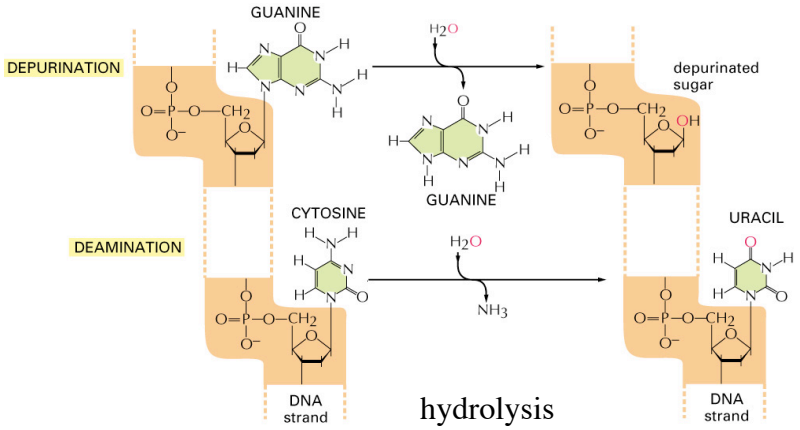
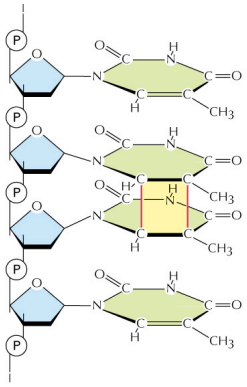
Common types of DNA damage



hydrolysis



pyrimidine intrastrand dimer

base modifications: methylation, oxidation
mispairs: mistakes in DNA synthesis
cross-linked nucleotides: intrastrand, interstrand covalent links
double-stranded DNA breaks

Different types of repair fix different types of damage

In increasing order of complexity of the problem:

- direct repair of specific modification
- base excision repair: missing or altered base
- (oligo)nucleotide excision repair:
  distortion of B-DNA with damage on one strand
- mismatch repair: both bases are OK but the combo is not
- interstrand cross-link or double-stranded break repair:
  both strands are damaged

Common forms of damage have devoted repair enzymes

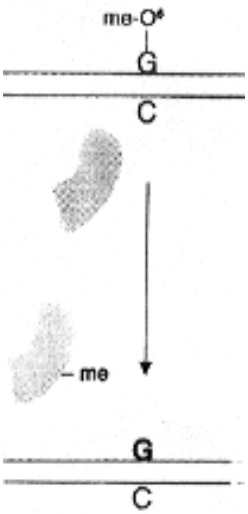
This is not ideal, because then new or unusual types of damage don't get repaired. But it can make repair of common damage efficient.

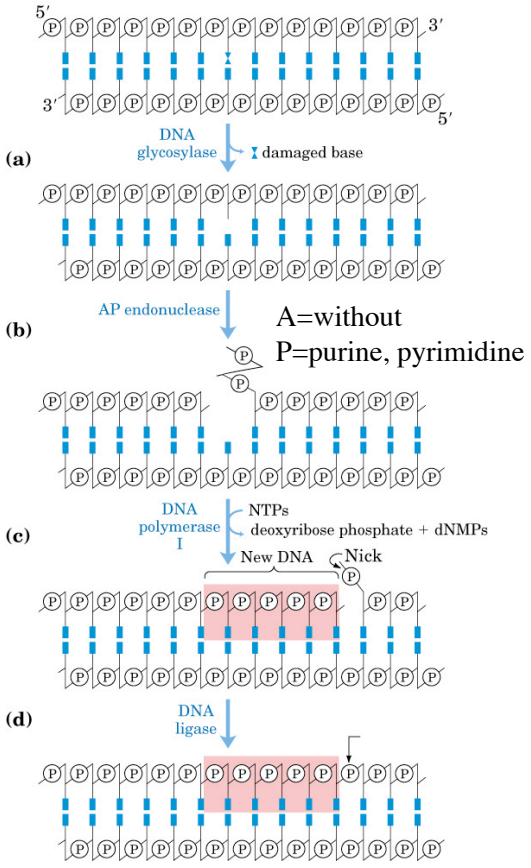
Two examples:

A specific methyltransferase enzyme can remove the methyl group from *O*^{6}-methylguanine, directly reversing the modification.

