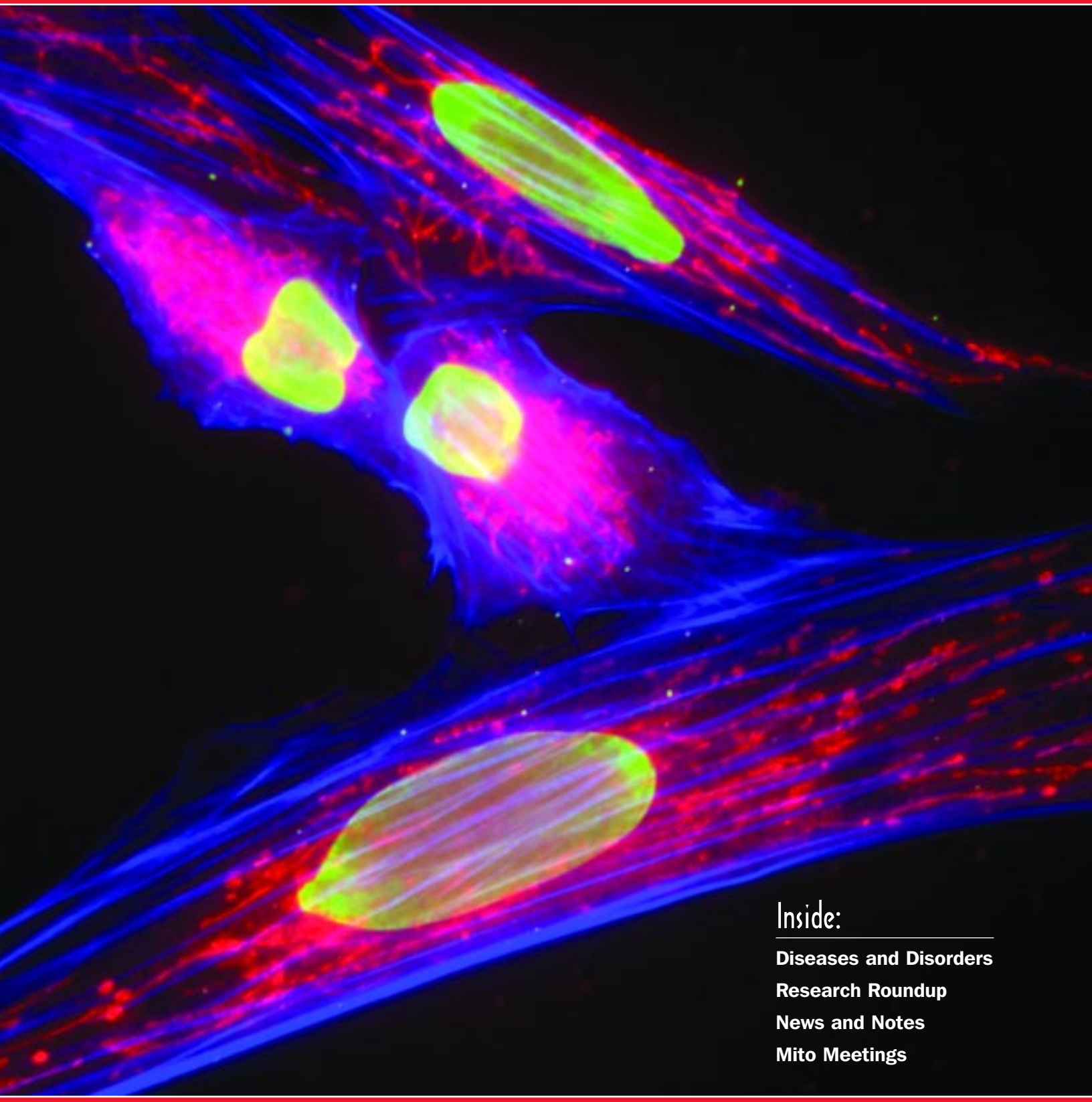
MitoMatters

The Official Newsletter of the Mitochondria Research Society



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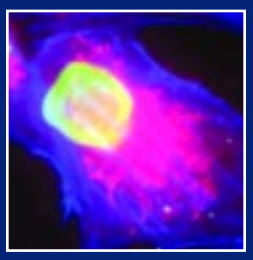

Dear Colleagues:

It's a pleasure to inform you that MRS together with The Mitochondrial Medicine Society organized the "Mitochondria 2003" meeting in June 12–14 this year. The meeting was a success. It was attended by approximately 200 scientists from around world. Our next meeting will be organized in 2004 in association with both the Mitochondrial Medicine Society (MMS) and the United Mitochondrial Disease Foundation. The exact date of the meeting is not finalized. Stay tuned for the date announcement. We would also like to inform you that the MRS executive board recently approve a two year term for the president and secretary. This was necessary in light of a two year term of MMS officers.

