

Rekombinacija in popravljanje DNA

Voet 3: poglavja 30.5 – 30.7

Stryer 5: poglavji 27.5 in 27.6

Krebs 3: poglavji 15 in 16

Rekombinacija

- homologna (=splošna): zamenjava homolognih segmentov med dvema molekulama DNA – poteka med mejozo pri evkariontih in pri nekaterih tipih popravljanja napak na DNA
- mestnospecifična: pri prokariontih (haploidni), kjer druga veriga DNA vstopi v celico (transformacija, transdukcija, konjugacija)
- somatska: v celicah imunskega sistema, kjer je potrebna velika pestrost zapisov (npr. za različna protitelesa)

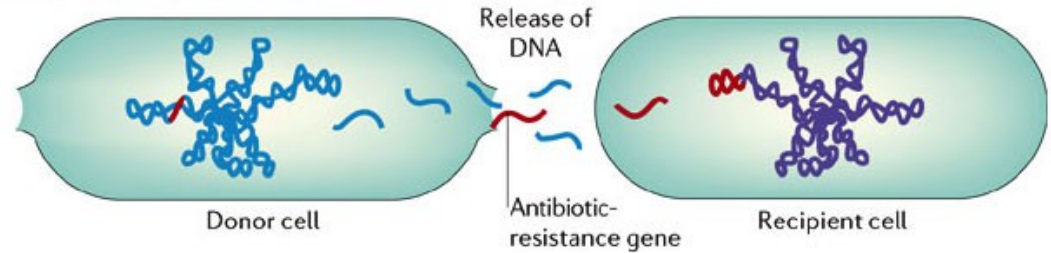
Prenosi DNA med prokarionti

Transformacija: sprejem tuje DNA iz okolice

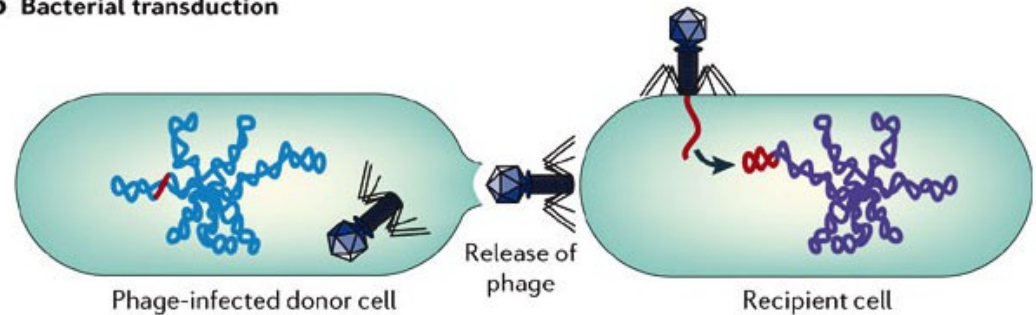
Transdukcija: bakteriofagi prinašajo DNA iz druge bakt. celice

Konjugacija: DNA prihaja iz druge celice iste ali druge vrste medtem, ko sta celici fizično povezani

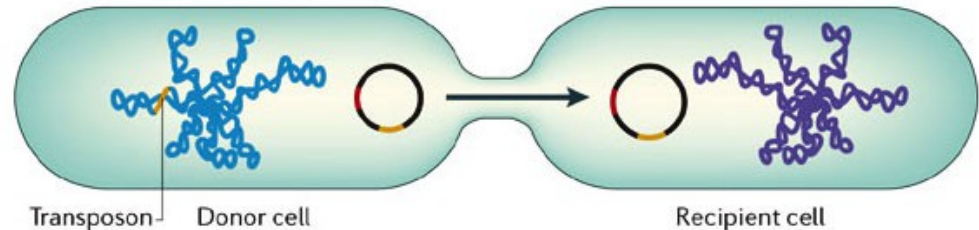
a Bacterial transformation



b Bacterial transduction

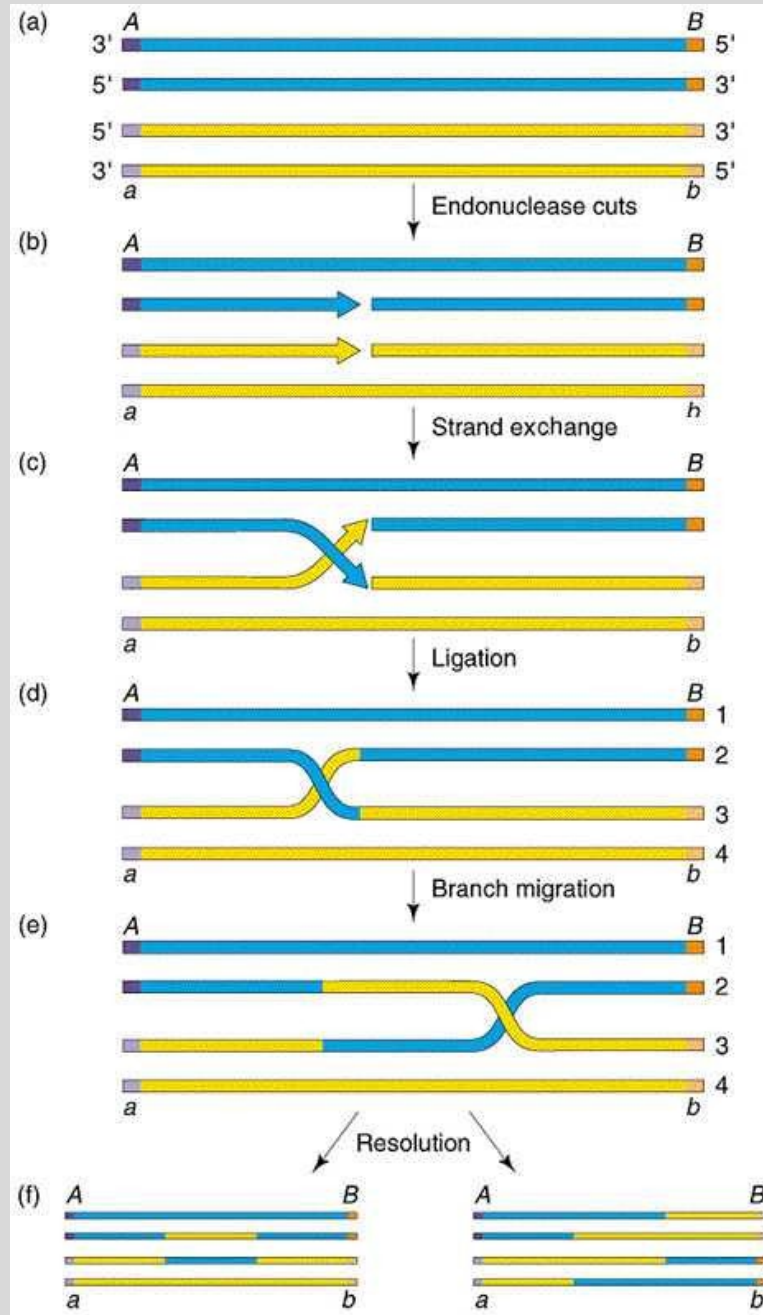


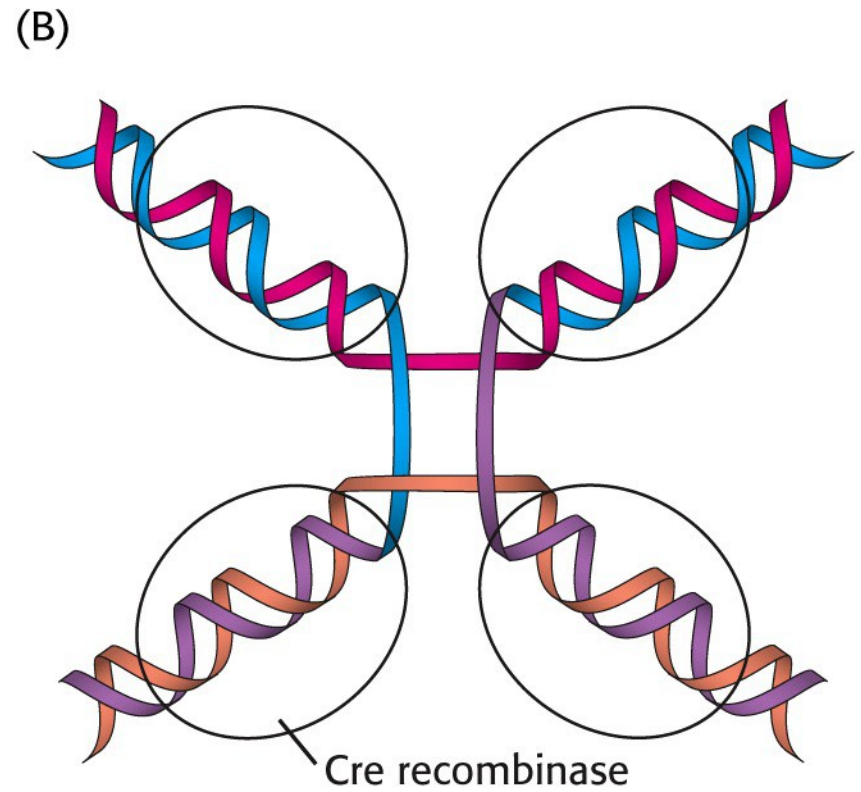
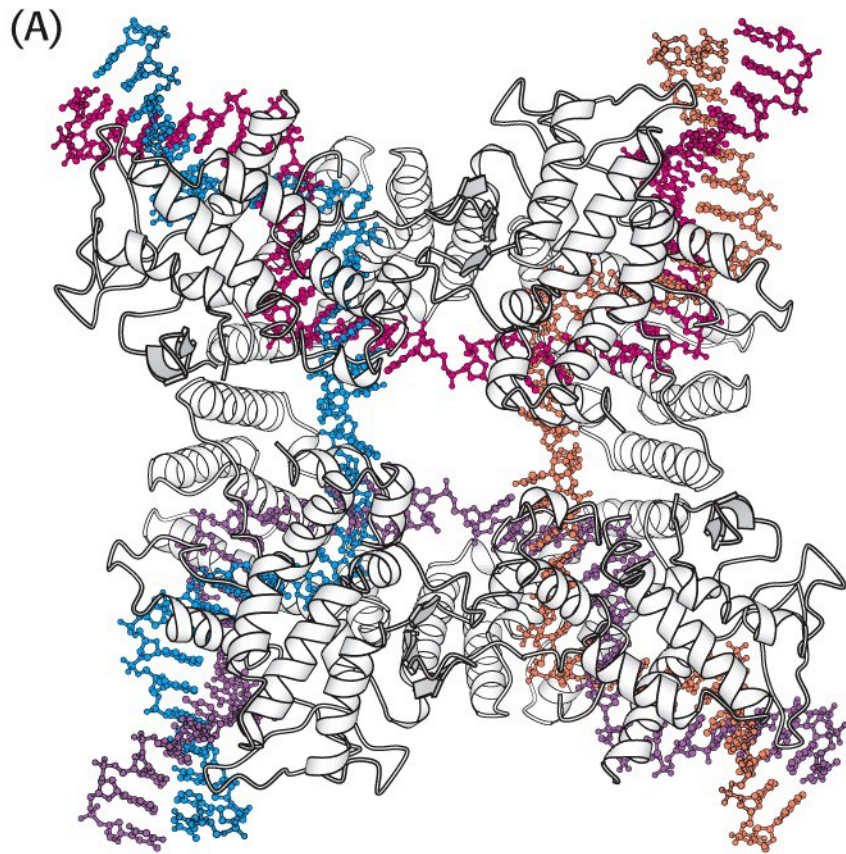
c Bacterial conjugation



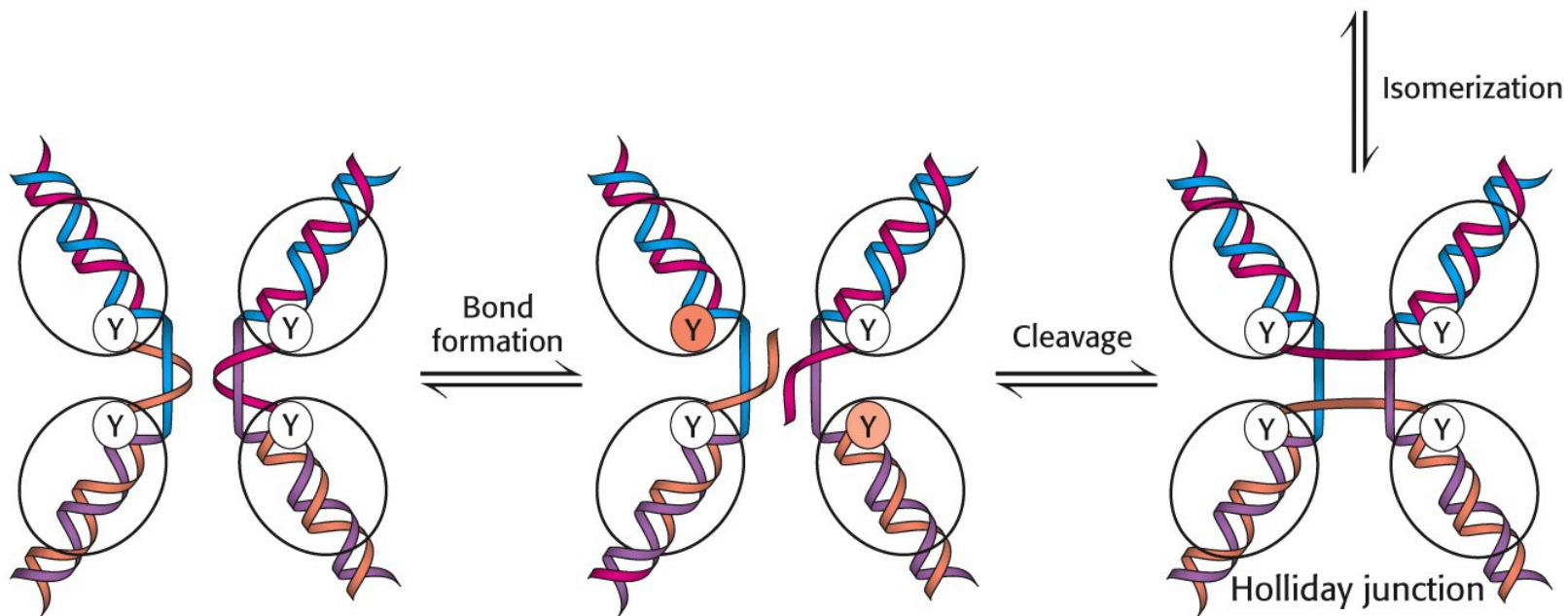
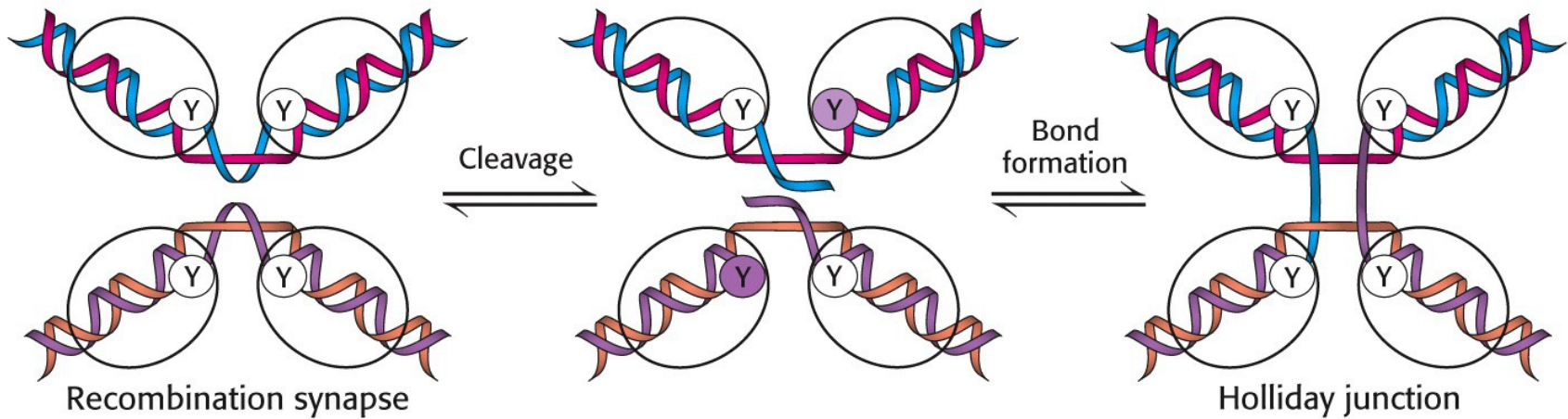
Rekombinacija /2

- intermediat imenujemo Hollidayevo križišče
- potrebna je aktivnost nukleaze in ligaze
- alternativna modela: Messelson-Raddingov in model popravljanja dvojnih prekinitev





Cre iz faga P1 deluje na tarčna mesta loxP (34 bp)



Rekombinaze:

encimi, ki katalizirajo izmenjavo verig DNA

- RecA (*E. coli*)
- RAD51 (*S. cerevisiae*)

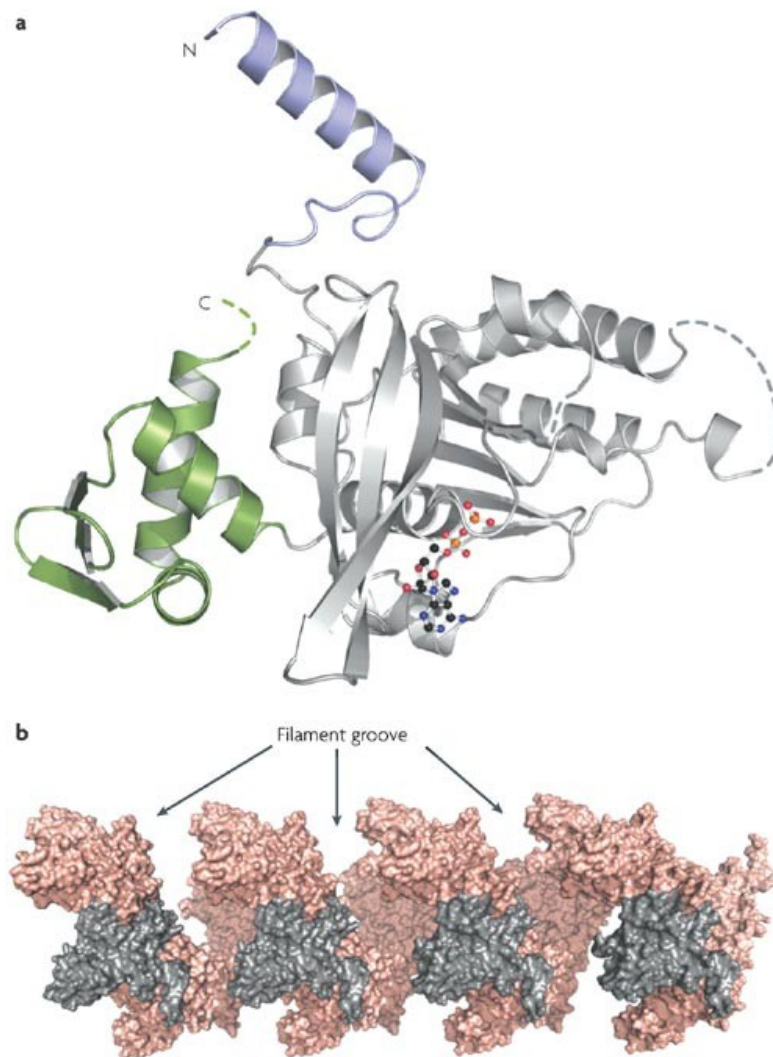
- Cre (fag P1)
- FLP / FRT
- ...

Pri *E. coli* mutante *recA*⁻ izmenjujejo DNA 10⁴-krat redkeje kot *recA*⁺.

