

Genome Editing and Engineering

Course No: BT-637



LECTURE-18

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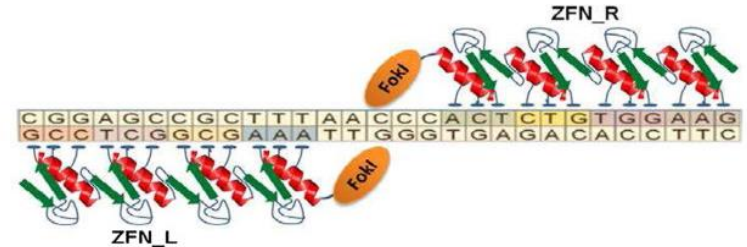
Indian Institute of Technology Guwahati

Genome Editing Tools

Protein-guided

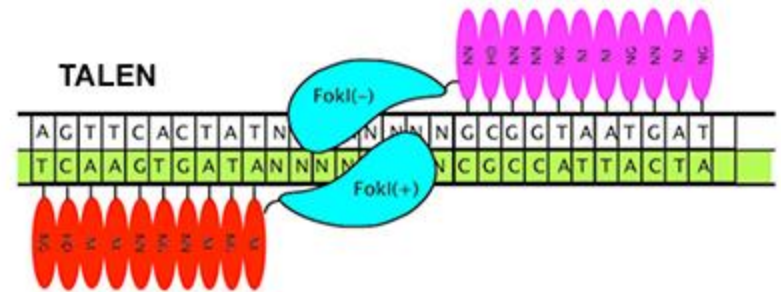
➤ Zinc finger nucleases

DNA binding domain (Zinc finger)
and DNA cleavage domain (FokI)



➤ TALENs

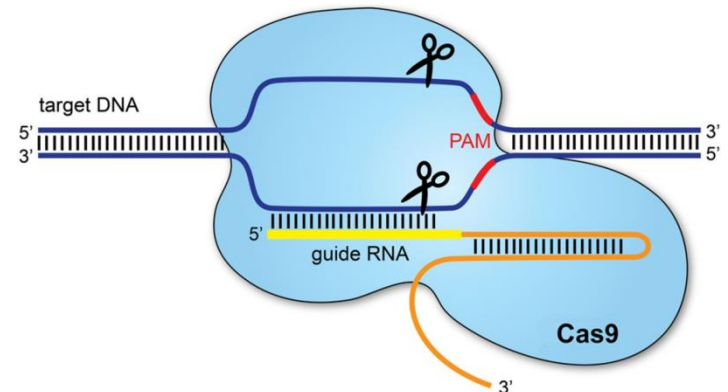
TAL (transcription activator like) effector
and DNA cleavage domain (FokI)
LTPEQVVAIAS^{HD}GGKQALETVQRLLPVLCQ/ (repeat)



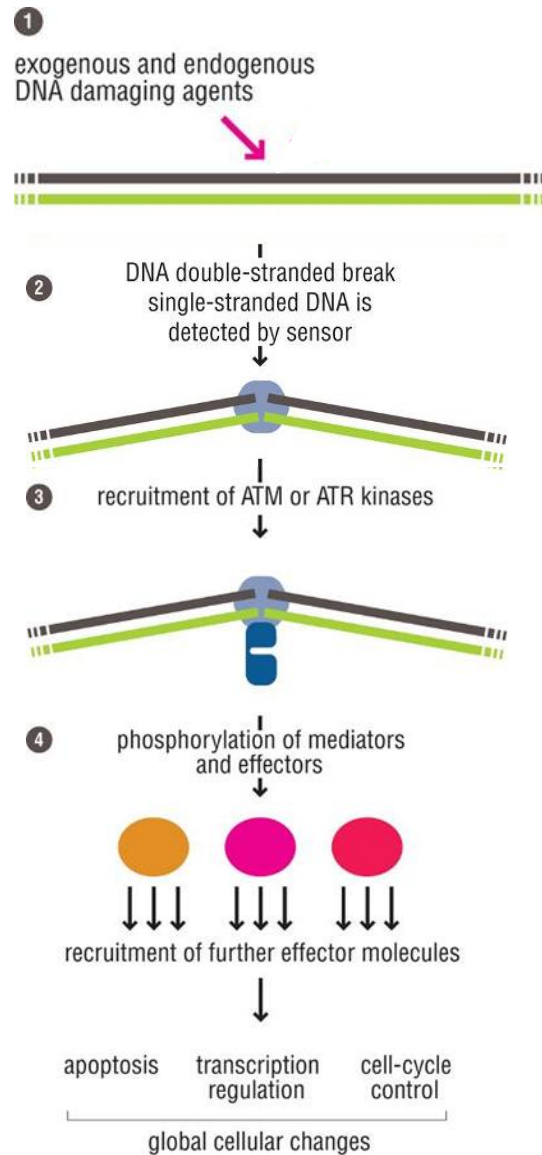
RNA-guided

➤ CRISPR/Cas system

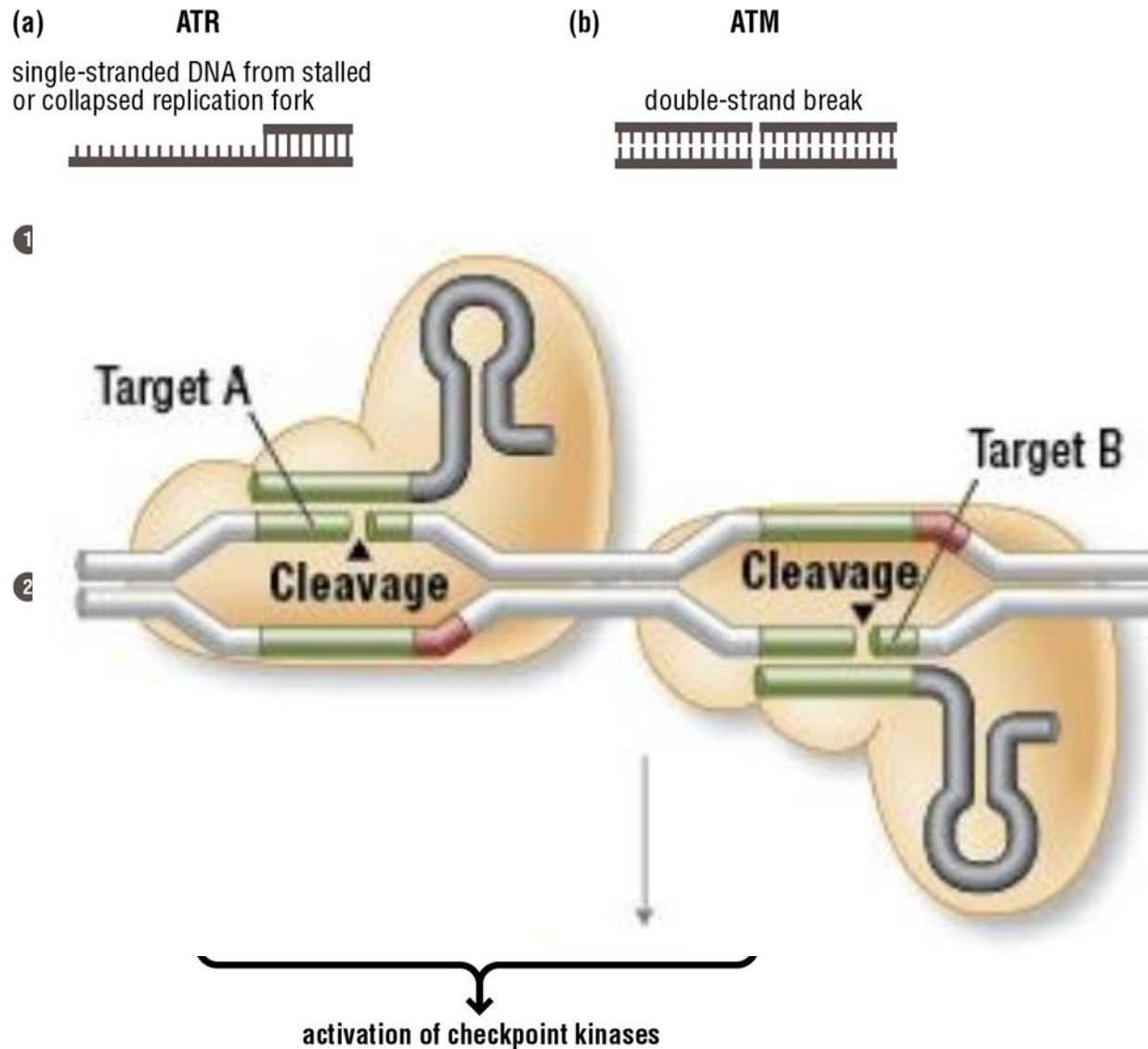
- RNA-guided, 20bp
- Efficient
- Easy, simple and affordable



