

Role of Macrophages in Hematopoiesis and Disease

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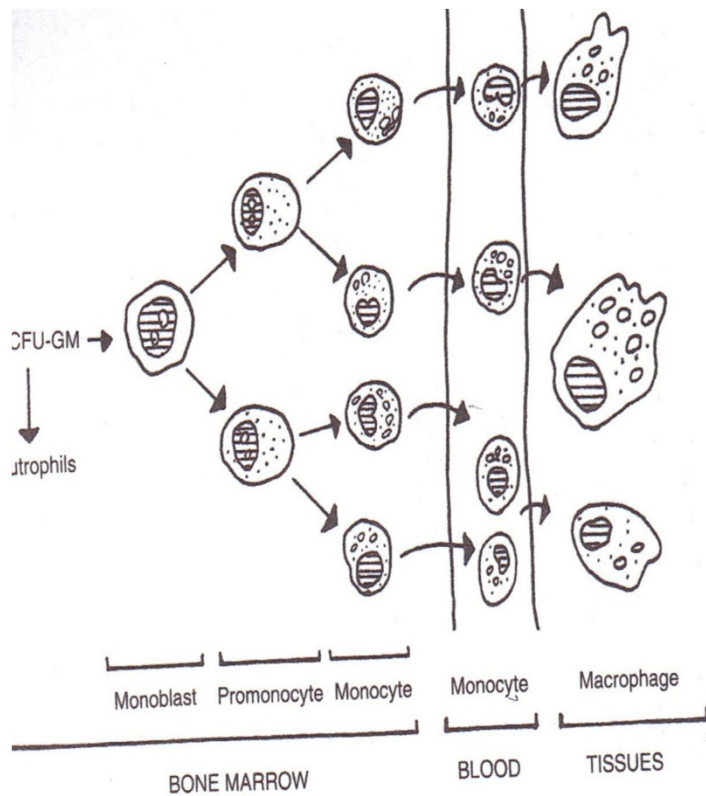
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Health

MACROPHAGES

- Remarkably versatile cells
- Participate in both innate and acquired immunity through a myriad of cytokines and low molecular secretory products
- Antigen presenting cells
- Important cells in hematopoiesis
- On the negative side, contribute to pathology of many chronic diseases ,including atherosclerosis and Alzheimers disease

Macrophage Origin and Function

Origin



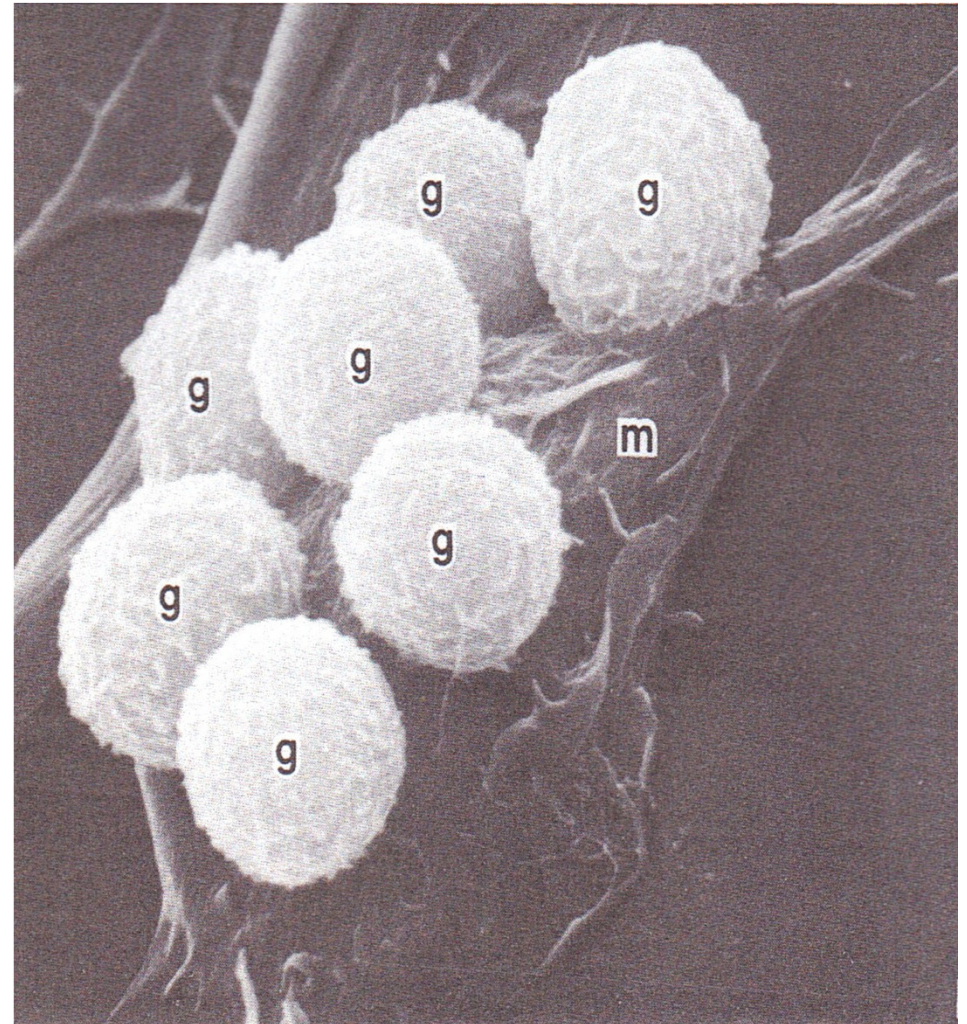
Function

During myelopoiesis, external differentiating signals regulate the expression of a set of transcription factors. The combined actions of these transcription factors subsequently determine the expression of myeloid-specific genes leading to the generation of monocytes and macrophages.

Blood monocytes are an intermediate stage which then further differentiate in tissues to various macrophage populations.

Bone Marrow Macrophages

- IL-1 from stromal macrophages signal to fibroblasts and endothelial cells to make growth factors for myelopoiesis and lymphopoiesis
- Physical interaction with developing granulocytes in granulocyte islets
- Bone marrow macrophages are target cells for drugs and environmental chemicals



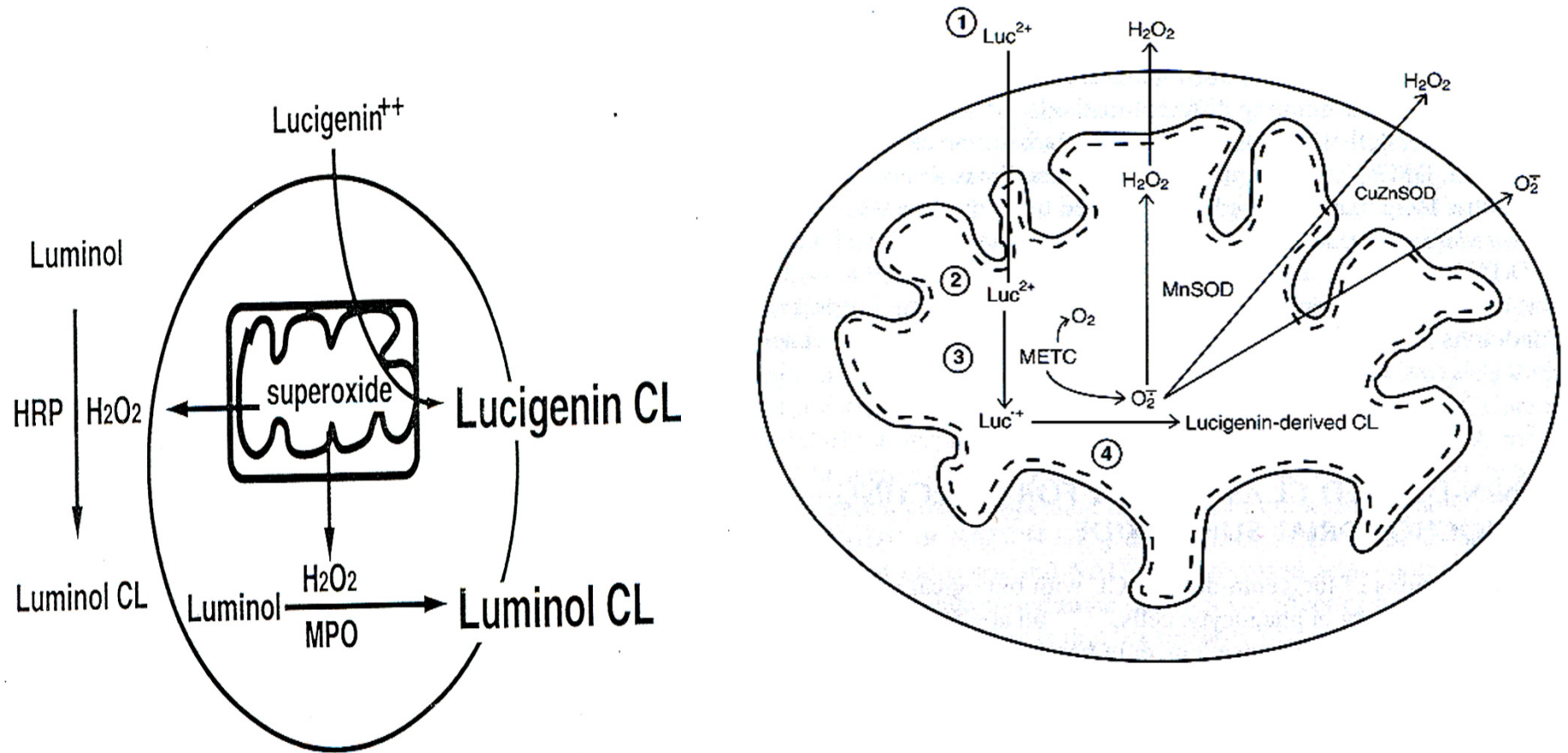
ROS and Myeloid Cells

- One of the distinguishing biochemical characteristics of PMNs and macrophages is their ability to synthesize and release **ROS** .
- One of the primary cellular sources of ROS in these cell types is a plasma membrane **NADPH oxidase**, which generates superoxide .
- While **myeloperoxidase** (MPO) is found in myeloid progenitor cells, MPO is found in the differentiated PMNs and monocytes but **not in macrophages** .
- Another biochemical and metabolic characteristic which distinguishes **macrophages** from PMNs and monocytes is their utilization of **mitochondrial respiration** for the generation of cellular energy

