LECTURE I

Metal Ions in Cellular (Patho)physiologies

Διατμηματικό Πρόγραμμα
Μεταπτυχιακών Σπουδών
στη Βιοανόργανη
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LABORATORY OF INORGANIC CHEMISTRY
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DEPARTMENT OF CHEMICAL ENGINEERING
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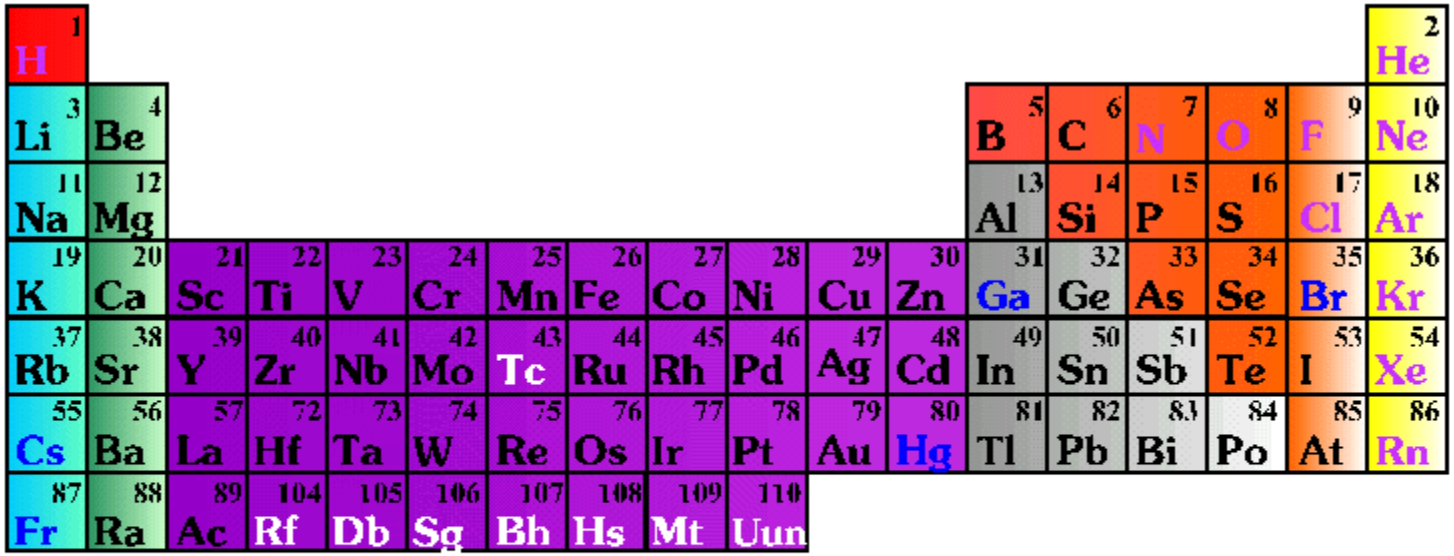
Outline of the presentation

- **Metals in the brain**
- **Cell physiology and pathology**
- **Oxidative stress and metal ions**
- **Morbidity**
- **Metals in Disease**
- **Metal ions in cellular pathophysiology with emphasis on neurodegeneration (Alzheimer, Parkinson, etc.)**

Periodic Table of elements



Description

Li	Solids	Cs	Liquids	Ar	Gases	No	Artificial
	Alkali metals		Alkaline earth metals		Transition metals		Rare earth metals
	Other metals		Noble gases		Halogens		Other non-metals

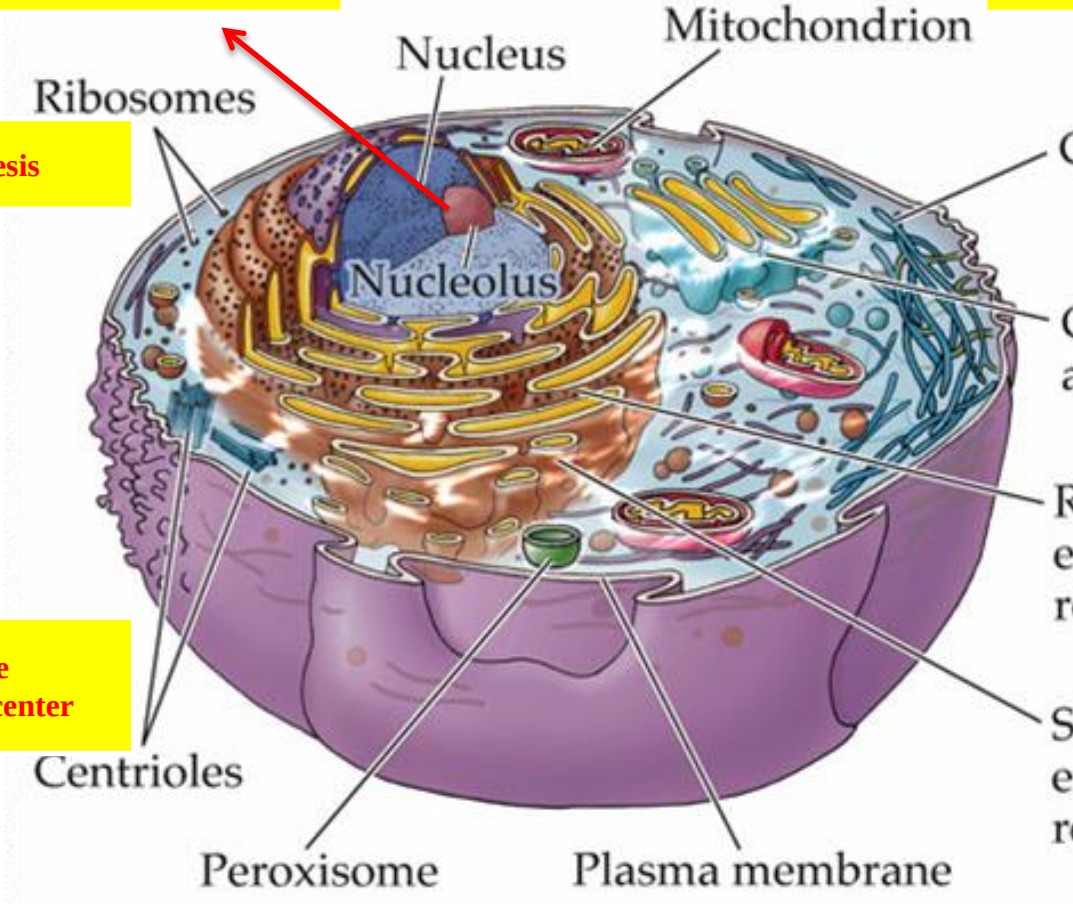
Metals in the brain

- **The importance of metal ions in neurotransmission and synthesis of neurotransmitters**
- **Spectroscopically silent K^+, Na^+, Mg(II), Ca(II), Zn(II)**
- **Spectroscopically accessible Fe(II,III), Cu(II), Mn(II,III), etc.**
- **Oxidative stress**
  - **Fenton metal ions (Cu(II), Fe(III), etc.)**
  - **Non-Fenton metal ions (Al(III))**
  - **The onset of neurodegenerative disease**

Structures in the cell where metal ions are encountered

rRNA synthesis

Energy – ATP production



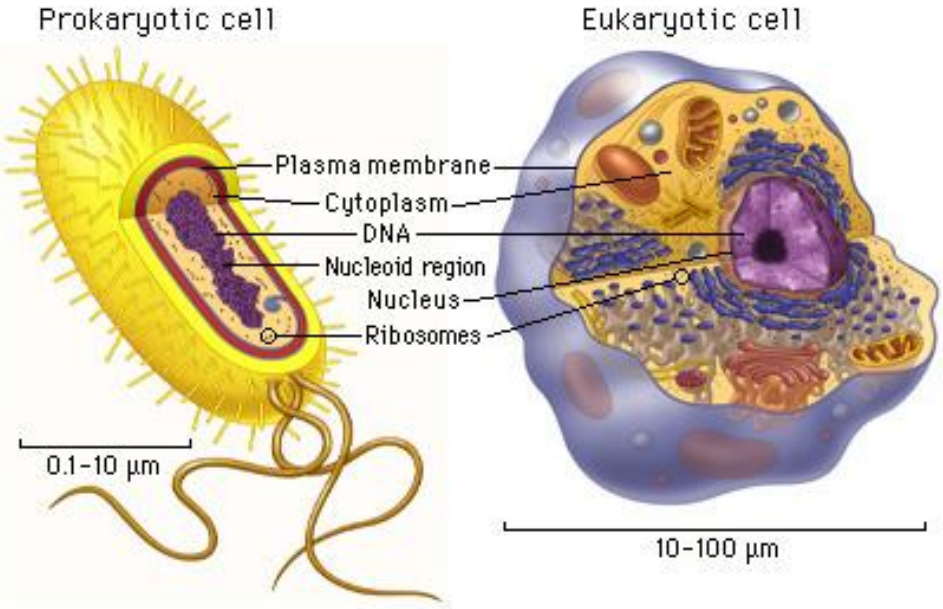
Protein synthesis

Terminal post-translational changes
Packing and transport

Protein synthesis and segregation Post-translational changes

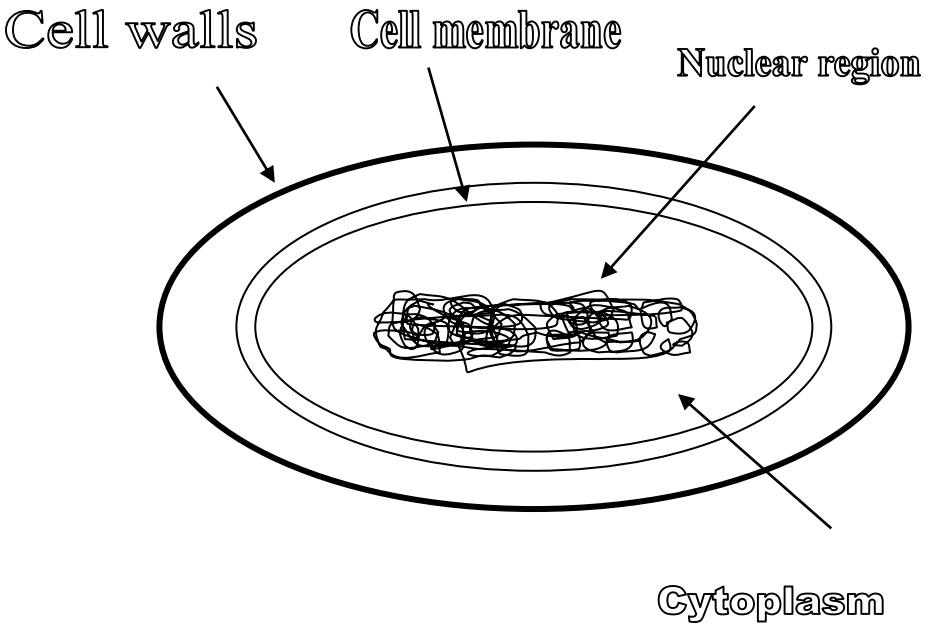
Microtubule polymerization center

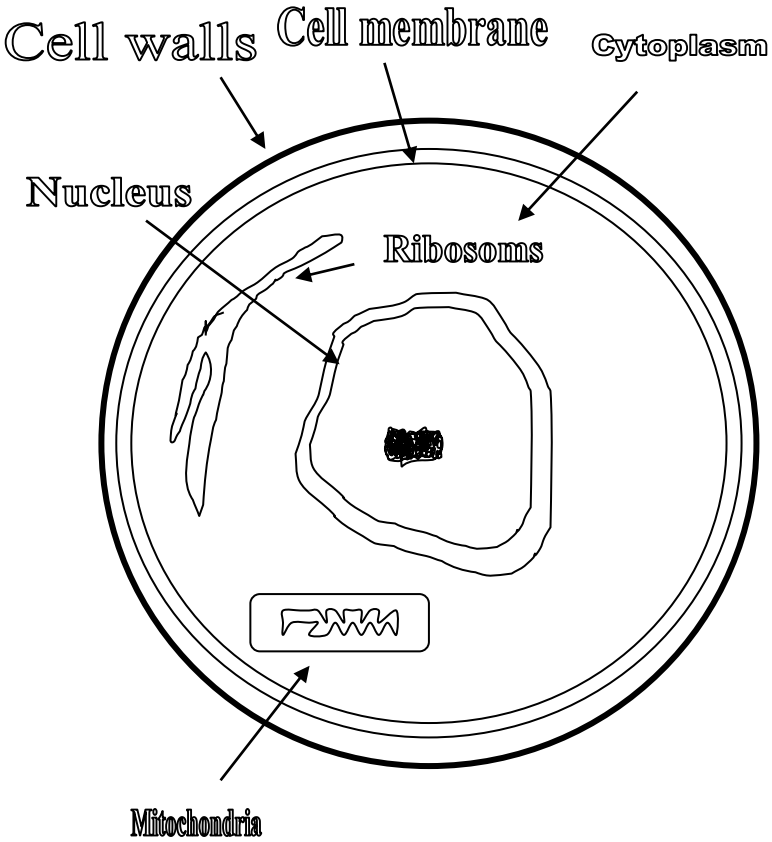
Detoxification and steroid synthesis



Common features in prokaryotic and eukaryotic cells

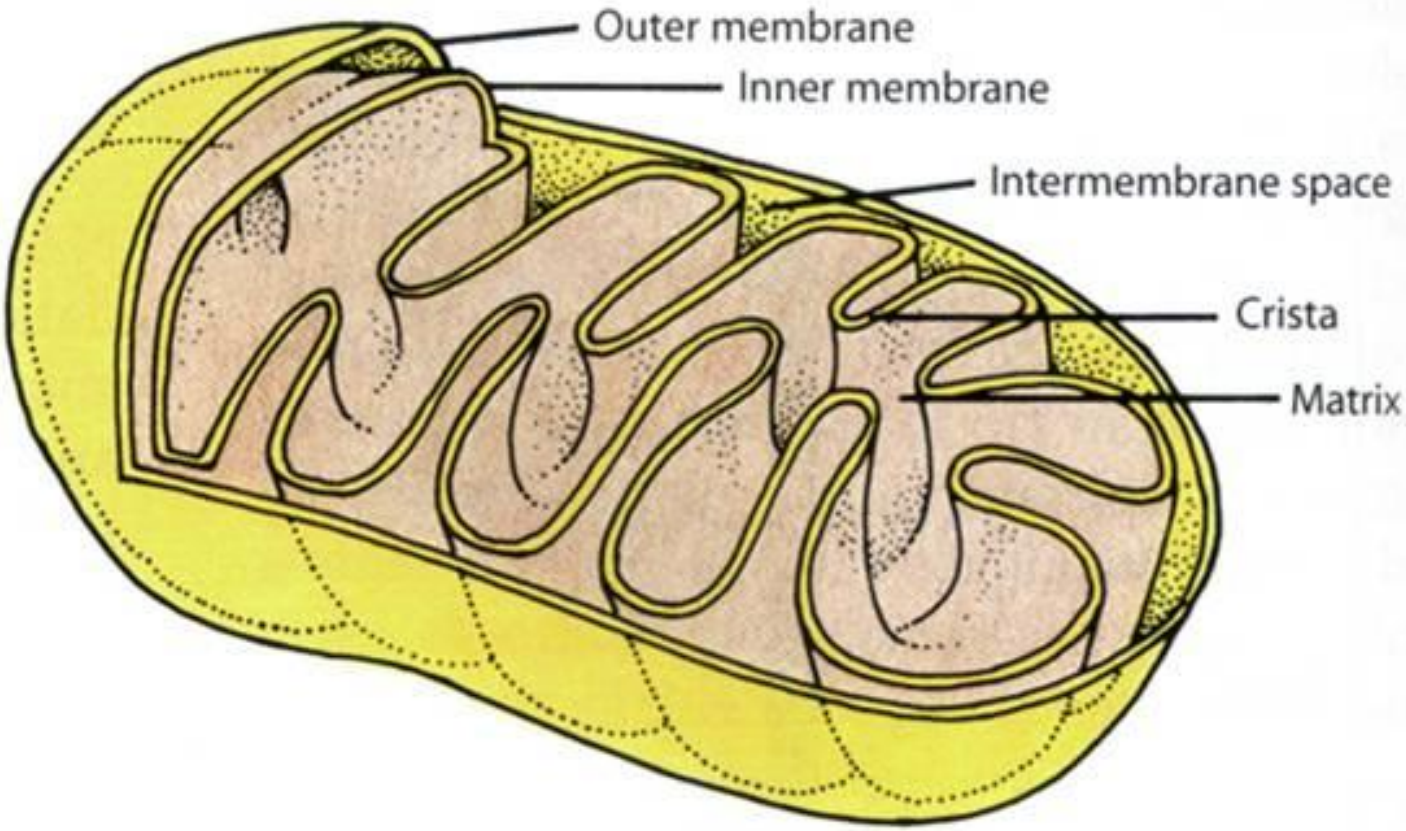
- **DNA**
- **Plasma membrane**
- **Cytoplasm**
- **Ribosomes**





Differences between prokaryotic and eukaryotic cells

- Cell size
- Nuclear membrane
- Transcription and Translation of DNA



Mitochondrion

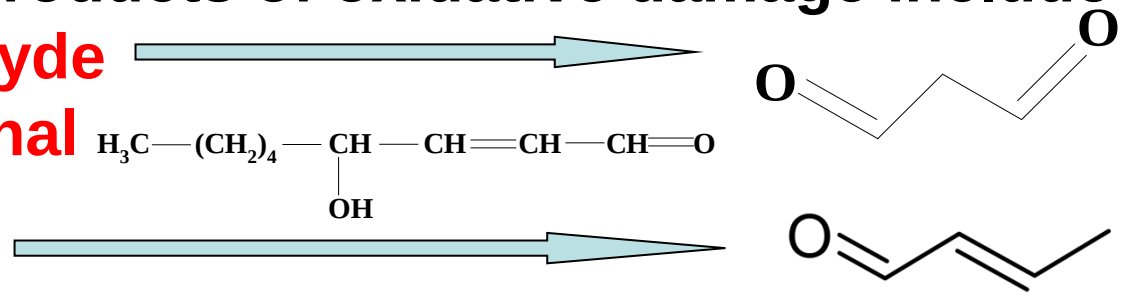
Oxidative Stress and metal ions

Redox active metal ions are linked to oxidative stress
Oxidative stress is associated with
- **Reactive Oxygen Species (ROS) and**
- **Reactive Nitrogen Species (RNS)**

ROS and RNS are linked to disease onset and propagation
Brain is very susceptible to oxidative damage

As a result of oxidative stress the following damage occurs:
- **Oxidative modification of amino acids**
- **Destruction of membrane phospholipids**

Low molecular mass products of oxidative damage include
- **MDA – Malondialdehyde**
- **HNE – Hydroxynonenal**
- **Crotonaldehyde**



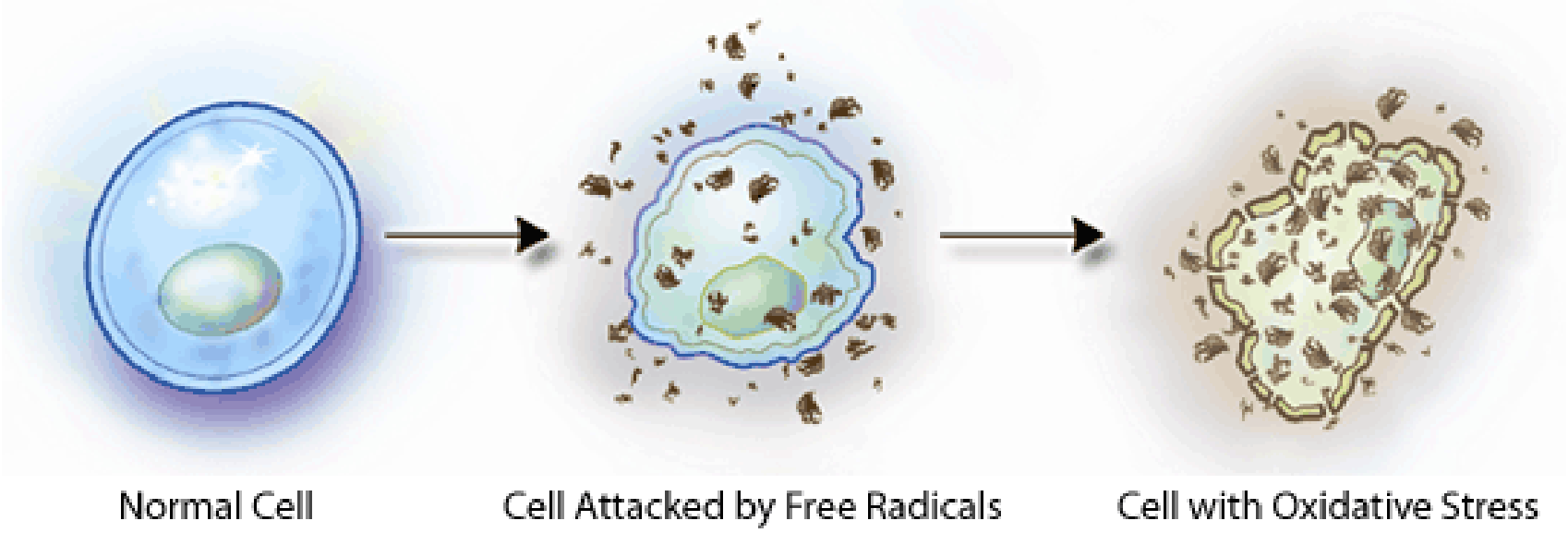
Oxidative Stress

The term Oxidative Stress relates to the disturbance of the balance between pro-oxidant and anti-oxidant processes in the physiology of the cell.

