Two Nicking Enzyme Systems Specific for Mismatch-containing DNA in Nuclear Extracts from Human Cells*

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We have identified two novel enzyme systems in human HeLa nuclear extracts that can nick at specific sites of DNA molecules with base mismatches, in addition to the T/G mismatch-specific nicking enzyme systems of Salmonella typhimurium, K., and Jiriczny, J. (1989) Nature 339, 234-236). One enzyme (called all-type) can nick all eight base mismatches with different efficiencies. The other (A/G-specific) nicks only DNA containing an A/G mismatch. The all-type enzyme can be separated from the T/G-specific and A/G-specific nicking enzymes by Bio-Rex 70 chromatography. Further purification on a DEAE-5PW column separated the A/G-specific nicking enzyme from the T/G-specific nicking enzyme. Therefore, at least three different enzyme systems are able to cleave mismatched DNA in HeLa nuclear extracts. The all-type and A/G-specific enzymes work at different optimal salt concentrations and cleave at different sites within the mismatched DNA. The all-type enzyme can only cleave at the first phosphodiester bond 5' to the mispaired bases. This enzyme shows nick disparity to only one DNA strand and may be involved in genetic recombination. The A/G-specific enzyme simultaneously makes incisions at the first phosphodiester bond both 5' and 3' to the mispaired adenine but not the guanine base. This enzyme may be involved in an A/G mismatch-specific repair similar to the Escherichia coli mutY (or micA)-dependent pathway.

DNA mismatches may arise from DNA replication errors, deamination of 5-methylcytosine, and DNA recombination. Recombination between homologous but not identical sequences generates mismatched heteroduplexes. Their repair may account for gene conversion, high negative interference, or map expansion (1, 2). In Escherichia coli and Salmonella typhimurium, mismatch repair directed by dam methylation is mediated through a DNA glycosylase and an apurinic/apyrimidinic (AP) endonuclease reaction (28, 29).

Recent discoveries support a common evolution of mismatch repair machinery among diverse organisms. Protein sequences of MutL of S. typhimurium (18), HexB of Streptococcus pneumoniae (19), and PMS1 of Saccharomyces cerevisiae (20) have conserved regions. Proteins with significant homology to the MutS protein of S. typhimurium were found in human and mouse tissue (21, 22). Also, a 100-kDa protein has been identified that binds A/C-, T/C-, and T/T-containing DNAs in human Raji cells (23). Thus, mammalian cells may use mechanisms similar to those found in prokaryotes to correct replication errors in favor of the parental strand (24).

In vitro repair systems directed by strand breaks have been established in nuclear extracts of HeLa and Drosophila cells (25). A specific repair system in human HeLa cells can repair deaminated 5-methylcytosines (26) and is equivalent to the T/G-specific pathway found in E. coli (6). Binding to and nicking of T/G-mismatch-containing DNA have been reported in nuclear extracts of HeLa cells (27, 28). The nicking of T/G-containing DNA is mediated through a DNA glycosylase and an apurinic/apyrimidinic (AP) endonuclease reaction (28, 29).

In this paper, we describe two novel nicking enzymes in HeLa nuclear extracts; one can recognize all eight mismatches and the other can only recognize A/G mismatches. These two enzymes can be distinguished from each other and from the T/G-specific nicking enzyme (28) by column chromatography and substrate specificity.

**EXPERIMENTAL PROCEDURES**

**DNA Preparations—**Eight 116-mer oligonucleotides (four upper and four lower strands, Fig. 1) were synthesized by a MilliGen 7500 DNA synthesizer and purified from 8% sequencing gels. The bases at position 51 of the upper strand and position 70 (counted from the 5' end) of the lower strand vary by A, C, G, or T. Two complementary 116-mer oligonucleotides were annealed to generate a heteroduplex DNA containing a mismatched base at position 51 (of the upper strand). The annealed duplexes were labeled at the 3' end on the upper or lower strand with DNA polymerase Klenow fragment and [α-32P]dCTP or [α-32P]dATP, respectively (30). After 25 min at 25 °C, the synthesis was completed by adding all four unlabeled deoxynucleo-

