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Speed matters: How subtle changes in DNA end resection rate affect repair

Pablo Huertas[1,2,*] and Andrés Cruz-García[1,2]

[1]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER); Sevilla, Spain; [2]Departamento de Genética; Universidad de Sevilla; Sevilla, Spain

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The contribution of BRCA1 (breast cancer 1) to the repair of broken DNA is well established, but its real role at the molecular level is less well understood. By developing a new high-resolution, single-molecule technique, we have now shown that BRCA1 accelerates the processing of DNA breaks that subsequently engage in homologous recombination.

DNA end resection consists of nucleolytic degradation with 5′–3′ polarity of a single strand of DNA on each side of a DNA break.[1] This step is critical for repairing broken DNA molecules, as the single-stranded DNA generated by DNA end resection is the substrate for the error-free repair pathway known as homologous recombination.[1,2] Proper DNA repair is essential to avoid cancer development, thus it is not surprising that mutations in genes involved in homologous recombination are common in many malignancies,[3] including the genes encoding BRCA1 (breast cancer 1) or RBBP8 (Retinoblastoma Binding Protein 8), best known as CtIP (CtBP-Interacting Protein).[4,5] In cancer development, as in many other natural and pathological biological processes, subtle defects can accumulate to yield relevant differences in the final outcome. However, the techniques currently available for studying these defects have limited the differences that can be measured. This is exemplified by studies to determine the contribution of the tumor suppressor gene *BRCA1* to DNA end resection: traditional techniques have both implicated and ruled out a role for BRCA1 protein (through its interaction with CtIP) in resection.[6-8] We reasoned that these apparent contradictions were mainly caused by technical limitations. Briefly, DNA resection in higher eukaryotes has previously been analyzed by the focal accumulation of the single-stranded DNA-protecting replication protein A (RPA) complex. However, rather than reflecting differences in the length of the resected DNA, this method reveals whether the ssDNA was long enough to accommodate a sufficient number of RPA complexes to form a visible focus (**Fig. 1**). This inability to discriminate between subtle differences in DNA resection has rendered a rather simplistic view within the field, with proteins categorized into 2 discrete groups as either essential or irrelevant for resection (**Fig. 1**).

With the application of a novel high-resolution technique, we can now observe shades of gray within this overly simplified black-and-white picture.[9] By modifying the DNA combing technique used for high-resolution replication analyses, we have created a new assay that can measure progression of resection at the level of individual DNA fibers.[9] We call this new approach Single Molecule Analysis of Resection Tracks, or SMART. By applying this technique, we have now begun to observe subtle effects in DNA end resection that were previously ignored but are relevant to the repair process in the long term. The SMART approach has the potential to drastically change the way we study resection, yet it is important to point out that the two techniques are complementary. Specifically, the SMART technique measures the speed of resection of the breaks that have initiated resection but does not take into consideration whether the number of resected breaks is the same. On the other hand, observing RPA foci formation better reflects the number of breaks that are resected, but not how fast they are processed.

With regard to the role of BRCA1, we could now characterize the function of the protein in DNA end resection in more detail. We clearly observed that resection takes place in the absence of BRCA1 but at a slower pace.[9] Thus, we conclude that the role of BRCA1 in DNA resection is to increase the processivity of this process. In fact, such a role is completely dependent on the interaction of BRCA1 with CtIP.[9] This subtle mechanistic effect, which might seem irrelevant, can in fact explain the long-term consequences of *BRCA1* mutations for cancer development.

The length of resected DNA regulates the mechanism of repair that will act on a DNA break.[1,2,10] Whereas classic non-homologous end joining can only occur on breaks with little or no processing, microhomology-mediated end joining requires resection to expose short tracks of homology.[10] Moreover, resection is essential for all types of recombinational repair,

