DNA damage and repair

Why is it important to study „DNA damage“?

DNA: the genetic material ensuring

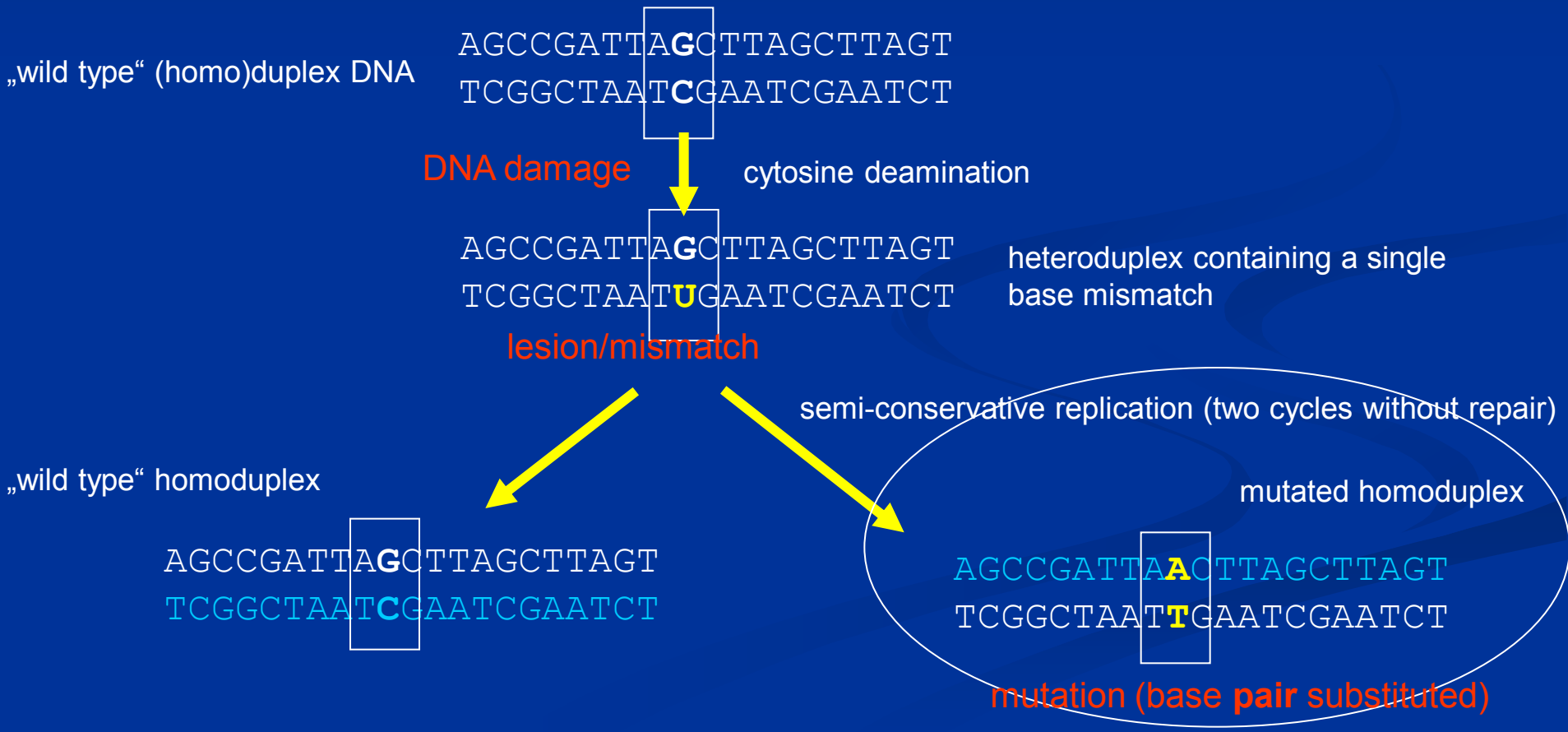
- preservation of the genetic information
- its transfer to progeny
- its transcription and translation into proteins

Damage to DNA may

- lead to change of the genetic information (mutation)
- affect gene expression
- **have severe health impacts**

DNA damage, mutation, lesion, mismatch…?

- mutation may arise from (among others) DNA damage which is not repaired prior to DNA replication, e.g..

