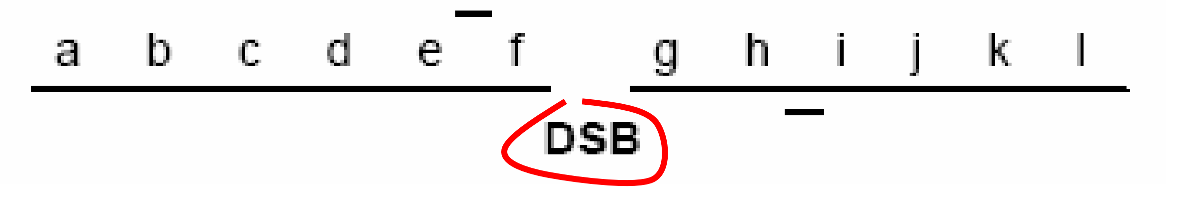
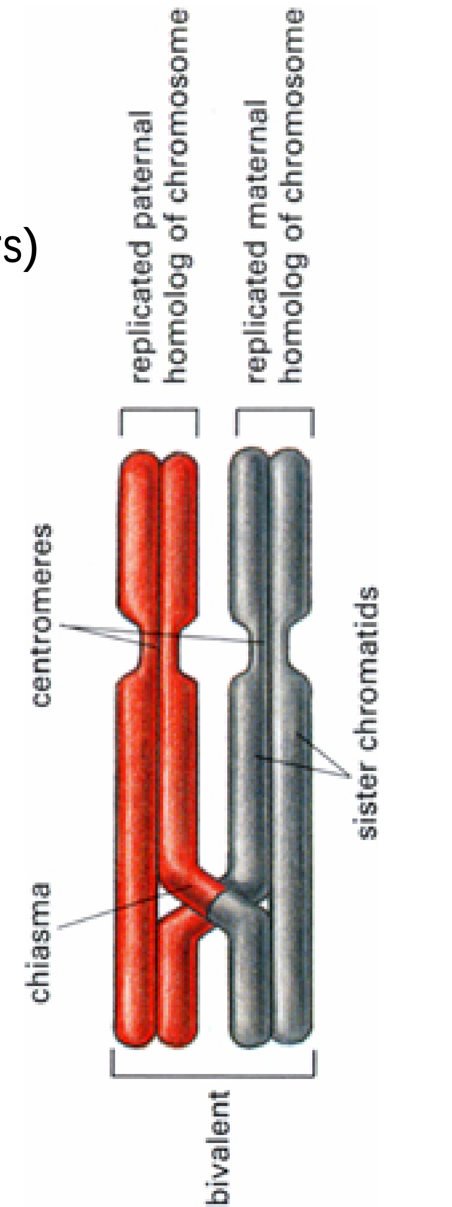
Double-strand breaks (DSBs), their repair and misrepair



DSBs in meiosis

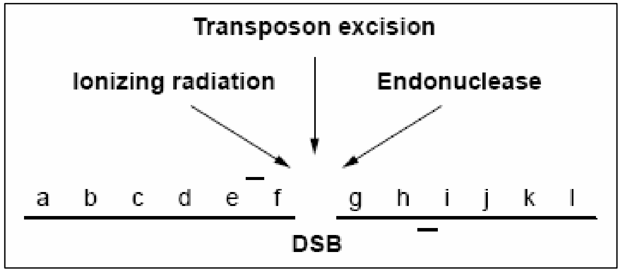
- necessary for homologous recombination (cross-overs)
- induced by the Spo11 topoisomerase



How are generated double-strand breaks

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!



- during meiotic 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 mitosis and their repair
(in somatic plant cells)

DSBs repair

non-homologous end joining (NHEJ)

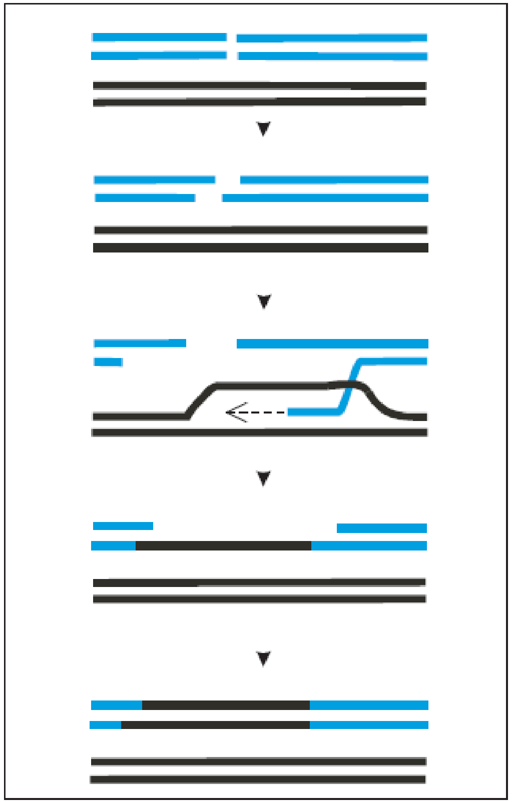
- also known as “illegitimate recombination”
- the broken ends are re-ligated directly
- often an error-prone process
- throughout the cell cycle (mainly G_1 phase)
- main mode of DSB repair in higher eukaryotes (somatic plant cells)

homologous recombination (HR)

