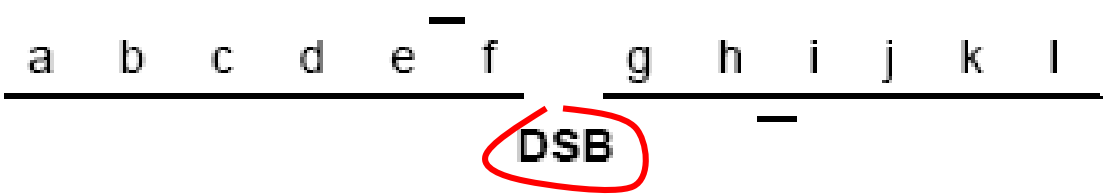
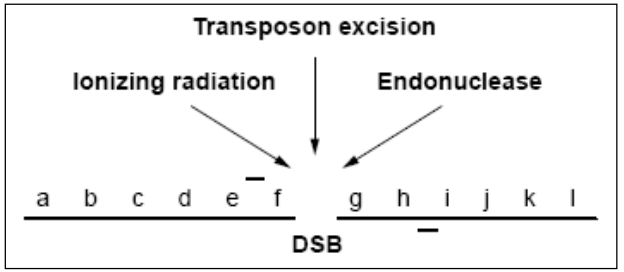
Double-strand breaks (DSBs), their repair and misrepair



How double-strand breaks are generated

DSBs are caused by several factors:

- arrest of replication and restart of DNA synthesis (replication forks tend to stall in regions of repeat elements - e.g. tRNA genes, retroposons, and telomeres); major source of DSBs!



- transposon excision
- during meiotic recombination (needed for recombination)
- mechanical pulling (e.g. in dicentric chromosomes)
- experimentally (radiation by X-rays, DSBs inducing chemicals, rare cutting restriction endonucleases, DNA transposons)

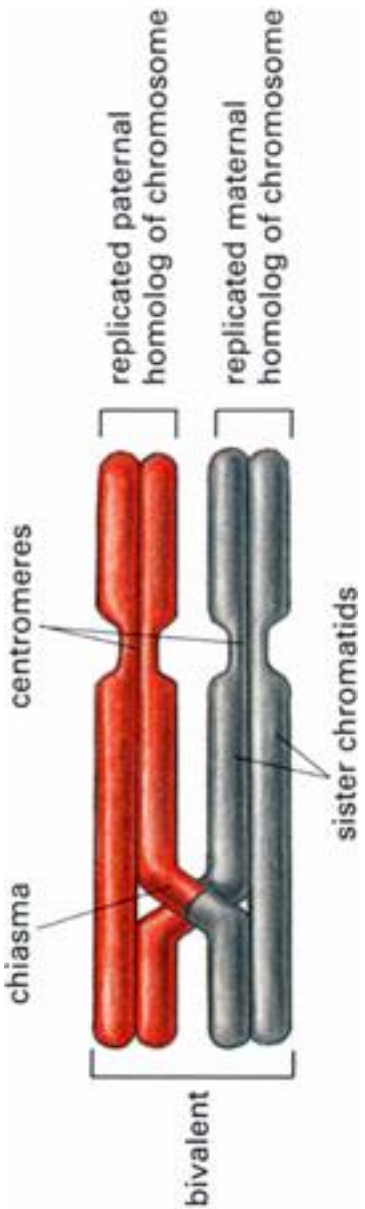
❖ in vegetative (mitosis) and generative cells (meiosis)

❖ DSBs have to be repaired before genomes are replicated (S phase)

❖ in plants, errors in DSB repair (DSBs misrepair) can have the evolutionary significance because changes in meristematic cells can be transferred to the offspring >>> **chromosome rearrangements**

DSBs in meiosis

- necessary for homologous recombination (cross-overs)
- induced by the Spo11 topoisomerase



