

occur in conventional allosteric proteins in which input and output activities are centralized in a single folded structure, and gating is mediated by subtle conformational shifts.

Domain recombination space sampled in these experiments proved functionally rich: Although constructs showed a range of different gating behaviors (negative-positive, integrating-nonintegrating, etc.), nearly all of them show some form of gating. Gating as an emergent property, therefore, does not appear to be extremely rare, as might be expected if only very precise domain arrangements yielded regulation. This modular framework, in addition to promoting switch protein evolution, could be used to engineer proteins with novel regulatory control and, in principle, novel cellular circuits.

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## Supporting Online Material

www.sciencemag.org/cgi/content/full/301/5641/1904/DC1

Materials and Methods

Figs. S1 to S5

Table S1

References and Notes

21 April 2003; accepted 21 August 2003

## An Age-Induced Switch to a Hyper-Recombinational State

Michael A. McMurray and Daniel E. Gottschling\*

There is a strong correlation between age and cancer, but the mechanism by which this phenomenon occurs is unclear. We chose *Saccharomyces cerevisiae* to examine one of the hallmarks of cancer—genomic instability—as a function of cellular age. As diploid yeast mother cells aged, an ~100-fold increase in loss of heterozygosity (LOH) occurred. Extending life-span altered neither the onset nor the frequency of age-induced LOH; the switch to hyper-LOH appears to be on its own clock. In young cells, LOH occurs by reciprocal recombination, whereas LOH in old cells was nonreciprocal, occurring predominantly in the old mother's progeny. Thus, nuclear genomes may be inherently unstable with age.

Age may be the greatest carcinogen: Cancer incidence increases exponentially near the end of human life (1). Chromosomal abnormalities are a hallmark of most tumors, and it is widely held that genomic instability is a prerequisite for tumorigenesis (2). In older individuals, there is evidence for increased genomic instability, even in noncancerous cells (3). Although numerous hypotheses exist to explain the association between aging and genomic instability (1), these have been difficult to test. To develop a mechanistic understanding of age-related genomic instability, we asked whether such a phenomenon occurs in a model biological system, the budding yeast *Saccharomyces cerevisiae*.

Heterozygosity was created in a diploid strain by the insertion of a marker gene in one

copy of a locus. Loss of heterozygosity (LOH) at the locus was detected when a genetic alteration occurred in which the marker was "lost." Although LOH in yeast can arise by multiple mechanisms, spontaneous LOH in wild-type cells occurs primarily through mitotic recombination (4). Recombination is presumed to be initiated by DNA damage along the chromosome and is typically accompanied by LOH at all centromere-distal loci (5, 6); accordingly, distal markers were more likely to undergo spontaneous LOH (table S1). Therefore, in order to maximize the chance of observing LOH events, we inserted markers distally on the two longest chromosome arms: at the *SAM2* locus on the right arm of chromosome IV and at the *MET15* locus on the right arm of XII, about 1 and 2 Mb, respectively, from their centromeres (7). Marker genes affecting colony color when lost were inserted at these loci (8, 9).

The number of daughter cells produced before death by a yeast (mother) cell defines her life-span (10). In order to determine whether genomic instability, manifested as LOH, was affected by a mother

cell's increasing age, we isolated by micro-manipulation every daughter cell produced from a mother and allowed each daughter to form a colony (11). When the life-spans of the mother cells were complete, daughter colonies were assayed for LOH by changes in colony color. LOH was readily observed in the progeny of aging mothers by the appearance of uniformly colored colonies, or colored sectors within colonies. LOH events resulting in sectorized daughter colonies were scored as half-, quarter- or eighth-sectors, which are consistent with the daughter cell or its progeny experiencing an LOH event one, two, or three generations after separation of the daughter from the original mother cell (Fig. 1, A and B).

Examination of these pedigrees revealed a marked change in LOH with the mother's age (Fig. 1C). Daughter colonies early in the life-span had no LOH events, whereas LOH was observed frequently in the colonies produced by daughters of old mothers.

The first LOH events observed in the pedigrees of individual mothers did not occur until the mothers had gone through 23 cell divisions (median value); this late onset was observed at both loci analyzed (Fig. 1D, open bars). However, once an LOH event was observed in a lineage, subsequent LOH events were much more frequent, occurring in every third to fourth daughter colony (Fig. 1, B and D, solid bars). The rate of LOH per cell division in old cells was ~40 to 200 times that of young cells (Table 1). The frequency of LOH remained constant as the mother cells continued to age: After the first event, there was no significant correlation between the age of the mother and the frequency of subsequent LOH events (*MET15*  $P = 0.69$  and *SAM2*  $P = 0.39$  for a nonparametric Spearman correlation coefficient).

