Genome Stability, Genetic Diseases and Cancer

Lecture 1 DNA damage. Damage Reversal. Base excision repair.
Mismatch repair

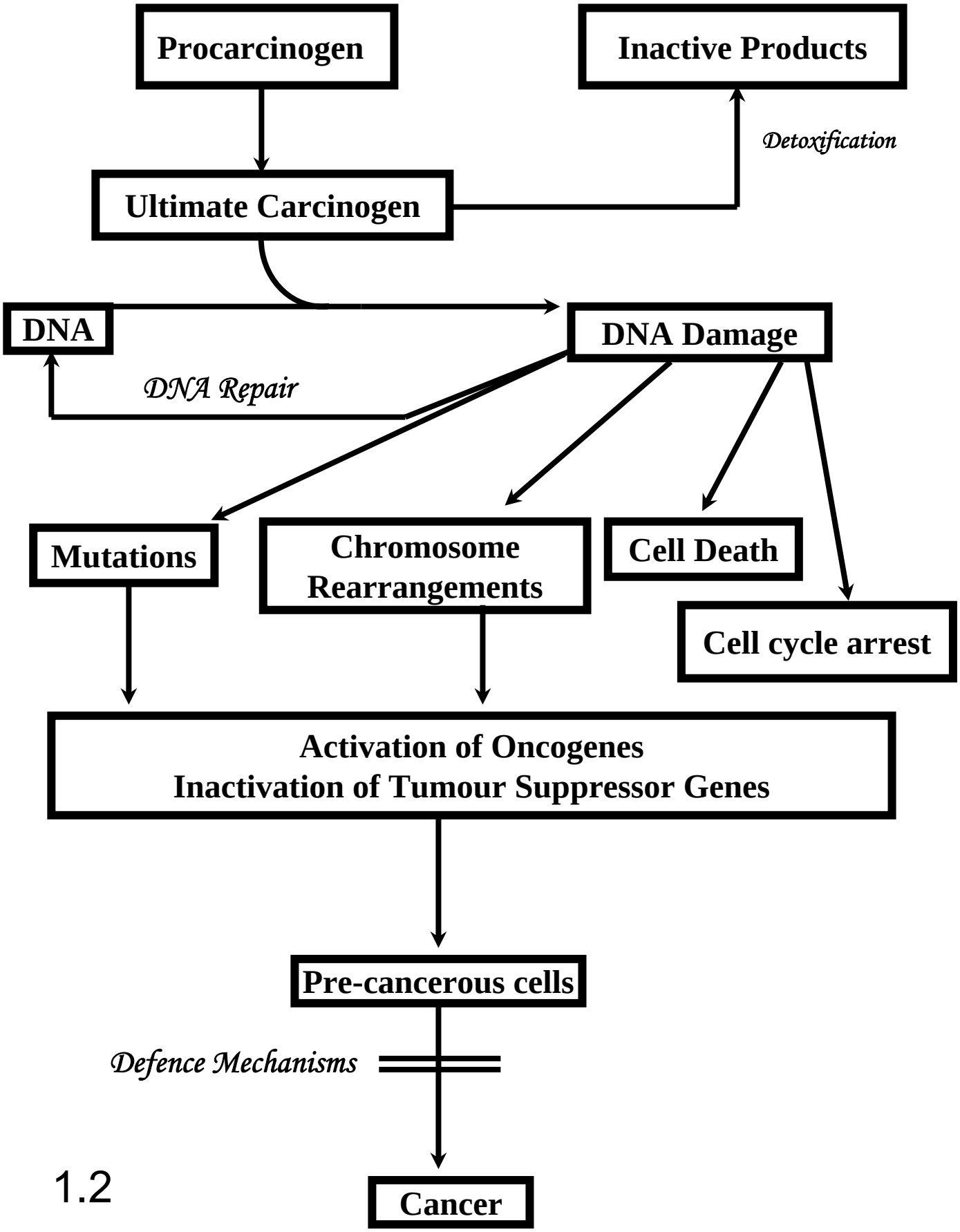
Lecture 2 Nucleotide excision repair: cellular and clinical aspects
Nucleotide excision repair: genes and proteins

Lecture 3 Replication of damaged DNA.
Mutagenesis and carcinogenesis

Learning outcomes (Lecture 1a)

Understanding:

- Different types of DNA damage
- Three examples of ways in which cells can reverse DNA damage in situ
- Basic mechanism of Base Excision Repair

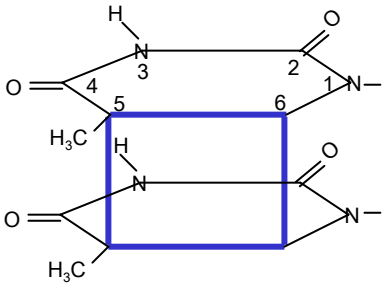

Procarcinogen
Inactive Products
Detoxification
Ultimate Carcinogen
DNA
DNA Damage
DNA Repair
Mutations
Chromosome Rearrangements
Cell Death
Cell cycle arrest
Activation of Oncogenes
Inactivation of Tumour Suppressor Genes
Pre-cancerous cells
Defence Mechanisms
Cancer

1.2

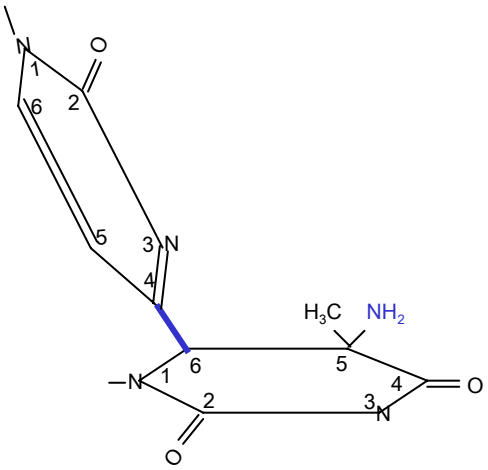
DNA Damage

	UV	Ionizing Radiation	Monofuctional Chemicals	Bifunctional Chemicals
Non distorting chemical damage	-	+	+	+ -
Minor distorting chemical damage	+	+	+	+ -
Major distorting chemical damage	+	-	+	+
Interstrand cross links	-	-	-	+
Strand breaks	-	+	+	+ -

Major UV photoproducts

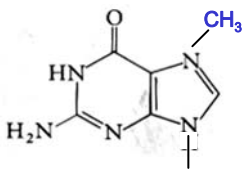


Cyclobutane pyrimidine dimer (CPD)

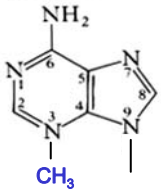


TC (6-4) photoproduct (6-4PP)

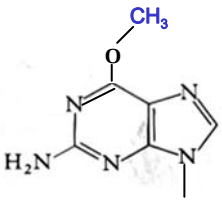
Methylated purines



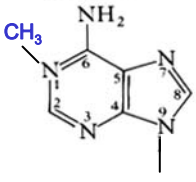
7-methylguanine



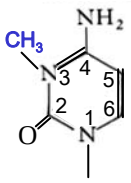
3-methyladenine



O-6-methylguanine



1-methyladenine



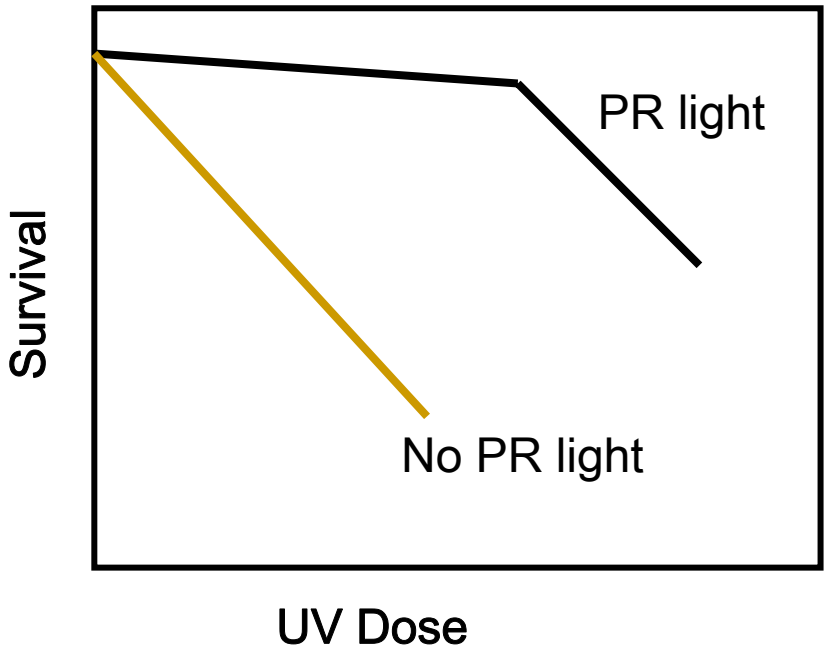
3-methylcytosine

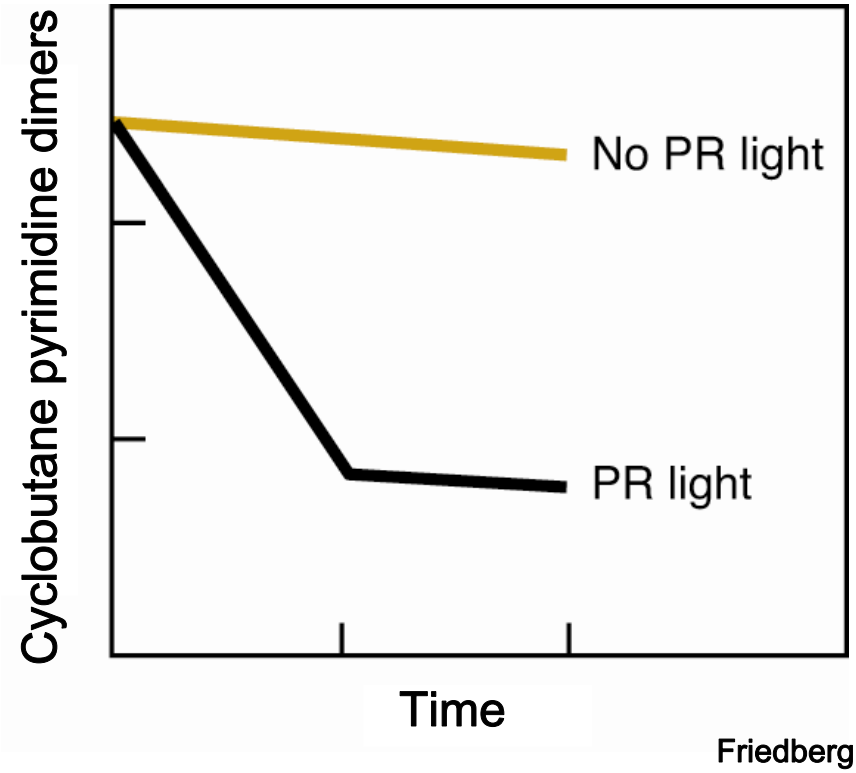
Aspects of DNA repair

1. **Initial damage**
2. **Repair of damage**
3. **Genes involved**
4. **Mechanism of action of gene products**
5. **Replication of unremoved damage. Cell cycle progression.**
6. **Biological consequences of damage, repair and failure to repair.**