DNA strands break



DNA strands break



Single DNA strand breaks

(a) **ATR**

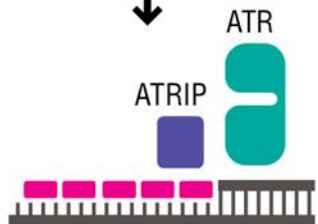
single-stranded DNA from stalled
or collapsed replication fork



1 detection of DNA damage
↓

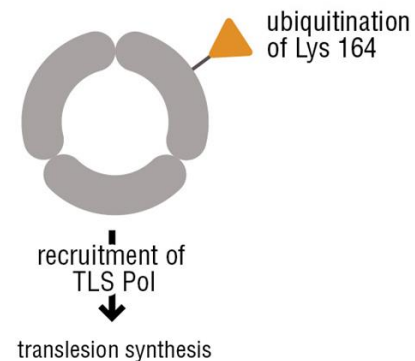
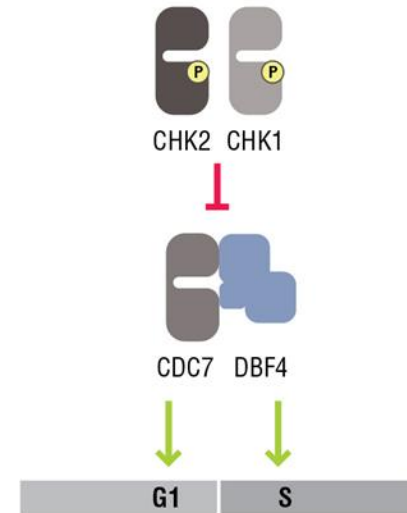
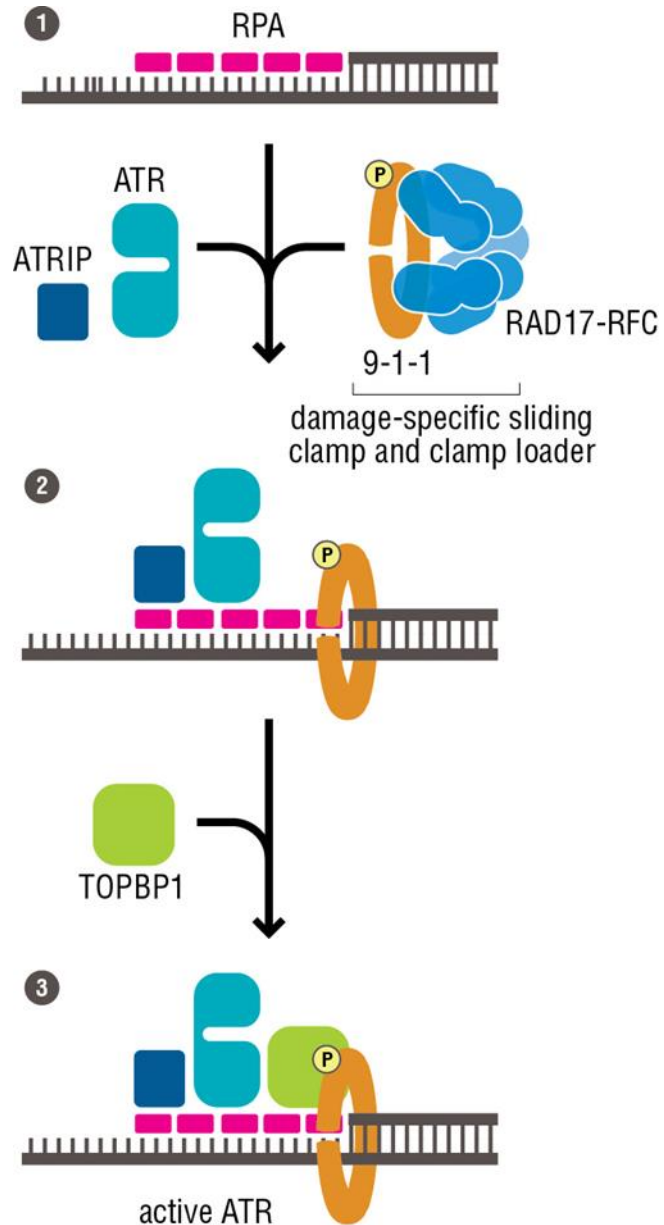


2 recruitment of
protein kinase ATR
↓

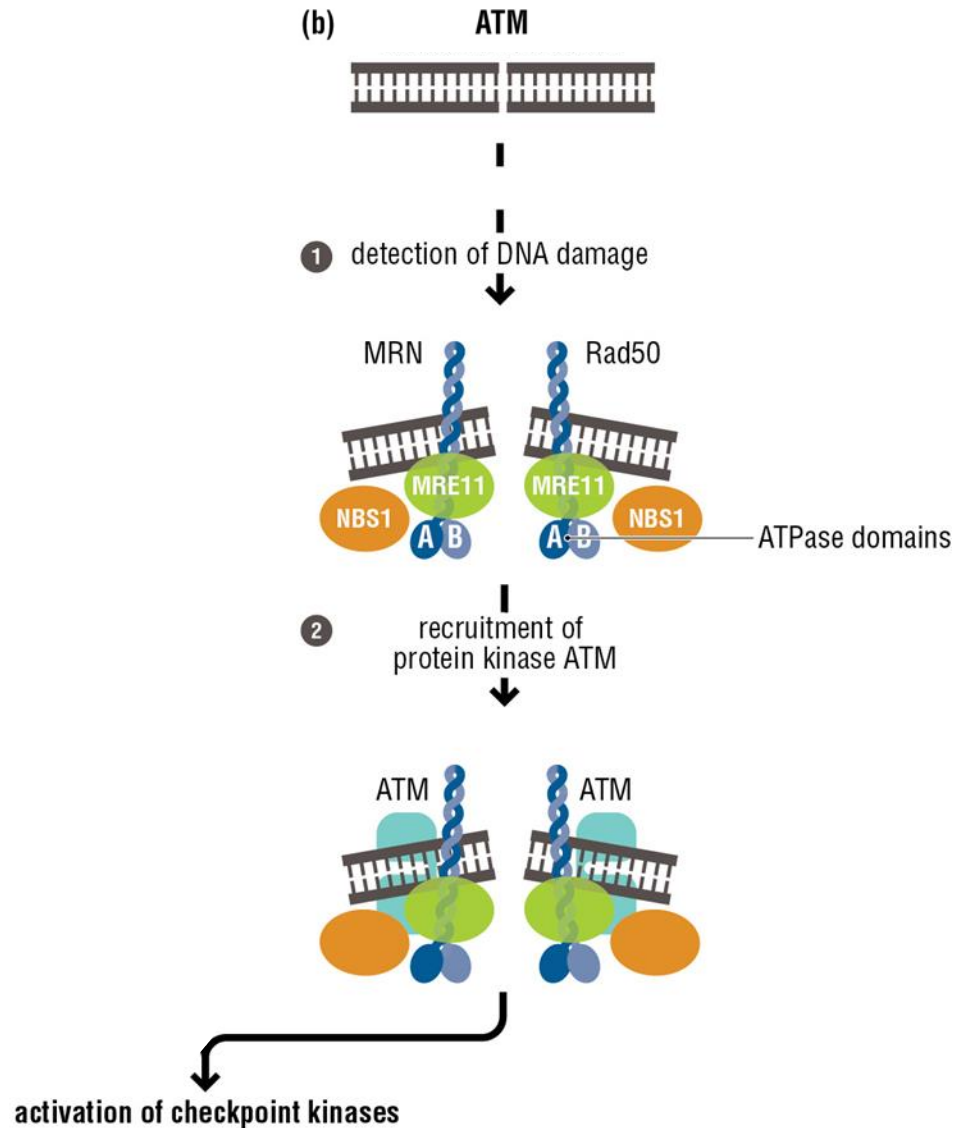


activation of checkpoint kinases

Single DNA strand breaks

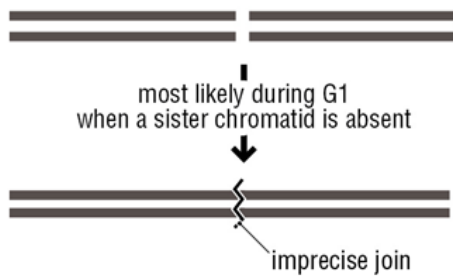


Double strands break

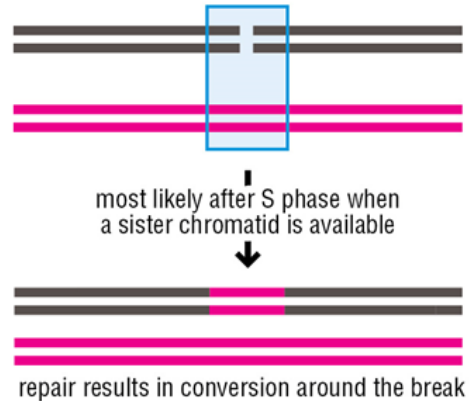


Double strands break

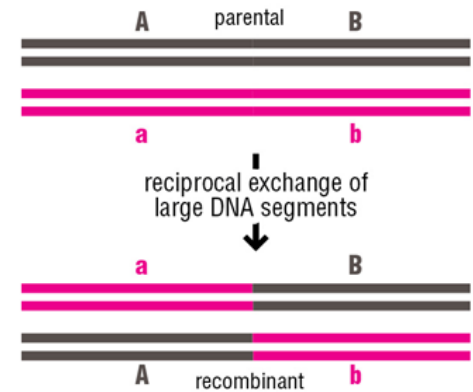
(a) non-homologous end-joining (NHEJ)



