Mechanism of non-DNA targeted mutagenesis: the role of intra cellular nucleotide pool

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1-2 Gy gamma Radiation

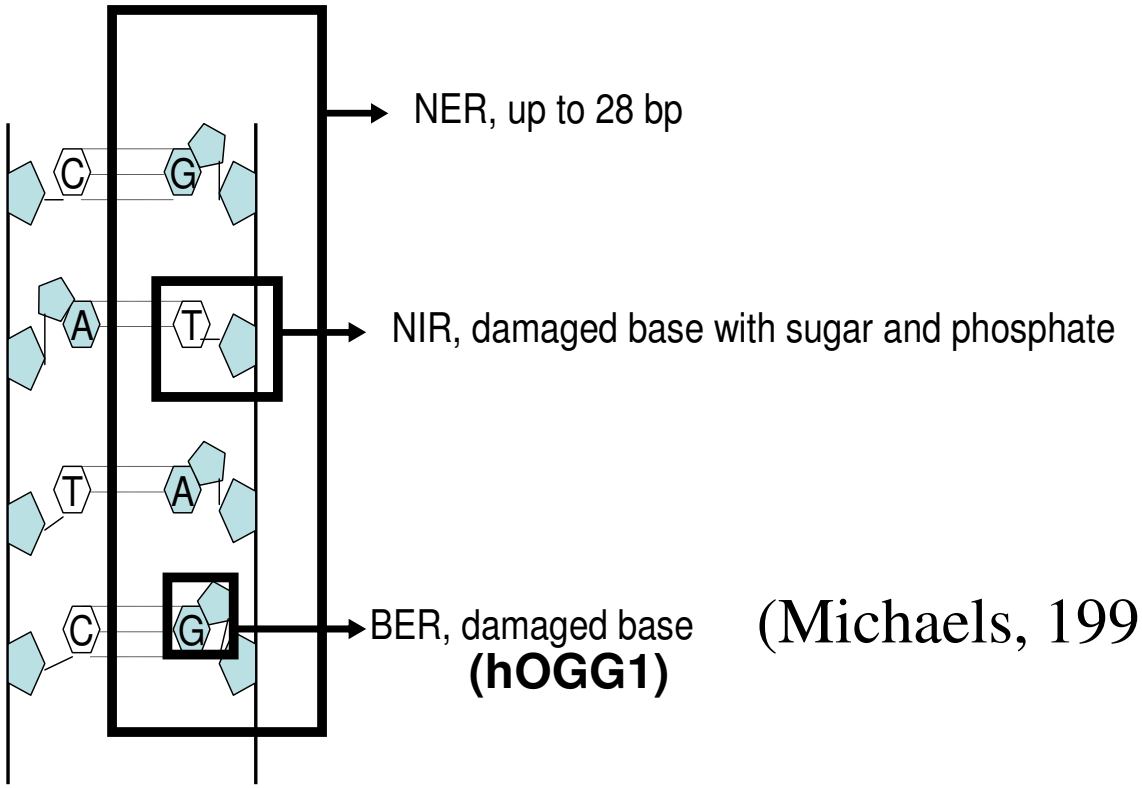
Incubation for repair

Detection of oxidized DNA base in serum

1 Gy

–20-40 DSB
–~1000 SSB
–~2000 base damages (500-700 8-oxo-dG)
–~10000 ionizations in a cell

8-Oxo-dG repair pathways

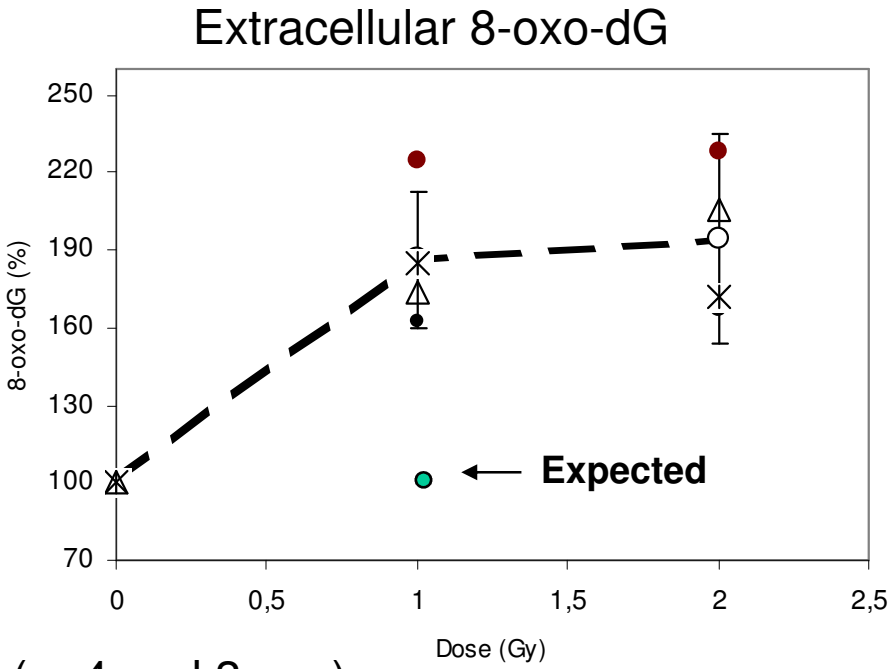


(Michaels, 1991, BM Ames 1991)

Repaired 8-oxo-dG is released to urine via blood serum where it can be detected.

Extracellular 8-oxo-dG as a sensitive marker for oxidative stress in vivo and in vitro

- Amount of 8-oxo-dG excreted by leukocytes, exposed to 1 Gy, is 35 times higher than what is expected to be formed in DNA.
- DNA is not the main source for extracellular 8-oxo-dG



(n=4 and 3 exp)

(***In vitro* study, HPLC**)

