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Mismatch repair in *Xenopus* egg extracts: DNA strand breaks act as signals rather than excision points

(*mutS*/replication/recombination)

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ABSTRACT **In *Xenopus* egg extracts, DNA strand breaks (nicks) located 3′ or 5′ to a mismatch cause an overall 3-fold stimulation of the repair of the mismatch in circular heteroduplex DNA molecules. The increase in mismatch repair is almost entirely due to an increase in repair of the nicked strand, which is stimulated 5-fold. Repair synthesis is centered to the mismatch site, decreases symmetrically on both sides, and its position is not significantly altered by the presence of the nick. Therefore, it appears that in the *Xenopus* germ cells, the mismatch repair system utilizes nicks as signals for the induction and direction of mismatch repair, but not as the start or end point for excision and resynthesis.**

Precise transmission of genetic information is dependent on enzymatic systems that detect and repair base pair mismatches in DNA. Mispaired bases arise from several events, such as replication errors, strand exchange between nonidentical sequences, and induced or spontaneous modification of nucleotides within the DNA helix. While mismatch repair systems have been identified in both prokaryotes and eukaryotes, they have been best characterized in bacteria (for review, see refs. 1–3).

Escherichia coli possesses at least three mismatch repair systems. The long patch methyl-directed repair, referred to as the MutHLS pathway, directs the correction of mismatches to the newly synthesized DNA strand via its undermethylated state at the d(GATC) ("Dam") sites (4–6); this system is also involved in the prevention of recombination between homologous but nonidentical sequences (7–9). In addition to this pathway, two specialized short-patch repair systems have been characterized: the Vsr system corrects specific G/T mismatches, including those arising from spontaneous deamination of 5-methylcytosine to thymine in the Dcm sequence (10, 11), whereas the MutY system acts on G/A mismatches that are inefficiently repaired by the MutHLS pathway (12–15). The MutHLS pathway has been reconstituted *in vitro* in a purified system and its mechanism largely elucidated (16). Direction of mismatch repair by DNA methylation is not a universal feature, though. For example, in *Streptococcus pneumoniae*, the long patch mismatch repair is directed by a strand discontinuity (for review, see ref. 17). The HexA and HexB gene products (homologs of MutS and MutL) are required for the mismatch-provoked removal of the incoming transforming DNA strand (17). A nick stimulates and directs mismatch repair even in *E. coli* where it can complement the requirement for the unmethylated GATC sequence and the MutH protein (18).

In *Saccharomyces cerevisiae*, the PMS/MLH- and MSH2-dependent system seems to be analogous to the bacterial methyl- or nick-directed repair systems in terms of specificity as well as homologies at the amino acid sequence level (19–23). Unlike bacteria, yeast encodes for gene families homologous to *mutS* (*msh*) and *mutL* (*mlh*) genes. Members of the *msh* and *mlh* families have also been identified in vertebrates. In human they have been shown to be involved in hereditary nonpolyposis colon cancer, one of the most common forms of tumor predisposition (24, 25).

In vitro systems have been developed showing that mismatch repair occurs as a long tract excision repair acting on all types of mismatches in extracts of *Xenopus laevis* eggs (26, 27), of *Drosophila* Kc, and of human cell lines (28, 29). Cell extracts have also been used to detect human proteins recognizing different mismatches (30, 31).

Discontinuities are present on the newly synthesized strand during DNA replication (32–34) as well as in intermediates in genetic recombination (single- or double-strand breaks). Hence, strand breaks have been hypothesized to be strand-discrimination signals for correction of replication errors and recombinational heteroduplexes by the mismatch repair system even in organisms that use unmethylated GATC sequences as signal (18, 35). Accordingly, *in vitro* studies with *Drosophila* and HeLa cell extracts have shown that a nick can provide strand direction in mismatch repair (28, 29). More recently, the mechanism of mismatch repair in HeLa cell extracts was found to be similar to that of the *E. coli* system in that it possesses a bidirectional excision process driven by a nick (36).

Strand discontinuities may be located either in the 3′ or 5′ direction of an incorrect base pair at the replication fork. It is thus of interest to examine both quantitatively and qualitatively the influence of a nick on the pattern of repair synthesis associated with a mismatch. We have examined the role of a nick on mismatch correction in *Xenopus* egg extracts and conclude that strand discontinuity stimulates and directs DNA mismatch repair, but seems to acts as a signal and not as a free end for excision and resynthesis.