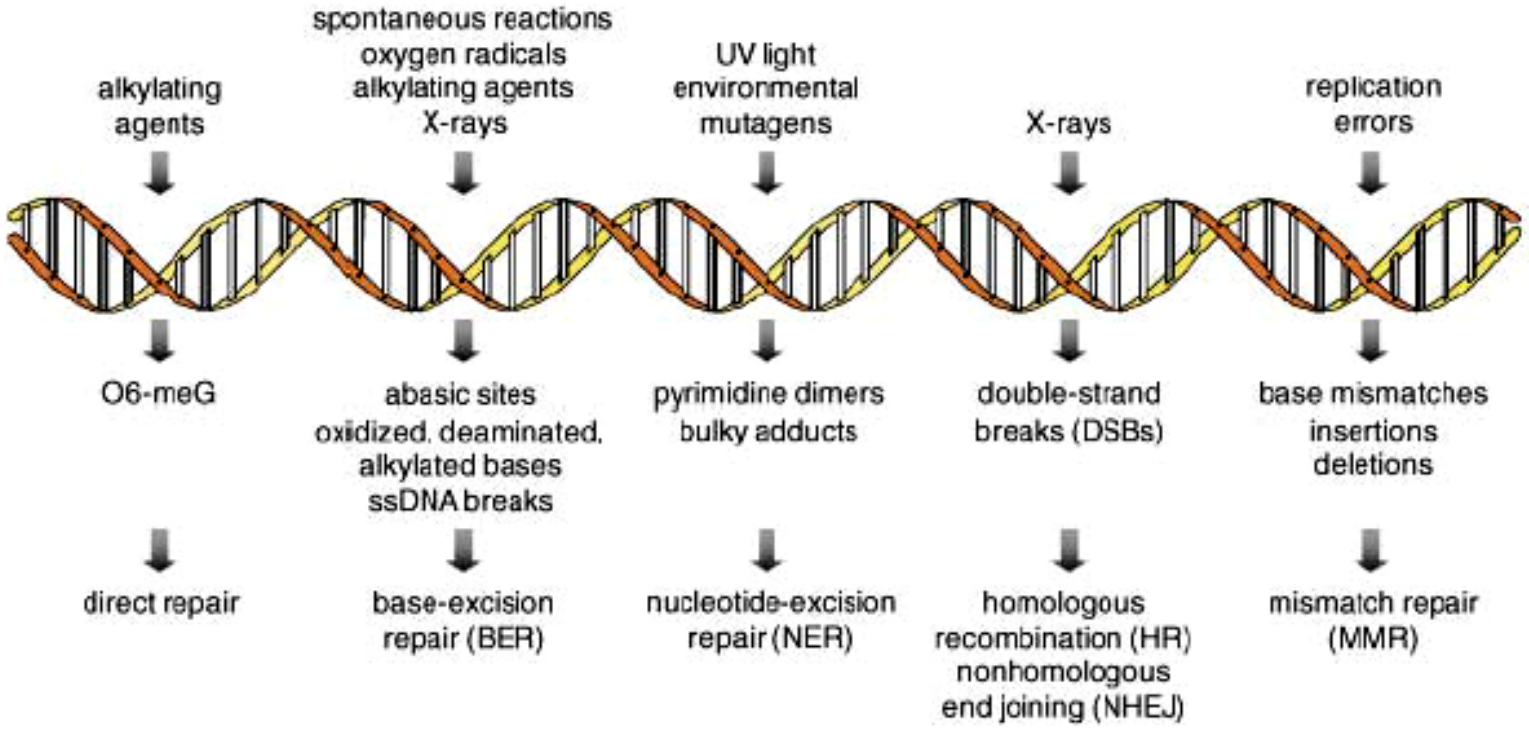


DNA damage, mutation, lesion, mismatch…?

- **mutations arise from unrepaired DNA damage** (or from replication errors)
- **damaged DNA is not mutated yet!** (damage is usually repaired in time i.e. before replication – lesions and/or mismatches are recognized by the reparation systems)
- **DNA with mutated nucleotide sequence does not behave as damaged!** All base pairs in such DNA are „OK“ (no business for the DNA repair machinery) but the **genetic information is** (hereditably) **altered.**

DNA in the cells is permanently exposed to various chemical or physical agents

- endogenous - products and intermediates of metabolism
- exogenous - environmental (radiation, pollutants)



Scharer, O. D. (2003) Chemistry and biology of DNA repair, *Angew. Chem. Int. Ed. 42*, 2946-74.

Most frequent products of DNA damage („lesions“)

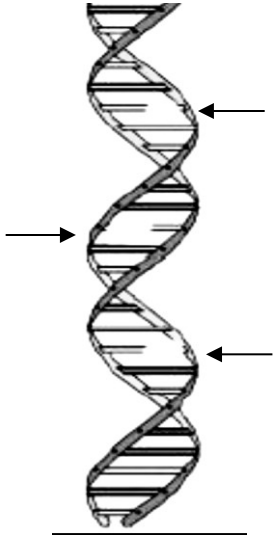
interruptions of DNA sugar-phosphate backbone

single-strand break

double-strand break

- reactive oxygen species
- action of nucleases
- consequence of base damage

interruption of the N-glykosidic linkage



abasic sites

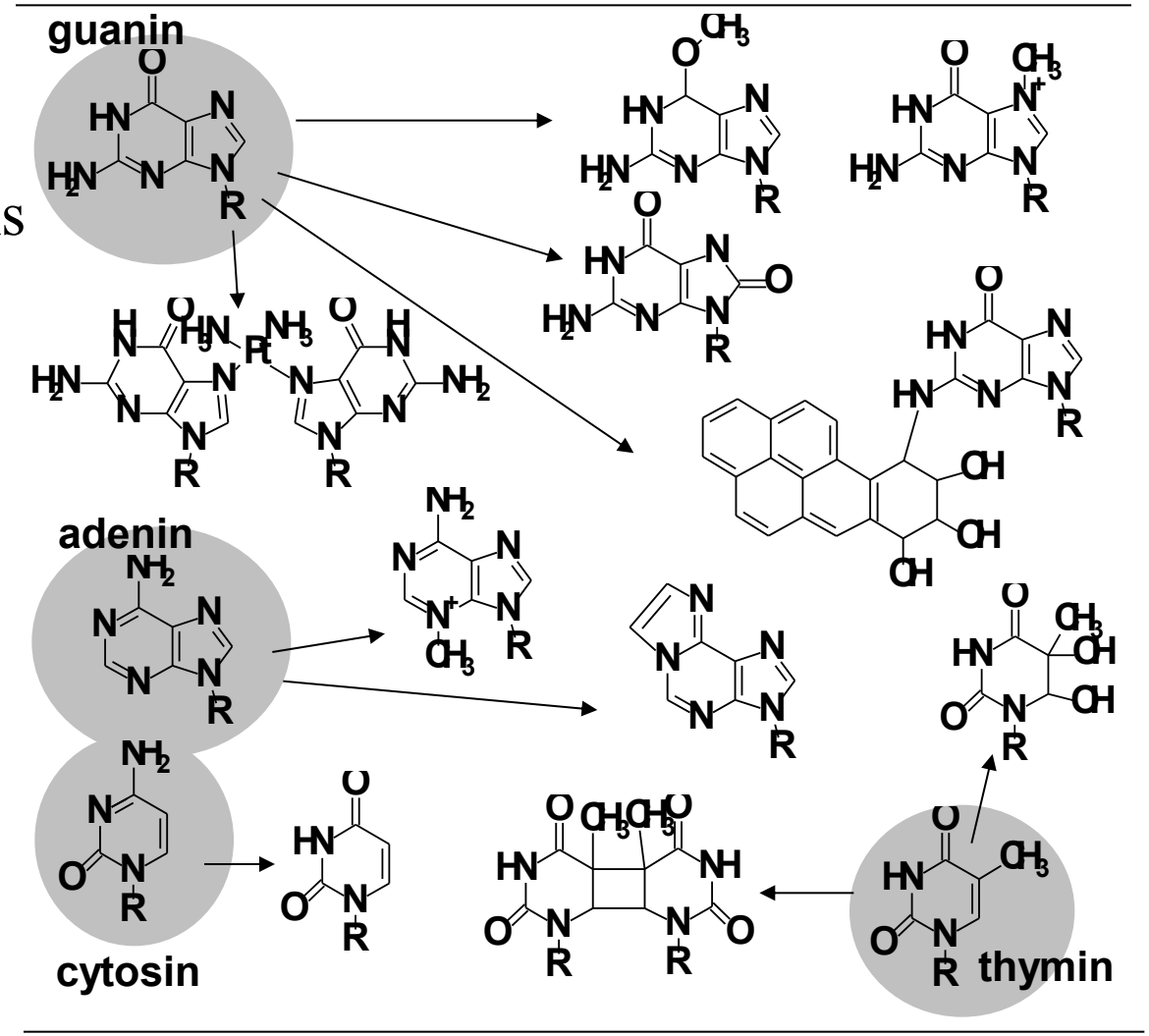
- spontaneous hydrolysis (depurination)
- consequence of base damage

