DNA Repair and Genomic Instability

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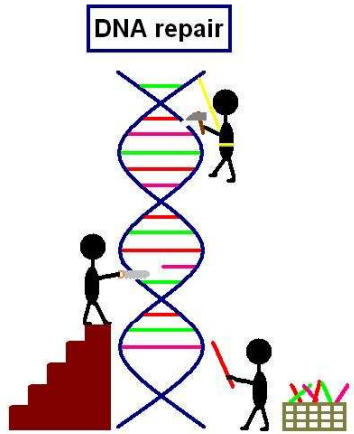
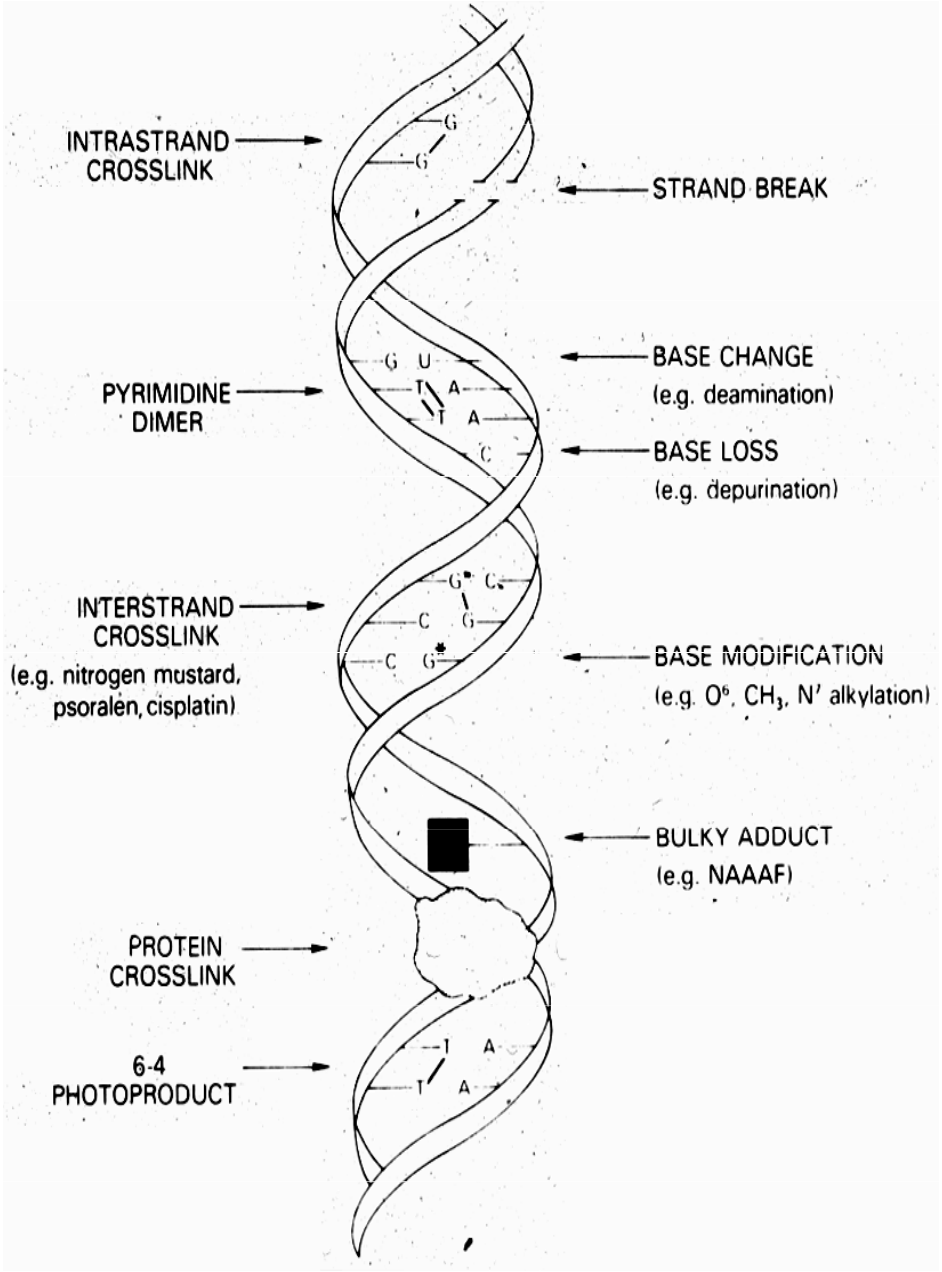
& Department of Biology



Jaký mechanismus se podílí na opravě dvouřetězcových zlomů?

Why do we study this?

Common Types of DNA Damage and Spontaneous Alterations



Exogenous Sources

UV (sunlight)
Pollution (hydrocarbons)

Smoking
Foodstuffs

Radiotherapy
Ionizing Radiation
X-rays

Chemotherapy
(Alkylating agents)
Cisplatin
Mitomycin C
Cyclophosphamide
Psoralen
Melphalan

Endogenous Sources

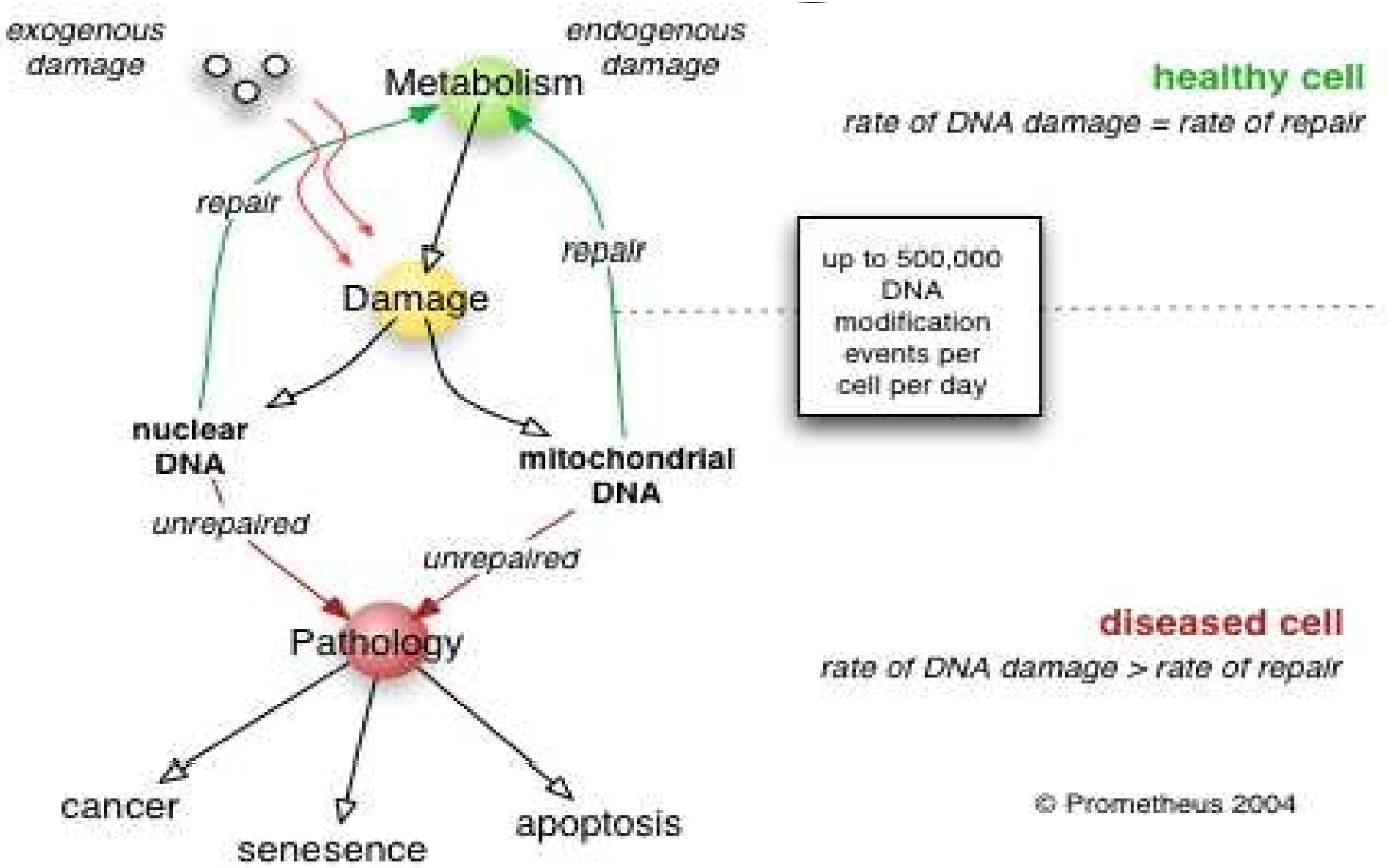
Oxidative damage by free radicals
(oxygen metabolism)
Replicative errors
Spontaneous alterations in DNA
Alkylating agents
(malondialdehyde)

DNA damage in human cell per day:

- loss of base – 26,000
- deamination of cytosin – 1 000
- alkylation of base – x 10 000
- dimerization of pyrimidins – 50 000
- ssDNA breaks – 100,000

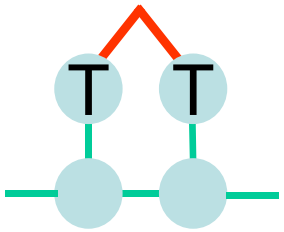
Total ~ 500 000 damage/day

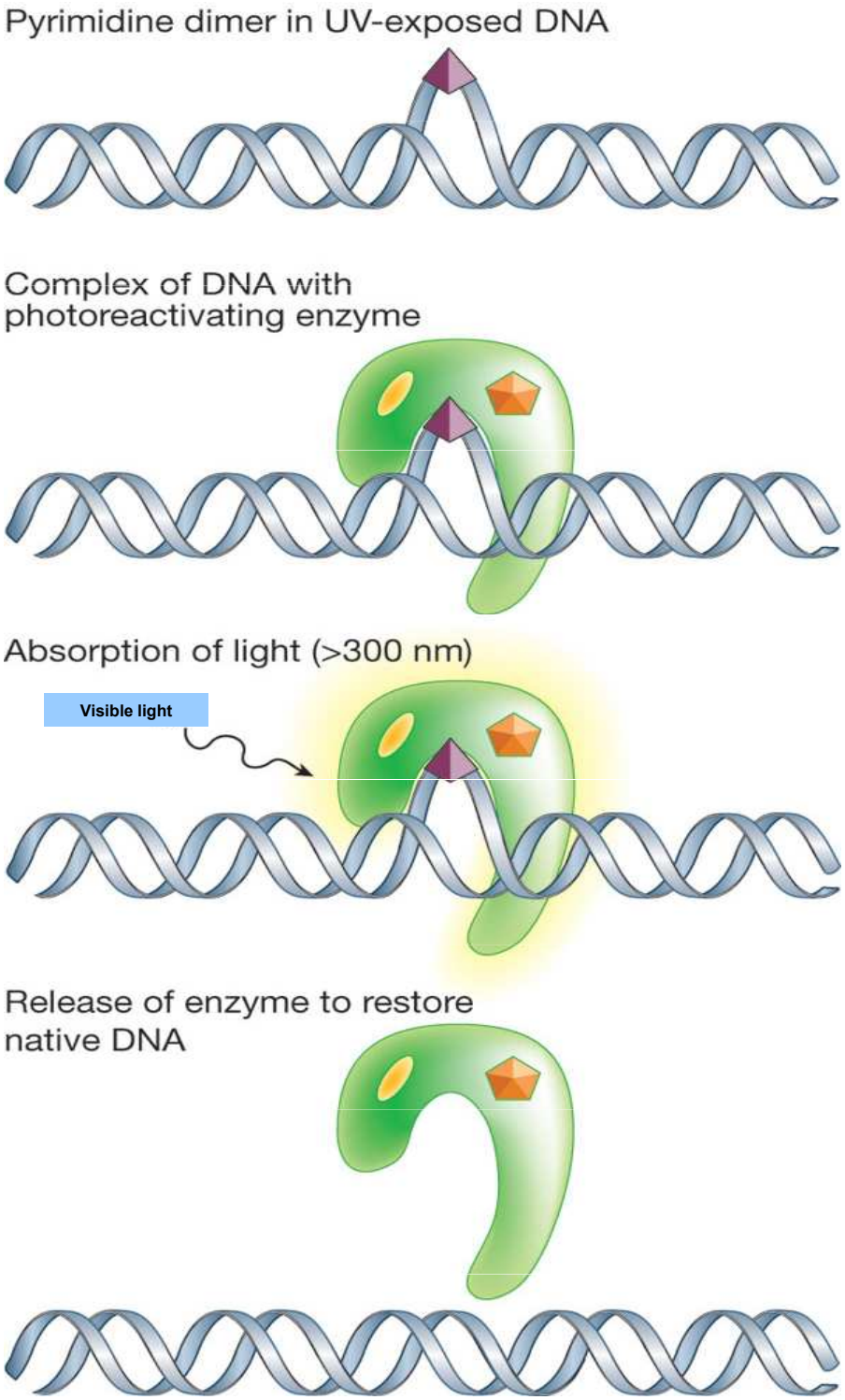
Failure to repair DNA damage:

