Biochemistry (Moscow), Vol. 70, No. 10, 2005, pp. 1157-1162. Translated from Biokhimiya, Vol. 70, No. 10, 2005, pp. 1403-1409.

Enhanced Activity of DNA Polymerase Iota in Mouse Brain Cells Is Associated with Aggressiveness

L. V. Gening[1], A. A. Kamensky[2], N. G. Levitskaya[2], A. N. Petrochenkov[1], I. I. Poletaeva[2], and V. Z. Tarantul[1]*

[1]Institute of Molecular Genetics, Russian Academy of Sciences, ul. Kurchatova 2, 123182 Moscow, Russia; fax: (7-095) 196-0221; E-mail: tarantul@img.ras.ru
[2]Biological Faculty, Lomonosov Moscow State University, 119899 Moscow, Russia; fax: (7-095) 939-2895; E-mail: kamensky@5.human.bio.msu.ru

Received August 5, 2004
Revision received September 16, 2004

Abstract—Recent studies performed with crude extracts of mouse tissues showed that the activity of DNA-polymerase iota (Pol ι) can be detected only in brain and testis extracts. To assess whether the activity of Pol ι is associated with animal behavior, we determined Pol ι activity in brain extracts of mice of two lines sharply differing in aggressiveness (RSB and RLB). We found that Pol ι activity in the mice with aggressive behavior was three times higher than in the less aggressive mice. The possible relationship between the activity of Pol ι and animal behavior is discussed.

Key words: DNA-polymerase iota, aggressiveness, animal behavior, brain, genome stability

Of all DNA polymerases known to date, DNA polymerase iota (Pol ι), which was discovered recently as a result of computed-based analysis of human genomic nucleotide sequences, exhibits the greatest ability for incorrect DNA synthesis [1]. In particular, unlike other DNA polymerases, Pol ι breaks the Watson–Crick rule and preferentially incorporates deoxyguanosine monophosphate into the growing DNA strand opposite to thymidine of the template [2-4]. The biological functions of Pol ι remain unknown because diseases or susceptibilities to diseases associated with mutations in the gene encoding this enzyme in humans have not been described.

We recently studied the activity of Pol ι immediately in mouse tissue extracts [5]. The analytical method used for this purpose was based on the ability of Pol ι to preferentially incorporate dGMP opposite to T of the template. The DNA polymerase primer extension reaction in the presence of a single nucleotide triphosphate, dGTP, performed with mouse cell extracts, showed that this ability was most pronounced in the brain and testis extracts. This result was quite unexpected because other authors showed that Pol ι mRNA is present in all mouse tissues studied and that its content in heart, pancreas, and testis is even greater than in brain [6].

We assumed that in brain cells Pol ι may play a specific role, possibly associated with a brain function such as behavior. To test this assumption, we analyzed the activity of Pol ι in two mouse lines contrasting with respect to inter-male aggressiveness, differing, in particular, in the response to neonatal injection of buspirone [7].

MATERIALS AND METHODS

Substrate for testing DNA polymerase activity. Two complementary oligodeoxyribonucleotides forming a duplex with protruding 5′ end upon hybridization [2] were used as substrates for testing DNA polymerase activity (Fig. 1). A 17-mer oligonucleotide was labeled with ^{32}P at the 5′ end, and brain extracts were obtained as described in [5]. Animals were sacrificed under standard conditions by a sharp dislocation of the cervical vertebrae. The brain was extracted with a spatula and homogenized as described in the same work.

Determination of DNA polymerase activity. The reaction was performed in 20 μl of a mixture containing 300 nM substrate (in 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM $MgCl_2$ and 1 mM dithiothreitol (DTT)), 5 μl of extract of the respective tissue, and 1 μM deoxynucleotide triphosphate at 37°C for 15 min with subsequent addition of an equal volume of a mixture of

* To whom correspondence should be addressed.

0006-2979/05/7010-1157

95% formamide, 50 mM EDTA, and 0.05% bromophenol blue.

