can damage biological macromolecules

Transition metals accelerate oxidant chemistry

Free iron and copper can catalyze radical formation

Risk

Unbound transition metals participate in redox cycling and oxidative reactions.

Control

Storage and transport proteins restrict the reactive free-metal pool.

Fenton chemistry generates hydroxyl radicals



The substrate

Hydrogen peroxide becomes dangerous when reactive ferrous iron is available.

The critical product

$\bullet\text{OH}$ is highly reactive and can rapidly modify adjacent molecules.

Physiologic functions versus injury

Controlled ROS generation

- Phagocyte antimicrobial reactions
- Regulated cellular signaling at low oxidant levels
- Oxidants are normally counterbalanced by scavengers

Excessive oxidative burden

- Accumulation of ROS exceeds detoxification
- Lipid, protein, and DNA injury develops
- Necrosis and apoptosis may follow severe damage

Cellular defenses act at three levels

Prevent formation

Metal-binding proteins limit radical-generating catalysis



Intercept oxidants

Small-molecule antioxidants scavenge reactive species

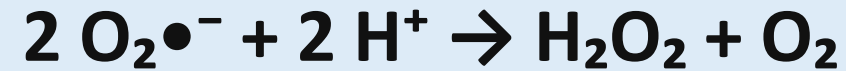


Enzymatic disposal

SOD, catalase, and glutathione peroxidase clear key oxidants

The layers are complementary, not interchangeable.

Superoxide can dismutate spontaneously



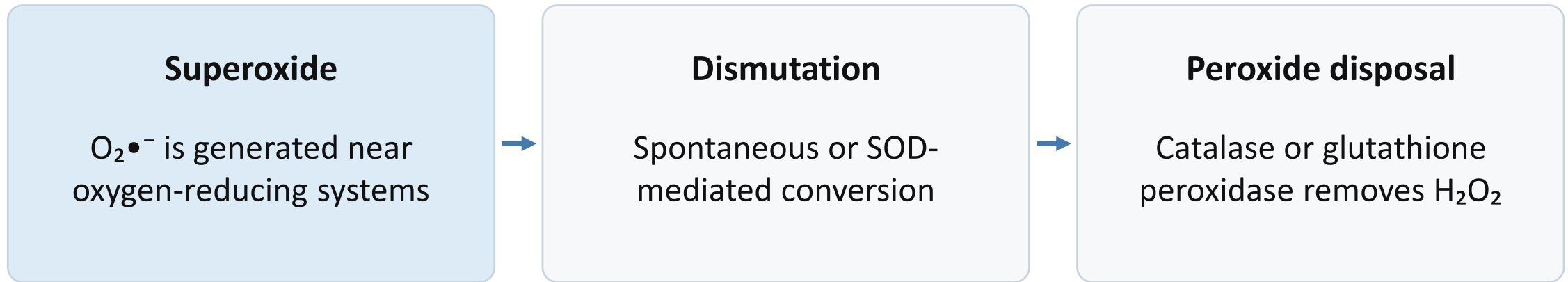
Spontaneous reaction

Superoxide is unstable and can decay in aqueous surroundings.

Important limitation

The reaction leaves H_2O_2 , which still requires cellular control.

Superoxide clearance is a two-stage problem



Lower superoxide concentration does not by itself establish complete detoxification.

Nonenzymatic defense categories

Defense	Textbook examples	Protective principle
Lipid-soluble antioxidant	Vitamins E and A	Antioxidant action within lipid-associated environments
Water-associated antioxidants	Ascorbic acid; glutathione	Interception of oxidants in cellular aqueous compartments
Metal-binding proteins	Ferritin, transferrin, lactoferrin, ceruloplasmin	Limit catalytically available Fe / Cu

Vitamin E and vitamin A are lipid-soluble

Lipid-soluble antioxidants contribute to radical control

Vitamin E

Named by Robbins among antioxidants capable of limiting free radical reactions.

