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Mismatch correction catalyzed by cell-free extracts of *Saccharomyces cerevisiae*

(mutagenesis/gene conversion/transition mutations/transversion mutations/repair synthesis)

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ABSTRACT **Heteroduplex DNA substrates containing a 4- or 7-base-pair insertion/deletion mismatch or each of the eight possible single-base-pair mismatches were constructed. Extracts of mitotic *Saccharomyces cerevisiae* cells catalyzed the correction of mismatched nucleotides in a reaction that required Mg^{2+} and had a partial requirement for ATP and the four dNTPs. The insertion/deletion mismatches and the A·C and G·T mismatches were repaired efficiently, while the six other single-base-pair mismatches were repaired poorly or at undetectable rates. Mismatch correction was accompanied by the specific incorporation of less than 20 nucleotides at or near the site of the repaired mismatch.**

Mispaired nucleotides in DNA are thought to arise by at least three different mechanisms. Misincorporation during DNA replication can lead to mispairs in DNA and mismatch correction can increase replication fidelity (1–4). Deamination of DNA bases can form mispairs, and such lesions can be repaired by *N*-glycosylases (5, 6). Mispaired bases can be formed during genetic recombination; and correction of mispairs, or the failure of correction, can explain gene conversion, post-meiotic segregation, localized negative interference, and map expansion (7–12).

Transformation experiments have provided direct evidence for mismatch correction (1–4, 13–17). In *Escherichia coli* several mismatch correction systems have been identified. The *E. coli dam*-instructed system has been postulated to repair mispairs produced during DNA replication and genetic recombination (1–4, 18). A second system will repair fully *dam* methylated DNA and may act during genetic recombination (19, 20). Both reactions involve excision/resynthesis tracts that are several thousand nucleotides long (1, 19, 20). The other mismatch correction reactions in *E. coli* appear to involve short excision tracts (refs. 19 and 21, R. Fishel and R.K., unpublished results). In *Streptococcus pneumoniae* mismatch correction is catalyzed by the *hex* system and strand specificity may be directed by the presence of breaks in the strand to be corrected (14, 15). In *Saccharomyces cerevisiae* the *PMS1, PMS2, PMS3*, and *PMS4* genes are thought to be involved in mismatch correction (ref. 22 and D. Bishop, R.K., M. Williamson, and S. Fogel, unpublished results). Because *S. cerevisiae* DNA is unmethylated, methylation is unlikely to play a role in this type of mismatch correction (23, 24). However, cytosine methylation may play a role in mismatch correction in mammalian cells (25).

The development of *E. coli in vitro* mismatch correction systems have provided assays for use in purifying the proteins required for mismatch correction (26, 27). This communication describes a *S. cerevisiae in vitro* mismatch correction system.

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EXPERIMENTAL PROCEDURES

Strains. The *E. coli* strain JM105 [Δ(*lac-pro*) *thi strA hsdR4 endA sbcB* F′ *traD36 proAB*$^+$ *lacI*^{q} *lacZ M15*] and a sample of M13mp11 covalently closed replicative form DNA (RFI) DNA (28) were from M. A. Osley (Dana–Farber Cancer Institute). The *E. coli* strain RS5033 [(PO of *HfrH*) *metB1 rel-1 str-400 azi-7 lacM5286 φ80dII thi-1, lacBK1 dam-4*] was from Paul Modrich (Duke University, Durham, NC). The yeast strain AP-1 *MATa/MATα ade1/ADE1 ade2-1/ade2-R8 ura1/URA1 his7/HIS7 lys2/LYS2 tyr1/TYR1 gal1/GAL1 CYH2/cyh2 CAN1/can1 LEU1/leu1*, which is a *pac1* strain, was from B. Byers (University of Washington) and LL20 *MATα his3-11,-15 leu2-3,-112 can1* was from J. Szostak (Massachusetts General Hospital, Boston, MA). Extracts prepared from both yeast strains gave identical results.

Enzymes. Restriction endonucleases and T4 DNA polymerase were from New England Biolabs and were used as suggested by the manufacturer. T4 DNA ligase was purified by an unpublished procedure.

