National Institute of Standards and Technology
Chemical Science and Technology Laboratory

Radiation Chemistry of DNA

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Effects of Ionizing Radiation

1. Direct effect

Ionization, excitation

2. Indirect effect

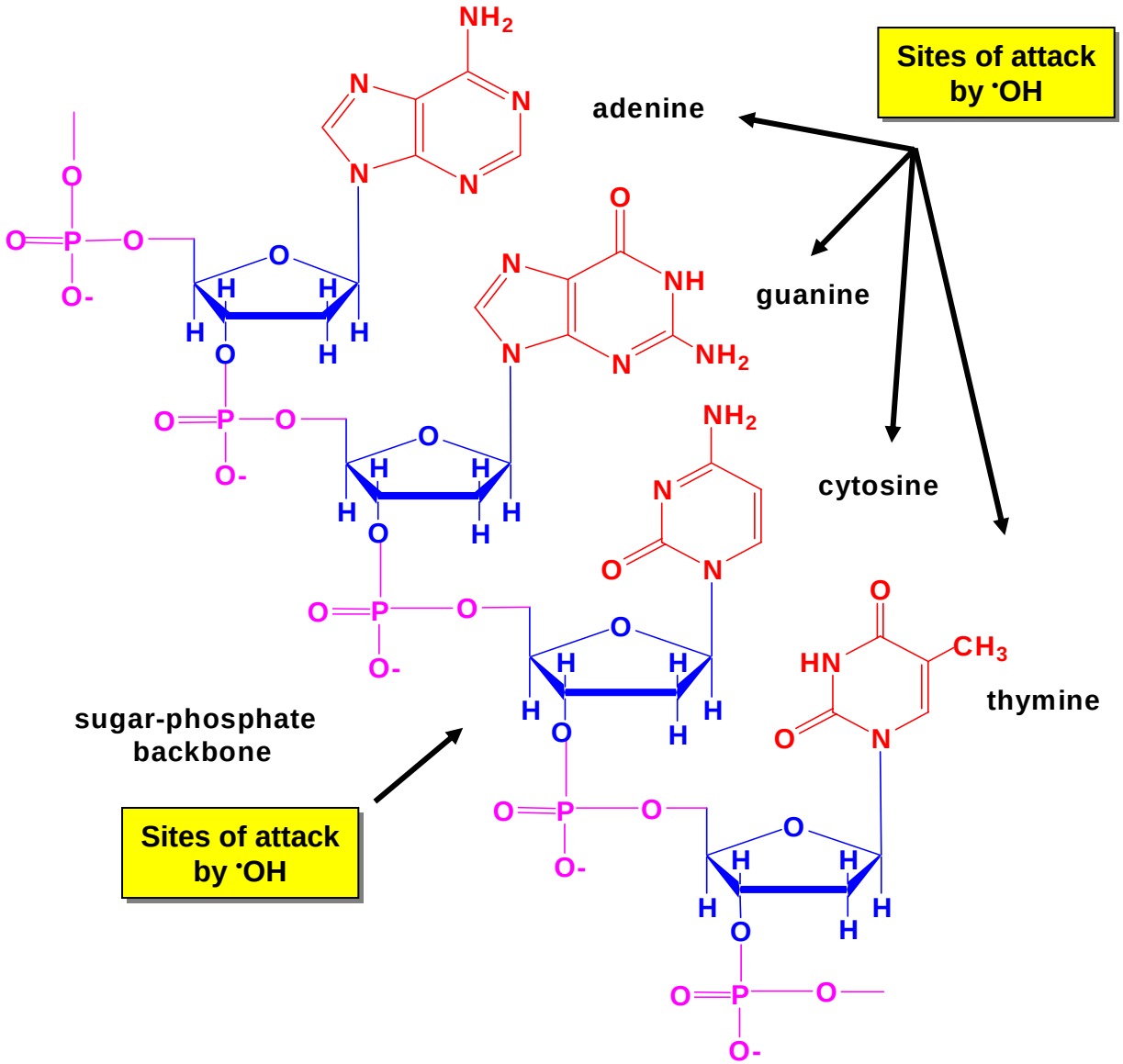
Reactions of the species formed from the solvent with the solute

Radiation chemistry of water

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^{\bullet}OH,\ e_{aq}^{-},\ H^{\bullet},\ O_2^{\bullet -},\ H_2O_2,\ H_2$$

Sites of radiation damage to DNA

DNA Damage



Types of DNA Damage by Free Radicals

- DNA base damage
- DNA sugar damage
- 8,5'-cyclopurine-2'-deoxynucleosides
- DNA-protein cross-links
- Single- and double-strand breaks
- Abasic sites

Rate constants (M^{-1} s^{-1}) of water radicals with DNA constituents

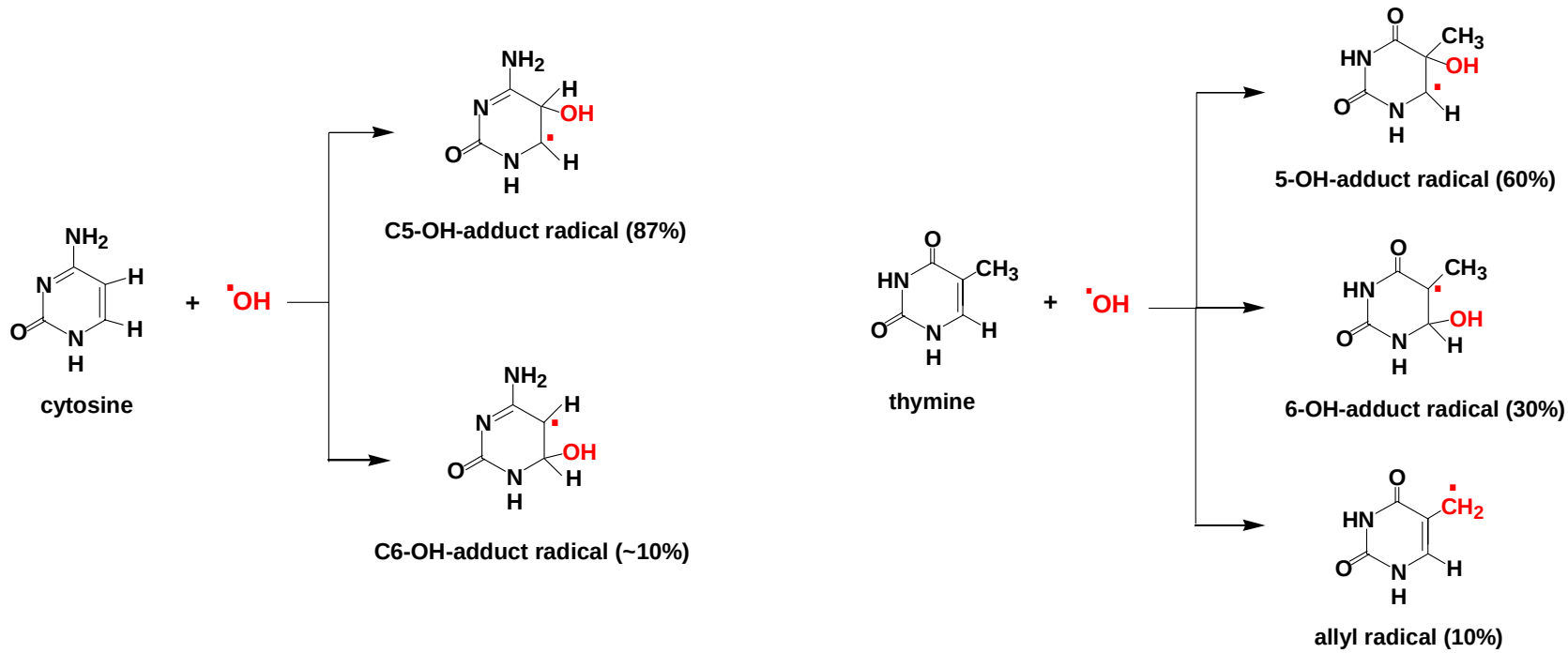
	$^{\bullet}OH$	e_{aq}^{-}	$H^{\bullet}$
Adenine	5×10^9	9×10^9	1×10^8
Guanine	9×10^9	1.3×10^{10}	-
Cytosine	4.5×10^9	1.3×10^{10}	1×10^8
Thymine	5×10^9	1.7×10^{10}	5×10^8
2-Deoxyribose	1.9×10^9	1×10^7	2.3×10^7
DNA	6×10^8	1.4×10^8	5×10^7

$A + B \rightarrow C + D$

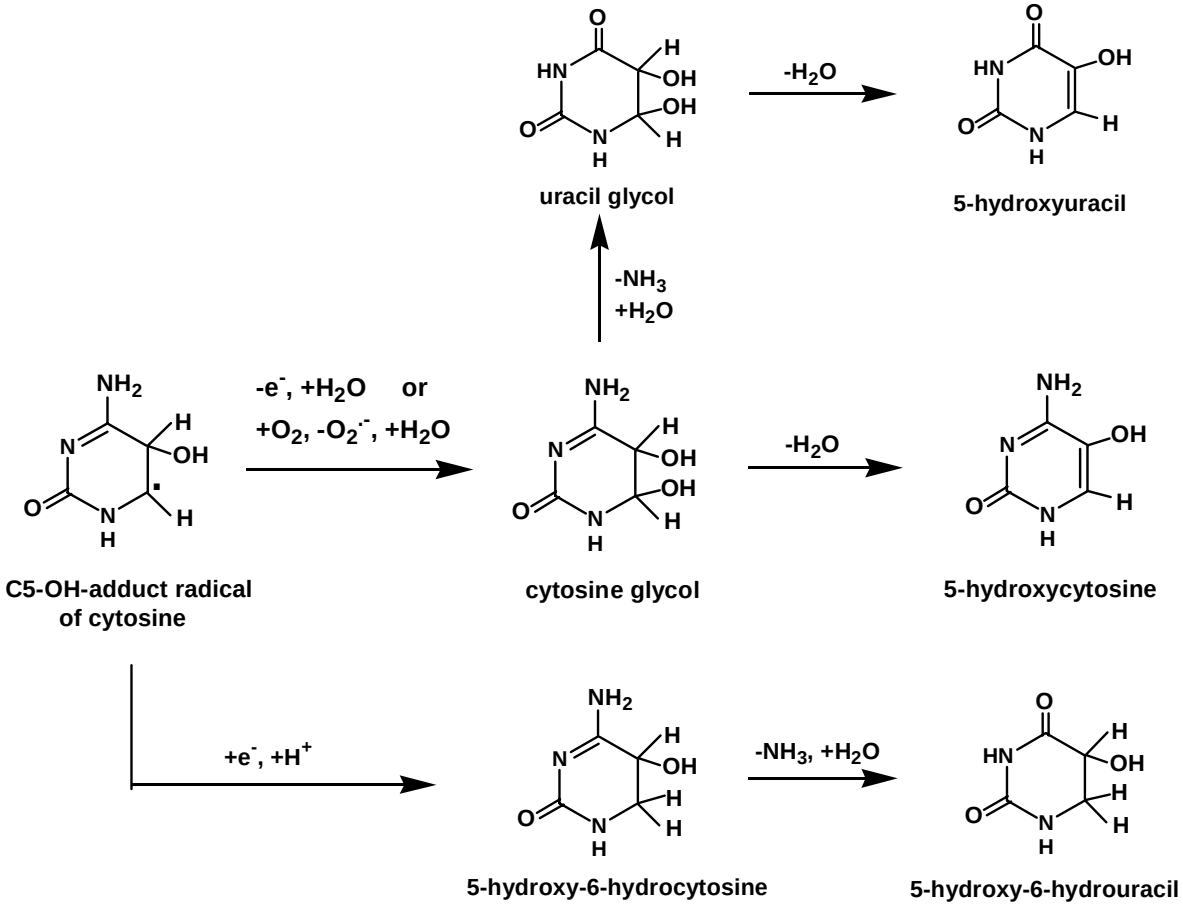
Rate = $-d[A]/dt = k[A][B]$ "second-order reaction"

k = rate constant (M^{-1} s^{-1})

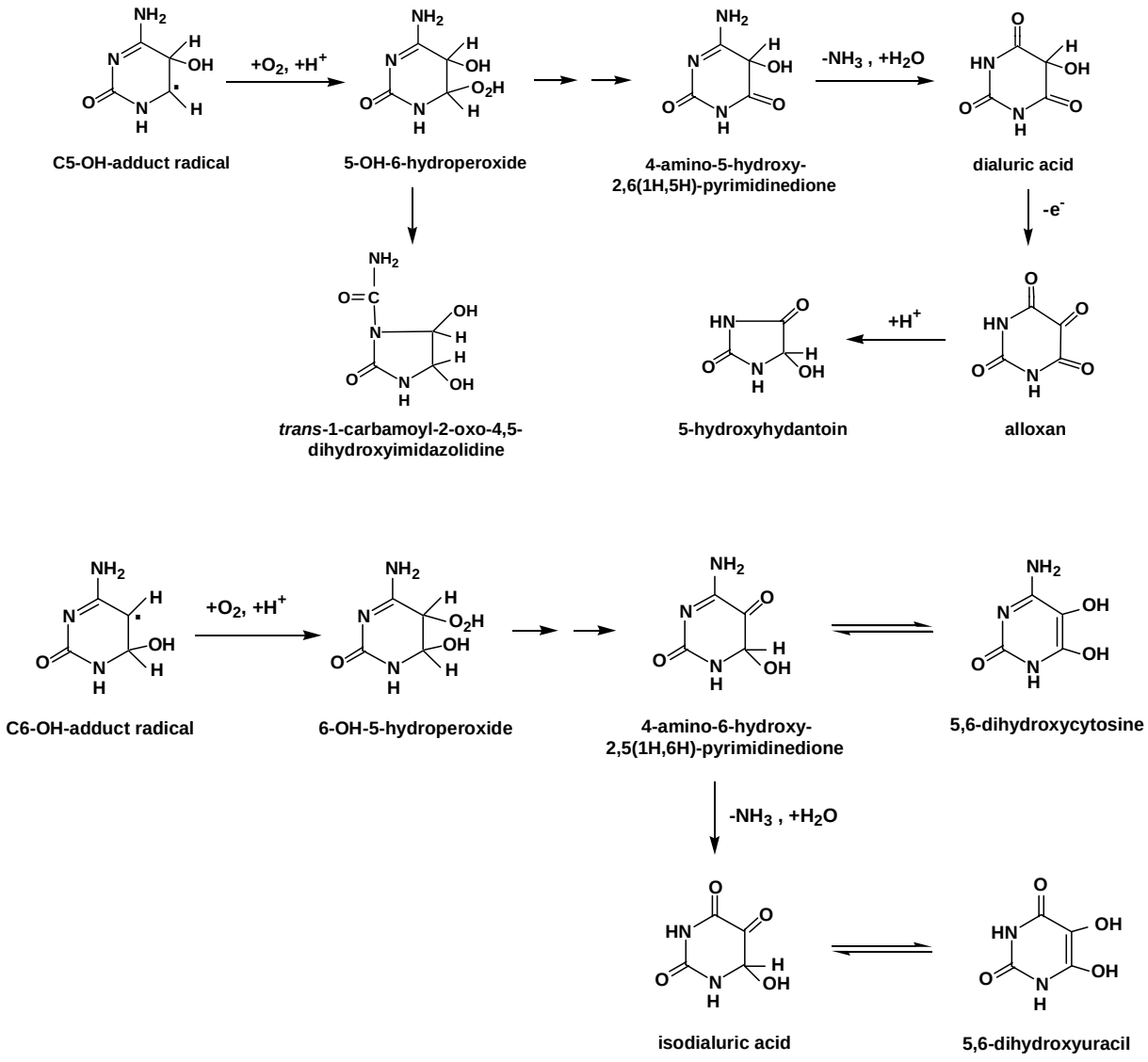
Reactions of hydroxyl radical with pyrimidines



