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Biochemical Basis of Disease

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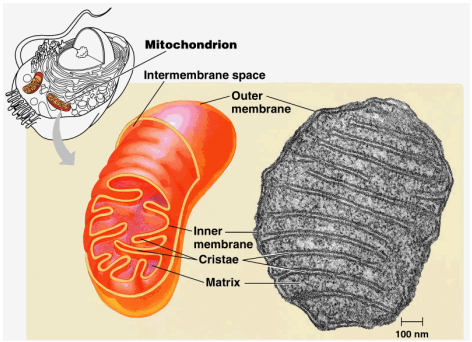
Outline of Lectures

Bioenergetics
mtDNA diseases
ROS-production
Ischaemia/reperfusion damage
Parkinson's disease
Immune response
Granulomatosis

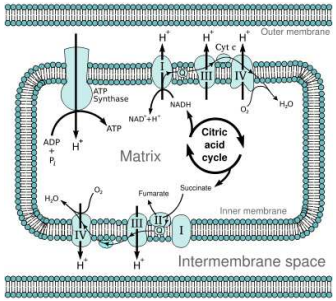
Mitochondria



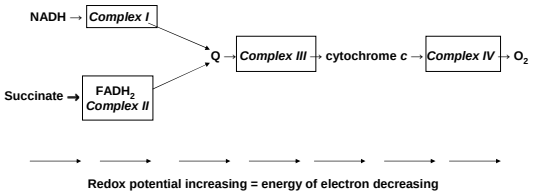
What are mitochondria?

- An intracellular organelle.
- There are 100 to 1000s of mitochondria/cell.
- All mitochondria come from the mother.
- Mitochondria have their own DNA.
- Found in all cell types, except the RBC.
- Major functions of mitochondria:
  - Makes energy in the form of ATP.
  - Programmed cell death (apoptosis).

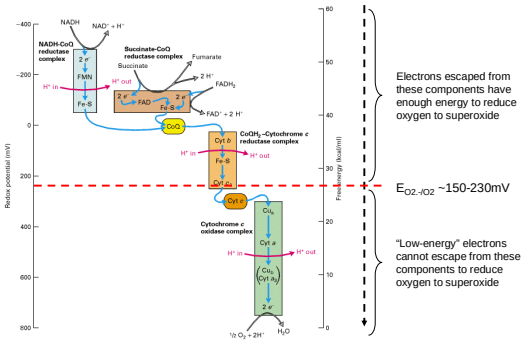
Mitochondrial respiratory chain



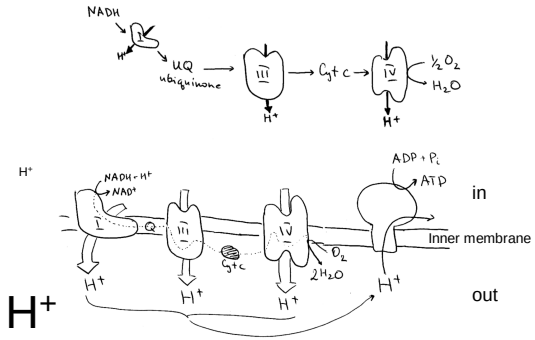
Mitochondrial respiratory chain



Mitochondrial respiratory chain



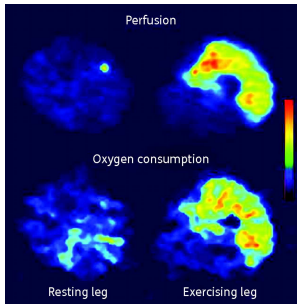
Electron transport chain and ATP synthase



Bioenergetics: Energy

- At rest, the average adult male will need 3.0 x 10^{18} molecules of ATP per second for normal organ functioning.
- The body produces and makes approximately 70 Kg of ATP daily (average adult male).
- The brain uses approximately 70% of all ATP produced.

Examples of parametric perfusion and oxygen uptake PET images during dynamic knee-extension exercise.



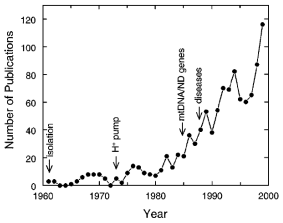
Sutinen J et al. J. Antimicrob. Chemother. 2010;65:1497-1504

Number of Mitochondria per cell

• Most somatic cells	100-10,000
• Lymphocyte	1000
• Oocytes	100,000
• Sperm	few hundred

- No mitochondria in red cells and some terminally differentiated skin cells

History: Disease



Mitochondrial disorders

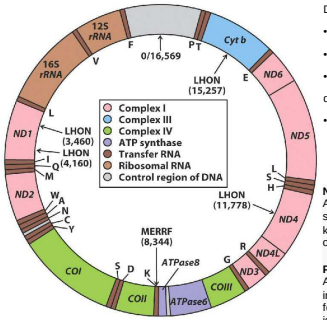
20% are due to mtDNA mutations (200 pathogenic mutations)

80% nuclear DNA mutations

Probably the most common neurometabolic diseases in childhood, Darin (2001)

Incidence of 1:5000 live birth (Smeitink 2006)

Mitochondrial DNA



Double stranded, circular

- No introns, 80 - 93% coding gene
- No repeats
- Lack histone and DNA repair mechanism → damage, mutations (free radicals)
- 37 gene: 22 tRNA, 2 rRNA & 13 protein

NUCLEOID
A dynamic complex that consists of several copies of mitochondrial DNA and key maintenance proteins within the organelle.

POLYCISTRONIC
A form of gene organization that results in transcription of an mRNA that codes for multiple gene products, each of which is independently translated from the mRNA.

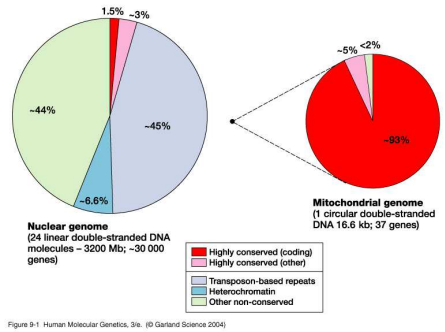
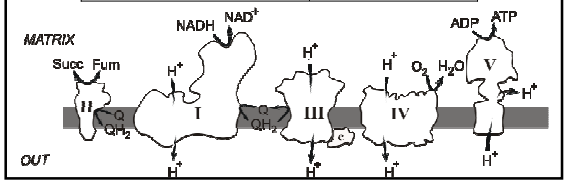


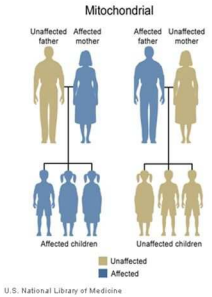
Figure 9-1 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Mitochondrially encoded subunits of respiratory chain enzymes

ENZYME	Genes
NADH:ubiquinone oxidoreductase / (complex I)	MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6
Ubiquinone:cytochrome c oxidoreductase / (complex III)	MT-CYB
cytochrome c oxidase / (complex IV)	MT-CO1, MT-CO2, MT-CO3
ATP synthase / (complex V)	MT-ATP6, MT-ATP8



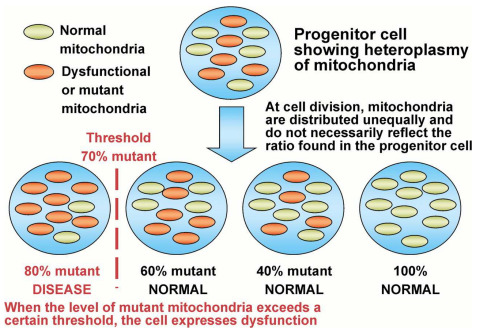
Maternal or mitochondrial inheritance

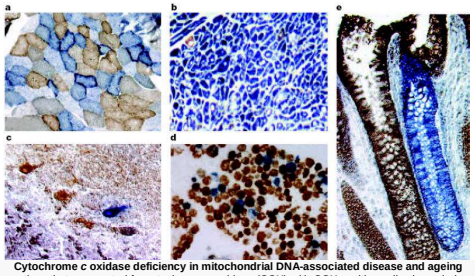


An affected woman transmits the trait to all her children. Affected men do not pass the trait to any of their offspring.

