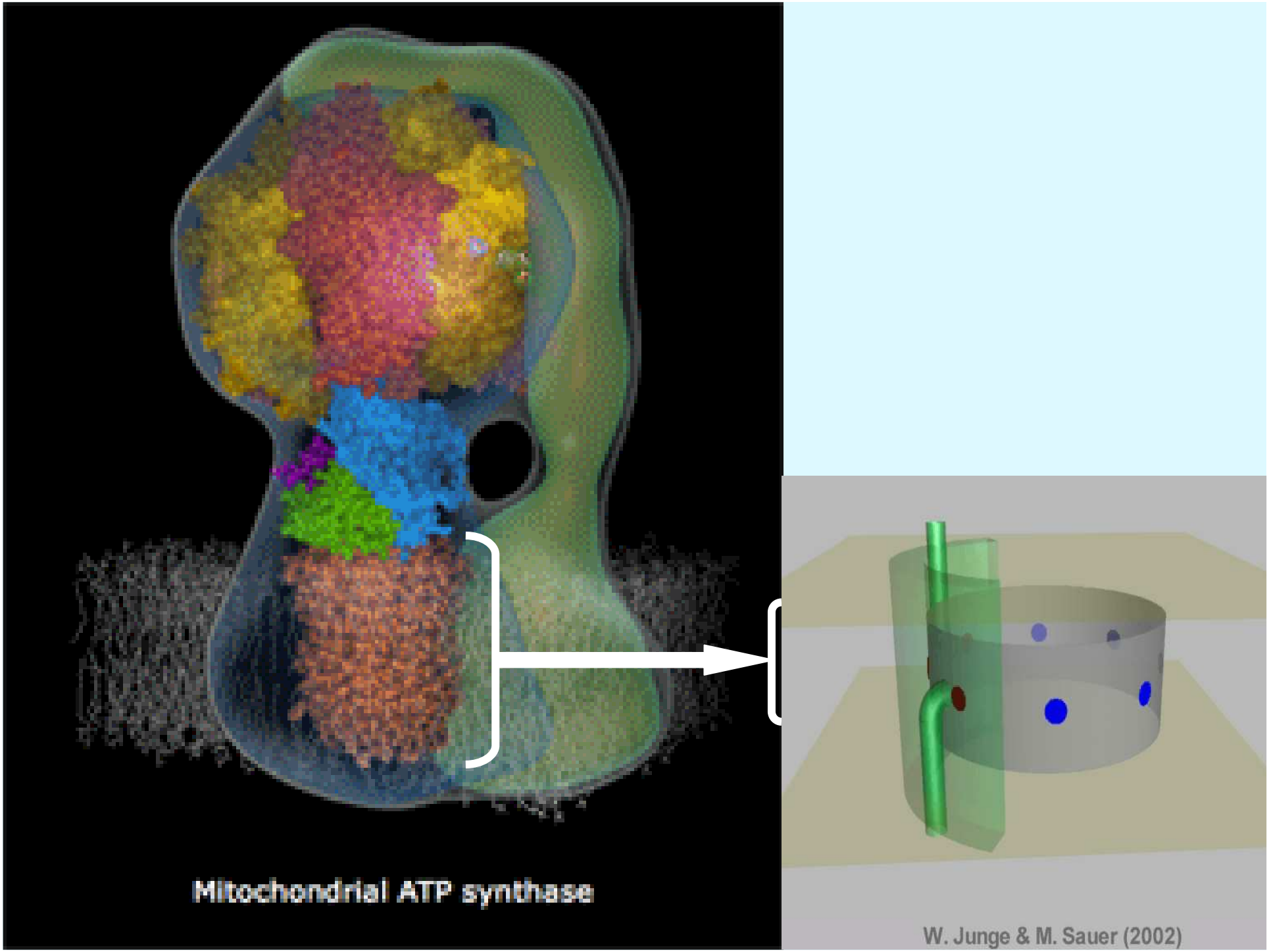

# Possibile ruolo energetico della mielina

Prospettive nello studio del sonno, della memoria e delle malattie neurodegenerative.

***Torino – 20 giugno 2011***

# (1)

# Sintesi di ATP


Mitochondrial ATP synthase
W. Junge & M. Sauer (2002)

# ATP SYNTHESIS BY ROTARY CATALYSIS

Nobel Lecture, December 8, 1997

by

JOHN E. WALKER

The Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, U. K.

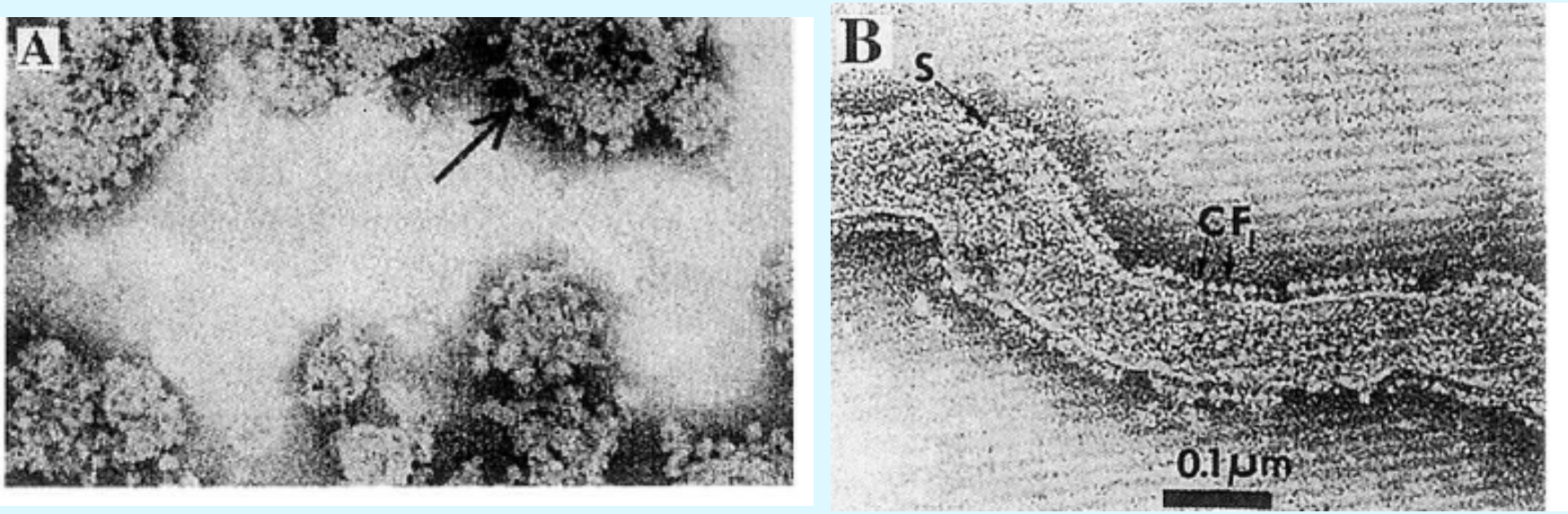
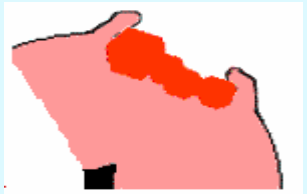



Figure 2. Knobs associated with biological membranes detected by electron microscopy in negative stain. (A), Inside-out vesicles from bovine heart mitochondria; (B), thylakoid membranes form pea chloroplasts;

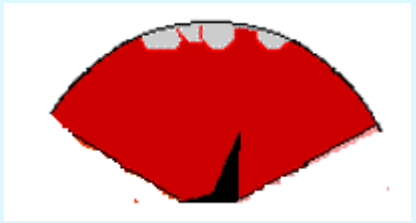
Conformazione

Light **L**



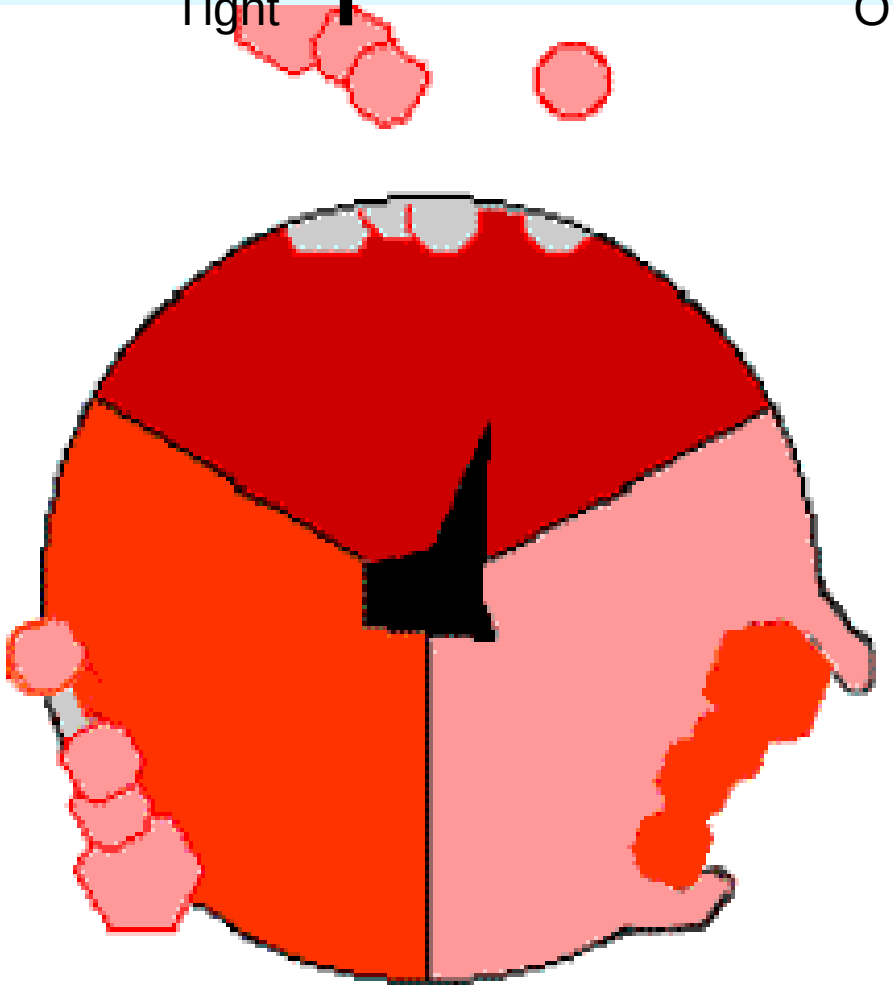
Conformazione

Tight **T**



Conformazione

Open **O**



# The Nobel Prize in Chemistry 1997

**"for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"**
**"for the first discovery of an ion-transporting enzyme, Na+, K+ -ATPase"**

## Paul D. Boyer

◔1/4 of the prize

USA

University of California
Los Angeles, CA, USA

b. 1918

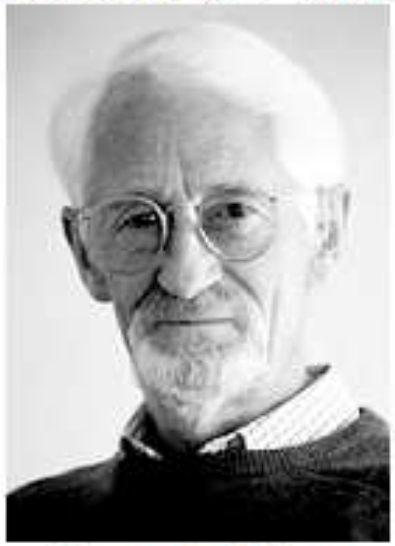
## John E. Walker

◔1/4 of the prize

United Kingdom

MRC Laboratory of Molecular Biology
Cambridge, United Kingdom

b. 1941



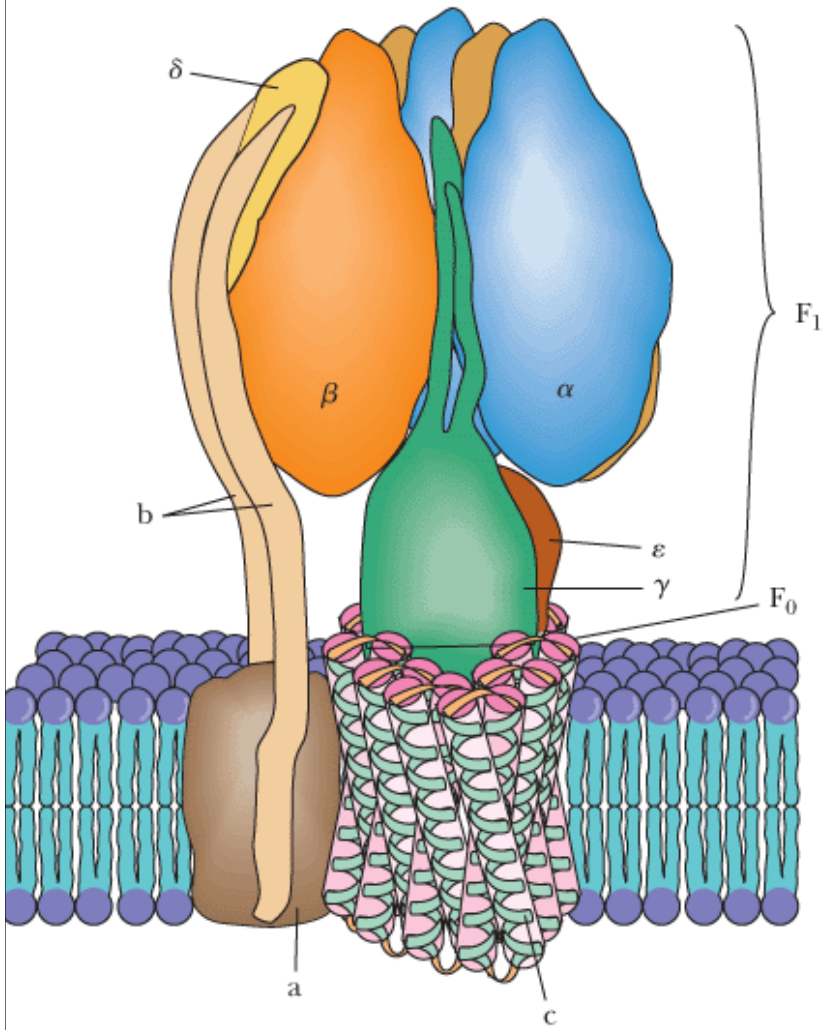
## Jens C. Skou

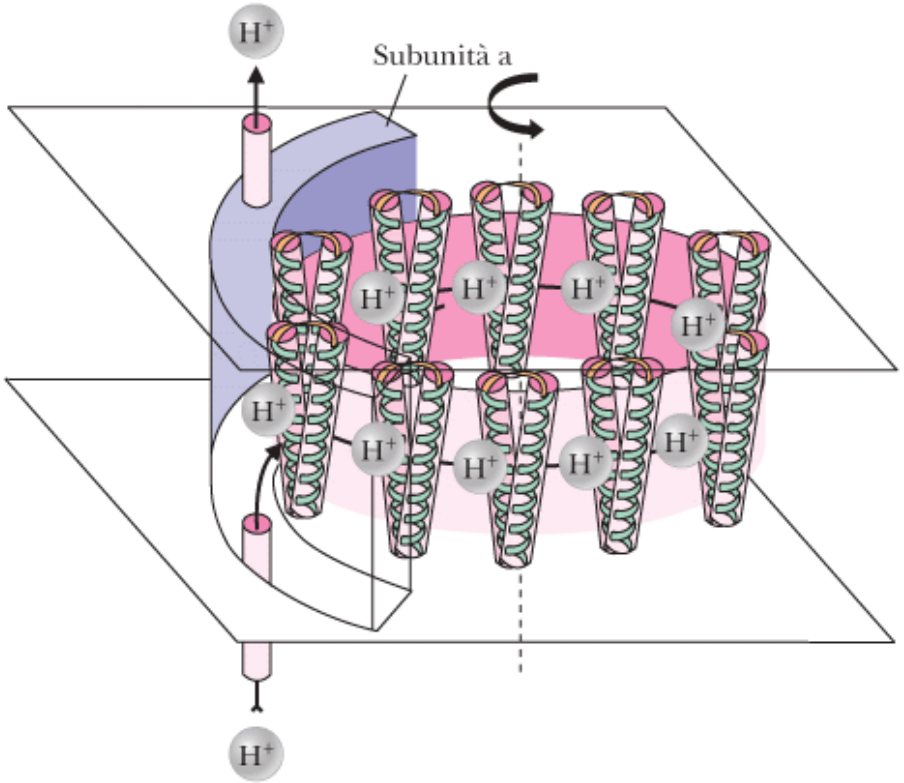
◑1/2 of the prize

Denmark

Aarhus University
Aarhus, Denmark

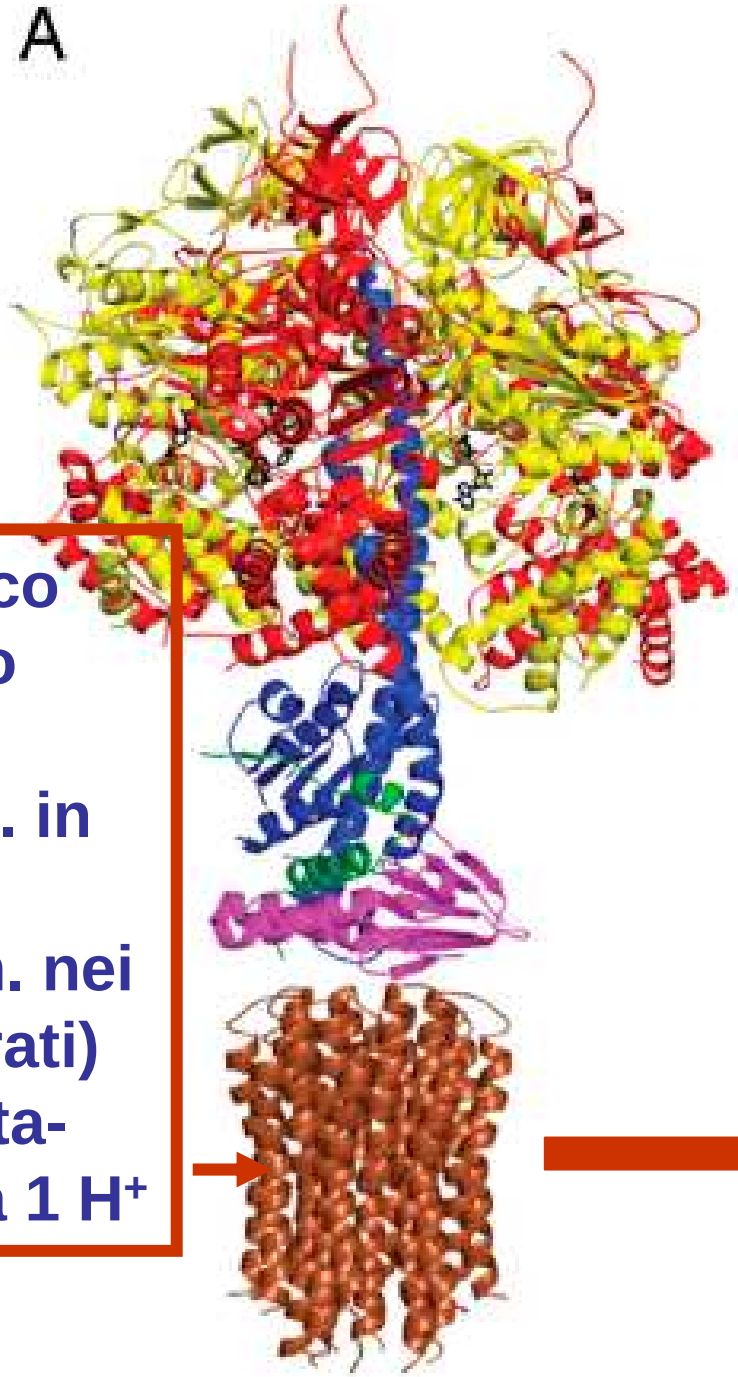
b. 1918

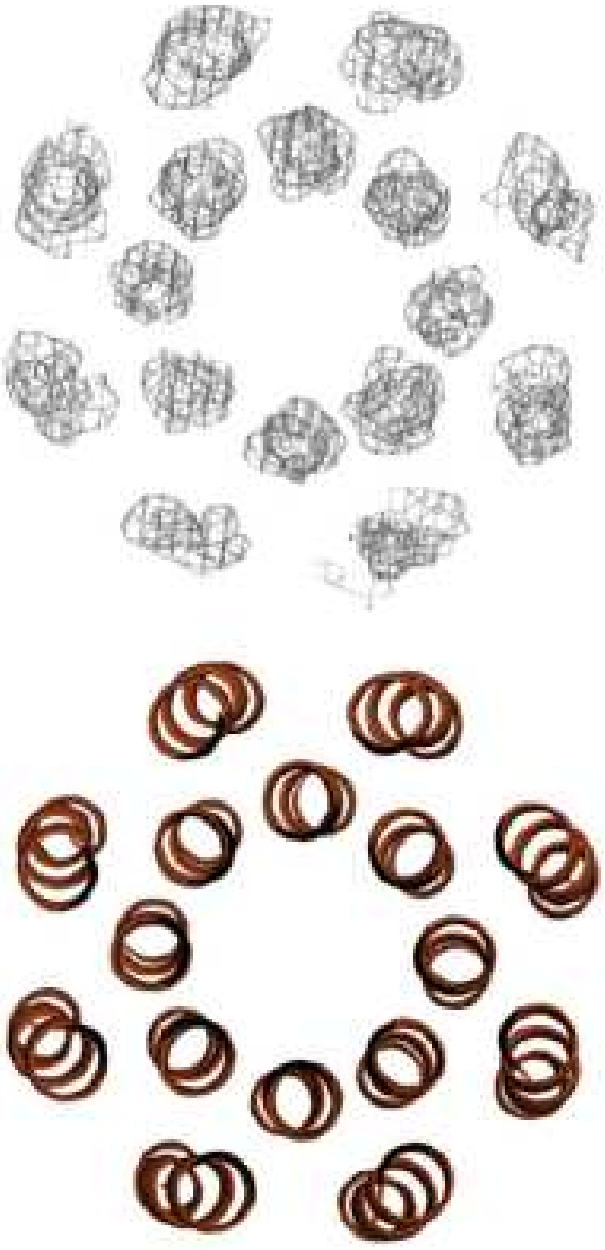

δ
β
α
b
ε
γ
F1
F0
a
c


H+
Subunità a
H+
H+
H+
H+
H+
H+
H+
H+
H+
H+
H+

A

B

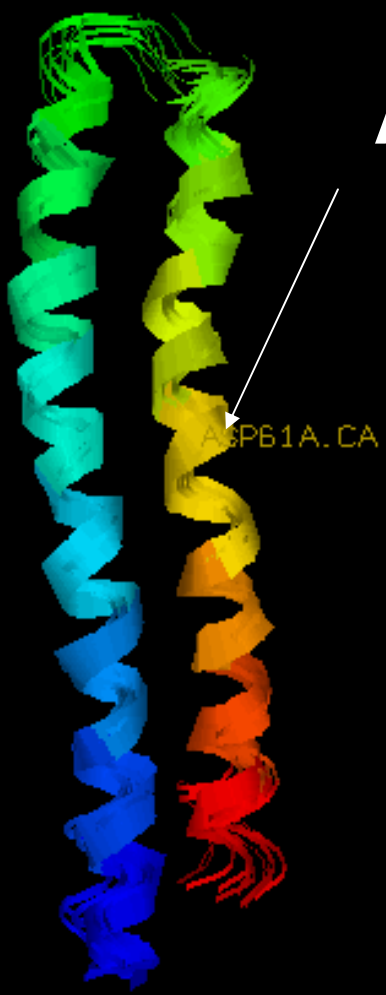




**Un unico residuo acido (aspart. in E.coli- Glutam. nei vertebrati) acquista- rilascia 1 $H^+$**

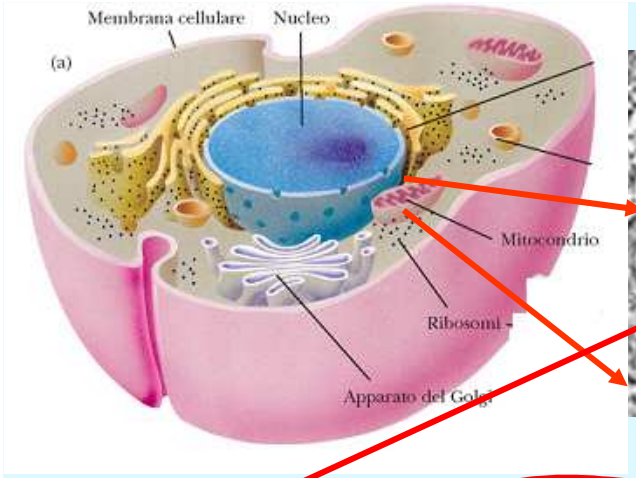
**Fo ATP synthase**

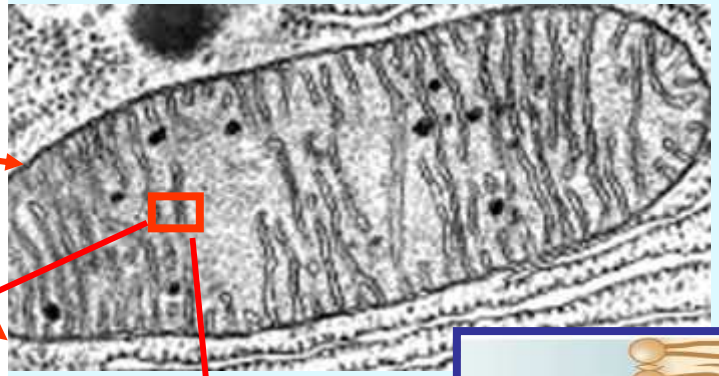
**Subunità c**

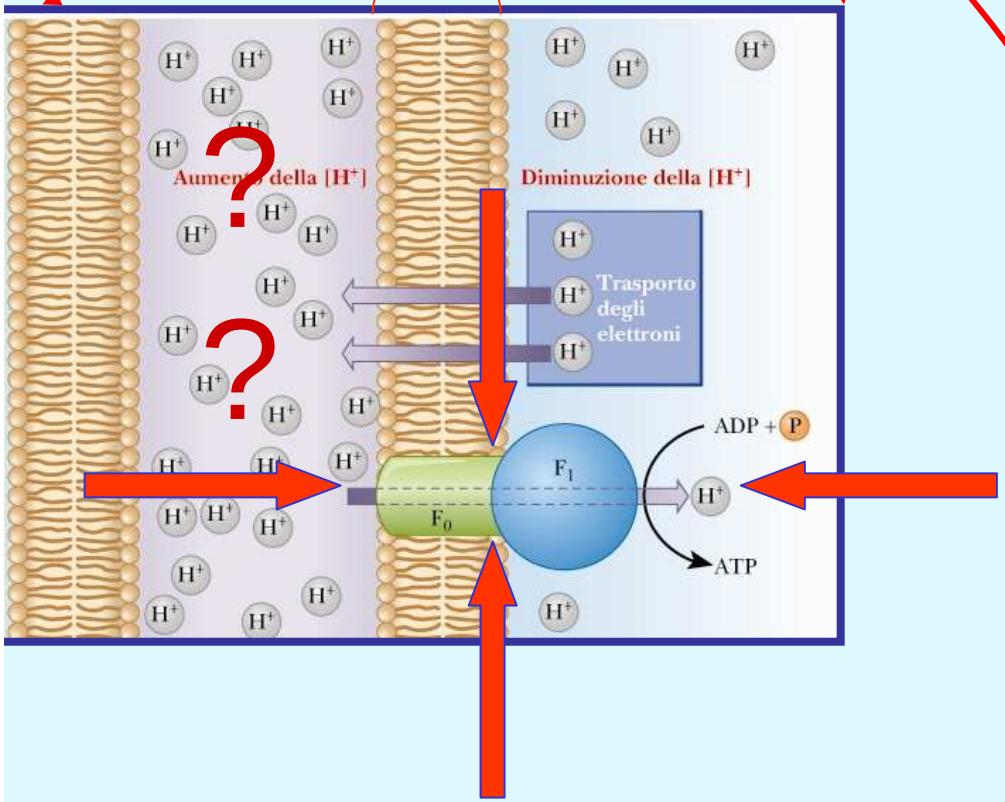


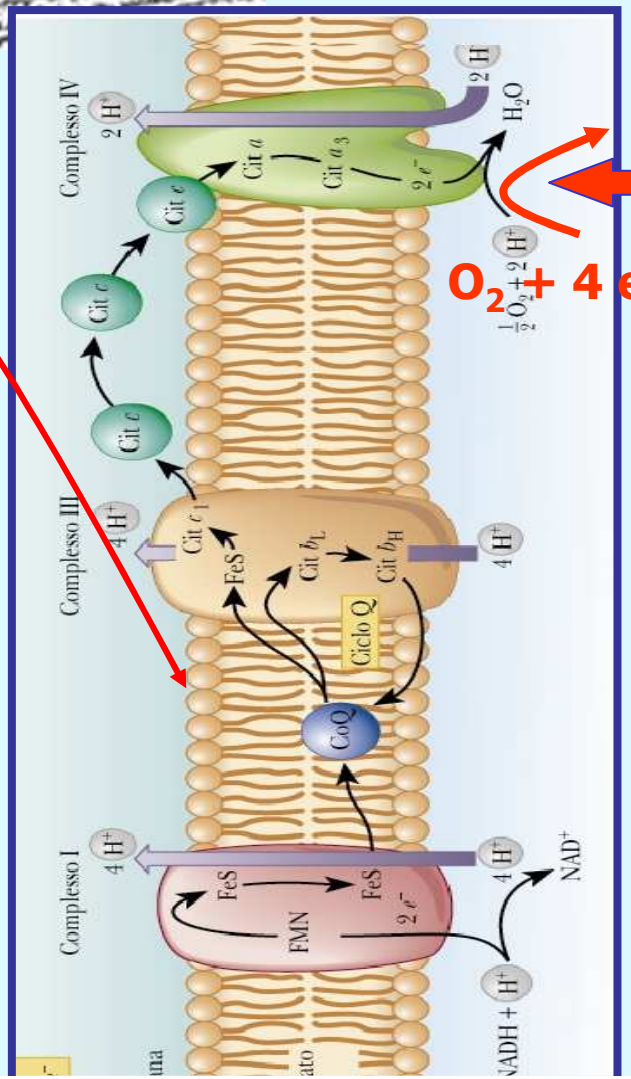

**Aspartico 61**

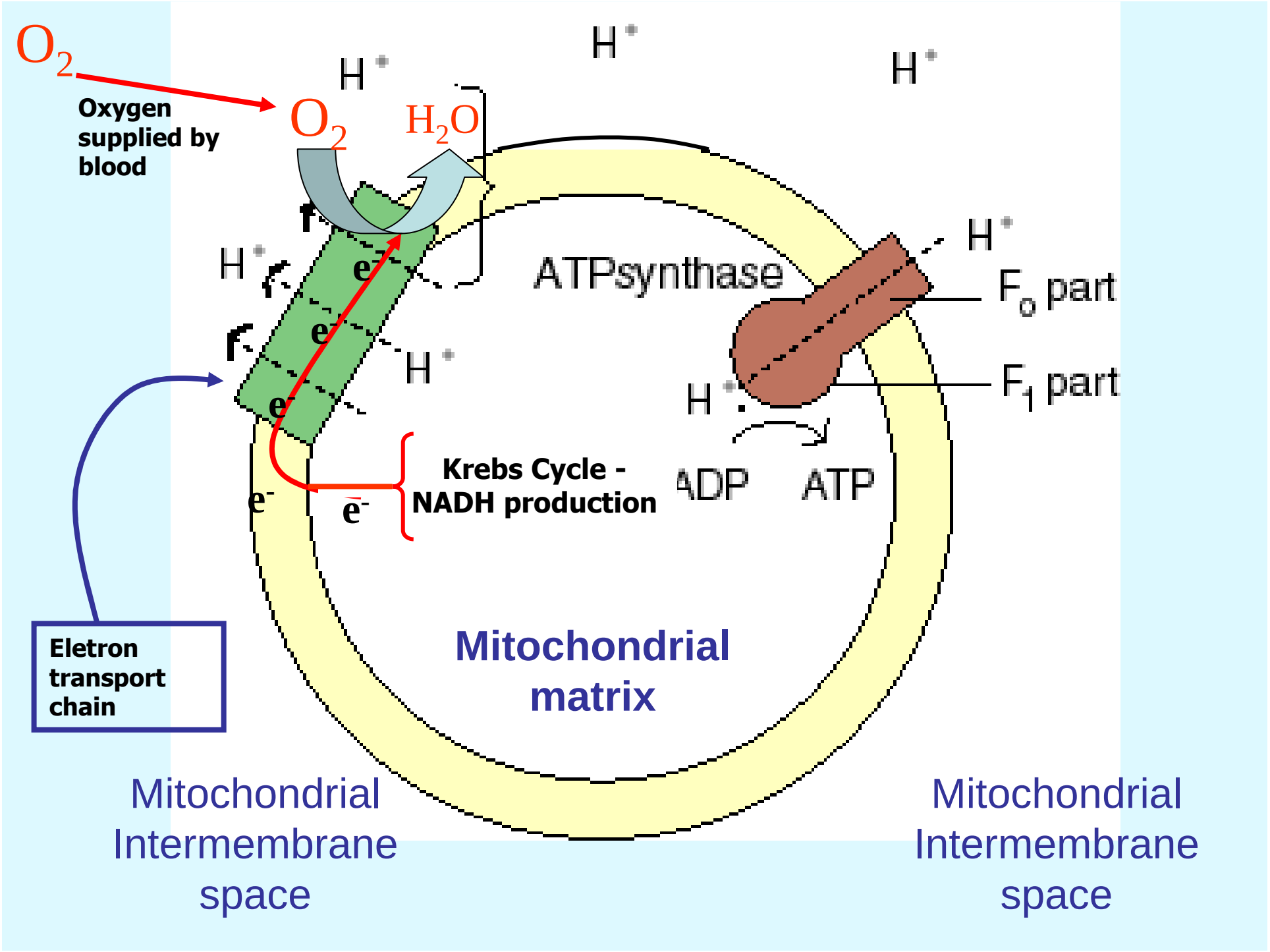
# Il Complesso IV (citocromo ossidasi) riduce l'Ossigeno

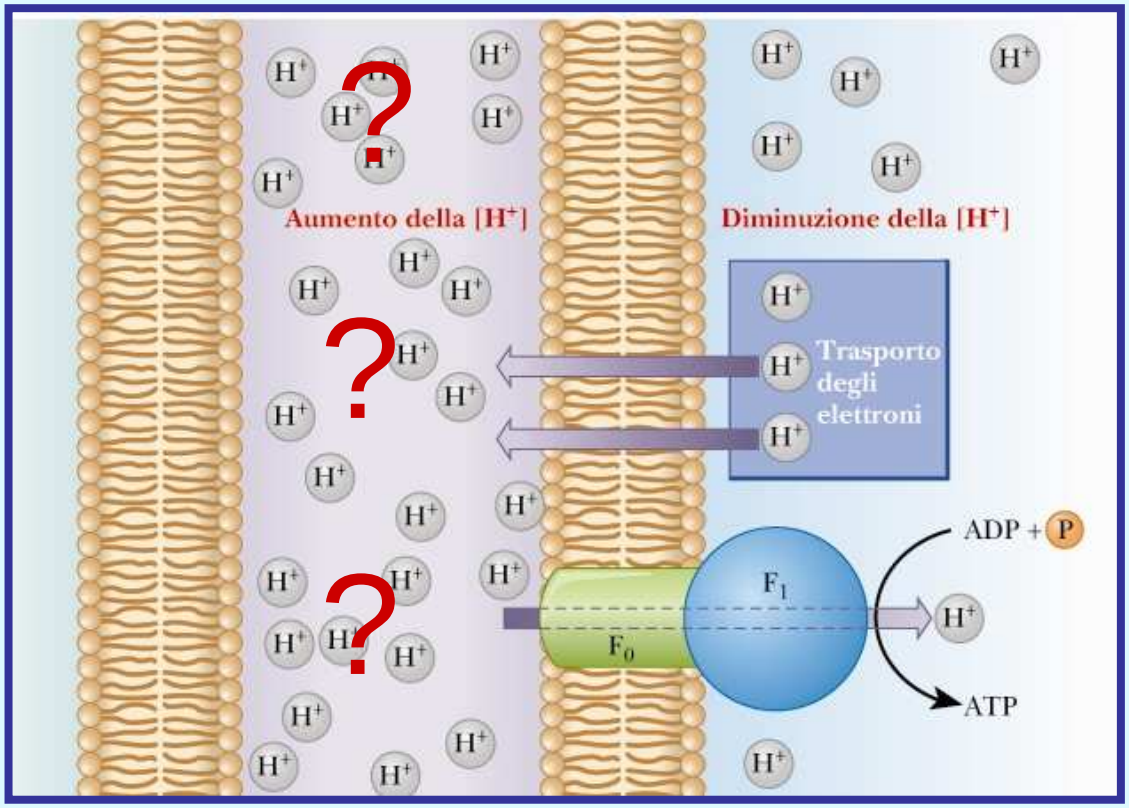





O2
Oxygen supplied by blood
O2
H2O
H+
H+
H+
H+
H+
H+
e-
e-
e-
e-
e-
ATPsynthase
Fo part
F1 part
Krebs Cycle - NADH production
ADP
ATP
Eletron transport chain
Mitochondrial matrix
Mitochondrial Intermembrane space
Mitochondrial Intermembrane space


Aumento della [H+]
Diminuzione della [H+]
Trasporto degli elettroni
ADP + P
F1
F0
ATP

(2)

# Translocation and lateral diffusion of **Protons**


Theodor Von Grutthus fu allievo di Gay-Lussac. Nutriva grande ammirazione per gli studi di Alessandro Volta.
1805 - Von Grotthus e Gay-Lussac migrarono sulle pendici del Vesuvio per effettuare studi sull'eruzione da poco avvenuta.
1805- Von Grotthus pubblica a Roma il famoso pamphlet che anticipa una sua famosa pubblicazione del 1806

1817- Theodore von Grotthus concluse che solo la luce che è assorbita da una molecola può produrre una modifica fotochimica in quella molecola.

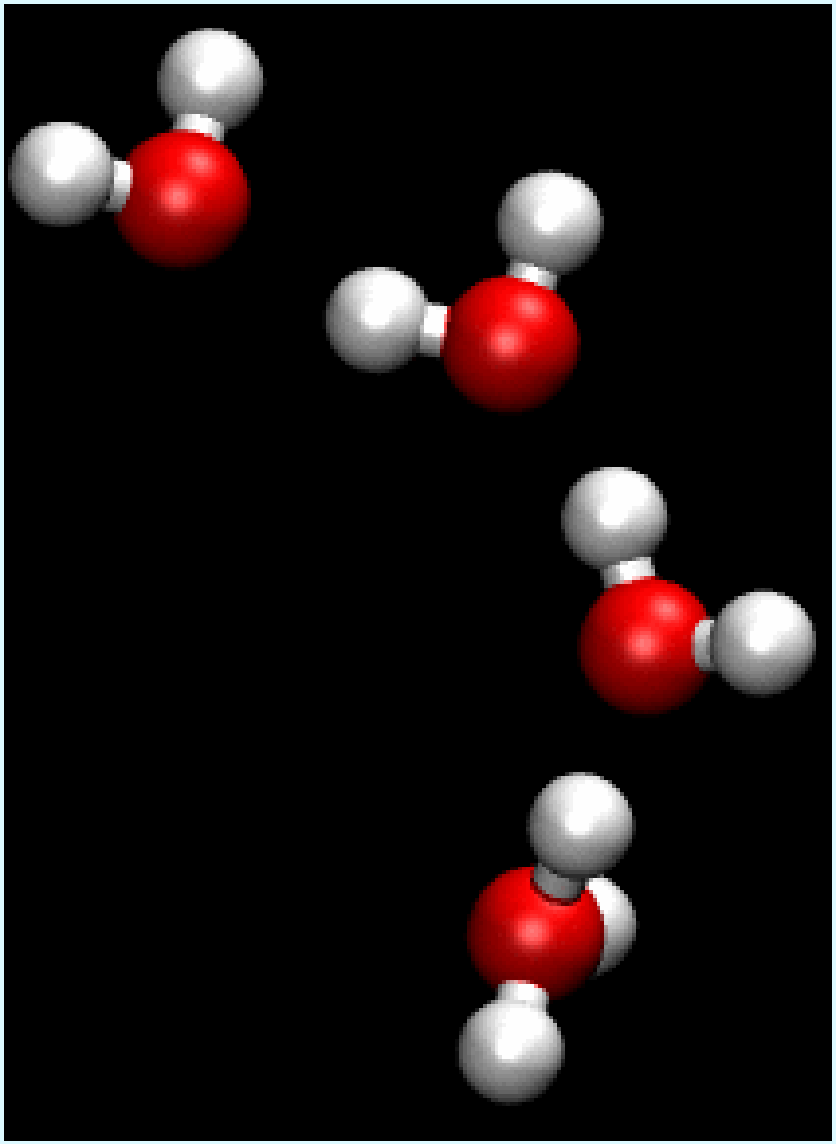
⇨ Teoria della decomposizione dell'acqua da parte dell'elettricità galvanica;

⇨ In analogia con i poli + e — della pila di Alessandro Volta, Von Grotthus formulò l'ipotesi che anche le molecole d'acqua possedessero poli positivi e negativi. In virtù del "semplice contatto tra gli elementi costitutivi (l'idrogeno **h** e l'ossigeno **o**), l'elettricità naturale è ripartita nelle molecole d'acqua in modo tale che h e o acquisiscano, rispettivamente, uno stato *positivo* ed uno stato *negativo"*.

Von Grotthus formulò la teoria della

**DIFFUSIONE STRUTTURALE DEL PROTONE**

# Diffusione strutturale del protone



**Proton diffusion according to the Grotthuss mechanism occurs much faster than molecular diffusion because it is uncoupled from the self-diffusion of its mass (Kreuer, 1996).**

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Biochimica et Biophysica Acta 1757 (2006) 913–930
2006
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Review

# Protons @ interfaces: Implications for biological energy conversion

Armen Y. Mulkidjanian [a,b,*], Joachim Heberle [c,d], Dmitry A. Cherepanov [e]

…The pulsed experiments revealed that proton exchange between the membrane surface and the bulk aqueous phase takes as much as about 1 ms, but could be accelerated by added mobile pH-buffers…

…The membrane-buried layers of these networks can eventually serve as a

storage/buffer for protons (proton sponges)…

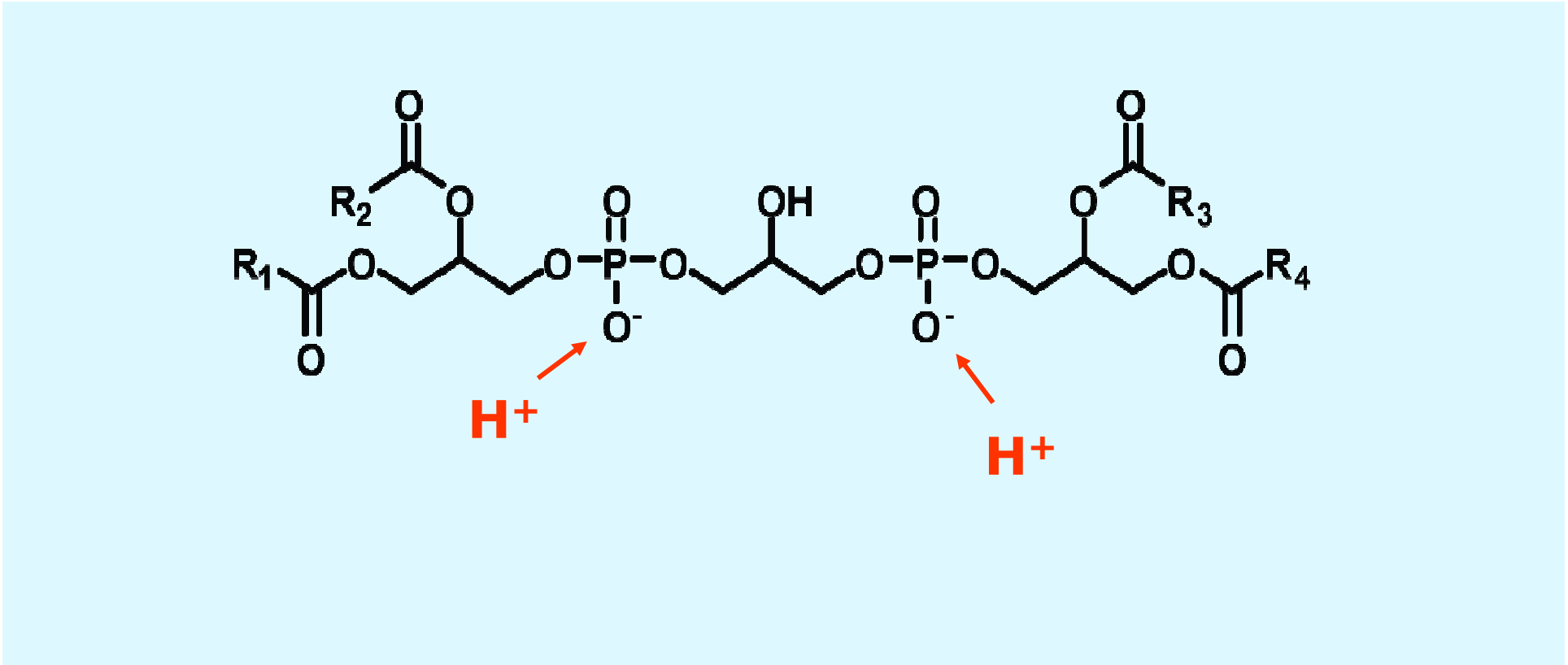
BIOPHYS. J. © Biophysical Society
Volume 50 August 1986 265–276

# DEPENDENCY OF ΔpH-RELAXATION ACROSS VESICULAR MEMBRANES ON THE BUFFERING POWER OF BULK SOLUTIONS AND LIPIDS

STEPHAN GRZESIEK AND NORBERT A. DENCHER
*Biophysics Group, Department of Physics, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33, Federal Republic of Germany*

…A very high contribution of lipid headgroups to the internal buffering power of the liposomes is observed, amounting to an equivalent phosphate buffer concentration of **110 mM**…

…The lipid headgroup region could represent a two-dimensional reservoir capable of **storage and rapid transfer** (Teissie et al., 1985) of protons from proton delivering reactions to proton consuming ones (**e.g., ATPsynthase**)…




FEBS 26508

FEBS Letters 528 (2002) 35–39

Minireview

# Cardiolipin: a proton trap for oxidative phosphorylation

Thomas H. Haines[a,*], Norbert A. Dencher[b]

[a] *Department of Chemistry, City College of the City University of New York, New York, NY 10031, USA*
[b] *Darmstadt University of Technology, Physical Biochemistry, 64287 Darmstadt, Germany*

Received 30 July 2002; revised 15 August 2002; accepted 19 August 2002

First published online 28 August 2002

# Cardiolipin

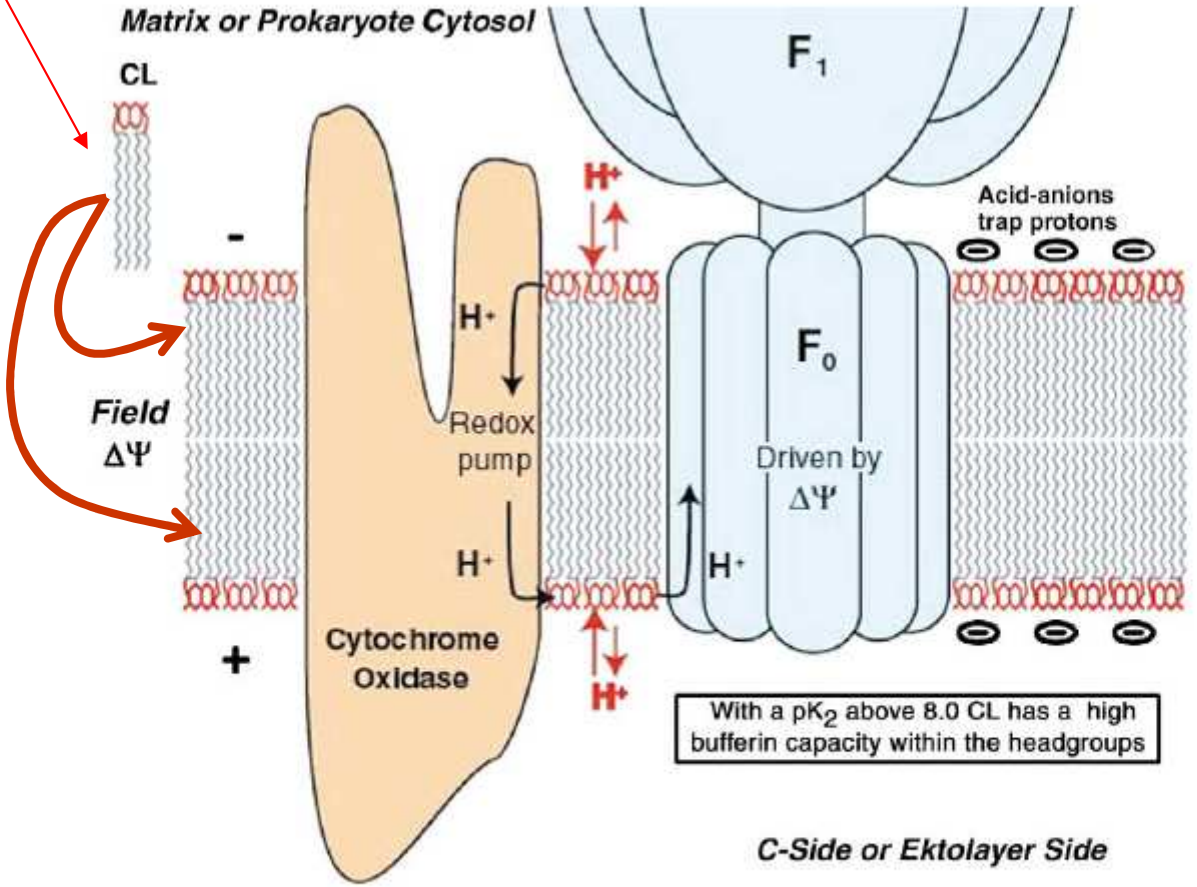
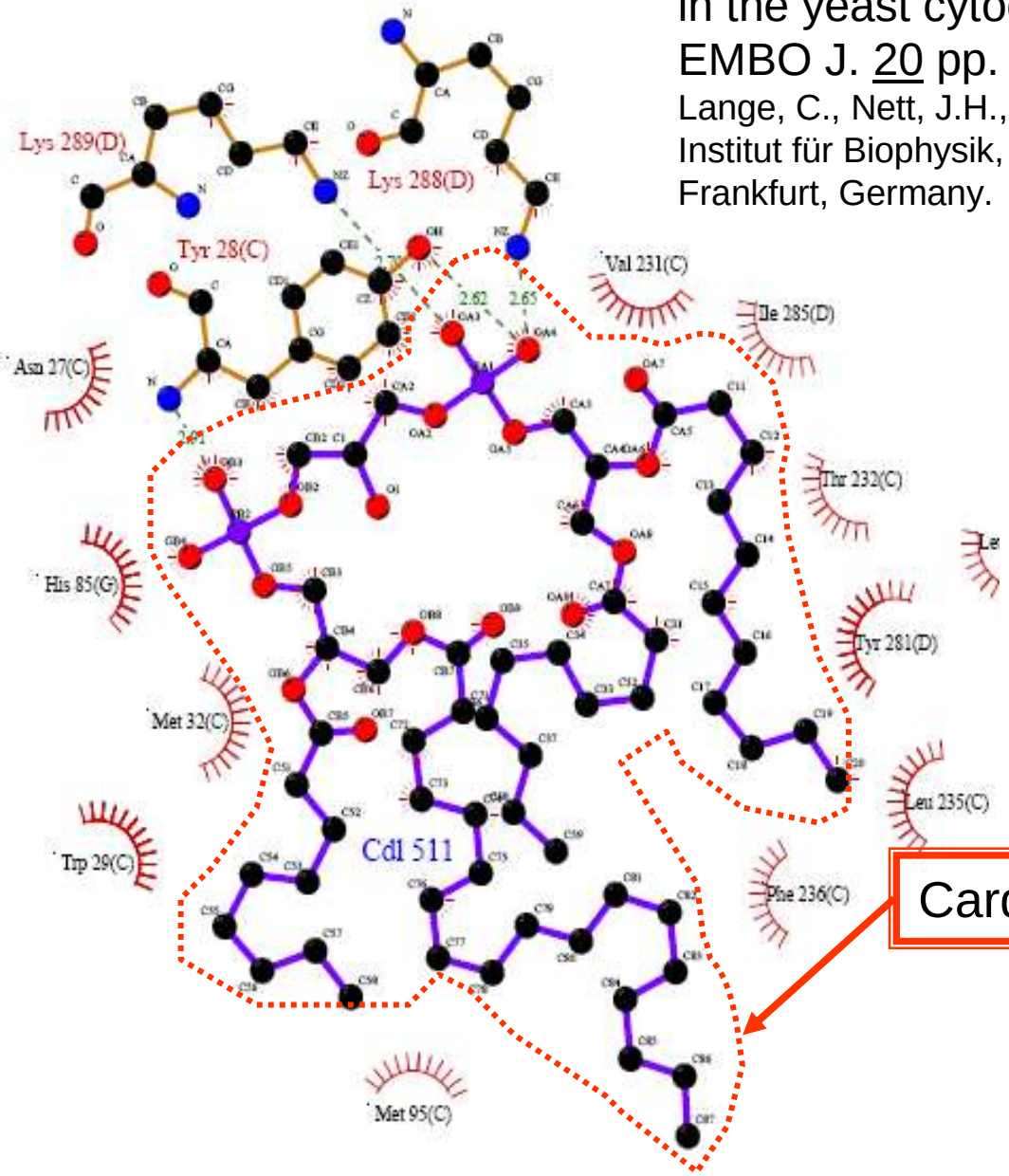


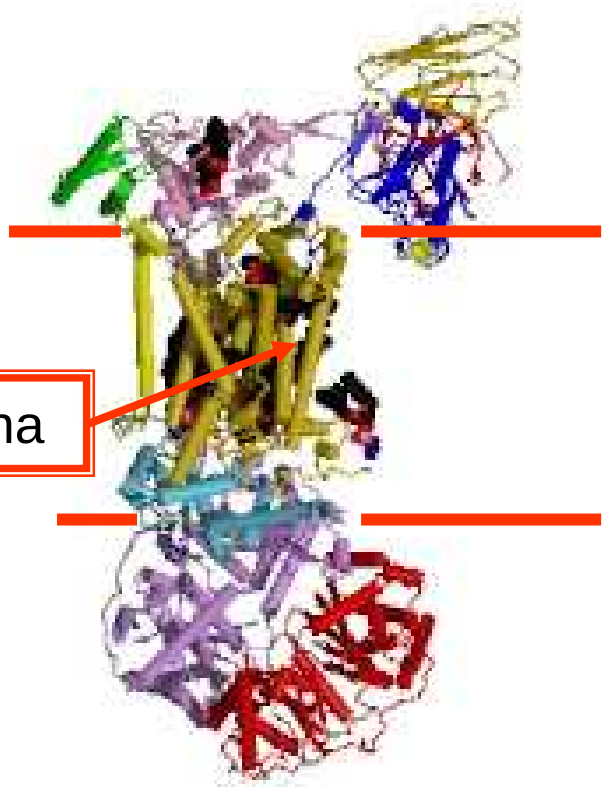

Fig. 3. A cartoon of the unique role of CL as a proton trap for protons in the headgroup domain during oxidative phosphorylation. Its pK (>8.0) is above that of water. It absorbs protons from the bulk phase at both high and low pH. All seven proteins of the oxidative phosphorylation system have high affinity binding sites for CL, suggesting a patch of the lipid may be formed in their presence. A redox-pumped proton need not leave the CL headgroup domain to be delivered to $F_0$. The model implies that it is not the proton, nor the ΔpH, but a single charge, driven by ΔΨ, that is used for ATP synthesis.

