Condensed History of DNA Repair with Emphasis on Small Lesion Repair – A Personal Perspective

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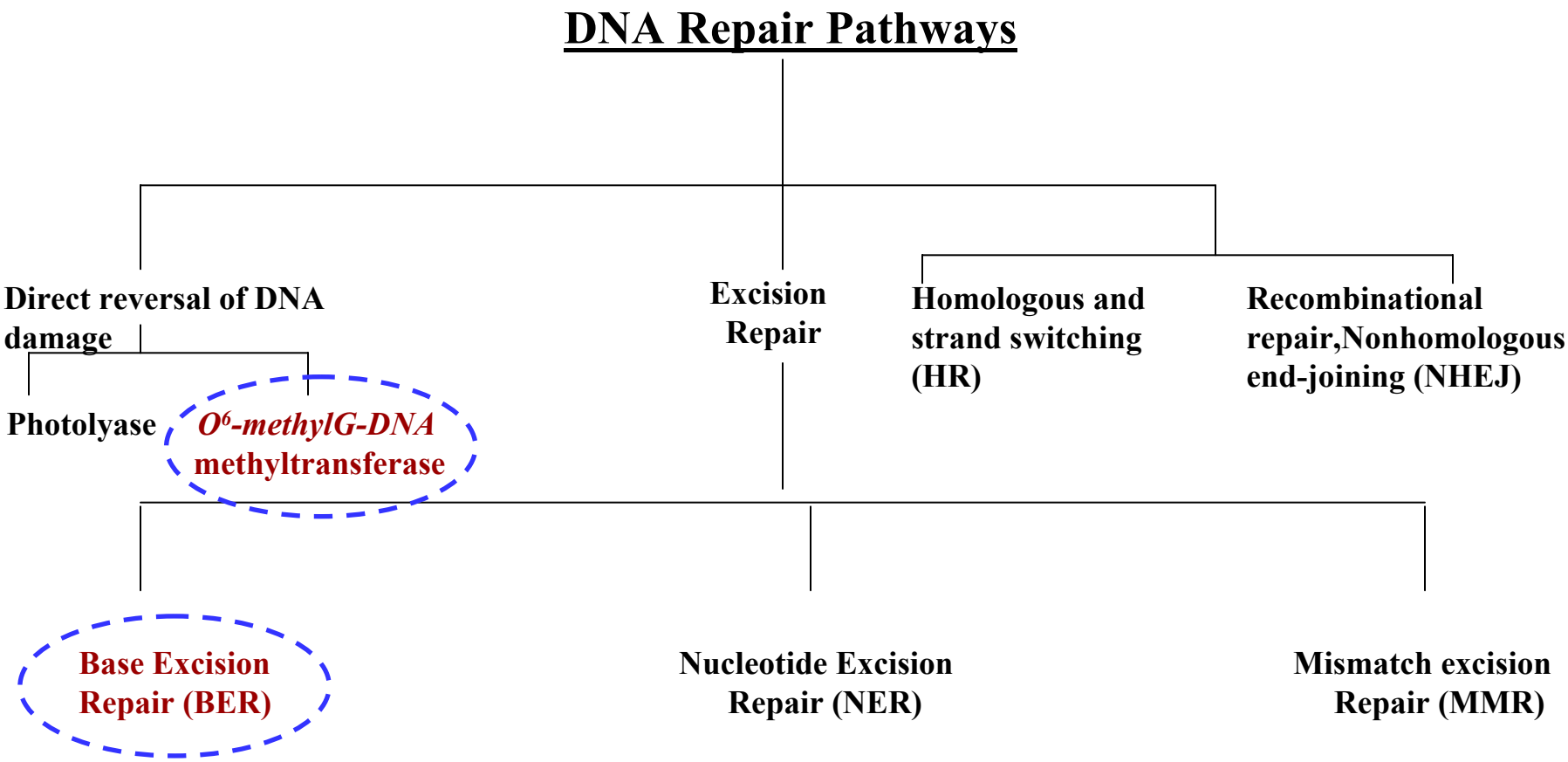
DNA Repair Videoconference
MD Anderson Cancer Center
Science Park
May 20, 2008

All history becomes subjective; in other words, there is properly no history, only biography.

Ralph Waldo Emerson

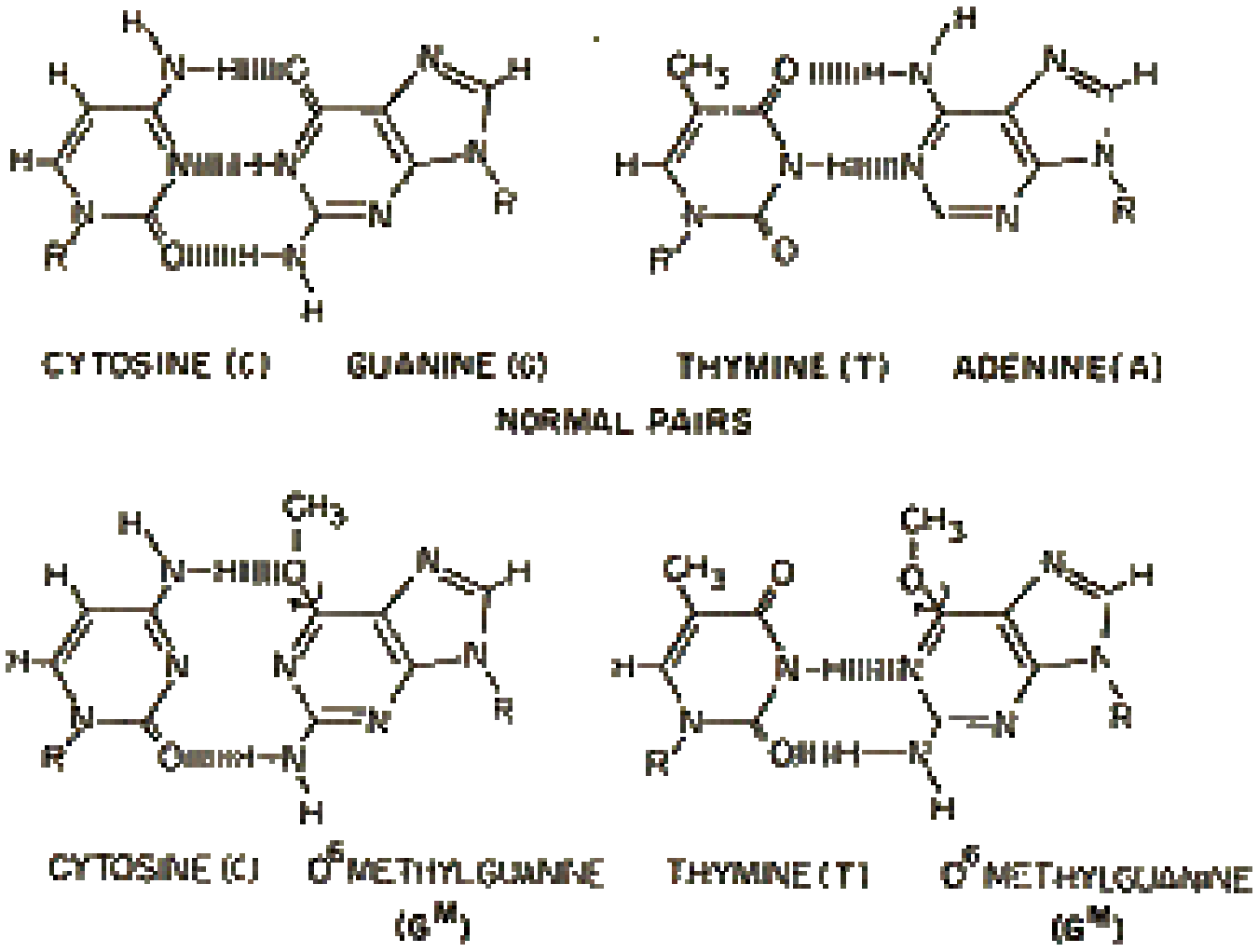
DNA Repair (A Recipe For Survival) Conserved Among All Organisms



Two Phases of DNA Repair Studies in Mitra Lab

1978 – 2000:	**Repair of alkylation damage in *E. coli* and mammalian genomes**
1992 – Present:	**Repair of oxidative damage in mammalian genomes.**

Mispairing of O^6-alkylguanine in DNA

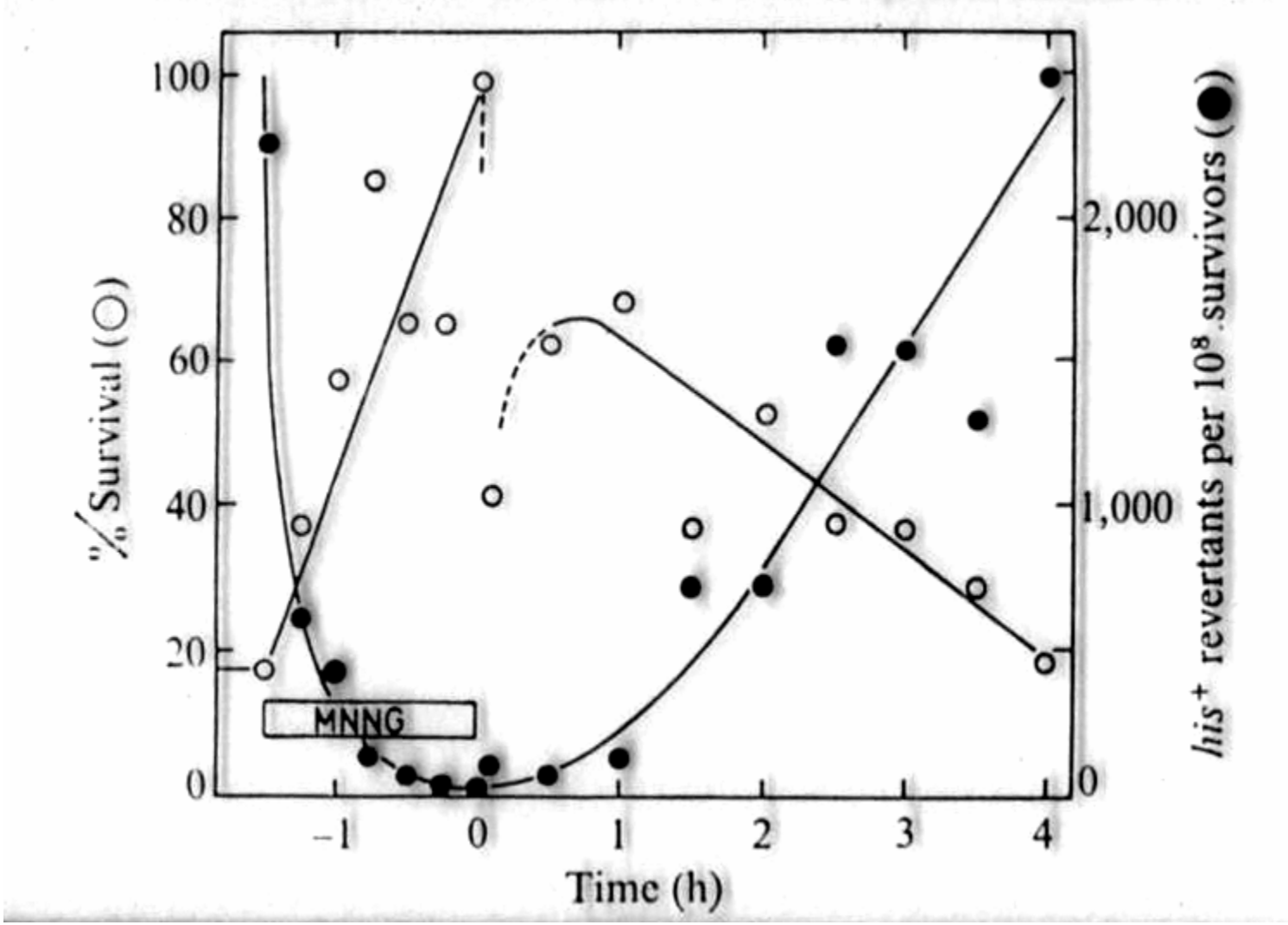


John Cairns': Seminal Contribution in Alkylation Damage Repair

* **Discovery of adaptive response and ada regulation in *E. coli*.**

* **Prediction of Ada as a suicide protein.**

Adaptive Response in *E. coli* to MNNG



(Samson and Cairns, 1977)

