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Mitochondrial Network Size Scaling in Budding Yeast

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Mitochondria must grow with the growing cell to ensure proper cellular physiology and inheritance upon division. We measured the physical size of mitochondrial networks in budding yeast and found that mitochondrial network size increased with increasing cell size and that this scaling relation occurred primarily in the bud. The mitochondria–to–cell size ratio continually decreased in aging mothers over successive generations. However, regardless of the mother’s age or mitochondrial content, all buds attained the same average ratio. Thus, yeast populations achieve a stable scaling relation between mitochondrial content and cell size despite asymmetry in inheritance.

The amount of mitochondria in the cell varies in response to metabolic demands (1), requiring active size regulation mechanisms. The mitochondrial to cell size ratio is relatively constant in mammalian cells (2) and two yeast species (3, 4). We used the budding yeast Saccharomyces cerevisiae to study how the relationship between mitochondrial and cell size is achieved in cells growing and dividing asymmetrically (fig. S1). Yeast mitochondria are three-dimensional (3D) networks of dynamic membrane-bound tubules localized at the cell periphery (5). To study mitochondrial size scaling, we developed a method to quantify the 3D skeletons of mitochondrial networks (Fig. 1A and fig. S2) using spinning disk confocal z-stacks of live yeast cells expressing mitochondrial matrix-targeted green fluorescent protein (GFP). We converted network length to mitochondrial volume (μm³), assuming a constant tubule diameter. Absolute network length accuracy was within 85% of manual measurements, with an average reproducibility of 96% (6).

We imaged time courses of live yeast cells growing for one to two generations and measured the total cell and mitochondrial volumes for mother and bud compartments. We found a strong correlation between total mitochondrial and cell volumes (Fig. 1B). However, when we compared mother and bud compartments separately, the constant mitochondrial volume ratio found in the population as a whole was dominated by the scaling in the bud (Fig. 1, C and D). Mitochondrial volume ratio in the buds increased and then

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leveled off when buds were about half their final size (Fig. 1E). Bud size reflects the progression of budding, suggesting that buds accumulate mitochondria until they reach a set point of mitochondrial volume relative to bud size. In contrast, in mothers the mitochondrial volume ratio decreased with increasing cell size (Fig. 1F). Mother size reflects generational age, suggesting that old mothers do not preserve the mitochondrial volume ratio set point that they inherited when they were buds.

Analysis of the dynamics of cell growth and mitochondrial accumulation during budding time courses revealed dramatic asymmetry in mitochondrial accumulation between mother and bud (Fig. 2). During budding, mothers experienced an overall loss of both mitochondrial volume and the resultant volume ratio, while at the same time mitochondrial content increased in the bud proportional to bud growth (Fig. 2 and figs. S3 and S4). The mitochondrial accumulation rate in mothers was most negative at the moment that it was greatest in the bud (fig. S3B), suggesting that the bud might gain mitochondria at the expense of its mother. Although first and 2+ generation mothers differed significantly in cell size and mitochondrial content at the start of budding, the average kinetics of both bud growth and mitochondrial accumulation in their buds was indistinguishable (Fig. 2C and fig. S3), resulting in the same average volume ratios at division (Fig. 2D). Thus, regardless of the initial size or mitochondrial content of mothers, they generate, on average, identical buds and do so with the same dynamics throughout budding.

Yeast cells displayed “mitochondrial content asymmetry” with a lower mitochondrial volume ratio in the mother than in the bud upon division (Fig. 2D). We asked whether mitochondrial content is replenished during the G1, unbounded, phase of the cell cycle. The mitochondrial volume ratio tended to increase for cells with lower, and decrease for cells with higher, initial volume ratios (Fig. 3A and fig. S5), suggesting a partial homeostatic restoration toward a unique mitochondrial volume ratio set point. We found no evidence that cells delayed G1 exit in response to a decreased volume ratio (fig. S6), but instead that cells may respond to deviations in the appropriate volume ratio by adjusting their rate of mitochondrial biogenesis (Fig. 3B and fig. S7). Analysis of mitochondrial content versus generational age (Fig. 3C) showed that sequential loss during budding and partial regain during G1 resulted in continual loss of mitochondrial volume ratio in aging mother cells (Fig. 3D and figs. S8 and S9). However, these much older mothers still generated buds with the same average volume ratio at division. By generating buds with identical mitochondrial content, the population can renew and maintain a narrow distribution of mitochondrial volume ratios despite asymmetry in the inheritance of mitochondrial content between mother and bud (fig. S10).