Most frequent products of DNA damage („lesions“)

base damage:

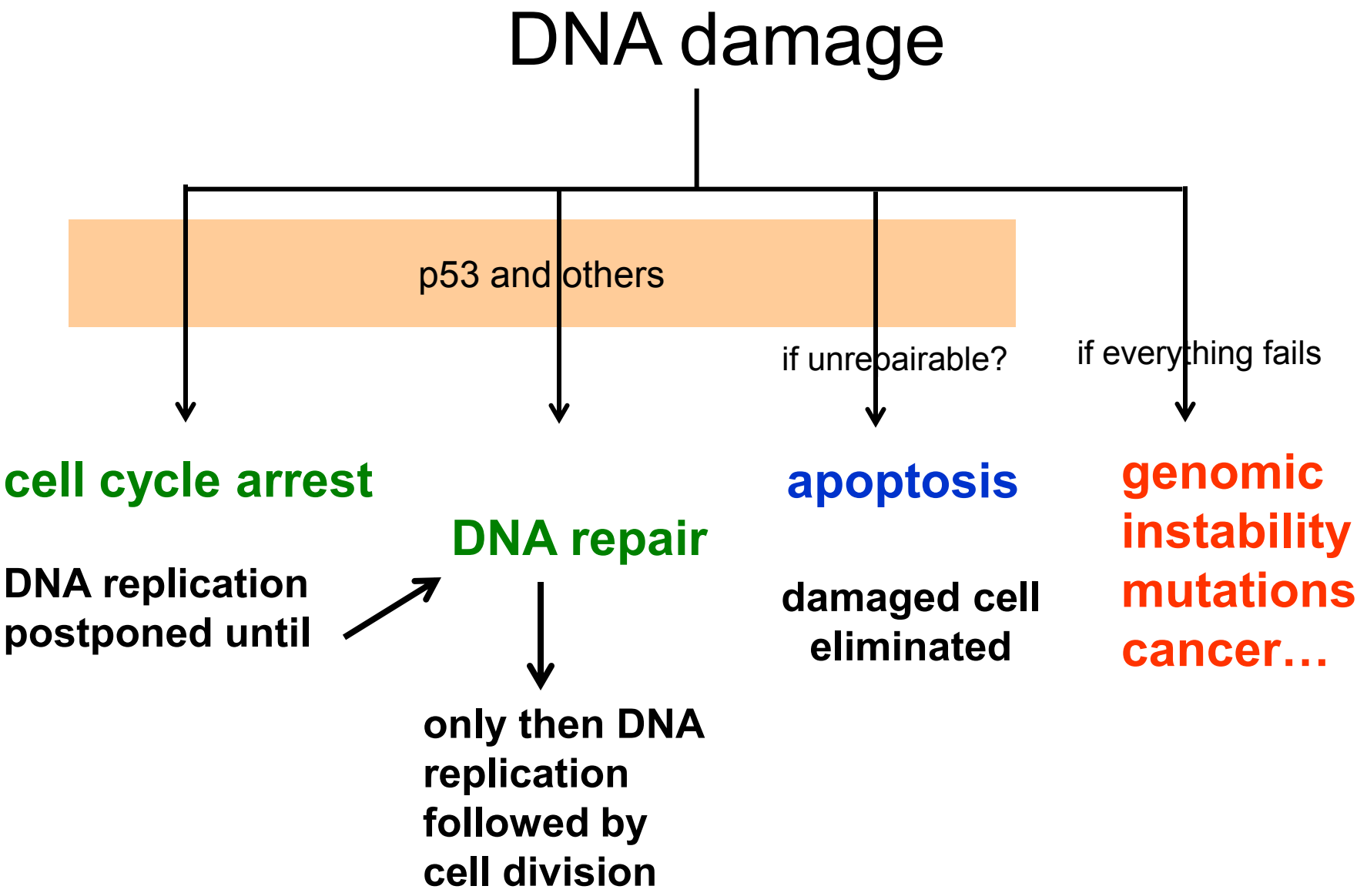
chemical modifications

- alkylation
- oxidative damage
- deamination
- damage by UV radiation (sunlight)
- metabilically activated carcinogens
- anticancer drugs



Importance of DNA repair

- estimated number of DNA-damage events in **a single human cell: 10^4-10^6 per day!!**
- only a small number of base pairs alterations in the genome are in principle sufficient for the induction of cancer
- DNA-repair systems must effectively counteract this threat
- in an adult human (10^{12} cells) about 10^{16}–10^{18} repair events per day

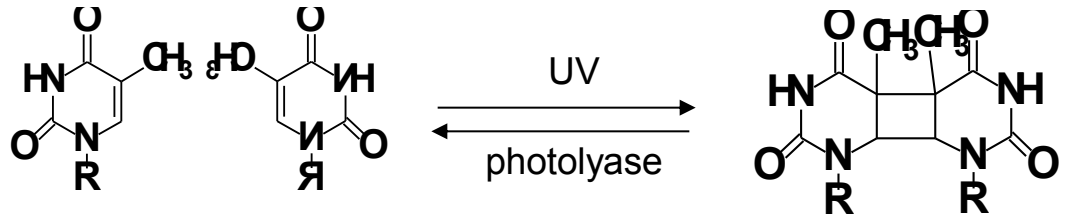

DNA damage
p53 and others
if unrepairable?
if everything fails
cell cycle arrest
DNA repair
apoptosis
genomic instability mutations cancer…
DNA replication postponed until
only then DNA replication followed by cell division
damaged cell eliminated