Vitamin A

Also listed among lipid-soluble antioxidant defenses.

The source lists these vitamins; It does not quantify their relative clinical effects.

Ascorbate and glutathione are small antioxidants

Ascorbic acid

- Listed among cellular nonenzymatic antioxidants
- Participates in the broader small-molecule defense against oxidants
- Source does not specify a disease-specific treatment effect

Glutathione (GSH)

- Present in cytosolic antioxidant defenses
- Also used as the reducing substrate by glutathione peroxidase
- Its oxidized form is GSSG

Two ways an antioxidant can protect cells

Before radical formation

- Block conditions that favor radical generation
- Bind catalytic metals and limit electron transfer opportunities
- Acts upstream of cellular damage

After a radical has formed

- Scavenge or inactivate reactive molecules
- Interrupt the opportunity for additional target reactions
- Acts after formation but before widespread injury

Metal sequestration protects without scavenging ROS

Iron and copper

Metals may donate or accept electrons



Protein binding

Transport/storage proteins
reduce reactive metal
availability



Less catalytic chemistry

Fewer opportunities for new
ROS generation

This defense prevents radical formation rather than metabolizing H_2O_2 .

Transferrin and ferritin

Transferrin

- Iron transport protein listed as a protective metal binder
- Reduces the freely reactive metal pool
- Works upstream of hydroxyl radical formation

Ferritin

- Intracellular iron-storage protein listed by Robbins
- Sequesters iron away from redox catalysis
- Does not substitute for peroxide-removing enzymes

Lactoferrin and ceruloplasmin

Lactoferrin

- Named among proteins that restrain metal reactivity
- Illustrates protective metal binding
- Distinct from enzymatic removal of superoxide

Ceruloplasmin

- Named among metal-handling proteins
- Helps minimize free-metal-mediated chemistry
- Included in the preventative antioxidant layer

Unbound versus protein-bound metal

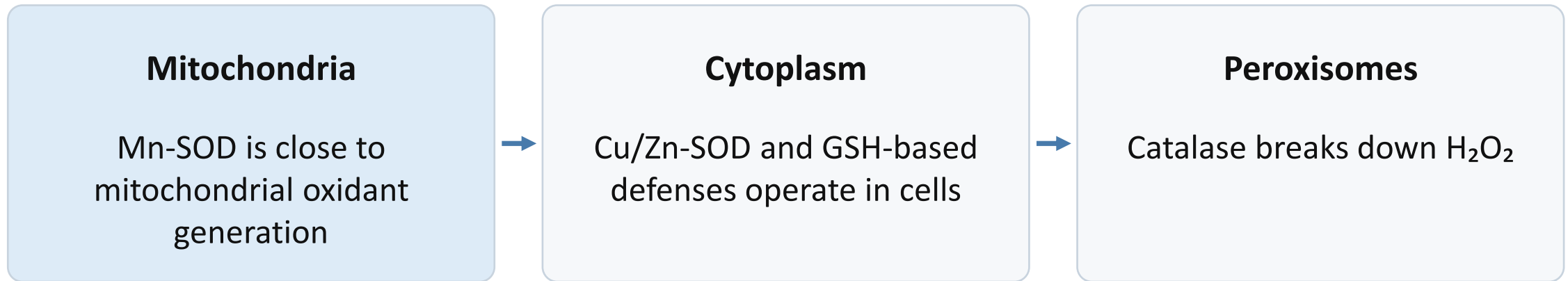
Reactive free metal

- May participate in reactions that form reactive oxygen intermediates
- Available Fe^{2+} supports Fenton chemistry
- Oxidant burden may increase

Protected protein-associated metal

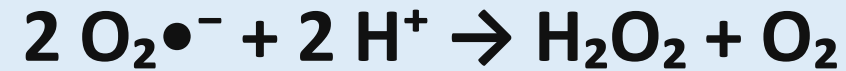
- Sequestration limits access to catalytic reactions
- Transport / storage becomes chemically safer
- Protection is indirect but mechanistically important

The enzyme defense system is compartmentalized



Location helps explain why oxidant control is organized, not random.