MATERIALS AND METHODS

Preparation of Egg Extracts. Extracts from unfertilized *Xenopus laevis* eggs were prepared as described (27). Protein concentration was between 30 and 45 mg/ml as determined by the method of Bradford (37).

DNA Heteroduplex Preparation. Circular heteroduplex molecules containing only one base pair mismatch at a defined position and a specific single-strand break (Fig. 1) were prepared as described (27) using M13 HK7 phage DNA, except that ligation and CsCl centrifugation steps were omitted and the following steps were added. (*i*) After the hybridization step, excess single-stranded circular DNA was eliminated by anion exchange chromatography (Qiagen, Chatsworth, CA) following the manufacturer's recommendations. (*ii*) The linear homoduplex DNA was hydrolyzed with exonuclease V from *Micrococcus luteus* (United States Biochemical) as recommended by the manufacturer. (*iii*) After extraction with phe-

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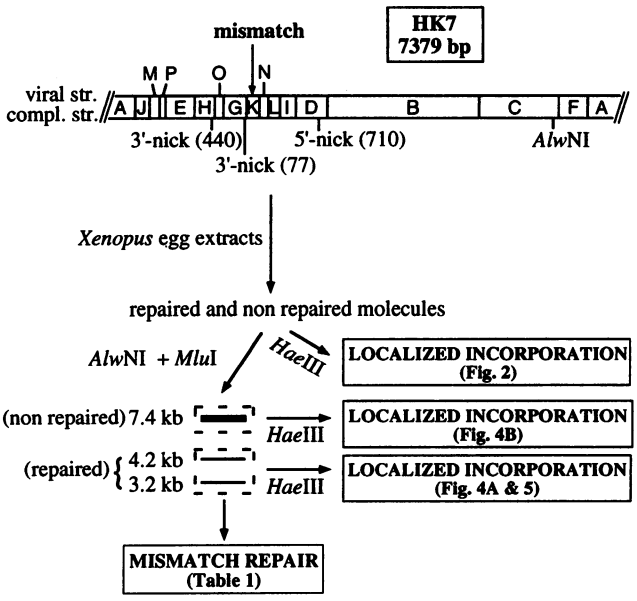

FIG. 1. Linear representation of the heteroduplex DNA substrate and flow chart of mismatch repair assays. The construction of the 7379-bp HK7 M13 derivative has been described previously (27). The mismatch is at position 6354, and the single-strand break is on the complementary strand at either 77 bp (position 6277, *Sac*I site) or 440 bp (position 5914, *Ava*II site) in the 3′ direction, or at 710 bp (position 7064, *Bgl*II site) from the mismatch in the 5′ direction. Letters refer to the *Hae*III-restricted fragments analyzed in Fig. 2. When the DNA products were additionally hydrolyzed with *Alw*NI and *Mlu*I before analysis (Figs. 4 and 5), fragments C and K were each split in two fragments called Cl and Cr, and Kl and Kr, respectively.

nol/chloroform followed by chloroform, and then precipitation with ethanol, the heteroduplex DNA was resuspended in 10 mM Tris·HCl/1 mM EDTA (pH 8.0) and purified by a second Qiagen chromatography. (*iv*) After precipitation, purified open circular heteroduplex was dissolved in 2 mM Tris·HCl/0.2 mM EDTA (pH 8.0). Control homoduplex DNA was similarly processed. Control closed circular heteroduplex was prepared as described (27).

The 7379-bp-long circular molecule derived from M13 phage DNA contains a mismatch at position 6354, and a nick in the 3′ direction at a distance of 77 (*Sac*I site) or 440 bases (*Ava*II site), or in the 5′ direction at 710 bases (*Bgl*II site) from the mismatch, on the complementary strand (Fig. 1). The single-strand break is always on the complementary strand. The first written base of the mismatch (e.g., C in C-A) is on the viral strand of the molecule and the second base (A) is on the nicked strand. The mispaired bases are within overlapping restriction sites such that repair of either strand can be detected and quantified by measuring the restored sensitivity to the relevant restriction enzyme, as described (27). Diagnostic enzymes for repair of the different mismatches used are as follows: C-A, *Mlu*I (C·G) and *Cla*I (T·A); C-C, *Mlu*I (C·G) and *Eco*NI (G·C); and G-T, *Eco*NI (G·C) and *Xmn*I (A·T).

