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SPOTLIGHT

Between sisters: Watching replication-associated recombinational DNA repair

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Amarh et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201803020) visualize for the first time the repair of double-strand breaks during DNA replication. As viewed by live-cell fluorescent imaging of *Escherichia coli*, repair of replication-dependent breaks is extraordinarily rapid and localized within the cell.

The process of DNA replication is vital to the survival of all forms of life. However, DNA replication is vulnerable to the formation of DNA double-strand breaks (DSBs). For example, each replication of the genome in a human cell generates ∼50 breaks with the potential, if mispaired, to promote genetic changes that can lead to cancer (Vilenchik and Knudson, 2003). DSBs generated during replication can be efficiently and faithfully repaired by homologous recombination with the sister chromosome, which immediately after replication is present in close proximity to the damaged site. This proximity should facilitate the homology search process that underlies homologous recombination reactions. We have not previously been able to observe the repair of replication-generated breaks because of the difficulty in inducing sister chromosome recombination at specific sites in vivo. Several investigators have observed DSB repair after cleavage by rare-cutting site-specific exonucleases (I-SceI; Lesterlin et al., 2014; Badrinarayanan et al., 2015). Such breaks are not confined to the replication fork, the site of most spontaneously generated breaks. Moreover, because efficient cutting by such enzymes cleaves both sister chromosomes, negating the possibility of repair, it is necessary to use inefficient conditions for cleavage such that only one sister is cut. In this issue, Amarh et al. use an elegant genetic system and present the first visualization of DSB repair between sister chromosomes broken during replication in *Escherichia coli*.

Amarh et al. (2018) have developed and studied a genetic system in the bacterium *E. coli* that allows only one sister chromosome to be cleaved with high efficiency at a specific site (Eykelenboom et al., 2008). Cleavage is replication dependent and generates a two-ended break, with the replication fork moving away from the site of the break. Subsequent homologous recombination reactions between sister chromosomes restore chromosome integrity and cell viability. The beauty of this system comes from the properties of the SbcCD endonuclease. SbcCD, a member of a group of enzymes that includes the eukaryotic Mre11–Rad50–Nbs1 (Xrs2) complex, incises DNA carrying inverted repeats that form hairpin DNA secondary structures (Connelly et al., 1998). In *E. coli*, structures eliciting SbcCD cleavage are formed only during replication and only on the sister chromosome formed by lagging-strand replication (Eykelenboom et al., 2008). Therefore, only one of the newly replicated sister chromosomes is broken; the other remains intact and will act as a source of DNA sequence homology to direct recombinational repair (Fig. 1). Despite a high efficiency of cleavage, there is no detectable loss of viability: all cells apparently survive this event.

In the system presented by Amarh et al. (2018), 246-bp inverted repeats are placed in the *lacZ* gene on the *E. coli* chromosome. Binding sites for fluorescently tagged repressor proteins (LacI-YPet and TetR-Cerulean) flank this palindromic cleavage site, allowing the intact locus and both ends of the break to be monitored in live cells. Expression of the SbcCD nuclease, which efficiently and rapidly cleaves the palindromic site, is controlled by an arabinose-inducible promoter. The group has previously shown that survival after SbcCD cleavage requires the RecABCD DSB pathway of homologous recombination: the RecBCD helicase/nuclease resects the broken DNA to form single-stranded termini, substrates for binding and strand exchange mediated by the RecA protein (homologous to Rad51 of eukaryotes). Other required factors include the Holliday junction processing proteins RuvABC and RecG along with the PriA replication restart protein (Eykelenboom et al., 2008). Other fluorescently tagged proteins allow the investigators to track the cellular position and timing of the appearance and disappearance of recombination intermediates (marked with RecA-mCherry) and replication complexes (marked by the tagged replication clamp YPet-DnaN). Growth conditions are such that there are distinct prereplication, replication, and postreplication periods of the cell cycle, with one chromosome replicated to two.

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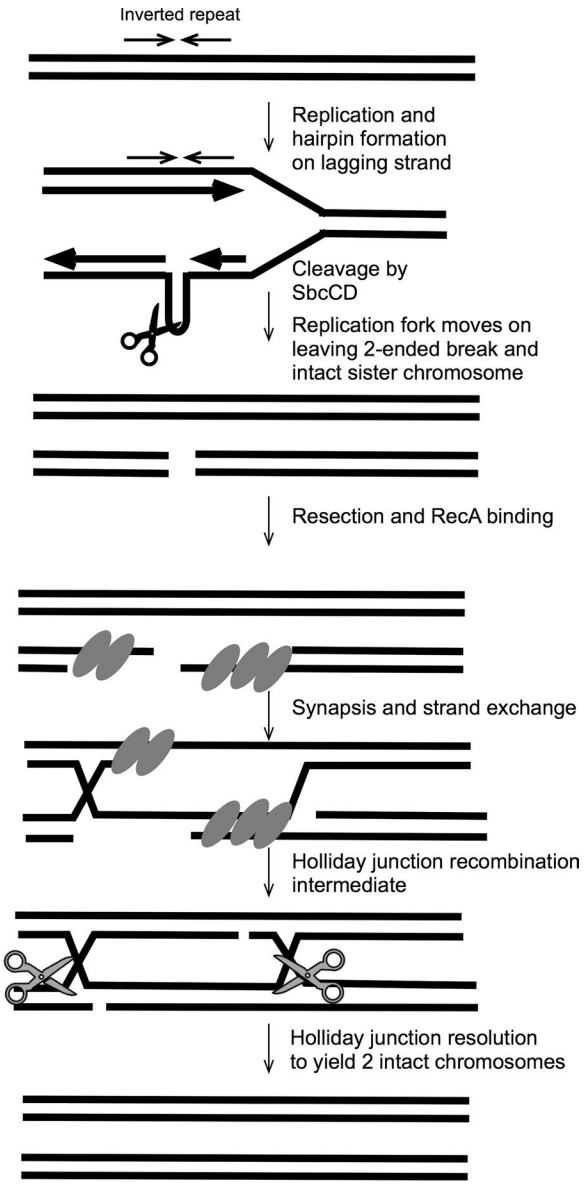

