**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**

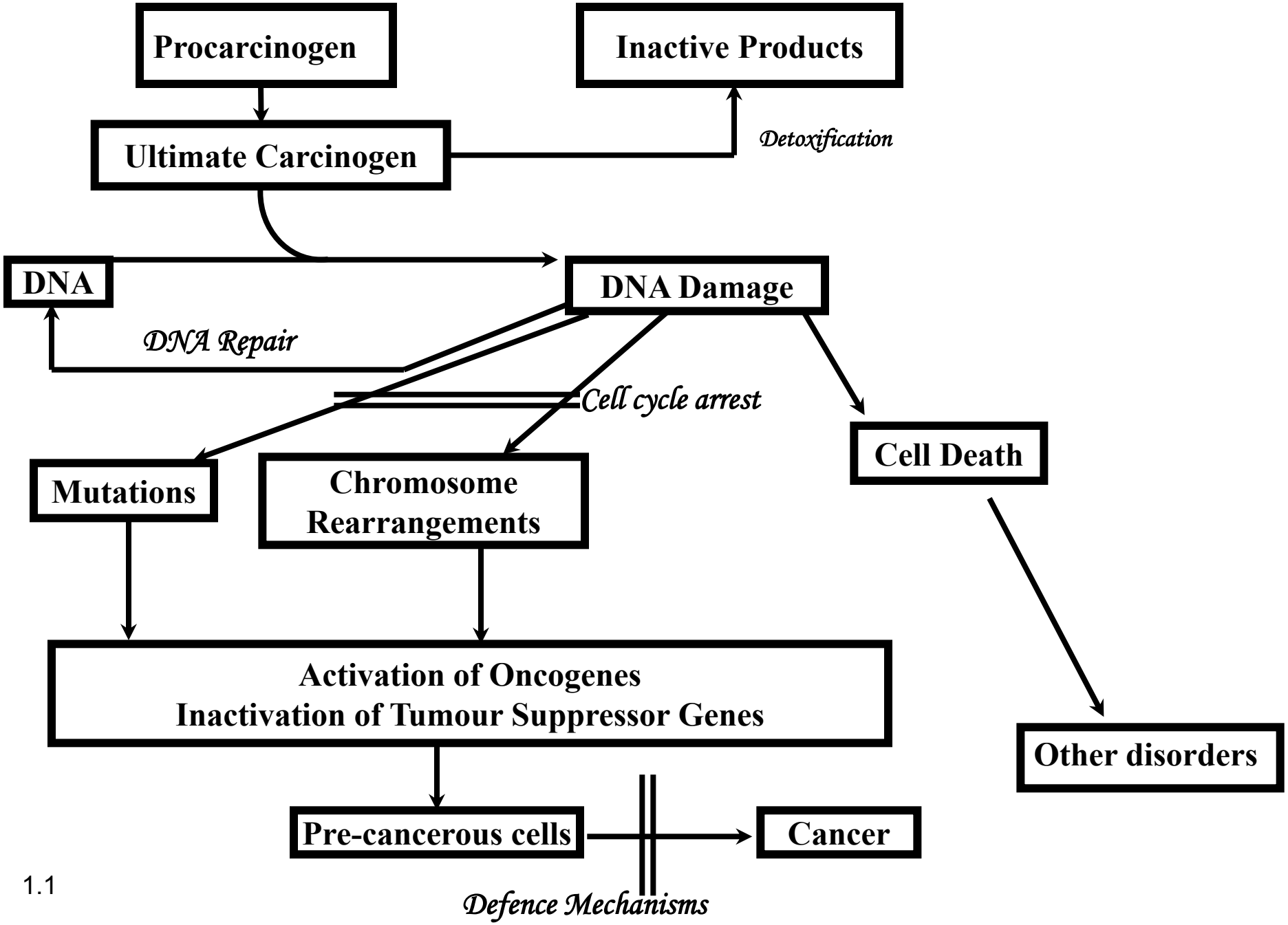
# Course Learning objectives

- To gain an understanding of the molecular mechanisms that maintain genome stability
- To appreciate the importance of this topic for human health.

# Learning outcomes (Lecture 1a)

Understanding:

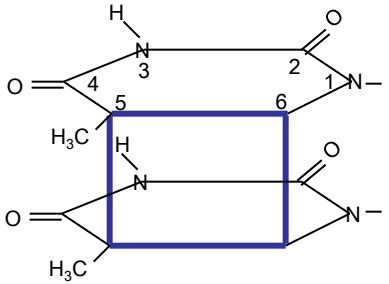
- Different types of DNA damage
- Three examples of ways in which cells can reverse damage in situ
- Basic mechanism of Base Excision Repair


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

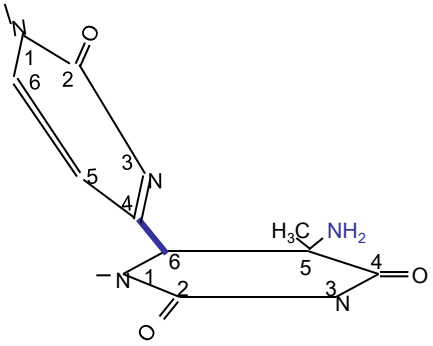
# DNA Damage

| | 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

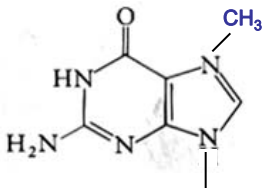


Cyclobutane pyrimidine dimer (CPD)

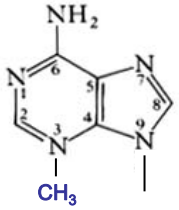


TC (6-4) photoproduct (6-4PP)