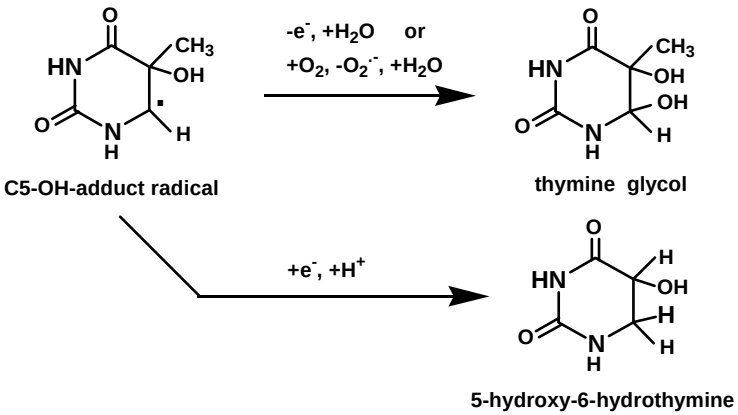
Formation of cytosine products

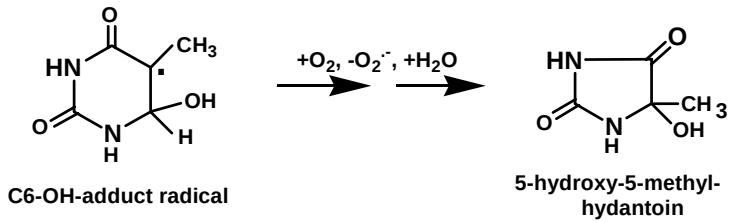


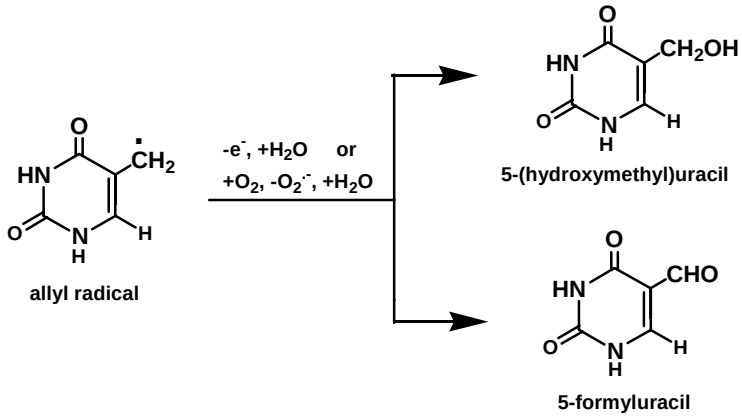
Formation of cytosine products



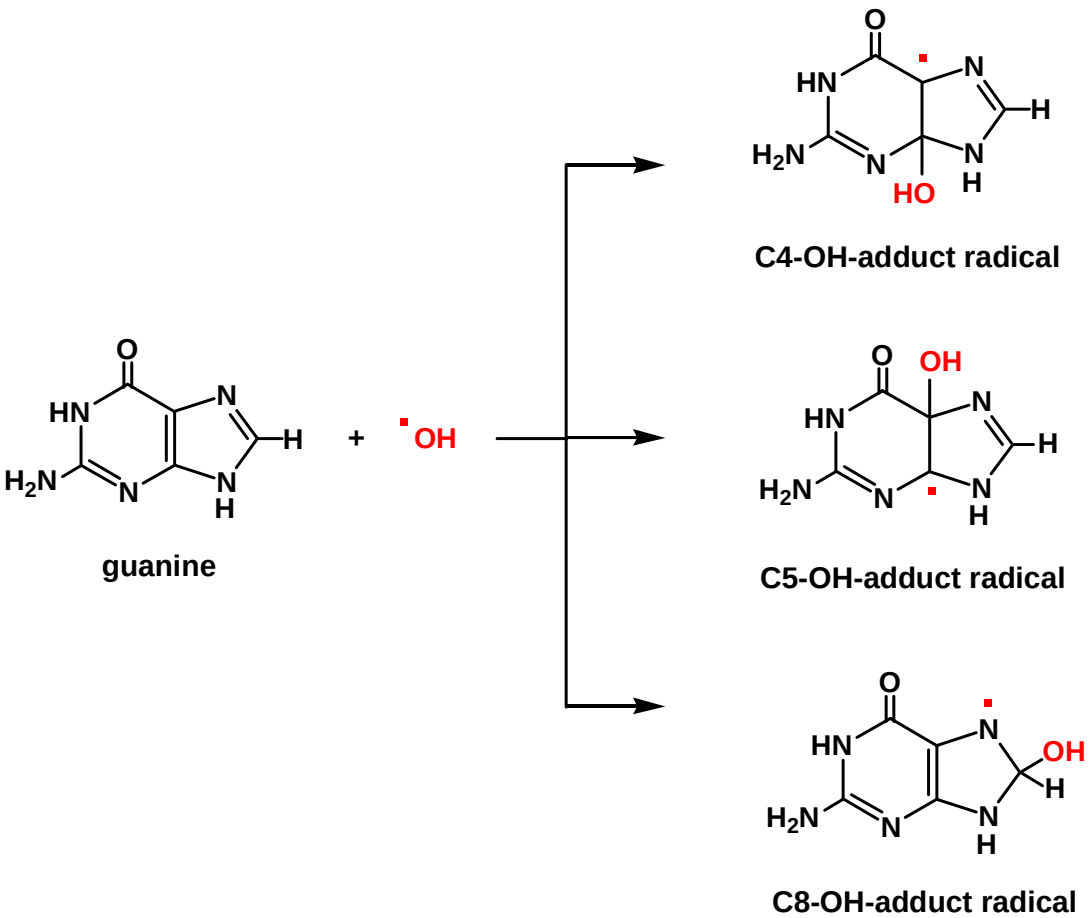
Formation of thymine products





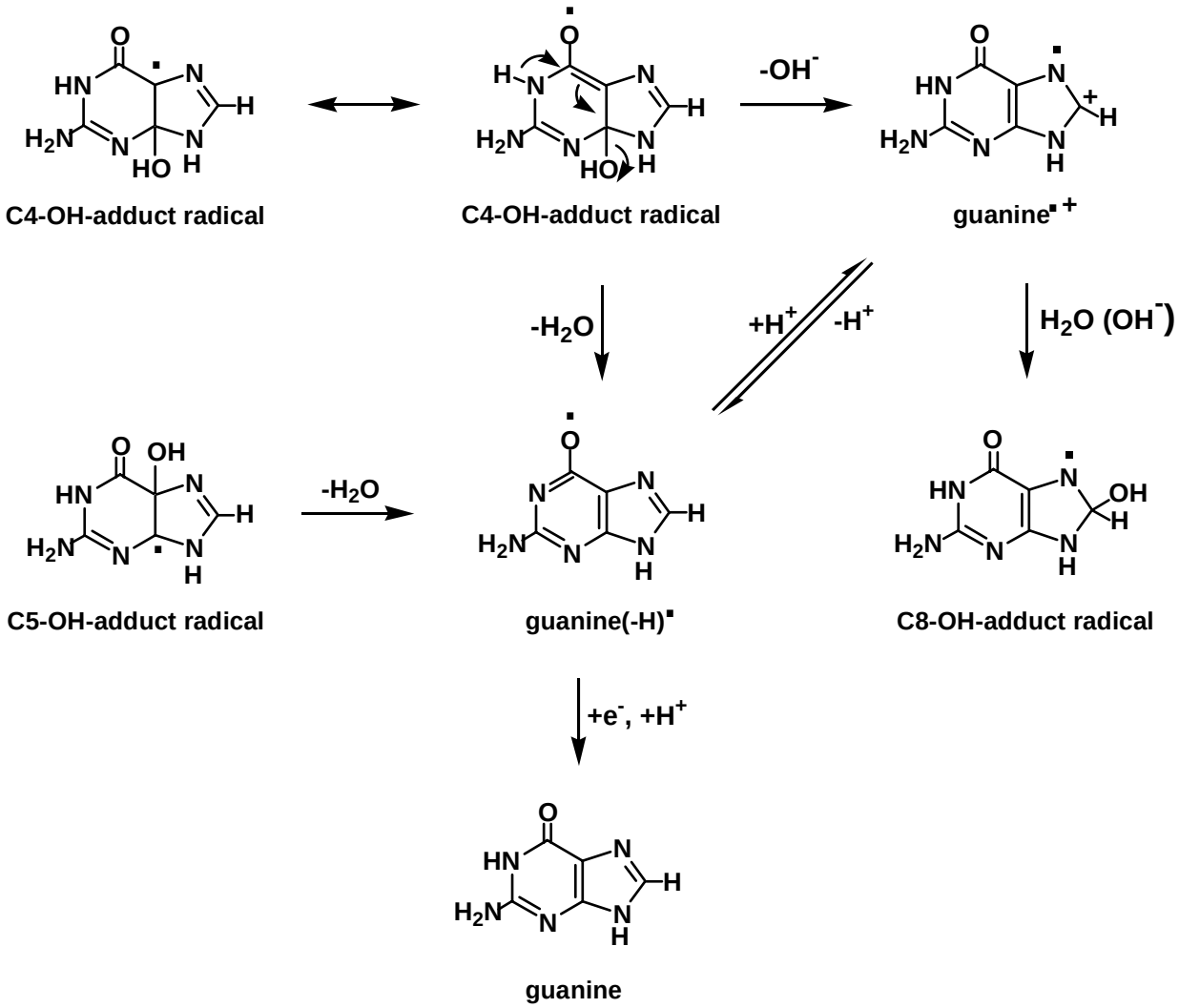


Reactions of hydroxyl radical with purines

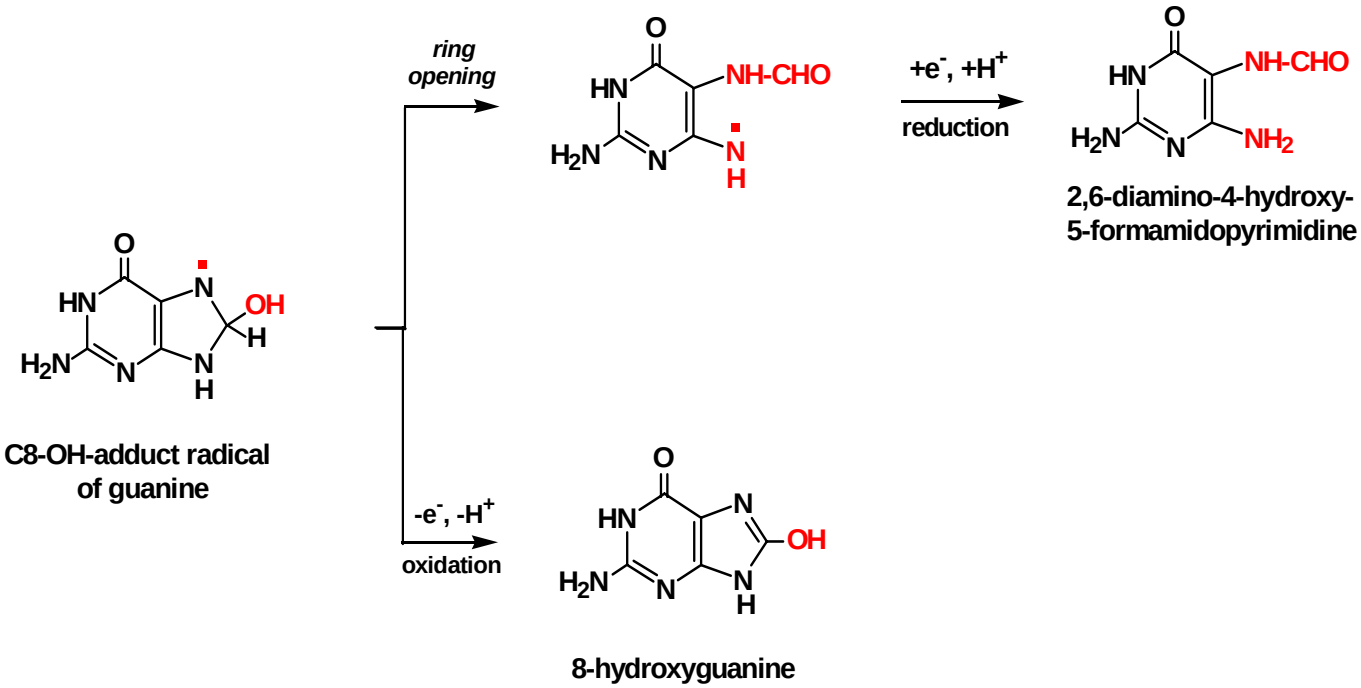


guanine

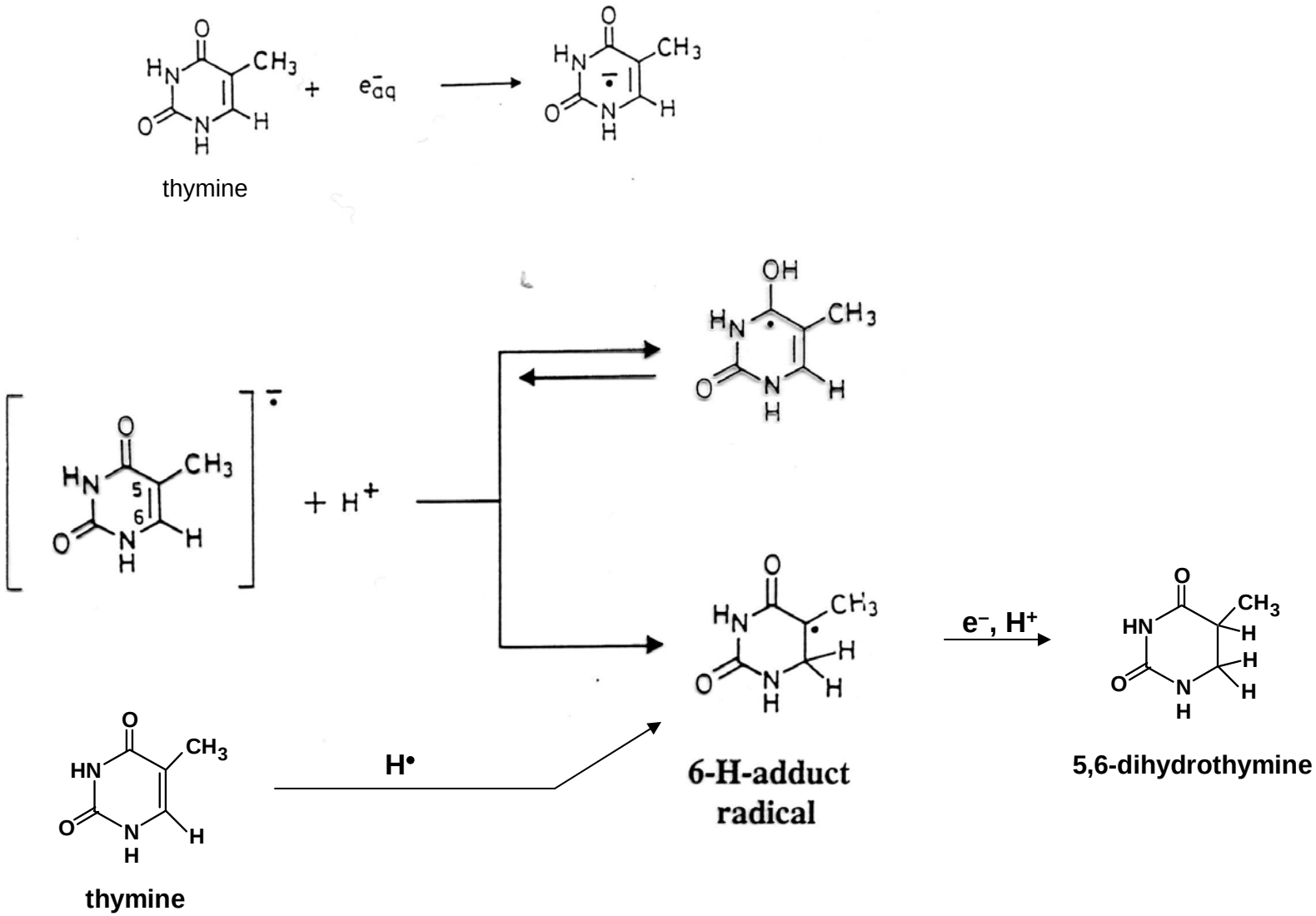
Formation of guanine products



Formation of guanine products



Reaction of e_{aq}^- with pyrimidines



Direct effect of ionizing radiation on DNA

$$\text{DNA} \xrightarrow{\text{ionizing radiation}} \text{DNA}^{+\bullet} + e^{-}$$

$$\text{DNA}^{+\bullet} + H_2O \longrightarrow \boxed{(\text{DNA(OH)})^{\bullet}} + H^{+}$$

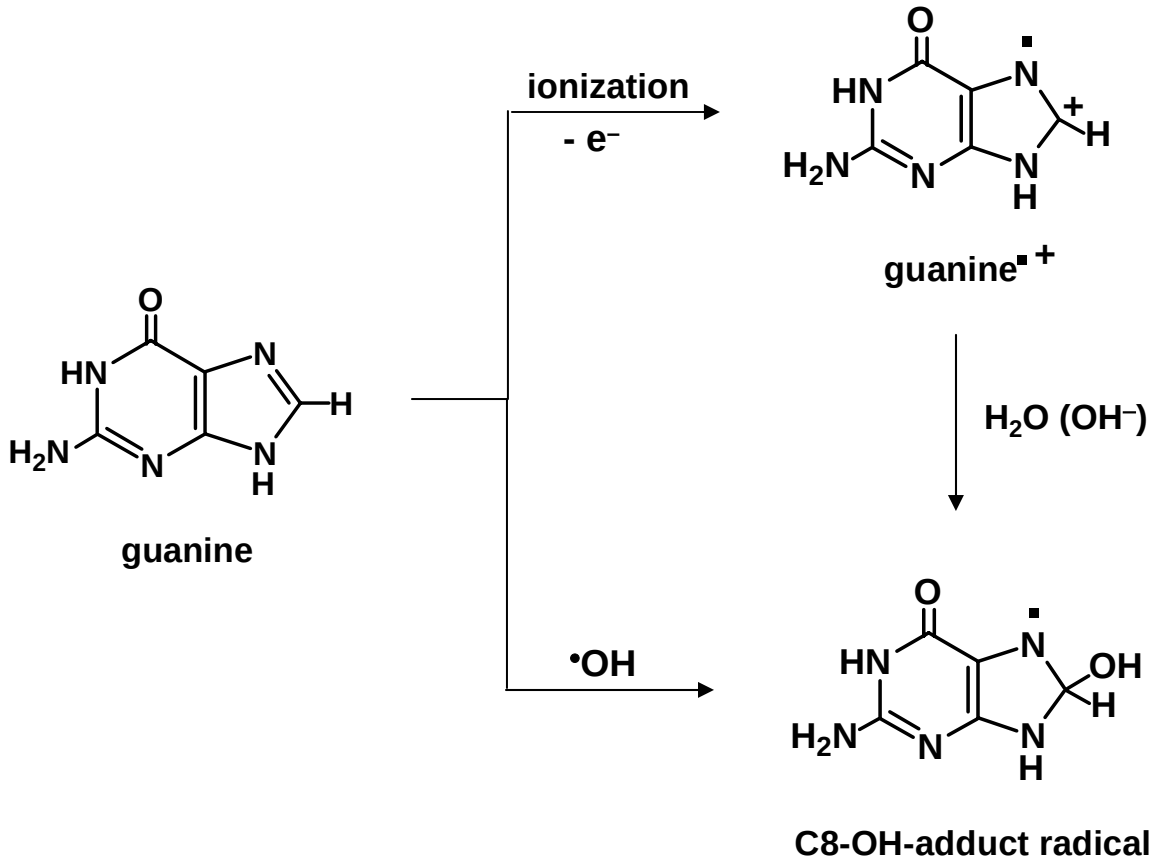
$$\text{DNA}^{+\bullet} \longrightarrow H^{+} + \boxed{(\text{DNA(-H)})^{\bullet}}$$

Indirect effect of ionizing radiation on DNA

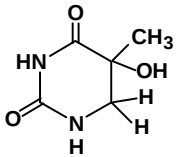
$$\text{DNA} + {}^{\bullet}\text{OH} \longrightarrow \boxed{(\text{DNA(OH)})^{\bullet}}$$

$$\text{DNA} + {}^{\bullet}\text{OH} \longrightarrow \boxed{(\text{DNA(-H)})^{\bullet}} + H_2O$$

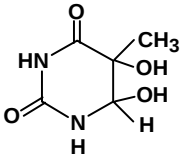
Direct effect of ionizing radiation on DNA



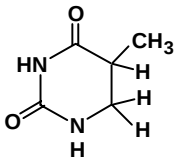
Products of the free radical-induced damage to DNA bases



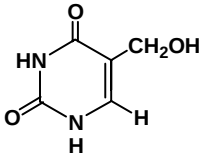
5-hydroxy-6-hydro-thymine



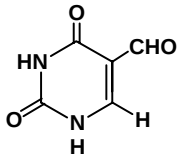
thymine glycol



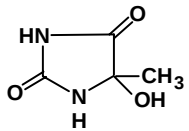
5,6-dihydrothymine



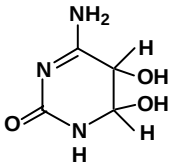
5-hydroxymethyl-uracil



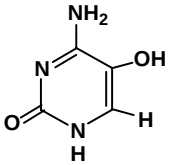
5-formyluracil



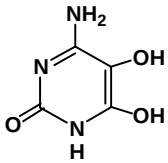
5-hydroxy-5-methyl-hydantoin



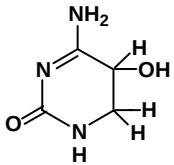
cytosine glycol



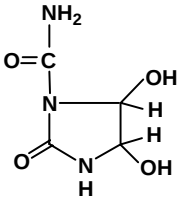
5-hydroxycytosine



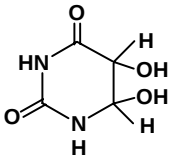
5,6-dihydroxycytosine



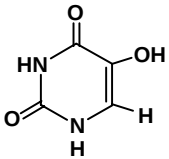
5-hydroxy-6-hydro-cytosine



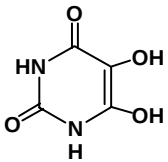
trans-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine



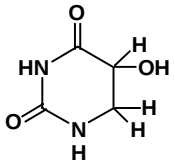
uracil glycol



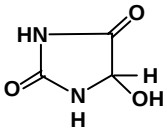
5-hydroxyuracil



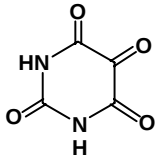
5,6-dihydroxyuracil



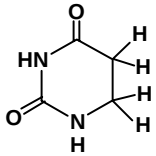
5-hydroxy-6-hydro-uracil



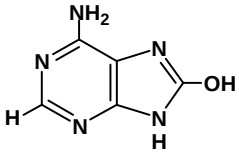
5-hydroxyhydantoin



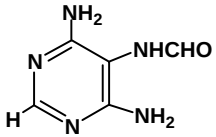
alloxan



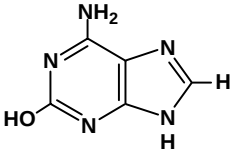
5,6-dihydrouracil



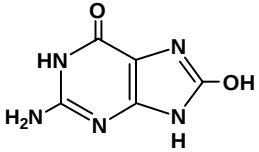
8-hydroxyadenine



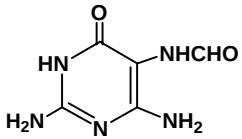
4,6-diamino-5-form-amidopyrimidine



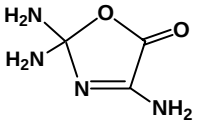
2-hydroxyadenine



8-hydroxyguanine

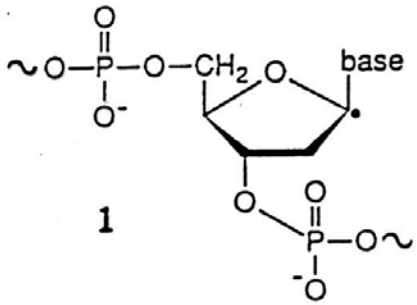


2,6-diamino-4-hydroxy-5-formamidopyrimidine



oxazolone

Radical formed be reactions of hydroxyl radical with the sugar moiety of DNA



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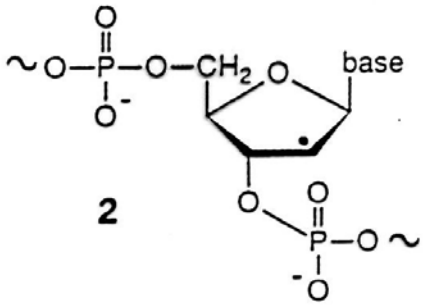

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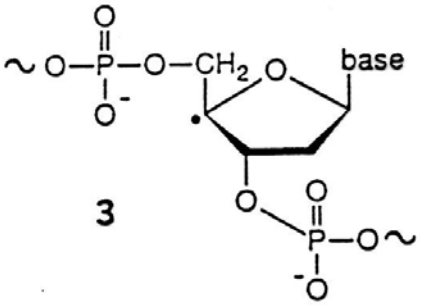

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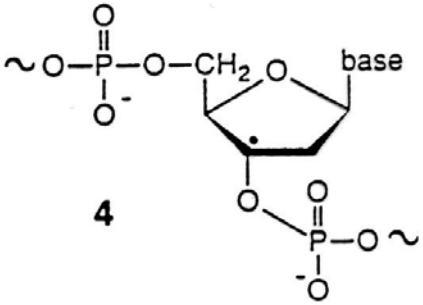

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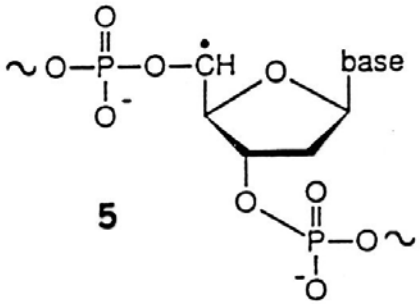
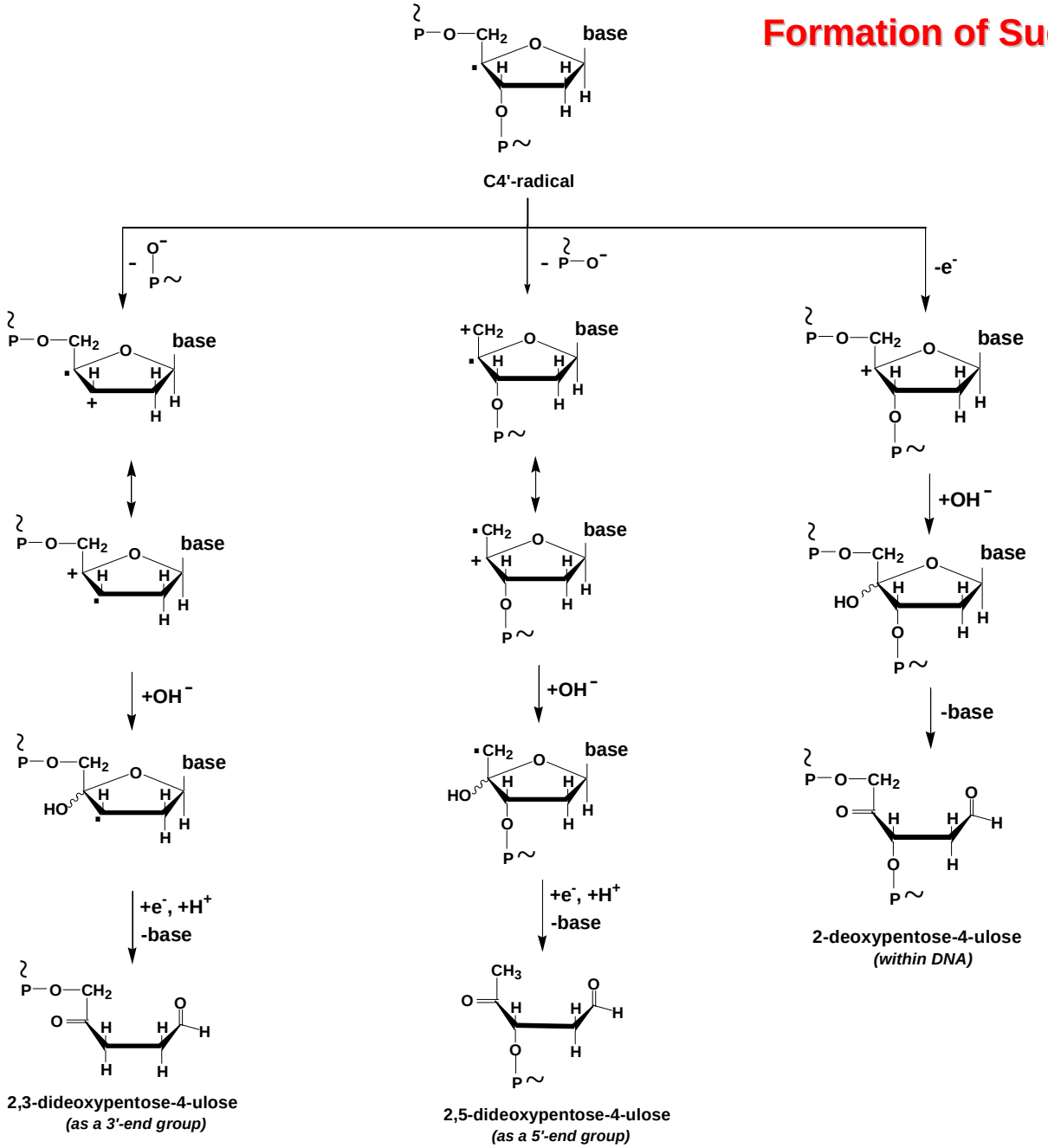
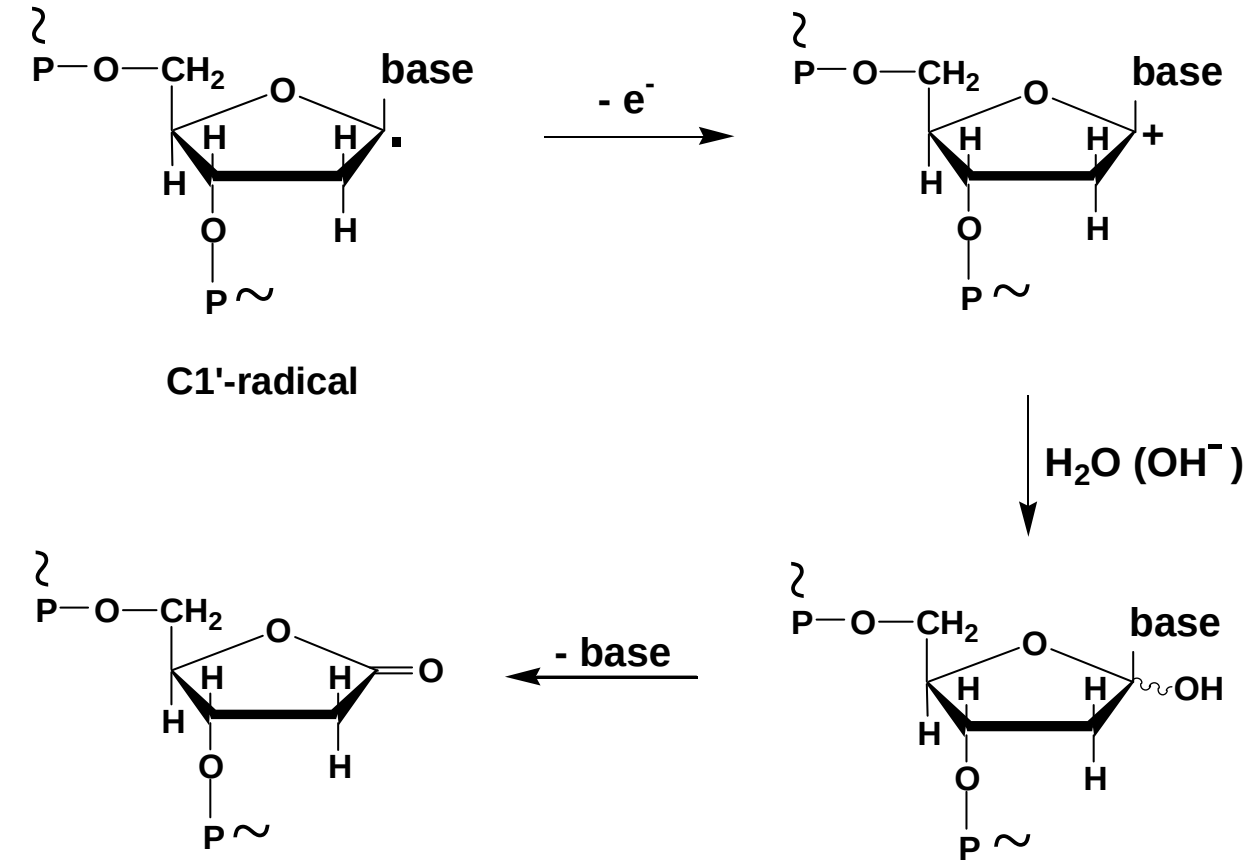


5

Formation of Sugar Products



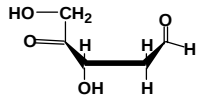
Formation of Sugar Products



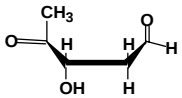
C1'-radical

2-deoxypentonic acid lactone
(within DNA)

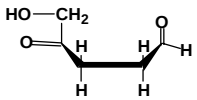
DNA Sugar Products



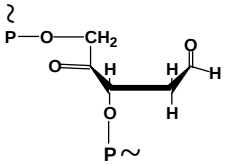
2-deoxypentose-4-ulose



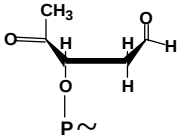
2,5-dideoxypentose-4-ulose



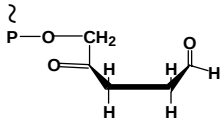
2,3-dideoxypentose-4-ulose



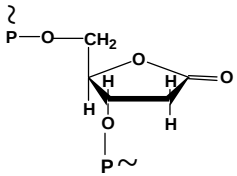
2-deoxypentose-4-ulose
(within DNA)



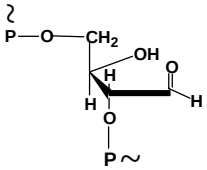
2,5-dideoxypentose-4-ulose
(as a 5'-end group)



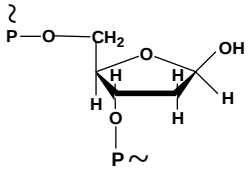
2,3-dideoxypentose-4-ulose
(as a 3'-end group)



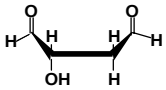
2-deoxypentonic acid lactone
(within DNA)



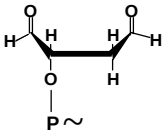
erythrose
(within DNA)



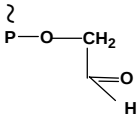
base-free site
(within DNA)



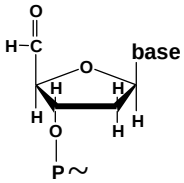
2-deoxytetrodialdose



2-deoxytetrodialdose
(as a 5'-end group)

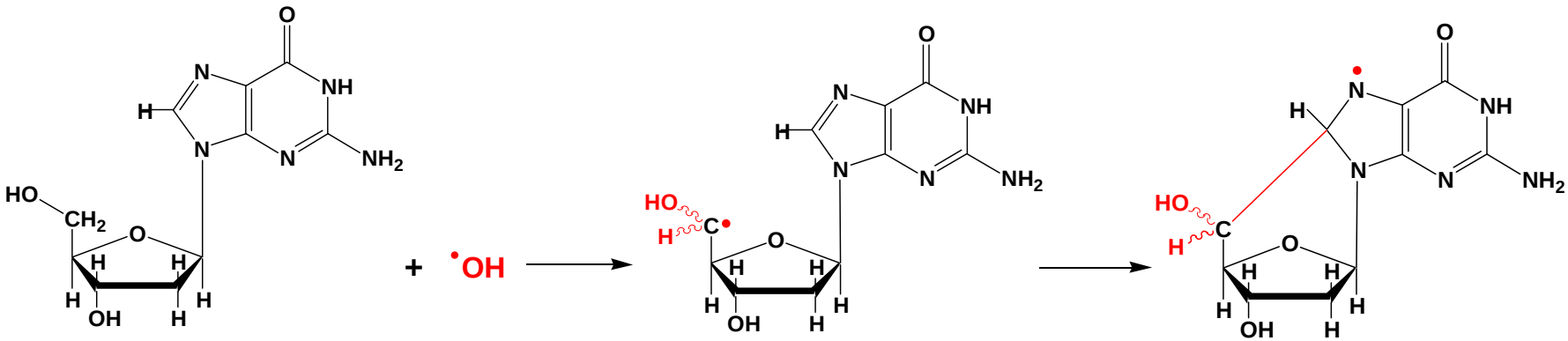


glycolic acid
(as a 3'-end group)

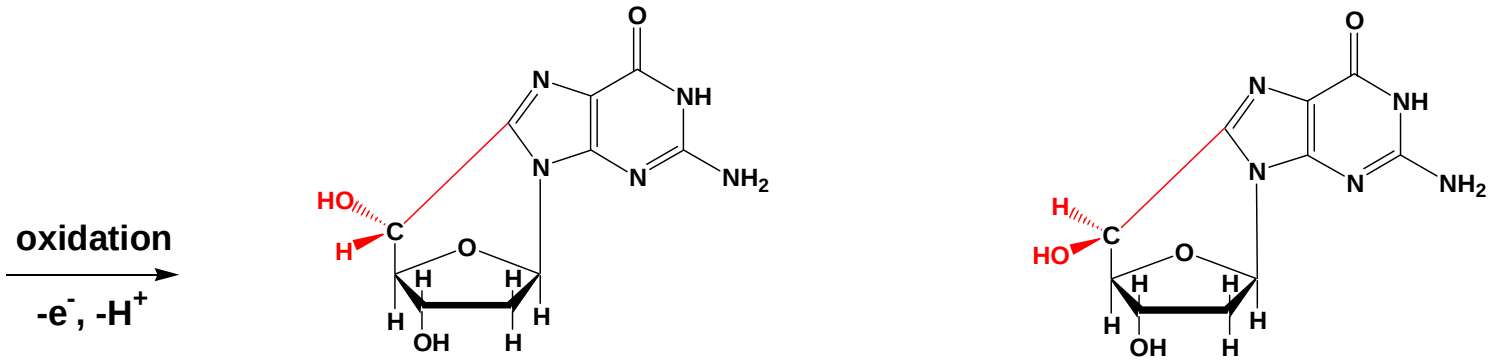


5'-aldehyde nucleoside
(as a 5'-end group)

Formation of 8,5'-cyclopurine- 2'-deoxynucleosides



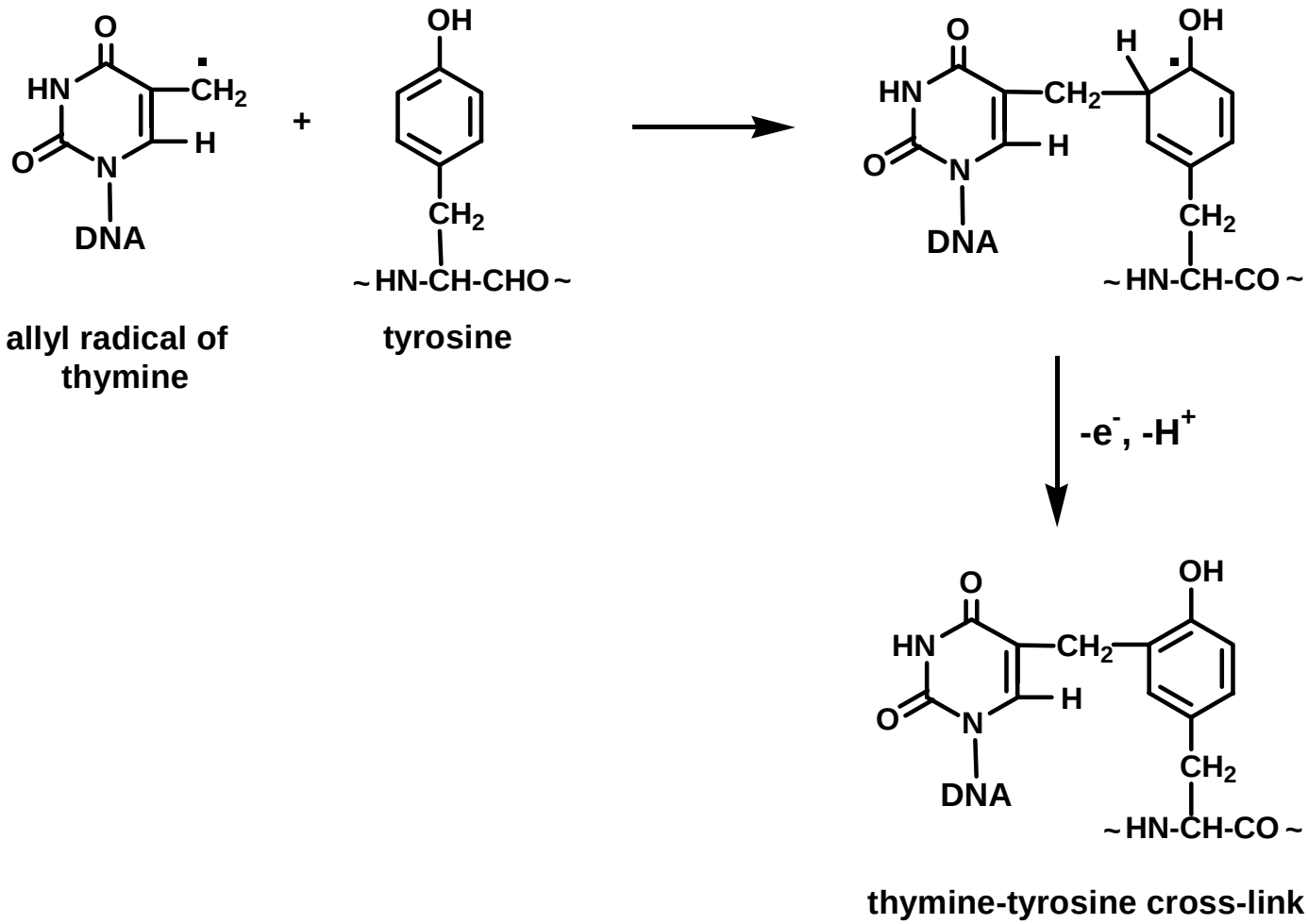
2'-deoxyguanosine



(5'R)-8,5'-cyclo-2'-deoxyguanosine

(5'S)-8,5'-cyclo-2'-deoxyguanosine

Formation of DNA-Protein Cross-Links



Biological effects of DNA lesions resulting from oxidative damage

Mutagenic lesions

DNA polymerase bypasses the lesion inserting a “wrong” intact base opposite the lesion.