Cell Lines and Macrophage Differentiation

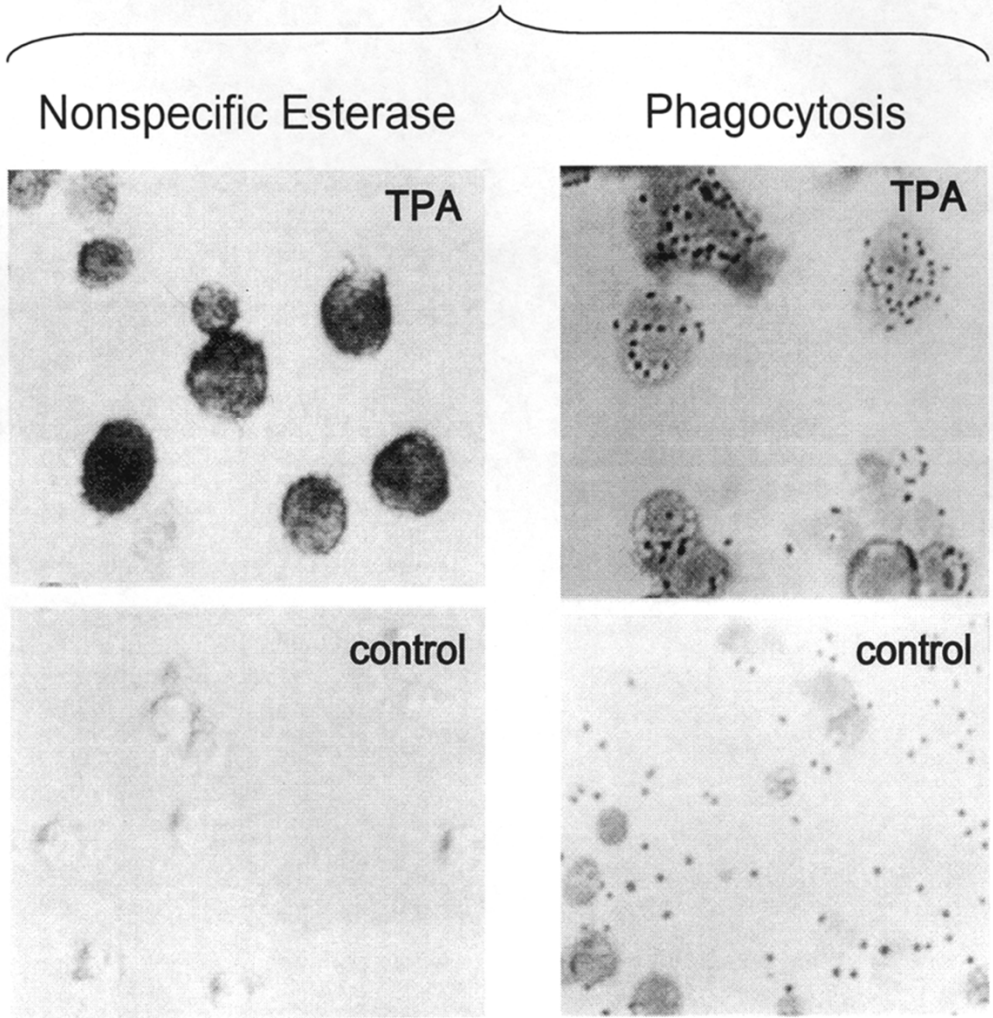
- To understand the molecular mechanisms underlying macrophage differentiation, investigators have turned to human myeloid cell lines such as HL-60, U937, THP-1 and **ML-1**. These cell lines have served as a basis for differentiation therapy.
- In vitro, these myeloid cell lines continuously proliferate in suspension culture, and can be induced to differentiate into macrophages by 12-O-tetradecanoylphorbol-13-acetate (TPA) or 1,25-dihydroxyvitamin D3.
- HL-60 cells can also be differentiated to PMNs by dimethyl sulfoxide or retinoic acid. As such, these cell lines have been widely used as cell models for studying the molecular and cellular aspects of myeloid differentiation.
- HL-60 cells do not differentiate to macrophages. However, HL-60 cells transfected with wild-type p53 gene differentiate to macrophages not PMNs

