DNA repair and gene targeting in plant end-joining mutants

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Chapter 1

General introduction: DNA repair and gene targeting

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1. Introduction

The genome is subject to various assaults by both exogenous environmental factors (e.g. ionizing radiation, chemicals and reactive oxygen species) and endogenous cellular events (e.g. transposition, meiotic double strand break formation). These assaults cause a wide range of genetic damage, such as base lesions, DNA single-strand breaks (SSBs) and DNA double-strand breaks (DSBs). Among these DNA lesions, DSBs are particularly detrimental, because both strands are damaged and it is, therefore, impossible to reconstitute the missing information from a complementary strand. Defects in the repair of DSBs may cause chromosomal aberrations and genomic instability, which can promote mutation, accelerate aging and induce cell death. Even a single unrepaired DSB may induce cell death (1).

In order to maintain genomic integrity and stability, organisms have evolved multiple DNA repair mechanisms. Cellular responses to DNA damage activate cell-cycle checkpoints, which can stop the cell cycle and provide time for the cell to repair the damage before division (2). Base lesions and SSBs can be detected and removed by nucleotide excision repair (NER), mismatch repair or base excision repair (BER) (3). The most harmful damage, DSBs, can be repaired by two types of pathways: homologous recombination (HR) and non-homologous end joining (NHEJ).

HR utilizes sequence homology to align and join the DNA ends of the break. It employs a homologous stretch of DNA on a sister chromatid as a template. The HR pathway mediates an accurate form of repair. On the other hand, NHEJ is a straightforward pathway that can rejoin the two ends independently of significant homology. It is an error-prone process with insertion or deletion of nucleotides as a result. HR is only operative during the S/G2 phases of the cell cycle when sister chromatids are present. By contrast NHEJ can function

in all phases of the cell cycle, but is mainly used in the G1 phase when HR is suppressed. HR and NHEJ operate in both competitive and collaborative manners, depending on the repair context and specific attributes of the broken DNA. HR is the predominant DSB repair mechanism in prokaryotes and lower eukaryotes. NHEJ seems the main DSB repair pathway in multicellular eukaryotes (e.g. mammals and plants). Most of the major factors involved in NHEJ were initially identified in mammals. Genome sequencing has led to the discovery of homologous NHEJ factors in other eukaryotes and prokaryotes. It indicates that NHEJ has been conserved during evolution (4).

In this chapter, I shall review first how the cell responds to DNA DSBs and repairs those breaks. Then the main regulatory mechanisms that affect the choice of DNA repair pathway throughout the cell cycle are discussed, and how they affect gene targeting is addressed. Finally I will give an outline of the thesis.

2. DNA Damage Checkpoints

DNA damage checkpoints are the cellular surveillance systems, which prevent damaged DNA from being converted into heritable mutations in order to maintain the genomic stability. The presence of DNA damage leads to the initiation of signal transduction cascades (Figure 1), which leads to chromatin remodeling, transcriptional responses, cell cycle arrest, DNA repair or in some cases apoptosis (5). Cell cycle arrest is necessary to provide the cell with enough time to repair the DNA lesions. Chromatin remodeling and transcriptional responses facilitate DNA repair, and increase the resistance to further damage.

The checkpoints are initiated by the transient recruitment of the MRE11/RAD50/NBS1 (MRN) complex in mammals and plants (6) or the equivalent MRE11/RAD50/Xrs2 (MRX) complex in yeast (7;8), to DSB sites, followed by the activation of phosphoinositide-3-kinase-related kinases (PIKKs). This group of proteins comprises the ataxia-telangiectasia mutated (ATM in mammals and plants; Tel1 in yeast), the ATM and Rad3 related (ATR in mammals and plants; Mec1 in yeast) and the catalytic subunit of DNA protein kinase (DNA-PKcs, which is lacking in yeast). In general, ATM and DNA-PKcs respond mainly to DSBs, whereas ATR is activated by single-stranded DNA (ssDNA) and stalled replication forks (9). These kinases are activated and recruited to DNA lesions by direct interactions with specific factors: NBS1/Xrs2 (for ATM/Tel1), ATR-interacting protein (ATRIP)/Ddc2 in yeast (for ATR/Mec1) and Ku80 (for DNA-PK) (10). Then these checkpoint kinases transmit and amplify the checkpoint signal by different phosphorylation events to different downstream effectors that are essential for the DNA-damage response and DNA repair, including phosphorylation of H2AX (γH2AX), mediator of DNA damage checkpoint 1 (MDC1), tumor protein 53 binding protein 1 (TP53BP1) and breast cancer 1 (BRCA1).

In general, there are two possibilities for the following steps (Figure 1). In the first possibility, ATM phosphorylates the histone H2AX (γH2AX) which forms foci covering many megabases of chromatin surrounding the DSBs within seconds of DNA damage (11).

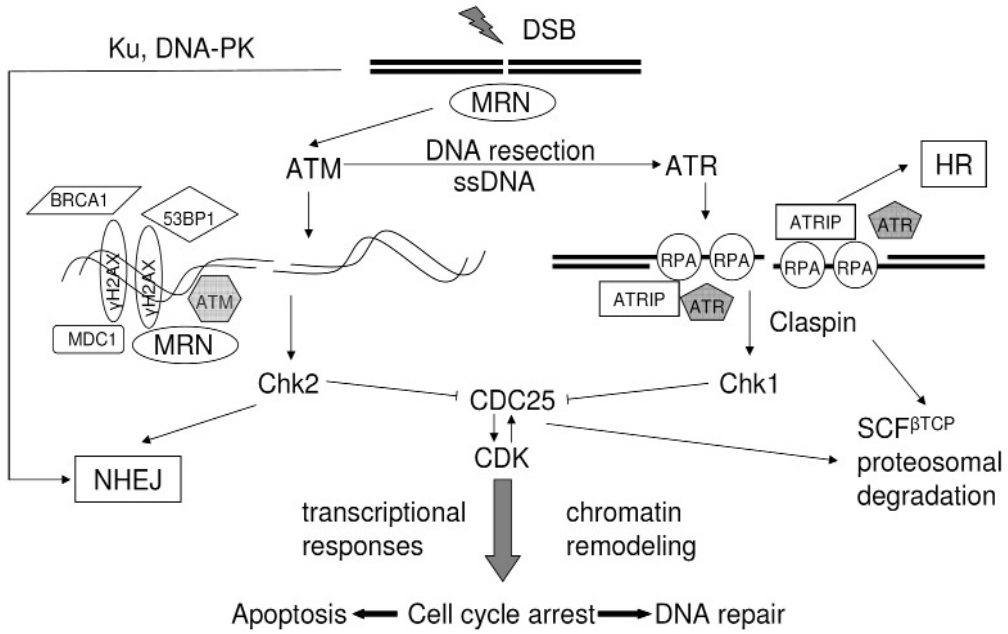

Figure 1. Responses to DNA Double Strand Breaks and checkpoint activation.
Ku and MRN complexes bind to DSBs and activate the PIKKs: DNA-PKcs and ATM respectively. The DNA-PK complex promotes NHEJ repair of DSBs which take place throughout the cell cycle. The MRN complex allows the activation of the checkpoint, mediated by ATM. ATM phosphorylates H2AX, which serves as a docking platform for MDC1, 53BP1, BRCA1, MRN and ATM itself. Active, phosphorylated ATM monomers are increased at the site of damage and activate the downstream signaling kinase Chk2. During S and G2 phases, DSBs are resected to ssDNA which is coated by RPA. The RPA coated ssDNA recruits the ATR-ATRIP complex to DNA lesions. With the help of Claspin mediator protein, ATR phosphorylates Chk1. Claspin maintains stable with the present of Chk1 and can be degradated via $SCF^{\beta TCP}$ proteosome. Both activated Chk2 and Chk1 phosphorylate CDC25, marking it for proteosomal degradation by $SCF^{\beta TCP}$ ubiquitin ligase. This regulates the activity of CDK, which controls cell cycle arrest and facilitates one of the following steps: apoptosis or DNA repair. Chk2 and Chk1 can also facilitate other processes such as damage induced transcription and chromatin remodeling. In general, the ATM pathway promotes NHEJ repair, while the ATR pathway facilitates HR repair.

Alternatively, γH2AX associates with the DSB-flanking chromatin regions, serving as a docking platform for MDC1, 53BP1, BRCA1, the MRN complex, and indeed ATM itself (12). The increased ATM phosphorylates and activates checkpoint kinase 2 (Chk2), or RAD53 in *Saccharomyces cerevisiae*, which induces the phosphorylation of CDC25A, marking it for proteosomal degradation by $SCF^{\beta TCP}$ ubiquitin ligase. This blocks the activation of the cyclin-dependent kinases (CDKs) and leads to cell cycle arrest. The G1-S cell phase is thus arrested to avoid the replication of damaged DNA. Recent research showed that Chk2 also appears to have a conserved function in the control of mitotic progression following DNA damage after the G2/M transition (13). CDKs orchestrate control of the cell cycle. The activation of CDKs is also regulated by the mitosis-inhibiting kinase Wee1. In the G1 phase, this ATM signaling pathway facilitates NHEJ. In the G2 phase, initial

activation of ATM is followed by activation by the ATR signaling pathway and repair by HR (14). In this case, the DSB is resected, leading to formation of ssDNA which is coated and stabilized by the replication protein A (RPA), and which recruits the ATR-ATRIP complex. ATR phosphorylates the Chk1 with the help of the Claspin mediator protein (12). Activated Chk1 phosphorylates CDC25A. This will cause S and G2 arrest. p53, a key player in DNA-damage checkpoints is also activated by Chk1 and Chk2.

To sum up, the checkpoint generates a broad spectrum of responses to DNA damage, leading to cell cycle arrest and DNA repair in animals. Although checkpoint activation is not essential for DSB repair, it modulates how the damage is repaired and whether HR or NHEJ is used for repair (15).

In plants, there is no functional CDC25 homolog identified yet. Mitosis-inhibiting kinase Wee1 activates DNA damage checkpoints in an ATM/ATR-dependent manner (16). Also many other genes, needed for checkpoints in mammals, have not been identified in plants yet (such as Chk1, Chk2, PLK, p53, Claspin, 53BP1, ATRIP and MDC1). Two kinds of CDKs have been identified in plants: CDKA and CDKB. They can control the cell cycle directly. These checkpoint genes are not essential for normal growth in plants, whereas they are essential in mammals (17).

3. Homologous Recombination (HR)

HR is a mechanism which uses DNA homology to direct DNA repair. It occurs in all life forms. In eukaryotes, HR is carried out by the Radiation sensitive 52 (Rad52) epistasis group of proteins, which were initially identified in *Saccharomyces cerevisae* from the genetic analysis of ionizing radiation (IR) hypersensitive mutants (18). The Rad52 epistasis group is composed of Rad50, Rad51, Rad52, Rad54, Rad55, Rad57, Rad59 and the MRX complex. Most of those proteins are well preserved among eukaryotes. Orthologs have been identified in mammals and plants (Table1), except for Rad55/57 and Rad59, which are functionally replaced by five Rad51 paralogs (19). All HR events are initiated by 5'-3' resection at the DSB end, which is facilitated by the MRX/MRN complex. The MRX/MRN complex plays a critical role in the early DSB response. It has 3'-5' exonuclease, single-strand endonuclease, and DNA unwinding activities and is involved in the 5'-3' resection of DSB ends to produce 3' single-strand overhangs. Sae2 in *S. cerevisiae* and CtIP in mammals are involved together with the MRX/MRN complex in the processing of DSB ends. Sae2 exhibits endonuclease activity on ssDNA and DNA strand transition and cooperates with MRX to cleave DNA hairpin structures. After this initial processing of the DSB resection, it is taken over by the exonuclease (Exo1) and the Sgs1 helicase (20). This may lead to a several-kbp-long region of single stranded DNA (ssDNA). Mammals and plants have orthologues of those proteins, suggesting a general mechanism for DSB end processing in eukaryotes.

Once 3'-ssDNA overhangs are generated, they are coated by RPA. RPA directly interacts with Rad52 in yeast, which recruits Rad51 to load on single-strand DNA by displacing

Table 1. HR factors in yeast, mammals and plants.

