

Uracil-DNA glycosylase is not implicated in the choice of the DNA repair pathway during B-cell class switch recombination

Nour Ghazzaoui, Hussein Issaoui, Alexis Saintamand, Yves Denizot, François Boyer

► **To cite this version:**

Nour Ghazzaoui, Hussein Issaoui, Alexis Saintamand, Yves Denizot, François Boyer. Uracil-DNA glycosylase is not implicated in the choice of the DNA repair pathway during B-cell class switch recombination. Cellular and molecular immunology, Nature Publishing Group/Chinese Society of Immunology, In press, 16 (1), pp.93-95. 10.1038/s41423-018-0034-y . hal-01812480

HAL Id: hal-01812480

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-01812480>

Submitted on 30 Aug 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Uracil-DNA glycosylase is not implicated in the choice of the DNA repair pathway during B-cell
2 class switch recombination.

3

4 Nour Ghazzoui^{1,2}, Hussein Issaoui^{1,2}, Alexis Saintamand³, Yves Denizot^{1,4}, François Boyer¹

5 ¹UMR CNRS 7276, INSERM U1262, University of Limoges, CBRS, rue Pr. Descottes, 87025
6 Limoges, France

7 ²N.G. and H.I. contributed equally to this work.

8 ³present address: INSERM U1236, Rennes 1 University, France

9 ⁴To whom correspondence should be addressed. Email: yves.denizot@unilim.fr

10 Running title: UNG and B-cell class switch recombination.

11

12 Acknowledgments: We thank Dr. Tomas Lindahl for sending us UNG-deficient mice. This work
13 was supported by ANR (projet EpiSwitch-3'RR 2016). N.G. was supported by a grant from
14 Association de Spécialisation et d'Orientation Scientifique (Lebanon) and the municipality of
15 Khiam (Lebanon). H.I. is supported by CORC (FJA/NP 2015-109) and University of Limoges. The
16 authors are "Equipe Labellisée LIGUE 2018". We thank the GénoLim platform and the Cytometry
17 platform of the University of Limoges for sequencing and cell sorting. F.B. is supported by
18 Fondation Partenariale de l'Université de Limoges and ALURAD.

19

20 The authors declare no conflict of interest.

21 Author contributions: Y.D designed research. N.G., H.I. and A.S. performed research. A.S., F.B and
22 Y.D. analysed data. A.S, F.B and Y.D. wrote the paper.

23

24 Mature B-cells express membrane IgM and IgD (of same specificity) through alternative splicing of
25 a pre-mRNA encompassing constant (C_μ) and C_δ genes. After encountering antigen, B-cells undergo
26 class switch recombination (CSR) that substitutes the C_μ gene with C_γ , C_ϵ or C_α , thereby generating
27 IgG, IgE and IgA antibodies with same antigenic specificity but new effector functions. The DNA-
28 editing enzyme activation-induced deaminase (AID) is essential to CSR by targeting switch (S)
29 regions preceding C_μ (namely the S_μ donor region) and C_γ , C_ϵ and C_α genes (namely the $S_{\gamma,\epsilon,\alpha}$
30 acceptor regions) (1, 2). CSR is controlled in *cis* by IgH locus super-enhancers (3) and in *trans* by a
31 wide spectrum of enzymes and proteins (1, 2). Among them the role of the uracil DNA glycosylase
32 (UNG) remains controversial. UNG is a key enzyme of the base excision repair (BER) component
33 that carries out faithful repair. Some authors estimate that during CSR the UNG enzymatic activity
34 removes the AID-induced dC to dU converted base of single strand DNA generating abasic sites
35 thus leading to DNA strand breaks (1). For other authors the role of UNG is to stabilize the S-S
36 synapse and to recruit DNA repair factors that facilitate the end-joining process (4, 5). Thus, the
37 classical non-homogenous end joining (c-NHEJ) pathway would be increased over the alternative
38 end joining (A-EJ) in UNG deficient mice (4) suggesting an intriguing role of UNG in favour of the
39 A-EJ way. These results were based on the analysis of few S_μ - $S_{\gamma 1}$ sequences obtained in UNG
40 deficient conditions due to technical limitations (conventional Sanger sequencing) to investigate S-
41 S junction molecular signatures. We recently reported a new computational tool (CSReport) for
42 automatic analysis of CSR junctions sequenced by high-throughput sequencing (6). We have used it
43 to analyze the rare S_μ - S_δ junctions form during IgD CSR (7, 8) and S_μ - $S_{\gamma 3}$, S_μ - $S_{\gamma 1}$ and S_μ - S_α
44 junctions in *wt* mice (9). We thus used CSReport and high-throughput sequencing to analyze the
45 molecular signature of S_μ - $S_{\gamma 3}$, S_μ - $S_{\gamma 1}$ and S_μ - S_α junctions in UNG deficient mice more in depth.

