Presentation

Introduction of DNA Recombination

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DNA Recombination

- Roles
- Types
- Examples

Biological Roles for Recombination

1. Generating new gene/allele combinations (crossing over during meiosis)
2. Generating new genes (e.g., Immuno-globulin rearrangement)
3. Integration of a specific DNA element
4. DNA repair

Practical Uses of Recombination

1. Used to map genes on chromosomes

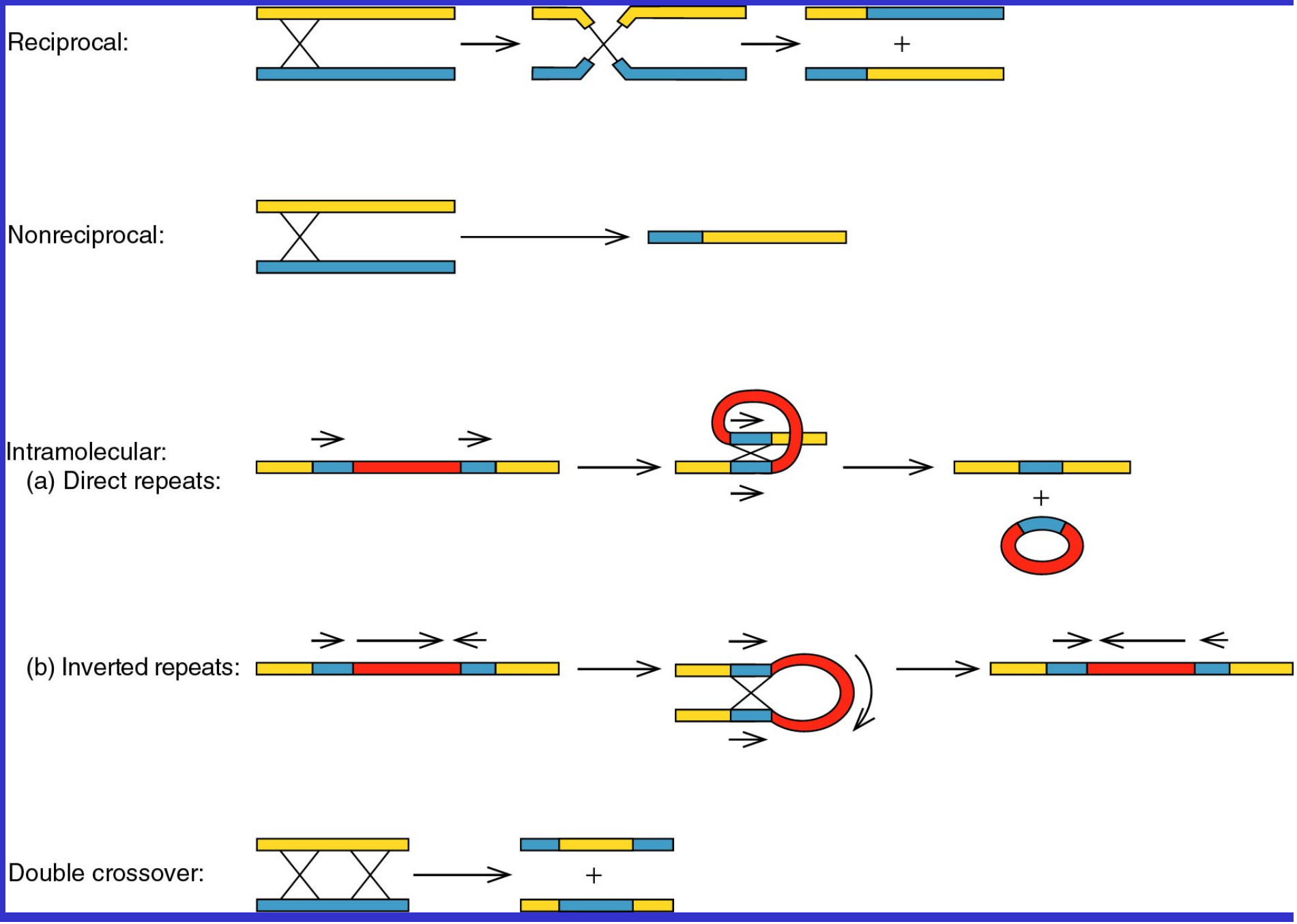
2. Making transgenic cells and organisms

Types of Recombination

1. Homologous - occurs between sequences that are nearly identical (e.g., during meiosis)
2. Site-Specific - occurs between sequences with a limited stretch of similarity; involves specific sites
3. Transposition – DNA element moves from one site to another, usually little sequence similarity involved