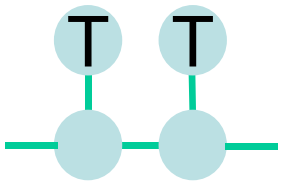


DNA Repair Pathways

1. **Direct reversals**
2. **Excision repair**
   - Base Excision Repair (BER)
   - Nucleotide Excision Repair (NER)
3. **Mismatch repair (MMR)**
   - replication errors
4. **Recombinational repair (HR and NHEJ)**
   - multiple pathways
   - double strand breaks and interstrand cross-links
5. **Tolerance mechanisms**
   - lesion bypass (TLS)
   - recombination







Repair by Direct reversal: photoreactivation

Damage Recognized:
Thymine dimers
6-4 photoproduct

Gene Products Required:
Photolyase

Related disease:
Photolyase not yet found in placental mammals

Excision Repair Pathways

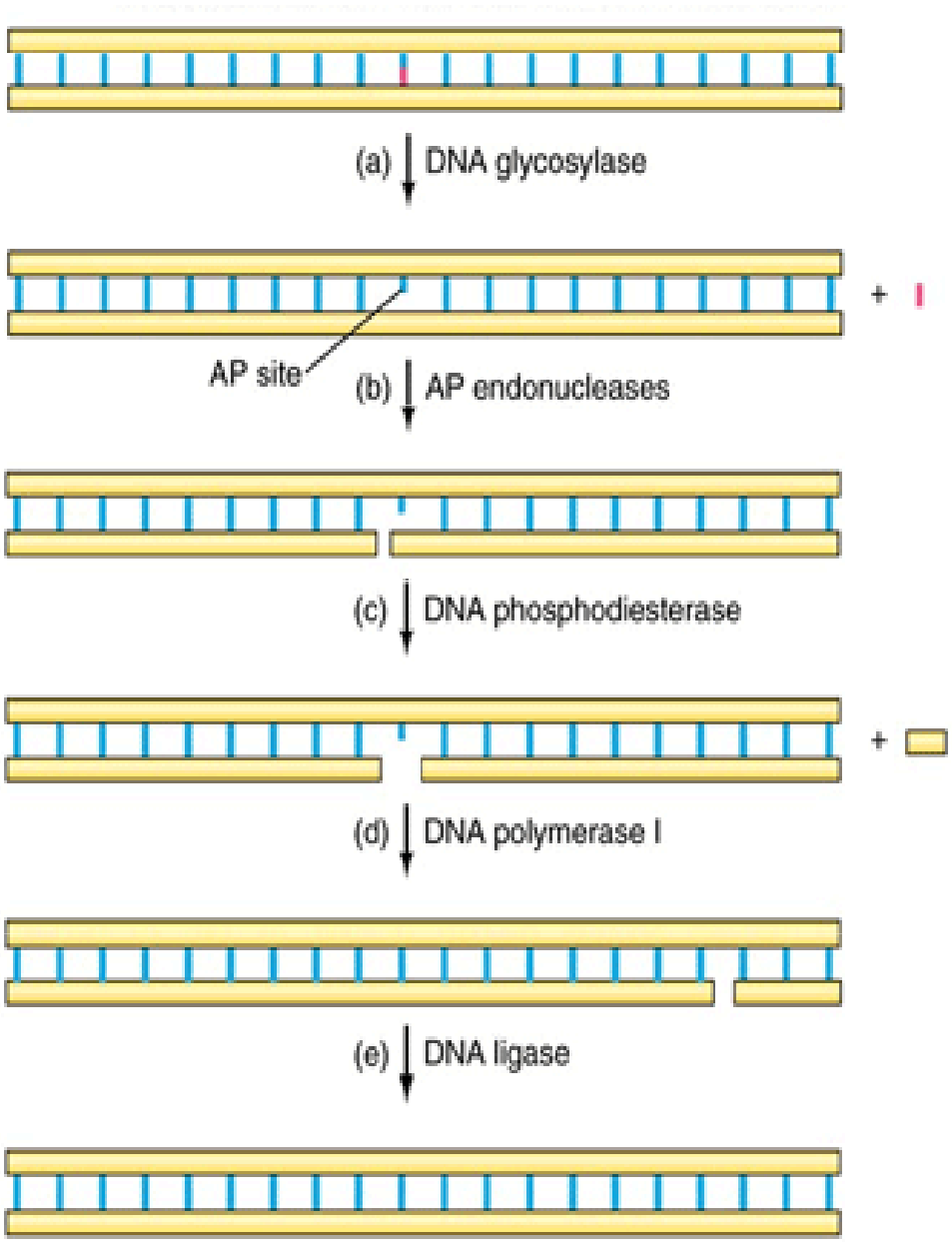
Base Excision Repair

- damaged bases are removed as free bases
- primarily responsible for removal of oxidative and alkylation damage
- most genes in pathway are essential
- thought to have an important role in aging and cancer

Nucleotide Excision Repair

- damaged bases are removed as oligonucleotides
- primarily responsible for removal of UV-induced damage and bulky adducts
- also removes ~ 20% of oxidative damage
- deficient in human disorders

E. coli Base Excision Repair (BER)



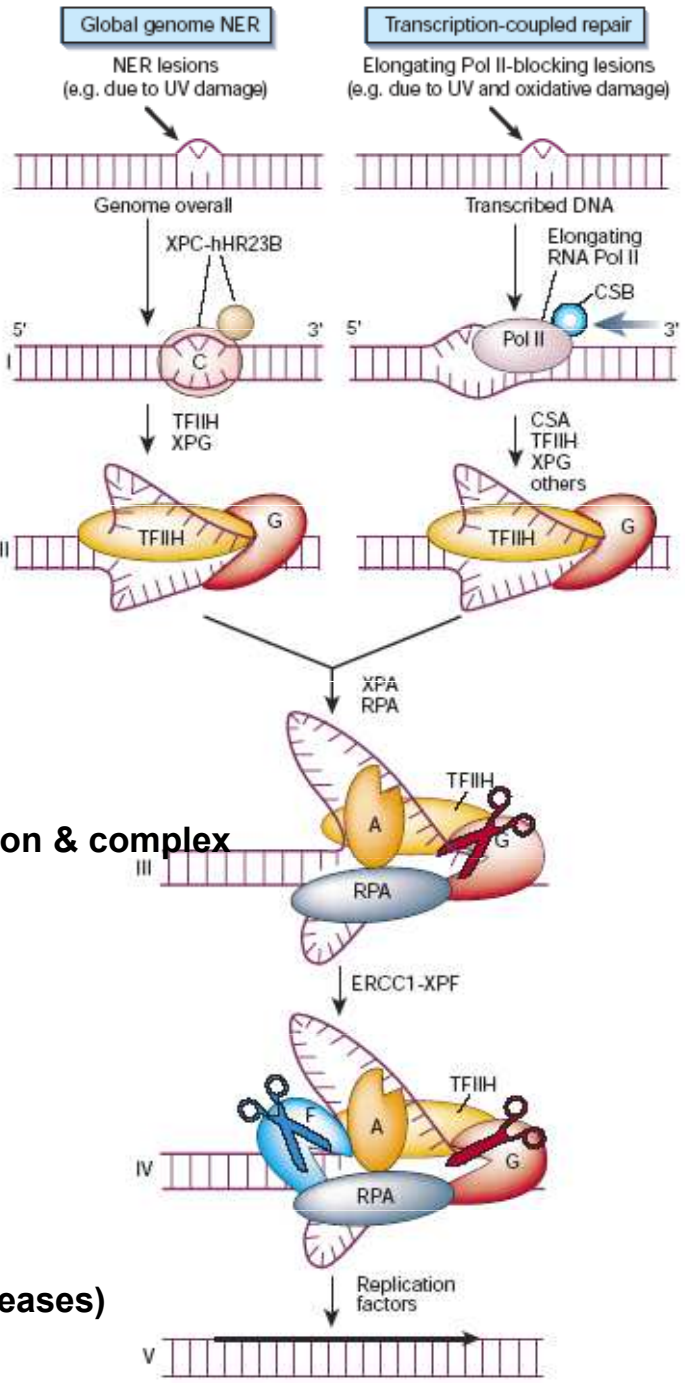
Damage Recognized:

- Base deamination
- Oxidative damage
- and other minor base modifications

Gene Products Required (5):

- Glycosylase
- AP endonuclease
- Phosphodiesterase
- DNA polymerase
- DNA Ligase

Nucleotide Excision Repair



Replication-coupled NER

XPC - damage recognition

CSA & CSB - role in processing RNAP II?
XPC not required

XPB & XPD - DNA helicases

XPA & RPA - damage validation & complex stabilization

XPG - 3' incision

ERCC1-XPF - 5' incision

(junction specific endonucleases)

E. coli

5' incision is 8 nuc. from lesion
3' incision is 4 nuc. from lesion

Mammals

5' incision is 22 nuc. from lesion
3' incision is 6 nuc. from lesion

Genetics of NER in Humans

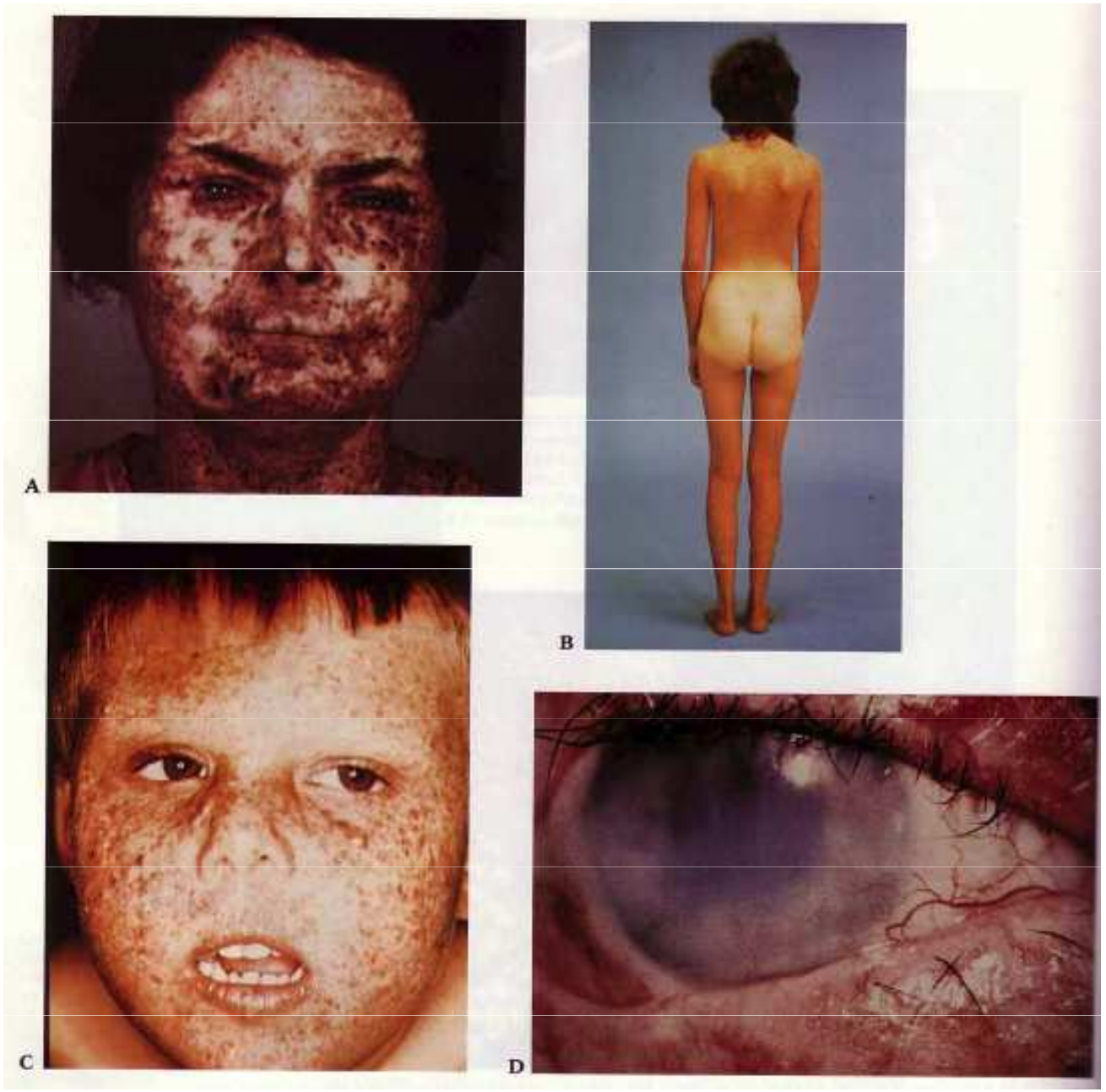
Xeroderma Pigmentosum (classical)

- Occurrence: 1-4 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: multiple skin disorders; malignancies of the skin; neurological and ocular abnormalities
- Biochemical: defect in early step of NER
- Genetic: autosomal recessive, seven genes (A-G)