Direct linkage to two interdependent processes

1. **Oxidation or Pro-oxidant activity**
2. **Reduction or Anti-oxidant activity**

Oxidative Stress in Cells



Free Radicals and Oxidative Stress: Free radicals attack normal cells causing damage known as oxidative stress. Over time, the build up of oxidative stress can lead to insulin resistance and diabetic neuropathy.

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

- **Free Radicals**

  $O_2^{-\cdot}$ $^{\cdot}HO_2$

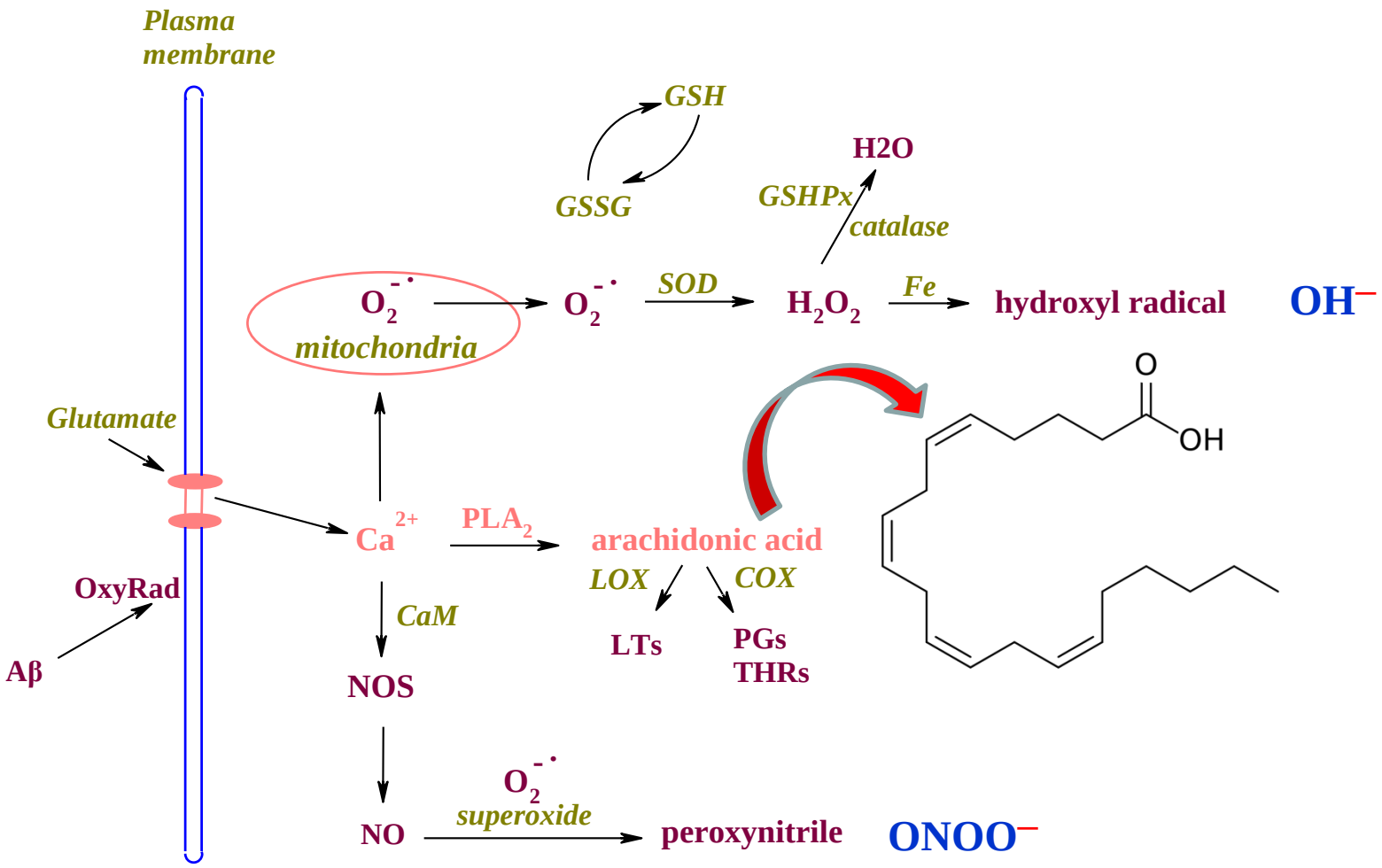
  $OH^{\cdot}$ $RO^{\cdot}$

  $ROO^{\cdot}$ $NO^{\cdot}$

- **Molecules being sources of ROS and RNS**

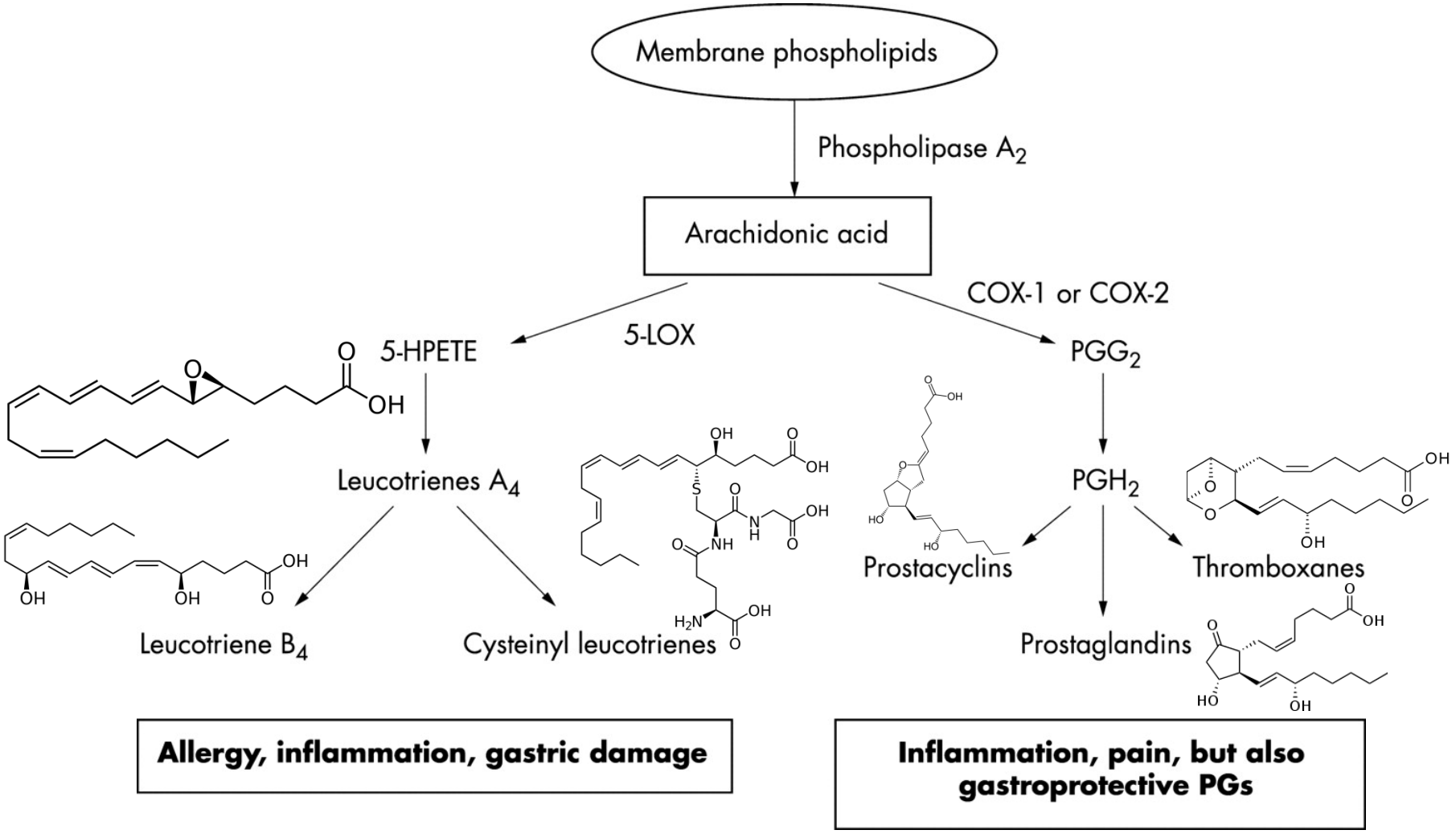
  $ONOO^-$ H_2O_2

  ROOH



SOURCES of REACTIVE OXYGEN AND NITROGEN SPECIES (ROS-RNS)

Products and enzymes of arachidonic acid metabolism involved in the inflammatory process.



J Martel-Pelletier et al. Ann. Rheum. Dis. 2003, 62, 501-509

ARD

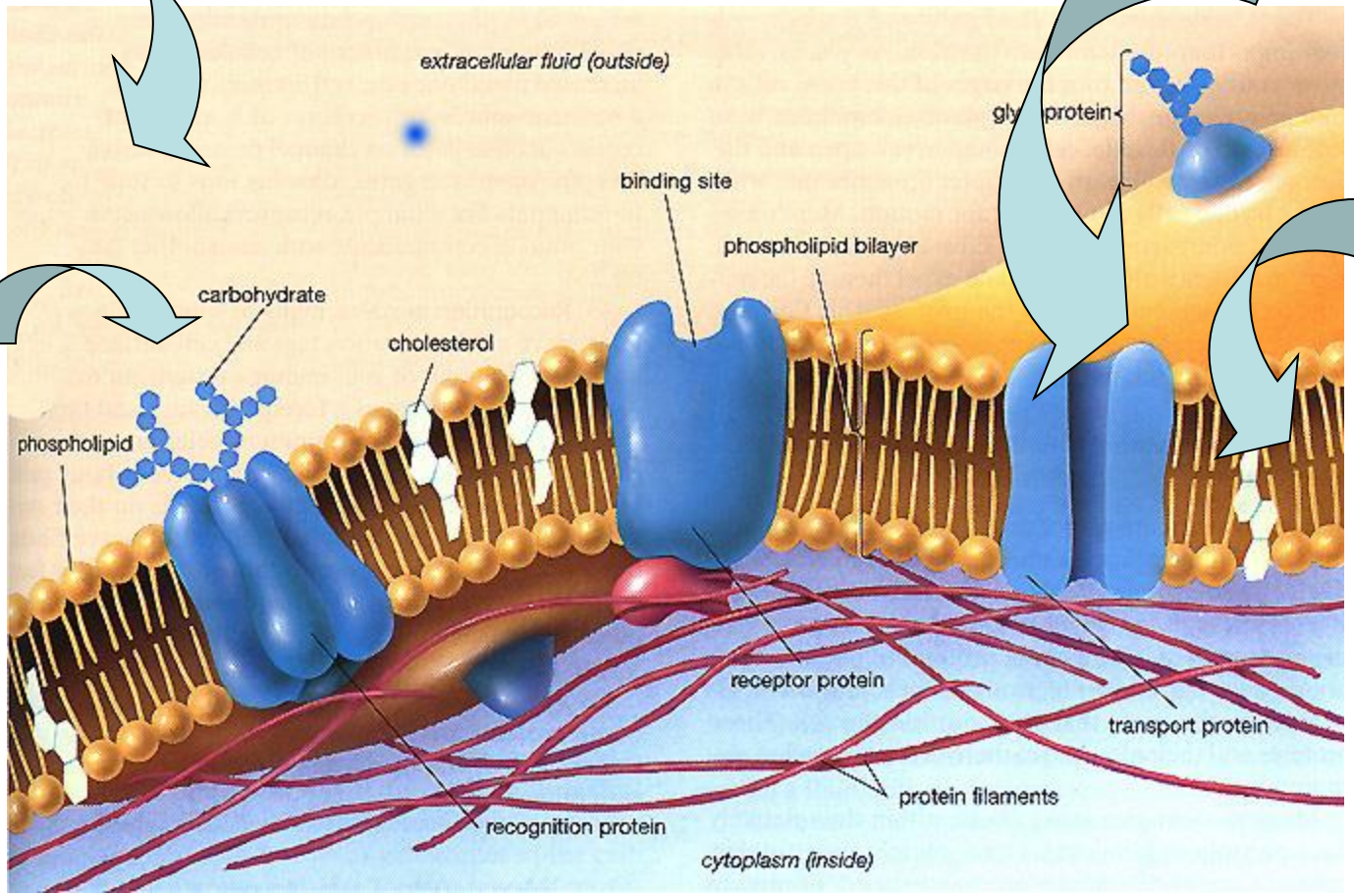
ROS GENERATION

H_2O_2

$O_2^{\cdot -}$

$OH^{\cdot -}$

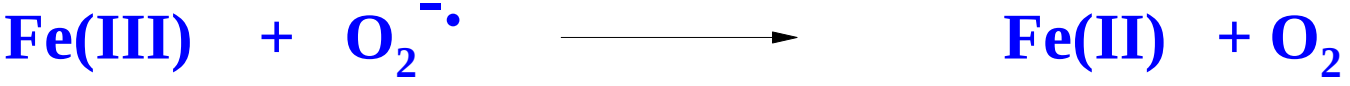
$\cdot OH_2$



Metal ion catalytic activity in Fenton reactions occurs according to the following series

$$Cu^{II} > (Cr^{III}) < Co^{II} < Fe^{III} < Mn^{II} < Ni^{II} < Zn^{II} < Cd^{II}$$

Fenton



Haber-Weiss

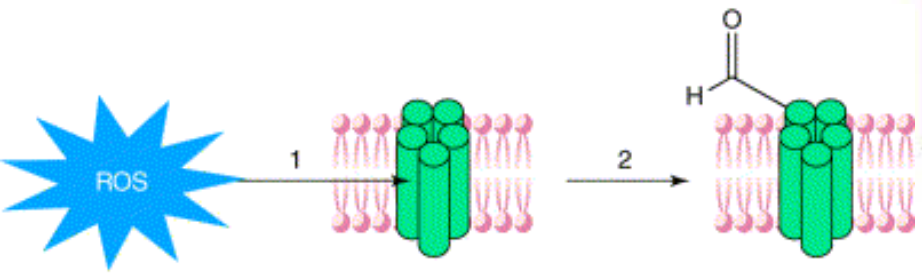
Fundamental metal ion catalyzed redox reactions leading to the formation of hydroxy radicals. The Cu(II)/Cu(I) metal ion pair behaves analogously.

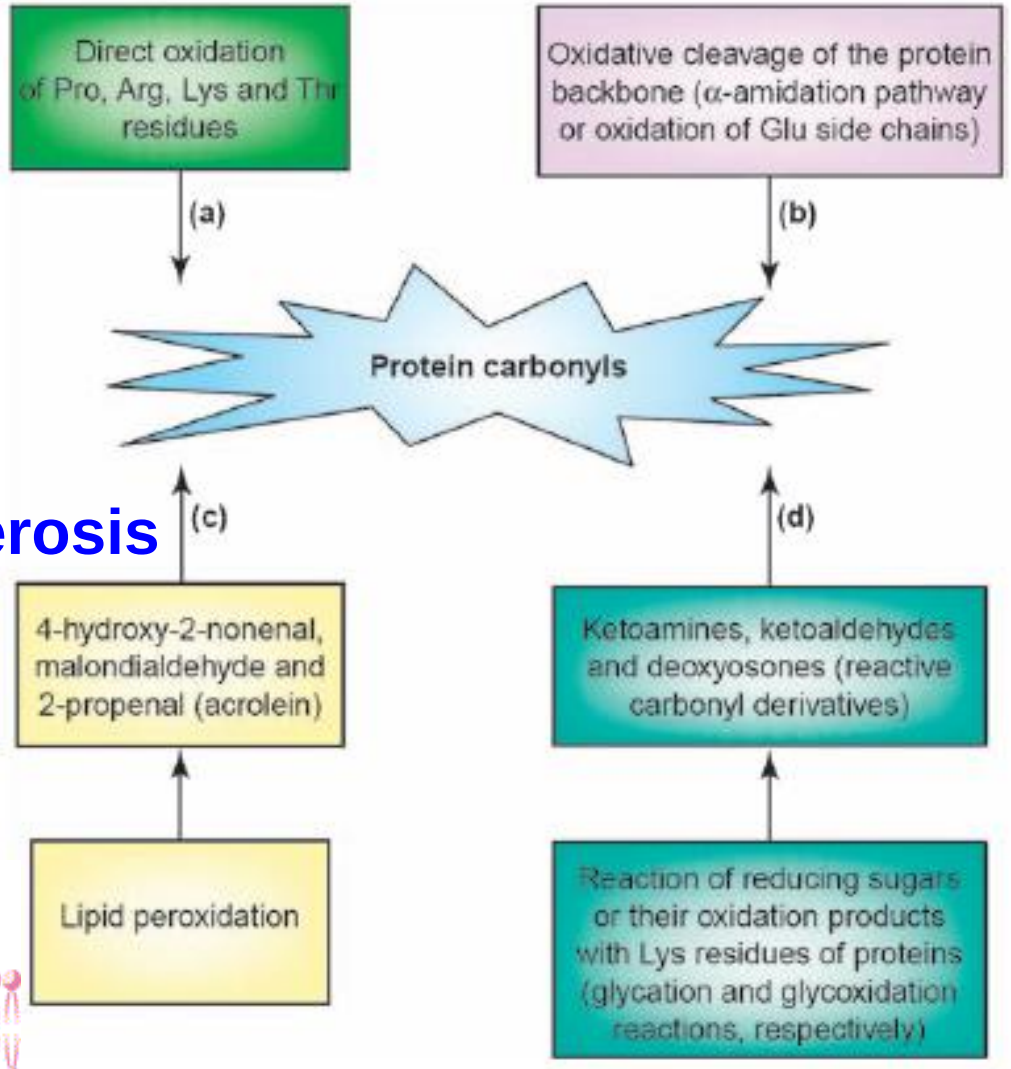
Oxidative damage through ROS leads to protein carbonyls

