Introduction to the Thematic Minireview Series: DNA double-strand break repair and pathway choice

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Environmental agents and reactive metabolites induce myriad chromosomal lesions that challenge the integrity of our genome. In particular, the DNA double-strand break (DSB) has the highest potential to cause the types of chromosome aberrations and rearrangements found in transformed and cancer cells. Several conserved pathways of DSB repair exist in eukaryotes, and these have been the subject of intense studies in recent years. In this Thematic Minireview Series, four leading research groups review recent progress in deciphering DSB repair mechanisms and the intricate regulatory network that helps determine the preferential engagement of one pathway over others.

Four conserved pathways of DNA double-strand break (DSB)[2] repair, namely, nonhomologous DNA end joining (NHEJ), alternate end joining (a-EJ), homologous recombination (HR), and single-strand annealing (SSA), operate in eukaryotic cells. The mechanism by which each of these pathways eliminates DSBs is distinct, although HR, SSA, and a-EJ share a common step in the initial lesion processing that yields long single-stranded (ss) DNA tails as a prerequisite. In WT cells, the majority of DSBs are removed via either NHEJ or HR, with a-EJ and SSA making only a minor contribution in this regard. Importantly, the types of products generated by the four repair mechanisms differ greatly, with HR being particularly adept at accurately restoring the original configuration of the injured DNA molecule. In contrast, the remaining three DSB repair mechanisms are mutagenic in nature, with a-EJ and SSA being the most deleterious in terms of DNA sequence loss during repair.

Advancements in delineating DSB repair mechanisms will have far-reaching implications for human health, as dysfunction in NHEJ and HR is associated with malignancies, and paradoxically, all four DSB pathways are capable of generating the types of chromosome rearrangements encountered in cancer cells. Importantly, HR also helps mediate the removal of interstrand DNA cross-links and is indispensable for meiotic chromosome segregation, and recent studies have implicated a subset of HR proteins in the protection of stressed DNA replication forks against spurious attrition by cellular nucleases (1). Likewise, NHEJ also serves an important function in the joining of DSBs that arise during V(D)J recombination and IgH class switch recombination (2).

In the first article, Her and Bunting (3) review how cells go about making decisions to engage any of the four DSB repair pathways. These decisions are in large part tied to the cell cycle, as cells in the G_1 stage are much less adept at resecting DSB ends, thereby rendering NHEJ the default repair mechanism therein. Besides direct exclusion of the resection machinery from DNA ends by the abundant NHEJ factor Ku, DNA resection is additionally restricted by the chromatin-binding factor 53BP1 (Rad9 in budding yeast) in G_1 cells. Recent studies have shown that 53BP1 nucleates the formation of a higher-order complex, termed Shieldin (Ref. 4 and references therein), that serves to prevent recruitment of end resection factors. Thus, Shieldin effectively "channels" the DNA break ends into NHEJ for rejoining (3, 4). With the onset of S phase, a series of post-translational modifications, including a cascade of protein phosphorylation and ubiquitin conjugation to key targets, occurs. These events culminate in the recruitment of the tumor suppressor complex BRCA1–BARD1, which, acting in an undefined manner, helps overcome the suppressive effects of Ku and Shieldin on resection so as to enable HR, a-EJ, and SSA.

In the second article, by Pannunzio, Watanabe, and Lieber (5), we are treated to an expert analysis of the biochemical mechanism and regulation of NHEJ. There has been a persistent misconception that NHEJ does not entail resection of the DNA ends to expose ssDNA. The authors go to considerable length to dispel this by emphasizing how NHEJ is often accompanied by a modest amount of DNA strand resection, the purpose of which is to generate ssDNA regions for the formation of a DNA hybrid of a few base pairs to facilitate the end-joining reaction. Such processing of DNA ends results in nucleotide loss or addition, which explains why broken DNA repaired by NHEJ is rarely restored to the original DNA sequence. Recent work has unveiled surprising mechanistic complexity of NHEJ. Starting with Ku and the recruitment of the nuclease Artemis in complex with the kinase DNA-dependent protein kinase, catalytic subunit (DNAPKcs), NHEJ can follow one of several alter-

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[2] The abbreviations used are: DSB, double-strand break; NHEJ, nonhomologous DNA end joining; a-EJ, alternate end joining; HR, homologous recombination; SSA, single-strand annealing; ssDNA, single-stranded DNA.

nate reaction paths depending on the nature of the DNA ends. As such, the NHEJ machinery possesses considerable plasticity, to allow it to effectively engage different DNA end configurations, resulting in multiple junctional outcomes during break repair.

The biochemical mechanism of HR and its DSB repair role are the subject of the third article, by Wright, Shah, and Heyer (6). Although HR is considered a high-fidelity repair tool, it can also generate chromosome rearrangements, *e.g.* chromosome arm translocations that might lead to undesirable phenotypic changes and cell transformation (7). Accordingly, HR is subject to multiple layers of regulation so as to avoid the formation of potentially pathological end products (3, 7). In metazoans, HR depends on several well-known tumor suppressors, including BRCA1 and BRCA2, that act to facilitate different stages of the repair reaction. The authors skillfully lead us through key yeast studies and parallel endeavors in higher eukaryotes that have yielded major insights into the execution and regulation of HR. Specifically, they discuss how different HR factors and their regulators function in concert to execute the DNA end resection, DNA homology search, DNA strand invasion, and repair DNA synthesis steps of HR. Moreover, we learn how, in mitotic cells, late HR intermediates are resolved by conserved mechanisms to prevent the formation of potentially deleterious crossover recombinants.

The fourth article, by Sallmyr and Tomkinson (8), focuses on the biochemical mechanism of a-EJ and SSA. As mentioned earlier, although these two repair pathways act infrequently in DSB elimination, both have the potential of generating DNA products that harbor a large deletion of sequences flanking the DNA break. Because a-EJ and SSA require the presence of long 3′-tailed ssDNA regions, they are dependent on DNA end resection that produces the ssDNA template needed for the assembly of the RAD51-containing HR machinery (3, 6). The notion that a-EJ and SSA are "pathological" oddities arising in cells deficient in HR or NHEJ has been dispelled by the finding that they operate under normal physiological conditions. As such, a-EJ and SSA should be viewed as "backup" pathways to NHEJ and HR. Understanding the mechanism of a-EJ has become a hot topic and will likely have therapeutic implications, because inhibition of this DSB repair system should, in theory, provide an effective means to kill tumor cells deficient in HR or NHEJ without affecting normal tissues.

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