Riparazione per excisione di basi (BER)

Rimuove le basi chimicamente modificate che distorcono localmente la doppia elica

Agisce su danni al DNA piuttosto limitati, quelli che si producono ogni giorno spontaneamente

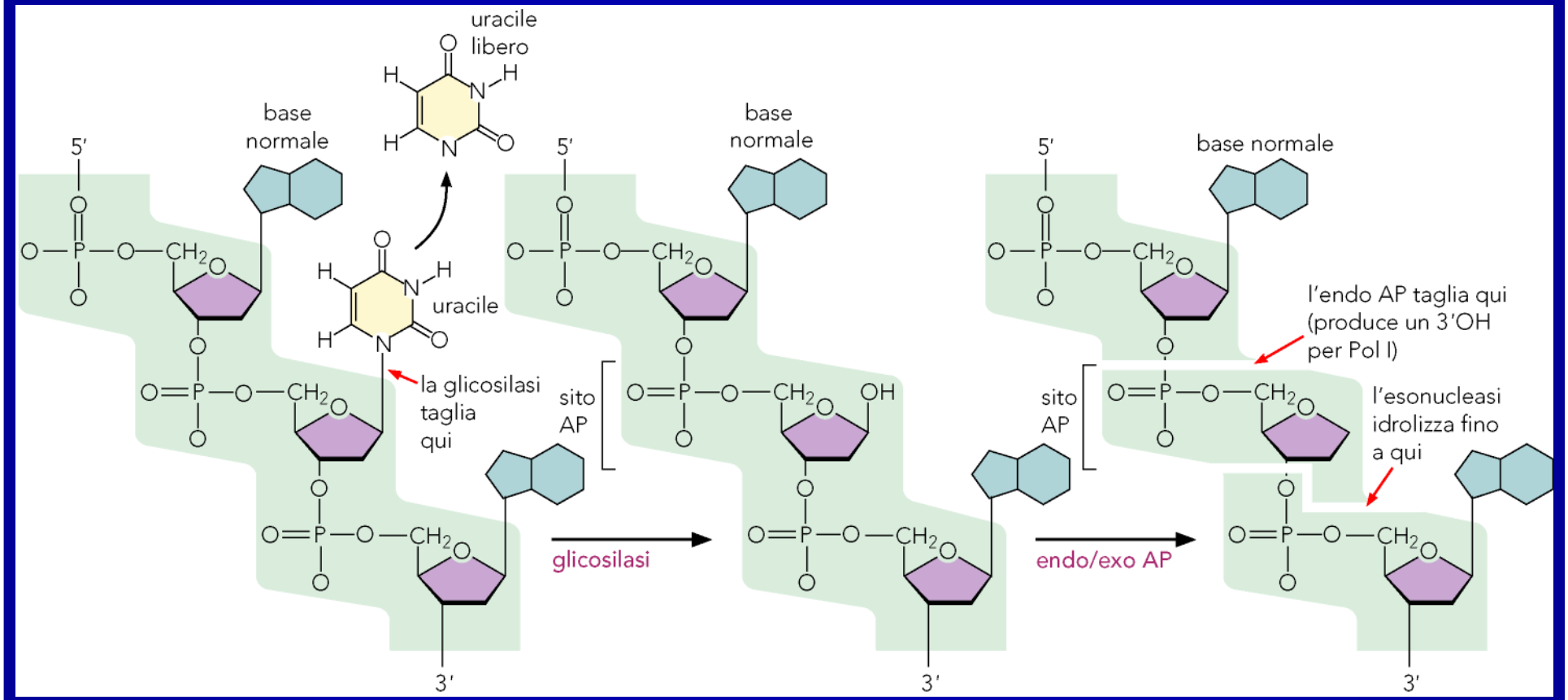
E' il meccanismo prevalente per la rimozione di lesioni che interessano il singolo filamento

- The Nobel Prize in Chemistry 2015 was awarded jointly to
- Tomas Lindahl,
- Paul Modrich and
- Aziz Sancar

"for mechanistic studies of DNA repair"

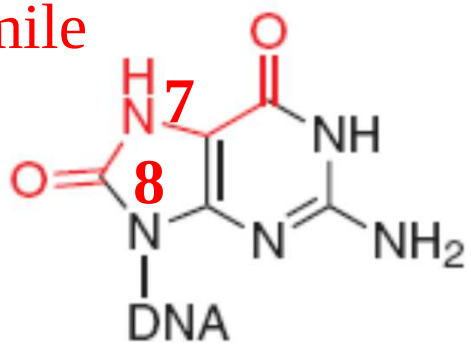
Tomas Lindahl

- In the early 1970s Tomas Lindahl demonstrated that DNA has limited chemical stability even in the absence of external physical assaults.
- Under physiological conditions DNA is subject to a number of chemical reactions such as hydrolytic deamination, oxidation and non-enzymatic methylation.
- These reactions modify the bases of DNA and as a consequence increase the risk for mutations.
- Tomas Lindahl used the term **DNA decay** to describe these processes and elegantly demonstrated that under physiological conditions, spontaneous hydrolytic DNA depurination occur at significant levels.
- The most fascinating discovery was the demonstration of high levels of spontaneous cytosine deamination under physiological conditions, which leads to the formation of uracil.
- Lindahl demonstrated that DNA is an inherently unstable molecule, subject to decay even under physiological conditions. Guided by this observation, Lindahl identified a completely new group of **DNA glycosylases** and described their role in base excision repair.

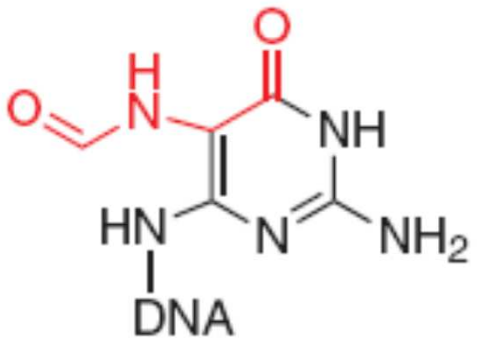

uracile
libero
base
normale
5′
uracile
la glicosilasi
taglia
qui
sito
AP
glicosilasi
base
normale
5′
OH
3′
sito
AP
endo/exo AP
base normale
5′
l'endo AP taglia qui
(produce un 3'OH
per Pol I)
l'esonucleasi
idrolizza fino
a qui
3′

