DNA Repair and Mutagenesis of Reactive Oxygen Species-Generated Lesions

Masaaki (Maki) Moriya
SUNY at Stony Brook

Contributors

SUNY

I.-Y. Yang, X. Liu, K. Hashimoto, R.L. Revine, S. Stein
G. Pandya, G. Chan
S. Attaluri, R. Rieger, M. C. Torres, S. Khullar, Y. Huang, F. Johnson
T. Zaliznyak, C. de los Santos
H. Miller, A. Grollman

UMN

Y. Lao, S. Hecht

Others

J.E. Cleaver, M. Cordeiro-Stone, F. Hanaoka, J.H.J. Hoeijmakers, H. Ohmori, Z. Wang, R. Woodgate

Generation of DNA Adducts by Reactive Oxygen Species

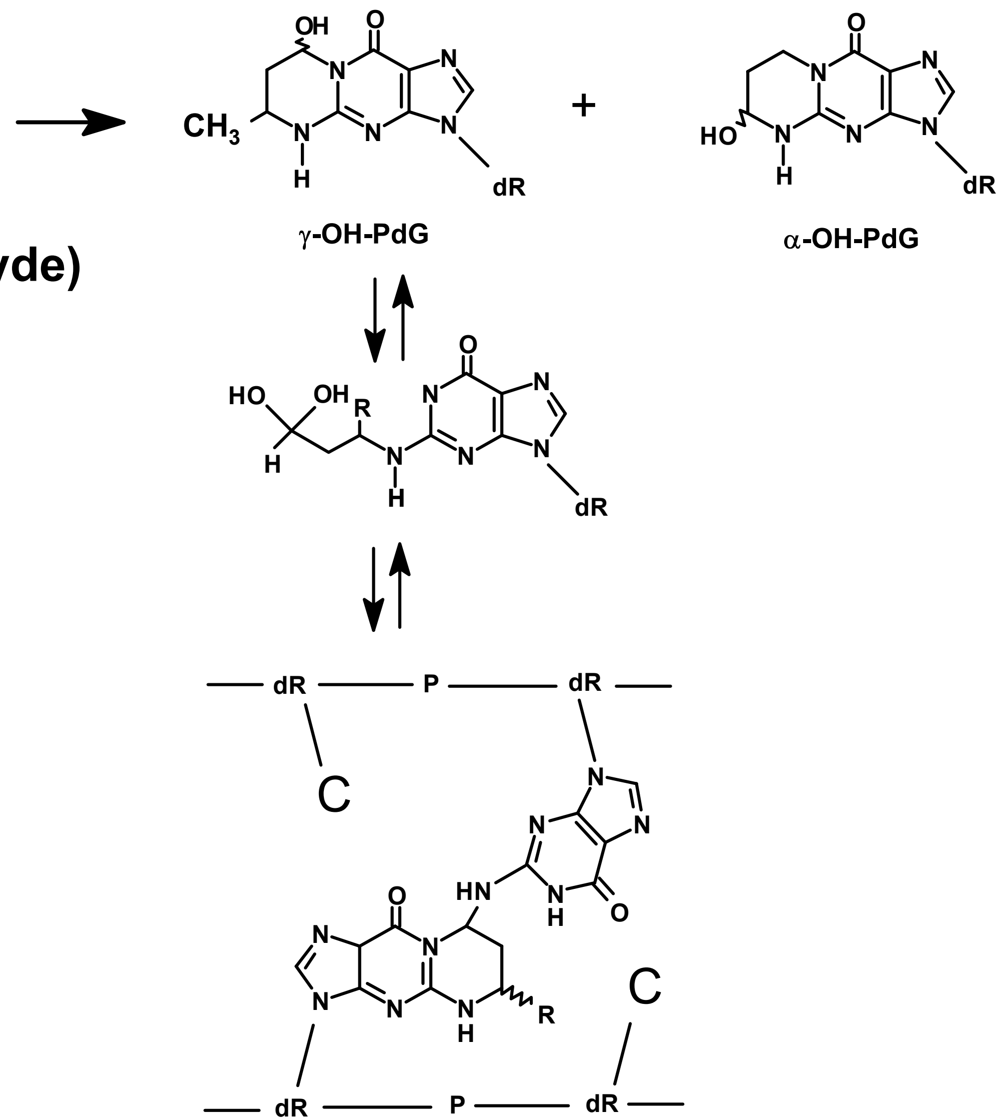
Reactive oxygen species → Lipid peroxidation Enals → Epoxides

Reactive oxygen species	Lipid peroxidation Enals	Epoxides
↓	↓	↓
DNA	DNA	DNA
↓	↓	↓
Oxidized bases	**Exocyclic propano and propeno adducts**	**Etheno adducts**
8-Oxo dG, dA	α and γ-OH-propano dGs	εdA, εdC, εdG
2-OH dA	M_1G, etc	Substituted εdN
Thymine glycol		etc
5-OH-methyl dC		
etc		

dG in DNA

+

Bifunctional aldehyde
(acrolein, crotonaldehyde)



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(SUNY) S. Hecht, Y. Lao (UMN)

Site-specific Procedure

1. Synthesis and purification of modified oligonucleotides
2. Incorporation into a vector
3. Introduction into a host cell
4. Recovery of progeny
5. Analysis for mutagenic and repair events

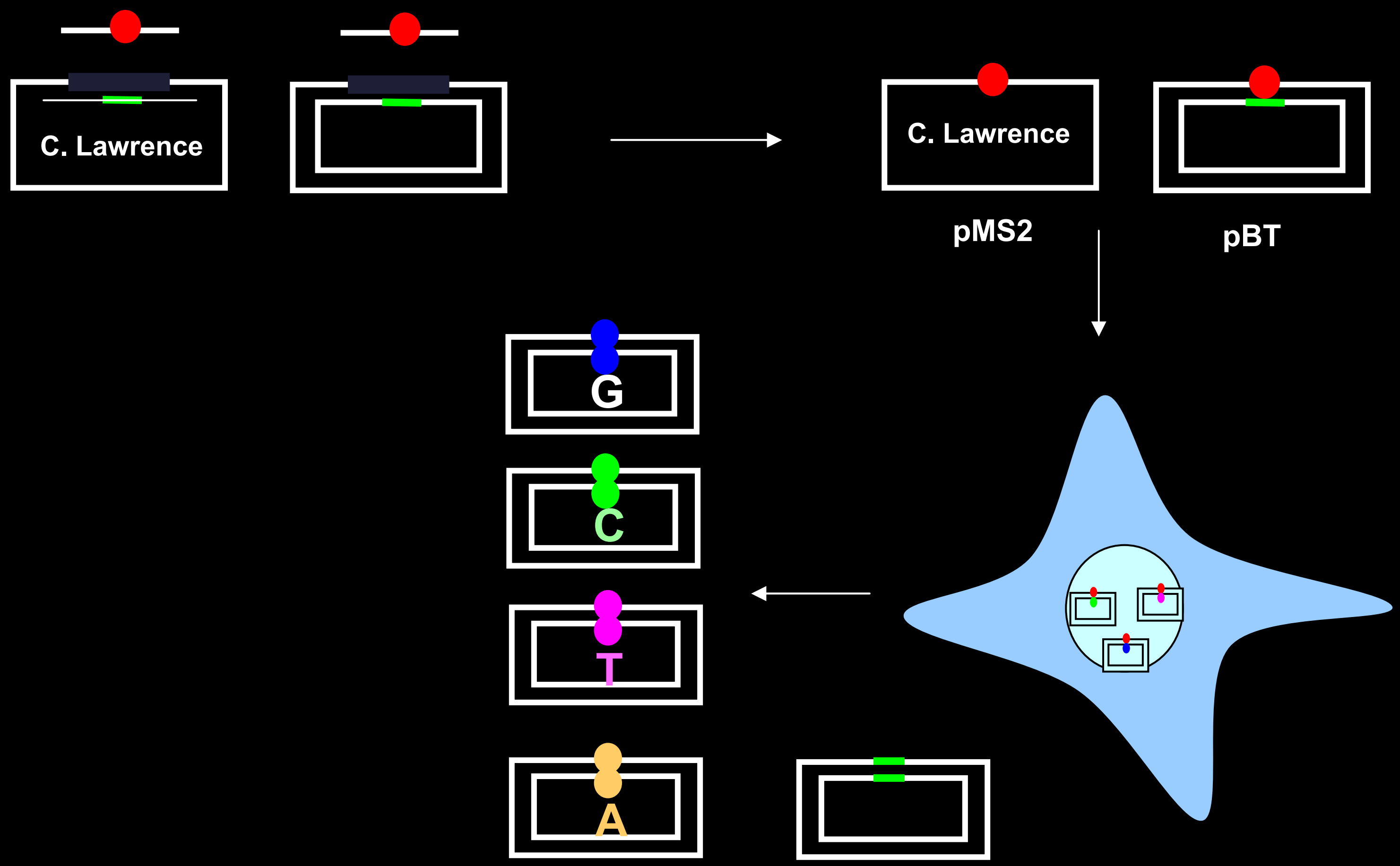
Obstacles to mutation studies

- DNA Repair
- DNA synthesis block

  Preferential replication of undamaged strand = Labor-intensive analysis for TLS events

