Il mantenimento dell'integrità del messaggio genetico è di fondamentale importanza per la vita

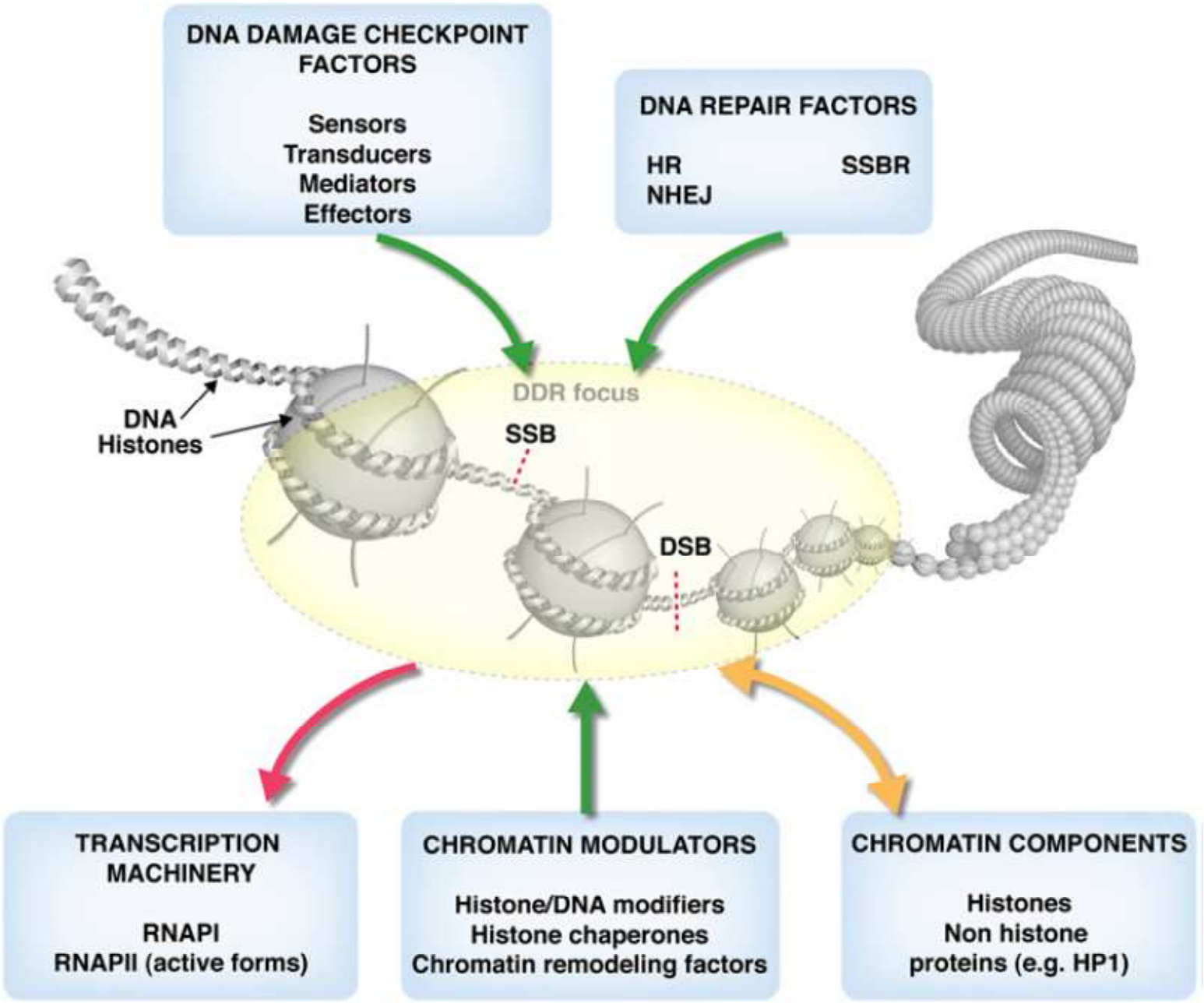
Fattori che possono alterare il DNA:

- **Errori di replicazione**
- **Instabilità del DNA (deaminazione, depirimidinazione, depurinazione)**
- **Specie reattive dell'ossigeno**

ENDOGENI

- **Trattamenti (radio- chemioterapia)**
- **Ambientali (agenti chimici, raggi UV)**

ESOGENI


DNA DAMAGE CHECKPOINT FACTORS
Sensors
Transducers
Mediators
Effectors
DNA REPAIR FACTORS
HR
SSBR
NHEJ
DNA
Histones
DDR focus
SSB
DSB
TRANSCRIPTION MACHINERY
RNAPI
RNAPII (active forms)
CHROMATIN MODULATORS
Histone/DNA modifiers
Histone chaperones
Chromatin remodeling factors
CHROMATIN COMPONENTS
Histones
Non histone proteins (e.g. HP1)

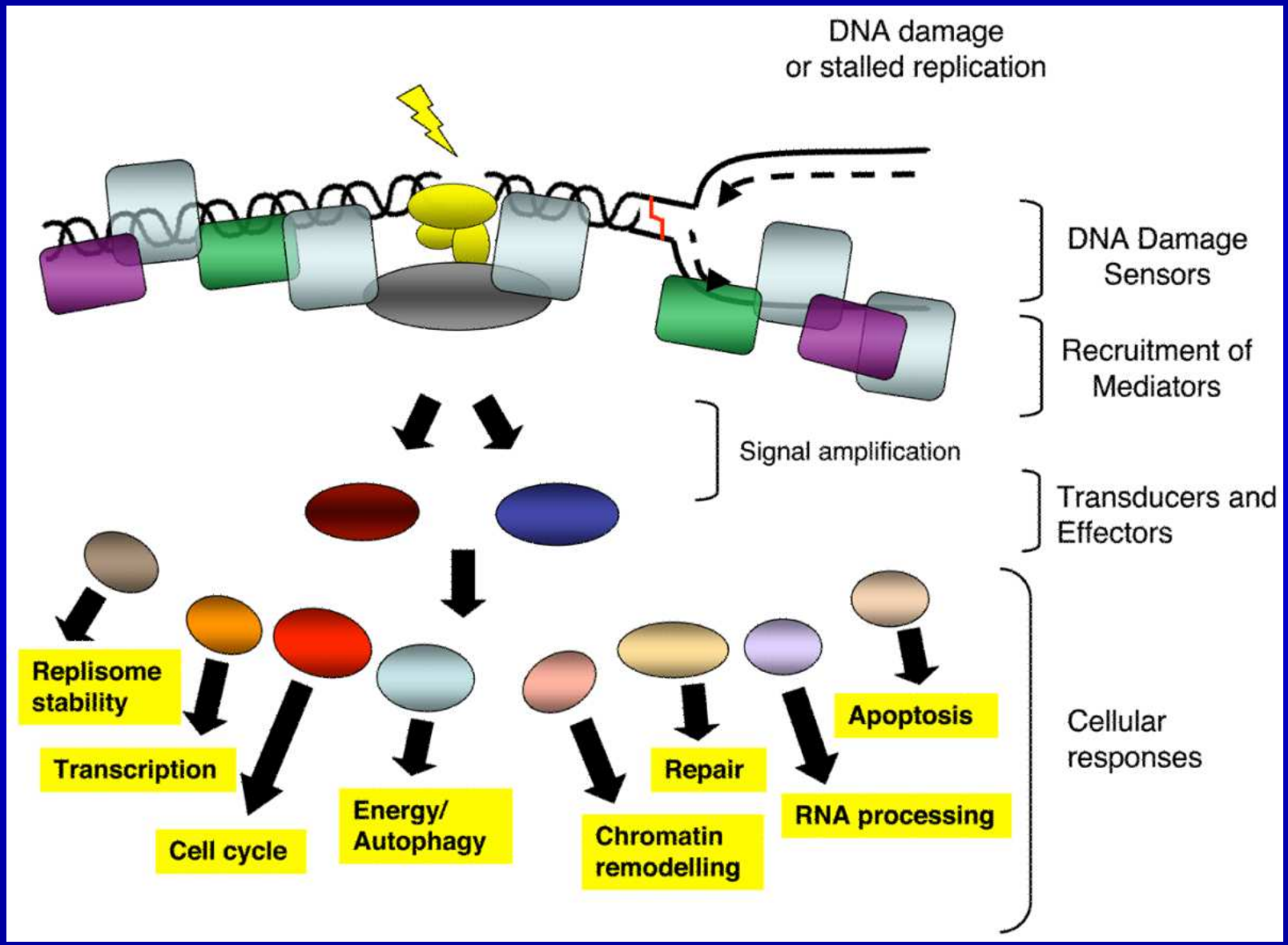

DNA damage
or stalled replication
DNA Damage Sensors
Recruitment of Mediators
Signal amplification
Transducers and Effectors
Cellular responses
Replisome stability
Transcription
Cell cycle
Energy/ Autophagy
Chromatin remodelling
Repair
RNA processing
Apoptosis

Table 1. *Factors involved in DNA strand break repair and damage signaling in budding yeast and mammals*

	Mammals	Yeast (*S. cerevisiae*)
DNA strand break repair		
NHEJ		
End binding		
End processing		
Ligation		
HR		
Resection		
Homologous pairing and strand exchange		
DNA synthesis		
HR resolvases		
Dissolution of HR intermediates		
SSBR		
Detection		
End processing		
Gap filling, ligation		
DNA damage signaling		
Sensors		
Transducers		
Mediators		
ATM signaling		
ATR signaling		
Effectors		

DDR mechanisms and components. See text for details.

DDR mechanism	Prime lesions acted upon
Direct DNA-lesion reversal	UV photo-products O^6 alkylguanine
Mismatch repair (MMR)	DNA mismatches and insertion/deletion loops arising from DNA replication
Base excision repair (BER) and single-strand break repair (SSBR)	Abnormal DNA bases, simple base-adducts, SSBs generated as BER intermediates, by oxidative damage or by abortive topoisomerase I activity
Nucleotide excision repair (NER)	Lesions that disrupt the DNA double-helix, such as bulky base adducts and UV photo-products
Trans-lesion bypass mechanisms	Base damage blocking replication-fork progression
Non-homologous end-joining (NHEJ)	Radiation- or chemically-induced DSBs plus V(D)J and CSR intermediates
Homologous recombination (HR)	DSBs, stalled replication forks, inter-strand DNA cross-links and sites of meiotic recombination and abortive Topoisomerase II action

Meccanismi di riparazione del DNA

Le cellule sono dotate di numerosi meccanismi specializzati per la riparazione del DNA, altamente conservati nell'evoluzione delle specie:

