COMMENTARY

Genomic instability and repair mediated by common repeated sequences

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If you happen to find a suspicious cell, say, in your soup, you may recognize to what species it belongs simply by looking at its chromosome configuration (karyotype) under the microscope. This approach works because most cells of most organisms have a stable genome, and gross chromosomal rearrangements (GCRs) such as translocations, inversions, and deletions of big chunks of chromosomes are relatively rare. This is in stark contrast to cancer cells, which exhibit abundant GCRs (1). When the borders of such rearrangements are mapped, they often belong to repetitive sequences, which are scattered across the genome of all organisms. Tandem repetitive sequences are also present at the ends of the eukaryotic chromosomes, forming the telomeres, which help replicate the genome and protect it from degradation (2). Interestingly, telomeric repeats can also be found at internal positions along the chromosomes in many organisms. These interstitial telomeric sequences (ITSs) often colocalize with chromosomal fragile sites and with endpoints of GCRs (3, 4). However, little is known about the mechanisms responsible for genome instability at interstitial telomeric sequences. In PNAS, Aksenova et al. (5) present a study in which the power of yeast genetics is harnessed to address this particular question. Using a sophisticated genetic trap, the authors measure the rate of GCR formation and characterize the molecular mechanisms leading to them. The results show a surprisingly high level of recombinational activity involving these repeated sequences.

To investigate the frequency at which GCRs involving ITSs occur, Aksenova et al. use a clever yeast genetic trick (Fig. 1*A*): A yeast strain carrying an intron-containing *URA3* gene (involved in uracil biosynthesis) on chromosome III is phenotypically Ura$^+$, because the intron is spliced efficiently. Ura$^+$ cells can grow on plates lacking uracil, but cannot grow on plates containing the toxic compound 5-fluoroorotic acid (5-FOA). Within this intron, they inserted an ITS-like sequence (composed of telomere repeats), still enabling proper splicing and expression of the *URA3* gene. If the tandem repeats are amplified beyond a certain size, or if, alternatively, a GCR event or a mutation occurs, the cells can become Ura$^-$ and 5-FOA resistant. The rate of 5-FOA–resistant cells is in the order of 10^{-8} per division in a strain with a small intron at the *URA3* gene. When 8 or 15 copies of the yeast telomeric sequences (TGTGTGGG) were inserted within the intron, this rate increased 20- and 125-fold, respectively. Interestingly, in 75% and 38% of these, respectively, the 5-FOA–resistant phenotype was due to point mutations affecting the coding capacity of the *URA3* gene and not by gross amplifications/rearrangements. Thus, the presence of telomeric repeats increases the rate of mutations in adjacent regions. The rest of the 5-FOA–resistant colonies carried complex chromosomal rearrangements (see below). Strikingly, small changes (additions or deletions of a single repeat) within the inserted telomeric sequences were very frequent and could be detected even in the absence of selection: These events happened at a rate of about 10^{-3} per cell division. As observed in other genomic regions that are hotspots for genomic rearrangements, the high rate of mutations and of small rearrangements suggest that double-strand breaks (DSBs) are often created at the internal telomeric repeats; their repair is accompanied by frequent insertions/deletions, mutations, and GCRs (Fig. 1*B*). This is in accordance with the fact that telomeric regions, being G-rich, are notoriously hard to replicate, and tend to stall the replication machinery, which can potentially create DSBs (6).

Analysis of GCRs Generated by the Presence of Internal Telomeric Sequences

Almost half of the 5-FOA–resistant colonies in the strain with 15 telomeric copies and a quarter of those with 8 copies carried gross chromosomal rearrangements. These were analyzed by a combination of pulse-field gel electrophoresis, PCR, comparative genome hybridization (CGH), and sequencing and were found to fall into four distinct categories. All of the events can be explained assuming that a DSB took place during replication of the internal telomeric sequences.

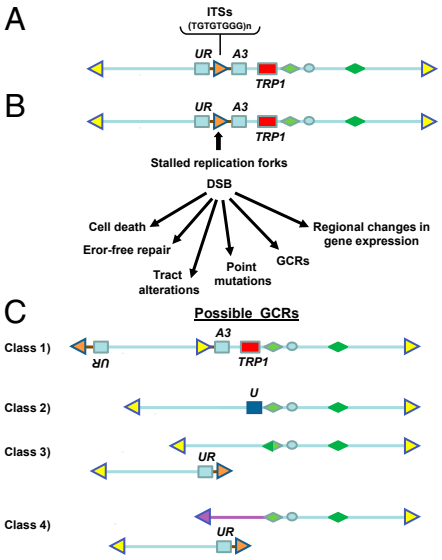

Fig. 1. Genomic consequences of the presence of ITSs in the genome. (*A*) Schematic description of the system used. Either 8 or 15 copies of the telomere sequence TGTGTGGG were inserted within an intron of the *URA3* gene and placed on chromosome III. Triangles represent telomeric sequences; circles represent centromeres; rhombs represent Ty elements (a yeast repeated sequence). (*B*) Schematic description of the consequences of replication stalling at an ITS. (*C*) Four types of gross chromosomal rearrangements described in the text. In class 1, cells carry a terminal inversion; class 2 represents a repair event using *URA3* and Ty sequences; and in classes 3 and 4, a linear fragment coexists with a healed chromosome that used internal Ty sequences (class 3) or Ty sequences in another chromosome (class 4) to heal.

