Does the telomere and telomerase influence repair pathway choice during antigenic variation in African trypanosomes?

Trypanosoma (T) brucei evades the host's immune system and persists in the body through a process known as antigenic variation, which is the stochastic change of *T. brucei*'s variant surface glycoprotein (VSG), and relies on homologous recombination. One of >2000 *VSG* genes is expressed from a bloodstream expression site located in an inherently fragile subtelomeric region, with the *VSG* adjacent to a telomere. The key enzyme responsible for telomeric maintenance is telomerase reverse transcriptase (TbTERT), which extends the telomere by 6-12 bp per division (1,2). Without telomerase activity (in *tert*$^{-/-}$ mutants), the telomere loses 3-6 bp per generation (3), leading to short telomeres that have been linked to increased VSG switching (4). However, whether TbTERT influences repair pathway choice or recombination events driving VSG switching remains unexplored.

Mutants with disrupted *TERT* were generated and a controlled double-strand break (DSB) was induced by the I-SceI endonuclease in the active expression site to examine the DNA damage response. We measured the length of the telomere using Southern blot and compared the parental and *tert*$^{-/-}$ mutants before induction of a break, to ensure that any differences in response were not due to initial telomere length variation. We saw that the initial telomere length was similar between the cell lines. Using VSGseq, we analysed *VSG*s used for switching 7 days post-break in *tert*$^{-/-}$ mutants. We observed a noticeable, 3-fold increase in number of *VSG*s used compared to parental cells. These results suggest that TERT may suppress access to the VSG archive during switching, while its deletion leads to hyper-recombinogenic state. Notably, this occurs independently of the early DNA damage response, as DNA resection and γH2A accumulation remained intact in *tert*$^{-/-}$ cells. We are now tagging known factors such as Rad51-3 and PPL2 using TurboID, which will be followed by quantitative mass spectrometry analysis to identify proteins that accumulate at the site of the DSB. It will allow us to determine whether DNA repair chromatin composition differs between *tert*$^{-/-}$ and parental cells, particularly when comparing subtelomeric and chromosome internal DSBs.

References

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