15P.10 Simultaneous ratiometric imaging of ATP and Ca^{2+} concentrations inside single living cells

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Calcium ion regulates many cellular processes. Previous study that employed firefly luciferase as a reporter of intracellular ATP concentration has revealed that histamine-induced increase of intracellular Ca^{2+} concentration induces increase of ATP concentration of both cytoplasm and mitochondria [1]. Because oligomycin inhibits the increase of ATP, Ca^{2+} must promote ATP synthesis in mitochondria. However, this experiment used ensemble of cells, and therefore it is still unclear how Ca^{2+} dynamics affects to intracellular ATP concentration at single cell level. We have recently reported a series of genetically-encoded Förster resonance energy transfer (FRET) indicator for ATP, called ATeam, which is based on CFP, YFP and ε subunit of bacterial F_oF_1-ATP synthase [2]. Ratiometric FRET imaging of cells expressing ATeam has enabled us to monitor ATP concentration of cytoplasm, nucleus or mitochondria at single cell level. However, it has been difficult to image intracellular ATP together with Ca^{2+} because there are significant overlaps of both excitation and emission spectra between ATeam and ratiometric calcium indicators (eg. Fura-2). Here, we report a red-shifted ATeam (GO-ATeam) that has GFP and OFP as a donor and an acceptor of FRET, respectively, instead of CFP and YFP. Because GO-ATeam is excited with longer wavelength light than the previous ATeam, it is compatible with a UV-excitable ratiometric calcium indicator, Fura-2. We simultaneously imaged ATP and Ca^{2+} concentrations of the same single living cells by loading Fura-2 calcium indicator into the cells expressing GO-ATeam. When Ca^{2+} spark was induced in the cells by histamine, mitochondrial also ATP elevated following Ca^{2+}. Intracellular Ca^{2+} typically returned to a basal level within 10 minutes after histamine stimulation. In contrast, most of cells retained high level of mitochondrial ATP for a much longer time (sometimes more than 30 min). This result strongly supports the idea that intracellular Ca^{2+} promotes long-term activation of energy metabolism in mitochondria [1].

References

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15P.11 Outer mitochondrial membrane protein degradation by the proteasome/ubiquitin system

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Protein turnover is used for regulatory processes and to eliminate superfluous, denatured or chemically inactivated polypeptides. Mitochondrial proteins may be particularly susceptible to damage induced by reactive oxygen species and several pathways of mitochondrial proteostasis have been illuminated. However, in contrast to matrix and inner mitochondrial membrane protein degradation, little is known about the turnover of integral outer mitochondrial membrane (OMM) proteins or the mechanisms involved. Our previous studies have demonstrated that the OMM proteins are under control of the ubiquitin (Ub)/proteasome system. Our new data indicate that in addition to the proteasome, various membrane steps of Ub-dependent degradation of the OMM proteins, including retrotranslocation through mitochondrial membrane(s) (through the activity of AAA-ATPase p97), are similar to those of the endoplasmic reticulum-associated degradation pathway. As an example we found a striking increase in ubiquitinated proteins, including Mfn2 and Tom20, of mitochondria isolated from cells expressing a dominant negative mutant of AAA-ATPase p97, p97QQ. Since expression of p97QQ inhibits retrotranslocation of polyubiquitinated proteins from the ER, these data suggest that p97 might function in a similar manner in retrotranslocation of polyubiquitinated proteins from the OMM. We have also identified a family of the OMM-associated E3 Ub ligases that are likely to control ubiquitination and degradation of the OMM proteins. One of these proteins, IBRDC2, an IBR-type RING-finger E3 Ub ligase, regulates the levels of Bax and protects cells from unprompted Bax activation and cell death. Downregulation of IBRDC2 induces increased cellular levels and accumulation of the active form of Bax. The ubiquitination-dependent regulation of Bax stability is suppressed by IBRDC2 down regulation and stimulated by IBRDC2 overexpression, both in healthy and apoptotic cells. These findings suggest the existence of an Ub- and IBRDC2-dependent apoptosis checkpoint safeguarding mitochondria from Bax-dependent damage and thus cell from unprompted apoptosis.

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15P.12 The influence of estradiol, epinephrine and cAMP on mitochondria energization and intracellular free Ca^{2+} concentration in lamprey hepatocytes

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The aim of the study was to clarify the role of epinephrine, estradiol and isobutiryl-cAMP in regulation of mitochondrial membrane potential ($\Delta\psi_{mit}$) and intracellular free Ca^{2+} concentration in lamprey hepatocytes during metabolic depression connected with starvation. TMRM (tetramethylrhodamine 0.5 μM) and $DiOC_6$ (3,3'-dihexyloxacarbocyanine iodide 1 nM) were used with laser confocal microscopy (Leica TCS SP5) and flow cytometry (Backman Coultrer EPIXC-XL) to investigate $\Delta\psi_{mit}$ in isolated hepatocytes. For measurement of Ca^{2+}_i concentration hepatocytes were loaded by 5 μM FURA 2-AM and were measured by spectrofluorophotometer Shimadzu RF-1501. In autumn lampreys (*Lampetra fluviatilis L.*) return from the Baltic sea to the river Neva where they spend all winter switching off exogenic feeding. In spring after the spawn lampreys die, because they are monocyclic animals. The liver of adult lamprey is a unique natural model witch demonstrates both the processes of metabolic depression (from November until March) and activation (April-May). $\Delta\psi_{mit}$ decreased from October till December. Under the treatment of epinephrine (10^{-5} M) in the middle of November the energization of mitochondria in hepatocytes increased. At the end of November the influence of epinephrine passed away, which seems to be connected with down-regulation of membrane receptors. However, at that time the introduction of isobutiryl-cAMP (10^{-7} M) sharply increased the fluorescence intensity TMRM and $DiOC_6$. In this period estradiol not influenced on $\Delta\psi_{mit}$. Intracellular free Ca^{2+} concentration increased from 60 nM in October to 130 nM in February. In period of metabolic depression (the Atkinson charge in lamprey liver did not exceed 0.2-0.3) cytosolic Ca^{2+} was very high while intracellular calcium stores