

1 Article

2 Estimation of The Mutagenic Potential of 8-oxoG in 3 Nuclear Extracts of Mouse Cells Using The “Framed 4 Mirror” Method

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10 **Abstract:** We propose an improved earlier described “mirror” method [1] for detecting in cell
11 nuclear extracts mutations that arise in DNA during its replication due to misincorporation of
12 deoxyadenosine-5'-monophosphate (dAMP) opposite 7,8-dihydro-8-oxoguanine (8-oxoG). The
13 method is based on the synthesis of a complementary chain (“mirror”) by nuclear extracts of
14 different mice organs on a template containing 8-oxoG inside and dideoxycytidine residue (ddC)
15 at the 3'-end. The “mirror” was amplified by PCR using primers part of which was non-
16 complementary to the template. It allowed obtaining the “framed mirror” products. The
17 misincorporation of dAMP in “framed mirror” products forms an *EcoRI* restriction site. The
18 restriction analysis of double-stranded “framed mirror” products allows a quantification of the
19 mutation frequency in nuclear extracts. The data obtained showed that the mutagenic potential of
20 8-oxoG markedly varied in different organs of adult mice and embryos.

21 **Keywords:** DNA lesions; 7,8-dihydro-8-oxoguanine (8-oxoG); mutagenic activity; method of
22 detection; cell nuclear extracts; mice organs and embryos

23

24 1. Introduction

25 One of the main causes of GC-AT transversion somatic mutations is such a DNA lesion as 8-
26 oxoguanine (8-oxoG) arising in the genome under the action of reactive oxygen species (ROS)
27 generated in cells in the processes of oxidative phosphorylation [2]. The intensity of the conversion
28 of this DNA lesion into a mutation is a key moment of aging and oncological, neurological and
29 some other diseases [3,4]. The presence of 8-oxoG is dangerous for the stability of the genome
30 because the entire known DNA polymerases insert, with different frequencies and along with a
31 correct dC, a mutant dA opposite this lesion. For replicative DNA polymerases, this insertion is
32 close to 100% [5]. These data show that mutations owing to 8-oxoG arise in the process of DNA
33 replication mostly due to the appearance of mutant dA residues in the nascent DNA instead of
34 correct dC.

35 Besides this, it is known that in mammalian cells there is a base excision repair (BER) pathways
36 that reduce the mutational burden of ROS, ensuring the correct and efficient repair of A:8-oxo G
37 mismatches and the removal of 8-oxoG lesions from the genome [3]. In 8-oxoG:dA mismatches, dA is
38 removed by MutYH glycosylase and DNA polymerase lambda for post-replicative repair [5].

39 The mutagenic potential of 8-oxoG was studied over decades by various authors using
40 different experimental systems. As early as 1991, Shibutani et al. [6] showed that the ratio of
41 incorporated dA to dC opposite 8-oxoG varied for different DNA polymerases. These authors
42 conducted the reaction of primer extension by purified DNA polymerases on an artificial template
43 containing 8-oxoG. The copies obtained were separated by electrophoresis in denaturing
44 polyacrylamide gel. It allowed to separate DNA molecules with a definite mutation from those

45 lacking this mutation, whereas DNA with other mutations was absent. Although the advantages of
46 this method were obvious, it was not used or improved over the last 28 years. The reason was
47 probably that separation of equal-sized DNA molecules that differed only in one nucleotide is
48 technically complicated.

49 In later experiments, a technically less complicated approach was used, where primer
50 extension assay with four parallel DNA polymerase reactions carried out simultaneously (each of
51 them with only one of four dNTPs) was usually performed to ascertain which nucleotide is
52 incorporated opposite a certain lesion [7-9]. However, in all these experiments, the primer
53 extension reaction products were not separated, and therefore no conclusion could be drawn
54 concerning the question if these lesions cause the misincorporation of only one type of nucleotides
55 and don't generate other mutations as well.

56 The most advanced is the method where, at first, a specific DNA polymerase is used to obtain
57 a copy of the template containing a DNA lesion. The obtained copy is then sequenced and studied
58 for mutations [10]. This method allows identifying nucleotides incorporated in the copy opposite
59 this or that DNA lesion, and to determine if there are closely located other mutations.