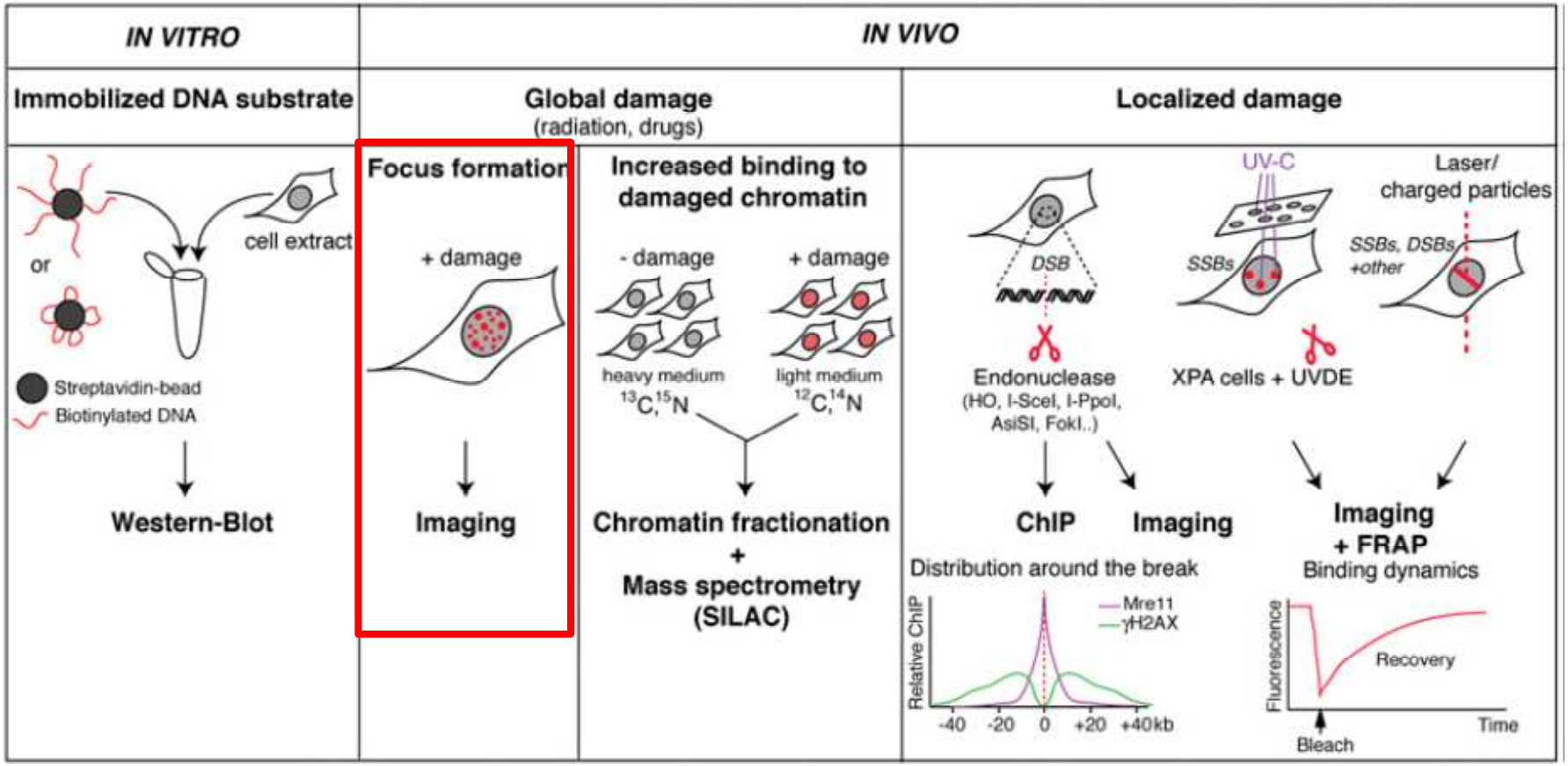
- Attività di proofreading della DNA polimerasi
- Riparazione per excisione di basi (BER)
- Riparazione per excisione di nucleotidi (NER)
- Riparazione degli errori di appaiamento (MMR)
- Riparazione per ricombinazione omologa (HRR)
- Riparazione per ricombinazione non omologa o Non Homologous End joining (NHEJ)

Table 2. *Factors involved in chromatin dynamics recruited to/dissociating from damaged chromatin in response to DNA breaks*

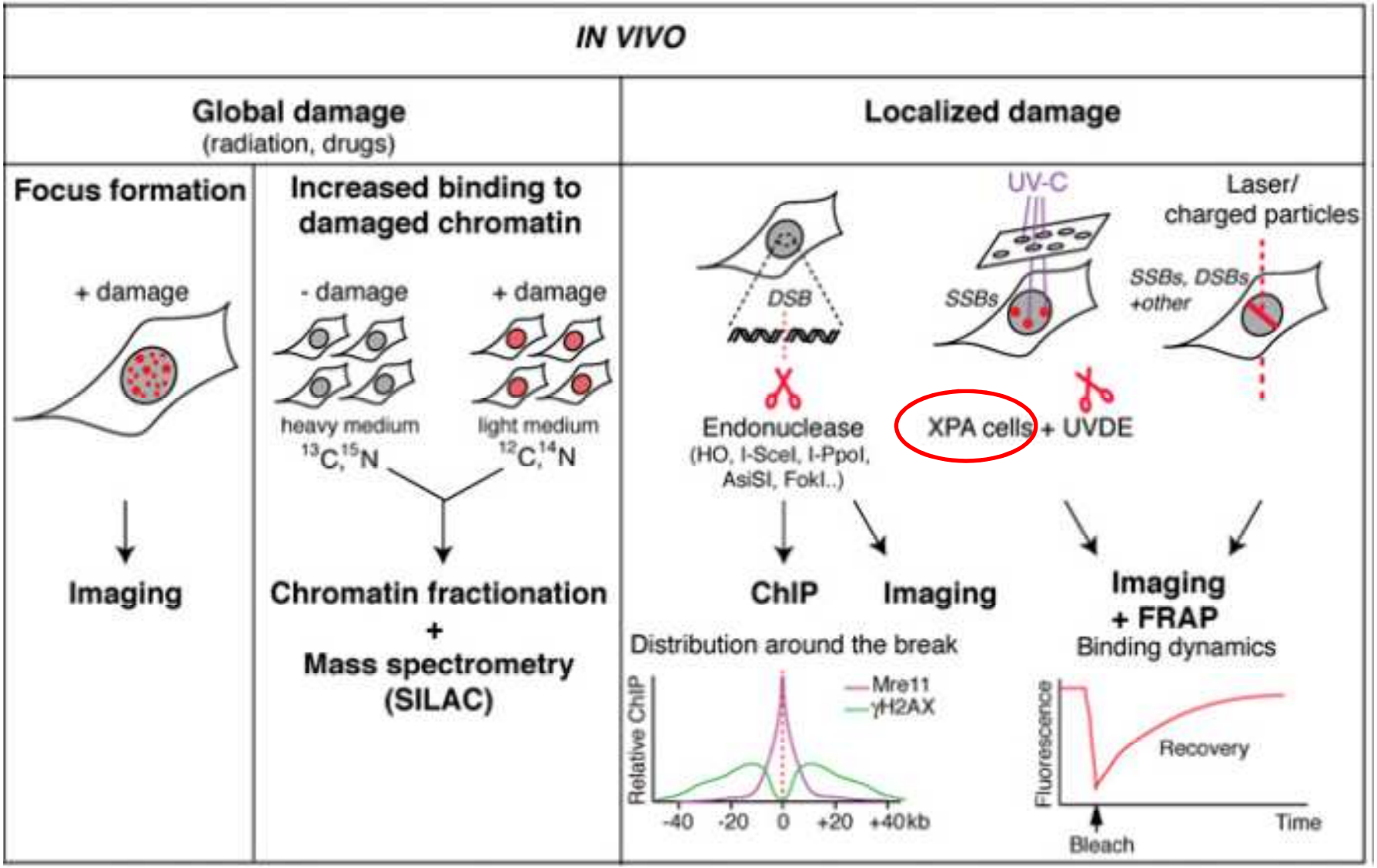
	Name	Organism	References
DNA methyltransferases			
	Dnmt1	Human, mouse	
	Dnmt3b	Human	
Histone-modifying enzymes			
Histone methyltransferase	EZH2	Human	
	PR-Set7/Set8	Human	
Histone acetyltransferase	Esa1	Budding yeast	
	Gcn5	Budding yeast	
	Hat1	Budding yeast	
	NuA4	Budding yeast	
	Tip60	Human	
Histone deacetylase	Hst1	Budding yeast	
	Rpd3	Budding yeast	
	Sir2	Budding yeast	
	HDAC1	Human	
	HDAC2	Human	
	HDAC4	Human	
	SIRT1	Human, mouse	
	SIRT6	Human	
Chromatin remodeling factors			
	INO80	Budding yeast	
	RSC	Budding yeast	
	SWI/SNF	Budding yeast	
	SWR1	Budding yeast	
	ALC1	Human	
	INO80	Human, mouse	
	ISWI	Human	
	NuRD	Human	
	p400	Human	
	SWI/SNF	Human	
Histone chaperones			
	CAF-1	Human	
	FACT	Human	

All listed factors are recruited to damaged chromatin, with the exception of FACT, which dissociates from chromatin upon DNA damage.

METODI


IN VITRO
IN VIVO
Immobilized DNA substrate
Global damage
(radiation, drugs)
Localized damage
Focus formation
Increased binding to
damaged chromatin
cell extract
or
Streptavidin-bead
Biotinylated DNA
+ damage
- damage
+ damage
heavy medium
$^{13}C,^{15}N$
light medium
$^{12}C,^{14}N$
DSB
UV-C
SSBs
Laser/
charged particles
SSBs, DSBs
+other
Endonuclease
(HO, I-SceI, I-PpoI,
AsiSI, FokI..)
XPA cells + UVDE
Western-Blot
Imaging
Chromatin fractionation
+
Mass spectrometry
(SILAC)
ChIP
Imaging
Imaging
+ FRAP
Binding dynamics
Distribution around the break
Relative ChIP
Mre11
γH2AX
-40
-20
0
+20
+40kb
Fluorescence
Recovery
Bleach
Time

METODI

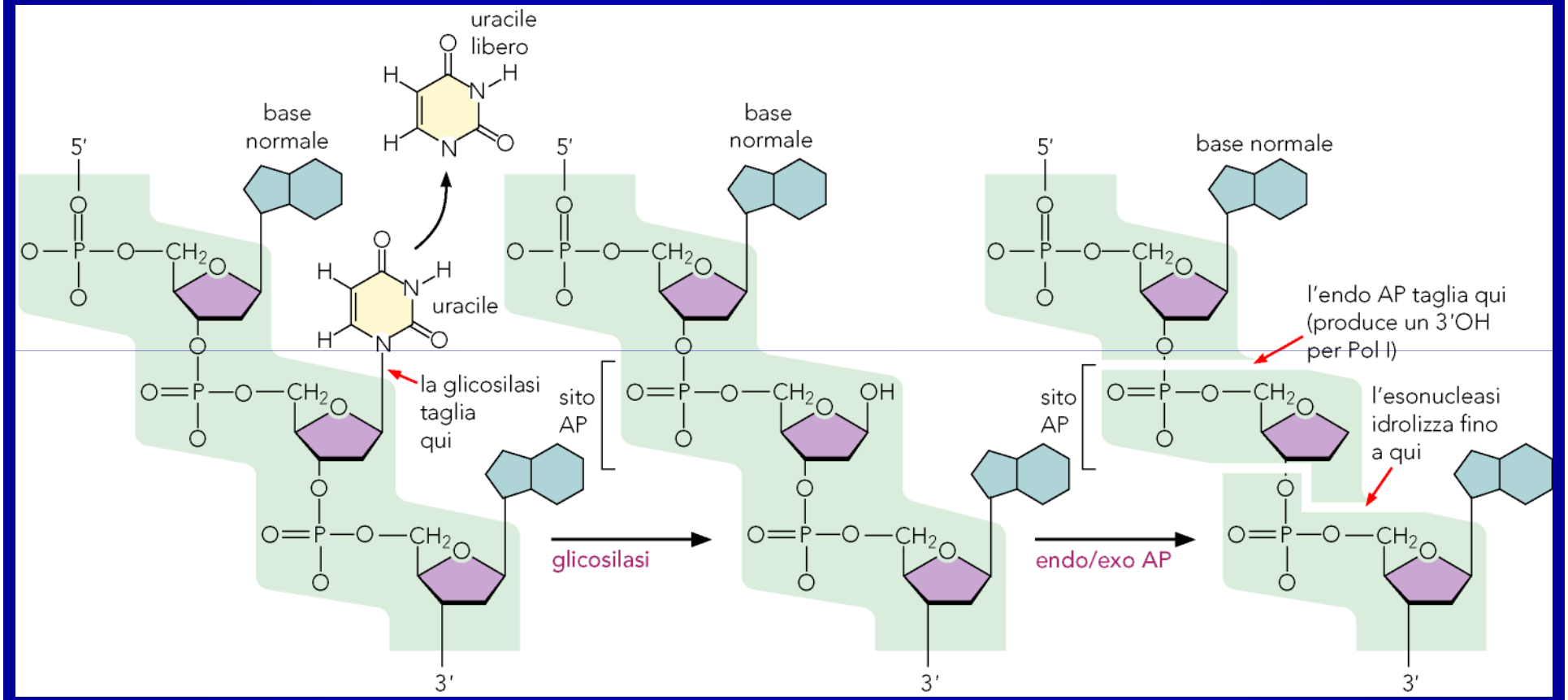

IN VIVO
Global damage
(radiation, drugs)
Localized damage
Focus formation
+ damage
Imaging
Increased binding to damaged chromatin
- damage
+ damage
heavy medium
$^{13}C,^{15}N$
light medium
$^{12}C,^{14}N$
Chromatin fractionation
+
Mass spectrometry
(SILAC)
DSB
UV-C
SSBs
Laser/
charged particles
SSBs, DSBs
+other
Endonuclease
(HO, I-SceI, I-PpoI,
AsiSI, FokI..)
XPA cells + UVDE
ChIP
Imaging
Distribution around the break
Relative ChIP
Mre11
γH2AX
-40
-20
0
+20
+40kb
Imaging
+ FRAP
Binding dynamics
Fluorescence
Recovery
Time
Bleach

