BACTERIAL CHROMOSOMES

Pol III picks up the pieces

George Marshall

The extreme resistance to ionizing radiation exhibited by *Deinococcus radiodurans* relies on a robust DNA repair system that reassembles its fragmented genome. Reassembly occurs through a coordinated series of DNA degradation and synthesis events, which, according to Slade and colleagues, the authors of a study published in *Cell*, involves the activity of DNA polymerase III (Pol III) in addition to DNA polymerase I (Pol I) and the recombination repair proteins RecA and RadA.

D. radiodurans can survive doses of radiation as high as 7 kGy, which shatter its 3.2 Mb genome into 20–30 kb fragments by introducing 100–150 double-strand breaks. The repair process requires diploidy to provide a template for repair and commences with an extensive exonucleolytic erosion of one strand of the DNA fragments to leave single-stranded overhangs. RecA, or its homologue RadA, is then thought to promote strand exchange, allowing the overhangs to be used to prime strand elongation in a process known as extended synthesis-dependent strand annealing (ESDSA). Elongated complementary strands then become annealed, re-establishing chromosomal contiguity. Previous work had shown that *D. radiodurans polA* mutants, which encode the Pol I polymerase, show substantial defects in synthesis and repair following irradiation.

Slade *et al.* found that following irradiation, exonucleolytic degradation of up to 40% of the genome and strand invasion by the resulting overhangs were both essential for DNA repair in a manner that depended on RecA and RadA; deletion of *recA* significantly reduced DNA degradation, whereas deletion of either *recA* or *radA* resulted in abolition of DNA repair synthesis. To determine whether DNA repair synthesis relied solely on the activity of Pol I, the authors irradiated cells from a *polA* mutant strain. Although genome repair was impeded, reassembly did occur and coincided with extensive DNA synthesis, indicating that another polymerase was involved in the repair process. Under normal conditions, Pol I functions to fill gaps in the DNA that arise during replication, repair and recombination events, whereas the bulk of DNA synthesis is carried out by the Pol III holoenzyme. To determine whether Pol III was involved in the repair pathway, Slade and colleagues tested a temperature-sensitive mutant of *dnaE* (*dnaE*^{ts}), which encodes the catalytic component of the Pol III holoenzyme. Shifting *dnaE*^{ts} cells to the restrictive temperature following irradiation abolished DNA repair synthesis, which indicated, for the first time, that Pol III activity is indispensable for DNA repair through the ESDSA pathway. The effectiveness of Pol III in the ESDSA repair process does appear to be limited by a requirement for Pol I: in *polA* mutant cells, genome repair was not complete even after an extended period of time. The authors propose a model in which DNA synthesis is initiated by Pol III and then elongated either by Pol I alone or by Pol III, with Pol I filling in any gaps that arise from excision repair of damaged bases.

Determining whether such a role for Pol III exists during DNA repair in other bacteria may help explain why *D. radiodurans* has such an unusually high tolerance for ionizing radiation.

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> “...Pol III activity is indispensable for DNA repair through the ESDSA pathway.”

ORIGINAL RESEARCH PAPER Slade, D., Lindner, A., Paul, G. & Radman, M. Recombination and replication in DNA repair of heavily irradiated *Deinococcus radiodurans*. *Cell* **136**, 1044–1055 (2009)