i) Class 1 consisted of a large (80 kb long) inversion, in which the normal telomeric sequences are now adjacent to half the *URA3* gene, and the other half of the gene is now at the telomere (Fig. 1*C*). Such a structure can be created by a DSB at the ITS, followed by resection of the ends, and annealing/ligation in the inverse orientation. It is thus likely that in about half of the cases, when the annealing is carried out in the normal orientation, such events will stay undetected or only result in gain/loss of a small number of telomeric repeats at the ITS. Interestingly, during the formation of the rearranged structures, the

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number of telomeric repeats increased from 15 to 40–60, resulting in the silencing of the adjacent *TRP1* gene. These experiments demonstrate how dynamic changes in chromosomal sequences may have profound consequences for gene expression and thus for the phenotype and fitness of the cells.

ii) Class 2 was probably created by repair of the two ends of the broken chromosome by homologous recombination, using a single template, the second copy of *URA3* present at its normal location on chromosome V (Fig. 1*C*). This ectopic recombination event, which creates a deletion in chromosome III, was facilitated by the presence, within the *URA3* allele on chromosome V, of another type of repeated sequence, a Ty element. These elements are retrotransposons that change location by transposition at extremely low levels (7) but are very actively involved in genomic rearrangements caused by homologous recombination (8, 9). With ∼35 copies of Ty elements per haploid genome, and several hundred copies of the 340-bp-long terminal repeats (LTRs), they represent a substantial fraction of the genome.

iii) In Class 3, a linear, acentric fragment of 80 kb is detected, and the centromere-containing fragment was healed by a break-induced replication (BIR) event that duplicated the intact distal chromosomal end (Fig. 1*C*). Again, chromosome healing was accomplished by taking advantage of Ty sequences with the right orientation in the genome.

iv) Class 4 is similar to class 3, with the difference that the Ty element used to heal the broken centromere-containing chromosome III was located on another chromosome, and thus a nonreciprocal translocation was created (Fig. 1*C*).

The 80-kb linear acentric plasmid carries telomeric sequences at its ends (one natural and one extended from the ITS in the *URA3* intron) and is surprisingly stable, suggesting that a partitioning mechanism able to segregate it correctly between mother and daughter cells may exist.

Repeated Sequences Break, Repeated Sequences Repair

The results of Aksenova et al. (5) show that repeated sequence elements in the genome play important roles in both disrupting and maintaining the stability of the genome. Simple repeats, such as the ITSs (which may themselves represent "scars" of previous chromosomal rearrangements and repair events), present a challenge to the replication machinery and promote the breakage of chromosomes (10). The cells use all of the tricks available to try to repair the broken chromosomes and to survive. Remarkably, repetitive sequences also play a central role in the attempts to patch the broken chromosomes.

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As ITSs promote genomic instability, they must be under a strong evolutionary pressure to change, so as not to create "trouble." One way in which cells deal with this problem is to change their chromatin configuration (4). Telomere position effect or variegation is common to all eukaryotic organisms, and ITSs are also found in a heterochromatic configuration (11). In this sense, the ITS analyzed in the present paper might be particularly active, as, being within an actively expressed gene, it is probably present in a euchromatic context. It will be interesting to compare its behavior to that of a similar natural heterochromatic sequence. The genetic control of ITS-promoted rearrangements is also extremely interesting: For example, class 1 events, which can be explained by a simple inversion, could be mediated by nonhomologous end joining (dependent on the ligase IV activity), by a recombinational mechanism requiring strand invasion of duplex GT repeats by the telomeric ssDNA (which should require the activity of Rad51), or by a simple annealing between resected ends [similar to single strand annealing (SSA), and therefore Rad52 dependent but Rad51 independent]. Other open questions to explore in the future are whether telomere-affecting genes (12, 13) may play some role in preventing the type of rearrangements described.

Conclusions

The study of Aksenova et al. (5) provides further evidence for the dynamic nature of genomes. The authors show that spontaneous lesions probably occur, at a single ITS, at a frequency above 10^{-3} per generation, and this is a clear underestimate, as only events that introduce a visible change can be detected in the current setting. If one multiplies this effect by the number of potential repeated sequences with effects on the replication efficiency, the total potential for genomic instability is astounding, and it is remarkable how efficient the DNA damage response and repair systems of the WT cells are in preventing genomic rearrangements. It is also quite remarkable that repetitive genomic sequences, which are the source of this instability, are also part of its solution.

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