

Division versus Fusion: Dnm1p and Fzo1p Antagonistically Regulate Mitochondrial Shape

Hiromi Sesaki & Robert E. Jensen

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Background: Mitochondrial Fusion & Division

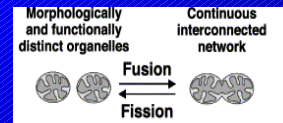


Figure adapted from: Westermann B. Mitochondrial membrane fusion. Biochim Biophys Acta. 2003 Aug 18; 1641 (2-3): 195-202.

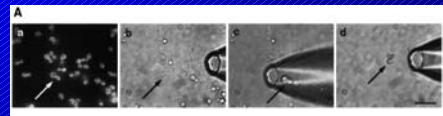
- Split & fuse continuously
- Part of normal growth & reproduction
- Can change shape & move around cell
- Prior study identified yeast homologue to FZO1, involved in mitochondrial fusion

Purpose of this Study

- Identify gene(s) controlling mitochondrial division
- Explore relationship between division & fusion

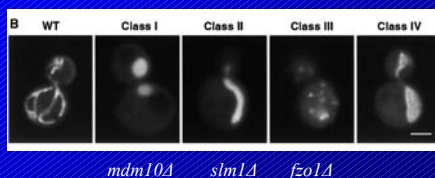
Methods: Mutant Screen

- Constructed strain YHS2
 - Under ADH1 promoter, expresses GFP+Cox4p (binds to mitochondrial matrix)
- Mutagenized with Ethane Methyl sulfate
- Screened & isolated mutants

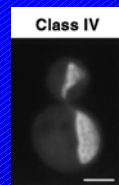


Methods: Mutant Screen

- Characterized & Classified Mutants



Closer Look at Class IV Mutants

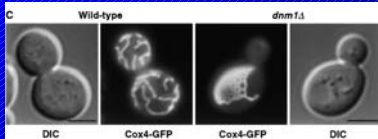


Mapping Study: all Class IV mutations linked to region of Chromosome XII centromere

****DNM1 maps to same location****

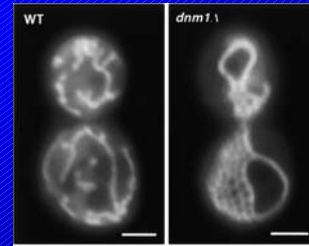
Class IV = *dnm1Δ*?

- Crossed Class IV cells to *dnm1Δ* strain
- Used plasmid with DNM1 to rescue phenotype in Class IV mutants
- Performed gene disruption of DNM1



Conclusion: All Class IV mutants are alleles of *dnm1Δ*

Mitochondrial Division in Budding Yeast



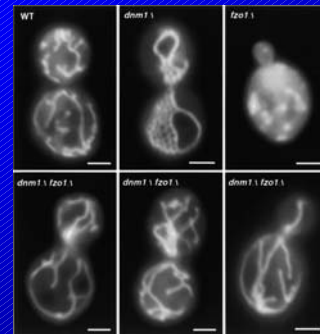
Observation: *dnm1Δ* Cells Are Defective in Mitochondrial Division

Fusion Vs. Division

- Observation: equilibrium = inverse relationship
- Prior study: *fzo1Δ* = fragmented mitochondria
- Idea: blocked division = fewer, larger mitochondria

****DNM1 controls division****

Fusion Vs. Division

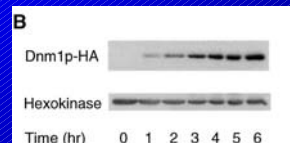


Dnm1p Vs. Mitochondrial Form

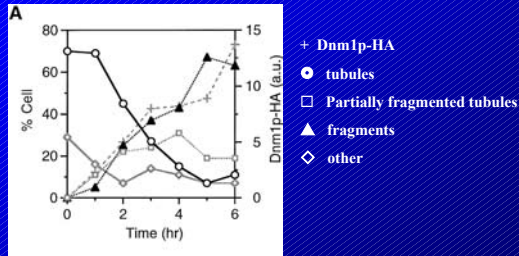
- Expressed Dnm1p in *dnm1Δfzo1Δ* cells
 - Induced expression of Dnm1p+HA epitope under GAL1 promoter
- Results: *dnm1Δfzo1Δ* cells
 - With galactose: rescued *dnm1Δ* phenotype
 - Without: *dnm1Δfzo1Δ* phenotype

Dnm1p Vs. Mitochondrial Form

- Moved cells from (-)gal to (+)gal
- Result: increased levels of Dnm1p



Dnm1p Vs. Mitochondrial Form

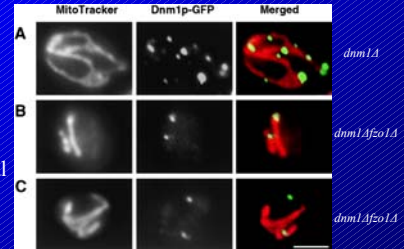


Localization of Dnm1p

- Traced Dnm1p+GFP in cells stained w/ MitoTracker

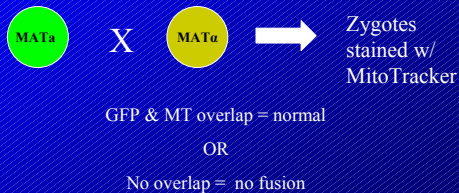
Results:

Dnm1p localizes to sites of mitochondrial division

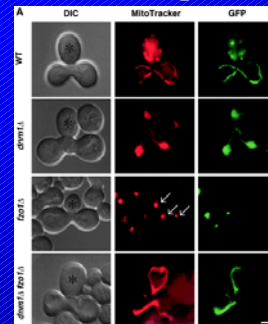


Dnm1p Vs. Fusion

- Suspicion: Double mutants have normal phenotype because Dnm1p blocks fusion
- Experiment: tracked fusion in mating cells



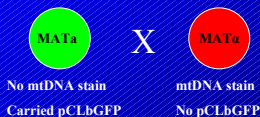
Dnm1p Vs. Fusion



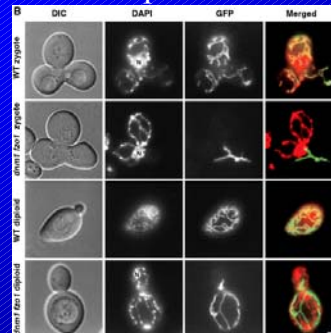
- Conclusion: *dnm1Δfzo1Δ* cells are deficient in mitochondrial fusion

Dnm1p Vs. Fusion

- Confirmed results with more sensitive assay
- Used pCLbGFP to visualize mitochondrial matrix
- DAPI stained mtDNA instead of using MitoTracker



Dnm1p Vs. Fusion



Conclusions

- DNM1 controls mitochondrial division
- *dnm1Δfzo1Δ* cells are deficient in both fusion & division
- DNM1 & FZO1 create equilibrium