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LOH was observed in a majority of the pedigrees: 25 of 40 mothers produced at least one LOH event. Those that did not were generally short-lived. Together, these results demonstrate that there is an age-induced onset of genomic instability in *S. cerevisiae*. Once an LOH event occurs in a pedigree, additional LOH is observed at a higher frequency for the duration of the mother's life-span. This suggests that as mother cells age, there is a switch from a state with a low spontaneous rate of LOH to a state of increased genomic instability.

Extrachromosomal ribosomal DNA circles (ERCs) accumulate in aging mother cells and have been proposed to cause replicative senescence (12). To determine whether ERCs were responsible for the observed age-induced genomic instability, we genetically altered the accumulation of ERCs in mother cells and determined the effect on LOH. Cells with a mutant *FOBI* gene have re-

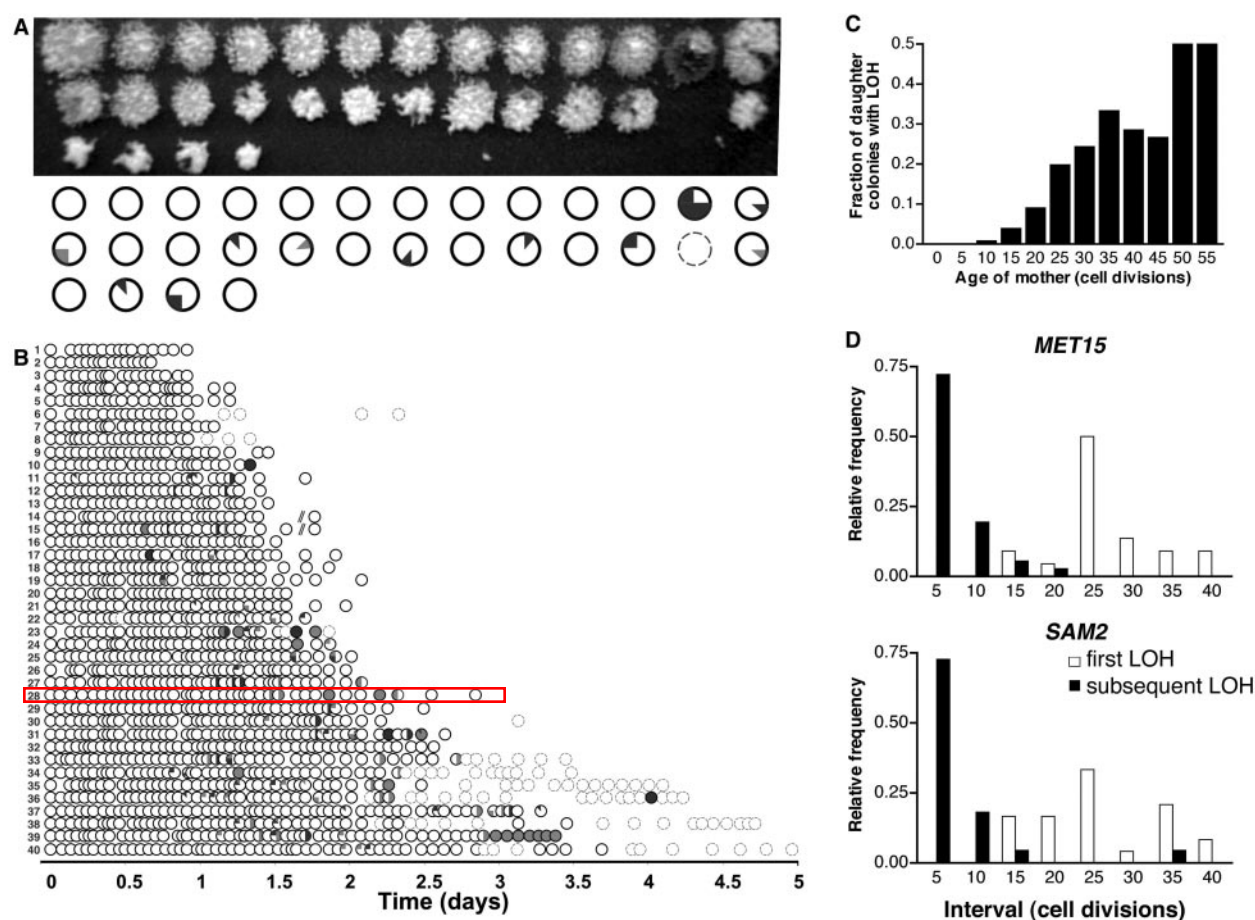
duced levels of recombination at the ribosomal DNA (rDNA) locus and thus accumulate fewer ERCs, whereas mutants in *SIR2* have higher rates of rDNA recombination and rapidly accumulate ERCs (13–15). Consistent with published results (13), deletion of both copies of the *FOBI* gene