46 Our research has been approved by our local ethics committee review board (Comité Régional
47 d'Ethique sur l'Expérimentation Animale du Limousin, Limoges, France) and carried according the
48 European guidelines for animal experimentation. Wild-type (*wt*) mice and UNG-deficient mice (a
49 gift from Dr. Tomas Lindahl, UK) were used. Single-cell suspensions of spleen cells were cultured

50 4 days at 1×10^6 cells/ml in RPMI 1640 with 10% fetal calf serum and 5 $\mu\text{g/ml}$ LPS, with (CSR
51 toward IgG_1) or without (CSR toward IgG_3) addition of 20 ng/ml IL-4 or 2 ng/ml $\text{TGF}\beta$ (PeproTech,
52 Rocky Hill, NJ) (CSR toward IgA) (3, 9, 10). Splenocytes were washed in PBS and stained with
53 various antibodies: anti-B220-PC5, anti-CD138-APC, anti-IgM PEcy7, anti-IgG₃-FITC, anti-IgG₁-
54 FITC and anti-IgA-FITC. Cells were analyzed on a Fortessa LSR2 (Beckman Coulter). In paralleled
55 experiments, stimulated splenocyte DNA was extracted for investigation of S_μ - S_{γ_3} , S_μ - S_{γ_1} and S_μ - S_α
56 junctions. As previously described in detail (6), junctions were PCR amplified. Libraries of 200bp
57 were prepared from the 1-2kb PCR products of S_μ - S_{γ_1} , S_μ - S_{γ_3} and S_μ - S_α amplification for Ion
58 Proton sequencing (“GénoLim platform” of the Limoges University, France). Sequenced reads were
59 then mapped to S_μ , S_{γ_1} , S_{γ_3} and S_α regions using BLAST algorithm. The computational tool
60 developed for experiments performs junction assembly, identifies breakpoints in S_μ , S_{γ_1} , S_{γ_3} and S_α ,
61 identifies junction structure (blunt, micro-homology, large-homology or junction with insertions)
62 and outputs a statistical summarization of identified junctions.

63 The UNG deficiency markedly reduces CSR toward IgG_3 (mean 0.1% vs 6.9%), IgG_1 (mean 1.9%
64 vs 14.7%) and IgA (mean 1.4% vs 2.5%) compared to *wt* mice (Fig. 1A). After DNA extraction the
65 molecular signatures of S_μ - S_{γ_1} , S_μ - S_{γ_3} and S_μ - S_α junctions were investigated. The structural profiles
66 of S_μ - S_{γ_1} , S_μ - S_{γ_3} and S_μ - S_α junctions (blunt, micro-homology, large-homology or junction with
67 insertions) for UNG-deficient and *wt* mice are reported in Fig. 1B. The distributions of IgG_1 , IgG_3
68 and IgA junctions in terms of distance from the forward PCR primer in S_μ and from of one of the
69 reverse primers in S_{γ_3} , S_{γ_1} and S_α are reported in Fig. 1C. Localizations of breakpoints within AID
70 hotspots (AGCT, WRCY, RGYW) and other motifs are shown in Fig. 1D (displayed along
71 specifically targeted segments within S regions).

72 Data indicated that if UNG deficiency markedly affected CSR efficiency, it did not significantly
73 affect the pattern of blunt vs micro/large-homology remaining junctions. Similarly the positions of
74 IgG_3 , IgG_1 and IgA junctions (in term of distance from S_μ and $S_{\gamma_1, \gamma_3, \alpha}$) and their colocation with

75 AID-attack motifs were not affected. Summarization of these 1568 independent junctions provided
76 description of CSR sequences in UNG-deficient mice at an unprecedented level. Until now only
77 few had been reported after cloning and subsequent sequencing by Sanger's method (4). In this
78 latter study, the panel of *wt* junctions was markedly different (in terms of blunt *vs* micro-homology
79 junctions) from ours and those of Panchakshari and coll. (2). Our results undoubtedly demonstrate
80 that CSR in UNG deficient conditions did not affect the balance between N-HEJ and A-EJ. These
81 results also show that μ - γ_1 , μ - γ_3 and μ - α CSR in UNG-deficient mice are regulated and that double
82 strand breaks for IgG1, IgG3 and IgA CSR are not random breaks. In conclusion our results
83 strengthen the hypothesis that during AID induced CSR, UNG in association with recombination
84 factors may facilitate the stabilization of the S-S synapse to facilitate an efficient recombination. In
85 contrast our results do not argue in favor of an UNG role in the recruitment of specific DNA repair
86 factors.

