# Radiation-induced clustered DNA damage: biological consequences

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# Clustered DNA Damage

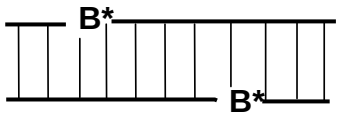
Clustered DNA damage occurs when two or more lesions are formed within one or two helical turns of the DNA

**DSB**

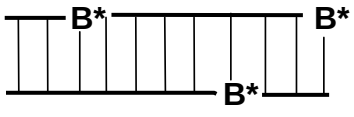
SIMPLE

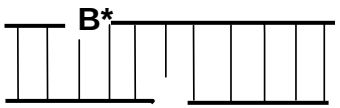
# Non-DSB Clustered DNA Damage

SIMPLE COMPLEX

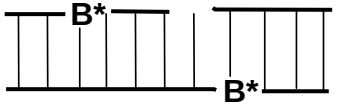



**Base Lesions**



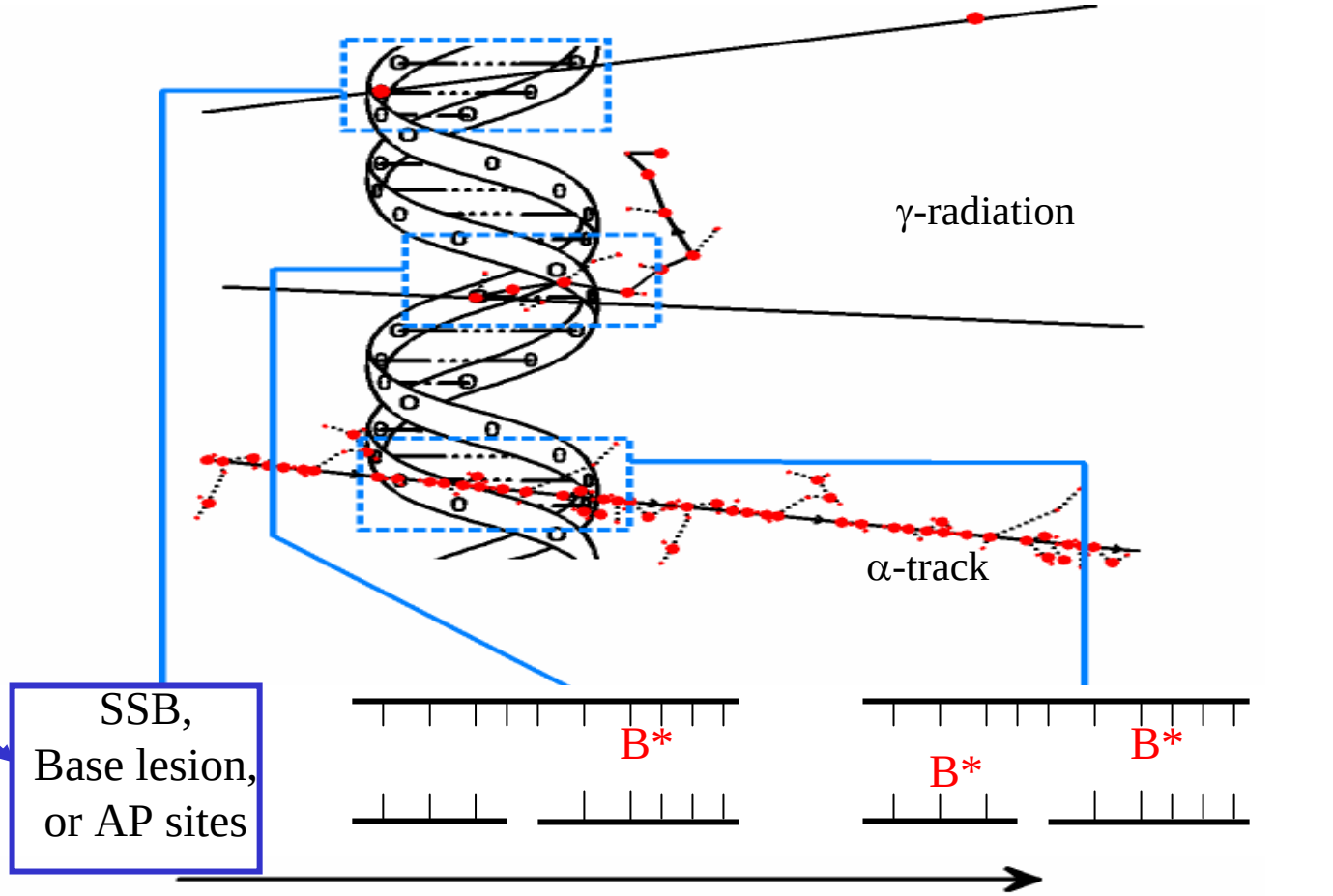




**Base Lesion(s) and SSB(s)**



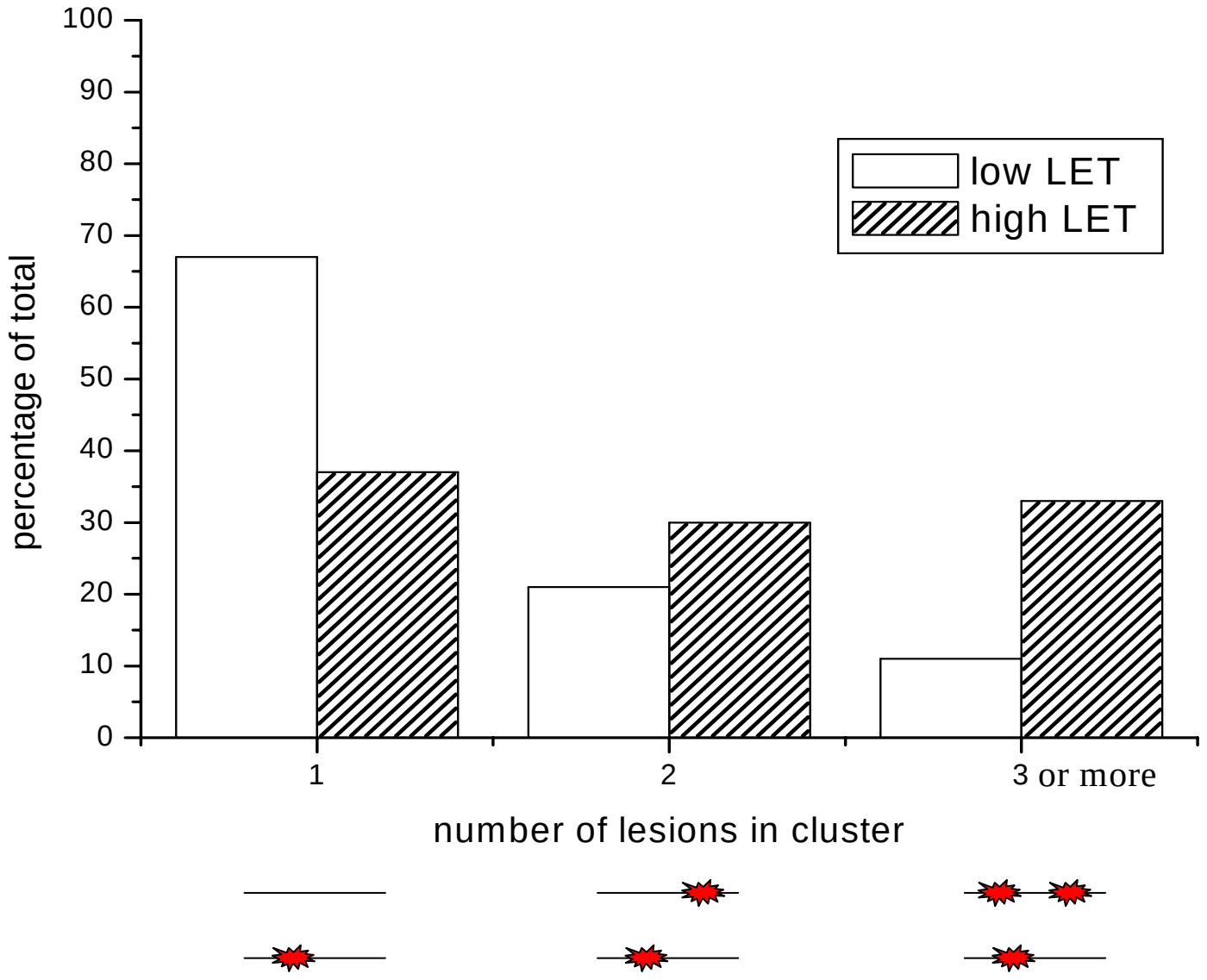
# Radiation tracks & DNA damage

Endogenous DNA damage
(base lesions, ssb, AP sites)
**(~2200 lesions/cell)**




**Cluster complexity increases with increasing ionisation density**

# Complexity of clustered DNA damage




Nikjoo

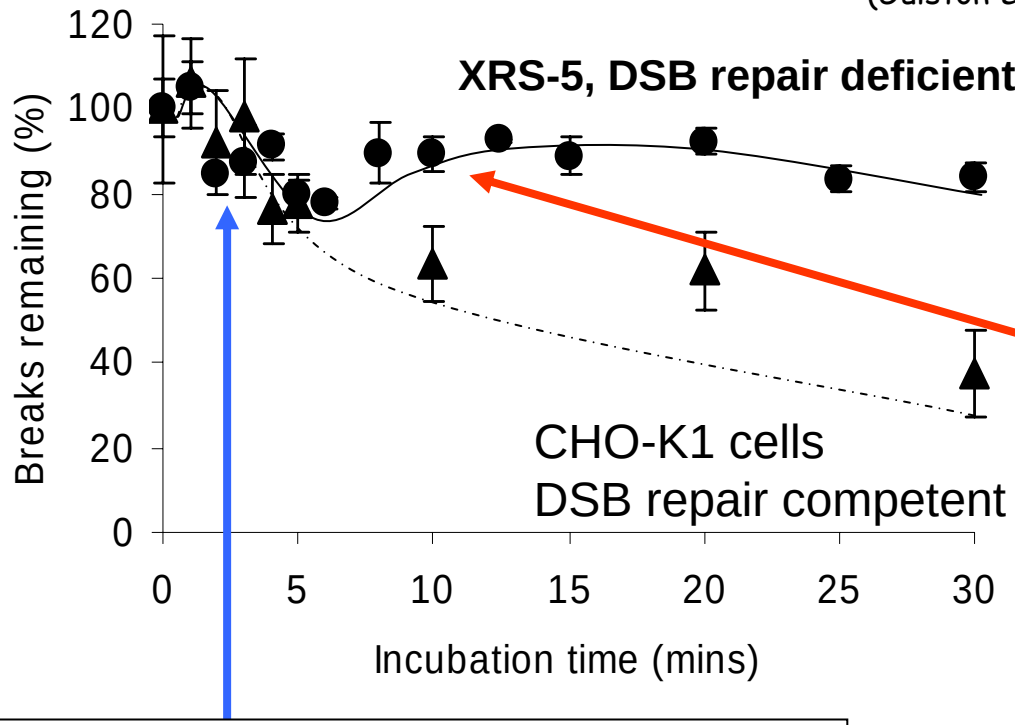
# low LET radiation in mammalian cells induces about 4x more non-DSB clustered DNA damage than prompt DSB