Mismatch Repair Reaction. Mismatch repair assay was performed as described (27). Incubations were at 22°C for 45 min. Standard mismatch repair reactions (20 μl) contained 20 mM Hepes·KOH (pH 7.4)/80 mM potassium glutamate/10 mM magnesium acetate/25 mM potassium acetate/1 mM dithiothreitol/70 μM of the four dNTPs (including endogenous pool of ≈50 μM, as estimated from determination of dCTP)/2 mM ATP/2.4% (wt/vol) sucrose/200 μg of bovine serum albumin per ml/20 μM (130 ng) heteroduplex DNA/70% (vol/vol) egg extract.

Localization of DNA Repair Synthesis. The reaction mixtures were as above except for the added [α-^{32}P]dCTP or [α-^{32}P]dATP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham), exogenous cold dNTPs were not added and the reactions were scaled up 3-fold. Typically the final specific activities were in the range of 3.5–4.8 cpm/fmol of total dATP. After incubation with *Xenopus* egg extracts and postreaction purification, DNA was digested with *Hae*III and the fragments separated by electrophoresis on 8% acrylamide gels. Gels were dried and the radioactivity incorporated in each *Hae*III fragment was quantified using a PhosphorImager (Molecular Dynamics). Analysis was as described (27). Localized incorporation of the labeled nucleotides was normalized for the nucleotide content and for the size for each fragment. Then, relative localized incorporation was calculated using a large fragment (usually fragment A or C) far away from the mismatch site as a reference fragment. Absolute localized incorporation is not relevant because it is not possible to compare global incorporation between different mismatches, since it depends on the initial quantity of input DNA and on the loss of DNA, not quantifiable, during postincubation procedures.

Separate analysis of the repaired and nonrepaired molecules (Figs. 3–5) was as follows: after extract incubation and postreaction purification, DNA (mixed population) was digested with *Alw*NI and the repair-diagnostic enzyme. Individual fragments were separated by agarose gel electrophoresis and purified with Gene Clean (Bio 101). Repaired and nonrepaired molecules were then separately digested with *Hae*III, and the fragments were resolved by electrophoresis on 8% polyacrylamide gels. Quantification and analysis were as above. For comparison purposes, plots of the closed circular and nicked molecules were matched by adjusting the relative incorporation of mismatch-containing fragments, which does not effect the corresponding patterns of the reaction products.

RESULTS

The aim of this work was to examine at a biochemical level how a mismatch repair reaction is triggered and performed in a particular eukaryotic cell extract. *Xenopus* eggs are extremely rich in enzymatic activities due to the accumulation of enzymes and substrates during oogenesis, sufficient for 12 cycles of DNA and cell duplication following fertilization, which occur in the absence of any transcription (38). Thus, they provide an interesting system to examine the mechanism of mismatch repair at a crucial stage of life in vertebrates. We have previously shown that DNA repair synthesis triggered by a mismatch is localized to the region around the mismatch (26, 27) and that it occurs with mismatch-specific efficiencies in extracts of *Xenopus* eggs. However, this *in vitro* mismatch repair occurred with similar efficiency on either strand of a closed circular heteroduplex, giving no clue to the strand-specific signaling in mismatch repair. We therefore undertook to determine whether a single strand break could serve as a signal for strand discrimination. We have previously described an assay that allows quantification of mismatch repair and physical mapping of the repair tract. This experimental system was further refined by the introduction of nicks at precise locations relative to the mismatch. We chose mismatches located at the same position but repaired with different efficiencies: C-A and G-T as examples of mismatches that are repaired efficiently, and C-C as a mismatch that is repaired inefficiently (27). The nick was located either in the 3′ or 5′ direction from the mismatch at the distance of 77 or 440 bp in the 3′ direction, and 710 bp in the 5′ direction (Fig. 1).

Mismatch Correction Is Stimulated by a Nick and Is Strand-Specific. Cleavage of the DNA substrate by a repair diagnostic enzyme (e.g., *Mlu*I for a C-A mismatch) gives rise, after gel electrophoresis, to two well-separated fragments (Fig. 1, see ref. 27). These fragments and their relative abundance indicate not only that the mismatch has been repaired, but also which base pair is repaired (i.e., on which strand repair took

place). Uncut DNA thus corresponds to unrepaired DNA as well as to DNA repaired toward a base pair that is resistant to *Mlu*I (i.e., T·A). The ratio of these two *Mlu*I-resistant populations can be further evaluated by using another repair diagnostic enzyme, *Cla*I, which cleaves the sequence with a T·A base pair. All the data are summarized in Table 1.

