SESSION 3

Organelle, Cell-to-Cell and Long-Distance Signalling

The mitochondrial pulsing as novel player in plant cell signaling system: modulation by cytoskeleton remodeling

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Mitochondria are well-known as multifaceted organelles, which play an essential role in cellular metabolism not only as regulated source of energy and 'building blocks' but due to their signaling function, especially under influence of environmental and endogenous stimuli. Owing to opened new possibilities in confocal fluorescent microscopy, non-invasive functional imaging of live-cell processes, including dynamics of signaling events, become possible to detect with high spatio-temporal resolutions in a real-time manner and at a single-organelle level. To date, there is striking stochastic phenomenon termed 'flickering' or 'pulsing' visualized as abrupt transient depolarization of mitochondrial inner membrane potential being, therefore, attributed to mitochondrial permeability transition. Such pulses are often coupled with another discrete dynamic events termed as 'mitoflashes' or reactive oxygen species (ROS) flashes. Despite different pharmacological manipulations, the triggering mechanisms of initiation of these phenomena remain obscure. Moreover, occurrence of discrete mitoflashes in individual plant mitochondria has not been yet clearly demonstrated at all. Objects-to-be-tested were etiolated early-growth seedlings of winter cereals (*Triticum aestivum* L., *Secale cereale* L.) undergone low positive temperatures as well as field plants during autumn acclimation and wintering. Using confocal laser-scanning microscopy in combination with the appropriate fluorescent dyes (TMRM and H_2DCF-DA), we first received a strong experimental evidence of reproducible pulses and mitoflashes for plant mitochondria displaying their inherent and universal feature in living cells. To elucidate a putative role of cytoskeleton in the regulation of the mitochondrial dynamic events, we investigated the influence of anti-actin (latrunculin B, 300 nM) and anti-microtubule (oryzalin, 10 μM) drugs on behavior of mitochondria. Both anti-cytoskeletal agents reversibly stopped moving of cytoskeleton-associated subpopulation of mitochondria due to arrest of cytoplasmic streaming, and this simultaneously evoked stimulation of the pulsing rate. For wheat coleoptile epidermal cells, we detected average rates of 3.3 and 7.4 pulses per 100 mitochondria per 1 min for untreated and latrunculin-treated samples, respectively. In rye cells, latrunculin-stimulating effect on the pulsing rate was more evident (up to 5-fold) in comparison with wheat ones that positively correlated with intensity of cytoplasmic streaming. We propose that cytoskeleton disassembling decreases intracellular ATP consumption leading to overreduction of the electron respiratory chain that, in turn, results in increase of mitochondrial dynamic incidents to avoid excessive ROS production. Interestingly, that added H_2O_2 (100 μM) induced the similar increase of the pulsing rates (up to 2) while the effect of oligomycin (6 μM), ATP-synthase blocker, was higher (3-fold). Cold treatment dropped mitochondrial pulsing activity; in this case latrunculin had marked pulsing-stimulating effect on rye cells in contrast with wheat ones. The latter is most probably caused by the relevant cytoskeleton reorganizations as well as different adaptive strategies of these species to temperature stresses. To sum up, the pulsing activity being inherent feature of mitochondrial dynamic behavior is regulated by cell metabolic and oxidative status directly and/or via cytoskeletal-mediated modulations and might serve as an integral optical 'readouts' reflecting functional stress-induced changes, and finally cell survivability.