DNA repair pathways

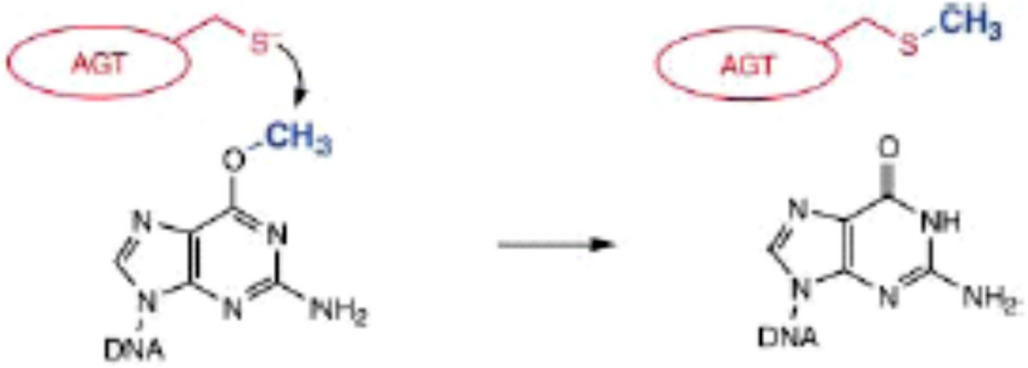
- direct reversal of damage
- base excision repair
- nucleotide excision repair
- mismatch repair
- repair of double strand breaks

Direct reversal of DNA damage

- photolyases: repair of cyclobutane dimers



- O6-alkylguanine transferase: reverses O6-alkylguanine to guanine by transferring the alkyl group from DNA to a reactive cysteine group of the protein



Base excision repair

- repair of damage by deamination (U, I), oxidation (8-oxoG), and alkylation
- initiated by **DNA glycosylases**, which recognize damaged bases and excise them from DNA by hydrolyzing the N-glycosidic bond
- substrate specificity of the glycosylases: developed to repair expectable „errors“?

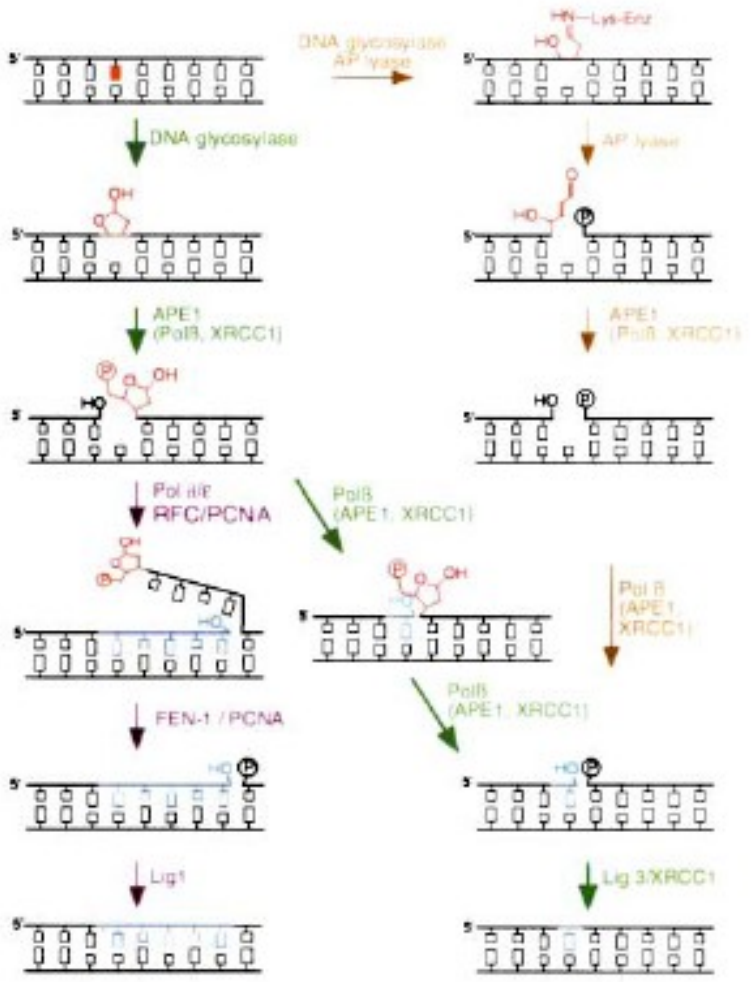
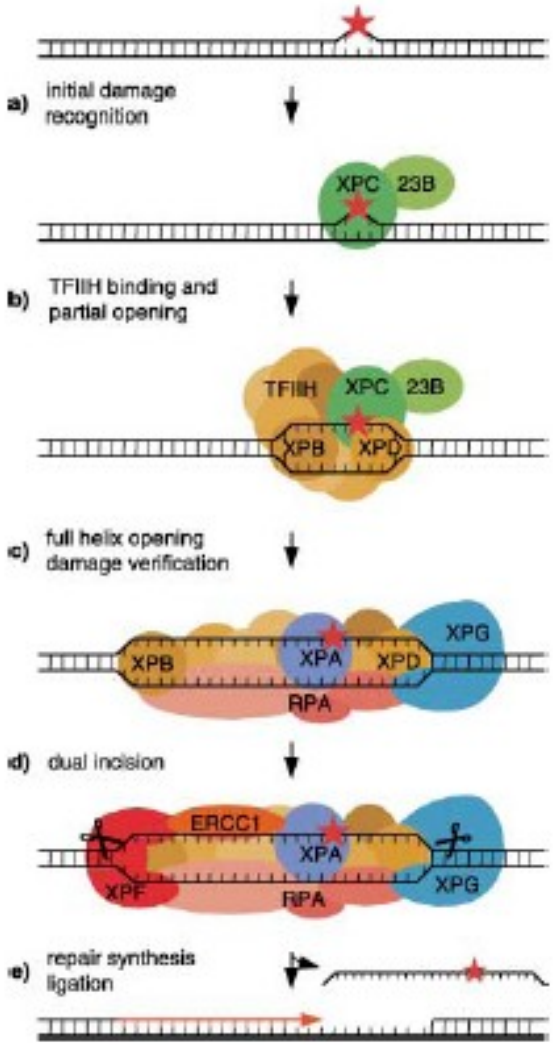


Table 1: Human DNA glycosylases

Enzyme	Most important substrate	AP lyase
UNG	U, 5-OH-U in ss/dsDNA	no
SMUG1	U, 5-OH-U in ss/dsDNA	no
TDG	U:G, T:G, εC	no
MBD4	U:G, T:G	no
OGG1	8-oxoG:C, fapy	yes
MYH	A:8-oxoG	no
NTH1	ox. pyrimidine, fapy	yes
NEI1	ox. pyrimidine, fapy	yes
AAG (MPG)	3-MeA, 7-MeG, εA, Hx	no

