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Base mismatch-specific endonuclease activity in extracts from *Saccharomyces cerevisiae*

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ABSTRACT

An endonuclease activity (called MS-nicking) for all possible base mismatches has been detected in the extracts of yeast, *Saccharomyces cerevisiae*. DNAs with twelve possible base mismatches at one defined position are cleaved at different efficiencies. DNA fragments with A/G, G/A, T/G, G/T, G/G, or A/A mismatches are nicked with greater effiencies than C/T, T/C, C/A, and C/C. DNA with an A/C or T/T mismatch is nicked with an intermediate efficiency. The MS-nicking is only on one particular DNA strand, and this strand disparity is not controlled by methylation, strand break, or nature of the mismatch. The nicks have been mapped at 2–3 places at second, third, and fourth phosphodiester bonds 5′ to the mispaired base; from the time course study, the fourth phosphodiester bond probably is the primary incision site. This activity may be involved in mismatch repair during genetic recombination.

INTRODUCTION

DNA mismatch repair is responsible for the correction of replication errors and deaminated cytosines. The lack of mismatch repair usually increases mutation rate and causes mutator phenotype (1). Mismatch repair and double-strand break repair are the favored mechanisms for gene conversions between DNA heteroduplexes generated during genetic recombination. Correction of DNA heteroduplexes could lead to a gene conversion event: the nonreciprocal segregation of two alleles during meiotic recombination (2). The biochemical mechanisms of mismatch repair are best known in procaryotes (3, 4, 5). In *Escherichia coli*, DNA mismatches are repaired either by a methylation-dependent pathway (3, 4, 5) or by methylation-independent A/G- (6–9) and T/G-specific pathways (10, 11, 12). The long-patch methylation-dependent repair specifically requires the gene products of *mutHLS* and assures that correction occurs on the newly synthesized DNA strands. It also can abort recombination intermediates between homologous but nonidentical sequences assuring high fidelity of homologous recombination (13, 14). The T/G-specific repair (10, 11, 12) converts T/G to C/G, is dependent of the *vsr* gene product (15), and is responsible for repairing deaminated 5-methylcytosines (16). The A/G-specific repair pathway corrects A/G mismatches to C/G base pairs unidirectionally and reduces the C/G-to-A/T transversions (6, 7, 8). This pathway requires the gene product of *mutY* (or *micA*), a DNA glycosylase (6, 8, 17, 18). The action of A/G specific glycosylase and an apurinic/apyrimidinic (AP) endonuclease produces a two-nucleotide excision on a DNA strand containing mispaired adenine ('A') but have no detectable incision on the mispaired guanine ('G') strand (19).

Transformation of DNA heteroduplexes combined with restriction site analysis has indicated that mismatch repair occurs in yeast, *Saccharomyces cerevisiae* (20–23). Different mismatches were corrected with different efficiencies in mitotic (20–23) and meiotic (24) yeast cells. Kolodner and cowokers concluded that A/A, T/T, and C/C mismatches were repaired less efficiently than other mismatches (20–22). Using similar analysis, Kramer et al. found that all mispairs were well corrected except C/C mismatch (23). C/C mismatches were also inefficiently repaired during meiotic recombination (24). It has been shown that *pms1* gene products are required for the mismatch correction (21, 22, 23). *pms* mutants have higher frequencies of post-meiotic segregation (5:3 or 3:5) and mitotic mutation (25, 26). Protein sequence of PMS1 of *S. cerevisiae* (26) contains conserved region to MutL of *S. typhimurium* (27), and HexB of *S. pneumoniae* (28). The yeast PMS-dependent pathway may be homologous to the bacterial methyl- or nick-dependent repair pathways.

A cell-free system has also been developed in yeast that is able to correct short insertion/deletion and some single-base-pair mismatches (29). In this in vitro system, A/C and G/T mismatches were repaired efficiently but transversion mismatches were repaired poorly. This repair is accompanied by a short repair tract and may be governed by the mismatch asymmetry.