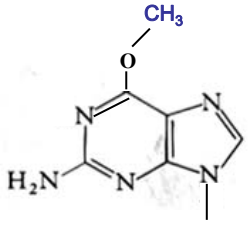
Methylated purines



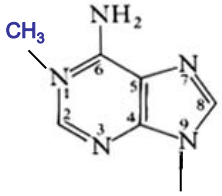
7-methylguanine



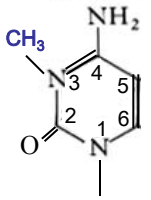
3-methyladenine



O-6-methylguanine



1-methyladenine



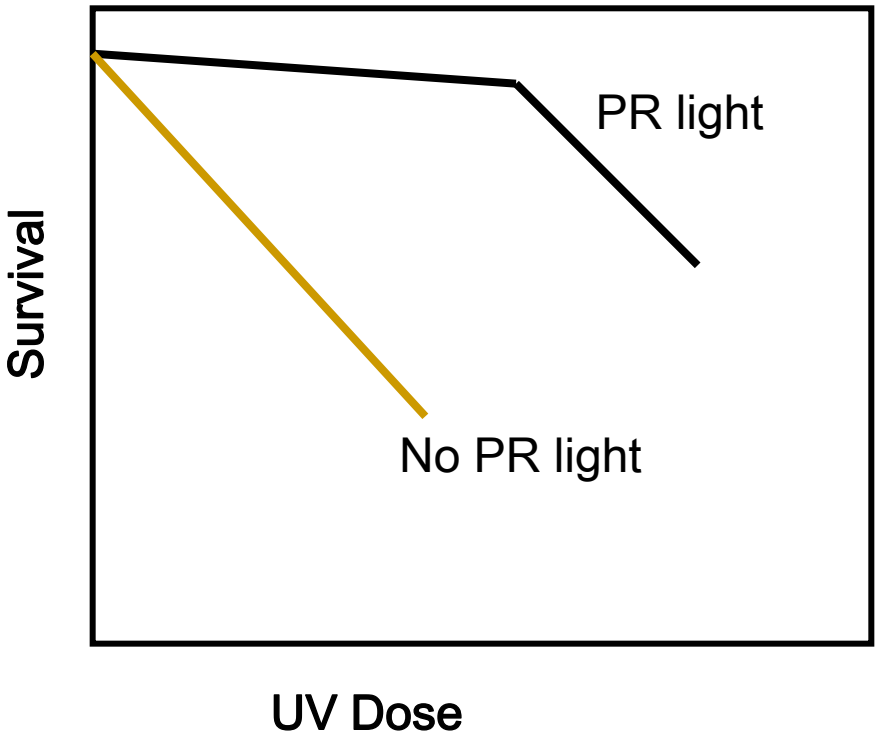
3-methylcytosine

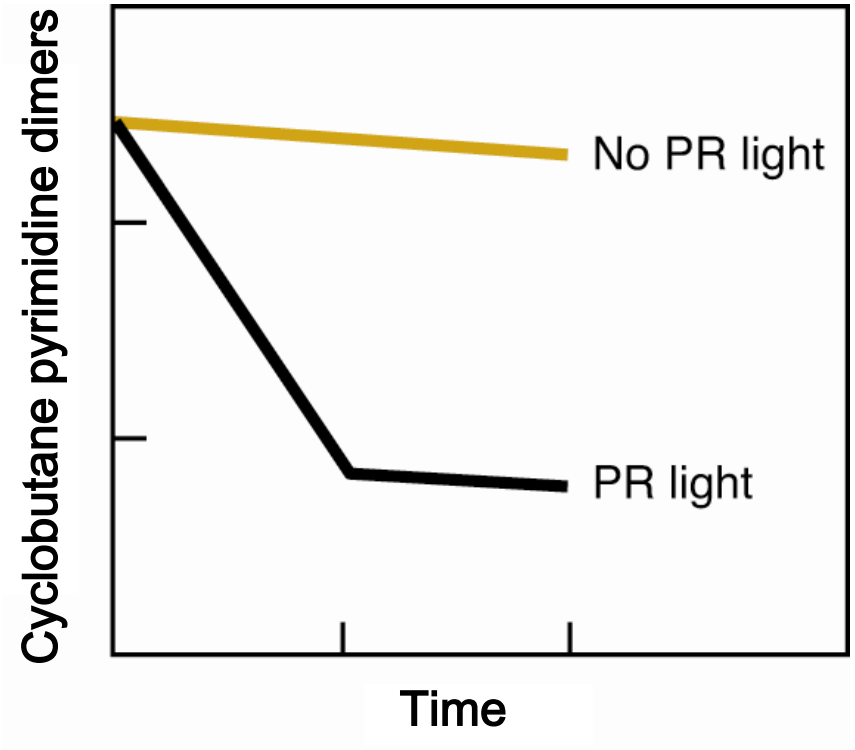
# Aspects of DNA repair

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

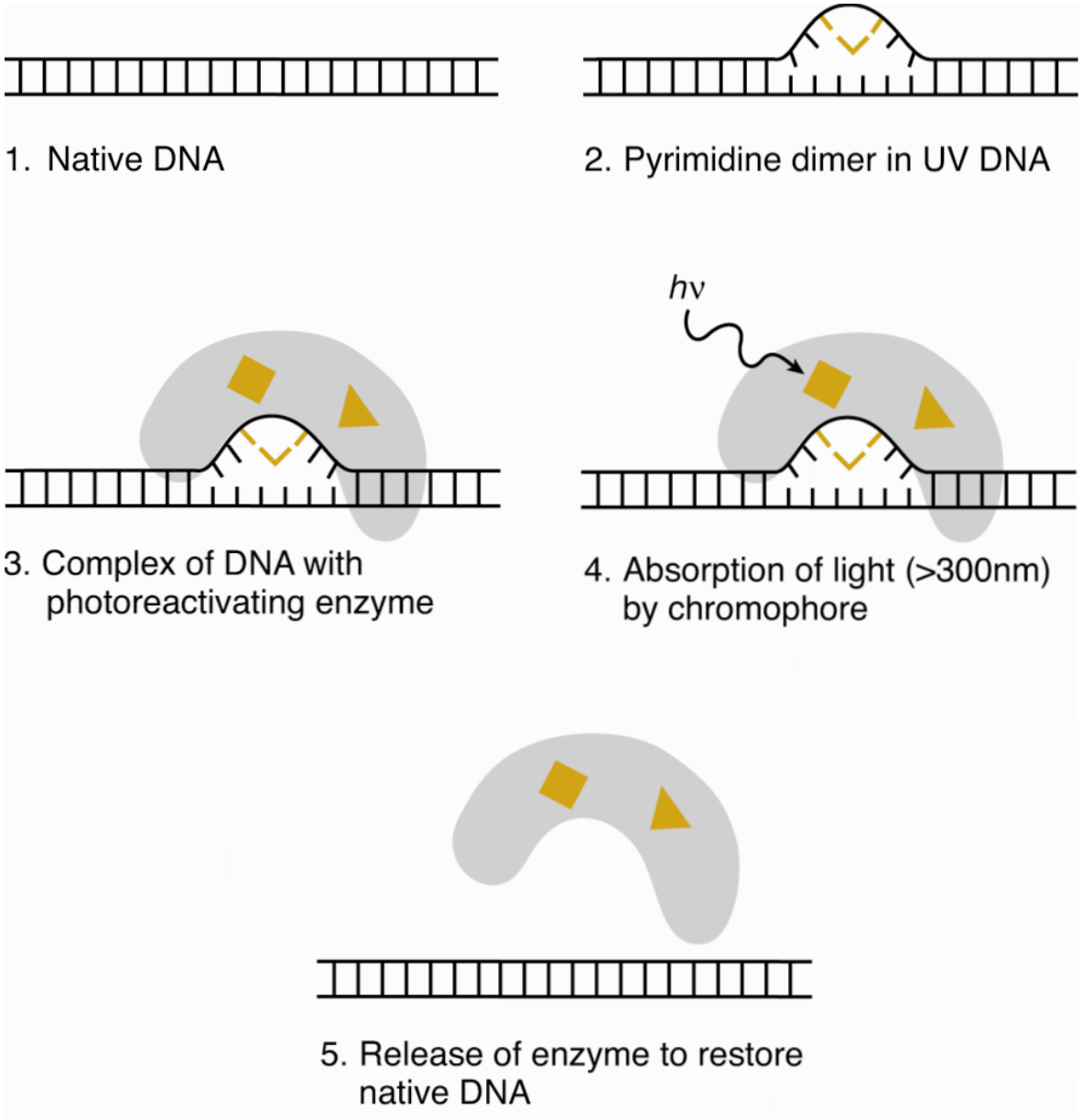
## 1. Photoreactivation




Friedberg et al, 2005
DNA Repair and Mutagenesis

# Photolyase mechanism

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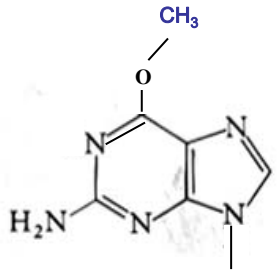
# Damage reversal

# 2. Repair of O6-methylguanine

Methylated purines




O-6-methylguanine

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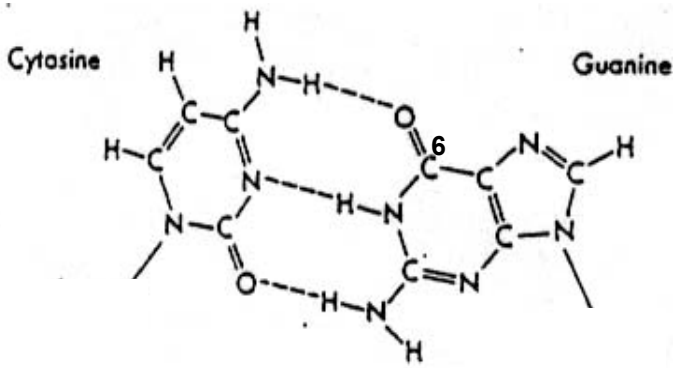
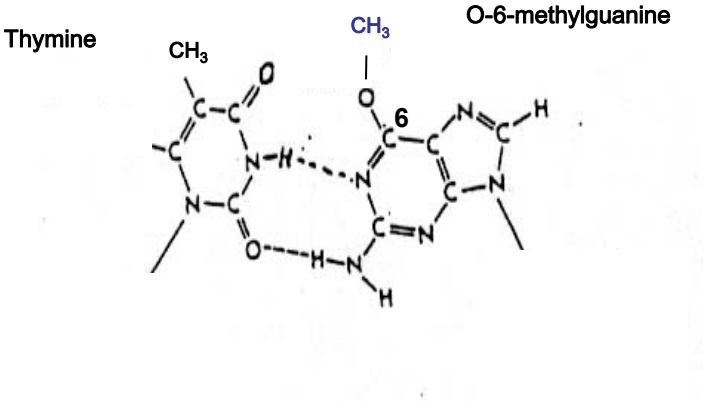