Purification and Analysis of Nucleic Acids. Oligonucleotides were synthesized on an Applied Biosystems 380A DNA synthesizer. The following oligonucleotides were used: 1, 5′ AGCTTGGTACGCGTGCTCTAGATCCGG 3′; 2, 3′ ACCATGCGCACGAGACCTAGGCCTTAA 5′; 3, 3′ ACCATGCGCACGAGCTCTAGGCCTTAA 5′; 4, 5′ AGCTTGGTACCCGTGCTCTAGATCCGG 3′; and 5, 5′ AGCTTGGTACGCGAGCTCTAGATCCGG 3′. Oligonucleotides 1, 4, and 5 were M13 viral strands and oligonucleotides 2 and 3 were M13 complementary strands. M13 viral DNA and M13 RFI DNA were purified essentially as described (28). The M13 DNA used in the experiments described below was obtained from the *dam-4 E. coli* strain RS5033 and was not methylated at its *dam* sites.

DNA samples were analyzed by electrophoresis through agarose slab gels run in 40 mM Tris·HCl, pH 7.9/5 mM sodium acetate/1 mM EDTA/ethidium bromide at 0.5 μg/ml. The gels were photographed on Polaroid Type 665 Positive/Negative film and quantitated using a densitometer. The amount of DNA present in a band was determined by comparison with known amounts of DNA included on every gel. In some cases, DNA was purified from individual gel bands as described (29).

Construction of M13 Mutants. Seven different mutant derivatives of M13mp11 were constructed as described below. A map of M13mp11 is presented in Fig. 1, and the DNA sequences of the polylinker region of the heteroduplex substrates are presented in Fig. 2.

A 4-base-pair (bp) insertion that inactivated the *Xba* I cleavage site of M13mp11 was constructed as described (30).

Abbreviations: bp, base pair(s); kb, kilobase(s); RFI, covalently closed replicative form DNA.

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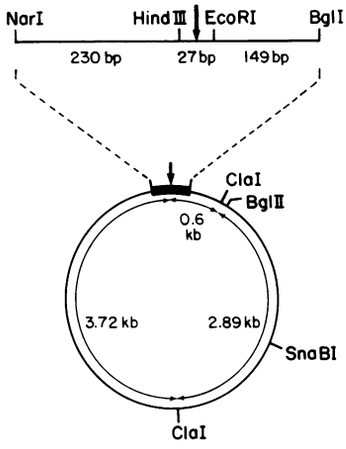

FIG. 1. Map of M13mp11-derived mismatch-correction substrates. The distances given on the circular map are the distances between the two *Cla* I sites and the site of the mispair that lies within the polylinker region found between the *Eco*RI and *Hin*dIII sites. The nucleotide coordinates of the two *Cla* I sites, the *Bgl* II site, and the *Sna*BI site are 6882, 2527, 6935, and 1268, respectively, on the standard M13mp11 map (28).

The DNA was used to transform *E. coli* JM105, and white plaques were screened for *Xba*I-resistant M13 RFI DNA (28). The sequence of this mutant was verified by DNA sequence analysis (31). This mutant is called mutant 6. A 7-bp deletion mutation inactivating the *Pst* I site of M13mp11 was similarly constructed except the 3'-single-stranded overhangs produced by digestion with *Pst* I were removed with T4 DNA polymerase prior to cyclizing the digested DNA. This mutant is called mutant 7.

	Site	Mutant
1 AGCTTGGGCTGCAGGTCGACTCTAGAGGATCC ACCCGACGTCCAGCTGAGATCTCCTAGG	— XbaI	6 M13mp11
2 AGCTTGGGTCGACTCTAGAGGATCCCCGGGCG ACCCAGCTGAGATCTCCTAGGGGCCCGC GACGTCC	— PstI	7 M13mp11
3 AGCTTGGTACGCGTGCTCTAGATCCGG ACCATGCGCACGAGACCTAGGCCTTAA	XbaI BamHI	1 2
4 AGCTTGGTACGCGTGCTCTGGATCCGG ACCATGCGCACGAGATCTAGGCCTTAA	BamHI XbaI	2 1
5 AGCTTGGTACGCGTGCTCTAGATCCGG ACCATGCGCACGAGCTCTAGGCCTTAA	XbaI XhoI	1 3
6 AGCTTGGTACGCGTGCTCGAGATCCGG ACCATGCGCACGAGATCTAGGCCTTAA	XhoI XbaI	3 1
7 AGCTTGGTACCCGTGCTCTAGATCCGG ACCATGCGCACGAGATCTAGGCCTTAA	KpnI MluI	4 1
8 AGCTTGGTACGCGTGCTCTAGATCCGG ACCATGGGCACGAGATCTAGGCCTTAA	MluI KpnI	1 4
9 AGCTTGGTACGCGTGCTCTAGATCCGG ACCATGCGCTCGAGATCTAGGCCTTAA	MluI SacI	1 5
10 AGCTTGGTACGCGAGCTCTAGATCCGG ACCATGCGCACGAGATCTAGGCCTTAA	SacI MluI	5 1