Transfection of DNA heteroduplexes has indicated that mismtch repair also occurs in higher eukaroytic cells (30, 31). Hare and Taylor has suggested that DNA methylation and the presence of nicks may control strand specificity of correction (30, 32). Mismatch repair has been shown in extracts of *Xenopus* eggs (33). In vitro repair systems directed by strand breaks have been established in extracts of *Drosophila* (34) and HeLa cells (34, 35). Also, a 100 kDa protein has been identified to bind A/C,

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T/C and T/T containing DNAs in human Raji cells (36). These findings suggest that higher organisms has a general, strand-specific mismatch repair system. Genes with significant homology to the *mutS* of *Salmonella typhimurium* were found in human and mouse cells (37, 38). However, the enzymes involved in the repair pathways have not been identified.

A T/G-specific repair system has been reported in human HeLa cells (39). This pathway is believed to repair deaminated 5-methylcytosines and is equivalent to the T/G-specific pathway found in *E. coli* (10). T/G mismatch-specific binding and nicking proteins have been identified in nuclear extracts of HeLa cells (40, 41). The nicking to T/G-containing DNA is mediated through a DNA glycosylase and an AP endonuclease reaction (41, 42). Recently, an A/G-specific endonuclease that can nick DNA fragments containing A/G mismatches on the 'A' strands but not the 'G' strands has also been identified in HeLa cells (43). This enzyme may be involved in an A/G mismatch-specific repair similar to the *E. coli mutY* (or *micA*)-dependent pathway (6, 8, 17).

In order to identify enzymes involved in mismatch repair pathways, we have investigated the ability of yeast extracts to nick DNA fragments containing defined single base mismatches. In this paper, we report one endonuclease activity in yeast extracts that can nick DNA fragments containing all twelve possible mispairs with different efficiencies. This nicking is specifically on one particular DNA strand and may be involved in genetic recombination.

MATERIALS AND METHODS

Preparation of cell extract

Cells of yeast, *Saccharomyces cerevisiae* JL6B (α, *cry*^{r}, *leu 2-3, leu 2-112, ura3-52, trp1*), were grown in 2 liters of YPD medium to an A_{590} of 0.8, harvested by centrifugation, and resuspanded with 40 ml of buffer containing 50 mM Tris-HCl pH 7.6, 0.1 mM EDTA, 0.5 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride. Cells were mixed with equal volume of glass beads (0.5 mm) and disrupted in a bead-beater (Biospec Products). After centrifugation at 5,000 g for 5 min to remove cell debris and glass beads, the supernatant (Fraction I) was treated with ammonia sulfate at 0.23 g/ml and centrifuged with 10,000 g for 30 min. The precipitate was resuspended in 1.2 ml of buffer A (20 mM KPO_4, pH 7.4, 0.5 mM dithiothreitol, 0.1 mM EDTA, 0.1mM phenylmethylsulfonyl fluoride and 10% glycerol) containing 0.05 M KCl, and dialyzed against 1 liter of the same buffer twice, 1.5 hours each. The protein extract (Fraction II) was stored in small aliquots at −80°C.

In one experiment, yeast extract (Fraction II) from 4-liter culture was applied onto a 3-ml DEAE-5PW column (Waters, Millipore Corp.) equilibrated with buffer A containing 0.05 M KCl. After washing with 6 ml of equilibration buffer, the column was eluted with a 30-ml linear gradient of KCl (0.05−1.0 M) in buffer A. Mismatch-specific nicking was eluted at 0.15 M KCl.

