European Science Foundation

Standing Committee for the European Medical Research Councils (EMRC)

ESF/EMRC EXPLORATORY WORKSHOP

Trends in Mitochondrial Pharmacology and Genetics

SCIENTIFIC REPORT

Warsaw-Mądralin, Poland

9-12 May 2003

Scientific Coordinators:

Jerzy Duszyński and Lech Wojtczak

Secretary:

Adam Szewczyk

Local Organising Team:

Joanna Szczepanowska, Krzysztof Zabłocki

Nencki Institute of Experimental Biology, Warsaw, Poland

Executive and scientific summary

Mitochondria play a central role in energy-generating processes within the cell. Apart from this important function, mitochondria are involved in such complex processes as apoptosis and cardioprotection. A rapidly expanding body of literature also suggests that mitochondrial dysfunctions play pivotal roles in neurodegenerative disorders ranging from Parkinson's to Huntington's to Alzheimer's diseases. Mitochondrial DNA mutations, whether inherited or acquired, cause impaired respiratory chain functioning. This, in turn, leads to decreased production of ATP, formation of free radicals and alterations in cellular calcium handling. These events may initiate peroxidation of mitochondrial DNA, proteins, and lipids, and opening of the mitochondrial permeability transition pore, an event linked to apoptotic cell death. Mitochondria are also targets for drugs such as antidiabetic sulphonylureas, immunosupressants, some antilipidemic agents, etc.

The purpose of the Workshop was to sum up recent state of our knowledge on mitochondrial functions within the cell with the numerous aspects of cell energetics, development and transformation. Special attention was put to mitochondria as a target for various therapeutically applied substances and to achievements in mitochondrial genetics and mitochondrially based diseases.

The Workshop was attended by 42 participants, including the Representative of the European Science Foundation and the Editor-in-Chief of *Toxicology: Mechanisms and Methods*, an international journal that offered to publish Workshop proceedings. Among these participants, who came from 14 countries, 36 were supported from the budget made available by ESF and 6 obtained support from other sources.

The Workshop comprised 31 lectures that have been grouped around ten specific topics as summarised below.

1. Mitochondria and metabolic signalling.

This section was devoted to the relationship between mitochondria and whole cell metabolism. Alfred Mejier gave the lecture about amino acids as regulators of many metabolic pathways and especially as amino acid-dependent signalling of insulin production. Jadwiga Bryła dealt with mitochondrial response to agents controlling glucose production in kidney-cortex tubules under normal and diabetic conditions.

2. Uncoupling proteins

Mitochondrial biogenesis and increased synthesis of the uncoupling protein 1 are hallmarks of the thermogenic recruitment process. Barbara Cannon presented results confirming function of one and original uncoupling proteins UCP-1 (which is localised only in brown adipose tissue) in nonshivering thermogenesis and showed that no proteins other than UCP-1 (not even proteins with high sequence similarity, such as UCP2 orUCP3) shared this ability. Others speakers, Francis Sluse and Wiesława Jarmuszkiewicz, were talking about regulation, functions and evolution of all uncoupling proteins in animal and plant kingdoms.

3. Mitochondrial permeability transition and its role in programmed cell death

Beyond a fundamental role in energy metabolism, mitochondria also play a key role in apoptosis. One of the events accompanying or preceding apoptotic cell death is a phenomenon called the mitochondrial permeability transition. Paolo Bernardi presented an overview of our understanding of the problem starting from experiments on isolated mitochondria up to investigations on pharmacological manipulation with the whole cell. Dieter Brdiczka presented his investigations on the role of the outer mitochondrial membrane pore and contact sites between the outer and the inner mitochondrial membranes in regulation of energy metabolism and apoptosis.

4. Mitochondria and the oxidative stress

Reactive oxygen species may modulate a variety of signalling pathway. In many cells the mitochondria constitute the primary source of reactive oxygen species. Under specific conditions mitochondria may produce these highly reactive and, in general, toxic agents at a rate that exceeds their scavenging capacity. A variety of evidence has implicated such situation as a potential basis for both degenerative diseases and ageing. This was the subject of Giorgio Lenaz's presentation who paid a special attention to the role of the respiratory complex I in this process.

