

## Session 9: Mitochondria and Degenerative Diseases – I. Animal Models

### **9-01. Knockdown of COX5a in zebrafish phenocopies aspects of human COX deficiency.**

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Zebrafish offer significant genetic, physiologic, and economic benefits as a model system in which to study human disease [1]. While mouse has been the traditional animal model of mitochondrial disease, we believe zebrafish will be a powerful new system in which to investigate outstanding questions in the field of mitochondrial pathophysiology [2].

In humans, defects in mitochondrial energy production, particularly by oxidative phosphorylation (OXPHOS), affect a wide variety of tissues including central and peripheral nervous system, ocular, cardiac, gastrointestinal, and skeletal muscle [2-3]. Deficiency in cytochrome c oxidase (COX), an integral part of the OXPHOS respiratory chain, is a component of many mitochondrial disorders [4]. We are using a morpholino antisense oligonucleotide to the COX5a subunit (COX5a-MO) to knockdown expression of COX5a and recapitulate human COX deficiency syndromes in zebrafish. Utilizing monoclonal antibodies we are able to follow tissue expression of COX5a in wildtype and MO-injected fish by immunohistochemistry and western blot, while monitoring the levels of COX using a monoclonal antibody against subunit 1 and total mitochondrial content in cells with an antibody to porin.

Preliminary results of COX5a knockdown in the developing zebrafish indicate that many of the tissues affected in humans with COX deficiency are also affected in zebrafish. Morpholino knockdown of COX5a results in stunted growth, motility impairment, pericardial edema, brain and eye abnormalities, failure to inflate the swim bladder, and no gastrointestinal tract development. The zebrafish model of COX deficiency will be useful for investigating the molecular and cellular basis of mitochondrial disease.

1. Grunwald D, Eisen J (2002) Headwaters of the zebrafish – emergence of a new model vertebrate. *Nat. Rev. Genet.* 3: 717-24.
2. Wallace D. (2001) Mouse models for mitochondrial disease. *Am. J. Med. Genet.* 106: 71-93.
3. Zeviani M (2001) The expanding spectrum of nuclear gene mutation in mitochondrial disorders. *Cell. Dev. Biol.* 12: 407-416.
4. DiMauro S, Bonilla E, Davidson M, Hirano M, Schon E (1998) Mitochondria in neuromuscular disorders. *Biochim. Biophys. Acta* 1366: 199-210.



### **9-02. APP transgenic mice exhibit mitochondrial dysfunction.**

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Mitochondrial dysfunction underlies many common age-related diseases, including Alzheimer's disease (AD). AD is characterized by two major histopathological hallmarks, extracellular plaques of fibrillar  $\beta$ -amyloid (A $\beta$ ) peptides and intracellular neurofibrillary tangles (NFT) composed of hyperphosphorylated tau protein [1,2]. In previous studies we could show that P301L tau transgenic mice exhibit mitochondrial

respiratory defects [3]. Furthermore, accumulation of A $\beta$  and oxidative stress seem to play central roles in the pathogenesis, by probably directly leading to mitochondrial dysfunction.

To investigate the contribution of A $\beta$  in AD-related neurodegenerative processes, we used isolated mitochondria of APP transgenic (tg) mice. These mice exhibit onset of A $\beta$  plaques at an age of 6 months, but intracellular A $\beta$  load is already increased at the age of 3 months. The non-physiological high levels of A $\beta$  found in transgenic mice lead to senile plaque formation like in their human counterparts, preceded by oxidative stress [4]. We detected decreased basal levels of mitochondrial membrane potential ( $\Delta\psi_m$ ) in tgAPP mice compared to littermate non-tg control mice. Hydrogen peroxide and the nitric oxide donor sodium nitroprussid damaged the cells significantly by decreasing  $\Psi_m$  in littermate non-tg control mice but not in tgAPP mice. Most probably, this is due to the preliminary insult caused by the chronic APP exposure. In addition, we observed decreased ATP levels behaving in a similar pattern after additional oxidative stress. Complementary, we observed a significant reduction of cytochrome c oxidase activity in 8 month old tg APP mice. In contrast, no differences in the NADH ubiquinone oxidoreductase activity between WT and tgAPP mice could be observed.

Our results further emphasize the important role of mitochondrial dysfunction in the pathogenesis of AD. Moreover, they indicate that A $\beta$  is already involved in these neurotoxic mechanisms before plaque formation occurs.