Haghdoost S. et al. Free Radic. Res. 2005

Chromatin structure

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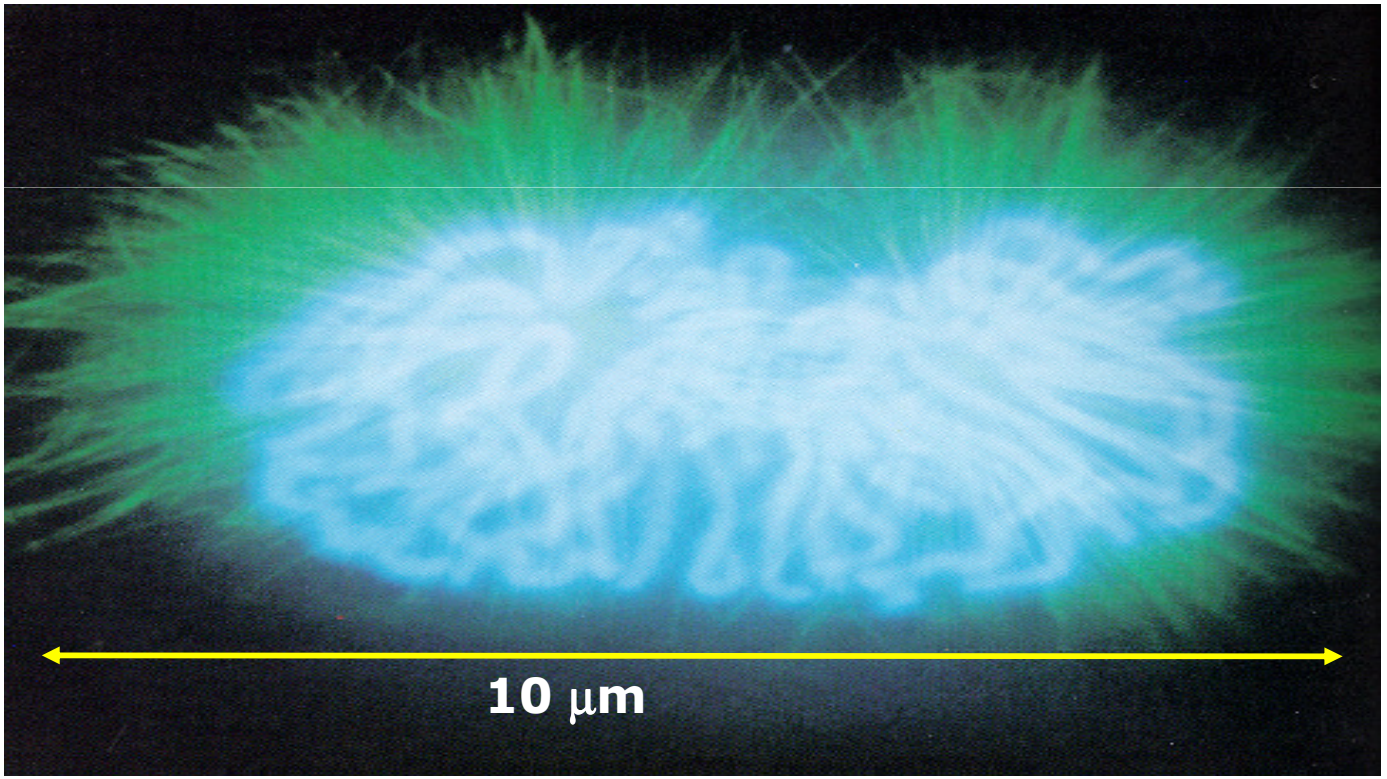
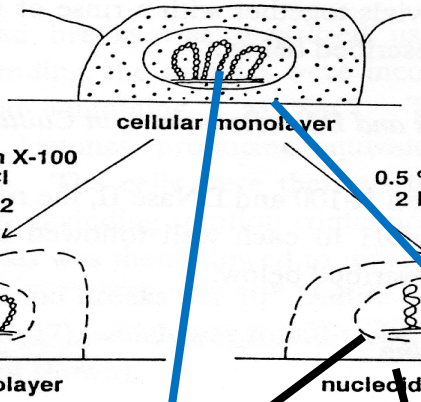
Number of bases	3×10^9
Chromosome nr 1	8.9 cm
DNA/cell	100 cm



