DNA REPAIR -
molecular mechanisms and biological consequences of DNA damage and repair

Take care of your DNA
as you do not know how long it will last

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To do …

DNA damage - sources, thymine dimer; single strand breaks; double strand breaks;

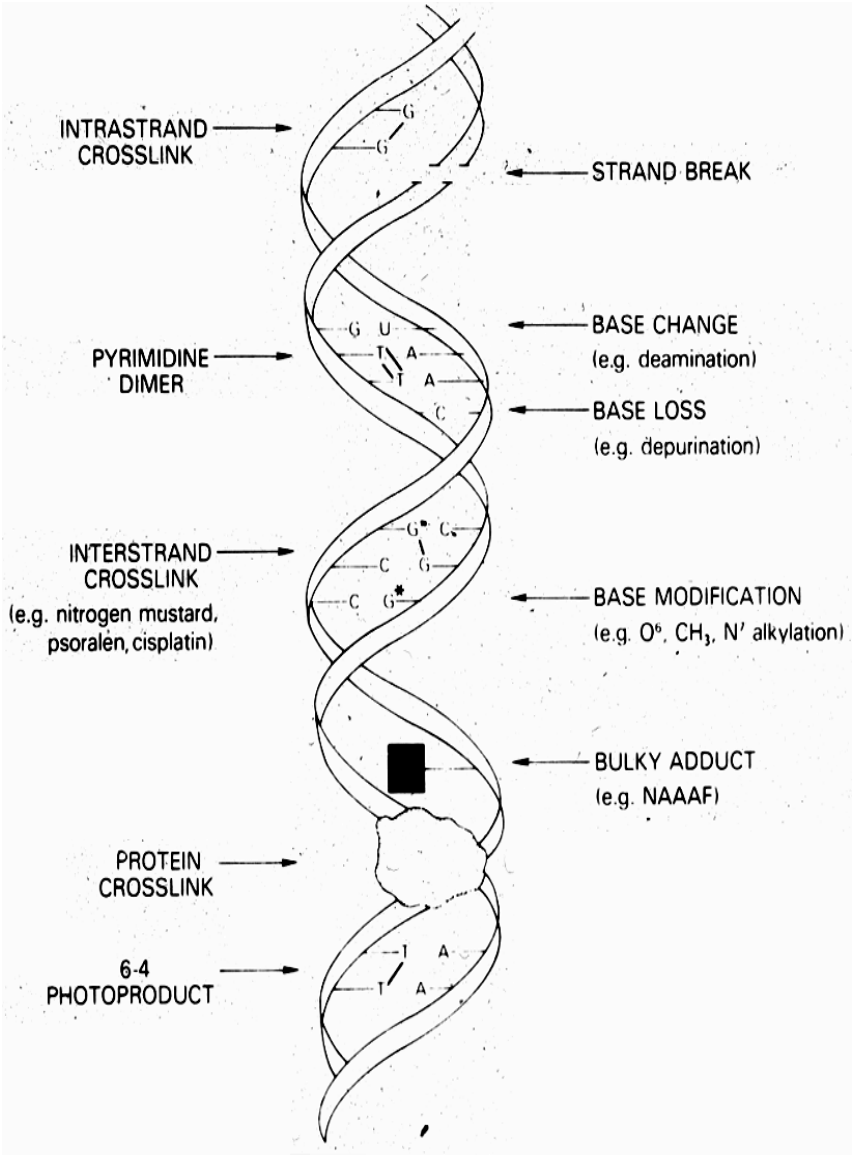
Mutations – definition and types of gene mutations

DNA repair - mechanisms of DNA repair; base excision repair; nucleotide excision repair; mismatch repair; DSB repair

Human disease linked with DNA repair - Xeroderma Pigmentosum, Cockayne, Bloom and Werner syndromes, Fanconi Aneamia

Mutations and epigenetic aberrations in cancer

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

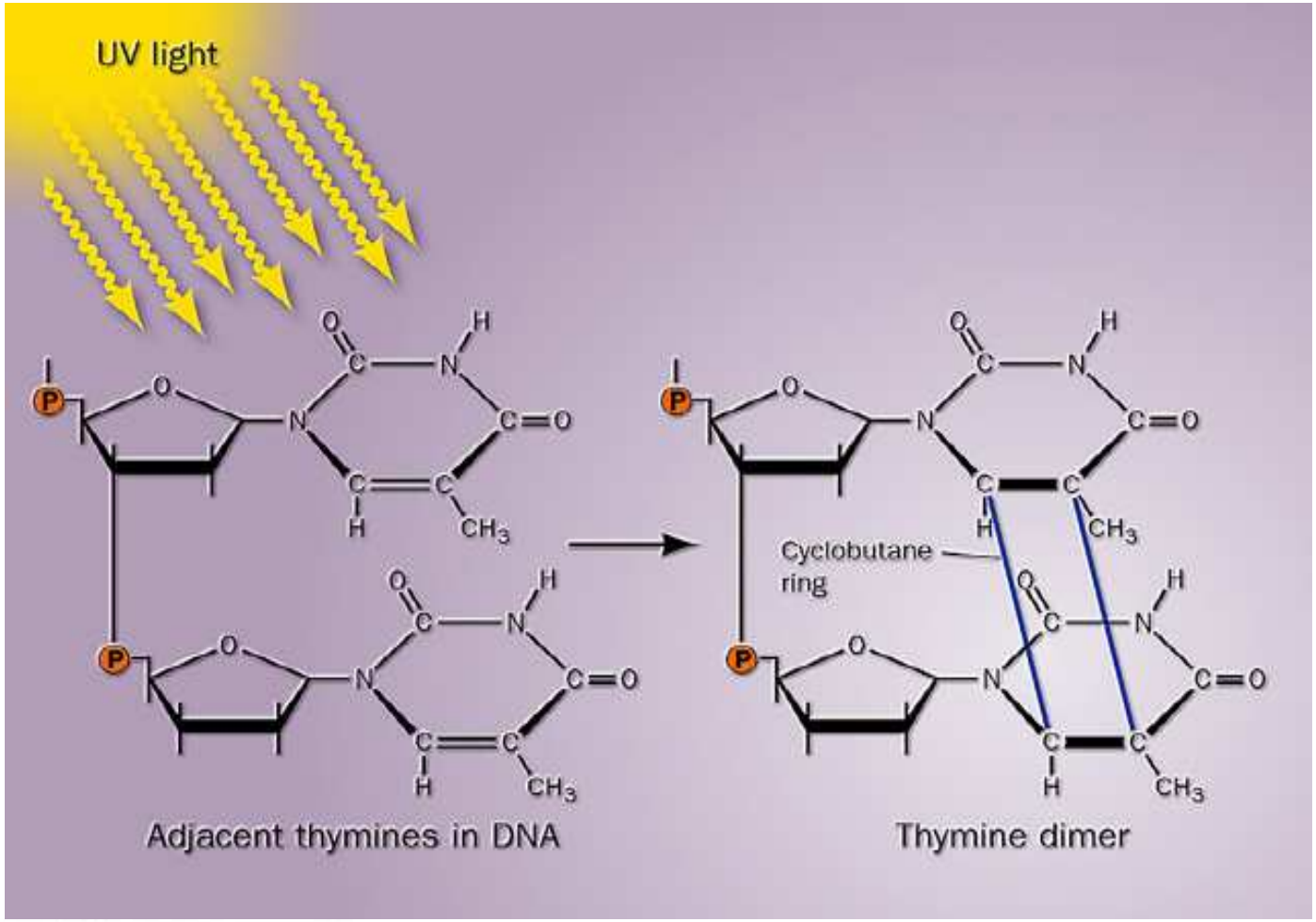
Oxidative damage by free radicals
(oxygen metabolism)
Replicative errors
Spontaneous alterations in DNA
Alkylating agents

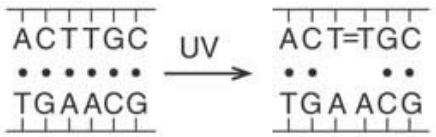
DNA damage in human cell per day:

- **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

UV-induced damage

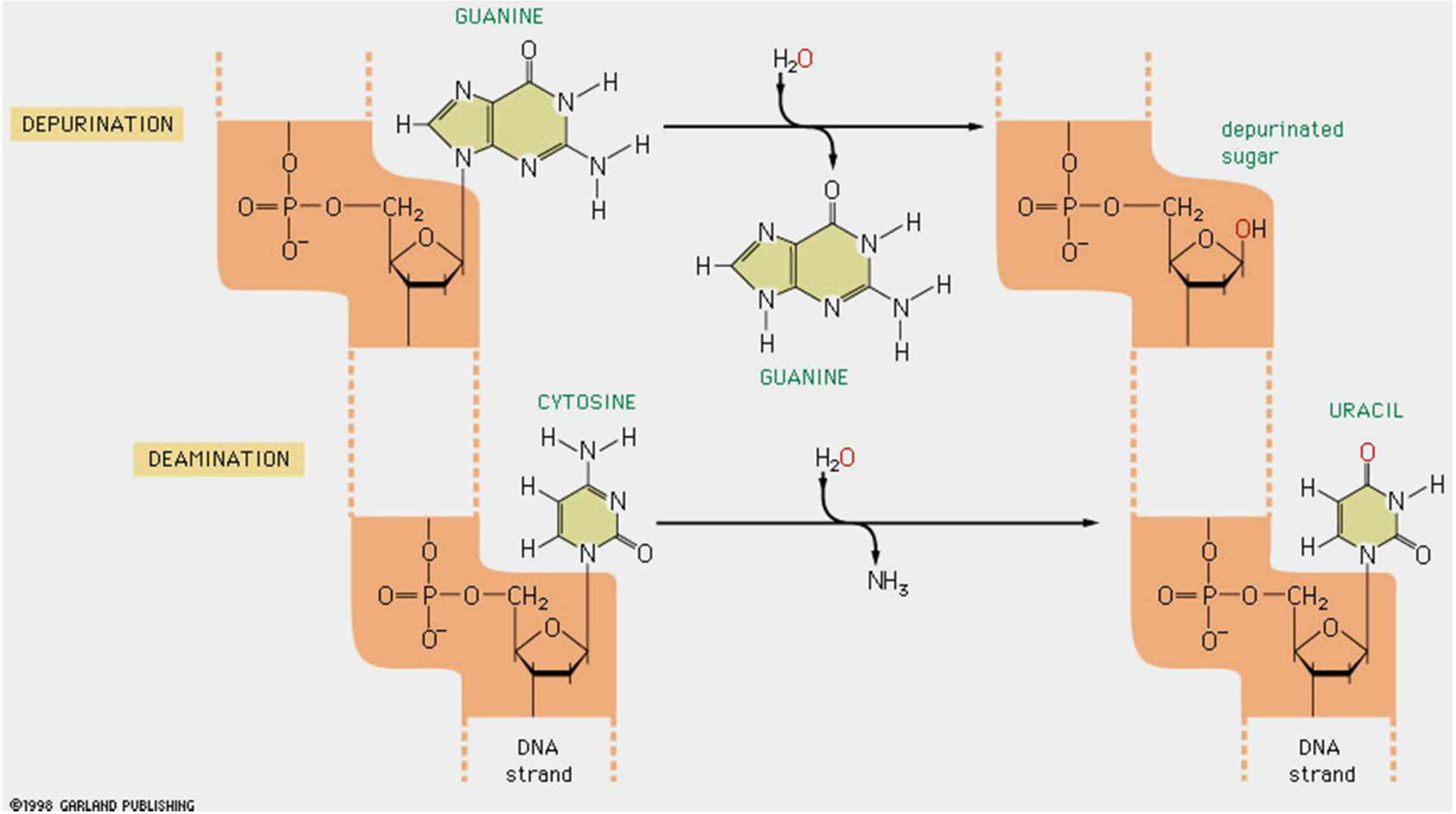




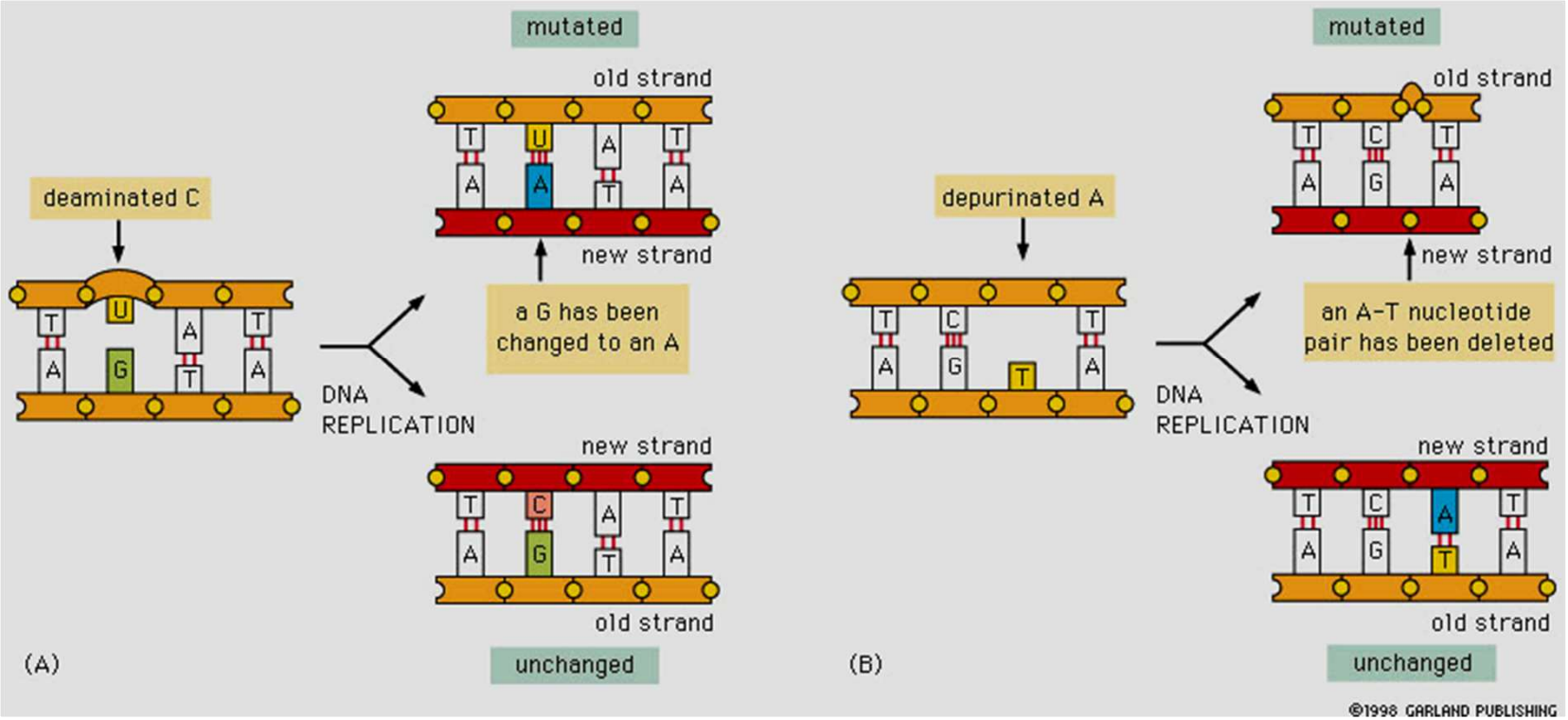
Covalent linkage between neiboring thymins

- **thymin dimers** (pirimidin dimers).

Loss of base and deamination



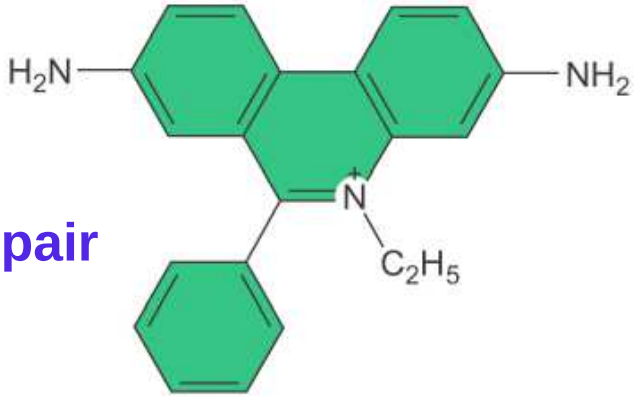
Biological consequences of deamination and depurination



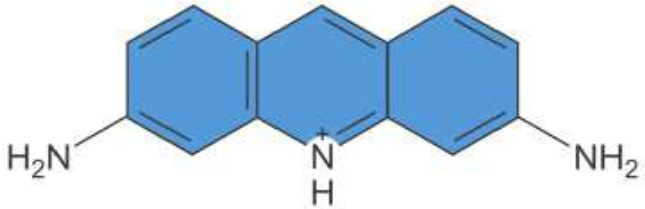
Deamination – **base exchnage** Depurination – **deletion**

