Aging and Genetic Instability in Yeast

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Abbreviations

ERC Extrachromosomal rDNA circle, DSB double-strand break, BIR break-induced replication, LOH loss of heterozygosity

Keywords

Saccharomyces cerevisiae, yeast, aging, senescence, lifespan, genetic instability, genomic instability.

“Teaser”

Recent findings suggest that yeast aging is accompanied by chromosomal instability, suggesting a direct link between cellular age and integrity of the genome.

Summary

There is a striking link between increasing age and the incidence of cancer in humans. One of the hallmarks of cancer, genomic instability, has been observed in all types of organisms. In the yeast *Saccharomyces cerevisiae*, it was recently discovered that during the replicative lifespan, aging cells switch to a state of high genomic instability which persists until they die. In considering these and other recent results, we suggest that accumulation of oxidatively damaged protein in aging cells results in the loss of function of gene products critical for maintaining genome integrity. Determining the identity of these proteins and how they become damaged represents a new challenge for understanding the relationship between age and genetic instability.

Introduction

As people approach middle-age they enter a period in which there is an exponential increase in the incidence of many types of cancer with each additional year [[1] – see Figure 1a]. The precise mechanism underlying the dramatic age-related rise of cancer remains unclear, but the importance of mutation in cancer presents a likely explanation [2]. Genetic alterations are both a hallmark of, and in some cases a prerequisite for, carcinogenesis [1,3]. It is hypothesized that a small number of genetic events — causing either activation of oncogenes or inactivation of tumor suppressor genes — precede each stage of tumorigenesis [2,4,5]. By middle age, sufficient cellular genomic damage may accumulate to initiate carcinogenesis.

However, the steady accumulation of mutations, based on rates of spontaneous mutation observed in human tissue culture cells, cannot account for the amount of widespread aneuploidy and other types of chromosomal instability that are present in most tumors [6]. This has led to the postulate that one of the early steps in cancer progression is a genetic change causing a switch to a higher than normal rate of mutation, creating a “mutator phenotype” that increases the likelihood of subsequent genetic events [7-9].

It has been difficult, if not impossible, to ascertain exactly how genetic alterations contribute to the age-related increase in cancer. For instance, do mutations arise at a constant rate throughout an individual’s life, or do mutation rates increase with age? There are a myriad of biological and clinical issues that confound this determination, including: clonal expansion, by which an early mutation provides a growth advantage and increases opportunities for subsequent mutations; tissue specific effects, in terms of both

the frequency of tumor detection and the inherent susceptibility of certain cell types to tumorigenesis; environmental challenges, including extrinsic (*e.g.*, carcinogens) and intrinsic (*e.g.*, hormones) factors that arise over a lifetime and can affect tumor formation; and the continual improvement of diagnostic methods. Similarly, examining non-cancerous tissues of individuals at various ages has not clearly defined whether increases in mutations in older individuals are due to a steady accumulation of events at an unchanged rate, or to an increase in the rate accompanying advanced age.

Yeast as a model system for genome stability changes with cellular age

Unicellular eukaryotes provide an opportunity to assess the effects of cellular aging on genetic stability without the confounding factors mentioned above. The yeast *Saccharomyces cerevisiae* is particularly amenable to such a task, having served as a powerful system to dissect the mechanisms that underlie the faithful transmission and replication of nuclear chromosomes. In fact, more is known about how genome integrity is maintained in this organism than in any other [10]. *S. cerevisiae* has also become an excellent model for the study of cellular aging [11]. Thanks to the fundamental asymmetry of budding as a method of mitotic cell division [12], a single mother cell can be distinguished from each of her daughters and monitored for age-dependent changes throughout her finite replicative lifespan. The combination of these two attributes has recently been exploited to gain new insight into changes in genome integrity as a function of cellular age.