1. Lee VM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* 24: 1121-1159.
2. Gotz J, Streffer JR, David D, Schild A, Hoernli F, Pennanen L, Kurosinski P, Chen F (2004) Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. *Mol. Psychiatry* 9: 664-683.
3. David DC, Hauptmann S, Scherping I, Schuessel K, Keil U, Rizzu P, Ravid R, Dröse S, Brandt U, Müller WE, Eckert A, Götz J (2005) Proteomic and functional analysis reveal a mitochondrial dysfunction in P301L tau transgenic mice. *J. Biol. Chem.* [Epub ahead of print].
4. Smith MA, Casadesus G, Joseph JA, Perry G (2002) Amyloid- $\beta$  and  $\tau$  serve antioxidant functions in the aging and Alzheimer brain. *Free Radic. Biol. Med.* 33: 1194-1199.

### **9-03. Proteomic and biochemical analyses of diabetic human and obese rodent skeletal muscle mitochondria reveals altered concentration, oxidation, and activity of respiratory chain proteins.**

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Altered mitochondria-related gene expression in skeletal muscle has been observed in Type 2 Diabetes (T2DM) patients compared to Insulin Sensitive (IS) individuals, implicating mitochondrial dysfunction in the development of insulin resistance (IR). Obesity-related and diabetes-related IR in skeletal muscle has been linked to intramyocellular lipid (IMCL) accumulation and impaired mitochondrial oxidative phosphorylation [1,2]. It is hypothesized that specific mitochondrial defects in skeletal muscle, including altered expression of individual respiratory chain complex proteins and increased reactive oxygen species (ROS) generation, are directly related to increased IMCL and insulin resistance.

Using proteomic and biochemical analyses, we have assessed whether there are altered expression, oxidative modification, and/or activity of respiratory chain protein complexes in 6 pairs of IS and T2DM patients matched for age, race, gender, and BMI and in an animal model of obesity using Zucker (fa/Br) obese rats. Mitochondrial preparations were subjected to 2-Dimension Blue-Native gel electrophoresis for optimal separation of membrane proteins [3,4]. Multiple proteins associated with respiratory

complexes I, III, IV, and V were found to be altered in T2DM compared to IS individuals and expression of complexes I and IV were decreased in the obese rat compared to lean littermates. Alterations in the ETC protein subunits could also increase ROS formation. Western blot analysis confirmed increased levels of oxidized mitochondrial thiols and increased protein carbonyl adducts in T2DM individuals compared to insulin sensitive controls and in the obese rodents compared to their controls which could further compromise function [5-7].

These abnormalities in respiratory chain protein expression and oxidation could affect function of respiratory complexes resulting in defects in substrate oxidation and accumulation of IMCL. The dramatic increases in respiratory protein oxidation in diabetes implicate increased ROS generation as a potential key mechanism for impaired insulin action and metabolism in insulin resistant humans.

1. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* 350: 664-671.
2. Machann J, Haring H, Schick F, Stumvoll M (2004) Intramyocellular lipids and insulin resistance. *Diabetes Obes. Metab.* 6: 239-248.
3. Schagger H, Cramer WA, von Jagow G (1994) Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. *Anal. Biochem.* 217: 220-230.
4. Brookes PS, Pinner A, Ramachandran A, Coward L, Barnes S, Kim H, Darley-Usmar VM (2002) High throughput two-dimensional blue-native electrophoresis: a tool for functional proteomics of mitochondria and signaling complexes. *Proteomics* 2: 969-977.
5. Kim JR, Yoon HW, Kwon KS, Lee SR, Rhee SG (2000) Identification of proteins containing cysteine residues that are sensitive to oxidation by hydrogen peroxide at neutral pH. *Anal. Biochem.* 283: 214-221.
6. Ramachandran A, Ceaser E, Darley-Usmar VM (2004) Chronic exposure to nitric oxide alters the free iron pool in endothelial cells: role of mitochondrial respiratory complexes and heat shock proteins. *Proc. Natl. Acad. Sci.* 101: 384-389.
7. Conrad CC, Choi J, Malakowsky CA, Talent JM, Dai R, Marshall P, Gracy RW (2001) Identification of protein carbonyls after two-dimensional electrophoresis. *Proteomics* 1: 829-834.