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G mismatch-specific repair has been identified in E. coli (10-13) and S. typhimurium (14). This mutY (or micA)-dependent pathway (15) acts on A/G mismatches to restore C/G base pairs exclusively, and in conjunction with MutT protein, also can reduce C/G-to-A/T transversions (16). Specific binding and nicking to DNA fragments containing A/G mispairs have been identified in E. coli extracts (16). The mechanism of the mutY (or micA)-dependent repair involves the action of a DNA glycosylase (17) followed by a 2-nucleotide excision and subsequent resynthesis (16).

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* A-L. Lu and D.-Y. Chang, manuscript in preparation.

† The abbreviations used are: AP, apurinic/apyrimidinic; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
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Identification of Base Mismatch-specific Endonucleases in HeLa Cells—In order to identify human enzymes that can nick mismatch-containing DNA fragments, we employed an assay similar to the specific nicking near mismatched bases of E. coli A/G endonuclease (16). Synthetic double-stranded DNA fragments containing different mismatches at one particular position (Fig. 1) were incubated with HeLa nuclear extracts and then fractionated on a denaturing sequencing gel. The two DNA strands in Fig. 1 were arbitrarily defined as upper and lower strands. Initially, nicking was assayed with DNA fragments containing T/G or A/G mismatches, using C/G-containing DNA as a control. In HeLa nuclear extracts, nicking activities could be detected to T/G- or A/G-containing DNA but not to homoduplex DNA (data not shown). Nicking of T/G-containing DNA has been shown to proceed through a DNA glycosylase-AP endonuclease pathway in nuclear extracts of HeLa cells (28, 29). Our results suggested that HeLa nuclear extracts might contain other mismatch-specific nicking enzymes. Therefore, these nuclear extracts were fractionated using a Bio-Rex 70 column and assayed for the nicking activities to mismatch-containing DNA substrates. As shown in Fig. 2, fractions 60–70 (Fraction II A) were able to nick T/G- or A/G-containing DNA substrate at the proximity of the mismatched site but not to A/- or C/G-containing DNA substrate. Fractions 110–130 (Fraction II B) could nick A/A-, T/G-, and A/G-containing DNA but not homoduplex DNA. Fraction II A had higher activity to T/G-containing DNA than to A/G-containing DNA. However, T/G-containing DNA was a poor substrate for Fraction II-B.

Further purification of Fraction II A on a DEAE-5PW column yielded two overlapping peaks of activity (Fig. 3). Peak fraction 28 showed nicking activity for T/G-containing DNA, and peak fraction 32 showed it for A/G-containing DNA. However, Fraction II-B could not be separated into subpeaks by DEAE-5PW chromatography (data not shown). Therefore, at least three mismatch-specific endonucleases can be observed in HeLa nuclear extracts.

A/G Mismatch-specific Endonuclease Is Present in HeLa Nuclear Extracts—When Fraction II A from the Bio-Rex 70 column was assayed with DNA substrates containing one of the eight mismatches, we found that only nick DNA containing T/G or A/G mismatches. We suspected that Fraction II A might contain one T/G-specific nicking enzyme (or a DNA glycosylase and an AP endonuclease) as reported by Wiebauer and Jiricny (28) and an A/G-specific enzyme similar to the E. coli mutY (or mica)-dependent A/G-nicking enzyme (16, 17). To prove this hypothesis, Fraction II A was further purified by DEAE-5PW chromatography. This fraction could be separated into two peaks (Fig. 3) with one T/G-specific and one A/G-specific enzyme. When these two enzymes were further separated by a third chromatographic step (heparin-agarose), the A/G- and T/G-specific endonuclease activities did not overlap each other (data not shown). The T/G-specific enzyme was proven to be a DNA glycosylase (data not shown), the same enzyme identified by Wiebauer and Jiricny (28), and was not further characterized. Fig. 4 (lanes 1–9) shows the A/G-specific enzyme has no nicking activity for T/G, A/A, T/T, G/C, C/A, C/T, or C/G-containing DNA. Furthermore, this A/G-specific nicking enzyme could only nick the "A" strand but not the "G" strand (Fig. 5). These properties are similar to the A/G-specific enzyme of E. coli.
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**Fig. 2.** Purification of mismatch-specific endonucleases from human HeLa nuclear extracts by Bio-Rex 70 chromatography. DNA containing A/A (a), T/G (b), A/G (c), or C/G (d) at position 51 was labeled at the 3’ end and assayed with fractions from the column. The cleaved fragment, after denaturation, was analyzed on an 8% sequencing gel that was then autoradiographed. Fractions (60-70) containing A/G and T/G nicking activities were pooled (Fraction II-A). Fractions (110-130, pooled as Fraction II-B) had nicking activities to A/A-, T/G-, and A/G-mismatched DNA.