BER: 8-OXOGUANINA

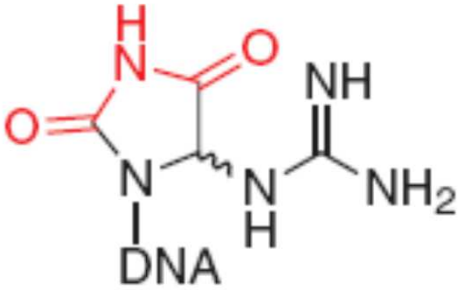
b T simile



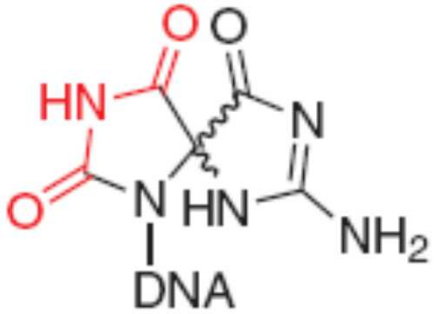
8-oxoG



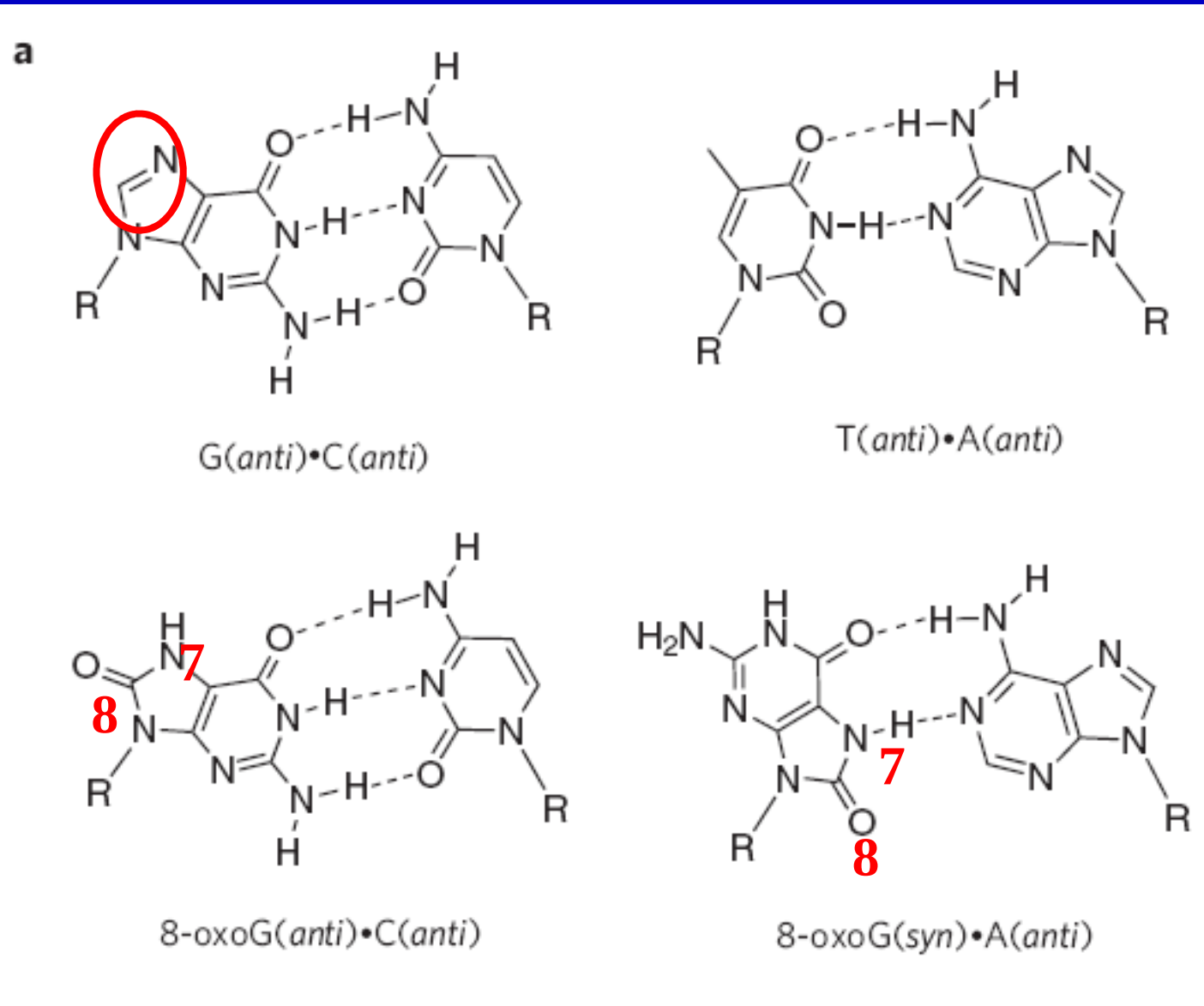
FapyG



Gh



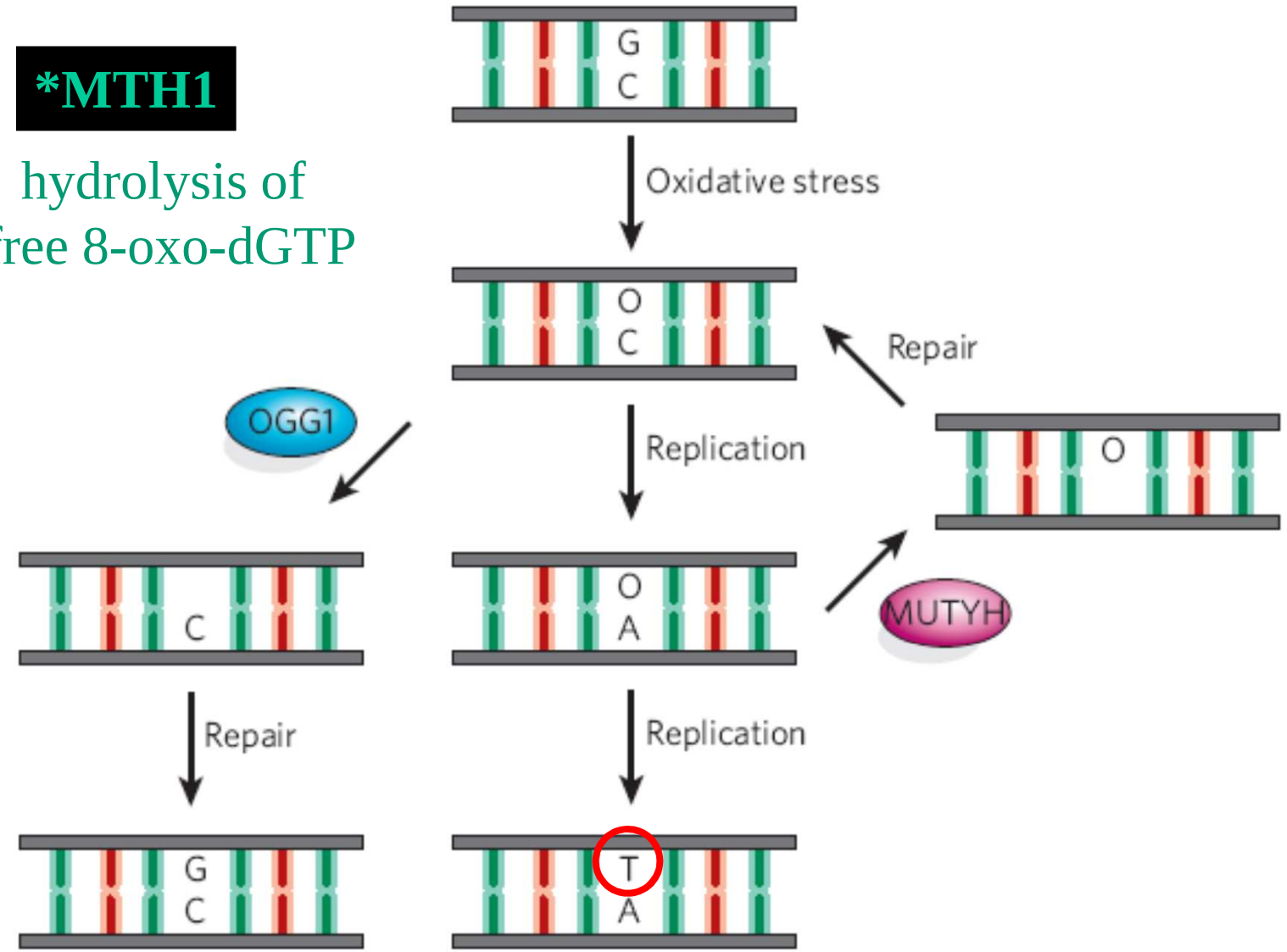
Sp

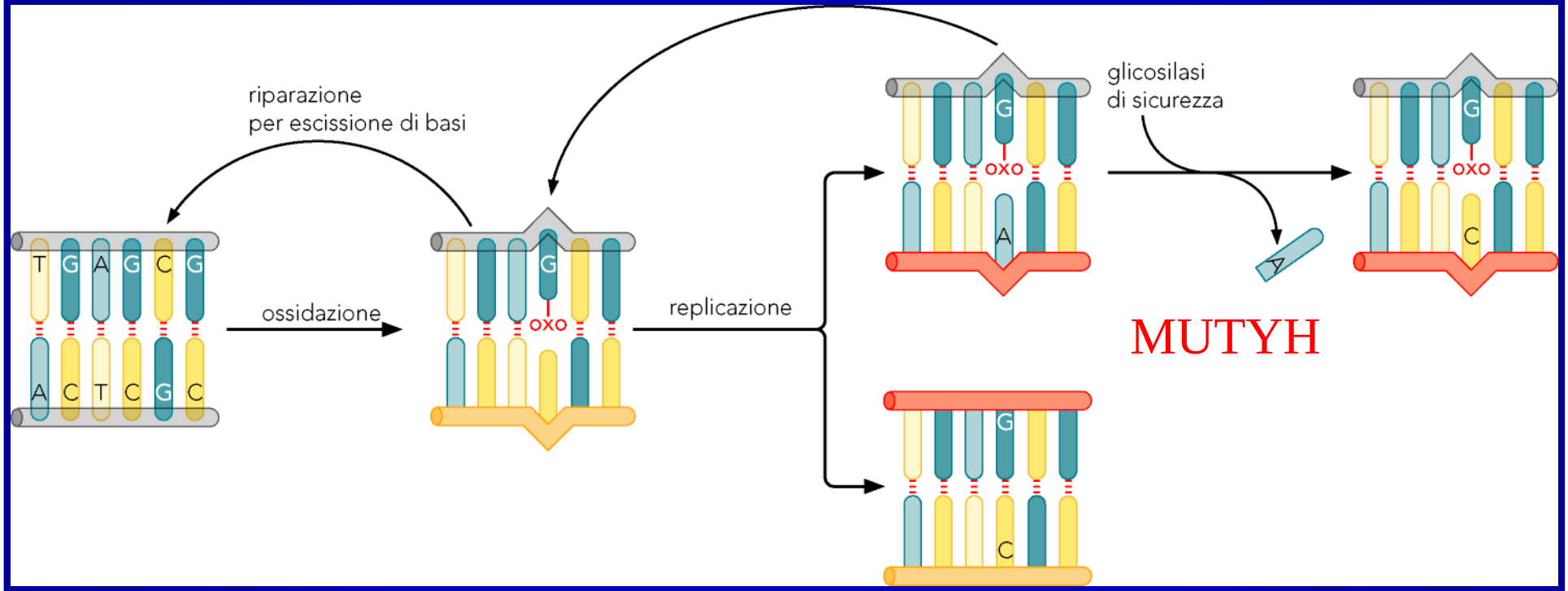

a
G(anti)•C(anti)
T(anti)•A(anti)
8
7
8-oxoG(anti)•C(anti)
7
8
8-oxoG(syn)•A(anti)

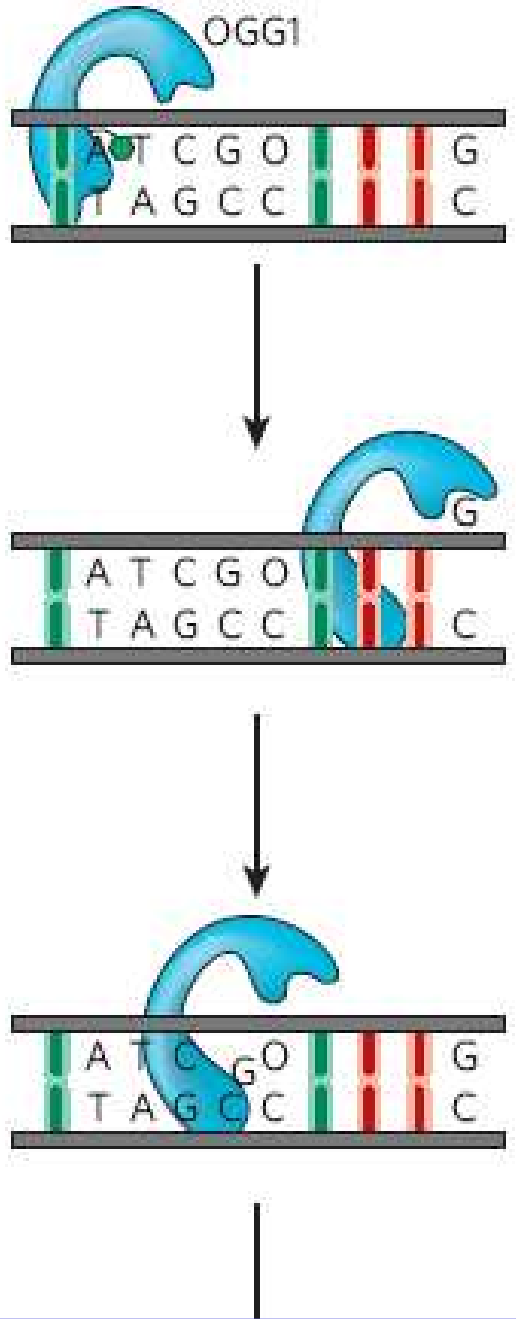
- **MutT and its human homologue MTH1 have an important role in preventing the incorporation of 8-oxoG, through hydrolysis of free 8-oxo-dGTP.**