In the three different heteroduplexes examined here, total repair of nicked circular molecules was found to be up to 3-fold higher than the repair of the covalently closed heteroduplex. There was no significant difference in the pattern or extent of repair when the nick was placed 77 bases or 440 bases from the mismatch in the 3′ direction. Consequently, repair data obtained with both constructions were pooled in Table 1. It is interesting to note that the presence of a nick in the 3′ direction did not alter the hierarchy of repair efficiency, which remained C-A ≈ G-T > C-C. A similar repair efficiency was observed when the nick was placed in the 5′ direction relative to the mismatch (Table 1, C-A mismatch). In the 24.9% or 5.5% open circular heteroduplexes that have been repaired, a strong bias favored the repair of the nicked (complementary) strand versus its template (the viral strand). Ninety percent and 87% of the repair was observed in the nicked strand for C-C and C-A mismatches, respectively, or a strand bias up to 10 to 1. Finally, the presence of a nick stimulated repair on that strand 5 fold (21.7% versus 4.1%; 5.0% versus 1.2%), while the intact strand repair remained basically unchanged (3.2% versus 4.9%; 0.5% versus 1.8%). Taken together, these results suggest that mismatch repair in *Xenopus* egg extracts is not only stimulated by the presence of a single strand break, but also strongly directed to the nicked strand.

Localization of Mismatch-Stimulated DNA Synthesis Is Centered to the Mismatch. To determine where repair DNA synthesis has actually occurred, purified DNAs were digested with *Hae*III, and the amount of incorporated radiolabel in each fragment was determined relative to a standard. Nucleotide incorporation concomitant to mismatch repair was found to be highly specific of the DNA region bearing the mismatch. The diagram of Fig. 2 shows the relative incorporation of labeled nucleotides as a result of DNA synthesis in 3′-nicked heteroduplexes bearing the C-A and C-C mismatches, and in their homoduplex counterpart (C-G). Molecules not repaired or repaired on the intact strand were not expected to contribute significantly to nucleotide incorporation (Table 1). Consequently, localized incorporation was measured in all molecules—i.e., a mixture of repaired and nonrepaired molecules. The presence of a nick did not stimulate detectable DNA synthesis in mismatch-free homoduplex DNA (Fig. 2*C*), probably because of efficient ligation in *Xenopus* egg extracts (39). However, nucleotide incorporation in C-A heteroduplex DNA was localized to the region bearing the mismatch, and could extend over several hundred bp (Fig. 2*A*). Thus, the DNA synthesis observed in our experiments appears to be associated

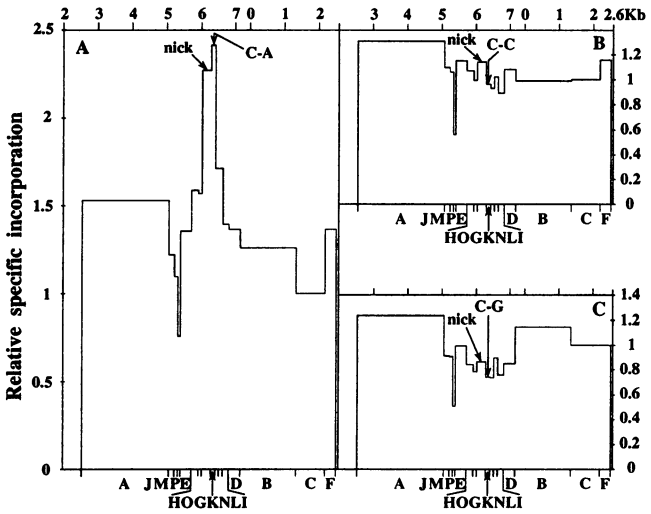

FIG. 2. Mismatch-localized DNA synthesis of open circular DNA heteroduplex or homoduplex molecules. (*A*) C-A mismatch. (*B*) C-G mismatch. (*C*) C-G complementary base pair. The nick and the mismatch are distant by 77 bp. Mismatch repair reactions were performed as described in the presence of [α-^{32}P]dATP (4.0–4.8 cpm/fmol). After purification, DNA was digested with *Hae*III and separated by electrophoresis on an 8% acrylamide gel. Nucleotide incorporation in the mixed population (containing repaired and nonrepaired molecules) was quantified using a PhosphorImager (Molecular Dynamics) and analyzed as described (26). Map positions of the fragments are indicated on the top axis. The position of the mismatch is within fragment K and the nick is at the *Sac*I site in fragment G. Nucleotide incorporations are relative to the incorporation in fragment C which is taken as a reference. Comigration of the fragments E and F on the gel prevented their resolution; half of the E-F incorporation in the E-F fragments was arbitrarily attributed to each fragment.

with the repair of the mismatch. The relative incorporation of nucleotides in the C-C-containing heteroduplex (Fig. 2*B*) does not seem to increase significantly in the vicinity of the mismatch, in agreement with a low repair efficiency of this mismatch (27).