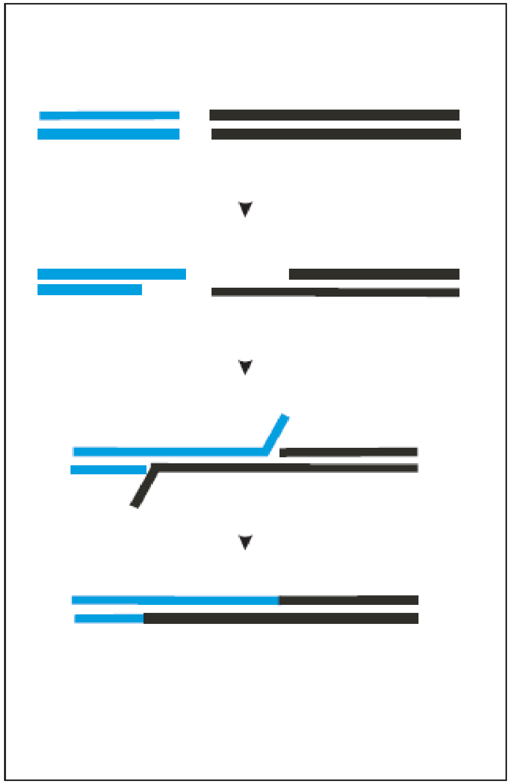
- uses sister chromatids as a template to rejoin DSBs
- error-free repair
- in late S-G_2 phase
- minor pathway

Models describing the pathways of DSB repair in somatic plant cells

synthesis-dependent strand annealing (SDSA)
mechanism



single-strand annealing (SSA)
mechanism



for details see Puchta (2005) JEB 56: 1-14

Different pathways of HR

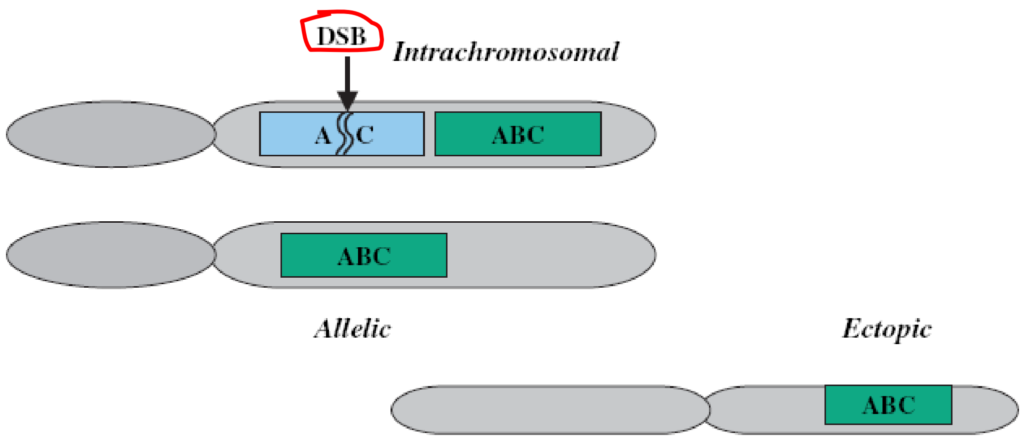
Homologous information for repair can be copied from:

- elsewhere in the genome (‘ectopic’)
- the homologue as in meiosis (‘allelic’)

} inter-chromosomal

- the same chromatid (chromosome)
- the sister chromatid (after replication)

} intra-chromosomal



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
- different classes of NHEJ repair events were characterized:

  a) the repair of the break was accompanied by incorporation of filler DNAs,

  b) the break ends were rejoined with or without deletions

⇩

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 ???

NHEJ vs. HR

- 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

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

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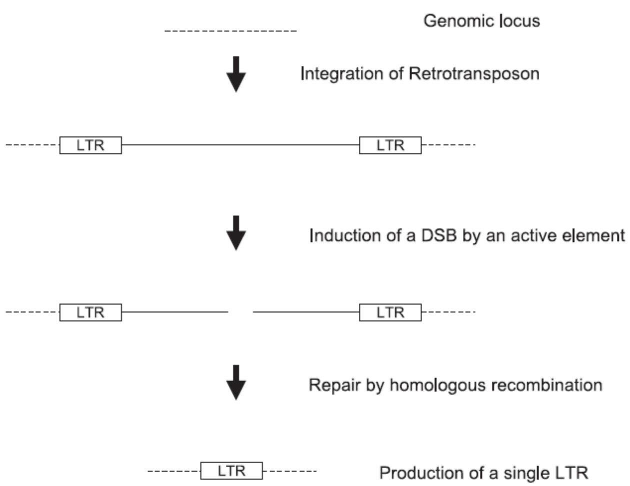
- DSB repair by **HR** might also influence genome organization

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

- 'single-strand annealing' 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



DSB repair and misrepair can lead to chromosome rearrangements...

(next lecture)