# The Intrinsic Fragility of DNA

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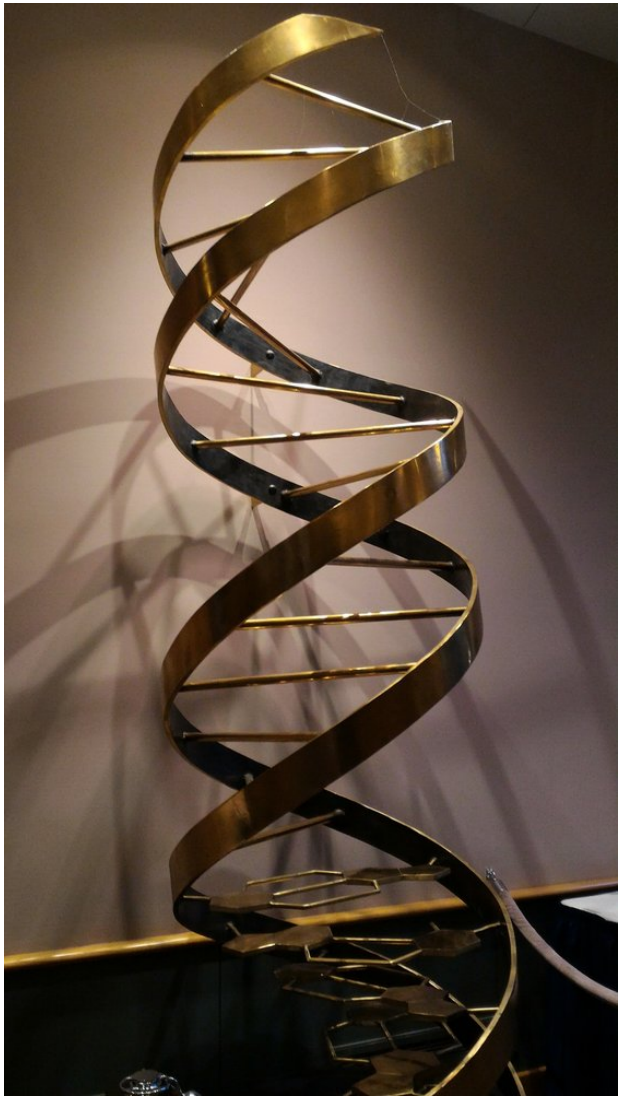
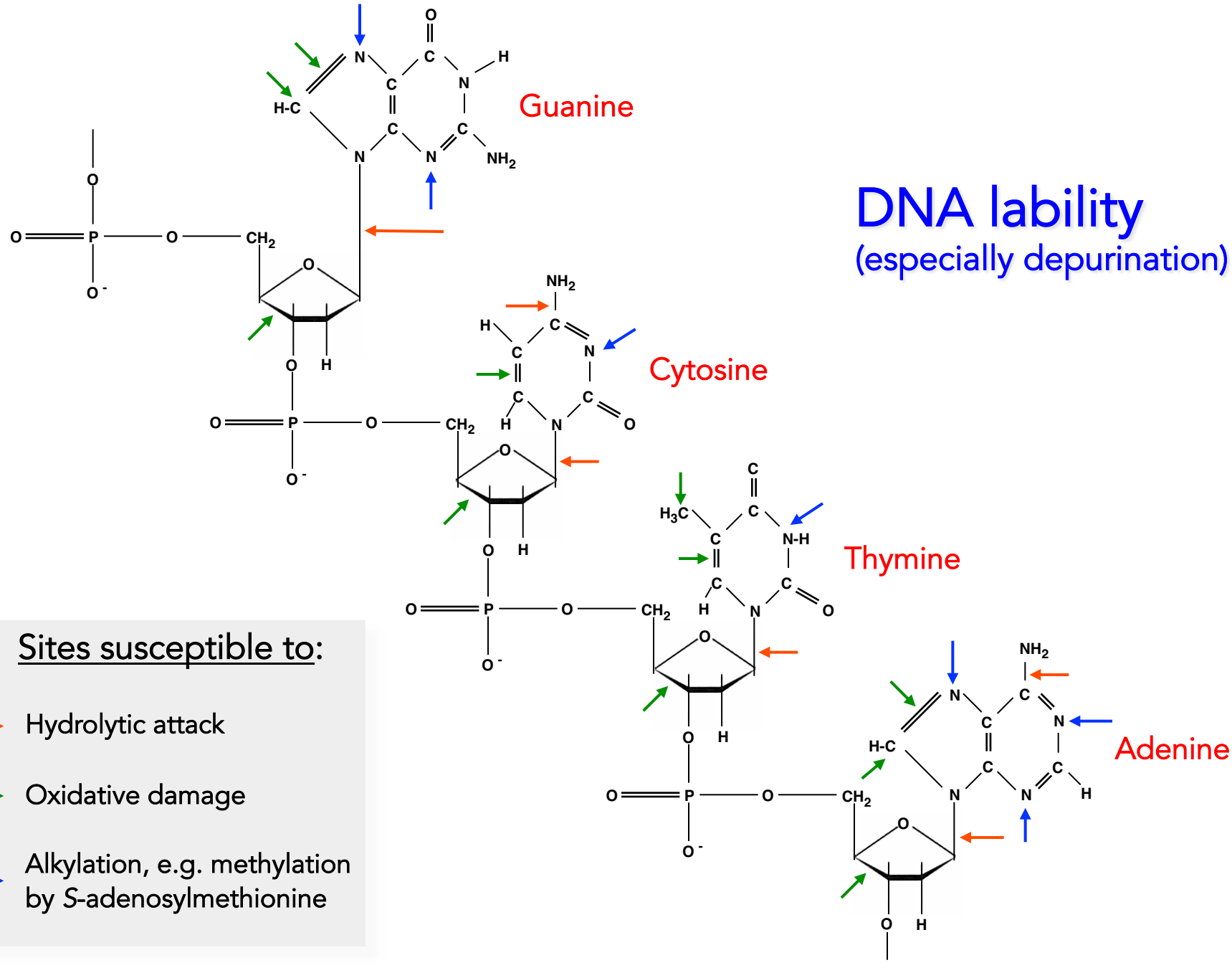
Nobel Lecture, December 8, 2015