Superoxide dismutase: The substrate and product



Substrate

Two superoxide anions are converted during dismutation.

Products

Hydrogen peroxide and oxygen; Peroxide requires downstream removal.

Two SOD systems

Enzyme	Primary localization	Relevant protective action
Enzyme	Primary localization	Relevant protective action
Mn-SOD	Mitochondria	Disposes of mitochondrial superoxide
Cu/Zn-SOD	Cytoplasm	Converts superoxide formed in cytoplasmic settings

Manganese superoxide dismutase

Mn-SOD protects the mitochondrial compartment

Why mitochondria?

Mitochondrial oxygen metabolism can form partially reduced oxygen species.

What Mn-SOD does

It promotes superoxide dismutation where this oxidant arises.

Copper–zinc superoxide dismutase

Cu/Zn-SOD provides cytoplasmic superoxide removal

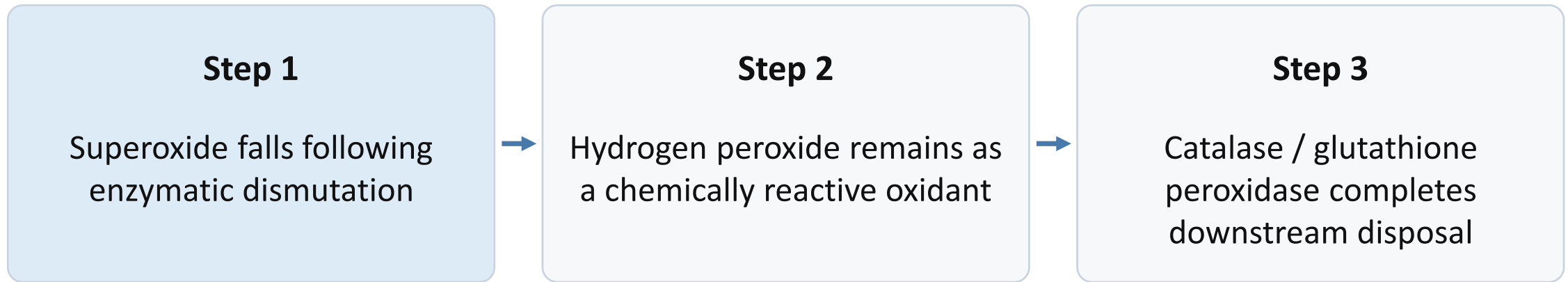
Substrate

Cytoplasmic $\text{O}_2\bullet^-$ is converted into H_2O_2 and oxygen.

Next step

Peroxide must subsequently be metabolized by appropriate scavenging systems.

SOD is necessary but not the endpoint



SOD and peroxide-metabolizing enzymes form a functional sequence.

Catalase decomposes hydrogen peroxide



Localization

Catalase is present in peroxisomes.

Protective role

Decomposition of H_2O_2 limits oxidant accumulation.

Peroxisomes contain catalase

Peroxisomal catalase converts H_2O_2 into stable products

Input

Hydrogen peroxide generated by cellular oxidase reactions or SOD-dependent conversion.

Output

Water and molecular oxygen; Catalytic removal of peroxide.

