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Report

Age-Dependent Usage of Double-Strand-Break Repair Pathways

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Summary

A DNA double-strand break (DSB) can be repaired by any of several alternative and competing mechanisms. The repaired sequences often differ from the original depending on which mechanism was used so that the cell's "choice" of repair mechanism can have profound genetic consequences. DSBs can accumulate with age [1, 2], and human diseases that mimic some of the effects of aging, such as increased susceptibility to cancer, are associated with certain defects in DSB repair [3, 4]. The premeiotic germ cells of *Drosophila* provide a useful model for exploration of the connection between aging and DNA repair because these cells are subject to mortality and other age-related changes [5], and their DNA repair process is easily quantified. We used Rr3, a repair reporter system in *Drosophila* [6], to show that the relative usage of DSB repair mechanisms can change substantially as an organism ages. Homologous repair increased linearly in the male germline from 14% in young individuals to more than 60% in old ones, whereas two other pathways showed a corresponding decrease. Furthermore, the proportion of longer conversion tracts (>156 bp) also increased nearly 2-fold as the flies aged. These findings are relevant to the more general question of how DNA damage and repair are related to aging.

Results and Discussion

The suspected link between DNA damage and aging [2–4, 7–16] raises the question of whether cells change their way of handling DNA damage as the organism ages. The complexity of DNA repair mechanisms leaves adequate room for such changes. For example, cells have multiple mechanisms for repairing double-strand breaks [17], implying a degree of flexibility in the repair process. All of these mechanisms rejoin the broken ends so that the cell cycle can proceed, but they differ in the likelihood and extent of sequence changes at the repair site. Therefore, any changes in the relative usage of DSB repair mechanisms will affect how DNA damage accumulates. In this report, we show that the relative usage of DSB repair pathways in at least one tissue type can change dramatically as the organism ages.

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Measuring Three Types of DSB Repair

Our approach was to use a reporter construct, Rr3 (Repair reporter 3), to monitor the relative usage of three DSB repair mechanisms in the germ cells of *Drosophila* males as they age [6]. It is a longitudinal study with each male being sampled multiple times in its lifetime. The three repair outcomes we measure are NHEJ (nonhomologous end-joining), SSA (single-strand annealing), and HR-h (homologous repair with the homolog as the template). NHEJ requires no extensive homology but often leaves small additions, deletions, or other sequence alterations at the repaired site [18]. SSA makes use of directly duplicated sequences flanking the break [19, 20] and is only used when such a duplication is available. One copy of the duplication and any interstitial sequence is lost in the course of the repair. HR involves conversion from a template duplex, which is frequently the sister chromatid or the homolog [21]. Here, we will distinguish between HR with the sister chromatid as template and HR with the homolog as template by using the abbreviations HR-s and HR-h. Figure 1A shows where our three measured quantities fit into a scheme of known DSB repair pathways.

In our experimental system, double-strand breaks are created at a specific site within the Rr3 construct (Figure 1B) by an endonuclease gene that is expressed continuously throughout development and aging. Each break can be repaired by SSA, NHEJ, or HR-h such that the repair products can be distinguished in the next generation by a combination of phenotypic and PCR scoring (see Experimental Procedures). Breaks can also be repaired by HR-s, but such events restore the cut site within Rr3 and thus enable further rounds of breakage and repair. The three measured frequencies represent the possible endpoints of this potentially cyclical process. Previous measurements with Rr3 [6] indicate that the frequencies of SSA, NHEJ, and HR-h usually total close to 100%, suggesting that most copies of Rr3 are cut at least once and are finally repaired by one of the three measured pathways. No further cutting by the endonuclease is possible after SSA, NHEJ, or HR-h has occurred and the endonuclease recognition site has been destroyed.

Figure 2 shows the scheme for monitoring the relative frequencies of the three possible repair outcomes throughout the fertile lifetime of individual males. In this scheme, each male provides a statistically independent estimate of the relative frequency for each outcome at multiple time points as he ages. Each time point consists of sperm sampled during a 1 week period. Sampling was stopped after 6 weeks when most of the males had died or become infertile. Only males that remained fertile for at least the first 3 weeks were used.