DSBs in in somatic plant cells and their repair

DSBs repair

non-homologous end joining (NHEJ)

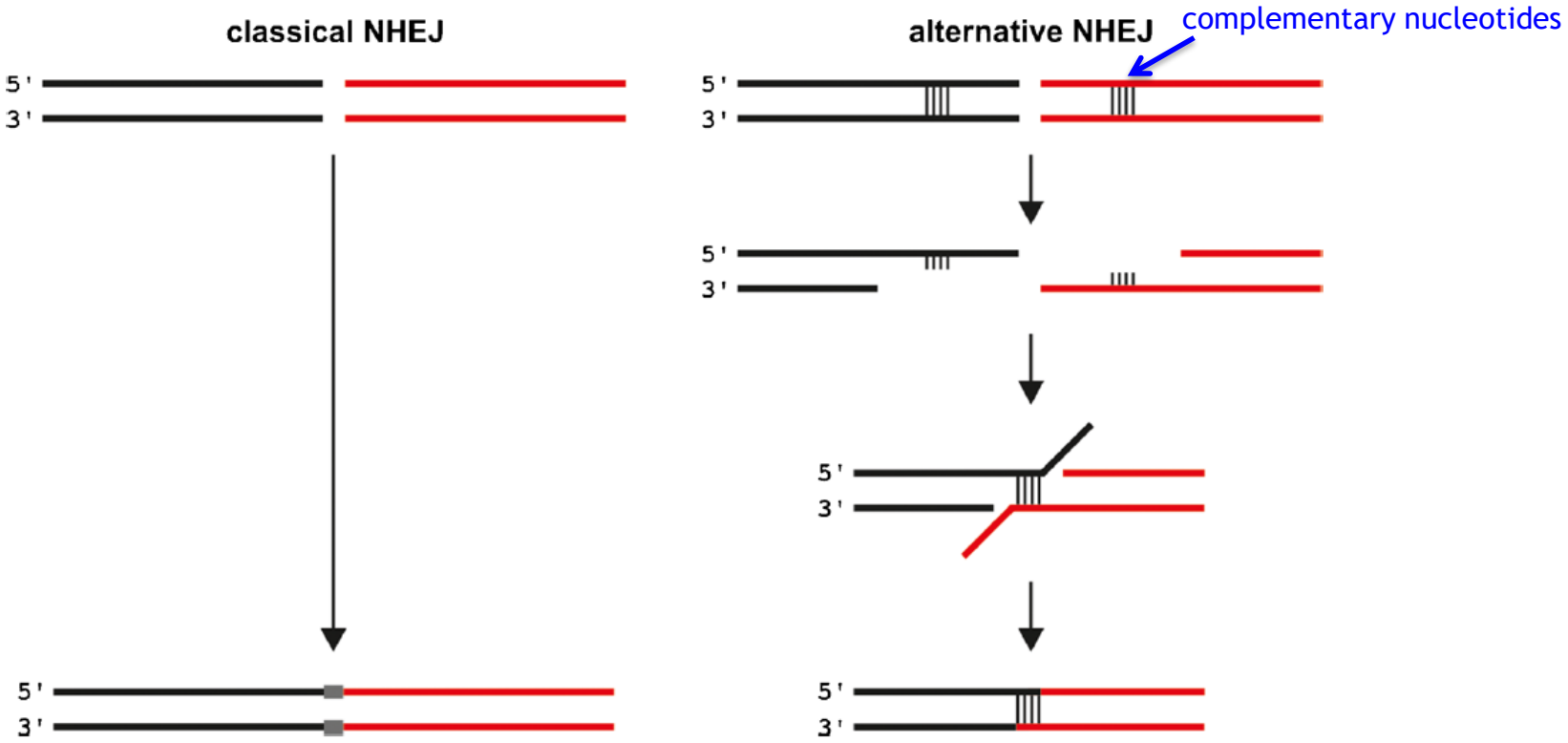
- also known as homology-independent recombination or **"illegitimate recombination"**
- **main mode of DSB repair** in higher eukaryotes (somatic plant cells)
- the broken ends are re-ligated directly
- often an error-prone process
- throughout the cell cycle (mainly G_1 phase)

homologous recombination (HR)