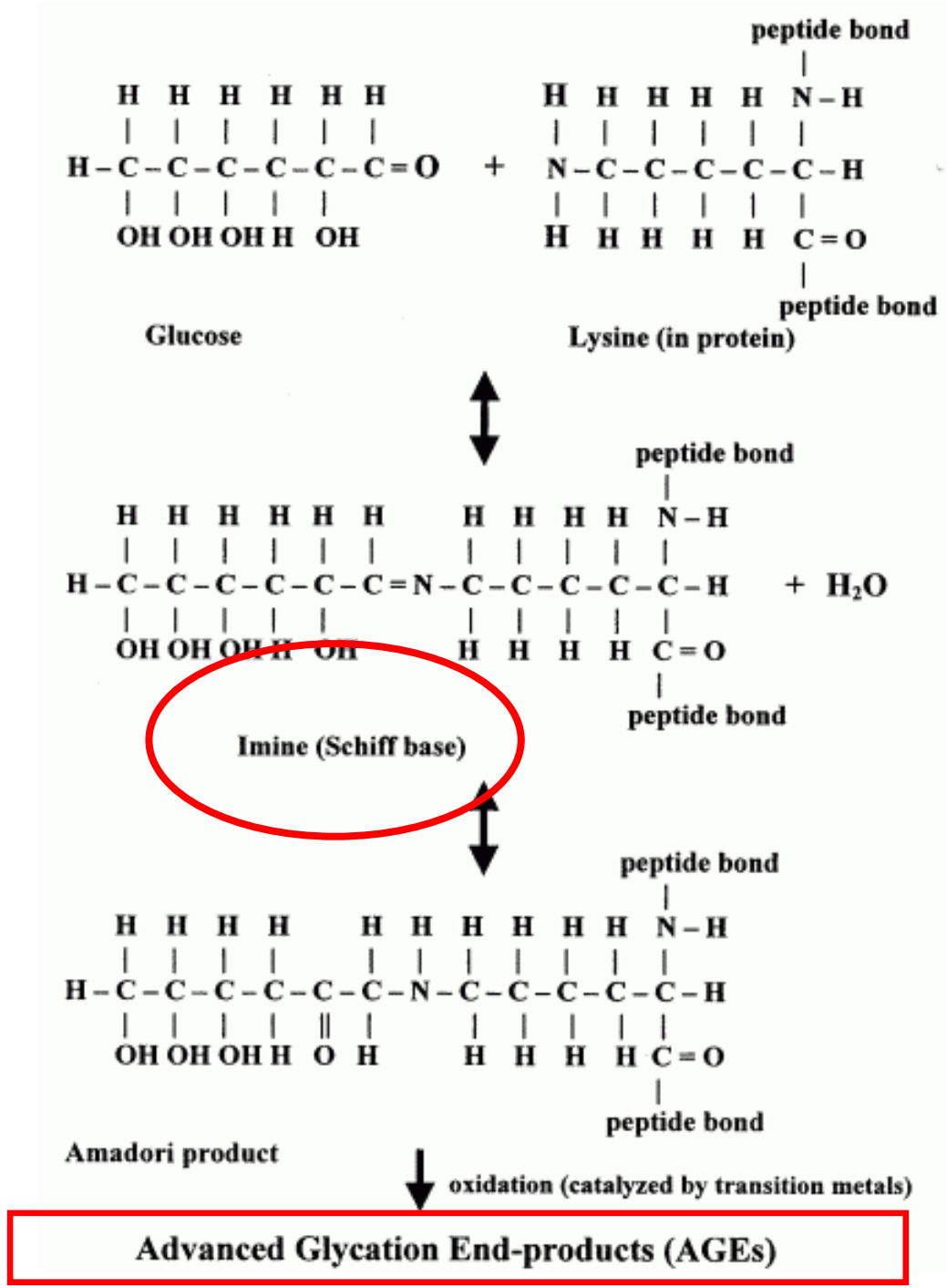
The result is

- **Misfolding of proteins**
- **β-sheet aggregation**
- **Accumulation**

- ❖ **Alzheimer's Disease**
- ❖ **Amyotrophic Lateral Sclerosis**
- ❖ **Parkinson's Disease**
- ❖ **Huntington's Disease**
- ❖ **Friedreich's Ataxia**

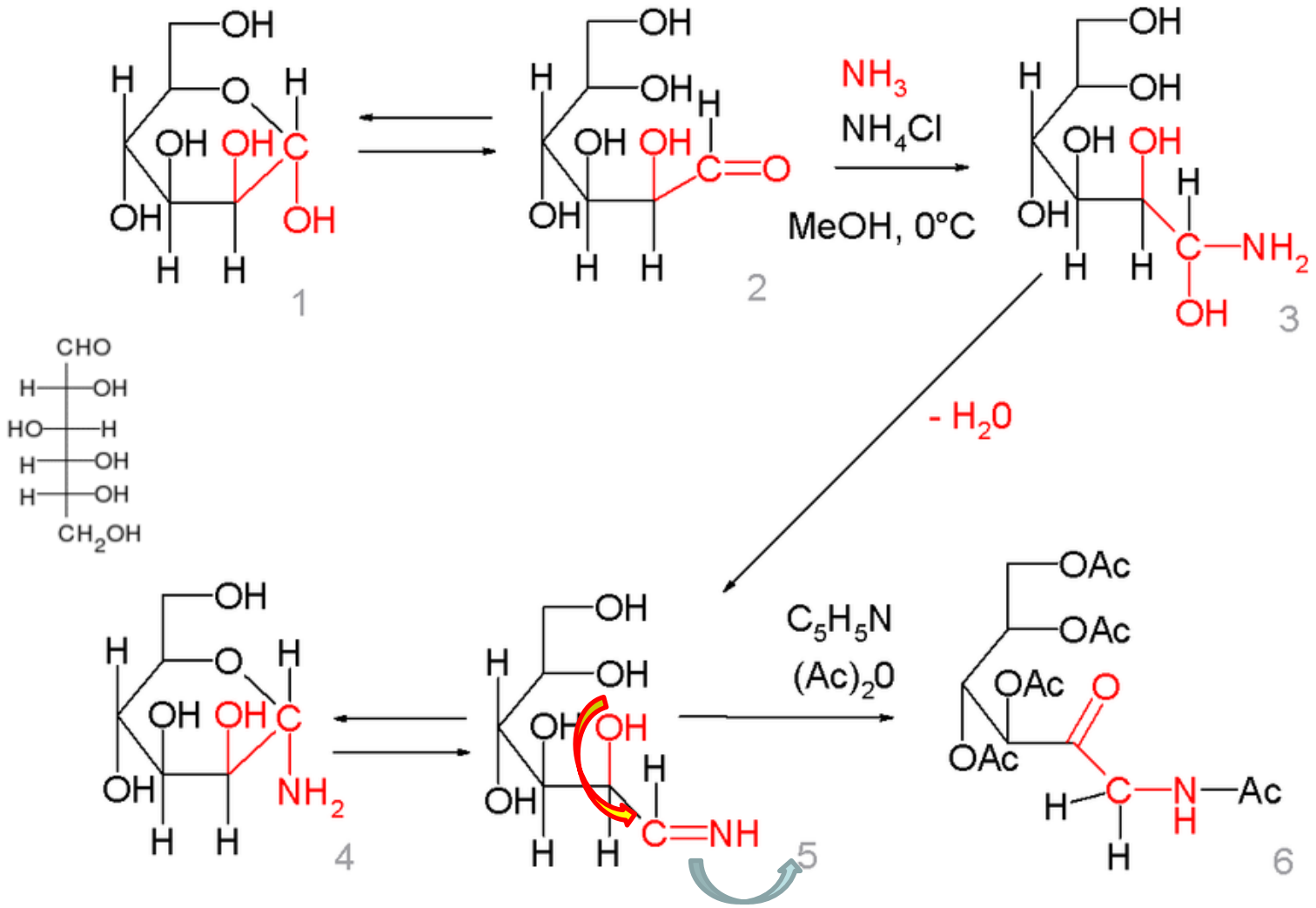




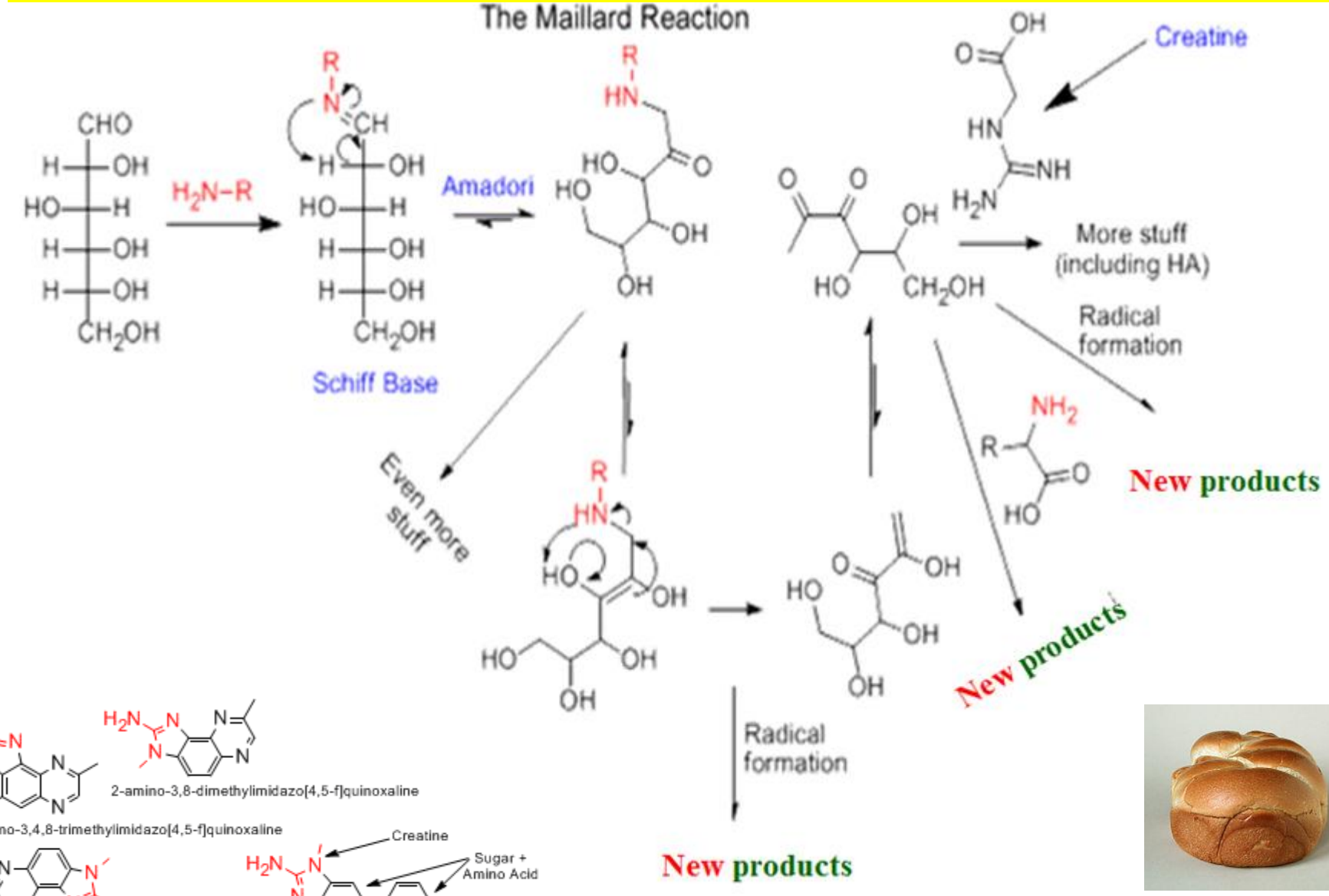


Interactions of ROS with proteins

Amadori reaction



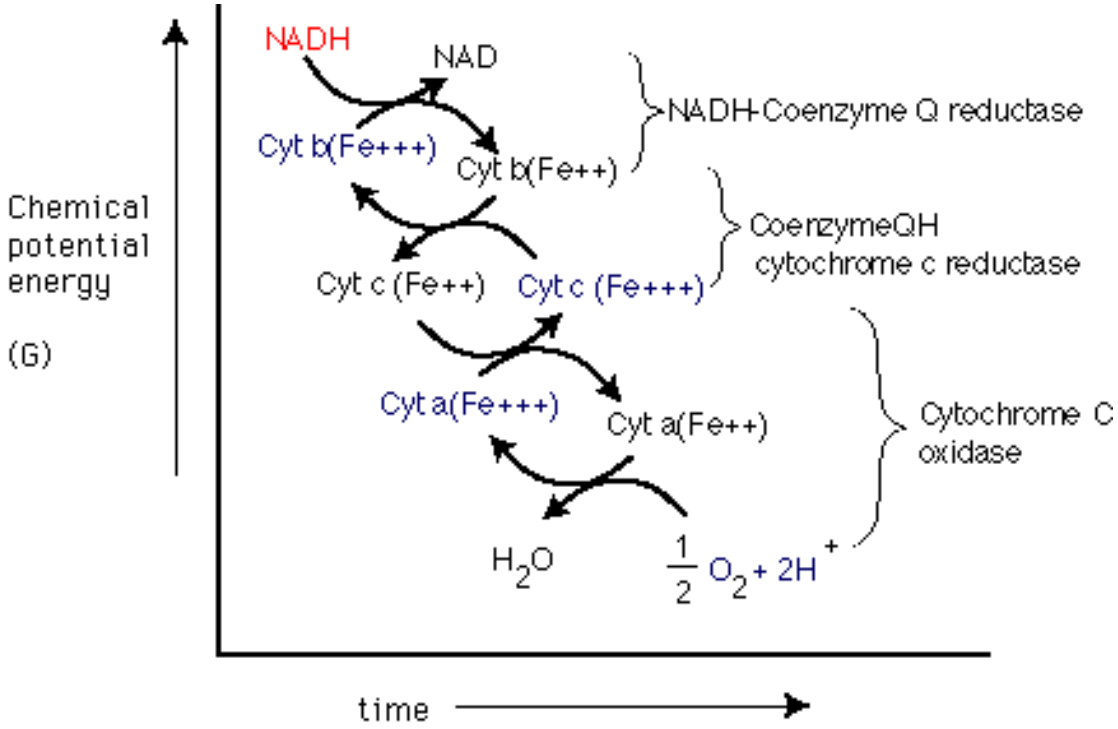
The Maillard reaction



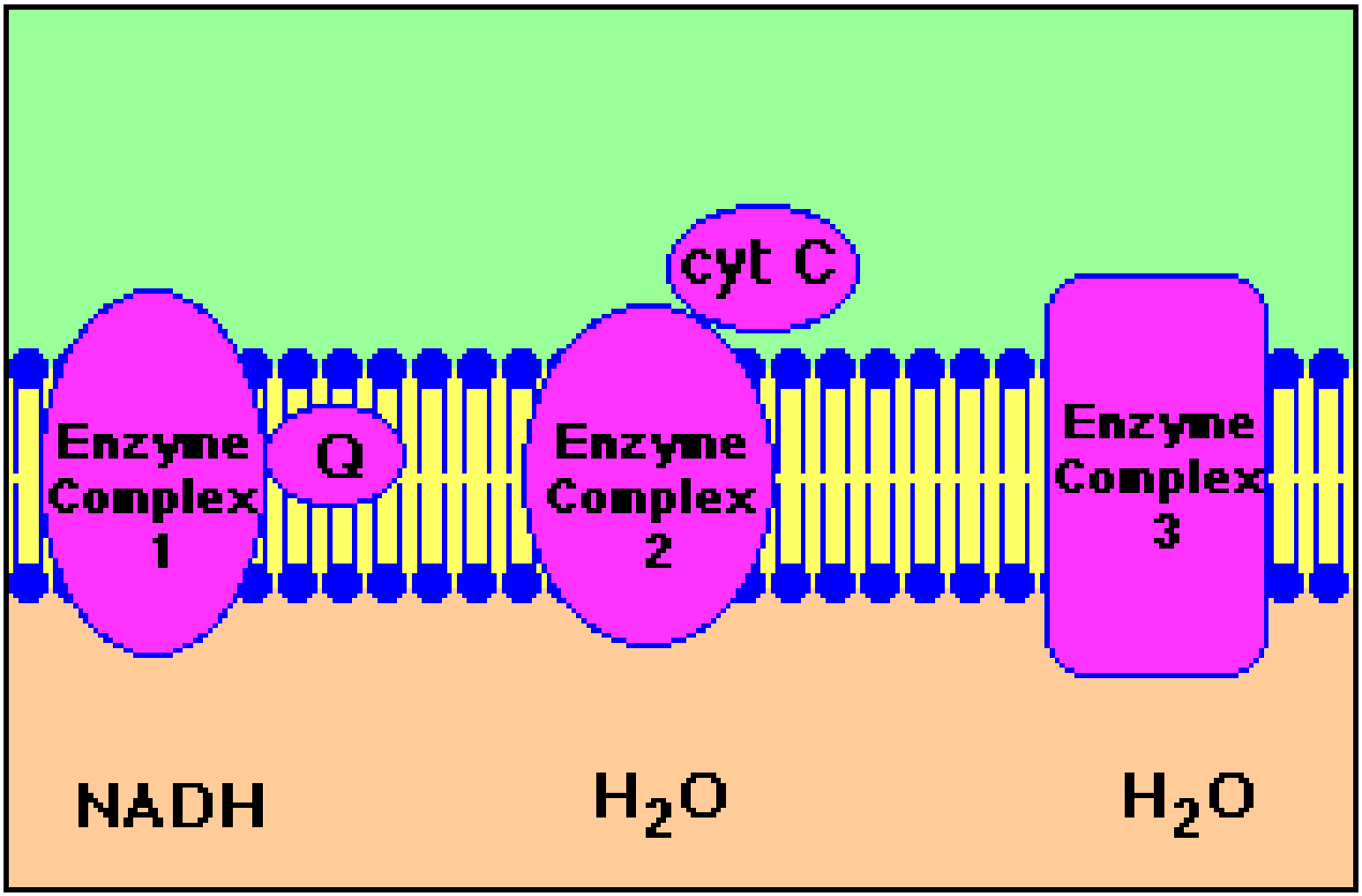
We are being cooked up!!!!

