15P.7 Fluorescence measurement of absolute mitochondrial membrane potential in adherent cultured cells

Akos A. Gerencser[1], Christos Chinopoulos[2], Matthew J. Birket[1], Martin Jastroch[1], David G. Nicholls[1], Martin D. Brand[1]
[1]*Buck Institute for Age Research, USA*
[2]*Semmelweis University, Department of Medical Biochemistry, Neurobiochemical Group, Hungarian Academy of Sciences, Szentagothai Knowledge Center, Hungary*
E-mail: agerencser@buckinstitute.org

An assay of in situ mitochondrial membrane potential ($\Delta\psi m$) within live cells matched with respirometry is an invaluable tool for kinetic analysis of the electron transport chain. All known $\Delta\psi m$ measurement techniques (fluorescence, voltammetric or isotopic) rely on the Nernstian distribution of lipophilic cations, such as the fluorescent tetramethylrhodamine methyl ester (TMRM). The accumulation of these cations in cells is not only a function of $\Delta\psi m$, but also of the plasma membrane potential ($\Delta\psi p$), the matrix to cell volume ratio, and its activity coefficients and binding in the matrix and the cytosol. When comparing different cell types or genetic manipulations, these parameters are not expected to be the same. We have developed a fluorescence microscopy based technology for absolute determination of $\Delta\psi m$ in millivolts and to monitor its changes in time in live adherent cells. $\Delta\psi p$ is followed with a lipophilic anionic plasma membrane potential indicator (PMPI). The $\Delta\psi p$-dependent distribution of the probes through the plasma membrane is modeled by electrostatic barrier limited diffusion, and a solution of this model is used to deconvolute $\Delta\psi p$ in time from changes of PMPI fluorescence intensity. Then, $\Delta\psi m$ is determined in millivolts by deconvoluting TMRM fluorescence taking in account the slow, $\Delta\psi p$-dependent redistribution and its Nernstian behavior. The electrostatic barrier model was verified by voltage clamp in perforated whole cell mode, combined with fluorescence imaging. The volume and activity coefficient ratios are determined in separate, purely fluorescence microscopic methods that minimize the uncertainty of the suboptical matrix to mitochondrion volume ratio. The absolute value of $\Delta\psi m$ is calculated from the efflux kinetics of TMRM following an acute and complete mitochondrial depolarization. Ultimately, time courses of fluorescence intensity are converted into millivolts with the minimal requirement of complete depolarization of the $\Delta\psi m$ and then of the $\Delta\psi p$ at the end of the assay. The calculations are performed by standard Excel functions. The technique has been validated on rat cortical neuronal cultures, yielding $\Delta\psi m$ values in accordance to earlier radioisotope studies. $\Delta\psi m$ determinations have been performed on INS-1E, HEK and PC12 cultures, and on human embryonic stem cells and derivatives.

doi:10.1016/j.bbabio.2010.04.372

15P.8 Mitochondria as potential regulators of mRNA life-span in mammalian cells

Daria Gudkova, Ganna Panasyuk, Ivan Nemazanyy, Valeriy Filonenko
Institute of Molecular Biology and Genetics NASU, Department of Cell Signalling, Ukraine
E-mail: d.o.gudkova@gmail.com

Processing bodies (PB) were recently discovered as cellular structures that are involved in the cytoplasmic processing of mRNAs and have critical roles in mRNA degradation and post-transcriptional gene silencing. One of the scaffold proteins of PB is RCD-8. RCD-8 was identified in our lab as a novel binding partner of CoA synthase, metabolic enzyme which is responsible for CoA synthesis in cells and is predominantly localized within outer membrane of mitochondria. Further exploration of RCD-8 in mammalian cells revealed that it may localize on mitochondria under normal conditions, and after stress treatment (osmotic, oxidative, mitochondrial etc) it released to the cytoplasm. In addition the efficacy of RCD-8/CoA synthase protein complex formation at stress conditions is greatly decreased. It was shown that C-terminus of RCD-8 is responsible for its localization in PB and according to our data involved in interaction with CoA synthase. Taking into consideration, that RCD-8 is necessary for the formation of processing bodies, we proposed that some proteins (and/or CoASy probably) could serve as repository of RCD-8 on mitochondria and in this way can regulate mRNA life-span. In other words, we assumed existing of some steric competition between these partners/CoASy and other proteins of processing bodies (e.g. DCP1/DCP2) for interaction with RCD-8. Next, we found out that down regulation of PI3K-dependent signaling by specific chemical inhibitor lead to strong reducing of RCD-8 amount on mitochondria comparing with untreated cells. So, we proposed that mitochondria may have additional functions, which consists in the regulation of cellular metabolism of mRNA, but precise mechanism of it remains to be determined.

doi:10.1016/j.bbabio.2010.04.373

15P.9 Regulation of mitochondrial respiration and apoptosis through phosphorylation of cytochrome *c*

Petr Pecina[1], Grigory G. Borisenko[2], Natalia A. Belikova[2], Yulia Y. Tyurina[2], Alena Pecinova[1], Icksoo Lee[1], Alejandro K. Samhan-Arias[2], Valerian E. Kagan[2], Maik Hüttemann[1]
[1]*Wayne State University, Center for Molecular Medicine and Genetics, Detroit, USA*
[2]*University of Pittsburgh, Center for Free Radical and Antioxidant Health and Department of Environmental and Occupational Health, Pittsburgh, USA*
E-mail: mhuttema@wayne.edu

We have recently shown that cytochrome *c* (Cytc) is tyrosine-phosphorylated *in vivo* on two distinct sites, Tyr97 in bovine heart and Tyr48 in bovine liver, suggesting that Cytc functions are regulated through cell signaling pathways in a tissue-specific manner (Lee *et al.*, 2006, *Biochemistry* **45**: 9121-9128; Yu *et al.*, 2008, *Biochim. Biophys. Acta* **1777**: 1066-1071). To study the role of Cytc tyrosine phosphorylation using an independent approach, we overexpressed and purified Tyr48Glu mutant Cytc, mimicking the *in vivo* Tyr48 phosphorylation found in cow liver, along with wild-type and Tyr48Phe variants as controls. The midpoint redox potential of the phosphomimetic mutant was decreased by 45 mV compared to control (192 mV vs. 237 mV). Similar to Tyr48 *in vivo* phosphorylated Cytc, direct kinetic analysis of the Cytc reaction with isolated cytochrome *c* oxidase revealed decreased *V*max for the Tyr48Glu mutant by 30% compared to wild-type or the Tyr48Phe variants. Interestingly, the phosphomimetic substitution resulted in major changes of Cytc functions related to apoptosis. The binding affinity of Tyr48Glu Cytc to cardiolipin was decreased by about 30% compared to wild-type or the Tyr48Phe variants, and Cytc peroxidase activity of the Tyr48Glu mutant was not inducible by cardiolipin, unlike the controls. Importantly, the Tyr48Glu Cytc failed to induce any detectable downstream activation of caspase-3. Our data suggest that *in vivo* Tyr48 phosphorylation might serve as an anti-apoptotic switch, and highlight the strategic position and role of the conserved Cytc residue Tyr48 in regulating multiple functions of Cytc.

doi:10.1016/j.bbabio.2010.04.374