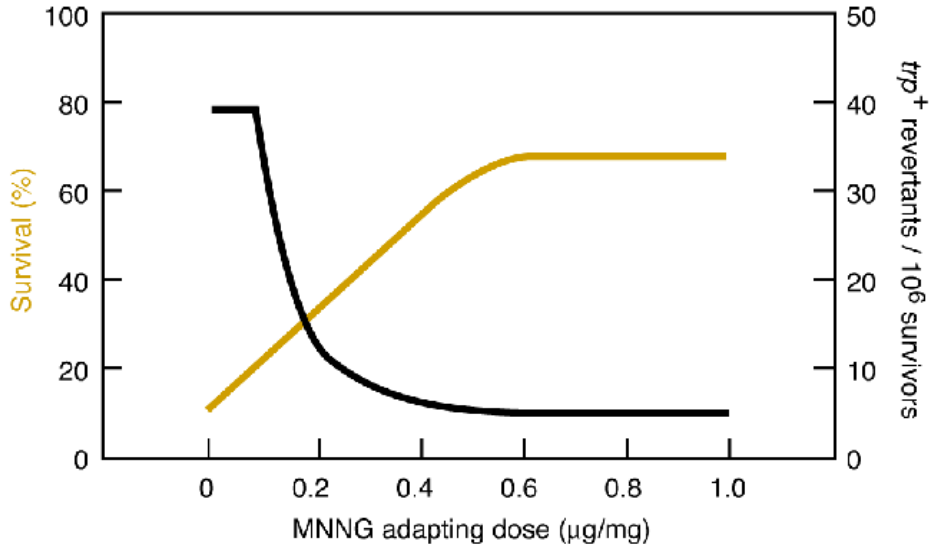

Mispairing of O-6-methylguanine with thymine

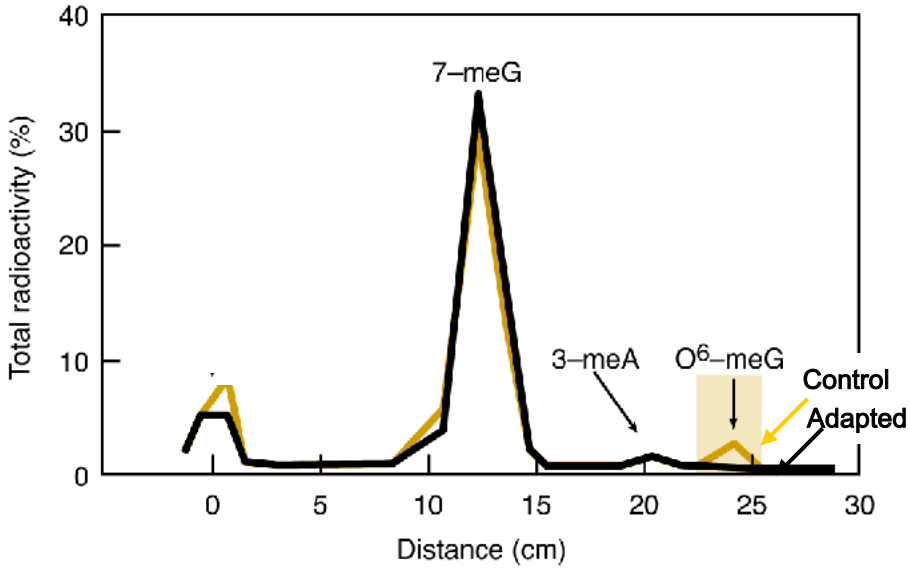
# Damage reversal

# 2. Repair of O6-methylguanine

## Adaptation

Treat *E.coli* with indicated dose of MNNG, then expose to high dose

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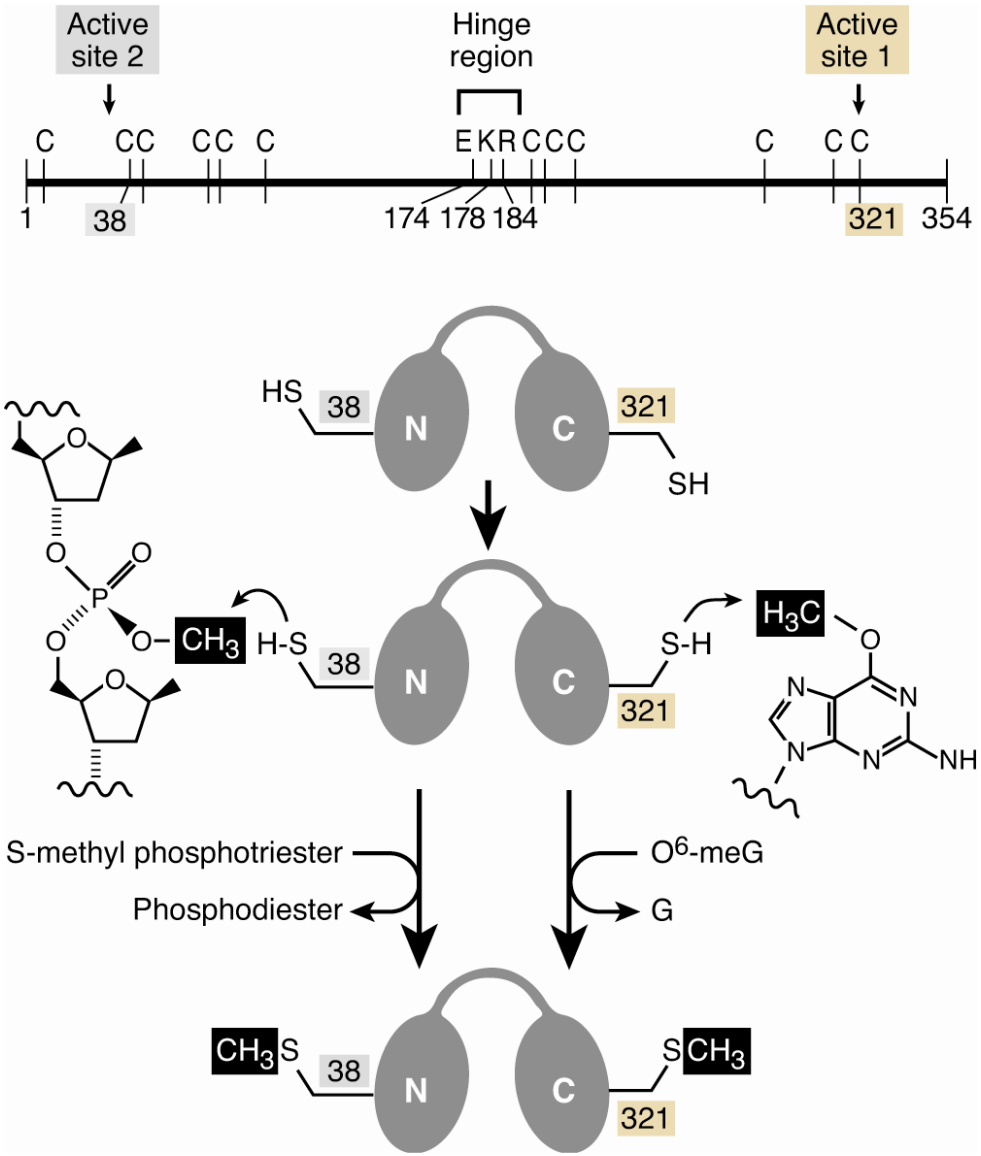
# Dual activities of Ada methyltransferase

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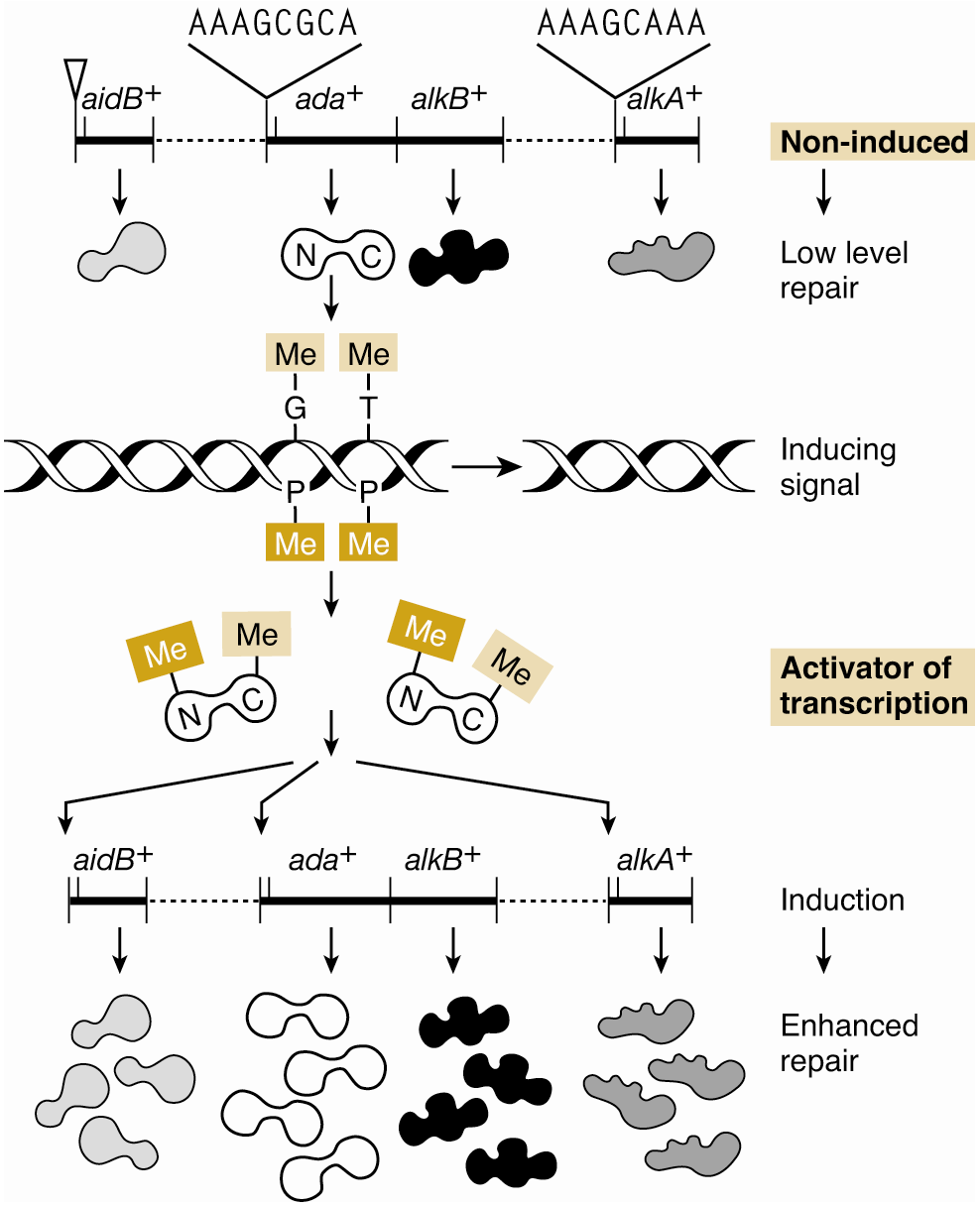
# Induction of *ada* gene



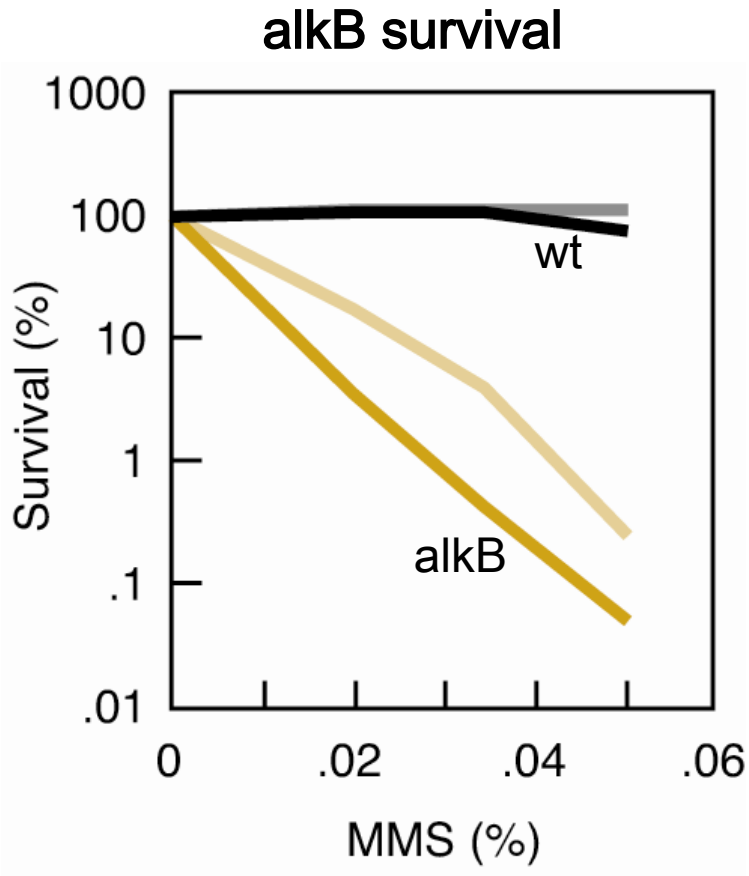

*Ogt* gene is not inducible

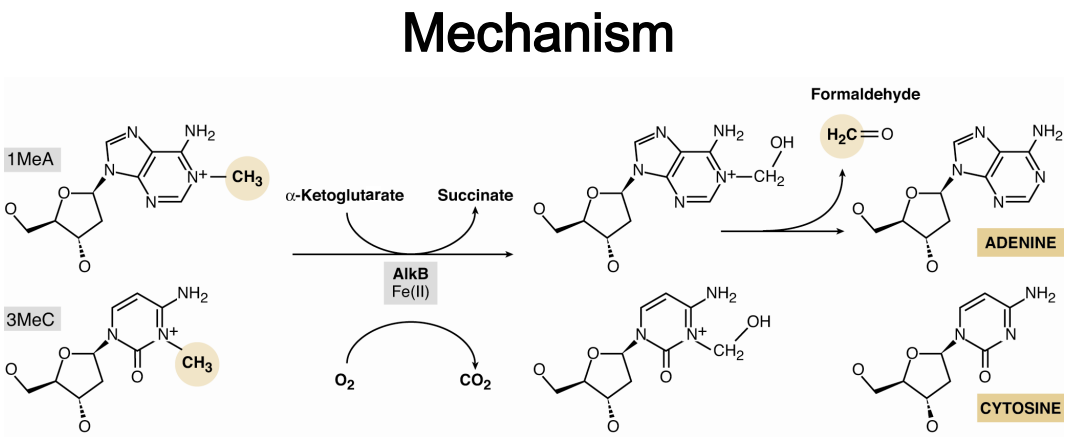
Friedberg et al, 2005
DNA Repair and Mutagenesis