KEY FINDINGS ABOUT MGMT

- Loveless (1969): O^6-alkylguanine as a mutagenic base adduct.
- Rajewsky, Kleihues (1974): Correlation of m^6G level to alkyl nitrosamine-induced tumors.
- Magee, Montesano, Pegg (1983-1989): Alkylating carcinogens, potential role of m6G.
- Samson and Cairns (1977): Adaptive response of *E. coli* to MNNG and discovery of inducible *ada* regulon.
- Lindahl, Mitra (1980): Characterization of Ada as m^6G-DNA methyltransferase (MGMT).
- Saffhill, Mitra, Essigmann, Barbacid (1979-1984): m^6G, a mutagenic lesion *in vitro* and *in vivo*.

KEY FINDINGS ABOUT MGMT (CONT'D)

- Lindahl (1983): Repair of primary CNU adduct by MGMT.
- Lindahl (1985): Characterization of *E. coli ada* gene.

  Strauss and Day (1980): Lack of m^6G repair in Mex $^-$/Mer $^-$ tumor cells.
- Samson (1990): Presence of MGMT in yeast.
- Mitra, Sekiguchi, Karran (1990): Cloning of mammalian MGMT cDNA.

KEY FINDINGS ABOUT MGMT (CONT'D)

- **Dolan, Pegg (1990): Identification of O^6-benzylguanine as a pseudosubstrate inhibitor of MGMT.**
- **Brent, Thomale, Rajewsky, Ikenaga (1985-1987): Correlation of MGMT level and CNU resistance.**
- **Brent, Mitra (1991): Cloning and characterization of hMGMT promoter.**
- **Mitra, Boldogh (1998-1999): Multiple signaling pathways for MGMT activation.**
- **Pieper and Erickson, Ikenaga, Brent, Mitra (1991-2000): Repression of MGMT gene due to CpG methylation.**

Key Discoveries in Alkylation Damage Repair

1980: Discovery of Ada and its reaction mechanism with a sensitive, quantitative assay (collaboration with B. C. Pal and Bob Foote).

1982: First evidence for *in vivo* mutagenesis due to misreplication of O^6-methylguanine (Dodson and Masker).

Discovery of MGMT activity in mammalian cells (collaboration with A. Pegg).

1984: First kinetic analysis of O^6-methylG replication by prokaryotic DNA polymerases (Snow, Ph.D. thesis).

1988: Quantitation of MGMT in various Mex^+ and Mex^- tumor cells (collaboration with Day, Yarosh, Ikenaga).

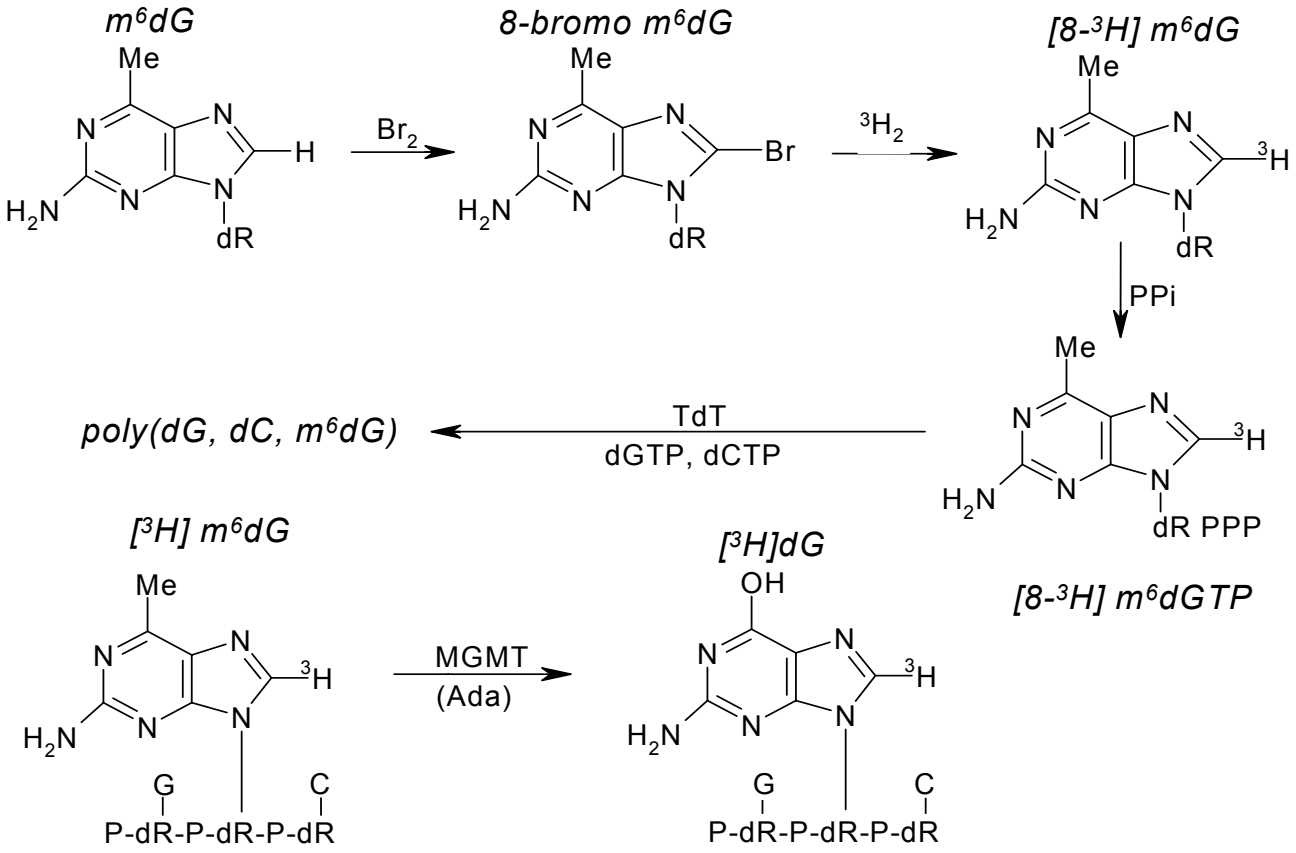
1990: Cloning of human MGMT cDNA by phenotypic complementation in *E. coli* (Tano, Shiota)

1991: Cloning of human MGMT promoter (collaboration with Tom Brent).
(1992: Cloning of human MPG cDNA)

1998: Regulation of MGMT by gluacorticoid and protein kinase C.

2000: Mechanism of MGMT extinction due to CpG methylation.

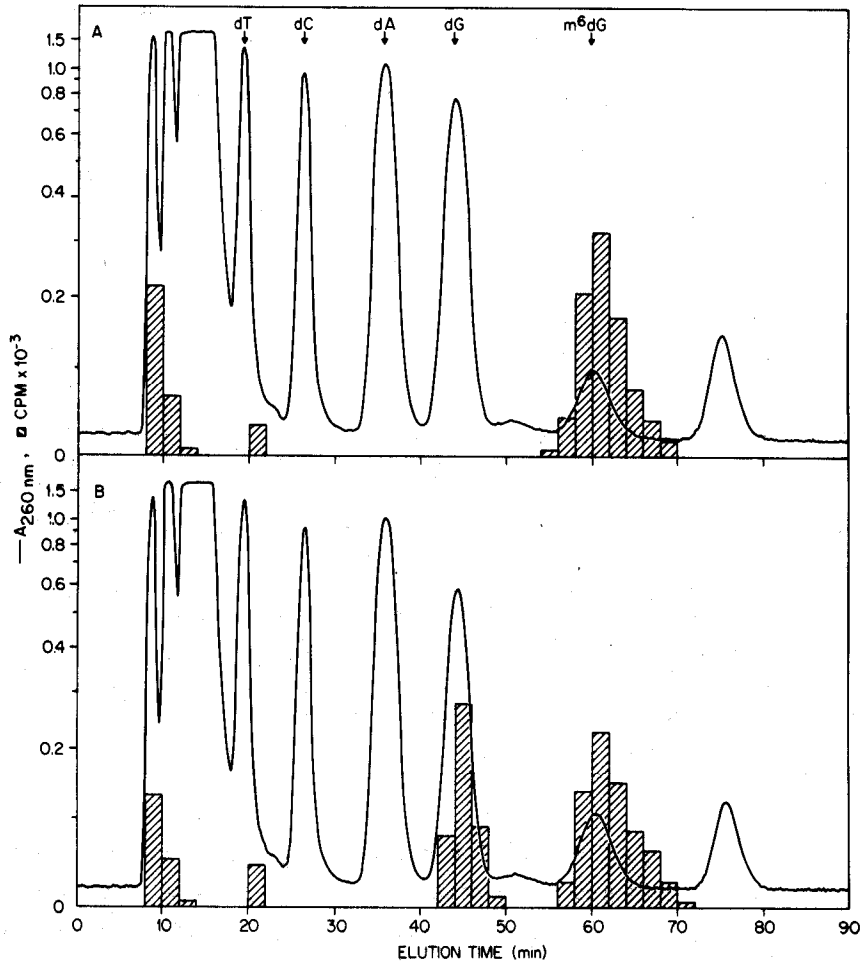
Synthesis of m^{6}G-containing DNA and Quantitation of MGMT



$$\text{No. of transferred methyl groups} = \frac{[^3\text{H}]\text{dG}}{[^3\text{H}]\text{dG} + [^3\text{H}]\ \text{m}^6\text{dG}} \times \text{number of m}^6\text{G residues}$$

(Foote *et. al.*, BBRC,1980)

MGMT assay with poly (dC, dG, m^{6}G)



Chromatographic analysis of enzymatic hydrolysates of recovered poly(dC,dG,[8-^{3}H]m^{6}dG) after incubation for 1 hr at 37° with extract from (A) nonadapted and (B) adapted cells. Each incubation contained 240 pmol (2600 cpm) of [^{3}H]m^{6}dG (contained in polymer) and 4 mg of cell extract protein. Background radioactivity of 22 cpm was subtracted from each fraction. The normal unlabeled deoxynucleosides arose from hydrolysis of endogenous DNA contained in cell extracts.

