

**MOLECULAR BIOLOGY**  
**FOS 730**

**RECOMBINATION & REPAIR**  
**OF DNA**

# MUTABILITY & REPAIR OF DNA

Mutation: change in DNA

Sources of mutation

- replication errors
  - misincorporation of bases due to “tautomeric flickering”
- chemical damage
  - spontaneous
  - induced

# REPLICATION ERRORS & THEIR REPAIR

## MUTATIONS

- base substitution
  - **point mutations**
    - *transitions*
      - pyrimidine-to-pyrimidine substitutions (T→C)
      - purine-to-purine substitutions (A→G)
    - *transversions*
      - pyrimidine-to-purine substitutions (T→G or A)
      - purine-to-pyrimidine substitutions (A→C or T)
- insertions or deletions of a small number of nucleotides
  - **frame shift mutations**
    - recombination errors
    - transposons

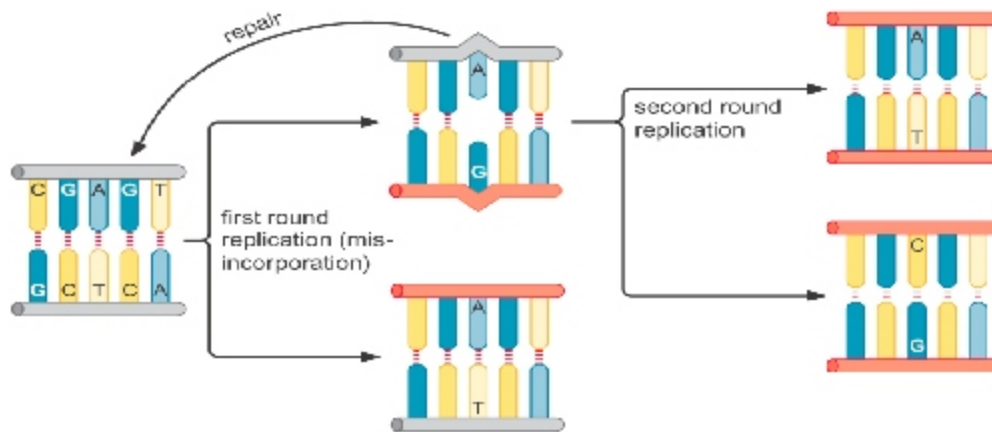
Spontaneous rate of mutation at a given site on a chromosome

- $10^{-6}$  to  $10^{-11}$  per round of replication
  - species and site specific
  - “hot spots”
    - DNA microsatellites
      - repetitive sequences errors due to “slippage” of DNA polymerase during replication
  - low frequency sites

# REPLICATION ERRORS & THEIR REPAIR

DNA polymerase (*E. coli*)

- inserts one incorrect nucleotide for every  $10^5$  nucleotides
  - due to tautomeric “flickering” of the bases
- **proofreading exonuclease** remove incorrectly base-paired nucleotides
  - degrade DNA in a 3'→5' direction
  - polymerase can add the correct nucleotide
    - error rate reduced to 1 mistake in every  $10^7$  base pairs added
    - *note* rate is further reduced to  $10^{10}$  post-replication mismatch repair



# REPLICATION ERRORS & THEIR REPAIR

## MISMATCH REPAIR SYSTEM

- repairs errors that escape proofreading
- *E. coli*
  - Mut S, L, H (mutator) system

# REPLICATION ERRORS & THEIR REPAIR

**MutS** dimer scans DNA for mismatches which distort DNA backbone

binds ATP conformational change in MutS bends the DNA

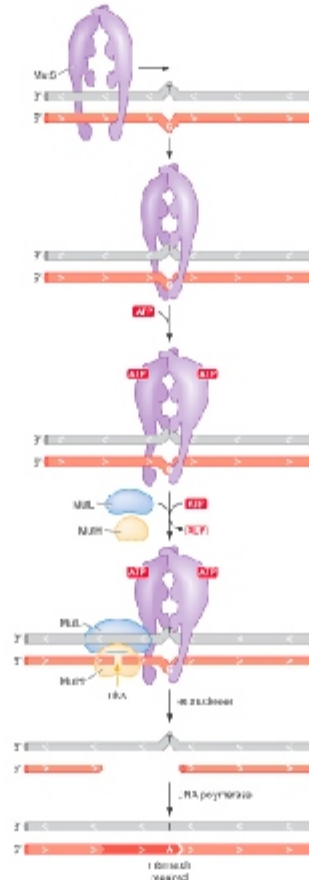
ATP•MutS complex recruits **MutL** & **MutH**  
ATP hydrolysis required for loading

MutL activates **MutH** **endonuclease** activity nicks one strand near the mismatch

DNA is unwound by **helicase UvrD** from the incision to the site of the mismatch

**exonuclease** digests displaced strand

gap filled in by **Pol III**  
nick sealed by **ligase**

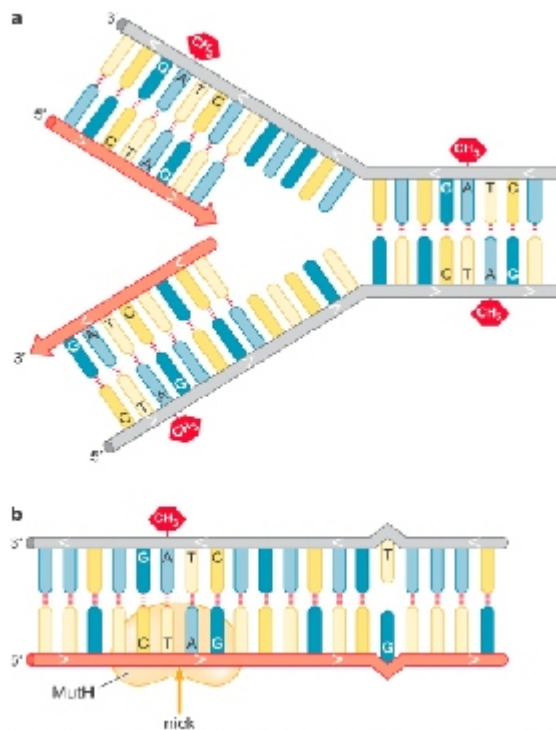


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# REPLICATION ERRORS & THEIR REPAIR

Recognition of damaged DNA strand (*E. coli*)

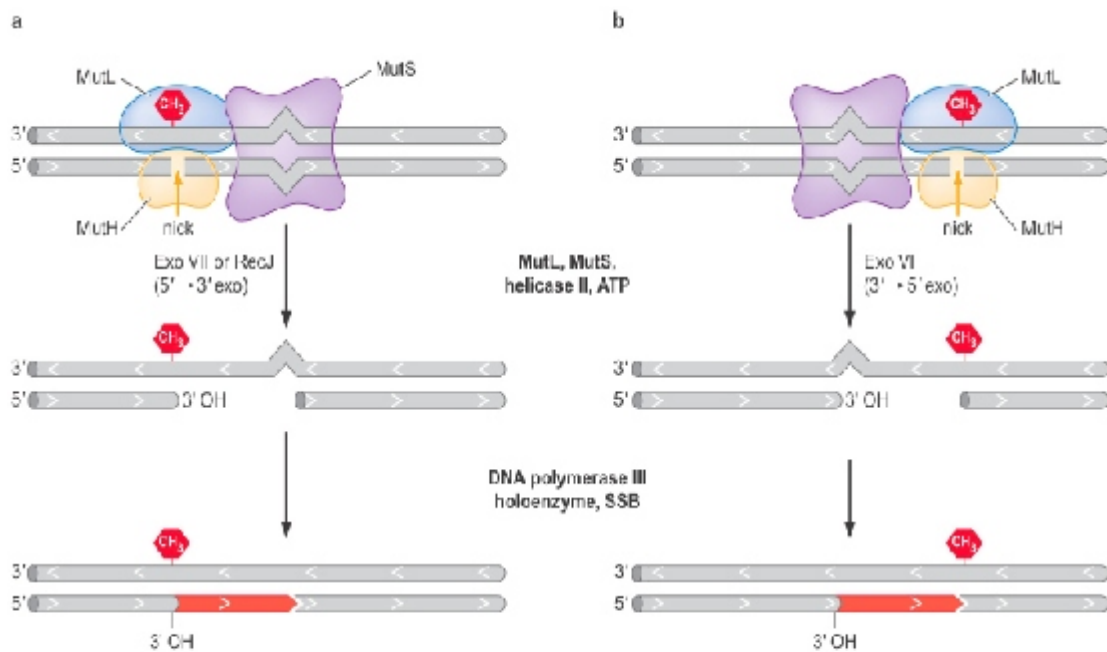
- **Dam methylase** methylates A residues on both strands of 5'-GATC-3' sequences
  - occur once every 4<sup>4</sup> or 256 bases
- during replication daughter duplexes will be **hemimethylated** for a few minutes
  - marks the newly synthesized strand



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# REPLICATION ERRORS & THEIR REPAIR

- activated MutH nicks unmethylated strand
  - cuts on either the side of mismatch
    - 5' side
      - exonuclease VII or Rec degrade DNA 5'→3' direction
    - 3' side
      - exonuclease I degrade DNA 3'→5' direction



## REPLICATION ERRORS & THEIR REPAIR

- eukaryotes *homologous system*
  - **MSH** (Mut S homologs), **MLH & PMS** (Mut L homologs)
  - lack Mut H homologs
    - use Okazaki fragments to distinguish newly synthesized DNA
    - nick between Okazaki fragments equivalent to nick in created by E. coli MutH