IRON IN PLANTS

➢ **Iron participates in the electron transport chains in photosynthesis and respiration.**



Electron Transport in Mitochondria



Generation of ROS forms of O_2

$$O_2 + e^- \longrightarrow O_2^{\cdot -}$$

$$O_2^{\cdot -} + H^+ \longrightarrow HO_2^{\cdot}$$

$$O_2^{\cdot -} + e^- \longrightarrow O_2^{2-}$$

$$O_2^{2-} + 2H^+ \longrightarrow H_2O_2$$

ROS IN OXIDATIVE STRESS

- A reactive oxygen species (ROS) generated by the cellular machinery under specific conditions (e.g. oxidative stress)
- Product of superoxide ($O_2^{-\cdot}$) processing in the mitochondria and the cytoplasm of all cells by the enzyme Superoxide Dismutase (SOD)

$$2O_2^{-\cdot} + 2\,H^+ \longrightarrow O_2 + H_2O_2$$

- Substrate for the catalase enzyme dismutating hydrogen peroxide to oxygen

$$2H_2O_2 \longrightarrow O_2 + 2\,H_2O$$

- Key agent in the generation of Fenton and Fenton-Weiss radicals promoting oxidative damage to tissues

$$Fe(II) + H_2O_2 \longrightarrow Fe(III) + OH^- + OH^{\cdot}$$

CHLOROPLAST

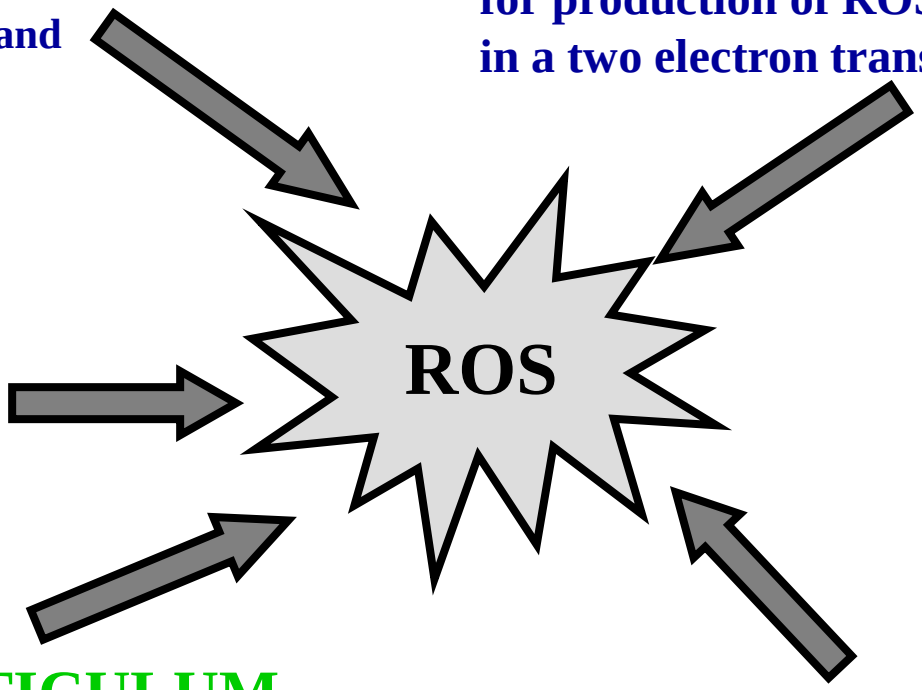
Formation of ROS during electron transfer in the chloroplast under NADP limiting conditions; excitation of O_2 from triplet to singlet state and by release of partially reduced oxygen products

MICROBODIES

Microbodies, like peroxisomes and glyoxysomes, are active sites for production of ROS like H_2O_2 in a two electron transfer process

MITOCHONDRIA

Generation of ROS in mitochondria affecting ETS by redox change in Fe and Cu in carriers; generation in Fe-S proteins and NADH dehydrogenase



ENDOPLASMIC RETICULUM

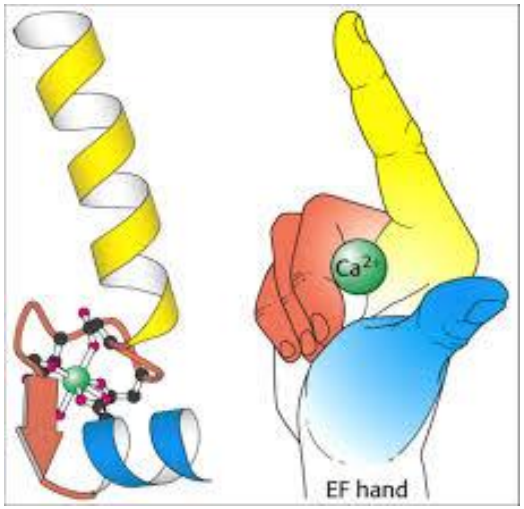
ROS like $O_2^{\cdot -}$ can be generated in the endoplasmic reticulum by univalent reduction of substrate like –RH and subsequent addition of triplet oxygen $^3O_2$ to form complex P_{450} –RHOO, which decomposes to P_{450} –RH releasing $O_2^{\cdot -}$

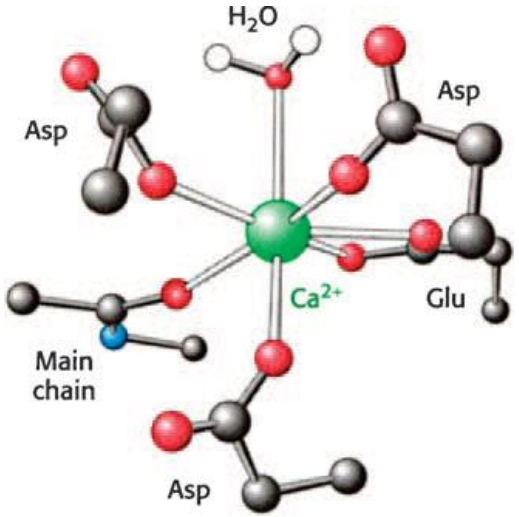
PLASMA MEMBRANE

Plasma membrane generates ROS like $O_2^{\cdot -}$ by superoxide generating NADP(H) oxidase via redox cycling certain quinones or nitrogenous compounds

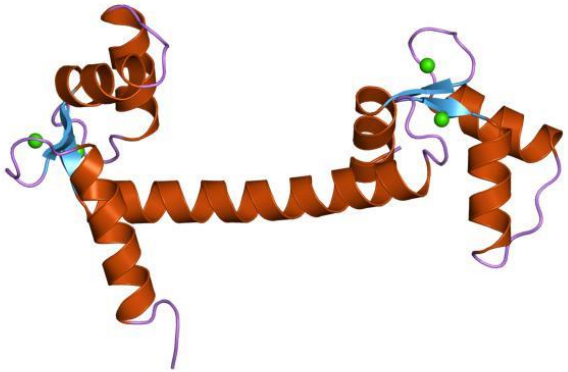
Calcium – Ca(II)
Structures – Molecules - Processes

- Ca-EF hands
- Ca(II) concentration in and out of the cell very important
- Ca(II)-linked molecules (peptides and proteins)
- Ca(II)-linked processes
- The effect on brain function or pathology

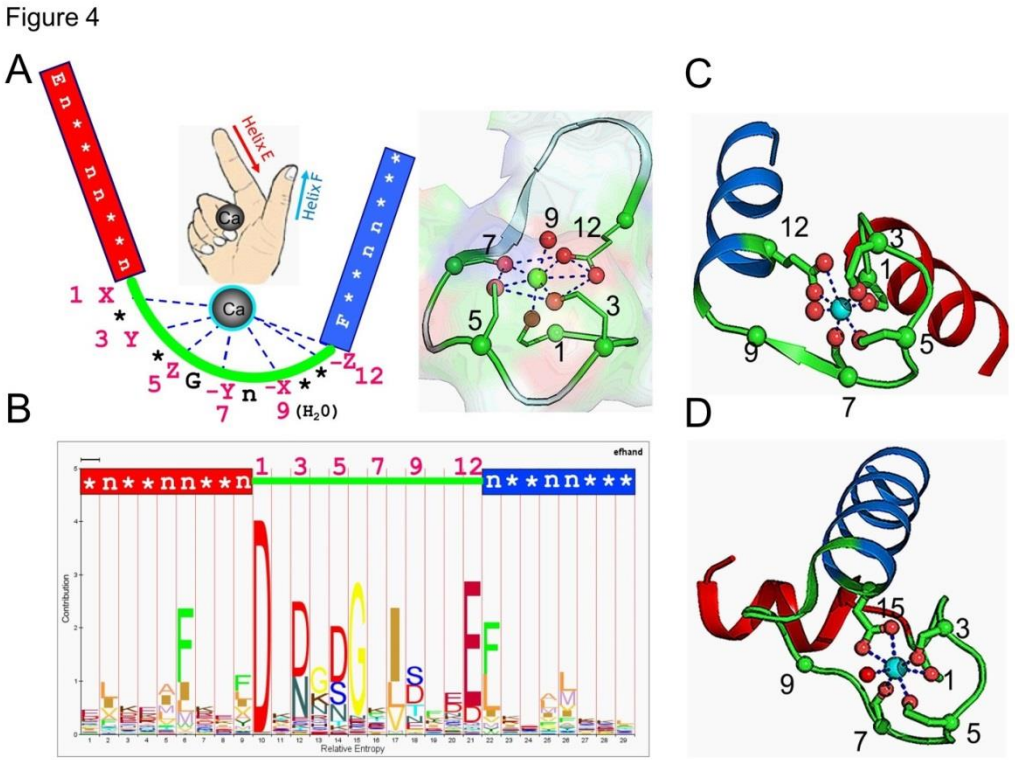




Calcium

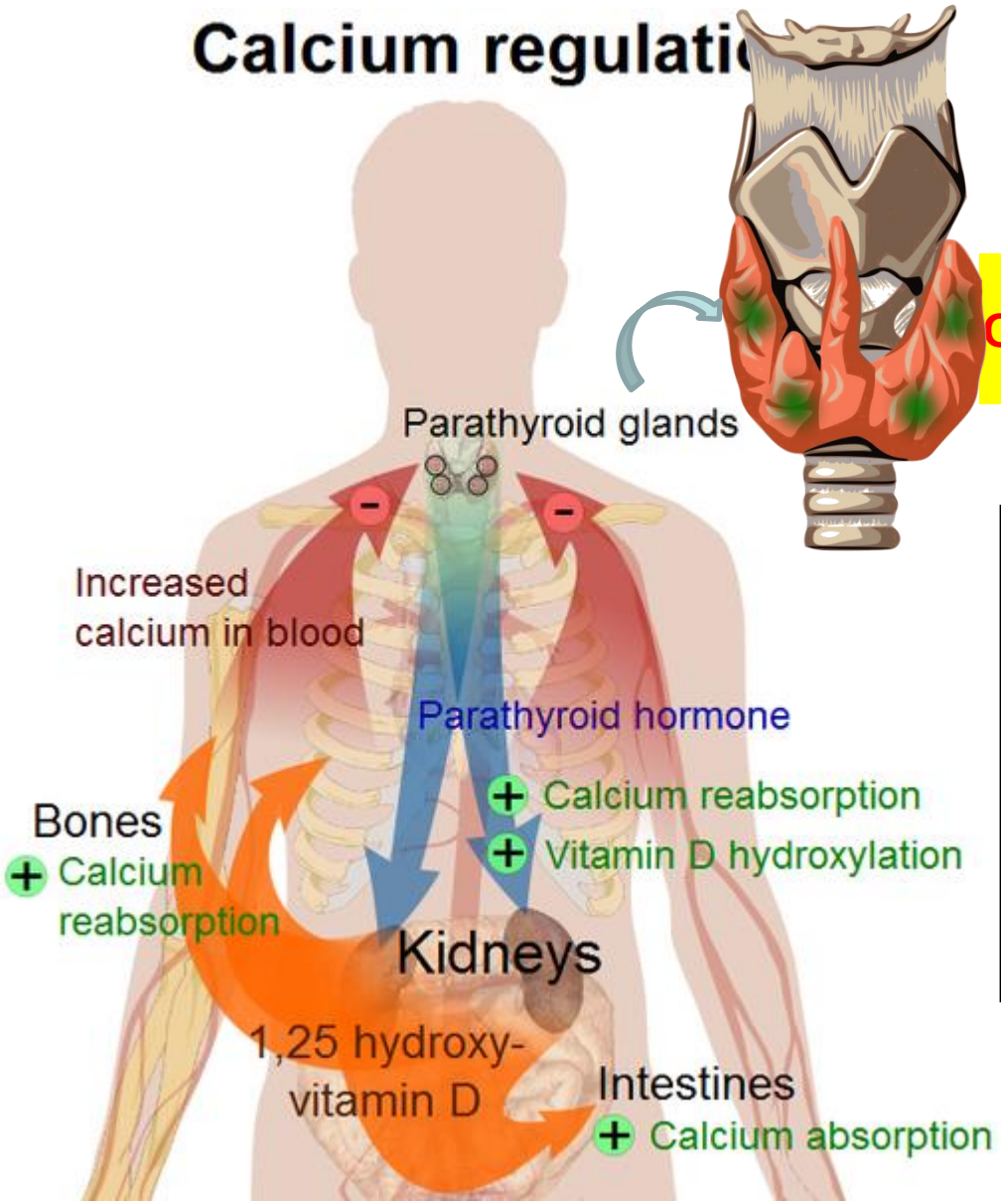


Calcium EF-hand
Structure of the recombinant
***Paramecium tetraurelia* calmodulin**



EF-hand Ca(II) binding motif

The EF hand is a helix-loop-helix structural domain found in a large family of calcium-binding proteins.

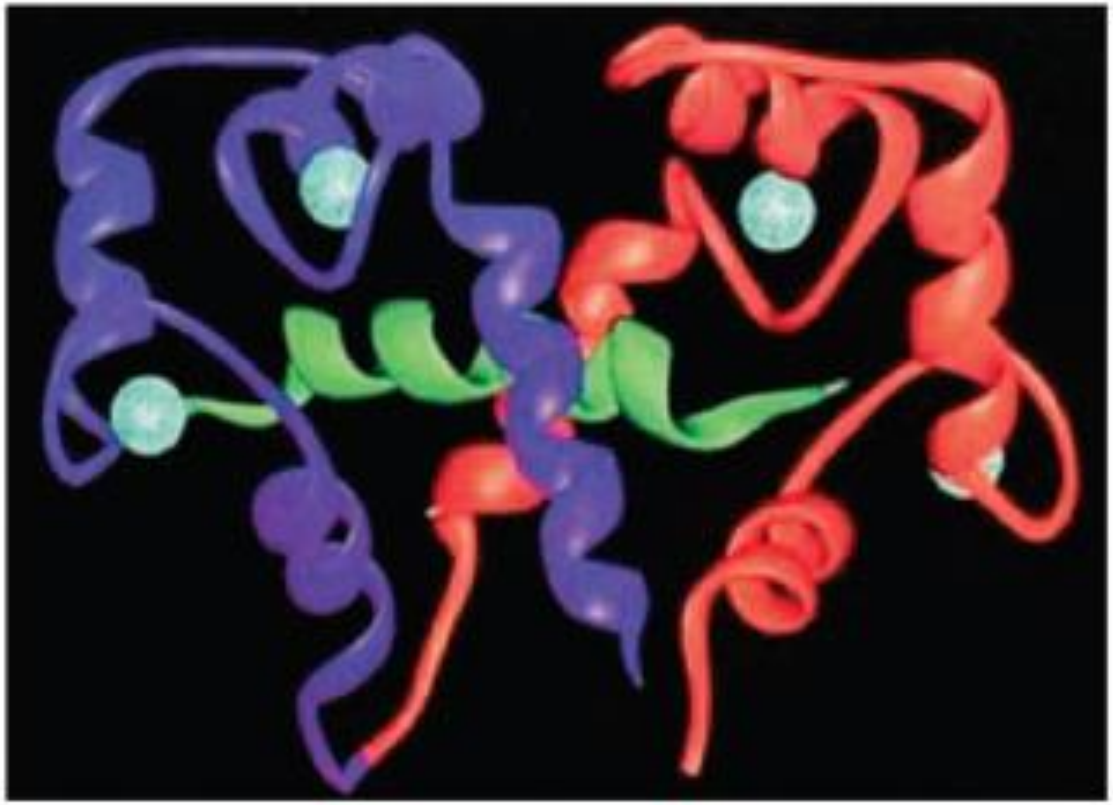


Calcium regulation in the human body

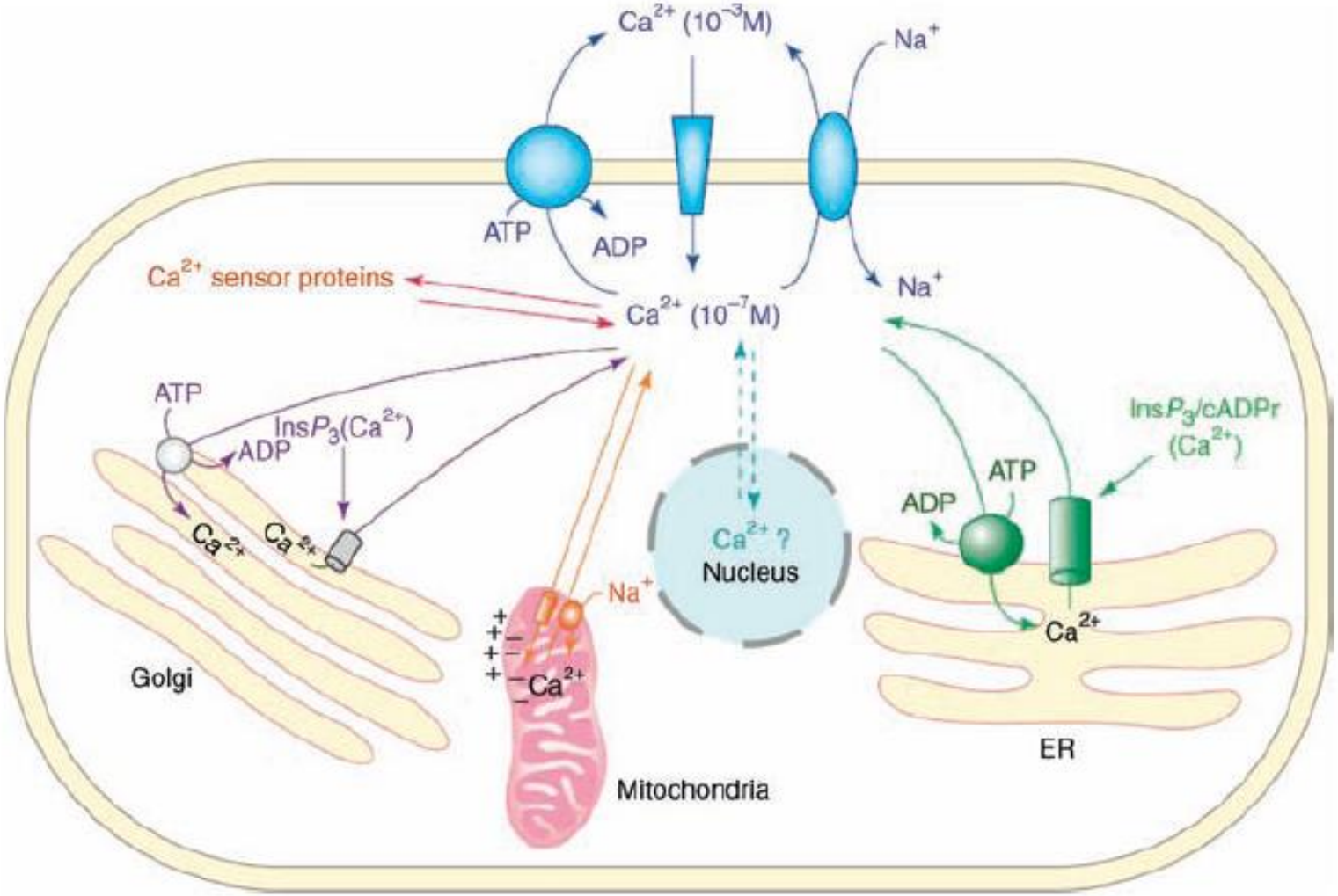
Hypercalcemia – Hypocalcemia

- When [Ca(II)] rises, the thyroid gland releases calcitonin.
- When [Ca(II)] falls the parathyroid gland releases parathyroid hormones.

Thyroids primarily influence the metabolic rate and protein synthesis. The hormones also have many other effects including those on development.

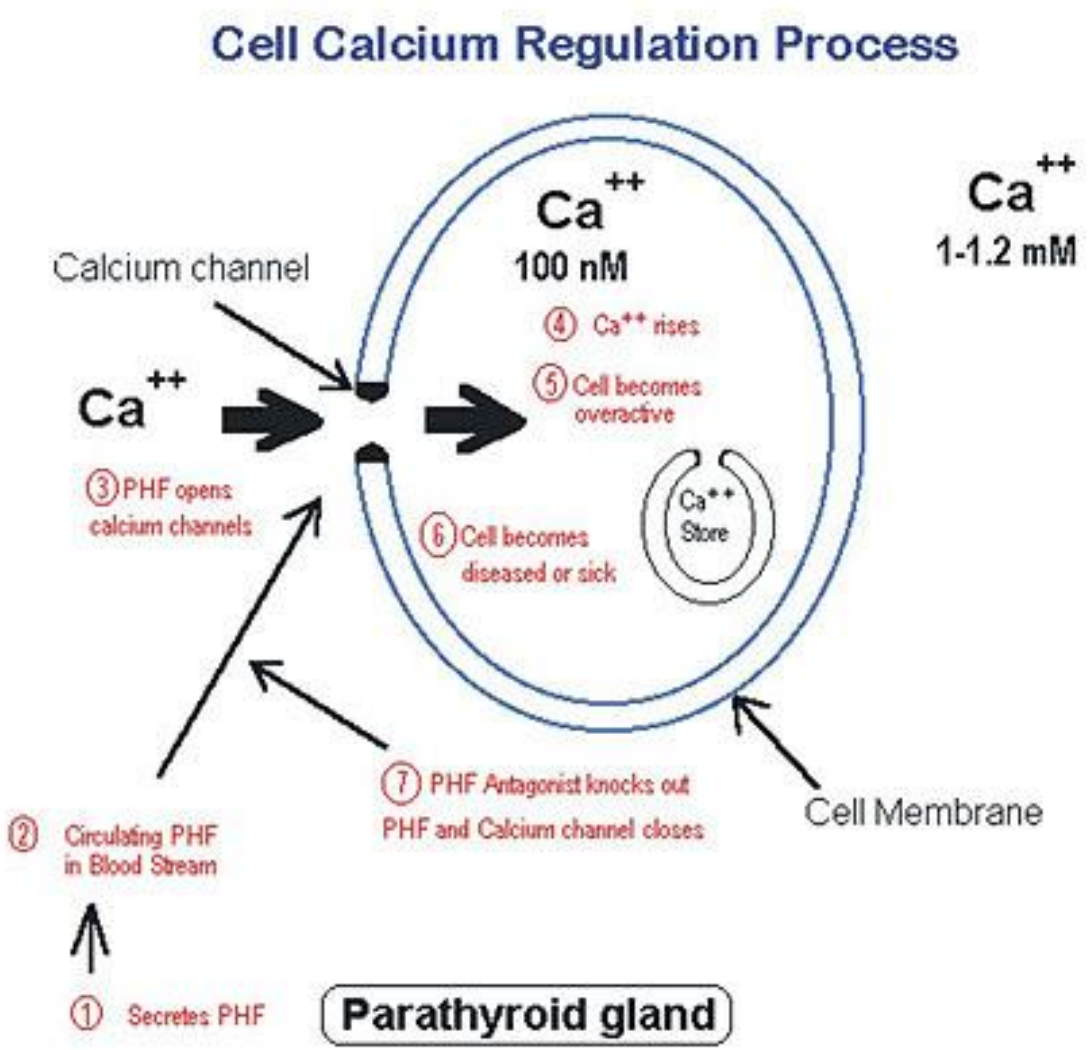


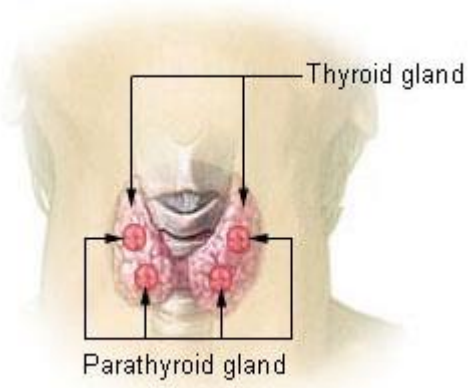
Ribbon diagram showing the NMR structure of $(Ca(II))_4$–calmodulin bound to a 26 residue target peptide. The two domains of calmodulin are in purple and red, the Ca(II) ions as spheres and the target peptide in green.