60 Recently we proposed a "mirror" method to study the mutagenic potential of 8-oxoG using
61 purified DNA polymerases [1]. At the first stage of this method, similar to the method used by
62 Targgart *et al.* [10], a specific DNA polymerase is used to obtain a copy of the template that we
63 called "mirror". The "mirror" method differs in that, instead of sequencing "mirror", the nucleotide
64 opposite the lesion is identified by simple restriction analysis, whereas other mutations can be
65 identified by the SSCP method. The "mirror" method allows analyses of much more material in a
66 short time without additional sequencing.

67 However, studies with purified DNA polymerases give only a partial idea of the real situation
68 *in vivo*. A more detailed information about the mutagenic potential of 8-oxoG in normal cells and in
69 various pathologies can be obtained from using cell extracts containing the whole set of DNA
70 polymerases and regulatory proteins. Such an approach facilitated determining the role in the
71 correction of DNA synthesis of not only DNA polymerases but also other proteins involved in DNA
72 replication. For example, experiments using model DNA template, purified DNA polymerases and
73 extracts of knocked down cells showed that accessory replication protein A and PCNA function as
74 molecular switches that activated the efficient incorporation of correct dC opposite 8-oxoG of the
75 template by DNA polymerase lambda, but blocked the incorporation of incorrect (mutant) dA [11].

76 Thus, on the one hand, certain DNA polymerases can, with a high probability, incorporate
77 mutant dA opposite 8-oxoG in the template, and, on the other hand, certain regulatory proteins
78 inhibit this and support the trend to incorporate correct dC. However, in contrast to purified DNA
79 polymerases, nuclear and cell extracts of mammalian cells contain not only the full set of enzymes
80 involved in DNA synthesis and reparation, but also many nucleases. They also have a lower ability
81 to support processive DNA synthesis on an artificial template. As a result, the synthesized DNA
82 fragments have different lengths, and only part of them can correspond to the full-size copy of the
83 template ("mirror"). All this complicates the use of the "mirror" method with cell extracts

84 In the present work, we used a modified "mirror" method ("framed mirror" method) that
85 allows to overcome the existing experimental difficulties and to determine the mutagenic potential
86 of 8-oxoG in nuclear extracts of different mouse cell organs. The results obtained showed that the
87 bypass of DNA lesions caused by 8-oxoG is different in different organs of adult animals and in
88 embryos.

89 2. Materials and Methods

90 *Mice and embryos*

91 The animals were treated in accordance with the European Society Council 86/609/EEC
92 Requirement concerning the use of animals for experimental studies. The extracts of mouse organs
93 were obtained from C57B1 strain mice (about 3 months of age) Mouse embryos were isolated on
94 day 12-16 of the fetal development.

95 *Preparation of nuclear extracts*

96 Cell nuclear extracts were prepared according to a slightly modified method described by
 97 Schreiber et al. [12]. All procedures were conducted at 0°C. Typically, 300 mg of a mouse organ was
 98 transferred into an Eppendorf tube filled with an equal volume of 20 mM Tris-HCl buffer, pH7.5,
 99 with 1 mM EDTA, 1 mM phenylmethylsulfonylfluoride (PMSF), and 1 mM DTT. The content of the
 100 tube was then homogenized with a Teflon pestle for 5 min. The homogenate was then centrifuged
 101 for 5 min at 14,000 rpm in an Eppendorf centrifuge. The pellet was resuspended in an equal volume
 102 of 20 mM Tris-HCl pH 7.5, 60 mM KCl, 1 mM EDTA, 1 mM PMSF and 1 mM DTT. Then it was
 103 homogenized for 5 min with a Teflon pestle and centrifuged for 5min at 14,000 rpm. The pellet was
 104 resuspended in an equal volume of 20 mM Tris-HCl pH 7.5, with 450 mM NaCl, 1.5 mM MgCl₂, 0.2
 105 mM EDTA, 1 mM PMSF, and 25% glycerol, and homogenized one more time. The homogenate
 106 obtained was incubated for 15 min at 0°C with stirring, and then centrifuged as described above.
 107 The last stage was repeated twice. Two fractions obtained by extraction in buffer with high salt
 108 concentration (450 mM NaCl) were poured together and dialyzed for 5 h against 300 ml Tris-HCl
 109 buffer pH 7.5, 100 mM KCl, 0.2 mM EDTA, 0.5 mM DTT and 0.1 mM PMSF. Aliquots of the
 110 dialysate were stored at -70°C. Protein concentration in the fractions (10-15 mg/ml) was measured
 111 by the Bradford method [13].

