DNA repair proteins - mechanisms and functions

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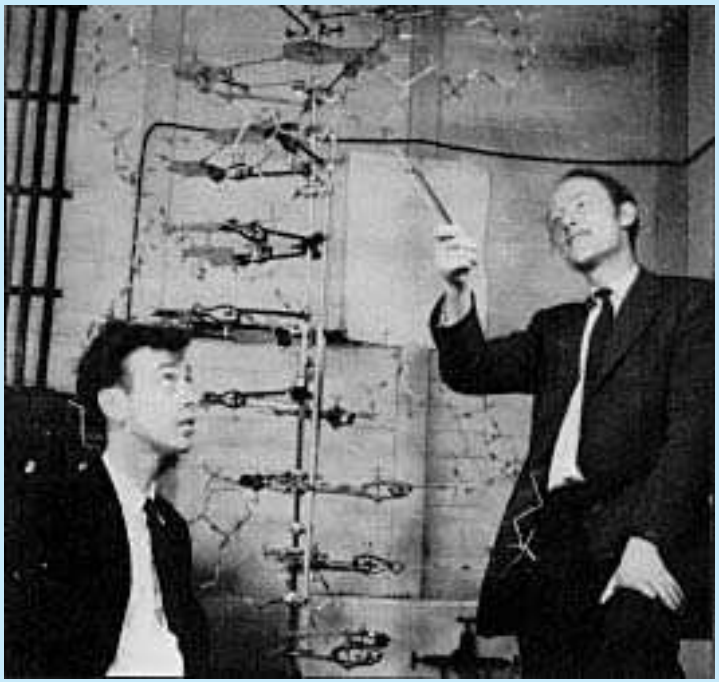
DNA was assumed to be inert and not subject to damage and repair

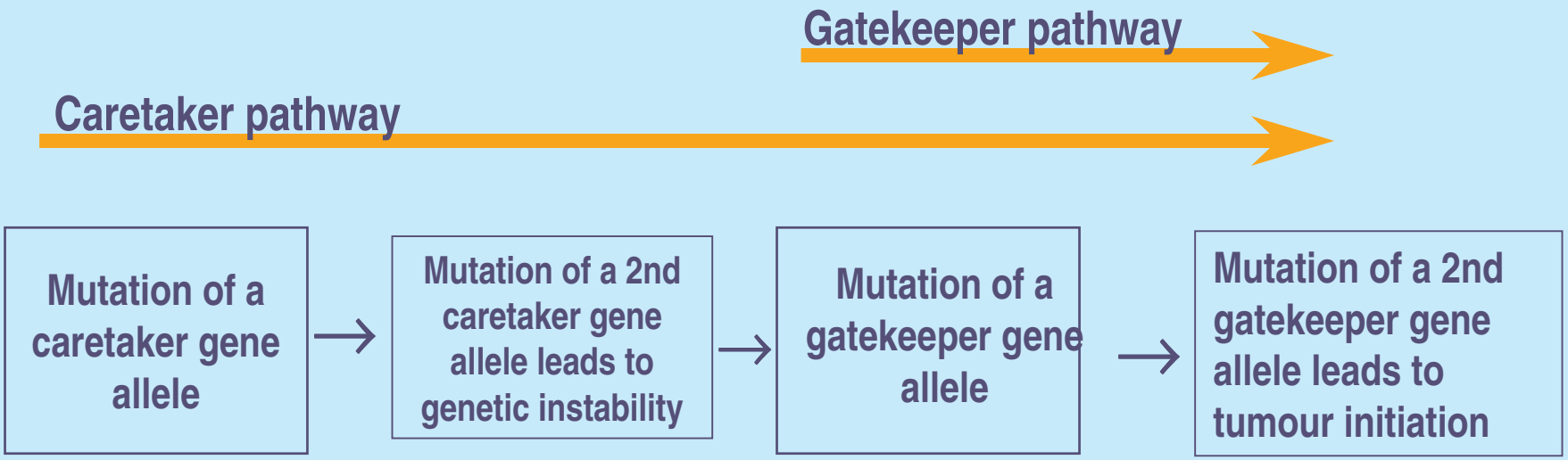


J. Watson F. Crick

J. Watson and F. Crick (1953): "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

F. Crick (1974): "We totally missed (in 1953) the possible role of enzymes in repair,…… I later came to realize that DNA is so precious that probably many distinct repair mechanisms would exist."

Mutations are required for malignant transformation, which follows two paths



Caretaker genes: Genes required for DNA repair, DNA replication and detoxification of carcinogens

Gatekeeper genes: Genes for protooncogenes and tumour suppressor genes

*Adapted from Kinzler and Vogelstein, Nature 386:761-763 (1997)

DNA REPAIR GENES AND CANCER

Rare human cancer forms:

Xeroderma pigmentosum - skin cancer; deficiency in one of at least seven different genes for nucleotide excision repair (NER)

Fanconi's anemia - leukemia and solid tumors; four complementation groups, chromosome breakage, genes not identified

Ataxia teleangiectasia - mostly lymphomas. Very sensitive to ionising radiation. Mutation in *ATM*-gene. Function not clear

Bloom's syndrome -many cancer forms. Mutation in *BLM*-gene, a RecQ helicase-gene homologue

More common human cancer forms:

Early onset hereditary breast cancer -Mutation in *BRCA2*-gene, responsible for 50% of these cancers. Brca2 protein may be an essential cofactor for HsRad51, involved in repair of double strand DNA breaks

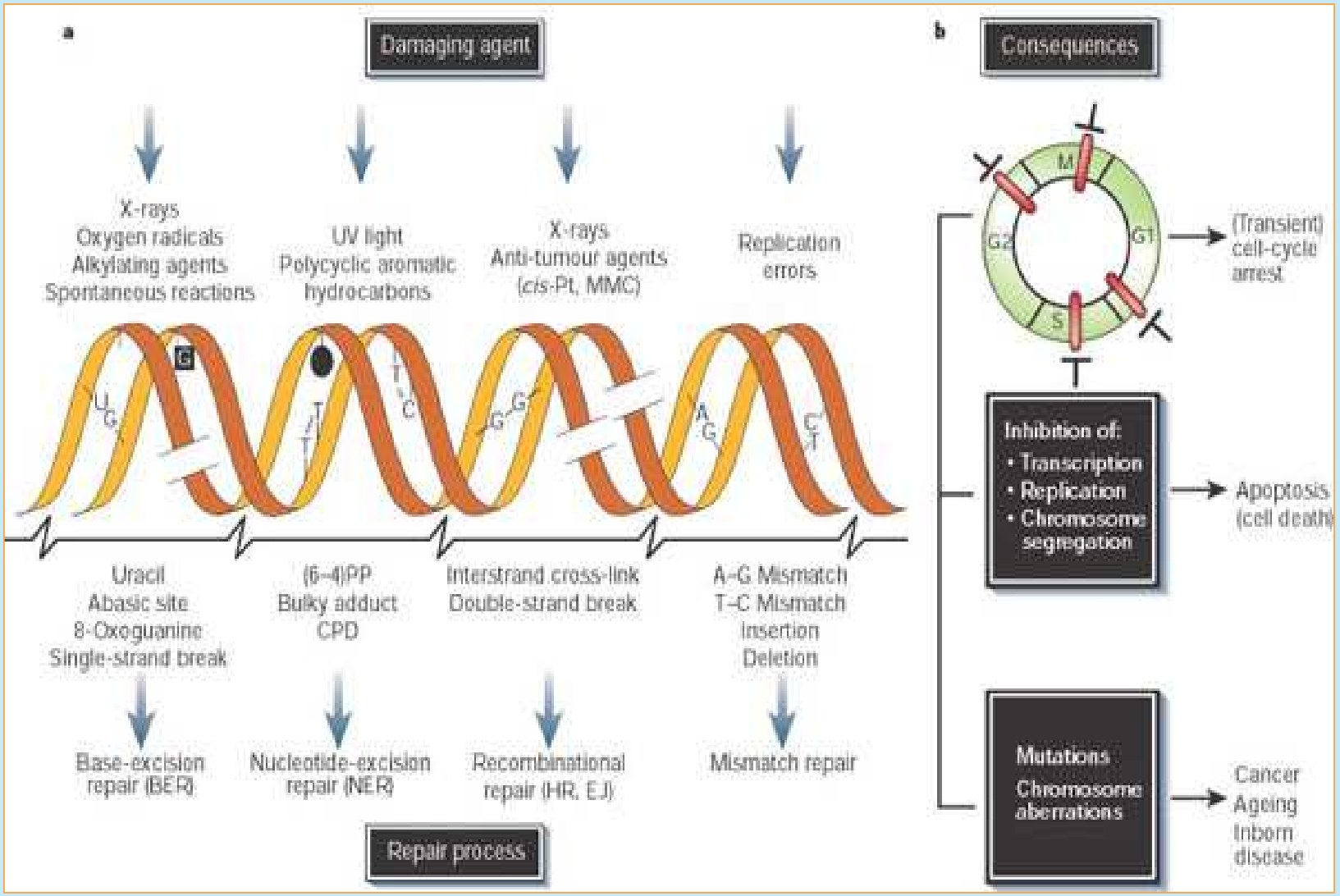
Hereditary nonpolyposis colorectal cancer (HNPCC) - 5% of all colorectal cancers. Deficient mismatch repair. Mutations in *hMSH2* (45%) or *hMLH1* (45%) most common

Hereditary colon cancer with polyposis - hMYH-mutations (Y165G and G382D), (not frequent)

Sporadic colorectal cancer – defective mismatch repair genes in 12-15% of all cases