  Formation of DSBs→ NHEJ →Deletion mutants
- Determination of targeted events

Site-specific mutagenesis approach



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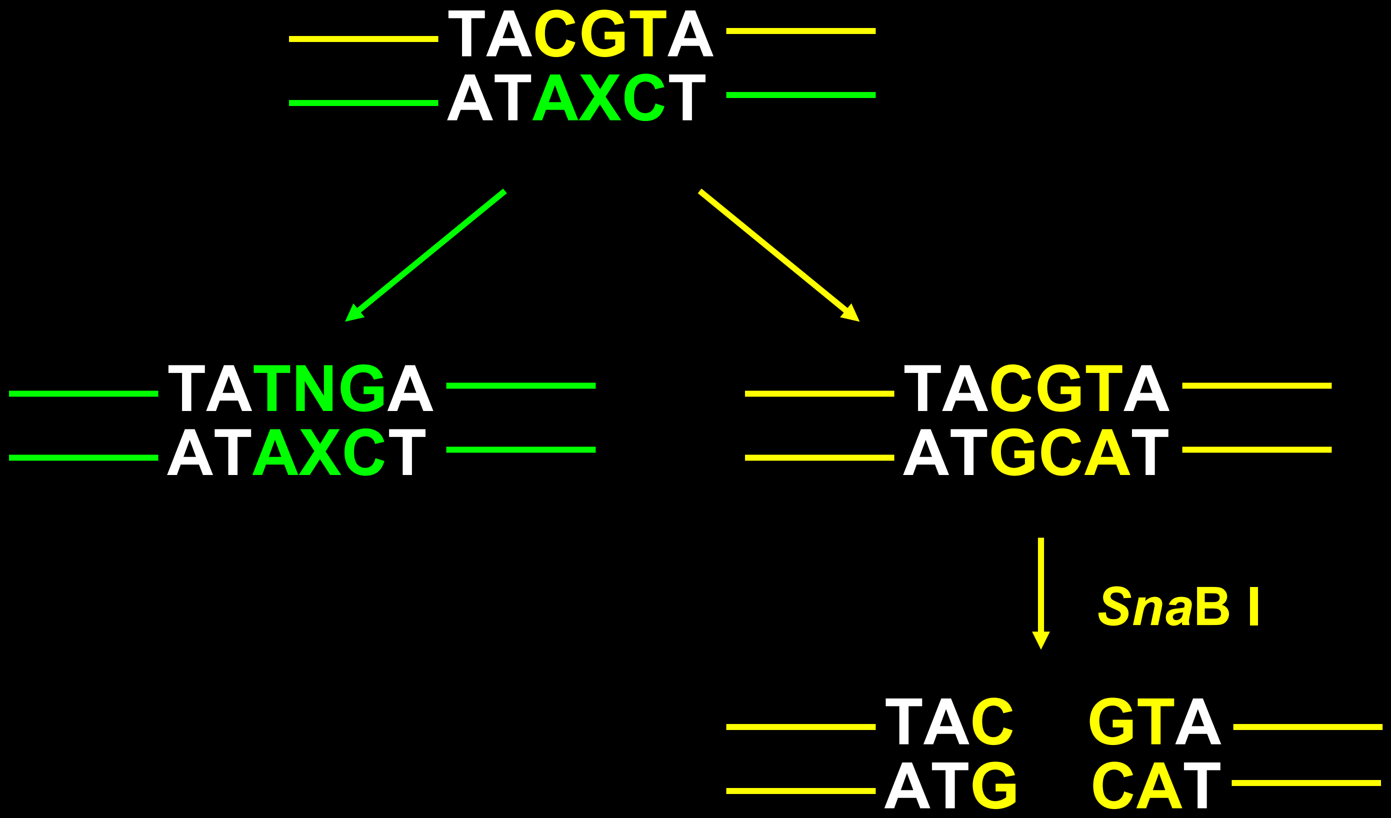
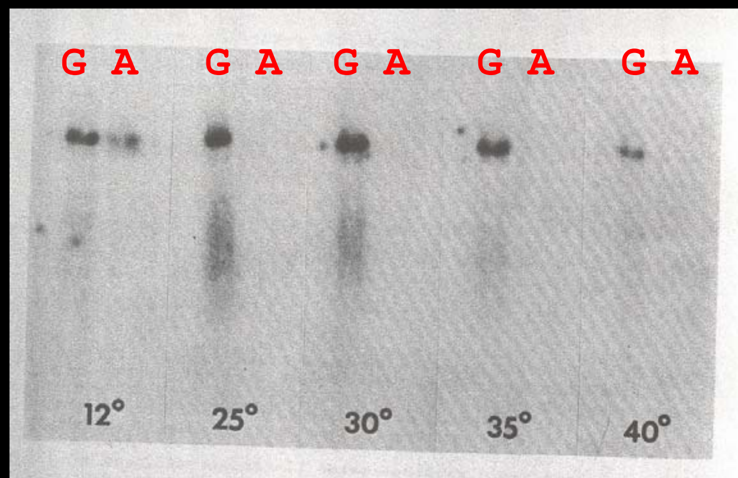
Site-specific mutagenesis strategy