Measuring ROS Through Chemilumigenic Probing

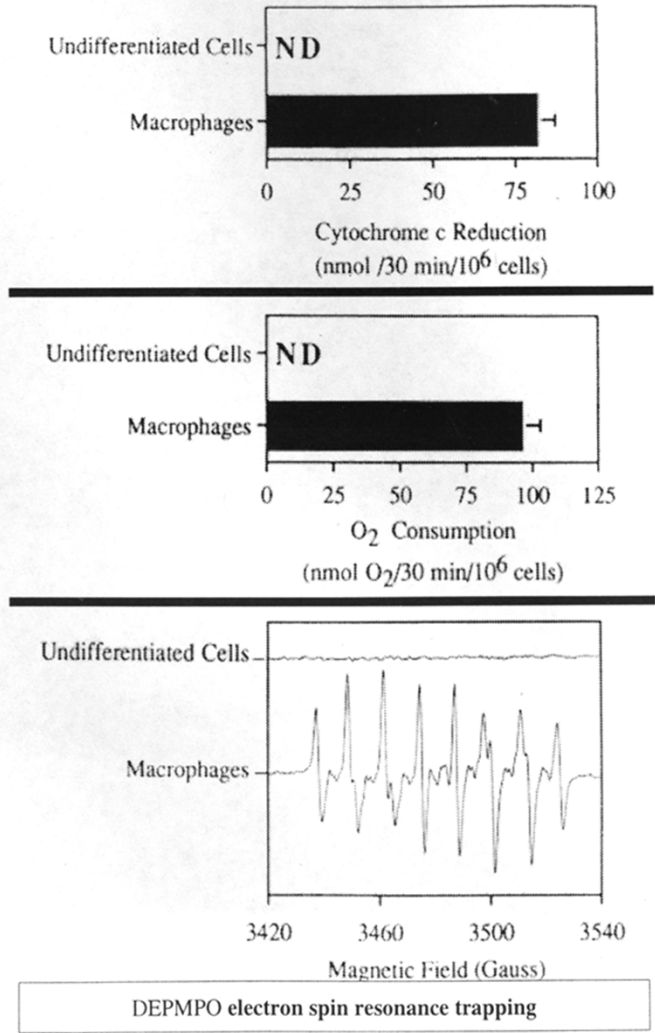


Measureable endpoints in the ML-1 differentiation model

Day 6



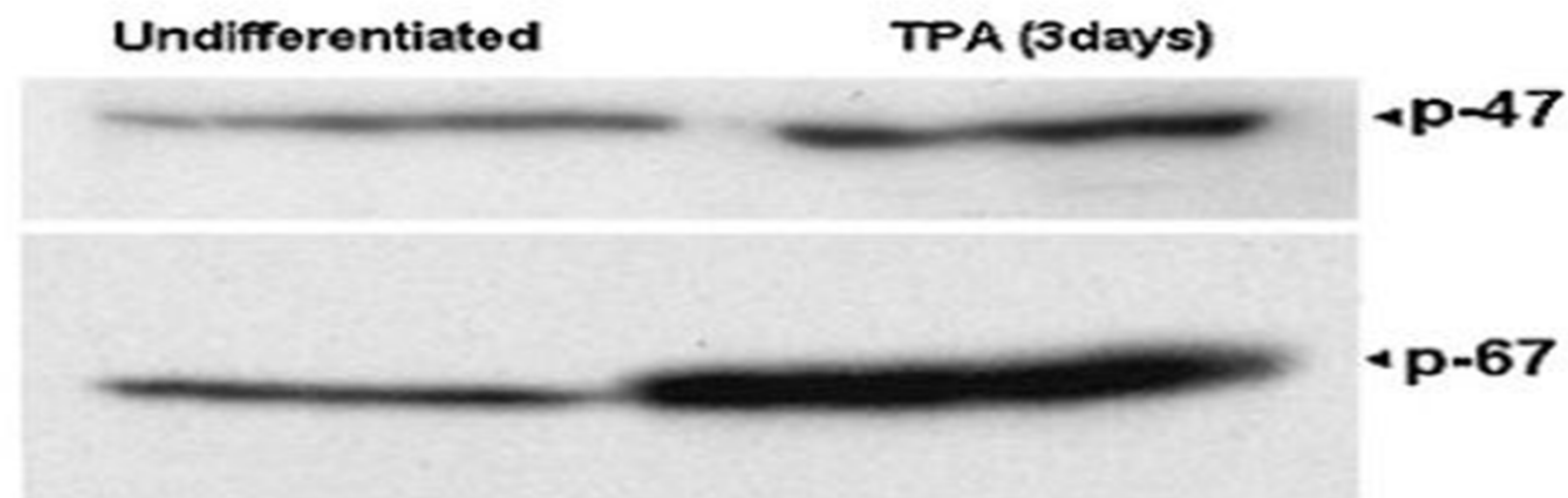
Day 7 NADPH oxidase activity



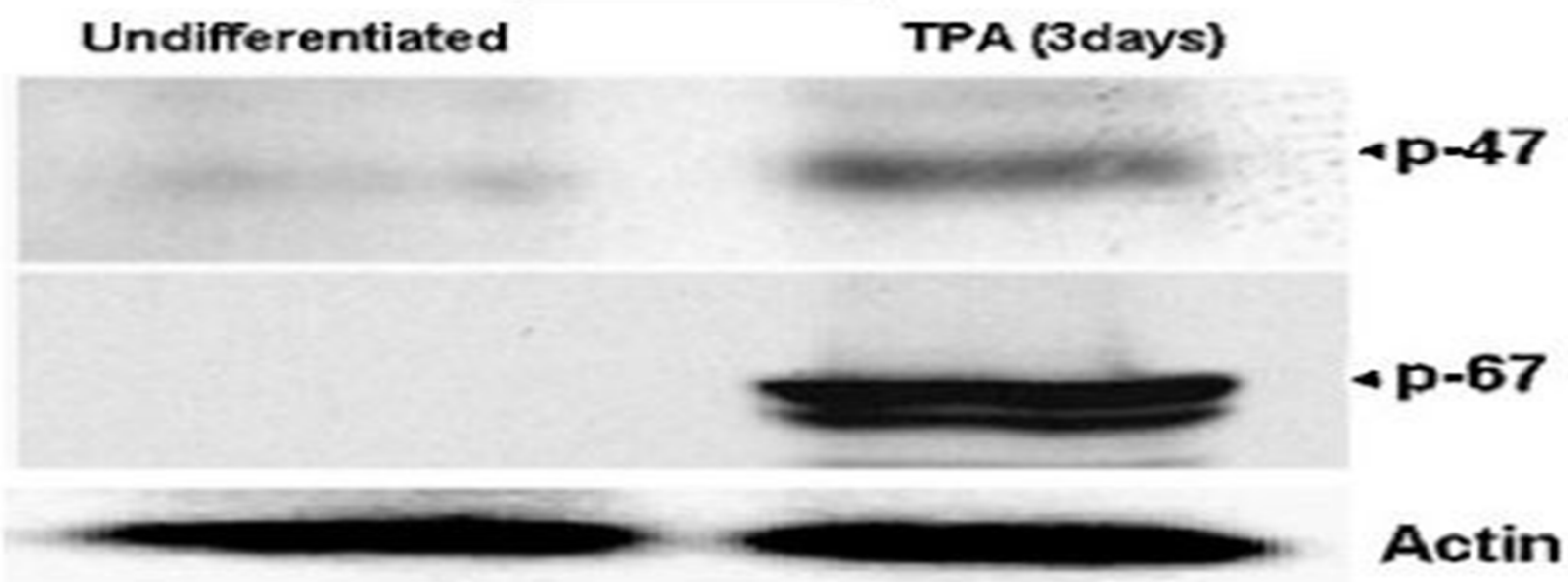
from Hongchin *et al.*, 1999.

from doctoral thesis by Yunbo Li, 1999.