***MTH1**

hydrolysis of
free 8-oxo-dGTP




riparazione
per escissione di basi
T G A G C G
A C T C G C
ossidazione
G
oxo
replicazione
G
oxo
A
glicosilasi
di sicurezza
A
G
oxo
C
G
C
MUTYH

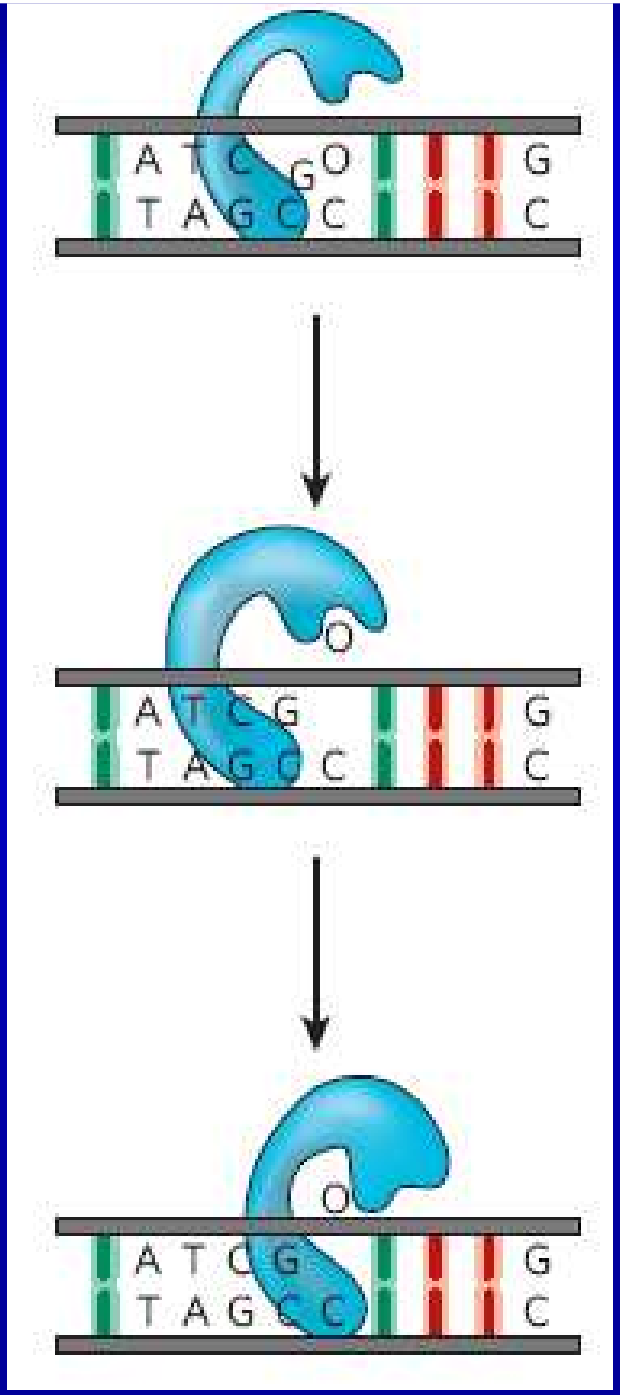


The 8-oxoG lesion search process.

- The enzyme tracks rapidly along DNA, inserting a ‘probe’ amino-acid residue (green hexagonPhe 114) at various base pairs to test the stability and/or deformability of the duplex.
- OGG1 samples millions of base pairs per second!!!!!!.

- OGG1 was found to move along the DNA with a diffusion constant approaching the theoretical upper limit for one-dimensional diffusion,indicating that OGG1 samples **millions of base pairs per second**.

- On the basis of these measurements, the estimated barrier to sliding is extremely small (0.5 kcal mol–1). The smaller barrier and the observed unbiased random movement of OGG1 on DNA suggest that OGG1 **rapidly searches along DNA as a consequence of brownian motion**.



The 8-oxoG lesion search Process (2).

- the 8-oxoG is extruded to the exosite and captured in the 8-oxoG-specific pocket, where it is excised from the DNA.

Una **DNA glicosilasi** (l'uomo ne possiede almeno 8, specifiche per varie lesioni) rompe il legame tra la base errata e il desossiriboso liberando la base

↓

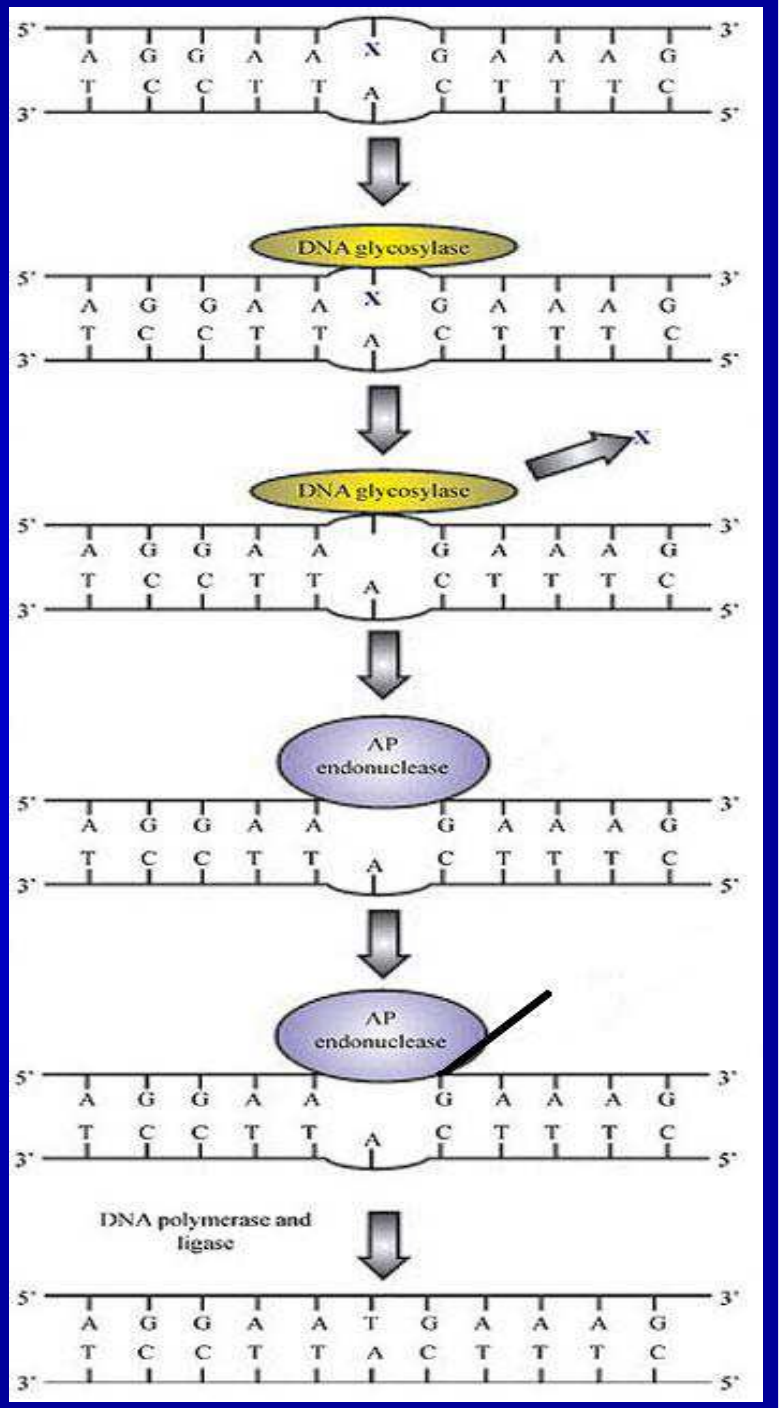
Formazione di un sito AP che viene riconosciuto da **APE1** (AP endonucleasi) → APE1 taglia il singolo filamento in 5' al sito AP