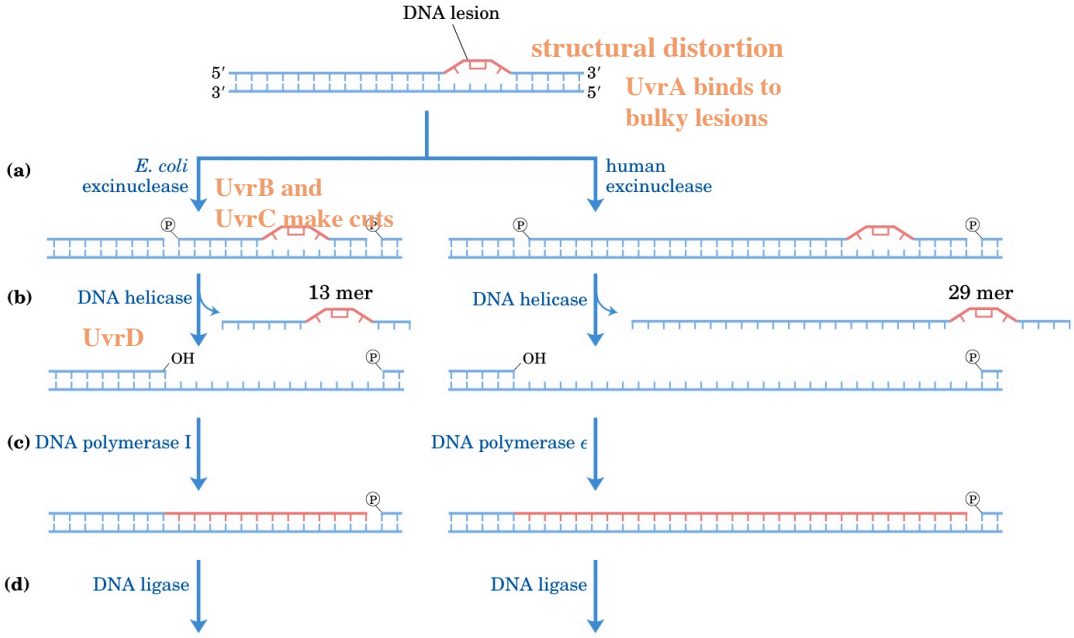
Specialized DNA polymerases are used to insert residues opposite damage sites.

O^6-meG would pair with TTP





DNA repair by the base-excision repair pathway (BER). **(a)** A DNA glycosylase recognizes a damaged base and cleaves between the base and deoxyribose in the backbone. **(b)** An AP endonuclease cleaves the phosphodiester backbone near the AP site. **(c)** DNA polymerase I initiates repair synthesis from the free 3' OH at the nick, removing a portion of the damaged strand (with its 5'→3' exonuclease activity) and replacing it with undamaged DNA. **(d)** The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.



Nucleotide-excision repair (NER). (a) Two excinucleases (excision endonucleases) bind DNA at the site of bulky lesion. **(b)** One cleaves the 5' and the other cleaves the 3' on either side of the lesion, and the DNA segment is removed with the aid of a helicase. **(c)** The gap is filled in by DNA polymerase, and **(d)** the remaining nick is sealed with DNA ligase.

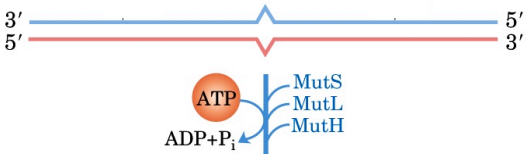
Xeroderma Pigmentosum (XP)

Human genes for NER are defective XP patients:

