DNA Mismatch Repair and Cancer

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DNA Damage

Concept: Any modification of DNA that alters its coding properties or its normal function in replication and transcription.

DNA damage arises:

1. Spontaneous (Endogenous): Generated without influence from outside the cell.
2. Environmental(Exogenous): Generated as a result of exposure to extra cellular agents.

Cause of Endogenous DNA Damage

Spontaneous Changes:

- Hydrolysis

  Depurination, depyrimidination, cytosine deamination, 5-methycytosine deamination

- Oxidation by ROS (reactive oxygen species)

  8-oxo guanine, ring-saturated primidine

- Nonenzymatic methylation by S-adenosylmethionnine

  7-methyguanine, 3-methyguanine, 1-methyladenine and 3-methylcytosine

DNA Metabolic Errors:

- Replicative synthesis errors

  Misincorporation of nucleotides, or incorporation of unusual or altered nucleotides

  Strand slippage at repeated sequences

- Collapse of replication forks or recombination intermediates

- Chromosome segregation problems $\rightarrow$ double-strand breaks

Endogenous DNA Lesions Arising and Repaired in A diploid Mammalian Cell in 24 Hours

Endogenous source	100% double-stranded DNA
Hydrolysis	
Depurination	18,000
Depyrimidination	600
Cytosine deamination	100
5-Methylcytosine deamination	10
Oxidation	
8-oxoG	~1,000–2,000
Ring-saturated pyrimidines (thymine glycol, cytosine hydrates)	~2,000
Lipid peroxidation products (M_1G, etheno-A, etheno-C)	~1,000
Nonenzymatic methylation by *S*-adenosylmethionine	
7-Methylguanine	6,000
3-Methyladenine	1,200
1-Methyladenine and 3-methylcytosine	ND[c]
Nonenzymatic methylation by nitrosated polyamines and peptides	
O^6-Methylguanine	20–100

[a]Data from reference 297.

[b]Estimates are for two 3×10^9 -bp genomes per cell.

[c]ND, none detected

Adapted from *DNA Repair and Mutagenesis, 2006*

Cause of Exogenous DNA Damage

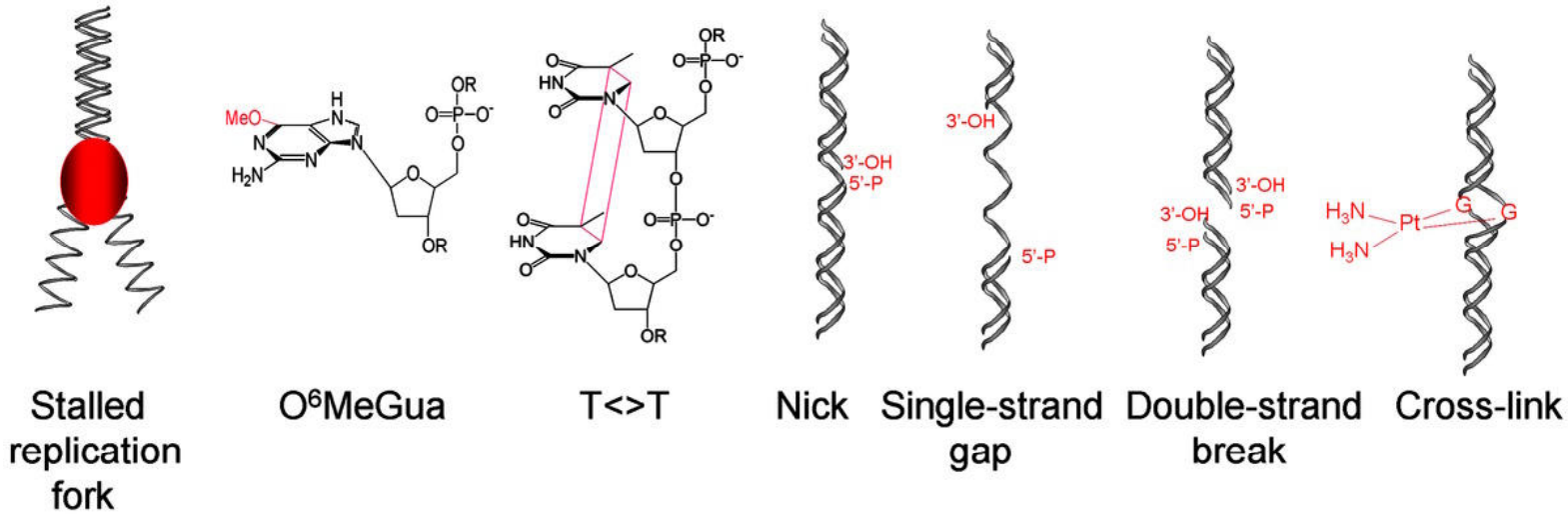
Radiations:

- Ionizing radiation: Radon, Rock and Soil, Cosmic and Medical X-rays
- UV radiation: Solar UV

Chemical Agents (mutagens and carcinogens)

- Tobacco usage
- Dietary consumption of components and contaminants
- Environmental contaminants
- Chemotherapeutic agents

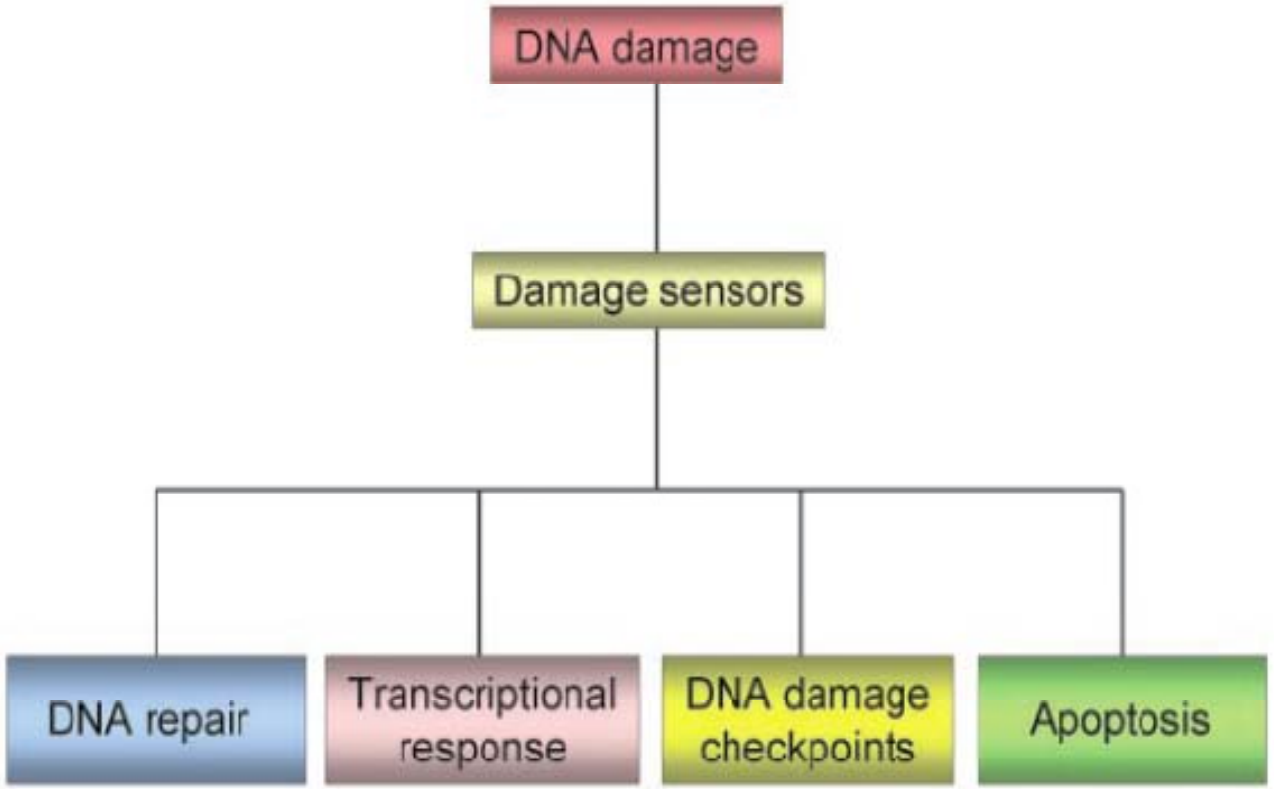
Major DNA Lesions



Stalled replication fork | O^6MeGua | T<>T | Nick | Single-strand gap | Double-strand break | Cross-link

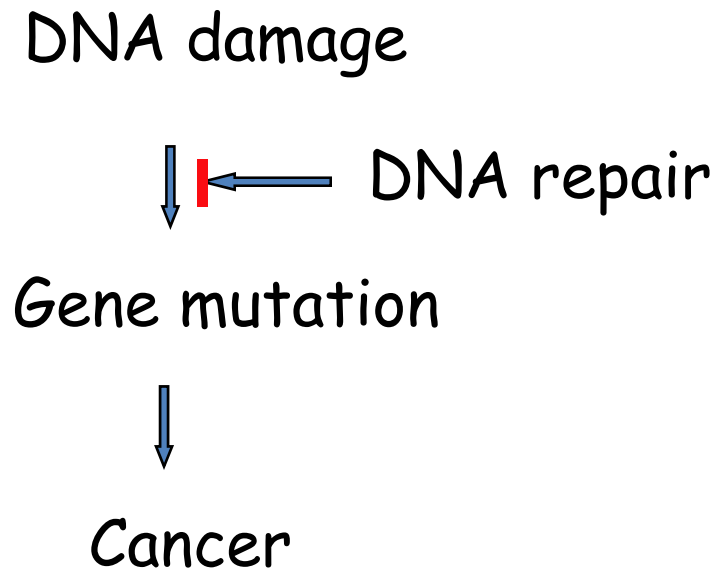
DNA lesions and structures that elicit DNA response reactions. Some of the base backbone lesions and noncanonical DNA structures that elicit DNA response reactions are shown. O^6MeGua indicates O^6-methyldeoxyguanosine, T<>T indicates a cyclobutane thymine dimer, and the cross-link shown is cisplatin G-G interstrand cross-link.