With this newsletter you will find the membership application/ renewal form. If you have not already renewed your membership, please do so as soon as possible. With your renewal you will continue to receive an uninterrupted subscription to the *Mitochondrion* journal, this newsletter as well as other membership benefits.

If you have not done already, please register at our Web site (www.mitoresearch.org). Soon we intend to distribute this newsletter electronically only. So if you do not register now, you may not receive the next newsletter.

Managing Editors

Time to renew your 2003 MRS membership and receive uninterrupted subscription to the *Mitochondrion* journal and this newsletter.

Front cover image: *Muntjac skin fibroblasts labeled with a mouse monoclonal anti-histone antibody and visualized with green-fluorescent Alexa Fluor® 500 goat anti–mouse IgG antibody. F-actin was labeled with blue-fluorescent Alexa Fluor® 350 phalloidin, and mitochondria were stained with red-fluorescent MitoTracker® Red CMXRos. Image contributed by Michael Janes, Molecular Probes Inc.*

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Use of Fluorescent Probes in Mitochondrial Research

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Mitochondrial physiology, beyond ATP generation, is increasingly a focus of research in cell biology. It is now clear that mitochondria play a pivotal role in programmed cell death and perhaps also in many disease pathologies. Many studies of mitochondrial function/dysfunction in these contexts require visualization of mitochondria in real time and within intact cells. A wide range of fluorescent probes capable of reporting chemical and physical changes within the mitochondria (or cell) have been developed for these purposes. Used correctly, these probes allow for the identification of individual and regional differences in the mitochondrial population, as well as spatial and temporal changes in response to stimuli. This article briefly reviews some of the fluorescent probes currently being used in studies of mitochondrial biology. An excellent review of this topic by Michael R. Duchen et al. appears in *Methods in Enzymology* (361:353–389, 2003).

Fluorescent probes used to study mitochondria are membrane permeant and can enter mitochondria directly through the phospholipid bilayer. Some such probes (e.g., Mito Tracker Green FM and MitoFluor Green) accumulate in mitochondria regardless of whether a membrane potential is present and are thus useful in visualizing aspects of mitochondrial reticular morphology, or mitochondrial movement. In many instances, fluorescent probes are used to assess alterations in mitochondrial membrane potential, such as might be associated with apoptosis. These probes are membrane permeant cations that accumulate in the matrix in a membrane-potential sensitive manner. Examples of these include rhodamine 123, TMRE, TMRM, JC1, DiOC6, and DASPMI. The specific properties and usages of these probes varies, but it is important to note that all are subject to artifact and misinterpretation. Some of these potential problems have been described by Ward et al. (2000), who have developed and made public mathematical models of probe behavior that are useful in interpreting experimental observations. Strengths and weaknesses of individual probes are also discussed at length by Duchen et al. (2003). Artifacts associated with fluorescent probes include inhibition of respiratory complexes, generation of reactive oxygen species (ROS) upon photoactivation, autoquenching, and non-specific localization within the cell. Many of these problems can be overcome by using sufficiently low concentrations of probe. Despite these complications, it is apparent that cautious use allows at least a semi-quantitative assessment of mitochondrial membrane potential in situ. Qualitative changes in membrane potential associated with cellular perturbations may also be discerned.

Many of the disease pathologies associated with mitochondrial dysfunction implicate bioenergetic dysregulation and overproduction of ROS as causal factors in the onset and progression of disease. Studies of these phenomena benefit from methods for assessing ROS production in the cytosol. A number of fluorescent probes are available to detect intracellular ROS production. Some are specific for individual ROS. For example, trans-1-(2'-Methoxyvinyl) pyrene is apparently specific for singlet oxygen. Other intracellular probes, such as dichlorodihydrofluorescein diacetate (H_2DCFDA) and dihydrorhodamine 123, react more nonspecifically with a variety of ROS and also reactive nitrogen species.