Intercalating mutagens fit between adjacent base pairs

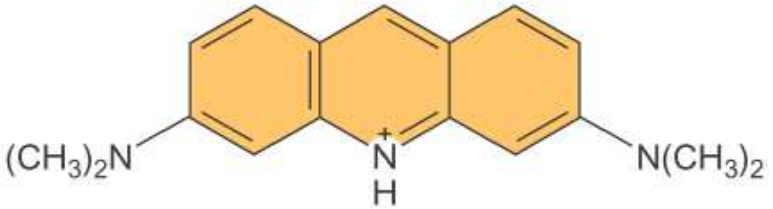
Same size as a base pair



ethidium



proflavin



acridine orange

- **cause base insertions, leading to translational frameshifts**

Mutations

Gene (point) mutations

Chromosomal (structural aberration of chromosomes)

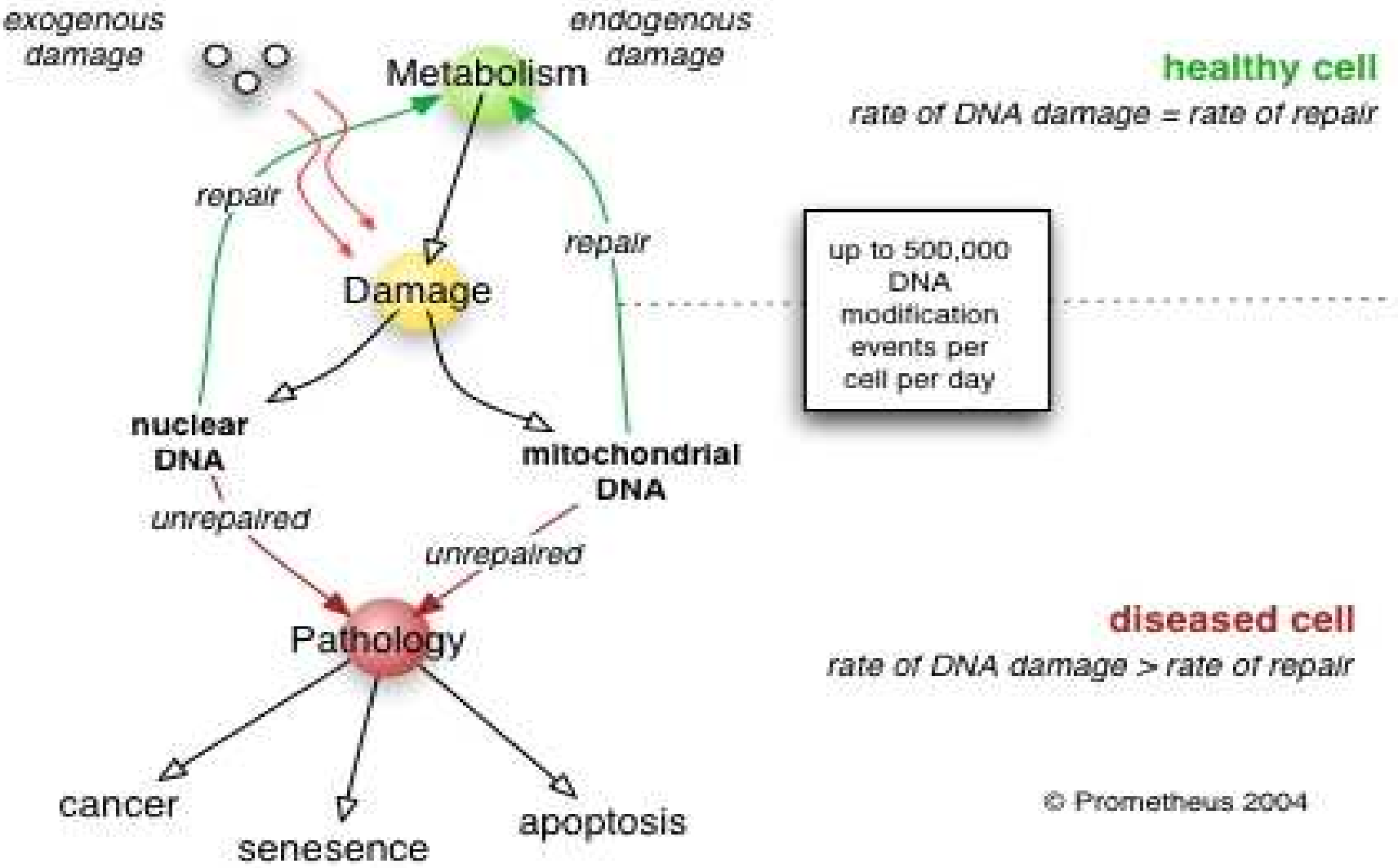
Genomic (numeric aberration of chromosomes)

Somatic versus germline mutations

Somatic mutations manifested in 1 cell – clone – not transmitted but can lead to cell death or **cancer transformation** – 1 individual is affected

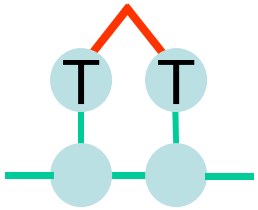
Mutations of germinal cells – carrying to offsprings – abortion or **inborn deffects** – Mutations affecting the germ cells are passed on to future generations.

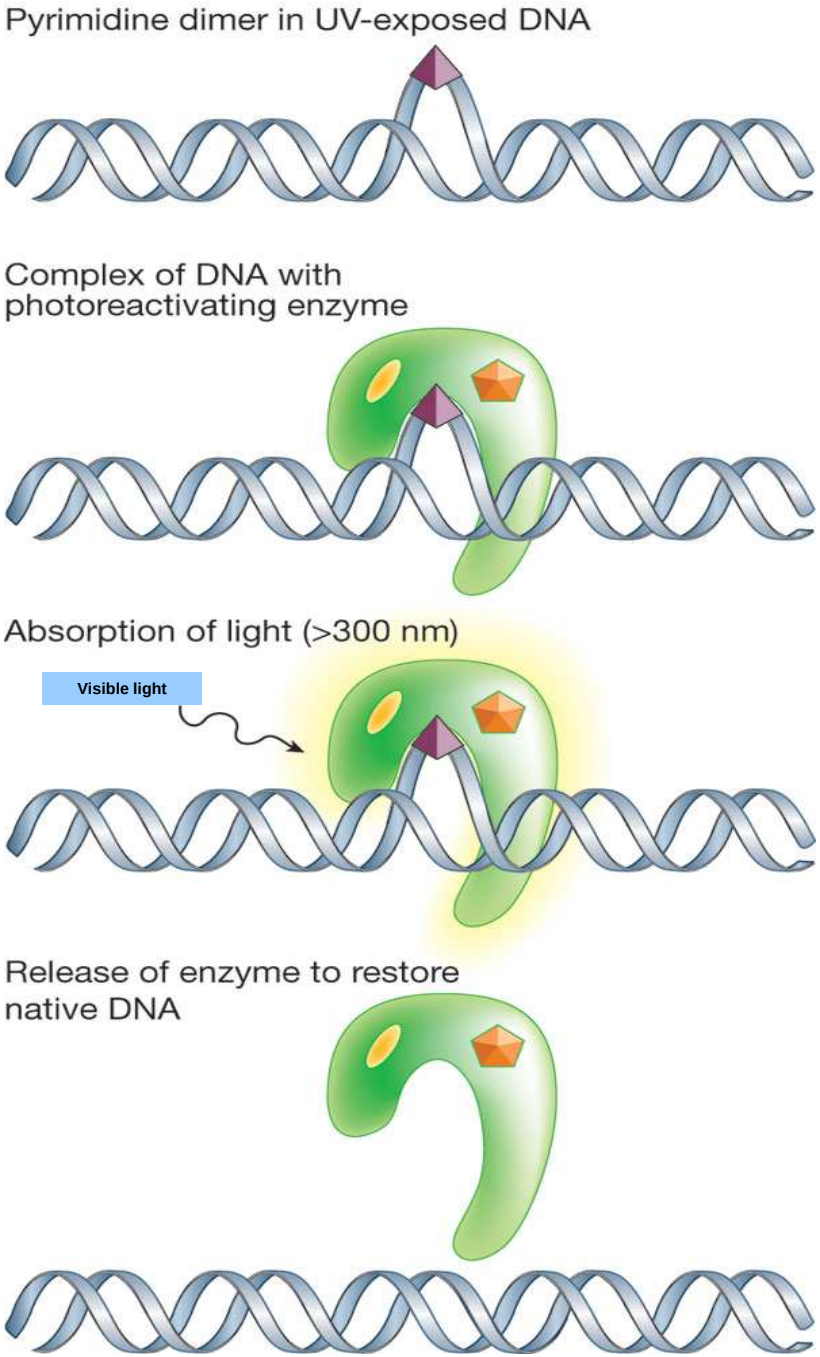
DNA damage and repair

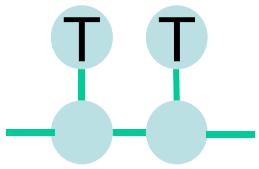


DNA Repair Pathways

1. **Direct reversals**
2. **Excision repair**
   - Base Excision Repair (BER)
   - Nucleotide Excision Repair (NER)
3. **Mismatch repair (MMR)**
   - replication errors
4. **Recombination 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

