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Unbalanced Growth, Senescence and Aging

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Abstract

Usually, cells balance their growth with their division. Coordinating growth inputs with cell division ensures the proper timing of division when sufficient cell material is available and affects the overall rate of cell proliferation. At a very fundamental level, cellular replicative lifespan—defined as the number of times a cell can divide, is a manifestation of cell cycle control. Hence, control of mitotic cell divisions, especially when the commitment is made to a new round of cell division, is intimately linked to replicative aging of cells. In this chapter, we review our current understanding, and its shortcomings, of how unbalanced growth and division, can dramatically influence the proliferative potential of cells, often leading to cellular and organismal aging phenotypes. The interplay between growth and division also underpins cellular senescence (i.e., inability to divide) and quiescence, when cells exit the cell cycle but still retain their ability to divide.

Keywords

Cell senescence; Quiescence; Aging; Cell growth; Protein translation; mTOR signaling; Asymmetric division; Hypertrophy and Cdk

8.1 Introduction

The two cells generated at the end of the cell cycle usually inherit sufficient amounts of essential constituents, ensuring their survival. Moreover, the composition of proliferating cells varies very little from generation to generation, implying that proliferating cells somehow balance their growth (increase in biomass) with their division. Since different levels of nutrients and growth factors sustain different rates of cell proliferation, cells have elaborate mechanisms to sense nutrient and growth signals, adjusting their metabolic and proliferative activity accordingly. A detailed mechanistic understanding of this coupling between growth and division has remained elusive. Nonetheless, properly coupling growth with division is thought to determine the rate at which cells proliferate [1–6].

Nutrient and growth factor limitations do not delay all cell cycle transitions uniformly. Instead, transit through some cell cycle phases is delayed disproportionately. Overwhelmingly, poor growth conditions prolong the G1 phase of the cell cycle, preceding initiation of DNA synthesis, while transit through the remaining cell cycle phases is not

delayed significantly [1, 2, 7–12]. In yeast, the point of commitment to a new round of cell division is called START [2] and in animal cells the Restriction Point [6]. Once cells pass through these points in late G1 phase, they will initiate and complete their division even if they encounter growth limitations [1, 2, 6, 12]. Hence, although there may be some nutrient and growth factor inputs in later stages of the cell cycle [13, 14], it is in G1 that cells delay committing to a new round of cell division in the face of weak growth prospects.

The consequences of uncoupling growth from division are profound and accompanied by changes in the size of cells (see Fig. 8.1 for a schematic). In this Chapter, we describe possible outcomes when growth and division are not balanced. We also discuss models that envision imbalances between growth and division as a critical component of senescence and aging mechanisms [15, 16]. Our discussion will include examples from animal models systems and unicellular organisms, especially the budding yeast *S. cerevisiae*.

8.2 Growth and Division: A Tight Balancing Act

Intuitively, it makes sense that for a cell to successfully divide and give rise to two viable cells, it must produce enough macromolecules, membranes, and organelles for the two cells that will arise at the end of cytokinesis. Since these cellular components are determinants of the cell's volume, it is not surprising that cell size has often been used as an "umbrella" metric for cell growth [3, 5, 14, 17, 18]. Lately, there is renewed interest in the development of methodologies that report accurately and precisely on the size of animal cells [5, 19–21]. In addition, asymmetric segregation of cellular constituents between two products of a mitotic cell division is also tightly regulated and will be discussed below.

Although cell size is a very useful "growth" metric, the events most closely associated with cell proliferation are anabolic processes that yield the macromolecules necessary to build new cells. Among those macromolecules, proteins are often considered the most important component of growth, and for good reason. The protein fraction of dry mass is ≈55% in *E. coli* [22] and ≈40–45% in the budding yeast *S. cerevisiae* [22, 23]. The protein content of mammalian cells varies in different tissues. For commonly used cell lines, such as mouse fibroblasts (NIH3T3 cells) or human HeLa cells, the protein molecules per unit volume is roughly the same as in budding yeast cells (1–2E + 06 proteins/fL; [22]). Also, a significant fraction of the proteome (>20%) is dedicated to making ribosomal proteins and translation factors that will, in turn, promote the synthesis of more proteins [24].