Specific roles of protein-phospholipid interactions in the yeast cytochrome bc1 complex structure. EMBO J. 20 pp. 6591-600, 2001

Lange, C., Nett, J.H., Trumpower, B.L., Hunte, C.Max-Planck-Institut für Biophysik, Heinrich-Hoffmann-Strasse 7, D-60528 Frankfurt, Germany. PDB ID: **1KB9**

Struttura 3D del Complesso respirat. III



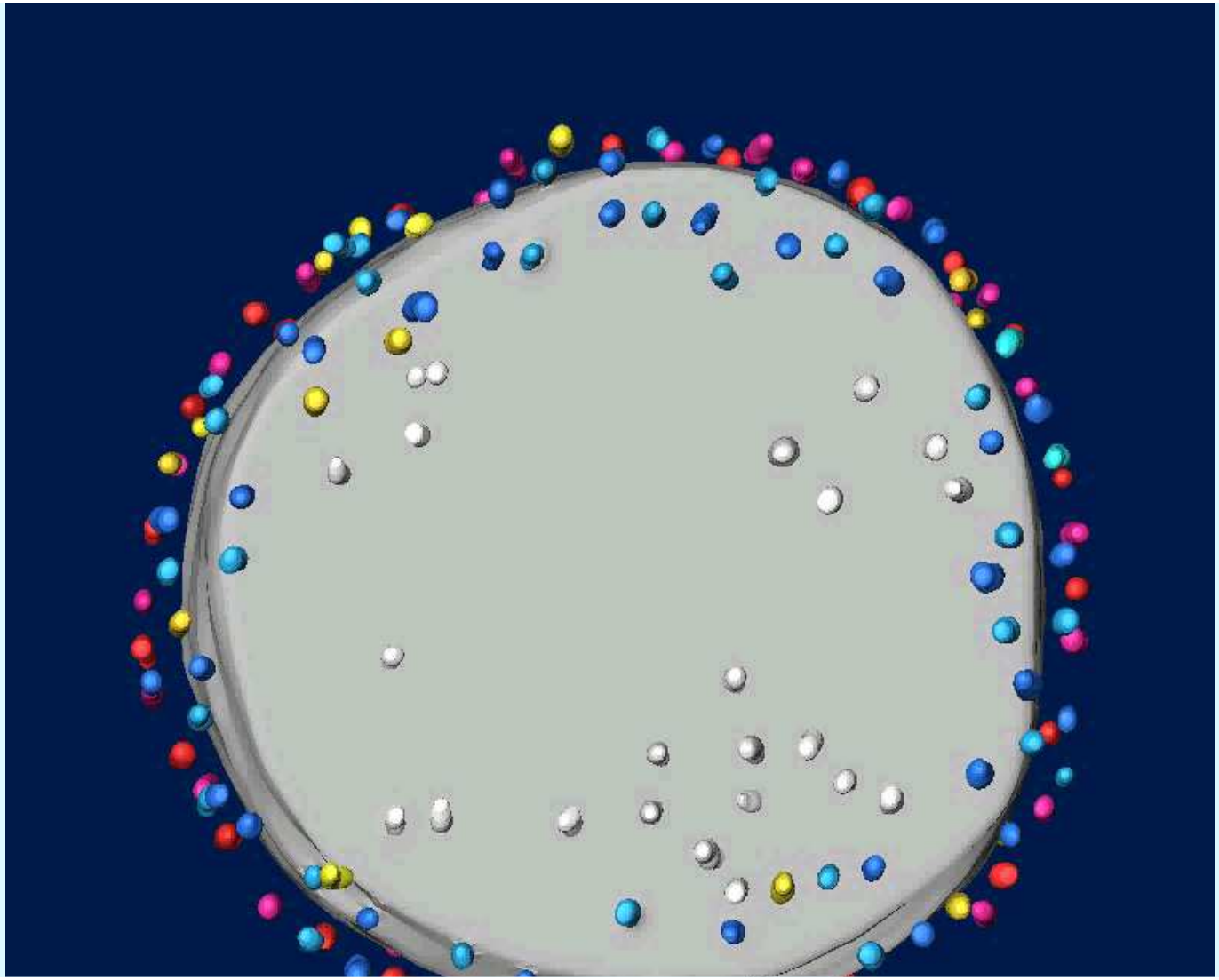



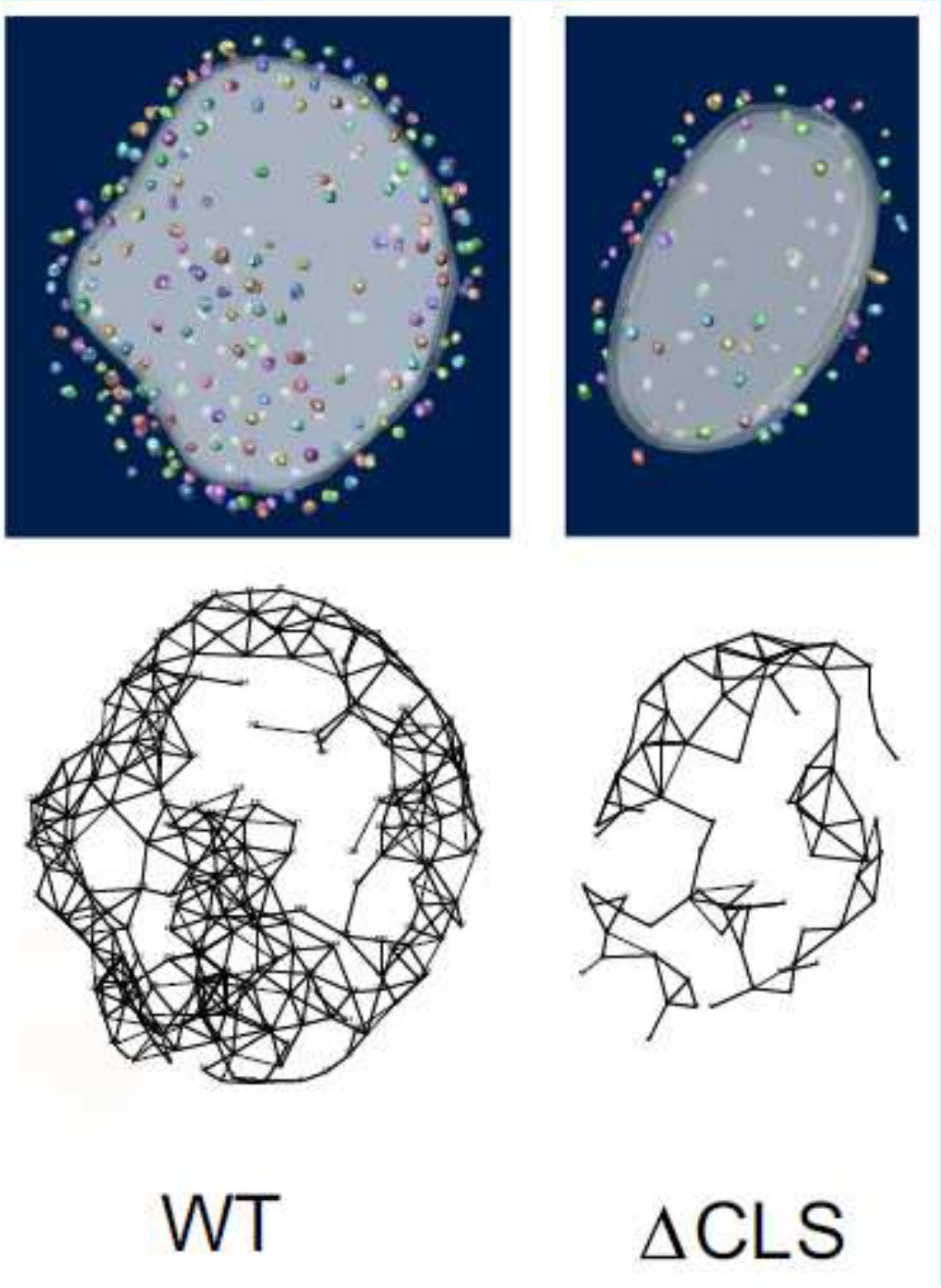
# Cardiolipin Affects the Supramolecular Organization of ATP Synthase in Mitochondria

Biophysical Journal Volume 100 May 2011 2184–2192

Devrim Acehan,[†‡] Ashim Malhotra,[§] Yang Xu,[§] Mindong Ren,[†] David L. Stokes,[†‡¶] and Michael Schlame[†§*]

[†]Department of Cell Biology, [‡]Skirball Institute, Structural Biology Program, and [§]Department of Anesthesiology, New York University School of Medicine, New York, New York; and [¶]New York Structural Biology Center, New York, New York




WT
ΔCLS

# Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria

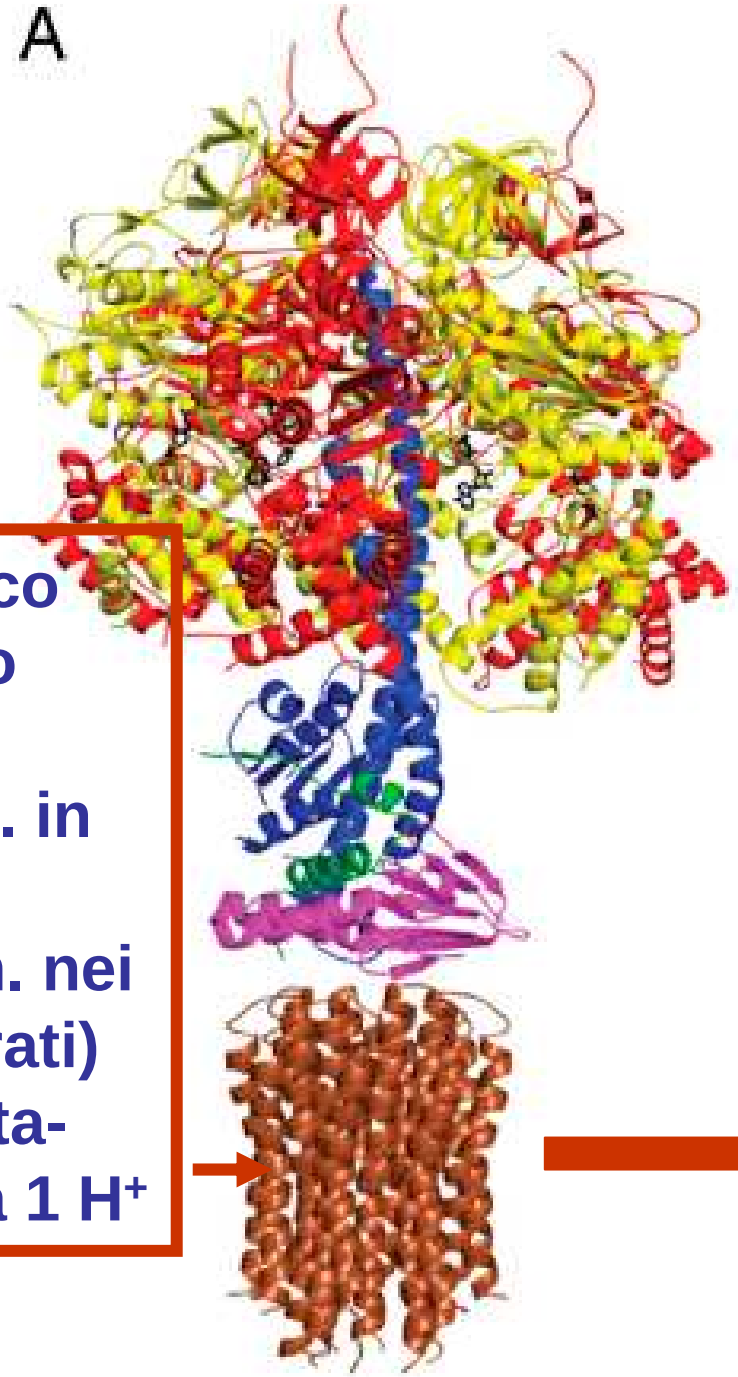
Ian N. Watt[a], Martin G. Montgomery[a], Michael J. Runswick[a], Andrew G. W. Leslie[b,1], and John E. Walker[a,1]

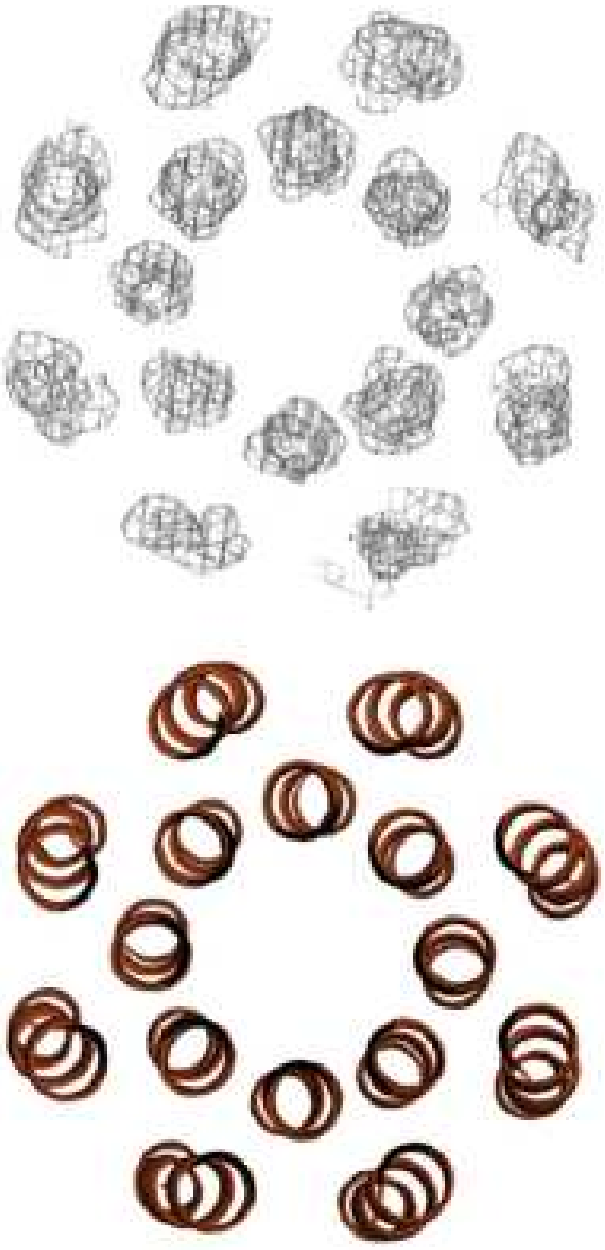
[a]The Medical Research Council Mitochondrial Biology Unit, Hills Road, Cambridge, CB2 0XY, United Kingdom; and [b]The Medical Research Co
Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, United Kingdom

PNAS | September 28, 2010 | vol. 107 | no. 39 | 16823–16827

A

B



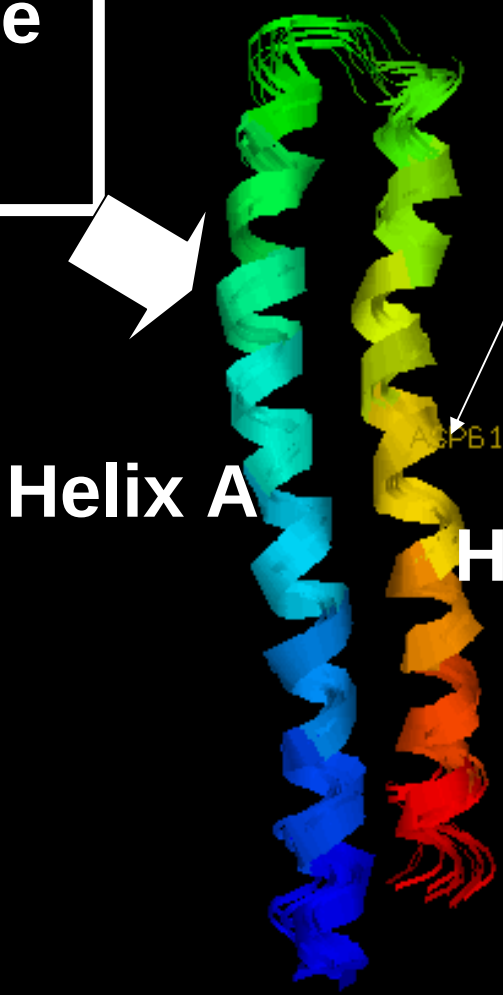


**Un unico residuo acido (aspart. in E.coli- Glutam. nei vertebrati) acquista- rilascia 1 $H^+$**

Helix A | Helix B

**Fo ATP synthase**

**Subunità c**

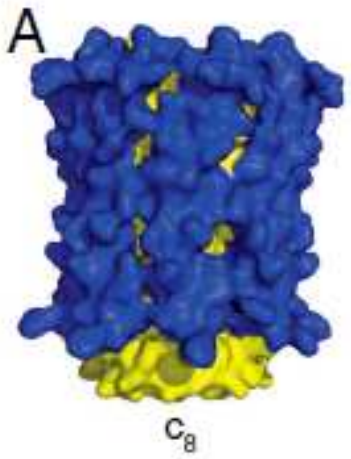


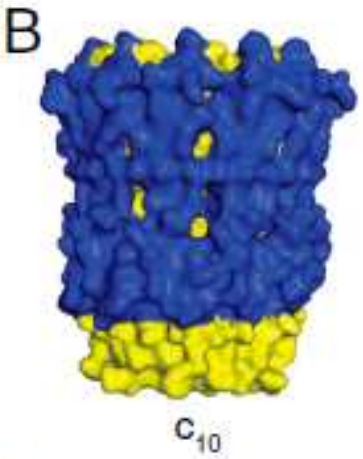

**Aspartico 61**

**Helix A**

**Helix B**

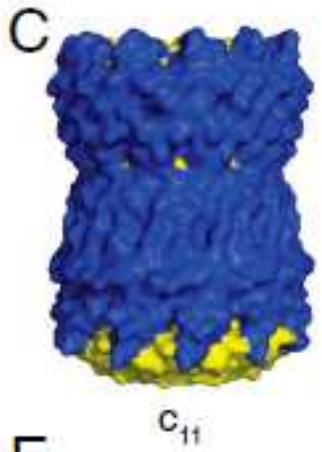
Bovine

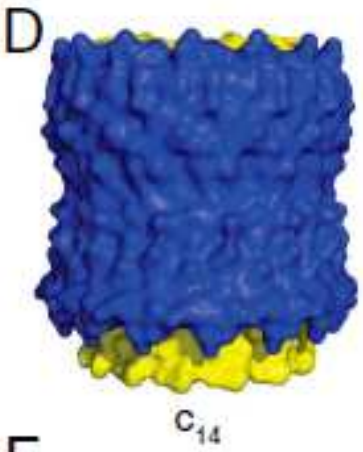



Spinacea oleracea

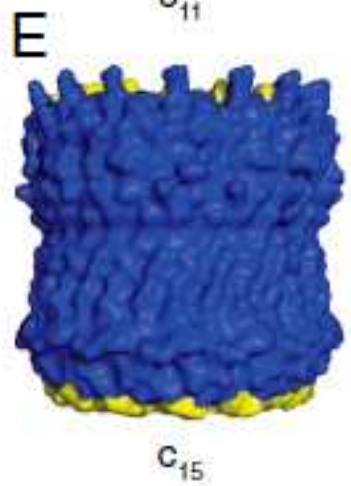
Yeast

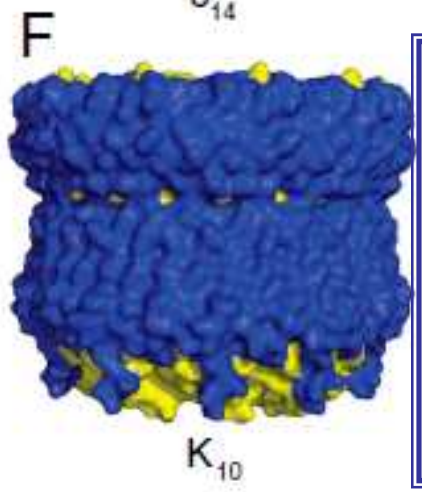



Spirulina platensi (Cianobatterio)

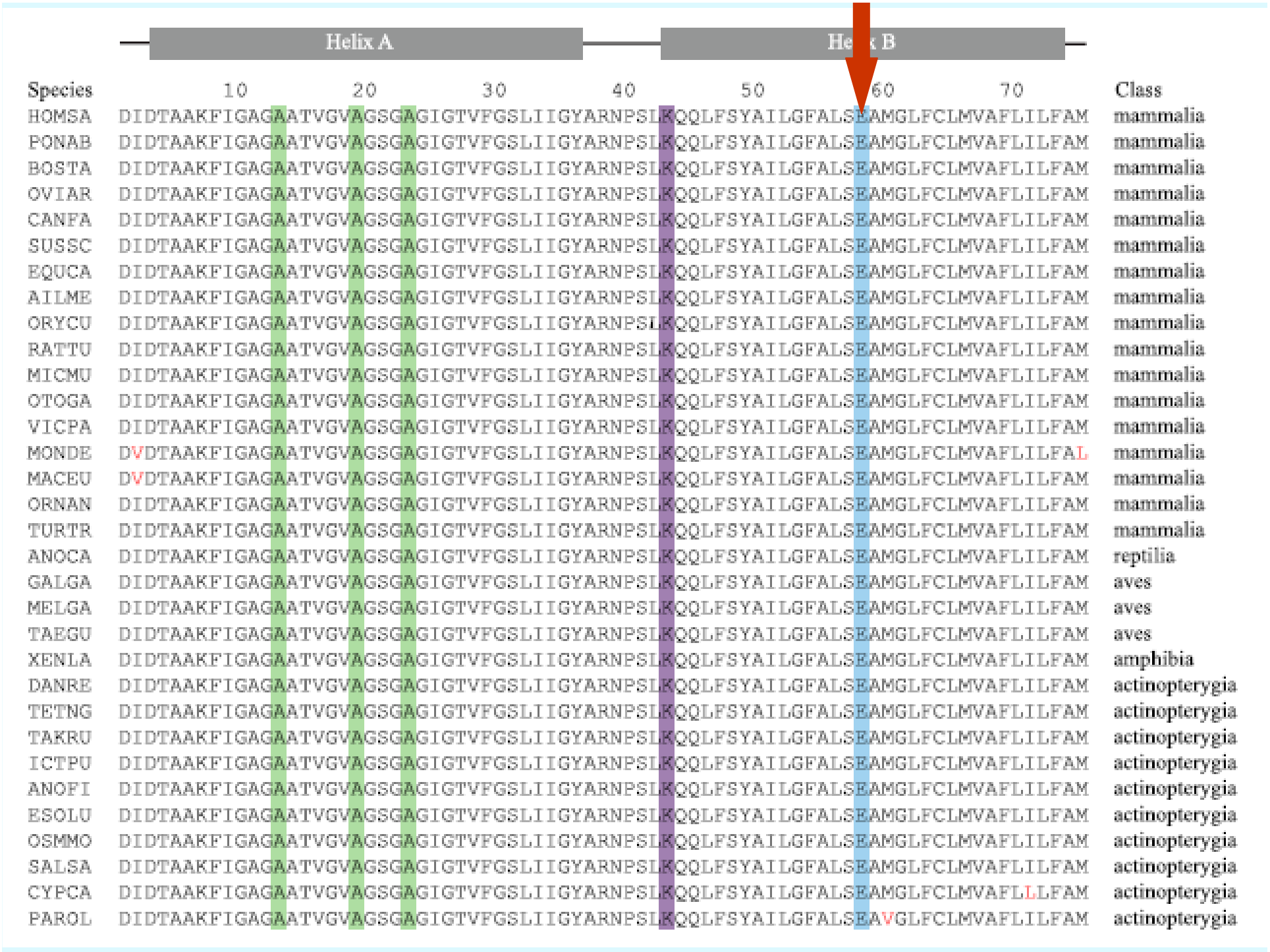
Ilyobacter tartaricus (anaerobico)

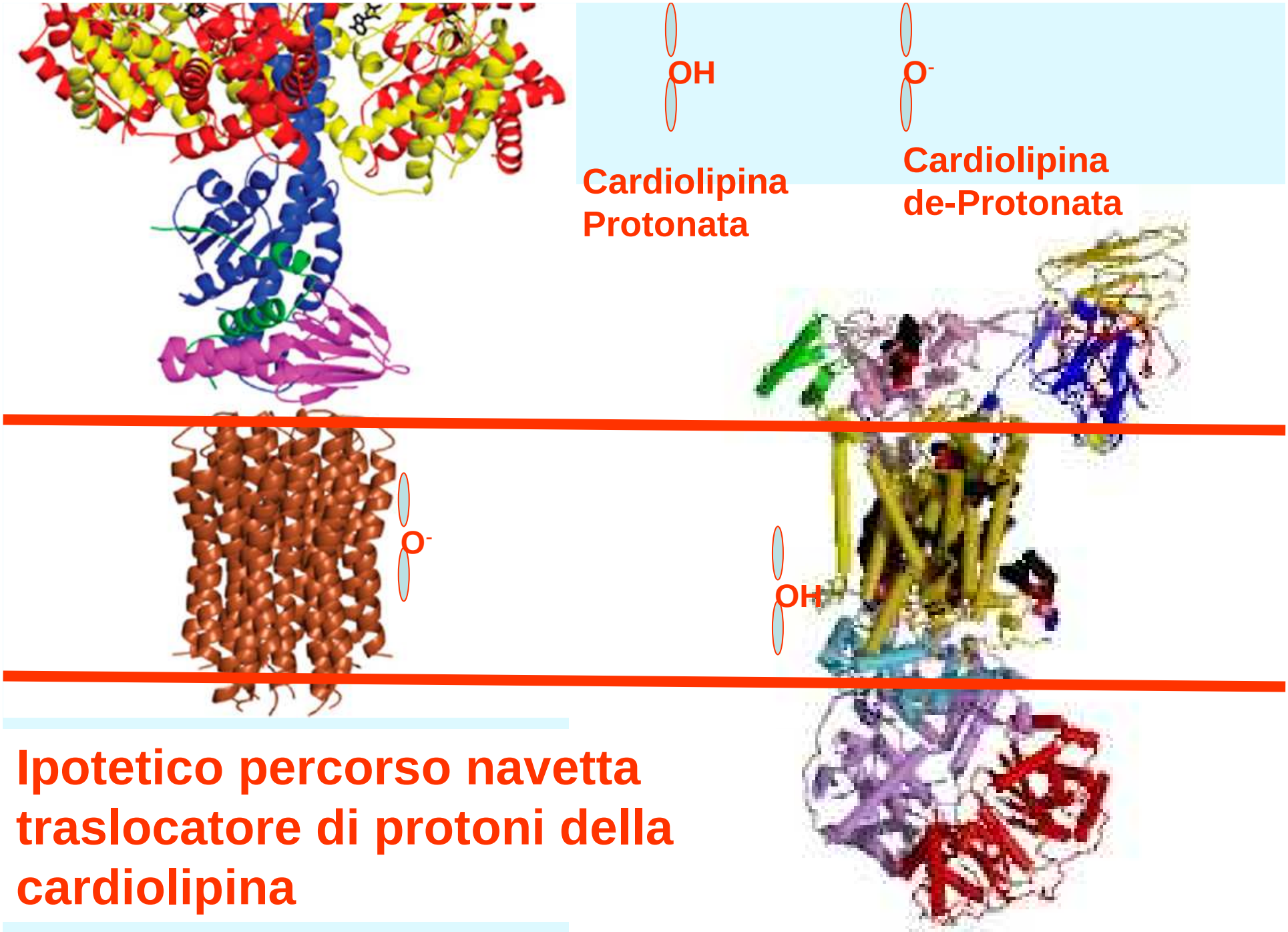



V-Type

Enterococcus hirae (Facoltativo)

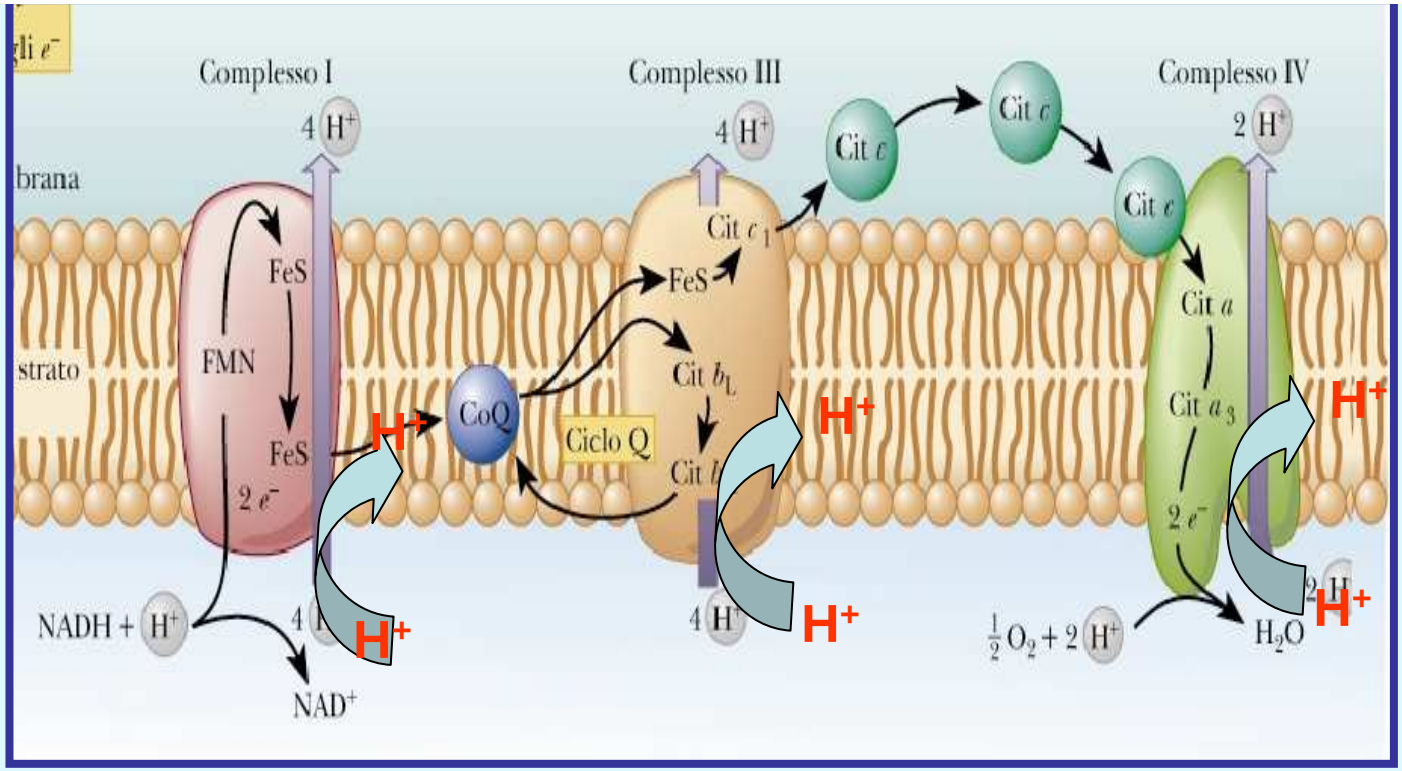

Helix A
Helix B
Species
10
20
30
40
50
60
70
Class
HOMSA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
PONAB DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
BOSTA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
OVIAR DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
CANFA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
SUSSC DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
EQUCA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
AILME DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
ORYCU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
RATTU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
MICMU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
OTOGA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
VICPA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
MONDE DVDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAL mammalia
MACEU DVDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
ORNAN DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
TURTR DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM mammalia
ANOCA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM reptilia
GALGA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM aves
MELGA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM aves
TAEGU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM aves
XENLA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM amphibia
DANRE DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
TETNG DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
TAKRU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
ICTPU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
ANOFI DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
ESOLU DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
OSMMO DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
SALSA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLILFAM actinopterygia
CYPCA DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAMGLFCLMVAFLLFAM actinopterygia
PAROL DIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARNPSLKQQLFSYAILGFALSEAVGLFCLMVAFLILFAM actinopterygia



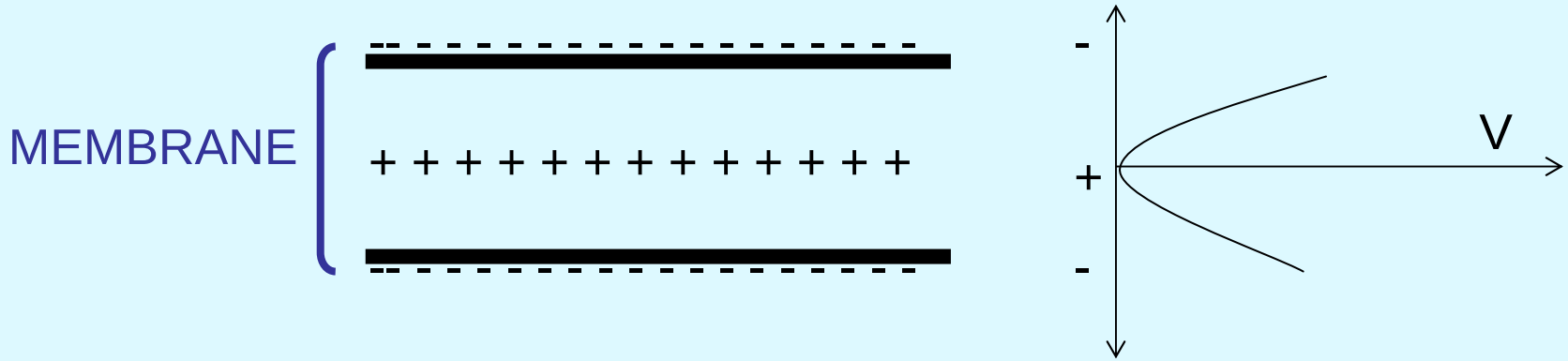

**Ipotetico percorso navetta traslocatore di protoni della cardiolipina**

# (3)

# Ipotesi di potenziali intramembrana




A hypothetical intramembranous asymmetric distribution implies the generation of an intramembrane potential .



Biophysical Journal Volume 84 April 2003 2768–2780

# The Effect of Asymmetric Surface Potentials on the Intramembrane Electric Field Measured with Voltage-Sensitive Dyes

Chang Xu and Leslie M. Loew

Department of Physiology and Center for Biomedical Imaging Technology, University of Connecticut Health Center, Farmington, Connecticut 06030

ABSTRACT Ratiometric imaging of styryl potentiometric dyes can be used to measure the potential gradient inside the membrane (intramembrane potential), which is the sum of contributions from transmembrane potential, dipole potential, and the difference in the surface potentials at both sides of the membrane. Here changes in intramembrane potential of the bilayer membranes in two different preparations, lipid vesicles and individual N1E-115 neuroblastoma cells, are calculated from the fluorescence ratios of di-4-ANEPPS and di-8-ANEPPS as a function of divalent cation concentration. In lipid vesicles formed from the zwitterionic lipid phosphatidylcholine (PC) or from a mixture of the negatively charged lipid phosphatidylserine (PS) and PC, di-4-ANEPPS produces similar spectral changes in response to both divalent cation-induced changes in intramembrane potential and transmembrane potential. The changes in potential on addition of divalent cations measured by the fluorescence ratios of di-4-ANEPPS are consistent with a change in surface potential that can be modeled with the Gouy-Chapman-Stern theory. The derived intrinsic 1:1 association constants of Ba and Mg with PC are 1.0 and 0.4 $M^{-1}$; the intrinsic 1:1 association constants of Ba and Mg with PS are 1.9 and 1.8 $M^{-1}$. Ratiometric measurements of voltage sensitive dyes also allow monitoring of intramembrane potentials in living cells. In neuroblastoma cells, a tenfold increase of concentration of Ba, Mg, and Ca gives a decrease in intramembrane potential of 22 to 24 mV. The observed changes in potential could also be described by Gouy-Chapman theory. A surface charge density of 1 $e^-$/115 $Å^2$ provides the best fit and the intrinsic 1:1 association constants of Ba, Mg, and Ca with acidic group in the surface are 1.7, 6.1, and 25.3 $M^{-1}$.

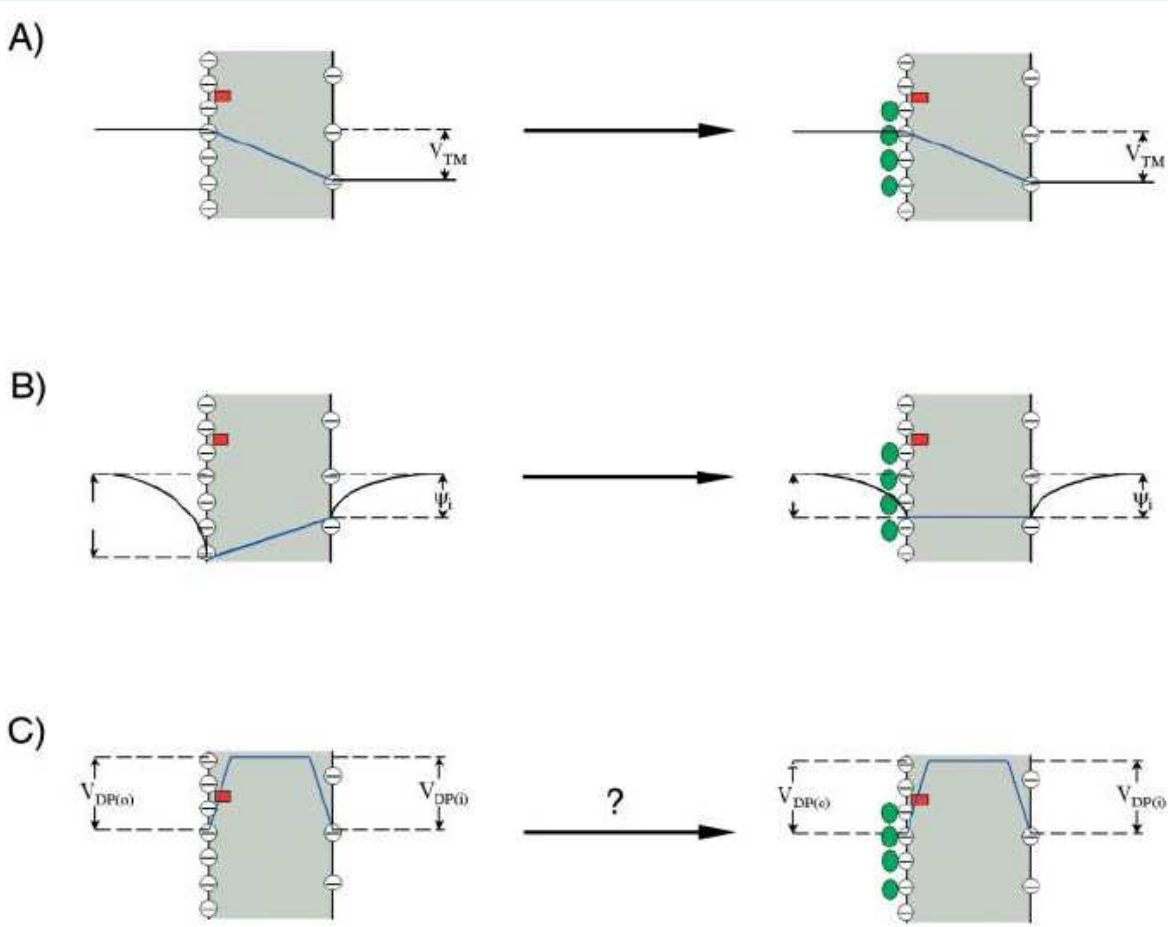
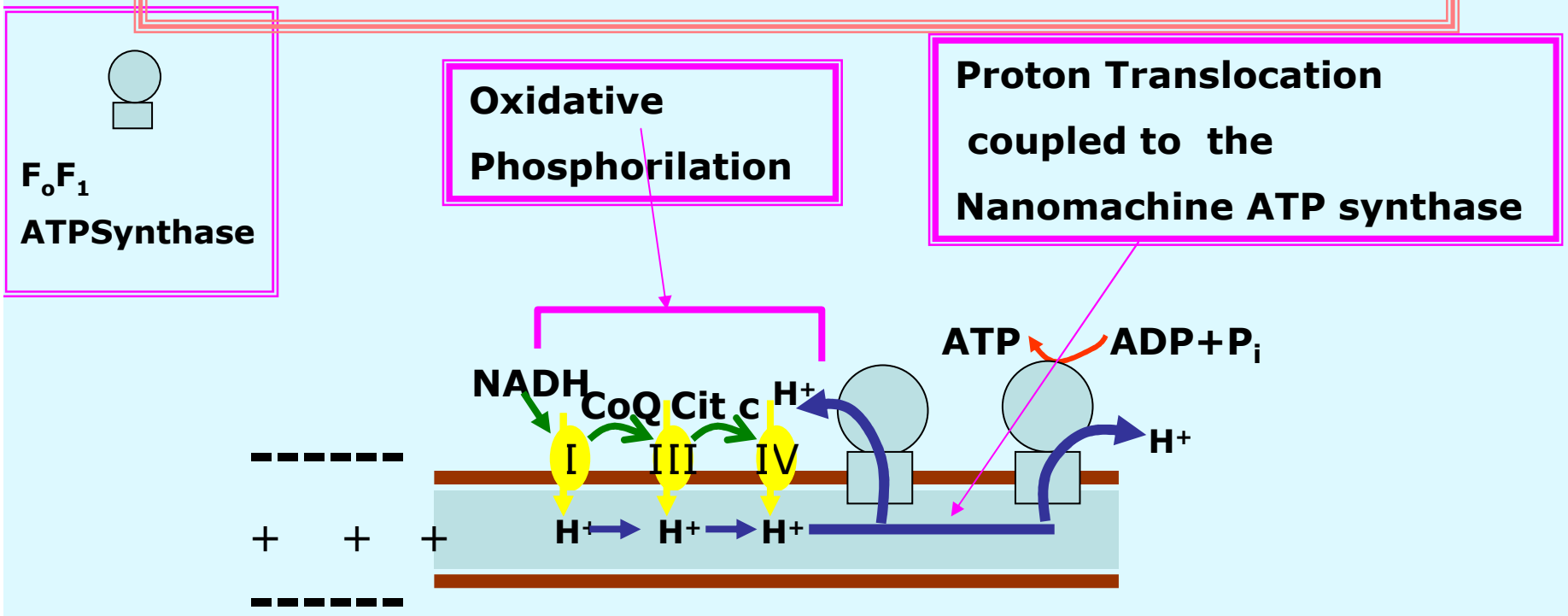



FIGURE 1 Effect of extracellular cations on the potential profile across a cell plasma membrane. The dye chromophores (*red rectangle*) is sensing the electric field (*blue line*) generated by transmembrane potential ($V_{TM}$) (*A*), the difference between the external surface potential ($\psi_o$) and internal surface potential ($\psi_i$) (*B*), and the dipole potential at the outer surface ($V_{DP(o)}$) (*C*). We define all potential differences as potential inside minus potential outside. When the negative charges on the outer surface are neutralized and bound by cations (*green oval*), the equilibrium values of $V_{TM}$ is unchanged. However, the $\psi_o$ is reduced, leading to an increase in intramembrane potential across the membrane, although $\psi_i$ is unchanged. The effect of divalent cation binding on dipole potential is relatively small, especially at the concentrations used in this study. Therefore, the change in intramembrane potential, $\Delta\Delta V$, which is calculated from Eq. 7 ($\Delta\Delta V = \Delta V_{TM} + \Delta(\psi_i - \psi_o) + \Delta V_{DP}$), is approximately equal to $-\Delta\psi_o$.

# *sorting-out Mono-side of protons*



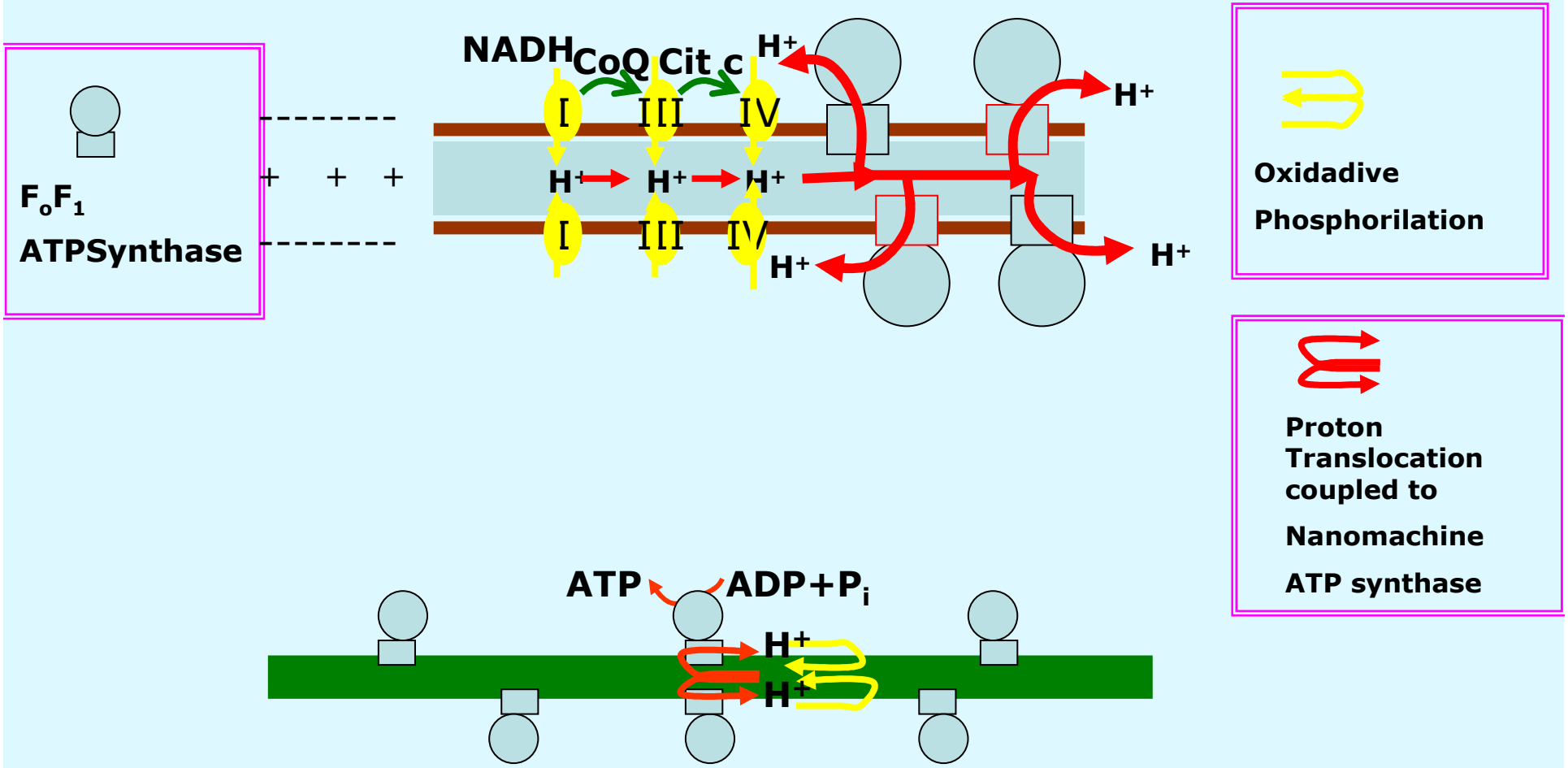

» Diffusion of protons (by proton-trap)

» Intramembrane Potential: positive at the centre of the membrane, negative on the two surphaces

- **With the assumptions that protons can display a lateral diffusion (free or carried by a phospholipid) it is possible to overcome the assumption that requires a closed compartment where the protons accumulate**

- **Implication: in any membrane with ATP synthase driven by proton diffusion, you can have the extramitocondrial synthesis of ATP.**

# *sorting-out Bi-side of protons*



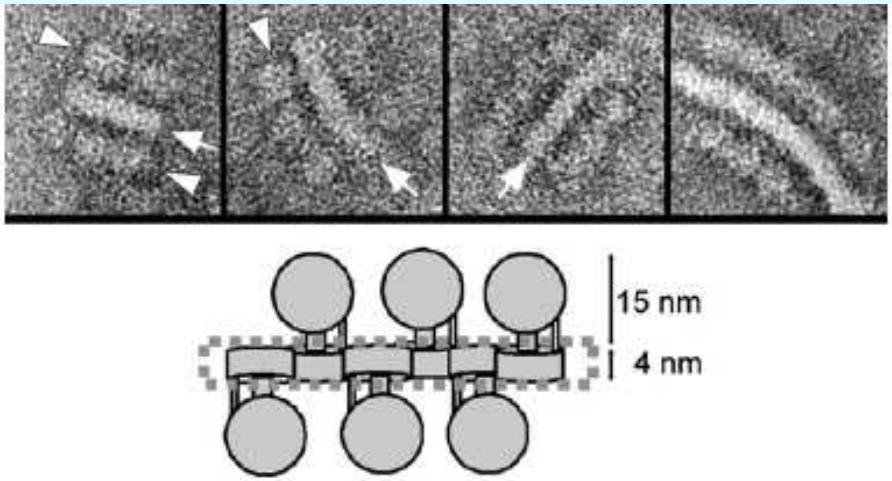
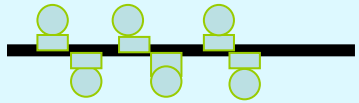



Fig. 2. TEM of bovine $F_1F_o$-ATP synthase reconstituted at low LPR. Electron micrograph: ATP synthase reconstituted with egg yolk PC at an LPR of 0.4 (w/w). Detergent (DDM) was removed by addition of Bio-Beads. Gallery: The areas in the electron micrograph marked by broken squares were magnified. Strings of $F_1F_o$-ATPase complexes in an up-down orientation are discerned. $F_1$ and $F_o$ domains are indicated by arrowheads and arrows, respectively. Bottom: Proposed model for the orientation of the ATP synthases in the imaged strings. Scale bar: 200 nm. Frame sizes of insets: 65 nm.