# 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 (A3)

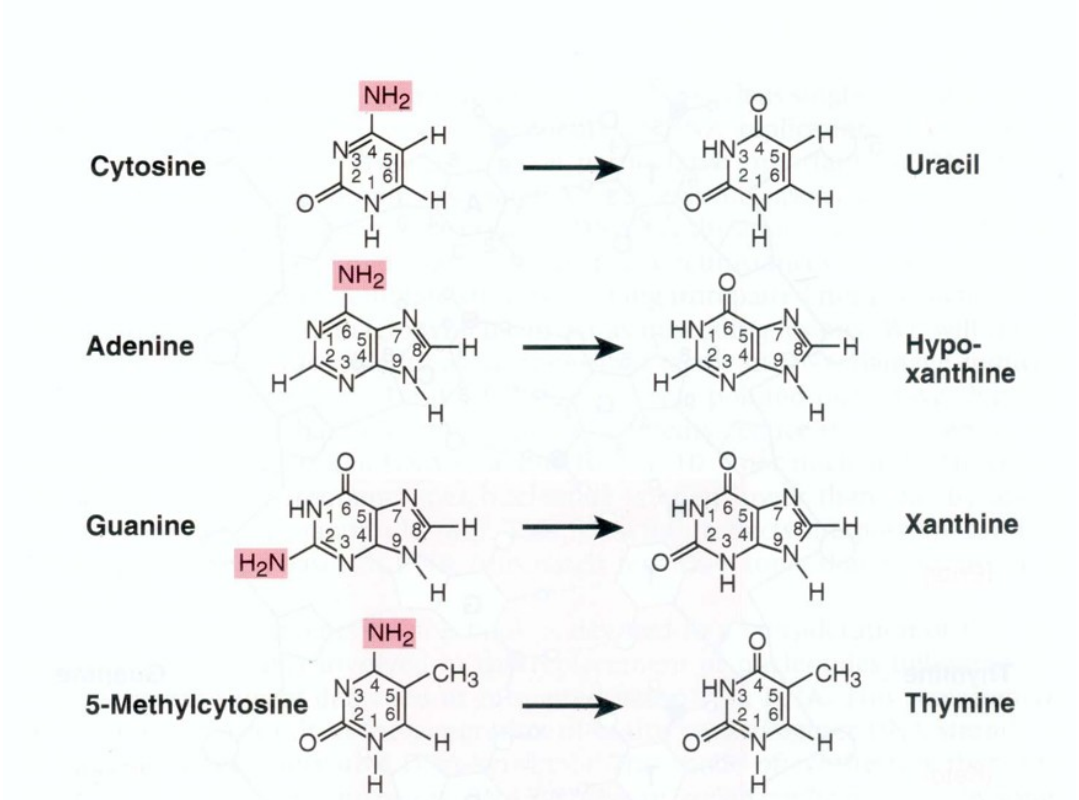


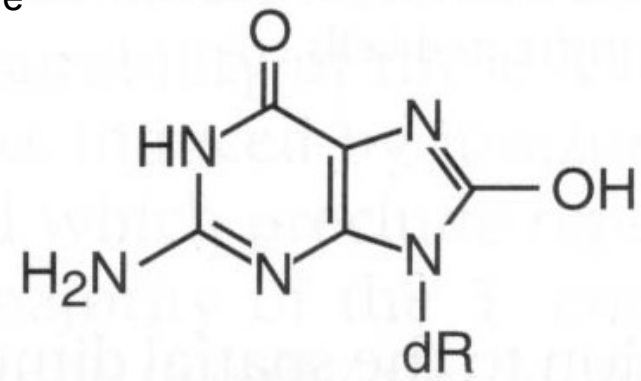

Friedberg et al, 2005
DNA Repair and Mutagenesis

# Base Excision Repair

# Deamination of bases

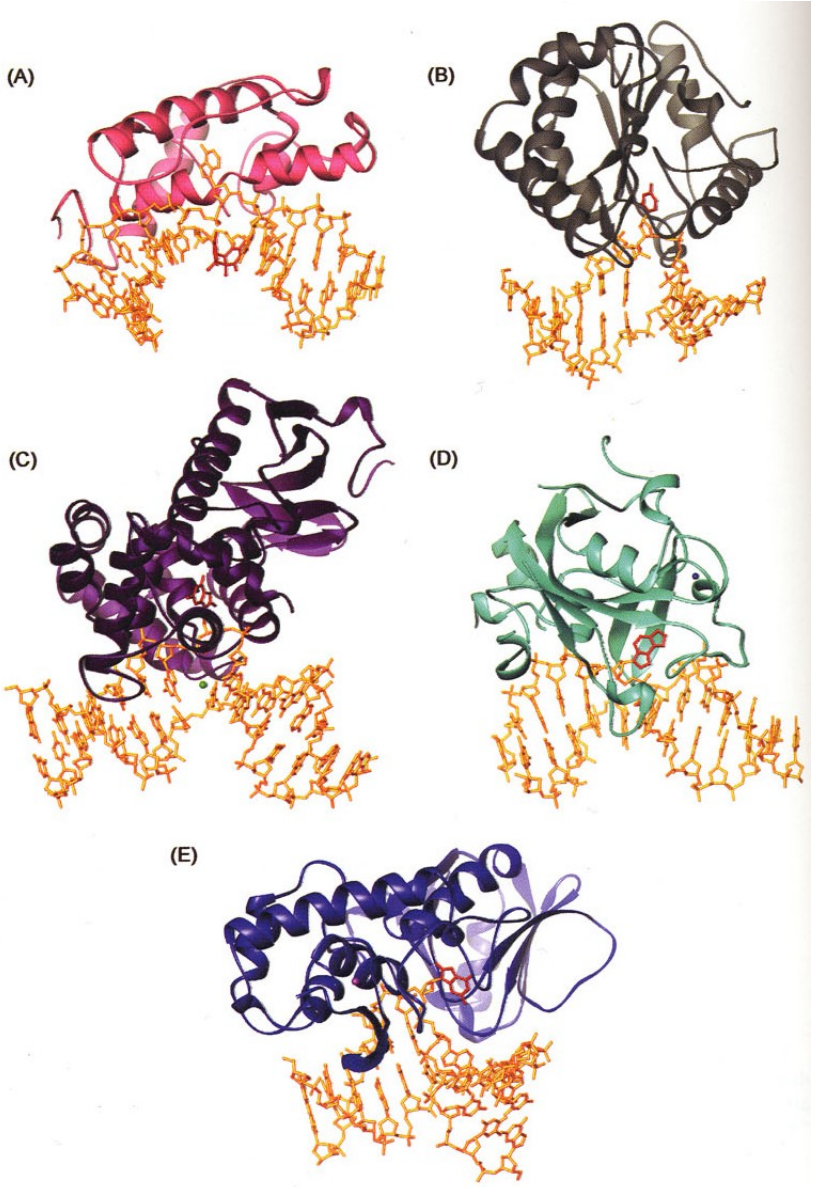



8-hydroxyguanine



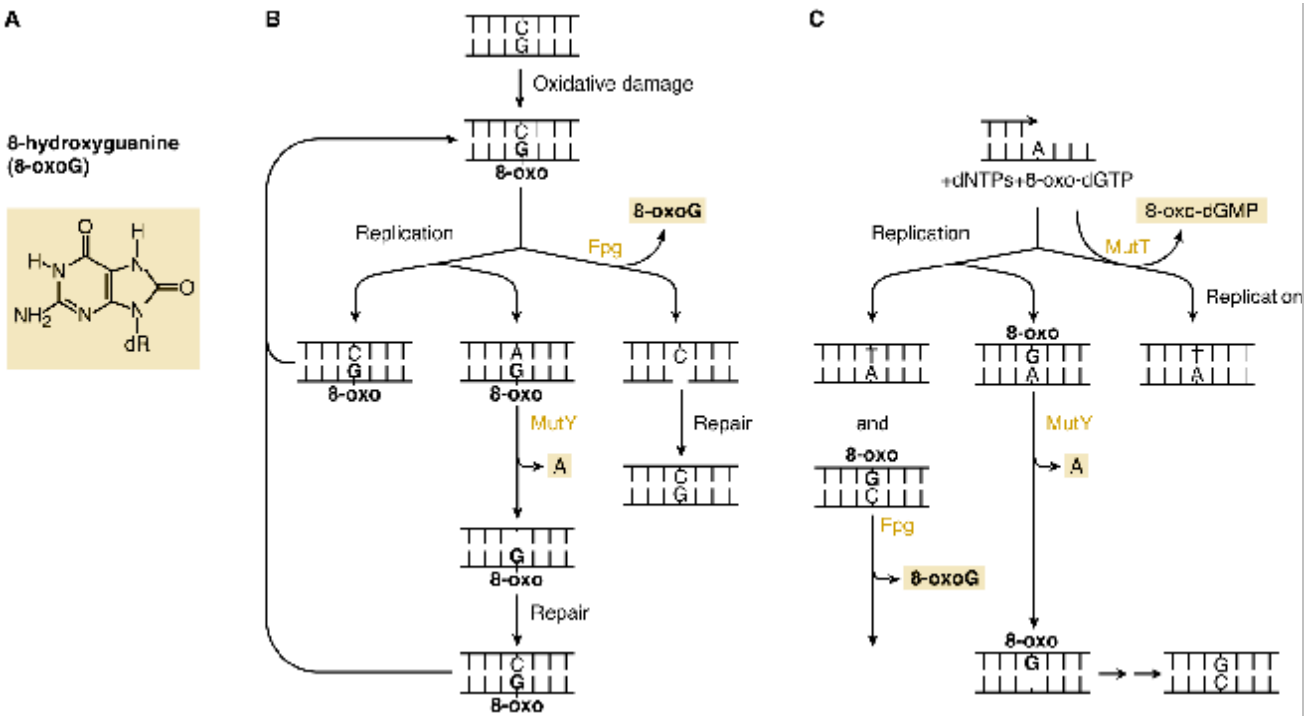
| DNA glycosylases | | | | |
|---|---|---|---|---|
| Enzyme | | Size (aa) | Chromosome location of gene | Altered base removed from DNA |
| *E. coli* | Human | | | |
| ung | UNG2 | 313 | 12q23-q24 | U and 5-hydroxyuracil (rep fork) |
| | MUG | 410 | 12q24.1 | U or T opposite G, ethenocytosine |
| | hSMUG1 | 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