Damage reversal

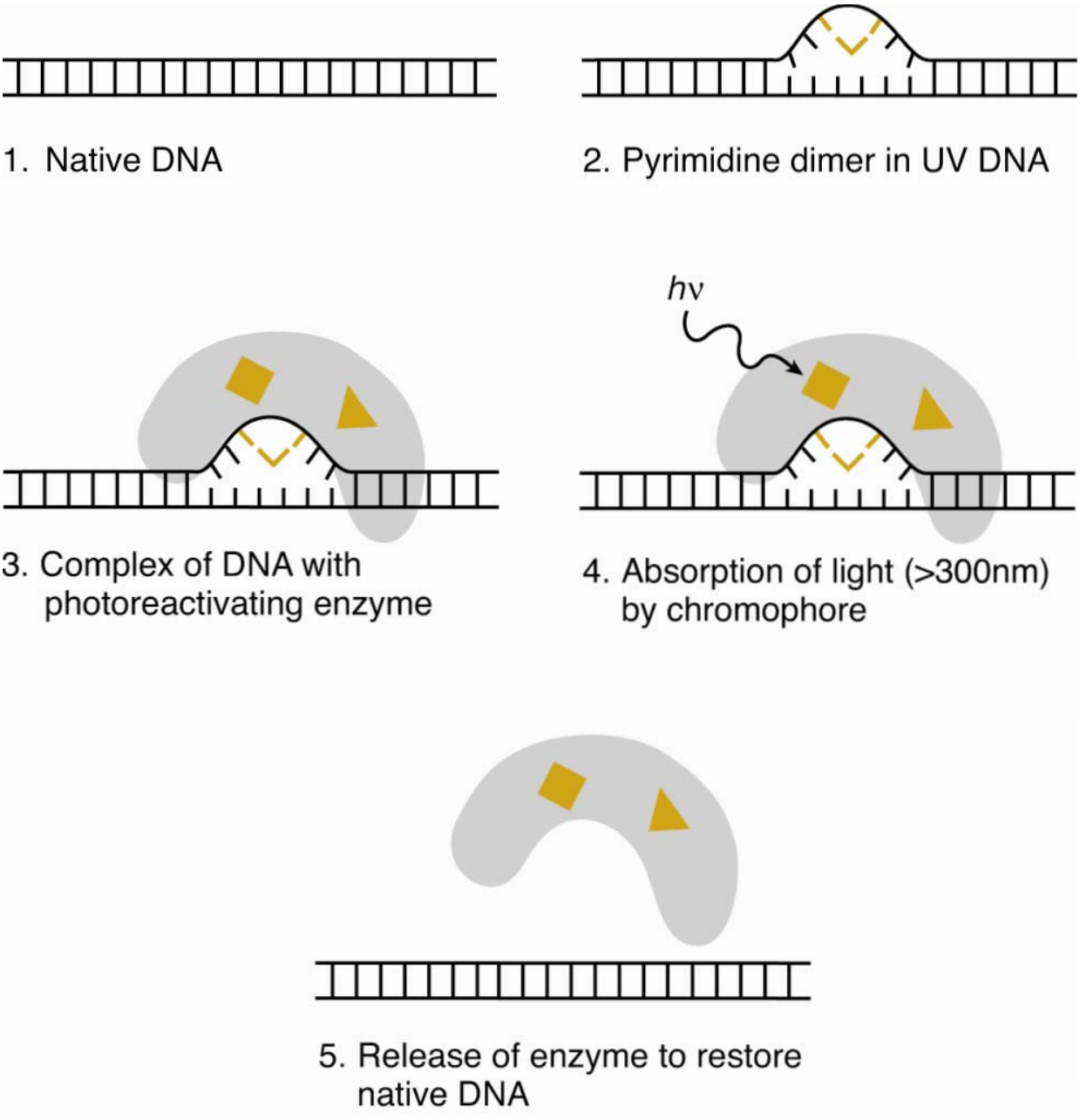
1. Photoreactivation





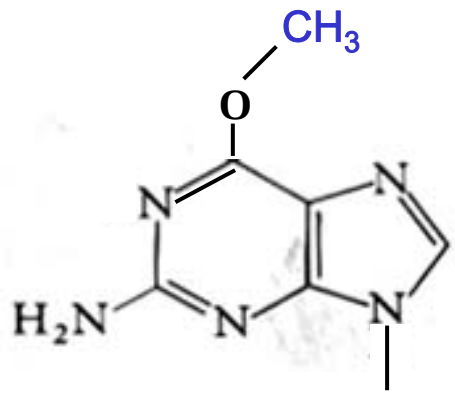
Friedberg et al, 2005
DNA Repair and Mutagenesis

