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DNA Repair: Corrections in the Golden Years

Genetic changes increase with the age of organisms, but the basis for this increase is unclear. A study has found that the major pathway of DNA repair is altered with age in the testes of male *Drosophila*, thus providing a powerful system to dissect the basis for age-related genomic changes.

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Despite claims to the contrary, it is inevitable that, as we get older, real physiological changes occur throughout our bodies. Many of the changes are obvious and we are reminded of them daily: skin goes from smooth and supple to brittle and wrinkled; hair grays and may even disappear; hearing and eyesight acuity degrade. But other changes occur that are less obvious, going unnoticed until we call upon a less frequently used function. We do not recover from injury as rapidly when we are older, we cannot run as fast, and our ability to procreate is compromised. In fact, there is a dramatic decline in the ability to have children as we age [1,2]. For those who do have babies later in life, there is an increased probability of genetic abnormalities in their offspring.

The decline in fecundity and increased genetic abnormalities with age has largely been attributed to genetic changes in the egg and sperm of the parents. Chromosome segregation errors, which lead to aneuploidy, appear to be the predominant reason for age-related defects in eggs [3], while there is little evidence for such a problem in sperm [4]. But older fathers are not completely off the hook as contributors to their child's genetic outcome; there is some suggestion that DNA damage and chromosome breaks may increase with age in sperm [5].

As they reported recently in *Current Biology*, Preston *et al.* [6] have discovered an interesting phenomenon that may shed light on changes that occur to sperm with age. They have obtained compelling evidence that, in sperm from male *Drosophila* flies, the predominant mechanism by which DNA double-stranded breaks are repaired changes as the male ages. The foundation for their conclusions is an assay developed earlier by the authors [7], which is based on assays developed in the yeast *Saccharomyces cerevisiae* to analyze DNA double-stranded break repair *in vivo* [8]. They use a genetic reporter construct that introduces an inducible site-specific double-stranded break in the DNA, created by the endonuclease I-*Sce*I, and provides a readout for the pathway by which double-stranded break repair occurs. While there are several routes to repair a double-stranded break, their assay readily distinguishes between three major types: non-homologous end-joining, single-strand annealing and homologous repair using the homologous chromosome as a template [9].

The I-*Sce*I endonuclease is expressed in all somatic cells, but when the double-stranded break is repaired in a way that eliminates the cleavage site, a 'signature' of the particular repair pathway that was used is created and is propagated in the DNA sequence of the repaired chromosome. The repair pathway signature is then detected by phenotypic and PCR analysis. While different tissues may repair the double-stranded break by different pathways, Preston *et al.* [6] focused on repair events that happened in the male germline lineage by mating male flies with the reporter construct to females that lacked the reporter construct and then analyzing repair signatures in the offspring. By examining all the offspring from a single male, they could determine the distribution and relative number of single-strand annealing, non-homologous end-joining and homologous repair events that occurred during sperm development in that male.

New sperm are made throughout the fertile life of a *Drosophila* male [10]. The testis contains an average of nine germline stem cells, which divide asymmetrically to give one cell that retains its stem cell identity and one that becomes a spermatogonium. This differentiated cell divides four additional times to produce a cyst of 16 interconnected spermatogonia that typically undergo meiosis and differentiate into individual spermatids. Exactly when in this process the double-stranded break repair events occur is unclear, but as described by Preston *et al.* [6], it does not appear to occur in the germ cells. Therefore, repair must occur in the subsequent stages of sperm development, but before the haploid genomes are packaged into spermatids.

In order to examine the effects of age on DNA repair in sperm development, Preston *et al.* [6] took 115 young male flies and mated each one with an independent 'harem' of virgin females. Seven days later, each male was introduced to a new harem of young virgins. This weekly opportunity was repeated throughout the fertile life of each male. They then analyzed the distribution of non-homologous end-joining, single-strand annealing and homologous repair in the offspring from each harem for each individual male. When the males were young, single-strand annealing was the predominate pathway of repair, accounting for ~55% of the events, while homologous repair was the lowest at ~14%.

