Reactive oxygen species and mitochondria

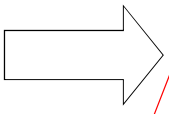
Reactive oxygen intermediates

Reactive oxygen intermediates

Reactive oxygen species

Free radicals

Oxyradicals



Superoxide radical $O_2^{\cdot -}$

Hydrogen peroxide H_2O_2

Hydroxyl radical $^{\cdot}OH$

Peroxynitrite $ONOO^-$

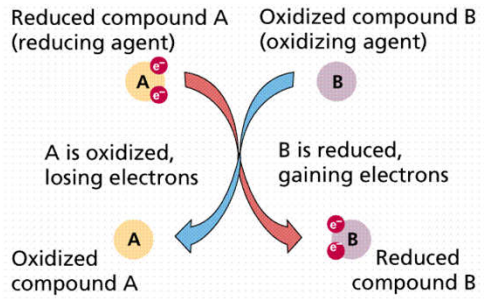
Hypoclorite $HClO$

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

$E^o_{A_{ox}/A_{red}} < E^o_{B_{ox}/B_{red}}$

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

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

Animals

Oxidation of food to obtain energy

Animals →

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

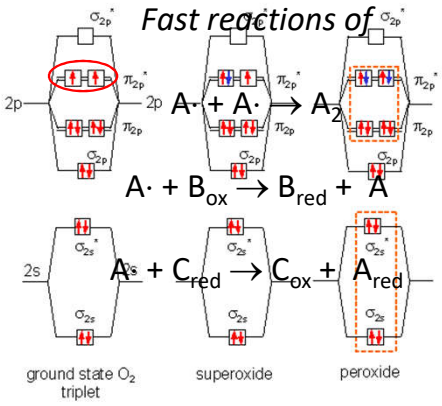
← Plants

What is radical?

Free radical – molecule containing unpaired electron(s)

Oxygen is diradical – contains two unpaired electrons

Fast reactions of



$A\cdot + A\cdot \rightarrow A_2$

$A\cdot + B_{ox} \rightarrow B_{red} + A$

$A\cdot + C_{red} \rightarrow C_{ox} + A_{red}$

Reactive oxygen intermediates

O_2	Oxygen
Energy transfer O_2 + energy → $^1O_2$	Singlet oxygen
One electron reduction of molecular oxygen $O_2 + 1e^- \rightarrow O_2^{\cdot-}$	Superoxide radical
SOD - Dismutation of superoxide radical $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2$	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) $O_2^{\cdot-} + NO \rightarrow ONOO^-$	Peroxynitrite
Myeloperoxidase reaction $H_2O_2 + Cl^- + H^+ \rightarrow H_2O + HClO$	Hypochlorite

Reduction of oxygen and superoxide

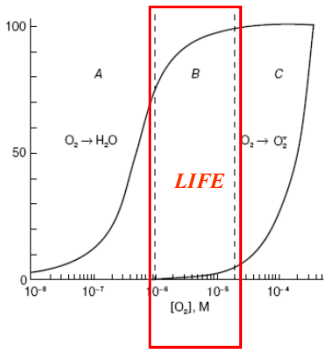
More than 90% of oxygen in our body is metabolised by cytochrome *c* oxidase = mitochondrial Complex IV

Enzymatic reaction

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

Non enzymatic "leak"

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



POLL:

Mitochondria are the main source of ROS.

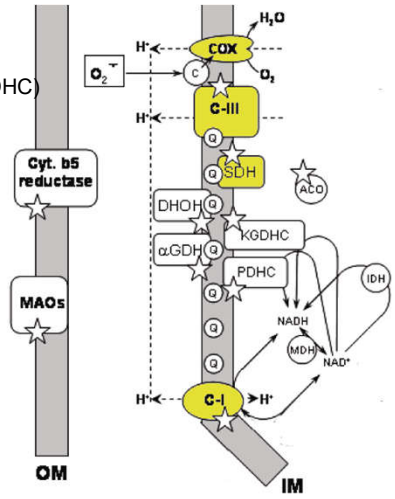
True or false?

Sites of ROS production in mitochondria

Inner membrane:
- Complexes I, II, III
- Dihydroorotate dehydrogenase (DHOH)
- α-Ketoglutarate dehydrogenase complex (KGDHC)
- Glycerol 3-phosphate DH (αGDH)

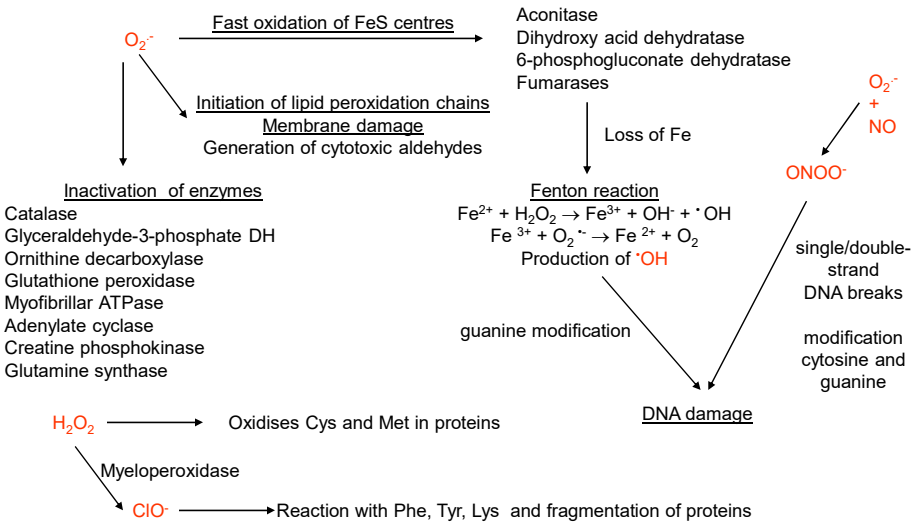
Outer membrane:
- Cytochrome *b*5 reductase
- Monoamine oxidases (MAOs)

Matrix: Aconitase (Aco)

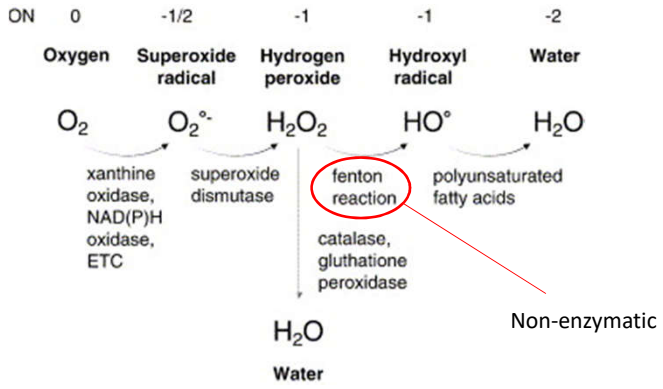


Andreev et al., 2005, Biokhimia

What is the damage?



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	$O_2^{\cdot-}$	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

ROS 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)

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, Issue of November 10, pp. 5753–5760, 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

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.*

(*Received* 20 *December* 1971)

Table 6. *Estimation of the effectiveness of the intracellular sources of H_2O_2 in rat liver*

For details see the text. Experimental values are taken from Tables 1–5 and Figs. 5 and 6.

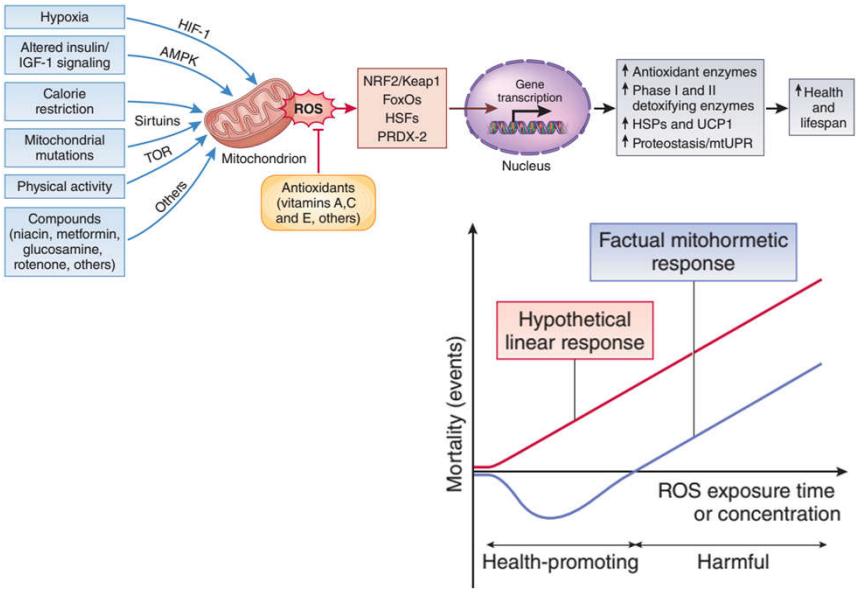
Fraction and substrate	Production of H_2O_2 (nmol/min per mg of protein)	Protein content (mg/g of liver)	Production of H_2O_2 (nmol/min per g of liver)
Mitochondria	—	25	—
Endogenous substrate	0.16		4
Succinate	0.50		12*
Microsomal fraction		25	
Endogenous substrate	0.13		3
NADPH	1.70		42*
Peroxisomes		20	
Intraperoxisomal endogenous substrate	2.2		44
+Supernatant	8.6		172
Estimated (see text)	5.0		100
Diffusing, estimated (see text)			30*
Supernatant		40	
Endogenous substrate	0.1		4*
Homogenate		140	
Endogenous substrate	0.27		38

* These values are considered as the physiological rates of H_2O_2 production.