# DNA DAMAGE

## Spontaneous

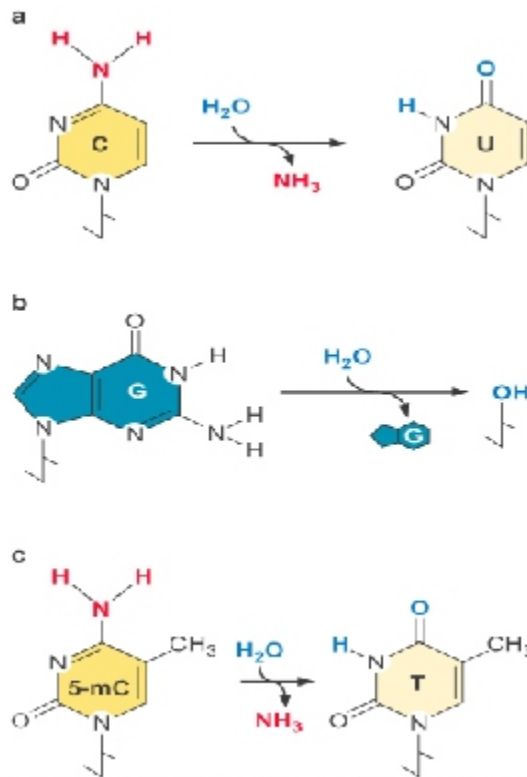
### - hydrolytic damage

#### - deamination

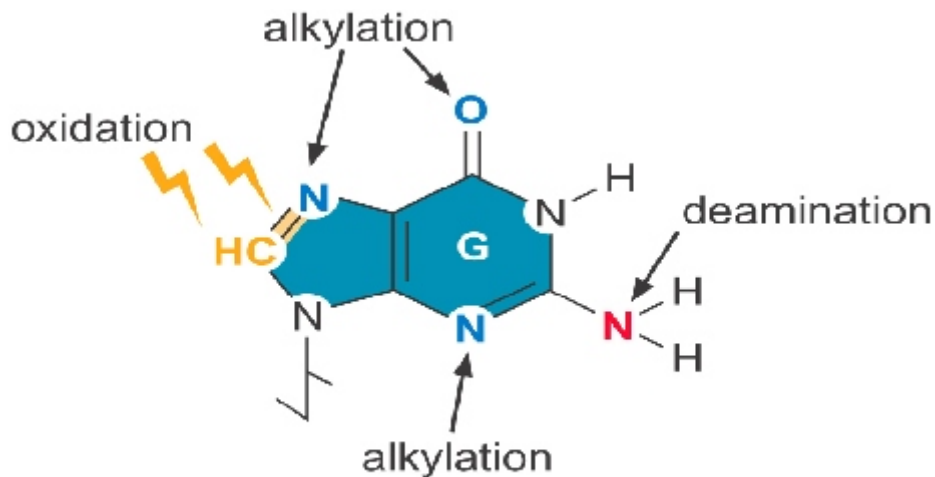
- cytosine to uracil (C→G to U→A)
- adenine to hypoxanthine (A→T to H→C)
- guanine to xanthine (still base pairs to C but with only two hydrogen bonds)
- 5-methyl cytosine to thymine (C→G to T→A)

#### - depurination/depyrimidination

- cleaves the N-glycosyl bond generating an abasic site



# DNA DAMAGE



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## Induced via *mutagens*

### - alkylation

- addition of methyl or ethyl groups to the bases or to the phosphates on the DNA backbone
- agents such as nitrosamines and N-methyl-N<sup>1</sup>-nitro-N-nitrosoguanidine

### - oxidation

- attack by reactive oxygen species ( $O^{2-}$ ,  $H_2O_2$  and  $OH\bullet$ ) produced by oxidizing agents and ionizing radiation
- oxidation of guanine generates 7,8-dihydro-8-oxoguanine (oxoG) which can base-pair with C or A

# DNA DAMAGE

## - radiation

- ultraviolet light

- wavelength of 260 nm strongly absorbed by the bases

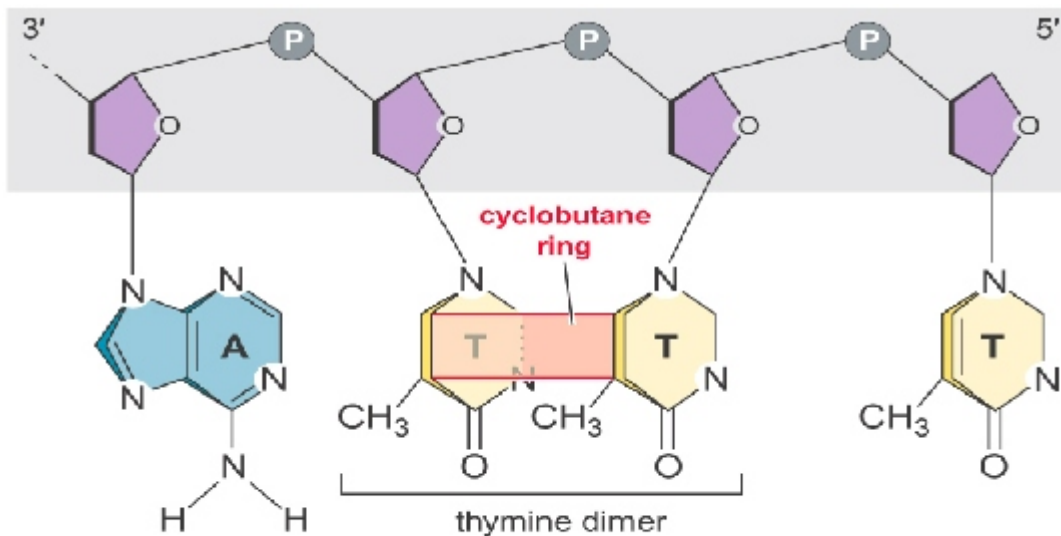
- photochemical fusion of adjacent pyrimidines on the same chain

- **thymine dimer**

- **cyclobutane** ring generated by covalent links

- between carbon atoms 5 and 6 on adjacent thymines

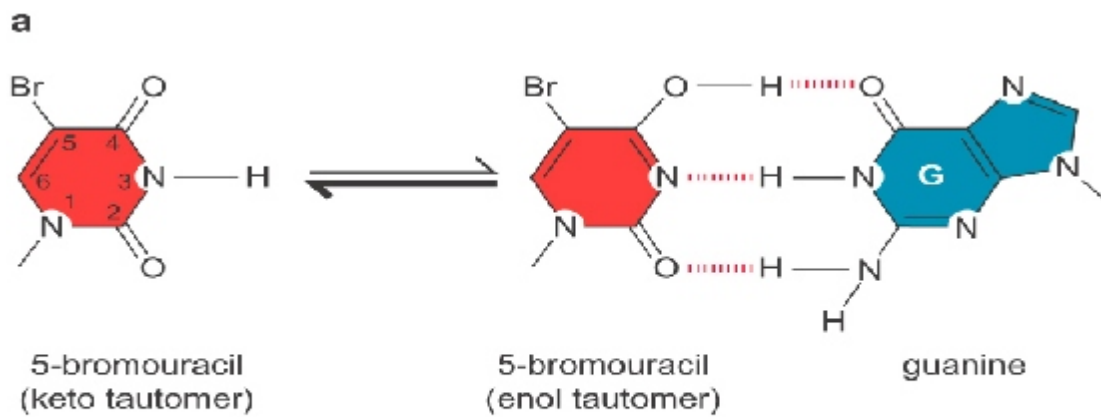
- gamma and X-rays (ionizing) cause ds breaks in DNA



# DNA DAMAGE

## Base analogs

- 5-bromouracil (thymine analog)
- tautomerization results in mispairing with guanine

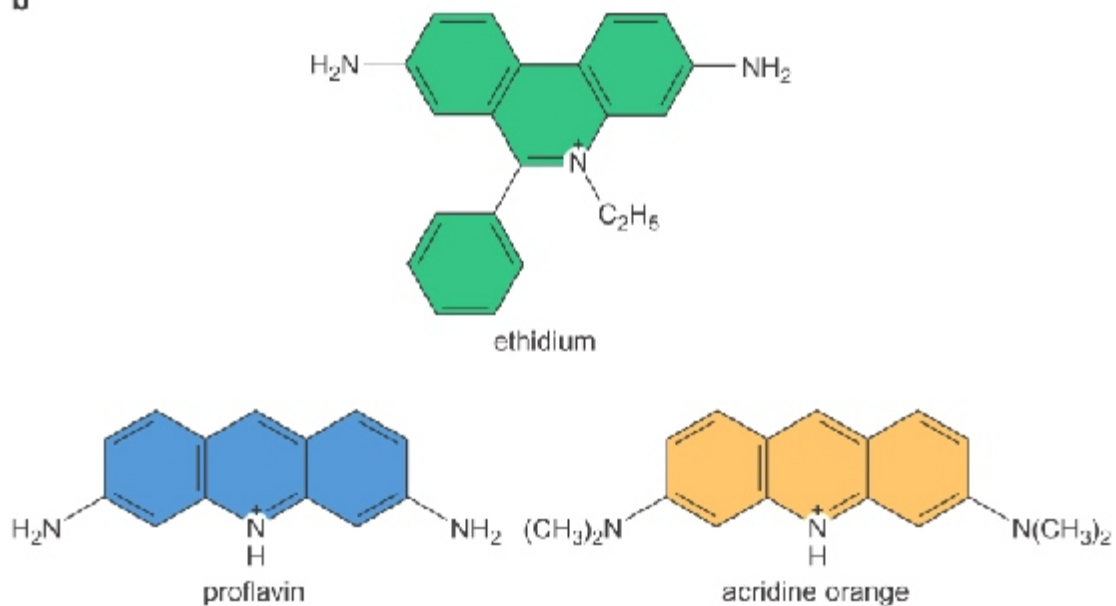


# DNA DAMAGE

## Intercalating agents

- flat molecules with polycyclic rings that bind to the DNA bases (slip in between the bases)
- proflavin, acridine orange, and ethidium
  - distort DNA template
  - errors during replication result in addition or deletion of a single or a few base pairs

b



## **REPAIR OF DAMAGED DNA**

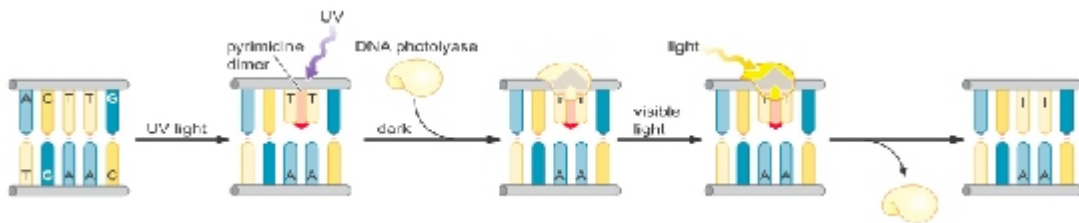
Several systems

- direct reversal
- excision repair
- recombinational repair (double-strand break repair)
- translesion DNA synthesis