The 5′ and 3′ Nicks Induce the Same Symmetrical Nucleotide Incorporation Pattern Centered to the Mismatch. Examination of the incorporation pattern of Fig. 2 suggested that the nick and the mismatch region were not connected in terms of DNA repair synthesis. In other words, nucleotide incorporation associated with mismatch repair may well be more intense at the mismatch than at the nick. To determine how much incorporation had occurred at the nick and at the mismatch, we improved the resolution power of our assay by increasing the distance between the nick and the mismatch.

Table 1. Repair efficiencies for open and closed circular heteroduplexes

Mismatch	Nick	Total repair, %	Repair on, %		Nicked strand/ total repair, %
			Nicked strand	Viral strand	
C-A	3′	24.9 ± 5.8	21.7 ± 4.3	3.2 ± 1.5	87
C-A*	5′	ND	17.9 ± 2.1	ND	—
C-A	None	9.0 ± 2.2	4.1 ± 1.0	4.9 ± 1.2	45
C-C	3′	5.5 ± 3.8	5.0 ± 3.7	0.5 ± 0.1	90
C-C	None	3.0 ± 1.4	1.2 ± 0.6	1.8 ± 0.8	40
G-T	3′	ND	22.2 ± 5.9	ND	—

The mismatches are in the same sequence context in the HK7 molecules. The nick on the complementary strand is either 77 or 440 nucleotides in the 3′ direction, or 710 nucleotides in the 5′ direction from the mismatch. Quantification of repair is as described (27). Results are the average of at least three experiments. ND, not determined.

*[α-^{32}P]dATP was the only exogenous deoxynucleotide added in the reaction.

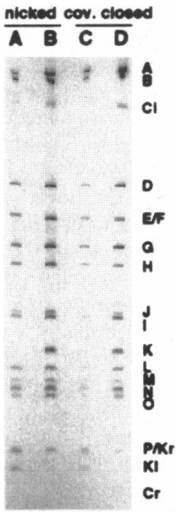

FIG. 3. Autoradiograph of a 8% polyacrylamide gel after incorporation of labeled nucleotides in C-A-containing heteroduplexes incubated with *Xenopus* egg extracts. Two experiments were analyzed on the same gel. The heteroduplex had either a nick in the 3′ direction at 440 nucleotides from the mismatch (lanes A and B) or was covalently closed (lanes C and D). Digestion by *Mlu*I yielded repair-diagnostic fragments upon agarose gel electrophoresis. After gel purification of *Mlu*I-generated fragments (lanes A and C) as well as *Mlu*I-resistant fragments (lanes B and D), DNAs were fragmented further by *Hae*III and submitted to a secondary polyacrylamide gel electrophoresis which allowed precise quantification of incorporation label in each fragment. The position of the mismatch is either in fragment K or between fragments Kl and Kr. The nick is in fragment H.

We introduced a nick 440 bases in the 3′ direction, or 710 bases in the 5′ direction, away from the mismatch in such a way that the nick-bearing and mismatch-bearing fragments were separated by two or three fragments, respectively. A typical result is presented in Fig. 3 and diagrams of the relative nucleotide incorporations are plotted in Figs. 4 and 5. The pattern of DNA synthesis in the C-A substrate was analyzed separately in the fraction of molecules repaired on the nicked strand (i.e., toward the C·G pair; Figs. 4*A* and 5 *A* and *B*), and in the remaining fraction (i.e., molecules nonrepaired and repaired on the other strand toward the T·A pair; Fig. 4*B*). For the nick-bearing DNA molecules repaired to the C·G pair (that is on the nicked strand), there is no significant shift of the repair synthesis in the region between the mismatch and the nick placed in either 3′ or 5′ direction (Figs. 4*A* and 5, solid lines). The virtually symmetric distribution of the synthesis around the mismatch is indistinguishable from that of the same heteroduplex molecule without nick (Figs. 4*B* and 5, dotted lines). DNA synthesis patterns were similar for the molecules repaired on the nicked strand (C·G) and for the remaining fraction of molecules (Fig. 4*B*): they all peaked at the position of the mismatch. Taken together, these results show that, in *Xenopus* egg extracts, a single-strand break stimulates mismatch repair of the nicked strand, but does not act a free end for excision–resynthesis in the repair reaction. Indeed, in the population of repaired molecules, the nick- and mismatch-stimulated DNA synthesis extends equally in both directions from the mismatch for several hundred bases, independently of the position of the nick.