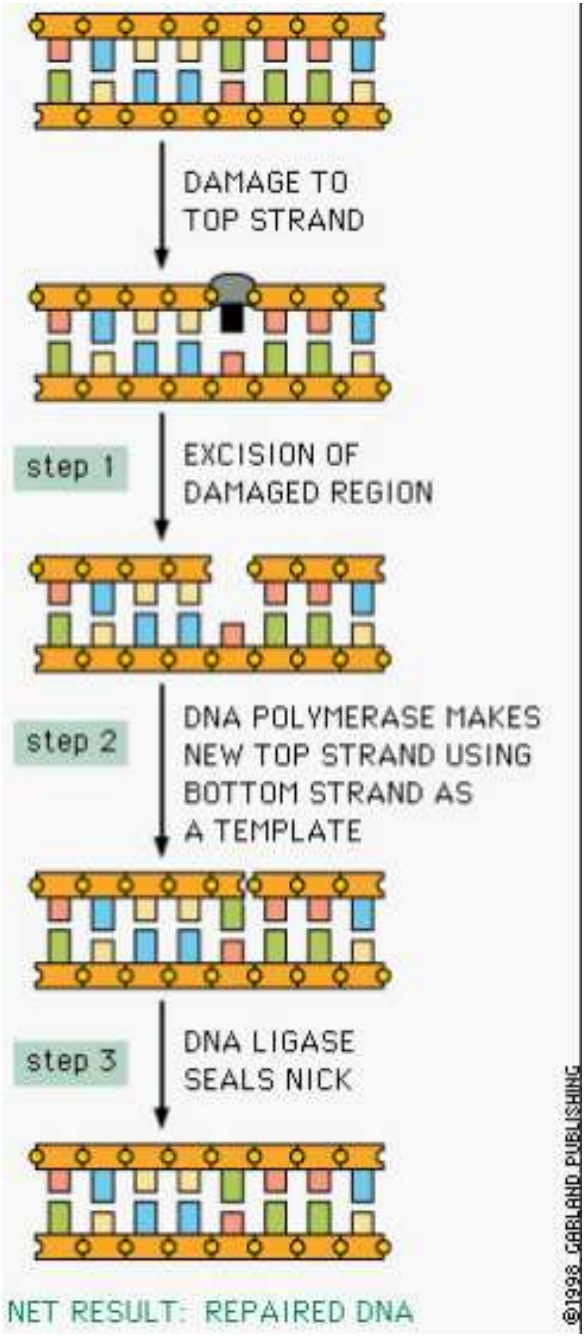
Related disease:
Photolyase not yet found in placental mammals

Base Excision Repair (BER)

1) Recognition and removal of lession
2) Gap filling
3) Joning DNA strands

Steps 2 and 3 are same in other DNA repair pathways.

Repair of deamination, oxidative damage and other small base lesions



Summary BER

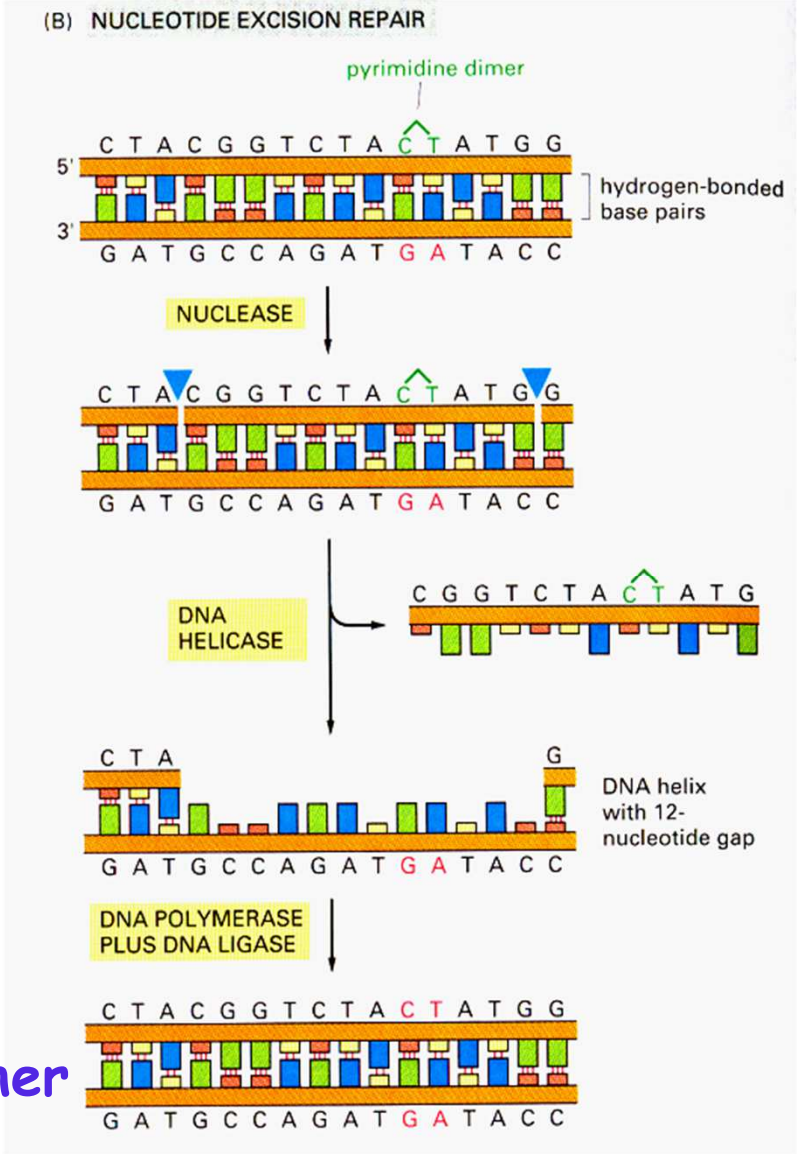
- Two pathways of global genomic repair
- Transcription-coupled pathway
- Many components of the global pathways are essential
- Defective TCR causes Cockayne's syndrome
- Repairs wide variety of base damage
    - oxidative damage
    - alkylation damage
    - ionizing radiation damage
    - incorrect base (deamination of cytosine to uracil)
    - abasic sites
    - some types of UV damage

Further reading: Fortini et al. Biochimie 85, 1053 (2003)