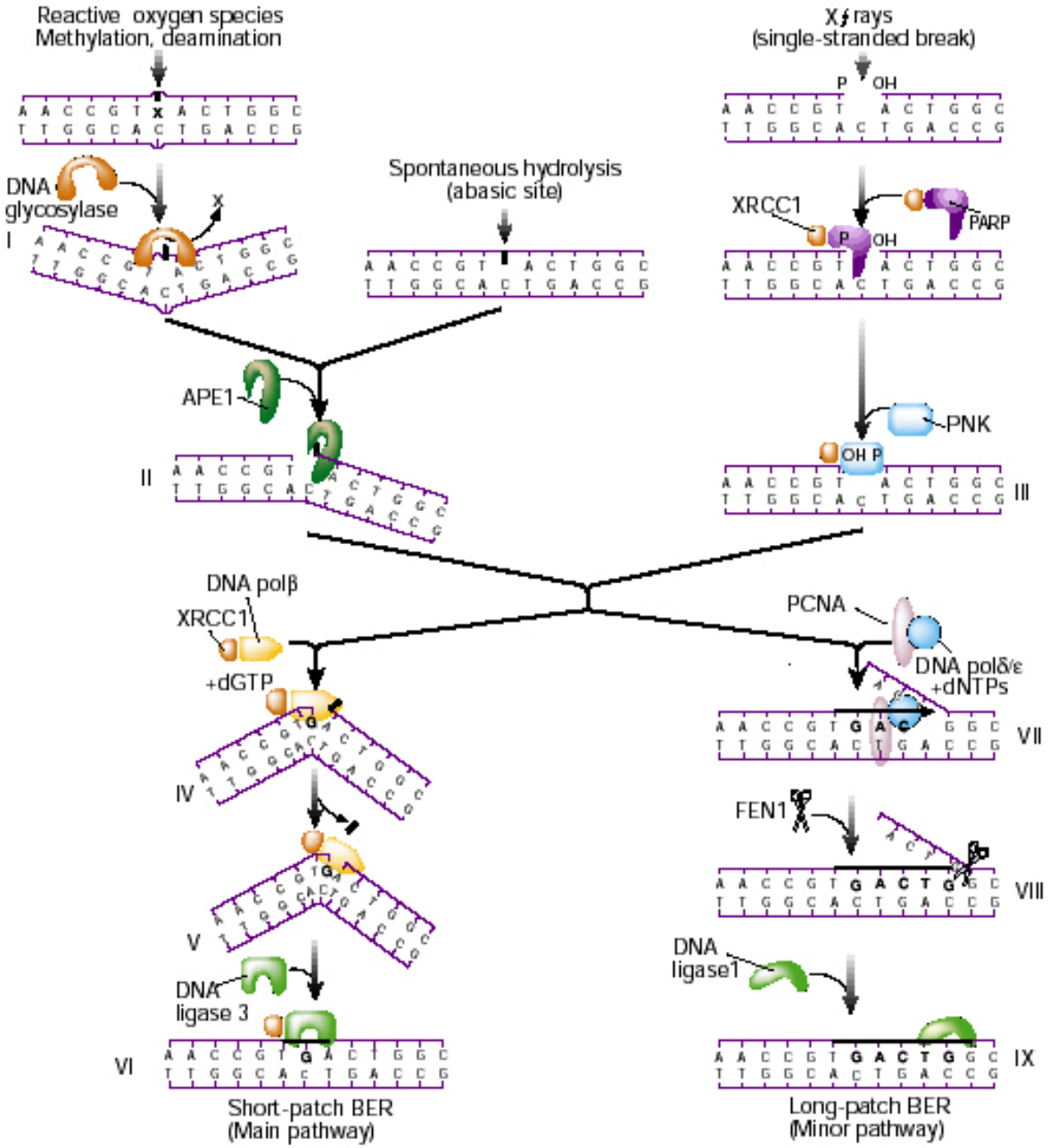



FROMME AND VERDINE, FIG. 4. (*continued*)

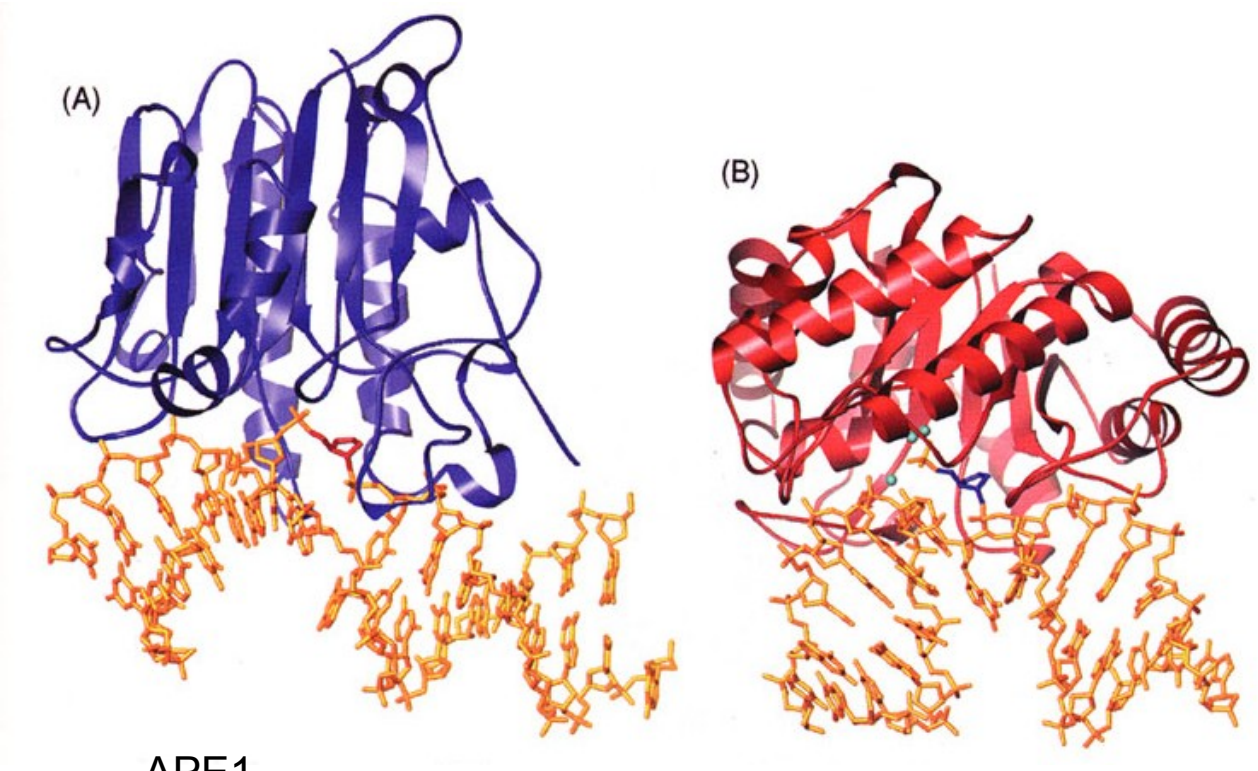
# Protection from 8-oxoguanine



# Base Excision-Repair



# 3-d structures of APendonucleases




APE1

ENDO IV

# Summary (Lecture 1a)

- DNA damage can cause distortions of different severity
- 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

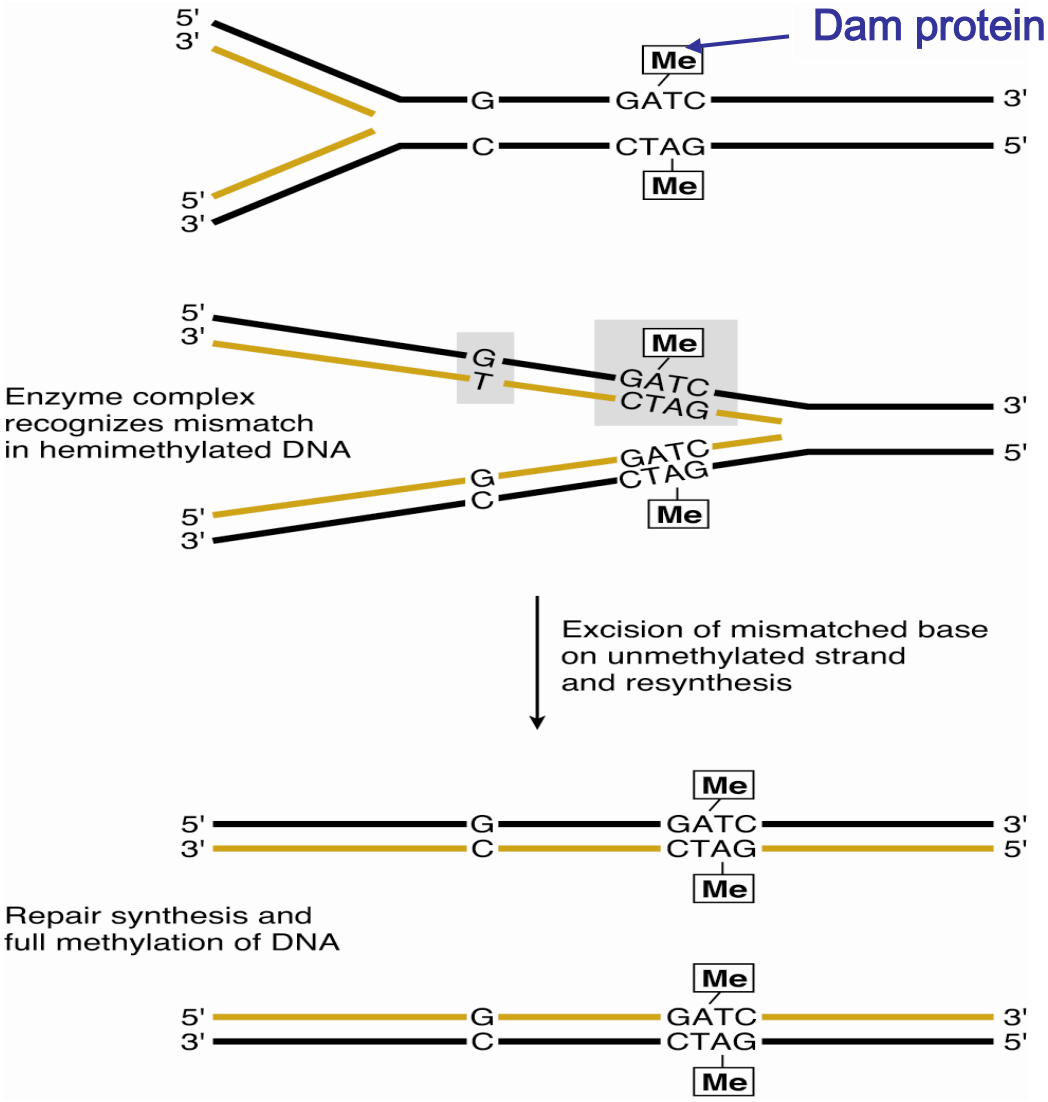
- But genomes are big: *E. coli* $3x10^6$ bp, mammals $3x10^9$

