Analysis of mitochondrial DNA (mtDNA) replication intermediates (RI) by two-dimensional (2D-AGE) agarose gel electrophoresis in *Saccharomyces cerevisiae* rho– mutants

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**Keywords:**

mitochondrial DNA, replication

**Abstract Body:**

*S. cerevisiae* can survive complete loss or extensive alterations of its mtDNA when a fermentable carbon source is provided. Mutants carrying small fragments of mt genome are called rho–. Rho– mtDNA is maintained as a short concatemeric remnant sequence, amplified to reach approximately the mass of wild type mtDNA. Some rho– mutants, called hypersuppressive (HS), retain an active ori element in their mtDNA. Competing transcription or recombination based models have been proposed to explain the priming of rho– mtDNA replication. The present work describes topological analyses of mtDNA in HS rho– strain by 2D-AGE. Importantly, deletion of RNA polymerase RPO41 did not change the structures of mtDNA intermediates downplaying the role of transcription. We also analyze specific changes in HS rho– mtDNA RI associated with helicases Pif1, Hmi1 and Irc3; recombinase Mhr1, resolvase Cce1, DNA packaging protein Abf2 and Mgm101. Our analyses did not reveal bubble structures associated with specific replication origins and the theta mode of replication. We detected Y-shaped (replication forks), X-shaped (Holliday junctions), complex branched molecules and structures containing ssDNA. Abundant recombination structures in the form of X-arcs and complex branched structures support the role of recombination in rho– mtDNA replication. We also detected σ-like molecules indicating that the rolling circle replication mode is possibly used for mtDNA propagation.