# REPAIR OF DAMAGED DNA

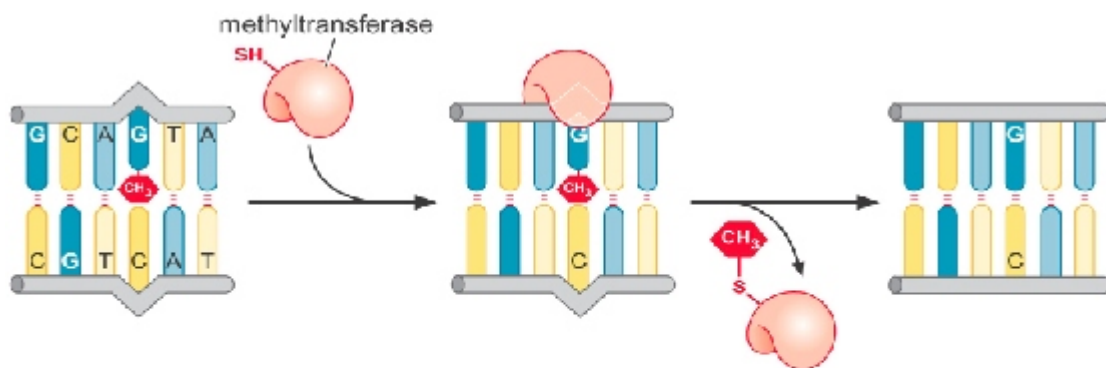
## Direct reversal of DNA damage

- **photoreactivation** direct reversal of pyrimidine dimers
  - DNA photolyase uses the energy from light to break the covalent bonds between adducts
- found in prokaryotes and eukaryotes (particularly plants)



## REPAIR OF DAMAGED DNA

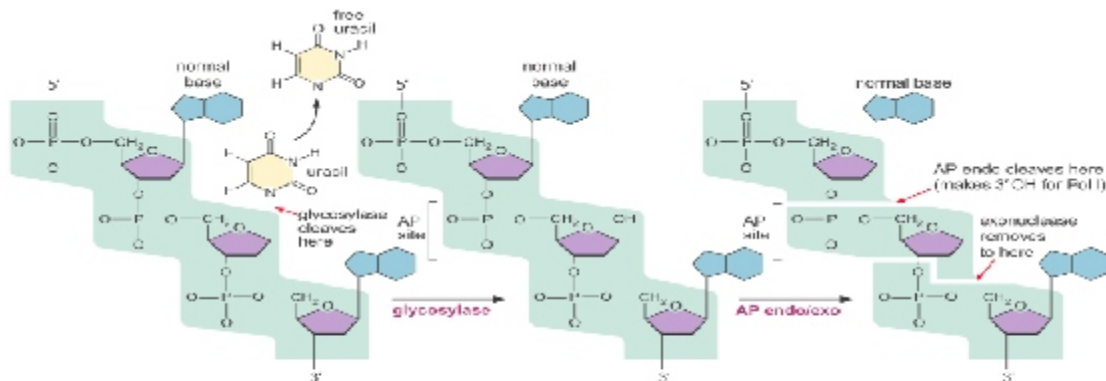
- removal of the methyl group from a methylated base
  - methylated base in O<sup>6</sup>-methylguanine by a methyltransferase
    - methyl group transferred to a cysteine residue on the enzyme
  - inactivates the enzyme (not catalytic)



# REPAIR OF DAMAGED DNA

## Base excision repair

- **glycosylase** recognizes and removes the damaged base by hydrolyzing the glycosidic bond
  - flip out base so that glycosyl bond is position in the active site
  - multiple glycosylases
    - lesion specific
- abasic sugar removed from the backbone by an
  - AP (apurinic/aprimidinic) endonuclease cleaves the backbone 5' to the AP site
    - free 3' OH for Pol I
  - exonuclease removes a stretch of DNA surrounding the damage
    - leaves a 5' phosphate
- repair by DNA polymerase and ligase
- prokaryotes and eukaryotes



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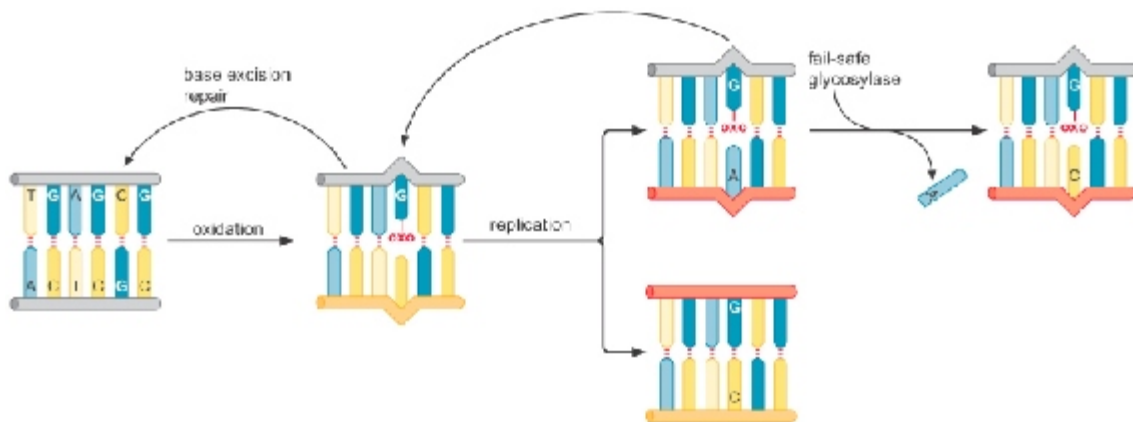
uracil glycosylase reaction

# REPAIR OF DAMAGED DNA

## Base excision repair

### Fail safe mechanism

- if lesion is not repaired before replication glycosylases repair
- oxoG:A base pair by removing A
- T:G mismatch repaired by removing T
  - arises from the deamination of 5'methyl-cytosine to T



# REPAIR OF DAMAGED DNA

## Nucleotide excision repair

- system recognizes distortions in the shape of the double helix
  - genome wide lesions
  - lesions on the template strand of DNA during transcription
    - transcription-coupled repair
- Uvr system in *E. coli*
- homologous XP (xeroderma pigmentosa) proteins in humans

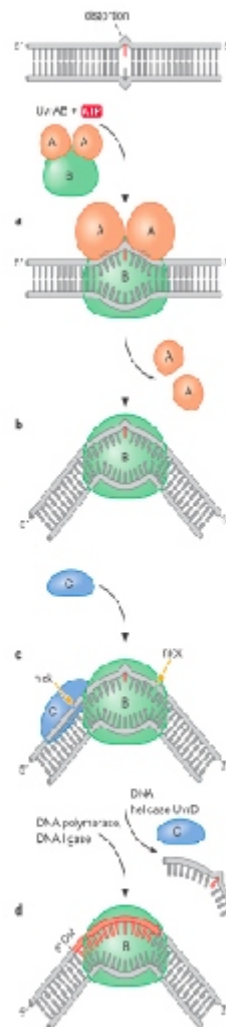
UvrA/UvrB complex scans DNA for distortions  
ATP required for assembly

UvrA detects damage & dissociates from DNA  
UvrB melts DNA creating a ss bubble

UvrB recruits UvrC  
UvrC endonuclease nicks the backbone 5' and 3' to the site of the lesion (12-13 residue long ss region)

UvrD helicase recruited  
Releases ss fragment

Repair by Pol I and ligase



# REPAIR OF DAMAGED DNA

## **Double-strand break (DSB) repair pathway**

- both strands damaged
- retrieves information from the sister chromatid
  - homologous recombination
- recA and recBCD in E. coil

Fail safe mechanism when damage occurs early in the cell cycle before synthesis of the sister chromatid

- nonhomologous end joining (NEJ)
  - does not involve homologous recombination
- two ends of broken DNA directly joined to each other by misalignment between the ss protruding from the broken ends
  - serendipitous microhomologies due to base pairing stretches as short as one base pair
- gaps filled in by DNA polymerase
- nicks sealed by ligase

# REPAIR OF DAMAGED DNA

## Translesion DNA synthesis

- bypass lesions encountered during replication
- Y family DNA polymerases (UmuC) in *E.coli*
  - error-prone
    - template dependent incorporation of nucleotides independent of base pairing
- genes encoding translesion polymerase are induced
  - expressed as part of the SOS response
    - regulon (global pathway that responds to DNA damage)

# HOMOLOGOUS RECOMBINATION

## **Homologous recombination** required

- genetic variation
  - recombination between the bacterial chromosome and DNA that enters via phage infection or conjugation
- retrieval of information lost through DNA damage
- restarting stalled or damaged replication forks
- repairing ds breaks in DNA
- critical for meiosis in eukaryotes
  - required for proper chromosome pairing
  - reshuffles genes between parental chromosomes
    - genetic variation