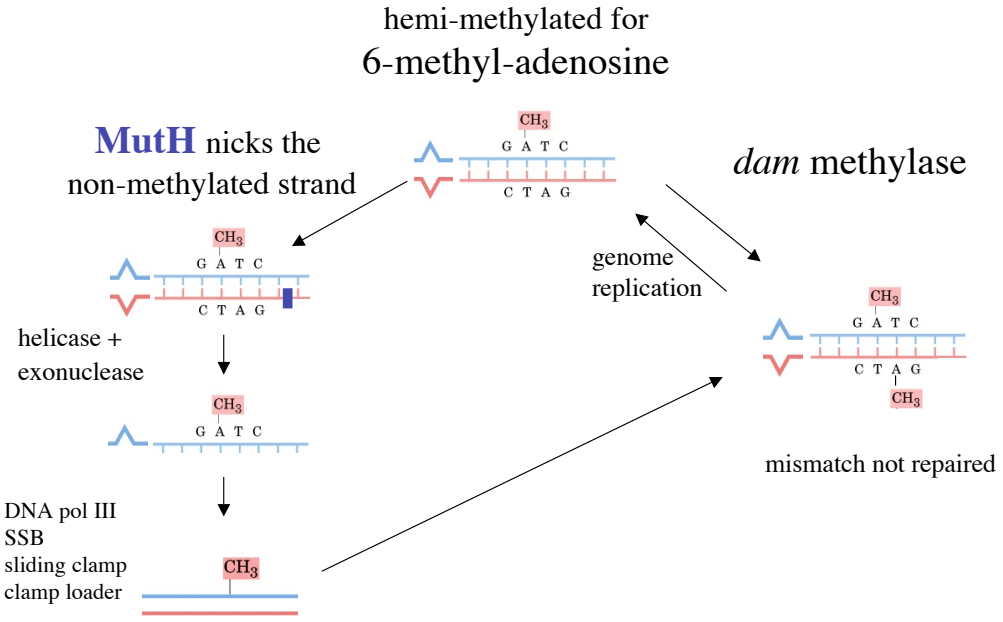
XP-A: damage recognition
XP-B: helicase
XP-C: DNA binding
XP-D: helicase
XP-F: 5' nuclease
XP-G: 3' nuclease

Yeast equivalents were discovered as genes important for surviving radiation (RAD genes)

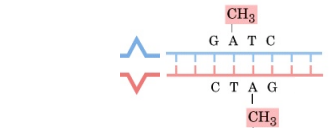
Mismatch repair: Which strand has the mutation?



Mut S binds mispair
Mut L links S to H
Mut H recognizes the correct strand

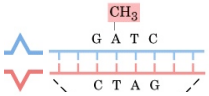


6-methyl-adenosine does not interfere with base-pairing
(it is NOT a sign of damage)