Xeroderma Pigmentosum (variant)

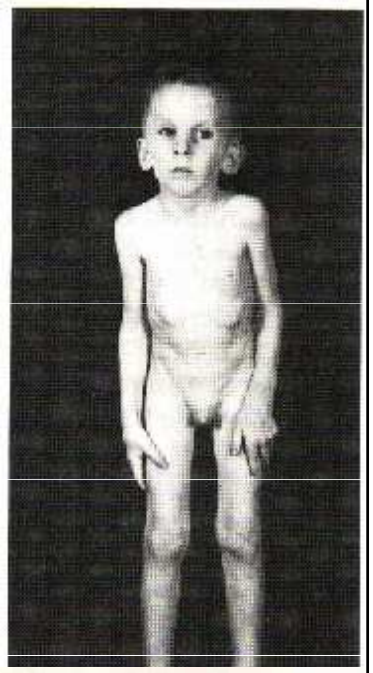
- Occurrence: same as classical
- Sensitivity: same as classical
- Disorder: same as classical
- Biochemical: defect in translesion bypass

Xeroderma Pigmentosum



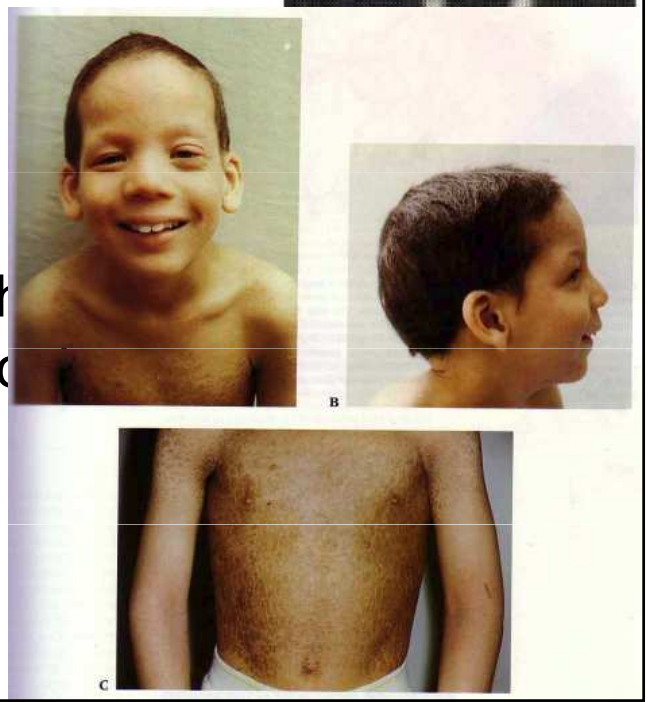
Genetics of NER in Humans

Cockayne's Syndrome



- Occurrence: 1 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: arrested development, mental retardation, dwarfism, deafness, optic atrophy, intracranial calcifications; (no increased risk of cancer)
- Biochemical: defect in NER
- Genetic: autosomal recessive, five genes (A, B and XPB, D & G

Trichothiodystrophy



- Occurrence: 1-2 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: sulfur deficient brittle hair, mental and growth retardation, peculiar face with receding chin, ichthyo (no increased cancer risk)
- Biochemical: defect in NER
- Genetic: autosomal recessive, three genes (TTDA, XPB, XPD)

DNA Mismatch Repair

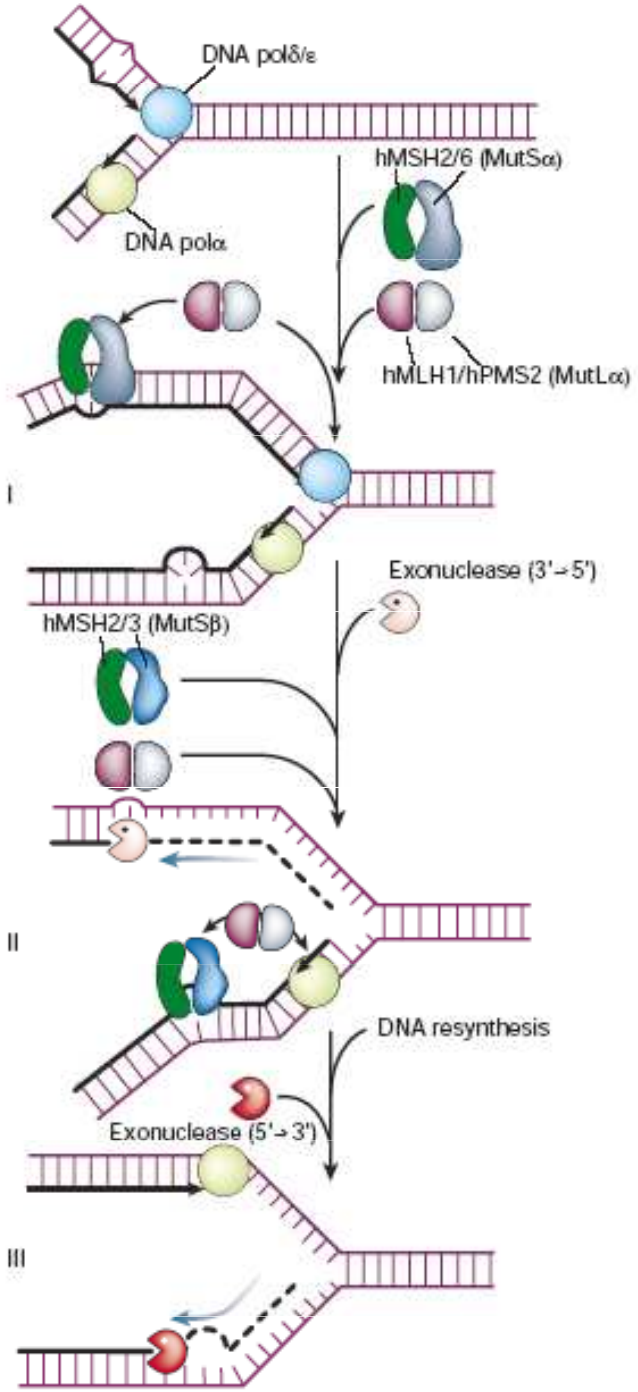
Repair of Replication Errors

Mechanisms for Insuring Replicative Fidelity

1. Base pairing	10^{-1} to 10^{-2}
2. DNA polymerases	10^{-5} to 10^{-6}
- base selection	
- proofreading	
3. Accessory proteins	10^{-7}
- single strand binding protein	
4. Mismatch correction	10^{-10}

Further reading: A. Bellacosa, Cell Death and Differentiation 8, 1076 (2001)
M. J. Schofield & P. Hsieh, Ann. Rev. Microbiol. 57, 579 (2003)

Mismatch Repair



Mismatch Repair Mutations in Hereditary Nonpolyposis Colon Cancer (HNPCC)

- MMR mutations in 70% of families
- MLH1 (50%), MSH2 (40%)
- Minor role for MSH6, PMS1, PMS2

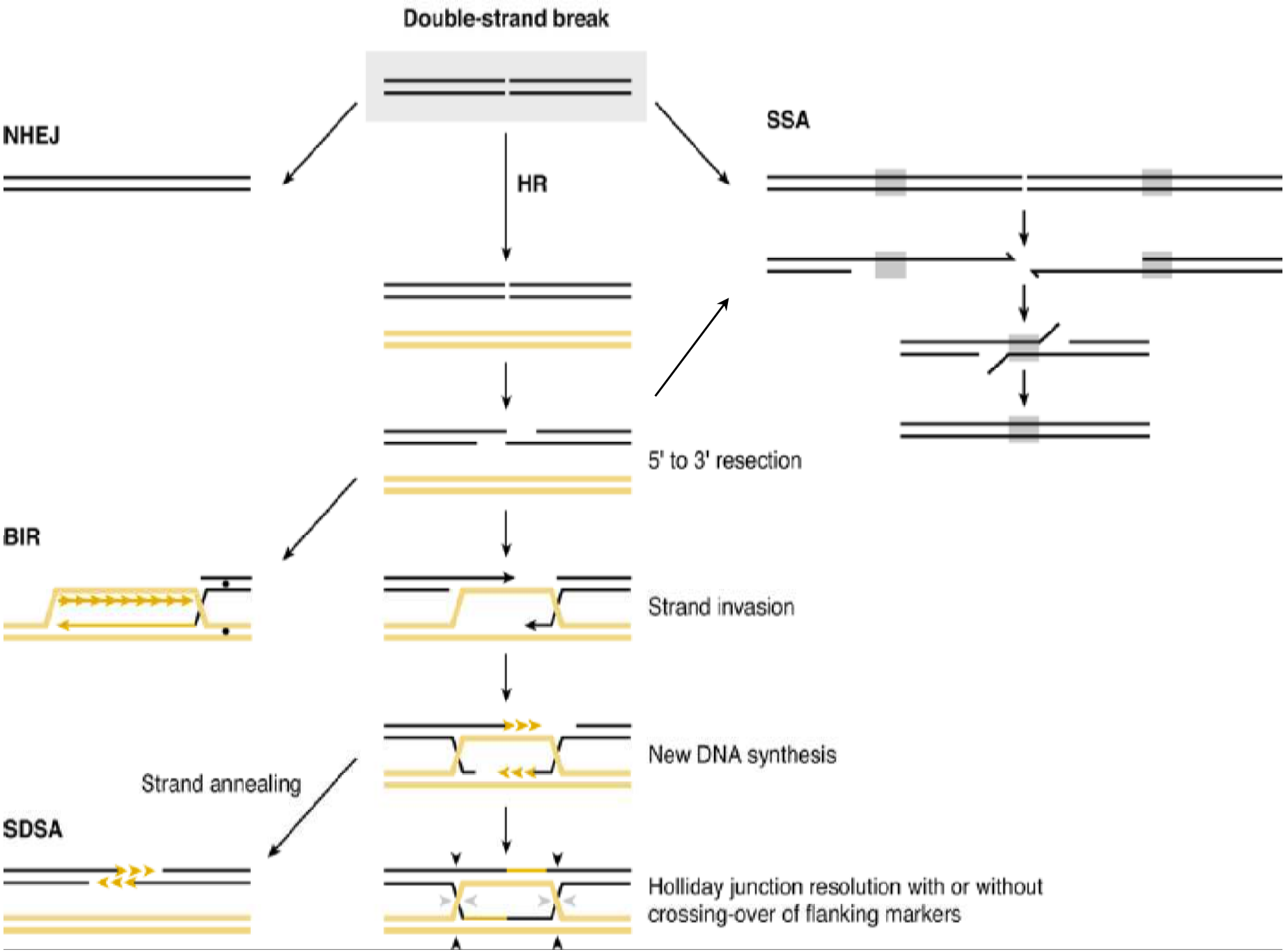
- Population prevalence 1:2851 (15-74 years)
- 18% of colorectal cancers under 45 years
- 28% of colorectal cancers under 30 years

Recombinational DNA Repair Mechanisms

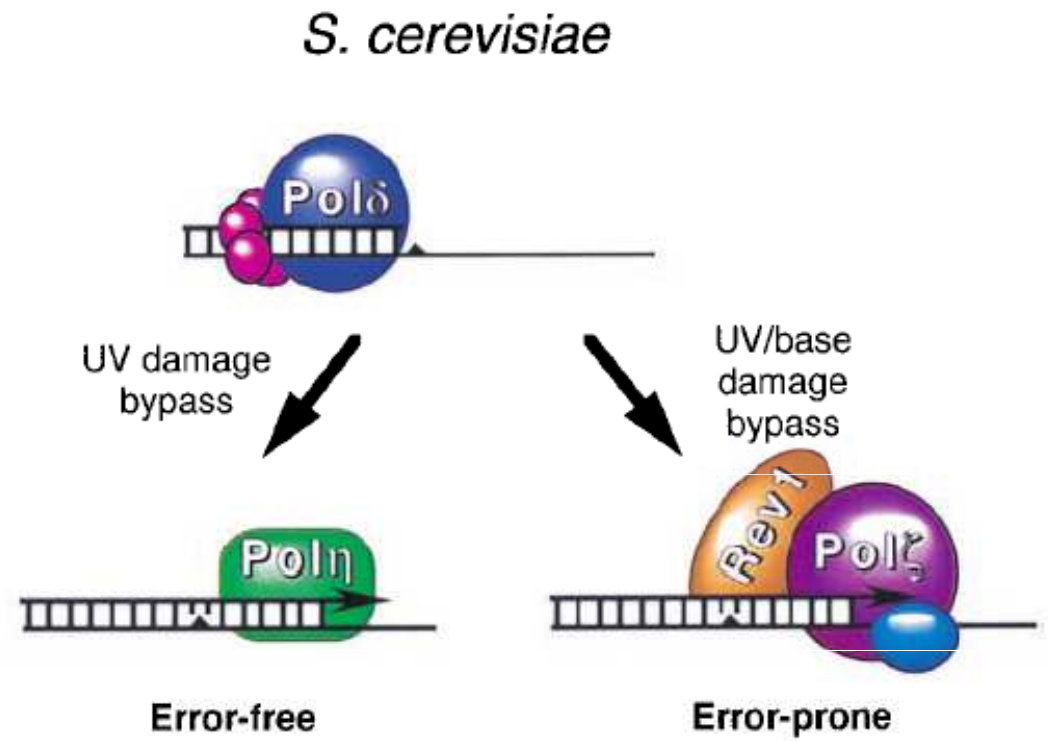
Lesions repaired

1. Double-strand breaks
2. Interstrand cross-links

Further reading: Paques and Haber, Microbiol. & Molec. Biol. Rev. 63, 349 (199


Double-strand break
NHEJ
HR
SSA
5' to 3' resection
BIR
Strand invasion
New DNA synthesis
Strand annealing
SDSA
Holliday junction resolution with or without
crossing-over of flanking markers