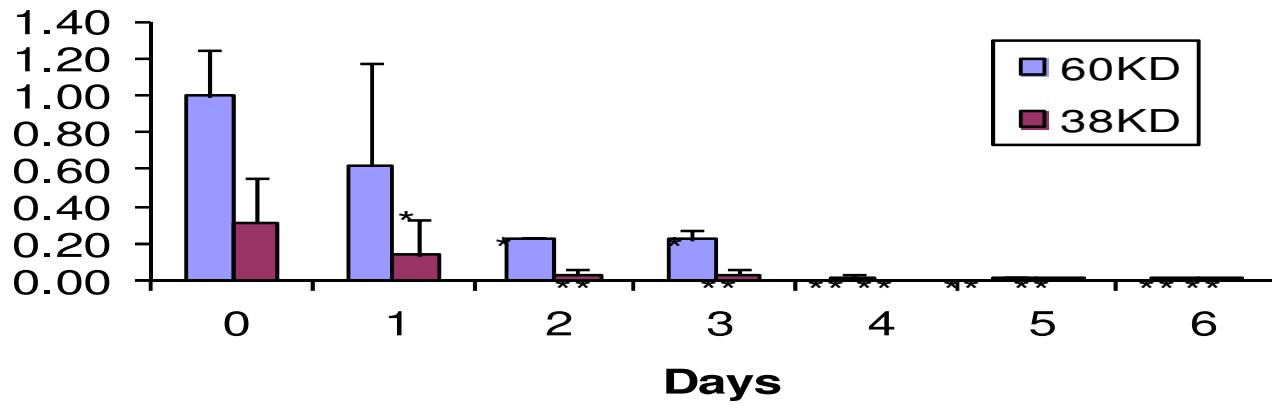
THP-1 Cells



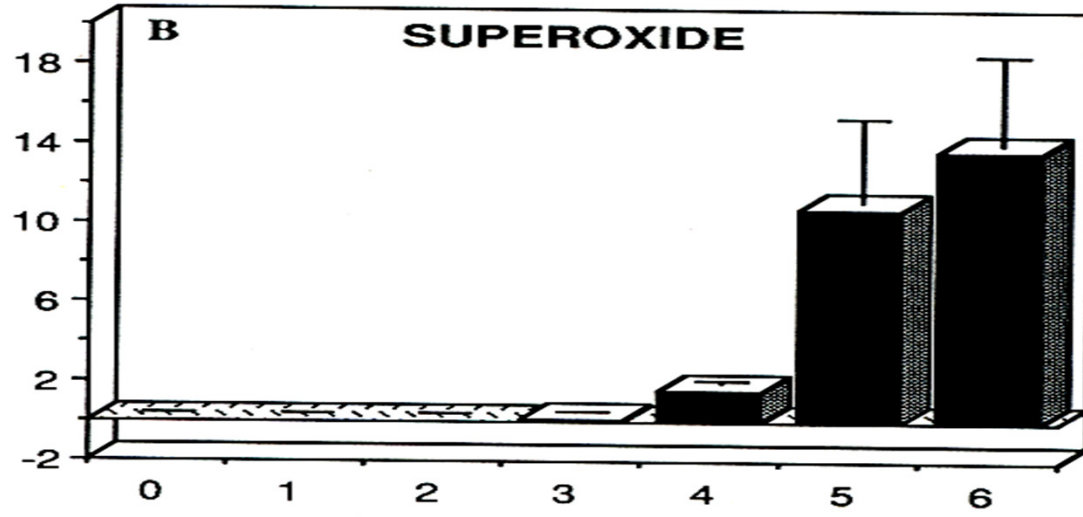
ML-1 Cells

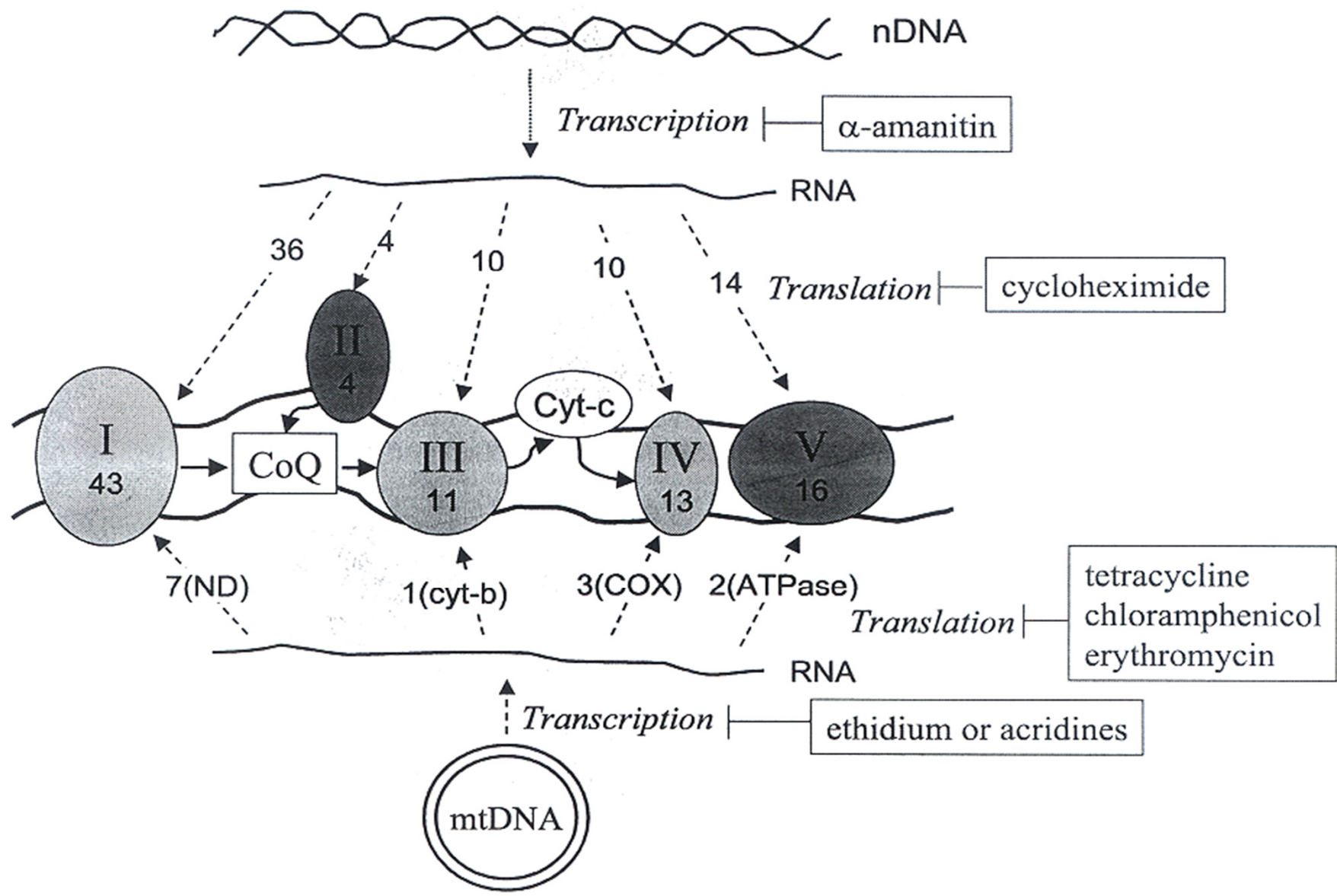


Relative Density of Protein Expression

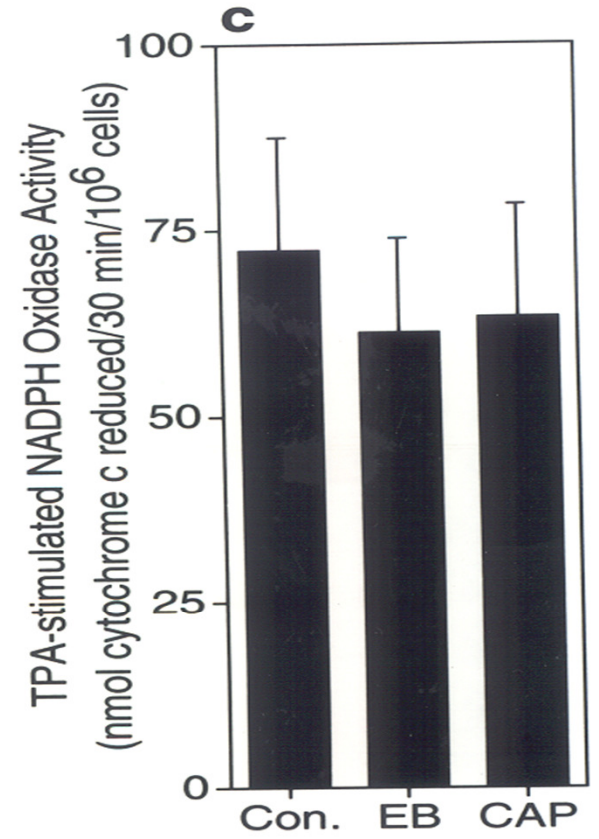
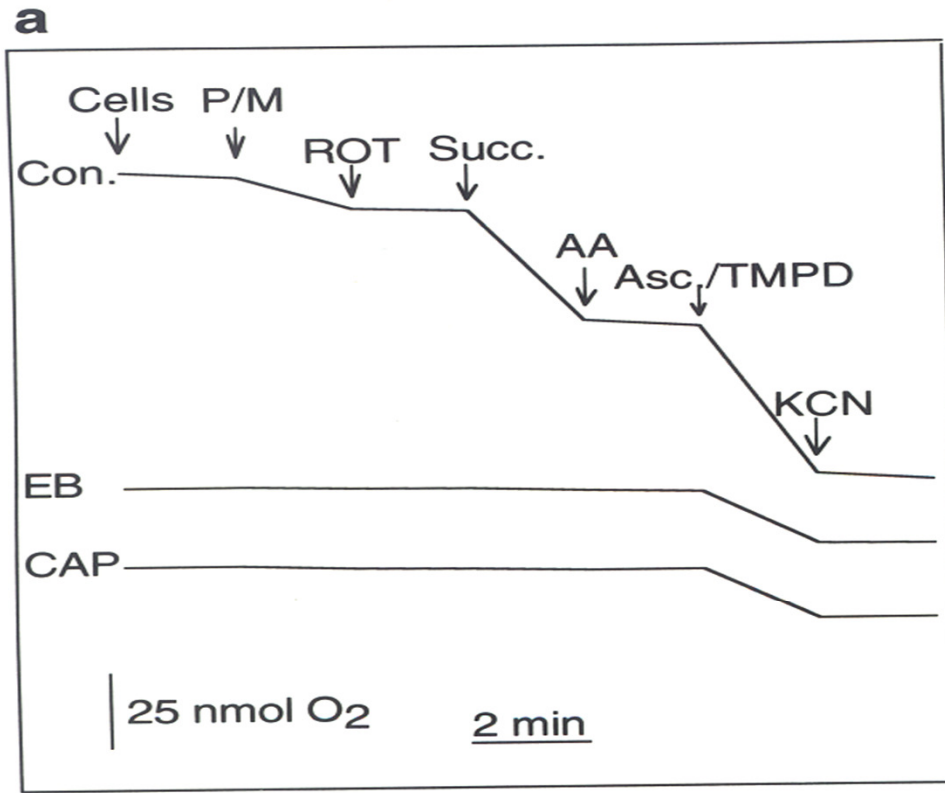


MITOCHONDRIA-DERIVED SUPEROXIDE
(Integrated LDCL X 10⁻⁶)

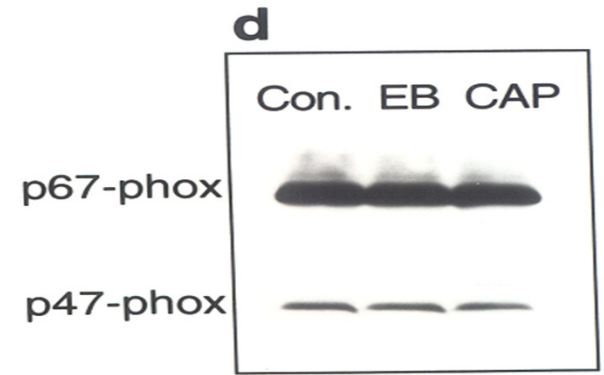
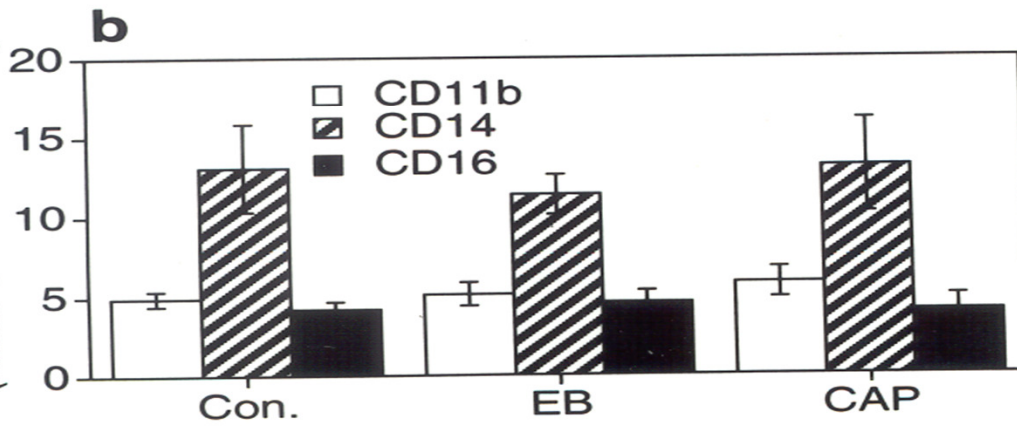