nearly doubled the life-span (fig. S1A). Nevertheless, the frequency and onset of age-induced LOH were unaffected in these cells. The median number of cell divisions in *FOBI*-deleted (*fob1Δ/fob1Δ*) mother cells before a first LOH event was detected was the same as in wild-type mothers: 25

**Table 1.** Age increases the rate of LOH. The rate of LOH at the *MET15* and *SAM2* loci was calculated for young and old cells as described (11). The 95% confidence interval (CI) is based on the Poisson distribution.

| Genotype           | LOH rate per 10,000 cell divisions (95% CI) |             |               |              |
|--------------------|---|-------------|---------------|--------------|
|                    | Young                                       |             | Old           |              |
|                    | <i>MET15</i>                                | <i>SAM2</i> | <i>MET15</i>  | <i>SAM2</i>  |
| Wild type          | 7 (5–10)                                    | 1 (0.5–2.0) | 300 (100–500) | 200 (50–400) |
| <i>fob1Δ/fob1Δ</i> | 7 (4–10)                                    | 1 (0.4–3.0) | 150 (90–230)  | 80 (30–200)  |
| <i>sir2Δ/sir2Δ</i> | 160 (120–200)                               | 1 (0.4–3.0) | 200* (50–300) | †            |

\*The *sir2Δ/sir2Δ* rate of *MET15* LOH in old cells was calculated by half-sector frequency. †No *sir2Δ/sir2Δ* mother cell produced more than a single daughter colony with a *SAM2* LOH event.



**Fig. 1.** Pedigree analysis reveals age-induced LOH. (A) Daughter colonies were replica-plated to media that permit the detection of loss at *MET15* (light gray) and loss at *SAM2* (dark gray) (8). Top: Colonies produced by the 12th to 41st (left to right, top to bottom) daughters of a single representative mother cell. Bottom: A schematic representation of the pedigree obtained from these colonies; circles represent colonies derived from individual daughter cells. Colonies with eighth-, quarter-, or half-sectors of colored cells are represented; two LOH events occurred in the 23rd daughter colony. Dashed circles represent daughter cells that

did not form a colony. (B) Pedigrees of 40 individual mother cells of strain UCC809, distributed on the y axis with the same representation as in (A); x-axis position indicates the time of separation of the daughter cell from the mother. Double lines precede colonies from daughter cells that could not be separated from the dead mother. (C) The fraction of daughter colonies with LOH events (*SAM2* or *MET15*) for a given mother's age. (D) The number of cell divisions before the first LOH event occurs or between subsequent LOH events at the *MET15* or *SAM2* locus is presented as a frequency distribution for 40 pedigrees.

## REPORTS

divisions at *MET15* and 24 divisions at *SAM2*. Also unchanged was the increased frequency with which subsequent LOH events occurred (Table 1 and fig. S1C). Overall, the absence of *FOB1* affected only the total number of LOH events observed; the longer life-span translated into 38 of 40 mothers displaying age-induced LOH, and nearly three times as many LOH events were detected after the initial event in a lineage. Thus, reducing the level of ERCs prolonged life-span, but did not delay the onset nor decrease the frequency of age-induced genomic instability.

Deletion of both copies of the *SIR2* gene reduced the average life-span of the LOH-detection strain by more than half (fig. S1A), as expected (14). In contrast to wild-type or *foi1Δ/foi1Δ* cells, *MET15* LOH occurred in *sir2Δ/sir2Δ* cells at a very high frequency, regardless of age (Table 1 and fig. S1C). There was little change in this frequency once a first LOH event occurred in a mother's pedigree (Table 1 and fig. S1C). At the *SAM2* locus, however, only a minority of mother cells produced even a single LOH event (8 of 35); no subsequent LOH event was observed in any lineage (15). This discrepancy is readily explained by a locus-specific recombination effect of

*SIR2*. In *sir2Δ/sir2Δ* cells, mitotic recombination is greatly increased within the rDNA (16). LOH at *MET15*, which is distal to the rDNA array, increased as well (Table 1); this recombination increase was restricted to chromosome XII and did not affect LOH at *SAM2* (Table 1). As *sir2Δ/sir2Δ* mother cells did not undergo a sufficient number of cell divisions (median life-span was 11 divisions) to achieve the age-induced increase in LOH, virtually no LOH was observed at *SAM2*. Overall, these data suggest that the onset and subsequent increased frequency of age-induced LOH operate on a different "clock" than does yeast replicative life-span. The aging-associated genetic instability represented by ERCs (17) is thus distinct from the age-induced LOH we observed, and ERCs are not responsible for the age-induced LOH.