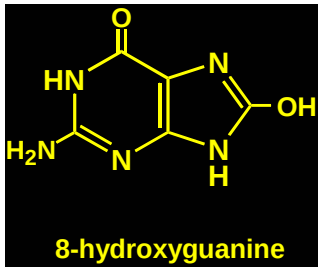
Mutagenic lesions influence replication fidelity, thereby causing mutations and contributing to disease processes.

Cytotoxic lesions

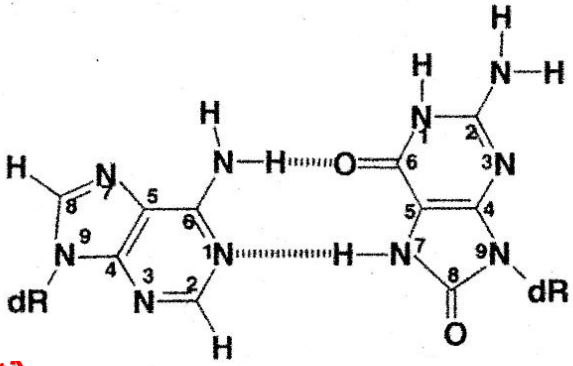
DNA polymerase is blocked by the lesion.

Cytotoxic lesions block DNA replication, gene transcription, or chromosome segregation, ultimately leading to cell death.

Base-pairing of 8-hydroxyguanine (8-OH-Gua)

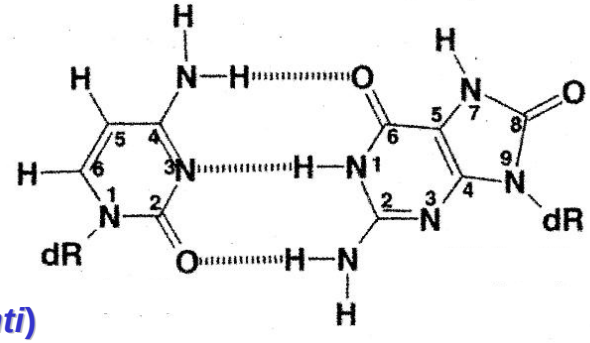


8-OH-Gua •Ade



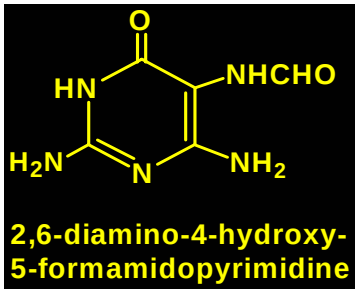
Ade (*anti*) 8-OH-Gua (*syn*)

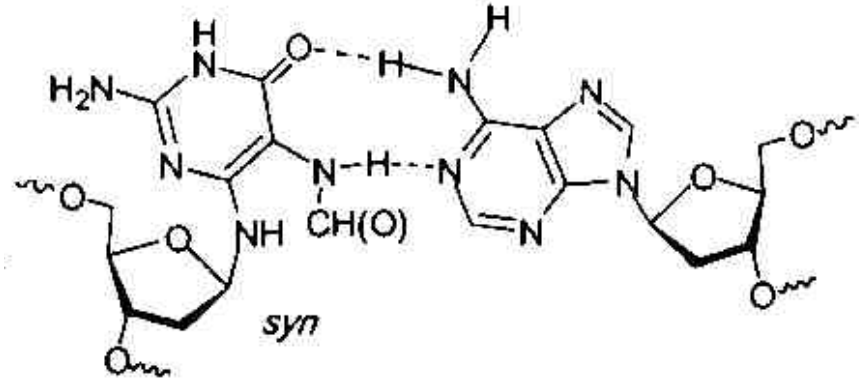
8-OH-Gua •Cyt

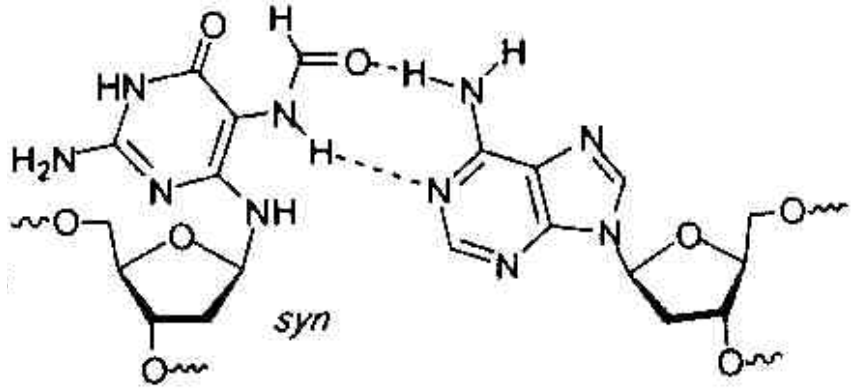


Cyt (*anti*) 8-OH-Gua (*anti*)

Base-pairing of 2,6-diamino-4-hydroxy-5-formamidopyridine (FapyGua)

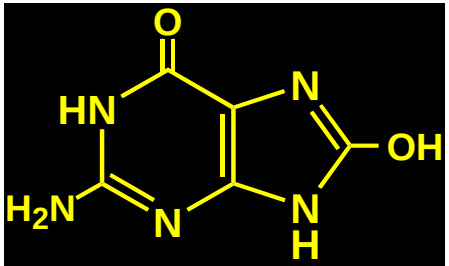




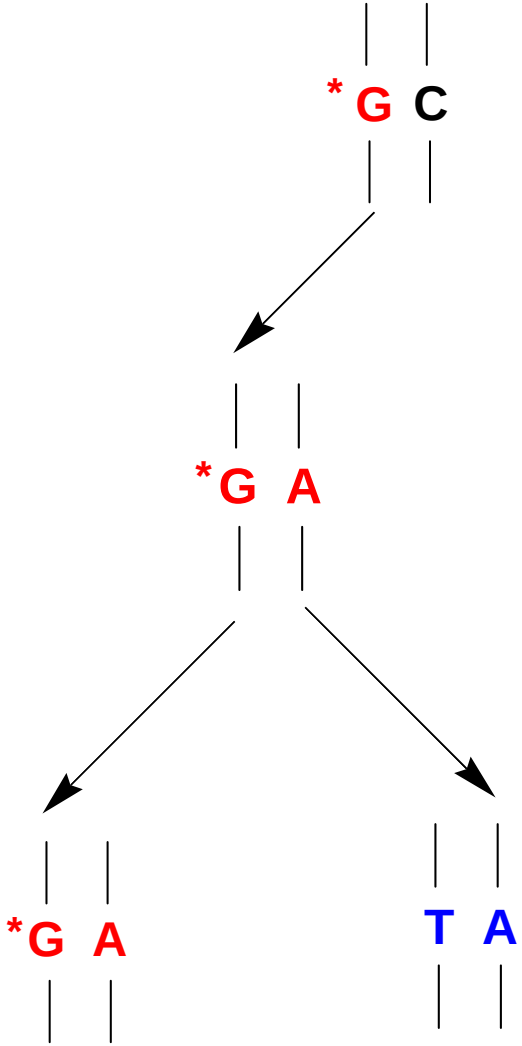


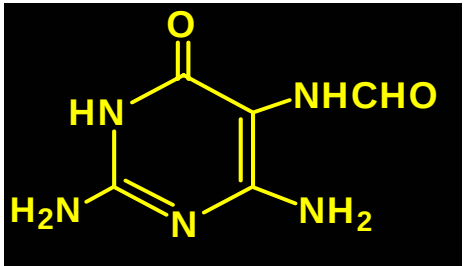
FapyGua•Ade

Mutation by 8-hydroxyguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine



8-hydroxyguanine



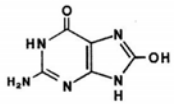
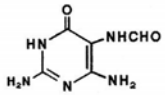
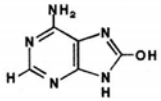
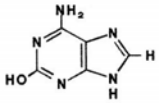
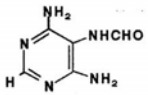
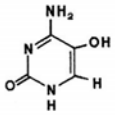
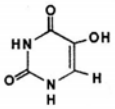
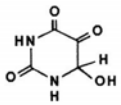
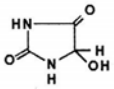
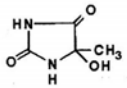


2,6-diamino-4-hydroxy-5-formamidopyrimidine

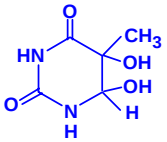
GC → TA

transversion

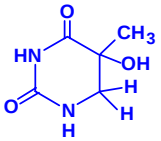
Mutations generated by products of free radical-induced damage to DNA

	Pairs with	mutation	
 8-hydroxyguanine	A	G → T	
 2,6-diamino-4-hydroxy-5-formamidopyrimidine	A	G → T	
 8-hydroxyadenine	C, G	A → G	A → C
 2-hydroxyadenine	A, G	A → T	A → C
 4,6-diamino-5-form-amidopyrimidine	A, G	A → T	A → C
 5-hydroxycytosine	A, C	C → T	C →G
 5-hydroxyuracil	A, C	C → T	C →G
 isodialuric acid	?		
 5-hydroxyhydantoin	?		
 5-hydroxy-5-methyl-hydantoin	?		

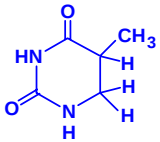
Cytotoxic lesions



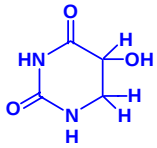
thymine glycol



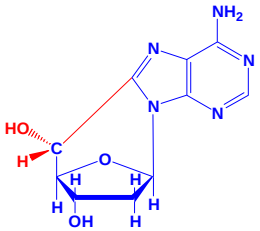
5-hydroxy-6-hydro-thymine



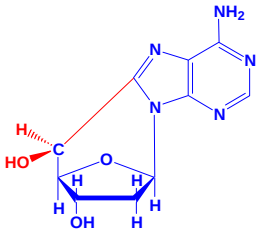
5,6-dihydrothymine



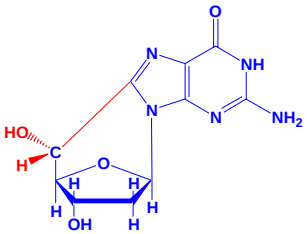
5-hydroxy-6-hydro-uracil



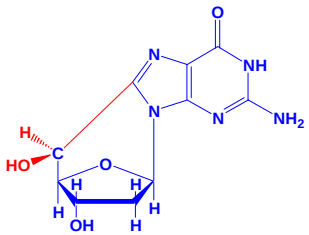
(5'R)-8,5'-cyclo-2'-deoxyadenosine



(5'S)-8,5'-cyclo-2'-deoxyadenosine



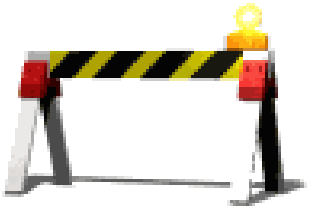
(5'R)-8,5'-cyclo-2'-deoxyguanosine

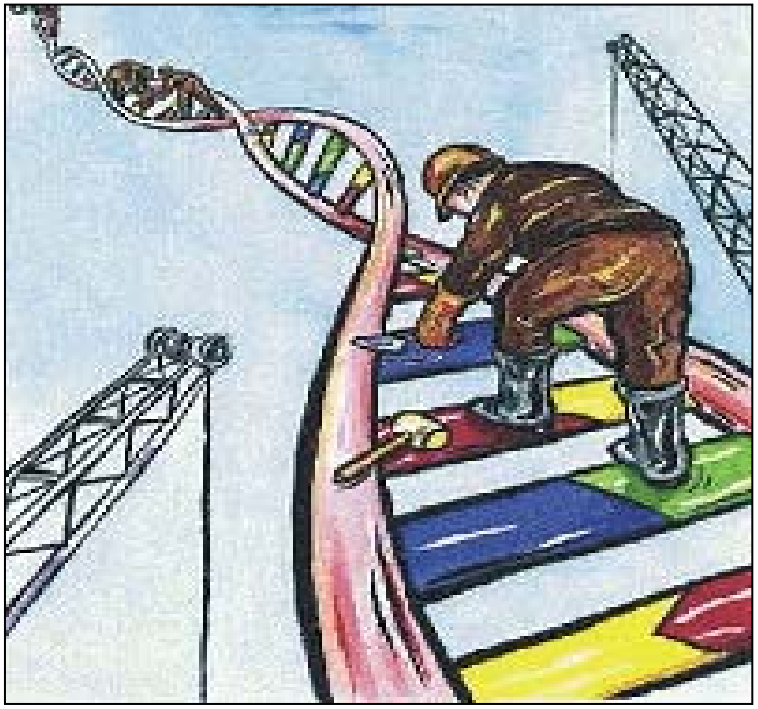


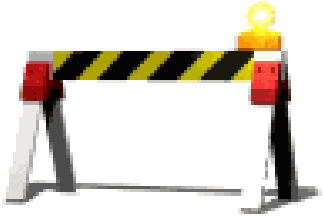
(5'S)-8,5'-cyclo-2'-deoxyguanosine

DNA Damage & Repair

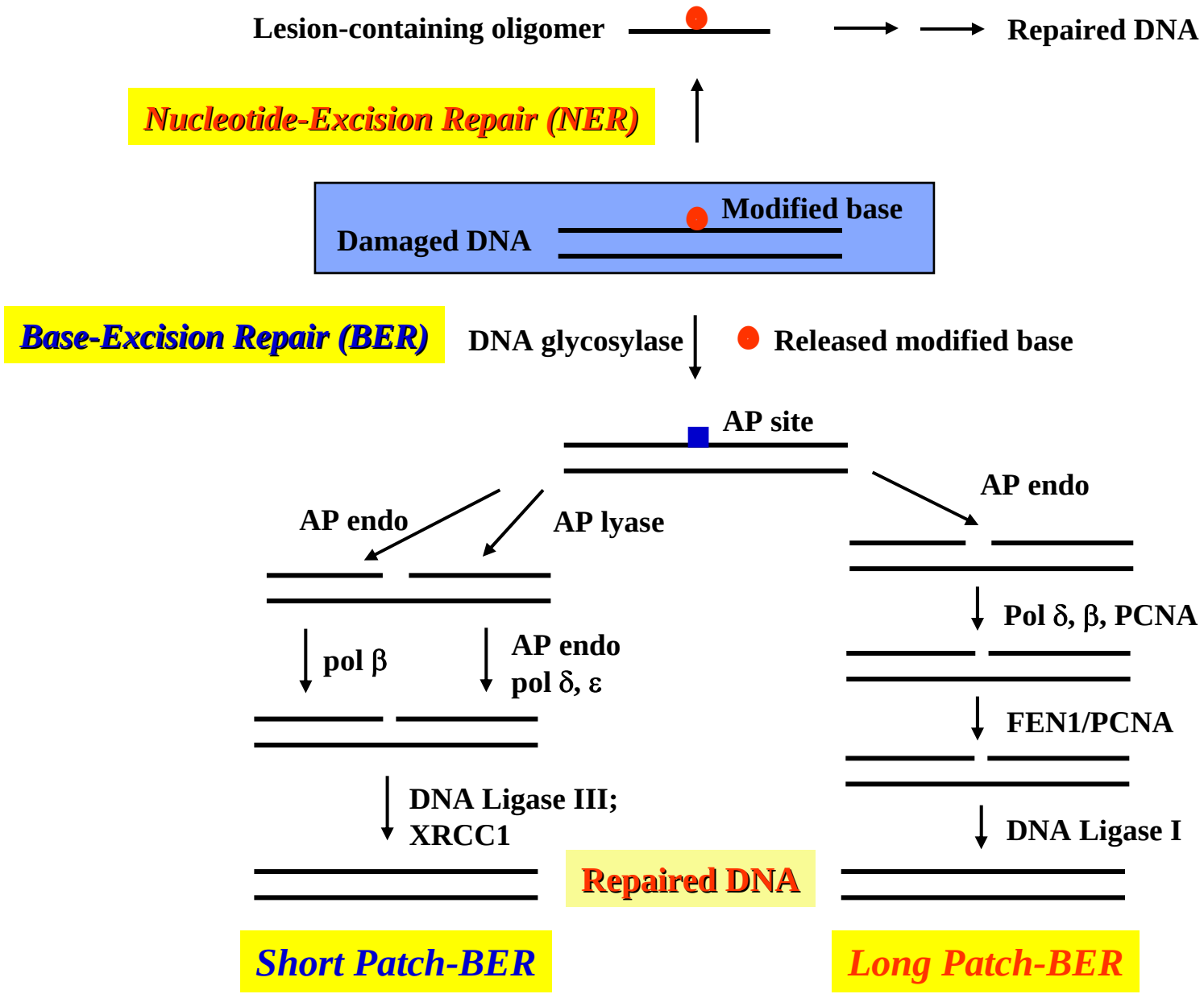
Understanding DNA Repair Mechanisms



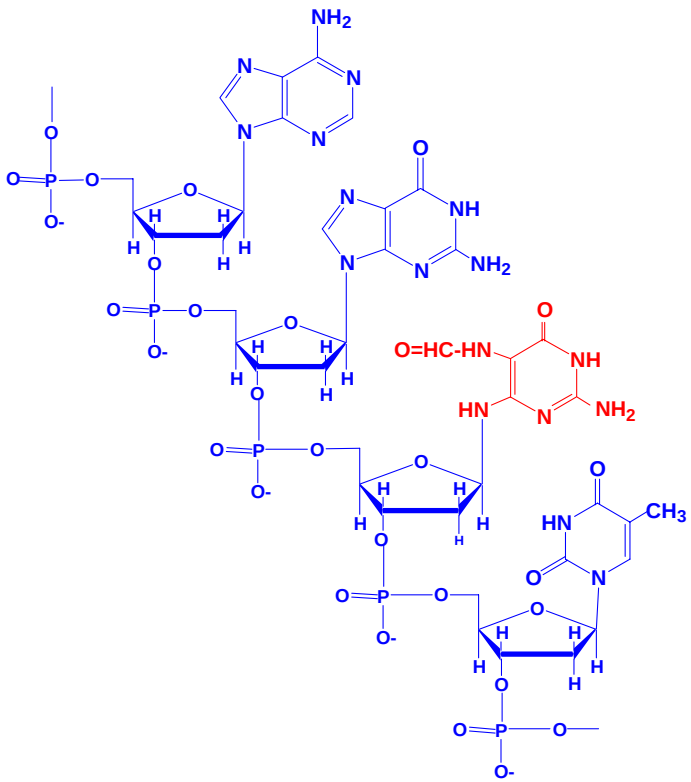




- **Interventions against mutagenic or cytotoxic DNA lesions aimed at preventing damage or increasing DNA repair capacity might protect from cancer and/or aging.**
- **Understanding DNA complex repair mechanisms would offer potential for drug development and pharmacological modulation of DNA repair efficiency.**
- **The ability to modulate DNA repair might lead to improvement and effectiveness of therapies.**