**Fig. 3.** Purification of A/G- and T/G-specific endonucleases by DEAE-5PW chromatography. Fraction II-A pooled (p) from the Bio-Rex 70 column was applied on a 4-ml DEAE-5PW column (Waters, Millipore Corp.). DNA containing T/G (a) or A/G (b) at position 51 was labeled at the 3’ end of the upper strand and assayed with fractions from the column as described in the legend to Fig. 2. T/G- and A/G-specific endonucleases peak at fraction 28 and fraction 32, respectively. Fractions (30-34) were pooled as Fraction III-B.

Concentration of NaCl dramatically affected the activity of the all-type nicking enzyme (Fig. 6). The nicking activities for T/G-specific, A/G-specific, and all-type nicking enzymes were decreased to 66, 21, and 0%, respectively, when they were assayed in buffer containing 80 mM NaCl compared with no NaCl. Salt concentration may have an effect on mismatch conformation or kinetic parameters governing the formation of protein-DNA complexes.

**Incision Sites of the A/G-specific and All-type Nicking Enzymes**—We have used the DNA substrates labeled at different ends and different DNA strands to determine the cleavage sites of the A/G-specific and all-type nicking enzymes. The denatured cleavage products were separated on a sequencing gel in parallel with a sequencing ladder generated by the Maxam and Gilbert chemical method (31). As shown in Fig. 7a, the cleavage product of all-type enzyme on A/G-containing DNA ran at the same position of the T<sup>30</sup> band of the sequencing ladder generated from C/G-containing DNA labeled at the 3’ end of the upper strand. We conclude that the all-type enzyme cleaves 5’ to the mispaired adenine. The cleavage product of T/G-specific enzyme ran at the same position of the C<sup>30</sup> band (sequencing ladder from C/G-containing DNA fragment). This incision site at the 3’ side of mispaired thymine is consistent with the result of Wiebauer and Jiricny (28). The A/G-specific enzyme cleaved at the same site as the T/G-specific enzyme (i.e. at the first phosphodiester bond 3’ to the mispaired base, data not shown). Using DNA substrates labeled at the 5’ end of the upper strand for A/G-specific nicking enzyme, a band migrating between A<sup>32</sup> and G<sup>32</sup> could be detected on a sequencing gel (Fig. 7b). According to the chemistry of the Maxam and Gilbert method, the site generated by the endonuclease was assigned between T<sup>30</sup>-A<sup>31</sup> and probably contains a 3’-hydroxyl group. Thus, the cleavage site was mapped to the first phosphodiester bond 5’ to the mispaired adenine. However, for the all-type nicking enzyme, when using DNA substrates labeled at the 5’ end of the upper...
FIG. 5. Strand specificity of A/G-specific enzyme. DNA containing A/G or G/A at position 51 (see Fig. 1) was labeled (presented as *) at the 3' end on the upper or lower strand with Klenow fragment of DNA polymerase I and [α-32P]dCTP or [α-32P]dATP, respectively. A/G-specific enzyme only nicked on the “A” but not the “G” strand. A nick on the 3' end-labeled upper strand gave a 69-nucleotide fragment, whereas a nick on the lower strand generated a 50-nucleotide band.