The reaction products were separated by electrophoresis in 20% polyacrylamide gel (acrylamide/bisacrylamide 30 : 1) with 7 M urea in Tris-borate buffer at 30 mA until the exit of bromophenol blue from the gel. After electrophoresis, the gel was enveloped in a polypropylene film and autoradiographed using a Storage Phosphor Screen (Molecular Dynamics, USA). The autoradiograph was scanned on a Storm 840 phosphorus imager (Molecular Dynamics). Data were analyzed using Image Quant 5.2 software.

Mouse lines. The experiments on determination of the activity of DNA polymerases in brain cell extracts were performed with two mouse lines, RSB and RLB. A comparative study of these lines, which was performed earlier, showed that RSB mice exhibit a greater level of inter-male aggressiveness and that a neonatal administration of buspirone increases this level. RLB were initially less aggressive, and neonatal administration of buspirone decreased the level of inter-male aggressiveness [7].

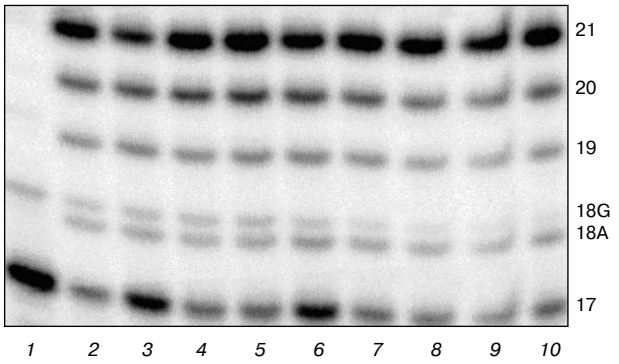

Fig. 2. Electrophoretic separation of oligonucleotide products obtained by incubation of the substrate with brain cell extracts of aggressive (lanes *2-7*) and control (lanes *8-10*) mice in the presence of dATP and dGTP. Lane *1* shows the same extract as lane *2* that was incubated in the presence of dGTP. The sizes of nucleotides are shown on the right: 17 nucleotides, the primer; 18A, the primer plus A at the 3′ end; 18G, the primer plus G at the 3′ end.

RESULTS

Earlier, we showed that activity characteristic of Pol ι can be detected in mouse brain extracts. To determine Pol ι activity in the brain of mice of different lines, we used an oligonucleotide complex consisting of a 30-nucleotide template and a 17-nucleotide primer (Fig. 1) as described in [2]. To quantify results, we slightly modified the conditions of the reaction in which mouse brain extracts were used as substrates. In the modified procedure, the mixture for DNA polymerase reaction contained not only dGTP but also dATP. The presence of dATP along with dGTP is required to prevent misincorporation of G opposite T by other DNA polymerases of brain cell extracts. Some DNA polymerases do it, although with a low efficiency, if the reaction mixture contains solely dGTP. An addition of dATP completely excludes this possibility because it is known that only Pol ι, despite of the presence of excess dATP, incorporates G opposite to T 10-30 times more effectively than A against T.

Because the oligonucleotide substrates contain T immediately opposite to the 3′ end of the primer, all DNA polymerases (except for Pol ι) should incorporate dAMP, whereas Pol ι under these conditions preferentially incorporates dGMP. Therefore, after electrophoretic separation of reaction products, the product produced by Pol ι can be identified and quantified relative to other products synthesized in the presence of crude extracts by other DNA polymerases.

5'-GGAAGAAGAAGTAT GTT-3'
3'-CC TTCTT CTT CATACAATCTTACTTCTTCC-5'

Fig. 1. Substrate of the DNA polymerase reaction (the upper oligonucleotide is labeled with ^{32}P at the 5' end).

As seen from the structure of the substrate, in the presence of dGTP and dATP, according to the Watson–Crick base-pairing rule, all DNA polymerases except for Pol ι should incorporate successively in the course of synthesis of the oligodeoxyribonucleotide AGAA to form a labeled 21-nucleotide product. However, if cell extracts contain Pol ι, G should be incorporated preferentially instead of the first A. Because Pol ι exhibits a low processivity, the synthesis of DNA strand is terminated at this stage. If the reaction mixture contains solely dGTP, only a small amount of dG is incorporated into the 19-nucleotide reaction product according to the Watson–Crick rule, because the template contains C in the corresponding position (Fig. 1). This may be observed in experiments performed both with purified Pol ι preparations [2] and mouse brain cell extracts (Fig. 2, lane *1*). Thus, the detection of the 18-nucleotide reaction product containing G at the 3′ end indicates that the extract contains the activity characteristic of Pol ι.

