MINIREVIEW

Role of asymmetric cell division in lifespan control in *Saccharomyces cerevisiae*

Ryo Higuchi-Sanabria, Wolfgang M.A. Pernice, Jason D. Vevea, Dana M. Alessi Wolken, Istvan R. Boldogh & Liza A. Pon

Department of Pathology and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Correspondence: Liza A. Pon, Department of Pathology and Cell Biology, Columbia University, 630 W. 168th St. P&S 14-442, New York, NY 10032, USA.
Tel.: (212) 305 1947;
fax: (212) 305 5498;
e-mail: lap5@columbia.edu

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Abstract

Aging determinants are asymmetrically distributed during cell division in *S. cerevisiae*, which leads to production of an immaculate, age-free daughter cell. During this process, damaged components are sequestered and retained in the mother cell, and higher functioning organelles and rejuvenating factors are transported to and/or enriched in the bud. Here, we will describe the key quality control mechanisms in budding yeast that contribute to asymmetric cell division of aging determinants including mitochondria, endoplasmic reticulum (ER), vacuoles, extrachromosomal rDNA circles (ERCs), and protein aggregates.

Introduction

Asymmetric cell division is the process whereby asymmetric inheritance of cellular components gives rise to two daughter cells that have different characteristics and fates. This process is essential for development and for maintaining stem and progenitor cell number and differentiation (Wang *et al.*, 2009; Oliaro *et al.*, 2010). It also occurs in unicellular organisms including bacteria (e.g. *Caulobacter* and *E. coli*) and yeast (e.g. *S. cerevisiae*) (Ackermann *et al.*, 2003; Stewart *et al.*, 2005; Radhakrishnan *et al.*, 2008; McFaline-Figueroa *et al.*, 2011). Asymmetric cell division can occur in response to either environmental or intrinsic cues. In eukaryotes, asymmetric cell division processes are dependent upon the cell polarity machinery and orientation of the mitotic spindle relative to fate-determining factors.

One important consequence of asymmetric cell division in *S. cerevisiae* is mother–daughter age asymmetry, the phenomenon whereby daughter cells or buds are born young, irrespective of the age of their mother cell. Aging in yeast is manifest in two different lifespan measurements, which model two distinct forms of cellular aging (Longo *et al.*, 2012). Replicative lifespan, the number of divisions a cell can undergo prior to reaching senescence, is a model for aging in division-competent cells. Chronological lifespan, the survival time of nondividing yeast cells in stationary phase, is a model for stress resistance in postmitotic cells. Asymmetric cell division in budding yeast produces cells with differing replicative lifespans; it is not known whether yeast division produces asymmetry in chronological lifespan.

Aging determinants that undergo asymmetric inheritance, including oxidatively damaged protein aggregates, mitochondria, vacuoles, extrachromosomal rDNA circles, and antioxidant activity, have been identified in *S. cerevisiae*. Moreover, emerging studies have revealed a role for the cell polarity machinery in the inheritance of these aging determinants. Specifically, the cytoskeleton can serve as a track or force generator for polarized movement of aging determinants from mother cells to buds and from buds to mother cells (Fehrenbacher *et al.*, 2004; Liu *et al.*, 2011). It also serves as a filter to prevent inheritance of damaged material from mother cells to buds

(Higuchi *et al.*, 2013). Anchorage of components at their delivery sites also promotes asymmetric inheritance of aging determinants (Swayne *et al.*, 2011). For example, in *S. cerevisiae*, higher functioning mitochondria, which promote lifespan, are trafficked to the bud tip and are retained at that site by anchorage to membranes in the bud tip. Interestingly, there is evidence that the cytoskeleton drives movement of anchorage proteins, or mRNAs that encode the anchorage proteins, to their site of action (Shepard *et al.*, 2003). Finally, the cytoskeleton is required both for creation of a diffusion barrier at the bud neck, which contributes to maintenance of asymmetry between mother cells and buds, and for generation of a rejuvenating environment within the bud (Erjavec & Nystrom, 2007; Clay *et al.*, 2014). Such rejuvenation processes can promote mother–daughter age asymmetry by acting to restore what physical sequestration fails to or cannot accomplish.

Here, we will discuss the mechanisms for establishing cell polarity in *S. cerevisiae*, determinants that are asymmetrically inherited during yeast cell division, the role of the polarity machinery in these events, and how asymmetric cell division promotes production of an immaculate daughter cell in yeast. We also discuss how some of the pathways identified in yeast may also act in mammalian systems to promote cellular function and fitness in organisms with longer lifespans, including humans.

Polarity establishment during cell division in *S. cerevisiae*

During cell division in budding yeast, a bud site is selected on the cell surface. Thereafter, the cytoskeleton is polarized toward that site, resulting in transport of cellular constituents from the mother cell to the bud, enabling bud growth. Here, we provide an overview of polarization of the actin, tubulin, and septin cytoskeletons during polarized growth and cell division in *S. cerevisiae*.

The first step in cell division in *S. cerevisiae* is selection of a bud site. This process is mediated by cortical tags that localize to the selected bud site and activate a GTPase-dependent signaling cascade. This, in turn, results in assembly of the cytoskeleton that reflects and reinforces the established polarity. Cdc42p, the small GTPase, is a critical component of the GTPase cascade (Park & Bi, 2007). It is activated by Bud1p/Rsr1p, another small GTPase, and its two regulatory partners, Bud2p and Bud5p. Their concerted action results in the establishment of two specialized cytoskeletal structures: the septin ring and the polarized actin cytoskeleton (Kang *et al.*, 2001). Cdc42p polarization at a single cortical site depends on several factors organized by the scaffold protein Bem1p, which binds to Cla1p, a p21-activated kinase, and to Cdc24p, a GEF for Cdc42p (Bi & Park, 2012).

Many effectors participate in the polarized organization of actin and septins. Some of the effectors are organized into a functional system. One of these is the polarisome, which is a small network of functionally related proteins that interact with each other at sites of polarized growth and regulate processes downstream of Cdc42p, such as polarized actin cable organization and exocytosis. This network includes Spa2p, a scaffold protein; Bni1p, a formin that catalyzes actin polymerization; Bud6p, which binds to actin monomers and promotes Bni1p activity; Pea2p, a protein with unknown function; and two GAPs, Msb3p and Msb4p, for the Rab protein Sec4p (Park & Bi, 2007). Other effectors such as Gic1/2p and Cla4p participate in septin recruitment and septin ring assembly at the putative bud site (Park & Bi, 2007).