Examples of Recombination

Fig. 22.1



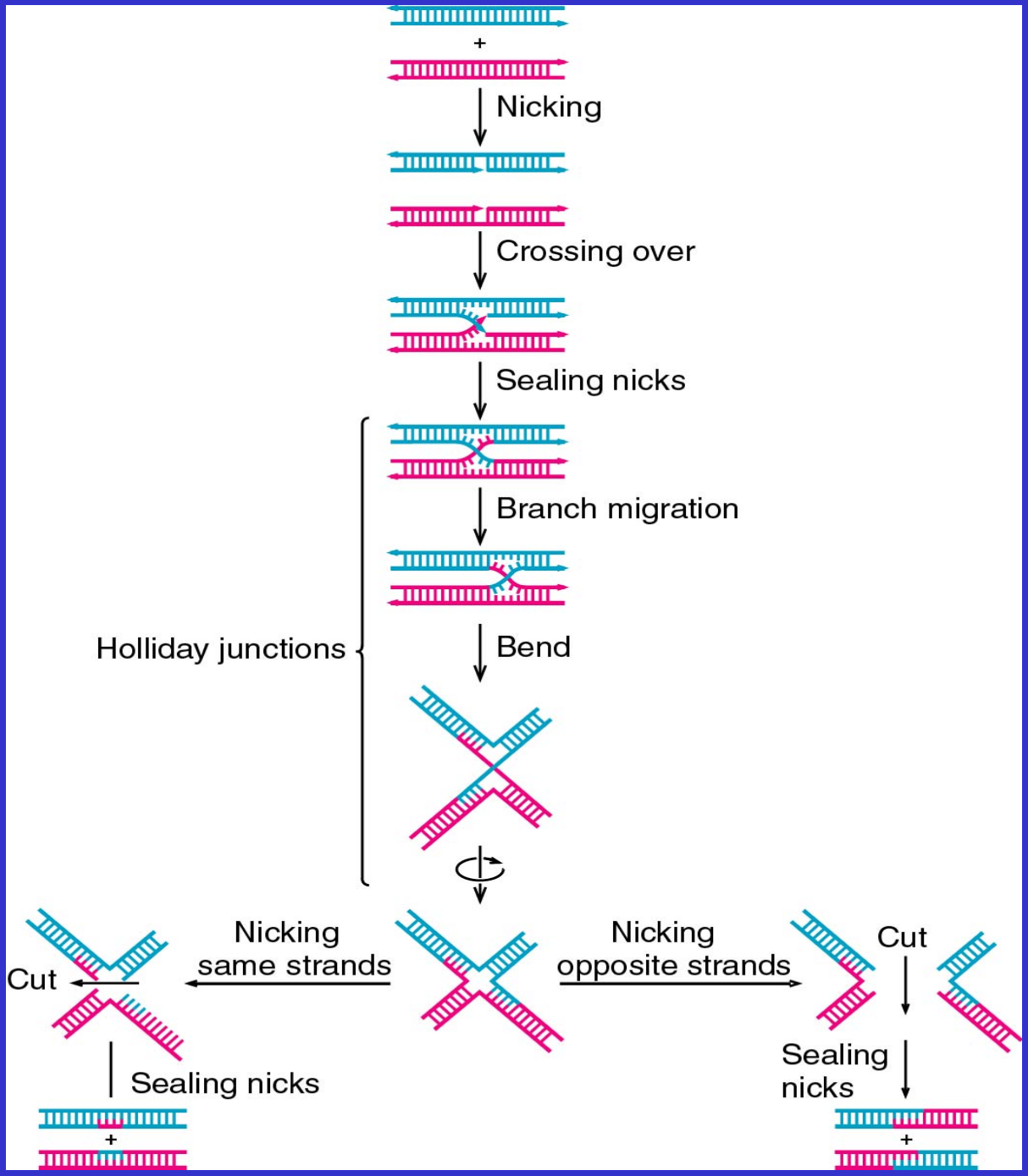
Holliday Model

