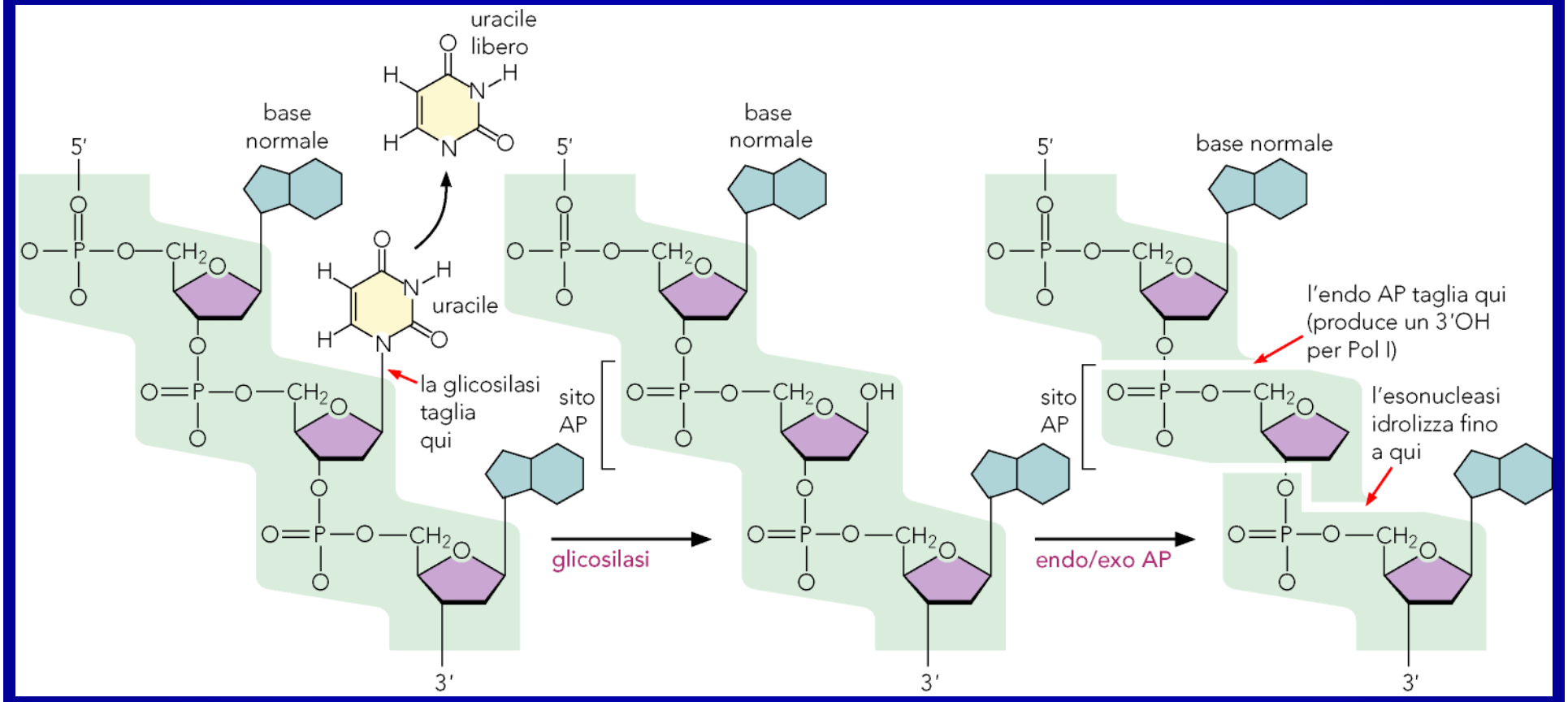
Riparazione per excisione di basi (BER)

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

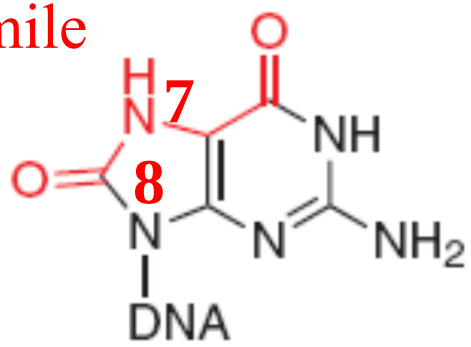
E' il meccanismo prevalente per la rimozione di lesioni che interessano il singolo filamento

Esempio: rimozione uracile dal DNA

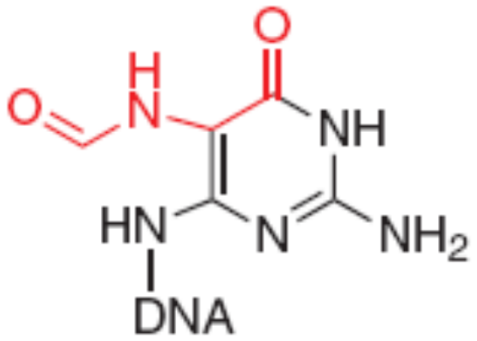


BER: 8-OXOGUANINA

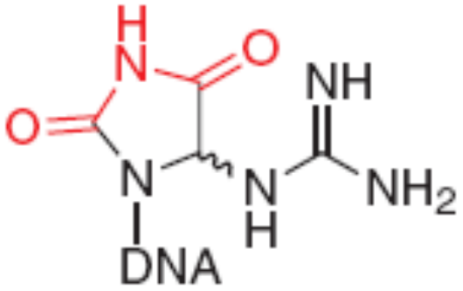
b T simile



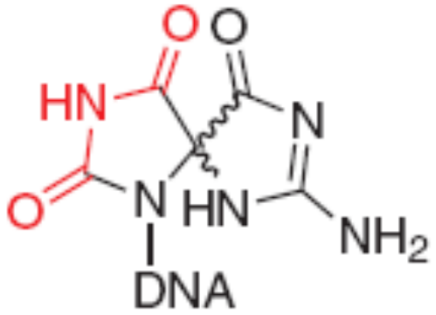
8-oxoG



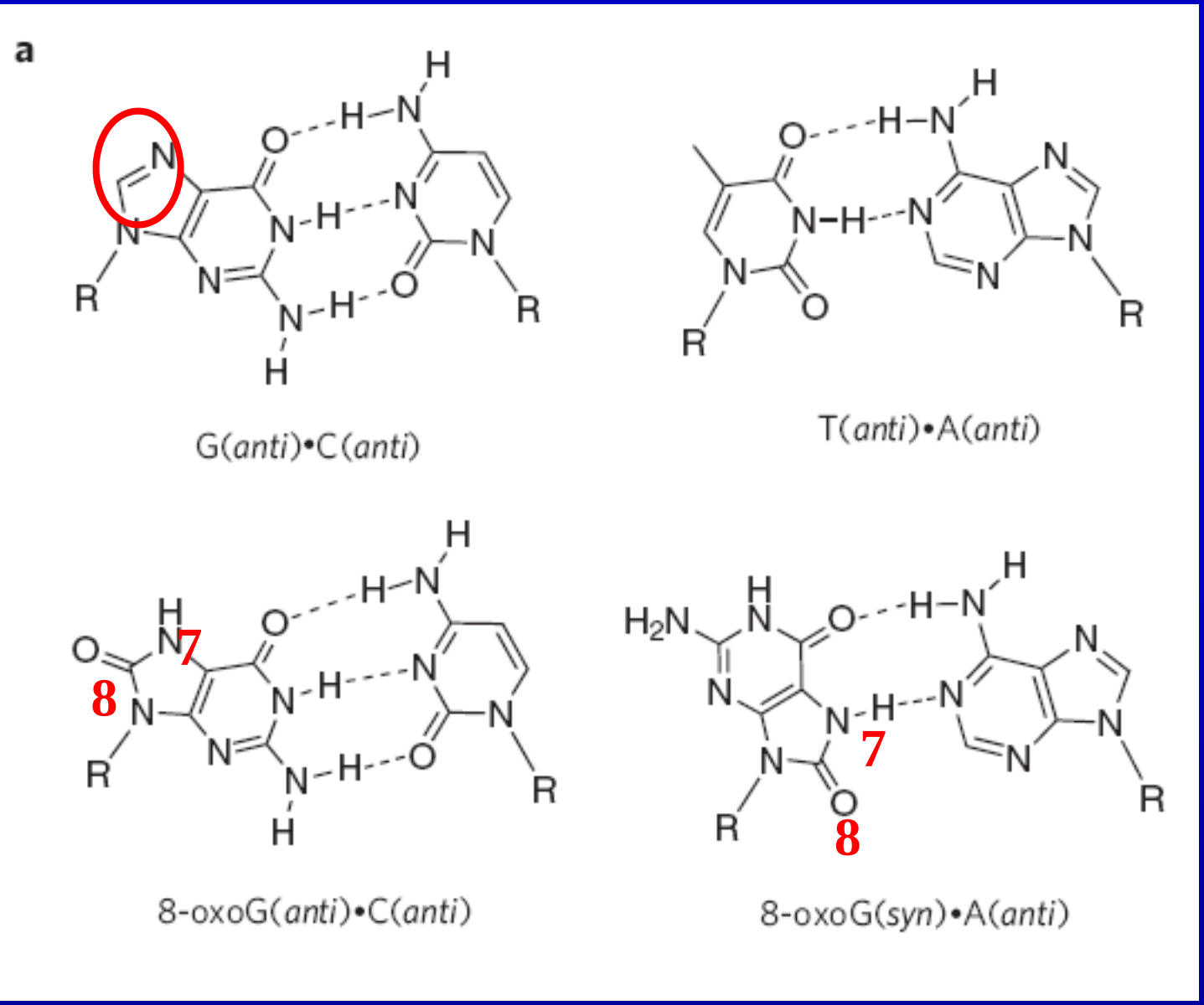
FapyG



Gh



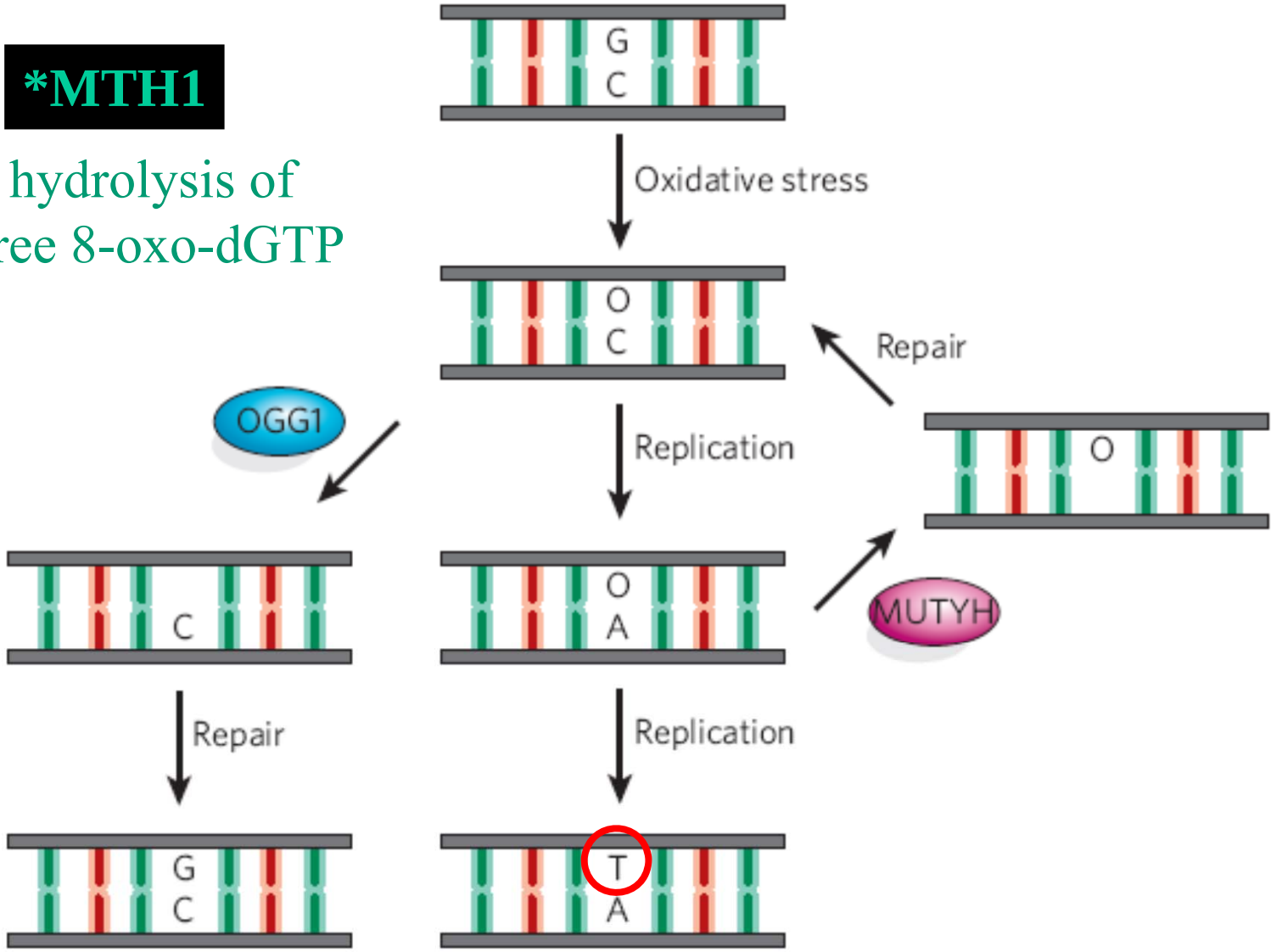
Sp

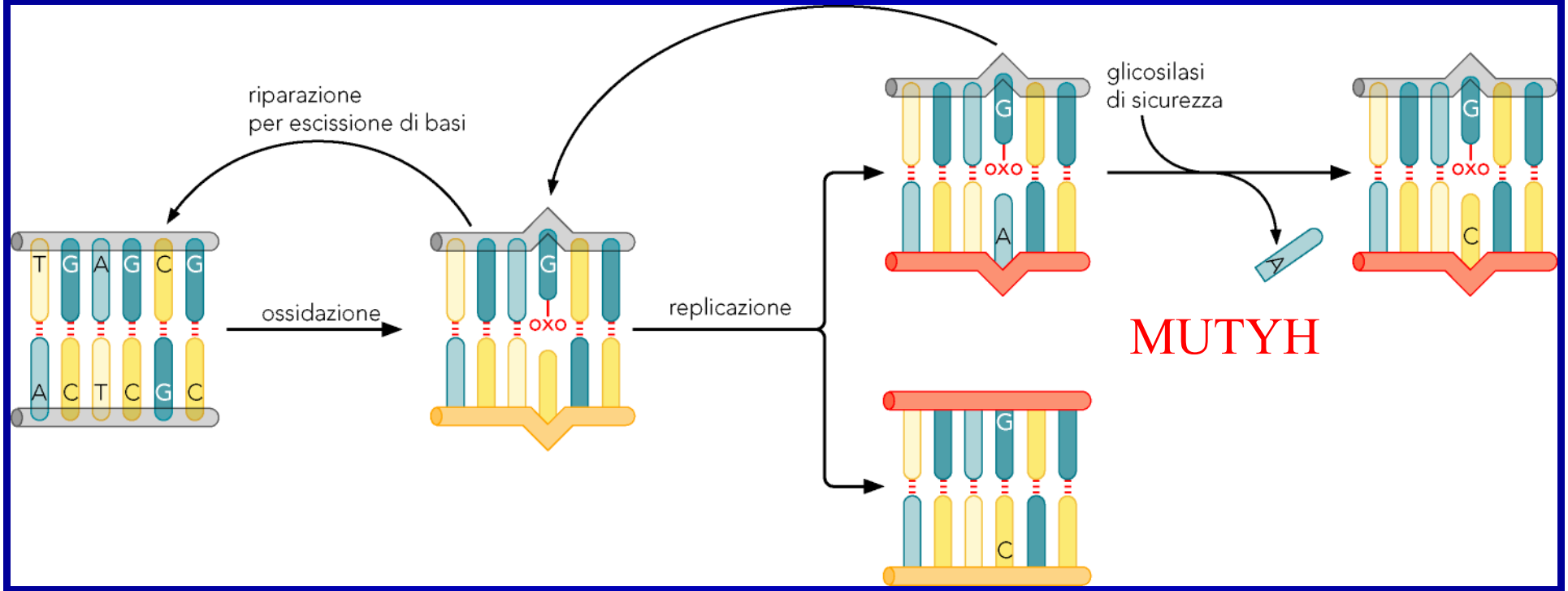

a
G(anti)•C(anti)
T(anti)•A(anti)
8
7
8-oxoG(anti)•C(anti)
7
8
8-oxoG(syn)•A(anti)