Saccharomyces cerevisiae	*Homo sapiens*	*Arabidopsis thaliana*	Locus in Arabidopsis and ref.	Function
Rad50	Rad50	AtRad50	At2G31970 (169-171)	DNA binding, DNA-dependent ATPase, complex with Mre11 and Xrs2/Nbs1, DSB ends processing, DNA-damage checkpoints
Mre11	Mre11	AtMre11	At5G54260 (169;172;173)	3'-5' exonuclease, complex with Rad50 and Xrs2/Nbs1, DSB end processing, DNA-damage checkpoints
Xrs2	Nbs1	AtNbs1	At3G02680 (6)	DNA binding, complex with Rad50 and Xrs2/ Nbs1, DSB end processing, DNA-damage checkpoints
Sae2	CtIP	AtCom1/ AtGr1	At3G52115 (174)	Endonuclease, DNA strand transition
Rad51	Rad51	AtRad51	At5G20850 (175-177)	RecA homologue, strand invasion
Dmc1	Dmc1	AtDmc1	At3G22880 (178;179)	Rad51 homologue
Rad52	Rad52	AtRad52 (22)		ssDNA binding and annealing, recombination mediator, interacts with Rad51and RPA
Rad54	Rad54	AtRad54	At3G19210 (180)	ATP binding, DNA binding, helicase activity, recombination mediator
Rad55-Rad57	-	-		ssDNA binding, recombination mediator
Rad59	-	-		single-strand annealing

Saccharomyces cerevisiae	*Homo sapiens*	*Arabidopsis thaliana*	Locus in *Arabidopsis* and ref.	Function
-	Rad51B	AtRad51B	At2G28560 (181;182)	ssDNA binding, recombination mediator
-	Rad51C	AtRad51C	At2G45280 (181;183;184)	
-	Rad51D	AtRad51D	At1G07745 (181;185)	
-	Xrcc2	AtXrcc2	At5G64520 (181)	
-	Xrcc3	AtXrcc3	At5G57450 (186)	
	Brca1	AtBrca1	At4G21070 (187;188)	checkpoint mediator, recombination mediator
-	Brca2	AtBrca2-1, AtBrca2-2	At4G00020 (178;179)	recombination mediator
Exo1	Exo1	At1G18090?	At1G18090?	exonuclease
Sgs1	BLM	AtRecQ4A	At1G10930 (189)	ATP binding, RecQ helicases

RPA. RAD51 is present in mammals. But RAD52 is not identified in mammals, BRCA2 fulfills the role of yeast Rad52 (19;21). Recently the orthologs of Rad52 were also identified in plants (22). Several possible homology directed repair subpathways have been postulated on the basis of the outcome of the recombination reaction: classical double-strand break repair (DSBR), synthesis-dependent strand-annealing (SDSA), single-strand annealing (SSA) and break-induced replication (BIR) (Figure 2) (23). DSBR was initially described to explain gene conversion and crossover events during meiosis (24). SDSA is based on mitotic DSB repair data in model organisms (25) and is thought to be the predominant mechanism to repair two-ended DSBs by HR. SSA may be utilized to repair a two-ended DSB, when a repeat sequence is present adjacent to the DSB. BIR has been described in yeast and may be used for one-ended DSBs to restart collapsed replication forks and elongate uncapped telomeres (26). In plants, there is evidence for the existence of SDSA, DSBR and SSA (27).

After 3'-end resection and Rad51 coating of the ssDNA, the nucleoprotein filament may invade into a homologous double stranded DNA (dsDNA) sequence and form a heteroduplex DNA intermediate which is called D-loop. This process occurs in the case of repair by the SDSA or classical DSBR subpathways (Figure 2). The "X" shaped structure formed at the border between the hetero- and homoduplex of a D-loop is called a Holliday Junction (HJ) (28). DNA is synthesized from the 3' end of the ssDNA beyond the original break site by D-loop migration to restore the missing sequence information. In the case of SDSA, the newly synthesized end of the invading strand is released by sliding the HJ toward

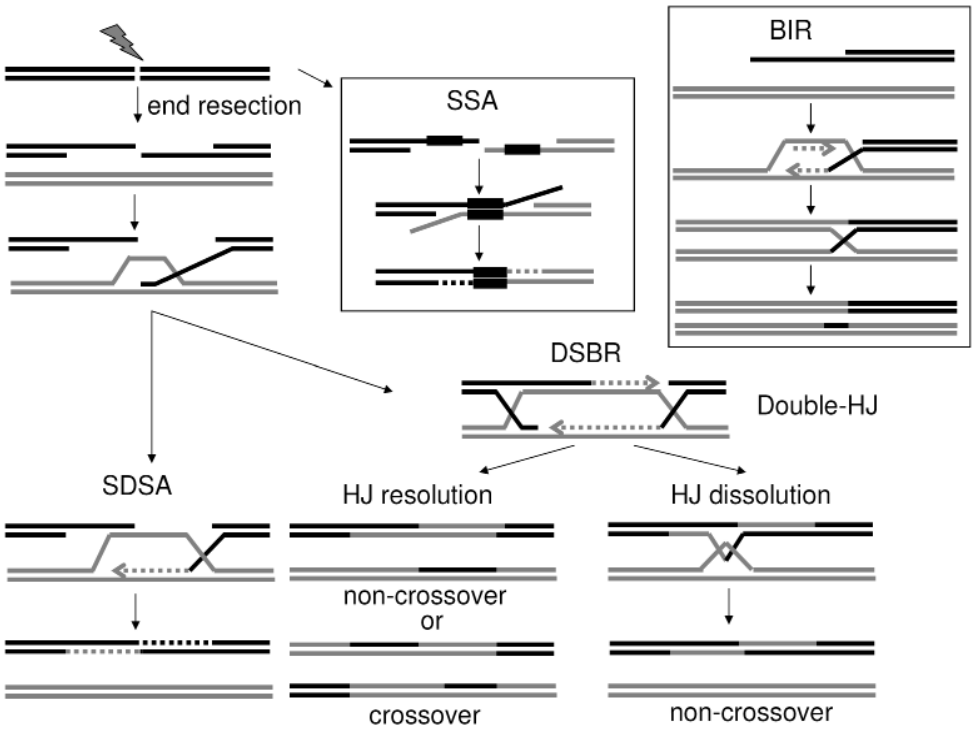

Figure 2. Models for DSBs repair via HR.
DSBs can be repaired by several HR pathways, including SDSA, DSBR, SSA and BIR. SDSA, DSBR and SSA are supposed to repair two-end DSBs. In those pathways, repair is initiated by end resection to provide 3'ssDNA overhangs. In the SDSA and DSBR pathways, the 3'ssDNA overhang invades into a strand with a homologous sequence by forming a D-loop, followed by DNA synthesis. In the SDSA pathway, the newly synthesized DNA forms a migrating replication bubble and is released from the template to anneal to the ssDNA on the other break end. The next step is gap-filling DNA synthesis and ligation. This will result in gene conversion. In the DSBR pathway, the other DSB end is also captured to form a double-HJ intermediate. The double-HJ structure can be resolved or dissolved in a non-crossover or crossover mode. DSBR can lead to gene conservation and crossover events. In the SSA pathway, the complementary DNA repeats (black boxes) serve to anneal the DSB ends and the noncomplementary overhangs are removed, followed by gap-filling and ligation. SSA produces a deletion between two sequence repeats. BIR is utilised to repair one-end DSBs. The single DSB end invades into a homologous strand, initiates a unidirectional DNA synthesis and replicates the entire homologous template arm. A single HJ is formed and is cleaved to repair the break with a duplication of the chromosome arm used as a template. BIR might result in a large-scale loss of heterozygosity, or a high mutation rate by template switching.

the 3' end. The displaced end can then anneal with the second resected DSB end. After removing flaps and filling-in gaps, the remaining nicks are ligated to complete this pathway. In the case of classical DSBR, both DNA ends invade a homologous chromosome to copy genetic information into the donor chromosome. The DNA joint molecule harbors two HJs that may be resolved to create a crossover or a non-crossover product. Whereas classical DSBR is used in meiosis for recombination between the two homologous chromosomes,

SDSA is used in somatic cells to restore DNA damage using the sister chromatid for repair.

In the case of SSA, the resected ends anneal to each other, which is possible when repeats are present near the DSB site (Figure 2). The protruding single-strand tails are removed by nucleases. Then gaps and nicks are filled in by DNA synthesis and ligation. SSA leads to permanent deletions, so it is error-associated.

For BIR, the DSB end is nucleolytically processed to a single-stranded tail that invades a homologous DNA sequence, followed by DNA synthesis to replicate the chromosome template (Figure 2). Unlike SDSA, for BIR a homologous sequence in a non-homologous chromosome is utilized as a template to initiate repair, and thus BIR can result in a non-reciprocal translocation.

4. Non-Homologous End Joining (NHEJ)

The basic NHEJ event is the direct joining of DSB ends, which are juxtaposed through end bridging, end-processed and ligated. NHEJ is a potentially less accurate mechanism for DSB repair, compared with HR. DNA end bridging occurs via protein-protein interactions between DNA end binding proteins, which bind directly to the DNA ends immediately after the breaking.

Most factors involved in the NHEJ pathway were initially identified in mammalian systems, such as the Ku heterodimer, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), DNA ligase IV (Lig4), XRCC4, XLF/Cernunnos (Table 2). The classical NHEJ (C-NHEJ) pathway utilizes Ku, DNA-PKcs, Lig4, XRCC4 and XLF/Cernunnos as central components; therefore it is also called DNA-PK-dependent NHEJ (D-NHEJ). C-NHEJ repairs rapidly a large proportion of DSBs. Recent findings show there are also one or several distinct alternative pathways, so-called backup NHEJ (B-NHEJ) pathways, which repair DSBs more slowly in the absence of the C-NHEJ factors. B-NHEJ pathways are Ku-independent, but utilize instead poly(ADP-ribose) polymerase (Parp) and DNA ligase III (Lig3) in mammalian cells. The most commonly discussed form of B-NHEJ is the microhomology-mediated end joining (MMEJ), which is mediated by a stretch of microhomologous base pairing of about 5 to 25 base pairs (bps).

There are two possibilities for the ligation of the juxtaposed DSB ends in NHEJ (Figure 3). Firstly, the ends can be ligated precisely. But the majority of DSBs generated by exposure to DNA damaging agents does not have ligatable termini and must be processed prior to ligation. In most cases, this will eventually produce deletions or insertions at the restored break site. Secondly, micro-homologous repeats surrounding the DSB ends may be aligned to repair the break by microhomology-mediated end joining (MMEJ). The mechanism is similar to SSA, but the homologous sequence used for MMEJ is only 5 to 25 bps, which is much shorter than the homology required for SSA. MMEJ will delete one of the repeats and the sequence between the repeats.

Table 2. NHEJ factors in yeast, mammals and plants.

Saccharomyces cerevisiae	*Homo sapiens*	*Arabidopsis thaliana*	Locus in Arabidopsis and ref.	Function
Ku70	Ku70	AtKu70	At1G16970 (151;190;191)	DSB end binding, protection and juxtaposition
Ku80	Ku80	AtKu80	At1G48050 (153;191)	
-	DNA-PKcs	-		protein kinase
Snm1/Pso	Artemis	AtSnm1?	At3G26680 (59;192)	DNA end processing, 5'-3'exonuclease, endonuclease
Pol4	Pol χ family	Pol λ	At1G10520 (60)	DNA end processing, filling in DNA gap
Tpp1?	PNK	-		DNA end processing, 3'-DNA phosphatase, 5'-DNA kinase
Tdp1	Tdp1	At5G15170	At5G15170 (193)	DNA end processing, 3'-DNA phosphatase
Dnl4	DNA ligase IV	AtLig4	At5G57160 (150;152)	ATP-dependent DNA ligase
Lif1	XRCC4	AtXRCC4	At3G23100 (152)	Complex with Lig4, DNA binding
Nej1	XLF/ Cernunnos	-		Lig4/XRCC4 binding
-	Parp1	AtParp1 (ZAP)	At2G31320 (194-196)	DNA binding, NAD+ADP-ribosyltransferase activity
-	Parp2	AtParp2 (APP)	At4G02390 (99;194-196)	DNA binding, NAD+ADP-ribosyltransferase activity
-	DNA ligase III	-		ATP-dependent DNA ligase
	XRCC1	AtXRCC1	At1G80420 (197)	Complex with Lig3
		AtLig6	At1G66730 (101)	ATP-dependent DNA ligase

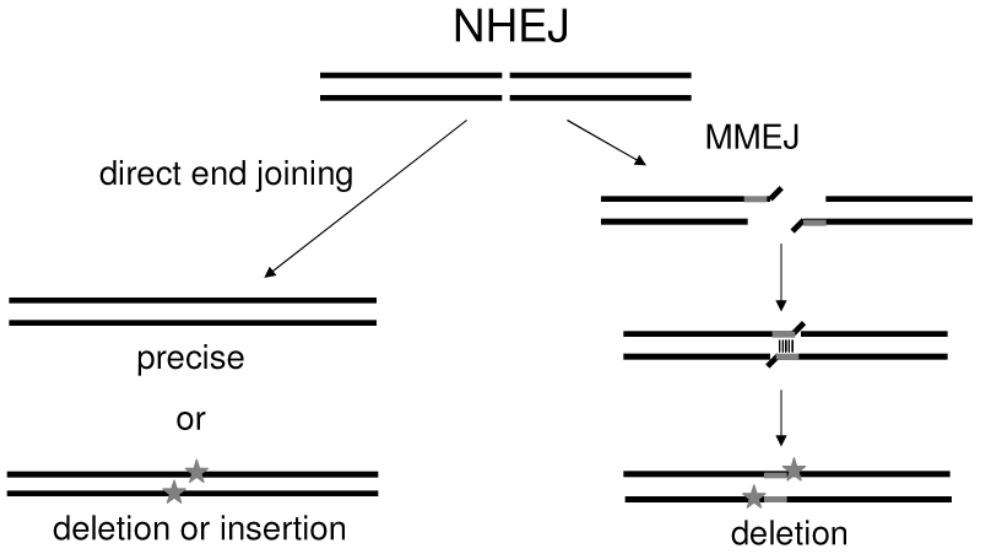

Figure 3. Ligation models of the juxtaposed DSB ends in NHEJ.
There are different ways for DSB ends to join: direct end joining or MMEJ. DSB ends can be joined precisely if the two ends are ligatable. But in most cases, the damaged ends are not ligatable, and they need end processing before ligation. This may induce deletions or insertions. For MMEJ, the ends are also processed to ssDNA, followed by strand annealing promoted by a short stretch of homologous sequence. Any non-paired flaps are removed and the ends are ligated. This will produce deletions.

