

The mystery of aging and rejuvenation — a budding topic

Thomas Nyström and Beidong Liu

In the process of yeast budding, an aged and deteriorated mother cell gives rise to a youthful and pristine daughter cell. This remarkable event offers a tractable model system for identifying factors affecting life expectancy and it has been established that multiple aging factors operate in parallel. Herein, we will highlight the identity of such aging factors, how they are asymmetrically segregated, and whether the knowledge of their deteriorating effects might be utilized to approach cellular and tissue rejuvenation in metazoans, including humans.

Addresses

Department of Chemistry and Molecular Biology, University of Gothenburg, Medicinargatan 9C, S-413 90 Göteborg, Sweden

Corresponding author: Nyström, Thomas (thomas.nystrom@cmb.gu.se)

Current Opinion in Microbiology 2014, 18: 61–67

This review comes from a themed issue on **Cell regulation**

Edited by **Cecilia Arraiano** and **Gregory M Cook**

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<http://dx.doi.org/10.1016/j.mib.2014.02.003>

“To get back my youth I would do anything in the world, except take exercise, get up early, or be respectable.”
— Oscar Wilde

Introduction

Nothing would alter human life more radically than the knowledge of how to delay or stop aging [1] and our fascination with this subject matter is perceptible in numerous works of literature. ‘Charlie and the great glass elevator’ serves as a charming example in which Willy Wonka, the manufacturer of superb candy, invents the elixir ‘Wonka-Vite’ to reverse aging of Charlie’s old and bedridden grandparents allowing them to help out in the chocolate factory [2]. While skeptics might maintain that assurances of drugs such as Wonka-Vite are appropriately confined to fiction and fairy-tales, the yeast *Saccharomyces cerevisiae* shows us that rejuvenation of old individuals is entirely feasible and was invented by nature rather than creative writers. Specifically, a mother cell of *S. cerevisiae* grows older with each generation and has a finite capacity to produce new cells — the hallmark of replicative aging (Figure 1a,b). Nevertheless, aged and deteriorated mother cells generate daughter cells that display a full replicative potential demonstrating that the clock of aging

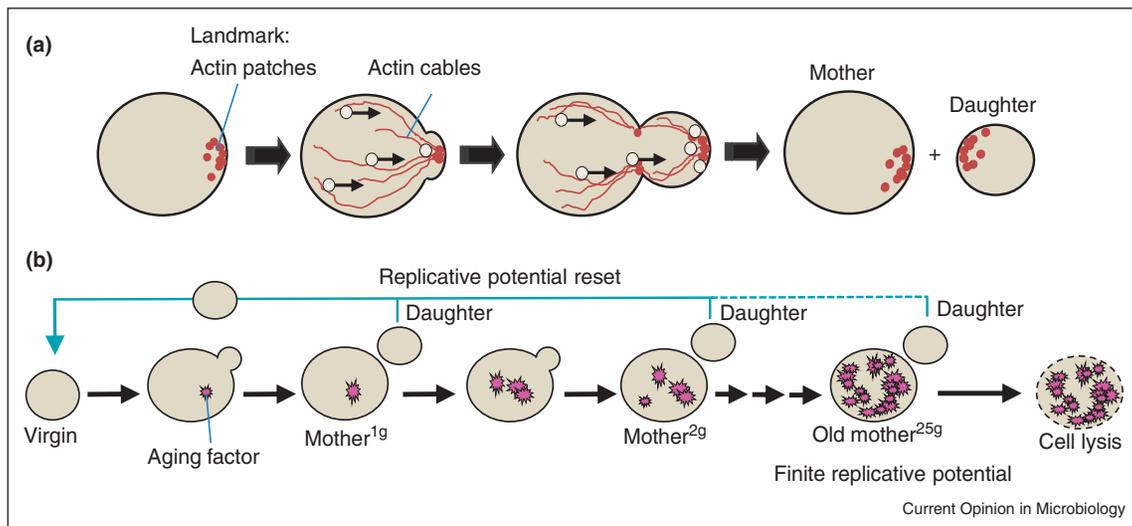
is not only stopped but also completely reset in these cells (Figure 1b). Work in the last decade suggests that this singularity encompasses spatial confinement, or filtering, of one or several aging factors. In this paper, we will review such data with special emphasis on extra chromosomal rDNA circles (ERCs), damaged/aggregated proteins, dysfunctional mitochondria, and defective vacuoles and, by extrapolations, discuss what the formula of a Wonka-Vite-like elixir might need to target to reverse aging also in metazoans.