Journal of Structural Biology 160 (2007

# Reconstitution of mitochondrial ATP synthase into lipid bilayers for structural analysis

Ignacio Arechaga [a,*], Dimitrios Fotiadis [b,*]

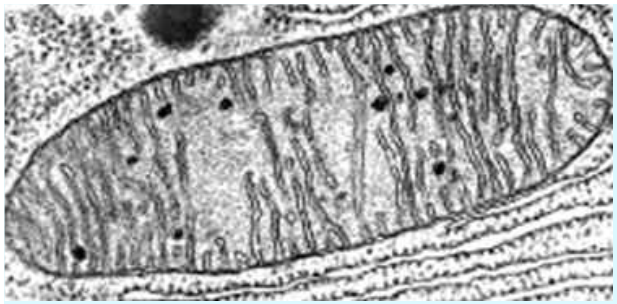
[a] *MRC Dunn Human Nutrition Unit, Hills Road, Cambridge, UK*
[b] *M.E. Müller Institute for Microscopy, Klingelbergstrasse 70, CH-4056, Basel, Switzerland*

Received 2 May 2007; received in revised form 10 July 2007; accepted 12 September 2007

(4)

question:

are mitochondria the ”exlusive” site of aerobic ATP synthesis ?



Mitochondria (and thylacoid disks) are considered the exclusive site of aerobic ATP synthesis. It is true?

**Objection n°1 :**

It is well known that in vitro mitochondria produce little ATP and that there is the need to energize them with pyruvate and to add cyclosporin-A, etc.), which seems anomalous.

Other cellular processes (DNA duplication, proteic synthesis, etc.) can procede *in vitro* with the same if not higher efficiency than *in vivo*.

**Objection n°2** :

How can ADP and/or ATP cross 4 membranes? ATP should be synthesized where it is utilized, like in bacteria.

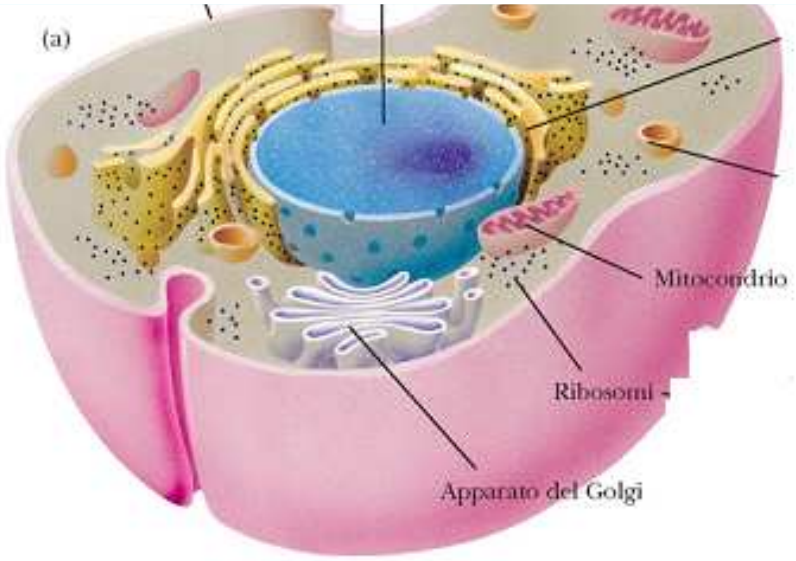
**Objection n°3** : when a symbiosis is estabilished there must be a mutual advantage especially when essential functions are involved.

ATP SYNTHESIS is the most essential function for a cell and it seems strange that the cell would demand to mitochondria such monopoly.

ATP Turnover :

procaryotes: ~ 20 sec

eukariotes : ~ 2 – 3 min




**<u>Objection n°4</u>** : If the great amount of oxygen elaborated by the cell came exclusively from mitochondria, these would produce so many free radicals to cause seroius damage to the particularly vulnerable mitochondrial DNA.

**ObJection n°5** : the ATP synthesizing apparatus fo mitochondria has a capacity similar to that of bacteria, therefore can only sustain its metabolic processes.
If the mitochondria should really produce all of the ATP required from the cell it should possess an ATP-synthesizing molecular machinery far more efficient.

MOLECULAR CELLULAR BIOCHEMISTRY
February 6, 1977 Volume 14, numbers 1-3

**GENETIC DETERMINATION OF THE MITOCHONDRIAL ADENINE NUCLEOTIDE TRANSLOCATION SYSTEM AND ITS ROLE IN THE EUKARYOTIC CELL**

Ladislav KOVAC, Jordan KOLAROV and Julius SUBIK

- The AdN translocation system serves not only to transport ATP synthesized within mitochondria into the cytosol but also to transport cytosolic ATP into the mitochondria when oxidative phosphorylation is not functioning.

- It assures appropriate concentrations of intramitochondrial ATP.

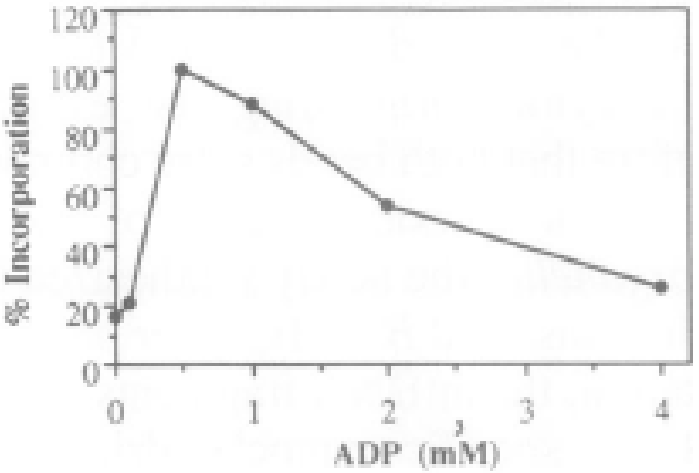
# Objection n°6 :

*Nucleic Acids Research, 1994, Vol. 22, No. 10* **1861**

## Highly efficient DNA synthesis in isolated mitochondria from rat liver

José A.Enríquez, Javier Ramos, Acisclo Pérez-Martos, Manuel J.López-Pérez and Julio Montoya*
Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain

**As the concentration of ADP increases, the intramitochondrial ATP concentration would be higher and the whole molecule could be replicated with the consequent appearance of the two BamHI fragments.**



**Objection n°7 :**

If di Ethidium Bromide is given to cultured cells (for ex. Hepa1-6 cells) these suffer a dramatic damage to mitochondrial DNA (before the nuclear DNA, which shows that mitochondrial DNA is little protected and displays a faster turnover) **and you get even more vital cells (and paradoxically) known as**

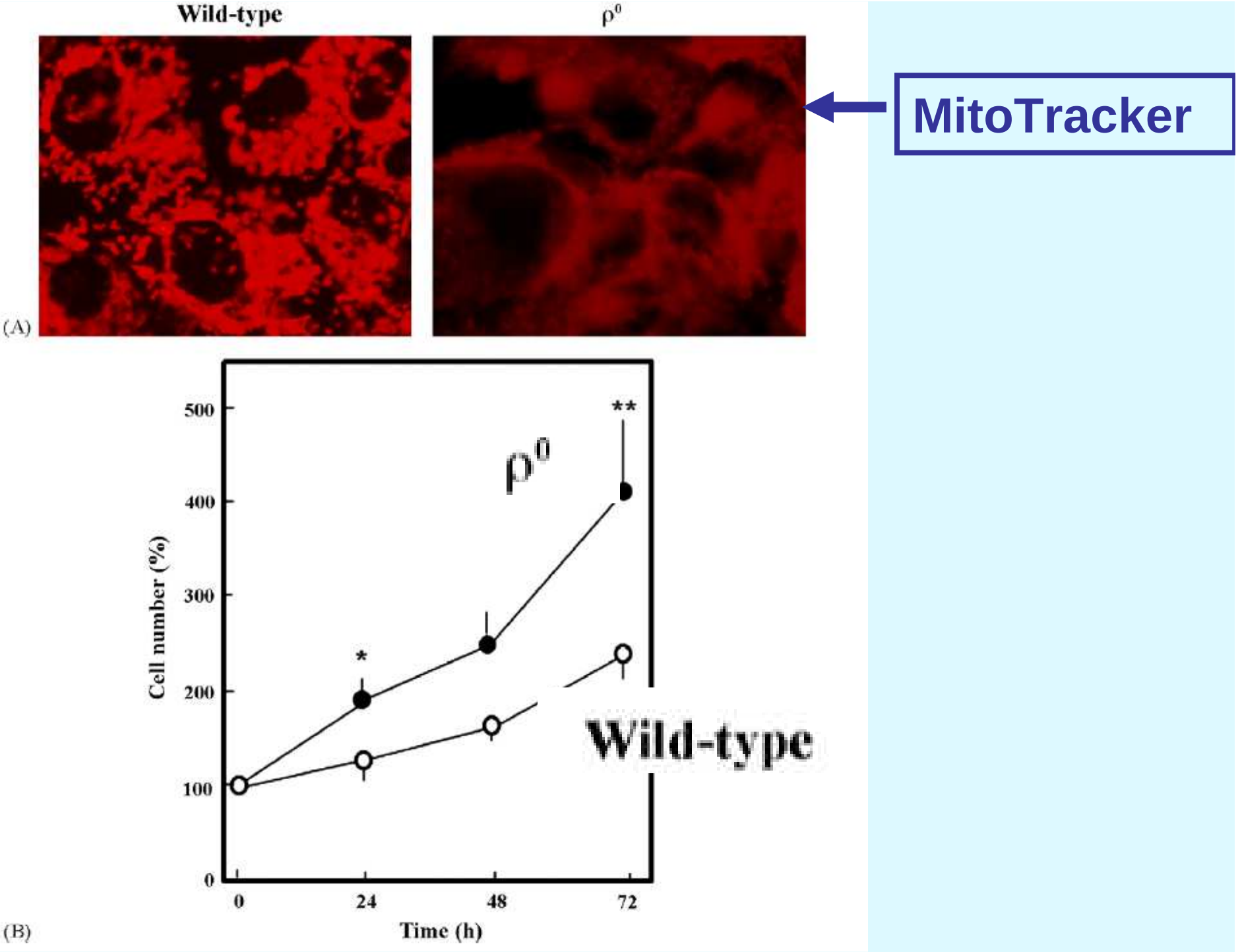
**Rho0 = Cells without MITOCONDRIA**

Hepatology Research **36** (2006) 209–216

# Lack of mitochondrial DNA enhances growth of hepatocellular carcinoma in vitro and in vivo

Anwarul Haque [a], Manabu Nishikawa [a,*], Wei Qian [a], Masayuki Mashimo [a], Masaki Hirose [a], Shuhei Nishiguchi [b], Masayasu Inoue [a]

[a] *Department of Biochemistry & Molecular Pathology, Osaka City University Medical School, Osaka 545-8585, Japan*
[b] *Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo 663-8131, Japan*


Wild-type
ρ⁰
(A)
MitoTracker
(B)
Cell number (%)
500
400
300
200
100
0
0
24
48
72
Time (h)
*
**
ρ⁰
Wild-type

**Objection nº8** : (evoluzione)

According to the theory of endo-symbiosis, housed bacteria would derive from the mitochondria (most likely enocytised) by anaerobes, however the CLASSICAL THEORY of endosymbiosis is not accepted by many evolutionists.

It’s difficult to think that the phagocytized bacteria would produce enough ATP for the primitive eukaryote. Is is called "*suicide hypothesis*" . It 's more likely that the bacteria in endosymbiosis had given the OXPHOS genes to the nucleus which then would localize them to facilities in those subcellular sites where ATP is needed.

# The origin of eukaryotes: the difference between prokaryotic and eukaryotic cells

*Proc. R. Soc. Lond.* B (1999) **266**, 1571–1577

**Tibor Vellai**[1*] **and Gábor Vida**[2]

Alternatively, the so-called 'hydrogen hypothesis' (Martin & Müller 1998) suggests that anaerobic archaebacteria (hosts) were originally strictly autotrophic and hydrogen dependent. Later, they were transferred under conditions characterized by the absence of hydrogen sources. This selective force made starving archaebacteria establish tight physical association with heterotrophic proteobacteria able to produce molecular $H_2$ through anaerobic fermentation. The symbionts feeding the hosts were surrounded by them completely, and were then lost or evolved into mitochondria, or into hydrogenosomes, explaining the anaerobic energy metabolism in eukaryotes. According to this hypothesis, the initial advantage of cellular merger was the excretion of molecular $H_2$ produced by the symbionts.

## In Summary:

Primordial eukaryotes (archaea) produced methane and took advantage of the first symbiotic bacteria because they were receiving hydrogen and, in return, it is plausible that the host would give ATP back to the bacteria that could not produce it efficiently, before the oxygen era.

When oxygen appeared bacteria became aerobic, and worked out oxidative phosphorylation. In the symbiotic association bacteria gave to their host the molecular machineries for the oxidative phosphorylation, that is still now likely assembled inside the mitochondria and exported in subcellular sites where it can be even more efficient.

**from:**
**Hackstein et al.- Zoology (Jena) -2001- 104(3-4):290-302**
**Hydrogenosomes: convergent adaptations of mitochondria to anaerobic environments.**

“…it is reasonable to assume that both, hydrogenosomes and mitochondria are evolutionary adaptations to anaerobic or aerobic environments, respectively.”

# Experimental data confirm the absence of mitochondrial production of ATP in some cellular types:

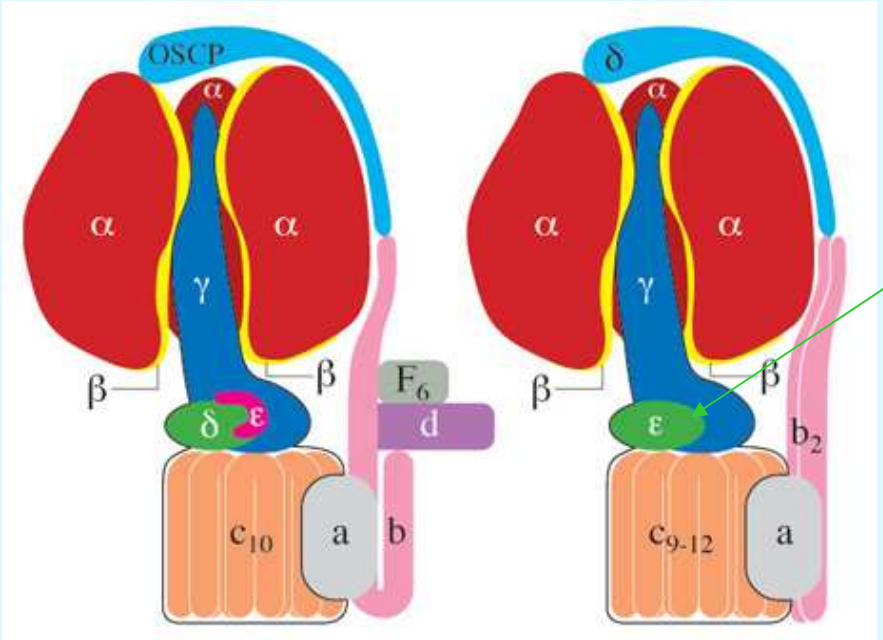
## Citation n°1: September 2009

PNAS | September 15, 2009 | vol. 106

# Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators

Hiromi Imamura[a,b,1], Kim P. Huynh Nhat[b], Hiroko Togawa[b], Kenta Saito[c], Ryota Iino[b], Yasuyuki Kato-Yamada[d], Takeharu Nagai[a,c], and Hiroyuki Noji[b,1]

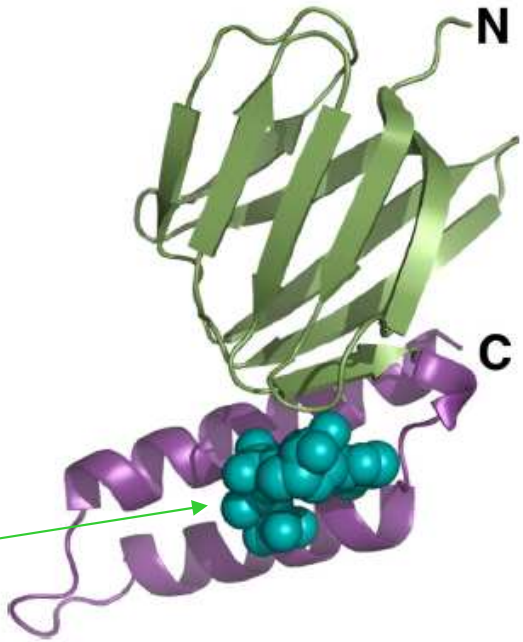
[a]Precursory Research for Embryonic Science, Japan Science and Technology Agency, 5 Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan; [b]Institute of Scier and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan; [c]Research Institute for Electronic Science, Hokkaido University Kita-20 Nishi-10, Kita-ku, Sapporo, Hokkaido 001-0020, Japan; and [d]Department of Life Science, Rikkyo University, 3-34-1 Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan




Mitochondrial ATP Synthase (Bovine)

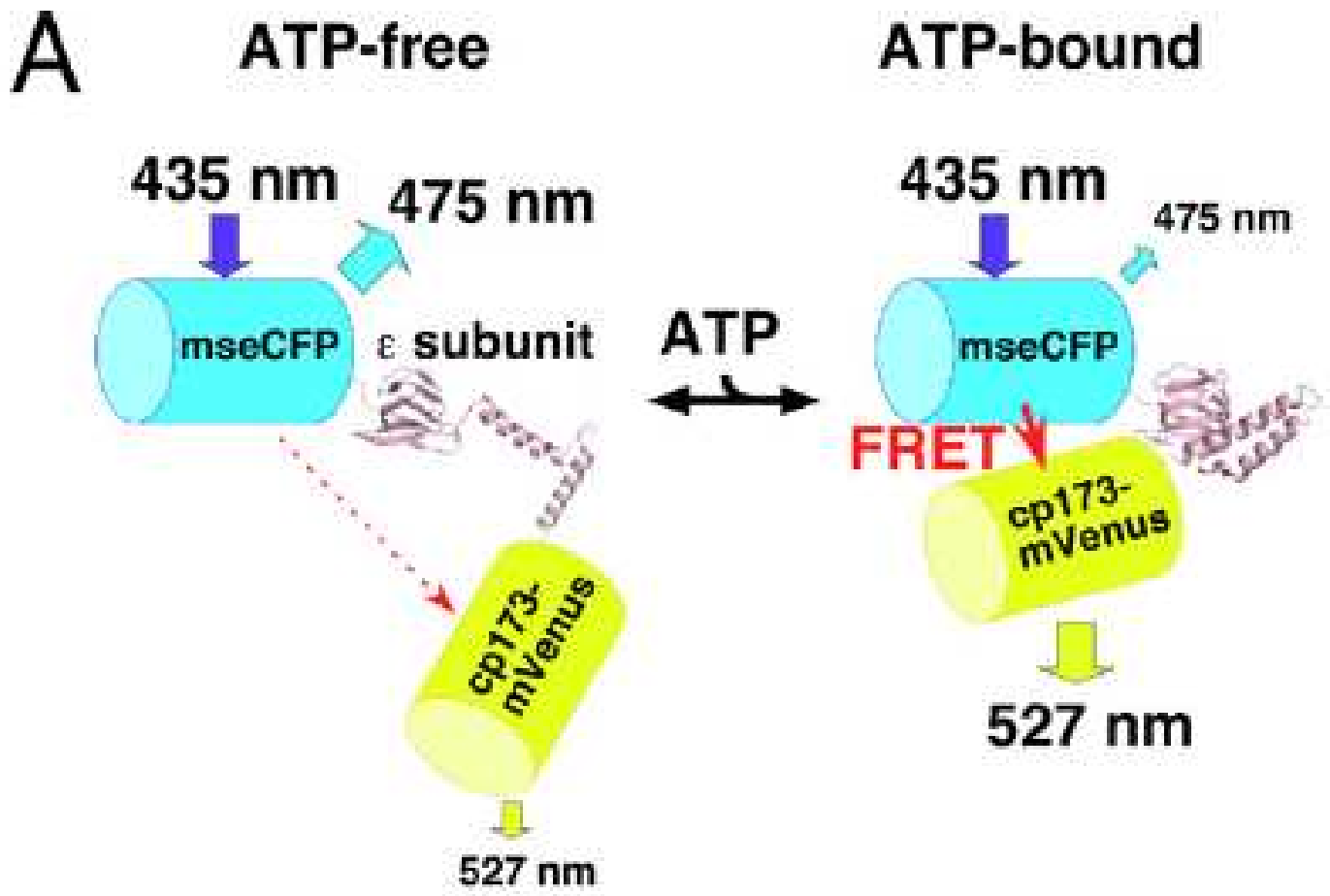
E.coli ATP Synthase

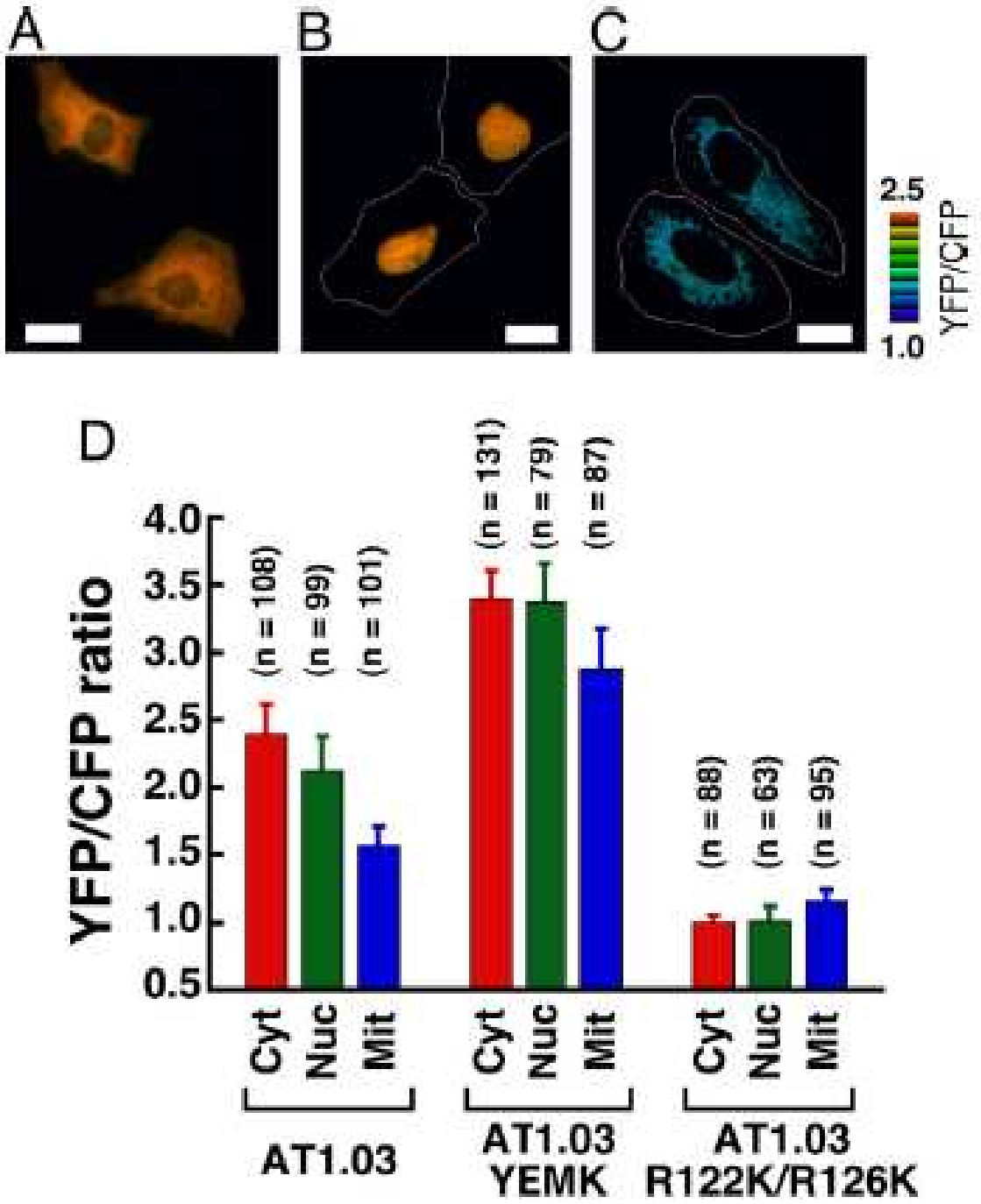
**ε Subunity of E.coli ATP-synthase**




ATP Molecule

# FRET: Fluorescence Resonance Energy Transfer






**...By targeting ATeams to different subcellular compartments, we unexpectedly found that ATP levels in the mitochondrial matrix of HeLa cells are significantly lower than those of cytoplasm and nucleus…**

Hoppe-Seyler's Z. Physiol. Chem.
Bd. 355, S. 378–393, März 1974

## Citation n°2: March 1974

# Measurement of the ATP/ADP Ratio in Mitochondria and in the Extramitochondrial Compartment by Fractionation of Freeze-Stopped Liver Tissue in Non-aqueous Media*

*Rembert Elbers, Hans W. Heldt, Peter Schmucker, Sibylle Soboll and Helga Wiese*

b) 25 % of the adenine nucleotides in the cell are contained in the mitochondria. In normoxic perfused liver the ATP/ADP ratio is much lower in the mitochondria than in the extramitochondrial compartment.

Table 3. Assay of adenine nucleotides in the mitochondria and in the extramitochondrial compartment of rat liver. Normoxic perfusion. Assays 39 and 39A were performed with the same liver. For details see Methods and text.

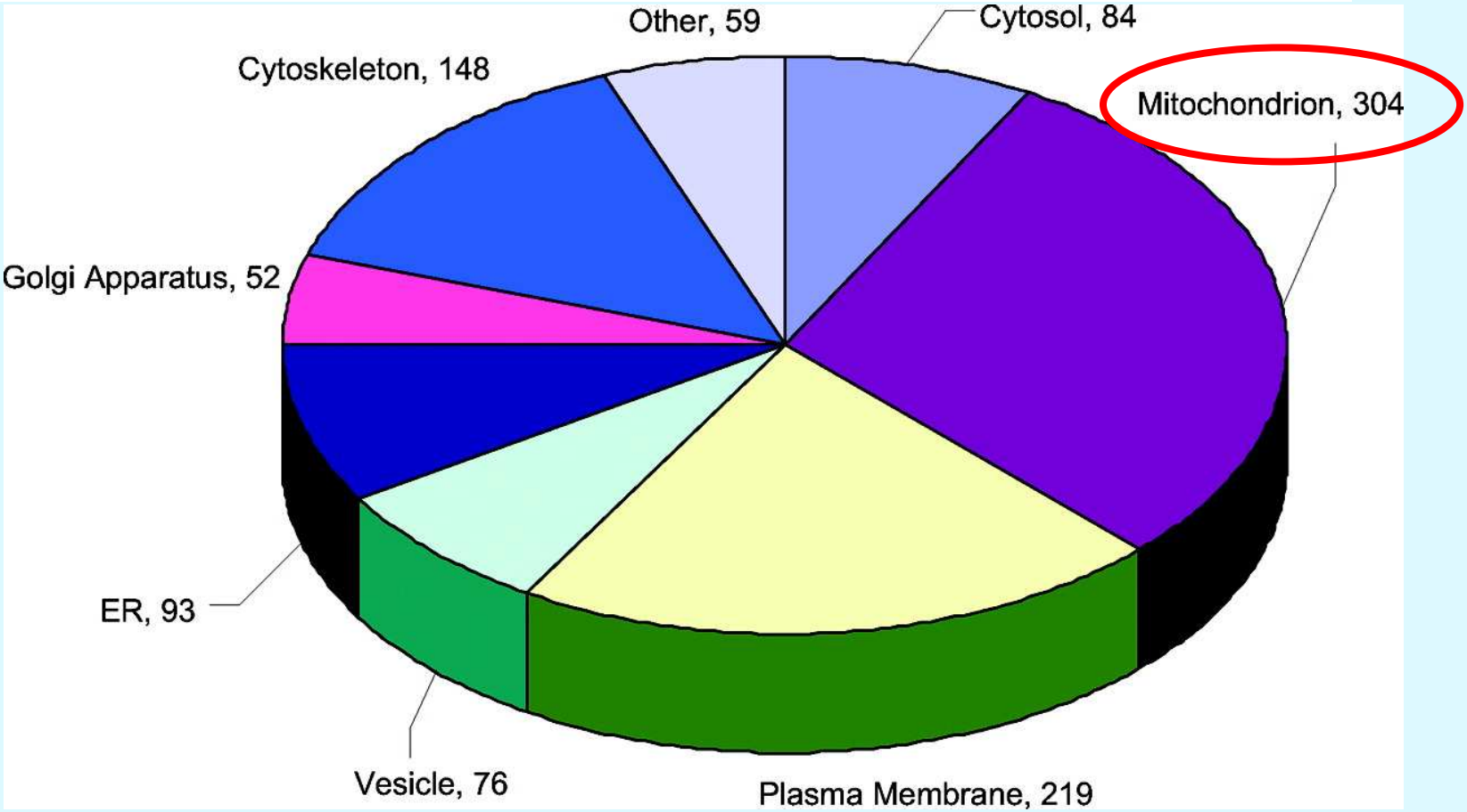
| Assay No. | | 39 | 39A | 41 | 59 |
|---|---|---|---|---|---|
| Liver, total | ATP | 12.6 | 12.9 | 13.5 | 11.2 |
| [nmol/mg total liver prot.] | ADP | 4.15 | 4.12 | 4.32 | 3.51 |
| | AMP | 2.38 | 2.29 | 1.70 | 1.20 |
| | Σ | 19.1 | 19.3 | 19.5 | 15.9 |
| | ATP/ADP | 3.04 | 3.13 | 3.12 | 3.18 |
| Mitochondria | ATP | 4.68 | 5.08 | 0.85 | 3.94 |
| [nmol/mg mitoch. prot.] | ADP | 6.32 | 5.71 | 7.11 | 6.15 |
| | AMP | 3.08 | 3.12 | 4.47 | 3.07 |
| | Σ | 14.1 | 13.9 | 12.4 | 13.2 |
| | ATP/ADP | 0.74 | 0.89 | 0.12 | 0.64 |
| | $(ADP)^2/(ATP \cdot AMP)$ | 2.77 | 2.05 | 13.3 | 3.13 |
| Extramitochondrial | ATP | 16.9 | 17.1 | 20.3 | 15.1 |
| compartment | ADP | 2.98 | 3.27 | 2.82 | 2.09 |
| [nmol/mg extramitoch. prot.] | AMP | 0.26 | 0.23 | 0.20 | 0.19 |
| | Σ | 20.1 | 20.6 | 23.3 | 17.4 |
| | ATP/ADP | 5.66 | 5.24 | 7.21 | 7.24 |
| | $(ADP)^2/(ATP \cdot AMP)$ | 2.02 | 2.71 | 1.95 | 1.52 |
| % of total adenine nuc. in mitochon. | | 27.2 | 25.3 | 23.0 | 26.4 |
| % of total prot. in mitochon. | | 35.4 | 35.1 | 36.2 | 31.9 |

# (5) Extramitochondrial ATP synthesis in plasmacell

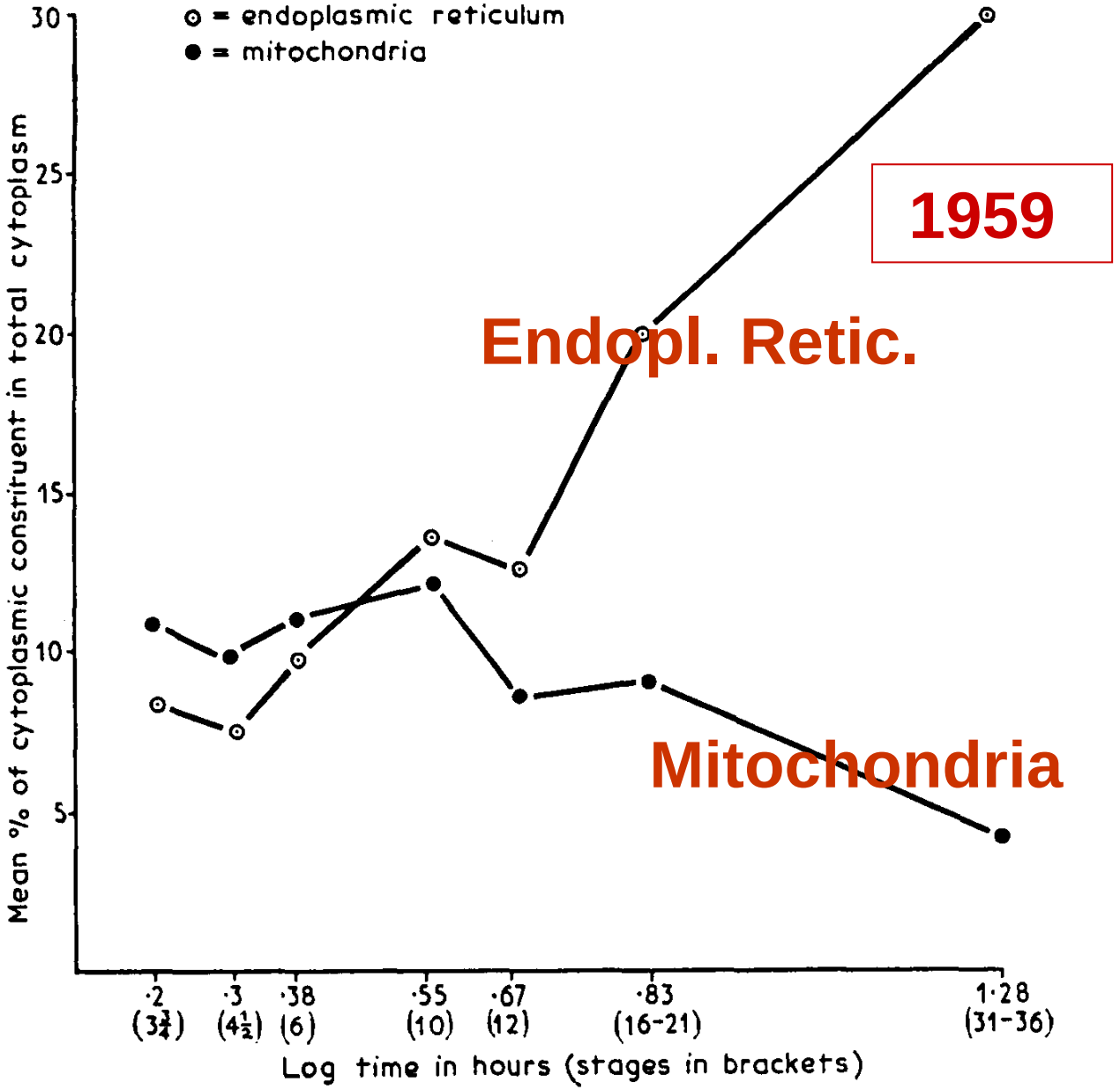
research articles Journal of proteome research 2008, 7, 1046–1054

# Proteomic Analysis of Mouse Brain Microsomes: Identification and Bioinformatic Characterization of Endoplasmic Reticulum Proteins in the Mammalian Central Nervous System

Stanley M. Stevens, Jr.,[†] R. Scott Duncan,[‡] Peter Koulen,[‡] and Laszlo Prokai*[,†]



The Development of the Nervous System in Chick Embryos,studied by Electron Microscopy
***by***
**RUTH BELLAIRS**
**[J. Embryol. exp. Morph. Vol. 7, Part 1, pp. 94-115, March 1959]**




TEXT-FIG. 2. Graph to show how the percentages of endoplasmic reticulum and of mitochondria in the cytoplasm vary during development. The way in which these percentages have been

Complex I (reduzction of ferricyanide):

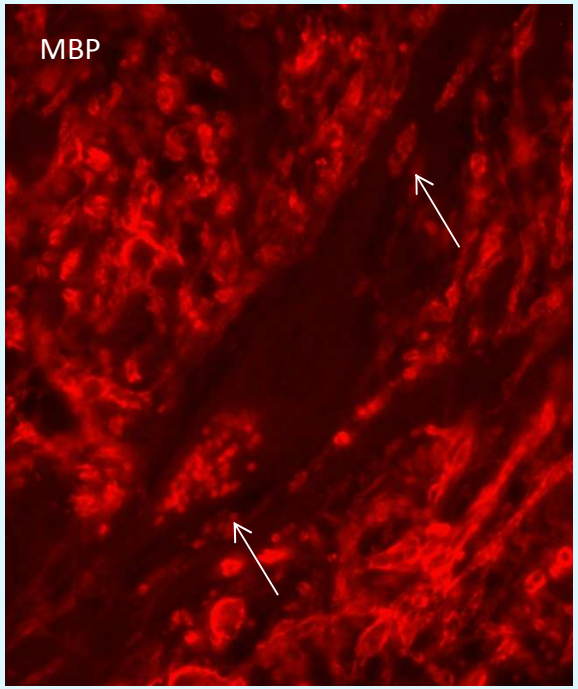
in **S9**: **3,76 U/mg** ----- In intact Mitochondria **3,47 U/mg**

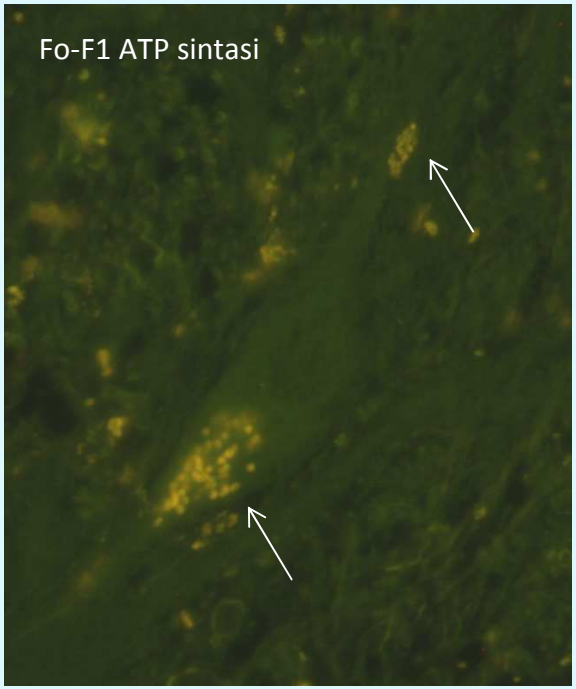
Complex IV (Cytocrome c - Fe++ Oxidation ):

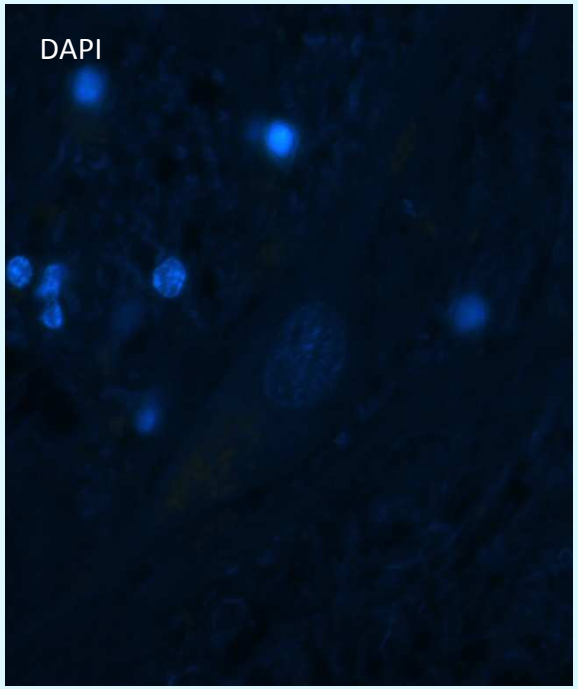
in **S9** : **0,09** In intact Mitochondria **0,14 U/mg**

**Measurements taken** on 20/09/ 2010 in DIPTERIS - Genoa

| | **Mitochondria bovine liver** | Inhibition % | **S9 bovine liver** | Inhibition % |
|---|---|---|---|---|
| Sample+ Nigericin (No added nutrients) | 194,55 µM $O_2$/min/mg | | 63,55 µM $O_2$/min/mg | |
| + NADH | 63,55 µM $O_2$/min/mg | | 202,5 µM $O_2$/min/mg | |
| + Rotenone | 7,95 µM $O_2$/min/mg | 87% | ~ 0 µM $O_2$/min/mg | ~ 100 % |
| + Succinate | 356 µM $O_2$/min/mg | | 603,5 µM $O_2$/min/mg | |
| + Antimycin | 55,6 µM $O_2$/min/mg | 84% | 234,25 µM$O_2$/min/mg | 61 % |
| + Ascorbate | 325,55 µM $O_2$/min/mg | | 412,9 µM $O_2$/min/mg | |
| + Cyanide | ~ 0 µM $O_2$/min/mg | ~ 100 % | ~ 0 µM $O_2$/min/mg | ~ 100 % |

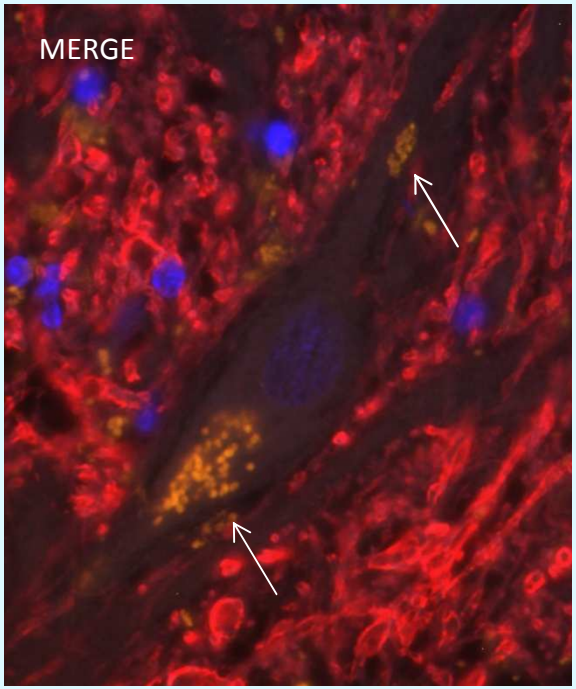



**Measurements taken**
on 23/09/ 2010 in
DIPTERIS - Genoa

L'immagine si riferisce ad una sezione di materia bianca umana. In molti corpi cellulari dei neuroni si è evidenziato il Golgi (frecce), marcato sia con l'anticorpo contro la MBP sia con l'anticorpo contro Fo-F1 ATP sintasi (un esempio è rappresentato da questa immagine) o COX (Complesso IV) e ND4L (Complesso I), a suggerire che le proteine mitocondriali possono essere esportate attraverso ER/Golgi.