**Discussion**

In this paper, we describe the preliminary characterization of two enzyme systems from human HeLa cells that recognize and nick mismatch-containing DNA fragments. The enzyme systems described here may involve more than one protein. One enzyme system is specific for DNA containing an A/G mismatch, and the other can nick DNA containing one of eight possible base mismatches. These two enzyme systems can be separated by chromatography from the T/G-specific nicking enzyme system, which was shown to consist of a DNA glycosylase and an AP endonuclease (28, 29).

Although we currently lack evidence demonstrating that the HeLa A/G-specific nicking is mediated by DNA glycosylase-AP endonuclease, the human A/G-specific enzyme is similar to the *E. coli* MutY DNA glycosylase and AP endonuclease system involved in A/G-specific repair (10-13). The high specificity to A/G mismatches and nicking to the “A” but not “G” strand are common for both enzyme systems. Both enzymes have no requirement for Mg2+ or ATP. Our results suggest that higher eukaryotes have A/G-specific repair pathways similar to those identified in bacteria (11, 13). As in bacteria, this pathway may be involved in correcting replication errors to prevent C/G-to-A/T transversions. This resembles the methyl-directed system of *E. coli* and *S. typhi*.

**FIG. 6.** The effect of salt concentration on the nicking activities of three mismatch-specific endonucleases. The DNA substrates containing an A/G (lanes 1-4) or T/G (lanes 5-6) mismatch were labeled at the 3' end of the upper strand and were incubated with A/G-specific (fraction 32 eluted from DEAE-5PW column, lanes 1 and 2), all-type (fraction 130 eluted from a Bio-Rex 70 column, lanes 3 and 4), or T/G-specific (fraction 28 eluted from a DEAE-5PW column, lanes 5 and 6) nicking enzyme. The reactions were carried out in 20 mM Tris-HCl (pH 7.6), 10 μM ZnCl2, 1 mM dithiothreitol, 1 mM EDTA, and 2.9% glycerol (lanes 1, 3, and 5) or containing 80 mM NaCl in addition (lanes 2, 4 and 6). The fraction 32 eluted from DEAE-5PW column was concentrated by Centricon 3 (Amicon) centrifugation.

**FIG. 7.** a, incision sites of the all-type mismatch and T/G-specific endonucleases on 3' end-labeled DNA. The upper arrow marked the cleavage product of A/G-containing DNA by the all-type enzyme (lane 5), and the lower arrow marked the product of T/G-containing DNA by the T/G-specific enzyme (lane 6). Homoduplex DNA was not cut by the nuclear extract (lane 7), which contained both enzymes. Experiments using DNA substrates labeled at the 3' end of the upper strand were performed as described in the legend to Fig. 2. The four lanes on the left (cleaved at G, C+T, C, and A+C, respectively) show a sequencing ladder of homoduplex DNA (C at position 51) by the method of Maxam and Gilbert (31). b, incision site of the A/G-specific endonuclease on 5' end-labeled DNA. DNA containing an A/G mismatch at position 51 was labeled at the 5' end of the upper strand (see Fig. 1) and cleaved with A/G endonuclease (Fraction III-B). The cleaved product (marked by an arrow) was analyzed on an 8% sequencing gel with G and A>C sequencing ladders of the same DNA.

**FIG. 8.** Incision sites (represented by arrows) of three mismatch-specific endonucleases from human HeLa cells. a, T/G-specific; b, A/G-specific; c, all-type mismatch endonucleases. X and Y represent A, C, G, or T at position 51 of the DNA substrates (Fig. 1) but are not complementary bases. The position of the nicking site of the T/G-specific endonuclease was determined by Wiebauer and Jiricny (28). The two nicking sites of the A/G-specific endonuclease were determined by 3' and 5' end-labeled A/G mismatch-containing DNA. There is no detectable incision at the “G” strand. The all-type mismatch endonuclease can nick all eight mismatched bases at the first phosphodiester bond 5' to the mispaired base on the upper strand.