-GGACTTTGTGGGATACCCTCGCTTT-   -GGACTTTGTAGGATACCCTCGCTTT-

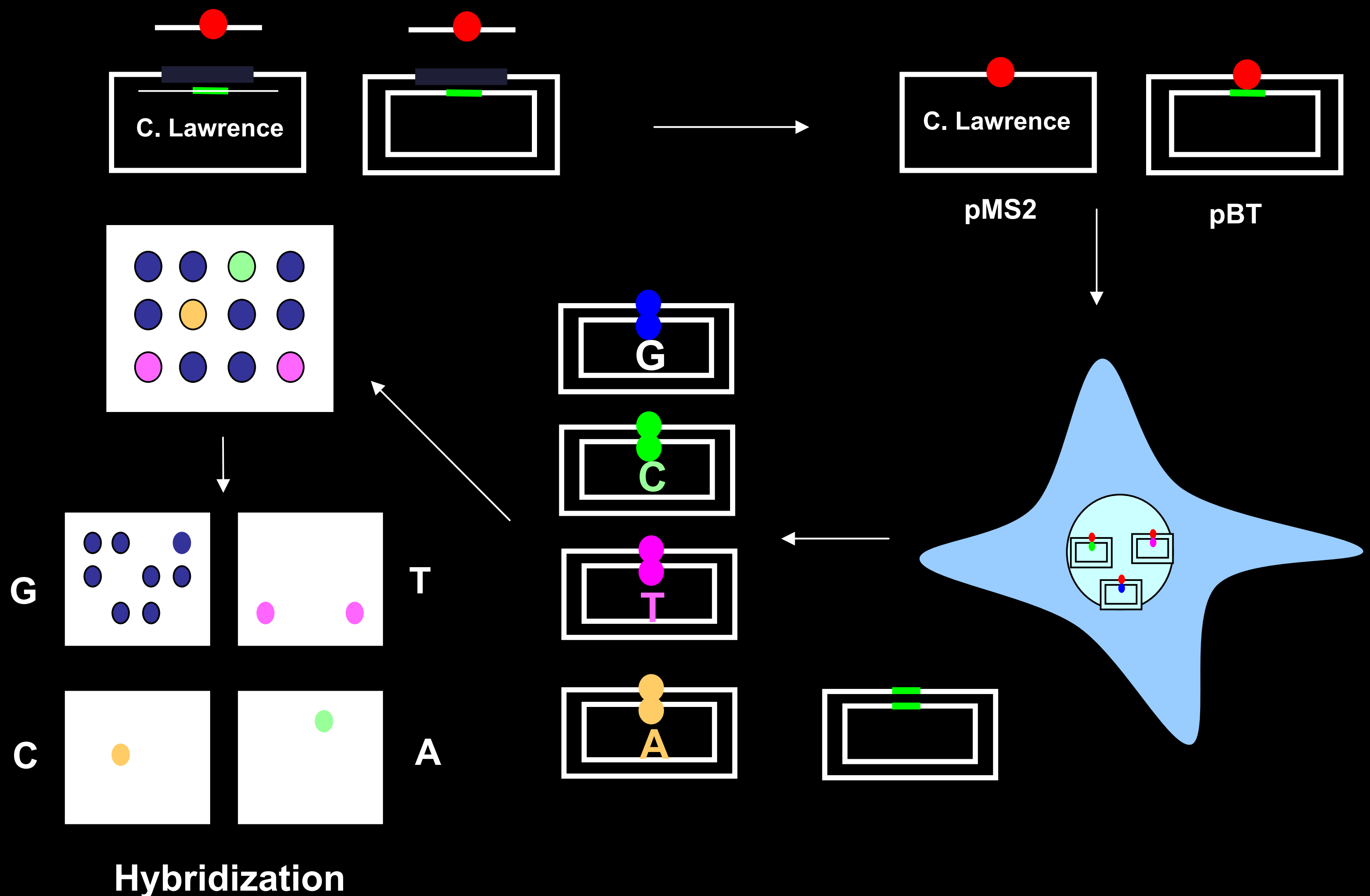
AACACCCTATGGGA-^{32}P



Hybridization temp. = 4(G+C) + 2(A+T) - 4

K. Itakura, 1979

Site-specific mutagenesis approach



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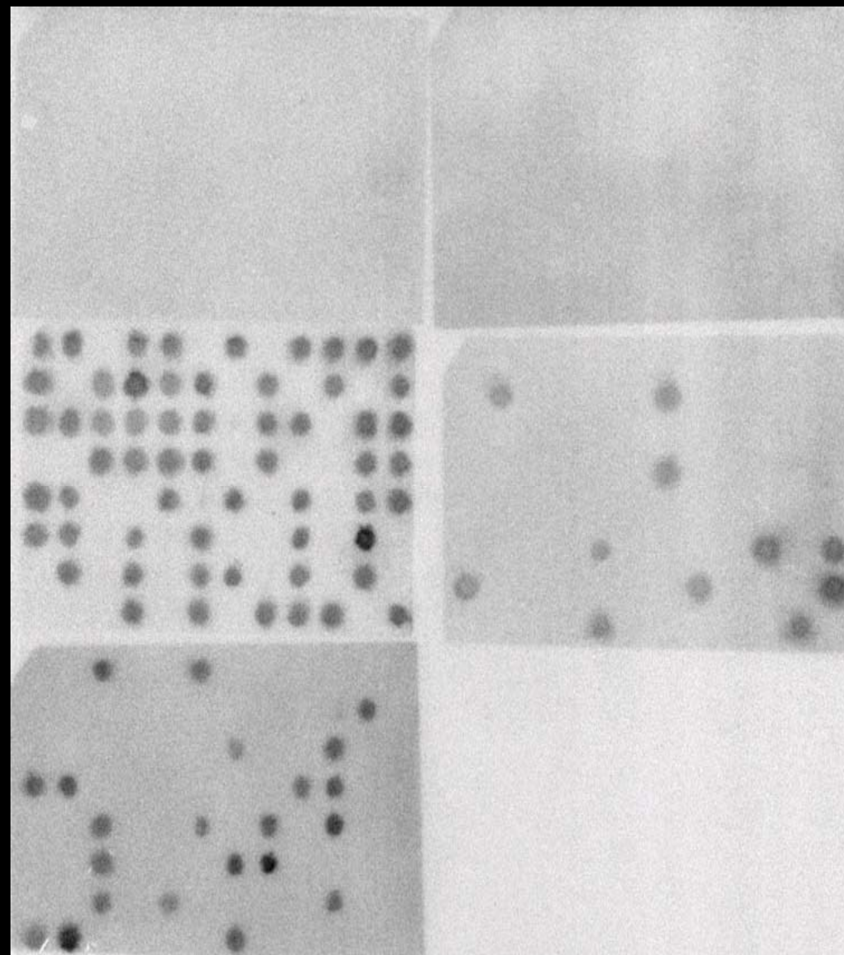
Oligonucleotide Probe hybridization

C

G

T



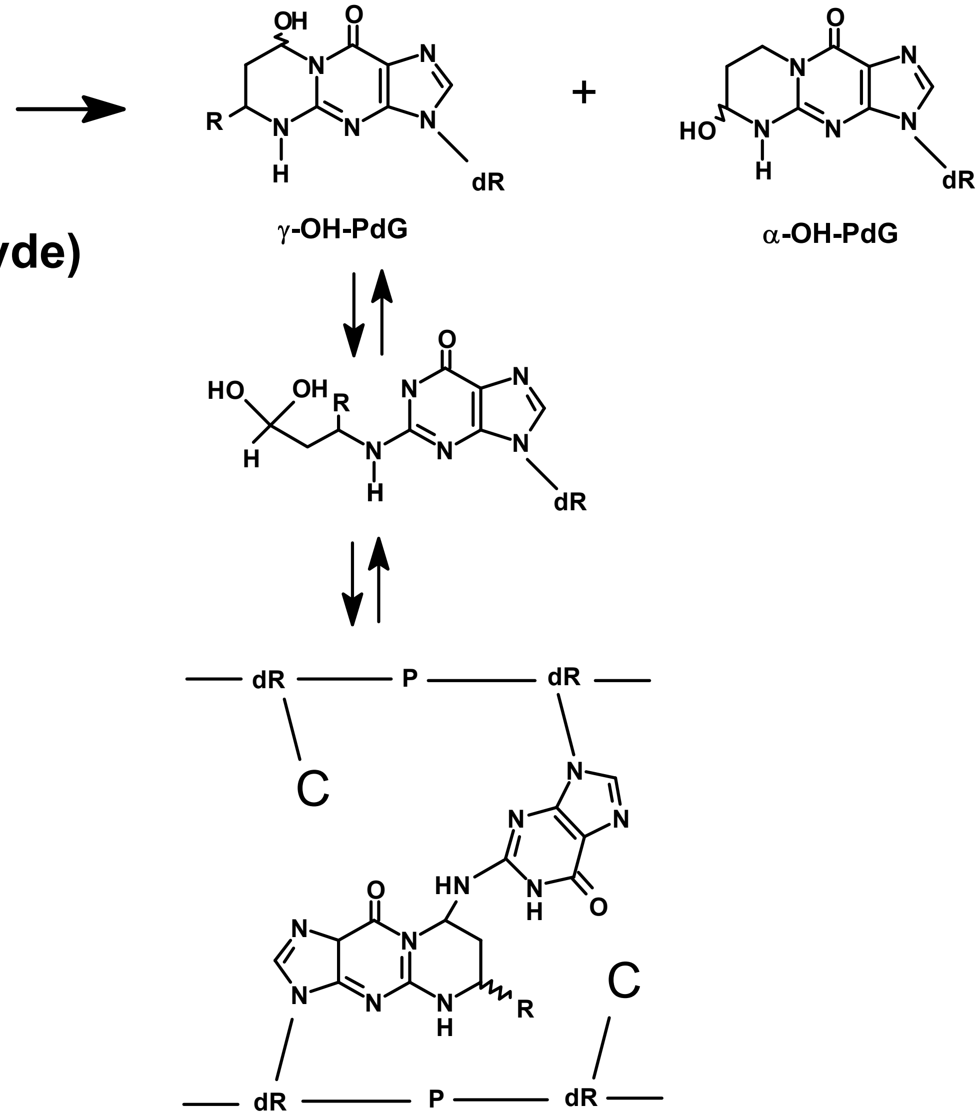
Δ

A

dG in DNA

+

Bifunctional aldehyde (acrolein, crotonaldehyde)