# HOMOLOGOUS RECOMBINATION

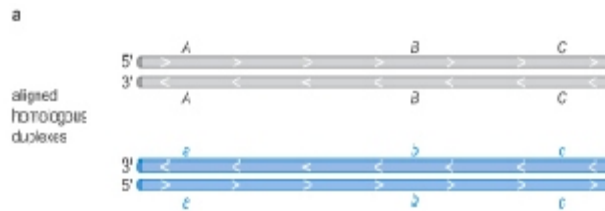
## MODELS

- several
- share key steps
  - alignment of two homologous molecules
    - sequences identical or nearly identical for a region of at least 100 bp
    - small regions of sequence differences
      - alleles
  - introduction of breaks in DNA
    - one or both strands
  - formation of initial short regions of base pairing
    - ss region of DNA from one parental molecule pairs with the complementary region in the homologous duplex
      - **strand invasion**
        - generate a cross structure called a **Holliday junction**
  - branch migration
  - resolution
    - cleavage of the Holliday junction to generate two separate duplex DNA molecules
    - joining of cut fragments

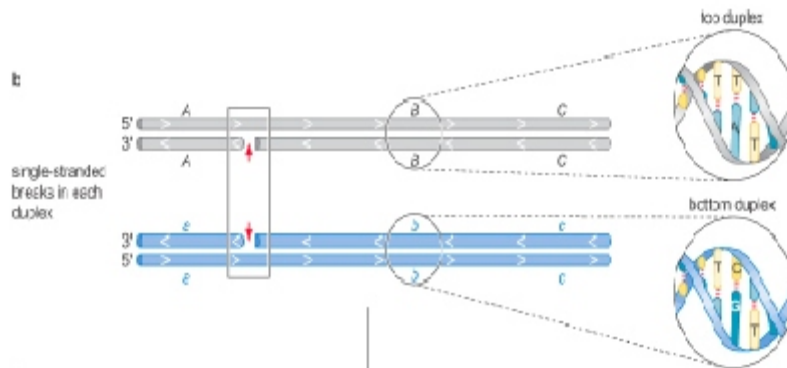
# HOMOLOGOUS RECOMBINATION

## HOLLIDAY MODEL

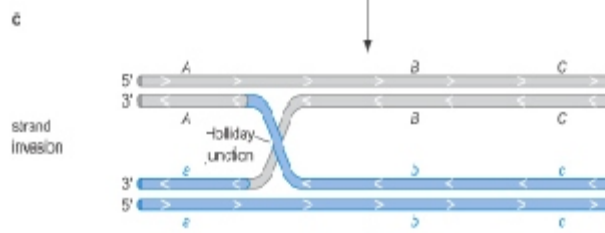
Alignment of homologous regions



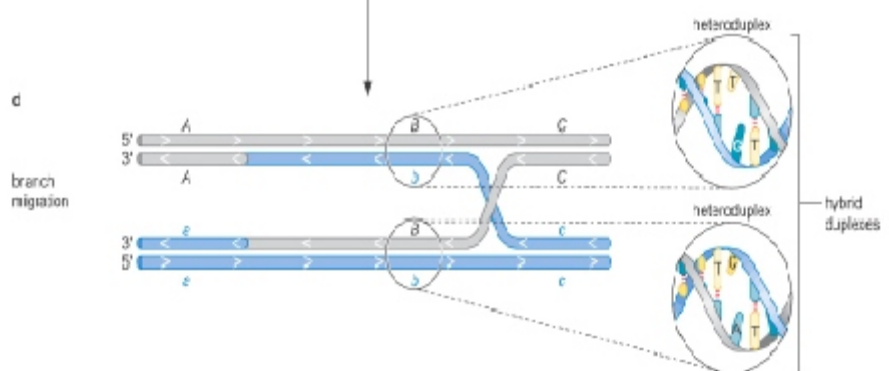
One strand of each duplex is nicked at an identical location



Nicked strands invade the duplex  
Holliday junction created



Branch migration of the Holliday junction  
Heteroduplex (one or a few sequence mismatches) regions generated



# HOMOLOGOUS RECOMBINATION

## HOLLIDAY MODEL: RESOLUTION

Resolution: cleavage and joining

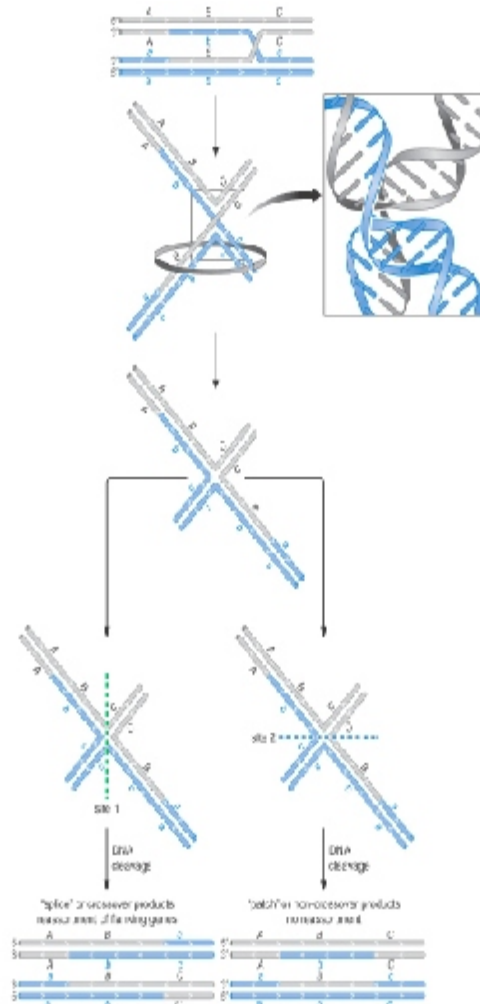
- occurs in one of two different ways which give rise to distinct classes of products
  - cleavage and joining of the two DNA strands that were *not broken* during the initial reaction generates **splice** or **crossover products** that results in reassortment of genes that flank the site of recombination
  - cleavage and joining of the two DNA strands that were broken during the initial reaction generates **patch products** or **non-crossover** products that contain a patch of hybrid DNA that does not result in reassortment of genes flanking the site of initial cleavage

# HOMOLOGOUS RECOMBINATION

## HOLLIDAY MODEL: RESOLUTION

cut unbroken strands

cut broken strands



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Splice products

Patch products

# HOMOLOGOUS RECOMBINATION

## DOUBLE-STRANDED BREAK REPAIR PATHWAY

Alignment of chromosomes  
Double-stranded break (DSB) in  
one duplex

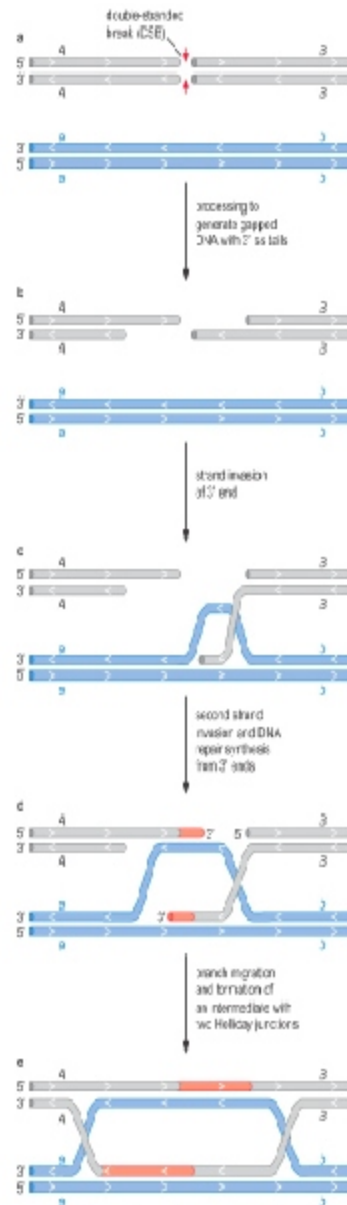
Ends processed by a DNA-  
cleaving enzyme to generate ss  
tails with 3' overhangs

Strand invasion by ss tails  
Both strands invade the  
homologous unbroken duplex

Sequence information lost by end  
processing is replaced by  
synthesis using the homologue as  
template

Nonreciprocal event  
Gene conversion- allele of a gene  
is lost and replaced by an  
alternative allele