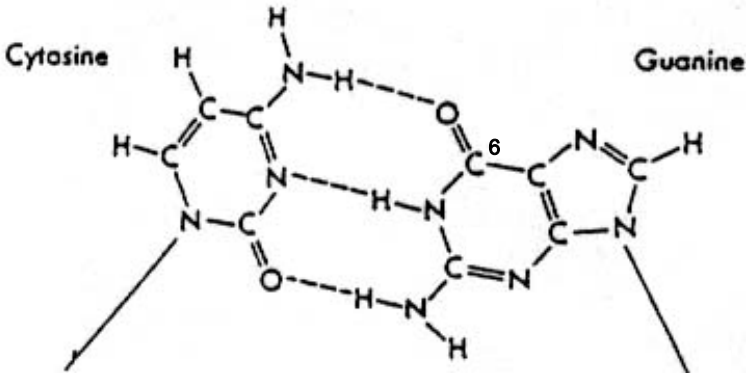
Photolyase mechanism

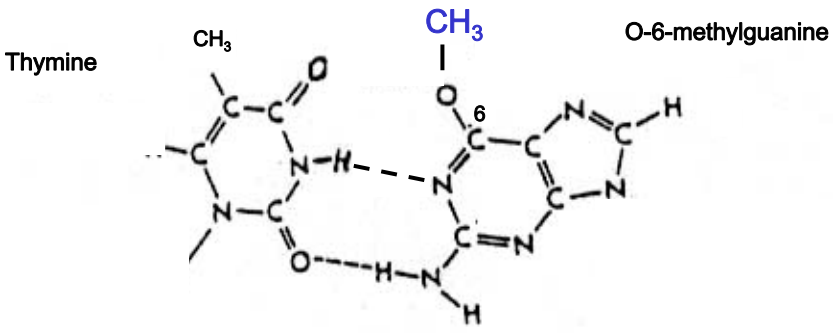


Friedberg et al, 2005
DNA Repair and Mutagenesis

Damage reversal
2. Repair of O6-methylguanine

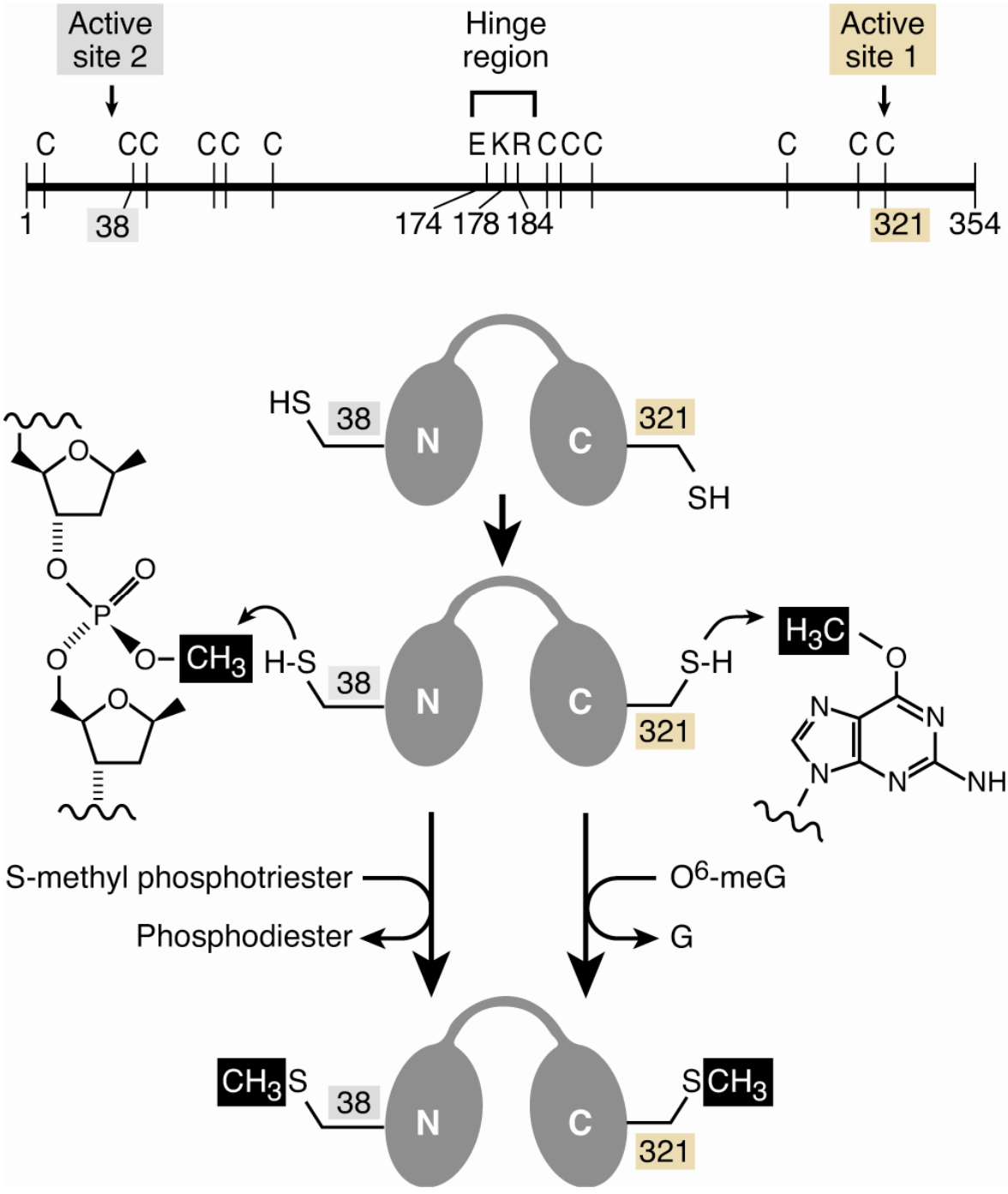






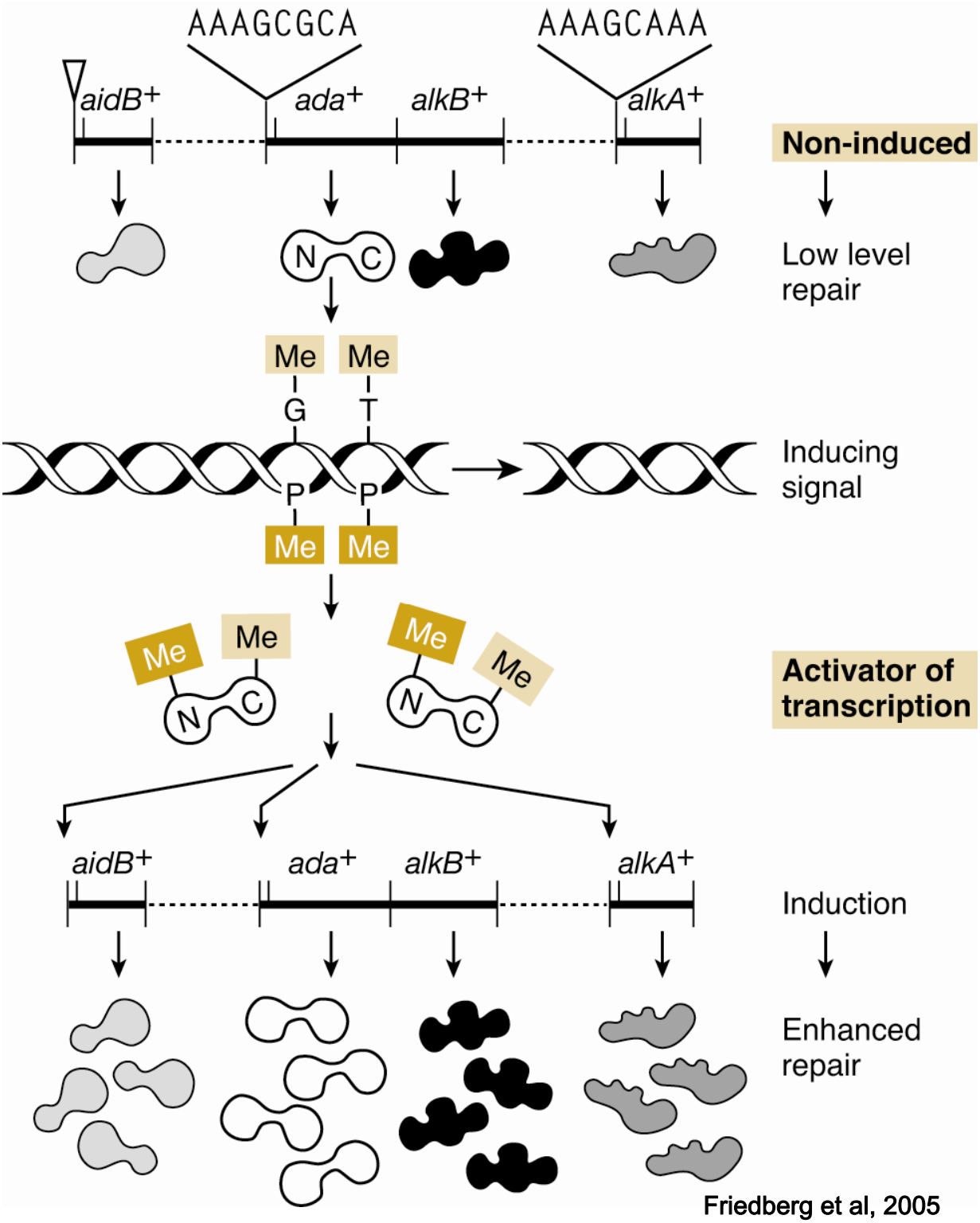
Mispairing of O-6-methylguanine with thymine

Dual activities of Ada methyltransferase



Friedberg et al, 2005
DNA Repair and Mutagenesis

Induction of *ada* gene



Friedberg et al, 2005
DNA Repair and Mutagenesis

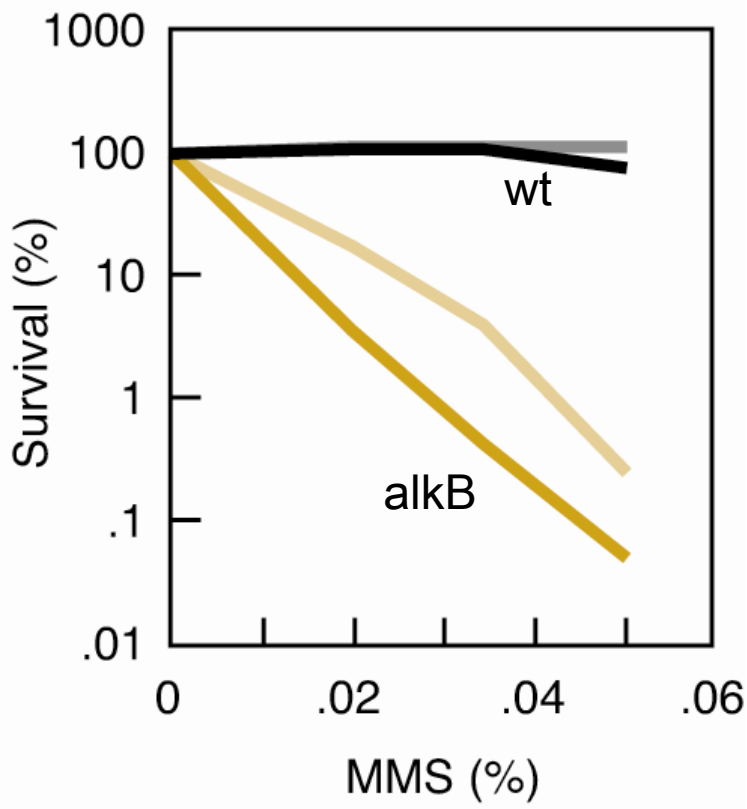
Ogt gene is not inducible

Alkyltransferases in mammalian cells

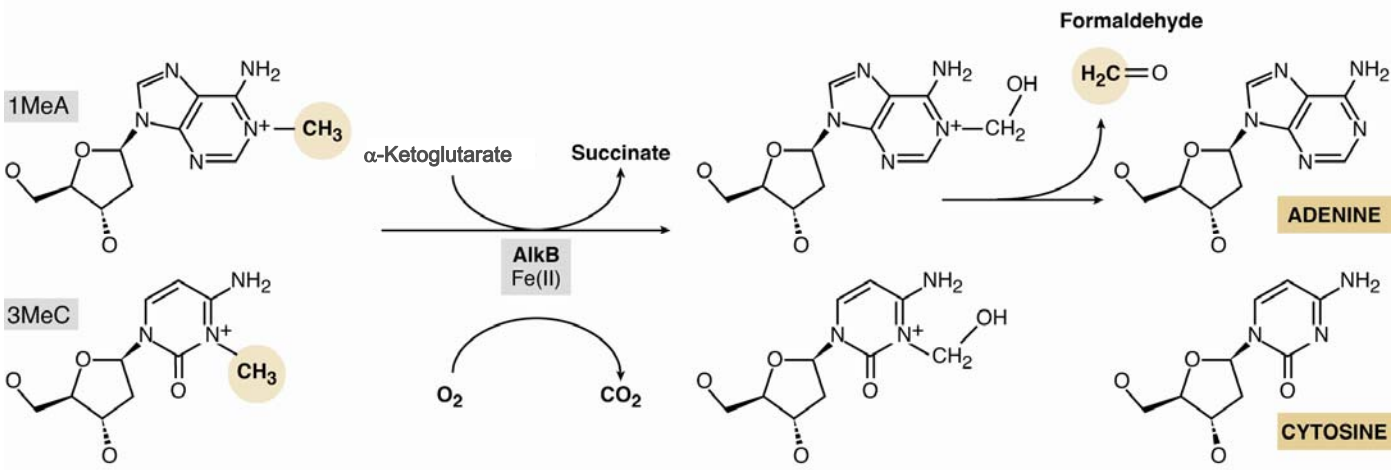
- Similar mechanism to *E. coli,* but for O-6-meG alone, like Ogt, not inducible.
- K/o mouse constructed, very sensitive to carcinogenesis by methylating agents.
- Conversely transgenic mice bearing MGMT gene are more resistant.
- Many cancer cell lines are Mex^-. MGMT silenced by methylation in about 50% of tumours.
- Mex^- cells are sensitive to killing and mutagenesis by alkylating agents.
- Many cancer therapy drugs are alkylating agents, eg temozolomide.
- Patrin2 binds MGMT and depletes it. Currently in clinical trials together with temozolomide.

Damage reversal
3. Oxidative demethylation (A4)

alkB survival



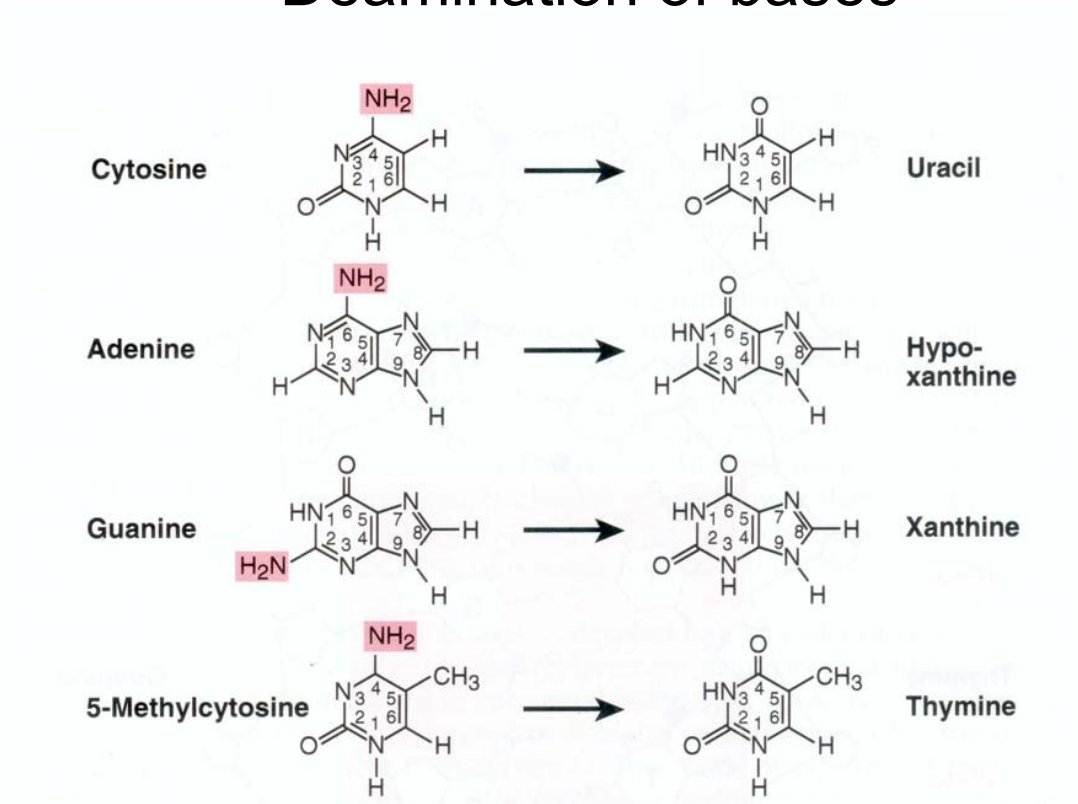
Mechanism



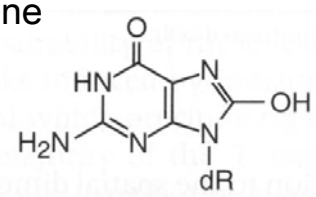
Friedberg et al, 2005
DNA Repair and Mutagenesis

