Characterization of the interplay between DNA repair and CRISPR/Cas9-induced DNA lesions

Anne Bothmer, Tanushree Phadke, Luis Barrera, Carrie Margulies, Hari Jayaram, Vic Myer, Cecilia Cotta-Ramusino

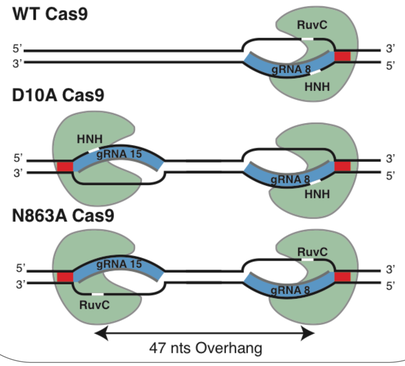
Editas Medicine • 11 Hurley Street • Cambridge, MA 02141

Background

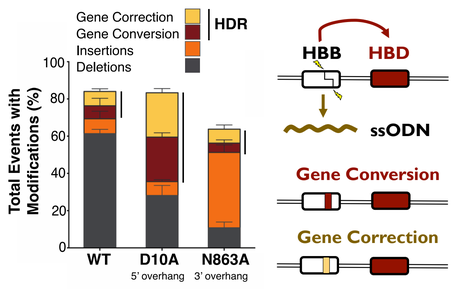
The CRISPR/Cas9 system provides a versatile toolkit for genome engineering that can introduce a variety of DNA lesions at specific genomic locations. However, a better understanding of the exact nature of these lesions and the repair pathways engaged as a consequence thereof is critical to realizing the basic research and therapeutic potential of this technology..

Here we characterize the DNA structures arising from the use of Cas9 variants directed to the endogenous human beta-globin locus. The different lesions arising from each Cas9 variant resulted in the engagement of different endogenous repair pathways.