R. Holliday (1964)

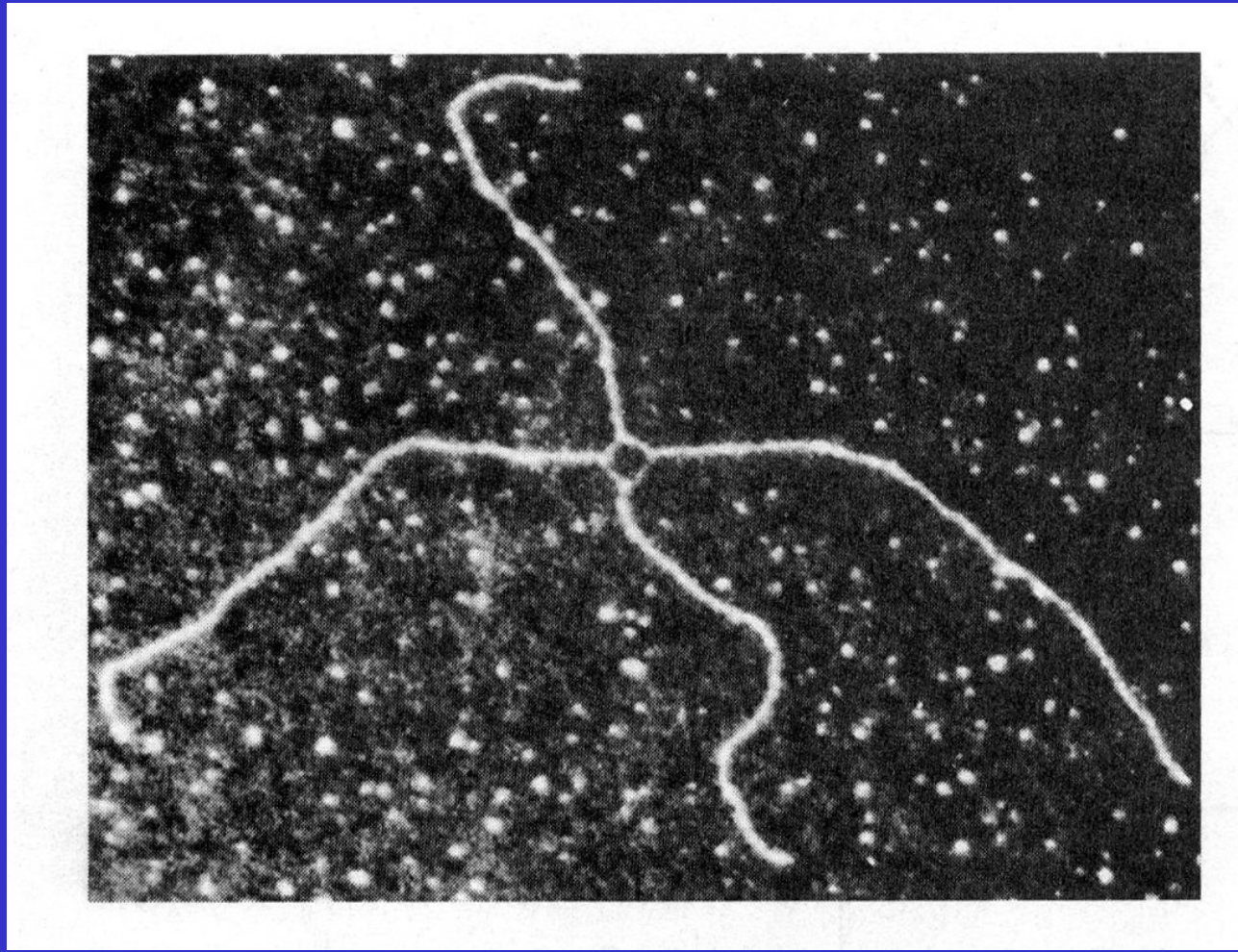
- Holliday Junctions form during recombination