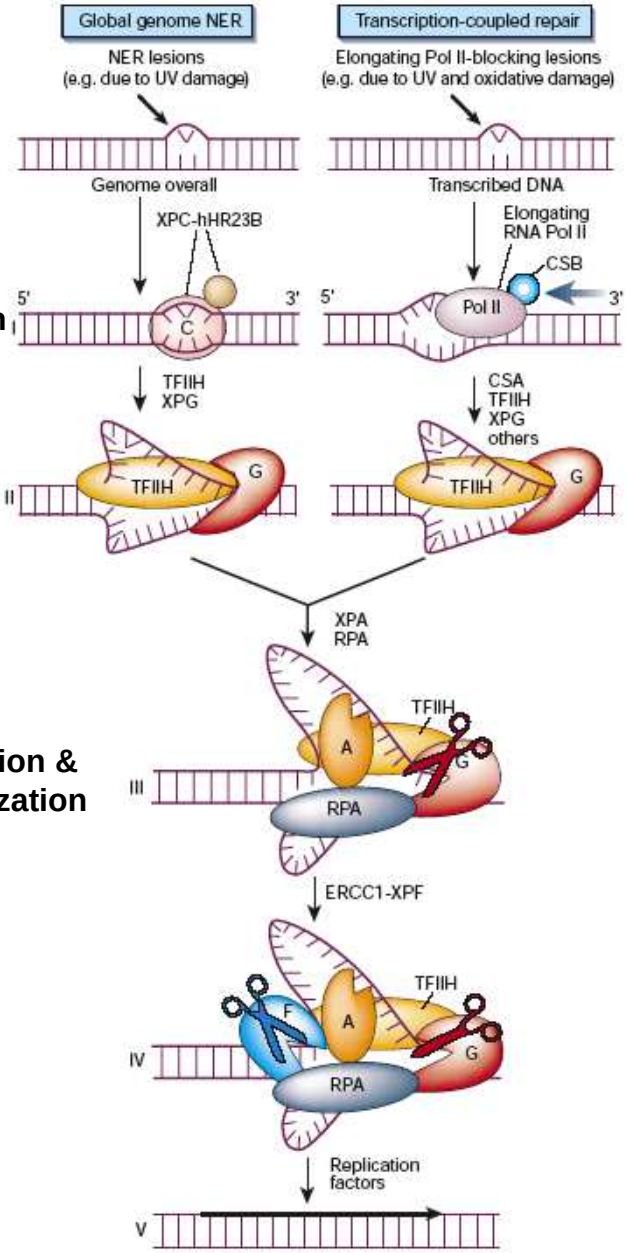
Nucleotide Excision Repair

1. Recognition by **protein factors**
2. Incision on each site from the lession (**nuclease**).
3. Unwinding of DNA (**DNA-helicase)**
4. Synthesis from the complementary strand (**DNA polymerase δ a ε**)
5. Joining DNA ends by **DNA ligase**

Repair of dimmers, crosslinks and other helix distorting damage



Mammalian NER Pathways



XPC - damage recognition

XPB & XPD - DNA helicases

XPA & RPA - damage validation & complex stabilization

XPG - 3' incision

ERCC1-XPF - 5' incision

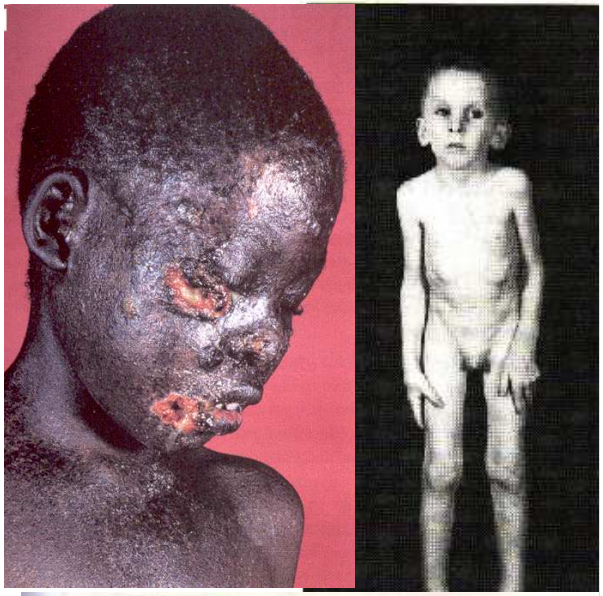
CSA & CSB - role in processing RNAP II?
XPC not required

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

Genetics of NER

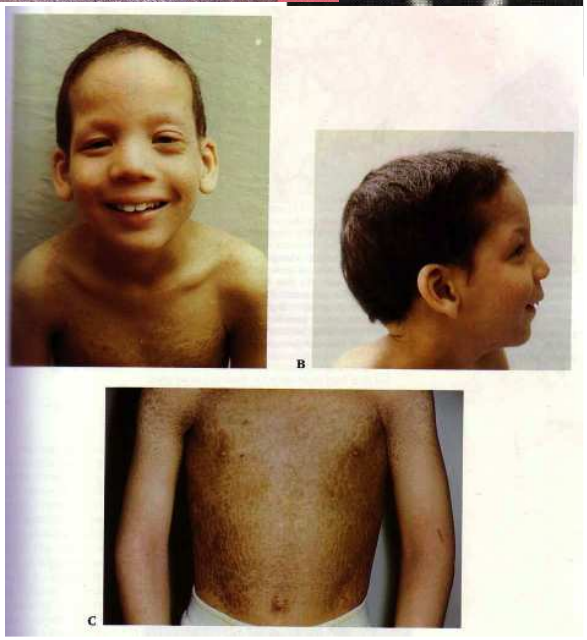
Xeroderma Pigmentosum

- Occurrence: 1-4 per million population
- Genetic: autosomal recessive, seven genes (XPA-G)
- Disorder: multiple skin disorders; malignancies of the skin; neurological and ocular abnormalities



Cockayne's Syndrome

- Occurrence: 1 per million population
- Genetic: autosomal recessive, genes (XPA, B, D & G)
- Disorder: arrested development, mental retardation, dwarfism, deafness, optic atrophy, intracranial calcifications; (no increased risk of cancer)



Trichothiodystrophy

- Occurrence: 1-2 per million population
- Genetic: autosomal recessive, (TTDA, XPB a D)
- Disorder: sulfur deficient brittle hair, mental and growth retardation, peculiar face with receding chin, ichthyosis; (no increased cancer risk)

Replication errors

- The human genome is 10^9bp (~ 2m) per cell
- During the course of our lives we synthesise a 'light year' (10^{16}m) of DNA
- During this talk, we will each synthesise ~ 20 billion metres of DNA.
- Mutations in any of a large number of genes can cause cancer...
- ... yet 2/3 of us will not get cancer!

DNA Mismatch Repair

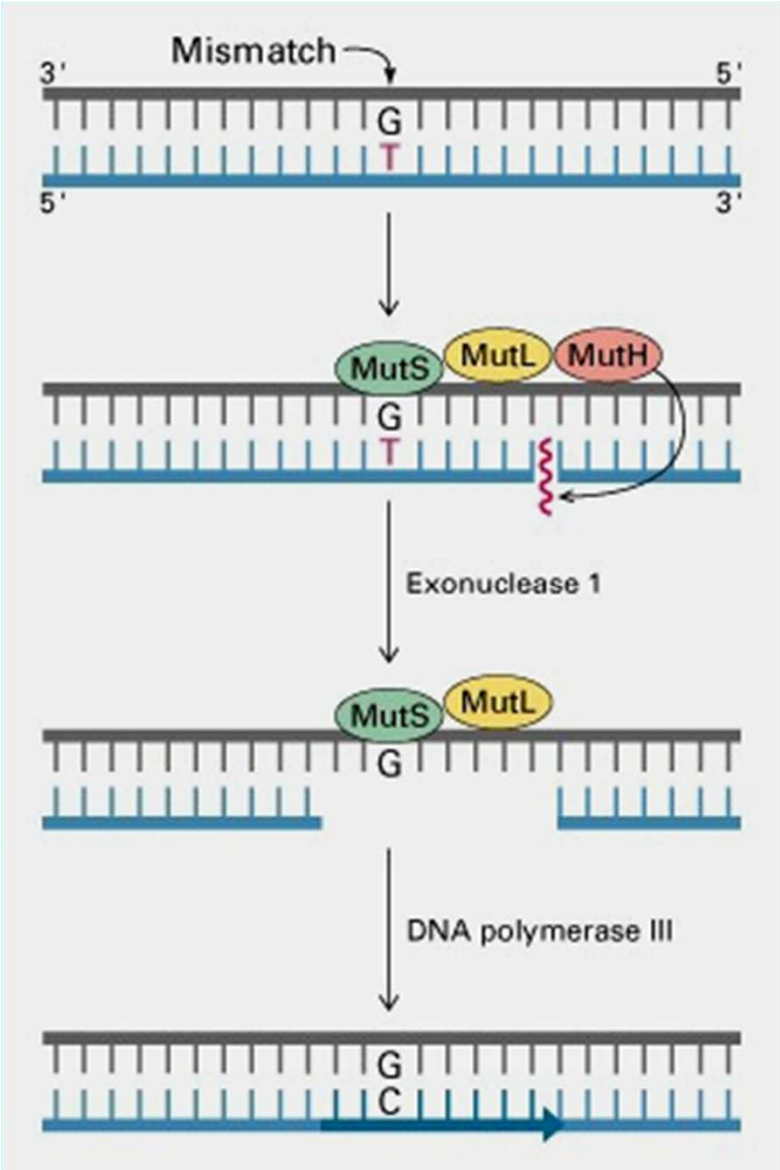
Mechanisms for Insuring Replicative Fidelity

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

1) MutS recognizes and binds at the site of a base pair mismatch
2) MutH is activataed to create a nick in newly synthesized strand
3) „Marked" strand is removed by exonuclease, resynthesized by DNA polymeraze a joined by DNA ligáse.