Riparazione per excisione di basi (BER)

Rimuove le basi chimicamente modificate che distorcono localmente la doppia elica

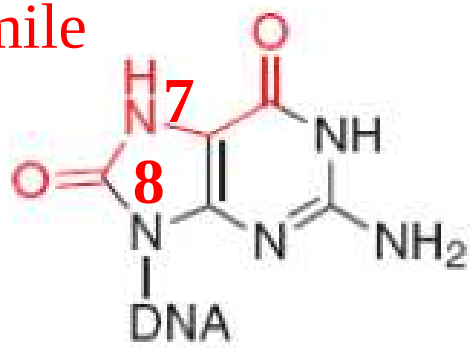
Agisce su danni al DNA piuttosto limitati, quelli che si producono ogni giorno spontaneamente

E' il meccanismo prevalente per la rimozione di lesioni che interessano la singola elica

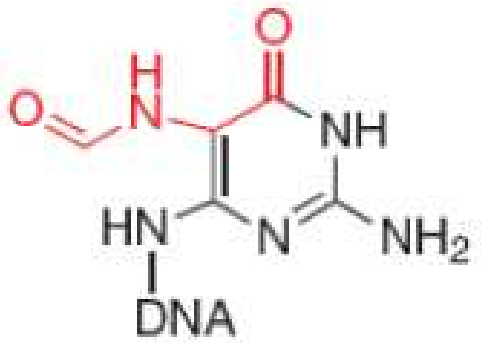

uracile
libero
base
normale
5′
uracile
la glicosilasi
taglia
qui
sito
AP
glicosilasi
base
normale
5′
OH
3′
sito
AP
endo/exo AP
base normale
5′
l'endo AP taglia qui
(produce un 3'OH
per Pol I)
l'esonucleasi
idrolizza fino
a qui
3′