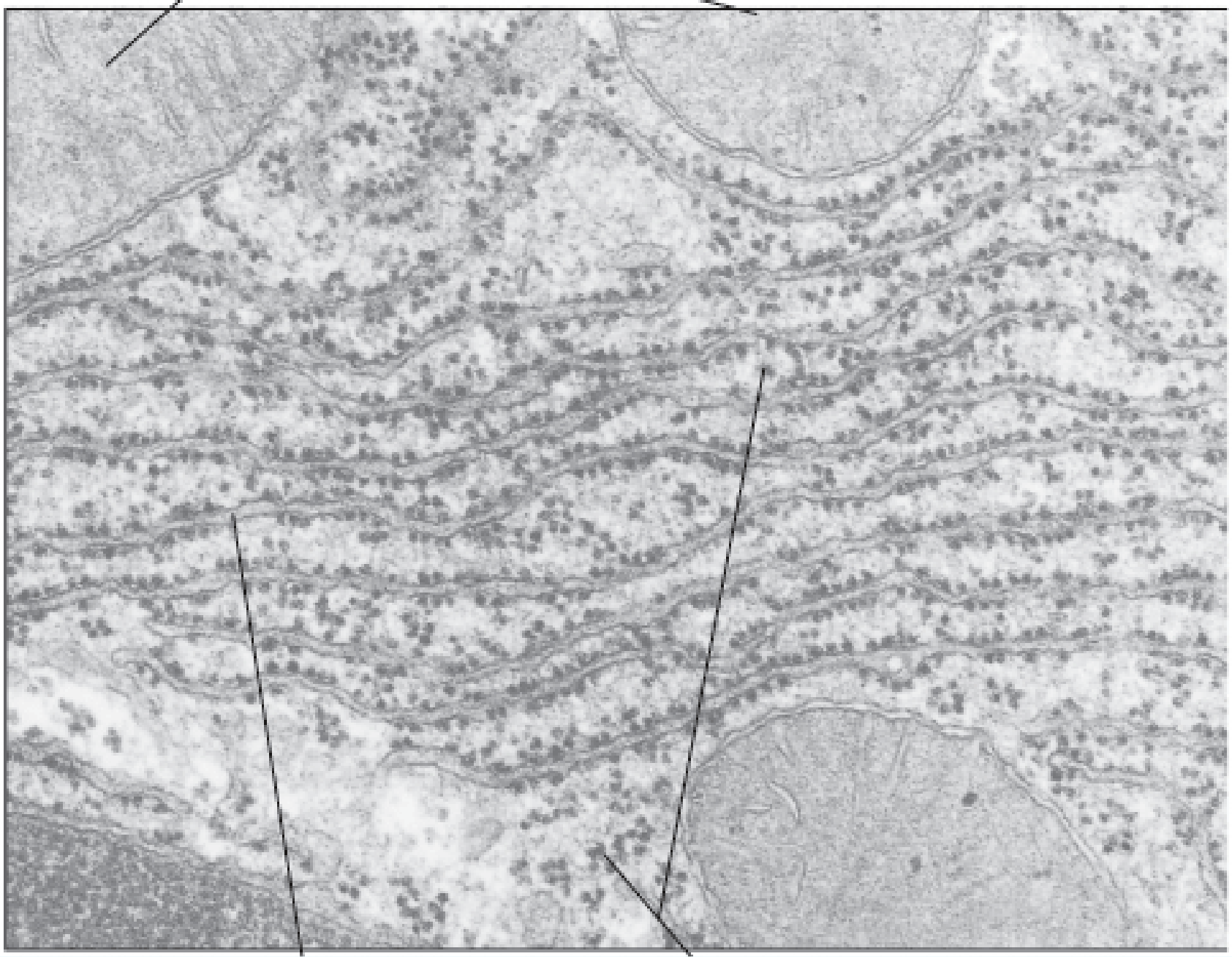



**Merge of:**

**MBP and**

**Fo-F1 ATPsynthase**

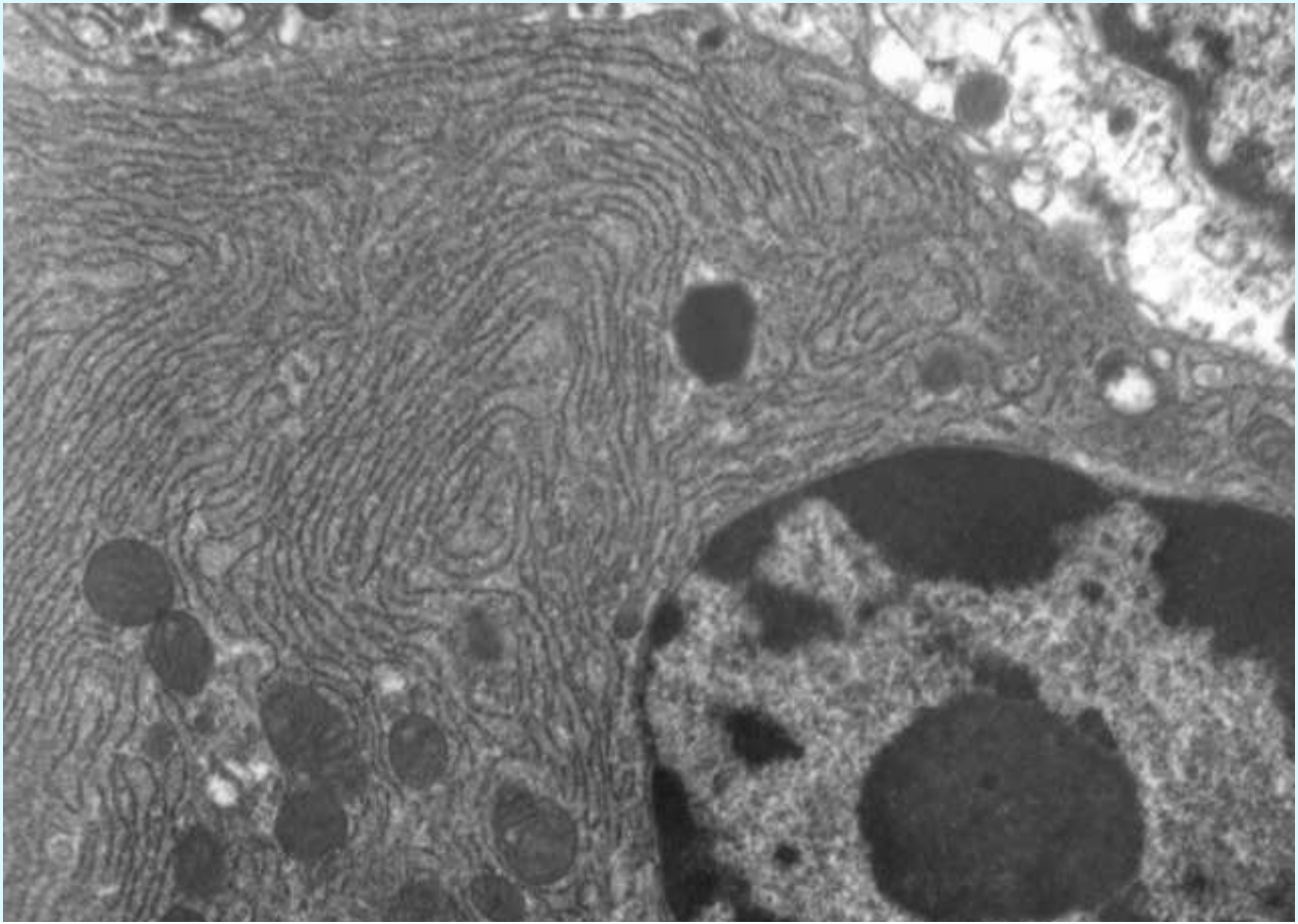
# Rat hepatocyte

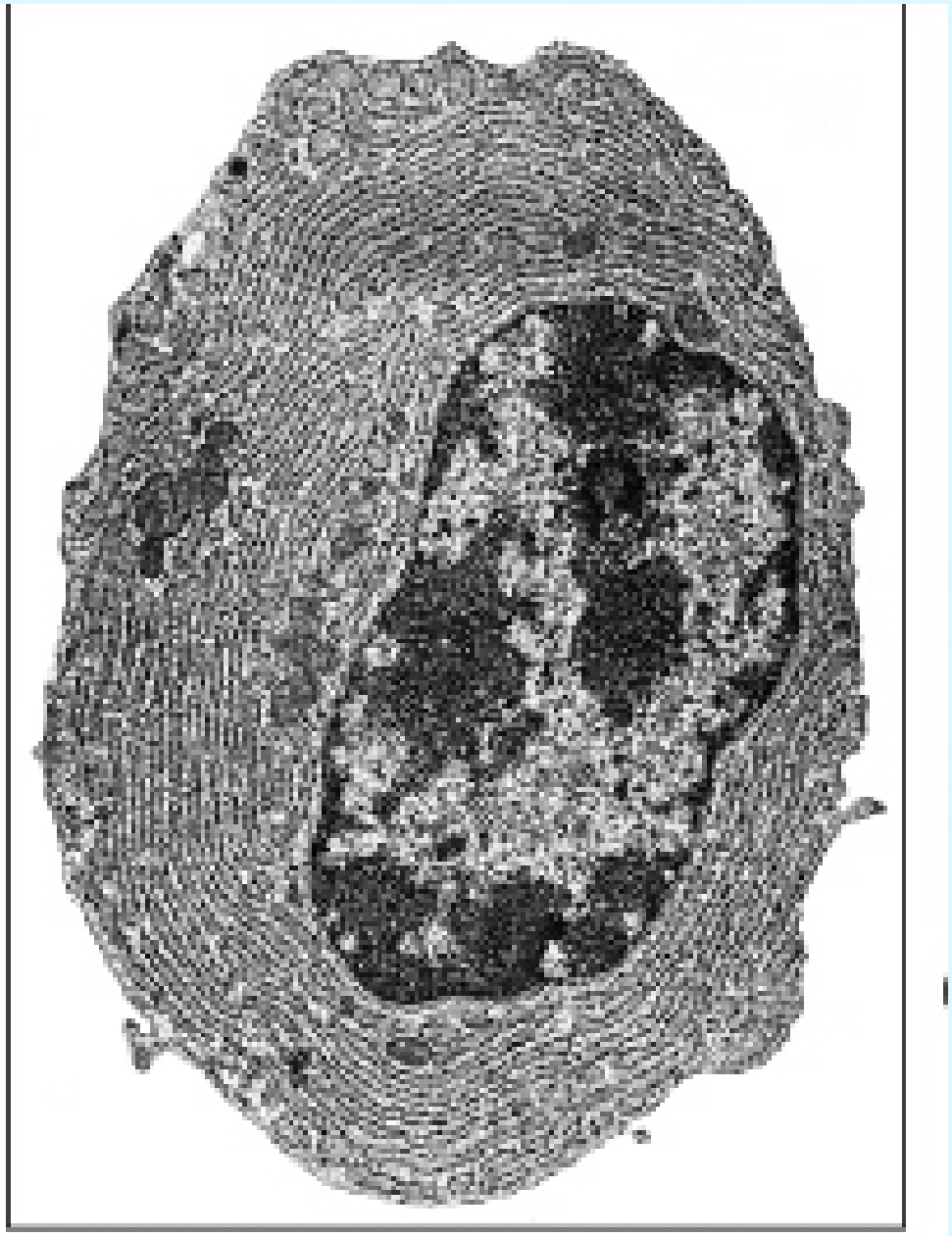



Per gent. conc. di Dr. Sue Ellen Gruber, Mt. Holyoke College

# Lung cell – TEM

**(from: *Rippel Electron Microscope Facility - Dartmouth College*)**





(B) cellula B effettrice (plasmacellula)

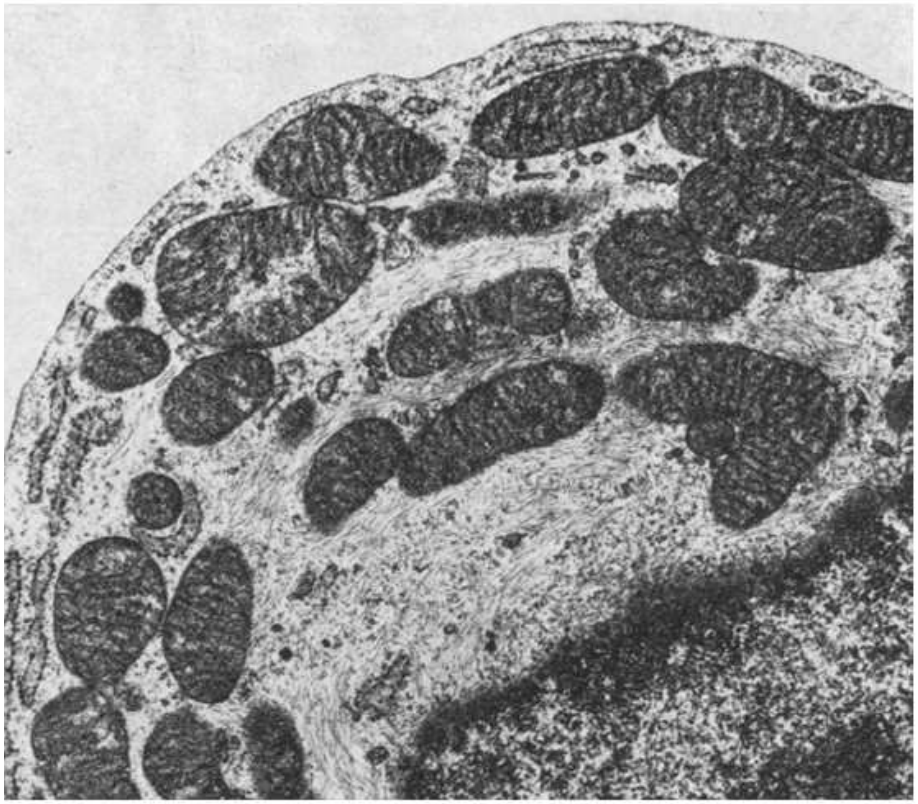
1 µm


Mature Plasmacell

Blut 35, 11-19 (1977)

**Transmission and Scanning Electron Microscopy Study on Plasma Cell Leukemia**

B. Klein, U. Lewinski, F. Shabtai, N. Freidin and M. Djaldetti



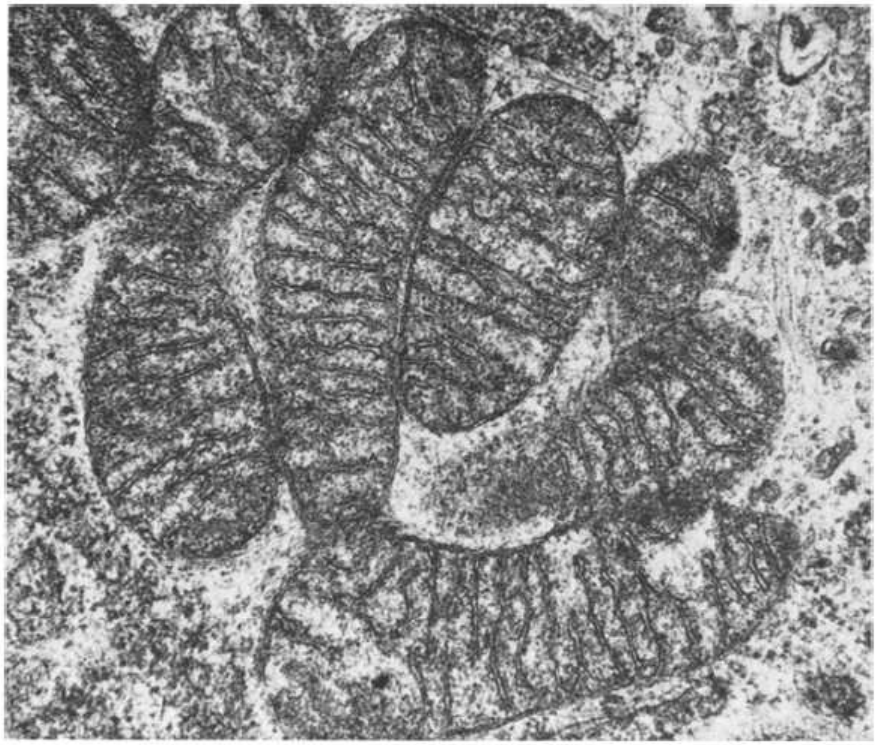
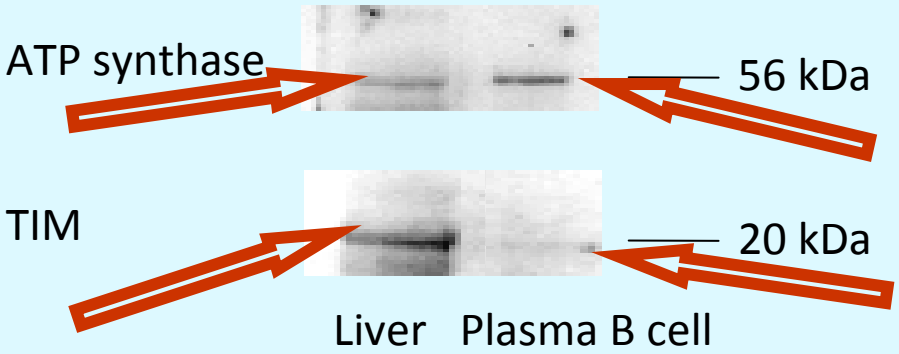


Fig. 6. Higher magnification of a portion of a plasma cell revealing mitochondrial abn
×49,720

# Oxygen consumption in plasma cells and mitochondria (mesured by Morelli et al.,on Genova, 4/05/2011 – unpublisched results)

| Oxygen consumption (μM $O_2$/min/mg) | | | | |
|---|---|---|---|---|
| Plasma cells | | Mitochondria + ciclosporin | Mitochondria no ciclosporin | Epathome culture |
| Sample (basal) | 27.8 ± 3.2 | 21,34 ± 2,92 | ND | ND |
| Pyruvate/Malate | 99.5 ± 8.4 | 60,63 ± 3,03 | 0,23 | 198 ± 12,5 |
| Succinate | 56.3 ± 6.2 | 91,58 ± 4,6 | 0,56 | ND |

# Western Blot Liver and Plasma Cell homogenates 24 - 5 - 2011

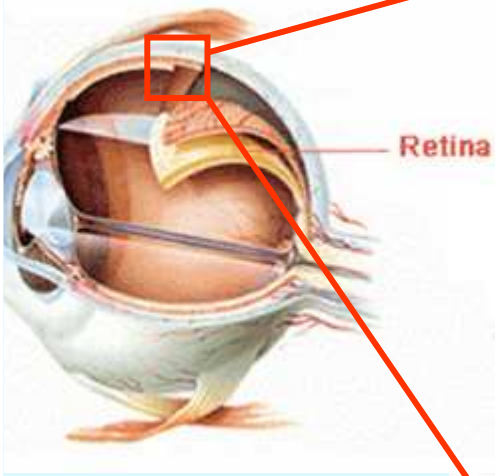


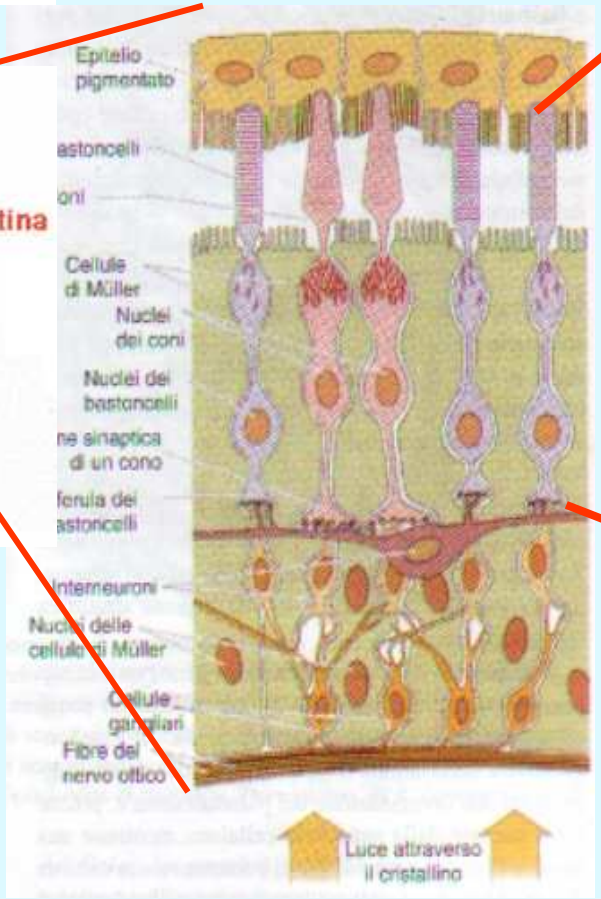

## Densitometric analysis

| | Liver (homogenate) | Plasma B Cell (homogenate) |
|---|---|---|
| ATP synthase | 1134 | 1256 |
| TIM | 6475 | 1995 |
| ATP synthase/TIM | 0,17 | 0,63 |

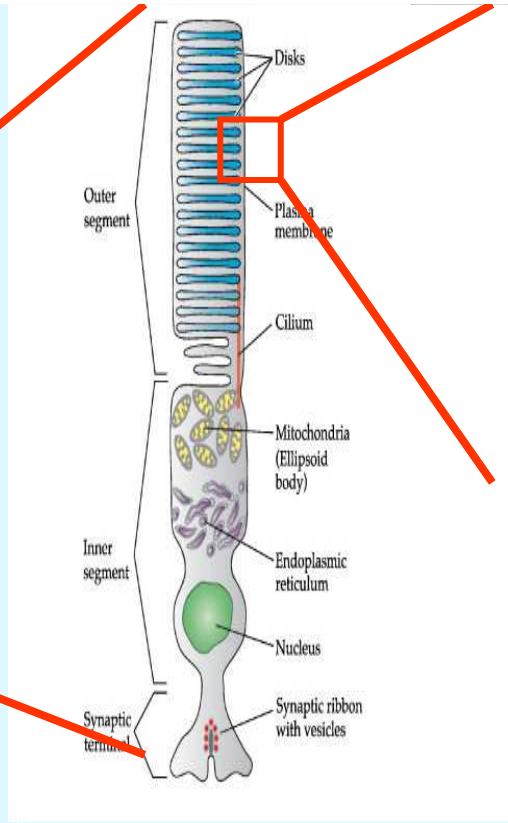
# (6) Extramitocondrial ATP Synthesis in photoreceptors

DIPARTIMENTO DI BIOLOGIA
UNIVERSITÀ DI GENOVA

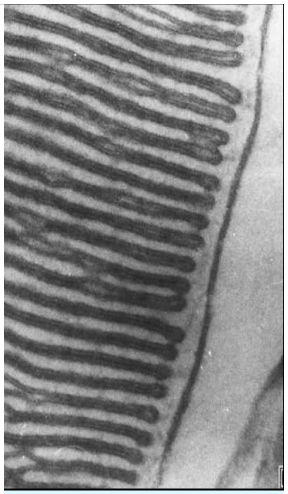



retina

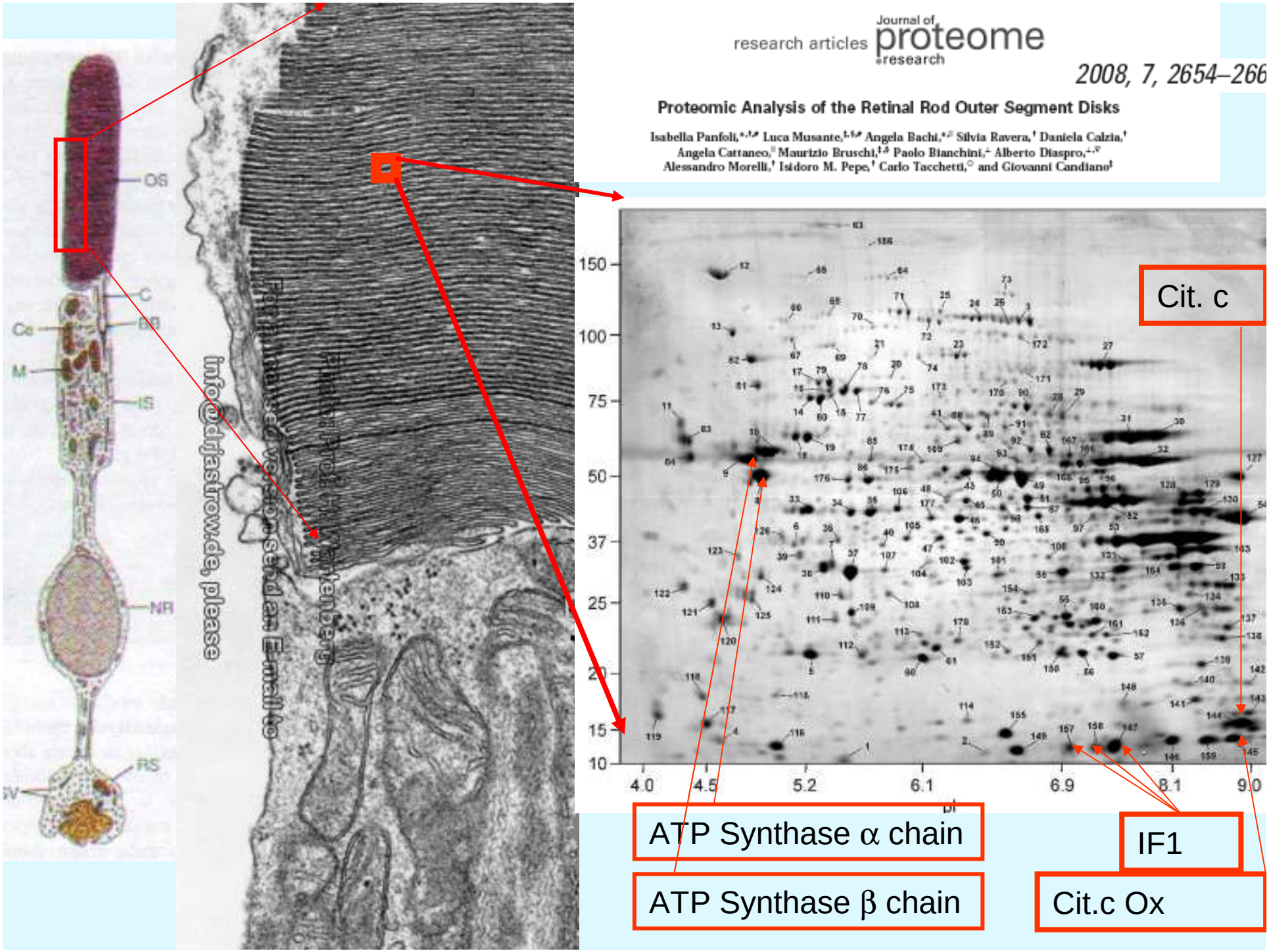



rod



Disks

**Vertebrate retinal rod photoreceptors, allowing vision at low light intensities use a modified cilium (ROS) for the detection of light. The ROS is packed with membrane sacs (disks), housing reactions of visual transduction**




research articles Journal of proteome research

2008, 7, 2654–266

**Proteomic Analysis of the Retinal Rod Outer Segment Disks**

Isabella Panfoli,[*,†,#] Luca Musante,[‡,§,#] Angela Bachi,[*,⊥] Silvia Ravera,[†] Daniela Calzia,[†] Angela Cattaneo,[∥] Maurizio Bruschi,[‡,§] Paolo Bianchini,[-] Alberto Diaspro,[-,▽] Alessandro Morelli,[†] Isidoro M. Pepe,[†] Carlo Tacchetti,[○] and Giovanni Candiano[‡]

September/October 2008 • Volume 13 Number 5
ISSN 1083-3668
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BIOMEDICAL OPTICS

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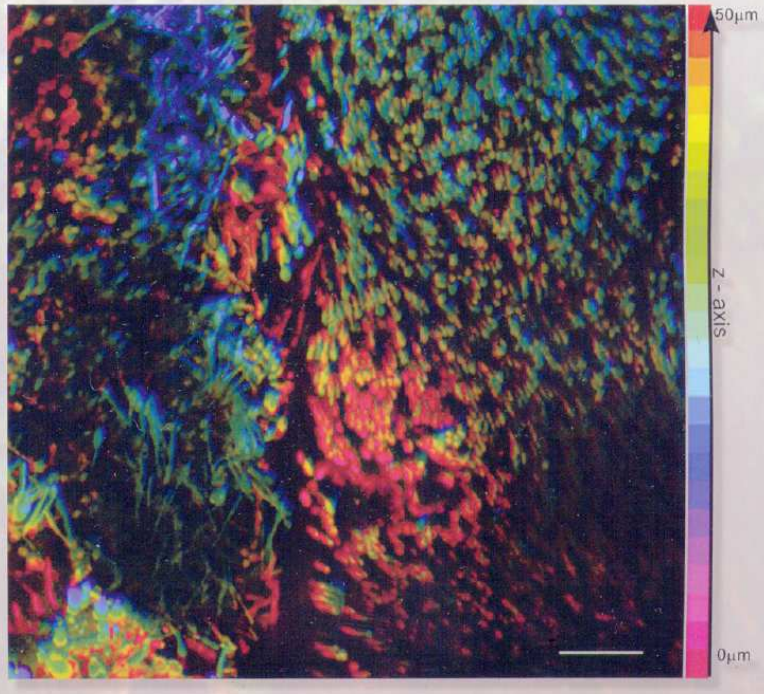




# Live imaging of mammalian retina: rod outer segments are stained by conventional mitochondrial dyes

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MicroSCoBiO Research Center
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16132 Genova, Italy

**Abstract.** The vertebrate retina is an array of "narrow-capture" photoreceptive elements of diverse cellular types that allow the fine spatial resolution characteristic of vision. Imaging of photoreceptors and of the whole retina has been previously reported; however, both were achieved exclusively after fixation. We report our development of a new technique for imaging live bovine retinas *ex vivo*. Using this technique, we conducted fluorescence confocal laser scanning microscopic imaging of bovine retinas. Eyecups were incubated with conventional fluorescent mitochondrial probes (MitoTracker and JC-1). Unexpectedly, we found that, besides the retinal mitochondria, the rod outer segments that are devoid of mitochondria were also stained. No other neuron was stained. Both protonophores, which decrease mitochondrial membrane potential, or inhibit electron transport strongly inhibited the selective association of dyes with both retinal rod outer segments and mitochondria. This is the first time that living rod outer segments were visualized by this technique. This finding may shed light on previous reports of the existence of a proton potential across the disk membranes and on the mechanism of the adenosine tri-phosphate (ATP) supply for phototransduction, which still requires investigation.  [DOI: 10.1117/1.2982528]

Keywords: confocal laser scanning microscopy; fluorescent dyes; mitochondrial probes; retina; imaging; JC-1; MitoTracker.

Paper 08068R received Feb. 26, 2008; revised manuscript received Apr. 29, 2008; accepted for publication May 2, 2008; published online Oct. 14, 2008.

## 1 Introduction

The retina comprises cells from the central nervous system (CNS) for the transduction of light messages into electrical signals.[1–3] The photoreceptors (rods and cones) are the sites of light capture,[4] while the retinal physiological repertoire is due to downstream diverse inner-retinal neurons.[3]

The rods, where photoreception in dim light begins,[3,5,6] consist of a rod inner segment (RIS) and a rod outer segment (ROS). The RIS contains the nucleus and other organelles. The ROS is composed of a stack of sealed, flattened membrane vesicles—the disks—that contain the photopigment rhodopsin (Rh) and are surrounded by the plasma membrane. The ROS is constantly renewed;[7,8] new disks are formed at the cilium of the outer segment from evaginations of the plasma membrane and are displaced toward the apical tip where old disks are shed by the rod pigmented epithelium

*Paolo Bianchini, Daniela Calzia and Silvia Ravera contributed equally to this work.

Address all correspondence to Isabella Panfoli, DIBIO, University of Genova, V. le Benedetto XV, 3 - Genoa, 16132 Italy; Tel: +39 010 3537397; Fax: +39 010 3538153; E-mail: Isabella.Panfoli@unige.it

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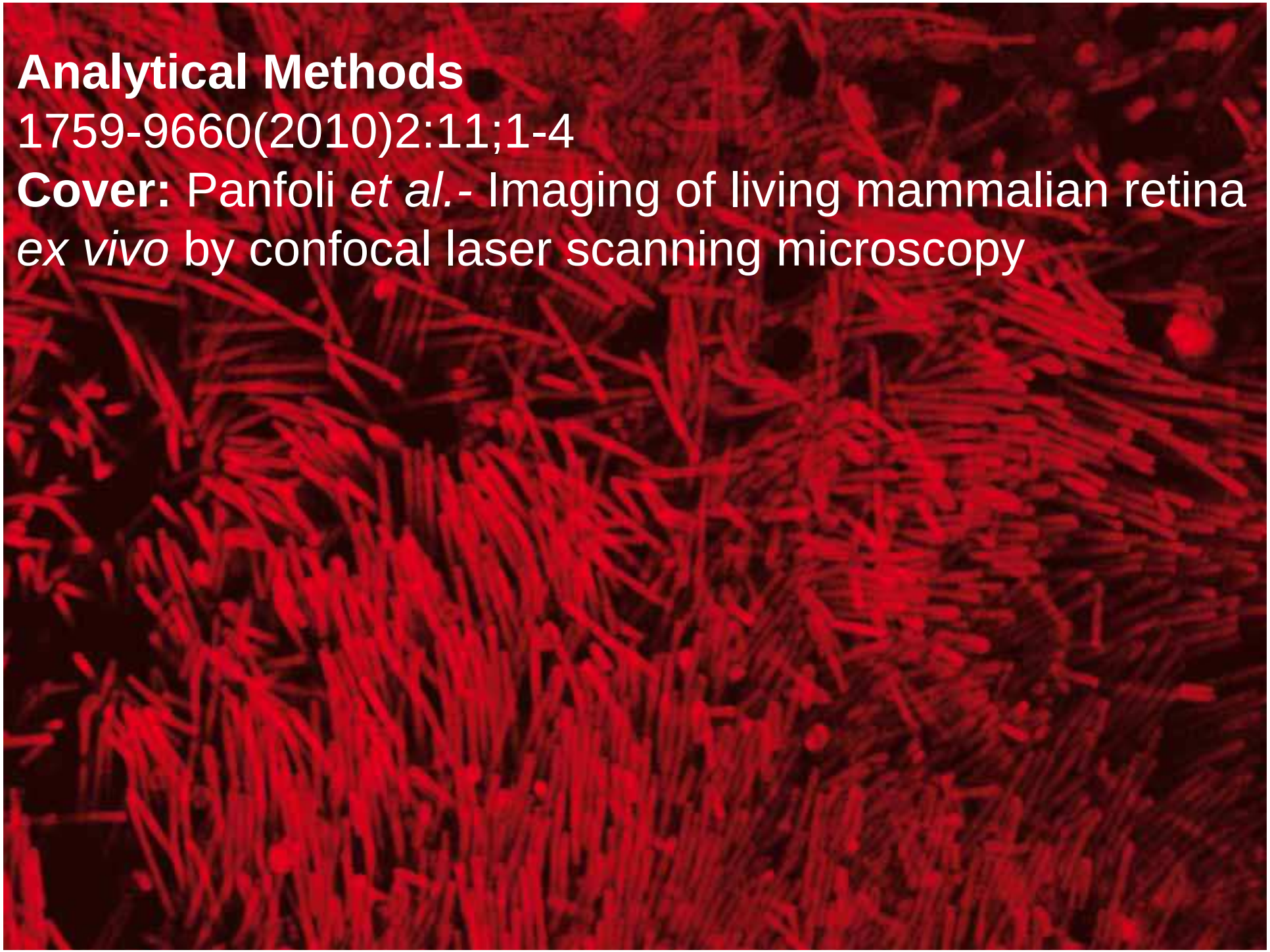
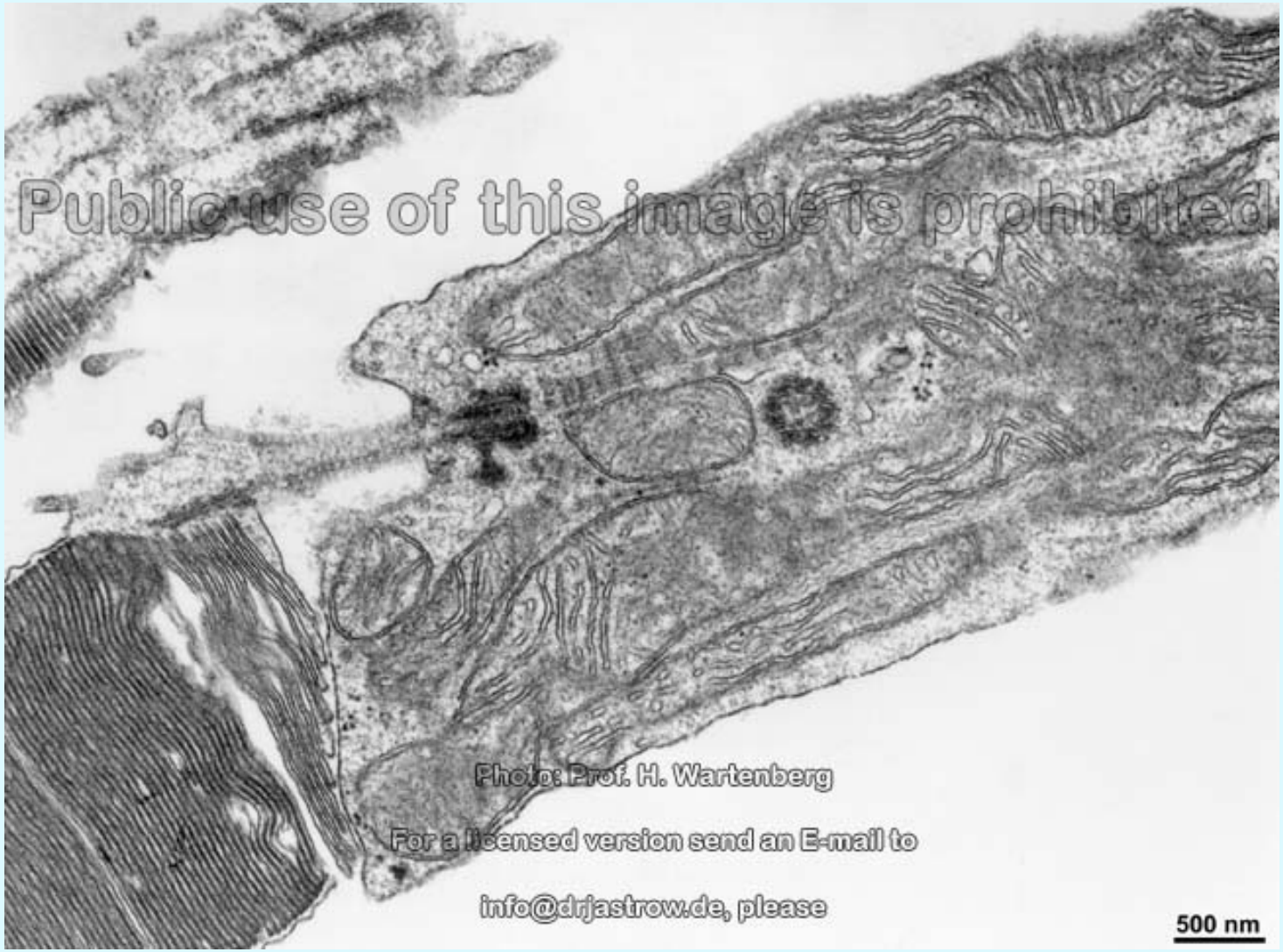

**Analytical Methods**
1759-9660(2010)2:11;1-4
**Cover:** Panfoli *et al.*- Imaging of living mammalian retina *ex vivo* by confocal laser scanning microscopy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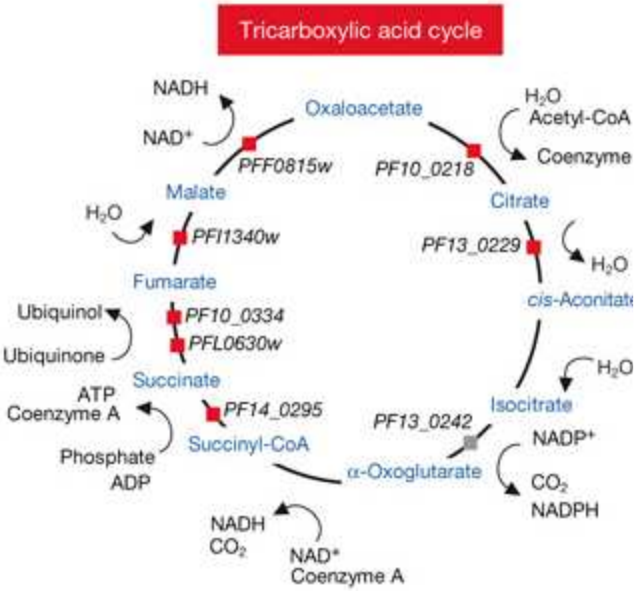
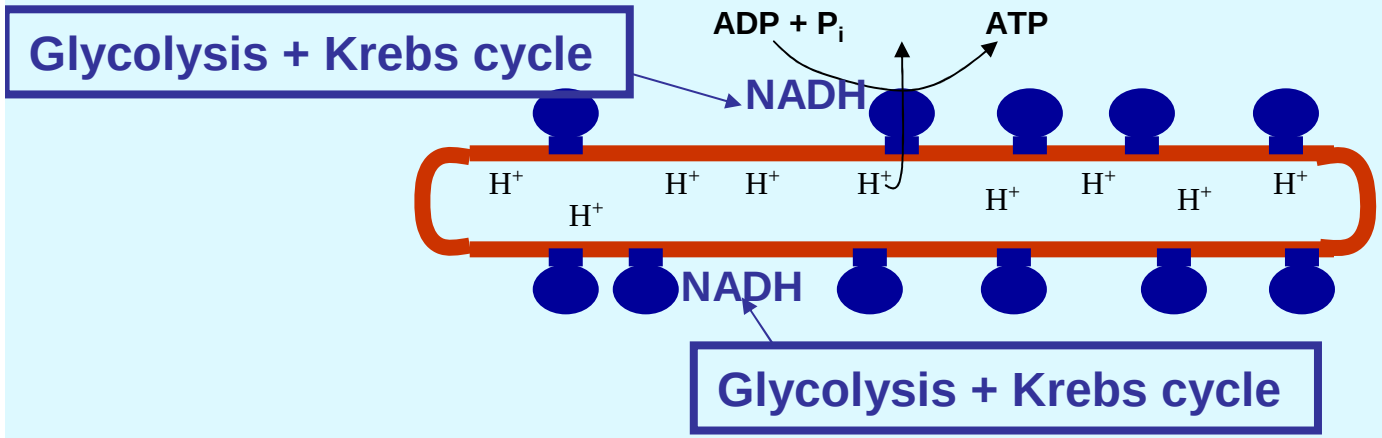
500 nm

# EXTRAMITOCHONDRIAL TRICARBOXYLIC ACID CYCLE IN RETINAL ROD OUTER SEGMENTS.

## Biochimie - Panfoli et al. - *Accepted, 23-5-2011*

| U /mg protein | | |
|---|---|---|
| **Enzymes** | **Mitochondria** | **ROS Cytosol** |
| Citrate synthase | 2,01±0,32 | 1,80±0,22 |
| Aconitase | 0,052±0,006 | 0,118±0,013 |
| Isocitrate dehydrogenase | 0,020±0,002 | 0,012±0,001 |
| αKetoglutarate dehydrogenase | 0,006±0,001 | 0,009±0,001 |
| Succinyl -CoA synthetase | 0,203±0,025 | 0,095±0,010 |
| Fumarase | 0,038±0,004 | 0,015±0,002 |
| Malate dehydrogenase | 1,5±0,2 | 4,4±0,6 |






**Both in thylakoid, and in vertebrate photoreceptor disks :**

⏩ $F_1F_o$ATP sintetasi is external. **ATP synthesis is located WHERE it IS USED.**

⏩ **The $H^+$ would accumulate inside absorbed by weak acid / bases in the disc that could act as buffer = energetic flywheel**

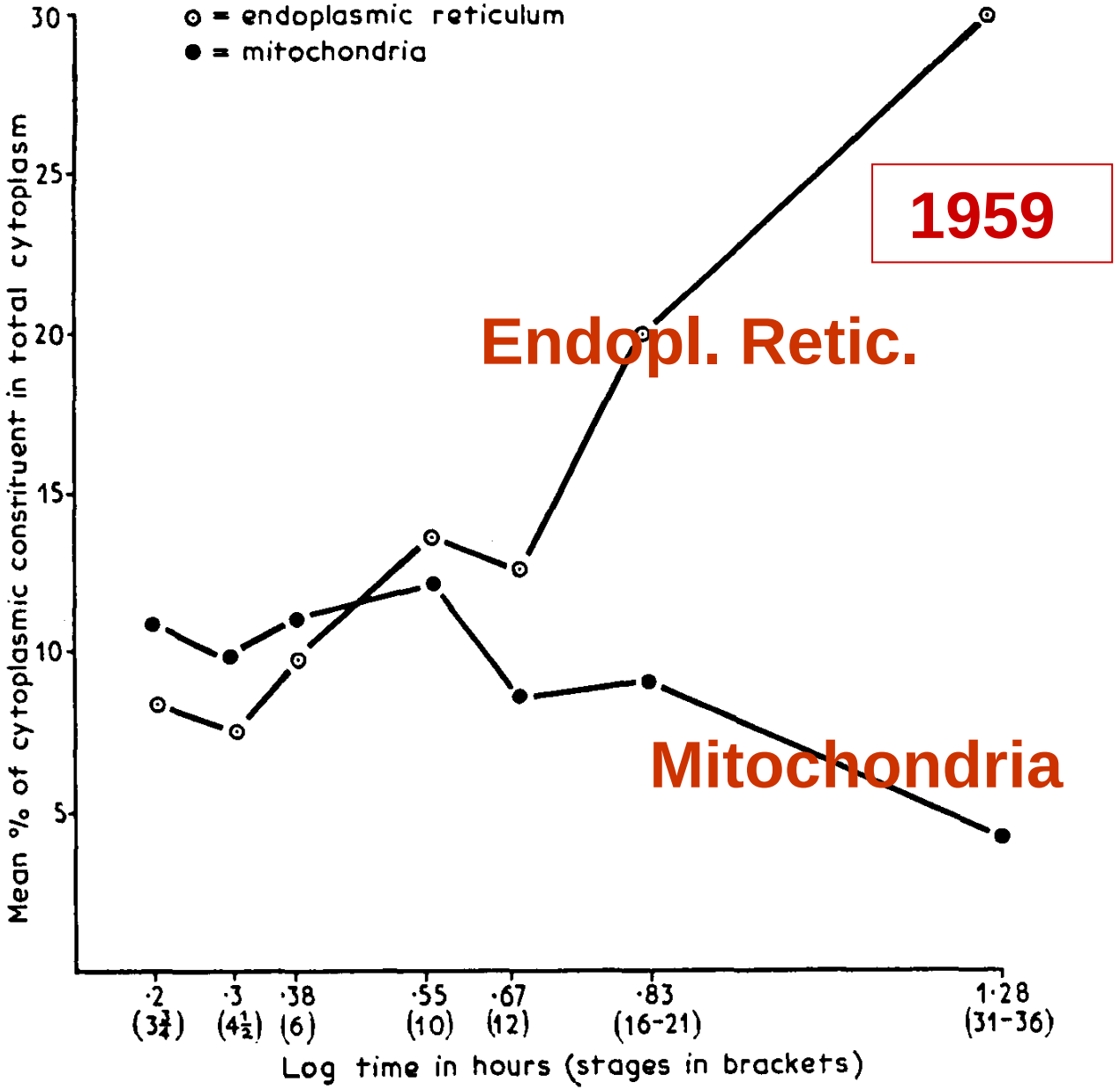
# ( 7 )
# Extramitocondrial ATP Synthesis in neuron

- **Considering that brain consumes much more oxygen than the other tissues it must have a great mitochondrial number. Instead…:**

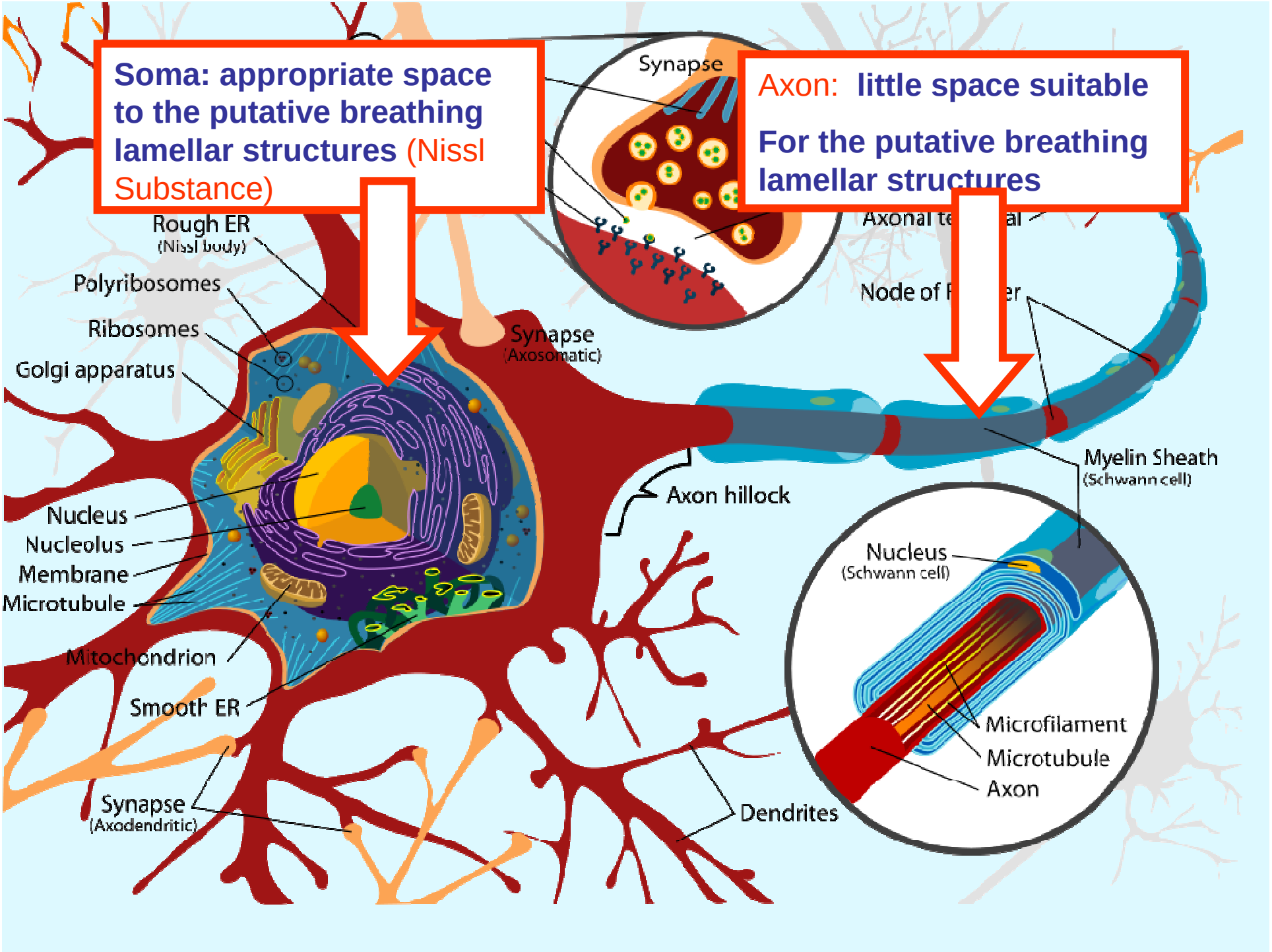
## Neuronal mitochondria:

- **Are *fewer* than in the other tissues.**
- **Have *smaller* dimensions.**
- **Have *smaller* cristal surface.**

The Development of the Nervous System in Chick Embryos,studied by Electron Microscopy
***by***
**RUTH BELLAIRS**
**[J. Embryol. exp. Morph. Vol. 7, Part 1, pp. 94-115, March 1959]**

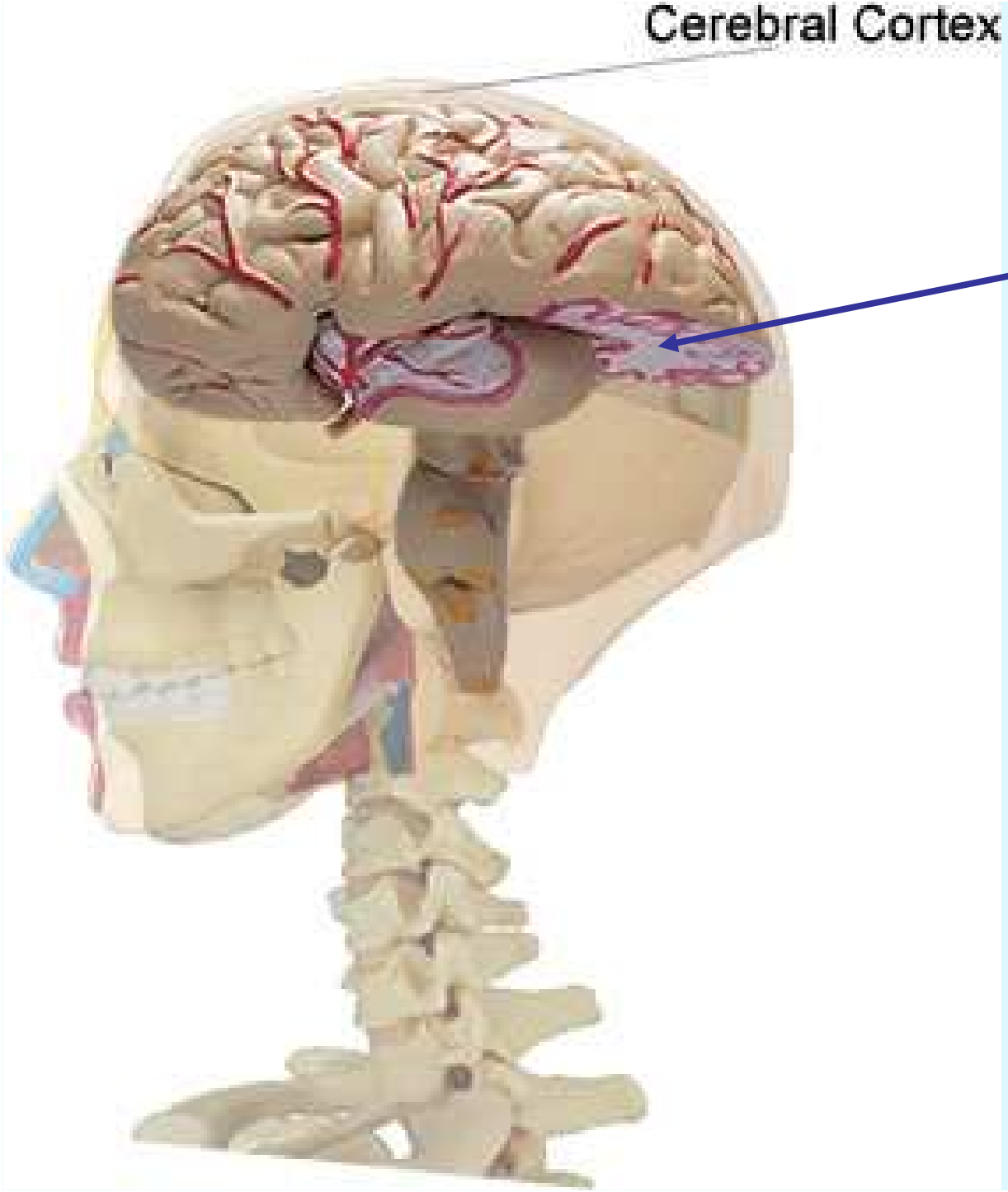



TEXT-FIG. 2. Graph to show how the percentages of endoplasmic reticulum and of mitochondria in the cytoplasm vary during development. The way in which these percentages have been


Soma: appropriate space to the putative breathing lamellar structures (Nissl Substance)
Axon: little space suitable
For the putative breathing lamellar structures
Synapse
Rough ER
(Nissl body)
Polyribosomes
Ribosomes
Golgi apparatus
Synapse
(Axosomatic)
Node of
Myelin Sheath
(Schwann cell)
Axon hillock
Nucleus
Nucleolus
Membrane
Microtubule
Mitochondrion
Smooth ER
Synapse
(Axodendritic)
Dendrites
Nucleus
(Schwann cell)
Microfilament
Microtubule
Axon