Migration of the two Holliday  
junctions

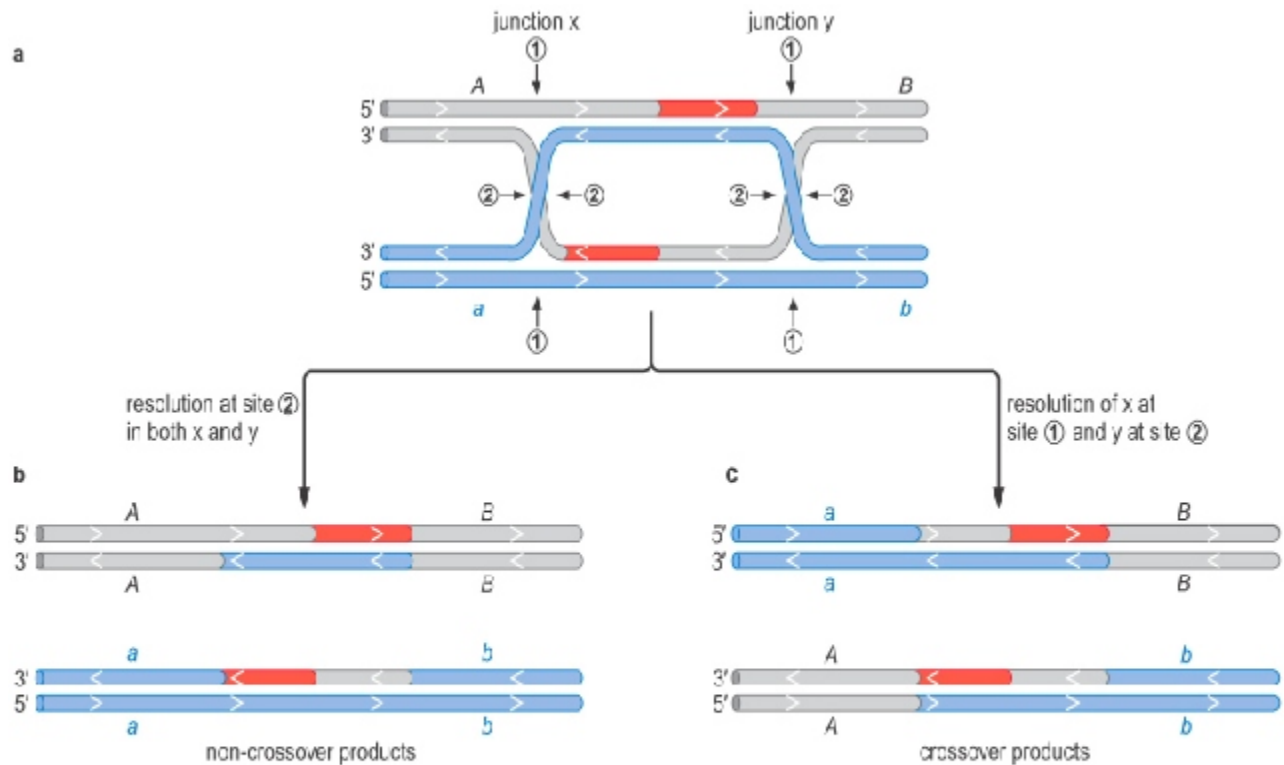


# HOMOLOGOUS RECOMBINATION

## DOUBLE-STRANDED BREAK REPAIR PATHWAY: RESOLUTION

### Resolution

- non-crossover products are generated if both junctions are cleaved in the same way



## **HOMOLOGOUS RECOMBINATION: PROTEIN PATHWAYS**

*E. coli*

- **RecBCD pathway**

- DSB-repair pathway

- no protein that introduces DSBs in DNA

- breaks generated as a result of damage or a failure of the replication fork

# HOMOLOGOUS RECOMBINATION: PROTEIN PATHWAYS

## RecBCD helicase/nuclease

Binds to site of ds break and tracks along DNA using energy of ATP hydrolysis

Rec B and D subunits are helicases each migrates on a different strand

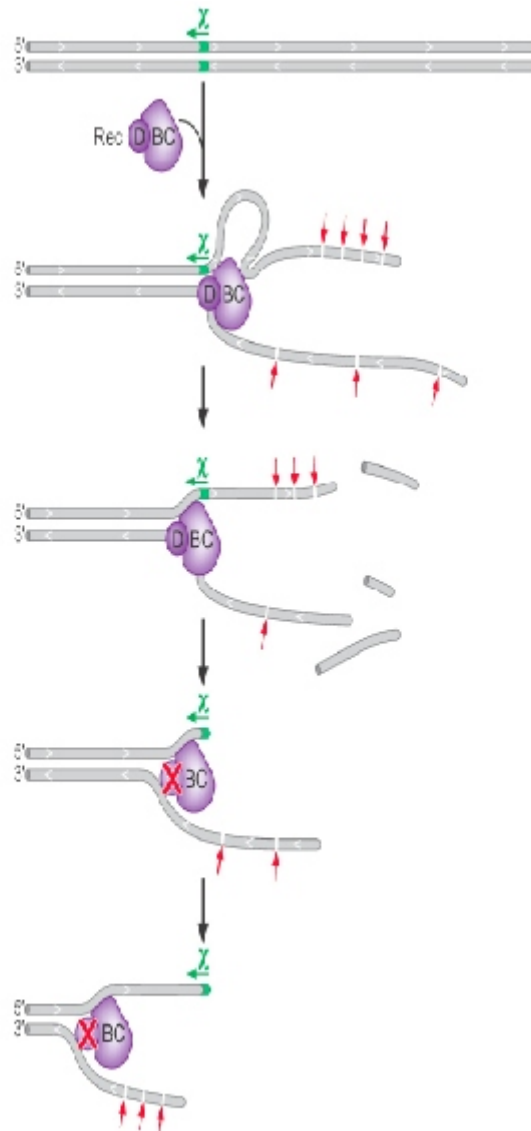
Cleavage activities controlled by **chi sites** (cross-over hotspot instigator, GCTGGTGG)

Sites function only in one orientation

Here chi site will only modify a RecBCD enzyme moving from right to left

After encounter with chi site RecD lost or inactivated

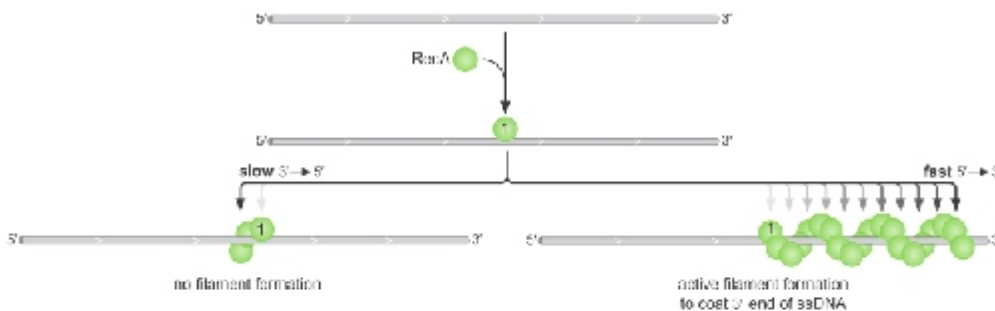
Differential rates of cleavage generate the 3' overhangs having chi sequences



# HOMOLOGOUS RECOMBINATION: PROTEIN PATHWAYS

## RecA

- strand-exchange protein
  - members in this family found in all organisms and phages
  - catalyzes the pairing of homologous DNA molecules
- active form is a protein-DNA filament
  - huge and variable in size
    - approximately 100 subunits of RecA and 300 nucleotides of DNA
  - accommodate one to four strands of DNA
    - DNA highly extended (1.5 fold that of B form)
- protein subunits bind cooperatively
  - more rapid on ss than ds DNA
  - filament grows in the 5' to 3' direction
    - DNA strands with 3' overhangs



# HOMOLOGOUS RECOMBINATION: PROTEIN PATHWAYS

## RecA filament

two binding sites: primary bound by first DNA (ss) molecule & secondary which can be occupied by a ds DNA

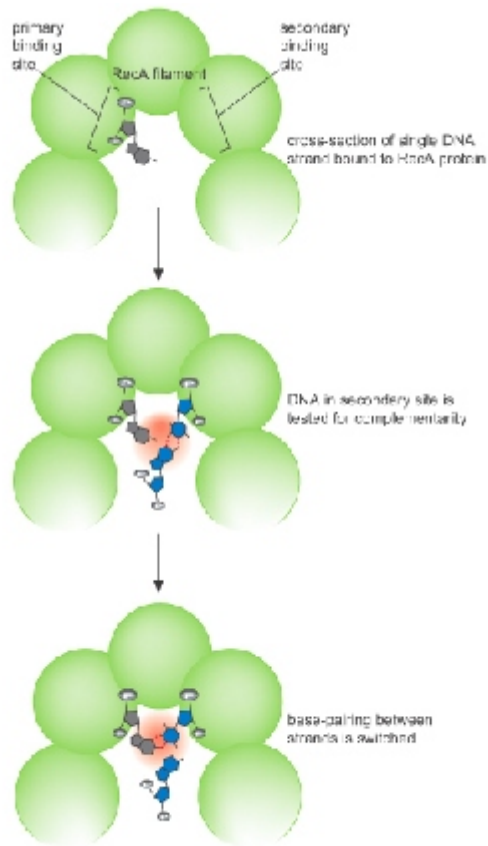
binding to secondary site is rapid, weak, transient and independent of DNA sequence

tested for complementarity with ss DNA in primary site  
match of 15 bp sufficient for strand exchange

joint molecule (RecA-three stranded DNA)

Strand exchange occurs: DNA in primary binding site becomes base paired with its complement in the secondary binding site

After strand exchange newly-paired strands intertwined to form a double helix.