**Fig. 5.** Strand specificity of A/G-specific enzyme. DNA containing A/G or G/A at position 51 (see Fig. 1) was labeled (presented as *) at the 3' end on the upper or lower strand with Klenow fragment of DNA polymerase I and [α-32P]dCTP or [α-32P]dATP, respectively. A/G-specific enzyme only nicked on the “A” but not the “G” strand. A nick on the 3' end-labeled upper strand gave a 69-nucleotide fragment, whereas a nick on the lower strand generated a 50-nucleotide band.
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murium and the hex pathway of S. pneumoniae (3–5). Thus, the three mismatch repair systems reported in bacteria are all present in higher organisms. The human all-type mismatch repair enzyme has a broad substrate specificity. It nicks all eight base mismatches but with different efficiencies. Similar enzymes have also been identified in calf thymus and yeast. These data suggest that the all-type enzyme is not the analog of bacterial MutS protein. First, the MutS protein from E. coli can bind to mismatched sites but has no catalytic activity. The MutS analog found in human and mouse by protein sequence homology search (21, 22) may be the same protein that binds A/C-, T/C-, and T/T-containing DNAs in human Raji cells (23). Second, the mismatch specificity also differs from the two enzyme systems. The E. coli MutS protein binds very well to DNA containing a T/G mismatch, but the G/T mismatch is the weakest substrate for the HeLa all-type repair enzyme. C/C mispair is repaird poorly in the E. coli methyl-directed (11, 13) and HeLa terminus-directed reactions (25) but is nicked very well by the HeLa all-type repair enzyme.

The unique property of the HeLa all-type nicking enzyme is its strand disparity. With respect to the DNA fragment shown in Fig. 1, the enzyme nicked only the upper strand, and no incision on the lower strand could be detected. This strand specificity is not directed by strand breaks or methylated DNA sequences because unmodified linear DNA substrates are used in these experiments. Preliminary data suggest that the neighboring DNA sequences influence the disparity. As far as we are aware, this type of enzyme has not been described in any organisms. One unsolved problem for this enzyme is that no nicking product could be observed by using DNA fragments labeled at the 5′ end of the cutting strands. There may be a contamination of a 5′-phosphatase or a 5′- to 3′-exonuclease that degrades the nicking product. After specific nicking at the 5′ side of the mismatched base, a 3′- to 5′-exonuclease also may act from this end and degrade the 5′-labeled product. Another possibility involves a mismatch-specific exonuclease that acts from the 5′ end toward the mismatched site with reaction stopping just before the mispair. Further purification and characterization are needed to address this question.

The human all-type mismatch nicking activity is functionally homologous to the resolvases from bacteriophage T4 (33, 34), yeast (35–37), calf thymus (38), and human (39) in two aspects. Both enzyme systems make an incision (or incisions) near the mismatched site or Holliday junction point, and cleavage occurs in one orientation depending on the neighboring sequences. In some respects, the Holliday junction may be viewed as two heteroduplex DNA molecules, each with one mismatched base pair. Some resolvases are active on heteroduplex loops (37, 40). However, the action of resolvase requires Mg2+, which is not essential for the human all-type mismatch repair enzyme. The incision sites were also different for both enzyme systems. The human all-type repair enzyme nicks at the first phosphodiester bond 5′ to the side of the mispaired base. The nicking sites of most resolvases, except yeast Endo X1 (35) and T7 endonuclease I (41), map to the 3′ side of the junction point or loop.

While the T/G-specific nicking activity may be involved in repairing deaminated 5-methylcytosines and the A/G-specific nicking may be involved in preventing C/G-to-A/T transversions, the function of human all-type nicking enzyme is not known. It may be involved in the gene conversion during genetic recombination. Reciprocal and unequal mitotic recombination between nonidentical repeated sequences generates heteroduplex DNA. Gene conversion may play a role in sequence homogenization or diversification. Mismatch repair in heteroduplex DNA formed from the V regions or pseudo-V genes of the immunoglobulin genes could generate antibody diversity (42, 43).