Sperm mitochondria are shed before entry of the sperm nucleus. All mitochondrial in the zygote are contributed by the egg cell.

Heteroplasmy





Cytochrome *c* oxidase deficiency in mitochondrial DNA-associated disease and ageing

Tissue sections that are reacted for cytochrome *c* oxidase (COX) with COX-positive cells shown in brown and COX-deficient cells shown in blue.

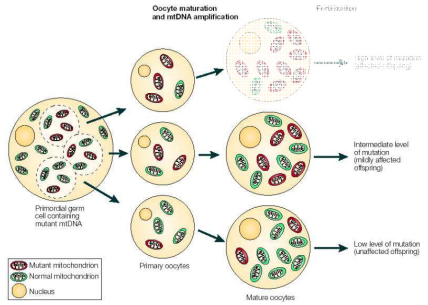
a Skeletal muscle from a patient with a heteroplasmic mitochondrial tRNA point mutation. The section shows a typical 'mosaic' pattern of COX activity

b Cardiac tissue (left ventricle) from a patient with a homoplasmic tRNA mutation that causes hypertrophic cardiomyopathy, which demonstrates an absence of COX in most cells.

c A section of cerebellum from a patient with an mtDNA rearrangement that highlights the presence of COX-deficient neurons.

d,**e** Tissues that show COX deficiency (**d**; extraocular muscles) and rapidly dividing cells (**e**; colonic crypt)

GENETIC BOTTLENECK



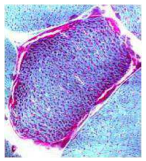
GENETIC BOTTLENECK: A temporary reduction in population size that causes the loss of genetic variation.

History: Disease

- *1962: Luft et al.* (J Clin Invest 1962;41:1776)
  - Described a woman having a hyper-metabolic state, structurally abnormal mitochondria, and abnormalities of oxidative phosphorylation.
- *1963: Nass and Nass* (J Cell Biol 1963;19:593)
  - Described mitochondrial DNA.

History

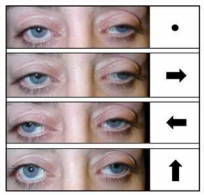
- *1963: Engle and Cunningham (Neurology 1963;13:919)*
  - Described ragged red fibers - clumps of diseased mitochondria accumulate in the subsarcolemmal region of the muscle fiber. They appear as "Ragged Red Fibers" when muscle is stained with modified Gömöri trichrome stain



Ophthalmoplegia

1988: First description of mitochondria DNA mutations, insertion-deletions and base substitutions, causing disease.

Kearns-Sayre/Chronic progressive external ophthalmoplegia (*Holt et al., Nature 1988;331:717*).
Leber's Heredity Optic Neuritis (*Wallace et al., Science 1988;242:1427*).



Some diseases associated with mitochondrial mutations

MERRF = Myoclonic Epilepsy with Ragged Red Fibres

MELAS = Myopathy, Epilepsy Lactic acidosis, Stroke-like episodes

LHON = Leber's Hereditary Optic atrophy

Kearn-Sayre syndrome (eye problems, heart block, ataxia i.e. loss of coordination)

Leigh syndrome (rare severe brain disease in infancy, heart problems)

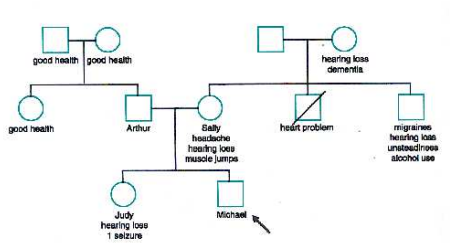
Investigations in clinic

Blood/CSF/Urine

1. Elevated CSF (fasting > 1.5 mmol/L) and blood lactate (fasting > 3 mmol/L /lactic acidosis
2. Elevated lactate/pyruvate ratio
3. Others (blood CK, myoglobinuria, blood/CSF alanine)
4. Urine organic acid (ethylmalonic aciduria, tricarbon excretion)

Imaging

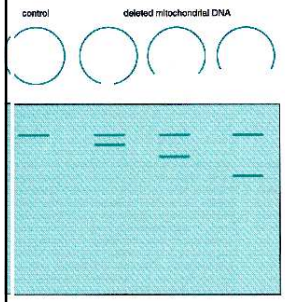
1. MRI/CT scan brain (abnormal signal or calcification in the basal ganglia; brain atrophy; bliateral striatal necrosis, cerebellar hypoplasia; infarct
2. MRS – metabolic alteration in the basal ganglia



Michael presented with muscle problems, epilepsy, lack of progress at school, difficulty with vision and hearing.

Diagnosed as MERRF aged 12 after muscle biopsy. At postition 8344 he has a change from A-G in most of the mitochondrial DNA from muscle and lymphocytes.The other relatives have different proportions of the same mutation, which is in the tRNA for lysine (*MT-TK*)

Diagnosis of deletions



Deletions of mitochondrial DNA in muscle biopsies from individuals with Kearns-Sayre syndrome. DNA was digested with restrictase, which cuts the mitochondrial genome at one site, resulting in a 16.5-kb fragments that is detected on a gel. Each individual with the syndrome has two populations of mitochondrial DNA: one of normal size and one of smaller size form

Zeiani M, Moraes CT DiMauro S et al. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. Neurology 1988; 38: 1339-1346)

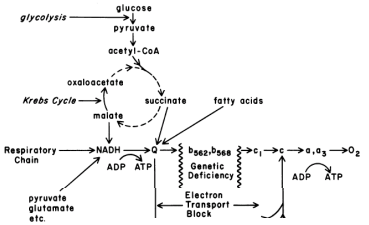
Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3529–3533, June 1984
Medical Sciences

^{31}P NMR study of improvement in oxidative phosphorylation by vitamins K_3 and C in a patient with a defect in electron transport at complex III in skeletal muscle

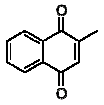
(metabolic diseases/exercise physiology/lactic acidosis/hypoxia/genetic deficiency)

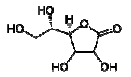
S. Eleff*†, N. G. Kennaway‡, N. R. M. Buist‡§, V. M. Darley-Usmar¶, R. A. Capaldi¶, W. J. Bank||, and B. Chance†

Case Report. The patient is a 17-year-old girl with an 8-year history of progressive muscle weakness associated with a ragged-red fiber myopathy and lactic acidosis.

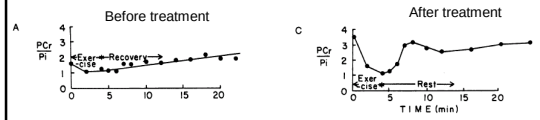


10 mg menadione and
1g Vit C every 6 h





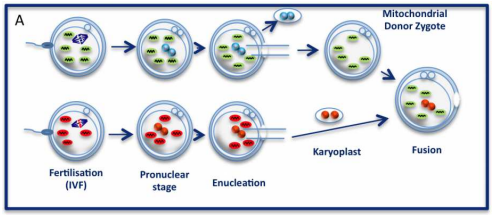
Response to Therapy



Subjective Response to Therapy. In the year prior to NMR testing, our patient's tolerance to exercise was severely compromised. She could walk only half a block or climb 5–10 steps without resting, and she frequently used a wheelchair. Exercising on the ergometer was fatiguing and she felt "tired." Within 24 hr of starting redox therapy, there was a marked contrast, the patient claiming to have "more energy." Within 2 days she ceased using her wheelchair, walking several blocks without stopping and no longer complaining of fatigue. Over the next few months, this improvement has been maintained; she can walk two blocks without tiring, can climb 30–40 steps, and has not used her wheelchair at all.