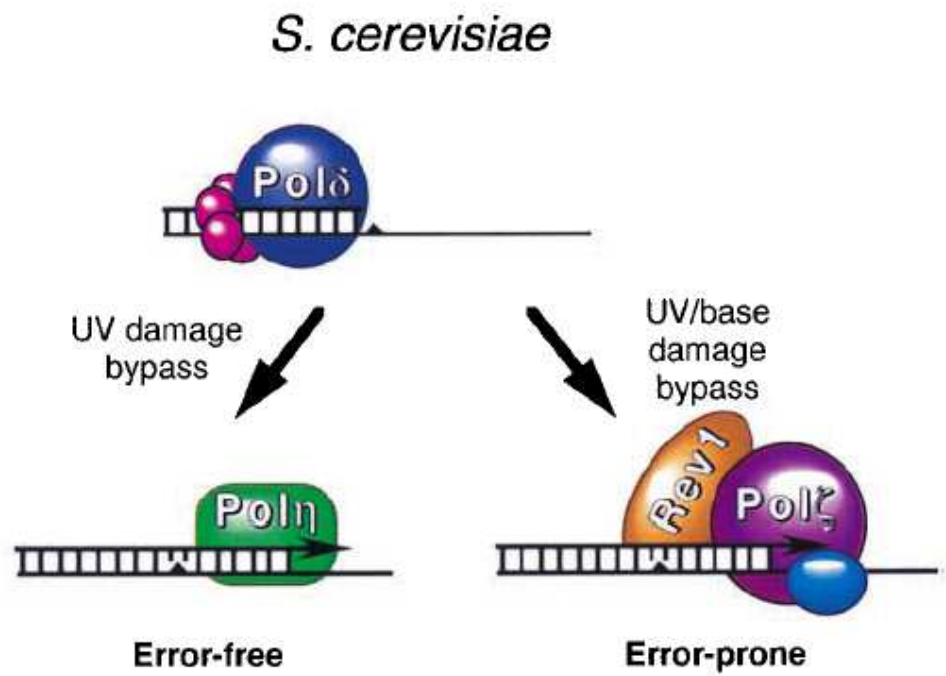


Mismatch Repair Mutations in Hereditary Nonpolyposis Colon Cancer (HNPCC)

- 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

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

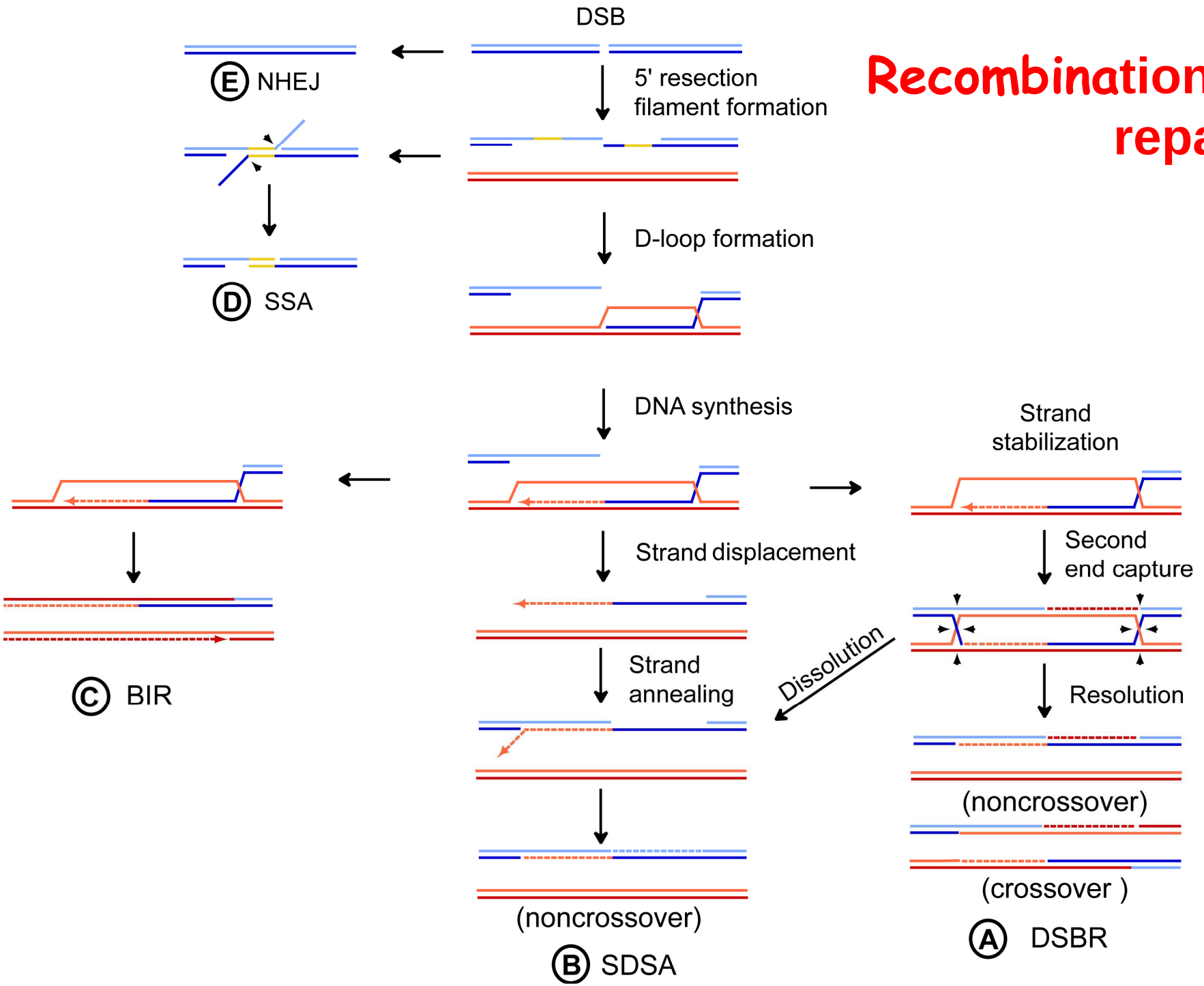
Double-strand breaks (DSB)

Generation of DSB:

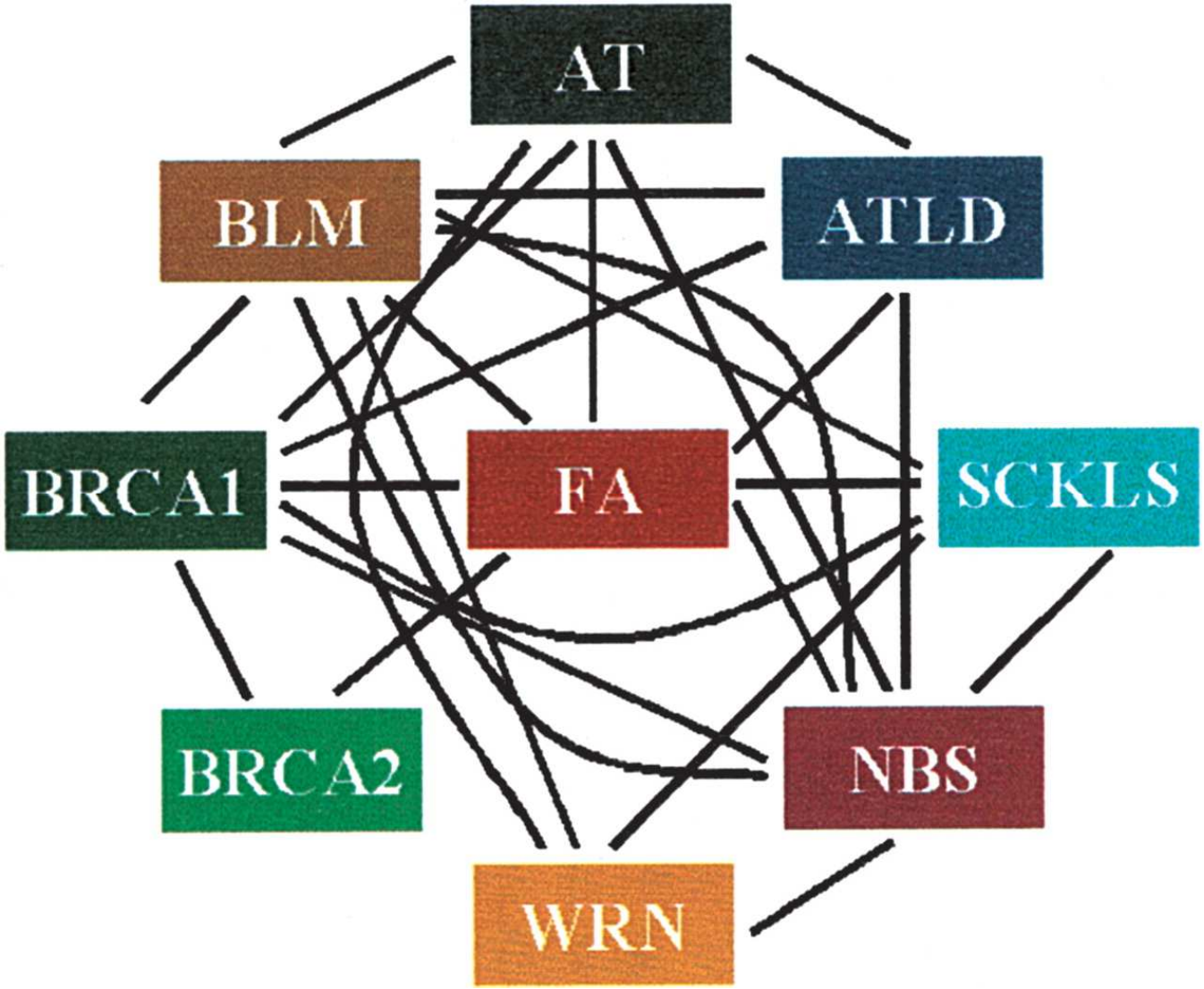
- Induced by radiation & chemicals
- During replication of damaged DNA template
- Initiation of meiotic recombination
- Part of immune reponse