- 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



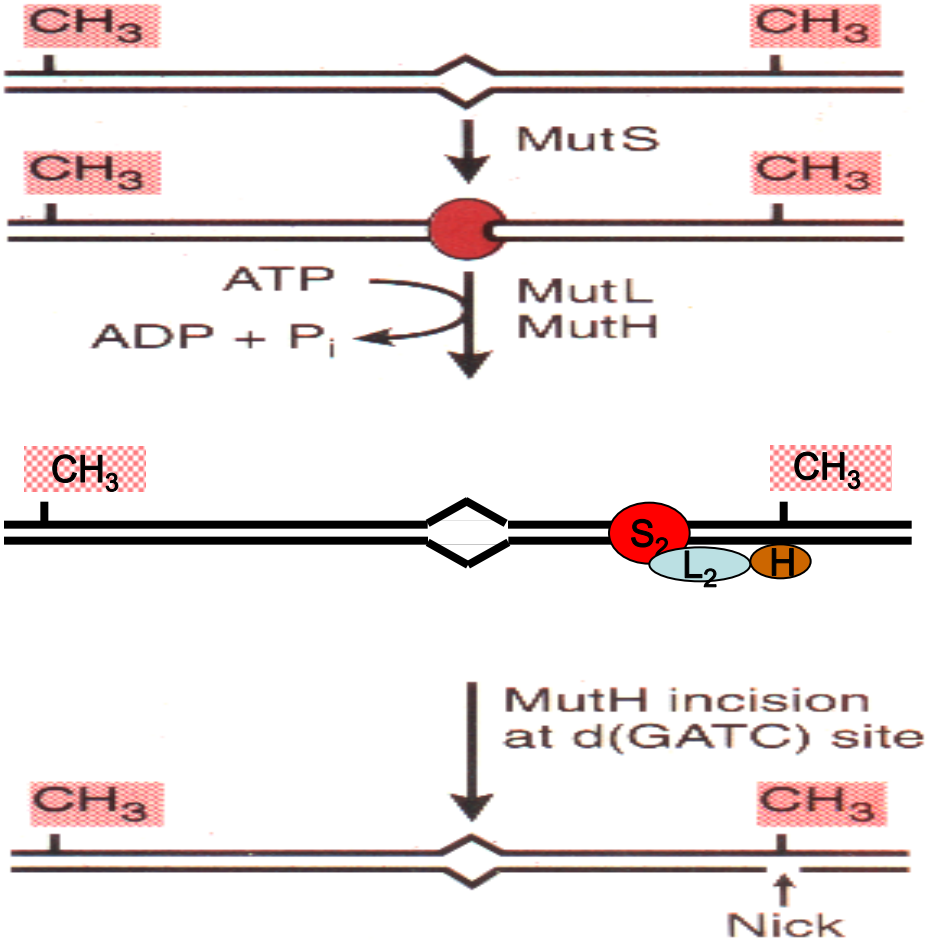

Friedberg et al, 2005
DNA Repair and Mutagenesis



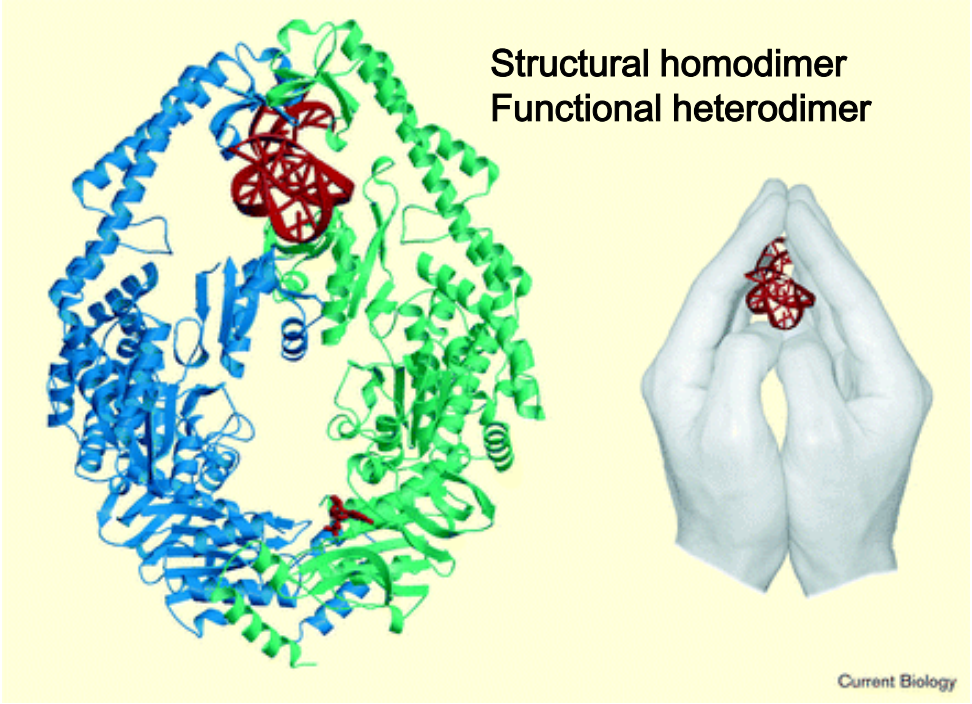
Dam$^-$ strains (methylation deficient) are mutators

# Mismatch recognition and strand discrimination in *E. coli*

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

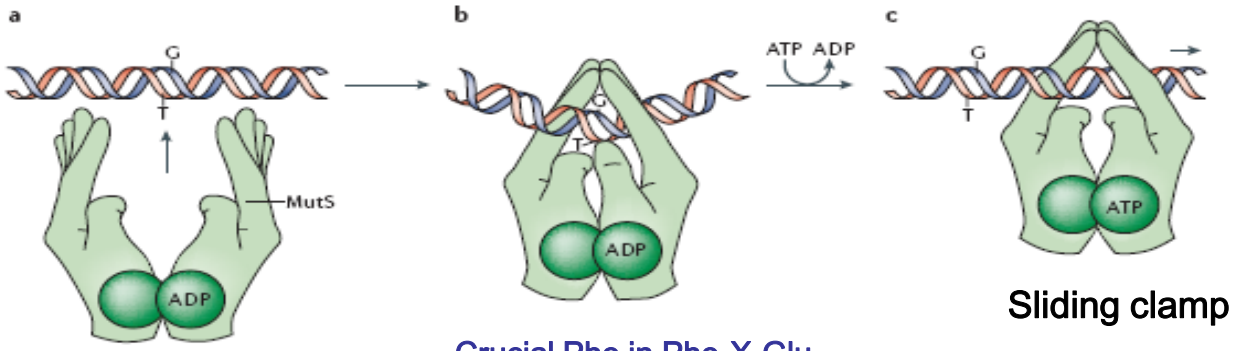


# 3-D structure of MutS




Jiricny, Current Biology, 2000

# Activities of MutS

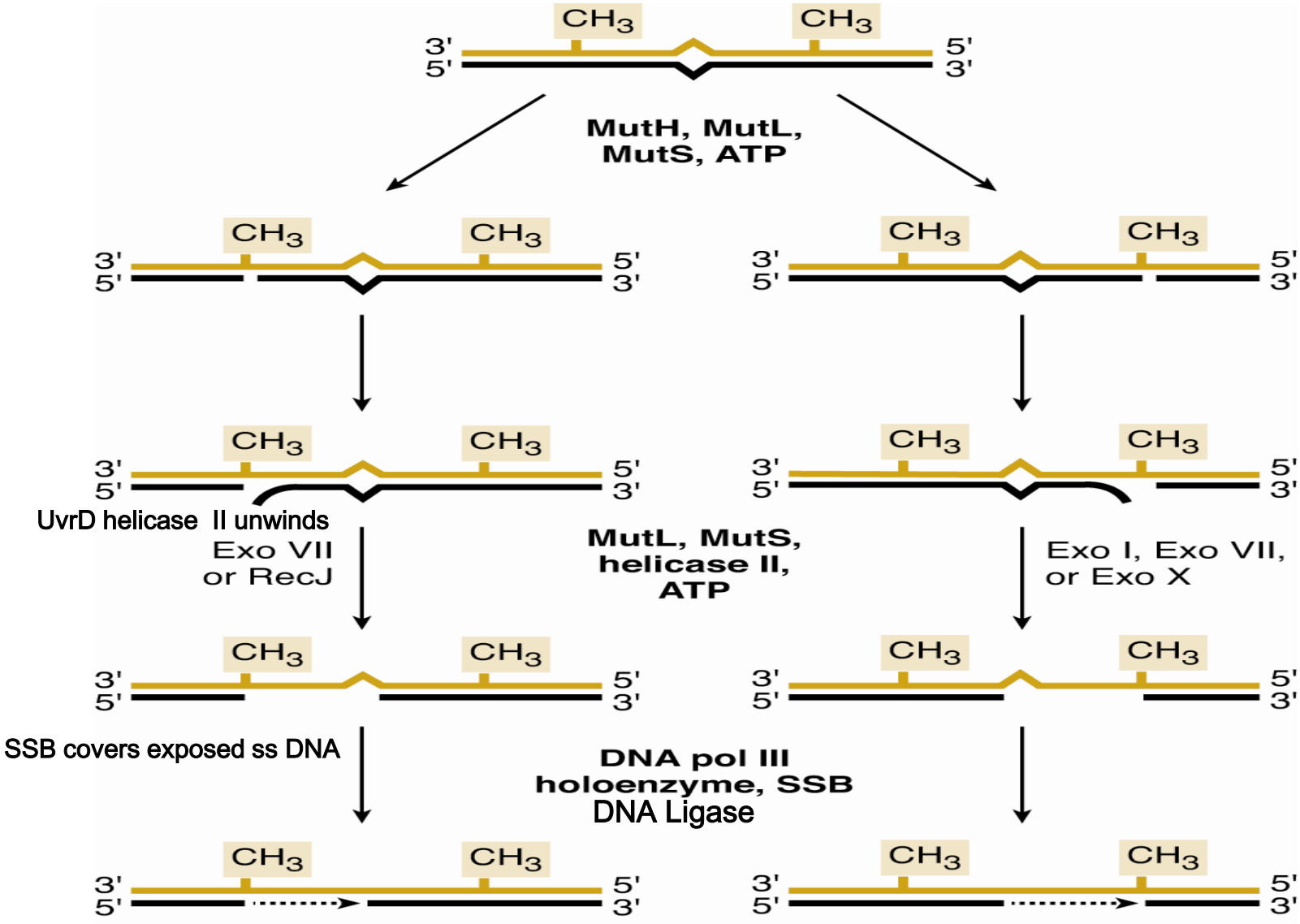



Sliding clamp

Crucial Phe in Phe-X-Glu contacts mismatch

Jiricny, NRMCB, 2006

# Late steps in MMR in *E. coli*






Friedberg et al, 2005
DNA Repair and Mutagenesis

# Eukaryotic 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

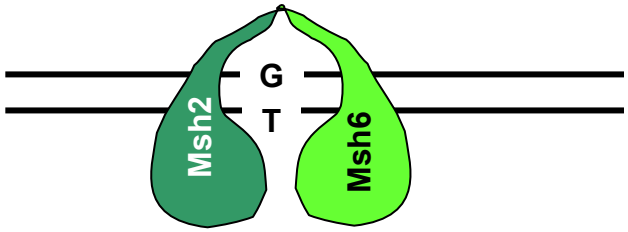
Strand discrimination based on nicks/ends in daughter DNA