To separate the 18-nucleotide reaction product containing dG at the 3′ end from a similar product containing dA at the 3′ end, after incubation the reaction mixture was fractionated by electrophoresis under denaturing conditions on a long (60-cm) plate of polyacrylamide gel. Incubation with the substrate yields both reaction products longer than 17 nucleotides and the products of primer degradation by nucleases, which are shorter than 17 nucleotides. Degradation products accounted for less than 30% of the total product of each reaction, with their composition and proportion being nearly constant as assessed by the results of numerous experiments. For this

Intensities of bands (%) shown on the electrophoregram in Fig. 2

DNA fragment, bp	Number of lane on the electrophoregram										
	2	*3*	*4*	*5*	*6*	*7*	I_{mean}	*8*	*9*	*10*	I_{mean}
17	12.8	39.2	11.42	10.58	28.88	12.03	19.2 ± 4.9	12.66	11.27	12.67	12.2 ± 0.5
18A	3.55	3.69	2.99	2.56	5.06	3.76	3.6 ± 0.3	3.89	3.54	3.94	3.8 ± 0.1
18G	2.18	2.84	1.64	2.12	1.7	0.92	1.9 ± 0.2	0.97	0.96	1.05	1.0 ± 0.02
19	6.81	7.77	5.17	5.29	6.48	6.26	6.3 ± 0.4	4.99	5.63	5.03	5.2 ± 0.2
20	15.56	15.95	16.56	15.48	16.39	14.57	15.7 ± 0.3	11.06	13.58	12.91	12.5 ± 0.7
21	59.1	30.55	62.23	63.97	41.5	62.46	53.3 ± 5.6	66.42	65.01	64.13	65.1 ± 0.6
18G/(18G + 18A)	38.0	43.4	35.4	45.2	25.0	19.6	34.4 ± 4.2	19.9	21.3	21.0	20.7 ± 0.4

Note: I_{mean}, mean value for group ± SE.

reason, our attention was focused only on the products of synthesis.

As seen in Fig. 2 (lanes *2-10*), the 18-nucleotide reaction products containing dG at the 3′ end of the synthetic primer migrate during electrophoresis somewhat slower than analogous dA-containing products and can be well separated from the latter. This feature allowed us to notice that the intensity of the band corresponding to the product containing G at the 3′ end was considerably greater in the samples incubated with the brain extracts of RSB mice (lanes *2-7*), which exhibited greater aggressiveness, than in the samples incubated with the brain extracts of RLB mice (lanes *8-10*).

The ratios between the intensities of bands corresponding to the oligonucleotides consisting of 17 to 21 nucleotides in samples *2-10* (Fig. 2) are summarized in the table. It can be seen that, in RLB mice, the intensities of bands of the same size are similar, whereas in RSB mice the intensities of the same bands differed and data scattering was greater. The high intensity of bands corresponding to the 17-nucleotide oligonucleotide (primer) was the most pronounced on lanes *3* and *6* (39.2 and 28.9%, respectively, as opposed to 12% in other lanes). Less intense bands in the same samples corresponded to the 21-nucleotide oligonucleotide (30.1 and 41.5%, respectively, as opposed to 60-65% in the other lanes). The 18G band, corresponding to the product appearing in the presence of brain extract of RLB mice, had an intensity of 1% (lanes *8-10*), whereas in the brain extracts of RSB mice it varied from 1% (lane *7*) to 2.8% (lane *3*).

Because the intensity of the band corresponding to the 18A product in experiments with RLB mice was stable in different samples, we decided to use it as an internal control and to measure the activity of Pol ι relative to the intensity of bands 18G/(18G + 18A). As seen from the table, in five out of six RSB mice (lanes *2-5*), in which this parameter varied from 35.4 to 45.2%, the Pol ι activity was twice as great as in RLB mice (lanes *8-10*), in which this parameter was not greater than 21%.