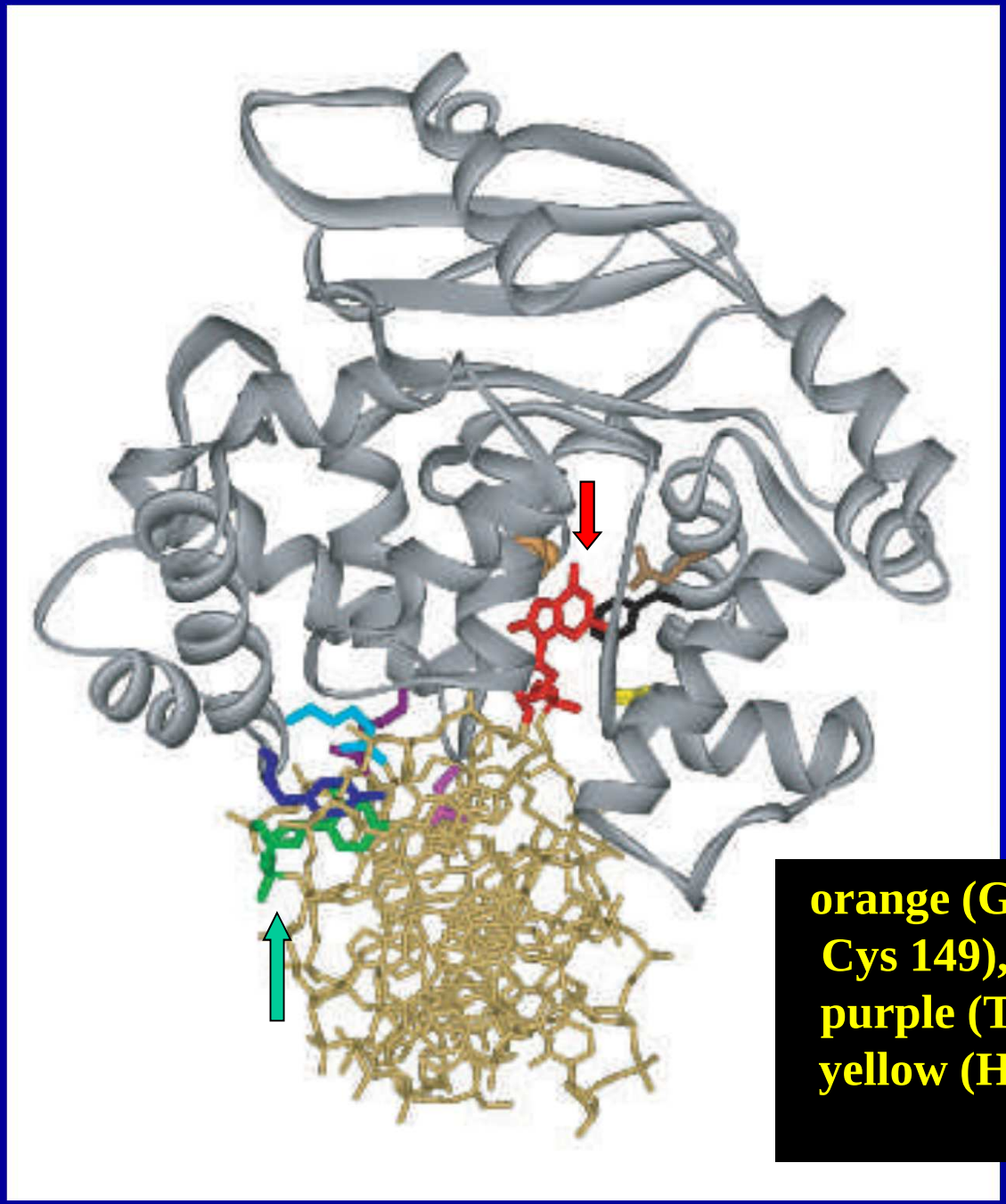
↓

La **DNA polimerasi** riempie il gap lasciato dalla glicosilasi usando come stampo l'elica parentale

↓

La **ligasi** richiude l'elica riparata

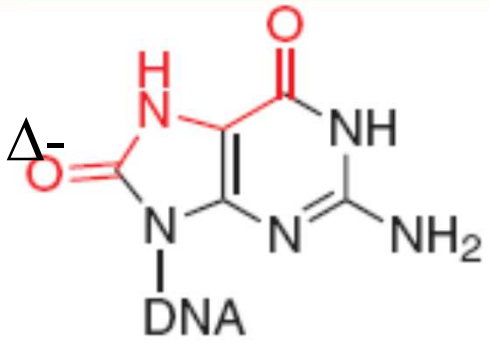




OGG1 LRC with 8-oxoG•C-containing DNA. 8-oxoG is shown in red, and the C in green.

orange (Gly 42), dark pink (Asn 149 or Cys 149), light purple (Arg 154), dark purple (Tyr 203), light blue (Arg 204), yellow (His 270), brown (Gln 315) and black (Phe 319).

lesion-recognition complexes (LRCs).



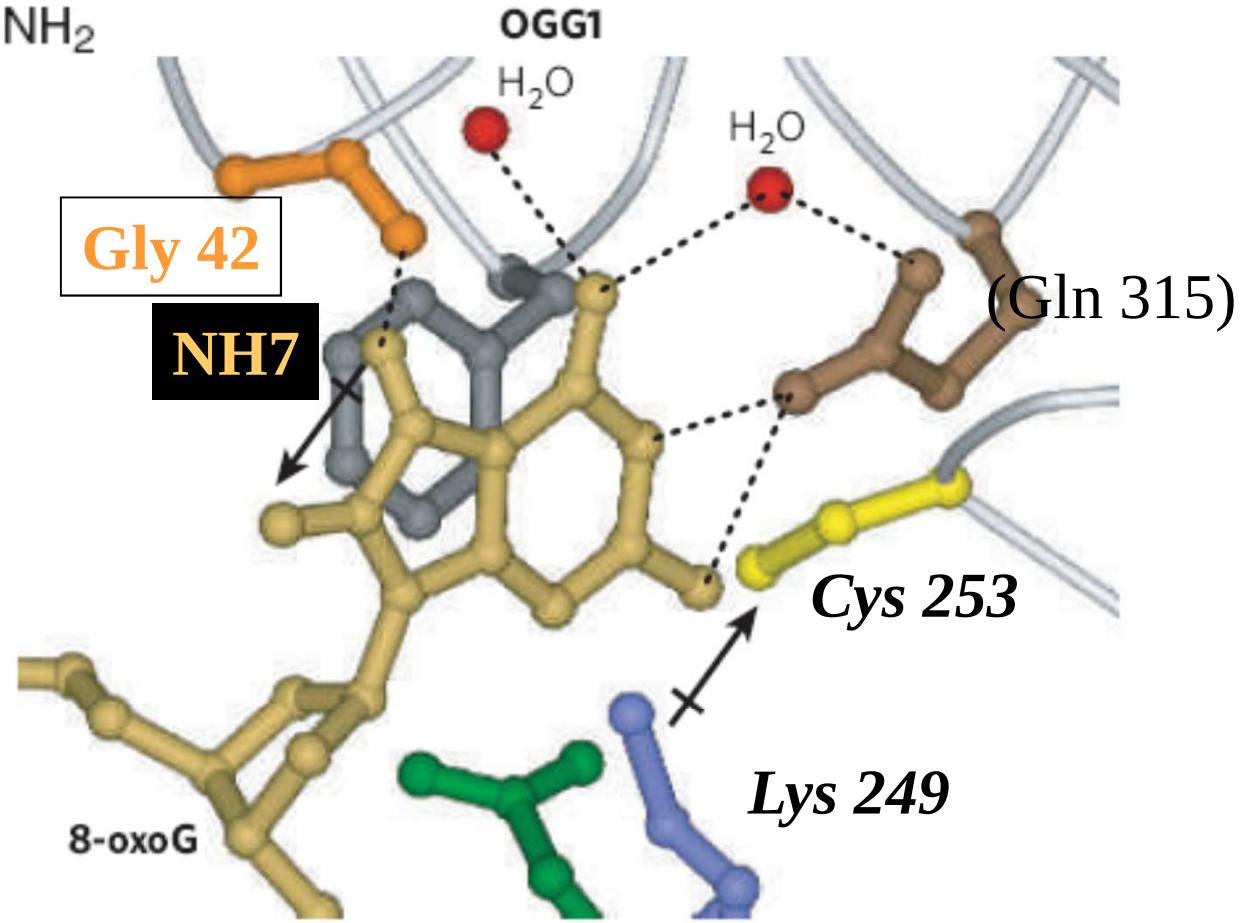
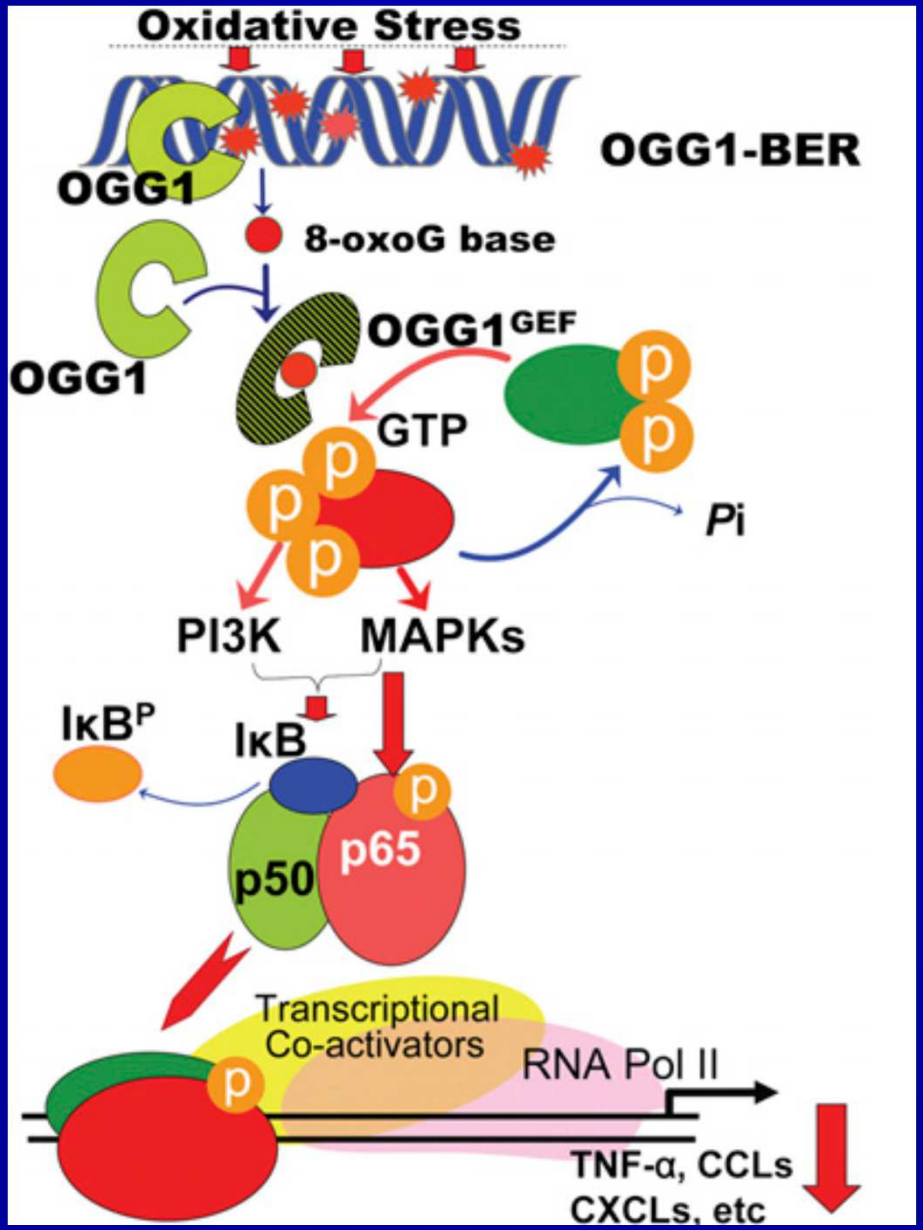