DNA heteroduplexes

To construct DNA molecules that contain defined single base mismatches, 116-mer oligonucleotides and their complementary strands (Figure 1) were synthesized by a Milligen 7500 DNA synthesizer and purified from 8% sequencing gels. The two DNA strands in Figure 1 were arbitrarily defined as upper and lower strands. Four oligonucleotides A16, G16, C16 and T16 are the upper strands of the DNA substrates and contain A, G, C, or T, respectively, at position 51. The complementary lower strands, A20, G16, C20 and T21, have A, G, C, and T at position 70 (counted from the 5′ end). These 8 oligonucleotides can generate all 16 possible combinations of base pairs (of which 12 contain mismatched bases) at position 51 (of the upper strand) by hybridization. The sequences of the oligonucleotides were designed to form 3′ recessed ends in duplex DNA molecules for subsequent 3′ end labeling (Figure 1).

DNA fragments were hybridized in 10 mM Tris-HCl, pH 7.6, 60 mM NaCl and 6.6 mM $MgCl_2$. After being heated at 90°C for 2 min, DNA fragments were annealed at 65°C for 30 min, then at 37°C for 30 min, and finally at room temperature for more than 10 min. The annealed duplexes were labeled at the 3′ ends on the upper or lower strands with a DNA polymerase Klenow fragment and [α-^{32}P] dCTP or [α-^{32}P] dATP, respectively (44). After 25 min at 25°C, the synthesis was completed by adding all four unlabeled deoxy-nucleoside-5′-triphosphates and incubated for an additional 5 min. The resulting filled-in duplex DNA is 120 bp in length. Alternatively, the upper strand was labeled at its 5′ end with T4 polynucleotide kinase and [γ-^{32}P] ATP before being annealed with the lower strand. Labeled DNA fragments were purified with G-25 Quick-Spin columns (Boehringer Mannheim).

Endonuclease nicking assay

Endonuclease activity was assayed similarly to the method of Lu and Chang (19). Protein extracts (30−50 μg of Frction II or 3 μl of DEAE-5PW fractions) were incubated with 10.7 fmol end-

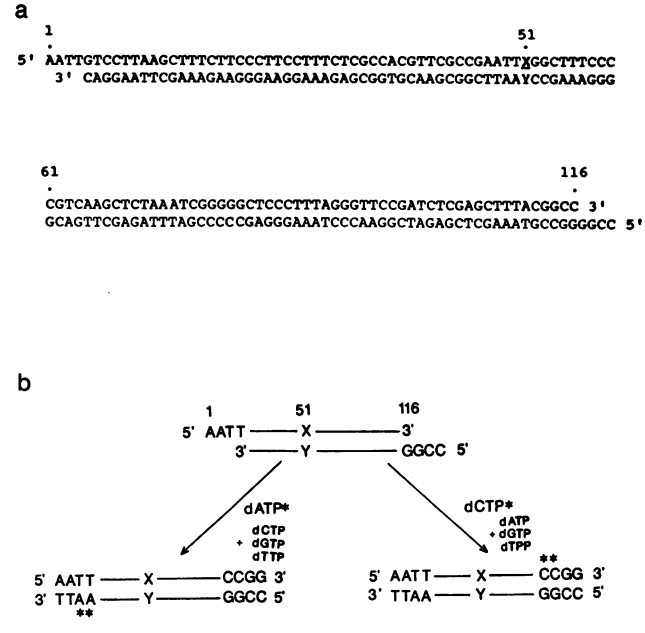

Figure 1. (a), Sequences of the mismatch-containing 116-mer DNA substrates. The bases at position 51 of the upper strand (X) and position 70 (counted from the 5′ end) of the lower strand (Y) vary by A, C, G, or T. (b), Labeling of DNA substrats with α-^{32}P nucleoside triphosphates at 3′ ends. The annealed duplexes from two oligonucleotides were labeled at the 3′ ends on the upper or lower strands with Klenow fragment of DNA polymerase I and [α-^{32}P] dCTP or [α-^{32}P] dATP (marked by *), respectively (44). After 25 min at 25°C, the synthesis was completed by adding all four unlabeled deoxynucleoside-5′-triphosphates and incubated for an additional 5 min. The resulting filled-in duplex DNA is 120 bp in length.