Increase in Homologous Repair during Aging

The results (Figure 3A and Table S1 in the Supplemental Data available online) show a marked change in relative pathway usage over time. Only 13.6% of the

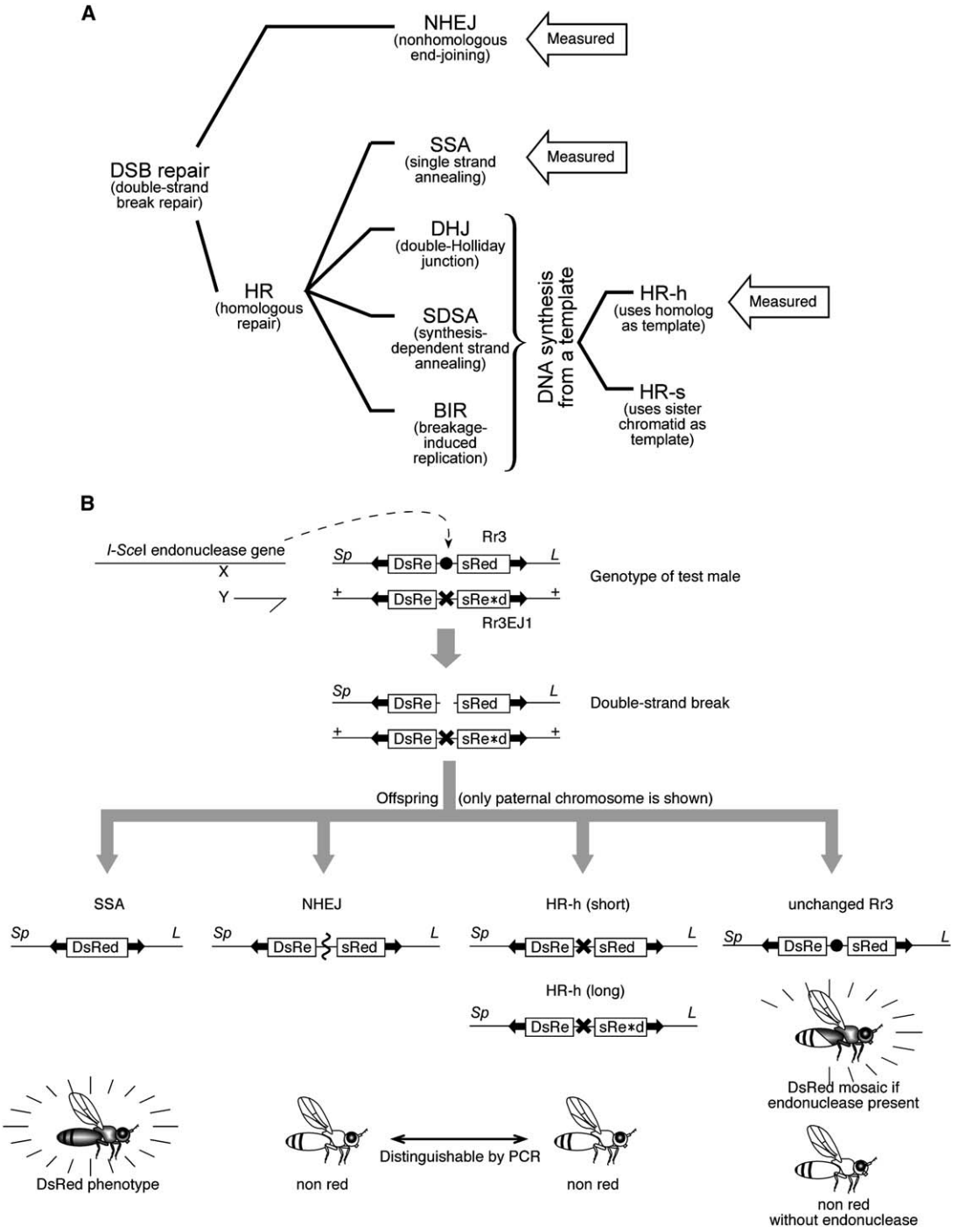

Figure 1. Measuring Relative Usage of DSB Repair Pathways

(A) The three measured quantities (NHEJ, SSA, and HR-h) shown in the context of known DSB repair pathways.

(B) Scheme used for measuring relative usage of DSB repair pathways [6]. Repair outcomes are measured in the germline of individual males that are heterozygous for the reporter construct, Rr3. This construct includes a nonfunctional copy of the DsRed gene that is interrupted by a recognition site (●) for the I-SceI endonuclease. It also contains a 147 bp duplication, symbolized by the letters sRe within DsRed. The other homolog carries a modified version of Rr3, called Rr3EJ1, in which the I-SceI cut site has been destroyed by the insertion of 14 bp and loss of 2 bp (represented by a thick black "X"). It also carries a 16 bp deletion (*) relative to Rr3 located 156 bp to the right of the former cut site. Dominant visible markers *Sternopleural* (*Sp*) and *Lobe* (*L*) were used to distinguish the homologs [36].

chromosomes sampled from the youngest males had been repaired by HR-h, making it the least frequent outcome of the three. As the flies aged, however, the frequency of HR-h increased steadily until it was the dominant outcome (60.9%) in samples from the oldest males. At the same time, SSA and NHEJ decreased proportionately so that the total of the three outcomes remained close to 100% throughout the 6 week period.