There are two F-actin-containing structures that persist through the cell cycle in *S. cerevisiae*: actin patches and actin cables. Actin patches are endosomes endowed with a coat of F-actin that form in the bud and are believed to be critical for recycling of membrane constituents during polarized secretion in the bud. Actin cables are bundles of F-actin filaments that align along the long axis of dividing cells from bud tip to mother tip and act as tracks and movement generators for cargo transport (Moseley & Goode, 2006).

The first evidence that actin cables serve as tracks for asymmetric cargo distribution during yeast cell division came from studies of Ash1p, a transcription repressor and cell fate determinant that localizes to the bud through most of the cell division cycle in *S. cerevisiae*. Here, asymmetric distribution is achieved, not by protein transport, but by mRNA transport. *ASH1* mRNA, which is sequestered in ribonucleoprotein particles, binds to the type V myosin, Myo4p, using the cargo adapters She2p and She3p, and uses the forces generated by Myo4p for transport from mother cells to buds (Munchow *et al.*, 1999; Takizawa *et al.*, 2000). More than 30 mRNAs use actin cables for asymmetric inheritance and localization to the bud tip including mRNAs that encode proteins that mediate binding of ER to the plasma membrane (*IST2, TCB2-3*), anchorage and retention of higher functioning mitochondria in the bud tip (*MMR1*), and stress response (Fig. 1) (Shepard *et al.*, 2003; Aronov *et al.*, 2007; Oeffinger *et al.*, 2007). The roles of proteins encoded by these mRNAs in promoting daughter cell fitness and mother–daughter age asymmetry are described below.

Although many cytoskeletal tracks are static, actin cables are dynamic structures that undergo continuous retrograde movement from the bud into the mother cell (Yang & Pon, 2002) (Fig. 2). Two primary forces drive

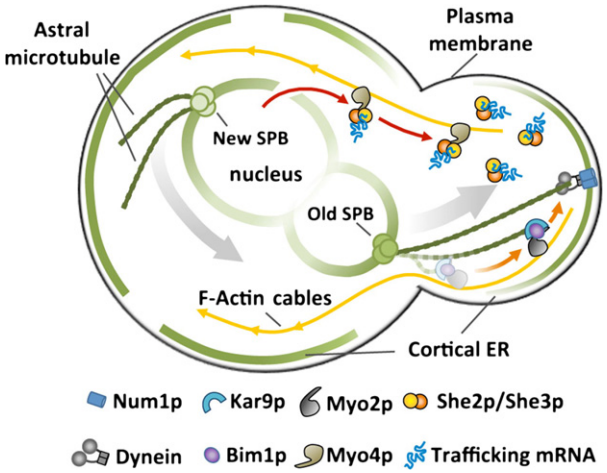

Fig. 1. Cellular cargo, such as mRNA and microtubules, undergo bud-directed movement along actin cables. mRNAs move to the bud tip along actin cable tracks, by binding to type V myosin motors via cargo adapters (She2p and She3p). Several of these bud-localized mRNAs encode proteins that promote asymmetric segregation, including Mmr1p essential in anchorage of mitochondria at the bud tip. The spindle apparatus aligns along the mother–bud axis using actin and microtubule-dependent force generators. Astral microtubules on the mitotic spindle bind to the type V myosin Myo2p using the cargo adapter Kar9p and the microtubule end-binding protein Bim1p, and move toward the bud tip using actin cables as tracks. This movement, together with pulling forces by dynein in the bud tip, results in alignment of the spindle apparatus along the mother–bud axis, which in turn leads to asymmetric cell division.

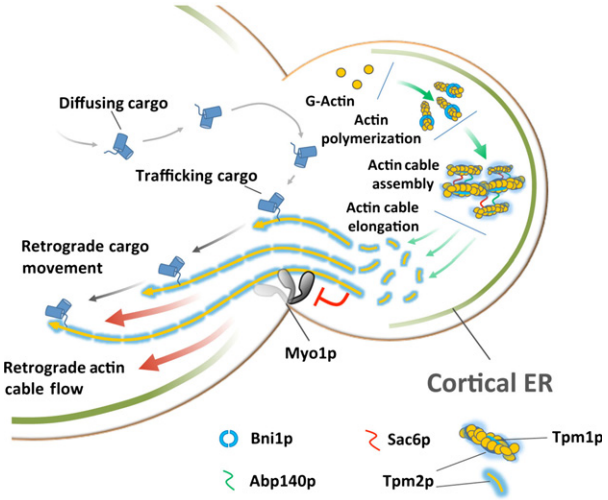

Fig. 2. Actin nucleation and Myo1p act in concert to drive retrograde actin cable flow. Actin cables are dynamic structures that undergo retrograde movement from their site of assembly in the bud tip and bud neck toward the tip of the mother cell distal to the bud. In this model of RACF, the formin protein, Bni1p, stimulates polymerization of G-actin into F-actin at the bud tip. A similar process is driven in the bud neck via the formin protein, Bnr1p (not depicted). F-actin then assembles into actin cables using the bundling proteins fimbrin (Sac6p) and Abp140p, and tropomyosins, Tpm1p and Tpm2p. These bundles are inserted at the tip of the actin cable in the bud tip, providing a pushing force for RACF. The type II myosin at the bud neck binds to actin cables in a Tpm2p-regulated manner and provides pulling forces for RACF. RACF drives movement from the bud toward the mother cell. During this process, cargos bind to dynamic actin cables and use them as 'conveyor belts' for retrograde movement.

this movement termed retrograde actin cable flow (RACF) (Huckaba *et al.*, 2006). First, there is a pushing force, that is, driven by elongation of actin cables by insertion of new material at the tip of the actin cable in the bud tip or bud neck. This pushing force requires formins (Bni1p and Bnr1p), which stimulate actin polymerization for actin cable assembly. It also requires resident actin cable proteins that assemble with F-actin into actin cables including actin bundling proteins, fimbrin (Sac6p) and Abp140p, and tropomyosins, Tpm1p and Tpm2p, which stabilize F-actin cables and regulate actin cable dynamics, respectively. A pulling force also drives RACF via a type II myosin motor, Myo1p, anchored at the bud neck. RACF is critical for asymmetric cell division and mother–daughter age asymmetry as a force generator for movement of aging determinants from buds to mother cells, and as a filter to prevent lower-functioning mitochondria from moving from mother cells to buds (Liu *et al.*, 2011; Higuchi *et al.*, 2013) (*vide infra*).

To ensure that chromosomes are segregated to both the bud and mother cell, the spindle must align with the mother–bud axis. This alignment depends on two cytoskeleton-dependent pathways. First, Kar9p acts early in the cell cycle and links the plus ends of astral microtubules to polarized cortical actin cables by interacting with Myo2p, a type V myosin, and Bim1p, the yeast equivalent of the microtubule binding protein EB1 (Yin *et al.*, 2000; McNally, 2013). Here, actin cables provide tracks for Myo2p-dependent movement of astral microtubules toward the bud tip, which results in alignment of the spindle apparatus along the mother–bud axis (Hwang *et al.*, 2003).