Liver tissue – estimated relative contribution of different organells

Total production H2O2 - 90 nmol/min per g w/w
Mitochondrial fraction – 12 nmol/min per g w/w ~13% only!

Mitohormesis



Ristow, 2014, Nat.Med.

ROS detoxification

Enzymatic:
- Superoxide dismutase (MnSOD)
- Catalase (Cat)

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

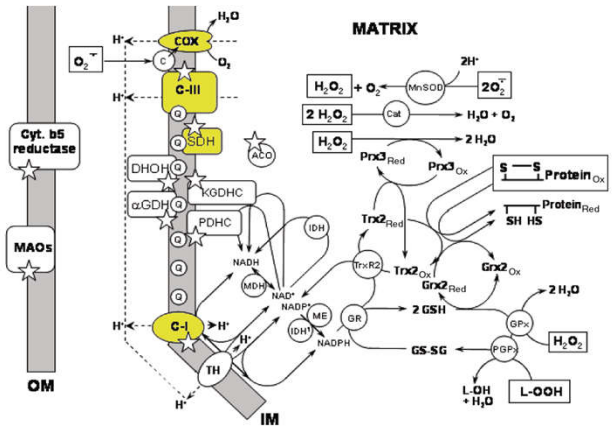
Glutaredoxin2 (Grx2)

Peroxiredoxins (Prx3) and other oxins.

Thioredoxin2 (Trx2)

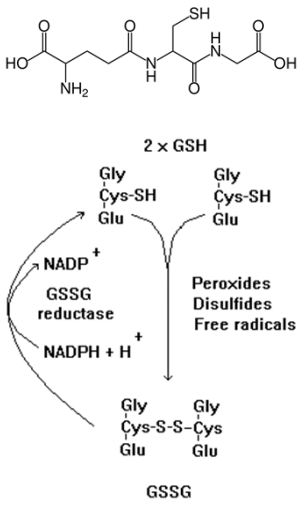
Thioredoxin2 reductase (TrxR2)

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



Andreyev et al., 2005, Biokhimia

Glutathione system



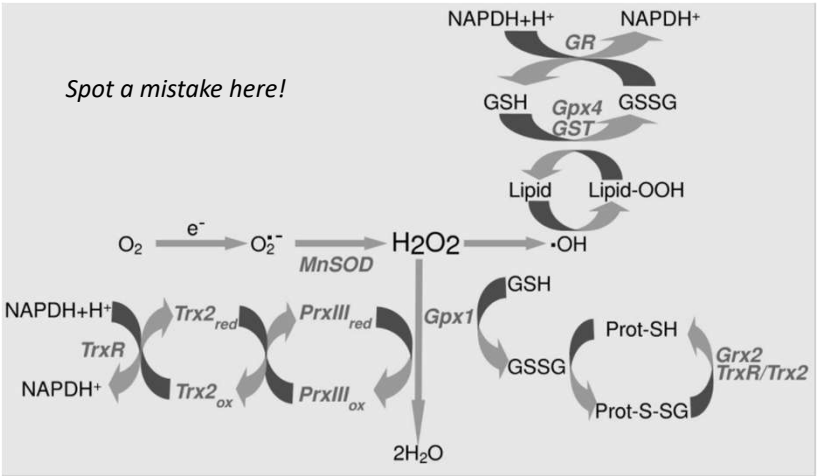
Mitochondria contain ~10% of total GSH in a cell but, due to the relatively small volume of the matrix, the concentration of GSH in mitochondrial matrix is higher than in the cytoplasm

Glutathione in matrix ~ 2-14 mM

GSH/GSSG~10

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

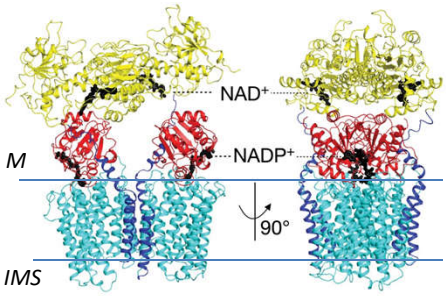
ROS metabolism in the matrix



Mari et al., 2009, Aniox.Red.Signal.

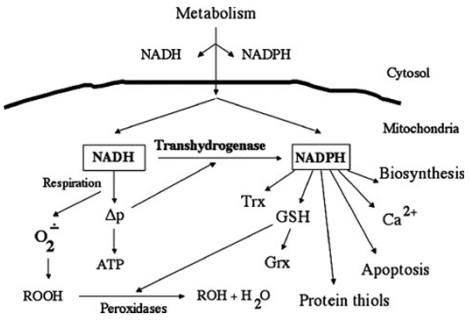
ROS metabolism in the matrix – NADPH (role of NNT)

$H^+_{out} + NADP^+ + NADH \leftrightarrow H^+_{in} + NADPH + NAD^+$



Proton-translocating transhydrogenase = Nicotinamide nucleotide transhydrogenase (NNT)
Use Δp to drive the reaction

Absent in C57BL/6 mice



Maintenance of matrix NADPH and thiol redox homeostasis

Kampjut&Sazanov, 2019, Nature

Rydstyrom, 2006, BBA

Superoxide dismutase

Fridovich & McCord 1969

Reaction:

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

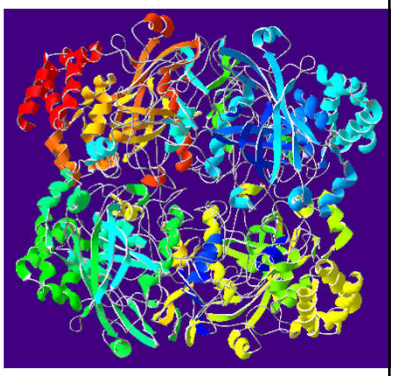
SOD1=Cu-Zn-SOD cytoplasmic - dimer 2x16 kDa
SOD2=Mn-SOD mitochondrial - tetramer 4x22.2 kDa
SOD3=Cu-Zn SOD extracellular - tetramer 4x33.8kDa

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'

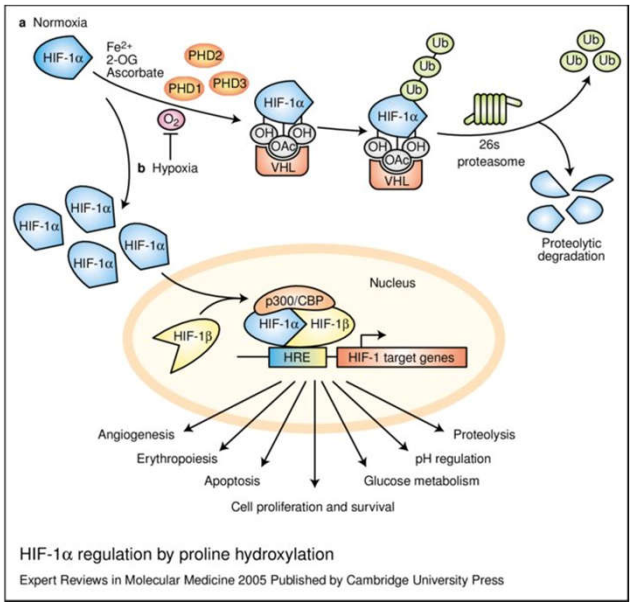
Reaction:

$2H_2O_2 \rightarrow 2H_2O + O_2$ 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!!!

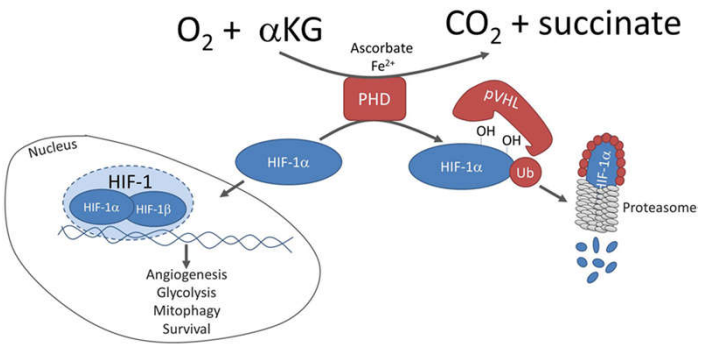
Story of HIF1α



HIF-1α regulation by proline hydroxylation

Expert Reviews in Molecular Medicine 2005 Published by Cambridge University Press

Prolyne hydroxylases are the main players



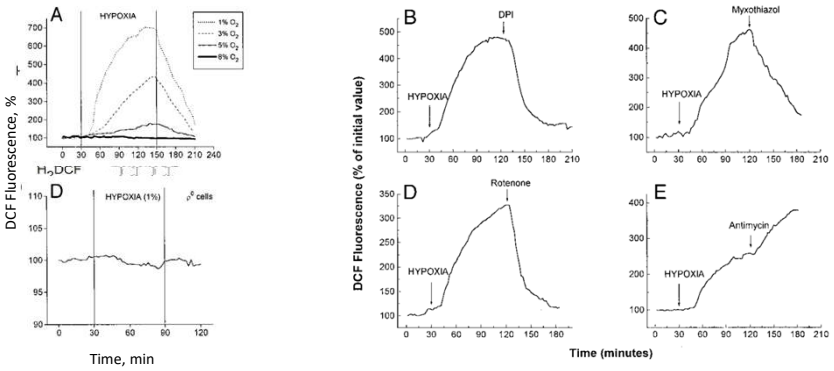
Iommarini et al., 2017, Front.Oncol.

