The Importance of Mitochondrial Morphology in *Saccharomyces cerevisiae*

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Objective

- To investigate how mitochondria in the yeast *Saccharomyces cerevisiae* interact with the endoplasmic reticulum and if this interaction effects mitochondrial morphology
- Specifically, see how the Mmm1 and Fzo1 proteins mediate these interactions

Background

- Mitochondria and endoplasmic reticulum interactions
  - Endoplasmic reticulum-mitochondrial encounter structure (ERMES)
  - Possible functions: phospholipid exchange, Ca+2 exchange, mitochondrial protein import, mtDNA maintenance

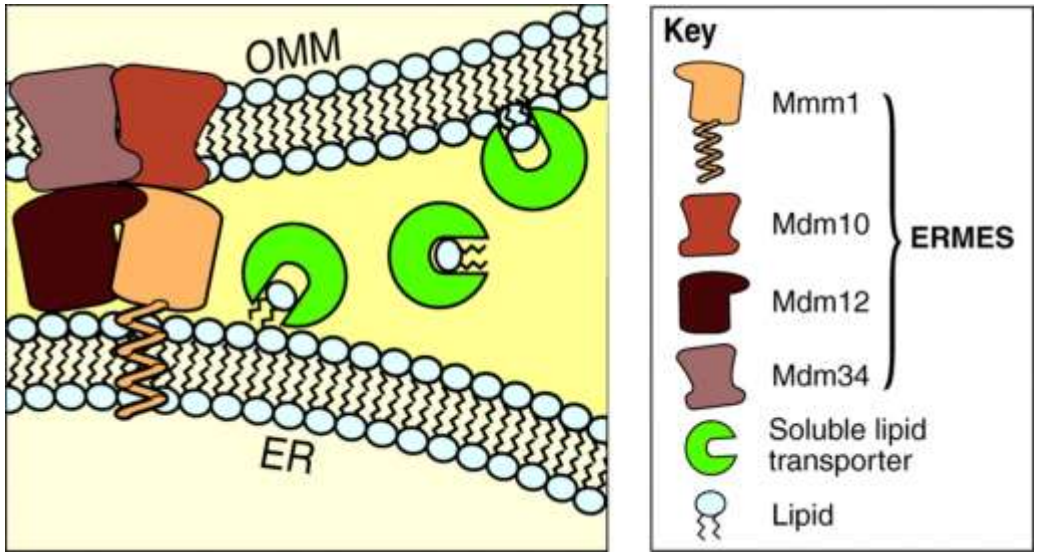
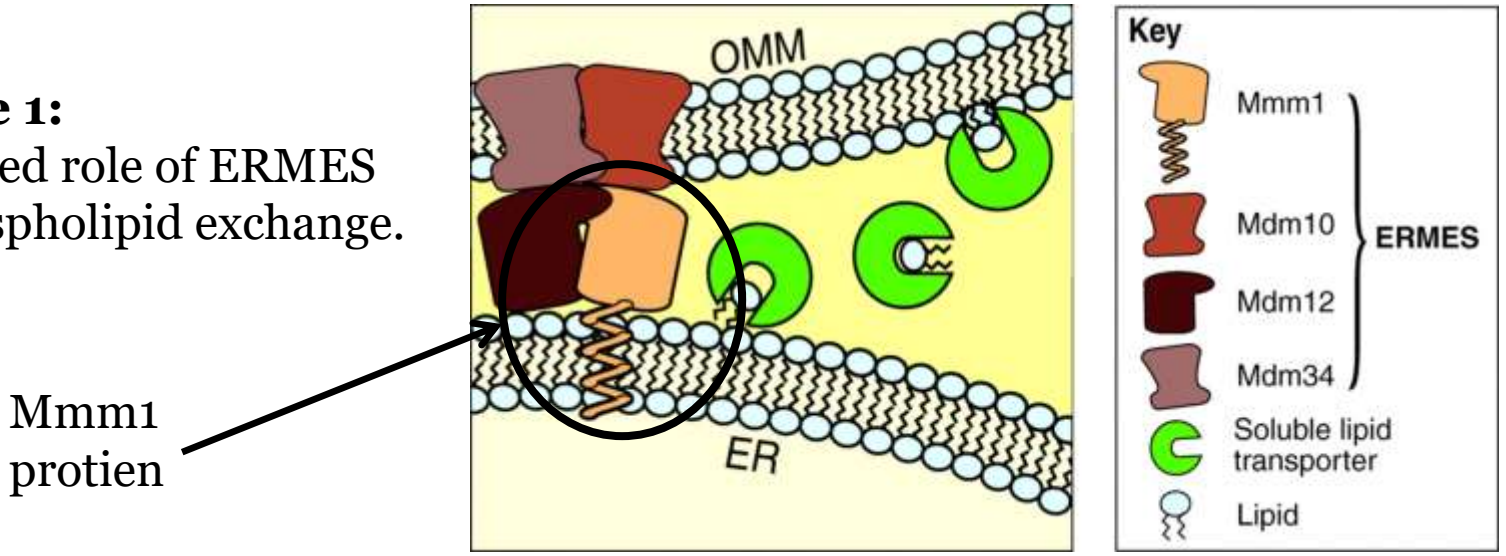


Figure 1
Proposed role of ERMES in phospholipid exchange.