Loss of heterozygosity (LOH) is an important event in the initiation and development of cancer [6], and is indicative of chromosome recombination, deletion or loss. In the recent study, a simple system was created in diploid yeast cells to monitor

LOH, taking advantage of two markers which, when lost, cause the yeast cells to change either from white to brown or from white to red [13]. This strain was then subjected to pedigree analysis, in which a mother cell is isolated on agar media, and each of her newborn daughter cells is successively moved to a different location and allowed to form a colony. These “daughter colonies” provide a retrospective life history of the mother cell: if a genetic change such as LOH occurs during the mother’s lifespan, it is detected by a color change in the daughter colonies.

An age-induced switch in genomic instability

In analyzing the complete pedigree of 40 mother cells whose median lifespan is ~30 cell divisions, a rather striking and unexpected resulted was observed. Daughter colonies produced from the first ~25 divisions of a mother display essentially no LOH (Figure 1). Thereafter, however, daughter colonies with LOH are frequently observed; on average, nearly every third daughter colony produced by mothers older than 25 had an LOH event ([13], Figure 1). This behavior suggests a “switch” of states in the mother: Once the first LOH event is observed in the pedigree of an aging mother cell, subsequent daughter colonies with LOH events occur at a constant frequency throughout the remainder of her lifespan. The likelihood of a mother yeast cell experiencing this “switch” to increased LOH in her progeny appears to increase exponentially with advancing age, analogous to the relationship between age and cancer in people.

Further analysis revealed that nearly all the age-induced LOH occurs by break-induced replication (BIR), suggesting that chromosomal damage — particularly a double-strand break (DSB) — is responsible for the increased genomic instability, rather than a decrease in the fidelity of mitotic chromosome segregation.

Mother knows best

Remarkably, although it is the age of the mother that appears to be responsible for the dramatic increase in the rate of LOH, the genome of the mother cell appears largely unaffected by this process. Instead, the vast majority of these age-induced LOH events affect the progeny of old mothers. Furthermore, many of the daughter colonies are sectored, suggesting the possibility that not only do the daughter cells display LOH, but their progeny may as well [13]. There are two implications of these findings. First, if we consider a yeast mother cell to be equivalent to a metazoan stem cell, then the mother "stem" cell may protect itself from DNA damage so that it retains the potential to produce daughter cells in the future that are "normal". Second, aging induces a hyper-recombinational state, and this state may be inherited by the daughters, granddaughters, and great-granddaughters of old mother cells (see discussion below).

Age-induced genomic instability is on its own "clock"

The replicative lifespan of yeast cells can be increased by certain mutations, illustrating the significant genetic component of aging in this organism [11]. Indeed, deletion of the *FOB1* gene nearly doubles the number of times a mother cell can divide [13]. However, extending lifespan this way does not affect the onset nor the frequency of age-induced LOH events: The first observed LOH events occur after ~25 divisions by the mother, and thereafter in every third daughter colony [13]. These longer-lived mothers simply generate more daughter colonies with LOH. Thus, in this instance, the effects of aging on genome stability are independent of the factors that limit the number of times a cell can divide (see below).

Is age-induced LOH caused by increased DNA damage in old cells?

A recent report examining genome-wide mRNA transcript levels of haploid mother cells that had been aged ~18 cell divisions revealed a profile consistent with elevated levels of DNA damage [14]. Such an observation would be consistent with the elevated level of LOH in older cells. However, this transcriptional response may be the result of an increase in DNA damaging events in old mother cells, or the accumulation of DNA damage due to a failure to properly repair normal levels of spontaneous damage (it has been estimated that a DNA double-strand break (DSB) forms once in every fifth cell division in normal yeast cells [15]). Future work directly assessing the frequency and types of genetic lesions and the repair and checkpoint capacity of old cells should help distinguish between these possibilities.

What causes the asymmetry of age-induced LOH?