Sutherland *et al*. PNAS, **97**, 103, (2000), Radiat Res. **157**, 611 (2002);
Jenner *et al*., Rad. Res, **156**, 590 (2001)
Gulston *et al*, Nucl. Acids Res. **30**, 3464 (2002)

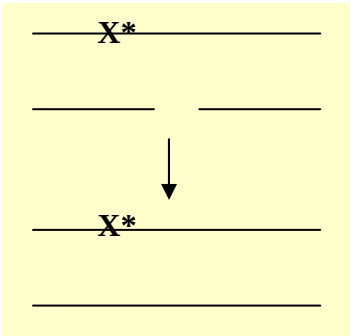
Does intracellular processing of clustered DNA damage sites give DSB?

# Non-DSB clustered DNA damage induced in XRS-5 & CHO-K1 Cells by γ-radiation are converted into DSB

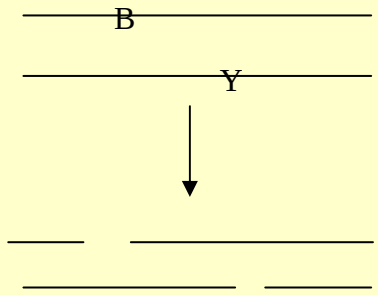
(Gulston *et al.* Nucl. Acids Res. **32**, 1602-1609 (2004))