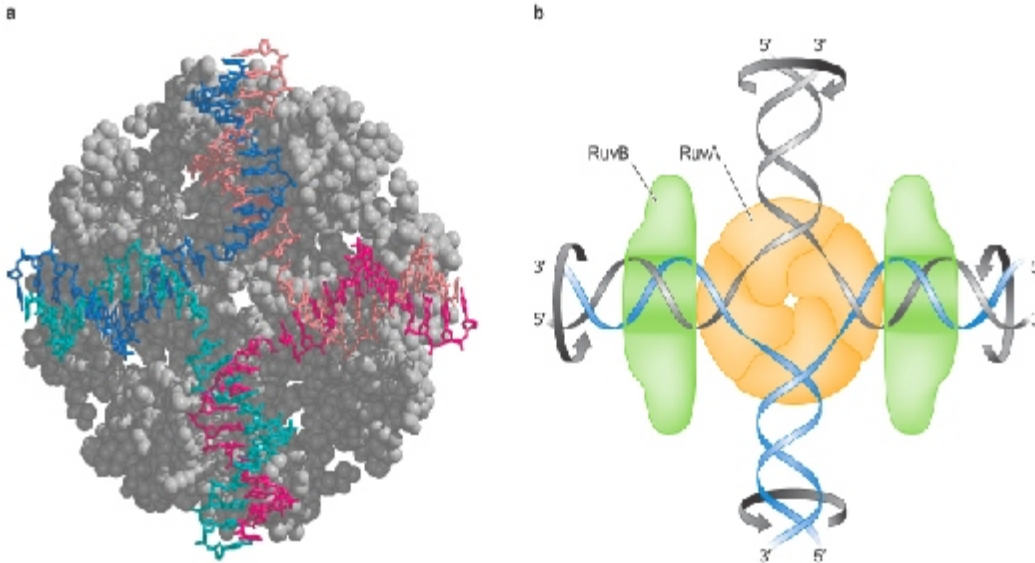


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# HOMOLOGOUS RECOMBINATION: PROTEIN PATHWAYS

## RuvAB complex

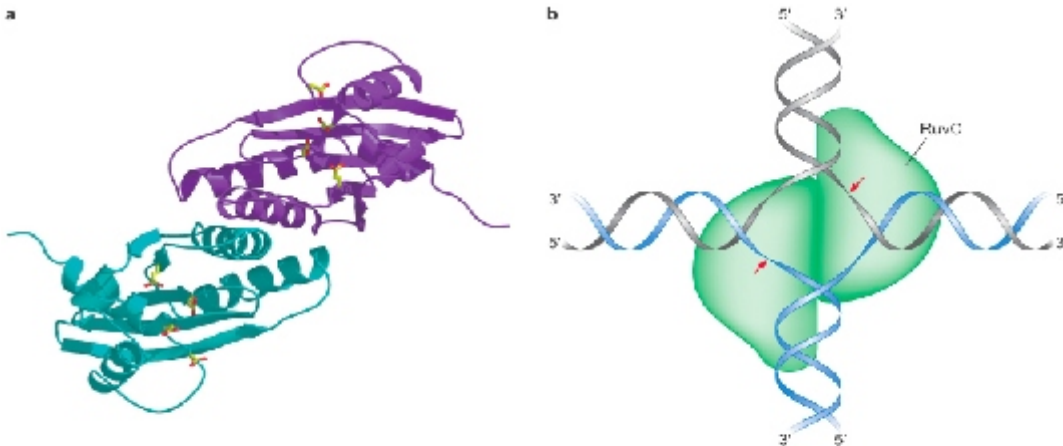
- RuvA<sub>4</sub>RuvB<sub>2</sub>
- **RuvA**
  - Holliday junction specific DNA binding protein
  - recruits RuvB
- **RuvB**
  - hexameric ATPase
  - provides energy to drive the exchange of base pairs for branch migration



# HOMOLOGOUS RECOMBINATION: PROTEIN PATHWAYS

## RuvC

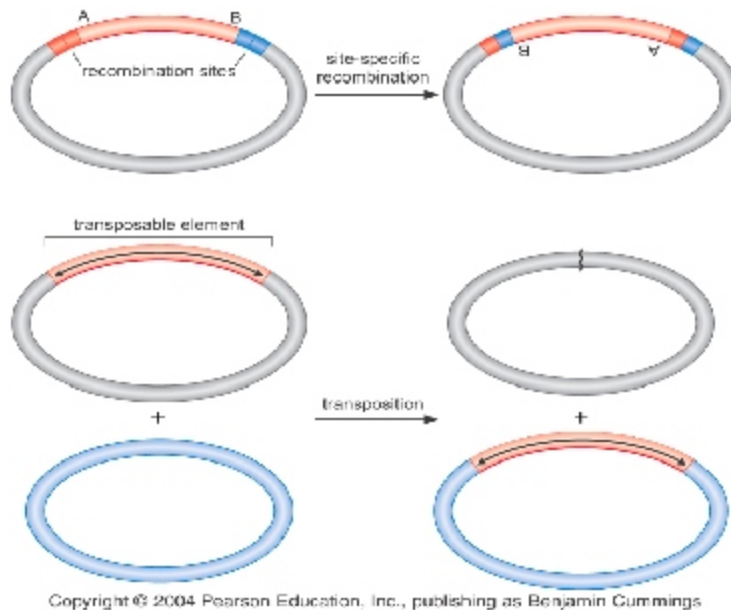
- dimer
- recognizes Holiday junction
- nicks two of the homologous strands that have the same polarity
  - consensus sequence 5'A/T-T-T-G/C
    - occurs once every 64 nucleotides
    - cleavage after second T
- cleavage results in DNA ends with 5' phosphates and 3'OH
  - substrates for DNA ligase



# SITE-SPECIFIC RECOMBINATION & TRANSPOSITION OF DNA

Two classes of genetic recombination:

- **conservative site-specific recombination (CSSR)**
  - between two defined sequence elements
- **transpositional recombination (transposition)**
  - between specific and nonspecific DNA sites



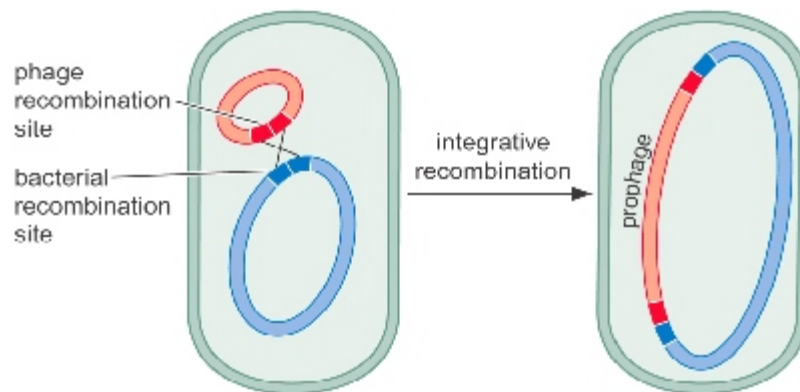
Share key mechanistic features:

- **recombinases** recognize specific sequences where recombination will occur within a DNA molecule
- formation of the **synaptic complex**
  - protein-DNA complex
  - cleavage and rejoining of DNA molecules to either invert a DNA segment or move a segment to another site

# CONSERVATIVE SITE-SPECIFIC RECOMBINATION

## Site-specific recombination

- occurs at specific DNA sequences in the target
- segment of DNA that will be moved carries **recombination sites**
  - short specific sequence elements
  - sites where DNA exchange occurs

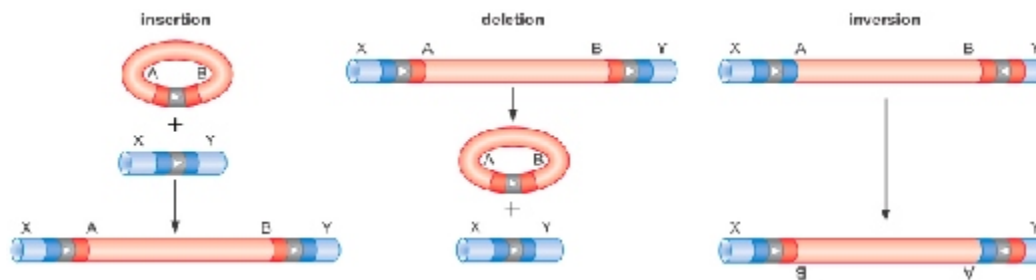


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## Integration of phage $\lambda$

# CONSERVATIVE SITE-SPECIFIC RECOMBINATION

CSSR generates three different types of arrangements depending on the organization of the recombination sites

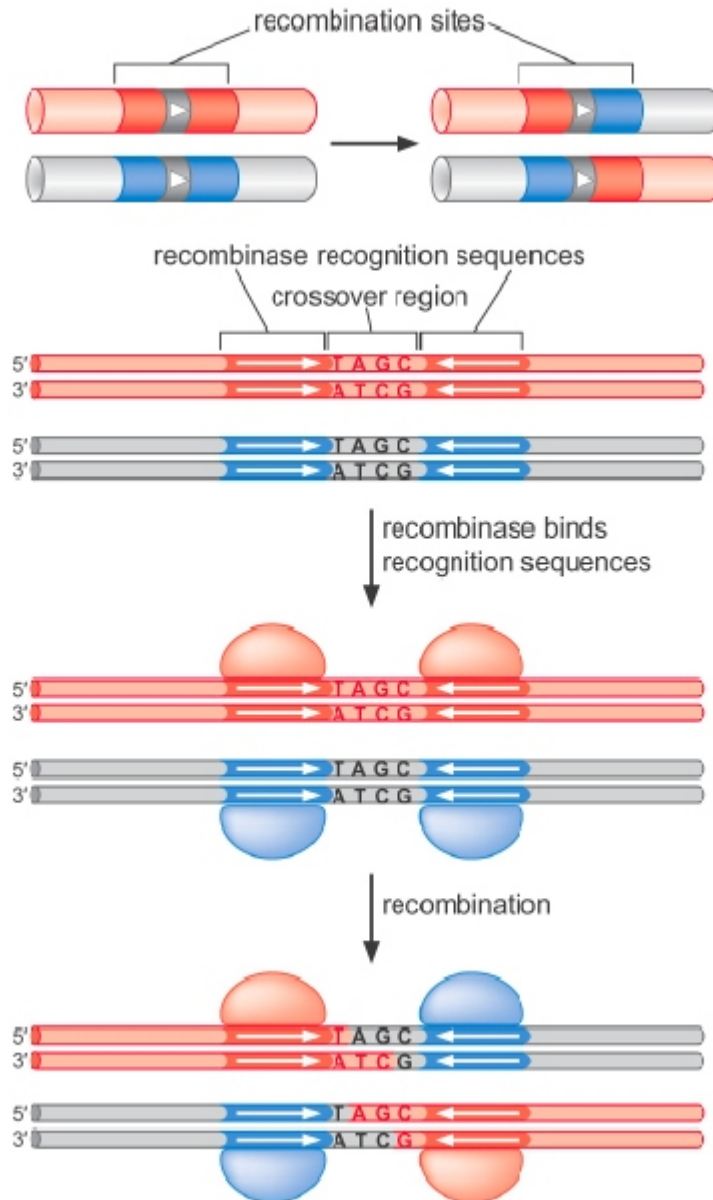


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## Recombination site

- pair of symmetrical **recombinase recognition sequences**
- central **crossover region**
  - short asymmetric sequence
    - polarity
  - cleavage and rejoining site
- orientation of two sites on a single molecule arranged as **direct or inverted repeats**
  - recombination between a pair of inverted repeats inverts the DNA segment between
  - recombination between a pair of direct repeats deletes the DNA segment between
  - recombination between sites on two different molecules inserts (integrates) DNA