Translesion Bypass DNA Polymerases

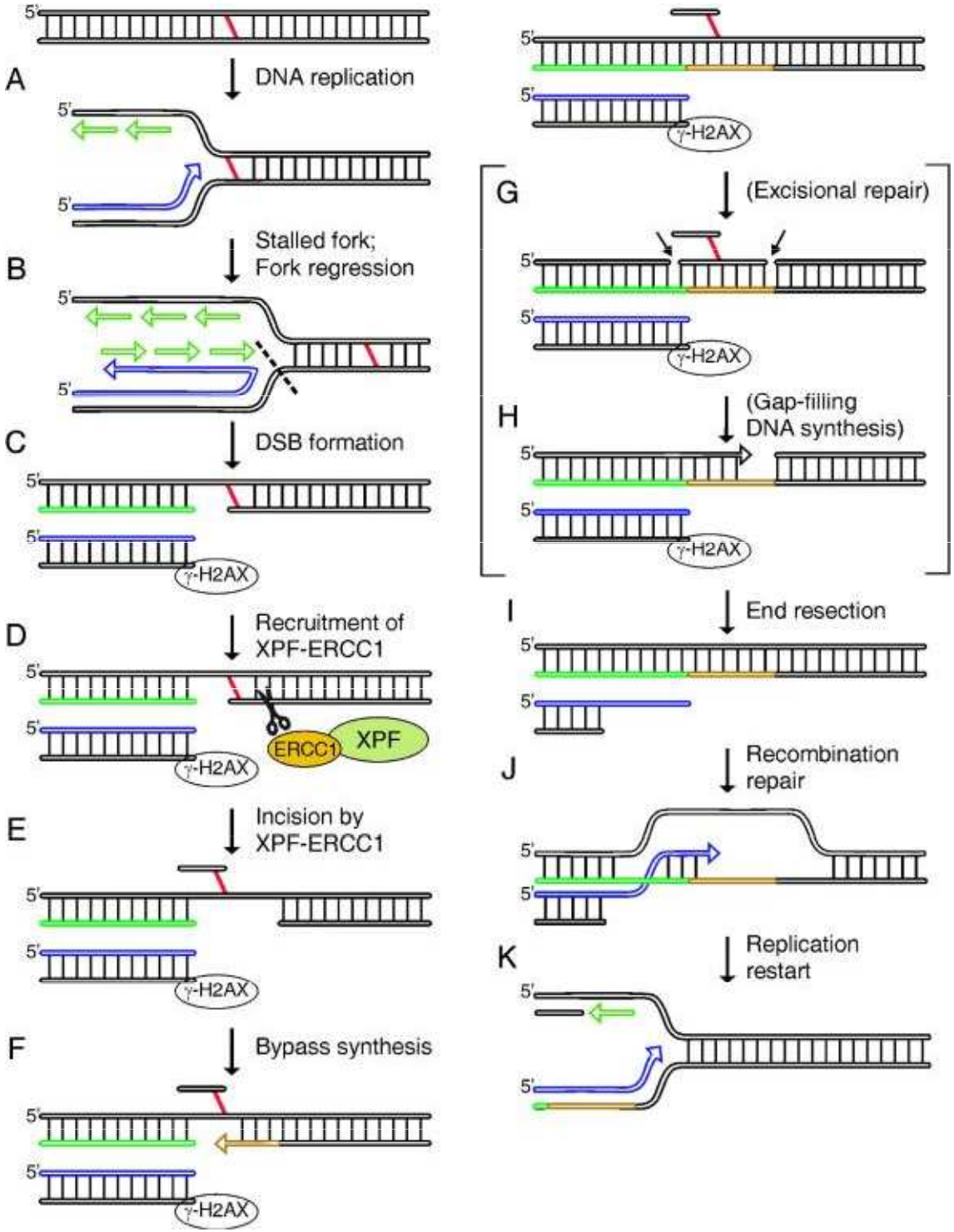


Pol eta
- inserts adenosines opposite TT dimers
- in general has low fidelity
- low processivity
- may be error-prone with other lesions
- Pol eta is a product of the XPV gene

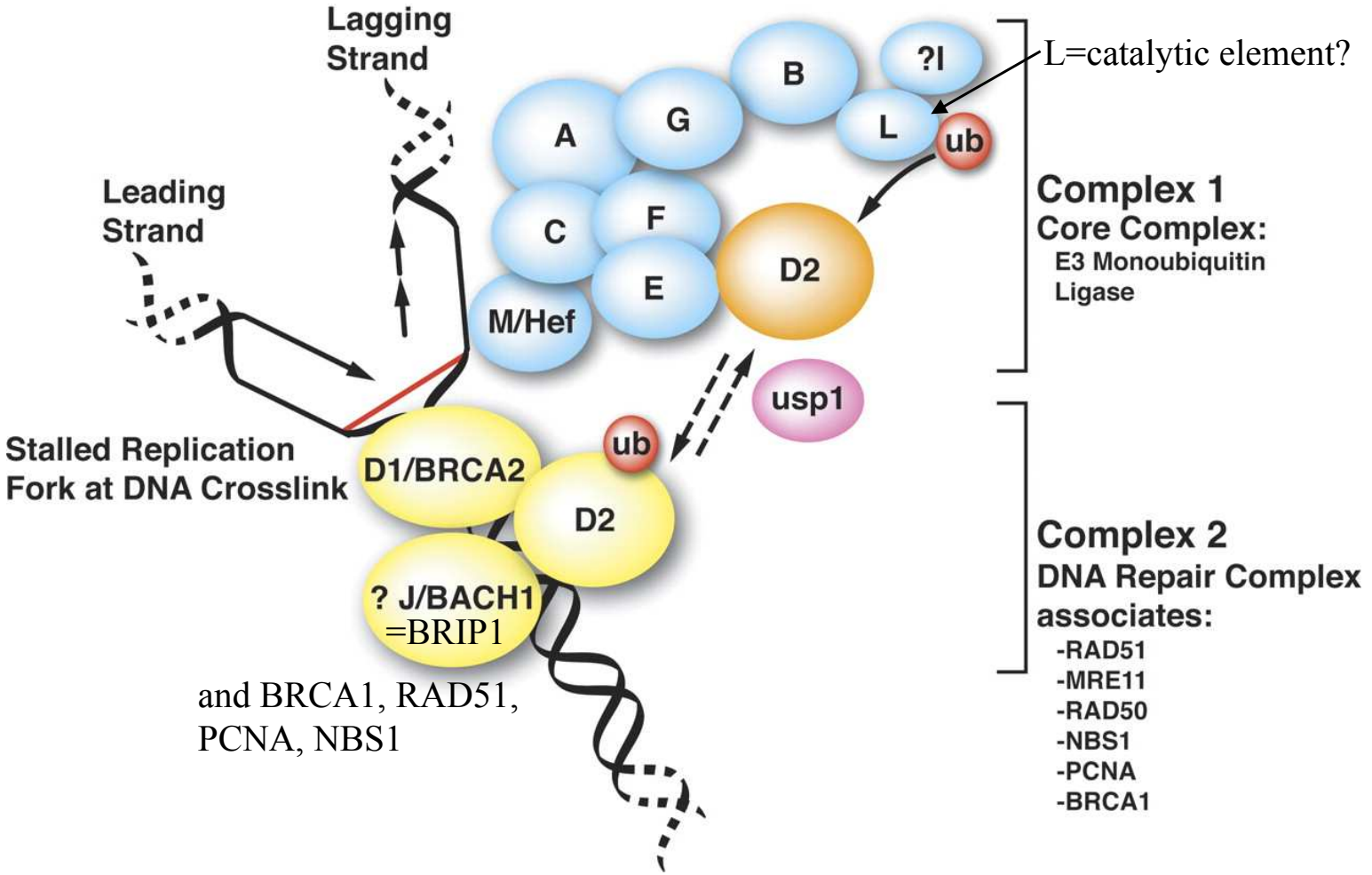
Pol zeta and Rev 1
- Rev 1 inserts random bases opposite dimer
- Pol zeta extends bypass by a few bases
- Both polymerases have low fidelity and low processivity

Cross-link repair

Model for the mechanism of DNA ICL repair in mammalian cells.



Schematic interaction of the FA pathway



Richard D. Kennedy et al. Genes Dev. 2005; 19: 2925-2940

Fanconi's Anemia

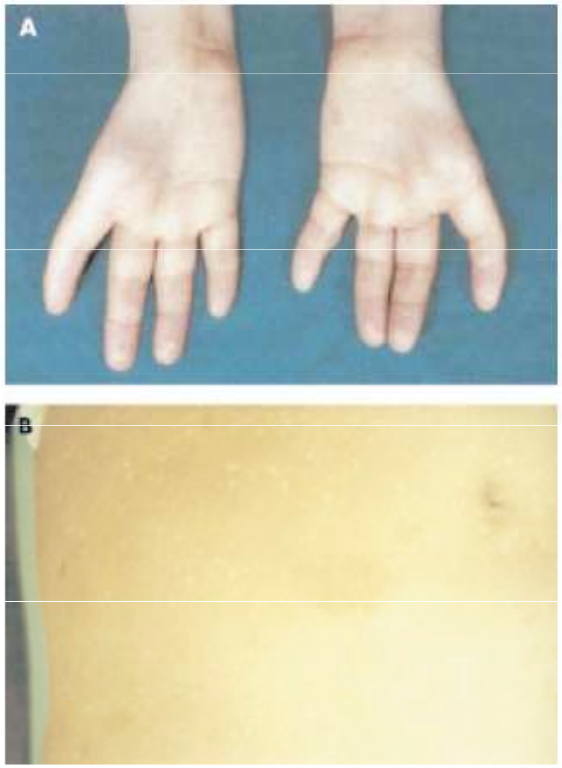


Figure 1 (A) Typical radial ray abnormalities and (B) café au lait patches and hypopigmentation, all common features in FA.

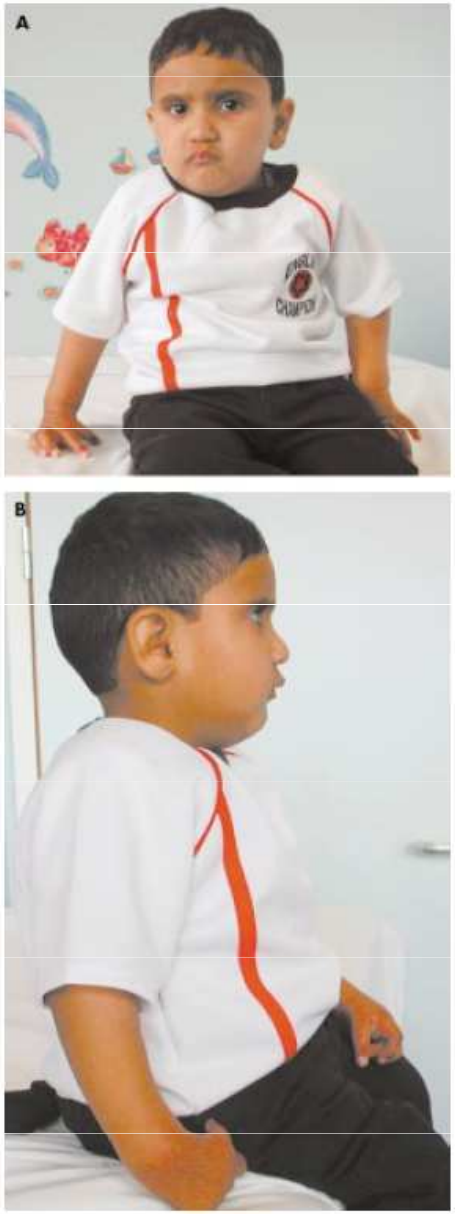


Figure 2 (A, B) A 3½ year old FA child showing radial ray abnormalities. Height and head circumference are both below the 3rd centile.