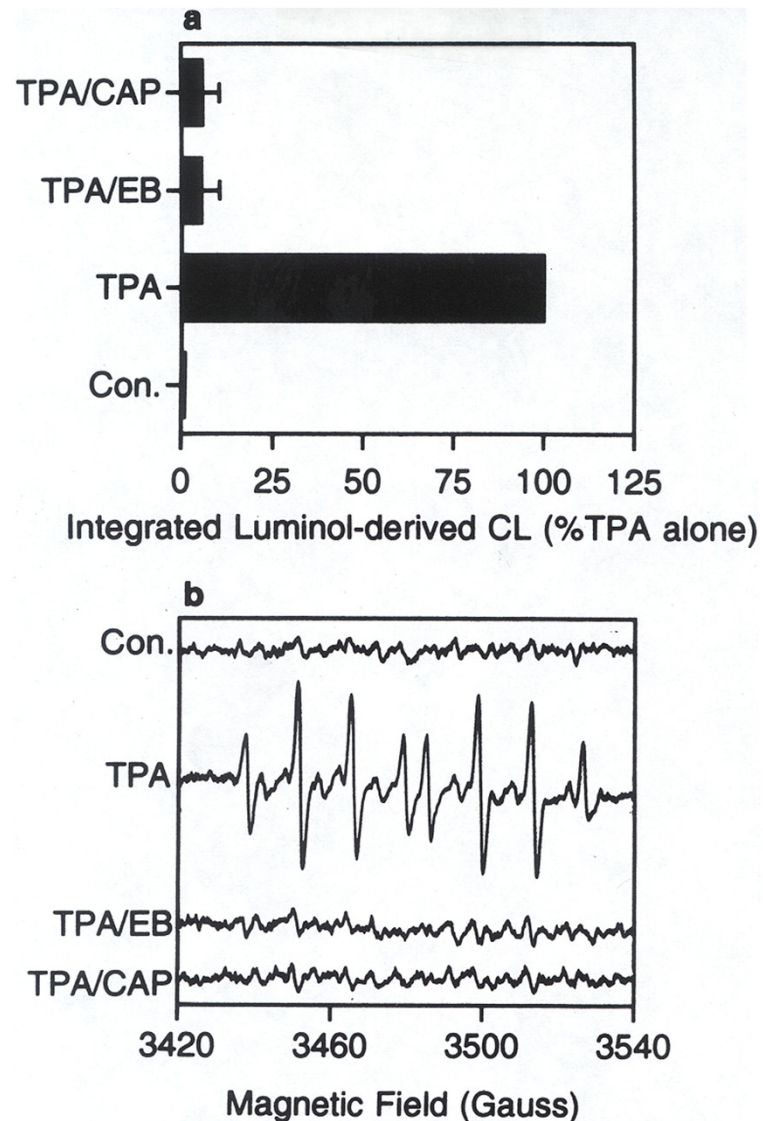
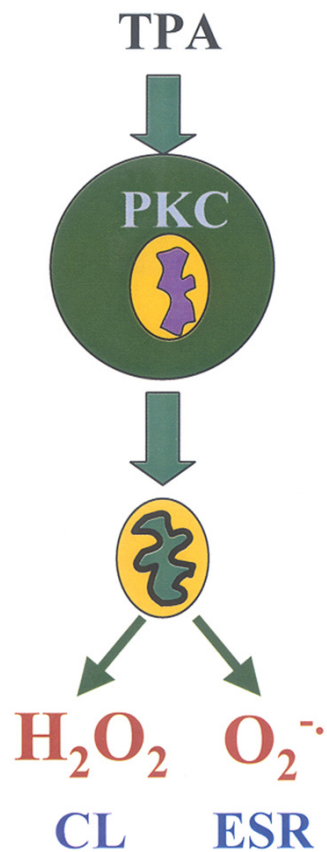
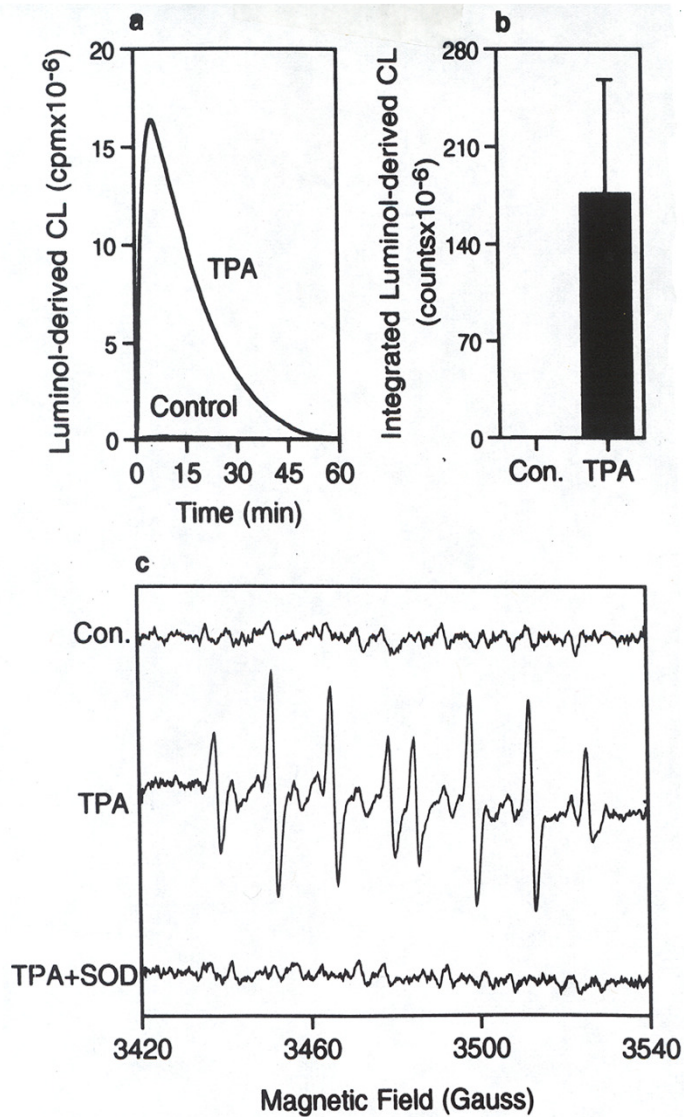
Substrate-supported Mitochondrial O₂ Consumption
in Digitonin-permeabilized Cells

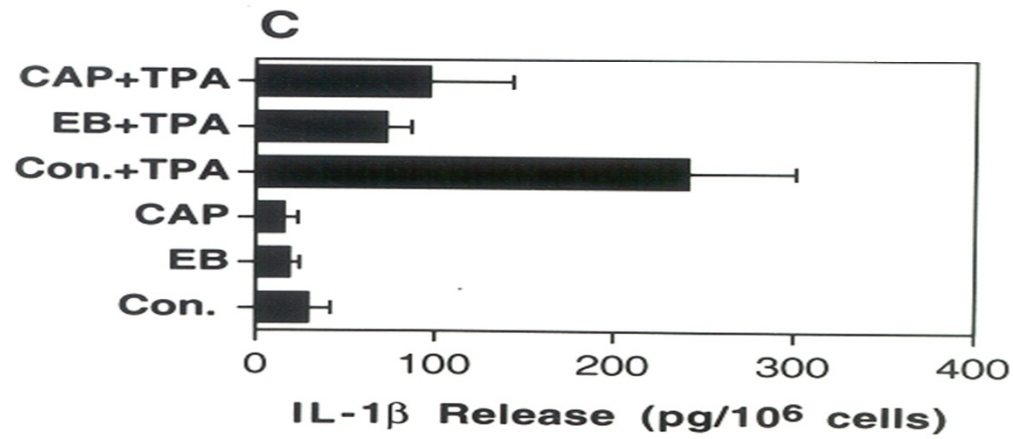
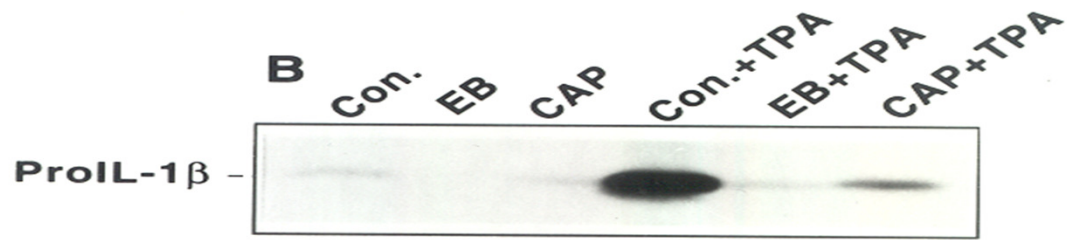
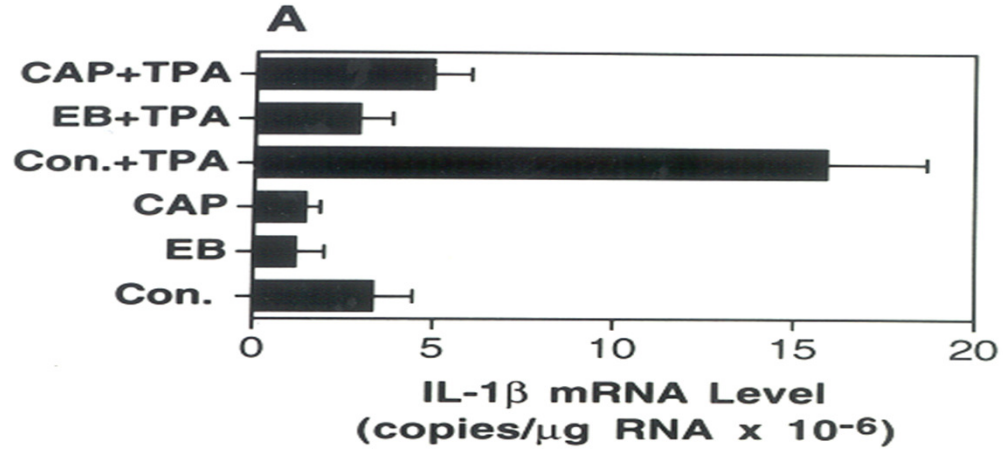


