

# **Molecular and Cellular Responses to Oxidative Stress and Changes in Oxidation-Reduction Balance**

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# *Oxidative Stress in Pathophysiology*

*Aging*

*Heart disease*

*Inflammation*

*Cancer*

# Generalized scheme for oxidative injury to macromolecules

**Reactive oxygen species**

```
graph TD; ROS[Reactive oxygen species] --> DNA["Nucleic acid damage, mutation, carcinogenesis"]; ROS --> Membrane["Membrane damage, Lipid peroxidation"]; ROS --> Protein["Protein damage, enzymes, receptors, transporters"]; ROS --> Polysaccharide["Polysaccharide damage, hyaluronic acid, arthritis"]; ROS --> Detoxifying["Detoxifying enzyme systems"];
```

**Nucleic acid damage,  
mutation, carcinogenesis**

**Membrane damage  
Lipid peroxidation**

**Protein damage  
enzymes, receptors  
transporters**

**Polysaccharide damage  
hyaluronic acid, arthritis**

**Detoxifying  
enzyme systems**

## *What is Oxidative Stress ?*

*Definition: Disturbance in the prooxidant-antioxidant balance in favor of the former*  
*Sies, 1985*

## *What are prooxidants ?*

- ROS, ROOH (ox-LDL), paraquat,  
adriamycin, etc

## *What are antioxidants ?*

- enzyme systems: SOD, catalase, GSH Px
- radical scavengers: ascorbic acid,  $\alpha$ -tocopherol  
GSH, selenium, etc

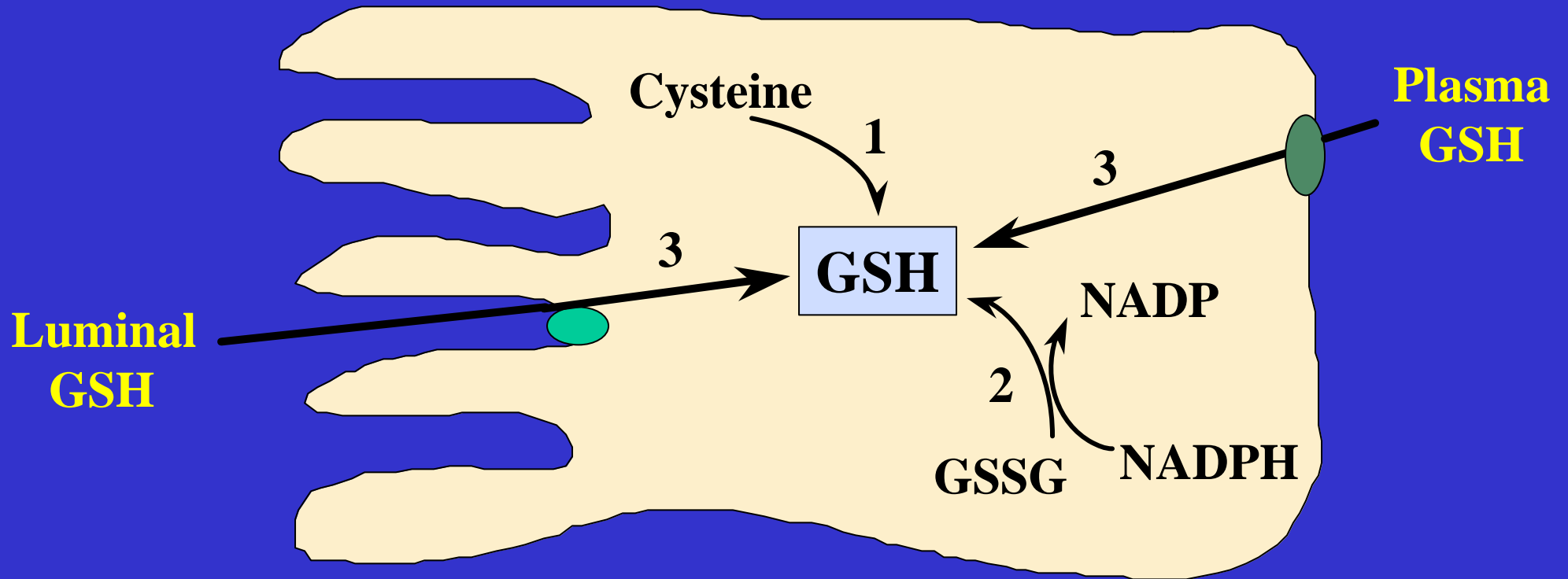
*Oxidative stress induces....*

*Cellular redox imbalance*

*Thiol redox: GSH, GSSG, protein sulfhydryls*

*Pyridine nucleotide redox: NADPH/NADP<sup>+</sup>  
NADH/NAD<sup>+</sup>*

## *Sources of cellular GSH*



## Differential GSH/GSSG imbalance under different pathophysiological conditions in different cell types

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Cell type	GSH/GSSG ratio	
	Control	Experimental
HUVECs (I/R)	9	0.6
CaCo-2 cells (peroxide)	60	15
HeLa cells (thiol oxidant)	50	0.4