112 *Templates and primers for "framed mirror" product synthesis.*

113 For the work, we used oligonucleotides synthesized by the firms "DNA synthesis" and
 114 "Eurogene", Moscow.

115 Templates:

116 **AXT/ddC** (with 8-oxoG (X) and dideoxycytidine (ddC)):

117 5'-GGGATCCTGCTGCCATAGGAAXTCTTGATTGGAAAGTCGACCTGddC-3'.

118 **45(T)** (with dT in the underlined *EcoRI* restriction site):

119 5'-GGGATCCTGCTGCCATAGGAAATCTTGATTGGAAAGTCGACCTGC-3'.

120 **45 (G)** (with dG instead of T in the *EcoRI* restriction site):

121 5'-GGGATCCTGCTGCCATAGGAAGTCTTGATTGGAAAGTCGACCTGC-3'.

122 **45(A)** (with dA instead of T in the *EcoRI* restriction site):

123 5'-GGGATCCTGCTGCCATAGGAAAATCTTGATTGGAAAGTCGACCTGC-3'.

124 **45(C)** (with dC instead of T in the *EcoRI* restriction site):

125 5'-GGGATCCTGCTGCCATAGGAACTCTTGATTGGAAAGTCGACCTGC-3'.

126 Primers:

127 **P1:** 5'-GTTGACCTACCCACACCATCCGCAGGTCGACTTTCCAATCAA-3'.

128 **P1t:** 5'-CY3-GTTGACCTACCCACACCATCC-3'.

129 **P2:** 5'-CATAATTACGAGCAATATGAAGGGATCCTGCTGCCATAGGAA-3'.

130 **P2t:** 5'-CATAATTACGAGCAATATGAA-3'.

131 All the oligonucleotides were purified by electrophoresis in polyacrylamide gel.

132 *Synthesis of the "framed mirror" products*

133 A general scheme of the "framed mirror" synthesis method is shown in Fig. 1.

134 Synthesis of the "mirror" by cell extracts on the AXT/ddC template was done in a 30µl reaction
 135 mixture containing 50 mM Tris-HCl pH 8.0, 50 mM KCl, 5 mM MgCl₂, 1 mM EDTA, 1 mM PMSF, 1

136 mM DTT, 50 µg/ml tRNA, 0.25 mM each of four dNTPs, 400 nM AXT/ddC template, 400 nM P1
 137 primer, and 100 µg protein of nuclear extract. The reaction mixture was incubated for 30 min at
 138 37°C. The reaction was terminated by adding EDTA up to a concentration of 10mM. The mixture
 139 was then diluted 100 times with reaction buffer. 10 µl of the diluted mixture was supplemented
 140 with 2X DreamTaq Master Mix (Thermo Scientific, Waltham, MA, USA) containing *Taq* polymerase
 141 and dNTP, 400 nM primer P2 having homology with the 3'-end of the "mirror". One cycle PCR
 142 with *Taq* polymerase (95 °C, 20 s; 95 °C, 20 s; 55°, 20 s; 72°, 20 s; 72°, 5 min) yielded a DNA chain
 143 complementary to the "mirror" (single-stranded "framed mirror"). At the next stage, this "framed
 144 mirror" was amplified. To this end, asymmetric PCR was carried out in the mixture with an overall
 145 volume of 50µl containing 5 µl of the mixture with the single-stranded "framed mirror", 500 nM
 146 primer P1t labeled withCY3, 5 nM primer P2t, and 25 µl 2X DreamTaq Master Mix under the
 147 following conditions: 95 °C for 20 s; 25 cycles of 95 °C for 20 s, then 60 °C for 20 s, then 72 °C for 20
 148 s; and 72 °C for 5 min. After this, the second strand of the "framed mirror" was synthesized in 50 µl
 149 of the reaction mixture containing 20 µl of the asymmetric PCR product, 25 µl of 2X DreamTaq
 150 Master Mix (Thermo Scientific, USA), and 500 nM primer P2t. The PCR reaction was performed as
 151 follows: 95 °C for 20 s; 2 cycles of 95 °C for 20 s, then 55 °C for 20 s, then 72 °C for 20 s; 72 °C for 5
 152 min. At the last stage, the obtained double-stranded "framed mirror" was cut with *EcoRI* at 37 °C
 153 for 2 h in the mixture containing 10 µl of the double-stranded "framed mirror", 2 µl of 10X *EcoRI*
 154 buffer (Thermo Scientific, USA), and 5 U of *EcoRI* (Thermo Scientific, USA).