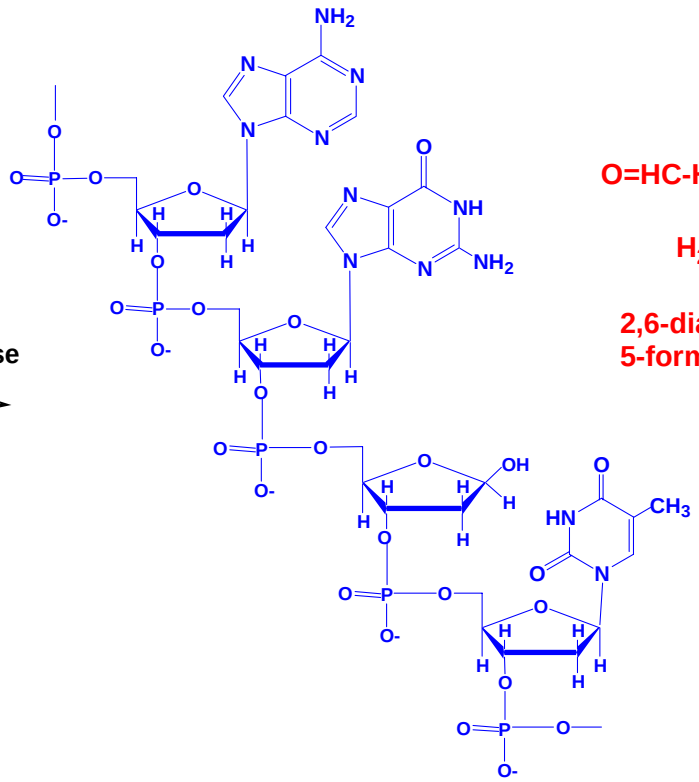

Lesion-containing oligomer
Repaired DNA
Nucleotide-Excision Repair (NER)
Modified base
Damaged DNA
Base-Excision Repair (BER)
DNA glycosylase
Released modified base
AP site
AP endo
AP lyase
AP endo
pol β
AP endo
pol δ, ε
DNA Ligase III;
XRCC1
Repaired DNA
Short Patch-BER
Pol δ, β, PCNA
FEN1/PCNA
DNA Ligase I
Long Patch-BER

Excision of a modified DNA base by a DNA glycosylase



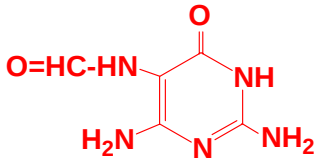
DNA glycosylase

$+H_2O$



DNA with an abasic site

free base

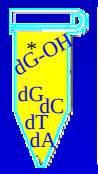


2,6-diamino-4-hydroxy-5-formamidopyrimidine

Gas Chromatography/Mass Spectrometry (GC/MS) with Isotope-dilution technique

Liquid Chromatography/Mass Spectrometry (LC/MS) with Isotope-dilution technique

DNA Analysis by GC/MS and LC/MS

	GC/MS		LC/MS
Step 1. Hydrolysis	**Acidic** (Formic Acid)	**Enzymic** (Fpg protein)	**Enzymic** (Nuclease P1, Phosphodiesterase I, Alkaline phosphatase)
	all free modified + all free intact bases	free modified bases (enzyme specific)	 all free modified + all free intact nucleosides
	↓	↓	↓
Step 2. Derivatization	**Pyridine & BSTFA**	**Pyridine & BSTFA**	✕
	↓	↓	↓
Step 3. Analysis	**GC/MS**	**GC/MS**	**LC/MS**

Analysis of DNA Base Damage by GC/MS

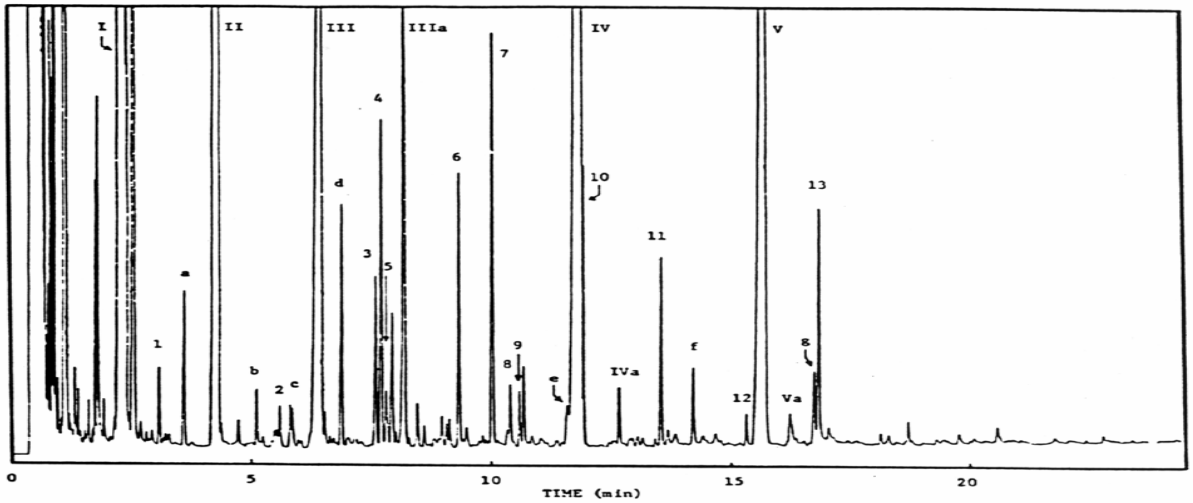


FIG. 1. GC separation of a trimethylsilylated HCOOH hydrolysate of irradiated calf thymus DNA. Dose: 330 Gy. Column: fused silica capillary (12 m, 0.2-mm i.d.) coated with crosslinked SE-54, programmed after 3 min at 100°C from 100 to 250°C with a rate of 7°C/min. For other details, see Materials and Methods. Peak identifications are given in Table 1.

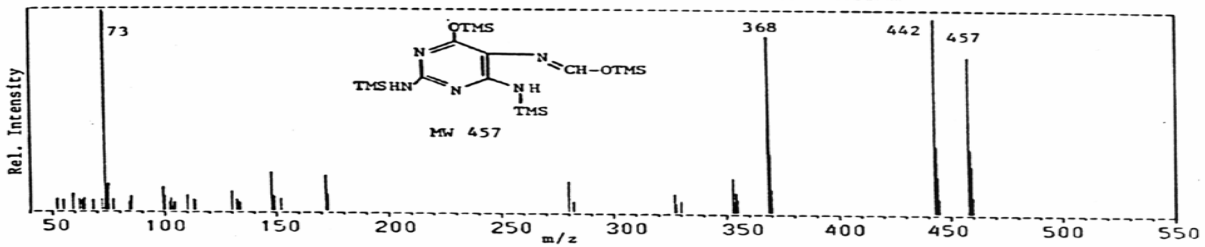


FIG. 7. Mass spectrum taken from peak 12 in Fig. 1.

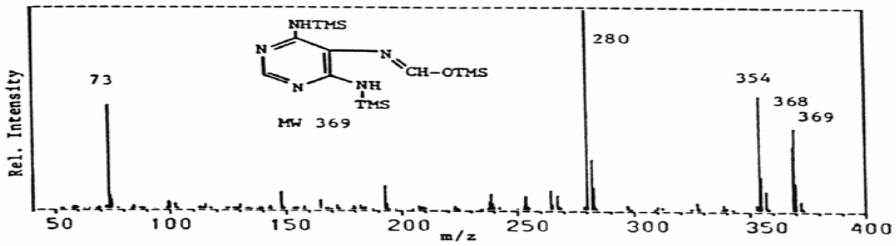
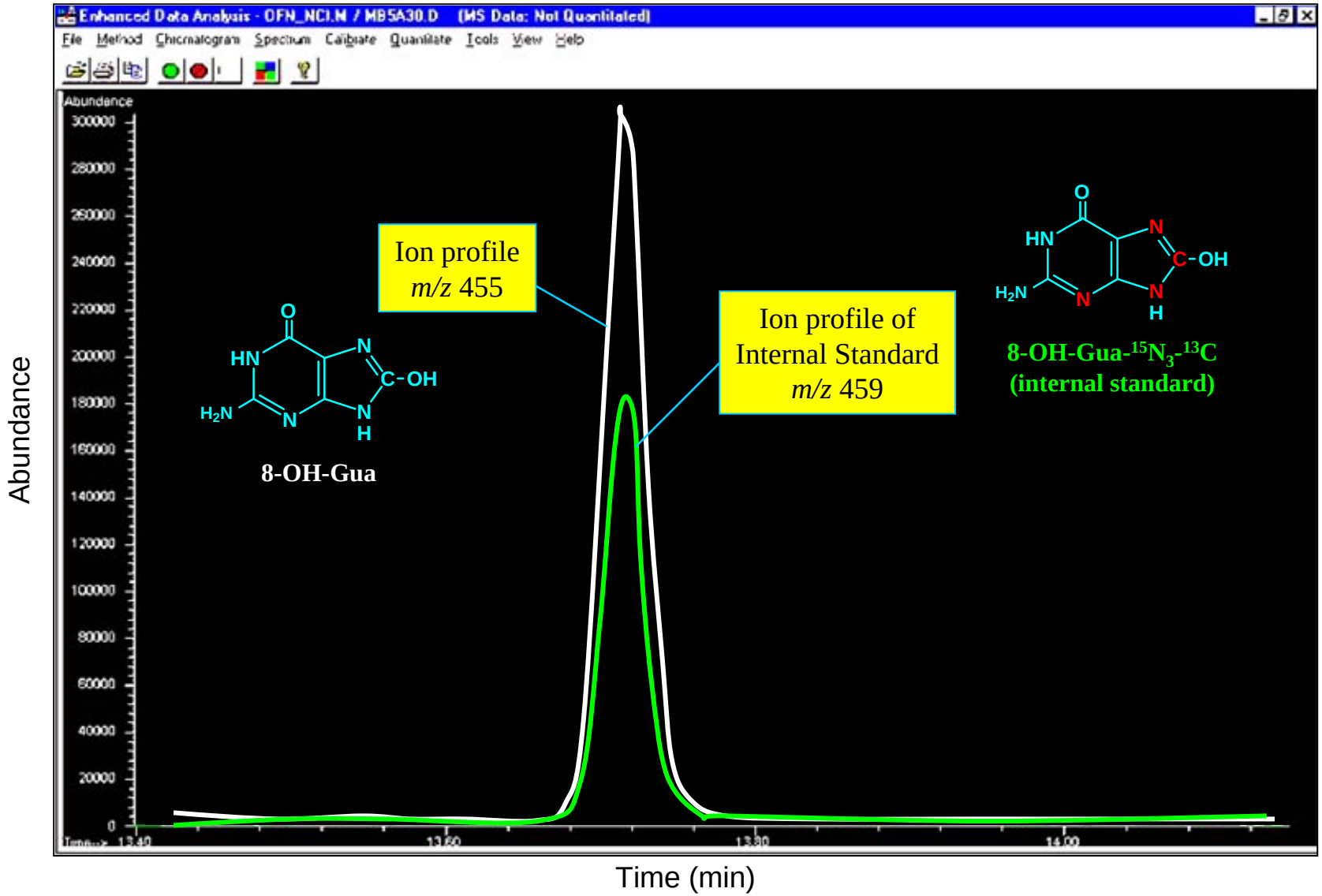
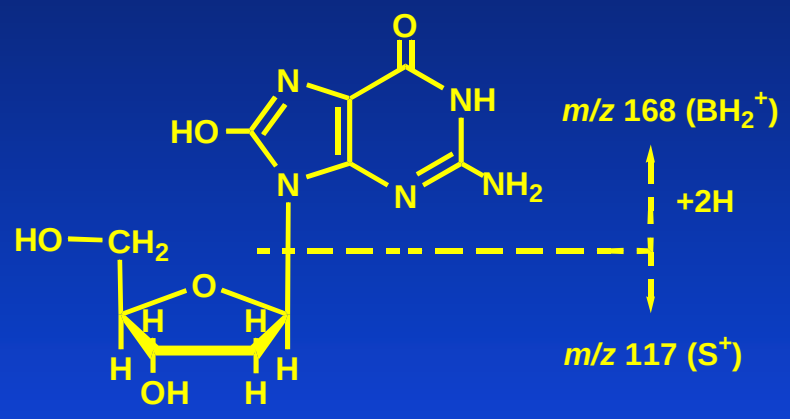


FIG. 5. Mass spectrum taken from peak 10 in Fig. 1.

Assurance of Precise Measurements (Isotope-Dilution Mass Spectrometry)



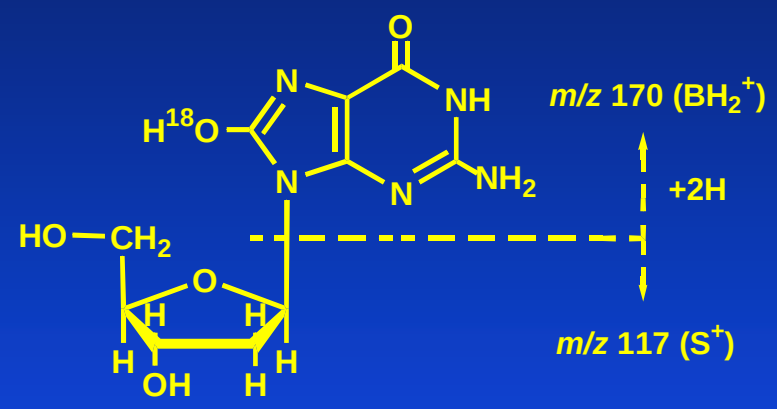
Fragmentation mechanism of 8-hydroxy-2'-deoxyguanosine



8-hydroxy-2'-deoxyguanosine

m/z 284 (MH^+)

m/z 306 (MNa^+)

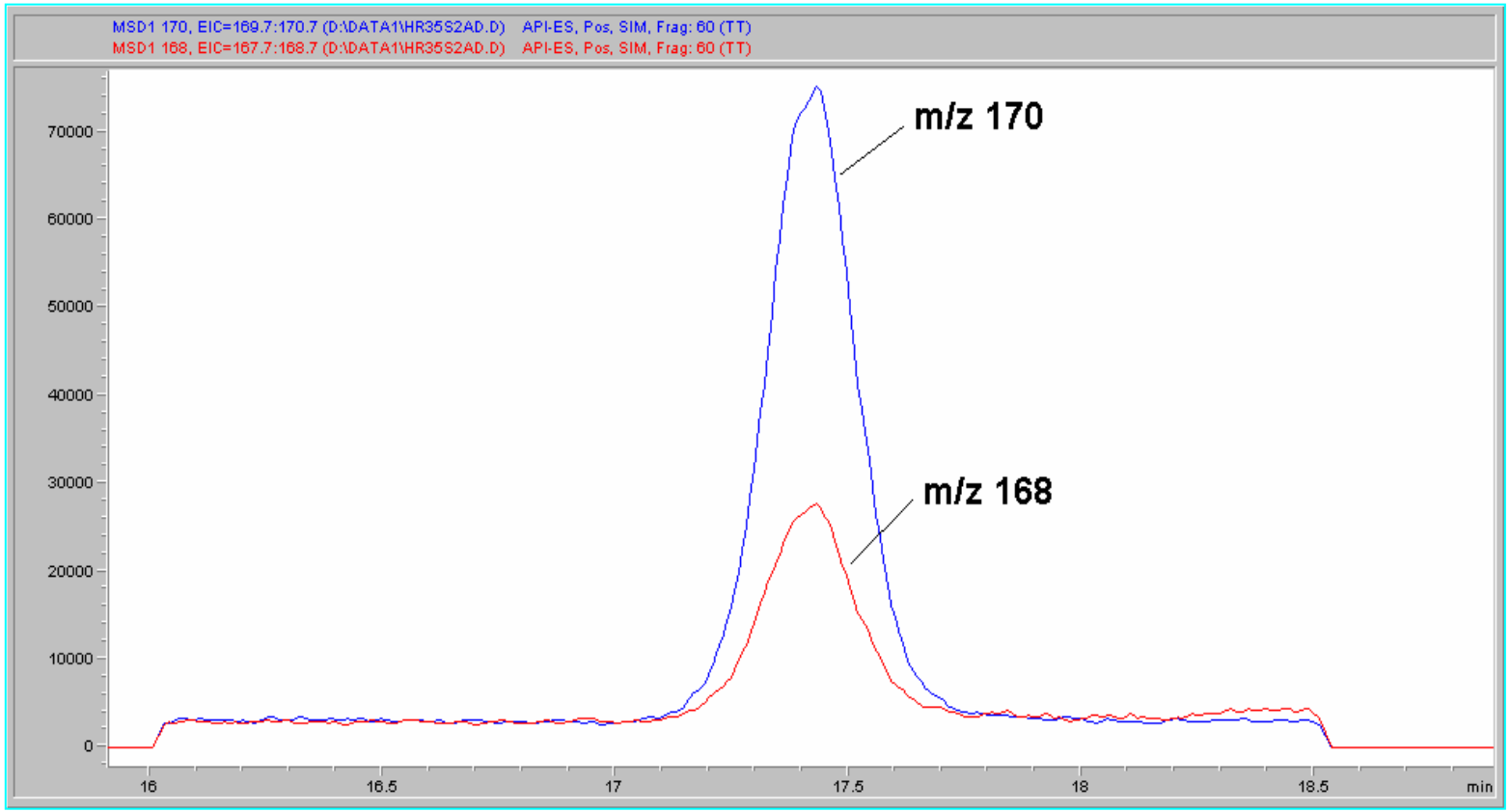


8-hydroxy-2'-deoxyguanosine-^{18}O

m/z 286 (MH^+)

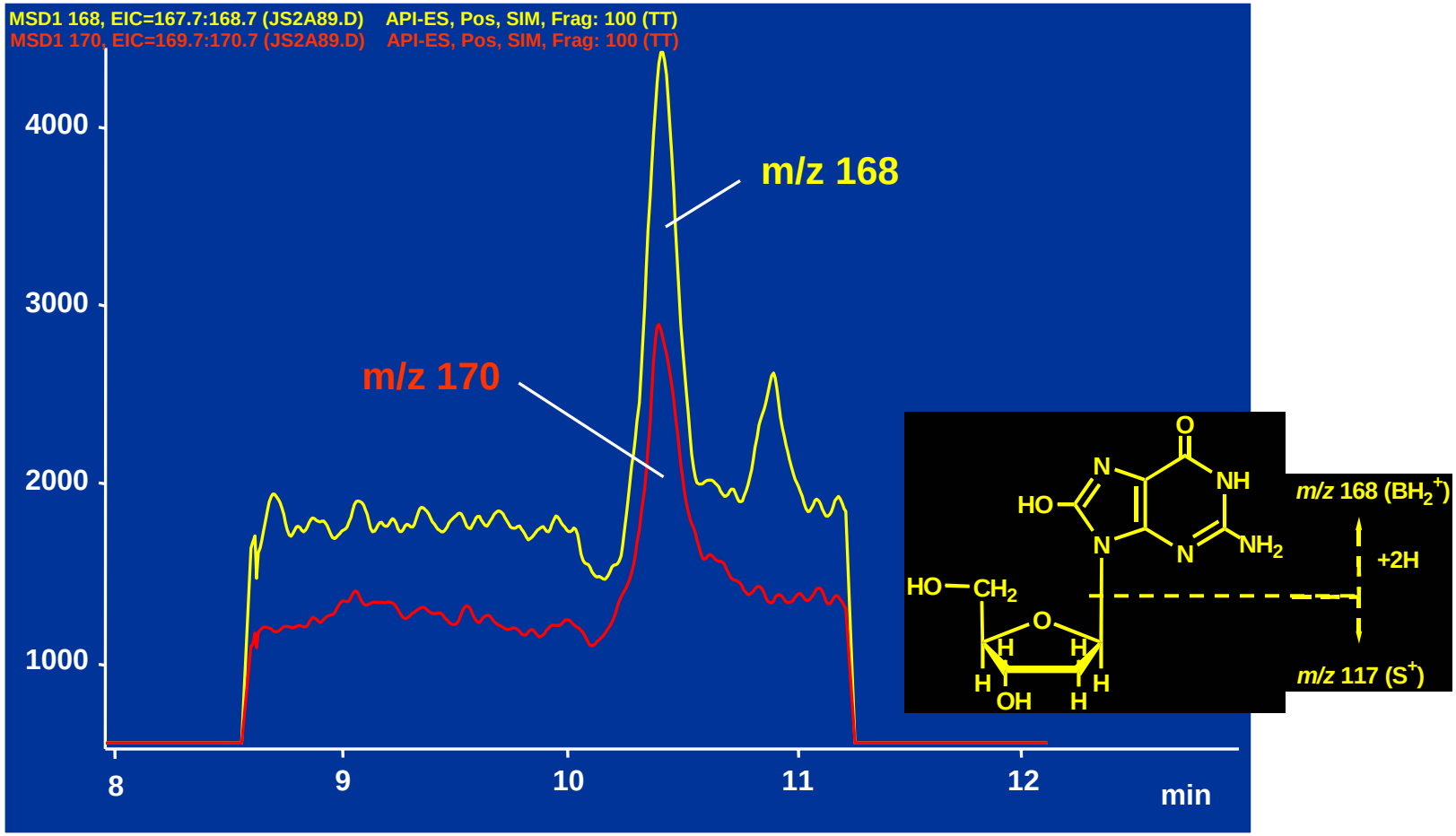
m/z 308 (MNa^+)