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

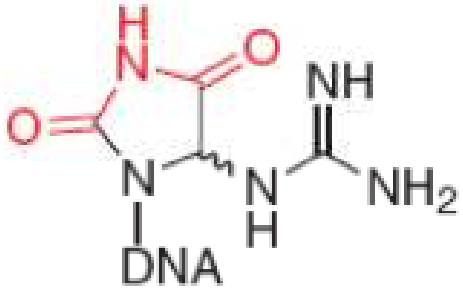
b T simile



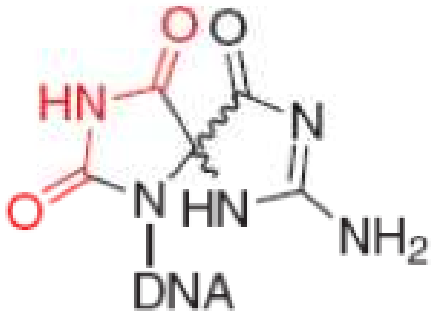
8-oxoG



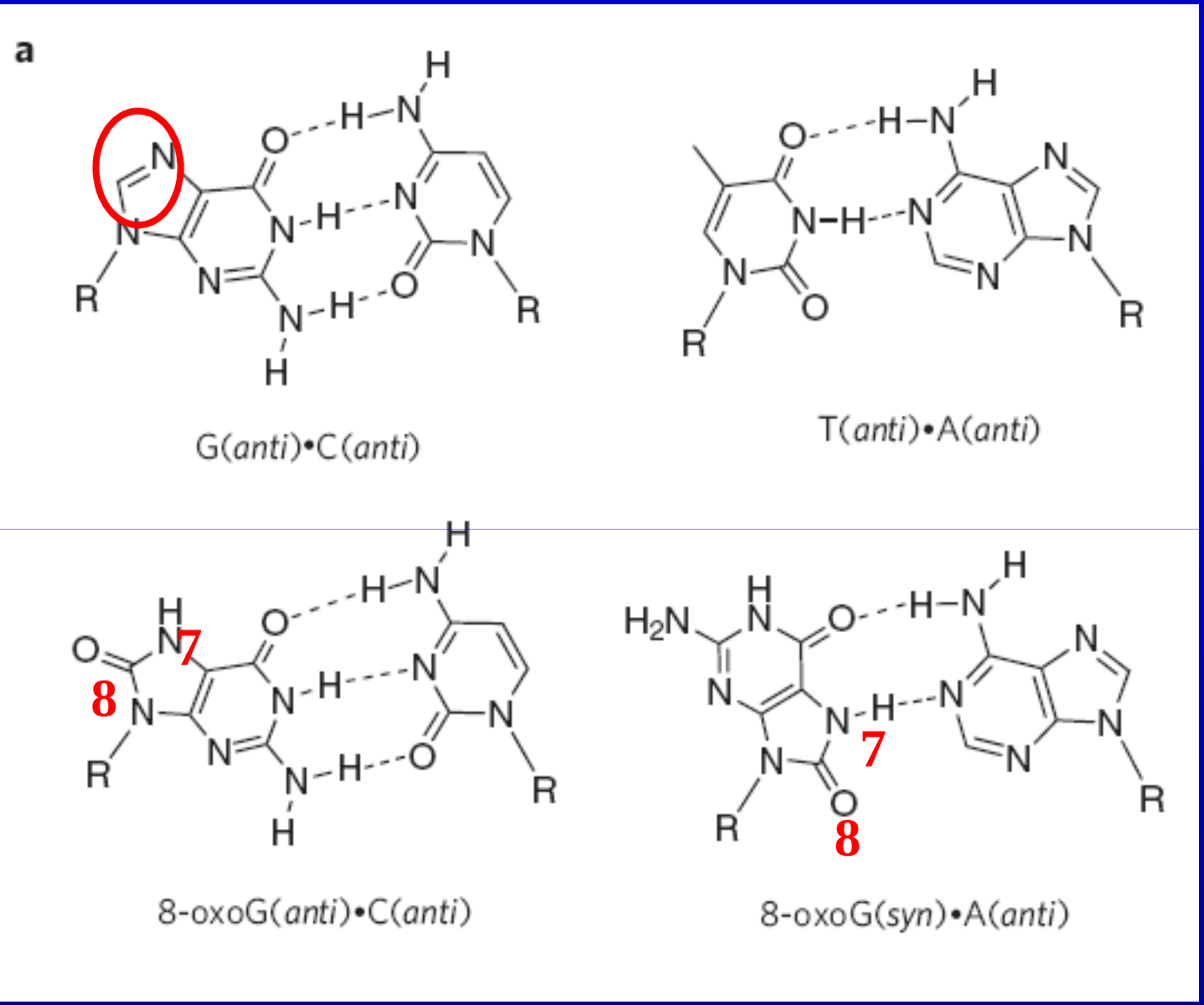
FapyG

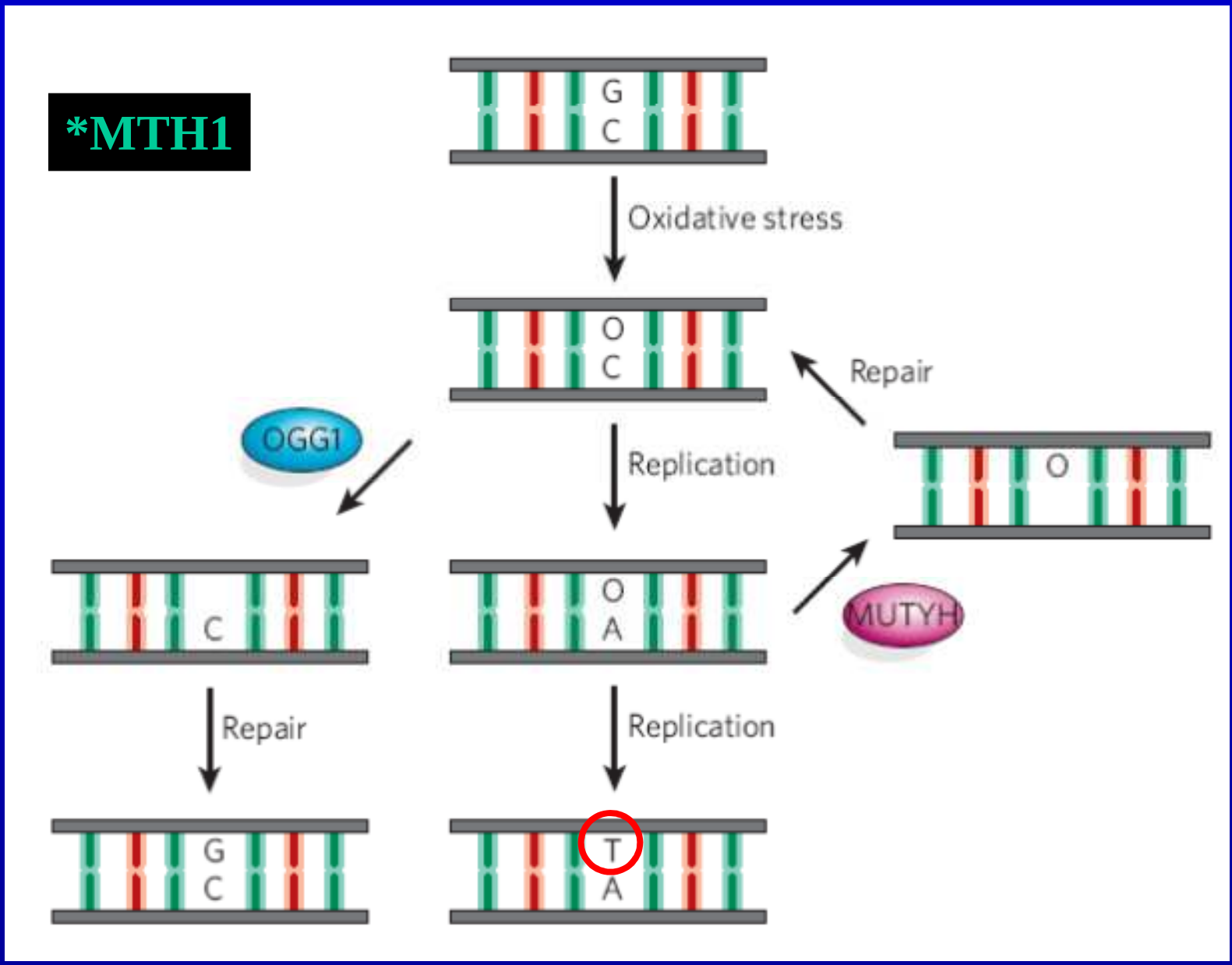


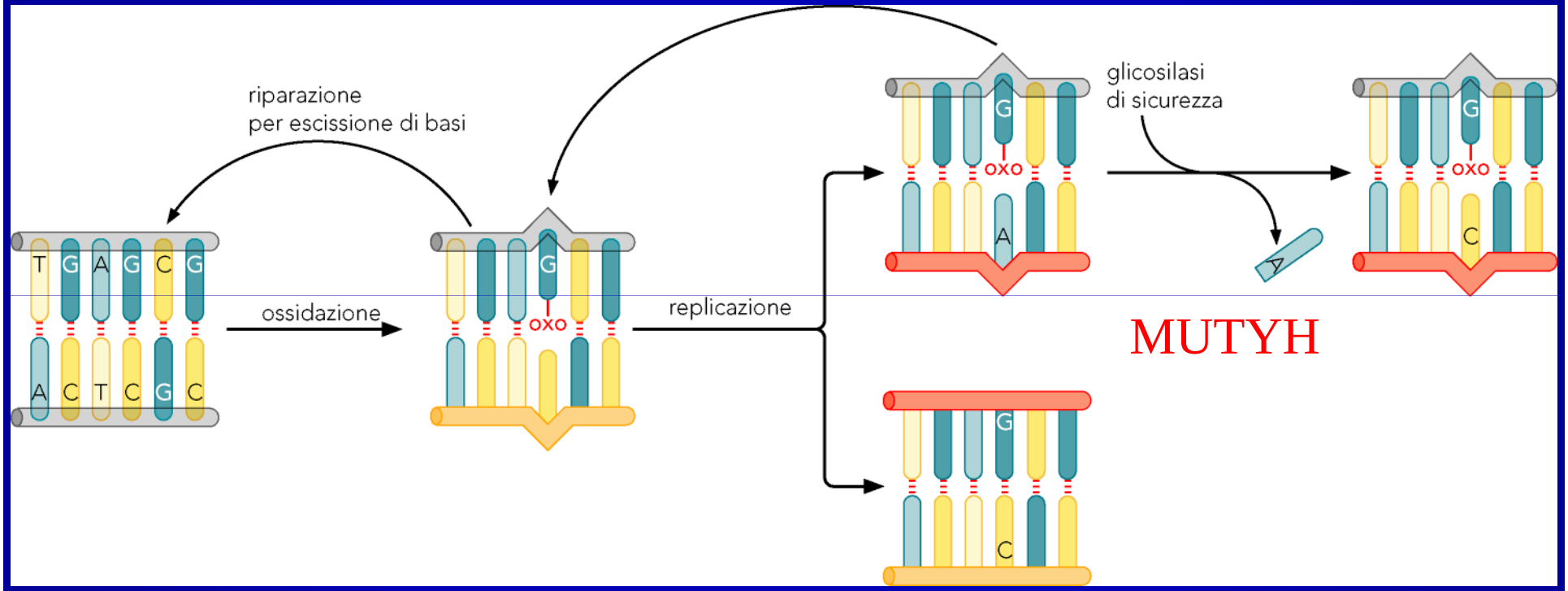
Gh

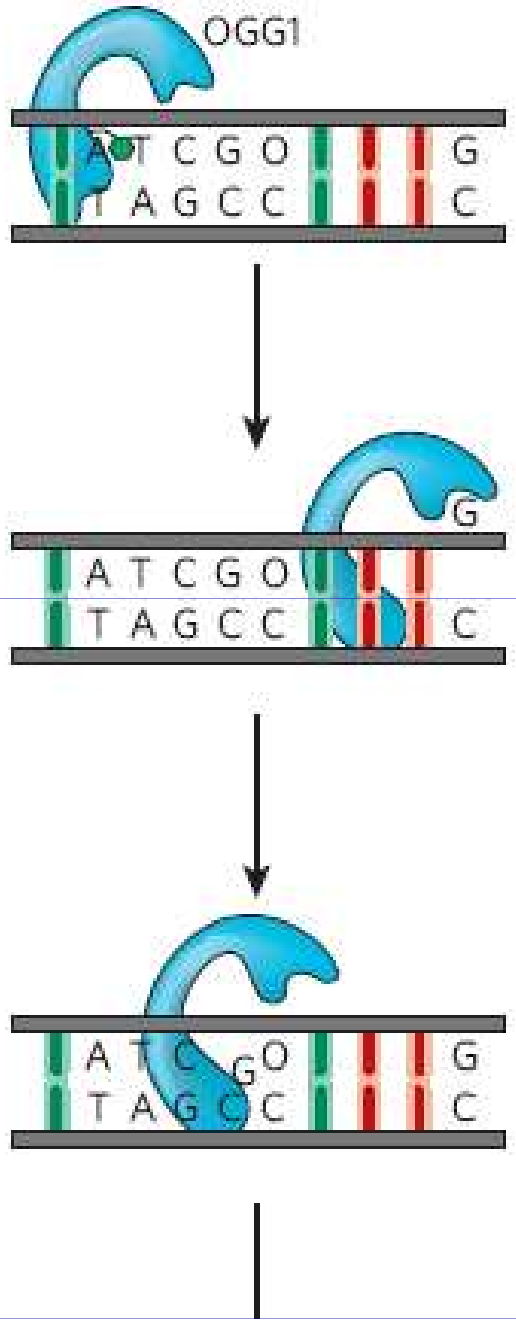


Sp


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

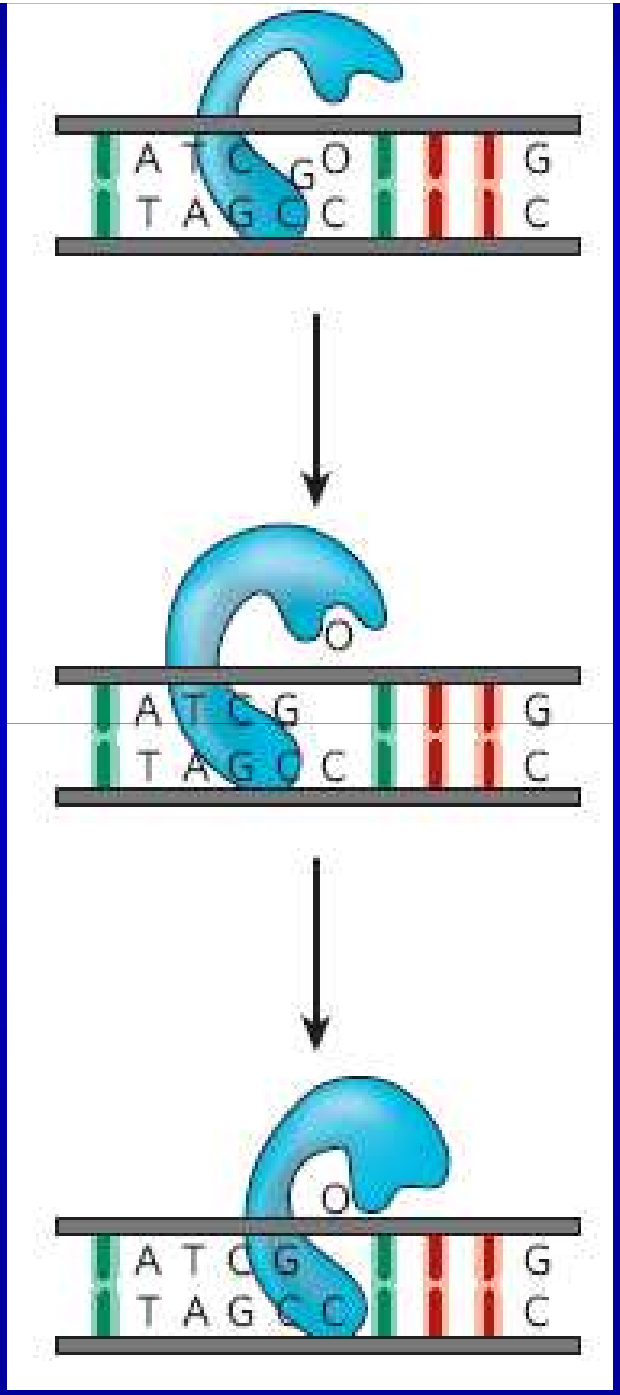

*MTH1
G
C
Oxidative stress
O
C
Repair
OGG1
Replication
O
C
O
A
MUTYH
Repair
Replication
G
C
T
A


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!!!!!!.



The 8-oxoG lesion search Process (2).

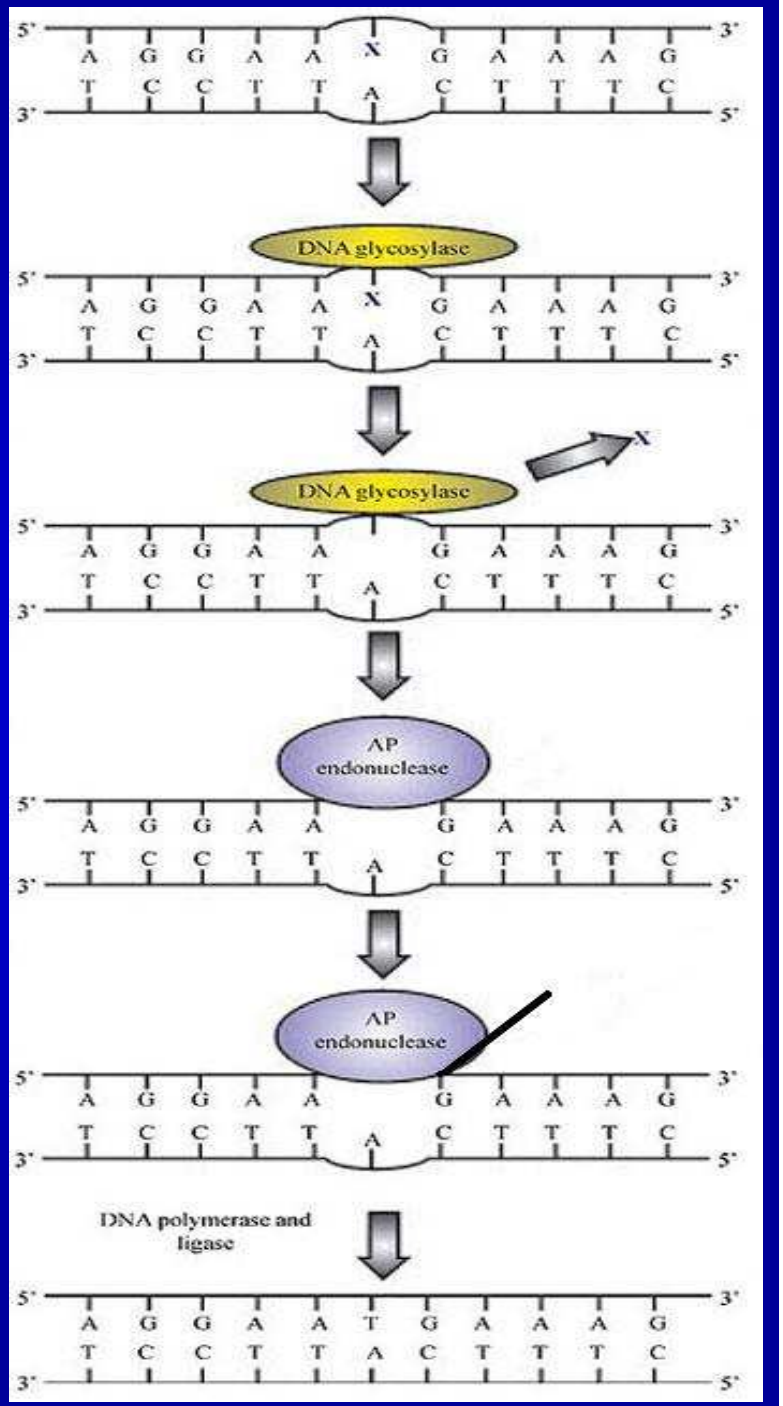
- the 8-oxoG is extruded to the exosite and captured in the 8-oxoG-specific pocket, where it is excised from the DNA.

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

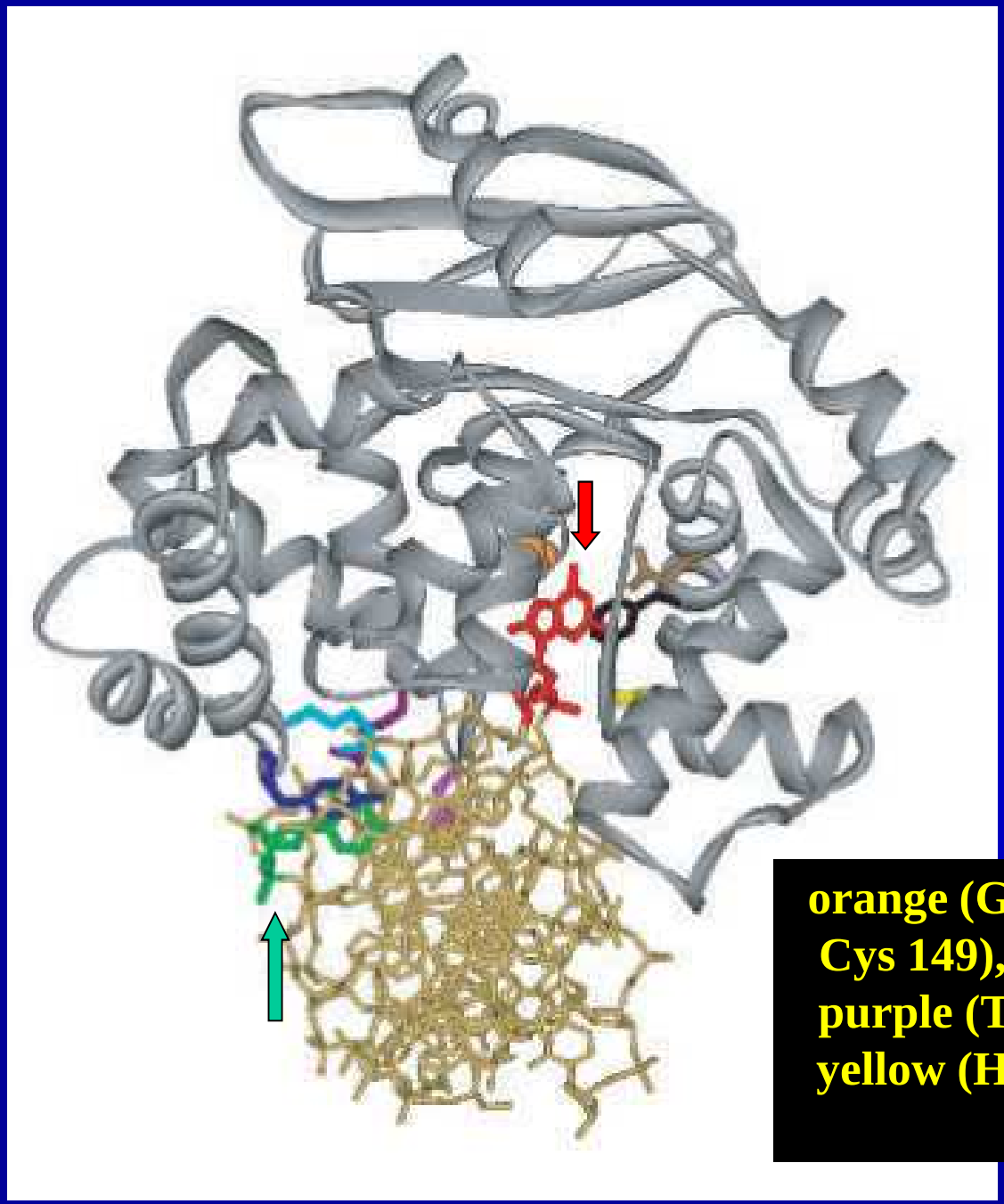
La **DNA polimerasi** riempie il gap lasciato dalla glicosilasi usando come stampo l'elica parentale