Unadapted cells

Adapted cells

(Foote *et. al.*,1980)

Number of O^6-methylguanine-DNA methyltransferase molecules in a wild-type and ada *E. coli* strains

Strain	Relevant genotype	No. of cells used in extract (10^9)	No. of O^6-methylguanine molecules demethylated (10^{11})	No. of methyltransferase molecules per cell	O^6-Methylguanine demethylated (pmol/mg of protein in extract)
F26	B/r *his thy*	5.0	2.0	40	1.09
AB1157	F⁻ *thr-1 leu-6 proA2 his-4 thi-1 argE lacY1 galK ara-14 xyl-15 supE44*	5.5	1.7	30	1.1
BS21	F26 *adc*	0.18	12	6,700	310
BS23	BS21 *ada*	18	4.1	23	0.54
PJ1	AB1157 *ada-1*	20	2.5	13	0.33
PJ6	AB1157 *ada-6*	11	2.6	24	0.51
BK2106	F⁻ *his tag-2 ada*	20	3.6	18	1.04

The residual MGMT activity in ada mutant is very likely to be due to constitutive Ogt

(Mitra *et.al.*, 1982)

MGMT levels in Mex$^+$ and Mex$^-$ HeLa Cells

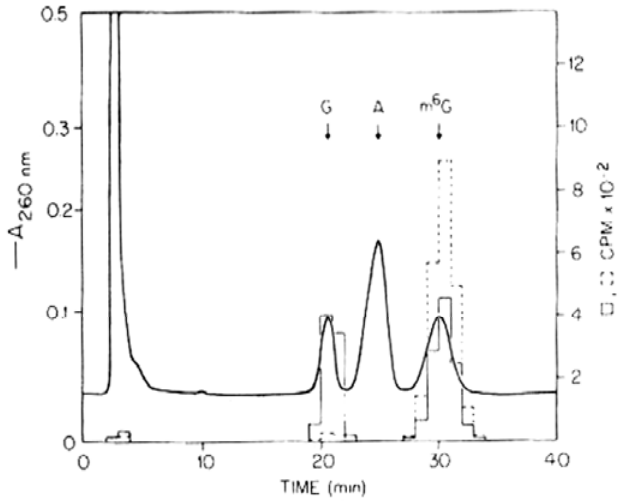


Fig. 1. Chromatographic analysis of acid-released purines from DNA after a 4-h incubation of poly(dC,dG,[8-^{3}H]m^{6}dG) with extract of 2.5 × 10^6 HeLa CCL2 cells (solid bar), or without extract (dashed bar). Details of the assay are given in Materials and Methods. Background radioactivity of 23 cpm was subtracted from each fraction. The elution positions of guanine (G), adenine (A) and O^6-methylguanine (m^{6}G) are indicated.

TABLE 1

O^6-METHYLGUANINE-DNA METHYLTRANSFERASE ACTIVITY OF HeLa CELL EXTRACTS

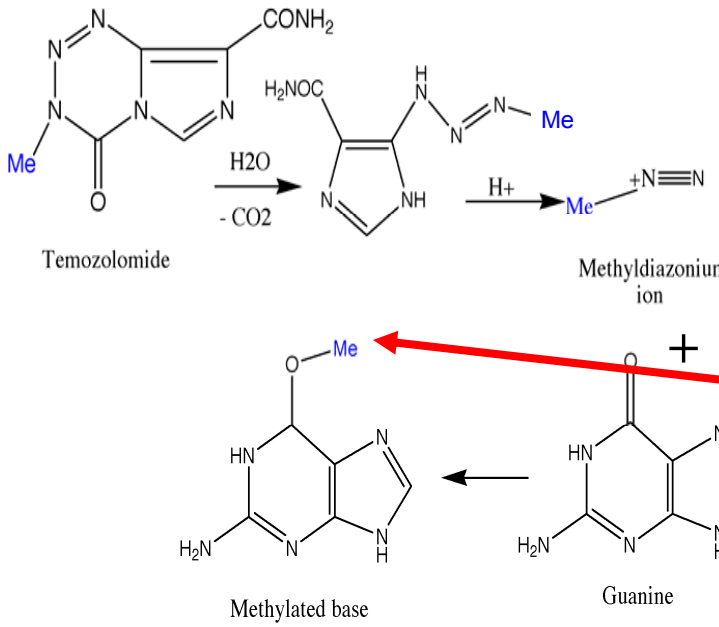
Strain	Extract No.	Cell equivalents of extract used in assay	pmoles of m^{6}G (in polymer) in assay	pmoles of m^{6}G demethylated	m^{6}G molecules demethylated per cell	pmoles m^{6}G demethylated per mg protein in extract
HeLa CCL2	1	2.0 × 10^6	1.0	0.32	96 000	0.49
	2	2.0 × 10^6	1.0	0.35	105 000	0.48
		2.0 × 10^6	10.0	0.38	114 000	0.53
HeLa S3	1	2.5 × 10^6	1.0	None detected	–	–
		1.0 × 10^8	1.0	None detected		–

Extracts were incubated with poly(dC,dG,[8-^{3}H]m^{6}dG) for 4 h as described in Materials and Methods, except that the assay of extract of 1.0 × 10^8 HeLa S3 cells was carried out in a 5.0-ml reaction mixture. Concentrations of HeLa CCL2 extracts were in the linear range of concentration dependence (Fig. 3).

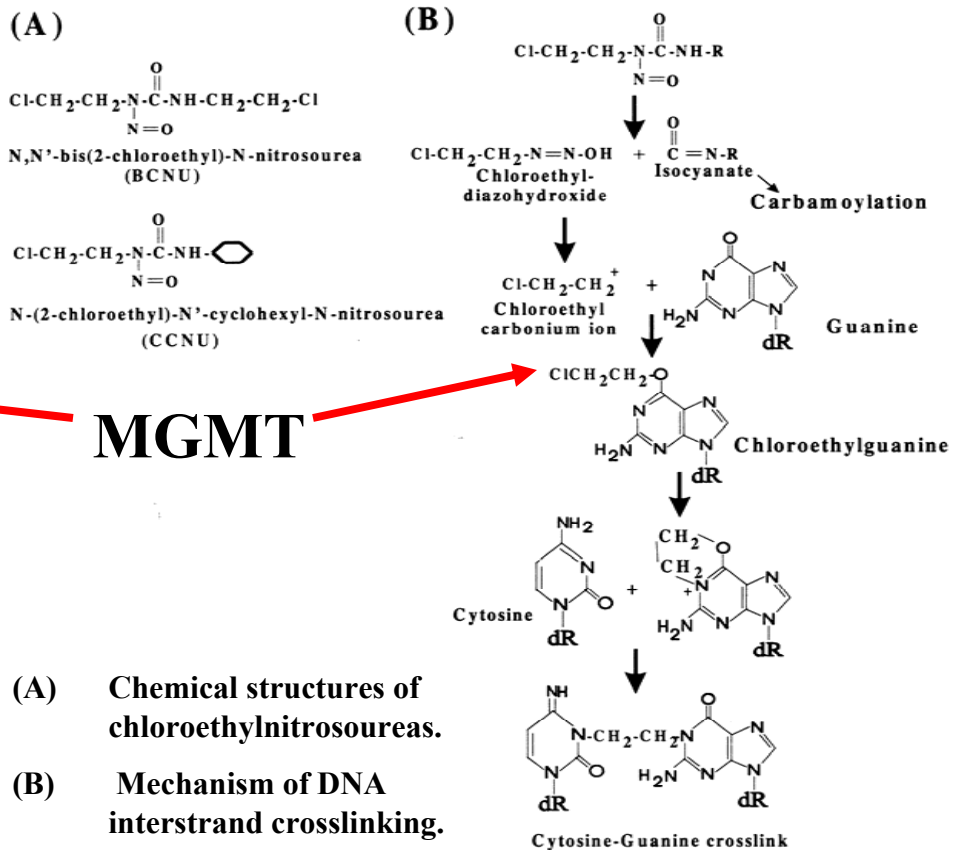
(Foote, 1980)

Formation of alkyl DNA adducts and crosslinks

Temozolomide (monofunctional)



Chloroethylnitrosoureas (bifunctional)



(A) **Chemical structures of chloroethylnitrosoureas.**

(B) **Mechanism of DNA interstrand crosslinking.**

(Anti-Cancer Drug Design 14:205-17, 1999)

O^6-methylguanine – A Pro-apoptotic DNA Lesion

* Marinus & Karran (1982) - m^6G could be repaired by the MMR pathway.

* Mitra (1990) - Cloning of MGMT human cDNA in *E. coli ada, based* on enhanced resistance to MNNG, indicates that MGMT substrates (m^6G or m^4T) are cytotoxic in *E. coli.*

* Thilly, Modrich, Karran, Kunkel, Day (1990-1995) – MMR-dependent cell death due to persistent m^6G in the genome. Recognition of m^6G•T pair by MutSα.