Somehow ROS are involved

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11715–11720, September 1998
Cell Biology

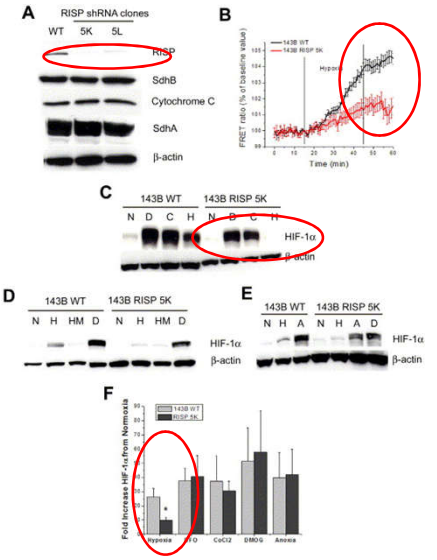
Mitochondrial reactive oxygen species trigger hypoxia-induced transcription

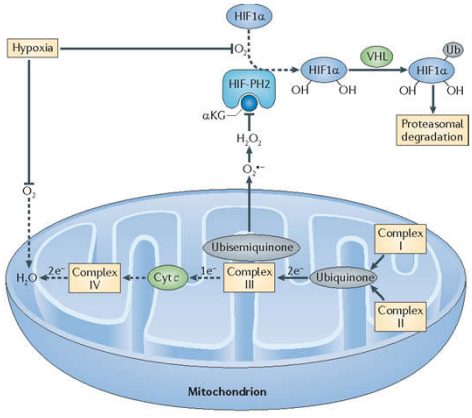
N. S. CHANDEL*, E. MALTEPE†, E. GOLDWASSER‡, C. E. MATHIEU*, M. C. SIMON§, AND P. T. SCHUMACKER*¶



Chandel et al., 1998, PNAS

Hypoxia-stimulated ROS in HIF-1α stabilization?





Guzy et al., 2005, Cell.Met

Nathan et al., 2013, Nat.Immun.

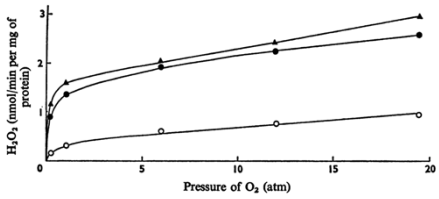
Mitochondria are the first to be affected by the lack of oxygen during hypoxia or tissue ischemia.

How does ROS generation by mitochondria depend on concentration of oxygen in a physiologically relevant range?

What is the dependence of the H_2O_2 release rate on oxygen concentration?

The Mitochondrial Generation of Hydrogen Peroxide
GENERAL PROPERTIES AND EFFECT OF HYPERBARIC OXYGEN

By ALBERTO BOVERIS* and BRITTON CHANCE
Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174, U.S.A.

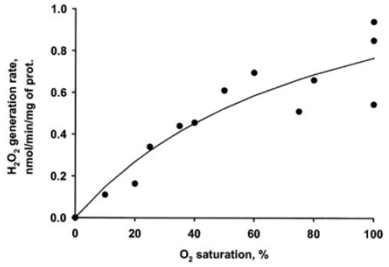


Hyperbaric oxygen

Pigeon heart mitochondria
Endogenous substrates

Characterization of Superoxide-producing Sites in Isolated Brain Mitochondria*

Alexei P. Kudin‡§, Nana Yaw-B. Bimpong-Buta§¶, Stefan Vielhaber‡, Christian E. Elger¶, and Wolfram S. Kunz¶‖



Hyperoxic oxygen

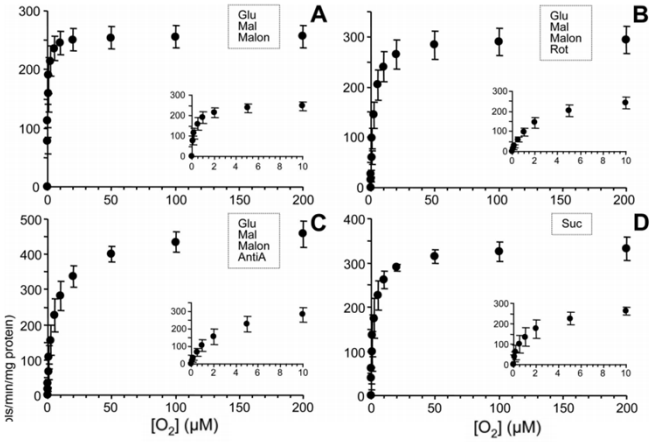
Rat brain mitochondria
Oxidation of succinate

What is the dependence of the H_2O_2 release rate on oxygen concentration?

Oxygen Sensitivity of Mitochondrial Reactive Oxygen Species Generation Depends on Metabolic Conditions[S]

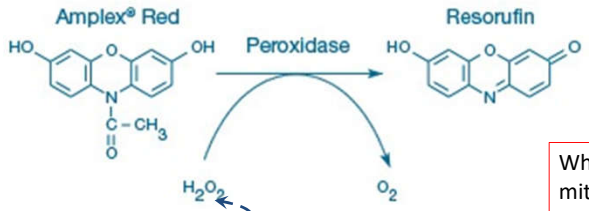
Received for publication, December 18, 2008, and in revised form, March 18, 2009 Published, JBC Papers in Press, April 14, 2009, DOI 10.1074/jbc.M809512200

David L. Hoffman‡ and Paul S. Brookes§,1



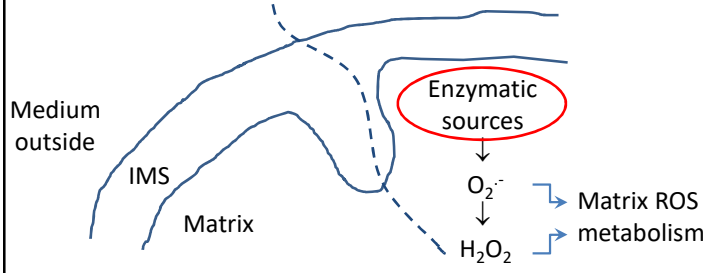
Liver mitochondria
Hyperbolic dependence in ALL conditions

Detection of ROS in intact mitochondria with Amplex UltraRed assay

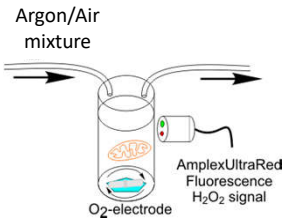


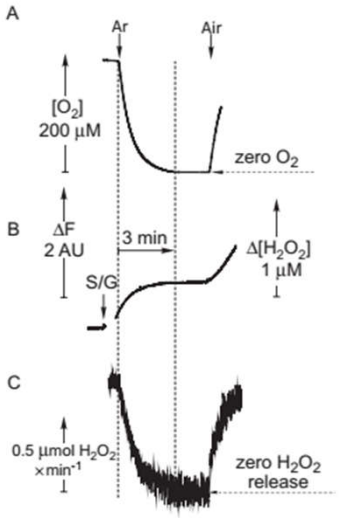
Excitation 525 nm
Emission 580 nm

What we measure in intact mitochondria is not «generation» of ROS, but «release» of H_2O_2