Figure 1. **Steps in recombinational repair of a SbcCD-delivered DSB.** Replication of an inverted repeat generates a hairpin DNA secondary structure on the lagging strand. The structure is recognized and cleaved by the SbcCD endonuclease while the replication fork moves onward. The broken ends are resected by RecBCD, allowing RecA to bind to the single-stranded regions. RecA promotes pairing and strand exchange between the broken ends and an intact sister chromosome, generating a branched intermediate known as a Holliday junction. Holliday junctions are resolved by cleavage, generating two intact sister chromosomes.

Amarh et al. (2018) track the appearance of the recombination protein RecA with respect to the cleaved locus. They observe that after cleavage is induced, transient RecA foci appear in close proximity to the repressor-marked cleavage locus. The RecA foci disappear, followed by the segregation of the two repressor-marked sister loci to opposite sides of the cell, which subsequently divides. The median duration of RecA foci is quite short, at 1.5 min. Contrary to studies in bacteria using rare-cutting endonucleases (Lesterlin et al., 2014; Badrinarayanan et al., 2015) or after exposure to DNA-damaging agents (Kidane and Graumann, 2005; Renzette et al., 2005), no larger elongated RecA bundle structure is observed: RecA appears as puncta. The persistence of RecA bundle structures in these latter studies and different conditions are also much, much greater, at 45 min or more. The short lifetime of RecA foci in the study by Amarh et al. (2018) implies that the RecA-dependent homology search, pairing, and strand-exchange reactions between a replication-dependent break and the sister chromosome are extraordinarily fast.

Several factors affect the in vitro stability of the filament formed by RecA binding to single-strand DNA in *E. coli* (Cox, 2007). Destabilizing factors include UvrD and RecX; in the system from Amarh et al. (2018), knockout of these genes does extend the lifetime of the observed RecA foci, although the effect is rather small. Δ*recX* increases the median lifetime to 2.1 min, and Δ*uvrD* affects only a subset of cells, increasing the lifetime to 4–6 min. A mutant in RecA-stabilizing factor DinI shortens the median RecA focus lifetime to 1.3 min, not significantly different from WT. At least in recombination reactions between replicating sister chromosomes, these factors have a minor impact.

This system can be used to examine the effect of DSB repair on the cell replication and division cycle. The duration of YPet-DnaN foci, indicative of ongoing replication, is not affected by SbcCD cleavage of the chromosome (69 vs. 68 min), a finding consistent with SbcCD cleavage behind the replication fork. RecA foci appear 2.5 min after the *lac* locus is replicated, as judged by the colocalization of YPet-DNA with the repressor-marked *lac* locus. *E. coli* replication initiates at a single origin and proceeds bidirectionally; given this duration of replication, we expect duplication of *lac* at 32 min after the initiation of replication, evident from the first appearance of a YPet-DnaN focus. This and measurements of the time after initiation when two *lac* loci first become visible allow us to estimate the time after replication that *lac* remains in cohesion with its sister locus, with and without ongoing DSB repair. Without cleavage, the time of cohesion is 18 min; with cleavage and subsequent repair, the time of cohesion is extended only modestly, to 24 min. Homologous recombination between sister chromosomes therefore has a minimal effect on cell cycle progression.

Confirming what has been seen previously (Mangiameli et al., 2017), Amarh et al. (2018) describe a single YPet-DnaN replisome focus in most cells undergoing replication, suggesting colocalization of the two bidirectional replication forks. This colocalization is not obligate as stable appearance of two replisome foci can be seen in a subset of cells; even colocalized replisomes separate transiently in other cells.

In this study, the researchers noted that repair of the break is localized within the cell. As compared with cells with no break, after SbcCD cleavage, the *lac* locus exhibits more constrained movement and is localized to mid-cell during and after the appearance of RecA foci. This indicates that ongoing DSB repair occurs at mid-cell and that, even after RecA-mediated chromosome synapsis is complete, the DNA remains locally constrained for some time. The replisome, marked with YPet-DnaN, does not always remain colocalized to mid-cell with *lac*

after cleavage, showing that this constraint to mid-cell is likely imposed by the repair reaction itself.

As observed with this elegant system, DSB repair between replicating chromosomes is extraordinarily rapid, barely perturbing the cell cycle. Therefore, bacteria have the capacity to accommodate inevitable breaks without slowing replication.

This study raises some interesting questions to be answered by future studies. What constrains repair reactions to mid-cell? How different are recombinational repair reactions between replicating versus nonreplicating chromosomes? Are RecA bundle structures (as opposed to foci) required for recombination at breaks formed outside the realm of replication? Or are these "off-pathway" structures? How much does sister chromosome cohesion contribute to efficient repair? Is cohesion merely topological intertwining of bacterial circular chromosomes, or is it protein mediated? Is repair this rapid in all bacteria? How different is this process in eukaryote cells?

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