Ion-current profiles at *m/z* 168 and 170 recorded during LC/MS-SIM analysis of an enzymic hydrolysate of DNA



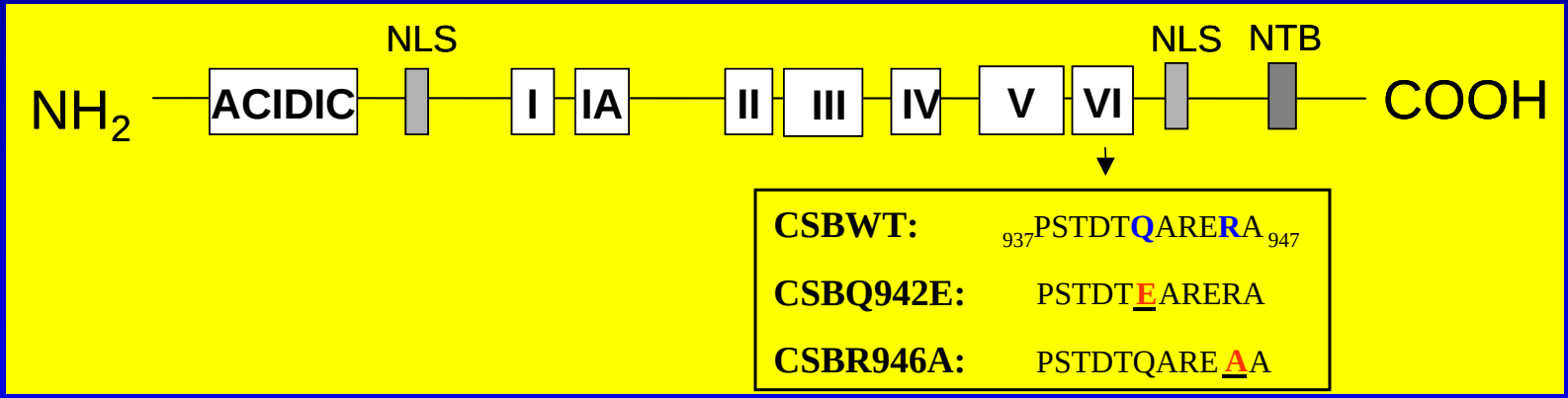
***m/z* 168: 8-hydroxy-2'-deoxyguanosine**
***m/z* 170: 8-hydroxy-2'-deoxyguanosine-^{18}O**

Measurement of 8-OH-dGuo in cells by LC/MS



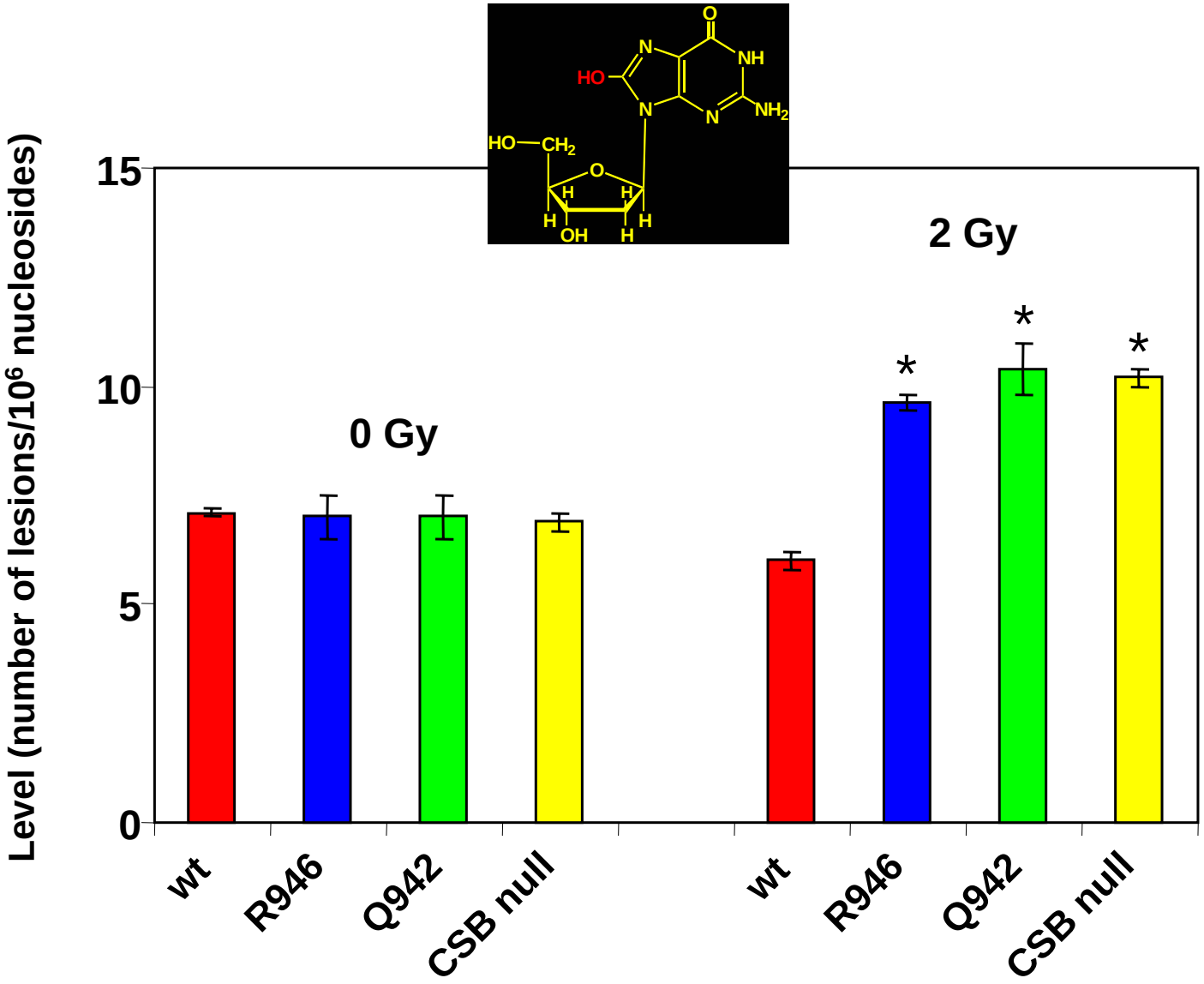
Ion current profiles at *m/z* 168 (BH_2^+) of 8-OH-dGuo and at *m/z* 170 (BH_2^+) of 8-OH-dGuo-^{18}O

Structure of the CSB protein (1493 amino acids, 168 kDa) and the location of the designed mutants

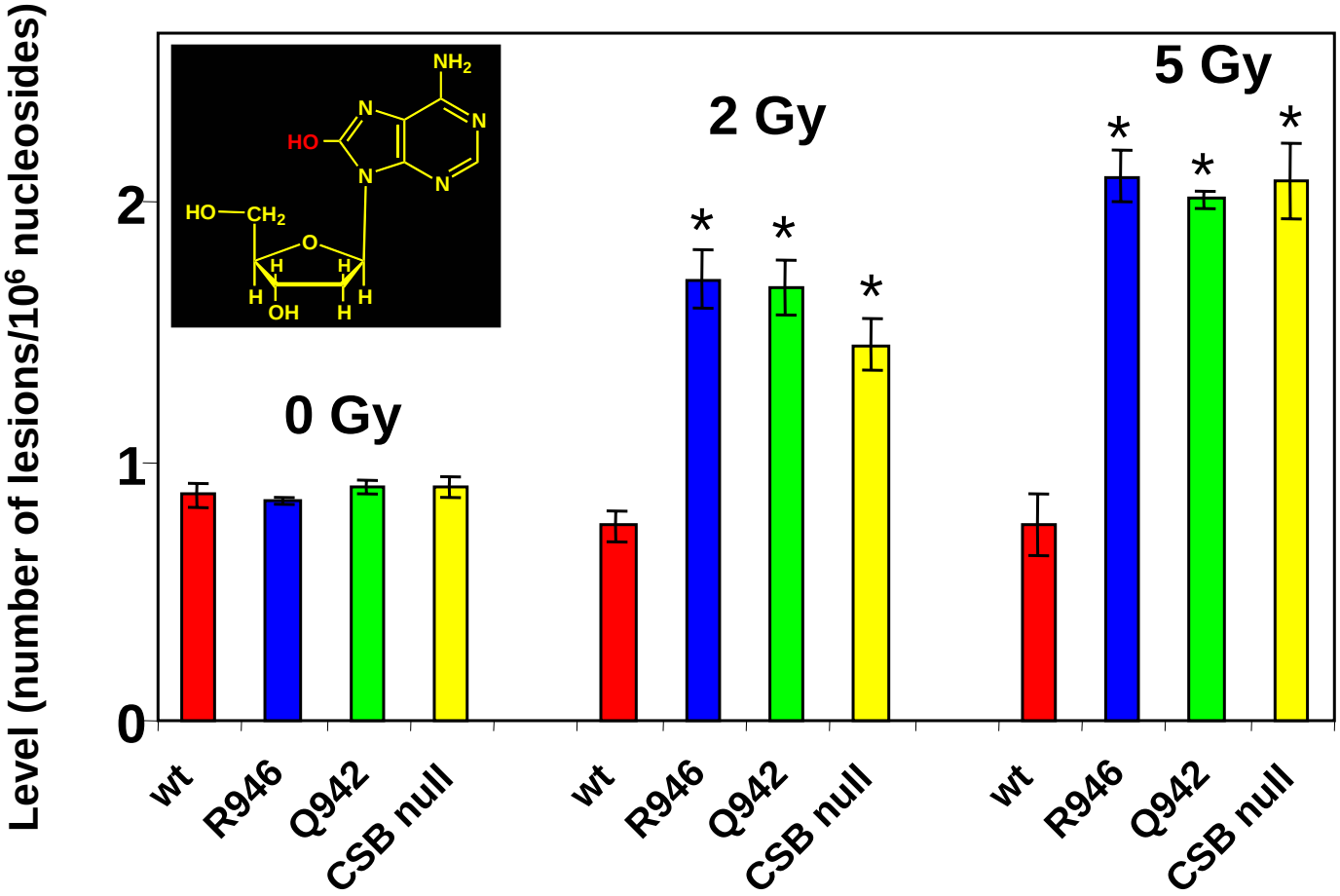


CSBQ942E	glutamine	→	glutamic acid
CSBR946A	arginine	→	alanine

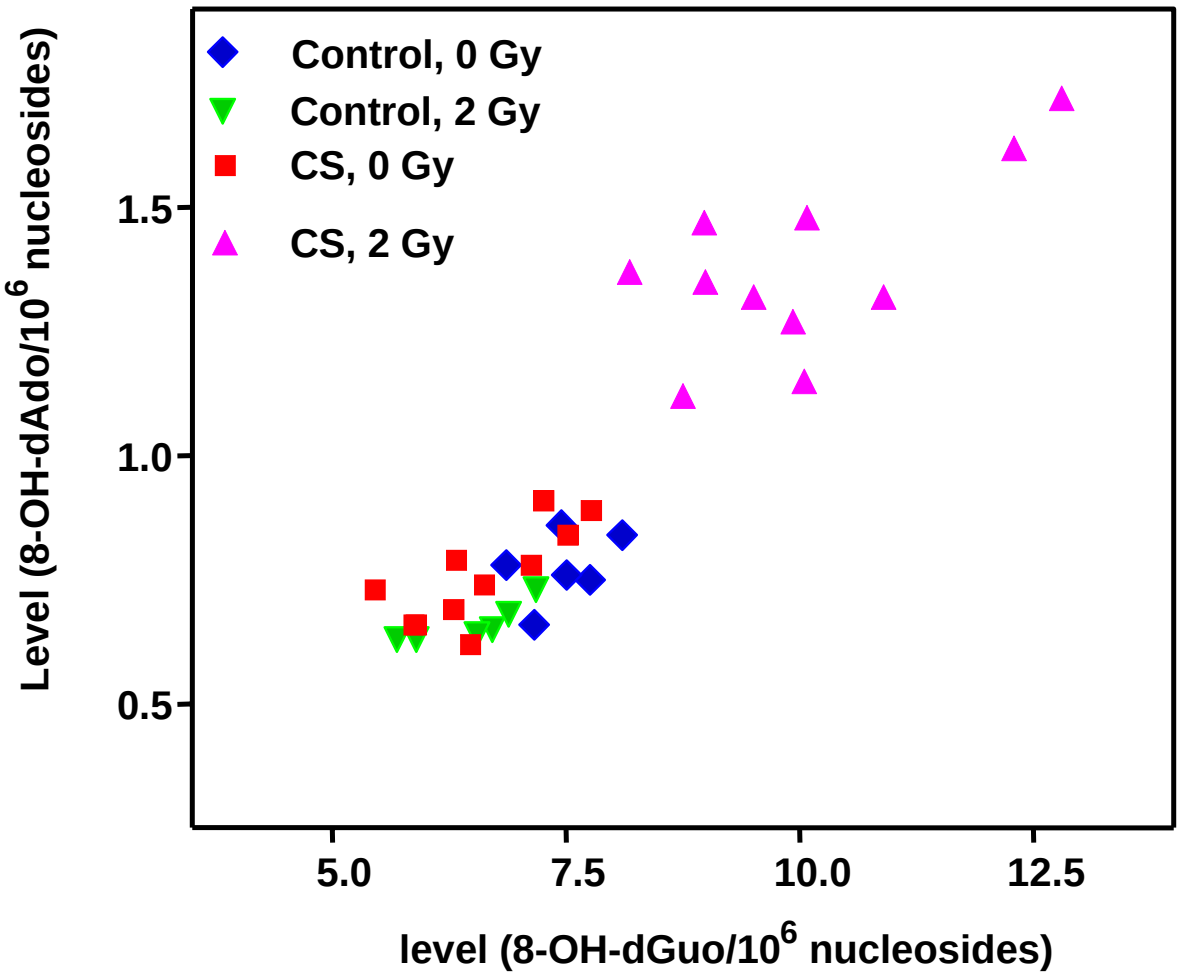
Cellular repair of 8-hydroxy-2'-deoxyguanosine in DNA of CS-B cells



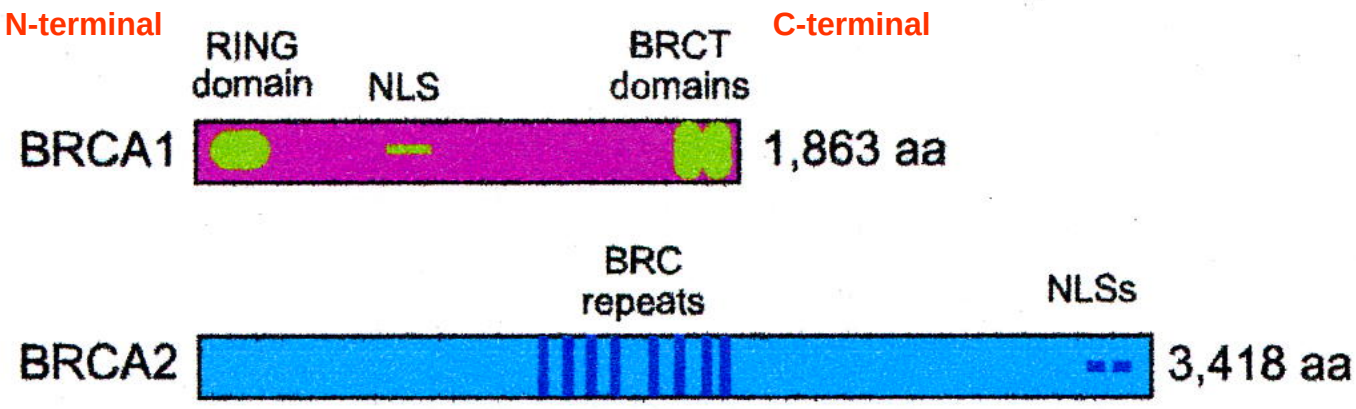
Cellular repair of 8-hydroxy-2'-deoxyadenosine in DNA of CS-B cells



Cellular repair of 8-hydroxy-2'-deoxyguanosine and 8-hydroxy-2'-deoxyadenosine in DNA of fibroblasts from CS-B patients



BRCA proteins



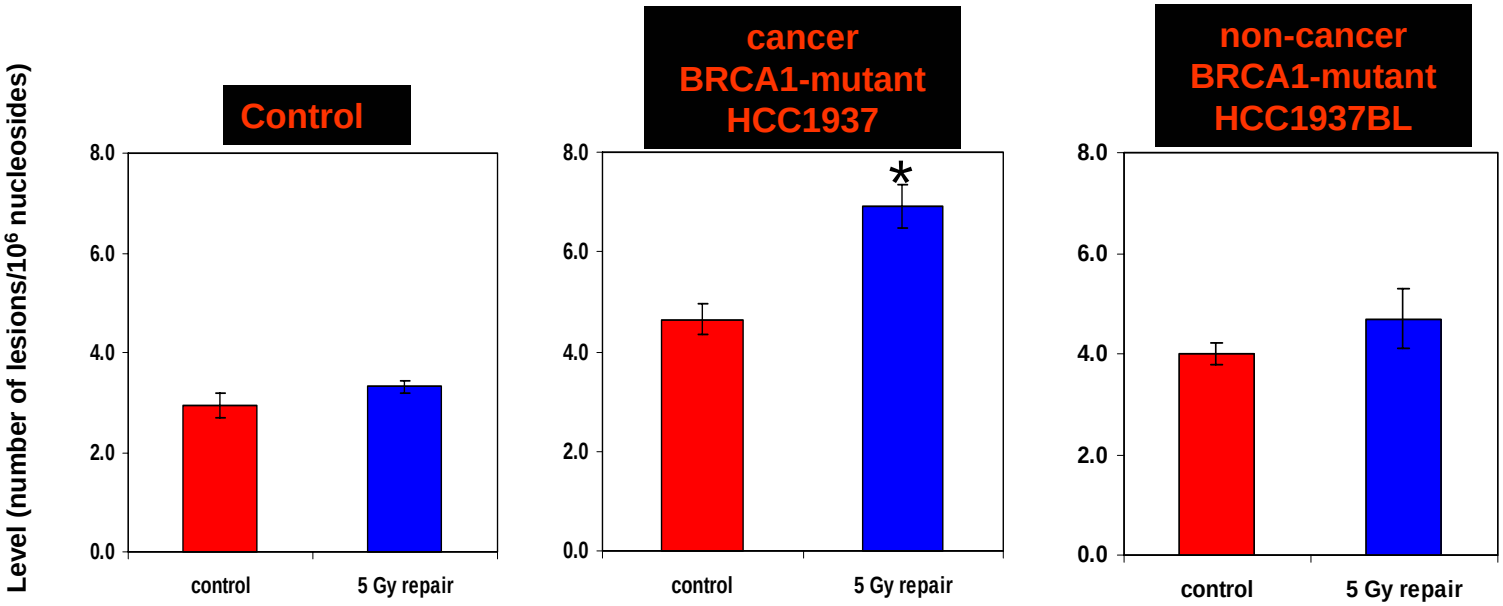
Cell lines

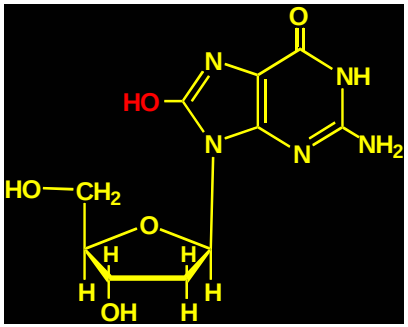
1. AG10097 is a non-malignant lymphoblastoid cell line that expresses wild type BRCA1 and BRCA2 proteins.