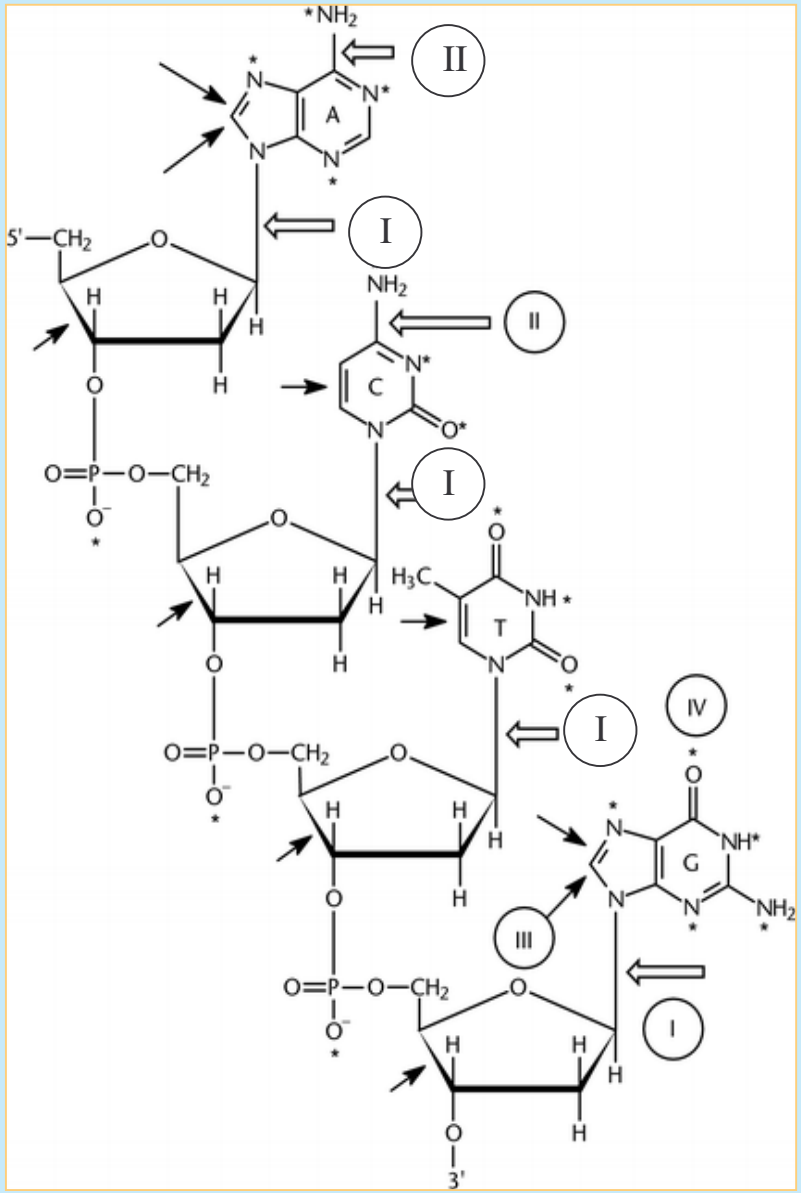
Sporadic lung cancer: - *Low activity of DNA glycosylase hOGG1 and some SNPs enhance risk, Mutations in other BER enzymes genes enhance risk of lung cancer in smokers (APE1 and XRCC1)*

DNA Damage and Repair - Overview



From: Hoeijmakers 2001, Nature 411:366-74.

SPONTANEOUS DAMAGE OCCURS FREQUENTLY



I. Loss of base:

Depurination → AP-site (10 000/cell/day)
Depyrimidination → AP-site (500/cell/day)

II. Deamination:

Adenine → Hypoxanthine (10/cell/day)
Cytosine → Uracil (500/cell/day)

III. Oxidative damage:

Guanine → 8-oxoGuanine (10 000/cell/day ?)
Thymine → Thymine glycol (500/cell/day ?)

IV. Alkylation damage:

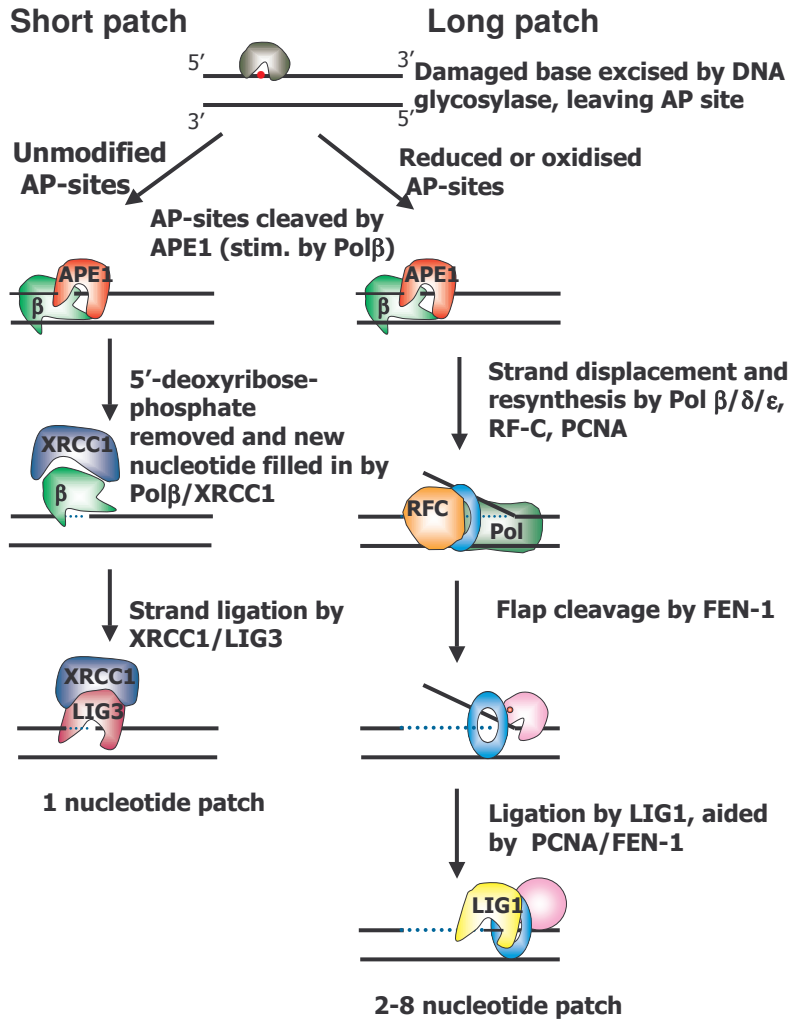
Guanine→ O^6-methylGuanine (spont./induced)
Adenine→ 3-methylAdenine (spont./induced)

Consequences:

1. **Mispairing in replication (mutation)**
2. **Block of replication (cytotoxic)**
3. **Block of transcription (cytotoxic)**

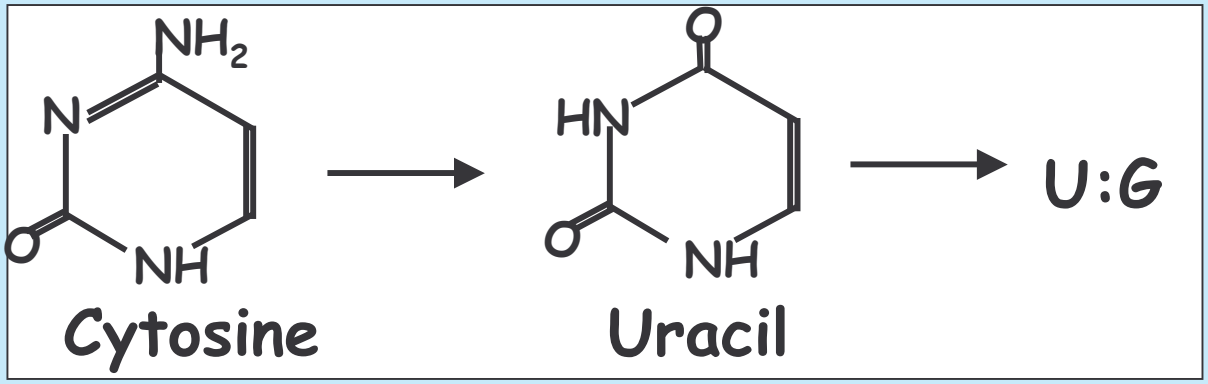
Base excision repair (BER)

- BER removes damaged or inappropriate bases that do not cause helix distortion
- Deficient BER may cause cancer and immune deficiency
- Important for development
  - polβ: embryonic lethal
  - XRCC1: embryonic lethal
- hMYH-def. : colorectal cancer
- hOGG1-def.: lung cancer
- hUNG: defect antibody maturation (defective CSR and SHM)
- mUNG: B-cell lymphoma (late)
- mUNG: Increased postischemic brain injury (Endres et al., 2004)



URACIL IN DNA

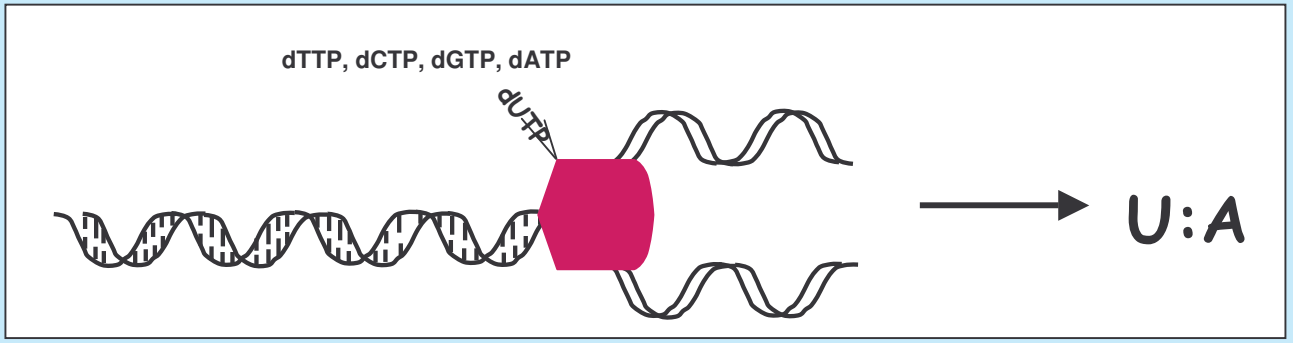
- Spontaneous deamination of cytosine



- Enzymatic deamination of C by AID in B-lymphocytes

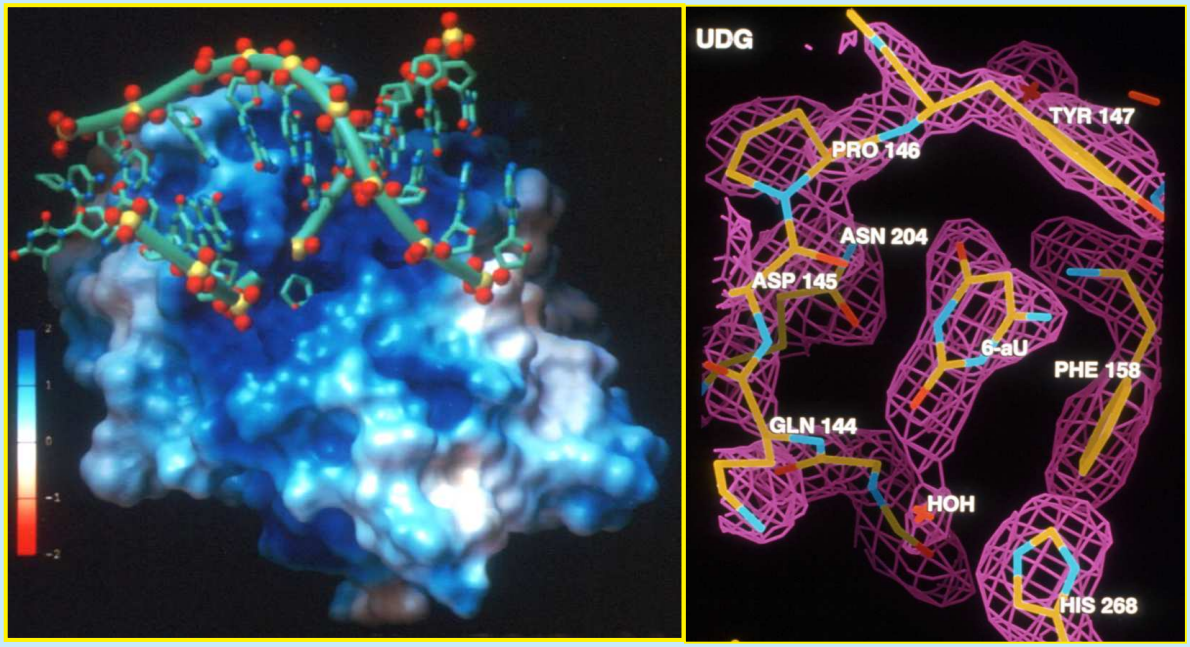
Enzymatic deamination of C to U is an early step in affinity maturation of antibodies - recently discovered

- Incorporation of dUMP during DNA replication (quant.dom.)