4.1 Classical Non-Homologous End Joining (C-NHEJ)

4.1.1 Detecting and tethering DSB ends

It is generally assumed that NHEJ is initiated by the binding of a heterodimeric complex (Ku) to both DNA ends at the DSB site. The Ku heterodimer is composed of a 70kDa and 80kDa subunit, termed Ku70 and Ku80 respectively (29). Ku can bind various types of DNA ends (hairpins, blunt ends and 5' or 3' overhangs) in a sequence independent fashion with a high affinity *in vitro* (29;30). It associates with the DSB ends immediately after the generation of the break (31). The redox conditions can regulate the DNA-Ku binding by changing the structure of Ku, but it is still unclear how this happens (32). The Ku70 and Ku80 heterodimer forms a ring structure that slides over the DSB ends in an ATP independent manner (29). Ku stabilizes the DNA ends to facilitate NHEJ repair and protects DSB ends from DNA 5'-end resection which is a prerequisite for HR repair (23). Ku also recruits other NHEJ factors (such as DNA-PKcs, Lig4/XRCC4/XLF) to DSB ends and serves as a scaffold for the assembly of the NHEJ synapse (33).

In mammals, the DNA-Ku complex recruits DNA-PKcs, a ~465kDa member of the PIKKs, to form the active DNA-PK holoenzyme. DNA-PKcs is a nuclear protein serine/threonine kinase. The flexible arm of the Ku80 C-terminal region extends from the DNA-binding core to recruit and retain DNA-PKcs at DSBs (34). Crystallography studies revealed that DNA-PKcs forms a large open-ring cradle to promote the DSB repair (35). Electron

microscopy studies suggested that DNA-PKcs functions as a DNA-end bridging factor to tether the broken ends for rejoining (36). Two DNA-PKcs molecules interact across the DSB with the Ku dimer in a synaptic complex (37). This interaction stimulates the kinase activity of DNA-PKcs (38). The active DNA-PK catalyzes autophosphorylation and phosphorylation of other downstream NHEJ proteins. DNA-Pkcs phosphorylation appears to be important for DNA repair. Upon autophosphorylation DNA-PKcs is released from the DNA ends by changing the conformation (34). This conformation change would make the DNA ends accessible for the processing enzymes and ligases (39). This suggested that DNA-PKcs might regulate NHEJ by phosphorylation.

In yeast and fungi, no homologues of DNA-PKcs have been identified. Biochemical evidence shows that the MRX complex takes the role of end bridging in the yeast NHEJ instead of DNA-PKcs (40). Rad50 contains a high-affinity DNA-binding domain and a split ATPase domain. A functional ATPase is formed when two Rad50 proteins associate. Rad50 may be able to bridge the DNA ends together (41). Mre11 interacts with yKu80 and Xrs2 interacts with the Lif1 cofactor of the Lig4 ligase (8). It seems that MRX enables formation of a stable NHEJ complex (23). MRX is the only protein complex that participates in both NHEJ and HR DSB-repair pathways in yeast (23) and thus, might regulate repair pathway utilization.

4.1.2 Processing DSB ends

DSBs may have various ends. In case of incompatible ends, DSB ends processing is required to remove non-ligatable end groups and other lesions prior to ligation. Processing may consist of resection by nucleases, filling DNA gaps by polymerases or addition of 5' phosphate groups by polynucleotide kinase (PNK) (39). Several accessory enzymes have been implicated in this process, including Artemis, Mre11, DNA polymerase Pol χ family members and PNK, as mentioned above.

In mammals, Artemis, a key end-processing enzyme, may be recruited to DSBs by interacting with DNA-PKcs (42). The activity of Artemis can be regulated via phosphorylation by DNA-Pkcs and ATM, suggesting that Artemis may participate in multiple aspects of the DNA damage response (14;42). Artemis is a 5'-3' exonuclease and also possesses an endonuclease activity in a DNA-PKcs-dependent manner to remove both 5' and 3' overhangs for NHEJ repair and cleave the DNA hairpins during V(D)J recombination (38). The nucleolytic processing of DNA ends might create small gaps that must be filled in by DNA polymerase Pol χ prior to DNA joining during NHEJ. The mammalian Pol χ family includes DNA polymerases β (Pol β), Pol μ, Pol λ and terminal deoxynucleotidyl transferase (TdT) (43). TdT is a unique template-independent polymerase, which can add random nucleotides during V(D)J recombination. Pol μ is template-dependent, and Pol β and Pol λ can synthesize in both template-independent and template-dependent manner (44). If DNA ends contain non-ligatable 5' hydroxyls and 3' phosphates, the mammalian PNK can modify those groups to facilitate the ligation (45). PNK possesses both 3'-DNA phosphatase

and 5'-DNA kinase activities and interacts with XRCC4 during NHEJ (23).

In yeast, the MRX complex is suggested to have the same function as the mammalian DNA-PKcs/Artemis complex (4). Mre11 is a nuclease and can remove hairpins and 3'-ssDNA overhangs at the ss/ds DNA junction. So Mre11 may be involved in end processing in NHEJ (7). Pol4 is the only Pol χ polymerase which acts in gap filling without strict dependence on the template during NHEJ in yeast (46;47). The yeast homologue of mammalian PNK, 3'-phosphatase-1 (Tpp1), lacks 5' kinase activity (48). Recent reports showed that 3' nucleosidase activity of tyrosyl-DNA-phosphodiesterase 1 (Tdp1) regulates the processing of DNA ends by generating a 3' phosphate and restricts the ability of polymerases from acting at DNA ends (49).

4.1.3 Ligating DSB ends

After the proper processing of DNA ends, NHEJ is completed by the final ligation step. This rejoining step is carried out by the complex of DNA ligase IV (Lig4) and XRCC4. A third essential component for the NHEJ ligation step is XRCC4-like factor (XLF, also named Cernunnos), which stimulates the activity of Lig4 and is required for NHEJ and V(D)J recombination (50;51).

Lig4 contains an ATP utilizing catalytic domain in the N-terminal region and two C-terminal BRCT domains. The two BRCT domains of Lig4 and the linker region between them interact with XRCC4 to form a stable complex (39;52). The BRCT motifs may also be involved in the interaction with the Ku complex (53). XRCC4 stabilizes Lig4 and stimulates its activity (38). The globular N-terminal head of XRCC4 interacts with the DNA helix (54). Like Ku proteins, XRCC4 acts as a scaffold to recruit other NHEJ factors, including the processing enzyme PNK. The Lig4/XRCC4 complex not only facilitates the ligation step but may also stimulate the DNA end processing. Lig4 has a high degree of substrate flexibility in the presence of XRCC4 and XLF. The complex can ligate both blunt ends and compatible overhangs. It can also ligate across gaps and incompatible ends with short overhangs (44). XLF has a similar structure as XRCC4, with which it interacts. The crystal structure of XLF reveals that it has an N-terminal globular coiled-coil head and a C-terminal stalk, which is suitable for the head-to-head interactions in a 2:2:1 XRCC4:XLF:Lig4 complex (55). XLF can enhance Lig4/XRCC4 ligation activity by promoting its re-adenylation, but XLF is dispensable for the stability of Lig4/XRCC4 complex suggesting that XLF is not essential for all DSB rejoining (56). Both XRCC4 and XLF are phosphorylated by ATM and DNA-PK, though phosphorylation seems to be not required for NHEJ (57;58).

Homologs of Lig4/XRCC4 have been identified in mammals, yeast and plants, suggesting that the mechanism by which the ligation step occurs is universal in eukaryotes. The counterpart of XLF has also been found in yeast and is named Nej1. But so far no XLF homolog has been identified in plants.

In plants, no affirmative homologues of DNA-Pkcs have been identified until now. This

suggests that Ku of plants may have more general functions in NHEJ pathway than that of mammals, and it could be that Ku or MRN bridge the two DNA ends together. The processing of DSB ends in NHEJ is largely unknown in plants. Plants contain an Artemis homologous protein known as Pso2p/Snm1p. However, due to the low similarity of amino acid sequence between Artemis and Pso2p/Snm1p, they may have different functions in DNA repair (59). DNA ends may be processed by the MRN complex to make them suitable substrates for DNA ligase in plants as that in yeast. The only member of the Pol χ family identified in plants is DNA Pol λ. It has a close similarity to mammalian Pol β and is supposed to function as a DNA repair enzyme in meristematic and meiotic tissues (60).

4.2 Backup Non-Homologous End Joining (B-NHEJ): Microhomology-Mediated End Joining (MMEJ)

Nowadays more and more evidence show that the majority of DSBs can be rejoined with slow kinetics in the absence of C-NHEJ core factors, such as DNA-PKcs, Ku70/Ku80 and Lig4/XRCC4, suggesting the existence of one or multiple alternative or backup pathways of NHEJ (61;62). The alternative pathways were well demonstrated in C-NHEJ deficient cells or in wild-type cells after the treatment with inhibitors against C-NHEJ factors (63-66). Compared to the extremely fast and efficient C-NHEJ, the alternative pathways are quite slow and error-prone (67). Defects in C-NHEJ are implicated in chromosomal translocation and gene instability (68-70). Many studies utilizing *in vitro* plasmid based end joining assays also have provided evidence for the existence of B-NHEJ (62;71;72). End joining is observed in the extracts of DNA-Pkcs deficient cells and in Ku-depleted extracts. Anti-Ku antibodies inhibit DNA end joining strongly only in the presence of DNA-PKcs, and Ku is also essential for the inhibition of DNA end joining by the DNA-PKcs inhibitor wortmannin (62). It suggests that Ku, cooperating with DNA-PKcs, directs joining of broken ends to C-NHEJ, at the same time suppressing B-NHEJ. The repair events via B-NHEJ preferentially use short stretches of homology (5~25bps) (65;73;74), and therefore this type of repair has also been called microhomology-mediated end joining (MMEJ). It seems that MMEJ is the dominant pathway among the B-NHEJ pathways. MMEJ induces small deletions and causes gene instabilities (75). Several proteins have been identified as participating in B-NHEJ in mammals: histone H1, poly(ADP-ribose) polymerase-1 (Parp1), DNA Ligase III (Lig3) and XRCC1 (76;77).

Histone H1 is a major structural component of chromatin with functions in DNA repair. Protein fractionation and *in vitro* end-joining assays showed that histone H1 enhances DNA-end joining strongly by activating Lig3 and Parp1, suggesting it is a putative B-NHEJ factor (78). Histone H1 has been shown to inhibit HR (79) and C-NHEJ (80). It binds to naked DNA and may juxtapose to form end to end polymers (81), and thus histone H1 may be an alignment factor operating in B-NHEJ (78).

Poly(ADP-ribose)polymerases (Parps) catalyze the covalent attachment of poly(ADP-ribose) units on amino acid residues of itself and other acceptor proteins using NAD^+ as

a substrate in order to modulate various cellular processes by poly(ADP)ribosylation (82). Among the 17 members of the Parp family, Parp1 and its close homologue Parp2 are activated in response to DNA damage (83). Parp1 has a high binding affinity for DNA SSBs and DSBs by its two zinc finger motifs. Binding to DNA leads to Parp1 automodification and subsequent release from DNA to allow the access of other repair proteins (76). There is plenty of evidence that Parp1 is implicated in SSB repair and base excision repair (BER) and thus prevents formation of DSBs during replication (83;84). Parp1 is probably also involved in B-NHEJ. The synapsis activity of Parp1 and the ligation activity of Lig3/XRCC1 were established via a two-step DNA *in vitro* pull down assay with nuclear extracts and recombinant proteins (85). In absence of DNA-PK or Lig4/XRCC4, DSB end joining activity was observed, which was dependent on Parp1 and Lig3/XRCC1. Recent reports also show that Parp1 facilitates B-NHEJ using microhomology (86). Parp1 binds to DNA ends in direct competition with Ku to regulate the utilization of C-NHEJ and B-NHEJ pathways (87). Parp1 is supposed to be a sensor of DNA breaks and may help to form the end synapsis. Parp2 may have a similar function as Parp1 (88). Parp2 has a higher affinity for gaps or flaps than SSBs, indicating that Parp2 is involved in later steps of the repair process (88). Parp1 regulates the activity of Pol β in long patch BER (89) and interacts with Mre11 for end processing in the restart of replication forks (90), suggesting that Pol β and MRN may also function in end processing during B-NHEJ.