2. HCC1937 is a primary ductal breast carcinoma cell line from a female breast cancer patient. This cell line is homozygous for *BRCA1* germline mutation that causes deletion of the C-terminus of BRCA1 yielding a truncated BRCA1 protein that lacks the BRCT domain.

3. HCC1937BL is an EBV-transformed B-lymphoblastoid cell line established from peripheral blood lymphocytes of the same patient. This cell line is heterozygous for the same *BRCA1* germline mutation and expresses full-length BRCA1 protein.

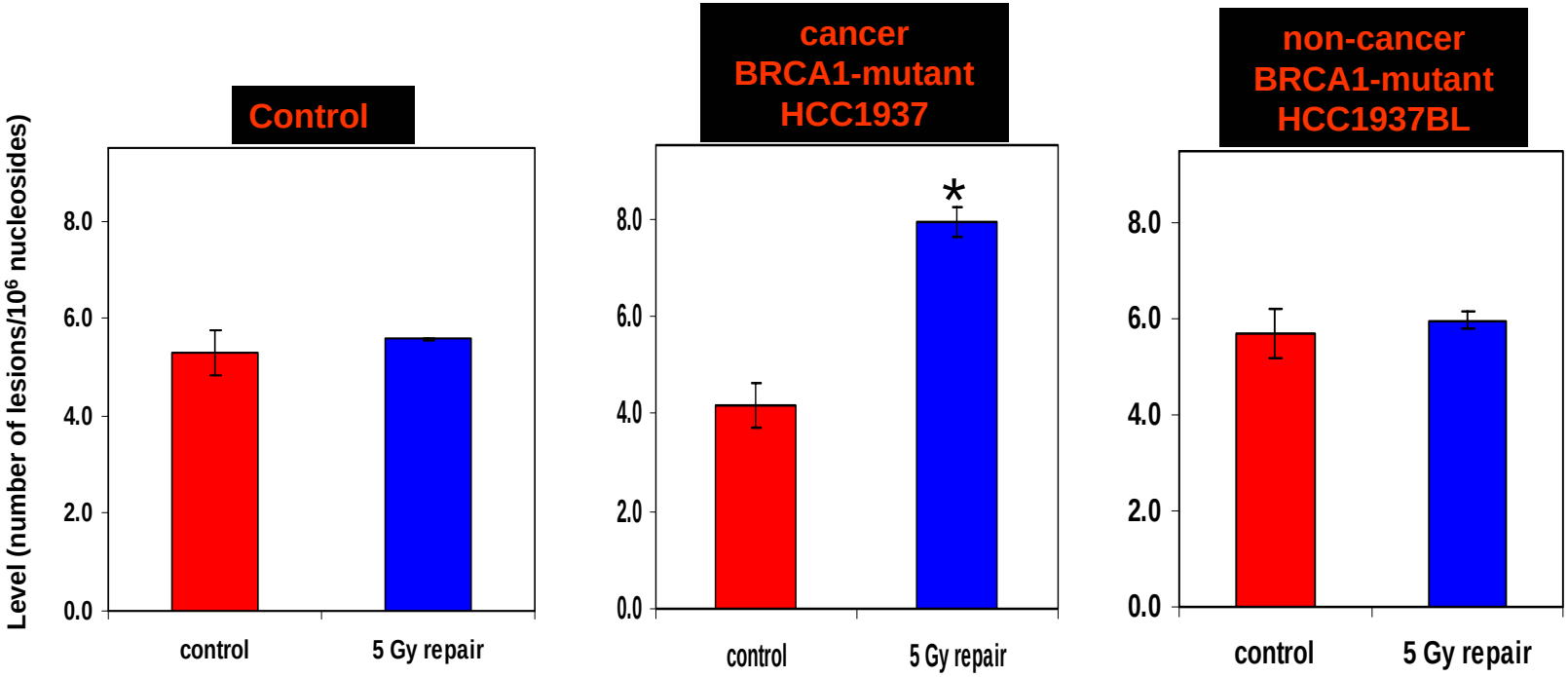
Cellular repair of 8-hydroxy-2'-deoxyguanosine in DNA of breast cancer cells with *BRCA1* mutation

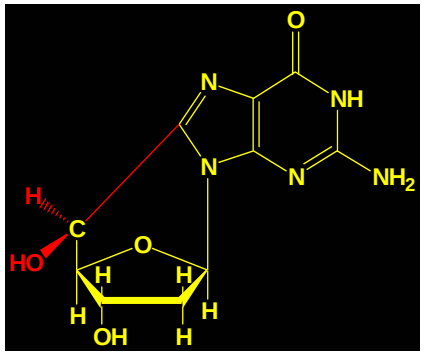




Nyaga, S.G., Trzeciak, A.R., Jaruga, P., Lohani, A., Barnes, J., Dizdaroglu, M. and Evans, M.K., *Molec. Cell. Biol.* (submitted)

Cellular repair of (5'S)-8,5'-cyclo-2'-deoxyguanosine in DNA of breast cancer cells with *BRCA1* mutation



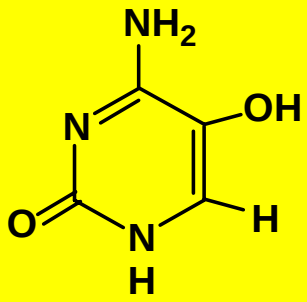


Nyaga, S.G., Trzeciak, A.R., Jaruga, P., Lohani, A., Barnes, J., Dizdaroglu, M. and Evans, M.K., *Molec. Cell. Biol.* (submitted)

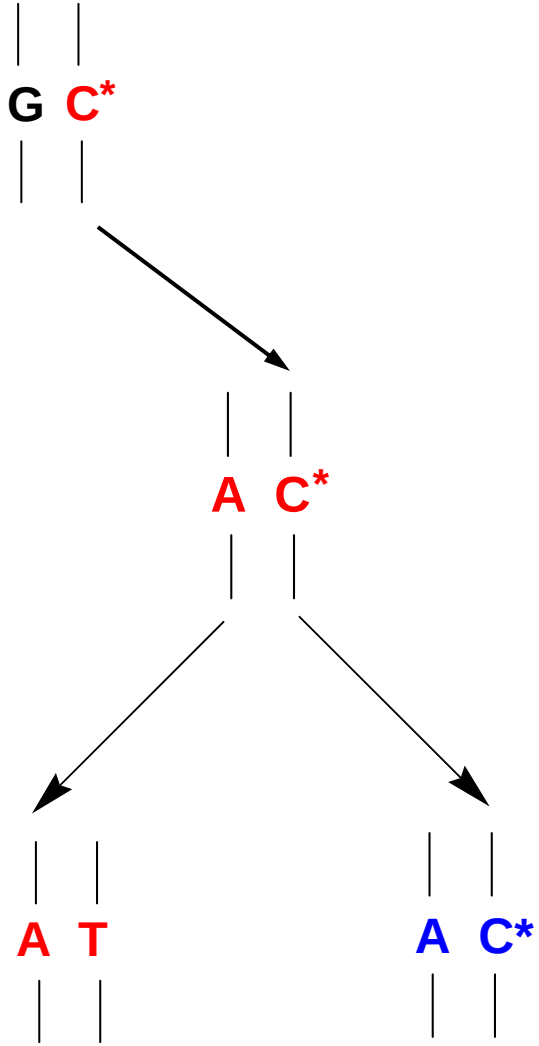
Types of DNA Damage by Free Radicals

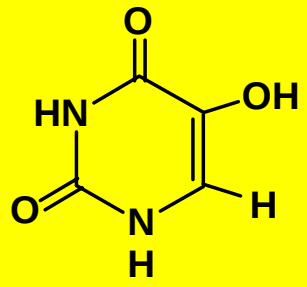
- DNA base damage
- DNA sugar damage
- 8,5'-cyclopurine-2'-deoxynucleosides
- DNA-protein cross-links
- Single- and double-strand breaks
- Abasic sites

Mutation by 5-hydroxycytosine and 5-hydroxyuracil



5-hydroxycytosine



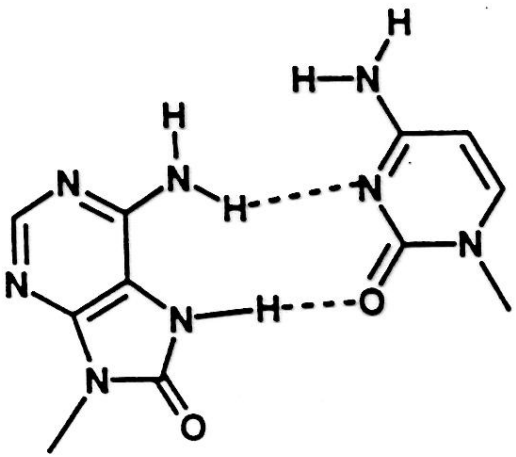


5-hydroxyuracil

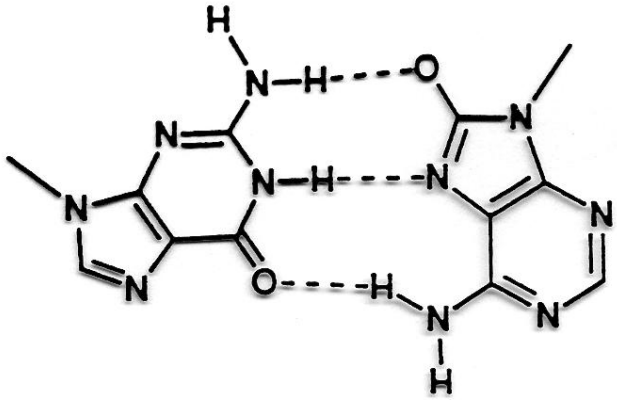
GC → AT

transversion

Base-pairing of 8-hydroxyadenine

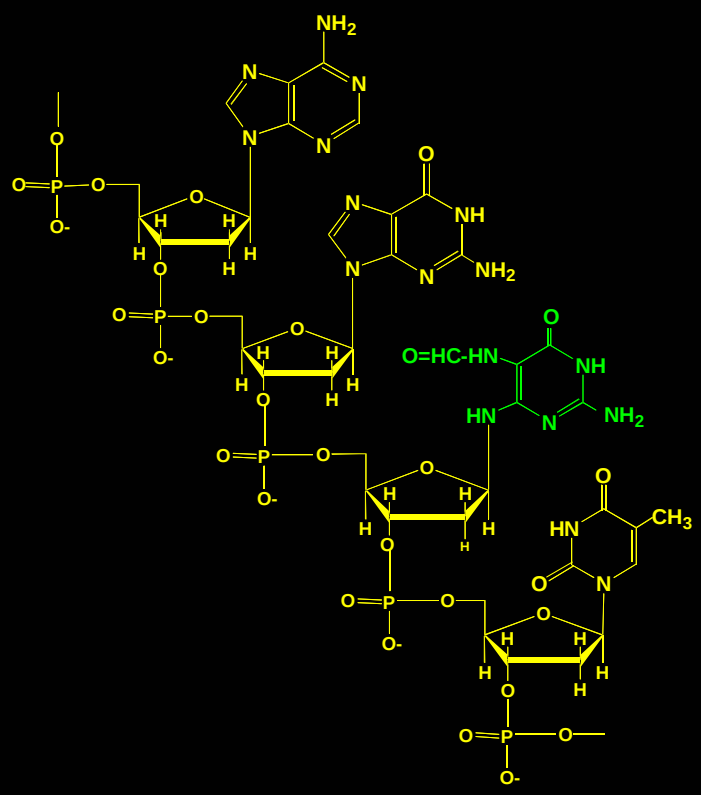


8-hydroxyadenine•cytosine



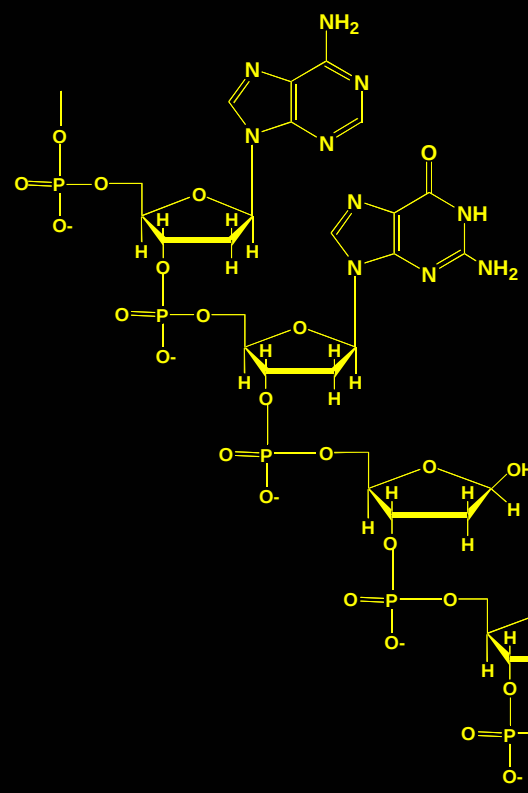
8-hydroxyadenine•guanine

Hydrolysis of a DNA lesion

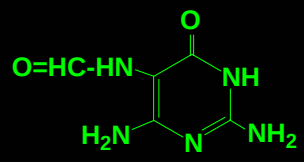


Acid or
DNA glycosylase

$+H_2O$



free base

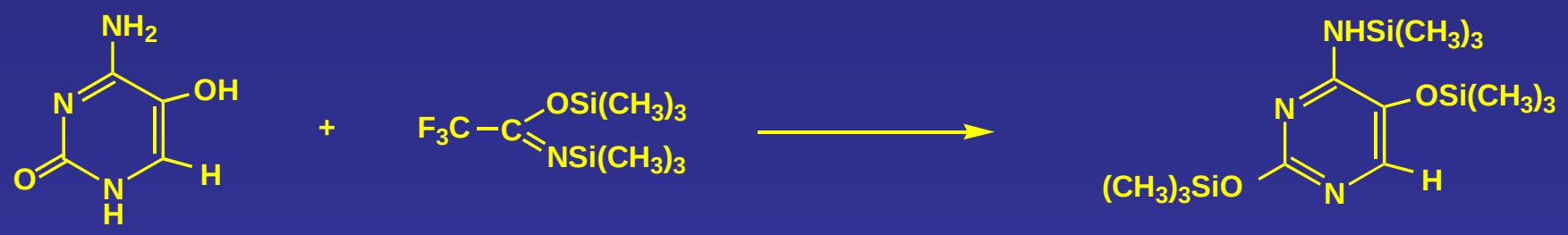


2,6-diamino-4-hydroxy-
5-formamidopyrimidine

DNA with an abasic site

Trimethylsilylation of DNA bases

8-hydroxyguanine



5-hydroxycytosine

GC/MS Analysis of DNA

1. Hydrolysis

   a. enzymatic hydrolysis
   b. acidic hydrolysis

2. Derivatization

Xeroderma pigmentosum (XP)

Human hereditary disease

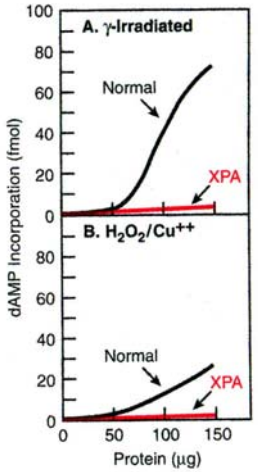
Deficiency in repair of oxidative DNA damage

- **XP patients are prone to the early onset of severe photosensitivity of the exposed regions of the skin, high incidence of skin cancers and frequent neurological abnormalities.**

- **Increased frequency of neoplasms affecting internal organs is also common.**

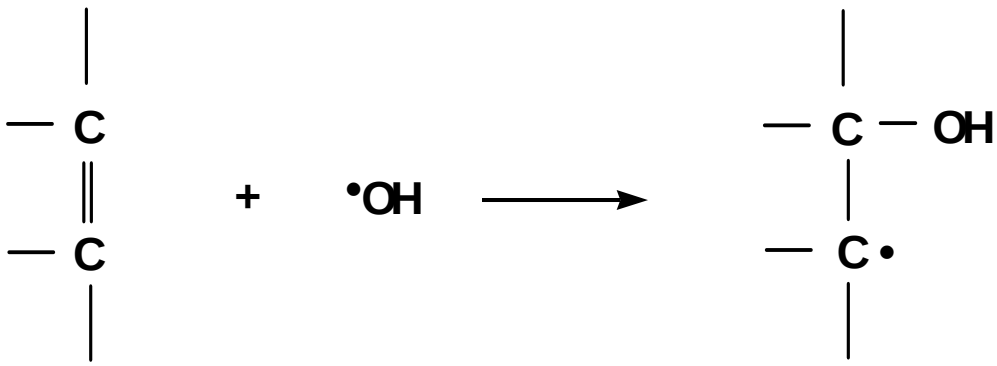
- **XP is characterized by defective *nucleotide-excision repair* of DNA damage.**

- **There is evidence that some components of oxidative DNA damage are not repaired in XP cells.**