155 *Electrophoretic analysis of EcoRI digestion products*

156 The products of the *EcoRI* digestion were analyzed by electrophoresis in 20% polyacrylamide
 157 gel with Tris-borate buffer pH 7.5, using 12-centimeter-long glass, at 18 mA current. The
 158 electrophoresis ran at 10°C for 4 h in the dark. The resulting gel was scanned with a Typhoon FLA
 159 9500, and the data were processed with ImageQuant™ v5.2 software.

160 *SSCP analysis of the single-stranded "framed mirror".*

161 Single-stranded products of asymmetric PCR were electrophoresed in 20% polyacrylamide gel
 162 with Tris-borate buffer, pH 7.5, using 25-centimeter-long glass, at 9 mA current. The electrophoresis
 163 ran for 35 h at 10°C in the dark. The gel was scanned with a Typhoon FLA 9500, and the data were
 164 processed with ImageQuant™ v5.2 software

165 **3. Results**

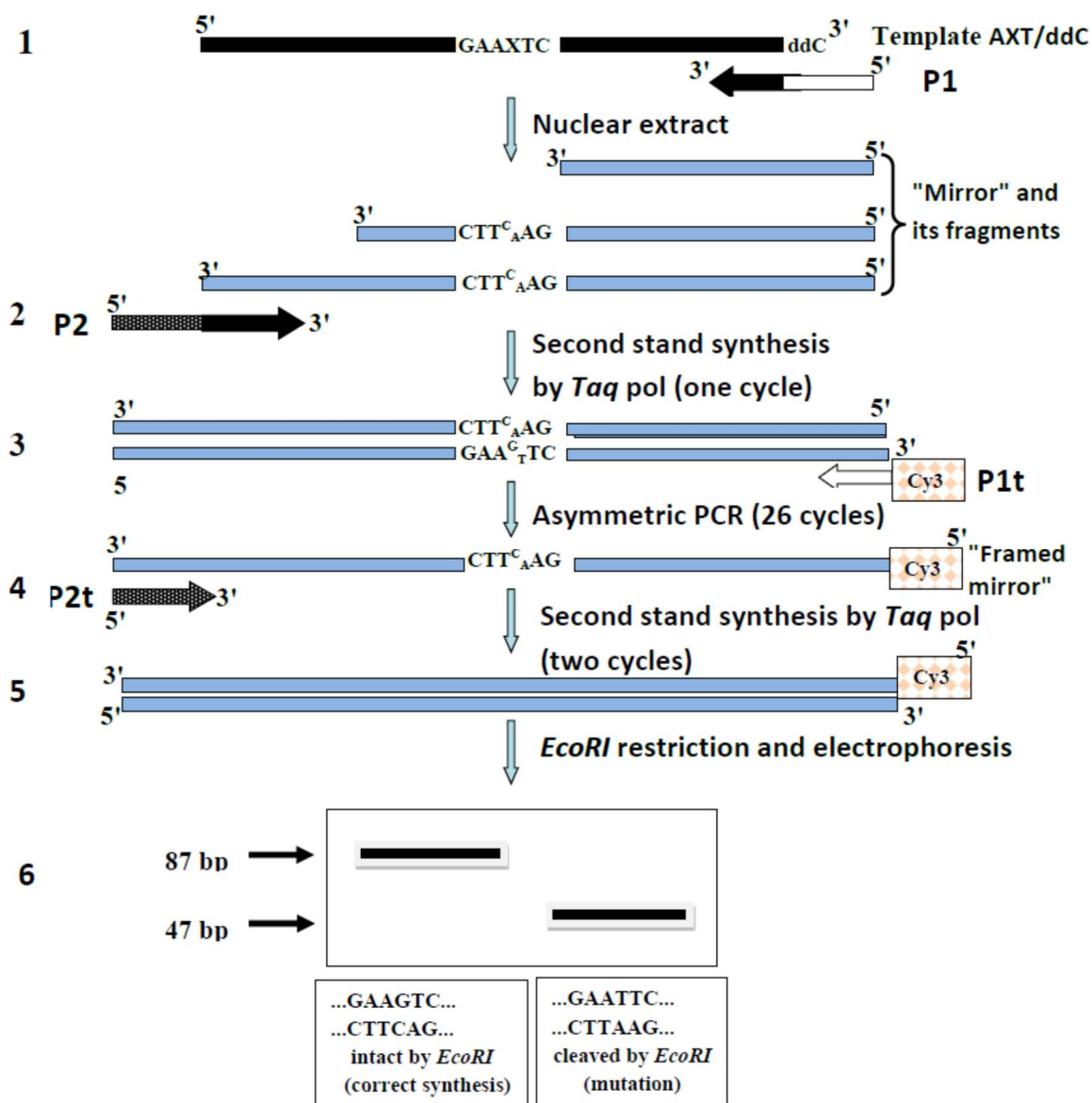
166 *The "framed mirror" method*

167 Earlier, we proposed a "mirror" method for studying mutagenic activity of purified DNA
 168 polymerases [1]. In the present work, to estimate the mutagenic activity of 8-oxoG in homogenates
 169 of nuclear extracts, we used a modified "mirror" method called "framed mirror". A modification
 170 was necessary because DNA-synthesizing activity in cell extracts is rather low. Besides, apart from
 171 DNA polymerases, cell extracts contain many other enzymes (nucleases, phosphatases, DNA
 172 glycosylases etc.) which can affect the structure of the DNA template under study. The extracts can
 173 also contain components able to affect PCR. Taking all this into account, we changed the "mirror"
 174 method as shown in Figure 1.

175 At stage 1, a "mirror" is synthesized with primer P1 (see Materials and Methods) on an