Therapy: Pronuclear Transfer

1 + 1 + 0.00001 ≠ 3



Any fertilised egg reaches a point where the nuclear DNA from both the sperm and the egg has formed two pronuclei that are visible under a normal light microscope. The pronuclei containing nuclear DNA from both parents can be taken from the fertilised egg and placed in a donated egg which has had its pronuclei removed. The donated egg with its healthy mitochondria and replaced nuclear DNA is then implanted in the mother as per standard IVF procedures (or mtDNA analysis).

Reactive oxygen intermediates

Reactive oxygen intermediates

Reactive oxygen species

Free radicals

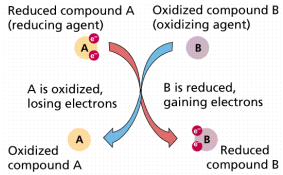
Oxyradicals

Superoxide radical	$O_2^{\cdot -}$
Hydrogen peroxide	H_2O_2
Hydroxyl radical	OH
Peroxynitrite	$ONOO^-$
Hypoclorite	HClO
Singlet oxygen	$^1O_2$

not a radical

Redox reactions

(reduction-oxidation reactions)



$A_{red} \rightarrow A_{ox} + 2e^-$

$B_{ox} + 2e^- \rightarrow B_{red}$

$A_{red} + B_{ox} \rightarrow A_{ox} + B_{red}$

Electrons transferred from A to B if

$E^o_{A=/A^{red}} < E^o_{B=/B^{red}}$

Reduction of oxygen

$CO_2 + H_2O \overset{+h\nu}{\leftrightarrow} \{CH_2O\} + O_2$

Photosynthetic organisms

Capture of solar energy to use it for reduction of carbon compounds

$\{CH_2O\} + O_2 \leftrightarrow CO_2 + H_2O + energy$

Animals

Oxidation of food to obtain energy

Animals

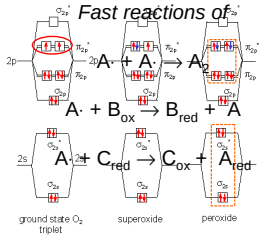
$O_2 + 4e^- + 4H^+ \leftrightarrow 2H_2O$

Plants

What is radical?

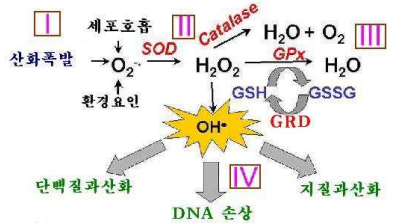
Free radical – molecule containing unpaired electron(s)

Oxygen is diradical – contains two unpaired electrons



Reactive oxygen intermediates

O_2	Oxygen
Energy transfer O_2 + energy → $^1O_2$	Singlet oxygen
One electron reduction of molecular oxygen $O_2 + 1e^- \rightarrow O_2^-$	Superoxide radical
SOD - Dismutation of superoxide radical $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2$	Hydrogen peroxide
Transition metal catalysed reactions (Fenton reaction) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$	Hydroxyl radicals
Reaction with nitric oxide (k~ 6.7×10^9) $O_2^- + NO \rightarrow ONOO^-$	Peroxynitrite
Myeloperoxidase reaction $H_2O_2 + Cl^- + H^+ \rightarrow H_2O + HClO$	Hypochlorite



Reduction of oxygen and superoxide

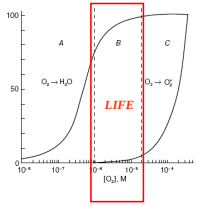
More than 99% of oxygen in body is metabolised by cytochrome c oxidase = mitochondrial Complex IV

Enzymatic reaction

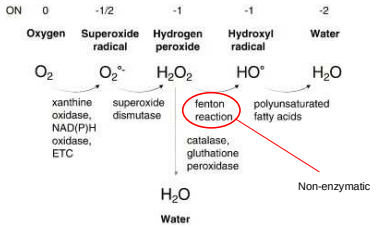
$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ four electron reduction $E^0_{H2O/O2}$ ~ +800mV

Non enzymatic "leak"

$O_2 + 1e^- \rightarrow O_2^-$ one electron reduction $E^0_{O2\cdot-/O2}$ ~-160mV



Reactive oxygen intermediates



Consecutive reduction of dioxygen yields reactive oxygen species. The conversion of dioxygen to superoxide requires energy. The following steps are exothermic.

Stability of reactive intermediates

Singlet oxygen	$^1O_2$	microseconds
Superoxide	O_2^-	seconds
Hydrogen Peroxide	H_2O_2	days
Hydroxyl radicals	$\cdot OH$	nanoseconds
Peroxynitrite	$ONOO^-$	~0.1 sec at pH 7

Superoxide is not membrane-permeable unless specific anion transport systems are present

Hydrogen peroxide is a membrane-permeable molecule

Discovery of superoxide radical and SOD

A blue copper protein was isolated in 1938 by Mann and Keilin from erythrocytes and liver.

Ubiquitous in animal tissues: hemocuprein, erythrocuprein, cerbrocuprein, hepatocuprein and cytocuprein

It began with the observation that inhibition of reduction of cytochrome *c* in xanthine oxidase reaction by a tissue extract is dependent on oxygen.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 243, No. 21, November 10, 1968
Printed in U.S.A.

The Reduction of Cytochrome *c* by Milk Xanthine Oxidase[*]

(Received for publication, June 14, 1968)

JOE M. MCCORD[‡] AND IRWIN FRIDOVICH[§]

cyt c^{3+} + hypoxanthine → cyt c^{2+} + xanthine

O_2 + hypoxanthine → $O_2^{\cdot-}$ + xanthine

$O_2^{\cdot-}$ + cyt c^{Fe3+} → cyt c^{Fe2+} + O_2

Superoxide dismutase: $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 \Rightarrow$ inhibition of cytochrome *c* reduction

Sources of superoxide

•Enzymatic (mitochondrial enzymes, microsomes (P450), NADPH oxidases, xanthine oxidase, etc.)

•Toxic compounds (paraquat, sulfa drugs, antimalarial drugs)
– these can be called "pro-oxidants"
•*Affecting* enzyme systems (i.e. activating the production of superoxide)
•Chemical reaction to *create* ROI (Fe^{2+}, paraquat)

•Ionising radiation

ROI production by mitochondria

FIRST OBSERVATION:

Biochemical Journal, Vol. 38, 1944

The Oxidative Metabolism of *Ascaris suis*

BY H. LASER, *The Molteno Institute, University of Cambridge*

(*Received 5 June* 1944)

Formation of H_2O_2 in the whole worm. **The fact that the raising of the oxidation rate of muscle pulp by the addition of M.B., or by an increase of the O_2 tension, caused the formation of H_2O_2, seemed to offer an explanation for the observation that worms die fairly quickly when brought into an atmosphere of pure O_2.**

Biochim. Biophys. Acta, 122 (1966) 157–166

ANTIMYCIN-INSENSITIVE OXIDATION OF SUCCINATE AND REDUCED NICOTINAMIDE–ADENINE DINUCLEOTIDE IN ELECTRON-TRANSPORT PARTICLES

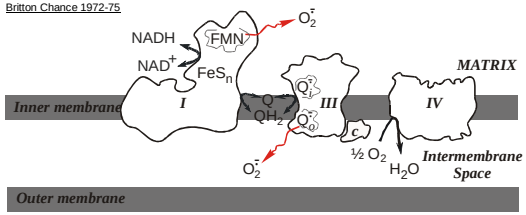
I. pH DEPENDENCY AND HYDROGEN PEROXIDE FORMATION

P. K. JENSEN

(Received December 7th, 1965)