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

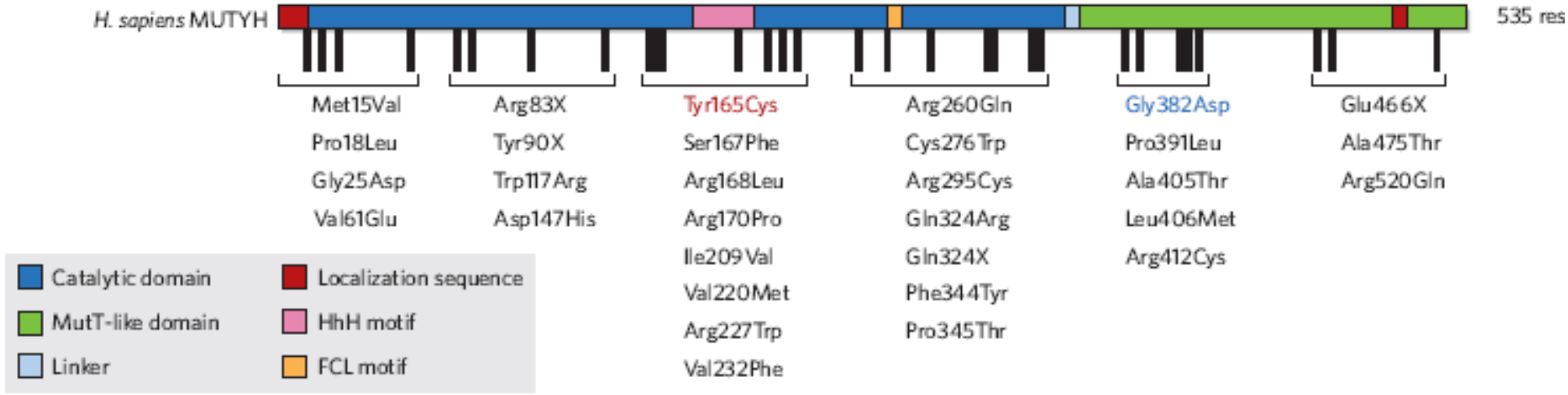
***MTH1**

hydrolysis of
free 8-oxo-dGTP



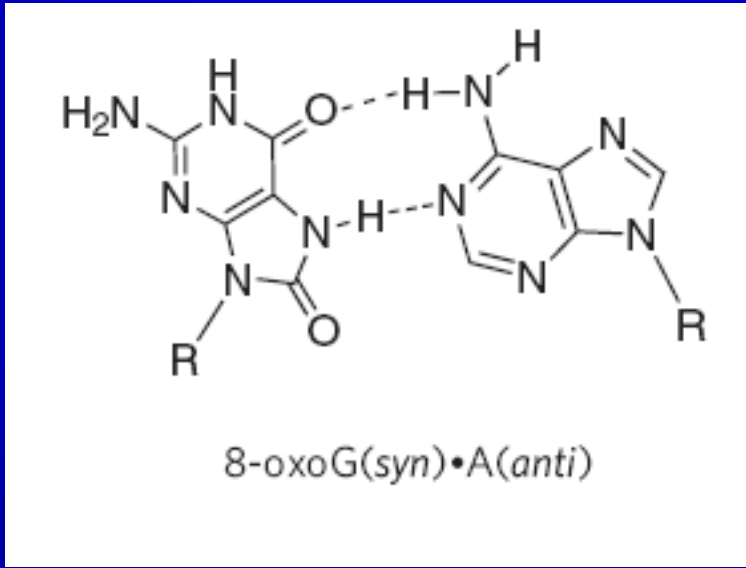

riparazione
per escissione di basi
T G A G C G
A C T C G C
ossidazione
G
oxo
replicazione
G
oxo
A
glicosilasi
di sicurezza
G
oxo
C
G
C
MUTYH

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**

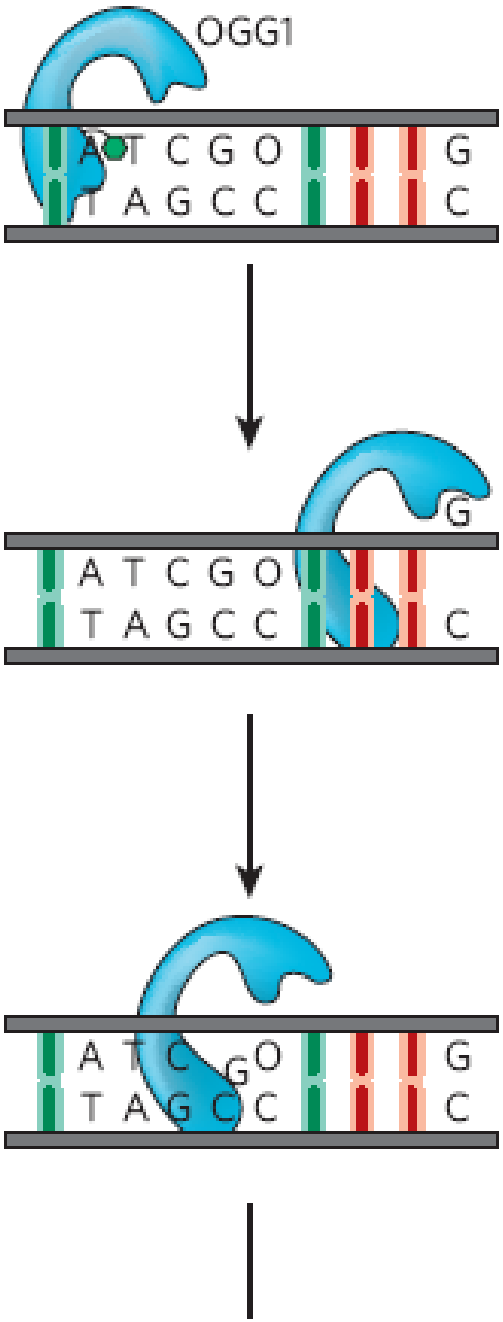
- Consistent with a global defect in 8-oxoG•A repair, a high proportion of tumours from patients with biallelic mutations in *MUTYH* have been observed to contain G-to-T transversions



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.

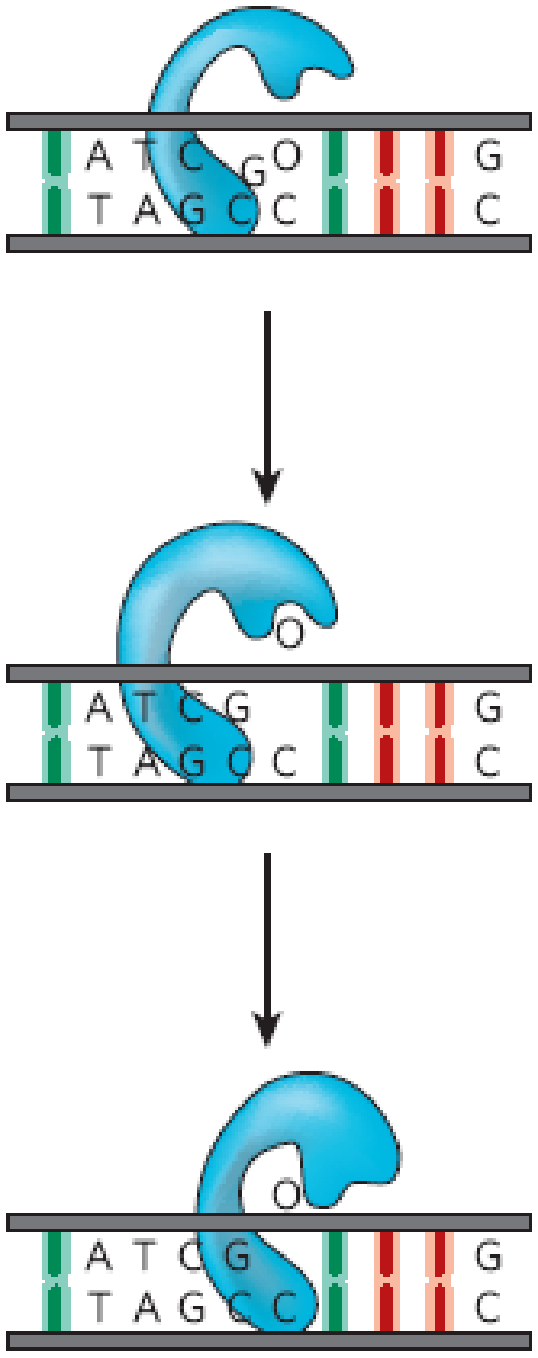


The 8-oxoG lesion search process.

- 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!!!!!!.

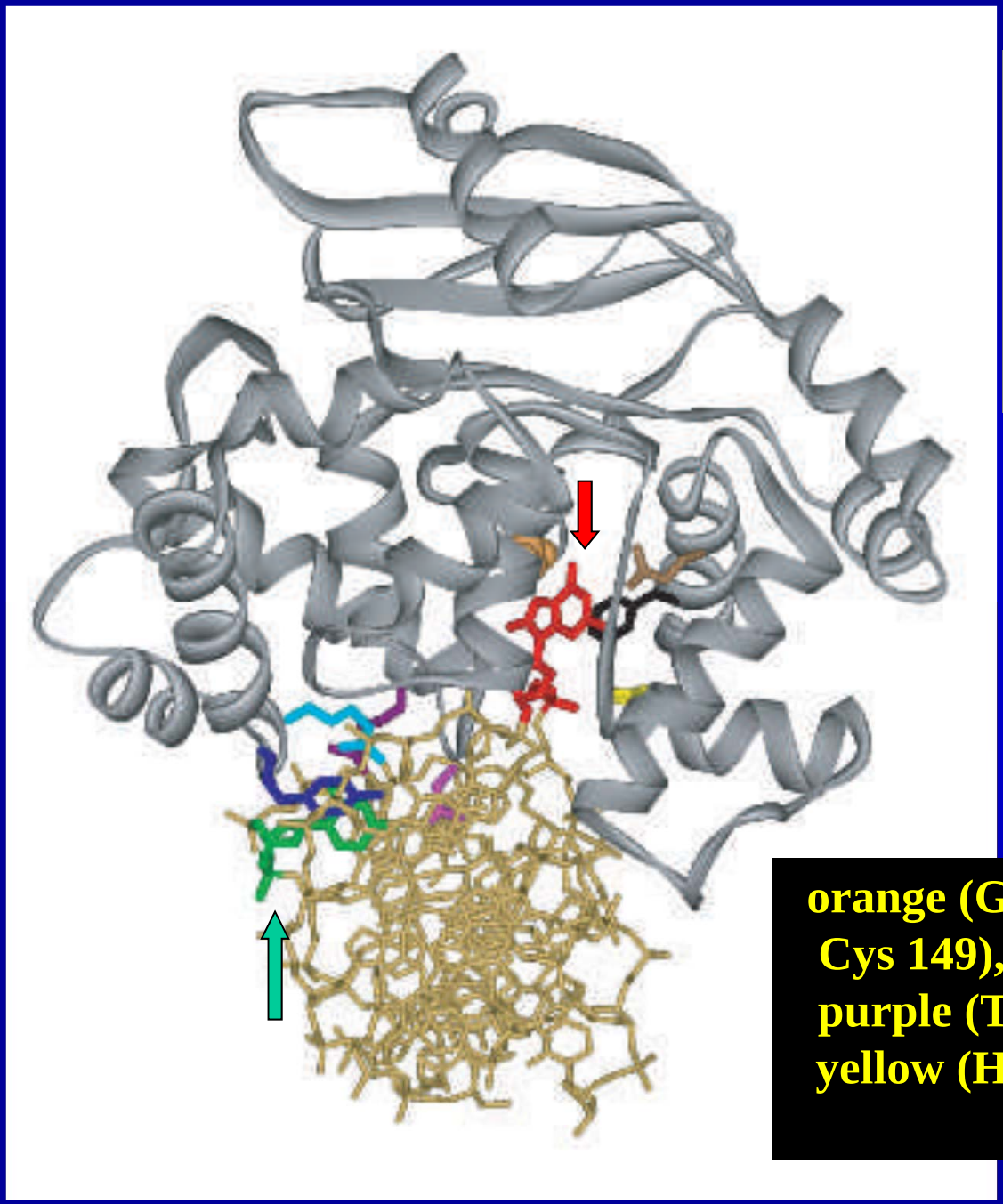
- OGG1 was found to move along the DNA with a diffusion constant approaching the theoretical upper limit for one-dimensional diffusion,indicating that OGG1 samples **millions of base pairs per second**.

- 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).

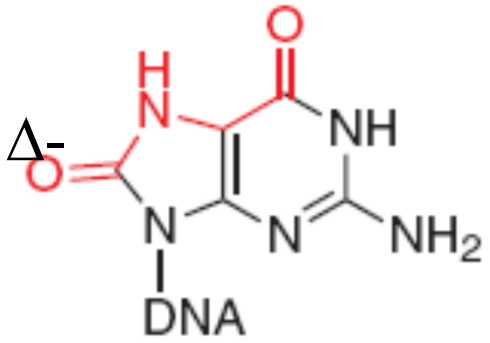
- the 8-oxoG is extruded to the exosite and captured in the 8-oxoG-specific pocket, where it is excised from the DNA.



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).



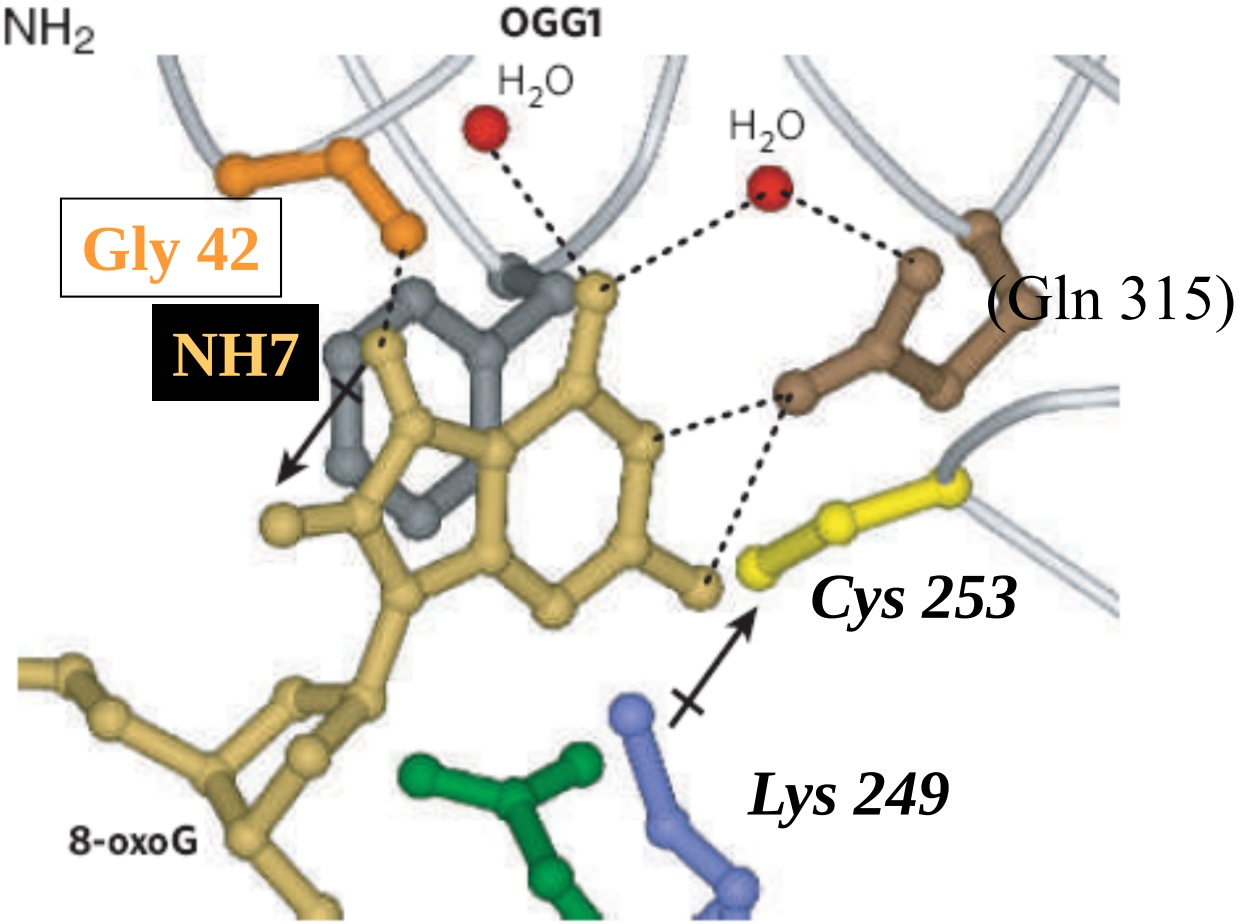
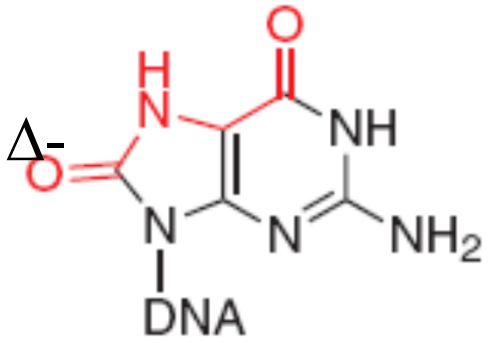
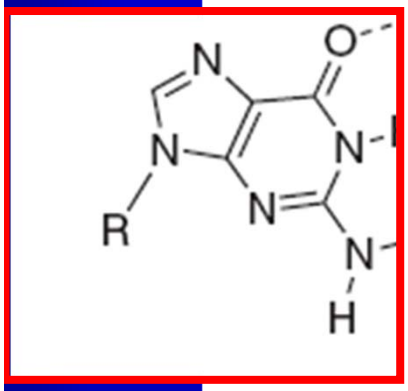


Figure 3 | Recognition of 8-oxoG by OGG1 observed in the LRC of OGG1 with 8-oxoG•C-containing duplexes. This is a view of the base-specific pocket

lesion-recognition complexes (LRCs).