 176 artificial template containing 8-oxoG in its middle and dideoxycytidine (ddC) at its 3'-end. Primer
 177 P1 consisted of two parts. Its 3'-end was complementary to the template and at this end DNA
 178 synthesis started. The remaining part was homologous to primer P1t (see Materials and Methods)
 179 and served for further amplification of the "mirror" with *Taq* polymerase irrespective of the matrix.
 180 The ddC located at the 3'-end of the template blocked the PCR synthesis of extra "mirrors".

181 At stage 2, the fragments of the "mirror" were filled in to obtain the full size "framed mirror"
 182 with a site complementary to primer P1t (Figure 1). Simultaneously, the "framed mirror" was
 183 converted into the double-stranded form with added sites homologous to primers P1t and P2t for

184 further amplification of the "framed mirror" irrespective of the template. One cycle of PCR with *Taq*
 185 polymerase yielded a double-stranded copy of the "framed mirror" (double-stranded "framed
 186 mirror") that could be amplified by PCR with primers Pt1 and Pt2 (Figure 1) non-homologous to
 187 the initial template.



188

189 **Figure 1.** Scheme of the "framed mirror" method.

190 1 – synthesis of the "mirror" on a template containing 8-oxoG. 2 – filling in fragments of the
 191 "mirror" to obtain full-size "framed mirror" and its conversion into the double-stranded form. 3 –
 192 asymmetric PCR of the double-stranded "framed mirror". 4 – filling in products of the asymmetric
 193 PCR to obtain double-stranded forms. 5 – restriction digestion of the double-stranded DNA
 194 fragments with *Eco*RI. 6 – electrophoretic analysis of the DNA fragments digested by *Eco*RI
 195 in polyacrylamide gel.