In addition to generating progeny with a high rate of LOH during their cell divisions (manifested as sectored daughter colonies), an aging mother yeast cell frequently produces buds that have lost heterozygosity [13]. However, as mentioned above, the old mother cell herself rarely experiences LOH. This paradox can be resolved by considering the predominant mechanism of age-induced LOH — break-induced replication (BIR) — and the situations in which it has been previously documented. Specifically, increases in BIR were observed in yeast cells in which separation of chromosomes during mitosis occurs in the presence of unrepaired DNA breaks. Such events — resulting either from the failure of [16], or adaptation to [17], the G_2/M DNA damage checkpoint — allow the separation of the two fragments of a broken chromosome into different cells without normal DSB repair [18]. One cell inherits the chromosome fragment containing the centromere (the “centric” fragment”), while the

other inherits the centromere-less (“acentric”) fragment (Figure 2). In these situations, each cell faces limited repair options, perhaps the best of which is BIR using the intact homologous chromosome as a template. For the cell with the centric fragment, this results in LOH at all loci centromere-distal from the break. By contrast, BIR initiated by acentric fragments rarely progresses past the centromere of the intact template [19]; as a result, these fragments may be degraded in the following S phase [20]. Thus, failure to arrest the cell cycle before mitosis predisposes cells with chromosome breaks to repair via BIR. Importantly, old mother cells do not appear to delay the cell cycle during divisions when LOH occurs in the bud [13], consistent with a failure to activate or execute the DNA damage checkpoint.

The final piece of the puzzle regarding the asymmetry of age-induced LOH lies in the behavior of acentric molecules. In an early application of yeast pedigree analysis, the inheritance of acentric plasmids was revealed to be inherently mother-biased [21]. If the same is true of acentric chromosome fragments, there is an intrinsic bias for an old mother to inherit the acentric fragment in the checkpoint failure situation described above. Because segregation of centromere-containing molecules is random, mothers will often inherit both fragments of the broken chromosome after mitosis, allowing for standard mechanisms of DSB repair, which rarely lead to LOH [22] (Figure 2). On the other hand, the daughter of an old mother will rarely inherit both chromosome fragments, due to the biased retention of the acentric fragment in the mother. This will result in increased rates of LOH by BIR in the following G_1 of the daughter (Figure 2). Thus, biased mitotic segregation of acentric chromosome fragments provides an explanation for the asymmetry of age-induced LOH.

Age-related events that are not responsible age-induced LOH

Characteristics of old mothers — increased size, slower division rate, abnormal nucleolar morphology, and sterility — are not the result of deleterious genetic events, as they are not stably inherited by the progeny of old mothers [11]. Nonetheless, a form of genetic instability is the basis of a model originally touted as an explanation for nearly every aspect of the yeast aging process [23]. Extrachromosomal copies of the genes encoding the ribosomal RNA accumulate in aging mother cells and have been proposed to inhibit cell division in old mothers [23]. Consistent with this view, manipulations that increase or decrease the levels of extrachromosomal rDNA circles (ERCs) result in decreased or increased lifespan, respectively [11]. However, the chromosomal recombination events that initiate ERC accumulation appear to occur early in life [24]; it is the mother-biased mitotic segregation of ERCs, not their excision from the chromosome, that allows for their exponential accumulation with age [24]. Thus, an age-associated increase in the rate of LOH is neither a requirement for, nor a prediction of, the ERC model. Indeed, as mentioned above, the onset and frequency of age-induced LOH in *fob1*Δ*/fob1*Δ mother cells, which have decreased levels of ERCs and correspondingly longer lifespans [13,25,26], is not delayed relative to that of wild type mother cells ([13], Figure 1a). Thus, genetic instability in the rDNA may be a cause of aging, but it is not responsible for age-induced genome-wide genetic instability.

Cultured human diploid fibroblasts are capable of a finite number of population doublings *in vitro* before they cease division, or “senesce” [27]. There is a molecular mechanism to explain this phenomenon: the failure to fully replicate chromosome ends in the absence of the enzyme telomerase causes a “crisis” that arrests cell division when

telomeres reach a certain critically short length [28]. Genome integrity is also typically compromised during crisis. Normally, telomeres maintain chromosome stability by protecting the ends of chromosomes [29]; in the absence of telomerase, cells invoke alternate recombination-based mechanisms to maintain their chromosomes, which can result in an increased frequency of genomic rearrangements [30]. Telomerase-deficient yeast cells also experience both replicative senescence and genetic instability; survival requires a mechanism of telomere maintenance based on recombination that can include higher rates of break-induced replication [31-34]. However, telomerase is expressed throughout the lifespan of wild type *S. cerevisiae* mother cells, telomere length is not affected by successive mitotic cell divisions [35], and transcriptional profiles of old wild type mother cells do not resemble the profile of telomerase deficient cells [14]. It is also worth noting that the tract lengths of age-induced BIR-mediated LOH events [13] are more consistent with random DSBs throughout the genome rather than telomere-mediated recombination events. Thus, short telomeres are not the cause of age-induced genomic instability in yeast.