Extra chromosomal rDNA circles (ERCs) — aging by self-inflicted poisoning

Extrachromosomal rDNA circles (ERCs) are generated by homologous recombination from 100 to 200 copies of tandem rDNA repeats and they replicate via the autonomously replicating sequence (ARS) in each such repeat. Several studies have demonstrated that ERCs, and other ARS plasmids, accumulate in aging mother cells, display mother-biased segregation, and causes deterioration of the mother cell [3,4]. It is not entirely clear how ERCs are poisoning the mother cell but recent data suggest that they might act as a sink for limiting replication factors [5*]. The rDNA repeats are transcriptionally silenced by Sir2 [6] and this protein deacetylase is counteracting yeast aging, in part, by limiting ERC accumulation [7].

Several potential models have been proposed for asymmetrical segregation of ERCs, including the proposal that ARS-containing episomes associate with fixed nuclear sites predominantly segregating to the mother [4]. In line with such models, ERCs associate with nuclear pore complexes (NPCs) and the septin ring works as a diffusion barrier for the retention of pre-exiting NPCs in the mother (Figure 2a) [8**]. In this scenario, ERCs stay in the mother cell by virtue of being tethered on structures, NPCs, that are themselves experiencing a mother-biased segregation (Figure 2a). However, other results indicate that pre-existing NPCs are freely inherited by daughters [9,10*]. On the basis of such results, a passive diffusion-based model has been proposed suggesting that the asymmetrical segregation of ARS plasmids is solely depending on the geometrical shape of the nucleus and the limited duration of mitosis [11]. This model, however, does not readily explain the behavior of a circular 1.45 kb ARS plasmid, called the TRP7 circle [12], which shows no detectable segregation bias [4] and that a small ARS plasmid (pYA3, 4.3 kb) displays a higher segregation bias to the mother than larger plasmids (e.g. pWJ52, 9.8 kb and pSZ106, 6.3 kb) [4]. Thus it is unclear whether the passive diffusion model, on its own,

Figure 1



(a) Yeast cell division — budding. Before outgrowth of a bud/daughter from the mother cell, a landmark determining where budding will take place is laid down and actin cytoskeletal patches are recruited to this site. Subsequently, actin cables are nucleated at the bud tip and bud neck and serve as tracks for the transport of constituents, building blocks, and organelles into the new daughter cell. After completion of cytokinesis, new landmarks are established adjacent to the old site of division in haploid cells. **(b)** Aging and rejuvenation. A virgin daughter cell can, after becoming a mother cell, on average produce between 25 and 30 daughter cells before entering terminal senescence. Yet, even when old (if not too close to its terminal stage), a mother cell will generate daughter cells of a full replicative potential. This is accomplished, in part, by the retention of critical aging factors in the mother cell compartment.

offers a sufficient explanation for the asymmetric segregation of ERCs and ARS-containing plasmids [13].

Mother cells as trashcans for aged and damaged proteins

Like ERCs, oxidatively damaged and aggregated proteins accumulate in mother cells with replicative age and experience mother-biased segregation [14,15,16,17]. This segregation requires the protein disaggregase Hsp104 [15,16,17] and Sir2 [14,15,18,19]. The role of both these proteins in establishing damage asymmetry has been linked to actin cable-dependent processes and the polarisome [16,17]; a complex at the tip of the daughter cell required for actin cable nucleation (Figure 2b).