5. Fatty acids, mitochondria and cell death

This subject was dealt by two speakers. Lech Wojtczak presented his studies on the role of nonesterified fatty acids and N-acylethanolamines in inducing permeability of the inner mitochondrial membrane to protons and in opening the unspecific permeability pore. Peter Schönfeld was talking about mitochondrial damage by phytanic acid as one of the possible mechanisms of the Refsum syndrome.

6. Mitochondria in nitric oxide- and cadmium cell death

Vilma Borutaite concentrated her presentation around the role of nitric oxide in induction of cell death. Nitric oxide or its derivatives inhibit mitochondrial respiration, decrease production of ATP and increase that of reactive oxygen species. Thus, nitric oxide can induce cell death by a variety of mechanisms, both related and unrelated to respiratory inhibition. Frank Thévenod presented the effect of cadmium ions on mitochondria. Cadmium can be taken up from the environment into the body through pulmonary and enteral pathways. Kidney tubule is a major target for chronic cadmium toxicity. Frank Thévenod has shown that cadmium causes mitochondrial swelling and release of cytochrome c.

7. Mitochondria in calcium signalling

Considerable evidence implicates mitochondria as a Ca^{2+} sequester at the expense of energy. Intramitochondrial Ca^{2+} is a major modulator of Ca^{2+}-sensitive metabolic reactions and also functions in modulating cytosolic Ca^{2+} transients. The first part this session was related to the role of mitochondria in modulating the release of calcium from endoplasmic reticulum (ER) and the influx of Ca^{2+} across the plasma membrane. Nicolas Demaurex studied Ca^{2+} fluxes between mitochondria and ER using “cameleon” indicators. He showed that some mitochondria were situated close to the sites of calcium release and that the distance between mitochondria and ER determined mitochondrial calcium signals and filling state of neighbouring ER regions. Thus, he proposed a functional relationship between mitochondria and ER. Rosario Rizzuto analysed molecular determinants and physiological role in mitochondrial calcium signalling. According to his results, efficient mitochondrial Ca^{2+} uptake depends on the preservation of high Ca^{2+} microdomains at the "mouth" of ER calcium release sites close to mitochondria.

The second part of this section was devoted the role of mitochondria in the regulation of calcium influx via store operated calcium channel. Krzysztof Zabłocki proposed a novel model that explains pH dependency of calcium influx into the cell on mitochondrial energy status. Joanna Szczepanowska observed no differences in the regulation of store operated calcium channel between heteroplasmic and homoplasmic osteosarcoma cell lines, although she found differences in mitochondrial and cytoskeletal organization within these cells.

8. Mitochondrial ion channels

Channels selective for potassium or chloride ions are present in the inner mitochondrial membrane. They probably play an important role in mitochondrial events such as formation of pH gradients and regulation of mitochondrial volume changes. Mitochondrial potassium could also be the target for pharmacologically active compounds such as

potassium channel openers and antidiabetic sulphonylureas. This was the subject of the presentations by Adam Szewczyk and Karol Ondriaš. The former one described the interaction of BK-type potassium channel openers with human glioma cells, whereas the latter one presented single channels recordings of mitochondrial potassium and chloride channels.

9. Mitochondrial RNA and DNA

Human mitochondrial DNA is a 16.6-kb circular DNA that contains only 37 genes. Twenty-two genes specify transfer RNAs and two specify ribosomal RNAs. Only 13 genes encode polypeptides, all of which are components of the respiratory chain oxidative phosphorylation system. Piotr Stępień presented mitochondrial RNA degradation and mitochondrial RNA metabolism. He suggested that yeast mitochondrial degradosom (composed of 2 subunits an RNAase and RNA helicase) RNAase activity is necessary for mitochondrial biogenesis and that degradosom is a central part of a mitochondrial RNA surveillance system responsible for degradation of aberrant and unprocessed RNAs. Magdalena Boguta presented results concerning termination of mitochondrial translation in yeast. In contrast to most other eukaryotic organisms, yeast can survive without respiration. This ability has been exploited to investigate nuclear genes required for expression of mitochondrial DNA. She also discussed the role of membrane-located factors responsible for quality control and turnover of mitochondrially-synthesized subunits as well as for assembly of respiratory complexes. Michel Rigoulet's lecture concerned the control of cellular mitochondrial content in growing yeast.