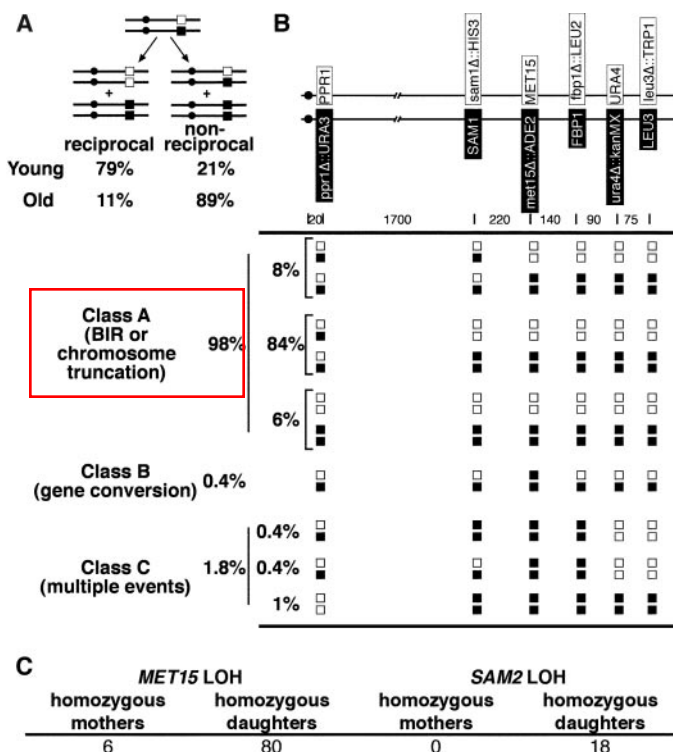
We characterized the mechanism of age-induced LOH by examining whether the age-induced LOH events were the result of chromosome loss. In addition to heterozygosity at the *SAM2* locus on the right arm of chromosome IV, the left arm was marked by heterozygosity at the *ho* locus (*ho/hoΔ::TRP1*). Both *ho* alleles were still present in all *SAM2* LOH clones. Similarly, no chromosome loss was detected when

LOH occurred at *MET15* [(15) and see below]. Thus, age-induced LOH is likely initiated by chromosomal damage and is not the result of chromosome nondisjunction during mitosis.

We next determined whether LOH occurred by reciprocal or nonreciprocal events. Reciprocal recombination (crossing-over) in heterozygous cells results in a mother cell homozygous for one allele and a daughter cell homozygous for the other allele (Fig. 2A). In nonreciprocal events, one of the pair (mother or daughter) loses heterozygosity, while the other remains heterozygous (Fig. 2A). Reciprocal and nonreciprocal LOH events are readily distinguished by examination of half-sector colonies (18, 19). We found that in young cells, spontaneous LOH at the *MET15* locus occurred primarily through crossing-over (Fig. 2A). In contrast, the age-induced LOH events occurred predominantly by a nonreciprocal pathway (Fig. 2A). These results identify a mechanistic difference between the pathway of mitotic recombination normally responsible for LOH in young cells and the pathway that results in increased levels of LOH as cells age.

Mechanisms of nonreciprocal LOH can be distinguished by examination of multiple heterozygous loci along a chromosome. In general, gene conversion, small deletion, and point mutation do not affect markers that are more than 10 kb from the locus where LOH is detected (20). Conversely, break-induced replication (BIR) or chromosome truncation results in the loss of all markers distal from a breakpoint. BIR achieves homozygosity by copying from the homologous chromosome, typically all the way to the telomere (21), whereas a chromosome truncation is manifested by hemizygosity of distal markers. In order to identify the mechanism of age-induced nonreciprocal LOH, a diploid strain was created with heterozygous markers at six loci, including *MET15*, distributed along ~2200 kb of the right arm of chromosome XII. Pedigree analysis of these mother cells revealed that age-induced LOH at *MET15* occurred with the same late onset, increased frequency, and nonreciprocal character that we found earlier (15). Furthermore, in ~99% of these LOH events, all the markers distal to *MET15* also underwent LOH, including the most distal marker more than 300 kb from *MET15* (Fig. 2B) (15). *MET15* LOH events initiated at various locations along the chromosome between *MET15* and the centromere (Fig. 2B). Quantitative Southern analysis demonstrated that in ~95% of the events, the loci experiencing LOH remained at two copies per cell (15). Thus, BIR was the predominant pathway of LOH, with ~5%