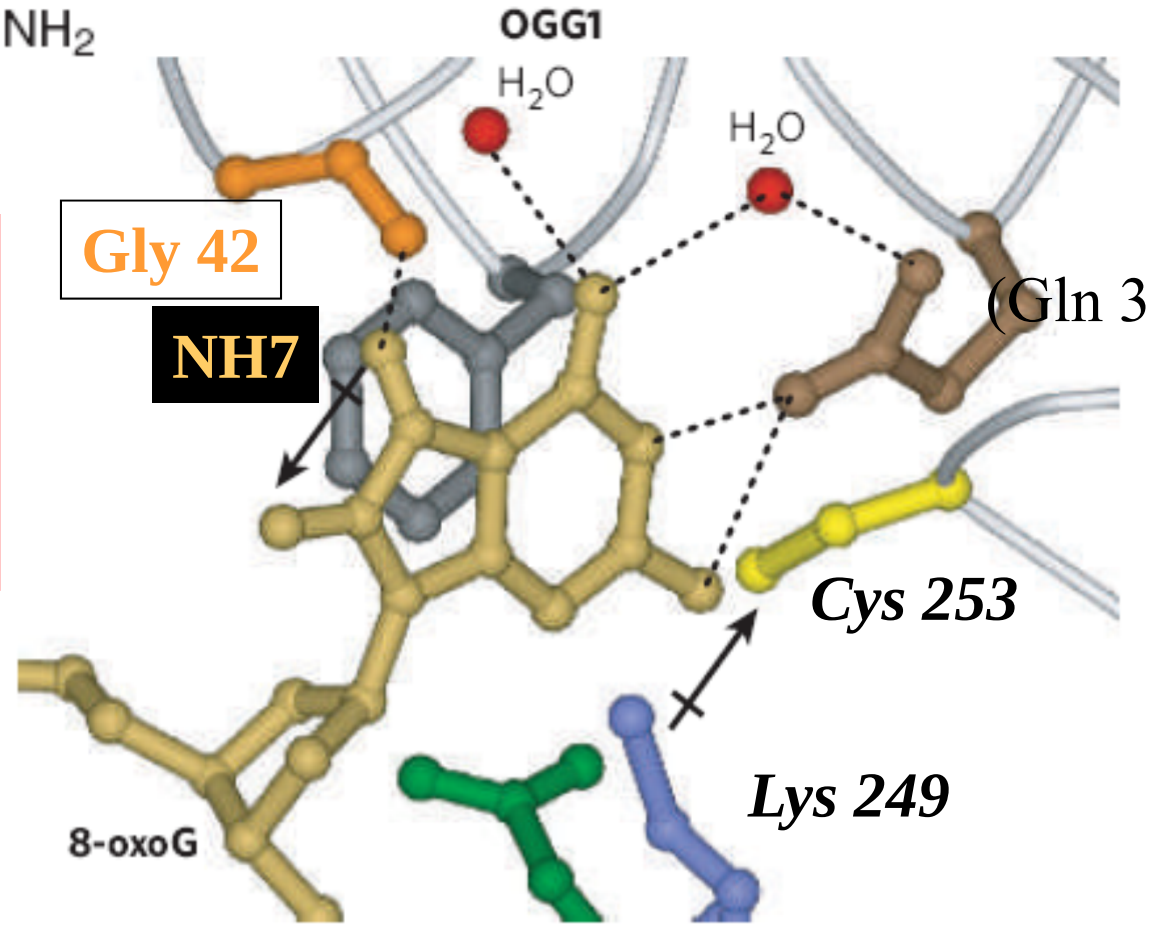


Figure 3 | Recognition of 8-oxoG by OGG1 observed in the LRC of OGG1 with 8-oxoG•C-containing duplexes. This is a view of the base-specific pocket

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

↓

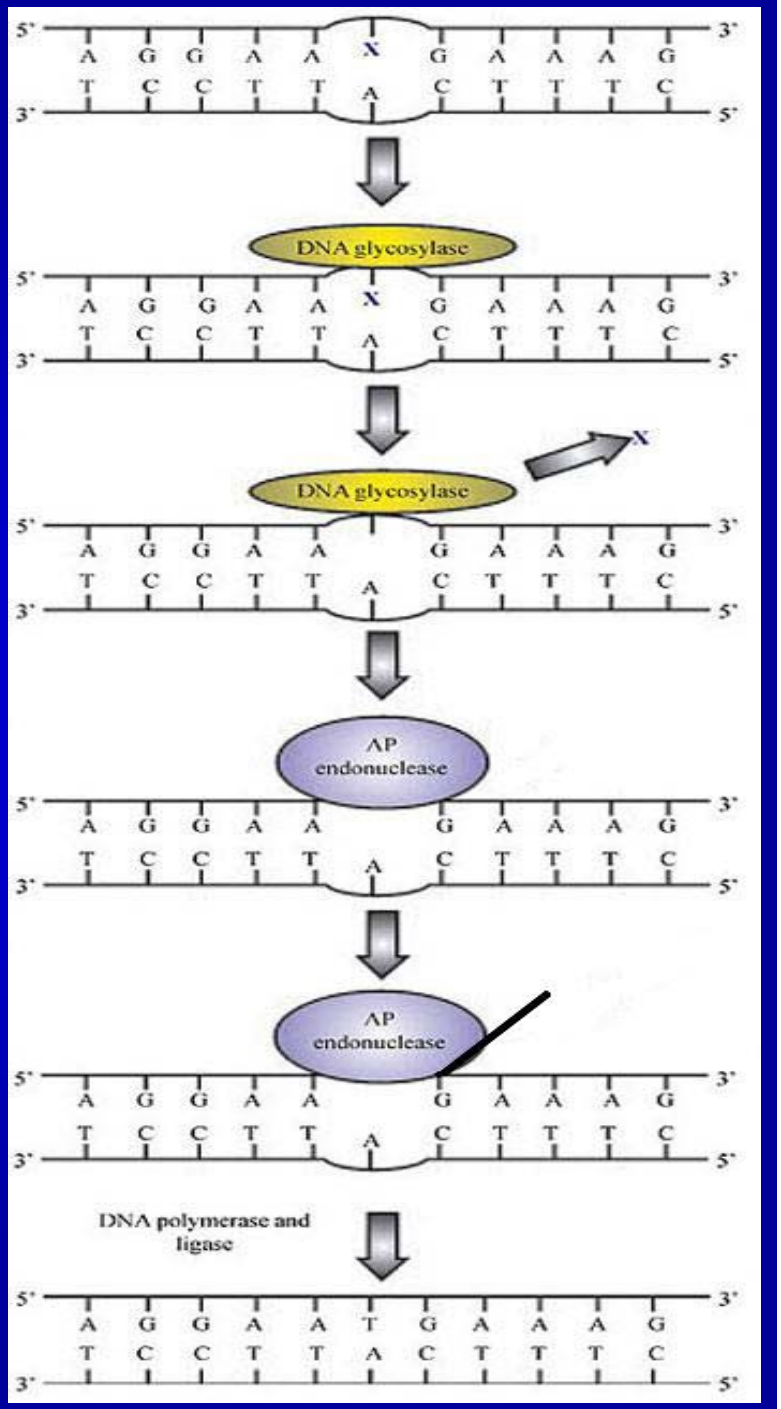
Formazione di un sito AP che viene riconosciuto da **APE1** (AP endonucleasi) → APE1 taglia il singolo filamento in 5' al sito AP

↓

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

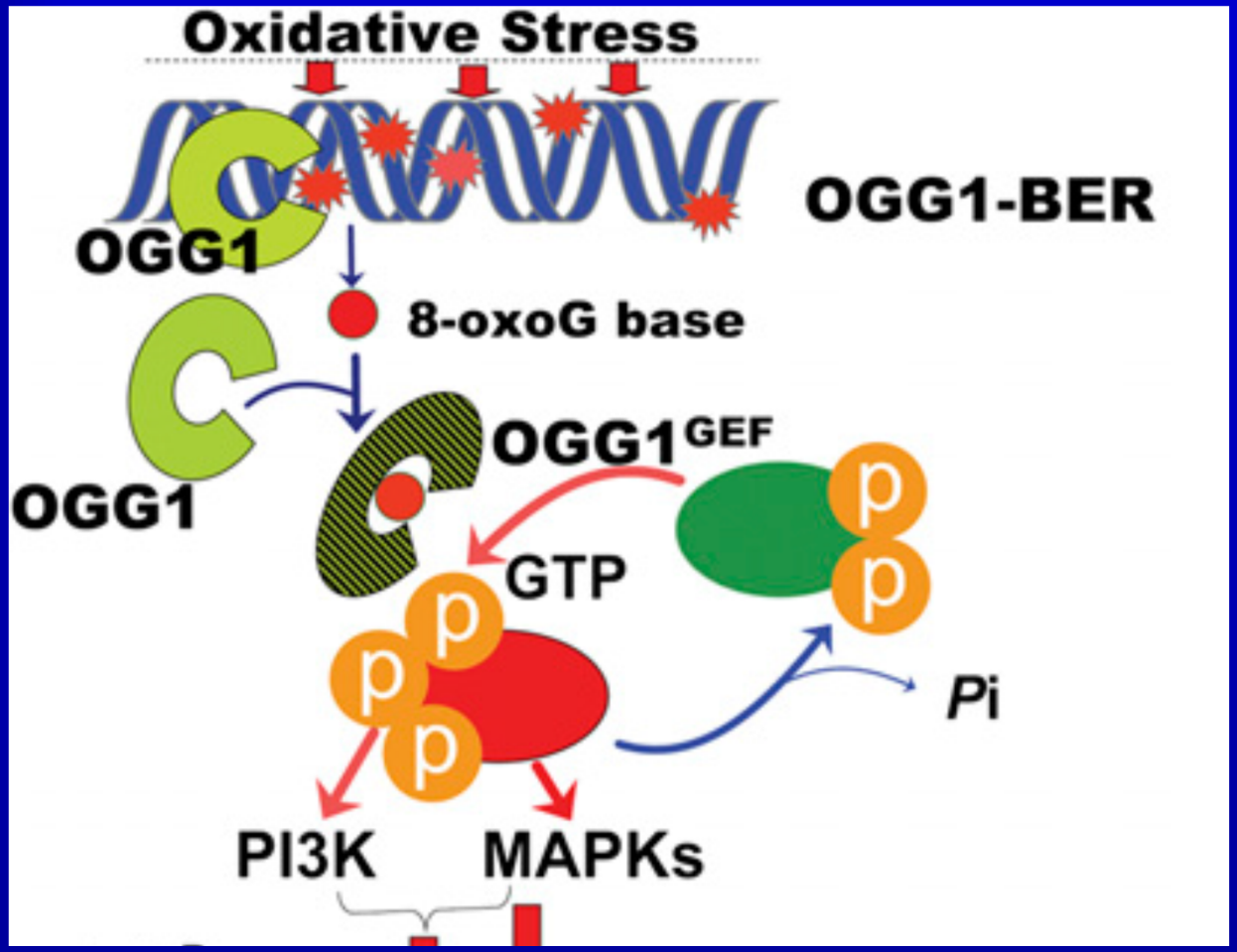
↓

La **ligasi** richiude l'elica riparata



OXOGUANINA/BER e INFIAMMAZIONE/ TRASCRIZIONE

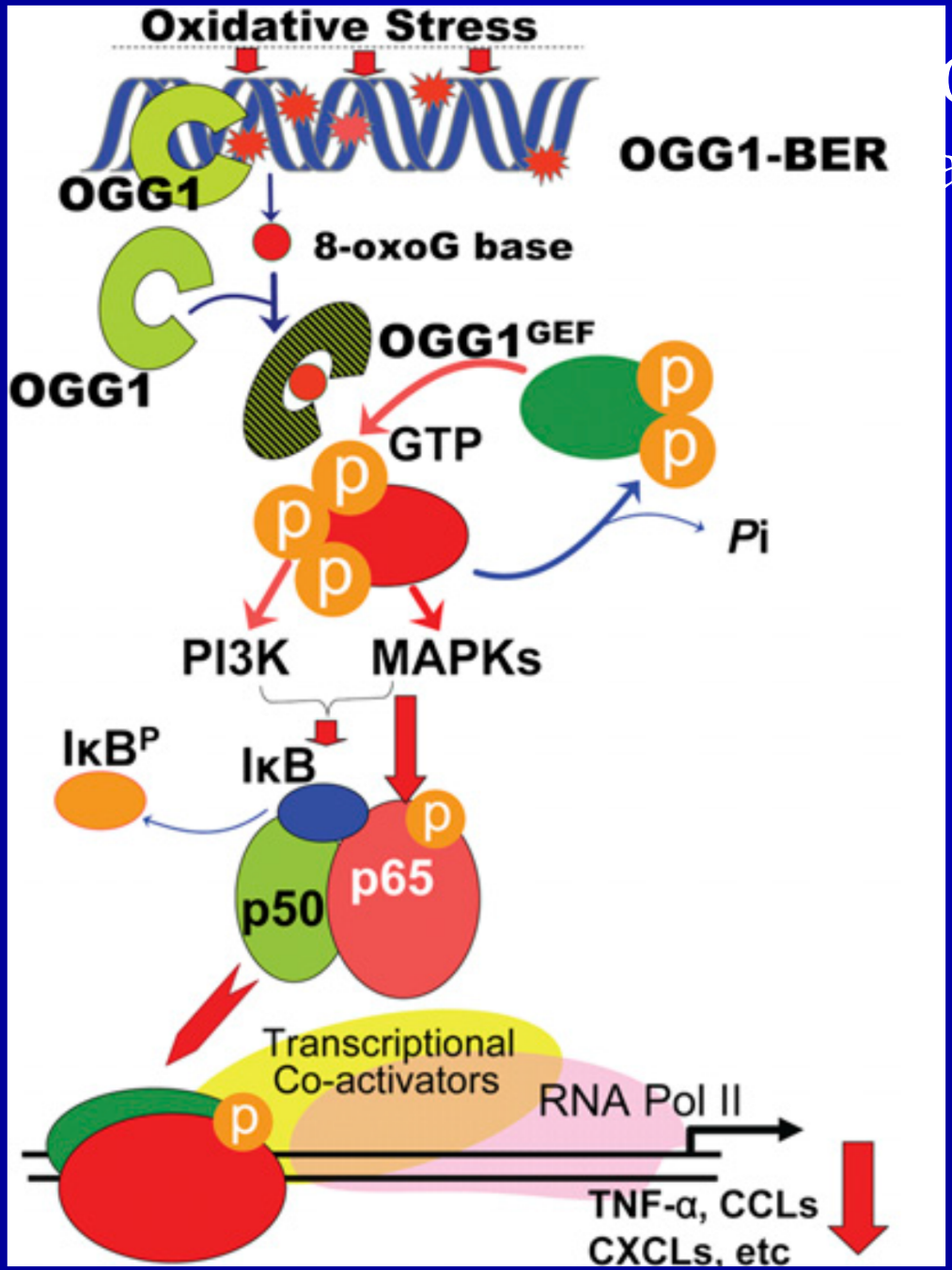
OGG1-initiated DNA base excision repair and **inflammation**



