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# Reduced Replication: A Call to ARMS

**In this issue of *Cell*, Lemoine et al. (2005) monitor chromosome instability in yeast cells with reduced levels of an essential replicative DNA polymerase. The authors identify a hotspot for chromosome aberrations reminiscent of fragile sites in human cells. This hotspot is composed of inverted Ty elements, which lead to a double-strand break under conditions of limited replication.**

The three R's of DNA metabolism—replication, repair, and recombination—are intimately interrelated to assure accurate transmission of genetic material from generation to generation. Changes in any of the components can greatly alter the ability of our cells to deal with external or internal environmental threats where the consequences include cancer and a variety of other diseases. Chromosomal DNA itself looms as one of the threats. If the three R's function improperly, at-risk DNA motifs (ARMS) such as repeated sequences and palindromes increase the likelihood of genetic alterations. Chromosome aberrations and aneuploidy are a major source of a variety of genetic diseases and carcinogenesis. There are many examples of non-B forming DNAs that are ARMs associated with genomic rearrangements in humans (Bacolla and Wells, 2004). Human chromosomal fragile sites are regions in which alterations in DNA metabolism, particularly replication, can increase the incidence of chromosomal aberrations (Glover et al., 1984; reviewed in Arlt et al., 2003). With this as a backdrop, a stimulating paper by Lemoine et al. in this issue of *Cell* investigated the impact of changes in replication on chromosomal stability and, in so doing, expanded the repertoire of approaches in the common baker's yeast for examining genome stability (Kolodner et al., 2002). They discovered that aberrations are frequent and that they are often associated with ARMs.

Healthy replication in yeast as well as human cells requires the DNA polymerases α, δ, and ε where α is required for replication initiation and the polymerases δ and ε replicate the leading and lagging strands. The Petes lab developed a highly regulatable DNA polymerase α and found that cells were able to grow even when the level was reduced over 10-fold. To register chromosomal changes, they capitalized on a classic system that reveals "illegitimate mating" due to loss of chromosome III (Chr III), or at least that portion of the chromosomal arm that contains the mating type locus. These losses enable cells to mate promiscuously to cells of the same mating type. The system has an advantage over haploid-based systems because regions containing essential genes are not excluded from analysis, as the altered chromosome is covered by a homolog in the resulting diploid. This allows detection of a wide variety of chromosome alterations in a large DNA region. The genetic detection of chromosomal changes is followed by molecular analysis with comparative genome hybridization (CGH) to DNA microarrays. CGH is commonly used to identify variations in amounts of DNA across individual chromosomes, thereby revealing chromosome aberrations. However, the resolution of events, particularly breakpoints, is much better with yeast because of the compactness of its genome. Changes in any chromosome are identified by differences in relative amounts of randomly primed chromosomal DNA from colonies of control versus stressed cells that hybridize with a nearly complete arrayed set of yeast coding sequences. Under- and overrepresented contiguous regions of the genome that could result from a variety of events including translocations (except reciprocal), deletions, duplications, and aneuploidy are detected. Because the density of genomic sequences on the microarry chips is high, sites of chromosomal disruptions can be approximated, and subsequent restriction digestion and Southern analysis reveal the breakpoints more precisely.

Lemoine et al. found that reduction in DNA polymerase α dramatically increased yeast promiscuity (>200-fold), consistent with the general view that altered replication can destabilize the genome. Genetic analysis pointed to gross chromosomal changes arising from complete or partial loss of Chr III. Included among the possible culprits are large internal deletions, deletions with generation of a telomere, translocation, and aneuploidy. CGH analysis revealed that the aberrations associated with Chr III did not occur randomly. Since many of the breakpoints were associated with Ty retrotransposons within Chr III and with Tys in other chromosomes or small *delta* remnants, recombinational interactions figure prominently in the induced chromosome aberrations. (Molecular sleuthing by Lemoine et al. revealed that the Chr III breakpoint regions actually contained suspected Ty elements.) Particularly interesting were events associated with a pair of closely spaced (283 bp) inverted Ty elements on Chr III. This led to a critical, highly revealing experiment that directly addressed the role of the inverted Tys. By changing the Ty orientation to direct repeats, the instability of Chr III associated with reduced replication was nearly eliminated. This clearly implicated the inverted Ty repeats in the Chr III fragility.

Why should inverted Tys be fragile sites when replication is reduced? The genetic activity of inverted repeats (IRs) has been extensively investigated from bacteria to animals (Lemoine et al. and references therein). IRs can be instrumental in their own destruction and can lead to a variety of genomic changes. For example, long artificial IRs similar in organization to that described by Lemoine et al. can generate large deletions and translocations in yeast via homologous recombination involving the region of the IR, and this instability is greatly increased by a defective polymerase δ (Lobachev et al., 1998). Another form of IR—a palindrome of 300 bp human *Alu* sequences inserted into the yeast genome—can lead to a closely associated double-strand break (DSB) and generation of inverted chromo-

some duplications (Lobachev et al., 2002). Using a similar approach, Lemoine et al. went on to show that the limited replication generated a DSB hotspot in the region of the inverted Ty elements. The reason(s) that altered replication has this effect remains unknown, although potential opportunities for intrastrand self-pairing and cruciforms are suggested in the synergistic genetic interaction between altered replication and inverted repeat ARMs (Lemoine et al., 2005; Lobachev et al., 1998). The replication-associated fragile sites in yeast have implications for human cells. Much of the human genome contains repeated DNAs. For example, *Alu*'s account for as much as 10% of the human DNA and they are frequently closely spaced and inverted, albeit diverged (see references in Lobachev et al., 2002).

It will be interesting to learn more about how replication defects lead to chromosome aberrations that are not IR associated, the roles for DNA replication and repair genes that have human homologs such as *RAD54, RAD51*, and the *MRE11*, although reductions in DNA polymerase $\delta$ appeared to have little effect on the IR-associated fragility. Surprisingly, overexpression of DNA polymerase $\alpha$ also increased Chr III associated changes, possibly by titrating out other replication components. This is reminiscent of decreased mismatch repair when the Msh3 component of the mismatch repair complex is overexpressed (Drummond et al., 1997). The results obtained with changes in expression implicate a finely tuned balance of at least some DNA metabolic proteins in genome stability. Possibly, cell-to-cell variation of these proteins or agents that lead to even modest changes in replication may be potential sources of chromosome aberrations, especially in the presence of ARMs of potential destruction.

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