Kornmann B , Walter P J Cell Sci 2010;123:1389-1393

- **Mmm1**
  - Maintenance of mitochondrial morphology protein 1
  - Part of the Endoplasmic reticulum-mitochondrial encounter structure (ERMES)

Figure 1:
Proposed role of ERMES in phospholipid exchange.



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- **Fzo1**
  - Fuzzy onion 1 protein
  - High molecular-mass transmembrane protein
  - Function in mitochondrial fusion in budding yeast
  - May also play a role in the mitochondrial-endoplasmic reticulum fusion

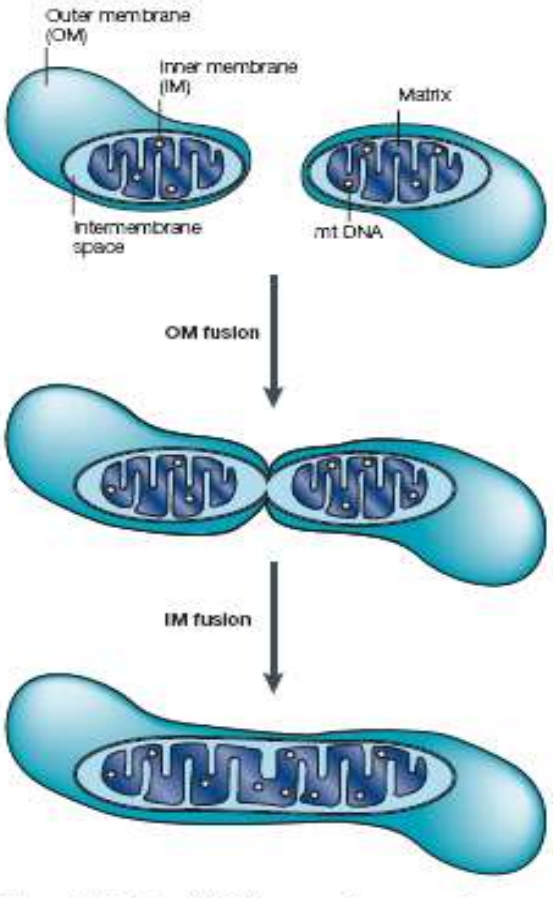

Figure 2:
Mitochondrial fusion

Mozdy, A.D. and Shaw, J.M. 2003 *Nature Reviews Molecular Cell Biology* **4**, 468-478

Method

- To see the mitochondrial morphology, transformed the TOM 70 GFP fusion into the yeast strains of interest
- TOM70 on the outer mitochondrial membrane
- GFP fluoresces under green light so can view with a fluorescence microscope

Yeast Strains Used

- **KDY 8 and 9**: The a and α strains containing DNA with the TOM70 GFP with a ura marker
- **THY23**: mmm1 deletion strain
- **KWY121**: fzo1 point mutant
- **KWY79**: fzo1 point mutant with an mmm1 deletion

Previous work on these strains

- **THY23:**
  - Significantly reduced growth on YPEG, loss of respiration
  - Loss of mtDNA
- **KWY121:**
  - Reduced growth on YPEG, reduced respiration
  - Some mtDNA loss
- **KWY79:**
  - Normal growth on YPEG, like wildtype, revertance of respiration
  - Retention of some mtDNA

Procedure

- Isolated genomic DNA from KDY8 and KDY10
- PCR
  - primers flanking 3.5 kb TOM70 GFP Ura 3
  - Taq Polymerase, 47^{0}C annealing temperature
- DNA gel electrophoresis
  - Verify product size of 3.1 kb
- Transform PCR into THY23, KWY121, KWY79
- Select cells using medium that doesn't contain uracil
- Viewed cells with a fluorescence microscope

Results: PCR Product on DNA gel

- 0.8% Agarose gel with ethidium bromide
- λ hindIII weight markers
- Product size 3.5 kb
- Confirms size was correct
- No evidence of contaminants