GDP→GTP exchange

RAS-GTP–driven signaling

Innate Inflammation Induced by the 8-Oxoguanine DNA



OGG1-initiated DNA
ase excision repair and

inflammation

GDP→GTP exchange

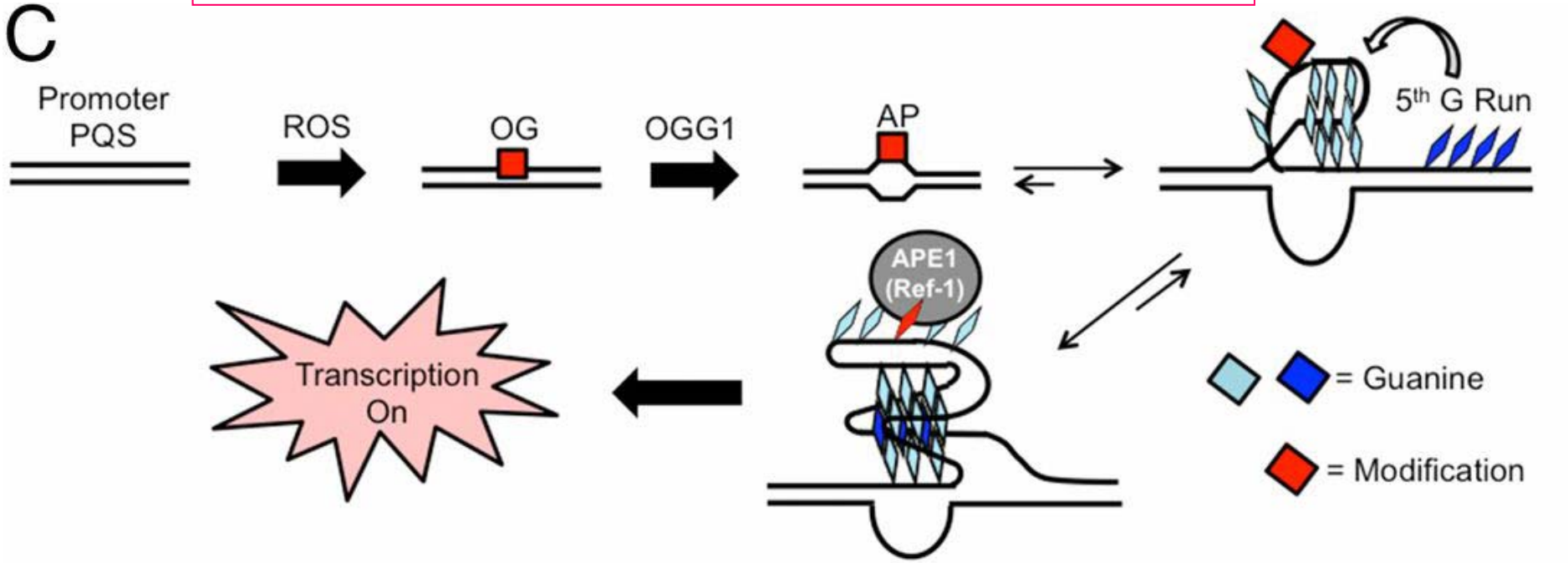
RAS-GTP–driven signaling

NF-kB activation

proinflammatory
chemokine/cytokine
expression

8-OxoG represents an epigenetic modification

potential G-quadruplex–forming sequences (PQS)

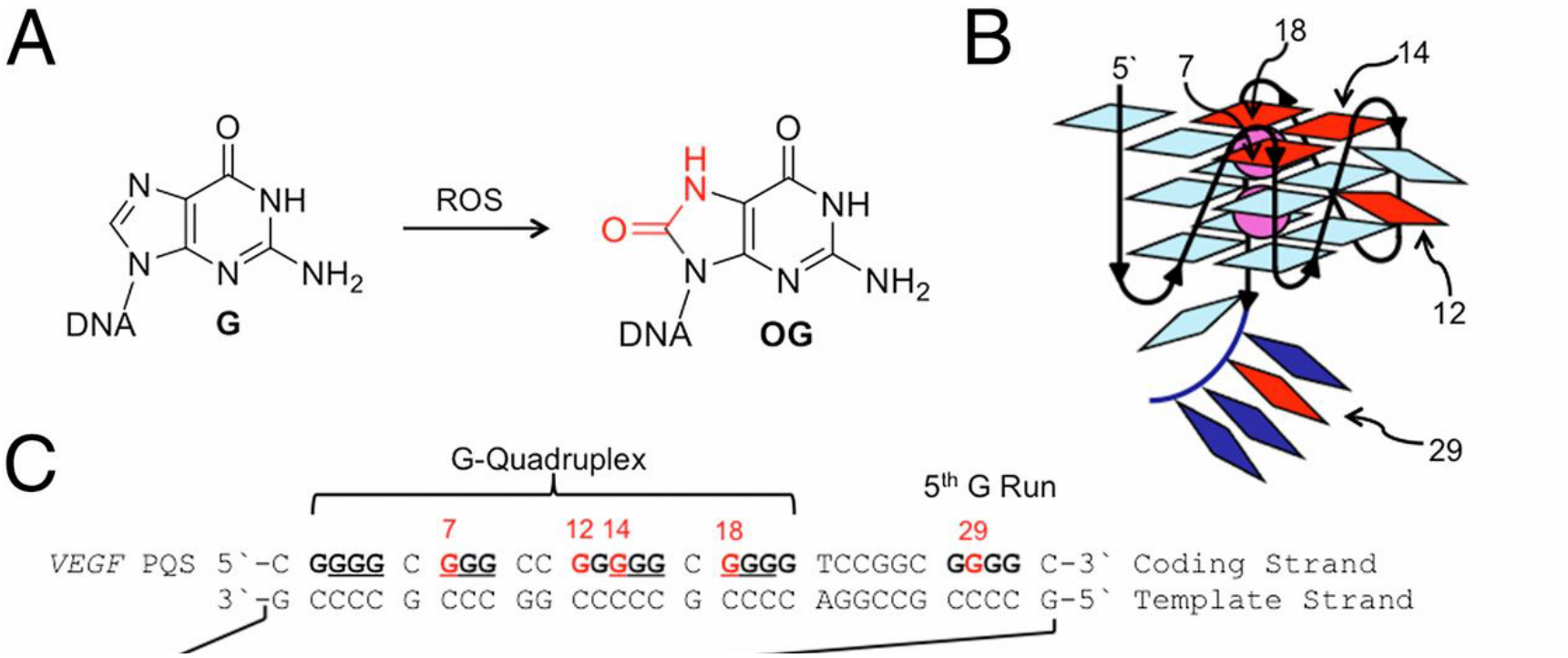


apurinic/apyrimidinic endonuclease 1 (APE1)

initiation of OG removal in the VEGF promoter PQS induces binding of APE1 and activation of transcription (MODEL)

Aaron M. Fleming et al. PNAS 2017;114:2604-2609

Oxidation of G in the VEGF PQS induces transcription.



potential G-quadruplex–forming sequences (PQS)

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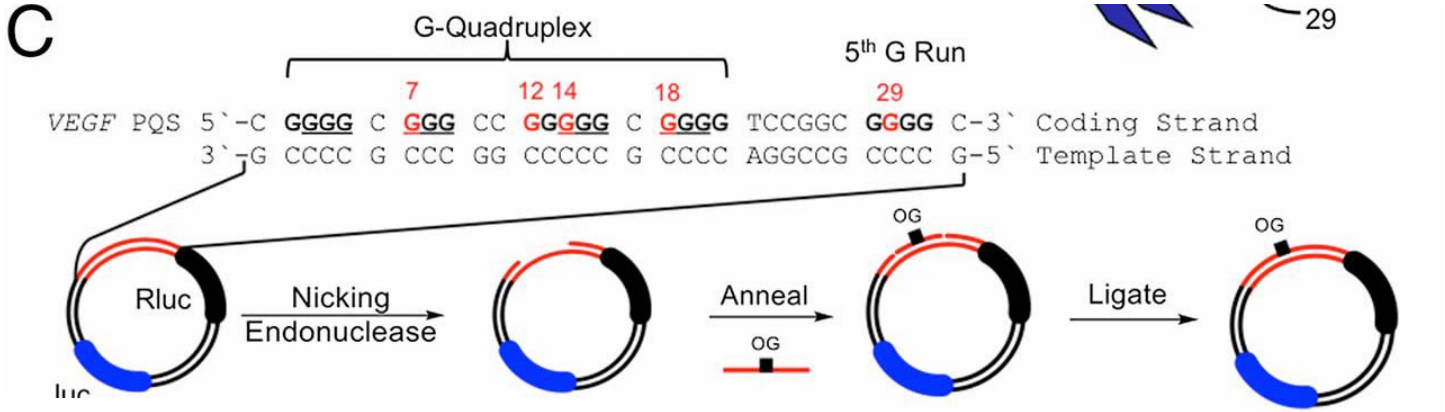
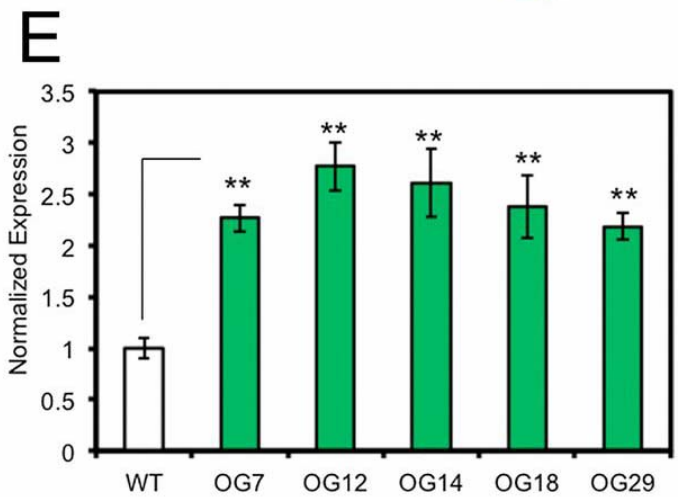


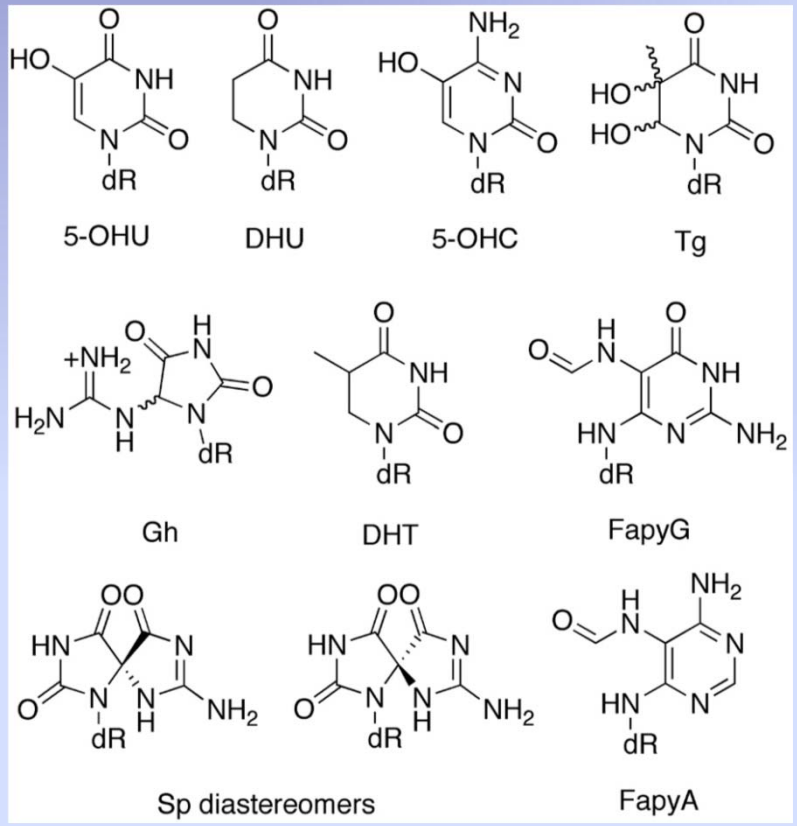


expression at 48 h posttransfection of OG-containing reporters in glioblastoma cells. WT plasmid with the VEGF PQS with undamaged Gs

Aaron M. Fleming et al. PNAS 2017;114:2604-2609

BER: neil1

Known substrates for the base excision repair glycosylase NEIL1.



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
5′ – TGT TCA TCA TGC TCXT GGT ATA TCC CAT – 3′
3′ – ACA AGT AGT ACG G C CCA TAT AGG GTA – 5′
A T
TTCT

Bulge
5′ – TGT TCA TCA TGC GTC X TC GGT ATA TCC CAT – 3′
3′ – ACA AGT AGT ACG CAG – AG CCA TAT AGG GTA – 5′

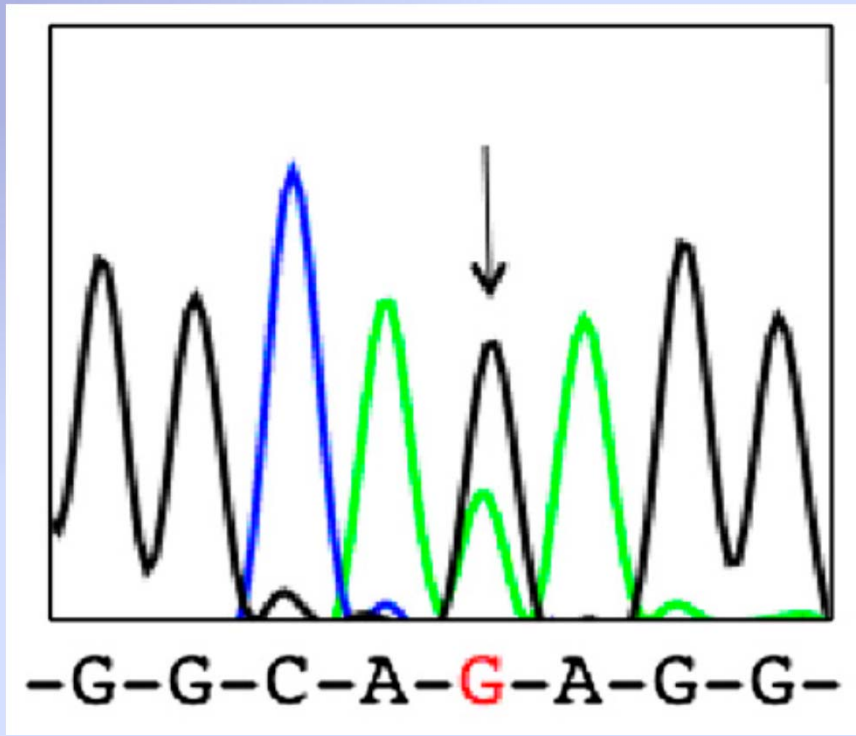
X = Gh, Sp, or Tg

- 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

NEIL1 mRNA sequencing



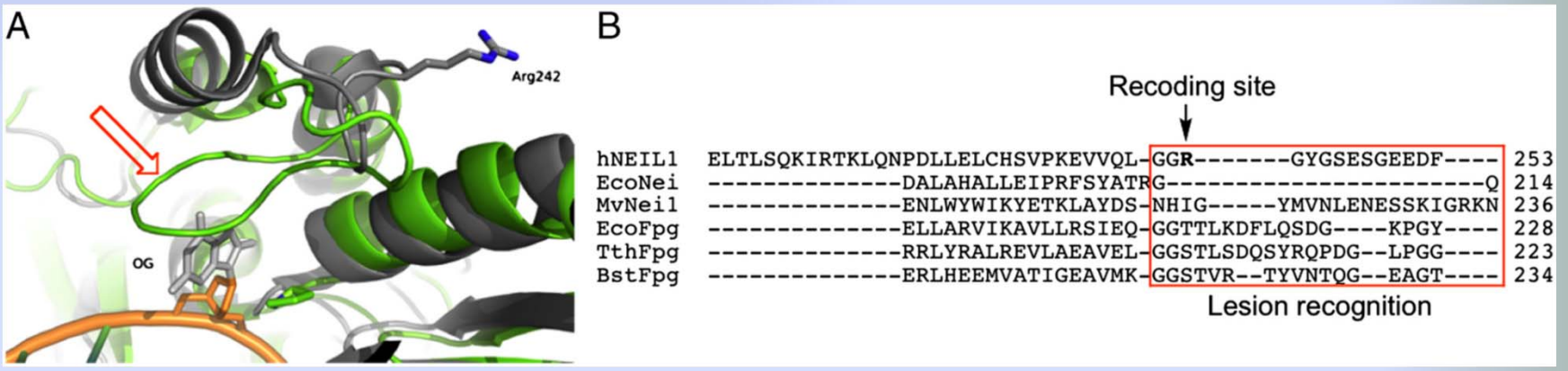
mRNA Editing

A to G

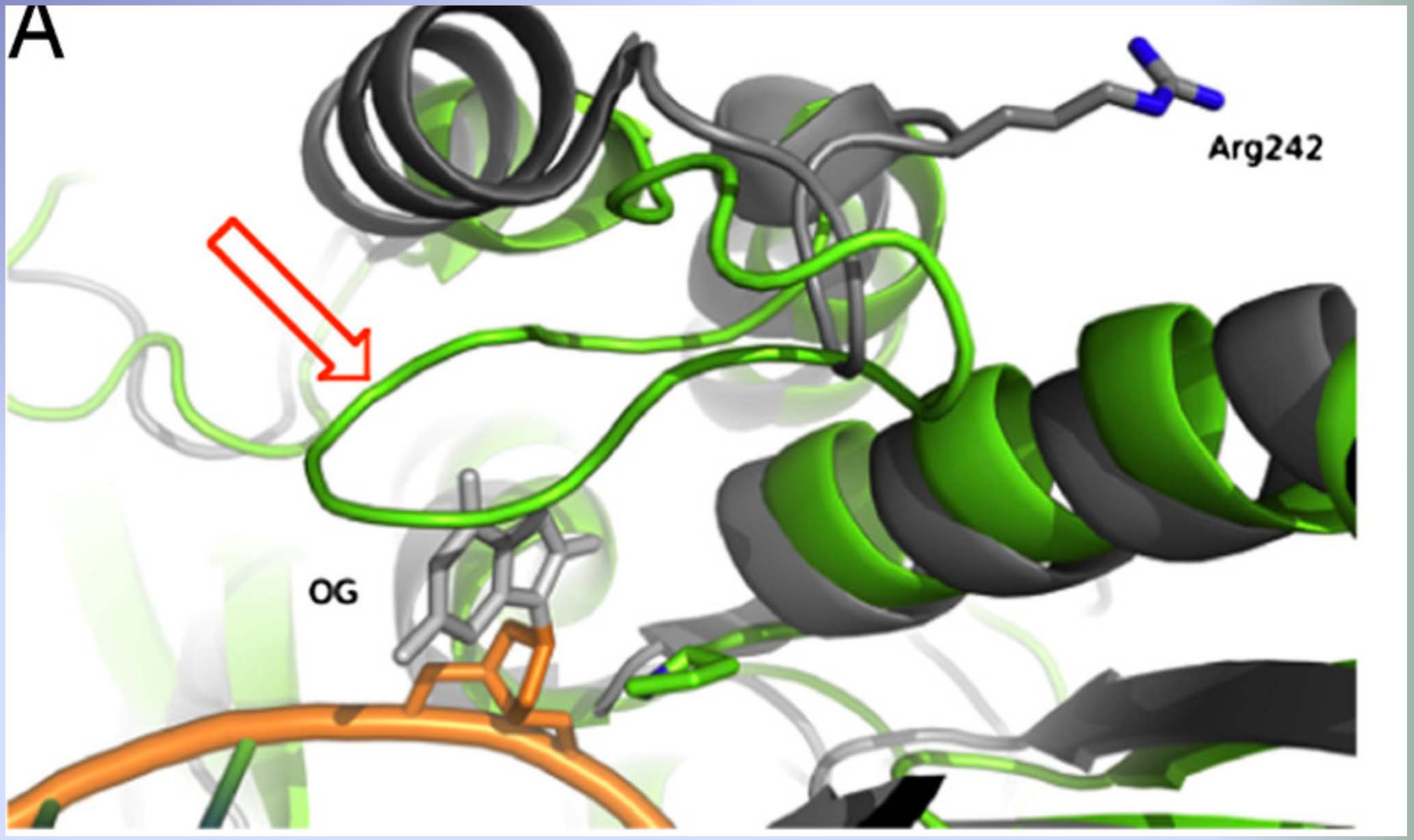
AAA to AIA (AGA)