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# Cellular sources of NADPH

## 1. Mitochondrial NADH transhydrogenation



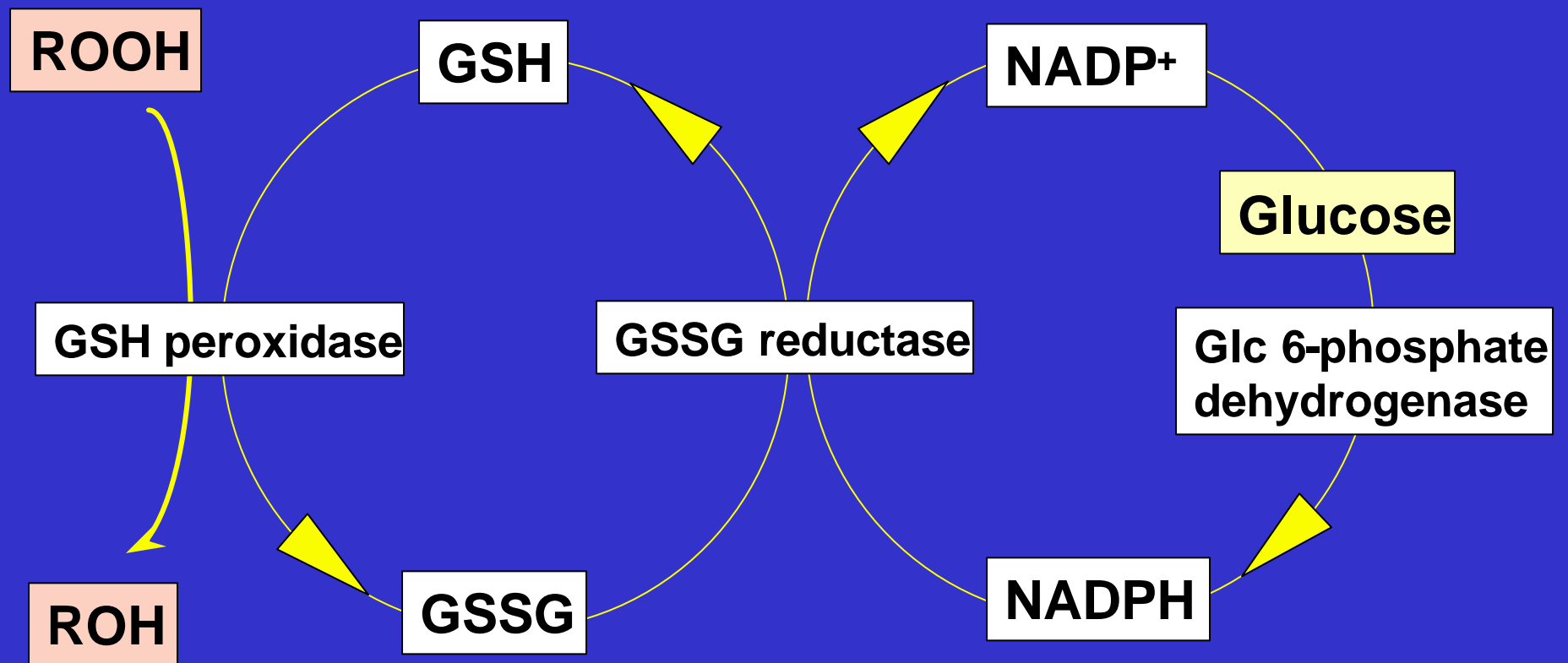
## 2. NADP<sup>+</sup> specific dehydrogenases



## 3. Pentose phosphate pathway



# Coupling of GSH redox cycle function to pentose phosphate pathway



## **Transient versus sustained GSH/GSSG imbalance: Survival versus death**

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<b>Condition</b>	<b>GSH/GSSG ratio</b>		<b>Outcome at 4h</b>
	<b>30'</b>	<b>4h</b>	
<b>Untreated</b>	<b>60</b>	<b>60</b>	<b>Survival</b>
<b>Diamide (normal glucose)</b>	<b>0.4</b>	<b>55</b>	<b>Survival</b>
<b>Diamide (low glucose)</b>	<b>0.4</b>	<b>0.6</b>	<b>Death</b>

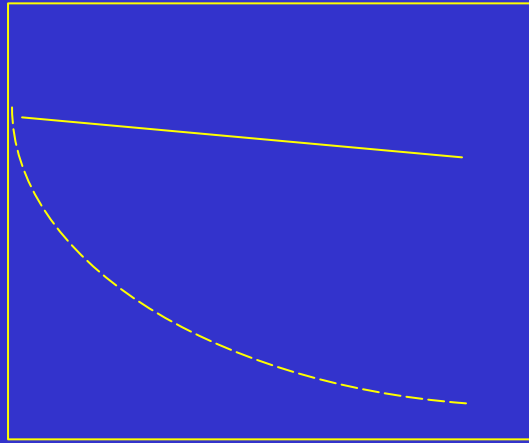
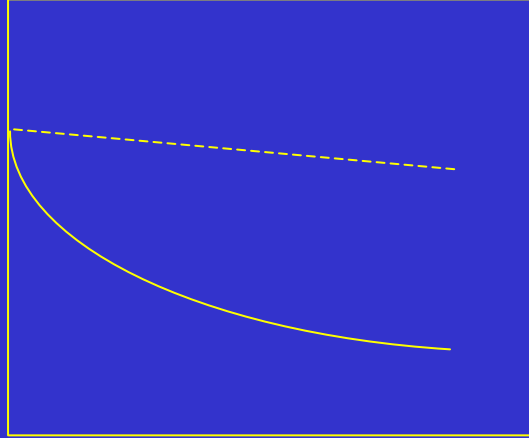
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## *Intracellular GSH pools*

- 1. Cytoplasmic pool - largest pool; origin from synthesis; redox homeostasis, cell integrity and protein function; thiol/disulfide exchange scavenge cytoplasmic ROS**
- 2. Mitochondrial pool - origin cytoplasm; scavenge mitochondrial ROS**
- 3. Nuclear pool - origin from cytoplasm; participate in redox control of gene expression**

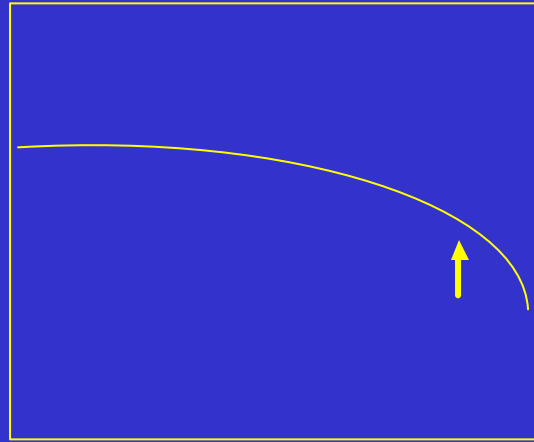
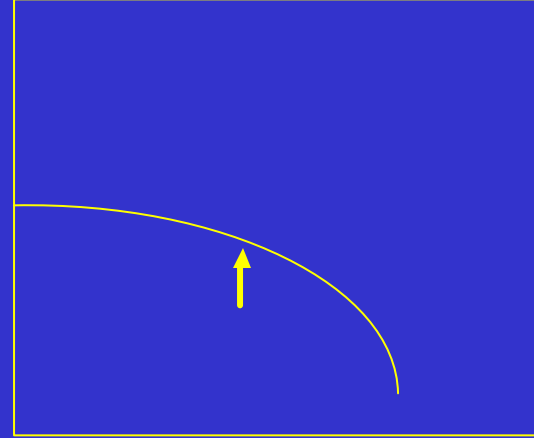
# Mitochondrial GSH and cell injury