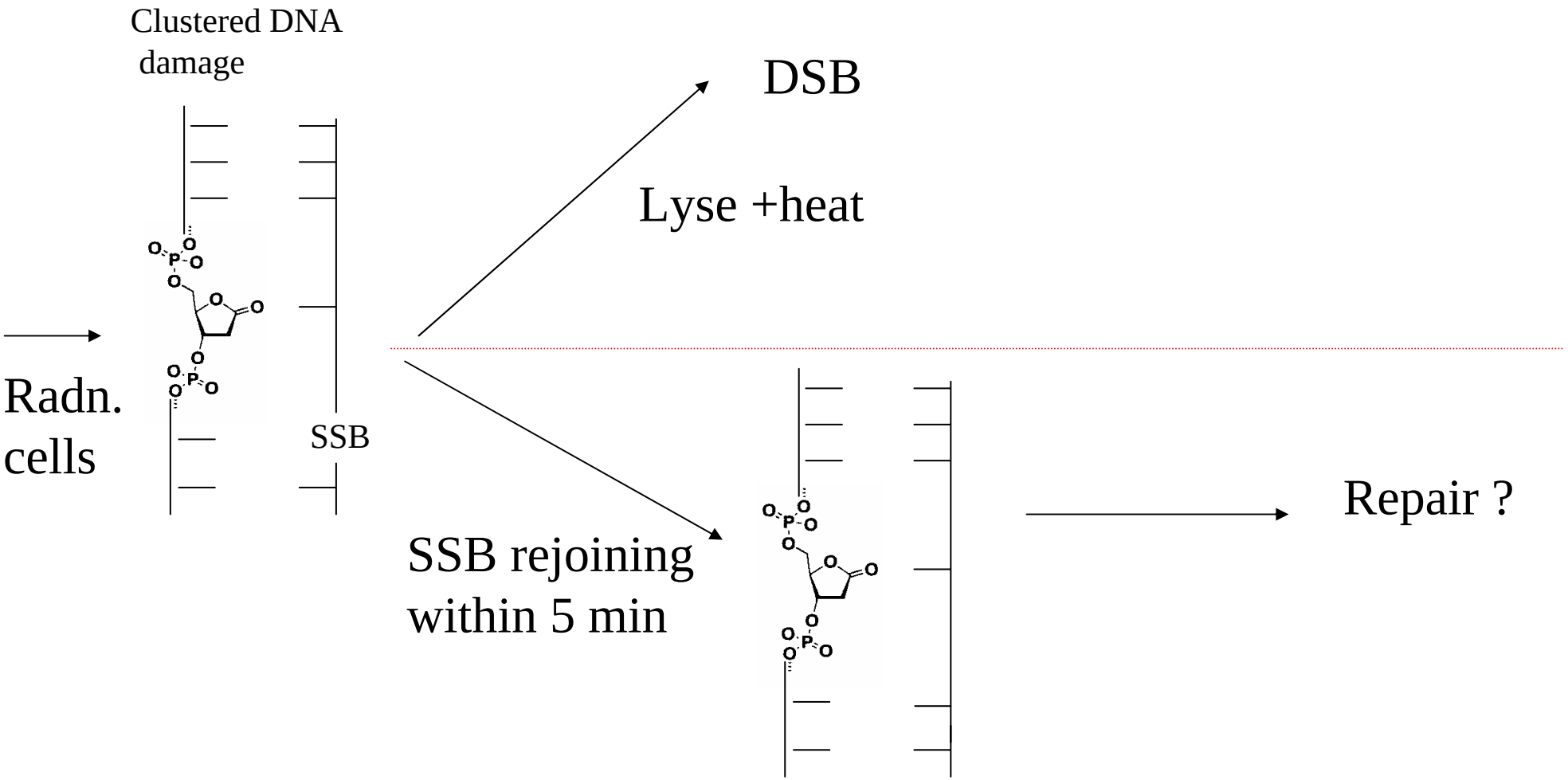

- Fast rejoining within 4 min independent of DSB repair process (NHEJ & HR)
-- heat-sensitive non-dsb clustered damage




Conversion of non-dsb clustered DNA Damage into DSB (candidates 2 opposed AP sites or AP/SSB)



# Conversion of Heat-labile sites into DSB



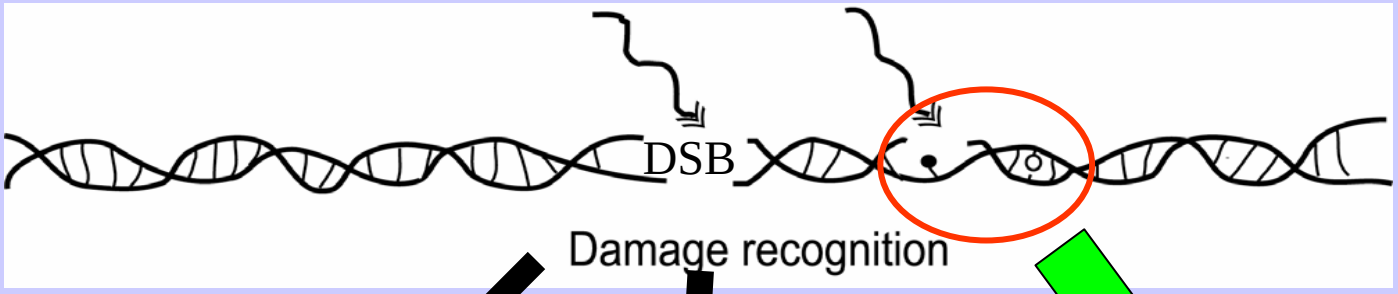
~10% of non-DSB clustered damage sites

are converted into DSB
(Gulston *et al.* Nucl. Acids Res. **32**, 1602-1609 (2004))