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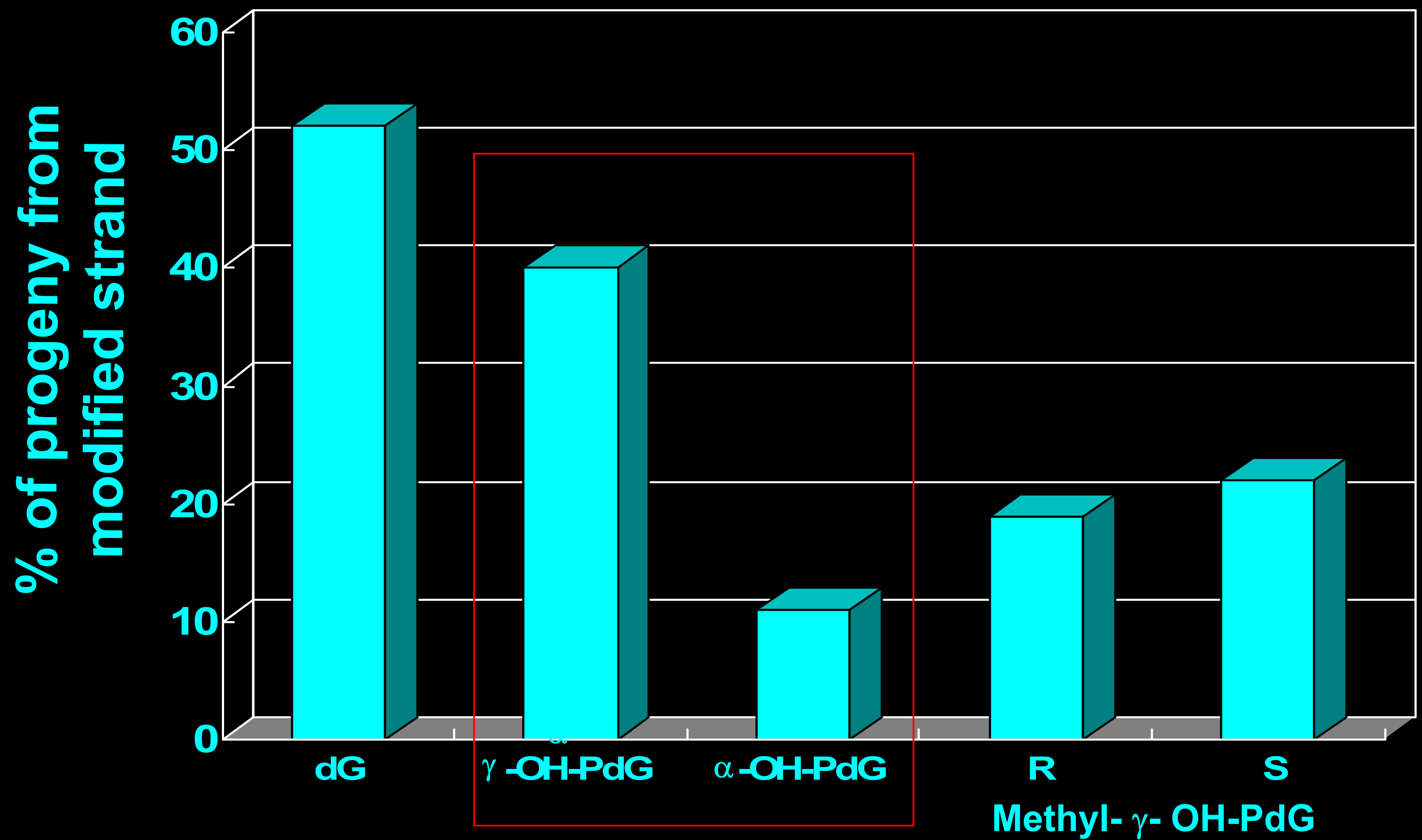
Efficiency of translesion synthesis across monoadducts in human XPA cells



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Mutagenicity of PdG adducts

Host	Adduct	Miscoding Frequency (%)	Miscoding Specificity
Human XPA cell	S isomer	10	T > C, A
	R isomer	5	T > A, C
	α-OH-PdG	11	T > C, A
	γ-OH-PdG	< 0.4	-----

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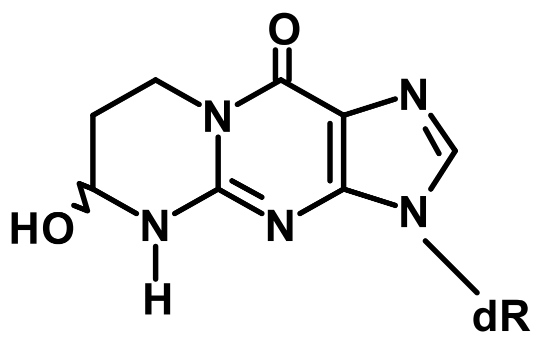
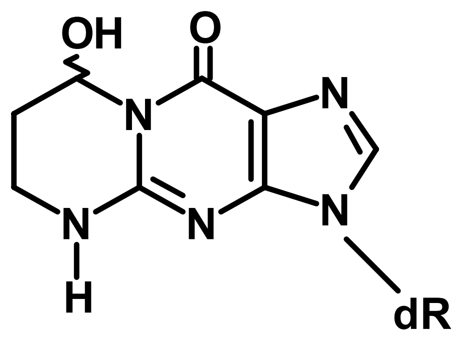
Genotoxic (point mutations) potency

= TLS efficiency x Fidelity

CH_3- γ -OH (*S*) > CH_3- γ -OH (*R*), α-OH >> γ – OH-PdG



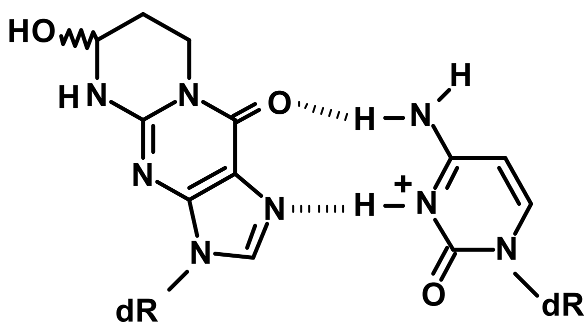
α(6)-OH-PdG



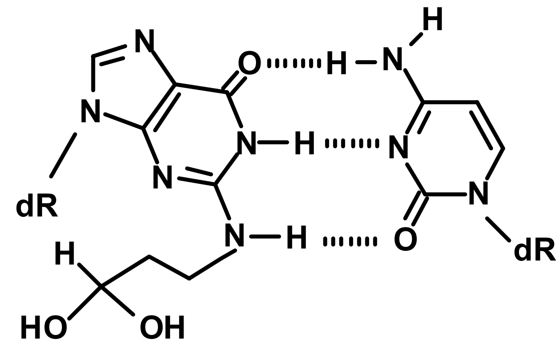
γ(8)-OH-PdG

S. Khullar, Y. Huang, F. Johnson (SUNY)

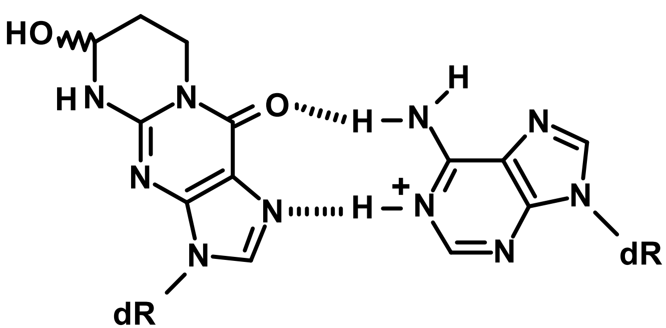
Non-mutagenic and mutagenic pairing



α-OH-PdG(*syn*) dC^{+}(*anti*)



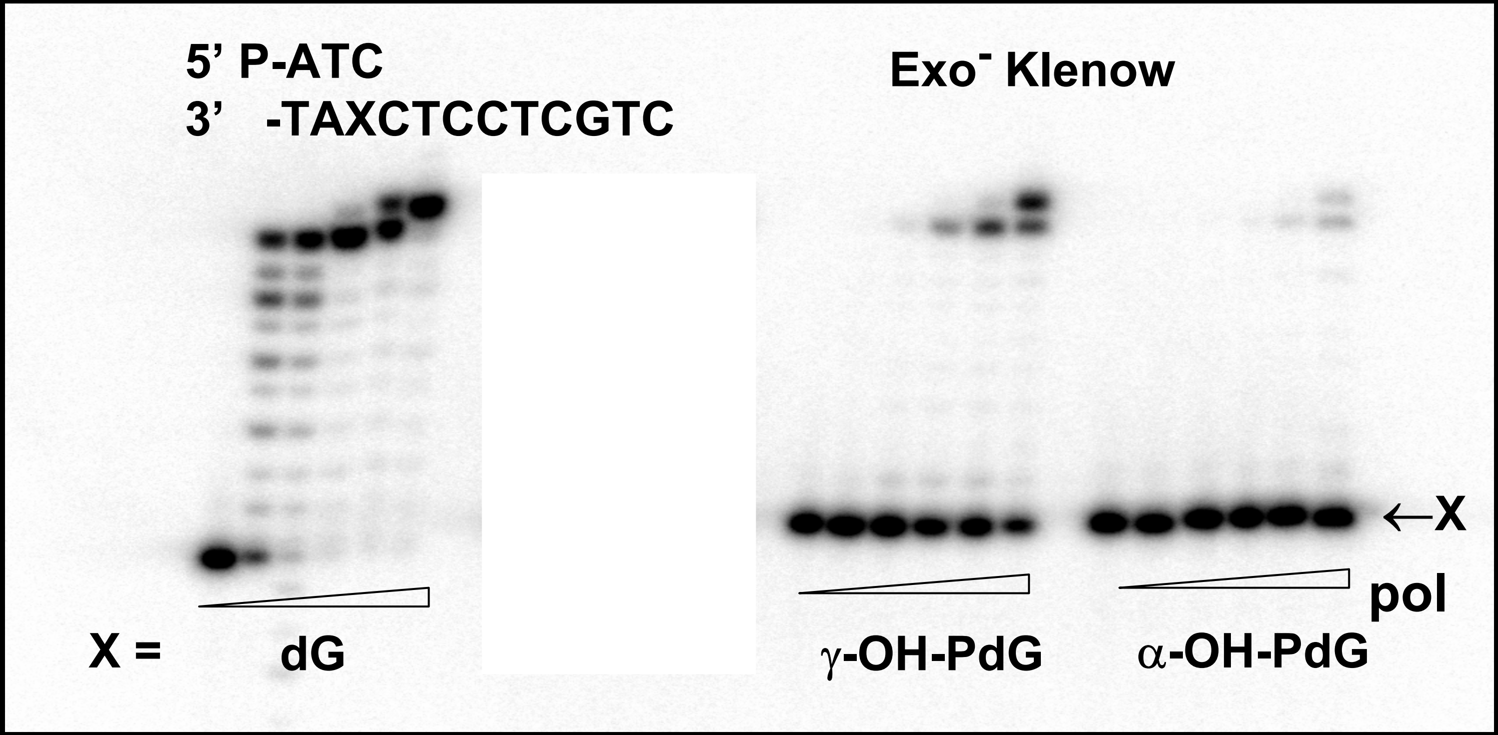
γ-OH-PdG(*anti*) dC(*anti*)