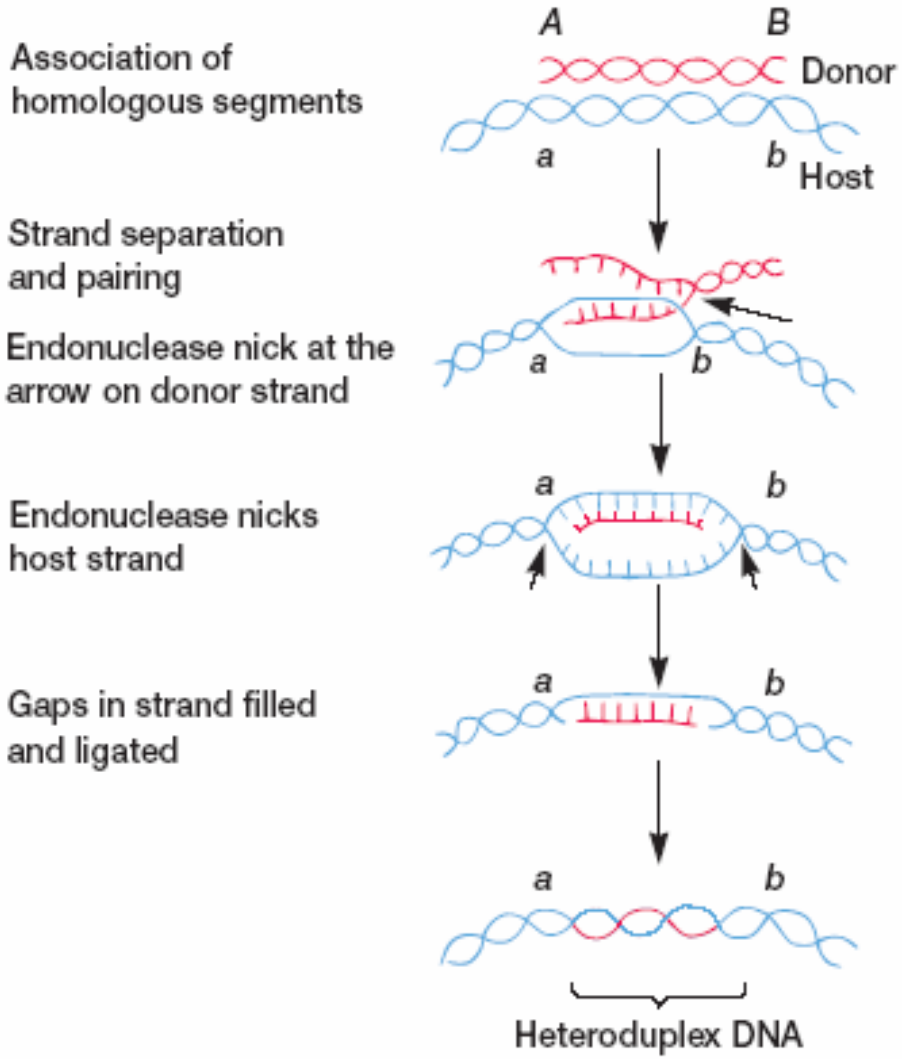
- HJs can be resolved 2 ways

patch



EM of a Holliday Junction w/a few melted base pairs around junction





Nonreciprocal recombination

The *recBCD* Pathway of Homologous Recombination

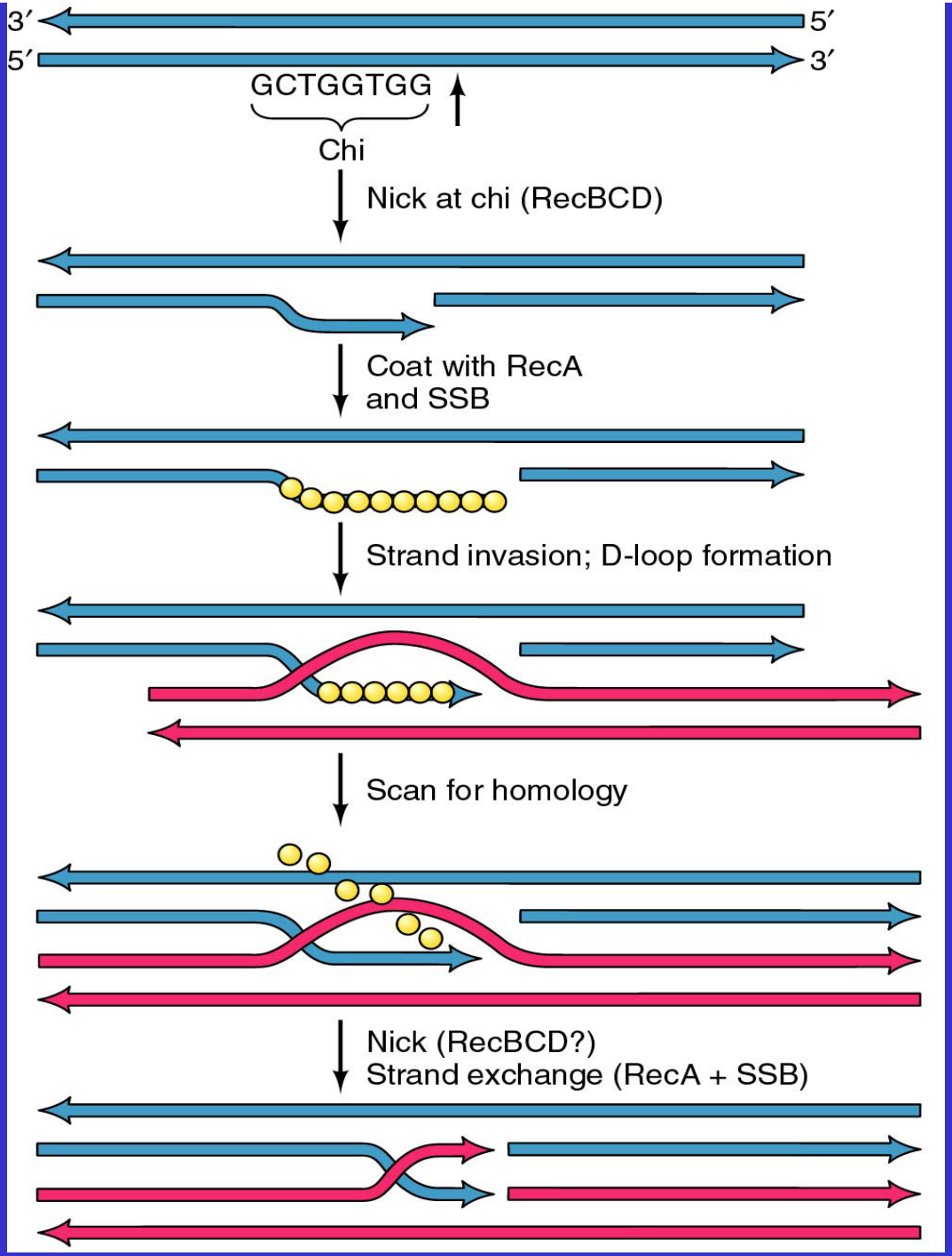
- 3 steps of strand exchange:

1. Pre-synapsis: *recA* coats single stranded DNA (accelerated by SSB, get more relaxed structure)
2. Synapsis: alignment of complementary sequences in SS and DS DNA

3. Post-synapsis or strand-exchange: SS DNA replaces the same strand in the duplex to form a new DS DNA (requires ATP hydrolysis)