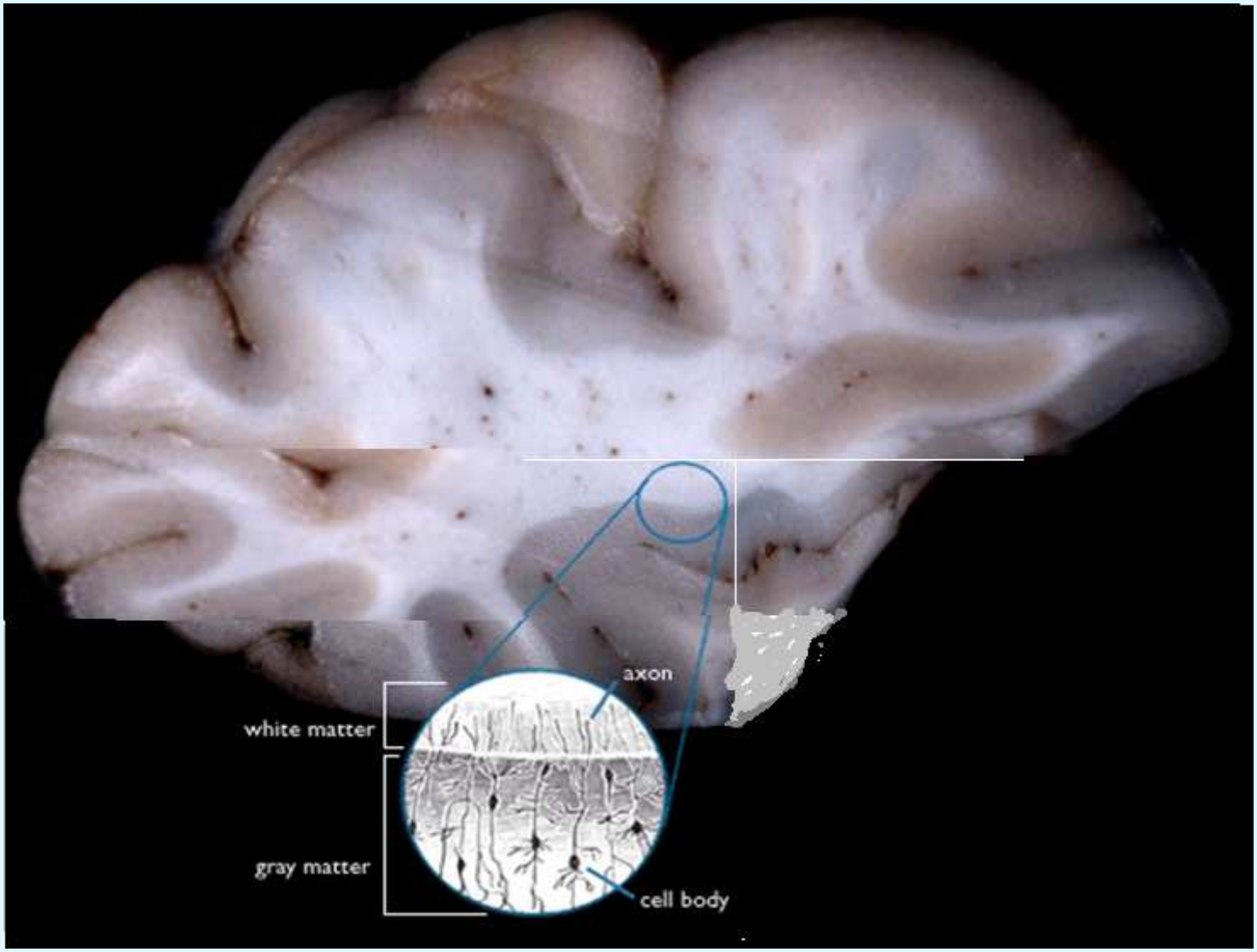
# ( 8 )

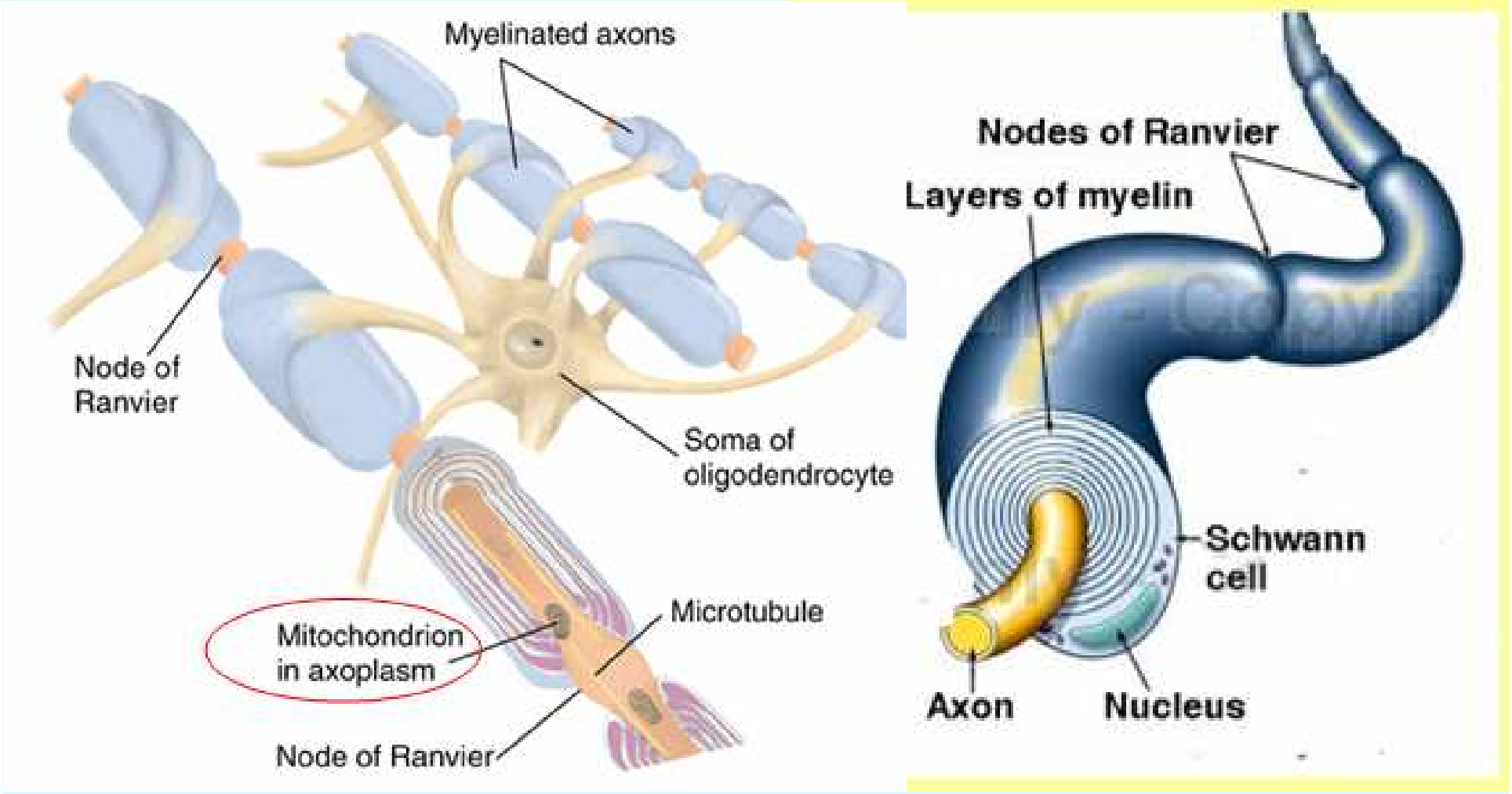
# Extramitocondrial ATP Synthesis in myelin

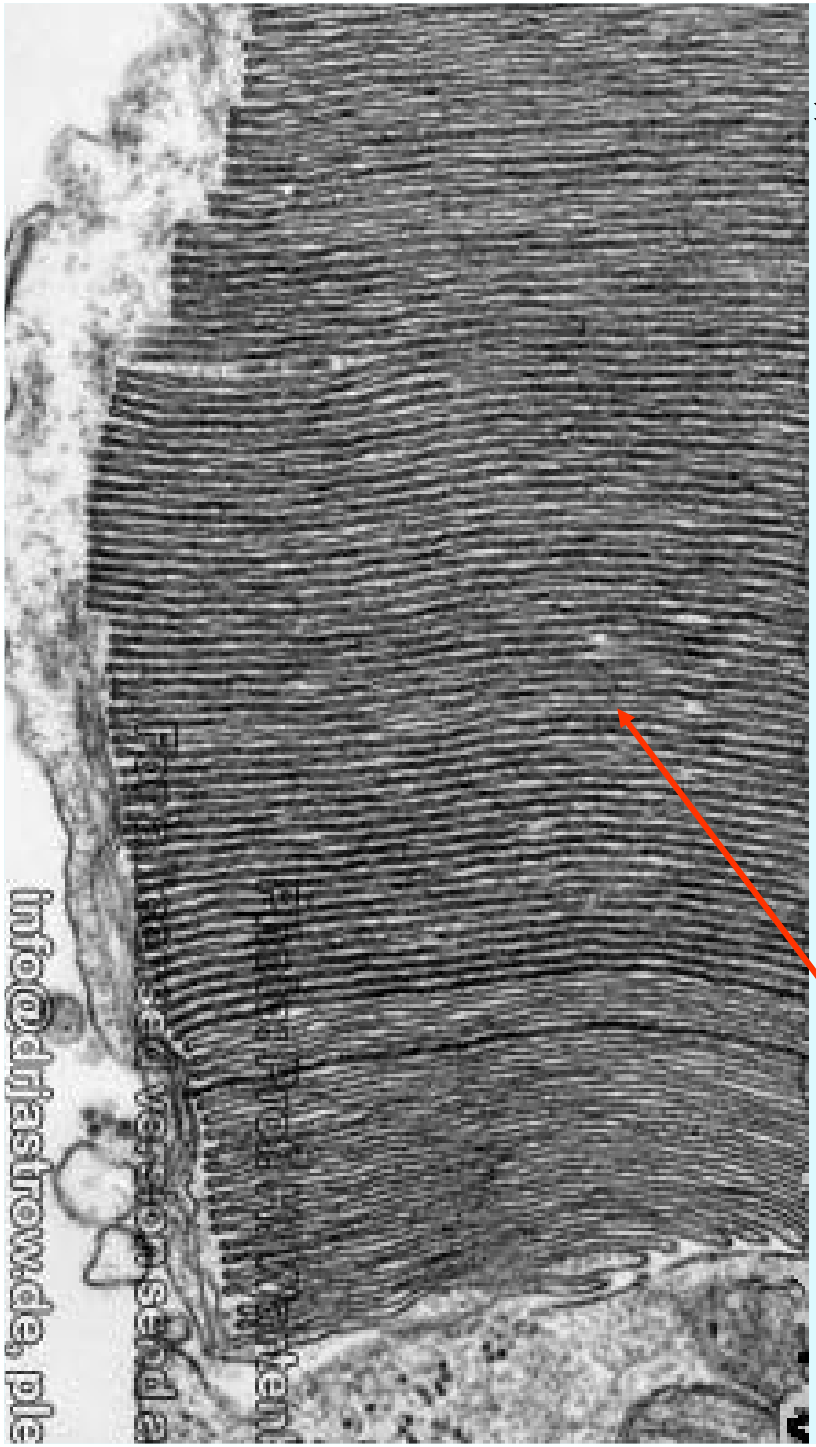



**White matter**

**~ 60 % of brain**


axon
white matter
gray matter
cell body


Myelinated axons
Node of Ranvier
Soma of oligodendrocyte
Mitochondrion in axoplasm
Microtubule
Node of Ranvier
Nodes of Ranvier
Layers of myelin
Schwann cell
Axon
Nucleus

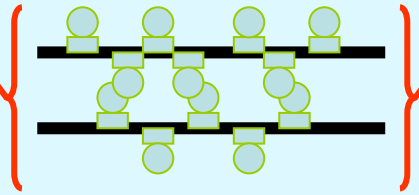


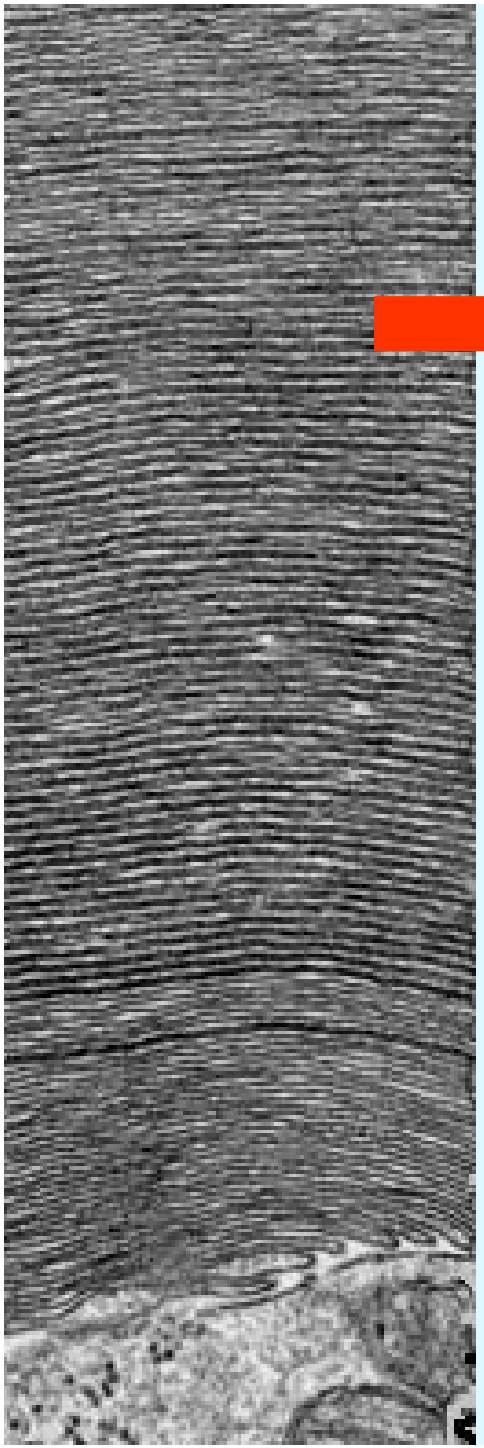
Interdiskal distance:

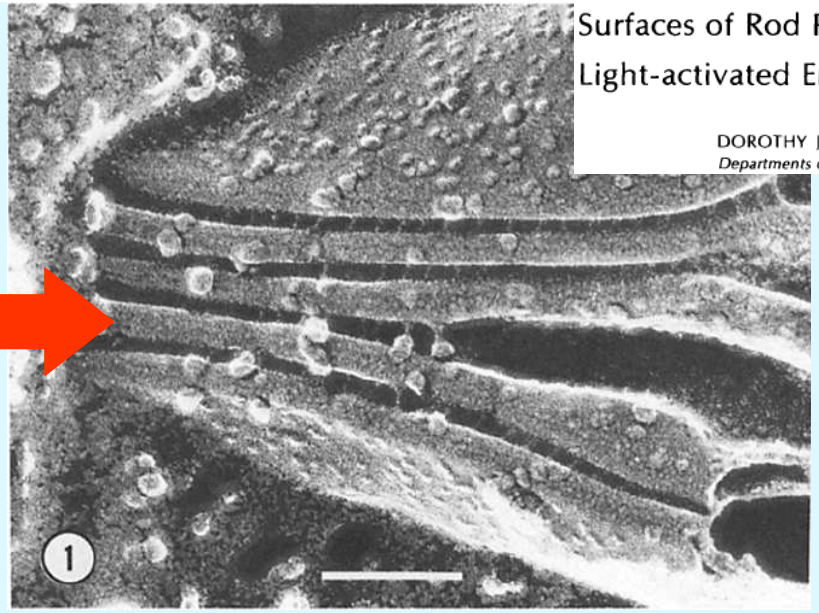
~35 nm

Interperiodal distance :

~25 nm







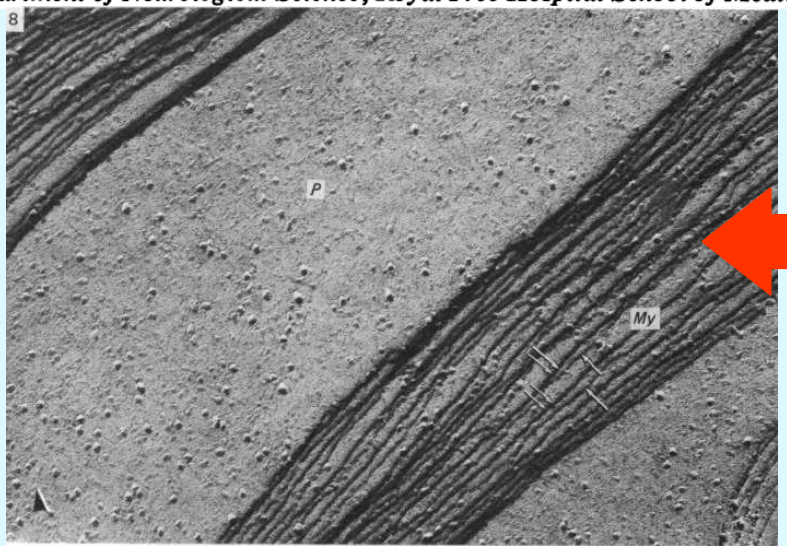

Surfaces of Rod Photoreceptor Disk Membranes:
Light-activated Enzymes **1982**

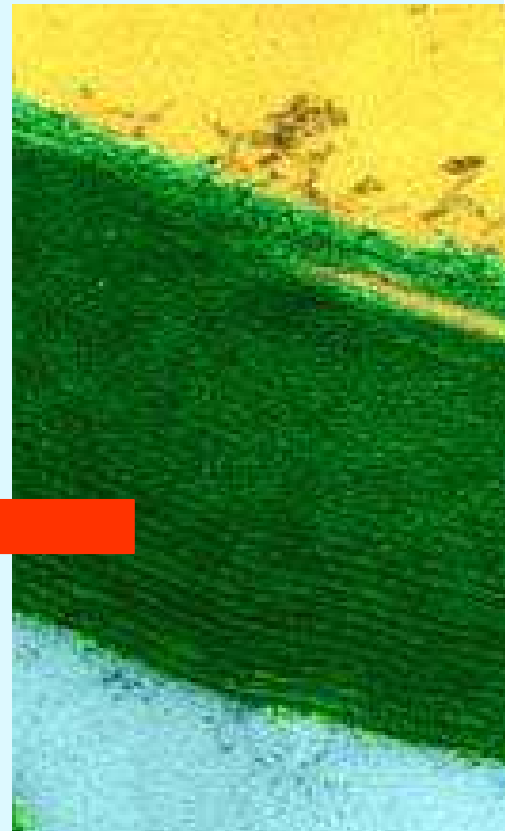
DOROTHY J. ROOF, JUAN I. KORENBROT, AND JOHN E. HEUSER
*Departments of Physiology and Biochemistry and Biophysics, University of California School*

**Freeze-fracture observations on human peripheral nerve***

**1986**

G. GABRIEL†, P. K. THOMAS†, R. H. M. KING†
C. STOLINSKI‡ AND A. S. BREATHNACH‡
†*Department of Neurological Science, Royal Free Hospital School of Medicine*

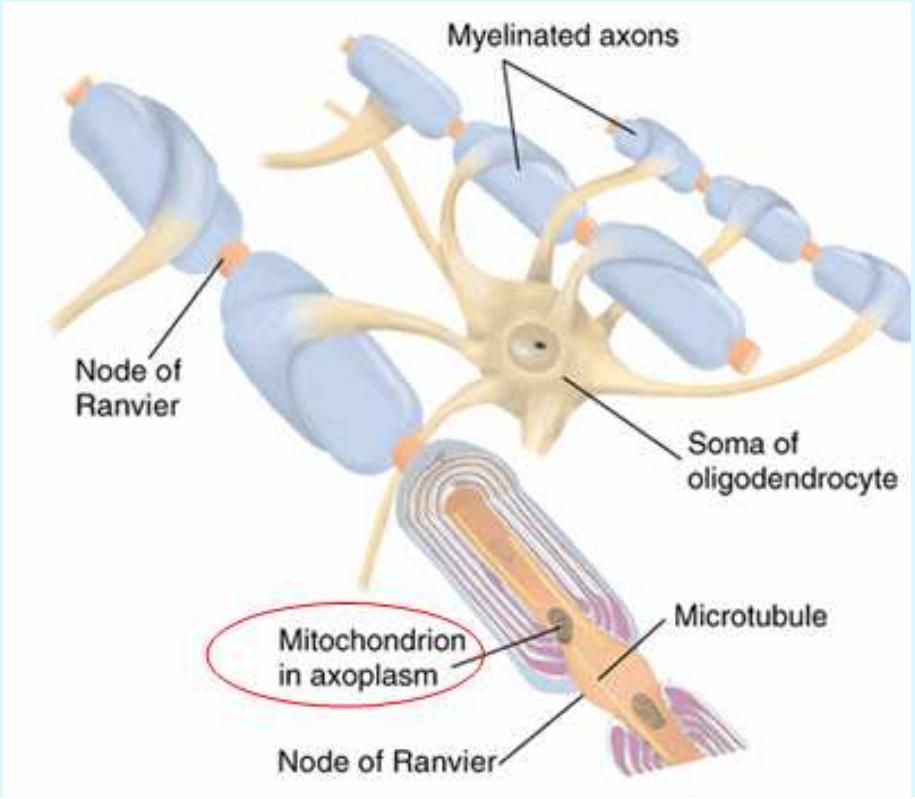


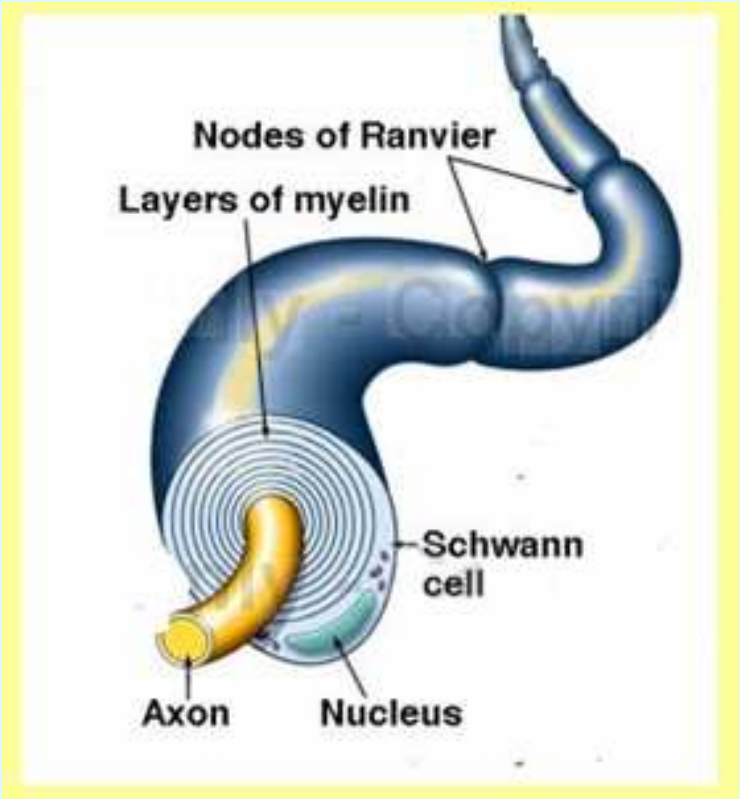



Our attention has focused to the finding of structures possible **vicariant of mitochondria**

# Myelin sheath

**Could be such structure, thanks to its enormous surface**



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possible **vicariant of mitochondria**

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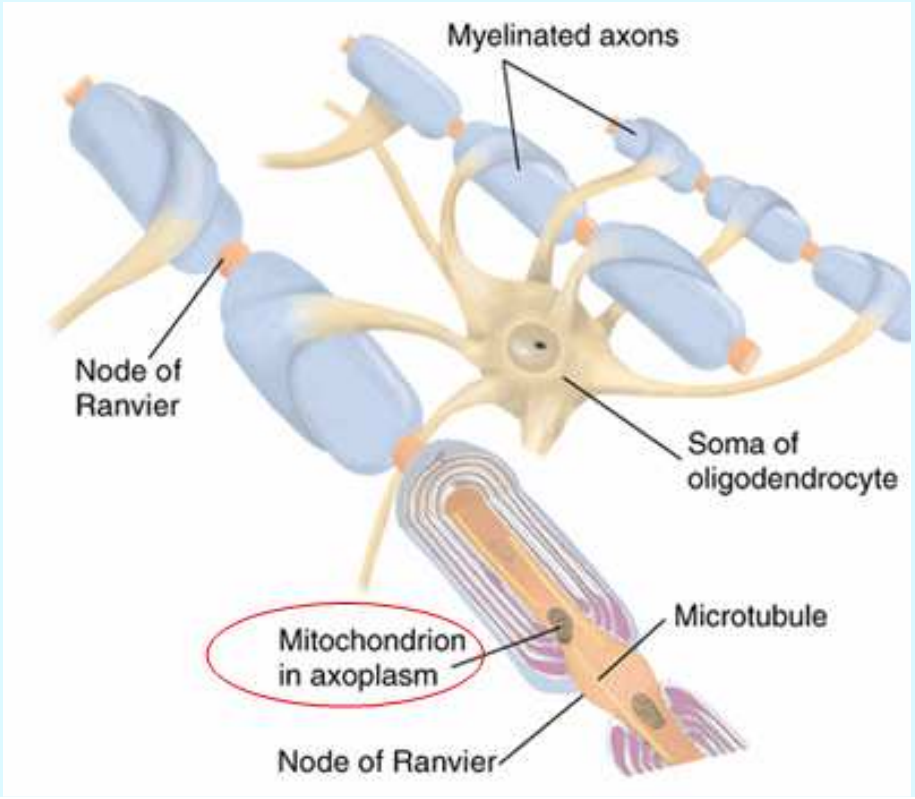
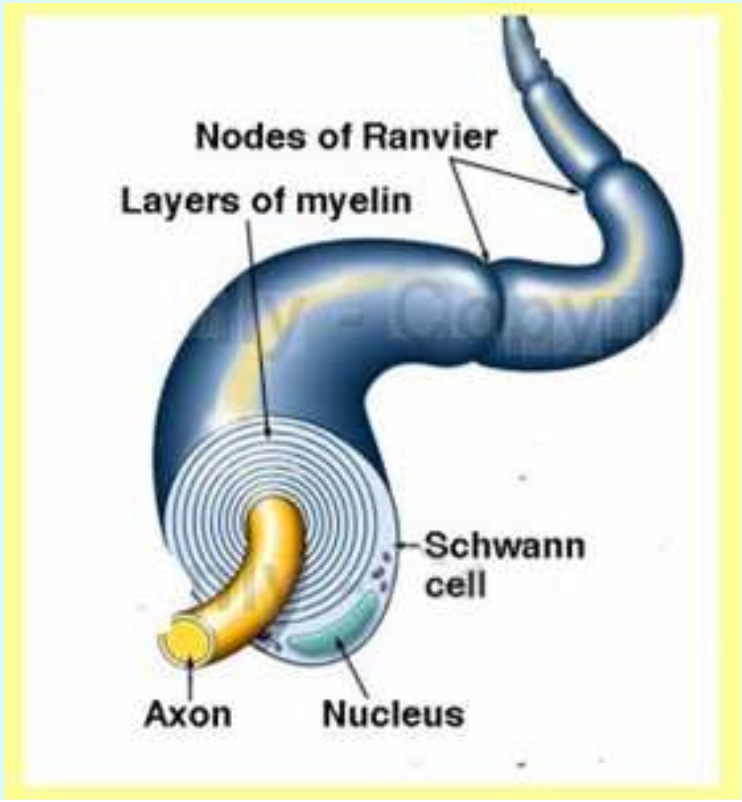
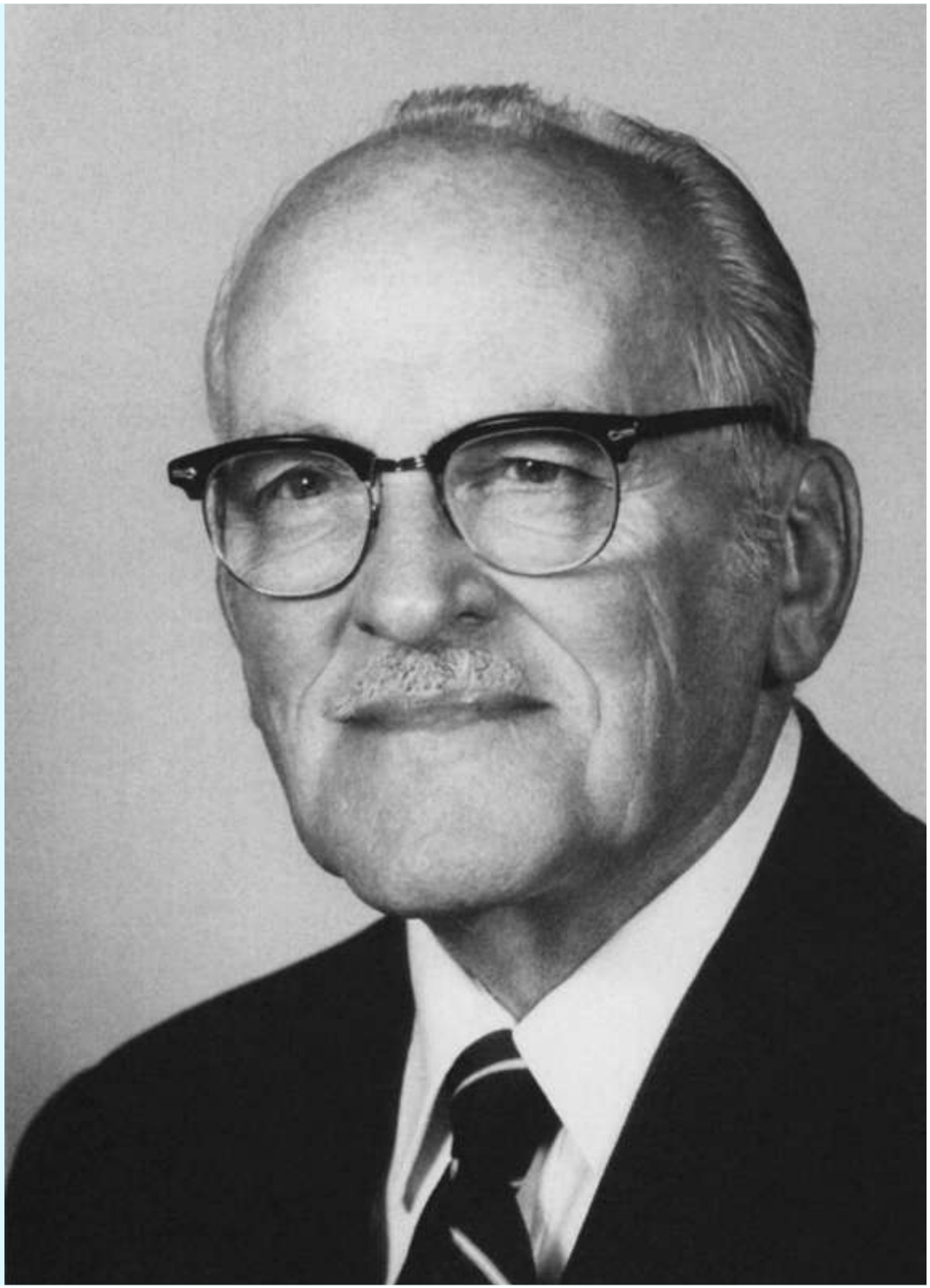


# Francis O. Schmitt

(1903–1995) Institute Professor at the Massachusetts Institute of Technology was pioneer in molecular biology and neuroscience.

The current head of the Department of Biology, Nobel laureate (1993, Med) Phillip A. Sharp, said: "Frank's leadership was critical in developing modern biochemistry and cellular biology at MIT and his spirit will be greatly missed."



Proceedings of the National Academy of Sciences of the United States of America

# *THE STRUCTURE OF THE SCHWANN CELL AND ITS RELATION TO THE AXON IN CERTAIN INVERTEBRATE NERVE FIBERS**

By Betty Ben Geren and Francis O. Schmitt

CHILDREN'S CANCER RESEARCH FOUNDATION; PATHOLOGY DEPARTMENT, HARVARD MEDICAL SCHOOL; AND DEPARTMENT OF BIOLOGY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY

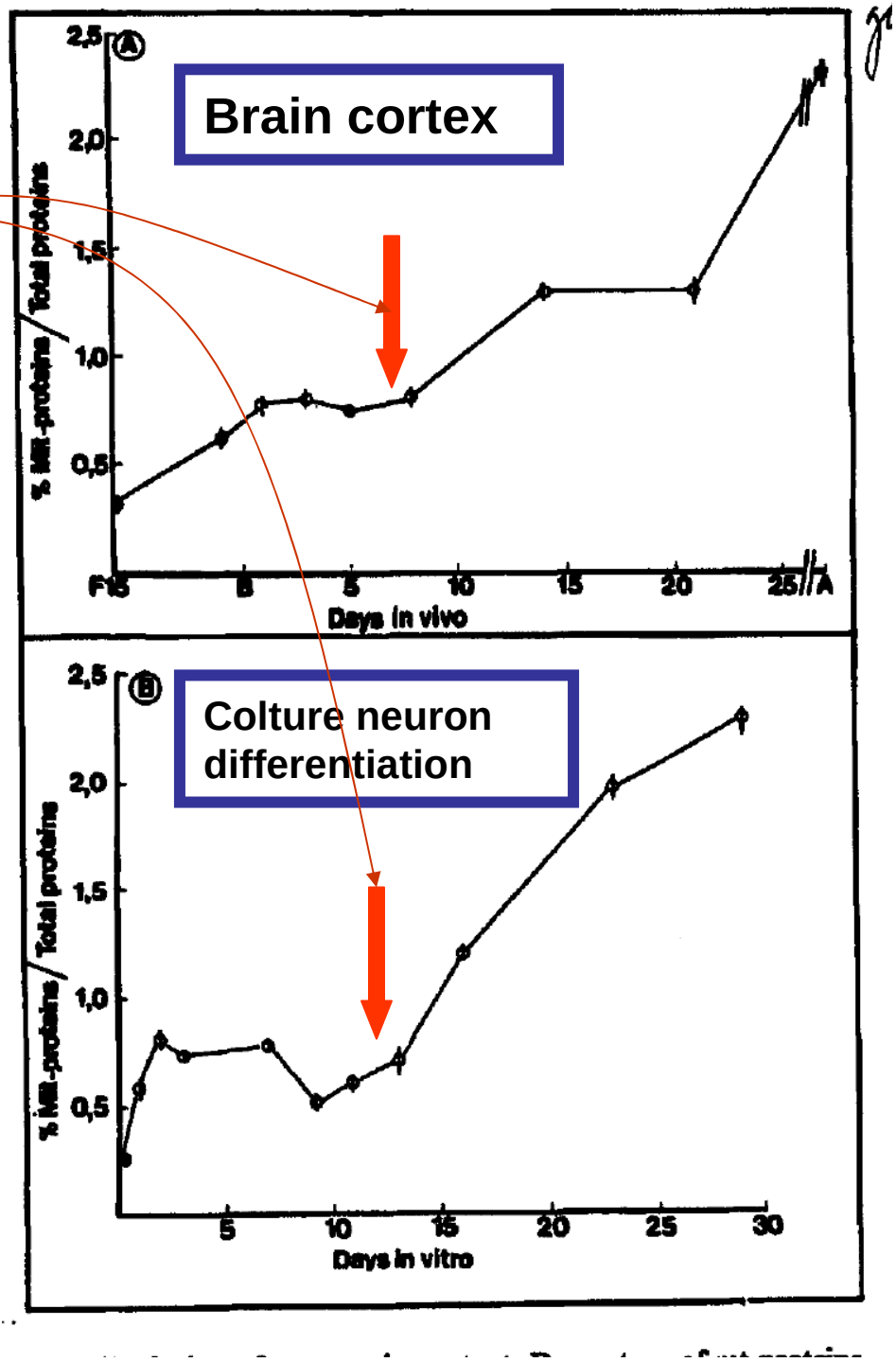
*Communicated June 23, 1954*

The concept of mitochondria as the power plant of the cell[25] has led morphologists to associate the presence of large numbers of mitochondria in tissue situations with the expenditure of large amounts of metabolic energy. The orientation of the sarcosomal mitochondria about the myofibrils is an example of such a case. If such a morphological diagnosis of function were applied to lobster and squid nerve fibers, one might suppose that the Schwann cell, spread out in a very thin layer around the axon, may be the site of high metabolic activity. The highly contorted axonal surface of the Schwann cell is in agreement with such a possibility. The high surface-to-volume relation in the Schwann cell in itself suggests high energy expenditure and the diffusion into the surface film of molecules presumably from Schwann cell protoplasm.

**Start**

**of glial cell proliferation**

Cordeau-Lossouarn, L., Vayssiere, J. L., Larcher, J. C., Gros, F.,& Croizat, B. (1991). Mitochondrial maturation during neuronal differen-tiation in vivo and in vitro. *Biology of the Cell, 71, 57–65.*



Mitochondrion 10 (2010) 143–150

PMID: 20005986

Contents lists available at ScienceDirect

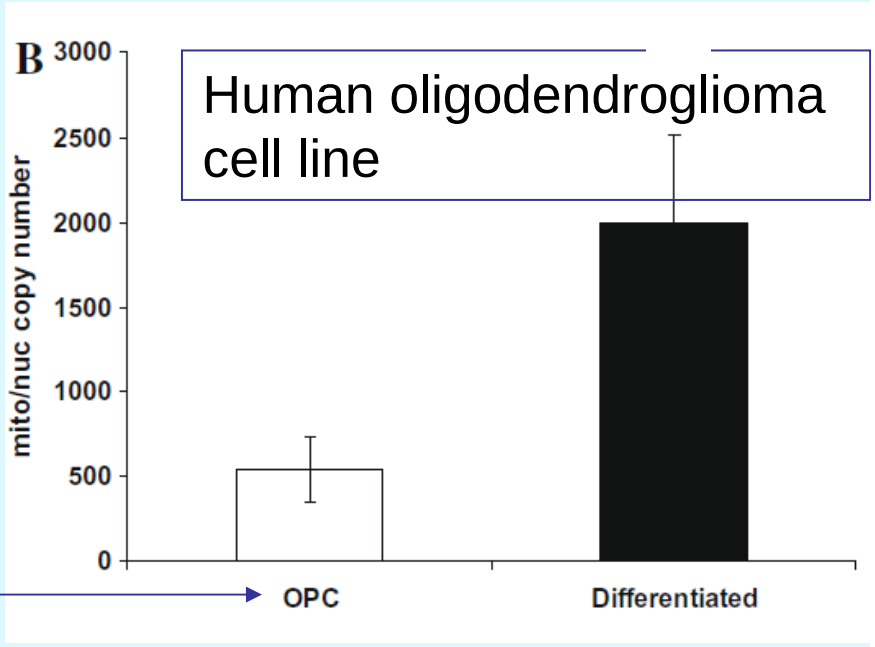
Mitochondrion

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

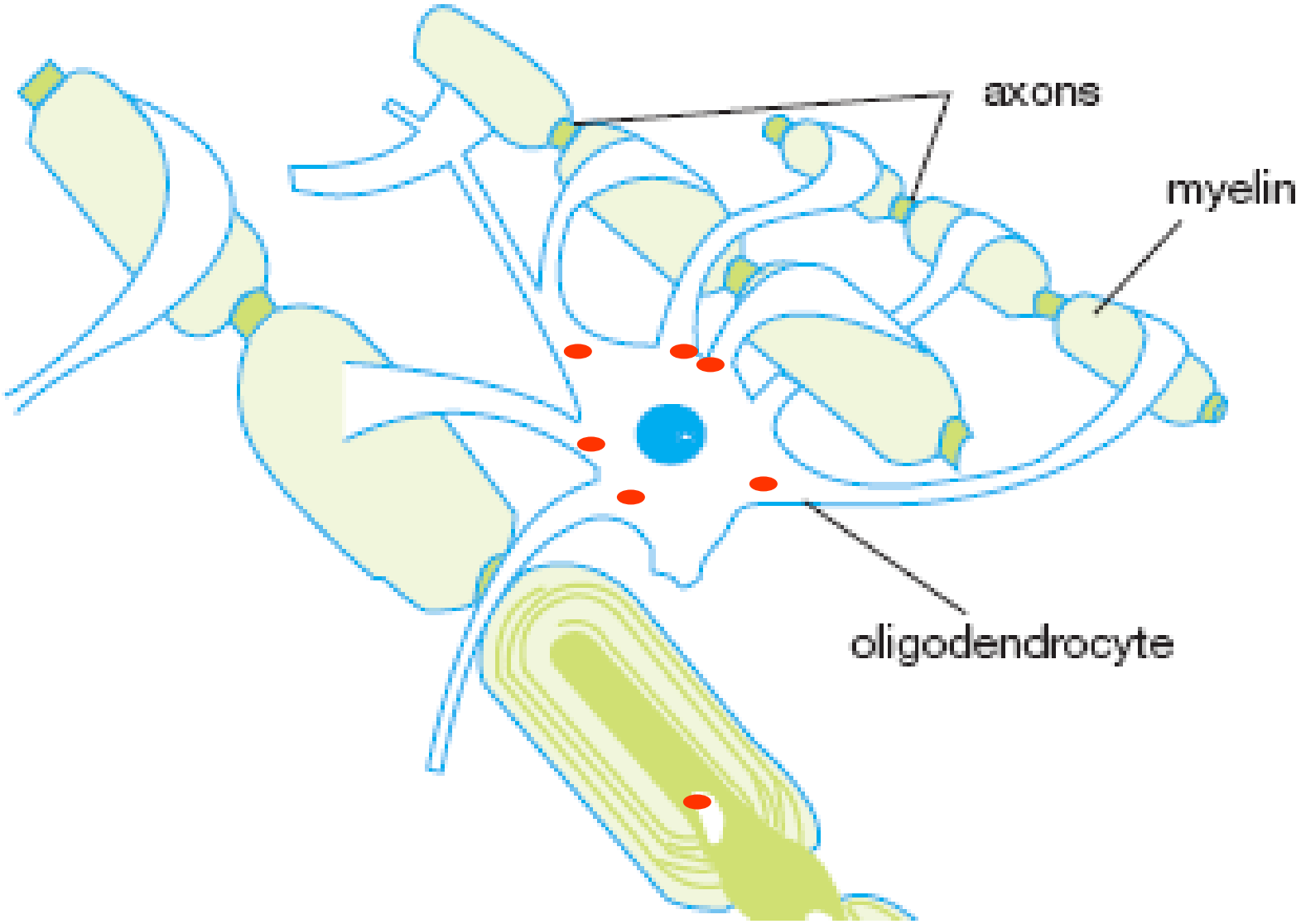
# Oligodendroglial differentiation induces mitochondrial genes and inhibition of mitochondrial function represses oligodendroglial differentiation

Robert Schoenfeld [a,1], Alice Wong [a,1], Jillian Silva [a], Ming Li [a], Aki Itoh [b], Makoto Horiuchi [b], Takayuki Itoh [b], David Pleasure [b], Gino Cortopassi [a,*]

**…mitochondrial transcripts and mtDNA are amplified during oligodendroglial differentiation, and differentiating oligodendroglia are especially sensitive to mitochondrial inhibition, suggesting mechanisms for demyelination observed in mitochondrial disease.**




**OPC:** Oligodendroglial Primary Cultures

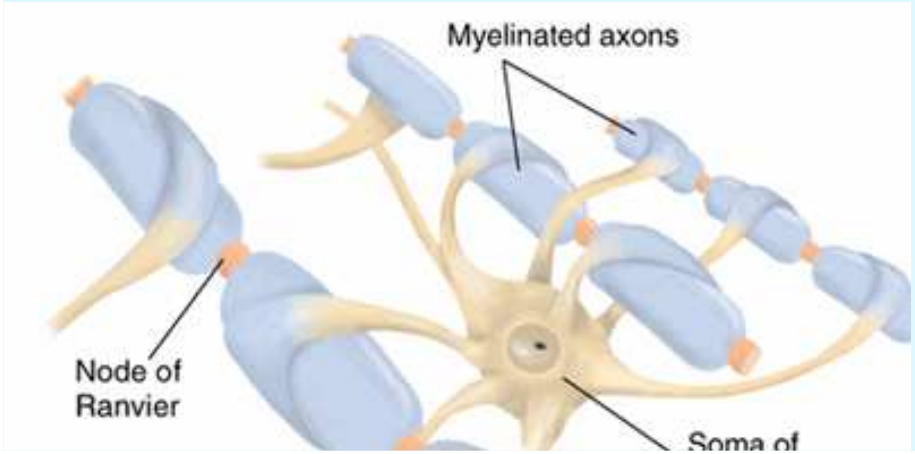

axons
myelin
oligodendrocyte

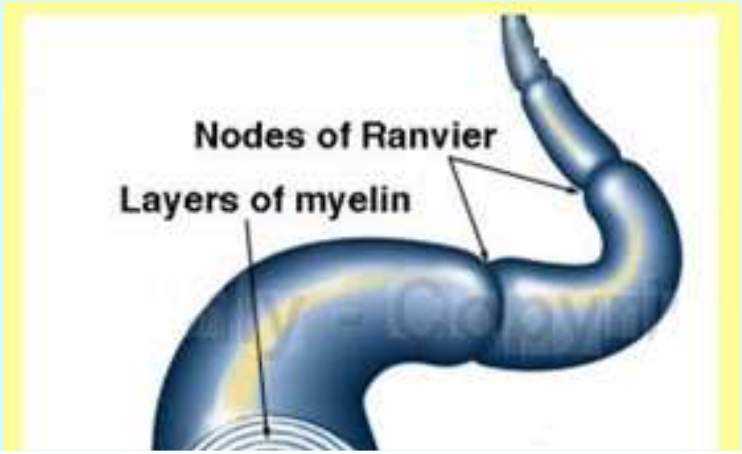
# An important task: OXYGEN trap and delivery in brain

→There is also the need that other neural and / or glial structures are appointed to the absorption of oxygen, because the brain does not contain (unlike other tissues, e.g. muscle), myoglobin, which acts as a slow reserve (flywheel ) of oxygen

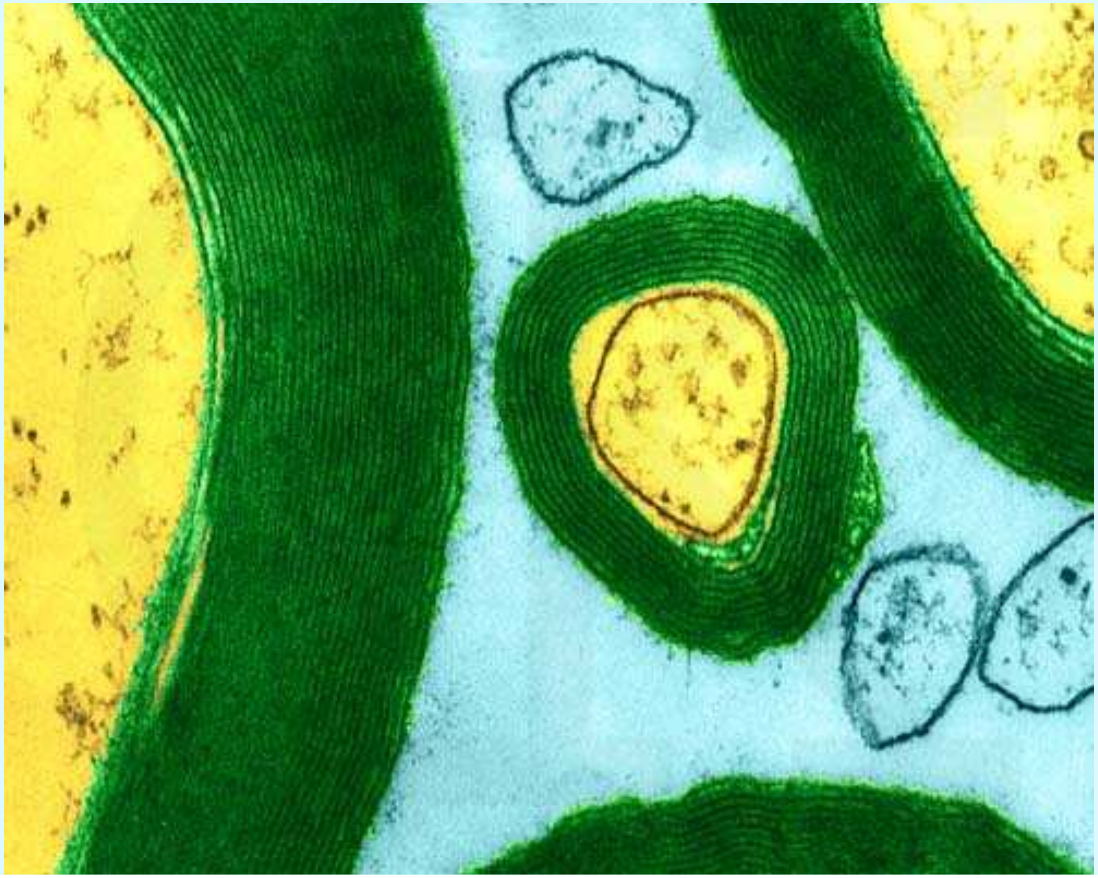
→To meet this need, these structures vicariant of myoglobin must have a big (huge) surface development

→Myelin sheath meets these requirements



**In fact, the myelin has a high (50-55%), % of neutral lipid (cholesterol, cerebrosides) in which oxygen can be dissolved at concentrations up to 4-5 times higher than water**



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JEB1401



# INTRACELLULAR OXYGEN DIFFUSION: THE ROLES OF MYOGLOBIN AND LIPID AT COLD BODY TEMPERATURE

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*Accepted 6 November 1997; published on WWW 24 March 1998*

Experiments with both seasonally cold-bodied fishes and polar fish species suggest that several factors combine to overcome these limitations in delivery of oxygen from the blood to the mitochondria. First, reductions in body temperature induce increases in mitochondrial density of Experiments with both seasonally cold-bodied fishes and polar fish species suggest that several factors combine to overcome these limitations in delivery of oxygen from the blood to the mitochondria. First, reductions in body temperature induce increases in mitochondrial density of oxidative muscle cells, reducing the mean diffusional pathlength for oxygen between capillaries and mitochondria. Second, cold body temperature in both temperate-zone and polar fishes is frequently correlated with a high content of neutral lipid in oxidative muscles, providing an enhanced diffusional pathway for oxygen through the tissue.

Table 1. *Effect of structural changes induced by thermal acclimation upon determinants of oxygen diffusion through aerobic skeletal muscle of striped bass* Morone saxatilis

**Oxygen Solubility**

| | Acclimation temperature | |
|---|---|---|
| | 5 °C ($N$=4) | 25 °C ($N$=5) |
| $D_{O_2} \times 10^6$ ($cm^2\ s^{-1}$) | 2.57±0.40 | 2.5±0.18 |
| $\alpha_{O_2} \times 10^2$ (ml $O_2$ $cm^{-3}$ $atm^{-1}$) | 6.64±0.27 | 3.59±0.20* |
| $K_{O_2} \times 10^6$ (ml $O_2$ $cm^{-1}$ $min^{-1}$ $atm^{-1}$) | 10.4±1.86 | 5.35±0.40 |

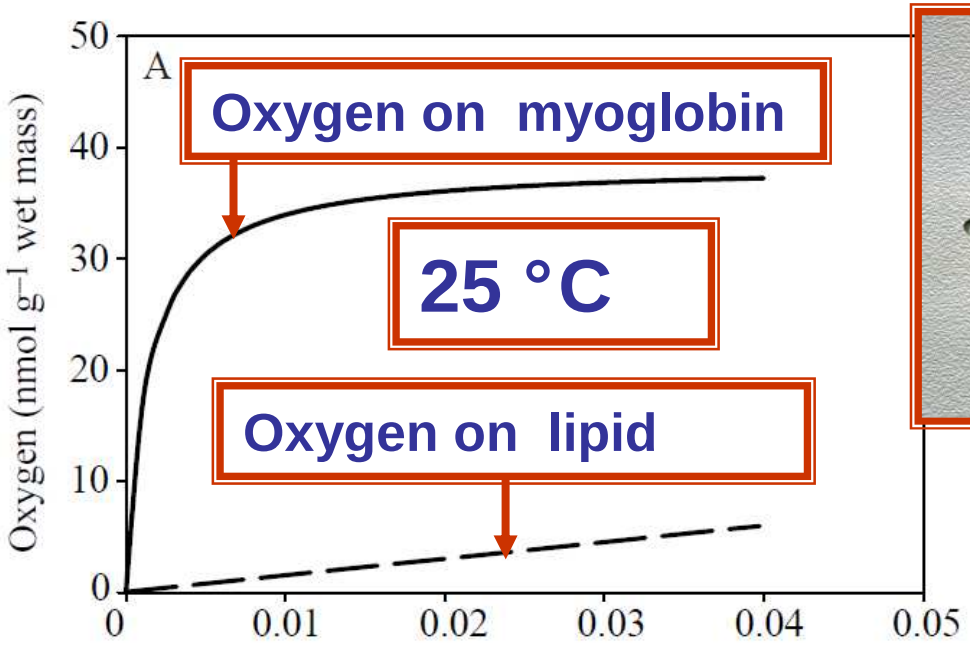
Values are means ± S.E.M. and were measured at an experimental temperature of 15 °C; $N$ is the number of animals.