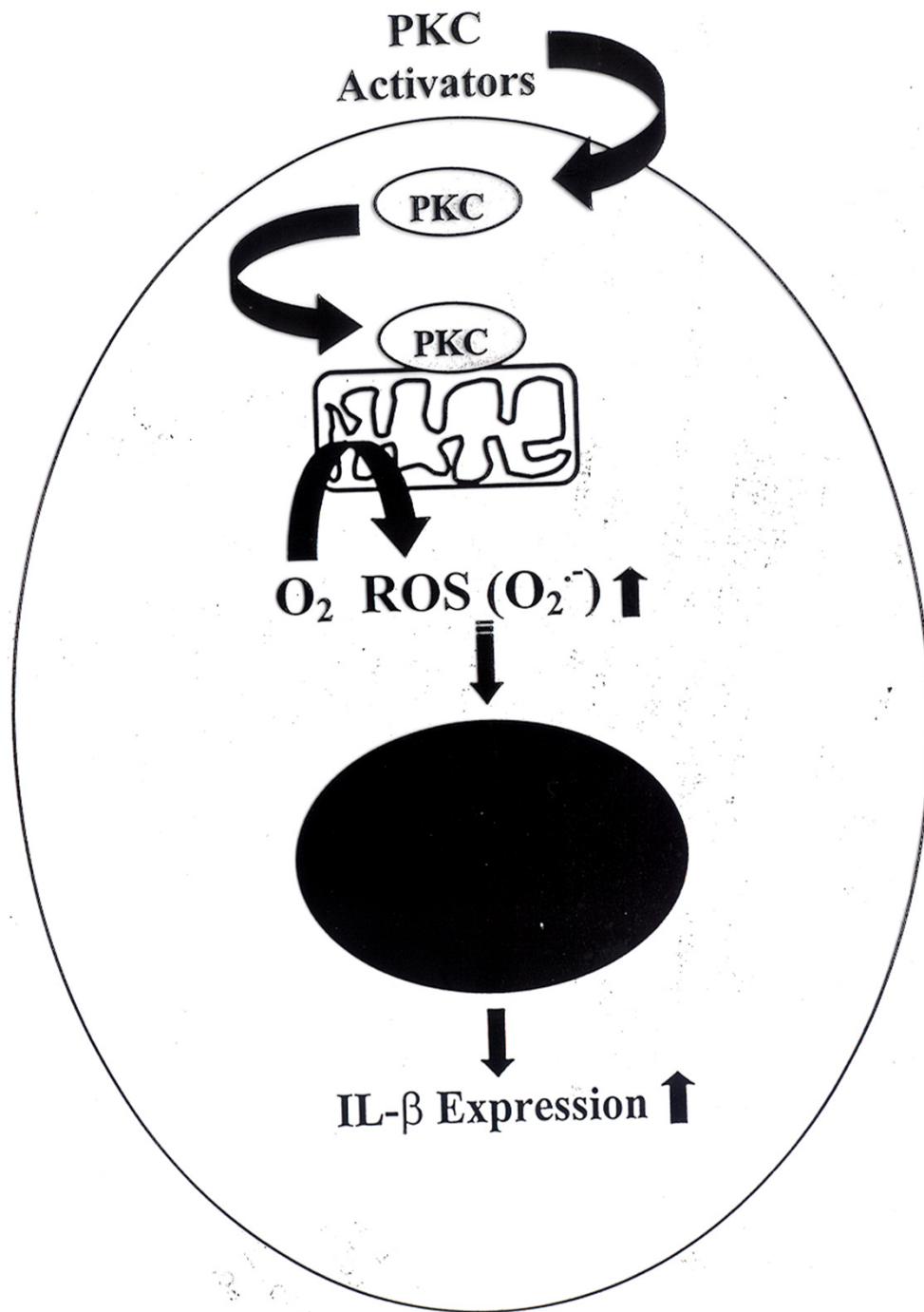
Cell Surface Marker Expression
(Mean Channel Number)



