Mitochondrial inheritance in *Saccharomyces cerevisiae* septin mutants

Kala Anderson, Sophia Porras, Sonia Giyanani, Nisha Giyanani, Matthew Goldstein and Patricia Melloy

Department of Biological and Allied Health Sciences, Fairleigh Dickinson University, Madison, NJ 07940

Introduction:

This study focuses on the potential role of septins in controlling organelle inheritance, specifically mitochondrial inheritance. Septins are essential for cytokinesis and are known to regulate many events required at the completion of cell division. The five mitotic septins (Cdc3p, Cdc10p, Cdc11p, Cdc12p, and Shs1p) form a filamentous ring that can act as a scaffold at the bud neck in *Saccharomyces cerevisiae* (for reviews: Gladfelter, et al., 2001; Marquardt, et al., 2019; Oh and Bi, 2011). Temperature-sensitive mutants of septin genes were first discovered in the original screen for cell division cycle mutants in budding yeast (Hartwell, et al., 1970; Hartwell, 1971). Mitochondrial inheritance in wild type yeast cells is controlled by a number of proteins regulating mitochondrial dynamics and mitochondrial transport along the actin cytoskeleton into the new bud (Peraza-Reyes, et al., 2010; Vevea, et al., 2014; Westermann, 2014). In this study, we use the temperature-sensitive mutants, *cdc3*, *cdc11*, and *cdc12*, to examine changes in mitochondrial inheritance and morphology occurring at non-permissive temperature to determine if the septin ring is required for proper mitochondrial inheritance.

Materials and Methods

Yeast media

Yeast strains were maintained on either YPD (YPD- Yeast Peptone Dextrose Media) or SD-TRP (SD-Synthetic defined media) agar plates at room temperature. Liquid cultures were grown overnight before imaging the next day on the fluorescence microscope. The absorbance was taken in a spectrophotometer to make sure the yeast growth was in mid-logarithmic phase. To observe cells at a non-permissive temperature, cells were grown at 23 degrees overnight and then shifted to 37 for 2 hours unless otherwise noted. We also tested the growth of our yeast strains on different carbon sources in which we used YPEG (YPEG-Yeast Peptone Ethanol and Glycerol) and YPD (YPD- Yeast Peptone Dextrose Media).

Mitochondrial staining

The mitochondria were observed by staining using MitoTracker Red. This fluorescent dye is commonly used for staining mitochondria in live cells (Catalogue #M7512, Life Technology/Fisher, Eugene, OR). MitoTracker was used at a final concentration of 15 nM. The cells were grown for 45 minutes in the dye and were washed three times with SD-TRP media before observing. The initial protocol for MitoTracker staining of yeast cells was found in Juneau, et al., 2009. Several MitoTracker concentrations were tested to find the lowest effective concentration for staining.

Microscopy

The prepared microscope slide with MitoTracker was observed using a DM5500 Leica Fluorescence Microscopy System (Buffalo Grove, IL). A Hamamatsu ORCA-ER camera (Bridgewater, NJ) was used for imaging.

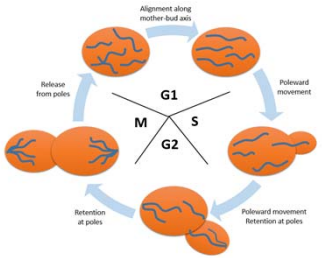

Figure 1: The mitochondrial inheritance cycle of budding yeast

During the G1 phase of the cell cycle, mitochondria align along the mother-bud axis and orient toward the site of bud emergence. From S phase through mitosis, mitochondria move linearly toward the tip of the bud or mother cell. Mitochondria are retained at the tip of the bud or mother cell until the end of the cycle -- where they are released and distributed evenly throughout the cytoplasm. Graphic inspired by Boldogh, et al., 2001

