

MALATTIE NEURODEGENERATIVE

- **progressiva** alterazione di funzioni di sistemi neuronali che alla fine portano alla **morte delle cellule neuronali**
- le malattie ad eziologia vascolare, metabolica, autoimmune **non** sono considerate neurodegenerative
- estrema **variabilità**:
 - processi altamente ristretti che coinvolgono specifici gruppi di cellule nervose o specifiche vie nervose, in aree definite del cervello
 - danni cellulari relativamente diffusi ad aree estese del SNC

Le **manifestazioni cliniche** dipendono dai sistemi neuronali coinvolti

Due tipi principali di sintomi clinici

✓ **DEMENZE:**

disordini cognitivi, associativi, caratteriali e di memoria

✓ **DISORDINI DEL MOVIMENTO:**

Ipercinesia, acinesia, paralisi

Molte delle malattie neurodegenerative presentano entrambe le classi di sintomi in qualche momento nel corso della progressione della malattia

CLASSIFICAZIONE DELLE MALATTIE NEURODEGENERATIVE

✓ *DEMENZE (degenerazioni corticali)*

- Malattia di Alzheimer
- Demenze a corpi di Lewy
- Demenze Fronto-temporali

✓ *DISORDINI DEL MOVIMENTO:*

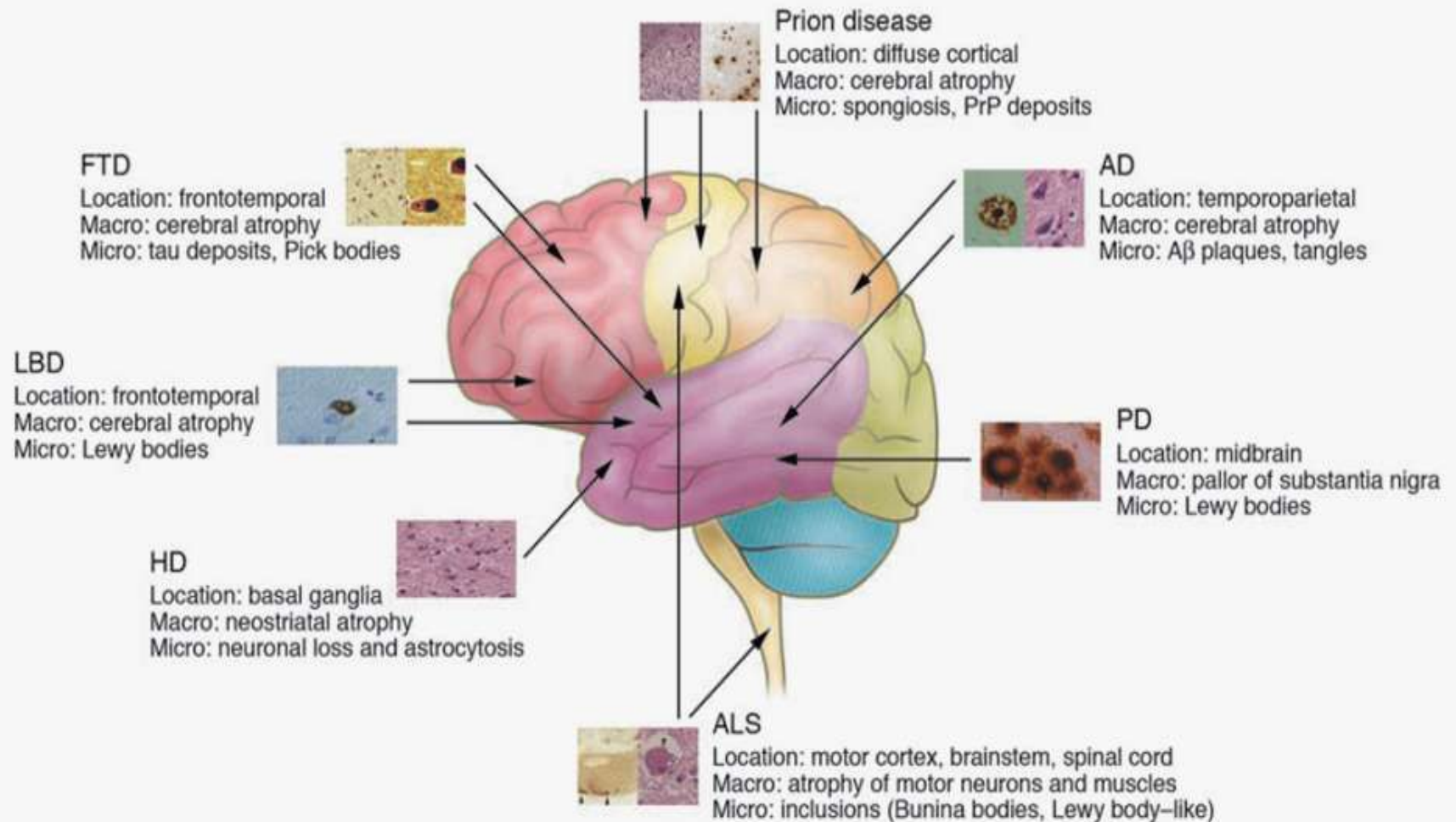
Sindromi acinetiche: Parkinson, Palsia supranucleare progressiva

Malattie dei sistemi motori: SLA, SMA

Sindromi ipercinetiche: Malattia di Huntington

Atassie cerebellari: genetiche/sporadiche

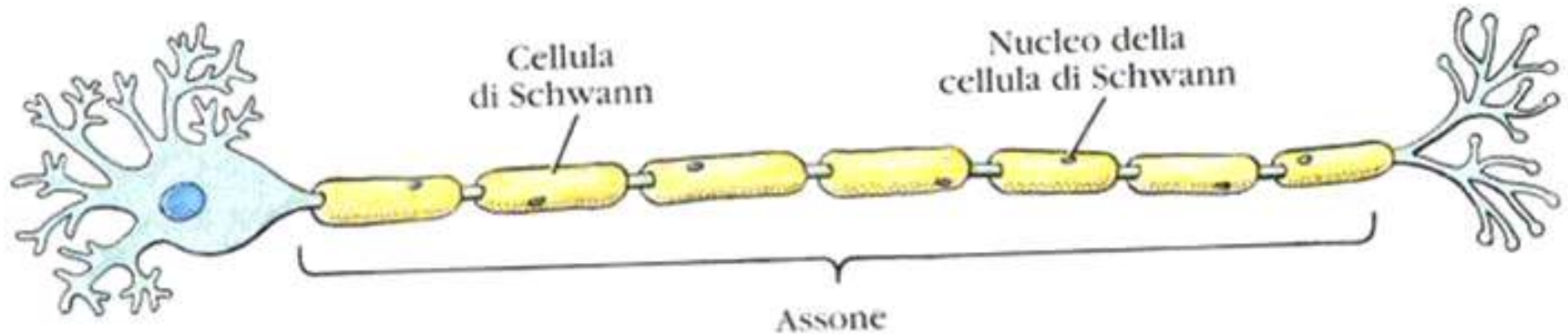
Localizzazione delle lesioni nel sistema nervoso



Le aree cerebrali (e più in generale il Sistema Nervoso) **non** sono formate da un unico tipo cellulare

I diversi tipi cellulari si associano tra loro da un punto di vista **morfologico** e **funzionale**

Il sistema nervoso è composto da **neuroni** e da altri tipi di cellule (non neuronali)



Neuroni: cellule specializzate per la trasmissione degli impulsi.

Dendriti prolungamenti riccamente ramificati e un lungo Assone fibroso.

I neuroni formano una **rete** di cellule che interagiscono fra di loro e con cellule non neuronali



neuroni

unipolari
bipolari
multipolari...

motoneuroni

interneuroni

glia

astrociti

microglia

oligodendrociti

cellule di Schwann

cellule ependimali

cellule satelliti

Il **dialogo** tra neuroni è reso possibile dalle loro due caratteristiche tipiche di eccitabilità e conducibilità.

Eccitabilità : capacità di reagire a stimoli esterni (fisici e chimici) che vengono convertiti in impulso nervoso.

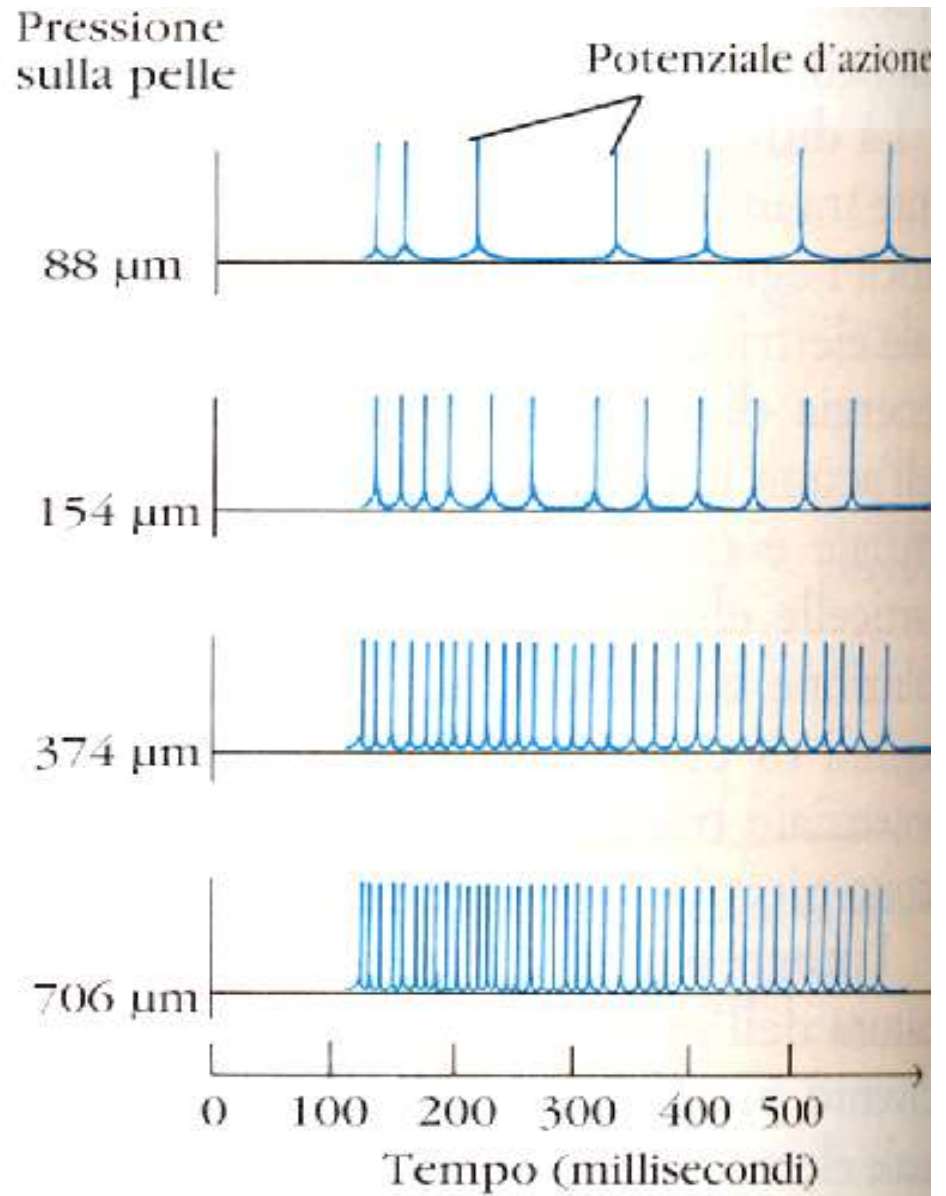
Conducibilità : l'impulso nervoso attivato in una cellula può essere trasmesso alle altre cellule in corrispondenza delle sinapsi.

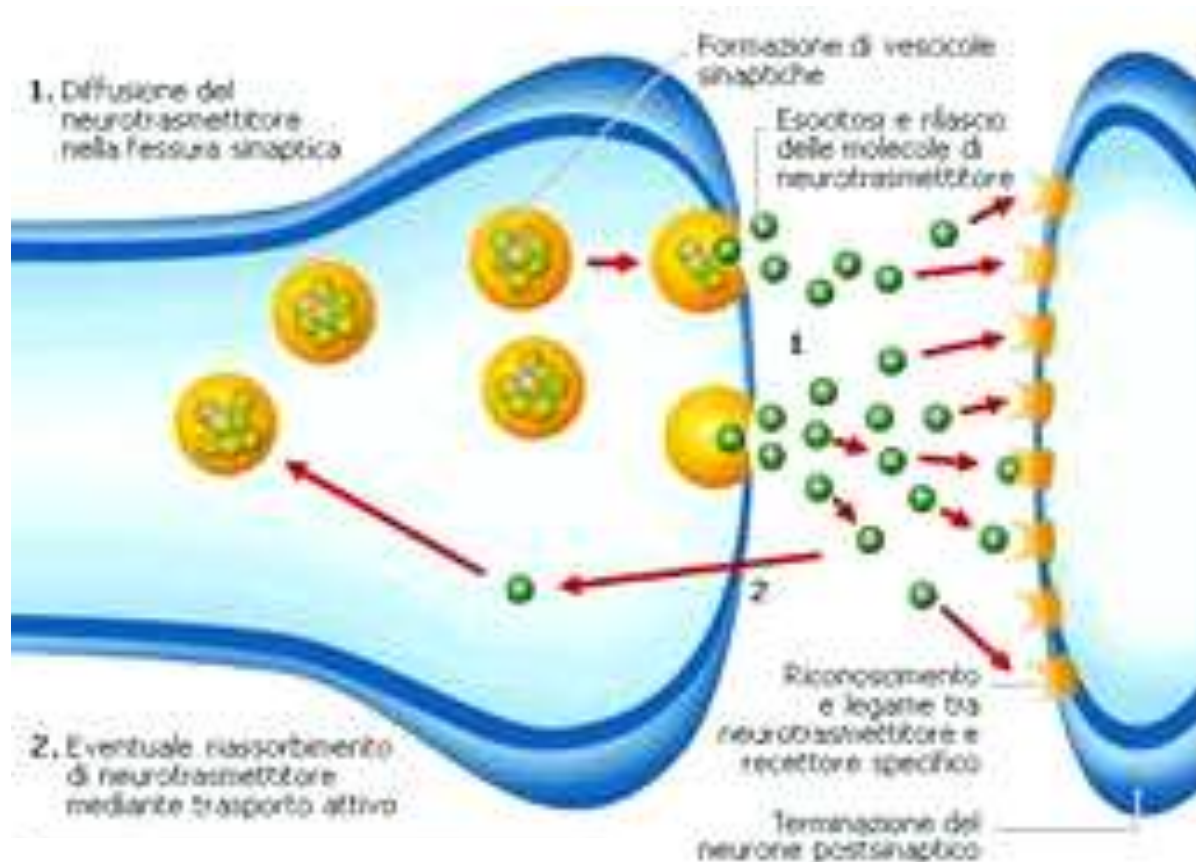
La sinapsi e la conduzione dell'impulso nervoso

- Le sinapsi sono siti dove gli impulsi nervosi passano da una cellula **presinaptica** (neurone) ad un'altra cellula **postsinaptica** (un neurone, una cellula muscolare o ghiandolare).
- Le sinapsi quindi permettono la comunicazione fra neuroni e fra questi e le cellule effettrici.