**Fig. 2.** Age induces a switch in the mechanism of LOH. (A) Schematic representation of reciprocal and nonreciprocal events leading to LOH. Open or solid squares represent two different alleles at the same locus. Phenotypes of half-sector colonies of strain UCC768, obtained either by plating cells in culture (young,  $n = 103$ ) or from daughter colonies of old mothers generated by pedigree analysis (old,  $n = 74$ ), were used to determine the proportion of reciprocal and nonreciprocal LOH events. (B) Strain UCC768 was subjected to pedigree analysis, and LOH events were analyzed further to identify the mechanism of *MET15* LOH. The genotype on the right arm of chromosome XII is diagrammed, with the distance in kilobases between marked loci indicated below the diagram. Genotypes of LOH clones ( $n = 270$ ) were determined by phenotype of the affected locus or by polymerase chain reaction (12). Open and solid squares represent the corresponding alleles at each locus. (C) The daughter bias of age-induced LOH is shown by the number of LOH events at the indicated locus that created homozygous mothers or homozygous daughters, identified by pedigree analysis. Data were pooled from pedigrees of five strains.





of the events resulting from a chromosomal truncation. This suggests that age-induced LOH is the result of an age-dependent increase in DNA double-strand breaks and/or a decrease in the normal repair of such damage.

Complete pedigree analysis also allowed us to determine whether LOH events occurred in mother or daughter cells. The continuous appearance of colored daughter colonies in a mother's life-span demonstrated that the mother cell had undergone LOH (e.g., Fig. 1B, mother 39). A completely colored daughter colony followed by daughter colonies with wild-type cells indicated that LOH occurred in the daughter cell only, while the mother remained heterozygous (e.g., Fig. 1B, mother 28). We presumed that BIR events would occur with equal likelihood in mother and daughter cells. In contrast to this expectation, we found a strong daughter bias (nearly 20-fold) for age-induced LOH at both the *MET15* and *SAM2* loci (Fig. 2C). This asymmetry presents an interesting biological situation for the mother cell, which may be viewed as a type of stem cell (22, 23). When age-induced LOH occurs, the genomic integrity of the mother appears to be maintained relative to the daughter, preserving the potential to produce subsequent daughters with a wild-type genome.

What defect causes age-induced LOH? Our mechanistic characterization allows us to eliminate several possibilities. Nondisjunction, leading to chromosome loss, is not responsible. Telomere length is not affected by replicative age in *S. cerevisiae* (24), precluding telomere loss as the cause of chromosomal instability. An increase in interchromosomal fusions, resulting in dicentric chromosomes, could produce substrates for BIR, but such events do not explain the daughter bias of age-induced LOH.

Rather, we postulate that aging mother cells accumulate damaged proteins over time (25), which effectively eliminates the normal function of a gene product required for genome integrity. This defect appears to thwart normal DNA damage detection, because, unlike young cells repairing an induced double-strand break (26), mother cell divisions producing a daughter with LOH lacked noticeable cell cycle delays or arrests (Fig. 1B).

The daughter bias of age-induced LOH may be the result of broken chromosomes that persist immediately after cell division. If the centromere-containing and acentric fragments are partitioned into separate nuclei, with the acentric fragment tending to remain in the mother cell (27), repair from the homolog by BIR (accompanied by LOH) will become the primary DNA repair option in the daughter cell.

Our results provide predictions about the mechanisms that underlie age-related genomic instability in eukaryotic cells, as well as a model system in which to test them. Ultimately, deeper understanding of this phenomenon in yeast may help to solve the link between oncogenesis and age in humans.