DNA Damage Response



DNA damage response reactions in mammalian cells

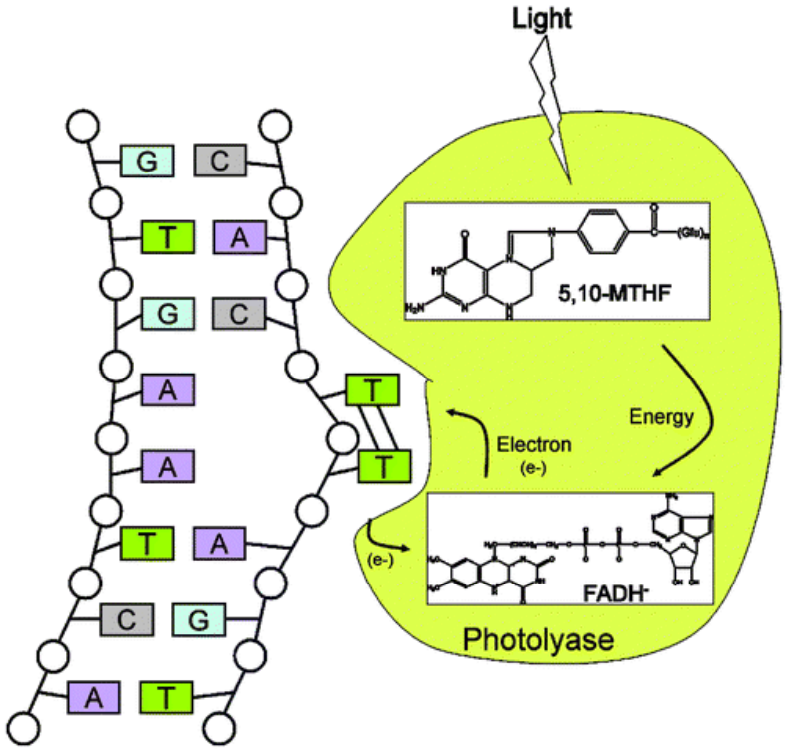
DNA Repair and Cancer



Major DNA Repair Pathways

- Reversal of base damage
- Base excision repair (BER)
- Nucleotide excision repair (NER)
- Transcriptional-coupled nucleotide excision repair (TC-NER)
- Translesion synthesis (TLS)
- Mismatch repair (MMR)
- Double strand break repair (DSBR)
  - Recombination repair
  - Non-homologous end joining

Direct Repair of Photodimer



• Photoreactivation was the first DNA repair pathway that was discovered.

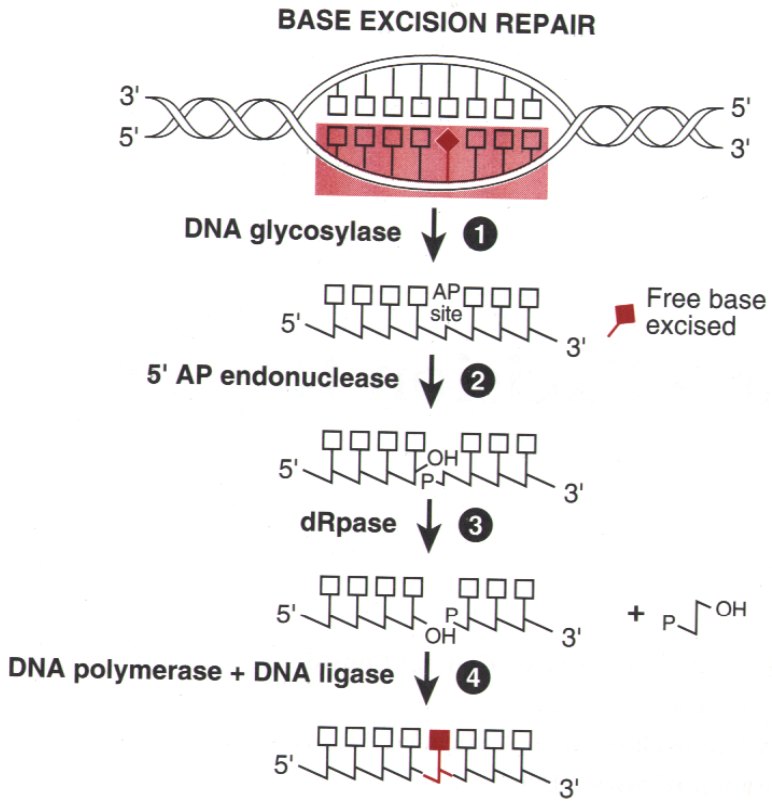
• Direct repair by **photoreactivation**. **Photolyase** binds to DNA containing a pyrimidine dimer in a light-independent reaction and flips the dimer out into the active site pocket. Catalysis is initiated by light.

• Simplest type of repair

• Single enzyme-catalyzed reaction

• Occurs for UV damage in E. coli and yeast and some eukaryotes but **not placental mammals**

Base Excision Repair (BER)

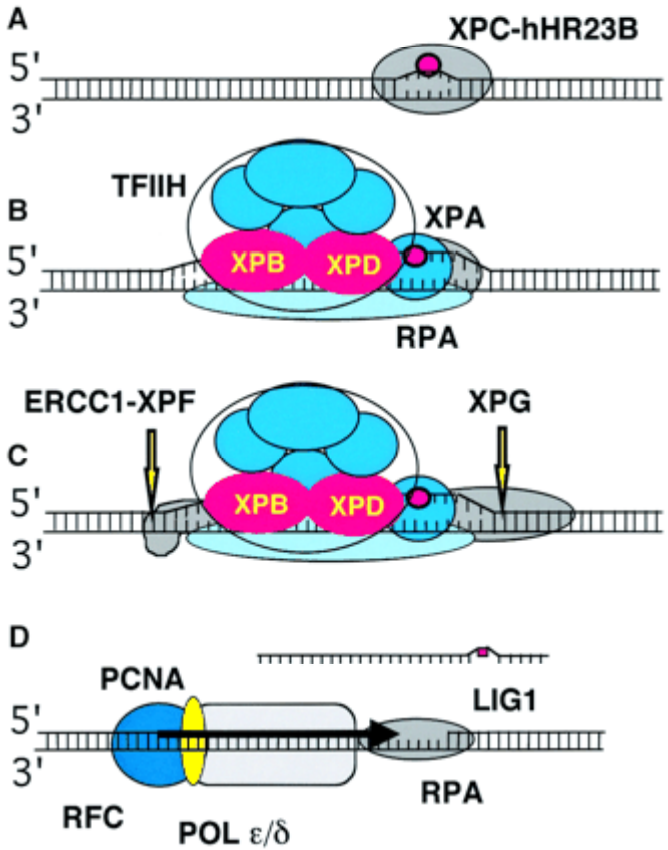


• DNA glycosylase removes the damaged base as a free base, generating another DNA damage called **apurinic** or **apyrimidinic** (**AP**) site.

•The removal of an AP site is initiated by a second class of BER enzymes called AP endonucleases, which specifically recognize AP site and produce incisions or nicks in duplex DNA by hydrolyzing the phosphodiester bonds immediately 5′ the AP site, resulting in a 5′ terminal deoxyribose-phosphate.

•An exonuclease called DNA **deoxyribophosphodiesterase** (**dRpase**) removes the sugar moiety. The single nucleotide gap can be filled by DNA polymerase.

Nucleotide Excision Repair (NER)



There are 8 xeroderma pigmentosum (**XP**) genes, called XP complementation groups (from **XPA** to **XPG**, and **XPV**).

• DNA damage is recognized by the **XPC-hHR23B** protein (*A*).

•An open bubble structure is then formed around a lesion in a reaction that uses the ATP-dependent helicase activities of **XPB** and **XPD** (two of the subunits of **TFIIH**) and also involves **XPA** and **RPA** (B).

• Formation of this open complex creates specific sites for cutting on the 3' side by the **XPG** nuclease and then on the 5' side by the **ERCC1-XPF** nuclease(*C*).

• After a 24- to 32-residue oligonucleotide is released, the gap is filled in by **PCNA**-dependent **POL** ε or δ and sealed by a DNA ligase, presumably **LIG1**(D).

Direct reversal and excision repair pathways mostly deal with damage to the **bases**

DNA Mismatch Repair (MMR)

- DNA MMR plays very important roles in maintaining genomic stability.

-MMR system is highly conserved through evolution.

- Loss of this system leads to an accelerated accumulation of mutations, and predisposes to certain types of cancers in human.

What is DNA Mismatch?

What is DNA mismatch?

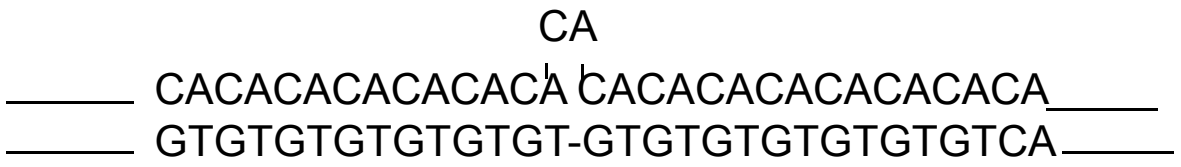
Non-Watson-Crick base pairs

Two classes of mismatches

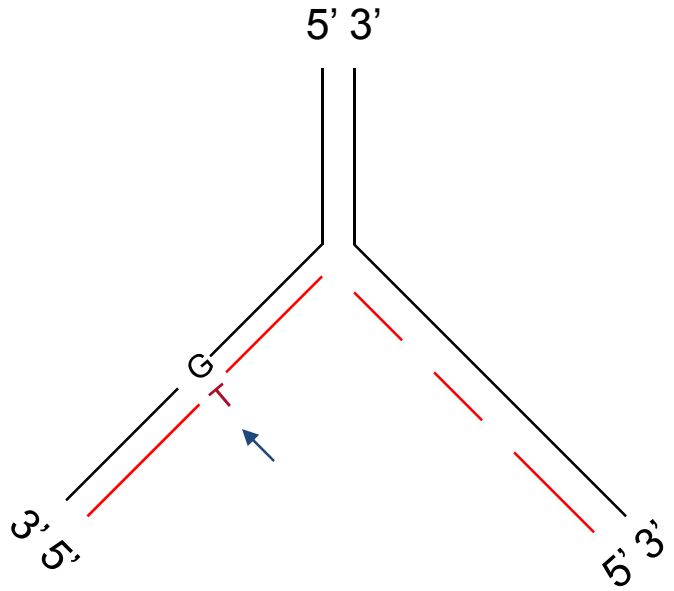
Base-base mismatch

G-T, A-C, A-A, A-G, G-G, C-C, C-T, T-T

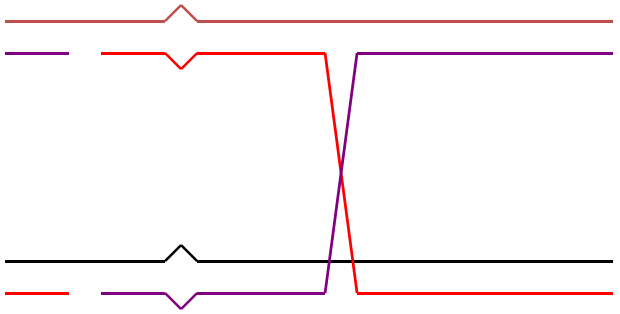
Insertion-deletion (unpaired) mismatch



Mismatch Derived from Normal DNA Metabolism



DNA replication errors



DNA recombination

Mismatch Repair in E. Coli

- Have been extensively studied in last thirty years and reconstituted in vitro in 1989 by Modrich's group.

- Methyl-directed and MutSLH-dependent

- Bi-directional process (either 5′ to 3′ or 3′ to 5′)

- Fairly broad substrate specificity (both base-base mismatch and a variety of ID mispairs.