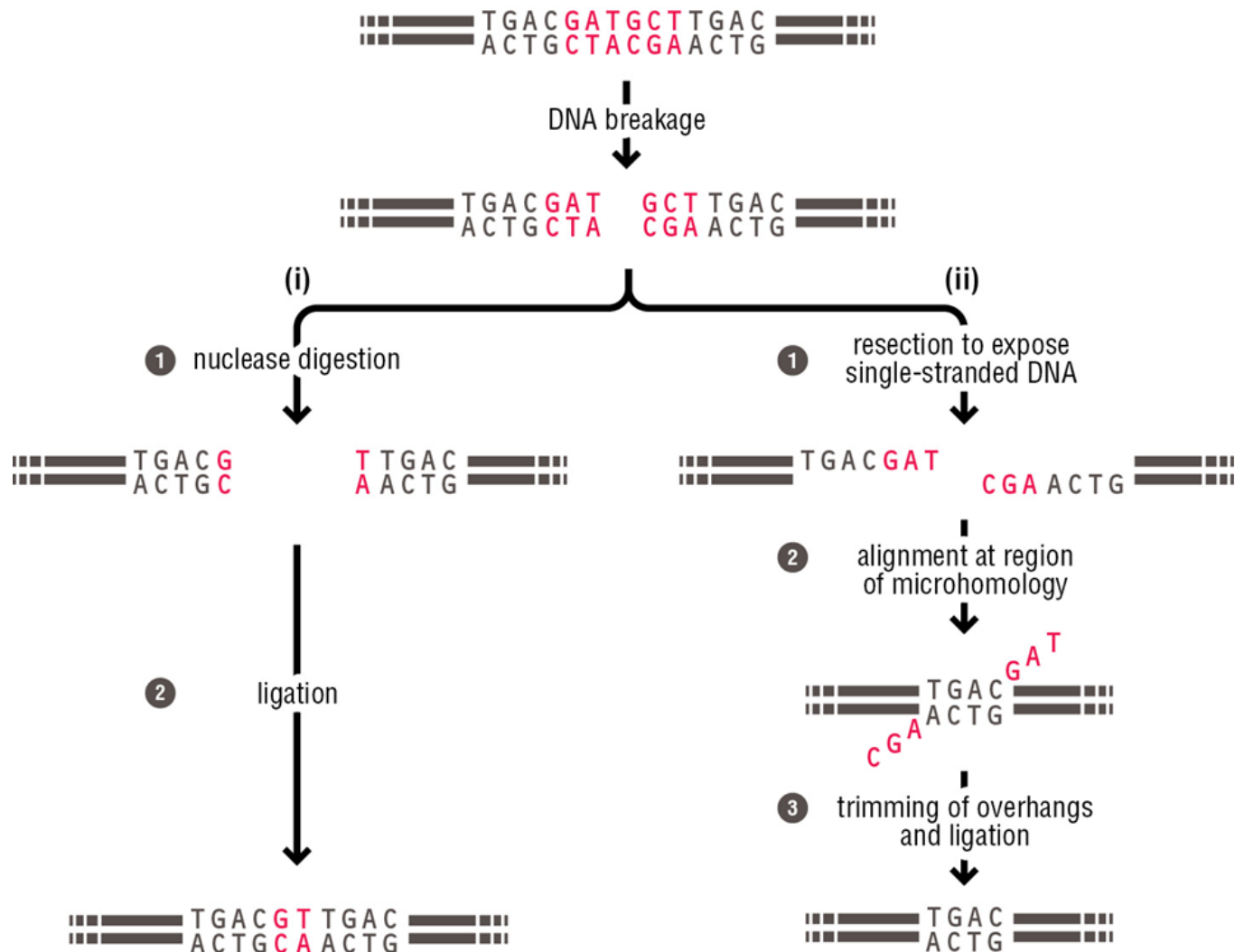
(b) homology-directed repair using homologous chromosome



(c) homologous recombination



Non-homologous end joining (NHEJ)



Non-homologous end joining (NHEJ)

(a) Table of proteins that mediate NHEJ in different organisms			
bacteria		eukaryotes	
functional component		<i>Saccharomyces cerevisiae</i>	multicellular eukaryotes
DNA end binding	Ku (30–40 kDa)	Ku 70/80	Ku 70/80
polymerase	POL domain of LigD	Pol4	Pol μ and λ
nuclease	?	Rad50:Mre11:Xrs2	Artemis: DNA-PKcs
kinase/phosphatase	phosphodiesterase domain of LigD	Tpp1 and others	polynucleotide kinase/ phosphatase and others
ligase	LIG domain of LigD	Nej1:Lif1:Dnl4	Ligase IV-XRCC4 XLF/Cernunnos

Non-homologous end joining (NHEJ)

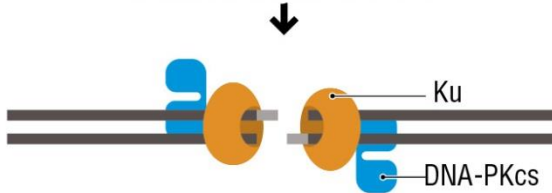
1 double-strand break and degradation



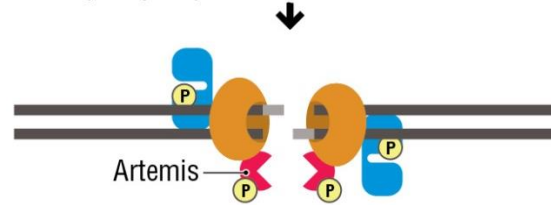
2 ends are partially resected by nuclease



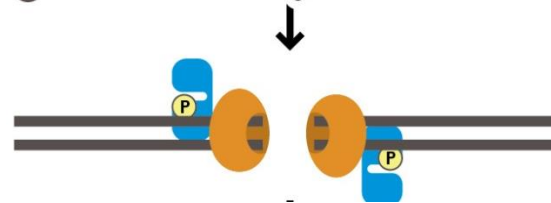
3 Ku heterodimers bind broken ends and recruit DNA-PKcs



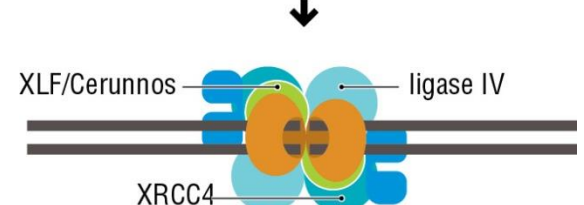
4 DNA-PKcs recruits and phosphorylates the nuclease Artemis



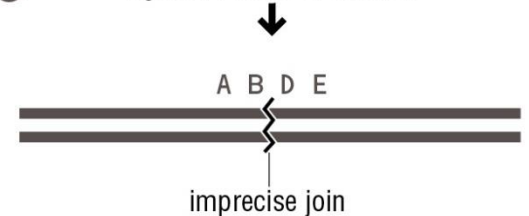
5 Artemis trims single-stranded tails

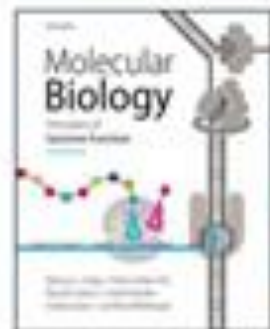


6 DNA ends are ligated by ligase IV in complex with XRCC4 and XLF/Cerunnos



7 ligation results in deletion



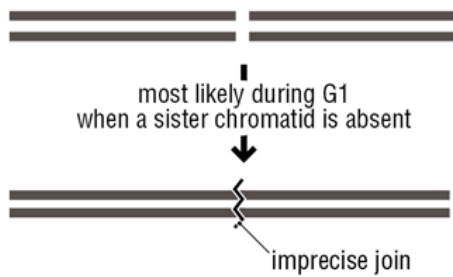


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Second Edition

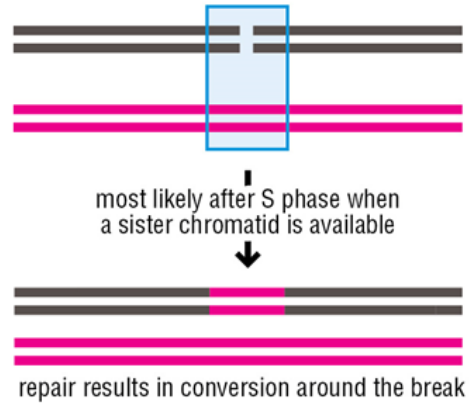
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Animation 12: **Non-homologous end joining**