DISCUSSION

We have investigated the effect of a single-strand break in the vicinity of a mismatch (77, 440, or 710 bp) on the repair of heteroduplex molecules in *Xenopus* egg extracts. Strand discontinuities in the DNA substrate appear to stimulate the mismatch repair, particularly for mismatches that were known to be efficiently repaired in covalently closed circular substrates (27). This stimulation is essentially accounted for by the enhanced repair of the nicked strand, suggesting that the strand discontinuities are responsible for the strand bias during mismatch repair *in vivo*. Previous studies with human and *Drosophila* cell extracts have already provided evidence for the strand directionality of mismatch repair by a single-strand break (28, 29). Thus, strand discrimination and stimulation of the mismatch repair system by the presence of a strand discontinuity seem to be ubiquitous characteristics of general mismatch repair systems (for a review, see refs. 1 and 2). In *E. coli*, unmethylated d(GATC) sequences from either side of the

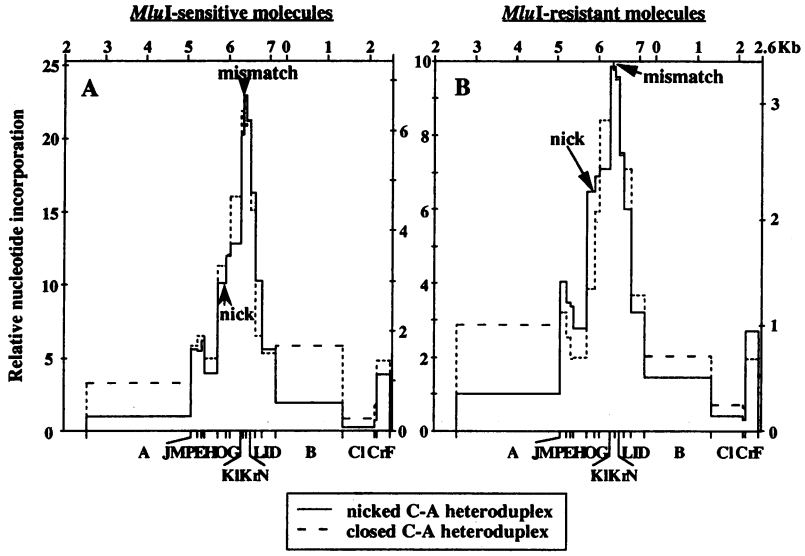

FIG. 4. Mismatch (C-A)-localized DNA synthesis in molecules with and without nick repaired on the complementary strand, and in nonrepaired molecules. (*A*) Relative nucleotide incorporation in the molecules repaired on the complementary nick-containing strand (to C·G). (*B*) Relative nucleotide incorporation in the nonrepaired molecules and in the molecules repaired to T·A. Legend is the same as in Fig. 2, except that the mismatch is between fragments Kl and Kr, the nick is at position 5914 in the fragment H (*Ava*II site), and molecules repaired on the complementary strand (*A*) were separated from those not repaired on this strand (*B*) (see *Materials and Methods*). The left *y* axis corresponds to nicked molecules (solid lines) and the right *y* axis corresponds to closed molecules (dashed lines). Nucleotide incorporation in fragment A is taken as reference.