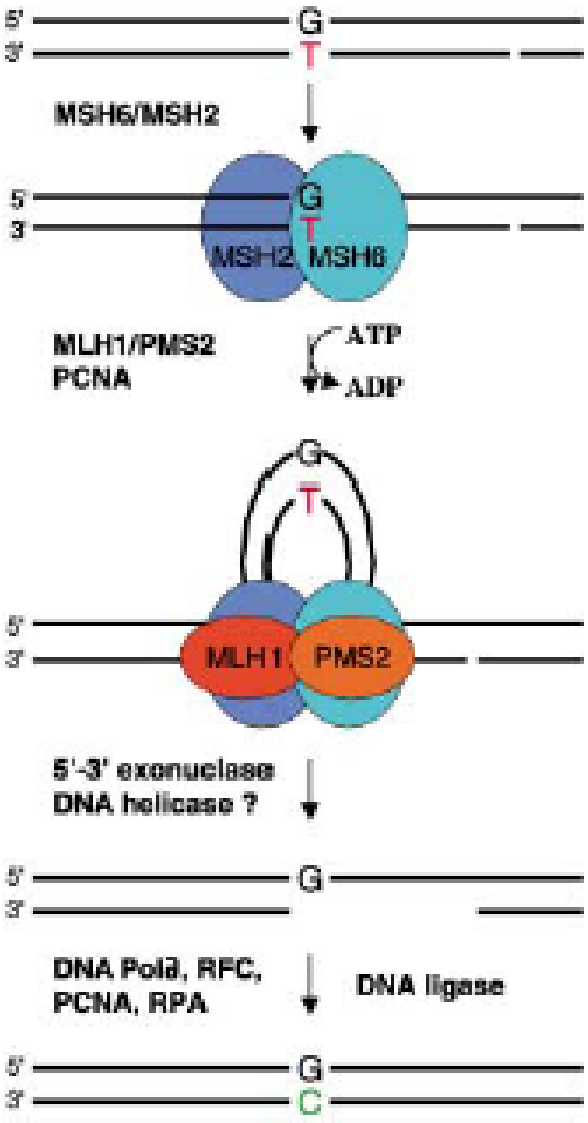
Nucleotide excision repair

- removes **bulky base adducts** (such as those formed by UV light, various environmental mutagens, and certain chemotherapeutic agents) from DNA
- broad substrate specificity: dealing with unexpected environmental DNA damaging agents
- excision of the damaged oligonucleotide
- then filling the gap & the sealing break



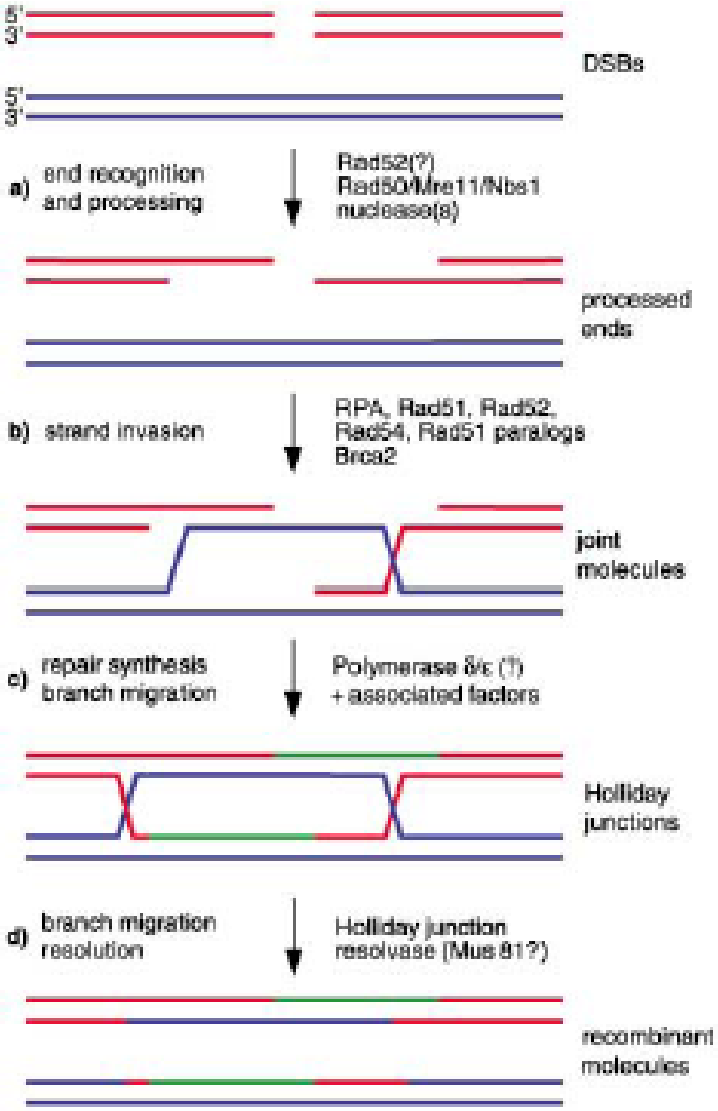
Mismatch repair

- dealing with **replication errors**
- polymerases introduce about one erroneous nucleotide per 10^5 nucleotide; their 3'→5'-exonuclease activity decreases incidence of the errors to $1:10^7$
- the MMR contributes to replication fidelity by a factor of 10^3 by removal of base-base mismatches, insertions and deletions (hence the resulting incidence of mutations due to erroneous replication is only $1:10^{10}$)
- the system must be able discrimitate between parental and daughter DNA strand!
- MutS binds to mismatches and insertion/deletion loops
- „repairosome" formation, removal of a part of the daughter strand by 5'→3'- exonuclease
- new DNA synthesis and ligation