Making ribosomal components and assembling them into functional ribosomes involves a broad repertoire of cellular constituents and processes [25–27]. In budding yeast, the cytoplasmic ribosome contains 78 ribosomal proteins encoded by the RP regulon of 138 genes. Note that 59 of the 78 yeast ribosomal proteins are encoded by pairs of very similar paralogs [28, 29]. The ribosomal proteins together with the four rRNAs (5S, 5.8S, 18S, and 25S) make up the ribosome. The rRNA genes are encoded by rDNA tandem repeats, whose number is dynamic (usually ≈100–200) and varies with growth conditions. Greater than 200 protein assembly and accessory factors are needed at many stages to put a functional ribosome together. Their expression is thought to be regulated coordinately, through the ribosome biogenesis (Ribi) regulon. In the Ribi regulon, one also finds the various tRNA

synthetases, rRNA processing and modifying enzymes, and translation factors, which collectively control translational capacity [30, 31]. Most of the cell's transcriptional activity is devoted to building and maintaining the translational machinery. Of all the RNA in the cell, 80% is rRNA, 15% is tRNA, and 5% is mRNA, and a large fraction of mRNA is devoted to ribosome synthesis [25, 32].

Transcription of RP genes alone is responsible for approximately 50% of all RNA PolII-mediated transcription initiation events. The energetic cost of making the translation machinery is astounding, consuming as much as ≈90% of the total energy of fast-proliferating yeast cells [25]. Estimates of the ribosome content of cells give an even more impressive view of the centrality of ribosome biogenesis in governing the growth of cells. From super-resolution, single-molecule imaging techniques, it seems that *E. coli* cells contain 30,000–50,000 ribosomes per fL [33]. Analogous quantitative measurements are lacking in eukaryotes, but prior estimates in yeast put the number at about 200,000 ribosomes per cell [25]. On average then, during one cell cycle lasting ≈100 min, a yeast cell must produce ≈2000 ribosomes per minute. Based on these metrics of the cellular economy, one can easily see why for decades protein synthesis has been viewed as *the* fundamental measure of cell growth in considerations of balancing growth with cell division [34].

Building and maintaining the ability to synthesize proteins is such a costly process that would be expected to influence if, and when, cells commit to a new round of cell division. The earliest evidence for specific effects on the cell cycle due to translational control was the isolation of budding conditional yeast *cdc* (cell division cycle) mutants in what turned out to be translation factors [2]. Hypomorphic mutations in translation initiation factors impair the capacity of cells to initiate a new round of cell division [12, 35–40]. Moreover, signaling pathways that control initiation of division, such as the Target of Rapamycin (TOR) pathway, may do so, at least in part, by regulating translation initiation. Loss of TOR function causes G1 arrest in mammalian cells [41, 42] and yeast [43, 44]. Conversely, overexpression of translation initiation factor eIF4E in mammals is oncogenic [45], and the translational output of TOR signaling is critical for cancer initiation [46]. Moreover, inhibiting translation elongation with cycloheximide also prolongs the G1 phase of the cell cycle [12, 47]. In budding yeast, cycloheximide reduces the newborn cell size [12, 47] and the rate at which cells increase in size [48]. It also increases the critical size threshold for START [47, 48]. Together, these results support the notion that a critical rate of protein synthesis is required for G1 transit and completion of START in budding yeast [49] and animal cells [50, 51].

If ribosome biogenesis and protein synthesis are such integral parts of cell growth, propelling cells to divide, how do cells control ribosome biogenesis? In all eukaryotes, the principal regulator of catabolic processes leading to energy production is protein kinase A (PKA), while the analogous "master" regulator of anabolic, biosynthetic processes is the TOR kinase (the TORC1 complex). As we will describe in subsequent sections, these two pathways have overlapping roles as determinants of cellular and organismal replicative potential and aging. In rapidly proliferating cells, however, for maximal growth and rates of cell division, both PKA and TORC1 are active, and they are both needed to activate ribosome biogenesis fully by derepressing the Ribi regulon [44, 52, 53].