La **ligasi** richiude l'elica riparata



- OGG1 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.

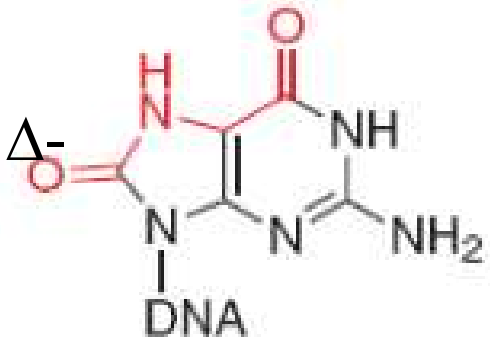
- On the basis of these measurements, the estimated barrier to sliding is extremely small (0.5 kcal mol–1). The smaller barrier and the observed unbiased random movement of OGG1 on DNA suggest that OGG1 rapidly searches along DNA as a consequence of brownian motion.



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).



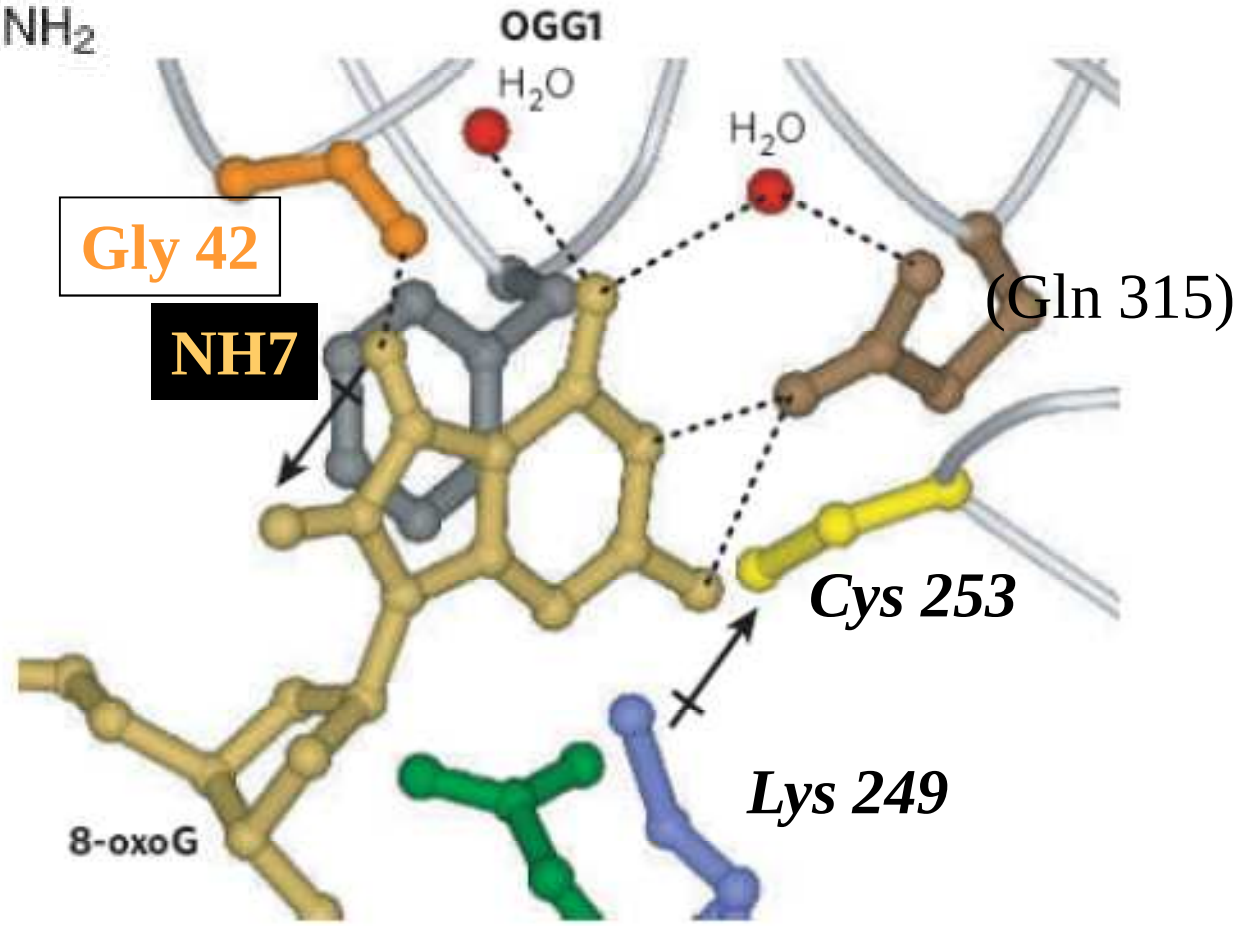
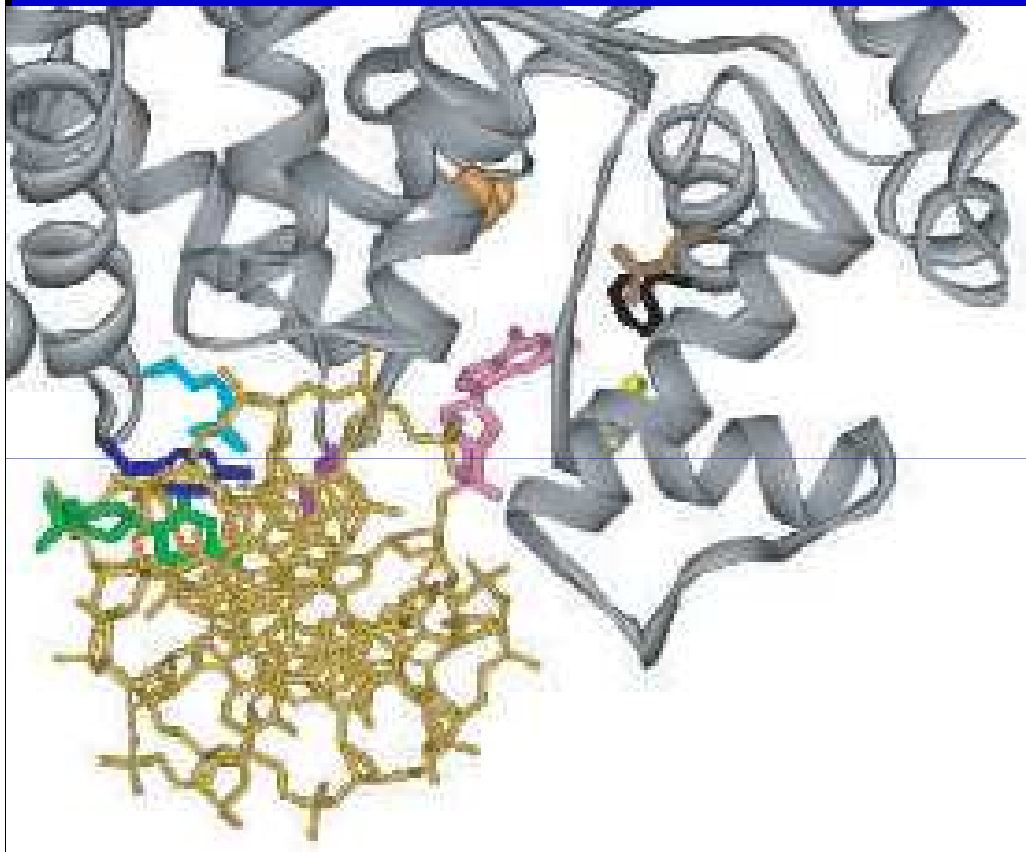
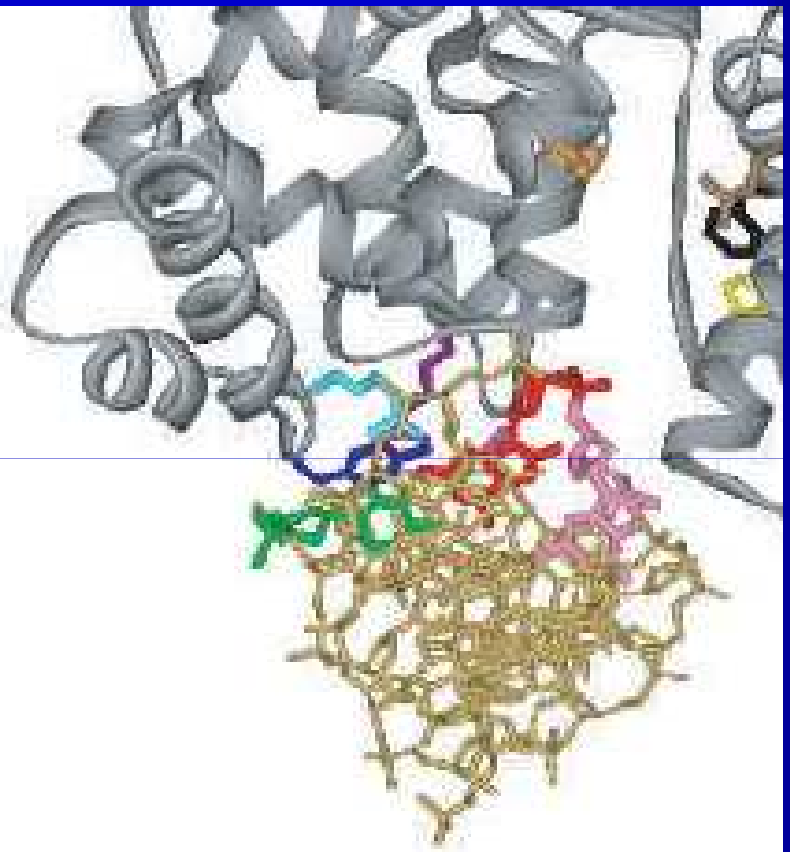


Figure 3 | Recognition of 8-oxoG by OGG1 observed in the LRC of OGG1 with 8-oxoG•C-containing duplexes. This is a view of the base-specific pocket