Failure to repair DSB:

- Cell death
- Chromosomal aberation
- Meiotic aneuploidy
- Immuno-deficiency



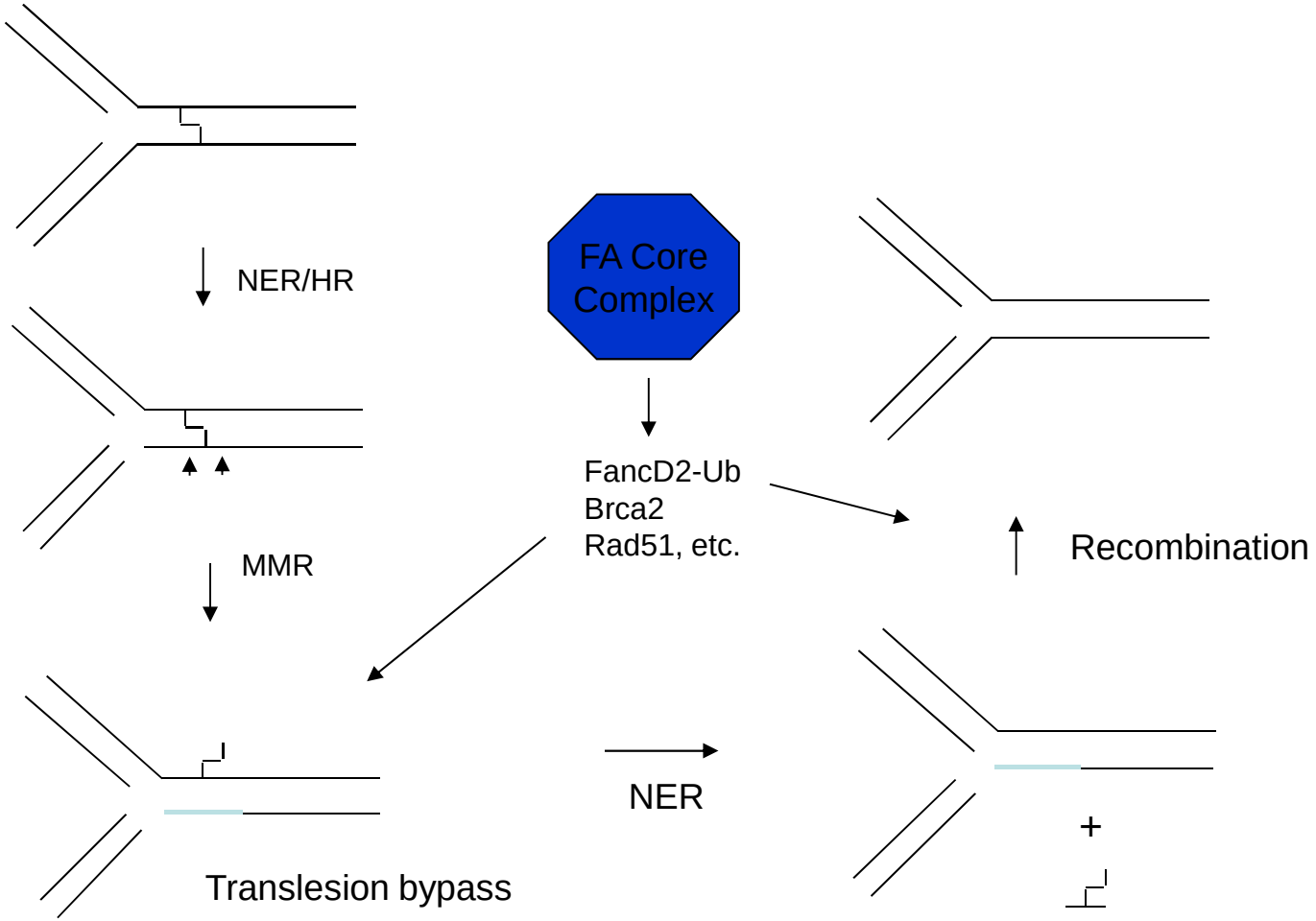
Recombinational repair



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

Model of ICL repair

(Interstrand CrossLinks)



Fanconi aneamie

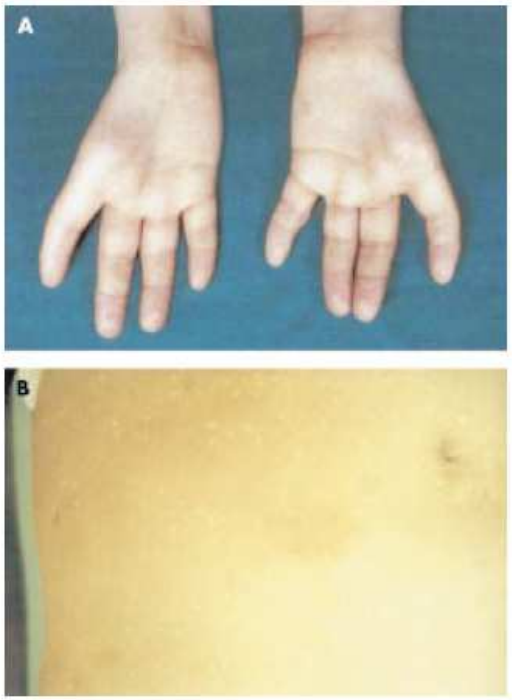


Figure 1 (A) Typical radial ray abnormalities and (B) café au lait patches and hypopigmentation, all common features in FA.

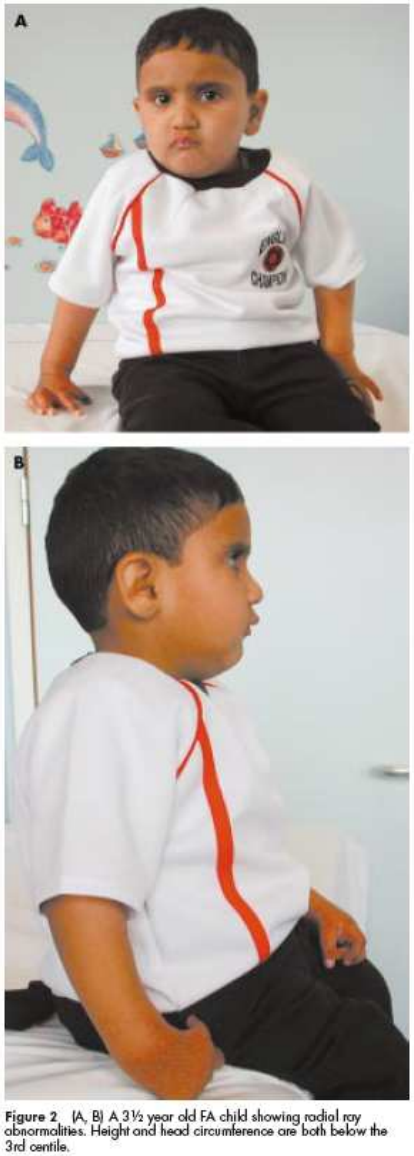


Figure 2 (A, B) A 3½ year old FA child showing radial ray abnormalities. Height and head circumference are both below the 3rd centile.