The *recBCD* Pathway of Homologous Recombination

Part I: Nicking and Exchanging

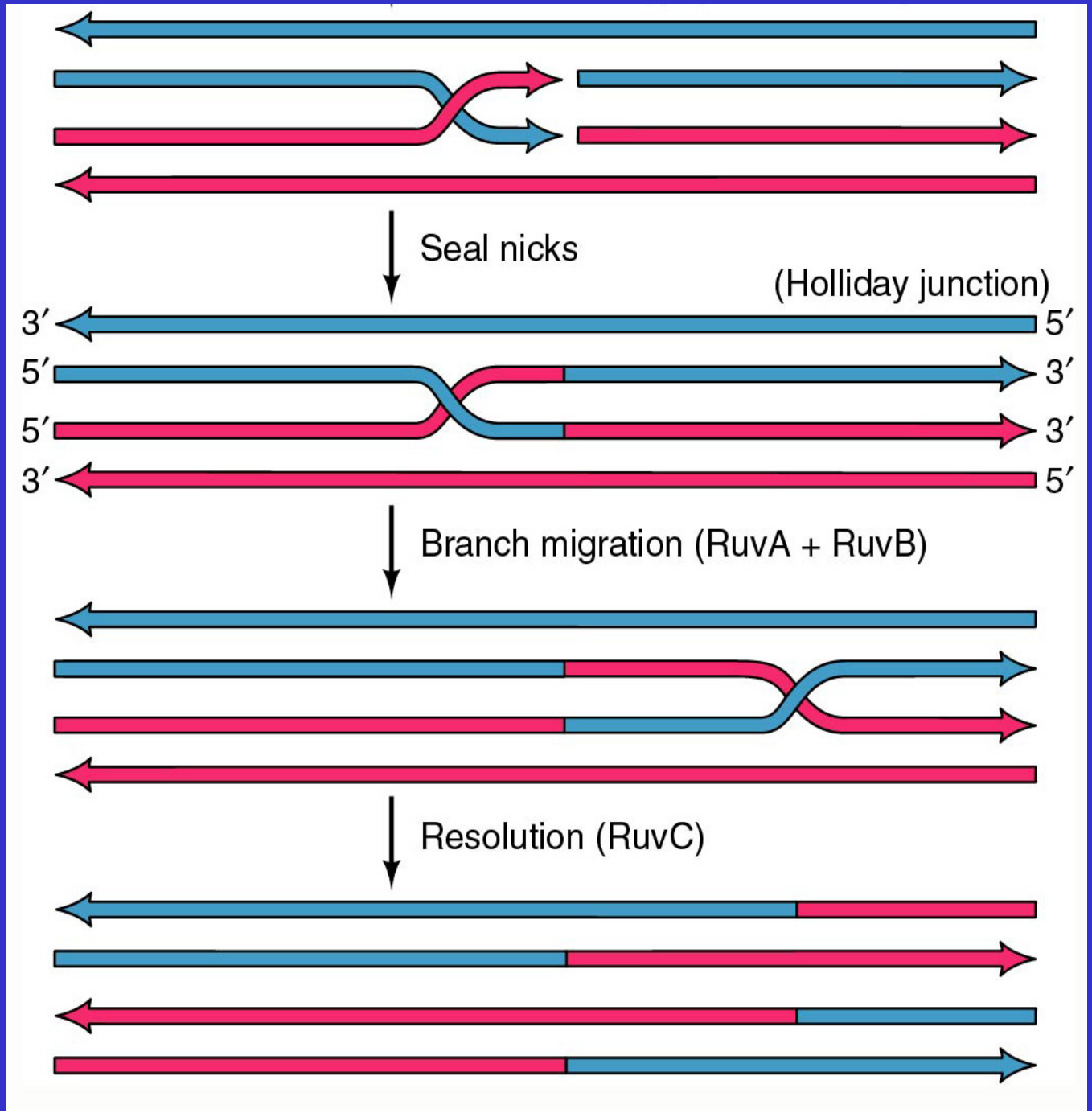


recBCD Pathway of Homologous Recombination
Part I: Nicking and Exchanging

1. A nick is created in one strand by *recBCD* at a *Chi* sequence (GCTGGTGG), found every 5000 bp.
2. Unwinding of DNA containing *Chi* sequence by *recBCD* allows binding of SSB and *recA*.
3. *recA* promotes strand invasion into homologous DNA, displacing one strand.
4. The displaced strand base-pairs with the single strand left behind on the other chromosome.
5. The displaced and now paired strand is nicked (by *recBCD?*) to complete strand exchange.

recBCD Pathway of Homologous Recombination

Part II: Branch Migration and Resolution



recBCD Pathway of Homologous Rec. Part II: Branch Migration and Resolution

1. Nicks are sealed → Holliday Junction
2. Branch migration (*ruvA* + *ruvB*)
3. Resolution of Holliday Junction (*ruvC*)