In yeast, replicative aging is defined by the number of buds that can be produced by one mother cell, indicative of the number of times it can progress through the cell cycle and undergo mitosis [54]. An increase in cell size (i.e., cellular hypertrophy) has been linked to replicative aging. The cellular hypertrophy model of aging was formulated to account for the yeast replicative aging, invoking the existence of a maximal cell size beyond which cells could not maintain division [55, 56]. According to the cellular hypertrophy model, the large cell size of old yeast mother cells is incompatible with some cellular function that is necessary for cell division. Every time mother cells divide, they increase in size until they reach the terminal, large size, at which point they will enter a state of proliferative arrest. The hypertrophy model predicts that small cells would be able to divide more times before reaching the terminal size, resulting in longer replicative lifespan. On the other hand, large cells will reach the terminal size after fewer divisions, having a shorter replicative lifespan. Overall, the model predicted that changes in replicative lifespan and cell size ought to be proportional. However, based on genome-wide measurements of cell size, we recently reported that the mean cell size of long-lived yeast mutants was not significantly different from the size of mutants that were not long-lived [57]. This finding is incompatible with the key prediction of the hypertrophy model that long-lived mutants would have a small overall cell size, allowing these cells to divide more times until they reach the terminal size and enter senescence. Therefore, based on these experiments in yeast, it seems that the cellular enlargement is not a primary determinant of aging.

The factors linked to cell growth and protein translation definitely affect aging, however. Reduced mTOR signaling leads to lifespan extension is yeast [58, 59], worms [60], flies [61], mice [62, 63], and initial studies suggest possibly humans [64, 65]. The mechanisms that underlie lifespan extension remain to be fully determined, partly due to the complexity of these signaling pathways, but evidence exists both for altered protein synthesis and enhanced protein turnover through autophagy [66]. The former comes from findings that reduced expression of several translation initiation factors and ribosomal components also lead to lifespan extension in a range of organisms [67]. It is clear at least for replicative lifespan in yeast (the number of times one cell can divide to produce daughter cells), however, that globally and uniformly inhibiting protein synthesis in insufficient to slow aging since cycloheximide is unable to mediate this effect [68]. This finding indicates that translational changes to specific mRNAs are likely conferring, at least in part, longevity phenotypes in mTORC1 and translation factor mutants.

Interestingly, one downstream factor linked to lifespan extension is *GCN4*, a transcription factor regulated itself by translation and dependent on the presence of small upstream open reading frames in its mRNA [68, 69]. Inhibition of mTORC1, reduced 60S ribosomal subunit levels and calorie restriction all lead to enhanced *GCN4* translation [70–74], which in yeast induces expression of stress and nutrient response pathways [69]. Loss of *GCN4* at least partially abrogates lifespan extension by these interventions. The mammalian ortholog of *GCN4*, *ATF4*, is also induced in cells and mice from a range of interventions conferring long lifespan [75, 76], arguing for conservation of this pathway.

Reduced mTORC1 signaling also leads to enhanced autophagy, which has been linked to lifespan extension as well in a range of conditions [77]. In non-vertebrates, for instance,

inhibition of autophagy is sufficient to block lifespan extension by mutants that affect translation [78–81]. Moreover, at least in flies and mice, induction of autophagy through overexpression of autophagy components is reported to lead to lifespan, and sometimes healthspan, extension [82–85].

The mTORC1 complex regulates transcription as well and this phenomenon has been studied extensively in yeast. For chronological aging, defined as survival in a post-replicative state, reduced mTORC1 signaling enhances longevity [59, 86], likely through mechanisms leading to enhanced transcription of a stress response transcription factor network driven by Msn2/4 and Gis1 [54]. Interestingly, reduced PKA signaling promotes chronological lifespan extension through overlapping mechanisms [87, 88].