# The Stability of DNA



# DNA lability
(especially depurination)




Sites susceptible to:

- Hydrolytic attack
- Oxidative damage
- Alkylation, e.g. methylation by *S*-adenosylmethionine

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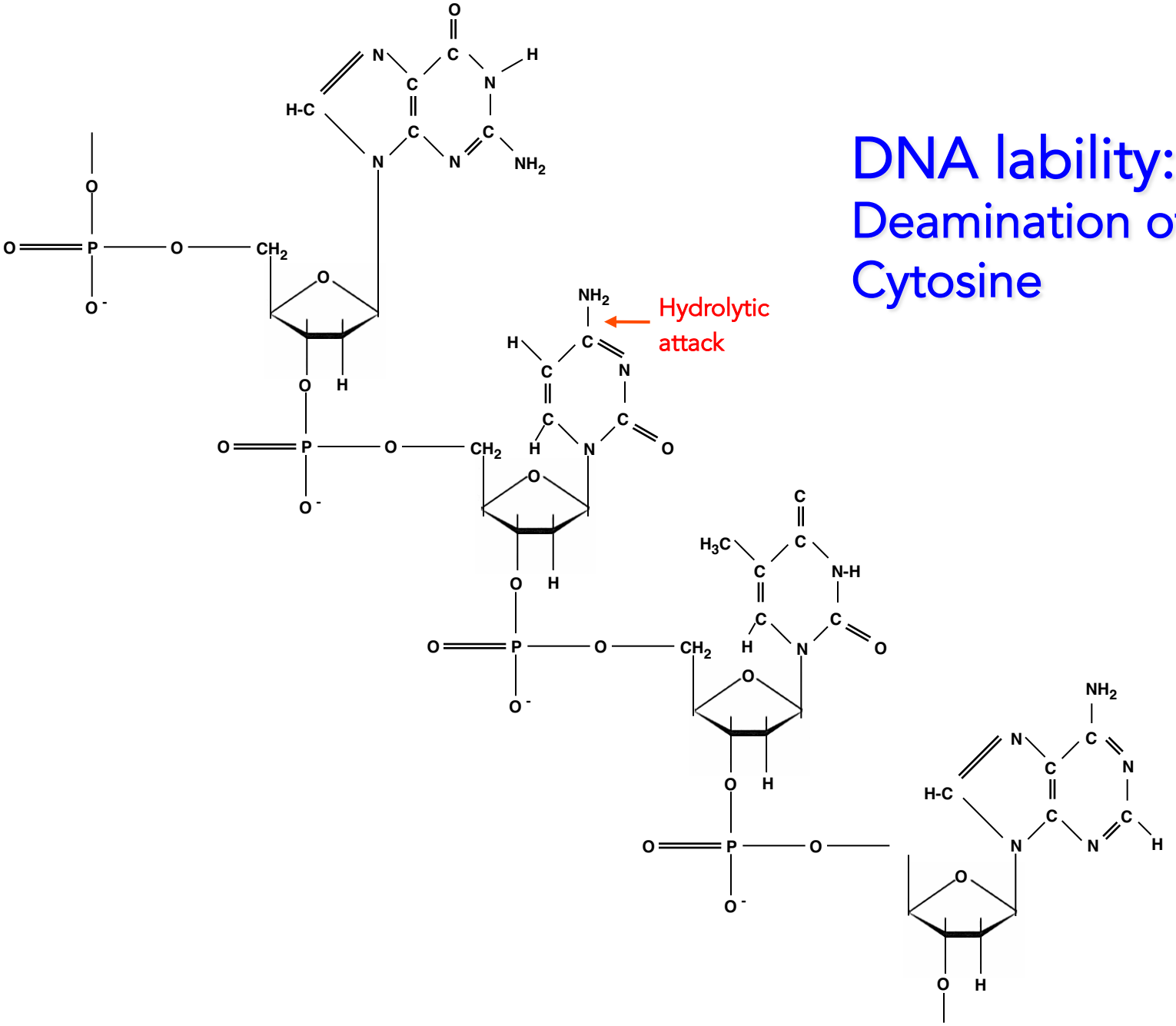
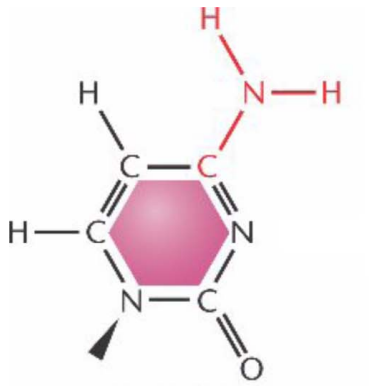
# DNA lability:
## Deamination of Cytosine