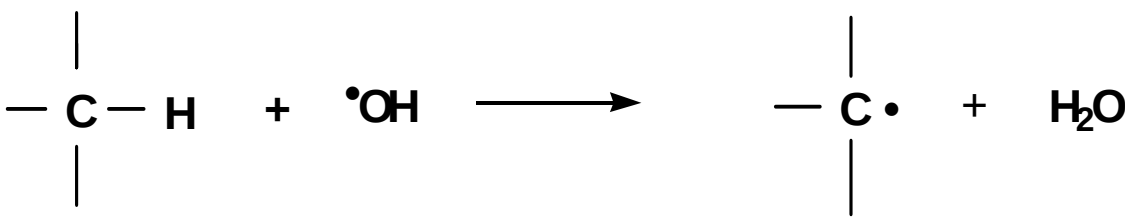


Satoh, M.S., Jones, C.J., Wood, R.D. and Lindahl, T., PNAS, 90, 6335-6339, 1993

Reactions of water radicals with substrates

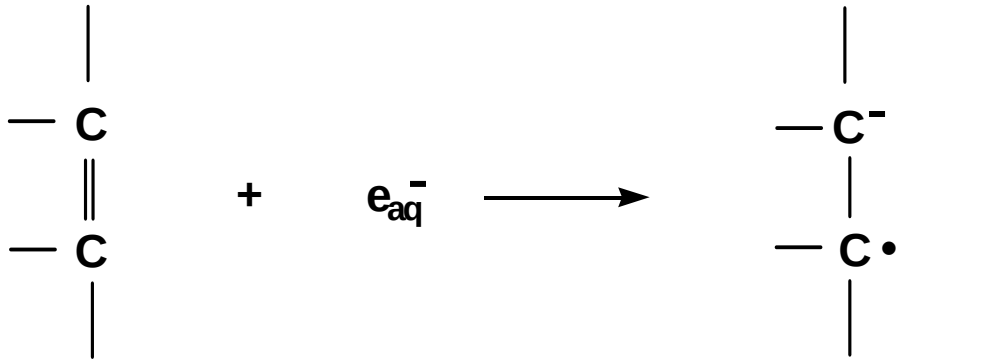


addition



abstraction

Reactions of water radicals with substrates



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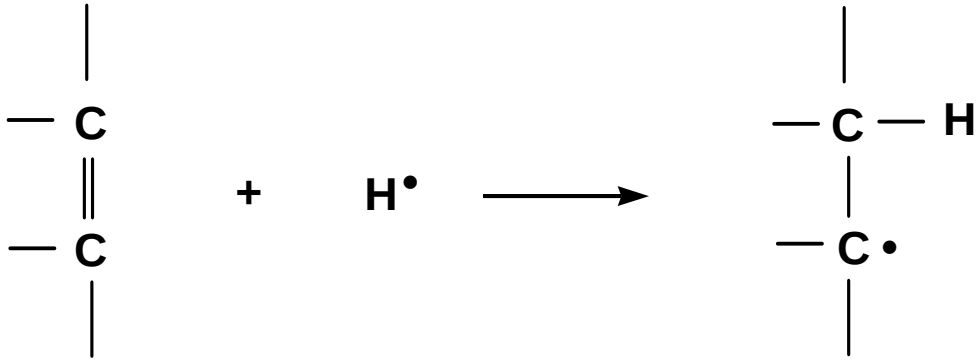
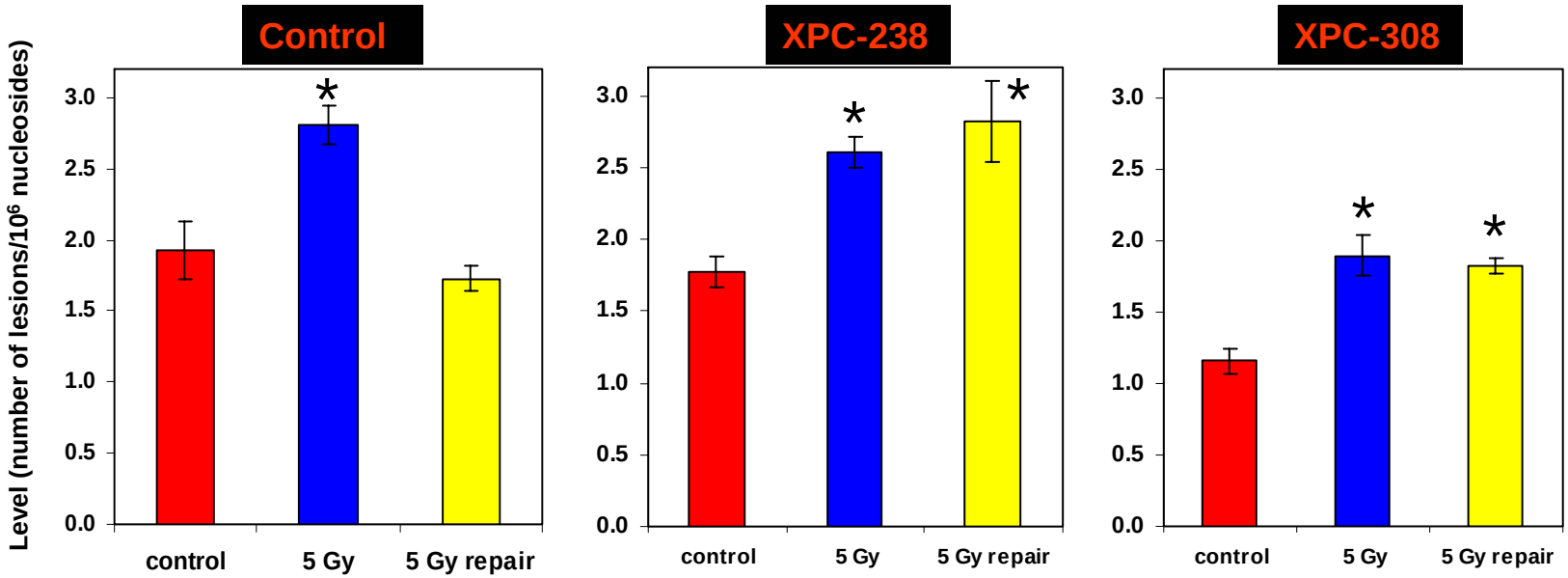
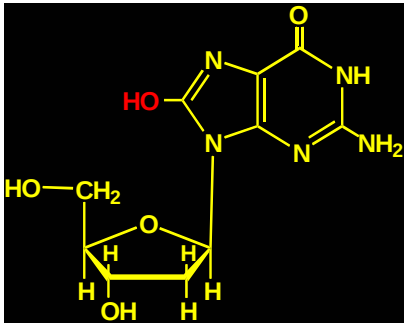


addition

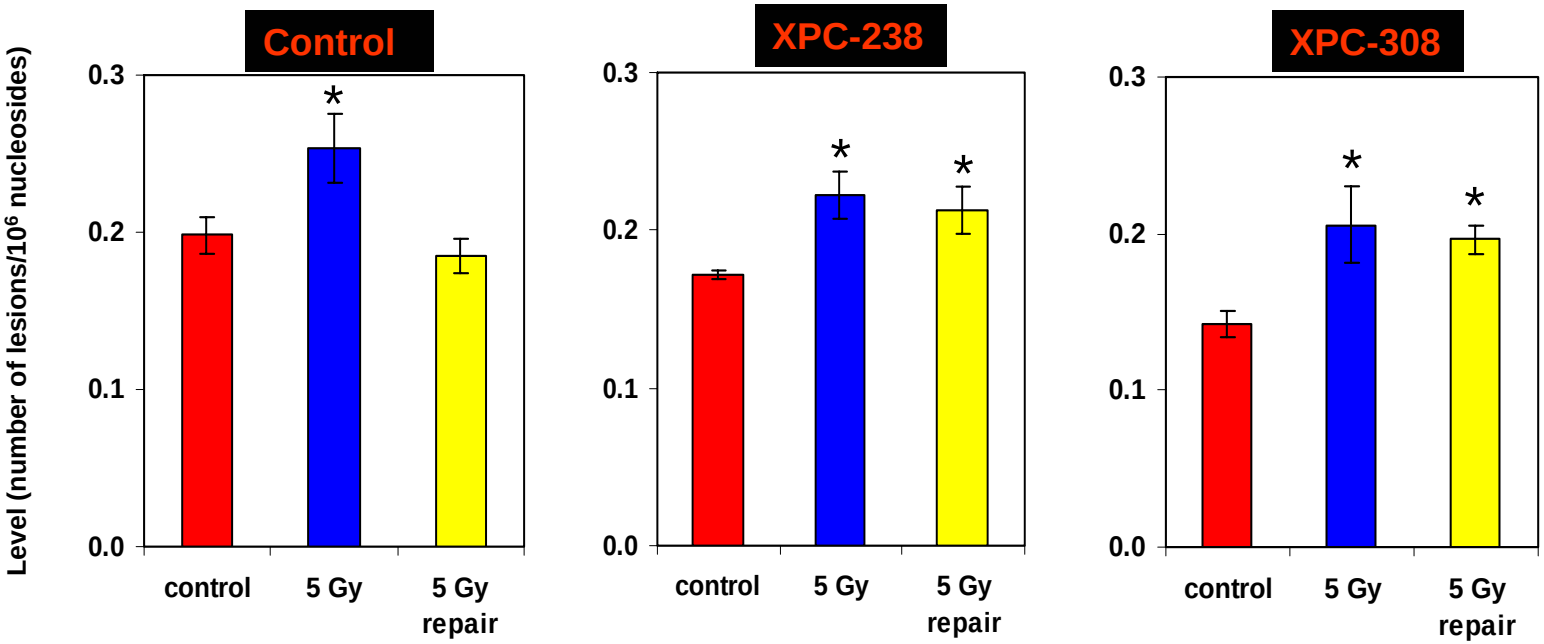
Cellular repair of 8-hydroxy-2'-deoxyguanosine in DNA of XPC keratinocytes

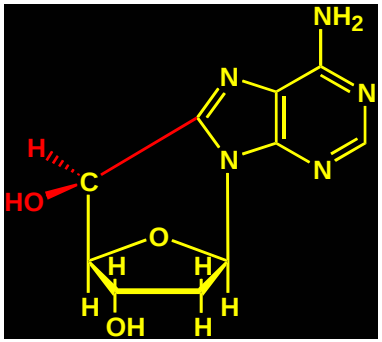




Dogliotti, E., Jaruga, P. and Dizdaroglu, M. et al. (in preparation)

Cellular repair of (5'S)-8,5'-cyclo-2'-deoxyadenosine in DNA of XPC keratinocytes





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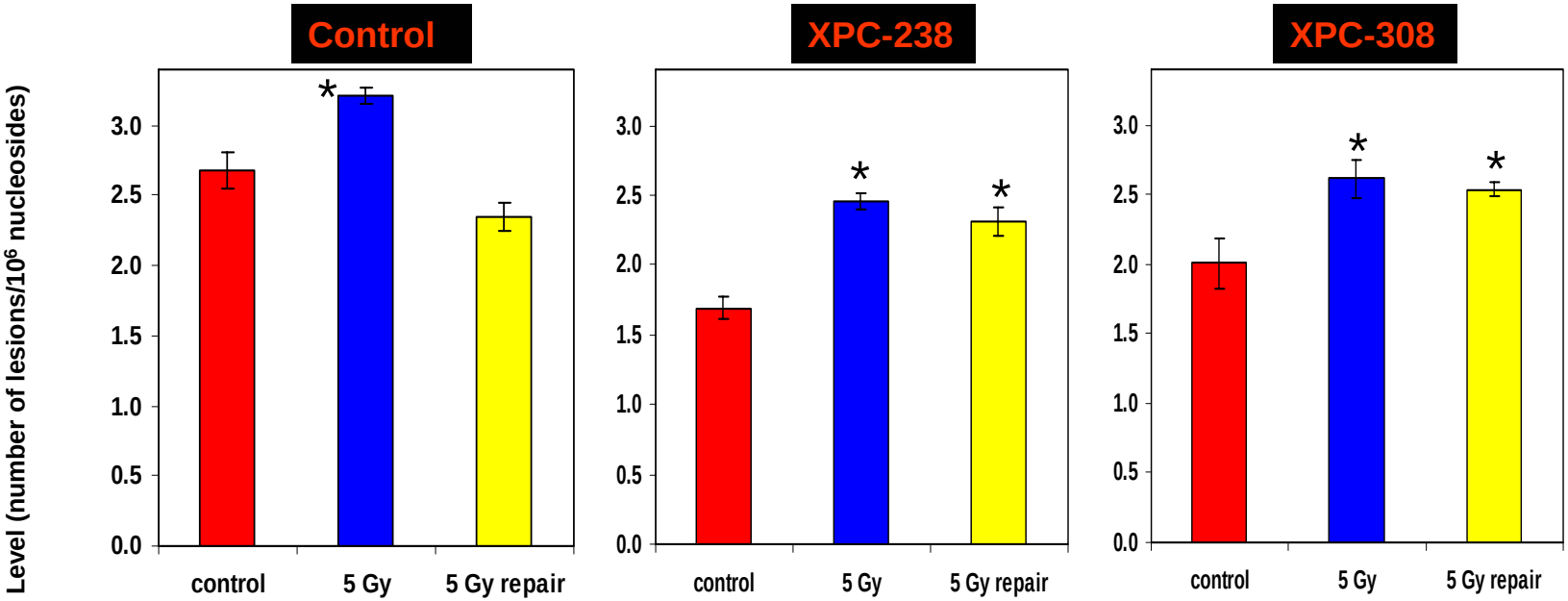

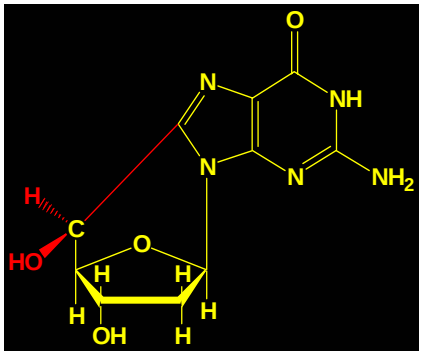

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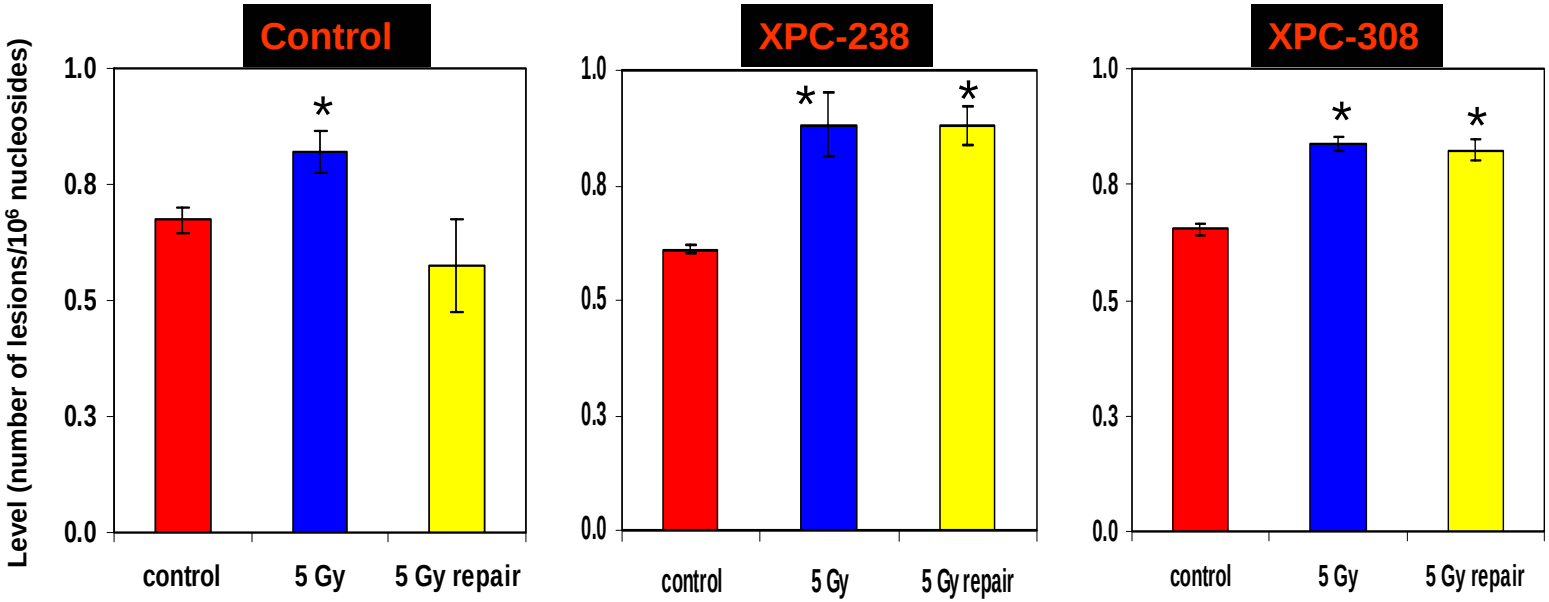
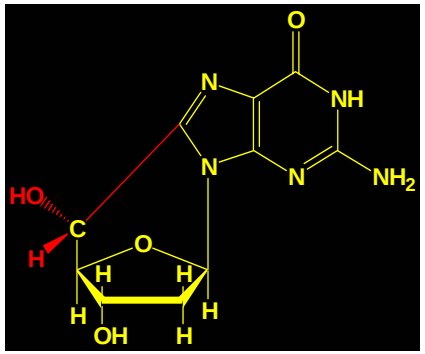
Cellular repair of (5'R)-8,5'-cyclo-2'-deoxyguanosine in DNA of XPC keratinocytes





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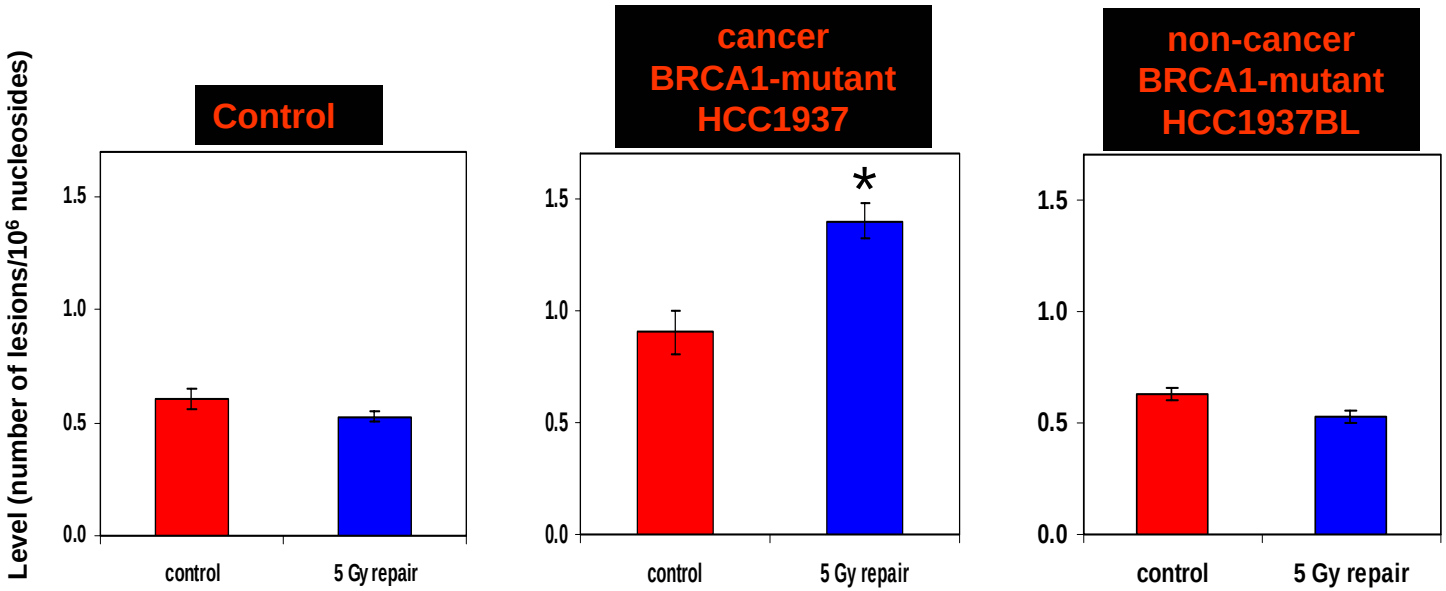
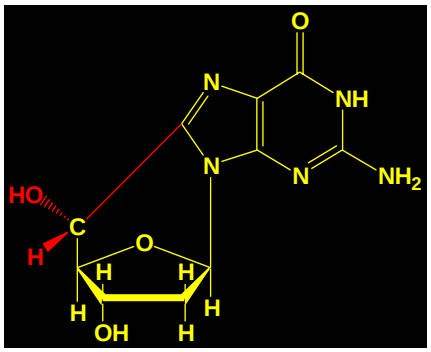
Cellular repair of (5'R)-8,5'-cyclo-2'-deoxyguanosine in DNA of breast cancer cells with *BRCA1* mutation





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Summary

- **We observed lower activity of DNA repair and the accumulation of major DNA lesions induced by oxidative DNA damage in human cells with mutations in *CSB*, *BRCA1* and *XPC* genes.**

- **The results demonstrate reduced capability of CS cells, *BRCA1*-mutant breast cancer cells and XPC cells in the cellular repair of oxidative DNA damage.**

- **The failure to repair the DNA lesions resulting from oxidative DNA damage might contribute to the pathogenesis of these diseases.**

- **Future therapeutic interventions might involve efforts to protect against oxidative DNA damage and to increase the capacity of cells to repair it.**