DISCUSSION

The human Pol ι gene was identified as a homolog of the yeast gene *RAD-30*, which, in turn, is homologous to the *E. coli* genes *Din B* and *Din C* [1]. The biochemical properties of the human and mouse enzymes encoded by this gene have been actively studied by different groups of authors, who used only homogeneous preparations of this enzyme obtained using Pol ι gene expression in insect and yeast cells [2-4, 6, 8].

Pol ι is characterized by an extremely incorrect copying of undamaged DNA and is able to continue synthesis even after mismatched bases at the 3′ end of the primer. A unique property of Pol ι consists in preferential incorporation of G opposite template T, which makes it able to accurately copy deaminated cytosines. Note that Pol ι sufficiently correctly copies template adenosines and exhibits the ability to eliminate the phosphate of deoxyribose, which is required during the repair of damaged DNA regions [9].

Pol ι belongs to the Y family of DNA polymerases [10]. The biochemical properties and genetic studies of some enzymes of this family confirmed that they play a key role in human and mammalian cells. For example, DNA polymerase eta (Pol η), the best characterized representative of this family, is able to overcome a substantial set of different DNA lesions in the course of synthesis [11-14]. Individuals with mutation in the gene encoding this enzyme exhibit an increased sensitivity to ultraviolet radiation and are susceptible to skin cancer [15]. Furthermore, Pol η is involved in somatic hypermutagenesis of antibodies, because during copying of undamaged templates it makes numerous mismatches [16-18]. As a result, the spectrum of mutations at variable

immunoglobulin genes is considerably changed in individuals with the Pol η gene malfunctions [19, 20]; those types of mutations that are generated by Pol η during DNA synthesis *in vitro* are absent in them [21, 22].

With respect to its properties, Pol ι is the closest to Pol η [1], although the ability of the former to eliminate some DNA lesions during DNA synthesis is strongly limited [9, 23]. For this reason, taking into account the fact that this enzyme contains activity eliminating the phosphate of deoxyribose, it can be assumed that Pol ι is most likely intended for specific ways of repair of damaged DNA regions, from which nitrogen bases are removed [24]. There exist only scanty data demonstrating that the probability of development of lung cancer of mice may depend on allelic variants of Pol ι present in different lines of these animals [25].

As a result of a pronounced tendency to incorrect DNA synthesis, Pol ι may be regarded as a candidate for an agent involved in mutagenesis of variable immunoglobulin genes. Indeed, it was shown that Berkitt's lymphoma cells with a homozygous deletion of both genes encoding Pol ι are characterized by a decreased level of somatic hypermutagenesis [26]. However, these data are inconsistent with the results of Donald et al., who showed that the level of somatic hypermutagenesis of variable immunoglobulin genes and the spectrum of mutations in mice whose Pol ι gene is damaged do not differ from those characteristic of mice with an intact Pol ι gene [27].

The study of the properties of various DNA polymerases in brain cells of adult animals suggests that these enzymes maintain the stability of the genome in these cells [28]. In the brain, the most actively expressed DNA polymerase is DNA polymerase beta (Pol β) [28, 29], which belongs to the X family and is able to repair DNA structure after the elimination of improper bases by DNA glycosylases. Besides Pol β, brain cells contain three other forms of DNA polymerases—alpha (Pol α), delta (Pol δ), and epsilon (Pol ε) [29]—which belong to the B family and play a key role in replication resumption after the stoppage of the replicative fork at damaged DNA sites [29].

Active transcription of a large number of loci—a characteristic feature of nerve cells—is apparently the major source of DNA lesions in brain cells. Recent data have shown that actively transcribed DNA regions are most prone to mutations [30], because breaks and gaps resulting from the double strand unwinding are formed in them with the highest probability.