Protein kinase A signaling has been connected to aging in multiple organisms [89]. In yeast, under maximal growth conditions in rich media, where both PKA and mTOR collaborate to drive protein synthesis, mutations leading to reduced PKA activity promote replicative and chronological lifespan extension [90, 91]. Although not studied as extensively in multi-cellular organisms, this phenomenon may also be conserved as mice lacking the protein kinase subunit RIIβ are long-lived [92, 93], as well as those lacking *ADCY5*, encoding type 5-adenylyl cyclase (AC5) that converts ATP into cAMP in turn activating PKA. These mice are stress resistant and experience a 30% increase in median lifespan [94, 95]. In addition, genetic variants leading to reduced production of the adenylyl cyclase-activating β2-adrenergic receptor, are prevalent in men from a Chinese centenarian population [95].

In conclusion, in balanced growth and division, the increased ribosome biogenesis and protein synthesis is coupled to cell division, maintaining the overall cellular homeostasis and macromolecular composition. These processes also have robust effects on aging, although the links are far from straightforward.

8.3 Asymmetric Segregation During Cell Division

When cells divide, their cellular constituents have to be divided between the two offspring and studies have started to address mechanisms underlying this partitioning. In mammalian cells, for instance primary fibroblasts in culture, division is symmetric and while partitioning may occur, it is hard to distinguish morphologically. Interestingly the culture of primary fibroblasts senescences at a similar number of population doublings, suggesting that with respect to cellular aging damaged molecules may not be partitioned specifically to one cell after division.

Yeast, being a single-celled organism, has to maintain continuous division in the colony in the face of the challenges of aging. By virtue of their division by budding, which produces a larger mother cell and a smaller bud that are easily distinguishable, yeast offers a great opportunity to detect differential segregation of cellular materials. While the mother cell ages, the daughter remains youthful, suggesting that damaged cellular constituents driving aging may remain in the mother cell [96]. Some components of the daughter cell, such as the cell wall are largely the result of new synthesis during division, representing one method of segregating old material to mothers. However, many cytoplasmic factors partition and

several aging factors are reported to remain in mothers. For instance, extrachromosomal rDNA circles (ERCs), small episomes containing rDNA repeats that drive aging possibly by competing for replication factors with chromosomal origins [97], are heavily partitioned toward mothers [98]. The mechanism likely relates to the closed mitosis of yeast, which maintain a nuclear structure. Originally nuclear pore association was proposed as a mechanism by which ERCs were retained in the mother, with the assertion that nuclear pores have very restricted access to daughters [99]. Later reports called that into question [100], and suggested that ERCs may simply not diffuse efficiency through the bud neck [101–103]. Thus geometry drives asymmetry.

Damaged aggregated proteins are also retained in the mother cells, likely through restriction of access to daughters and also by active transport of damaged molecules from daughters to back to mothers [96]. The former process again has been reported to involved both active retention and limits to passive diffusion to daughters, while the latter likely involves the actin cytoskeleton and requires Sir2, a protein deacetylase linked to aging [104]. The mechanisms underlying these processes remain to be fully elaborated.

Mitochondria are reported to undergo asymmetric inheritance in yeast, with fitter mitochondria finding their way to daughters [105]. More work is required to access whether and how partition occurs in this and other organelles. In fact, cellular processes to maintain asymmetry may be broader that we currently appreciate as recent single cell based screens have identified hundreds of asymmetrically partitioned proteins during budding. One screen identified 74 proteins partitioned to mothers and 60 to daughters [106]. Interestingly, strains lacking genes for the mother-specific proteins are more likely to have an enhanced lifespan. Whether it comes to individual proteins, damaged protein aggregates, extrachromosomal rDNA circles or organelles, evidence suggests that asymmetry breaks down with the age of the mother and this is consistent with observations that daughters from old mothers do not enjoy a full replicative lifespan.

Of course, asymmetry in cell division has massive impacts during development and cell differentiation throughout the mammalian organism. A classic example is an adult stem cell that divides to produce another stem cell and a cell committed to a differentiation pathway. Asymmetry of cellular constituents plays a role in defining cell fate in this context and it is highly likely that damaged molecules are partitioned to the more committed cell [107]. Clearly cell- autonomous and–non autonomous mechanisms are in play and it will be intriguing to determine to what extent the more elaborated mechanisms in yeast are conserved with the cell autonomous mechanisms.