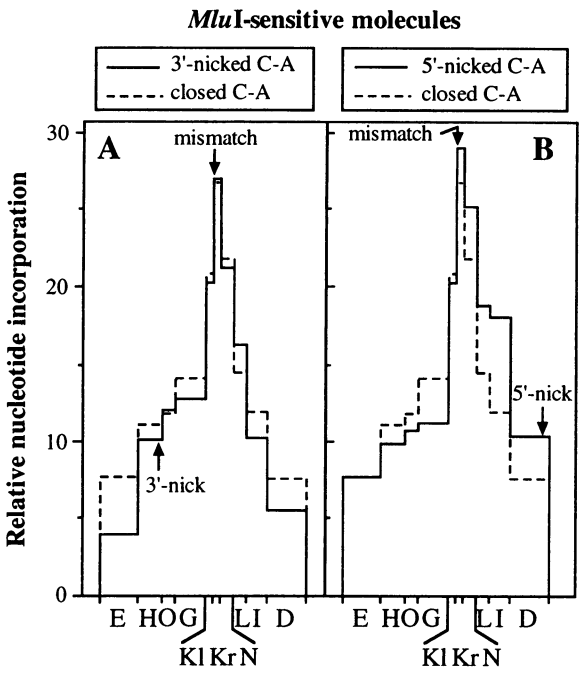

FIG. 5. Mismatch (C-A)-localized DNA synthesis on the nick-bearing strand. (*A*) Molecules with a nick located 440 bp 3′ to the mismatch. (*B*) Molecules with a nick located 710 bp 5′ to the mismatch. In each case the molecules with a nick (solid lines) are compared with those without a nick (i.e., closed circular; dashed lines). Description of the graphs is as described in the legend to Fig. 3.

mismatch are incised by the mismatch repair proteins to initiate the excision process (40, 41). In contrast, methylation of d(CCGG) sequences in a M13 substrate by *Hpa*II methylase has no effect on efficiency and strand bias of mismatch repair in *Xenopus* egg extracts (M. Petranovic, P.B., and M.R., unpublished results).

Our analysis of the mismatch-associated DNA repair synthesis reported here is in good agreement with the ability of a nick to stimulate mismatch repair and with the correlation of repair synthesis to the repair efficiency. Indeed, in the nicked molecules, the C-A heteroduplex shows more repair synthesis around the mismatch than the C-C heteroduplex (Fig. 2). Moreover, when the repaired molecules are analyzed separately, there is a higher peak of incorporation at the mismatch site in the purified molecules that are repaired on the nicked strand to the C-G pair than in those that are not repaired to C-G (Fig. 4). If the nick acted as a free end for excision and resynthesis associated with mismatch repair, then an alteration of the pattern of DNA synthesis would be expected because of the 5′ to 3′ polarity of DNA synthesis. However, the pattern of incorporation, centered to the mismatch region, was basically the same in the presence and absence of a single-strand break and spanned a few hundred bases around the mismatch (Figs. 4*A* and 5). Yet, there was a 5-fold stimulation of repair by the nick toward the C-G pair for the 3′ nick (Table 1, repair of C-A or C-C to the viral strand, molecules with versus without nick) and therefore the majority of molecules repaired to C-G should have undergone the nick-stimulated and nick-directed repair.

The same profile of the localized synthesis is obtained for the totality of incubated molecules and for those repaired on the nicked strand (Figs. 2, 4*A*, and 5). This means that this mismatch-targeted synthesis is not attributed to unrepaired intermediates. Three lines of evidence suggest that the excess molecules repaired via nick-stimulated events did not escape our analysis—e.g., because of an incomplete repair synthesis. Since there are three and four *Hae*III restriction sites between the mismatch and the nick, an excess of repaired nicked molecules with the gap in this region would create a deficit of label to the left of the mismatch position in Fig. 4*A*. Yet, all 18 bands resolved on the acrylamide gel are equally discrete and located at identical positions for both intact and nicked substrates (Fig. 3). Furthermore, blotting under denaturing and native conditions, followed by hybridization with the labeled substrate DNA probe, showed no evidence of gapped DNA material (data not shown). The same is true for unrepaired molecules (results not shown). In a control experiment, we have first separated and purified the supercoiled and relaxed reaction products of an initial 3′-nicked substrate, then separated the repaired molecules in each product and determined the pattern of repair synthesis as in Figs. 4 and 5. The majority of the supercoiled products were repaired whereas a minority of relaxed products were repaired (results not shown). The repair synthesis pattern is the same in supercoiled and nicked reaction products and shows no evidence of skewing toward the site of the repaired nick for the supercoiled products. Finally, the observation that neither 3′ nor 5′ nicks changed significantly the pattern of DNA repair synthesis, while the majority of analyzed repair events were nick-stimulated, is not consistent with a repair synthesis starting or ending at, or around, the nick. If the nick acted as the free end for excision–resynthesis, then for the 5′ nick, the 5′ to 3′ DNA synthesis is expected to proceed from the nick toward the mismatch. Therefore, the labeling should occur first at the nick site and be as intense as around the mismatch site. This was not observed, since the labeling pattern was very similar for the 5′ nicked, 3′ nicked, and unnicked substrates (Figs. 4*A* and 5).

