MONITORING OF COMPLEX I SUPEROXIDE GENERATION BY USE OF
CONFOCAL MICROSCOPY

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Mitochondria belong to a few primary sources of superoxide and hence contribute significantly to reactive oxygen species (ROS) production in the cell. ROS were implicated in determining an organism’s lifespan, aging, and pathology of many diseases. It is also important to have reliable tools for in situ monitoring of certain mitochondrial ROS. We have developed a new method based on confocal microscopy and fluorescent probe MitoSOX for monitoring the excessive superoxide (over MnSOD) production released to the mitochondrial matrix of in situ HEPG2 cells (Jm).

We attempted to characterize this superoxide release by Complex I under the conditions of retarded forward electron transport and retarded proton pumping. Inhibition of electron transport by rotenone significantly increased Jm; uncoupler attenuated it back to the basal levels, but not when proton pumping was inhibited. Thus, maximum superoxide formation proceeds when simultaneous inhibition of electron transport and proton pumping (by inhibition or by high potential) takes place.

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