E. Coli MMR Components

MutS:

- 97 kDa, Homodimer
- Mismatch recognition protein
- ATPase activity

MutL:

- 70 kDa, Homodimer
- Matchmaker protein
- Weak ATPase activity

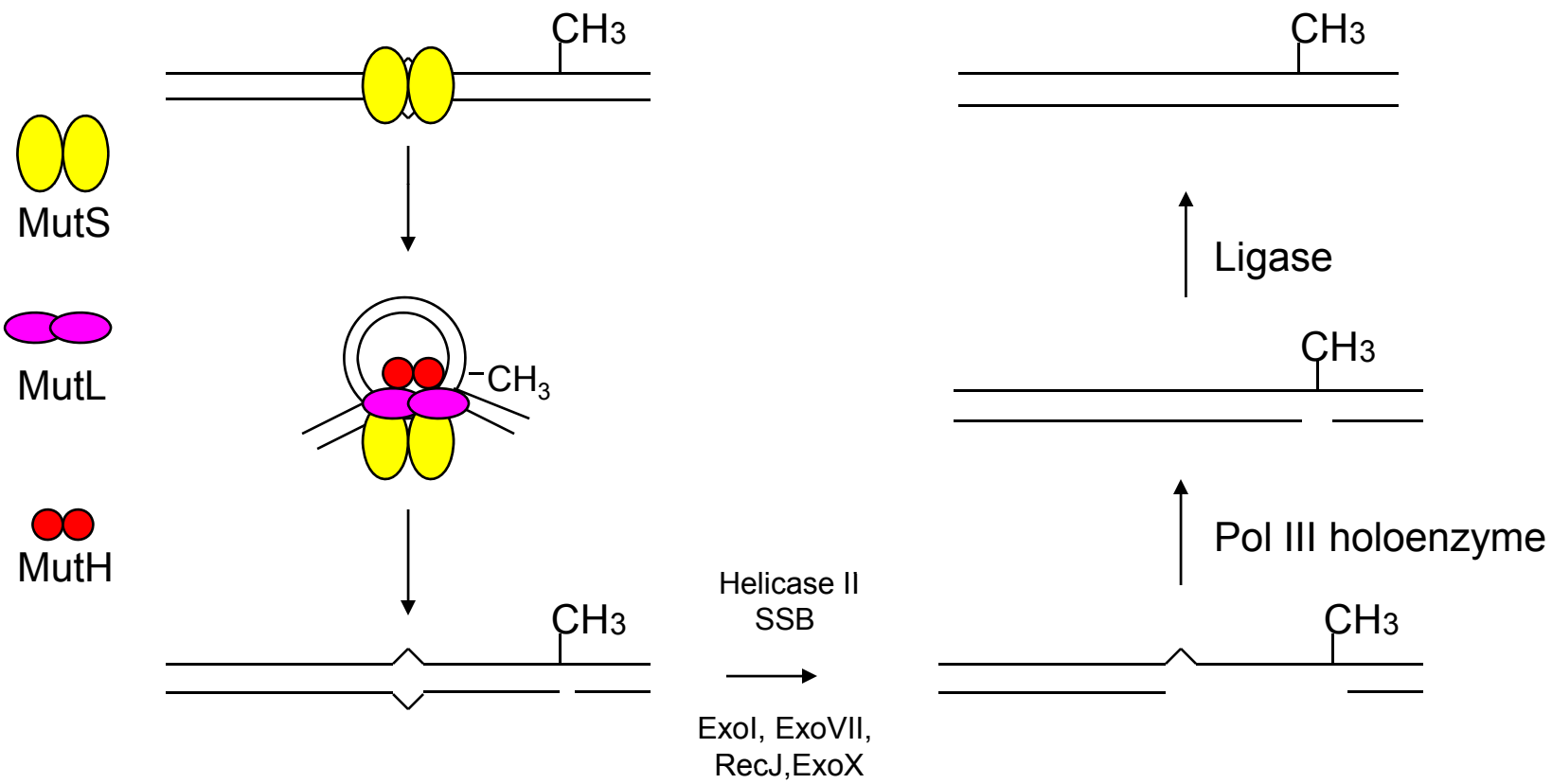
MutH:

- 23 kDa
- Endonuclease activity

Others:

- DNA helicase (MutU/UvrD)
- Exonuclease (Exo I, Exo VII, Exo X and Rec J)
- SSB (single strand binding protein)
- DNA polymerase III holoenzyme
- DNA ligase

Mismatch Repair in E. Coli



MMR in Human

- MutL and MutS-dependent. Human MutL and MutS are heterodimers.

- Bi-directional process (either 5′ to 3′ or 3′ to 5′).

- Strand specificity (nick-directed).

- Fairly broad substrate specificity (both base-base mismatch and a variety of IDL mispairs).

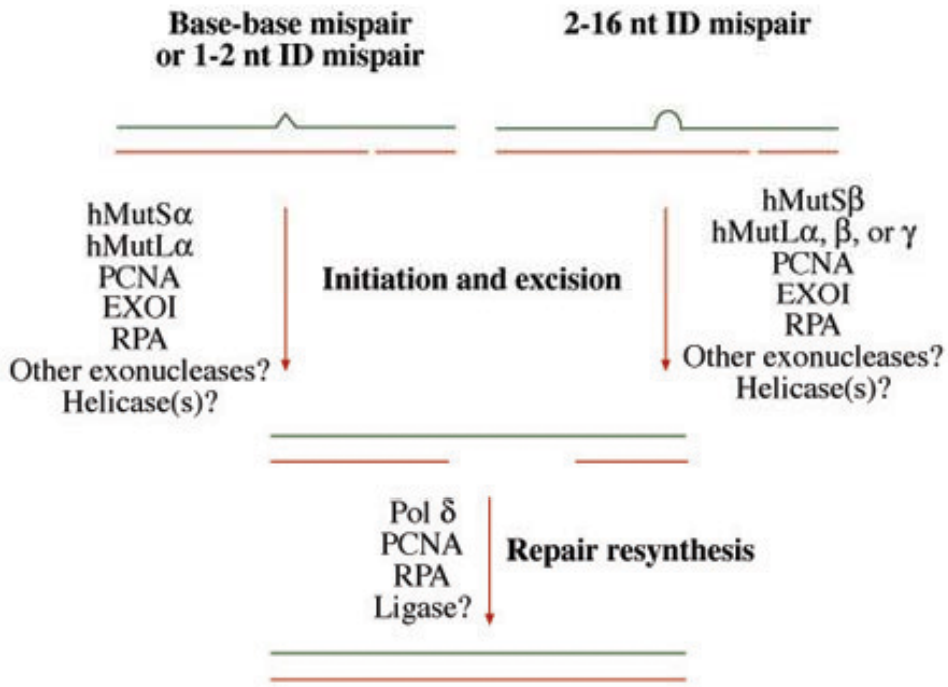
Human Mut Homologs

E. coli	Human
$(MutS)_2$	MutSα: MSH2, MSH3, MSH6; MutSβ: MSH2, MSH3
$(MutL)_2$	MutLα: MLH1, PMS1; MutLβ: MLH1, PMS2; MutLγ: MLH1, MLH3
MutH	?

MMR Components in E.coli and Human

E. coli	Human
MutS	MutSα, MutSβ
MutL	MutLα, MutLβ, MutLγ
MutH	?
UvrD	?
ExoI, ExoVII, ExoX, RecJ	ExoI (others?)
SSB	RPA
Pol III holoenzyme	Pol δ (α, ε?)
DNA ligase	DNA Ligase I
	PCNA RFC HMGB1

Human Mismatch Repair



The human MMR system can bi-directionally process all eight base-base mismatches and 1-16 nt ID mispairs. The repair process in each case involves repair initiation, excision, and resynthesis. Except for mismatch recognition, where hMutSα and hMutSβ are required to distinguish specific substrates as indicated, activities required for the remaining steps of the reaction are believed to be the same for the processing of base-base mismatches and ID mispairs.

Main Functions of MMR

- Repair function

  Correcting heteroduplexes and insertion-deletion

- Apoptosis function

  Eliminating damaged cells from body by promoting apoptosis

Microsatellite Sequence and its Instability

Key Terms

Microsatellite:

Simple repetitive DNA sequences are called microsatellite.

For example: $(A)_n$
$(CA)_n$
$(CAG)_n$
$(CAGA)_n$

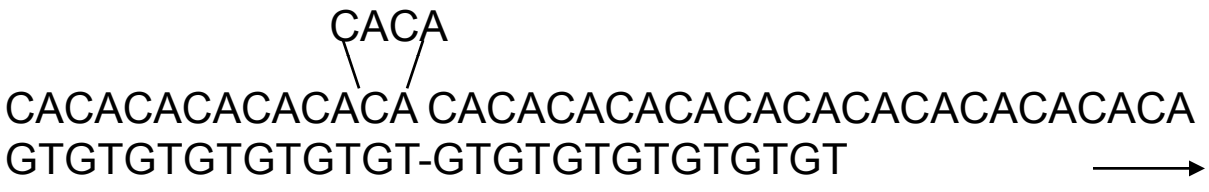
There are hundreds and thousands of microsatellite sequences in our DNA, and they are located mostly in **non-coding regions, but also in coding region.**

Microsatellite instability (MSI):

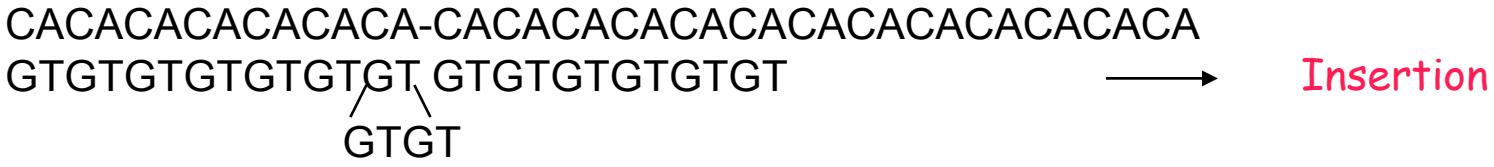
Alterations in repeat numbers of microsatellite sequences from one generation to another.