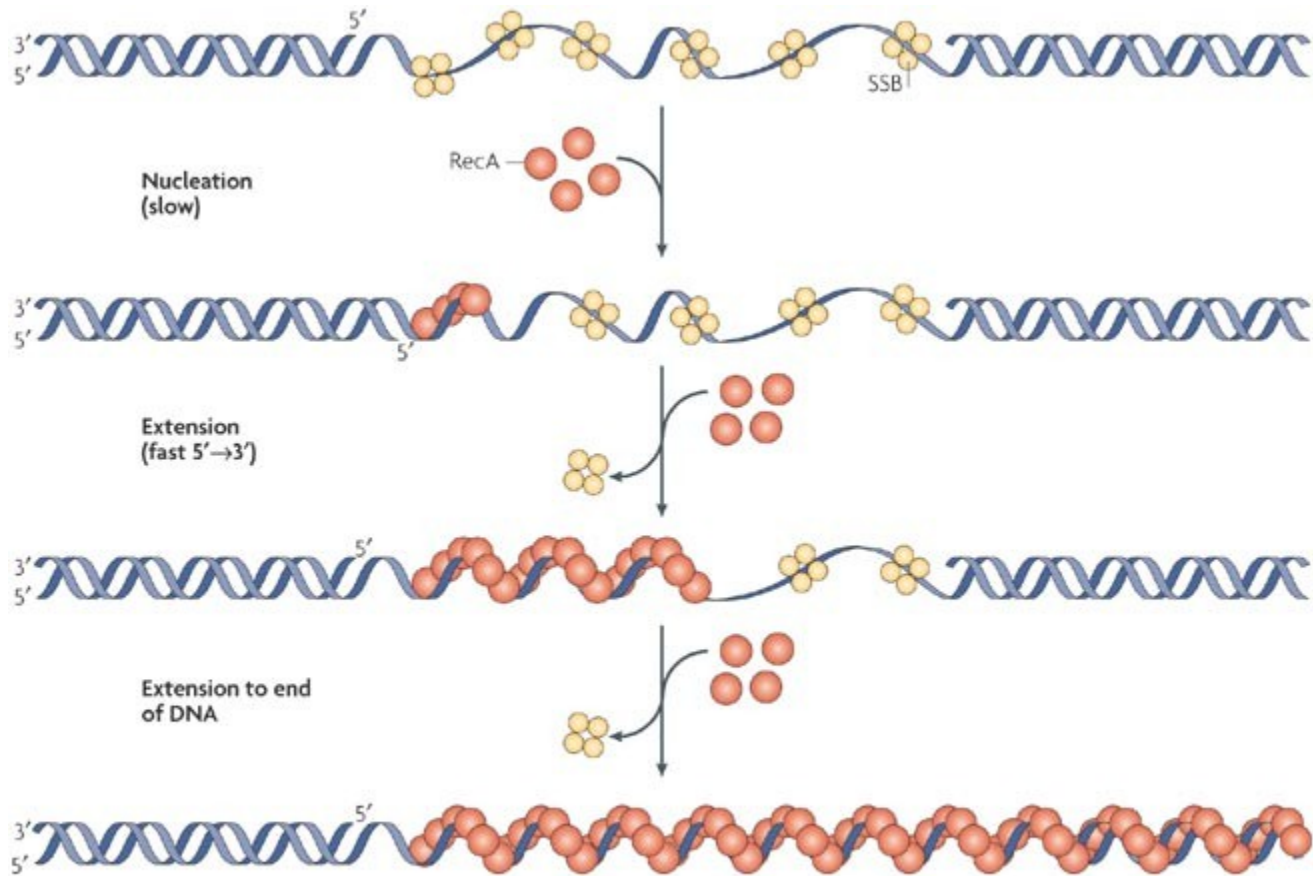
RecA se veže na DNA (ds ali ss) neodvisno od zaporedja – predpogoj je zareza z vsaj 1 nt ssDNA – in naredi filamente, dolge tudi >1000 monomernih enot. Vežejo se na homologna mesta in ob hidrolizi ATP katalizirajo izmenjavo verig.

~6 monomerov se veže na 1 zavoje dsDNA, ki se razvleče in vsebuje ~19 bp.

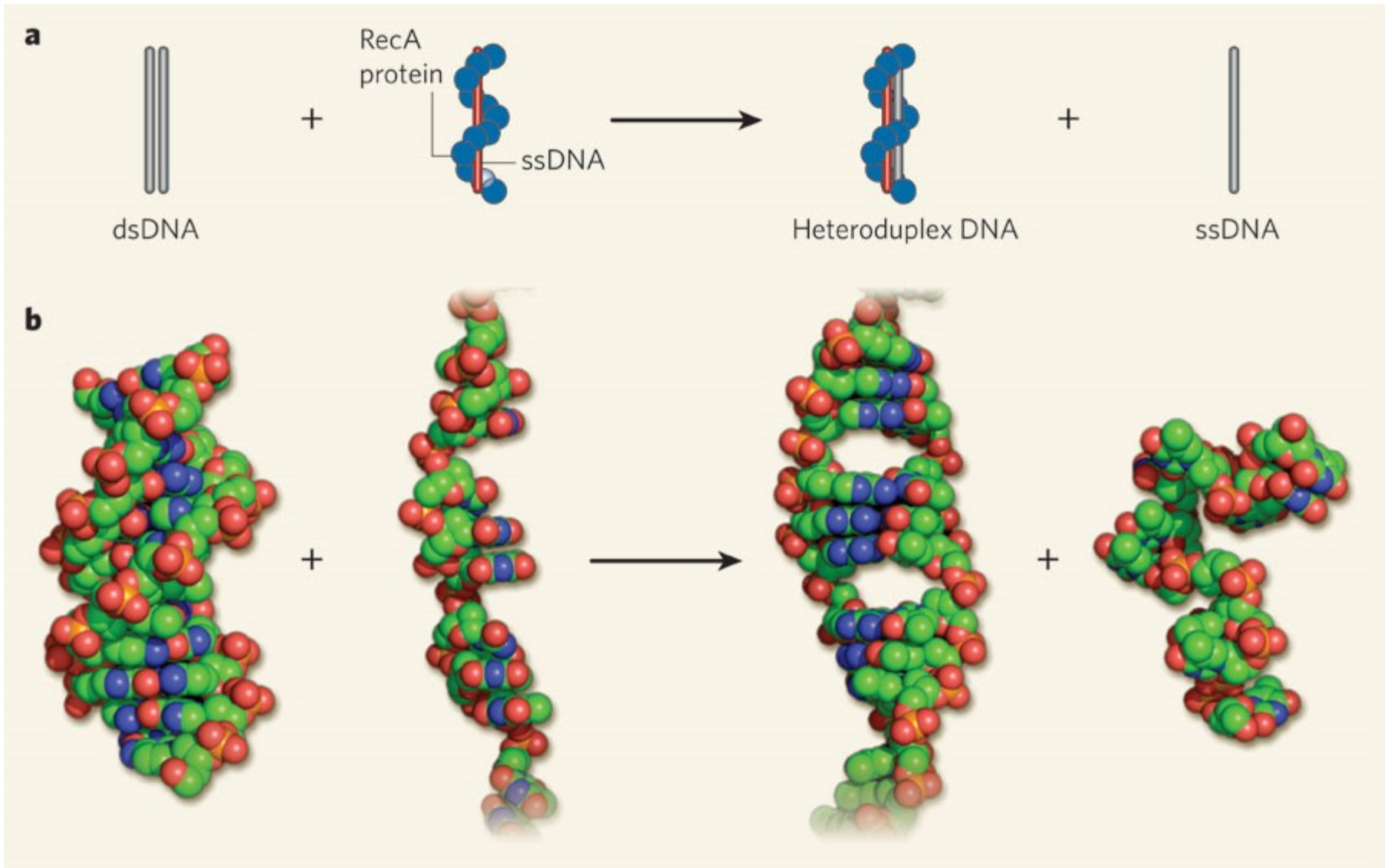
Struktura rekombinaze RecA in sestavljanje filamentov

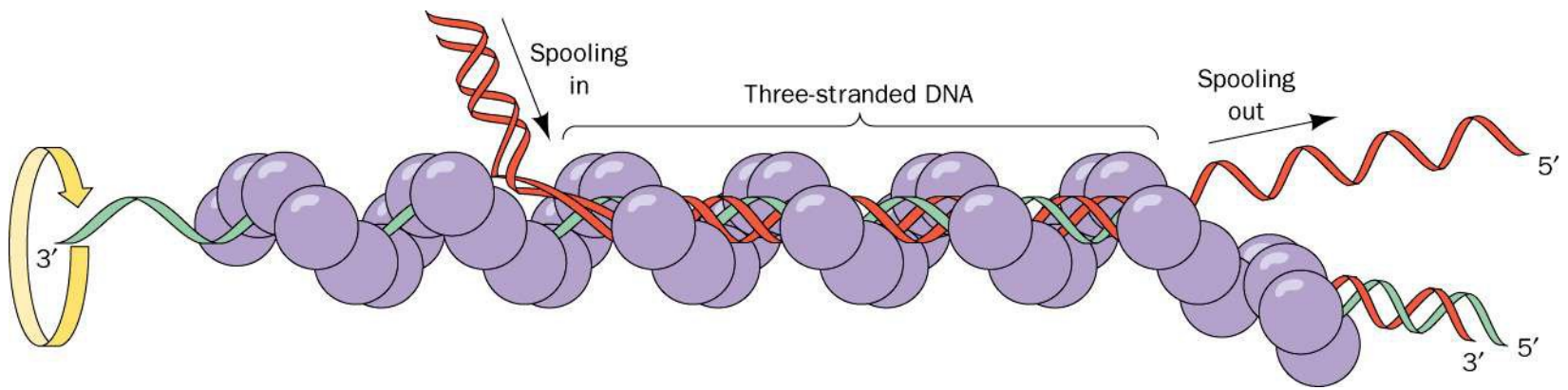
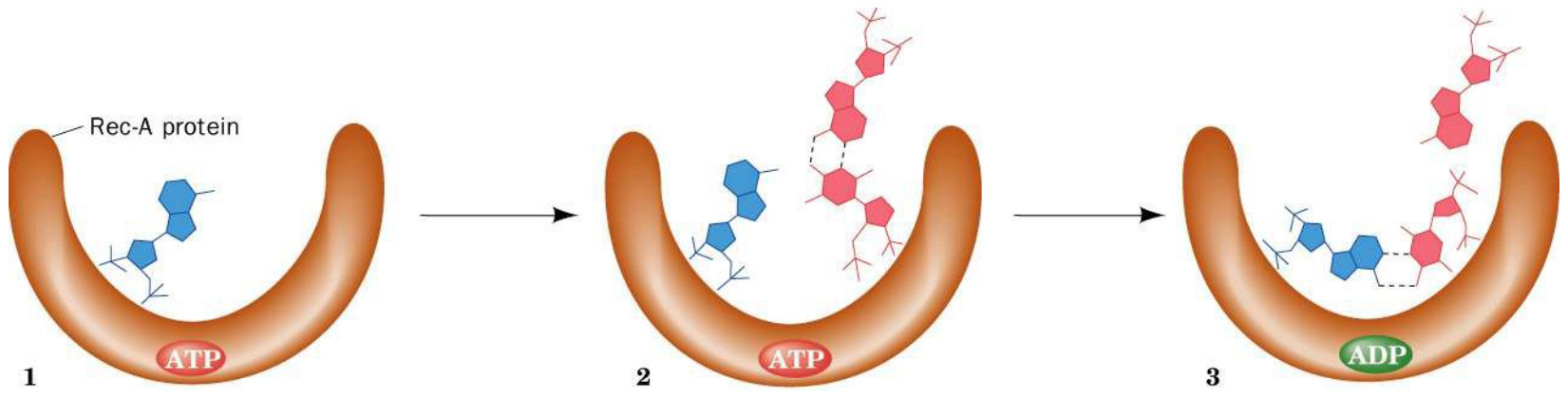


Nukleoproteinski filament z RecA

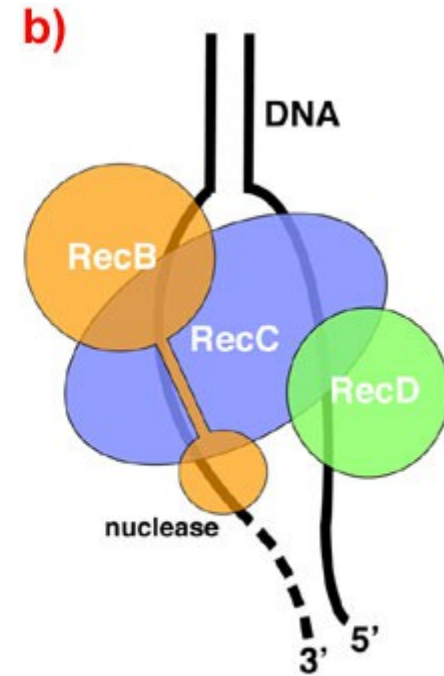
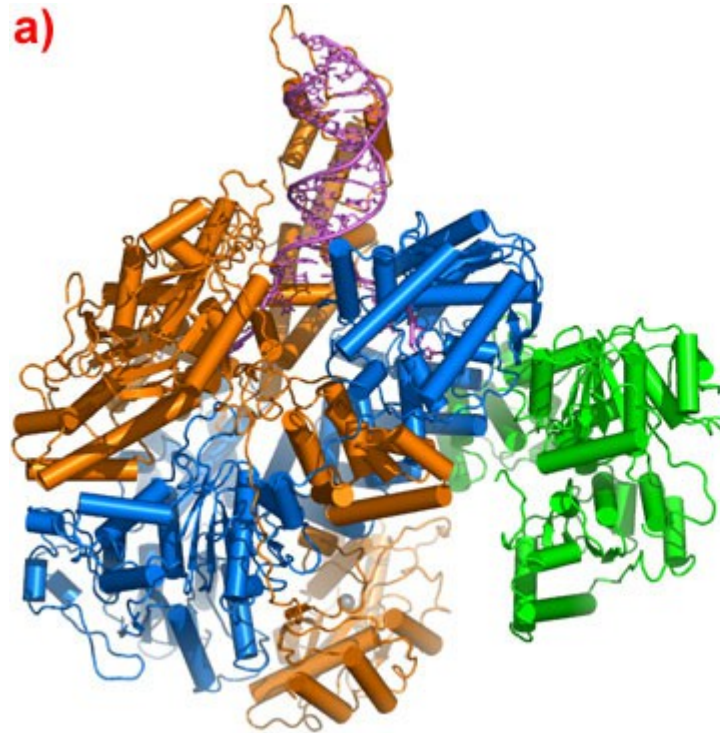
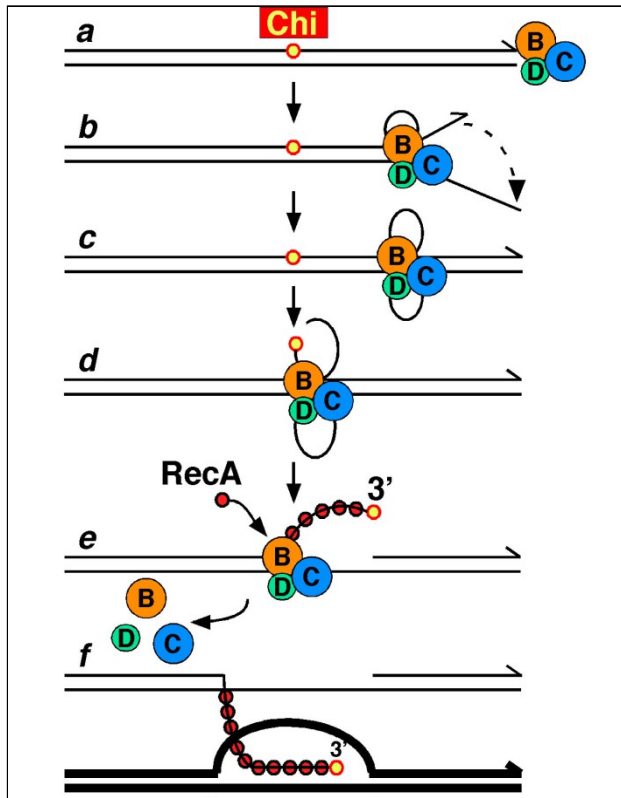


Izmenjava verig s posredovanjem RecA



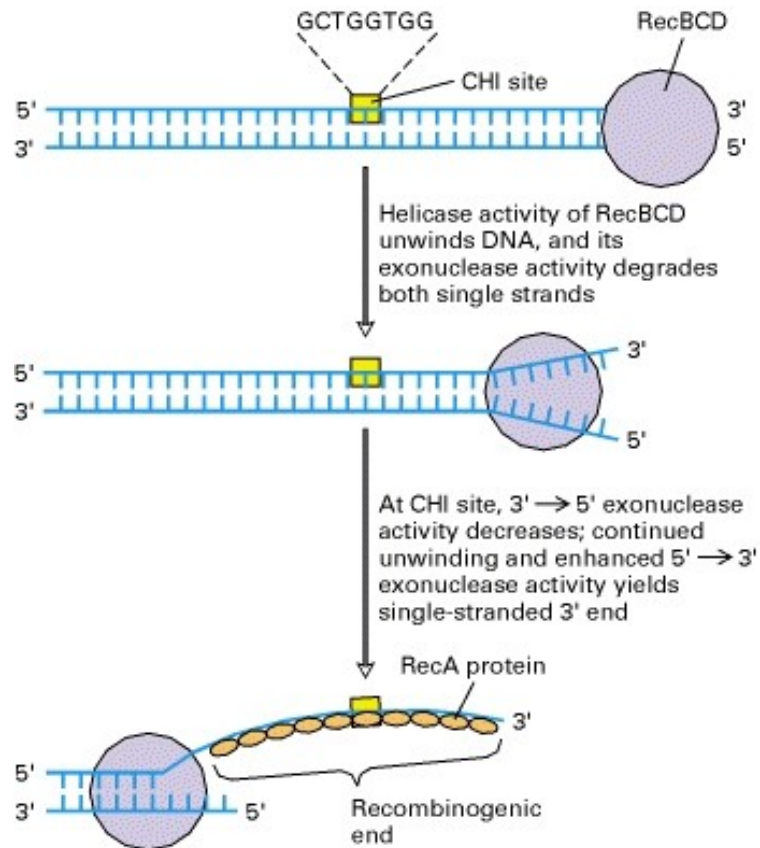


Iniciacija rekombinacije: RecBCD

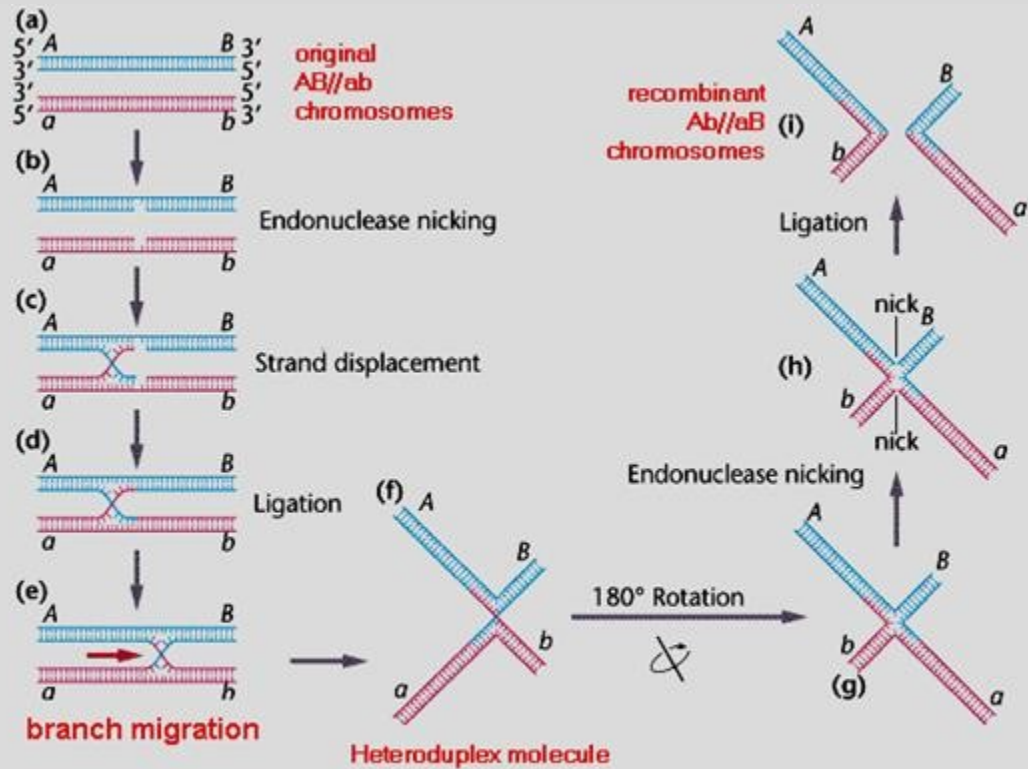


330 kDa, helikaza + nukleaza

Mehanizem delovanja RecBCD



mesta hi: pri fagu lambda pogosta mesta začetka rekombinacije, pri *E. coli* na ~5 kb