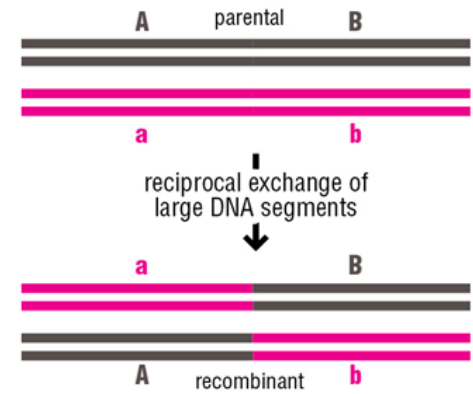
(a) non-homologous end-joining (NHEJ)



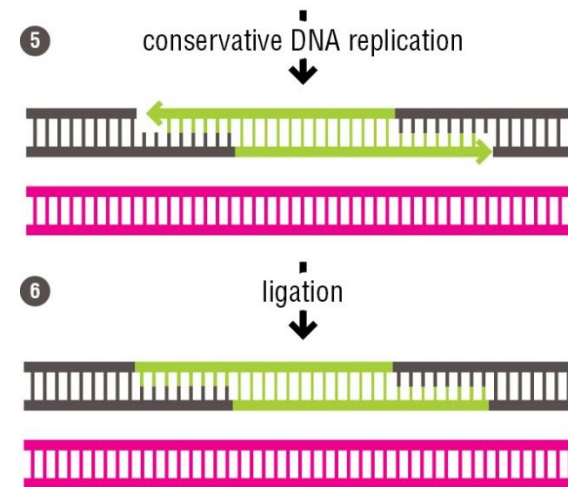
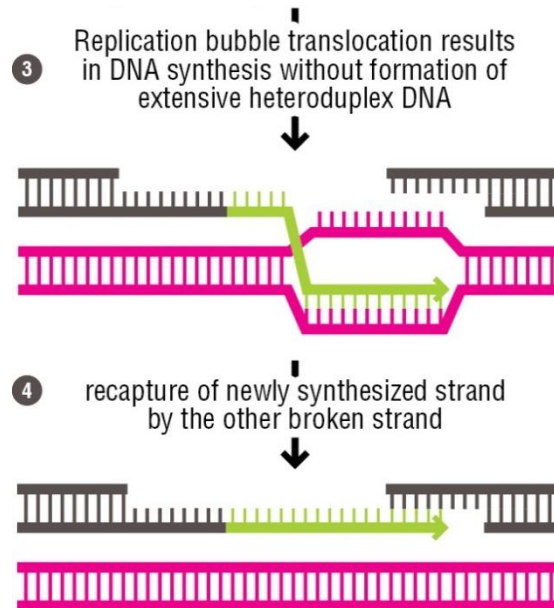
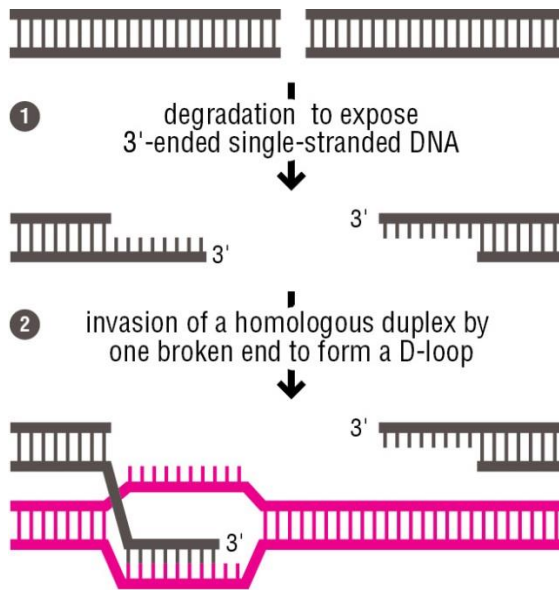
(b) homology-directed repair using homologous chromosome



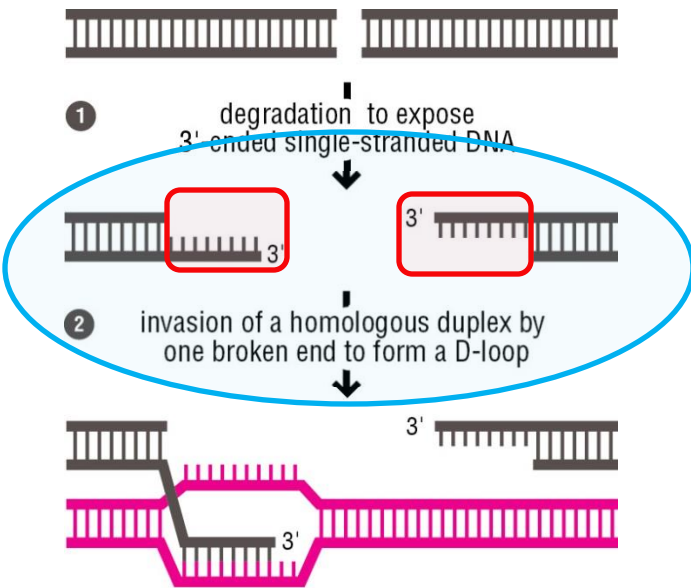
(c) homologous recombination



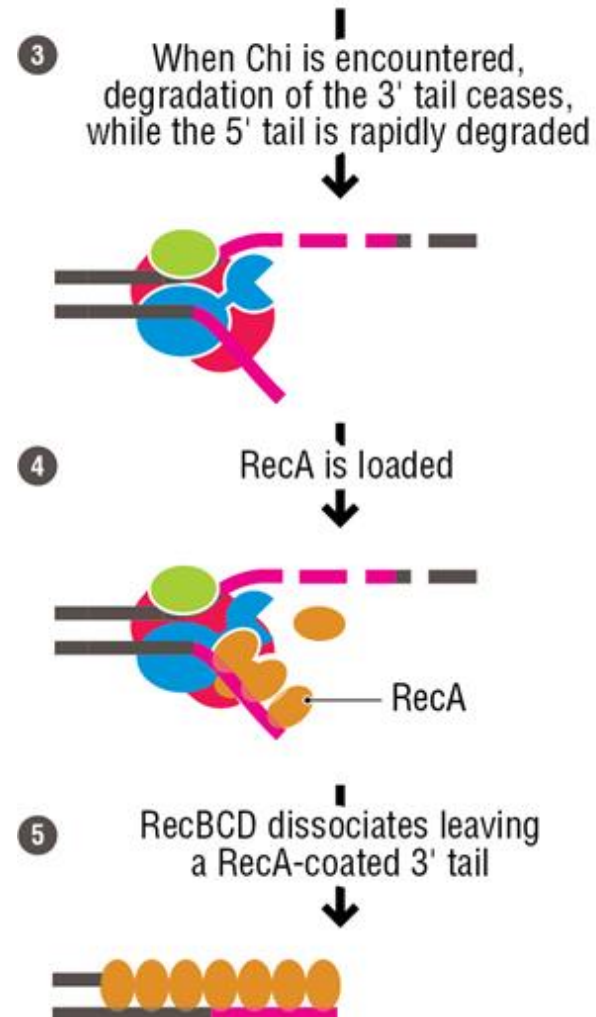
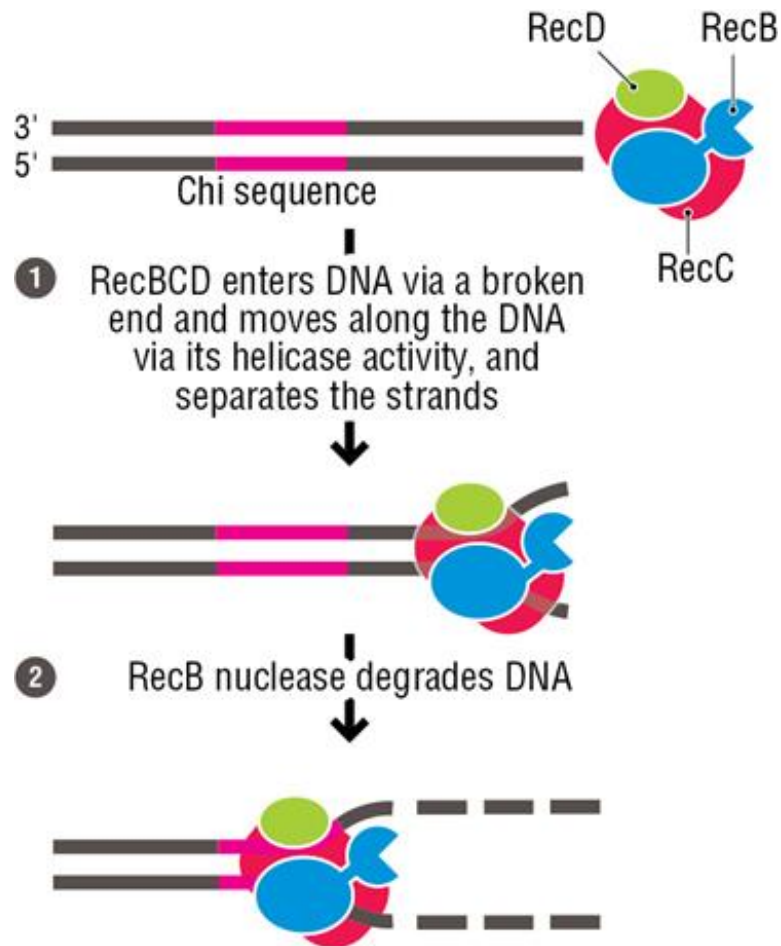
Homology-directed Repair



