Finding the Phountain of Youth

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GMR Deyter

All living organisms have an expiration date that is unavoidable due to ageing. Ageing is defined as a “gradual change in an organism that leads to increased risk of weakness, disease, and death” (Merriam-Webster Dictionary) and occurs at the organismal as well as cellular level. Within cells, mitochondria are the energy producing powerhouse organelles that become dysfunctional as cells age and are important contributors to the ageing process.

Many characteristics of aged cells and the biochemical pathways that are involved in ageing are evolutionarily conserved from single-celled yeast to humans. Yeast are therefore ideally suited, with the ease of genetic manipulation, for studying the processes that contribute to ageing. Adam Hughes, a post-doctoral fellow in the laboratory of Daniel Gottschling (Basic Sciences Division), set out to uncover mechanisms that lead to mitochondrial demise in aged yeast cells. His studies were aided by a procedure that was developed in the Gottschling Lab called the Mother Enrichment Program that enriches yeast cultures for mother cells by eliminating the proliferation potential of daughter cells.

The authors first determined when mitochondria exhibited signs of perturbed function by analyzing membrane fragmentation and decreased membrane potential. Yeast cells undergo an average of 28 divisions, but signs of mitochondrial dysfunction were evident early in the ageing process (86% of cells at 8 divisions). Since 250 genes in yeast have been identified that regulate mitochondrial structure, the authors surmised that the overexpression of gene products that have decreased function in aged cells might rescue the mitochondrial defects. Only 2 of the 250 overexpressed gene products, namely Vma1 and Vph2, strongly rescued the mitochondrial defects of aged cells. Both proteins are required for the activity of the vacuolar V-ATPase, an evolutionarily conserved protein complex that acidifies the yeast vacuole lumen (lysosome in metazoa).

Hughes and Gottschling then tested if vacuolar pH changed as cells aged and found a steady decline in vacuolar acidity during the first 4 cell divisions that remained low thereafter. Vacuolar acidity decreased before mitochondrial dysfunction, indicating that changes in vacuolar pH might influence mitochondrial function. This idea was confirmed by showing that the overexpression of Vma1 and Vph2 increased vacuolar acidity and mitochondrial function. Also, young cells with defective V-ATPase activity were shown to have mitochondrial defects similar to aged cells

indicating a critical role for vacuolar pH in the ageing process. The V-ATPase mutant also had a reduced lifespan and, conversely, the overexpression of V-ATPase components increased lifespan.

A critical question remained: How does vacuolar pH influence mitochondrial function? The vacuole is a site of protein degradation as well as ion and amino acid storage, all of which are influenced by vacuolar lumen pH. The authors discovered that defective vacuolar neutral amino acid storage and not protein degradation resulted in mitochondrial dysfunction. By overexpressing a vacuolar transporter that increases neutral amino acid vacuole storage to prevent mitochondrial dysfunction and increase lifespan, the researchers confirmed a critical link between vacuolar function and ageing.

Calorie restriction in eukaryotes increases lifespan through conserved nutrient-sensing kinase-cascade pathways. Hughes and Gottschling found that aged yeast grown in low calorie media had higher vacuolar acidity, lower mitochondrial dysfunction, and a longer lifespan compared to yeast grown in high calorie carbon sources. Yeast mutants with defective nutrient-sensing kinase activity (and increased lifespan) exhibited greater vacuolar acidity indicating that conserved nutrient-sensing pathways regulate the activity of two organelles: vacuoles and mitochondria.

Dietary amino acid limitation has been shown to increase the lifespan of a variety of organisms, so the defective uptake of amino acids in aged-yeast vacuoles (and potentially in metazoan lysosomes) likely places a large burden on mitochondrial function and hastens cell death. Future investigations will determine exactly how increased intracellular amino acid levels negatively affect mitochondrial activity. Altogether, these data drive an impressive advancement in understanding how calorie restriction increases lifespan.

Hughes AL, Gottschling DE. 2012. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature*492(7428):261-5.

To learn more about the mother enrichment program (MEP) see:

Lindstrom DL, Gottschling DE. 2009. The mother enrichment program: a genetic system for facile replicative life span analysis in Saccharomyces cerevisiae. *Genetics* 183(2):413-22.

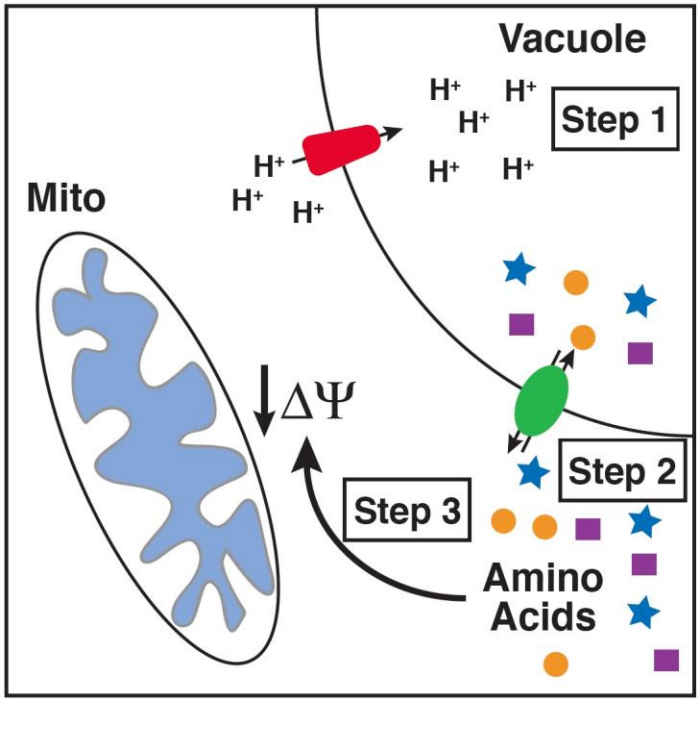

Image obtained from manuscript

Figure: An early-age neutralization of vacuolar acidity alters amino acid storage and mitochondrial function. Early in the budding yeast aging process, the vacuolar pH becomes more basic (Step 1). The decreased intra-vacuolar proton concentration negatively affects vacuolar amino acid uptake and storage (Step 2), leading to increased intracellular amino acid levels. The imbalance in amino acid homeostasis then perturbs mitochondrial function by an as-yet elusive mechanism (Step 3).