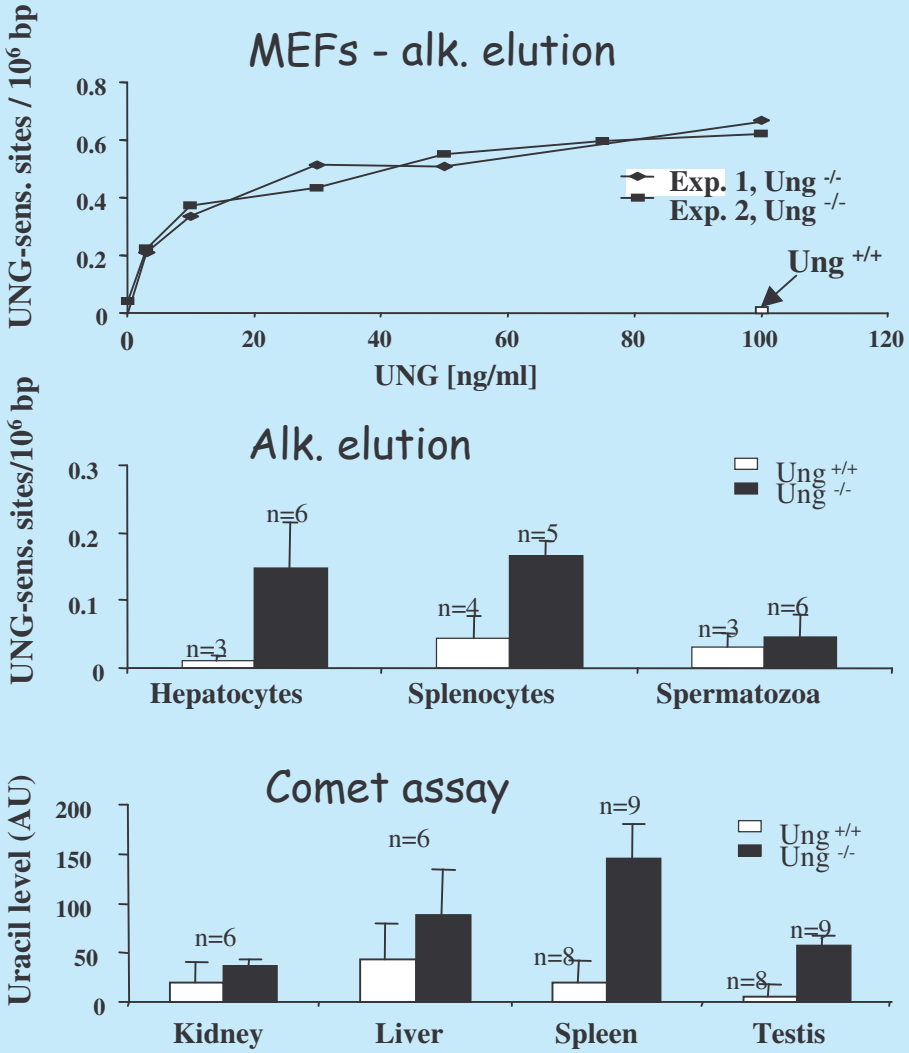
How much uracil in DNA ?

How important is nuclear UNG2 for removal of uracil ?



The structure of UNG catalytic domain is 10 years in 2005
Catalytic domain of UNG common to UNG1 and UNG2
Flipping of uracil into tight fitting pocket

Uracil in DNA analyzed by alkaline elution or comet assay



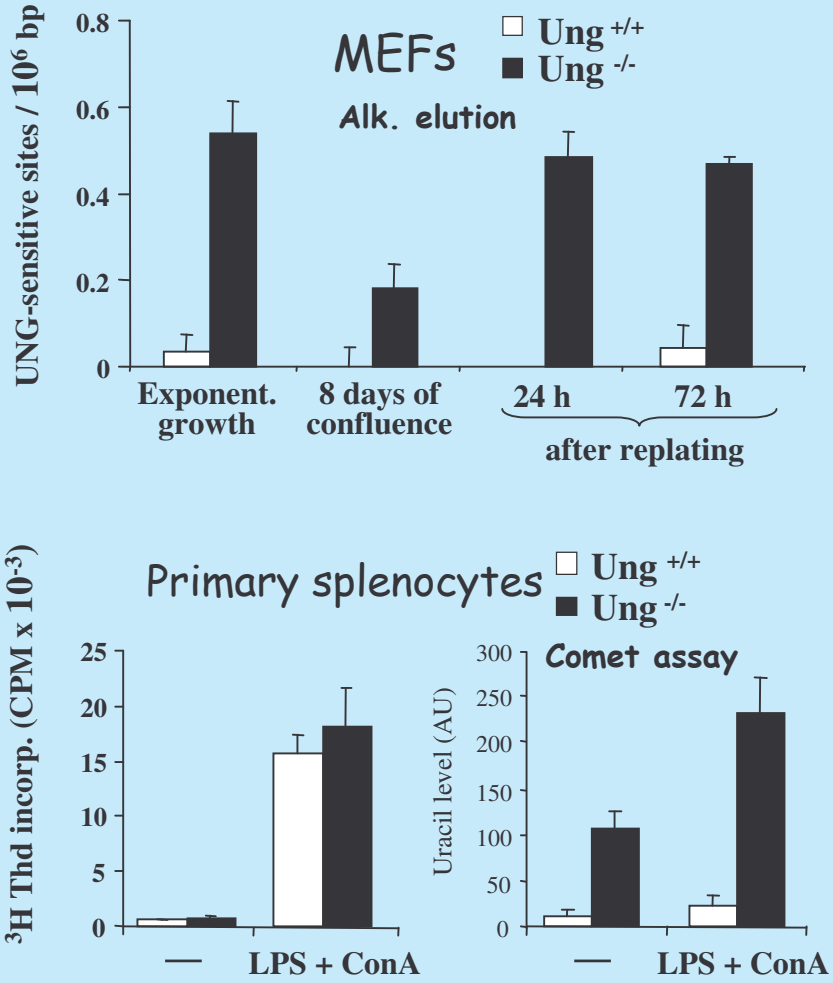
***Ung*$^{-/-}$**

MEFs: 0.6 per 10^6 bp , or ~3600 per dipl. genome;
Liver: ~900 per dipl. genome
Cell specific
Low in spermatozoa

***Ung*$^{+/+}$**

MEFs: Below detection level (0.02 ± 0.05 per 10^6 bp);
At least 10-fold lower than *UNG*$^{-/-}$

More uracil in DNA of proliferating than in nonproliferating cells



Conclusions

Ung2 is very important for removal of uracil from DNA, but even when Ung2 is not present uracil is eventually removed

Higher uracil content in proliferating cells points to dUMP incorporation as a major quantitative source

Ung2 very important for removal incorporated uracil

This does not exclude a role in repair of deaminated cytosine

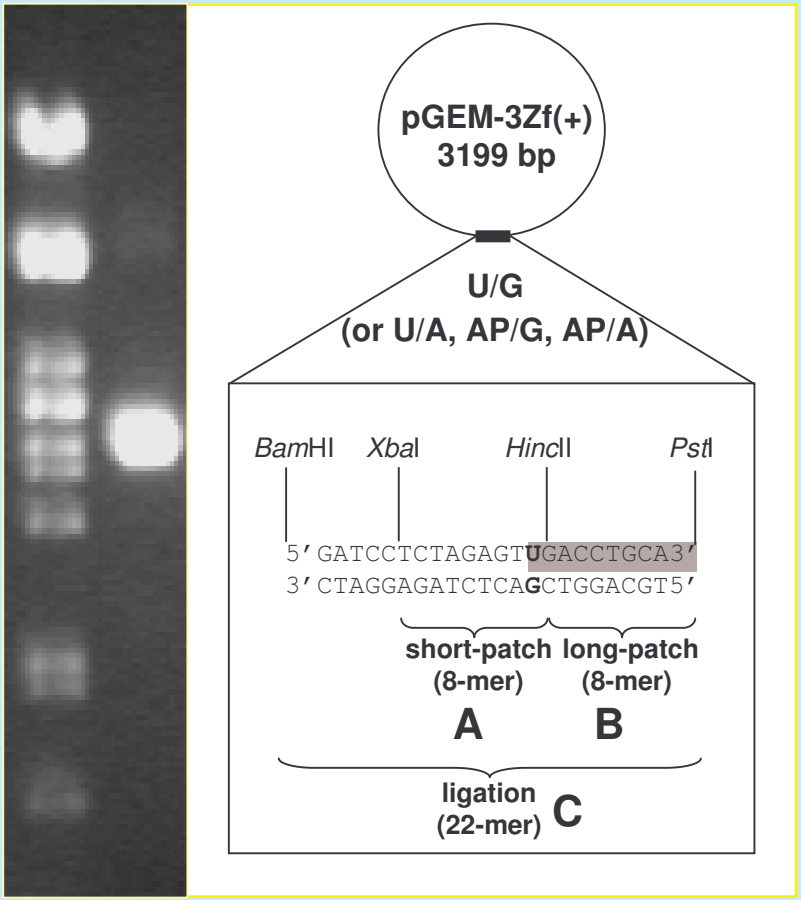
Hypothesis:

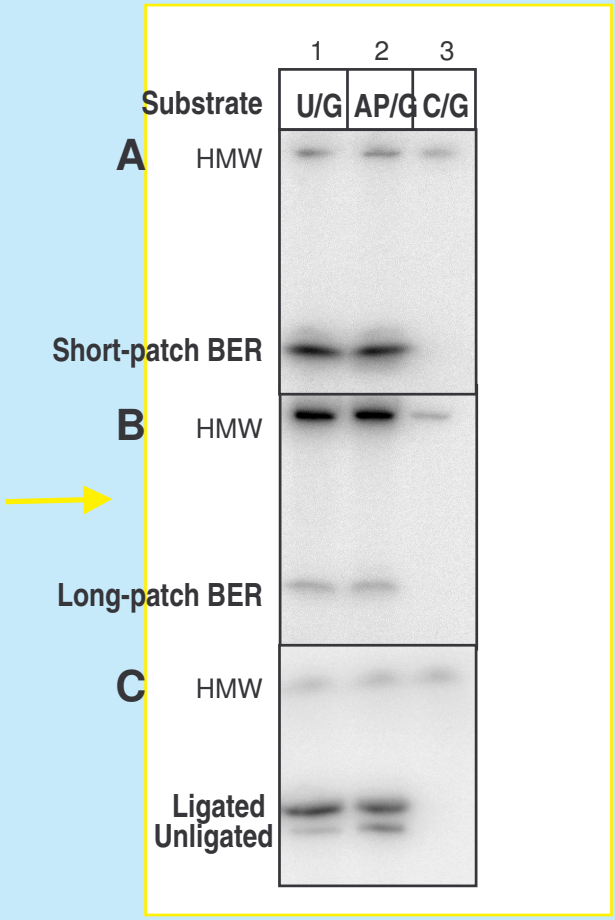
Does BER take place in organized, preformed complexes?