Base Excision Repair

Deamination of bases

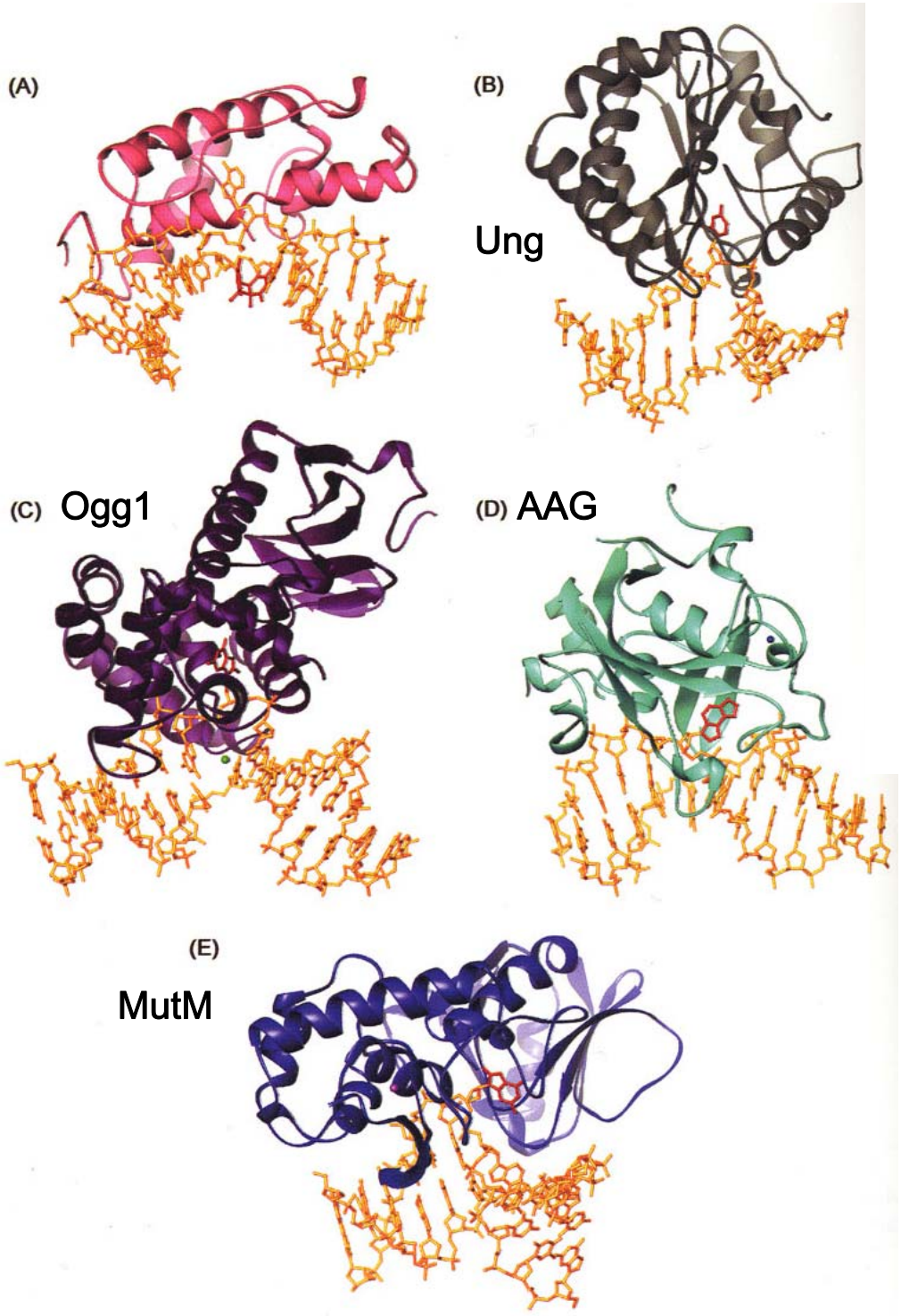


8-hydroxyguanine



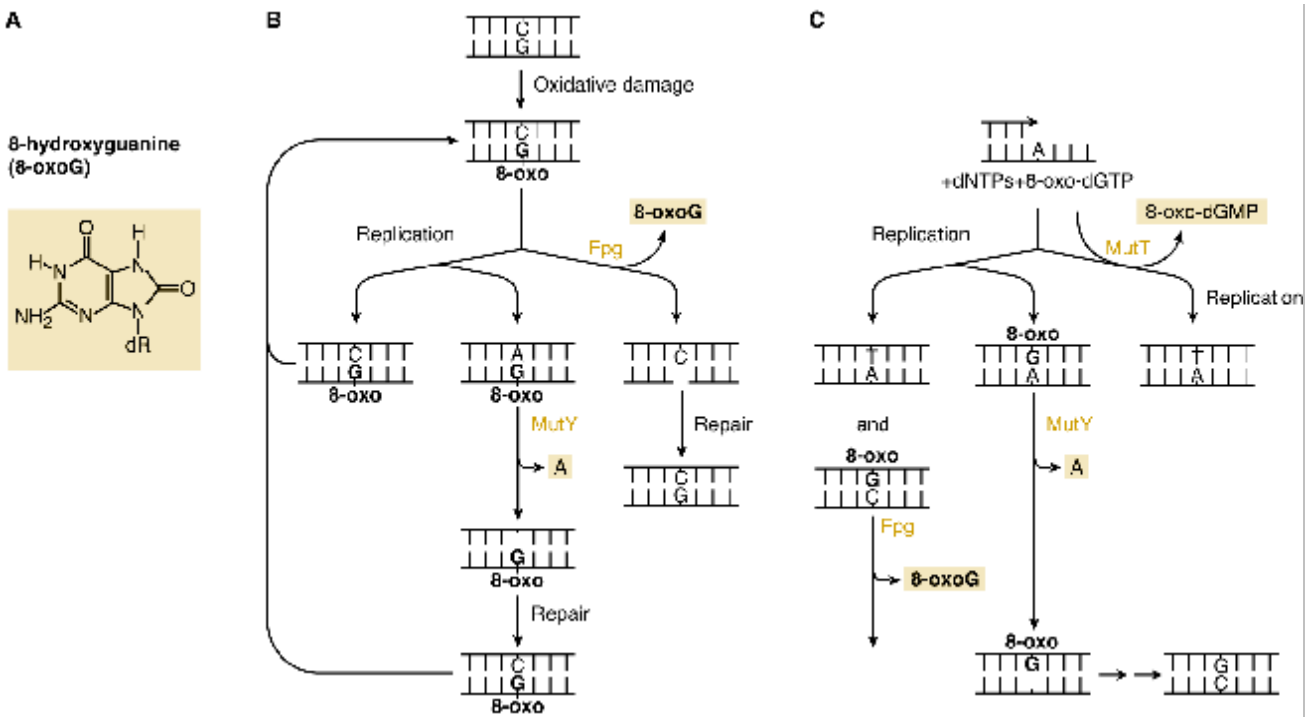
DNA glycosylases				
Enzyme		Size (aa)	Chromosome location of gene	Altered base removed from DNA
E. coli	Human			
ung	UNG2	313	12q23-q24	U and 5-hydroxyuracil (rep fork)
	MUG	410	12q24.1	U or T opposite G, ethenocytosine
	hSMUG1	270	12q13.1-q14	U (from G:U mismatches)
	MBD4	580	3q21	U or T opposite G at CpG sequences
Fpg (MutM)	hOGG1	345	3p25	8-oxo G opposite C, formamidopyrimidine
MutY	MYH	521	1p32.1-p34.3	A opposite 8-oxo G
Nth	hNTH1	312	16p13.2-	Thymine glycol, cytosine glycol, dihydrouracil, formamidopyrimidine
AlkA and Tag	AAG	293	16p (near telomere)	3-MeA, ethenoadenine, hypoxanthine
Nei	Neil 1			Oxidised pyrimidines (rep fork)
	Neil2			Oxidised pyrimidines
	Neil3			

3-d structures of glycosylases

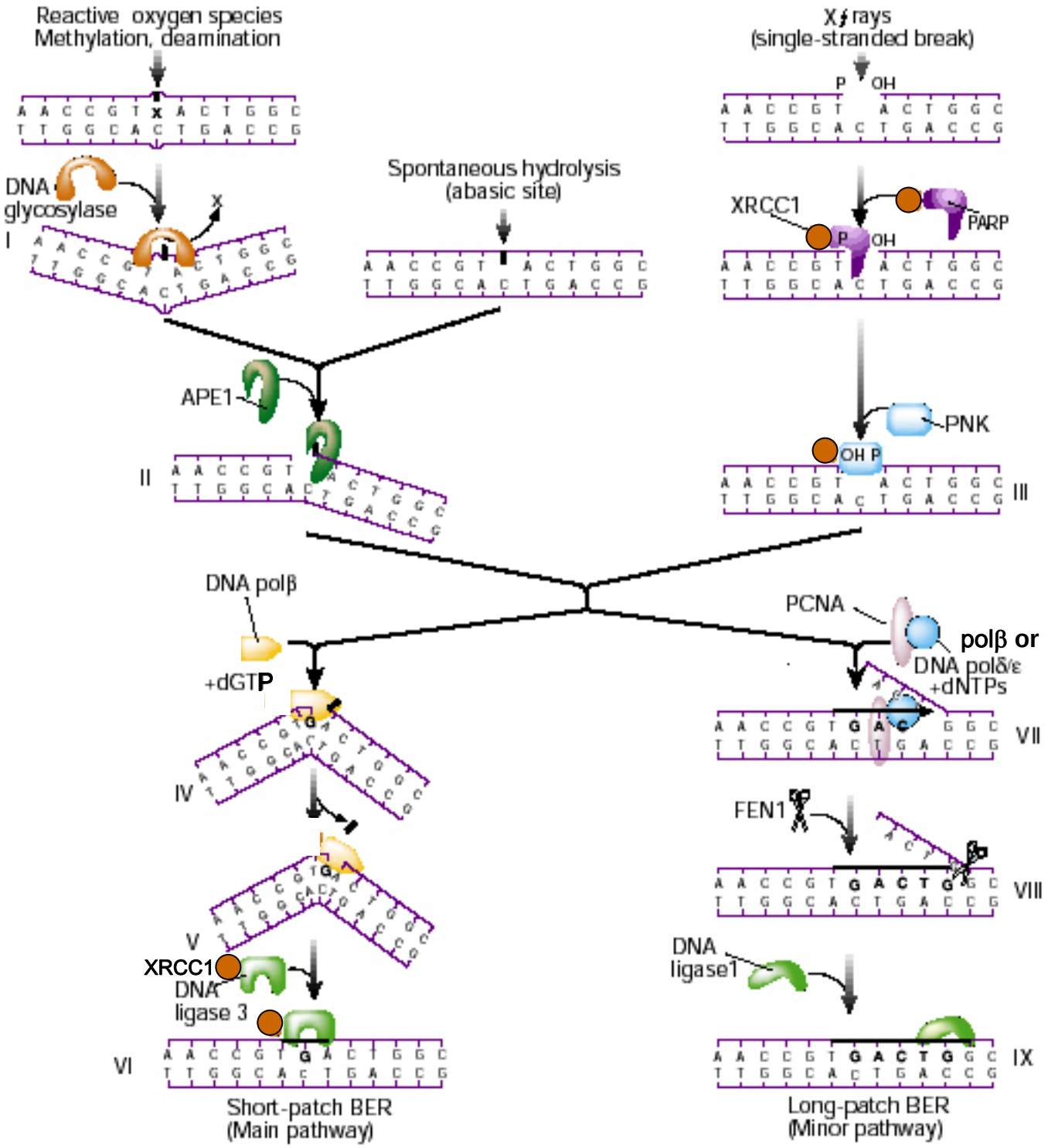


FROMME AND VERDINE, FIG. 4. (*continued*)