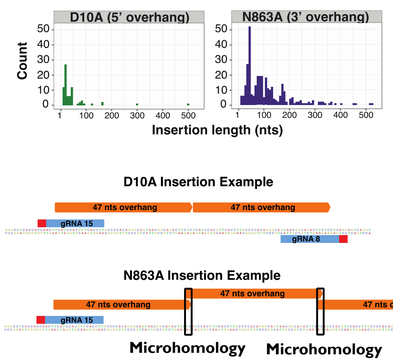
Schematic of Cas9 variants

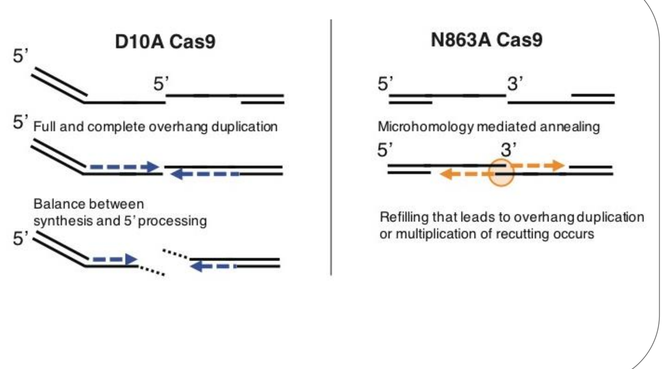


Overall modification frequency

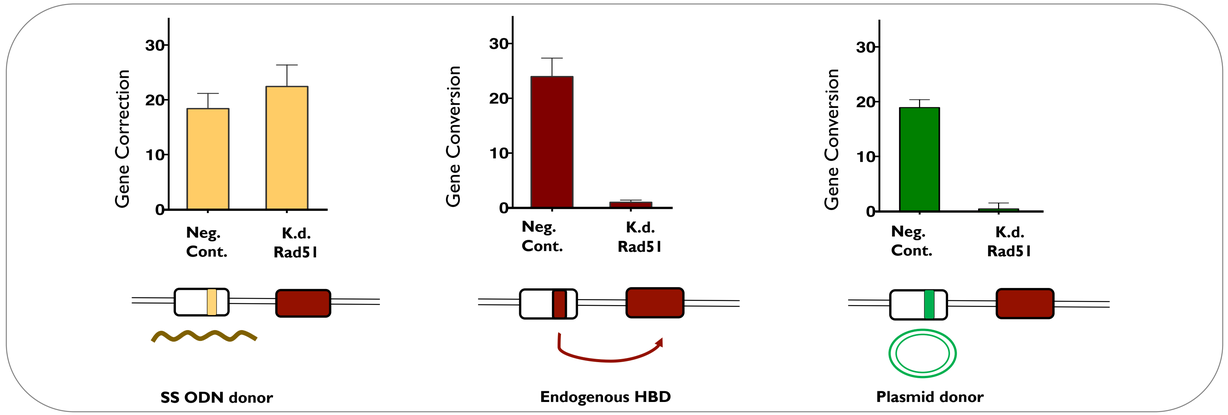


Mechanism of generation of insertions differs between D10A and N863A

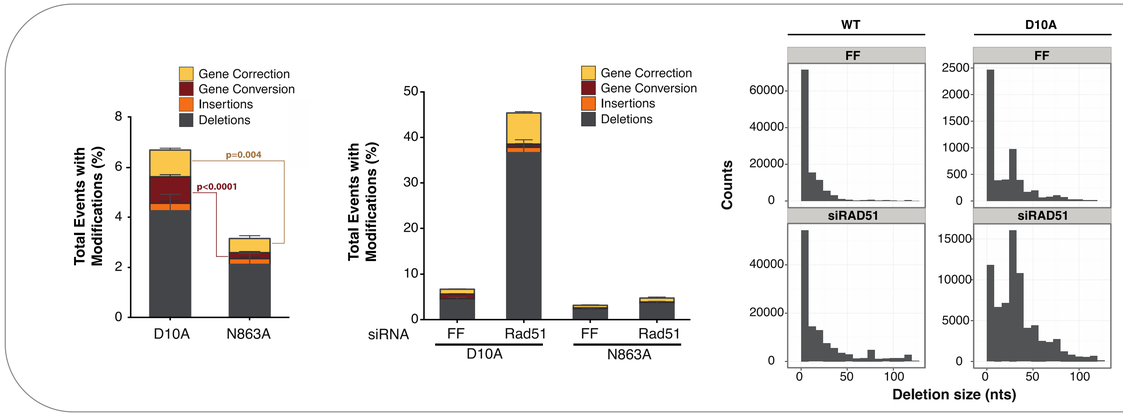




Different donors engage different repair pathways

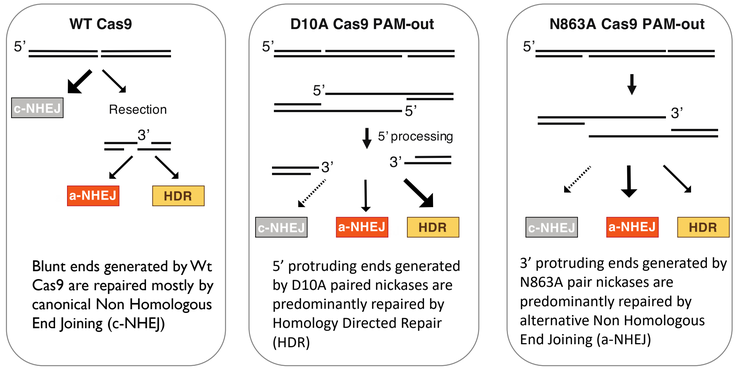


Single nicks generated by D10A or by N863A activate different repair pathways

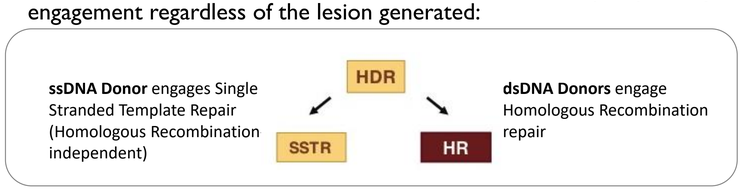


Conclusions

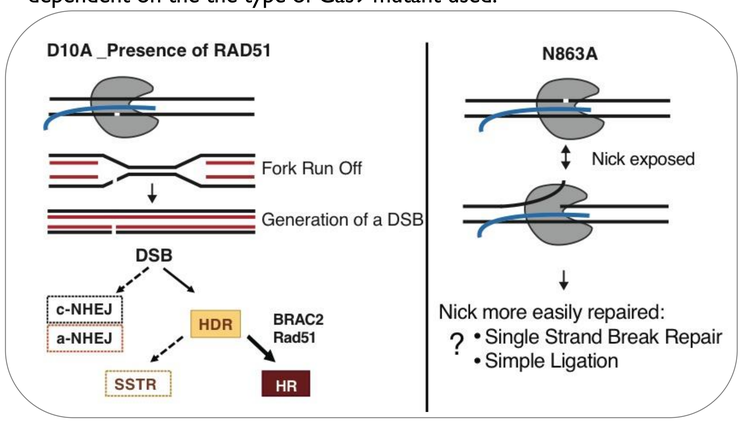
1. WT-Cas9 and Cas9 paired nickases led to the activation of double-strand break (DSB) response pathways at similar rates and the presence and polarity of the overhang structure is a determinant of DSB-repair pathway choice.



2. The nature of the donor is an important determinant in repair pathway engagement regardless of the lesion generated:



3. Similarly, individual Cas9-induced nicks activate different repair pathways dependent on the the type of Cas9 mutant used:



This detailed characterization of repair pathway choice in response to CRISPR/Cas9-induced lesions enables a more deterministic approach to the design of genome engineering strategies for the creation of model systems and, ultimately, novel human therapeutics.