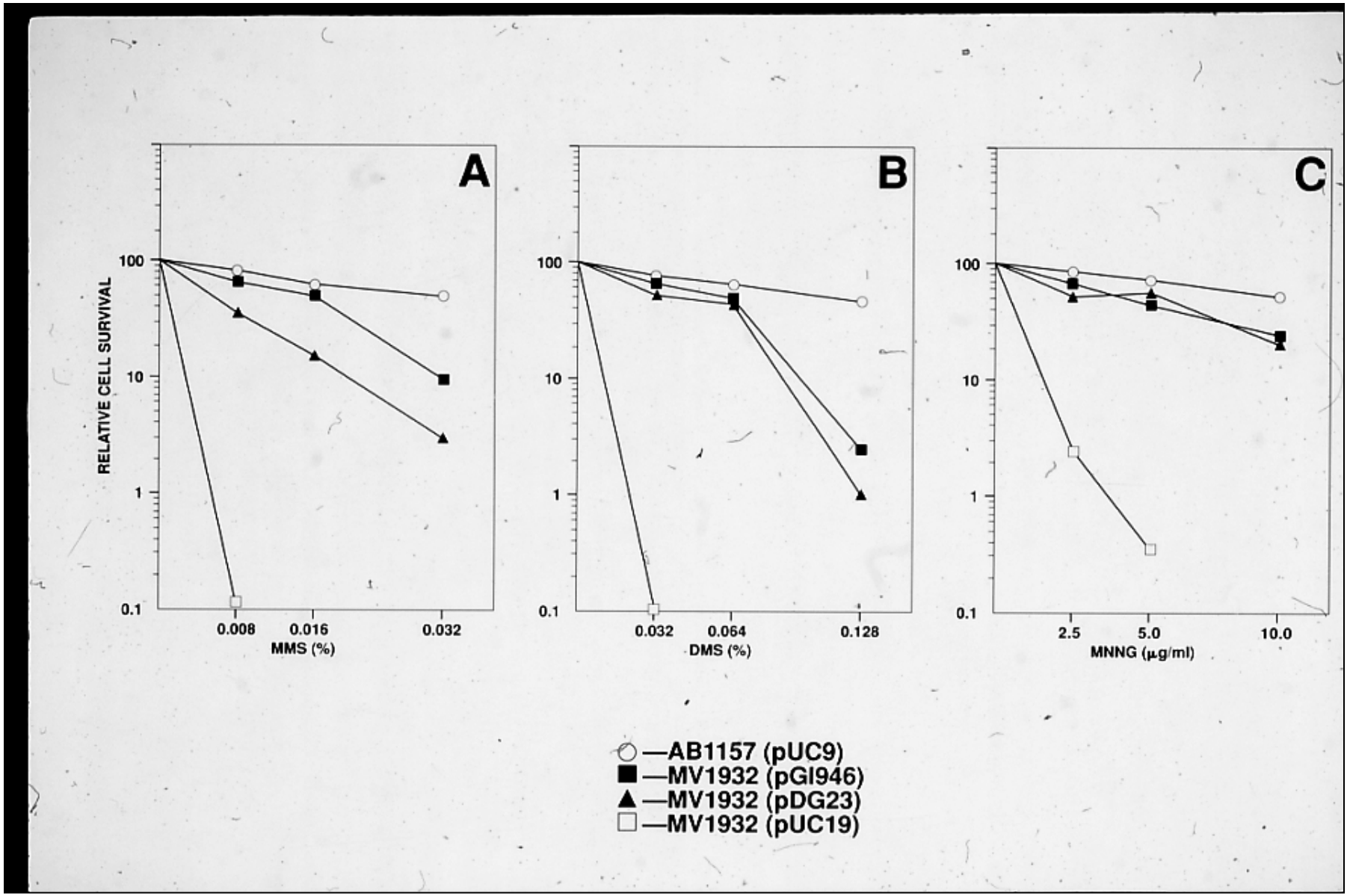
* Kaina, Samson, Sekiguchi (1997-1999) - m^6G triggers apoptosis in MGMT null ES cells and Mex^-/Mer^- fibroblasts and lymphoblastoids.

* Kaina (2004) - m^6G-induced apoptosis could be intrinsic (mitochondria-mediated) or ligand-mediated (Fas/p53-dependent).

Cloning of human MGMT cDNA by phenotype complementation in *E. coli*

(Tano, Shiota *et al*., *PNAS,* 1990*)*

MNNG resistance of *E. coli ada* expressing hMGMT



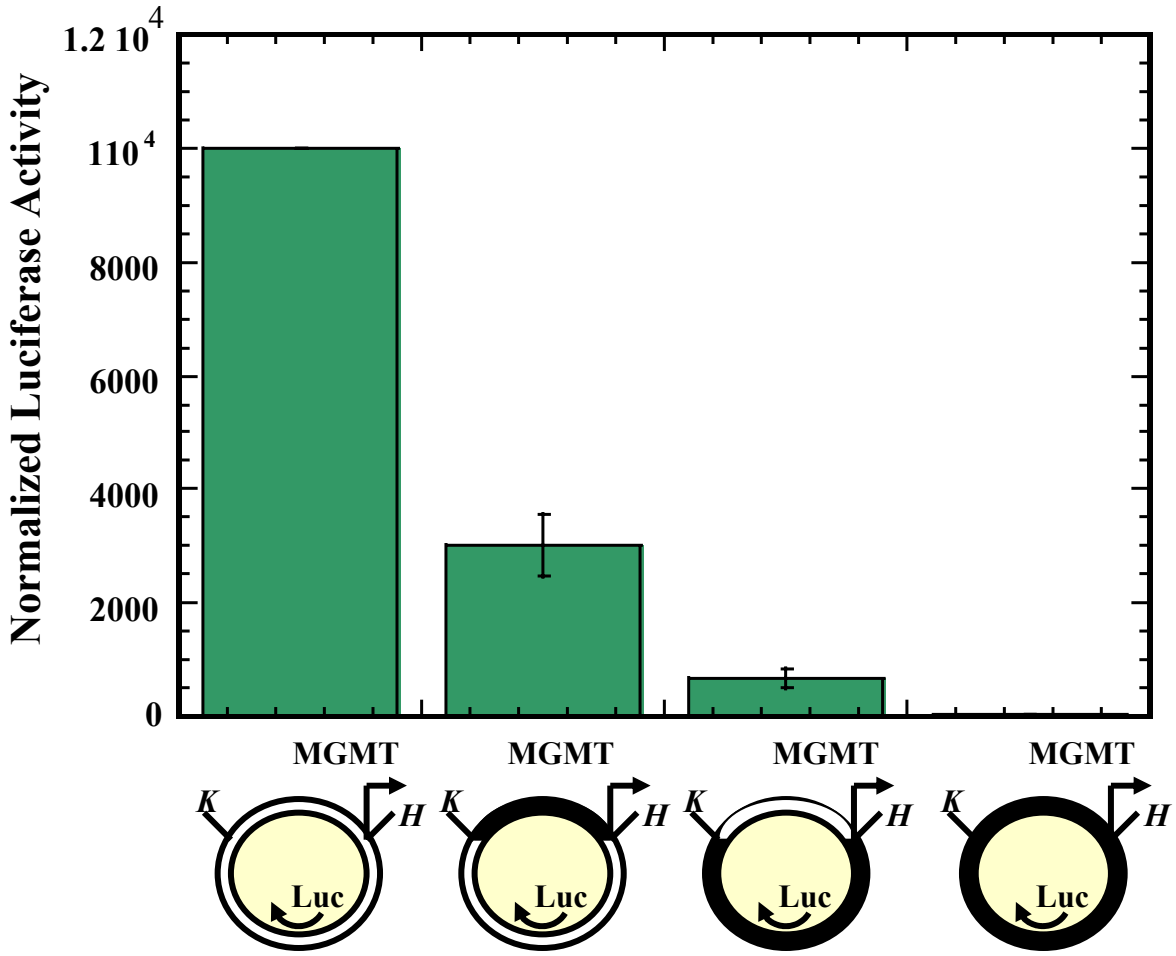
(Tano *et. al.*, 1989)

Mammalian MGMT and its regulation

- **MGMT level varies widely in a time- and cell-specific fashion.(Range 10^5 molecules/cell in hepatocytes to < 10^3 molecules/cell in lymphocytes)**

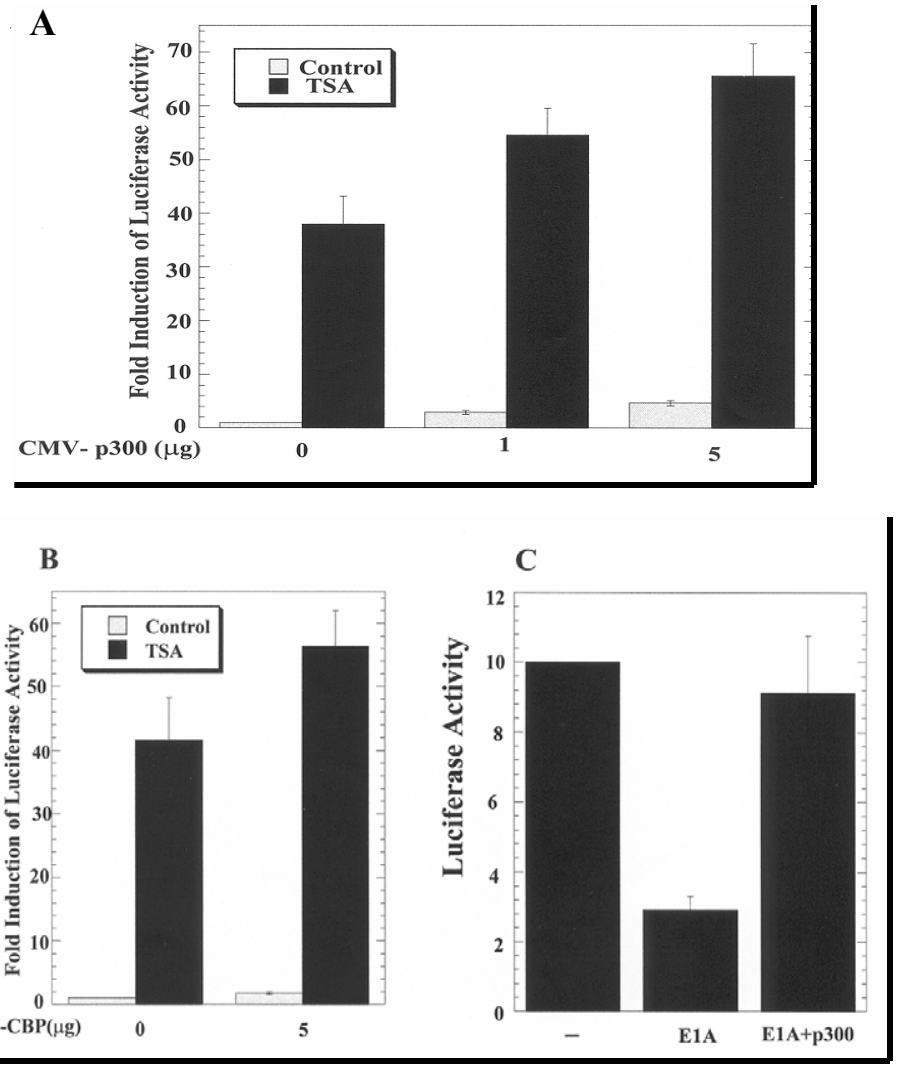
- **Certain primary tumors without apparent alteration of MGMT gene sequence have undetectable MGMT activity (<200 molecules/cell). Other tumors have >10 fold higher levels of MGMT relative to their progenitor cells.**

Transcriptional Effects of Methylation of MGMT Promoter and Body of the Plasmid



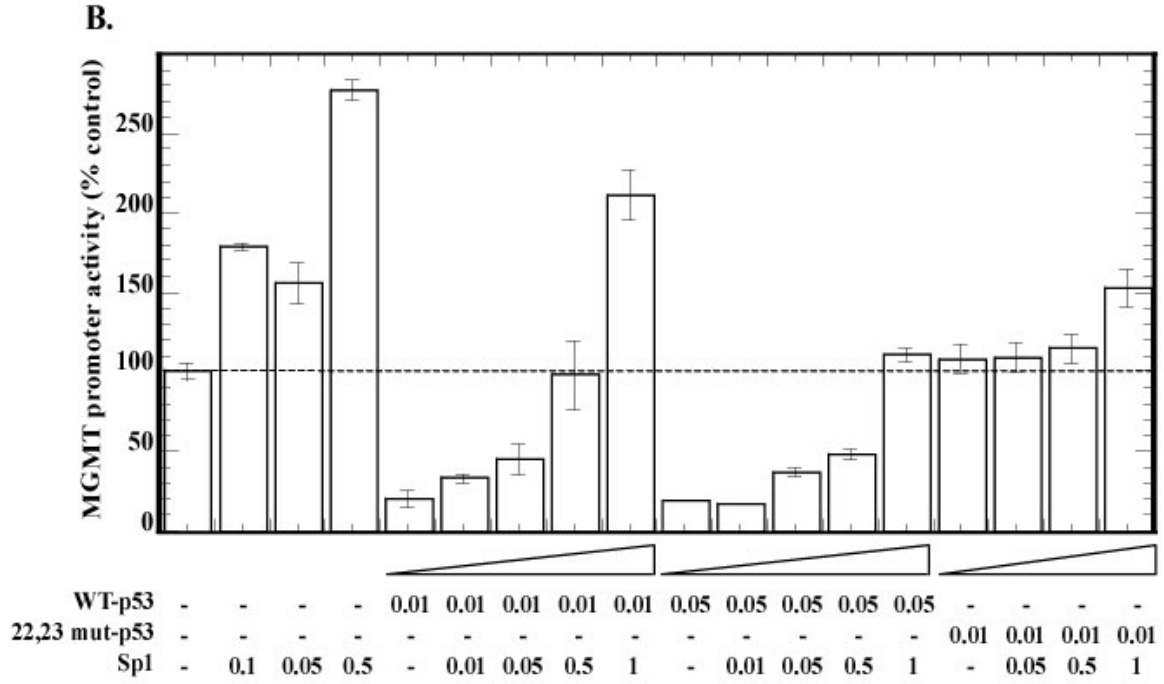
(Bhakat *et.al.*, 2003)