Complex I and III are the main sources of superoxide production in respiratory chain

Britton Chance 1972-75

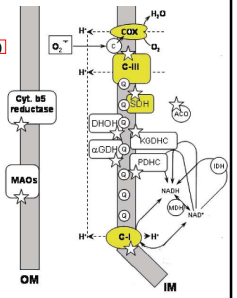


Additional sites of superoxide production in mitochondria

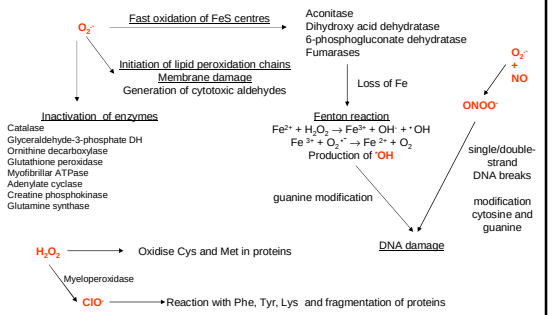
Inner membrane: **Complex II, aka Succinate dehydrogenase (SDH)**
Dihydroorotate dehydrogenase (DHOH)
α-Ketoglutarate dehydrogenase complex (KGDHC)
Dehydrogenase of α-glycerophosphate (αGDH)

Outer membrane: **Cytochrome *b*5 reductase**
Monoamine oxidases (MAOs)

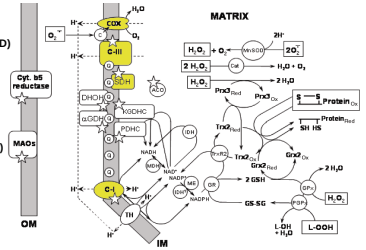
Matrix: **Aconitase (Aco)**



What is the damage?



ROS detoxification



Enzymatic:

Superoxide dismutase (MnSOD)
Catalase (Cat)

Glutathione (GSH/GS-SG)
+Glutathione reductase (GR)
+Glutathione peroxidase (GPx)
+Phospholipid hydroperoxide glutathione peroxidase (PGPx)

Glutaredoxin2 (Grx2)

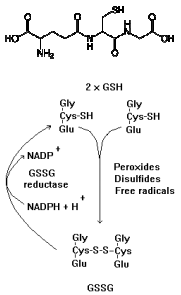
Peroxiredoxins (Prx3) and other oxins.

thioredoxin2 (Trx2)

Thioredoxin2 reductase (TrxR2)

Non enzymatic:
α-tocopherol
ascorbic acid
uric acid
cytochrome c

Glutathione system



Mitochondria contain ~10% of total GSH in a cell but, due to the relatively small volume of the matrix, the concentration of GSH in mitochondrial matrix is higher than in the cytoplasm

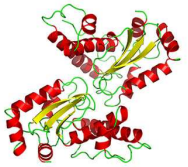
Glutathione in matrix ~ 2-14 mM

GSH/GSSG~10

If GSH/GSSG ratio falls it is indication of oxidative stress

Superoxide dismutase

Fridovich & McCord 1969



Reaction:

$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2$ Rate - 10^9 M^{-1} s^{-1}

SOD1=Cu-Zn-SOD cytoplasmic - dimer 2x16 kDa
SOD2=Mn-SOD mitochondrial - tetramer 4x22.2 kDa
SOD3=Cu-Zn SOD extracellular - tetramer 4x33.8kDa

Redox centres - metal atoms

Knock outs:

SOD2 $^{-/-}$ - neonatal death
SOD1 $^{-/-}$ or SOD3 $^{-/-}$ - no acute phenotype

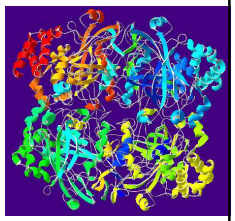
Catalase

Louis Jacques Thénard 1811

Discovery of H_2O_2 'eau oxygene'

Reaction:

$2H_2O_2 \rightarrow 2H_2O + O_2$ Rate - 10^9 M^{-1} s^{-1}



Tetramer 4x500kDa
Redox centres – haem group

Acatalasemia - total loss of catalase activity in RBC – only lesions in oral cavities!!!

Original data: are mitochondria the main source of ROS?

Biochem. J. (1972) **128**, 617–630
Printed in Great Britain

The Cellular Production of Hydrogen Peroxide

By ALBERTO BOVERIS,* NOZOMU OSHINO and BRITTON CHANCE
Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19104, U.S.A.

In liver homogenate:

•H_2O_2 Production: 90 nmol of H_2O_2/min per g of liver
~1% of total O_2 consumption

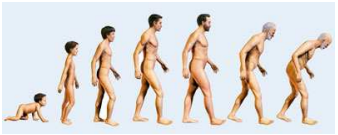
Microsomes -	***50%***
Peroxisomes -	***35%***
Mitochondria -	***10-15%***
Cytosol -	***5%***

H_2O_2 generation – H_2O_2 removal = H_2O_2 emission

How much ROS escapes from the detoxification?

Why mitochondria kill us at the end?

Mitochondrial free radicals theory of ageing



Mitochondria make ROS as a byproduct of enery metabolism
⇓
ROS damage mitochondrial DNA
⇓
More mutations occure
⇓
Proteins encoded in mtDNA cannot perform their functions anymore
⇓
Mitochondria cannot produce enough energy
⇓
Energy crisis and DEATH ✞

Ugly exception: Naked rat mole

Naked mole rats live ~30 years (not 3-4 years as other rodents)

They are mammals but their temperature is not constant

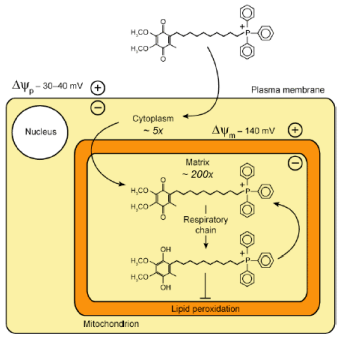
Has only 100 hairs

Underground colonies are organized like an insect community around a single breeding queen and workers and soldiers.

Ugly exception: Naked rat mole

Will antioxidant therapy help?

MitoQ therapy



What else?

<u>Neutrophil function</u>

•Sterilization of microbes

•Generation of signals that attract more neutrophils

•Induction of a macrophage-based programme that switches the state of damaged epithelium from pro-inflammatory and non-replicative to anti-inflammatory and replicative.

Increased pain sensitivity (hyperalgesia)

Recent studies:

Radical scavengers or SOD mimetics attenuate hyperalgesia

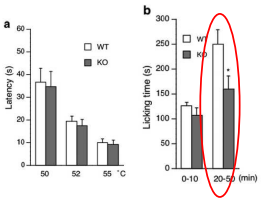
Inactivation of SOD increases pain reception

Increased pain sensitivity (hyperalgesia)

The Journal of Neuroscience, September 17, 2008 • 28(38):9486–9494

Reactive Oxygen Species Derived from NOX1/NADPH Oxidase Enhance Inflammatory Pain

Masakazu Ibi,[1] Kuniharu Matsuno,[1] Dai Shiba,[2] Masato Katsuyama,[1] Kazumi Iwata,[1] Tomoko Kakehi,[1] Takayuki Nakagawa,[3] Kazunori Sango,[4] Yasuhito Shirai,[5] Takahiko Yokoyama,[2] Shuji Kaneko,[3] Naoaki Saito,[5] and Chihiro Yabe-Nishimura[1]



(**a**) No difference in response to thermal stimuli as measured by tail flick

(**b**) Formalin injection to induce pain response measured as licking time
Phase 1 – direct stimulation of pain receptors
Phase 2 – induced inflammation elicits pain response

ROS from NADPH-oxidase increase pain via protein kinase c dependent pathway in dorsal root ganglia neurons.

Primary effect of ROS is cysteine residue oxidation in something (???)