R to K

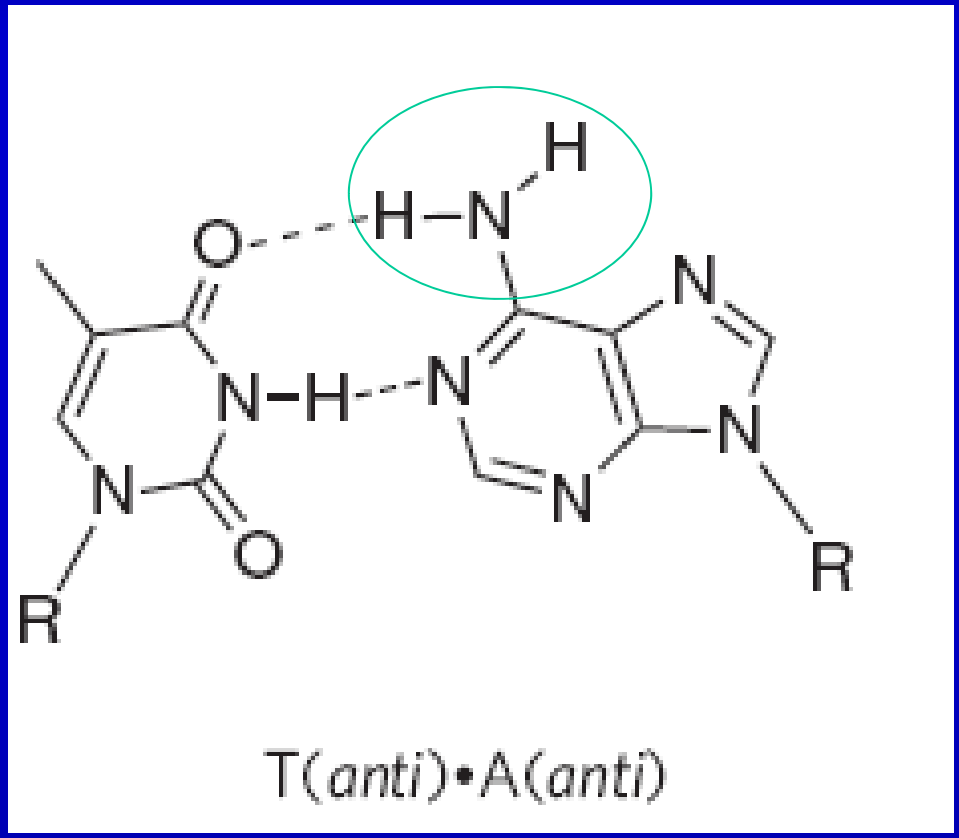
(A) Superposition of human NEIL1 structure (dark gray) with that of E. coli Fpg (green) bound to 8-oxoguanine-containing DNA. Red open arrow indicates lesion recognition loop of Fpg.
(B)B) Sequence alignment of Fpg/Nei family of DNA repair glycosylases indicating the position of the hNEIL1 recoding site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



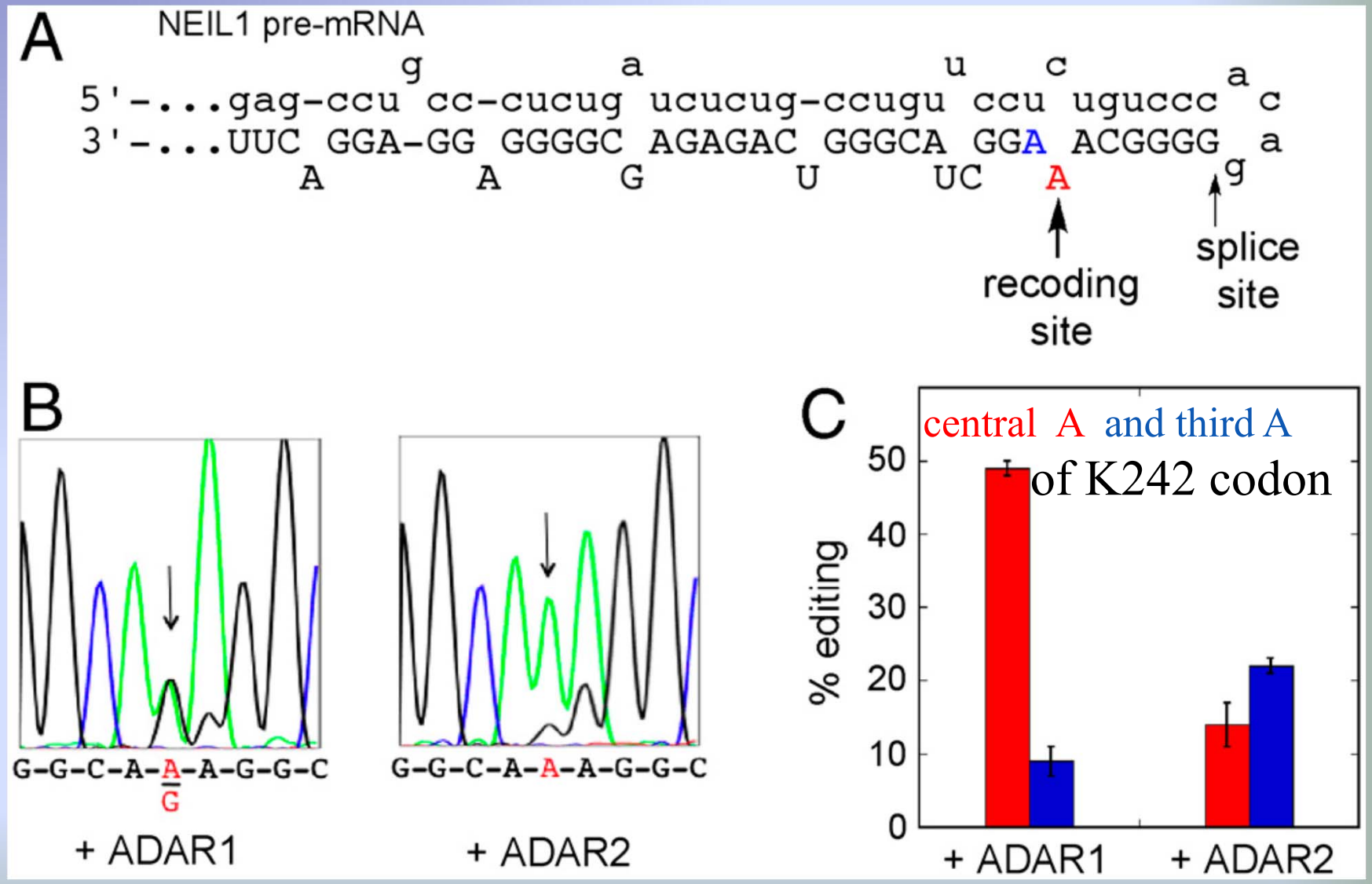
Superposition of human NEIL1 structure (dark gray) with that of E. coli Fpg (OGG1, green) bound to 8-oxoguanine-containing DNA.



T(*anti*)•A(*anti*)

ADAR catalizza la deaminazione ossidativa di specifiche adenine

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

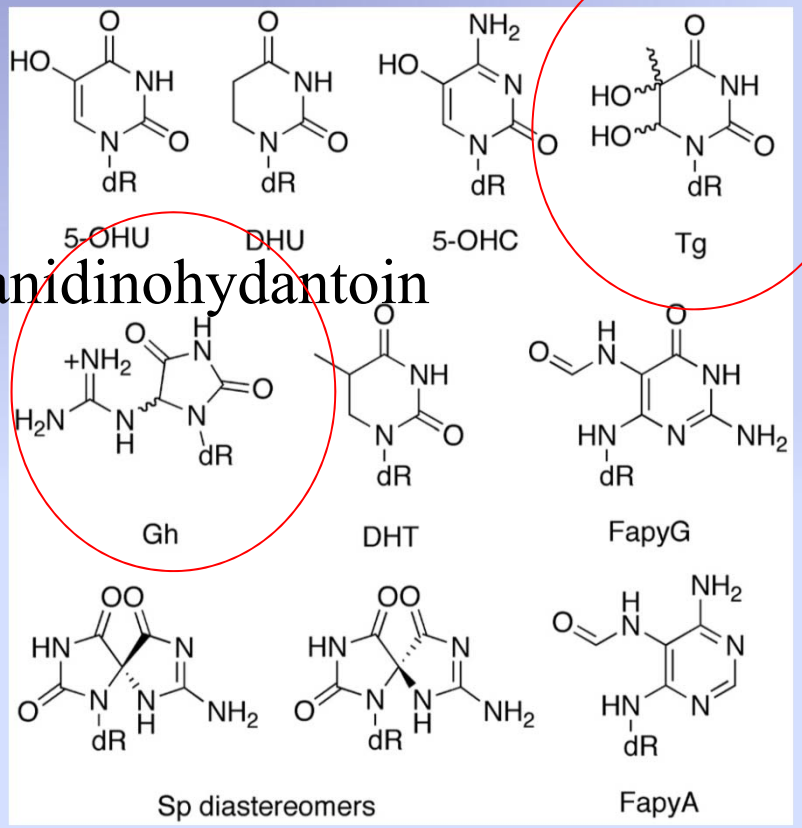


Editing of the pre-mRNA for the DNA repair enzyme NEIL1 causes a lysine to arginine change in the lesion recognition loop of the protein.

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′

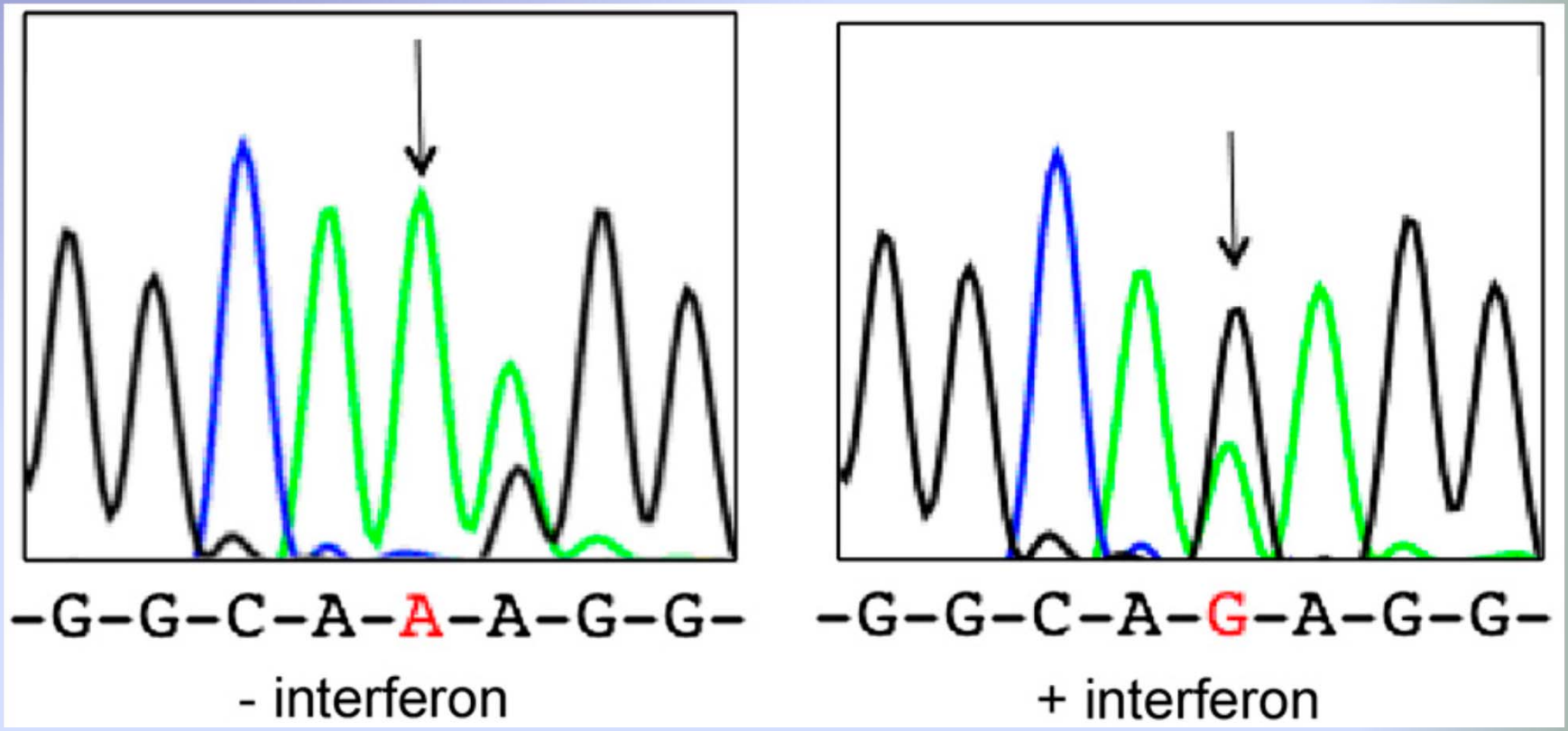
X = Gh, Sp, or Tg

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.