10. Mitochondrial diseases and the role of mitochondria in neurodegenerative diseases

Mitochondrial defects occur in a wide variety of degenerative diseases, ageing and cancer. Mitochondrial DNA has a very high mutation rate. When a mutation arises, cells initially contain a mixture of wild type and mutant mtDNAs, a state known as heteroplasmy. The same mtDNA mutation can produce markedly different symptoms among members of the same family. Jiři Zeman spoke about segregation of mtDNA mutations and their impact on activities of the respiratory chain complexes. He observed immaturity of the mitochondrial energy-generating system and suggested that significant development of mitochondria energy metabolism occurs during the last 3 months of prenatal development. Wolfram Kunz presented his investigation on mitochondrial dysfunction in epilepsy, while Xavier Leverve described the action of an antidiabetic drug in terms of its action on complex I of the respiratory chain. Josef Houštěk made an overview of deficiency in mitochondrial ATP synthase. Pathogenic mutations have been found in only one subunit of this enzyme.

Maternally inherited Leigh syndrome is associated with high levels (>90%) of point mutation in A6 subunit. Lower levels (~ 70-90%) result in a different disorder called NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa). Thus variation in the percentage of mutant mtDNAs between patients must change the ATP output and cause variations in clinical symptoms. Affected children with a mutation load higher than 90% usually do not survive after infancy. Ewa Bartnik presented mitochondrial DNA mutation in cancer and ageing. Mutations and deletions in mtDNA have been observed in various tumours and in ageing lymphocytes. Cristina Ugalde spoke about "mitochondrial" diseases involving deficiency in complex I. This defect leads to a wide variety of clinical symptoms. The end of the session was marked by Rodrigue Rossignol's lecture on the "control strength" of various points of oxidative phosphorylation and their possible involvement pathogenic mutations in mitochondrial DNA.

* * * * * * * * * * * * * * *

Twenty one presentations have been recorded, by authors' permission, on compact disc and will be made available to all participants. Some presentations will appear in form of short experimental papers and mini-reviews in a special issue of *Toxicology: Mechanisms and Methods* (Taylor & Francis, Philadelphia PA).

Trends in Mitochondrial Pharmacology and Genetics

FINAL PROGRAMME

Friday 9th May 2003

16.15	**Opening of the Workshop (chair: J. Duszyński, L. Wojtczak)**
	Presentation of the European Science Foundation C.E. Sekeris (ESF - European Medical Research Councils - EMRC)
16:30 - 17:00	**Insulin-dependent signalling: regulation by amino acids and energy** A.J. Meijer
17:00 - 17:30	**Do mitochondria respond to the potential therapeutic agents controlling glucose production in kidney-cortex tubules of normal and diabetic rabbits?** J. Bryła
17:30 - 18:00	**UCP1 - regulation and physiological significance** B. Cannon
	Coffee break
18:30 - 19:00	**New regulatory phenomenon for the uncoupling of oxidative phosphorylation by UCP3 in intact mitochondria** F.E. Sluse
19:00 - 19:30	**Uncoupling proteins outside the animal and plant kindoms: functional and evolutionary aspect** W. Jarmuszkiewicz
20:00	*Welcome reception*

Saturday 10th May 2003

Chair: Z Drahota, P. Stępień

09:00 - 09:30	**The mitochondrial permeability transition: from in vitro artifact to pharmacological target** P. Bernardi
09:30 - 10:00	**Complexes between outer mitochondrial membrane pore and adenine nucleotide translocator: function in regulation of energy metabolism and apoptosis** D. Brdiczka
10:00 - 10:30	**The mitochondrion as source and target of free radicals: implications in aging and diseases** G. Lenaz
10:30 - 11:00	**Possible role of fatty acids and N-acylethanolamines in apoptosis** L. Wojtczak
	Coffee break
11:30 - 12:00	**Phytanic acid toxicity – implications on the permeability of the inner mitochondrial membrane to ions** P. Schönfeld
12:00 - 12:30	**Mitochondria in nitric oxide-induced cell death** V. Borutaite
12:30 - 13:00	**Control of life and death calcium signals by mitochondria** N. Demaurex
	Lunch