- after replication: uses sister chromatids as a template to rejoin DSBs (most frequently)
- **minor pathway**
- error-free repair
- in late S-G_2 phase

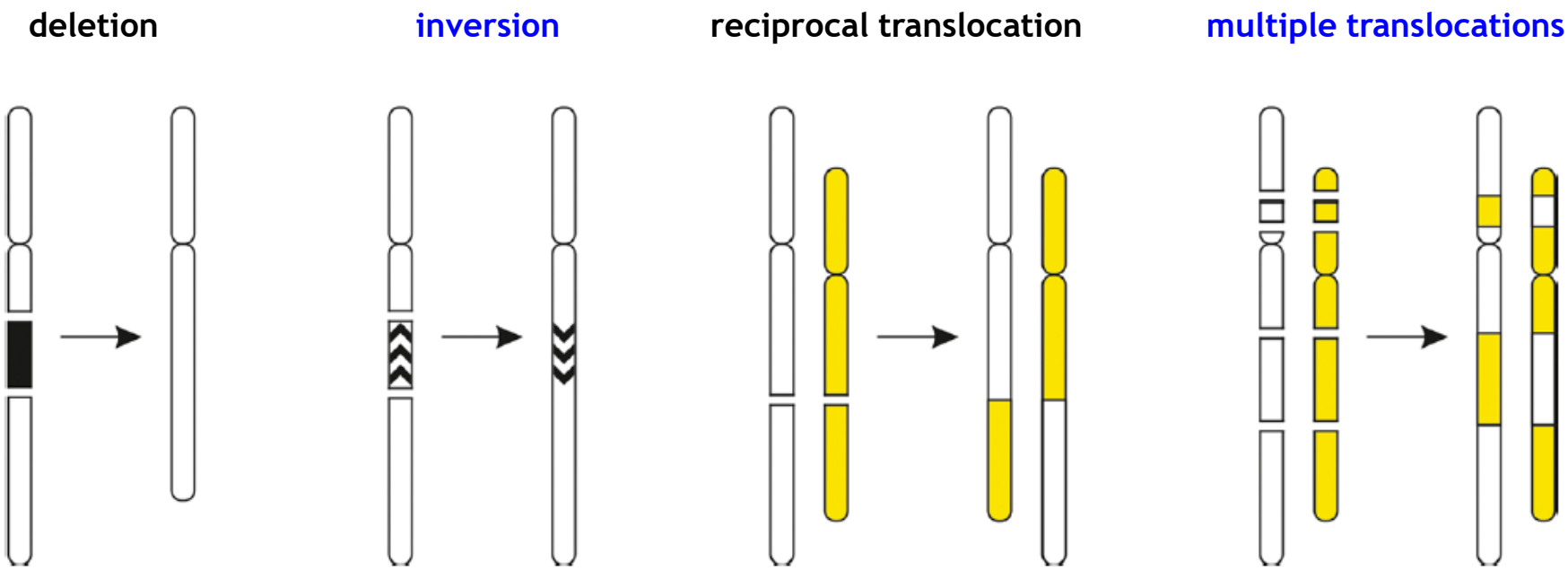
The two main pathways of non-homologous end joining (NHEJ) in plant cells

microhomology-mediated end joining (MMEJ)



DNA lost but some DNA can be inserted (filler DNA)

NHEJ-mediated rearrangements



NHEJ in plant somatic cells

- NHEJ seems to be the main mode of DSB repair in higher eukaryotes
- NHEJ might lead, in some cases, to genomic changes (deletions, insertions or various kinds of genomic rearrangements)
- genomic alterations in meristematic cells can be transferred to the offspring
- **alternative NHEJ can mediate genome size loss**