Answer:

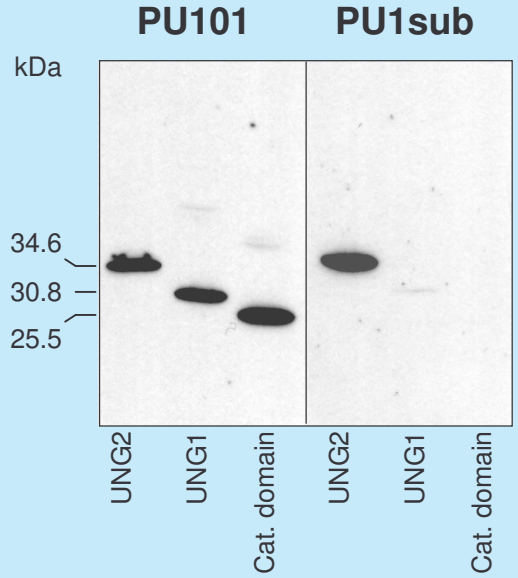
Probably yes

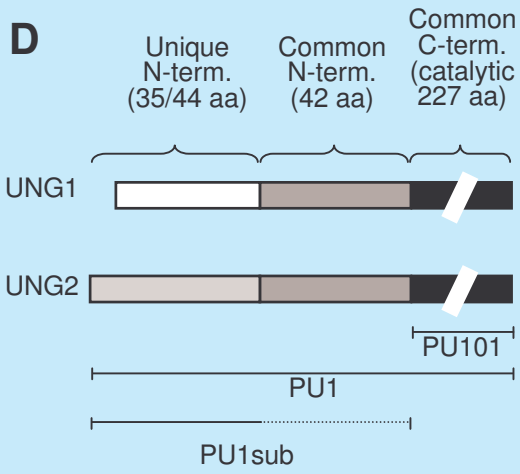
Assay for short- and long patch BER





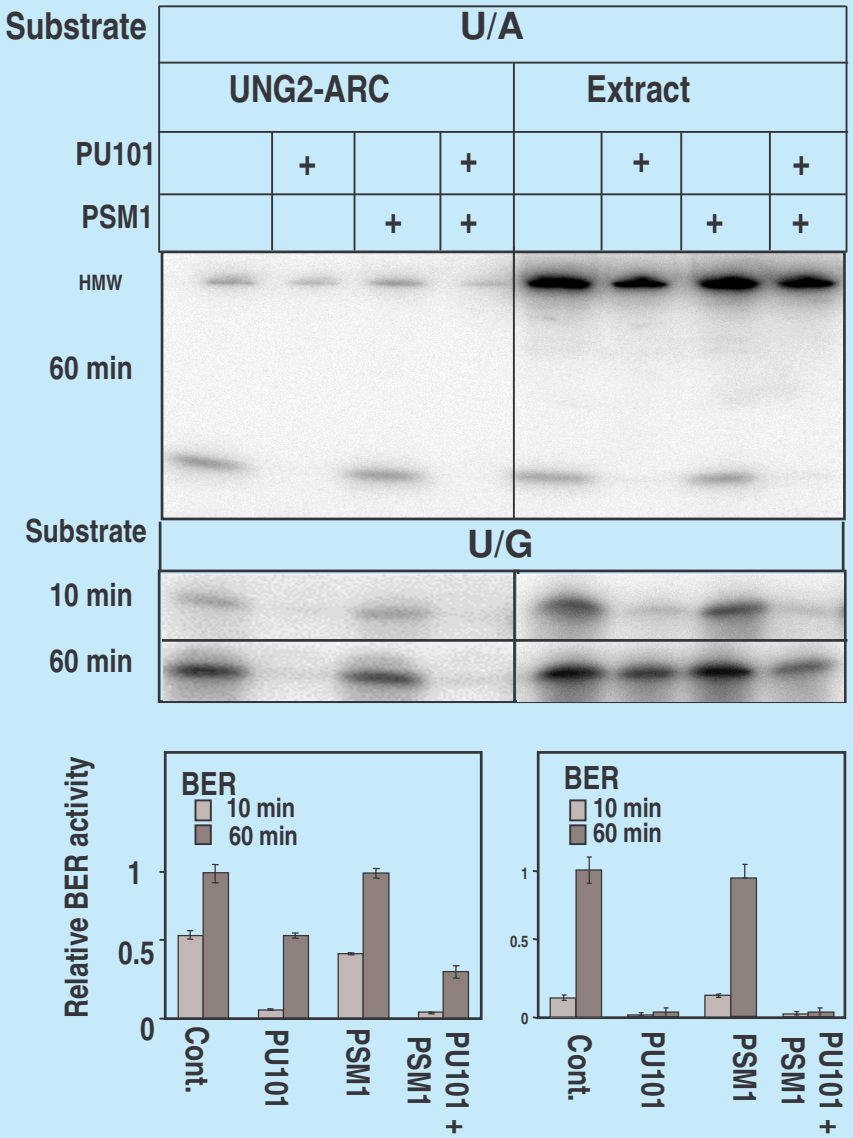
BER COMPLEX - AFFINITY ISOLATION BY UNG2 ANTIBODIES





1. Prepare cell extract from HeLa cells
2. Mix extract with PU1sub-Dynabeads (paramagnetic) (PU1sub is specific for N-terminal non-catalytic part of UNG2 and does not affect UNG-activity)
3. Wash beads 4 times with excess buffer
4. Incubate beads with plasmid containing uracil, AP-site or nick at defined position, dNTPs, [^{32}P]dCTP/dTTP, ATP and appropriate buffer
5. Isolate DNA, cleave with restriction enzyme, analyze by PAGE for short-patch or long patch BER

UNG2 is the major enzyme for U/A and "sole" enzyme for U/A



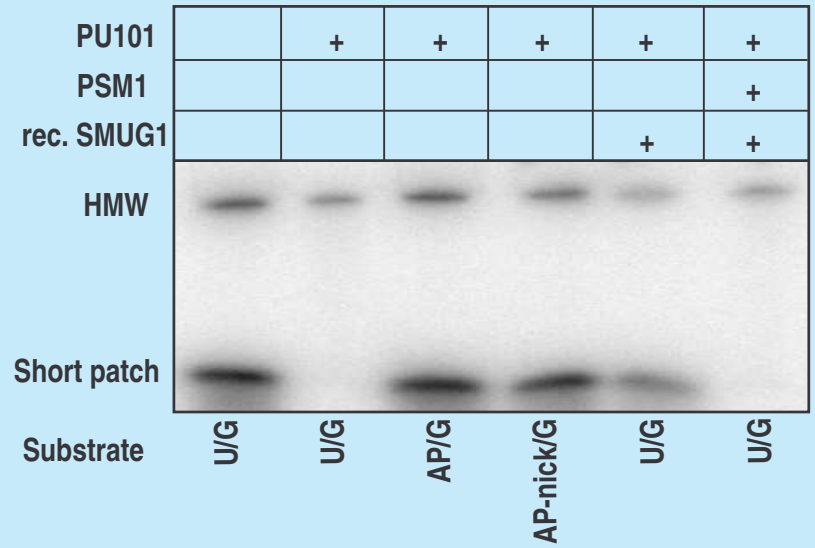
PU101 (anti-UNG) completely inhibits U/A repair and U/G repair by repairosome (UNG2-ARC)

PSM1 (anti-SMUG1) has no effect on U/A or U/G repair in repairosome

PU101 also completely inhibits U/A repair in extracts, but only partially inhibits U/G repair

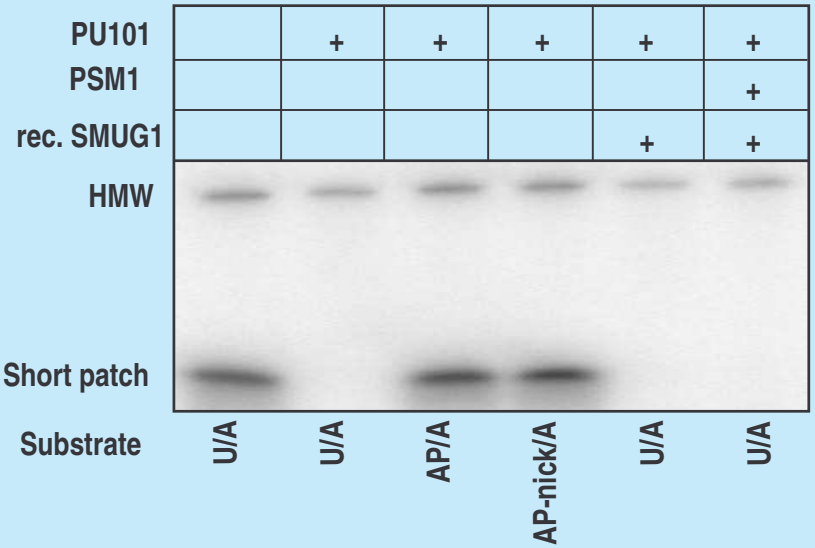
Even in extracts anti-SMUG1 has a partial effect on U/G repair

Recombinant SMUG1 complements UNG2 in repair of U/G, but not U/A in repair complex



Recombinant SMUG1 partially restores U/G repair by repairosome inhibited by UNG-antibody

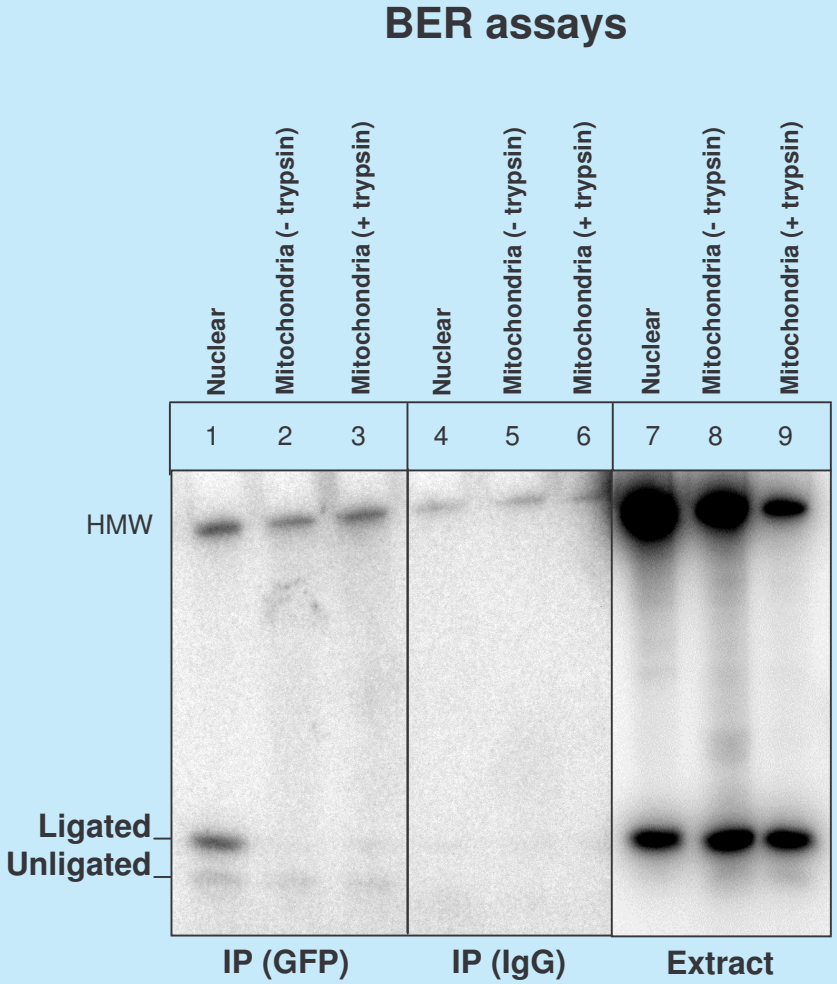
Repair of abasic sites and nicks is not affected by UNG-antibodies