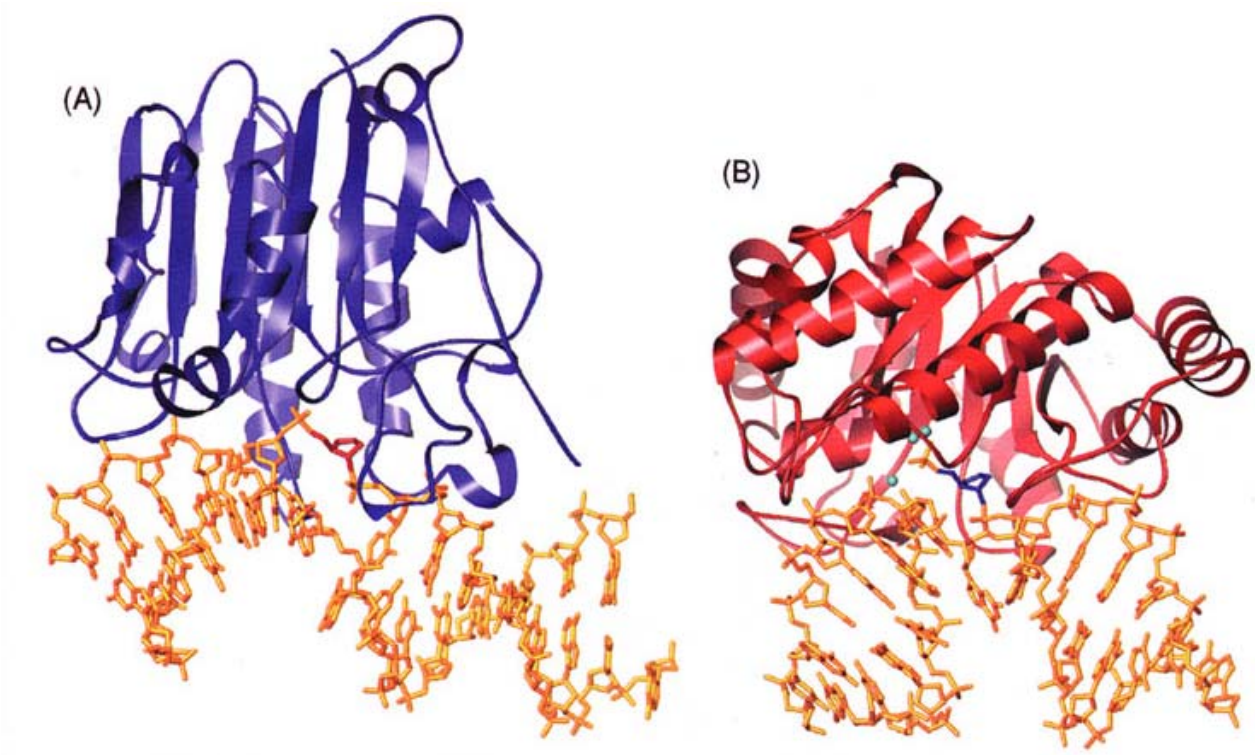
Protection from 8-oxoguanine



Base Excision-Repair



3-d structures of APendonucleases



APE1

ENDO IV

Summary (Lecture 1a)

- DNA damage can cause distortions of different severity
- With visible light, UV damage is repaired by photoreversal (not in placental mammals)
- O6-methylguanine is repaired by a specific methyltransferase
- 1-methyladenine and 3-methylcytosine are repaired by oxidative demethylation
- Spontaneous lesions are removed by Base Excision Repair

Learning outcomes (Lecture 1b)

Understanding:

- Detailed mechanism of mismatch repair in *E. coli* and eukaryotes
- How mismatch repair is important both for cancer protection and cancer therapy

Mismatch Repair (A5, A6)

- DNA polymerases replicate DNA very faithfully.
  Accurate insertion
  Associated 3'-5' exonuclease for proof-reading
  Error rates c. 10^{-6} or less

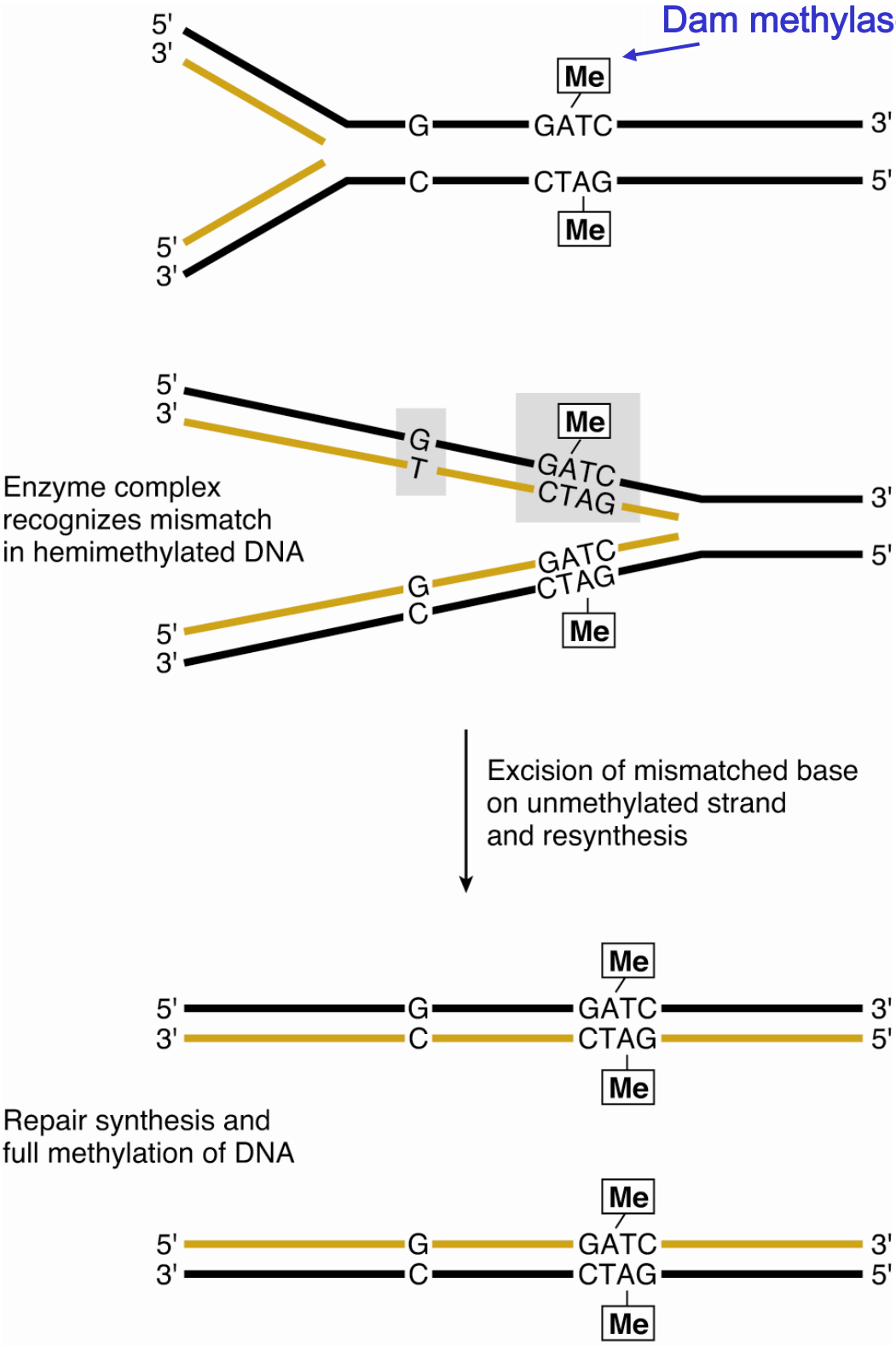
- But genomes are big: *E. coli* $3x10^6$ bp, mammals $3x10^9$

- Errors can be single base mismatches or small insertions or deletions caused by base slippage

- Mismatches are repaired by the MMR system which recognises the mismatched bases

- But there's a problem

Methylation-directed mismatch repair

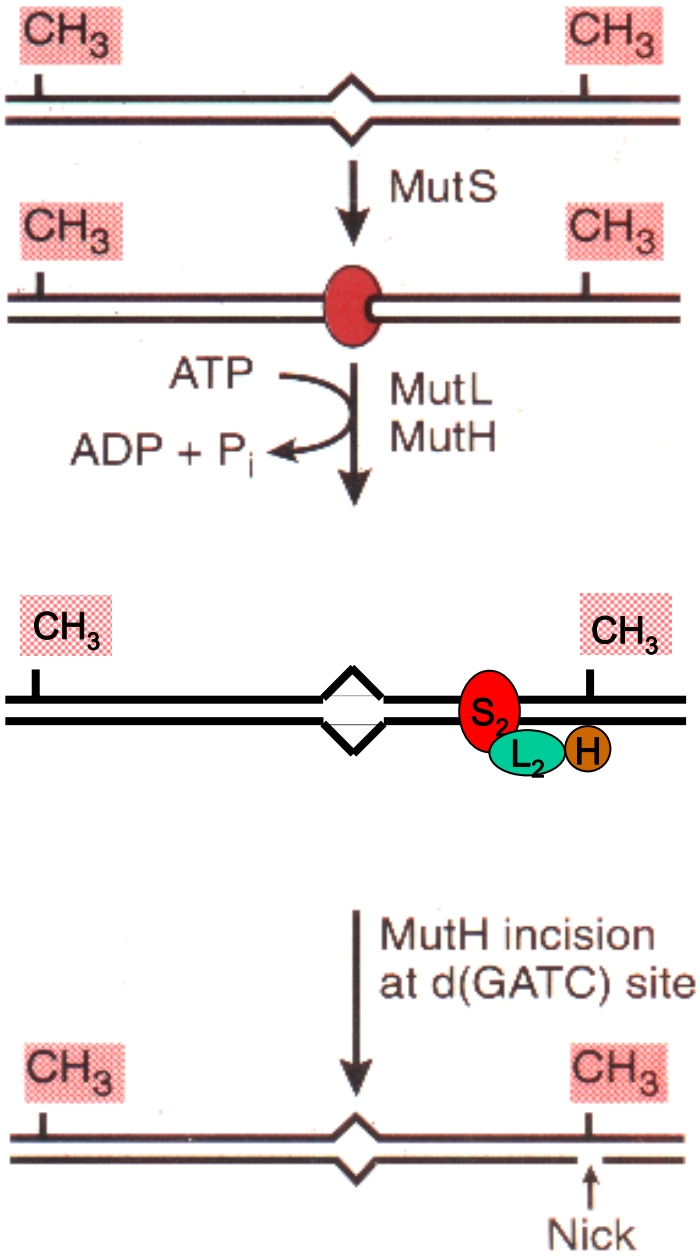


Friedberg et al, 2005
DNA Repair and Mutagenesis

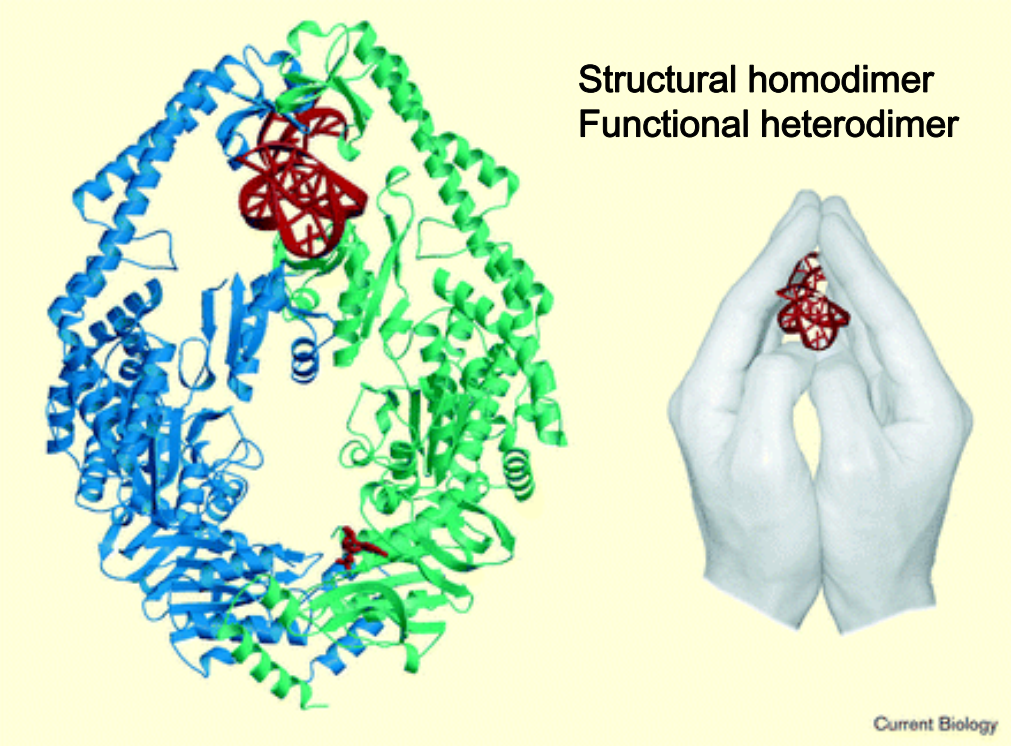
Dam$^-$ strains (methylation deficient) are mutators