As the males aged, however, the ratios changed dramatically. Homologous repair events increased with each week, so that by six weeks of age (the effective limit of male fertility) homologous repair represented ~60% of the events, while the proportion of single-strand annealing and non-homologous end-joining events had decreased. Commensurate with the increase in homologous repair events, the tract length, or amount of DNA sequence copied from the homologous chromosome, also

increased. In fact, almost the entire increase in homologous repair was the result of the longer tract events. Taken together, these quantitative changes in repair indicate that there is a qualitative change in the lineage of sperm-producing cells as they age. As all interesting observations tend to do, this study raises a whole new spectrum of questions which, using this experimentally tractable system, have the chance to be addressed.

What causes the age-dependent shift in the spectrum of double-stranded break repair? In considering this issue it is worth viewing the non-homologous end-joining, single-strand annealing and homologous repair pathways as representing three different DNA repair machineries that are all competing for the same substrate — a double-stranded break. With this in mind, the simple answer is that aging causes a change in the relative activity of these DNA repair machinery. But how these changes occur is what must be understood to ultimately link the mechanics of aging to this phenomenon. Here are a few of the possibilities. Components of the homologous repair pathway might become overexpressed or post-translationally modified to increase their activity. Conversely, single-strand annealing and non-homologous end-joining components might lose their activity with age. Components of these pathways might become damaged with age, for example by post-translational modifications associated with aging cells such as carbonylation or glycosylation [11]. If any of these explanations were true, then interest would turn to understanding what causes such an age-related change in regulation.

Aging cells have also been reported to contain increased levels of damaged DNA [12]. This, in turn, could lead to compensatory DNA repair mechanisms to handle the extra DNA damage, or one of the pathways may simply become overwhelmed, leaving homologous repair to primarily handle the induced double-stranded break repair. But then what leads to the increased damage? This may be the result of increased production of agents that increase damage, or a failure of the damage repair system to function properly. Alternatively, the affect may be even more indirect. As male flies age, the cell division timing slows down in germline stem cells [13]. Where it has been examined, non-homologous end-joining appears to act predominantly in G1 phase of the cell cycle, while homologous recombination is the primary repair path in S and G2/M phases [14,15].

Might the age-related change in DNA repair in *Drosophila* represent a fundamental process in all eukaryotes? Very likely it does. One of the obvious situations in which this may be important is during the dramatic increase in cancer with age [16]. And it may be most relevant to those tissues/organs in which cells are regularly renewed from a stem cell compartment. For instance, the spectrum of genomic rearrangements in leukemia patients who develop the disease later in life are quite distinct from those who have the disease before the age of 56 [17]. While there are many hypotheses to explain this difference, it may be based on the same underlying causes of the genomic changes seen in the Preston *et al.* [6] study.

The change in DNA repair with age during sperm development in *Drosophila* is quite analogous to age-induced loss-of-heterozygosity during the replicative lifespan of the yeast *S. cerevisiae* [18]. In yeast, there is an increase in genomic instability with age, and while loss-of-heterozygosity events occur predominantly by a reciprocal recombination pathway, in the age-induced loss-of-heterozygosity it occurs by a non-reciprocal recombination pathway, break-induced replication. The assay used by Preston *et al.* [6] does not detect a change in spontaneous genomic instability; nevertheless it may reflect that a similar aging-related process is occurring in *Drosophila* and yeast. It is also worth noting that, just as Preston *et al.* [6] were able to determine that the repair of the induced double-stranded break did not generally occur in the stem cells, in the age-induced loss-of-heterozygosity events in yeast, the loss-of-heterozygosity was predominantly manifested in the daughters of old mother yeast cells, not the mothers themselves (yeast mother cells have been equated with metazoan stem cells) [19].