Values reported for $K_{O_2}$ are calculated from data where $\alpha_{O_2}$ and $D_{O_2}$ were obtained from the same individual. Because $K_{O_2}$ is a calculated value, statistical comparisons between treatment groups were not performed. An asterisk indicated a significant difference between treatment groups *$P$<0.001.

Data are from Desaulniers *et al.* (1996).

$D_{O_2}$, diffusion coefficient for oxygen; $\alpha_{O_2}$, solubility coefficient for oxygen; $K_{O_2}$, diffusion constant for oxygen.

1 atm = 101.3 kPa.




***Morone saxatilis***

**Persico spigola**

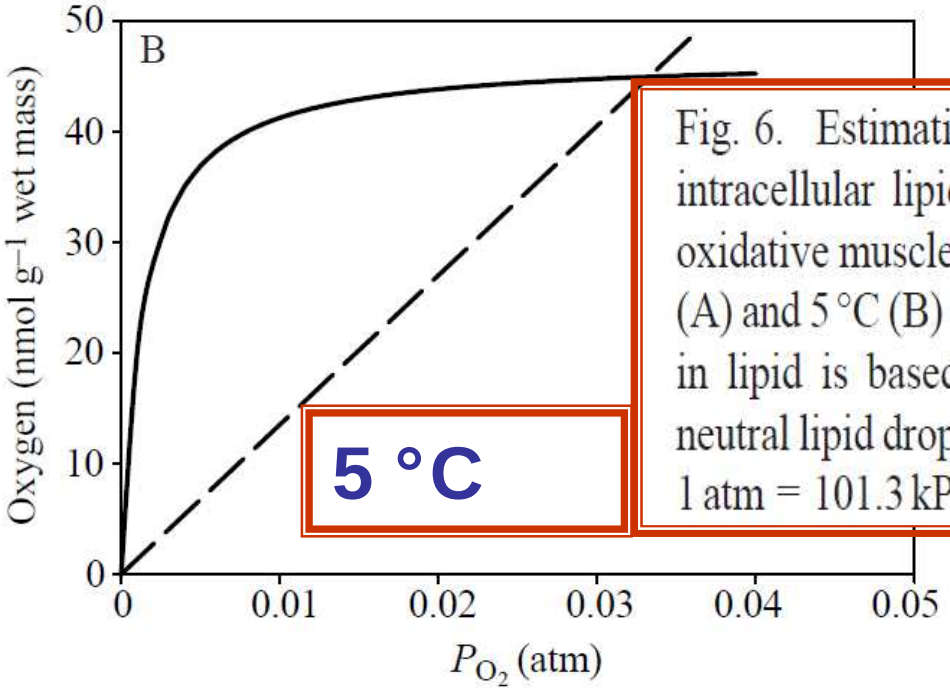



Fig. 6. Estimation of the amount of oxygen associated with intracellular lipid (dashed line) and with myoglobin (solid line) in oxidative muscle of striped bass *Morone saxatilis* acclimated to 25 °C (A) and 5 °C (B) as a function of $P_{O_2}$. The amount of oxygen dissolved in lipid is based solely upon the volume of anatomically discrete neutral lipid droplets. Figure is adapted from Desaulniers *et al.* (1996). 1 atm = 101.3 kPa.

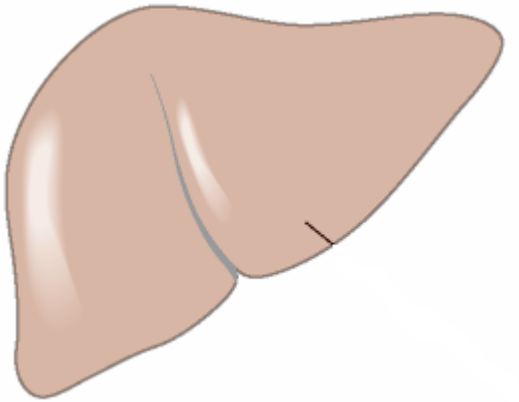
## $O_2$ consumed by 1 $cm^3$ liver:

## ~ 0,16 μmol/min

Absorbed by ~ 5 $m^2$ mitochondrial cristae
(surface of 1 $cm^3$ liver)

$O_2$ consumato da
**1500 gr fegato:**

~ **5** ml / min



## $O_2$ consumed by 1 $cm^3$ brain:

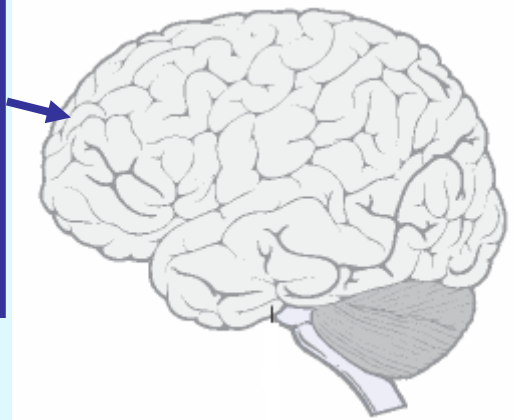
## ~ 1,6 μmol/min

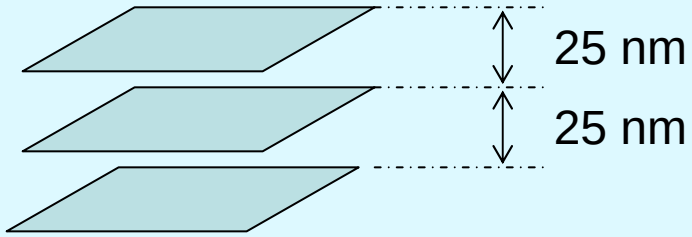
**The surface of the ridges of the brain mitochondria measured with stereological measurements in 1 cm3 of brain is** ~ 1,2 $m^2$

**It 's a totally inadequate surface!**
**If 0.16 μmol / min require 5$m^2$ then 1.6 μmol (10 times!) 50 $m^2$ or surface area of mitochondrial cristae are theoretically required which is indeed 1.2 $m^2$**

$O_2$ consumato da
**1400 gr cervello:**

~ **50** ml / min






# Development of myelin Surface

⇨ In 1 $\mu m^3$ of myelin thereare ~ 40 layers of 1 $\mu m^2$ x 2
(*major dense lines* have 2 sides at the distance of ~25 nm.)

⇨ 40 x 2 x 1 $\mu m^2$ = 80 $\mu m^2$. ⇨ 1 $\mu m^3$ = 80 $\mu m^2$ .

But: 1 $cm^3$ = 1 x $10^{12}$ $\mu m^3$

⇨ So **in 1 $cm^3$** = $10^{12}$ x 80 $\mu m^2$ = 67 . $10^{12}$ x $10^{-12}$ $m^2$

= **80 $m^2$**. in mitochondria: **25 m2**

⇨ myelin represents only ~ 50 % of white matter .

⇨ myelin surface in 1 $cm^3$ of white matter

becomes 80 x 0,5 = **40 $m^2$**

very colse to **50 $m^2$** necessary.

# PROTEOMIC STUDIES – 1 (2004)

## Proteomic mapping provides powerful insights into functional myelin biology

Christopher M. Taylor*, Cecilia B. Marta*, Robert J. Claycomb*, David K. Han†, Matthew N. Rasband*, Timothy Coetzee*‡, and Steven E. Pfeiffer*§

Departments of *Neuroscience, MC 3401, and †Physiology, MC 3501, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3401

www.pnas.org/cgi/doi/10.1073/pnas.0400922101 PNAS | March 30, 2004 | vol. 101 | no. 13 | 4643–4648

| | | |
|---|---|---|
| 57 | γ Enolase† | P17183 |
| 58 | ATP synthase β chain† | P56480 |
| 59 | Vesicle amine transport protein 1† | Q8R3G0 |
| 60 | Tubulin β-2 chain‡§ | P05217 |
| 61 | Tubulin α-2 chain§ | P05213 |
| 62 | Sep2 fragments† | Q9ESF7 |
| 63 | CAP 1† | P40124 |
| 64 | ATP synthase α chain† | Q03265 |

"In addition, and initially surprising to us, a number of mitochondrial proteins were identified… It is plausible that at least part of this energetic demand is supplied by local "**myelin mitochondria**." That mitochondria have been implicated in several neurodegenerative diseases accompanied by loss of myelin adds additional interest to these findings."

*Expert Rev. Proteomics* 8(2), (2011)

EXPERT REVIEWS

# Proteomics unravels the exportability of mitochondrial respiratory chains

*Expert Rev. Proteomics* 8(2), 231–239 (2011)

Isabella Panfoli[1],
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Maurizio Bruschi[2],
Giovanni Candiano[2]
and
Alessandro Morelli[†1]

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[†]*Author for correspondence:*

Expression of F1Fo-ATP synthase, which generates the majority of cellular ATP and is believed to be strictly confined to mitochondria, has recently been identified in ectopic locations, together with the four complexes of oxidative phosphorylation (OXPHOS) or enzymes from the Krebs cycle. Identification of these proteins has mostly been accomplished by proteomic methods and mass spectrometry – techniques that hold great promise in increasing our understanding of the proteome. The ectopic presence of ATP synthase has variably been attributed to contamination of the sample or to its action as a cell-surface receptor for apparently unrelated ligands, but OXPHOS proteins have sometimes been found to be catalytically active in oxidative phosphorylation, as they were true components of the system under investigation. The present article focuses on how mass spectrometry can increase our understanding of the proteome of subcellular membranes. We review the recent evidence for an extra-mitochondrial expression of OXPHOS by proteomics studies, highlighting what we can learn by combining these data.

# Krebs Cycle Enzymes

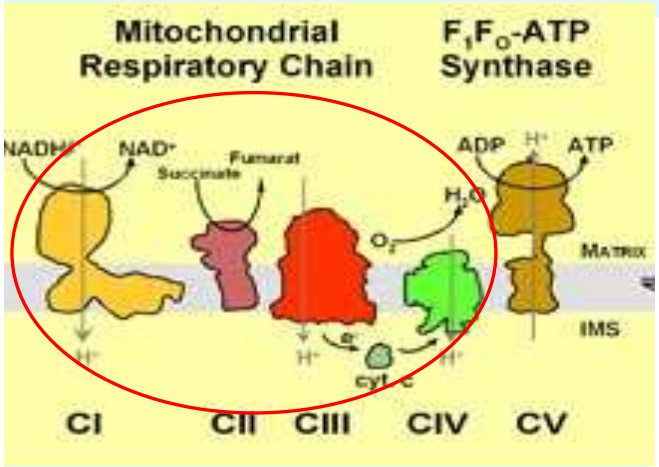
# in Mitochondria and in Myelin

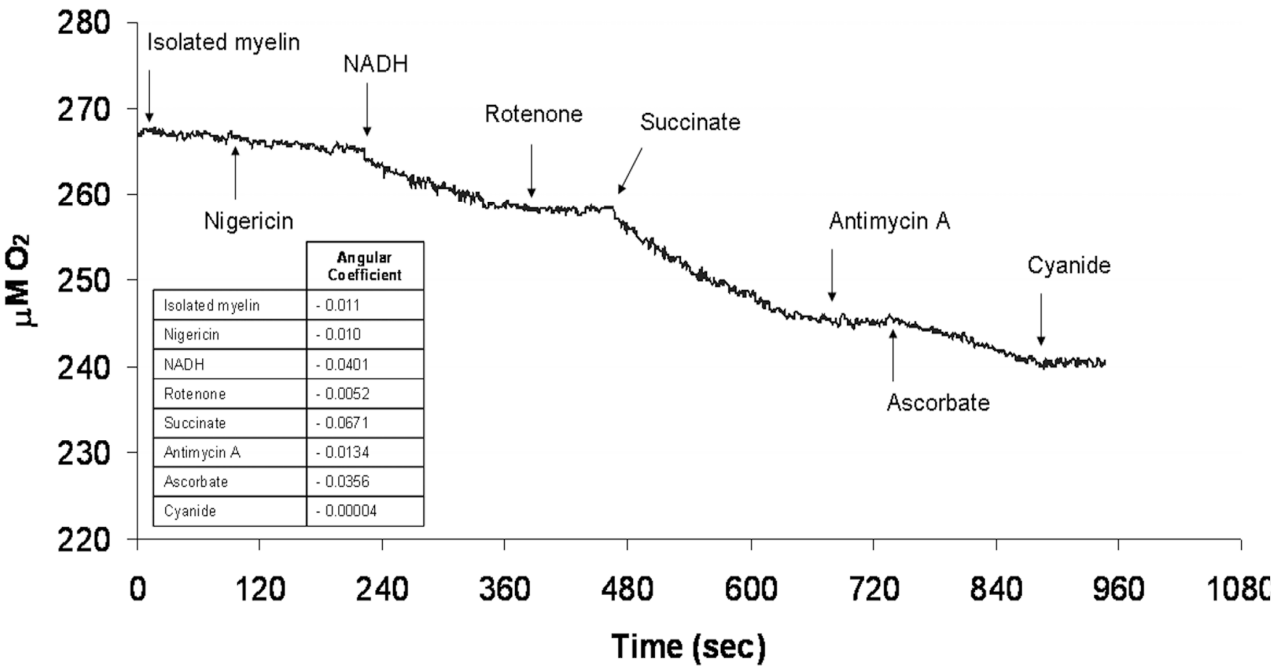
Measurements taken in Biology Dep. University of Genova.

November - December 2009

| | mU/mg (nMoli substrato consumato/min/mg prot) | | | |
|---|---|---|---|---|
| | **Mitochondria** | **homogenate. brain** | **Raw Myelin** | **Myelina** |
| **Citrate Synthase** | **220 ± 20** | 60 ± 6 | 80 ± 9 | **140 ± 20** |
| **Aconitase** | **30 ± 5** | 60 ± 7 | 80 ± 10 | **90 ±** |
| **Isocitric Dehydrog** | **13 ± 2** | 11 ±1 | 8 ± 1 | **11 ± 2** |
| **α-ketoglut Dehydr** | **7 ± 1** | 4 ± 0,5 | 5 ± 0,5 | **6 ± 1** |
| **Succinyl CoA Synth.** | **320 ± 30** | 115 ± 11 | 155 ± 16 | **210 ± 23** |
| **Succinic Dehyrog** | **12 ± 2** | 9 ± 1 | 6 ± 0,7 | **6 ± 0,7** |
| **Fumarase** | **900 ± 0,7 87** | 556 ± 0,7 55 | 753 ± 73 | **820 ± 86** |

# RESPIRATION RATE IN ISOLATED MYELIN



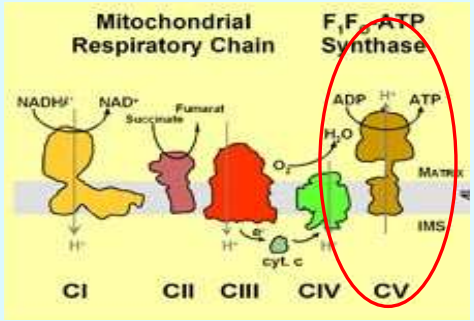



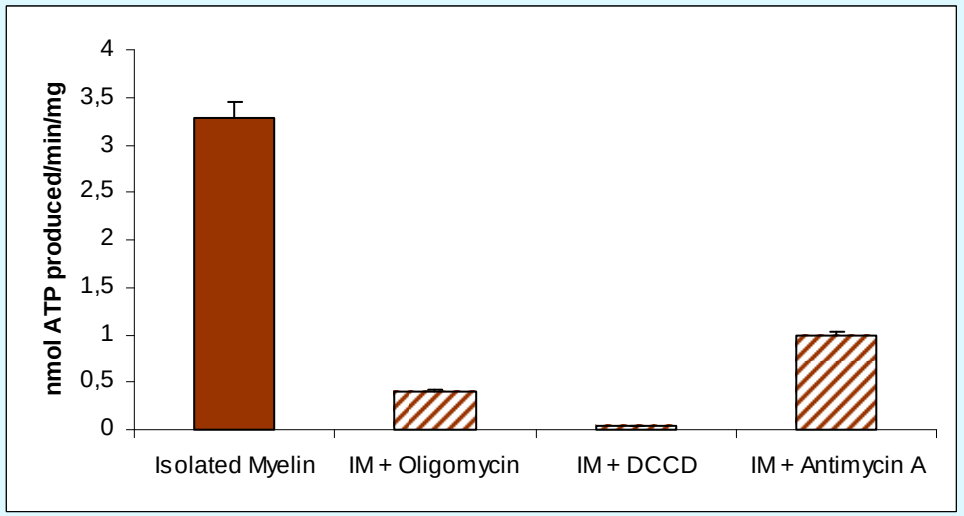

| | Angular Coefficient |
|---|---|
| Isolated myelin | - 0.011 |
| Nigericin | - 0.010 |
| NADH | - 0.0401 |
| Rotenone | - 0.0052 |
| Succinate | - 0.0671 |
| Antimycin A | - 0.0134 |
| Ascorbate | - 0.0356 |
| Cyanide | - 0.00004 |

| | Oxygen consumption (µM $O_2$/min/mg) | | |
|---|---|---|---|
| **Samples** | **Complex I + III + IV** | **Complex II + III + IV** | **Complex IV** |
| Forebrain homogenate | 42.37 ± 2.12 | 64.95 ± 3.24 | 38.25 ± 1.91 |
| Crude Myelin | 38.54 ± 1.93 | 62.05 ± 3.11 | 35.4 ± 1.77 |
| Isolated Myelin | 39.06 ± 1.97 | 61.38 ± 3.14 | 35.12 ± 1.76 |
| Mitochondria-enriched fraction | 60.63 ± 3.03 * | 91.58 ± 4.58 | 52.68 ± 2.64 |

* In this case the Complex I + III + IV activity was measured with addition of pyruvate/malate and cyclosporin A

# ATP SYNTHESIS IN ISOLATED MYELIN




**a/b subunits ATP Synthase**


| | 1 Mitochondria-enriched fraction | 2 Forebrain Homogenate | 3 Crude Myelin | 4 Isolated Myelin |
|---|---|---|---|---|
| α subunit Fo-F1 ATP Synthase (57 kDa) | 5,4 | 4,8 | 4,5 | 4,5 |
| β subunit Fo-F1 ATP Synthase (53 kDa) | 5,1 | 4,9 | 4,6 | 4,4 |

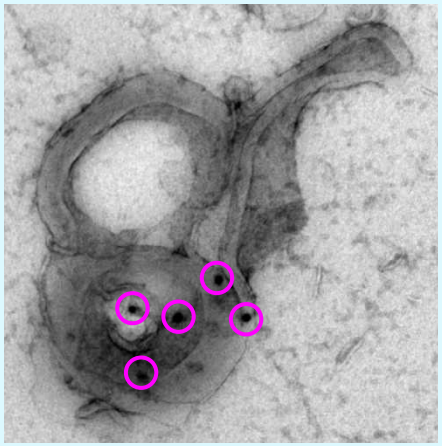
| Samples | nmol ATP produced/min/mg |
|---|---|
| Forebrain Homogenate | 4,02 ± 0,21 |
| Crude Myelin | 3,29 ± 0,15 |
| Isolated Myelin | 3,43 ± 0,17 |
| Mitochondria- enriched fraction + Cyclosporin A | 6,75 ± 0,34 |
| Mitochondria-enriched fraction | 0,37 ± 0,02 |

**45***

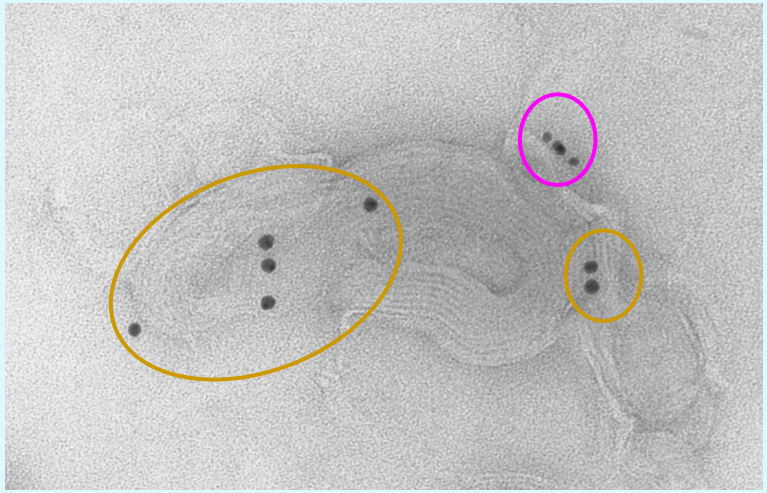
* Misura effettuata dic. 2009

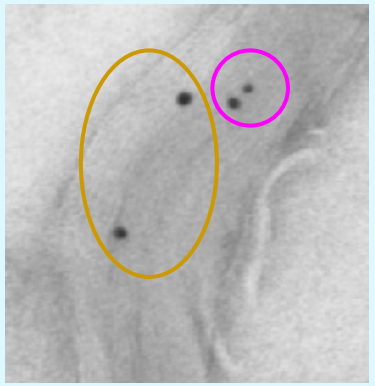
# TEM IMAGING

**α/β subunits ATP Synthase**

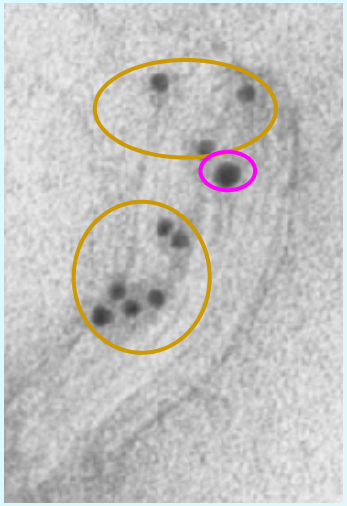


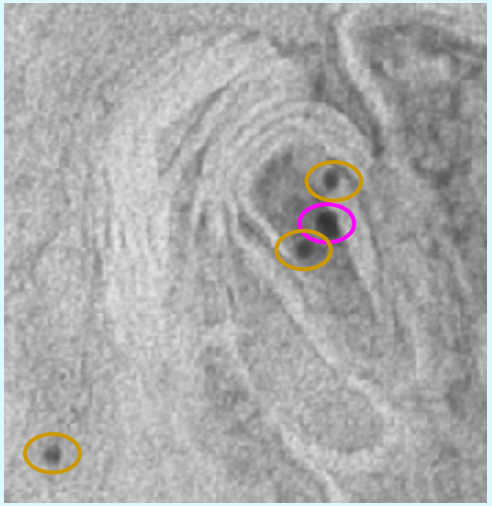
**α/β subunits ATP Synthase (10 nm)+ MBP (15 nm)**



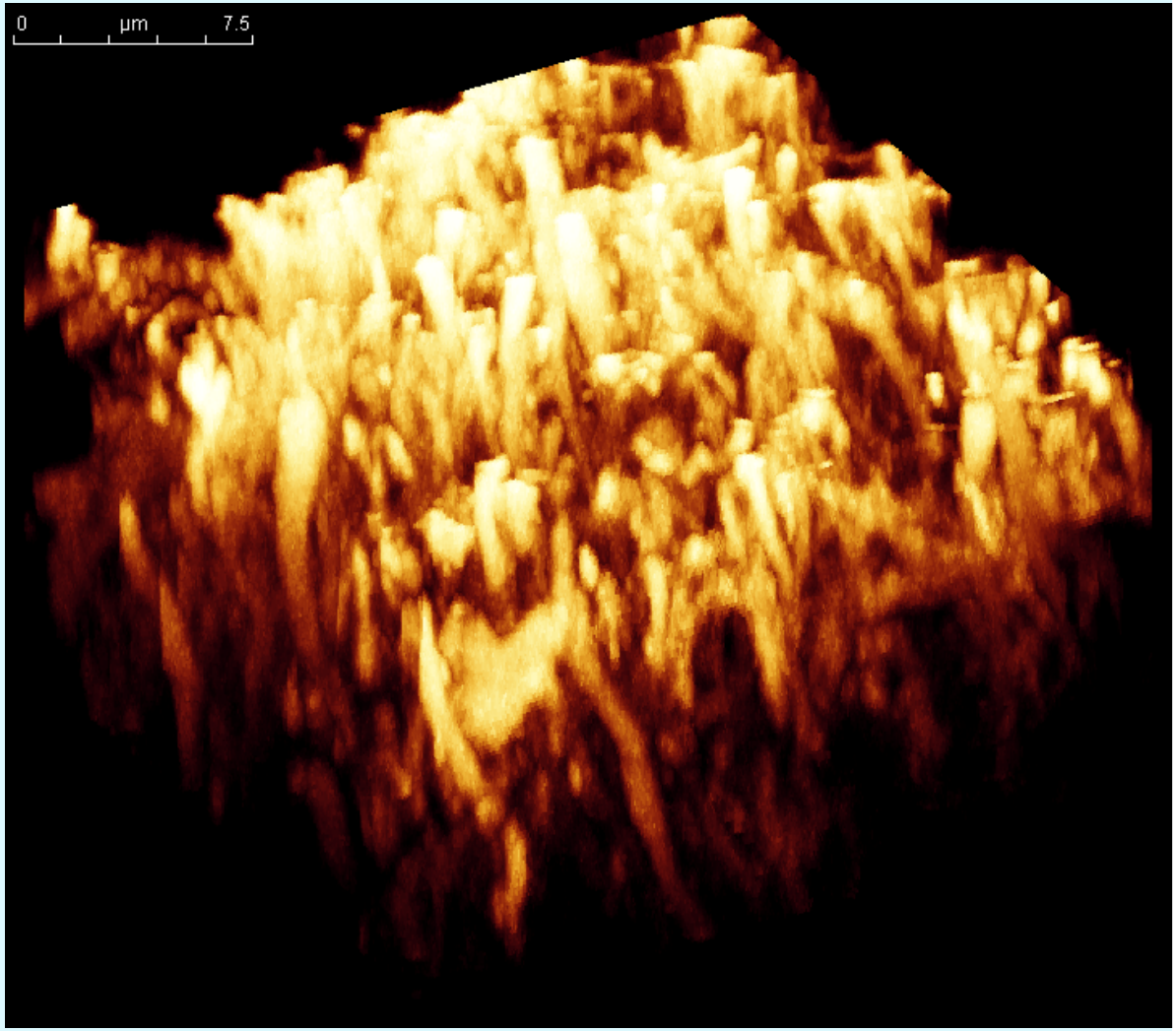


**α/β subunits ATP Synthase (15 nm) + MBP (10 nm)**





By a new technique that we have developed (Bianchini, 2008), myelin vesicles and optical nerves were incubated with MitoTracker Deep Red 633, a fluorescent dye that stains actively respiring membranes. After incubation, the samples were analyzed by CLSM.



The International Journal of Biochemistry & Cell Biology 41 (2009) 1581–1591
Available online 22 January 2009

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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


# Evidence for aerobic ATP synthesis in isolated myelin vesicles

Silvia Ravera[a], Isabella Panfoli[a], Daniela Calzia[a], Maria Grazia Aluigi[a], Paolo Bianchini[b], Alberto Diaspro[b], Gianluigi Mancardi[c], Alessandro Morelli[a,*]

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Salvatore Di Mauro
Evaluated 11 Jan 2010

**Evidence for aerobic ATP synthesis in isolated myelin vesicles.**

Ravera S, Panfoli I, Calzia D, Aluigi MG, Bianchini P, Diaspro A, Mancardi G, Morelli A
*Int J Biochem Cell Biol* 2009 Jul **41**(7):1581-91

**This study is not just fascinating but potentially revolutionary because it shows convincing evidence of extramitochondrial oxidative ATP production in isolated myelin vesicles (IMVs). This 'heretical' finding is not confined to isolated subcellular particles, but good evidence is provided by ex vivo confocal laser scanning microscopy and immunohistochemistry that a 'respiratory chain (RC)-like' system is present in the myelin sheath of the optic nerve. Their data led the authors to conclude that myelin may be a 'respiring wrap' providing ATP to the axon.**

The first part of this work is based on classical subcellular fractionation of bovine brain myelin and shows that IMVs utilize oxygen to produce ATP by using a proton gradient. Mitochondrial contamination is convincingly excluded, and the coexistence of ATP synthase and myelin basic protein (MBP) is documented in both IMVs and intact optic nerves. While the presence of mitochondrial RC components in extramitochondrial membranes (endoplasmic reticulum, plasma membrane, and even myelin) is not new (and the authors refer to the relevant literature), it is fair to say that the function of these 'foreign' proteins had not been fully clarified.

In this article, it is cogently suggested that myelin may be an extension of the mitochondrial redox apparatus, indeed a "mitochondrial inner membrane-like membrane" (to quote the authors), which would reconcile the high energy demand of the brain with its scarcity of mitochondria.
To support their argument, the authors stress the structural similarity between myelin and the inner mitochondrial membrane, especially the common presence of cardiolipin, a phospholipid of crucial functional importance for the RC. How does this RC-like apparatus get to the myelin sheath? The authors suggest that it is synthesized 'orthodoxically' in the inner mitochondrial membrane, after which mitochondria fuse with developing myelin sheaths, thus transferring 'ready-to-use' RC components. Although this process remains to be documented, it is in keeping with our increasing knowledge about mitochondrial dynamics. Obviously, this new vision of myelin as a 'respiring wrap' for the axon raises all kinds of possible pathogenic scenarios in which myelin RC defects may be involved, but this is another story. Strengths of the study include the solid biochemical data and convincing ex vivo studies of the optic nerve. Confirmatory data are needed, and the pathogenic role of 'respiratory defects' of myelin in demyelinating or neurodegenerative diseases is a hypothetical but exciting possibility. The studies presented here should be extended to human brain samples from patients with leukoencephalopathies, e.g. those associated with known RC defects.

( 9 )

# Gap juntions in myelin for radial Adenosin Nucleotide diffusion

**Gap Junctions between Cells Expressing Connexin 43 or 32 Show Inverse Permselectivity to Adenosine and ATP**

**Gary S. Goldberg‡§, Alonso P. Moreno¶, and Paul D. Lampe**

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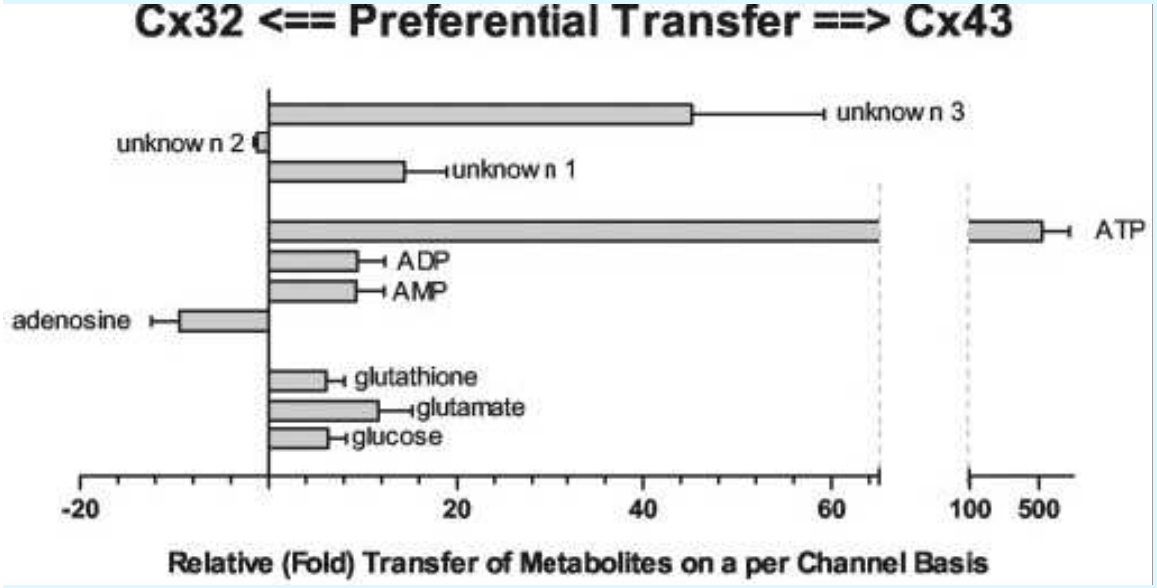
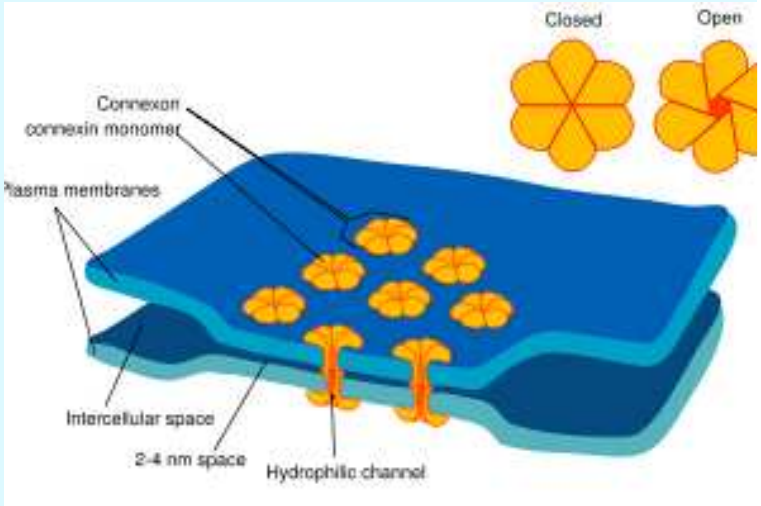
*the ¶Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, Indiana 46202, and*

*Fred Hutchinson Cancer Research Center, Seattle, Washington 98109*

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 39, Issue of September 27, pp. 36725–36730, **2002.**



# Myelin is rich in Gap-Junctions

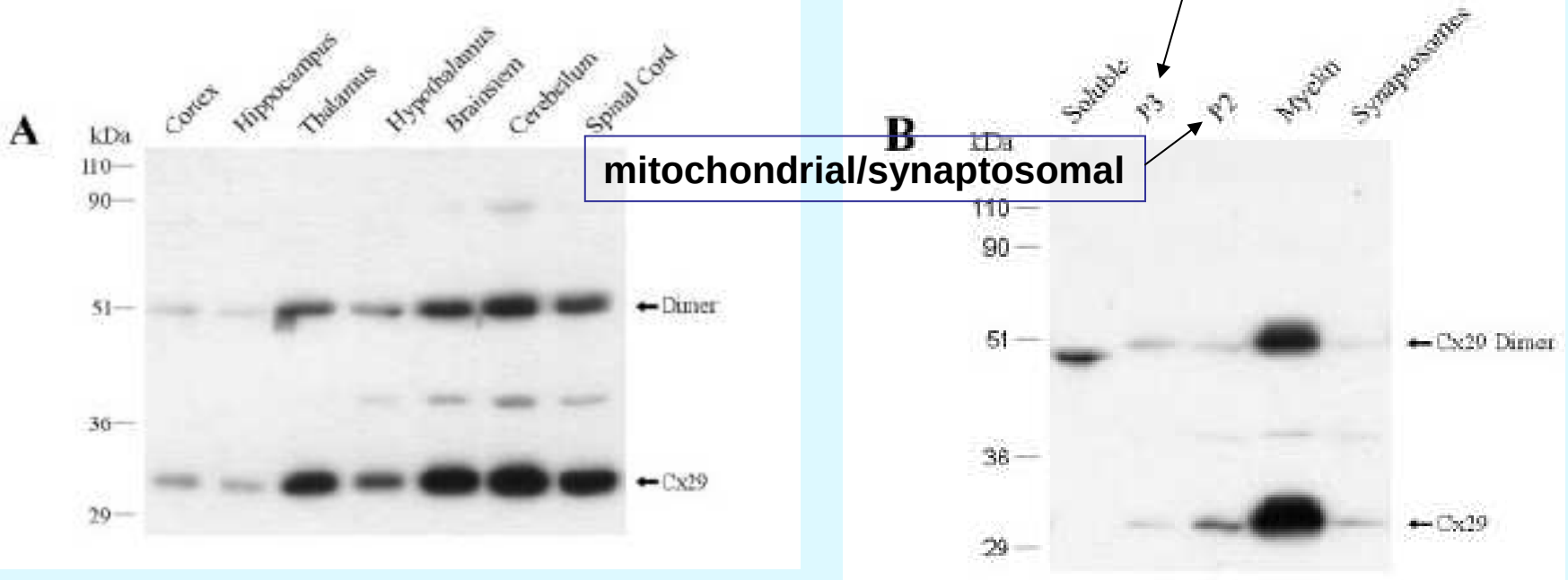
THE JOURNAL OF COMPARATIVE NEUROLOGY 464:356–370 (2003)

## Connexin29 and Connexin32 at Oligodendrocyte and Astrocyte Gap Junctions and in Myelin of the Mouse Central Nervous System

JAMES I. NAGY,[1]* ANDREI V. IONESCU,[1] BRUCE D. LYNN,[1] AND JOHN E. RASH[2]
[1]Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 3J7, Canada

Microsomal membrane

mitochondrial/synaptosomal

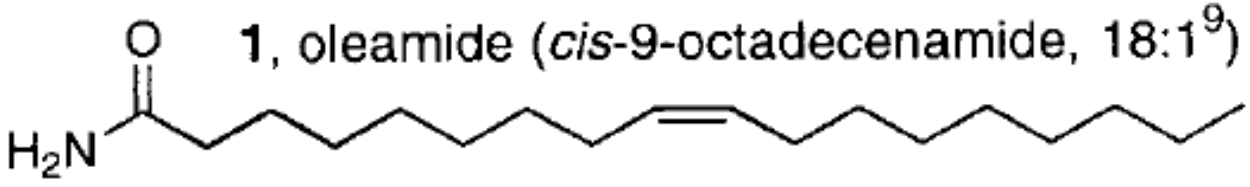


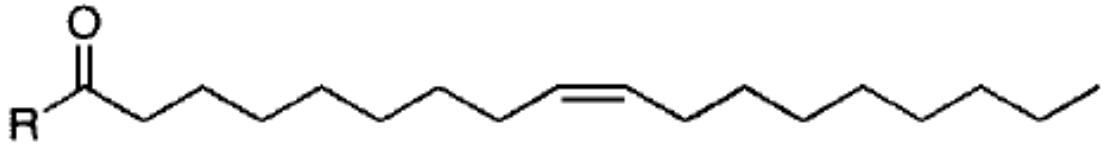
*Proc. Natl. Acad. Sci. USA*
Vol. 95, pp. 4810–4815, April 1998
Chemistry

# Chemical requirements for inhibition of gap junction communication by the biologically active lipid oleamide

DALE L. BOGER*†, JEAN E. PATTERSON*, XIAOJUN GUAN‡, BENJAMIN F. CRAVATT*, RICHARD A. LERNER*, AND NORTON B. GILULA‡

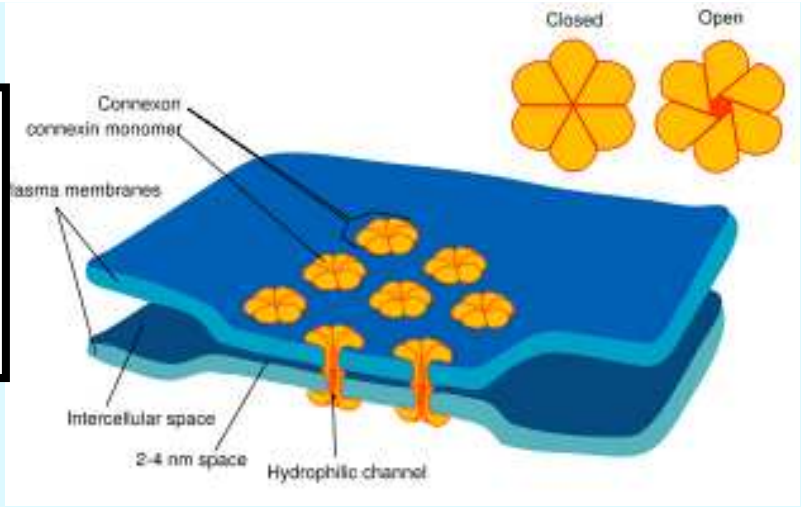
Departments of *Chemistry and ‡Cell Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines
La Jolla, CA 92037



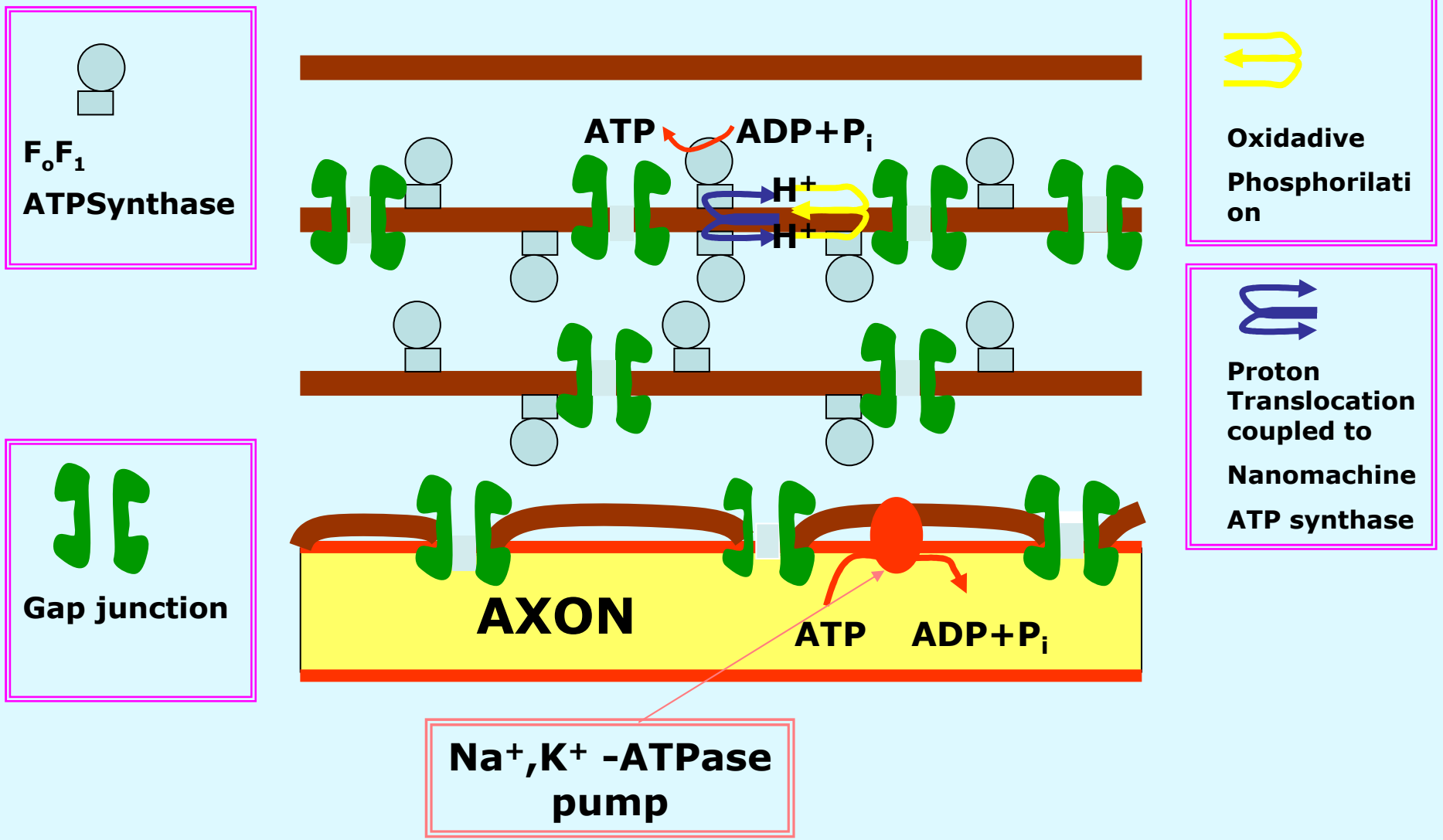

| Agent (R) | % inhibition†, μM | | |
|---|---|---|---|
| | 100 | 50 | 20 |
| $NH_2$ (oleamide) | 100 | 100 | 100 |
| OH (oleic acid) | 0 | 0 | 0 |

# Test of ATP production with Oleamide, an inhibitor of Gap-Junctions




| Sample | ATP produced (nmol/min/mg) |
|---|---|
| Vesicl Myelin (+ DMSO) | 3,5 ± 0,3 |
| Vesicl Myelin + Oleamide 50 μM (in DMSO) | 0,5 |
| Vesicl Myelin + Oleic Acid 50 μM (in DMSO) | 3,5 ± 0,3 |

# Hypothesis of Energized Myelin Sheath



J. Physiol. (1949) 108, 315–339

612.816.3

# EVIDENCE FOR SALTATORY CONDUCTION IN PERIPHERAL MYELINATED NERVE FIBRES

By A. F. HUXLEY and R. STÄMPFLI

*From the Physiological Laboratory, University of Cambridge, and the Physiological Institute, Berne*

**Another difficulty is that, according to many authors (e.g. Maximow & Bloom, 1942; Grundfest, 1947), the myelin sheaths of fibres of the central nervous system are uninterrupted except at points where the fibres branch. If this is true, the saltatory theory cannot apply to central fibres.**

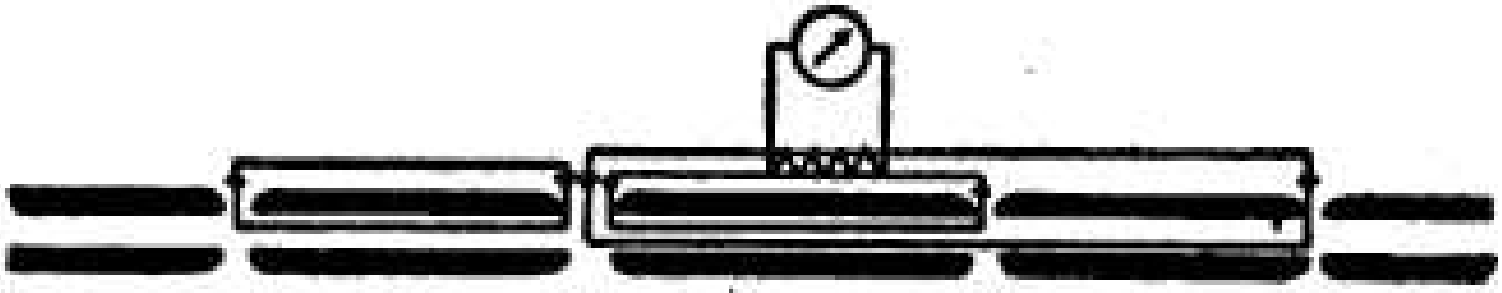
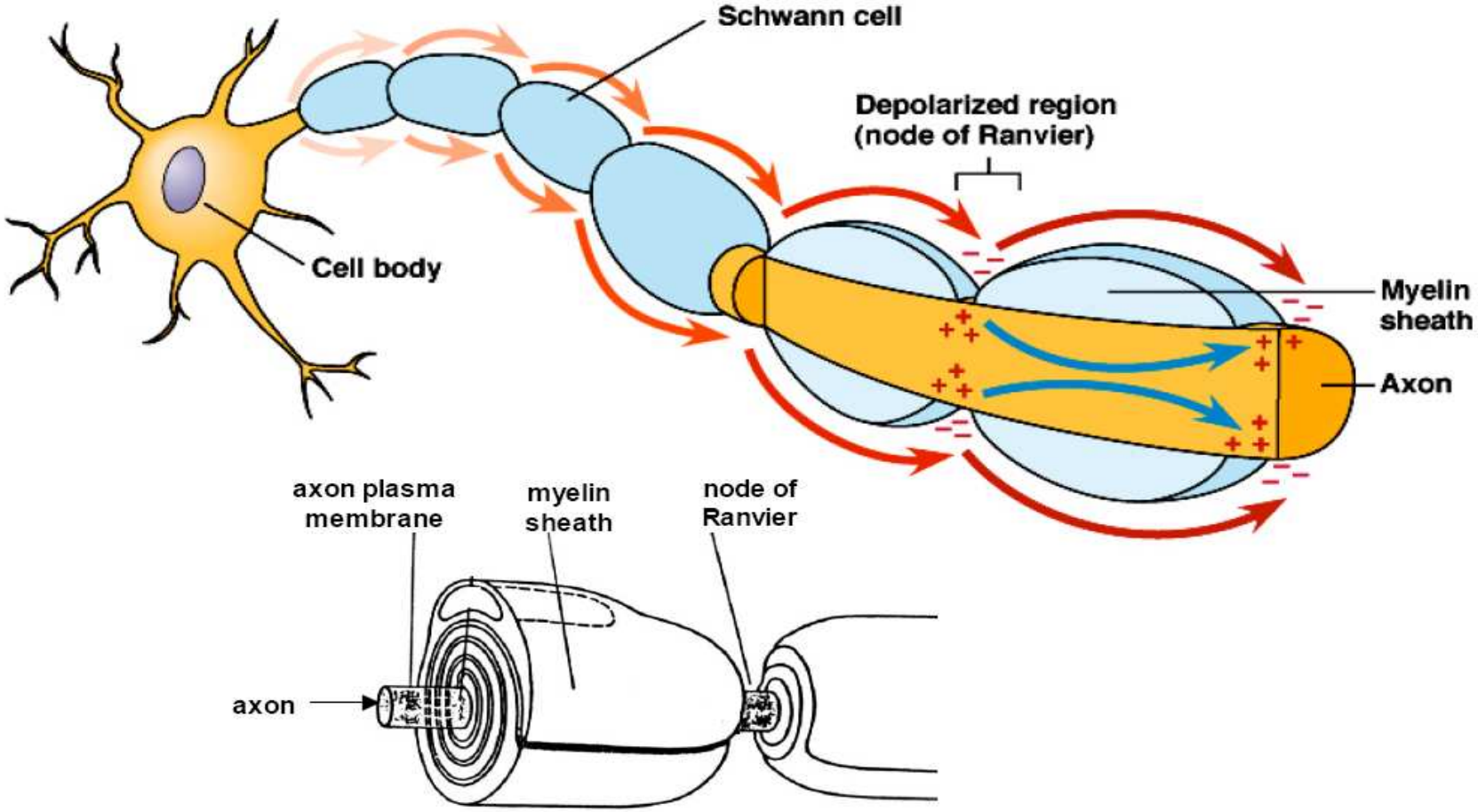