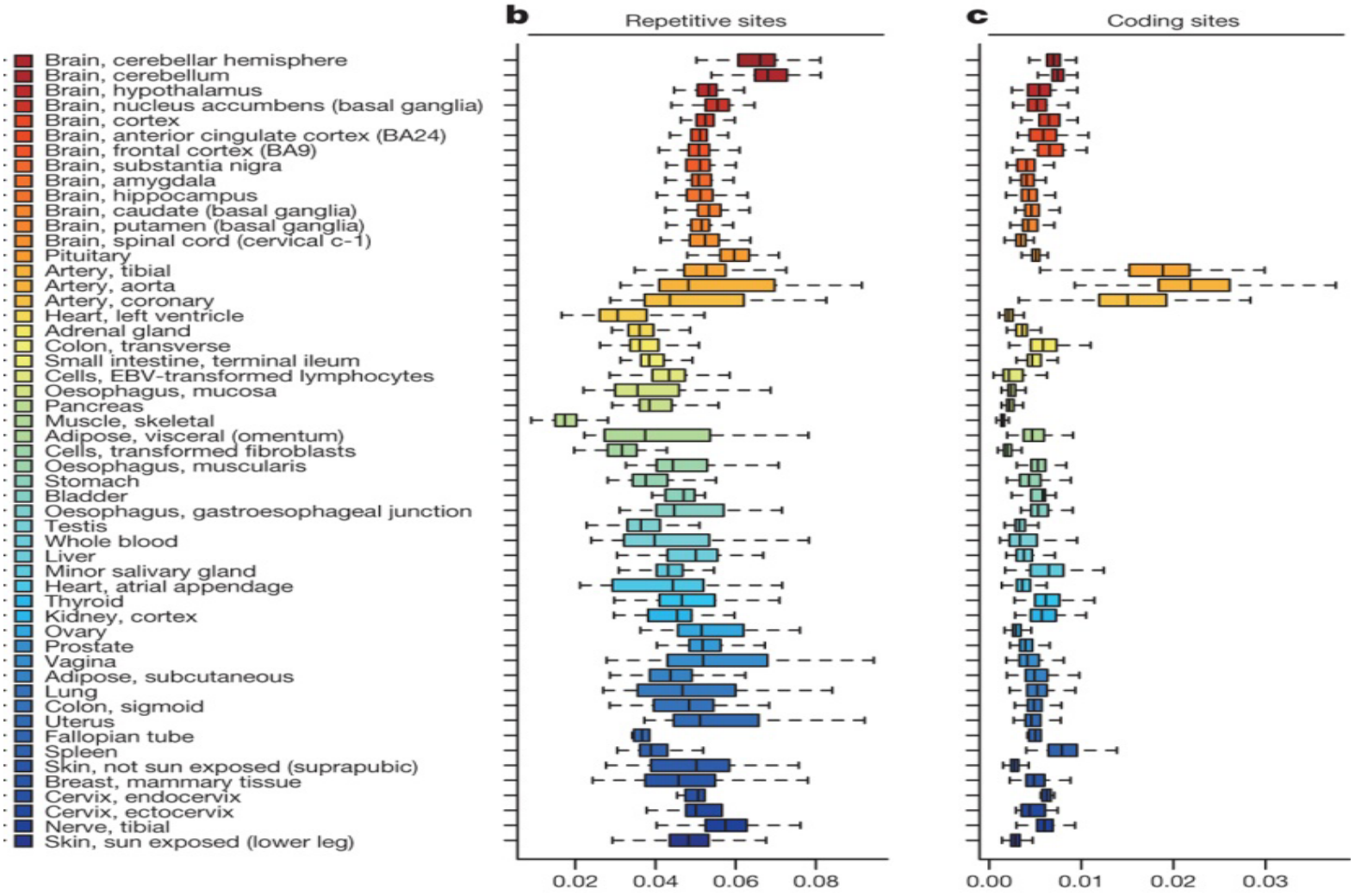
Three human ADAR
(adenosine deaminase acting on RNA)-family members

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

ADAR2 localizes predominantly in the nucleolus

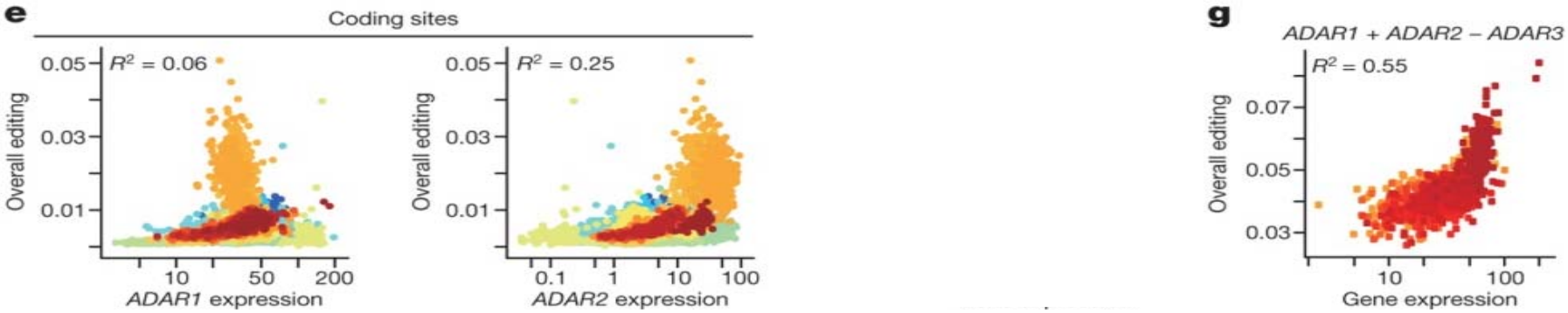
The Genotype-Tissue Expression Consortium multi-tissue RNA editome

editing levels of repetitive (b) or non-repetitive (c) coding sites in various human tissues



M H Tan *et al. Nature* **550,** 249–254 (2017) doi:10.1038/nature24041

nature



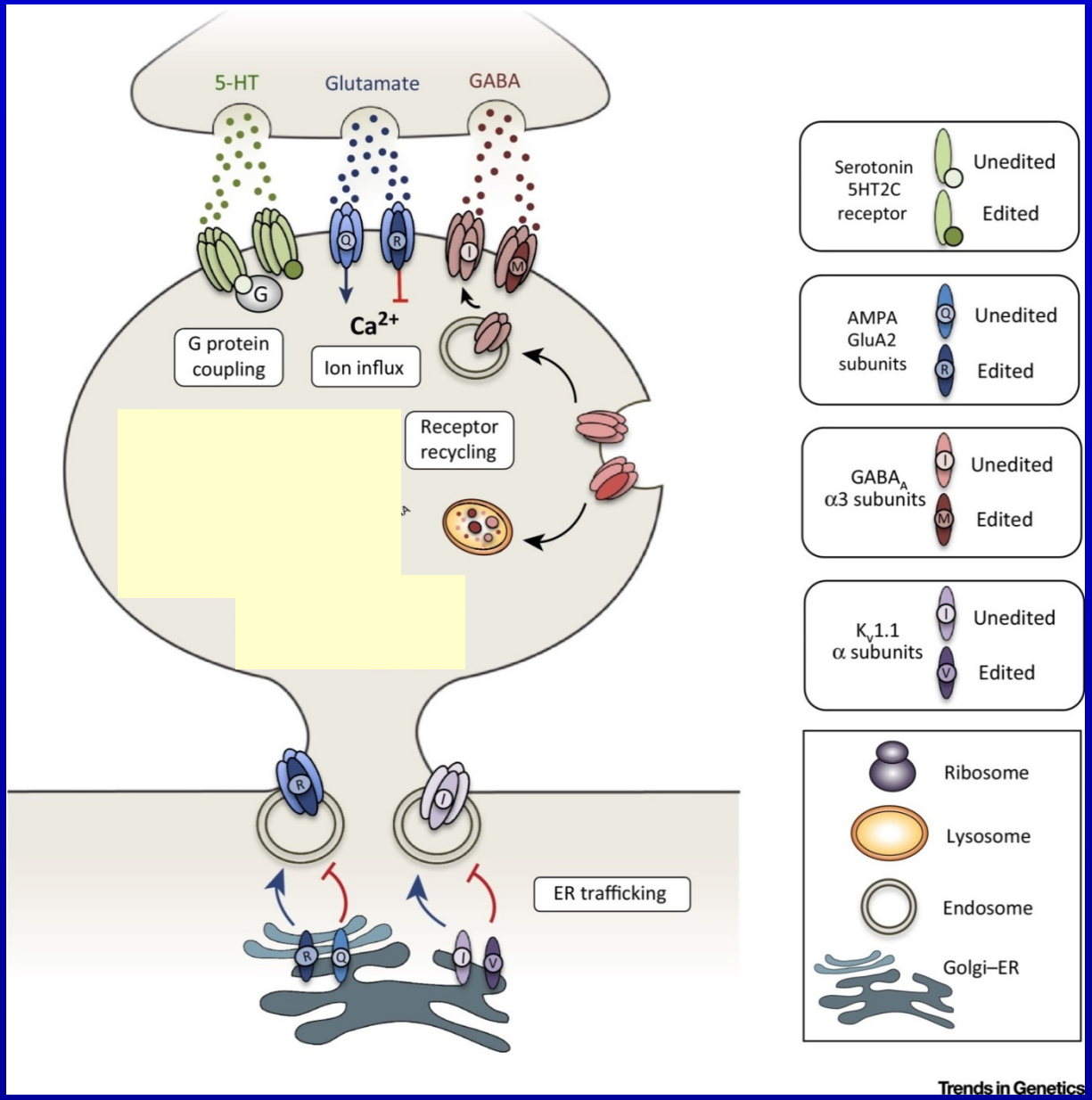
Correlations between expression levels of ADAR1/2 and overall editing levels of non-repetitive (e) coding sites
g, Correlation of ADAR1 and ADAR2 expression with overall editing of all sites in the brain tissues

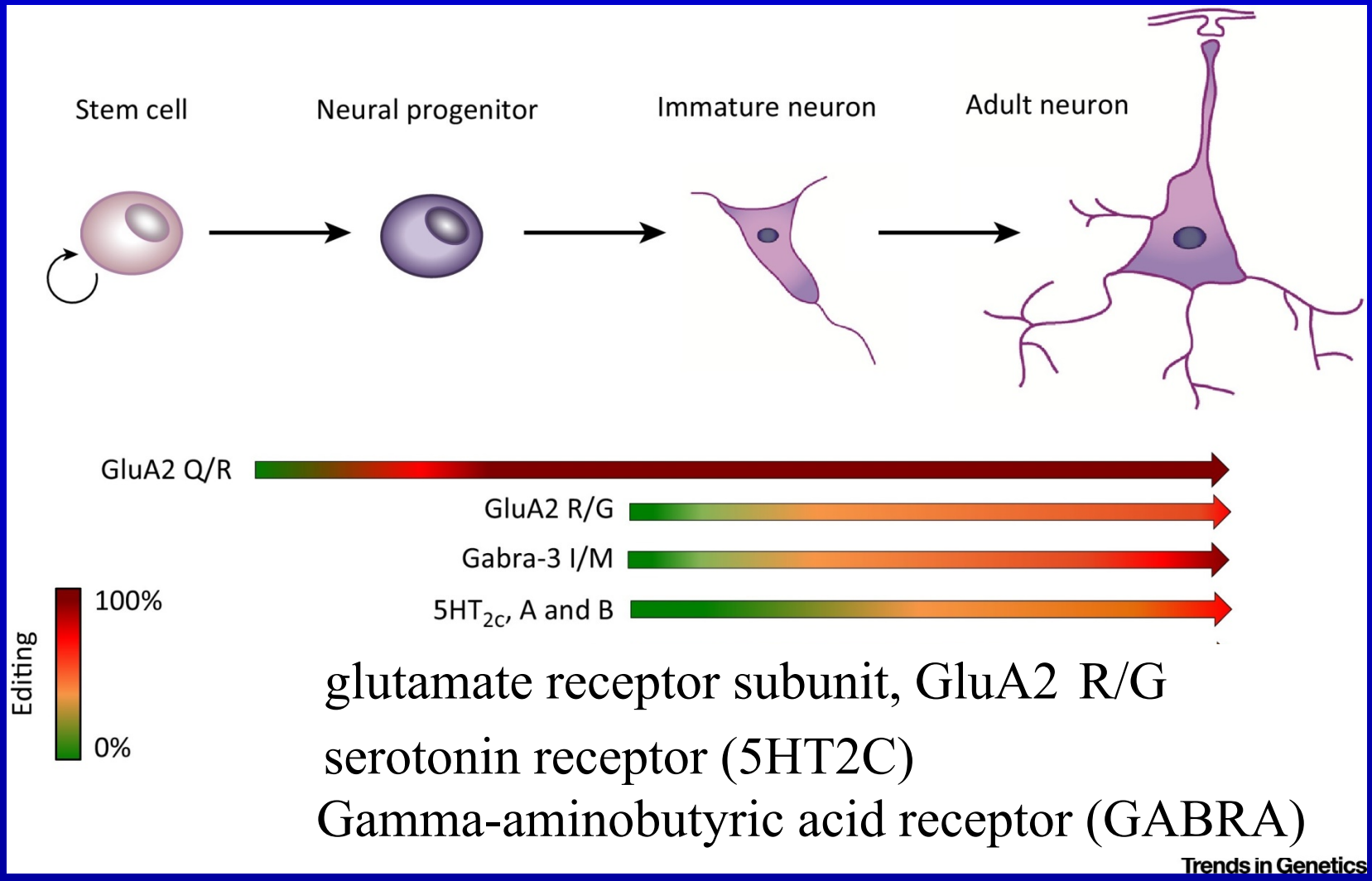
The Genotype-Tissue Expression Consortium multi-tissue RNA editome

M H Tan *et al. Nature* **550,** 249–254 (2017) doi:10.1038/nature24041

nature

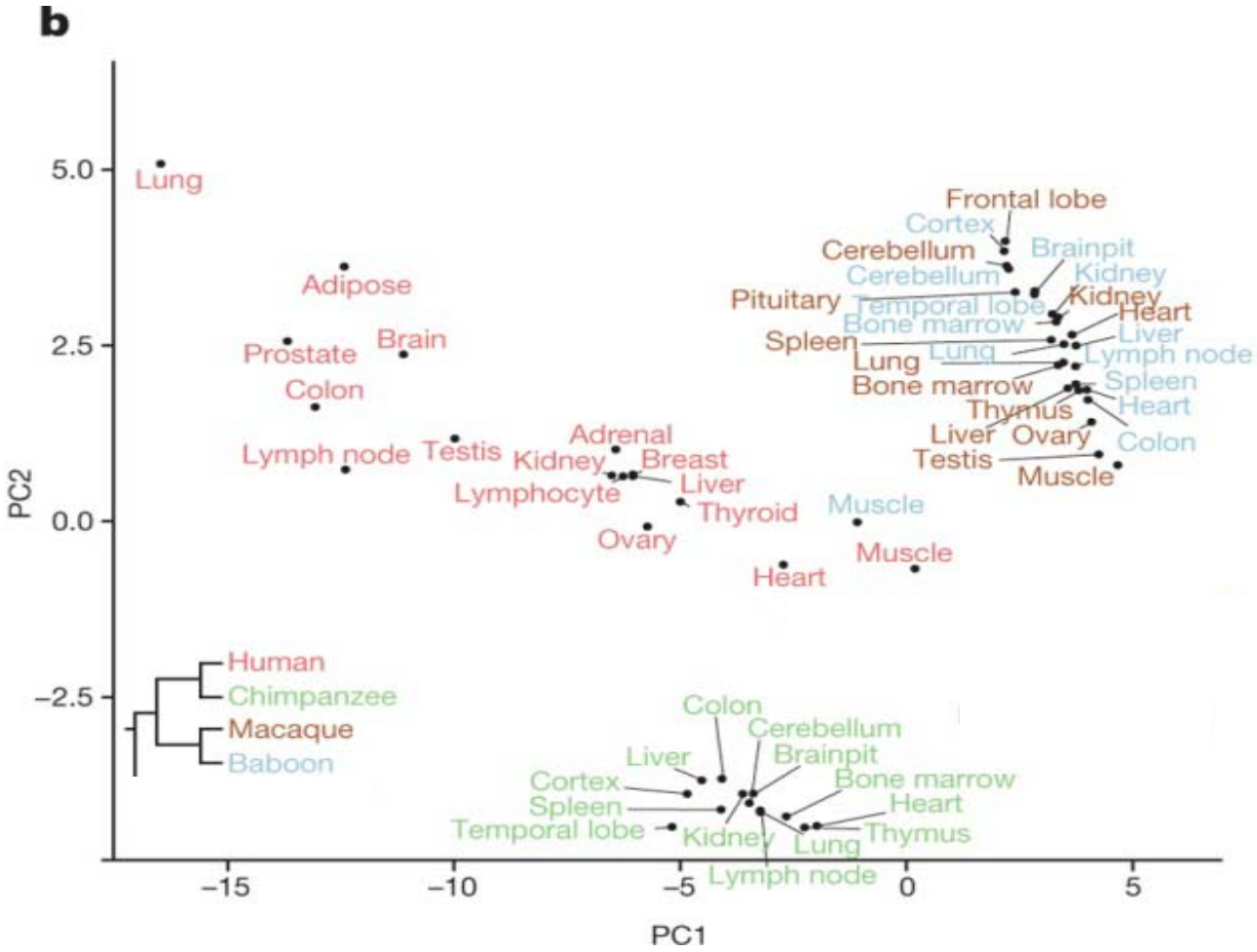
RNA Editing Events Contribute to Synaptic Plasticity