labeled DNA fragments in a reaction mixture (10 μl) containing 20 mM Tris-HCl pH 7.6, 80 mM NaCl, 1 mM dithiothreiol, 5 mM $MgCl_2$, and 2.9% glycerol. After incubation at 30°C for 30 min, the DNA fragments were extracted once with phenol and purified by ethanol precipitation. The samples were redissolved in 3 μl of 90% (v/v) formamide, 10 mM EDTA, 0.1% (w/v) xylene cyanol, and 0.1% (w/v) bromophenol blue. DNA was denatured at 90°C for 3 min and applied to a standard 8% polyacrylamide-8.3 M urea sequencing gel for electrophoresis (45).

RESULTS

Identification of base-mismatch specific nicking enzymes in yeast extracts

Short-patch mismatch repairs are involved in correcting A/G (6−9) and T/G (10, 11, 12) mismatches in *E. coli* and require incisions and resynthesis near the mismatched sites. In order to search for short-patch repair pathways in yeast, we assayed the yeast extracts for specific nicking near mismatched bases. In the assay, radiolabeled DNA containing defined base mismatches (Figure 1) were incubated with yeast extracts (Fraction II) and the reaction products were identified as shorter fragments on a denaturing sequencing gel. Synthetic 116-nucleotide DNA fragments were designed to form a duplex with a single mismatch at position 51. The four ends of the duplexes can be uniquely labeled either at 5′ or 3′ ends. Initially, nicking was assayed with 3′ end-labeled DNA fragments containing A/G or T/G mismatches, using C/G-containing DNA as a control. As shown in Figure 2, A/G-and T/G-containing DNA could be nicked by the yeast extracts to generate 2−3 cleavage products (arrows) that were not evident in the C/G-containing DNA. Therefore, nicking enzymes specific to DNA fragments containing mismatches could be detected in yeast extracts. The mismatch-specific nicking activity could be attributed by multiple endonucleases or the combined action of multiple enzymes. We refer thisnicking as mismatch-specific (MS)-endonuclease activity.

Mismatch-specific nicking catalyzed by yeast extracts was found to be critically dependent on the method of preparation of the extract and the reaction conditions. Requirements for the yeast mismatch-specific endonuclease(s) are shown in Table 1. The endonuclease activity required Mg^{2+} for cleavage. Omission of NaCl from reactions reduced the activity to 18% of the reaction containing 80 mM NaCl. Ca^{2+} had no effect on the activity while Zn^{2+} reduced the activity to 73%.

Because Mg^{2+} is present in the reaction, nuclease activities can be detected. To minimized backgroud nuclease activities, the

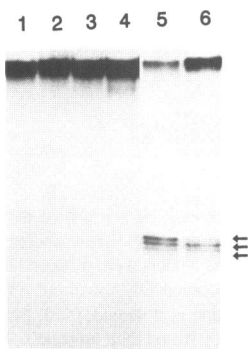

Figure 2. The cleavage assay of A/G- (lane 5) and T/G- (lane 6) containing DNA with yeast protein extracts. DNA duplex containing C/G, A/G or T/G at position 51 was labeled at the 3′ end of the upper strand and assayed with yeast extracts (Fraction II). The cleavage products, after denaturation, were analyzyed on an 8% sequencing gel (45) that was then autoradiographed. DNA fragment without mismatch (C/G, lane 4) was used as a control. Samples in lanes 1 (C/G), 2 (A/G), and 3 (T/G) are not treated with the extract. Arrows marked the positions of mismatch-specific cleavage products.

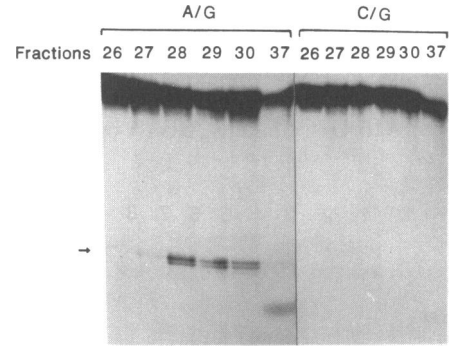

Figure 3. Purification of yeast MS-endonuclease activity by DEAE-5PW chromatography. DNA containing C/G (left panel) and A/G (right panel) at position 51 was labeled at the 3′ end and assayed with fractions (as numbered) from the column. The cleaved fragment, after denaturation, was analyzed on an 8% sequencing gel (45) that was autoradiographed.