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#### Supporting Online Material

[www.sciencemag.org/cgi/content/full/301/5641/1908/DC1](http://www.sciencemag.org/cgi/content/full/301/5641/1908/DC1)

Materials and Methods

Fig. S1

Tables S1 and S2

References and Notes

6 June 2003; accepted 6 August 2003

## Essential Roles for Ecdysone Signaling During *Drosophila* Mid-Embryonic Development

Tatiana Kozlova\*† and Carl S. Thummel

Although functions for the steroid hormone ecdysone during *Drosophila* metamorphosis have been well established, roles for the embryonic ecdysone pulse remain poorly understood. We show that the EcR-USP ecdysone receptor is first activated in the extraembryonic amnioserosa, implicating this tissue as a source of active ecdysteroids in the early embryo. Ecdysone signaling is required for germ band retraction and head involution, morphogenetic movements that shape the first instar larva. This mechanism for coordinating morphogenesis during *Drosophila* embryonic development parallels the role of ecdysone during metamorphosis. It also provides an intriguing parallel with the role of mammalian extraembryonic tissues as a critical source of steroid hormones during embryonic development.

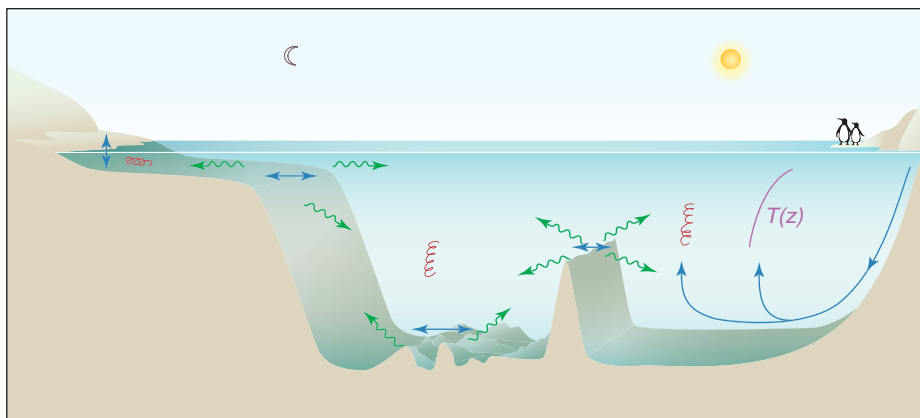
Ecdysone triggers the programmed cell death of obsolete larval tissues and signals cell shape changes in the imaginal discs during *Drosophila* metamorphosis, transforming the body plan of the insect from a crawling larva into an adult fly. ("Ecdysone" in this paper refers to physiologically active ecdysteroids.) Ecdysone signal-

ing is mediated by a heterodimer of two nuclear receptors, EcR (NR1H1) and the *Drosophila* RXR ortholog USP (Ultraspiracle, NR2B4) (1). Characterization of *EcR* and *usp* mutants at the onset of metamorphosis has revealed similar lethal phenotypes, indicating that these factors act together to transduce the hormone signal (1). A high titer ecdysone pulse also occurs midway through embryonic development, peaking during germ band retraction (GBR) (2). GBR, dorsal closure (DC), and head involution (HI) compose the major morphogenetic movements that form the body plan of the first instar larva. Ecdysone is required for cuticle deposition during late embryogenesis (3, 4), but functions for ecdysone at earlier stages of embryonic

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**Tides in the ocean.** (Left) The to and fro of tidal currents generates internal waves at the edge of the continental shelf and over topographic features in the deep ocean. These internal waves can lead to turbulence and mixing. (Right) This mixing plays a role in maintaining a gradual transition between the sun-warmed surface layer of the ocean and the upwelling cold, dense water formed at high latitudes.  $T(z)$  denotes the temperature profile as a function of depth  $z$ .

recession of the Moon at 38 mm/year (4). Another 0.5 TW or so comes from tides driven by the Sun. Second, and this is more revolutionary, the tidal maps have shown that, while most of the energy is indeed lost in shallow seas, close to 1 TW is lost in the deep ocean over rough topography (9).

The simple picture of ocean circulation presented above has also been challenged (10), with much greater roles given to wind-driven ocean circulation, air-sea interaction, and 100-km-scale eddies. In this scenario, only about 0.6 TW of mixing energy is required to reproduce the observed ocean structure. The true energy requirement may lie between the two estimates of 0.6 and 2 TW, but internal waves generated by the tides do seem to be more important than those generated by the wind. The latter are estimated to carry about 0.5 TW into the deep ocean (11), and a small contribution may also come from internal waves

generated as low-frequency, nontidal, currents flow over rough seafloor topography.

Measurements of current over mid-ocean ridges confirm the importance of internal tides (12), and the surface signature of their propagation away from ridges is also evident from satellite altimetry (13). At smaller scales, deep-ocean observations of turbulence and mixing show a variation over the spring-neap cycle (between large tides near full and new moon and smaller tides near the Moon's quarters) as the main tidal currents over rough topography wax and wane (2). Moreover, a revival of theoretical models developed in the 1970s (14), as well as new computer models (15), support the idea that a considerable amount of energy is converted into internal tides in the deep sea, eventually leading to turbulence and mixing.