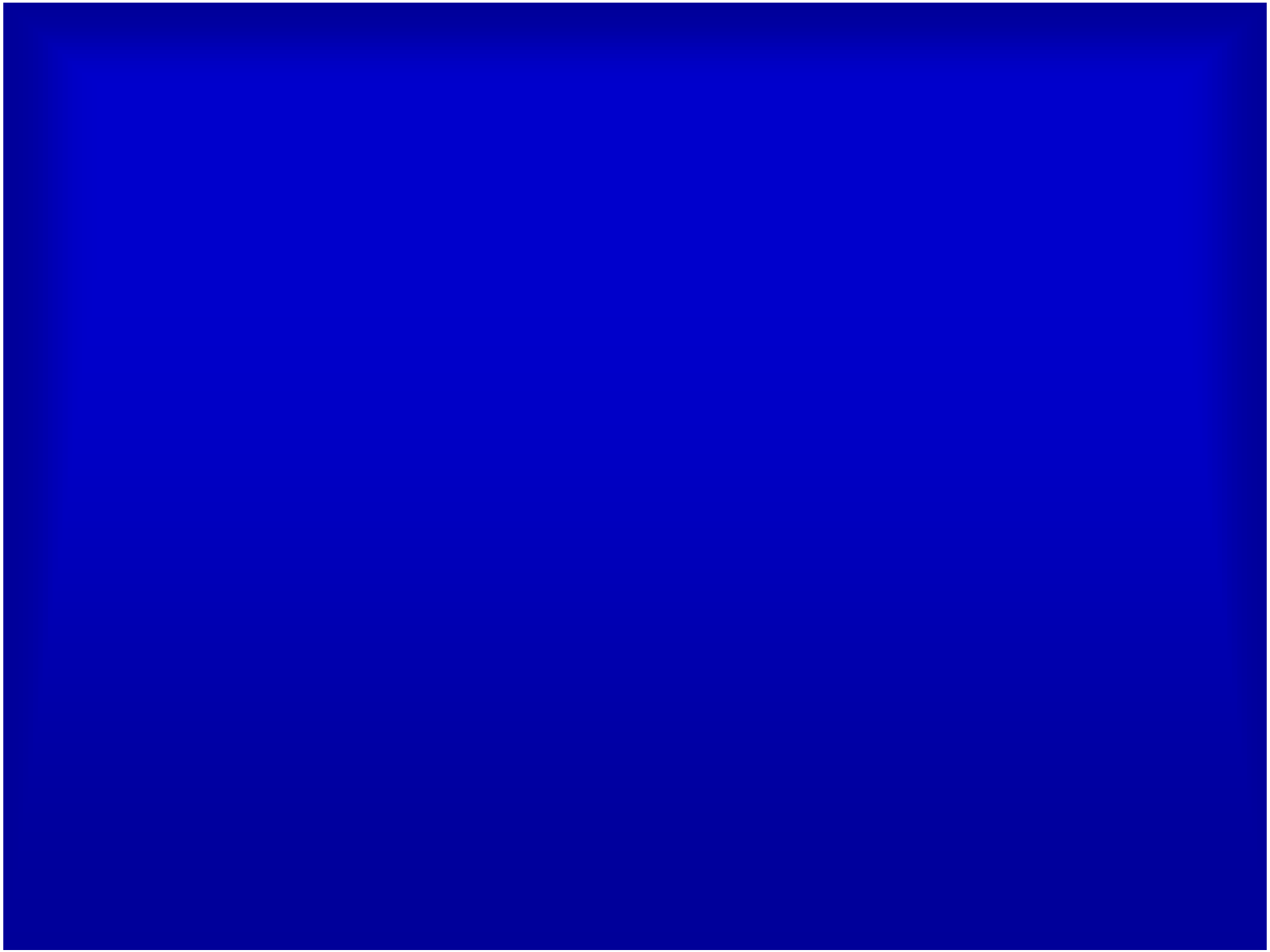
RNA Editing Is Differentially Regulated During Neuronal Differentiation and Maturation

Comparison of A-to-I editing between human and non-human primate tissues

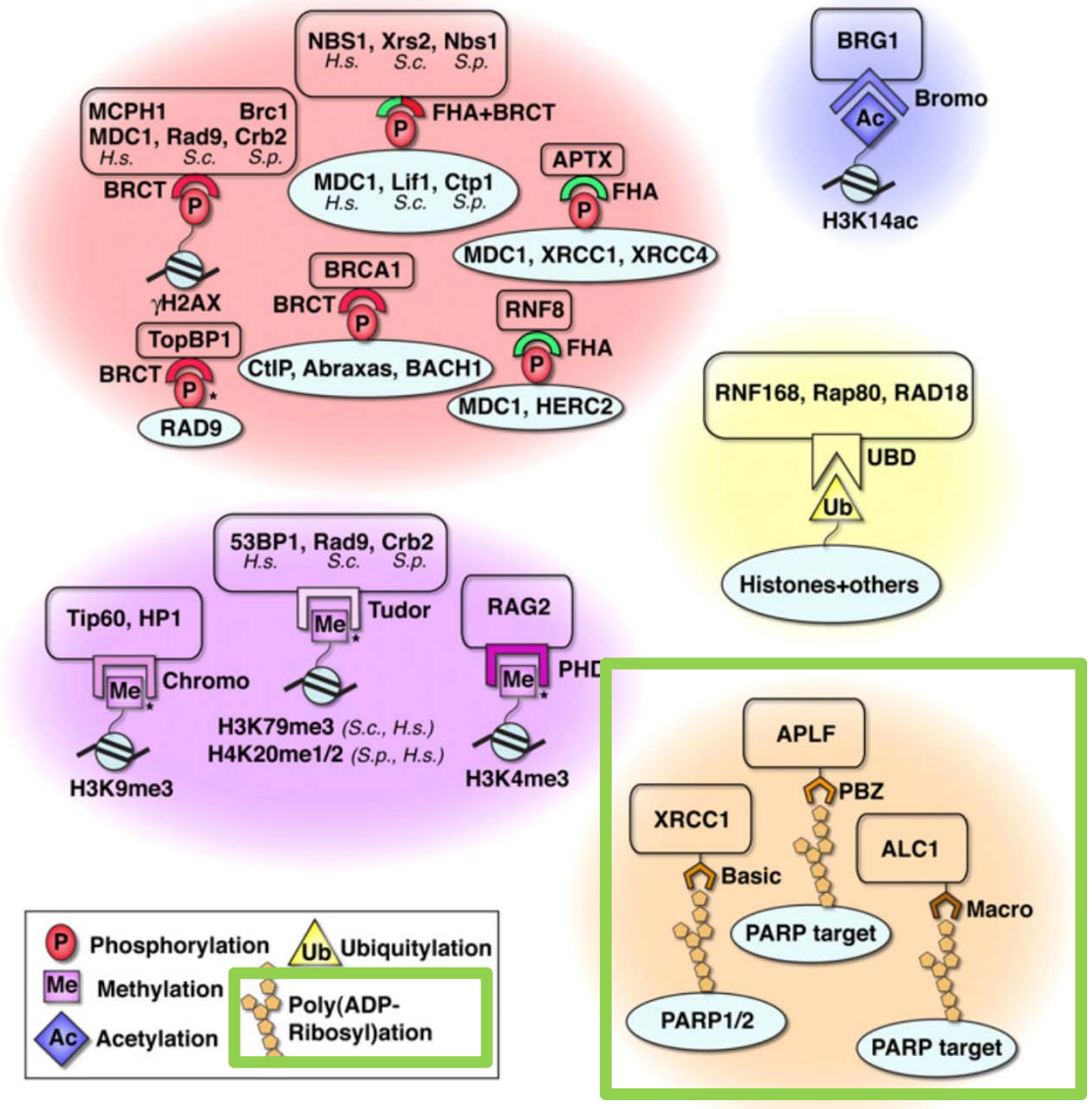


M H Tan *et al. Nature* **550,** 249–254 (2017) doi:10.1038/nature24041

nature

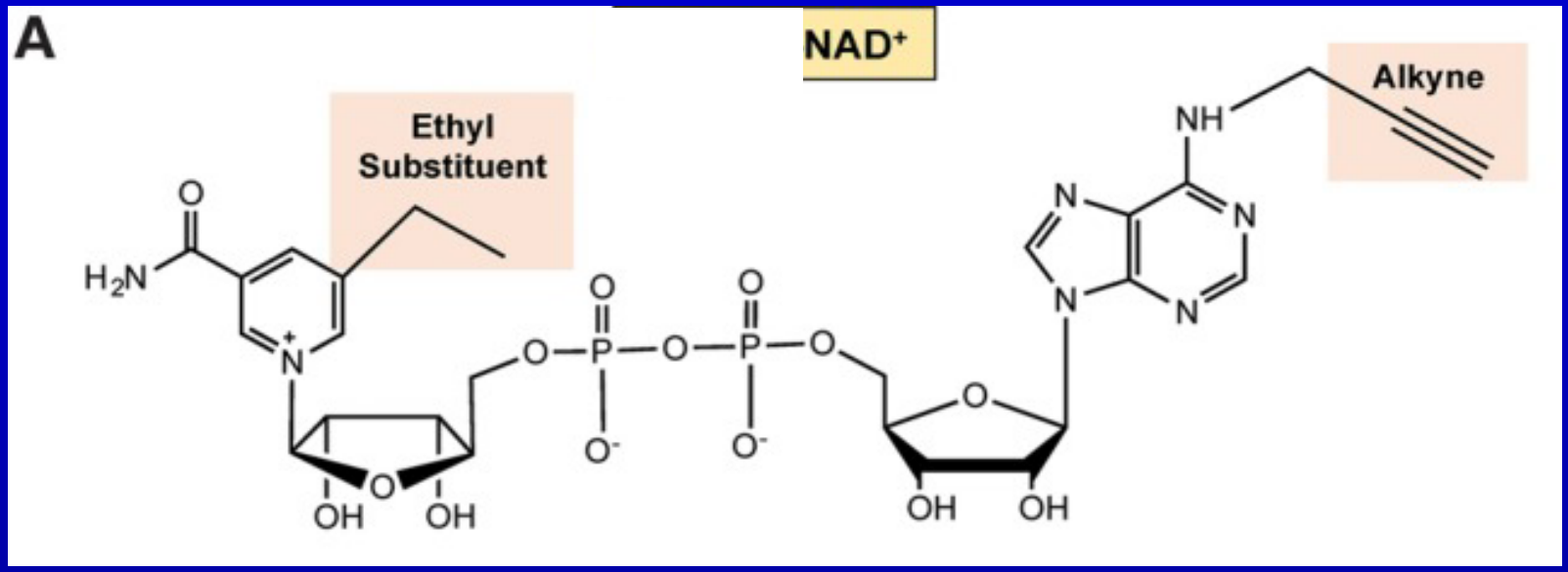


Specialized binding modules for recognition of post-translational modifications (PTMs) at DNA breaks



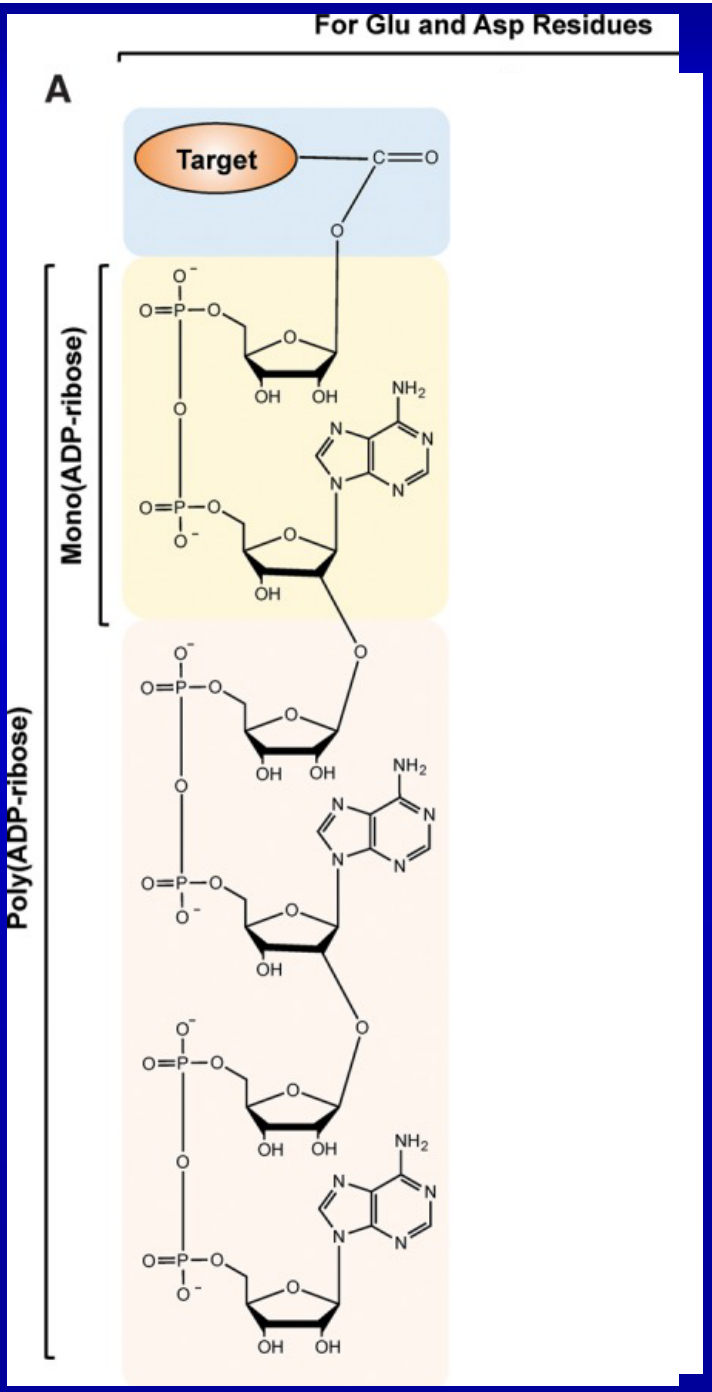
PolyADP-ribosylation

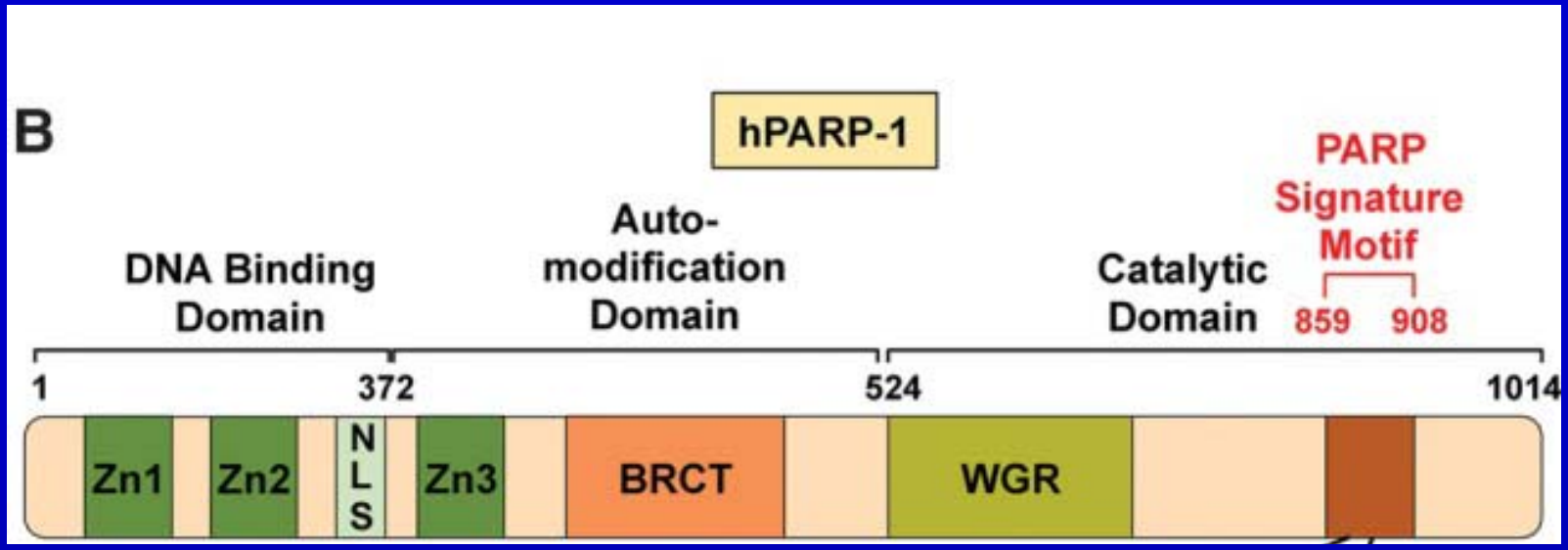
- PolyADP-ribosylation of proteins is a posttranslational modification mediated by **poly(ADP-ribose) polymerases PARPs**



PARPs use NAD+ as substrate to form the negatively charged polymer of poly(ADP-ribose) (PAR)

the negatively charged polymer of polyADP-ribose

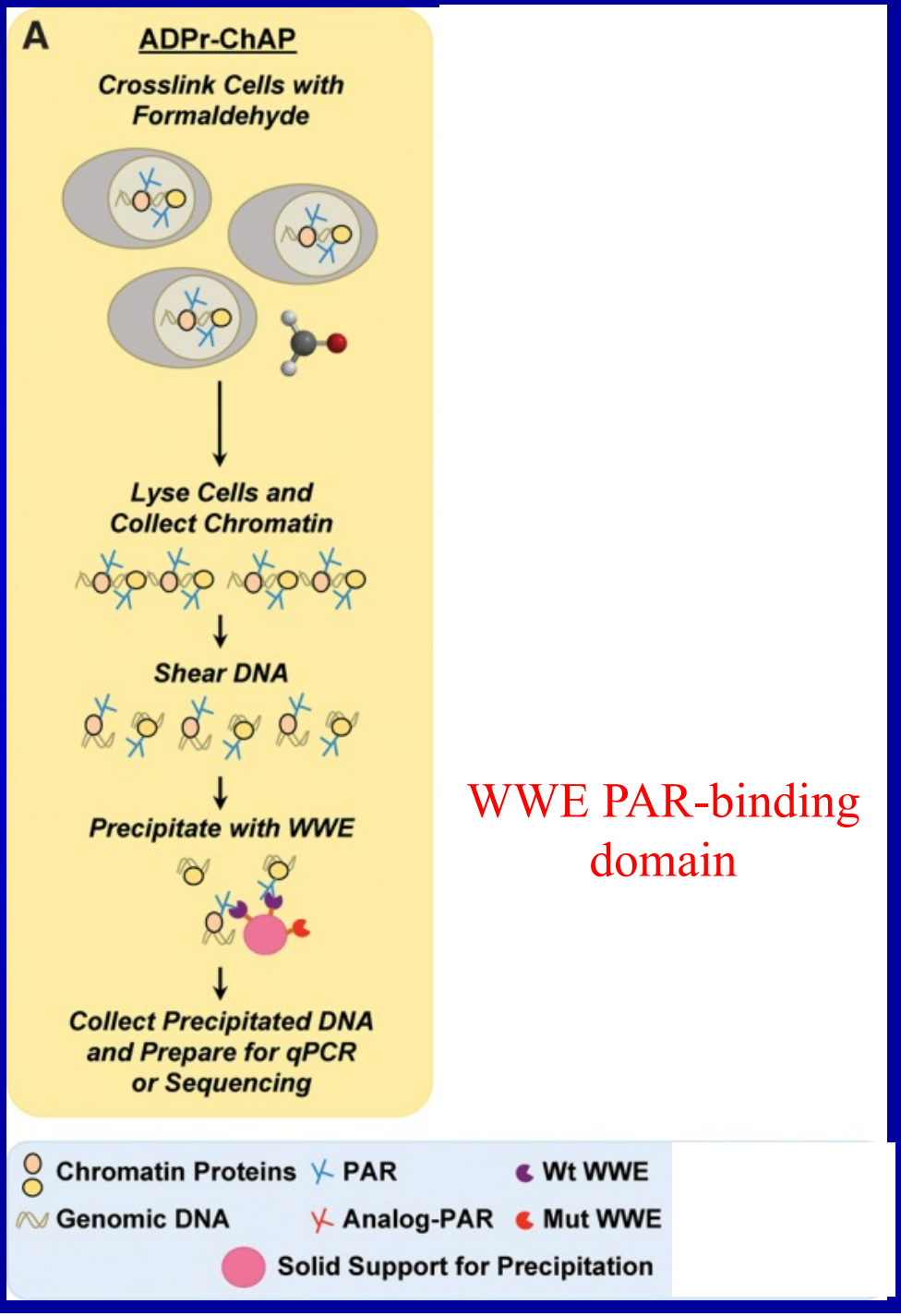




After DNA damage, PARP-1 is responsible for approximately 90% of the total cellular PARylation activity.