Mitochondrial (--) or cellular (--) GSH



Time →

Cell viability



Time →

## *What is Oxidative Stress ?*

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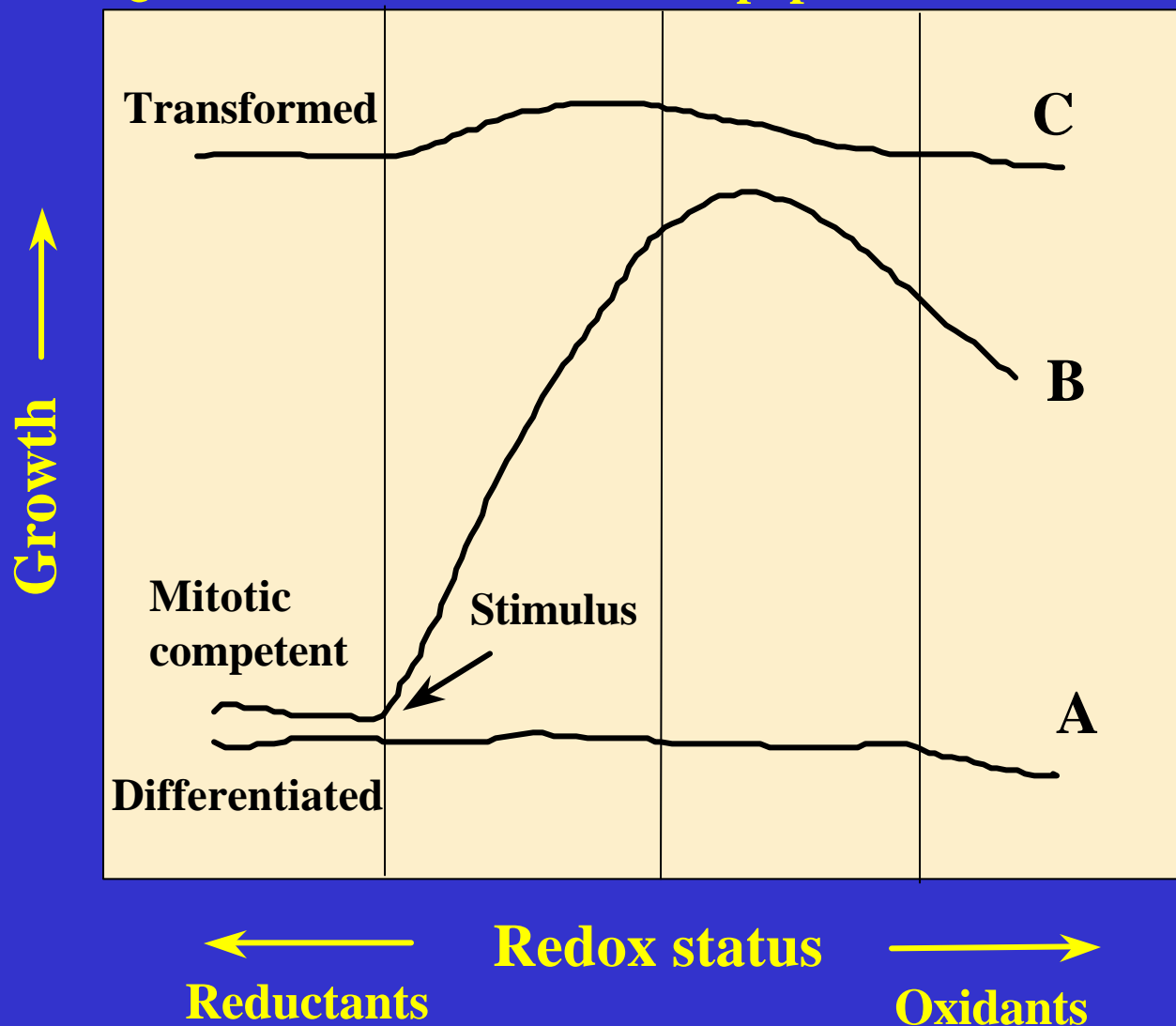
*Sies, 1985*

*Working definition: should take into consideration that the oxidant imbalance in a tissue must lead to potential damage (or altered cell responses)*

*Sies, 1991*

# Cellular responses to oxidative stress

Quiescence Proliferation Apoptosis Necrosis

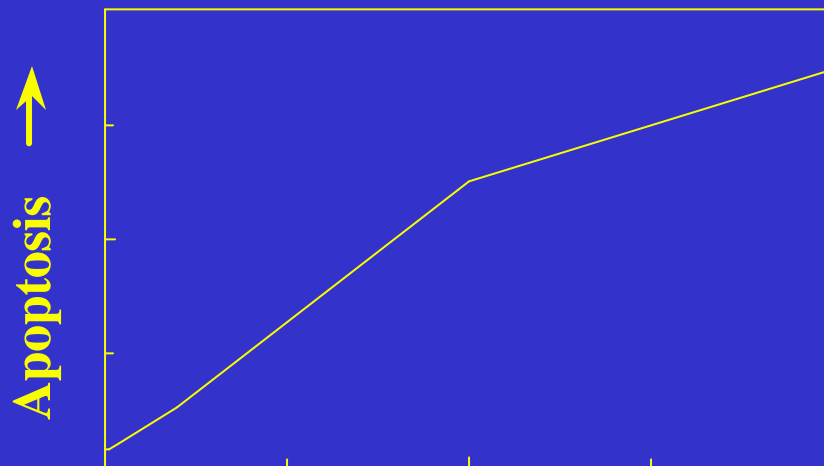
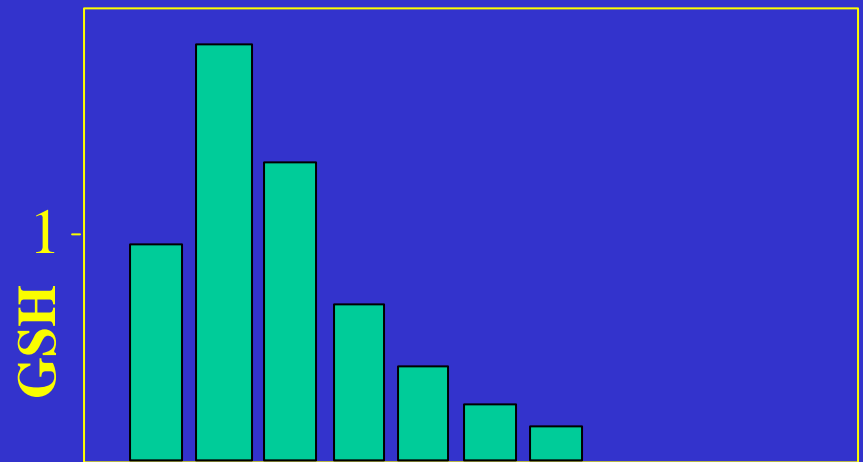
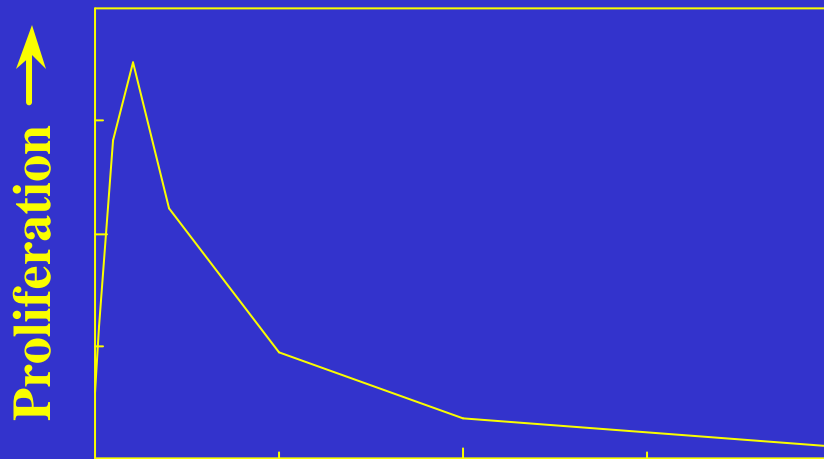