1C = 157 Mb

1C = 4.5 Gb

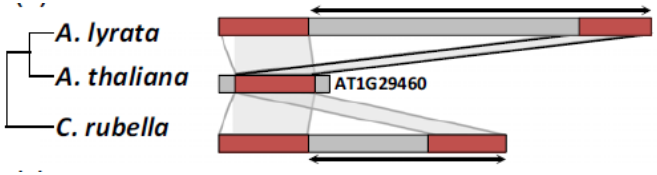
Arabidopsis vs. tobacco (genome size larger in tobacco)

- tobacco: almost every second deletion event is accompanied by the insertion of filler sequence
- Arabidopsis: no insertions
- overall length of the deletions is about one-third shorter in tobacco than in Arabidopsis

>>> inverse correlation between genome size and the medium length of deletions

>>> ??? species-specific differences in DSB repair pathways can contribute to the evolution of eukaryotic genome size ???

- *A. thaliana* (157 Mb) has lost **6**× more introns than *Arabidopsis lyrata* (210 Mb) since the divergence of the two species but gained very few introns

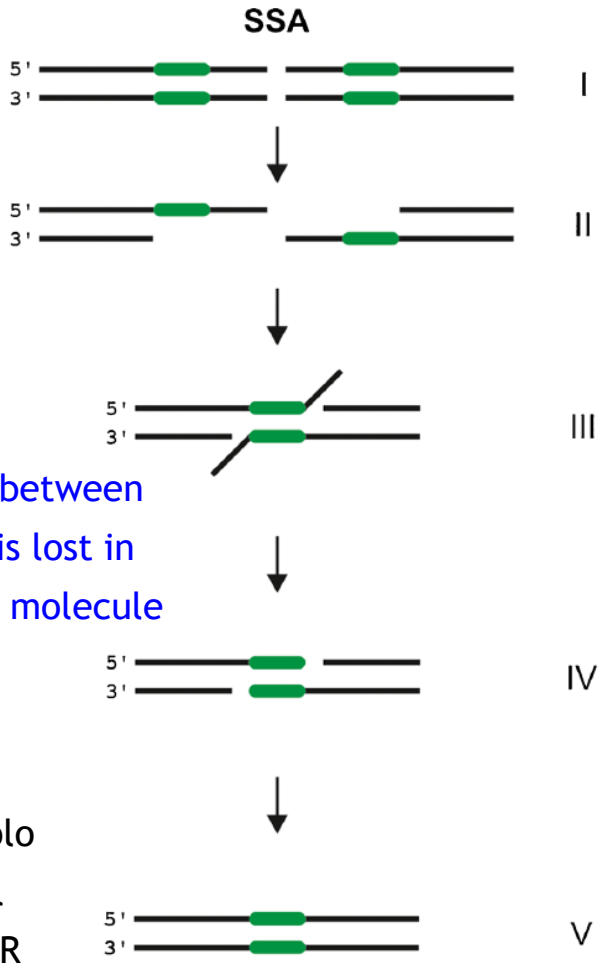


Homologous recombination

The two main pathways of **homologous recombination** in somatic plant cells

relevant term:
gene conversion

single-strand annealing



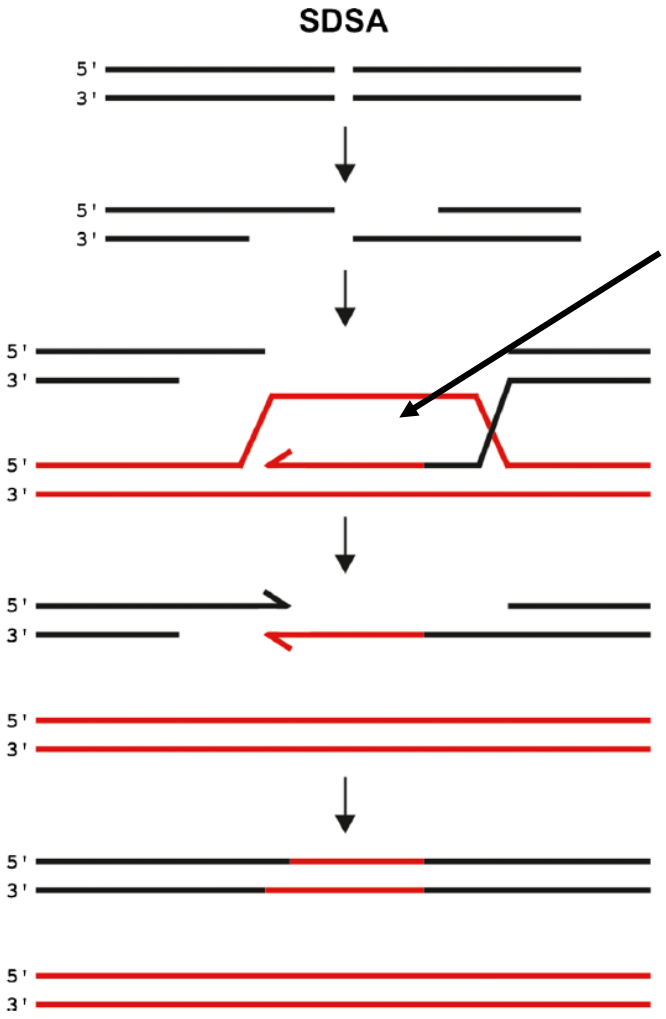
Deletions!

all sequence information between the repeats is lost in the rejoined molecule

DNA loss

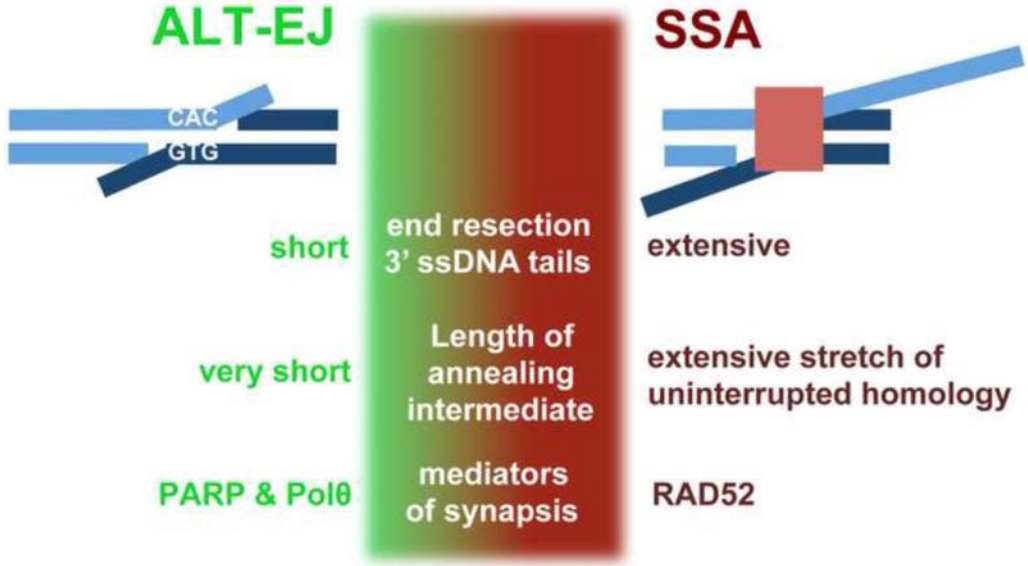
(evidence: solo long terminal repeats of LTR retrotransposons) → **genome size decrease**

synthesis-dependent strand annealing (template needed)