Mismatch recognition and strand discrimination in *E. coli*

MutH, MutL and MutS$^-$ strains are mutators

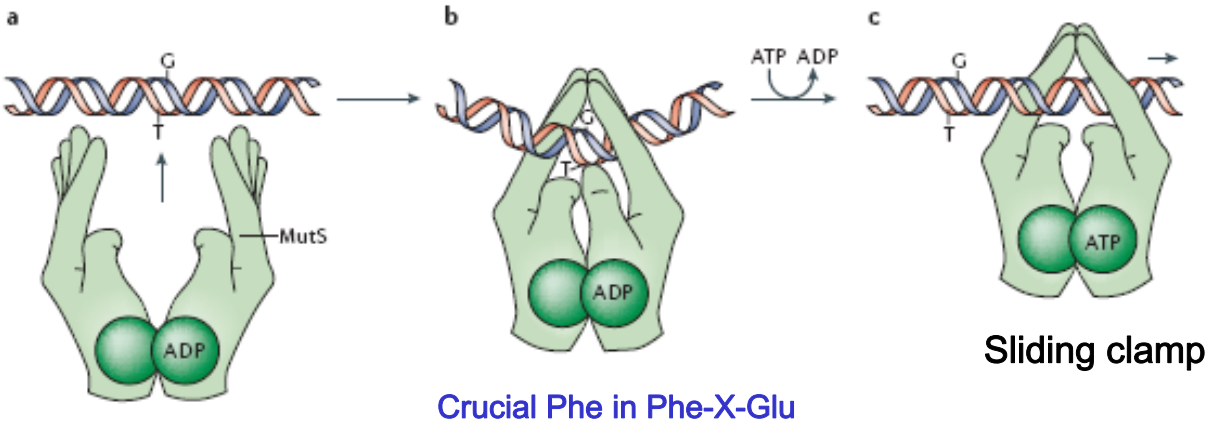


3-D structure of MutS



Jiricny, Current Biology, 2000

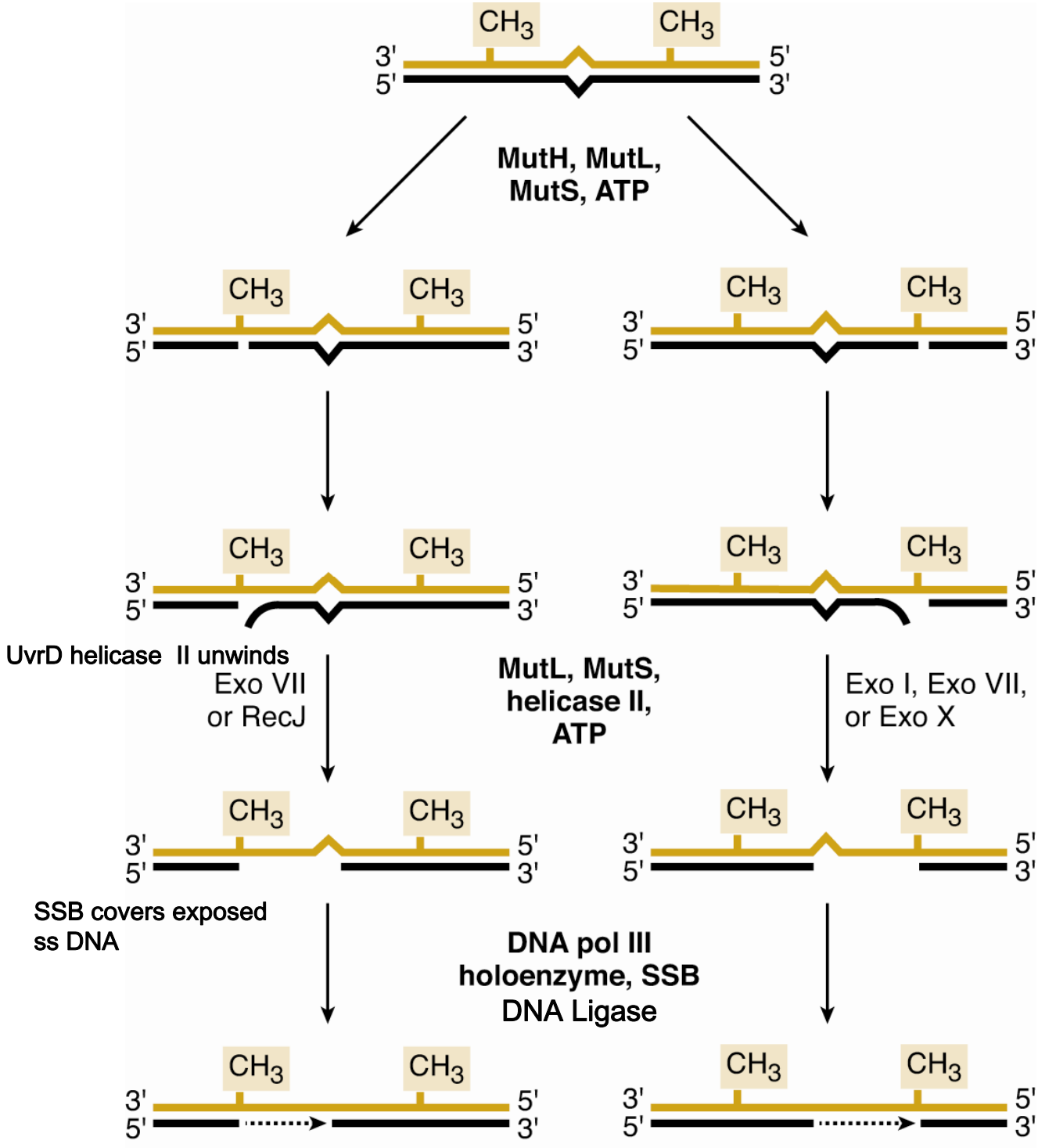
Activities of MutS



Crucial Phe in Phe-X-Glu contacts mismatch

Jiricny, MRMCB, 2006

Late steps in MMR in *E. coli*



Friedberg et al, 2005
DNA Repair and Mutagenesis

Human homologues of MutH,L,S

MutS:

Msh2	MMR
Msh3	MMR
Msh4	Meiosis
Msh5	Meiosis
Msh6	MMR

MutL:

Mlh1	MMR
Mlh2	?
Mlh3	MMR
Pms1	?
Pms2	MMR (= Pms1 in yeast)

MutH:

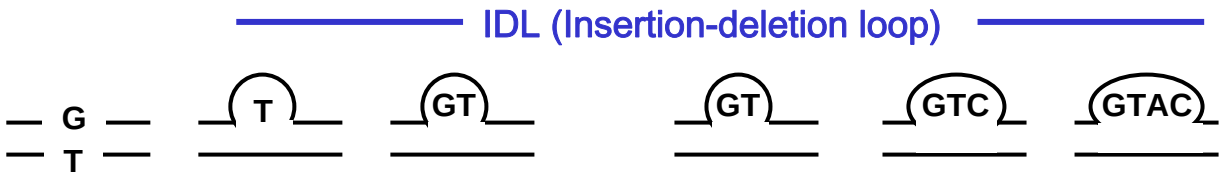
No homologues

Neither yeast nor Drosophila has methylated DNA

Strand discrimination based on nicks/ends in daughter DNA

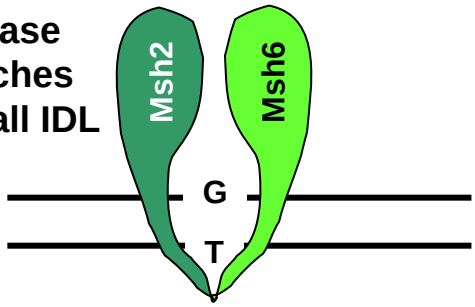
MMR proteins interact with PCNA at replication fork

Mismatch Repair in eukaryotes

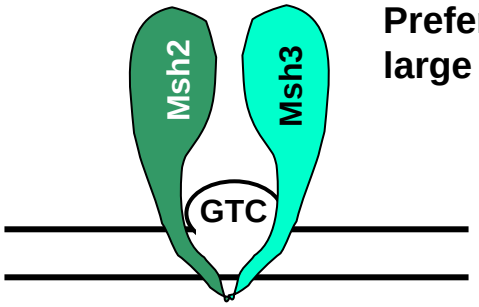


Primary recognition

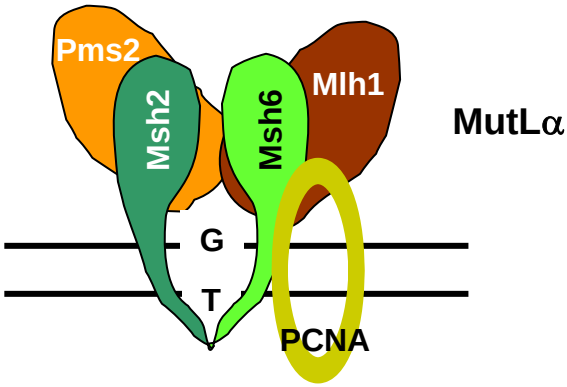
MutSα
Preference for single base mismatches and small IDL