Sinapsi elettriche
Sinapsi chimiche

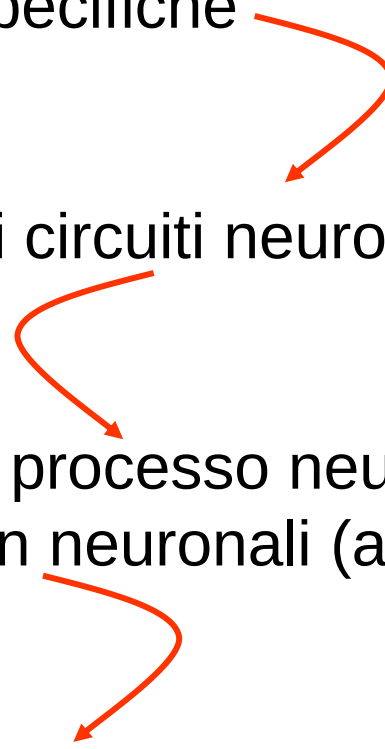
L'informazione viaggia
nel sistema nervoso
mediante trasmissione
di **impulsi elettrici** lungo
fibre conduttrici
(dendriti, assoni)





Nelle sinapsi chimiche i segnali elettrici vengono convertiti in segnali chimici utilizzando mediatori chimici, o **neurotrasmettitori**

Le malattie neurodegenerative sono un **processo dinamico**

- Lesioni cellulari specifiche
 - Interessamento di circuiti neuronali collegati
 - Modificazione del processo neurodegenerativo ad opera di cellule non neuronali (astrociti e microglia)
 - Progressione temporale (da mesi ad anni)
- 
- ```
graph TD; A[• Lesioni cellulari specifiche] --> B[• Interessamento di circuiti neuronali collegati]; B --> C[• Modificazione del processo neurodegenerativo ad opera di cellule non neuronali (astrociti e microglia)]; C --> D[• Progressione temporale (da mesi ad anni)];
```

I meccanismi biochimici che sottintendono alle malattie neurodegenerative sono  
(sorprendentemente) **pochi, inter-connessi** e  
(sorprendentemente) **comuni** alle diverse malattie

### *Malattie a carattere*

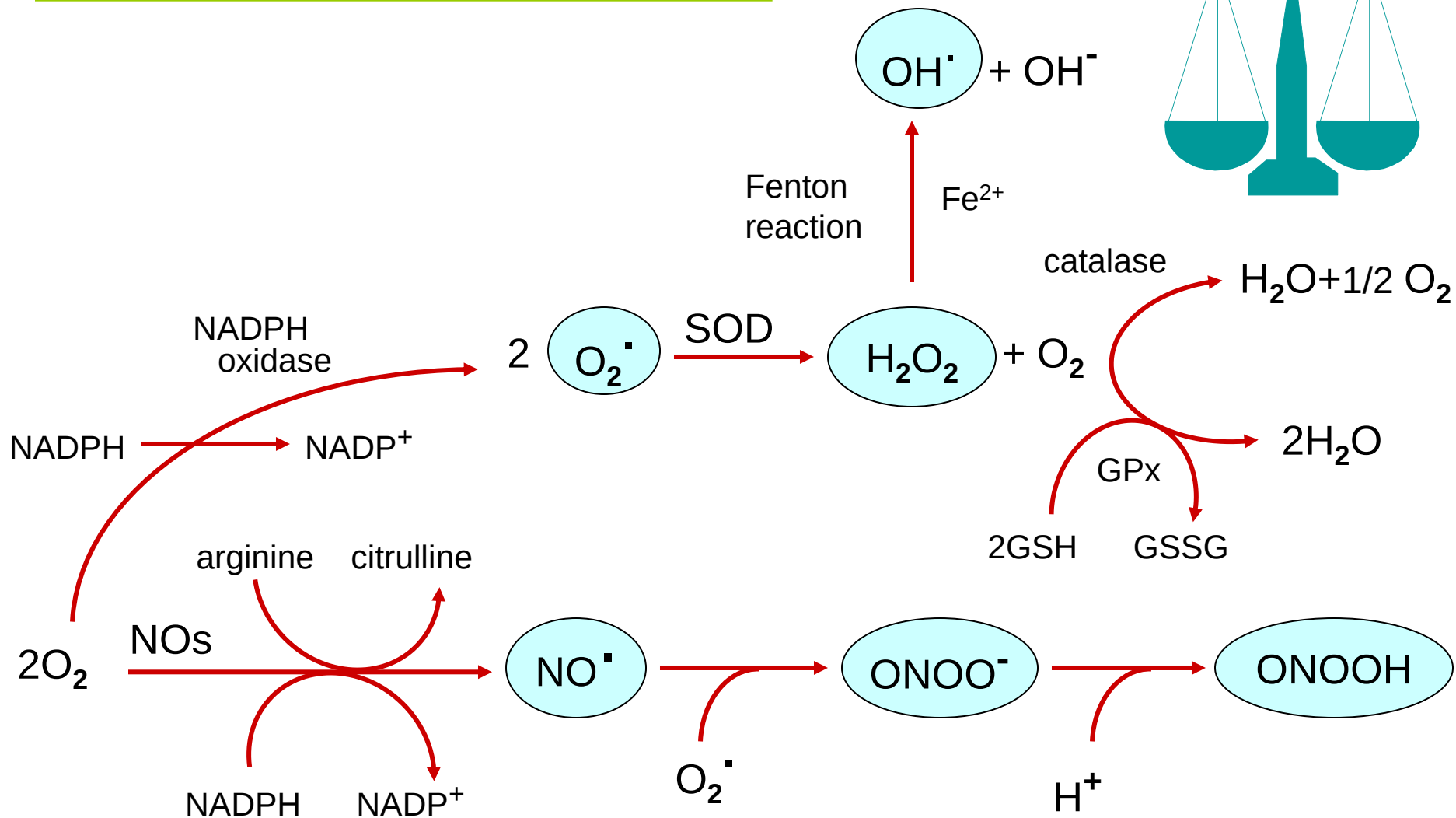
**Familiare** (causa genetica, più casi nella stessa famiglia)

**Sporadico** (causa “ambientale”, casi isolati)

- 
- stress ossidativo
  - disfunzione mitocondriale
  - aggregazione proteica
  - difetti del trasporto assonale
  - eccitotossicità

# Meccanismi – 1 – Stress ossidativo

# “Reactive” Oxygen Species

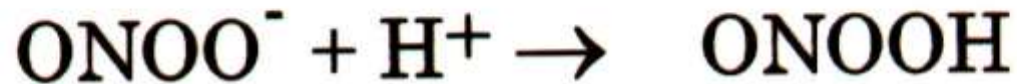
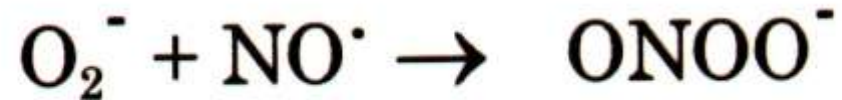


# “Nitrate” Oxygen Species

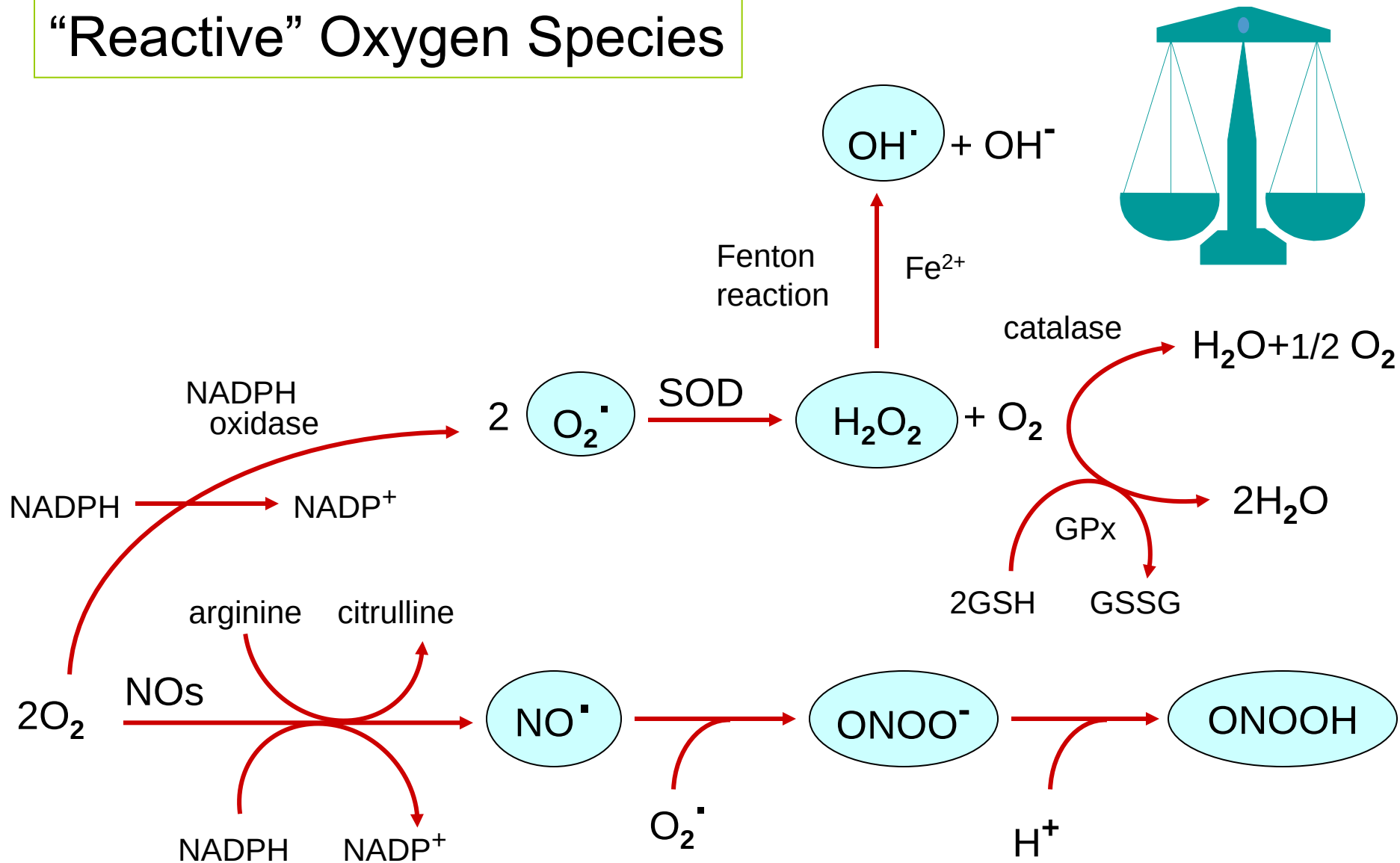
## La reazione di Fenton



## La formazione di perossinitrito



# “Reactive” Oxygen Species



# “Nitractive” Oxygen Species

# Clinical conditions in which involvement of oxidative stress has been demonstrated

## **Inflammatory-immune disease**

Autoimmune diseases

Rheumatoid arthritis

## **Degenerative syndromes**



Ageing

Cataract

Retrolental fibrosis

## **Neurodegenerative syndromes**



Alzheimer's disease



Parkinson's disease

Progressive supranuclear palsy



Huntington's disease

Down's Syndrome

Atherosclerosis



Amyotrophic lateral sclerosis



Prion disease

## **Drug and toxin-induced reactions**

Cancer (smoke, chemicals, asbestos etc.)

Iron overload

Alcoholism

Haemolytic syndromes

Lung emphisema

Toxicity from drugs (Bleomycin, paraquat, adramycin...)

Radiation injury

Ischemia-reperfusion states (myocardial, brain, intestinal infarction)



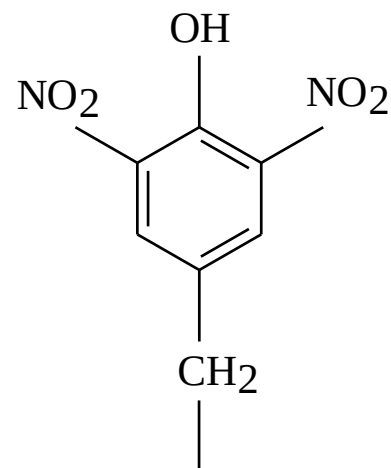
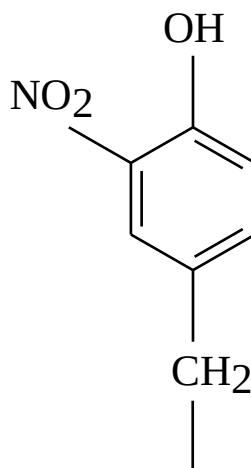
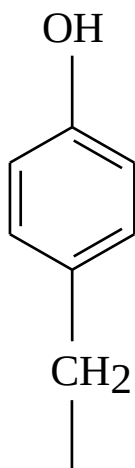
## conseguenze dello stress ossidativo

- ✓ Carbonilazione delle proteine
- ✓ Perossidazione dei lipidi di membrana
- ✓ Nitrazione delle proteine
  
- ✓ Ossidazione del mtDNA
- ✓ Ossidazione del DNA nucleare
- ✓ Ossidazione dell'RNA