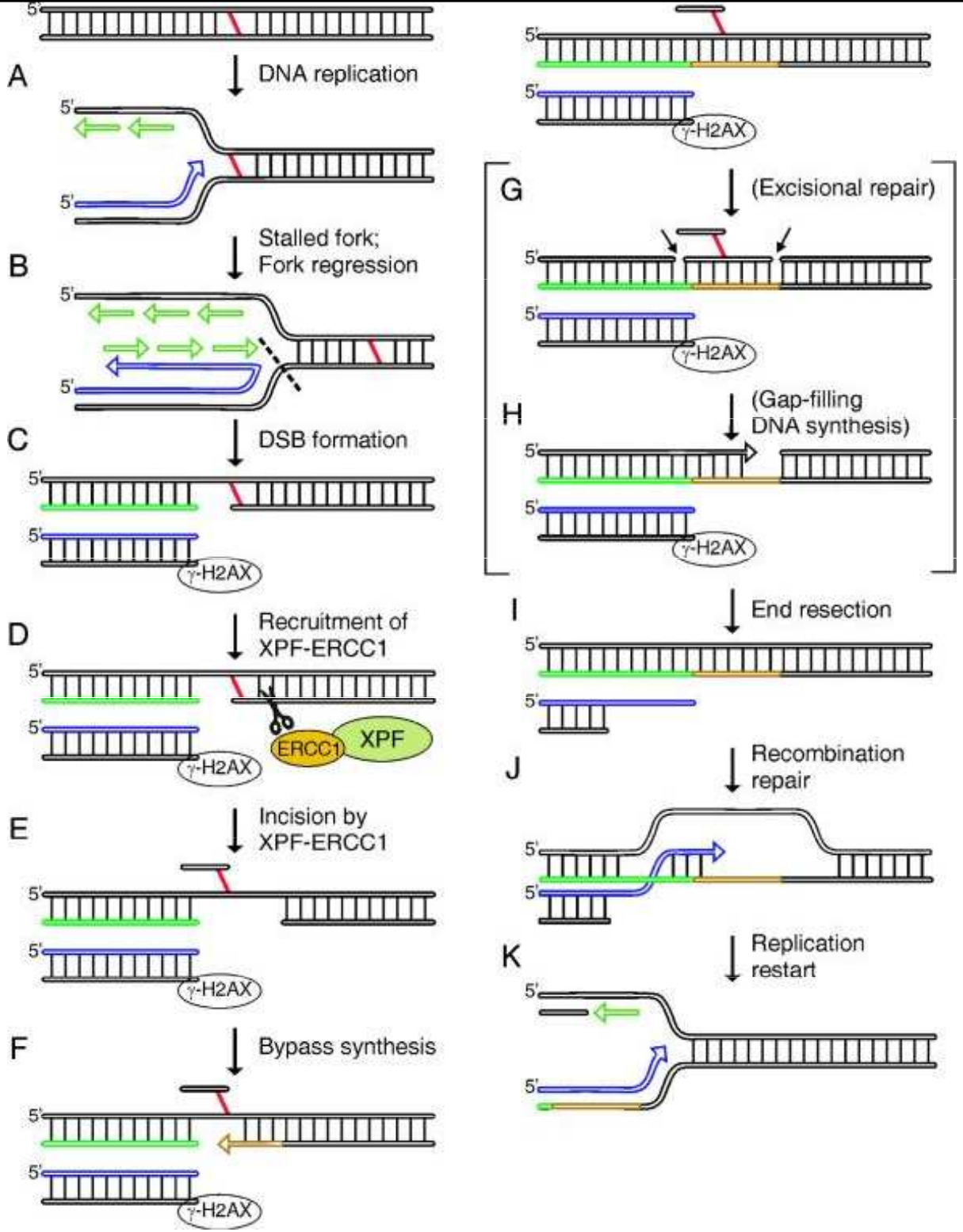
Congenital abnormalities
- skeletal
- skin pigmentation
- short stature
- male genital
- mental retardation
- cardiac abnormalities
- hearing

Cancer
- myeloid leukemia
- solid tumors

13 genes in FA
BRCA2 is deficient in FA-D1

Review: Tischkowitz & Hodgson, J. Med. Genet. 40, 1 (20

Cross-link repair



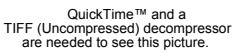
What do we study?

DNA double-strand breaks (DSB)

- Induced by ionizing radiation & chemicals
- Arise when replicating a damaged template
- Serve as the initiator of meiotic recombination
- Part of immune response

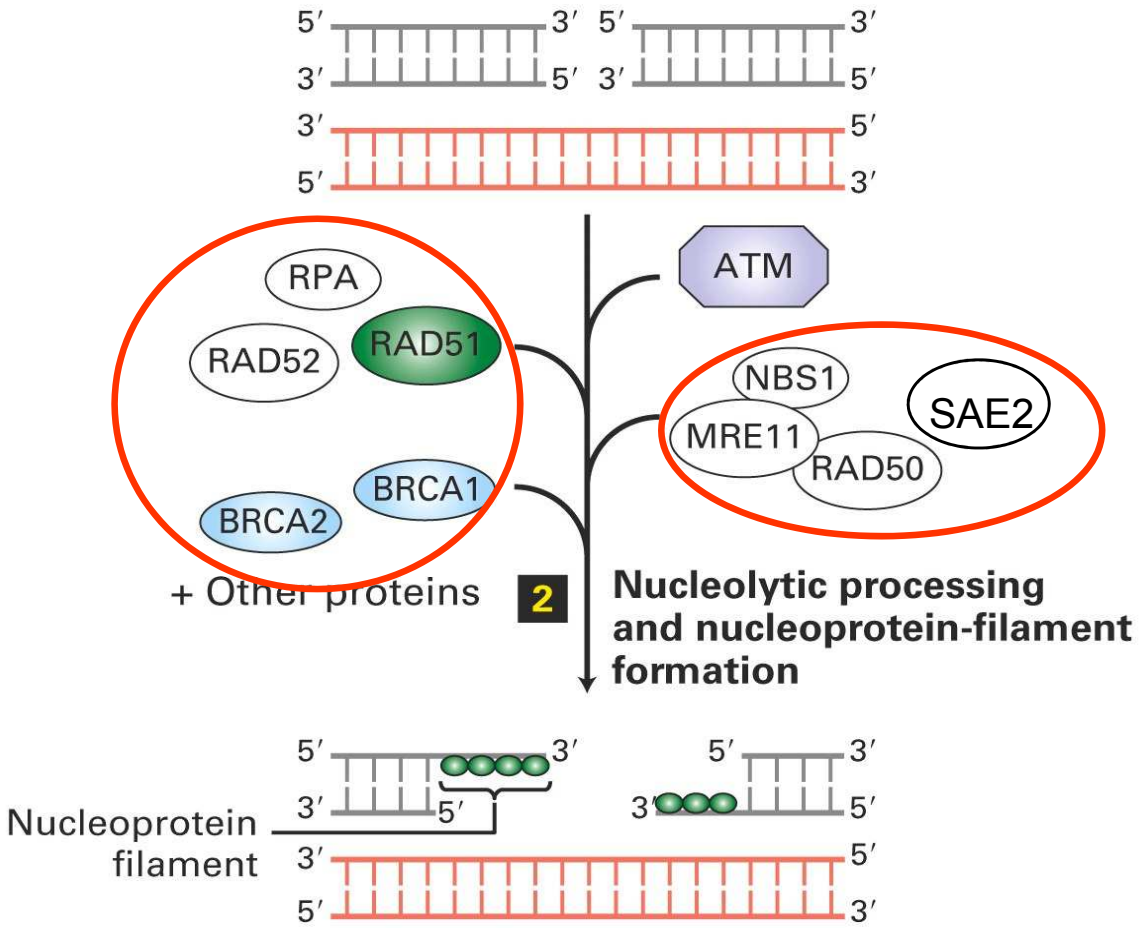
Failure to properly process DSBs

- **Cell death**
- **Chromosomal aberrations**
- **Meiotic aneuploidy**
- **Immunodeficiency**

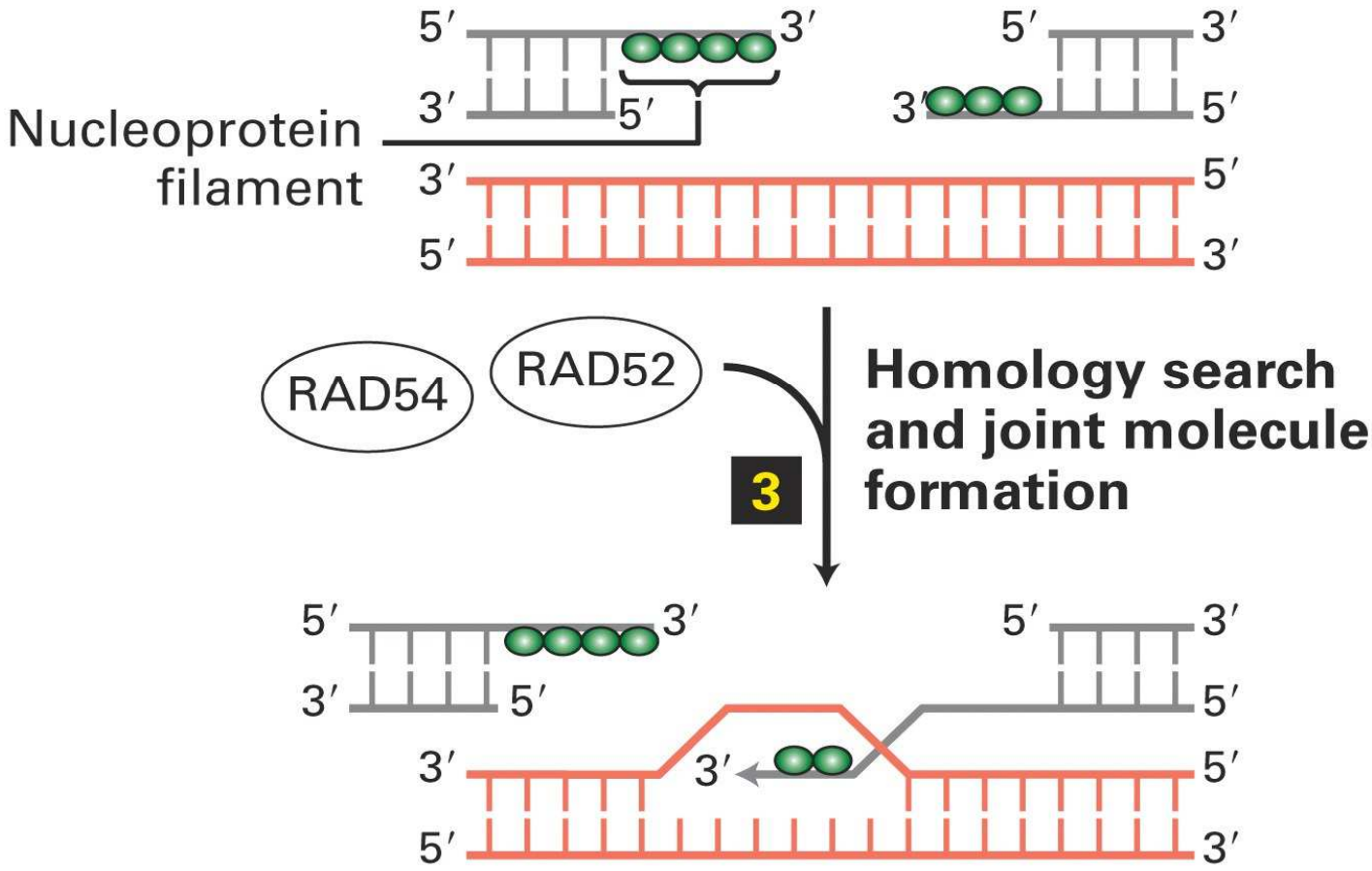


Adapted from Surralles et al., Genes Dev. (2004)