Table 1. Effects of ions and EDTA on the activity of mismatch-specific endonuclease on A/G-containing DNA fragments[a]

Conditions of Reactions	Relative Activity (%)[ξ]
1. Control*	100
2. − Mg^{2+}	0
3. − NaCl	18
4. + Ca^{2+} (2mM)	95
5. + Zn^{2+} (2mM)	73
6. + EDTA (1mM)	118

[a] Results represent the average of at least three experiments.
[ξ] 100% nicking is defined as the formation of 6.2 fmol of cleavage product.
* Assay contains 20 mM Tris-HCl pH 7.6, 80 mM NaCl, 1 mM dithiothreiol, 5 mM $MgCl_2$, and 2.9% glycerol.

Table 2. Mismatch specificity of yeast mismatch-specific endonuclease[a].

Mismatched DNA	Input DNA being nicked* fmole	%
1. A/G	6.2	58.0
2. T/G	3.8	35.5
3. A/C	2.2	20.6
4. T/C	0.7	6.1
5. G/A	4.9	46.1
6. G/T	3.4	31.8
7. C/A	0.8	7.1
8. C/T	1.1	10.7
9. A/A	3.7	34.5
10. G/G	4.8	44.6
11. T/T	2.4	22.5
12. C/C	0.7	8.1
13. C/G	0.0	0.0

[a] Results represent the average of at least three experiments.
* In each reaction, 0.2 ng (10.7 fmole) of labeled DNA was treated with 50 μg of yeast extract.

whole cell extracts (Fraction I) from actively growing cells were treated with 35% ammonia sulfate for Fraction II. Nucleases can be further separated from the mismatch-specific nicking by column chromatography. On a DEAE-5PW column, the mismatch-specific nicking peaked at fraction 28 while the nucleases peaked at fraction 37 (Figure 3).

Mismatch specificities

From the results of Figure 2, we expected that yeast extracts may contain A/G- or T/G-specific nicking enzymes because A/G-to-C/G and T/G-to-C/G pathways have been found in human cells (41, 43). DNA fragments labeled at the 3′ end on the top strand (Figure 1) with twelve possible mismatches (including A/C, G/T, A/G, and C/T in two orientations) were cleaved with the yeast Fraction II. Surprisingly, the yeast extract could nick DNA containing all possible mismatches but with different efficiencies (Table 2). Mismatched DNA can be classified into three groups according to the endonuclease activity. The first group including A/G, G/A, T/G, G/T, G/G, and A/A could be nicked with high efficiencies in which more than 30% of the substrates were cleaved. The second group included A/C and T/T of which 20% were nicked. The third group (C/T, T/C, C/A and C/C) was poorly nicked.

Strand specificity

To investigate further the present of A/G- and T/G-specific nicking activities in yeast extracts, we constructed DNA molecules with A/G or T/G mismatches in reversed orientations of the two strands (A/G vs G/A and T/G vs G/T). For example, the molecules of A16/G16 contains A/G (A on the upper strand) mismatch at the position 51 and the molecules of G19/A20 contains G/A (G on the upper strand) mismatch at the same position. The yeast Fraction II can nick 3′ end-labeled DNA fragments containing A/G or T/G mismatches on the 'A' or 'T' strands but not on the 'G' strands (Figure 4, compare lanes 1 and 5 and lanes 2 and 6). However, when DNA with G/A or G/T mismatches were used as substrates, the 'G' strands were nicked but not the 'A' or 'T' strands (Figure 4, lanes 3, 4, 7, and 8). These results are in constrast to the A/G or T/G-specific enzymes found in *E. coli* and HeLa cells that unidirectionally nick A/G- or T/G-containing DNAs at A or T strands, respectively (17, 19, 41, 43). Our results suggested that the upper strands not the lower strand of DNA fragments with mismatches (Figure 1) were subject to mismatch specific nicking. Nicking favored to the upper DNA strand was also observed in the experiments using DNA fragments containing A/A, T/T, G/G or C/C mismatch at the same position (Figure 5). Here C/C was only nicked at a limited amount (also see Table 2). Since these four substrates contained identical nucleotides at the mismatched