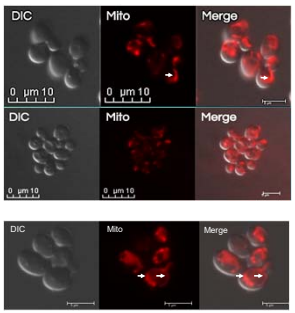

Figure 2: Localization of mitochondria in wild type cells

MitoTracker staining was optimized in wild type cells before conducting experiments using mutants. Shown here are cells stained with either 30nM (top row) or 60nM (middle row) MitoTracker dye. Our working concentration was eventually lowered to 15nM. Staining was optimized to prevent vacuolar staining and staining artifacts. MitoTracker staining was compared to mitochondrial localization using a CFP marker, TRX3-CFP, data not shown. This fluorescent marker was not bright enough to use on its own for experiments. In the bottom row, wild type cells were imaged after shifting to an elevated temperature, 37°C. White arrows indicate mitochondria near the cell cortex or bud neck.

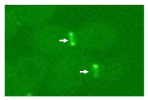

Figure 3: Localization of septin ring in budding yeast (arrows)

Hypothesis: Mitochondrial distribution, inheritance and morphology will be disrupted in septin mutants

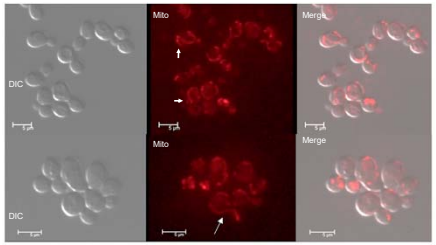

Figure 4: Mitochondrial localization in *cdc12-6* septin mutant at permissive temperature

Shown are normal filamentous mitochondria networks. Arrows indicate normal localization of mitochondria near the cell cortex

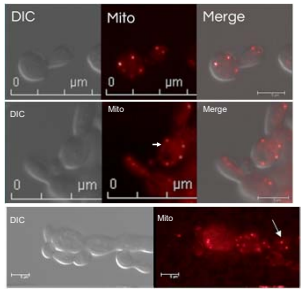

Figure 5: Localization of mitochondria in *cdc12-6* mutant at non-permissive temperature (37°C)

Abnormal mitochondrial localization is seen. Instead of the typical filamentous network of mitochondria, there are punctate spots of mitochondria as indicated by the white arrows.

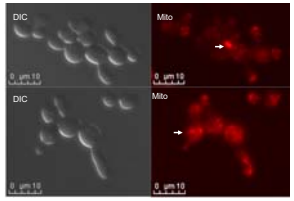

Figure 6: Localization of mitochondria in the *cdc3-3* septin mutant grown at elevated temperature (37°C)

Abnormal mitochondrial localization is seen. Instead of the typical filamentous network of mitochondria, there are punctate spots of mitochondria as indicated by the white arrows.

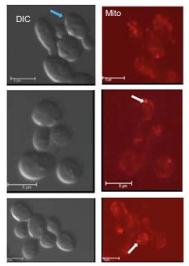

Figure 7: Mitochondrial localization of the septin mutant *cdc11* at non-permissive temperature (37°C)

Shown is an abnormal mitochondrial localization and yeast shape. The blue arrow indicates the yeast bud's spoon-shaped distortion due to a failure of cytokinesis. The white arrows indicate punctuated mitochondrial dots.

Table 1: Mitochondrial localization patterns based on cell cycle stage in wild type yeast at elevated temperatures (37°C).

Categories of mitochondrial localization	G1 Phase	S Phase	G2/M Phase
Mitochondria concentrated near bud neck	0	0	0
Mitochondria concentrated near cell cortex	29 (54%)	1 (4%)	9 (22%)
Mitochondria spread evenly throughout the cell body	22 (41%)	0	15 (37%)
Mitochondria localized to the bud neck and cell cortex	0	8 (30%)	2 (5%)
Mitochondria at bud neck with a trail to the cell cortex across the cell body	0	16 (60%)	12 (29%)
Abnormal, punctate localization (> 1 spot)	3 (6%)	2 (7%)	3 (7%)
TOTAL CELLS	54	27	41

Table 2: Mitochondrial localization patterns based on cell cycle stage in septin mutants (*CDC11-6*) at elevated temperatures (37°C).