## Oxidative stress induces:

- A - terminally differentiated cells to die by apoptosis or necrosis
- B - mitotically competent cells to proliferate, but at higher oxidative stress, cells will die by apoptosis or necrosis
- C - highly proliferative tumor or transformed cells to die by apoptosis or necrosis

Aw (1999) *Am J. Clin Nutr* 70:559

# Differential responses of cells to oxidation/reduction imbalance: Proliferation or apoptosis



- Oxidant challenge induces cell oxidative stress
- Mild oxidative stress enhances proliferative activity
- At higher oxidant stress, cells die by apoptosis

Oxidant →

## Signaling molecules regulated by cellular redox

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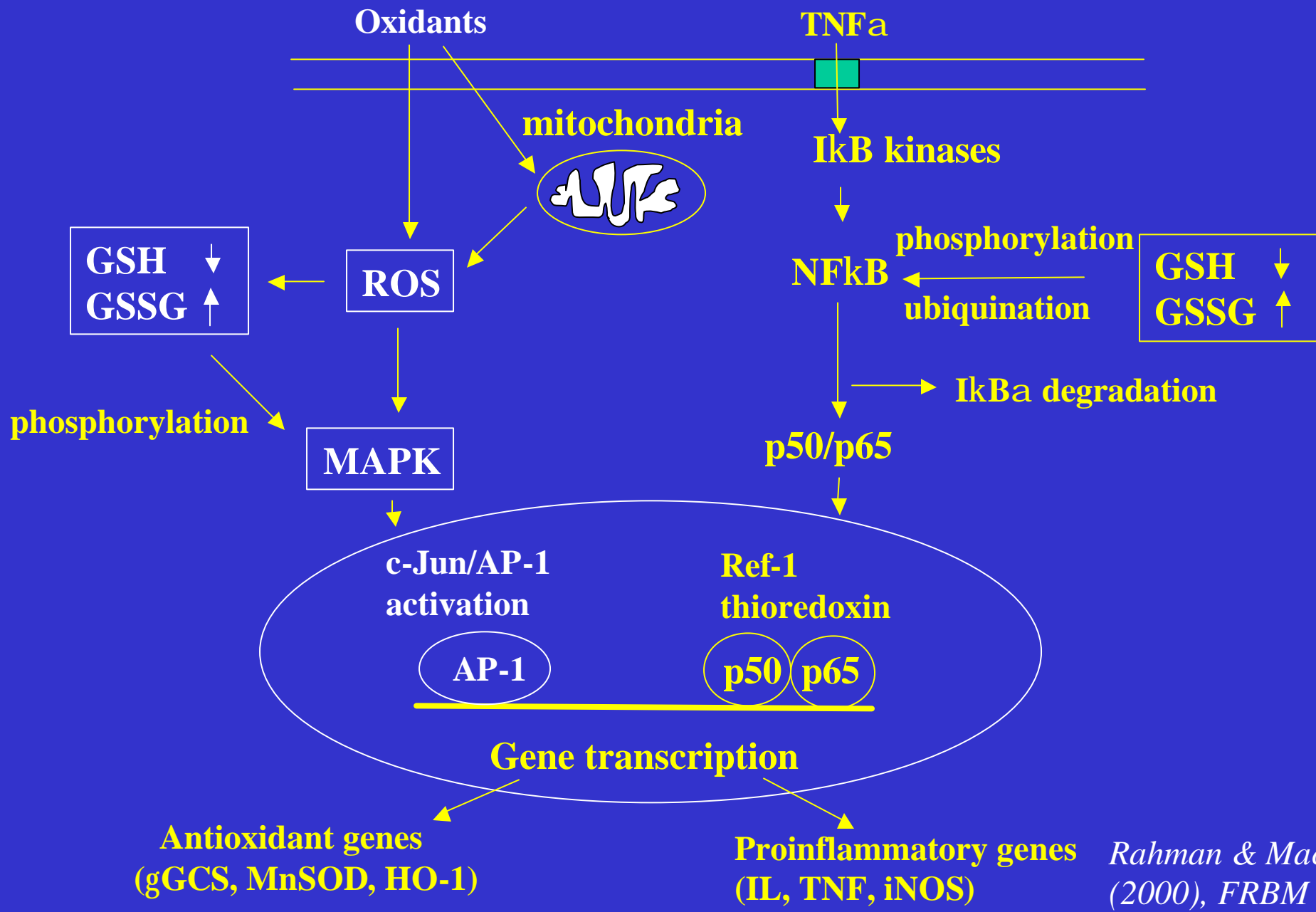
### Signaling molecule