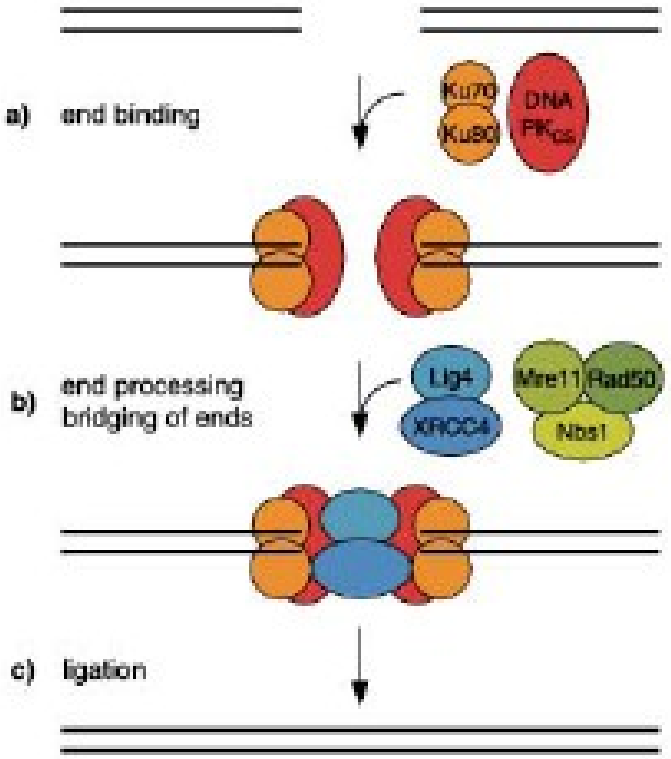
Repair of double strand breaks

- consequences of DSBs can be very severe (chromosome aberrations)
- two repair pathways:
- homologous recombination: an intrinsically accurate repair pathway that uses regions of DNA homology (such as sister chromatids) as coding information.



Repair of double strand breaks

- consequences of DSBs can be very severe (chromosome aberrations)
- two repair pathways:
- non-homologous end joining: conceptually simple pathway that involves the religation of broken ends (without using a homologous template
- less accurate: may loss of a few nucleotides at the damaged DNA ends



Examples of techniques used to detect DNA damage

1. Techniques involving complete DNA hydrolysis followed by determination of damaged entities by chromatography or mass spectrometry

HPLC: 8-oxo guanine determination

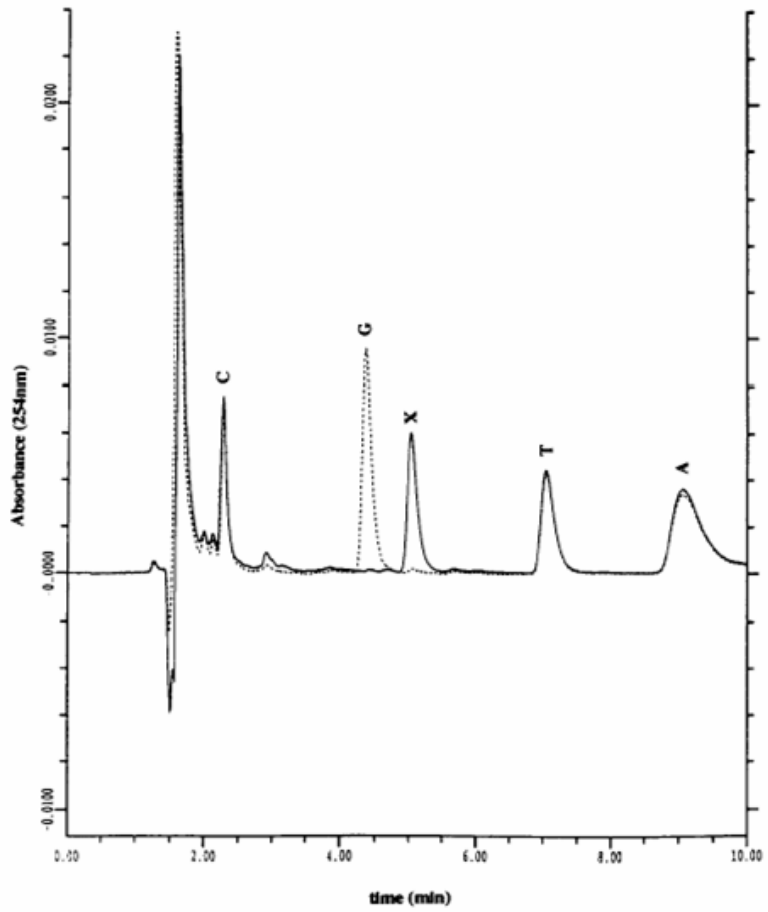


Fig. 4. Effect of guanase on bases derived from a formic acid hydrolysate of calf thymus DNA. Samples were HPLC with UV detection prior to (---) and following (—) guanase treatment as described in Materials and Methods. G, guanine; X, xanthine; T, thymine; A, adenine.

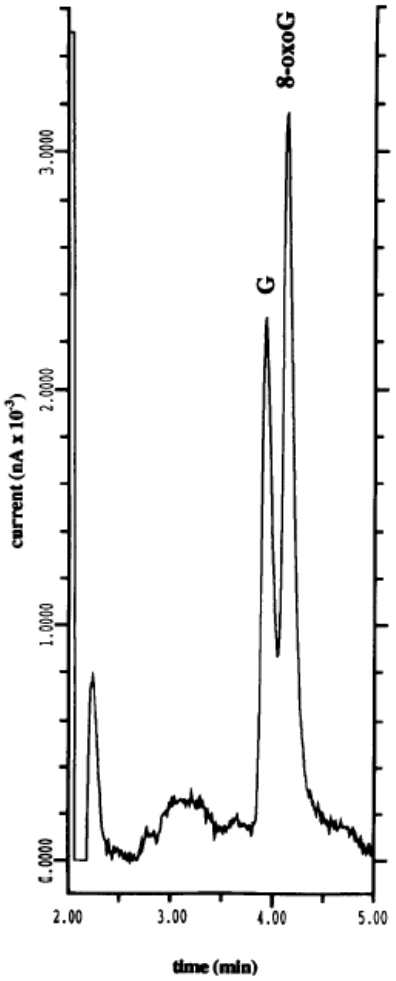
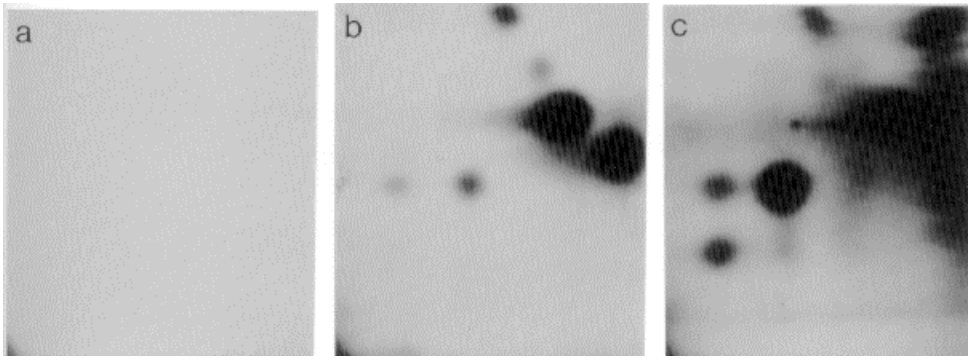


Fig. 1. Reversed-phase HPLC analysis, using electrochemical detection at +600 mV, of a solution containing 500 nM guanine (G) and 40 nM 8-oxoguanine (8-oxoG). Chromatographic conditions were as described in Materials and Methods except the mobile phase was 50 mM sodium acetate, 1 mM EDTA, pH5.1 containing 2% methanol.