Displacement (D-) loop OR double Holliday junction (not shown)

a restored dsDNA molecule without changing sequence of the donor locus



key distinctions between ALT-EJ and SSA, with diagrams of the annealed/synapsed intermediate for each pathway. Distinct features include the extent of end resection involved and hence the length of 3′ ssDNA that is cleaved during processing of this intermediate, the length of the homologous repeat involved in the annealing, and the relative role of PARP and Polθ *versus* RAD52. The precise boundaries of these features that define ALT-EJ *versus* SSA remain unclear. Indeed, we represent the possibility that these features exist along a spectrum with a middle region that could potentially be repaired by either pathway. For example, it is conceivable that intermediate lengths of annealed homology are repaired by either ALT-EJ or SSA (i.e. mediated by PARP and Polθ, or RAD52). Similarly, a moderate extent of resection could be sufficient to reveal such intermediate lengths of flanking homology. However, it is also possible one or more of these features have defined boundaries that distinguish repair by ALT-EJ *versus* SSA.

Bhargava R, Onyango DO, Stark JM. Regulation of Single-Strand Annealing and its Role in Genome Maintenance. *Trends Genet*. 2016;32(9):566–575. doi:10.1016/j.tig.2016.06.007

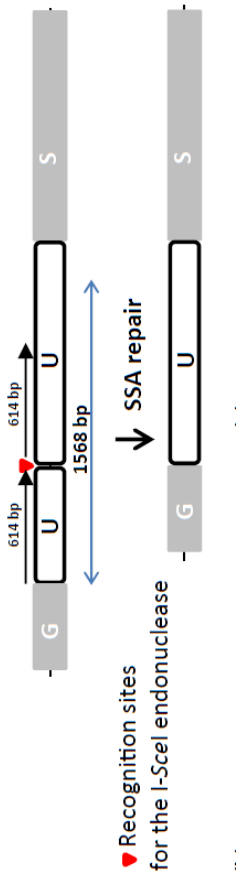
HR: single-strand annealing (SSA)

The role in genome size increase/decrease.

1C = **157 Mb**

1C = **5 500 Mb**

DSBs experimentally induced >>> DSB repair



A. thaliana: deletion size and frequency considerably larger (than in barley)

HR via SSA (and NHEJ) can contribute to evolutionary genome shrinking.

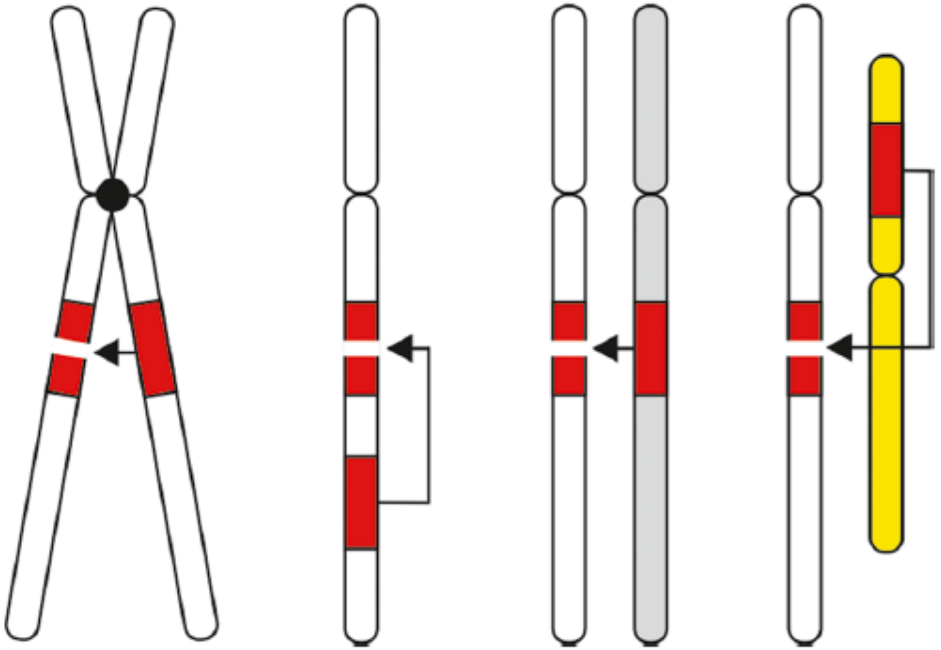
~70% of retrotransposon sequences in the *A. thaliana* genome are no longer autonomous