α-OH-PdG(*syn*) dA^{+}(*anti*)

T. Zaliznyak, C. de los Santos (SUNY)

Extension from C terminus opposite adducts



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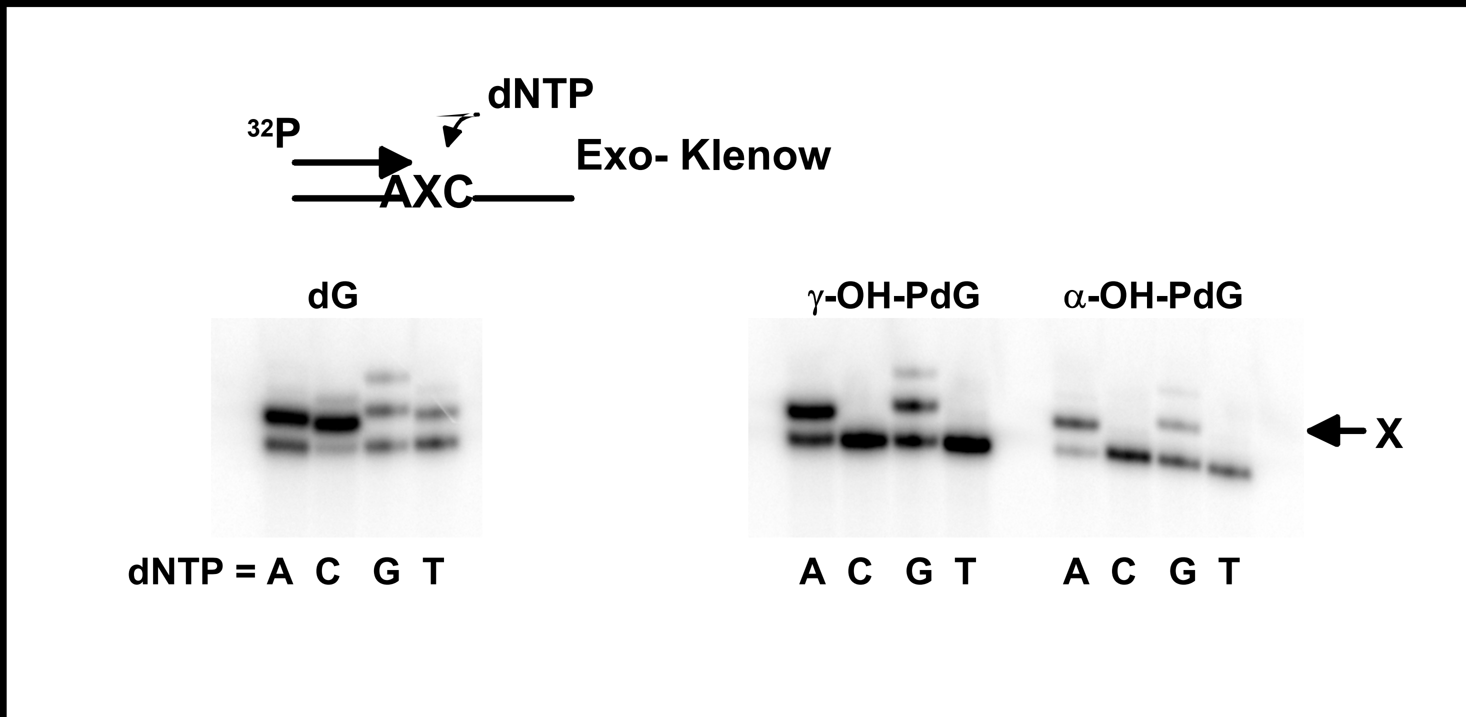
Incorporation of dNTP opposite a DNA adduct



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Eukaryotic DNA polymerases

Name	Pol family	Function(s)
Pol α	B	Priming DNA synthesis
Pol β	X	Base excision repair
Pol γ	A	mtDNA replication/repair
Pol δ	B	DNA replication/repair
Pol ε	B	DNA replication/repair
Pol η	Y	Translesion synthesis
Pol κ	Y	Translesion synthesis
Pol ι	Y	Translesion synthesis
Pol ζ	B	Translesion synthesis
REV 1	Y	Translesion synthesis
Pol θ	A	DNA cross-link repair
Pol μ	X	NHEJ
Pol λ	X	DSB repair, BER?
Pol σ	X	Sister chromatid cohesion

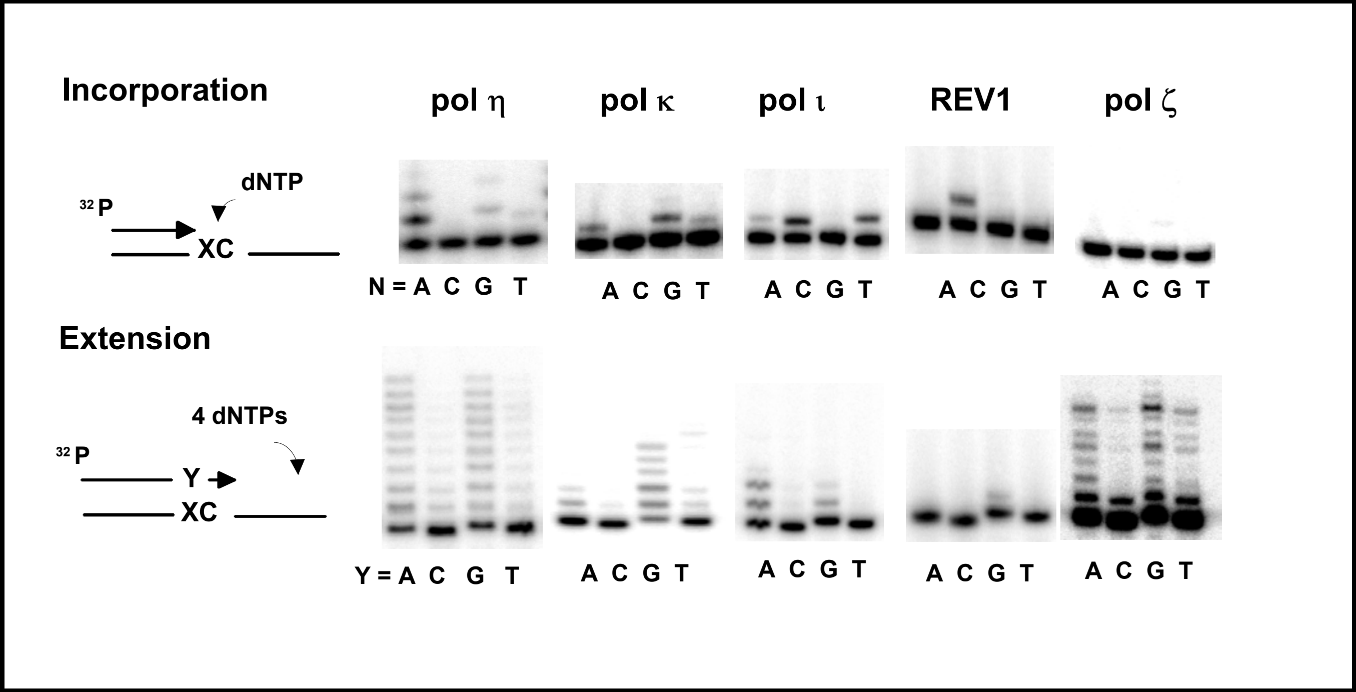
Pol η contributes to α-OH-PdG→T mutations