Figure 3 | Recognition of 8-oxoG by OGG1 observed in the LRC of OGG1 with 8-oxoG•C-containing duplexes. This is a view of the base-specific pocket



OGG1-initiated DNA base excision repair and **inflammation**

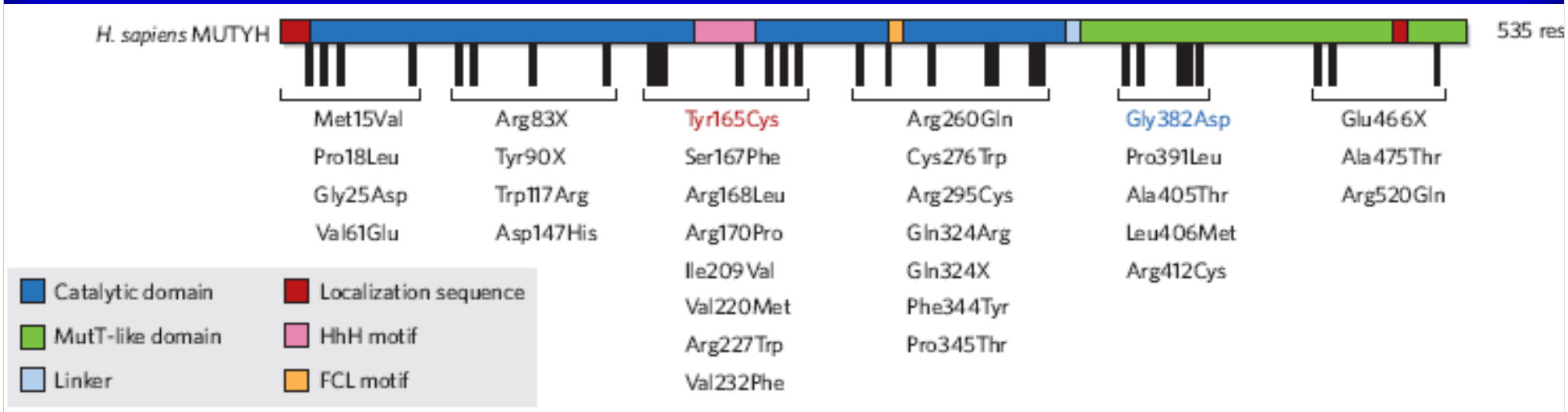
GDP→GTP exchange

RAS-GTP–driven signaling

NF-kB activation

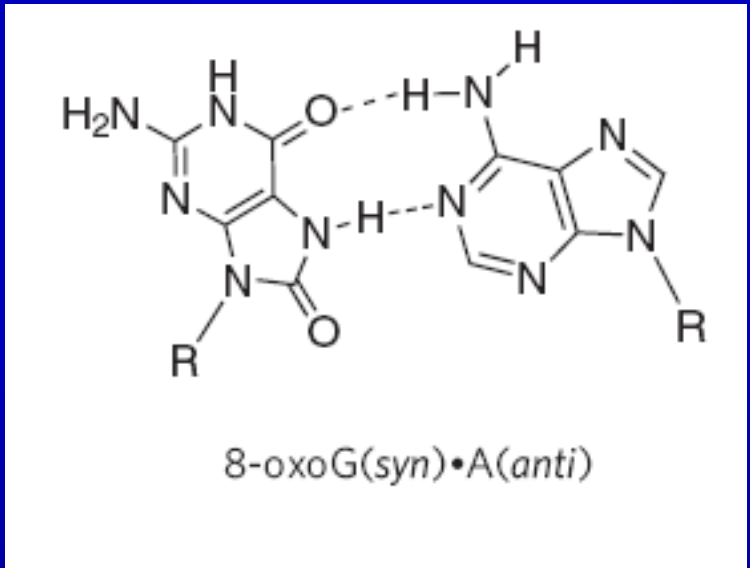
proinflammatory chemokine/cytokine expression

Germline mutations observed in *MUTYH* in individuals with polyposis



- **DNA-binding motifs: helix–hairpin–helix (HhH) motif and the Fe–S cluster loop (FCL) motif**

- Consistent with a global defect in 8-oxoG•A repair, a high proportion of tumours from patients with biallelic mutations in *MUTYH* have been observed to contain G-to-T transversions



8-oxoG(*syn*)•A(*anti*)

Uomini e Topi….

- mice that are deficient only in MUTYH do not show any atypical properties
- However, crossing MUTYH-deficient mice with multiple intestinal neoplasia (*Apc*Min/+) mice, which carry a nonsense mutation in *Apc*, resulted in greater intestinal tumorigenesis than in *Ap*cMin/+/*Mutyh*+/+ mice.

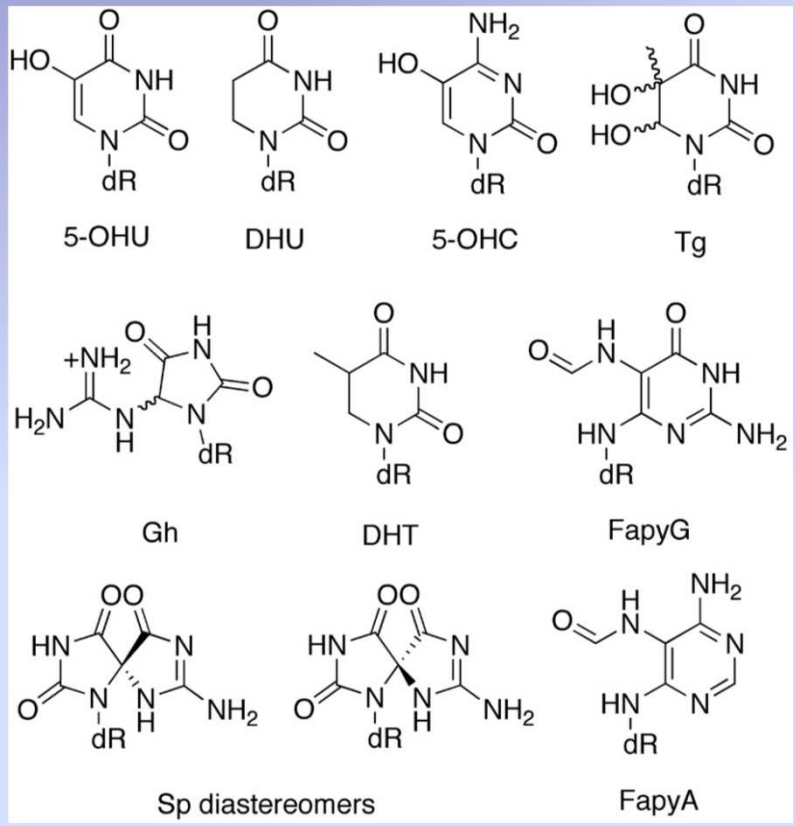
neil1

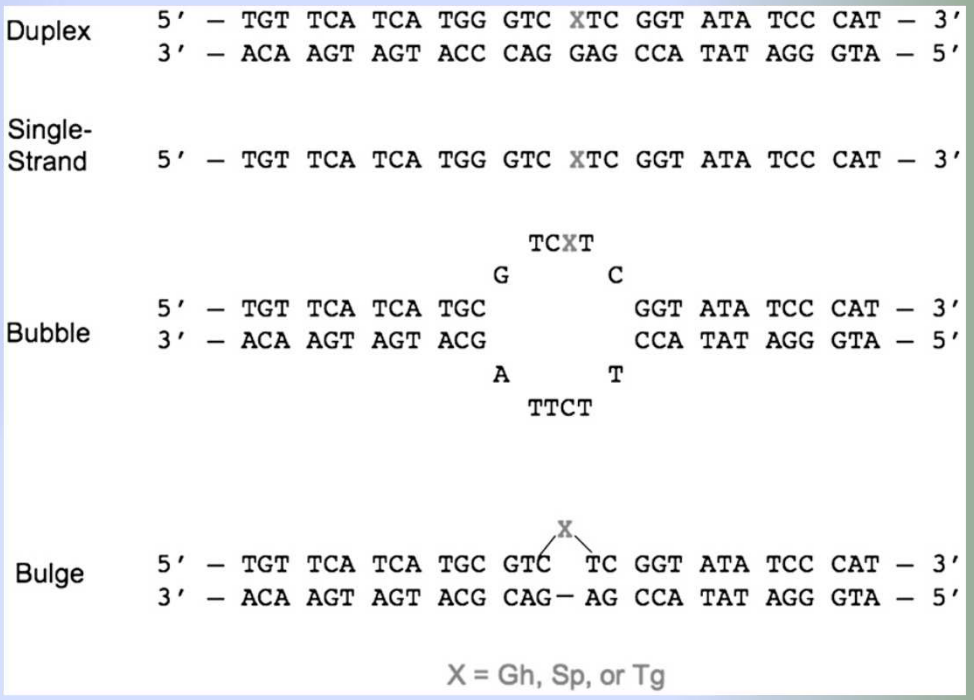
- RNA editing changes the lesion specificity for the DNA repair enzyme NEIL1