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### Effect of oxidants

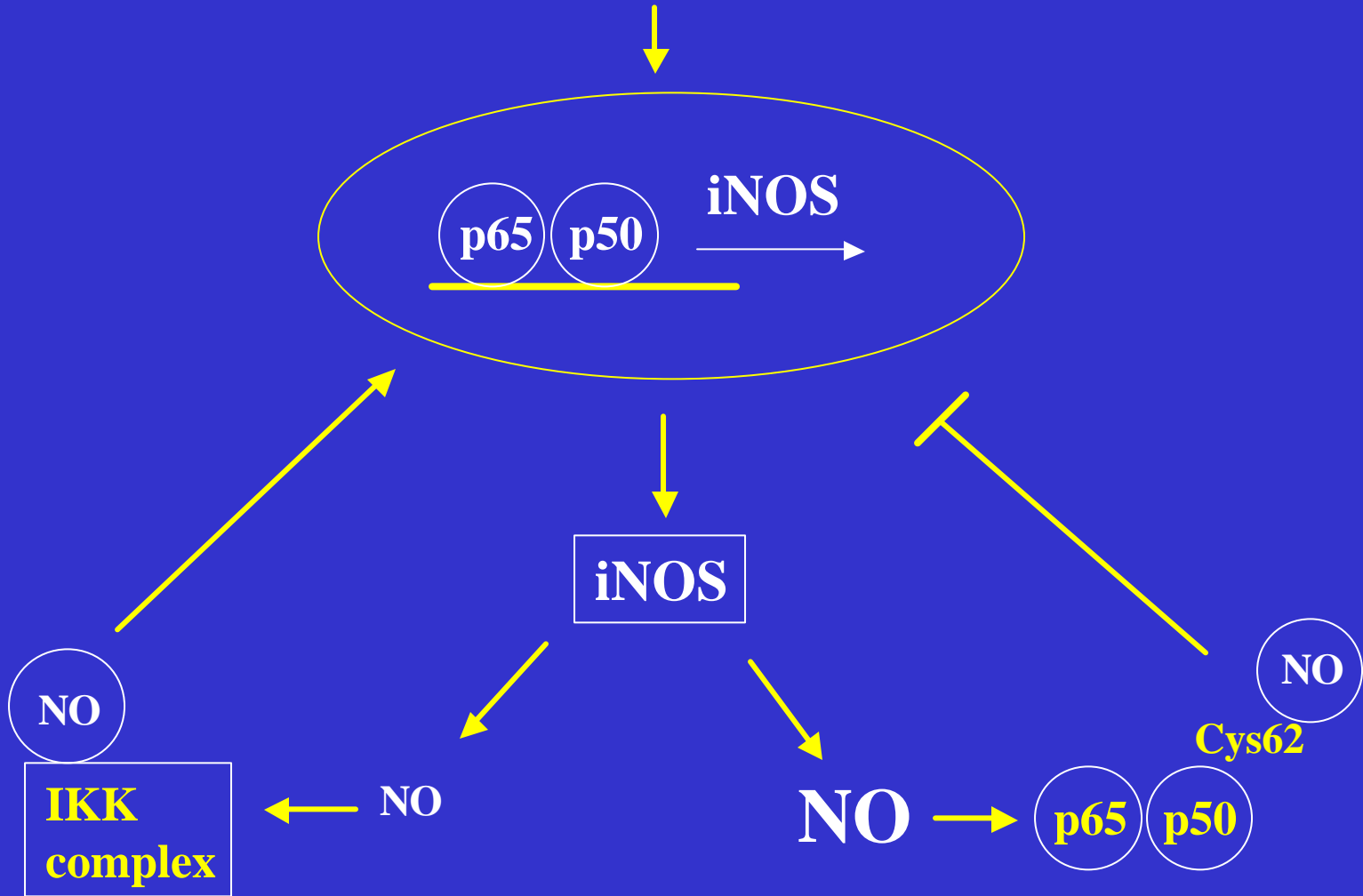
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- |                                   |                         |
|-----------------------------------|-------------------------|
| • Protein tyrosine kinase         | activation              |
| • Protein tyrosine phosphatase    | inactivation            |
| • Protein serine/threonine kinase |                         |
| MAPKs                             | activation              |
| PKC                               | activation/inactivation |
| • Small G protein                 | activation              |
| • Ca <sup>2+</sup> signal         | activation              |
| • Transcription factors           |                         |
| NFkB, AP-1                        | inactivation/activation |
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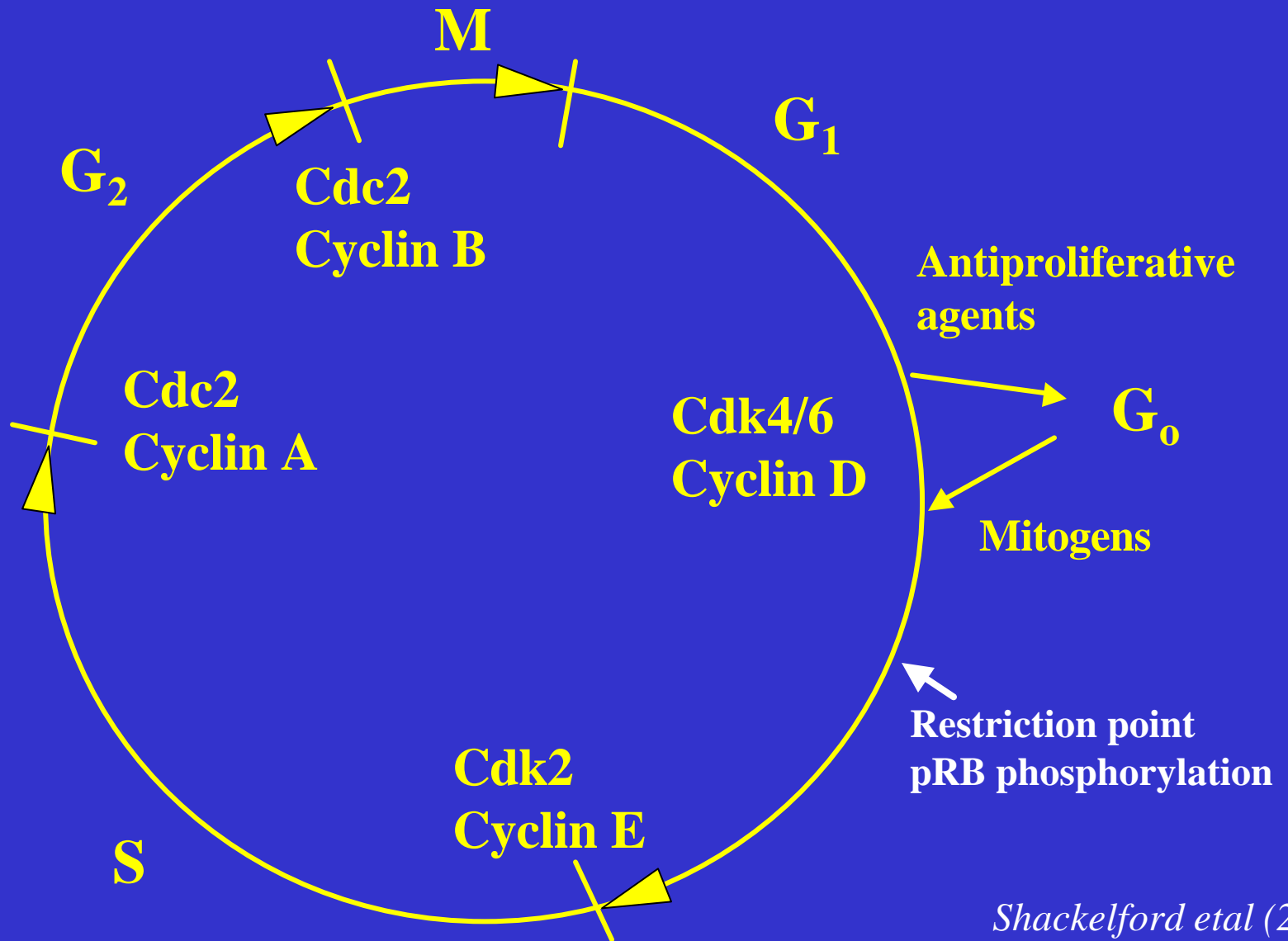