Glutathione peroxidase uses reduced glutathione



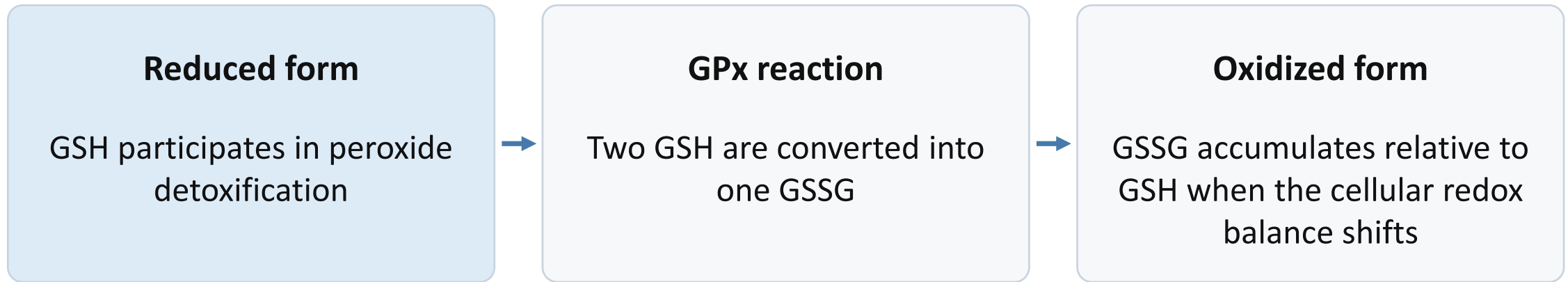
Electron donor

GSH supplies reducing equivalents during detoxification.

Oxidized product

GSSG is oxidized glutathione; The reaction reduces peroxide to water.

Glutathione cycles between redox states



GSH/GSSG status as a useful indicator of oxidative state.

Reduced versus oxidized glutathione

GSH: Reduced form

- A reactant in the glutathione-peroxidase reaction
- Represents reducing capacity available for H_2O_2 breakdown
- Used up as substrate during this reaction

GSSG: Oxidized form

- Product formed when glutathione is oxidized
- Read with GSH to understand cellular oxidation status
- An increased GSSG/GSH ratio indicates a more oxidizing state

The glutathione redox ratio is an indicator

GSSG/GSH reflects the oxidative state of the cell

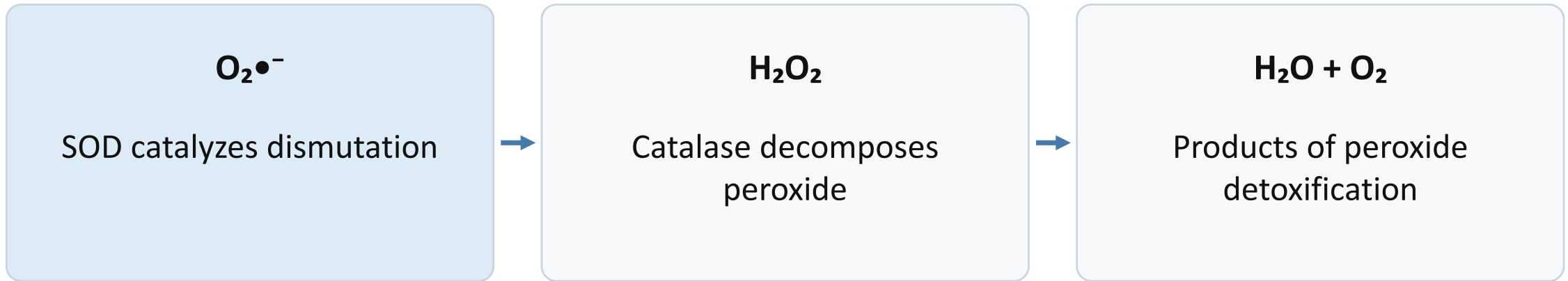
Higher oxidized fraction

A relative shift toward GSSG indicates a more oxidized glutathione pool.