Protective effect of chromatin structure

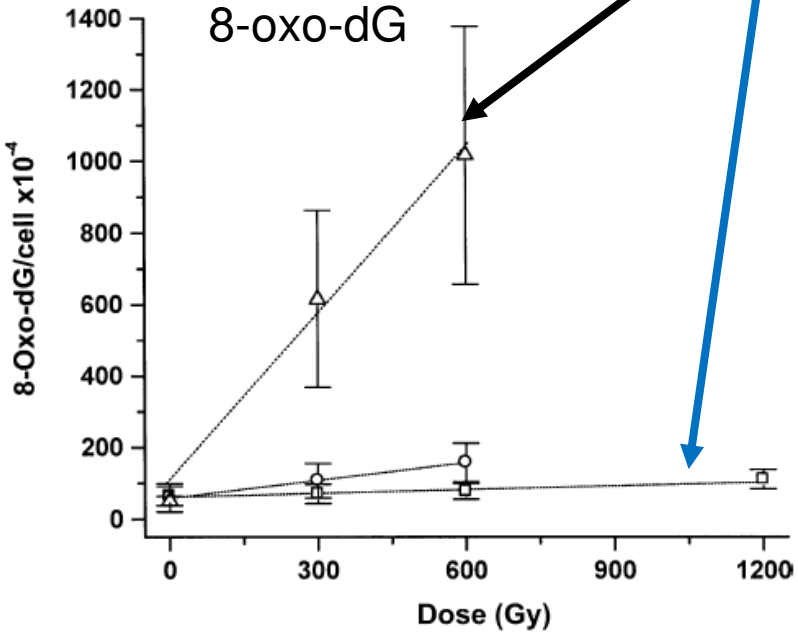
Ljungman, M. et al., 1991



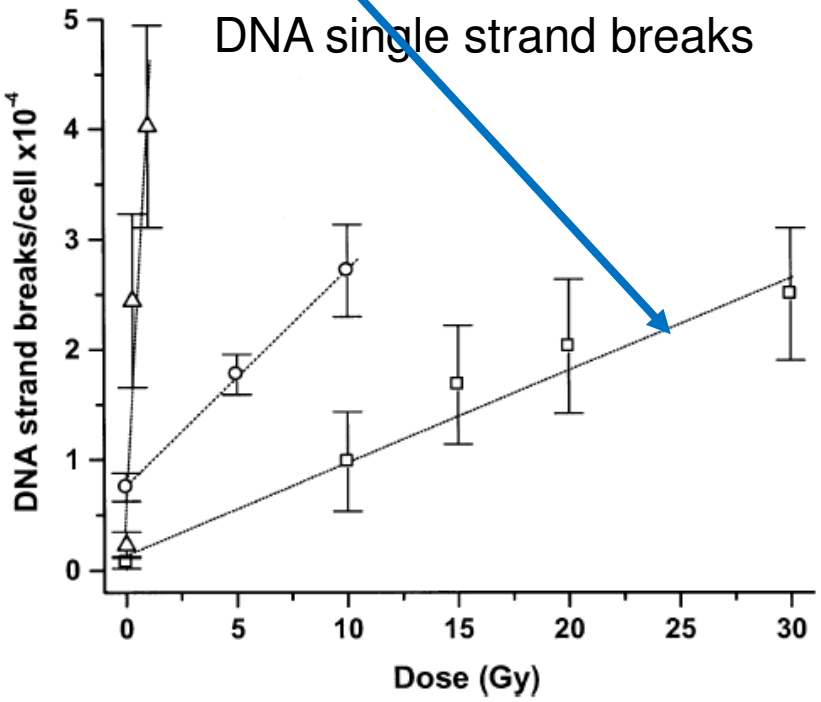
Histone free

Svoboda and Harms-Ringdahl, 2005

8-oxo-dG



DNA single strand breaks



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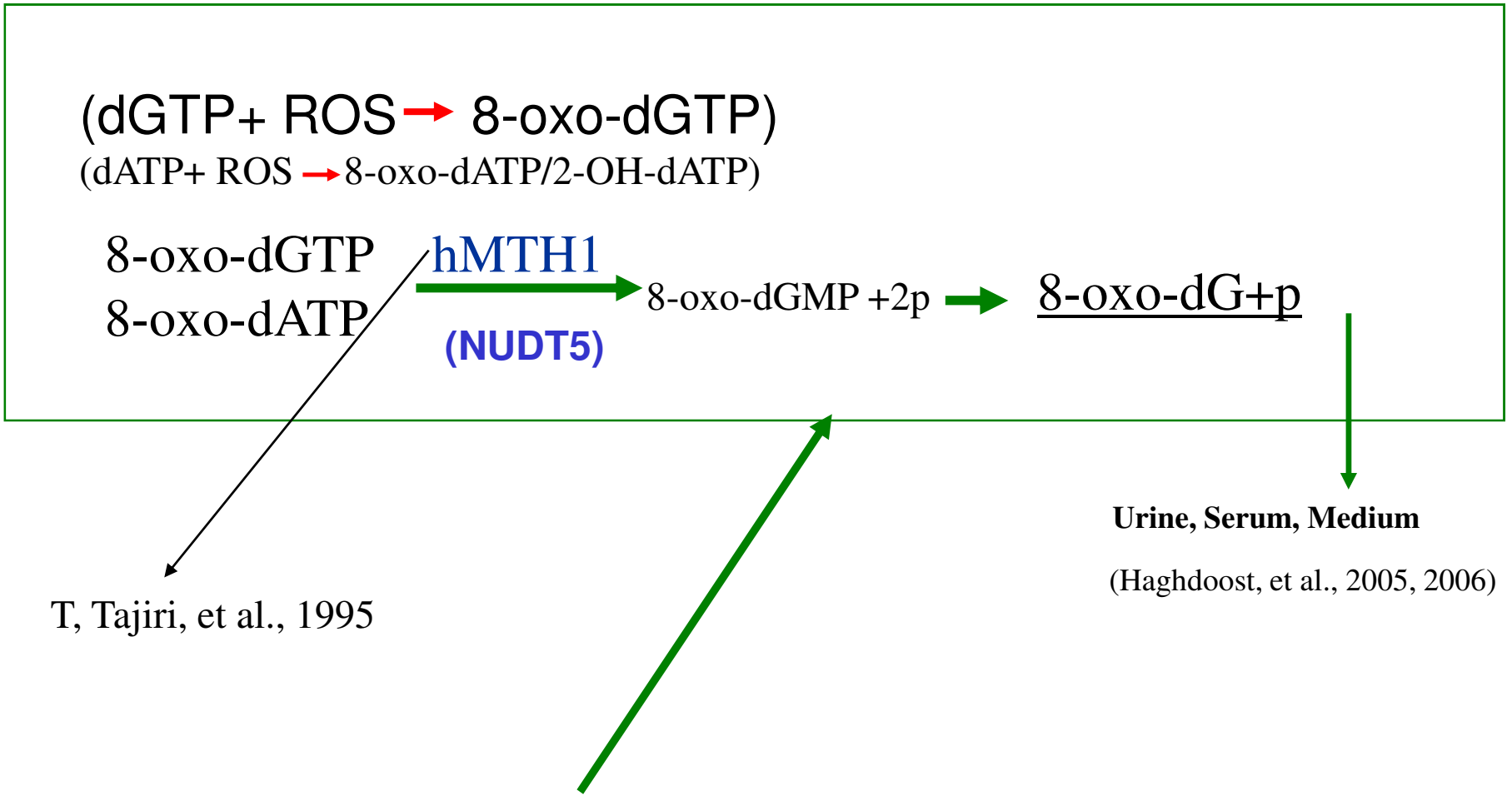
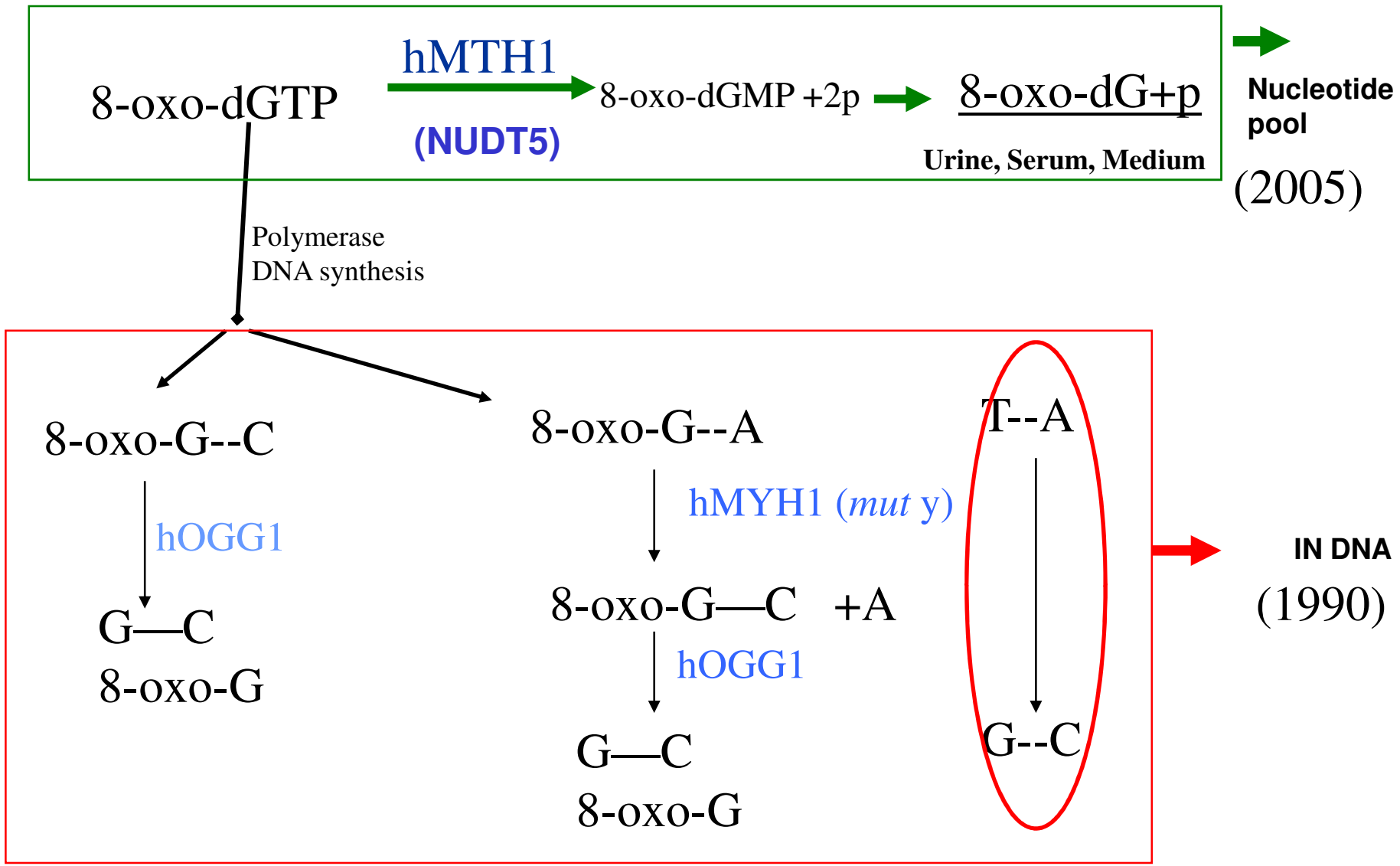
Summary

- Increase in extracellular 8-oxo-dG observed after in vitro irradiation of whole blood
- This yield
  - saturates above a dose of 1 Gy
  - is signifcantly different between individuals.
- Indications for a radiation induced stress response that would work primarily on the dNTP pool