The basic concepts of Ca(II) homoeostasis [Carafoli (2004)].

Ca(II) homeostasis

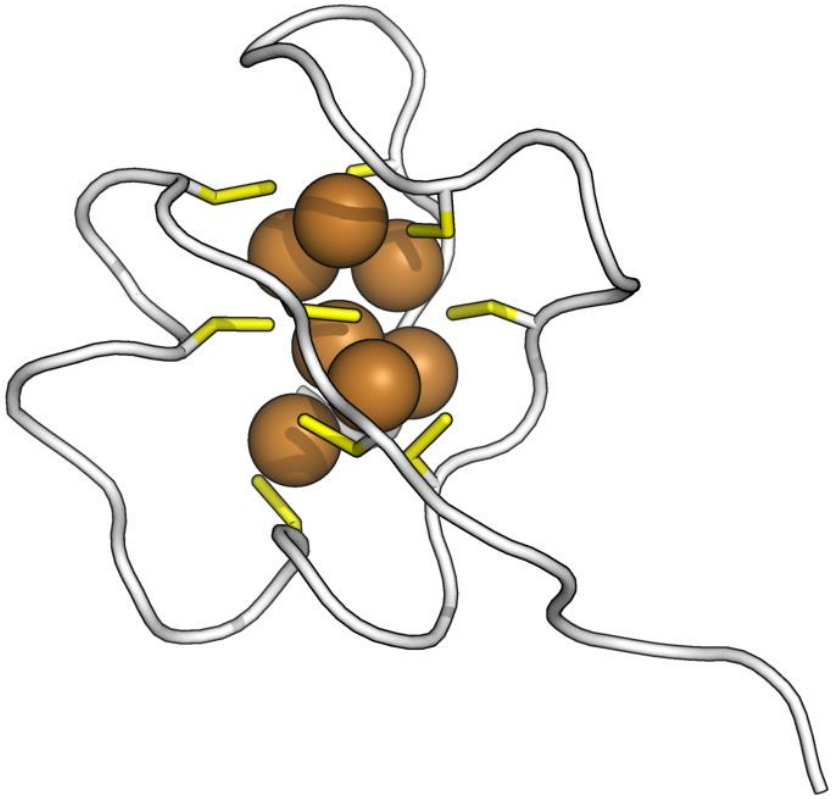




Parathyroid Hypertensive Factor, PHF

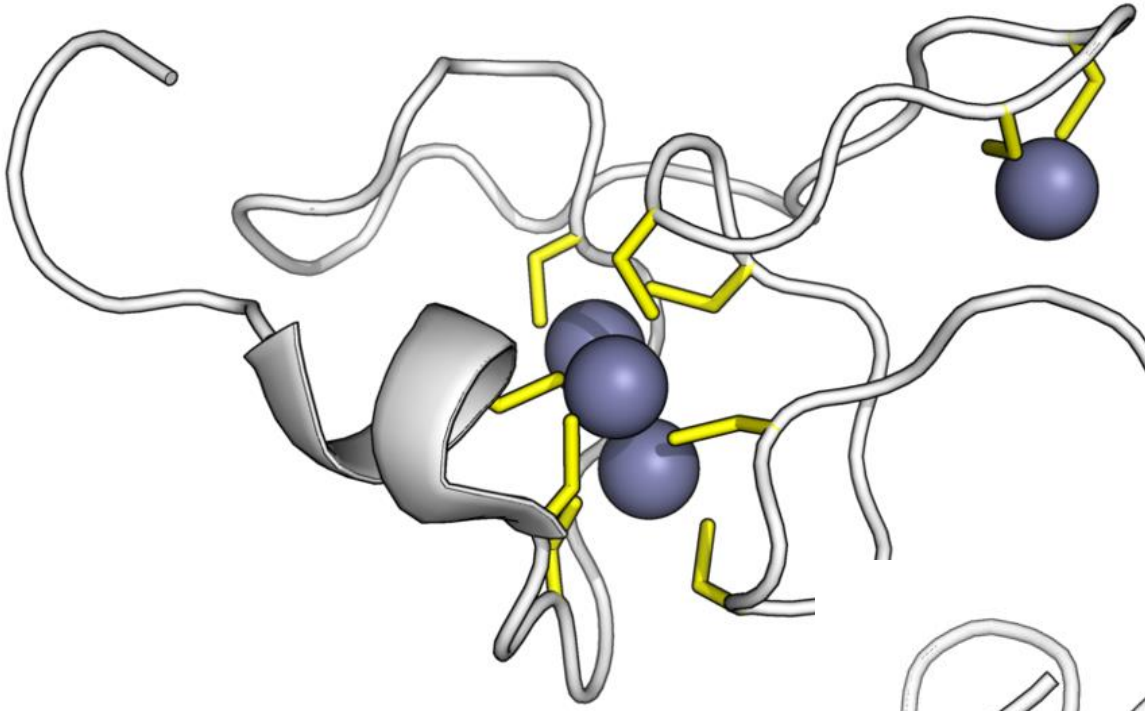
Zinc

- It is a neurometal connected to brain functions
- Regulates brain development during fetal and post-natal life.
- Linkage to stress and anxiety (animal studies)
- Excess linked to neuronal cell death
- Associated with changes in **metallothioneins (MTs)** and stress in the hippocampus
- Mutations in proteins linked to spinal muscular atrophy.

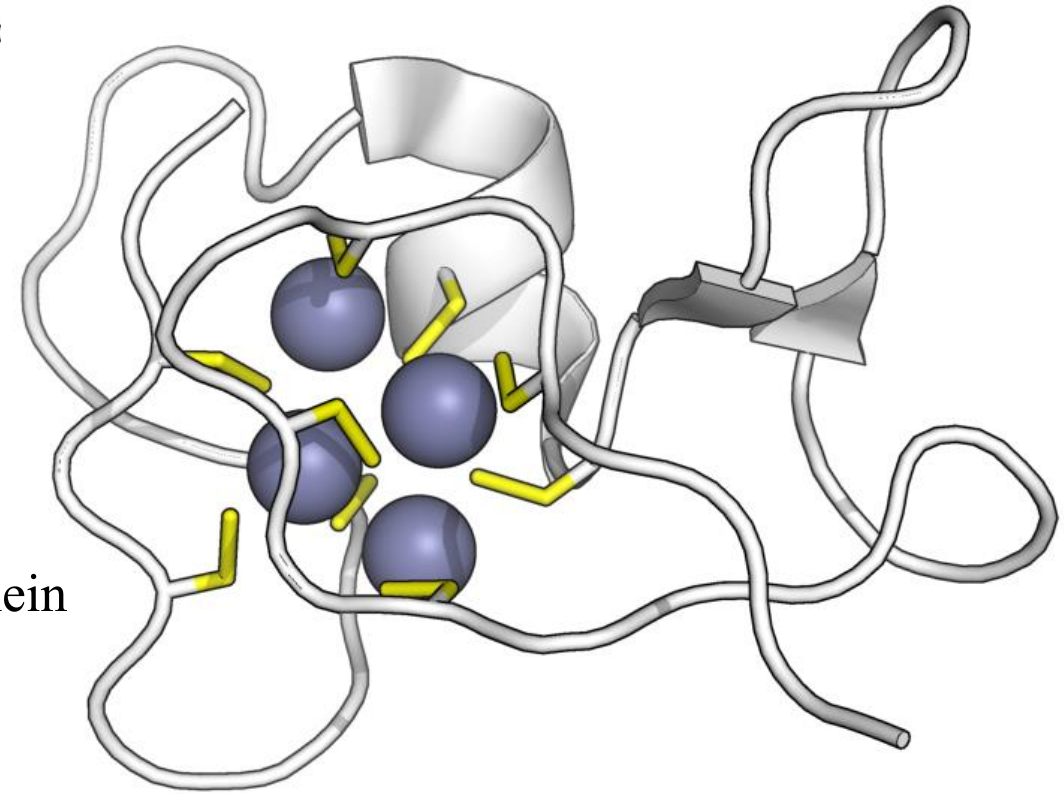


Saccharomyces cerevisiae MT metallothionein bound to **copper** ions.

Metallothioneins (MT) belong to a family of cysteine-rich, low molecular weight (MW 500-14000 Da) proteins. They are localized in the membrane of the Golgi apparatus. MTs have the capacity to bind both **physiological** (such as zinc, copper, selenium) and **xenobiotic** (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its **cysteine residues**, which represent nearly **30%** of its constituent amino acid residues. Experimental data suggest MTs may provide **protection against metal toxicity**, be involved in zinc and copper regulation, and provide **protection against oxidative** stress. There are four main isoforms expressed in humans: **MT1, MT2, MT3, and MT4**. In the human body, large quantities are synthesized primarily in the liver and kidneys.

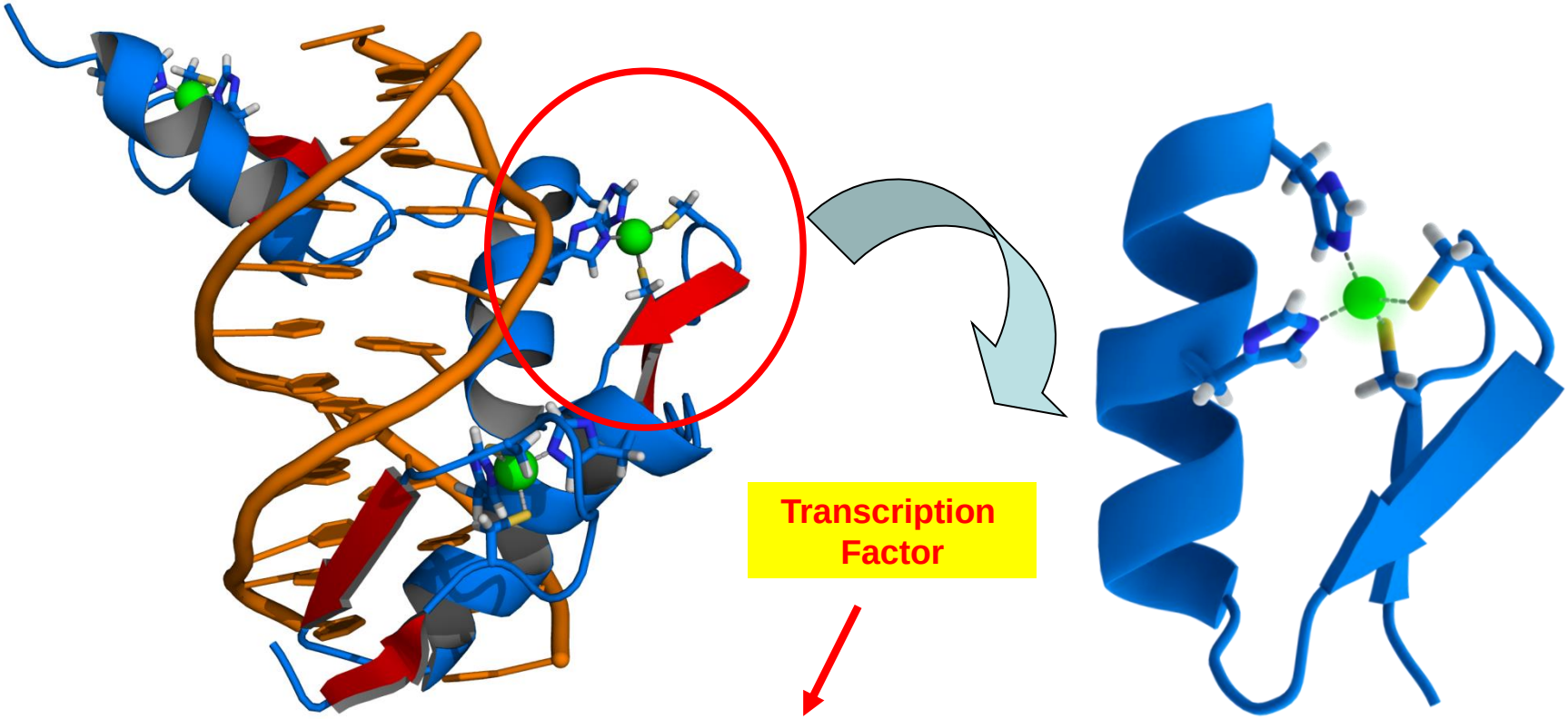


Beta-E-domain of **wheat** Ec-1 metallothionein bound to zinc ions



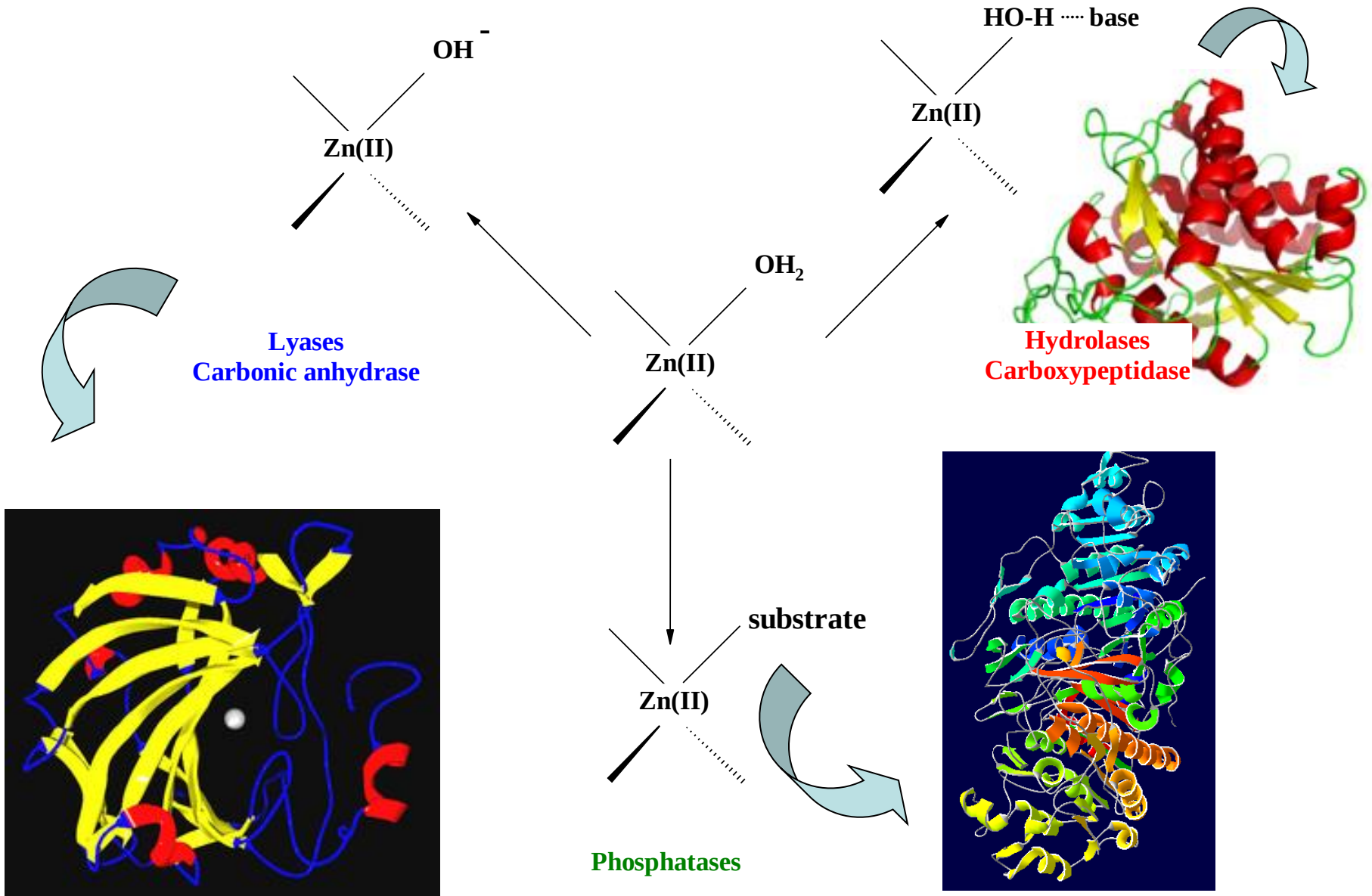
Cyanobacterial SmtA metallothionein bound to zinc ions.

Zinc fingers



Cartoon representation of the protein Zif268 (blue) containing three zinc fingers in complex with DNA (orange). The coordinating amino acid residues of the middle zinc ion (green) are highlighted.

