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Abstracts

S7 Mitochondrial Proteomics

Lectures

7L.1 Structural and functional changes induced by tyrosine nitration in cytochrome *c*, a bi-functional protein

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Tyrosine nitration is one of the most common post-transcriptional modifications of proteins, so affecting their structure and function. Respiratory cytochrome *c*, with 4–6 tyrosine residues, is an excellent case study as it is a well-known protein playing a double physiological role in different cell compartments. On one hand, it acts as electron carrier within the mitochondrial respiratory electron transport chain and, on the other hand, it serves as a cytoplasmic apoptosis-triggering agent. First, we have analyzed the nitration-induced changes in secondary structure, thermal stability, heme environment, alkaline transition and molecular dynamics of the five monotyrosine mutants of human cytochrome *c* — which have all their tyrosine residues but one replaced by phenylalanines. The resulting data, along with the functional analyses of the mutants, suggests that the specific nitration of Tyr46 and Tyr48 — which are both close to the heme propionate groups — and that of the solvent-exposed Tyr74 impairs the electron transfer to (horse) cytochrome *c* oxidase, enhances the peroxidase activity of cytochrome *c* and blocks its ability to activate caspase-9. In addition, a comparative proteomic analysis with human, algal and plant cytochrome *c* – and cell extracts from the respective organisms – is under way, so as to identify novel proteins that could act as physiological partners of cytochrome *c* under normal or programmed cell-death conditions. The finding of new protein partners of cytochrome *c* in differently evolved organisms will help us to understand, in a global way, the function of non-nitrated and nitrated cytochrome *c* in cell metabolism.

References

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7L.2 Metabolic rearrangements following AOX silencing and AOX over-expression in alga *Chlamydomonas rheinhardtii*: A proteomic approach

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In the present work we have isolated by RNA interference and characterized at the functional and the proteomic levels a *Chlamydomonas reinhardtii* (C.r.) strain devoid of the mitochondrial alternative oxidase (AOX). We have also performed a proteomics study of the soluble mitochondrial proteome of C.r. cells grown on nitrate over-expressing AOX compared to cell grown on ammonium. Our studies highlight how C.r. cells adapt their metabolism in front of AOX deprivation or up-regulation. The AOX-deficient strain displays a doubling of the cell volume and biomass (protein, starch and chlorophyll content), a significantly higher ROS production and no change in total respiration rate and in photosynthesis efficiency. Comparative proteomics at mitochondrial and cellular levels allows us to identify the molecular adaptations underlying this phenotype. Our analysis indicates a strong up-regulation of the ROS scavenging systems and important quantitative modifications of proteins involved in the primary metabolism, namely an increase of enzymes involved in anabolic pathways and a concomitant general down-regulation of enzymes of the main catabolic pathways. The results obtained with C.r. grown on nitrate/ammonium (up-regulation of AOX) show important proteomic modifications mostly related to primary metabolism. For instance we could note an up-regulation of some TCA cycle enzymes and a down-regulation of complex III together with an up-regulation of l-arginine and purine catabolism enzymes, possibly leading to a higher ubiquinone pool reduction level and to a higher ROS production by nitrate-grown cells respectively. Accordingly, a higher hydrogen peroxide production by mitochondria has been measured in nitrate-grown cells and we highlighted a subsequent induction of ROS scavenging systems. Hence, in nitrate-grown cells, AOX may play a dual role: (1) lowering the ubiquinone pool reduction level, then decreasing ROS production and (2) permitting the export of mitochondrial reducing power under the form of malate for nitrate and nitrite reduction in the cytosol and in the chloroplast. This role of AOX in the mitochondrial plasticity makes logical the localization of *Aox1* in a nitrate assimilation gene cluster.

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