Dependence of H_2O_2 release rate on oxygen concentration

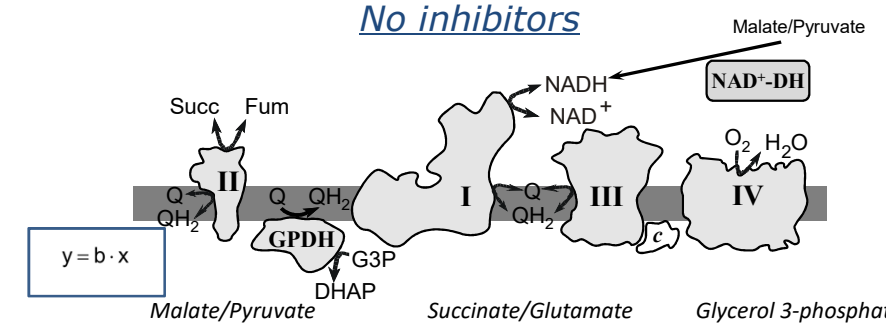


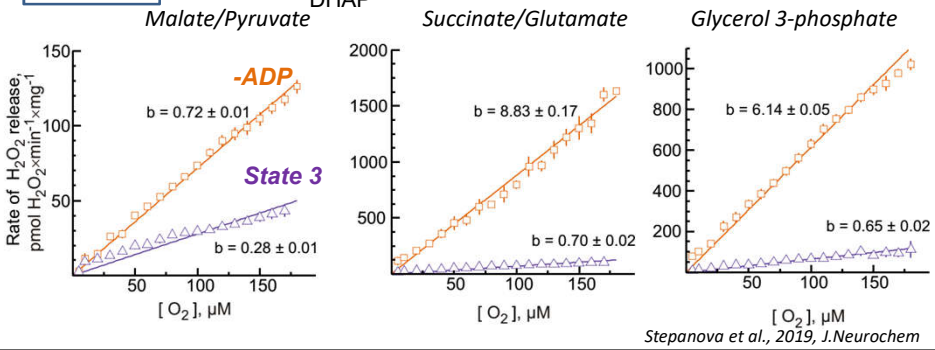


Stepanova et al., 2020, Meth.Mol.Biol

H_2O_2 release rate on oxygen concentration

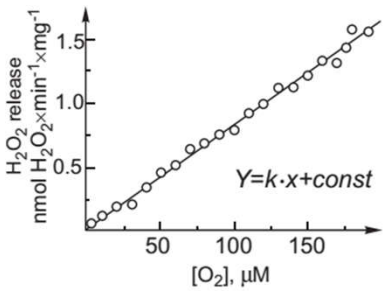
No inhibitors

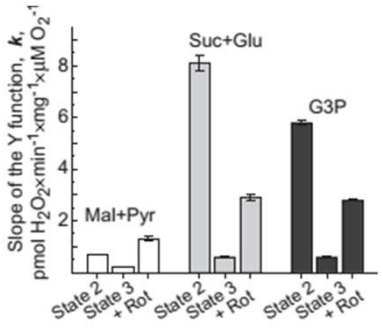




Stepanova et al., 2019, J.Neurochem

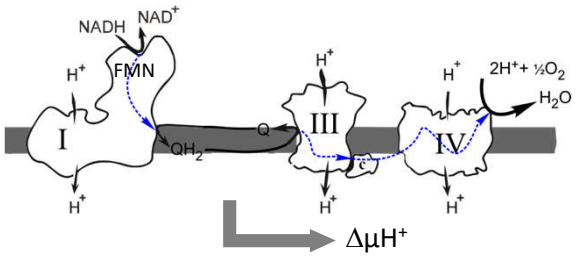
The highest rate of H_2O_2 release is when succinate is oxidized in conditions of so-called reverse electron transfer



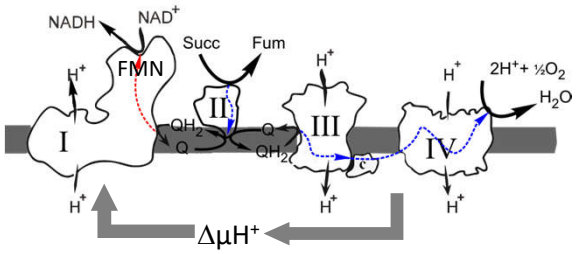


Stepanova et al., 2020, Metho.Mol.Biol

Forward and reverse electron transfer in mitochondria



Forward:
NADH:Q oxidoreduction
(oxidation of
malate/pyruvate)



Reverse:
QH_2:NAD^+ oxidoreduction
supported by potential
(oxidation of succinate)

Reverse electon transfer

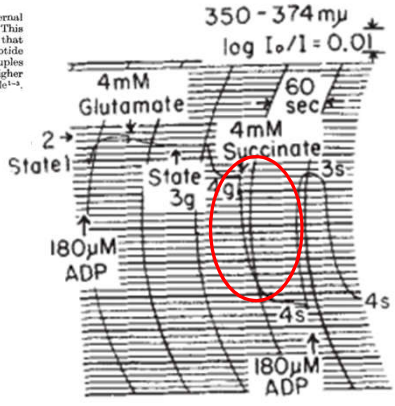
ENERGY-LINKED REDUCTION OF MITOCHONDRIAL PYRIDINE NUCLEOTIDE

By Prof. BRITTON CHANCE and Dr. GUNNAR HOLLUNGER*

Johnson Research Foundation, University of Pennsylvania, Philadelphia

IN thermodynamic computations of reactions in biological systems[1] it has been customary to assume that the formation of reduced pyridine nucleotide proceeds in an exergonic reaction in which the free energy of the oxidation-reduction couple ($\Delta H_{2'} \rightarrow A$) is sufficiently negative with respect to reduced pyridine nucleotide itself that no external energy source is required to drive the reaction[1]. This article presents experimental results suggesting that the reduction of mitochondrial pyridine nucleotide can be accomplished by oxidation-reduction couples having potentials several hundred millivolts higher than that of the pyridine nucleotide couple[1-3].

*Permanent address: University of Lund, Sweden.



Potential-driven reduction of NAD^+ by succinate

LETTER

doi:10.1038/nature13909

Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS

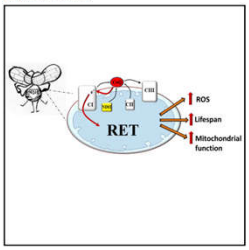
Edward T. Chouchani[1,2*], Victoria R. Pell[2*], Edoardo Gaude[3], Dunja Aksentijević[4], Stephanie Y. Sundier[5], Ellen L. Robb[1], Angela Logan[1], Sergiy M. Nadtochiy[6], Emily N. [illegible], Anna J. Dare[1], Andrew M. James[1], Sebastian R[illegible], Sean M. Davidson[10], Michael R. Duchen[5], Kou[illegible], Christian Frezza[3], Thomas Krieg[2] & Michael P. [illegible]

Article

Cell Metabolism

Mitochondrial ROS Produced via Reverse El[illegible] Transport Extend Animal Lifespan

Graphical Abstract



Authors

Filippo Scialò, Ashwin S[illegible], Daniel Fernández-Ayala, Jose Antonio Enríquez, Michael P. Murphy, Alb[illegible]

Correspondence

Alberto.Sanz@newcastl[illegible]

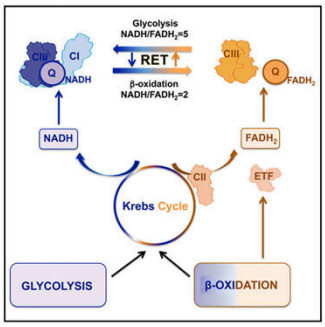
In Brief

Increasing mitochondri[illegible] specifically through con[illegible] health and extends lifes[illegible] show that the site at wh[illegible] produced determines th[illegible] increasing mtROS speci[illegible] electron transport throu[illegible] complex I delays aging[illegible] age-related diseases in[illegible]

Cell Reports

The CoQH2/CoQ Ratio Serves as a Sensor of Respiratory Chain Efficiency

Graphical Abstract



Authors

Adela Guarás, Ester Perales-Clemente, Enrique Calvo, ..., Plácido Navas, Jesús Vazquez, Jose A. Enríquez

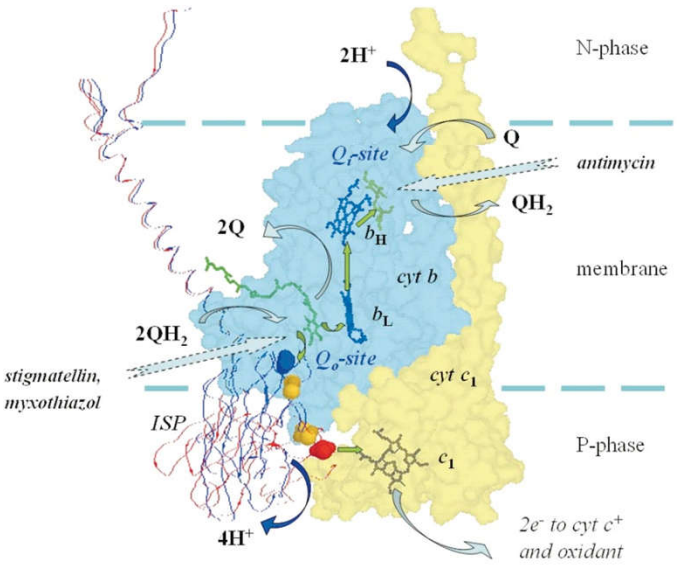
Correspondence

jaenriquez@cnic.es

In Brief

Guarás et al. show how the mitochondrial electron transport chain (mtETC) is optimized to better oxidize different nutrients or fuels using the reducing status of ubiquinone as a metabolic sensor and ROS generated by complex I by reverse electron transport as an executor.

In the absence of inhibitors H_2O_2 release by brain mitochondria is linearly dependent on oxygen concentration during both non–phosphorylating and phosphorylating (State 3) respiration with any substrate



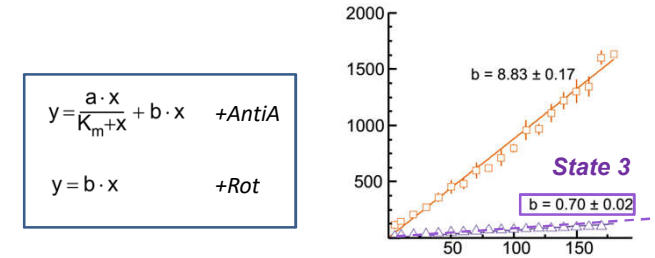
$QH_2 + 2\ cyt\ c^{+} + 2H_N^{+} \leftrightarrows Q + 2\ cyt\ c + 4H_P^{+}$