Unlike other DNA ligases, Lig3 appears to be unique to vertebrates. Lig3 is recruited to SSBs by preferentially interacting with automodified Parp1 (91). Thus Lig3 is considered to be involved in SSB repair and BER (92;93). Lig3 is also found to play a major role in end joining by using extract fractionation of Hela cells (94). DNA end joining activity can be reduced by knocking down Lig3 in Lig4-deficient mouse embryo fibroblasts (94). This indicates that Lig3 has a potential role in B-NHEJ pathway. XRCC1 interacts with Lig3 and is required to stabilize Lig3 (95). XRCC1 interacts with several other BER and SSB repair factors as well, such as Parp1 and Pol β (92), suggesting that XRCC1 may act as a scaffold for the protein assembly (96).

MMEJ has also been found in yeast which is repressed by Ku70 (97), suggesting that there may be similar backup NHEJ pathway in yeast. Specific B-NHEJ proteins have not been identified in yeast as yet. Although Parp activity is absent in yeast, human Parp1 expression in *Saccharomyces cerevisiae* leads to the inhibition of growth due to its effects on ribosome biogenesis (98). Other proteins may replace the function of Parp in yeast.

Like in mammals, there may be backup pathways of NHEJ in plants. Homologues of Parp1 and Parp2 have been identified in plants. One is the classical Zn-finger-containing polymerase (ZAP) and the other is APP/NAP, which is structurally different from the classical Parp and is lacking the N-terminal Zn-finger domain (99). APP/NAP is a DNA-dependent poly(ADP-ribose) polymerase and the expression of APP/NAP is induced by deficiency of Lig1 (99). Microarray data also showed that APP/NAP displayed transcriptional induction in the presence of bleomycin and in the *Atku80* mutant (100). All these observations

point to the implication of APP/NAP in DNA repair. Though no homologues of Lig3 has been identified in plants, a novel ATP-dependent DNA ligase, named Lig6, is found uniquely in plants. Bonatto *et. al.* (101) found that Lig6 has a high homology with Lig1 and is hypothesized to function in BER and B-NHEJ. Recently the work of Waterworth *et. al.* (102) showed that the *Atlig6* mutant was hypersensitive to X-ray and delayed in seed germination.

5. Regulation of DSB repair pathways

As discussed above, eukaryotes have two primary pathways to repair DSBs: HR and NHEJ. Both of them play an important role in maintaining genome stability and preventing the consequent disorders, such as the loss of genetic information, chromosomal translocations, cell death and diseases like cancer. How do cells modulate the respective usage of those pathways? Since different pathways generate distinct products, it is an important and compelling question. Also when the control mechanisms are well studied, ways to regulate them may be found, and used for instance to increase the usage of HR, which will facilitate gene targeting. These control factors may also become helpful as biomarkers for cancer detection and as targets for cancer therapy.

Different organisms prefer different pathways to repair DSB. Yeast tends to use HR, whereas higher eukaryotes like plants and mammals mainly use NHEJ in somatic cells. This is based on the observation that NHEJ-deficient yeast is not sensitive to IR unless HR is also deficient. In contrast, NHEJ-deficient mammals and plants are hypersensitive to IR whether HR is operative or not (23;103). Higher eukaryotes have larger and more complex genomes and enormous amounts of repetitive DNA, which may be the reason that NHEJ is preferred in order to prevent mistakes leading to translocation. The choice between the different forms of DSB repair also depends on the cell type, phase of the cell cycle and developmental stages of the organism. HR is more efficient in diploid than in haploid yeast due to template availability and the absense of Nej1, which is involved in NHEJ (104). Though most higher eukaryotes predominately utilize NHEJ in somatic cells, Chicken B cells (DT-40) possess a highly efficient HR machinery (105). Mouse embryonic stem (ES) cells are more prone to HR, than primary cells (106). HR is the predominant mode of DSB repair during early neural development, and NHEJ takes over the function at the later stage (107). DSB repair mechanisms are developmentally regulated in plants. Rad51 activity drops and Ku70 activity increases after germination, therefore, the rate of HR decreases with plant age in Arabidopsis (108).

It is well documented that NHEJ is active in all phases of the cell cycle, whereas HR is mainly restricted to the S and G2 phases of the cell cycle and directed to the utilization of the new sister chromatid as a template (104). This means that the cell cycle stage could be a decisive factor for the selection of the DSB repair pathway when a DSB is generated. Cell cycle progression is primarily controlled by cyclin-dependent kinases (CDKs) (109).

In yeast, Cdc28/Clb (Cdk1/cyclin B) is activated in the S/G2 phases and is suppressed in G1 phase. In the S/G2 phases, active CDK facilitates the DNA resection of DNA ends, which is the initiation step of HR. DSB ends are resected in 5'-3'- direction to generate ssDNA, which can anneal with a homologous sequence to effect HR, or if homology is not available, MMEJ. It was shown that CDKs phosphorylate Sae2 (budding yeast)/Ctp1 (fission yeast) and the mammalian orhologue CtIP to promote DSB resection and HR cooperatively with MRX/MRN in the S/G2 phases (110-112). The endonuclease activity of Sae2 for DSB resection is also regulated by the Cdc28/Cdk1-mediated phosphorylation in a cell-cycle dependent manner (112). Yun et al. (111) reported that CtIP is required for both HR in the S/G2 phases and MMEJ in G1 in the DT40 cell line. In mammals, CtIP is also found to regulate DSB resection by interaction with MRN and BRCA1 in the S/G2 phases (113). Phosphorylated CtIP recruits BRCA1 to DNA damage sites and increases the level of ssDNA (111). In the late M/G1 phases, CDK phosphorylates BRCA2, resulting in disassociation of the Rad51-BRCA2 complex, and in blocking HR (114).

During the cell cycle, the components of different DSB repair pathways seem to compete for the selection of DSB repair pathway. Ku is always the first factor recruited to DNA ends due to its high affinity for DNA ends. Ku protects the ends from resection and interferes with HR during the whole cell cycle. HR factors may also interact with the Ku-bound ends. Indirect evidence showed that in the S/G2 phase, HR factors, such as CtIP1 and BRCA1, are highly activated by phosphorylation, which facilitates HR over NHEJ. In the G1 phase, the Ku complex recruits the Lig4 complex to stabilize Ku and ligate the DNA ends via NHEJ. Biochemical evidence has shown that Parp physically interacts with Ku and decreases the affinity of Ku to DSBs so as to favor HR (106;115). Studies with DT40 cells implicate that Parp1 and the post-replicative repair protein Rad18 independently promote HR and suppress NHEJ (116;117). Parp1 also competes for DNA ends with Ku to enhance B-NHEJ in G2 (87;118). Recent results from studies with human somatic cells indicate that Ku suppresses other DSB repair pathways (HR and B-NHEJ), suggesting that Ku could be a critical regulator of DSB repair choice (74).

In summary, there is a crosstalk between all the DSB repair pathways. The cell cycle progression could be the key regulator for the switch between NHEJ and HR. The regulation involves among others the availability of repair templates, the activity of CDKs, DNA end resection and protein competition. HR and NHEJ work in a competitive and cooperative manner to maintain the genome stability.

6. Gene Targeting (GT)

Gene targeting (GT) is a technique by which endogenous genes in the genome are modified using HR with a transgene. It has broad applications. It is a powerful tool for studying gene function by the inactivation or modification of specific genes, and it is also a potential means for gene therapy and biotechnological improvement of crop plants. The implementation

of HR-based GT relies not only on the design of the sequence homology between the exogenous transforming DNA molecule (the donor) and the chromosomal DNA (the target), but also on the DNA-repair system used by the target cell. Efficient GT has been achieved in yeast, fungi and some cell lines, such as mouse embryonic stem cells, chicken DT40 cells, and human Nalm-6 pre-B cells (119;120). However, GT is inefficient in plants, and most other animal and human cell lines. How to improve the frequency of GT events in these organisms is a big issue and challenge.

There are several strategies to select and detect gene targeting events, one of which, the positive/negative selection (P/NS) scheme, proved to be very successful. In the P/NS strategy, the targeting construct contains a positive selectable marker placed in between homologous regions and a negative selectable marker gene placed outside the homology (121). It enriches for gene targeting events by selection for the positive selection marker and against the negative selection marker, thus reducing transformants in which the transgene had integrated by non-homologous recombination. The Herpes simplex virus thymidine kinase gene (HSV-TK) and the diphtheria toxin A-chain gene (DT-A) have been commonly used as negative selection markers in mammals (121;122). Also in plants several negative selection marker genes have been tested (123-125) and the results depended on the species and the developmental stage.

Based on the mechanism of GT, there are two general ways to increase the frequency of GT events. The first one is to genetically modify the organisms in order to facilitate the HR pathway by either increasing the HR components or reversely by blocking the NHEJ pathway. The second is to introduce specific DNA breaks in the target sequence so as to increase the chance of recombination at the target locus.Sufficient homology between the targeting vector and the target locus obviously is also important. In murine embryonic cells, a dramatic increase in the targeting frequency can be observed by an increase of homology in a range between 2 and 10kb (126). If the area of homologous DNA is reduced to below 1kb in length, gene targeting is strongly diminished (127). The need for homology is saturated by ~14kb (126).

6.1 Facillitating the HR pathway

Several reports showed that elevation of the expression of exogenous HR components, such as Rad51, Rad52 and Rad54, can enhance gene targeting frequencies (128-131). Previous reports also showed that HR could be enhanced by expression of bacterial RecA provided with a nuclear localization signal in mammals and plants (132;133). Enhanced gene targeting was also observed by overexpression of Rad51 in murine embryonic stem cells (128). In the yeast *Saccharomyces cerevisiae*, yRad52 and yRad51 are required for the targeted integration of *Agrobacterium* T-DNA via HR (134). Di Primio *et al.* (129) expressed the yRad52 in human cells and this increased the frequency of gene targeting by 37 fold. They proposed that yRad52 has a greater affinity for ssDNA to promote strand exchange than hsRad52, suggesting that the yeast protein could be used as a tool to enhance gene targeting. Recently

Kalvala et al. (130) followed up on this finding by delivering yRad52 directly into the human cells by the fusion of yRad52 to the arginine-rich domain of the HIV TAT protein (tat11), which is known to permeate cell membranes. The recombinant yRAD52tat11 still maintained the ability to bind ssDNA and promote intrachromosonal recombination after entering the nuclei of the cells. By using this approach, a 50-fold increase of gene targeting was observed. This approach of expression of yeast HR proteins to improve gene targeting was also utilized in plants. Shaked *et al.* (131) expressed the yRad54 in Arabidopsis plants and gene targeting was analyzed using a high-throughput assay based on visual screening of GFP in seeds. An increased frequency of gene targeting by 27 fold on average was found. yRad54, a member of the Swi2/Snf2 family of ATP-dependent chromatin remodeling factors, promotes strand invasion in HR. Disruption of the *Rad54* gene reduced gene targeting and increased radiation sensitivity in various species (135;136). Thus Rad54-like activity may be a limiting factor in gene targeting. All of these observations indicated that elevated expression of the factors involved in HR could be potentially useful to increase gene targeting. Whether it can be utilized in other organisms like livestock or crop plants, is still unknown.

Since random DNA integration in yeast can be suppressed by a deficiency in NHEJ (137), the number of gene targeting events may be increased after disruption of NHEJ. So far, this has been well manifested in lower eukaryotes, such as yeast and several species of filamentous fungi (134;137-145). Unlike the yeast *Saccharomyces cerevisiae*, other yeasts and fungi preferably use NHEJ over HR for DNA integration, leading to a low efficiency of gene targeting. This can be dramatically improved to 80%~90% after the abolishment of the NHEJ system, compared to less than 10% in the wild-type background (137;138;140-142;145). Like in fungi, NHEJ is also the predominant DNA repair pathway in plants and animals. A similar approach was used to improve gene targeting in plants and animals. Since the NHEJ components have important functions in early mammalian development, viable NHEJ mutants are hard to obtain and most research has therefore been done only with cell lines. In human somatic HCT116 cells with the $Ku70^{+/-}$ genotype, the frequency of gene targeting was increased by 5-10 fold compared to that in wild-type cells, and the result was affirmed by using RNA interference (RNAi) or short-hairpinned RNA (shRNA) strategies to deplete Ku70 in the cells of wild-type background (146). The GT frequency was further increased by 30 fold in the $Ku70^{+/-}$ cells of combined with the RNAi of Ku70. Recently, a 33-fold increase in gene targeting was also observed using the RNAi treatment of Ku70 and XRCC4 in human somatic HCT116 cells (147). The targeted integration was enhanced by Lig4 knock-out in chicken DT40 cells, but not in human Nalm-6 cell lines indicating that the impact of NHEJ factors on gene targeting varies between cell types (148). In murine embryonic stem cells, the gene targeting frequencies were increased by 3 fold in *parp1* knockout cells, but not in *ku80* or *DNA-PKcs* knockout cells, suggesting that Ku80 and DNA-PKcs would also be involved in HR or that a B-NHEJ is activated (149). Few reports described similar experiments in plants until now. Unlike mammals, most of

the NHEJ mutants in plants have no obvious phenotype under normal growth conditions (150-154), which gives us more chances to study the effects of these NHEJ factors on gene targeting. Tanaka et al. (155) claimed highly efficient gene targeting in an Arabidopsis *Atlig4* mutant by introducing double strand DNA fragments to plant cells with a particle gun. However, this result is probably the result of PCR artefacts.