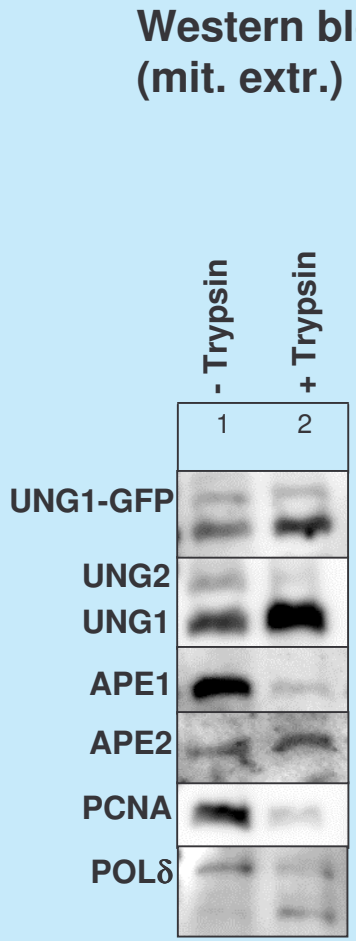


Recombinant SMUG1 does not restore U/A repair by repairosome inhibited by UNG-antibody

Repair of abasic sites and nicks is not affected by UNG-antibodies

Mitochondria are competent in BER, but mitochondrial BER proteins do not form a complex





Conclusions – BER complexes

1. Nuclear BER proteins can be isolated as complexes that carry out complete BER

2. Mitochondrial BER does not seem to require stable repair complexes

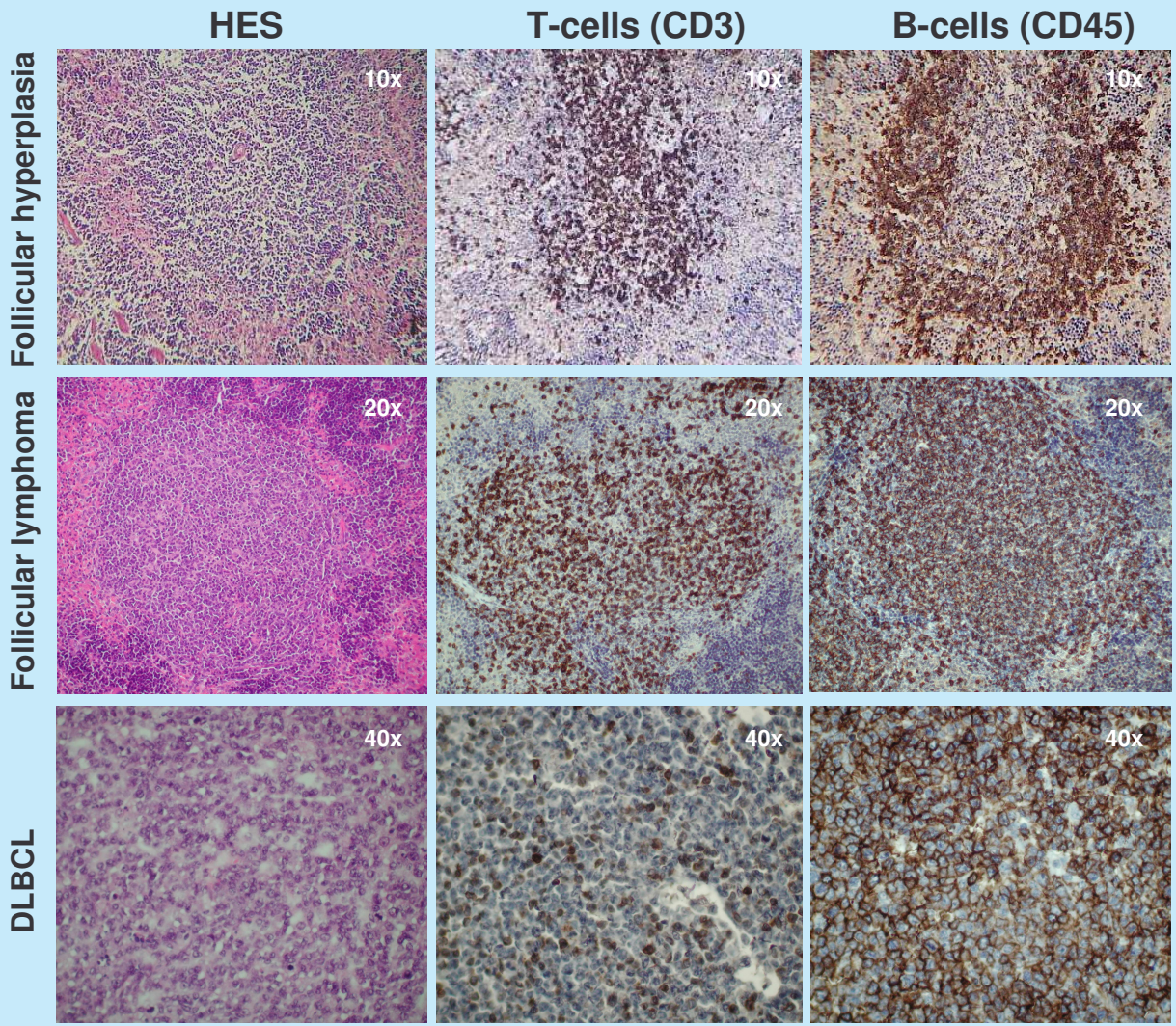
UNG knockout mice

- Develop normally and are fertile
- Accumumulate uracil in the genome
- Defective in post-replicative uracil removal
- Develop B-cell lymphomas (30-fold↑) late in life (all Nilsen et al., 2000, 2003)
- Abnormal somatic hypermutation (SHM) and class switch recombination (CSR) in B-cells (Rada et al., 2002)
- Increased postischemic brain injury (Endres et al., 2004)

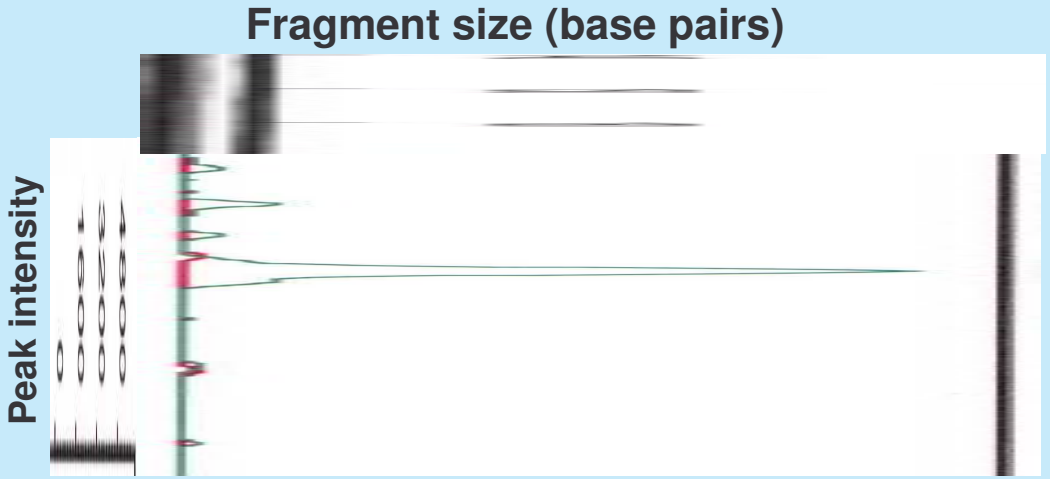
Ung $^{-/-}$ MICE WITH MACROSCOPIC LYMPHOMA

Diagnosis (n=22)	No.	Average age (months) ± SD
Follicular hyperplasia	3	21.0 ± 8.5
Follicular lymphoma	6	19.5 ± 5.6
Diffuse large B-cell lymphoma (DLBCL)	13	20.9 ± 3.5

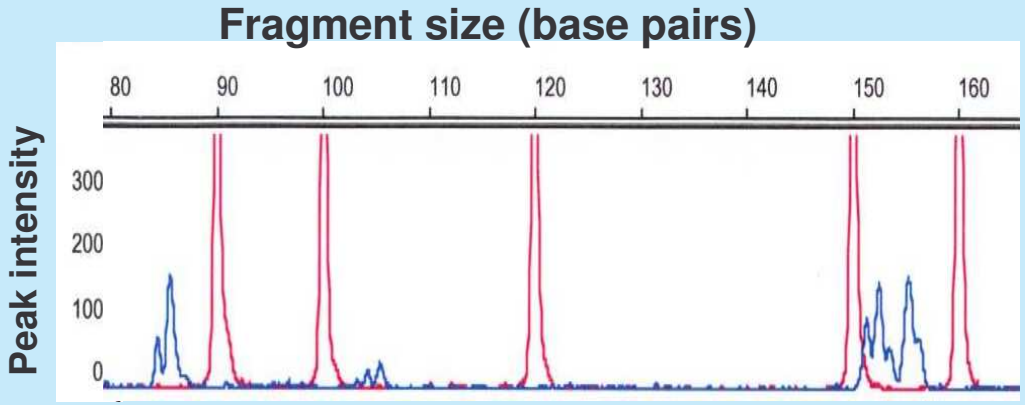
HISTOPATHOLOGY OF HYPERPLASIA AND LYMPHOMA