Mechanism of MSI

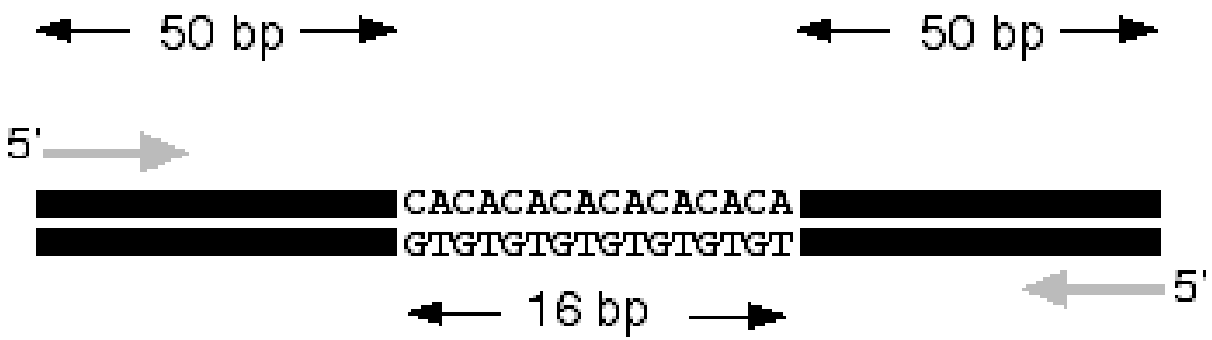
Loop out in the template strand



Loop out in the primer strand

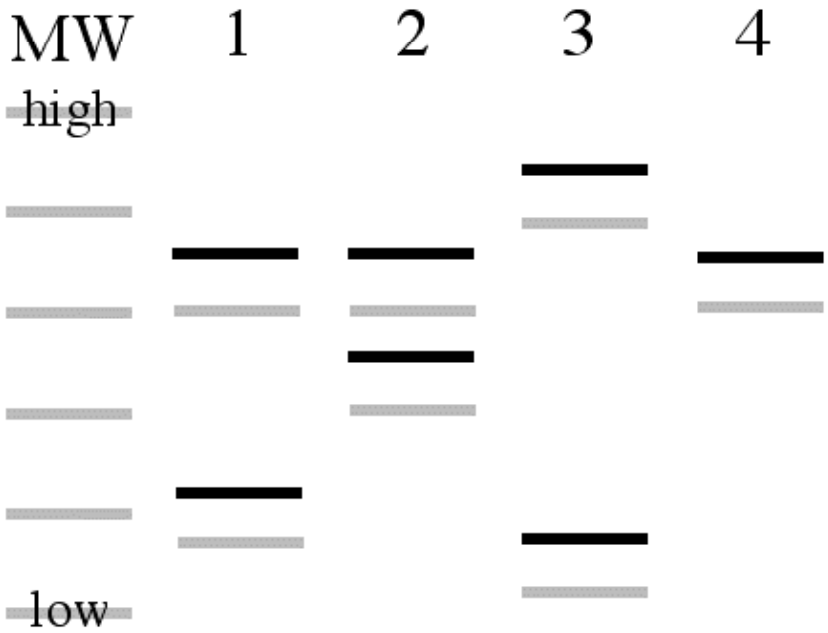


Detecting MSI from Genomic DNA



The most common way to detect microsatellites is to design PCR primers that are unique to one locus in the genome and that base pair on either side of the repeated portion. Therefore, a single pair of PCR primers will work for every individual in the species and produce different sized products for each of the different length microsatellites. There are more than thousand microsatellite sequences in our genome.

Detecting MSI from Genomic DNA



The PCR products are then separated by either gel electrophoresis. The size of the PCR product can be determined and thus how many times the dinucleotide "CA" was repeated for each allele.

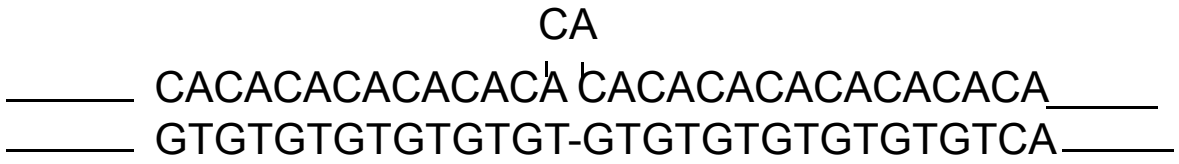
MMR Repairs Two Classes of Mismatches

Two classes of mismatches

Base-base mismatch

G-T, A-C, A-A, A-G, G-G, C-C, C-T, T-T

Insertion-deletion (unpaired) mismatch



Defective MMR Cause MSI

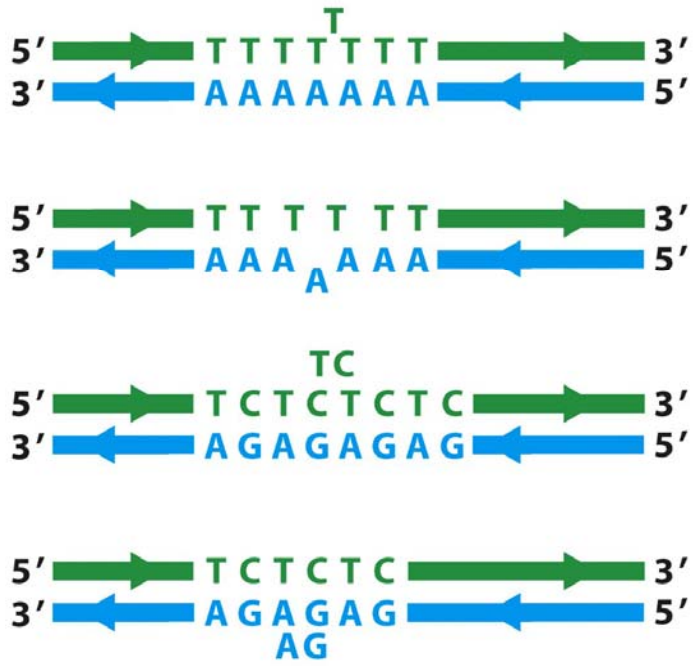


Figure 12-8a The Biology of Cancer (© Garland Science 2007)

A. DNA polymerase occasionally stutter or skip a base (bases) within a repeating sequence of DNA (microsatellite sequence)

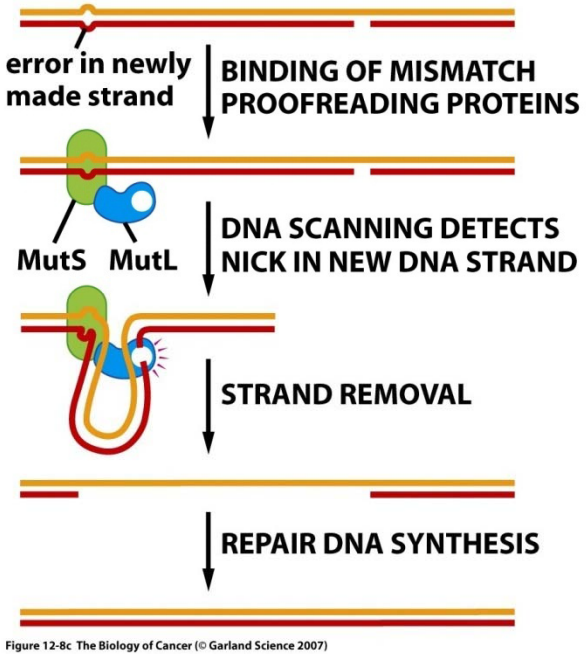


Figure 12-8c The Biology of Cancer (© Garland Science 2007)

B. MMR recognizes and repairs the mistakes made by polymerase.

Defective MMR cause MSI. MSI usually use as a hallmark of MMR deficiency.

From the Biology of Cancer by Weinberg

MSI in Coding Sequence

- Microsatellite sequence is very common in gene coding region.
- Genes containing a mononucleotide microsatellite are particularly prone to inactivation in MSI tumorigenesis.

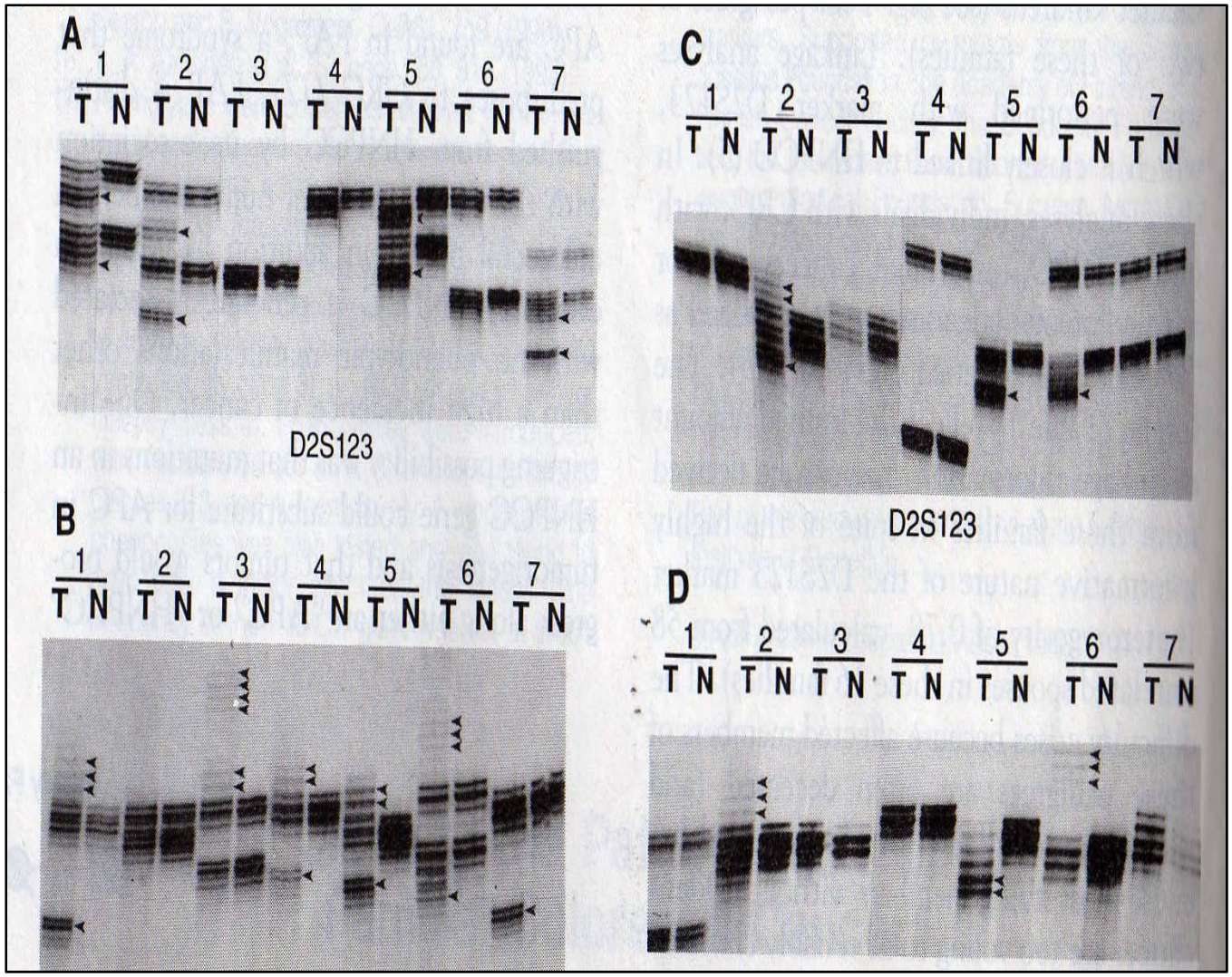
Examples:

TGF β RII (A_{10} repeat)
BAX (G_8 repeat)
CASPASE5 (A_{10} repeat)
Chk1 (A_9 repeat)
Also in MMR genes, such as MSH3 and MSH6

Defective MMR and Colon Cancers

	HNPCC	Sporadic
Population incidence	~1 in 500	1 in 20
Microsatellite instability	>90% of kindreds	13%
MMR gene mutations	70%	~65% of CRC with MSI
MSH2	45%	40%
MSH3	0%	
MSH6	0.5%	
MLH1	48%	60%
PMS1	One family	
PMS2	5%	
MLH3	?	

Loss of MMR function is the genetic basis of hereditary colorectal colon cancer (HNPCC) and some sporadic colon cancer.



A and B: Dinucleotide repeat polymorphisms in normal and tumor tissue from HNPCC patients. C and D: Dinucleotide repeat polymorphisms in normal and tumor tissue from sporadic colon cancer patients.