Chair: D. Brdiczka, J. Bryła

14:30 - 15:00	**Mitochondrial calcium signalling: molecular determinants and physiological role** R. Rizzuto
15:00 - 15:30	**The role of mitochondria in the regulation of calcium influx into Jurkat cells** K. Zabłocki
15:30 - 16:00	**Role of defective mitochondria in the regulation of Ca^{2+} influx into osteosarcoma cells** J. Szczepanowska
	Coffee break
16:30 - 17:00	**The role of mitochondria in cadmium-induced nephrotoxicity** F. Thévenod
17:00 - 17:30	**Complexity of single channels in mitochondrial membranes** K. Ondriaš
17:30 - 18:00	**Mitochondria as targets for potassium channel effectors** A. Szewczyk
	Dinner
20:00 - 22:00	**Round table discussion:** **"Mitochondria in the 6th Framework Programme"**

Sunday 11th May 2003

Chair: E. Bartnik, J. Nedergaard

09:00 - 09:30	**Carrier and channel properties of the mitochondrial transporters: Physiology and pathology?** E Rial
9:30 - 10:00	**Targeting mitochondria in cardioprotection** A.P. Halestrap
10:00 - 10:30	**Channels of the outer membrane of Saccharomyces cerevisiae mitochondria: cooperation and regulation** H. Kmita
10:30 - 11:00	**Mitochondrial RNA degradation** P. Stępień
	Coffee break
11:30 - 12:00	**Termination of mitochondrial translation in yeast** M. Boguta
12:00 - 12:30	**Control of cellular mitochondrial content in growing yeast** M. Rigoulet
12:30 - 13:00	**Segregation of mtDNA mutations and their impact on the activities of respiratory chain complexes** J. Zeman
	Lunch

Chair: C.E. Sekeris, A. Szewczyk

14:30 - 15:00	**The antidiabetic drug metforrmin is a mild inhibitor of complex 1 which inhibits cellular death** X.M. Leverve
15:00 - 15:30	**The role of mitochondria in epilepsy - implications for neurodegenerative diseases** W.S. Kunz
15:30 - 16:00	**ATP synthase diseases** J. Houštěk
	Coffee break
16:30 - 17:00	**Mitochondrial DNA mutations in cancer and aging** E. Bartnik
17:00 - 17:30	**Genetic and biochemical characterization of human complex I deficiency** C. Ugalde
17:30 - 18:00	**The control of oxidative phosphorylation. From theory to diagnosis** R. Rossignol
19:00	*Closing of the Workshop and farewell dinner*

Monday 12th May 2003

09:00	*Transfer of the participants to the Bio-Campus "Ochota" and visits to the laboratories of the Nencki Institute, the Biological Faculty of the University of Warsaw and the Institute of Biochemistry and Biophysics.*

Final List of Participants

Convenors:

1. **Jerzy Duszyński**
   Email: jdus@nencki.gov.pl

2. **Lech Wojtczak**
   Email: lwac@nencki.gov.pl

Secretary:

3. **Adam Szewczyk**
   Email: adam@nencki.gov.pl

Local Organising Team:

4. **Joanna Szczepanowska**
   Email: j.szczepanowska@nencki.gov.pl

5. **Krzysztof Zabłocki**
   Email: k.zablocki@nencki.gov.pl

   Nencki Institute of Experimental Biology
   3 Pasteur Str.
   02-093 Warsaw
   Poland
   Tel: +48 22 6593072
   Fax: +48 22 8225342

ESF Representative:

6. **C.E. Sekeris**
   Institute of Biological Research and Biotechnology
   National Hellenic Research Foundation
   48 Vassileos Constantinou Avenue
   116 35 Athens
   Greece
   Email: international@eie.gr

Participants:

7. **Ewa Bartnik**
   Department of Genetics
   University of Warsaw and Institute of Biochemistry and Biophysics
   Polish Academy of Sciences
   Pawińskiego 5a
   02-106 Warsaw
   Poland
   Email: ebartnik@ibb.waw.pl