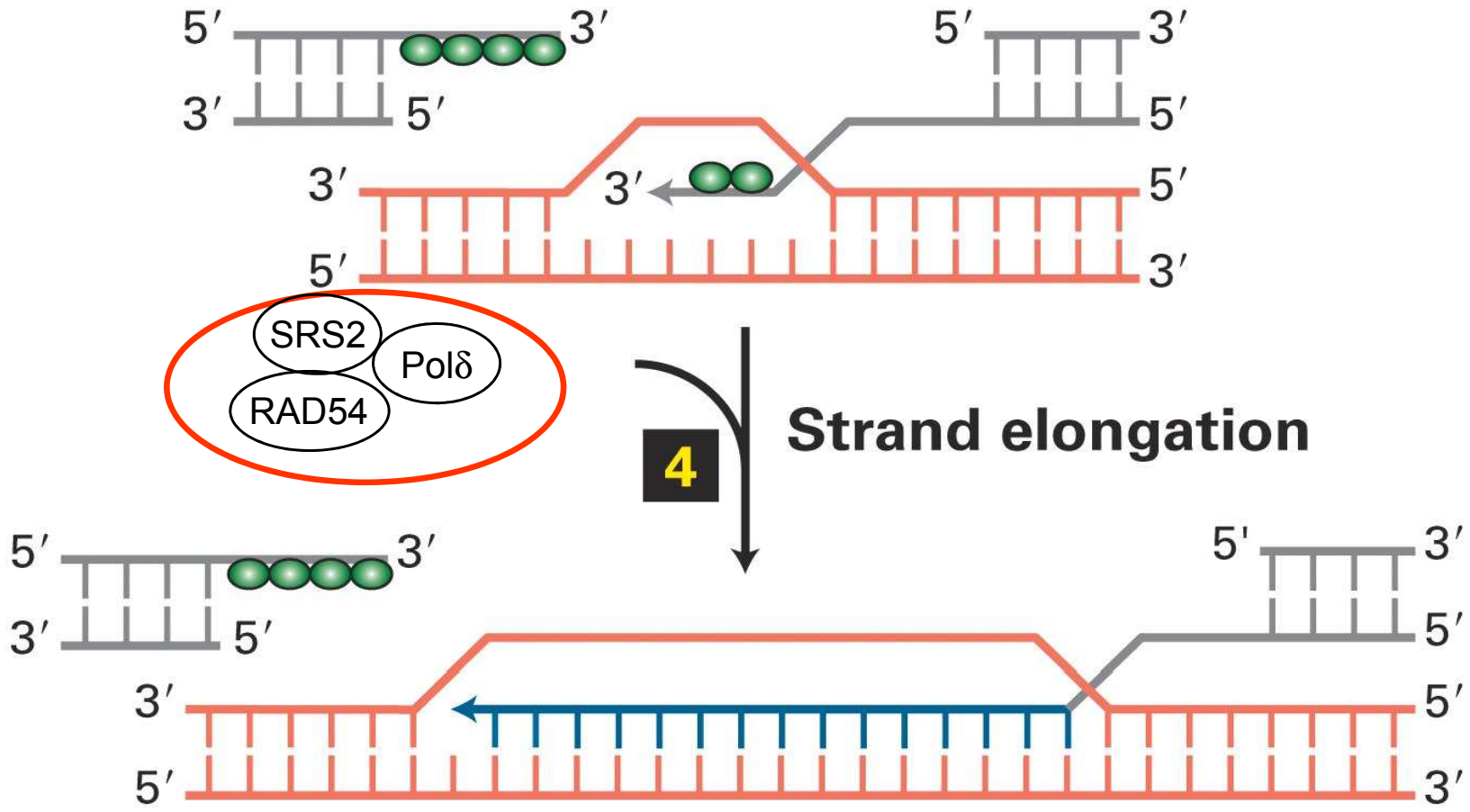
End processing



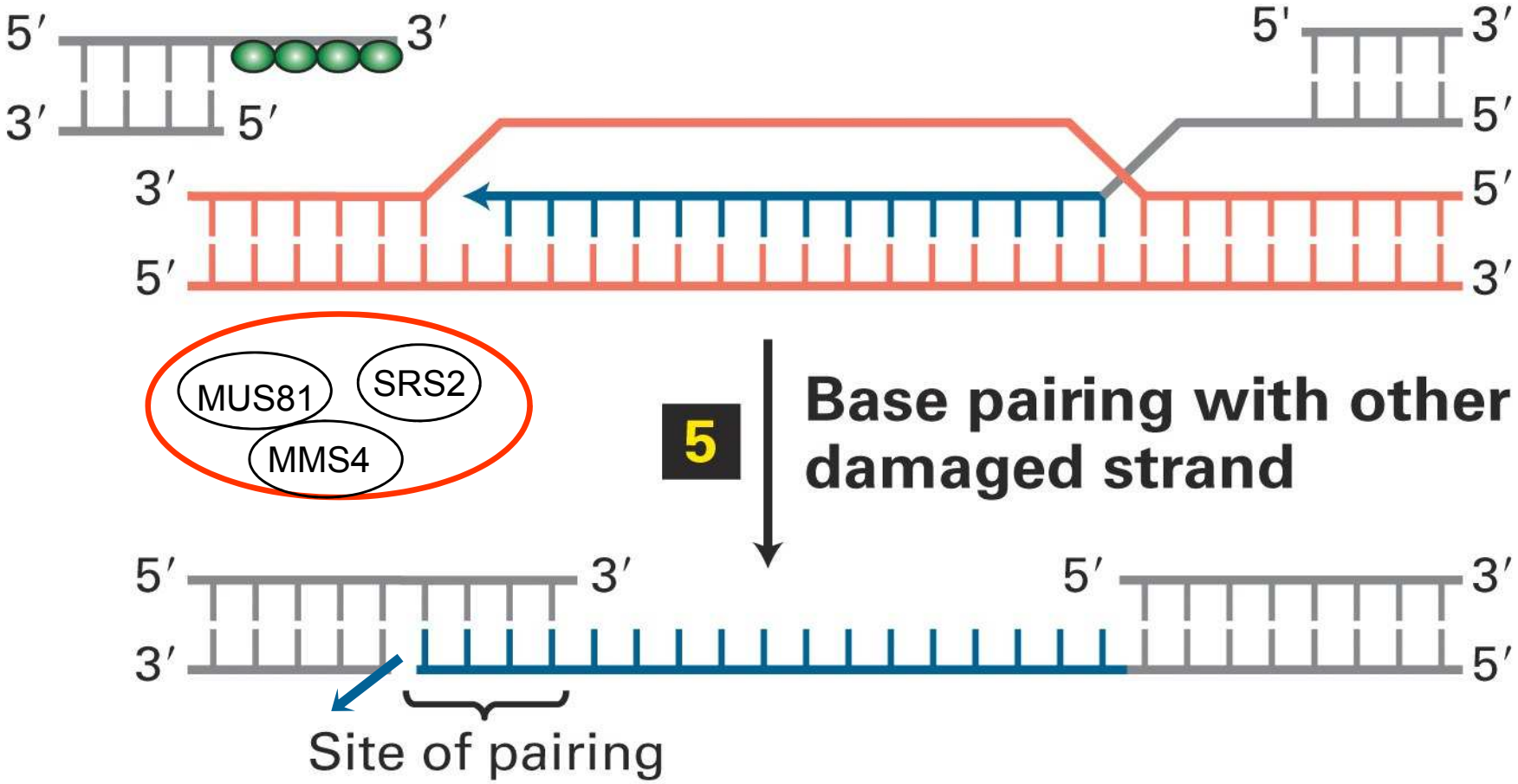
Homology search



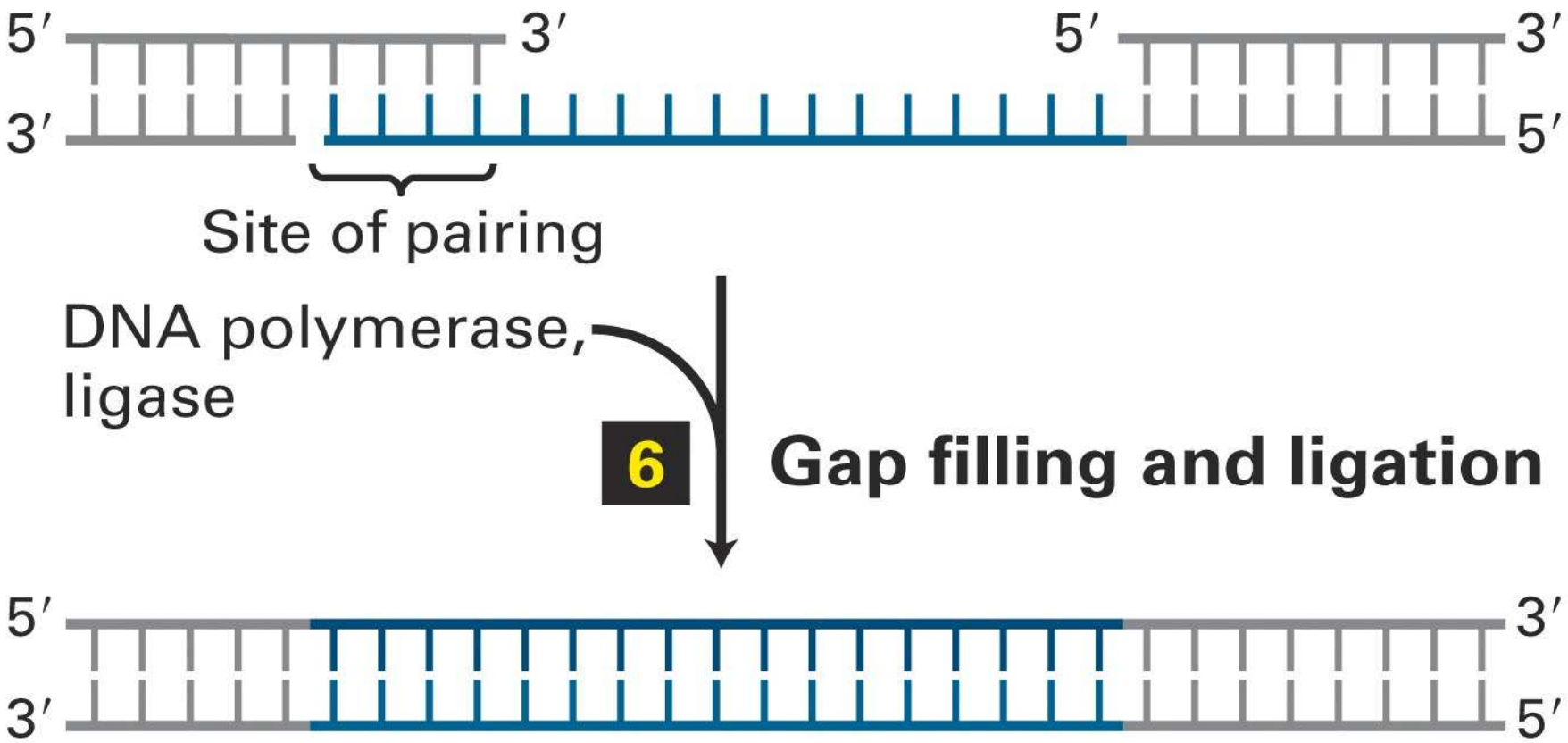
DNA repair synthesis



Resolution


5′
3′
3′
5′
5′
3′
3′
5′
5′
3′
5′
3′
MUS81
SRS2
MMS4
5
Base pairing with other damaged strand
5′
3′
3′
5′
5′
3′
5′
3′
Site of pairing

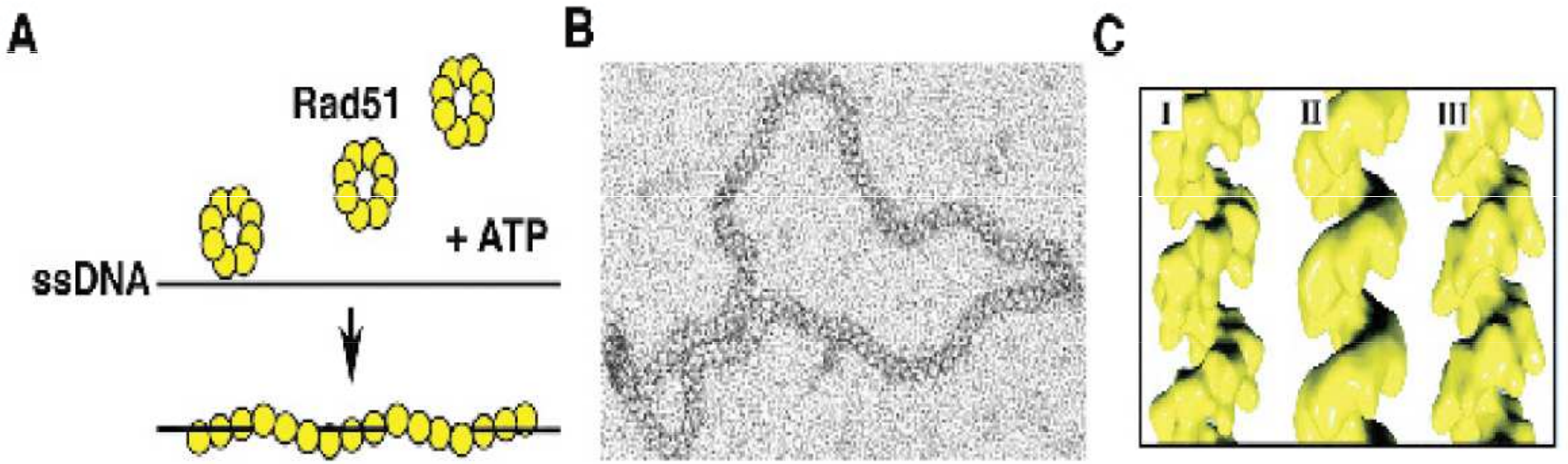
Ligation


5′
3′
3′
5′
5′
3′
Site of pairing
DNA polymerase,
ligase
6
Gap filling and ligation
5′
3′
3′
5′