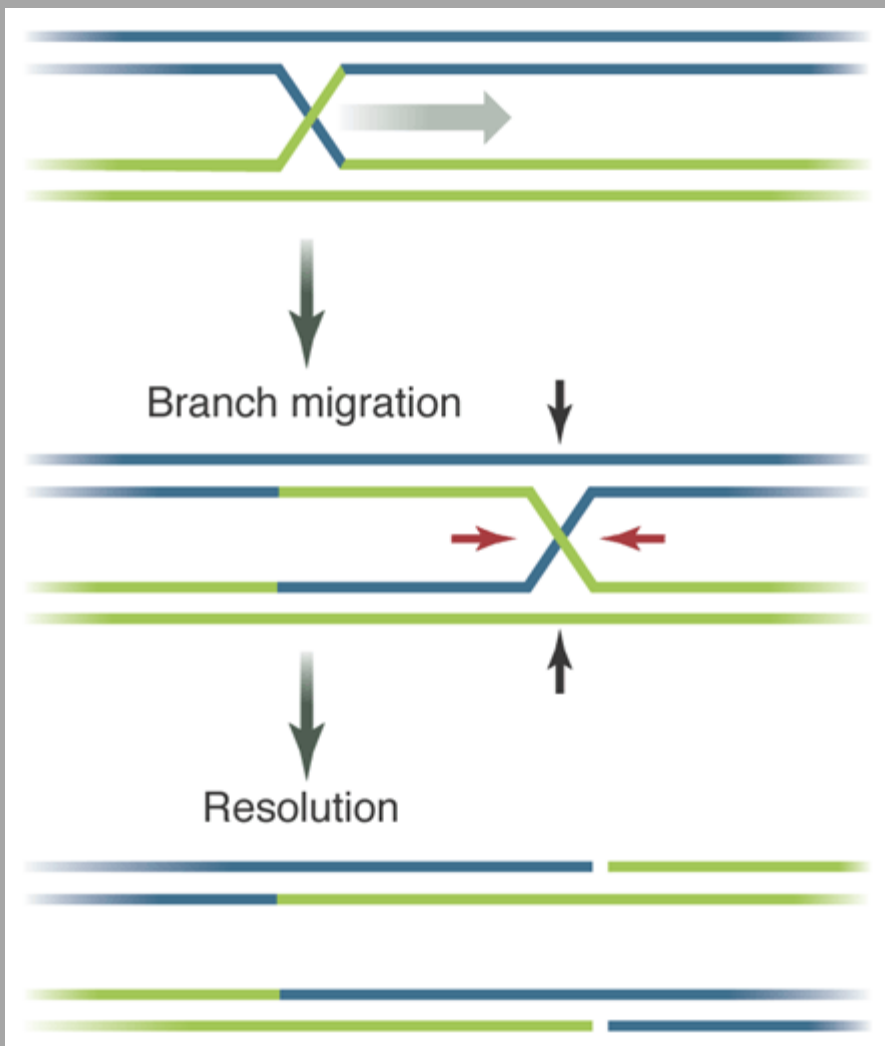
Holliday-Whitehouse model of recombination (after Klug & Cummings 1997)

RuvAB

Branch migration

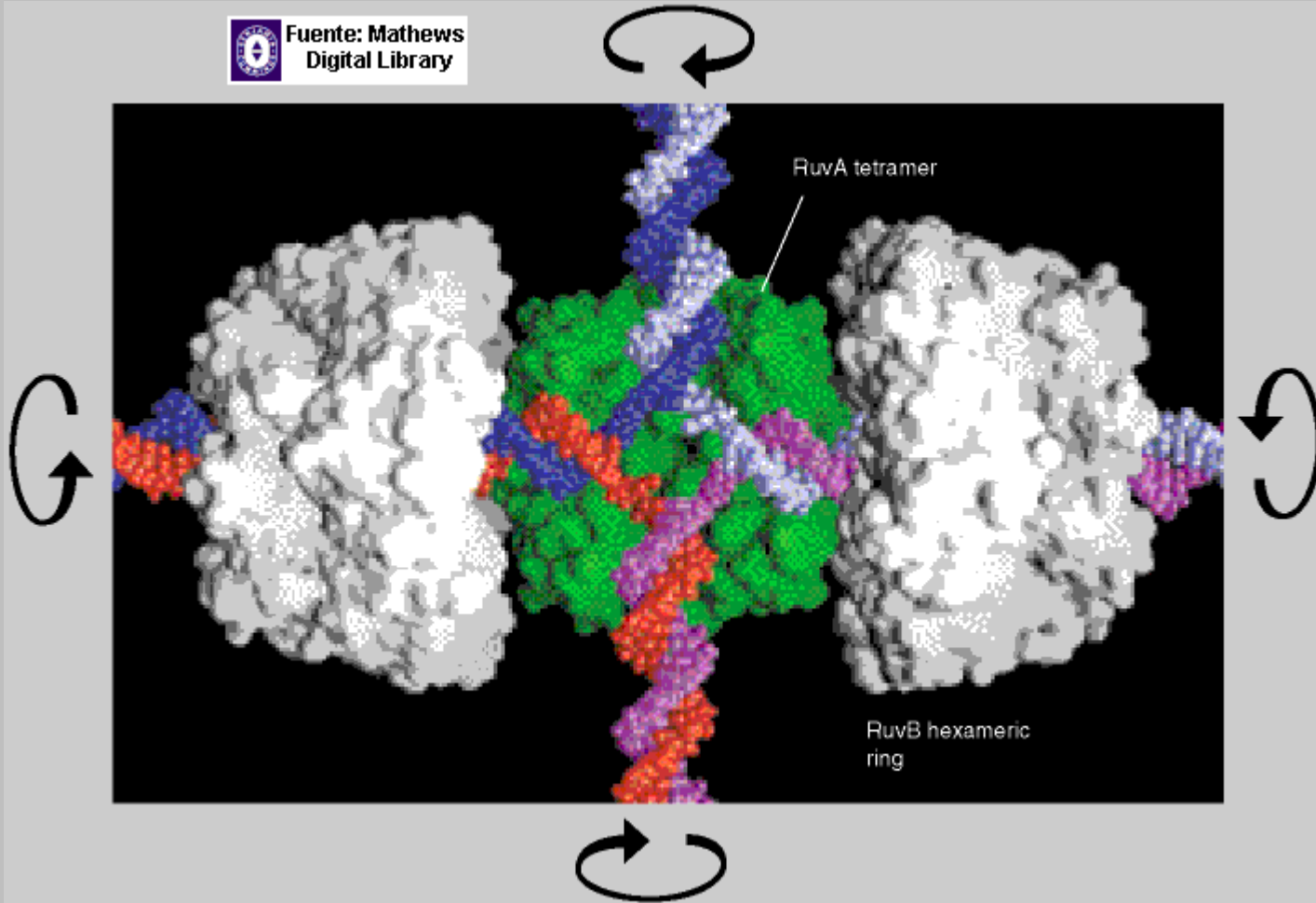
„resolvaza“
RuvC

Resolution





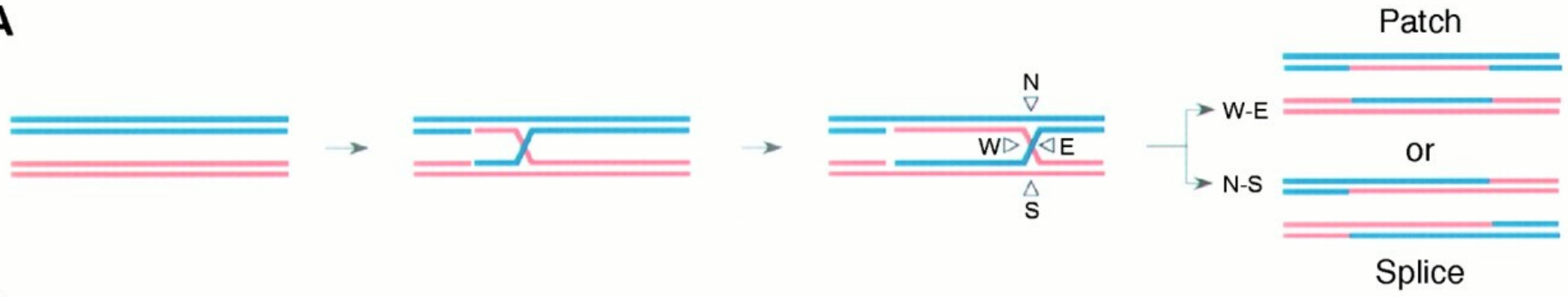
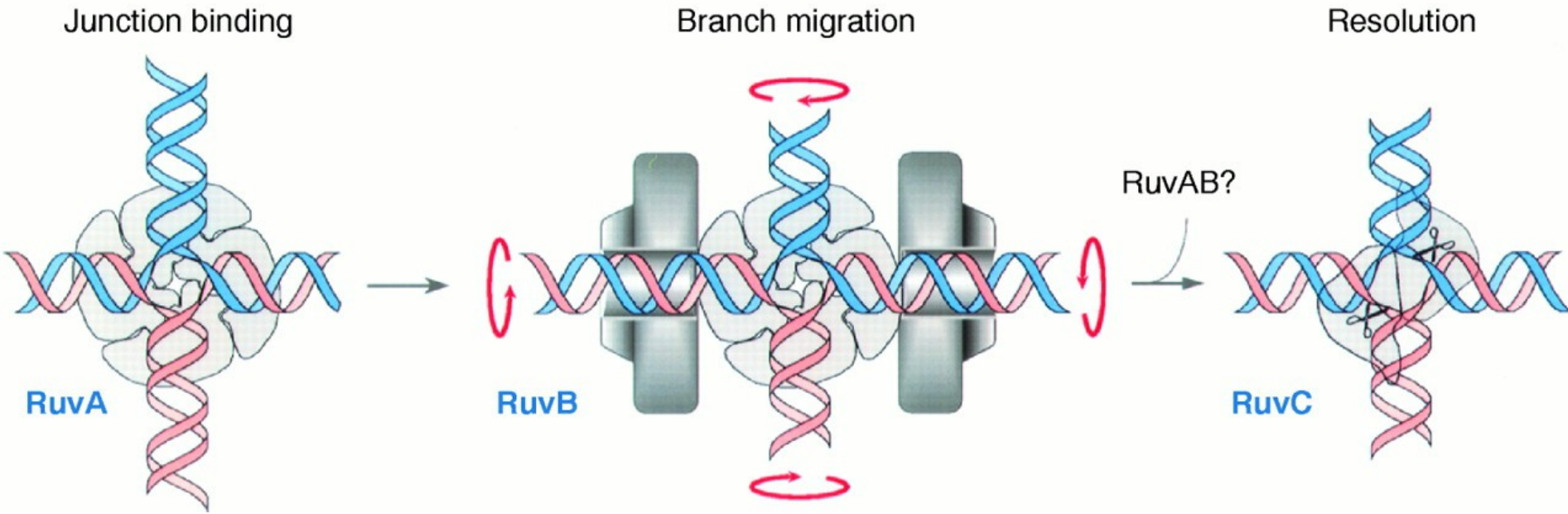
Fuente: Mathews
Digital Library



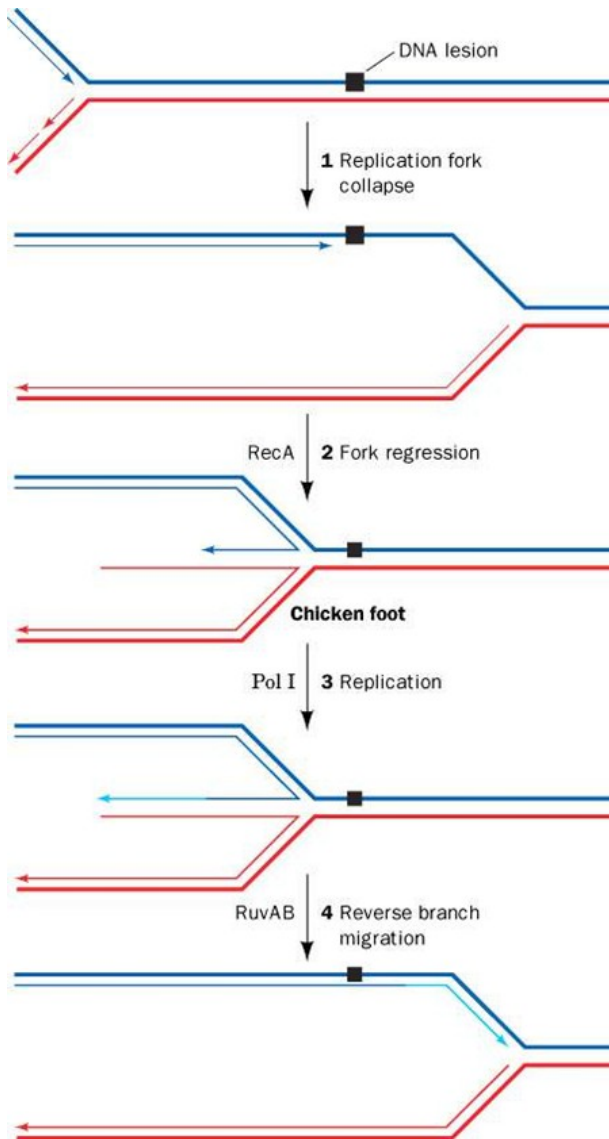
RuvA: veže RuvB na DNA

RuvB: helikaza, premika mesto prekrižanja po DNA

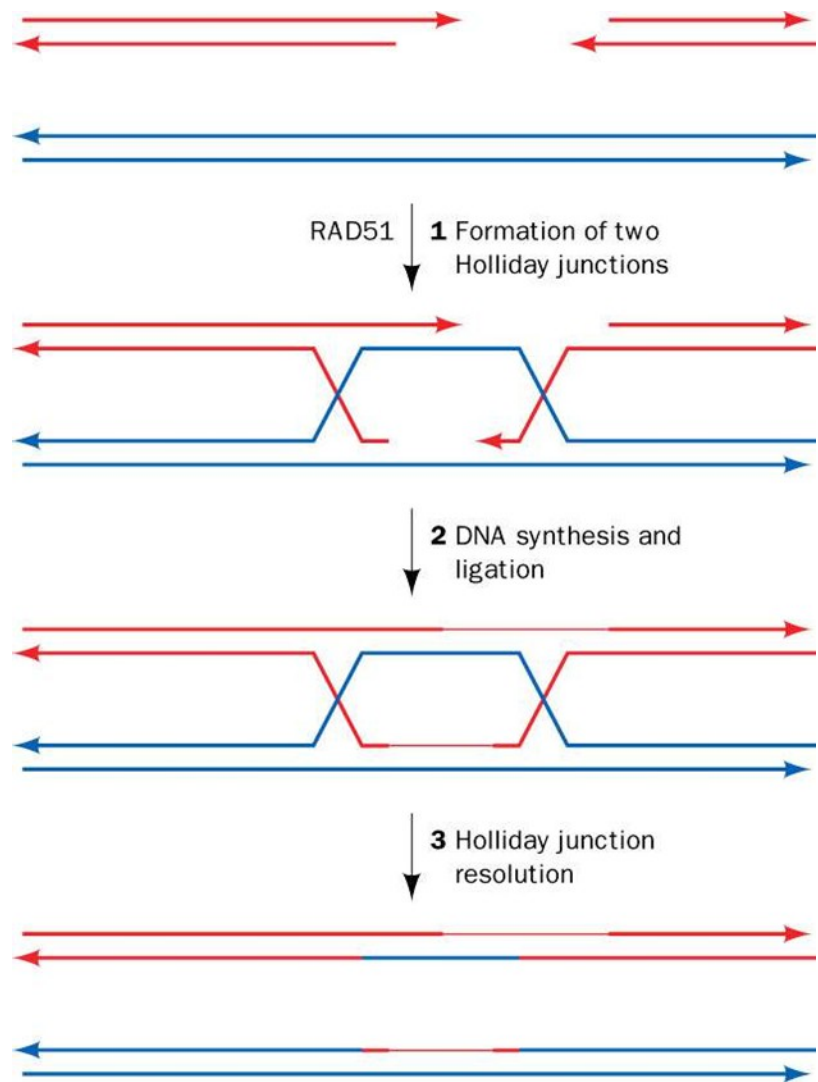
(RuvC: nukleaza, razcepi križišče)