Le coppie GC non sono tutte uguali

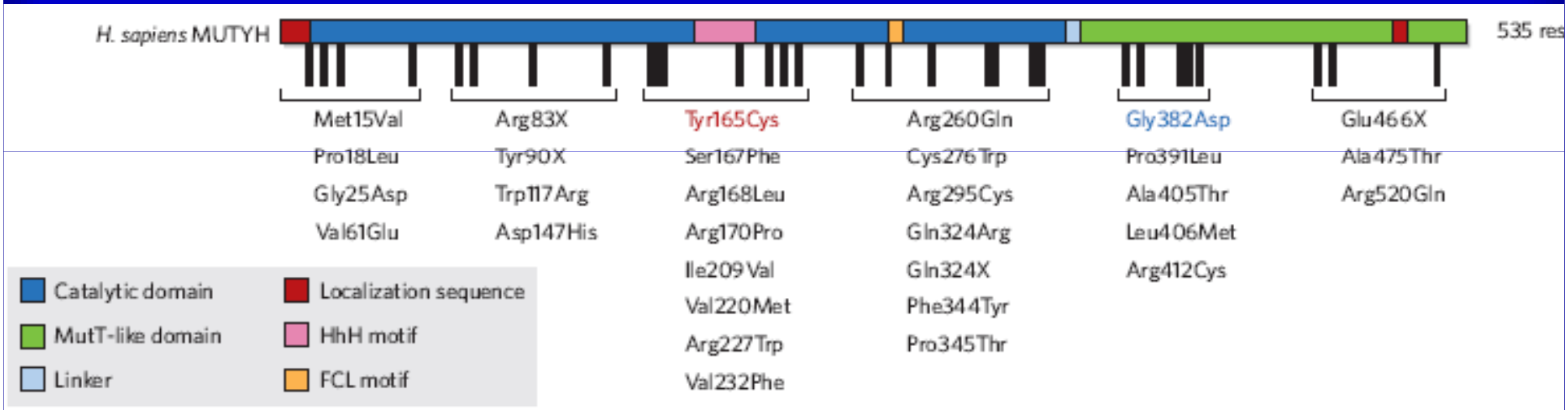


OGG1 interrogating a G•C base pair. The target G in shown in light pink, and the target C in green



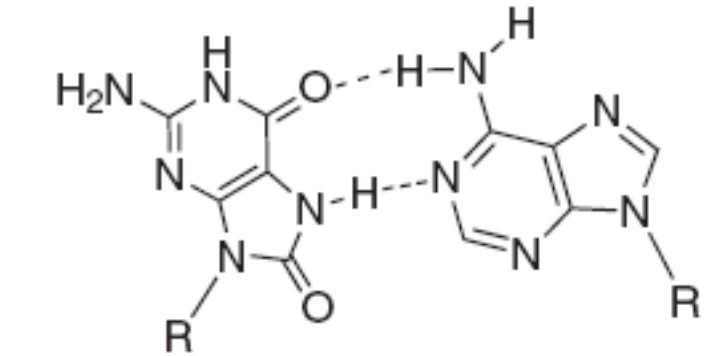
OGG1interrogating a G•C base pair adjacent to an 8-oxoG lesion. 8-oxoG isshown in red, the target G in light pink, and the target C in green

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 *Apc*Min/+/*Mutyh*+/+ mice.

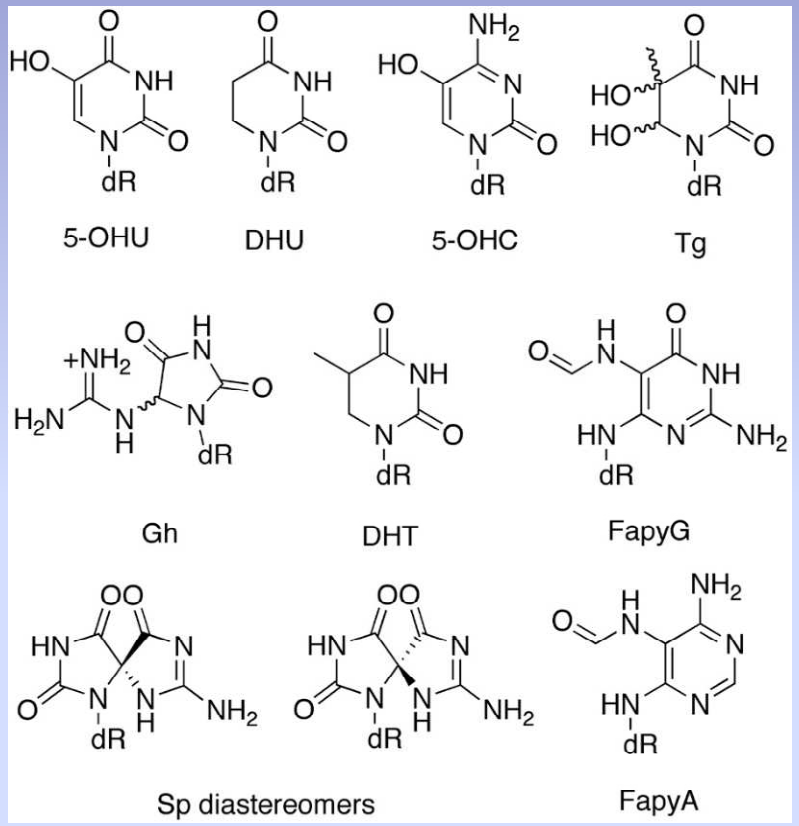
neil1

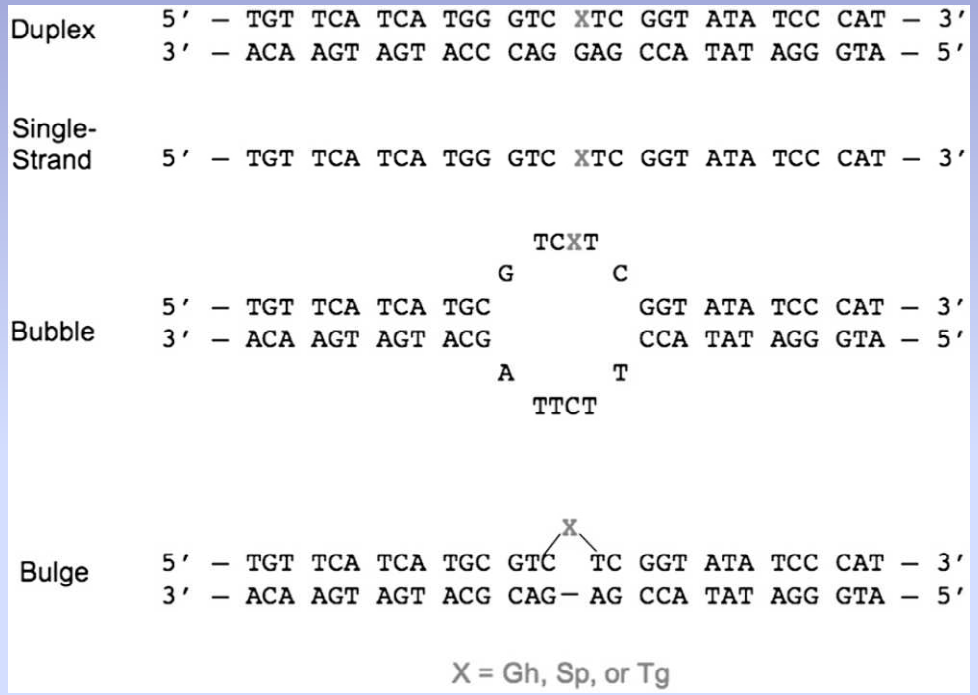
- RNA editing changes the lesion specificity for the DNA repair enzyme NEIL1

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)

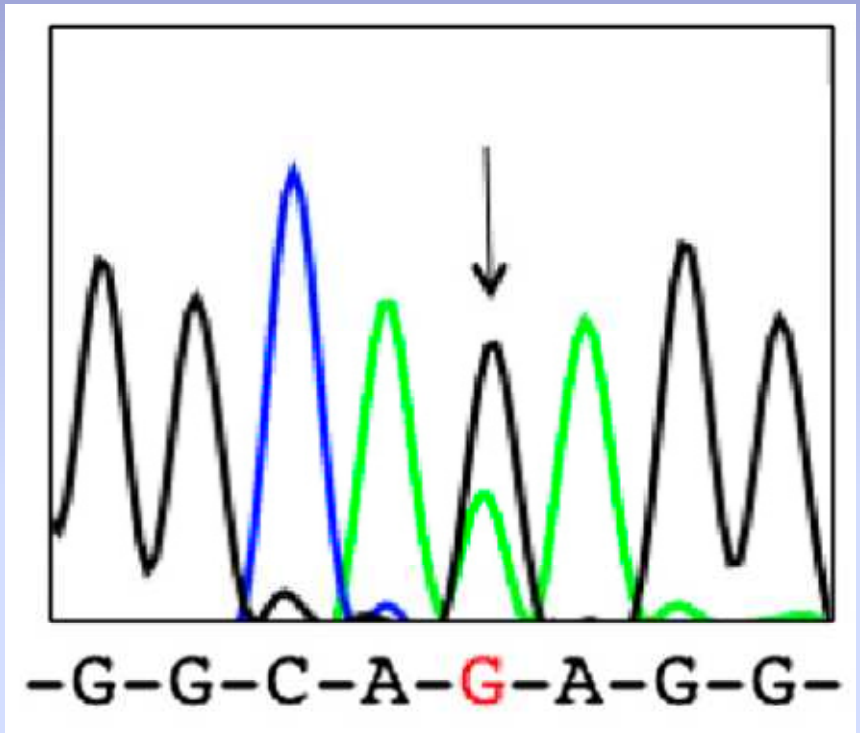
NEIL1 plays a key role in the initiation of base excision repair of oxidized base lesions by catalyzing the cleavage of the N-glycosidic linkage to the 2'-deoxyribose

Known substrates for the base excision repair glycosylase NEIL1.





NEIL1 mRNA sequencing



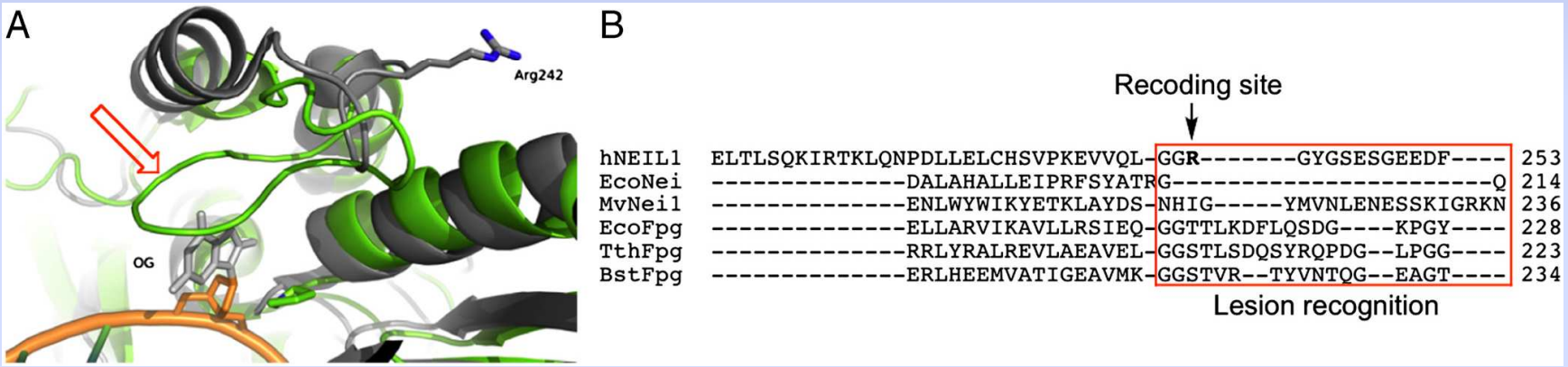
mRNA Editing

A to G

AAA to AIA (AGA)