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

Cancer
- myeloid leukemia
- solid tumors

15 genes in FA
BRCA2 is deficient in FANCD1

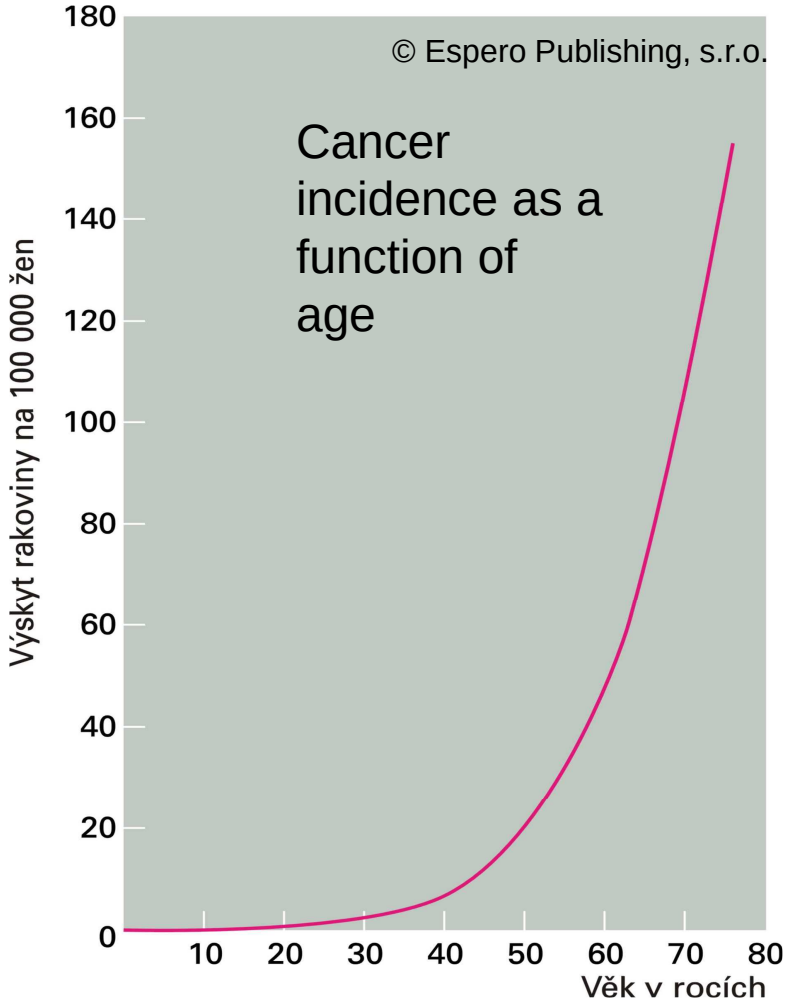
Review: Tischkowitz & Hodgson, J. Med. Genet. 40, 1 (2(

Summary for DNA Repair

Pathway	Error-free	Error-prone
Direct reversal	+	
NER	+	
BER	+	
Gene conversion	+	
SSA		+
BIR	+	
NHEJ		+
MMR	+	
Lesion bypass	+	+

Mutations and cancer

- **10^{16} cell divisions** in human body during the live
- Environments withouth mutagens **10^{-6}** chance of mutation/cell division = **10^{10}** mutations each day



!!! 1 mutation is not sufficient; most of mutations are repaired !!!

Epigenetic aberrations and cancer

corelation between increased histone acetylation and augmented transcription

Global DNA hypomethylation – striking feature of neoplasia-chromosomal instability, transcription of genes that were silenced

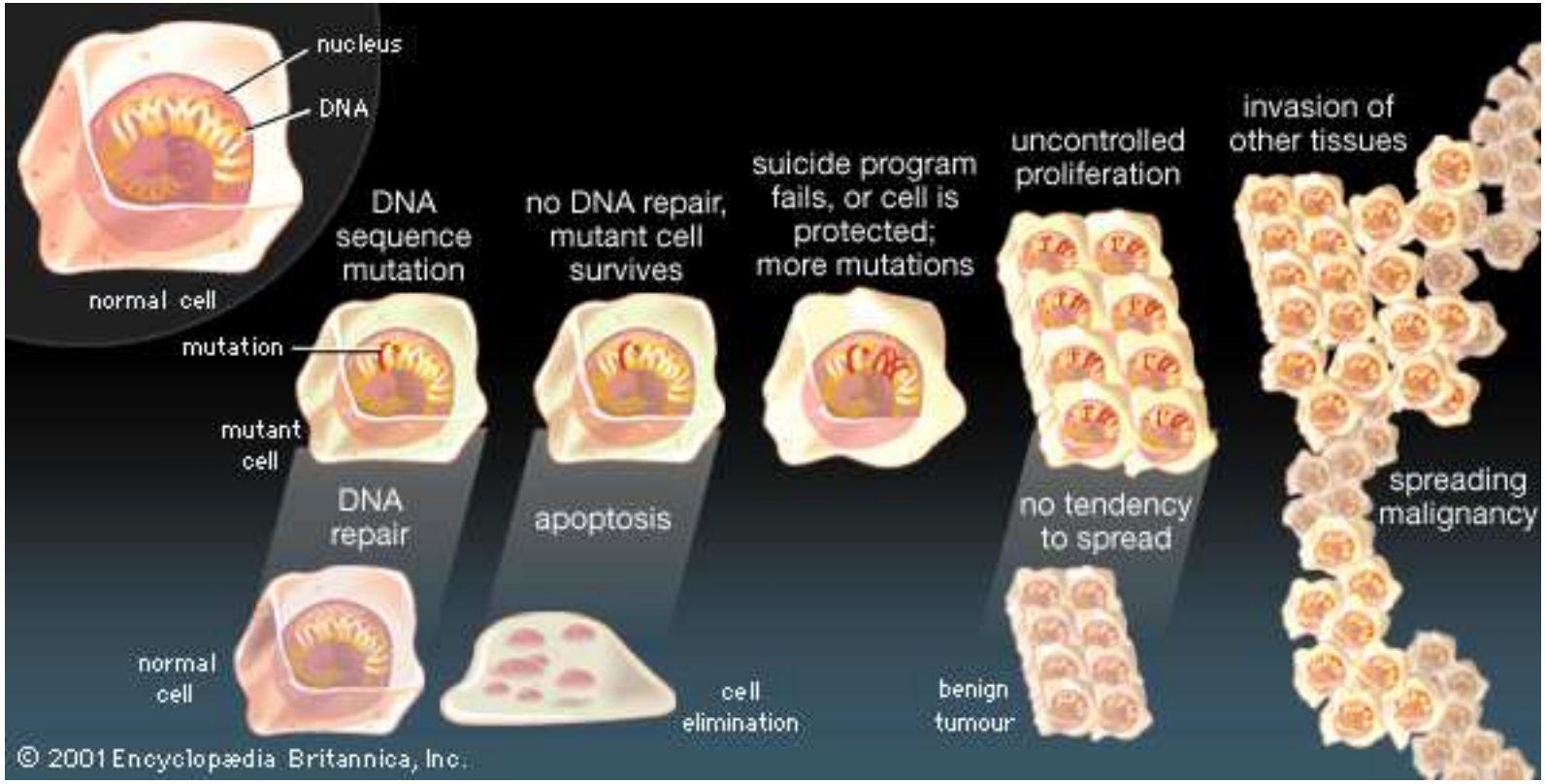
DNA hypermethylation and **chromatin hypoacetylation** of tumour-supressor genes (RB1) - cancer

Hypomethylation of specific genes –oncogenes

Chromatine alterations – post-translational histone modifications

Loss of imprinting – activation of the normally silenced allele or silencing of the normally active allele

Mutations and cancer



Future perspectives

Future perspectives

Synthetic lethal approach

**(*i.e.* BRCA2-patients with PARP inhibitors,
MLH1-patients with Pol γ and β inhibitors)**

Thank you for your attention

and take care of your DNA !!!!

Epigenetic DNA modifications

Epigenetic: something that affects a cell, organ or individual without directly affecting its DNA.

Non-sequence-based alterations that are inherited through cell division

DNA methylation – a covalent modification that can occur at cytosines within CpG-rich regions of DNA and is catalysed by DNA methyltransferases. Lower DNA accesibility to transcriptional complex – no transcription.

Histone modifications – post-translational modification, i.e. acetylation, methylation, phosphorylation…