A**B**

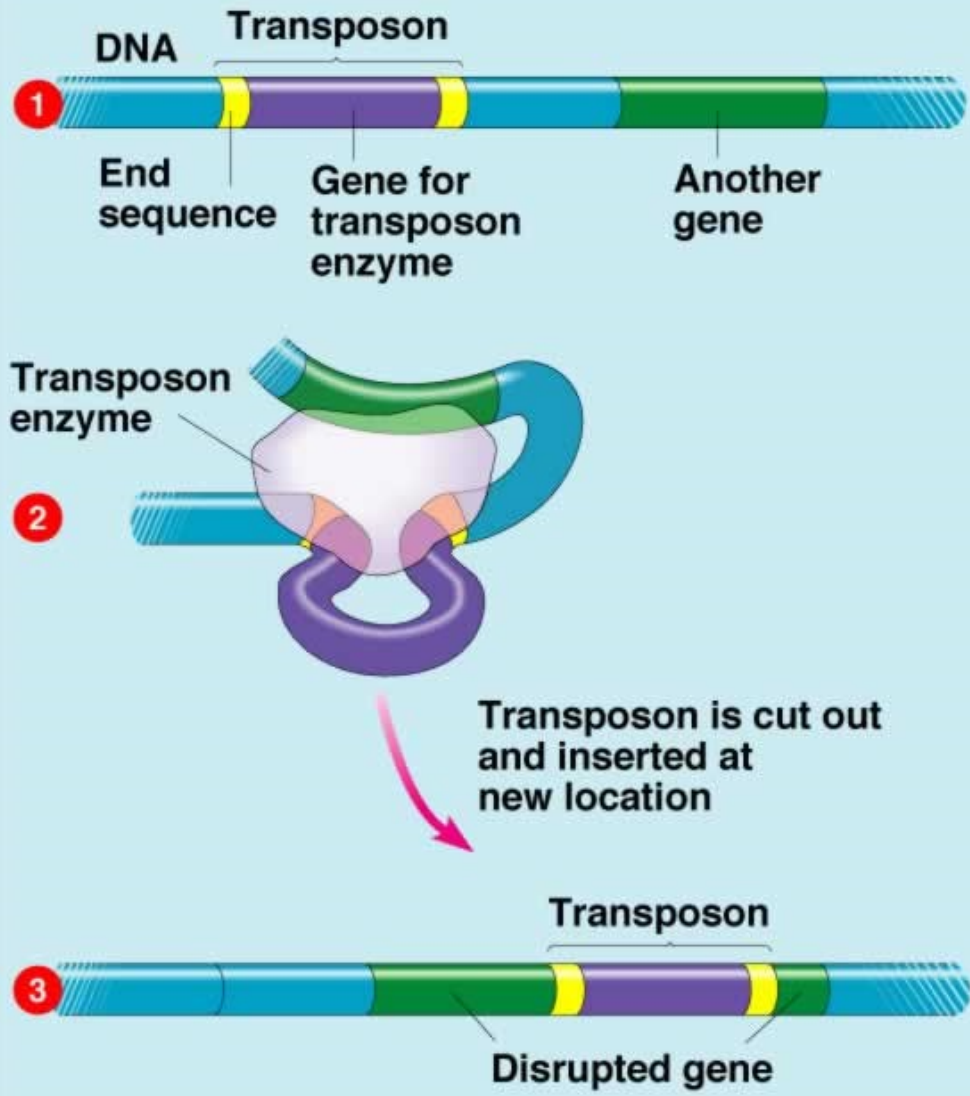
RuvABC: ‚resolvosom‘



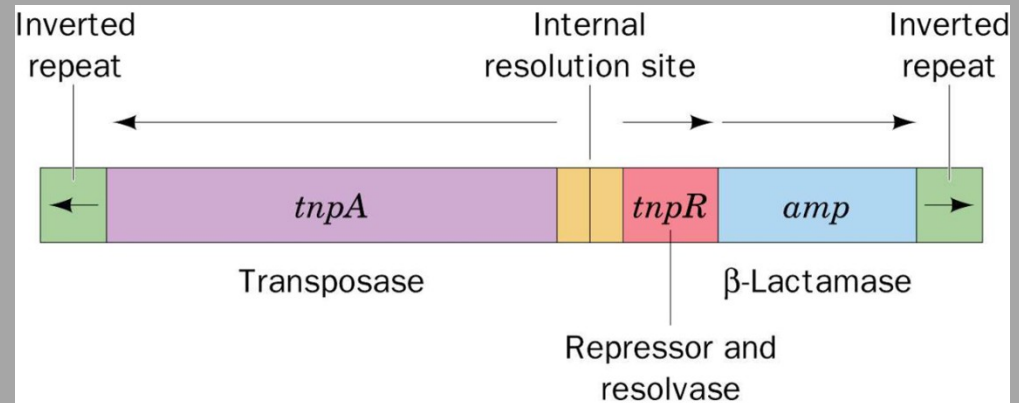
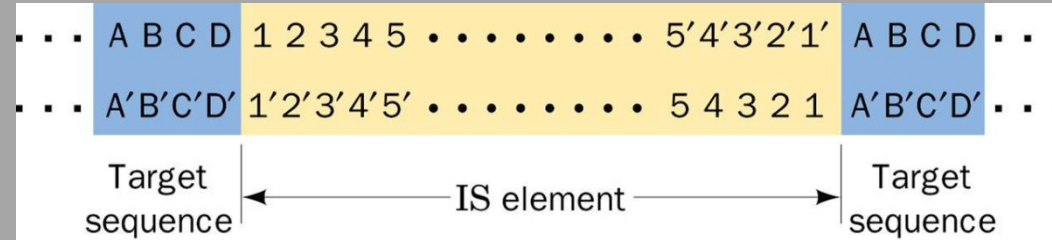
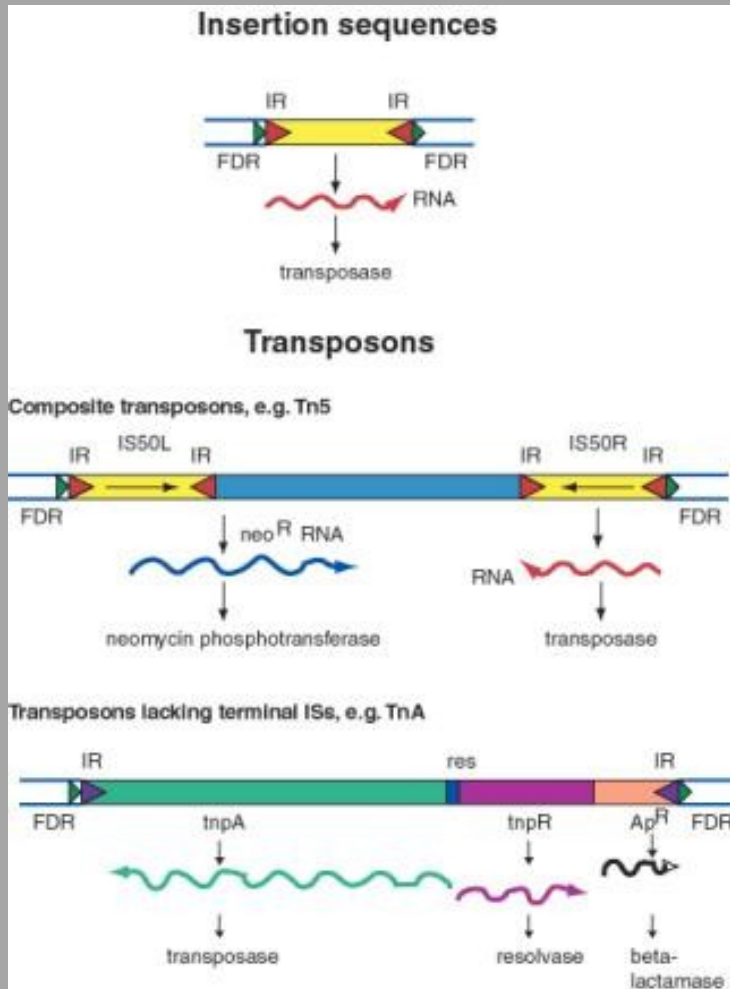
Rekombinacijsko popraviljanje zastoja na replikacijskih vilicah



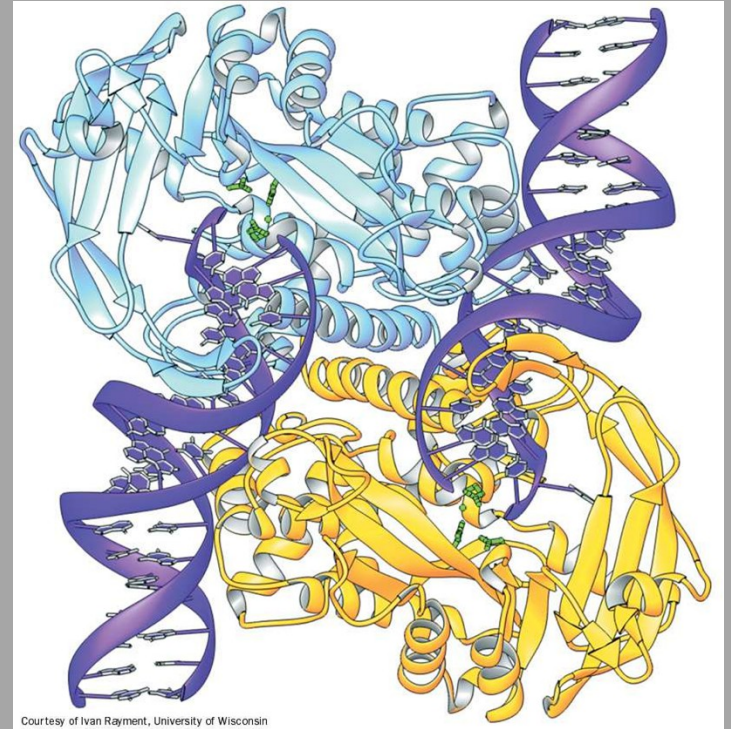
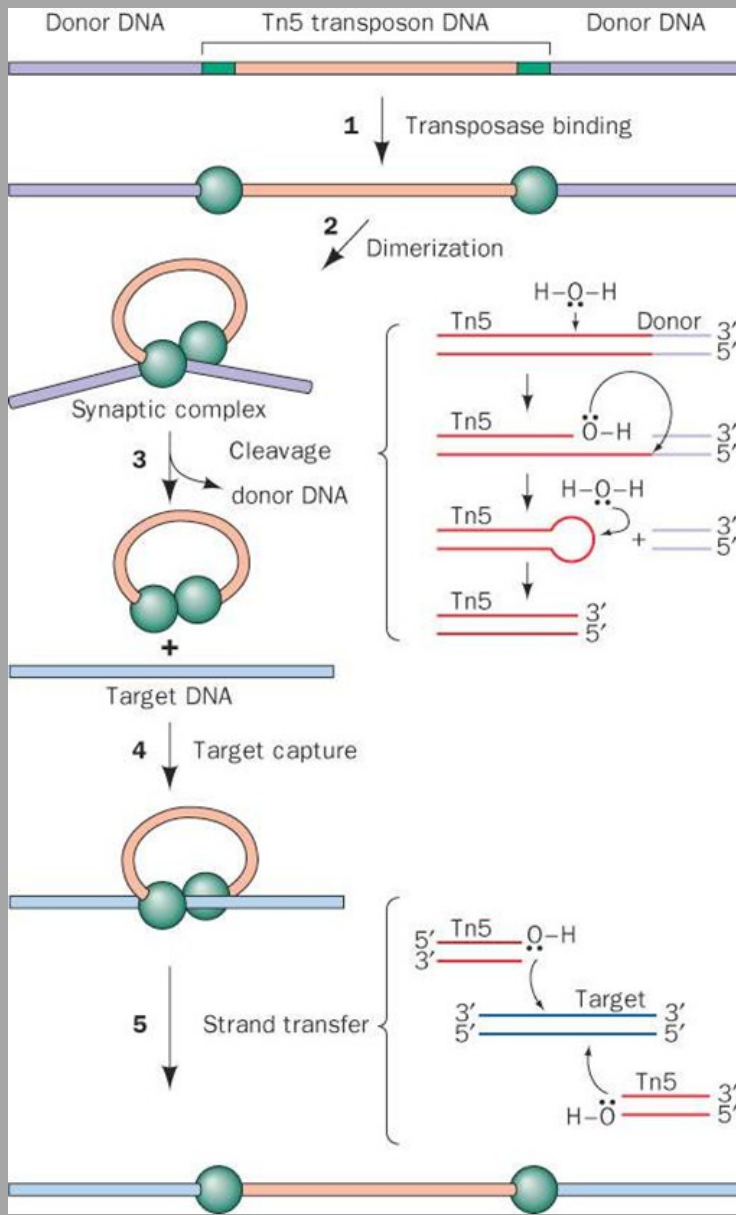
Popraviljanje prekinitve dveh verig s povezovanjem homolognih koncev



Insercijska zaporedja in transpozicijski elementi



Tn3

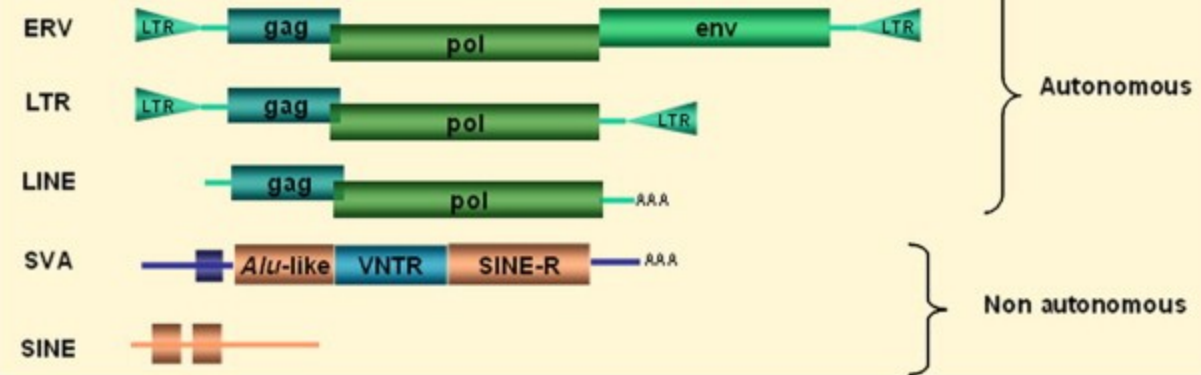


Mehanizem 'izreži in prilepi', ki ga katalizira transpozaza Tn5.

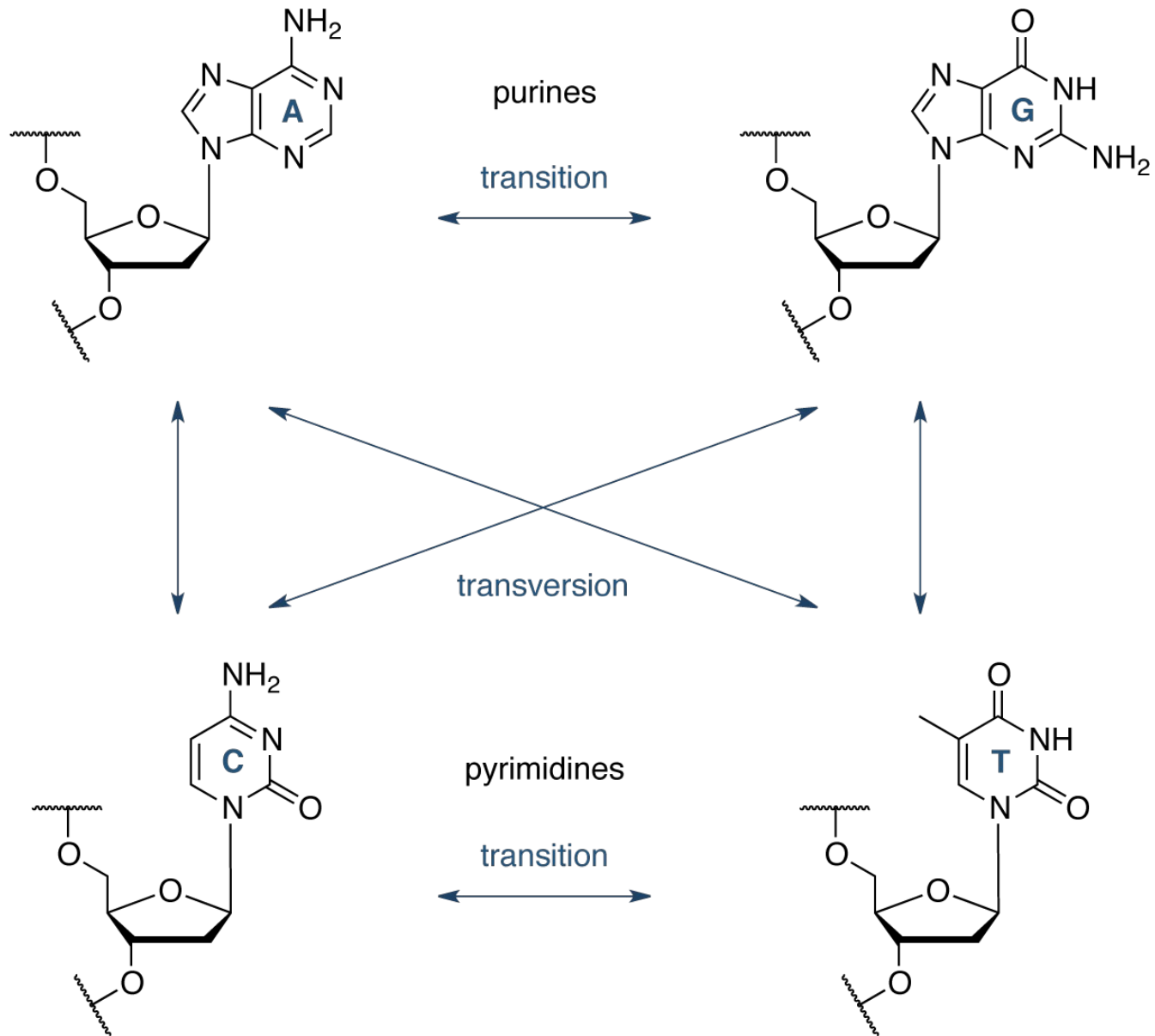
Class II : DNA transposons



Class I : Retrotransposons



Gene 454(1-2), 2010, 1-7



Vrste mutacij

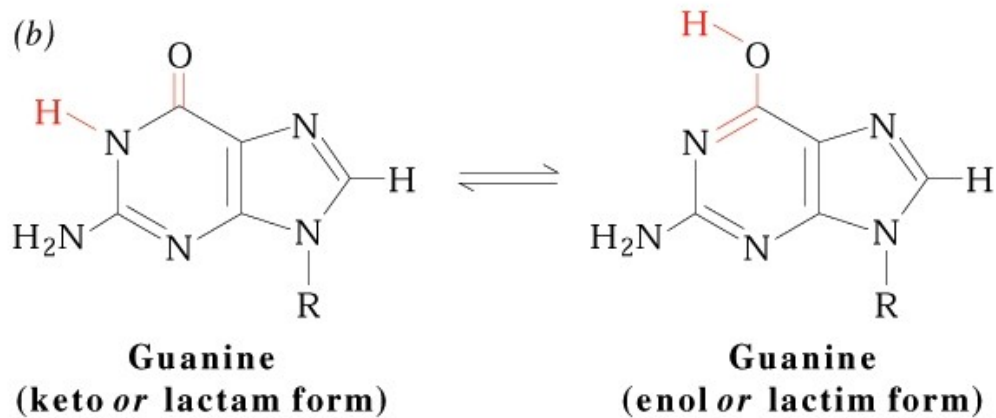
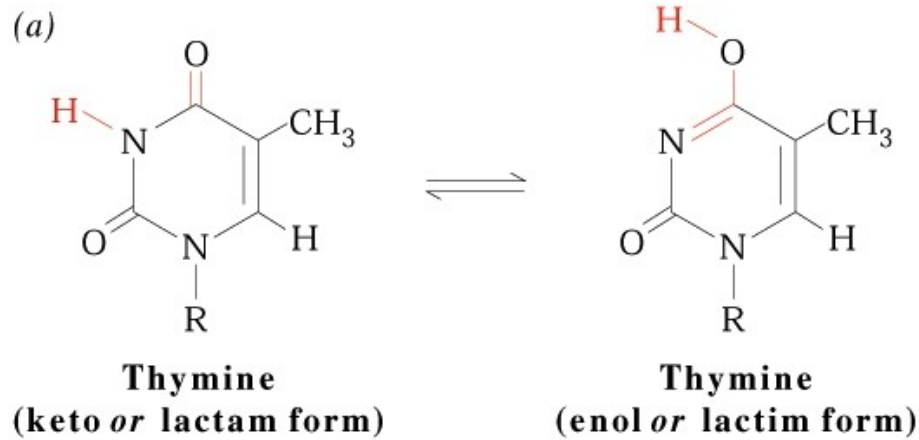
- **substitucije baz:** tranzicije/transverzije
- delecije 1 ali več baz
- insercije 1 ali več baz

tranzicije: pirimidin z drugim pirimidinom, purin z drugim purinom

transverzije: pirimidin s purinom ali obratno

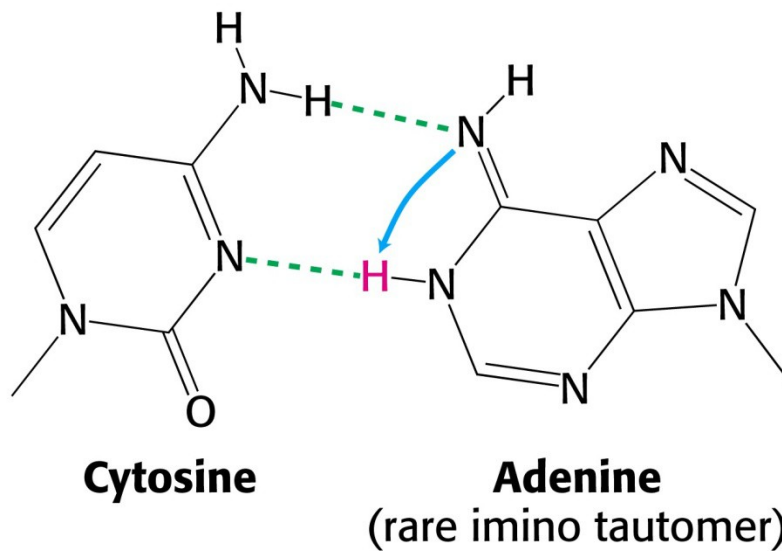
Tautomerizacija stranskih skupin na bazah

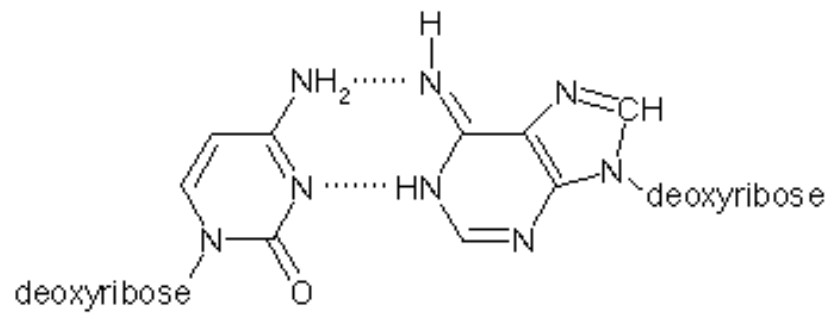
keto (-C=O) → enol (=C-OH)



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Tranzicije zaradi tautomerizacije str. skupin na bazah:
amino (-NH₂) → imino (=NH) ⇒ A ... T → A ... C

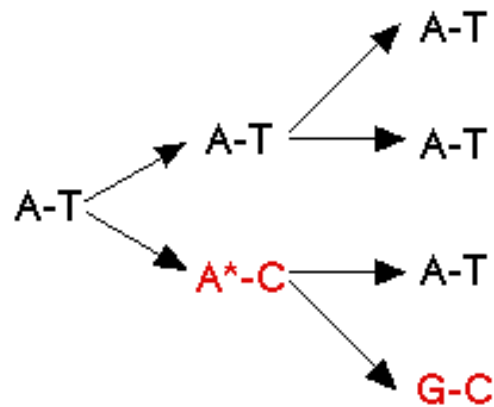




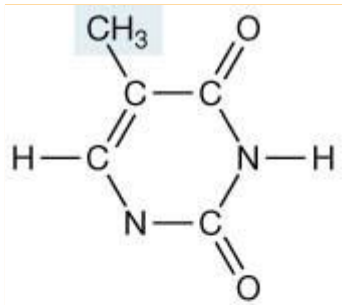
Cytosine

Tautomer of Adenine

Tautomerization of an adenine residue can result in the transition of an A-T base pair to a G-C base pair:



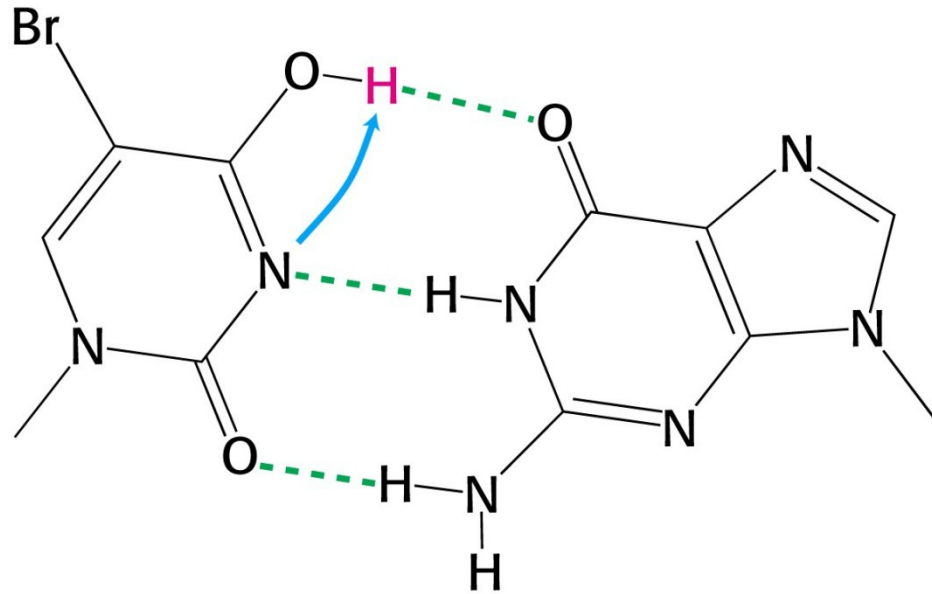
Tranzicije zaradi tautomerizacije baznih analogov:



Thymine

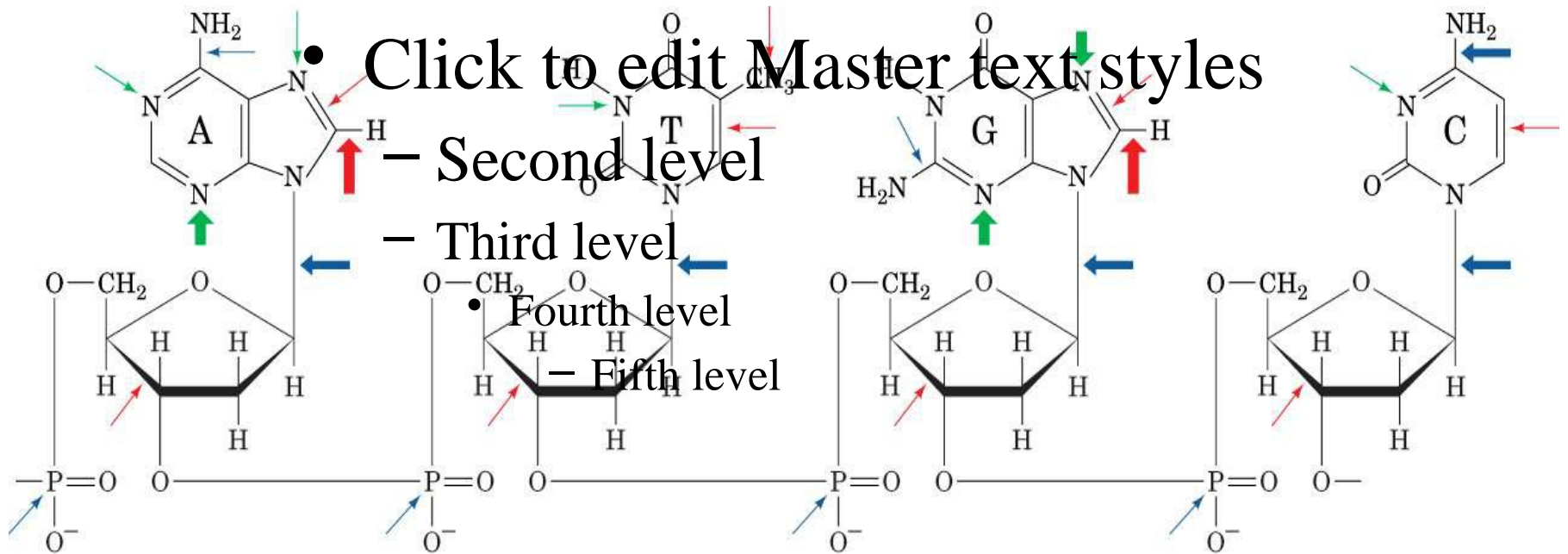


5-Bromouracil



5-Bromouracil
(enol tautomer)

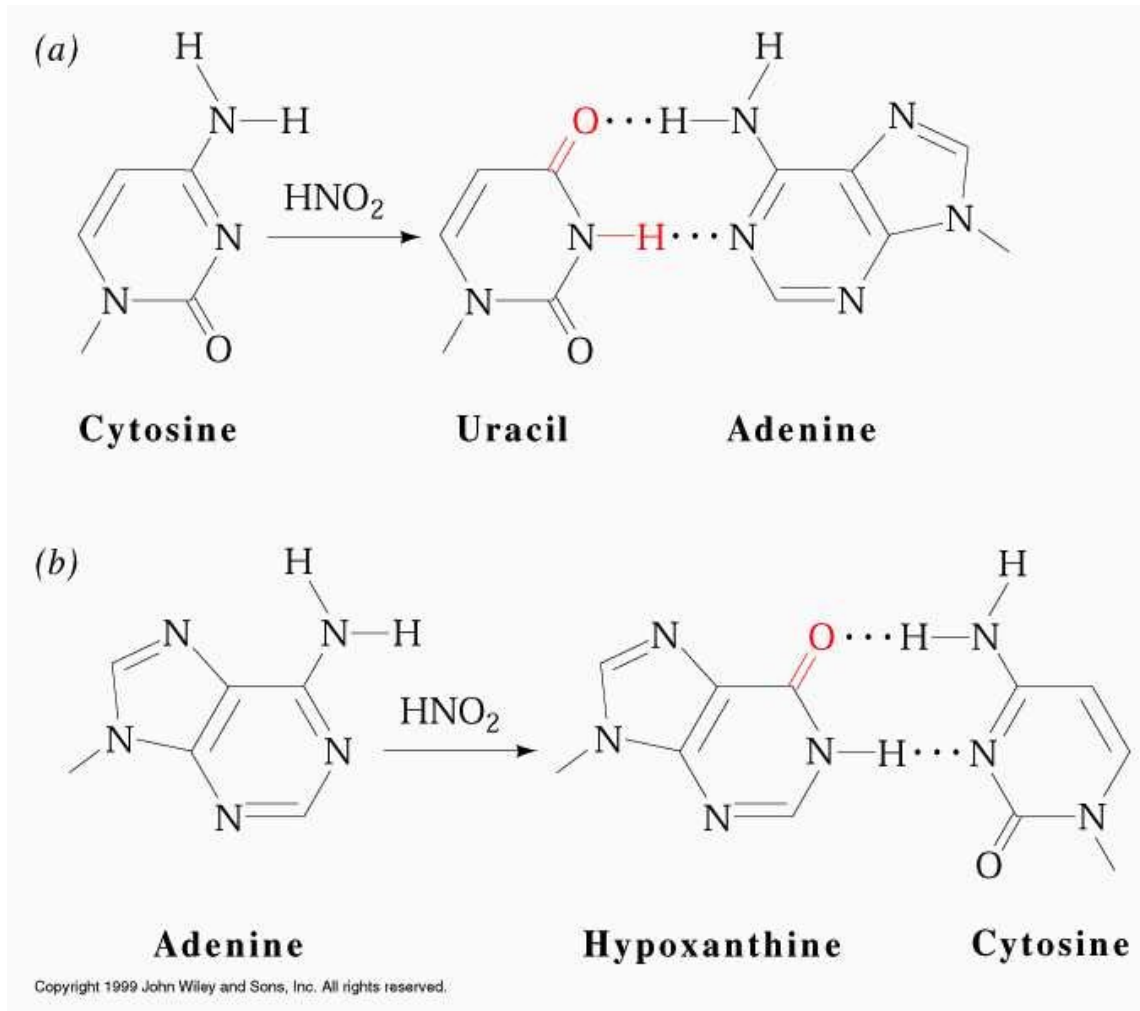
Guanine



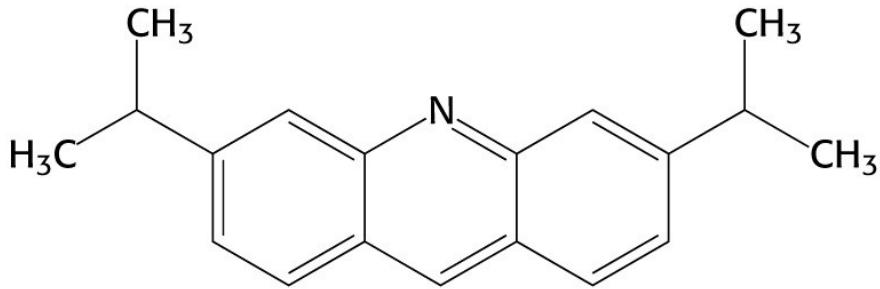
Poškodbe in mesta, občutljiva za kemične spremembe DNA *in vivo*.
 rdeče: oksidacija, modro: hidroliza, zeleno: metilacija

Kemični mutageni

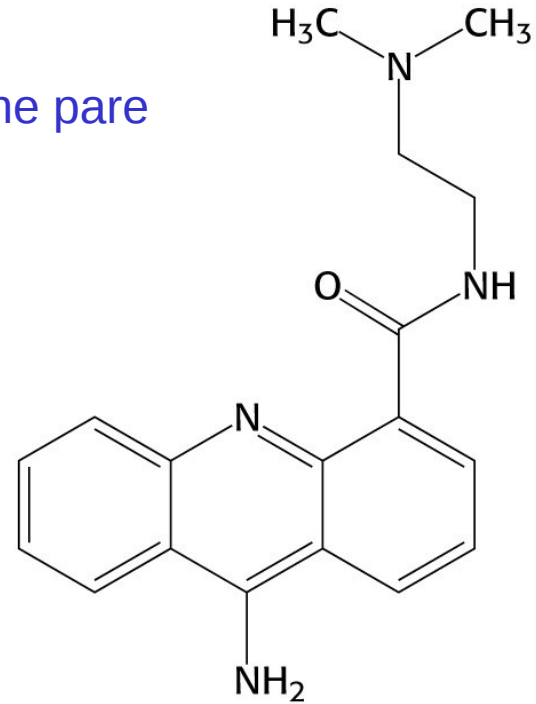
oksidativna deaminacija C → U, A → hipoksantin, G → ksantin



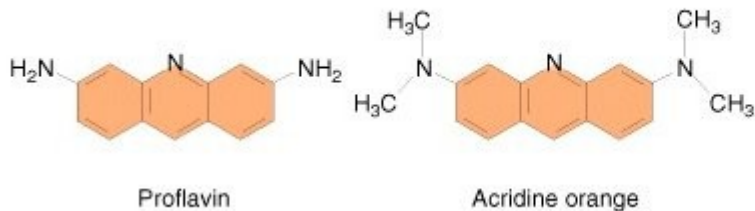
Insercije zaradi interkalacije:
ploske organske molekule se vrivajo med bazne pare



Acridine orange

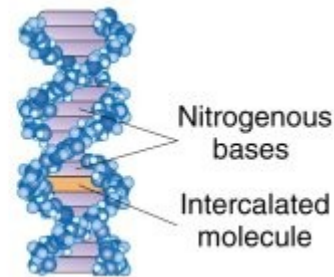


9-Amine-(N-(2-dimethylamino)-ethyl)acridine-4-carboxamide



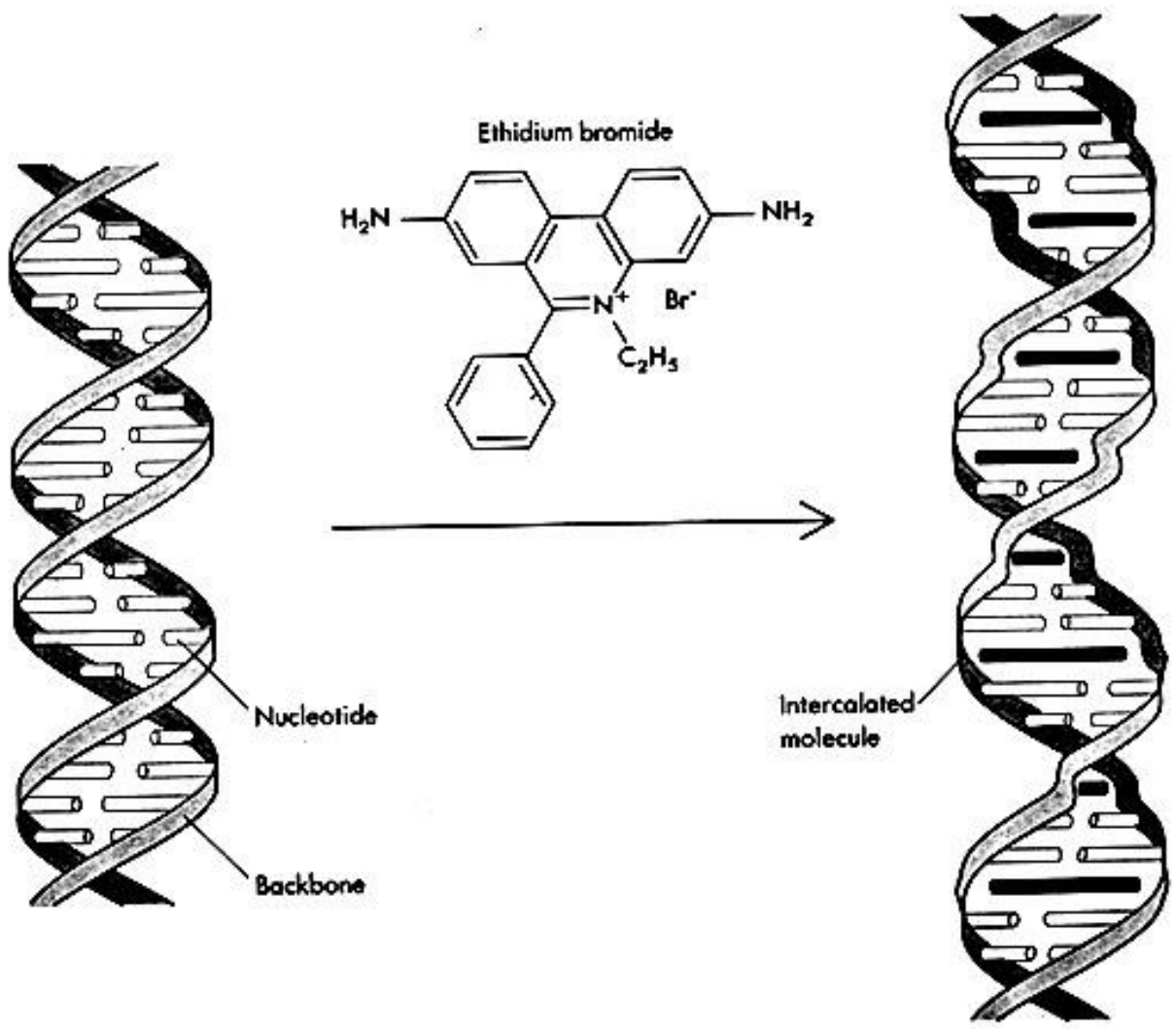
Proflavin

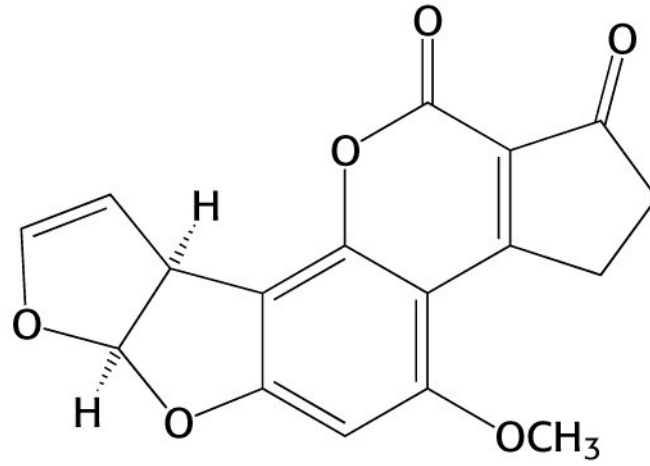
Acridine orange



Nitrogenous bases

Intercalated molecule

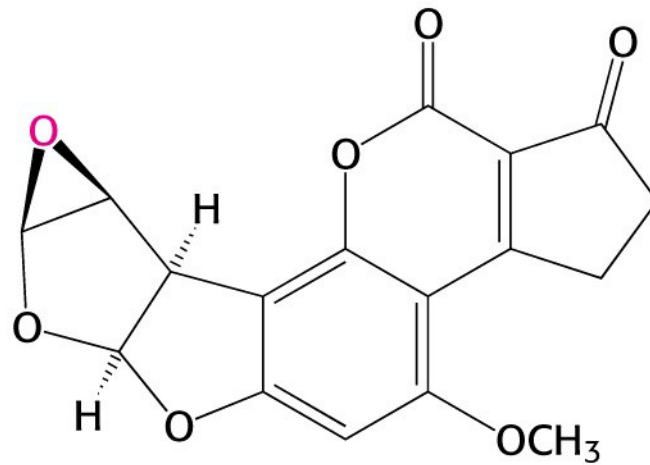




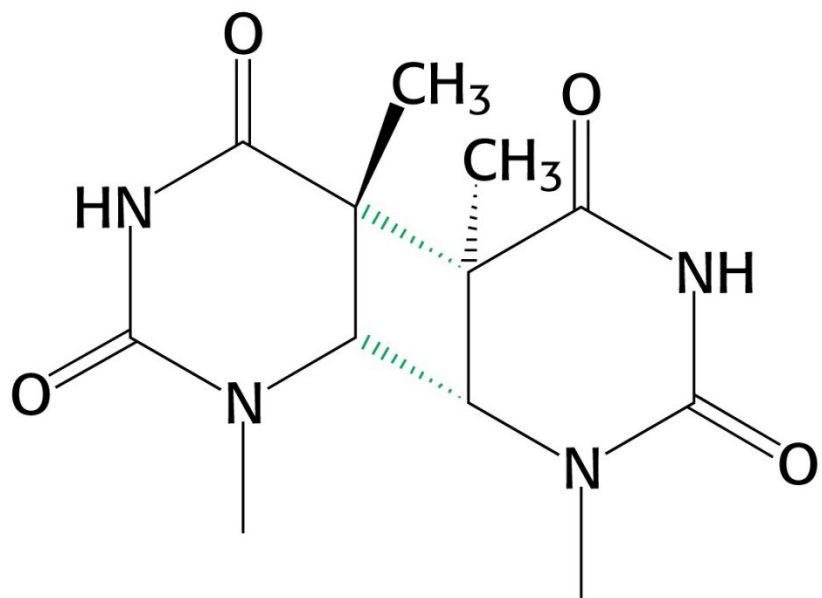
Aflatoxin B₁



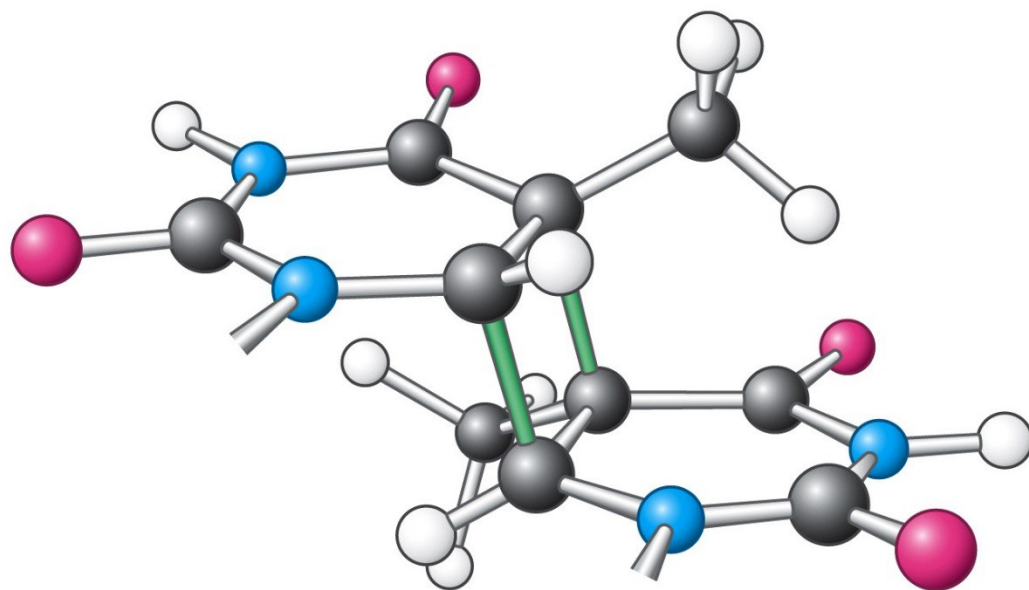
Cytochrome P450



Active DNA-modifying agent



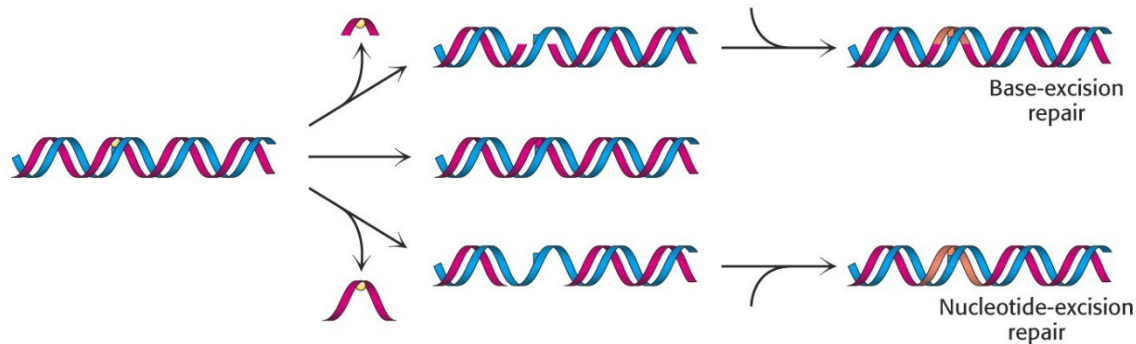
Thymine dimer



Mehanizmi popravljanja okvar na DNA:

NAPAKE NA 1 VERIGI

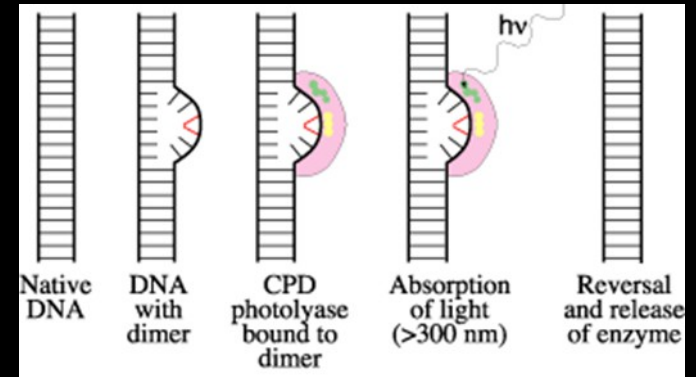
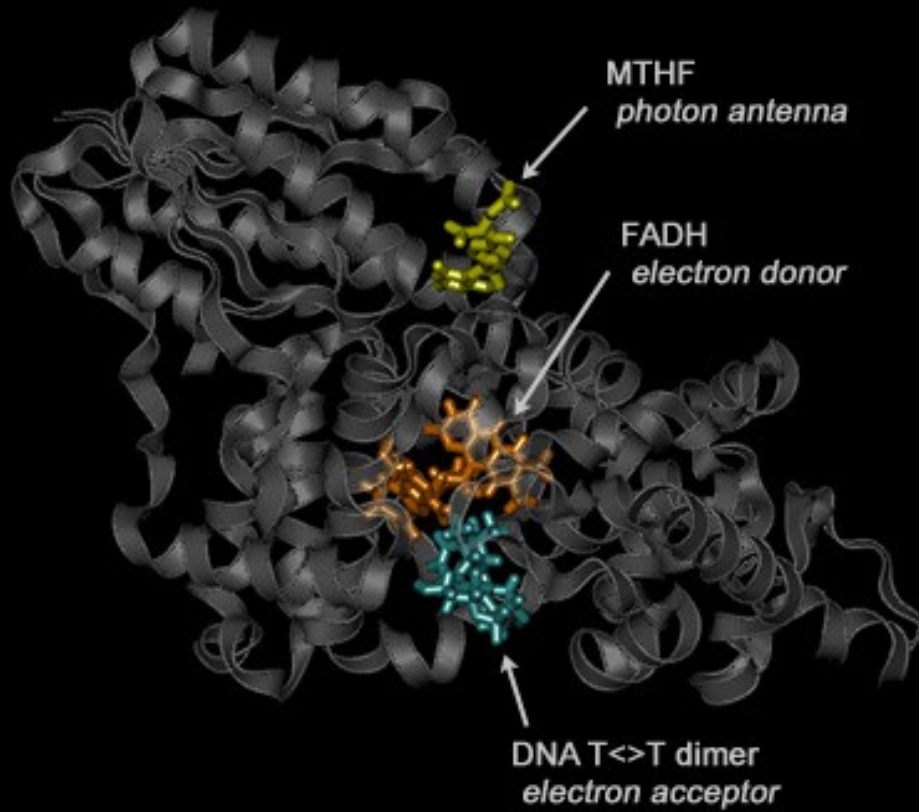
- direktno popravljanje (npr. T-dimerov, metiliranih G)
- popravljanje z izcepom baze (BER) – če je baza kemično modificirana
- popravljanje z izcepom nukleotida (NER) – če napaka pomeni distorzijo dvojne vijačnice
- **popravljanje neujemanja (MMR) – nesparjeni bazni pari po replikaciji**



NAPAKE NA 2 VERIGAH

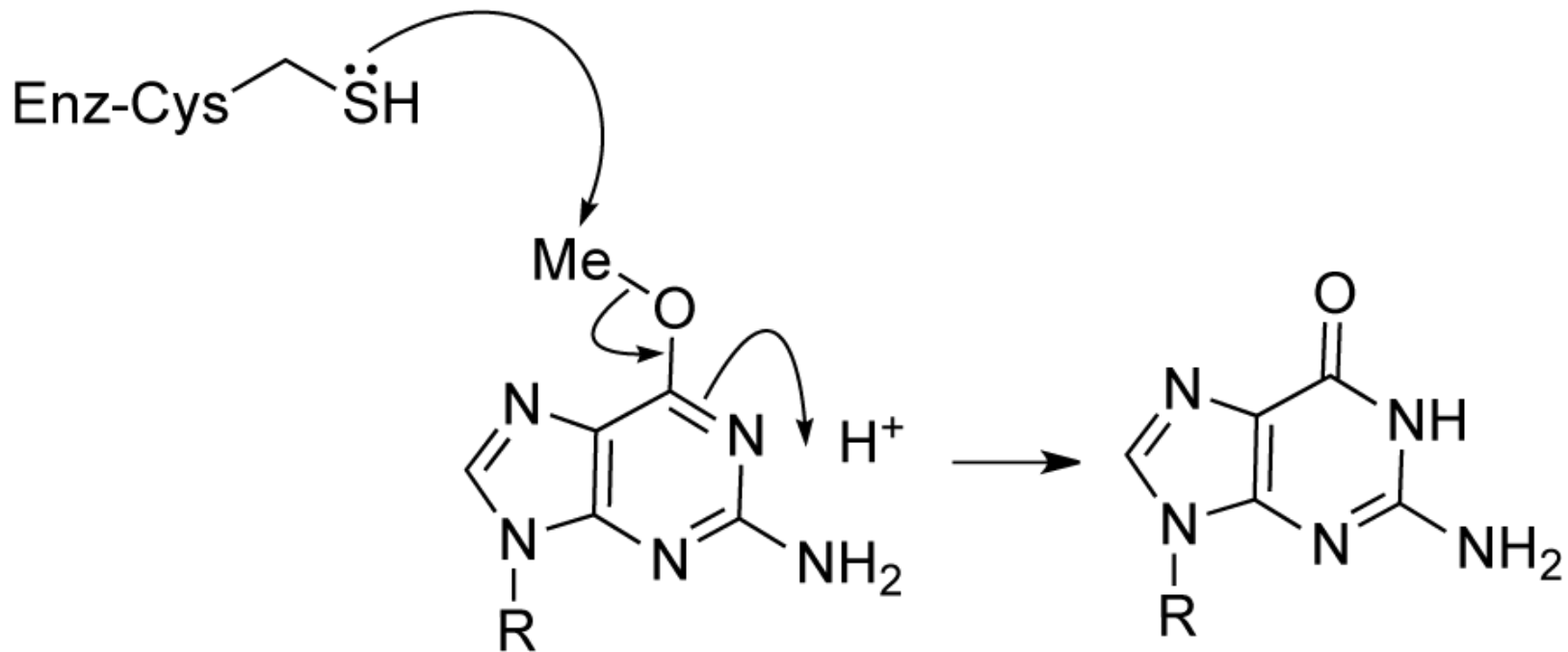
- nehomologno povezovanje koncev (NHEJ)
- homologna rekombinacija

DNA Photolyase from *E. coli*



MTHF= N,N-meteniltetrahidrofolat

Popravljanje kemično modificiranih baz – direktno popravljanje



Odstranjevanje metilne skupine z metilgvanina s samomorilskim encimom O(6)-metilgvanin metiltransferazo (MGMT). Encim se v reakciji metilira in ob tem inaktivira.



Nature 315 (6020), 604-606, 1985

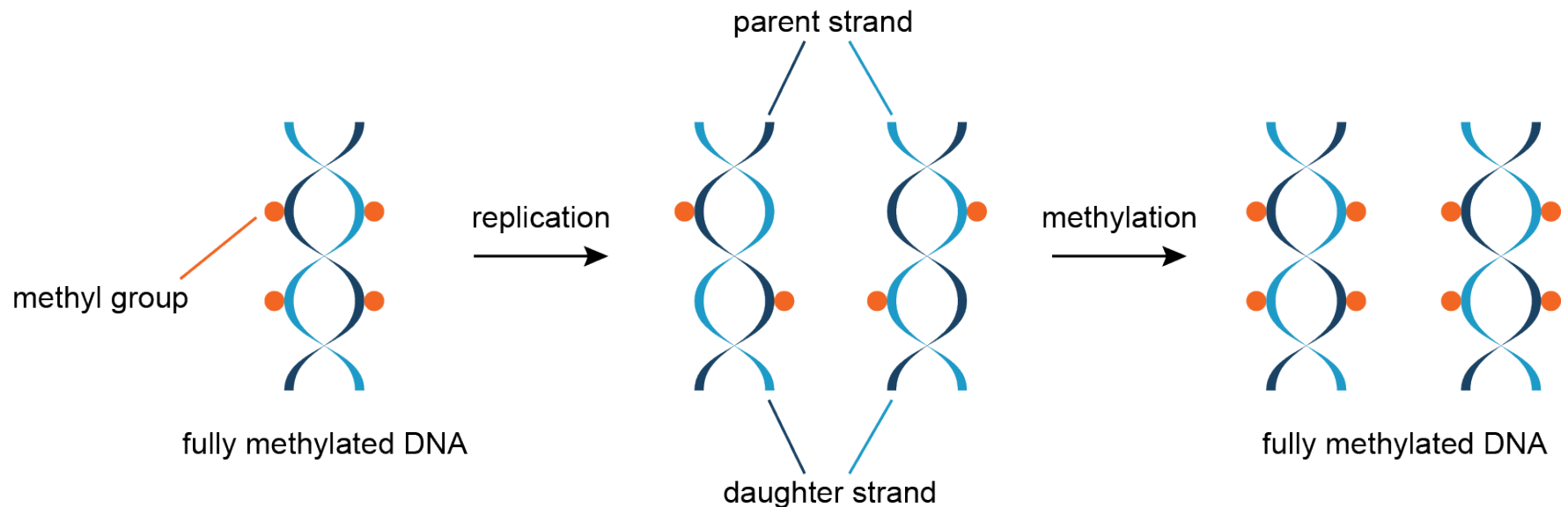
Izvihane baze na mestu mutacije: ,base flipping‘

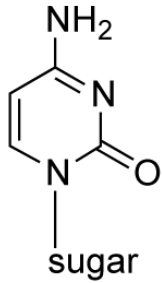
Prepoznavanje hčerinske in starševske verige

Starševska veriga je metilirana, hčerinska pa sprva še ne (*E. coli*).

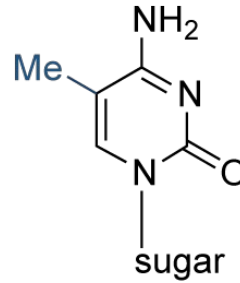
Pregledovalni proteini morda delujejo predvsem na mestih zarez med delnimi produkti podvojevanja genoma (predvsem pri zastajajoči verigi in pri evkariontih).

Popolno metilacijo kasneje, po morebitnem popraviljanju napak, katalizirajo vzdrževalne metiltransferaze.

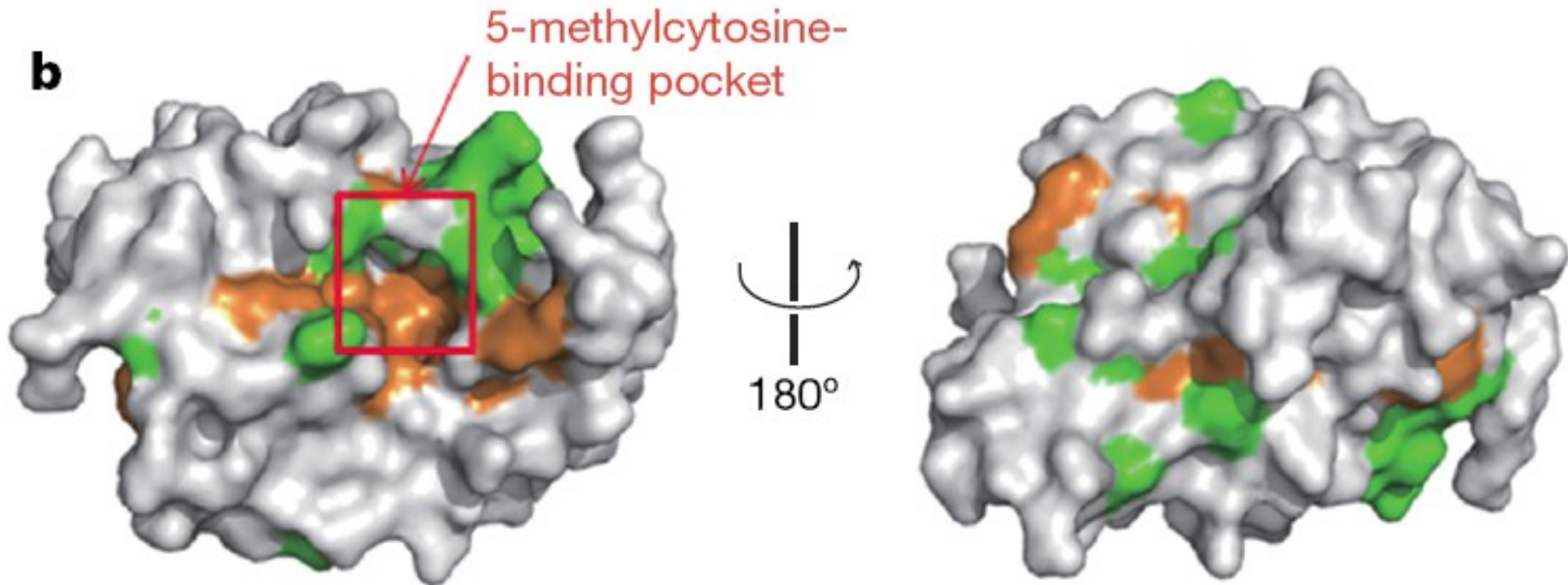




cytosine (C)



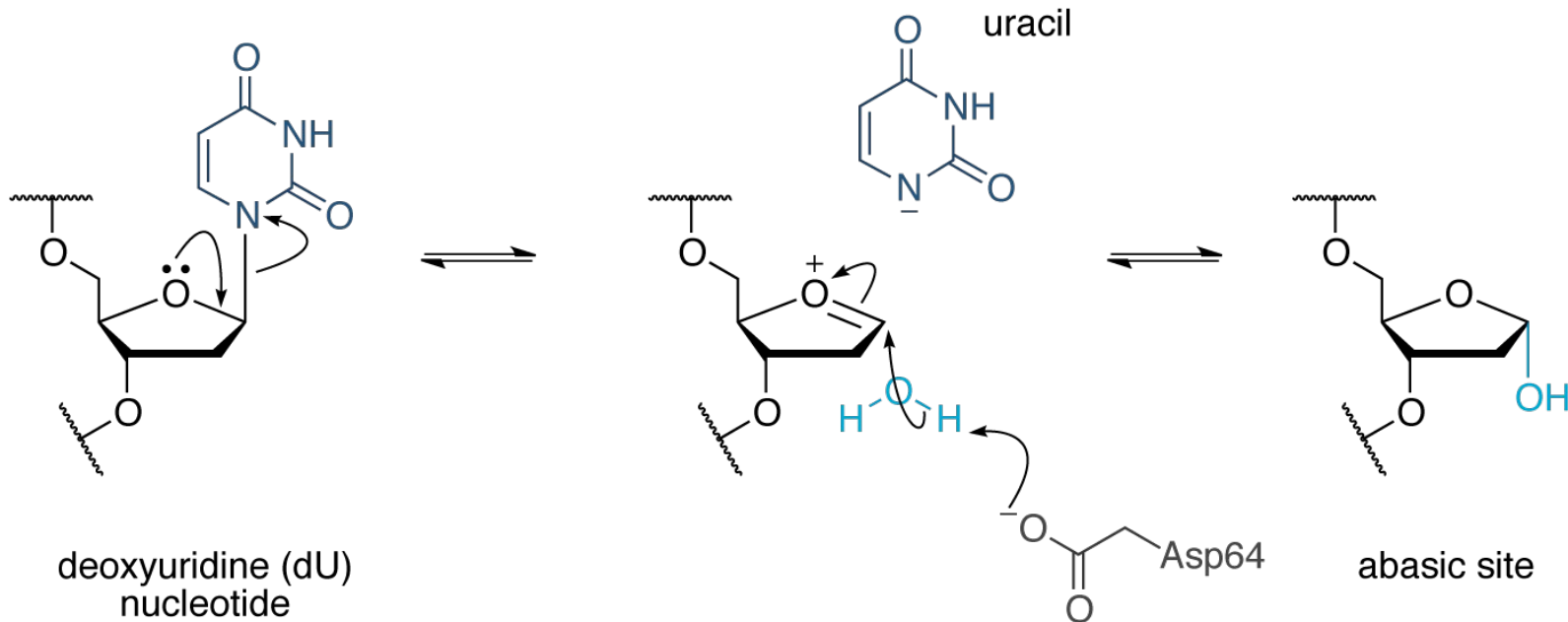
5-methylcytosine (mC)



Protein UHRF1 prepozna hemimetilirana mesta na DNA in nanja usmerja DNA-metiltransferazo I. Interakcija z DNA poteka preko domene SRA, ki so ji določili strukturo v prosti obliki in v kompleksu z DNA.