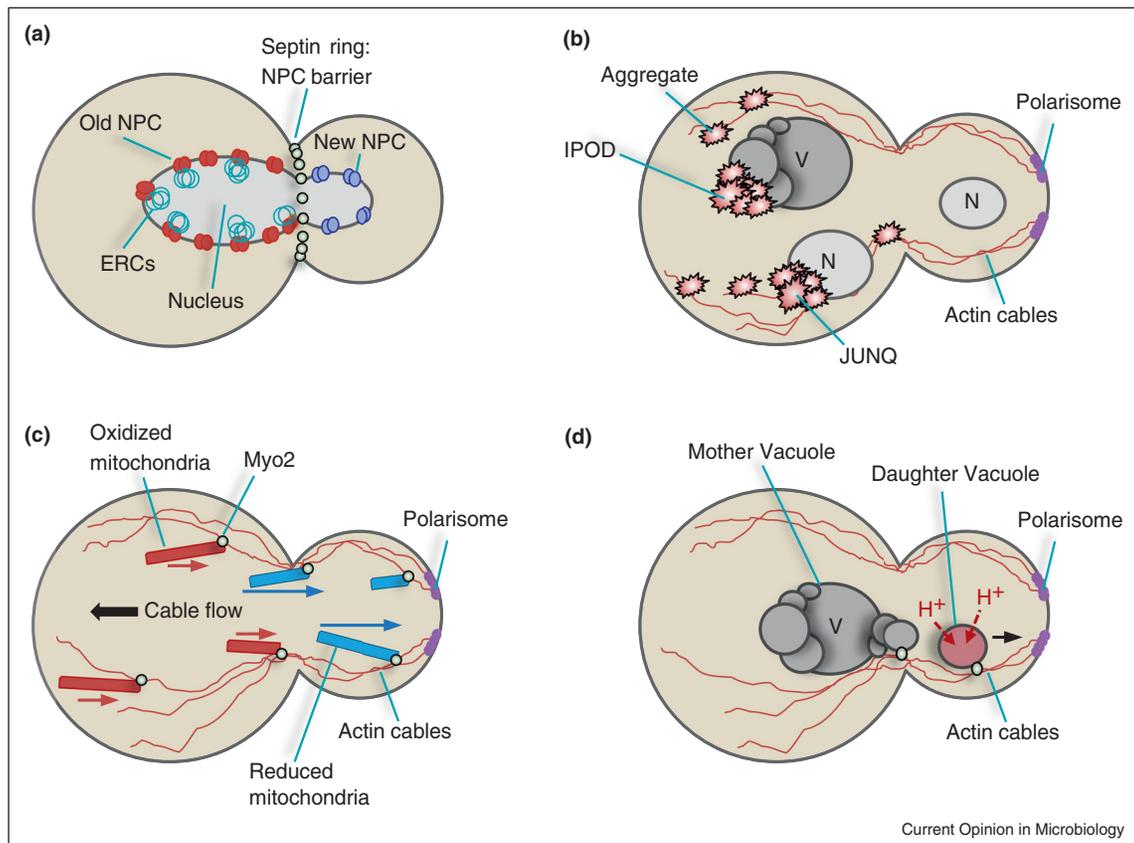
The role of actin cables in protein damage retention has been suggested to be the result of aggregates (and prions) associating with the actin cytoskeleton preventing their free diffusion into the daughter (Figure 2b) [16,17,20^{••},21[•],22]. The control of damage inheritance is dependent also on the spatial deposition of damaged/unfolded proteins into specific protein inclusion bodies (IBs), such as IPOD (perivacuolar inclusion) and JUNQ (juxtannuclear inclusions) [23,24^{••},25[•]] (Figure 2b). Apart from requiring Hsp104, formation of peripheral IPODs needs actin-cables and the small heat-shock protein Hsp42 [26[•]], suggesting that the actin cytoskeleton might be imperative in two interconnected processes required for asymmetrical inheritance; aggregate tethering [16]

and IPOD formation (Figure 2b) [26[•]]. It should be noted that proteins, such as the Huntingtin (Htt103Q) disease protein that does not form IBs, are also subjected to a mother-biased, polarisome-dependent, segregation [20^{••}] demonstrating that both IBs and small aggregates are subjected to inheritance control (at least in the presence of Hsp104; see [24^{••}]).