#### **9-04. Hypertriglyceridemia in transgenic mice is associated with higher mitochondrial resting respiration and increased whole body CO<sub>2</sub> production and body temperature.**

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High plasma levels of triglycerides lead to an increase in liver mitochondria resting respiration rate and predispose to mitochondrial permeability transition (MPT) [1]. In the present study, we demonstrate that spleen lymphocytes isolated from hypertriglyceridemic transgenic mice also present resting oxygen consumption rates 54 % higher when compared to spleen lymphocytes isolated from control mice. No significant alterations in the transmembrane mitochondrial potential or reactive oxygen species production could be detected through DHE or H<sub>2</sub>DCF-DA fluorescence method. Fibrate treatment reduced triglyceride plasma levels by 50 % and normalized liver mitochondrial resting respiration. However, insulin treatment, which reduced triglyceride plasma levels by 30 %, did not correct the liver mitochondrial respiratory control in transgenic mice. When submitted to oxidative stress, liver mitochondria isolated from transgenic mice showed higher susceptibility to lipid peroxidation induced by Fe(II)/citrate than control liver mitochondria. In agreement with the results of mitochondria resting respiration (isolated and *in situ*), whole mice CO<sub>2</sub> production rate was higher in the hypertriglyceridemic transgenic than in control mice (14.8 ± 1.1 vs. 12.6 ± 1.5 g·kg<sup>-1</sup>·h<sup>-1</sup>, *P*<0.05, respectively). In addition, body temperature was also higher in transgenic than in control mice (37 ± 0.4 °C vs. 36.3 ± 0.2 °C, *P*<0.05, respectively). We propose that this faster catabolism may represent a regulated

adaptation to oxidize excess free fatty acids in these hypertriglyceridemic transgenic mice.

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1. Alberici LC, Oliveira HCF, Bighetti EJ, de Faria EC, Degaspari GR, Souza CT, Vercesi AE (2003) Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition. *J. Bioenerg. Biomembr.* 35: 451-457.



### **9-05. A study of cytotoxicity of phytanic acid in mitochondria and astrocytes isolated from rat brain - possible mechanism in Refsum disease.**

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In Refsum disease, a peroxisomal genetic disorder, the branched-chain fatty acid phytanic acid (3,7,11,15-tetramethylhexadecanoic acid; Phyt) accumulates at high levels (up to 1 mM) throughout the body. Clinical features suggest that Phyt exerts severe cytotoxicity, mostly in tissues with a high energy turnover. We studied the influence of non-esterified Phyt on various parameters of the energy metabolism in rat brain by using isolated mitochondria and brain cells.

Single-cell analysis applied to isolated hippocampal astrocytes reveals that Phyt (100  $\mu$ M) drastically increases the inflow of extracellular  $\text{Ca}^{2+}$ , exerts strong depolarization of mitochondria *in situ*. Furthermore, cell viability of cultured astrocytes was significantly reduced after a 5h-exposure to Phyt. All these changes were not seen with the unbranched palmitic acid.

Isolated mitochondria become strongly deenergized by Phyt, applied at low concentrations (5 – 20  $\mu$ M, i.e. 5 – 20 nmol/mg of mitochondrial protein). In energized mitochondria, deenergization is mainly due to protonophoric activity of Phyt. In addition, Phyt decreases state 3 respiration by inhibition of the electron flow within the respiratory chain and inhibition of the ADP/ATP exchange across the inner membrane.

As an important functional consequence of these findings, mitochondria preloaded with small amounts of  $\text{Ca}^{2+}$  (100 nmol/mg protein) become highly sensitized to rapid membrane permeability transition (MPT), even when only low concentrations of Phyt (below 5  $\mu$ M) are applied. Depolarisation of the inner mitochondrial membrane and locking the ADP/ATP carrier in the matrix conformation most likely account for this sensitization to MPT.

Moreover, the interaction of Phyt with components of the respiratory chain raises strongly mitochondrial superoxide generation ( $\text{O}_2^{\bullet-}$ ), an observation which is not seen with palmitic acid. Interaction of Phyt with complex I mostly contributes to Phyt-related  $\text{O}_2^{\bullet-}$  generation. This conclusion is supported by (i) inhibition of NADH-ubiquinone oxidoreductase and (ii) decreased reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). In addition, similar to antimycin A (complex III-inhibitor), Phyt increases complex III-related  $\text{O}_2^{\bullet-}$  generation.

In conclusion, the observed harmful effects of Phyt on brain mitochondria and on astrocytes support the hypothesis that clinical features of Refsum disease are directly related to pathologically increased levels of Phyt.

