

***Yarrowia lipolytica*, a yeast genetic system to study mitochondrial complex I**

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The obligate aerobic yeast *Yarrowia lipolytica* is a powerful new model for the structural and functional analysis of mitochondrial complex I. The respiratory chain of *Y. lipolytica* contains complexes I-IV, one single “alternative” NADH-dehydrogenase (NDH2) and a non-heme alternative oxidase (AOX). Because the NADH binding site of NDH2 faces the mitochondrial intermembrane space rather than the matrix, complex I is essential in *Y. lipolytica*. Nevertheless, complex I deletion strains could be generated after redirecting NDH2 to the matrix side by N-terminally attaching the targeting sequence of a matrix protein. To allow fast and efficient purification of complex I by affinity-chromatography, a hexa-histidine tag was C-terminally attached to the NUGM (30 kDa) subunit and the his-tagged allele integrated into the genome by homologous replacement with the resident gene copy. The purified complex I has lost most of its NADH:ubiquinone oxidoreductase activity, but is almost fully reactivated by adding 400-500 molecules of phosphatidylcholine per complex I. The established set of genetic tools has proven useful for the site-directed mutagenesis of individual subunits of *Y. lipolytica* complex I. Characterization of a number of mutations already allowed for the identification of several functionally important amino acids demonstrating the usefulness of this approach.

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