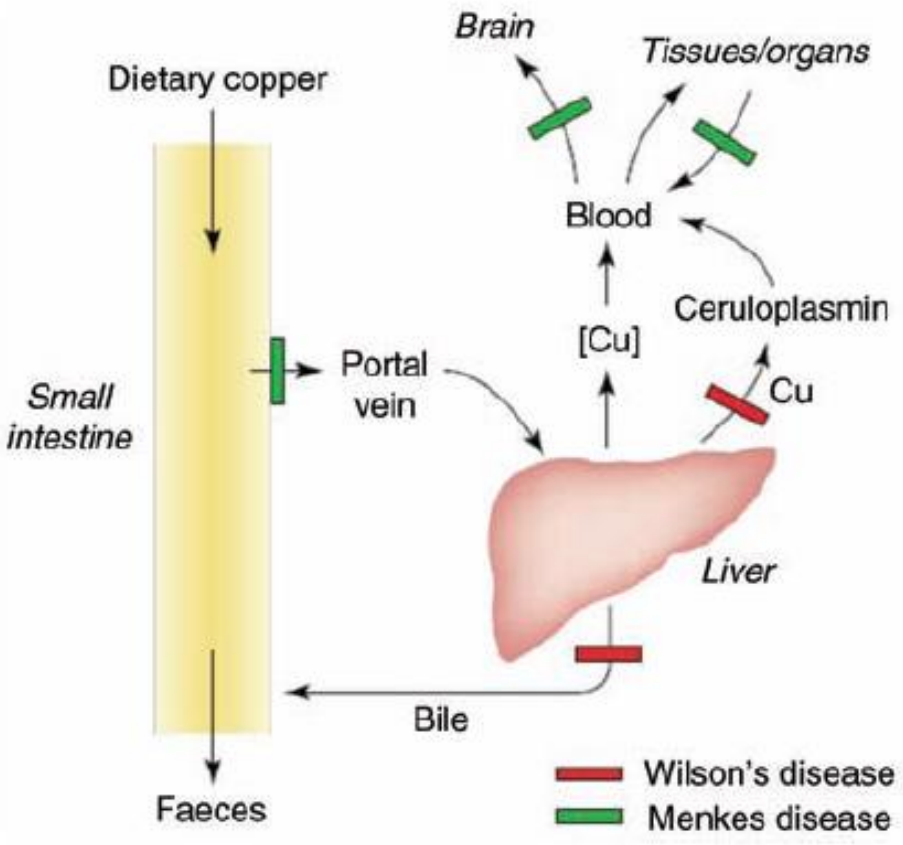
Zn(II): An electrophile turning nucleophile



Copper

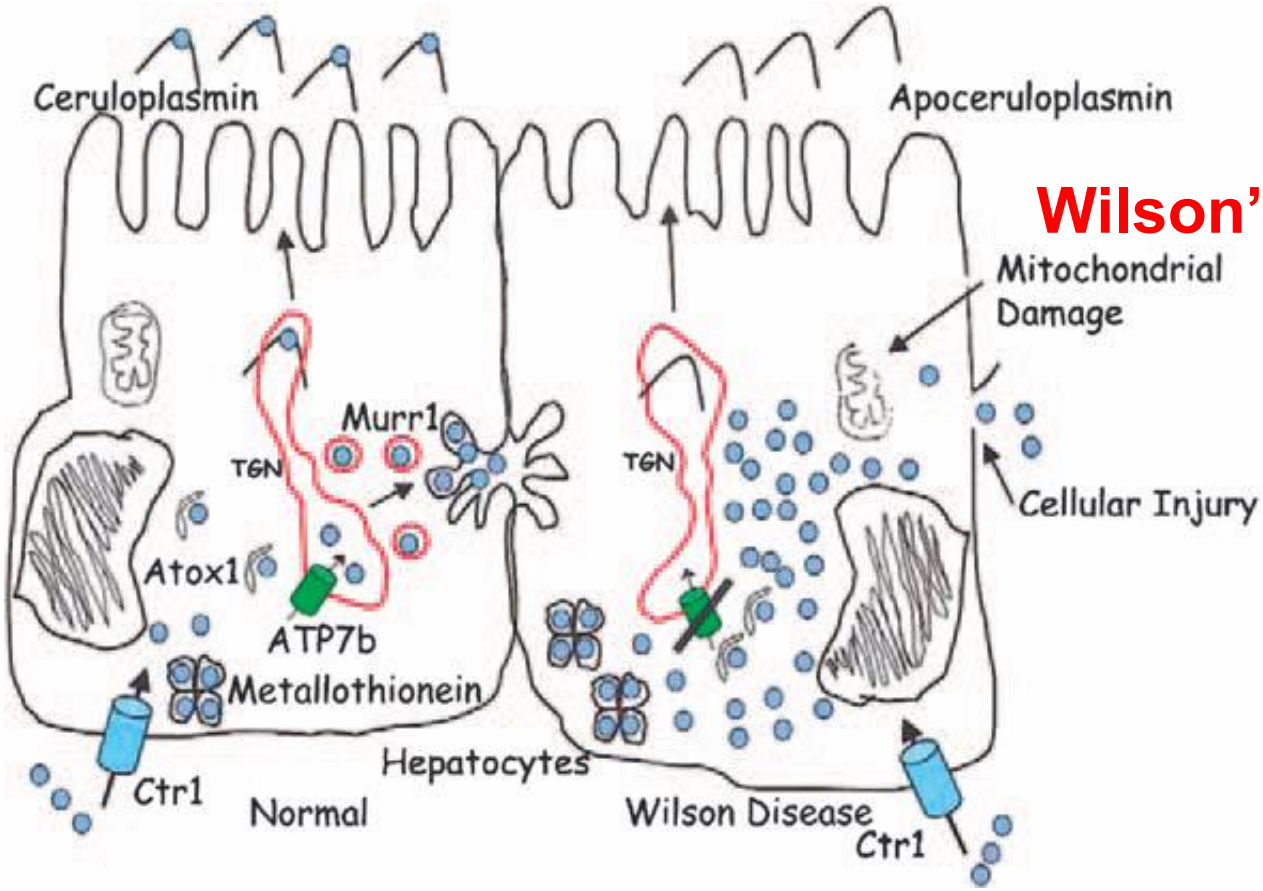
- Essential for normal brain function
- Deficiency linked to brain structural damage (myelin malformation)
- Excess is linked to redox activity (**oxidative stress**)
- Present in numerous metalloproteins linked to immune system (e.g. **S**uper **O**xide **D**ismutase -**SOD**)
- Dysregulation linked to **Wilson**'s and **Menkes** disease

Wilson's disease



Pathways of copper that are blocked in Menkes and Wilson's disease (from Crichton and Ward, 2006).

Disorders of copper metabolism



Proteins involved in copper uptake, incorporation into ceruloplasmin and biliary excretion in normal and Wilson's disease hepatocytes (From Crichton and Ward, 2006).

Aceruloplasminaemia

- **Ceruloplasmin thought to be a ferroxidase (Catalyzes oxidation of Fe(II) to Fe(III)).**
- **Neurodegenerative disease linked to absence of ceruloplasmin function due to mutations in the gene.**
- **Disruption of iron homeostasis and accumulation of iron in the brain and the liver.**

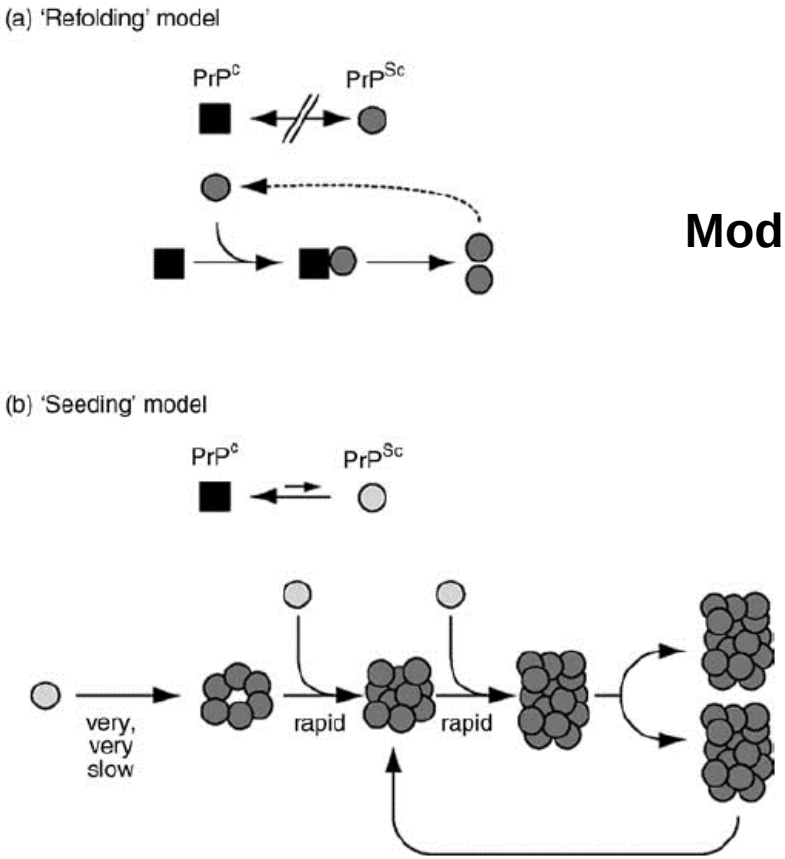
Ceruloplasmin

Ceruloplasmin is molecule containing copper and catalyzing the oxidation of Fe(II) to Fe(III)

$$Fe(II) \longrightarrow Fe(III) + e^-$$

Creutzfeldt-Jacob Disease (CJD)

- Fatal neurological disorders in humans discovered some 80 years ago
- Scrapie – Fatal disorder in sheep
- Transmissibility was established in the 1930's
- Bovine Spongiform Encephalopathy in cattle and link to CJD
- Causative agent is the prion protein.
- Intimate relationship to copper metabolism

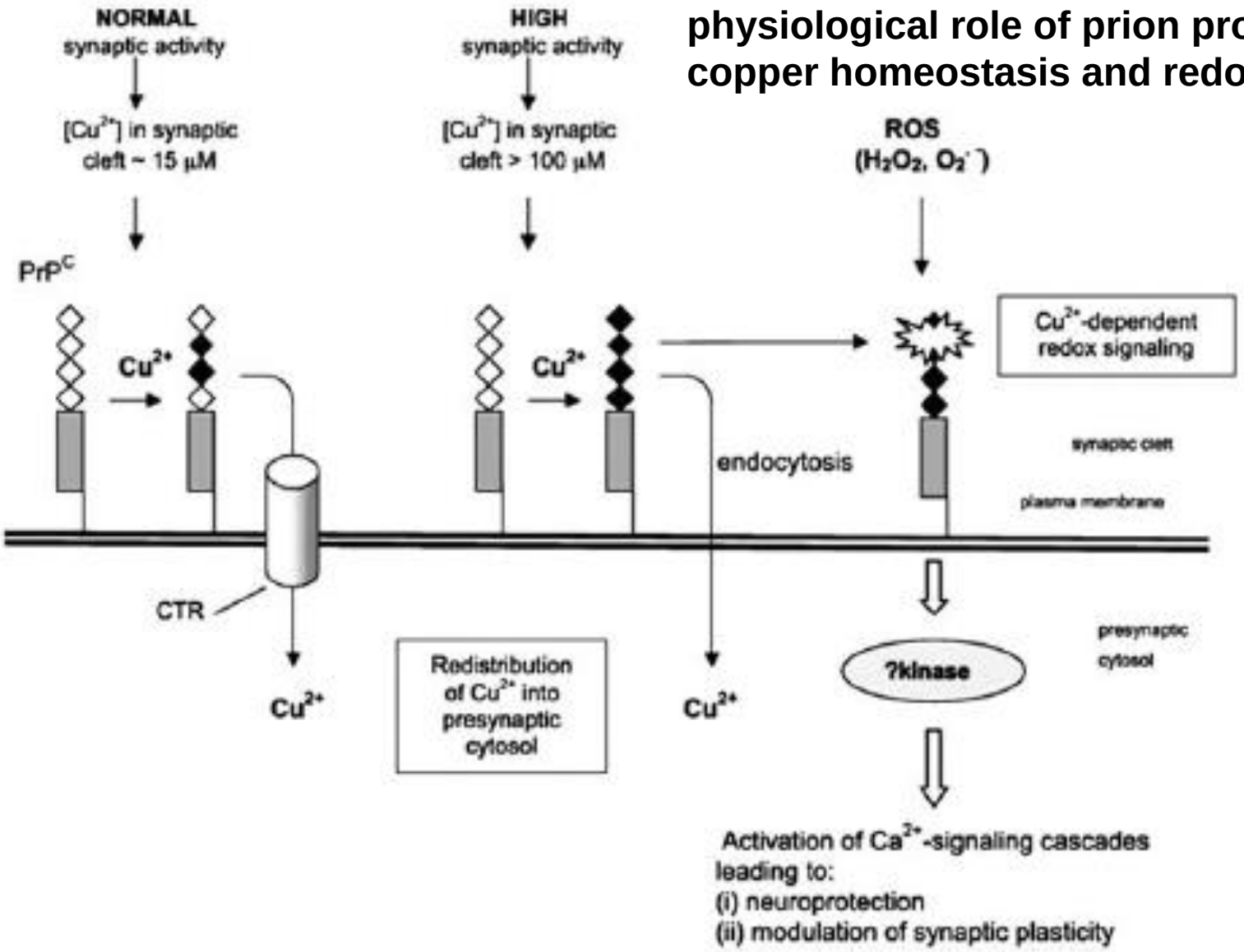


Models for the conversion of PrP^{c} to PrP^{Sc}.

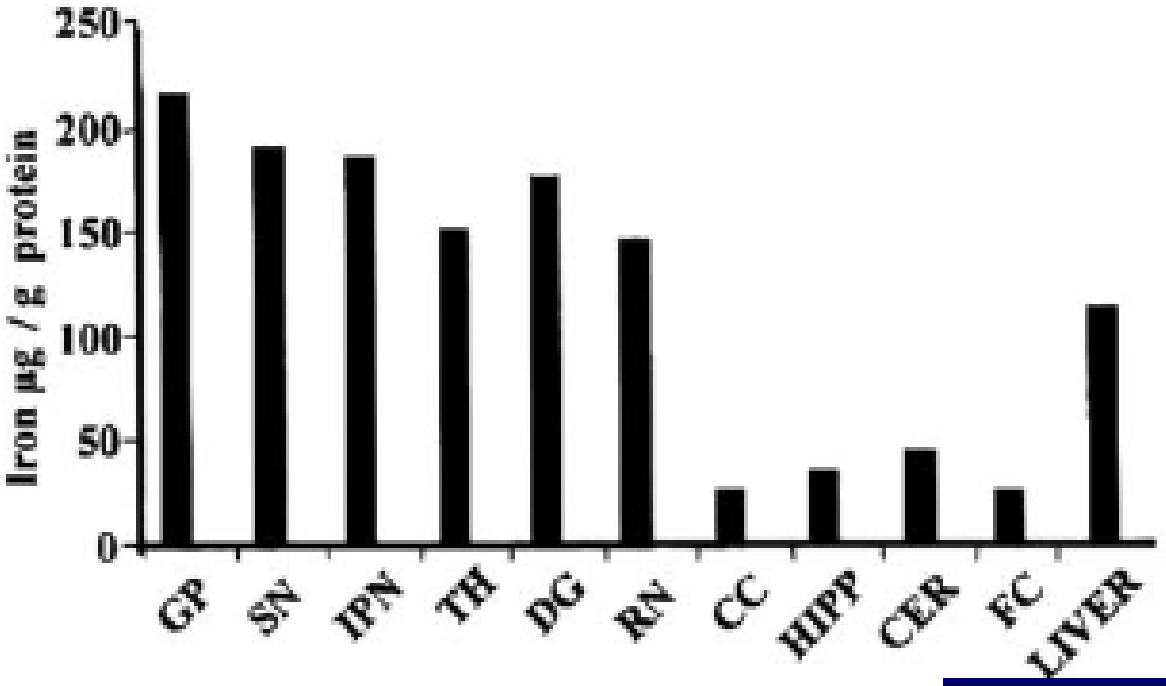
Conversion from an α-helical form in PrP^{C} to a β-sheet conformation in PrP^{Sc}

CJD link with Cu(II)

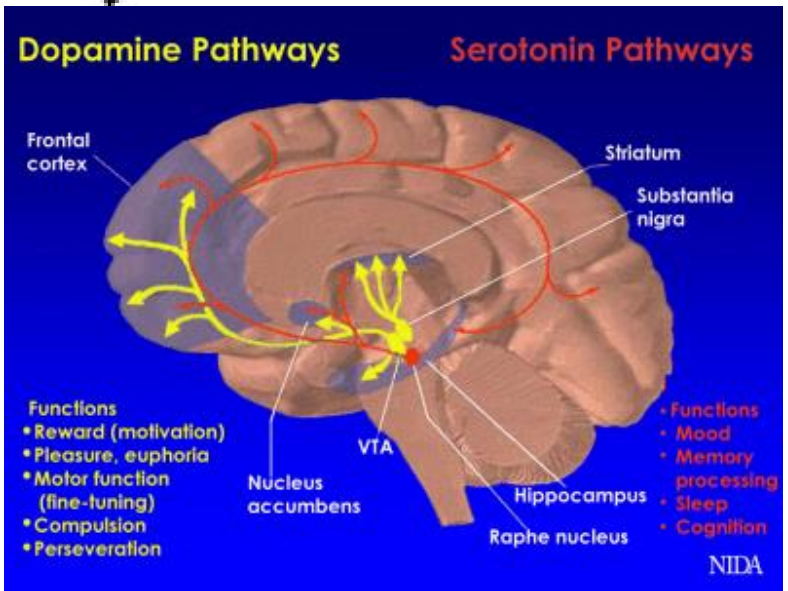
Schematic representation of the physiological role of prion protein (Prp^c) in copper homeostasis and redox signaling.



Iron in the Brain



Distribution of iron in human brain. GP, globus pallidus; SN, substantia nigra; IPN, interpeduncular nucleus; TH, thalamus; DG, dentate gyrus; RN, red nucleus; CC, cerebral cortex; HIPP, hippocampus; CER, cerebellum; FC, frontal cortex.



Receptor mediated endocytosis

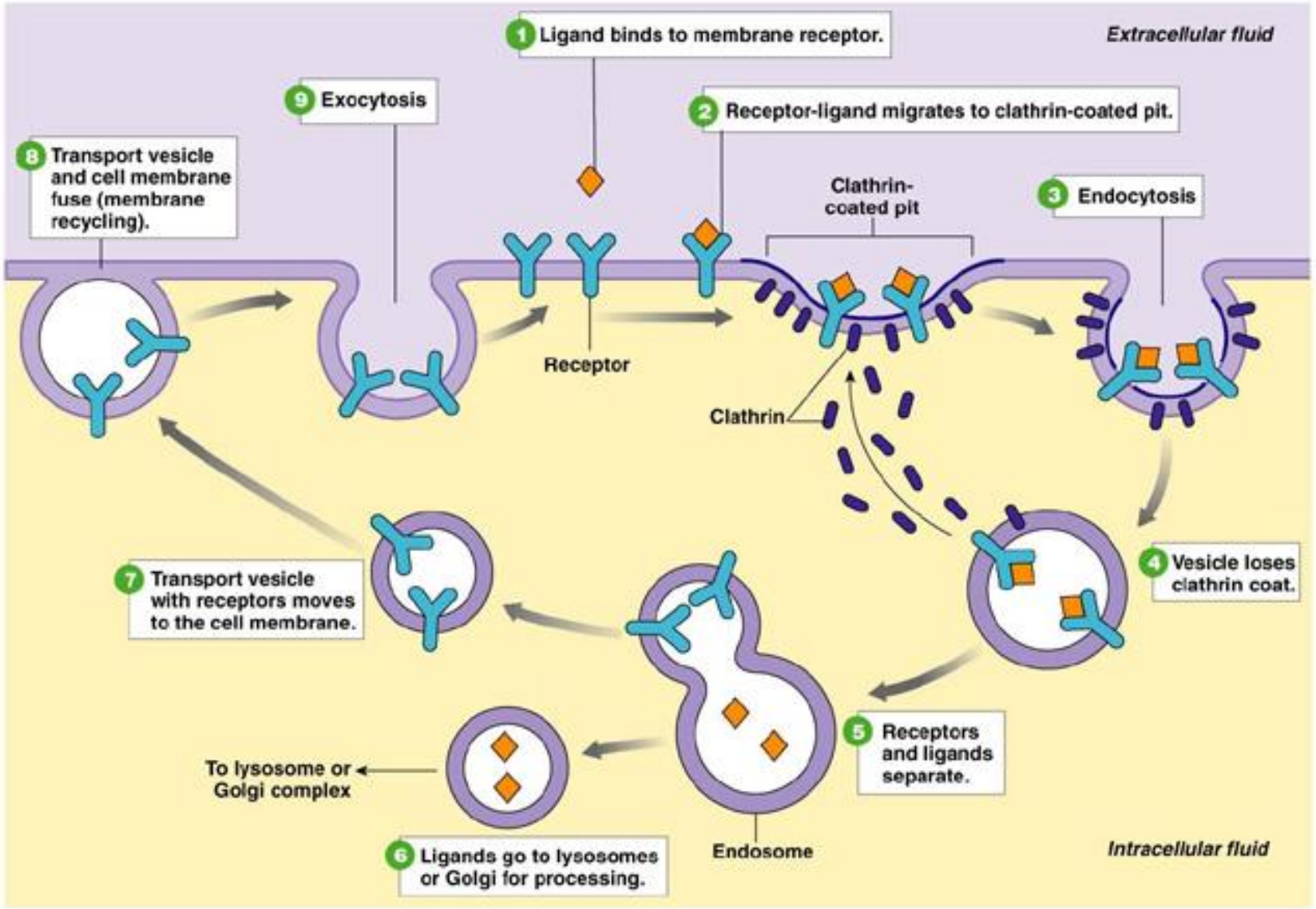
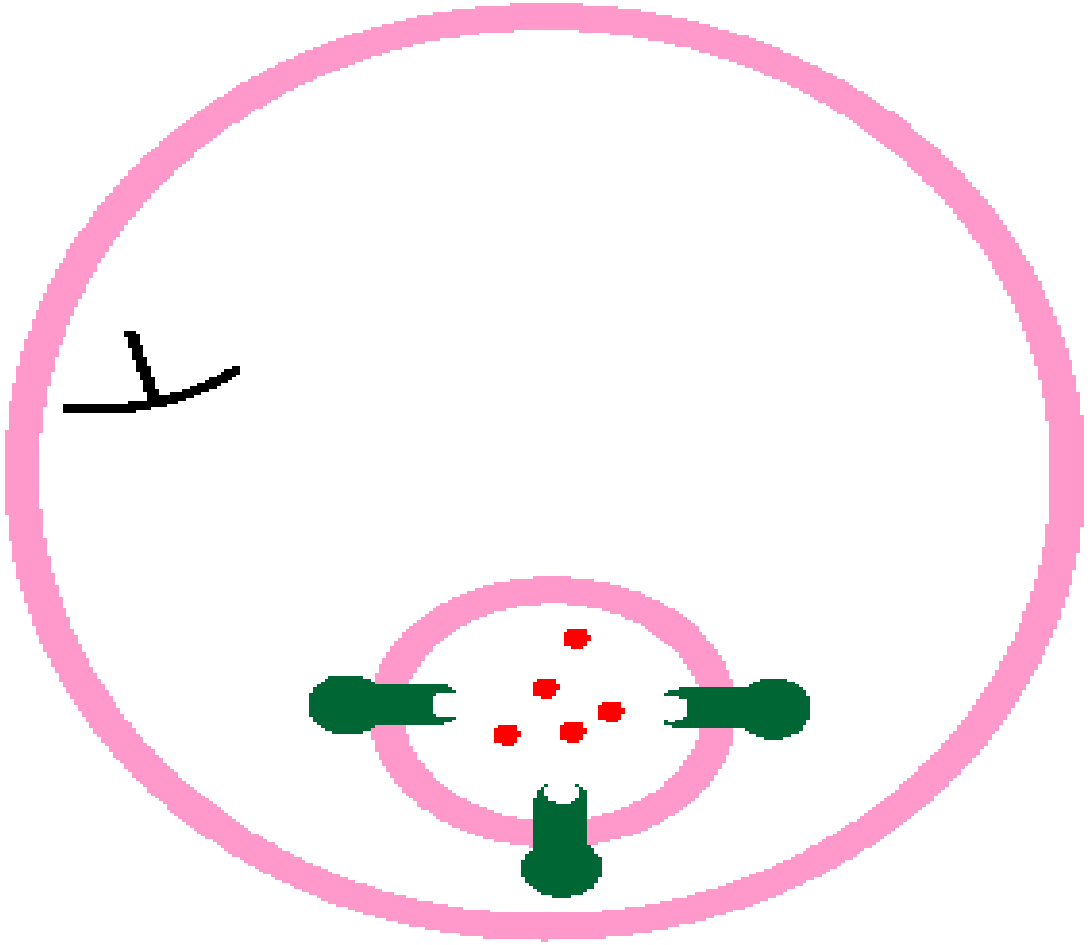