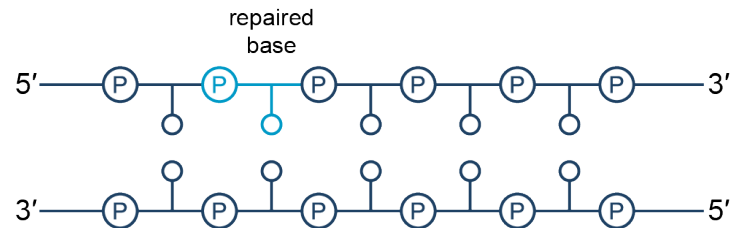
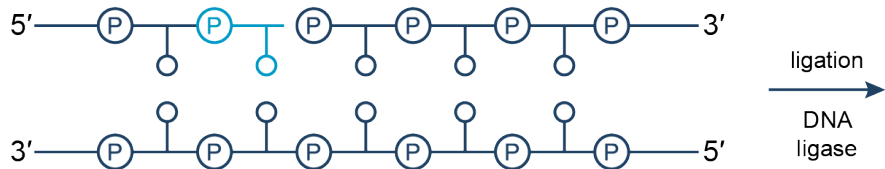
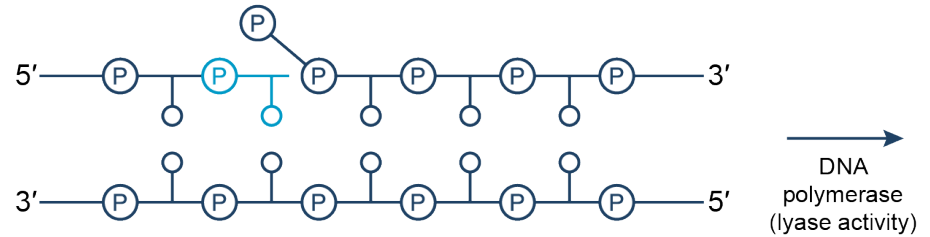
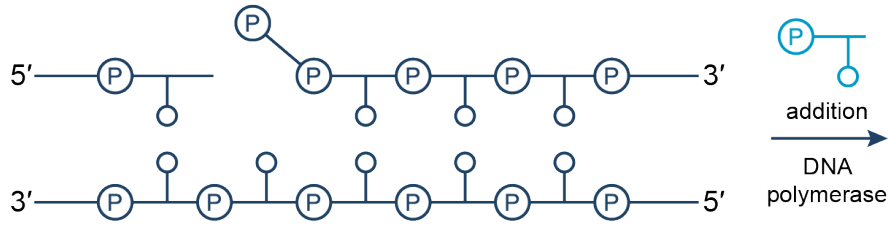
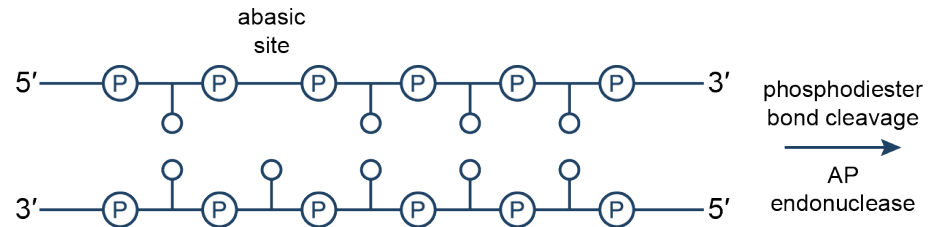
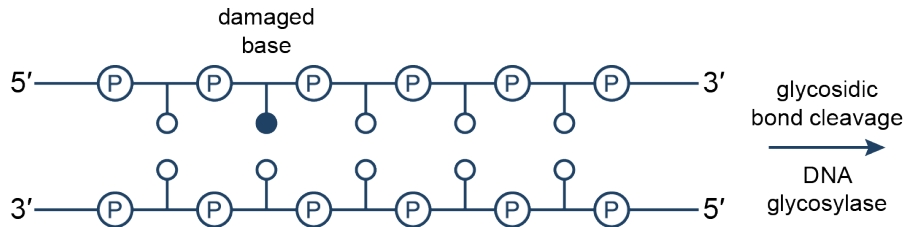
Popravljanje z izcepom baze (BER)

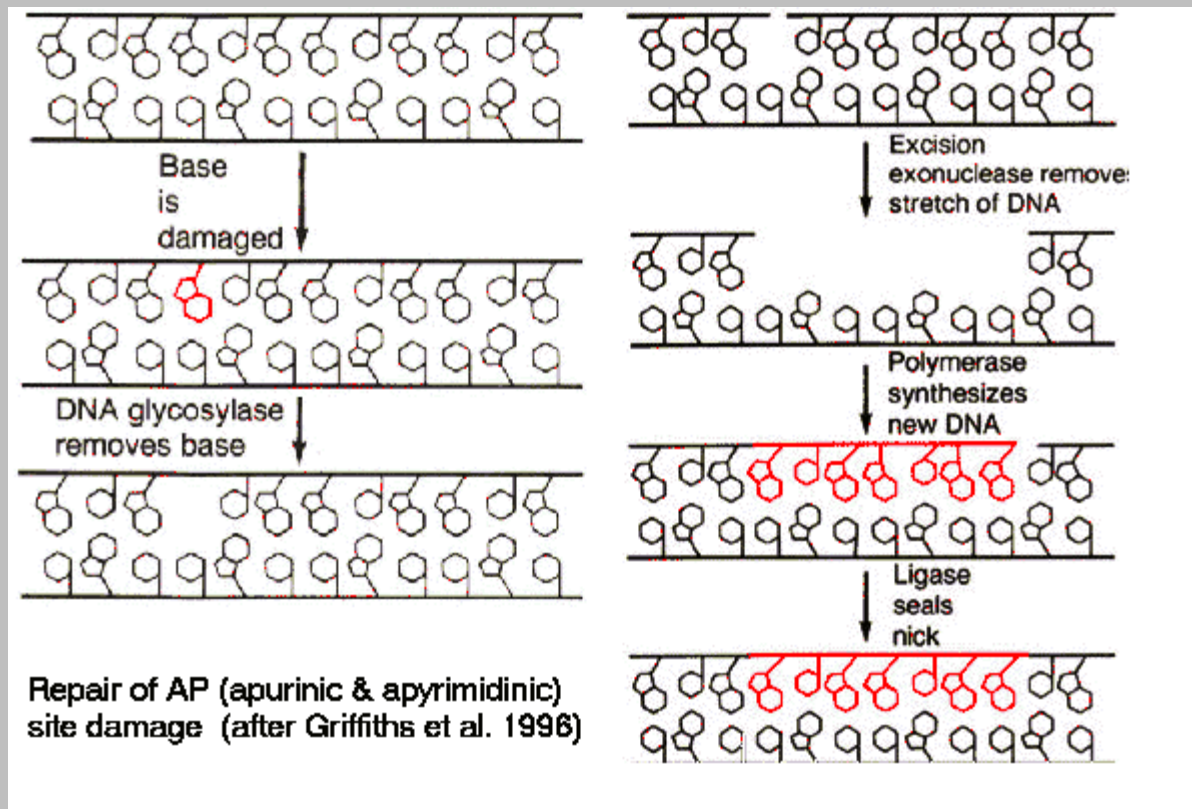
1. Okvarjeno bazo odstrani DNA-glikozilaza (za različne baze različne). S tem nastane abazično (AP) mesto na verigi.



Popravljanje z izcepom baze

2. Manjkajoči del se zapolni po delovanju AP-endonukleaze, DNA-polimeraze in DNA-ligaze.

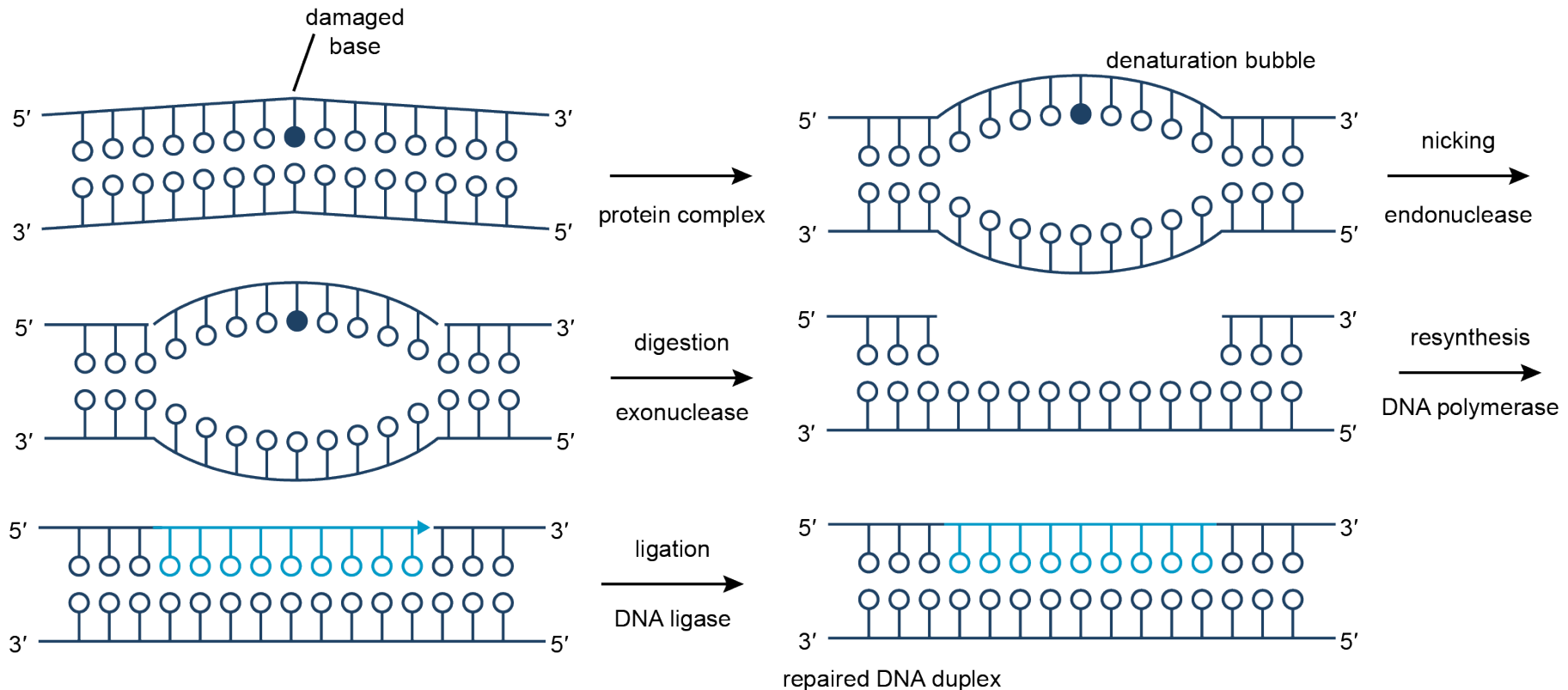




Popravljanje z odcepom baze:
alternativni mehanizem z zamenjavo več nt

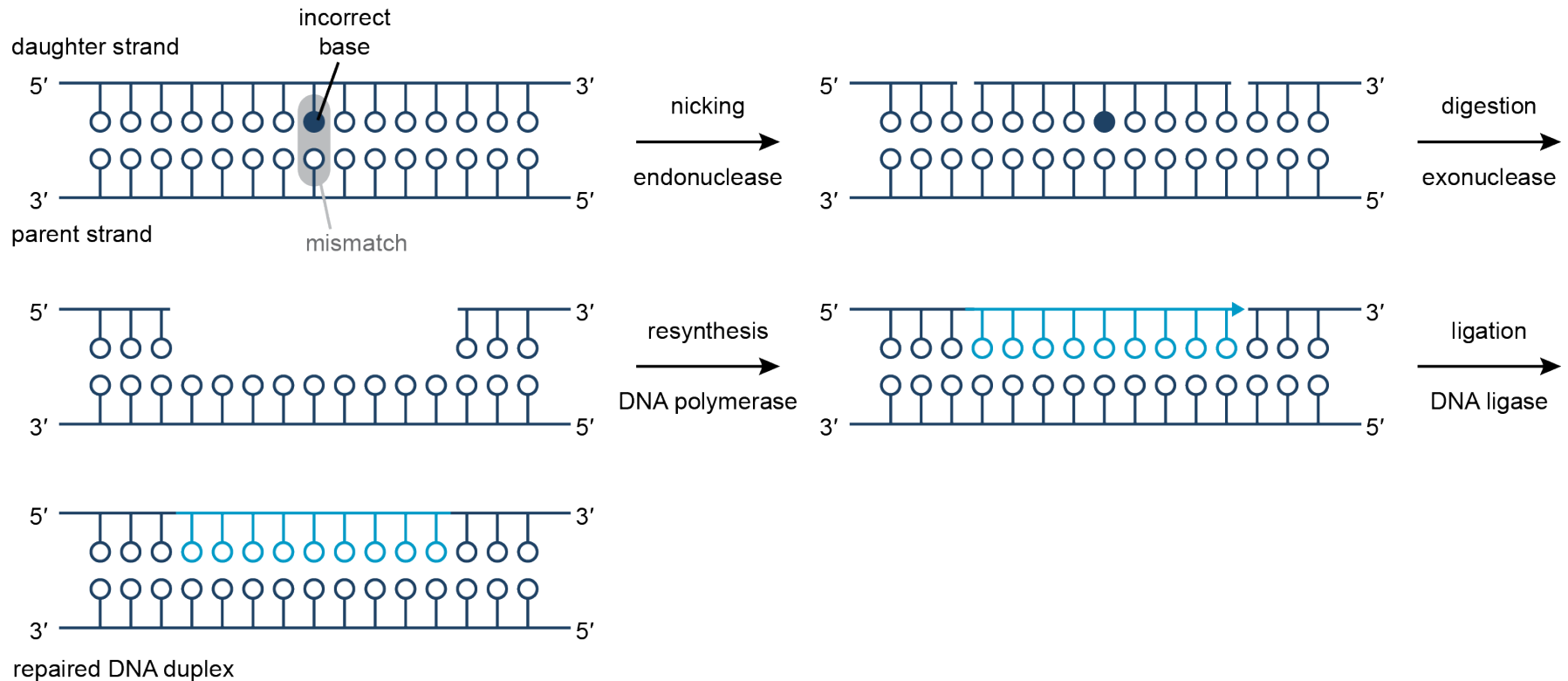
Popravljanje z izcepom nukleotida (NER)

Napake v obliki dvojne vijačnice prepoznajo proteini, nato nastane ‚denaturacijski mehurček‘, dolg ~30 bp. Pride do uvedbe dveh zarez, izcepa vmesnega segmenta in do popravljanja z DNA-polimerazo in ligazo.

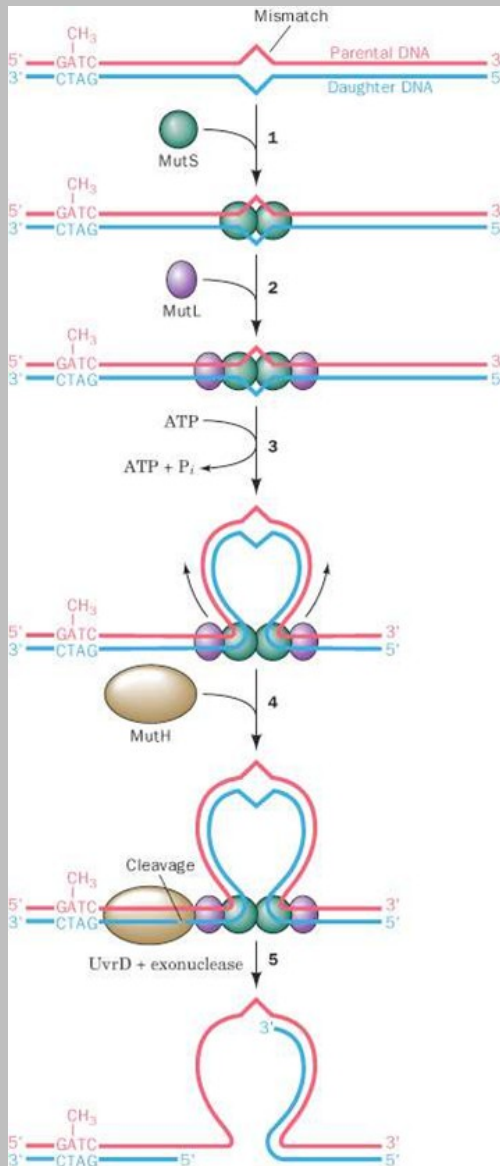


Popravljanje neujemanja (MMR)

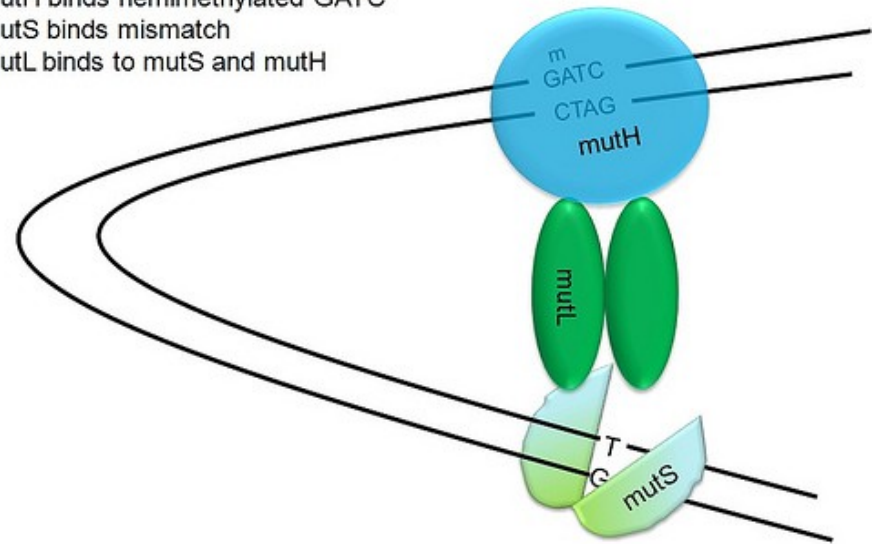
Proteini prepoznajo nepravilni bp. Na nemetilirani verigi (hčerinski) endonukleaza zareže na obeh straneh mutacije, eksonukleaza odstrani vmesni del. DNA-polimeraza in ligaza zapolnita vrzel.



Popravljanje neujemanja pri *E. coli*



mutH binds hemimethylated GATC
mutS binds mismatch
mutL binds to mutS and mutH



Prepoznavanje mesta mutacije

Dimer MutS (MutS2) prepozna napačno sparjeno bazo na hčerinski verigi in se veže nanjo.

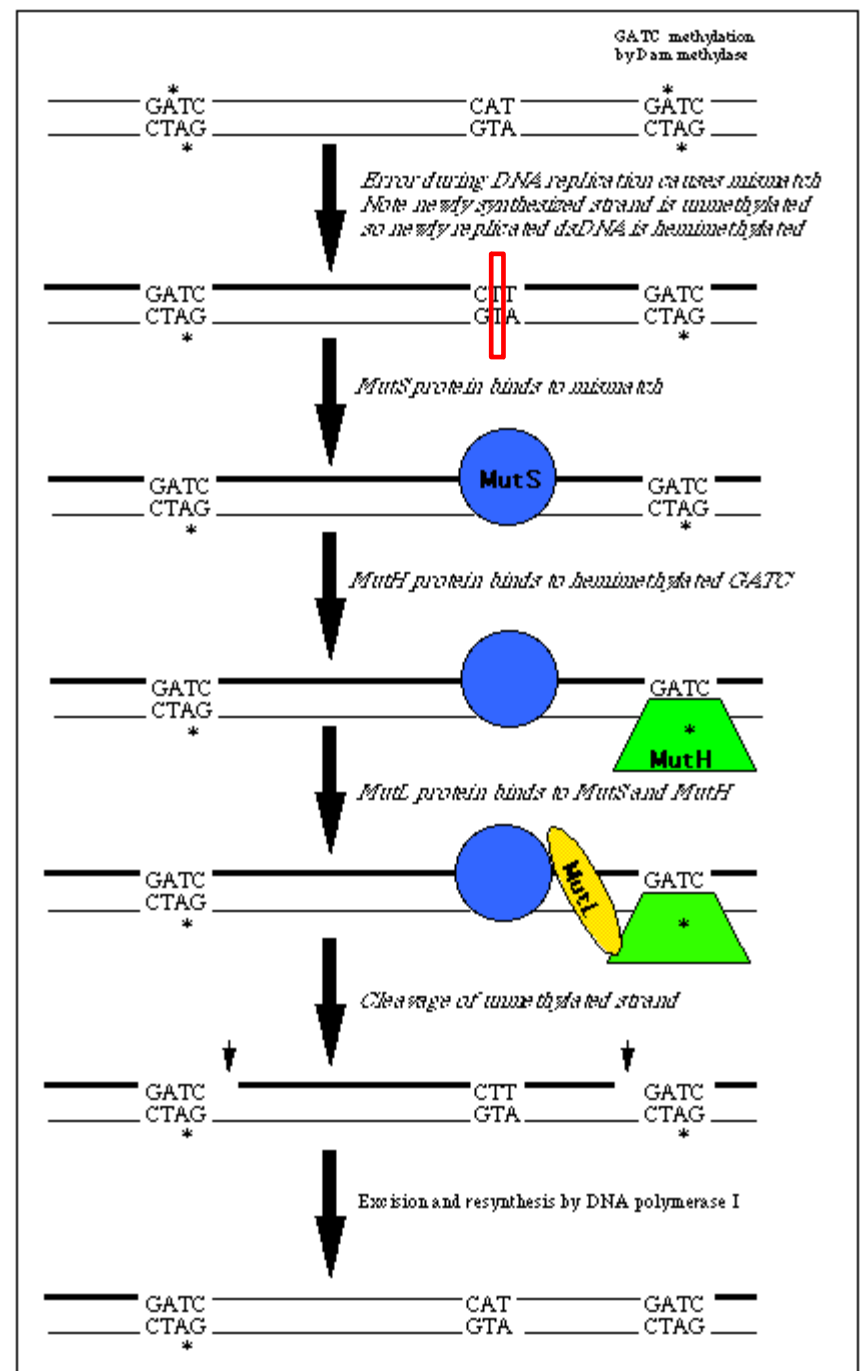
MutH se veže na hemimetilirana mesta, aktivira pa se po stiku z dimerom MutL (MutL2), ki se veže na MutS2-DNA.