196 Filling in the "mirror" fragments was necessary because cell extracts could not always form
 197 full-size copies of the template. Still, the size of most fragments formed in cell nuclear extracts
 198 showed that lesions of the template were mainly bypassed, and their filling in with *Taq* polymerase
 199 allowed to identify the nucleotide incorporated in the extracts opposite 8-oxoG.

200 At stage 3, asymmetric PCR with the double-stranded "framed mirror" and P1t primer was
 201 performed. This primer was not homologous to the template, and it was the only primer with a Cy3

202 label at its 5'-end. This PCR yielded Cy3-labeled single-stranded DNA fragments representing
 203 copies of the double-stranded "framed mirror". The reaction did not form copies of the template
 204 itself due to the absence of a site homologous to P1t primer and the presence of the ddC-block at the
 205 3'-end of the template. Additionally, the presence of other than incorporation dA opposite 8-oxoG,
 206 mutations that could arise under the action of cell extracts was checked using the SSCP method.

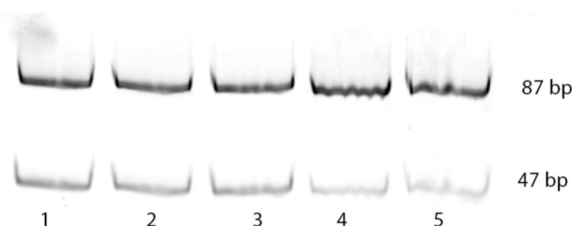
207 At stage 4, to measure the mutagenic potential in extracts more accurately, the single-stranded
 208 fragments were filled in with *Taq* polymerase and P2t primer to create double-stranded forms
 209 (Figure 1).

210 At stage 5, double-strand "framed mirror" was treated with *EcoRI* to reveal the mutation in
 211 the replication over the lesion.

212 At stage 6, the products of the *EcoRI* treatment were analyzed in non-denaturing
 213 polyacrylamide gel.

214 *Study of the mutagenic potential of 8-oxoG in organs of adult mice and their embryos*

215 The developed by us "framed mirror" method was used in the present work to determine the
 216 frequency of incorporation in the growing DNA chain of the mutant dA or correct dC opposite 8-
 217 oxoG by nuclear extracts of different mouse organs. The initial template was designed so that the
 218 incorporation of correct dC opposite 8-oxoG would give a 87 bp PCR product lacking *EcoRI* sites.
 219 On the contrary, the incorporation of mutant dA at the same position should give a PCR product
 220 that could be split with *EcoRI* into two fragments, 40 and 47 bp long. However, since the PCR
 221 product was labeled with Cy3 at one end, only 47 bp long fragment was visible on the
 222 electrophoregram. Figure 2 represents an example of electrophoretic separation of double-strand
 223 products synthesized in nuclear extracts of five different mouse organs and treated with *EcoRI*.



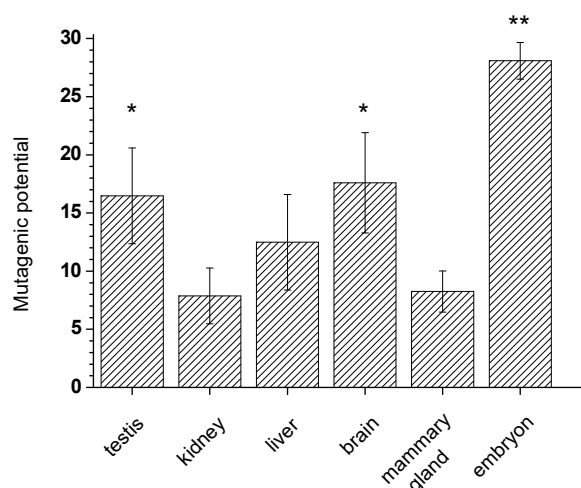
224
 225 Figure 2. Electrophoregram of the double-stranded products obtained in the extracts of cell nuclei and digested
 226 with *EcoRI*: 1 – testis, 2 - liver, 3 - brain, 4 – kidney, 5 - mammary gland. The percent of 47 bp band was taken
 227 as the mutagenic potential of 8-oxoG.