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REFERENCES

1. Fincham, J. R. S., and Holliday, R. (1970) Mol. & Gen. Genet. 109, 309–322
2. Holliday, R. (1974) Genetics 78, 273–287
3. Claverie, J.-P., and Lacks, S. A. (1986) Microbiol. Rev. 50, 133–165
4. Modrich, P. (1987) Annu. Rev. Biochem. 56, 435–466
5. Radman, M., and Wagner, R. (1988) Annu. Rev. Genet. 20, 523–538
6. Lieb, M. (1983) Mol. & Gen. Genet. 181, 118–125
7. Lieb, M., Allen R., and Reed, D. (1986) Genetics 114, 1041–1060
8. Raposa, S., and Fox, M. S. (1987) Genetics 117, 381–390
9. Jones, M., Wagner, R., and Radman, M. (1987) Cell 50, 621–626
10. Au, N. G., Cabrera, M., Miller, J. H., and Modrich, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9163–9166
11. Liu, A.-L., and Chang, D. (1989) Genetics 118, 593–600
12. Radicella, J. P., Clark, E. A., and Fox, M. S. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9674–9678
13. Su, S.-S., Lahtve, R. A., Au, K. G., and Modrich, P. (1988) J. Biol. Chem. 263, 6829–6833
14. Lebovitz, R., and Korner, A. (1985) J. Biol. Chem. 260, 2494–2499
15. Lieb, M., Allen R., and Read, D. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7698–7703
16. Au, A.-L., and Chang, D. Y. (1988) Cell 54, 805–812
17. Au, K. G., Clark, S. M., Miller, J. H., and Modrich, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8726–8730
18. Mankovich, J. A., McIntyre, C. A., and Walker, G. C. (1989) J. Bacteriol. 171, 3825–3831
19. Furdinonne, M., Martin, B., Mejean, V., and Claverie, J.-P. (1989) J. Bacteriol. 171, 5323–5338
20. Kramer, W., Kramer, B., Williamson, M. S., and Fogel, S. R. (1989) J. Bacteriol. 171, 3393–3398
21. Fuji, H., and Shimasda, T. (1989) J. Biol. Chem. 264, 10057–10064
22. Linton, J. P., Yen, J.-Y., Selleby, E., Chen, Z., Chinsky, J. M., Liu, K., Kellens, R., and Crousse, G. F. (1989) Mol. Cell. Biol. 9, 3058–3072
23. Stephenson, C., and Karran, P. (1989) J. Biol. Chem. 264, 21177–21182
24. Riet, B. T., and Taylor, J. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7350–7354
25. Hughes, J. J., Jr., Clark, S., and Modrich, P. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5887–5891
26. Brown, T. C., and Jiriczyn, J. (1988) Cell 60, 945–950
27. Jiriczyn, J., Hughes, M., and Rudkin, B. B. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8890–8894
28. Wiebe, R., and Jiriczyn, J. (1989) Nature 339, 254–236
29. Jiriczyn, K., and Jiriczyn, J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5842–5845
30. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
31. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 496–500
32. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1499
33. Kengen, B., and Garbers, M. (1981) Eur. J. Biochem. 115, 123–131
34. Lilly, D. M. J., and Kemper, B. (1984) Cell 36, 413–422
35. West, S. C., and Korner, A. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6445–6449
36. Snydman, S. L., and Kolodner, R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7247–7251
37. Jensch, F., Kosak, H., Seeman, N. C., and Kemper, B. (1989) EMBO J. 8, 4325–4334
38. Elborough, K. M., and West, S. C. (1990) EMBO J. 9, 2931–2936
39. Waldman, A. S., and Liskay, R. M. (1988) Nucleic Acids Res. 16, 10249–10256
40. Kellf, S., and Kemper, B. (1988) EMBO J. 7, 1357–1358
41. Dickie, P., McFadden, G., and Morgan, A. R. (1987) J. Biol. Chem. 262, 14826–14836
42. Maizels, N. (1989) Trends Genet. 5, 4–8
43. Becker, R. S., and Knight, K. L. (1990) Cell 63, 987–997