Majority of non-DSB clustered damage sites do not yield DSB in cells

# How are clustered DNA damage sites processed using cell extracts?

# DNA damage repair pathways for radiation damage




Homologous recombination

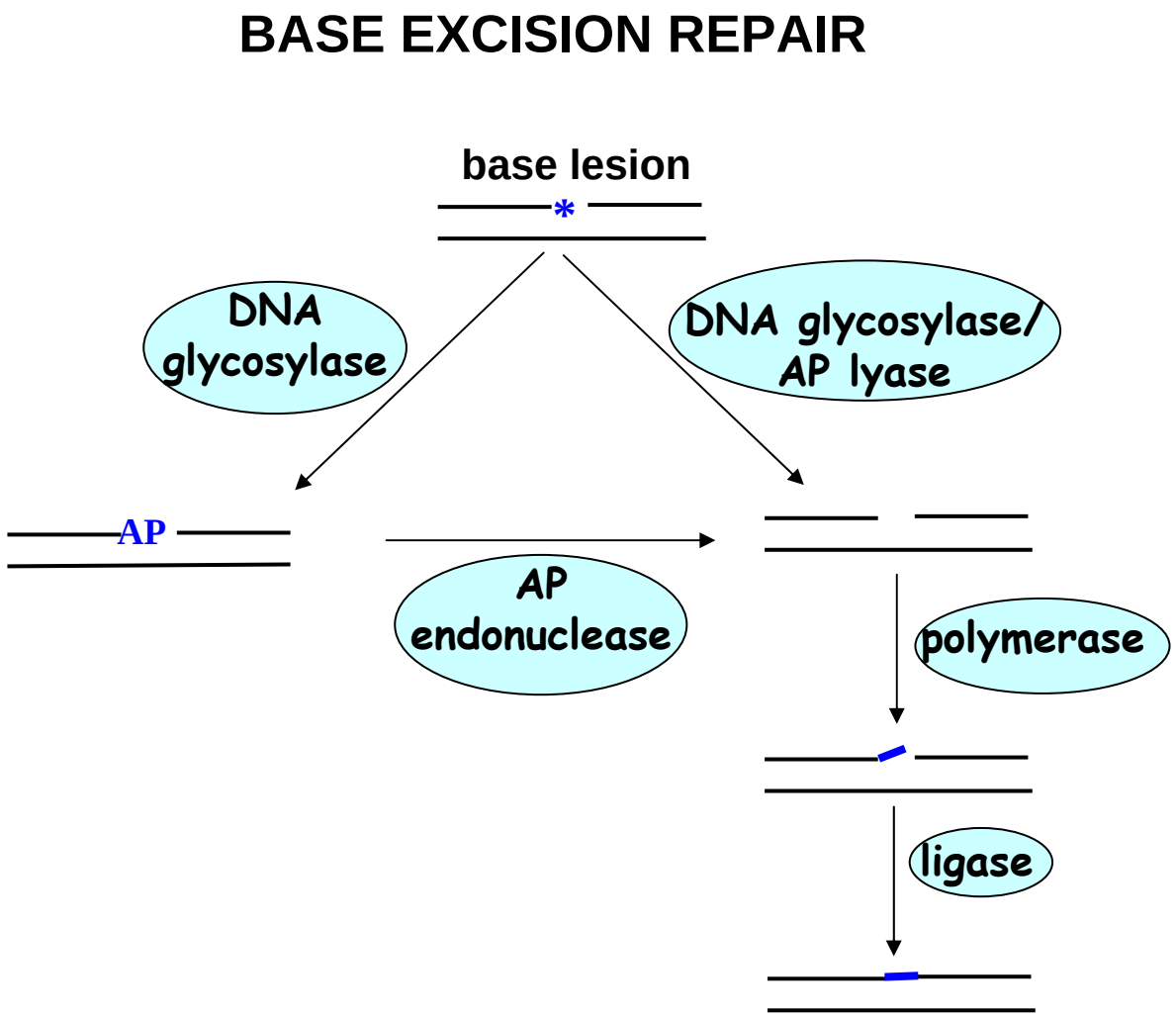
Non-homologous end joining

Clustered DNA damage

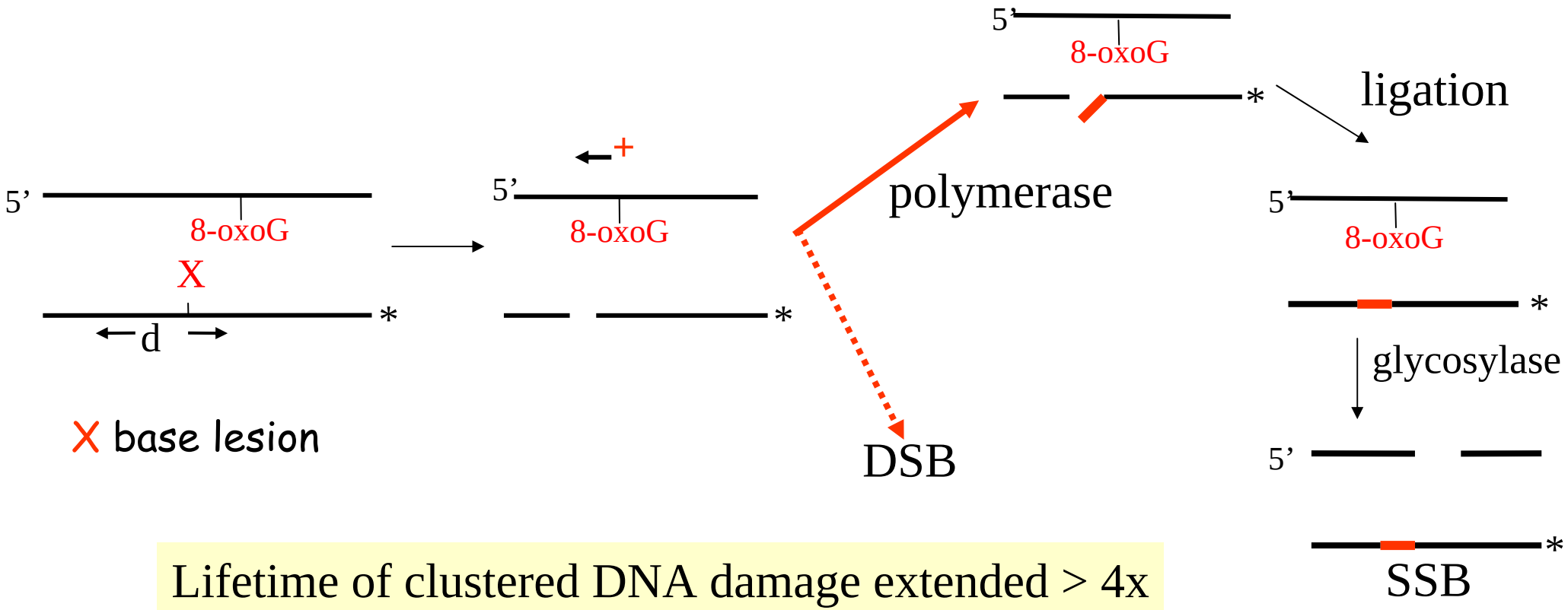
Base excision repair

# Is the processing of clustered DNA damage compromised?

Use of cell extracts to study repair



# SSB rejoins prior to excision of 8-oxoG: minimises DSB formation

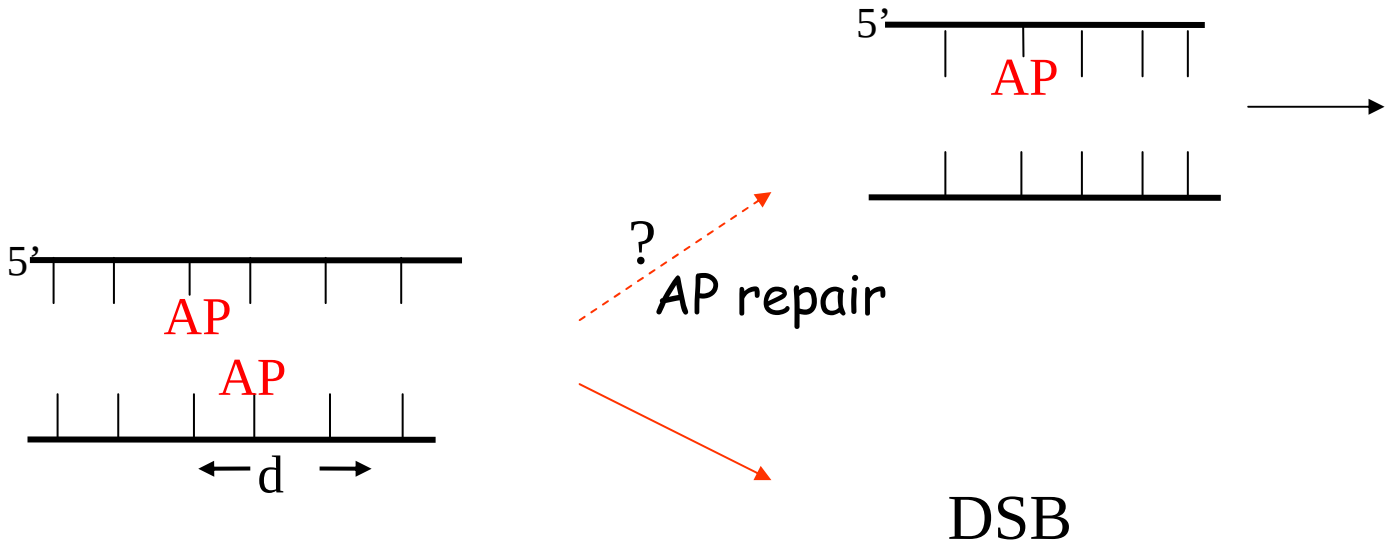



Lifetime of clustered DNA damage extended > 4x

Stalled replication due to extended lifetime of cluster