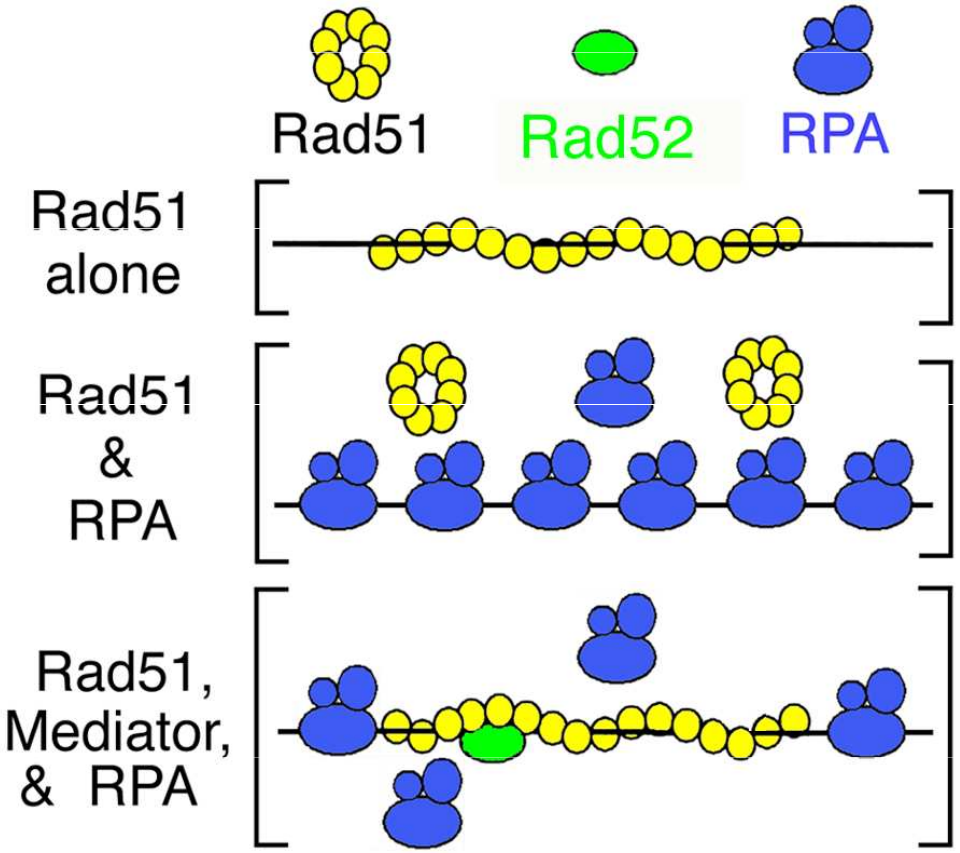
How do we study this?

Examples -
Regulation of recombination?

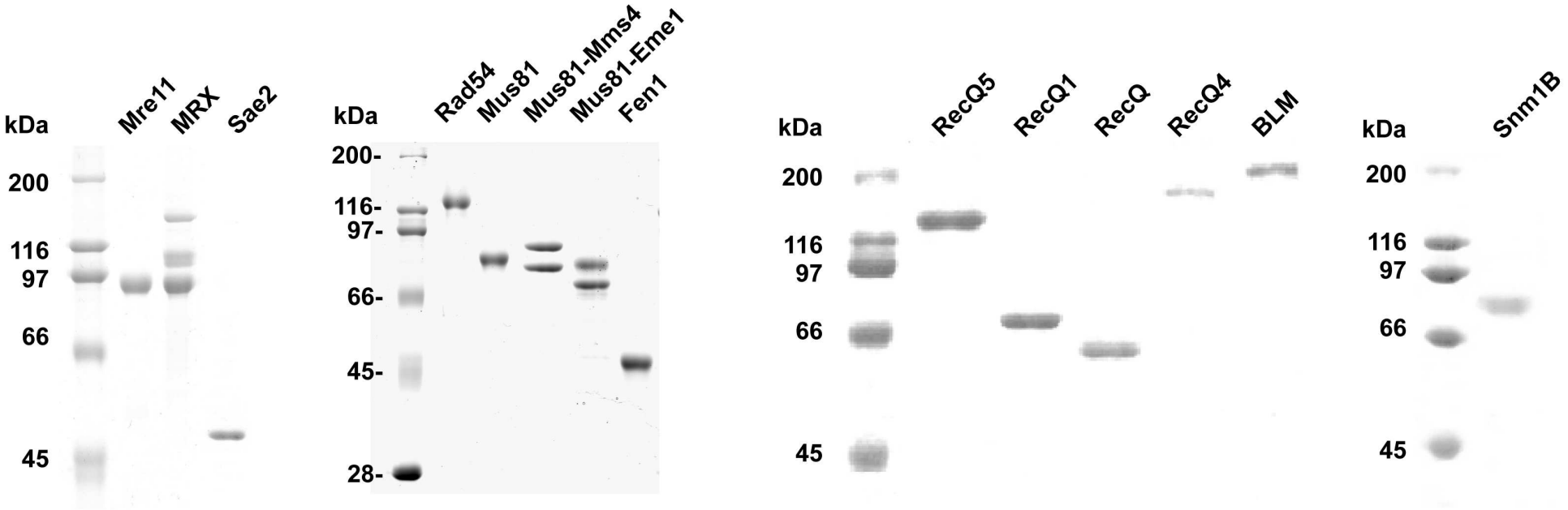
Presynaptic Rad51 filament



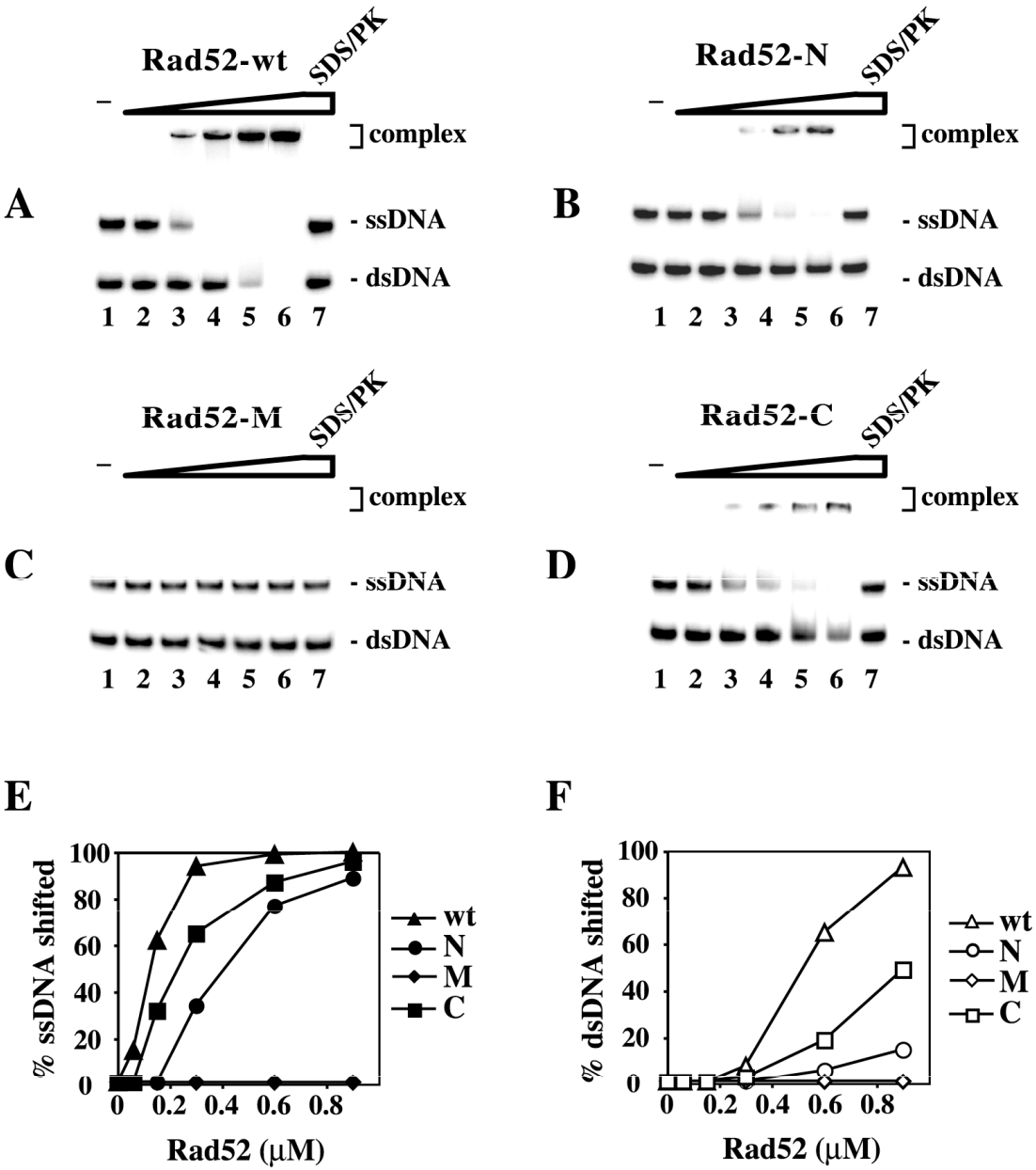
Positive regulation - Mediator proteins



Proteins

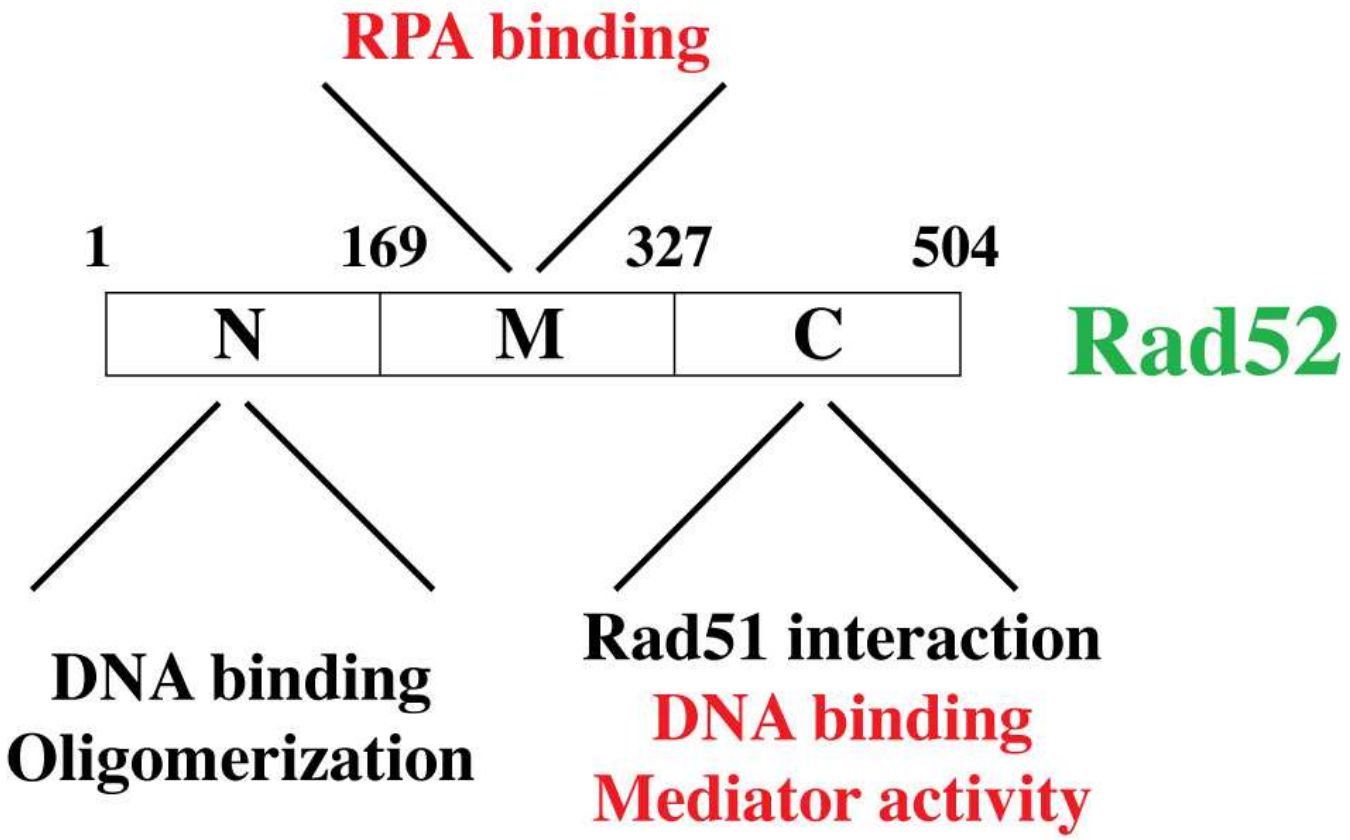

kDa
Mre11
MRX
Sae2
200
116
97
66
45
kDa
Rad54
Mus81
Mus81-Mms4
Mus81-Eme1
Fen1
200-
116-
97-
66-
45-
28-
kDa
RecQ5
RecQ1
RecQ
RecQ4
BLM
200
116
97
66
45
kDa
Snm1B
200
116
97
66
45