1. Wanders RJA, Jakobs C, Skjeldal OH (2001) Refsum disease. In: The metabolic and molecular bases of inherited disease. 8th ed (Scriver CR, Beaudet AL, Sly WS, Valle D, eds), McGraw-Hill, New York: 3303-3321.
2. Schönfeld P, Kahlert S, Reiser G (2004) In brain mitochondria the branched-chain fatty acid phytanic acid impairs energy transduction and sensitizes for permeability transition. *Biochem. J.* 383: 121-128.
3. Kahlert S, Schönfeld P, Reiser G (2005) The Refsum disease marker phytanic acid, a branched chain fatty acid, affects  $\text{Ca}^{2+}$  homeostasis and mitochondria, and reduces cell viability in rat hippocampal astrocytes. *Neurobiol. Dis.* 18: 110-118.

### **9-06. Mitochondrial biogenesis in rat embryo during placentation process.**

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Mitochondrial biogenesis is a complex event that requires the coordinated regulation of both mitochondrial and nuclear genome by several transcriptional activators and coactivators [1]. Although important advances in this field have been achieved, the molecular pathways are not well known. In this sense, the mitochondria of rat embryo during placentation are a suitable model to further understand the mitochondrial proliferation-differentiation process, due to the important oxidative metabolism activation that takes place at this stage of development [2]. Thus, the gene expression of some proteins involved in mitochondrial replication, transcription and function, such as mitochondrial single strand DNA binding protein (mtSSB) [3], mitochondrial transcription factor A (TFAM) [4] and cytochrome c oxidase subunit I (COXI) respectively, have been investigated in rat embryo throughout gestational days 11, 12 and 13. We have shown that during the period studied there was a reduction in mtSSB mRNA levels accompanied by a great decrease in cellular mitochondrial DNA content (mtDNA). In addition to that, an important rise in the ratio between TFAM and mtDNA, and also in COXI relative gene expression was observed on gestational day 13. All these results together suggest that during the placentation period the rat embryo mitochondria reduce their proliferation and enter a differentiation stage by increasing their transcriptional activity probably through the physiological TFAM function as a mitochondrial transcription factor. To sum up, the present study supports the fact that embryo development is a physiological condition where mitochondrial biogenesis is well illustrated. Therefore, the current model could be of great interest for further understanding many unknown aspects of mitochondriogenesis, which should help understand the pathophysiology of mitochondrial diseases.

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1. Kelly DP, Scarpulla RC (2004) Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev.* 18: 357-368.
2. Shepard TH, Muffley LA, Smith LT (1998) Ultrastructural study of mitochondria and their cristae in embryonic rats and primate (*N. nemestrina*). *Anat. Rec.* 252: 383-392.
3. Hoke GD, Pavco PA, Ledwith BJ, Van Tuyle GC (1990) Structural and functional studies of the rat mitochondrial single strand DNA binding protein P16. *Arch. Biochem. Biophys.* 282: 116-124.
4. Gaspari M, Falkenberg M, Larsson NG, Gustafsson CM (2004) The mitochondrial RNA polymerase contributes critically to promoter specificity in mammalian cells. *Embo J.* 23: 4606-4614.

### **9-07. The placental phenotype of a model of mitochondrial dysfunction in mice.**

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Maternal nutrition is known to affect fetal growth and development and by restricting protein intake studies have demonstrated several metabolic abnormalities leading to insulin resistance. Pups maintained on a low protein diet during lactation exhibit a lower growth weight and a reduced insulin response to glucose challenge. Proteome analysis has revealed altered expression of 70 proteins by fetal malnutrition including proteins related to mitochondrial energy transfer and glucose metabolism. Placental size is increased and a higher placental/birth weight ratio is seen in gestational diabetes. In mothers with anaemia produce larger placentas and in smoking mothers there is a reduced placental weight but increased ratio of placental weight to birthweight.

A common variant in human mtDNA, at bp 16189, has been associated with type 2 diabetes, thinness at birth [1] and high placental weight in humans [2]. The T to C transition lies in the major non-coding region which contains control sequences for replication and transcription and is a bidirectional origin of replication [3] and produces a polyC tract.

We have modeled the 16189 variant of mild mitochondrial dysfunction in mice by administering the mitochondrial inhibitor, zidovudine (AZT). This is a viral reverse transcriptase inhibitor used for the treatment of HIV infection. One prominent side effect of AZT is mtDNA depletion as a result of inhibition of the mitochondrial gamma polymerase. We used four groups of mice: control (C), on either AZT (AZT), a low protein diet (LPD), or a combination of both (AZTLPD).