H_2O_2 release rate on oxygen concentration

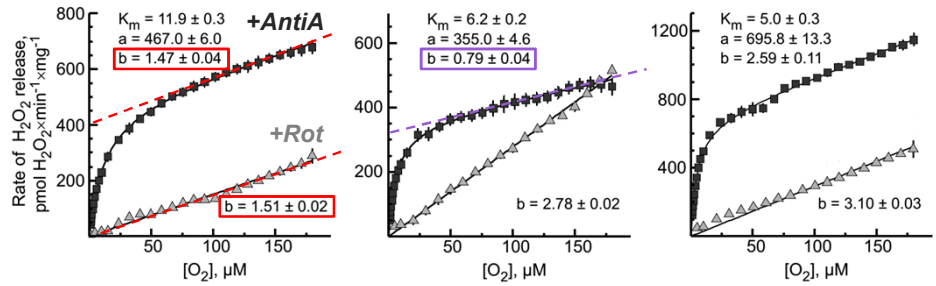
Effect of rotenone and antimycin A

$y = \frac{a \cdot x}{K_m + x} + b \cdot x$ *+AntiA*

$y = b \cdot x$ *+Rot*



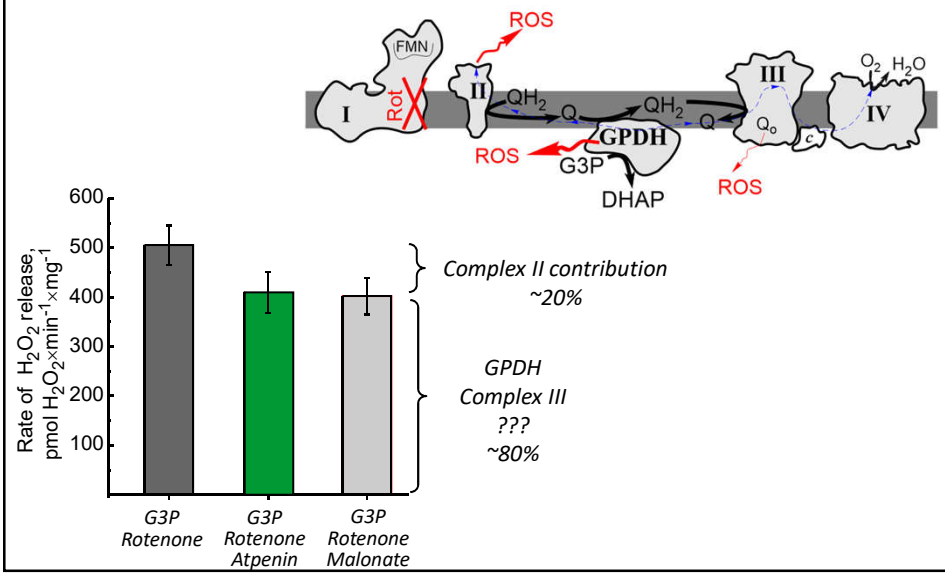
Malate/Pyruvate *Succinate/Glutamate* *Glycerol 3-phosphate*



What is the reason for hyperbolic dependence of ROS generation at complex III in the presence of antimycin A?

H_2O_2 release rate and glycerol 3-phosphate oxidation in the presence of rotenone

Effect of complex II inhibition



H_2O_2 release rate and glycerol 3-phosphate oxidation in the presence of rotenone

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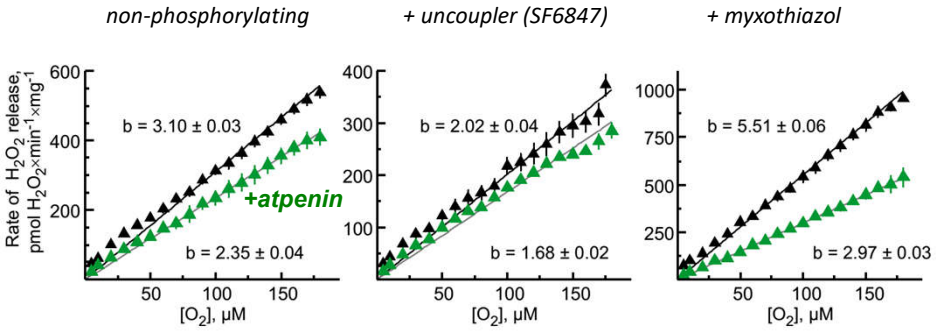


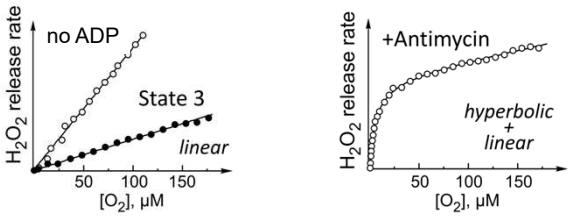
When the quinone pool is reduced, complex II contributes to ROS production even in the absence of its substrate succinate

1. In the absence of inhibitors with any substrate used H_2O_2 release by brain mitochondria is linearly dependent on oxygen concentration.

2. The dependence is still linear in the presence of rotenone or myxothiazol

3. ***Complex III*** contribution to overall H_2O_2 release is significant only in the presence of antimycin A. In this case, the oxygen dependence manifests mixed (hyperbolic+ linear) kinetics

4. When the quinone pool is reduced, ***complex II*** can contribute to ROS generation even in the absence of its substrate succinate



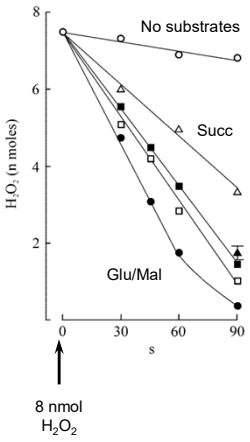
Source or sink?

Respiration-dependent Removal of Exogenous H_2O_2 in Brain Mitochondria

INHIBITION BY Ca^{2++}

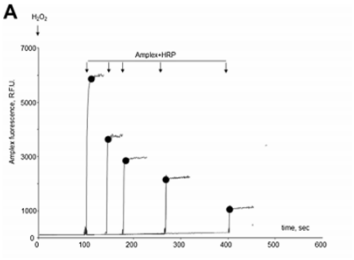
Received for publication, July 25, 2003, and in revised form, Novemb
Published, JBC Papers in Press, November 20, 2003, DOI 10.1074/jbc.3

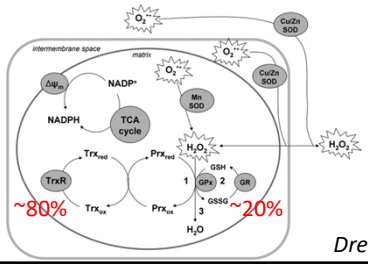
Franco Zoccaratoł, Lucia Cavallini, and Adolfo Alexandre



Scavenging of H_2O_2 by mouse brain mitochondria

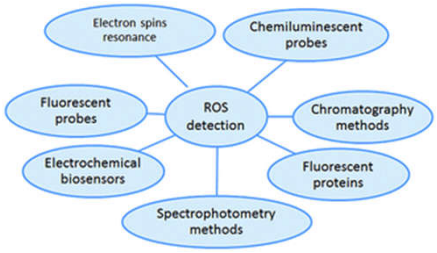
Anatoly A. Starkov · Alexander Yu Andreyev · Steven F. Zhang · Natalia N. Starkova · Maria Korneeva · Mikhail Syromyatnikov · Vasily N. Popov





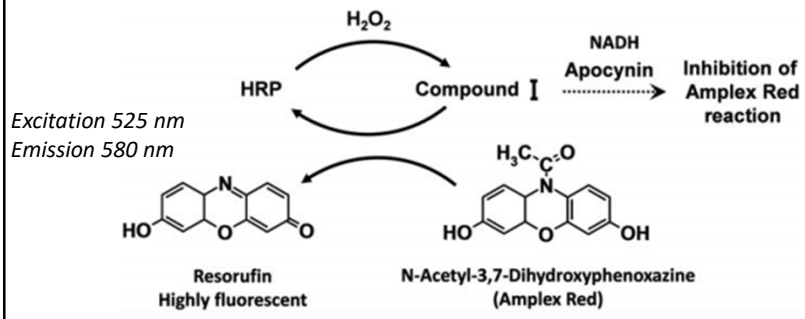
Drechel&Patel, 2010, JBC

ROS detection



1. In vitro absorbance/fluorescence probes – for homogenates, isolated mitochondria or purified/reconstituted enzymes
2. EPR probes
3. Absorbance/fluorescence cellular probes
4. Fluorescent-based proteins
5. Exomarkers (endpoint MS detection)
6. Footprints of ROS

Detection of H_2O_2 in intact mitochondria with Amplex UltraRed/Red assay (in vitro)



Affected by NADH
SOD should be present
Light sensitivity
Calibration by stadnard H2O2

Resorufin is Complex I inhibtitor
Autooxidation of Amplex Red
[Amplex Red] <10μM
Check for PMSF sensitivity

Liver mitochondria and Amplex Red Assay

Free Radical Biology and Medicine 90 (2016) 173–183

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

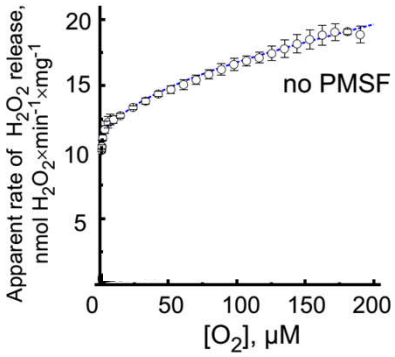
journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Carboxylesterase converts Amplex red to resorufin: Implications for mitochondrial H_2O_2 release assays

Satomi Miwa[a], Achim Treumann[b], Amy Bell[a], Giulio Vistoli[c], Glyn Nelson[a,d], Sam Hay[e], Thomas von Zglinicki[a,*]

Succinate/Glutamate



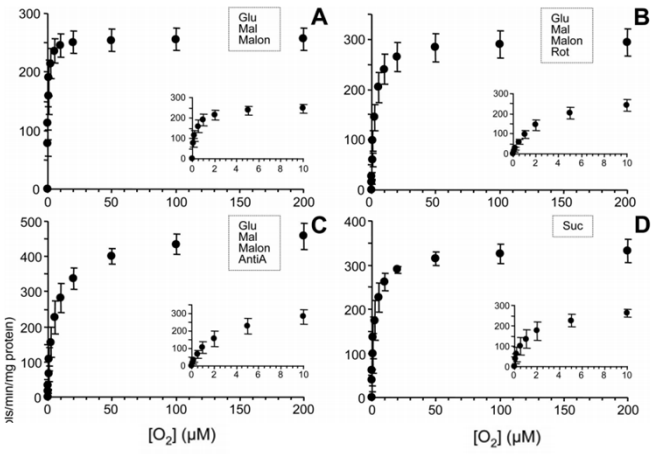
Unlike brain mitochondria, preparation of liver mitochondria catalyse H_2O_2-independent conversion of Amplex Ultra Red to resorufin by matrix carboxylesterase sensitive to phenylmethylsulfonyl fluoride (PMSF).