87

88 **References**

- 89 1. Chaudhuri J, Alt FW. Class-switch recombination: interplay of transcription, DNA deamination
90 and DNA repair. *Nat Rev Immunol* 2004; 4: 541-552.
- 91 2. Panchakshari RA, Zhang X, Kumar V, Du Z, Wei PC, Kao J et al. DNA double-strand break
92 response factors influence end-joining features of IgH class switch and general translocation
93 junctions. *Proc Natl Acad Sci USA* 2018; 115: 762-767.
- 94 3. Saintamand A, Rouaud P, Saad F, Rios G, Cogné M, Denizot Y. Elucidation of IgH 3' region
95 regulatory role during class switch recombination via germline deletion. *Nature Commun* 2015; 6:
96 7084.
- 97 4. Yousif AS, Stanlie A, Mondal S, Honjo T, Begum NA. Differential regulation of S-region
98 hypermutation and class-switch recombination by noncanonical functions of uracil DNA
99 glycosylase. *Proc Natl Acad Sci USA* 2014; 111: E1016-E1024.

- 100 5. Yousif AS, Stanlie A, Begum NA, Honjo T. Opinion: uracil DNA glycosylase (UNG) plays
101 distinct and non-canonical roles in somatic hypermutation and class switch recombination. *Int*
102 *Immunol* 2014; 26: 575-578.
- 103 6. Boyer F, Boutouil H, Dalloul I, Dalloul Z, Cook-Moreau J, Aldigier JC et al. CSReport: a new
104 computational tool designed for automatic analysis of class switch recombination junctions
105 sequenced by high-throughput sequencing. *J Immunol* 2017; 198: 4148-4155.
- 106 7. Ghazzoui N, Issaoui H, Saintamand A, Boyer F, Denizot Y. Analysis of IgD CSR junctions by
107 high-throughput sequencing. *Immunol Lett* 2017; 188: 86-88.
- 108 8. Issaoui H, Ghazzoui N, Saintamand A, Denizot Y, Boyer F. IgD class switch recombination is not
109 controlled through the immunoglobulin heavy chain 3' regulatory region super-enhancer. *Cell Mol*
110 *Immunol* 2017; 14: 871-874.
- 111 9. Issaoui H, Ghazzoui N, Saintamand A, Denizot Y, Boyer F. High throughput sequencing reveals
112 similar molecular signatures for class switch recombination junctions for the γ and α isotypes. *Cell*
113 *Mol Immunol* 2018 Mar 23. doi: 10.1038/s41423-018-0025-z. [Epub ahead of print]
- 114 10. Issaoui H, Ghazzoui N, Saintamand A, Carrion C, Oblet C, Denizot Y. The IgH 3' regulatory
115 region super-enhancer does not control IgA class switch recombination in the B1 lineage. *Cell Mol*
116 *Immunol* 2018; 15: 289-291.

117

118 **Legends to Figure**

119

120 **Figure 1: Class switch recombination in UNG-deficient and *wt* mice.**

121 A: IgG₁, IgG₃ and IgA CSR in UNG-deficient and *wt* mice. Spleen cells were LPS-cultured 4 days
122 with (CSR toward IgG₁) or without (CSR toward IgG₃) addition of IL-4 or TGF β (CSR toward IgA).
123 Cells gated on B220⁺ and/or CD138⁺ cells were investigated with anti-IgMPECy5, anti-IgG₃-FITC,
124 anti-IgG₁-FITC and anti-IgA-FITC antibodies. One representative experiment out of three to five
125 for each genotype is shown. B: Structure profiles of S $_{\mu}$ -S $_{\gamma 1}$, S $_{\mu}$ -S $_{\gamma 3}$ and S $_{\mu}$ -S $_{\alpha}$ junctions in UNG-

126 deficient and wt mice. Junctions are classified in terms of junction types (junction with insertions,
127 blunt junction, junction with micro- or large-homology). Number in parenthesis indicates number of
128 junctions of each type. Junction profile is not significantly different in *wt* and UNG-deficient mice
129 ($p=0.65$, $p=0.15$ and $p=0.28$ for $S_{\mu}-S_{\gamma 1}$, $S_{\mu}-S_{\gamma 3}$ and $S_{\mu}-S_{\alpha}$ respectively; chi-squared test). Results are
130 pooled from three to five mice per experiments. C: Breakpoint localizations in $S_{\mu}-S_{\gamma 1}$, $S_{\mu}-S_{\gamma 3}$ and
131 $S_{\mu}-S_{\alpha}$ junctions in *wt* and UNG-deficient mice (same junctions as in Figure 1B). D: Motif targeting
132 in $S_{\mu}-S_{\gamma 1}$, $S_{\mu}-S_{\gamma 3}$ and $S_{\mu}-S_{\alpha}$ junctions. Stars identified breakpoint positions in AGCT, WRCY and
133 RGYW AID hotspots and others (same junctions as in Figure 1B).

Figure 1