6.2 Introducing DSBs in the target

Targeted recombination will be enhanced by the introduction of genomic DSBs at the target site. This has been demonstrated in a wide range of animal and plant species (156). Meganucleases, which can generate DSBs at a specific large recognition site in the genome, are preferably used for this purpose. Meganucleases are sequence specific endonucleases with long recognition sequences (12-45 bp), including not only the natural homing nucleases, such as HO and I-SceI, but also the artificial endonucleases such as Zinc Finger Nucleases (ZFNs).

HO of budding yeast can induce HR by creating a DSB at the MAT locus to switch the mating type (157). I-SceI, encoded by a mitochondrial intron of *Saccharomyces cerevisiae*, can help to convert intronless alleles into alleles with an intron (158). Though gene targeting can be increased considerably by the use of the natural homing endonucleases (156;159;160), its application is so far limited to artificial target homing endonucleases recognition sites that are present in the target sites. Recently, however, a combinatorial approach was reported to redesign homing endonucleases to match with target sites that are naturally present in the genome (161).

Artificial ZFNs do not need the pre-insertion of recognizing sites into the target genome, making them useful as a novel genomic tool. ZFNs consist of the nonspecific DNA cleavage domain of the *Fok*I enzyme and a specific DNA binding domain composed of several engineered Cys_2-His_2 zinc fingers. The cleavage activity of the *Fok*I domain requires dimerization. The DSB can be generated in a spacer sequence (5~7bp) between the two ZFN monomers binding sites when they bind together in a tail to tail configuration. Since one single ZF recognizes 3 bases of DNA sequence, a heterodimer of two ZFN, each containing 3 to 6 ZFs, could recognize 18bp to 36bp target site, which is enough to define a unique sequence in most organisms statistically. ZFNs have been successfully used for GT experiments in a lot of organisms, such as *Drosophila*, *Xenopus*, *Caenorhabditis elegans*, zebrafish, human cell lines, Arabidopsis, tobacco and maize. The increase of GT frequencies vary from 10 to 10000 among different organisms and cell types (162-165). The activity and the expression level of ZFNs in the target cells also influence the GT efficiency. There are still some problems that need to be solved with this strategy. One issue is about the specificity and the toxicity of the ZFN (166-168). If ZFNs are not extremely specific and can cut the genome at off-target sites, they will cause instability of the genome and be toxic. The safety of ZFNs needs to be improved by optimizing the design of the ZFNs' structure or regulating the expression level and duration of the presence of ZFNs in the target cells.

7. Outline of the thesis

Non-homologous end joining (NHEJ) is the predominant pathway for the repair of DNA double strand breaks in higher eukaryotes, such as plants and mammals. This pathway has been well characterized in yeast and mammals, but in plants no detailed analysis has been described. In mammals, it includes the classical NHEJ (C-NHEJ), which is dependent on Ku proteins, and the backup NHEJ (B-NHEJ), which is dependent on the Ku proteins and is much less characterized (61-63;94). In plants, the major components of the C-NHEJ have been identified (19). The NHEJ pathway is used for random integration of T-DNA in yeast. Inactivation of NHEJ in yeast and fungi prevented integration by non-homologous end joining (134;145). As a consequence, T-DNA integration could only occur by homologous recombination (HR; gene targeting). The first objective of this project was to investigate the NHEJ pathway in plants and to find out whether the B-NHEJ pathway exists in plants using *Arabidopsis thaliana* as a model. The second objective was to analyze whether gene targeting frequencies increased in the absence of NHEJ as had been found in yeast and fungi (134;137;145).

Several NHEJ single mutants were ordered from the Salk collection for our research, such as *Atku70*, *Atku80*, *Atlig4*, *Atmre11*, *Atparp1*, *Atparp2* and *Atlig6*. They were functionally characterized in this thesis. Some double and triple mutants were also obtained by crossing the single mutants. In **chapter 2**, the T-DNA insertion single mutants (*Atku70*, *Atku80*, *Atlig4*), which are deficient in the main components of C-NHEJ, are described. Together with *Atku70* (Ws) and *Atmre11* (Ws), which had been characterized by our lab previously, they were all tested for the frequency of T-DNA integration and gene targeting.

As it was reported that Parp1 and Parp2 not only are involved in SSB DNA repair, but also in the B-NHEJ pathway in mammals (83;85;87), the orthologs of Parp proteins have been identified in plants as well (99), DNA repair in *Atparp1* and *Atparp2* mutants was studied in **chapter 3** and **chapter 4**. **Chapter 3** describes the characterization of the *Atparp1* and *Atparp2* mutants. The results show that also in plants the AtParp proteins play an important role in SSB repair and that they are also involved in B-NHEJ using areas of micro-homology. **Chapter 4** focuses on how exactly the DNA ends join in the NHEJ mutants, which were deficient in C-NHEJ, B-NHEJ or both. To this end, the *Atp1p2* double mutant and the *Atp1p2k80* triple mutant were obtained by crossing. The results are in accord with **chapter 3** showing that AtParp proteins facilitate MMEJ, and also suggest that AtKu plays a role in DNA end protection and thus prevents MMEJ and HR. Unexpectedly, the *Atp1p2k80* triple mutant still has the ability of end joining indicating that there must exist even a third pathway for end joining besides C-NHEJ and B-NHEJ.

DNA ligase 3 (Lig 3) has been reported to ligate the DNA ends for DNA repair in the B-NHEJ pathway in mammals (94). Since no ortholog of Lig3 was found in plants, a plant specific DNA ligase 6 (Lig6) was postulated to function in B-NHEJ. **Chapter 5** presents the

study on the two *Atlig6* mutants showing that *Atlig4lig6* double mutants still ligate DNA ends and integrate T-DNA. The *in silico* analysis of DNA ligases in plants revealed another possible candidate, which is possibly involved in B-NHEJ.

Reference List

1. Rich,T., Allen,R.L. and Wyllie,A.H. (2000) Defying death after DNA damage. *Nature*, **407**, 777-783.
2. Su,T.T. (2006) Cellular responses to DNA damage: one signal, multiple choices. *Annu. Rev. Genet.*, **40**, 187-208.
3. Nagy,Z. and Soutoglou,E. (2009) DNA repair: easy to visualize, difficult to elucidate. *Trends Cell Biol.*, **19**, 617-629.
4. Hefferin,M.L. and Tomkinson,A.E. (2005) Mechanism of DNA double-strand break repair by non-homologous end joining. *DNA Repair (Amst)*, **4**, 639-648.
5. Clemenson,C. and Marsolier-Kergoat,M.C. (2009) DNA damage checkpoint inactivation: adaptation and recovery. *DNA Repair (Amst)*, **8**, 1101-1109.
6. Waterworth,W.M., Altun,C., Armstrong,S.J., Roberts,N., Dean,P.J., Young,K., Weil,C.F., Bray,C.M. and West,C.E. (2007) NBS1 is involved in DNA repair and plays a synergistic role with ATM in mediating meiotic homologous recombination in plants. *Plant J.*, **52**, 41-52.
7. Zhang,X. and Paull,T.T. (2005) The Mre11/Rad50/Xrs2 complex and non-homologous end-joining of incompatible ends in *S. cerevisiae*. *DNA Repair (Amst)*, **4**, 1281-1294.
8. Matsuzaki,K., Shinohara,A. and Shinohara,M. (2008) Forkhead-associated domain of yeast Xrs2, a homolog of human Nbs1, promotes nonhomologous end joining through interaction with a ligase IV partner protein, Lif1. *Genetics*, **179**, 213-225.
9. Branzei,D. and Foiani,M. (2008) Regulation of DNA repair throughout the cell cycle. *Nat. Rev. Mol. Cell Biol.*, **9**, 297-308.
10. Falck,J., Coates,J. and Jackson,S.P. (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*, **434**, 605-611.
11. Nakamura,A.J., Rao,V.A., Pommier,Y. and Bonner,W.M. (2010) The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks. *Cell Cycle*, **9**, 389-397.
12. Bartek,J. and Lukas,J. (2007) DNA damage checkpoints: from initiation to recovery or adaptation. *Curr. Opin. Cell Biol.*, **19**, 238-245.
13. Varmark,H., Kwak,S. and Theurkauf,W.E. (2010) A role for Chk2 in DNA damage induced mitotic delays in human colorectal cancer cells. *Cell Cycle*, **9**, 312-320.
14. Beucher,A., Birraux,J., Tchouandong,L., Barton,O., Shibata,A., Conrad,S., Goodarzi,A.A., Krempler,A., Jeggo,P.A. and Lobrich,M. (2009) ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *EMBO J.*, **28**, 3413-3427.
15. O'Driscoll,M. and Jeggo,P.A. (2006) The role of double-strand break repair - insights from human genetics. *Nat. Rev. Genet.*, **7**, 45-54.
16. De Schutter,K., Joubes,J., Cools,T., Verkest,A., Corellou,F., Babiychuk,E., Van Der Schueren,E., Beeckman,T., Kushnir,S., Inze,D. *et al.* (2007) Arabidopsis WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint. *Plant Cell*, **19**, 211-225.
17. Cools,T. and De Veylder,L. (2009) DNA stress checkpoint control and plant development. *Curr. Opin. Plant Biol.*, **12**, 23-28.
18. Symington,L.S. (2002) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiol. Mol. Biol. Rev.*, **66**, 630-70.

19. Bleuyard,J.Y., Gallego,M.E. and White,C.I. (2006) Recent advances in understanding of the DNA double-strand break repair machinery of plants. *DNA Repair (Amst)*, **5**, 1-12.
20. Mimitou,E.P. and Symington,L.S. (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature*, **455**, 770-774.
21. Lisby,M. and Rothstein,R. (2009) Choreography of recombination proteins during the DNA damage response. *DNA Repair (Amst)*, **8**, 1068-1076.
22. Samach,A., Melamed-essudo,C., Avivi-ragolski,N., Korchia,J., Pietrokovski,S. and Levy,A.A. Plant RAD52 Homologs: Identification and Activity. Plant DNA repair and recombination 2010. 64. 3-2-2010. Ref Type: Conference Proceeding
23. Pardo,B., Gomez-Gonzalez,B. and Aguilera,A. (2009) DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship. *Cell Mol. Life Sci.*, **66**, 1039-1056.
24. Szostak,J.W., Orr-Weaver,T.L., Rothstein,R.J. and Stahl,F.W. (1983) The double-strand-break repair model for recombination. *Cell*, **33**, 25-35.
25. San,F.J., Sung,P. and Klein,H. (2008) Mechanism of eukaryotic homologous recombination. *Annu. Rev. Biochem.*, **77**, 229-257.
26. Llorente,B., Smith,C.E. and Symington,L.S. (2008) Break-induced replication: what is it and what is it for? *Cell Cycle*, **7**, 859-864.
27. Bray,C.M. and West,C.E. (2005) DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. *New Phytol.*, **168**, 511-528.
28. Helleday,T., Lo,J., van Gent,D.C. and Engelward,B.P. (2007) DNA double-strand break repair: from mechanistic understanding to cancer treatment. *DNA Repair (Amst)*, **6**, 923-935.
29. Walker,J.R., Corpina,R.A. and Goldberg,J. (2001) Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature*, **412**, 607-614.
30. Smith,G.C. and Jackson,S.P. (1999) The DNA-dependent protein kinase. *Genes Dev.*, **13**, 916-934.
31. Mari,P.O., Florea,B.I., Persengiev,S.P., Verkaik,N.S., Bruggenwirth,H.T., Modesti,M., Giglia-Mari,G., Bezstarosti,K., Demmers,J.A., Luider,T.M. *et al.* (2006) Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. *Proc. Natl. Acad. Sci. U. S. A*, **103**, 18597-18602.
32. Bennett,S.M., Neher,T.M., Shatilla,A. and Turchi,J.J. (2009) Molecular analysis of Ku redox regulation. *BMC. Mol. Biol.*, **10**, 86.
33. Yano,K., Morotomi-Yano,K., Adachi,N. and Akiyama,H. (2009) Molecular mechanism of protein assembly on DNA double-strand breaks in the non-homologous end-joining pathway. *J. Radiat. Res. (Tokyo)*, **50**, 97-108.
34. Hammel,M., Yu,Y., Mahaney,B.L., Cai,B., Ye,R., Phipps,B.M., Rambo,R.P., Hura,G.L., Pelikan,M., So,S. *et al.* (2010) Ku and DNA-dependent protein kinase dynamic conformations and assembly regulate DNA binding and the initial non-homologous end joining complex. *J. Biol. Chem.*, **285**, 1414-1423.
35. Sibanda,B.L., Chirgadze,D.Y. and Blundell,T.L. (2010) Crystal structure of DNA-PKcs reveals a large open-ring cradle comprised of HEAT repeats. *Nature*, **463**, 118-121.
36. Yaneva,M., Kowalewski,T. and Lieber,M.R. (1997) Interaction of DNA-dependent protein kinase with DNA and with Ku: biochemical and atomic-force microscopy studies. *EMBO J.*, **16**, 5098-5112.
37. DeFazio,L.G., Stansel,R.M., Griffith,J.D. and Chu,G. (2002) Synapsis of DNA ends by DNA-dependent protein kinase. *EMBO J.*, **21**, 3192-3200.
38. Mahaney,B.L., Meek,K. and Lees-Miller,S.P. (2009) Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochem. J.*, **417**, 639-650.
39. Weterings,E. and Chen,D.J. (2008) The endless tale of non-homologous end-joining.

