

Two aspartic acid residues in subunit PSST are essential for catalytic activity in complex I of *Yarrowia lipolytica*.

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Complex I catalyzes electron-transfer from NADH to ubiquinone and translocates protons across the inner mitochondrial membrane. The PSST subunit most likely carries iron-sulfur cluster N2 which has been proposed to play a crucial role in ubiquinone reduction and proton pumping (1-2). To explore the function of this subunit, we have generated site-directed mutants of all highly conserved acidic residues in the PSST subunit of *Yarrowia lipolytica*(3). Mutation D99N has only 5% of the wild type catalytic activity and D115N only 8%. In both cases complex I is stably assembled and no significant change in the EPR spectra of cluster N2 or other iron-sulfur centers was observed. Almost identical results were obtained if the aspartates were changed to glutamate.

In our structural model of the PSST and 49kDa subunits, based on homology to known X-ray structures of [Ni-Fe] hydrogenases (4), the two residues are located not far from cluster N2 at the interface between the two subunits. Remarkably, both are close to D458 of the 49kDa subunit. Mutations at this position exhibit pronounced resistance to hydrophobic inhibitors of complex I (5). We suggest that D99 and D115 play a critical role in the catalytic mechanism of complex I.

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