FIG. 2. Nucleotide sequences of the polylinker region of different mismatch-correction substrates. Substrates 1 and 2 utilized the M13mp11 polylinker region, and substrates 3–10 utilized a different 27-bp polylinker region. The latter polylinker is "in frame" so that in those cases where it does not contain a termination codon, the phages obtained will yield blue plaques. The upper strand is always the viral strand obtained from the indicated mutant phage. The lower strand is always the complementary strand from RFI DNA obtained from the indicated mutant. The underlined nucleotides and the listed restriction endonuclease cleavage site in each strand indicate the cleavage site sequence in each strand that is inactivated by the mispair.

Single-base substitution mutations were constructed by inserting oligonucleotide duplexes between the *Eco*RI and *Hin*dIII sites of M13mp11 and recovering mutant phage from transformants. Oligonucleotide duplexes were prepared by mixing oligonucleotides 1 and 2, 1 and 3, 3 and 4, and 3 and 5 such that each mixture contained 0.2 nmol of each oligonucleotide, 50 mM Tris·HCl (pH 8.0), and 10 mM $MgCl_2$ in a volume of 20 μl. The tubes were placed in a tray containing H_2O at 100°C, and the tray was allowed to cool at room temperature for 2 hr to reanneal the oligonucleotides. Then 50 fmol of a duplex oligonucleotide and 25 fmol of 7.19-kilobase (kb) *Hin*dIII–*Eco*RI fragment of M13mp11 DNA were incubated in a 50-μl reaction containing 50 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM ATP, and 50 pyrophosphate exchange units of T4 DNA ligase per ml for 2 hr at 20°C. Then 5-μl 0.2 M EDTA, pH 8.0/1 μl of *E. coli* B tRNA at 5 mg/ml/44 μl of 10 mM Tris·HCl, pH 8.0/1 mM EDTA was added, and the DNA was purified by phenol extraction and ethanol precipitation. The DNA was used to transform *E. coli* JM105, and plaques were screened for mutant RFI DNA by restriction mapping (28). The mixture of oligonucleotides 1 and 2 yielded the *Mlu*I^s *Xba*I^s *Bam*HI^r *Xho*I^r mutant 1 from the white plaques as this mutant contains a TAG stop codon. The blue plaques from this transformation yielded the *Mlu*I^s *Xba*I^r *Bam*HI^s *Xho*I^r mutant 2. The mixture of oligonucleotides 1 and 3 yielded the *Mlu*I^s *Xho*I^s *Xba*I^r *Bam*HI^r mutant 3 from the blue plaques. The mixtures of oligonucleotides 3 and 4 and 3 and 5 yielded the *Mlu*I^r *Kpn*I^s *Sac*I^r *Xba*I^s mutant 4 and the *Kpn*I^r *Mlu*I^r *Sac*I^s *Xba*I^s mutant 5, respectively, from the white plaques. By not phosphorylating the oligonucleotides, the insertion of multiple oligonucleotides was prevented.

Construction of Heteroduplex DNA. M13 RFI DNA at 100–300 μg/ml was digested with *Bgl* II or *Sna*BI. Then 1 M Tris·HCl, pH 8.0, and 0.5 M EDTA, pH 8.0, were added to concentrations of 100 mM and 10 mM, respectively. A 5- to 10-fold excess of the appropriate M13 viral DNA was added, and then 2 M NaOH was added to a concentration of 200 mM. After 10–20 min at room temperature the solution was neutralized by addition of 1 M Tris·HCl, pH 8.0, to a concentration of 200 mM. The DNA concentration ranged from 0.3–1 mg/ml depending on the experiment. The DNA was annealed by incubating it at 65°C for 45–60 min, and then the DNA solution was dialyzed sequentially against two 2-liter changes of 10 mM Tris·HCl, pH 8.0/1 mM EDTA. The DNA was then incubated with T4 DNA ligase as described above and digested with the restriction endonuclease that will digest the original M13 RFI DNA but not the heteroduplex DNA. The covalently closed circular heteroduplex DNA was then purified by equilibrium centrifugation in CsCl/ethidium bromide density gradients. For substrate 7 (Fig. 2), the open circular heteroduplex DNA was purified by agarose gel electrophoresis prior to the ligation step, and the final restriction endonuclease digestion step was omitted because *Sac* I will nick this substrate DNA at a low rate.