Cell Res., **18**, 114-124.
40. Chen,L., Trujillo,K., Ramos,W., Sung,P. and Tomkinson,A.E. (2001) Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. *Mol. Cell*, **8**, 1105-1115.
41. Riha,K., Heacock,M.L. and Shippen,D.E. (2006) The role of the nonhomologous end-joining DNA double-strand break repair pathway in telomere biology. *Annu. Rev. Genet.*, **40**, 237-277.
42. Ma,Y., Pannicke,U., Schwarz,K. and Lieber,M.R. (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell*, **108**, 781-794.
43. Nick McElhinny,S.A. and Ramsden,D.A. (2004) Sibling rivalry: competition between Pol X family members in V(D)J recombination and general double strand break repair. *Immunol. Rev.*, **200**, 156-164.
44. Lieber,M.R. (2008) The mechanism of human nonhomologous DNA end joining. *J. Biol. Chem.*, **283**, 1-5.
45. Chappell,C., Hanakahi,L.A., Karimi-Busheri,F., Weinfeld,M. and West,S.C. (2002) Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. *EMBO J.*, **21**, 2827-2832.
46. Daley,J.M., Palmbos,P.L., Wu,D. and Wilson,T.E. (2005) Nonhomologous end joining in yeast. *Annu. Rev. Genet.*, **39**, 431-451.
47. Daley,J.M. and Wilson,T.E. (2008) Evidence that base stacking potential in annealed 3' overhangs determines polymerase utilization in yeast nonhomologous end joining. *DNA Repair (Amst)*, **7**, 67-76.
48. Vance,J.R. and Wilson,T.E. (2001) Uncoupling of 3'-phosphatase and 5'-kinase functions in budding yeast. Characterization of Saccharomyces cerevisiae DNA 3'-phosphatase (TPP1). *J. Biol. Chem.*, **276**, 15073-15081.
49. Bahmed,K., Nitiss,K.C. and Nitiss,J.L. (2010) Yeast Tdp1 regulates the fidelity of nonhomologous end joining. *Proc. Natl. Acad. Sci. U. S. A*, **107**, 4057-4062.
50. Buck,D., Malivert,L., de,C.R., Barraud,A., Fondaneche,M.C., Sanal,O., Plebani,A., Stephan,J.L., Hufnagel,M., le,D.F. *et al.* (2006) Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell*, **124**, 287-299.
51. Ahnesorg,P., Smith,P. and Jackson,S.P. (2006) XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell*, **124**, 301-313.
52. Recuero-Checa,M.A., Dore,A.S., rias-Palomo,E., Rivera-Calzada,A., Scheres,S.H., Maman,J.D., Pearl,L.H. and Llorca,O. (2009) Electron microscopy of Xrcc4 and the DNA ligase IV-Xrcc4 DNA repair complex. *DNA Repair (Amst)*, **8**, 1380-1389.
53. Costantini,S., Woodbine,L., Andreoli,L., Jeggo,P.A. and Vindigni,A. (2007) Interaction of the Ku heterodimer with the DNA ligase IV/Xrcc4 complex and its regulation by DNA-PK. *DNA Repair (Amst)*, **6**, 712-722.
54. Sibanda,B.L., Critchlow,S.E., Begun,J., Pei,X.Y., Jackson,S.P., Blundell,T.L. and Pellegrini,L. (2001) Crystal structure of an Xrcc4-DNA ligase IV complex. *Nat. Struct. Biol.*, **8**, 1015-1019.
55. Li,Y., Chirgadze,D.Y., Bolanos-Garcia,V.M., Sibanda,B.L., Davies,O.R., Ahnesorg,P., Jackson,S.P. and Blundell,T.L. (2008) Crystal structure of human XLF/Cernunnos reveals unexpected differences from XRCC4 with implications for NHEJ. *EMBO J.*, **27**, 290-300.
56. Riballo,E., Woodbine,L., Stiff,T., Walker,S.A., Goodarzi,A.A. and Jeggo,P.A. (2009) XLF-Cernunnos promotes DNA ligase IV-XRCC4 re-adenylation following ligation. *Nucleic Acids Res.*, **37**, 482-492.
57. Yu,Y., Mahaney,B.L., Yano,K., Ye,R., Fang,S., Douglas,P., Chen,D.J. and Lees-

Miller,S.P. (2008) DNA-PK and ATM phosphorylation sites in XLF/Cernunnos are not required for repair of DNA double strand breaks. *DNA Repair (Amst)*, **7**, 1680-1692.
58. Yu,Y., Wang,W., Ding,Q., Ye,R., Chen,D., Merkle,D., Schriemer,D., Meek,K. and Lees-Miller,S.P. (2003) DNA-PK phosphorylation sites in XRCC4 are not required for survival after radiation or for V(D)J recombination. *DNA Repair (Amst)*, **2**, 1239-1252.
59. Bonatto,D., Brendel,M. and Henriques,J.A. (2005) *In silico* identification and analysis of new Artemis/Artemis-like sequences from fungal and metazoan species. *Protein J.*, **24**, 399-411.
60. Uchiyama,Y., Kimura,S., Yamamoto,T., Ishibashi,T. and Sakaguchi,K. (2004) Plant DNA polymerase lambda, a DNA repair enzyme that functions in plant meristematic and meiotic tissues. *Eur. J. Biochem.*, **271**, 2799-2807.
61. Nussenzweig,A. and Nussenzweig,M.C. (2007) A backup DNA repair pathway moves to the forefront. *Cell*, **131**, 223-225.
62. Wang,H., Perrault,A.R., Takeda,Y., Qin,W., Wang,H. and Iliakis,G. (2003) Biochemical evidence for Ku-independent backup pathways of NHEJ. *Nucleic Acids Res.*, **31**, 5377-5388.
63. Wachsberger,P.R., Li,W.H., Guo,M., Chen,D., Cheong,N., Ling,C.C., Li,G. and Iliakis,G. (1999) Rejoining of DNA double-strand breaks in Ku80-deficient mouse fibroblasts. *Radiat. Res.*, **151**, 398-407.
64. DiBiase,S.J., Zeng,Z.C., Chen,R., Hyslop,T., Curran,W.J., Jr. and Iliakis,G. (2000) DNA-dependent protein kinase stimulates an independently active, nonhomologous, end-joining apparatus. *Cancer Res.*, **60**, 1245-1253.
65. Verkaik,N.S., Esveldt-van Lange,R.E., van Heemst,D., Bruggenwirth,H.T., Hoeijmakers,J.H., Zdzienicka,M.Z. and van Gent,D.C. (2002) Different types of V(D)J recombination and end-joining defects in DNA double-strand break repair mutant mammalian cells. *Eur. J. Immunol.*, **32**, 701-709.
66. Wang,H., Zeng,Z.C., Perrault,A.R., Cheng,X., Qin,W. and Iliakis,G. (2001) Genetic evidence for the involvement of DNA ligase IV in the DNA-PK-dependent pathway of non-homologous end joining in mammalian cells. *Nucleic Acids Res.*, **29**, 1653-1660.
67. Kuhfittig-Kulle,S., Feldmann,E., Odersky,A., Kuliczkowska,A., Goedecke,W., Eggert,A. and Pfeiffer,P. (2007) The mutagenic potential of non-homologous end joining in the absence of the NHEJ core factors Ku70/80, DNA-PKcs and XRCC4-LigIV. *Mutagenesis*, **22**, 217-233.
68. Karanjawala,Z.E., Grawunder,U., Hsieh,C.L. and Lieber,M.R. (1999) The nonhomologous DNA end joining pathway is important for chromosome stability in primary fibroblasts. *Curr. Biol.*, **9**, 1501-1504.
69. Ferguson,D.O., Sekiguchi,J.M., Chang,S., Frank,K.M., Gao,Y., DePinho,R.A. and Alt,F.W. (2000) The nonhomologous end-joining pathway of DNA repair is required for genomic stability and the suppression of translocations. *Proc. Natl. Acad. Sci. U. S. A*, **97**, 6630-6633.
70. Lieber,M.R. (2010) NHEJ and its backup pathways in chromosomal translocations. *Nat. Struct. Mol. Biol.*, **17**, 393-395.
71. Cheong,N., Perrault,A.R., Wang,H., Wachsberger,P., Mammen,P., Jackson,I. and Iliakis,G. (1999) DNA-PK-independent rejoining of DNA double-strand breaks in human cell extracts in vitro. *Int. J. Radiat. Biol.*, **75**, 67-81.
72. Feldmann,E., Schmiemann,V., Goedecke,W., Reichenberger,S. and Pfeiffer,P. (2000) DNA double-strand break repair in cell-free extracts from Ku80-deficient cells: implications for Ku serving as an alignment factor in non-homologous DNA end joining. *Nucleic Acids Res.*, **28**, 2585-2596.
73. Simsek,D. and Jasin,M. (2010) Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. *Nat.*

Struct. Mol. Biol., **17**, 410-416.
74. Fattah,F., Lee,E.H., Weisensel,N., Wang,Y., Lichter,N. and Hendrickson,E.A. (2010) Ku regulates the non-homologous end joining pathway choice of DNA double-strand break repair in human somatic cells. *PLoS. Genet.*, **6**, e1000855.
75. McVey,M. and Lee,S.E. (2008) MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. *Trends Genet.*, **24**, 529-538.
76. Iliakis,G. (2009) Backup pathways of NHEJ in cells of higher eukaryotes: cell cycle dependence. *Radiother. Oncol.*, **92**, 310-315.
77. Liang,L., Deng,L., Nguyen,S.C., Zhao,X., Maulion,C.D., Shao,C. and Tischfield,J.A. (2008) Human DNA ligases I and III, but not ligase IV, are required for microhomology-mediated end joining of DNA double-strand breaks. *Nucleic Acids Res.*, **36**, 3297-3310.
78. Rosidi,B., Wang,M., Wu,W., Sharma,A., Wang,H. and Iliakis,G. (2008) Histone H1 functions as a stimulatory factor in backup pathways of NHEJ. *Nucleic Acids Res.*, **36**, 1610-1623.
79. Downs,J.A., Kosmidou,E., Morgan,A. and Jackson,S.P. (2003) Suppression of homologous recombination by the *Saccharomyces cerevisiae* linker histone. *Mol. Cell*, **11**, 1685-1692.
80. Kysela,B., Chovanec,M. and Jeggo,P.A. (2005) Phosphorylation of linker histones by DNA-dependent protein kinase is required for DNA ligase IV-dependent ligation in the presence of histone H1. *Proc. Natl. Acad. Sci. U. S. A*, **102**, 1877-1882.
81. Thomas,J.O., Rees,C. and Finch,J.T. (1992) Cooperative binding of the globular domains of histones H1 and H5 to DNA. *Nucleic Acids Res.*, **20**, 187-194.
82. Schreiber,V., Dantzer,F., Ame,J.C. and de Murcia,G. (2006) Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol.*, **7**, 517-528.
83. Woodhouse,B.C. and Dianov,G.L. (2008) Poly ADP-ribose polymerase-1: an international molecule of mystery. *DNA Repair (Amst)*, **7**, 1077-1086.
84. Woodhouse,B.C., Dianova,I.I., Parsons,J.L. and Dianov,G.L. (2008) Poly(ADP-ribose) polymerase-1 modulates DNA repair capacity and prevents formation of DNA double strand breaks. *DNA Repair (Amst)*, **7**, 932-940.
85. Audebert,M., Salles,B. and Calsou,P. (2004) Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. *J. Biol. Chem.*, **279**, 55117-55126.
86. Robert,I., Dantzer,F. and Reina-San-Martin,B. (2009) Parp1 facilitates alternative NHEJ, whereas Parp2 suppresses IgH/c-myc translocations during immunoglobulin class switch recombination. *J. Exp. Med.*, **206**, 1047-1056.
87. Wang,M., Wu,W., Wu,W., Rosidi,B., Zhang,L., Wang,H. and Iliakis,G. (2006) PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. *Nucleic Acids Res.*, **34**, 6170-6182.
88. Yelamos,J., Schreiber,V. and Dantzer,F. (2008) Toward specific functions of poly(ADP-ribose) polymerase-2. *Trends Mol. Med.*, **14**, 169-178.
89. Sukhanova,M., Khodyreva,S. and Lavrik,O. (2010) Poly(ADP-ribose) polymerase 1 regulates activity of DNA polymerase beta in long patch base excision repair. *Mutat. Res.*, **685**, 80-89.
90. Bryant,H.E., Petermann,E., Schultz,N., Jemth,A.S., Loseva,O., Issaeva,N., Johansson,F., Fernandez,S., McGlynn,P. and Helleday,T. (2009) PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. *EMBO J.*, **28**, 2601-2615.
91. Okano,S., Lan,L., Tomkinson,A.E. and Yasui,A. (2005) Translocation of XRCC1 and DNA ligase IIIalpha from centrosomes to chromosomes in response to DNA damage in mitotic human cells. *Nucleic Acids Res.*, **33**, 422-429.
92. Caldecott,K.W., Aoufouchi,S., Johnson,P. and Shall,S. (1996) XRCC1 polypeptide

interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. *Nucleic Acids Res.*, **24**, 4387-4394.