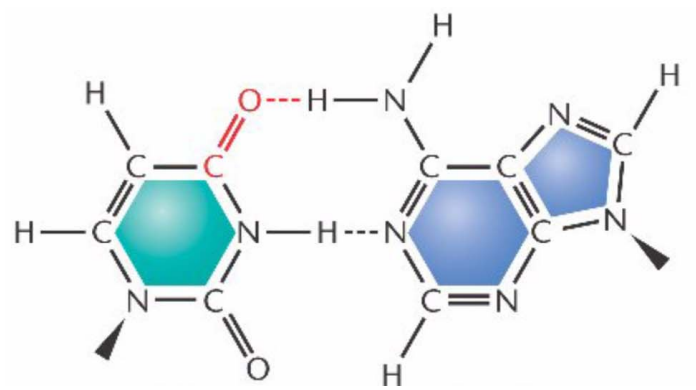
# Deamination of Cytosine Changes Coding Specificity



cytosine

(normally base pairs with guanine)

deamination



uracil

adenine

# Spontaneous DNA lesions in a Mammalian Cell

(number of altered nucleotides in a $3x10^9$ bp genome of double-stranded DNA after 24h at 37°C)

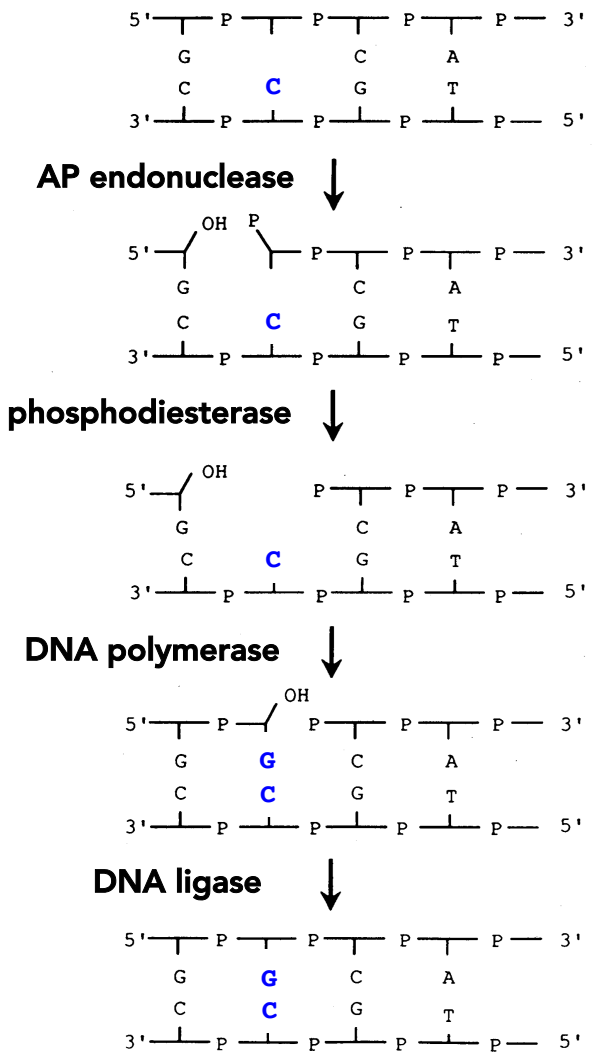
| | 100% dsDNA |
|---|---|
| **Hydrolysis** | |
| Depurination | 9000 |
| Depyrimidination | 300 |
| Cytosine deamination | 50 |
| 5-Methylcytosine deamination | 5 |
| **Oxidation** | |
| 8-Hydroxyguanine (8-oxoG) | 500-1000 |
| Ring saturated pyrimidines (thymine glycol, cytosine hydrates) | 1000 |
| Lipid peroxidation products ($M_1G$, etheno-A, etheno-C) | 1000 |
| **Non-enzymatic methylation by S-adenosylmethionine** | |
| 7-Methylguanine | 3000 |
| 3-Methyladenine | 600 |
| 1-Methyladenine/3-Methylcytosine | 10-20 |