**Fig. 1. Diagram illustrating principle of method.**

impulse. The principle of the method was suggested by Mr A. L. Hodgkin of Cambridge, who pointed out that, if current can enter or leave the axis cylinder only at the nodes of Ranvier, the current along the axis cylinder must be the same at all points in any one internode at any one moment. If a single fibre is used and the recording system passes no appreciable current, the longitudinal current outside the fibre must be equal and opposite to that in the axis cylinder. This external current can be detected by raising the external

## How does myelination help an action potential move along a neuron?

# SALTATORY CONDUCTION:




**But some dubts on “insulation” from myelin…**

# Effect of myelination on the conduction velocity of optic nerve fibres

**1991**

D. J. Tolhurst and P. R. Lewis

Velocity as Rushton Theory

Myelinated fibres

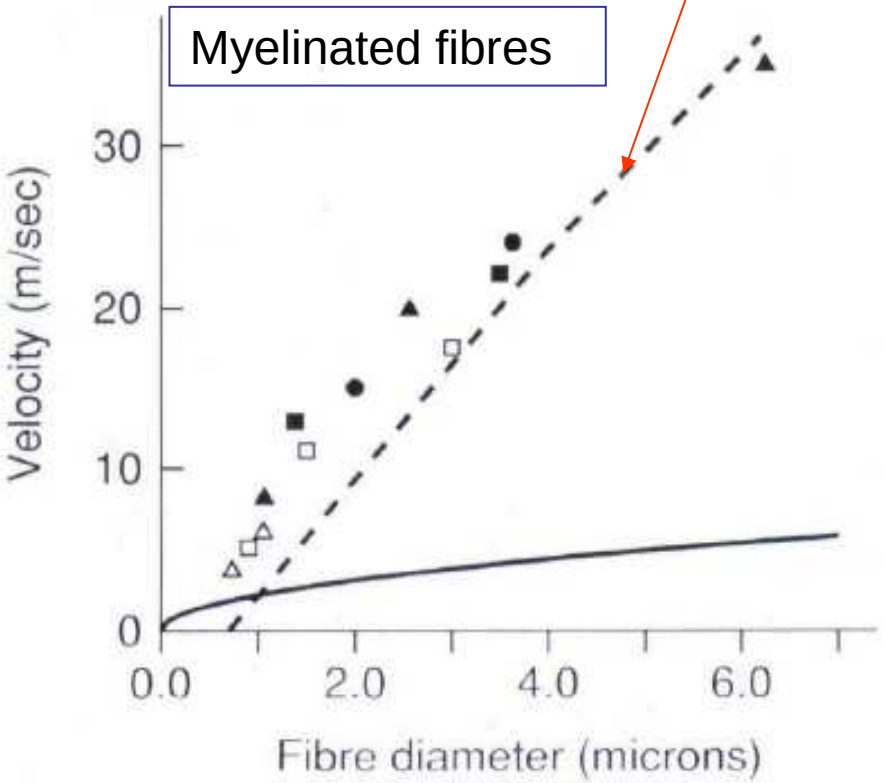



**Figure 1** The conduction velocity of myelinated fibres in the optic nerve or optic tract is plotted against their diameter, including the thickness of the myelin. The dashed curve shows Rushton's prediction of the relationship for myelinated fibres, while the continuous curve is the prediction for unmyelinated fibres. Values are shown for: ▲, cat; ■, macaque; ●, ferret; □, rat; △, hamster. See text for details of sources

Non Myelinated fibres

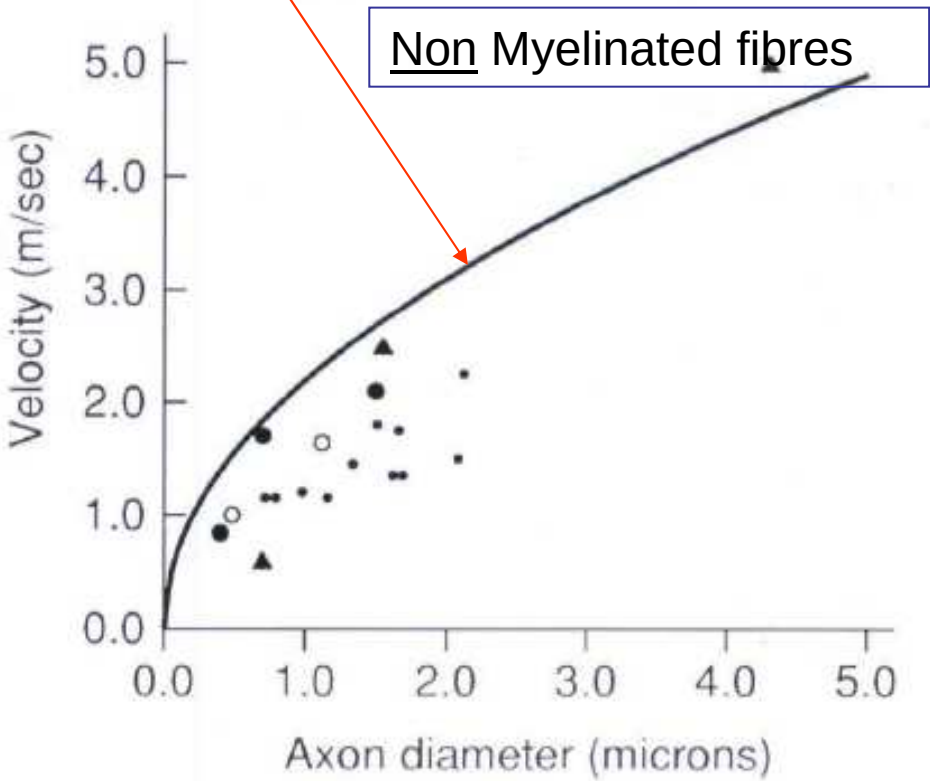



**Figure 2** The conduction velocity of unmyelinated intra-retinal axons is plotted against their diameter. The continuous curve is Rushton's prediction of the relationship for unmyelinated axons. Values are shown for cat (▲, ●) and for macaque (○): ▲, average values reported by Hsiao *et al.*[15]; ●, Stanford[16]; • show the 11 individual measurements reported by Stanford[17]

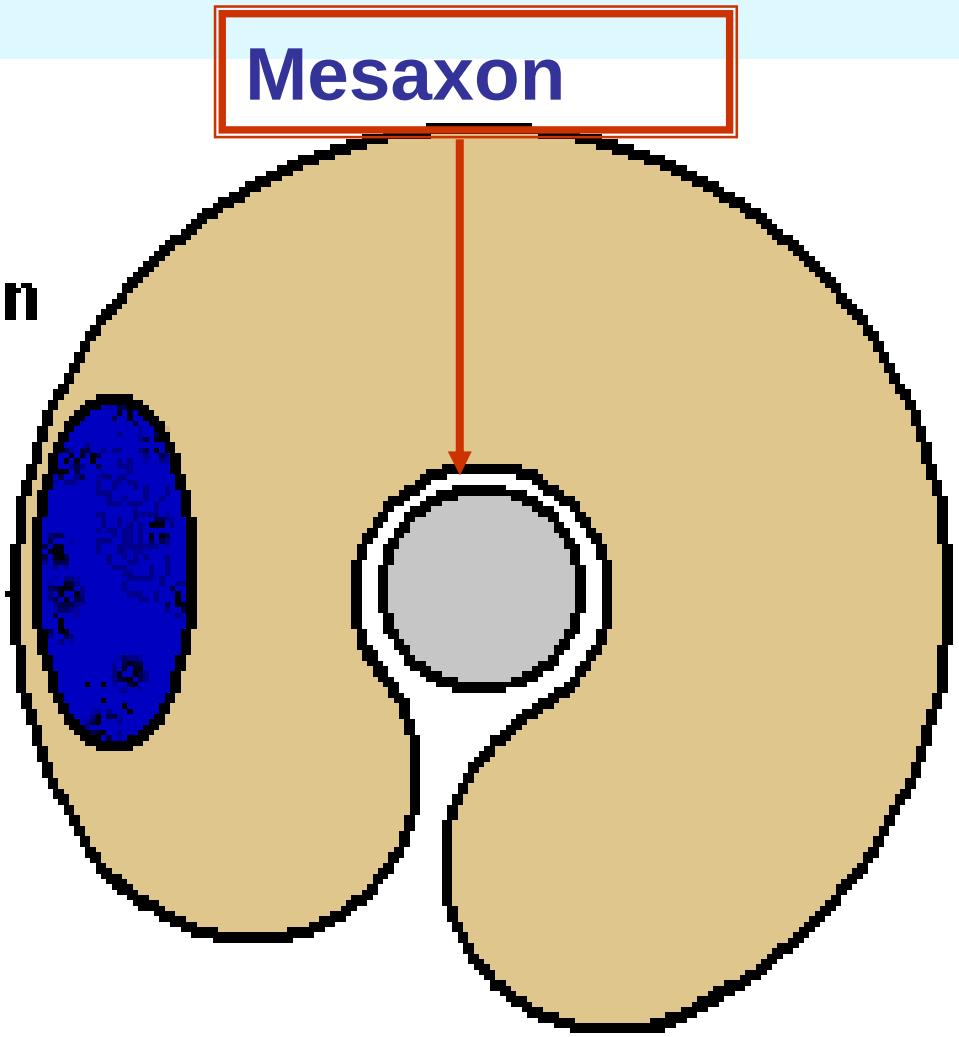
**Main objections against the hypothesis of an "insulating" myelin:**

1) The axon is not a conductor of “electric current" because: electric current is a flux of electrons that moves with a speed that can be more than half of light velocity, as normally takes place in metals (eg copper).

2) As “electric current” does not exist in an axon there will be no insulating substance.

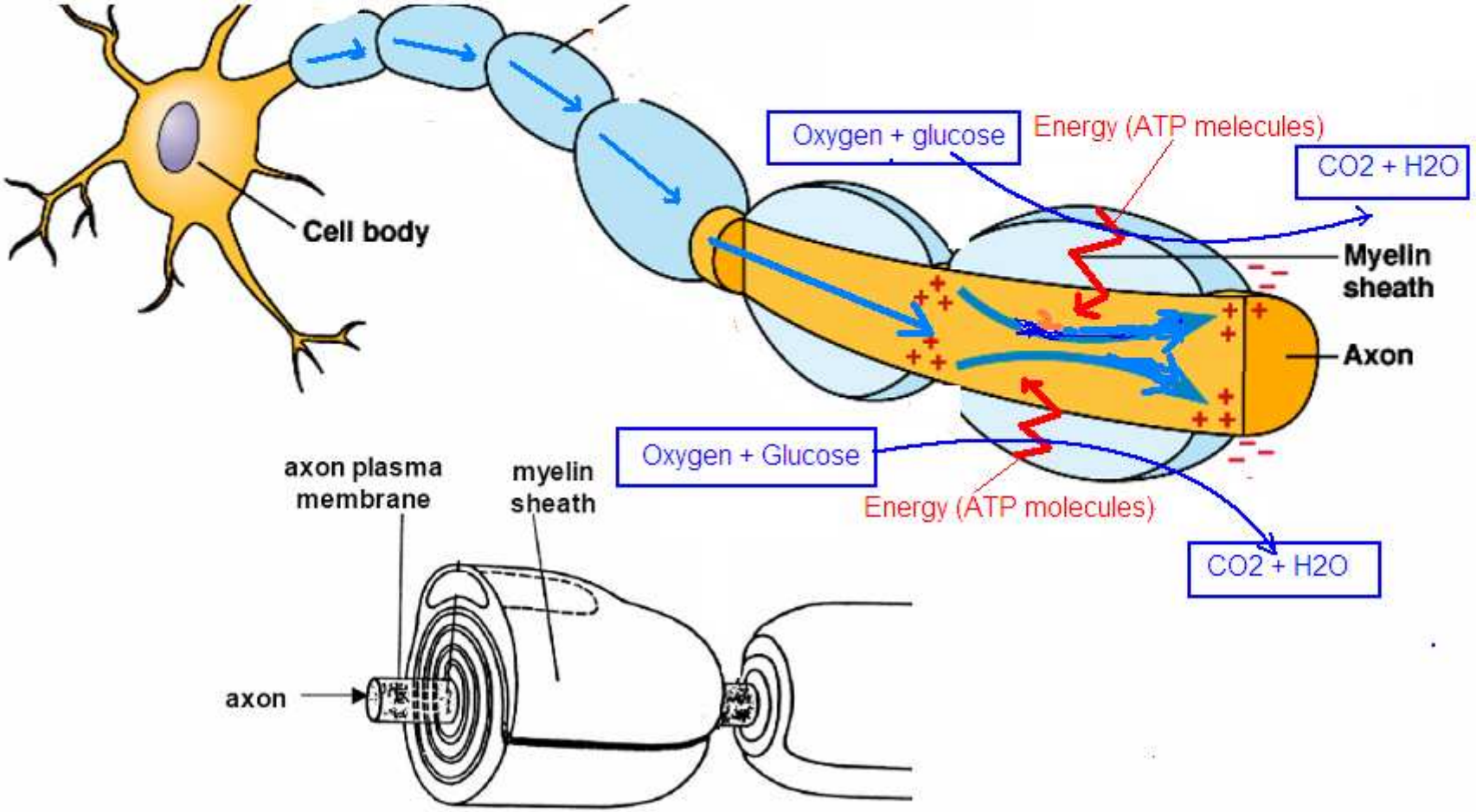
3) Even though myelin was “insulating” it should be in intimate contac with the axon, but this is not the case, in fact the **MESAXON,** a liquid-filled space, separates the axon from myelin.

Myelination of
a peripheral axon



**How does myelin sheath substain the rapid movement of signal along the nerve?**

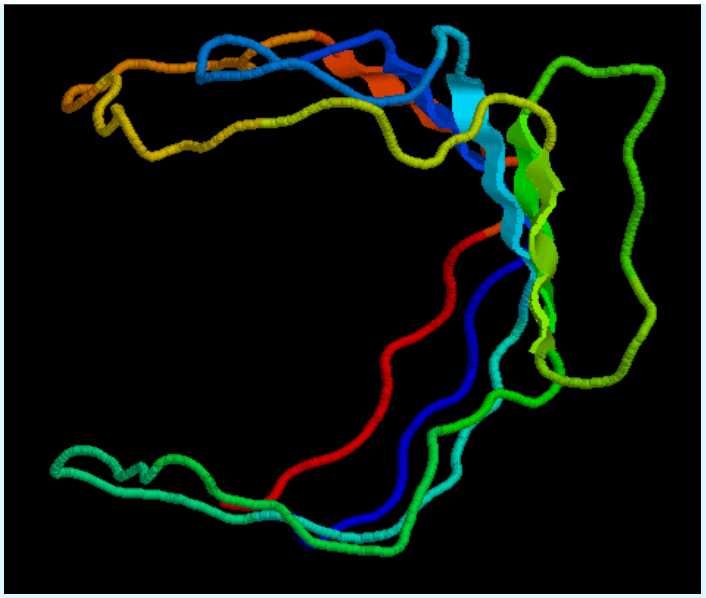
**There are experimental evidences that nerve energy (ATP molecules) is oversupplied by myelin. ATP is produced in myelin sheath trough glucose combustion.**



# (10)

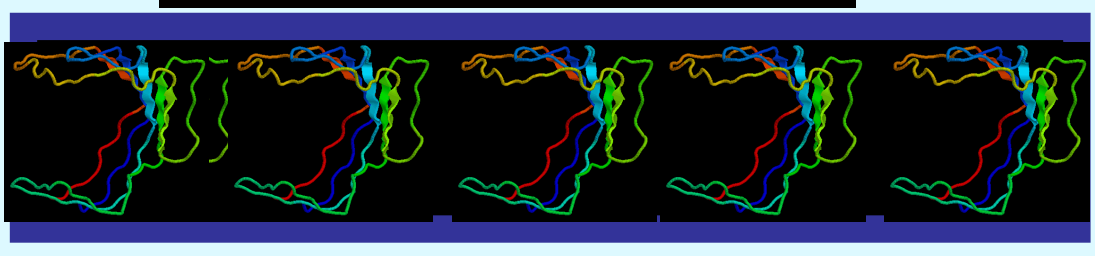
## Hypothesis of

1) Energizing myelin sheath;
2) Central role of MBP as chemiosmotic buffer.



Structure 3D of Myelin Basic Protein

**diameter ~ 4 nm**



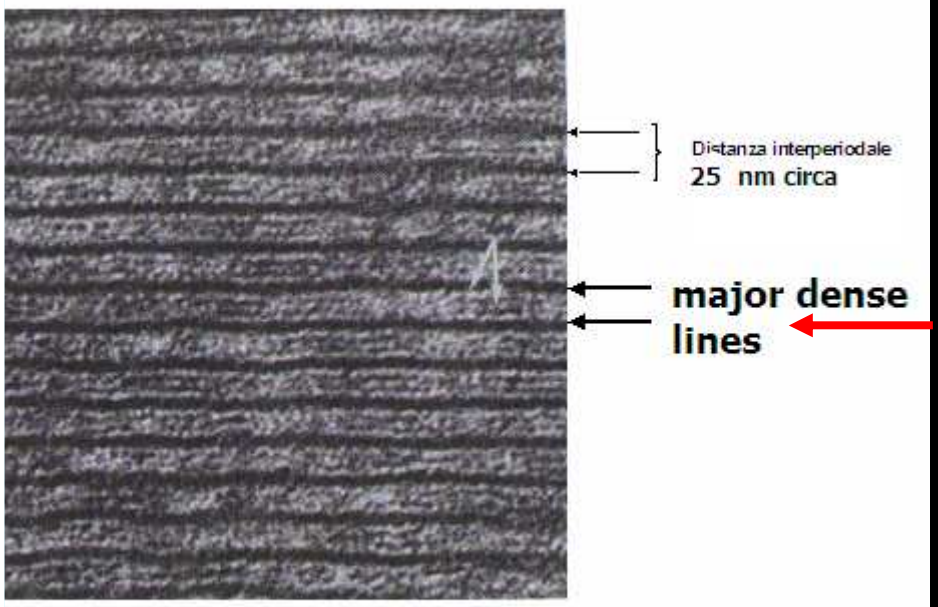
**Major dense line ~ 5 nm**

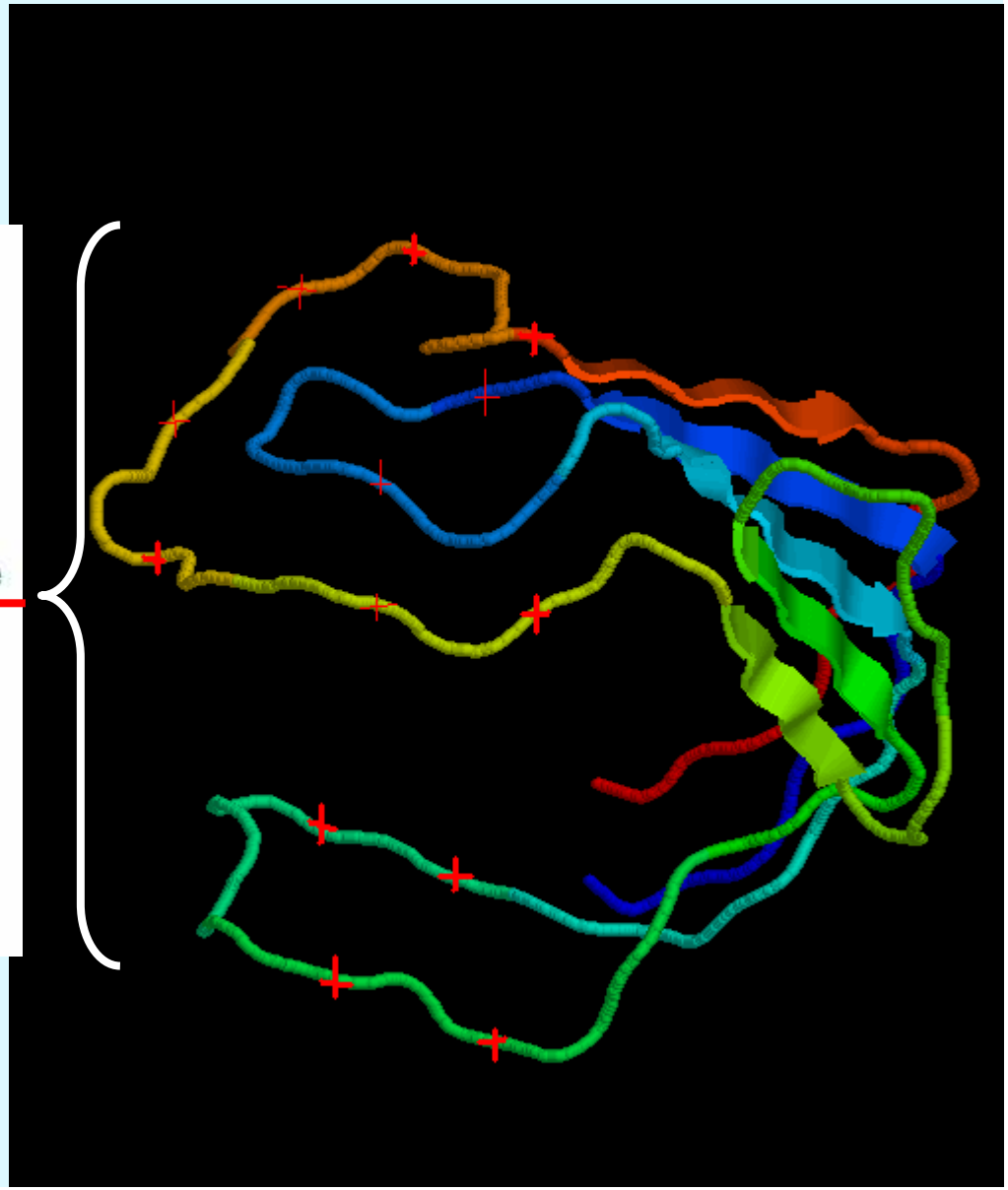
## Hypothesis:

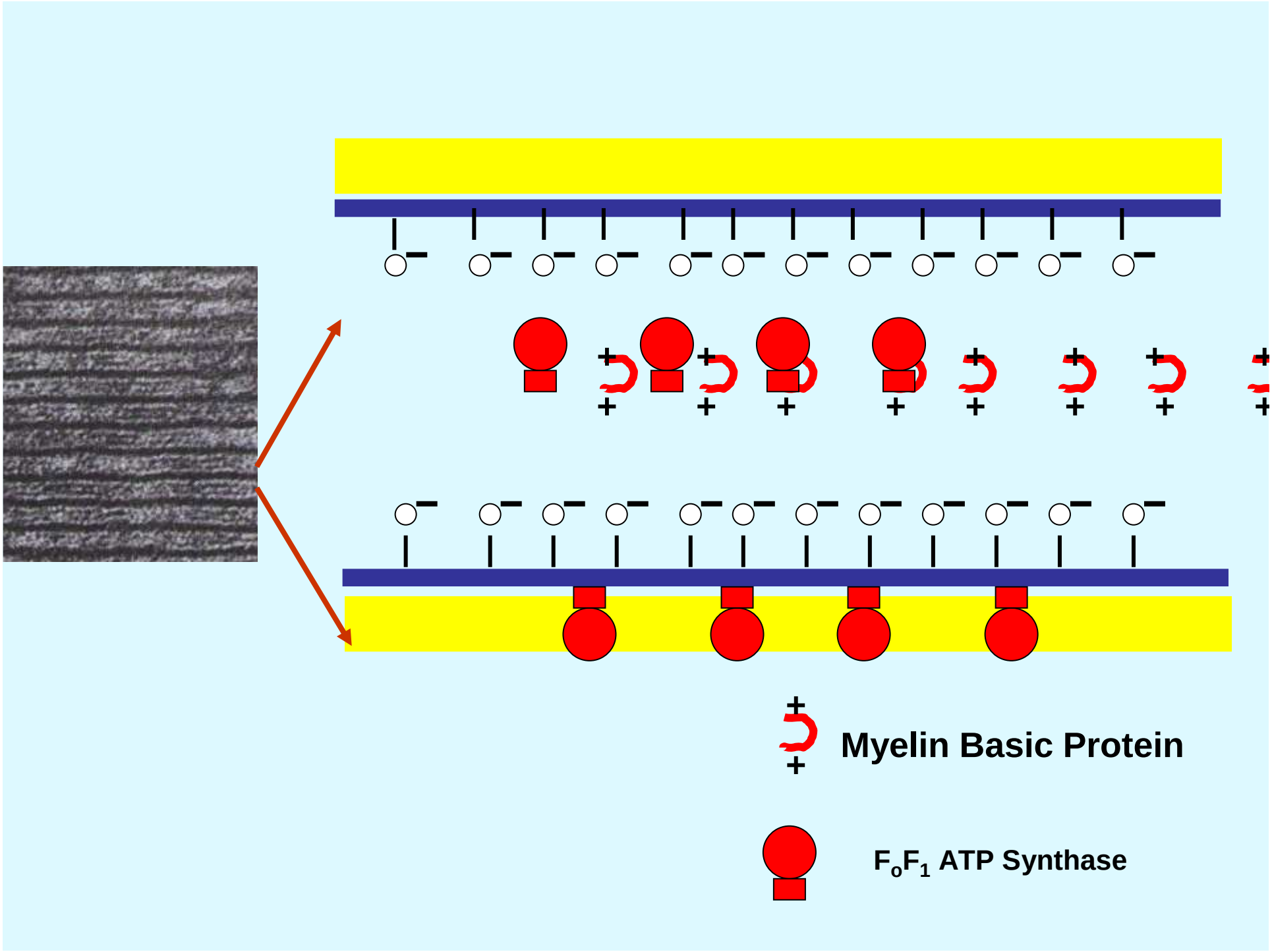
Myelin Basic Protein may act as a

*laminar chemiosmotic buffer.*

# Myelin Basic Protein is abundant (~ 30 % total proteins) and is inserted in the major dense line.




Myelin Basic Protein
$F_oF_1$ ATP Synthase

The existence of

# Laminar Chemiosmotic Buffer (LCB)

introduces a 3rd possibility of biologic energetic reserve, besides:

-Glucidic reserve (starch , glicogen)

-lipidic reserve (lipids)

N.B.: the energetic reserve represented by LCB has a physical nature, while the others have a chemical nature.

# (11)
# Myelination

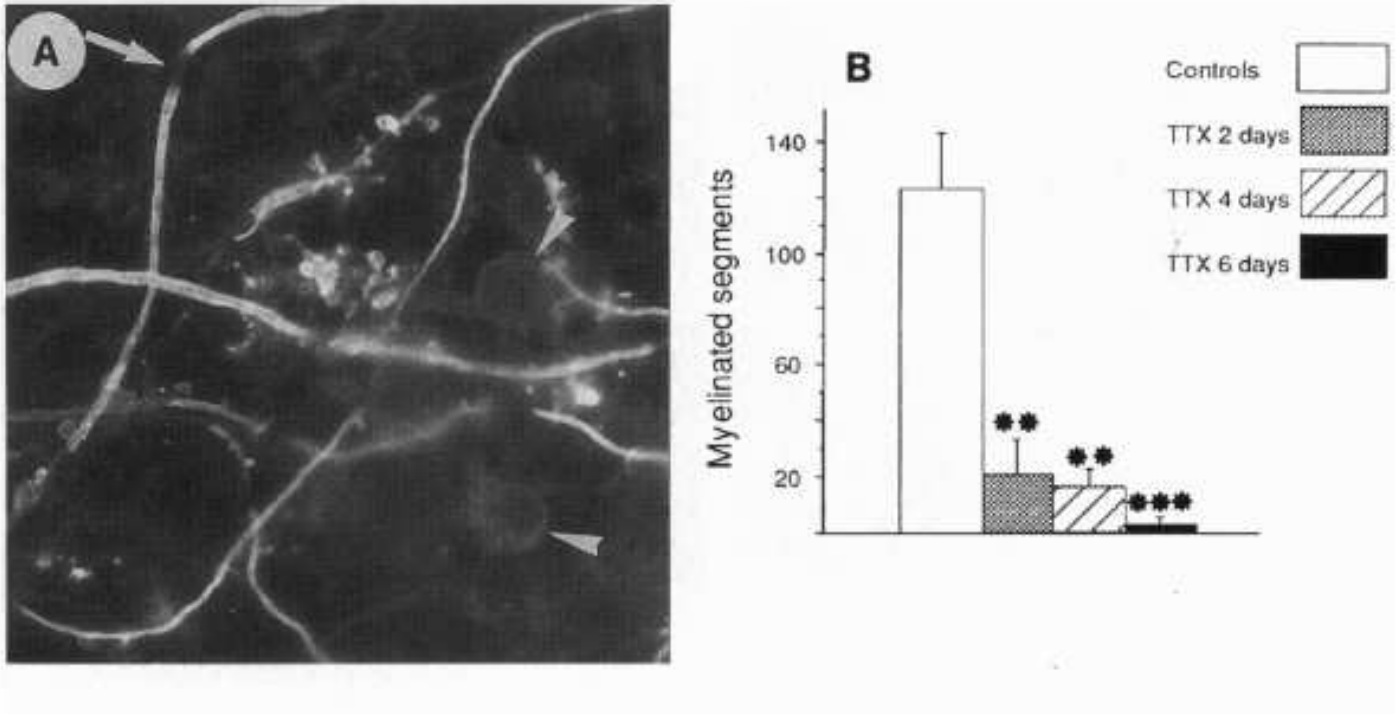
*Proc. Natl. Acad. Sci. USA*
Vol. 93, pp. 9887–9892, September 1996
Neurobiology

# Induction of myelination in the central nervous system by electrical activity

C. DEMERENS*, B. STANKOFF*, M. LOGAK*, P. ANGLADE†, B. ALLINQUANT‡, F. COURAUD§, B. ZALC*¶, AND C. LUBETZKI*

Laboratoire de Neurobiologie Cellulaire, Moléculaire et Clinique, Institut National de la Santé et de la Recherche Médicale, *Unité 134 and †Unité 289, Hôpital de la Salpêtrière, Université Pierre et Marie Curie, 75651 Paris, Cedex 13, France; ‡Ecole Normale Supérieure, Centre National de la Recherche Scientifique, Unité de Recherche Associée 1414, 26 rue d'Ulm, 75005 Paris, France; and §Laboratoire de Neurobiologie des Canaux Ioniques, Institut National de la Santé et de la Recherche Médicale, Unité 374, Faculté de Médecine Nord, 13916 Marseille, Cedex 06, France

tetrodotoxin which can blocks the firing of neurons.



**Tetrodotoxin (TTX) blocks the firing of neurons.**
**α-scorpion toxin (α-ScTX) increase the firing of neurons.**

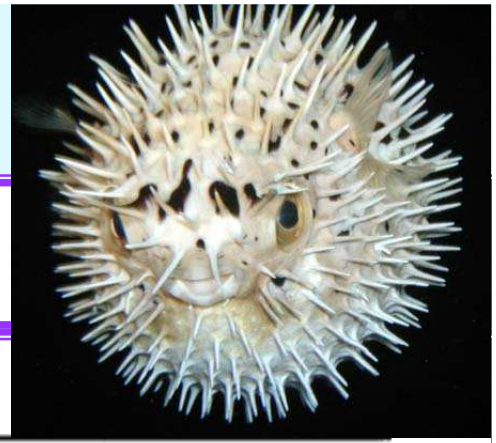


Table 1. The effect of TTX, α-ScTX, and $K^+$ on myelination in cultures

| Factor added | Time added, DIV | Myelinated segments, % controls | MBP-positive cells, % controls |
|---|---|---|---|
| TTX ($10^{-6}$ M) ($n$ = 3) | 9 | 39.4 ± 16.2 | 92.6 ± 17.3 |
| TTX ($10^{-6}$ M) ($n$ = 3) | 12 | 2.4 ± 1.1 | 159.6 ± 13.8 |
| α-ScTX ($10^{-8}$ M) ($n$ = 5) | 8 | 241 ± 44 | 119.8 ± 22.9 |
| $K^+$ (15 mM) ($n$ = 3) | 12 | 2.9 ± 1.4 | 80.1 ± 11.8 |
| TTX + $K^+$ (15 mM) ($n$ = 3) | 12 | 1.2 ± 0.6 | 93.8 ± 15.3 |

Myelinating cultures were as in Fig. 1. At various times after seeding, TTX, α-ScTX (toxin II from *Androctonus australis* Hector), $K^+$, or TTX plus $K^+$ were added to the culture media. Treatment lasted 2 days, after which excess reagents were thoroughly eliminated by washing as described (25). The total number of myelinated segments and of MBP-expressing oligodendrocytes per coverslip was evaluated at 21 DIV after immunolabeling with the anti-MBP antibody. Results are expressed as percent of values observed in control cultures (mean ± SEM).

Neuron 49, 823–832, March 16, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.neuron.2006.02.006

# Astrocytes Promote Myelination in Response to Electrical Impulses

Tomoko Ishibashi, Kelly A. Dakin, Beth Stevens, Philip R. Lee, Serguei V. Kozlov, Colin L. Stewart, and R. Douglas Fields,
Nervous System Development and Plasticity Section National Institute of Child Health and Human Development Bethesda, Maryland 20892

…**ATP** released from axons firing action potentials inhibits myelination by Schwann cells by arresting their development at an early stage (Fields and Stevens, 2000).

---

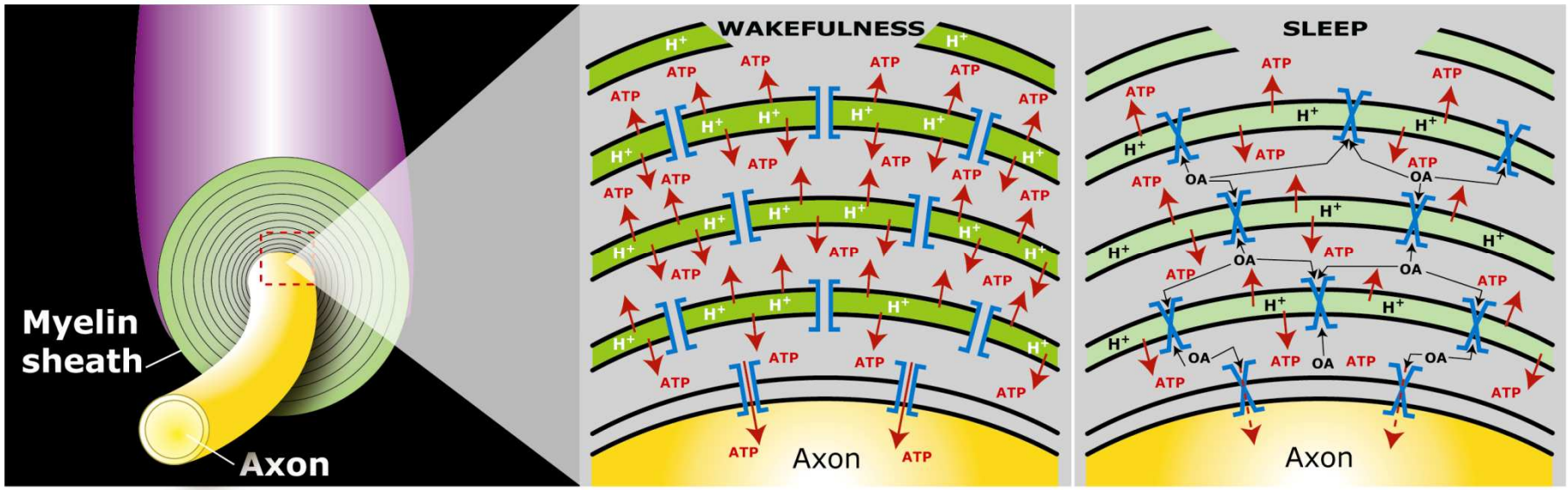
In the CNS, **adenosine**, presumably generated by the hydrolysis of extracellular ATP released from axons, inhibits proliferation of oligodendrocyte progenitor cells (OPCs), and stimulates their differentiation into a premyelinating stage, thus increasing myelination (Stevens et al., 2002)…

(12)

# Assumptions on the energetic basis of sleep

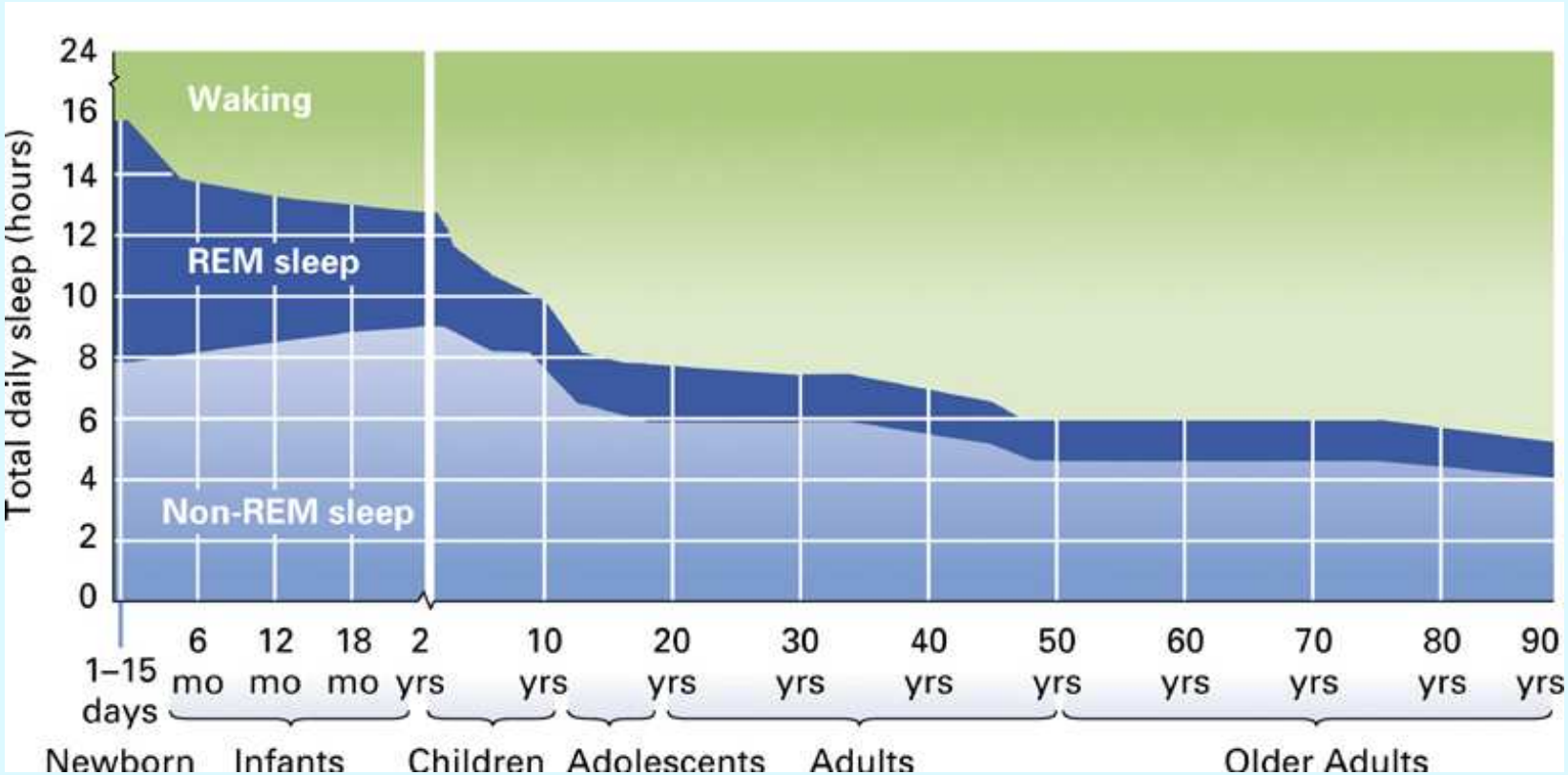
# Does sleep serve to "recharge“ myelin?

## Model of myelin sheath containing Myelin Basic Protein with function of laminar chemiosmotic buffer: a hypothesis on the role of MBP in the basic mechanisms of sleep / wake

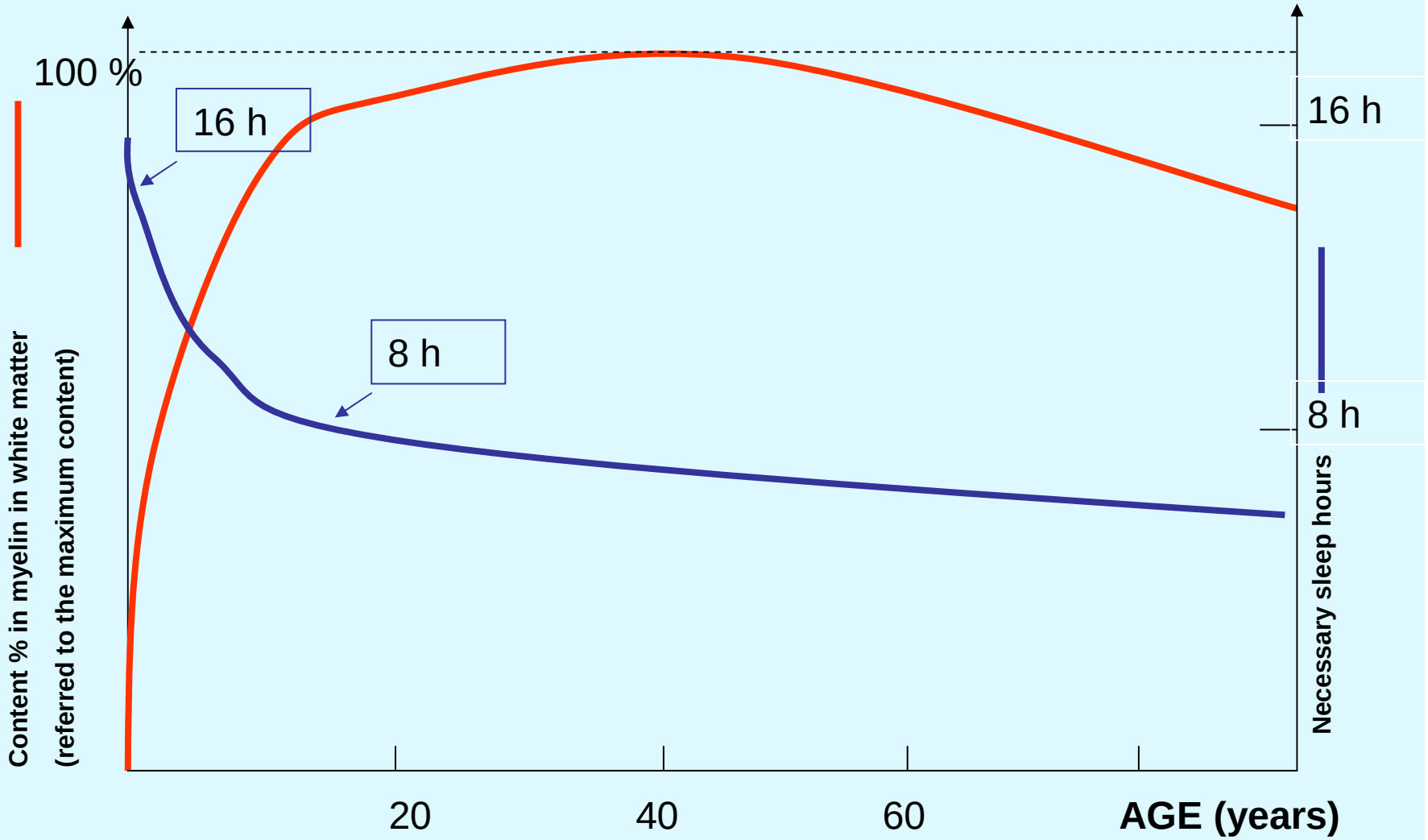



**OA = Oleammide**

# Sleep Across the Human Life Span



- **The cognitive ability increase with myelin**
- **The hours of sleep decrease as the myelin increases**



# The sleep:

For the vast majority of biological functions, there is a satisfactory explanation,connecting the structure of the organ to its function and vice versa. For ex. a muscle exerts a mechanical action, as there are molecules that slide over each other and receive chemical energy from other molecules and clearly identified

For the brain we do not know how it used the considerable energy it uses. About sleep, which is one of the most striking features of the brain, we have no explanation to date.

It 'been said that if sleep did not have a solid functional justification, it would be an extravagance of evolution and / or nature itself.

**Among the many theories about sleep, the energetic one is still considered the most, but its molecular and functional evidence, if any, is absolutely to define and / or to discover.**

- **During sleep, glucose burning is almost the same as during wakefulness.**
- It’s known that during sleep the nervous system as a whole consumes less energy.
- **It 'possible that the excess energy is accumulated in the form of protons stored in solution in neutral lipid and/or in myelin basic protein**

# *Sleep Neuro energetics*

Kalinchuk AV, ei al. Local energy depletion in the basal forebrain increases sleep.

*Eur J Neurosci 2003;17(4):863-9.*

Energy depletion in localized brain areas seems to mimick sleep need[31]: interestingly, infusion of 2,4-dinitrophenol (DNP), an uncoupler which prevents the synthesis of ATP, in the basal forebrain of rats induced increases in non-REM sleep that was comparable to those induced by sleep deprivation.

## Oleamide Synthesizing Activity from Rat Kidney

***IDENTIFICATION AS CYTOCHROME C***

**William J. Driscoll, Shalini Chaturvedi, and Gregory P. Mueller**

THE JOURNAL OF BIOLOGICAL CHEMISTRY , August 3, 2007

Naturally occurring oleamide was shown to accumulate in the cerebrospinal fluid of sleep deprived cats and to produce a profound sleep-like state when administered to rodents.. **These findings led to the proposal that oleamide directly contributes to the biochemical mechanisms underlying the drive to sleep** . More recently, oleamide was reported to modulate gap junctions.

# Soleep-Memory: Conclusions

- During the conscious waking the energy demand exceeds that mechaanically possible. There is a conscious activity in neocortical gray matter that utilizes most of the energy produced at the time by burning / breathing. Then there is a discernible physical activity of the unconscious in the white matter that extabilishes a huge number of connections between different sites of the cortex

- This activity during waking consumes the energy reserves represented by the accumulation of protons in the chemiosmotic laminar buffer of myelin and the feeling of sleep is due to poor energy supply fed by the axon myelin.

- neuronal activity requires a lot of energy intake. Biological systems use chemical energy in the form of ATP.

- With sleep we refill the proton and the myelin is energized, because the available energy is almost equal to that of wakefulness but during sleep it is mostly devoted to recharging, since the needs of the conscious system have decreased significantly.

- **It can be assumed that the memory is an energization of axonal connections by the white matter,  in light of the fact that sleep strengthens the memory.**

Sleep Medicine 12 (2011) 200–200

Contents lists available at ScienceDirect

Sleep Medicine

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

Letter to the Editor

## *from* Morelli, Ravera & Panfoli

**Myelin sheath: A new possible role in sleep mechanism**

pivotal role in the axon surrounding, allowing the nerve to transmit its impulses rapidly. However, there is growing evidence
that myelin has also an as yet unexplained neuro-trophic role. We reported that the respiratory chain components are expressed in myelin, outside of mitochondria [1]. These components would generate a proton gradient across myelin membranes to support ATP synthesis by an Fo-F1 ATP synthase. This may explain how in demyelinising diseases, as Multiple Sclerosis (MS), myelin loss causes an axonal necrosis.

Recently, Gallup et al [2] described sleep problems and frequent yawning in MS patients. During sleep, the glucose consumption by brain is very similar to that in wakefulness, even though the neuronal energetic demand is low. We have envisaged an involvement of myelin sheath. Imagine that myelin sheath acts as a proton (H+) buffer capacitor, thank to the abundance of myelin basic protein (MBP) and phospholipids, whose exceptional buffering capacity of phospholipids was demonstrated [3].

junctions, formed by connexins 32 [5], that seems to transport mainly ATP [6], likely from myelin to the axon.

This potential would be used by myelin to produce energy, during the wake period. In turn, sleep would be induced by a “discharge” of H+ in myelin sheath and wakefulness by a “complete recharge” of myelin sheath. The sleep need correlates to age. In fact, newborn and children have a higher sleep need than the adults. This may depend on myelinogenesis, that begins after born and goes on until 22-25 years age. Under these conditions, myelin may be less competent in accumulating energy= H+ and generate the need for sleep. Interestingly, sleep is also regulated by oleamide, an endogenous hypnotic compound that increases before the sleep, decreases before the wake up and is accumulated in cerebral spinal fluid of sleep-deprived animals [4]. Interestingly, oleamide closes the myelin gap junction, formed by connexin 32 [5], that seem to transport mainly ATP [6], likely from myelin to the axon.

# (13)
# Myelin
## Nerve transmission

## Cognitive

## Memory.

Neurobiology of Aging **31** (nov. 2010) pp. 1912–1926

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

# Cognition in healthy aging is related to regional white matter integrity, but not cortical thickness

David A. Ziegler[a,*], Olivier Piguet[a], David H. Salat[b], Keyma Prince[a], Emily Connally[a], Suzanne Corkin[a,b]

[a] *Department of Brain & Cog* [illegible] *lge, MA 02139, United States*
[b] *Athino* [illegible] *d States*

Neurobiology of Aging 31 (2010) 1912–1926

Cortical thinning occurred primarily in primary sensory and motor cortices, whereas WM changes were localized to regions underlying association cortices. Further, in Older Adults (OA), we found a striking pattern of region-specific correlations between measures of cognitive performance and WM integrity, but not cortical thickness. Specifically, cognitive control correlated with integrity of frontal lobe WM, whereas episodic memory was related to integrity of temporal and parietal lobe WM. Thus, age-related impairments in specific cognitive capacities may arise from degenerative processes that affect the underlying connections of their respective neural networks.

Review

# White matter in learning, cognition and psychiatric disorders

June 2008

Trends in Neuroscience – 31, (7) pp 361-370

R. Douglas Fields

Nervous System Development and Plasticity Section, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

**Experience changes white matter**

Myelination is a developmental process, but it has been known for decades that myelination of the human brain continues into the third decade of life [11], and this can now be tracked by noninvasive brain imaging [17]. However, the significance of this was not fully appreciated. If myelin is simply insulation, why is the process not completed by birth?

Myelination is nearly completed by birth in animals, such as horses [28] or wild mice (Acomys) [29], which are precocial and can walk and feed independently soon after birth, but in humans, myelination is delayed and the process extends at least through the first 20 years of life.

The prolonged period of myelination in humans coincides with the same period when the human cerebral cortex undergoes massive remodeling of synaptic connections, which are understood to modify the brain according to experience. This raises the possibility that myelin might participate in optimizing information processing through experience.