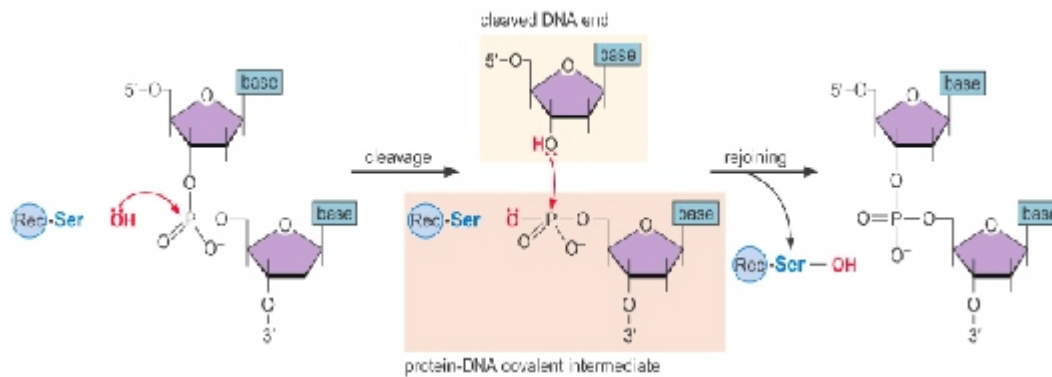
# CSSR STRUCTURES



# SITE-SPECIFIC RECOMBINASES

## CSSR recombinases

- cleave DNA generating a protein-DNA intermediate
  - conserves energy of cleaved phosphodiester bond
  - DNA strands joined by a reversal of the cleavage process
    - every DNA bond broken is resealed by the protein
- two classes
  - **serine recombinases**
  - **tyrosine recombinases**

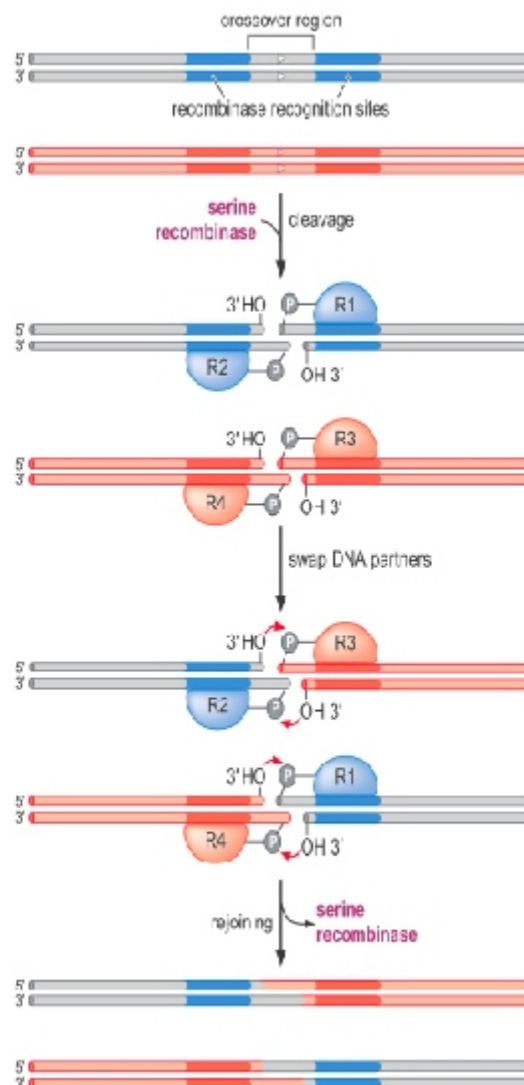


# SITE-SPECIFIC RECOMBINASES

**Serine recombinases** cleave all four strands prior to strand exchange- one molecule recombinase protein/cleavage  
- tetramer

Cleavage of two individual strands of one duplex staggered by two bases

two base region forms a hybrid duplex in the recombinant proteins



# SITE-SPECIFIC RECOMBINASES

**Tyrosine recombinases** cleave and rejoin two DNA strands

first and then cleave and rejoin the other two strands

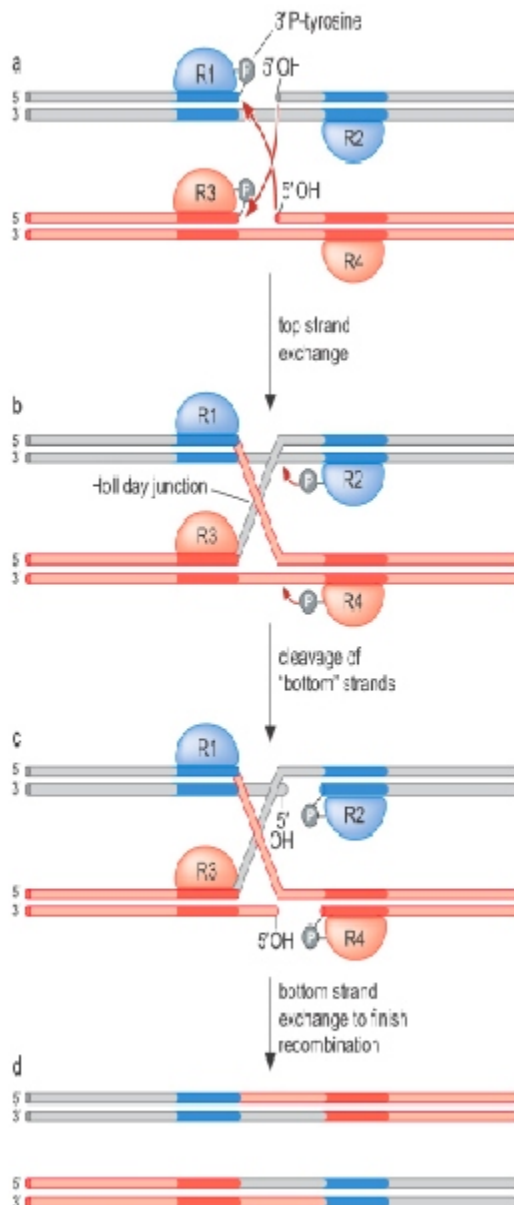
-one molecule recombinase protein/cleavage

- tetramer

Cleavage by R1 and R3 subunits

Strand exchange and rejoining generates a Holliday junction

Second recombination event undoes Holliday junction



# SITE-SPECIFIC RECOMBINASES

## TYROSINE RECOMBINASES: MECHANISM OF DNA EXCHANGE

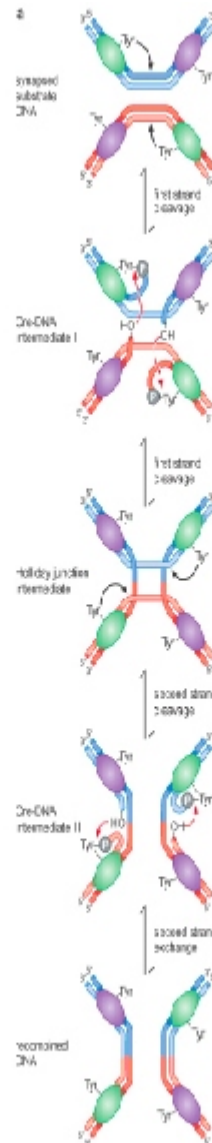
Cre-*lox* structures

Cre recombinase (phage P1)  
circularizes linear genome during  
infection

green = active conformation

purple = inactive conformation

recombination sites are called *lox*  
sites

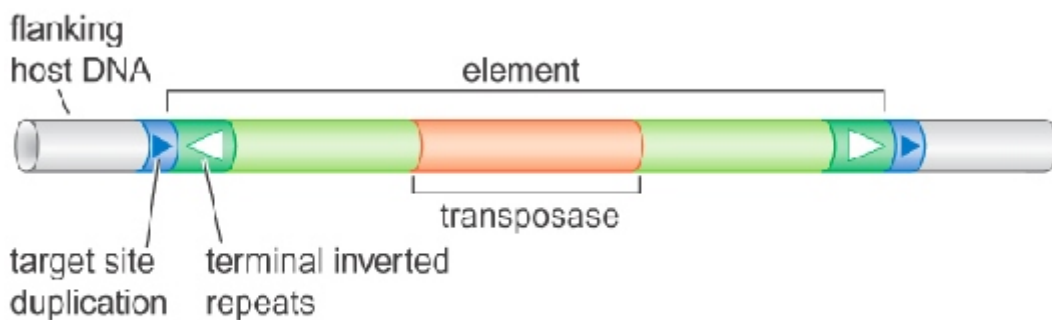


# TRANSPOSITION

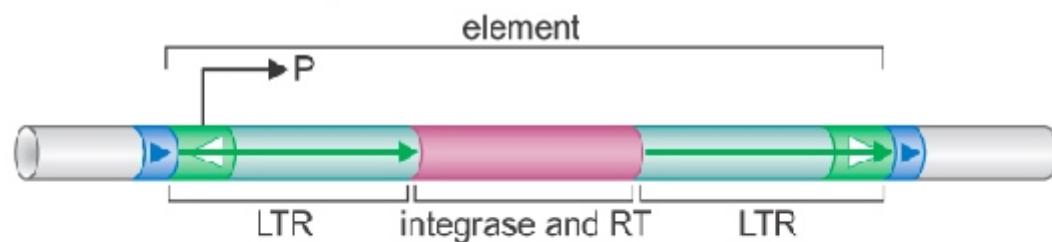
## Transposable elements

- DNA transposons
- RNA transposons
  - viral-like retrotransposons
    - LTR transposons
  - poly-A retrotransposons
    - nonviral retrotransposons

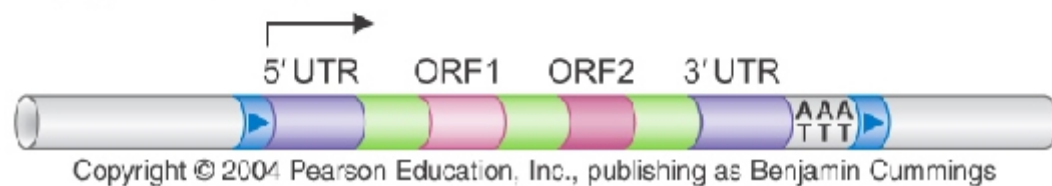
### a DNA transposons



### b viral-like retrotransposons/retroviruses



### c poly-A retrotransposons



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ORF1= RNA-binding enzyme; ORF2 enzyme with RT & endonuclease activities