MMR proteins interact with PCNA at replication fork

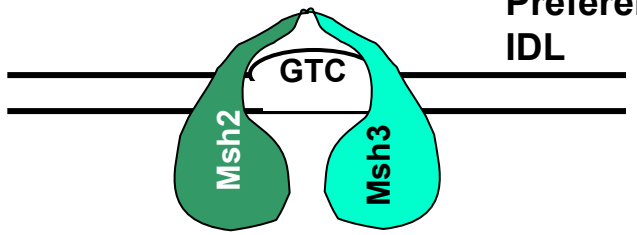
# Mismatch Repair in eukaryotes


## Primary recognition

MutSα
Preference for single base mismatches and small IDL

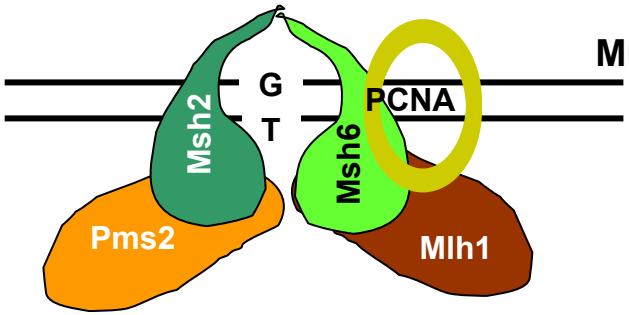


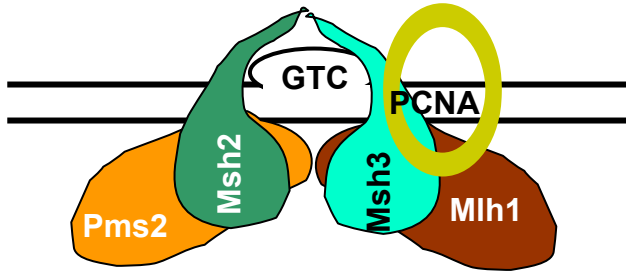

MutSβ
Preference for large IDL




## Secondary recognition

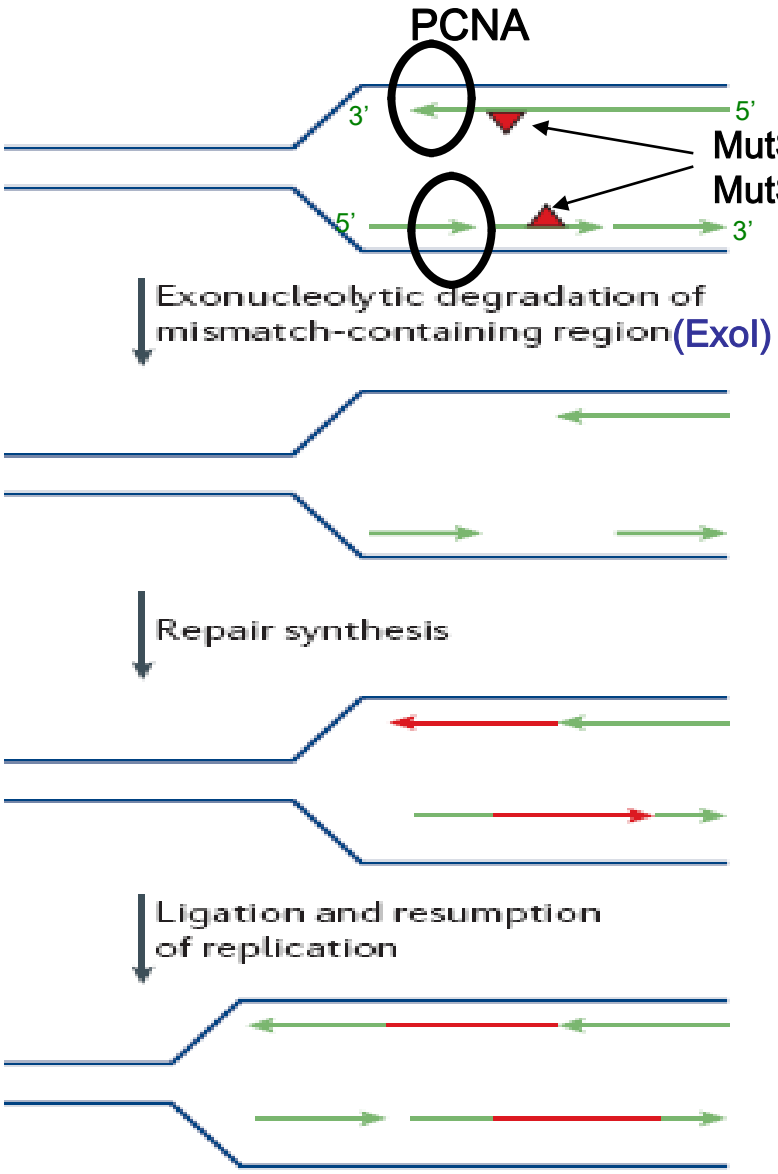
MutLα




## Removal and restoration

# Mismatch Repair in eukaryotes

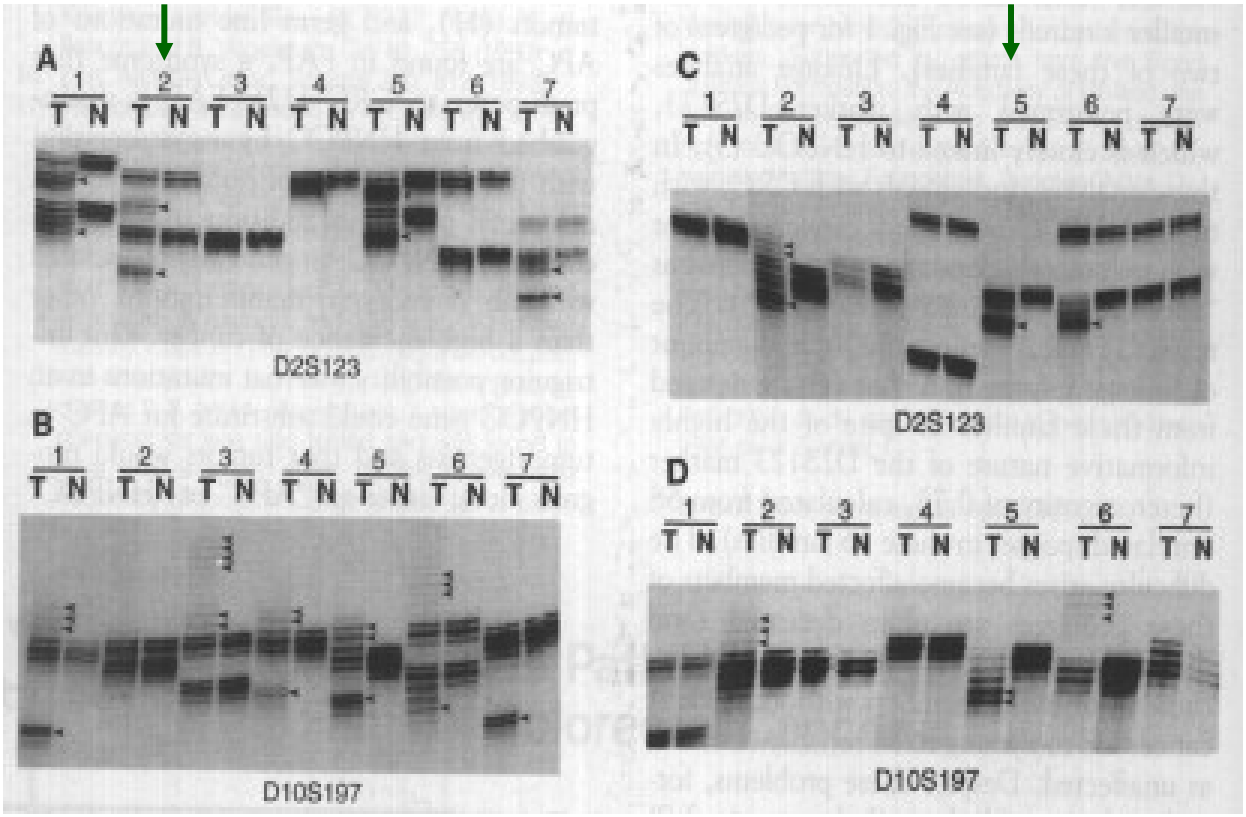



Leading strand: MutLα (Pms2 subunit) cleaves on 5' side of mismatch.
Exo1 degrades 5' to 3'
Lagging strand: Exo1 degrades 5' to 3'

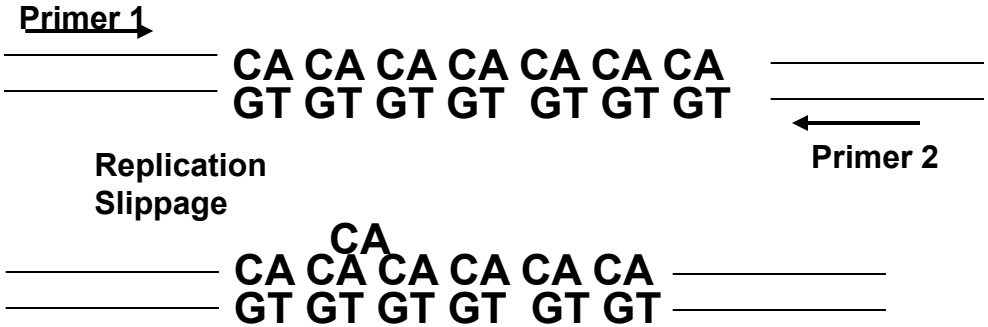
Modified from Jiricny, Nature Rev Mol Cell Biol 2006 (A5)