A number of fluorescent dyes that generate ROS within the cell are also available. These molecules may produce specific ROS and/or localize to specific intracellular compartments. For example, tetrabromorhodamine 123 localizes to the mitochondrial matrix where it generates singlet oxygen and can thus be used to direct an oxidative insult specifically to the mitochondrial matrix.

The fluorescent probes described above are an incomplete list of presently available materials. Membrane permeant fluorescent probes sensitive to many other intracellular and intramitochondrial compounds, including calcium and chloride, are also available and have been used extensively. As mitochondrial research becomes

increasingly a focus of cell biology, the need for tools that can be employed to investigate mitochondrial function in the intact, living cell grows. Fluorescent probes are one such tool, and used appropriately, they should be instrumental in continuing to uncover new and interesting aspects of mitochondrial physiology in living cells.

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Ward M.W., Rego A.C., Frenguelli B.G., and Nicholls D.G., 2000. Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. *J Neurosci.* 20, 7208–19.

Duchen, M.R., Surin, A., and Jacobson, 2003. J. Imaging mitochondrial function in intact cells. *Meth. Enzymol.* 361, 353–389.

Research Roundup

The untold story of mice in museums: The notion that mitochondrial DNA evolves faster than nuclear DNA is commonplace among geneticists. However, "fast," in evolutionary terms, is a relative concept since significant changes in relative distributions of haplotypes can take thousands of years. In their recent paper, Pergams and colleagues demonstrated that mtDNA evolves at an incredibly fast rate. By comparing mtDNA sequences from 150 old mouse skin samples from museum collections and mice caught alive in the same area last year, they found that the most common genotype 100 years ago is now extremely rare, demonstrating how fast mtDNA can evolve.

Pergams, O.R., Barnes, W.M., Nyberg, D., 2003. Mammalian microevolution: Rapid change in mouse mitochondrial DNA. *Nature* 423, 397.

Straight to the point: One aspect that has hindered the development of specific therapies for mitochondria-associated diseases is the difficulty in efficiently delivering drugs to the intra-mitochondrial compartment. Taking advantage of the chemical properties of certain compounds that specifically accumulate in mitochondria, Smith and colleagues demonstrated that high intra-mitochondrial concentrations of one particular compound of interest can be achieved by feeding mice with conjugates of a drug with mitochondria permeable molecules, such as tetraphenylphosphonium. This observation opens up the development of safer and more specific drugs for mitochondrial dysfunction.

Smith, R.A.J.; Porteous, C.M.; Gane, A.M.; Murphy, M.P., 2003. Delivery of bioactive molecules to mitochondria in vivo. *PNAS* 100 (9), 5407.

The new diet advice: uncouple. A possible involvement of uncoupling proteins in controlling obesity has long been postulated, but only indirect evidence existed. Now more direct evidence for a role of UCP2 and 3 in body mass have emerged with the work of Horvath and colleagues. They have created transgenic mouse lines expressing human UCP 2 and 3. Out of 12 lines, one was significant leaner than non-transgenic, and another was almost significant. These results shed light on the role of mitochondria in metabolic control as they provide new possible targets for drug interventions for obesity, a disease that has become a serious public health concern in the past two decades.

Horvath, T.L.; Diano, S. et al., 2003. Uncoupling proteins-2 and 3 influence obesity and inflammation in transgenic. *International J. of Obesity* 27 (4), 433.

Glowing proteins. Identifying new mitochondrial proteins could lead to better understanding of many biological processes related to normal metabolism or to disease. This is, however, a laborious job and the risk of contamination by proteins from other subcellular compartments has always been high. Recently, Ozawa and co-workers reported the development of a new screening method of large cDNA libraries, based on the reconstitution of split-enhanced green fluorescent protein (EGFP) by protein splicing of DnaE. This allows for the faster and more accurate identification of mitochondrial-targeted proteins, which represents a big step in better understanding mitochondria biology.

Ozawa, T.; Sako, Y.; Sato, M.; Kitamura, T.; Umezawa, Y., 2003. A genetic approach to identifying proteins. *Nat. Biotech* 26 (4), 287.