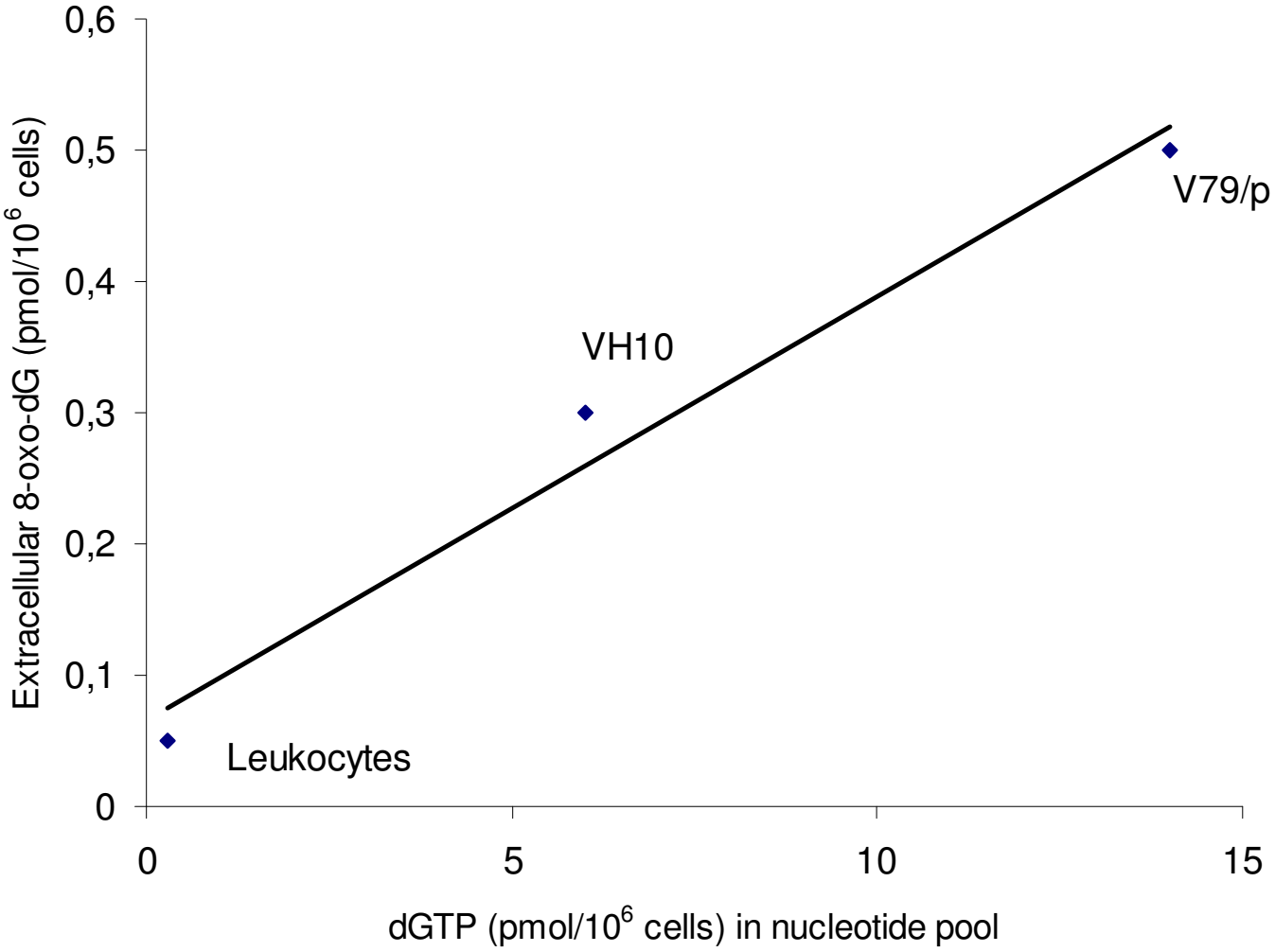


Nucleotide pool cleaning up system



2-OH-dA: AT-GC
8-oxo-dG: TA-GC

Pool size and extracellular 8-oxo-dG



Haghdoost S. et al, Free rad. Bio. Med. 2006

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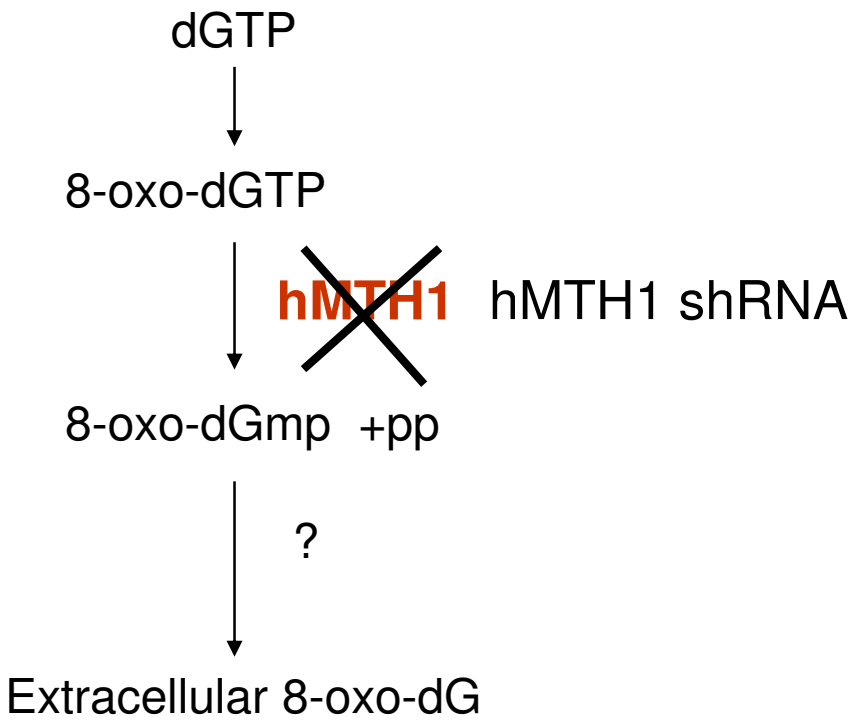
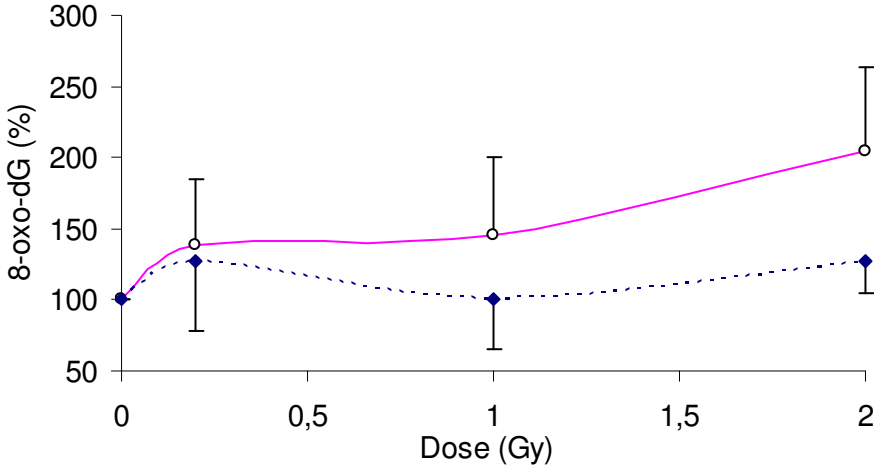
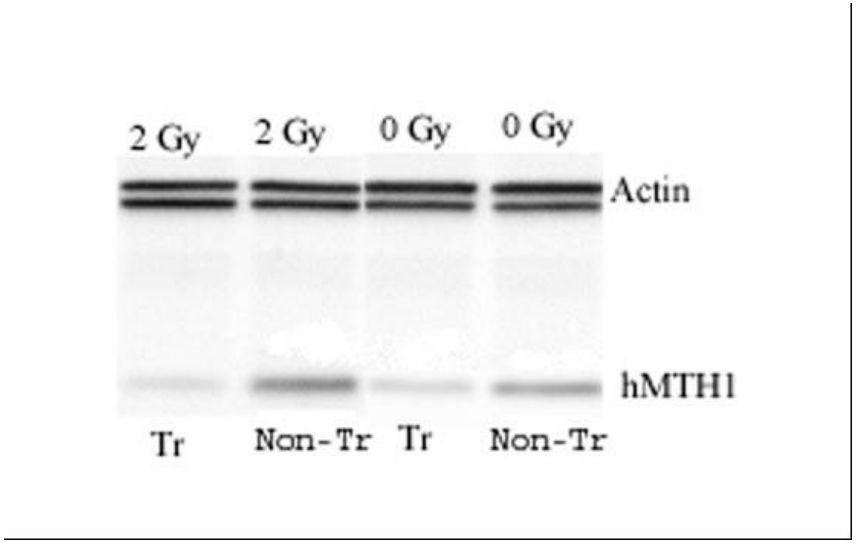
dNTP sanitization