Activation of MGMT Promoter by Ectopic Expression of p300 and CBP



(Bhakat *et.al.*, 2000)

MGMT promoter activity, downregulated by WT-p53, is restored with co-transfection of Sp1



- **Expression of p53 (mutant or WT) does not affect expression of Sp1**
- **Sp1 co-transfection restored (1 kb) MGMT promoter activity in a dose dependent manner, but no effect observed with mutant p53**

(Bocangel, Bhakat)

Revelation (1992)

Oxidative damage is the mother of all damage!

Oxidative damage of the genome is extremely complex, and is primarily repaired via the base excision repair (BER) pathway.

Background of Oxidative DNA Damage

- Many investigators provided critical information regarding genotoxicity of reactive oxygen species.
- Early discoveries include identification of 8-oxoguanine as a major oxidized base lesion (Nishimura, Floyd).
- Multitude of other DNA oxidation products characterized and quantitated (Dizdaroglu, Cadet, Ames).
- DNA strand breaks due to sugar oxidation and base loss and by radiation (Ward, Breen).

Repair of Small Adducts, Modified and Inappropriate Bases via BER

- Lindahl initiated characterization of BER with discovery of U-DNA glycosylase (UDG)
- U(T) repair with multiple UDGs : BER prototype (Krokan, Verdine, Jiricny)
- Long-patch BER (Dogliotti, Matsumoto, Wilson, Bohr): Distinct subpathway

Repair of Oxidized Bases (and Single-strand Breaks) via BER

- Discovery of E.coli oxidized base-specific DNA glycosylases Fpg, Nth and Nei (Laval, Lindahl, Wallace, Grollman)
- Characterization of AP endo vs. AP lyase (Verly)
- Cloning of yeast and mammalian OGG and OGG null mouse (Bioteaux, Seeberg, Lindahl, Nishimura, Grollman)

Key Discoveries in Repair of Oxidative Damage In Mammalian Genome

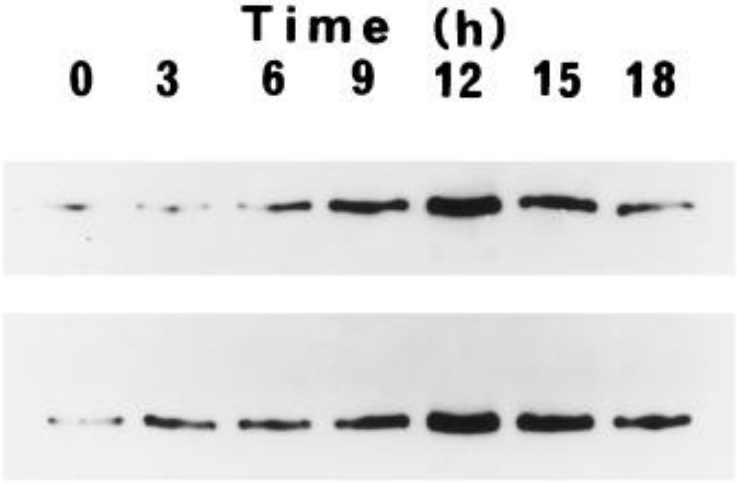
1998: Cloning and characterization of human NTH1 (collaboration with S. Seki and S. Ikeda)

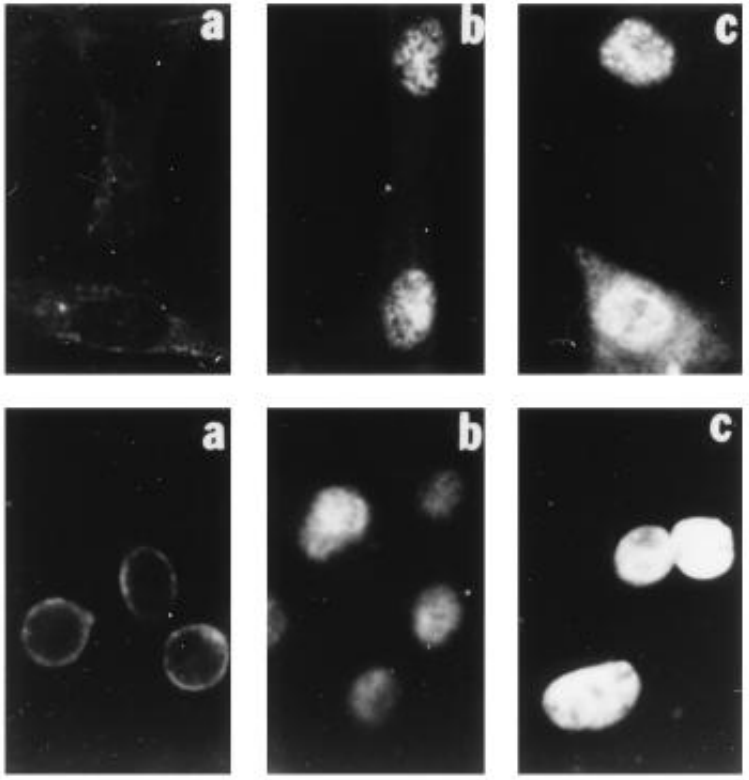
Activation of human APE1 and its nuclear translocation due to oxidative stress (Ramana)

2001: Non Michaelis Menten kinetics of OGG1 --- strong affinity for product (Jeff Hill dissertation)

2002: Discovery of Nei orthologs NEIL1 and NEIL2 (and NEIL3) in human cells (collaboration with Tapas Hazra, Wah Kow and T. Izumi)

Oxidative stress-induced activation and nuclear translocation of APE1 in human cells





(Ramana, C. V. et al., PNAS,1998)

Affinity of OGG1 and APE1 for repair substrates and products

Enzyme	Substrate/ Product	K_d^{app} (nM)
OGG1	8-oxoG·C	23.4 ± 3.4
OGG1	AP site	2.8 ± 0.2
OGG1	ß-elimination	223 ± 19
OGG1	3'-OH	20.7 ± 2.5
APE1	AP site	1.7 ± 0.2
APE1	ß-elimination	72.7 ± 6.6
APE1	3'-OH	4.3 ± 0.5

All duplex oligonucleotides have identical sequences, except for the lesion opposite cytosine.

(Hill, J. W. et al., NAR, 2001

2003: Discovery of single-stranded DNA as NEILs' substrate (Dou, Hazra)

Discovery of APE1 acetylation and of its co-repressor activity in parathyroid hormone expression (Bhakat, Izumi)

2004: Discovery of single nucleotide (SN)-BER catalyzed by NEIL1 that is APE1-independent and PNK-dependent (Wiederhold Ph.D. thesis)

Discovery of binary interaction between NEIL1 and all downstream BER proteins (except PNK)

2005: Discovery of essentiality of both repair and acetylation dependent trans-acting functions of APE1 in somatic cells which could act independently Izumi, Brown)

2006: Discovery of NEIL repair complexes able to carry out complete BER. Oxidative stress-induced activation of NEIL1 (Hazra)

2007: Mutator phenotype induced by NEIL deficiency (Hazra)

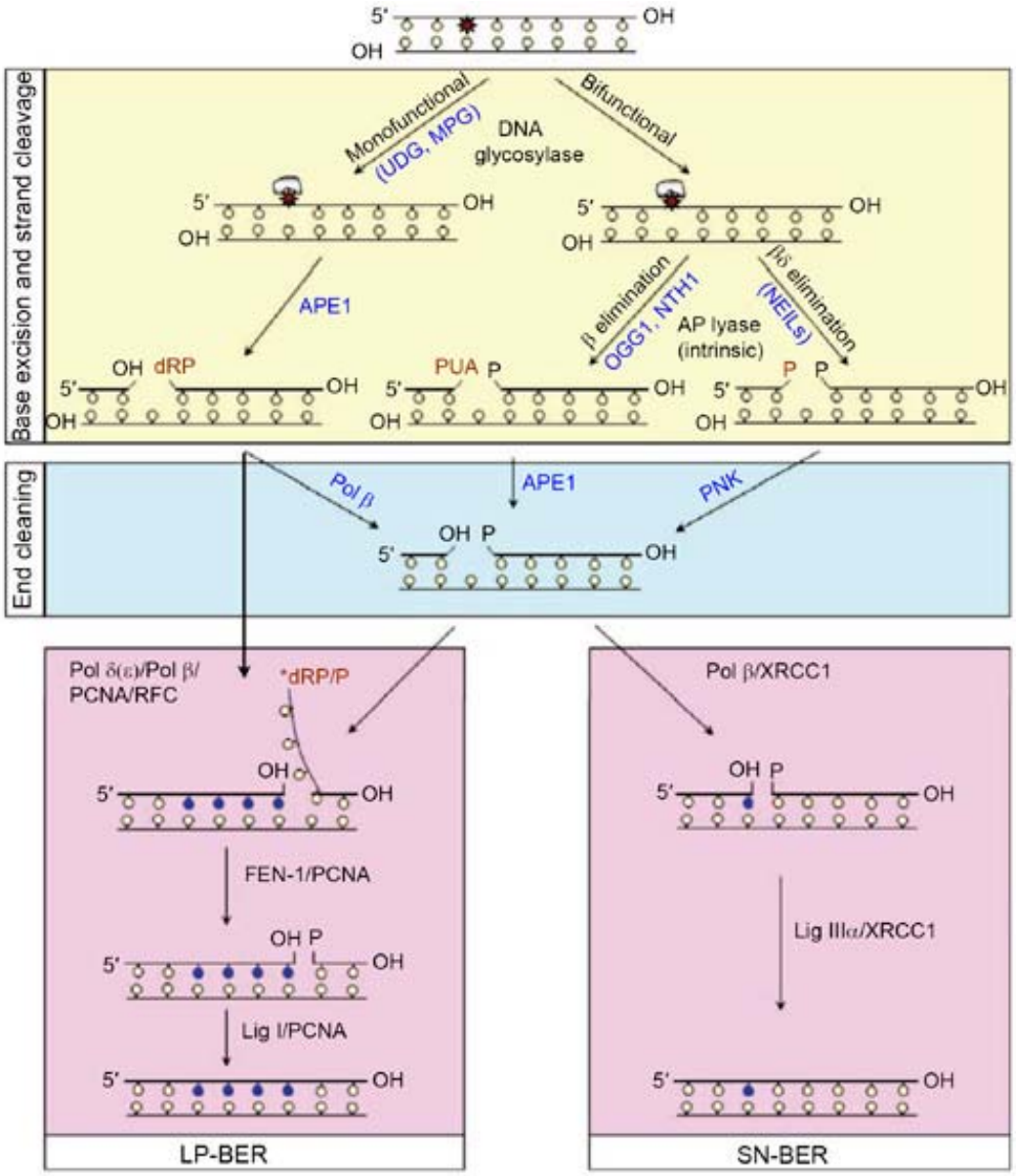
Characterization of NEIL1 interactome: stable interaction with both SN- and LP-BER and also non BER proteins (Hegde, Das, Theriot)

Characterization of a common interaction interface in NEIL1 dispersable for glycosylase activity but required for complete repair. (Hegde, Wiederhold)

Comparative Features of OGG1/NTH1 and NEIL1/NEIL2 NEILs discovered based on sequence homology.