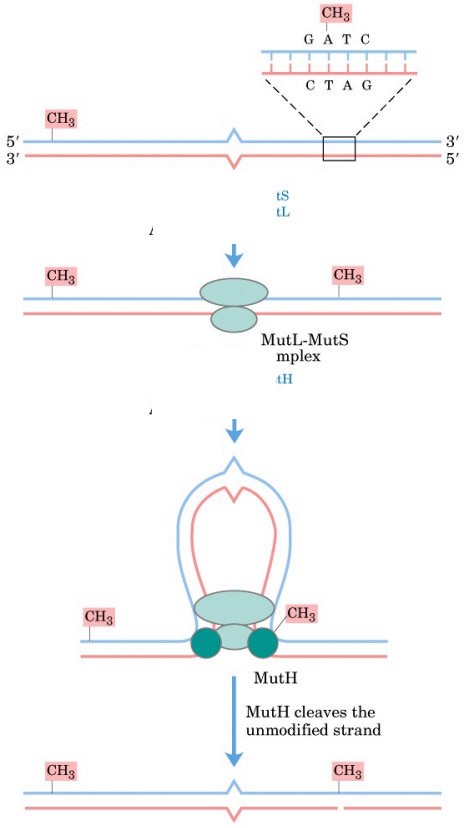


methylation by *dam*

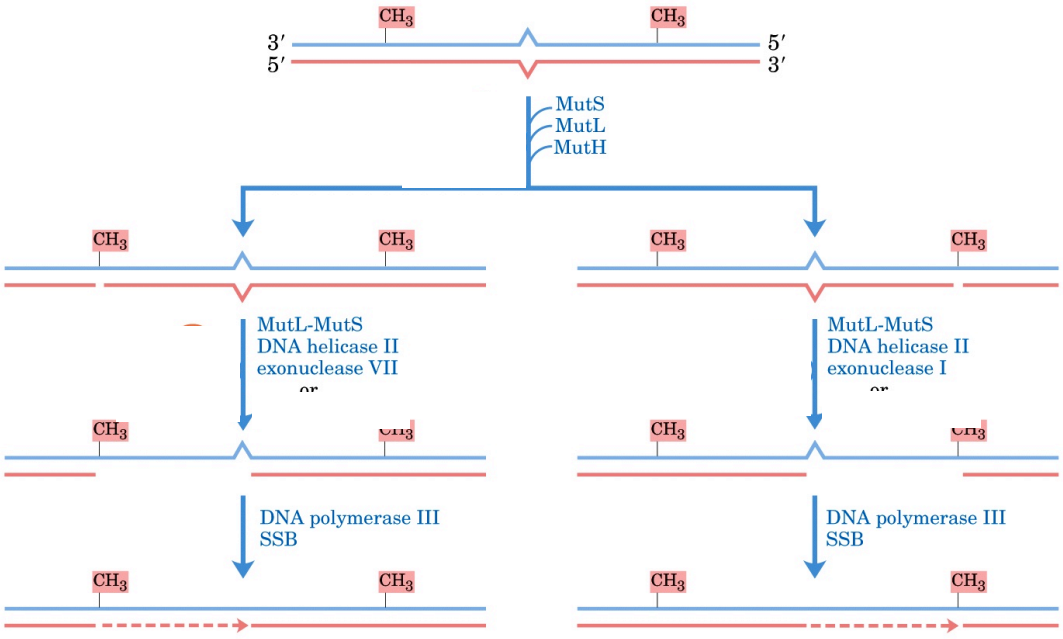
genome replication



transient hemi-methylation



Model for the early steps of methyl-directed mismatch repair. The proteins involved in this process in *E. coli* have been purified. Recognition of the sequence (5')GATC and of the mismatch are specialized functions of the MutH and MutS proteins, respectively. The MutL protein forms a complex with MutS at the mismatch. DNA is threaded through this complex such that the complex moves simultaneously in both directions along the DNA until it encounters a MutH protein bound at a hemimethylated GATC sequence. MutH cleaves the unmethylated strand on the 5' side of the G in the GATC sequence. A complex consisting of DNA helicase II and one of several exonucleases then degrades the unmethylated DNA strand from that point toward the mismatch.



Completing methyl-directed mismatch repair. The combined action of DNA helicase II, SSB, and exonucleases removes a segment of the new strand between the MutH cleavage site and a point just beyond the mismatch. The exonuclease used depends on the location of the cleavage site relative to the mismatch. The resulting gap is filled in by DNA polymerase III, and the nick is sealed by DNA ligase.

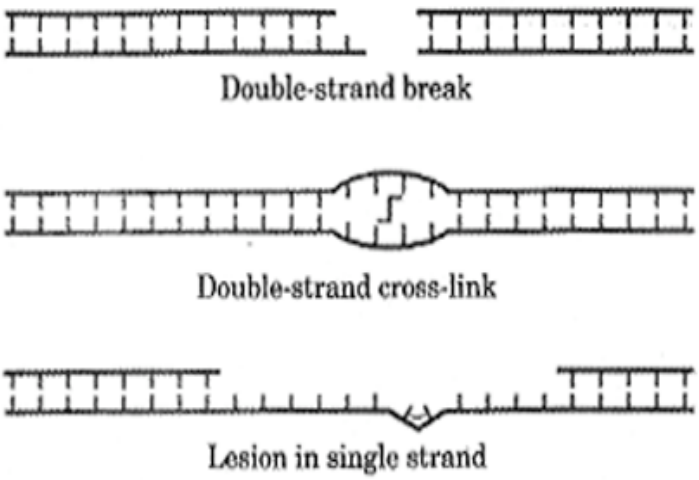
Hereditary Non-polyposis Colon Cancer (HNPCC)

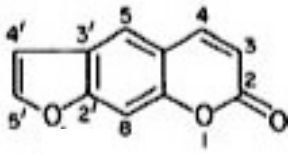
HNPCC results from mutations in genes involved in DNA mismatch repair, including:

- several different MutS homologs
- Mut L homolog
- other proteins: perhaps they play the role of MutH, but not by recognizing hemi-methylated DNA (no 6meA GATC methylation in humans, no dam methylase)

