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## Structure Determination of Complex I from *Yarrowia lipolytica*

**Topic:** 7C Oxidative Phosphorylation ; 7B Electron Transfer Systems

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Complex I (NADH-dehydrogenase) is the largest and least understood enzyme in the mitochondrial respiratory chain. It couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. The ~1 MDa eukaryotic enzyme is composed of up to 46 subunits. The strictly aerobic yeast *Yarrowia lipolytica* has been established as a model organism to study eukaryotic complex I. It has an alternative NADH dehydrogenase that has been genetically altered to complement for complex I deficiencies.

In a previous study the catalytic center has been localized in the peripheral region of the matrix arm by immuno-electron microscopy and image processing. This location disfavours functional models that postulate a direct coupling mechanism between electron transfer and proton translocation. The new position, however, would be consistent with a mechanistic model involving conformational changes.

We have determined the three-dimensional structure of complex I from *Y. lipolytica* from electron micrographs of stain embedded samples (ammonium molybdate and phosphotungstic acid). We analyzed large random conical data sets, used extensive classification and calculated the three-dimensional structures of many classes. Complex I shows a high degree of variability. In all reconstructions complex I has a highly detailed matrix arm with globular regions that may represent groups of subunits. A thin connecting link reaching from the protrusion of the 49 kDa subunit to one or two protrusions (connectors) extending from the membrane arm towards the matrix space is common to most classes. The connecting region between the matrix and membrane arm shows an opening that may form the access channel for ubiquinon. The major variations observed are the flexibility of the link between the 49 kDa subunit and the membrane arm and variations in the orientation and size of the connectors.

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