	Nth Type		Nei Type	
	OGG1	NTH1	NEIL1	NEIL2
Size (kD)	38	36	43	36
DNA Substrate	Duplex	Duplex	Bubble SS Duplex	Bubble SS Duplex
Downstream Enzyme	APE1	APE1	PNK	PNK
Conserved motif	HhH	HhH	H2TH	H2TH
Catalytic residue	Lys 249	Lys 212	Pro1	Pro1
Cell Cycle Dependence	None	None	S-phase Specific	None
Dispensable Sequences	C-terminal 20 & N-terminal 10 residues	N-terminal 80 residues	C-terminal 100 residues	C-terminal 10 residues
AP lyase	β-elimination (3′ dRP)		βδ lyase (3′ phosphate)	

(Hazra, *PNAS*, *JBC*, 2002)



A schematic illustration of BER subpathways for damaged bases and DNA strand breaks.

The damaged base is represented as a star (*). Divergent base excision steps converge to common steps for end processing, followed by repair DNA synthesis (represented as blue dots) and strand sealing. Pol β could also be involved in LP-BER by collaborating with FEN-1. Other details are discussed in the text.

(Hegde, M. L, Hazra, T. K, Mitra, S. *Cell Res.* 18:27–47, 2008)

Pathway Complexity in BER

* Multiple DNA glycosylases ↔ Multiple Base Lesion Substrates

* SN(SP)-BER vs. LP-BER

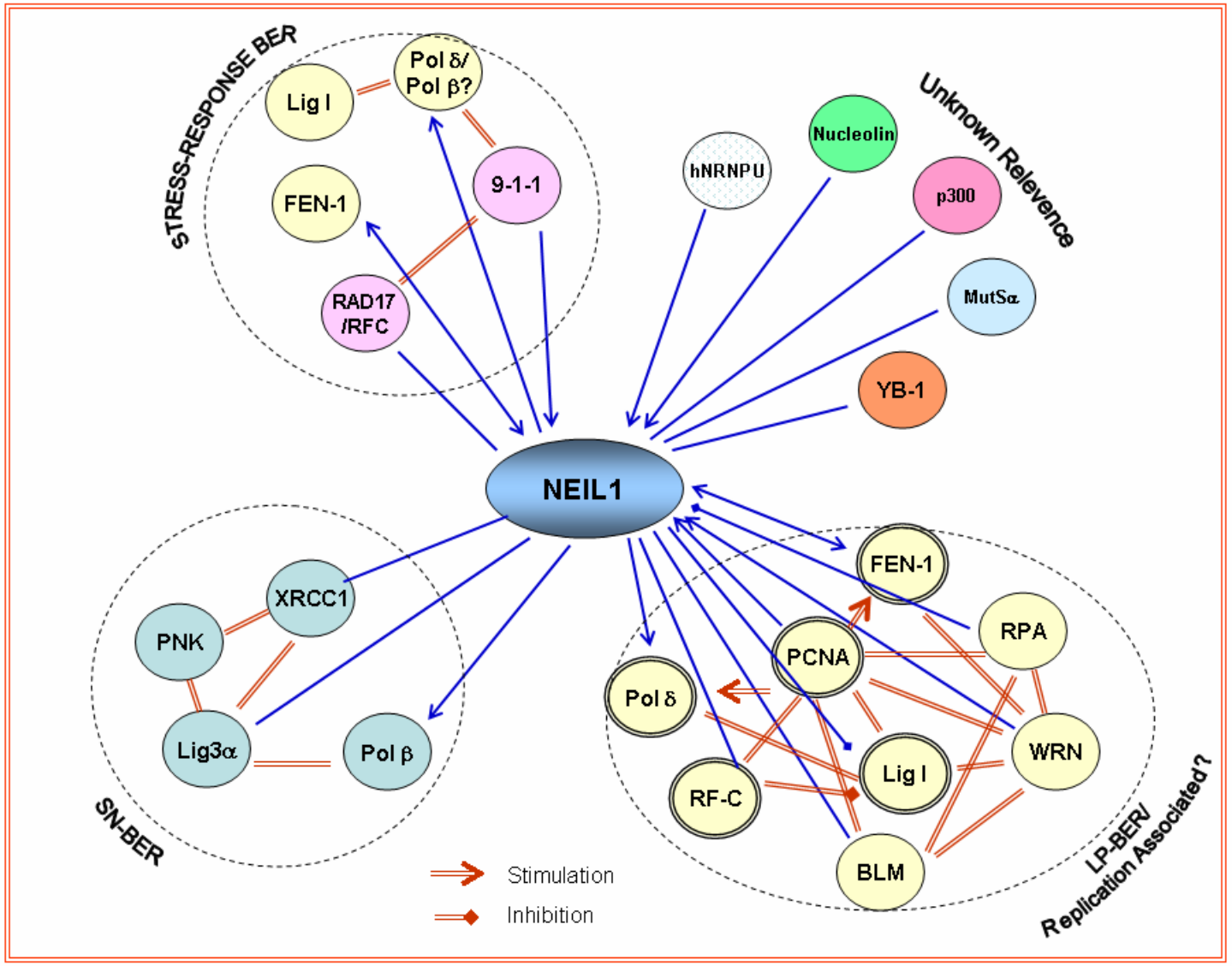
* Multiple AP-endonucleases

* Dispensability of APEs

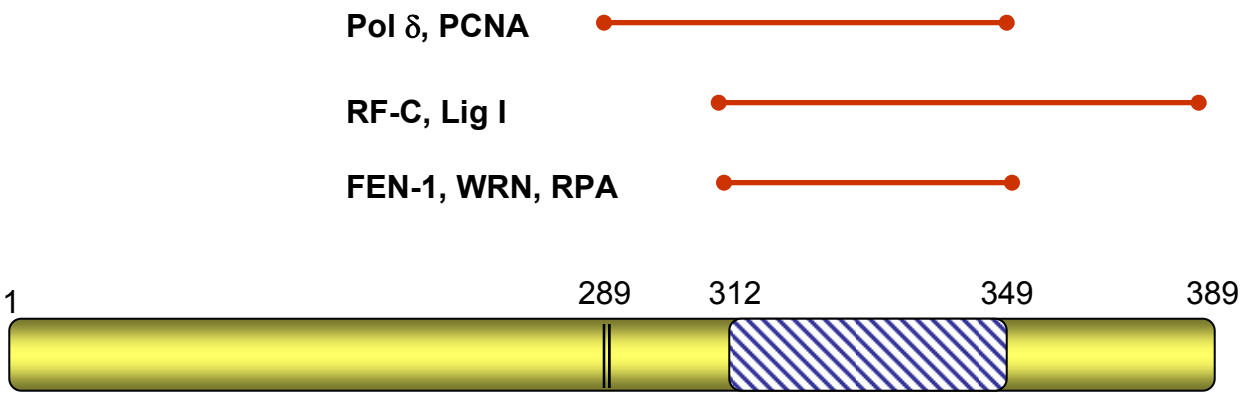
Emerging Concepts in Oxidized Base Repair In Mammalian Genomes

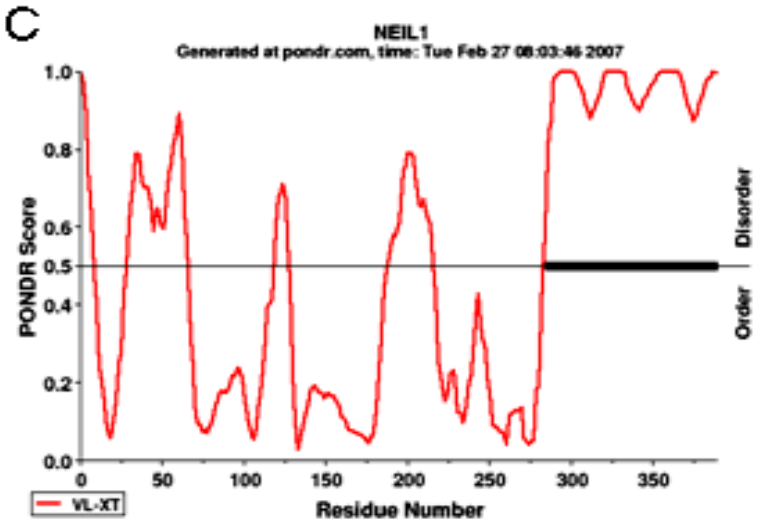
* Stable complexes of BER proteins (Berosomes) that can carry out complete repair
* Distinct complexes of the same DNA glycosylase (NEIL1) to carry out SN-BER vs. LP-BER
* NEIL1 utilizes a short peptide segment dispensable for activity as the common interaction interface for all partners
* Amount of complex modulated by stress or growth signal
* Potential role of nonBER proteins in repair
* Diverse replication proteins enhance NEIL1's product release

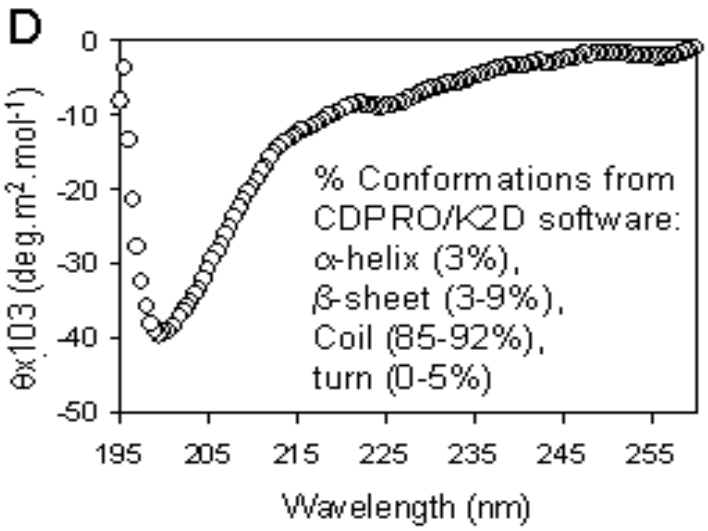
NEIL1 Interactome



NEIL1's disordered C- terminal region dispensable for activity but required for protein interaction







(Hegde, 2008)

Mammalian APE1:
A multifunctional essential protein

Unlike the prototype Xth, APE1 has a N-terminal extension required for organelle targeting and also for its transcriptional regulatory function.