8.4 Quiescence: Not Dividing, but Keep on Ticking

Cells can enter a quiescent state, in response to a range of signals, in which they do not divide, but maintain a metabolically active state and can resume the capacity to divide, when conditions permit. When cells adopt a differentiated state they exit the cell cycle, sometimes permanently [108]. In all eukaryotes, cyclin-dependent kinase (Cdk) protein complexes are at the core of the cell division machinery [109]. Initiation of cell division requires an increase in Cdk activity. Later cell cycle transitions also need high Cdk activity, while a drop

in Cdk activity triggers exit from mitosis. Cdks are Ser/Thr protein kinases, similar in structure to most kinases [110]. However, all Cdks are active only when they bind other activating proteins, such as cyclins. Cdk activity is further regulated by phosphorylation or binding of additional protein subunits. These layers of control can raise or lower overall Cdk activity, depending on the phosphorylated Cdk residue, or the interacting protein, in each case.

Changes in Cdk activity underlie transitions from resting to proliferative cellular states in health and disease. Indeed, high Cdk activity contributes to most proliferative disorders, including cancer cell development [111, 112]. On the other hand, low Cdk activity is associated with terminal differentiation [113], and accompanies poor organ regeneration, for example, in hepatic [114], cardiac [115], neuronal [116], or appendage tissues [117].

It is clear that in quiescent cells there is a strong albeit potentially reversible block in cell division. Maintaining the *potential* to divide, however, is a key feature that distinguishes quiescent from senescent cells. This concept was put to the test almost two decades ago, in a particularly lucid experiment. Microinjection of pre-formed active Cdk protein complexes was sufficient to initiate cell division in quiescent human fibroblasts, in the absence of growth factors [118].

In quiescent cells, the block in cell division is also accompanied by a profound reprogramming of cellular metabolism. The cells remain metabolically active, enabling them to stay alive (e.g., quiescent yeast cells) or perform the functions prescribed by their terminally differentiated state. Interestingly, balanced downregulation of the master “growth” signaling pathways we described above, the PKA and the TOR pathways, is observed in quiescent yeast cells [119], and this is important for chronological lifespan extension [119], which is the period of time a cell can remain viable in a non-proliferative state.

More recently, it was reported that quiescent cells have a massively re-organized chromatin structure [120]. In yeast cells entering quiescence, the conserved lysine deacetylase Rpd3p establishes a repressive transcriptional state, reducing by ≈30-fold steady-state mRNA levels [120]. Cells lacking Rpd3p also have a 2–3 fold reduction in their mean chronological lifespan [120]. The replicative lifespan of these cells, however, is not affected [121]. This is not surprising since there is no significant overlap of gene deletions that extend lifespan in both the chronological and replicative lifespan assays [122], at least under the assay conditions tested.

Interestingly, however, there are connections between the two types of yeast aging, as chronologically aged cells have reduced replicative lifespan when returned to the cell cycle [123–125]. This is clearly linked to metabolic state, as dietary restriction during the replicative phase of this experiment results in suppression of the short lifespan [126]. Quiescent cells certainly accumulate damage, but once a quiescent cell reenters the cell cycle, this damage may stay with the mother cell [127]. In that scenario, the proliferative capacity and fitness of the *population* as a whole would be maintained. While this nice

model needs further testing, what is clear is that growth and division are still balanced in the quiescent state, and homeostasis is maintained (see Fig. 8.1, the second case from top).

In quiescence, the down-regulation of TOR and PKA leads to significantly reduced ribosome biogenesis and overall protein synthesis [128]. Cell growth and metabolic activity is generally low in quiescent cells [129]. But because this is happening in the context of cell cycle arrest [129, 130], the general properties and macromolecular composition of quiescent cells remain stable and they are easily recognized [16, 128]. Overall, quiescence likely represents a physiological extreme in the normal range of balancing growth with division, a case where both growth and division are coordinately downregulated.