Clonality analysis - examples



Monoclonal

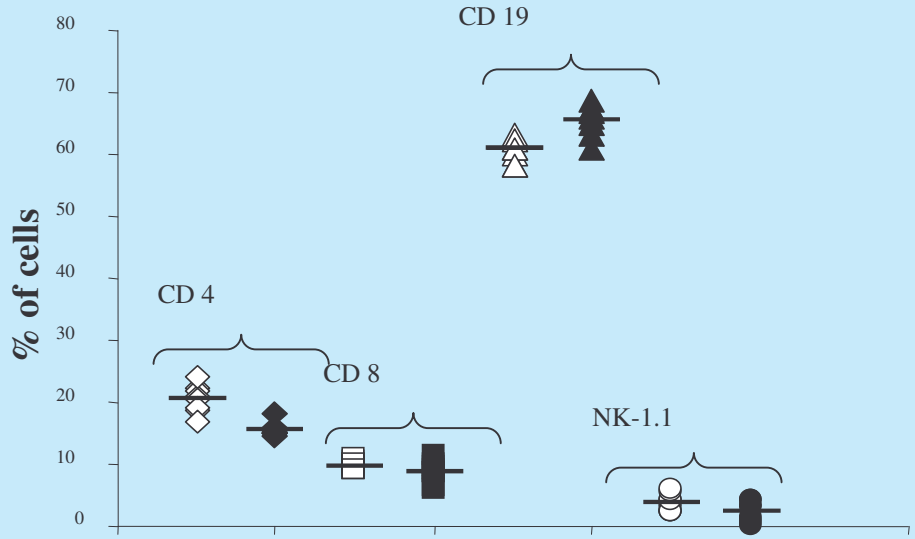


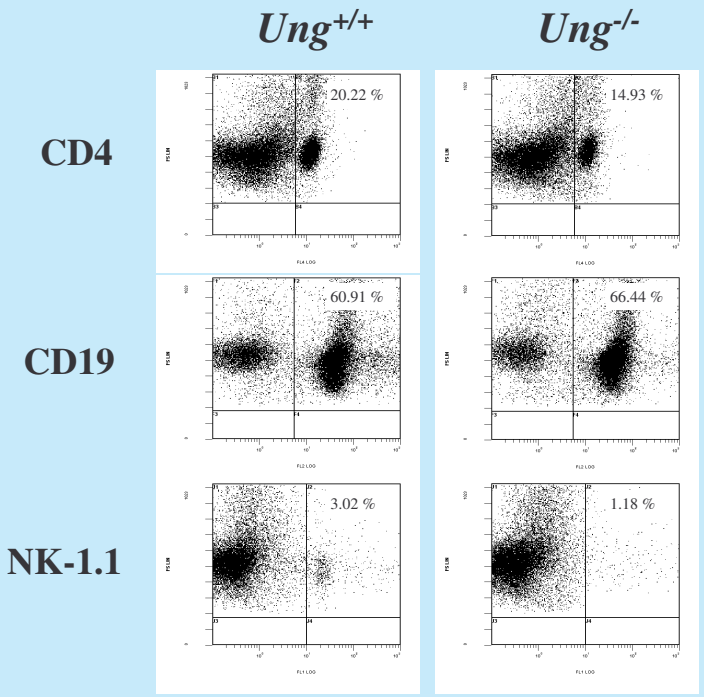
Polyclonal

Clonality of hyperplasias and lymphomas

Diagnosis\Clonality	Mono	Bi	Poly	n.d.
Hyperplasia	1	0	1	1
Follicular lymphoma	5	0	0	1
Diffuse large B-cell lymphoma	6	2	0	5

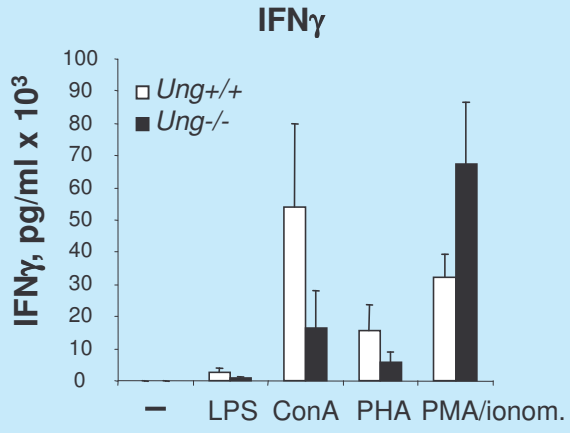
Imbalanced leukocyte populations already in in very youngUng-deficient mice (10-12 weeks)

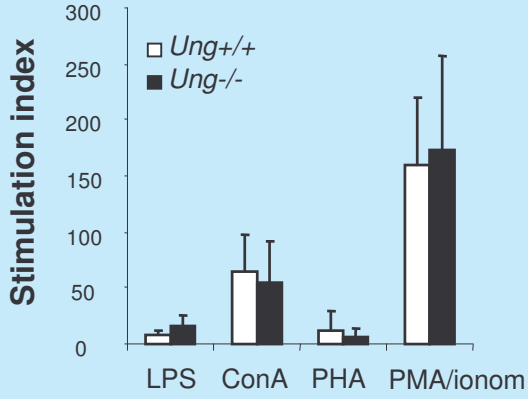


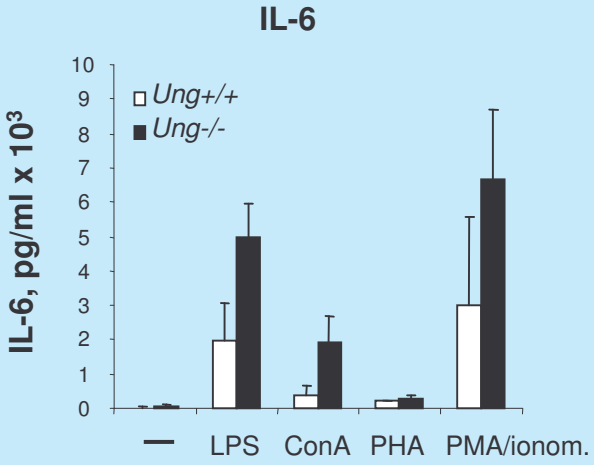


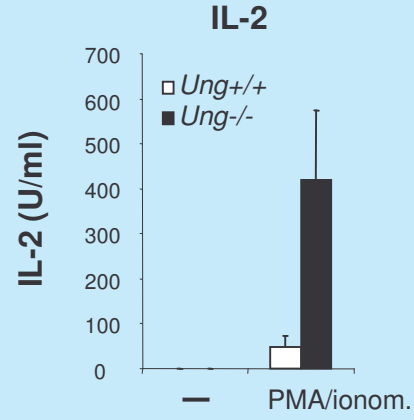
	CD4	CD8	CD19	NK-1.1
Ung$^{+/+}$	20.6	9.7	60.8	3.7
Ung$^{-/-}$	15.6	8.6	65.7	2.2