If both strands are damaged, there is

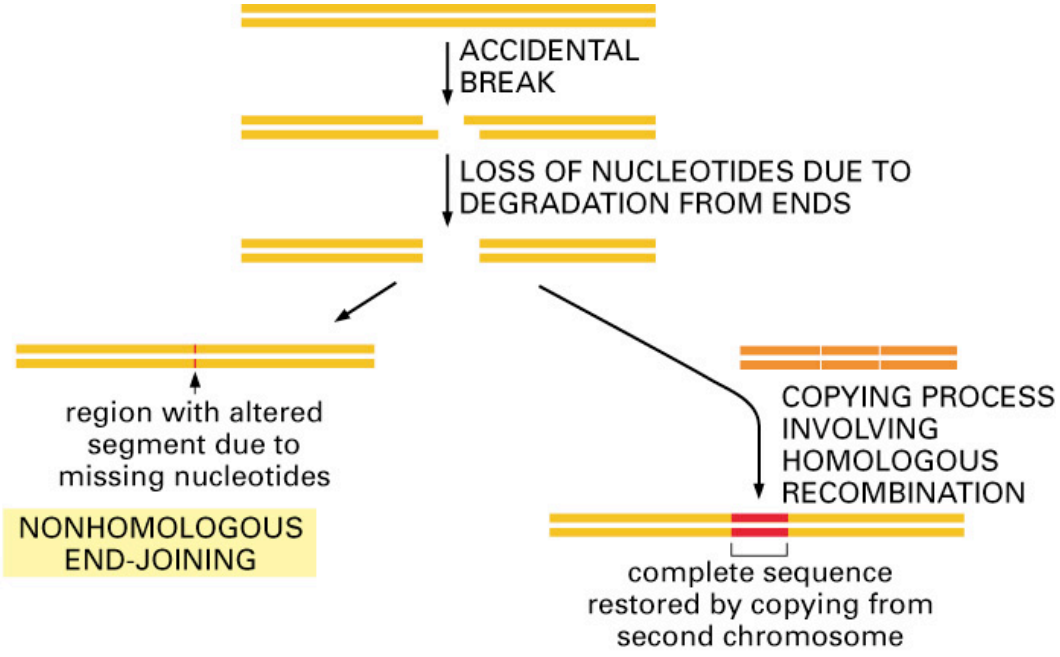
NO TEMPLATE FOR REPAIR!