B



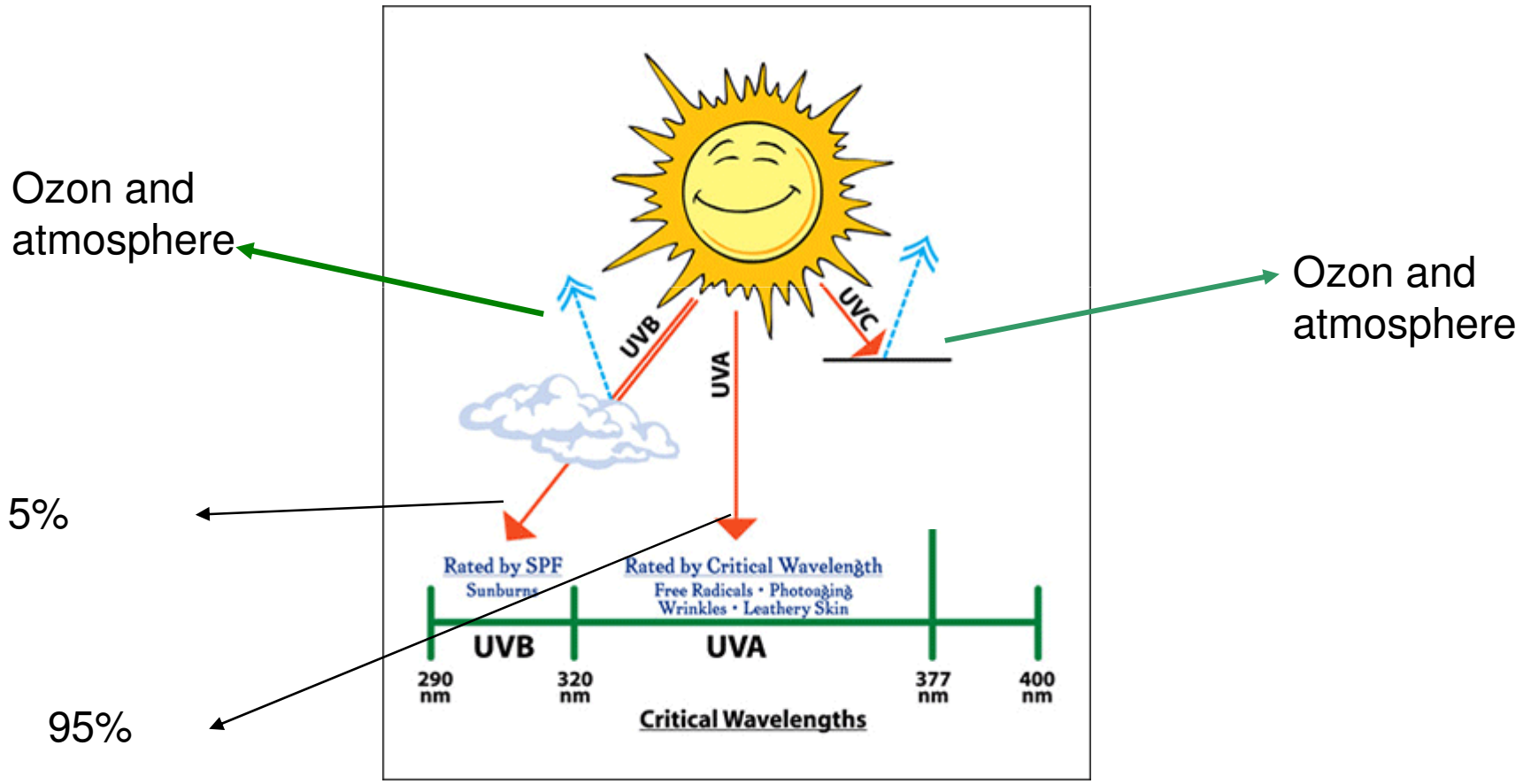
Extracellular levels of 8-oxo-dG in VH10 cells KD in MTH1





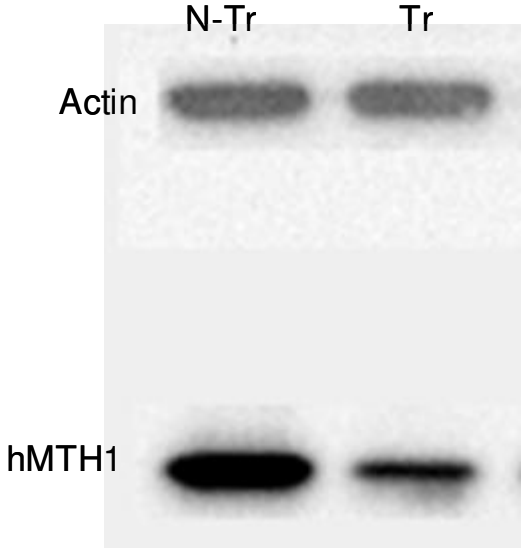
Haghdoost S. et al, Free rad. Bio. Med. 2006