Building a model to explain age-induced LOH

How does a mother yeast cell suddenly switch to an elevated level of LOH as she ages? We can begin by considering an interesting trait common among aging-related phenotypes (see above): Daughters of old mothers inherit the characteristics of aged cells. For instance, they divide slowly and only a few more times, and are increased in size [36,37]. However, youthful phenotypes are restored in the great, great-granddaughter of an old mother [36,37]. Similarly, age-induced LOH occurs in the daughters, granddaughters, and perhaps great-granddaughters of an old mother cell (see above).

However, when progeny cells > 20 generations removed from an old, genetically unstable mother cell are examined for LOH, they display low rates of LOH indistinguishable from that of isogenic cells derived from a young mother [M.A.M and D.E.G., manuscript in preparation]. That is, after many cell divisions through the daughter lineage, the switch to the hyper-recombinational state is reset, and genomic stability restored. Thus, age-induced LOH is not the result of a permanent genetic alteration that creates a persistent "mutator phenotype".

To explain why daughters of old mothers inherit aging phenotypes, it was proposed that mother cells accumulate a "senescence factor" with age ([36,37]; Figure 3), causing breakdown of normal cell function when amounts of the factor reach a certain critical threshold. Daughters are unaffected by the mother's age until another threshold is attained, at which point daughters of old mothers begin to inherit senescence factor and display the phenotypes of old cells. These phenotypes eventually disappear in the distant progeny of old mothers, as the senescence factor is "diluted out" by mother-biased asymmetric segregation. Because ERCs (see above) display mother-biased segregation and are inherited by the daughters of old mothers, they are a good candidate for a factor responsible for defining lifespan [23]. However, as discussed above, ERCs are not responsible for age-induced LOH [13]. Thus the actual senescence factor impinging on the genetic stability of aging mothers and their progeny (Figure 3) remains unknown.

However, a recent discovery by Aguilaniu, et al. [38] provides another possible explanation for the senescence factor. Carbonylated proteins (a common, irreversible result of oxidative damage) are preferentially localized in the mother cell after cytokinesis [38]. Accordingly, damaged proteins accumulate to high levels in old mother

cells [38]. Furthermore, this asymmetry of inheritance breaks down with age: the daughters of old mothers inherit high levels of damaged proteins [38]. In this way, the behavior of oxidatively damaged proteins fit precisely with the requirements for a senescence factor.

We propose that damaged versions of nuclear proteins critical for maintaining chromosome integrity accumulate in old cells. Once enough of the protein is in a damaged form, it acts as a "dominant negative" [39] and interferes with normal function, initiating an increased frequency of LOH (Figure 3). As damaged protein continues to accumulate with increasing age of the mother cell, it spills over into the daughter cell, and she too begins to display increased LOH. However, the amount of damaged protein is diluted away in further progeny (granddaughters, great-granddaughters, etc.), because the damaged protein is preferentially maintained in mother cells (for reasons that are unknown; [38]), and new protein synthesis likely occurs in daughter cells.

More recent findings provide a bit more support for this model and a way to test it. Caloric restriction (CR) — a manipulation that extends lifespan in yeast and many other organisms [40,41] — also decreases levels of oxidatively damaged protein in old mother cells [42]. Thus, examining age-induced LOH under CR addresses an obvious prediction. Furthermore, CR was recently shown to operate in a pathway distinct from those involving *SIR2* and *FOB1* [43], both of which regulate lifespan, but do not participate in age-induced genetic instability [13].