TGFβ RII and BAX mutations in Colon Cancer

Mutations in *TGFBR2*, *BAX*, or simultaneously in both genes in sporadic colorectal tumors with different degrees of MSI

	No mutation		Mutation in one gene		Mutation in both genes		
Variable	*n*	%	*n*	%	*n*	%	*P*
MSS	121	100	0	0.0	0	0.0	<.001
MSI-L	17	94.4	1	5.6	0	0.0	
MSI-H	0	0.0	13	81.2	3	18.8	

Abbreviations: H, high; L, low; MSI, microsatellite instability; MSS, microsatellite stability.

MSI and Non-Colorectal Cancers

Tumor	Frequency (%)
Endometrial	22
Ovarian	5
Gastric	60
Breast	10-20
Lung	34
Prostate	10
Lymphoma/Leukemia	52

Phenotypes of MMR-deficient knockout mice

Gene	MSI	Tumor	Fertility
MSH2	Yes	Lymphoma, GI, skin and other tumors	Yes
MSH3	Yes	GI tumors	Yes
MSH6	Low instability in dinucleotide repeats	Lymphoma, GI and other tumors	Yes
MLH1	Yes	Lymphoma, GI, skin and other tumors	No
PMS1	Mononucleotide repeats only	None	Yes
PMS2	Yes	Lymphoma and sarcoma	Male only
MLH3	Yes	Not available	No

Main Functions of MMR

- Repair function

  Correcting heteroduplexes and insertion-deletion

- Apoptosis function

  Eliminating heavily damaged cells from body by promoting Apoptosis.

MMR Deficiency and Drug Resistance

MMR-proficiency cells are 10 to 100-fold more sensitive than matched MMR-deficiency cells to a lot of cancer therapeutic drugs, such as cisplatin, MNNG, 6-thioguanine, indicating the MMR system also involved in the signaling of cell cycle arrests and cell death.

MutSa Binds to Damaged DNA

O^6-methylguanine (MNNG, procarbazine, temozolomide)

Cisplatin adducts

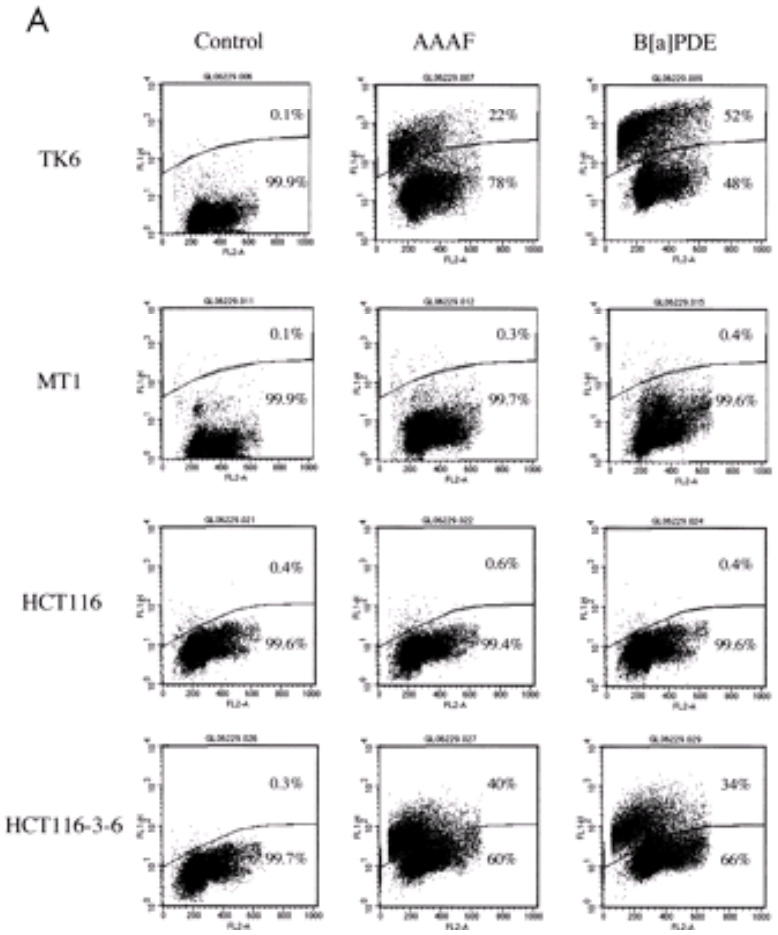
8-Hydroxyguanine (oxidative damage)

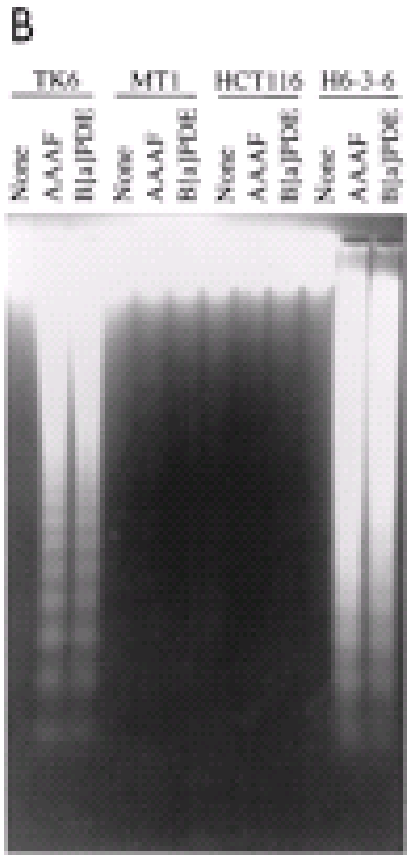
Aminofluorene adducts

Adducts of polycyclic aromatic hydrocarbon (B[a]P, B[c]Phe)

UV dimer

Mismatch Repair-Dependent Apoptosis





Mol Cell Biol. 1999 19(12):8292-301

AAAF: N-acetoxy-2-acetylaminofluorene.

B[a]PDE: benzo[a]pyrene-7,8-di-hydrodiol-9,10-epoxides.

Mechanism of MMR-Mediated Apoptosis

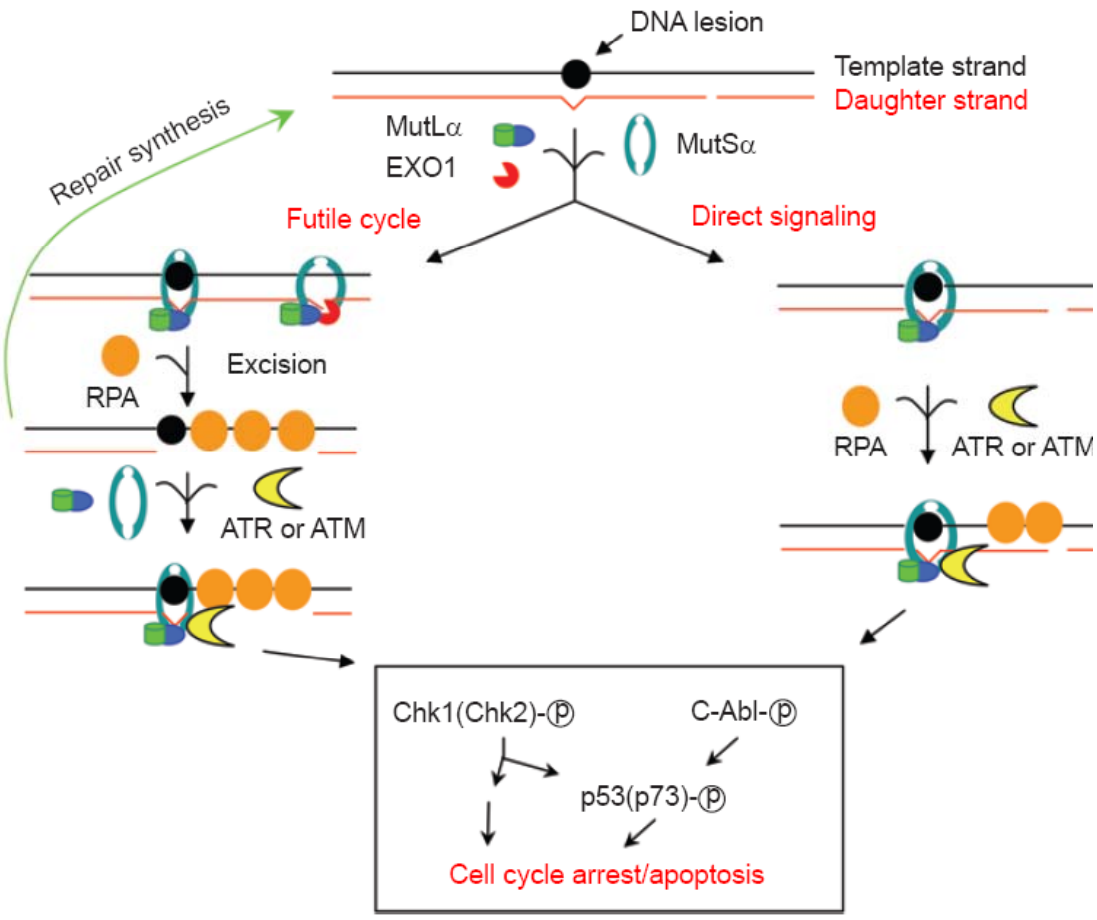
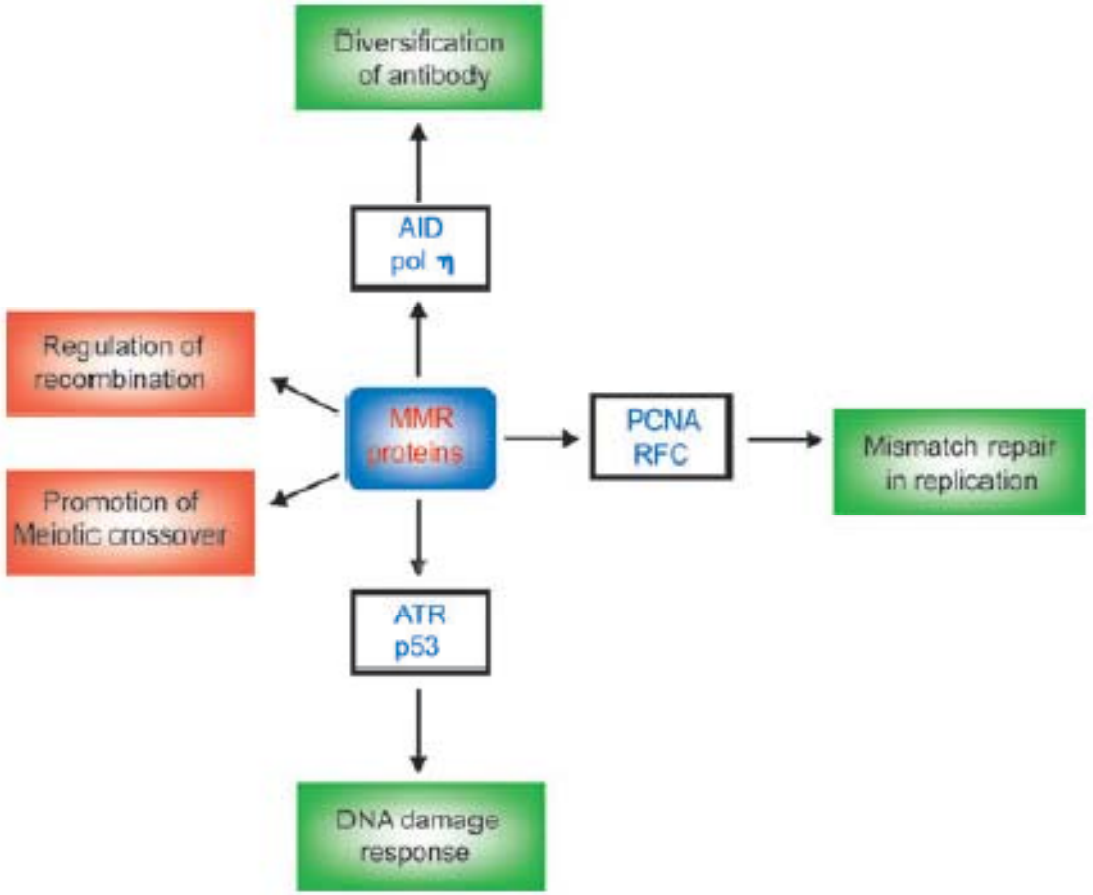
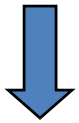
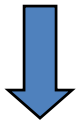