ROI and blood clotting

tissue factor ⇒ activation of NOX in smooth muscle

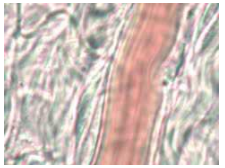
⇓

ROI generation

Platelet aggregation is abolished by catalase

⇓

Collagen-induced platelet aggregation is associated with a burst of H_2O_2 – it acts like a second messenger via arachidonic acid and phospholipase pathways



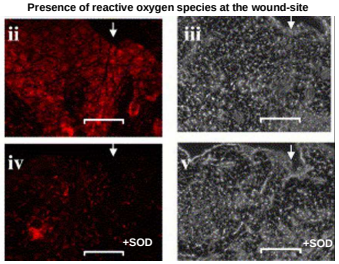
μM H_2O_2 concentration induces neutrophil chemotaxis

ROI control reaction of macrophages and neutrophils to many growth factors, etc

ROI and wound healing

Sashwati et al., *Mol.Ther.* 2006, 13, 211-220

Presence of reactive oxygen species at the wound-site



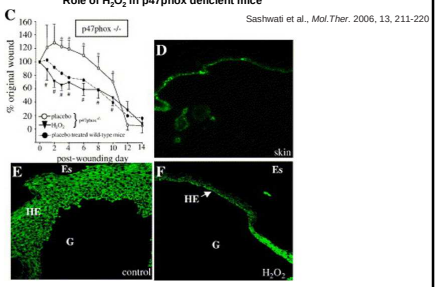
ROS production Granulation of the tissue

Healing is better in absence of SOD

ROI and wound healing

Role of H_2O_2 in p47phox deficient mice

Sashwati et al., *Mol.Ther.* 2006, 13, 211-220

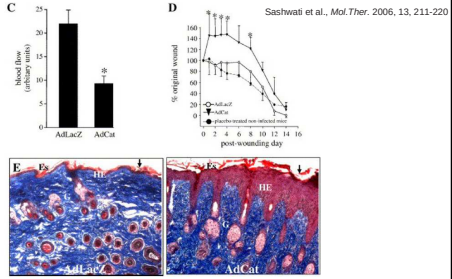


Higher expression of keratin 14 in control side (E) compared to H_2O_2-treated side (F) indicating healing is ongoing on the control side, while H_2O_2 treated side shows keratin 14 expression comparable to normal skin (D)

ROI and wound healing

Catalase over-expression impairs healing

Sashwati et al., *Mol.Ther.* 2006, 13, 211-220



Masson trichrome staining sections of regenerated skin at the wound-site. AdCat side (right) shows broader HE region indicative of incomplete (vs. control on left) regeneration of skin, consistent with slower closure. The wound-edge is marked with an arrow. Es, eschar; G, granulation tissue; HE, hyperproliferative epithelium.

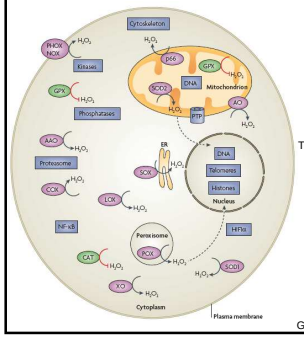
ROI and wound healing

NOX1 induces angiogenic switch - H_2O_2 signalling in wound:

- induction of matrix metalloprotease (MMP) - formation of actin filaments=scaffold
- induction of VEGF vascular endothelial growth factor
- chemotaxis

H_2O_2 signalling - new direction of research

H_2O_2 in cell

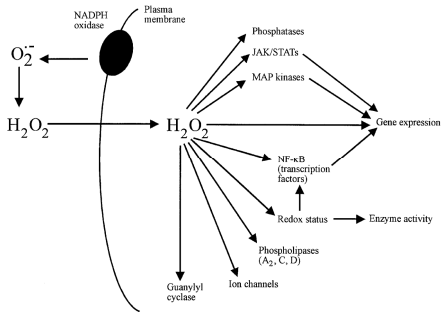


SOURCES: phagocytic oxidases (PHOX)
NADPH oxidases (NOX)
superoxide dismutases (SOD2)
Mitochondrial $p66^{Shc}$ (p66)
amine oxidase (AO)
peroxisomal oxidases (POX)
sulphydryl oxidase (SOX)
amino-acid oxidases (AAO)
cyclooxygenease (COX)
lipid oxygenase (LOX)
xanthine oxidase (XO)
superoxide dismutases (SOD1)

TARGETS: actin,
myosin,
tubulin (cytoskeleton),
different kinases,
several Ser/Thr and Tyr phosphatases,
proteasome,
mitochondrial permeability transition pore
mitochondrial and nuclear DNA,
transcription factors such as HIF1α or
nuclear factor (NF)-κB
histones and telomeres

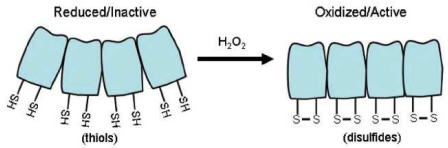
Giorgio et al., 2007, Nat.Mol.Cell.Biol.Rev., Vol. 8, 722

H_2O_2 signalling



Hancock, Desikan, Neill Biochem. Soc. Trans. (2001) 29, 345–350

Primary mechanism of H_2O_2 signalling

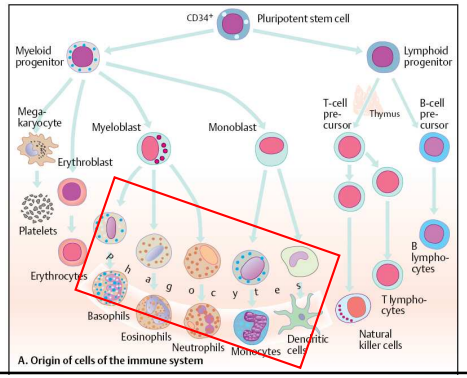


Conclusions

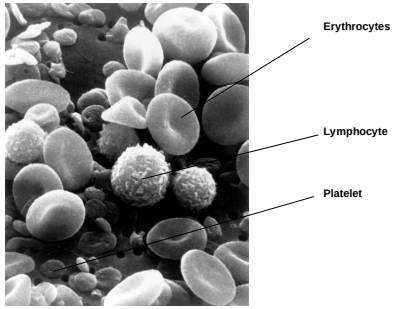
1. ROI provide primary immune response to infectious agents by rapid destruction of microbes and infected host cells.
2. ROI provide propagation of the signal and activation of the adaptive immune response via antigen-presenting cells
3. ROI may initiate a programme that switches to anti-inflammatory and replicative mode – wound healing.
4. ROI can play a role in established signal transduction pathways inside the cell.
5. ROI are not alone – there are reactive nitrogen species too.

If nature gives you lemons, make lemonade

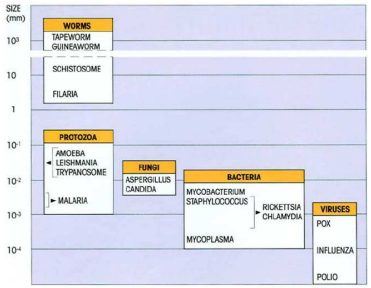
Origin of cells of the immune system



Blood cells



Infectious agents which confronts the immune system



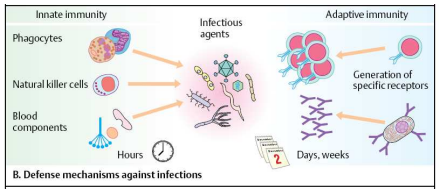
Specificity

Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci U S A.* 2000 97:8841-8848.

"A downside of highly specific recognition as a pillar of the immune response is that a microbe can sometimes escape recognition by altering a molecular feature that flags it, such as the order of monomers in its polymers. The advantage of using ROI for defence is that a microbe cannot readily evade them by dispensing with their targets, because the targets are atomic rather than macromolecular."