While the data reviewed suggest that mother-biased segregation of protein damage is an actin/organelle-dependent process, Zhou *et al.* [27] based on aggregate tracking experiments and modeling, suggest that asymmetric inheritance is a purely passive outcome of aggregates' random diffusion and dictated solely by the diameter of the bud neck and how long this neck is open (generation time). A counterpoint was made by Spokoini *et al.* [24^{••}] demonstrating that the aggregates analyzed in the Zhou *et al.* [27] study are IPOD and JUNQ inclusions, which cannot diffuse freely as they are attached to the surface of the vacuole and nucleus [24^{••}].

Yeast cells deficient in protein disaggregase activity [15] and 26S proteasome activity [28[•]] age prematurely whereas elevated proteasome activity extends lifespan [28[•]]. In addition, it has recently been shown that protein aggregates formed during mother cell aging obstruct proteasomal activity, which may give rise to a feed-back catastrophe loop in protein homeostasis [29[•]]. However, it should be noted that direct evidence for damaged/aggregated

Figure 2



Asymmetrical inheritance of aging factors. **(a)** Model for ERC retention: In this model ERCs are associated with the nuclear pore complex (NPC) [8**]. The pre-existing NPCs are retained in the nucleus on the mother-cell side by a barrier made up of the septin ring at the bud neck [8**]. Thus, transmission of ERCs into the daughter nucleus is restricted and new NPCs are made de novo in the part of the nucleus entering the daughter cell. **(b)** Model for retention of protein aggregates and inclusions: Aggregates are suggested to associate with the actin cytoskeleton restricting their diffusion [16]. In addition, unfolded/damage proteins are conveyed to IPOD and JUNQ deposition sites at the surface of the vacuole and nucleus, respectively. During budding, the daughter is predominantly inheriting the inclusion-free organelles [24**]. **(c)** Model for the enrichment of healthy mitochondria into daughter cells: Reduced (healthy) mitochondria move faster than oxidized ones with the Myo2 motor protein against the retrograde cable flow [36**]. As a result reduced mitochondria are enriched in the daughter cell [36**]. The polarisome is the site for actin nucleation at the tip of the daughter cell and the cables are thus pushed away from this site towards the mother cell. **(d)** Restoration of pH control in inherited vacuoles: Vacuoles, like mitochondria, are transferred to the daughter cell as Myo2 cargo moving on actin cables. The vacuole entering the daughter retains its ability to acidify the vacuolar lumen [38**]. This is different from a filtering mechanism and points towards the internal milieu of the daughter cell being sufficiently different to allow proton import into the vacuole.

proteins being *bona fide* aging factors in yeast are still pending. This is because lifespan extension by boosting 26S proteasome activity [28*] could be due to the destruction of other proteins (e.g. regulators of cell cycle progression) than damaged ones.

Filtering feeble mitochondria to reset the clock of aging

Malfunctioning mitochondria accumulate in yeast mother cells during replicative aging [30,31,32,33]. The emergence of these dysfunctional mitochondria is not only a consequence but also a cause of aging [30] and mitochondrial segregation is required to establish cellular age asymmetry [33]. Recent findings suggest that this age asymmetry is linked to daughter cells primarily inheriting

the healthier mitochondria from the mother cell [34,35] and that this process includes a mitochondrial filtering mechanism [36**]. This filtering device exploits retrograde flow of actin cables: Reduced (healthy) mitochondria move faster against the actin flow than oxidized (dysfunctional) ones resulting in an enrichment of healthy mitochondria in the daughter cell [36**] (Figure 2c). Increasing the rate of retrograde actin flow extends replicative lifespan in a mitochondria-dependent manner [36**].

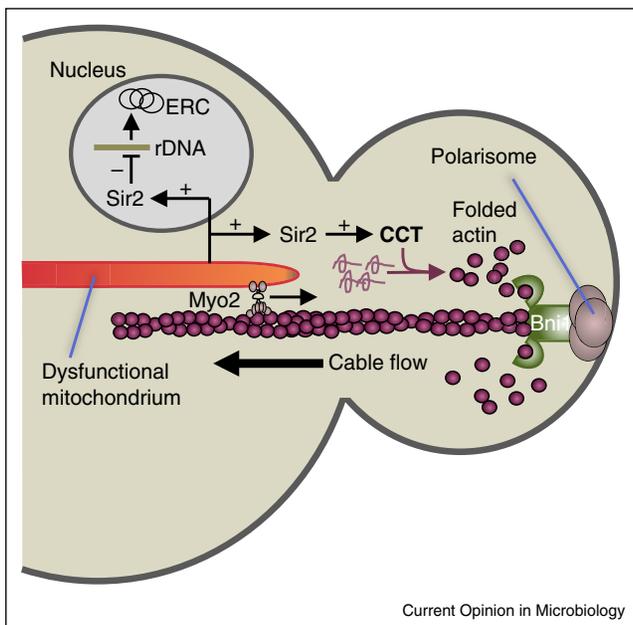
Similar to the inheritance of oxidized proteins [14] and protein aggregates/inclusions [16], asymmetrical segregation of oxidized mitochondria is dependent on Sir2 dosage: The removal of *SIR2* decreases the velocity of