Mismatch Correction Assays. Growth of cells and preparation of mitotic extracts were as described except that 14 mM 2-mercaptoethanol was included during the incubation with Zymolyase (32, 33). Assays were carried out in 50 μl containing 35 mM sodium Hepes buffer (pH 7.8); 10 mM $MgCl_2$; 2 mM gluthathione; 2 mM spermidine; 5 mM ATP; 0.2 mM each CTP, GTP, and UTP; 0.01 mM each of the 4 dNTPs; 1 mM NAD; bovine serum albumin at 100 μg/ml; and 0.4 μg of substrate DNA. Cell-free extract was included at optimal amounts that ranged from 80 to 120 μg of protein per ml. Incubation was at 26°C for 2 hr unless otherwise indicated. Then 5 μl of 0.1 M EDTA, pH 8.0, was added, and the DNA was purified by phenol extraction and ethanol precipitation. The DNA was then analyzed by restriction endonuclease digestion and agarose gel electrophoresis.

RESULTS

Substrates and Assays for Detecting Mismatch Correction. The assay for mismatch correction uses the observation that mismatched nucleotides located within a restriction endonuclease recognition sequence renders the sequence resistant to digestion (19, 26). Our substrates are heteroduplex derivatives of M13mp11 that contain mismatched nucleotides within the polylinker region located between the single *Eco*RI and *Hin*dIII sites of M13mp11 (Fig. 1). Digestion of the substrate DNA with *Cla* I and the restriction endonuclease, whose recognition site is inactive because of the mismatched nucleotide(s), will yield 4.3- and 2.9-kb fragments. Similar digestion of DNA in which the recognition sequence has been restored by mismatch correction will yield 3.7-, 2.9-, and 0.6-kb fragments.

The nucleotide sequences of the polylinker region of the 10 different substrates used are presented in Fig. 2. Substrates 1 and 2 contain a 4-nucleotide insertion mismatch and a 7-nucleotide deletion mismatch that inactivate the *Xba*I site and *Pst*I site of M13mp11, respectively. Substrates 3–10 contain a different polylinker region that is 27 bp long, and each contains a different single-base mispair. These eight single-base substitution substrates were designed so they contain two overlapping restriction endonuclease cleavage sites that differ by 1 nucleotide in the overlap region and contain a single mispair that inactivates both sites. Repair of a single-base substitution mismatch in one direction will produce one restriction endonuclease cleavage site and repair in the opposite direction will produce a different restriction endonuclease cleavage site. This feature allows us to assay for repair in both possible directions using the same substrate DNA.

Detection of Mismatch Correction *in Vitro*. The results presented in Fig. 3 indicate that incubation of substrate 1 with an extract of LL20α cells converted a portion of the DNA to an *Xba* I-sensitive form and yielded a 3.7-kb fragment in addition to the 4.3- and 2.9-kb fragments (Fig. 3, lanes 3 and 4). The expected 0.6-kb fragment was observed but was produced in insufficient amounts to be visible in Fig. 3. Digestion of the product DNA with *Xmn* I and *Xba* I or with *Bgl* I and *Xba* I confirmed that the new *Xba* I cleavage site was located at the site where the mismatched nucleotides were originally present (data not shown). Analysis of the product DNA by digestion with only *Cla* I both before (Fig. 3, lane 5) and after (Fig. 3, lanes 6 and 7) incubation with the extract indicated that the substrate DNA was not linearized at the site of the mismatched nucleotides during the reaction.

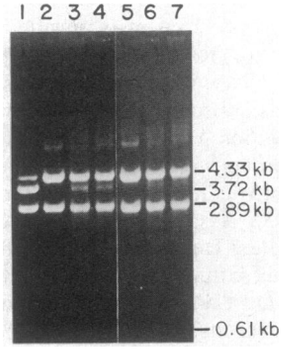

FIG. 3. Detection of mismatch correction. Reactions included extracts prepared from LL20α cells and substrate 1 (Fig. 2). Lane 1, markers produced by digesting M13mp11 RFI DNA completely with *Cla* I and partially with *Xba* I. Lanes 2 and 5, no extract. Lanes 3 and 6, extract at 100 μg/ml. Lanes 4 and 7, extract at 200 μg/ml. In lanes 2, 3, and 4, the product DNA was digested with *Cla* I and *Xba* I. In lanes 5, 6, and 7, the product DNA was digested with *Cla* I.