Host	α-OH-PdG →G, T, A, C				MF(%)
	G	T	A	C	
XPA	242	17	4	7	10.4
XPV (CTag)	371	2	2	0	1.1
XPV (XP30RO)	232	0	0	0	< 0.5
XPV- *mXPV*	316	17	4	2	6.8*

* $P < 0.001$

I.- Y. Yang (SUNY)

In vitro translesion synthesis catalyzed by specialized polymerases



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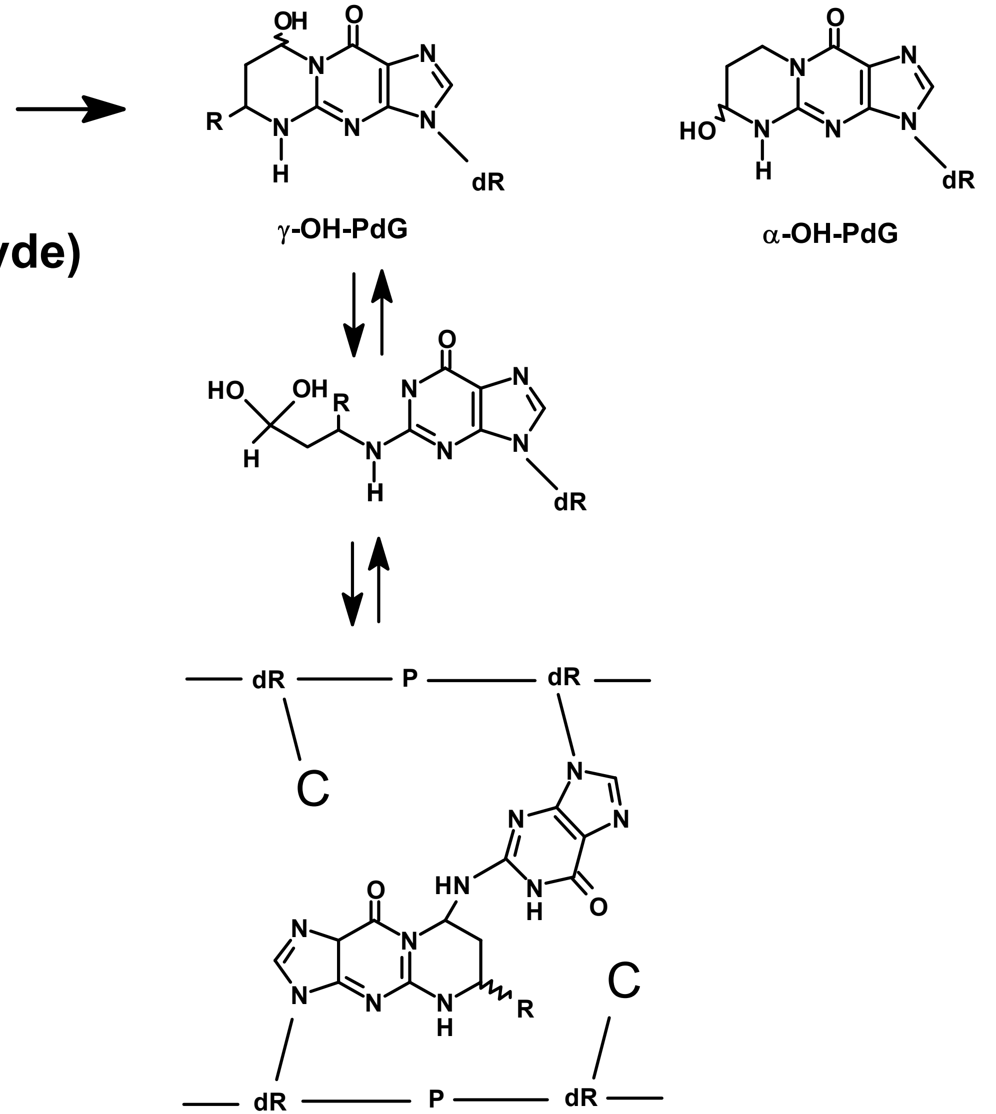
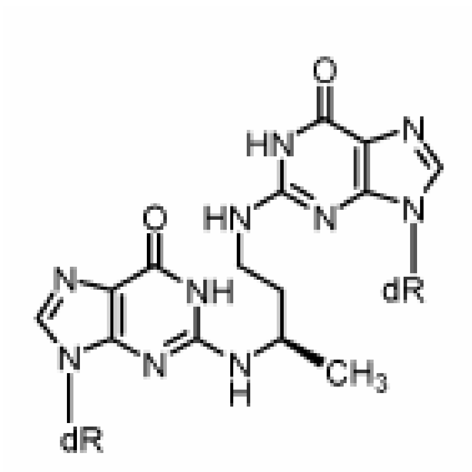
dG in DNA

+

Bifunctional aldehyde
(acrolein, crotonaldehyde)



Model ICL



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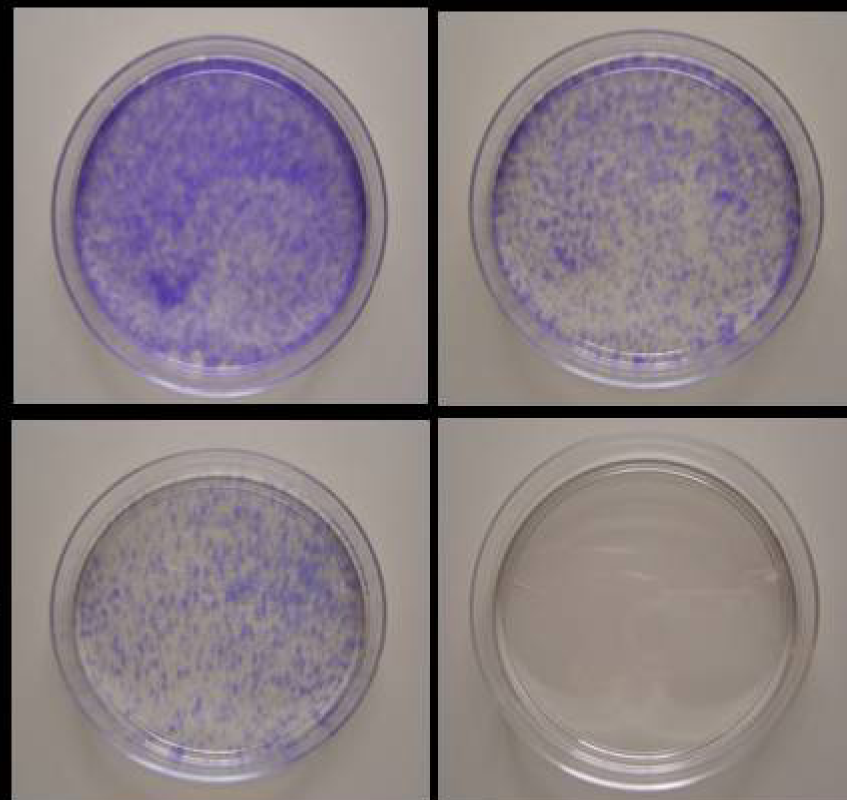
Unmodified plasmid in XPA