News and Notes

Most Downloaded Articles from Mitochondrion (April–December 2002)

1. Immo E. Scheffler, A century of mitochondrial research: achievements and perspectives, *Mitochondrion* 1 (2001), 3–31.
2. Tamir Dagan, Craig Sable, June Bray, and Mariana Gerschenson, Mitochondrial dysfunction and antiretroviral nucleoside analog toxicities: what is the evidence? *Mitochondrion* 1 (2002), 397–412.
3. Abstracts, *Mitochondrion*, 1 (2001), S1–S101.
4. Giuseppe Attardi, Role of mitochondrial DNA in human aging, *Mitochondrion* 2 (2002), 27–37.
5. Daciana H. Margineantu, W. Gregory Cox, Linda Sundell, Steven W. Sherwood, Joseph M. Beechem, and Roderick A. Capaldi, Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines, *Mitochondrion* 1 (2002), 425–435.
6. Xia Li and James M. May, Catalase-dependent measurement of H_2O_2 in intact mitochondria, *Mitochondrion* 1 (2002). 447–453.
7. James A. Dykens, Beth Fleck, Soumitra Ghosh, Michelle Lewis, Gonul Velicelebi, and Manus W. Ward, High-throughput assessment of mitochondrial membrane potential in situ using fluorescence resonance energy transfer, *Mitochondrion* 1 (2002), 461–473.
8. Karen Setterfield, Andrew J. Williams, Jennifer Donald, et al., Flow cytometry in the study of mitochondrial respiratory chain disorders, *Mitochondrion* 1 (2002), 437–445.
9. N. Karoline Scheffler, Scott W. Miller, Amy K. Carroll, et al., Two-dimensional electrophoresis and mass spectrometric identification of mitochondrial proteins from an SH-SY5Y neuroblastoma cell line, *Mitochondrion* 1 (2001), 161–179.
10. Cristina Ugalde, Marieke J. H. Coenen, Murtada H. Farhoud, et al., New perspectives on the assembly process of mitochondrial respiratory chain complex cytochrome c oxidase, *Mitochondrion* 2 (2002), 117–128.
11. Daciana H. Margineantu, Ruth M. Brown, Garry K. Brown, Andrew H. Marcus, and Roderick A. Capaldi Heterogeneous distribution of pyruvate dehydrogenase in the matrix of mitochondria, *Mitochondrion* 1 (2002), 327–338.
12. Daniela A. Bota and Kelvin J. A. Davies, Protein degradation in mitochondria: Implications for oxidative stress, aging and disease: A novel etiological classification of mitochondrial proteolytic disorders, *Mitochondrion* 1 (2001), 33–49.
13. Robert Terkeltaub, Kristen Johnson, Anne Murphy, and Soumitra Ghosh, Invited review: The mitochondrion in osteoarthritis, *Mitochondrion* 1 (2002), 301–319.
14. Roman A. Eliseev, Karlene K. Gunter, and Thomas E. Gunter, Bcl-2 sensitive mitochondrial potassium accumulation and swelling in apoptosis, *Mitochondrion* 1 (2002), 361–370.
15. M. Pilar Bayona-Bafaluy, Patricio Fernandez-Silva, and Jose A. Enriquez, The thankless task of playing genetics with mammalian mitochondrial DNA: A 30-year review, *Mitochondrion* 2 (2002), 3–25.
16. Mitochondria 2001 Meeting, San Diego, California, February 28–March 2, 2001, *Mitochondrion* 1 (2001), 87–116.
17. Kim Jae-Heup, E. Eizirik, S. J. O'Brien, and W. E. Johnson, Structure and patterns of sequence variation in the mitochondrial DNA control region of the great cats, *Mitochondrion* 1 (2001), 279–292.
18. Kazuto Nakada, Tomoko Ono, and Jun-Ichi Hayashi, A novel defense system of mitochondria in mice and human subjects for preventing expression of mitochondrial dysfunction by pathogenic mutant mtDNAs, *Mitochondrion* 2 (2002), 59–70.
19. Patrick F. Chinnery, Inheritance of mitochondrial disorders, *Mitochondrion* 2 (2002), 149–155.
20. Michael S. Lee and Barbara C. Levin, MitoAnalyzer, a computer program and interactive web site to determine the effects of single nucleotide polymorphisms and mutations in human mitochondrial DNA, *Mitochondrion* 1 (2002), 321–326.
21. Hua Yuan, Martha Mutomba, Immo Prinz and Roberta A. Gottlieb, Differential processing of cytosolic and mitochondrial caspases, *Mitochondrion* 1 (2001), 61–69.