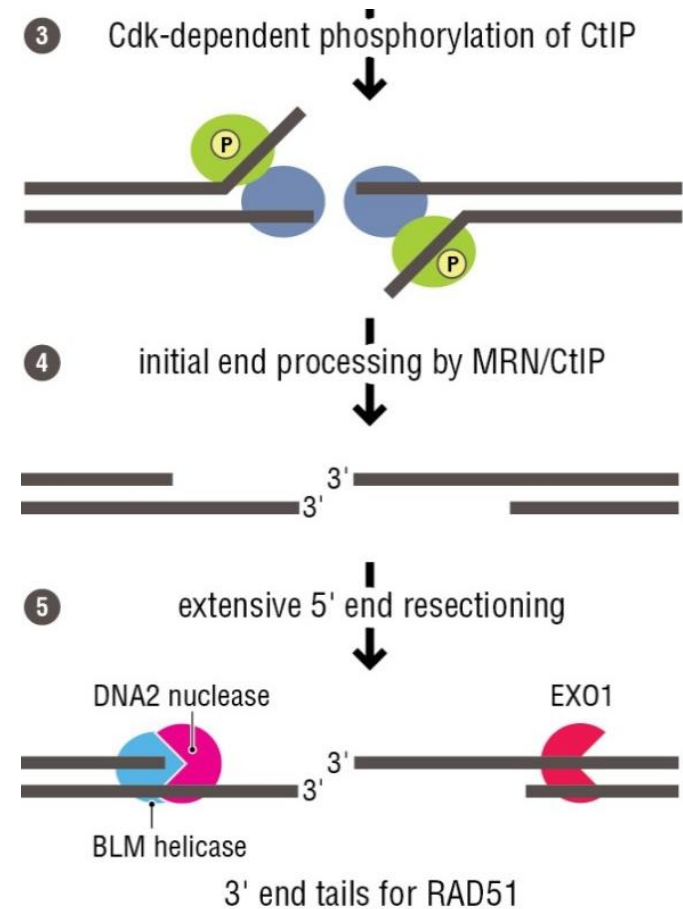
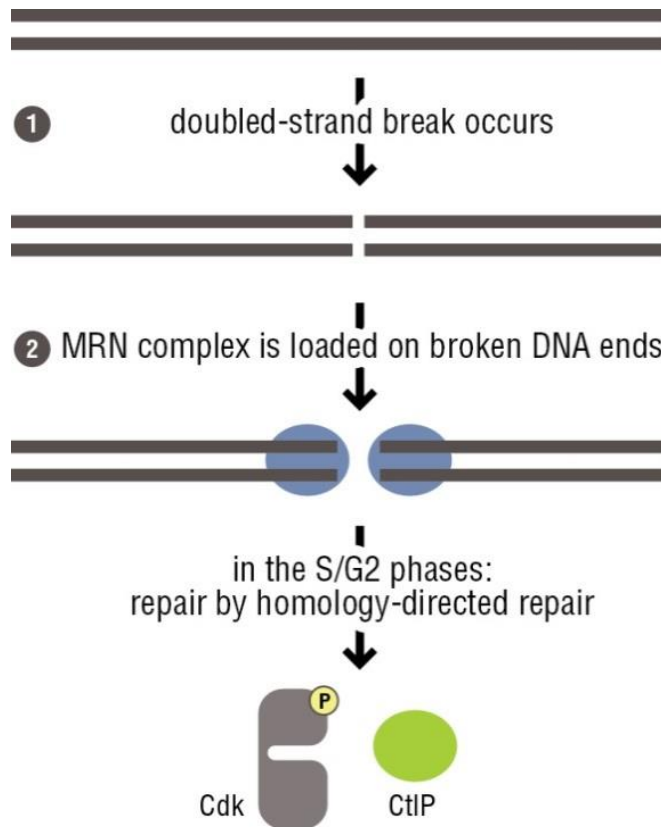
Generation of 3' single strand



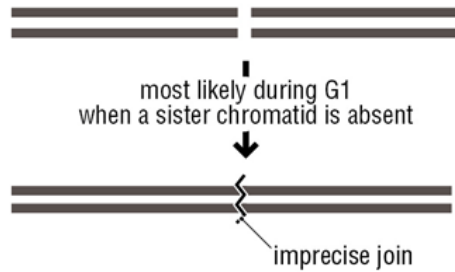
Generation of 3' single strand



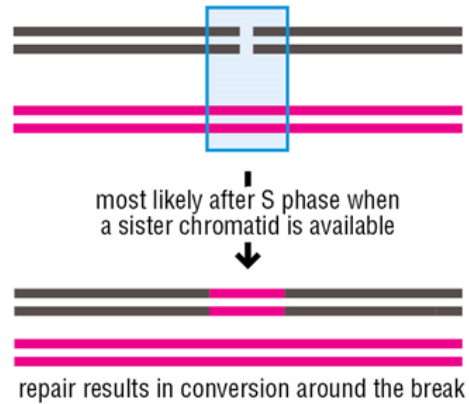
Generation of 3' single strand



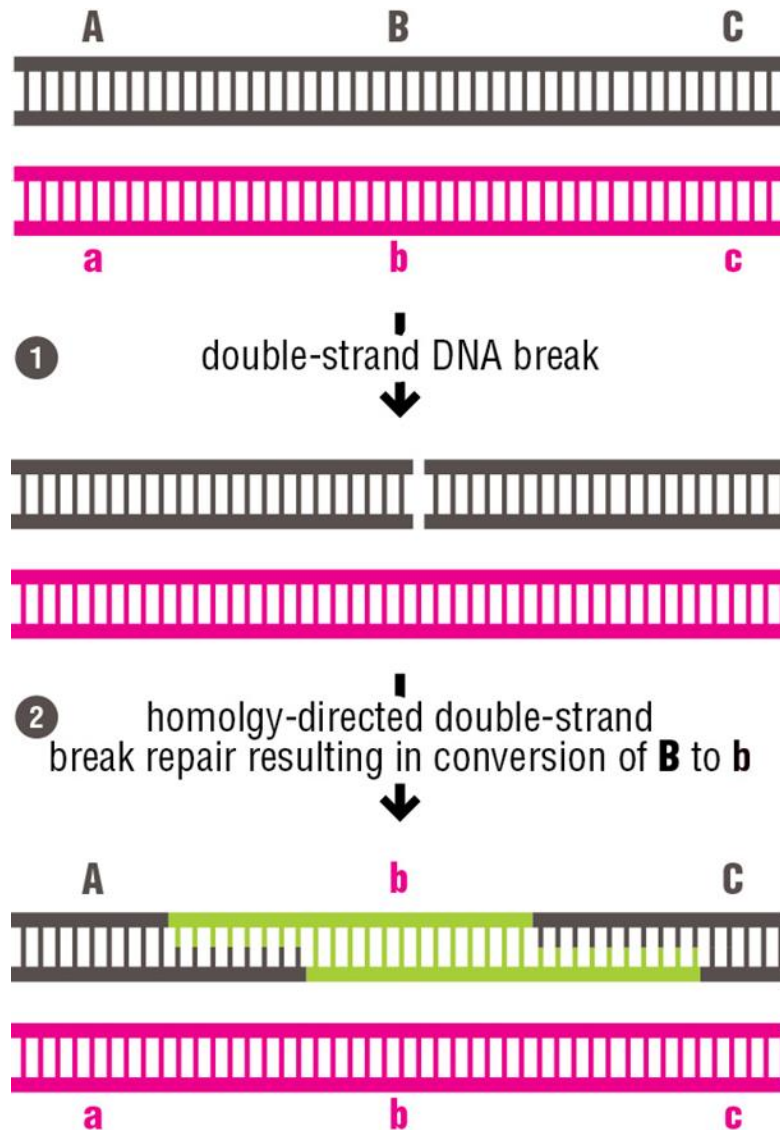
(a) non-homologous end-joining (NHEJ)