Interpretation limit

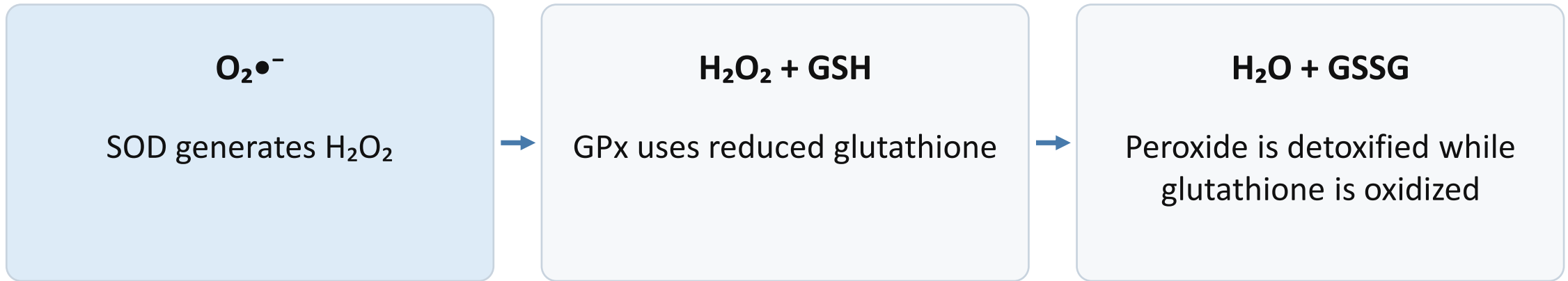
The ratio is an indicator of redox state, not a stand-alone diagnosis of a particular disease.

Integrated pathway: SOD followed by catalase



Two enzymes manage sequential oxidants produced by oxygen metabolism.

Integrated pathway: SOD followed by GPx



The GSH/GSSG balance supplies information about the redox burden.

Defense distribution across cellular locations

Location	Source-aligned antioxidant system	Chemical task
Mitochondria	Manganese-SOD; glutathione peroxidase	Restrict superoxide and peroxide
Cytoplasm	Copper–zinc-SOD; GSH-related systems	Limit cytoplasmic oxidative stress
Peroxisomes	Catalase	Break down H ₂ O ₂
Storage / transport compartments	Iron- and copper-binding proteins	Restrict metal-catalyzed radical formation

Compare the major removal pathways

Mechanism	Acts primarily on	Main outcome
Spontaneous dismutation	Superoxide ($\text{O}_2\bullet^-$)	$\text{H}_2\text{O}_2 + \text{O}_2$
Superoxide dismutase	Superoxide ($\text{O}_2\bullet^-$)	$\text{H}_2\text{O}_2 + \text{O}_2$
Catalase	Hydrogen peroxide	$\text{H}_2\text{O} + \text{O}_2$
Glutathione peroxidase	Hydrogen peroxide + GSH	$\text{H}_2\text{O} + \text{GSSG}$
Antioxidants / metal binding	Radicals or their upstream formation	Intercept oxidants / limit new ROS

Antioxidant protection has finite capacity

Generation increases

More reactive molecules
appear per unit time



Removal is outpaced

Free radicals persist or
propagate reactions

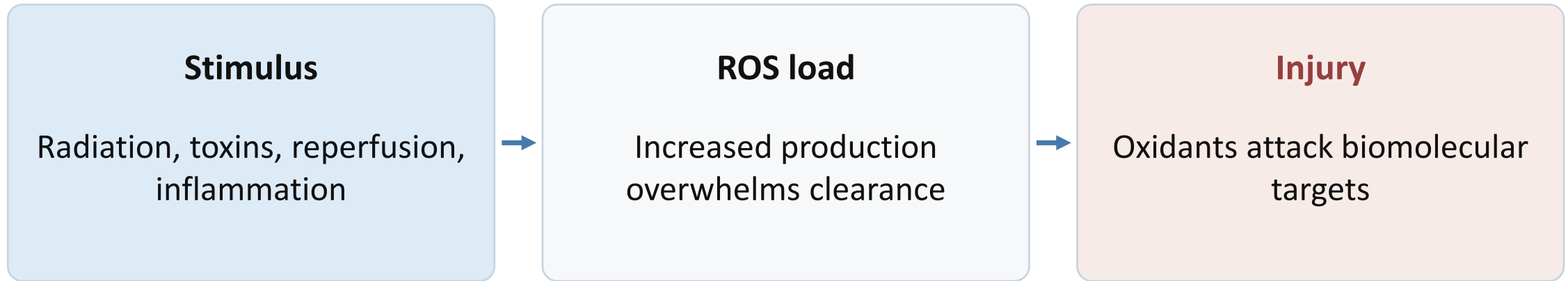


Macromolecular injury

Membrane, protein, and DNA
damage may occur

This imbalance as oxidative stress.

