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Alterations in the supramolecular interactions of respiratory chain complexes and enhanced superoxide production by the cytochrome b Y278C mutation which causes a multisystem disorder

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Respiratory complex III (CIII) is the central complex of the respiratory chain. In its native form, CIII is dimeric, and is closely associated in varying proportions with CI and CIV to form supramolecular structures, called supercomplexes [1]. The occurrence of such supercomplexes as structural and functional entities has been recently documented [1,2]. However, the physiological implications of such specific supramolecular interactions are not yet fully understood. Mutations in cytochrome *b*, which is the only CIII subunit encoded by the mitochondrial genome, are among the least common abnormalities identified to date in humans [3]. Recent studies on the bacterial enzyme proposed that specific amino acids at the Q_o site of CIII might provide protective mechanisms against oxidative damage. Interestingly, the conserved Tyr residue at position 302 of *Rhodobacter capsulatus* cytochrome *b* is critical for this process [4]. The same mutation at position 278 of human cytochrome *b* (p.Y278C, m.15579A>G) has also been encountered in a patient with severe exercise intolerance and multisystem disorder [5]. Here we have utilized cybrids bearing the homoplasmic p.Y278C mutation to dissect the biochemical alterations in CIII activity, ROS production and supercomplexes assembly/stability. Despite a dramatic reduction in CIII activity, and in CIII-driven ATP synthesis, the CI and I + III activities were less affected, and the rate of ATP synthesis driven by CI or CII substrates was only partially reduced. Accordingly, mutated cybrids maintained the mitochondrial potential in the presence of oligomycin. Remarkably, the p.Y278C mutation enhanced superoxide production and perturbed glutathione homeostasis. Finally, examination of respiratory supercomplexes revealed that dimeric CIII and III_2IV_1 were markedly decreased, whereas supercomplexes $I_1III_2IV_n$ increased. These findings suggest that the deleterious effects of cytochrome *b* p.Y278C mutation are mitigated when CIII is assembled into the supercomplexes $I_1III_2IV_n$ and underline the importance of supramolecular interactions between respiratory complexes on disease manifestations associated with this mutation.

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18P3

Control of electron transport routes through redox-regulated redistribution of respiratory complexes

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Both respiratory and photosynthetic electron transport are crucial for energy conversion in plants and many microbes. In cyanobacteria, respiratory electron transport takes place in close proximity to photosynthetic electron transport, because the complexes required for both processes are located within the thylakoid membranes [1,2]. This allows "unorthodox" electron transport pathways in which electrons cross over from respiratory donors to photosynthetic acceptors, or vice versa [3]. The balance of electron transport routes is crucial for cell physiology [4], yet the factors that control the predominance of particular pathways are poorly understood. Here we use a combination of tagging with green fluorescent protein and confocal fluorescence microscopy in live cells of the cyanobacterium *Synechococcus elongatus* PCC 7942 to investigate the distribution on submicron scales of two key respiratory electron donors, type-I NAD(P)H dehydrogenase (NDH-1) and succinate dehydrogenase (SDH) [5]. When cells are grown under low light, both complexes are concentrated in discrete patches in the thylakoid membranes, about 100–300 nm in diameter and containing tens to hundreds of complexes. Exposure to moderate light leads to redistribution of both NDH-1 and SDH such that they become evenly distributed within the thylakoid membranes. The effects of electron transport inhibitors indicate that redistribution of respiratory complexes is triggered by changes in the redox state of an electron carrier close to plastoquinone. Redistribution does not depend on de novo protein synthesis, and it is accompanied by a major increase in the probability that respiratory electrons are transferred to photosystem I rather than to a terminal oxidase. These results indicate that the distribution of complexes on the scale of 100–300 nm controls the partitioning of reducing power and that redistribution of electron transport complexes on these scales is a physiological mechanism to regulate the pathways of electron flow.

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Identification of potential biomarkers for complex III deficiency by 2D-DIGE proteomic approach

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Mitochondrial complex III deficiency is a relatively infrequent disorder that underlies a wide range of neuromuscular and multi-