The high rate of DNA decay implies that

repair mechanisms must exist

and are continually active under physiological conditions

# Repair of Abasic Sites in DNA

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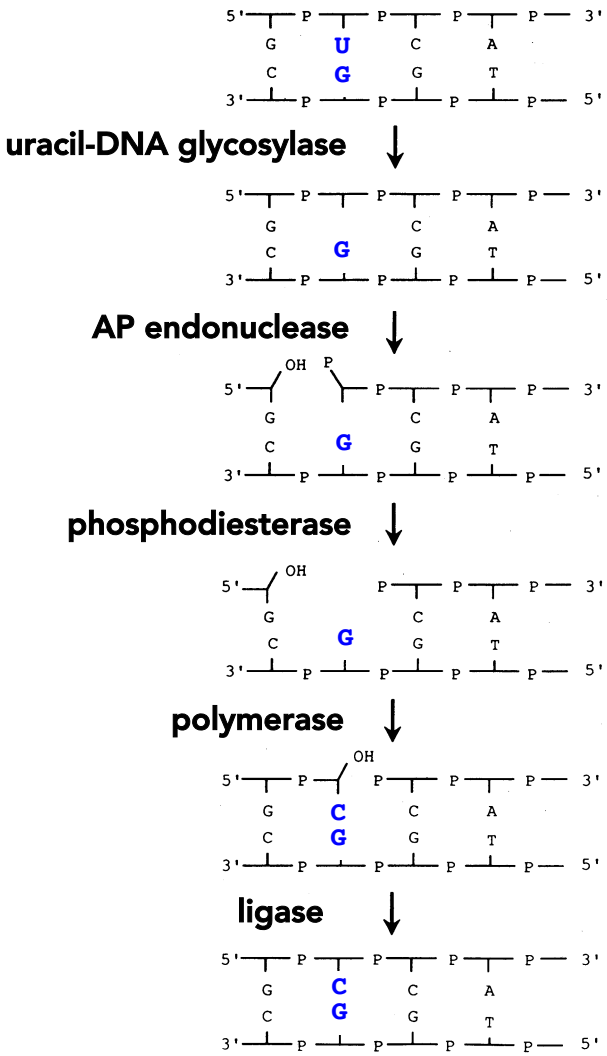
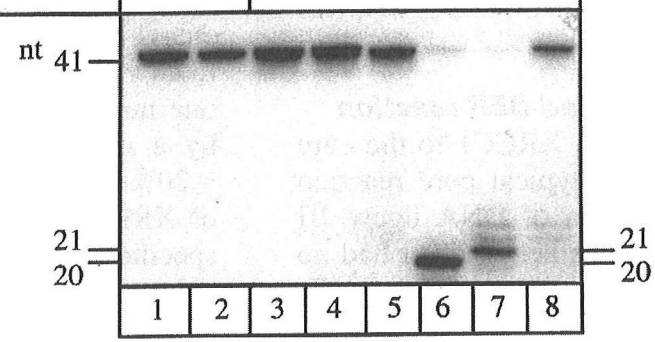
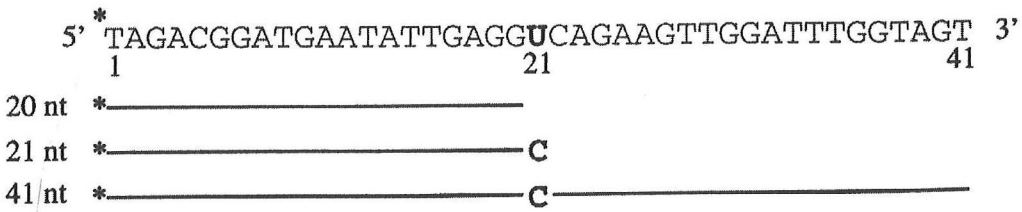
# Reconstitution of Base Excision Repair with Purified Human Proteins

- DNA glycosylase removes a damaged base, to create an AP site
- The first such enzyme discovered was uracil-DNA glycosylase



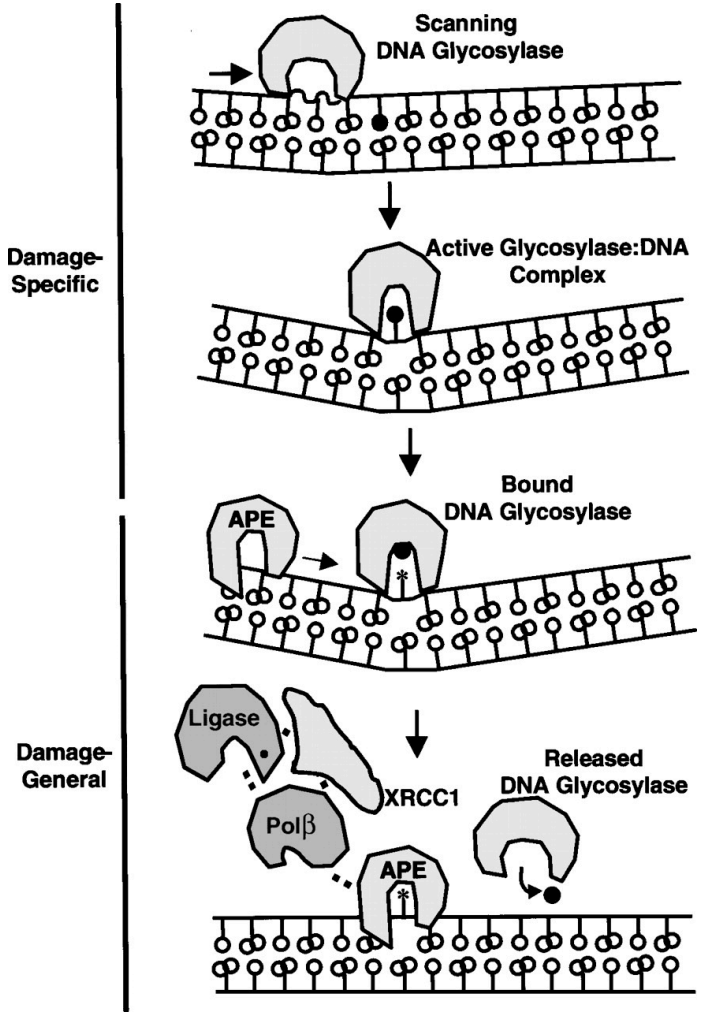

| Substrate DNA | wild type | | uracil containing | | | | | |
|---|---|---|---|---|---|---|---|---|
| UDG | – | + | – | – | + | + | + | + |
| HAP 1 | – | + | – | + | – | + | + | + |
| pol β | – | – | – | + | + | – | + | + |
| ligase III | – | – | – | – | – | – | – | + |




Kubota et al., EMBO J 1996

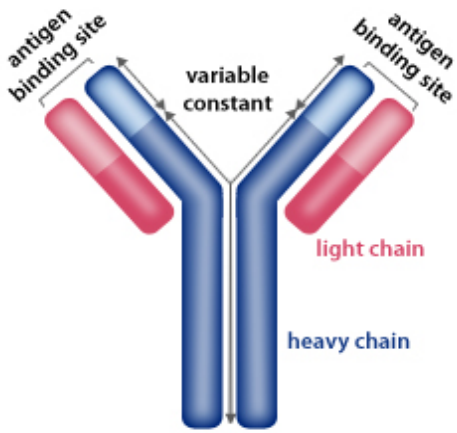
# Model for Base Excision Repair in Human Cells