Hopeful candidates

Which proteins become damaged in an age-dependent manner and lead to increased LOH? A simple way of identifying such candidates may be looking for mutants

in young cells that mimic the age-induced LOH phenotype: an ~100-fold increase in LOH occurring predominantly by BIR in daughter cells. While a great deal is known about mutants that cause genomic instability, few have been examined for their effect on LOH, and none has been examined for the daughter bias in BIR. Nevertheless, some genome integrity mutants have been analyzed for their effects on aging. Specifically, mutations in the DNA replication/recombination/repair genes *RAD50* [44], *RAD52* [44], *SGS1* [45,46], or *DNA2* [46] cause the accelerated appearance of multiple characteristics of old wild type mothers. Importantly, these aging phenotypes are not due to elevated levels of ERCs [44,46,47], to which aging phenotypes in wild type mothers had previously been attributed [23]. Assessing the role in age-induced LOH of these candidates awaits further analysis.

Conclusions

The recent discovery that *S. cerevisiae* cells undergo an age-induced switch to high levels of LOH suggests the intriguing possibility that a similar process may occur in other eukaryotic cells, particularly those — such as stem cells — that share the qualities of a mother yeast cell: asymmetric divisions and a finite replicative lifespan. We present models to explain the breakdown of genome integrity with age in the hope that they will provide a framework for identifying the molecular alterations responsible for age-induced genetic instability. This represents a new and exciting direction for yeast aging research.