Categories of mitochondrial localization	G1 Phase	S Phase	G2/M Phase	One projection	Two or more projections
Mitochondria concentrated near bud neck	0	1 (0.76%)	0	0	0
Mitochondria concentrated near cell cortex	3 (2.27%)	1 (0.76%)	0	1 (0.76%)	0
Mitochondria localized to bud neck and cell cortex	0	4 (3.03%)	0	0	0
Mitochondria at bud neck with a trail to the cell cortex across the cell body	1 (0.76%)	5 (3.79%)	0	2 (1.52%)	0
Mitochondria spread evenly throughout the cell body	10 (7.58%)	3 (2.38)	0	10 (7.58%)	0
Abnormal, punctate localization (> 1 spot)	5 (3.78%)	10 (7.56%)	3 (2.27%)	55 (41.7%)	18 (13.6%)
TOTAL CELLS IN EXPERIMENT	132				

Tables 1 and 2: Cell counts of different mitochondrial localization patterns seen in wild type (Table 1) and *cdc12-6* septin mutant cells (Table 2) grown at 37°C. Cells were categorized based on cell cycle stage.

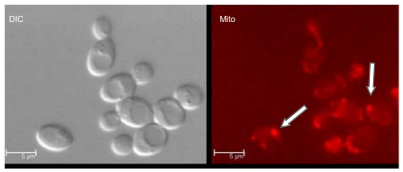

Figure 8: Mitochondrial staining of the mitochondrial fission mutant *mgm1-5* grown at elevated temperature (37°C) for 45 minutes

To determine if the punctate spots of MitoTracker staining seen in our septin mutants are comparable to mitochondrial fragments seen in known mitochondrial mutants, we looked at the mutant *mgm1-5*. Arrows indicate fragmented mitochondria forming aggregates, similar to phenotype seen in septin mutants. The *mgm1-5* strain was a gift from Dr. Jodi Nunnari and is described in Wong, et al., 2000.

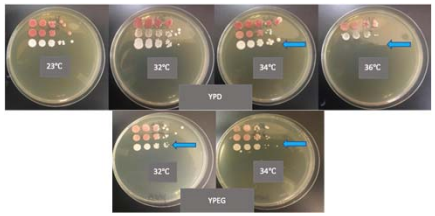

Figure 9: Serial dilutions to test yeast growth on different carbon sources

Photos of four different yeast strains on agar plates at the temperatures of 23°C,32°C, 34°C and 36°C on YPD plates (top row) and 32°C and 34°C on YPEG (bottom row) are shown. In the plates, the top row shows the wild type and the second row shows the *mgm1-5*, followed by *cdc11* in the third row. All strains grew similarly on the YPD and YPEG media, except for the *cdc11* strain which failed to grown at 36°C (blue arrow). We plan to repeat this experiment at a broader range of temperatures and using replica plating.

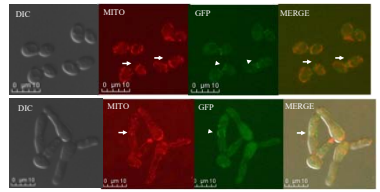

Figure 10: Comparison of localization of mitochondria and septin ring during cell division (using *cdc12-6* mutant)

Mitochondria were observed using the MitoTracker stain (white arrows) and the septin ring was visualized using SHS1-GFP (arrowheads, septin strains from Dr Erfei Bi). Note the proximity of the mitochondria to the septin ring. Cells were grown at permissive temperature (top row) or at 33°C (bottom row)

Summary:

- At non-permissive temperature, septin mutants displayed abnormalities in their mitochondrial morphology in the form of punctate spots rather than the normal reticular network near the cell cortex
- These mitochondrial abnormalities looked similar to the fragmentation and aggregation of mitochondria seen in the *mgm1-5* mutant

Future directions:

- Complete comparison of mother /daughter localization of mitochondria in wild type versus septin mutant yeast
- Complete time-lapse analysis (Figure 10) following septin ring and mitochondria
- Examine mitochondria in time-lapse analysis after longer incubation of septin mutants at non-permissive temperature (example: after 3, 4, 5 hours at 37°C)
- Retesting growth of strains on glycerol plates using replica plating technique or another method to examine mitochondrial function

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