Late in the cell cycle, a second pathway directs the minus end-directed microtubule motor, dynein, to exert pulling force onto the spindle, which also contributes to spindle alignment. To achieve the pulling force, dynein must be transported to and anchored at the cortex of the bud tip. This process is driven by astral microtubules, dynamic microtubules that extend and retract from the microtubule-organizing center, called the spindle pole body (SPB), and make contact with the cell cortex. Dynein is transported to the plus end of astral microtubules by Bik1p-Kip2p complexes, or in their absence, by Bim1p (Sheeman *et al.*, 2003; Carvalho *et al.*, 2004; Caudron *et al.*, 2008). Dynein is then delivered to the bud tip by dynamic astral microtubules and is retained at

that site by binding to Num1p (Markus & Lee, 2011). The BAR-like domains on Num1p are critical for assembly of Num1p into cortical patches and for interaction of Num1p with dynein (Tang *et al.*, 2012). Dynein then binds to and generates pulling forces on astral microtubules, which results in alignment of the spindle along the mother–bud axis. Recent evidence has shown that proper localization of Num1p is dependent on the ER diffusion barrier (Chao *et al.*, 2014) (*vide infra*).

Interestingly, the spindle itself is asymmetrically inherited. Duplication of the SPB gives rise to a new SPB and an old SPB. The old SPB is always inherited by the bud. A recent study showed that this age-dependent inheritance of the SPB is specified by a SPB component, Nud1p, through the Mitotic Exit Network (MEN) pathway (Hotz *et al.*, 2012). In addition, Kar9p localizes only to microtubules that emanate from the old SPB toward the bud (Liakopoulos *et al.*, 2003). This asymmetric localization requires SUMOylation and Cdk1p-dependent phosphorylation of Kar9p (Leisner *et al.*, 2008), which is stabilized by MEN-dependent phosphorylation of Kar9p (Hotz *et al.*, 2012).

The septin cytoskeleton also contributes to asymmetric cell division and mother–daughter age asymmetry. Septins are a subfamily of GTP-binding proteins that form higher order structures, including filaments, rings, and cage-like structures (Pan *et al.*, 2007; Bertin *et al.*, 2008, 2012; Garcia *et al.*, 2011; Saarikangas & Barral, 2011; Mostowy & Cossart, 2012). The septins localize to the prebud site forming unorganized patches that reorganize into a single cortical ring (Iwase *et al.*, 2006; Merlini & Piatti, 2011). The single septin ring later rearranges into an hourglass structure. As the bud grows and at the initiation of cytokinesis, the septins form a double ring surrounding the actomyosin ring (Cid *et al.*, 2001; Lippincott *et al.*, 2001). After cytokinesis, a single septin ring is retained in both the mother and daughter cells, marking the site of cell separation (Cid *et al.*, 2001). The septins act as a scaffold for contractile ring assembly and primary septum formation, two processes that drive cytokinesis in budding yeast. The septins also establish a diffusion barrier at the bud neck, which is critical for asymmetric cell division, as described below.

Asymmetric inheritance of aging determinants in cell division in *S. cerevisiae*

Retention of damaged protein aggregates in mother cells

As cells age, oxidatively damaged proteins begin to accumulate, forming toxic protein aggregates. These protein aggregates are preferentially retained in the mother cell, which promotes mother cell aging and production of a daughter cell with a full replicative lifespan (Aguilaniu *et al.*, 2003; Erjavec *et al.*, 2007; Liu *et al.*, 2010). This asymmetric inheritance is established by two mechanisms: one sequesters damaged protein aggregates in the mother cell, and the other removes aggregates from the daughter cell. Both processes are important for promoting cellular health and lifespan (Hill *et al.*, 2014; Song *et al.*, 2014). Elevation of the type I metacaspase, Mca1p, promotes clearance of aggregates in daughter cells and extends lifespan (Hill *et al.*, 2014). In addition, Sir2p, the founding member of the Sirtuin family of lifespan regulators, has genetic interactions with proteins that are required for asymmetric inheritance of protein aggregates and promote longer lifespan (Song *et al.*, 2014). These studies provide evidence that protein aggregates are bona fide aging determinants and not just a marker of aging.

Damaged or aggregated proteins are sequestered in two quality control compartments, the juxtanuclear quality control compartment (JUNQ) and the insoluble protein deposit (IPOD) (Kaganovich *et al.*, 2008). Under certain stress conditions, misfolded proteins accumulate in JUNQs, which are puntate perinuclear structures. In contrast, IPODs are sites where aggregation-prone, insoluble proteins are sequestered and are found near the vacuole (Spokoini *et al.*, 2012). Hsp104p, a disaggregase that binds to misfolded proteins and promotes their refolding or degradation when refolding cannot occur, has been localized to IPODs (Kaganovich *et al.*, 2008; Spokoini *et al.*, 2012).

Hsp104p, the actin cytoskeleton and Sir2p are all required for asymmetric inheritance of protein aggregates (Erjavec & Nystrom, 2007; Tessarz *et al.*, 2009; Orlandi *et al.*, 2010; Spokoini *et al.*, 2012). Deletion of *HSP104* results in a breakdown of damage asymmetry and failure of protein stress foci to undergo degradation or form inclusions, while its overexpression partially rescues defects seen in *sir2*Δ (Erjavec *et al.*, 2007; Spokoini *et al.*, 2012). Destabilization of the actin cytoskeleton or actin cables results in loss of the asymmetric segregation of protein aggregates, including Hsp104p-associated oxidized protein aggregates and Huntingtin aggregates (Liu *et al.*, 2011). Moreover, cells lacking Sir2p exhibit increased protein carbonylation, premature protein aggregate formation and failure to retain damaged proteins in the mother cell. A genomewide screen in *S. cerevisiae* revealed a link between Sir2p and proteins required for polarization of the actin cytoskeleton (Spa2p, Pea2p, Bud6p, Cdm1p, Myo2p) and proteins involved in assembly, elongation, and retrograde flow of actin cables (Bni1p) (Liu *et al.*, 2011; Song *et al.*, 2014). Consistent with this, deletion of Sir2p destabilizes actin cables and inhibits RACF

(Liu *et al.*, 2011; Higuchi *et al.*, 2013). This raises the possibility that Sir2p may function in asymmetric inheritance of protein aggregates through effects on the actin cytoskeleton and RACF.