"The infectious challenges faced by the immune system are so diverse and dire that they can only be met by a response in which collateral damage occurs as a matter of course"

Specificity and time



What triggers the immune response?

Danger theory

A theory that the trigger for mounting an immune response consists of an injury to host cells, resulting in the release of alarm signals that activate antigen-presenting cells.

Pattern-recognition theory

A theory that the trigger for mounting an immune response consists of the recognition of "microbial non-self" molecules by receptors expressed by innate immune cells.

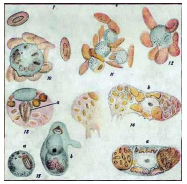
Ongoing detection of signals that report injury and signals that report infection

The integration of signals of two or more distinct classes derived directly or indirectly from tissue damage and the presence of a genome different from the host

Propagation and activation and programming of antigen-presenting cells

Phagocytosis

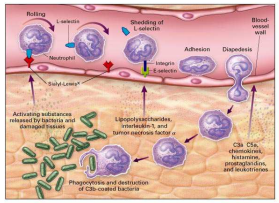
From L'Immunité dans dans les Maladies Infectieuses
(Immunity in infectious diseases) 1901



In l883, he observed that fungal spores can be attacked by the blood cells of Daphnia

Elie Metchnikoff

Activation of neutrophils



Delves PJ, Roitt IM. The immune system. First of two parts. *N. Engl. J. Med.* 2000, 343(1):37-49.

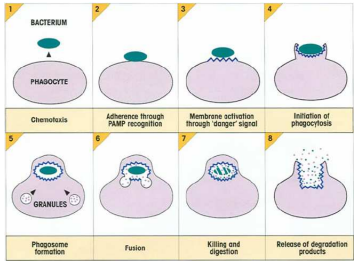
Rolling of Neutrophil

~~The Hunt for Red October~~

Neutrophil chasing a bacterium

Video by David Rogers at Vanderbilt University 1950s.

Phagocytosis



Neutrophils that sense tissue damage but fail to catch a bacterium release everything into the extracellular space (15-45 min)

Phagocytosis

Respiration burst

Am. J. Physiol. 1932, 103: 235-236

THE EXTRA RESPIRATION OF PHAGOCYTOSIS

C. W. BALDRIDGE AND R. W. GERARD

From the Department of Physiology, University of Chicago

April 14, 1932. Two-tenths cubic centimeter dog leucocyte paste in 0.6 cc. dog serum; 0.1 cc. N/10 NaOH in inset; onset, 0.2 cc. Ringer, plus $\frac{1}{10}$ ink or sarcina suspension.

TIME IN MINUTES	OXYGEN CONSUMPTION OF LEUCOCYTES, IN PER CENT INITIAL VALUE		
70	100	100	100
After tipping	India ink	Sarcina in saline	Saline
15	93	423	154
45	95	128	104
105	100	81	88
165	75	78	98

Superoxide production during respiration burst

NATURE November 11, 1961 Vol. 192

BIOCHEMICAL ASPECTS OF PHAGOCYTOSIS

By Dr. G. Y. N. IYER, D. M. F. ISLAM and Prof. J. H. QUASTEL, F.R.S.

In the consideration of the various factors that may operate in bringing about bactericidal action during phagocytosis, the possibility that hydrogen peroxide is formed during this process must be taken into account.

The Journal of Clinical Investigation Volume 52 March 1973

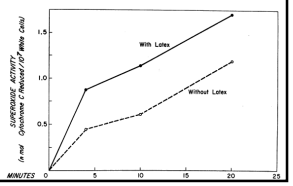
Biological Defense Mechanisms

THE PRODUCTION BY LEUKOCYTES OF SUPEROXIDE, A POTENTIAL BACTERICIDAL AGENT

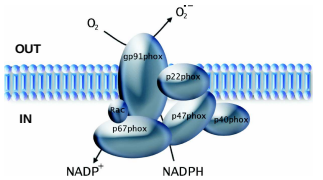
Bernard M. Babior, Ruby S. Kipnes, and John T. Curnutte

From the Thorndike Memorial Laboratory, Harvard Medical Unit, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02118

Superoxide is the initial product during respiratory burst



NADPH oxidase (phox)



Six subunits

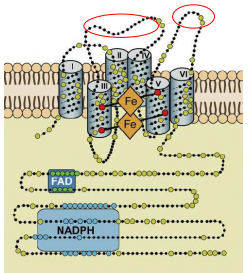
K_m^{NADPH} ~ 40 µM

K_m^{NADH} ~ 2.5 mM

$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^{\cdot-}$

Highest production in polymorphonuclear leukocytes
½ in macrophages

gp91phox (NOX2)



gp91phox = NOX2

FAD

Two haem cytochrome b_{558} ~ -245mV

4-6 transmembrane domains

N and C-terminus are facing cytoplasm

Mature protein ~70-90kDa

⇒ Highly glycosylated

After glycosidase ~55kDa

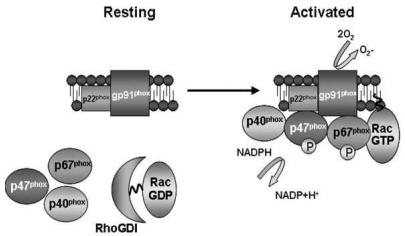
Carbohydrates attached to Asn residue

gp91phox is unstable in absence of p22phox

Localised in specific granules

Activation of NADPH oxidase

Wilkinson *et al. Journal of Neuroinflammation* 2006 **3**:30



Activation of the phagocytic NADPH oxidase complex. Stimulation of the phagocyte induces the parallel activation of oxidase components within the cytoplasmic vesicles. This activation causes the conversion of Rac into an active GTP-bound form and the phosphorylation of $p47^{phox}$ and $p67^{phox}$. These subunits then translocate to the membrane where they interact with $p22^{phox}$ and $gp91^{phox}$ to initiate reactive oxygen production. During activation vesicles fuse with the membrane.

Myeloperoxidase

Haem-containing protein

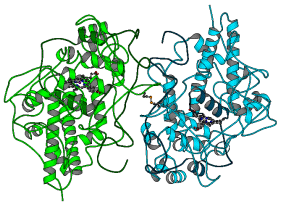
Stored in azurophilic granules

Tetramer (150 kDa) composed of 2 light chains and 2 heavy chains.

Produced as a single chain precursor and subsequently cleaved into a light and heavy chain.

1-5 % of dry weight of the cells

Very basic protein pI~10 – coat pyogenic bacteria



$H_2O_2 + Cl^- + H^+ \rightarrow H_2O + HClO$

$HClO \leftrightarrow H^+ + OCl^-$ (pK~7.53)

at low pH:
$Cl^- + HClO \rightarrow OH + Cl_2$

Nitric oxide synthase (iNOS)

L-Arginine $+2O_2$ $+3/2NADPH + 3/2H^+ \leftrightarrow$ Citrulline $+2H_2O + 3/2NADP+NO$

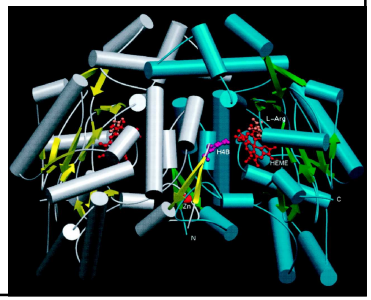
De novo transcription-biosynthesis

Induction by interferon-γ

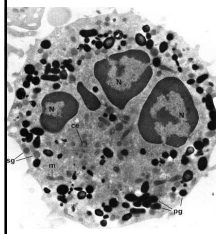
Redox centres:
FAD and two FMN
Haem
Tetrahydrobiopterin