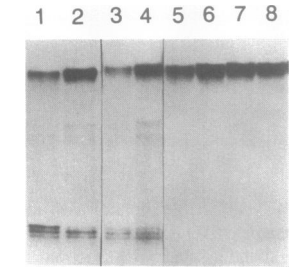

Figure 4. DNA fragments containing A/G (lanes 1 and 5), T/G (lanes 2 and 6), G/A (lanes 3 and 7), or G/T (lanes 4 and 8) mismatches were cleaved by the yeast extract (Fraction II). Mismatch-specific nicking products could be detected with DNA substrates labeled at upper strands (lanes 1−4), but not with DNA substrates labeled at lower strands (lanes 5−8).

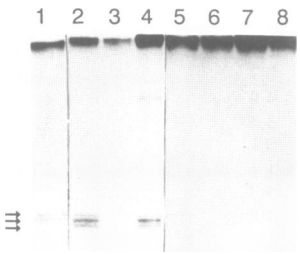

Figure 5. DNA containing A/A (lanes 1 and 5), G/G (lanes 2 and 6), C/C (lanes 3 and 7), or T/T (lanes 4 and 8) mismatches were used for the cleavage reactions with Fraction II. DNA substrates were labeled either at upper strands (lanes 1−4) or at lower strands (lanes 5−8).

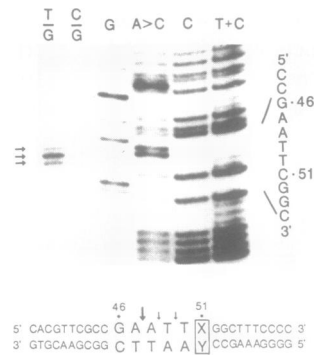

Figure 6. Incision sites (represented by arrows) of the yeast MS-nicking. The positions of nicking were determined by 3′ end labeled DNA containing a T/G mismatch (top panel). Sequencing ladders were produced by the methods of Maxam and Gilbert (45) with 3′ end-labeled DNA without a mismatch (C at position 51). The mismatch-specific nicking is at second, third, fourth phosphodiester bonds 5′ to the mispaired base on the top strand (bottom panel). The fourth phosphodiester bond 5′ to the mispaired base may be the primary incision site (represented by a larger arrow) because it occurs first in the time course study (Figure 7). There is no detectable incision at the lower 'G' strands. X/Y marks the mismatched site as in Fig. 1.

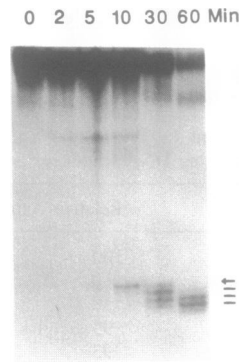

Figure 7. Time course study of yeast mismatch-specific cleavage. DNA containing A/G mismatch was treated with 35 μg of yeast extract (Fraction II) at different times as indicated. Arrow marked the band that appeared before other mismatch-specific cleavage products (indicated by bars).

sites, the preference of upper strand for the endonuclease cleavage was not dictated by the mismatch asymmetry. The strand specificity cannot be directed by strand breaks or methylation either because unmodified linear DNA substrates are used in these experiments.