# Microsatellite instability in tumour tissue from HNPCC

## (Hereditary non-polyposis colon carcinoma) Lynch Syndrome



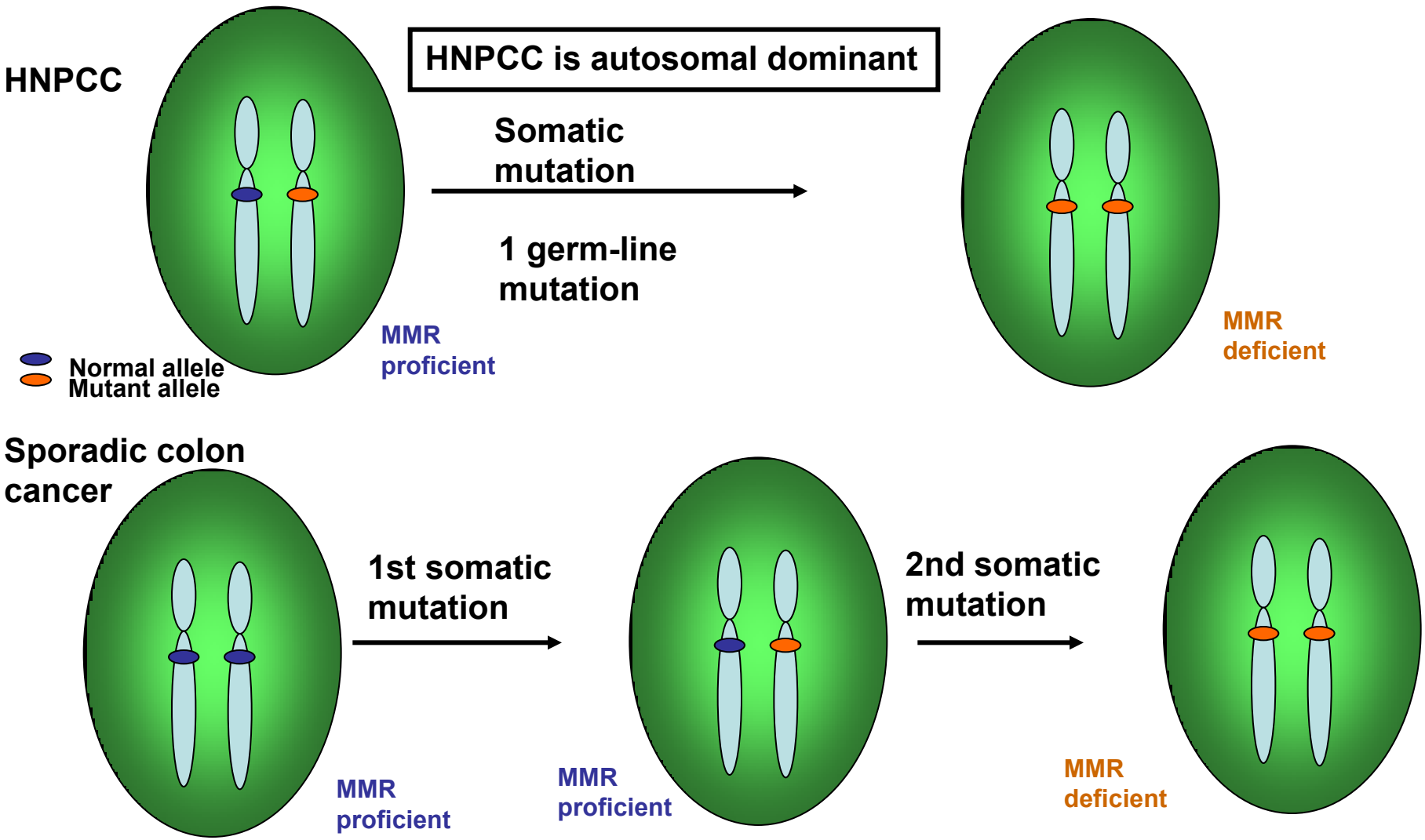

Aaltonen, et al.
*Science*
260, 812 (1993)



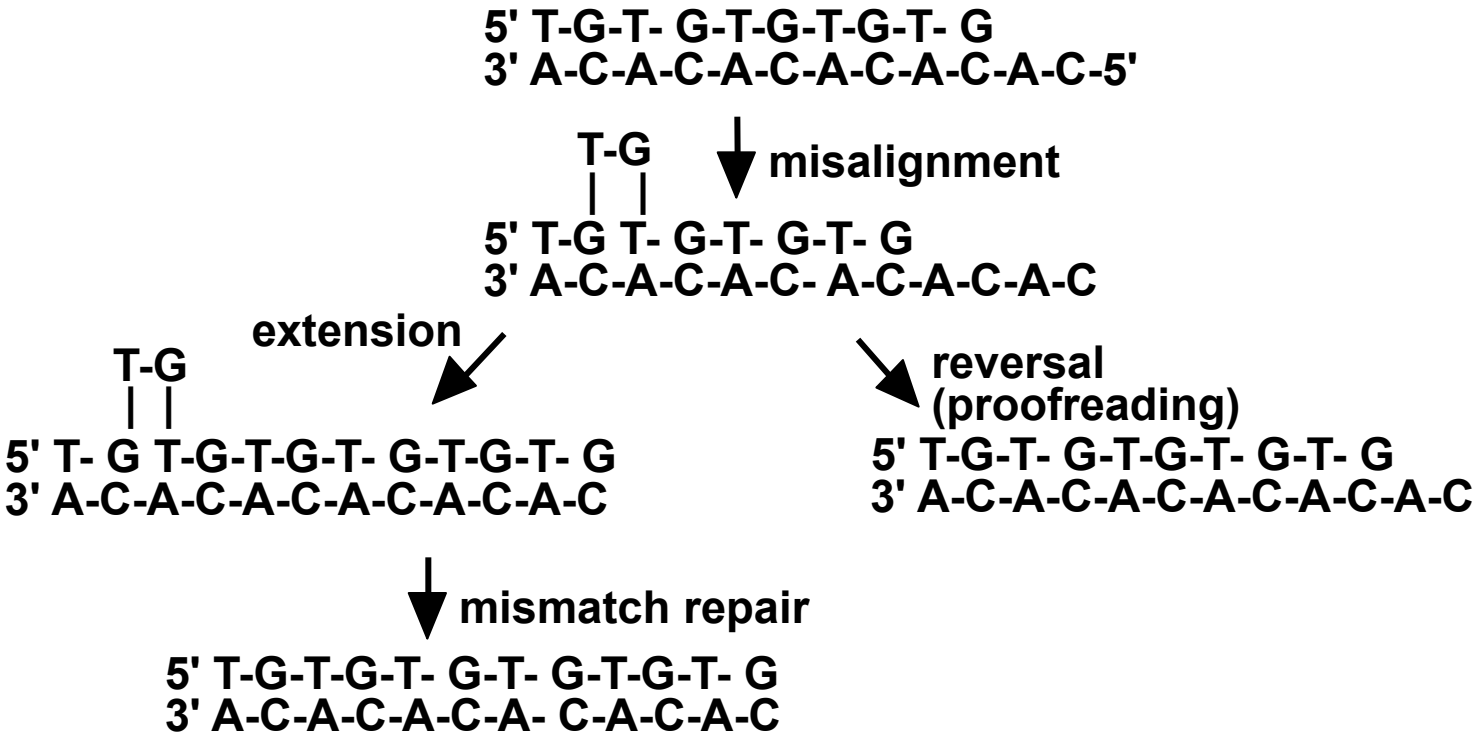
# 2-hit tumour suppressor model

**Most HNPCC result from mutations in hMsh2 or hMlh1**

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

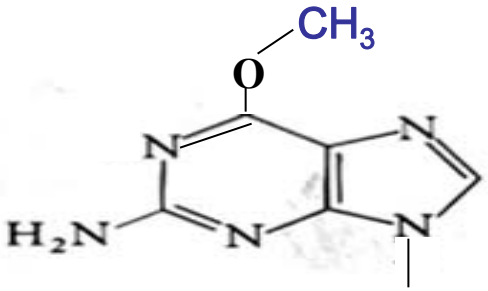


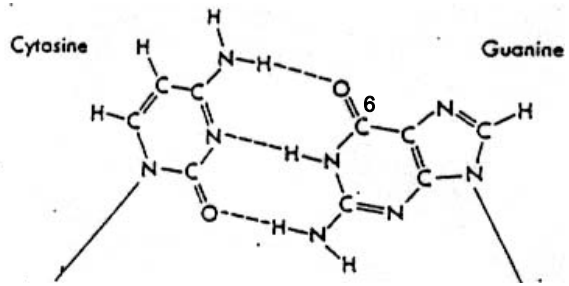
# Microsatellite instability results from loss of Mismatch Repair

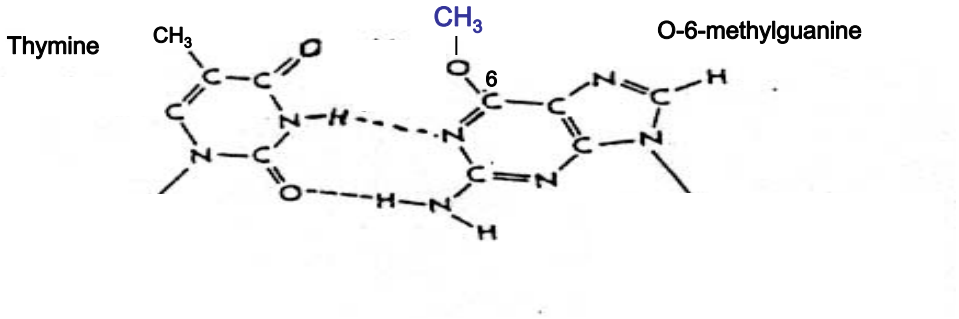



- Microsatellite instability is a useful diagnostic tool. It's not the cause of the cancers
- Cancers arise from high rate of single-base mismatches during replication
- These lead to high frequency of somatic mutations
- Why only in colon? Not known

# Damage reversal
# 2. Repair of O6-methylguanine



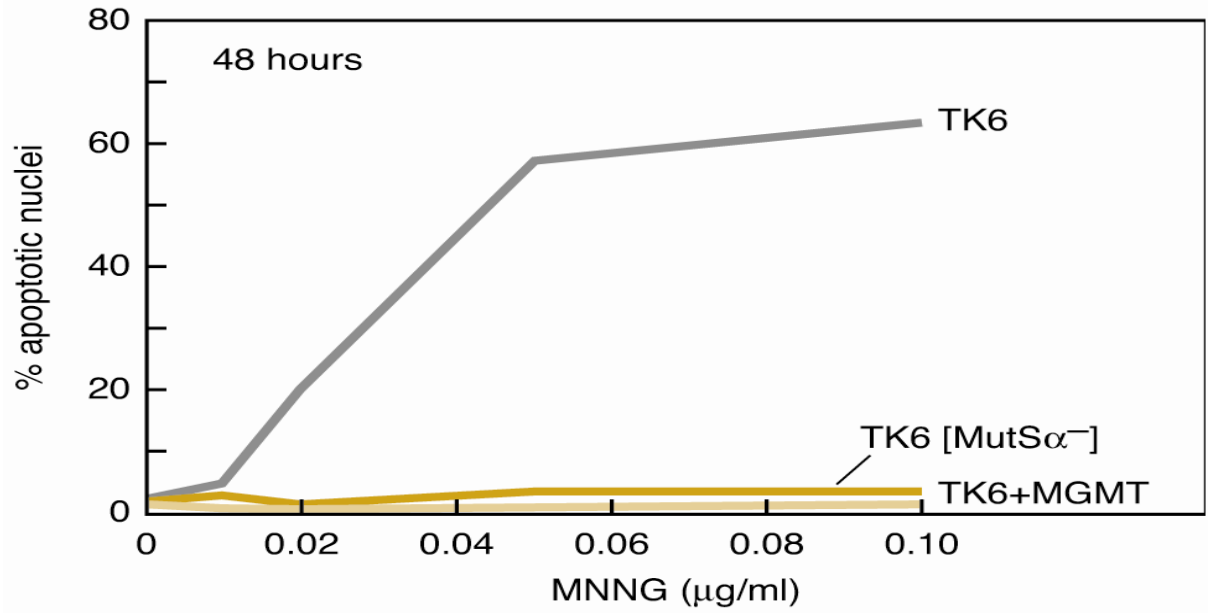

Mispairing of O-6-methylguanine with thymine

# 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