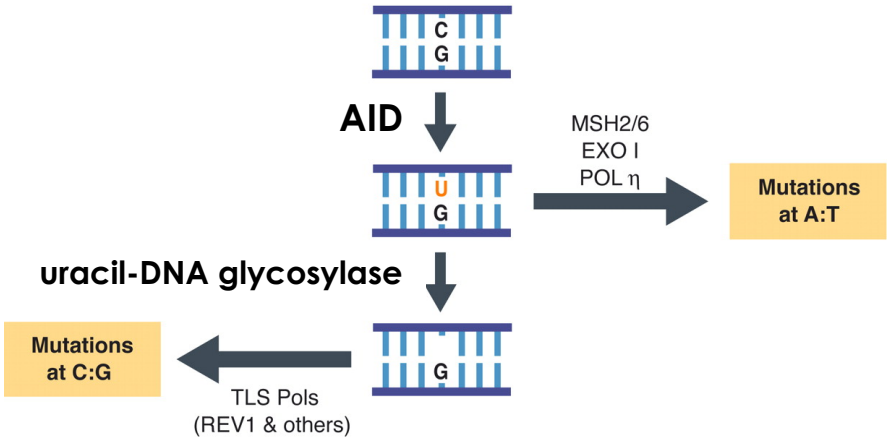



Mol, Pankh, Putman, Lo & Tainer Ann. Rev. Biophys. Biomol. Struct. 1999

# When DNA Damage is a Good Thing:

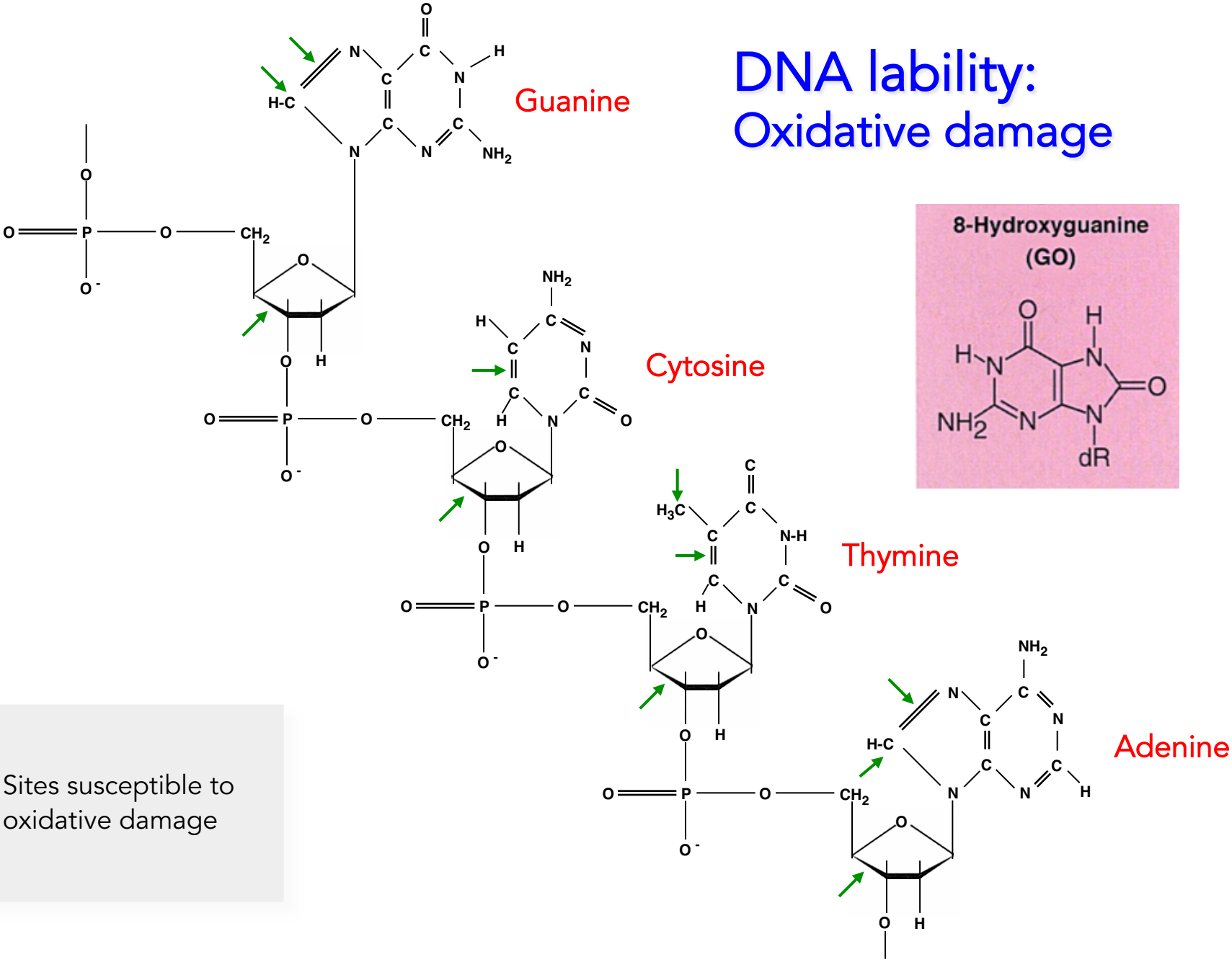
## Generation of Antibody Diversity by Somatic Hypermutation