The experiment presented in Fig. 4 shows the time course of repair of an A·C mispair. The results indicate that the A·T and G·C reaction products were produced in equal amounts. Quantitation of the A·C to A·T reaction indicated that after a short lag period the reaction had linear kinetics for 90 min at 26°C (Fig. 4*B*). Additional analysis (data not shown) showed that repair of the A·C mispair had a K_{max} of 4.2×10^{-10} M and a V_{max} of 7.1×10^{-15} mol/50-μl assay per 90 min under these conditions.

Reaction Requirements and Reaction Specificity. The requirement for different components present in a reaction was determined (Table 1). Mismatch correction required Mg^{2+} and had a partial requirement for ATP and the four dNTPs. The reaction was inhibited by *N*-ethylmaleimide and was partially inhibited by ATP[S] and dideoxyTTP. p[CH_2]ppA, coumermycin A1, and aphidicolin had no effect.

The specificity of the mismatch correction system was determined (Table 2). These results show that A·C and G·T mispairs and insertion/deletion mispairs were repaired efficiently, while the other six single-base mispairs were corrected less efficiently or were not corrected at all. Mixing experiments indicated that a poorly repaired mispair-containing substrate did not inhibit the repair of an efficiently repaired mispair. These experiments also indicated that the product DNA would have been digested to completion had it been formed. In the case of the A·C mispair the A·T and G·C products were formed in equal proportions, while the G·T mispair yielded five times more G·C product than A·T product. The deletion of 4 nucleotides occurred three times as frequently as the insertion of 7 nucleotides. The C·C, A·A, and T·T mispairs also showed repair asymmetry although they were repaired at lower efficiencies than the G·T and insertion/deletion mispairs. These results indicate that the *in vitro* mismatch correction system specifically recognizes mispaired nucleotides rather than repairing them at random.

Analysis of Mismatch Correction Specific DNA Synthesis. In initial experiments the incorporation of [^{3}H]dTTP into DNA during mismatch correction was measured. Newly synthesized DNA equivalent to 0.5% of the input DNA was formed (data not shown). This synthesis was independent of the

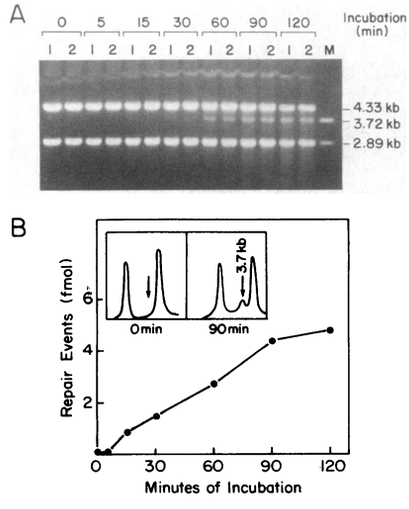

FIG. 4. Time course of repair of an A·C mispair. Reactions were carried out for the indicated times. Substrate 3 (Fig. 2) was used, and an extract of AP-1 cells was present at a concentration of 100 μg/ml. (*A*) Lanes 1: the product DNA was digested with *Cla* I and *Xba* I to detect the A·T-containing product. Lanes 2: the product DNA was digested with *Cla* I and *Bam*HI to detect the G·C-containing product. Lane M: 4.6 fmol of M13mp11 RFI DNA molecules digested with *Cla* I and *Xba* I. (*B*) The amount of A·T-containing product formed (lanes 1) was quantitated by densitometry. (*Inset*) The densitometer tracings from the 0- and 90-min samples.