Incision sites

Yeast extracts (Fraction II) seem to contain a 5′-exonuclease or phosphatase that can remove the labeled phosphate group at the 5′ end of DNA, therefore we were unable to use 5′ end-labeled substrates. Using DNA substrates labeled at the 3′ ends of the upper strands, the positions of cleavage can be mapped by comparison to the sequencing ladders. With T/G mismatch-containing DNA as substrate, three cleavage products (Figure 6, top panel) migrated at the same positions of A_{47}, A_{48}, and T_{49} of sequencing ladders generated by Maxam and Gilbert method (45). These sites were mapped between nucleotides $A_{47}A_{48}$, $A_{48}T_{49}$, and $T_{49}T_{50}$ that are the fourth, third, and second phosphodiester bonds 5′ to the mispaired base (Figure 6, bottom panel). A time course of the MS-endonuclease cleavage with Fraction II showed that the largest cleavage product appeared earlier than the shorter fragments and the largest product disappeared with the increace of the shorter fragments at longer reaction time (Figure 7). We suggest that some 5′-exonucleases in crude yeast extracts (Fraction II) may act on the largest fragment to generate the shorter fragments in the presence of Mg^{2+}. Cleavage predominantly at the fourth and third phosphodiester bonds was detected with fraction 28 from DEAE column (Figure 3). Therefore the primary nicking site may be located between A_{47} and A_{48}.

DISCUSSION

In this paper, we describe one mismatch-specific nicking in yeast extracts. This nicking could result by multiple endonucleases or the combined action of multiple enzymes. Nicking was observed on only one DNA strand at the 5′ side of all mismatches although the activities are lower for T/C, C/A and C/C mismatches. In our assays this is the major activity in yeast extracts that makes specific incisions on mismatch-containing DNA. Although there may be more than one mismatch-specific endonuclease, we are unable to detect A/G- and T/G-specific nicking activities in yeast extracts because G/A- or G/T-containing DNA fragments (G on the upper strand as in Figure 1) labeled at the 'A' or 'T' strand were not nicked (Figure 4). As reported in *E. coli* and human HeLa cells, enzymes specific to A/G or T/G mismatches do not react to other mismatches and make incisions only on the 'A' or 'T' strand, respectively (17, 19, 41, 43). Preliminary data supported this notion that yeast extracts contained limited A/G- and T/G-specific endonuclease activities by the failure to detect more than one mismatch-specific endonuclease activity by chromatographic separation (Chang and Lu, unpublished results). The yeast MS-endonuclease activity is probably not the analog of bacterial MutS activity because the MutS protein from *E. coli* can bind to mismatched sites but has no catalytic activity (46).

Although yeast extract may contain a 5′ to 3′ exonuclease or phosphatase that degrades the 5′ end-labeled DNA, the mismatch-specific cleavage we observed is unlikely caused by a 5′ to 3′ exonuclease that stalls on the 5′ side of most mispairs. First, DNA without a mismatched base is not degraded to the extent as DNA with an A/G or T/G mismatch (compare the intact band of lane 4 to lanes 5 and 6 in Figure 2). Second, The enzyme has different specificities to DNA with different mismatches. DNA fragments with C/T, T/C, C/A, and C/C mismatches are poor subtrates. In competition experiments, labeled A/G-containing DNA is competed by the same unlabeled DNA more than by the unlabled DNA with C/C or no mismatch (Chang and Lu, unpublished results). Third, the mismatch-specific cleavage products can only be detected on the top strand but not on the bottom strand (Figure 4). It is unlikely that a 5′ to 3′ exonuclease can selectly degrade one DNA strand but not the other. Finally, non-specific nuclease activity can be separated from mismatch-specific cleavage by DEAE-5PW (Figure 3) or Mono S (Pharmacia LKB Biotechnology Inc.) chromatographies (Chang and Lu, unpublished results).