^{32}P-postlabeling: analysis of base adducts



-Np-Np-Np-Np-Xp-Yp-Zp-
xenobiotic-modified DNA

↓

Enzymatic hydrolysis of DNA to 3'-mononucleotides using micrococcal endonuclease and spleen phophodiesterase

↓

Np + Np + Np + - - - + Xp + Yp + Zp + - -
Normal 3'-mononucleotides | Adducted 3'-mononucleotides

↓

Adduct enrichment by selective removal of normal 3'-mononucleotides
a) nuclease P1
b) n-butanol extraction

↓

Xp + Yp + Zp + - - - - -

↓

Radiolabel adducted 3'-mononucleotides with ^{32}P

↓

*pXp + *pYp + *pZp + - - - - -

↓

Multidimensional thin-layer chromatography of adducts on PEI-cellulose

↓

Autoradiography or storage phosphor imaging to determine the distribution of radioactivity on the chromatograms

↓

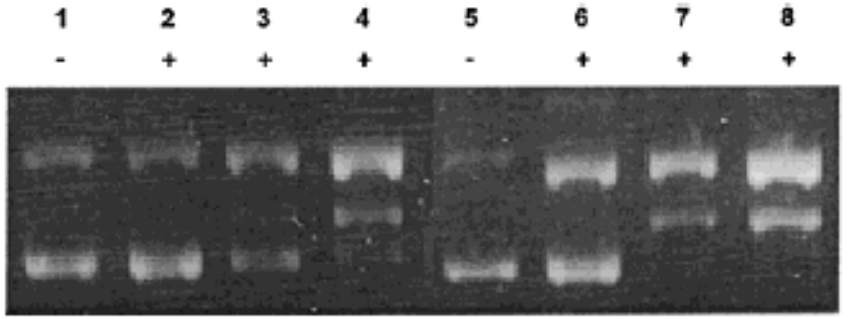
Quantitation of the radioactive areas on the chromatograms

1. Techniques involving complete DNA hydrolysis followed by determination of damaged entities by chromatography or mass spectrometry

2. Monitoring of changes in whole (unhydrolyzed) DNA molecules: electrophoretic and immunochemical techniques

detection of strand breaks:

relaxation (and/or linearization) of plasmid supercoiled DNA



scDNA (intact)

ocDNA (ssb)

linDNA (dsb) (damaged)

Figure 10. Relaxation of supercoiled pBR322 plasmid DNA by Fe(II)•deglycoBLM **11**. Lane 1, DNA + 10 μM deglycoBLM; lane 2, 1 μM deglycoBLM; lane 3, 5 μM deglycoBLM; lane 4, 10 μM deglycoBLM; lane 5, 10 μM deglycoBLM **11**; lane 6, 1 μM deglycoBLM **11**; lane 7, 5 μM deglycoBLM **11**; lane 8, 10 μM deglycoBLM **11**. Lanes 2–4 and 6–8 also contained 10 μM Fe^{2+}; essentially no cleavage was observed in the presence of Fe^{2+} alone.

„comet assay“ (dsb)

Fig.1 Unexposed control. Bundle of DNA (No-Tail)

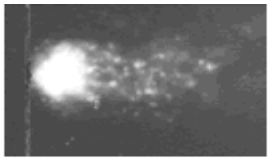


Fig.2 X-ray calibration 25.6 rads. DNA breaks are very obvious

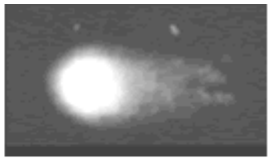
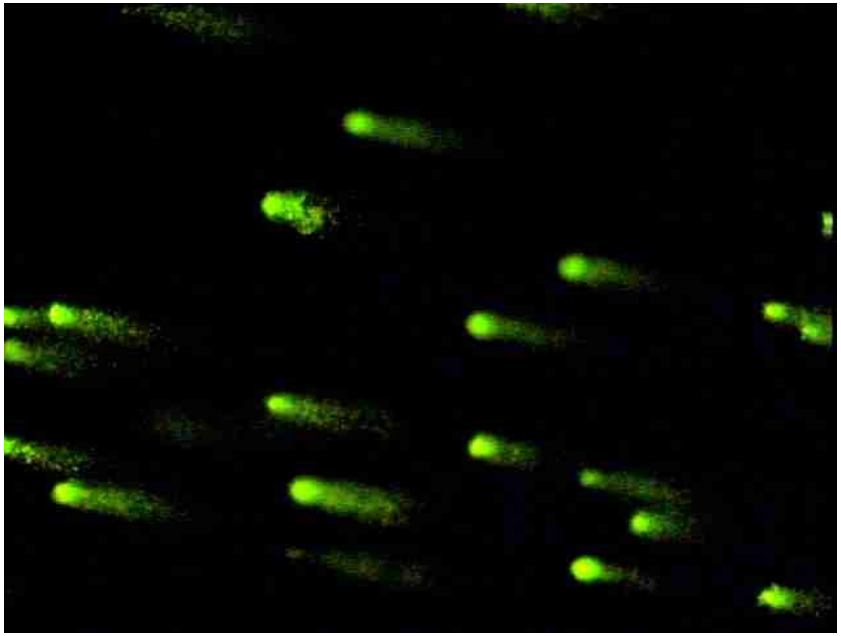


Fig.3 Cell Phone level microwave exposure 2hrs 2.45GHz reaching so called Safe SAR levels