Perhaps the most exciting aspect of the Preston *et al.* [6] work is that it defines an aging-dependent phenomenon in an area of biology — double-stranded break repair — about which we have a great deal of knowledge. Thus, it sets the stage to critically examine the link between genome integrity and the aging process, and the promise to explain this link in *Drosophila*, using its treasure trove of experimental tools and rich history of successfully dissecting difficult biological problems.

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Evolution: The Ecological Reverberations of Toxic Trace Elements

A recent study of plants that accumulate selenium from soils illustrates how plant defenses can be sequestered and presumably exploited defensively by herbivores that have co-evolved selenium resistance.

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The discrepancy between the time scales of a scientist's life (and funding) and evolutionary processes makes the study of evolution in long-lived organisms in real-time a challenge. Consequently, researchers prefer to study extreme selection pressures that force organisms to adapt quickly, such as the adaptation of plants to growth on soils with toxic levels of trace elements. Heavy-metal pollution and the urgent need to develop strategies for cleaning contaminated soils have motivated research into the physiology and ecology of such plants [1,2].

How strong selective pressures on contaminated soils can lead to reproductive isolation of adjacent populations in a relatively short time is well documented [3]. Several hypotheses have been proposed to explain the evolutionary advantage of adapting to such unfavorable conditions [4]. The lack of competition from other plants or the absence of attack from pathogens may allow metal-resistant plants to thrive in toxic waste dumps. But the secondary benefits of learning to cope with toxins may be just as important. The accumulation of toxic trace elements in plant tissue may equip plants with effective defenses against insect herbivores either directly or indirectly, by activating defense-related signaling cascades [4]. Several studies have shown that such elemental defenses exist, but their consequences for co-evolving species are unknown.

Freeman *et al.* [5] report in this issue of *Current Biology* how higher trophic levels are influenced by strong selective pressures from toxic-element stress. They have shown that the selenium-hyperaccumulating plant *Stanleya pinnata*, native to the western United States, is well defended against two common generalist pests, the diamondback moth (*Plutella xylostella*) and the cabbage white butterfly (*Pieris rapae*). Larvae fed on diets with selenium concentrations as high as those of hyperaccumulating *S. pinnata* plants die, and adult moths avoid ovipositing on selenium-rich plants. Yet in nature, these selenium-rich plants suffer herbivore damage from a formerly unknown variety of *P. xylostella*, which has obviously adapted by disarming the elemental defense. These insects thrive on a selenium-rich diet and do not show any oviposition- or feeding-deterrence. Moreover, they can accumulate about four times more selenium in their body tissues as can non-resistant varieties. Such an accumulation may influence the moth's predators or parasitoids: Freeman *et al.* [5] also analyzed the co-occurring parasitic wasp *Diadegma insulare* and found a correspondingly high amount of selenium, indicating co-evolution at the third trophic level.

Although selenium is an essential trace element for many species, it becomes toxic at high levels because of its similarity to sulfur and its consequent assimilation into selenocysteine. Selenocysteine replaces cysteine during protein biosynthesis, which leads to protein misfolding and severe toxicity. One mechanism by which detoxification occurs in selenium-resistant plants is the inactivation of selenocysteine by methylation. Such plant-derived methylselenocysteine is usually demethylated again after ingestion by herbivores, causing severe intoxication. In their analysis of seleno-compounds in all three species, however, Freeman *et al.* [5] found that the selenium-resistant varieties accumulate the inactive methylselenocysteine, whereas the selenium-sensitive varieties accumulate toxic selenocysteine. A decrease in demethylase activity may be the key adaptation; such a loss of activity would in general be a disadvantage, as it prevents the conversion of methyl-cysteine, which occurs in several Brassicaceae species, to cysteine, but with a methylselenocysteine-rich diet, the loss of activity might prove advantageous.

That *P. xylostella* has evolved resistance to toxic seleno-compounds is not surprising, as it