We have previously shown that AZT reduces mtDNA copy number in liver of offspring ( $P=0.021$ ). LPD decreased birthweight and litter size ( $P=0.012$  and  $0.01$  respectively). A combination of AZT with LPD decreased birthweight and litter size and increased fasting glucose and insulin compared with untreated controls. Our preliminary data shows that LPD in combination with AZT significantly increases placental size ( $P=0.039$ ). We are now investigating this further by exploring placental histology and gene expression.

1. Casteels Ong K, Phillips D, Bendall H, Pembrey M (1999) Mitochondrial 16189 variant, maternal restraint of fetal growth and impaired glucose tolerance/type 2 diabetes *Lancet* 353: 1499-1500.
2. Morten KJ, Parker E, Bain SC, Cockington RA, Phillips DI, Poulton J (2004) A common mtDNA variant is associated with high placental weight and thinness at aged 20. *Diabet. Med.* 21 (suppl P14).
3. Yasukawa T, Yang MY, Jacobs HT, Holt IJ (2005) A bidirectional origin of replication maps to the major noncoding region of human mitochondrial DNA. *Mol. Cell.* 18: 651-662.

### **9-08A. Role of mitochondrial dysfunction in mechanism of reversible metabolic depression of over-wintering lamprey liver during pre-spawning migration.**

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We have conducted an investigation with the aim to clarify mechanism(s) of metabolic depression observed in poikilothermic animals during over-wintering period. In this study we have elucidated the role of mitochondria in reversible metabolic depression of hepatocytes of Baltic lamprey (*Lampetra fluviatilis* L.) taking place in the last year of its life cycle. It is known that in autumn the lamprey migrates from the Gulf of Finland to the Neva River (North-Western Russia) and switches off the exogenous feeding during all period of pre-spawning migration. Using isolated mitochondria as a model, we have revealed clear-cut seasonal variations of the main bioenergetical parameters of the lamprey liver. These changes indicate that the metabolic depression observed during the last winter of the lamprey's life cycle is mediated by prolonged reversible mitochondrial dysfunction. The dysfunction is found to manifest itself in: (1) the very low activity of mitochondrial respiratory chain, especially of its complex I, (2) low oxidative phosphorylation, (3) decreased content of mitochondrial adenine nucleotides, (4) high level of reduced pyridine nucleotides, and (5) leaky mitochondrial membranes. The sharp activation of oxidation and phosphorylation in the lamprey liver mitochondria followed by spawning and death of the animal is observed in spring. The possible causes of the phenomenon and its difference from that taking place under oxidative stress are discussed. An amazing analogy between some molecular mechanisms underlying the metabolic depression in lamprey liver cells and those in cells of patients suffering from mitochondrial encephalomyopathies, neurodegenerative diseases, sepsis, poisoning, and cancerogenesis is revealed [1-3].

1. Luft R (1995) The development of mitochondrial medicine. *Biochim. Biophys Acta.* 1271: 1-6.
2. Gellerich FN, Trumbeckaite S, Mueller T, Deschauer M, Chen Y, Gizatullina Z, Zeiz S (2004) *Mol. Cell. Biochem.* 256/257: 391-405.

- Greenamyre JT, Sherer TB, Betarbet R, Panov AV (2001) Complex I and Parkinson's disease. *IUBMB Life*. 52: 135-141.

### **9-09A. Effect of thallium (I) ions on isolated rat liver mitochondria in the presence of nonactin.**

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It is known that the inner mitochondrial membrane is lightly permeable to  $Tl^+$  ions [1]. Transport of  $Tl^+$  in energized mitochondria occurs via the electrophoretic uniport mechanism [2,3]. It was found that nonactin being ionophore stimulates transport of  $Tl^+$  in mitochondrial matrix [3]. However, the mechanisms of effect of nonactin on mitochondria are not studied completely.

Effects of  $Tl^+$  on isolated rat liver mitochondria were studied. Both uptake of  $^{204}Tl^+$  in the presence of various nonactin concentrations in a medium and action of  $Tl^+$ -nonactin complex on uptake of  $^{137}Cs^+$  with valinomycin were tested in succinate-energized mitochondria. Besides, transport of  $Tl^+$  in mitochondria was evaluated from swelling of mitochondria incubated in the  $TlNO_3$  or  $Tl$ -acetate media. Action of  $Tl$ -acetate and nonactin on mitochondrial enzymes containing active SH-groups was estimated from their effects on the state 3, state 4, or 2,4-dinitrophenol uncoupled respiration of mitochondria.