228

229 As seen in Figure 2, treatment with *EcoRI* gave in all cases two DNA fragments: a 87 bp long
 230 fragment due to the incorporation of correct dC, and 47 bp long fragment resulting from the
 231 incorporation of mutant dA. Numeric values of the mutagenic potential for each extract were
 232 derived from the band intensities.

233 Figure 3 shows averaged values of the 8-oxoG mutagenic potential in different mouse organs.
 234 One can see that the potential in nuclei of testes and the brain (16-18%) is higher than in kidneys,
 235 liver and mammary gland (8-13%). Similar experiments with cell nuclear extracts of mouse embryos
 236 showed that the potential in these extracts was markedly (~25%) higher than in organs of adult
 237 mice. Also, it was roughly the same for embryos of different age (12-16 days).

238

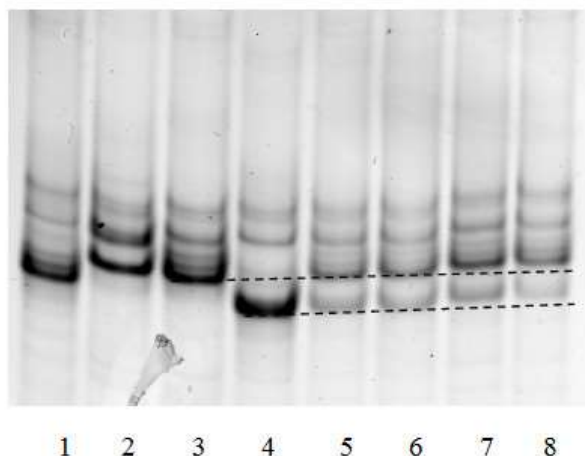


239

240 **Figure 3.** Values of the 8-oxoG mutagenic potential (percentage of the incorporated mutant dA
 241 relative to all incorporated nucleotides) measured by the “framed mirror” method in nuclear
 242 extracts of various mouse tissues and embryos. P values relative to mammary gland were * $P < 0.02$;
 243 ** $P < 0.001$ (averaged from at least three experiments).

244 *SSCP analysis of the single-strand “framed mirror”*

245 The developed by us “framed mirror” method allows to not only determine the frequency of
 246 dA incorporation opposite 8-oxoG in cell nuclear extracts, but also to test for the presence or
 247 absence in mammalian cells of factors capable of generating other mutations during translesion
 248 synthesis. Their presence should change the banding pattern of the electrophoresed single-strand
 249 “framed mirror” obtained by asymmetric PCR at stage 3 of the method. Figure 4 exemplifies such
 250 an electrophoresis of samples obtained in analysis of different mouse organs.



251

252 Figure 4. Separation in non-denaturing polyacrylamide gel of the products synthesized by Taq
 253 polymerase on control templates (1-4) (see Materials and Methods), and in nuclear extracts of
 254 different organs on the template containing 8-oxoG (AXT/ddC) (5-8). Templates: 1 - 45T, 2 - 45G, 3
 255 - 45C, 4 - 45A; organs: 5 - the brain, 6 - testes, 7 - liver, and 8 -mammary gland.

256 It can be seen that patterns of bands 5-8 correspond to only the patterns of *Taq* polymerase
 257 synthesis on control templates 45C and 45A. Extra bands due to possible deletions or insertions
 258 were not observed. This, together with a detailed analysis of band intensities, showed that the only
 259 mutation that could be generated by 8-oxoG within the genome was the incorporation of mutant
 260 dA opposite this lesion.

261 **4. Discussion**

262 There are some reports that DNA replication is not only coordinated with cell proliferation but
263 is also regulated uniquely in some cell types and organs. The differential regulation of DNA
264 synthesis requires a crosslink between DNA replication and differentiation [14]. These data suggest
265 that sets of enzymes involved in the DNA synthesis during differentiation and proliferation are
266 different in different cell types. Therefore, the mutagenic potential of different DNA lesions may
267 depend on the cell type and developmental stage of an organism. However, this type of studies is
268 practically absent. Also, there are no data for the frequency of mutant dA incorporation opposite 8-
269 oxoG instead of correct dC in mammalian cells.