93. Cappelli,E., Taylor,R., Cevasco,M., Abbondandolo,A., Caldecott,K. and Frosina,G. (1997) Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. *J. Biol. Chem.*, **272**, 23970-23975.
94. Wang,H., Rosidi,B., Perrault,R., Wang,M., Zhang,L., Windhofer,F. and Iliakis,G. (2005) DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. *Cancer Res.*, **65**, 4020-4030.
95. Caldecott,K.W., McKeown,C.K., Tucker,J.D., Ljungquist,S. and Thompson,L.H. (1994) An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. *Mol. Cell Biol.*, **14**, 68-76.
96. Ellenberger,T. and Tomkinson,A.E. (2008) Eukaryotic DNA ligases: structural and functional insights. *Annu. Rev. Biochem.*, **77**, 313-338.
97. Decottignies,A. (2007) Microhomology-mediated end joining in fission yeast is repressed by pku70 and relies on genes involved in homologous recombination. *Genetics*, **176**, 1403-1415.
98. Tao,Z., Gao,P. and Liu,H.W. (2009) Studies of the expression of human poly(ADP-ribose) polymerase-1 in *Saccharomyces cerevisiae* and identification of PARP-1 substrates by yeast proteome microarray screening. *Biochemistry*, **48**, 11745-11754.
99. Babiychuk,E., Cottrill,P.B., Storozhenko,S., Fuangthong,M., Chen,Y., O'Farrell,M.K., Van,M.M., Inze,D. and Kushnir,S. (1998) Higher plants possess two structurally different poly(ADP-ribose) polymerases. *Plant J.*, **15**, 635-645.
100. West,C.E., Waterworth,W.M., Sunderland,P.A. and Bray,C.M. (2004) Arabidopsis DNA double-strand break repair pathways. *Biochem. Soc. Trans.*, **32**, 964-966.
101. Diego Bonatto, Martin Brendel and Joao Antonio Pegas Henrique (2005) A new group of plant-specific ADP-dependent DNA ligases identified by protein phylogeny, hydrophobic cluster and 3-dimensional modelling. *Functional Plant Biology*, **32**, 161-174.
102. Waterworth,W.M., Masnavi,G., Bhardwaj,R.M., Jiang,Q., Bray,C.M. and West,C.E. (2010) A plant DNA ligase is an important determinant of seed longevity. *Plant J.*, **63**, 848-860.
103. Siede,W., Friedl,A.A., Dianova,I., Eckardt-Schupp,F. and Friedberg,E.C. (1996) The *Saccharomyces cerevisiae* Ku autoantigen homologue affects radiosensitivity only in the absence of homologous recombination. *Genetics*, **142**, 91-102.
104. Shrivastav,M., De Haro,L.P. and Nickoloff,J.A. (2008) Regulation of DNA double-strand break repair pathway choice. *Cell Res.*, **18**, 134-147.
105. Yamazoe,M., Sonoda,E., Hochegger,H. and Takeda,S. (2004) Reverse genetic studies of the DNA damage response in the chicken B lymphocyte line DT40. *DNA Repair (Amst)*, **3**, 1175-1185.
106. Sonoda,E., Hochegger,H., Saberi,A., Taniguchi,Y. and Takeda,S. (2006) Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. *DNA Repair (Amst)*, **5**, 1021-1029.
107. Orii,K.E., Lee,Y., Kondo,N. and McKinnon,P.J. (2006) Selective utilization of nonhomologous end-joining and homologous recombination DNA repair pathways during nervous system development. *Proc. Natl. Acad. Sci. U. S. A*, **103**, 10017-10022.
108. Boyko,A., Zemp,F., Filkowski,J. and Kovalchuk,I. (2006) Double-strand break repair in plants is developmentally regulated. *Plant Physiol*, **141**, 488-497.
109. Aylon,Y. and Kupiec,M. (2005) Cell cycle-dependent regulation of double-strand break repair: a role for the CDK. *Cell Cycle*, **4**, 259-261.
110. Limbo,O., Chahwan,C., Yamada,Y., de Bruin,R.A., Wittenberg,C. and Russell,P. (2007)

Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. *Mol. Cell*, **28**, 134-146.
111. Yun,M.H. and Hiom,K. (2009) CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature*, **459**, 460-463.
112. Huertas,P., Cortes-Ledesma,F., Sartori,A.A., Aguilera,A. and Jackson,S.P. (2008) CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature*, **455**, 689-692.
113. Sartori,A.A., Lukas,C., Coates,J., Mistrik,M., Fu,S., Bartek,J., Baer,R., Lukas,J. and Jackson,S.P. (2007) Human CtIP promotes DNA end resection. *Nature*, **450**, 509-514.
114. Esashi,F., Christ,N., Gannon,J., Liu,Y., Hunt,T., Jasin,M. and West,S.C. (2005) CDK-dependent phosphorylation of BRCA2 as a regulatory mechanism for recombinational repair. *Nature*, **434**, 598-604.
115. Galande,S. and Kohwi-Shigematsu,T. (1999) Poly(ADP-ribose) polymerase and Ku autoantigen form a complex and synergistically bind to matrix attachment sequences. *J. Biol. Chem.*, **274**, 20521-20528.
116. Hochegger,H., Dejsuphong,D., Fukushima,T., Morrison,C., Sonoda,E., Schreiber,V., Zhao,G.Y., Saberi,A., Masutani,M., Adachi,N. *et al.* (2006) Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells. *EMBO J.*, **25**, 1305-1314.
117. Saberi,A., Hochegger,H., Szuts,D., Lan,L., Yasui,A., Sale,J.E., Taniguchi,Y., Murakawa,Y., Zeng,W., Yokomori,K. *et al.* (2007) RAD18 and poly(ADP-ribose) polymerase independently suppress the access of nonhomologous end joining to double-strand breaks and facilitate homologous recombination-mediated repair. *Mol. Cell Biol.*, **27**, 2562-2571.
118. Wu,W., Wang,M., Wu,W., Singh,S.K., Mussfeldt,T. and Iliakis,G. (2008) Repair of radiation induced DNA double strand breaks by backup NHEJ is enhanced in G2. *DNA Repair (Amst)*, **7**, 329-338.
119. Adachi,N., So,S., Iiizumi,S., Nomura,Y., Murai,K., Yamakawa,C., Miyagawa,K. and Koyama,H. (2006) The human pre-B cell line Nalm-6 is highly proficient in gene targeting by homologous recombination. *DNA Cell Biol.*, **25**, 19-24.
120. Capecchi,M.R. (2005) Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat. Rev. Genet.*, **6**, 507-512.
121. Mansour,S.L., Thomas,K.R. and Capecchi,M.R. (1988) Disruption of the proto-oncogene *int-2* in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature*, **336**, 348-352.
122. Yagi,T., Ikawa,Y., Yoshida,K., Shigetani,Y., Takeda,N., Mabuchi,I., Yamamoto,T. and Aizawa,S. (1990) Homologous recombination at *c-fyn* locus of mouse embryonic stem cells with use of diphtheria toxin A-fragment gene in negative selection. *Proc. Natl. Acad. Sci. U. S. A*, **87**, 9918-9922.
123. Czako,M. and Marton,L. (1994) The herpes simplex virus thymidine kinase gene as a conditional negative-selection marker gene in *Arabidopsis thaliana*. *Plant Physiol*, **104**, 1067-1071.
124. Czako,M. and An,G. (1991) Expression of DNA coding for diphtheria toxin chain a is toxic to plant cells. *Plant Physiol*, **95**, 687-692.
125. Terada,R., Johzuka-Hisatomi,Y., Saitoh,M., Asao,H. and Iida,S. (2007) Gene targeting by homologous recombination as a biotechnological tool for rice functional genomics. *Plant Physiol*, **144**, 846-856.
126. Deng,C. and Capecchi,M.R. (1992) Reexamination of gene targeting frequency as a function of the extent of homology between the targeting vector and the target locus. *Mol. Cell Biol.*, **12**, 3365-3371.
127. Thomas,K.R., Deng,C. and Capecchi,M.R. (1992) High-fidelity gene targeting in

embryonic stem cells by using sequence replacement vectors. *Mol. Cell Biol.*, **12**, 2919-2923.
128. Dominguez-Bendala,J., Priddle,H., Clarke,A. and McWhir,J. (2003) Elevated expression of exogenous Rad51 leads to identical increases in gene-targeting frequency in murine embryonic stem (ES) cells with both functional and dysfunctional p53 genes. *Exp. Cell Res.*, **286**, 298-307.
129. Di Primio,C., Galli,A., Cervelli,T., Zoppe,M. and Rainaldi,G. (2005) Potentiation of gene targeting in human cells by expression of *Saccharomyces cerevisiae* Rad52. *Nucleic Acids Res.*, **33**, 4639-4648.
130. Kalvala,A., Rainaldi,G., Di Primio,C., Liverani,V., Falaschi,A. and Galli,A. (2010) Enhancement of gene targeting in human cells by intranuclear permeation of the *Saccharomyces cerevisiae* Rad52 protein. *Nucleic Acids Res.*, **38**, e149.
131. Shaked,H., Melamed-Bessudo,C. and Levy,A.A. (2005) High-frequency gene targeting in Arabidopsis plants expressing the yeast RAD54 gene. *Proc. Natl. Acad. Sci. U. S. A*, **102**, 12265-12269.
132. Shcherbakova,O.G., Lanzov,V.A., Ogawa,H. and Filatov,M.V. (2000) Overexpression of bacterial RecA protein stimulates homologous recombination in somatic mammalian cells. *Mutat. Res.*, **459**, 65-71.
133. Reiss,B., Klemm,M., Kosak,H. and Schell,J. (1996) RecA protein stimulates homologous recombination in plants. *Proc. Natl. Acad. Sci. U. S. A*, **93**, 3094-3098.
134. van Attikum,H. and Hooykaas,P.J. (2003) Genetic requirements for the targeted integration of *Agrobacterium* T-DNA in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **31**, 826-832.
135. Essers,J., Hendriks,R.W., Swagemakers,S.M., Troelstra,C., de,W.J., Bootsma,D., Hoeijmakers,J.H. and Kanaar,R. (1997) Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell*, **89**, 195-204.
136. Bezzubova,O., Silbergleit,A., Yamaguchi-Iwai,Y., Takeda,S. and Buerstedde,J.M. (1997) Reduced X-ray resistance and homologous recombination frequencies in a RAD54-/- mutant of the chicken DT40 cell line. *Cell*, **89**, 185-193.
137. van Attikum,H., Bundock,P. and Hooykaas,P.J. (2001) Non-homologous end-joining proteins are required for *Agrobacterium* T-DNA integration. *EMBO J.*, **20**, 6550-6558.
138. Krappmann,S., Sasse,C. and Braus,G.H. (2006) Gene targeting in *Aspergillus fumigatus* by homologous recombination is facilitated in a nonhomologous end- joining-deficient genetic background. *Eukaryot. Cell*, **5**, 212-215.
139. Ninomiya,Y., Suzuki,K., Ishii,C. and Inoue,H. (2004) Highly efficient gene replacements in Neurospora strains deficient for nonhomologous end-joining. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 12248-12253.
140. Takahashi,T., Masuda,T. and Koyama,Y. (2006) Enhanced gene targeting frequency in ku70 and ku80 disruption mutants of *Aspergillus sojae* and *Aspergillus oryzae*. *Mol. Genet. Genomics*, **275**, 460-470.
141. Nielsen,J.B., Nielsen,M.L. and Mortensen,U.H. (2008) Transient disruption of non-homologous end-joining facilitates targeted genome manipulations in the filamentous fungus *Aspergillus nidulans*. *Fungal. Genet. Biol.*, **45**, 165-170.
142. Villalba,F., Collemare,J., Landraud,P., Lambou,K., Brozek,V., Cirer,B., Morin,D., Bruel,C., Beffa,R. and Lebrun,M.H. (2008) Improved gene targeting in Magnaporthe grisea by inactivation of MgKU80 required for non-homologous end joining. *Fungal. Genet. Biol.*, **45**, 68-75.
143. Schorsch,C., Kohler,T. and Boles,E. (2009) Knockout of the DNA ligase IV homolog gene in the sphingoid base producing yeast Pichia ciferrii significantly increases gene targeting efficiency. *Curr. Genet.*, **55**, 381-389.
144. Abdel-Banat,B.M., Nonklang,S., Hoshida,H. and Akada,R. (2010) Random and targeted