= probably as the consequence of SSA (truncated retroelements and solo LTRs)

= these elements cannot contribute to genome expansion

Vu et al. (2017) New Phytol 214

Templates for homologous recombination (HR) via SDSA (needs a template)



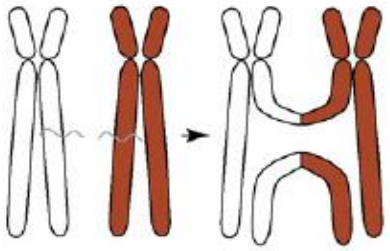
sister chromatid
(S and G2 phase)

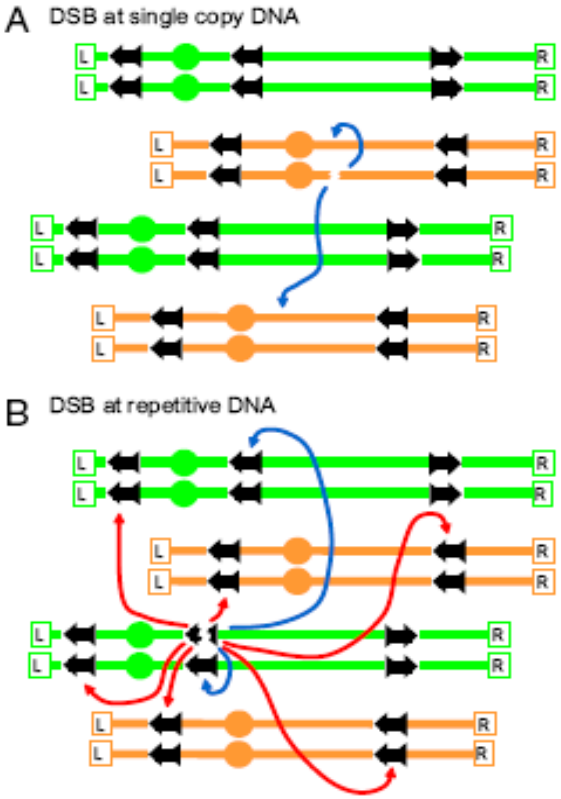
Intra-
chromosomal
homology

homologous
chromosome
(allelic sequence)

ectopic sequences
(heterologous chromosomes)

DSB repair and misrepair can lead to chromosome rearrangements





repair using a sister chromatid or homologue

repair using a sister chromatid or homologue
AND
ectopic recombination (generating chromosome abberations)

ectopic DSB repair competes with repair using the sister chromatid or homologue

Argueso et al. (2008) *PNAS* 105

NHEJ vs. HR (short summary)

- in somatic plant cells, DSBs are mainly repaired by **NHEJ**

- the NHEJ repair can be associated with deletions, but also insertions due to copying genomic sequences from elsewhere into the break; chr. rearrangements can be generated

- inverse correlation of deletion size to genome size (Arabidopsis vs. tobacco) >>> NHEJ might contribute significantly to evolution of genome size

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- DSB repair by **HR** might also influence genome organization

- intra-chromosomal HR is frequent (sequences in close proximity to the break), inter-chromosomal HR (allelic and ectopic) is hardly used for repair

- a 'single-strand annealing' (SSA) mechanism of HR that leads to sequence deletions between direct repeats is particularly efficient >> might explain the accumulation of single LTRs of retroelements in some plant genomes (?evolution of genome size?)