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Summary
Double-strand breaks (DSBs) are one of the most lethal forms of DNA damage. DSBs can occur during normal cellular metabolism or can be induced by external factors, and highly threaten genomic integrity and cell survival (Deriano and Roth 2013). To prevent this, cells have evolved complex and highly conserved systems to detect these lesions, signal their presence, trigger various downstream events and finally bring about repair. Two main pathways are used for DNA DSB repair: Homologous Recombination (HR) and Non-Homologous End-Joining (NHEJ). Both of them function together to maintain genome integrity. The HR pathway precisely restores the genomic sequence of the broken DNA ends. This requires the sister chromatid as a template for accurate repair. In contrast, NHEJ promotes direct ligation of the DSB ends without the requirement for sequence homology and may result in small insertions and deletions at the break site. Both pathways are highly conserved throughout eukaryotic evolution but their relative importance may be different depending on the stage of the cell cycle or the cell type. Unicellular eukaryotes with small genomes such as yeast (Saccharomyces cerevisiae) mostly rely on HR to repair DSBs, whereas in higher eukaryotes, like mammals and plants with large genomes containing many repeat sequences, the NHEJ pathway is the predominant repair pathway. At least two NHEJ pathways have been identified: the classic NHEJ pathway (c-NHEJ) and the backup-NHEJ pathway (b-NHEJ) also called alternative-NHEJ (a-NHEJ) or microhomology-mediated end-joining (MMEJ).

Agrobacterium tumefaciens is widely used as a vector to produce genetically modified plants. Agrobacterium-mediated genetic transformation involves the transfer of T-DNA from its tumor-inducing plasmid to the host cell nucleus, where it integrates into the plant genome. However, the molecular mechanism of T-DNA integration is still unclear. T-DNAs can integrate at artificially induced DSBs, which suggests that DSB repair mechanisms are probably involved in T-DNA integration in plants (Salomon and Puchta 1998). Moreover, it was shown in our lab that Agrobacterium T-DNA integration in yeast (S.cerevisiae) depends on NHEJ proteins (van Attikum et al. 2001; van Attikum and Hooykaas 2003). Arabidopsis NHEJ mutants have subsequently been studied for T-DNA integration. However, the results obtained by different research groups were variable and revealed either no or limited negative effects (Friesner and Britt 2003; van Attikum et al. 2003; Gallego et al. 2003; Li et al. 2005). Disruption of multiple DNA repair pathways at the same time did not eliminate transformation (Jia et al. 2012; Mestiri et al. 2014; Park et al. 2015), suggesting that there must be other unknown proteins and pathways that mediate T-DNA integration in plants. Furthermore, a recent study showed that the Arabidopsis Polymerase θ (Pol θ ) ortholog Tebichi (Teb) is essential for T-DNA integration (Kregten et al. 2016).

In Chapter 1, I review the current knowledge of the DNA damage response, the DSB repair pathways and their regulation, how these repair pathways affect DNA repair and Agrobacterium-mediated T-DNA integration and the artificial nuclease techniques for inducing DSBs. Compared to mammals, in which the NHEJ pathways have been well defined, there is still much to learn about NHEJ repair pathways in plants.

Chapter 2 describes the involvement of Parp3 and Xrcc1 in DNA repair and the effect of a combination of deficiencies of different NHEJ factors on T-DNA integration in Arabidopsis. Homozygous parp3 and xrcc1 mutants were isolated and characterized, and
parp1parp3, ku80xrcc1 double mutants and the parp1parp2parp3 triple mutant were obtained by crossing. The results from DNA damaging treatments showed that Parp3 and Xrcc1 are involved in DNA repair. We further examined transient and stable root transformation frequencies of these mutants together with the ku70, ku80, parp1, parp2, parp1parp2, lig4, lig6 and lig4lig6 mutants, which were characterized by our lab previously, after co-cultivation with Agrobacterium. The aim of this study was to investigate NHEJ pathways and analyze whether Agrobacterium-mediated transformation efficiency was affected by the absence of NHEJ factors in Arabidopsis. Deficiency in either the c-NHEJ or b-NHEJ pathway, did not lead to a significant decrease in root transformation. However, the ku80xrcc1 and ku80parp2 mutants showed a significant decrease in stable root transformation efficiency. However, no significant differences were observed in transient transformation. These results indicate that the known NHEJ repair pathways are required for optimal T-DNA integration, but that there must still be other important factors and/or pathways involved in T-DNA integration in Arabidopsis.