*Correspondence to: Pablo Huertas; Email: pablo.huertas@cabimer.es
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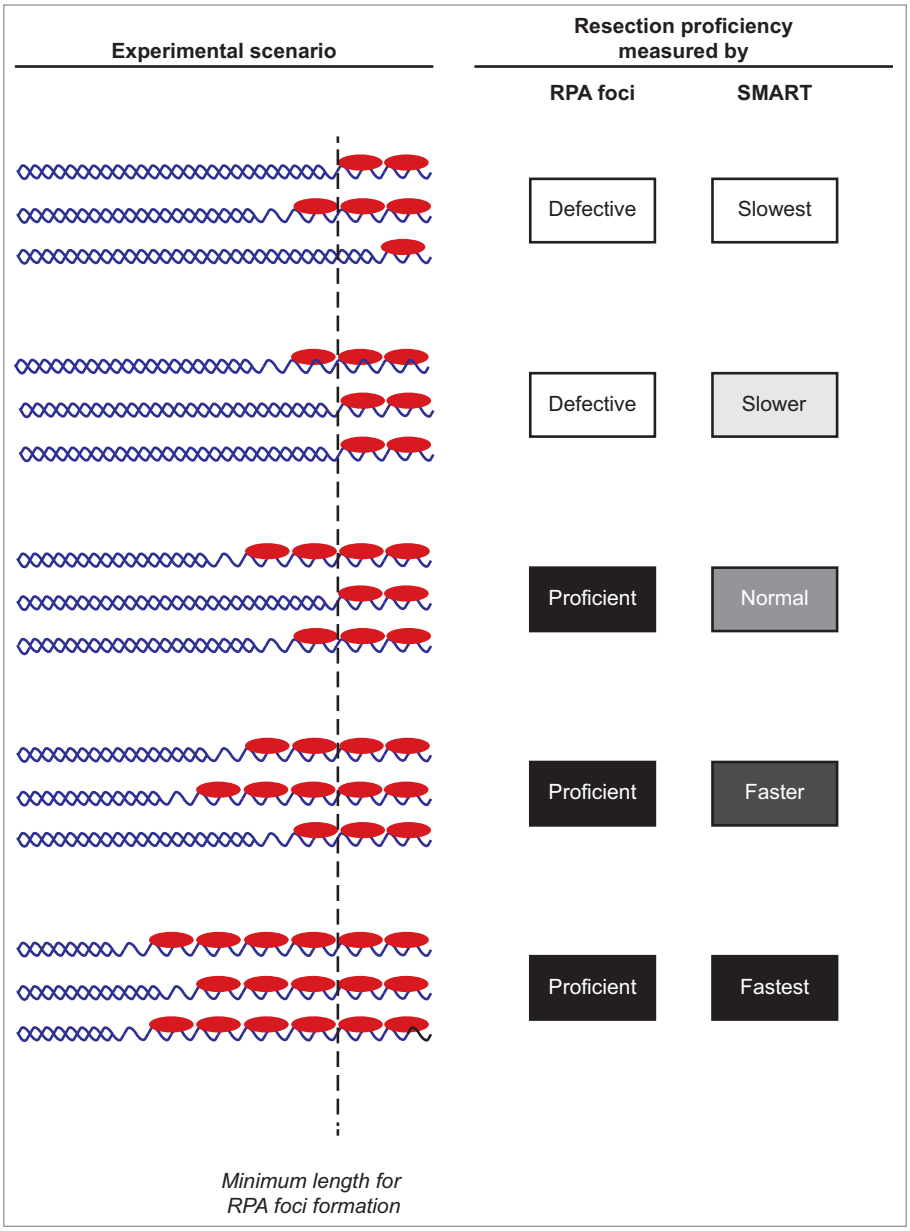

Figure 1. Single molecule analysis of resection tracks (SMART). Comparison of the sensitivity of RPA foci versus SMART as a measurement of DNA end resection. Five different hypothetical experimental scenarios with increasing levels of resection are shown. For RPA (replication protein A) foci, only 2 categories could be established: cases that form RPA foci (proficient) and those in which the length of resected DNA is too short to accumulate enough RPA to form a visible focus (defective). However, using the SMART (Single Molecule Analysis of Resection Tracks) technique, a more accurate measurement of the extent of resection in each case can be established, allowing a greater number of differences to be observed. Note that not all breaks in each population are resected equally, therefore RPA foci formation reflects the most common occurrence in each cell. In contrast, the SMART technique measures each single resection event in a population.

but each subpathway requires different amounts of resected DNA.[1,2,10] For example, limited resection will enable the newly synthesized DNA to catch the resected DNA to form a Holliday Junction, hence favoring recombination in which crossovers might occur.[1,2,10] On the other hand, extensive resection will increase the chances of non-allelic repeats situated in the same chromosome to become engaged in the mutagenic recombination pathway known as single-strand annealing (SSA) that will always cause deletions in the chromosomes.[1,2,10]

Beyond this characterization of the role of BRCA1 in DNA end resection and recombination, our work has yielded other observations with implications in our research field. For example, a main conclusion of our work is that resection in higher eukaryotes resembles the mechanism observed in yeast more closely than previously thought.[9] Thus, CtIP, which was previously described as totally essential for DNA end resection,[1] acts like its yeast counterparts Sae2 (sporulation in the absence of SPO11 2) in *Saccharomyces cerevisiae* and Ctp1 (CtIP-related protein) in *Schizosaccharomyces pombe*: it facilitates resection, but resection might still occur at a slower rate in its absence.[1,9] Moreover, the availability of a new high-resolution technique will expand our simplistic view of the process by allowing us to discover subtle effects in DNA end resection that have previously been ignored. This includes the first opportunity to measure resection rates. Another scenario that was previously impossible to study but can now be addressed is when resection is limited to the same number of cells but progresses either faster or slower. It would be impossible to observe such effects by simply scoring the number of cells that show RPA foci, but these differences will be readily observed using SMART. Thus, this new technique could open an entire new field of possibilities for study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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