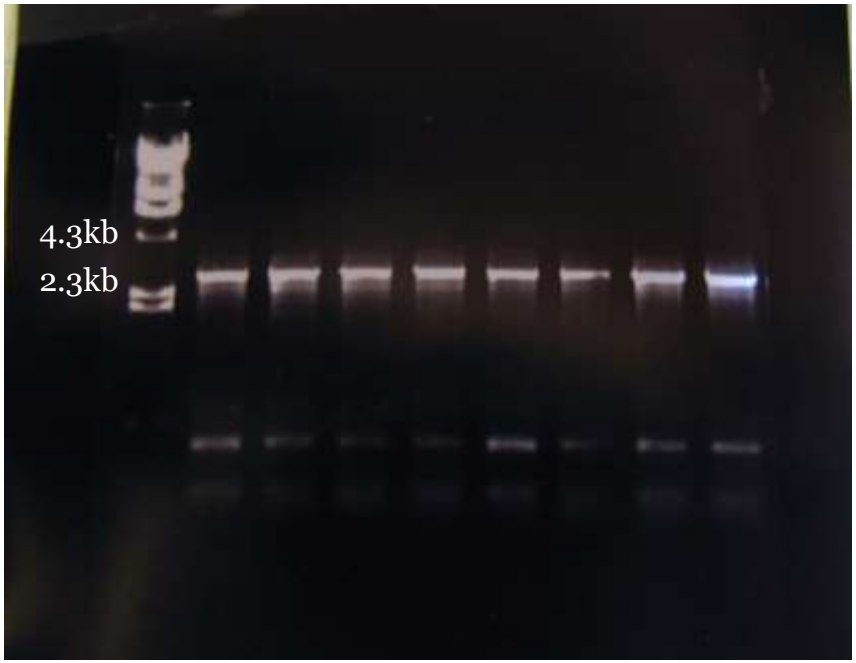

Figure 3:
PCR product DNA size determination using electrophoresis gel

Results: Strain mitochondrial morphology

All strains were successfully transformed with theTOM70 GFP marker and all strains successfully fluoresced under green light stimulation

- **THY23:**
  - Mitochondria were concentrated into a few round shaped clumps or one smooth round clump
- **KWY121:**
  - A more extended network with multiple fragmented mitochondria
- **KWY79:**
  - Condensed regions of mitochondria but clumps were smaller and more abundant than seen in THY23

Discussion of Strain Mitochondrial Morphologies

- The mmm1 deletion strain had a loss of the extended mitochondrial morphology usually seen in wildtype cells
- The fzo1 point mutant strain showed only a slight loss of the extended network
- The mmm1 deletion and fzo1 point mutant strain also lost the extended network but to a lesser extent than the mmm1 deletion strain

Comparing Respiration, mtDNA Retention and Mitochondrial Morphology

Strain	Respiration	mtDNA retention	Mitochondrial Morphology
Mmm1 deletion	Complete loss of Respiration	Loss of mtDNA	Complete loss of wildtype mitochondrial morphology
Fzo1 Point Mutant	Some loss of Respiration	Some mtDNA loss	Some loss of wildtype mitochondrial morphology
Mmm1 deletion with Fzo1 Point Mutation	Less loss of Respiration that the Mmm1 deletion	Less mtDNA loss than Mmm1 deletion	Less loss of wildtype mitochondrial morphology than Mmm1 deletion

- The changes in mitochondrial morphology that occurred in these strains correlate to the way these strains lost mtDNA and ability to respire
- Revertance seen with respect to mtDNA loss and ability to respire for the mmm1 deletion with the fzo1 point mutant correlates to revertance in the mitochondrial morphology

Discussion of the GFP method

- Marking the outer mitochondrial membrane TOM protein and not the mitochondrial matrix was a successful way to visualize the mitochondrial morphology of these strains
- If GFP had marked the interior of mitochondria, it would have been difficult to visualize because those cells that lack mtDNA do not import proteins well

Conclusion

- There appears to be a correlation of mtDNA retention and ability to respire to mitochondrial morphology
- Further research must be conducted to better understand the relationship in function between fzo1 and mmm1
- Putting the GFP marker on the outer mitochondrial membrane method proved to be a good way to look at mitochondrial morphology and this can now be applied to other strains lacking mtDNA to assess their mitochondrial morphology

References

- **Kornmann, B. and Walter, P.** (2010) ERMES-mediated ER-mitochondria contacts: molecular hubs for the regulation of mitochondrial biology. *J. Cell Science* **123**, 1389-1393.

- **Mozdy, A.D. and Shaw, J.M**. (2003) A fuzzy mitochondrial fusion apparatus comes into focus. *Nature Reviews Molecular Cell Biology* **4**, 468-478