There are two possibilities for how proper mitochondrial volume ratio is achieved in the bud: a passive mechanism not modulated as a function of mitochondrial content, where scaling would arise inherently [e.g., allometric growth (8)], or an active mechanism capable of sensing and feedback. In the passive case, a delay in mitochondrial inheritance would lead to fewer mitochondria entering the bud and a decreased volume ratio in the newborn mothers. In the active case, cells could sense and compensate for the delay to achieve the target volume ratio in the bud upon division. In △ptl1 mutants, which exhibit delayed appearance of mitochondria in the bud (6, 9, 10), we found that while buds were delayed in mitochondrial inheritance (figs. S11 and S12), their volume ratio increased rapidly such that volume ratios in △ptl1 and wild-type buds were indistinguishable at division (Fig. 4, A and B), consistent with an active mechanism. Meanwhile,
mother mitochondrial content increased transiently, presumably because mitochondria could not be redistributed into the buds. Mother-daughter mitochondrial content asymmetry was thus eliminated in the absence, and was enhanced with overexpression, of Ypt11p (Fig. 4C), suggesting that Ypt11p contributes to this asymmetry. However, in all three cases, the proper volume ratio was achieved in the bud at division. We thus propose that yeast cells can somehow sense mitochondrial accumulation kinetics in the bud and respond to ensure that the target volume ratio is achieved in time for division. The compensatory behavior observed in Δypt11 mutants likely involves one or both of the remaining mitochondrial inheritance pathways (6, 10), which themselves may also contribute to mother-daughter mitochondrial content asymmetry.

Δypt11 buds could potentially reach the proper mitochondrial volume ratio by slowing their growth and “waiting” for the delayed mitochondrial content to catch up. We analyzed time courses of Δypt11 cells and found that buds indeed grew at slower rates and for a longer time before division (Fig. 4D). Mutants with the longest budding durations exhibited greatly decreased mitochondrial volume ratios at the time when normal Δypt11 cells would divide (Fig. S13). However, Δypt11 cells divided with buds of a smaller size than wild-type buds, suggesting, unexpectedly, that cell size may be affected by mitochondrial inheritance kinetics. Yeast cell size is known to respond to changes in metabolism brought about by nutritional environment (11), suggesting perhaps that a delay in mitochondrial inheritance somehow altered the cell’s metabolic state even though its environment remained unchanged.

We tested whether simply delaying redistribution of mitochondria into the bud could alter the age-dependent loss of volume ratio observed in wild-type mothers (Fig. 3D). Aging Δypt11 mothers lost mitochondrial volume ratio much more slowly than wild-type mothers (Fig. 4E and fig. S14). Replicative life-span distributions of Δypt11 mutants (6) were similar to Δmmr1 mitochondrial inheritance mutants (12). The Δypt11 distribution was statistically bimodal (6), consisting of a shorter-lived population, not seen in wild type, and a longer-lived population with both a slightly increased average and significantly increased maximum life span compared with wild type (Fig. 4F and fig. S15) (6). Because of their decreased life spans, short-lived Δypt11 cells represent a decreasing proportion of the aging Δypt11 population (e.g., 19 and 5% of cells at generations 6 and 10). Thus, the Δypt11 cells with increased mitochondrial volume ratios at later generations consisted almost entirely of the second population with a longer life span than wild-type cells. Thus, in addition to the quality of mitochondria retained in the mother (12), their quantity may also contribute to their replicative life spans.

The failure of mother cells to maintain mitochondrial volume ratio suggests that they either cannot sense reduced content or are unable to compensate for the loss. Failing to preserve her own mitochondrial volume ratio could be the cost incurred for generating the healthiest, fittest offspring upon division.

References and Notes
6. Materials and methods are available as supplementary materials on Science Online.

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Supplementary Materials
www.sciencemag.org/cgi/content/full/338/6108/822/DC1
Materials and Methods
Figs. S1 to S21
References (12–32)
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