

**P014** The role of Glu272 in ubiquinol oxidation of mitochondrial cytochrome  $bc_1$  complex

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The cytochrome  $bc_1$  complex is a dimeric enzyme of the inner mitochondrial membrane that links electron transfer from ubiquinol to cytochrome  $c$  by a protonmotive Q cycle mechanism in which ubiquinol is oxidized at center P in the enzyme, and ubiquinone is rereduced at center N. The mechanism of bifurcated electron transfer upon ubiquinol oxidation and the proton exit routes are still debated. E272 of the conserved PEWY loop of cytochrome  $b$  has been suggested as ligand in the enzyme-substrate complex and as proton acceptor in parallel proton-electron transfer towards heme  $b_L$ . E272D and E272Q mutants support the importance of the residue for correct ubiquinol oxidation, showing effects such as lowered ubiquinol cytochrome  $c$  reductase activity, elevated bypass reactions, and altered  $K_M$  for ubiquinol. However, E272 is not fully conserved. We suggested that in  $\beta$ - and  $\gamma$ -proteobacteria, in which the PEWY glutamate is substituted with valine or proline, a glutamate equivalent to yeast H253 is conserved, which could take over the proton transfer function. To challenge this hypothesis, substitutions of H253 have been constructed with E272 wild type background and also as double mutation with substitutions of E272. Characterization of the variants will be presented.