Chapter 3 describes experiments done to reveal a putative role of Lig1a in DNA repair. The lig1a mutant was isolated and characterized, and the lig4lig1a double mutant and the lig4lig6lig1a triple mutant were obtained by crossing. Genotoxic tests showed that lig1a must have a role in repair as lig4lig6lig1a was more sensitive to DNA damage than lig4lig6. Also the comet assay revealed more DNA damage in the triple mutant. These results suggested that Lig1a probably plays a role in DNA repair when Lig4 and Lig6 are both mutated.

Chapter 4 describes an important function of Mre11 for b-NHEJ pathways involved in T-DNA integration. Our results from yeast-two-hybrid analysis showed that the interaction domain necessary for the formation of a Mre11-Rad50 complex is still present in the Mre11-2 protein, but absent in Mre11-4. This probably explains the phenotypic differences between the two mutants. In order to study whether Mre11 also functions in b-NHEJ in plants, the ku80mre11-2 double mutant was obtained by crossing. We found that the ku80mre11-2 double mutant was more sensitive to DNA damage and exhibited more cell death in the roots than each of the single mutants. Furthermore, the root transformation assays showed that the transformation frequencies of the single mutants were not significantly different from the wild type. However, the ku80mre11-2 double mutant is fully resistant to Agrobacterium mediated T-DNA integration. These results suggested that Ku80 and Mre11 are involved in two different pathways of T-DNA integration, each of which is not essential, but together are responsible for all T-DNA integrations in plants.

Chapter 5 focuses on how exactly the DNA ends join in the Arabidopsis NHEJ mutants in vivo, which were deficient in c-NHEJ, b-NHEJ or both. TALENs and CRISPR/Cas9 were used for DSB-mediated targeted mutagenesis in the cruciferin 3 (CRU3) and protoporphyrinogen oxidase (PPO) genes. Both nucleases were expressed and successfully induced DSBs in ku80, parp1parp2 and ku80parp1parp2 mutants. The results from footprint analyses showed that larger deletions predominated after DSB repair in ku80 and ku80parp1parp2 mutants. Furthermore, templated insertions were observed at the repair junctions more frequently in ku80 and ku80parp2 mutants than in wild type and parp1parp2 mutants, although such insertions were found in all four genotypes. These results indicate a shift to a more error-prone back up repair mechanism of DSB repair in the absence of Ku80
and that other Parp-independent back up pathways exist probably involving Pol θ mediated end-joining (TMEJ) responsible for the template insertions.

In short, the studies described in this thesis showed that back-up error-prone NHEJ repair pathways, together with classical NHEJ, are involved in Agrobacterium-mediated T-DNA integration. Mre11 could be a key player in this process, and together with Ku80 they are responsible for all T-DNA integration in plants. A model summarizing the main results described in this thesis is shown in Figure 1.

**Figure 1.** Model for Agrobacterium-mediated T-DNA integration in Arabidopsis. DSBs are recognized by Ku heterodimers or the MRN complex. Once Ku or MRN binds to the break ends, other factors including Polymerase θ (Pol θ), ligases, resection enzymes and unknown proteins are recruited to the break sites. One of these essential factors, Pol θ is responsible for the attachment of the single-stranded T-DNA left border (LB) to the plant genome by using a few bases of homology to prime DNA synthesis from the 3’ end. Similar to the LB, the activities of Ku, MRN and unknown proteins may be involved in the ligation of the T-DNA right border (RB) to the other end of the break.
References