There are no published data on the activity of Pol ι in brain cells. Our results showed that the activity of this enzyme in mouse brain cells is significantly greater than in the majority of other organs [5]. This finding suggests that the high activity of Pol ι in mouse brain cells should play a functional role. Similarly to Pol β, the dominant DNA polymerase in the brain, it contains activity eliminating the phosphate of deoxyribose and can substitute it in *in vitro* experiments, in which uracil is eliminated from DNA in the presence of uracyl-*N*-glycosylase, apurinic endonuclease, and DNA ligase [24]. The activity of Pol ι related to the preferential incorporation of G opposite template T may serve for repairing damage occurring in cells as a result of deamination of methylcytosines, which are converted into thymidines. Pol ι is able to restore the original context provided that it incorporates guanidines opposite these thymidines. However, the function of this enzyme in normal cells remains obscure.

The modern conception on the genetic mechanisms that determine inter-male aggressiveness—the trait by which the mouse lines RSB and RLB reliably differ—is based both on simple interlinear comparisons [31 32] and on the data on this trait in hybrids [33]. There is no doubt that inter-male aggressiveness is controlled by a great number of genes. This was shown, for example, using the modern method of qualitative trait locus (QTL) analysis, which is based on revealing genetic polymorphism by micro- and minisatellite sequences [34]. It was also shown that some genes associated with the manifestation of this trait are located in the Y chromosome [35]. However, it is obvious that genetic variability of inter-male aggressiveness should be associated with polymorphism at the genes of the enzymes catalyzing the synthesis and degradation of the main neurotransmitters, such as glutamate, GABA, and monoamines. This assumption was corroborated experimentally [36-38], in particular, using mice in which the genes encoding the respective proteins were knocked down [39, 40].

Apparently, the proposed method of testing Pol ι activity gives only an approximate estimate of the total amount of the active enzyme in cells. On the other hand, the advantage of this method is the possibility of simultaneous monitoring activities of all DNA polymerases contained in a cell extract, which can hardly be done using any other approach. The fact that the activity of Pol ι was only detected in the extracts of brain and testis cells can be accounted for by the posttranscriptional regulation of the synthesis of this protein, because tests with mRNA showed that the greatest amount of it is present in testis, heart, and pancreas cells [6].

Thus, the presence of Pol ι activity in cell extracts often does not correlate with the content of its mRNA. Possibly, specific activators of Pol ι function in the cells of some organs (testis and brain) are still undiscovered. The results of this study are indicative of an increased level of Pol ι activity against the background of other DNA polymerases in the brain of RSB mice.

A distinct trend can be traced in the ratio between the intensities of bands 18G/(18G + 18A), mostly varying in the range from 5 to 20% (but not exceeding 20%) in mice of other lines (data not shown), whereas in the line of aggressive mice studied this index varied from 19 to 45% (table).

The results of this study show that the 18-nucleotide product containing G at the 3′ end accounts for 1% of all products of DNA polymerase reaction catalyzed by brain cell extracts of RLB mice (with normal aggressiveness). In some RSB mice with aggressive behavioral pattern, this index was several times higher. This means that Pol ι contained in brain cell extracts of mice of this line exhibits the ability to incorporate G opposite T with a probability of 10^{-1}-10^{-2}.

An increase in Pol ι activity in brain cells of RSB mice could be explained by more intensive transcription at certain loci, which entails activation of the mechanisms of DNA repair with the use of Pol ι. However, as seen from our data (Fig. 2, table), the activities of DNA polymerases other than Pol ι do not increase in brain cells of RSB mice, as could be expected should our assumption be true. Furthermore, in the samples shown on lanes *3* and *6*, there was a very intense band corresponding to the primer, whereas the intensity of the band corresponding to the 21-nucleotide product was decreased, indicating a decrease in activities of DNA polymerases other than Pol ι.

Apparently, in the majority of mammalian tissues, the molecular mechanisms with the involvement of Pol ι either do not function or are activated only in certain situations. The analysis of cell extracts of mouse liver, heart, lungs, spleen, and pancreas, performed under the same conditions as the analysis of brain cell extracts, did not reveal Pol ι activity [5], although other DNA polymerases were detected well under these conditions.

