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Mitochondria Tether Protein Trash to Rejuvenate Cellular Environments

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Protein damage segregates asymmetrically in dividing yeast cells, rejuvenating daughters at the expense of mother cells. Zhou et al. now show that newly synthesized proteins are particularly prone to aggregation and describe a mechanism that tethers aggregated proteins to mitochondria. This association constrains aggregate mobility, effectively retaining and sorting toxic aggregates away from younger cells.

Accumulation of damaged macromolecules accompanies aging in all organisms and is considered a major factor contributing to degeneration of cells and tissues. Asymmetric distribution of molecular debris during cell division represents a conserved cellular strategy limiting the spread of protein damage over time and minimizing levels of damage inherited by younger cells. This process is best studied in the baker's yeast *Saccharomyces cerevisiae*, in which asymmetric segregation during bud formation is documented for extrachromosomal rDNA circles (ERCs), oxidatively damaged (carbonylated) proteins, and protein aggregates composed of misfolded conformers. These species accumulate in aged cells or during proteotoxic stress. Pioneering work by the Nystrom group shows that carbonylated proteins and protein aggregates are largely retained in mother cells, freeing younger cells (daughter buds) of damage and resetting replicative potential in the bud (Aguilaniu et al., 2003; Liu et al., 2010). Disruption of asymmetric damage inheritance in yeast mutants lacking either the Sirtuin Sir2, a factor centrally involved in aging, or the disaggregase Hsp104, which efficiently resolves aggregates after stress, correlates with shorter replicative lifespan, reflected by fewer cell divisions prior to senescence (Erjavec et al., 2007). Asymmetric segregation of cellular trash is therefore linked, though indirectly, to daughter rejuvenation at the expense of the older mother cell. In this issue, Zhou et al. (2014) describe a direct link between protein trash sorting and mitochondrial inheritance.

The mechanism of damage retention has been the focus of intense research and controversy, resulting in conflicting models. In one model, the retention of carbonylated (Erjavec et al., 2007; Tessarz et al., 2009) and aggregated (Liu et al., 2010) proteins relies on a functional actin cytoskeleton. Nystrom and coworkers show that protein aggregates are tethered to the actin cytoskeleton. Retrograde flow of actin cables, nucleating at the polarisome at the tip of daughter cells, either prevents aggregates entering the bud or clears the bud from aggregates. This is an active process involving the actin-associated motor protein Myo2 and Hsp104 (Liu et al., 2010; Song et al., 2014) (Figure 1). Li and coworkers, however, used quantitative particle tracking in yeast cells and failed to show any directional bias in aggregate movement (Zhou et al., 2011). Instead, they observed a random walk of aggregates with some degree of confinement. The inference is that the constrained mobility of aggregates and narrowness of the bud neck combined are sufficient to retain aggregated proteins in the mother cell, suggesting an essentially passive process. The work published by Zhou et al. now broaches two related key issues: first, the source of aggregating proteins and second, the basis of confined aggregate mobility.

This work pinpoints newly synthesized proteins that, upon proteotoxic stress, become tethered to mitochondria (and initially also the endoplasmic reticulum) for retention in the mother cell. The experimental model used involves protein aggregation triggered by heat shock and visualized by the GFP-tagged disaggregase Hsp104, which allows the tracking of endogenous aggregates while dispensing with specific misfolded protein reporters. Remarkably, inhibition of protein synthesis abrogates formation of detectable aggregates, even in the case of thermolabile proteins that misfold at increased temperatures. This indicates that newly synthesized proteins are particularly vulnerable to proteotoxic stress, represent a major source of aggregating protein species, and determine the site of initial aggregation of unstable proteins.

With regard to aggregate mobility, fluorescence microscopy and serial sectioning electron microscopy analyses carried out by Zhou et al. reveal that protein aggregates specifically associate with endoplasmic reticulum (ER) and mitochondria. Most protein aggregates (90%) appear to form initially at the surface of the ER. Here, aggregates also become linked to mitochondria and frequently appear first at ER-mitochondria contact sites distinct from ERMES (Figure 1). Aggregate capture by mitochondria increases upon stress relief, suggesting a sustained aggregate-trapping function of mitochondria.

Zhou et al. show that the tethering of protein aggregates constrains aggregate mobility, which also prevents aggregate segregation to daughter cells.