A comprehensive recent observational program (3) has shown that at the Hawaiian Ridge, local internal tides can lead to a

peak-to-trough range in the depth of density surfaces of as much as 300 m, with intense local mixing. As expected from theory, however, most of the energy generated radiates away. This is also thought to be true of internal tides generated over the less dramatic but more extensive areas of fracture zone topography (14).

It remains unclear, both theoretically and observationally, whether this radiated energy feeds internal waves and turbulence throughout the ocean or is lost in further encounters with the seafloor. Thus, a clear picture and understanding of the vertical and horizontal patterns of ocean mixing is still lacking. But it is clear that ocean tides do much more than cause the rise and fall of sea level. The effects of internal tides must be represented correctly in computer models of ocean circulation and climate. They may also help us to understand the history of the Earth and Moon. The study of ocean tides, with its long and fascinating history (5), is again at the center of attention.

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#### CELL BIOLOGY

## An Age of Instability

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If there's one thing we've learned from the last 50 years of research on bakers' yeast, it is not to underestimate how much this tiny fungus can tell us. We now know that yeast provide key insights into such complex human disorders as variant Creutzfeldt-Jacob disease, Parkinson's disease, and cancer. Even so, it is not hard to imagine the skepticism facing Mortimer and Johnston in the 1950s as they tried to

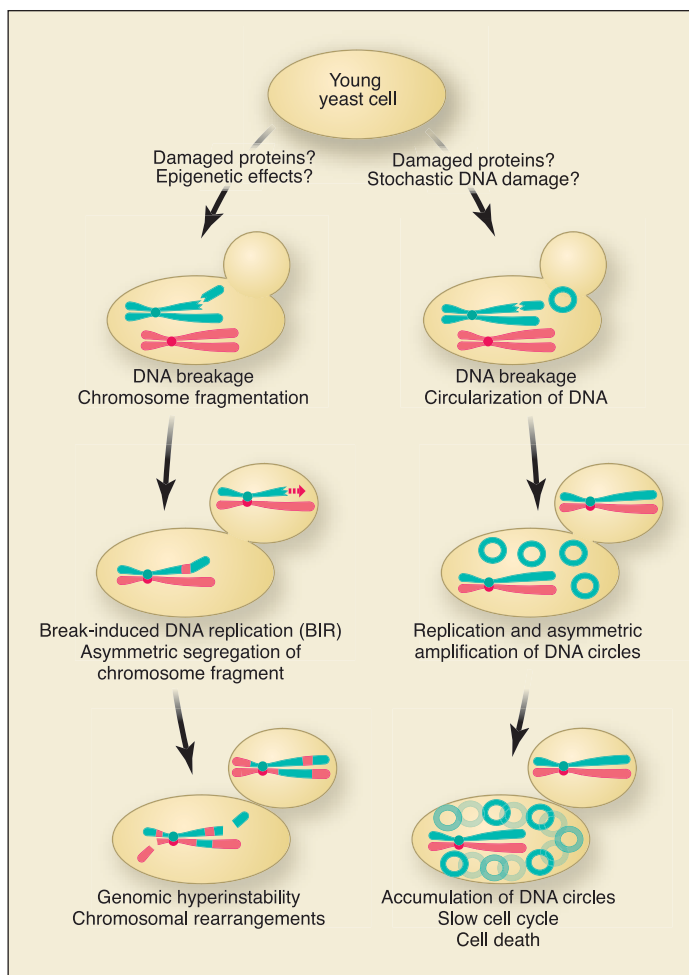
convince the scientific community that this unicellular organism might be useful for understanding human aging (1). Less skepticism should greet the report by McMurray and Gottschling (2) on page 1008 of this issue. These investigators show that yeast cell aging is accompanied by increased genetic instability, a hallmark of cancer. This finding might help researchers to understand the link between cancer and old age in humans.

In the final decades of life, one's chance of developing cancer rises exponentially (3). In fact, at age 70 the risk of developing

cancer is more than 10 times the risk three decades earlier. It is tempting to think that cancer occurs later in life because of a steady accumulation of mutations. Certainly, cells isolated from the elderly have more chromosomal abnormalities than cells from the young. But the story is not so simple because rates of spontaneous mutation are too low to account for the extensive genome rearrangements found in tumors (3). Experiments in mice have confirmed the suspicion that mutation rates increase with age (4).