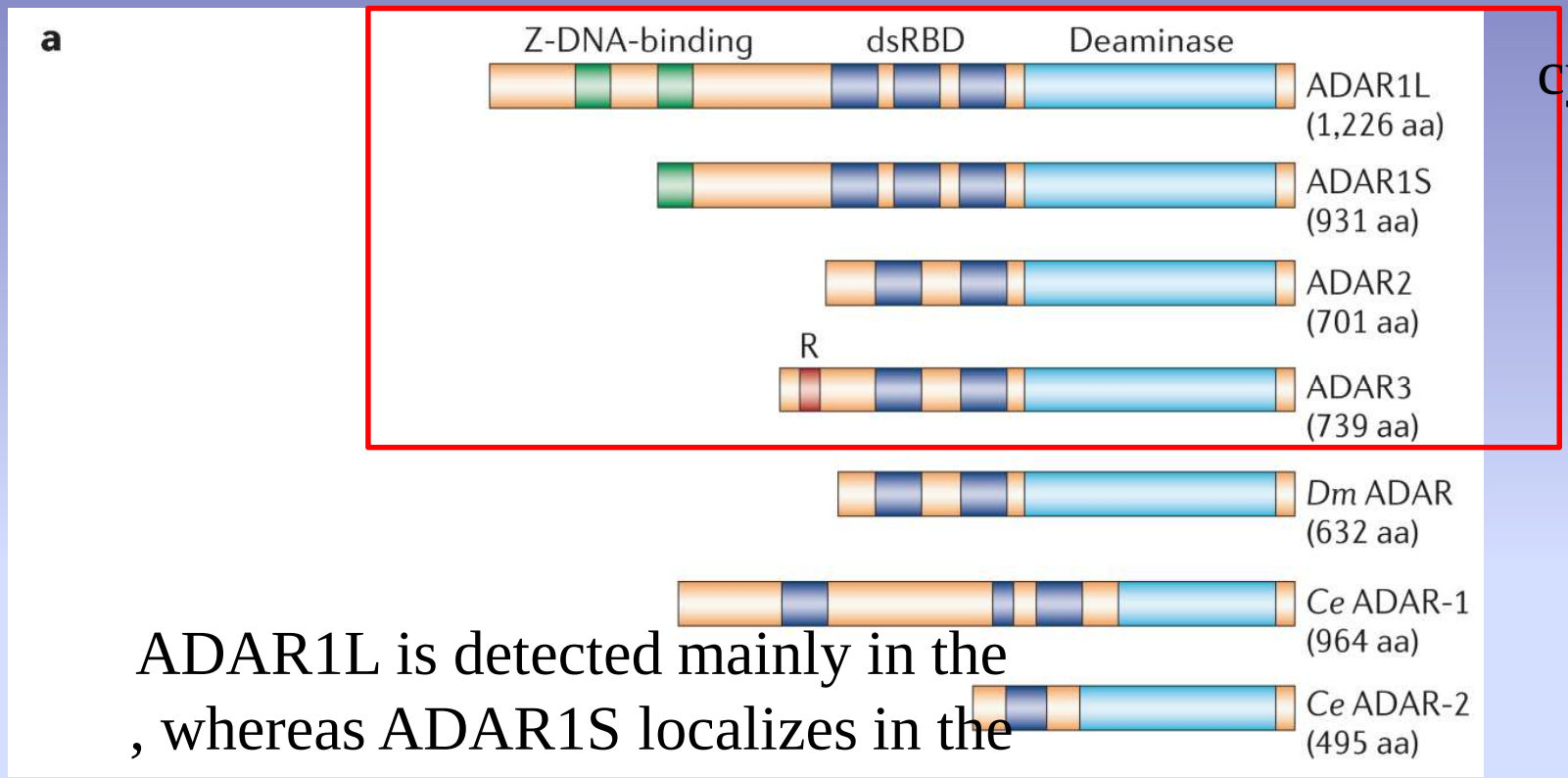
R to K

(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.

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



cytoplasm

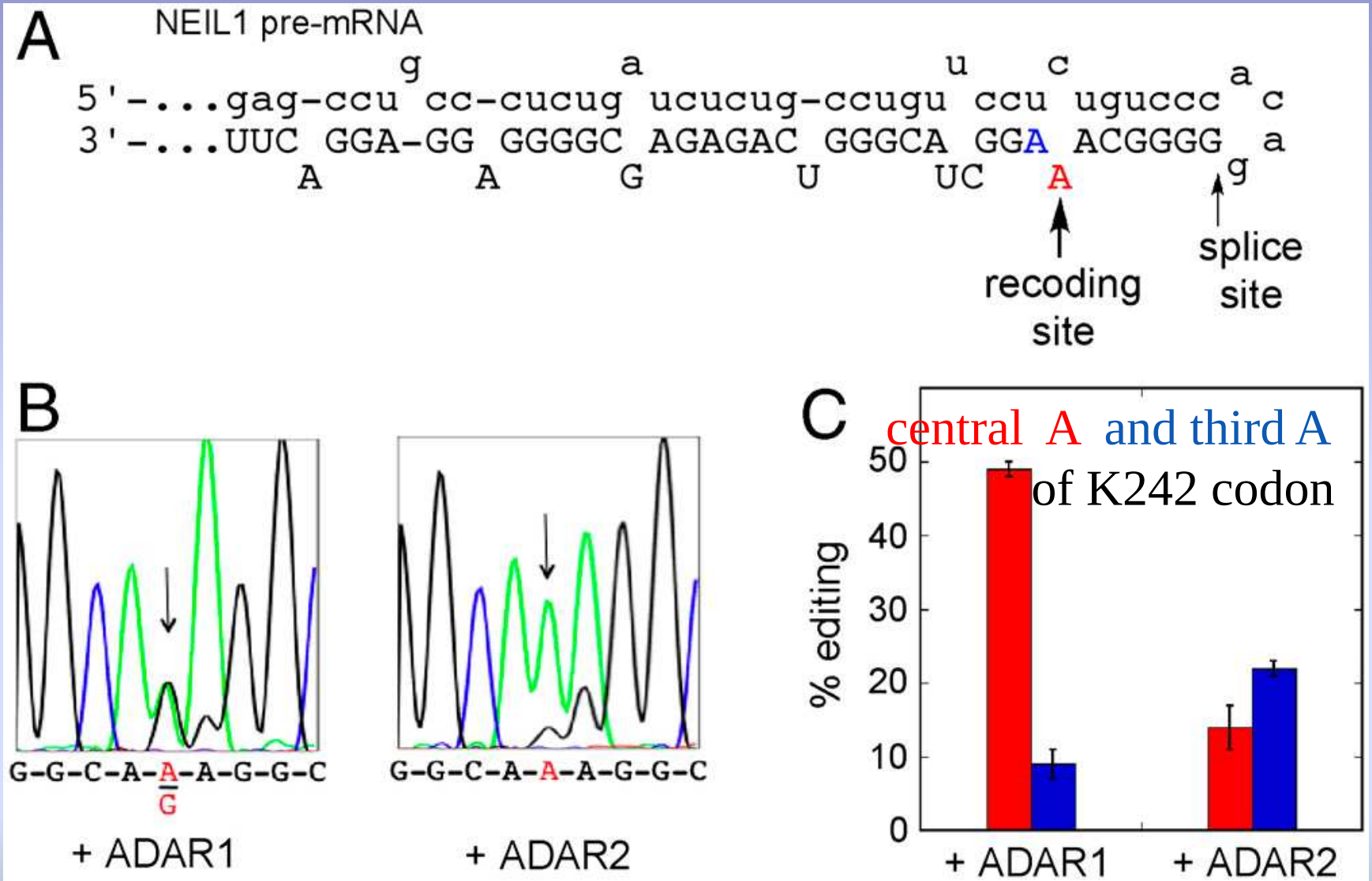
nucleo

and nuc

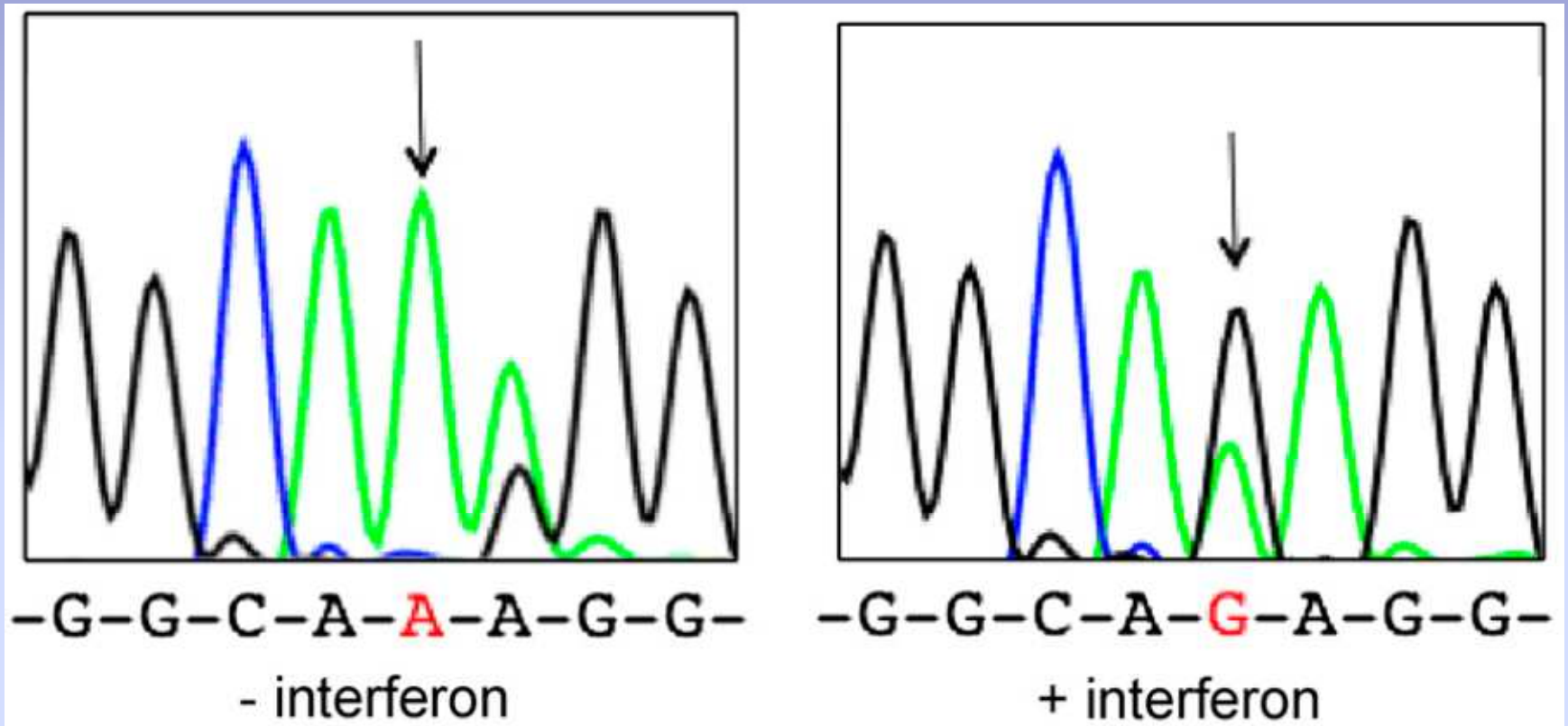
nucleolus

ADAR1L is detected mainly in the , whereas ADAR1S localizes in the s[40,44,45]. ADAR2 localizes predominantly in the nucleolus[44,46]

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.

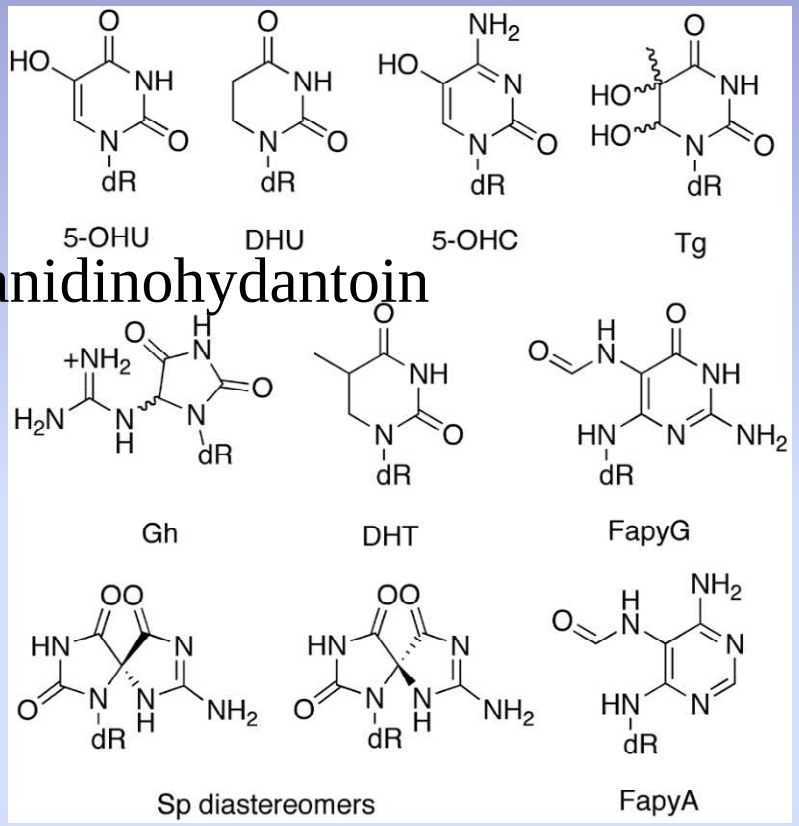


NEIL1 editing in response to IFN-α.
(Left) Sequence at the recoding site in NEIL1 cDNA from U87 human glioblastoma cells cultured in the absence of IFN-α.
(Right) NEIL1 cDNA sequence from U87 cells treated with IFN-α.