Figure 2 Models for MMR-dependent DNA damage signaling. The "futile DNA repair cycle" model (left) suggests that DNA adducts (solid black circle) induce misincorporation, which triggers the strand-specific MMR reaction. Since MMR only targets the newly synthesized strand for repair, the offending adduct in the template strand cannot be removed, and will provoke a new cycle of MMR upon repair resynthesis. Such a futile repair cycle persists and activates the ATR and/or ATM damage signaling network to promote cell cycle arrest and/or programmed cell death. The direct signaling model proposes that recognition of DNA adducts by MSH-MLH complexes allows the proteins to recruit ATR and/or ATM to the site, activating the downstream damage signaling.

Various Functions of Mismatch Repair Proteins



Tumor Suppression of MMR

	Wild-type cells	MMR mutant cells
Biosynthetic errors	Correction	Hypermutable
DNA damage	Apoptosis	Survival with mutation
		
Consequence	Mutation-free genome	Tumorigenesis

Summary

1. Defects in MMR are the genetic basis for certain types of cancer.

2. MMR maintains genomic stability via at least two distinct pathways.

   Repair biosynthetic errors.

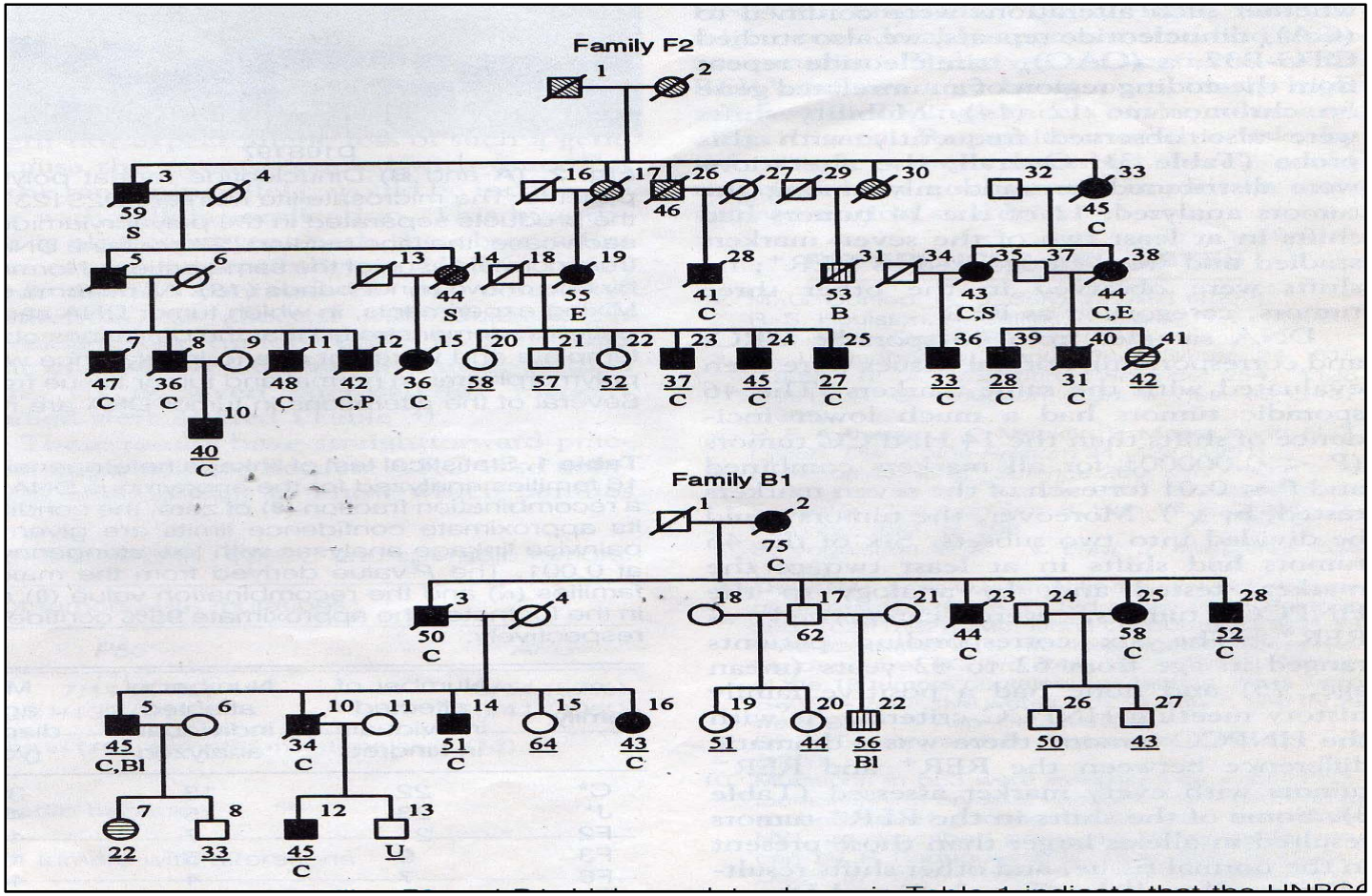
   Signaling cell cycle arrest and apoptosis.

Paper #1:

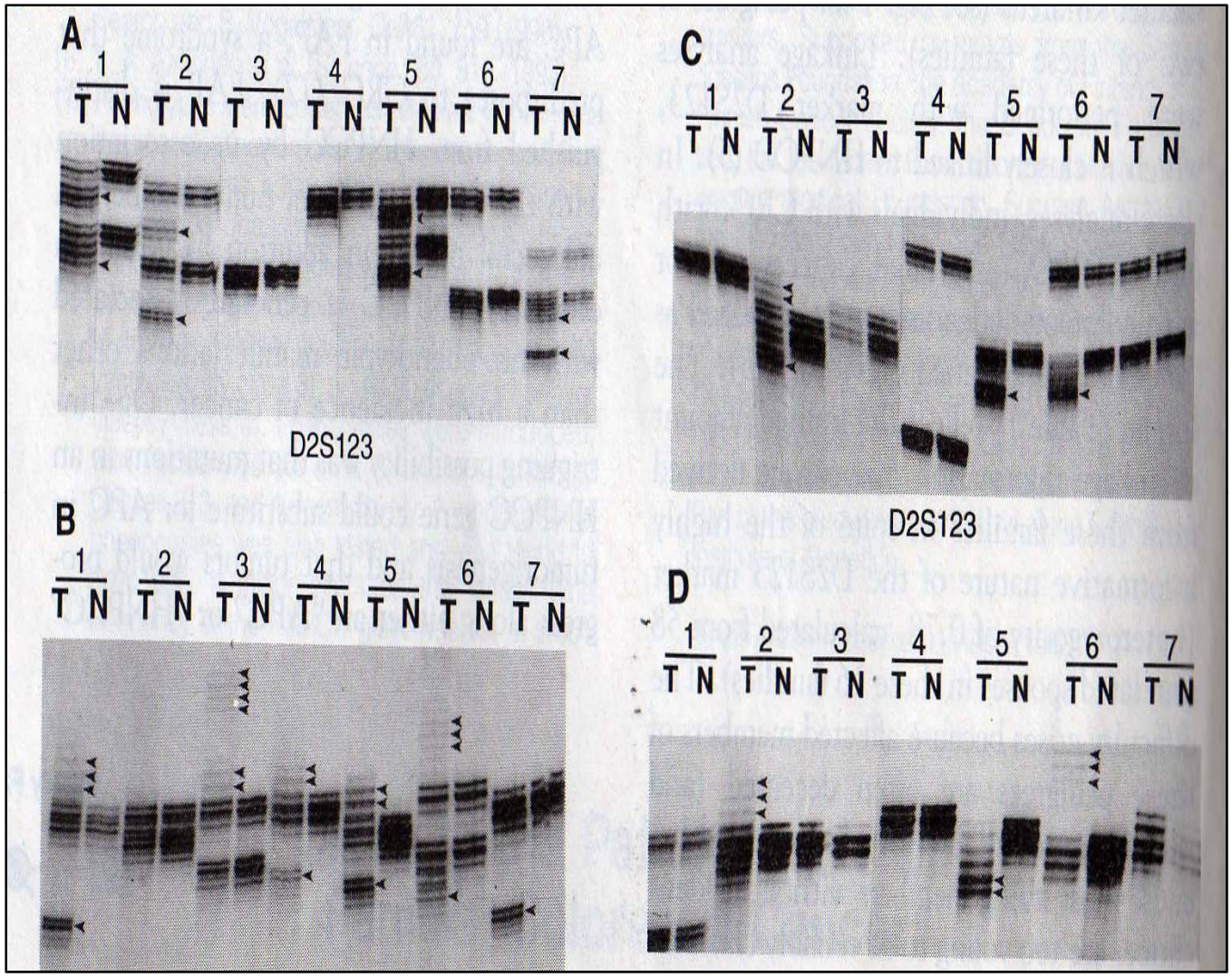
Clues to the Pathogenesis of Familiar Colorectal Cancer

Science (1993) , 260, 812-816

by Bert Vogelstein and Albert de la Chapelle



Pedigrees of HNPCC (Hereditary non-polyposis colorectal cancer) families F2 and B1. HNPCC is a heritable autosomal dominant disease, which is defined by the presence of colorectal cancer at least three family members in two successive generations.



A and B: Dinucleotide repeat polymorphisms in normal and tumor tissue from HNPCC patients. C and D: Dinucleotide repeat polymorphisms in normal and tumor tissue from sporadic colon cancer patients.

Table 3. Microsatellite alterations in HNPCC and sporadic tumors.

Tumor type*	Number of tumors	Percentage of tumors with alterations						
		D2S123	D2S147	D2S119	D11S904	D13S175	D10S197	CTG-B37
HNPCC								
RER$^+$	11†	91	55	55	55	64	91	55
RER$^-$	3‡	0	0	0	0	0	0	0
Total	14	71	43	43	43	50	71	43
Sporadic								
RER$^+$	6	67	83	83	67	100	83	67
RER$^-$	40	0	0	0	0	0	2	0
Total	46‡	9	11	11	9	13	13	9

*RER$^+$ tumors were defined as those with alterations in at least two of the indicated microsatellite markers.
†Nine colorectal carcinomas, one colorectal adenoma, and one ovarian carcinoma. ‡All colorectal carcinomas.