Comet Tail = DNA Damage

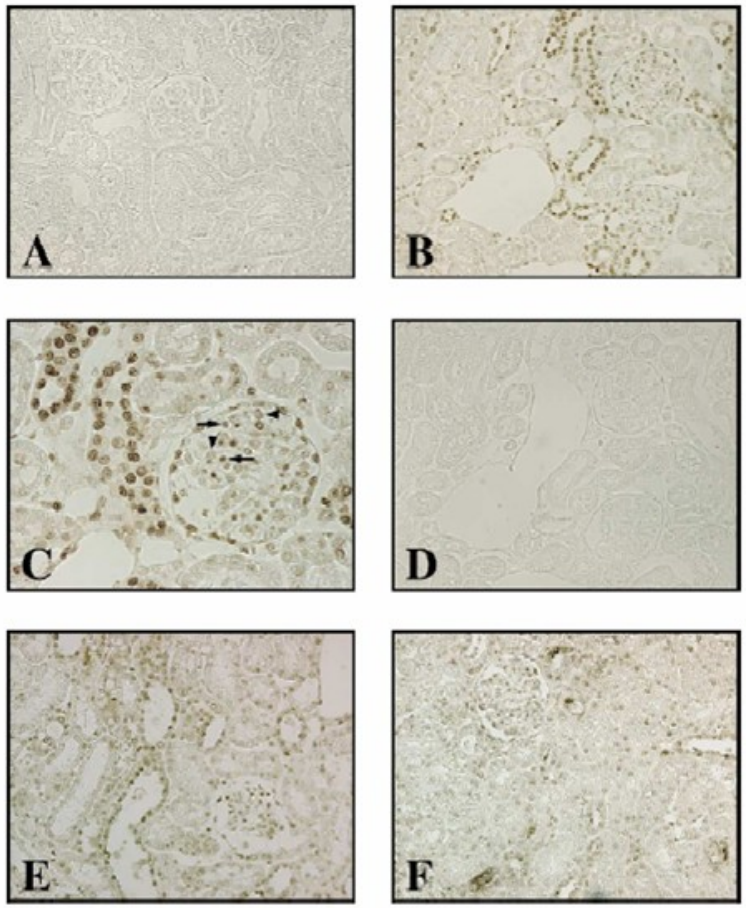
„alkaline elution assay“ (ssb + alkali-labile sites)



imunochemical techniques

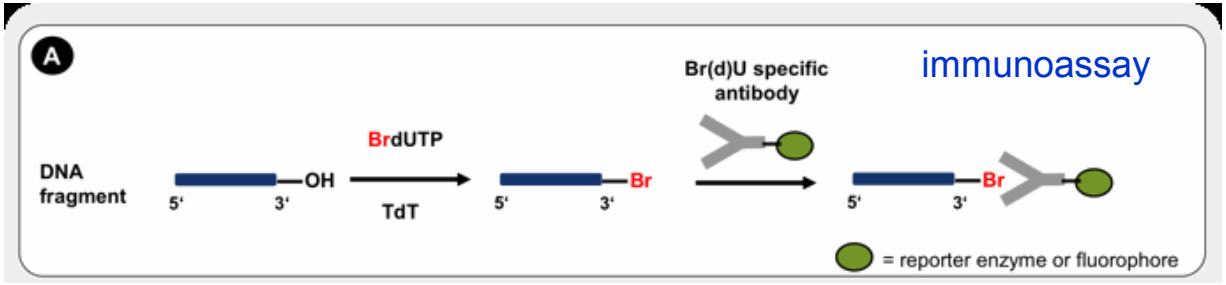
when antibodies against the adducts available

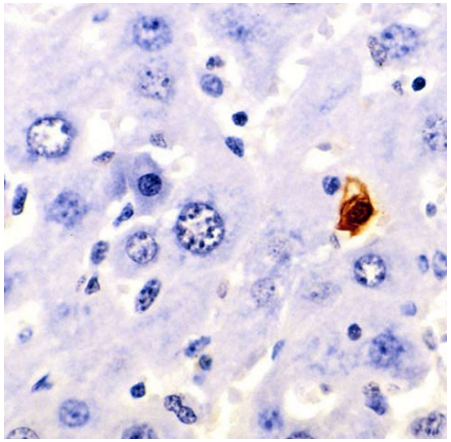
- ELISA
- *In situ* techniques



8-oxo guanine detection *in situ* in kidney tissue

TUNEL assay

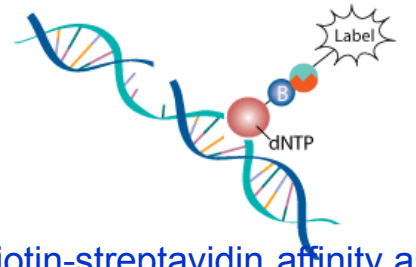




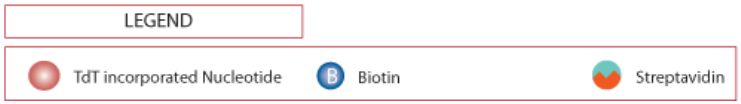
determination of 3‘-OH ends

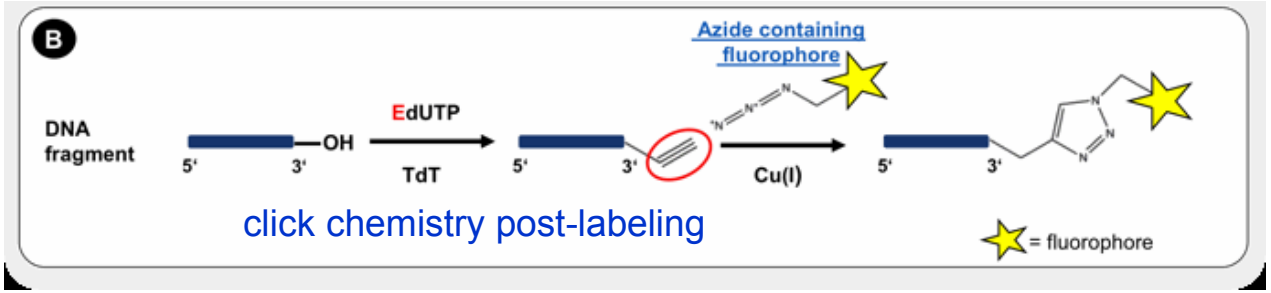
end labeling with terminal deoxynucleotidyl transferase

apoptosis indication



biotin-streptavidin affinity assay





click chemistry post-labeling