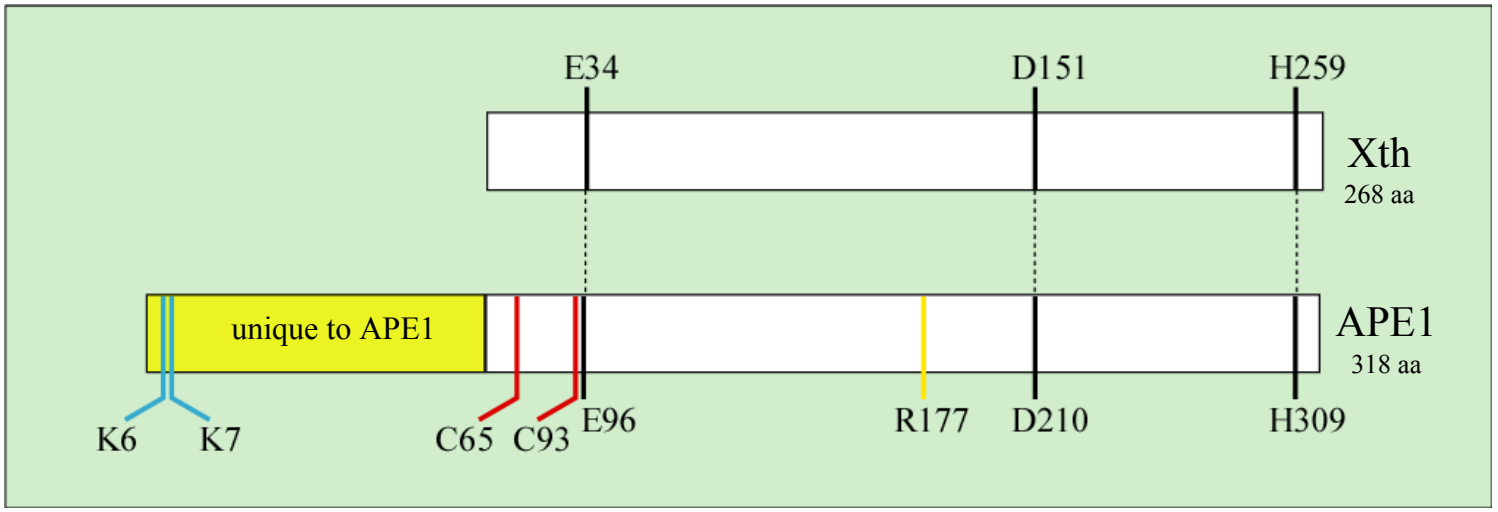
Background on Mammalian APE1
Cloning of APE1 (Demple, Seki, Hickson, 1991).

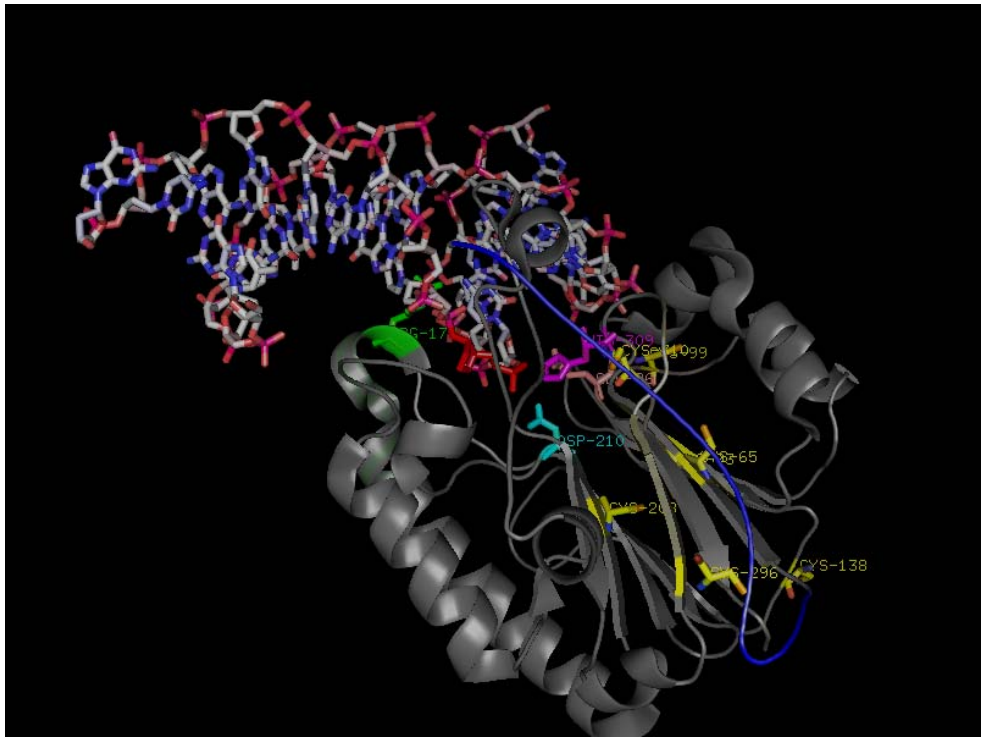
* N-terminal 61 aa residues are dispensable for AP-endonuclease/3′ phosphodiesterase activity (Izumi, 1998).

* APE1 was discovered as a reductive activator of of C-Jun (with C65), and named Ref-1 (Curran, 1992).

* Ref-1, identified as negative regulator of parathyroid hormone, binds to the negative Ca^{2+} response element (nCaRE) in promoter of this and other genes including APE1 itself (Okazaki, 1994; Izumi, 1996).

Conserved and unconserved residues in Xth and APE1





APE1 with

- N-ter (40-60)
- CYSs
- E96
- R177
- D210
- H309
- APsite

Mol et al, 2000

APE1 is Essential for Mouse Embryo Survival

* APE1 nullizygous embryos die very early (Curran, Friedberg, Chen).

* No APE1 null cell line is established. (This is surprising that *E. coli* and yeast mutants lacking both APEs are viable.)

* Ref-1 active site Cys (C64) is not required for viability (Curran).

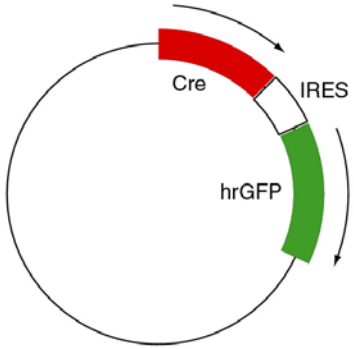
APE1 is Essential for Somatic Cells

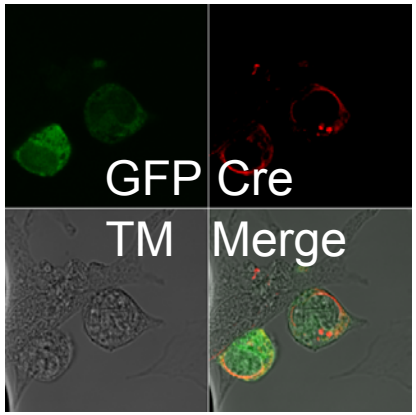
Strategy: Embryo fibroblasts of (-/-, tg) mouse embryos were established with or without transformation with T-antigen (p53 -/+).

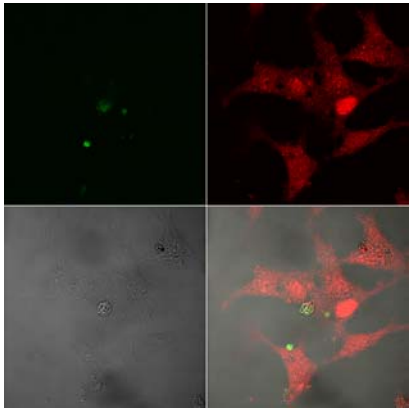
Cre expression plasmid was microinjected into the nuclei to delete hAPE1 transgene.

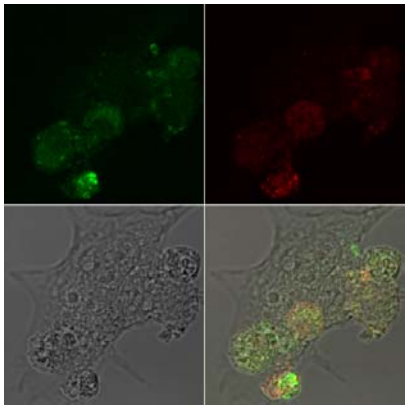
(Izumi, 2005)