The simplest interpretation of these results is that the nick serves as a strand-direction signal for repair that occurs by the same mechanism in initially covalently closed and in nicked substrates. The mechanism could involve imprecise nicking by a special mismatch-stimulated endonuclease to the left and/or to the right of the mismatch, mostly on the order of hundred nucleotides from the mismatch. Mismatch-dependent DNA synthesis was also detected in human cell extracts, but in the study of Holmes *et al.* (28) the fragmentation of the molecules did not allow distinction between the relative incorporation in the region of the nick versus that of the mismatch. Thomas *et al.* (29) fragmented more precisely the heteroduplex molecule and observed, in the mixture of repaired and nonrepaired molecules, higher nucleotide incorporation in the nick-containing fragment than in the fragment containing the mismatch, which is not expected for a mismatch-stimulated event since the nick was in the 3′ direction to the mismatch. Fang and Modrich (36) analyzed the excision tracts associated with the repair reaction in HeLa cell extracts and concluded that, under conditions of severely inhibited DNA synthesis, the excision tracts appear to extend from the nick to the mismatch area irrespective of the strand polarity. Our model is different from the one proposed by Fang and Modrich (36) using human HeLa cell line extracts who suggested that the nick is the end-point of excision and resynthesis. The difference may be due to the fact that we do not disrupt the repair process (DNA synthesis is not inhibited), and we measure a later stage of repair (synthesis versus excision product). Alternatively, it may be that only the *Xenopus*, or only the germ line (unfertilized egg), mismatch repair uses nicks as signals.

It is obvious that the mismatch repair synthesis in *Xenopus* egg extracts is most intense at, and close to, the mismatch site irrespective of the presence of a repair-stimulating distal nick (Figs. 4 and 5). The pattern of nucleotide incorporation stimulated by the mismatch appears symmetrical (i.e., the intensity falls off sharply on both sides as a function of the distance from the mismatch), suggesting either a bidirectional and symmetrical process or an ensemble of unidirectional repair events extending to either direction with similar probabilities. In a previous study, the distribution of nucleotide

incorporation was found to be much narrower among purified repaired molecules than in the total population (26). This was accounted for by the presence of a large fraction of unrepaired molecules that have undergone some mismatch-stimulated DNA synthesis. These observations suggest that the most efficient repair events are associated with DNA synthesis tracts that are shortest and closest to the mismatch. The same conclusion holds for the mismatch repair events stimulated and directed by a distal nick. A nick as a strand signal and activator is not without precedent: it has been postulated first by M. Fox for the repair of heteroduplex by the Hex system in *S. pneumoniae* (42), and it was demonstrated experimentally in the replication-coupled transcription system in bacteriophage T4 which is activated by a DNA tracking mechanism that is strand-directed by a nick (43). Furthermore, recent experiments on the mechanism of lagging strand replication by *E. coli* Pol III holoenzyme demonstrated that the replicase cycles from one DNA site to another via preassembled DNA sliding clamps. The authors suggested that the clamp, left on the DNA at the internal DNA termini (e.g., those of the Okazaki fragments) may be harnessed by other machineries coordinated with chromosome replication, for example the repair and recombination systems, and used as a signal for the newly synthesized strand (44, 45). Therefore, editing of DNA replication and recombination processes by the mismatch repair components could be accomplished using strand discontinuities as strand discrimination signals (1). Indeed, our results suggest a "passive" role for the nick acting as a signal in the strand directed repair, rather than a free end for excision and resynthesis.

The recent findings that (*i*) the mouse male meiosis is unaffected by MSH2 deficiency, whereas the PMS2 deficiency causes disordered chromosome pairing and meiotic sterility (46–48), and (*ii*) two meiosis-specific *mutS* homologs (MSH4 and MSH5) affect only crossovers in yeast meiosis (49, 50), suggest that our finding may indeed be a characteristic of the germ-line mismatch repair processes. A nick-signaled localized mismatch repair would prevent gene conversion but not crossovers, whereas a nick-to-mismatch excision would abort the initiated recombination. Perhaps all mitotic recombination is prevented by somatic mismatch repair and only meiotic gene conversion is suppressed by meiotic mismatch repair, thus permitting meiotic crossovers, chromosomal disjunction, and fertile intraspecies meiosis.

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