Lomax *et al.* DNA Repair, **3**, 289-299 (2004)

# TWO AP sites generally give DSB when processed using cell extracts




Lomax *et al.* Biochemistry in press (2004)

- If the clustered damage is still present at replication, then stalled replication may give rise to mutations and cancer.

- Mutation spectrum depends on cluster complexity?

# Are non-DSB clustered DNA damage sites mutagenic?

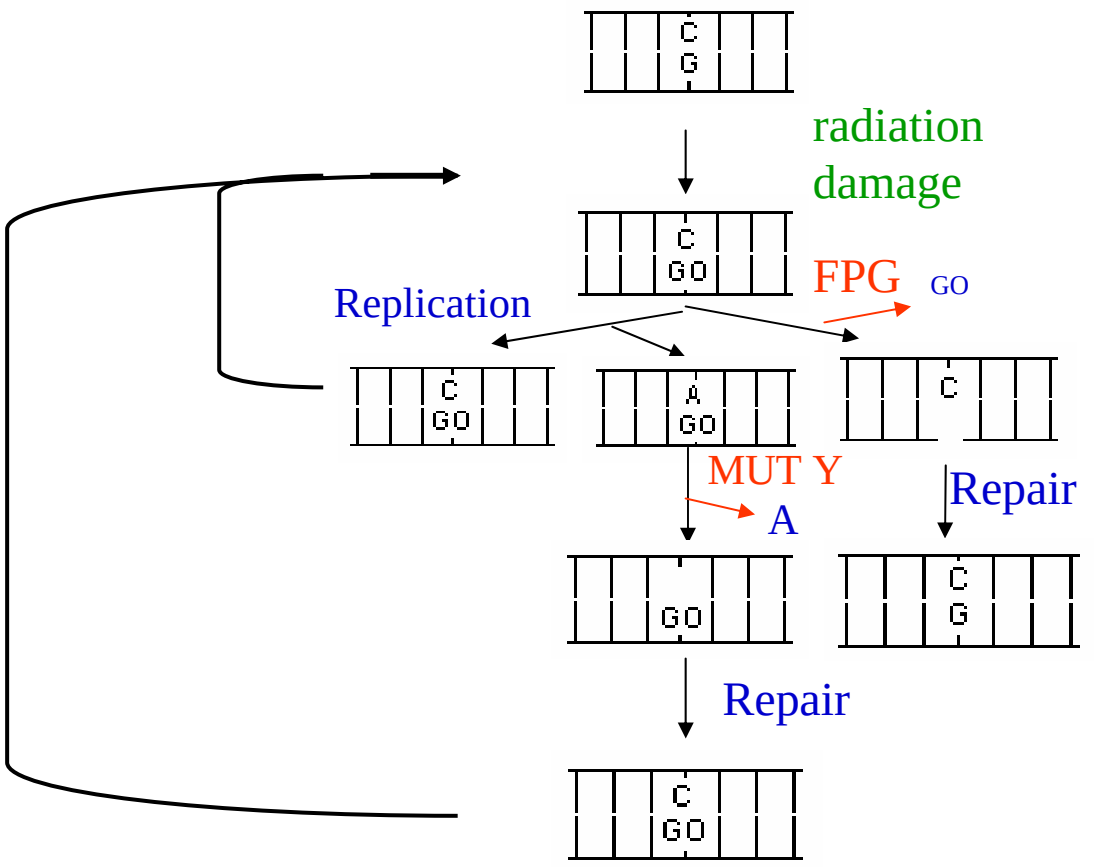
- the mutagenic potential of clustered damage DNA constructs containing the common oxidative lesion 8-oxoG, following processing by *E. coli*
- Using *E. coli* strains *fpg*, *mutY* and *fpg mutY*

