THE DYNAMIC PHENOMENA OF PLANT MITOCHONDRIA: FUNCTIONAL IMAGING *IN VIVO*

Динамические феномены митохондрий растений: функциональный имиджинг *in vivo*

Abdrakhimova Y.R.[1,2], Abdrakhimov F.A.[3]

[1] *Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia*
[2] *Kazan Federal (Volga region) University, Kazan, Russia; yoldez.abdrahimova@kpfu.ru*
[3] *Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center of Russian Academy of Sciences, Kazan, Russia*

Mitochondria play a key role in cellular metabolism providing for growth and develop processes with energy and building blocks as well as in its regulation under irregular environmental changes. Fundamental feature of respiratory machinery is to generate mitochondrial inner membrane potential ($\Delta\Psi m$) by proton extruding from the matrix coupled with electron flux through the electron transport chain; a reverse proton-driving force is well-known to be used for ATP synthesis and other vital intracellular needs. Although constitutive, single-electron leakage from the respiratory chain gives by-products as primary reactive oxygen species (ROS), mainly superoxide radical, there are multiple ROS-scavenging systems including mitochondrial ones to support cellular ROS balance. For a long time, ROS elevation is commonly interpreted as unfavorable consequence of antioxidant inefficiency often resulting in oxidative stress and even cell death. To date, a novel, paradigm-shifting, concept is that ROS overproduction underlies not only oxidative imbalance, but also active and signaling nature dynamic events such as recently discovered extracellular ROS/Ca^{2+} waves and discrete mitochondrial flashes, named 'mitoflashes'.

In contrast to the flashless basal ROS production, bursting ROS generation is so brief and highly localized being undetectable for determination by the routine methods. There were revolutionary, innovative improvements in fluorescence microscopy for the last two decades given a great advance in higher spatial and temporal resolutions that allowed researchers to visualize a quantal event at the level of individual mitochondrion. Although the phenomenon of mitochondrial flashes has been discovered as transient fluorescence increasing of ROS-biosensors, at the present time the interpretation of the data, particularly received by using cpYFP, are disputed. According to pH hypothesis supported by data from *Arabidopsis*, mitoflashes seem to reflect rather transient matrix alkalization than changes in ROS signal (Schwarzländer et al., 2012). Thus, the phenomenon of mitoflashes in plant mitochondria has not been yet clear demonstrated that may be accounted for serious, still insurmountable limitations of plant-adjusted imaging approaches due to intrinsic structural and functional features of plant cell (e.g., cell wall, plastids, intensive cyclosis, and etc.). Another striking quantal event, flicker or pulse, is accompanied by abrupt, transient (over the second ranges) depolarization of $\Delta\Psi m$, which dynamics can be monitored using the cationic lipophilic fluorescent dye, tetramethyl rhodamin methyl ester (TMRM), at the single-mitochondrion level in a real-time manner.

Using confocal laser-scanning microscopy in combination with appropriate fluorescent dyes (DCF and TMRM), we clarified whether these phenomena could be demonstrated *in vivo* and, if so, might they reflect functional activity of experimental systems and their stress-induced responses? For elucidation of the correlation with each other, the dynamics were investigated after double-labeling of samples (intact epidermal cells) and subsequent multitracking analysis. Objects-to-be-tested were etiolated early-growth seedlings of different agricultural species from mono- and dicotyledons undergone low positive temperatures as well as field winter cereals during autumn acclimation. It should be noted that incidents of the phenomena have been shown for all studied objects. Thus, we firstly have received a strong experimental evidence of reproducible mitoflashes and flickers of plant mitochondria *in vivo* displaying *per se* fundamental and universal properties of the organelle of living cells. Moreover, diverse modes of dynamics of mitochondrial ROS bursting and membrane pulsing, and also their relationships, have been revealed, compared to animal analogs. The features of the processes were widely modulated in response to stress treatments and some pharmacological manipulations, among them blocking of cytoskeleton polymerization. According to obtained results, there was an average rate of 10.2 pulses per min per 100 mitochondria or per 1000 μm^2 cell area (680 mitochondria from n=12 cells/coleoptiles) for winter wheat (*Triticum aestivum* L., cv. Mironovskaya 808) seedlings under control conditions. After low positive temperature treatments (2-3^{0}C, 3d), the pulsing activity dropped to 0.8 and 0.4 per 100 mitochondria and 1000 μm^2, accordingly (845 mitochondria from n=14 cells/coleoptiles). This fact unambiguously indicates that pulsing frequency depends on the metabolic activities of cells and also the cytoskeleton state, the changes of which have been observed concurrently via reversible inhibition of mitochondrial movement in response to cold treatments. It should be stressed that despite different pharmacological manipulations both another's and our own, the causal, triggering mechanisms of initiation of these processes remain obscure. To sum up, though being an array of challenging questions, the approaches for functional imaging are a new 'devises' readable the multifaceted processes of mitochondrial bioenergetics and ROS signaling, and so the dynamic quantal events may serve as a reliable 'digital' readout reflecting mitochondrial and cellular energy metabolism and its stress-induced changes *in toto*.