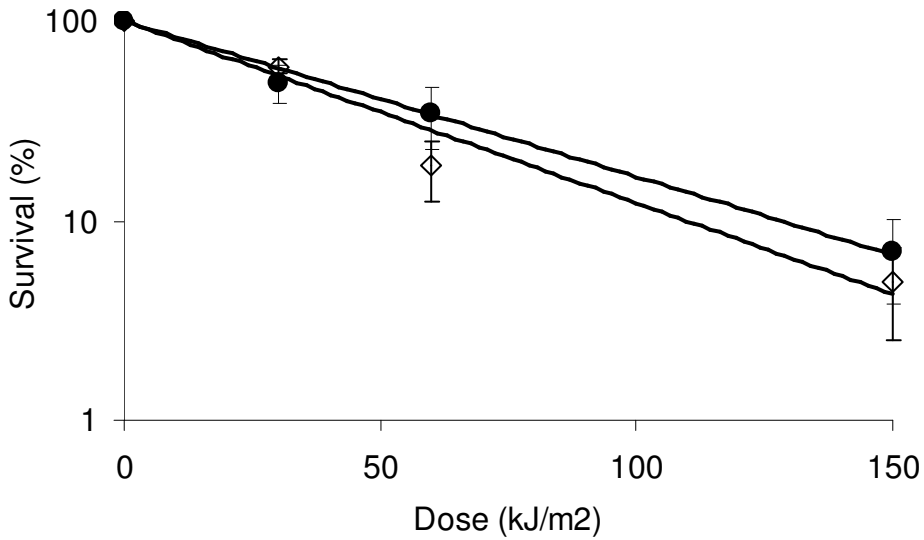
UVA and oxidative stress



www.greengoods.com

Clonogenic survival of TK6 cells KD in MTH1

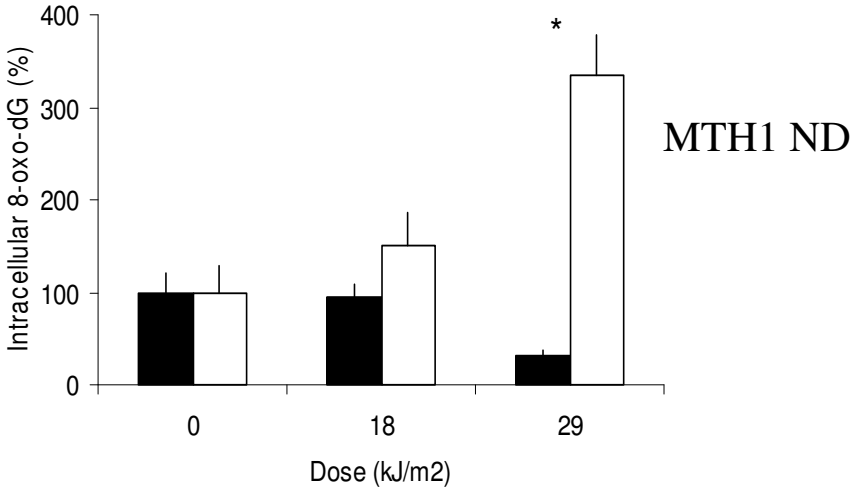




Fotouhi, et. al. Mut. Res. 2011

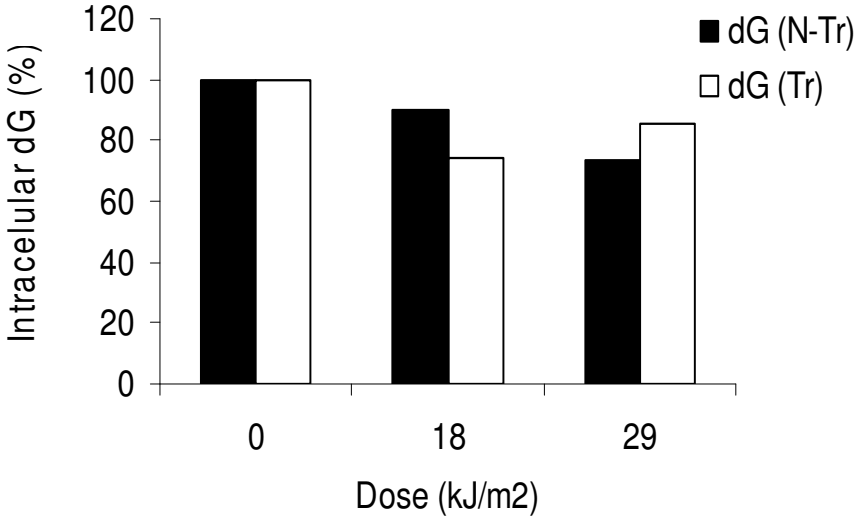
TK6 cells KD in MTH1

8-oxo-dGTP in cytoplasm

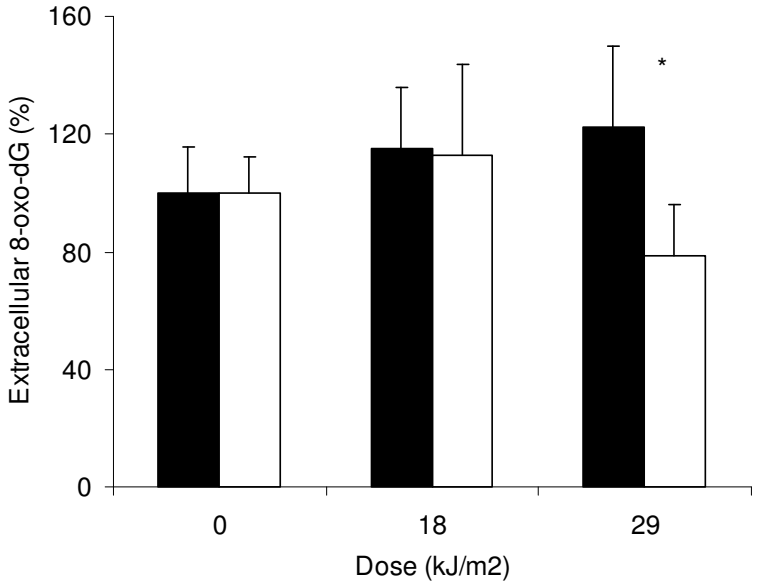