Modified plasmid in XPA

Replication competent plasmid

Replication incompetent plasmid

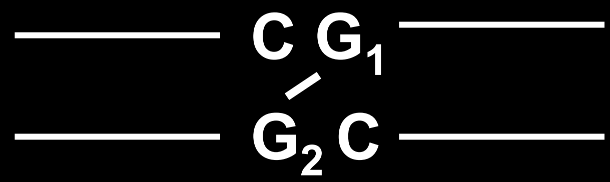


Selected for blasticidin S

X. Liu, I.-Y. Yang (SUNY)

Repair events in human cells

XPA, NER+: 2~3% T



NER+: ~0.7% T

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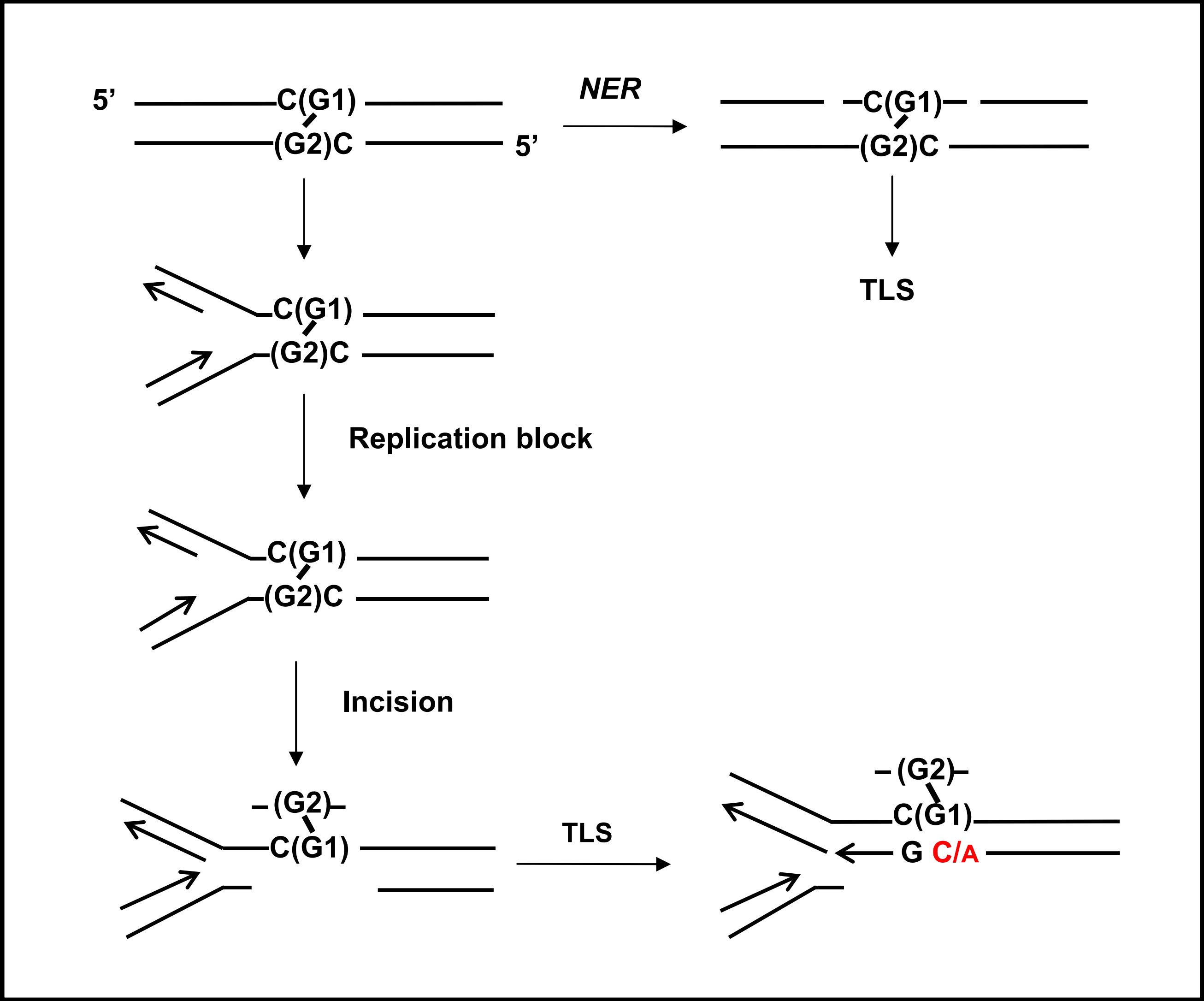
Replication-dependent and -independent ICL repair