ABNORMAL CYTOKINE PRODUCTION IN Ung-DEFICIENT MICE

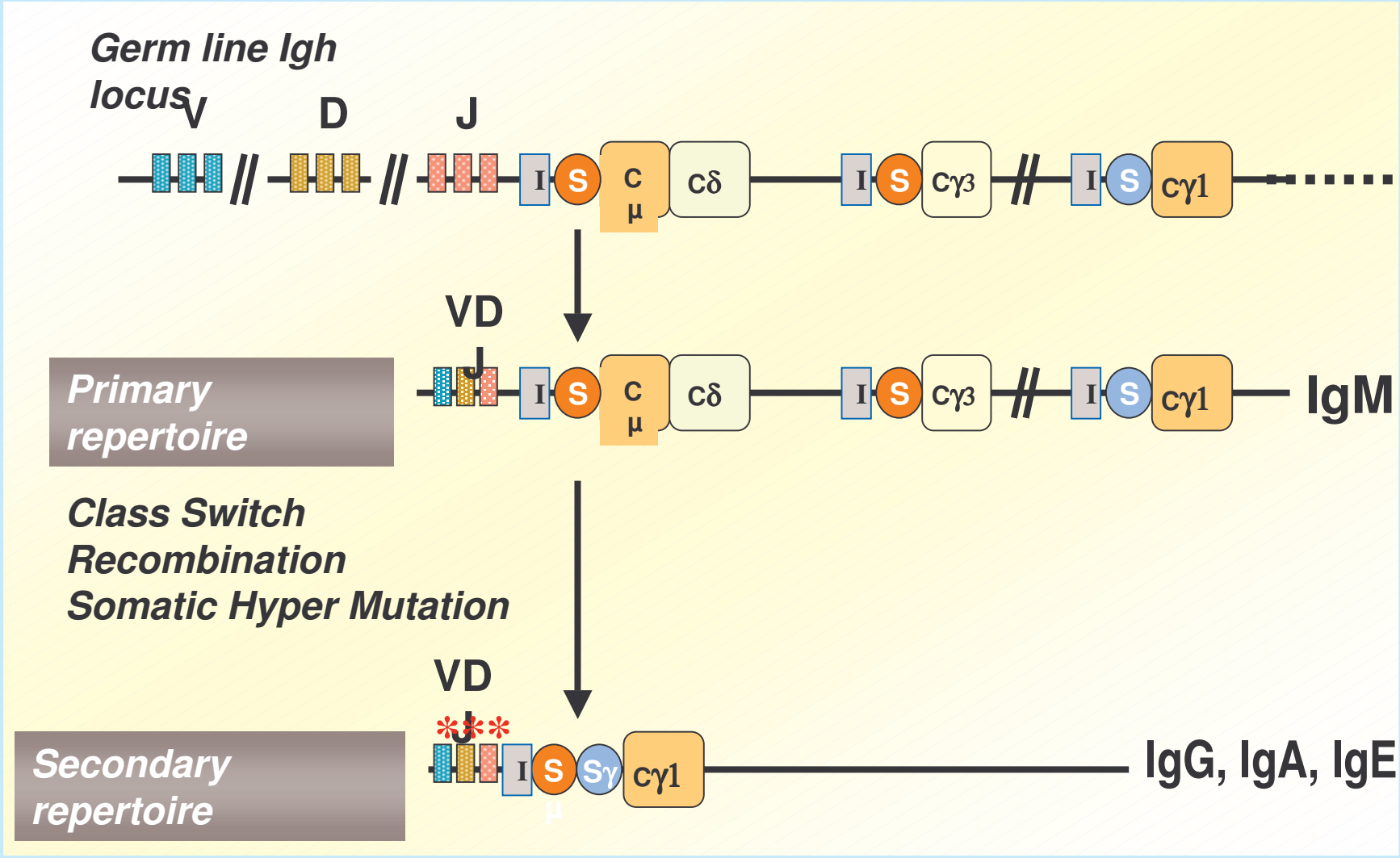






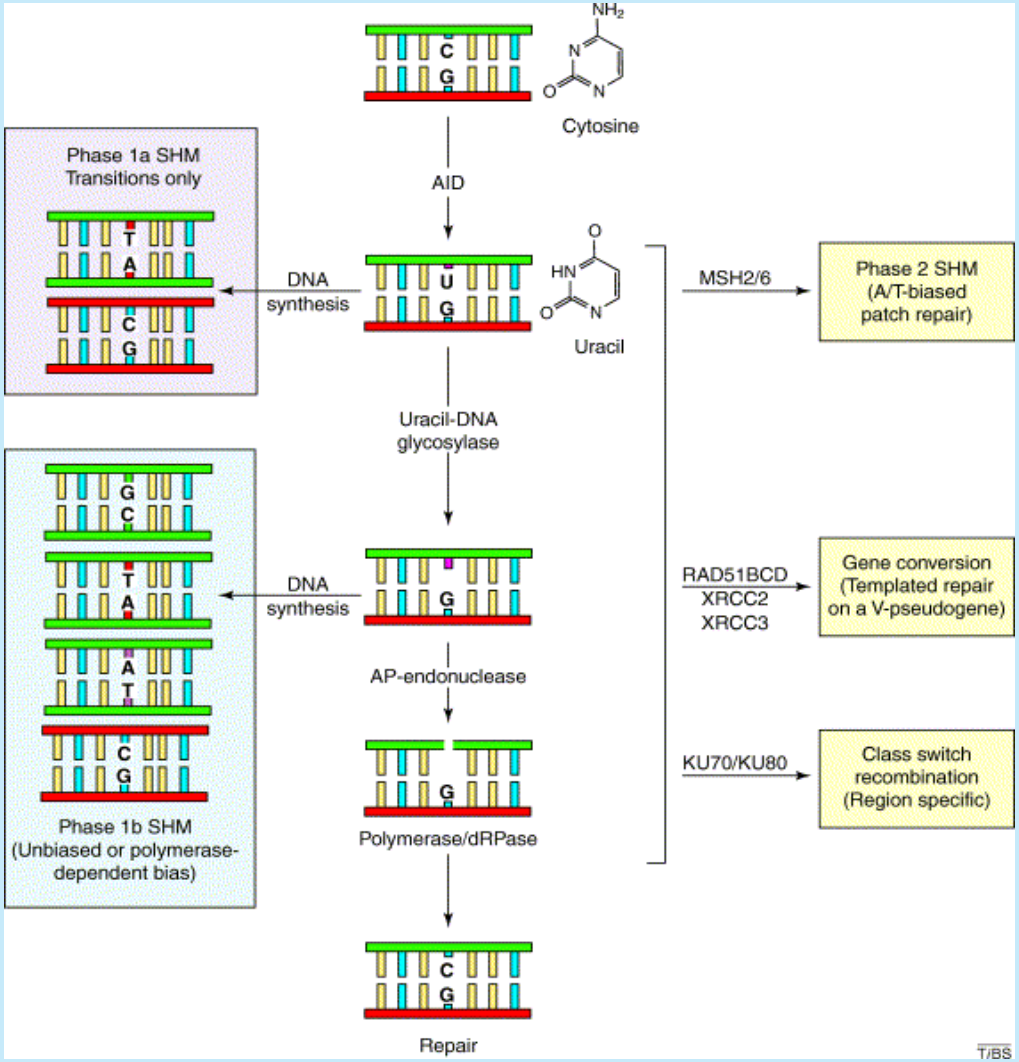


Generation of antibody repertoire



(Slide prepared by Dr. Anne Durandy, Paris)

Functions of AID and DNA repair proteins in SHM and CSR
UNG2 has a critical role in SHM and CSR



From Neuberger et al., 2003 Trends Biochem. Sci. 28:305-312

Apparently AID and UNG are more important than mismatch repair proteins.

UNG2 precedes functions of many other repair factors in SHM and CSR

In humans, CSR is more compromised than SHM when *UNG* is mutated

UNG2 is important for normal SHM and *essential* for CSR in humans
Patients suffer from HyperIGM syndrome (Imai et al., 2003, Nature Immunology 4:1023-1028)

HIGM patients – their UNG2 proteins

MIGQKTLYSF FSPSPARKRH APSPEPAVQG TGVAGVPEES GDAAAIPAKK APAGQEEPGT 60

PPSSPLSAEQ LDRIQRNKAA ALLPLAARNV PVGFG**ESWKK HLSGEF**GK**PY FIKLMGFVAE** 120

ERKHYTVYPP PHQV**FTWT**QM CDIKDVK**VVI LG**QDPYHGPN QAHGLCFSVQ RPVPPP**PSLE** 180

NIYKELSTDI EDFVHPGHGD L**SGWAK**QG**VL LL**NAVLTVRA HQANSHKERG **WEQFTDAVVS** 240

WLNQNSNGL**V FLLW**G**SYAQK KGSA**IDRKRH **HVLQT**AHPSP LSVYRGFFGC R**HFSKTNELL** 300

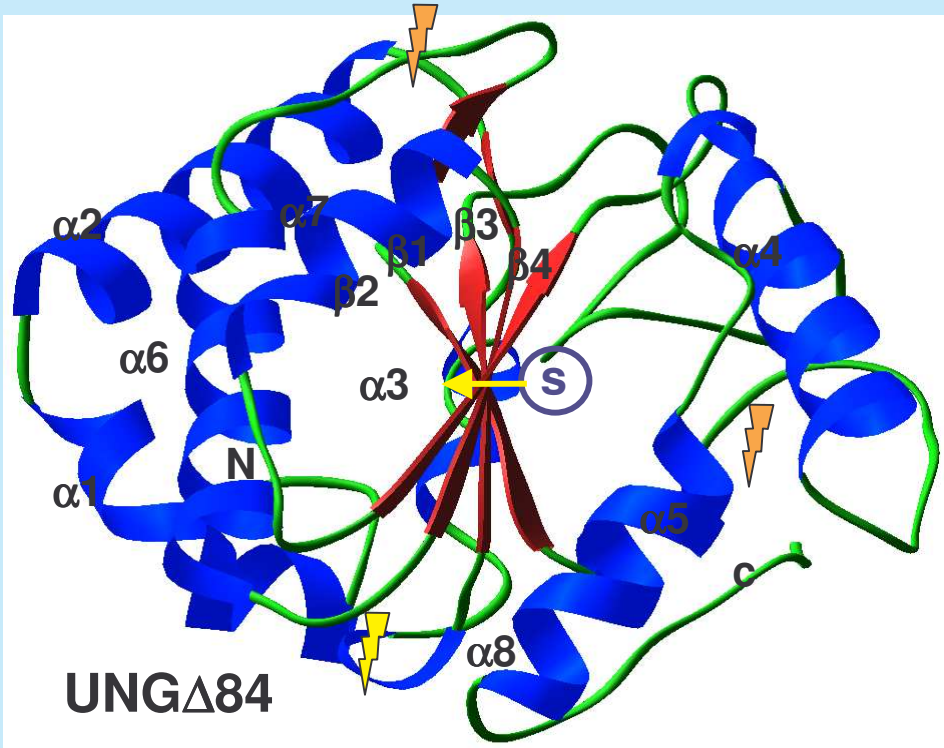
QKSGKKPIDWKEL

α1 α2 α3 β1 α4 α5 β2 α6 β3 α7 β4 α8 S

P1 frameshift,
stop at 141 and 224

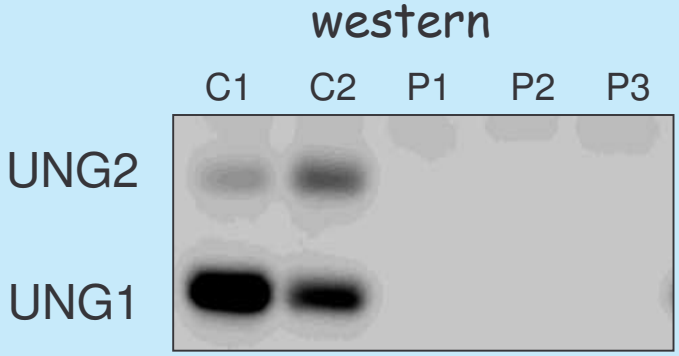
P2 S F251S

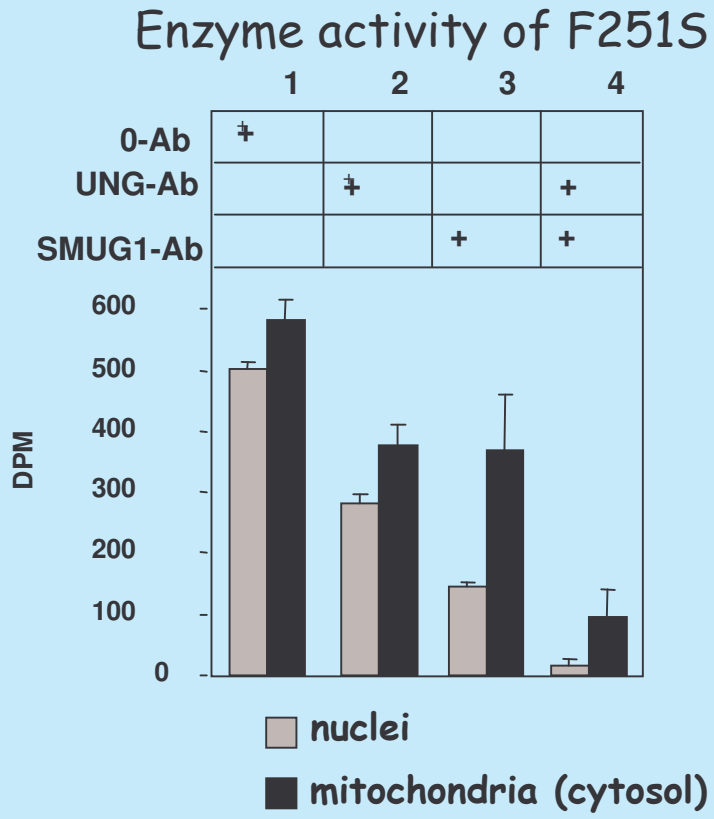
P3 frameshift,
stop at 159



No UNG1 or UNG2 activity is detectable in HIGM-syndrome patients with mutated *UNG*-gene, but minor enzyme activity (~ 0.4%) is detectable in cells carrying the F251S missense mutation.

Problem: When expressed in *E. coli,* UNG F251S i fully active and stable. Why is it absent in human cells?

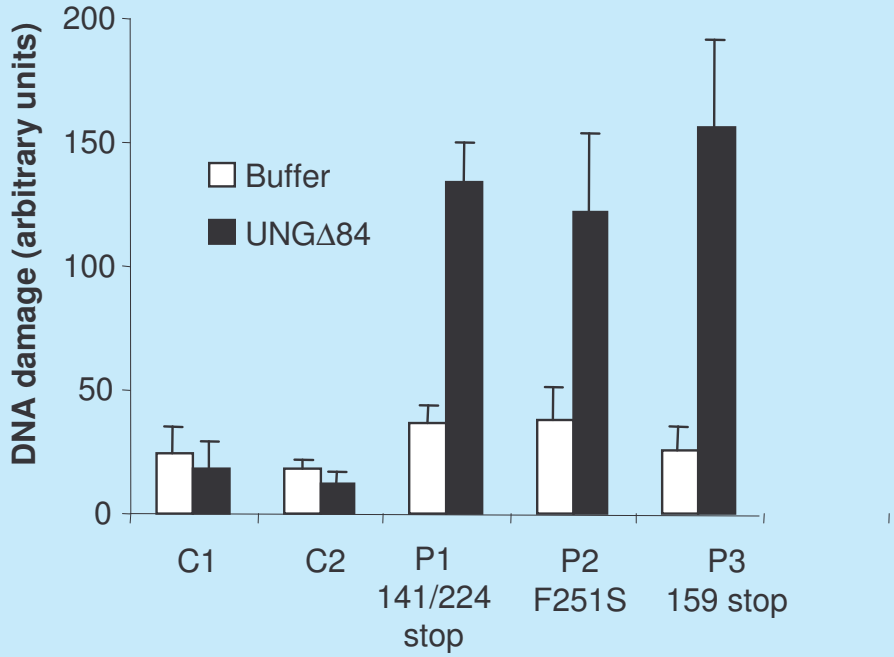




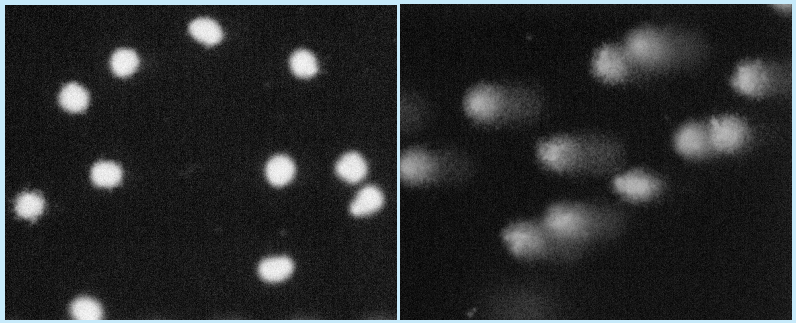
UNG mutations increase cellular uracil levels

Comet assays on B-cells; two controls and 3 patients.