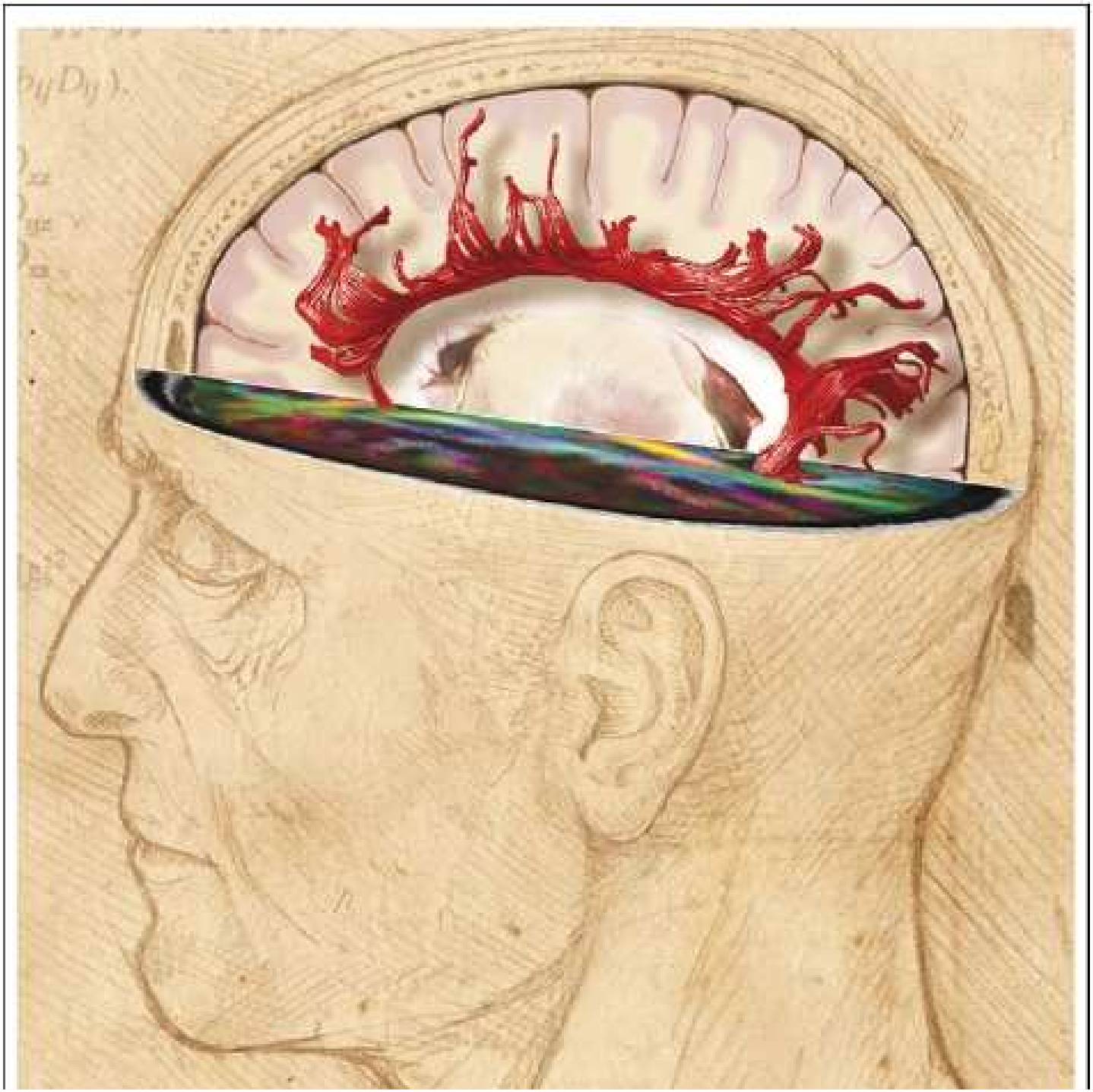


**Figure 2.** Neuroimaging reveals changes in white matter structure in the human brain. White matter (white) comprises half of the human brain and consists of bundles of myelinated axons connecting neurons in different brain regions. Gray matter (pink) is composed of neuronal cell bodies and dendrites concentrated in the outer layers of the cortex. Microstructural changes in white matter can be revealed by specialized MRI brain imaging techniques such as diffusion tensor imaging (DTI). This method analyzes the fractional anisotropy (FA) of proton diffusion in tissue, which is more restricted in white matter than in gray matter. The anisotropy increases with increased myelination, fiber diameter and axon compaction. The degree of anisotropy is represented on a pseudo color scale as shown in the human brain scan in the horizontal plane of the image above, where the major white matter tracts are revealed against a black background of low fractional anisotropy [107]. These data can be used to calculate the probable anatomy of white matter fiber bundles in living brain, a process called tractography. An example from human brain imaging is shown above as red filamentous bundles radiating out from the corpus callosum. Fiber orientation is calculated from the eigenvectors defining proton diffusion in three dimensions in each voxel. Using algorithms, the principal eigenvalue vector is connected to the next voxel to trace the fiber structure and orientation in white matter tracts [108]. Changes in white matter structure are seen by DTI in association with many neuropsychiatric disorders, cognitive function and during learning. FA image courtesy of Carlo Pierpaoli, NICHD, NIH, and DTI tractography, courtesy of Derek K. Jones, School of Psychiatry, Cardiff University. Illustration by Lydia Kibiuk, Medical Arts, NIH.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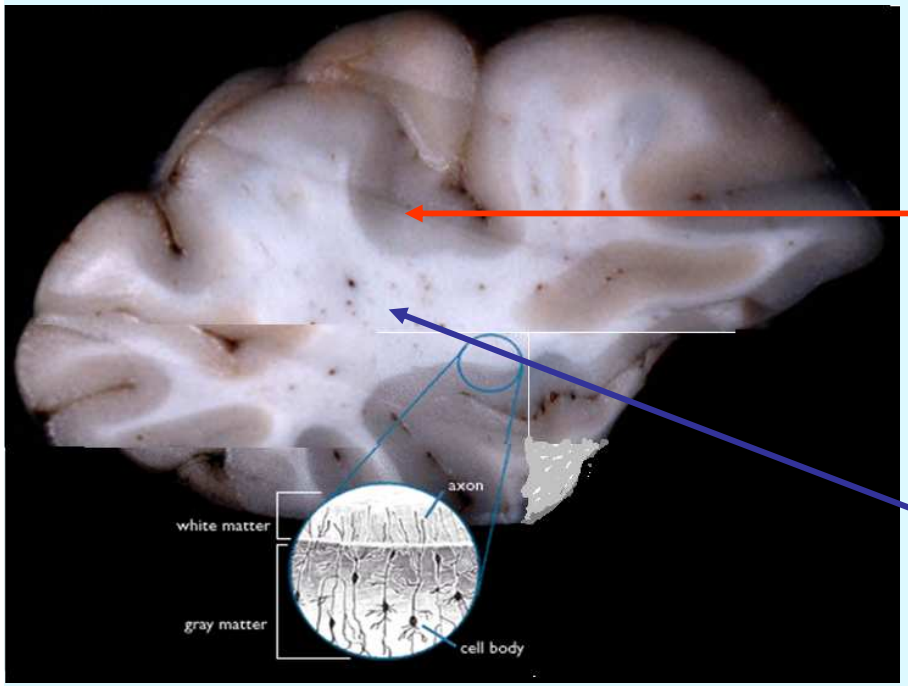
# Regional cerebral oxygen utilization and blood flow in normal man using oxygen-15 and positron emission tomography

R. S. J. Frackowiak*, T. Jones*, G. L. Lenzi** and J. D. Heather*

Acta neurol. scandinav. 62, 336–344, 1980



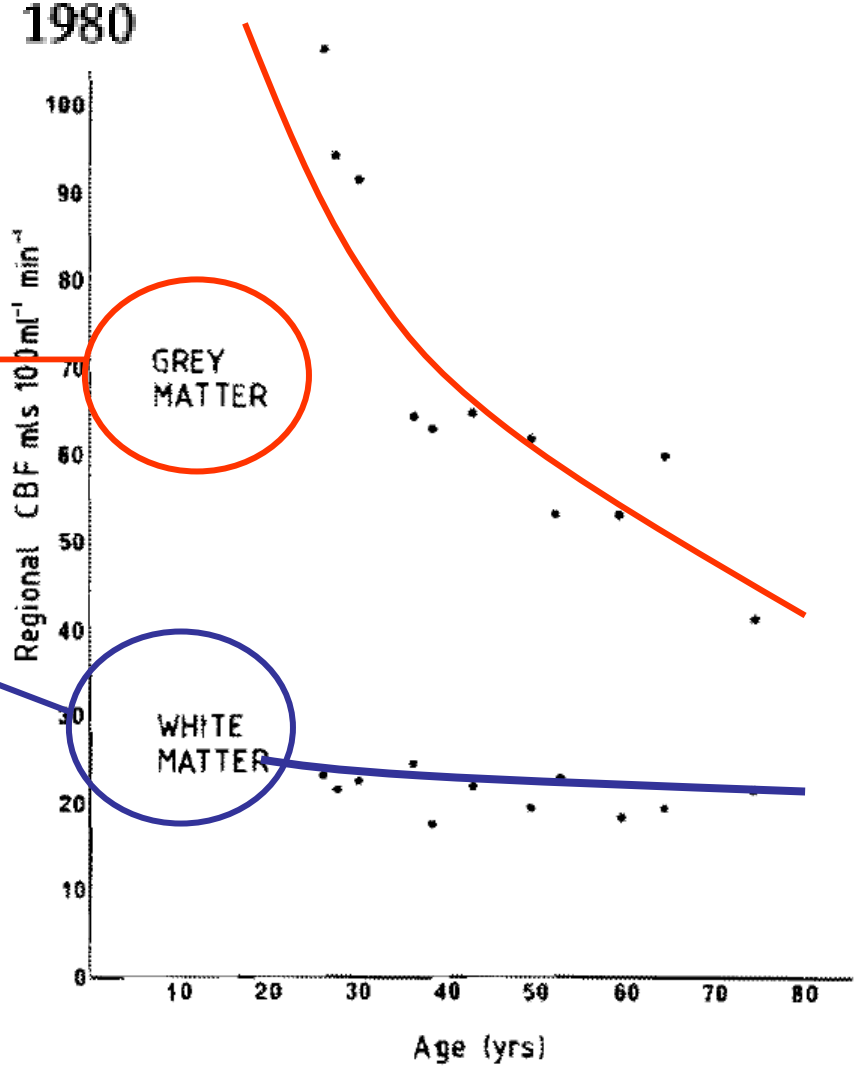



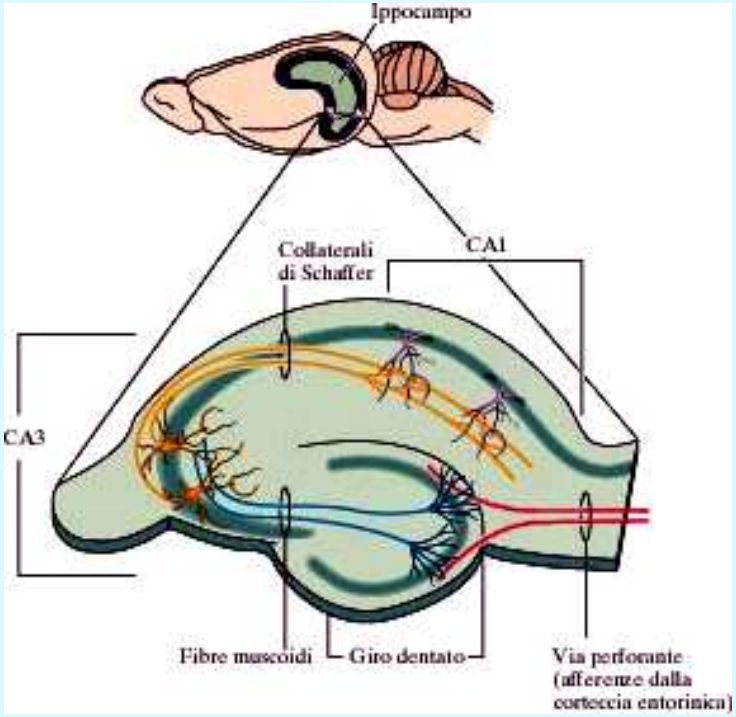
*Table 1. Regional grey and white matter values for CBF and $CMRO_2$ in 11 normal volunteers with mean cerebral BF and $MRO_2$ calculated assuming 60:40 grey:white composition of normal brain. Sampling sites for measurement of regional values are described in the text.*

| Age (years) | Grey matter | | White matter | | Calculated cerebral means | |
|---|---|---|---|---|---|---|
| | $CMRO_2$ ml $O_2$ 100 ml$^{-1}$ min$^{-1}$ | CBF ml 100 ml$^{-1}$ min$^{-1}$ | $CMRO_2$ ml $O_2$ 100 ml$^{-1}$ min$^{-1}$ | CBF ml 100 ml$^{-1}$ min$^{-1}$ | $CMRO_2$ ml $O_2$ 100 ml$^{-1}$ min$^{-1}$ | CBF ml 100 ml$^{-1}$ min$^{-1}$ |
| 26 | 9.74 | 107.2 | 2.34 | 23.6 | 6.78 | 73.8 |
| 27 | 8.65 | 94.8 | 1.59 | 21.7 | 5.83 | 65.6 |
| 30 | 6.28 | 91.9 | 1.62 | 22.6 | 4.42 | 64.2 |
| 36 | 7.00 | 64.5 | 1.81 | 24.5 | 4.98 | 48.7 |
| 38 | 6.72 | 63.3 | 1.94 | 17.5 | 4.81 | 45.0 |
| 43 | 5.47 | 65.2 | 2.05 | 22.0 | 4.10 | 47.9 |
| 49 | 5.99 | 62.2 | 2.01 | 19.8 | 4.40 | 45.2 |
| 52 | 4.26 | 53.3 | 1.92 | 23.1 | 3.32 | 41.2 |
| 59 | 4.92 | 53.4 | 1.70 | 18.6 | 3.63 | 39.5 |
| 64 | 5.14 | 60.4 | 1.62 | 19.0 | 3.73 | 43.8 |
| 74 | 4.87 | 41.0 | 2.01 | 21.6 | 3.73 | 33.2 |
| $\bar{x}$:45 | 6.28 | 68.9 | 1.87 | 21.3 | 4.52 | 49.8 |

**Peso medio del cervello: ~1.500 gr.**

**4,52 x 15 = 68 ml $O_2$/ min**

# The hippocampus



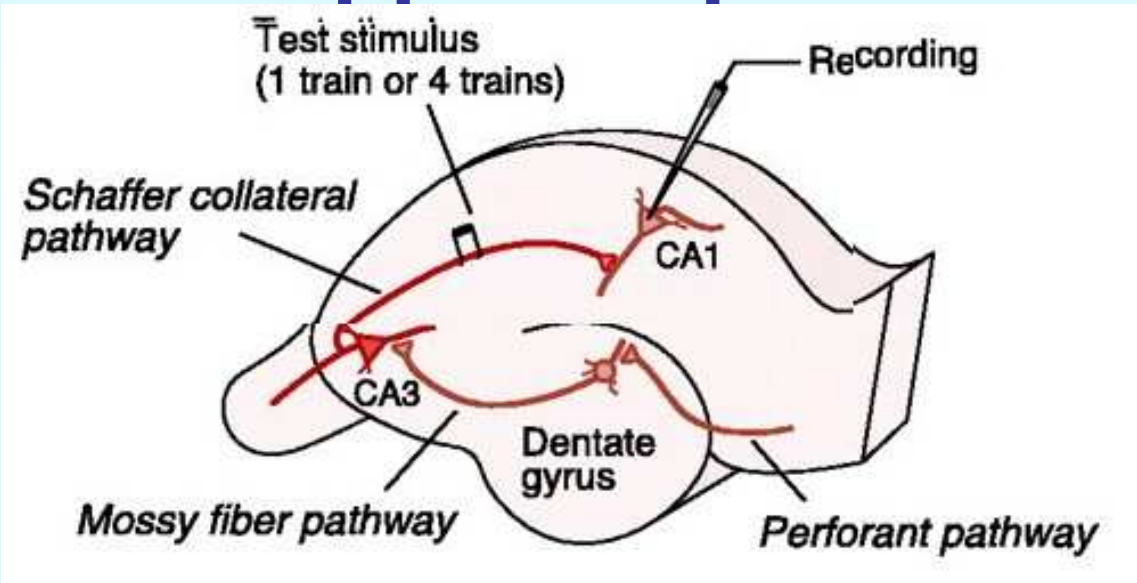

The hippocampal circuits consist of excitatory synapses that use glutamate as a neurotransmitter, which can interact with different subclasses of receptors (NMDA, AMPA, kainate, mGluRs)

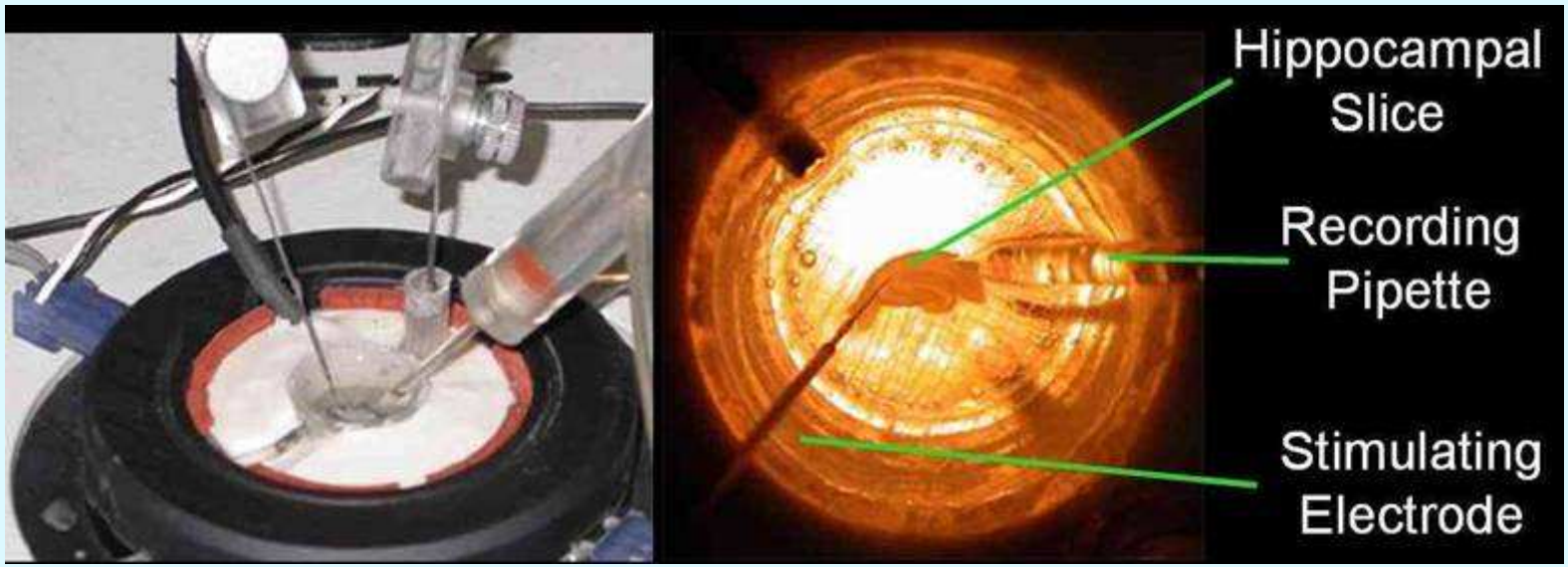
⇨ Bilateral hippocampal lesions result in deficits in the ability to consolidate forms of explicit but not implicit memory

⇨ The hippocampus is activated during certain types of learning (PET in humans)

⇨ Hippocampal lesions in rodents lead to deficits in learning different types of tasks, especially spatial ones

# Electrophysiological recordings from hippocampal slices



Neurochem Research - 2011 May 8 [Epub ahead of print]

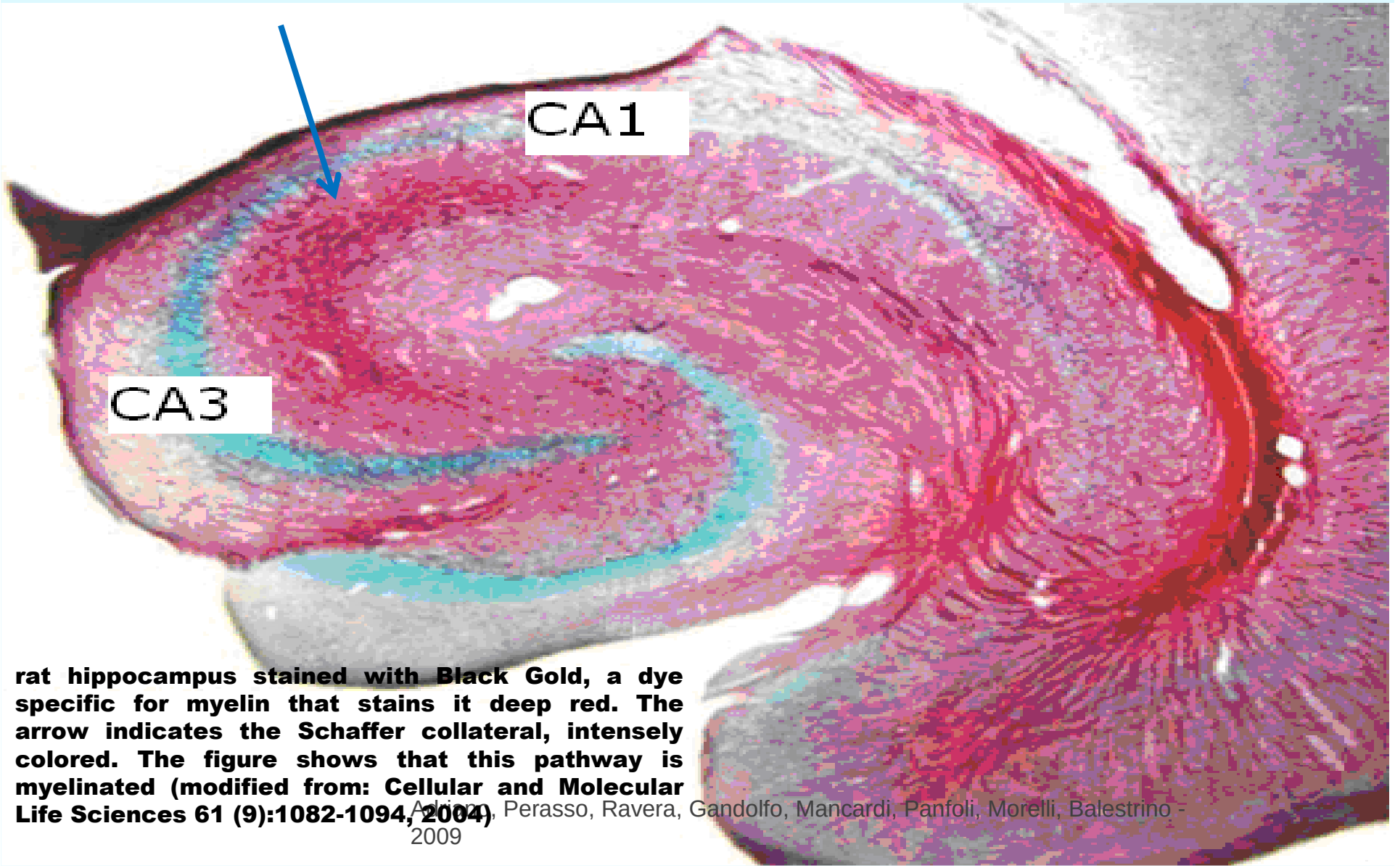
Neurochem Res
DOI 10.1007/s11064-011-0488-0

ORIGINAL PAPER

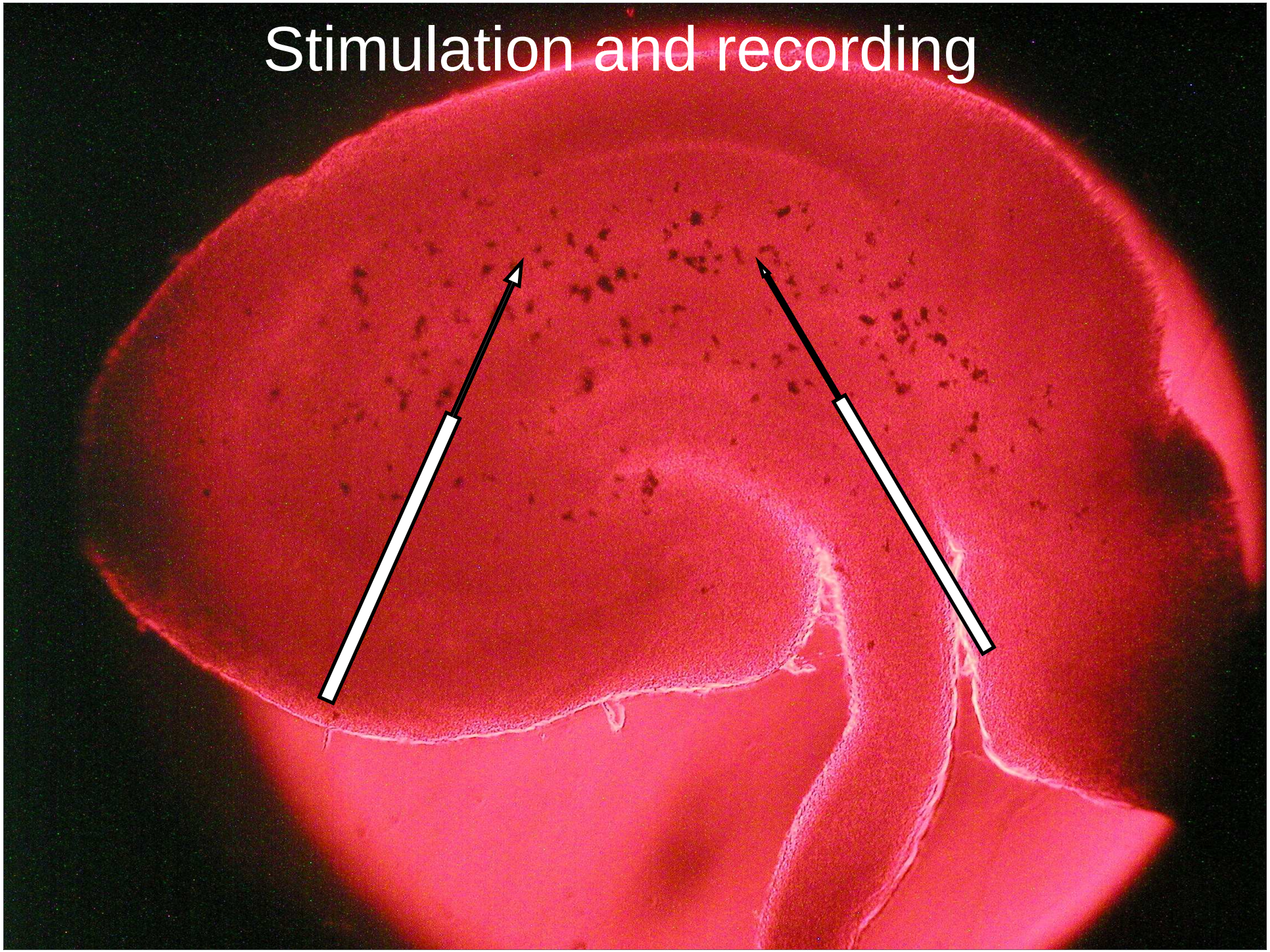
# A Novel Hypothesis About Mechanisms Affecting Conduction Velocity of Central Myelinated Fibers

Enrico Adriano · Luisa Perasso · Isabella Panfoli · Silvia Ravera · Carlo Gandolfo · Gianluigi Mancardi · Alessandro Morelli · Maurizio Balestrino

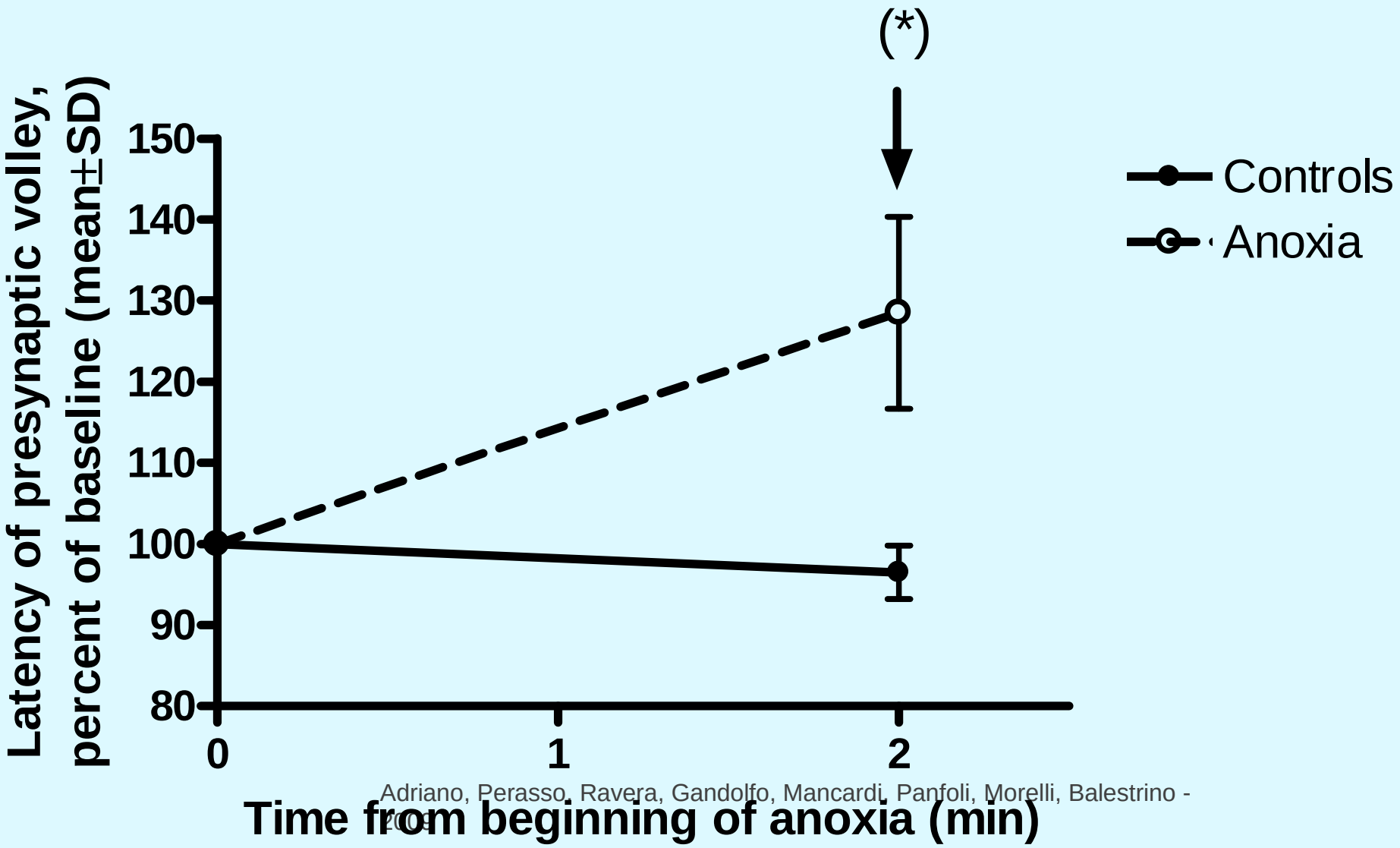
# WE USED AS EXPERIMENTAL MODEL THE STIMULATION AND RECORDING OF 'NERVOUS IMPULSE AT THE SCHAFFER COLLATERAL , A CENTRAL NERVOUS MYELINATED PATHWAY




**rat hippocampus stained with Black Gold, a dye specific for myelin that stains it deep red. The arrow indicates the Schaffer collateral, intensely colored. The figure shows that this pathway is myelinated (modified from: Cellular and Molecular Life Sciences 61 (9):1082-1094, 2004)**

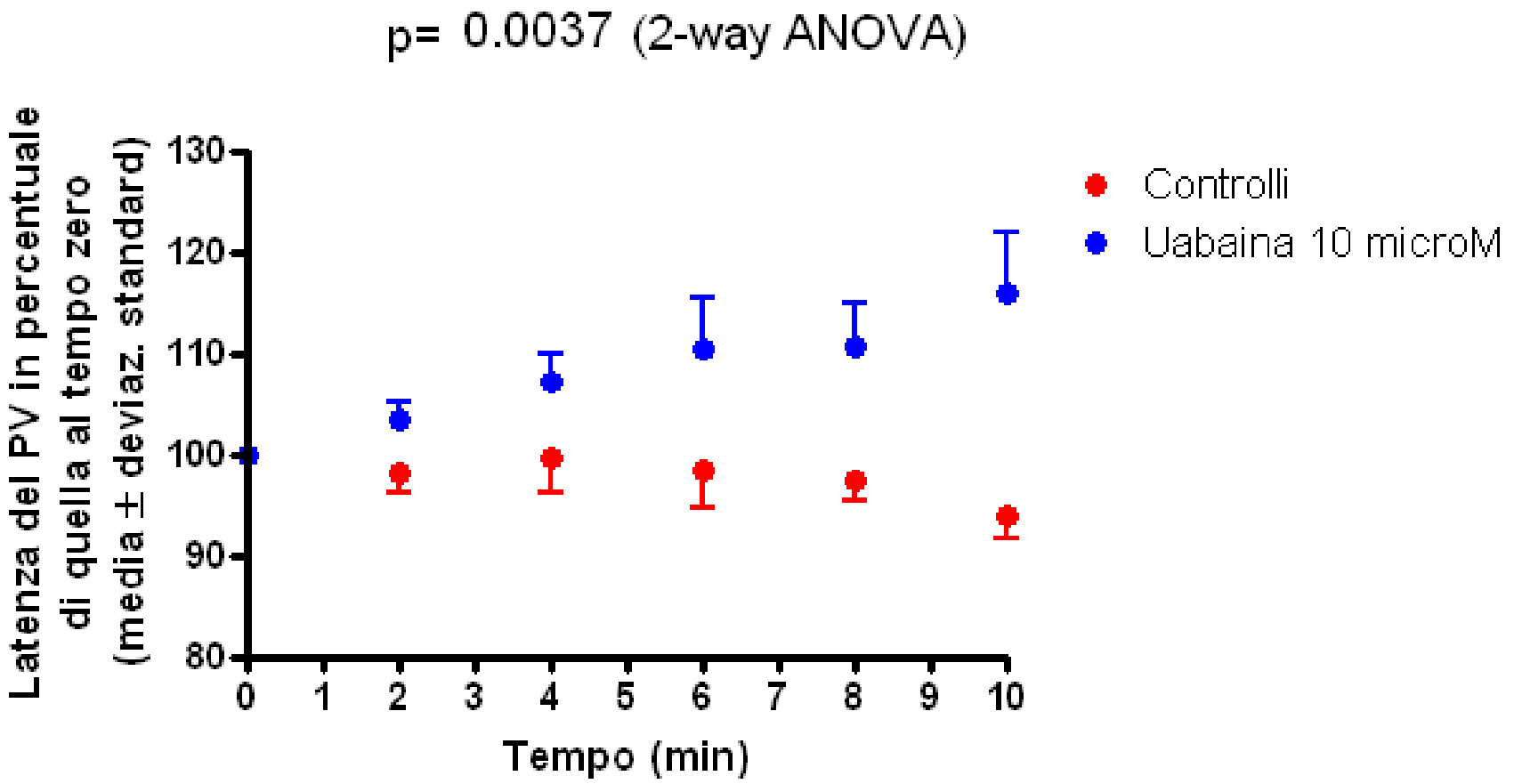

Stimulation and recording

# Anoxia and velocity of conduction



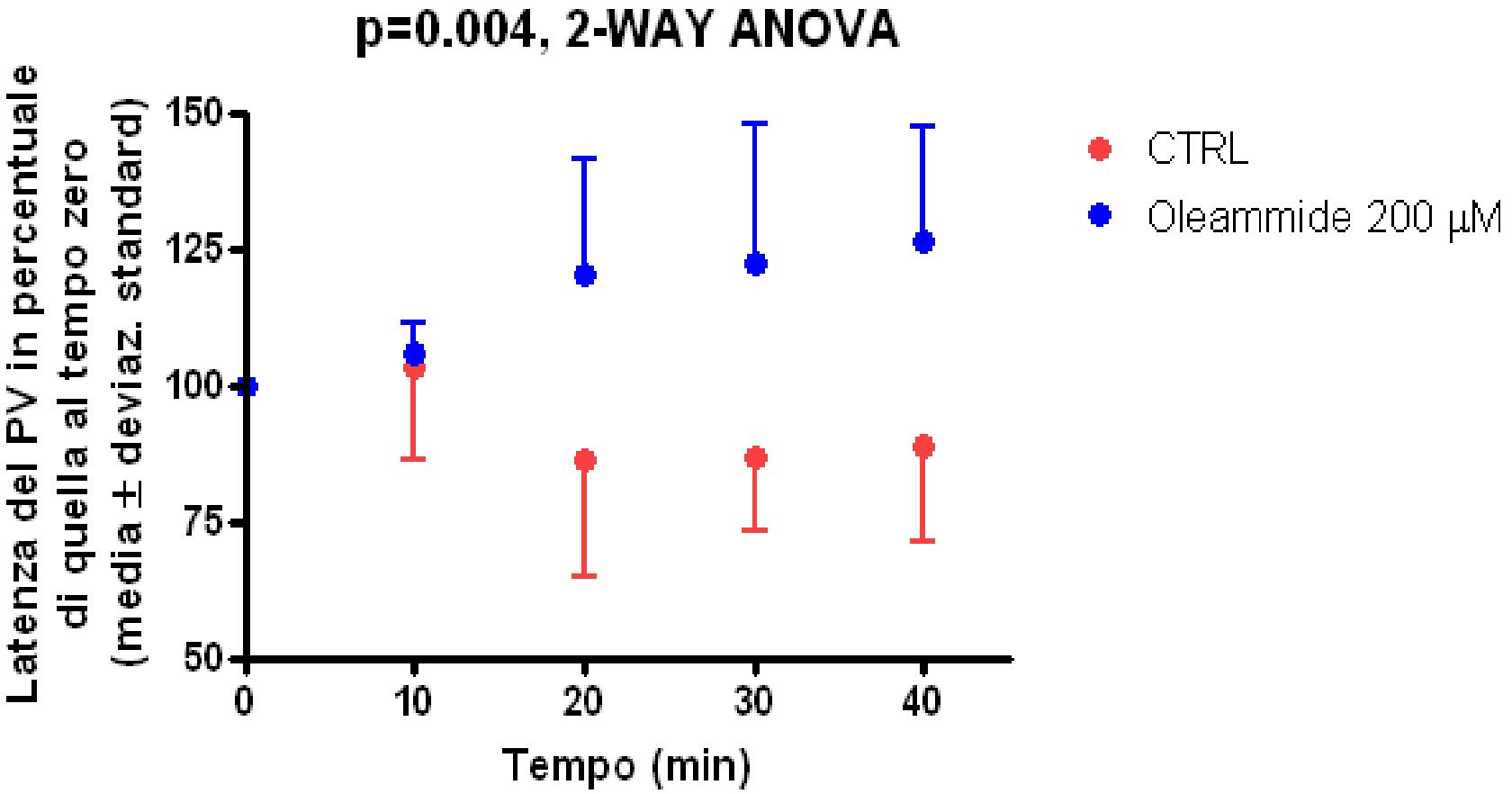

Adriano, Perasso, Ravera, Gandolfo, Mancardi, Panfoli, Morelli, Balestrino - 2008

# Inhibition of Na / K ATPase with uabaina reproduces the effects of ischemia and slows the conduction velocity in the Schaffer collateral




Adriano, Perasso, Ravera, Gandolfo, Mancardi, Panfoli, Morelli, Balestrino - 2009

# The experiment mimics the effect of ouabain and 'ischemia: Blocking of Gap-Junctions between neurons and glia with Oleammide is compatible with a transfer of ATP from glia to axons.




Adriano, Perasso, Ravera, Gandolfo, Mancardi, Panfoli, Morelli, Balestrino - 2009

Lab. Biochemistry, DIPTERS- University of Genoa - May 2011

# Assumptions on memory and energy storage

Since the beginning of '900, it was assumed that the memory was associated with the accumulation of energy.

→**Anoxia has as immediate consequence the loss of part or all of the memory** .

→ **Cognitive skills are associated with myelination**

→ **Given that sleep strengthens the memory, and the fact that sleep is likely in close connection to myelin energization, for the transitive property we can associate memory to myelin energization**

→. A new theory is proposed:

## Hypothesis: the memory can result from energization of the myelin of a neural network.

# This hypothesis is supported by links to mitochondria.:

→ Since many components in myelin are mitochondrial, we hypothesize that mitochondria may be linked to memory.

→ This hypothesis is not entirely daring. There are several reports (see slide This hypothesis is not entirely daring. There are several reports (see next slide) in line with this link: mitochondria-memory.

MOLECULAR AND CELLULAR BIOLOGY, Mar. 2010, p. 1357–1367 Vol. 30, No. 6
0270-7306/10/$12.00 doi:10.1128/MCB.01149-09


# Mitochondrial DNA Toxicity in Forebrain Neurons Causes Apoptosis, Neurodegeneration, and Impaired Behavior[▽]

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# Normal mitochondrial respiratory function is essential for spatial remote memory in mice

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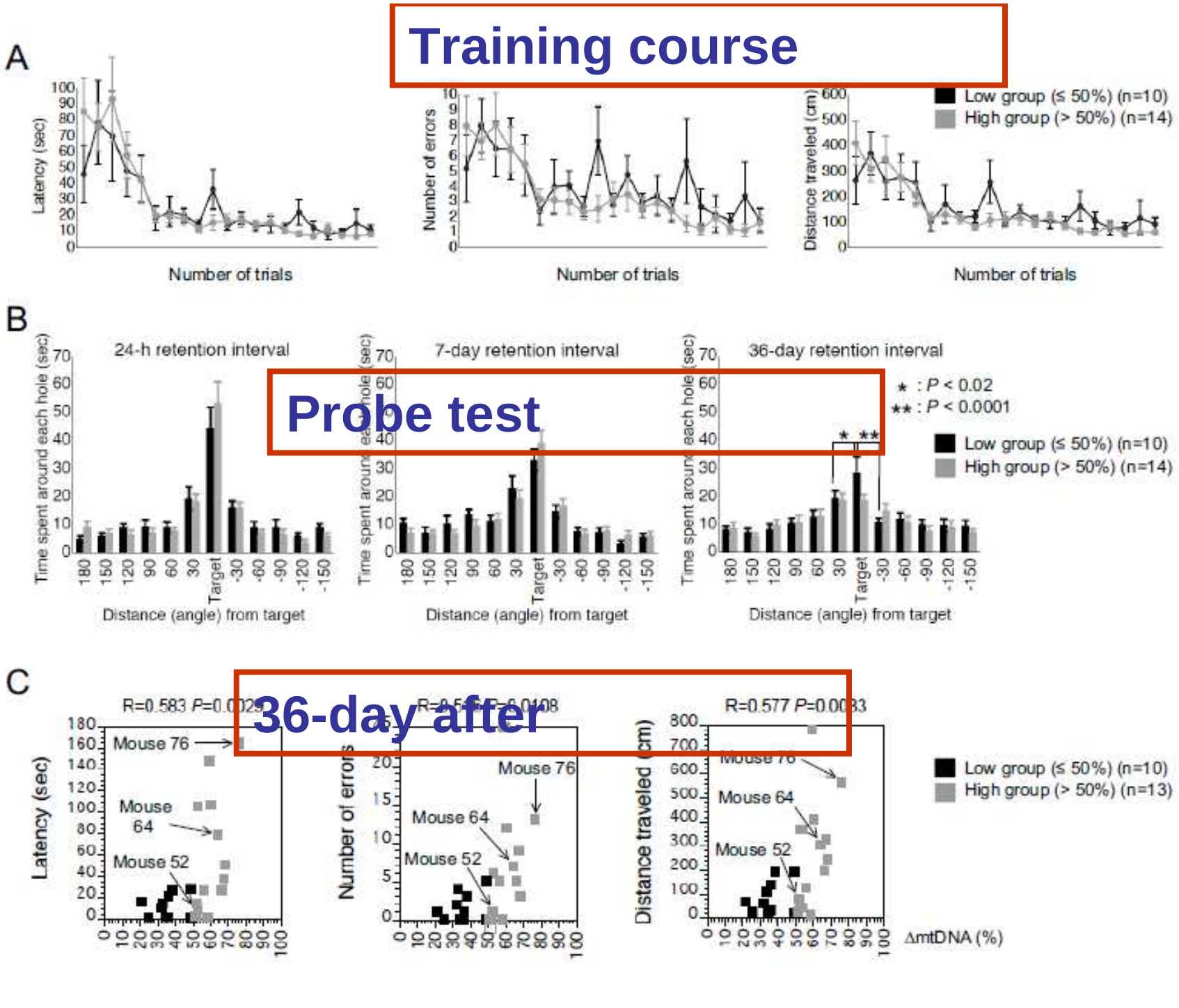
**Figure 3**

**Spatial learning and memory analyses using Barnes circular maze test in mito-mice**. (A) Training course. Latency ($P = 0.7381$), number of errors ($P = 0.5051$), and distance traveled ($P = 0.7885$) to target hole for low (black) and high (gray) groups were plotted. (B) Probe tests at 24-h, 7-day, and 36-day after the last training. There was no significant difference in the probe test at 24-h (between the target hole and right side hole, $P < 0.005$ in the low and $P < 0.001$ in the high groups; between the target hole and left side hole, $P < 0.05$ in the low and $P < 0.002$ in the high groups) and 7-day retention intervals (between the target and right side holes, $P < 0.001$ in the low and $P < 0.001$ in the high groups; between the target and left side holes, $P < 0.05$ in the low and $P < 0.002$ in the high groups). In the probe test at the 36-day retention interval, single and double asterisks indicate significant differences between the target and right side holes in the low group ($P < 0.02$ in the low and $P = 0.9804$ in the high groups) and between the target and left side holes in the low group ($P < 0.0001$ in the low and $P = 0.2106$ in the high groups), respectively. (C) A single retraining after the probe test at the 36-day retention interval. Each score of individual mito-mice in the low (black) and high (gray) groups were plotted against the proportion of ΔmtDNA in the tails at age 4 weeks. Mice 52, 64, and 76 carried 52%, 64%, and 76% ΔmtDNA, respectively. Pearson's product-moment correlation coefficients and the associated probabilities are indicated as R and *P*, respectively. All values are means ± SE.

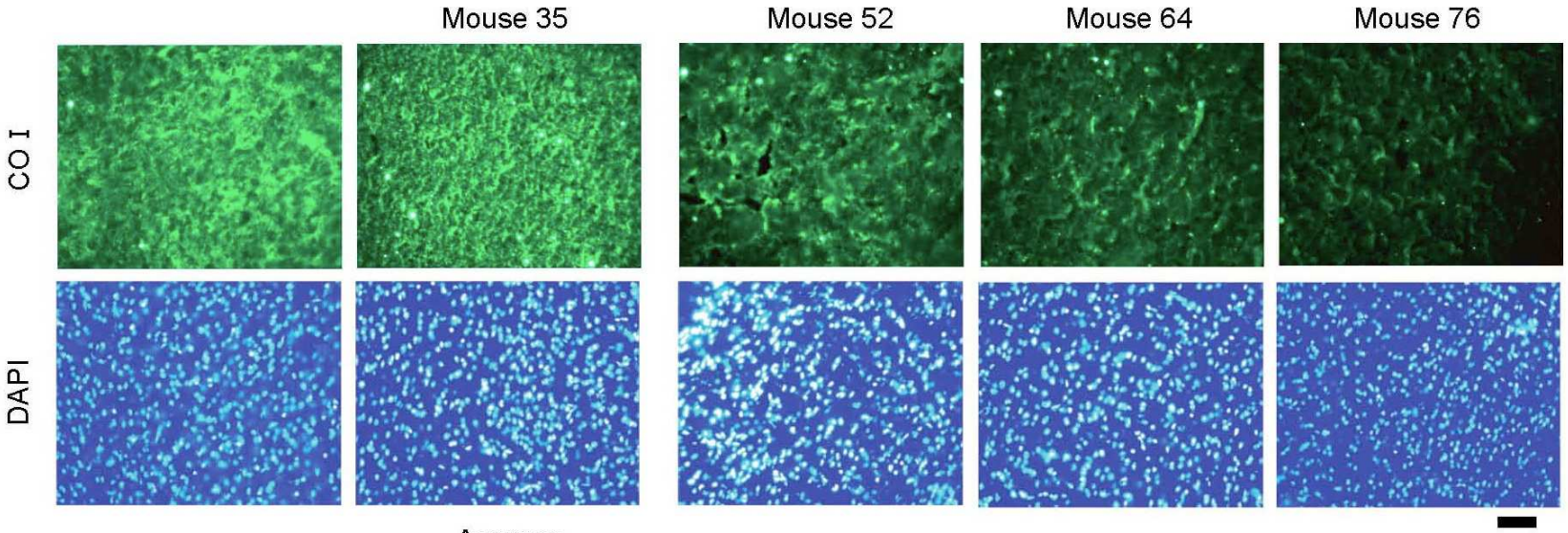

Training course
A
Latency (sec)
Number of trials
Number of errors
Number of trials
Distance traveled (cm)
Number of trials
Low group (≤ 50%) (n=10)
High group (> 50%) (n=14)
B
Probe test
24-h retention interval
7-day retention interval
36-day retention interval
Time spent around each hole (sec)
Distance (angle) from target
Target
* : P < 0.02
** : P < 0.0001
Low group (≤ 50%) (n=10)
High group (> 50%) (n=14)
C
36-day after
R=0.583 P=0.0029
R=0.577 P=0.0033
Latency (sec)
Number of errors
Distance traveled (cm)
Mouse 76
Mouse 64
Mouse 52
ΔmtDNA (%)
Low group (≤ 50%) (n=10)
High group (> 50%) (n=13)

http://en.wikipedia.org/wiki/Barnes_maze

**The Barnes maze is a tool used in psychological laboratory experiments to measure spatial learning and memory. The test subjects are usually rodents such as mice or lab rats, which either serve as a control or may have some genetic variable or deficiency present in them which will cause them to react differently to the maze.**

**Video: http://www.youtube.com/watch?v=MBwoYJ7MdI8**

| | Low group (n=10) | | | High group (n=12) | | |
|---|---|---|---|---|---|---|
| Tissue | Tail (4 weeks) | Brain (8.5 months) | Skeletal muscle (8.5 months) | Tail (4 weeks) | Brain (8.5 months) | Skeletal muscle (8.5 months) |
| ΔmtDNA load | 35.3% ± 2.8% | 36.9% ± 3.3% | 64.5% ± 3.5% | 60.4% ± 2.8% | 57.4% ± 2.0% | 82.5% ± 0.9% |
| Increased proportion | n.d. | 1.6% ± 2.8% | 29.2% ± 3.7% | n.d. | - 3.0% ± 1.6% | 22.1% ± 2.7% |




| | Average in low group (n=10) | Mouse 52 | Mouse 64 | Mouse 76 | Average in high group (n=12) |
|---|---|---|---|---|---|
| Latency (sec) | 12.6 | 15 | 79 | 165 | 59.8 |
| Number of errors | 1.6 | 1 | 7 | 13 | 6.7 |
| Distance traveled (cm) | 86.6 | 81 | 303 | 567 | 269.3 |

B) Relationship between mitochondrial respiratory function and phenotypic expression of impaired spatial remote memory

**These studies allow to conclude that spatial memory is impaired significantly by a decrease of the components of mitochondrial respiratory complexes encoded by mitochondrial DNA.**

*We therefore succeeded for the first time in showing experimental evidence that a high load of pathogenically mutated mtDNA and the resultant mitochondrial respiration deficiencies, in the absence of severe mitochondrial disease phenotypes, are responsible for the impairment of spatial remote memory.*

# parental transmission of behavior

*Anim. Behav.*, 1997, **54**, 559–570

## Genetic correlates of social behaviour in wild chimpanzees: evidence from mitochondrial DNA

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*Department of Anthropology, Harvard University*


protein
FIRST EYD
27/09/2010

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