# Markers of oxidative damage

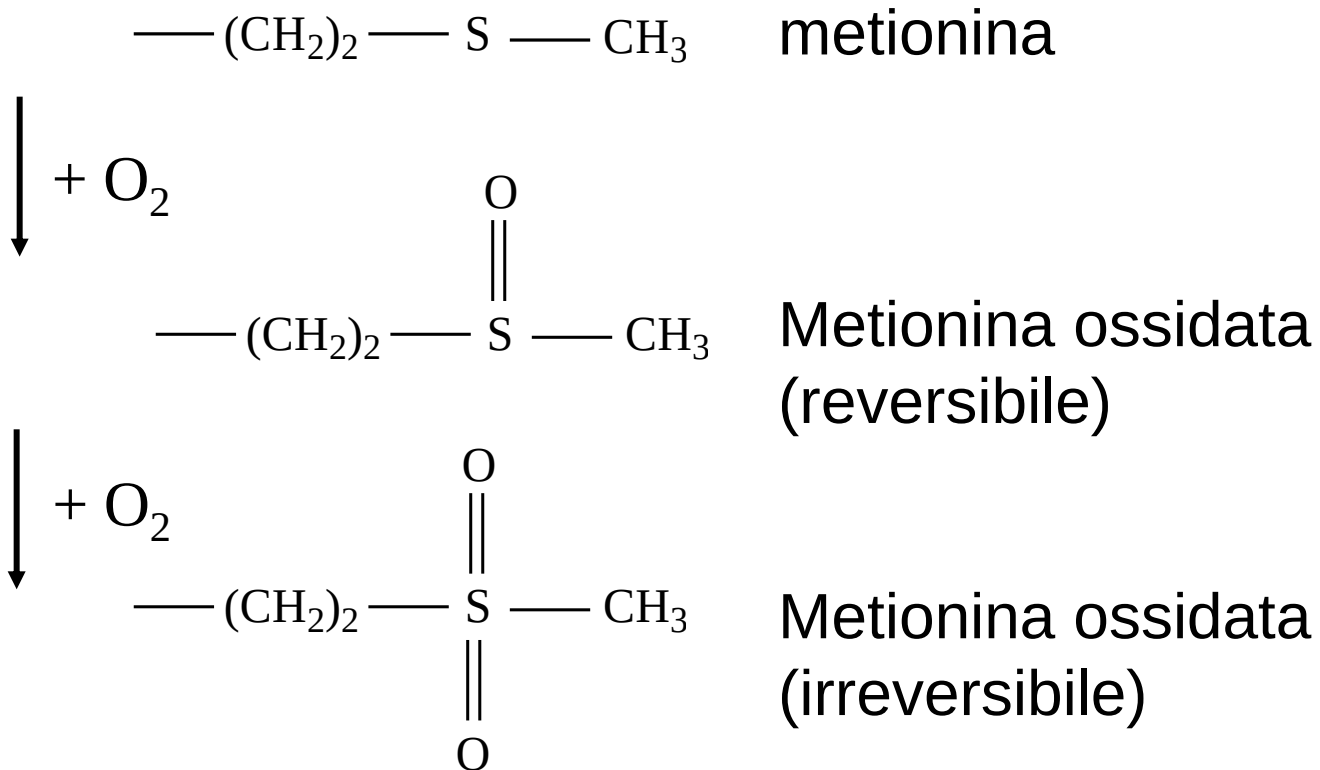
- > GSH : GSSG ratio
- > Protein nitration/oxidation
- > Free protein carbonyls
- > Lipid peroxidation adducts
- > Mitochondrial DNA oxidation adducts
- > Nuclear DNA oxidation adducts
- > Cytoplasmatic RNA oxidation adducts

# Tyrosine nitration

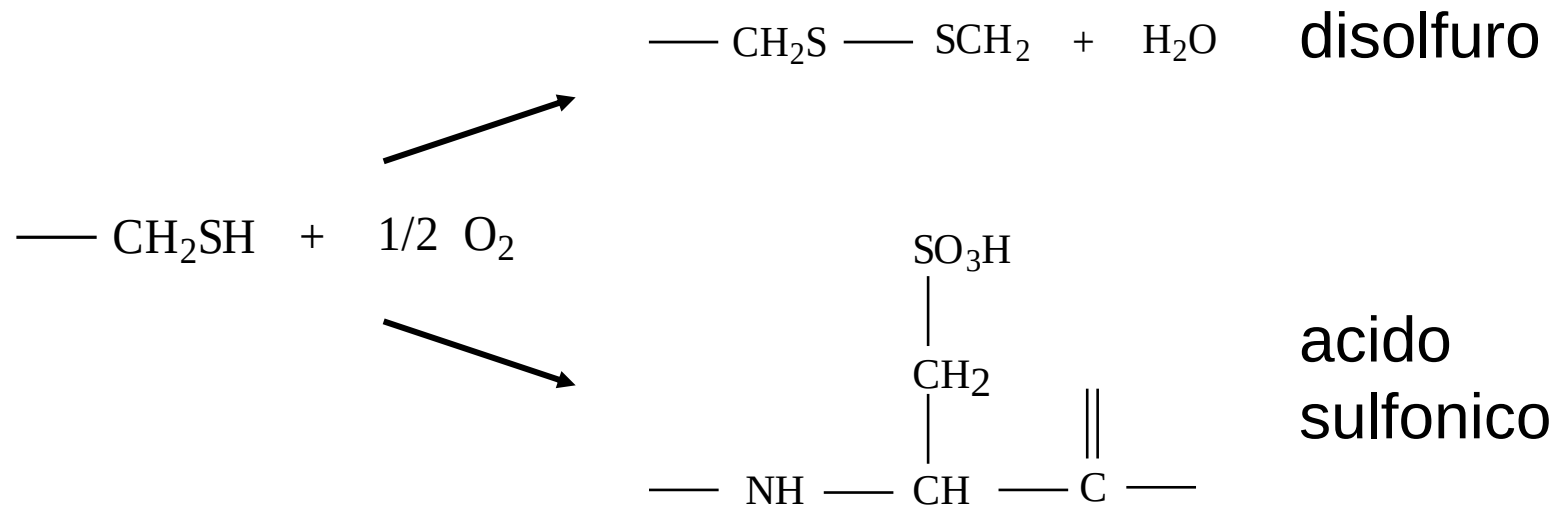


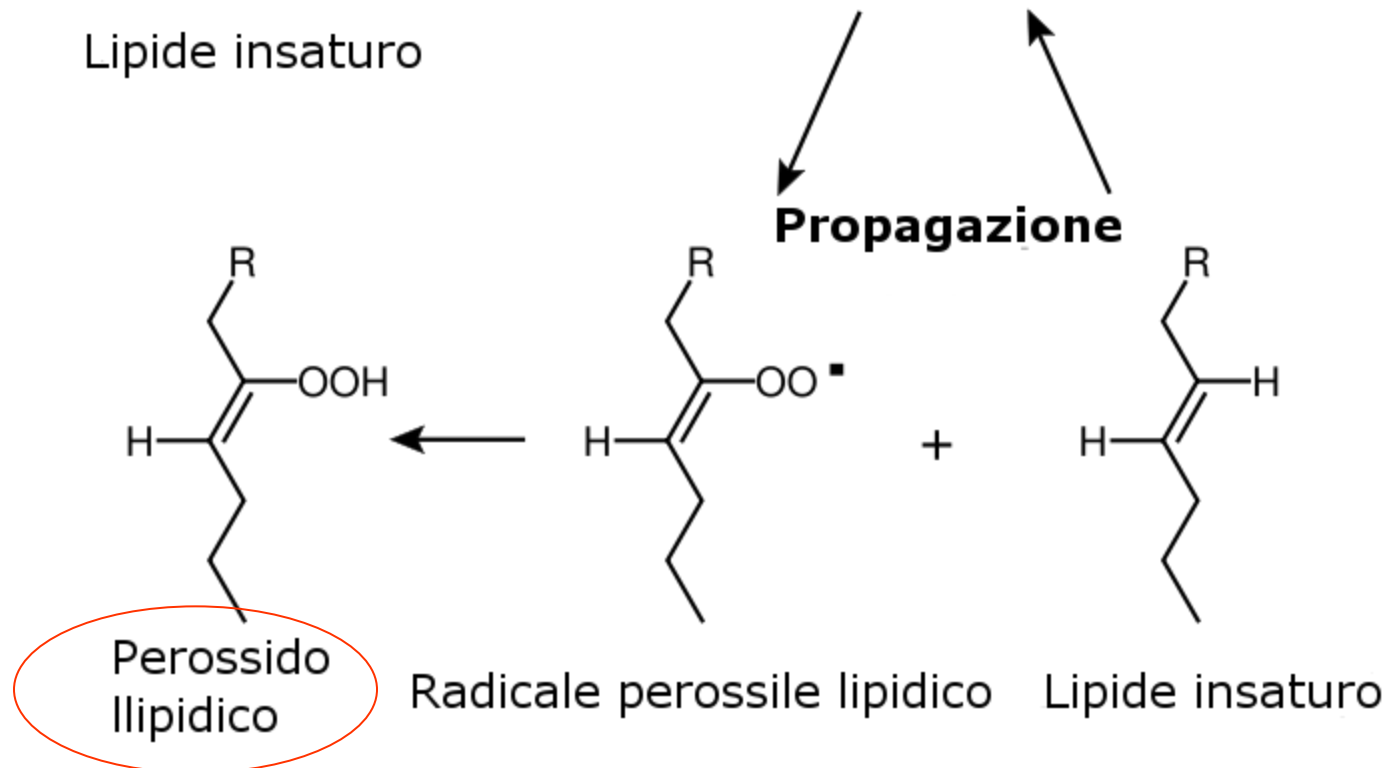
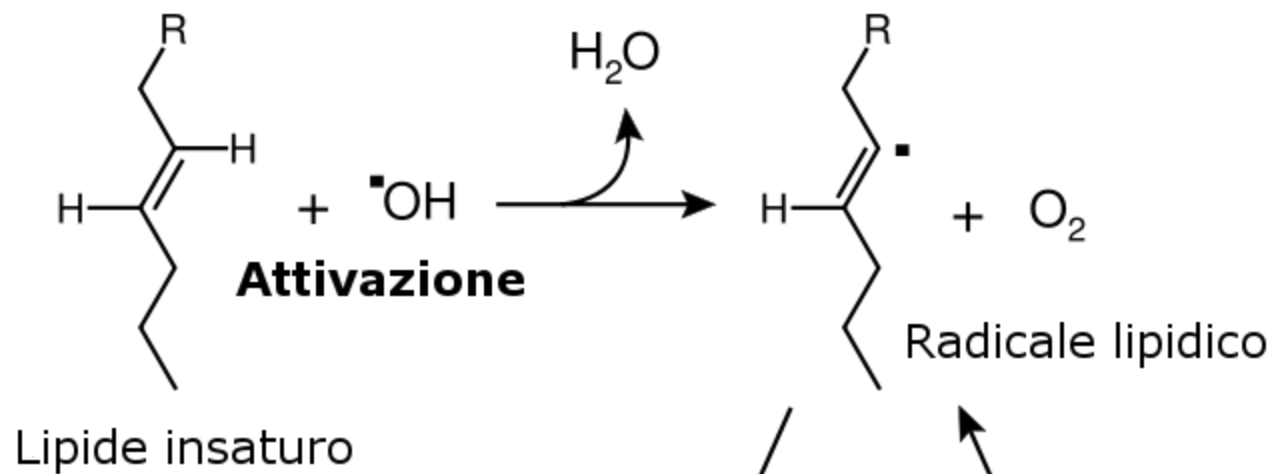
|                 | pK <sub>app</sub> -OH | λ <sub>max</sub> (nm) |
|-----------------|-----------------------|-----------------------|
| Tyrosine        | 10.1                  | 274.5                 |
| ε-nitrotyrosine | 7.2                   | 360                   |