8.5 Senescence: Growing Desperately, with No Possibility of Ever Dividing Again

It has become clearer in recent years that cell cycle arrest can come in different flavors, especially in the context of unabated cell growth. If a cell continues to make proteins and other macromolecules at a high rate in the face of a cell cycle block, then there are only a few possible outcomes. (1) The cell must find ways to get rid of the large excess (e.g., lysosomal degradation, secretion). (2) The cell must somehow accommodate the extra macromolecular amounts within its boundaries, inevitably leading to increased cell size. In fact, the above are typical properties of senescent cells [128, 131] (described in more detail below) and exemplify a clear case of unbalanced growth and division (see Fig. 8.1, the third case from top). The strong growth of senescent cells (often the result of oncogenic stimulation), is not balanced with cell division. Instead, it persists in the face of stable cell cycle blocks.

An important component in the cell cycle arrest of quiescent and senescent cells is the accumulation of Cdk inhibitor molecules. The kinds of Cdk inhibitors employed in each case, however, are different. The cell cycle arrest of quiescent or fully differentiated cells is usually imposed by members of the $p27^{KIP1}$ family of Cdk inhibitors, which inhibit multiple cyclin/Cdk complexes by interacting with both the cyclin and the Cdk. On the other hand, in senescent cells there is a buildup of $p16^{INK4}$ Cdk inhibitors, which bind to monomeric Cdk4/6 and reduce cyclin binding affinity [128, 131].

Likewise, while both in quiescent and senescent cells there is an accumulation of tumor suppressors that broadly inhibit transcription associated with entry into the cell cycle, the molecular players are different in each case. Quiescence is associated with the pRB-like proteins p107 and p130, which interact with the transcription factor E2F during G1 phase to inhibit G1/S transcription and commitment to division. Instead, senescent cells have high levels of pRB, and there is also a buildup of p53, a regulator of multiple processes (e.g., DNA damage response) that impinge on the cell cycle [128, 131]. Hence, the molecular effectors of the cell cycle arrest are different. Furthermore, the exit point of the cell cycle may be different in quiescent vs. senescent cells. Quiescent cells uniformly exit the cell cycle before initiation of DNA replication in G1 phase [128, 131]. G1 arrest is also common in senescence. Surprisingly, however, in several cases senescent cells appear to have a permanent G2 phase block in later stages of the cell cycle [128, 132–135].

The unbalanced growth and division observed in senescence is associated with a variety of phenotypes typical of extremely stressed cells. The exact signatures are still a matter of debate [128]. In addition to the cell cycle markers we mentioned above, other traits associated with senescence often include: short or dysfunctional telomeres, lysosomal stress and expression of β-galactosidase, DNA damage response, stress granule formation, hyper-secretory functions, formation of heterochromatic foci, and the senescence-associated secretory phenotype (SASP) [128, 131, 136]. Overall, senescent cells have been aptly compared to automobiles that simultaneously attempt to accelerate (i.e., hyperactive growth pathways) and stop (i.e., strong, permanent cell cycle block), putting the cell on its way to a highly stressed, irreversibly aged state [15, 16, 137].

The phenomenon of cell senescence was discovered more than 50 years ago and it was almost immediately hypothesized to be associated with organismal aging [138]. While it has been clearly established that cell senescence serves as a tumor suppressive mechanism [131], support for the aging theory has waxed and waned over the years. Currently, it is buoyed by a series of recent studies linking cell senescence to aging in mice.

A principle argument against a role for cell senescence in aging has been that even in old individuals, only a small fraction of cells within a tissue appear to be senescent. How could a phenomenon affecting only a small percentage of cells seriously impair an entire tissue? This question has been potentially resolved with the discovery and characterization of the SASP, wherein senescent cells secrete a novel panel of factors in part comprised of inflammatory cytokines that can have potent paracrine and endocrine effects on non-senescent cells [139, 140]. Moreover, a better understanding has emerged regarding the events that can drive cellular senescence. These now include a wide range of cellular stresses [131], which are associated with chronic diseases of aging, suggesting that aging events may drive cell senescence that in turn promote increased aging.

Senescent cells do accumulate with aging and the Cdk inhibitor, $p16^{INK4}$, has been proposed as a biomarker of aging [128]. Indeed, in selected T cell populations, $p16^{INK4}$ levels do show a statistically significant predictive value for human age. In addition, panels of inflammatory cytokines have been proposed as aging biomarkers and these may be at least in part related to the SASP. Several recent studies have reported mechanistic insights into SASP induction in senescent cells. Several pathways appear to be involved, including those related to cell growth, such as mTOR, and cell proliferation, such as p53. Rapamycin suppresses aspects of the SASP, but must be delivered continuously to have this effect [141]. This is in contrast to organismal aging, where a transient three-month exposure to rapamycin in middle age is sufficient to extend the lifespan of mice [142, 143].