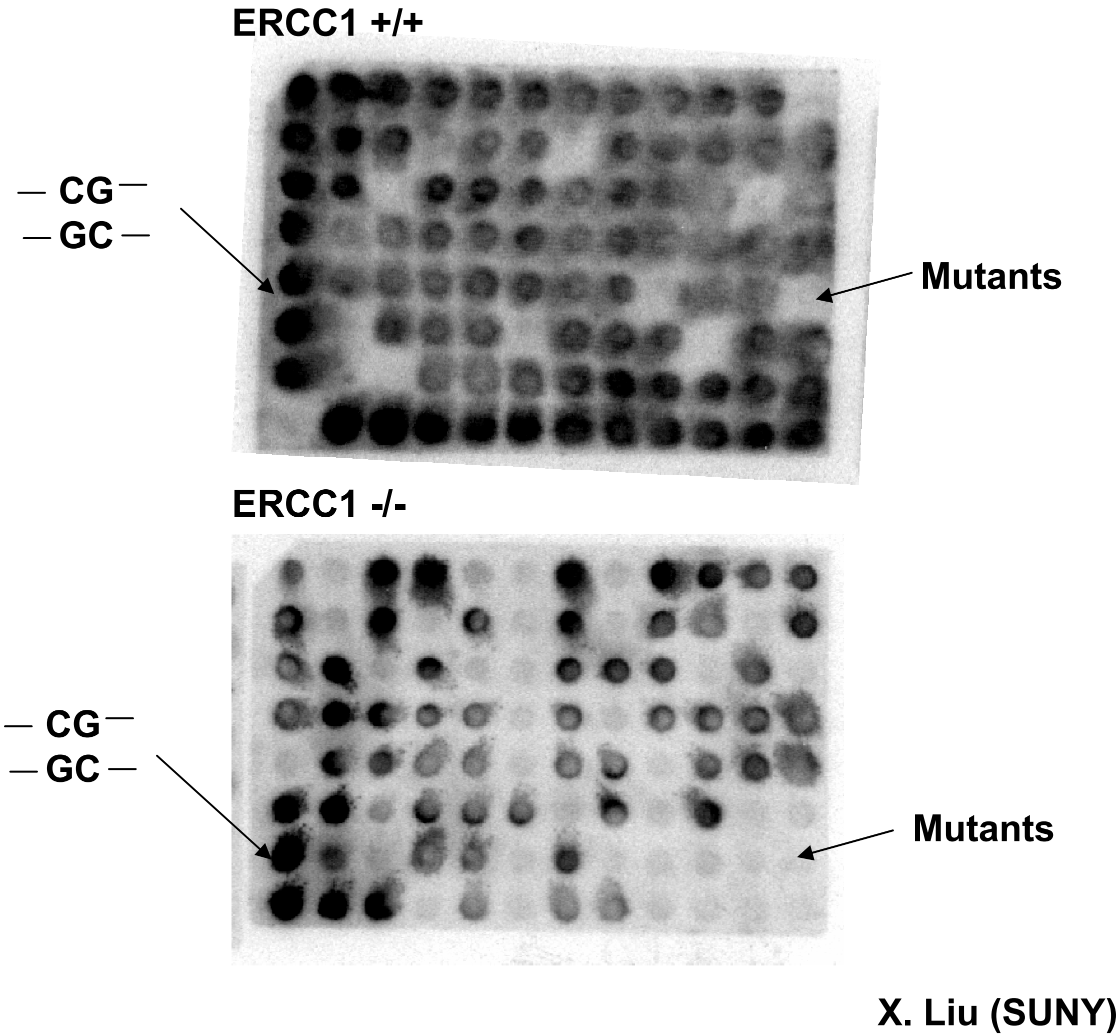


Limitation of human cells as a host for mechanistic studies

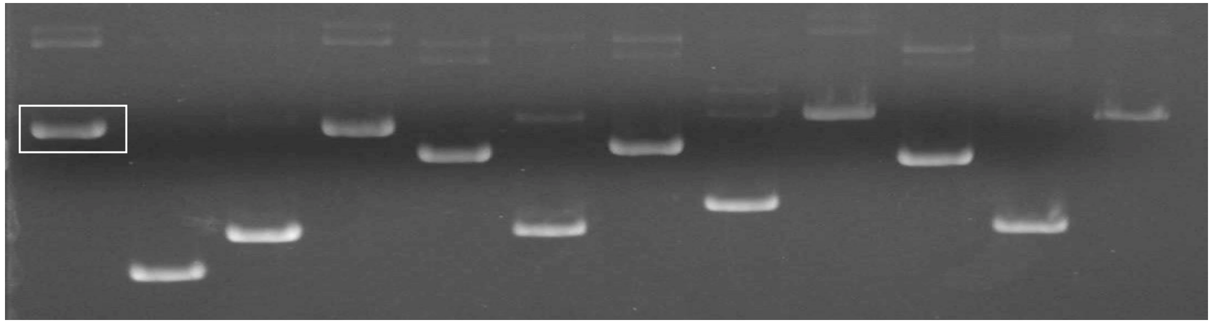
- Lack of various mutant cell lines

↓

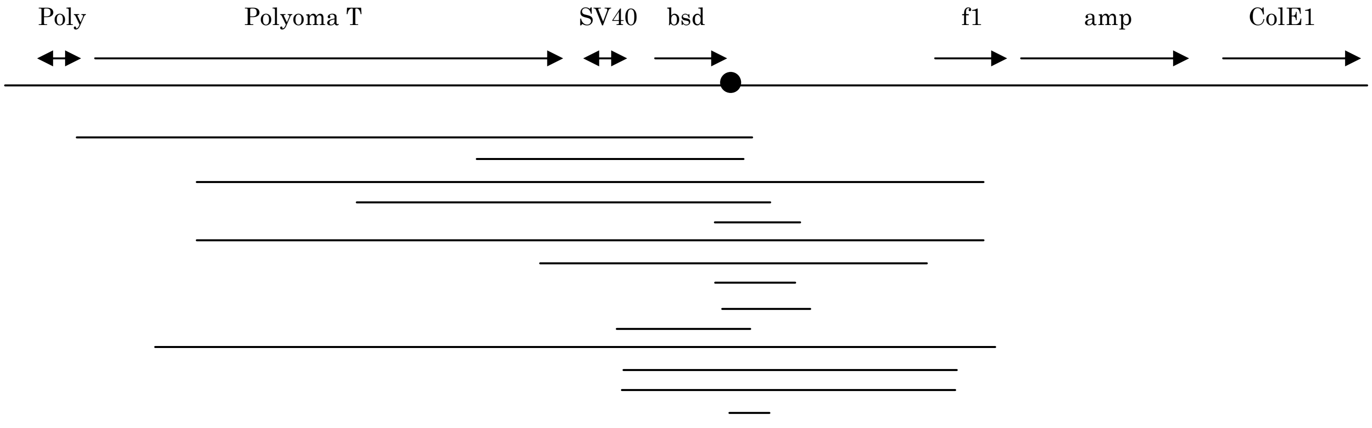
Use of RNAi or gene KO mouse cells as a host



ERCC1 +/+
CG
GC
Mutants
ERCC1 -/-
CG
GC
Mutants
X. Liu (SUNY)

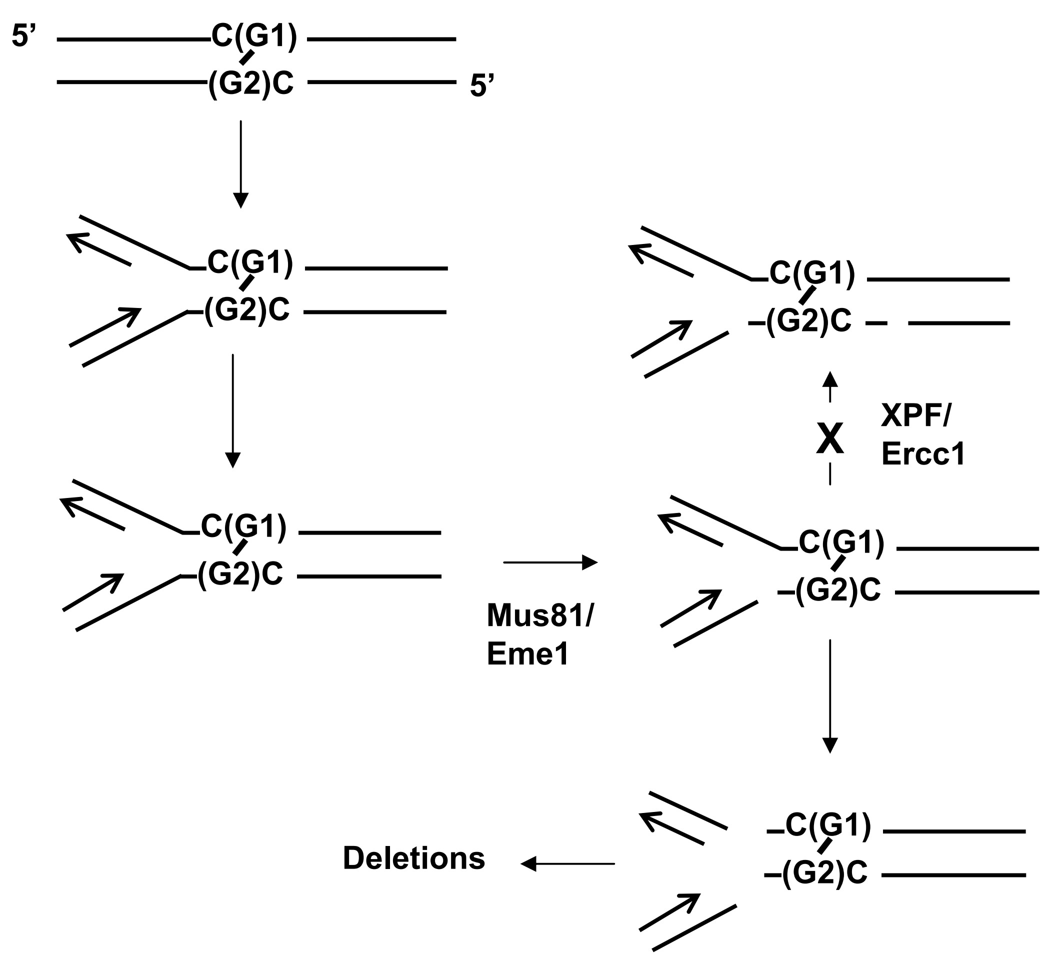


Plasmid map



Location and size of deletion

X. Liu (SUNY)


5'
C(G1)
(G2)C
5'
C(G1)
(G2)C
C(G1)
(G2)C
C(G1)
(G2)C
Mus81/
Eme1
C(G1)
(G2)C
X
XPF/
Ercc1
C(G1)
(G2)C
Deletions

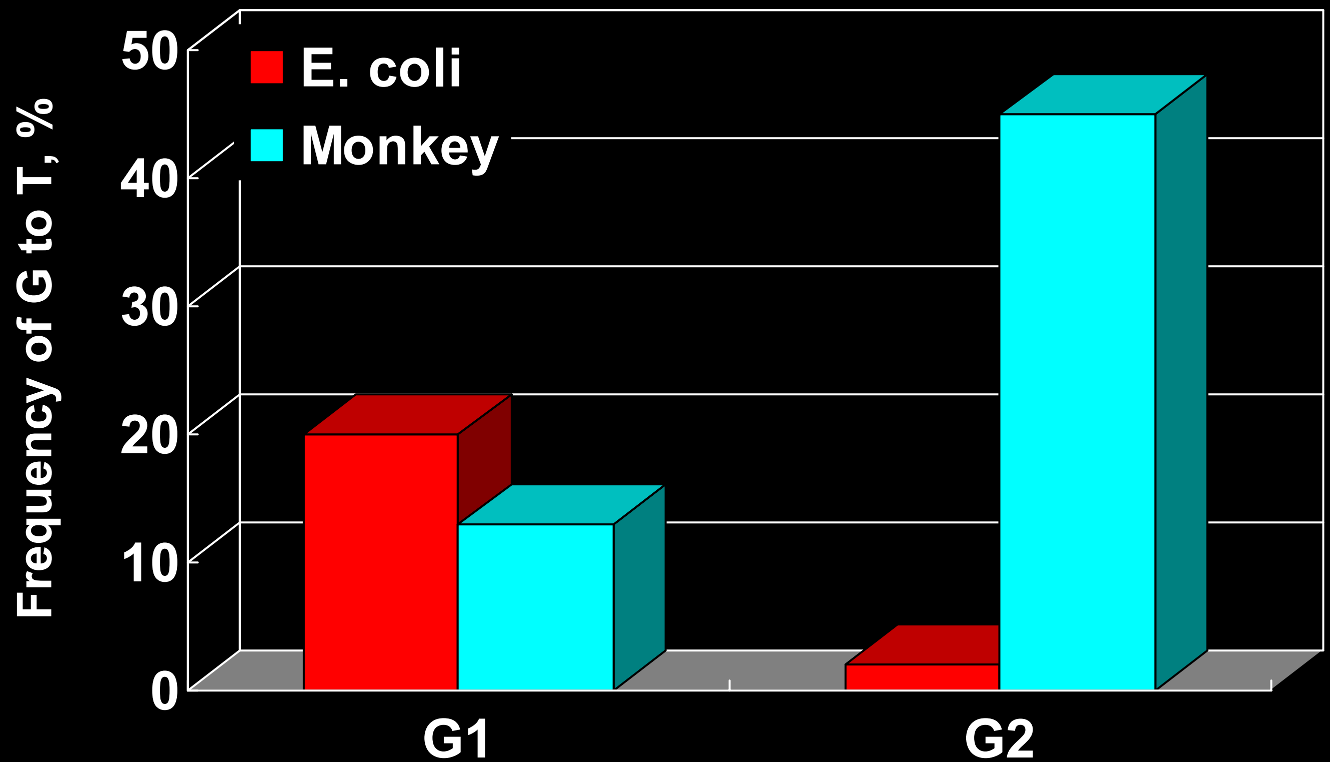
Site-specific approach

Characterization of genotoxicity of DNA adducts

Clear relationship between cause and effect

Quite labor-intensive

Effects of sequence context on B[a]P-dG-induced G $\rightarrow$ T mutations



5’ CTG$_1$G$_2$CC 3’

Moriya (SUNY)