

dependent mitochondrial impairment and stabilization of hypoxia-inducible factor (HIF)-1 α , which synergize to activate glycolysis and generate large quantities of ATP. The upregulation of glycolysis is completely dependent on NO and HIF-1 α . Furthermore, HIF-1 α stabilization is biphasic, with a reactive oxygen species-dependent early phase (1-2 h after activation) and later phase associated with the release of NO. We now demonstrate, using TMRM fluorescence and time-lapse confocal microscopy, that activated M Φ maintain a high mitochondrial membrane potential ($\Delta\Psi_m$) despite the complete inhibition of respiration by NO. The high $\Delta\Psi_m$ is maintained by utilization of a significant proportion (approx 30%) of total glycolytically-generated ATP and is achieved by the reverse functioning of F₀F₁-ATP synthase and adenine nucleotide translocase (ANT). Treatment of activated M Φ with inhibitors of either of these enzymes (oligomycin or bongkrekic acid), but not with inhibitors of the respiratory chain complexes, led to a collapse in $\Delta\Psi_m$ and to an immediate increase in intracellular [ATP]. This collapse of $\Delta\Psi_m$ was biphasic, with a rapid initial drop of approx 30% followed by a gradual decline, and was associated with translocation of Bax from cytosol to the mitochondria, release of cytochrome c into the cytosol, activation of caspases 3 and 9, and apoptotic cell death. Our results indicate that during inflammatory activation "glycolytically competent cells" such as M Φ utilize significant amounts of the glycolytically-generated ATP to maintain $\Delta\Psi_m$ and thereby prevent apoptosis. It remains to be investigated whether the diversion of energy for this purpose, which is also likely to occur in tissues, favours or is detrimental to the successful outcome of an inflammatory response.

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15P.19 Swapping mutants of voltage dependent anion channel highlight the functional importance of the N-terminal and confers anti-ageing features to the cell

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Voltage-dependent anion channels (VDACs) are the most abundant proteins of the mitochondrial outer membrane that form hydrophilic pore structures. VDAC constitutes the main pathway through which metabolites are exchanged between the cytosol and mitochondria and also serves as a site for the docking of cytosolic proteins, such as hexokinase. The structure of mammalian VDAC1 has been recently solved by NMR and crystallization experiments [1]. It shows a rather compact transmembrane channel, formed by 19 amphipathic β strands connected by short turns and loops, with the striking addition of the N-terminal moiety structured with α -helix segments. The N-terminal sequence is located inside the channel, forming a partial obstruction of the wide pore. CD and NMR spectroscopy showed that the 20 aa N-terminal peptide needs a special environment to form an ordered α -helix secondary structure. In addition it was shown that the deletion of the N-terminal does not change the mitochondrial targeting of the protein [2]. In higher eukaryotes three VDAC isoforms exist, but only VDAC1 and VDAC2 are well characterized. On the contrary VDAC3 has been poorly studied and does not show an evident pore-forming ability [3]. In this work, we provide insight into the function of the isoform 3 by exchanging the N-terminal sequence of the human VDAC3 with the homologous sequences of human VDAC1 and

VDAC2. The activity of the wild type and chimeric proteins was monitored in $\Delta por1$ yeast strain. Results obtained in complementation assay, oxidative stress resistance, chronological aging, ROS production and mitochondrial membrane activity measurements, outline the importance of the N-terminal moiety of VDAC isoforms in the function of the protein. Surprisingly swapping mutants show a doubled lifespan in comparison with wild type yeast strains.

References

- [1] Ujwal R. *et al.* (2008) *Proc Natl Acad Sci U.S.A.* **105**: 17742-17747.
- [2] De Pinto V. *et al.* (2007) *ChemBiochem* **8**: 744-756.
- [3] De Pinto V. *et al.* (2010) *Biochem. Biophys. Acta-Bioenerg.* Feb 4. [Electronic publication ahead of print].

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15P.20 Analysis of cellular function by use of cell-penetrating oxygen sensing probes

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Mitochondria are involved in many metabolic pathways such as production of ATP or the Krebs cycle. Dysfunction of the mitochondria can lead to many disorders such as neurodegenerative diseases or metabolic disorders. Therefore, monitoring of intracellular oxygen (as well as the other parameters such as Ca²⁺ or K⁺) can be used to assess mitochondrial function, as most of cellular oxygen is consumed by the mitochondria [1, 2]. Sensing of intracellular oxygen in mammalian cell lines can be performed by use of Pt-porphyrin based probes and phosphorescence quenching method. One of the examples of this approach is the recently constructed phosphorescent probes based on Pt(II)-coproporphyrin I (PtCP) dye. These probes were tailored in a manner to pass cell membrane by conjugation to cell-penetrating peptides. Cationic and hydrophobic nature of these probes eliminates the need for transfection reagents [2]. One such probe is PEPP0 which comprises an uncharged PtCP derivative covalently linked to bactenecin 7 (15-24) peptide. The uptake and toxicity of PEPP0 was studied on PC12, HCT116, SH SY5Y and HeLa cells. Fluorescence microscopy imaging has revealed cytoplasmic localization of this probe with a pattern resembling the mitochondrial one. It was found that probe uptake by HCT116 cells, was largely due to endocytosis mechanisms; whereas it was endocytosis, temperature and ATP independent in PC12 cells. Respirometric experiments with mitochondrial inhibitors and uncouplers were performed on differentiated PC12 cells transfected with PEPP0 probe using time-resolved fluorescence (TR-F) measurements (phosphorescence lifetime based sensing of O₂) and different levels of external (atmospheric) hypoxia. This allowed observation of localized deoxygenation of the cells. This probe chemistry and measurement approach utilizes microplates and standard TR-F plate reader, which allows high throughput analysis. Overall, the new intracellular probes are useful for the monitoring of oxygen in cultures of adherent mammalian cells.

References

- [1] Pieczenik SR, Neustadt J (2007) *Exp. Mol. Pathol.* **83**: 84-92.
- [2] Dmitriev RI *et al.* (2010) *Anal. Biochem.* **398**: 24-33.

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