The precise role of actin cables in asymmetric inheritance of protein aggregates is controversial. Hsp104p-GFP labeled oxidized protein aggregates exhibit linear, polarized movement from buds toward mother yeast cells (Liu *et al.*, 2010, 2011; Spokoini *et al.*, 2012). These studies also revealed that Hsp104p and Huntingtin aggregates colocalize with actin cables and that destabilization of actin cables by deletion of the tropomyosin, Tpm1p, results in loss of the asymmetric distribution of oxidized protein aggregates. Other studies revealed that cargos, like endosomes (actin patches) and mitochondria, can bind to actin cables and use the force of RACF to drive their movement from buds to mother cells (Fehrenbacher *et al.*, 2004; Huckaba *et al.*, 2004). Together, these findings support the model that RACF generates asymmetric distribution of protein aggregates by driving their movement from buds to mother cells (Fig. 3a).

On the other hand, Zhou *et al.* used a particle-tracking algorithm to follow the patterns and trajectories of these aggregates and concluded that damaged protein aggregates undergo stochastic, rather than directed, diffusion (Zhou *et al.*, 2011). They propose that the difference between mother and bud sizes and the narrow width of the neck are sufficient for retention of protein aggregates in the mother cell (Fig. 3b). However, a recent study indicates that increasing cell cycle time or increasing mother cell size, which should decrease or promote diffusion-dependent retention of protein aggregates in mother cells, respectively, has no effect on the asymmetric distribution of protein aggregates (Song *et al.*, 2014).

On the other hand, it is possible that the stochastic movements detected by Zhou and colleagues may be those of inclusions, like IPODs or JUNQs, that are bound to organelles, have limited movement and are retained in mother cells (Spokoini *et al.*, 2012; Nystrom & Liu, 2014). RACF may help establish asymmetry by trafficking damaged proteins and smaller stress foci to these IPODs, JUNQs, or similar inclusions, which are then retained in the mother cells by their association with its corresponding organelles. Yet to be determined is how the inclusions, such as IPODs and JUNQs, are retained in the mother cell during inheritance of the vacuole, nucleus, and potentially other organelles.

The integrity and dynamics of the actin cytoskeleton also has implications for cellular health in nondividing cells. Mice that lack the actin depolymerizing protein cofilin2 exhibit accumulation of cytotoxic protein aggregates and defects in actin organization and skeletal muscle maintenance (Gurniak *et al.*, 2014). While the causal relationship of these two phenotypes remains to be investigated, it is possible that protein aggregates normally undergo cytoskeleton-dependent movement to be sequestered and confined in nondividing mammalian cells. Indeed, mechanisms involving dynein-dependent retrograde transport along microtubules have been implicated in formation of damaged protein inclusions called aggresomes in mammalian cells (Garcia-Mata *et al.*, 1999; Kopito, 2000). These aggresomes accumulate around centrosomes and may potentially act as a center to concentrate damaged proteins for resolution by clearance pathways, such as autophagy (Johnson & Englund, 1998).

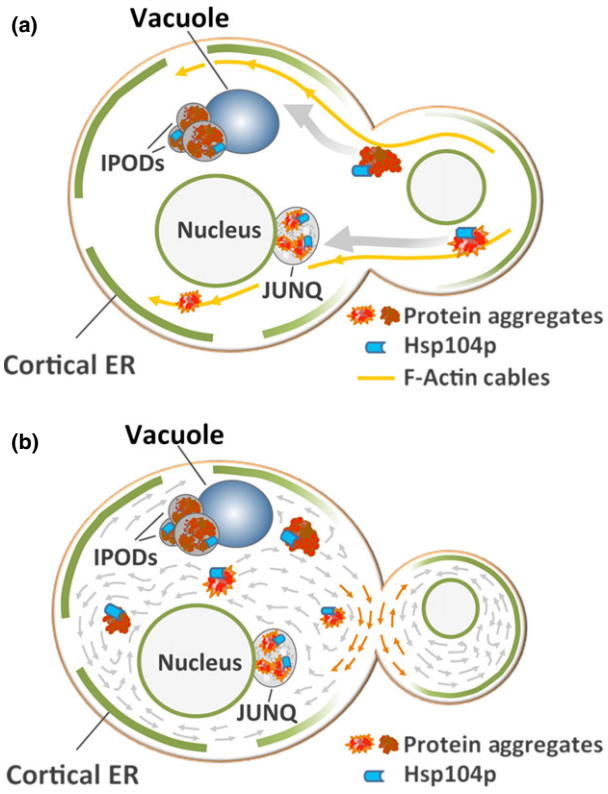

Fig. 3. The directed movement and stochastic diffusion models of protein aggregate retention in mother cells. (a) One model for actin cable function in clearance of protein aggregates from buds. Here, protein aggregates move in a directed, RACF-dependent fashion from buds toward mother cells and are sequestered at IPODs and JUNQs within the mother cell. (b) According to the stochastic diffusion model of protein aggregate movement, protein aggregates undergo random diffusion. Because the mother cell is much larger than the bud and the bud neck is a tight bottleneck, large protein aggregates that form in the mother cell are retained in the mother and are not inherited by the bud.

Inheritance of fitter mitochondria by buds

The mitochondrion is an essential organelle acting as the hub of energy production, central metabolism, and other

activities, such as calcium signaling and regulation of apoptosis. Accumulation of mitochondrial damage leads to loss of function of the organelle and is implicated in aging and related diseases such as neurodegeneration and muscle myopathies (McFaline-Figueroa *et al.*, 2011; Lopez-Otin *et al.*, 2013; Ramadasan-Nair *et al.*, 2014; Stauch *et al.*, 2014). Dysfunctional mitochondria are characterized by lower ATP production, decreased membrane potential ($\Delta\psi$), loss of or damage to mitochondrial DNA (mtDNA), increased ROS production, and more oxidizing redox states (Green *et al.*, 2011; McFaline-Figueroa *et al.*, 2011; Park & Larsson, 2011; Hughes & Gottschling, 2012).

Mitochondria undergo fusion and fission events, and recent studies have shown that mitochondria in *S. cerevisiae* are not a single, continuous reticulum (McFaline-Figueroa *et al.*, 2011). Specifically, fluorescence loss in photobleaching (FLIP) experiments indicates that mitochondria in large buds are physically distinct from mitochondria in mother cells. Moreover, use of biosensors to assess mitochondrial function in living yeast revealed that mitochondria in the bud have lower levels of reactive oxygen species (ROS) and are more reducing compared with mitochondria in mother cells. Thus, mitochondria, known aging determinants, undergo asymmetric inheritance, based on their functional state, during yeast cell division (McFaline-Figueroa *et al.*, 2011; Higuchi *et al.*, 2013).