Important findings:

The most of familial cancers (HNPCC) and some subtype of sporadic colon cancer had widespread alterations in short repeated DNA sequence, suggesting that numerous replication errors had occurred during tumor development and may be caused by a common mechanism.

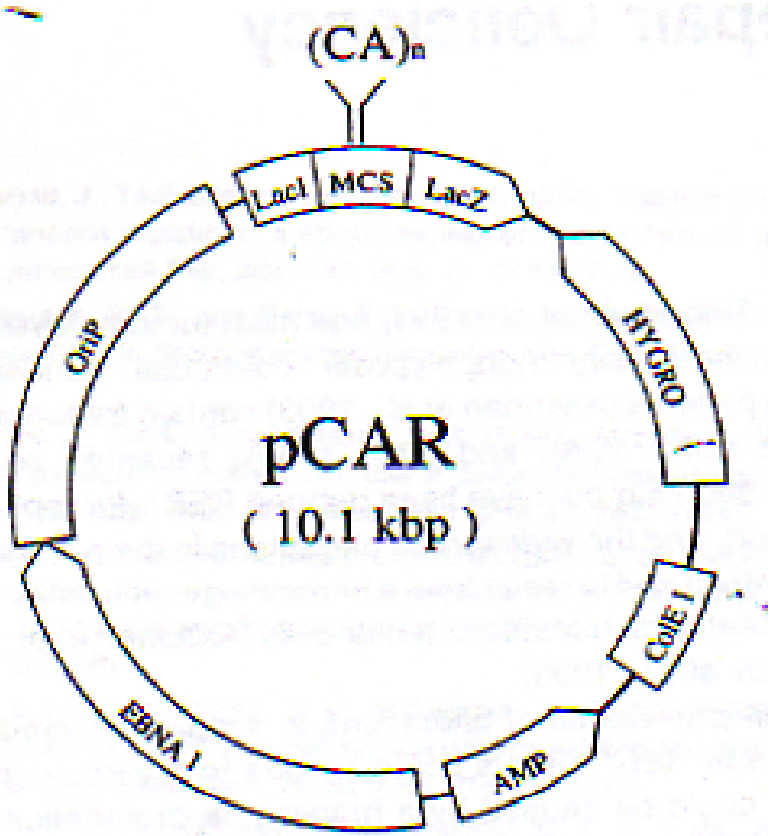
Paper #2:

Hypermutability and Mismatch Repair Deficiency in RER$^+$ Tumor Cells

Cell, 75, 1227-1233 (1993)

By Bert Vogelstein and Paul Modrich's group

RER$^+$ (Replication Error Positive) = MSI$^+$ (Microsatellite Instability)



Shuttle Vectors for (CA)n Mutability Assay.

Insertion and deletion in the (CA)n tract during replication in recipient cells result in restoration of frame, restoring β-galactosidase activity to reporter.

The vector was transfected into two colorectal cancer cell lines, **H6** (RER^+) and **SO** (RER^-). DNA were isolated and transfected to *E.coli* cells to detect β-galactosidase activity. Insertion or deletions in the $(CA)_{14}$ tract during replication of the vector in recipient cells could therefore result in restoration of frame, restoring β-galactosidase activity to the reporter.

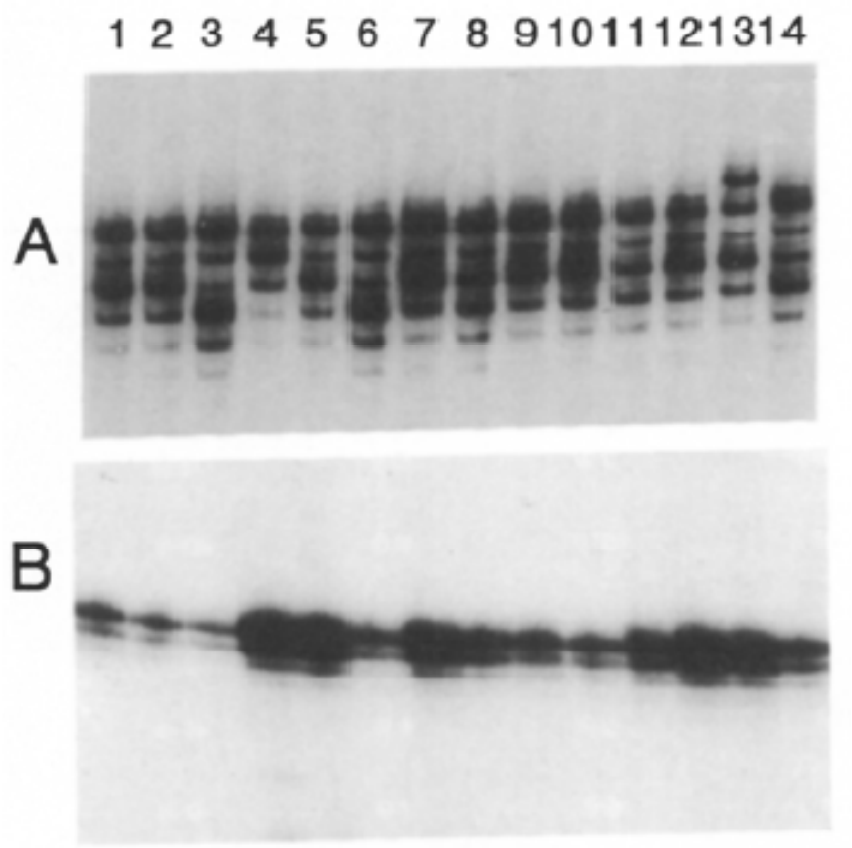
Table 1. Hypermutability of Exogenous Sequences

	H6				SO			
	pCAR1		pCAR2		pCAR1		pCAR2	
Vector	Percent[a]	Colonies Tested[b]	Percent[a]	Colonies Tested[b]	Percent[a]	Colonies Tested[b]	Percent[a]	Colonies Tested[b]
Experiment 1	1.1	744	1.2	256	0.0	1,488	0.14	1,392
Experiment 2	1.0	896	2.7	800	0.17	7,888	0.13	11,056
Experiment 3	0.42	944	3.2	252	0.071	1,400	0.18	1,136
Experiment 4	1.6	620	1.7	892	0.058	10,384	0.082	9,712
Total	0.97	3,204	2.2	2,200	0.094	21,160	0.12	23,296

[a] Percent β-galactosidase-positive colonies.
[b] Total colonies tested.

One copy of $(CA)_{14}$: pCAR1. Two copies of $(CA)_{14}$: pCAR2.

The mutability of $(CA)_{14}$ repeats is 10 times higher in H6 (RER^{+}) cells than in SO (RER^{-}) colon tumor cells.



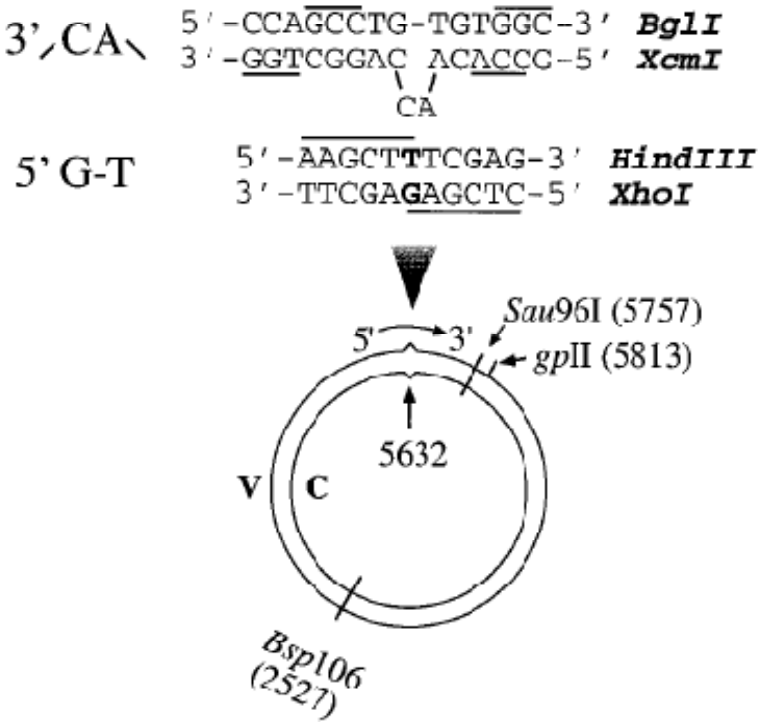
Microsatellite alterations in H6 or SO subclones. DNA from subclones of H6 or SO cells was used as template for PCR reaction with primer specific for the AFM164xe3 marker.

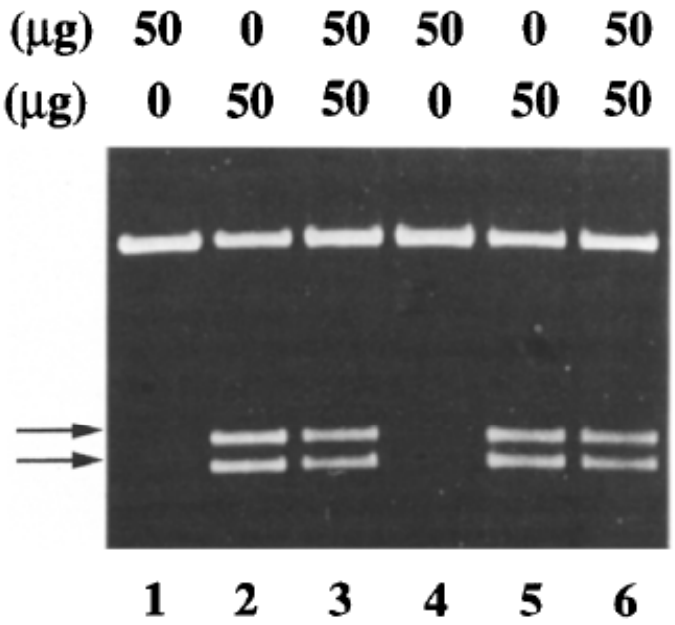
Table 2. Mutability of Endogenous Sequences

Cell Line	Clone	Number of Subclones Analyzed	Percent Subclones with Variants*	
			AFM164xe3 Marker	*AFM212xg5* Marker
H6	A	113	4.4	7.1
H6	B	154	11	8.4
SO	A	100	0	0
SO	B	40	0	0

* Variants had new alleles (not found in the parental clone) constituting at least 25% of the total alleles observed in the subclone.

H6 and SO cells were subcloned from four clones. DNA was then purified from 40-154 such subclones and analyzed for microsatellite alterations within (CA)n-containing markers.





DNA heteroduplex substrates used in MMR assay. The heteroduplex were constructed from the f1MR phage series to contain a G-T mismatch or a CA dinucleotide insertion/deletion mismatch . Both substrates have a nick on V or C strand. The substrates are resistant to restriction enzyme hydrolysis at the site of mismatch; However, repair of the substrate forms a homoduplex product that is sensitive to a restriction enzyme digestion.

H6 (RER^+) cell extract is defective in repairing mismatch substrate.