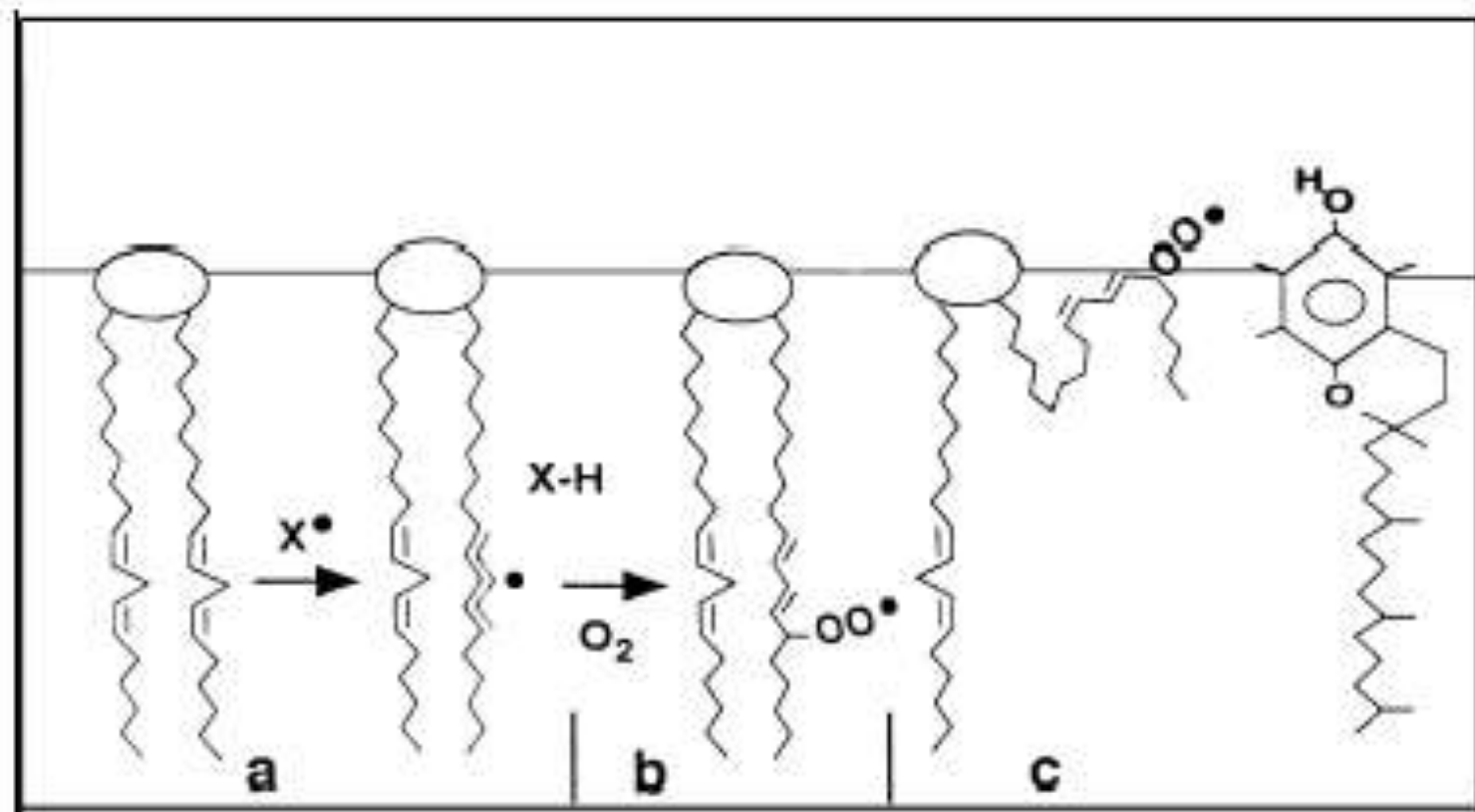
# Methionine oxidation

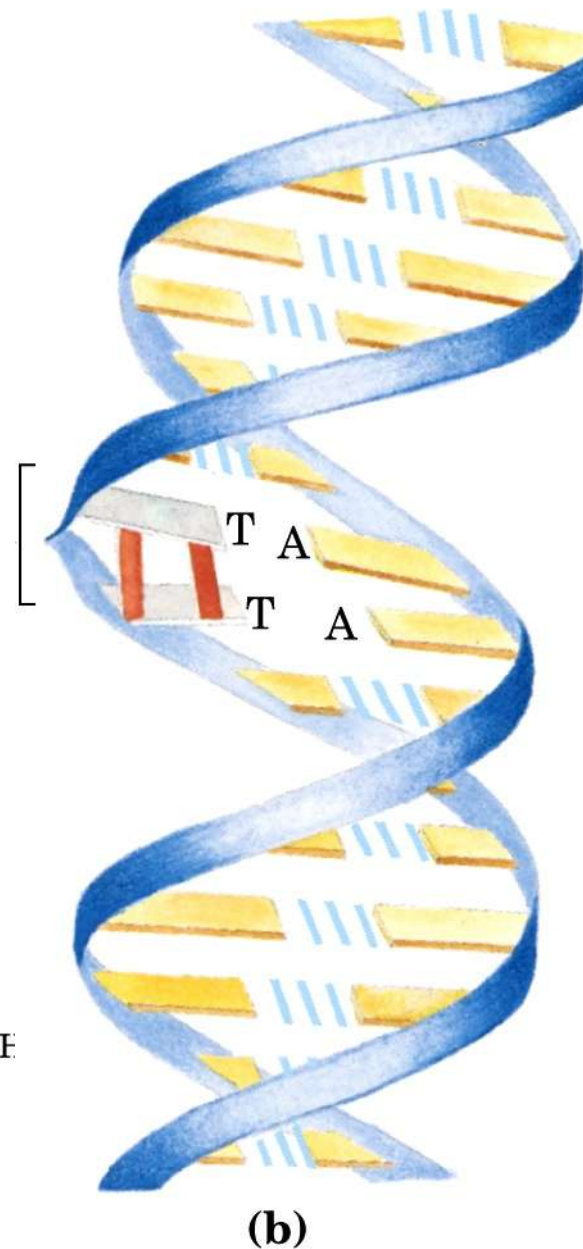
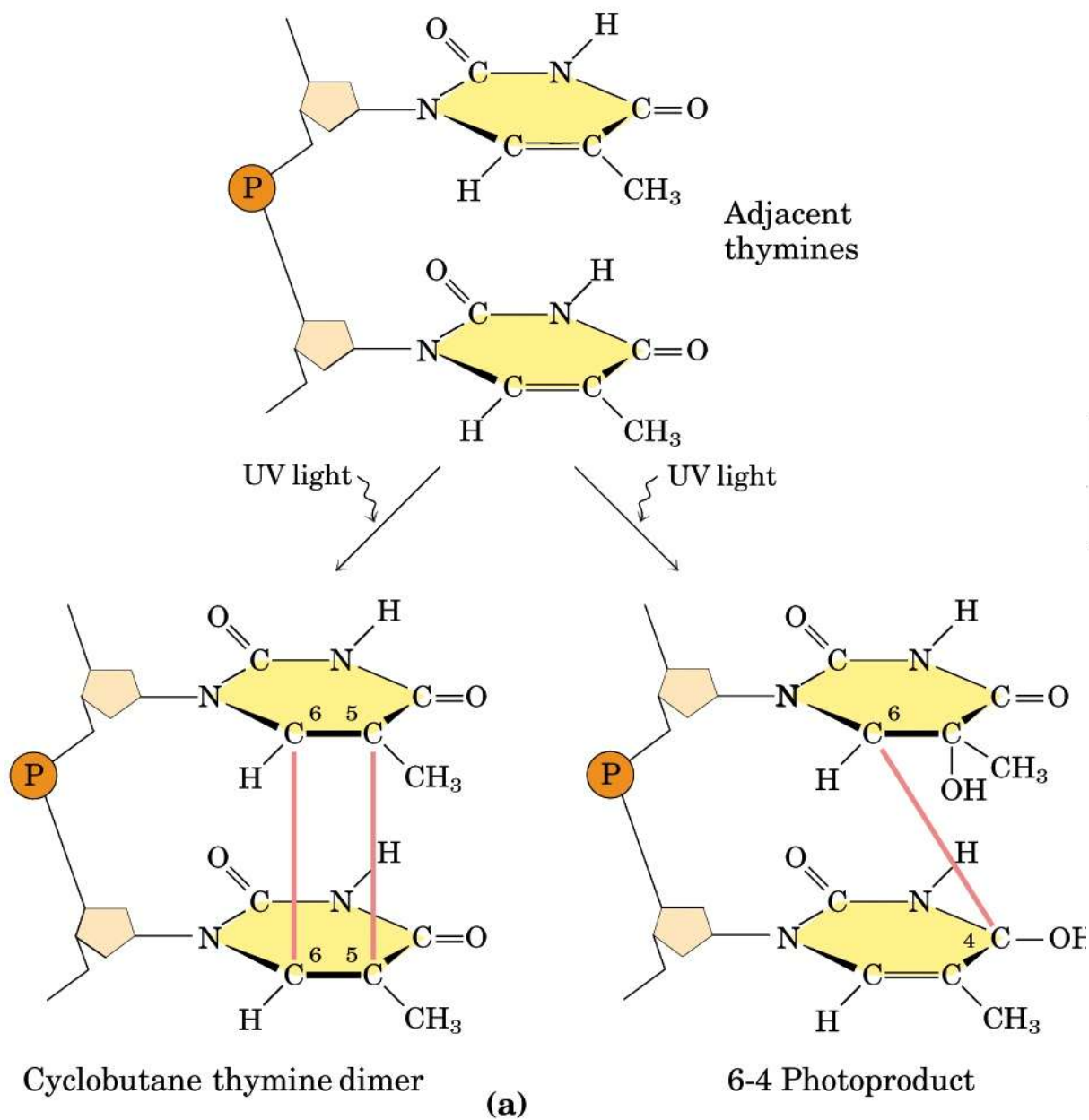


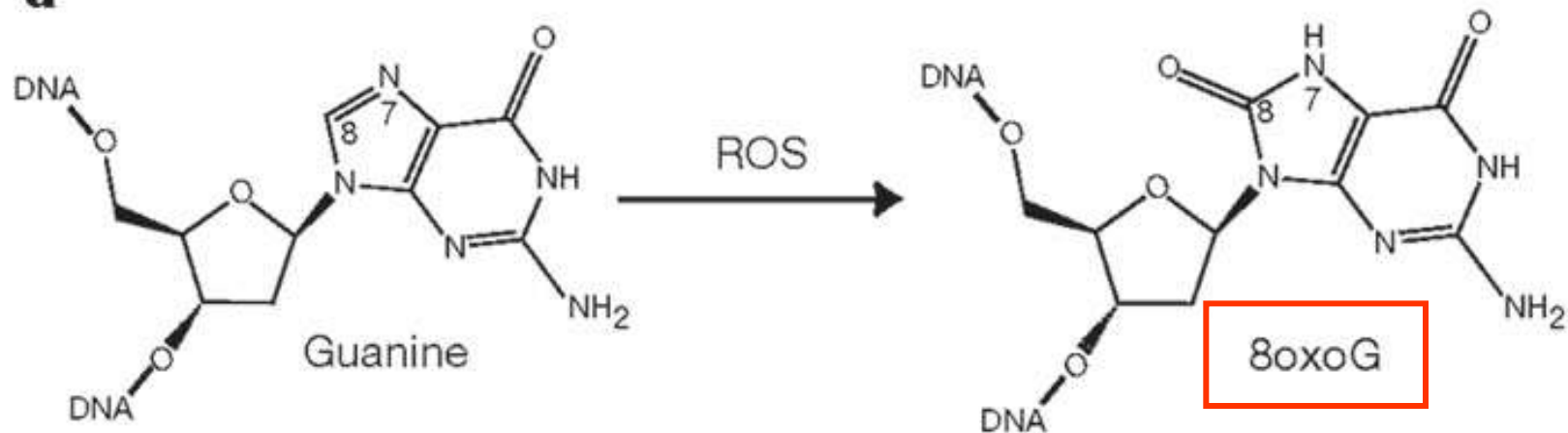
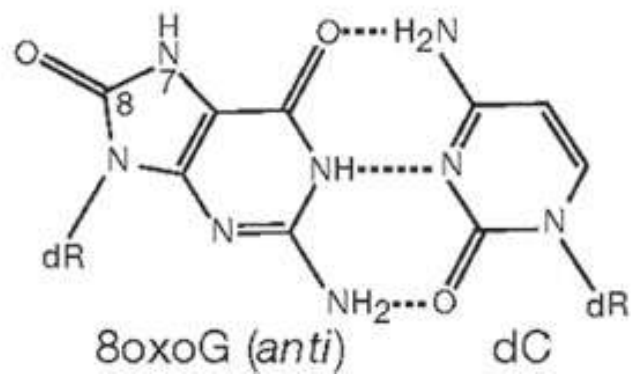
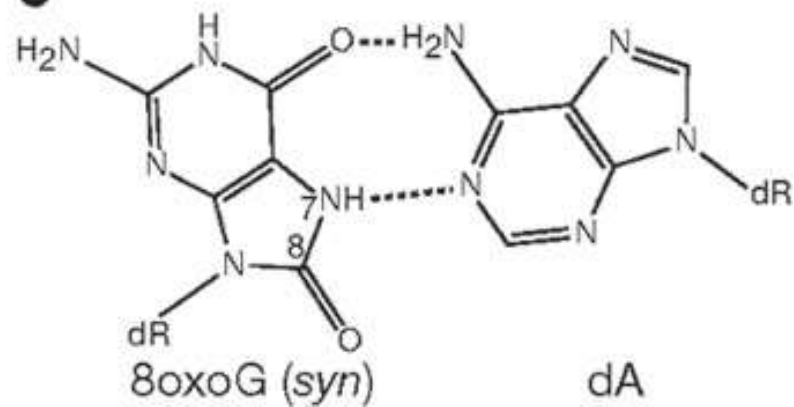
# Cysteine oxidation

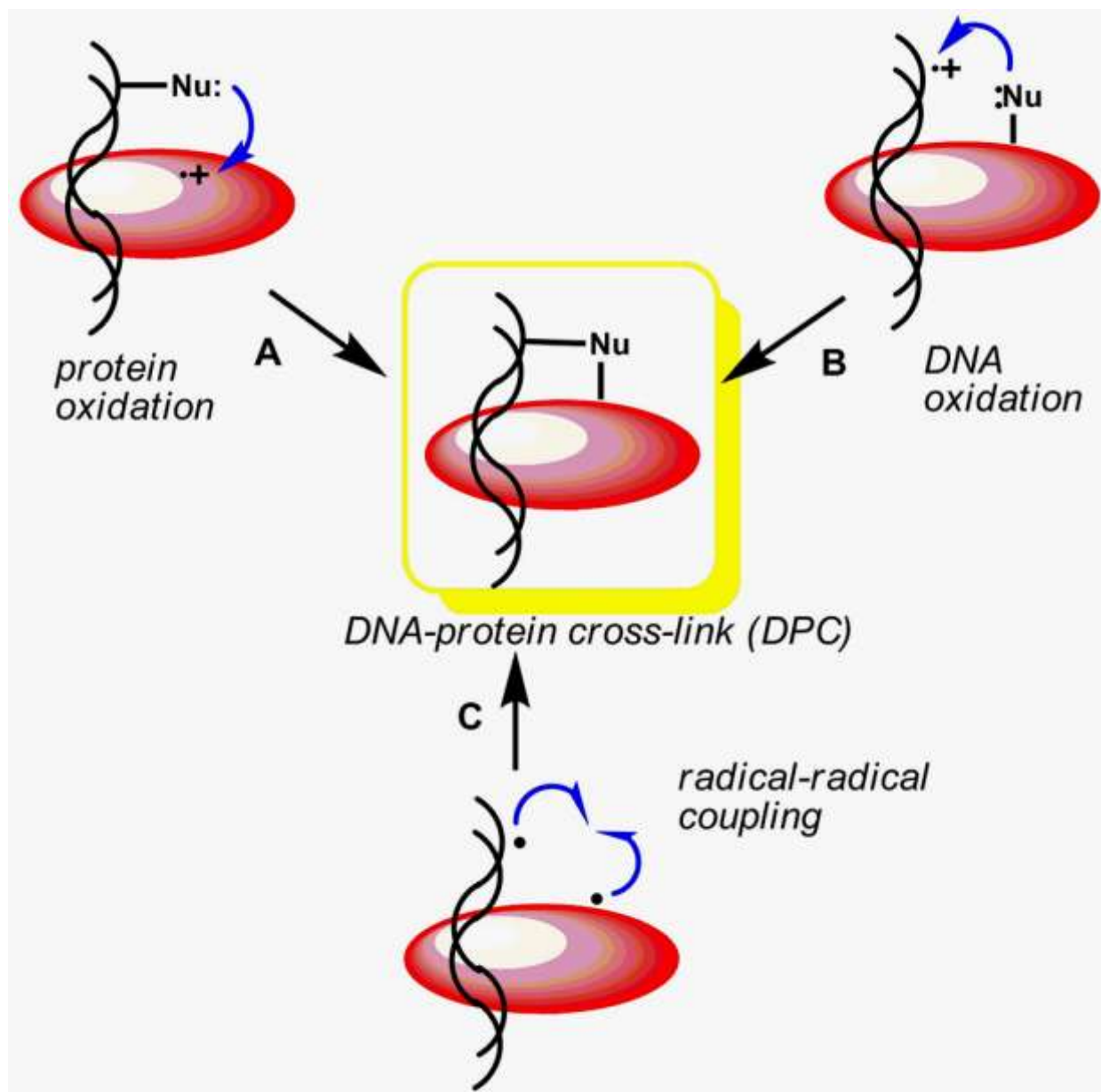








**a****b****c**



Oxidative stress is the product of **imbalance** in a three-box-system

- 1 - **Source** of reactive oxygen species
- 2 - **Defense** molecules
- 3 - **Target** tissue

# Defense against oxidative stress occurs at three levels

1- Source of ROS



Prevention



2- Defense molecules



Interception



3- Target tissue



Repair

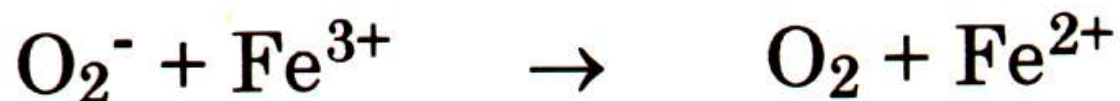
# Antioxidant defense 1.