Role of the actin cytoskeleton in aging through effects on asymmetric inheritance of high from low functioning mitochondria

As the direction of RACF is from buds to mother cells, mitochondria effectively 'swim upstream' against the opposing force of RACF to move from mother cells to buds. Therefore, RACF may contribute to the asymmetric inheritance of mitochondria by serving as a filter to prevent damaged and dysfunctional mitochondria from entering the bud during inheritance and/or as a force generator to drive movement of low functioning mitochondria out of buds. To test this hypothesis, Higuchi *et al.* (2013) assessed the effect of altering the rate of RACF on mitochondrial function, replicative lifespan, and cellular healthspan. RACF flow rates were decreased by deletion of Myo1p, the myosin that generates pulling forces on actin cables, or increased by deletion of Tpm2p, the tropomyosin that inhibits binding of Myo1p to actin cables.

These studies revealed that increasing the rate of RACF results in an increase in mitochondrial fitness. These mitochondria were more reduced and exhibited higher velocities of bud-directed movement compared with mitochondria in wild-type cells. Here, the redox state of the organelle was measured using redox-sensitive green fluorescent protein (roGFP), that is, targeted to the mitochondrial matrix (Vevea *et al.*, 2013). Mito-roGFP contains two surface-exposed cysteines that undergo environment-dependent oxidation and reduction, which in turn changes the excitation spectrum of the protein. Increasing RACF rates also promoted asymmetric inheritance of the organelle. Conversely, reducing the rate of RACF had the opposite effects. Thus, these studies revealed a role for the actin cytoskeleton in mitochondrial quality control and asymmetric inheritance of mitochondria, and the mechanism underlying these processes. As many cargos use actin cables for movement into and out of the bud, RACF may affect the asymmetric distribution of other cargos.

Increasing the rate of RACF also results in extension of replicative lifespan and increased cellular healthspan (i.e. decrease in mean generation time), and decreasing RACF rates has the opposite effect. Moreover, increasing the rate of RACF does not extend lifespan in yeast bearing a deletion of mitochondrial DNA (mtDNA) and the associated loss of mitochondrial respiratory activity. Thus, actin dynamics affects lifespan through its effects on mitochondria (Fig. 4). Finally, these studies revealed that deletion of *SIR2* results in a decrease in the rate of RACF and

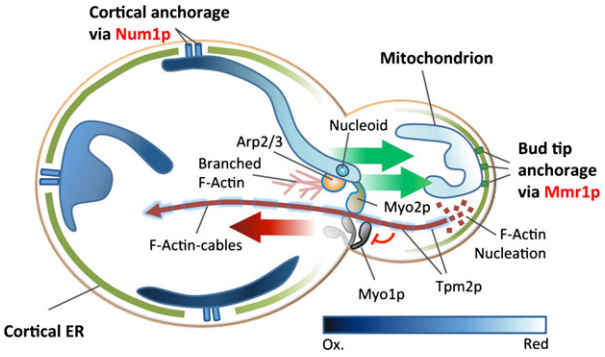

Fig. 4. RACF and anchorage of mitochondria in the bud tip contribute to the asymmetric segregation of fit from less fit mitochondria during yeast cell division. RACF acts as a filter to prevent inheritance of less fit, dysfunctional mitochondria into the bud. One possible mechanism for this effect is that fitter mitochondria may be better able to recruit drivers of anterograde movement and can overcome the force of RACF and enter the bud. Therefore, only higher functioning mitochondria move to the bud tip. Once they reach that site, they are anchored to cER in the bud tip by Mmr1p. Together, these two mechanisms generate asymmetry where fitter, higher functioning mitochondria are preferentially inherited, while damaged, dysfunctional mitochondria are retained in the mother. Lighter colors indicate higher functioning mitochondria and darker colors indicate lower-functioning mitochondria.

mild overexpression of *SIR2* has the opposite effect. This finding, that Sir2p regulates RACF, raises the possibility that Sir2p controls lifespan, in part, through its effect on actin dynamics.

Retrograde flow is an emerging topic of interest in mammalian cells, particularly in neurite growth in neurons (Ilani *et al.*, 2009; Flynn *et al.*, 2012; Zhang *et al.*, 2012). Indeed, retrograde actin flow occurs in the growth cone of developing neurites and loss or perturbation of actin dynamics results in defects in microtubule extension necessary for neurite formation (Flynn *et al.*, 2012). An ongoing question is whether retrograde flow of actin networks or bundles can contribute to cellular asymmetry of mitochondria and other cellular components in mammalian cells, similar to effects found in yeast cells. It is conceivable that high energy-demanding processes, such as growth cone extension, would require high concentrations of functional mitochondria for ATP production and calcium regulation at that site.

Site-specific anchorage controls asymmetric mitochondrial inheritance and lifespan

As a natural counterpart to mitochondrial transport, mechanisms to arrest transport or anchor mitochondria at specific subcellular areas play essential roles in adapting mitochondrial distribution to cellular needs and directly interface with pathways of mitochondrial quality control (Schwarz, 2013; Naon & Scorrano, 2014). Recent studies support a role for anchorage of mitochondria in the bud tip in asymmetric inheritance of the organelle and lifespan control. Visualization of mitochondria in living yeast cells revealed that they accumulate in the bud tip by anchorage to a limited number of sites in the bud tip by binding to cortical ER (cER), a network of ER tubules and sheets, that is, anchored to the plasma membrane (Swayne *et al.*, 2011).

These studies also revealed a role for Mmr1p in anchorage of mitochondria in the yeast bud tip. Mmr1p is a DSL-family tethering protein that undergoes asymmetric inheritance during yeast cell division: the protein and mRNA encoded by the *MMR1* gene localize to the bud tip. Myo4p, and the cargo adapters, She2p and She3p, drive movement of *MMR1* mRNA to the bud (Shepard *et al.*, 2003). Myo2p, another type V myosin, and a protein phosphatase, Ptc1p, which affects the phosphorylation state of Mmr1p, are required for localization of *MMR1*-encoded protein to the bud tip (Itoh *et al.*, 2004; Swayne *et al.*, 2011). Moreover, deletion of *MMR1* abolishes accumulation and anchorage of mitochondria at the bud tip. Finally, Mmr1p localizes at the interface of mitochondria and cER at the bud tip and is recovered with both organelles upon cellular fractionation. These studies support a role for Mmr1p in docking mitochondria in the bud tip by tethering mitochondria to cER at that site (Swayne *et al.*, 2011).

Interestingly, *mmr1*Δ cells also have defects in asymmetric segregation of mitochondria, replicative lifespan, and mother–daughter age asymmetry. Deletion of *MMR1* gives rise to two populations of yeast cells: a short-lived population of cells with more oxidizing mitochondria and increased mean generation time, and a long-lived population with decreased mean generation time and mitochondria of superior redox state and reduced ROS levels. The majority of short-lived population of *mmr1*Δ cells fails to give rise to daughter cells. However, the daughters of long-lived *mmr1*Δ cells have replicative lifespans that are significantly shorter than that of their mother cells (McFaline-Figueroa *et al.*, 2011). These studies provide additional links between mitochondrial function and asymmetric inheritance of the organelle with lifespan. They also provide a role for Mmr1p in this process, as a docking protein that anchors and retains higher functioning mitochondria in the bud tip.