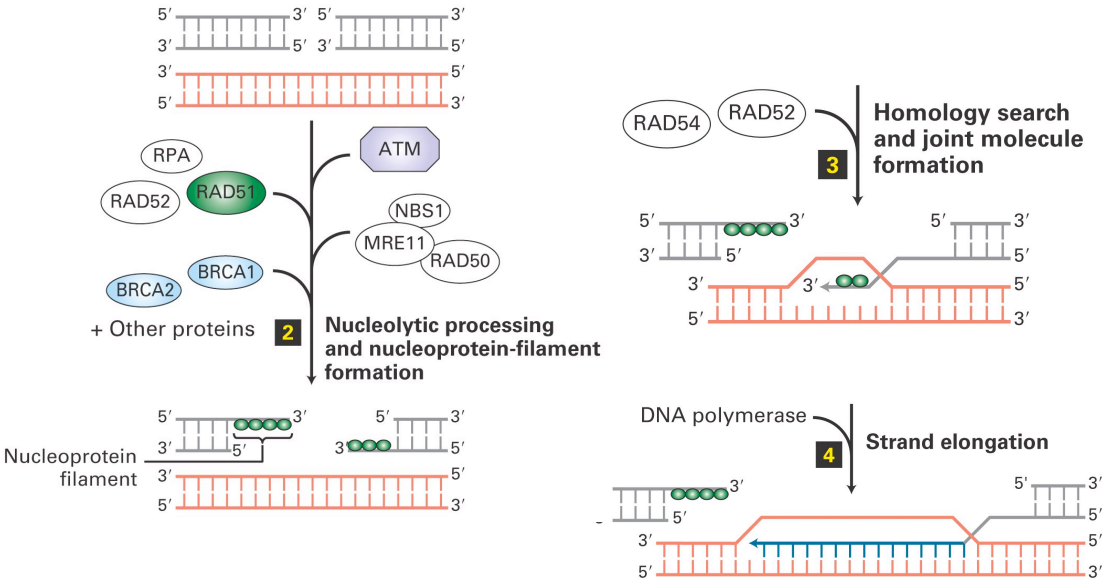


1 Psoralen

Alternatives for repair of a dsDNA break

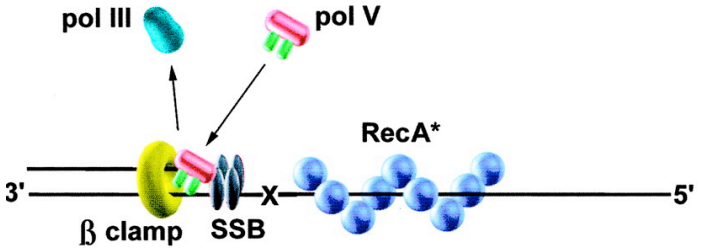


Repair by copying the homologous chromosome

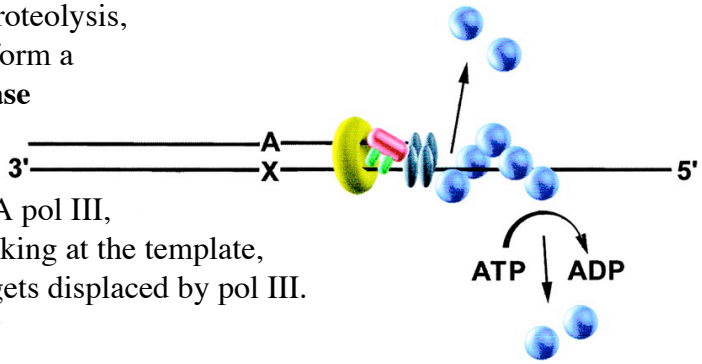


(this slide has the human DSBR machinery. we will return to this topic in detail)

If all else fails (in bacteria such *E. coli)* and no homologous strand of DNA can be found, cells induce **the SOS response**



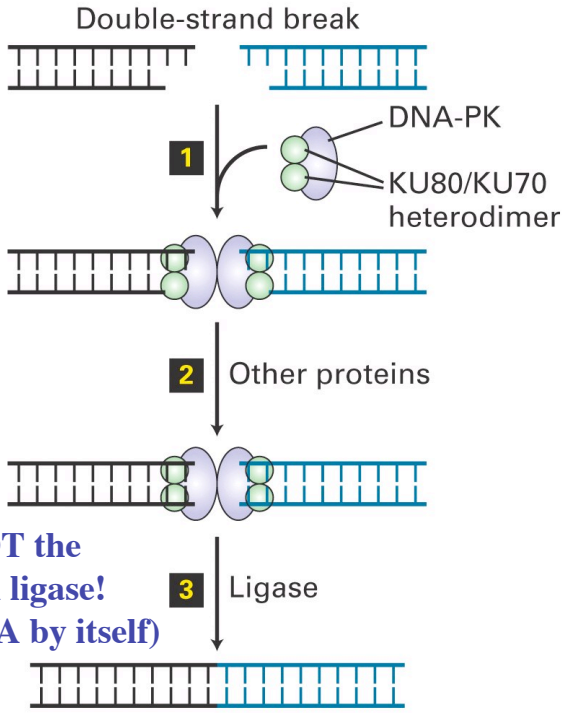
Something happens to mild-mannered RecA (*), which activates a protease, which cleaves transcriptional repressors, which makes UmuD and UmuC proteins, and also activates UmuD by proteolysis, at which point these subunits form a **highly error-prone polymerase pol V (UmuD'$_2$UmuC),**



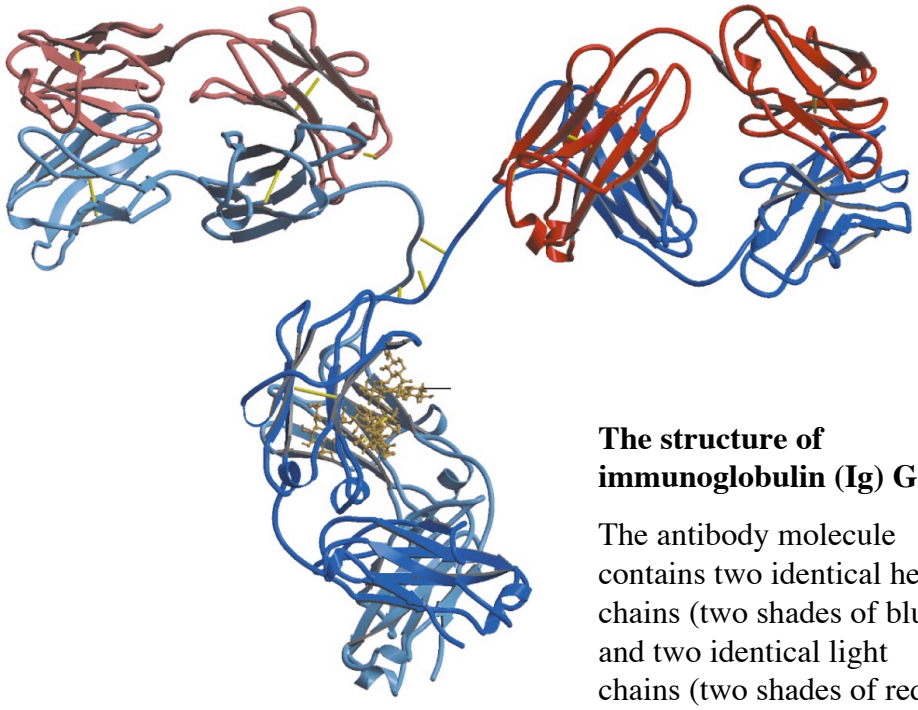
which then finds a stalled DNA pol III, adds dNTPs without really looking at the template, and after adding a few dNTP gets displaced by pol III.