actin cable flow allowing the inheritance of oxidized mitochondria whereas Sir2 overproduction is doing the opposite [36**]. These results are in line with Sir2 affecting the rate of actin folding by modulating the activity of the chaperonin CCT [16]. Thus, Sir2-deficiency might limit the availability of substrates — properly folded actin — for the polarisome, which results in diminished actin nucleation at the bud tip, reduced retrograde actin flow, and increased inheritance of unhealthy mitochondria (Figure 3). These findings are intriguing also in view of the fact that dysfunctional, translation-deficient mitochondria elevate Sir2 activity [37*]. Possibly, such enhanced Sir2 activity, like Sir2 overproduction [36**], could result in increased retrograde flow of actin cables ensuring that the filtering process is boosted upon demand to ensure the rejuvenation of the progeny, that is, when mitochondrial decline is sensed upon aging (Figure 3).

Vacuole acidity — the genesis of aging

Age-related loss of genome heterozygosity is caused by a prior decline in mitochondrial function during replicative aging [31]. The sequence of aging events was recently traced further back to its origin through identification of

Figure 3



A hypothetical model for feedback control between mitochondrial function and mitochondrial inheritance. Dysfunctional mitochondria trigger increased Sir2-dependent genomic silencing leading to lifespan extension [37*]. Possibly, as Sir2 modulates the activity of the CCT chaperonin [16], such an enhanced Sir2 activity could increase the retrograde flow of actin cables by boosting CCT-dependent folding of actin monomers [16]; the substrate of the polarisome form (Bni1). An increased flow of actin cables would prevent Myo2-dependent inheritance of dysfunctional mitochondria generated in the aging mother cell.

genes that postpone the onset of the age-associated mitochondrial defects [38**]. Intriguingly, this analysis pointed towards an early functional decline of another organelle — the vacuole. Specifically, vacuole pH increases early in the lifespan of the yeast mother cell and results in the subsequent loss of mitochondrial $\Delta\Psi$ control [38**]. Interestingly, while the vacuole of the mother cell displays a dysfunctional pH control, the one inherited by the daughter cell regains its acidic pH upon entering the progeny [38**] (Figure 2d). This is different from the filtering of functional and dysfunctional mitochondria (Figure 2C) as it is the daughter cell environment/constitution that is required for proper vacuolar pH control rather than the vacuole itself.

The interconnected control of vacuolar and mitochondrial functions appears mediated by vacuolar storage of neutral amino acids. The import and storage of neutral amino acids in the vacuole requires proper vacuolar acidification [38**]. Considering that neutral amino acids are catabolized by mitochondria, it was suggested that an excess of cytoplasmic amino acids released from dysfunctional vacuoles places an overpowering demand on proton-dependent mitochondrial carrier processes leading to a collapse in mitochondrial $\Delta\Psi$ control [38**]. Emerging questions are, why does vacuolar pH control fail in the mother cell in the first place, are other aging factors foregoing and triggering vacuolar collapse, how is pH control reset in the vacuole inherited by the daughter cell, and are other aging factors than dysfunctional mitochondria accumulating as a direct consequence of the early decline in vacuolar pH control?