## Prevention :

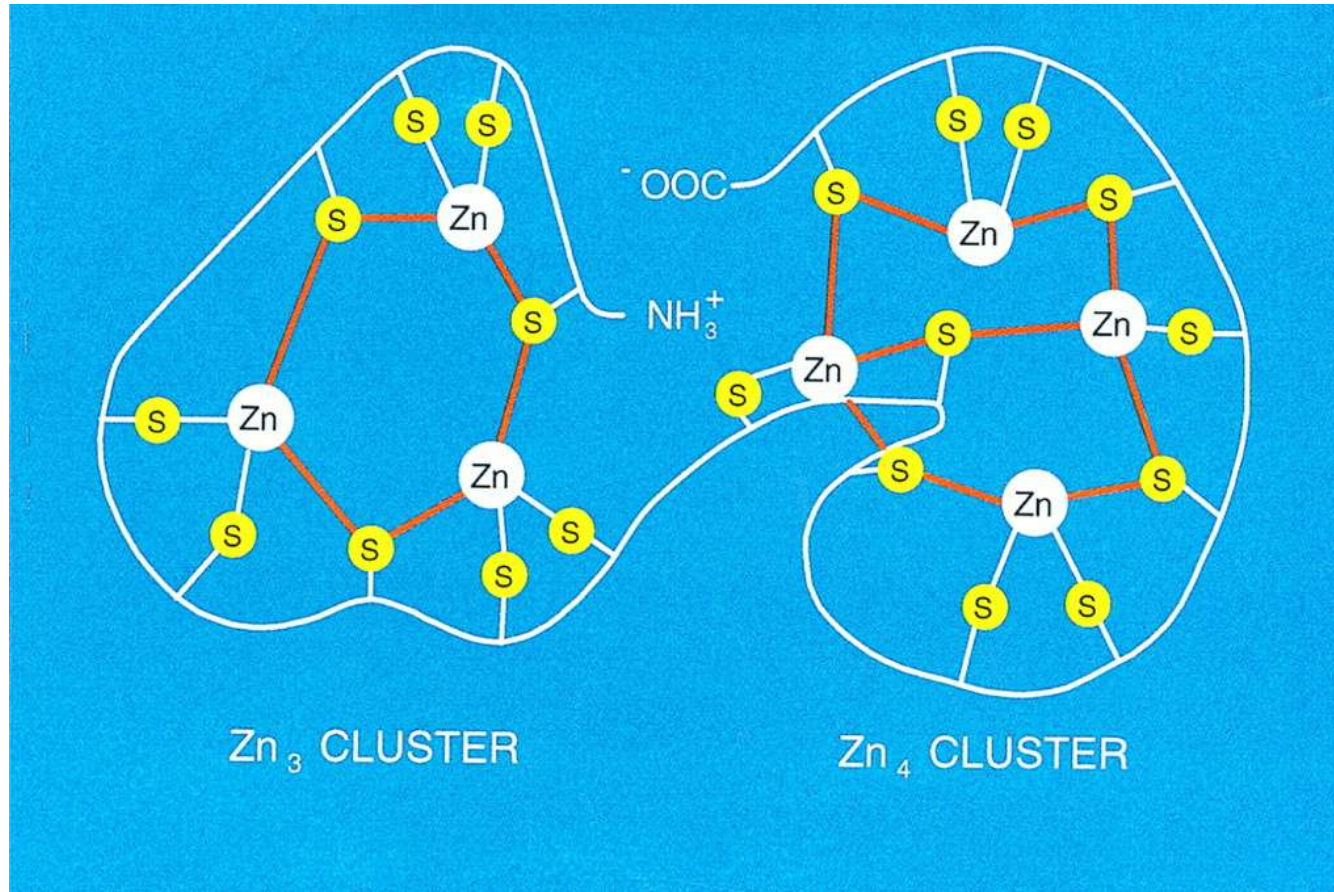
Metal sequestration  
and transport

Metallothionein, GSH, ferritin,  
transferrin, ceruloplasmin

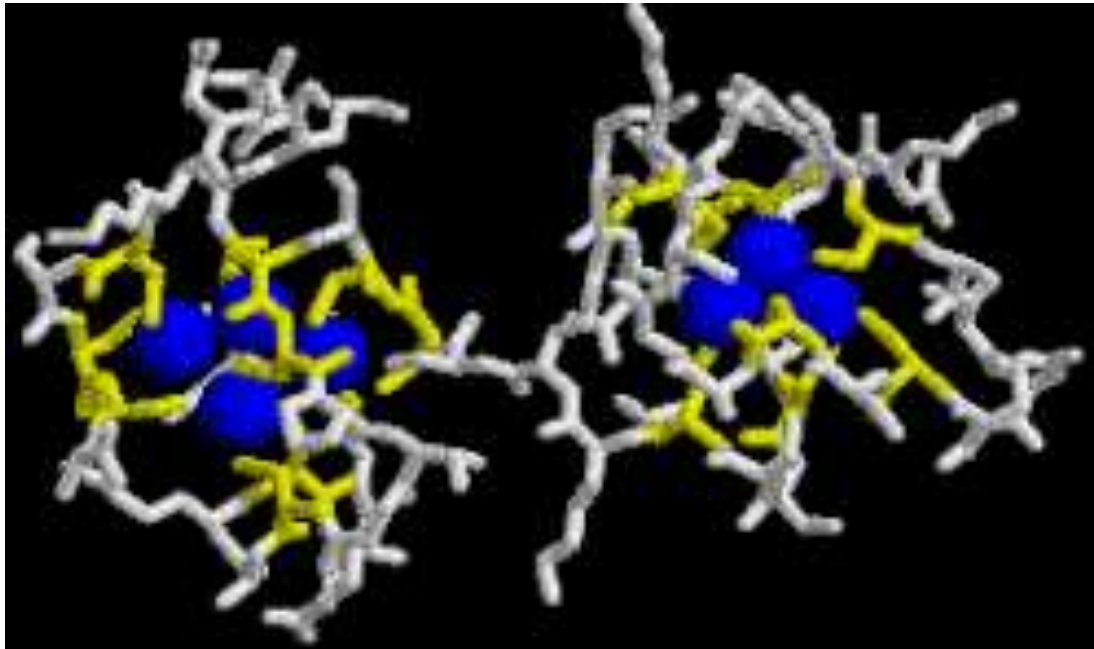
La reazione di Fenton  
avviene in presenza di metalli



Metallothionein (MT) is a family of cysteine-rich, low molecular weight (MW ranging from 500 to 14000 Da) proteins.



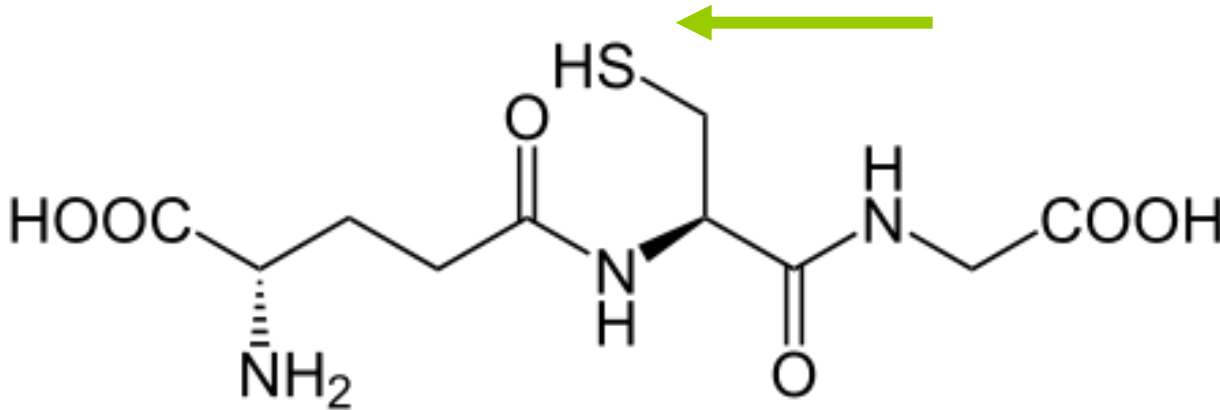
Metallothionein bind physiological (such as Zn, Cu, Se) and xenobiotic (such as Cd, Hg, Ag, As) **heavy metals** through the thiol group of its cysteine residues



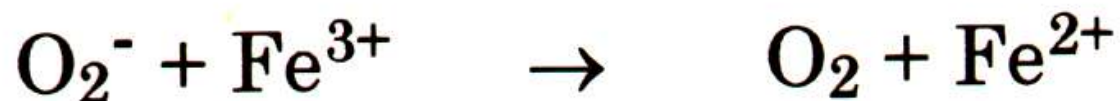
# Glutathione (up to 5 mM in the liver)

$\gamma$ -L-glutamyl-L-cysteinyl-glycine

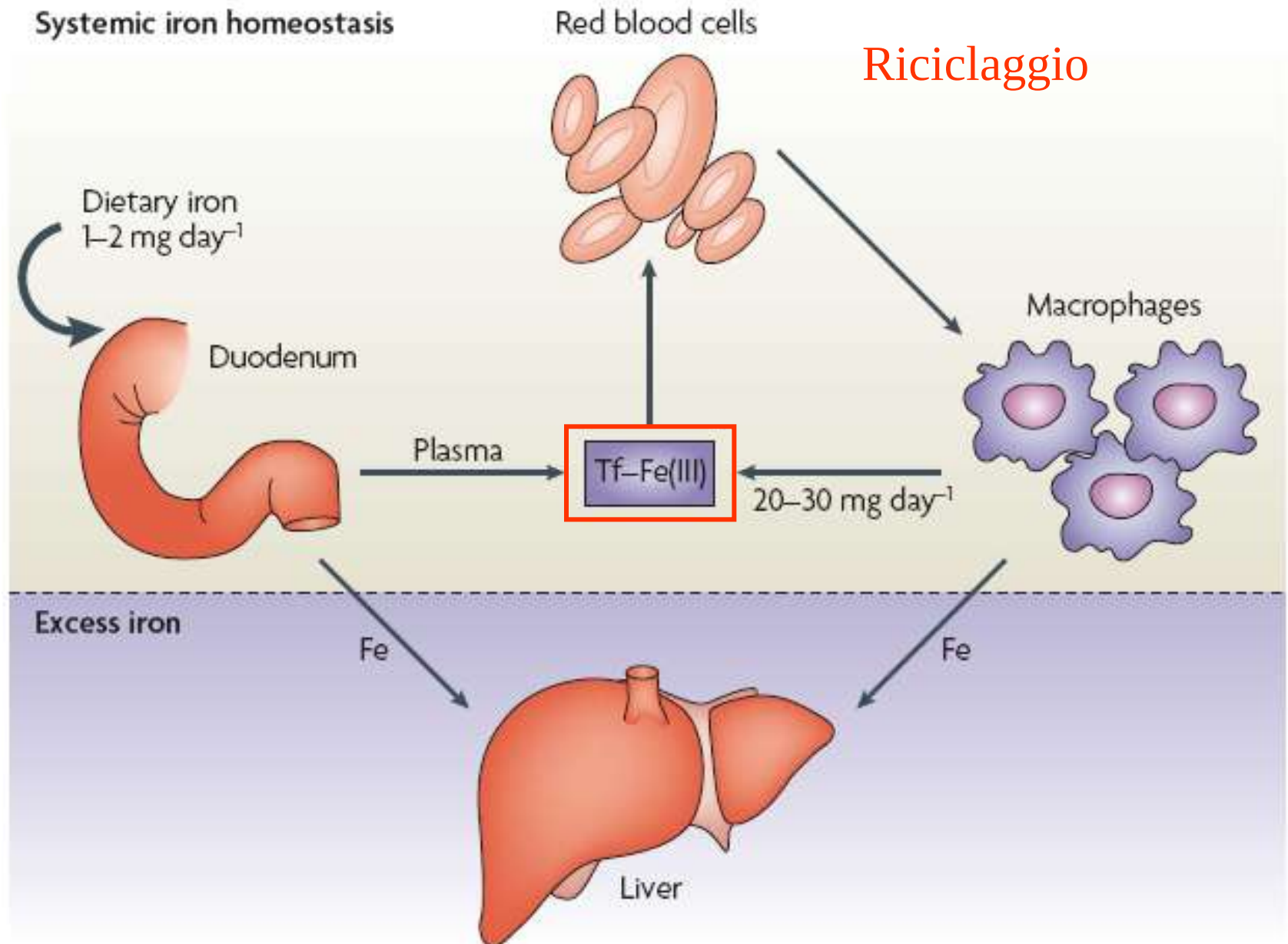
reduces disulfide bonds formed within proteins to cysteines by serving as an electron donor



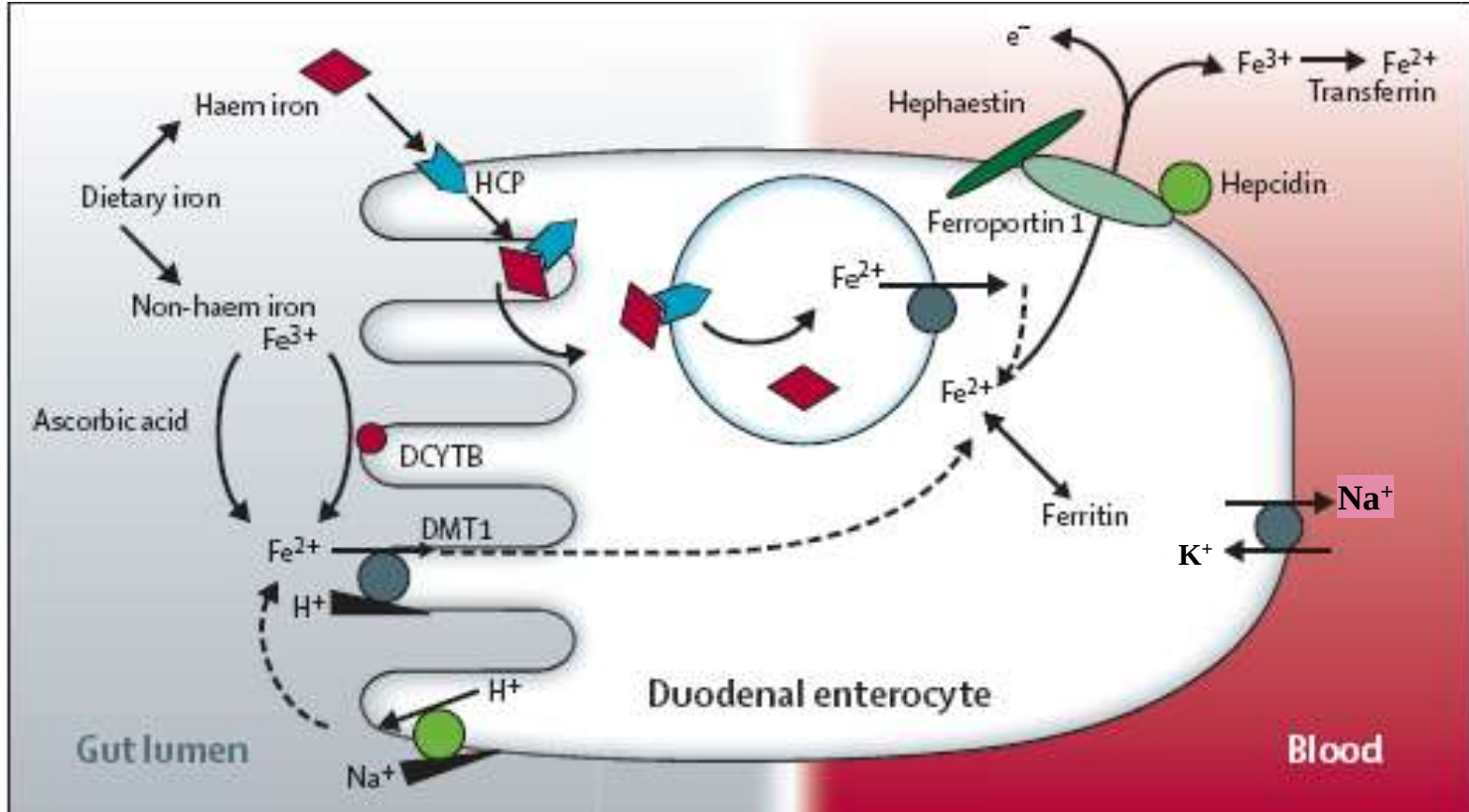
La reazione di Fenton  
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# Omeostasi sistemica del ferro - transferrina