The Rab-like protein, Ypt11p, has been implicated in facilitating retention of mitochondria at the bud tip by promoting the inheritance of cER. Ypt11p localizes to cER in the bud and is required for normal inheritance of cER (Buvelot Frei *et al.*, 2006). Deletion of *YPT11* results in defects in anchorage of mitochondria in the bud tip, which are less severe than those observed upon deletion of *MMR1* (Boldogh *et al.*, 2004; Lewandowska *et al.*, 2013). *ypt11*Δ cells exhibit aging defects that are similar to those observed in *mmr1*Δ cells. Deletion of *YPT11* gives rise to a long-lived and short-lived population of yeast cells (Rafelski *et al.*, 2012). While Mmr1p and Ypt11p appear to have a functional relationship in mitochondrial anchorage, recent evidence suggests they may also have partially redundant roles in anterograde mitochondrial transport by linking mitochondria to Myo2p (Chernyakov *et al.*, 2013; Westermann, 2014). Their direct role in mitochondrial trafficking is still under investigation, but it is clear that through their role in mitochondrial anchorage, they can counterbalance the effects of mitochondrial motility and ultimately affect inheritance.

Given the critical roles of Mmr1p and Ypt11p in anchorage at the bud tip, the recent identification of Num1p as a maternal anchorage protein (Lackner *et al.*, 2013) sparked considerable interest in the role of maternal mitochondrial retention during cell division. Num1p binds the plasma membrane via its C-terminal pleckstrin homology (PH) domain and forms cortical patches, which localize to the mother cell through most of the cell cycle (Klecker *et al.*, 2013). Deletion of *NUM1* results in

defects in peripheral localization of mitochondria along the maternal cellular cortex as tubules (Lackner *et al.*, 2013). Mitochondria in these mutants were also more mobile, suggesting a role in physical anchorage and retention of mitochondria in the mother cell. Yet to be determined is whether there are other proteins that contribute to retention of mitochondria in mother cells, and how this process affects mitochondrial quality control during inheritance, lifespan, and mother–daughter age asymmetry.

Overall, these findings indicate that RACF serves as a filter to prevent low functioning mitochondria from being inherited by the bud by generating force that can drive movement of mitochondria from buds back to the mother cell. They also support a role for RACF and anchorage of mitochondria in the bud tip in asymmetric inheritance of mitochondria, mother–daughter age asymmetry, and lifespan control. Thus, during normal asymmetric inheritance in yeast, mitochondria not only have to 'swim against the stream' of RACF but they also need bud tip anchorage to 'reach the shore' (Fig. 4).

Role of diffusion barriers at membranes in the bud neck in asymmetric cell division

Diffusion barriers in the plasma membrane

As described above, > 30 mRNAs undergo actin-dependent transport to the bud in *S. cerevisiae*. At least 10 of the proteins encoded by these mRNA are asymmetrically distributed including Mmr1p, the protein that mediates anchorage of high functioning mitochondria in the bud tip, and Ist2p, Tcb2p, and Tcb3p, three of the six proteins that mediate anchorage of cER to the plasma membrane (Shepard *et al.*, 2003). Equally important, most of the proteins that are encoded by bud tip-targeted mRNAs and asymmetrically distributed are membrane-associated. Early studies on Ist2p revealed a diffusion barrier in the plasma membrane at the bud neck that contributes to asymmetric distribution of proteins encoded by bud-targeted *IST2* mRNA (Takizawa *et al.*, 2000). Fluorescence recovery after photobleaching (FRAP) revealed that GFP-Ist2p freely diffuses in the membrane of the bud, but does not diffuse across the bud neck in wild-type yeast. However, shift of a temperature sensitive septin mutant (*cdc12-6*) from permissive to restrictive temperature results in loss of the asymmetric distribution of GFP-Ist2p. These studies support the model that septins are required for formation of a diffusion barrier in plasma membrane at the bud neck that maintains the asymmetric distribution of plasma membrane proteins synthesized in or transported to the bud.

Diffusion barriers in the ER

Fluorescence loss in photobleaching (FLIP) experiments on fluorescent proteins targeted to the ER lumen or membrane revealed that there is also a barrier at ER membranes near the bud neck (Luedeke *et al.*, 2005; Chao *et al.*, 2014; Clay *et al.*, 2014). Furthermore, recent work showed that misfolded ER and Golgi proteins are retained in the mother cell by a diffusion barrier formed at the bud neck (Clay *et al.*, 2014). Production and maintenance of the diffusion barrier is dependent on the concerted effort of several factors (Luedeke *et al.*, 2005; Chao *et al.*, 2014; Clay *et al.*, 2014; Wong *et al.*, 2014). First, a septin ring is formed and recruits cell polarization proteins including the Ras family protein Bud1p and its GTP/GDP exchanger, Bud5p. These proteins activate polarisome proteins, such as Pea2p and Bud6p, a protein that contributes to actin cable formation and assembly, via the Rho-family GTPase protein Cdc42p and its guanine nucleotide exchange factor, Cdc24p. This then results in the accumulation of sphingolipids at the bud neck, which act – either directly or indirectly as a scaffold for secondary proteins – to prevent lateral diffusion of misfolded ER proteins from mother to daughter cell (Fig. 5). A recent study proposed a novel role for the septin Shs1p in recruiting Scs2p and Epo1p to the septin ring to maintain the ER diffusion barrier (Chao *et al.*, 2014). Scs2p is

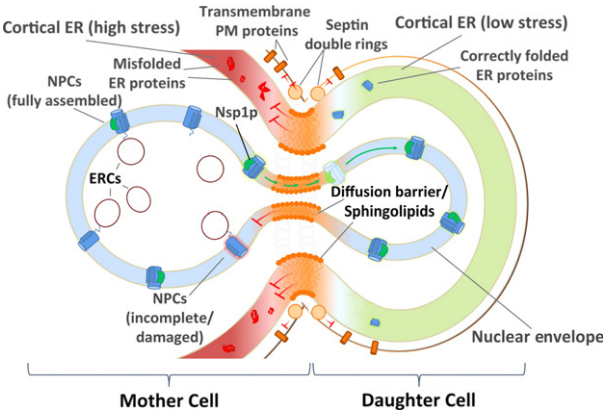

Fig. 5. The septin cytoskeleton and sphingolipids serve as a diffusion barrier in the ER, plasma membrane, and nuclear envelope. Septins form a ring at the bud neck, which allows recruitment of polarization proteins and accumulation of sphingolipids at that site. The concerted effort of these factors produces a diffusion barrier, which prevents free diffusion of transmembrane – and potentially some luminal – proteins within the ER, plasma membrane, and nuclear envelope at the bud neck. This diffusion barrier may promote asymmetric segregation of factors, including ERCs, NPCs, and damaged ER proteins, such that damage is retained in the mother (depicted in red) and daughter cells inherit only healthy and fully functional cellular components (depicted in green).