Whole transcriptome sequence analysis from various human tissues identified over 200 possible A to I editing sites in non repeat sequences, including a site predicted to cause recoding in the mRNA for the DNA repair enzyme NEIL1 (lysine 242 AAA codon edited to AIA codon for arginine)

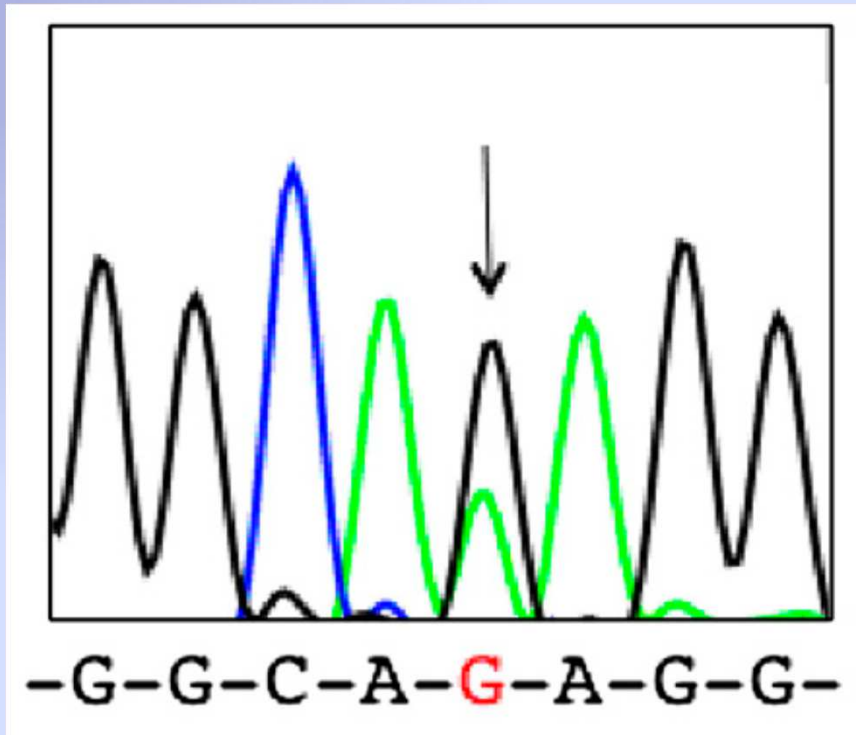
NEIL1 plays a key role in the initiation of base excision repair of oxidized base lesions by catalyzing the cleavage of the N-glycosidic linkage to the 2'-deoxyribose

Known substrates for the base excision repair glycosylase NEIL1.





NEIL1 mRNA sequencing



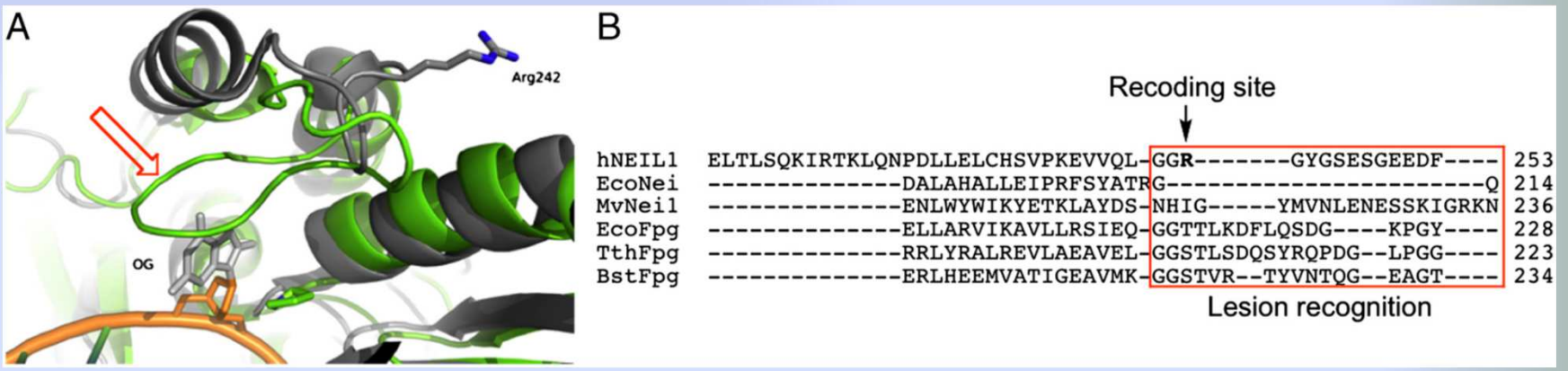
mRNA Editing

A to G

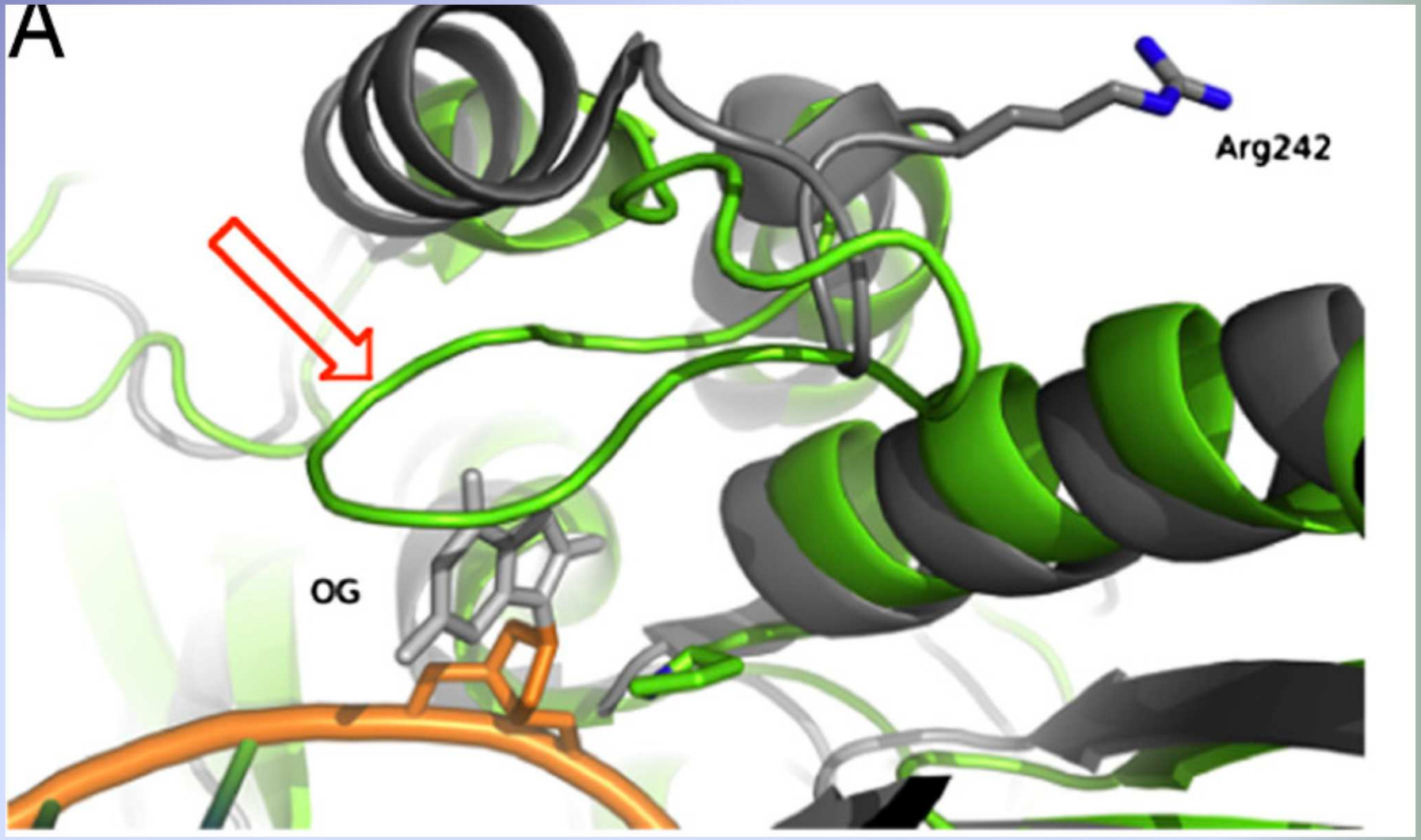
AAA to AIA (AGA)

R to K

(A) Superposition of human NEIL1 structure (dark gray) with that of E. coli Fpg (green) bound to 8-oxoguanine-containing DNA. Red open arrow indicates lesion recognition loop of Fpg.
(B)B) Sequence alignment of Fpg/Nei family of DNA repair glycosylases indicating the position of the hNEIL1 recoding site and lesion recognition loop .