PKC Activation Leads to Production of Mitochondrial ROS







GF 1092X03

Go 6976

Myristoylated PKC Pseudosubstrate

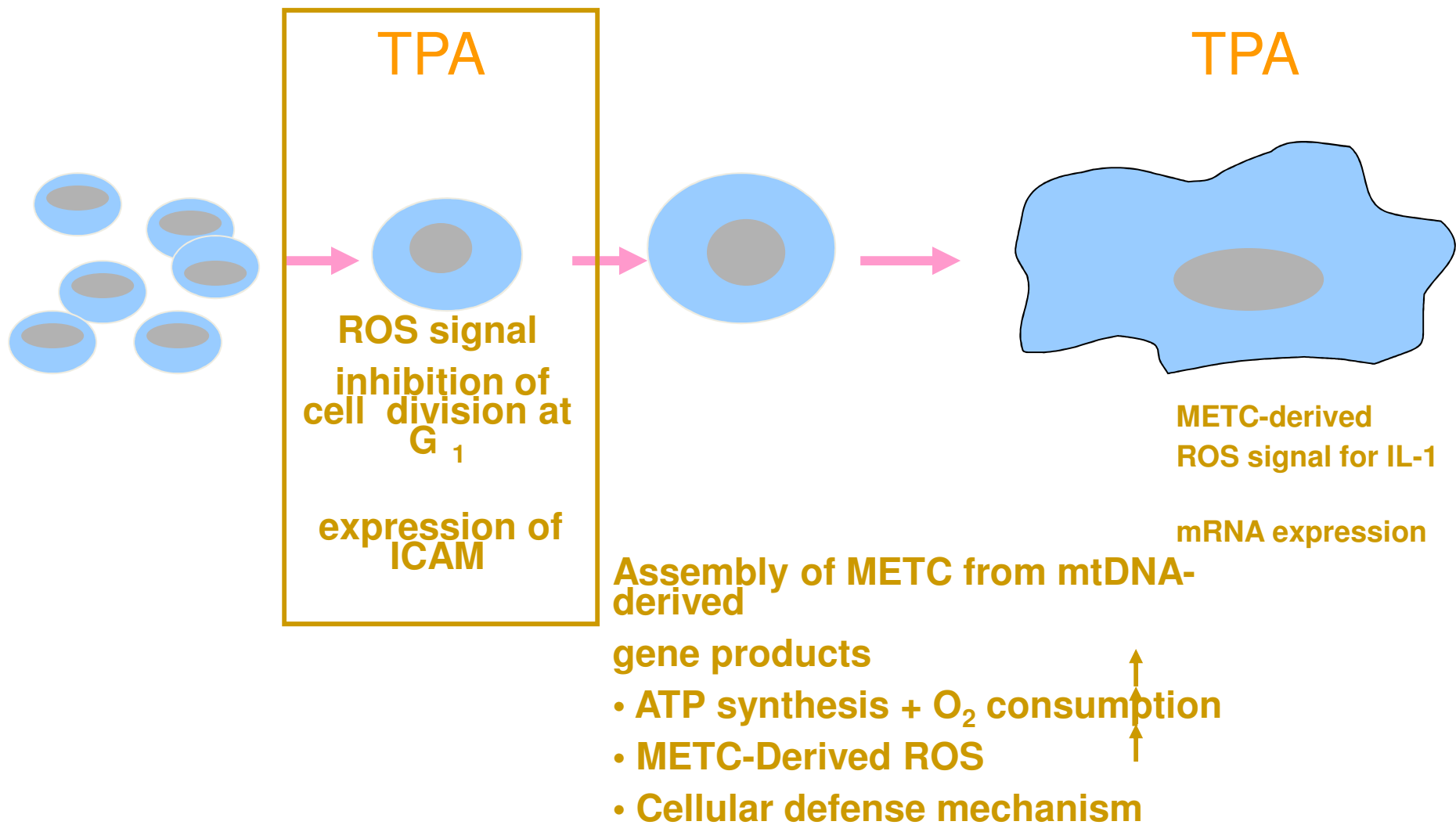
TEMPO

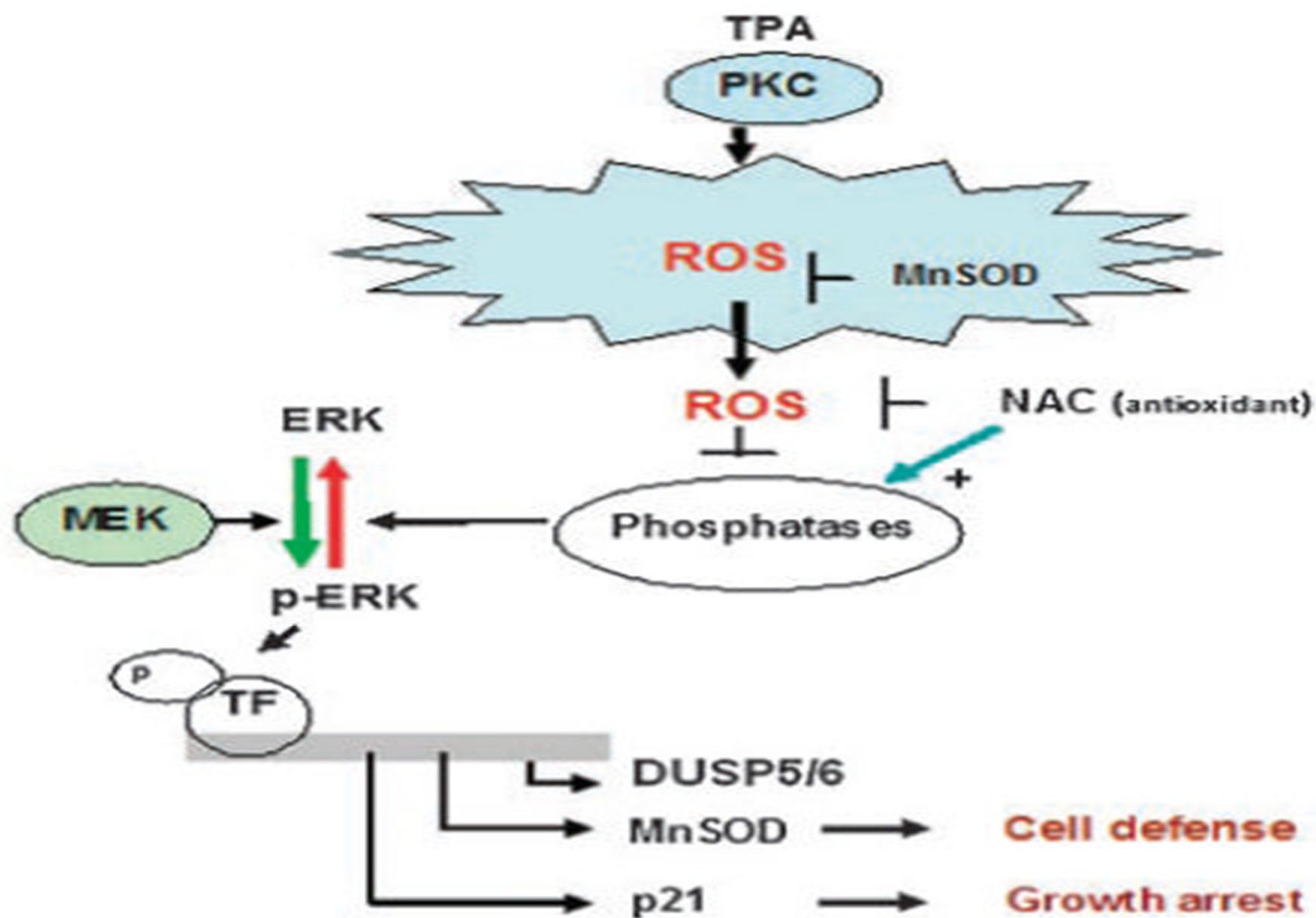
METC-Deficient Cells

DPI

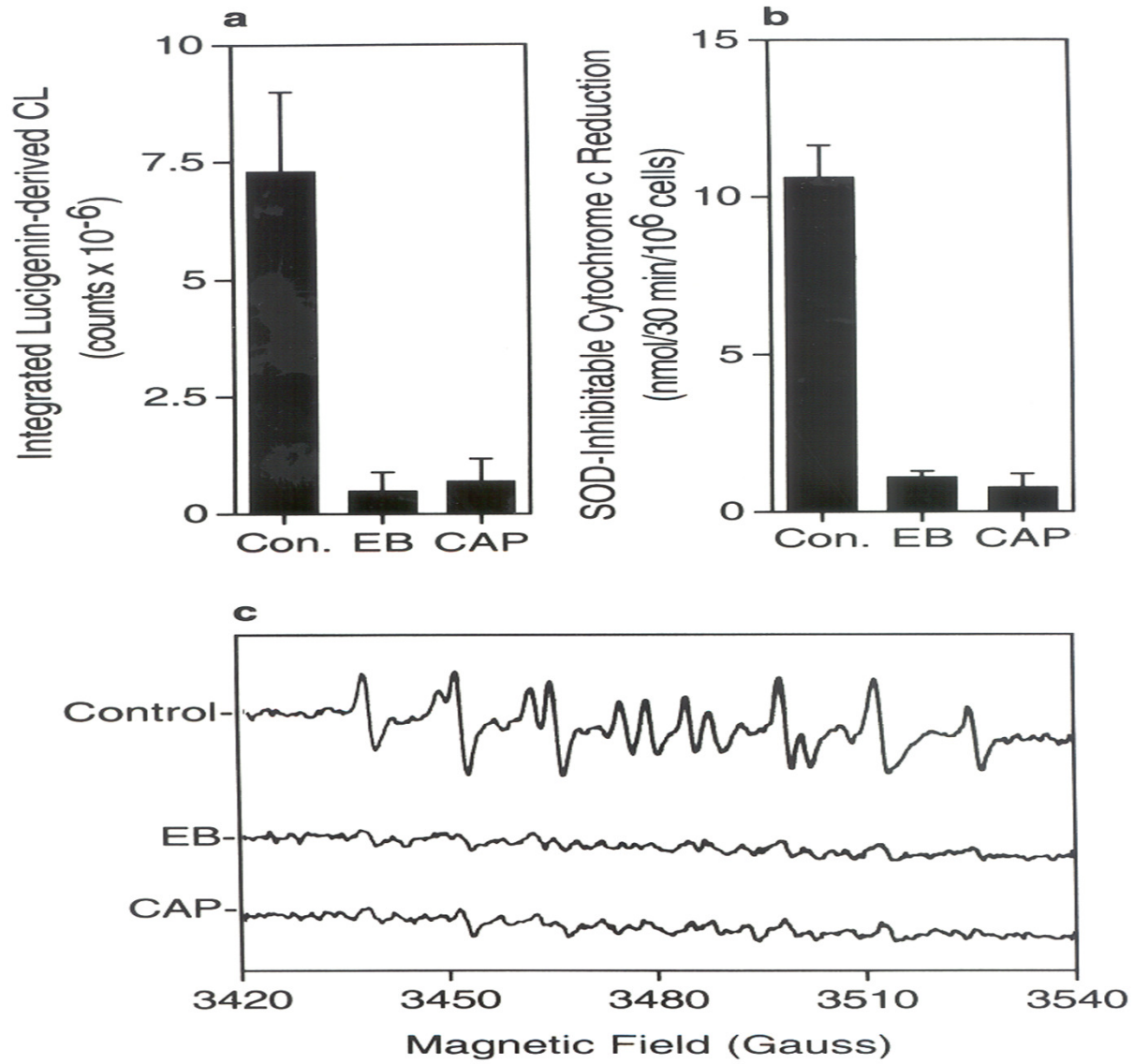
MnSOD Overexpression

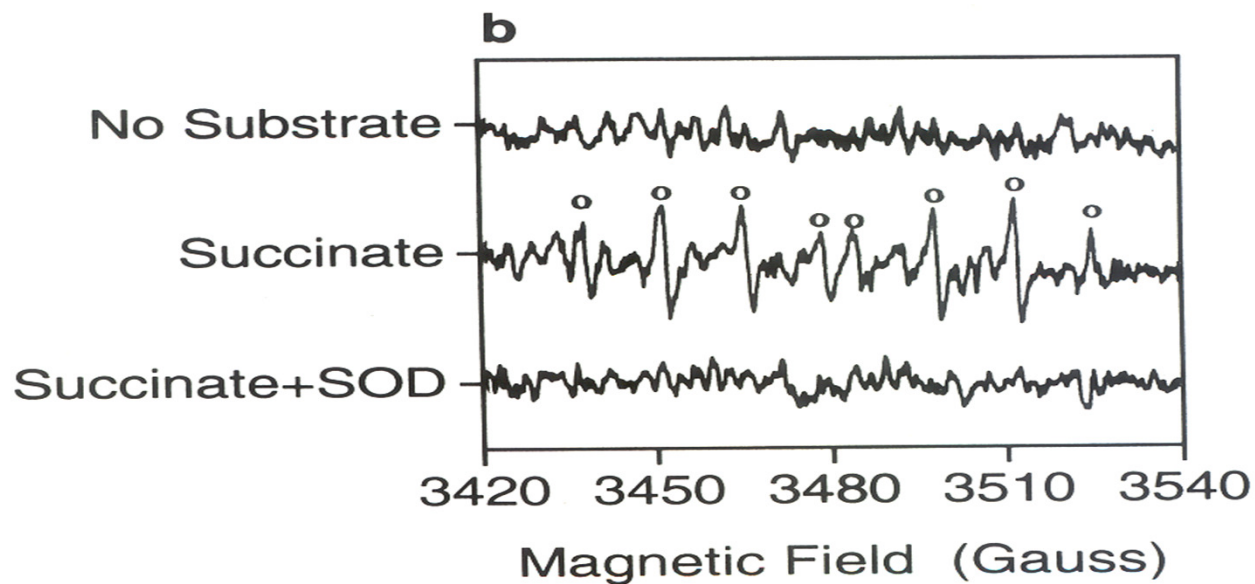
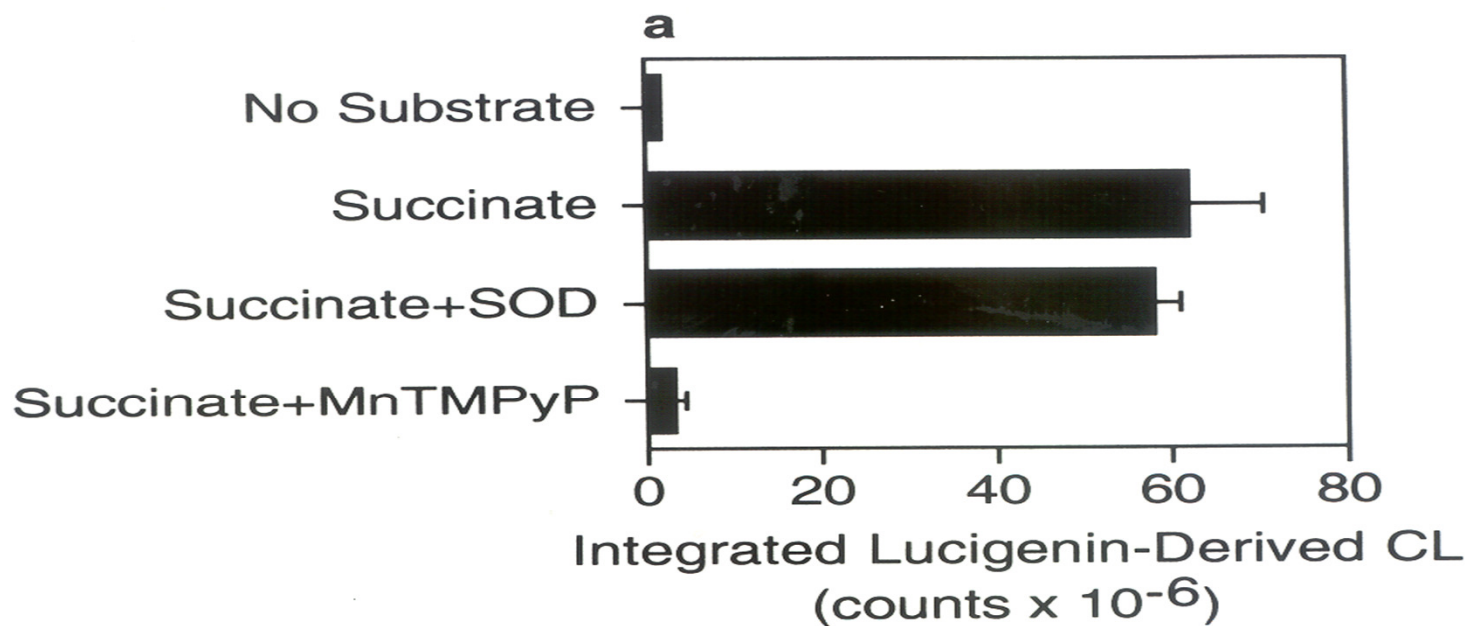
**ROS Signaling Is also
Involved in the
Differentiation of ML-1 Cells
to Macrophages**