8. **Paolo Bernardi**
   CNR Unit for the study of Biomembranes
   University of Padova
   Viale Giuseppe Colombo3
   35121 Padova
   Italy
   Email: bernardi@bio.unipd.it

9. **Magdalena Boguta**
   Institute of Biochemistry and Biophysics
   Polish Academy of Sciences
   Pawińskiego 5a
   02-096 Warsaw
   Poland
   Email: magda@poczta.ibb.waw.pl

10. **Vilmante Borutaite**
    Department of Biochemistry
    University of Cambridge
    Tennis Court Road
    Cambridge CB2 1QW
    United Kingdom
    Email: vb207@mole.bio.cam.ac.uk

11. **Dieter Brdiczka**
    Universität Konstanz
    Fakultät für Biologie
    Postfach 5560
    78434 Konstanz 1
    Germany
    Email: Dieter.Brdiczka@uni-konstanz.de

12. **Jadwiga Bryła**
    University of Warsaw
    Institute of Biochemistry
    Department of Metabolic Regulations
    Miecznikowa 1
    02-096 Warsaw
    Poland
    Email: bryla@biol.uw.edu.pl

13. **Barbara Cannon**
    The Wenner-Gren Institute
    The Arrhenius Laboratories F3
    Stockholm University
    106 91 Stockholm
    Sweden
    Email: barbara.cannon@wgi.su.se

14. **Stefan Chłopicki**
    Department of Pharmacology
    Medical College of the Jagiellonian University
    ul. Grzegórzecka 16
    31-531 Kraków
    Poland
    Email: mfschlop@cyf-kr.edu.pl

15. **Nicolas Demaurex**
    Department of Physiology
    University of Geneva Medical Center
    1211 Geneva,
    Switzerland
    Email: Nicolas.Demaurex@medicine.unige.ch

16. **Zdeněk Drahota**
Department of Bioenergetics
Institute of Physiology
Academy of Sciences of the Czech Republic
Vídeňská 1083
142 20 Prague 4
Czech Republic
Email: drahota@biomed.cas.cz

17. **Andrew P. Halestrap**
University of Bristol
Department of Biochemistry
School of Medical Sciences
Bristol BS8 1TD
United Kingdom
Email: a.halestrap@bristol.ac.uk

18. **Josef Houštěk**
Department of Bioenergetics
Institute of Physiology
Academy of Sciences of the Czech Republic,
Vídeňská 1083
142 20 Prague 4
Czech Republic
Email: houstek@biomed.cas.cz

19. **Wiesława Jarmuszkiewicz**
Department of Bioenergetics
Institute of Molecular Biology and Biotechnology
Adam Mickiewicz University
Fredry 10
61-701 Poznań
Poland
Email: wiesiaj@main.amu.edu.pl

20. **Anna Kiersztan**
University of Warsaw
Institute of Biochemistry
Department of Metabolic Regulations
Miecznikowa 1
02-096 Warsaw
Poland

21. **Hanna Kmita**
Department of Bioenergetics
Institute of Molecular Biology and Biotechnology
Adam Mickiewicz University
Fredry 10
61-701 Poznań
Poland
Email: kmita@main.amu.edu.pl

22. **Wolfram S. Kunz**
Department of Epileptology
University Bonn Medical Center
Sigmund-Freud-Str. 25
53105 Bonn
Germany
Email: Wolfram.Kunz@ukb.uni-bonn.de

23. **Giorgio Lenaz**
Dipartimento di Biochimica “G.Moruzzi”
Universita di Bologna
Via Irnerio 48
40126 Bologna,
Italy
Email: lenaz@biocfarm.unibo.it

24. **Xavier M. Leverve**
Laboratoire de Bioénergétique Fondamentale et Appliquée
Université Joseph Fourier
Département de Médecine Aiguë Spécialisée
Hôpital Albert Michallon
BP 217
38043 Grenoble Cedex
France
Email: xavier.leverve@ujf-grenoble.fr

25. **Alfred J. Meijer**
Department of Biochemistry
Academic Medical Centre
University of Amsterdam
Amsterdam
The Netherlands
Email: A.J.Meijer@amc.uva.nl