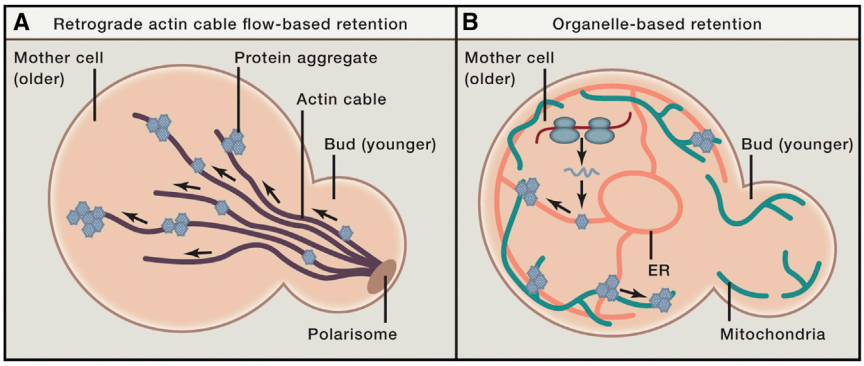

Figure 1. Asymmetric Inheritance of Aggregated Proteins in Yeast
(A and B) Protein aggregates are either tethered to actin cables (A) or endoplasmic reticulum (ER) and mitochondria organelles (B). Retrograde flow of actin cables retains aggregated proteins in the mother cell or clears the bud from aggregates (A). The association of aggregates with mitochondria constrains aggregate mobility and prevents their leakage to daughter cells (B).

Mitochondria, however, are dynamic organelles that rapidly extend into the outgrowing bud during cytokinesis and cell division. This at first glance presents a paradox, but the authors show that bud-inherited mitochondria are largely devoid of aggregates. Older and more oxidized mitochondria are also preferentially retained (Higuchi et al., 2013), suggesting a convergence of functions. This supports the broader concept of an interrelated cellular program for segregating damage away from younger cells.

A limited genetic screen provides further evidence that tethering to mitochondria contributes to asymmetric inheritance of aggregates. Decreased association of mitochondria with protein aggregates is linked to higher aggregate mobility and reduced retention in the mother. This profile is displayed by yeast cells lacking Fis1, a factor required for mitochondrial fission. Aggregates do not colocalize with sites of mitochondrial fission, however, hinting that Fis1 might have a further, previously unrecognized function relating to aggregate tethering. However, several different yeast fission mutants, including fis1Δ cells, exhibit a slightly increased replicative lifespan (Scheckhuber et al., 2007) despite segregating protein aggregates more evenly. The relationship between protein damage segregation and lifespan is therefore more complex than suggested by current models.

Finally, naturally formed or heat-stress-induced aggregates no longer colocalize with mitochondria in aged yeast cells, suggesting that aggregate sorting deteriorates with age. This seems a chicken-and-egg question, as it is under precisely these conditions that the sorting and retention system would be expected to operate. Does collapse of damage segregation, then, trigger aging? This failure is accompanied by abnormal morphology of mitochondria, which fragment and no longer form a tubular network in old cells. Whether aged yeast cells fail to segregate protein aggregates asymmetrically remains a crucial but unsettled question.

The concept of organelle-based retention of protein aggregates has been previously described for aggregates associated with vacuoles and nuclei (Spokoini et al., 2012), though Zhou et al. could not reproduce vacuolar tethering here. The findings presented by Zhou et al. nonetheless recalibrate the original passive model of aggregate retention to include specific active organization of protein aggregates, which is dependent on mitochondria and factors, including Fis1.

Several linked questions emerge relating to this updated organelle-retention model. Which components mediate tethering of protein aggregates to the ER and mitochondria? Are older, potentially less functional, mitochondria selected for tethering targets in the first place, allowing for simultaneous retention of damaged organelles and aggregated proteins in older cells? Which cellular system ensures that mitochondria associated with aggregates do not leak into daughter cells? Does the same retention mechanism operate in stem and progenitor cells of the metazoa exhibiting asymmetric damage distribution?

The organelle-retention model does not easily accommodate or link to the alternative aggregate retention model centered on actin cytoskeleton functions. Zhou et al. show that inhibition of actin polymerization by the drug Latrunculin A reduces aggregate mobility but does not affect aggregate association with mitochondria. However, increasingly oxidized and less functional mitochondria are preferentially retained in mother cells, in a process controlled by retrograde actin cable flow (Higuchi et al., 2013). This points to further cellular sorting systems that regulate and impact cellular rejuvenation and lifespan. Clarification of the role of the actin cytoskeleton in mitochondrial inheritance might well end up dovetailing both models for cellular rejuvenation by mitochondrial trash sorting.

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