To test the role of senescence in aging, two different strategies were employed to conditionally ablate senescent cells, both related to the specificity of $p16^{INK4}$ expression in this cellular condition. Findings in these mice appear promising as ablation of senescent cells is linked to partial suppression of pathology in a mouse progeria model, the BubR1 mice [144], and can extend the lifespan and some healthspan parameters in normal mice [145].

The connection between BubR1 and progeria is interesting in its own right as the gene encodes a component of the mitotic spindle assembly checkpoint, which prevents cells from initiating anaphase if one or more kinetochores are not attached to the mitotic spindle [146]. Mice hypomorphic for BubR1 rapidly develop aging features, including kyphosis, cachexia, and cataracts [147, 148]. They also have a severely reduced lifespan. With age, BubR1 expression declines in a number of tissues, suggesting that reduced expression of the protein late in life may contribute to normal aging [147]. Moreover, overexpression of BubR1 delays aspects of aging [149]. A potential unifying model is that reduced BubR1 expression leads to mitotic defects, driving cell senescence and that the senescent cells drive aging phenotypes through the SASP or other mechanisms [144]. Ablation of senescent cells improves a range of healthspan parameters.

The promise of research in senescence has led to drug discovery approaches designed to specifically kill senescent cells. Several candidates have already emerged, and these compounds have shown efficacy in preclinical models of chronic disease states [150–153]. While the clinical work remains to be done, the last 10 years have seen cell senescence emerge as one pathway likely to modulate organismal aging and many new pathways of therapeutics for age-associated diseases.

8.6 Division Without Growth

In the classic experiments by Hartwell and colleagues, it was established that in most cases growth controls cell division and not the other way around [1, 2, 12]. Stopping cell growth will also stop cell division, but stopping cell division does not usually stop cell growth (as displayed in senescent cells, see discussion in the previous section). From these principles, it follows then that cell division in the absence of growth is untenable, at least when the mass of the daughter cells is reduced below a threshold necessary to sustain their viability. This is precisely what happens during the early embryonic cell cycles after fertilization until the mid-blastula transition ([154]; see Fig. 8.1, last case). At the mid-blastula transition, before the re-establishment of the normal somatic cell cycles, the block in cell division is imposed by Cdk inhibitors. In mutants lacking these inhibitors, cells usually undergo just one extra division [154–156]. Overall, these early embryonic cell cycles do not necessarily violate the fundamental need to balance growth with division. They just reflect the fact that growth needs have been satisfied during oogenesis.

8.7 Outlook

In yeast, it is implicit that aging, both replicative and chronological, must be linked to critical cell cycle decisions. Balancing cell growth with division to maintain cellular homeostasis is a critical component of this process, whether cells are dividing or in a non-proliferative state. The key pathways that coordinate cell growth signals are intimately linked to aging in yeast, and considerable evidence suggests that they have conserved effects on aging in multicellular organisms. Therefore, continued efforts to understand yeast aging in the context of cell growth and division are likely to continue to inform about human aging. A major challenge now in yeast is to understand aging at the systems level, taking a holistic approach to integrate the contributions of different aging mechanisms and pathways

in order to model the aging condition. This approach involves combining large-scale studies, including transcriptomics and epistasis network analysis, with directed studies with the goal of establishing as complete as possible a picture of single cell aging that can set the stage for similar studies in multicellular organisms.

In the multi-cellular context, a major challenge has been to understand the links between aging at the level of the organism and (causal?) changes to cells in the aging body. In that context, cell senescence has emerged as a major candidate driver of aging processes. Major insights in this arena have led to the identification of candidate therapeutics to kill senescent cells as means of offsetting or treating age-related chronic diseases. The next few years will help define the merits of this new therapeutic route based on aging studies.