Patients with truncated UNG-proteins, as well as Phe251Ser have increased uracil in the genome



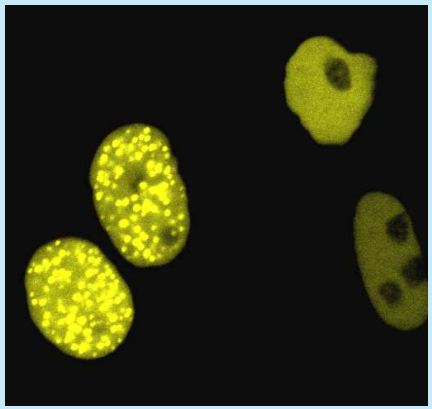
All growing cells from patients have comet tails, thus uracil content is increased in all cell cycle phases



C1 P3

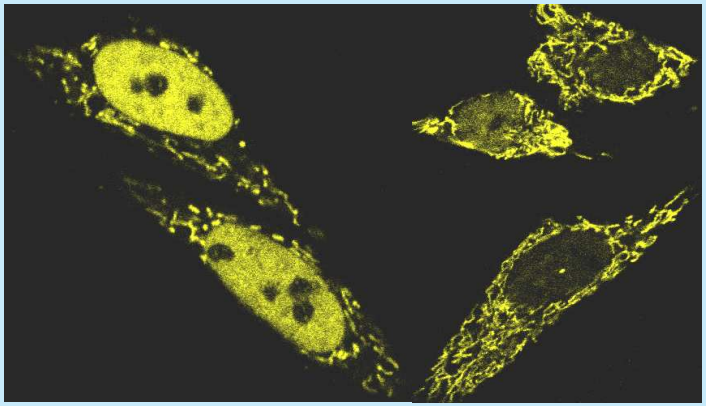
Localisation of UNG2 mutants from HIGM patient

Control



UNG2-EYFP
Two S-phase cells with UNG2 in nuclear foci

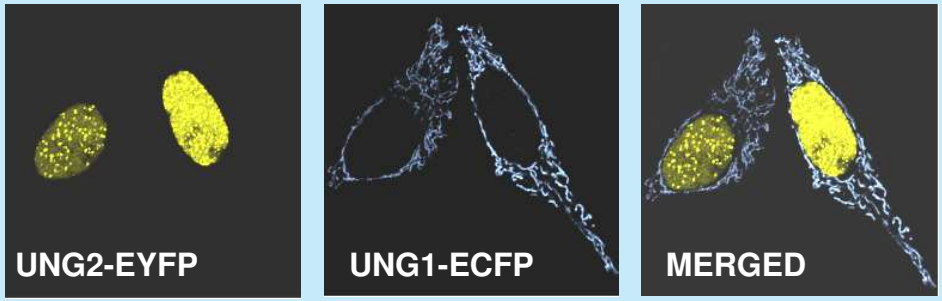
Phe251Ser



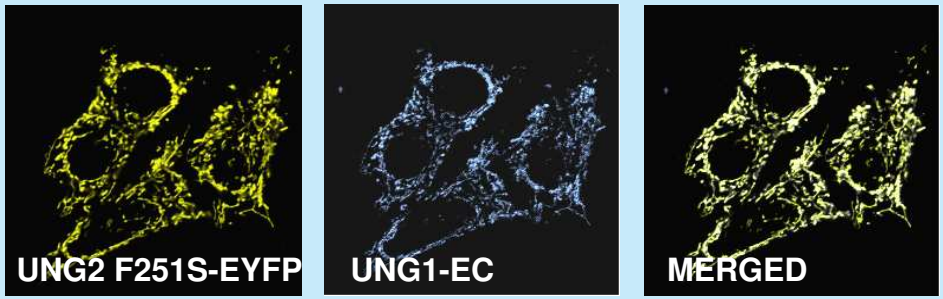
UNG2 F251S-EYFP
Localisation varies - some cells have mutated protein in both nucleus and cytoplasm, others mainly in cytoplasm

Conclusion: When overexpressed as EYFP-tagged protein, Phe251Ser is abnormally sorted. Why ? NLS not affected

UNG2 mutants from HIGM patients co-expressed with wild type UNG1



Yellow and blue tag does not affect the sorting of the proteins; normal sorting of both.



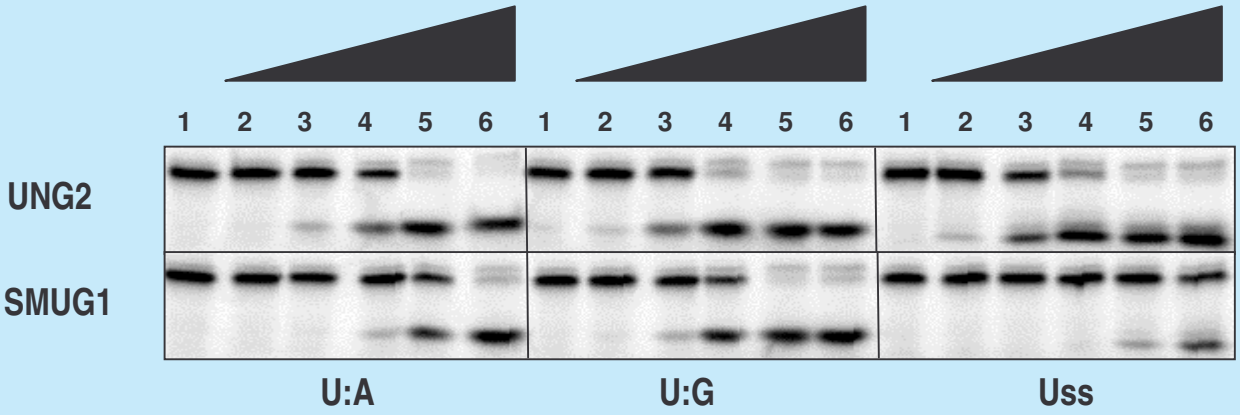
When co-expressing Phe251Ser with UNG1, all mutant protein is found in the cytoplasm where it co-localises with UNG1. After longer incubations F251S disappears, but wild type is stable

Conclusions:

The cellular sorting of the Phe251Ser mutant is clearly abnormal and disappears after longer incubations

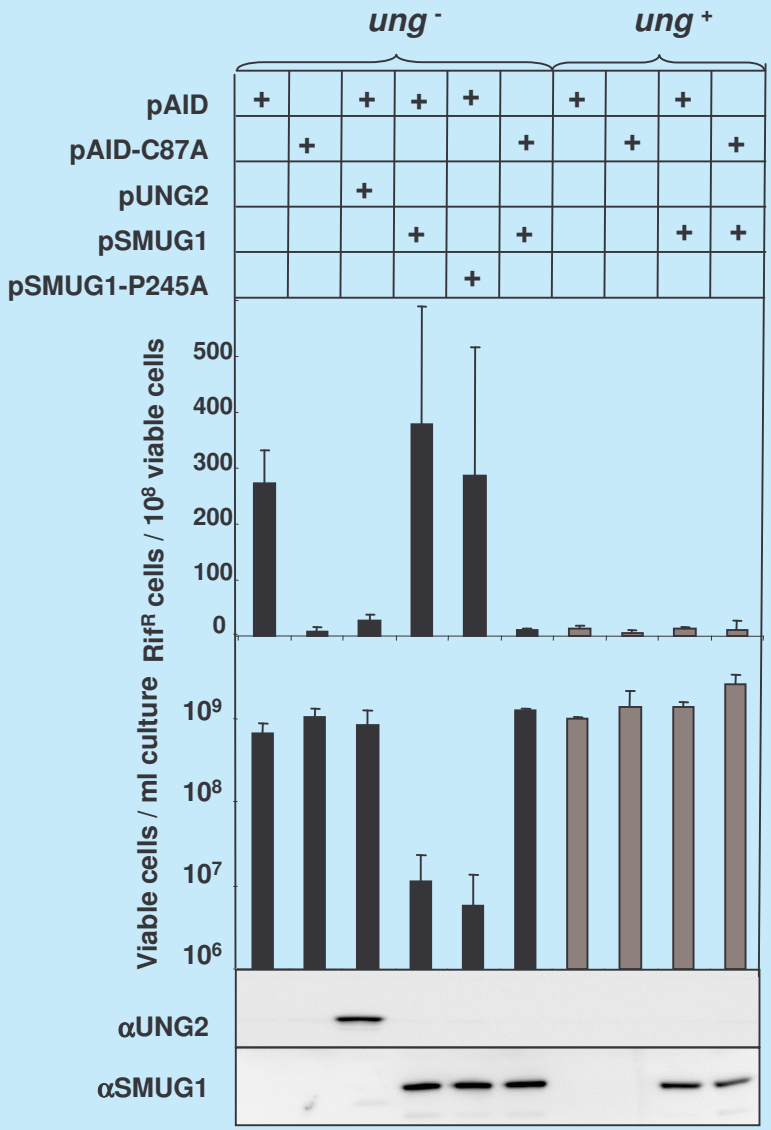
Hypothesis: UNG2 F251S forms dimers with UNG1 and transported to mitochondria, where it is degraded. The lack of nuclear UNG2 causes the hyperIGM syndrome in the patient.

UNG2 is ~1000-fold more efficient than SMUG1 in removal of uracil from single stranded DNA



AID only deaminates cytosine in ssDNA, probably explaining the inability of SMUG1 to complement UNG2 in UNG-deficient cells. In addition, SMUG1 is poorly expressed in B-cells

Mutagenicity of AID expressed in *E. coli* is abolished by UNG2, but not by SMUG1 which is also toxic in an *ung*⁻ background



AID is mutagenic, but not cytotoxic in Ung-deficient cells

AID-C87A, an inactive mutant, has no effect

UNG2 abolishes mutagenicity of AID, but SMUG1 does not

AID is not cytotoxic

Surprisingly, SMUG1 is cytotoxic in Ung-deficient, but not Ung-proficient cells

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