26. **Tomaš Mračeк**
Department of Bioenergetics
Institute of Physiology
Academy of Sciences of the Czech Republic,
Vídeňská 1083
142 20 Prague 4
Czech Republic

27. **Jan Nedergaard**
The Wenner-Gren Institute
The Arrhenius Laboratories F3
Stockholm University
106 91 Stockholm
Sweden
Email: jan@metabol.su.se

28. **Karol Ondriaš**
Institute of Molecular Physiology and Genetics
Slovak Academy of Sciences
Vlarska 5
83334 Bratislava
Slovak Republic
Email: umfgonds@kramare.savba.sk

29. **Daniele Penzo**
CNR Unit for the study of Biomembranes
University of Padova
Viale Giuseppe Colombo3
35121 Padova
Italy

30. **Eduardo Rial**
Centro de Investigaciones Biolgicas - CSIC
Velazquez 144
28006 Madrid
Spain
Email: rial@cib.csic.es

31. **Michel Rigoulet**
CNRS
1 rue Camille Saint-Saens
33077 Bordeaux Cedex
France
Email: michel.rigoulet@ibgc.u-bordeaux2.fr

32. **Rosario Rizzuto**
Dipartimento di Medicina Sperimentale e Diagnostica
Sezione Patologia Generale
Università di Ferrara
Via Borsari 46
44100 Ferrara
Italy
Email: rzr@dns.unife.it

33. **Rodrigue Rossignol**
Université Bordeaux 2
146 rue Leo-Saignat
33076 Bordeaux Cedex
France
Email: Rodrigue.Rossignol@phys-mito.u-bordeaux2.fr

34. **Peter Schönfeld**
Otto-von-Guericke-Universität
Institut für Biochemie
Leipziger Straße 44
39120 Magdeburg
Germany
Email: peter.schoenfeld@medizin.uni-magdeburg.de

35. **Francis E. Sluse**
Laboratory of Bioenergetics
Institute of Chemistry B6c
University of Liège
Sart Tilman
4000 Liège
Belgium
Email: f.sluse@ulg.ac.b

36. **Piotr Stępień**
Department of Genetics
University of Warsaw
and Institue of Biochemistry and Biophysiscs
Polish Academy of Sciences
Pawińskiego 5a,
02-106 Warsaw
Poland
Email: stepien@ibb.waw.pl

37. **Frank Thévenod**
Institut für Physiologie und Pathophysiologie
Medizinische Fakultät
Universität Witten/Herdecke
Stockumer Str. 12 (Thyssenhaus)
58448 Witten
Germany
Email: frank.thevenod@uni-wh.de

38. **Cristina Ugalde**
University Children's Hospital Nijmegen
Metabolic Disorders-435
P.O. Box 9101
6500 HB Nijmegen
The Netherlands
Email: C.Ugalde@cukz.umcn.nl

39. **Mariusz R. Więckowski**
Dipartimento di Medicina Sperimentale e Diagnostica
Sezione Patologia Generale
Università di Ferrara
Via Borsari 46
44100 Ferrara
Italy
Email: wcm@unife.it

40. **Barbara Zabłocka**
Medical Research Center
Polish Academy of Sciences
Pawińskiego 1
02-096 Warsaw
Poland
Email: zablocka@cmdik.pan.pl

41. **Jiři Zeman**
Department of Pediatrics
Center for Integrated Genomics
Charles University
Prague
Czech Republic
Email: jzem@lfl.cuni.cz

Toxicology: Mechanisms and Methods

42. **Rakesh Dixit**
Editor-in-Chief
Merck & Co, Inc.
Research Laboratories
WP45A-201
770 Sumneytown Pike
West Point, PA 19486
USA
rakesh_dixit@merck.com

Statistical information on participants:

Belgium	**1**
Czech Republic	**4**
France	**3**
Germany	**4**
Greece	**1**
Italy	**5**
Poland	**14**
Slovakia	**1**
Spain	**1**
Sweden	**2**
Switzerland	**1**
The Netherlands	**2**
United Kingdom	**2**
USA	**1**
----------	**----------**
Total number	**42**