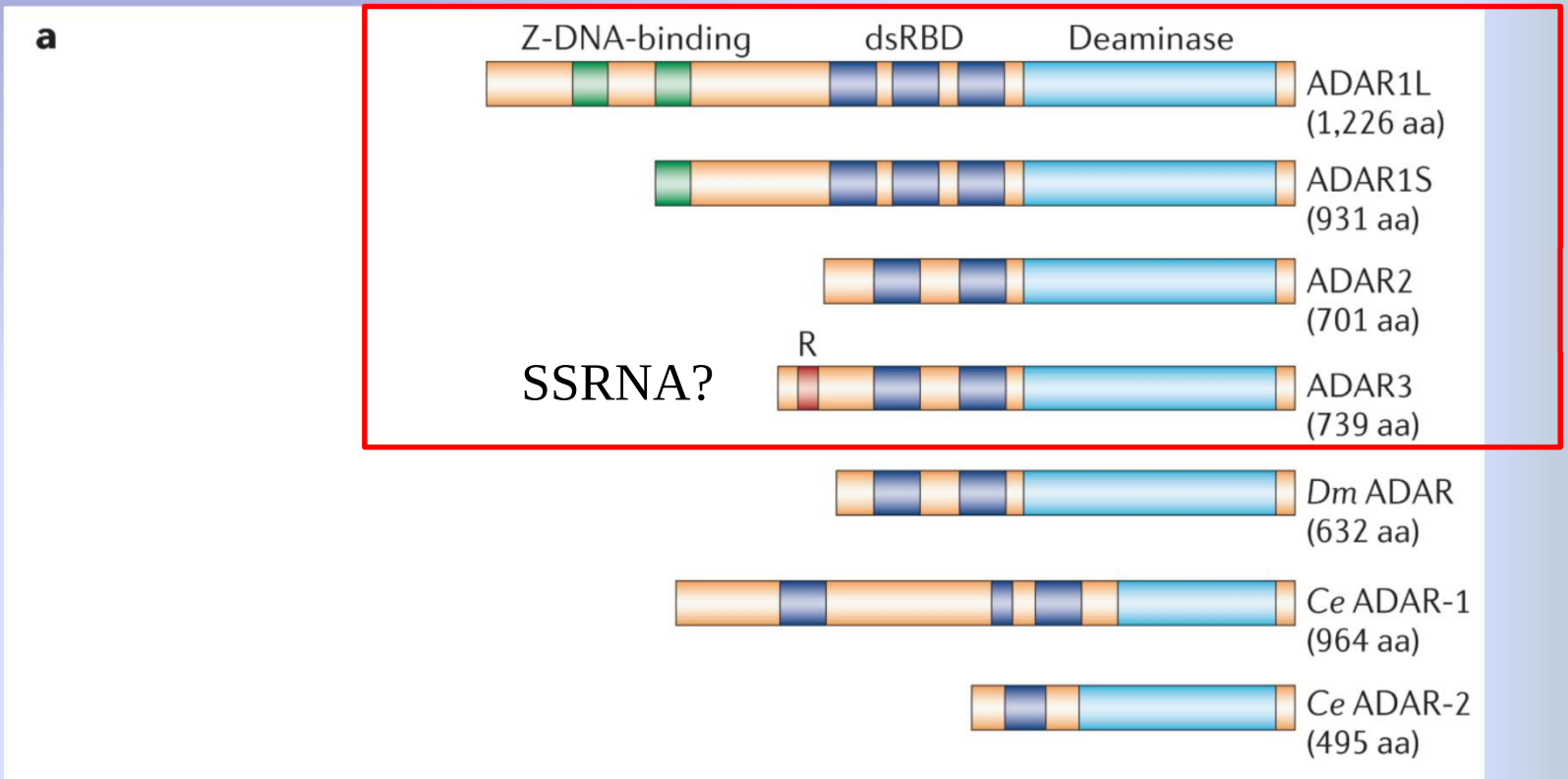


Editing of the pre-mRNA for the DNA repair enzyme NEIL1 causes
a lysine to arginine change in the lesion recognition loop of the protein.



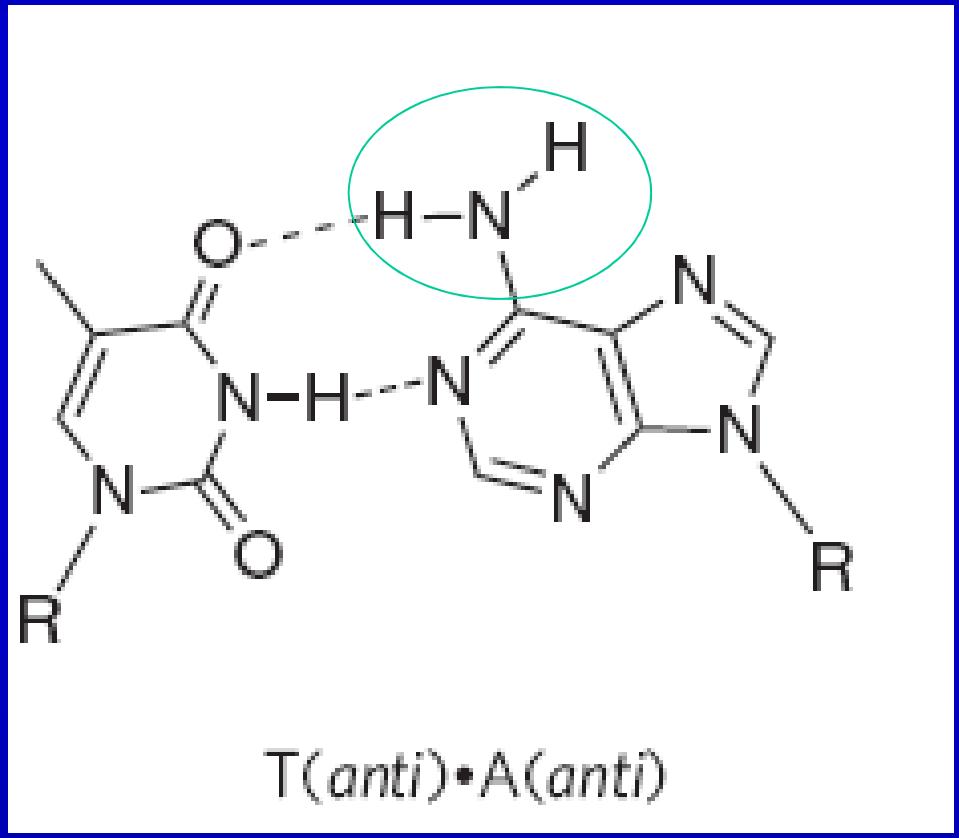
Superposition of human NEIL1 structure (dark gray) with that of E. coli Fpg (green) bound to 8-oxoguanine-containing DNA.

Three human ADAR (adenosine deaminase acting on RNA)-family members



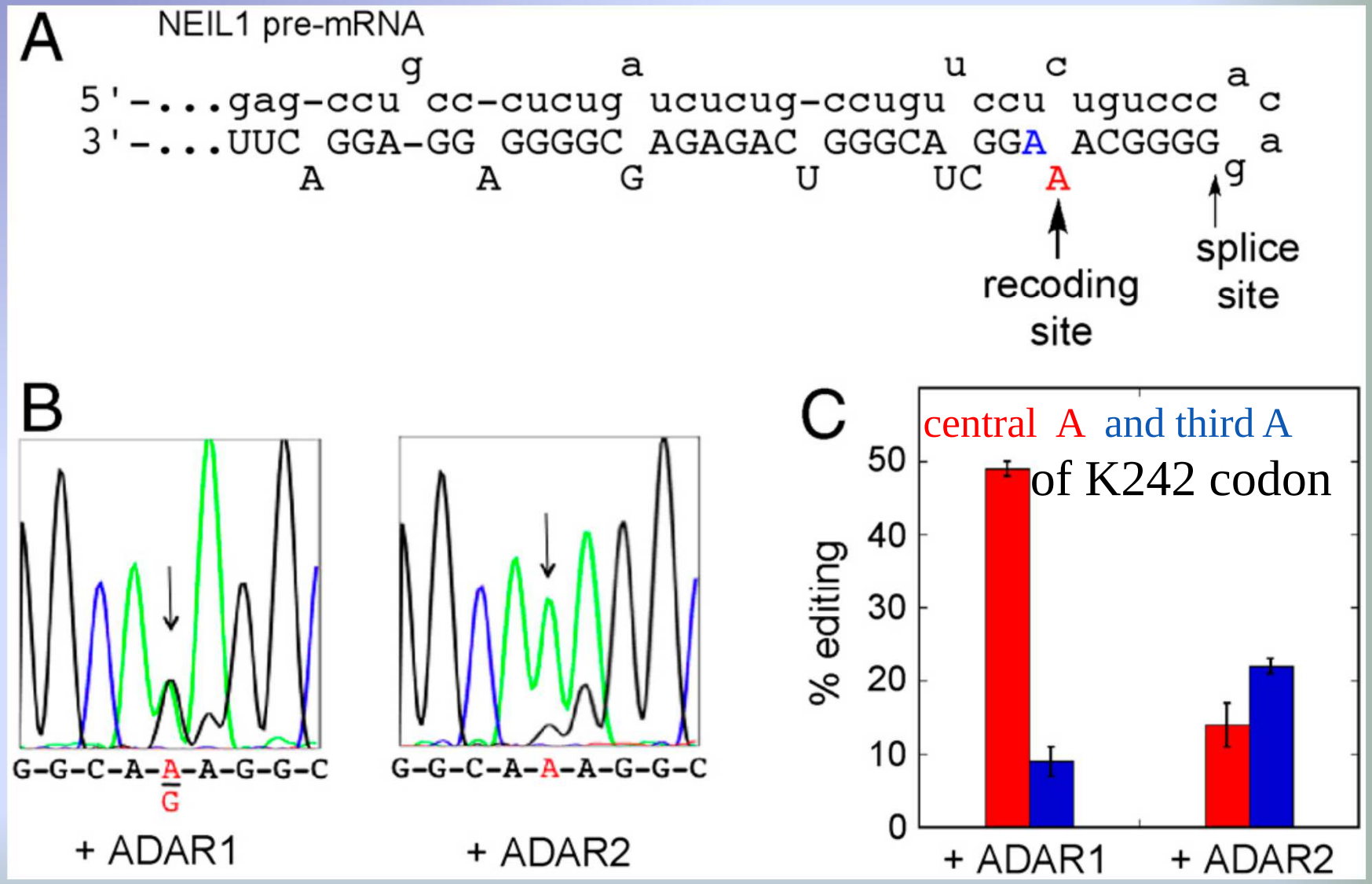
ADAR1L is detected mainly in the cytoplasm, whereas ADAR1S localizes in the nucleoplasmand nucleolus