Non-Homologous End Joining (NHEJ)

- Human cells do this quite well
- Required for antibody diversity: breaks at specific loci are repaired sloppily, so that each cell has a different gene sequence



DNA ligase IV, NOT the the DNA replication ligase! (this one can't bind DNA by itself)

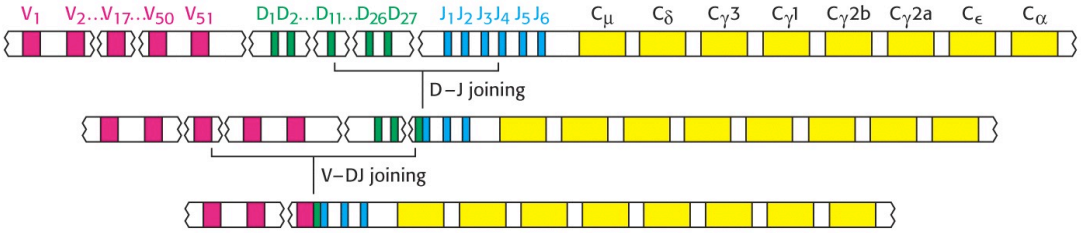


The structure of immunoglobulin (Ig) G.

The antibody molecule contains two identical heavy chains (two shades of blue) and two identical light chains (two shades of red)

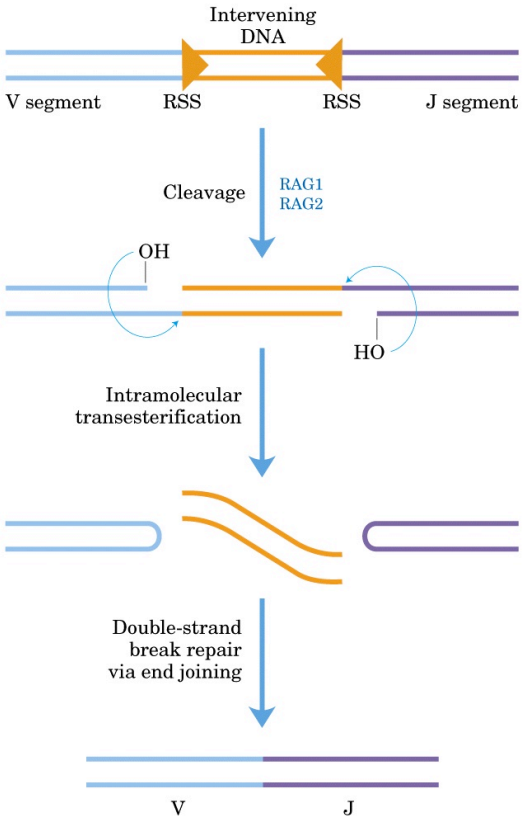
Any given cell makes only one heavy chain and one light chain, but each cell makes different heavy and light chains.

A heavy chain genomic locus in the germline must rearrange to give



... to give an expressed heavy chain in an antibody-producing B-cell

VDJ joining partners are variable. Other events switch the constant region (yellow) and set off locus hypermutation (mutagenesis), most cells die but some show improved antigen binding affinity.



Mechanism of immunoglobulin (Ig) gene rearrangement: irreversible excision of sequences.

The RAG1 and RAG2 proteins bind to a pair of recombination signal sequences (RSS) in INVERTED orientation and cleave one DNA strand between the RSS and the V + J segments to be joined. The liberated 3' hydroxyl then attacks a phosphodiester bond in the other strand to create hairpins at the 'coding ends' and breaks at the 'signal ends'. The resulting hairpin ends on the V and J segments are opened and then rejoined by NHEJ. The RSS ends are joined, but the excised circle is not maintained.

RAG1 and RAG2 are a former transposase, harne
purpose.

Error-prone repair of the V/D/J joins generates additional diversity.