an integral ER membrane protein and one of the six proteins that links cER to the plasma membrane. It also controls PIP4 metabolism. Epo1p binds to both Scs2p and Shs1p and may therefore tether cER to the septins. Mutations that disrupt the interaction or functions of Shs1p, Scs2p, and Epo1p result in free diffusion of ER membrane proteins between mother and bud cells, suggesting that this ER-septin complex contributes to the ER diffusion barrier.

These findings support that model that a diffusion barrier at ER membranes in the bud neck of *S. cerevisiae* prevents movement of ER proteins between the bud and mother cell, and a role for the septins and sphingolipids in generating that diffusion barrier. Septins also localize to the dendritic spines of neurons where there is highly restricted diffusion of ER proteins (Tada *et al.*, 2007; Cui-Wang *et al.*, 2012). Thus, septins may also contribute to ER diffusion barriers in higher eukaryotes.

Disruption of any of the proteins required for the ER diffusion barrier results in inheritance of misfolded ER proteins by the daughter cell. It also affects lifespan under conditions of ER stress produced by deletion of *YOS9*, an ER quality control lectin. *BUD1*, a bud site selection gene, and *SUR2*, which encodes a sphingolipid biosynthetic enzyme, are both required for the ER diffusion barrier at the bud neck. Deletion of either protein rescues lifespan in *yos9*Δ cells (Clay *et al.*, 2014). Bud1p and Sur2p have additional functions other than control of the diffusion barrier in ER at the bud neck, which may be the cause of the characterized lifespan effects. However, these findings raise the possibility that this diffusion barrier contributes to lifespan control in yeast undergoing ER stress through effects on the asymmetric distribution of unfolded ER proteins (Obeid *et al.*, 2002).

Diffusion barriers in the nuclear envelope

One of the first identified markers of replicative age in *S. cerevisiae* is the accumulation of extrachromosomal ribosomal DNA circles (ERCs) in the nucleus of mother cells (Sinclair & Guarente, 1997; Steinkraus *et al.*, 2008). rDNAs consist of tandem repeat arrays. Recombination within rDNAs results in copy number loss and generation of ERCs. Several findings support a link between ERCs and lifespan control. Expression of high-copy episomal DNAs reduces replicative lifespan (Falcon & Aris, 2003). Moreover, deletion of *SIR2*, which results in premature formation of ERCs by increasing rDNA recombination rates, results in decreased lifespan (Kaeberlein *et al.*, 1999). Thus, age-associated increases in ERCs may decrease lifespan. On the other hand, deletion of *FOB1*, a gene encoding a replication fork block protein, results in decreased rDNA recombination and ERC formation and increases lifespan (Kaeberlein *et al.*, 1999). Similarly, loss of Sgf73p, a component of the SAGA complex, which counteracts Sir2p function in rDNA recombination, has been shown to significantly increase lifespan (McCormick *et al.*, 2014).

How ERCs specifically contribute to aging and ultimately senescence in yeast is still unknown. They may sequester essential replication factors, transcription factors, or repair proteins, making them unavailable for endogenous functions. Alternatively, there is evidence that rDNA instability, which gives rise to ERCs and are caused by ERC accumulation, is an aging determinant. Ganley *et al.* find that ERC levels are not linked to lifespan in yeast. Instead, they find that conditions that promote or reduce rDNA instability decrease and increase lifespan, respectively. They also find that multicopy episomal plasmids can induce rDNA instability and that rDNA instability is preferentially reduced in mother cells (Ganley *et al.*, 2009). This study supports the model that rDNA instability contributes to lifespan control, presumably by reducing ribosome quality and quantity (Kobayashi, 2008; Ganley *et al.*, 2009). Moreover, as ERCs are episomes, they may also contribute to lifespan control through effects on rDNA stability. Indeed, accumulation of ERCs in mother cells may contribute to instability of rDNA, which occurs in mother but not daughter cells.

The underlying mechanism promoting asymmetric inheritance of ERCs is controversial. There is evidence that septin-dependent diffusion barriers in the nuclear envelope inhibit the passage of ERCs to the daughter cell in *S. cerevisiae* (Shcheprova *et al.*, 2008; Clay *et al.*, 2014). The diffusion barrier in the nuclear envelope is similar but not identical to that found in the ER. Nuclear envelope diffusion barriers require septins, sphingolipids, and Bud6p, but do not require Bud1p. Shcheprova *et al.* (2008) find that the diffusion barrier in the nuclear envelope at the bud neck prevents passage of nuclear pore complexes (NPCs) from the mother cell to the bud and that binding of ERCs to NPCs may be responsible for retention of ERCs in the mother cell. Consistent with this, other groups find that a subset of newly synthesized NPC proteins associate with the ER and are preferentially inherited by the bud and that this process requires the actin cytoskeleton, Myo2p, and a subcomplex containing the NPC protein Nsp1p. They also find that damaged NPCs or NPCs that lack essential components, such as Nup57p, Nup82p, or Nsp1p, cannot enter the bud and are retained in the mother cell (Colombi *et al.*, 2013; Makio *et al.*, 2013).

However, other studies indicate that a subset of NPCs, old NPCs, can be inherited by daughter cells (Khmelinskii *et al.*, 2010, 2012; Makio *et al.*, 2013) and that tethering of plasmids to the NPC does not result in asymmetric

segregation of the plasmids (Khmelinskii *et al.*, 2011). Moreover, visualization of episomes in living yeast confirms that they are retained in the mother cell nucleus, but freely diffuse in the nucleoplasm and are depleted at the nuclear envelope (Gehlen *et al.*, 2011). Thus, there is evidence that episomes do not associate with NPCs. Indeed, these studies find that the geometry of the nucleus and short length of the yeast cell division cycle are critical for retention of episomes in mother cells.