Theoretically, increased activity of Pol ι in brain cells of aggressive mice, tested only at the biochemical level, can cause considerable changes in DNA structure of at least a certain group of cells. However, any direct evidence for this is absent. Nevertheless, the presence of such activity supports the hypothesis that immunological mechanisms are involved in the formation of long-term memory, as proposed by I. P. Ashmarin and S. A. Titov in 1988 [41]. In this case, the role of Pol ι is to ensure the hypermutagenesis of variable regions of genes encoding hypothetical “memory antibodies”.

This study was supported by a grant for physicochemical biology from the Presidium of the Russian Academy of Sciences, a grant from the Government of Moscow, and by the Russian Foundation for Basic Research (project No. 02-04-48493-a).

REFERENCES

1. McDonald, J. P., Rapic-Otrin, V., Epstein, J. A., Broughton, B. C., Wang, X., Lehmann, A. R., Wolgemuth, D. J., and Woodgate, R. (1999) *Genetics*, **60**, 20-30.
2. Zhang, Y., Yuan, F., Wu, X., and Wang, Z. (2000) *Mol. Cell. Biol.*, **20**, 7099-7108.
3. Johnson, R. E., Washington, M. T., Haracska, L., Prakash, S., and Prakash, L. (2000) *Nature*, **406**, 1015-1019.
4. Tissier, A., McDonald, J. P., Frank, E. G., and Woodgate, R. (2000) *Gene Dev.*, **14**, 1642-1650.
5. Gening, L. V., Petrochenkov, A. N., Reshetnyak, A. B., Andreeva, L. E., and Tarantul, V. Z. (2004) *Biochemistry* (Moscow), **69**, 435-440.
6. Frank, E. G., Tissier, A., McDonald, J. P., Rapic-Otrin, V., Zeng, X., Geahart, P. J., and Woodgate, R. (2001) *EMBO J.*, **20**, 2914-2922.
7. Markina, N. V., Shilova, O. B., Perepelkina, O. V., Boyarishnova, O. S., Fedotova, I. B., Bizikoeva, F. Z., Gichenok, I. V., and Poletaeva, I. I. (2004) *Dokl. Akad. Nauk*, **396**, 1-3.
8. Vaisman, A., and Woodgate, R. (2001) *EMBO J.*, **20**, 6520-6529.
9. Vaisman, A., Frank, F. G., McDonald, J. P., Tissier, A., and Woodgate, R. (2002) *Mutat. Res.*, **510**, 9-22.
10. Ohmori, H., Friedberg, E. C., Fuchs, R. P., Goodman, M. F., Hanaoka, F., Hinkle, D., Kunkel, T. A., Lawrence, C. W., Livneh, Z., Nohmi, T., Prakash, L., Prakash, S., Todo, T., Walker, G. C., Wang, Z., and Woodgate, R. (2001) *Mol. Cell.*, **8**, 7-8.
11. Johnson, R. E., Prakash, S., and Prakash, L. (1999) *Science*, **283**, 1001-1004.
12. Washington, M. T., Johnson, R. E., Prakash, S., and Prakash, L. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 3094-3099.
13. Masutani, C., Kusumoto, R., Iwai, S., and Hanaoka, F. (2000) *EMBO J.*, **19**, 3100-3109.
14. Yuan, F., Zhang, Y., Rajpal, D. K., Wu, X., Guo, D., Wang, M., Taylor, J. S., and Wang, Z. (2000) *J. Biol. Chem.*, **275**, 8233-8239.
15. Cleaver, J. E., and Carter, D. M. (1973) *J. Invest. Dermatol.*, **60**, 29-32.
16. Washington, M. T., Johnson, R. E., Prakash, S., and Prakash, L. (1999) *J. Biol. Chem.*, **274**, 36835-36838.
17. Matsuda, T., Bebenek, K., Masutani, C., Hanaoka, F., and Kunkel, T. A. (2000) *Nature*, **404**, 1011-1013.
18. Johnson, R. E., Washington, M. T., Prakash, S., and Prakash, L. (2000) *J. Biol. Chem.*, **275**, 7447-7450.
19. Zeng, X., Winter, D. B., Kasmer, C., Kraemer, K. H., Lehmann, A. R., and Gearhart, P. J. (2001) *Nat. Immunol.*, **2**, 537-541.
20. Yavuz, S., Yavuz, A. S., Kraemer, K. H., and Lipsky, P. E. (2002) *J. Immunol.*, **169**, 3825-3830.
21. Rogozin, I. B., Pavlov, Y. I., Bebenek, K., Matsuda, T., and Kunkel, T. A. (2001) *Nat. Immunol.*, **2**, 530-536.
22. Pavlov, Y. I., Rogozin, I. B., Galkin, A. P., Aksenova, A. Y., Hanaoka, F., Rada, C., and Kunkel, T. A. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 9954-9959.
23. Tissier, A., Frank, E. G., McDonald, J. P., Iwai, S., Hanaoka, F., and Woodgate, R. (2000) *EMBO J.*, **19**, 5259-5266.
24. Bebenek, K., Tissier, A., Frank, E. G., McDonald, J. P., Prasad, R., Wilson, S. H., Woodgate, R., and Kunkel, T. A. (2001) *Science*, **291**, 2156-2159.
25. Wang, M., Devereux, T. R., Vikis, H. G., McCulloch, S. D., Holliday, W., Anna, C., Wang, Y., Bebenek, K., Kunkel, T. A., Guan, K., and You, M. (2004) *Cancer Res.*, **64**, 1924-1931.