# Metabolismo del ferro nell'enterocita



DMT1= Divalent Metal Transporter, un simporto con i protoni

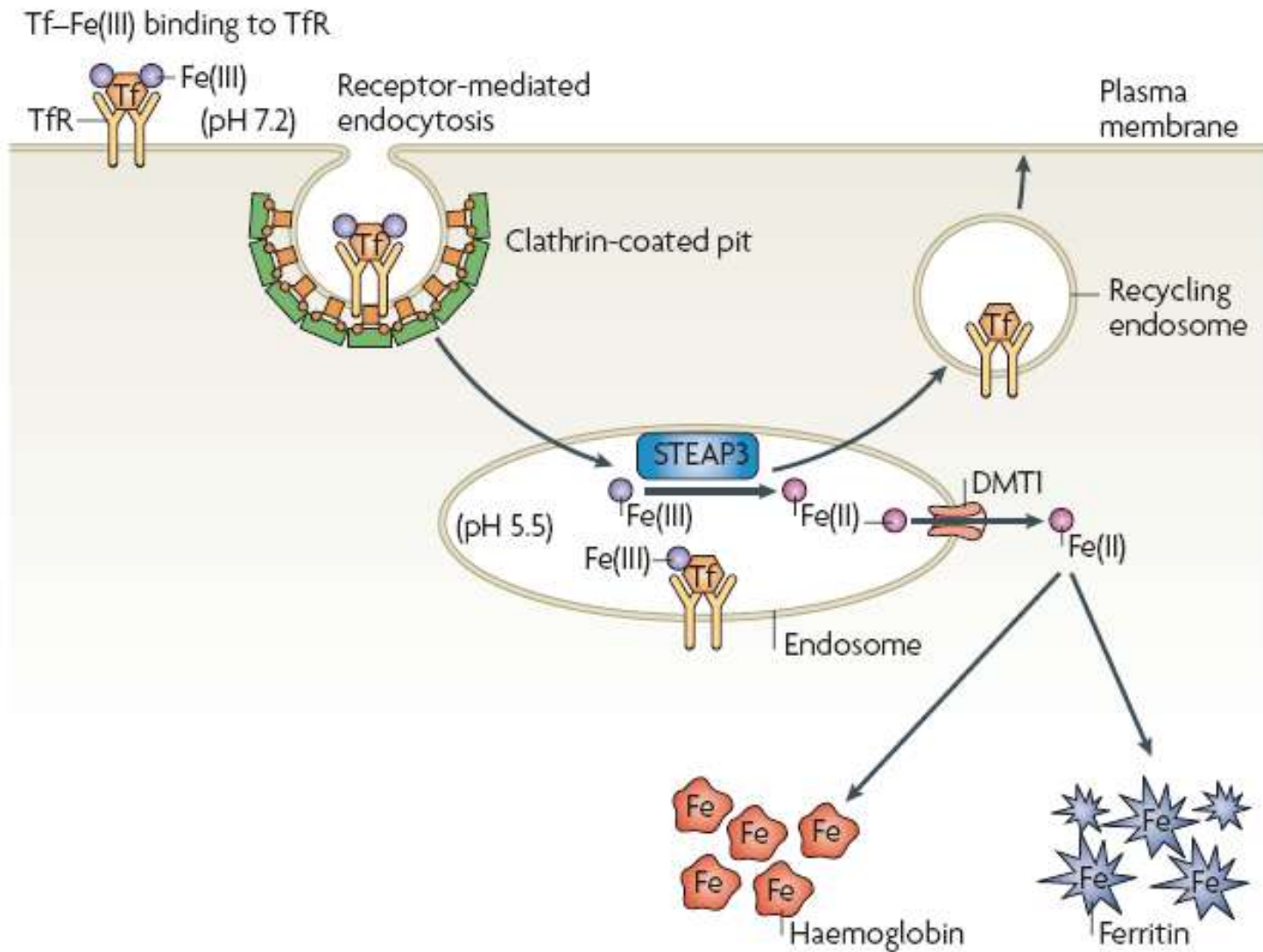
Tf = transferrina

DCYTB = Duodenal cytochrome b, ferredossidasi

HCP = Heme Carrier Protein

Hephaestina = ferrossidasi contenente rame

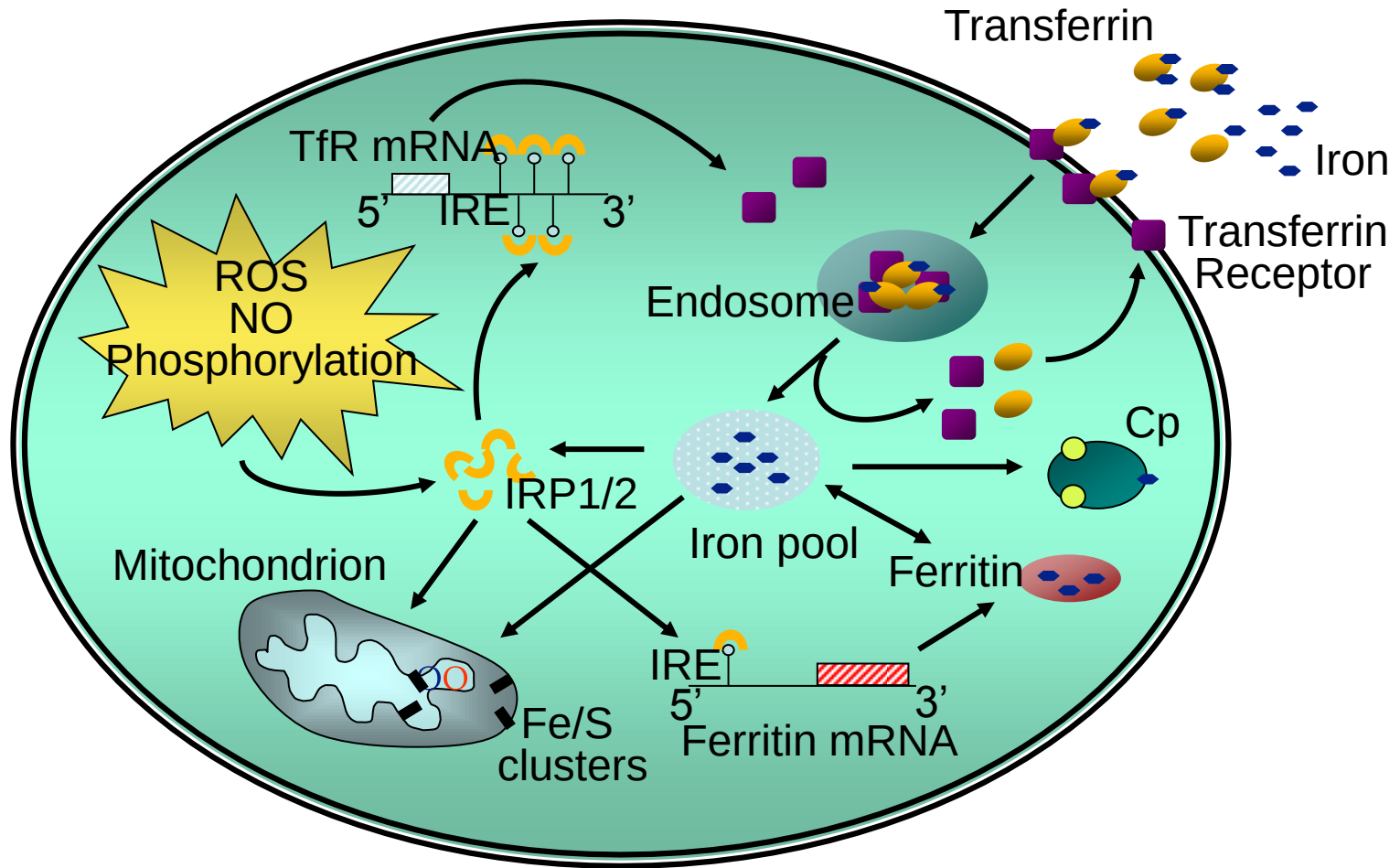
Hepcidina = peptide regolatorio, ormone



STEAP 3 è una ferro-reduttasi

DMT1 è un trasportatore di metalli bivalenti

# Ferritin/transferrin in Fe-handling



## Elementi di controllo del ferro nel mitocondrio

Nei mitocondri avviene parte della biosintesi dell'eme e dei centri Fe-S

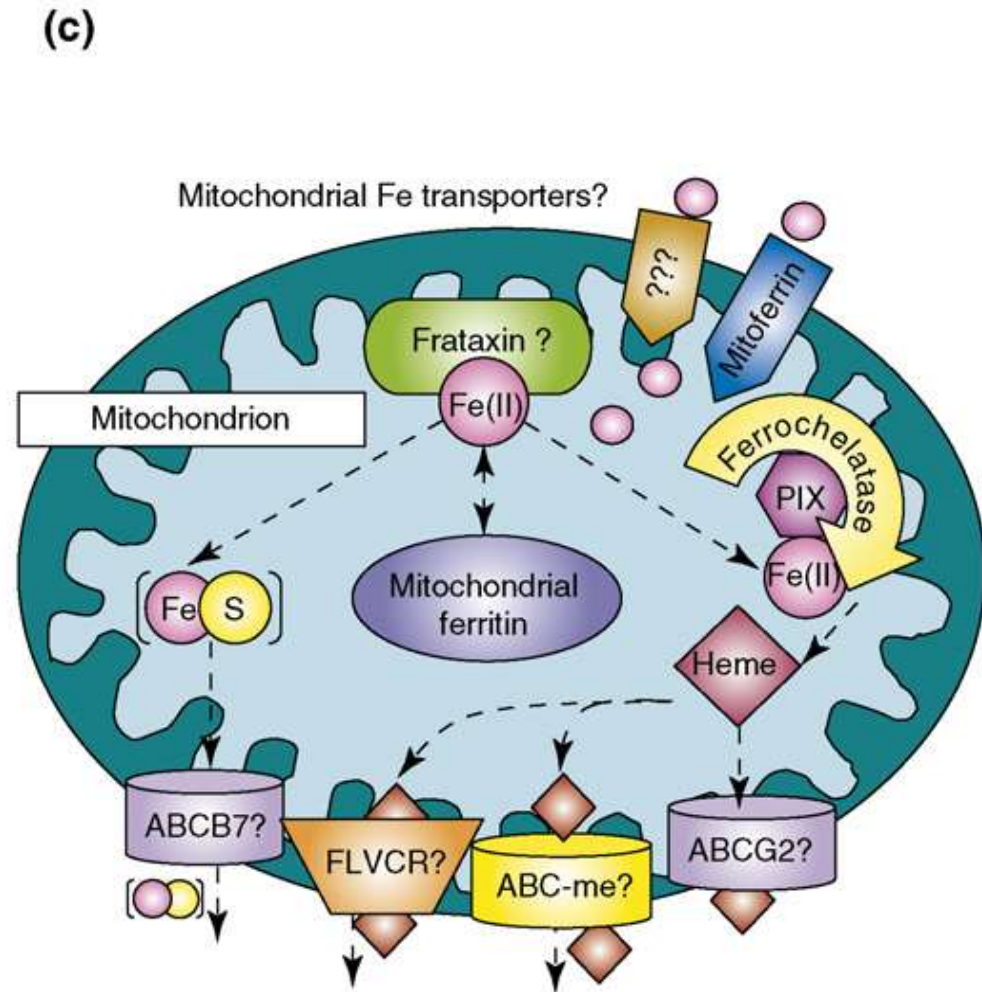
## Mitoferrina: ingresso del ferro

**Fratassina:** distribuendo il ferro nel mitocondrio regola le biosintesi di Fe-S e eme

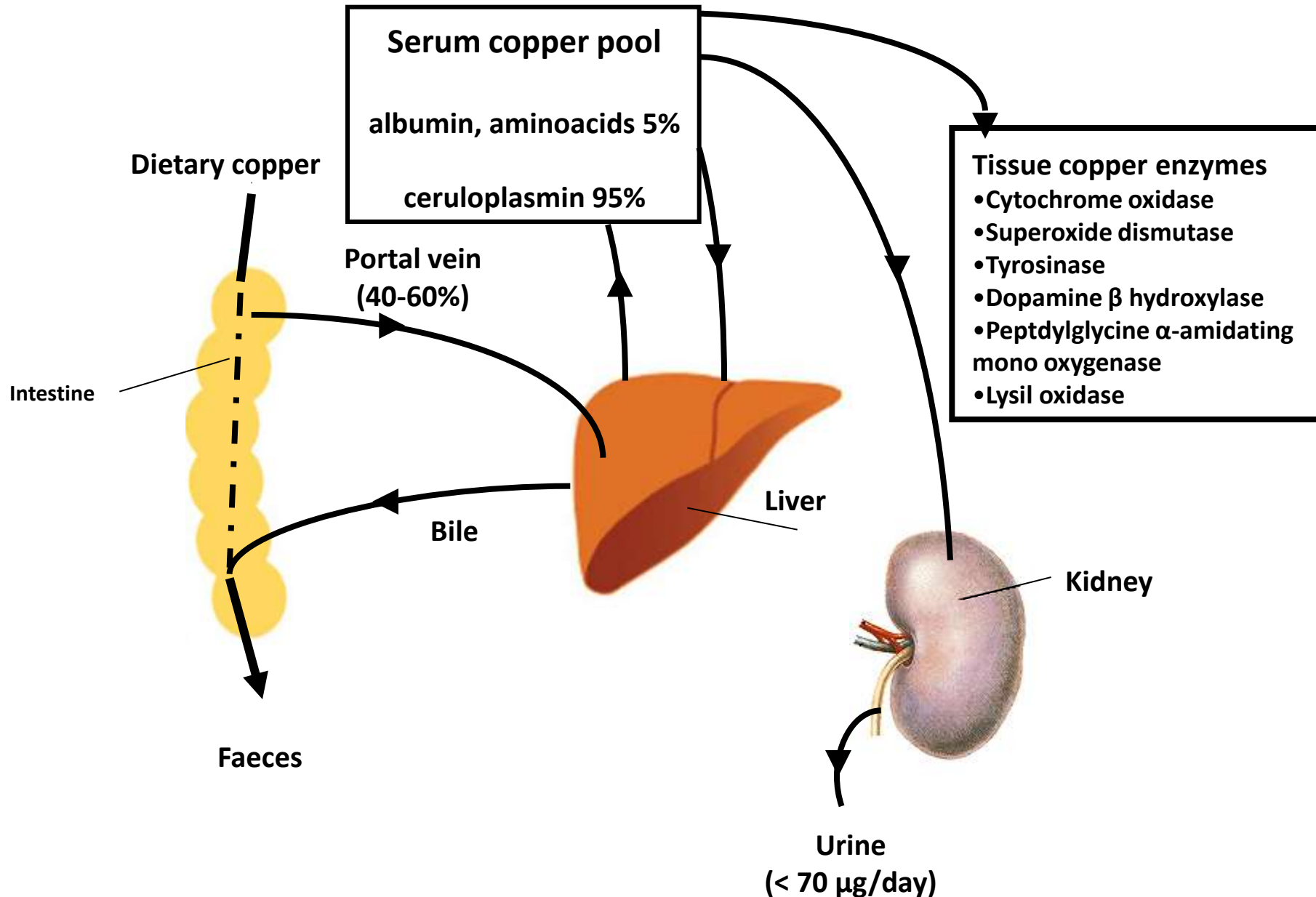
## Ferritina mitocondriale: deposito di ferro