In the mechanisms of mismatch repair, the enzyme systems need to recognize a mismatched site and direct repair to one particular strand. In the methylation- or nick-dependent repair pathway, the methylation or nick provides a signal for the repair enzymes (3, 4, 5, 34, 35). Repair is directed to the unmethylated or nick-containing DNA strands. In the A/G- or T/G-specific pathway, the repair is controlled by the mismatch itself (6−12, 38, 41, 43). The repair enzymes may recognize the mismatch configuration and repair A/G or T/G mismatches to C/G base pairs. The yeast MS-endonuclease activity can act on all twelve base mismatches with different efficiencies and always on the same strand regardless of the mismatch type. This unique strand bias is not contolled by methylation, nick, or nature of the mismatch. Preliminary data suggest that the neighboring sequence environments have effects on the disparity (Chang and Lu, unpublished results). The reciprocal change of four nucleotides on each side of an A/A mismatch between two DNA strands did not alter the strand preference but reduced the yeast MS-endonuclease activity. However, an A/A mismatch at different position and thus with defferent sequence environment is not recognized by the MS-endonuclease activity. Thus, the yeast MS-endonuclease activity may be directed by the neighboring sequences surrounding the mismatch.

Similar mismatch-specific enzyme activities (called all-type) have also been identified in human HeLa cells (43) and calf thymus (Yeh and Lu, unpublished results). Both enzyme systems have broad substrate specificity and the same strand disparity. Like the reaction of the yeast enzyme, the strand discrimination of HeLa and calf thymus all-type endonucleases is not determined by the species or orientations of nucleotides at the mismatched site. However, the yeast MS-endonuclease activity is different from the all-type enzyme acticities of HeLa and calf thymus in several ways. First, by chromatography, the HeLa all-type endonuclease can be separated from the A/G and T/G-specific nicking enzymes that are not detectable in yeast extracts. Second, the optimal conditions of reactions are quite different. The HeLa all-type enzyme does not need Mg^{2+} and the presence of NaCl reduces activity dramatically, while yeast endonuclease activity absolutely requires Mg^{2+} and NaCl can enhance enzyme activity. Third, the incision site of the HeLa all-type endonuclease is mapped at the first phosphodiester bond 5′ to the mispaired base while the yeast mismatch-specific nicking is at two to three places at second, third and fourth phosphodiester bonds 5′ to the mispaired bases (the fourth phosphodiester bond may be the primary incision site because it occurs first in the time course study). This suggests the two enzyme systems interact to the mismatched sites differently. Finally, mismatch specificity is also different for the two enzyme systems. Particularly, C/C and C/A are nicked well by the HeLa endonuclease (43) but are not good

substrates for the yeast MS-endonuclease activity and T/G is nicked poorly by the human all-type enzyme but is nicked well by the yeast extract.

The yeast MS-endonuclease activity could participate in one short-patch mismatch repair pathway. One short-patch mismatch repair system that can correct short insertion/deletion and transition mismatches has been described by Muster and Kolodner (29). However, the efficiencies of yeast mismatch-specific nicking (Table 2) are not parallel with the repair efficiencies measured by the in vitro assay (29). For example, A/C and C/A are not nicked well but are repaired with high efficiency. A/G, G/G, and A/A transversions are nicked well but are not repaired in vitro. If the yeast MS-endonuclease activity is involved in the mismatch repair described by Muster and Kolodner (29), different neighboring sequences may have an effect on the efficiencies. It is also possible that yeast MS-endonuclease activity is required in another repair pathway not being described.

The yeast MS-endonuclease activity may be involved in the gene conversion during genetic recombination. Failure to repair mismatches generated during meiotic recombination can lead to post-meiotic segregation in frequencies of 5:3 or 3:5. It is suggested that short patch repair can cause hyper-recombination of specific markers by creating patchwork sequences on the repaired strand (13, 14). Short patch repair by the MS-endonuclease activity may cause hyper-recombination to generate diversity during genetic recombination in yeast. While further investigation of the biological roles of yeast MS endonuclease activity is necessary, the identification of a mismatch-specific nicking should facilitate elucitation of the repair mechanisms in this organism.

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