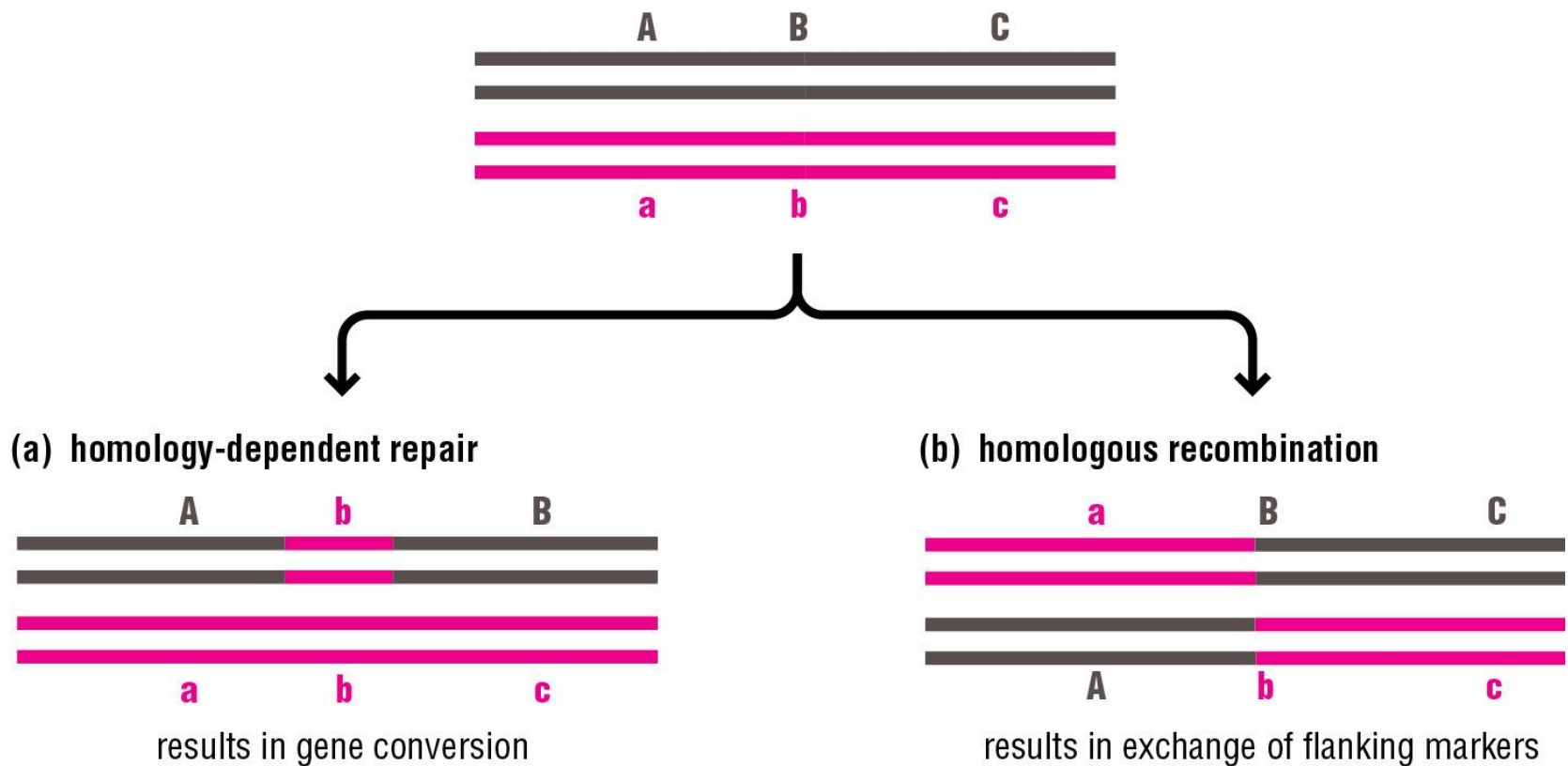


(b) homology-directed repair using homologous chromosome

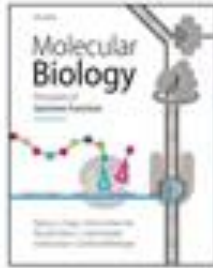


Homology directed DSR





- Homology-directed repair (a) and homologous recombination (b) have different outcomes
- Homology-directed repair results in gene conversions whereas homologous recombination exchanges DNA and results in new genetic combinations



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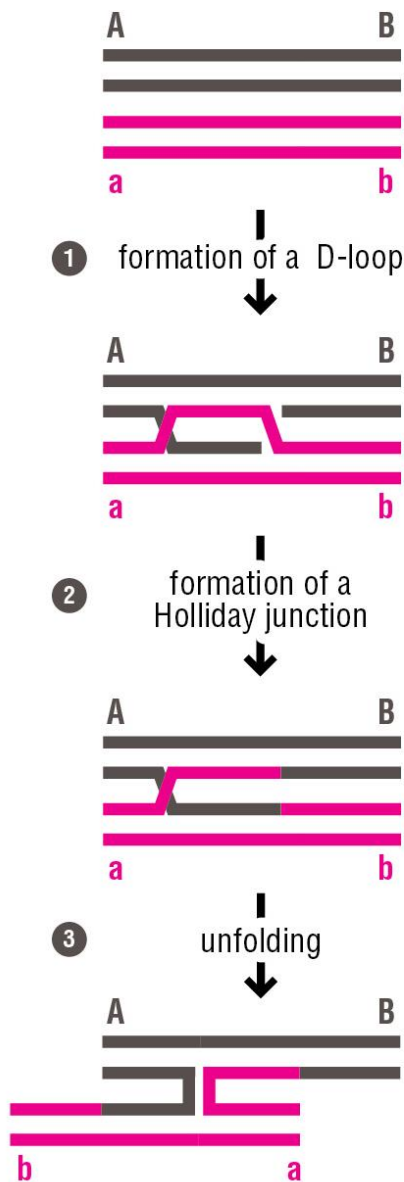


Animation 13: **Homology-dependent double strand break repair**

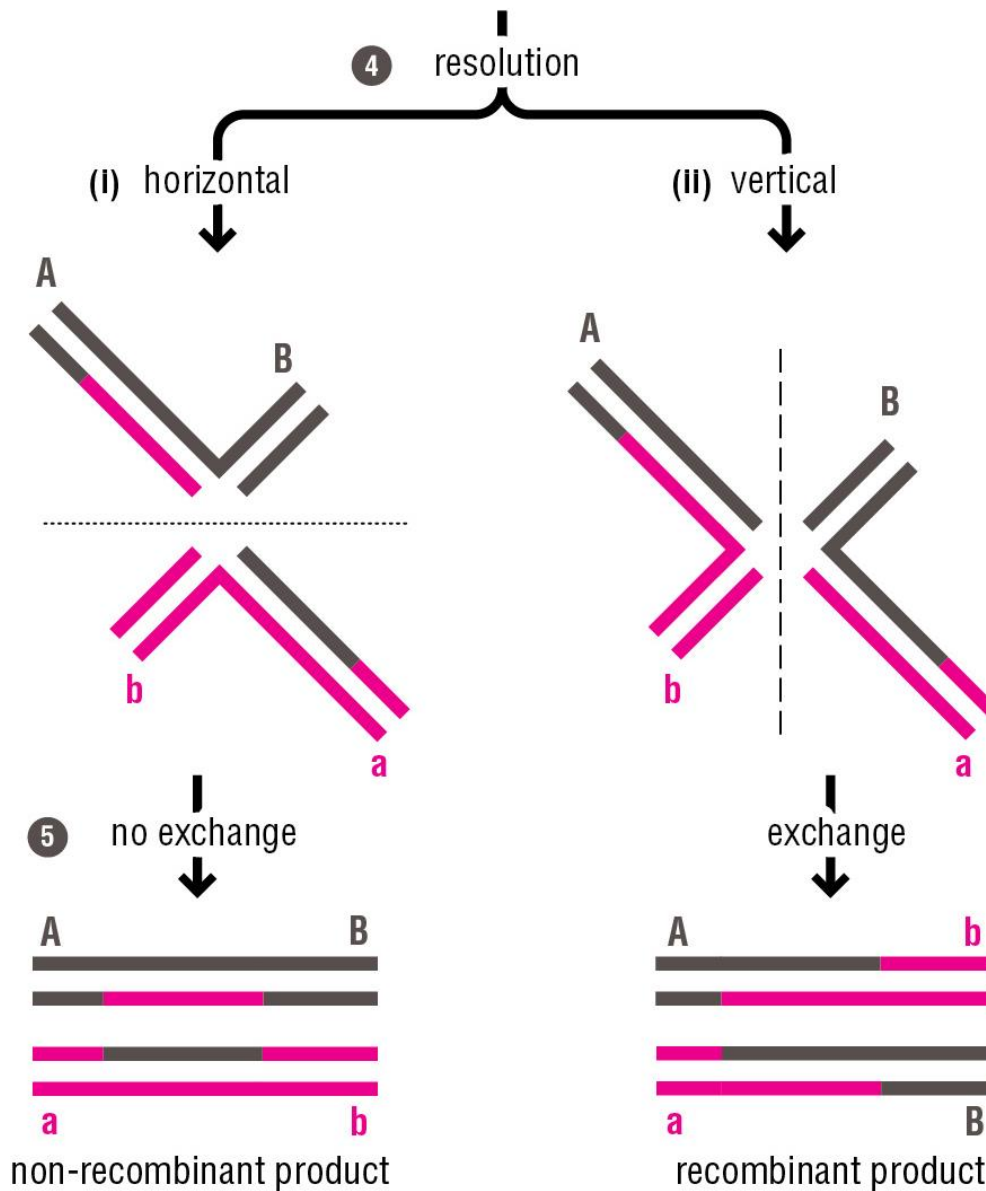
Animation produced by Connor Hendrich
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Conclusions of Lecture-18