Stepanova, unpublished data

Oxygen Sensitivity of Mitochondrial Reactive Oxygen Species Generation Depends on Metabolic Conditions[S]

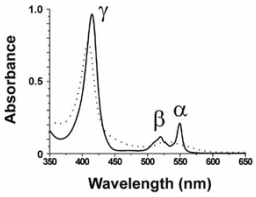
Received for publication, December 18, 2008, and in revised form, March 18, 2009 Published, JBC Papers in Press, April 14, 2009, DOI 10.1074/jbc.M809512200

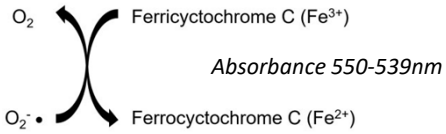
David L. Hoffman[‡] and Paul S. Brookes[§,1]

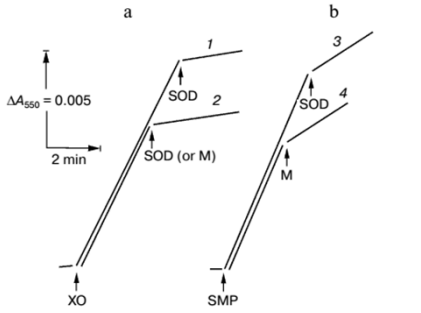


Most of the studies with Amplex Red were performed on liver mitochondria without PMSF = useless!

Detection of superoxide in intact mitochondria with (acetylated) cytochrome *c* (in vitro)







Grivennikova et al., 2005, BBA

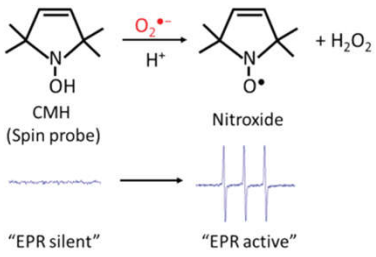
Original Fridovich method *Specific for superoxide* *Large amount of $O_2^{\cdot -}$ required*

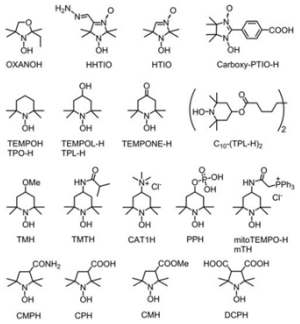
Can be oxidized/reduced by the respiratory chain => SOD-sensitivity should be checked

Acetylated Cyt c is less cross reactive with RC

Affected by oxypurinol, L-NAME, apocynin….

Detection of ROS by spin probes by EPR





Many molecules with different hydrophobicity

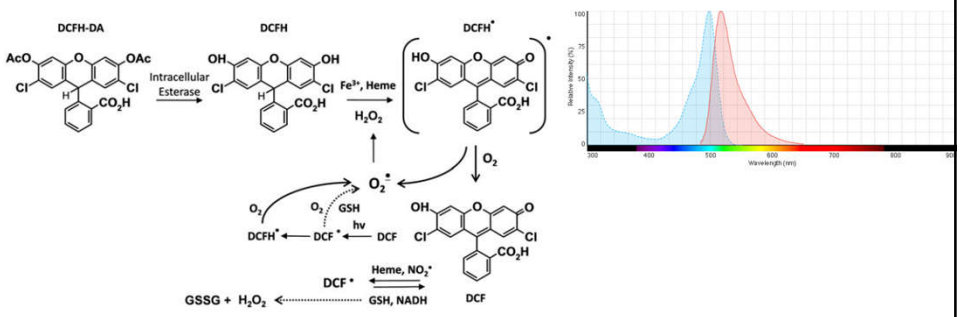
Very sensitive to $O_2^{\cdot -}$ and peroxynitrite *Can be membrane-permeable or targeted*

Requires expensive equipment and highly skilled staff

Painful samples preparation *SOD-sensitivity should be checked*

Trapping can be slower than scavenging *Transition metals increase oxidation*

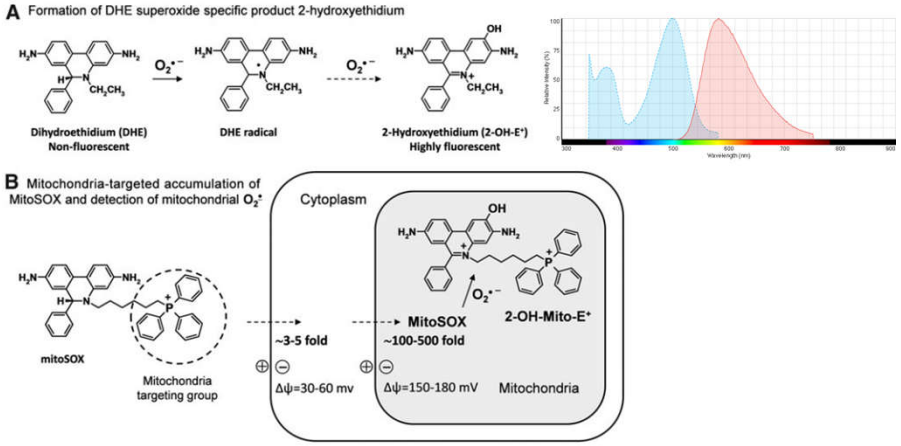
Fluorescent probes: dichlorodihydrofluorescein



Dikalov et al., Ant.Red.Signal, 2014

The editorial board of Free Radicals in the Biology and Medicine journal stated that DCFH should not be used as a reliable measure of H_2O_2

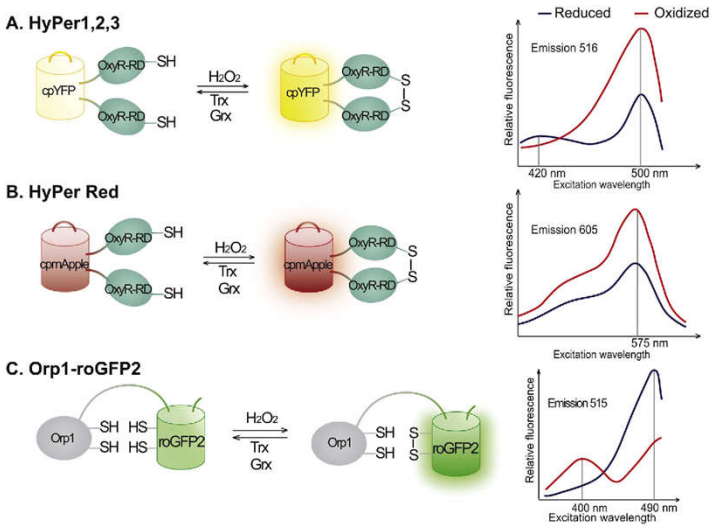
Fluorescent probes: dihydroethidium (DHE) and mitoSOX.



MitoSOX < 2μM
Fluorescent detection (~400/560 nm) should be confirmed by HPLC
Complex chemistry, light sensitivity, autooxidation

Dikalov et al., Ant.Red.Signal, 2014

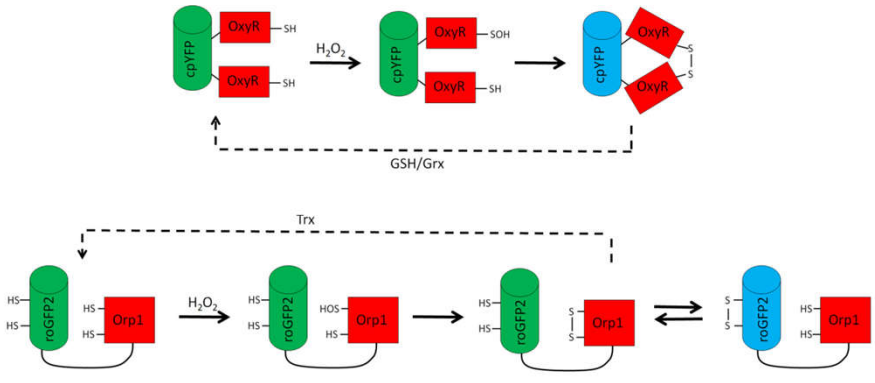
Fluorescent proteins



Genetically encoded – can be specifically targeted
Redox state rather than H_2O_2
Careful controls are needed
pH sensitive

Bilan et al., Free Rad.Med.Biol., 2018

Fluorescent proteins



Do not react with H_2O_2 only!