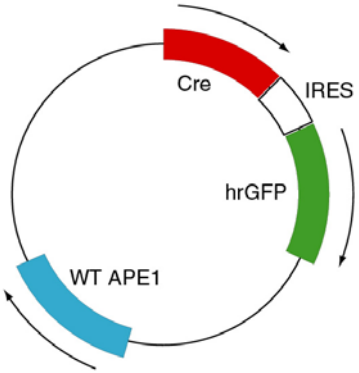
APE1 coexpression prevents apoptosis

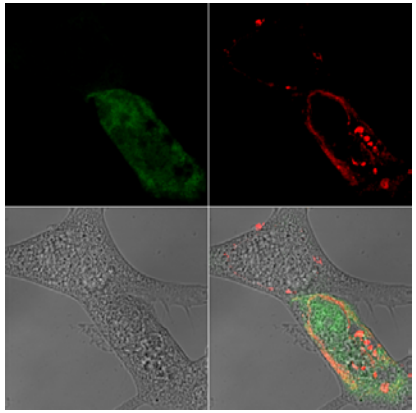


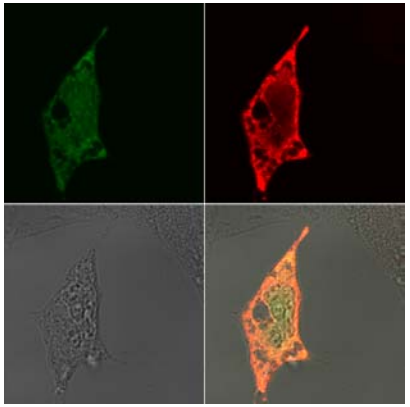


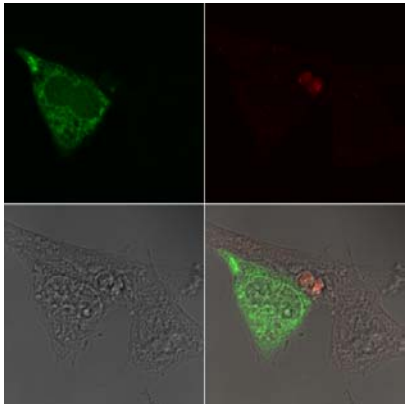












Transfection -> fixation -> staining

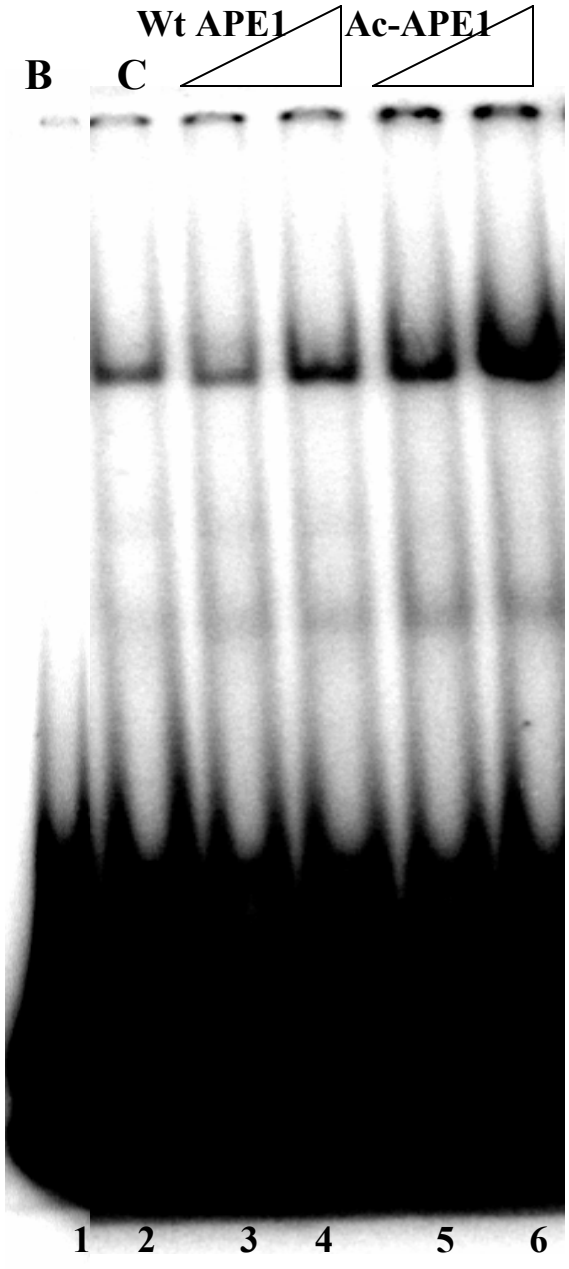
(Izumi, 2005)

Acetylation of Mammalian APE1

APE1 is acetylated by p300 at K6/K7 which is required for transacting function (Bhakat, 2003).

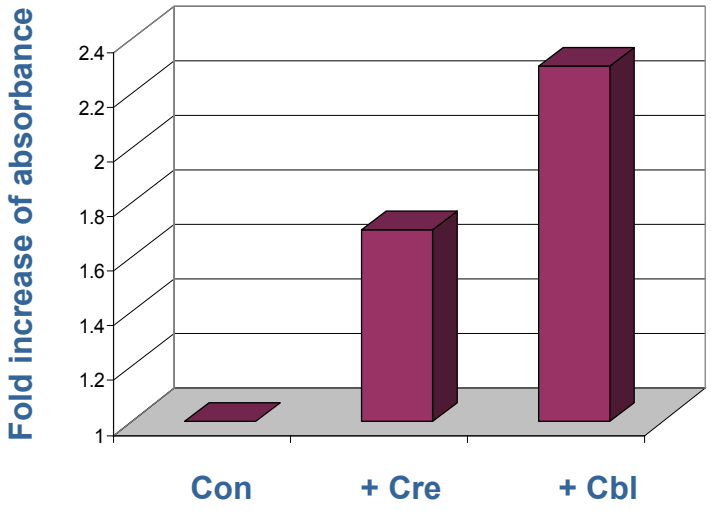
Both APE and acetylation-dependent activities of APE1 are essential and could be separately provided *in trans* to prevent apoptosis.

Acetylation of APE1 increases nCaRE-B binding *in vitro*

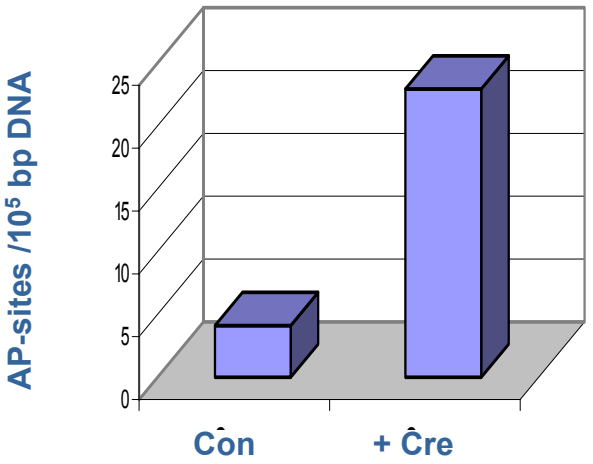


(Bhakat et al, 2003)

Characterization of Cre-induced apoptosis in MEF^{null}



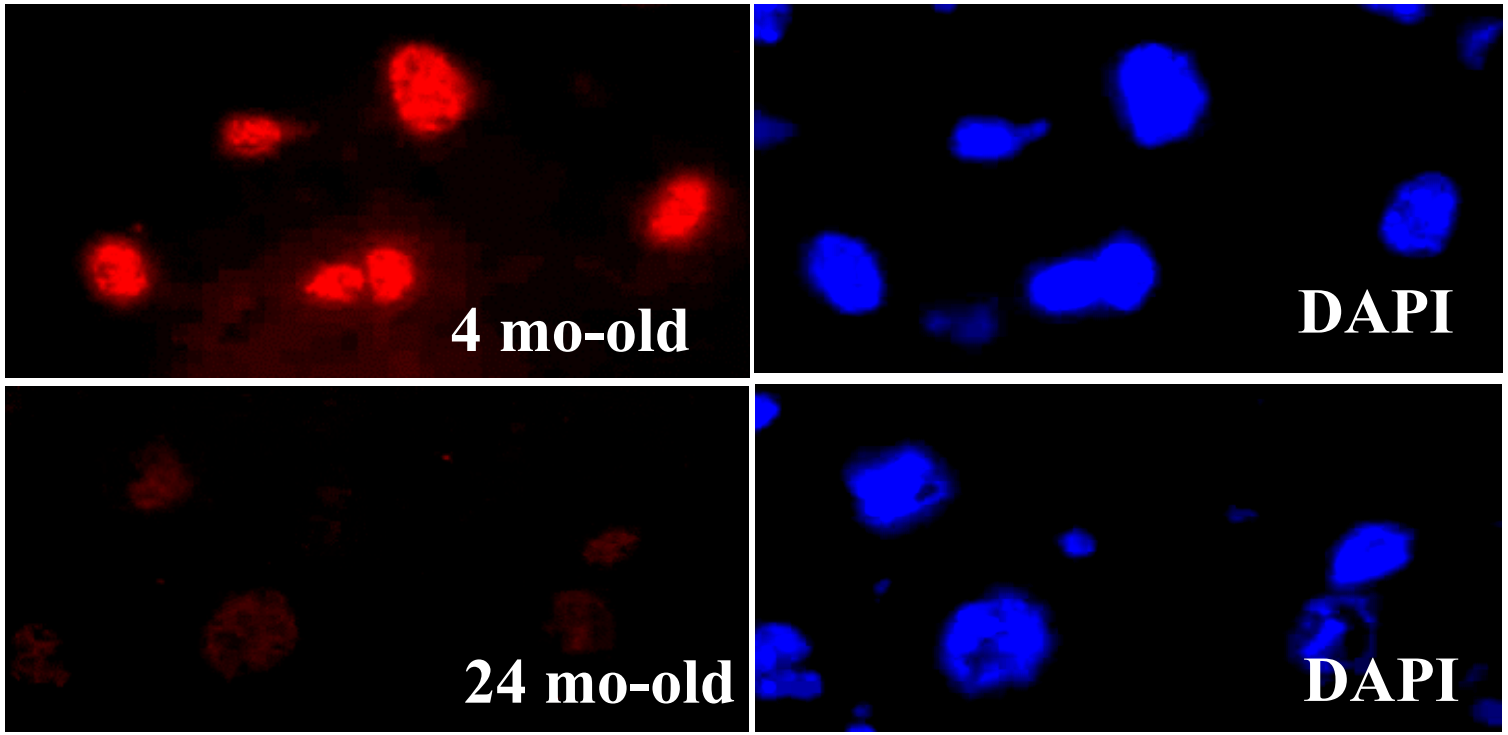
Assay for chromatin fragmentation



Assay for AP sites in DNA

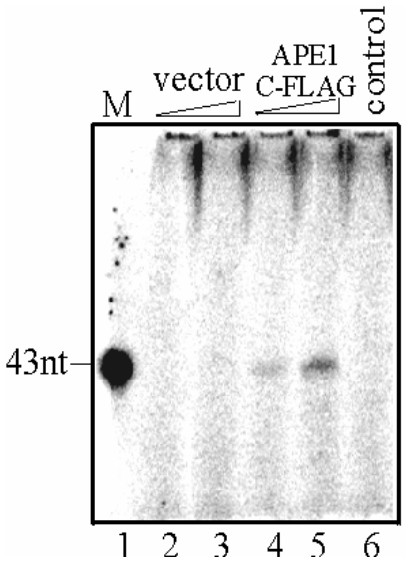
(Chattopadhyay, 2005)

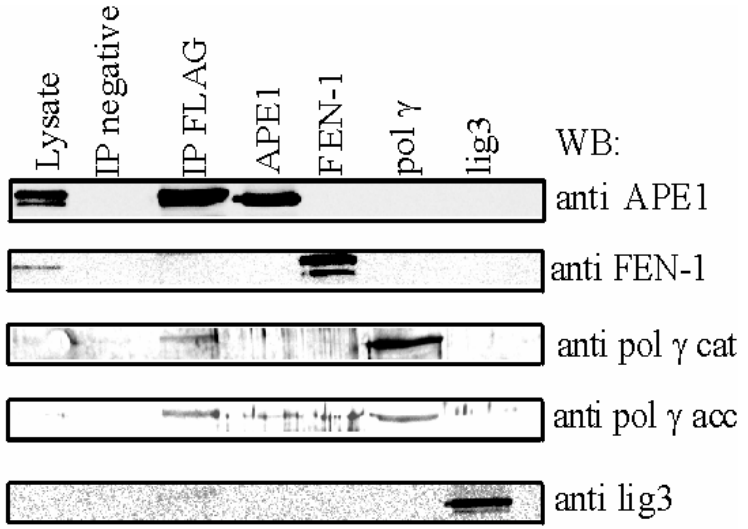
Age dependent decrease in acetylated APE1 level in mouse hepatocytes



(Szczesny et al, 2006)

Mitochondrial APE1 immunocomplex proficient in LP-BER





As in the nucleus, BER protein in mitochondria form repair active complex (berosome).

(Szczesny et al, 2008)

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