- **Nucleases = DNA Breaks**
- **DNA breaks = single / double strands**
- **Single strand breaks = ATR**
- **Double strands break = ATM**
- **DSB = NHEJ / HDR**
- **HDR= sister chromatids / homologues strands**



- Homologous recombination involves formation and resolution of Holliday junctions
- Holliday junctions form through invasion of another duplex and formation of a D-loop (1)
- The junction unfolds by rearrangement of the DNA into a cross-like structure (2, 3)
- This structure must then be resolved



- Holliday junctions rearrange into a cross-like structure with two axes of symmetry

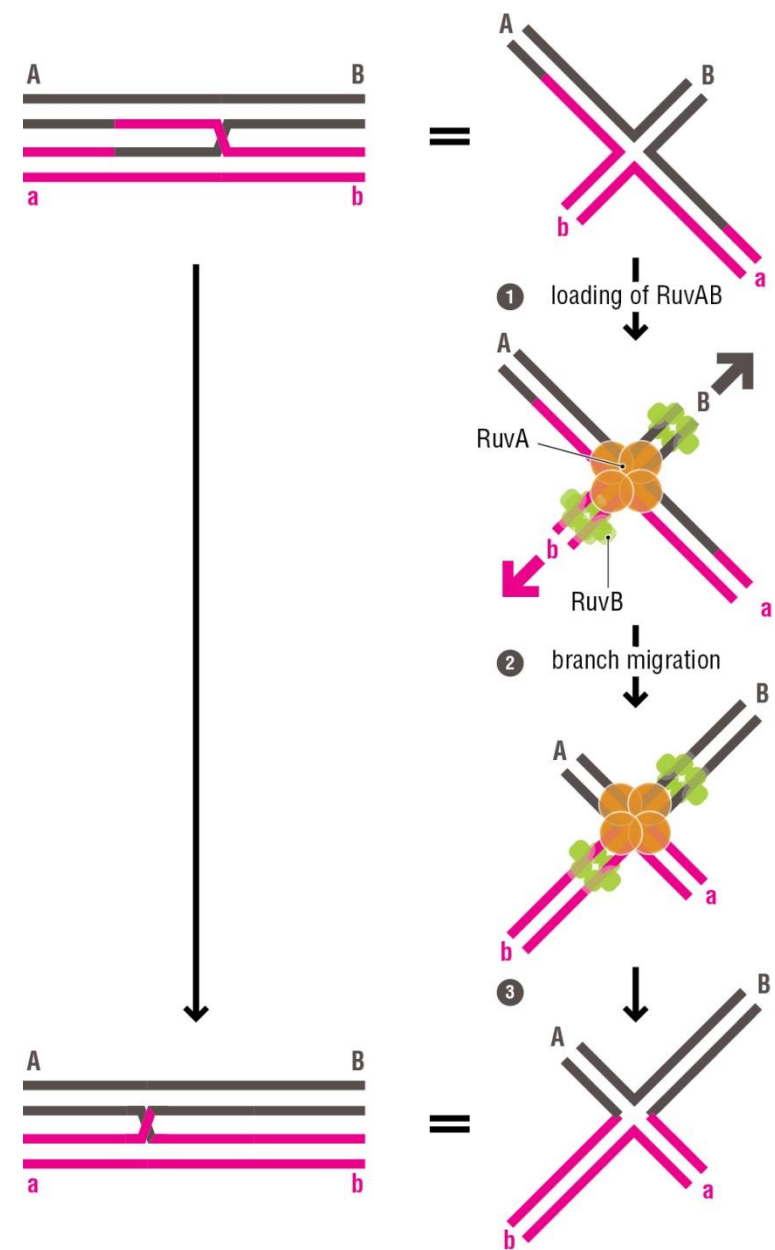
- Cleavage of the junction by resolvases can occur in either plane (4, i & ii)

- The direction of cleavage directs whether there is no exchange of DNA (5 i) or exchange and production of a recombinant (5ii)

- If there is no exchange, the parent strands remain together (A with B and a with b)

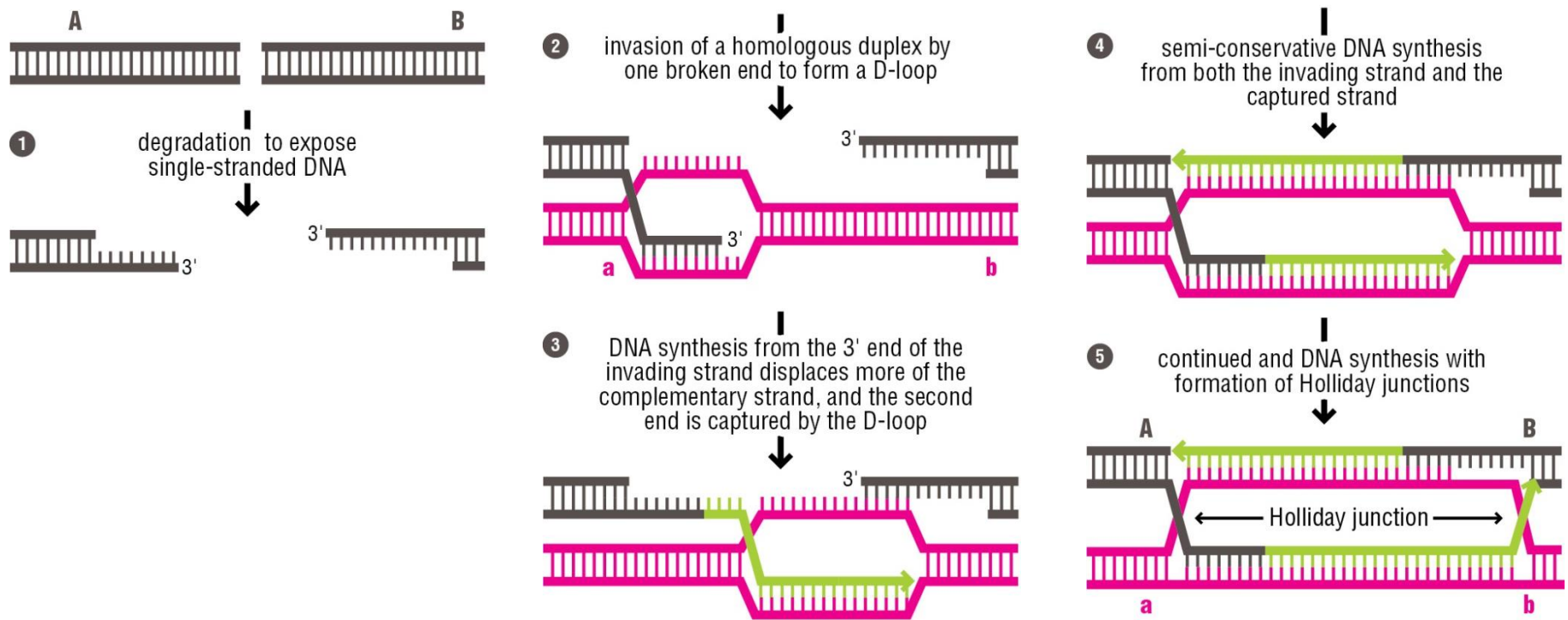
- If exchange occurs, parent strands are broken and become linked to one another (i.e., A and b are on the same molecule)

- Holliday junctions can move along the DNA – this is branch migration
- Migration when recombination is between homologous chromosomes rather than sister chromatids changes the amount of heteroduplex DNA present
- Branch migration in homologous chromosomes requires specific helicases
- Bacterial RuvA holds the junction in an open conformation (1). RuvB is a helicase, and breaks and re-forms base pairings to move the Holliday junction. RuvC is a resolvase
- Depending on the junction movement, more (top) or less (bottom) heteroduplex DNA is present