Rahman & MacNee  
(2000), FRBM 28, 1407

# Inflammatory mediators



# Cell cycle and cyclin/Cdk complexes



*Shackelford et al (2000)  
FRBM, 28: 1389*

## Oxidant effects on cell cycle checkpoint function

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Oxidant	Checkpoint	Effect
H <sub>2</sub> O <sub>2</sub>	G <sub>1</sub> phase	suppresses S phase entry by G <sub>1</sub> cells inhibit cyclin E/cdk2 activity
DEM	S phase	delay G <sub>1</sub> and S phase progression G <sub>2</sub> arrest
DEM tBH	G <sub>2</sub> phase	suppresses G <sub>2</sub> ----> M inhibit cyclin B/cdc2 activity

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## **GSH promotes cell proliferation**

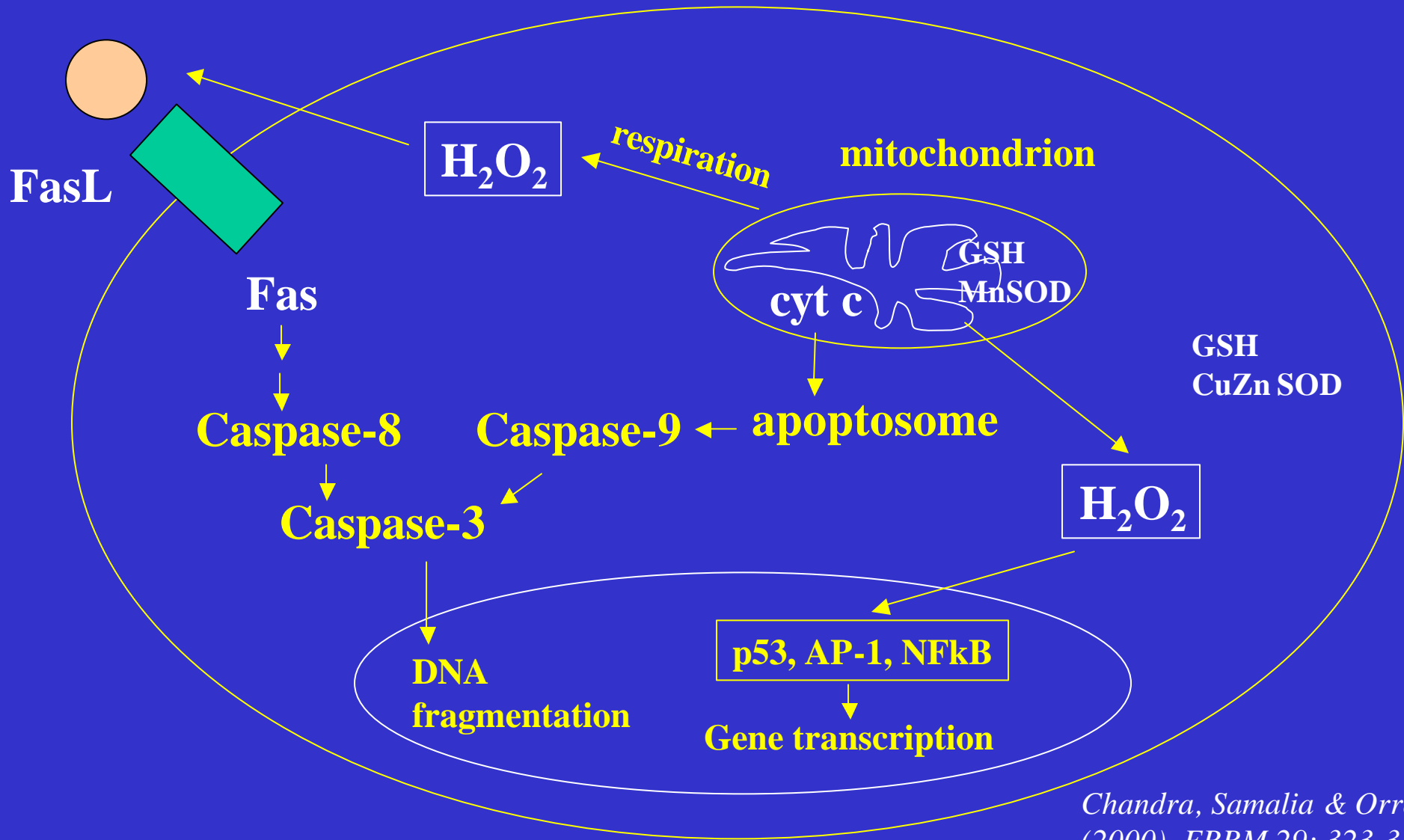
- **Growth factor stimulation of cell entry into S phase and DNA synthesis associated with GSH elevation**
- **GSH effects in proliferation are associated with autophosphorylation of GF receptor and stimulation of PKC**
- **Recent studies show that CaCo-2 cell proliferation is associated with changes in *extracellular* redox potential that is distinct from intracellular redox status**