map genome-wide ADP-ribosylation
ADP-ribose chromatin affinity precipitation

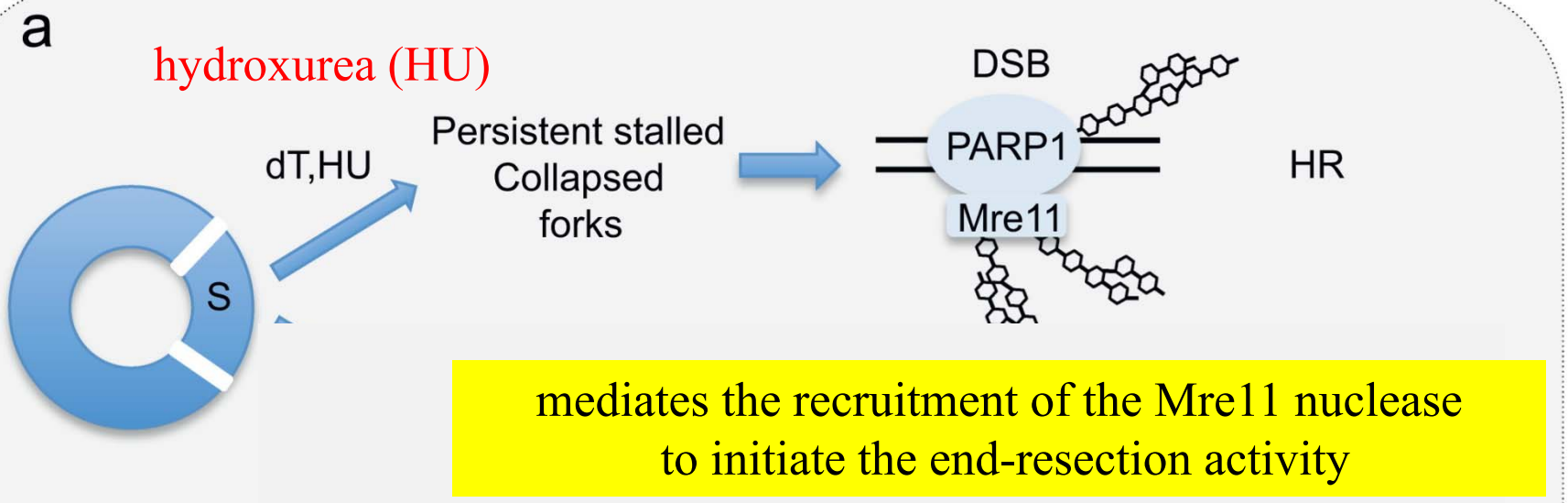
ADPr-ChAP)



WWE PAR-binding domain

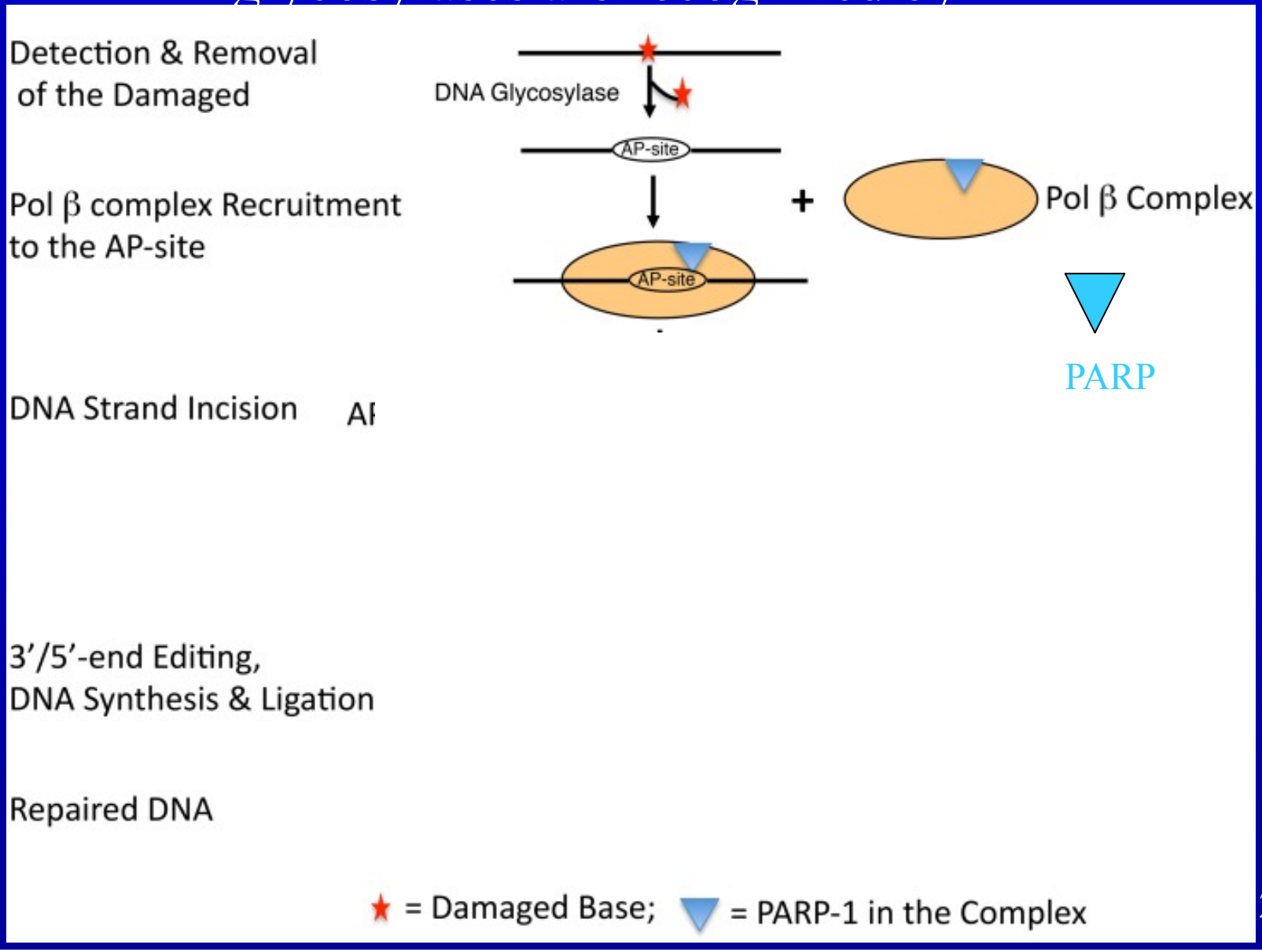
Bartolomei et al. (2016)

PARP1 is involved in HR-dependent repair of DSB at disrupted replication forks



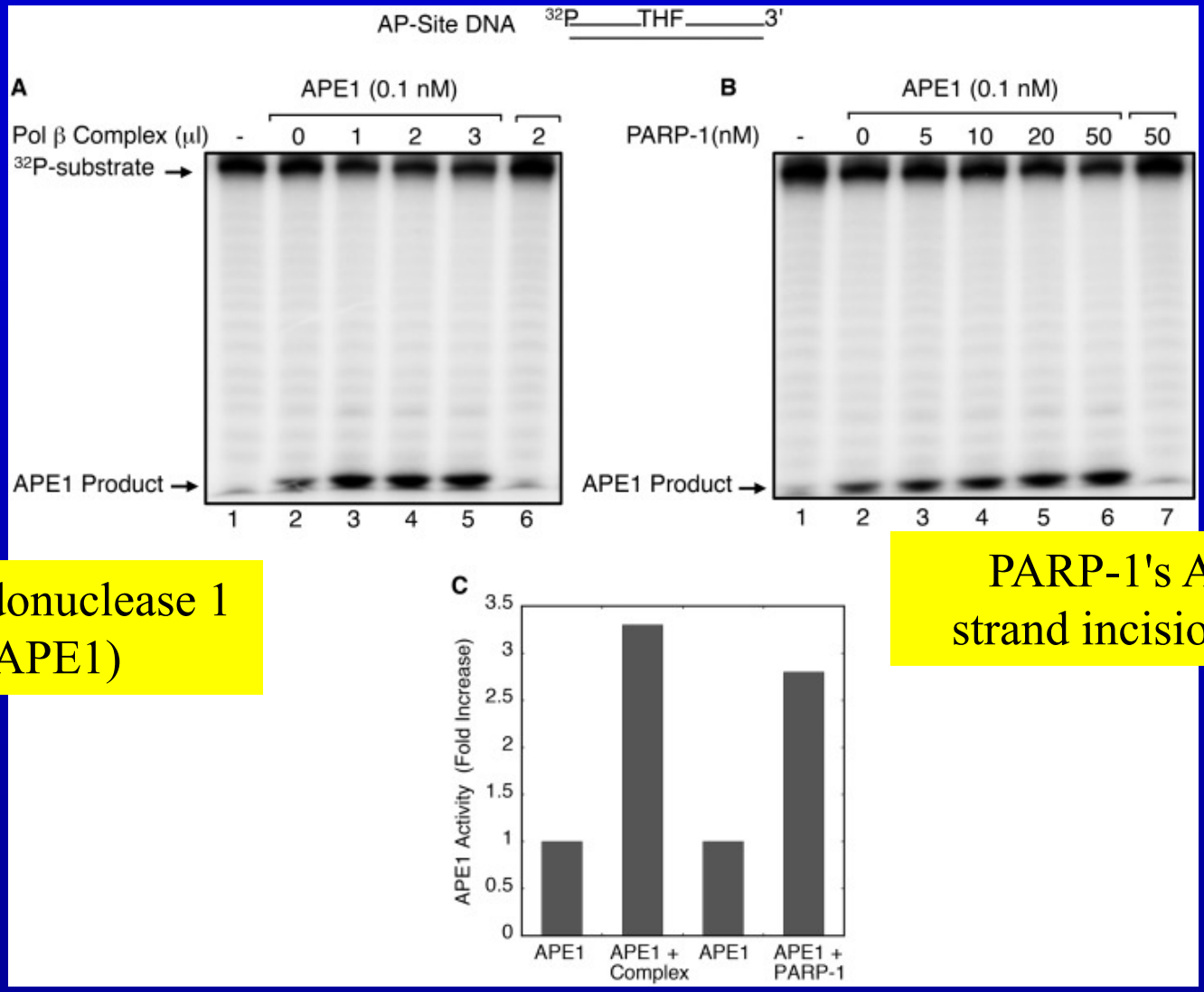
mediates the recruitment of the Mre11 nuclease to initiate the end-resection activity

AP-site lesions in DNA formed by DNA N-glycosylases are recognized by PARP-1



PLoS One. 2015; 10(5)

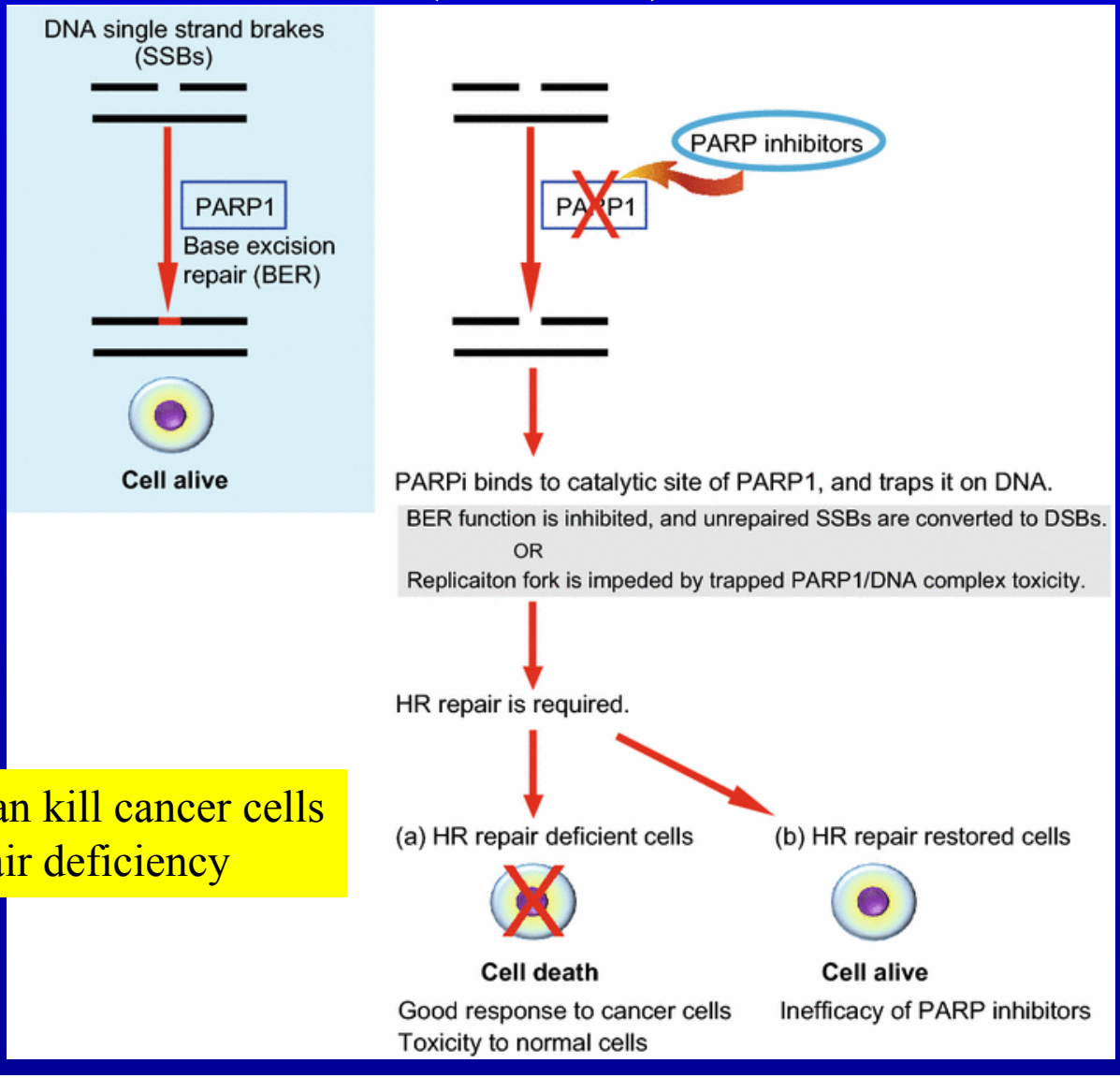
Stimulation of APE1 activity by the pol β complex or purified PARP-1



AP endonuclease 1 (APE1)

PARP-1's AP lyase strand incision activity

Inhibition of poly (ADP)-ribose polymerase1 (PARP1)



PARP inhibitors can kill cancer cells with HR repair deficiency

Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation

N Engl J Med. 2017 Aug 10;377(6

:

- Olaparib is an oral poly(adenosine diphosphate-ribose) polymerase inhibitor that has antitumor activity in patients **with metastatic breast cancer and a germline BRCA mutation.**

- CONCLUSIONS

Among patients with metastatic breast cancer and a germline BRCA mutation, olaparib monotherapy provided a significant benefit over standard therapy; median progression-free survival was **2.8 months longer** and the risk of disease progression or death was 42% lower with olaparib monotherapy than with standard therapy (Funded by AstraZeneca)