# DNA TRANSPOSONS

## Cut-and -paste mechanism

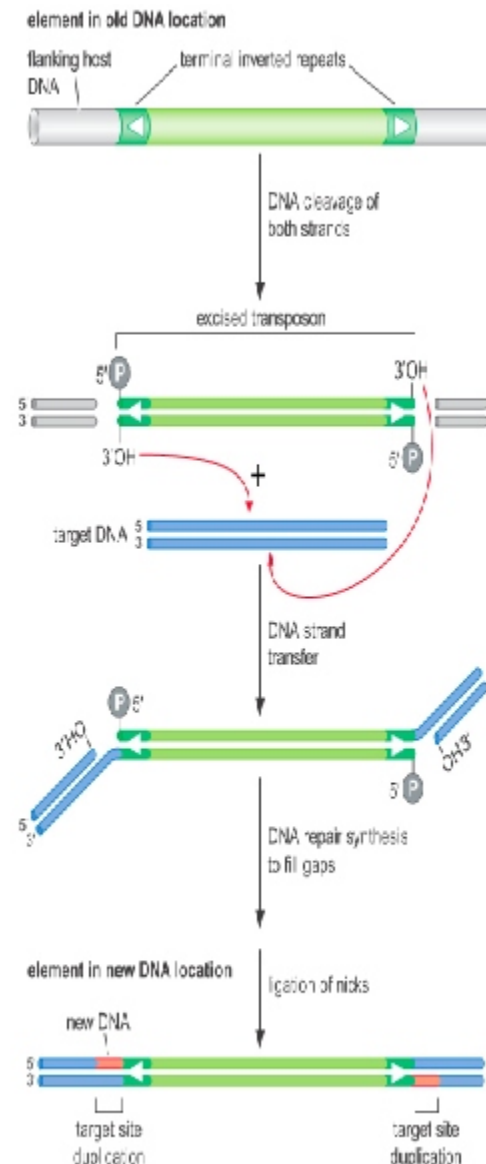
**excision** by transposase  
two nicks per strand

3' OH groups attack target  
DNA

Sites on two strands separated  
by a few nucleotides  
(staggered nicks)

**DNA strand transfer**  
transposon covalently joined  
to target DNA via one step  
transesterification reaction  
called

DNA repair & ligation



# DNA TRANSPOSONS

## Replicative mechanism

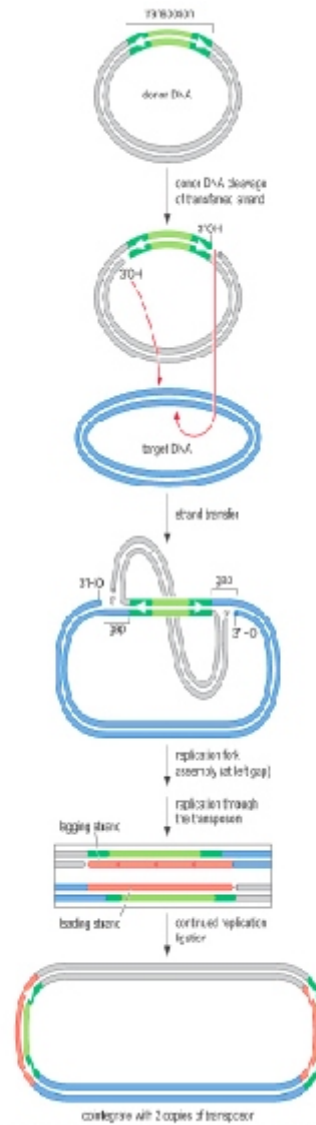
cleavage by transposase  
one nick per strand

attack by 3'OH groups and strand  
transfer

cointegrate formation

replication fork assembly at one  
fork  
replication through transposon  
sequence

Results in two copies of  
transposon



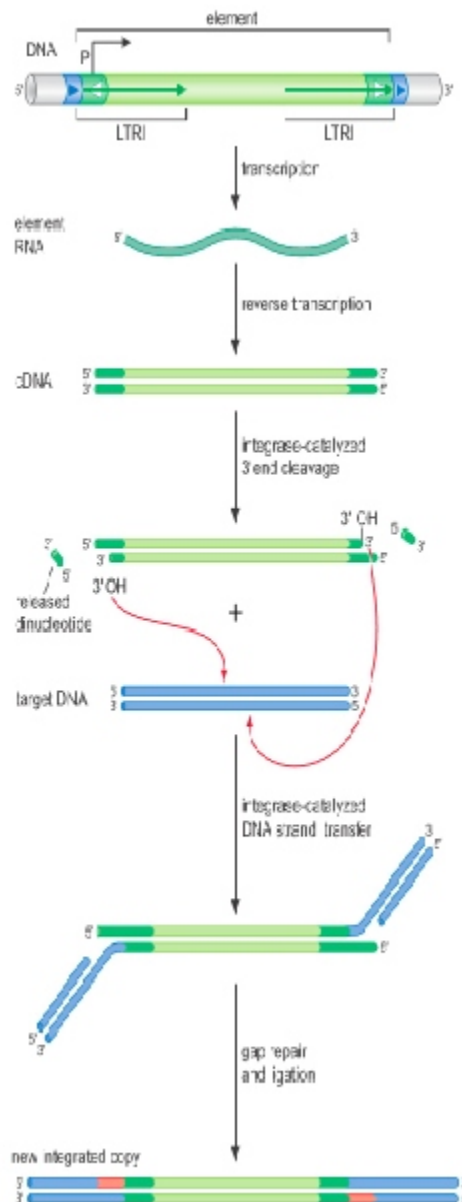
# VIRAL-LIKE RETROTRANSPOSONS

transcription of the retrotransposon DNA sequence into RNA

reverse transcription into cDNA

integrase recognition  
3'cleavage  
strand transfer

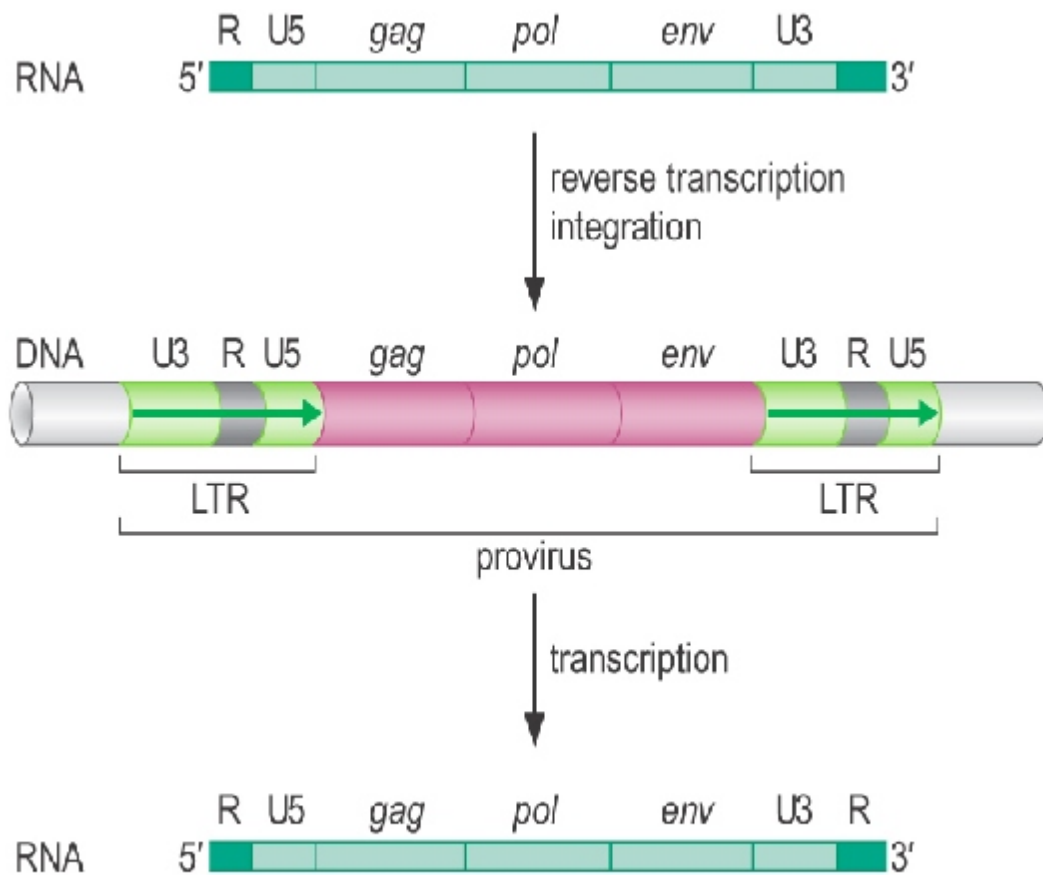
gap repair and ligation



## RETROVIRAL RNA & cDNA

Viral-like retrotransposons have a similar sequence organization

- *pol* gene encodes reverse transcriptase/RNAaseH and integrase activities



# POLY-A-RETROTRANSPOSONS

## Target site primed reverse transcription

cellular RNA polymerase initiates transcription of an integrated LINE (long interspersed nuclear element)

mRNA translated to produce products of two ORFs that bind to the 3' end of their mRNA

ORF2 DNA endonuclease and reverse transcriptase activities

protein-mRNA complex binds to a T-rich site in the target DNA

proteins initiate cleavage in the target DNA

RNA:DNA hybrid formation

3'OH at DNA end which forms the primer for reverse transcription of the element RNA to produce cDNA (first and second strand synthesis)

DNA joining and repair