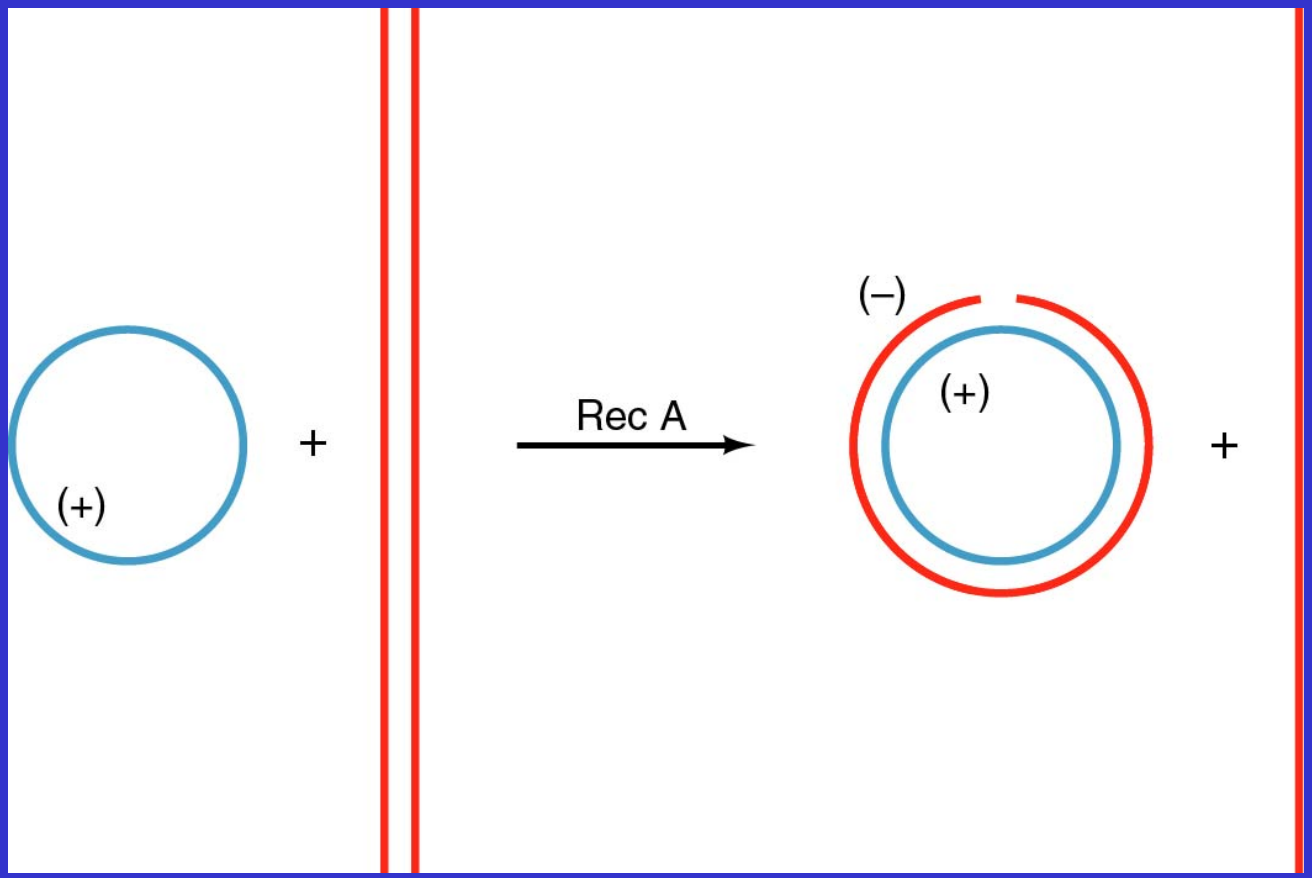
RecBCD : A complex enzyme

- *RecBCD* has:

1. Endonuclease subunits (*recBCD*) that cut one DNA strand close to Chi sequence.
2. DNA helicase activity (*recBC* subunit) and
3. DNA-dependent ATPase activity
   - unwinds DNA to generate SS regions