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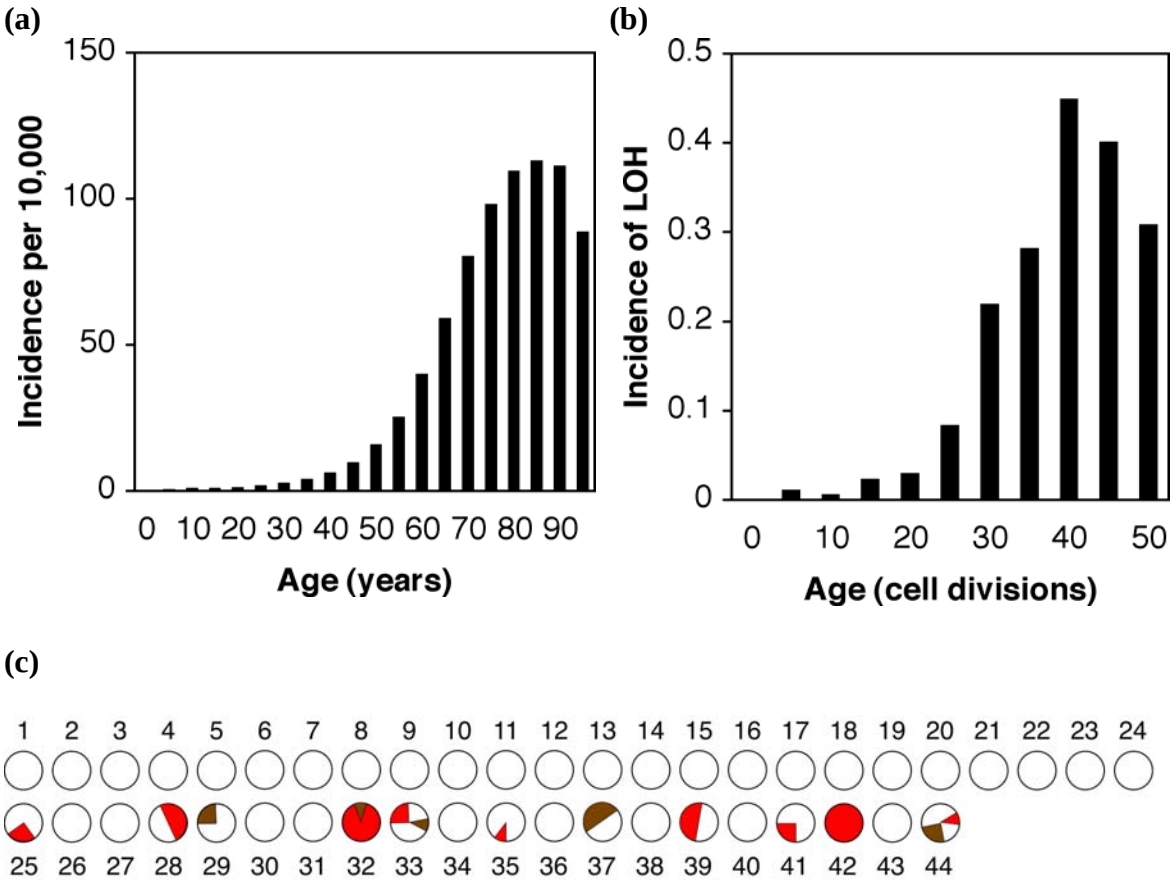
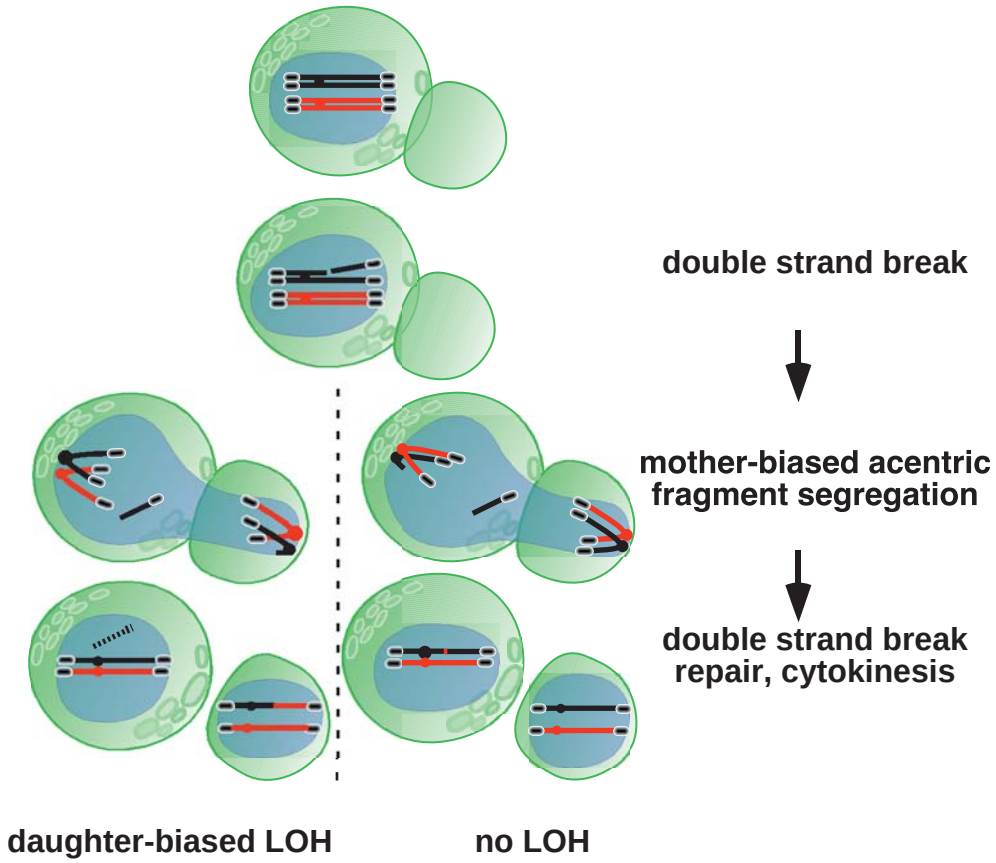
Figure 1.



A function of age: Human cancer and genetic instability in *Saccharomyces cerevisiae*. (a) The incidence of cancer increases dramatically with age in humans. Shown are probabilities of developing cancer by 5-year interval collected by SEER [48] from 1998-2000 for all types of cancer. (b) The incidence of loss of heterozygosity (LOH) in diploid budding yeast cells increases with similar kinetics during replicative aging. Shown are probabilities of producing a daughter cell that gives rise to a colony containing an LOH event, grouped by 5-division intervals for a cohort of 39 wild type diploid mother cells [M.A.M and D.E.G., unpublished results]. (c) Detection of age-induced LOH events by pedigree analysis. Each circle represents the colony produced by successive daughters of a single mother cell, the succession is labeled with numbers.