Pathways for active daughter cell rejuvenation

Previous studies revealed that higher functioning mitochondria, that are more reducing and have less ROS, are preferentially inherited by buds through multiple generations of yeast cell division, and that defects in this process affects lifespan and mother–daughter age asymmetry (McFaline-Figueroa *et al.*, 2011; Higuchi *et al.*, 2013). These studies and others also revealed that mitochondrial function in mother cells and buds declines with age as early as two generations, despite the fact that mitochondria in buds are fitter compared with those in their associated mother cells (McFaline-Figueroa *et al.*, 2011; Higuchi *et al.*, 2013). Thus inheritance of the fittest mitochondria contributes to, but is not sufficient for, generation of fully functional mitochondria in daughter cells. Below, we describe two mechanisms for daughter cell rejuvenation that may promote the function of mitochondria and other cellular constituents in daughter cells.

Activation of peroxidases in bud cells rejuvenates them postcytokinesis

One mechanism for daughter cell rejuvenation is increasing antioxidant activity (Erjavec & Nystrom, 2007). Specifically, Erjavec and Nystrom show that the levels of cytosolic peroxidase, Ctt1p, hydrogen peroxide, and superoxide are similar in mother cells and their associated buds. However, immediately after cytokinesis and separation of buds from mother cells, there is a twofold increase in catalase activity and a precipitous decrease in ROS levels in daughter cells, but not in mother cells. Daughter cell-specific activation of catalase does not occur in *sir2*Δ cells or in cells treated with Latrunculin-A, a drug that disrupts the actin cytoskeleton. These findings indicate that a rejuvenating environment, which reduces oxidative stress, is generated in daughter cells after they have separated from mother cells. As mitochondria in buds exhibit a decrease in redox state and increase in superoxide levels as a function of mother cell age, this rejuvenating environment in daughter cells can promote the function of mitochondria and other daughter cell constituents that are damaged by mitochondrial ROS.

Future studies will reveal the role of Sir2p and the actin cytoskeleton in this daughter cell-specific rejuvenation mechanism. However, recent work revealed that *S. cerevisiae* undergoes rejuvenation that resets the aging clock during sporulation, the meiosis-driven conversion of diploid cells to haploid spores (Unal *et al.*, 2011). Specifically, as old mother cells undergo sporulation, they exhibit an increase in Hsp104p aggregates and ERCs. However, after completion of meiosis and sporulation, the spores have decreased Hsp104p-protein aggregates and ERCs, and replicative lifespans that are similar to that of spores from young mother cells. These studies also revealed that Ndt80p, a sporulation-specific transcription factor, is required for resetting the aging clock after sporulation. Interestingly, expression of Ndt80p in yeast undergoing vegetative, mitosis-driven growth is sufficient to extend the lifespan of old yeast cells. Thus, it is possible that genes regulated by Ndt80p may contribute to catalase rejuvenation after completion of mitotic cell division in *S. cerevisiae*.

Vacuolar reacidification in bud cell rejuvenation

Vacuole fitness is associated with its acidity, which declines early with age in *S. cerevisae* (Hughes & Gottschling, 2012). It is not clear what causes this decline in vacuole function; however, it is clear that this has implications in aging. Indeed, mouse models of Alzheimer's disease exhibit decreased autophagy due to loss of lysosomal acidity (Wolfe *et al.*, 2013). In addition, overexpression of *VMA1*, the catalytic subunit of the vacuolar H1-ATPase, results in increased and prolonged vacuolar acidity and an increase in replicative lifespan in budding yeast (Hughes & Gottschling, 2012). Using $DiOC_6$ to monitor mitochondrial $\Delta\psi_m$, these studies confirmed previous findings that mitochondria in mother cells and buds also undergo a decline in function with early age. Interestingly, overexpression of *VMA1* also reduces the age-associated decline in mitochondrial $\Delta\psi_m$.

Perhaps surprisingly, vacuolar function in degradation of cellular constituents does not contribute to its function in lifespan control. Rather, loss of vacuolar acidity results in the progressive accumulation of neutral amino acids, including glutamine, within the cytosol. Mitochondria internalize neutral amino acids using $\Delta\psi_m$-dependent transport processes, which decreases mitochondrial fitness and induces fragmentation of the organelle (Hughes & Gottschling, 2012). Additionally, the buildup of neutral amino acids increases TORC1 activity, which has

pro-aging consequences (Hughes & Gottschling, 2012; Schmidt & Kennedy, 2012).

Interestingly, these studies also revealed that vacuoles undergo asymmetric inheritance. Specifically, vacuoles in mother cells undergo a decline in acidity (Schmidt & Kennedy, 2012). However, vacuoles in daughter cells remain acidic irrespective of the age of the mother cell (Henderson *et al.*, 2014). This asymmetry in vacuolar acidity is promoted by the inherent asymmetry in the plasma membrane proton ATPase, Pma1p, which pumps protons out of the cytosol and antagonizes vacuolar acidity by decreasing the pool of cytoplasmic protons available for import into the vacuole via vacuolar ATPases. Pma1p is retained in aging mother cells and its absence in the bud increases the availability of protons in the bud cytosol, allowing reacidification of the vacuole specifically in the bud (Henderson *et al.*, 2014). Control of vacuolar acidity may be another mechanism for rejuvenation of mitochondria and other aging determinants in yeast daughter cells.

Concluding remarks

Successful proliferation of the single-celled *S. cerevisiae* is dependent on 'resetting' the age of the daughter cell. Aberrant and dysfunctional proteins and organelles are retained in the mother cell while the daughter inherits highly functional organelles and a higher proportion of proteins and their mRNAs involved in stress response and repair. Mitochondria, the vacuole, ERCs, ER, mRNA, and damaged protein aggregates are all asymmetrically inherited during division in budding yeast. This asymmetry is dependent on the actin, microtubule, and septin cytoskeletons. Several important questions are yet to be answered regarding key mechanisms that result in asymmetry. For example, while there are key aging determinants that are retained in the mother cell including ERCs and damaged protein aggregates, the mechanisms that result in this asymmetry are still poorly understood. What mechanism underlies actin-dependent retention of damaged protein aggregates in the mother cell? Are there other still unidentified aging determinants that are also asymmetrically segregated via the RACF filter? How are ERCs asymmetrically segregated? Is their retention dependent on septin-mediated asymmetric segregation of NPCs or on nuclear envelope geometry and cell cycle duration?

There are also outstanding questions regarding rejuvenation events in the bud. Some factors have been identified including activation of catalases, reacidification of vacuoles in the bud, and active transport of mRNAs to the bud. Still to be discovered are the mechanisms involved in rejuvenation of the bud. Is activation of catalases and other protective proteins involved in quality control essential for bud rejuvenation, and if so, how does this activation occur? What is the mechanism underlying the retention of the plasma membrane proton-pumping ATPase, Pma1p, in mother cells? Further investigations of these complex issues will lead to a greater understanding of asymmetric cell division and its contribution to aging. It will also lay the foundation for future studies in mammalian cells that also undergo polarized growth and asymmetric cell divisions.

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