RecA

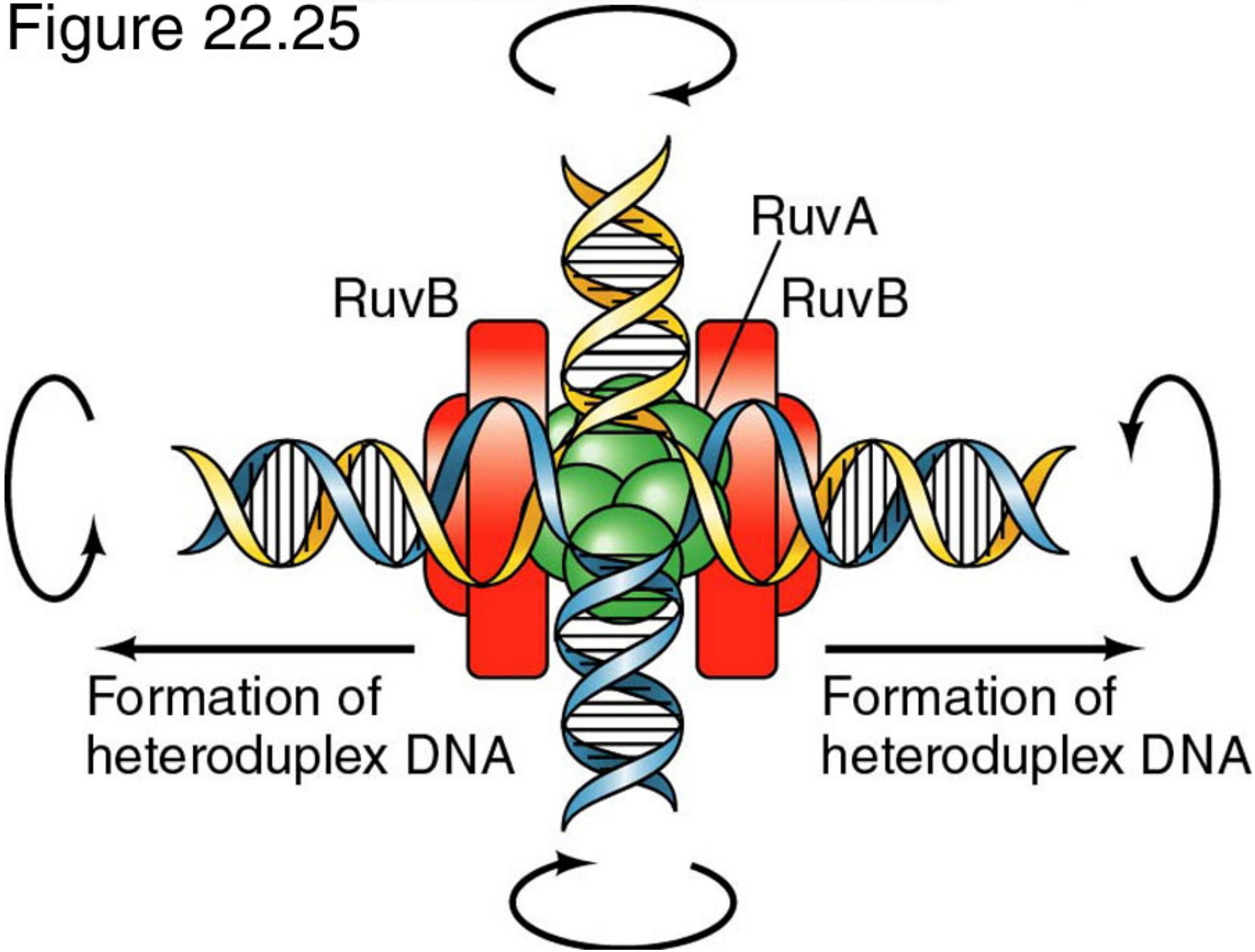
- 38 kDa protein that polymerizes onto SS DNA 5’-3’
- Catalyzes strand exchange, also an ATPase
- Also binds DS DNA, but not as strongly as SS

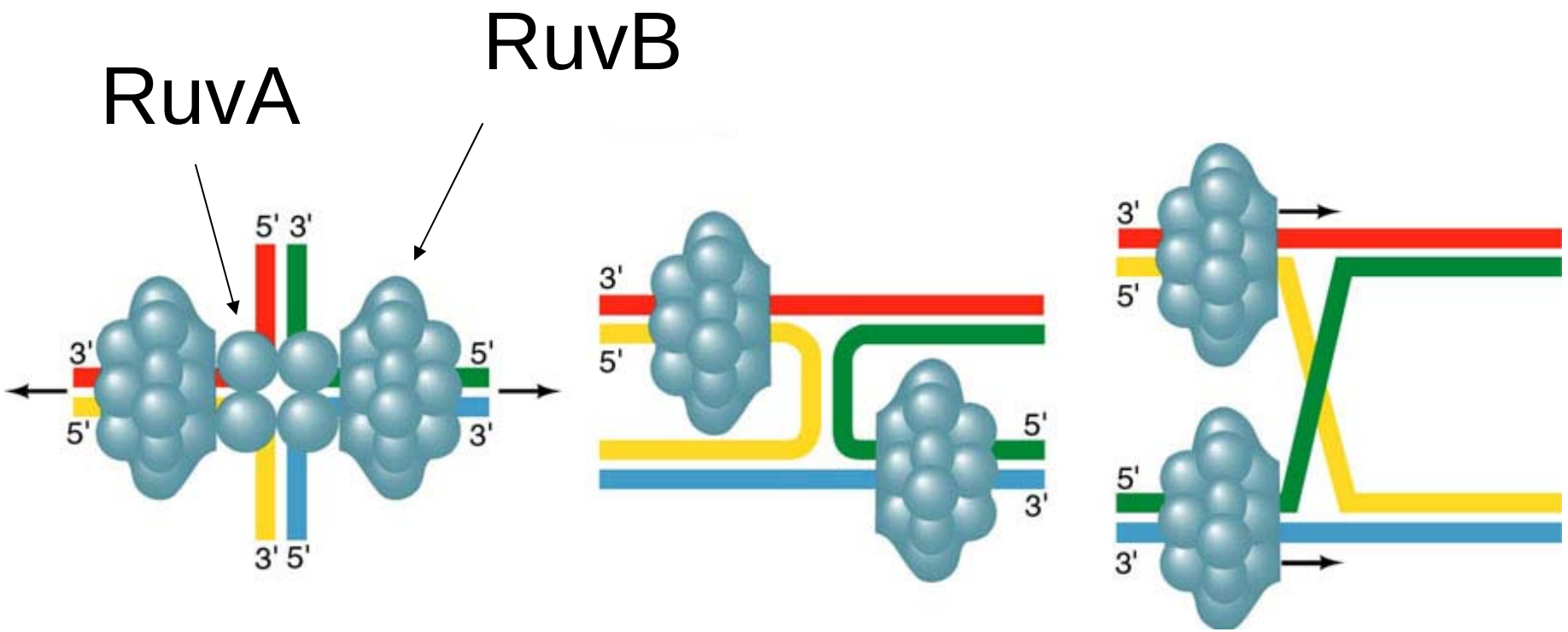


RuvA and *RuvB*

- DNA helicase that catalyzes branch migration
- *RuvA* tetramer binds to HJ (each DNA helix between subunits)
- *RuvB* is a hexamer ring, has helicase & ATPase activity
- 2 copies of *ruvB* bind at the HJ (to *ruvA* and 2 of the DNA helices)
- Branch migration is in the direction of *recA* mediated strand-exchange