Fig. 5-24

Transferrin receptor (TfR)-mediated endocytosis



Receptor-mediated endocytosis of Fe(III)

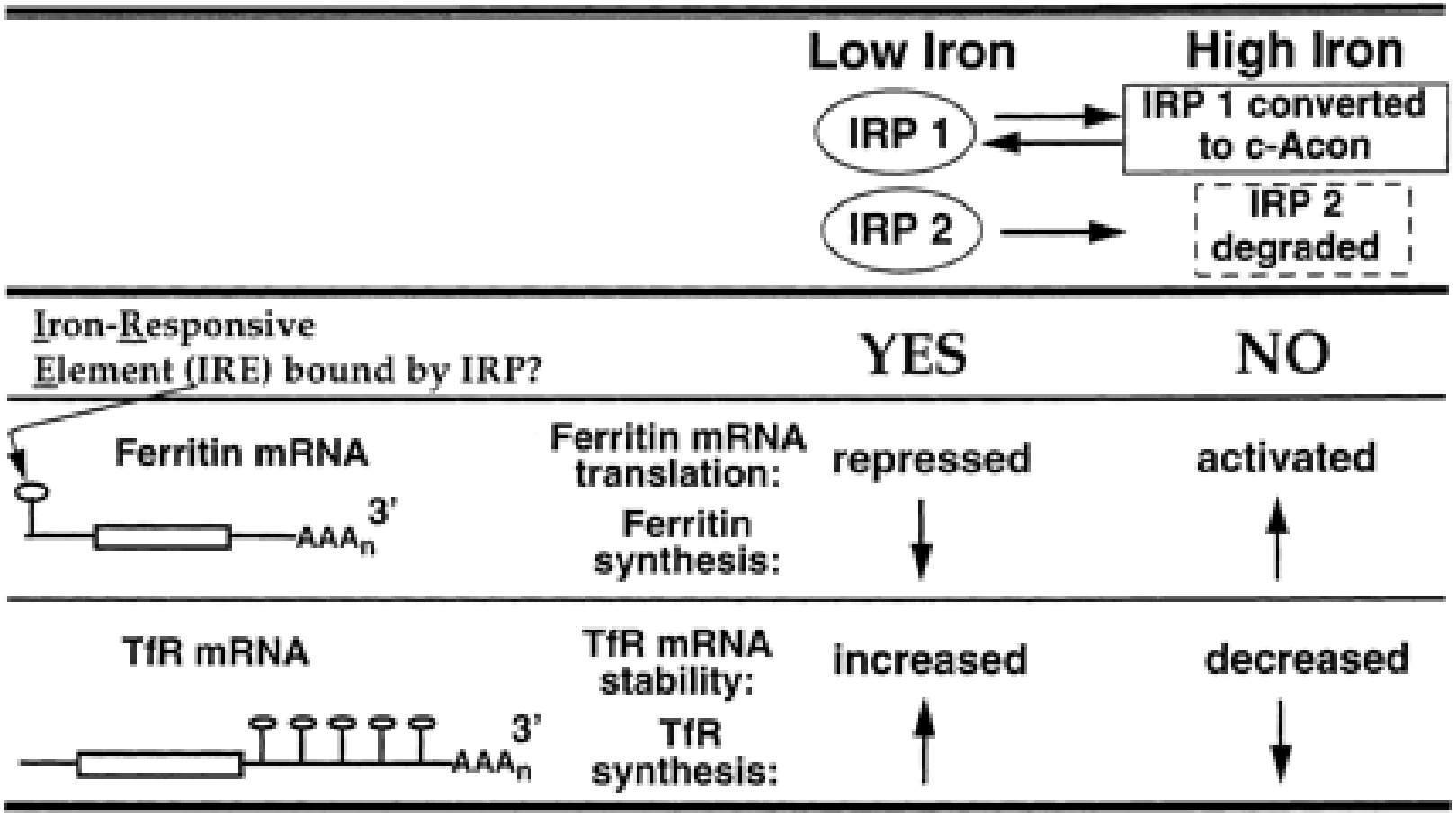
mRNA in the regulation of bio-iron

- mRNA is the main target of iron-induced changes in the expression of ferritin and TfR in animals. mRNA regulation depends on a family of non-coding sequences called **IREs (Iron Responsive Elements).**

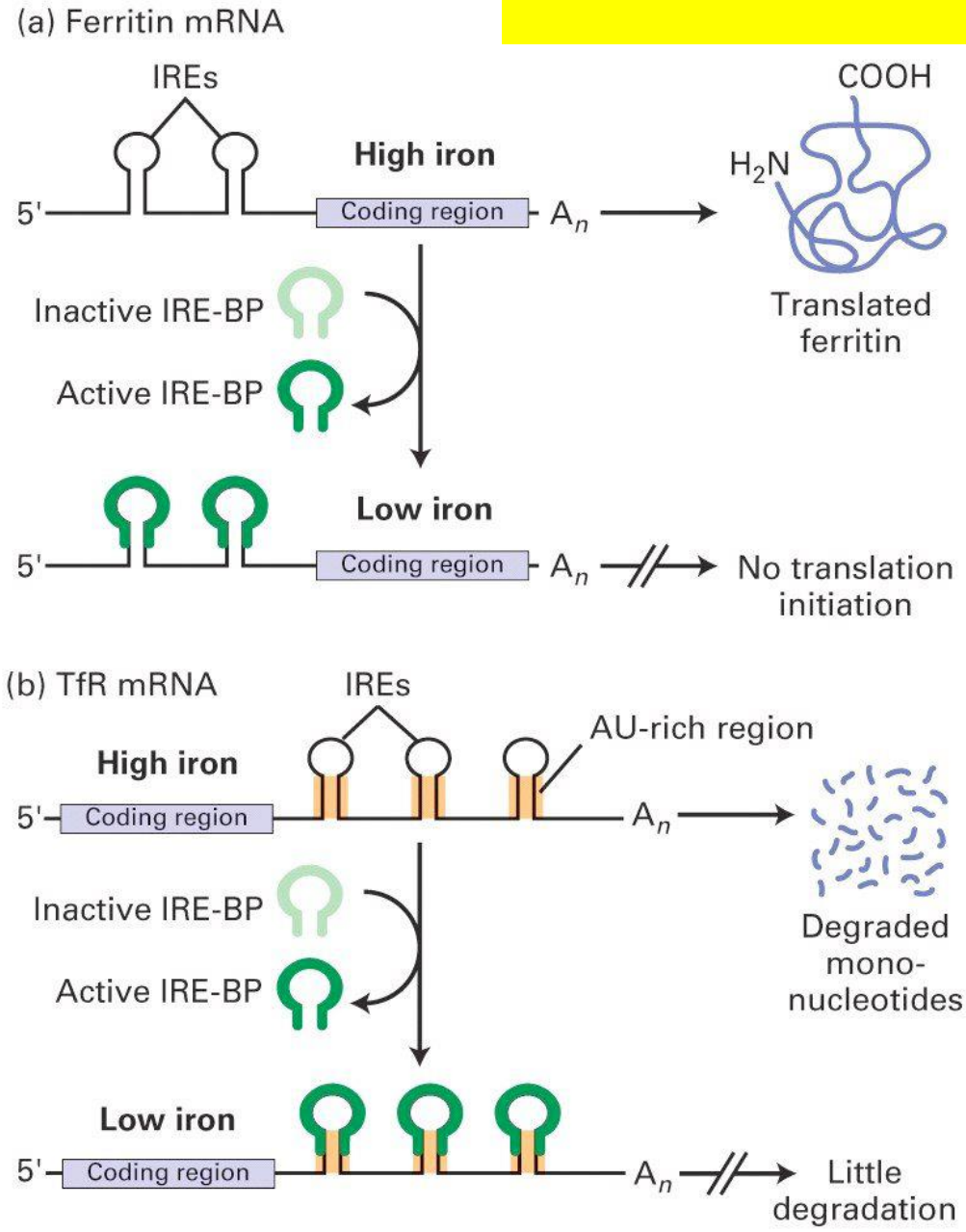
- IREs, that regulate expression, are rich in G/C and found in the 5’ end of mRNA. IREs responsible for the instability of mRNA are rich in A/U. They are encountered in the 3’ untranslated region of mRNA and contain the normal sequence AUUUA, frequently seen in many mRNA conversion elements.

- All IREs have a secondary structure reflecting a folded hairpin with a common terminal hairpin sequence CAGUG/C. The ternary structure emerges from interactions with proteins and nucleases.

IRON RESPONSIVE ELEMENTS - IRON RESPONSIVE PROTEINS

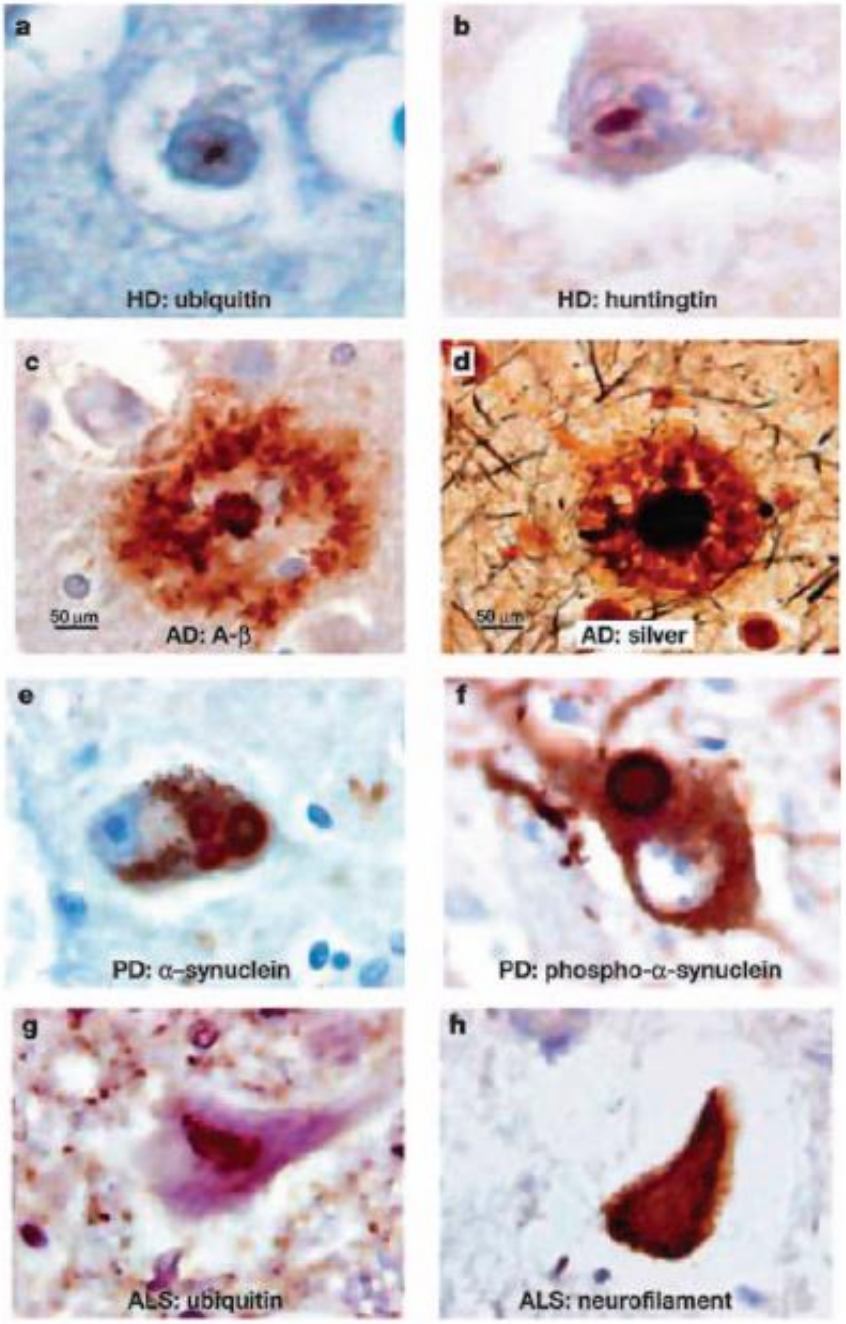


mRNA Ferritin regulation



Redox Metal Ions – Oxidative Stress and neurodegenerative disorders

Characteristic inclusion bodies in neurodegenerative diseases, all labeled with antibodies (except (**d**)) as indicated.
(a) and (b) HD intranuclear inclusion labeled for **ubiquitin** and **huntingtin** (cerebral cortex).
(c) and (d) AD neuritic plaque labeled with Aβ (cerebral cortex) and silver stained.
(e) and (f) PD, Lewy bodies labeled for α-synuclein and phosphorylated α-synuclein (substantia nigra).
(g) and (h) ALS labeled with ubiquitin and neurofilaments (medulla oblongata).



Parkinson's Disease

- There is a two fold increase in iron content in the brain
- There is no up-regulation of ferritin expression

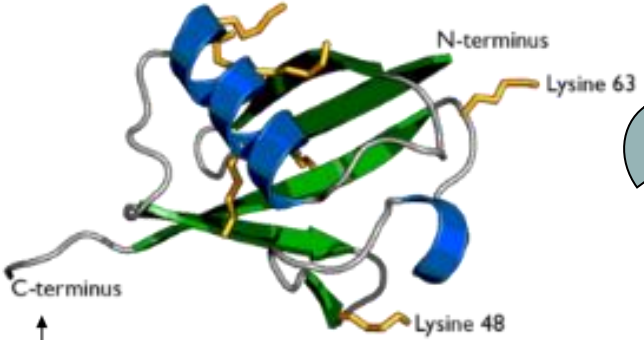
Molecules associated with the disease are

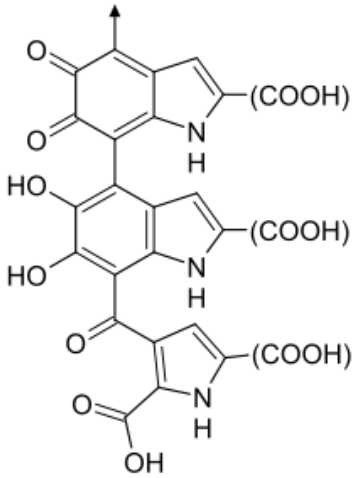
- α-synuclein
- Parkin
- Ubiquitin carboxy-terminal hydroxylase

Mutations in synuclein lead to fibril structures eventually yielding fibrils and Lewy bodies – hallmarks of the disease

Neuromelanin-iron complex in dopaminergic neurons in PD

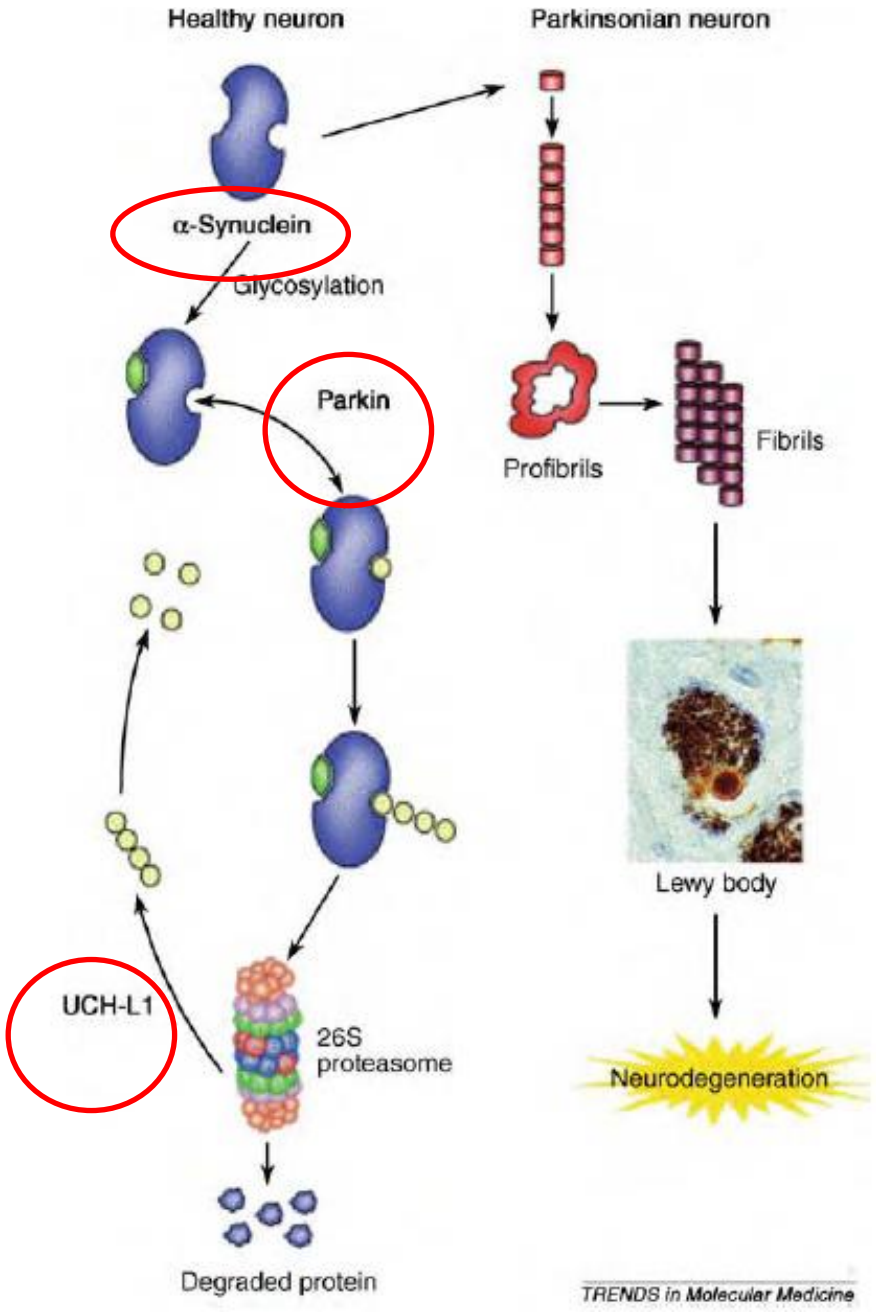
The three protein model in Parkinson's disease