Colored shapes represent sectors of cells having experienced LOH, where red and brown designate LOH at different loci.

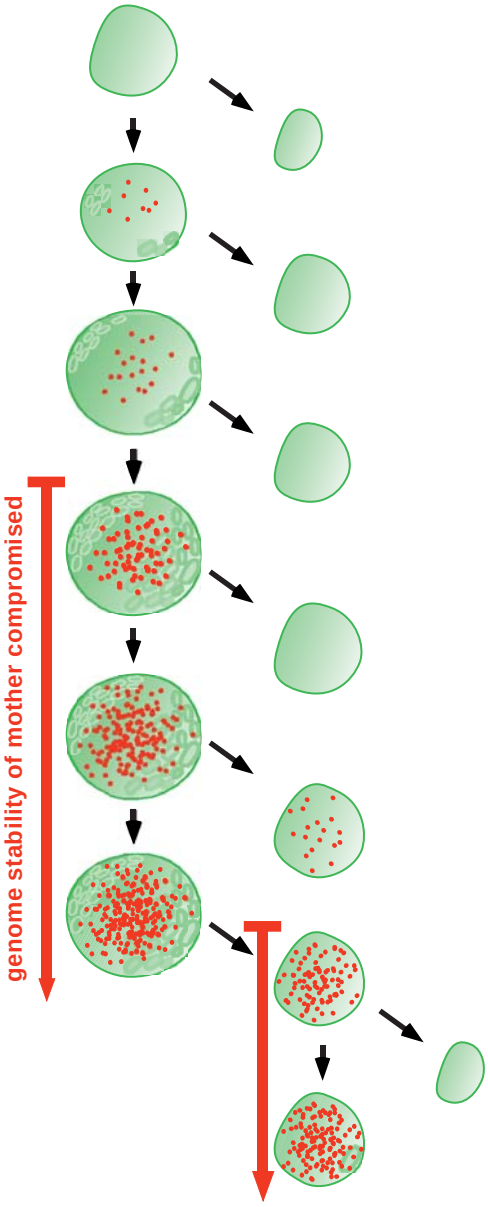
Figure 2.



A model for asymmetric age-induced LOH. A diploid mother yeast cell is depicted with two homologous chromosomes (red and black – centromeres are filled circles) contained within the nucleus (blue). The cell wall is shown in green and bud scars are depicted. Top, a mother cell after DNA replication with duplicated chromosomes. A double strand break (DSB) in one sister chromatid of the black chromosome is followed by mitosis without repair, resulting in two potential outcomes. On the left, the broken centromere-containing chromosome fragment segregates to the daughter; on the right, it segregates to the mother. In both cases, the acentric chromosome fragment remains in the mother cell after cytokinesis. On the left, the two fragments of the broken chromosome are separated by mitosis, and repair of the broken centromere-containing fragment occurs

by break-induced replication (BIR), resulting in duplication from the homologous chromosome of all sequences centromere-distal to the break. The acentric fragment remaining in the mother cell is shown to be degraded (dashed), but could have other fates. On the right, where the mother inherits both fragments, DSB repair by non-homologous end-joining or local gene conversion without crossing over preserves both alleles at distal loci (no LOH). Note that, prior to DNA replication, gene conversion accompanied by crossing over would not cause LOH.

Figure 3.



Model for the accumulation and inheritance of a senescence factor that affects genome stability as yeast age. On the left, a mother yeast cell undergoes replicative aging, producing a virgin daughter cell at each division, shown on the right. A hypothetical senescence factor (red dots) accumulates in the mother at each cell division, reaching high concentrations after many successive divisions. Past a certain threshold concentration, daughter cells begin to inherit senescence factor. Past another threshold

(red arrows), the concentration of senescence factor begins to negatively impact the genome stability of the cell; the daughter of an old mother may be affected, and begins accumulating more senescence factor once it becomes a mother. However, the granddaughter of an old cell (far right) does not inherit senescence factor, and retains normal genome stability.