Figure 22.25

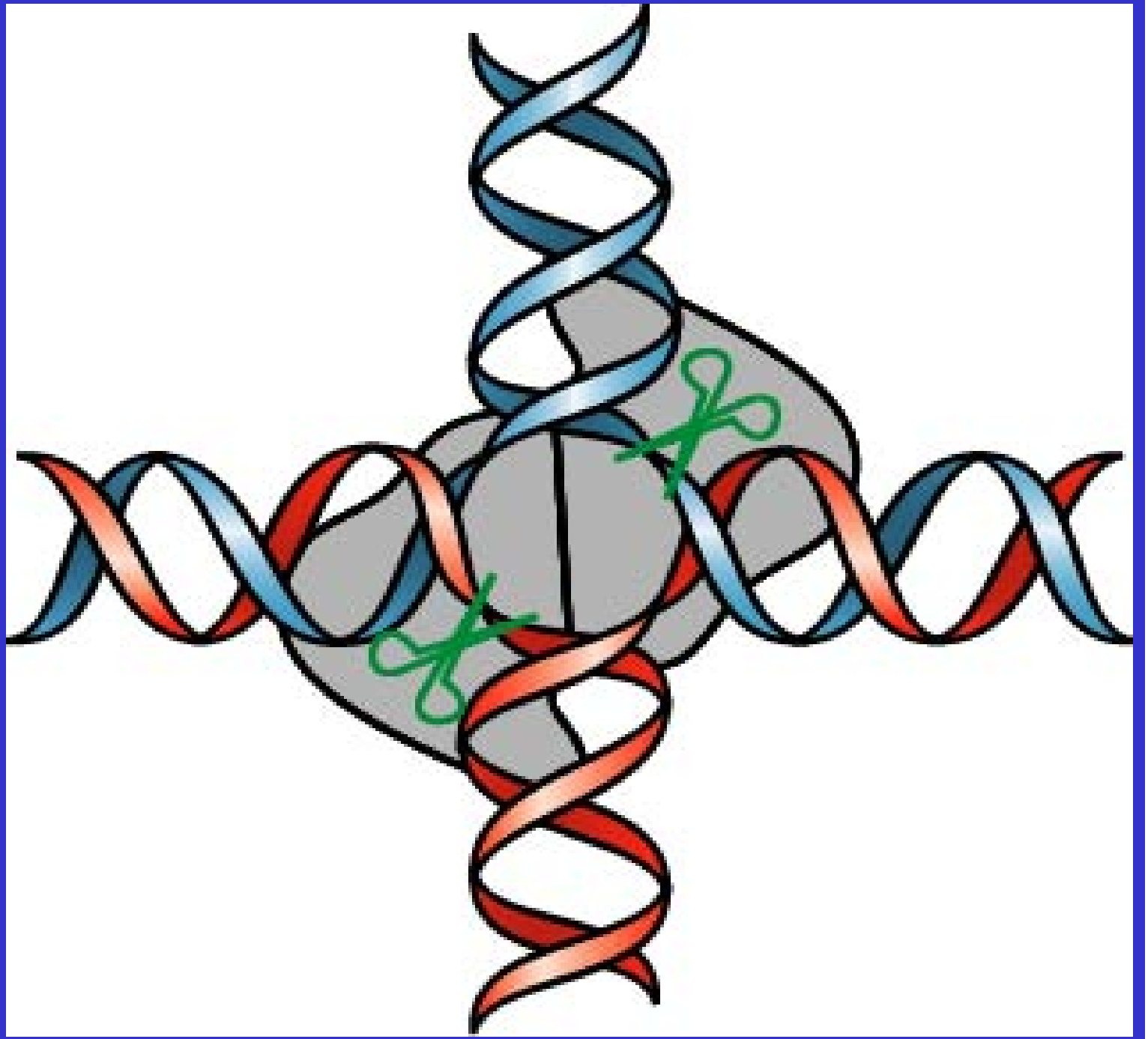



RuvA
RuvB
5' 3'
3'
5'
3' 5'
3'
5'
5'
3'
3'
5'
5'
3'
5'
3'
3'
5'

RuvC : resolvase

- Endonuclease that cuts 2 strands of HJ
- Binds to HJ as a dimer
- Consensus sequence: (A/T)TT ↓ (G/C)
  - occurs frequently in *E. coli* genome
  - branch migration needed to reach consensus sequence!

RuvC bound to Holliday junction



Thanks