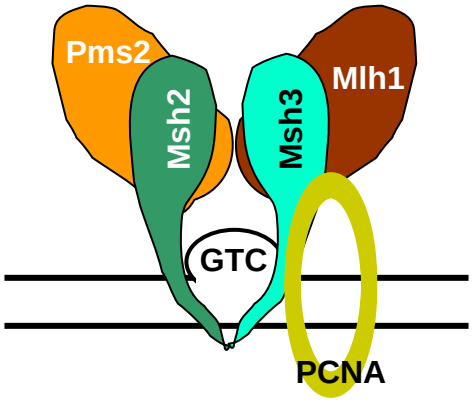
MutSβ
Preference for large IDL



Secondary recognition

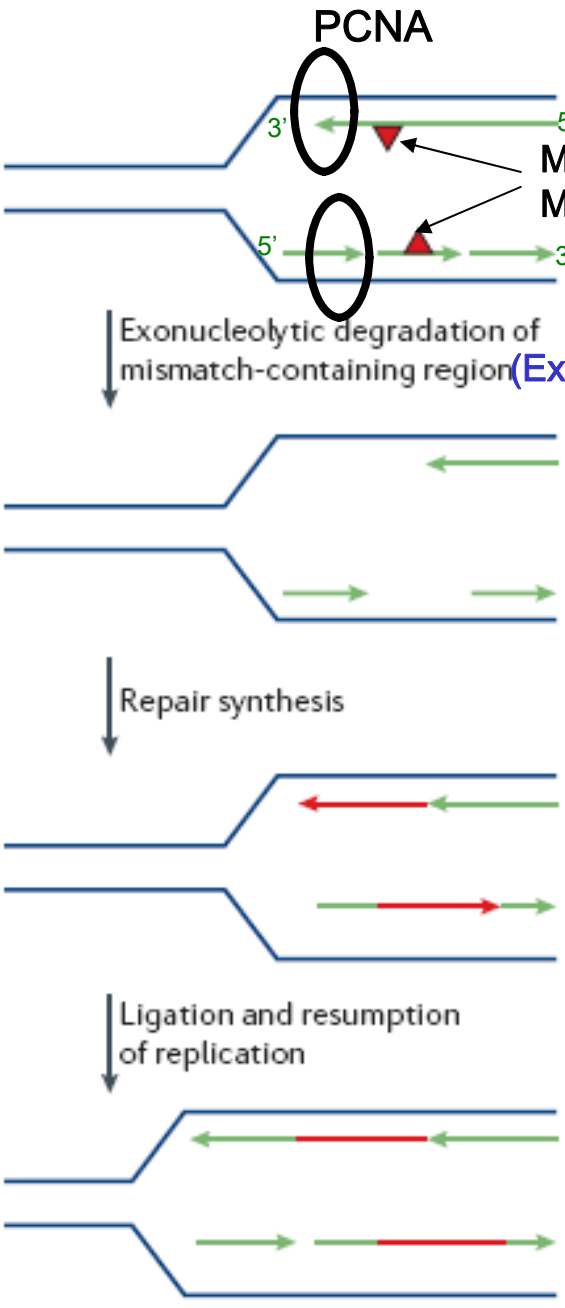


MutLα



Removal and restoration

Mismatch Repair in eukaryotes



MutSα or β binds mismatch
MutSα/MutLα translocates to end

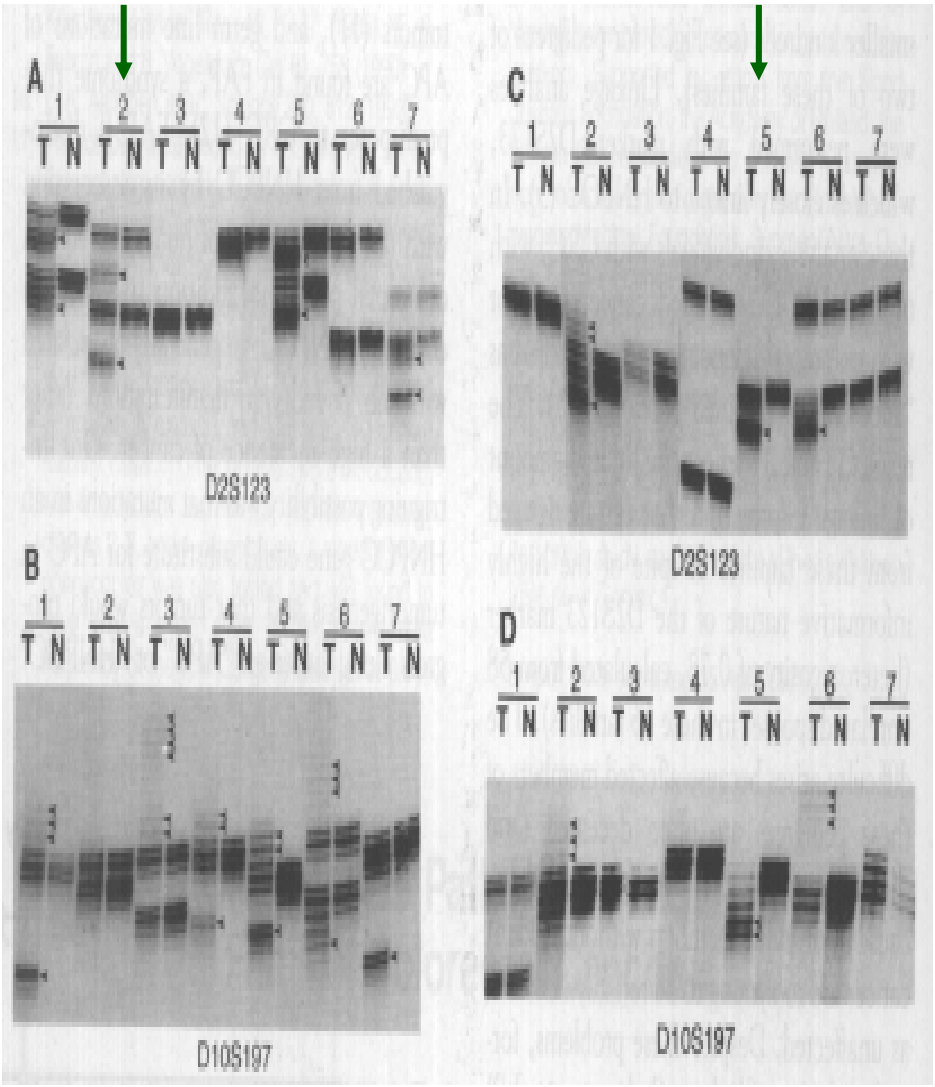
(ExoI)

Leading strand: MutLα (Pms2 subunit) cleaves on 5' side of mismatch. Exo1 degrades 5' to 3'

Lagging strand: Exo1 degrades 5' to 3'

Modified from Jiricny, Nature Rev Mol Cell Biol 2006

Microsatellite instability in tumour tissue from HNPCC (Hereditary non-polyposis colon carcinoma)



Aaltonen, et al. *Science* 260, 812 (1993)

Primer 1 →

CA CA CA CA CA CA CA

GT GT GT GT GT GT GT

← Primer 2

7 microsatellite repeats

Replication Slippage

1.23

CA

CA CA CA CA CA CA

GT GT GT GT GT GT

6 repeats

2-hit tumour suppressor model

Most HNPCC result from mutations in hMsh2 or hMlh1

Extracts of **tumour cells** are deficient in MMR of dinucleotide loops and single base mismatches

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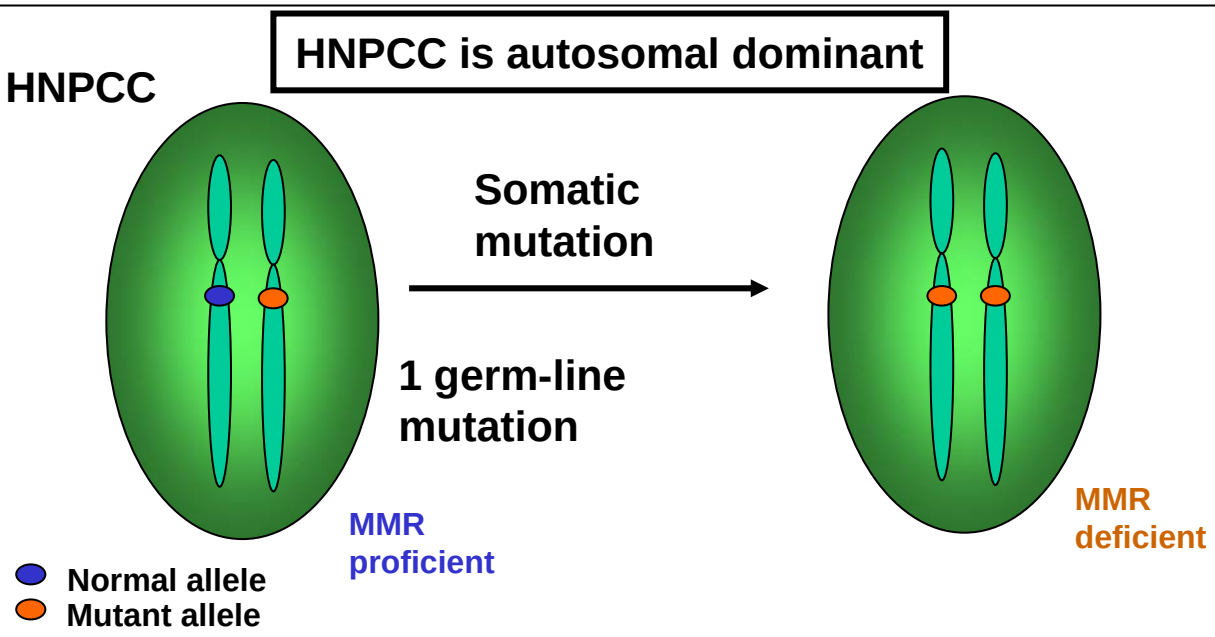
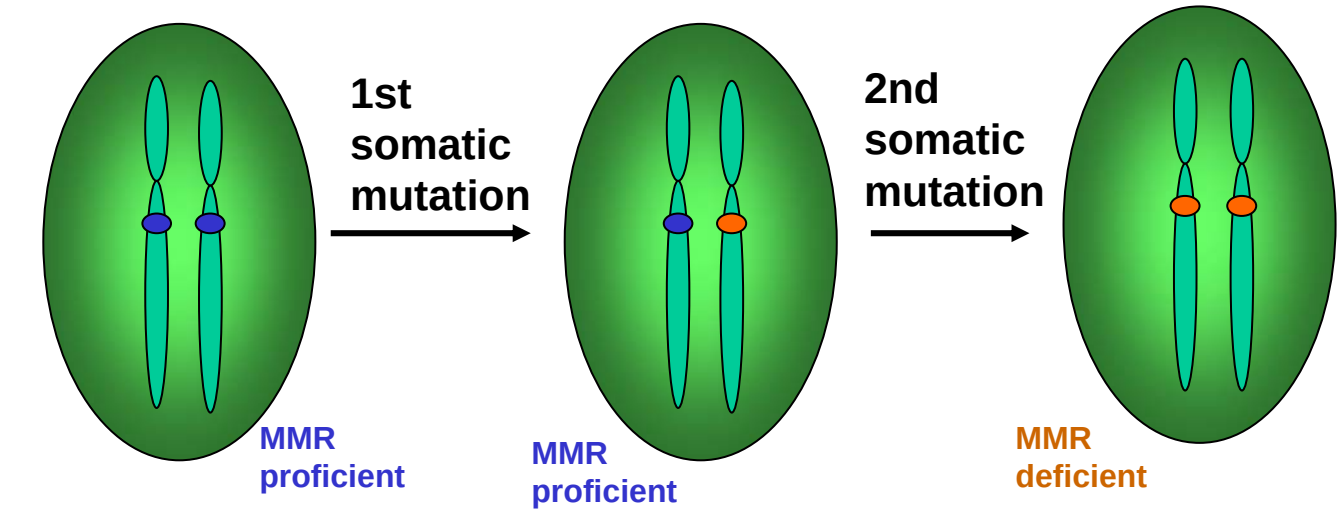
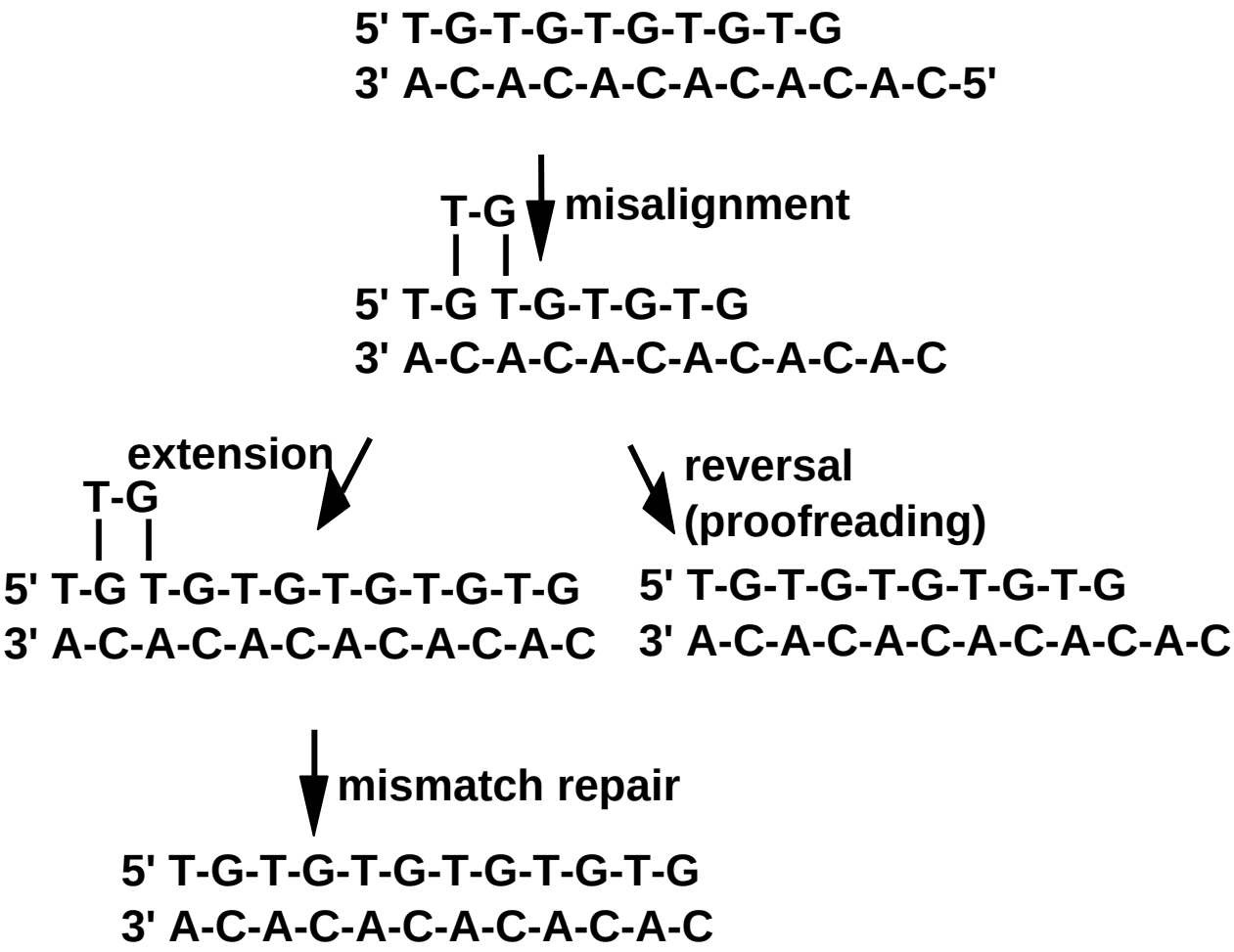