**MutM (Fpg)** - An 8-oxoG DNA glycosylase which removes 8-oxoG paired with cytosine.

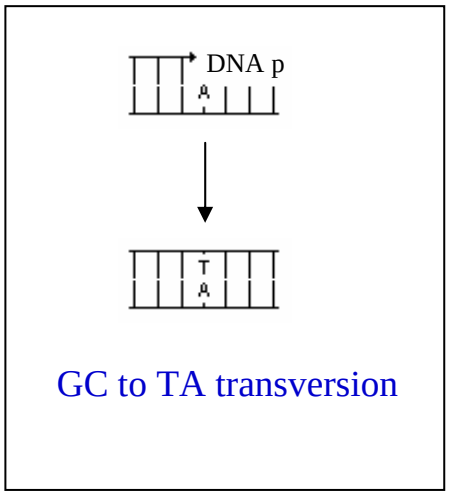
**MutY** - An adenine DNA glycosylase which removes misincorporated adenine residues opposite 8-oxoG

Pearson *et al.*, Nucl. Acids Res., **32**, 263-270 (2004)

# The roles of mut Y and fpg in BER.

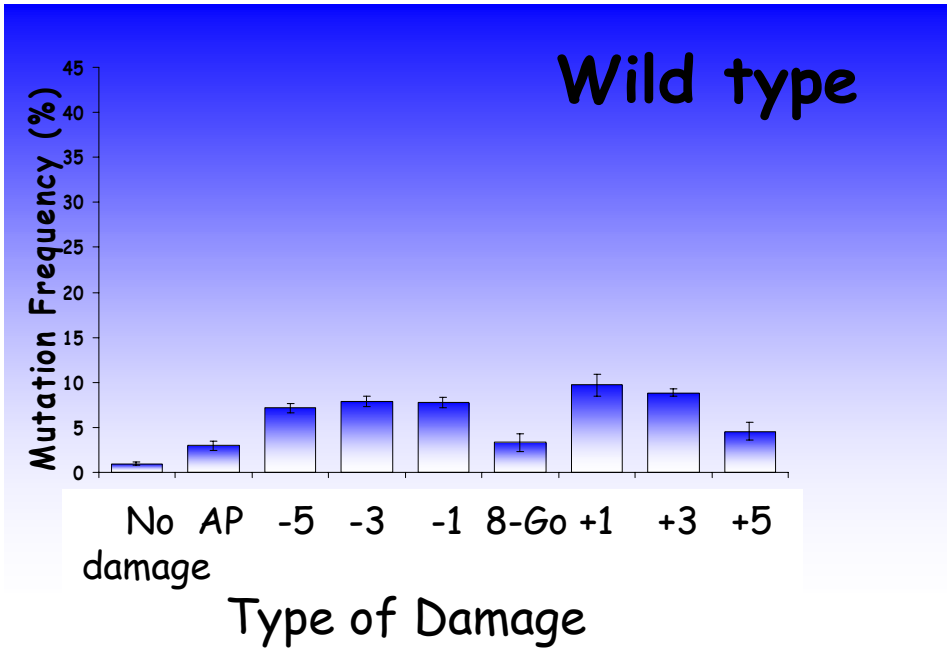


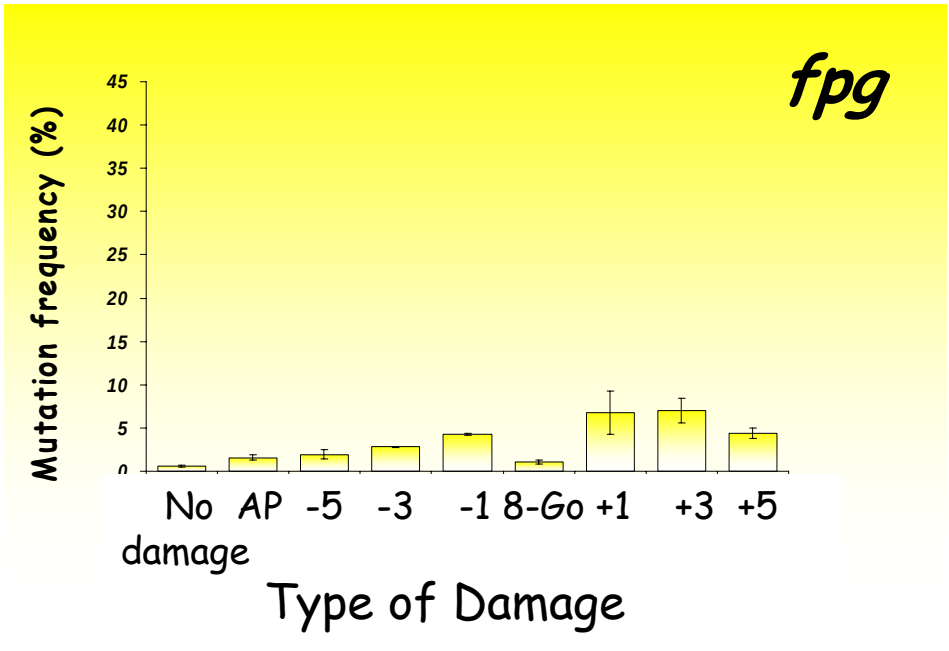

•DNA glycosylases remove the damaged base from the deoxyribose-phosphate backbone. Producing an AP site.