Neuberger & Rada, J. Exp. Med. 2007

# DNA lability:
## Oxidative damage

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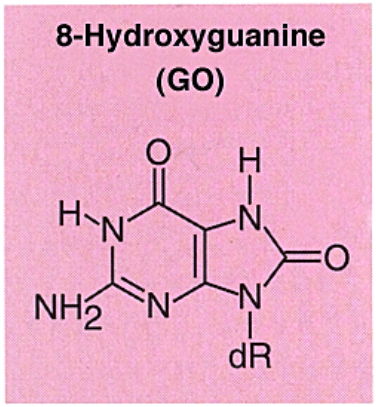
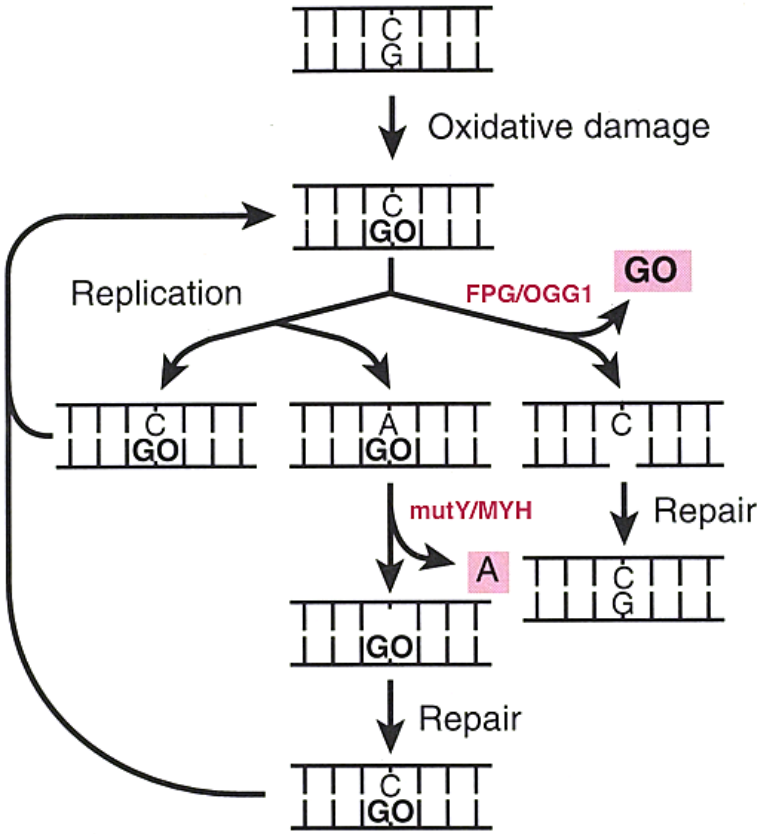
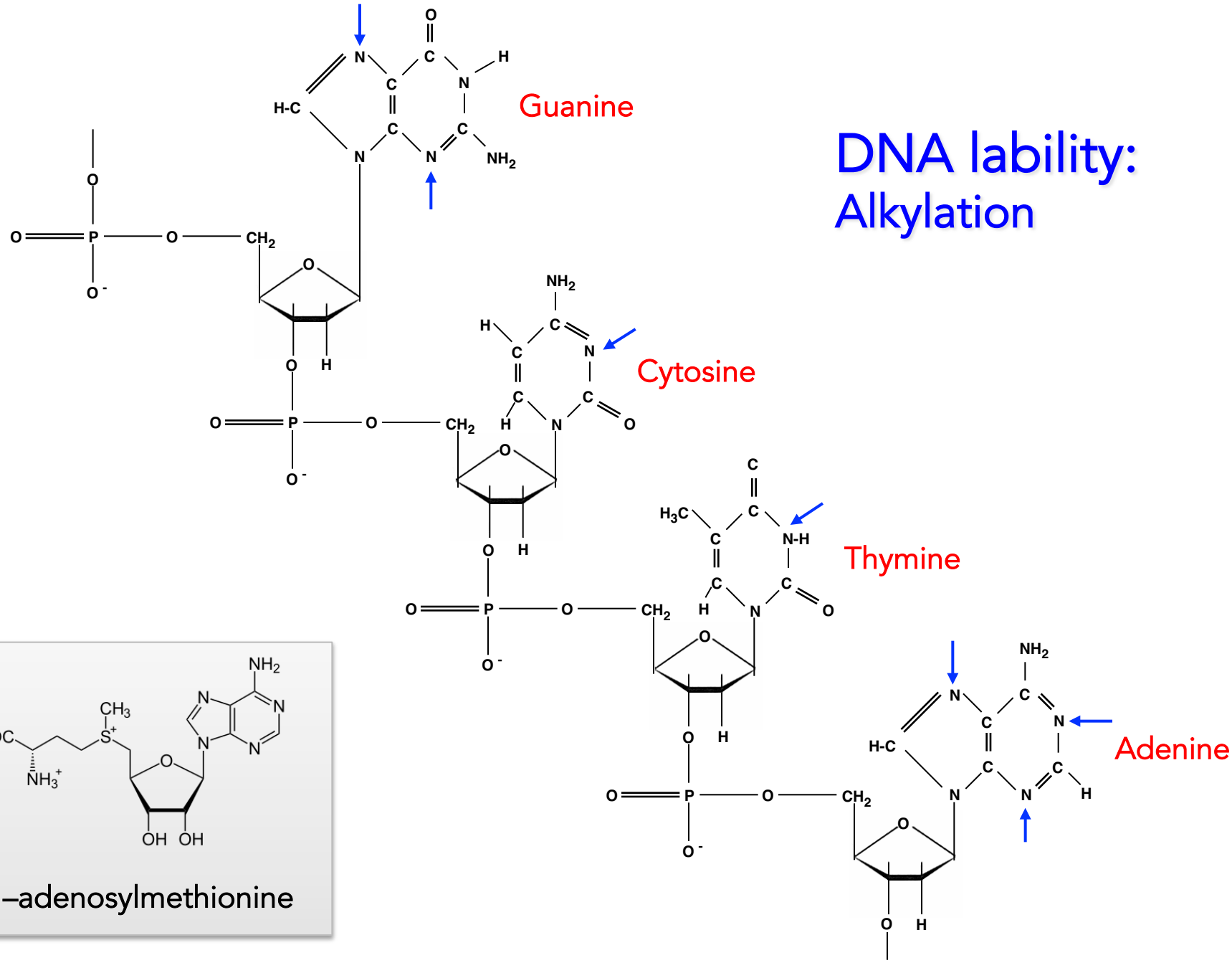
# Oxidative Damage to DNA:

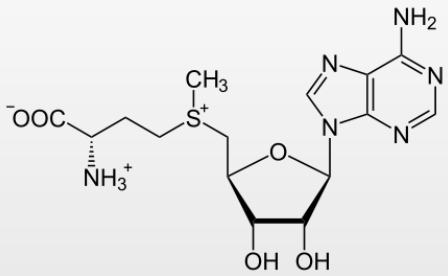
## Repair of Highly Mutagenic Lesions



# DNA lability:
## Alkylation



*S* –adenosylmethionine

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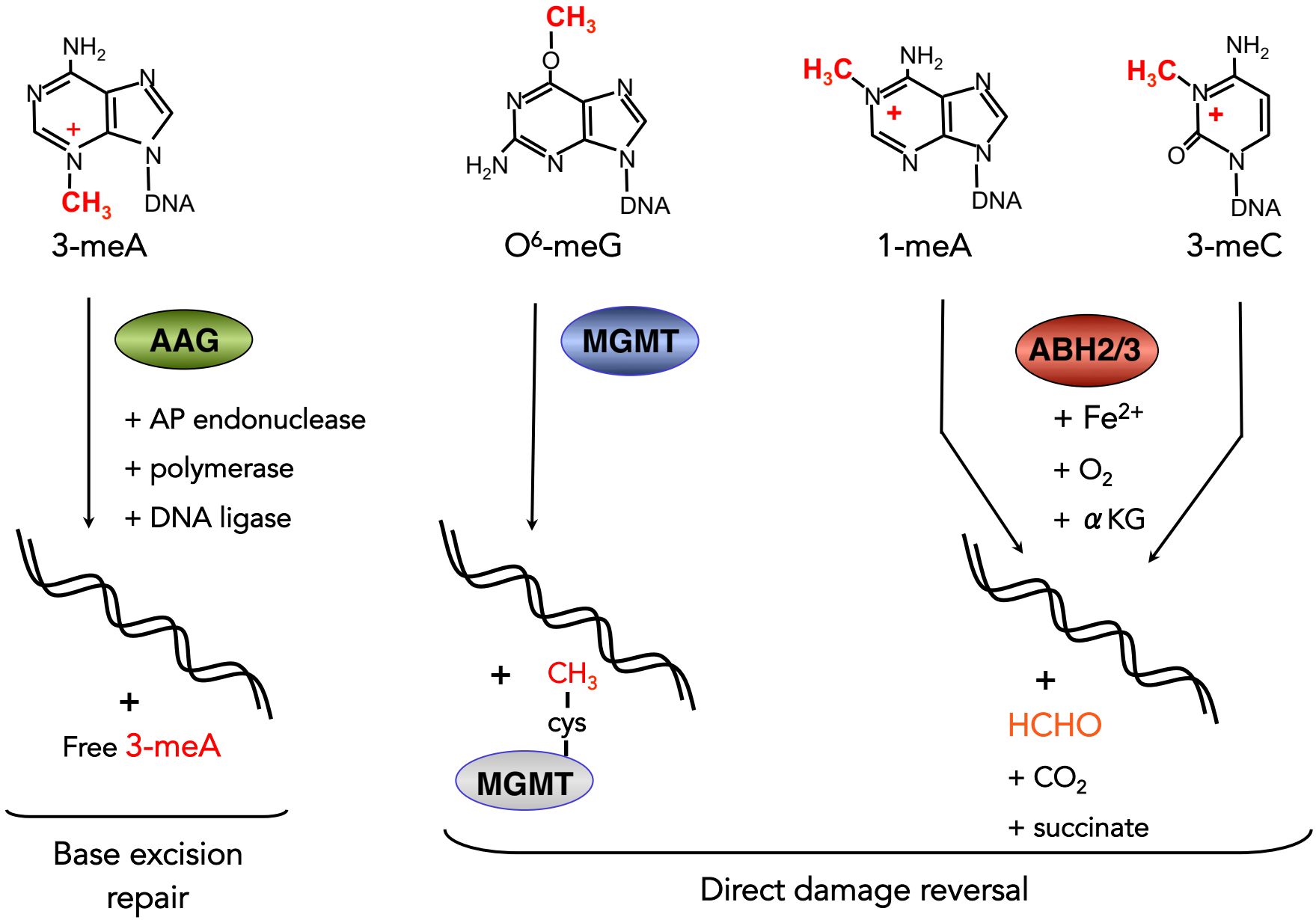
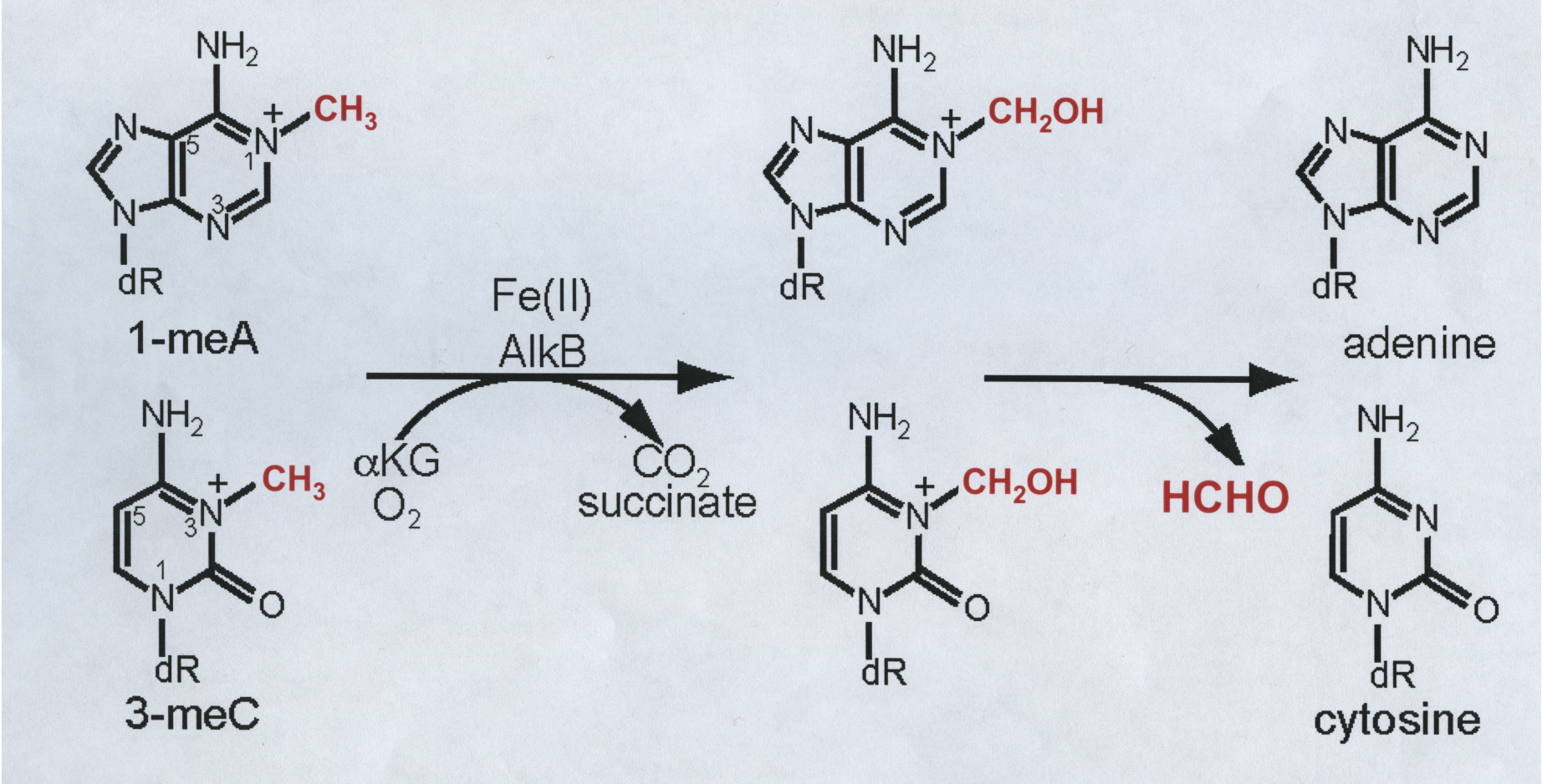
# Three Mechanisms that Repair Methylated DNA Bases