Oxidative stress: Increased production



Oxidative stress: Decreased removal

Defense deficit

Lower effective scavenging
capacity



Oxidant persistence

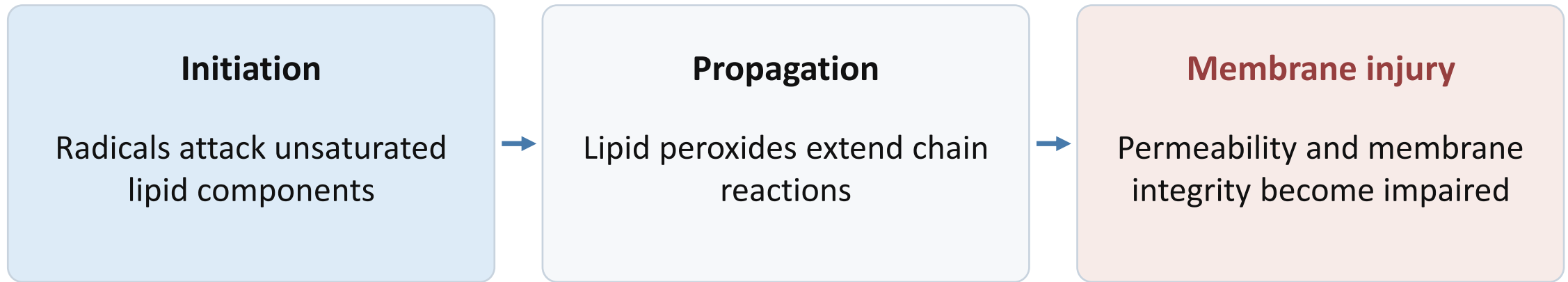
Normal or elevated ROS is
inadequately cleared



Injury

Oxidative modification
proceeds despite continued
generation

Uncontrolled ROS can peroxidize membrane lipids



This is a core downstream consequence of insufficient free-radical removal.

Membranes are high-value antioxidant targets

Lipid peroxidation compromises cell and organelle membranes

Mechanistic link

Membrane phospholipid modification destabilizes barriers and organelles.

Possible consequences

Membrane dysfunction contributes to worsening cellular injury and necrosis.

Free radicals chemically modify proteins

Oxidative attack

Amino-acid side chains /
backbones are modified



Structural effects

Cross-linking and unfolding
can follow



Functional loss

Enzymes and structural
proteins may malfunction

Proteins: Immediate and downstream effects

Immediate oxidative changes

- Side-chain oxidation
- Covalent interprotein cross-links
- Oxidation of protein backbones

Cell-level consequences

- Altered enzyme active sites
- Misfolded or unfolded protein accumulation
- Enhanced proteasomal degradation