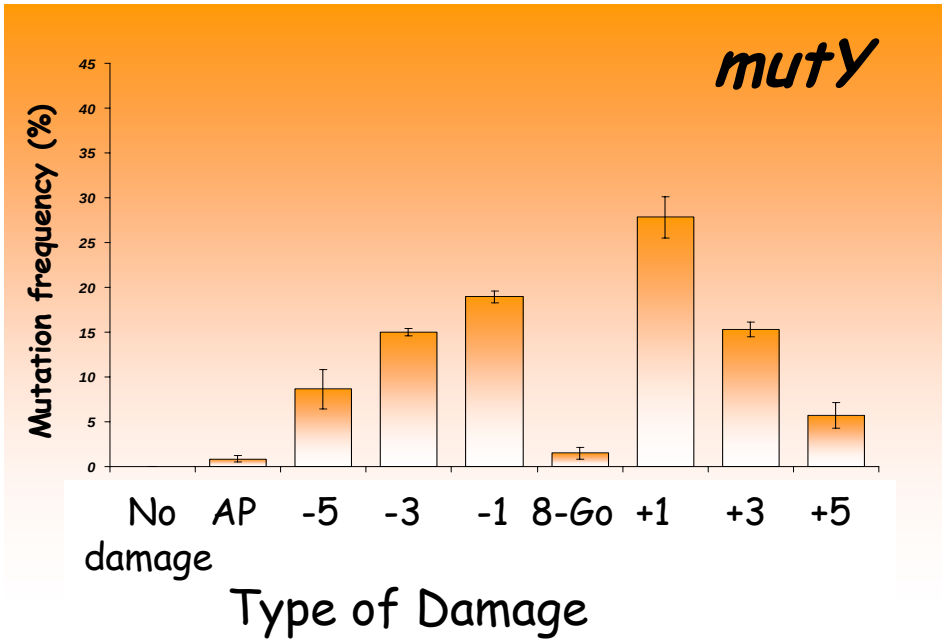


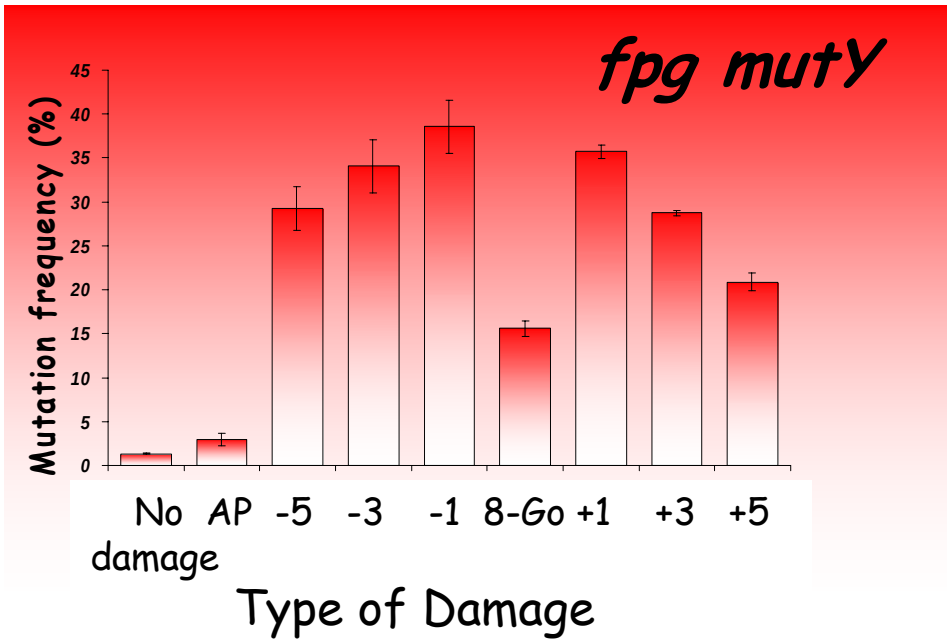

After Friedberg et al (1995).

# Mutation frequency on type of clustered DNA damage



- When a 8-oxoG lesion within a cluster is present at replication the probability increases that mis-pairing events are not corrected.

*mutY* = ↑ mutation frequency

*fpg mutY* = ↑↑ mutation frequency

**CLUSTERED DNA DAMAGE IS BIOLOGICALLY SIGNIFICANT**

Pearson *et al.*, Nucl. Acids Res., **32**, 263-270 (2004)

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