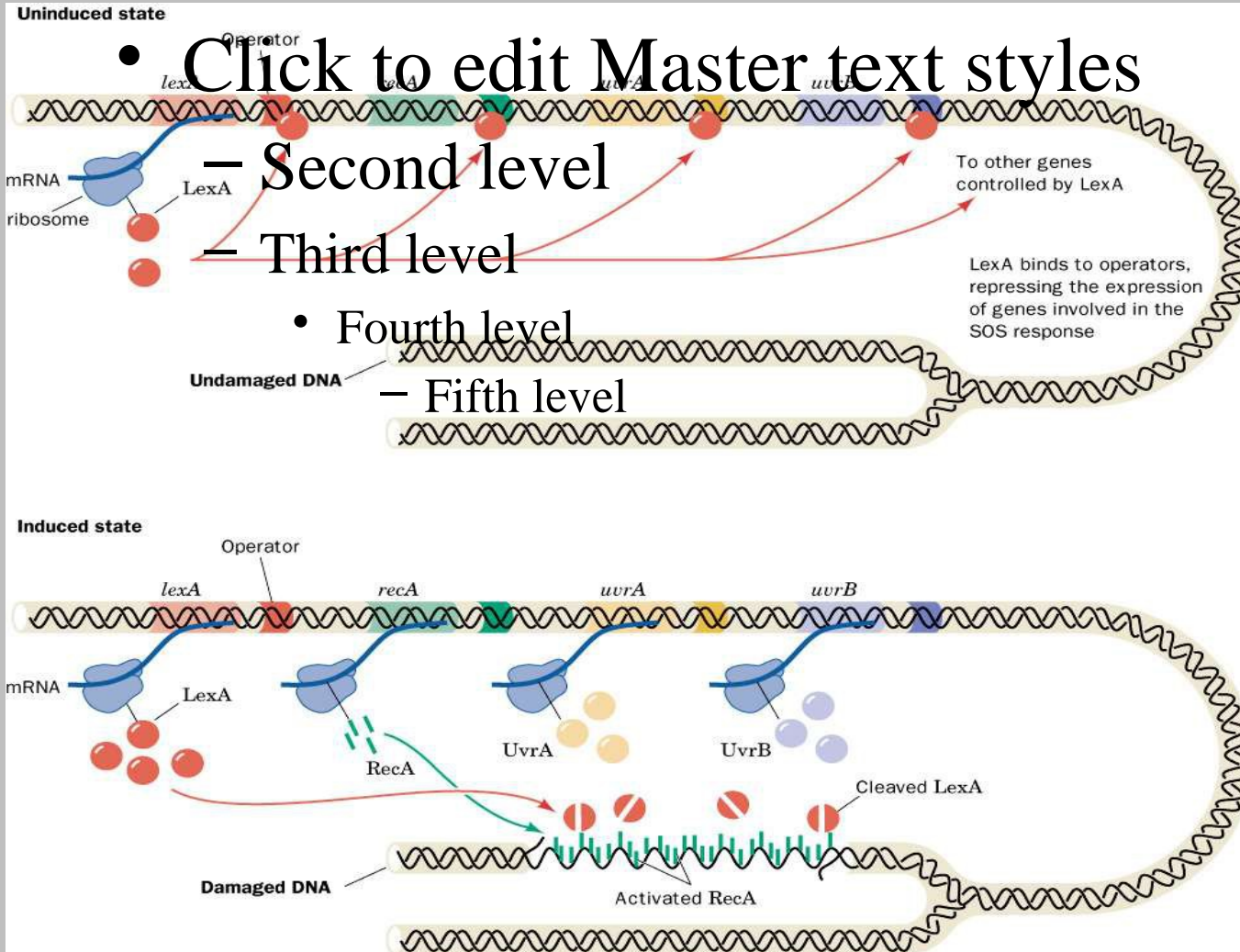
Na DNA naredi zanko in skenira, kje je najbližje metilacijsko mesto d(GATC), kar je lahko tudi ~1 kb stran.

Kompleksu sledi eksonukleaza in razgradi DNA.

Izreže se regija, ki je daljša kot samo do mesta mutacije.

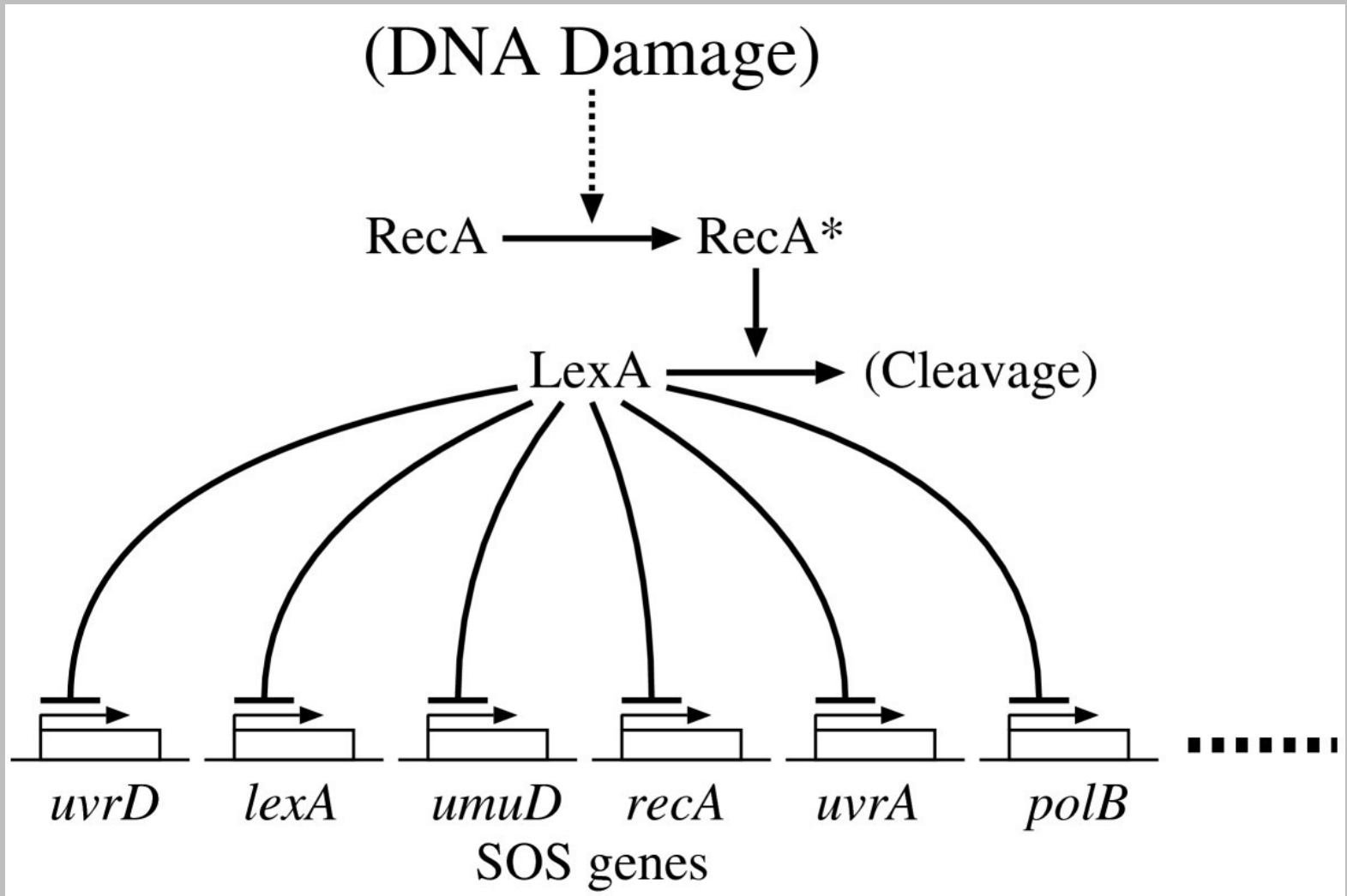
DNA-polimeraza sintetizira novo komplementarno verigo. Končno vrzel zapolni DNA-ligaza. Metilaza Dam metilira hčerinsko verigo.



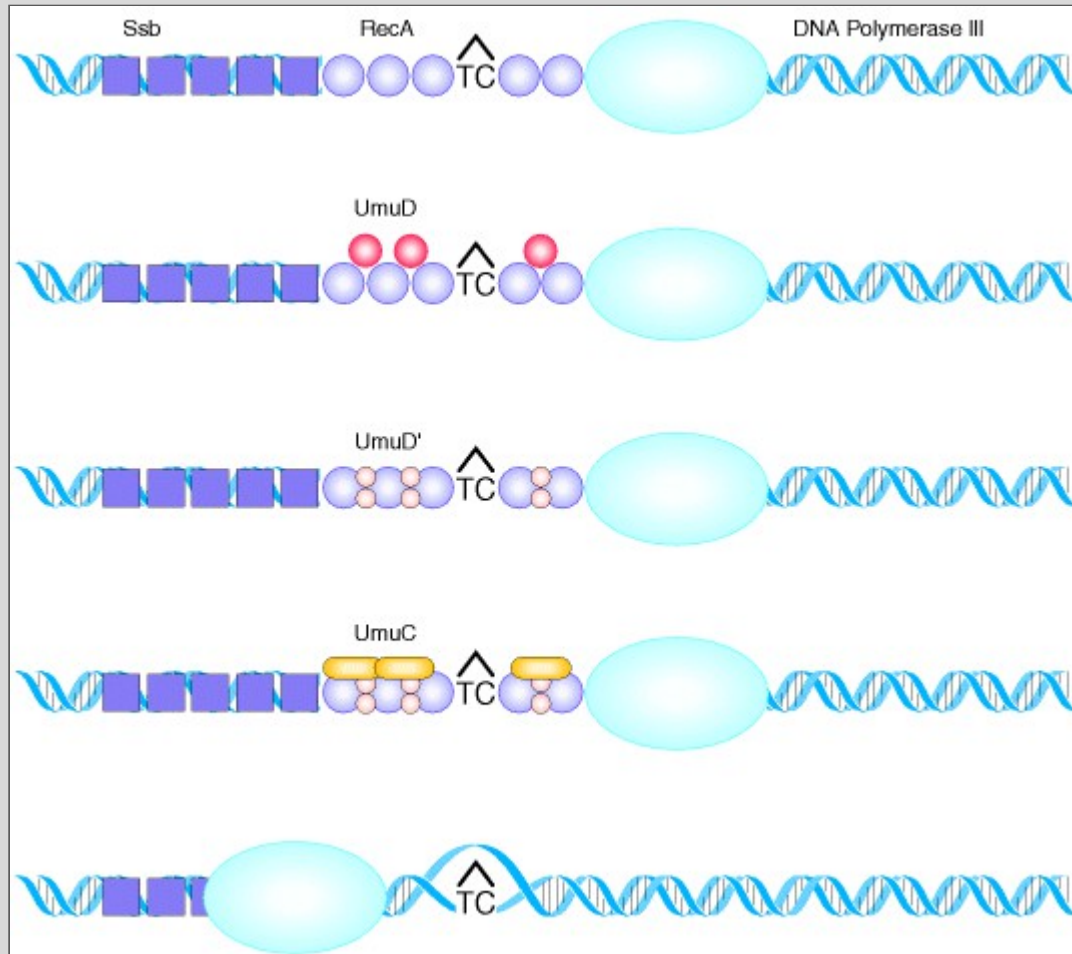


Uravnavanje SOS-odgovora pri *E. coli*

Delovanje LexA

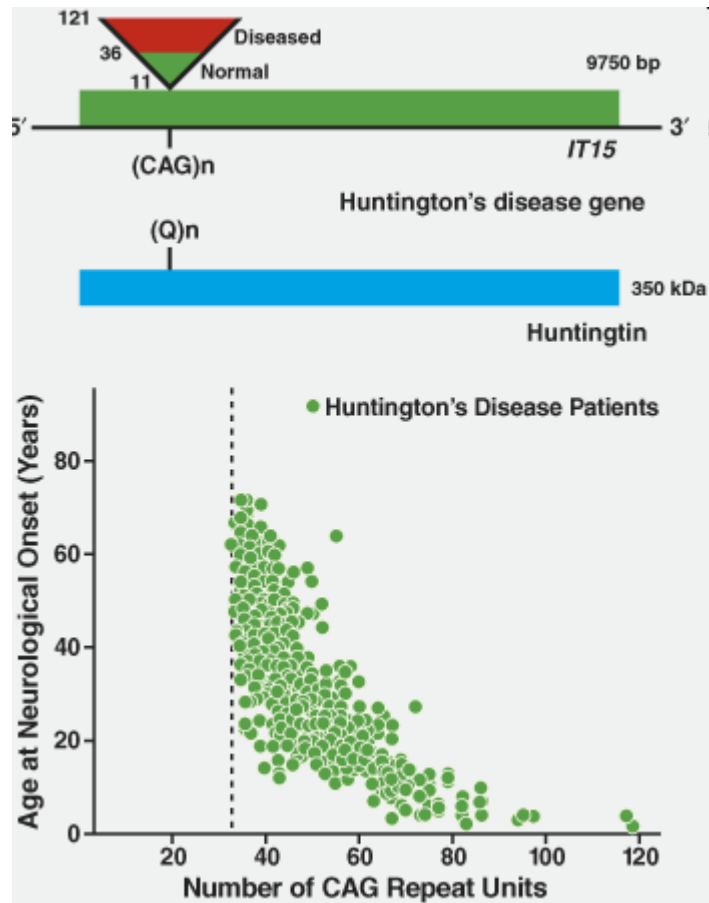


Princip SOS - popravljanja

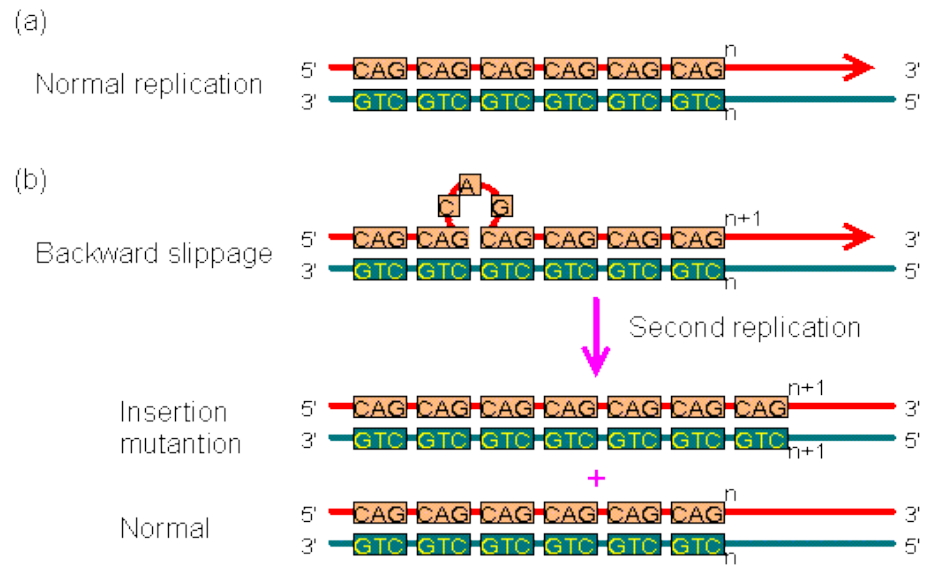


DNA-polIII se ustavi na mestu mutacije (dimer TC). Na mestih za mutacijo so vezani SSB in RecA v obliki filameta. Ker popusti blokada LexA, se sintetizira UmuD. Ta se procesira do aktivne oblike UmuD', ki pritegne UmuC. Ta kompleks (UmuD'2C) je DNA-pol V brez eksonukleazne aktivnosti in nadaljuje sintezo preko mutiranega mesta.

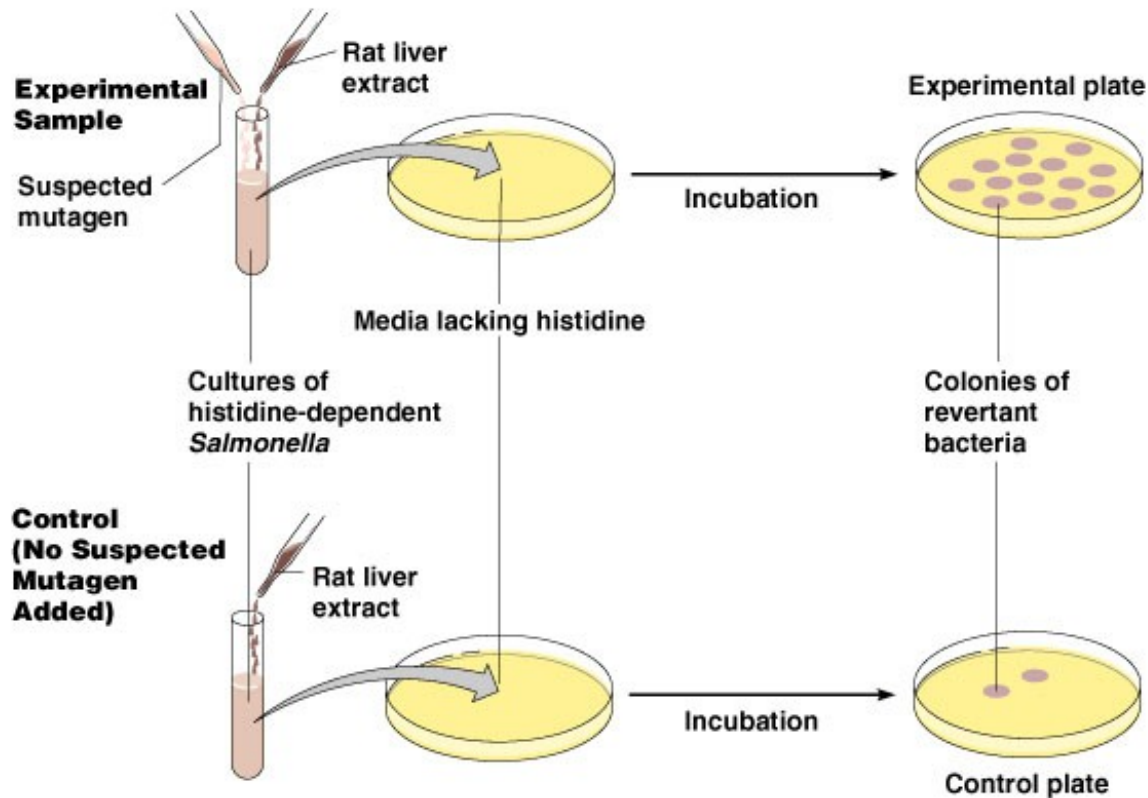
Za organizem je mutacija bolj sprejemljiva kot prekinitve sinteze.



<http://www.gladstone.ucsf.edu/gladstone/files/publicaffairs/mono15fig3.gif>



<http://www.web-books.com/MoBio/Free/Ch7F3.htm>



- 1 Two cultures are prepared of *Salmonella* bacteria that have lost the ability to synthesize histidine (histidine-dependent).
- 2 The suspected mutagen is added to the experimental sample only; rat liver extract (an activator) is added to both samples.
- 3 Each sample is poured onto a plate of medium lacking histidine. The plates are then incubated at 37°C for two days. Only bacteria whose histidine-dependent phenotype has mutated back (reverted) to histidine-synthesizing will grow into colonies.
- 4 The numbers of colonies on the experimental and control plates are compared. The control plate may show a few spontaneous histidine-synthesizing revertants. The test plates will show an increase in the number of histidine-synthesizing revertants if the test chemical is indeed a mutagen and potential carcinogen. The higher the concentration of mutagen used, the more revertant colonies will result.