# Oxidative stress and redox control of apoptosis

*“Paradigm for oxidative stress and apoptosis  
are constantly being redefined and honed”*

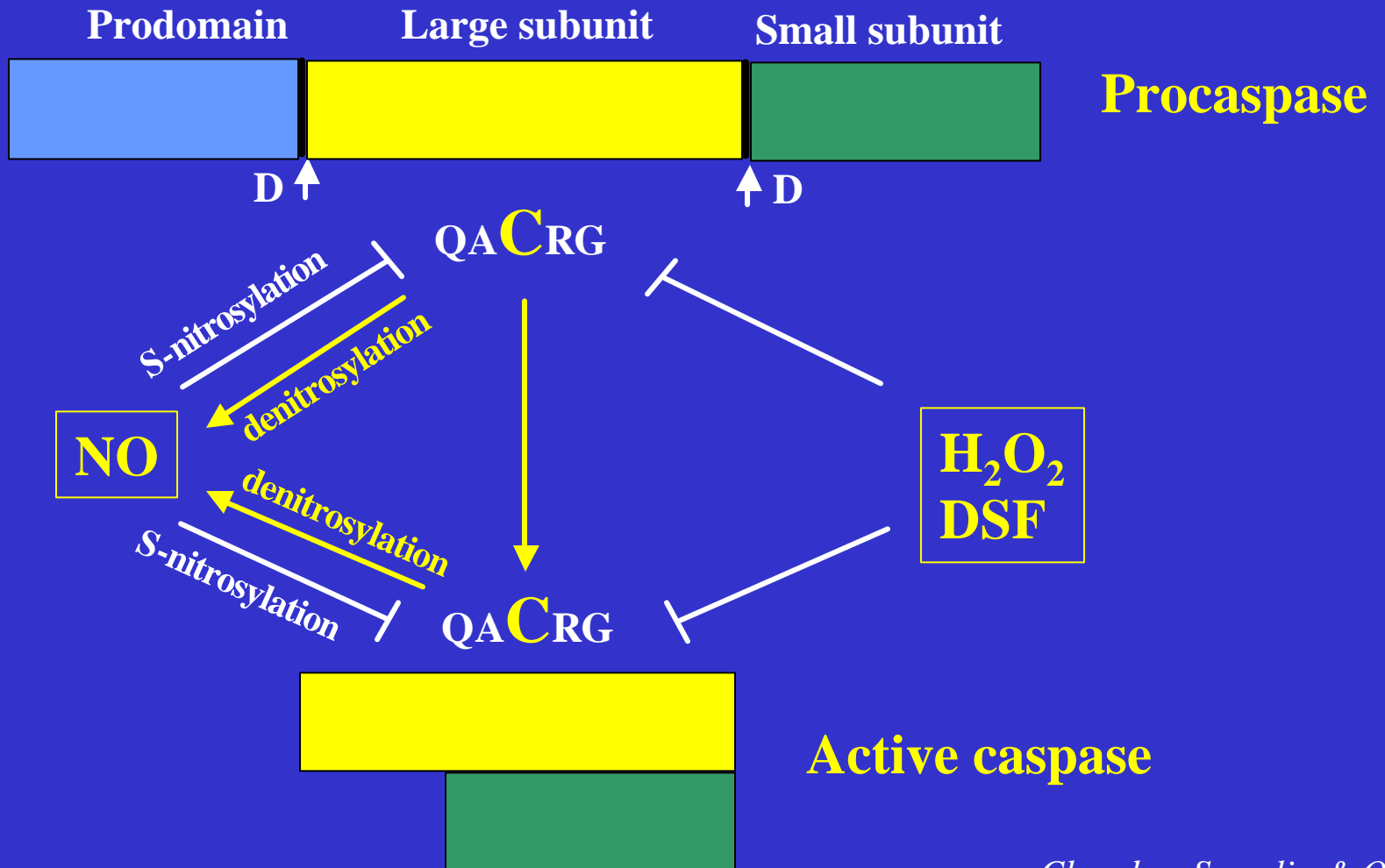
*Chandra, Samalia & Orrenius  
(2000), FRBM 29: 323-333*

# ROS and their interaction with the apoptotic pathway



Chandra, Samalia & Orrenius  
(2000), *FRBM* 29: 323-333

# ROS modulation of caspase

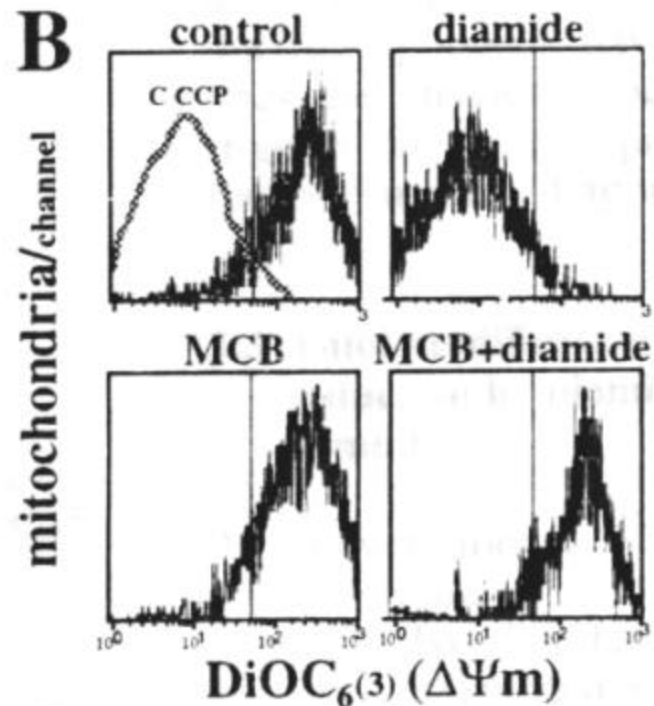
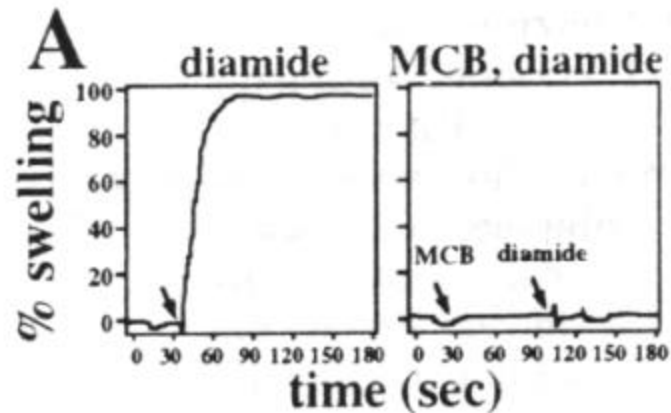