DNA binding



Seong et al. *J. Biol. Chem.*, 2008

Function of Rad52 protein

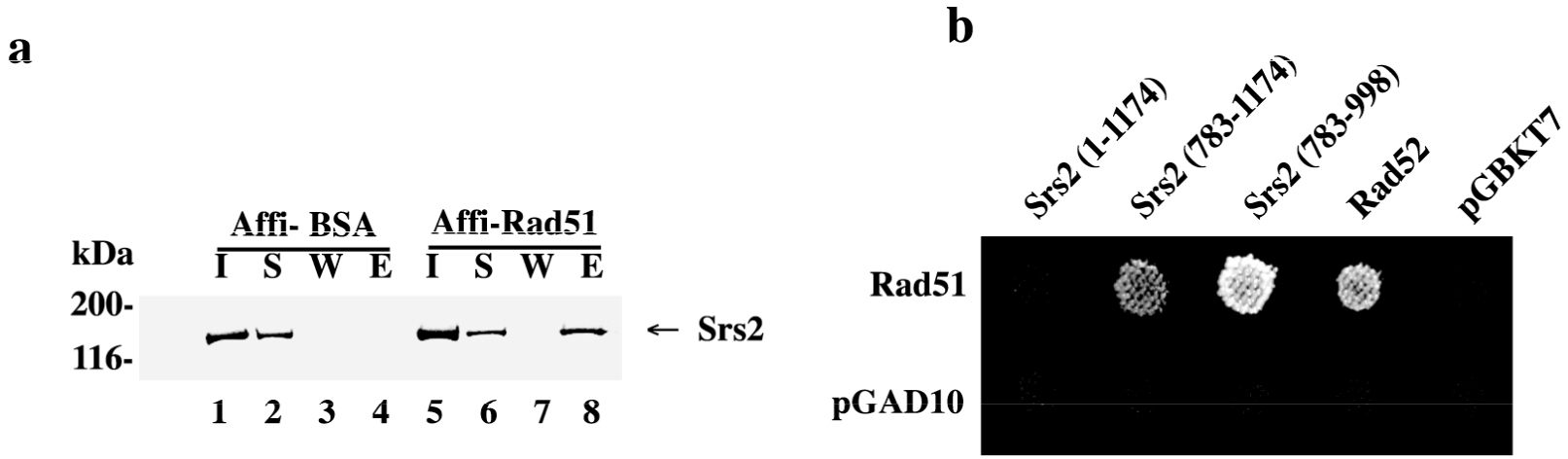


Negative regulation - *Recombination can be harmfull to cells:*

- **Can interfere with normal repair**
- **Elicits strong cell cycle responses**
- **Causes cell death**

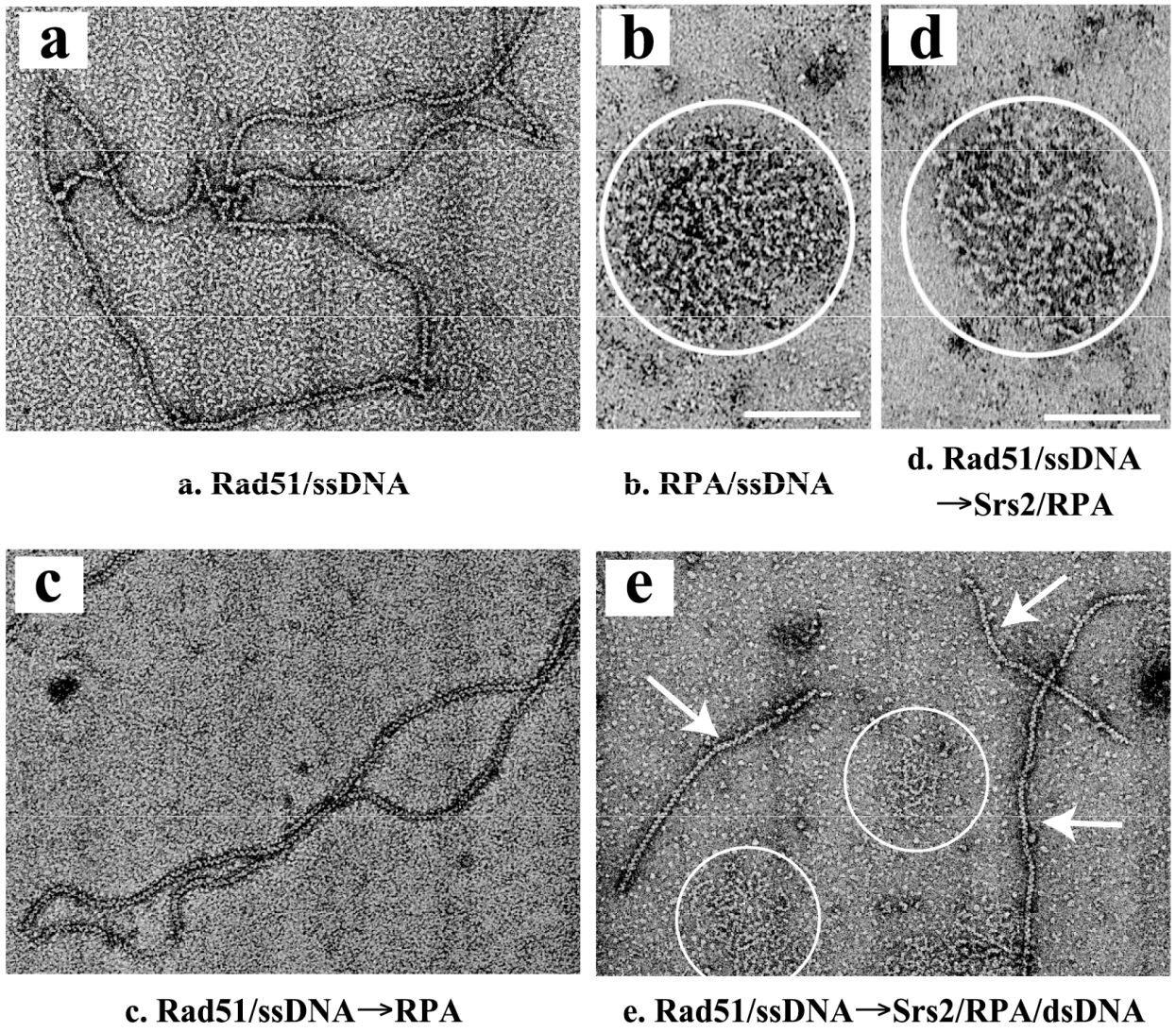
Cells have ways to prevent untimely recombination!

Srs2 binds Rad51



Krejci et al. *NATURE*, 2003

EM of Rad51 filaments



a. Rad51/ssDNA

b. RPA/ssDNA

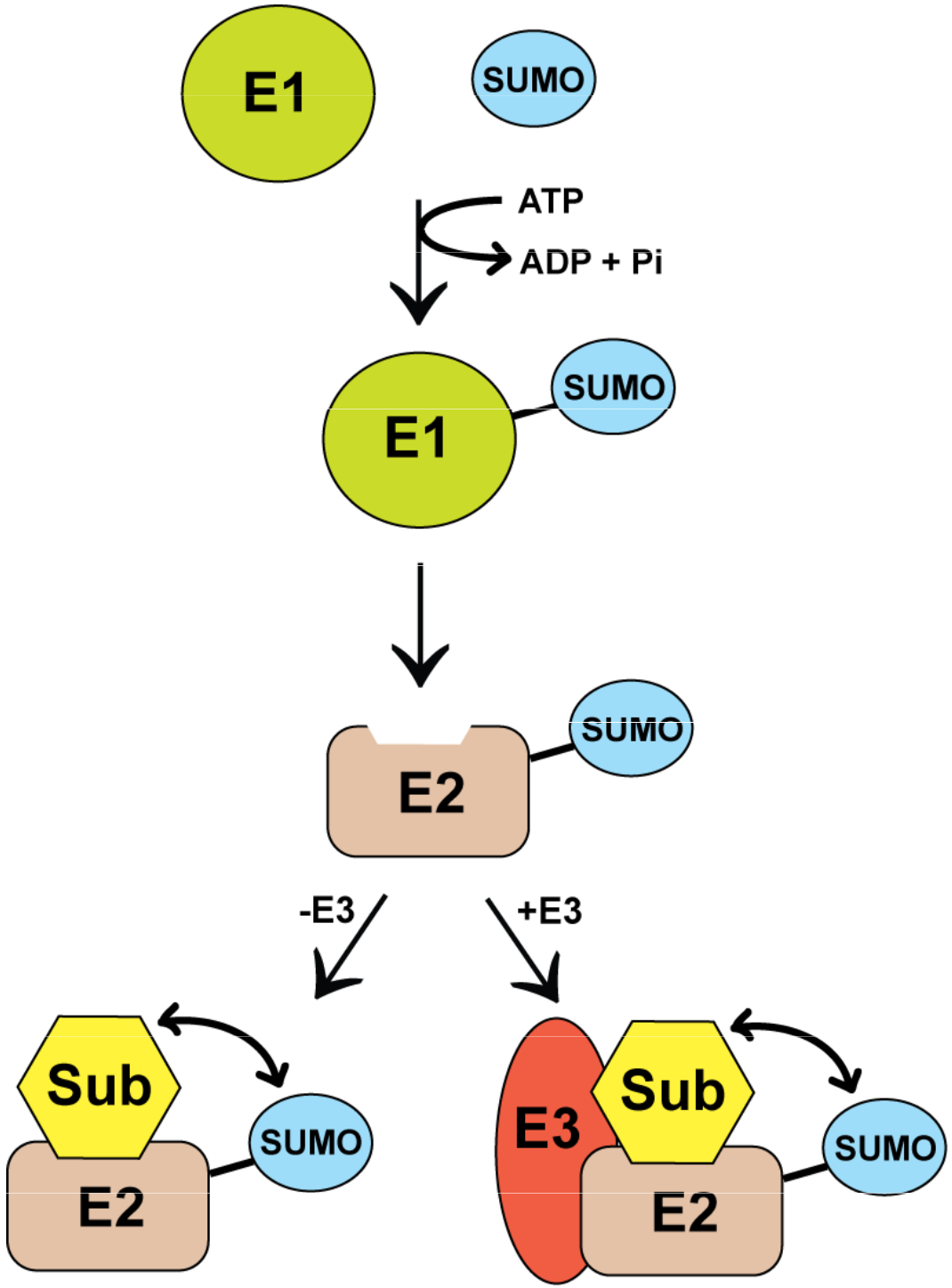
d. Rad51/ssDNA →Srs2/RPA

c. Rad51/ssDNA→RPA

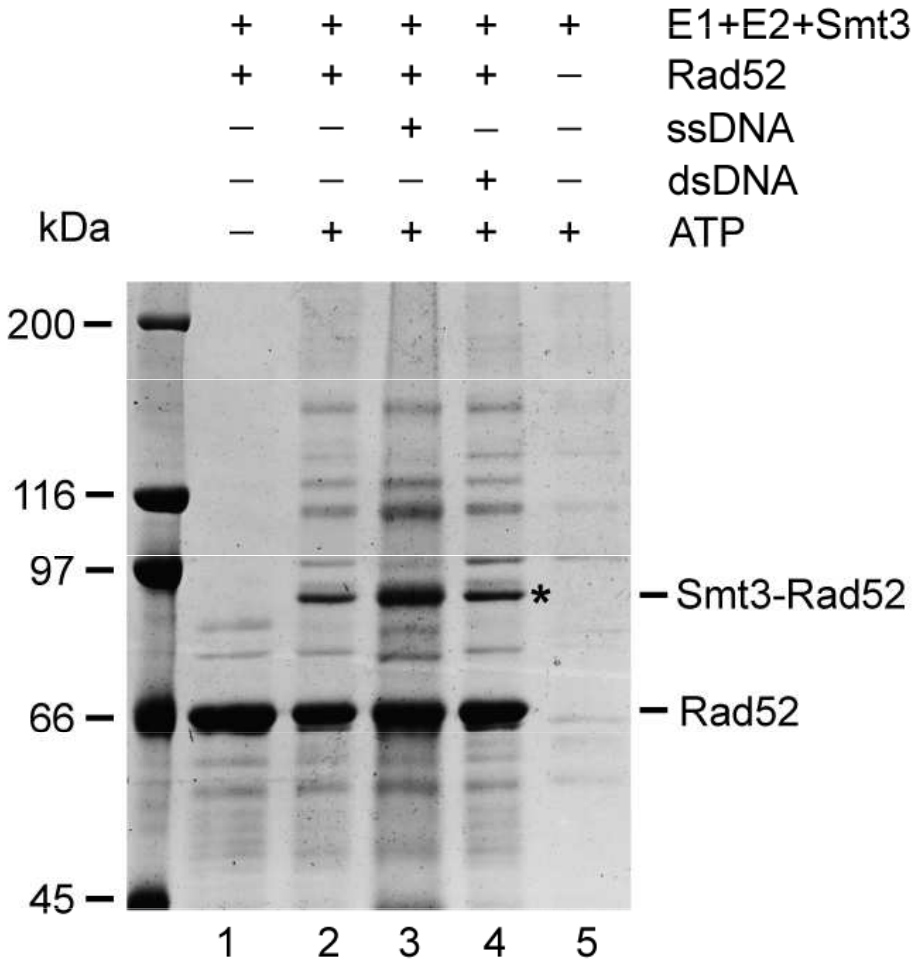
e. Rad51/ssDNA→Srs2/RPA/dsDNA

Krejci et al. *NATURE*, 2003

Protein modification by SUMOylation



Rad52 is SUMOylated



V. Altmannova

Quality control mechanism