# Oxidative Demethylation of 1-meA and 3-meC by AlkB




The molecular mechanism employed for the demethylation of DNA is also used to demethylate histones

# The Intrinsic Fragility of DNA:

## Group-specific Reagents Causing DNA Damage in Cells

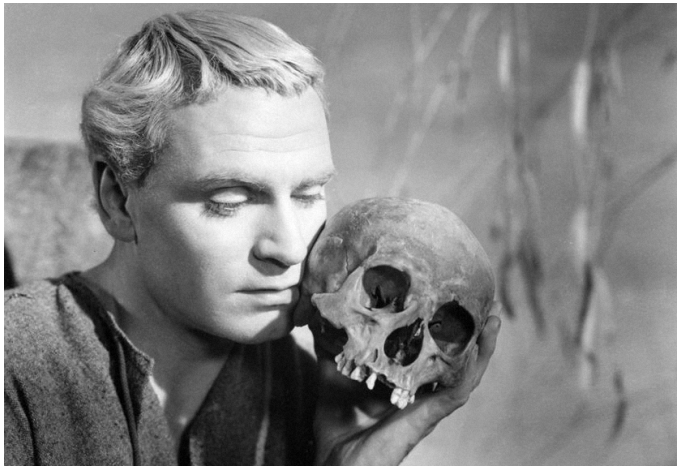
Water (55 M in cells!)

Reactive oxygen

S-adenosylmethionine (SAM)

Small reactive molecules – e.g. formaldehyde

- The diversity of lesions requires a diversity of repair enzymes
- Many DNA lesions, and the corresponding repair systems, may remain to be discovered



**Hamlet Act 5:**

**William Shakespeare**

HAMLET
How long will a man lie i' the earth ere he rot?

GRAVEDIGGER
I' faith -- he will last you some eight year
Or nine year: a tanner will last you nine year.

HAMLET
Why he more than another?

GRAVEDIGGER
Why, sir, his hide is so tanned with his trade, that
He will keep out water a great while; and your
*Water is a sore decayer* of your wretched dead body