Conclusion — a recipe for Wonka-Vite

As described herein, a successful recipe for a yeast elixir would have to contain ingredients that stop, or reverse, formation of ERCs, damaged and aggregated proteins, dysfunctional mitochondria, and defective vacuoles. When considering metazoans, it is unlikely that extra chromosomal circular DNA (eccDNA), like ERCs, are a cause of aging although elevated levels of eccDNA have been detected in patients suffering from Werner syndrome, a premature aging disorder [39]. This does not rule out sirtuins as potentially targets in a Wonka-Vite-type elixir as Sir2 affects other aging factors than ERCs and because mammalian sirtuins mediate some of the effects of caloric restriction and play key roles in age-related diseases [40].

There is ample evidence for aberrant and aggregated species of proteins affecting the rate of metazoan aging and triggering age-related neurological diseases [41,42]. For instance, compounds that target protein amyloids have been shown to cause a robust extension of *Caenorhabditis elegans* lifespan [43*], suggesting that therapeutic/chemical means of approaching age-related protein homeostasis may prove effective.

Similar to damaged/aggregated proteins, accumulating evidence suggest that metazoan aging is causatively linked to the age-related enrichment of dysfunctional mitochondria [44]. The mechanism, however, by which such dysfunctional mitochondria arise and affect aging might be multifactorial: One such mechanism in mice is the transmission of maternally originated mtDNA mutations to the progeny and the clonal expansion of such errors during development [44,45**]. Another mechanism, as described herein, is mitochondrial dysfunction caused by a previous collapse in vacuolar pH control [38**]; a means of mitochondrial breakdown that does not, presumably, act through accumulated mtDNA mutations. Regardless of the mechanisms involved, it appears essential to keep mitochondria in pristine shape to ensure longevity. However, if mitigating the functional decline of mitochondrial can extend lifespan in metazoans, like yeast [30], remains to be established.

Metazoan lysosomes are, like yeast vacuoles, used for waste disposal by processes including autophagy. Ectopic inhibition of autophagy triggers cellular/tissue deterioration resembling that observed during aging and aging is often accompanied by a reduced autophagic capacity [46,47]. Moreover, caloric restriction (CR), Sirtuin 1 activation, inhibition of insulin/insulin growth factor signaling, and rapamycin/resveratrol administration are only effectively extending lifespan in the presence of a fully functional autophagic machinery [46]. Whether age-related diminished autophagy is linked to failures in internal lysosomal pH control and if this is causing down-stream effects on mitochondrial function also in an autophagic-independent manner, as in yeast [38**], is not known but would be interesting to address. In addition, although surrounded by near-unresolvable caveats (see [46]), targeting macroautophagy in therapeutic or nutritional gerontology remains an interesting possibility to pursue.

Another potentially interesting in-road to therapeutic gerontology is to target the process of asymmetrical segregation of damage: Asymmetrical, polar, partitioning of damaged proteins is not unique for budding yeast but operates also in adult stem and progenitor cells [48]. For example, intestinal stem cells rids themselves of oxidatively damaged proteins such that most of the damage is inherited by the progeny [49*]. In contrast, asymmetrical cell division of both female germline and neuroblast stem cells results in most of the damage being retained in the progenitor stem cell [49*]. In all cases, the cell receiving most damage is the one with the shortest life expectancy [49*]. Currently, it is not known whether this damage segregation is subjected to an age-related decline, and if so, if therapeutically boosting the ability to maintain asymmetrical inheritance is beneficial in old individuals. More work is clearly needed to establish how and whether damage segregation contributes to tissue renewal and longevity in higher organisms.

Finally, a word of caution with respect to rejuvenation elixirs: Charlie's old and bedridden grandparents, predictably, got greedy and took a much higher dose of Wonka-Vite than required resulting in two of them becoming toddlers. The third, Georgina, disappeared altogether having become 'minus two'. As a result, Mr. Wonka was forced to invent an aging spray to get Georgina back into existence again. So, while Roald Dahl reveals no hints as to the required ingredients of a rejuvenation tincture, he offers us, as always, thought-provoking insights on human nature [2].

Acknowledgements

Work in the Nyström and Liu laboratories are supported by grants from the Swedish Research Council (TN and BL), the Knut and Alice Wallenberg Foundation (Wallenberg Scholar; TN), ERC (TN), the Swedish Cancer Society (TN and BL), and Stiftelsen Olle Engkvist Byggmästare Foundation (BL).

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