NO is membrane-permeable

In macrophages more than in neutrophils



Location in neutrophil



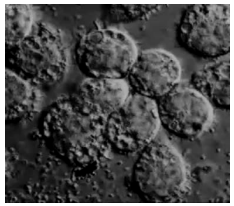
At least two types of granules

Azurophil granules (primary)	Specific granules (secondary)
0.5 µm 1500 per cell	0.2 µm 3000 per cell
Lysozyme – breaks cell wall Myeloperoxidase - hypoclorite Defensins – pore forming Serporocidins - protease BPI – increases permeability	Lysozyme - breaks cell wall NADPH-oxidase - superoxide Alkaline phosphatase Lactoferrin – iron binding Transcobalamin - binds Vit B_{12}

Work inside phagocytosis of particles

Work outside exocytosis

pH in the phagosome

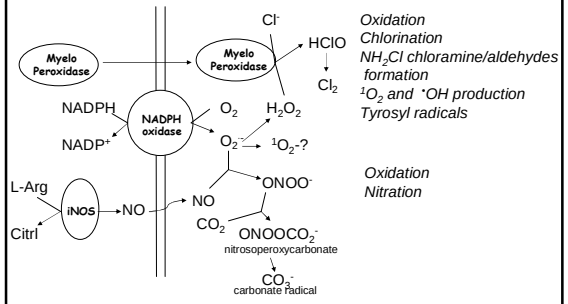


Intraphagosomal pH monitored with pH-sensitive fluorescent pHRODO dye

Rise (7.5-7.8, minutes) and fall (5.0-7.0, hours)

What could be the mechanisms and purpose for acidification?

Neutrophil antimicrobal system



Oxidation
Chlorination
NH_2Cl chloramine/aldehydes formation
$^1O_2$ and $^{\bullet}OH$ production
Tyrosyl radicals

Oxidation
Nitration

Cytoplasm

Intraphagosomal or extracellular space

Neutrophils and macrophages

Polymorphonuclear neutrophils – highest production of ROI – kill pyogenic bacteria and 30-70% of H_2O_2 is used for HClO formation. Short lifetime: 12 h - 2 days.

Macrophages (mononuclear phagocytes) 1/3 –1/2 of ROI - combat bacteria, protozoa or viruses living in the host. Long lifetime: months.

Macrophages produce more RNI than neutrophils

Table 1. Antimicrobial products of human phagocytes that are delivered to phagosomes

Product	Neutrophils*	Macrophages†
ROI	+	+
RNI	+	+
Myeloperoxidase	+	–
Lactoferrin	+	–
Bacterial permeability increasing factor	+	–
Serprocidins (elastase, cathepsin G, protease 3, azurocidin)	+	–
Phospholipase A2	+	–
Cathelicidin	+	–
Lysozyme	+	–
Defensins (HNPs) 1, 2, 3, 4	+	–

Nathan C, Shiloh MU. *Proc Natl Acad Sci U S A.* 2000 97:8841-8848.

Non-phagocytic oxidase

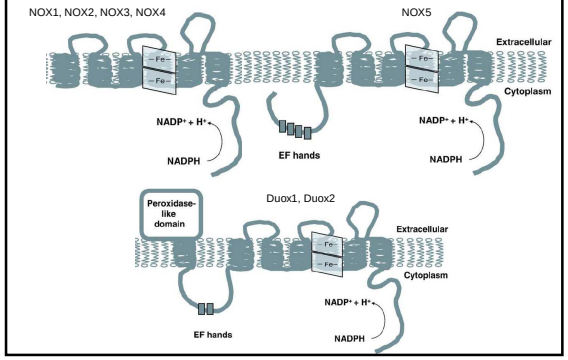
Enzyme systems similar to the phagocyte NADPH oxidase exist in many other cells.
Indication - ROS generation by fibroblasts in gp91phox deficient patients

TABLE 2. *Tissue distribution of NOX enzymes*

Bedard&Krause 2007, *Physiol.Rev.*, 85:245-313

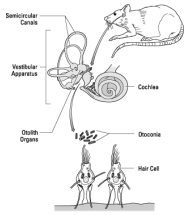
	High-Level Expression	Intermediate- to Low-Level Expression
NOX1	Colon	Smooth muscle, endothelium, uterus, placenta, prostate, osteoclasts, retinal pericytes
NOX2	Phagocytes	B lymphocytes, neurons, cardiomyocytes, skeletal muscle, hepatocytes, endothelium, hematopoietic stem cells, smooth muscle
NOX3	Inner ear	Fetal kidney, fetal spleen, skull bone, brain
NOX4	Kidney, blood vessels	Osteoclasts, endothelium, smooth muscle, hematopoietic stem cells, fibroblasts, keratinocytes, melanoma cells, neurons
NOX5	Lymphoid tissue, testis	Endothelium, smooth muscle, pancreas, placenta, ovary, uterus, stomach, various fetal tissues
DUOX1	Thyroid	Airway epithelia, tongue epithelium, cerebellum, testis
DUOX2	Thyroid	Salivary and rectal glands, gastrointestinal epithelia, airway epithelia, uterus, gall bladder, pancreatic islets

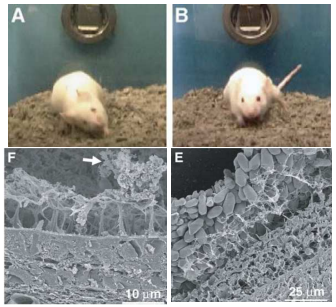
Non-phagocytic oxidase



NOX3 deficient mice (head-slant mice)

Kiss et al., 2005, *Curr.Biol.*, 16:208-213





Nox3-derived ROS cross-link extracellular proteins through the formation of disulfide bridges, leading to the formation of a protein precipitate, which later serves as nucleus in the calcification of otoconia.

??

Functions of NOXs

NOX1 – unknown – microbicidal activity in colon?

NOX2 – neutrophils and macrophages phagocytosis

NOX3 – vestibular function
originally was not detected in adult tissues

NOX4 – unknown – (aka Renox)
major source of ROS in endothelial cells

NOX5 – unknown – EF hand = calcium dependence
found in lymphocyte ⇒ lymphocyte signalling ??
in developing spermatocytes

Thyroid oxidases= NOX + peroxidase domain without haem + EF-hand

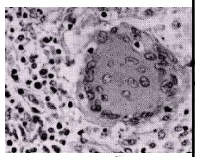
Duox1
Duox2 – mutations ⇒ hypothyroidism ⇒ hormone biosynthesis
Inhibition of Duox ⇒ impaired ability to eliminate bacteria

Chronic granulomatous disease

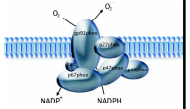
1957- gingivitis, swollen lymph nodes and nonmalignant granulomas

tumour-like mass consisting of a central area of activated macrophages surrounded by activated lymphocytes
+
severe, repeated infections of Gram-negative, *Staphylococcus*, fungi

Infancy ⇒ early death 1 in 200 000
1/3 of the deaths are caused by *Aspergillus* infection



1967-microbicidal defect in neutrophils found in CGD patients
Absence of respiratory burst



Defects in NADPH oxidase:

gp91phox (NOX2)	60% (generally – absence of flavocytochrome)
p47phox	30%
p67 phox	5%
p22phox	5%
Ras2	1 case

gp91phox (NOX2) in X-chromosome ⇒ males are affected

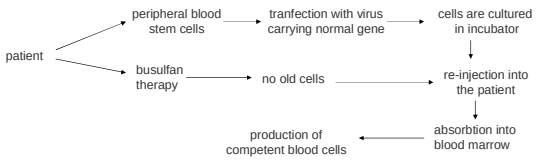
But normal immune response to *Pneumococcus* !

\+ Defects in NADPH synthesis:
Glucose-6-phosphate dehydrogenase (G6PD) deficiency - very rare

Chronic granulomatous disease treatment

Antibiotics trimethoprim-sulfamethoxazole
Fungicide itraconazole, voriconazole
Interferon-γ = Actimmune® - increase NADPH oxidase level in neutrophils ???
activation of different NADPH oxidase in monocytes ???