gene integrations through the control of non-homologous end joining in the yeast *Kluyveromyces marxianus*. *Yeast*, **27**, 29-39.
145. Kooistra,R., Hooykaas,P.J. and Steensma,H.Y. (2004) Efficient gene targeting in *Kluyveromyces lactis*. *Yeast*, **21**, 781-792.
146. Fattah,F.J., Lichter,N.F., Fattah,K.R., Oh,S. and Hendrickson,E.A. (2008) Ku70, an essential gene, modulates the frequency of rAAV-mediated gene targeting in human somatic cells. *Proc. Natl. Acad. Sci. U. S. A*, **105**, 8703-8708.
147. Bertolini,L.R., Bertolini,M., Maga,E.A., Madden,K.R. and Murray,J.D. (2009) Increased gene targeting in Ku70 and Xrcc4 transiently deficient human somatic cells. *Mol. Biotechnol.*, **41**, 106-114.
148. Iiizumi,S., Kurosawa,A., So,S., Ishii,Y., Chikaraishi,Y., Ishii,A., Koyama,H. and Adachi,N. (2008) Impact of non-homologous end-joining deficiency on random and targeted DNA integration: implications for gene targeting. *Nucleic Acids Res.*, **36**, 6333-6342.
149. Dominguez-Bendala,J., Masutani,M. and McWhir,J. (2006) Down-regulation of PARP-1, but not of Ku80 or DNA-PKcs', results in higher gene targeting efficiency. *Cell Biol. Int.*, **30**, 389-393.
150. van Attikum,H., Bundock,P., Overmeer,R.M., Lee,L.Y., Gelvin,S.B. and Hooykaas,P.J.J. (2003) The Arabidopsis AtLIG4 gene is required for the repair of DNA damage, but not for the integration of *Agrobacterium* T-DNA. *Nucleic Acids Res.*, **31**, 4247-4255.
151. Bundock,P., van Attikum,H. and Hooykaas,P.J.J. (2002) Increased telomere length and hypersensitivity to DNA damaging agents in an Arabidopsis KU70 mutant. *Nucleic Acids Res.*, **30**, 3395-3400.
152. West,C.E., Waterworth,W.M., Jiang,Q. and Bray,C.M. (2000) Arabidopsis DNA ligase IV is induced by gamma-irradiation and interacts with an Arabidopsis homologue of the double strand break repair protein XRCC4. *Plant J.*, **24**, 67-78.
153. West,C.E., Waterworth,W.M., Story,G.W., Sunderland,P.A., Jiang,Q. and Bray,C.M. (2002) Disruption of the Arabidopsis AtKu80 gene demonstrates an essential role for AtKu80 protein in efficient repair of DNA double-strand breaks in vivo. *Plant J.*, **31**, 517-528.
154. Friesner,J. and Britt,A.B. (2003) Ku80- and DNA ligase IV-deficient plants are sensitive to ionizing radiation and defective in T-DNA integration. *Plant J.*, **34**, 427-440.
155. Tanaka,S., Ishii,C., Hatakeyama,S. and Inoue,H. (2010) High efficient gene targeting on the AGAMOUS gene in an ArabidopsisAtLIG4 mutant. *Biochem. Biophys. Res. Commun.*, **396**, 289-293.
156. Paques,F. and Duchateau,P. (2007) Meganucleases and DNA double-strand break-induced recombination: perspectives for gene therapy. *Curr. Gene Ther.*, **7**, 49-66.
157. Kostriken,R., Strathern,J.N., Klar,A.J., Hicks,J.B. and Heffron,F. (1983) A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. *Cell*, **35**, 167-174.
158. Jacquier,A. and Dujon,B. (1985) An intron-encoded protein is active in a gene conversion process that spreads an intron into a mitochondrial gene. *Cell*, **41**, 383-394.
159. Haber,J.E. (2006) Transpositions and translocations induced by site-specific double-strand breaks in budding yeast. *DNA Repair (Amst)*, **5**, 998-1009.
160. D'Halluin,K., Vanderstraeten,C., Stals,E., Cornelissen,M. and Ruiter,R. (2008) Homologous recombination: a basis for targeted genome optimization in crop species such as maize. *Plant Biotechnol. J.*, **6**, 93-102.
161. Grizot,S., Epinat,J.C., Thomas,S., Duclert,A., Rolland,S., Paques,F. and Duchateau,P. (2010) Generation of redesigned homing endonucleases comprising DNA-binding domains derived from two different scaffolds. *Nucleic Acids Res.*, **38**, 2006-2018.
162. Weinthal,D., Tovkach,A., Zeevi,V. and Tzfira,T. (2010) Genome editing in plant cells by

zinc finger nucleases. *Trends Plant Sci.*, **15**, 308-321.
163. Cathomen,T. and Joung,J.K. (2008) Zinc-finger nucleases: the next generation emerges. *Mol. Ther.*, **16**, 1200-1207.
164. de Pater,S., Neuteboom,L.W., Pinas,J.E., Hooykaas,P.J. and van der Zaal,B.J. (2009) ZFN-induced mutagenesis and gene-targeting in Arabidopsis through *Agrobacterium*-mediated floral dip transformation. *Plant Biotechnol. J.*, **7**, 821-835.
165. Durai,S., Mani,M., Kandavelou,K., Wu,J., Porteus,M.H. and Chandrasegaran,S. (2005) Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. *Nucleic Acids Res.*, **33**, 5978-5990.
166. Bibikova,M., Golic,M., Golic,K.G. and Carroll,D. (2002) Targeted chromosomal cleavage and mutagenesis in Drosophila using zinc-finger nucleases. *Genetics*, **161**, 1169-1175.
167. Porteus,M.H. (2006) Mammalian gene targeting with designed zinc finger nucleases. *Mol. Ther.*, **13**, 438-446.
168. Szczepek,M., Brondani,V., Buchel,J., Serrano,L., Segal,D.J. and Cathomen,T. (2007) Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. *Nat. Biotechnol.*, **25**, 786-793.
169. Daoudal-Cotterell,S., Gallego,M.E. and White,C.I. (2002) The plant Rad50-Mre11 protein complex. *FEBS Lett.*, **516**, 164-166.
170. Gallego,M.E., Jeanneau,M., Granier,F., Bouchez,D., Bechtold,N. and White,C.I. (2001) Disruption of the Arabidopsis RAD50 gene leads to plant sterility and MMS sensitivity. *Plant J.*, **25**, 31-41.
171. Bleuyard,J.Y., Gallego,M.E. and White,C.I. (2004) Meiotic defects in the Arabidopsis rad50 mutant point to conservation of the MRX complex function in early stages of meiotic recombination. *Chromosoma*, **113**, 197-203.
172. Puizina,J., Siroky,J., Mokros,P., Schweizer,D. and Riha,K. (2004) Mre11 deficiency in Arabidopsis is associated with chromosomal instability in somatic cells and Spo11-dependent genome fragmentation during meiosis. *Plant Cell*, **16**, 1968-1978.
173. Bundock,P. and Hooykaas,P.J.J. (2002) Severe developmental defects, hypersensitivity to DNA-damaging agents, and lengthened telomeres in Arabidopsis MRE11 mutants. *Plant Cell*, **14**, 2451-2462.
174. Uanschou,C., Siwiec,T., Pedrosa-Harand,A., Kerzendorfer,C., Sanchez-Moran,E., Novatchkova,M., Akimcheva,S., Woglar,A., Klein,F. and Schlogelhofer,P. (2007) A novel plant gene essential for meiosis is related to the human CtIP and the yeast COM1/SAE2 gene. *EMBO J.*, **26**, 5061-5070.
175. Doutriaux,M.P., Couteau,F., Bergounioux,C. and White,C. (1998) Isolation and characterisation of the RAD51 and DMC1 homologs from *Arabidopsis thaliana*. *Mol. Gen. Genet.*, **257**, 283-291.
176. Markmann-Mulisch,U., Wendeler,E., Zobell,O., Schween,G., Steinbiss,H.H. and Reiss,B. (2007) Differential requirements for RAD51 in *Physcomitrella patens* and *Arabidopsis thaliana* development and DNA damage repair. *Plant Cell*, **19**, 3080-3089.
177. Li,W., Chen,C., Markmann-Mulisch,U., Timofejeva,L., Schmelzer,E., Ma,H. and Reiss,B. (2004) The Arabidopsis AtRAD51 gene is dispensable for vegetative development but required for meiosis. *Proc. Natl. Acad. Sci. U. S. A*, **101**, 10596-10601.
178. Siaud,N., Dray,E., Gy,I., Gerard,E., Takvorian,N. and Doutriaux,M.P. (2004) Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. *EMBO J.*, **23**, 1392-1401.
179. Dray,E., Siaud,N., Dubois,E. and Doutriaux,M.P. (2006) Interaction between Arabidopsis Brca2 and its partners Rad51, Dmc1, and Dss1. *Plant Physiol*, **140**, 1059-1069.
180. Osakabe,K., Abe,K., Yoshioka,T., Osakabe,Y., Todoriki,S., Ichikawa,H., Hohn,B. and Toki,S. (2006) Isolation and characterization of the RAD54 gene from *Arabidopsis*

thaliana. *Plant J.*, **48**, 827-842.
181. Bleuyard,J.Y., Gallego,M.E., Savigny,F. and White,C.I. (2005) Differing requirements for the Arabidopsis Rad51 paralogs in meiosis and DNA repair. *Plant J.*, **41**, 533-545.
182. Osakabe,K., Abe,K., Yamanouchi,H., Takyuu,T., Yoshioka,T., Ito,Y., Kato,T., Tabata,S., Kurei,S., Yoshioka,Y. *et al.* (2005) Arabidopsis Rad51B is important for double-strand DNA breaks repair in somatic cells. *Plant Mol. Biol.*, **57**, 819-833.
183. Li,W., Yang,X., Lin,Z., Timofejeva,L., Xiao,R., Makaroff,C.A. and Ma,H. (2005) The AtRAD51C gene is required for normal meiotic chromosome synapsis and double-stranded break repair in Arabidopsis. *Plant Physiol*, **138**, 965-976.
184. Abe,K., Osakabe,K., Nakayama,S., Endo,M., Tagiri,A., Todoriki,S., Ichikawa,H. and Toki,S. (2005) Arabidopsis RAD51C gene is important for homologous recombination in meiosis and mitosis. *Plant Physiol*, **139**, 896-908.
185. Durrant,W.E., Wang,S. and Dong,X. (2007) Arabidopsis SNI1 and RAD51D regulate both gene transcription and DNA recombination during the defense response. *Proc. Natl. Acad. Sci. U. S. A*, **104**, 4223-4227.
186. Bleuyard,J.Y. and White,C.I. (2004) The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis. *EMBO J.*, **23**, 439-449.
187. Kosarev,P., Mayer,K.F. and Hardtke,C.S. (2002) Evaluation and classification of RING-finger domains encoded by the Arabidopsis genome. *Genome Biol.*, **3**, RESEARCH0016.
188. Block-Schmidt,A.S., Dukowic-Schulze,S., Wanieck,K., Reidt,W. and Puchta,H. (2010) BRCC36A is epistatic to BRCA1 in DNA crosslink repair and homologous recombination in *Arabidopsis thaliana*. *Nucleic Acids Res.*,**39**,146-154.
189. Hartung,F., Suer,S. and Puchta,H. (2007) Two closely related RecQ helicases have antagonistic roles in homologous recombination and DNA repair in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A*, **104**, 18836-18841.
190. Riha,K., Watson,J.M., Parkey,J. and Shippen,D.E. (2002) Telomere length deregulation and enhanced sensitivity to genotoxic stress in Arabidopsis mutants deficient in Ku70. *EMBO J.*, **21**, 2819-2826.
191. Tamura,K., Adachi,Y., Chiba,K., Oguchi,K. and Takahashi,H. (2002) Identification of Ku70 and Ku80 homologues in *Arabidopsis thaliana*: evidence for a role in the repair of DNA double-strand breaks. *Plant J.*, **29**, 771-781.
192. Molinier,J., Stamm,M.E. and Hohn,B. (2004) SNM-dependent recombinational repair of oxidatively induced DNA damage in *Arabidopsis thaliana*. *EMBO Rep.*, **5**, 994-999.
193. Lee,S.Y., Kim,H., Hwang,H.J., Jeong,Y.M., Na,S.H., Woo,J.C. and Kim,S.G. (2010) Identification of tyrosyl-DNA phosphodiesterase as a novel DNA damage repair enzyme in Arabidopsis. *Plant Physiol*, **154**, 1460-1469.
194. Doucet-Chabeaud,G., Godon,C., Brutesco,C., de Murcia,G. and Kazmaier,M. (2001) Ionising radiation induces the expression of PARP-1 and PARP-2 genes in Arabidopsis. *Mol. Genet. Genomics*, **265**, 954-963.
195. De Block,M., Verduyn,C., De Brouwer,D. and Cornelissen,M. (2005) Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *Plant J.*, **41**, 95-106.
196. Vanderauwera,S., De Block,M., Van de Steene,N., van de Cotte,B., Metzlaff,M. and Van Breusegem,F. (2007) Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction. *Proc. Natl. Acad. Sci. U. S. A*, **104**, 15150-15155.
197. Petrucco,S., Volpi,G., Bolchi,A., Rivetti,C. and Ottonello,S. (2002) A nick-sensing DNA 3'-repair enzyme from Arabidopsis. *J. Biol. Chem.*, **277**, 23675-23683.