26. Faili, A., Aoufouchi, S., Flatter, E., Gueranger, Q., Reynaud, C. A., and Weill, J. C. (2002) *Nature*, **419**, 945-947.
27. McDonald, J. P., Frank, E. G., Plosky, B. S., Rogozin, I. B., Masutani, C., Hanaoka, F., Woodgate, R., and Gearhart, P. J. (2003) *J. Exp. Med.*, **198**, 635-643.
28. Raji, N. S., Krishna, T. H., and Rao, K. S. (2002) *Int. J. Dev. Neurosci.*, **20**, 491-496.
29. Rao, K. S., Annapurna, V. V., and Raji, N. S. (2001) *Ann. N. Y. Acad. Sci.*, **928**, 113-120.
30. Datta, A., and Jinks-Robertson, S. (1995) *Science*, **268**, 1616-1619.
31. Miczek, K. A., Maxson, S. C., Fish, E. W., and Faccidomo, S. (2001) *Behav. Brain Res.*, **125**, 167-181.
32. Sluyter, F., van Oortmerssen, G. A., de Ruiter, A. J., and Koolhaas, J. M. (1996) *Behav. Genet.*, **26**, 489-496.
33. Maxson, S. C. (1996) *Behav. Genet.*, **26**, 471-476.
34. Brodkin, E. S., Goforth, S. A., Keene, A. H., Fossella, J. A., and Silver, L. M. (2002) *J. Neurosci.*, **22**, 1165-1170.
35. Maxson, S. C., Ginsburg, B. E., and Trattner, A. (1979) *Behav. Genet.*, **9**, 219-226.
36. Guillot, P. V., Sluyter, F., Crusio, W. E., and Chapouthier, G. (1999) *Neurogenetics*, **2**, 171-175.
37. Mejia, J. M., Ervin, F. R., Baker, G. B., and Palmour, R. M. (2002) *Biol. Psychiatry*, **52**, 811-821.
38. Tordjman, S., Carlier, M., Cohen, D., Cesselin, F., Bourgoin, S., Colas-Linhart, N., Petiet, A., Perez-Diaz, F., Hamon, M., and Roubertoux, P. L. (2003) *Behav. Genet.*, **33**, 529-536.
39. Popova, N. K., Skrinskaya, Yu. A., Amstislavskaya, T. G., Vishnevetskaya, G. V., Seif, I., and de Maier, E. (2000) *Zh. Vyssh. Nervn. Deyat.*, **50**, 9991-9998.
40. Demas, G. E., Kriegsfeld, L. J., Blackshaw, S., Huang, P., Gammie, S. C., Nelson, R. J., and Snyder, S. H. (1999) *J. Neurosci.*, **19**, RC30.
41. Ashmarin, I. P., and Titov, S. A. (1988) *Nauch. Dokl. Vyssh. Shkoly. Biol. Nauki*, **3**, 30-42.