IN VIVO IMAGING OF MITOCHONDRIAL ROS FLASHES AND CHONDRIOME DYNAMICS IN THE WHEAT NON-GREEN ORGAN CELLS

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IN VIVO ИМИДЖИНГ 'ВСПЫШЕК' МИТОХОНДРИАЛЬНЫХ АФК И ДИНАМИКИ ХОНДРИОМА В КЛЕТКАХ НЕФОТОСИНТЕЗИРУЮЩИХ ОРГАНОВ ПШЕНИЦЫ

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The fundamental feature of respiratory machinery is to generate mitochondrial inner membrane potential ($\Delta\Psi_m$) by extrusion of protons from the matrix coupled with electron flux through the electron transport chain, and constitutive, single-electron leakage from the respiratory chain gives by-products, such as reactive oxygen species (ROS). For a long time, stress-induced ROS elevation has been interpreted as a prevailing sign of oxidative stress caused by mostly antioxidant inefficiency and resulted in cell damage and even death. To date, ROS are assigned a dual part, not only harmful one, but also as stress signalling molecules necessary for successful adaptation to variable environmental conditions. However, subcellular regulation of ROS production and vice versa ROS-dependent regulation of organellar morphofunction remains largely obscure [1]. Striking discrete events of high-located mitochondrial ROS overproduction are ROS flashes, the so-called mitoflashes [2, 3]. Contrary to the basal flashless ROS generation, a bursting mode is discrete and very brief in order to

be detectable by using the routine methods and therefore it requires the application of high-resolution live-imaging at the single-organelle level. At the same time, the existence of these mitoflashes described in animal mitochondria by means of circularly permuted yellow fluorescent protein [3] are still disputed because of their high pH sensitivity hence dramatically bursts of its fluorescence seem to reflect rather transient matrix alkalinisation than changes in ROS overproduction [4]. Although mitoflashes were initially described in cardiac myocyte mitochondria by using the synthetic ROS indicator, such as 2,7-dichlorodihydrofluorescein diacetate (DCF) [2] which has relative insensitivity to pH ranging from 6.0 to 9.5.

Another dynamic phenomenon, transmembrane potential pulsing, was detected by means of tetramethyl rhodamine methyl ester (TMRM) because their accumulation is directly $\Delta\Psi_m$ - dependent. In opposite to mitoflashes [4], pulsing were unambiguously characterised for plant cells as abrupt short-term (typically ~ 20 s) and stress-induced fluctuation of $\Delta\Psi_m$, though merely for Arabidopsis [5].

In our experiments, wheat (*Triticum aestivum* L., winter cv. Mironovskaya 808) seedlings were grown hydroponically on a tap water at 23-25°C for 3d in the dark. Field wheat plants were grown under natural conditions and had well-developed tillering nots responsible for plant winter survival. To establish whether these dynamic phenomena are intrinsic properties of plant mitochondria, we applied multi-tracking analysis for double-loaded samples by incubation in 0.5 μM TMRM and 10 μM DCF for 30 min. Live-cell imaging in a real-time manner allowed us to visualise coordinated flickerings of $\Delta\Psi_m$ and ROS production having been similar for mitochondria of wheat etiolated seedling coleoptile cells (Fig.) as well as the tillering nods cells. Typical curve of pulses had 3 distinct phases, such as abrupt decrease in fluorescent intensity (FI) to background fluorescence, then the delay phase with different durations depending on variety of experimental manipulations, and - finally - the recovery phase with reversion to the baseline (Fig.).

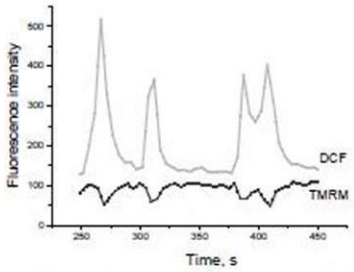

Figure The temporal relationship between changes in fluorescent intensities of TMRM and DCF in a single mitochondrion in the wheat coleoptile cell during monitoring in a real-time manner.

We confirmed that relatively short-term (30-50 s) changes in DCF FI were abrupt and transient, with more than 3-5-folds rise from the basal level and had inverse mirror relationship with pulsing dynamics (Fig.). Thus, high reliable negative Pearson's correlation coefficients between changes in FI of DCF and TMRM suggest the close functional interrelation between observed dynamic events both before and after cold treatment (Fig.). In contrast to mostly irreversible mode of DCF oxidation in animal mitochondria [2, 3], we detected a periodic character of fast cyclic oscillations of DCF FI (Fig.). Notably, the flickering activity was more inherent for immobile mitochondria, frequently physically contacted with the plasma membrane, than for mobile ones. From our results obtained by means of *in vivo* imaging approaches, cold acclimation evoked both a decrease in the dynamic activity and an increase in chondriome heterogeneity via the occurrence of mobile vermiform or disc-shaped organelles with a reduced ability to produce ROS in a flash manner. Previously, using transmission electron microscopy, we described *in situ* dramatic chondriome heterogeneity owing to expanded and unusual forms of mitochondria occurred in the tillering node cells of the field wheat plants during autumn adaptation [6].

To summarise, through living-cell imaging of mitochondrial morphological and functional dynamics in the wheat different non-green organ cells, we first have obtained significant experimental evidence of the periodic and mitochondria-originating dynamic phenomena of membrane potential pulsing - ROS flashing which depended on mitochondrial behaviour. In our report, it will be discussed the possible physiological meaning of the chondriome morphofunctional alterations as well as some challenges and limitations of using fluorescent probes to detect ROS production of mitochondrial origin, including *in vitro* [7].

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REDOX METABOLISM IN THE APOPLAST OF LICHENIZED FUNGI: MECHANISMS AND ROLES IN LICHEN BIOLOGY

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