Reactive species can damage nuclear DNA

DNA oxidation

ROS alter DNA chemical structure



Lesions

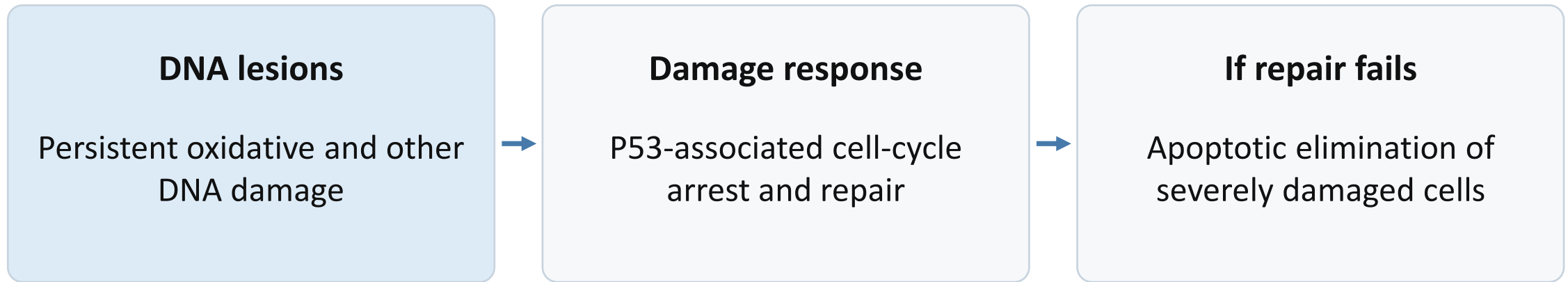
Strand breaks, cross-links, and adducts



Consequences

Damage responses; Potential mutation if not corrected

DNA damage initiates protective decisions



The source connects DNA damage, p53 responses, and apoptosis.

ROS injury can lead to multiple death pathways

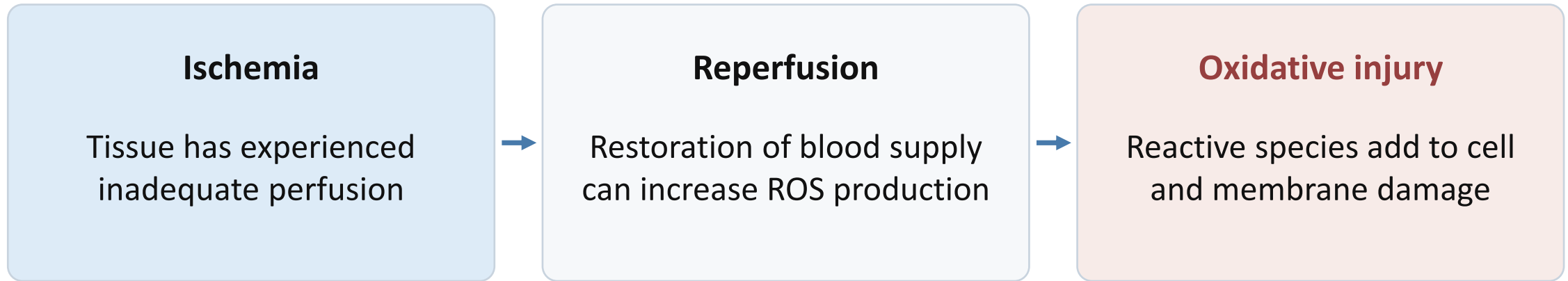
Necrosis

- Extensive cellular and membrane injury can culminate in necrosis
- Oxidative destruction contributes to loss of cell integrity

Apoptosis

- ROS can also initiate programmed cell-death pathways
- DNA damage signaling is one established route toward apoptosis

Ischemia-reperfusion: A setting for oxidative injury



External causes illustrate distinct ROS routes

Radiation injury

- Radiation can split cellular water
- •OH radicals are generated
- DNA and other cell components are vulnerable

Chemical injury

- Metabolism of some chemicals generates radicals
- CCl₄ can yield •CCl₃ radicals
- Not all toxic radicals are oxygen-derived

Inflammatory ROS: Useful and potentially harmful

Host defense

- NADPH oxidase deliberately produces oxidants
- Phagocytes use ROS to kill microbes
- Production is spatially and temporally regulated

Collateral injury

- Inflammatory ROS can contribute to surrounding tissue damage
- Protection depends on effective oxidant control
- Excess oxidative stress may injure host cells

Oxidative stress is implicated in chronic disease

A persistent oxidant imbalance has broad pathologic relevance

Textbook-associated processes

Cell injury, aging, cancer, and selected degenerative disorders.

Scientific caution

An association with oxidative stress does not by itself establish a specific therapy or causal contribution in every case.