22. Carla M. P. Cardoso, Leonor M. Almeida, and Jose B. A. Custodio, 4-Hydroxytamoxifen is a potent inhibitor of the mitochondrial permeability transition, *Mitochondrion* 1 (2002, 485–495.

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25. Abhijit Mukhopadhyay, Baoxian Wei, Steven J. Zullo, Lauren V. Wood, and Henry Weiner, In vitro evidence of inhibition of mitochondrial protease processing by HIV-1 protease inhibitors in yeast: a possible contribution to lipodystrophy syndrome, *Mitochondrion* 1 (2002), 511–518.

Mito Meetings

SEPTEMBER 2–6, 2003
IXTH INTERNATIONAL CONGRESS OF INBORN ERRORS OF METABOLISM, Brisbane, Australia
Visit **http://www.iciem.org/** for details.

SEPTEMBER 12–15, 2003
3RD CONFERENCE ON MITOCHONDRIAL PHYSIOLOGY Schröcken, Vorarlberg, Austria
Visit **http://www.uibk.ac.at/event/mip/** for details.
Contact: Erich Gnaiger, Kathrin Renner
Email: mip@uibk.ac.at, Fax: +43 512 504 4625

SEPTEMBER 17–20, 2003
2ND INTERNATIONAL MEETING ON YEAST APOPTOSIS, Smolenice, Slovak Republic
Visit http://www.fns.uniba.sk/~kbi/imya for details.
Contact: Jordan Kolarov, Department of Biochemistry, Faculty of Science Comenius University, Bratislava 84215, Slovak Republic
E-mail: Jordan Kolarov,
Phone: +421 2 60296539, Fax: +421 2 60296452

OCTOBER 23–25, 2003
INTERNATIONAL SYMPOSIUM ON FREE RADICALS AND HEALTH, Molecular Intervention and Protection of Life Style Related Diseases, Tohoku University of Community Service and Science, Sakata City, Yamagata Prefecture, Japan.
E-mail: midori@koeki-u.ac.jp

OCTOBER 29–NOVEMBER 2, 2003
3RD EUROPEAN METABOLIC COURSE
The Department of Metabolic and Endocrine Disorders and the Laboratory of Paediatrics and Neurology at the University Children's Hospital Nijmegen are organizing a meeting in collaboration with the Orphan Europe Academy. The course is designed for paediatricians with 2 to 5 years clinical experience in the metabolic field. It is pitched at a high level and restricted to 33 participants.
Contact: Guilaine Arduin, manager, Orphan Europe Academy, Orphan Europe Immeuble le Wilson - Cedex 70, 92046 Paris la Défense, France
E-mail: Guilaine Arduin
Tel: 33.1.47.73.94.20, Fax: 33.1.49.00.18.00

JUNE 30–JULY 4, 2004
6TH EUROMIT MEETING
Contact: Jan Smeitink, M.D., Ph.D.
Nijmegen Center for Mitochondrial Disorders, Department of Pediatrics,University Medical Center Nijmegen, Geert Grooteplein 10, P.O. BOX 9101, 6500 HB Nijmegen, The Netherlands.
E-mail: Jan Smeitink
Tel: 0031-24-3614430, Fax: 0031-24-3616428

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Membership benefits include:

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2. Subscription to *MitoMatters* newsletter highlighting new products/tools relevant to mitochondria research and developments in research, prevention, diagnosis, and treatment of mitochondrial diseases
3. MRS member directory
4. Reduced rate of job posting at MRS web site
5. Reduced registration fee in national and international meetings and workshops organized by MRS

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Please specify

Primary field of interest: ❒ Biochemistry ❒ Evolution ❒ Molecular Biology
❒ Biophysics ❒ Forensics ❒ Pharmacology
❒ Cell Biology ❒ Genetics ❒ Toxicology ❒ Other: _____
Please specify

Current research: _____

_____

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Membership fee for MRS is $50. To join, please submit a personal check or money order drawn in U.S. dollars and made payable to The Mitochondria Research Society, or pay by credit card. If paying by credit card, please fill out the credit card details below:

❒ Visa ❒ MasterCard

Credit card number _____ Expiration date _____

Signature _____ Date _____

Send or fax application to: The Mitochondria Research Society, P.O. Box 1952, Buffalo, NY 14221, USA; fax: 716-845-1047. Thank you.

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