The molecular basis of this increase in mutation rate is still under debate. The most popular explanation is the "mutator phenotype," which is thought to arise when genes required for preventing or repairing DNA damage are mutated, leading to runaway DNA instability (5). Although

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**Two mid-life crises for yeast.** Aging yeast cells switch to a state of DNA hyperinstability characterized by an increased rate of DNA breakage and recombination (2) (left). This process is independent of that limiting yeast life-span (right), which depends on the amplification and accumulation of toxic circular DNA molecules (6).

this is probably a major part of the story, some researchers argue that it fails to explain fully the gross chromosomal abnormalities and tissue distribution of most adult cancers (3). Experiments investigating a decrease in DNA-repair capacity in old animals have yielded equivocal results because of technical difficulties. Other factors thought to increase the rate of mutation over time include an accumulation of damaged proteins in cells, altered gene regulation, and changes in the tissue surrounding cells (3, 4).

The difficulty of studying aging in mammals makes the new work in yeast appealing. The life-span of budding yeast is typically defined as the number of daughter cells an individual mother cell produces before dying. McMurray and Gottschling set out to determine whether yeast cells, like mammals, also experience an age-dependent increase in mutation rate. They engineered yeast mother cells so that the colonies produced by their daughters

long-lived cells produced more than 60 offspring, requiring the investigators to pluck daughter cells for 5 days without a break—a heroic effort.

Fortunately, the effort paid off. Daughters of old cells were 40 to 200 times more likely to lose a marker gene than daughters of young cells. And after the initial loss, subsequent losses also increased in frequency. In this way, the researchers uncovered an age-dependent, genetic instability switch in yeast. Interestingly, it was predominantly the daughters and not the mothers that tended to lose the marker gene. By analyzing the chromosomes of the daughter-cell colonies, the authors inferred how the genes were being lost. Old mothers are apparently predisposed to DNA damage, and large sections of their chromosomes can break off. These fragments preferentially stay with the mother cell so the daughter is forced to repair the damage by re-extending a broken chromosome using the homologous chromosome

served as miniature colored “pie charts” that displayed the genetic history of the colony’s founders. For example, if a daughter cell produced a half-brown/half-white colony, this meant that the daughter had lost one of the marker genes (a measure of genetic instability) after her first cell division. If the daughter colonies were all brown instead of white, then the mother must have lost her marker gene at some point and passed on this genetic defect to her progeny.

One advantage of yeast is their short life-span: A yeast cell divides about 20 times, approximately once every 2 hours. A major disadvantage is that determining the life-span of microscopic cells is no easy task. A researcher must spend many days sampling cells with a fiber-optic needle connected to a microdissection apparatus. In the new work, the time of birth for each daughter cell was also recorded. Some

as a template, in a process called break-induced replication (see the figure). The authors raise the intriguing possibility that this bias toward preserving the mother cell’s genome might have implications for how the genomes of human stem cells are preserved.

Next, the researchers asked whether this DNA-instability switch contributes to yeast aging. A major cause of yeast aging stems from DNA exchanges between the highly repetitive ribosomal RNA genes (6). These events generate circular, replicating forms of ribosomal DNA known as ERCs that accumulate and eventually kill the mother cell. The authors found that suppressing ERC formation extended the mother cell’s life-span, consistent with previous reports, but did not affect the timing of the genetic instability switch. It appears that there are at least two independent clocks in yeast cells: one that determines life-span and another that controls the DNA-instability switch (see the figure). In mammals, this distinction may not be as clear-cut because, unlike yeast, rogue mutant cells can kill the organism by forming tumors.

Biological switches like the one that triggers DNA instability often indicate one of two mechanisms. Either there is an underlying process that crosses a critical threshold (for example, the shortening of telomeric ends of human chromosomes, leading to sudden growth arrest), or there is a positive-feedback loop that amplifies small changes (for example, the mutator phenotype). In the case of yeast, telomere loss and the mutator phenotype are unlikely to be root causes because yeast telomeres do not shorten with age and the hyperinstability state is not stably inherited.

Perhaps old cells accumulate damaged proteins, which decrease the cell’s ability to protect and repair DNA. This model fits with the recent finding that oxidized proteins accumulate preferentially in old yeast cells (7). Of course, yeast may surprise us with a new mechanism. Amid this speculation, one thing is certain: Only when we know the underlying causes of this phenomenon, can we truly predict how relevant it will be to humans. But if history is anything to go by, we can look forward to learning more lessons about aging and cancer from this tiny fungus.

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