Known substrates for the base excision repair glycosylase NEIL1.

thymine glycol

guanidinohydantoin



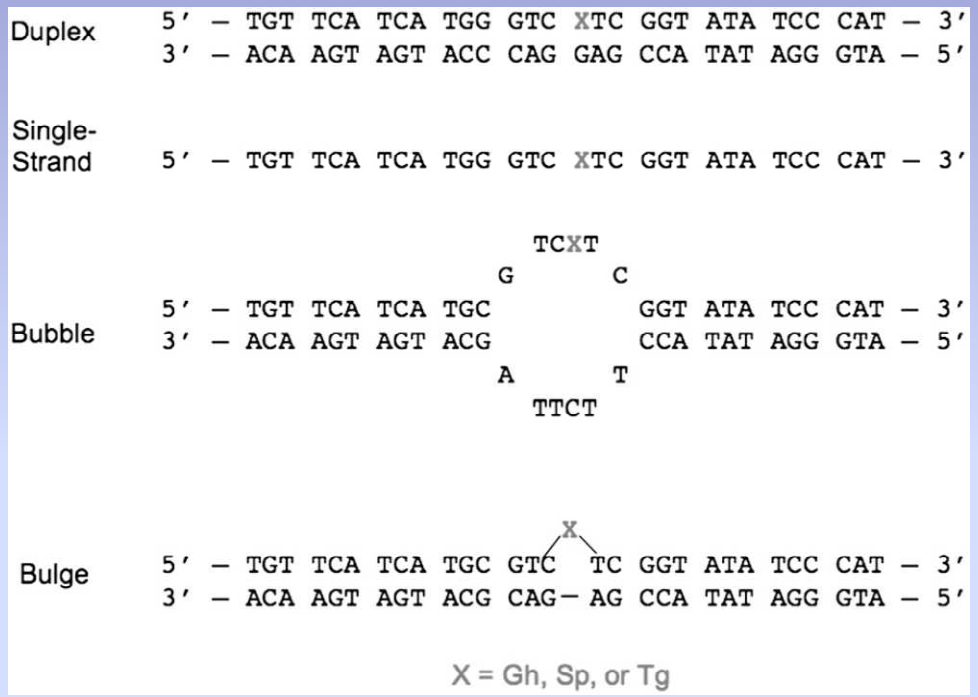


Table 1. Rate constants (k_g)* of base removal by edited versus unedited NEIL1

	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 from duplex DNA 30 times more slowly than the form encoded in the genome,
- whereas editing enhances repair of the guanidinohydantoin lesion by NEIL1.

- 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 unique regulatory mechanism for DNA repair and extend our understanding of the impact of RNA editing.

Riparazione per excisione di nucleotidi (NER)

Rimuove le basi modificate da agenti chimici che alterano drasticamente la singola elica e ripara i danni indotti da UV, ad esempio dimeri di Timina (l'uomo non possiede la fotoliasi)

Almeno 30 proteine coinvolte

Le proteine più importanti sono i prodotti dei sette geni XP-A→XP-G, di cui sono ormai note le caratteristiche molecolari e la funzionalità

XPC e **HHR23B** riconoscono e legano il DNA danneggiato

↓

Reclutamento di **TFIIH**, le cui subunità con attività elicasica (**XPB** e **XPD**) allentano la doppia elica

↓

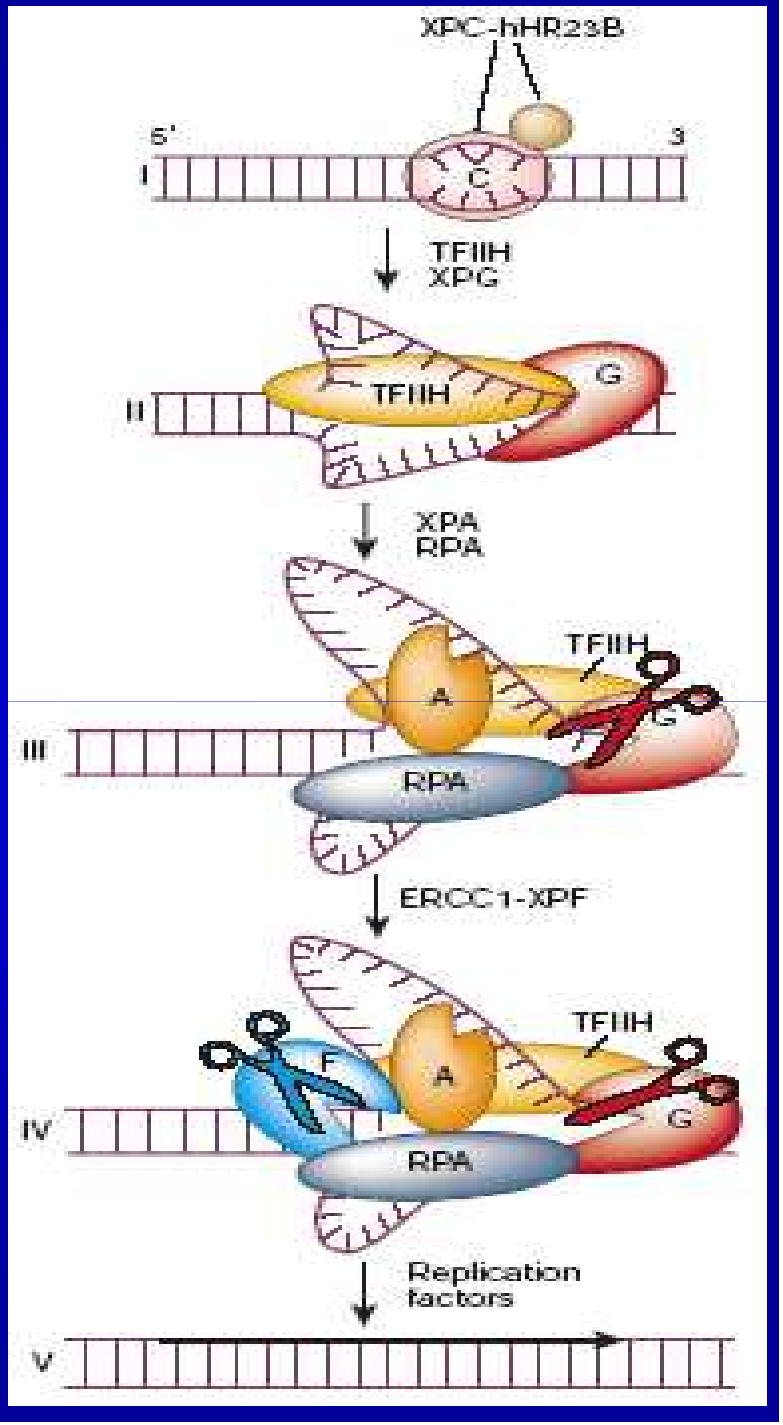
XPA e **RPA** legano il complesso e stabilizzano l'elica

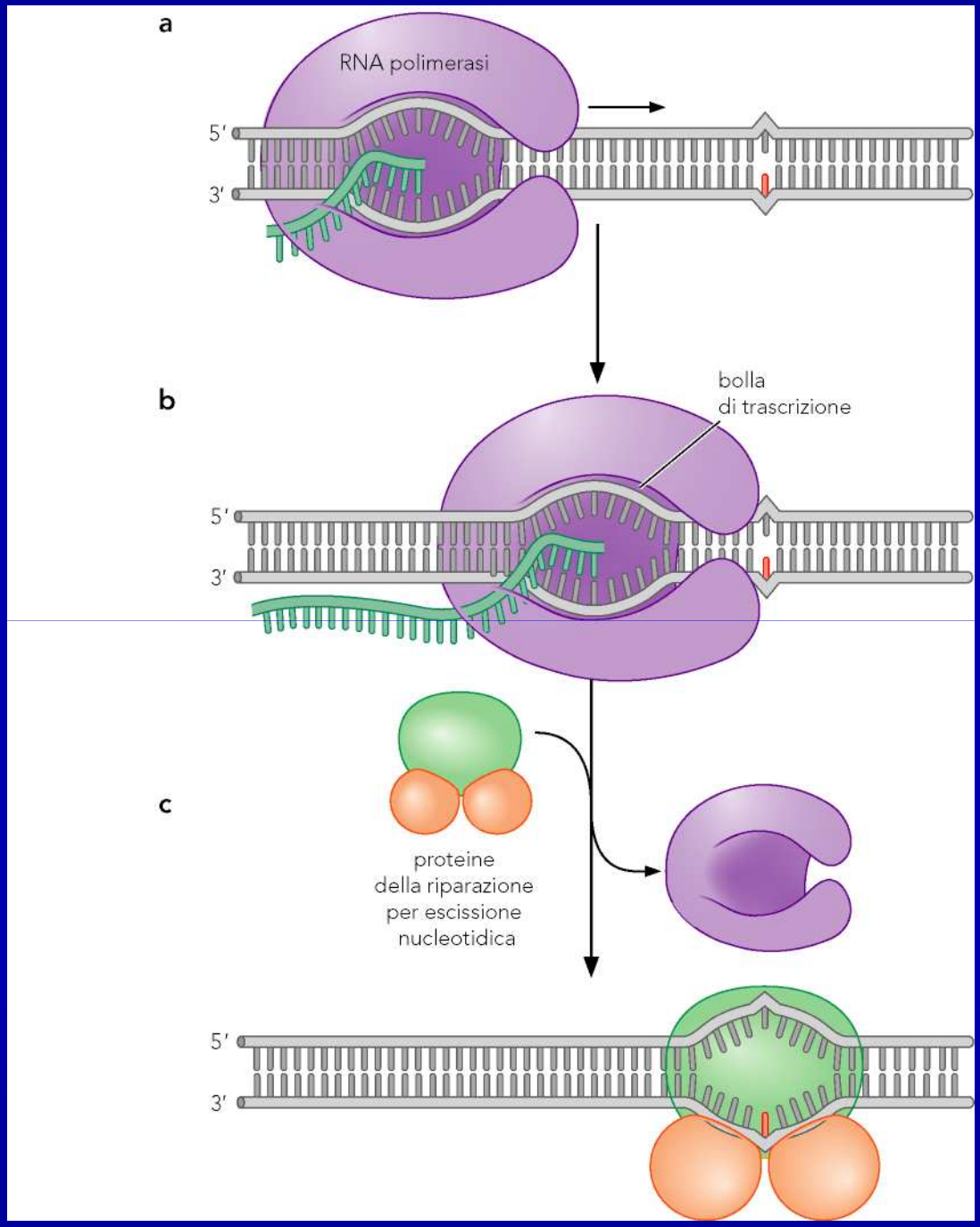
↓

XPF-ERCC1 e **XPG** tagliano la catena danneggiata

↓

Il gap è riempito dalla **DNA polimerasi** e da una **ligasi**




a
RNA polimerasi
5′
3′
b
bolla
di trascrizione
5′
3′
c
proteine
della riparazione
per escissione
nucleotidica
5′
3′

Mutazioni in un gene XP → inattivazione del NER → Xeroderma pigmentoso, patologia rara ($1/10^5$-10^6), sensibilità alla luce solare, aumentata probabilità (1000X) di avere carcinomi cutanei

NER è attivo soprattutto nei confronti dei danni fotochimici → l'esposizione agli UV in pazienti con XP causa accumulo di mutazioni a livello cutaneo e sviluppo di tumori a carico della pelle

20% pazienti con XP → neurodegenerazione, non causata dai raggi UV (non arrivano ai neuroni)

IPOTESI: accumulo di danni al DNA indotti dai radicali liberi, prodotti dai neuroni in sviluppo

Dati sperimentali: Katsumi et al, 2001

Fibroblasti umani mascherati da una membrana porosa e trattati con raggi UV → induzione fotoprodotti, la cui evoluzione è stato monitorata nel tempo mediante immunofluorescenza