The aim is controlled redox physiology

Adequate ROS removal—not complete ROS elimination

Protective purpose

Detoxification prevents excessive damage to lipids, proteins, and DNA.

Physiologic requirement

Some ROS are useful in host defense and cellular signaling.

Checkpoint: Which pathway removes H_2O_2 ?

A cell has converted superoxide to hydrogen peroxide. Which reaction directly detoxifies the remaining peroxide?

- A. Superoxide dismutase generates more H_2O_2
- B. Catalase yields H_2O and O_2
- C. Iron-dependent Fenton chemistry produces $\bullet\text{OH}$
- D. Free iron is released from storage proteins

Checkpoint: Mechanistic answer

B. Catalase decomposes H_2O_2 into water and oxygen.

SOD processes superoxide, but its reaction produces peroxide rather than finishing removal.

Fenton chemistry generates hydroxyl radicals; Unbound iron therefore increases oxidative hazard.

Application: Mitochondrial oxidant burden

Consider a hypothetical cell with excess mitochondrial $O_2\bullet^-$. Which enzyme is directly positioned to accelerate its dismutation?

A. Catalase in peroxisomes

B. Manganese superoxide dismutase

C. Copper–zinc superoxide dismutase in cytoplasm

D. Transferrin-mediated iron transport

Application: Match location to reaction

B. Manganese-SOD acts in mitochondria.

Mitochondrial respiration can form superoxide; Mn-SOD converts $O_2\bullet^-$ into H_2O_2 and O_2 .

Cytoplasmic Cu/Zn-SOD performs analogous chemistry elsewhere; The peroxide still needs removal.

Application: Transition-metal catalysis

Consider two otherwise similar systems containing H_2O_2 . One has more freely reactive Fe^{2+} . What is the expected chemical risk?

- A. Less hydroxyl-radical formation
- B. Greater potential for Fenton-generated $\bullet\text{OH}$
- C. Direct conversion of H_2O_2 into GSSG without enzymes
- D. No change because iron cannot participate in ROS formation

Application: Explain the iron effect

B. More available Fe^{2+} permits more Fenton chemistry.

Fe^{2+} reacts with H_2O_2 to generate Fe^{3+} , hydroxyl radical, and hydroxide.

Binding proteins such as ferritin and transferrin lower metal availability for radical-forming reactions.

Frequent conceptual errors

Incorrect inference	More accurate interpretation
H ₂ O ₂ is a free radical	H ₂ O ₂ is ROS, but lacks an unpaired electron
SOD finishes oxidant detoxification	SOD forms H ₂ O ₂ ; catalase or GPx is still needed
Metal-binding proteins are ROS-degrading enzymes	They prevent metal-catalyzed ROS formation
Every ROS molecule is harmful	Controlled ROS participates in microbial killing and signaling
GSSG alone identifies a particular disease	GSSG/GSH indicates cellular redox state, not disease specificity

Integrating the complete antioxidant defense

Formation / amplification

Respiration, inflammation,
radiation, reactive metals



Control / removal

Metal binding; Antioxidants;
SOD → catalase or GPx



Pathologic threshold

Persistent radicals damage
lipids, proteins, and DNA

Protective control is multilayered and coordinated.

Core principles to retain

- Free radicals are limited by both spontaneous decay and active cellular defenses.
- Nonenzymatic antioxidants and metal-binding proteins reduce the effective oxidant burden.
- SOD generates H_2O_2 from $\text{O}_2\bullet^-$; Catalase and glutathione peroxidase dispose of H_2O_2 .
- Excess formation or inadequate removal permits oxidative damage to membranes, proteins, and DNA.

How to reason through an oxidative-injury problem

- Identify which reactive species is accumulating and where it is generated.
- Ask whether the deficit lies in production control, radical scavenging, or peroxide disposal.
- Match each enzyme to its substrate, reaction product, and cellular location.
- Connect the unresolved oxidant to a specific macromolecular target and cellular consequence.