

■ MOLECULAR BIOLOGY

Doubling Up: How a DNA Sequence Becomes a Palindrome

Ratray AJ, Shafer BK, Neelam B, and Strathern JN. A mechanism of palindromic gene amplification in *Saccharomyces cerevisiae*. *Genes Dev* 19: 1390–99, 2005.

DNA palindromes (inverted repeats with little or no spacer) are genetically unstable structures because they can form cruciforms in the DNA, thereby disrupting normal processes such as replication, transcription, and repair. Palindromes are rare in normal cells. However, they are common in tumor cells and are associated with gene amplification. The mechanism by which palindromic gene amplification occurs has been a mystery because the instability of palindromes makes them intractable to molecular studies. In our article, we described the analysis of DNA palindromes generated as an aberrant but fairly common (6%) by-product of DNA double-strand break (DSB) repair in yeast. This detailed analysis has led us to propose a mechanism for how a single copy sequence is initially duplicated to generate a palindrome.

We had previously identified a yeast strain that tolerates DNA palindromes and developed a recombination substrate that produces this class of aberrant product as a readily identifiable byproduct of DNA DSB repair. Provided with the ability to generate dozens of independent palindromes, we developed a method to sequence palindromic DNA in the hope that analysis of the junction at the palindromic center would provide information about the mechanism of palindrome formation. In fact, the structure of the junctions supports a novel view of palindrome origin that involves a foldback and self-priming step. Previously, it was presumed that the junctions were formed by non-homologous end-joining (NHEJ). We demonstrated that palindrome formation is independent of NHEJ functions such as through the Ku complex or DNA ligase IV.

We induced a site-specific DSB in a recombination substrate and selected aberrant repair events that disrupted a nearby gene. Physical and genetic assays identified cells with palindromic gene duplications. To sequence the palindromic junctions, we first chemically modified the DNA with sodium bisulfite, which converts cytosine to uracil. This disrupts the intrastrand complementarity of the palindrome, allowing PCR primers (homologous to the modified DNA) to anneal for amplification and sequencing. Alignment of the original starting sequence with the junction sequence indicated that all of the 24 independent junctions analyzed mapped to only 7 sites and shared a characteristic hairpin structure—an

example is shown in **Figure 1, part A**. That is, there was a short hairpin (4–6 bp; shown in red) present at the point of sequence divergence. Furthermore, the novel sequence in the palindromes was precisely complementary to the sequence preceding the first inverted repeat (shown in green). Therefore, the new junction must have been formed by an intramolecular foldback between the 4–6 bp inverted repeats, which in turn served as a primer for new DNA synthesis. Indeed, all 24 junctions fell within a 400 bp region of the target gene and mapped only to sequences capable of forming short hairpins, even though hundreds of other short inverted repeats with longer spacers were present in the same 400 bp.

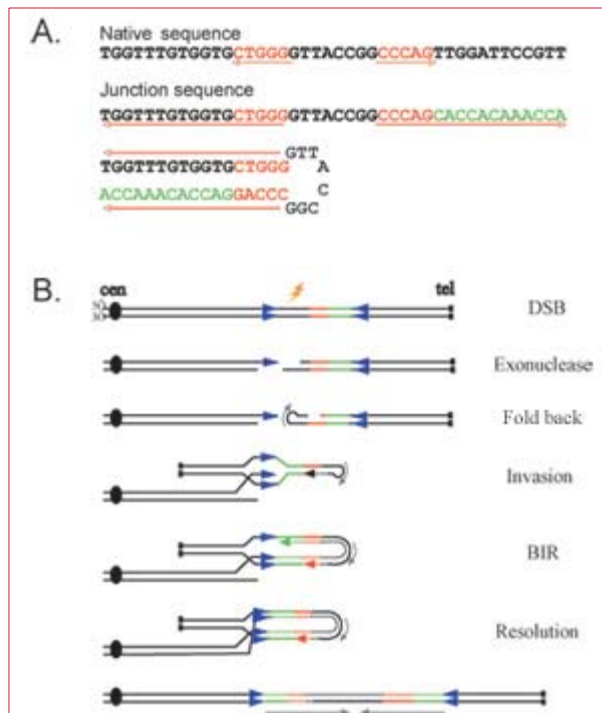


Figure 1. A) Sequence analysis of a palindromic junction: The native sequence prior to DNA double strand break (DSB) induction (top row), the deduced sequence of the palindromic junction obtained by sodium bisulfite sequencing (middle), and the fold-back priming event presumed to have taken place to produce the palindromic junction (bottom). B) Model for intrachromosomal palindrome formation. After the introduction of a DSB, exonucleolytic processing of the ends reveals a short hairpin that can fold back intramolecularly and prime new DNA synthesis. The remaining broken end can invade the other end via short dispersed inverted repeats, leading to break-induced replication (BIR) that proceeds around the newly created hairpin end, thus duplicating the sequence as a palindrome. Holliday junction resolution of the BIR intermediate leads to a long intrachromosomal palindromic duplication. The palindrome can then extrude into a cruciform, triggering a new DSB, initiating a new cycle of amplification (not shown). Gray arrows indicate extent of duplication. Note that if the other broken arm of the chromosome were to experience fold-back priming instead, this would lead to a hairpin-capped chromatid, which upon replication would resemble a sister chromatid fusion event (a common cytological observation in cells undergoing amplification). Cen and tel refer to

centromeric and telomeric regions of the chromosome, respectively.