It was found that uptake of  $^{204}Tl^+$  by energized mitochondria was enhanced by an increase of nonactin concentration in the incubation medium. On the other hand, uptake of  $^{137}Cs^+$  by energized mitochondria was markedly decreased in the presence of  $Tl^+$  with nonactin in the medium. Nonactin accelerated swelling of nonenergized mitochondria in the  $TlNO_3$  medium. At the same time, contraction of mitochondria after their succinate energization was markedly retarded in the presence of nonactin. In the medium containing 25-50 mM  $Tl$ -acetate, swelling of mitochondria before and after succinate administration and mitochondrial contraction after oxygen depletion were accelerated in experiments with nonactin. The state 4 respiration of mitochondria in the  $Tl$ -acetate medium was 2.5-fold increased in the presence of nonactin. The state 3 or 2,4-dinitrophenol uncoupled mitochondrial respiration was not affected by nonactin. The problems of nonactin effects on active and passive transport of  $Tl^+$  across the inner mitochondrial membrane and importance of the transmembrane potential ( $\Delta\psi_m$ ) on the transport processes are discussed [4].

- Saris NE, Skulskii IA, Savina MV, Glasunov VV (1981) Mechanism of mitochondrial transport of thallos ions. *J Bioenerg Biomembr.* 13: 51-59.
- Melnick RL, Monti LG, Motzkin SM (1976) Uncoupling of mitochondrial oxidative phosphorylation by thallium. *Biochem. Biophys. Res. Commun.* 69: 68-73.
- Skulskii IA, Saris NE, Savina MV, Glasunov VV (1981) Uptake of thallos ions by mitochondria is stimulated by nonactin but not by respiration alone. *Eur. J. Biochem.* 120: 263-266.
- Korotkov SM, Nikitina ER, Glazunov VV, Yagodina OV (2005) Effect of thallium ions on isolated mitochondria of rat liver in the presence of nonactin. *Doklady Biochem. Biophys.* 401: 97-103.





**MiP2003 - Cheese and wine reception at the "Vorarlberger Alpmuseum uf m Tannberg", Batzen**

Left: Annelies (chef of Hotel Mohnenfluh, serving the selection of fine Austrian wines); below sunscreen: Jakob Troppmair, Marisol Quintero, Veronica Hollis behind Graham Kemp (cutting cheese), Domenico Boffoli, Eiji Takahashi (cutting cheese); besides sunscreen: Josef Houstek, Zdenek Drahota; standing in row: Marek Böhm, Joan McEwen, Stefanie Jarolim (sitting), Frédéric Bouillaud, Sebastian Vogt, Daniela Curti, Petr Jezek (behind), Cecilia Giulivi, Julian Pakay, Petr Pecina (behind), Nazzareno Capitanio, Jordi Bermudez (behind); sitting at the hut: Sergio Papa, Peter Schönfeld, Andrea Dlasková (far right); standing in front left: Irina Shabalina, Adrian Lambert and Franz Hartner (behind), Mikhail Vyssokhik, Jaap Keijer, Maik Hüttemann (behind); around front bench: Alena Vojtiskova, Olga Matejková, Pavel Jesina (sitting), Ondrej Kuda (standing); left of car: Fernanda Ferreira, Zemfira Gizatullina, Jerzy Duszynski, Dieter Brdiczka, Bernhard Kadenbach (sitting); in front of car: Hans van Beek and Michael Verkhovsky (standing), Oliver Speer and Ove Eriksson (sitting).



Left, left to right: Annelies (Chef of Hotel Mohnenfluh), Marisol Quintero, Veronica Hollis, Domenico Boffoli, Jakob Troppmair, Graham Kemp, Marek Böhm, Marina Jendrach, Eiji Takahashi, Joan McEwen, Stefanie Jarolim, Frédéric Bouillaud, Sebastian Vogt, Alena Vojtiskova, Daniela Curti, Petr Jezek, Cecilia Giulivi.

Bottom: Sergio Papa demonstrates expertise in cutting the 1-year old alpcheese, produced in the neighbouring new *Alp Batzen*. Bottom left: Julian Pakay and Nazzareno Capitanio (behind), Sergio Papa, Sylvia Schramm-Strolz (hostess of Hotel Mohnenfluh) and Veronica Hollis (left of table); Mark Hüttemann and Susanne Arnold (sitting behind table); Marisol Quintero, Michael Verkhovsky, Chris Cooper, Steven Hand, Barbara Santoro (sitting). Bottom right: Domenico Boffoli, Joan McEwen, Sergio Papa, Sebastian Vogt, Daniela Curti, Petr Jezek, Cecilia Giulivi.