ADAR2 localizes predominantly in the nucleolus



T(*anti*)•A(*anti*)

In vitro editing: Sequence of products from reaction of 1 μM human ADAR

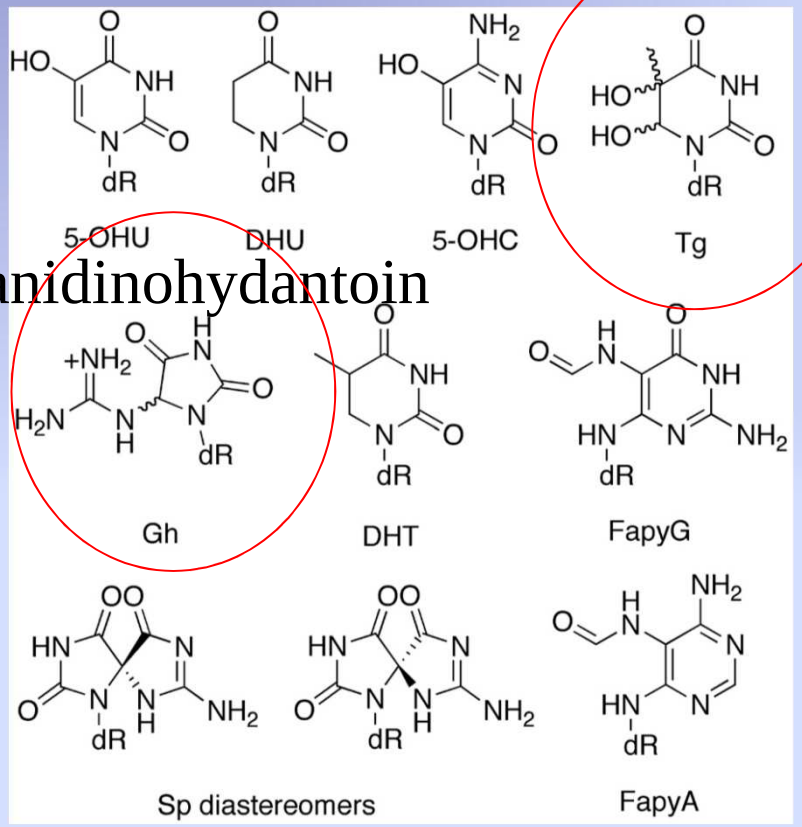


Editing of the pre-mRNA for the DNA repair enzyme NEIL1 causes a lysine to arginine change in the lesion recognition loop of the protein.

Known substrates for the base excision repair glycosylase NEIL1.

thymine glycol

guanidinohydantoin



Duplex	5′ – TGT TCA TCA TGG GTC XTC GGT ATA TCC CAT – 3′ 3′ – ACA AGT AGT ACC CAG GAG CCA TAT AGG GTA – 5′
Single-Strand	5′ – TGT TCA TCA TGG GTC XTC GGT ATA TCC CAT – 3′
Bubble	TCXT G C 5′ – TGT TCA TCA TGC GGT ATA TCC CAT – 3′ 3′ – ACA AGT AGT ACG CCA TAT AGG GTA – 5′ A T TTCT
Bulge	X 5′ – TGT TCA TCA TGC GTC TC GGT ATA TCC CAT – 3′ 3′ – ACA AGT AGT ACG CAG – AG CCA TAT AGG GTA – 5′

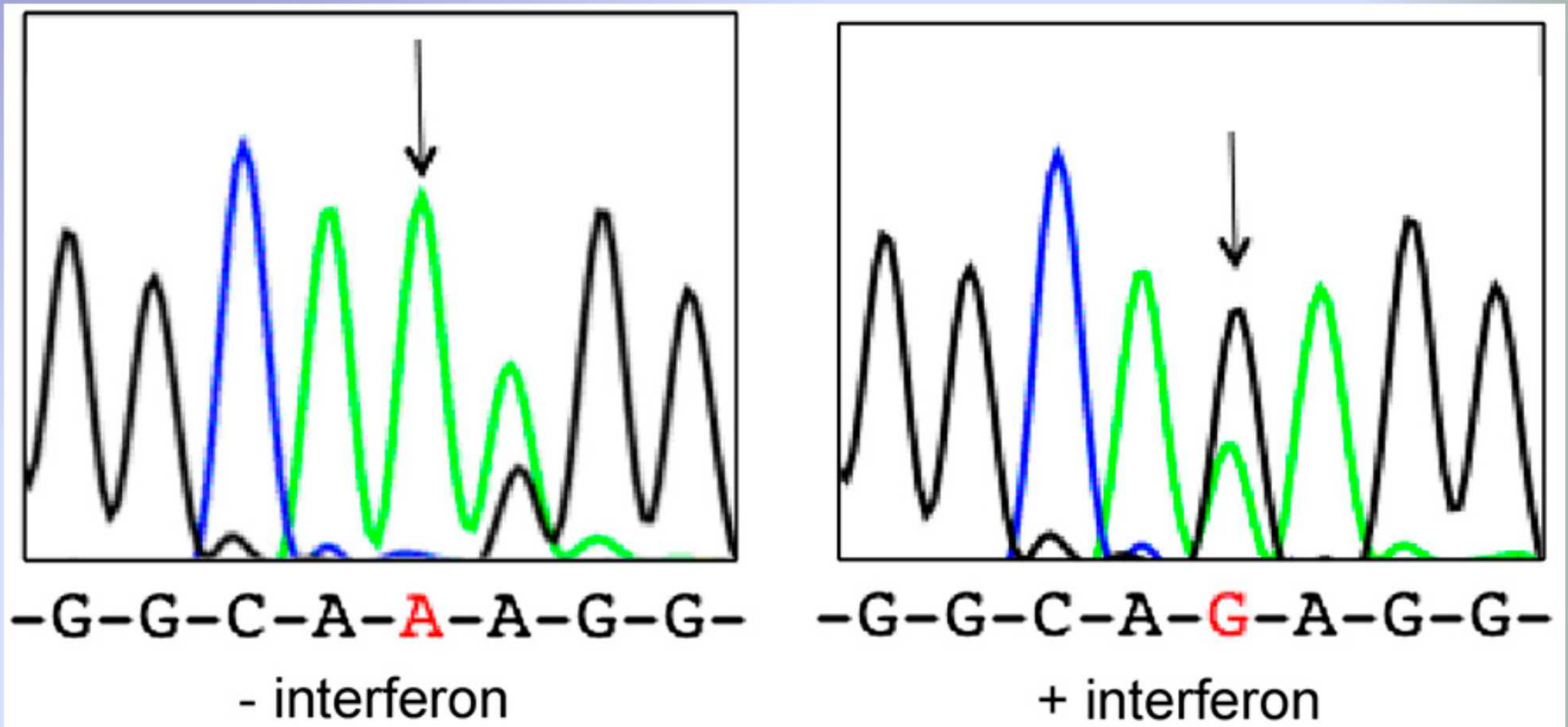
X = Gh, Sp, or Tg

Table 1. Rate constants (k_g)* of base removal by edited versus unedited NEIL1

	Tg†			Gh‡		
Context§	Unedited	Edited	Ratio¶	Unedited	Edited	Ratio
Duplex (X: G)	76 ± 10	2.5 ± 0.1	30	130 ± 20	370 ± 40	0.4
Single strand	0.6 ± 0.1	0.02 ± 0.01	30	1.2 ± 0.1	2.4 ± 0.6	0.5
Bulge	1.4 ± 0.1	0.04 ± 0.02	35	5.0 ± 0.6	13 ± 1	0.4
Bubble	1.2 ± 0.1	0.06 ± 0.02	20	30 ± 6	94 ± 8	0.3

*Rate constants in min^{-1} measured under single-turnover conditions (20 nM substrate, 200 nM enzyme) at 37 °C. Reactions wi
go to completion; slow reactions rates were determined based on initial rate rather than complete fitting of the progres
†Tg paired with G. Rate constants in the same duplex paired with A for edited and unedited NEIL1 are 1.3 ± 0.1 min^{-1}, and 53
ratio is 40.

- The two forms of NEIL1 have distinct enzymatic properties.
- The edited form removes thymine glycol Tg from duplex DNA 30 times more slowly than the form encoded in the genome,
- whereas editing enhances repair of the guanidinohydantoin Gh lesion by NEIL1.



NEIL1 editing in response to IFN-α.
(Left) Sequence at the recoding site in NEIL1 cDNA from U87 human glioblastoma cells cultured in the absence of IFN-α.
(Right) NEIL1 cDNA sequence from U87 cells treated with IFN-α.

Sommario Neil 1

- ADAR1-catalyzed editing of the NEIL1 mRNA causes the genomically encoded AAA lysine codon, corresponding to amino acid position 242 in the lesion recognition loop of the protein, to be converted to a codon for arginine.
- The two forms of the NEIL1 protein (edited and unedited) have distinct enzymatic properties with changes observed for both glycosylase activity and lesion specificity.
- Editing occurs in a hairpin duplex structure formed near the intron 5/exon 6 boundary in the NEIL1pre-mRNA.
- Furthermore, NEIL1 mRNA recoding is regulated extracellularly by interferon, as predicted for an ADAR1-catalyzed reaction.
- These results suggest a regulatory mechanism for DNA repair based on RNA editing.

Deciphering the functions and regulation of brain-enriched A-to-I RNA editing Nat Neurosci. 2013.

- Adenosine-to-inosine (A-to-I) RNA editing, in which genomically encoded adenosine is changed to inosine in RNA, is catalyzed by adenosine deaminase acting on RNA (ADAR).
- This fine-tuning mechanism is critical during normal development and diseases, particularly in relation to brain functions.
- A large number of RNA editing sites have recently been identified as a result of the development of deep sequencing and bioinformatic analyses.
- Deciphering the functional consequences of RNA editing events is challenging.