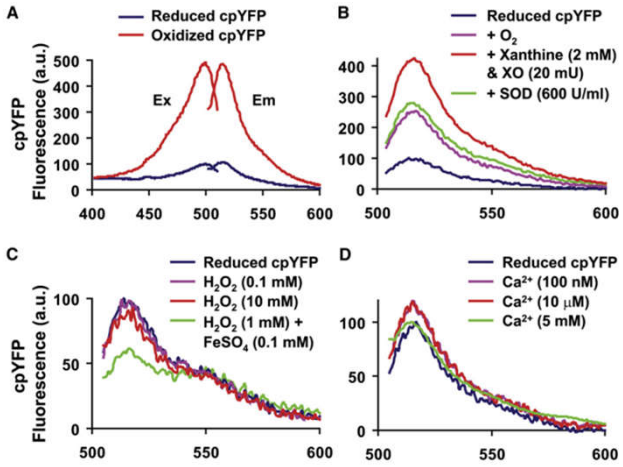
Val Laurel&Dick, Mol.Cell, 20186

A story of one artefact – mitochondrial flashes

Superoxide Flashes in Single Mitochondria

Cell

Wang Wang,[1,*] Huaqiang Fang,[4] Linda Groom,[5] Aiwu Cheng,[2] Wanrui Zhang,[4] Jie Liu,[4] Xianhua Wang,[4] Kaitao Li,[4] Peidong Han,[4] Ming Zheng,[4] Jinhu Yin,[3] Weidong Wang,[3] Mark P. Mattson,[2] Joseph P.Y. Kao,[6] Edward G. Lakatta,[1] Shey-Shing Sheu,[5] Kunfu Ouyang,[7] Ju Chen,[7] Robert T. Dirksen,[5] and Heping Cheng[4,*]



A story of one artefact – mitochondrial flashes

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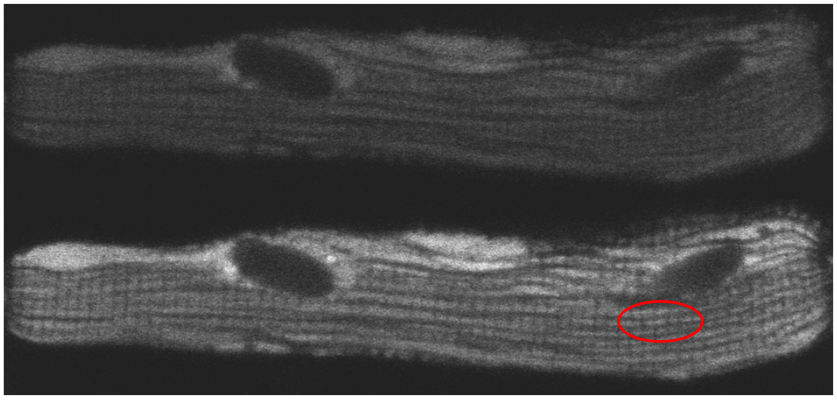
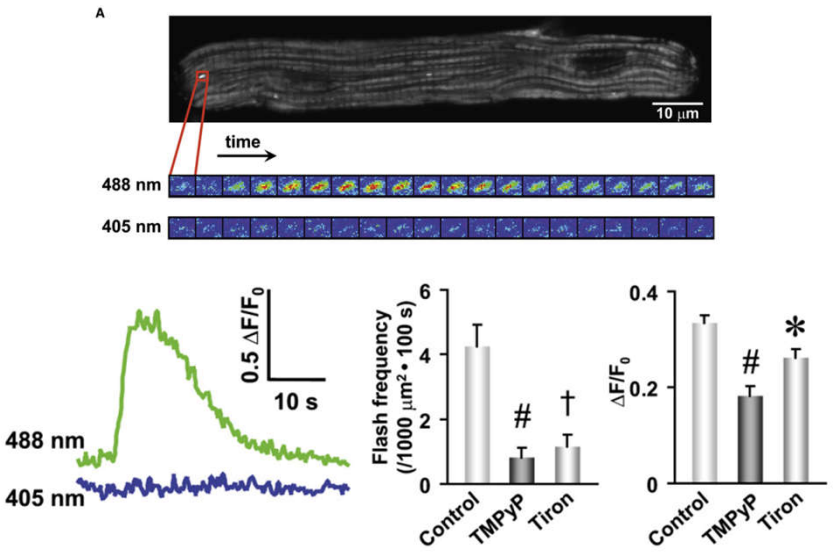


A story of one artefact – mitochondrial flashes

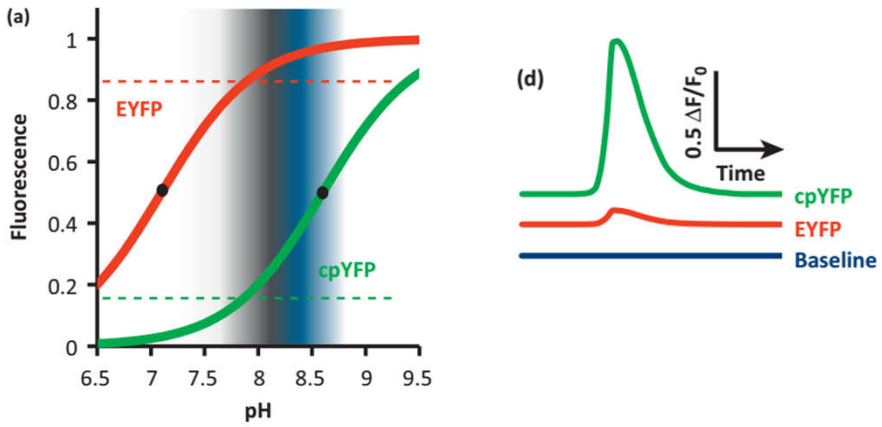


Opinion

Cell PRESS

Mitochondrial 'flashes': a radical concept repHined

Markus Schwarzländer[1], Michael P. Murphy[2], Michael R. Duchen[3], David C. Logan[4], Mark D. Fricker[5], Andrew P. Halestrap[6], Florian L. Müller[7], Rosario Rizzuto[8], Tobias P. Dick[9], Andreas J. Meyer[1] and Lee J. Sweetlove[5]



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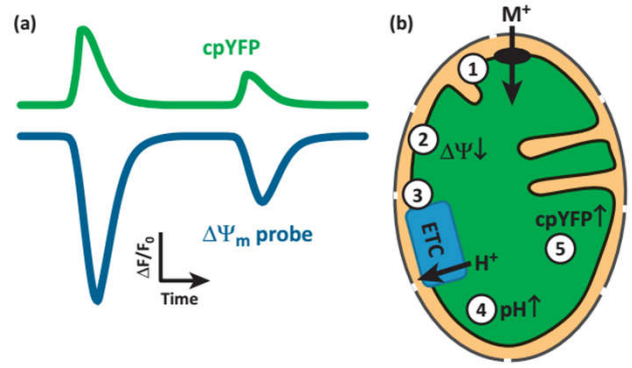


TRENDS in Cell Biology

Mitochondrial flashes: new insights into mitochondrial ROS signalling and beyond

Tingting Hou, Xianhua Wang, Qi Ma and Heping Cheng

Cell Research (2011) 21:1295-1304.

www.nature.com/cr

ORIGINAL ARTICLE

Imaging superoxide flash and metabolism-coupled mitochondrial permeability transition in living animals

Huaqiang Fang[1, 2, *], Min Chen[1, *], Yi Ding[1, *], Wei Shang[1], Jiejia Xu[1], Xing Zhang[3], Wanrui Zhang[1], Kaitao Li[1], Yao Xiao[1], Feng Gao[3], Shujiang Shang[1], Jing-Chao Li[2], Xiao-Li Tian[1], Shi-Qiang Wang[2], Jingsong Zhou[4], Noah Weisleder[3], Jianjie Ma[5], Kunfu Ouyang[6], Ju Chen[6], Xianhua Wang[1], Ming Zheng[1], Wang Wang[7], Xiuqin Zhang[1], Heping Cheng[1, 2]

August 5, 2011
ed in the U.S.A.

Superoxide Flashes

EARLY MITOCHONDRIAL SIGNALS FOR OXIDATIVE STRESS-INDUCED APOPTOSIS[*][S]

Received for publication, March 23, 2011, and in revised form, May 29, 2011 Published, JBC Papers in Press, June 9, 2011, DOI 10.1074/jbc.M111.241794

Qi Ma[‡§1], Huaqiang Fang[¶1], Wei Shang[¶], Lei Liu[‡§], Zhengshuang Xu[‖], Tao Ye[‖], Xianhua Wang[¶], Ming Zheng[¶2], Quan Chen[‡‡§§], and Heping Cheng[¶]**

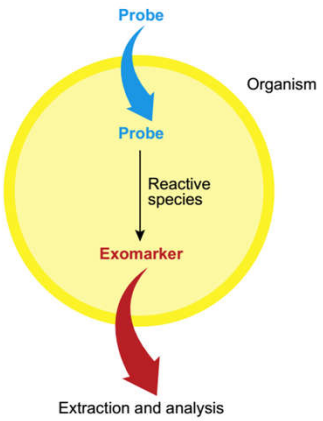
*From the [‡]Joint Laboratory of Apoptosis and Cancer Biology, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China, the [¶]Institute of Molecular Medicine and State Key Laboratory of Biomembrane and Membrane Biotechnology, Peking University, Beijing 100871, China, the [§]Graduate University of Chinese Academy of Sciences, Beijing 100049, China, the [‖]Laboratory of Chemical Genomics, Peking University Shenzhen Graduate School, Shenzhen 518055, China, the **Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China, and the [‡‡]Tianjin Key Laboratory of Protein Sciences, College of Life Sciences, Nankai University, Tianjin 300071, China*