A model for the formation of an intrachromosomal palindromic duplication based on our data is shown in **Figure 1, part B**. Key steps involve the processing of one side of the DSB to reveal a hairpin, followed by fold-back priming and a round of break-induced replication (BIR) of the hairpin into a DNA palindrome.

The genetic backgrounds that allow stabilization and recovery of palindromes are cells deficient in any one of the highly conserved genes of the Mre11/Rad50/Xrs2 (MRX) complex (the human ortholog of Xrs2 is Nbs1), or its modulator, Sae2. MRX is required for telomere maintenance, some non-homologous end-joining events, and recovery from cell cycle arrest after treatment of cells with DNA damaging agents (among other roles). Furthermore, MRX is involved in destabilizing palindromes, presumably by the known hairpin cleaving nuclease activity of Mre11. Perhaps a major role of MRX is, in fact, to prevent hairpin fold-back priming from occurring at the site of a broken replication fork.

We do not yet know whether the same type of mechanism is operating to create the common palindromic arrays associated with tumorigenesis, but our research opens the door to begin analyzing junctions directly from tumor cells, as well as studying the mechanism and gene requirements for their formation in yeast cells.

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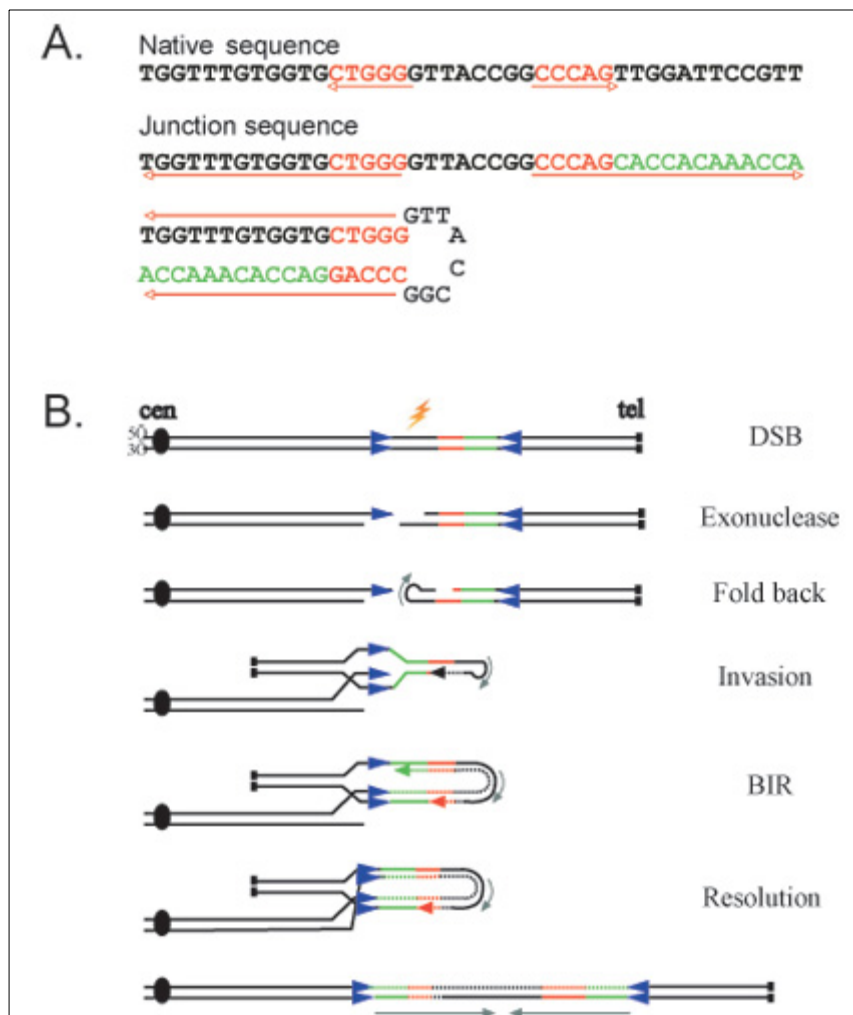


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