More broadly, aging is linked to several pathways involved in cell growth and specifically in protein synthesis and turnover. It is clear for instance that reduced mTORC1 signaling leads to lifespan extension, but further work needs to be done to identify whether aging benefits come from altered protein translation, increased turnover of damaged macromolecules, suppression of the SASP, or some other mechanism. Moreover, it is important to identify in what tissues reduced mTOR signaling, and other pathways such as PKA, promotes longevity. With dramatic increases in the aging population and new insights from research on aging and longevity, the promise is there for major new advances that could refocus medical care toward interventions that slow aging and keep people healthy longer. Understanding links between cell growth, division and aging are integral to achieving this goal.

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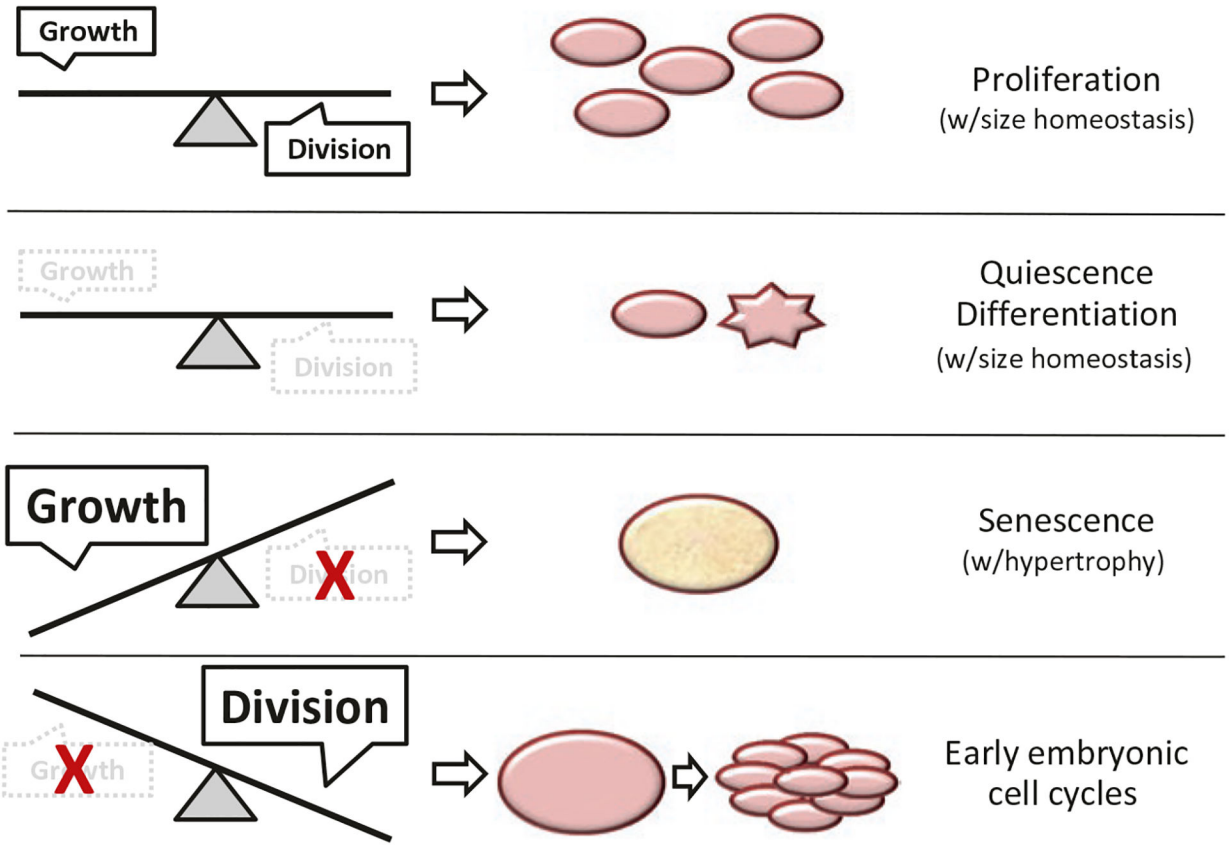

Fig. 8.1.

Schematic representation of all possible outcomes when growth and division are balanced or unbalanced