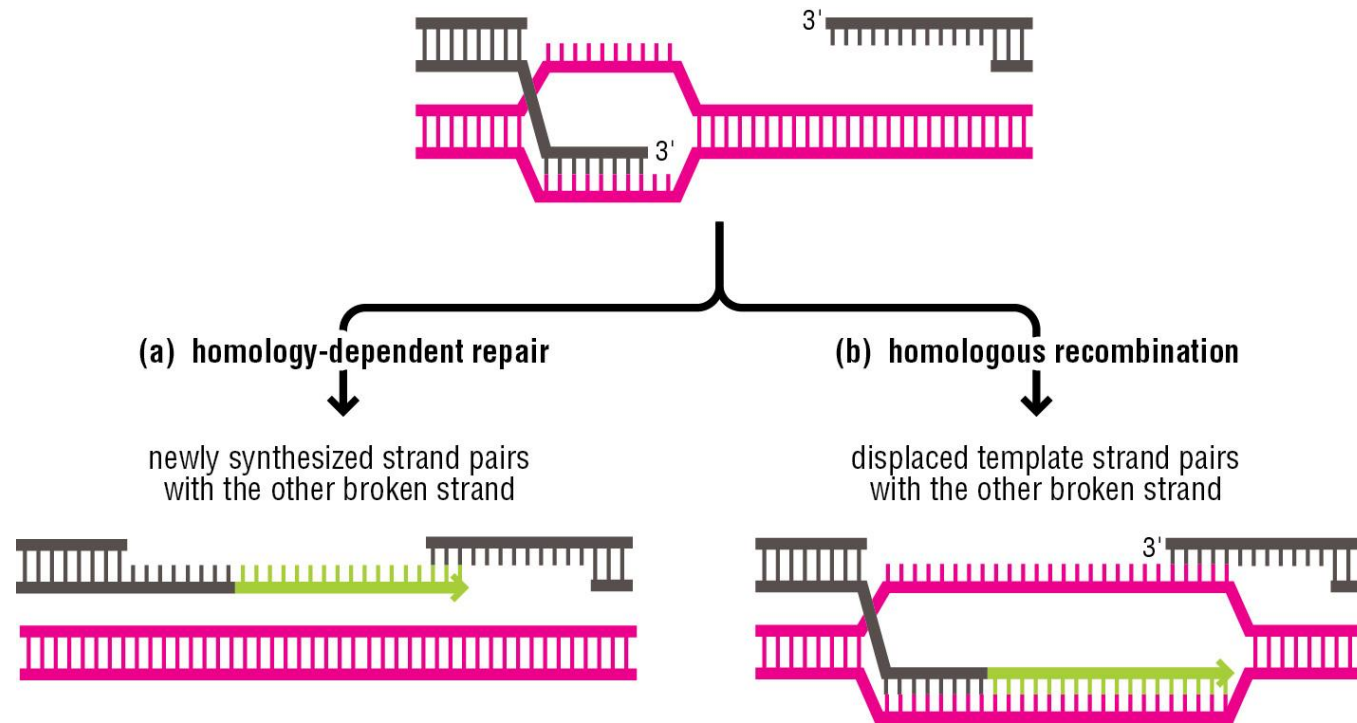


16.8: Homologous Recombination

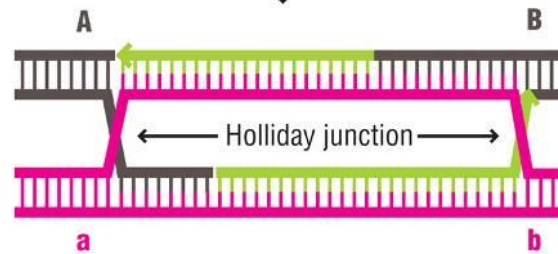
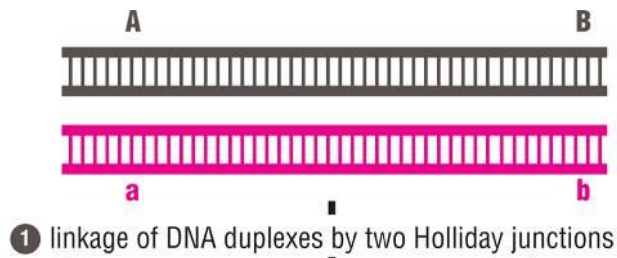
Figure 16-17



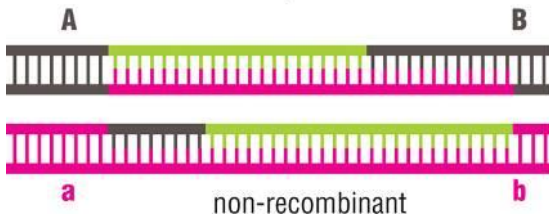
- Homologous recombination is initiated by a double strand break (1)
- DNA is resected and the 3' tail invades the opposing duplex (2)
- DNA is made (green) from the the invading strand (3)
- The other strand in the D-loop is captured by the other end of the double-strand break, and synthesis occurs (4)
- Two intact dsDNA regions joined by two Holliday junctions are formed (5)



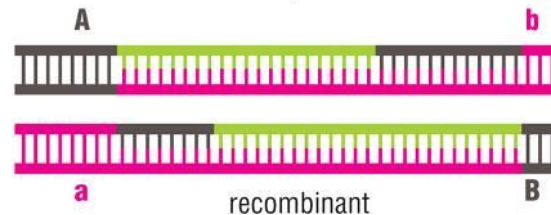
- Homology-directed repair and homologous recombination are similar: the key difference relates to the activity of the second end of the double-strand break
- In homology-directed repair (a) the second end of the double-strand break captures the newly synthesized DNA primed from the first end and the two duplexes do not become linked
- In homologous recombination (b), the second end of the double-strand break captures the displaced strand of the D-loop – the two duplexes are linked



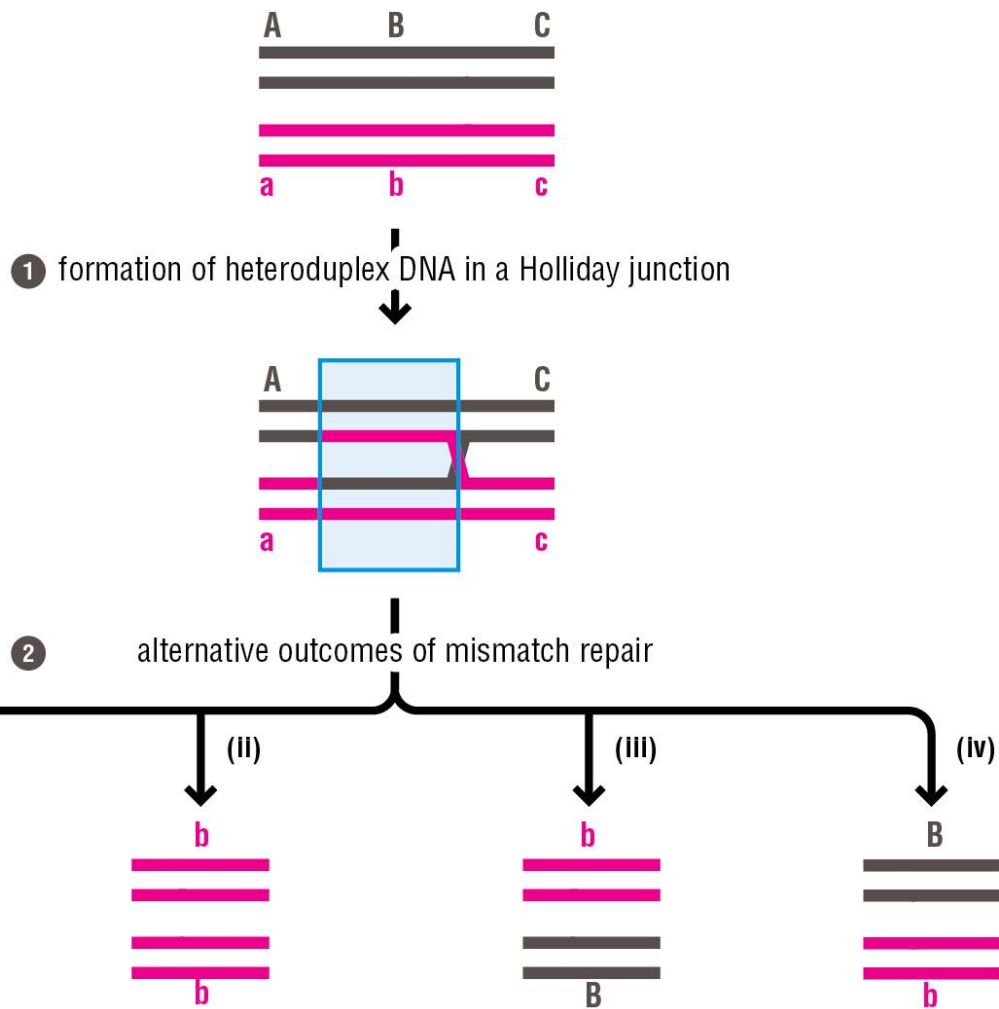
2 Holliday junction resolution and separation of the DNA duplexes



Holliday junction resolution and separation of the DNA duplexes



- Holliday junction resolution determines whether recombination occurs
- If the two junctions are resolved in the same direction (i), no recombination occurs (i.e. A and B are still on the same molecule)
- Resolution in different directions (ii) creates recombinant molecules (i.e., A and B are now on different molecules – A and b are linked instead)



- Holliday junction resolution can lead to changes in the gene sequence
- If heteroduplex forms between strands that have slightly different sequences, mismatch correction can use either strand as a template for repair
- Several possible outcomes are possible (i-iv), depending on the template strand used for repair

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Thank You!