MTH1 ND

dG (TP, MP, DP) in cytoplasm



8-oxo-dG in medium



Fotouhi, et. al. Mut. Res. 2011

Mutation frequency induced by UVA in transfected and non-transfected TK6 cells

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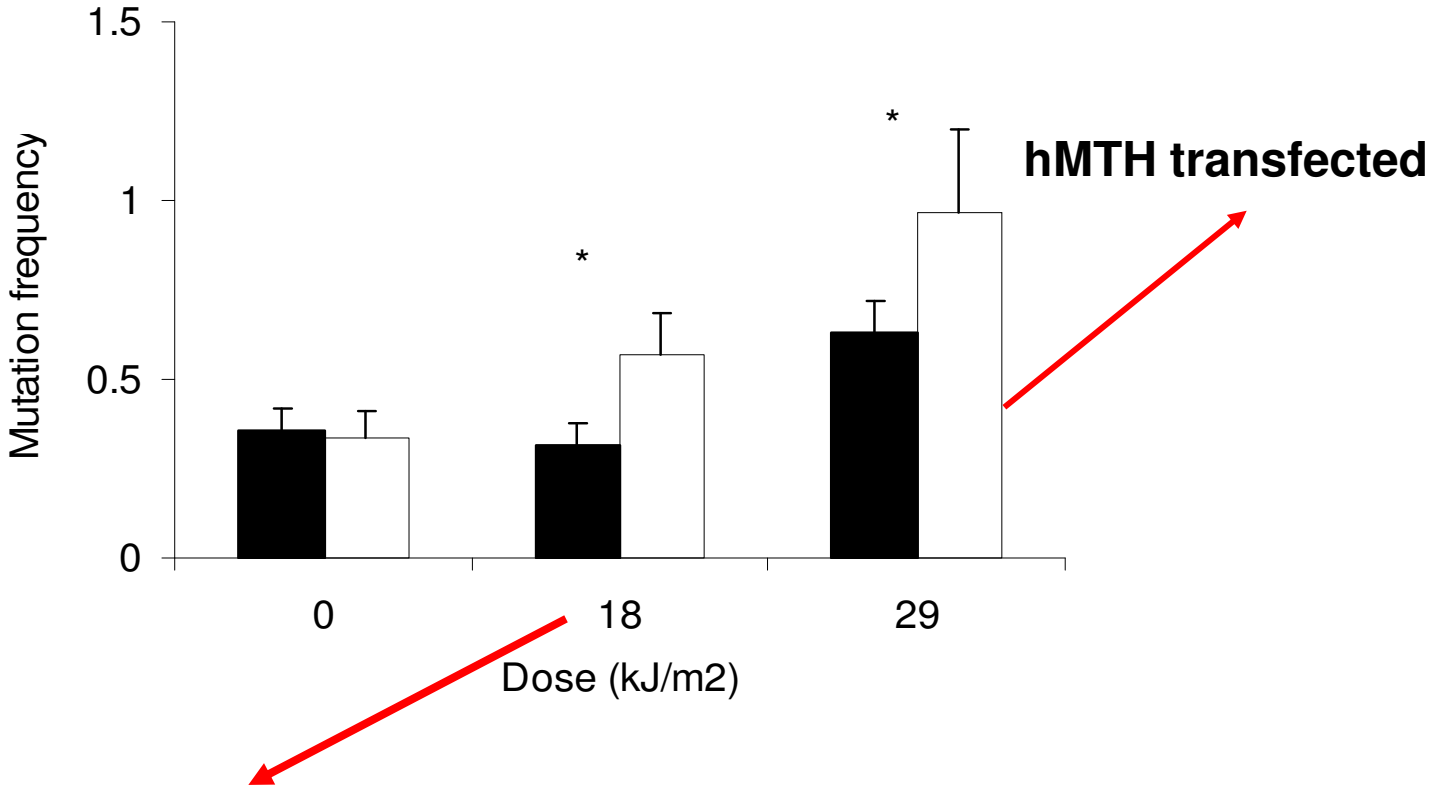
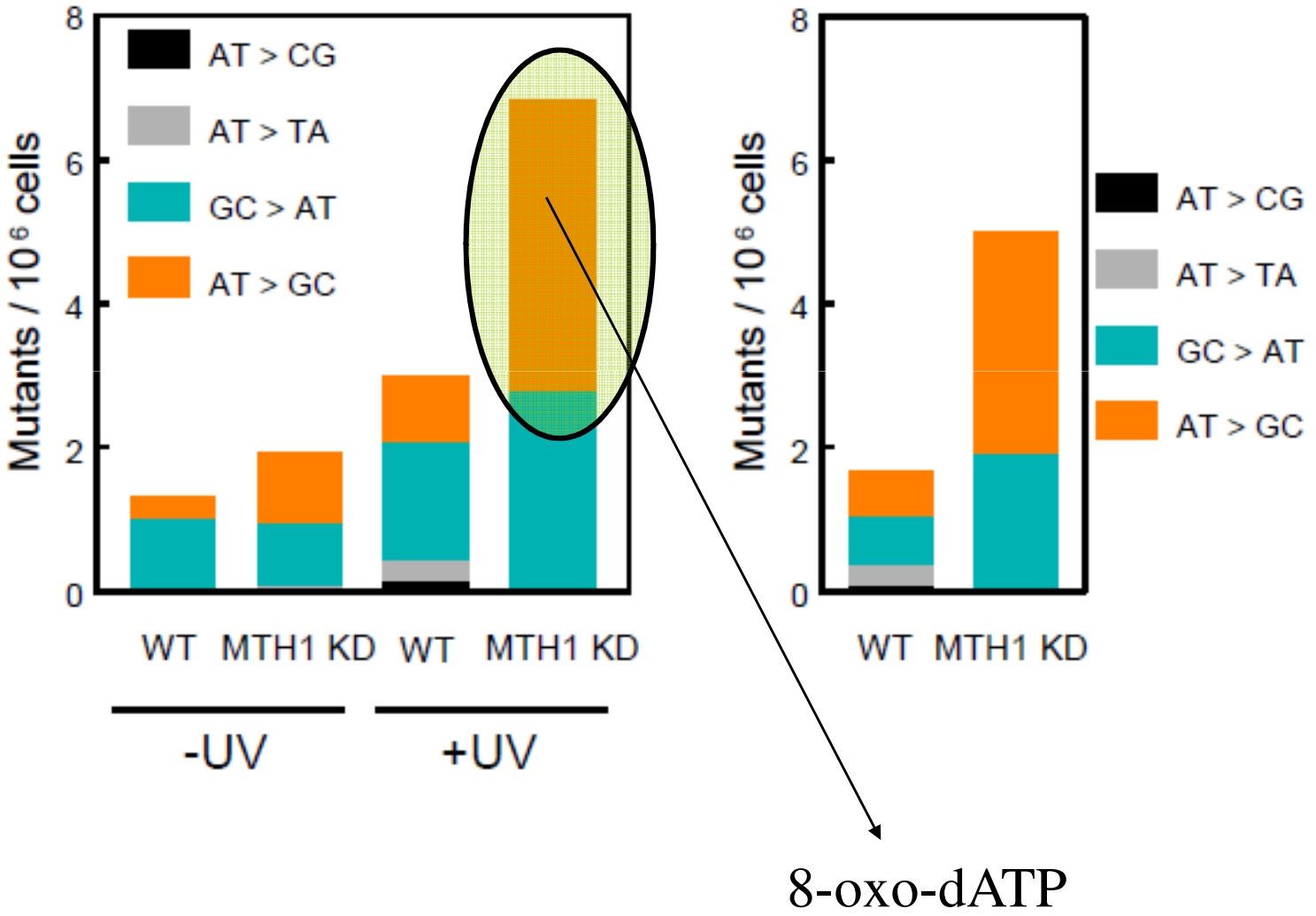
A. Fotouhi et al. Mut. Res. 2011