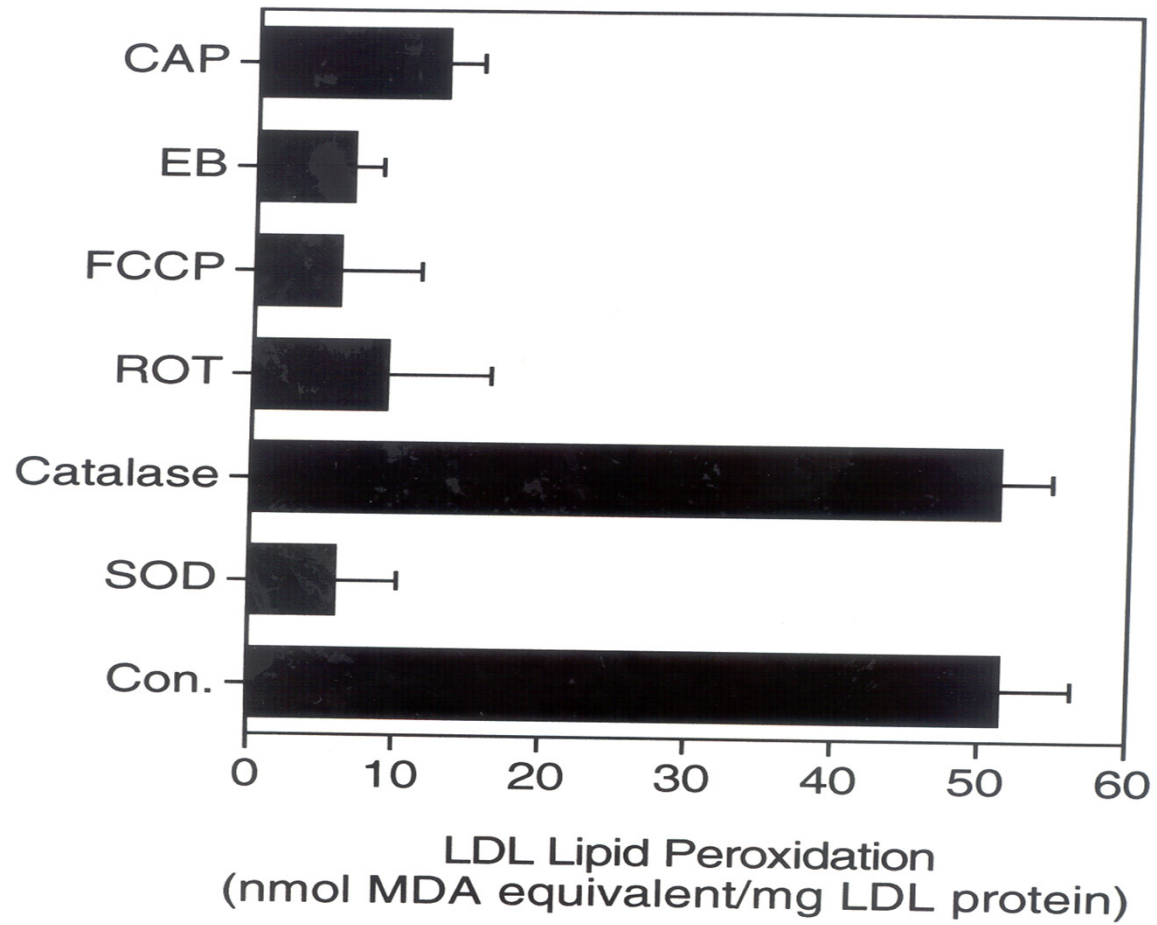


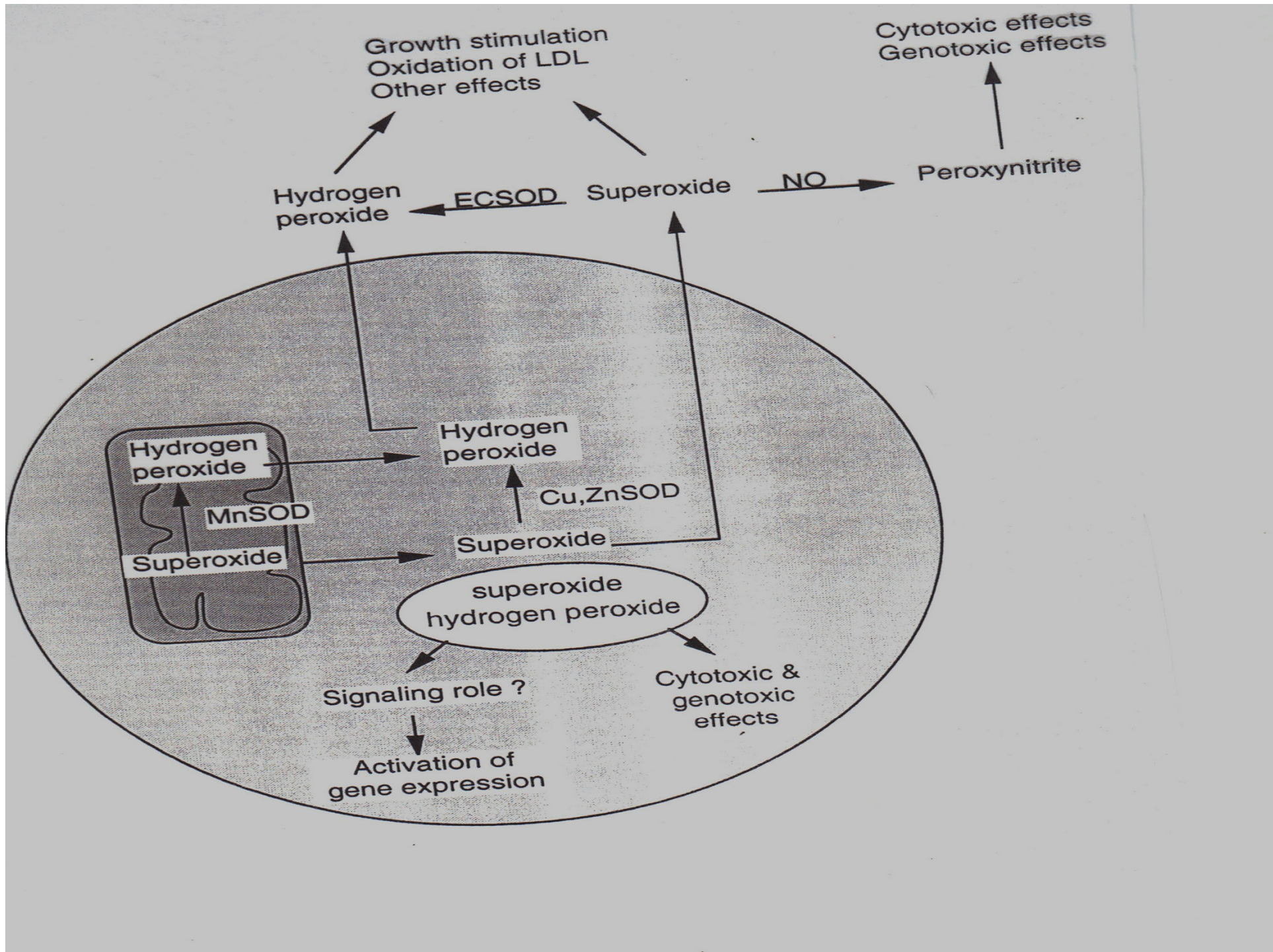


**Superoxide Can Exit from
Resting Differentiated
ML-1 Cells!!!!**









Acknowledgements

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- Hong Zhu
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