**Ferrochelatasi:** inserisce il Fe nella protoporfirina IX

I fattori coinvolti nell'estrusione  
dei centri Fe-S e dell'eme  
dal mitocondrio non sono del tutto compresi

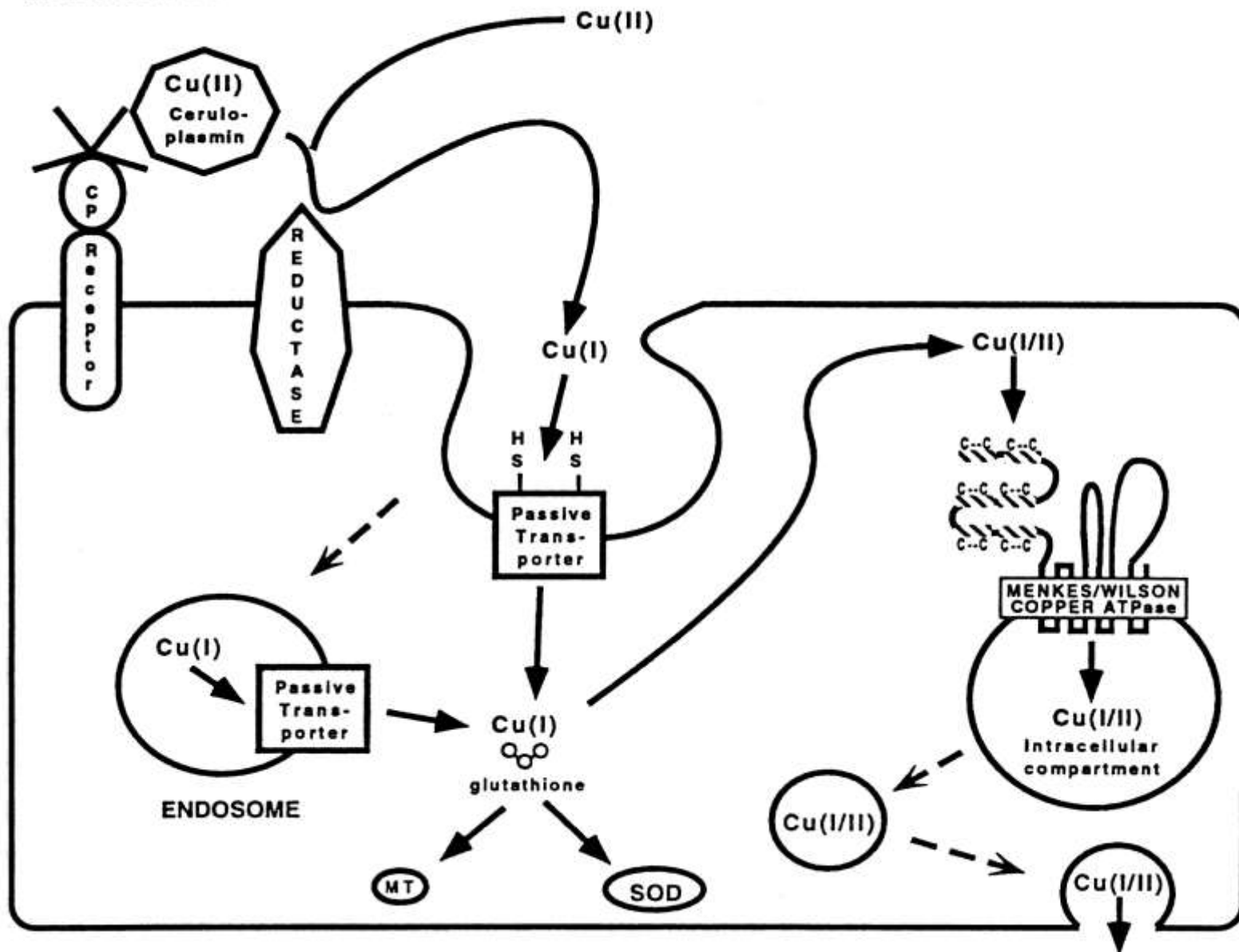


**Copper = 110 mg / total body**



**CERULOPLASMIN-MEDIATED PATHWAY**

**FREE COPPER PATHWAY**



# Rete di trasportatori del rame nelle cellule umane

**Steap:** reduttasi del rame

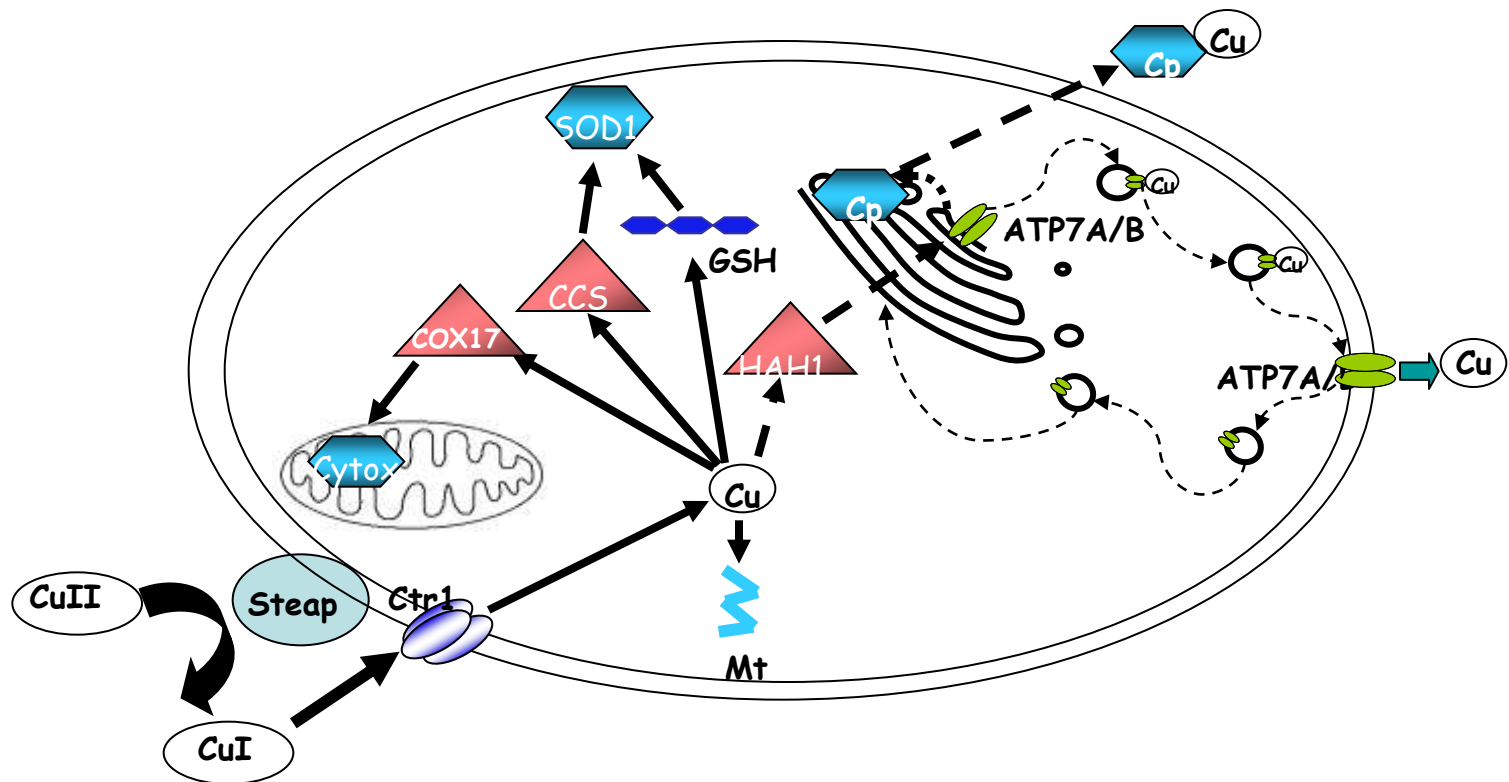
**Ctr1** = Copper transporter 1, fa entrare  $\text{Cu}^+$  nelle cellule

**Cox 17** = fa entrare il Cu nel mitocondrio

**CCS** = Copper Chaperone for Superoxide dismutasi: dona il Cu alla SOD1

**Mt** = metallothioneina, lega il rame in eccesso su residui di Cys

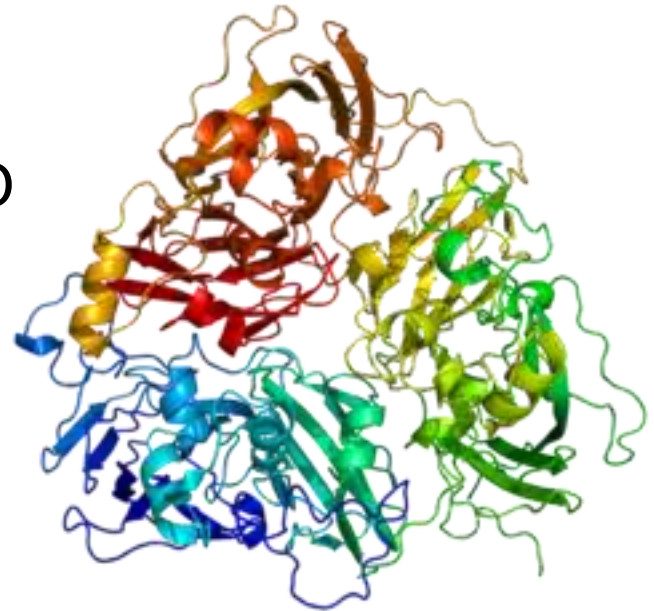
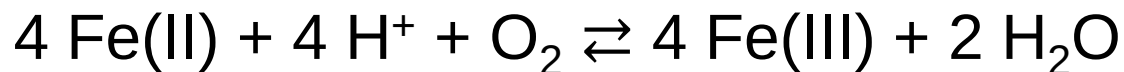
**ATP7A o ATP7B** = pompe di membrana, eliminano l'eccesso di Cu; donano il Cu alle proteine a rame che vengono secrete (es. Ceruloplasmina)



# Ceruloplasmin in Fe and Cu-handling

Ceruloplasmin is a ferroxidase that in humans is encoded by the CP gene. It is synthesized in the liver and contains 6 atoms of copper

Ceruloplasmin is the major **copper-carrying** protein in the blood, and in addition plays a role in **iron** metabolism.



# Aceruloplasminemia

## Genetics

3q 23-35 (CP)

Autosomal recessive

Frequency unknown

## Clinical

Adult onset,

Dementia,

Dystonia,

Diabetes,

Retinal degeneration

## Laboratory

Serum Cu, Cp ↓ ;

Liver Fe ↑↑

## Defect

Fe export from RE cells

## Treatment

Unknown

## Wilson disease

## Menkes disease

### Genetics

13q14.3

Autosomal recessive

1 in 30 000

Xq13.3

X-linked

1 in 300 000

### Clinical

Onset late in childhood and  
long life expectancy

Mainly liver (cirrhosis),  
Kayser-Fleisher ring

Onset before birth

Grey matter degeneration,  
Abnormal hair, hypothermia,  
Hypopigmentation,  
tortuous arteries

### Laboratory

Serum Cu, Cp ↓ ;

Liver Cu ↑↑

Serum Cu, Cp ↓ ;

Liver Cu ↓ ↓

### Defect

Biliary Cu excretion

Placental, GI Cu transport

### Treatment

Chelation very effective

No effective treatment

# Copper-binding proteins in humans – 1.

|                               | Biological Function                              | Consequence of Deficiency or Defect                                                          |
|-------------------------------|--------------------------------------------------|----------------------------------------------------------------------------------------------|
| Metallothionein               | Cu-sequestration                                 | Cu toxicity                                                                                  |
| Cu/Zn SOD                     | Free radical detoxification;<br>Cu-sequestration | Oxidative damage of cell components                                                          |
| Cytochrome c oxidase          | Electron transport in mitochondria               | Symptoms of deficiency of ATP: myopathy, ataxia, seizures                                    |
| Lysyl oxidase                 | Crosslinking of collagen and elastin             | Connective tissue symptoms: vascular rupture and torsion<br>Loose skin and joints, emphysema |
| Dopamine $\beta$ -hydroxylase | Catecholamine production                         | Hypothalamic imbalance                                                                       |

## Copper-binding proteins in humans – 2.

|                                    |                                        |                                            |
|------------------------------------|----------------------------------------|--------------------------------------------|
| Tyrosinase                         | Melanin production                     | Depigmentation                             |
| Peptidylglycine<br>Monooxygenase   | Bioactivation of<br>Peptide hormones   | Malfunction of several<br>peptide hormones |
| Ceruloplasmin                      | Ferroxidase, Cu transport              | Anemia                                     |
| Hephaestin                         | Iron egress from intestines            | Sex-linked anemia                          |
| Clotting Factor, V, VIII           | Blood clotting                         | Bleeding tendency                          |
| Angiogenin                         | Induction of blood<br>vessel formation | Defective blood<br>vessel development      |
| Prion protein                      | Unknown                                | Prion disease                              |
| $\beta$ -amyloid precursor protein | Unknown                                | Familial Alzheimer's Disease               |