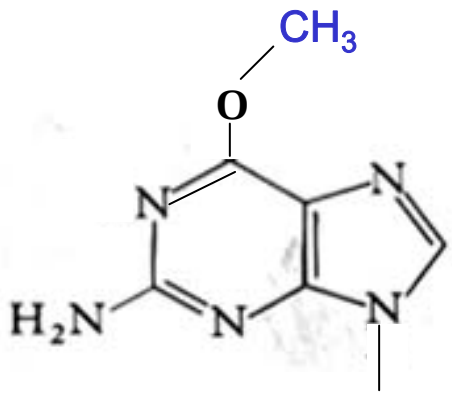


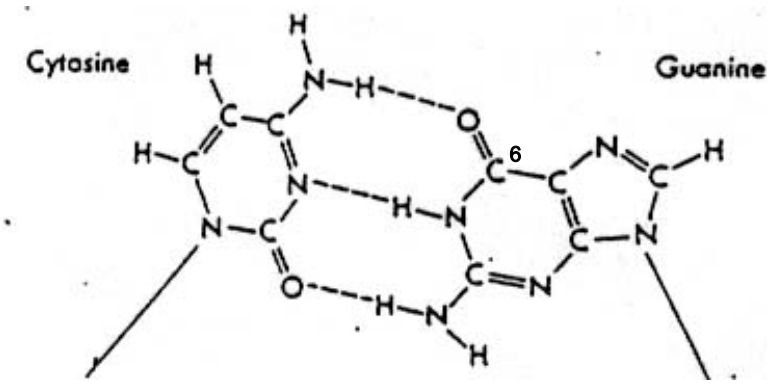
Microsatellite instability results from loss of Mismatch Repair

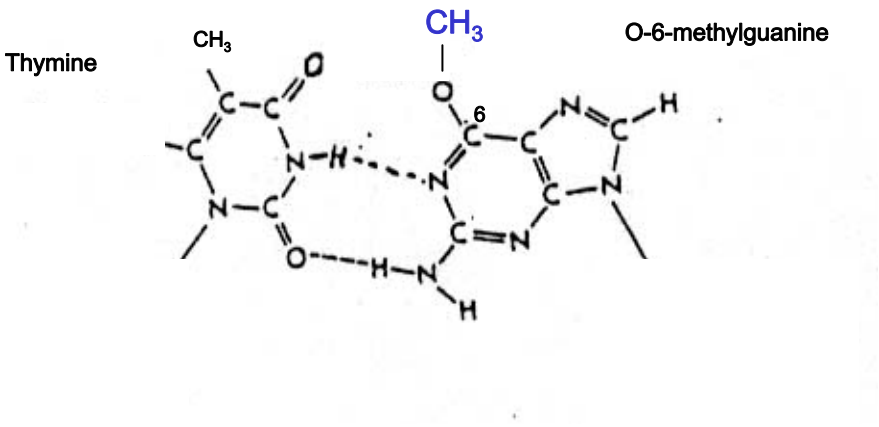


- Microsatellite instability is a useful diagnostic tool. It's not the cause of the cancers
- Cancers arise from high rate of single-base mismatches during replication
- These lead to high frequency of somatic mutations
- Why only in colon? Not known

Damage reversal
2. Repair of O6-methylguanine







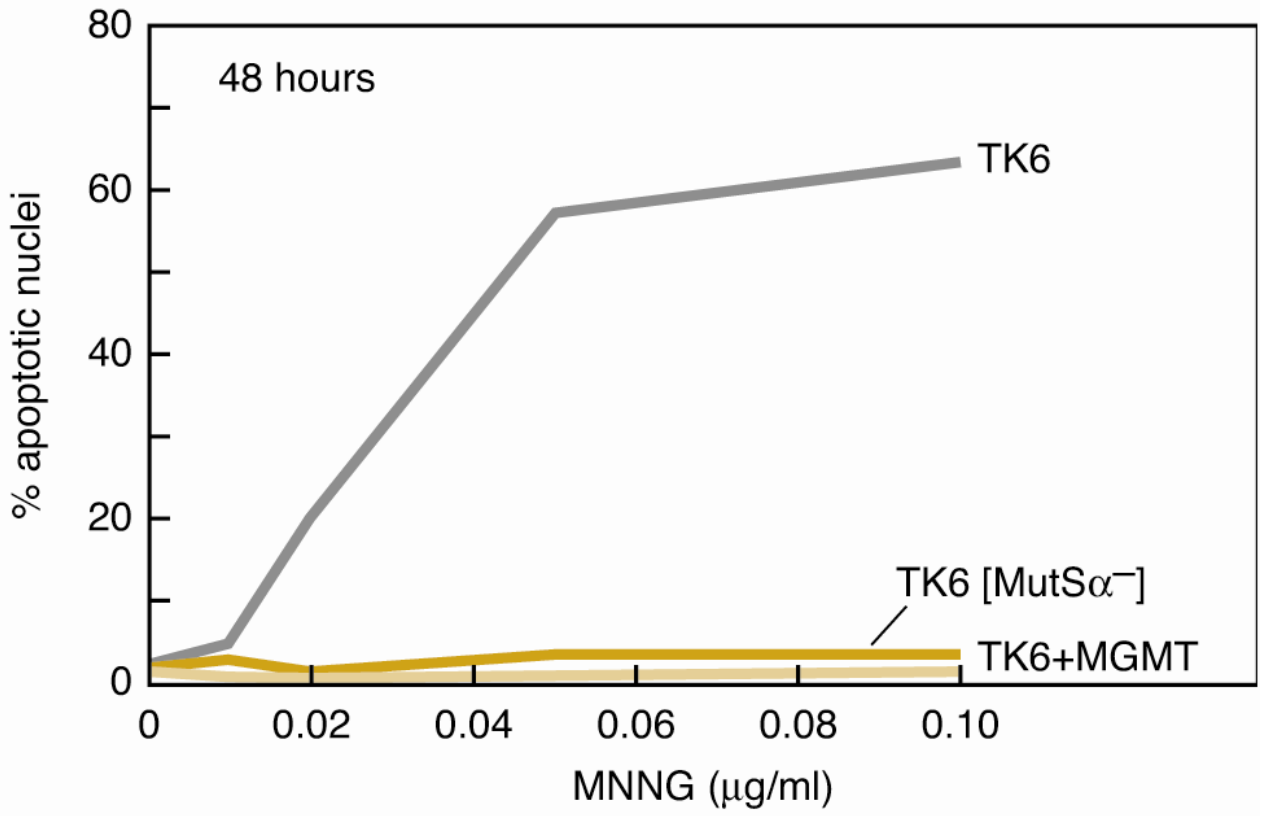
Mispairing of O-6-methylguanine with thymine

MMR and resistant tumours

- In many tumour cells MGMT is silenced. So alkylating agents are good for therapy.
- But often develop resistance.

- Select for alkylation-resistance in cells.
- MGMT not restored. O6-MeG remains in DNA. Instead cells have lost one of the MMR genes.
- Implies MMR somehow sensitises cells to alkylation damage.
- Result of futile cycles. O6-MeG:C and O6-MeG:T both recognised as mismatches.
- C or T opposite O6-MeG removed by MMR and replaced with C or T. Futile cycles.
- Results in cell cycle arrest or apoptosis

Loss of MMR protects against MNNG apoptosis



Friedberg et al, 2005
DNA Repair and Mutagenesis

MMR and cancer

MMR deficiency

- Increases cancer susceptibility (HNPCC)
- Results in resistance to cancer therapy

Summary (Lecture 1b)

- Mismatches are repaired by the Mut(H),L,S system
- Mismatches are recognised by MutS and its homologues
- Strand discrimination is brought about by methylation in *E. coli* and nicks/ends in daughter strands in eukaryotes
- MMR deficiency leads to HNPCC and is detected by microsatellite instability
- Loss of MMR results in resistance to alkylating agents