Equal to 1h sunshine on the French riviera at noon

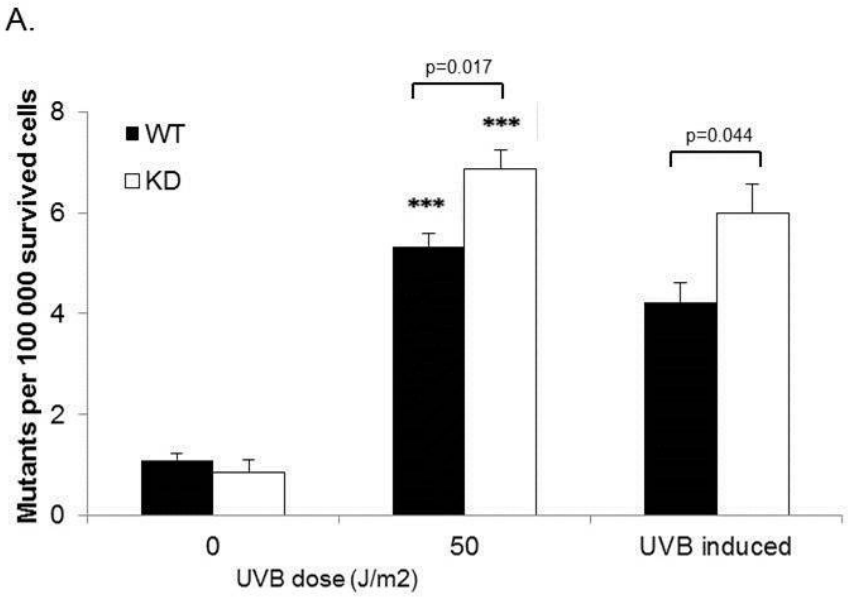
Point mutations induced by UVA



Fotouhi, A. et al, Mut. Res. 2013

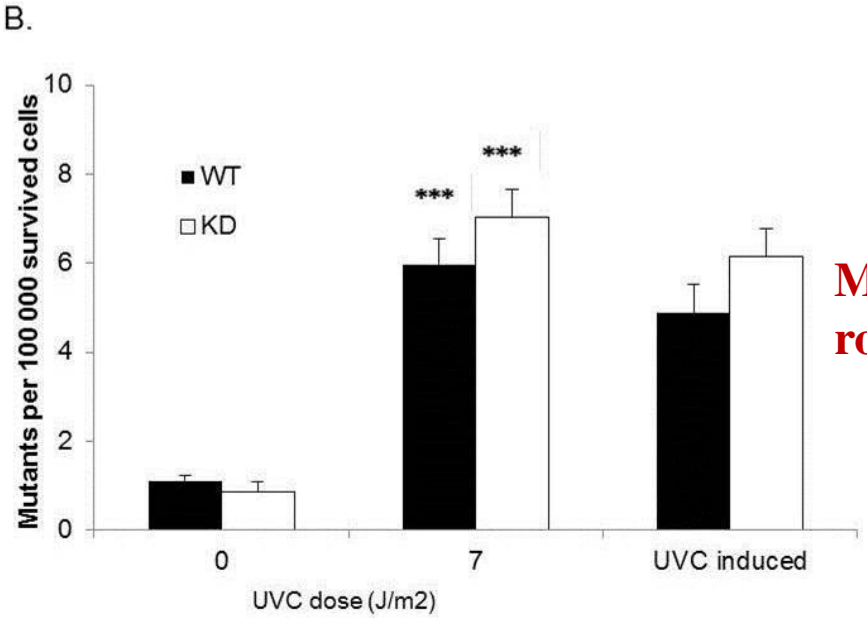
Mutation rate: the role of MTH1

UVB



LD50 dose

UVC



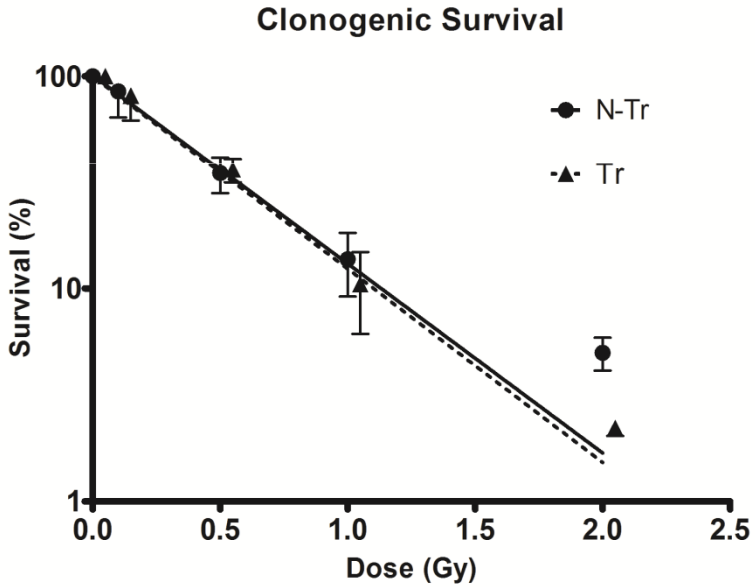
MTH1 har minor role in UVC mutagenecity

Exposure of the MTH1-transfected cells to UVA:

- MTH1 has no effect on suvival (UVA, B, C)
- High 8-oxo-dGTP in cytoplasm,
- Low 8-oxo-dG in the medium
- High mutation rate

- Exposure to Gamma radiation?

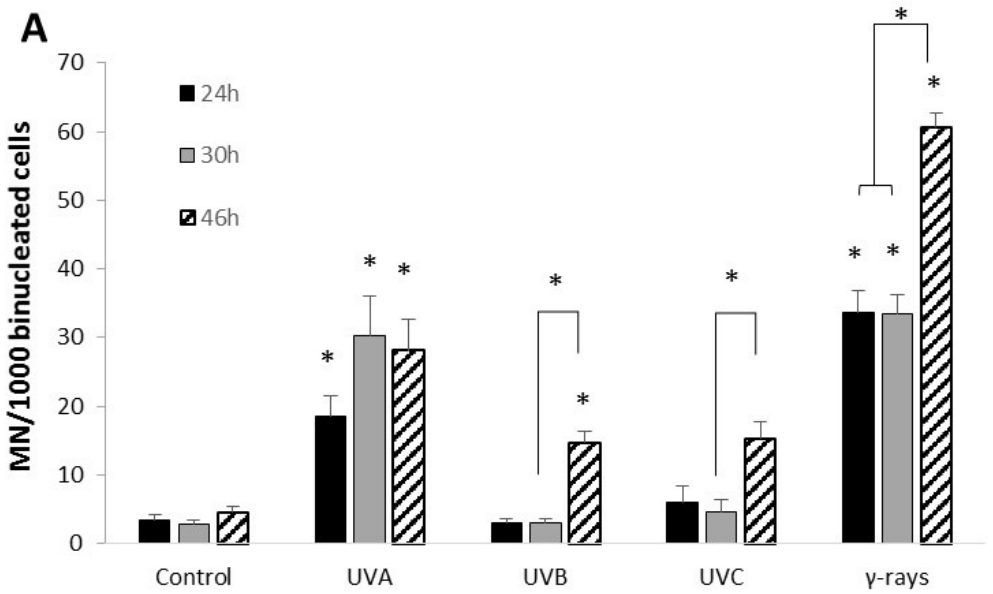
Gamma radiation



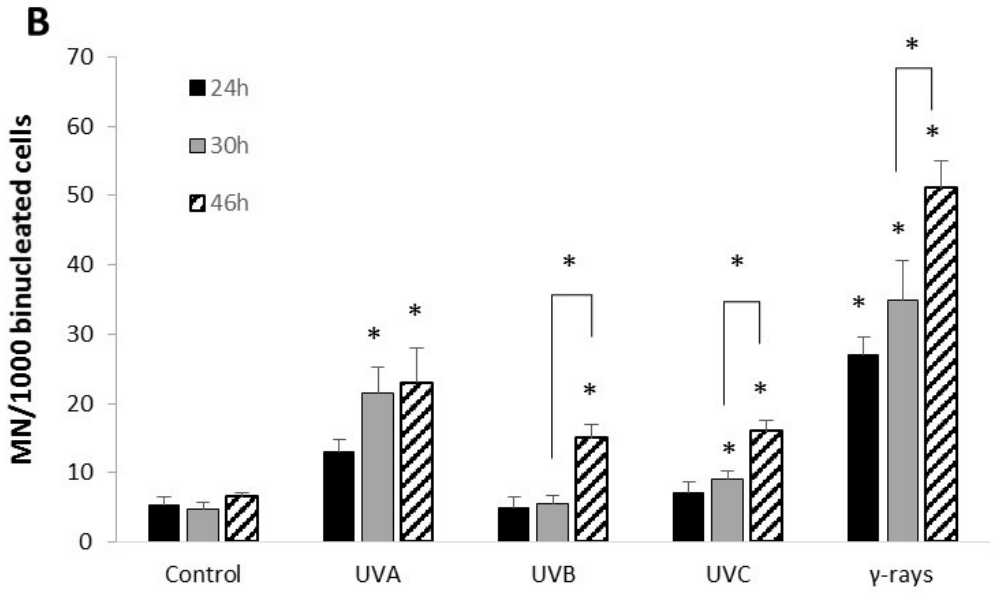
Shakeri-Manesh, S. et. al. Rad. Env. Biophys. 2014

Effect of MTH1 on micronuclei induction: UVA, B, C and gamma radiation

LD50



MTH1 +



MTH1 -

Final summary

dNTP (NTP?) is a significant mutagenic target for free radicals particularly for UVA.

MTH1 does not protect cells from radiation induced chromosomal damages

MTH1 does not influense survival of the cells exposed to UV and Gamma radiation

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Radiobiology groups at SU