Gene therapy



http://www.cgd.org.uk

Gene knock-out approach

Classical approach: Phenotype = the function of a gene product = nonredundancy.

redundancy ≠ dispensability, especially for genes competing with other genomes

Many null mutations do not yield phenotypes because the range of conditions tested is narrow; only 10-20 pathogens tested but in reality is different

- redundant only against certain pathogens (CGD and P*neumococcus*)
- antimicrobial mechanisms work synergistically (in phagosome: $O_2^{\bullet-}$, H_2O_2, HClO, NO, lactoferrin (Fe), serprocidins (proteins), phospholipase (membrane), lysozyme (cell wall), defensins (channels), cathelicidin (cationic)

Redundancy and synergy are essential features of the immune system

Knock-outs

Knock out mice

	susceptible	worse in WT
gp91^{phox-}	*Salmonella typhimurium* *Aspergillus fumigatus* *Staphylococcus aureus*	surgically-induced brain injury edema
MPO^{-}	*Candida albicans*	
iNOS^{-}	*Mycobacterium tuberculosis* *Leishmania* Ectromelia virus Coxsackie B3 virus	*Mycobacterium avium* Influenza A virus

gp91^{phox-} /iNOS^{-} die of spontaneous infection unless grown in sterile conditions+antibiotics

Human deficiencies

gp91^{phox-} CGD

MPO^{-} no phenotype, unless in combination with diabetes mellitus

iNOS^{-} not known

Relevance of mice experiments to humans:

The same stimuli induce iNOS in mice but fail to do so in human macrophages, depending on many conditions like location (blood or tissue), way of culturing, healthy or infected donor.

ROI and RNI

Table 4. Parallels between ROI and RNI

Feature	ROI	RNI
Primary catalyst	Multisubunit flavocytochrome	Multisubunit flavocytochrome
Substrates	O_2, NADPH	O_2, NADPH, L-arginine
Primary product	Inorganic radical (O_2^-)	Inorganic radical ('NO)
Actions at low levels	Activate or inhibit receptors, enzymes, transcription factors	Activate or inhibit receptors, enzymes, transcription factors
Actions at high levels	Cause mutagenesis, apoptosis, necrosis	Cause mutagenesis, apoptosis, necrosis
Basis of cellular resistance	SODs; catalase; peroxiredoxins; redox cycles involving glutathione, thioredoxin, glutaredoxin, trypanothione, ovothione, mycothione; methionine sulfoxide reductase; ascorbate; γ-tocopherol; urate; α-keto acids	Under study

Nathan & Shiloh, *PNAS*, 2000, 97, 8841-8848

Mechanisms of inflammatory neurodegeneration

Brain inflammation occurs behind the blood–brain barrier in the absence of leucocytes (particularly neutrophils, monocytes, macrophages, B-cells and T-cells) and antibodies
≠inflammation in the periphery

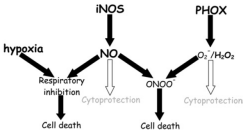
pathogens or damage —activation→ Microglia = resident brain macrophages → → activation of iNOS

Two models of cell death

1. NO inhibits mitochondrial respiration via inhibition of cytochrome *c* oxidase.
Synergizes with hypoxia

2.Synergy iNOS with *phox*.
Activation iNOS or *phox* alone = no cell death
Together = disappearance of NO, formation of ONOO-.

NO may be toxic even if *phox* is inactive (superoxide from mitochondria), i.e. Parkinson's disease:
Complex I is nitrosated+catalase is inhibited by NO



Brown, *Biochem.Trans*, 2007, 35,1119-1121

Parkinson's disease and mitochondrial Complex I

"Peter's good as dead anyway," Molly said. "In another twelve hours, he'll start to freeze up. Won't be able to move, his eyes is all."

"Why?" Case turned to her.

"I poisoned his sh*t for him," she said. "Condition's like Parkinson's disease, sort of."

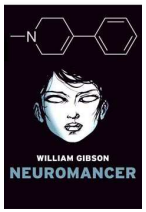
3Jane nodded. "Yes. We ran the usual medical scan, before he was admitted." She touched the ball in a certain way and it sprang away from Molly's hands. "Selective destruction of the cells of the substantia nigra. Signs of the formation of a Lewy body. He sweats a great deal, in his sleep."

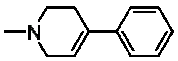
"Ali," Molly said, ten blades glittering, exposed for an instant. She tugged the blanket away from her legs, revealing the inflated cast. "It's the meperidine. I had Ali make me up a custom batch. Speeded up the reaction times with higher temperatures. N-methyl-4-phenyl-1236," she sang, like a child reciting the steps of a sidewalk game, "tetra-hydro-pyridene."

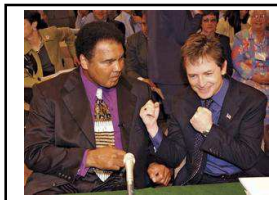
"A hotshot," Case said.

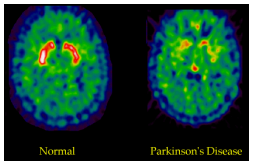
"Yeah," Molly said, "a real slow hotshot."

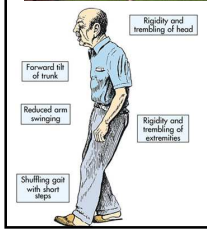
"That's appalling," 3Jane said, and giggled.

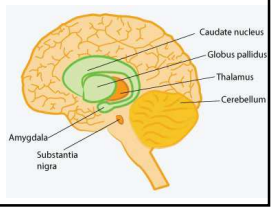




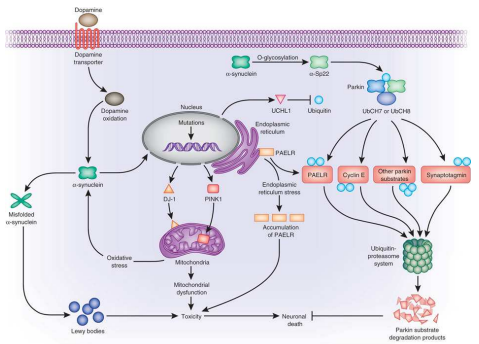




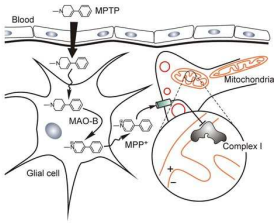




After decades of research, a single cause for Parkinson's disease has not been found and is unlikely to emerge.

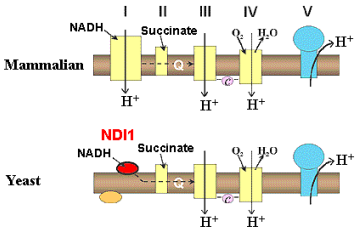


Parkinson's disease and mitochondrial Complex I inhibition in MPTP poisoning

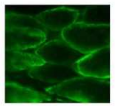


Animal models of Parkinson's disease based on complex I inhibition are very common

Molecular remedy of complex I defects

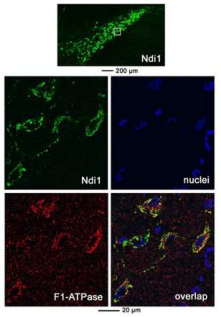


Molecular remedy of complex I defects



[Above] Ndi1 expressed in the mouse skeletal muscle.

[Right] Ndi1 expressed in the substantia nigra of rat brain. Confocal images after immuno-staining with antibody to Ndi1 (green), F1-ATPase (red) and nuclei (blue). It is clearly seen that Ndi1 is localized to mitochondria.



Prevention of apoptosis of cells treated with rotenone by Ndi1 expression