Different *locus* – different repair bias

Laboratory of gene conversion

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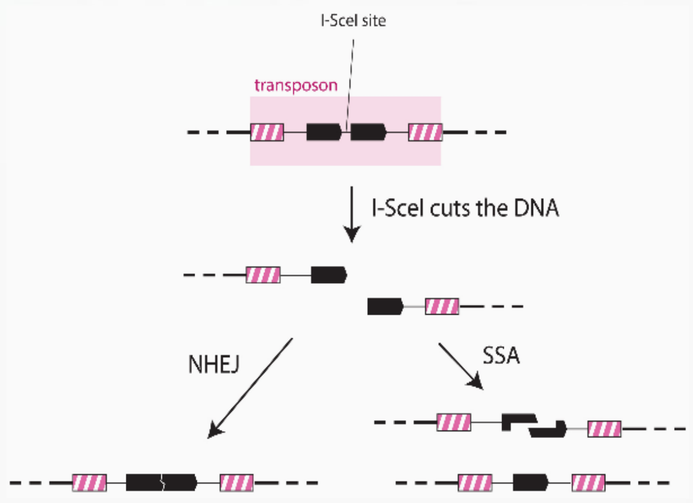
In the nucleus, chromosome breaks can be repaired in two major ways. In the first, called Non Homologous End Joining, the DNA ends are simply put back together. In the second, called Homologous Recombination, the repair process is more complex and involves exchange of DNA between chromosomes.

In our project we have made different experiments to understand how the cell chooses which repair pathway to use and what influences this choice.

Methods

- Restriction of DNA
- PCR
- Electrophoresis
- Transfection
- Microscopy
- Cell culture



Experiment 1: DNA repair pathway in mouse ES cells.

1.Integration of a reporter for DNA repair in mouse embryonic stem cells using a transposon. The DNA is cut by the I-SceI enzyme and repaired by SSA or NHEJ pathway *in vivo*.
2.Second restriction of DNA with I-SceI *in vitro* to get rid of transposons, which were not cut *in vivo*. Repaired DNA strands cannot be cut again because the I-SceI site is mutated in the process.
3.PCR to amplify the cut and repaired DNA fragments. PCR does not amplify the DNA molecules which were cut in the previous step.
4.Electrophoresis to determine the ratio of DNA strands to repair by NHEJ and SSA pathways.

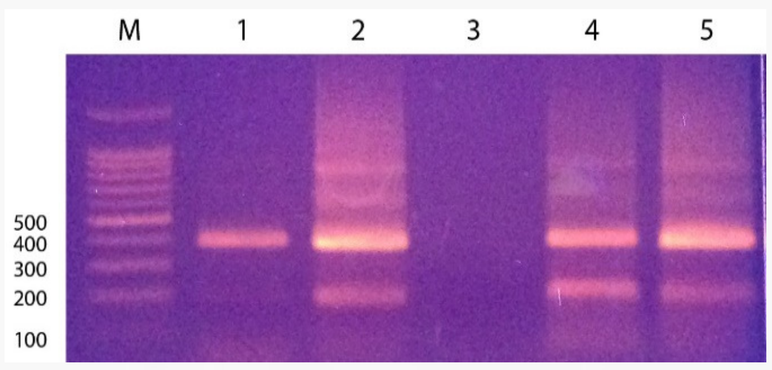


Figure 1. *PCR amplification and electrophoresis of the reporter after repair. M: marker of molecular weight. 1. No DNA control. 2. PCR on DNA not cut by I-SceI in vivo (control). 3-5: PCR on DNA cut by I-SceI in vivo from different cells.*

Transposons containing the restriction site for I-SceI are integrated randomly in the genome. The outcome of DNA repair are different ratios of NHEJ (400 bp) and SSA (200 bp) in different cell clones. It means that the location of the chromosome gap affects the repair pathway (figure 1).

Experiment 2: Visualizing SSA in HEK293 cells

1.Plasmid with the GFP sequence
2.Plasmid with the GFFP sequence
3.I-SceI expression plasmid
4.Plasmid with the GFFP sequence and I-SceI expression plasmid
The fourth sample of DNA is cut and repaired.

The GFFP gene contains an internal repeat flanking an I-SceI restriction site, which disrupts the coding sequence. Co-transfecting GFFP and I-SceI plasmids in HEK293 cells (top) restores the native structure of the GFP gene after repair through SSA. The restored GFP gene renders the cells fluorescent. This does not happen when only the GFFP plasmid is transfected (bottom right). Transfecting a native GFP plasmid (bottom left) yields maximum fluorescence.

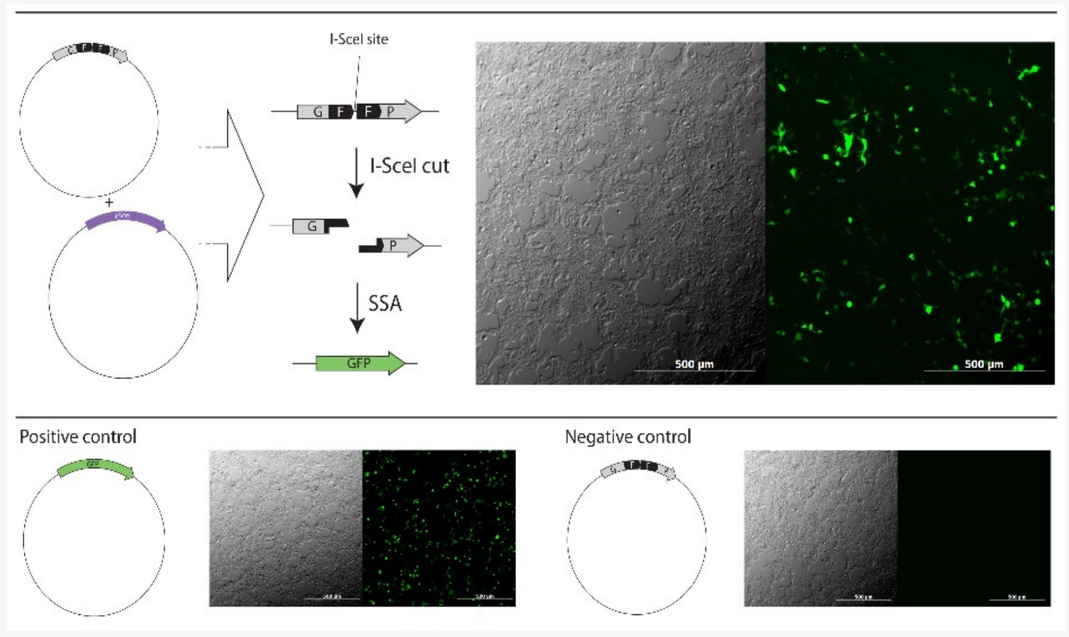


Figure 2. *Transfected HEK293 cells observed under the microscope.*

Conclusion: **our results raise the question how the cell chooses the DNA repair pathway.**

We would like to thank the foundation Dynasty and the Institute of Cell Biophysics.