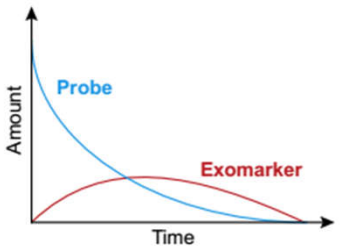
Exomarkers for ROS

Using exomarkers to assess mitochondrial reactive species *in vivo*

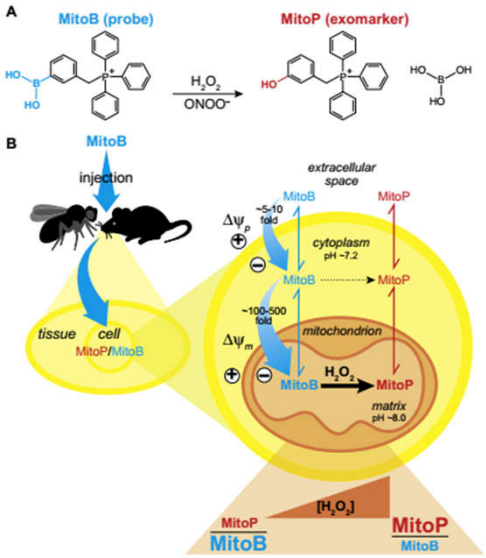
Biochimica et Biophysica Acta

Angela Logan [a], Helena M. Cochemé [a,b], Pamela Boon Li Pun [a], Nadezda Apostolova [c], Robin A.J. Smith [d], Lesley Larsen [d], David S. Larsen [d], Andrew M. James [a], Ian M. Fearnley [a], Sebastian Rogatti [a], Tracy A. Prime [a], Peter G. Finichiu [a], Anna Dare [a], Edward T. Chouchani [a], Victoria R. Pell [e], Carmen Methner [e], Caroline Quin [f], Stephen J. McQuaker [f], Thomas Krieg [e], Richard C. Hartley [f], Michael P. Murphy [a,*]





Using exomarkers to assess mitochondrial reactive species *in vivo* Biochimica et Biophysica Acta



Reviews to read

Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc). 2005 Feb;70(2):200-14. doi: 10.1007/s10541-005-0102-7.

Andreyev AY, Kushnareva YE, Murphy AN, Starkov AA. Mitochondrial ROS Metabolism: 10 Years Later. Biochemistry (Mosc). 2015 May;80(5):517-31. doi: 10.1134/S0006297915050028.

Michael P Murphy. 2009, How mitochondria produce reactive oxygen species Biochem J . 2009 Jan 1;417(1):1-13. doi: 10.1042/BJ20081386.

Ristow, 2014, Mitohormesis explains ROS-induced health benefits. Nature Medicine

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

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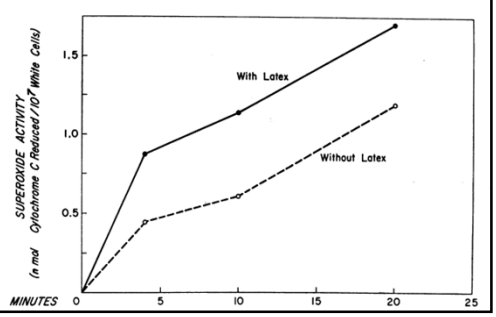
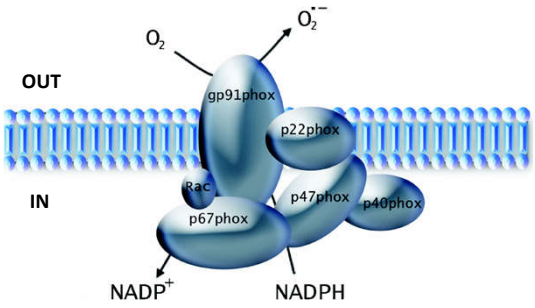
Superoxide is the initial product during respiratory burst



NADPH oxidase (phox)



Six subunits

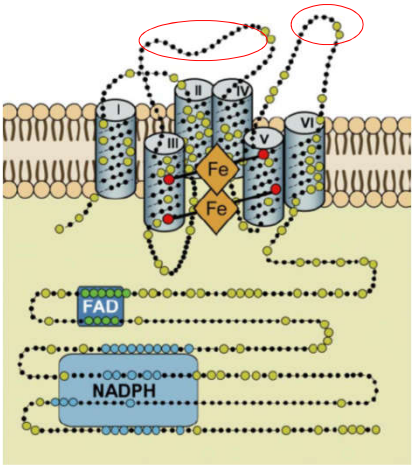
K_m^{NADPH} ~ 40 μM

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

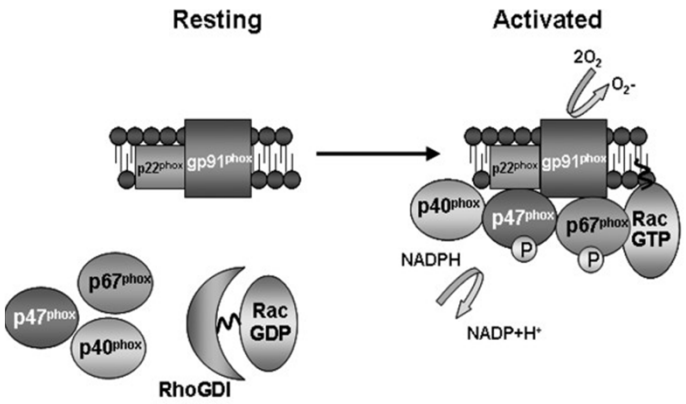
Carbohydrates attached to Asn residue

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

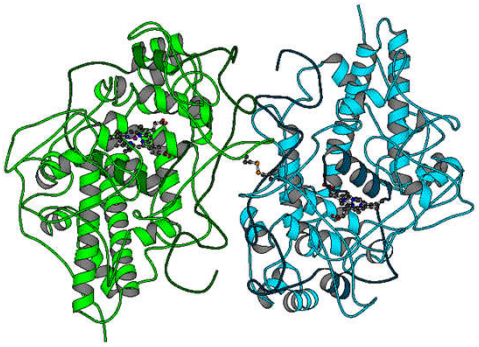
Stored in azurophilic granules

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 $+2H_2O + 3/2NADP+NO$

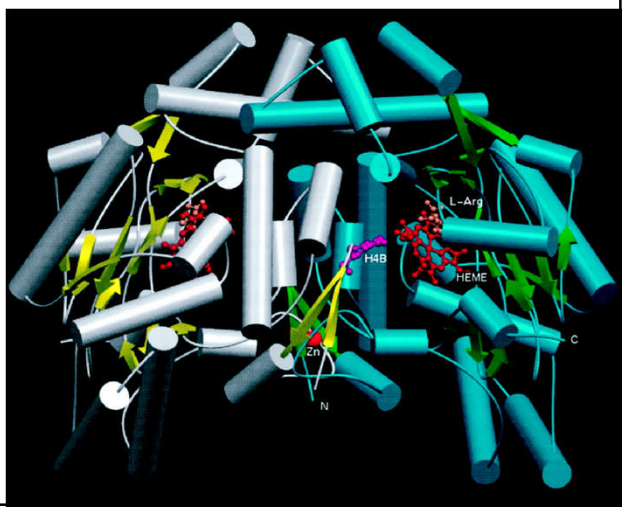
De novo transcription-biosynthesis

Induction by interferon-γ

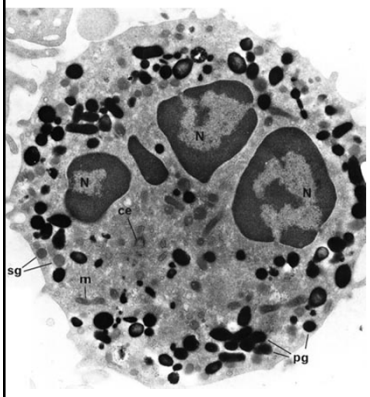
Redox centres:
FAD and two FMN
Haem
Tetrahydrobiopterin

NO is membrane-permeable

In macrophages more than in neutrophils



Location in neutrophil



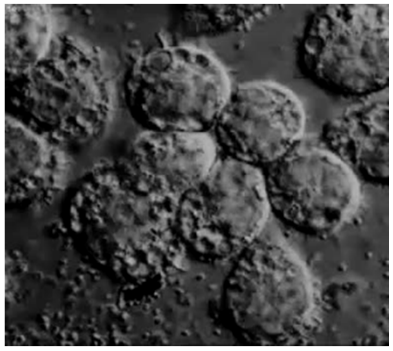
At least two types of granules

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

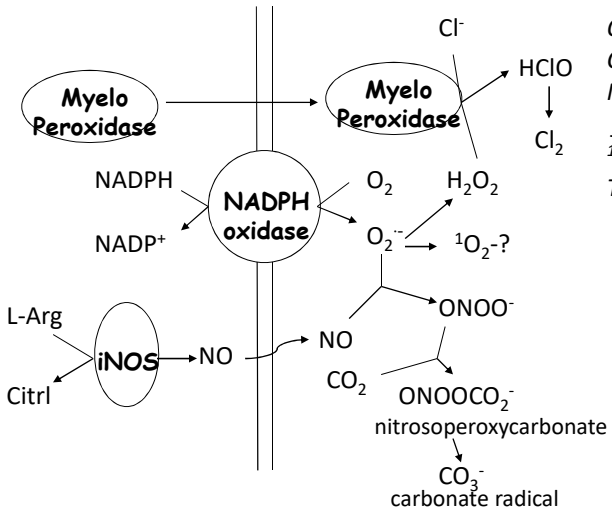


Intraphagosomal pH monitored with pH-sensitive fluorescent pHRODO dye

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