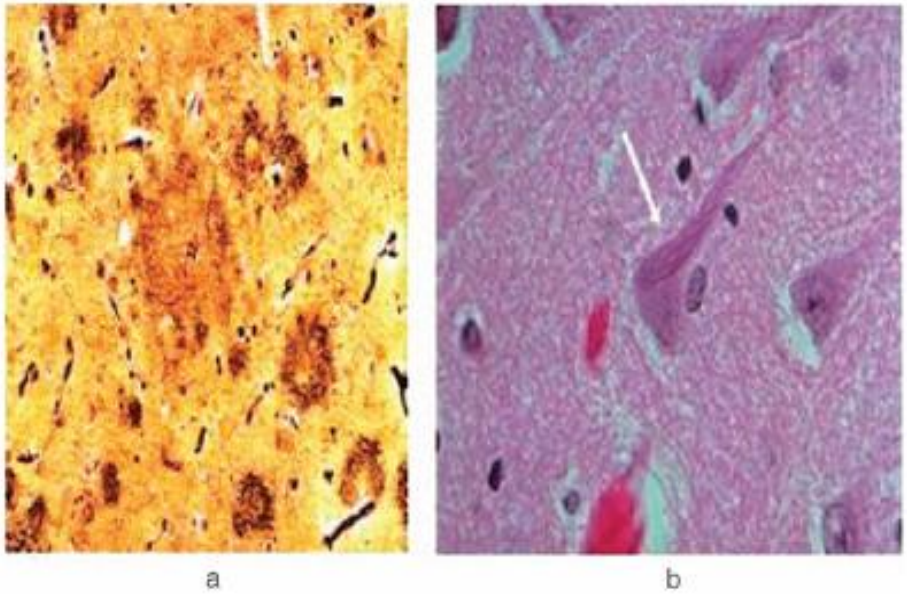
Fe(III)

(Eu)Melanin



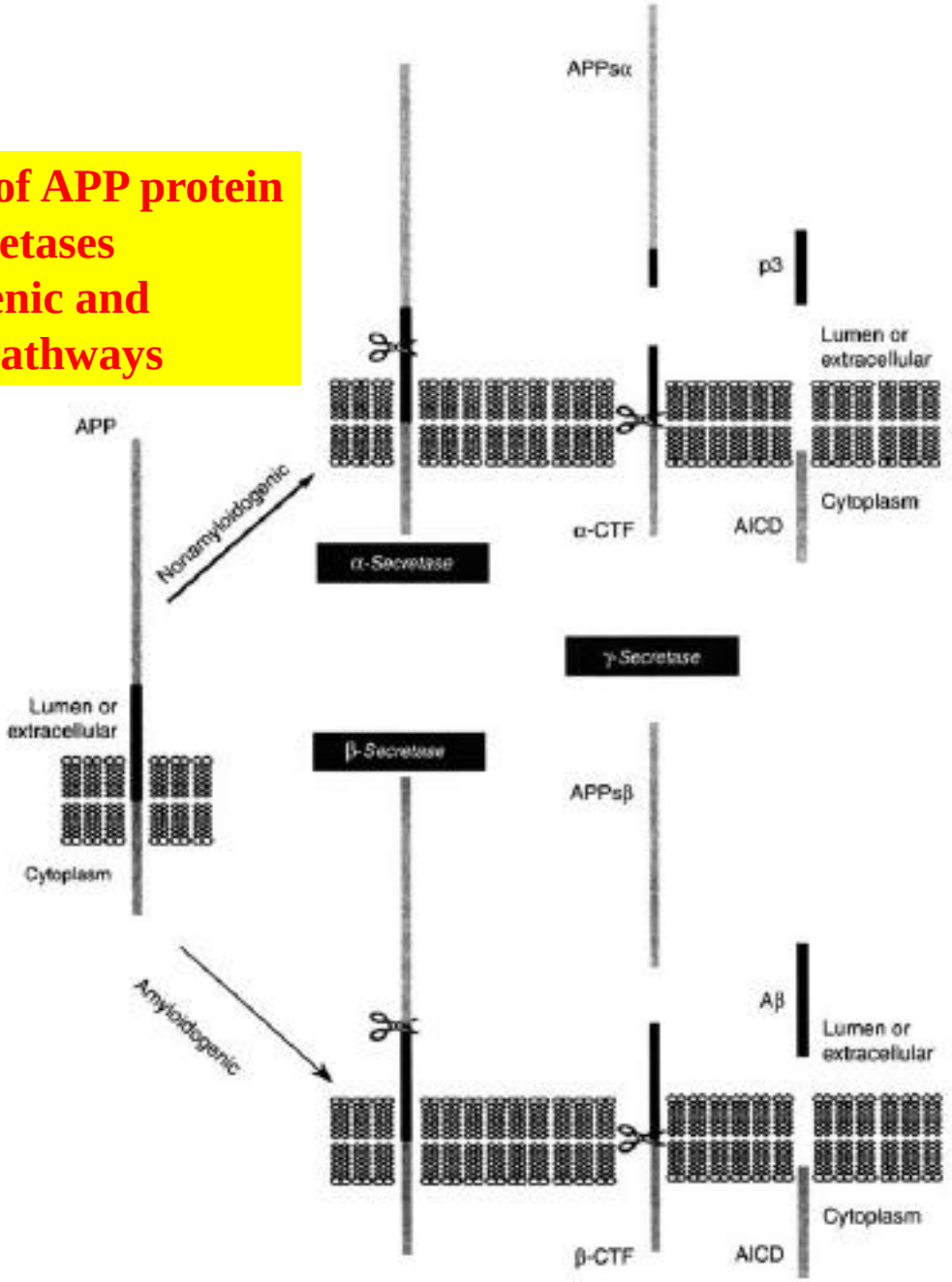
Alzheimer's Disease

- No effective treatment
- Memory loss and Progressive decline in cognitive and motor functions
- Females are more susceptible than males



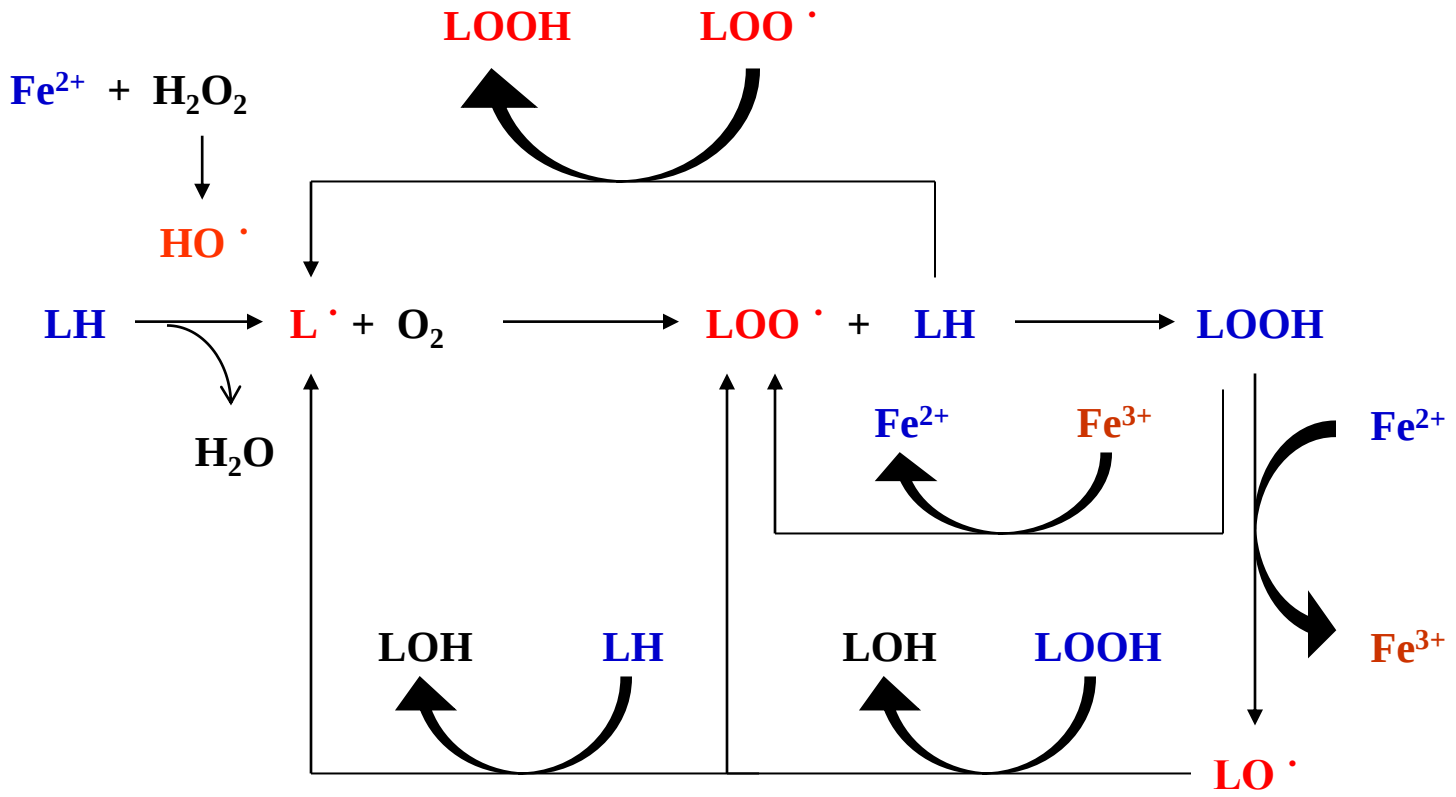
(a) Characteristic histo-pathological findings of Alzheimer's disease are senile plaques — a collection of degenerative pre-synaptic endings with astrocytes and microglia. Plaques are stained with silver stains and are of varying size.
(b) Neurofibrillary tangles of Alzheimer's disease. The tangles are present as long pink filaments in the cytoplasm. Each is composed of cytoskeletal intermediate filaments.

Proteolytic processing of APP protein by α, β, γ-secretases
Non-amyloidogenic and amyloidogenic pathways



The detrimental effects of metal ions in disturbing the physiology of natural defense. Relevance to health and disease states

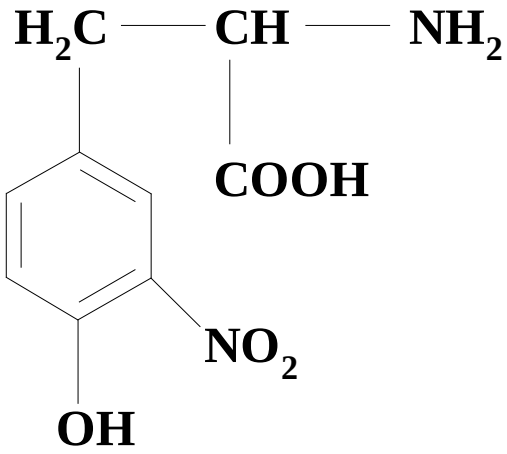
The effect of metal ions in the oxidative destruction of biological structures (e.g. lipid membranes)



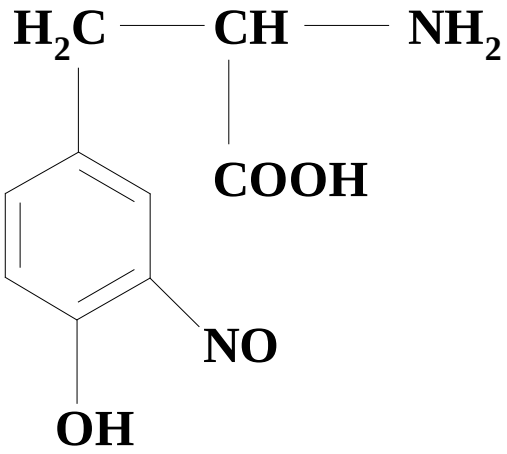
Oxidative Damage in Alzheimer's Disease

Target Molecule	Type of Modification
Advanced Glycation End	Glycation
Protein	Nitration-Carbonyls
Lipids	Hydroxynonenal-Acrolein
Nucleic Acids	8-Hydroxyguanosine

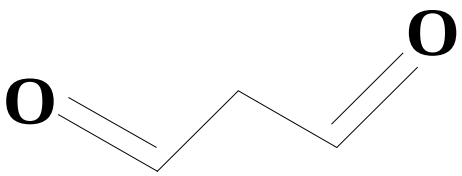
Products of Oxidative Stress



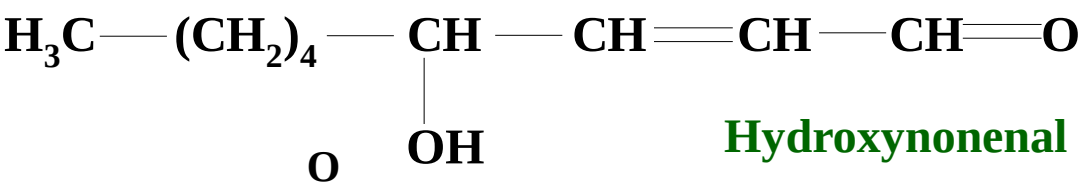
Nitrotyrosine



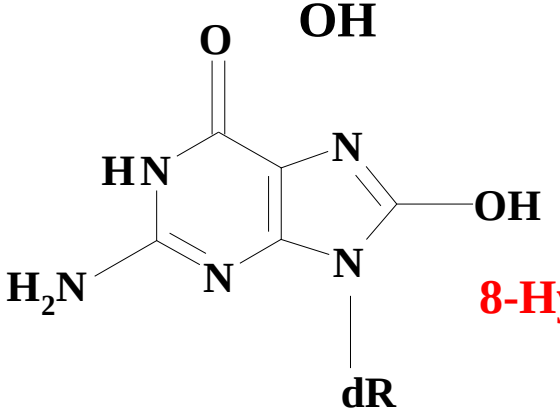
Nitrosotyrosine



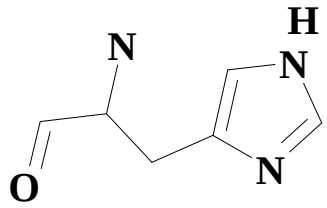
Malondialdehyde



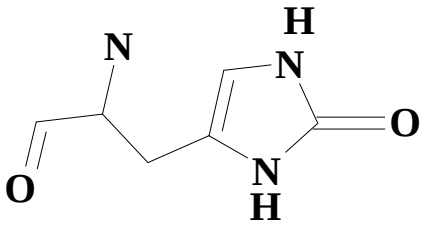
Hydroxynonenal



8-Hydroxyguanosine

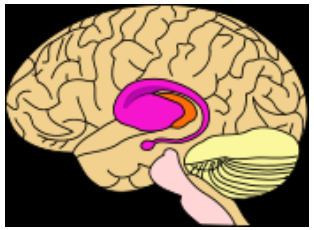


Histidine oxidation



Huntington's Disease

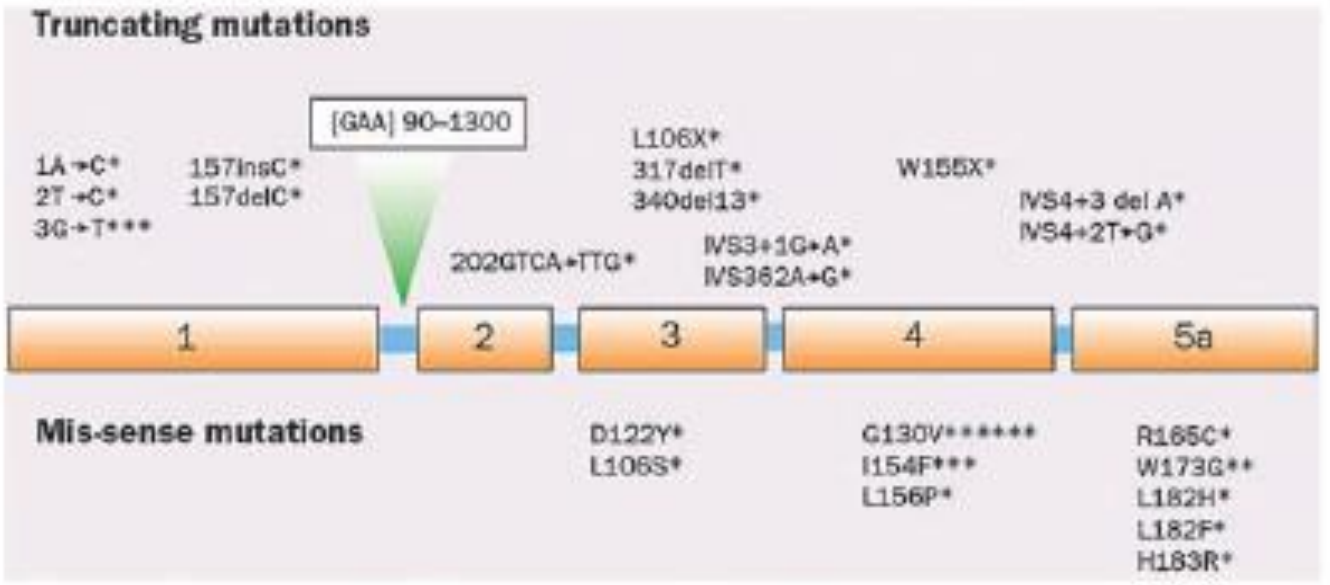
- Family of diseases intimately linked with the expansion of the CAG codon yielding extended polyglutamine (polyQ) tracts in the expressed protein
- **Bradykinesia,** slowness of speech, rigidity and dystonia
- The most sensitive region is the **striatum**



- The age and length of disease depends on the length of polyQ
- The protein is **huntingtin** (180 kb, 3144 amino acids)
- Brain iron dysregulation has been found in HD

Friedreich's Ataxia

- Common hereditary ataxia.
- Cerebral ataxia linked to clumsiness, sensory loss, foot deformity, etc.
- Differs from HD in that the expansion of triplet repeats takes place in non-coding regions.
- Frataxin mRNA decreases and so does the 210 amino acid protein FRDA.
- Link to iron dysregulation (accumulation) in mitochondria



Conclusions

- Metal ions are crucial to cellular physiology
- Metal ion chemistry at the biological level follows the basic fundamental principles of chemical reactivity with variable structure organic substrates
- Redox active (iron, copper) and inactive metal ions (Na^+, K^+, Ca(II), Mg(II)) play structural and catalytic roles in their host biological molecules
- Signaling in all of its manifestations involves either metal ions or metal ions incorporated in biological hosts, thereby controlling structure-function relationships
- Biological metal ion hosts regulate metal ion reactivity so as to meet the demands of the cellular host(s). That reactivity pertains to extracellular, cytosolic as well as nuclear genetic molecular materials
- Accumulation or depletion of neurometal ions inflicts serious problems upon the organelles, processes, and cells, thereby precipitating numerous diseases in the entire hierarchy of organisms. Notable among all those diseases are neurodegenerative diseases in humans.
- Given the complexity of the interactions involved and the repercussions inflicted upon cells, research in the field remains a challenge for the future and a goal for the society.