Table 4. H6 Cells Are Deficient in Repair of Base–Base Mismatches

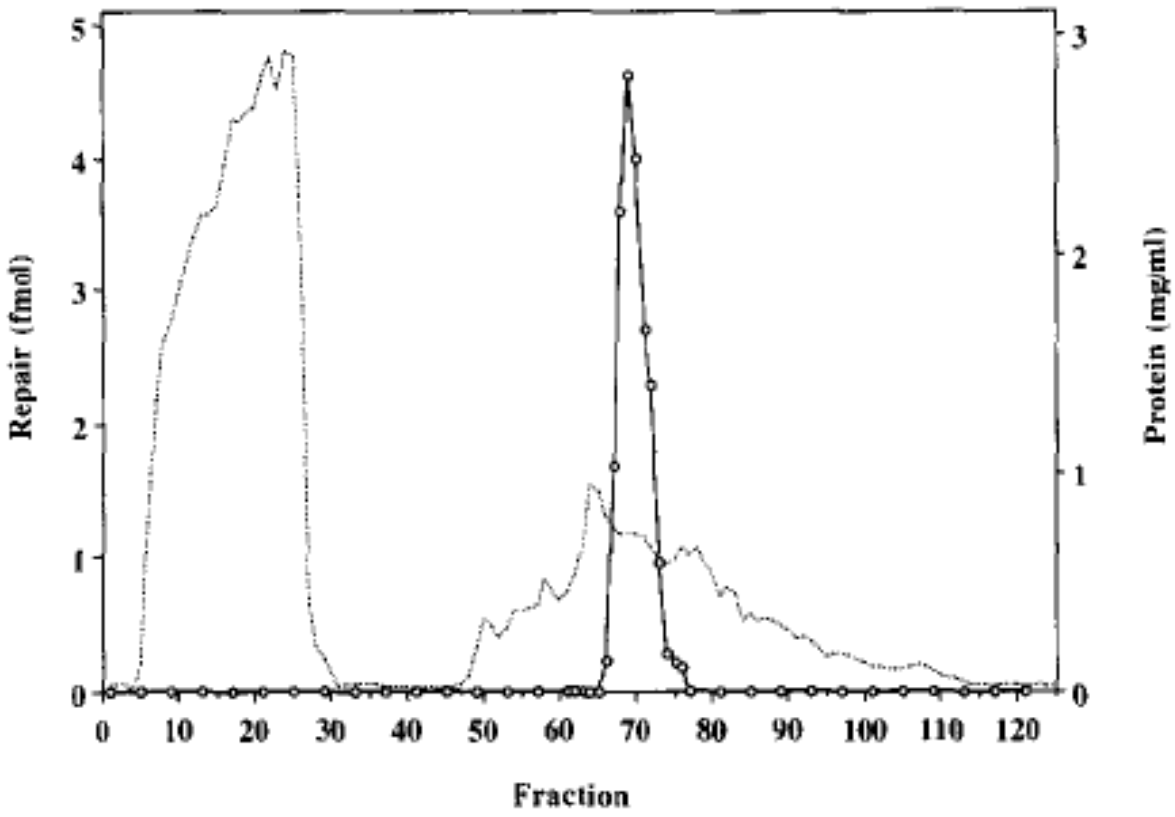
	Repair of Sau96I-Nicked Heteroduplexes (fmol)	
Mismatch	SO Cell Line	H6 Cell Line
G–T	12	<0.3
A–C	8.4	<0.3
G–G	10	1.7
A–A	8.4	0.7
A–G	6.5	0.7
T–T	7.9	<0.3
C–T	8.5	<0.3
C–C	6.3	0.3

H6 (RER^+) cell extract can not repair any base-base mismatched substract.

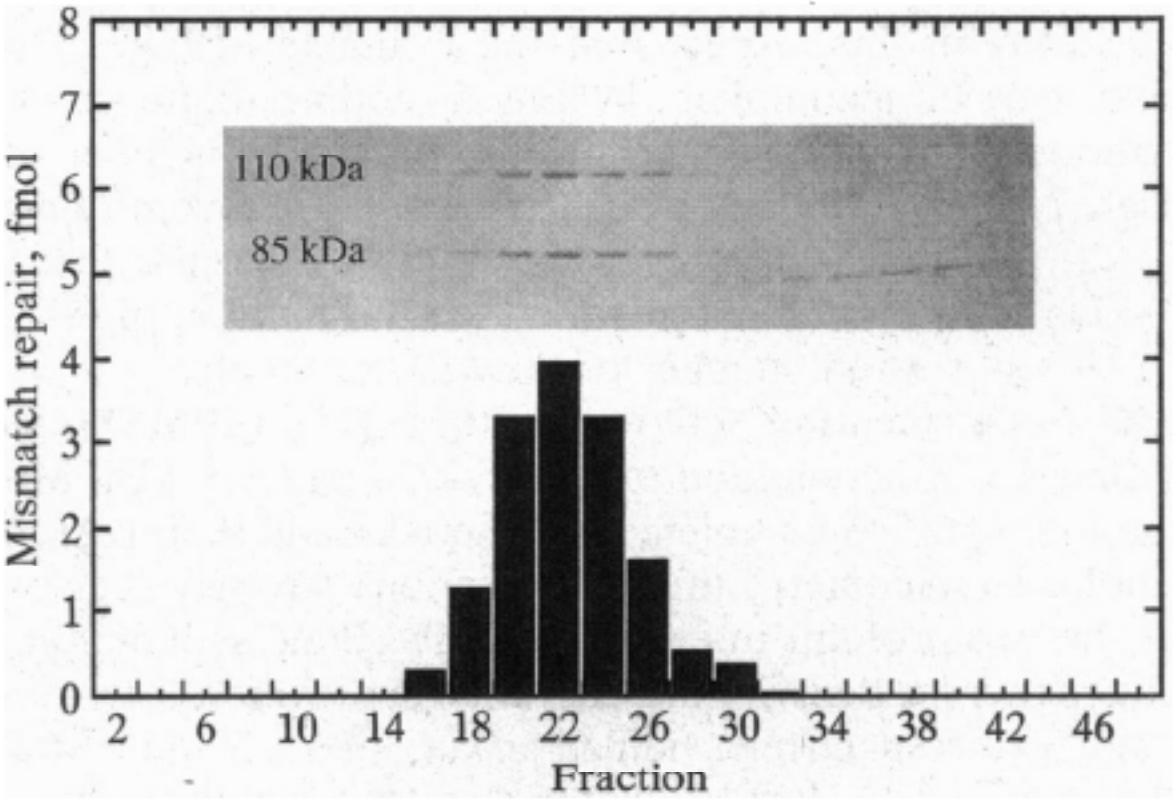
Table 3. H6 Cells Are Deficient in Repair of Slipped-Strand Mispairs

Mismatch	Restriction Markers	Repair (fmol) Sau96I Nick SO	Repair (fmol) Sau96I Nick H6
5′-CCAGCCTG-TGTGGC 3′-GGTCGGAC ACACCG AC	BglI–XcmI	9.1	<0.3
TG 5′-CCAGCCTG TGTGGC 3′-GGTCGGAC-ACACCG	XcmI–BglI	7.0	1.4
5′-CCAGCCTG-CTGGGC 3′-GGTCGGAC GACCCG GAC	BglI–XcmI	13	<0.3
CTG 5′-CCAGCCTG CTGGGC 3′-GGTCGGAC-GACCCG	XcmI–BglI	13	3.6
5′-CCAGCCTG- -TGGC 3′-GGTCGGAC ACCG ACAC	PflMI–XcmI	12	0.8
5′-AAGCTTG(CA)$_{20}$GTCTAGA 3′-TTCGAAC(GT)$_{19}$CAGATCT	–	9.2	<0.3
5′-AAGCTTG(CA)$_{19}$GTCTAGA 3′-TTCGAAC(GT)$_{20}$CAGATCT	–	5.6	0.5

H6 cells are defective in repairing slipped-strand mismatches.

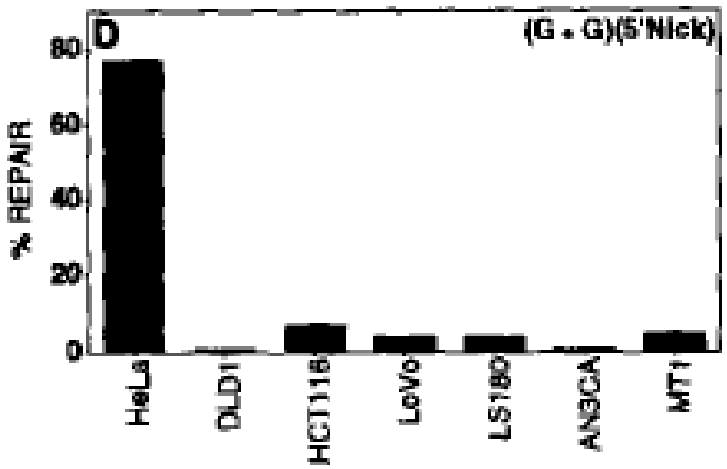


A Hela nuclear component restores mismatch repair to H6 nuclear extracts. Hela cell nuclear extract was fractionated by chromatography on phosphocelluose. Samples (2 μl) of each fraction were assayed for their ability to restore mismatch repair to H6 nuclear extract on CA insertion substrates. Mismatch repair is shown by open circles, while protein concentration is indicated by the dotted line.



Purification of MutLa from Hela-S3 cell extract.

Cell line	Original ref.	Type of cell	RER phenotype[a]
HeLa	23	Cervical carcinoma	–
SW480	24	Colorectal carcinoma	–
TK6[c]	25	Transformed lymphoblast	–
DLD1	26	Colorectal carcinoma	+
HCT116	27	Colorectal carcinoma	+
LoVo	28	Colorectal carcinoma	+
LS180	29	Colorectal carcinoma	+
AN_3CA	30	Endometrial carcinoma	+
HEC59	31	Endometrial carcinoma	+
MT1	32	Mutant derivative of TK6	+



RER phenotypes and their repair efficiency.

Important Findings:

This paper demonstrated that the mutation rate of (CA)n repeats in RER^+ tumor cells is at least 100-fold higher than that in RER^- (MSI-)tumor and the extract of RER^+ (MSI+) cells are completely defective in the repair of base-base and ID mispairs. A biochemical basis for this phenotype was identified in this paper.

MOLECULE OF THE YEAR

DNA Repair Works Its Way to the Top

The hard-working DNA repair enzymes preserve genetic information, guard against cancer, and unite basic cell biology, cancer research, and toxicology

1994
Molecule of
the Year

The following papers will help you understand the mechanism of DNA MMR in prokaryotic and eukaryotic cells

Lahue, R.S., Au, K.G., and Modrich, P. (1989) DNA mismatch correction in a defined system. **Science**, 245: 160-164

Zhang, Y., Yuan, F., Presnell, S., Tian, K., Gao, Y., Tomkinson, A., Gu, L., and Li, G.-M. (2005) Reconstitution of human DNA mismatch repair in a purified system. **Cell** 122, 693-705

Kadyrov, F.A., Dzantiev, L., Constantin, N., and Mordich, P. (2006) Endonucleolytic Function of MutLalpha in human mismatch repair. Cell 128:126 (2): 239-41.