## Antioxidant defense 2.

### Interception :

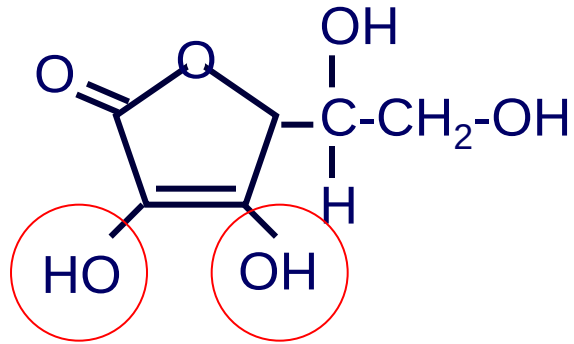
Non catalytic (scavengers)

Vit.E, Vit.C., urate, GSH,  
bilirubin, flavonoids

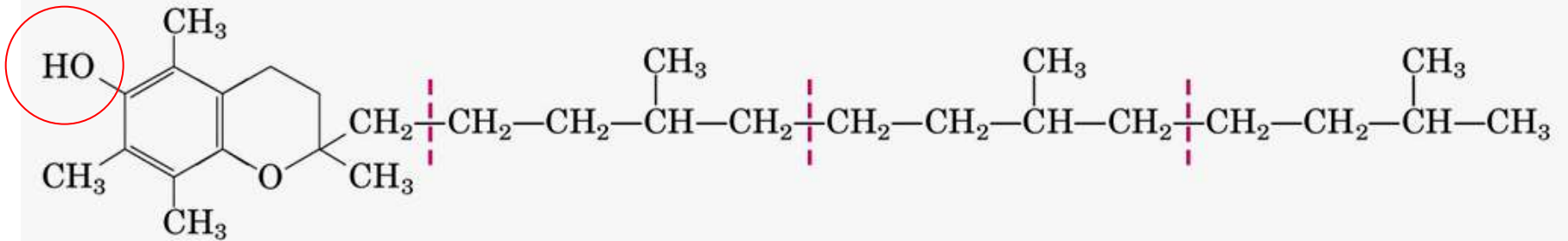
Catalytic (enzymes)

Superoxide dismutase,  
catalase, peroxidases

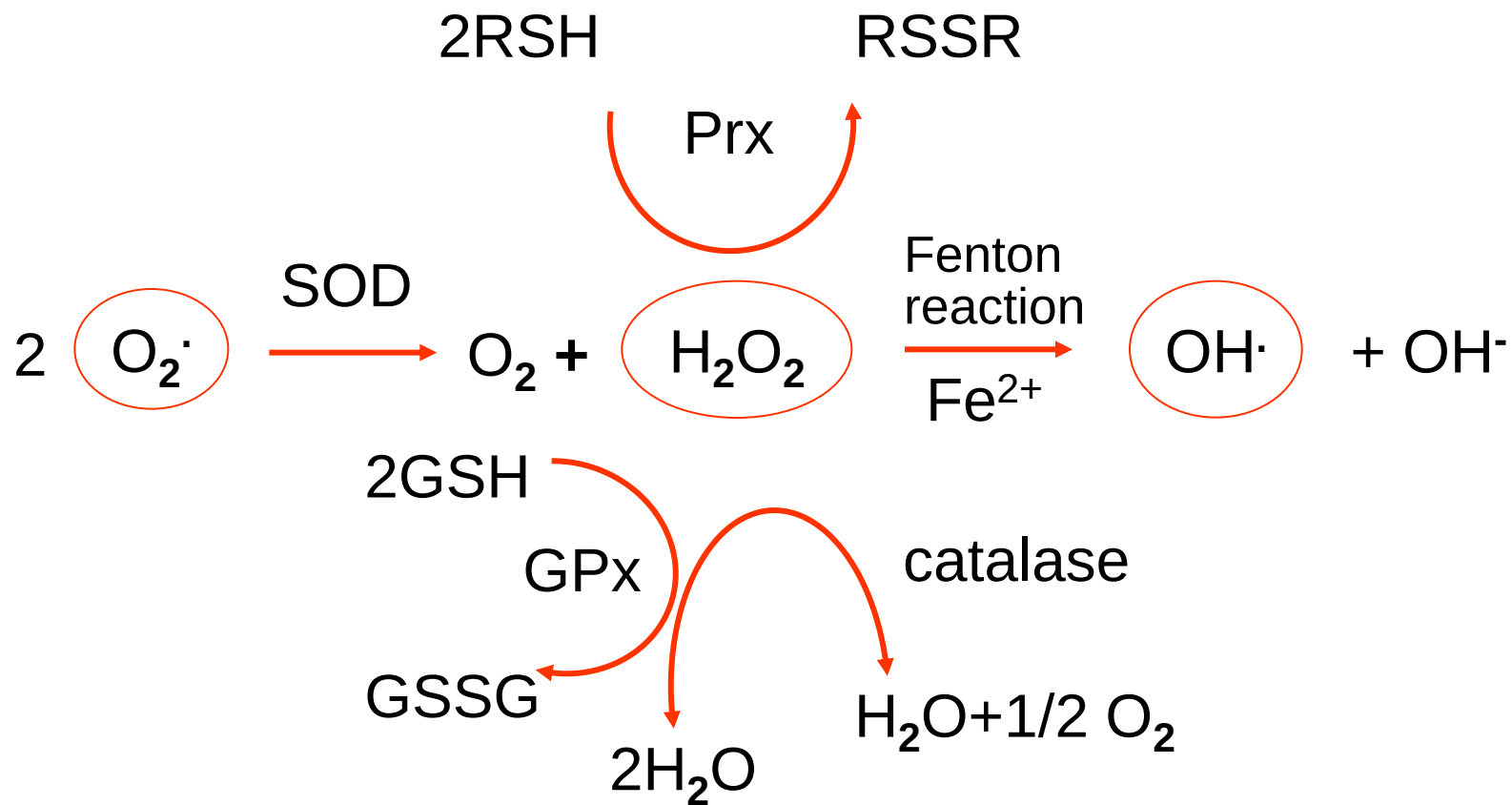
# Antioxydant vitamines



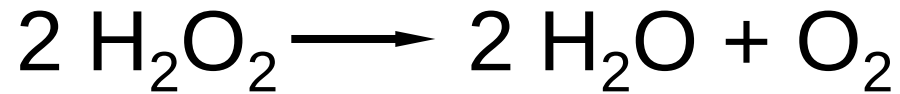
## Vitamin C



## Vitamin E



# Catalase



# Glutathione peroxidase



Se-dependent  
Se-independent

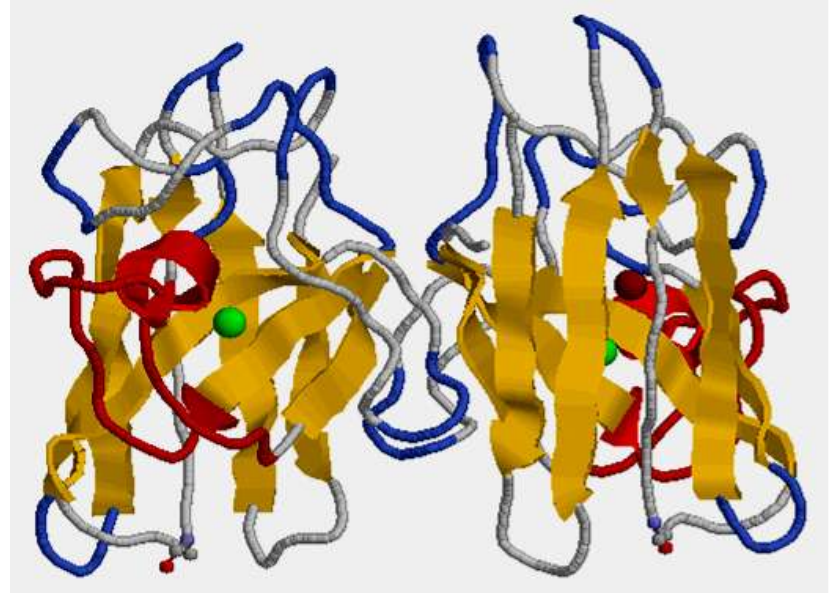
# Superoxide dismutase



SOD1 = Cu,Zn SOD

SOD2 = Mn SOD

SOD3 = EC-Cu,Zn SOD



## Antioxidant defense 3.

### Repair :

- DNA repair (mitochondrial DNA ?)
- Protein degradation (ubiquitine/proteasome)
- ? Lipid turnover
- RNA degradation

# Factors contributing to **brain vulnerability** to oxidative stress

## **1. Excess of production**

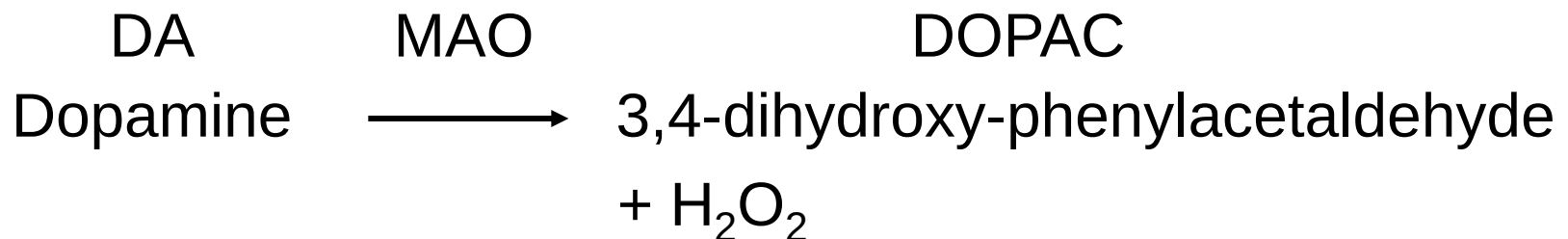
- High rate of oxidative metabolic activity
- Endogenous generation of oxygen radicals by specific neurochemical reactions
- Increased deposition of transition metal with age

## 1. High rate of oxidative metabolic activity in the brain

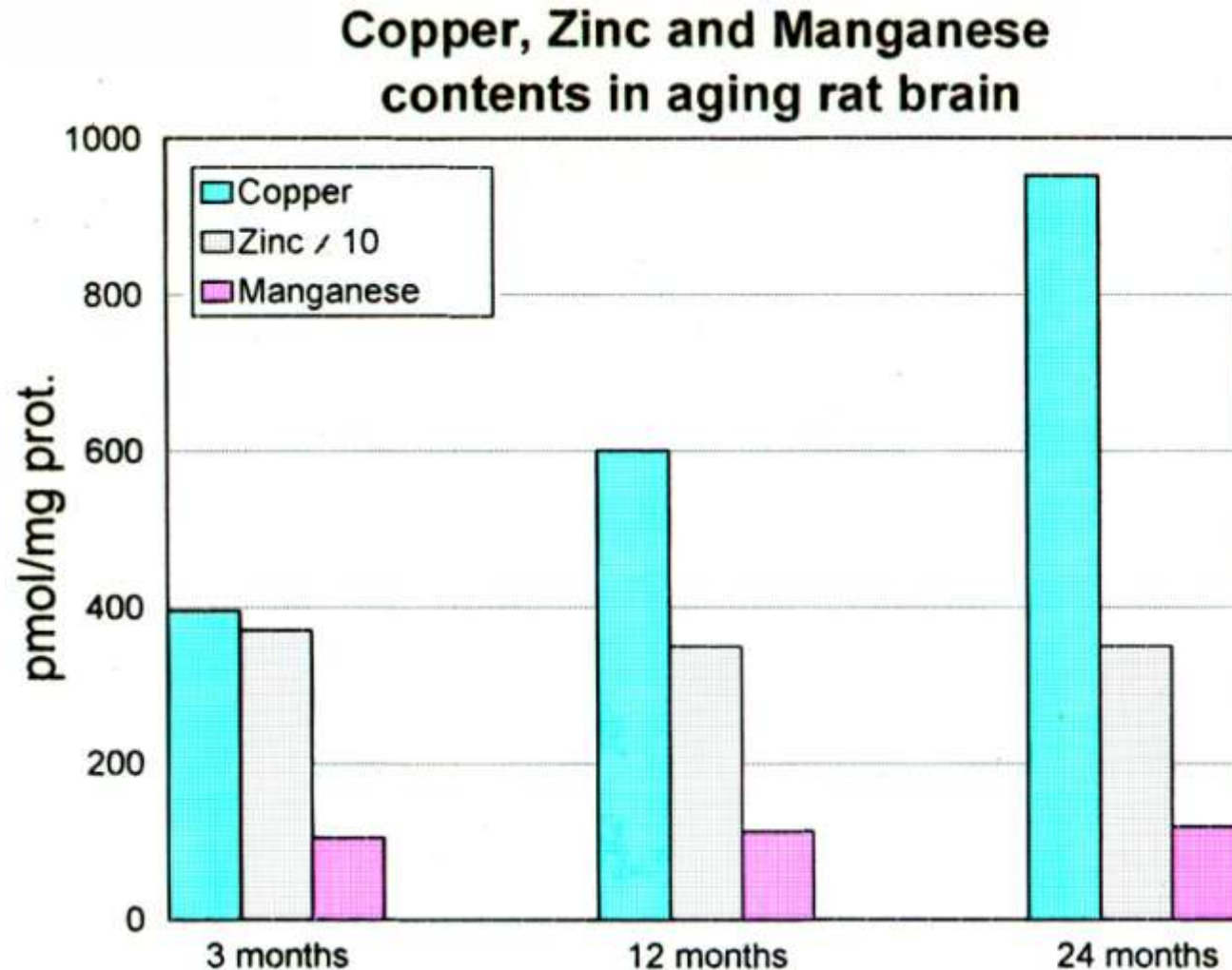
- High oxygen consumption
- High dependence from mitochondrial metabolism

## 2. Endogenous generation of oxygen radicals by specific neurochemical reactions

### *Dopamine oxidation*



### 3. Increased deposition of transition metal with age



# Factors contributing to **brain vulnerability** to oxidative stress

## 2. Deficit of defense

- Relatively **low levels** of protective antioxidant enzymes (catalase) with respect to other tissues like liver or blood

# Factors contributing to **brain vulnerability** to oxidative stress

## 3. Propensity of the target

- High concentration of readily oxidisable substrates (i.e. membrane PUFA)
- High ratio of membrane surface area to cytoplasmic volume
- Neuronal anatomical network vulnerable to disruption
- Neuronal cells are non-replicating