Chandra, Samalia & Orrenius  
(2000), *FRBM* 29: 323-333

## **Evidence that support a link between cellular GSH and apoptosis not necessarily associated with ROS**

- Decreases in cell GSH associated with initiation of apoptosis by glucocorticoid (thymocytes) and serum withdrawal (fibroblasts)
- GSH protects against dopamine-induced neuronal apoptosis
- GSH increases linked to BcL-2 expression; BcL-2 expression redistribute GSH to nucleus to preserve nuclear redox status
- Induction of apoptosis (Jurkat, HepG2, astrocytes) linked to active GSH efflux
- Thiols regulate mitochondrial permeability transition

## Diamide-mediated PT in isolated thymocyte mitochondria

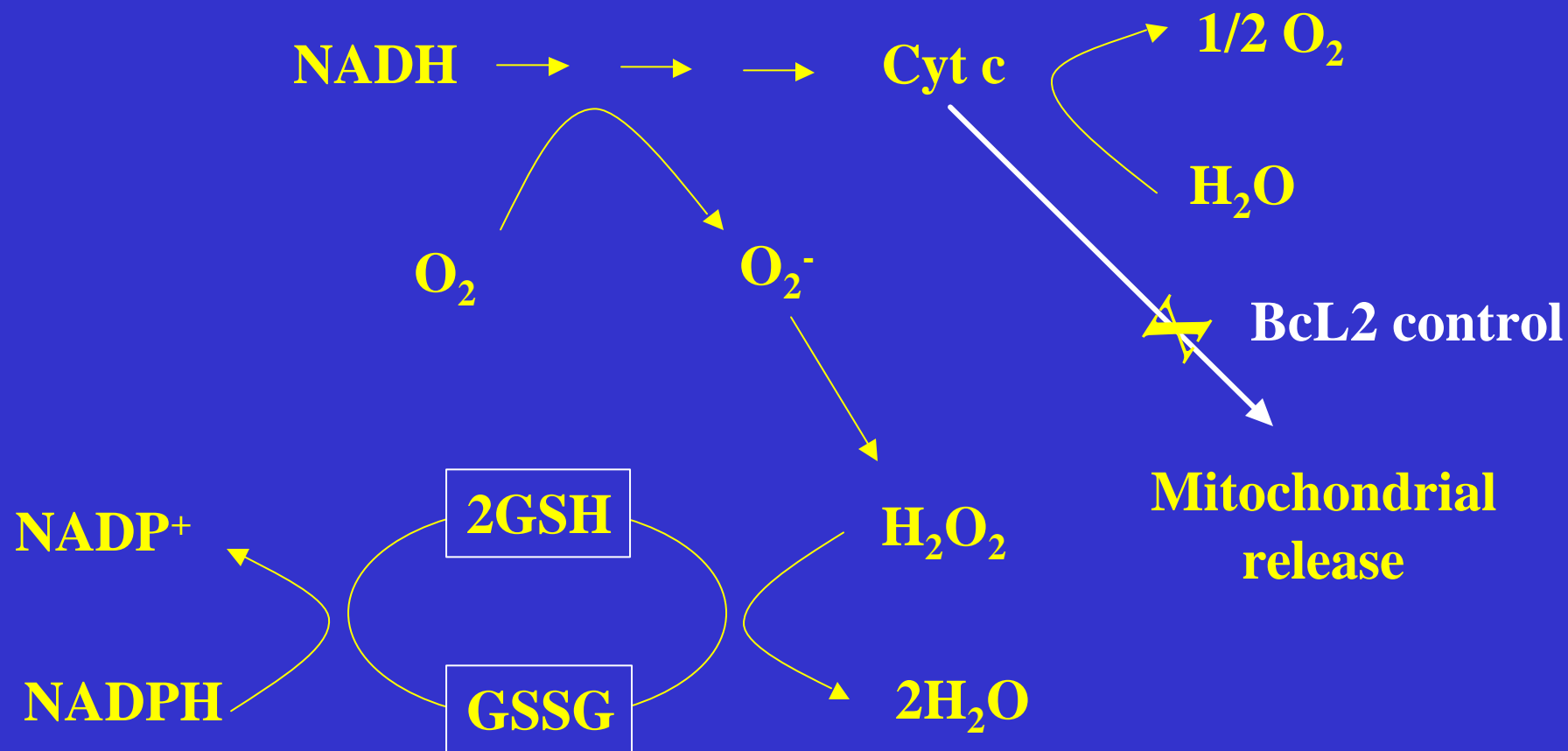


A - Effect of diamide and MCB (monochlorobimane) on mitochondrial amplitude swelling

B - Effect of diamide and MCB on the mitochondrial  $\Delta\Psi$

- Thiol cross-linking with diamide induces mitochondrial PT
- Thiol substitution by monovalent MCB impedes formation of disulfide bridges between vicinal thiols

## BcL-2-dependent control of cellular thiol-disulfide status



## Summary

**Regulation of cell survival or death by oxidative stress and redox is a complex process. Depending on the severity and duration of stress, cells exhibit proliferative or apoptotic responses that are mediated by a variety of different complex and often interacting signaling pathways**

*“Paradigm for oxidative stress and **cell responses** are constantly being redefined and honed”.....*

**Our challenge lies not in the conceptual acceptance of the hypotheses, but rather in the experimental documentation of the proposed associations**