270 In the present work, we used a new (“framed mirror”) method to study the mutagenic
271 potential of 8-oxoG lesion in different organs of mouse embryos and adult mice. To this end, we
272 used homogenates of cell nuclei instead of purified enzymes. We believed that such a system was
273 closer to the situation *in vivo*. The informativeness and efficiency of this system was demonstrated
274 in a number of experiments. For example, earlier we used cell homogenates to reveal alterations in
275 the DNA synthesis and reparation during the development of loach *Misgurnus fossilis*[15]. Analysis
276 of sea urchin embryo extracts [16] allowed the authors to reveal temporal heterogeneity of DNA
277 repair processes in animal development.

278 The data obtained in the present work showed that the mutagenic potential of 8-oxoG was
279 different in mouse embryos and organs of adult mice. It is widely accepted that mutation frequency
280 is significantly associated with proliferative activity. A relatively high mutagenic potential in
281 nuclear extracts of embryos can be therefore explained by the intense proliferation in embryonic
282 development. In organs of adult mice, the mutagenic potential was the highest in extracts of the
283 brain and testes. The high potential in cell extracts of testes might be also explained by intense
284 proliferation of cells and the presence of active replicative DNA polymerases that often incorporate
285 dA opposite 8-oxoG within a “mirror” template. However, the relatively high mutagenic potential
286 in cell extracts of the brain poorly agrees with the accepted view that cells of the brain practically do
287 not proliferate and contain quantitatively prevalent DNA polymerase beta that has a relatively low
288 mutagenic potential during bypassing of 8-oxoG lesion [17, 18].

289 It can be suggested that DNA polymerase activity in the brain differs from that in other organs.
290 In particular, we showed that, in contrast to most other organs, in extracts of mouse brain cells t-
291 stop after incorrectly inserted dT could be overpassed by DNA polymerase iota [19].

292 Another interesting result of this work is a higher mutagenic potential of 8-oxoG in mouse
293 embryos compared to organs of adult animals. It suggests that mutagenic activity of DNA lesions is
294 changed during ontogenesis. This finding well corresponds to our earlier data. In particular, we
295 showed that loach larvae virtually lacked the capacity for error correction of DNA duplexes
296 containing a mismatched nucleotide [15]. Also, analysis of incorrect activity of error-prone DNA
297 polymerase iota in *M. musculus* ontogeny demonstrated considerable changes in its activity, which
298 was the highest during prenatal development of most organs and decreased in the adult body [20].

299 Thus, the results obtained here suggest that (1) the mutation frequency at embryonic
300 developmental stages is higher than in organs of adult organisms, and (2) the mutagenic activity in
301 organs of adult animals is not strictly associated with proliferative activity of cells.

302
303 **Author Contributions:** A.A.V., I.V.M and K.Y.K. performed the experiments, L.V.G. and V.Z.T.
304 wrote the manuscript.

305
306 **Acknowledgments:** This study was supported by the Russian Foundation for Basic Research
307 (grants 16-04-00475 and 18-04-00733) and Russian Academy of Sciences (grant Molecular and
308 Cellular Biology). This study was performed using equipment of the Center of Cellular and Genetic
309 Technologies of the Institute of Molecular Genetics, Russian Academy of Sciences. We thank Boris
310 Glotov for critically reading and providing feedback on the manuscript.

311
312 **Conflicts of Interest:** The authors declare no conflicts of interest.

313 **Abbreviations and Notations**

- 314 8-oxoG - 7,8-dihydro-8-oxoguanine
 315 AXT - single-stranded DNA template containing 8-oxoG
 316 ATT - single-stranded DNA template containing T in place of 8-oxoG
 317 AGT single-stranded DNA template containing G in place of 8-oxoG
 318 BER – base excision repair
 319 dAMP- deoxyadenosine-5'-monophosphate
 320 ddC - dideoxycytidine
 321 DP - direct primer
 322 PMSF - phenylmethylsulfonylfluoride
 323 RP - reverse primer
 324 ROS - reactive oxygen species
 325 SSCP - single-strand conformational polymorphism

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