Table 1. Reaction requirements

	Relative repair activity, %
Complete reaction mixture	100*
$-MgCl_2$	5
−ATP	64
−dNTPs	51
−CTP, GTP, UTP	94
−NAD	91
−Spermidine	81
+1 mM ATP[S]	54
+1 mM p[CH_2]ppA	95
+0.01 mM d_2TTP	65
+Coumermycin A1 (5 μg/ml)	95
+2 mM *N*-ethylmaleimide	5
+Aphidicolin (50 μg/ml)	95

Assays were carried out as described except that the indicated omissions and additions were made. Substrate 1 of Fig. 2 was used, and the formation of the *Xba* I-sensitive product was measured. Cell-free extract of LL20α cells was present in all reactions at a final concentration of 100 μg/ml.
*100% repair is defined as the formation of 4.6 fmol of repair product.

presence of a mismatch. This indicated (*i*) that the amount of mismatch-correction-specific DNA synthesis was low compared to random nonspecific DNA synthesis and (*ii*) that the mismatch-correction-associated repair tracts were short compared to the length of the substrate DNA.

The distribution of newly synthesized DNA formed during mismatch-correction reactions was determined by restriction mapping. Repair reactions containing [α-^{32}P]dATP were carried out with substrates containing an A·C mispair, a T·C mispair, or no mispair, and the amount of radioactivity present in the 27-bp *Hin*dIII–*Eco*RI mispair containing fragment and 149-bp *Eco*RI–*Bgl* II fragment located clockwise from the 27-bp fragment (see Fig. 1) was determined (Fig. 5

Table 2. Specificity of repair

Substrate*	Mispair*	Product*	Relative repair, %†	Repair events, fmol
1	4-bp insertion/deletion	Deletion	100	4.6
2	7-bp insertion/deletion	Insertion	38	1.8
3	A·C	A·T	88	4.0
		G·C	91	4.2
4	G·T	G·C	50	2.3
		A·T	8	0.4
5	T·C	T·A	14	0.6
		G·C	11	0.5
6	G·A	G·C	<3	<0.1
		T·A	<3	<0.1
7	C·C	C·G	<3	<0.1
		G·C	10	0.5
8	G·G	G·C	<3	<0.1
		C·G	<3	<0.1
9	T·T	T·A	<3	<0.1
		A·T	20	0.9
10	A·A	A·T	<3	<0.1
		T·A	10	0.5

Repair reactions were carried out except that the indicated substrates and an extract of AP-1 cells at a final concentration of 96 μg/ml were present in individual reactions.
*The substrates and mispairs are exactly as described in Figs. 1 and 2. The products were detected by digesting the product DNA with appropriate restriction endonucleases as indicated in Fig. 2.
†100% repair is defined as the formation of 4.6 fmol of repair product.

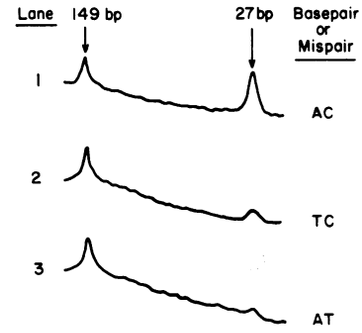

FIG. 5. Distribution of repair DNA synthesis after mismatch correction. Reactions contained [α-^{32}P]dATP (New England Nuclear) at a specific activity of 2.4×10^5 cpm/pmol. After incubation with extract of AP-1 cells present at 96 μg/ml, the DNA was purified by phenol extraction and ethanol precipitation. Then it was digested with *Hin*dIII, *Eco*RI, and *Bgl* I and fractionated by electrophoresis through a 14% acrylamide gel in 90 mM Tris/Borate, pH 8.3/2.5 mM EDTA. The radioactivity was visualized by autoradiography, and a densitometer tracing of the relevant portion of the autoradiogram is presented. The other portions of the autoradiogram were identical to each other and are not shown. Lane 1, reaction with substrate 3 containing an A·C mispair. Lane 2, reaction with substrate 5 containing a T·C mispair. Lane 3, reaction with mutant 1 RFI DNA containing an A·T base pair.

and Table 3). The results show that approximately equal incorporation was observed in the 27-bp fragment when it contained no mispair or the poorly repaired T·C mispair and that six times more incorporation was observed when the 27-bp fragment contained the efficiently repaired A·C mispair. Equal amounts of incorporation in the 149-bp fragment were observed with all three substrate DNAs. Incorporation into the 230-bp *Hin*dIII–*Nar* I fragment located counterclockwise from the 27-bp fragment (Fig. 1) was also measured. The results (Table 3) showed that equal amounts of incorporation into the 230-bp fragment was observed with all three substrates. Thus, the mismatch-correction-specific DNA synthesis did not appear to have extended clockwise past the *Eco*RI site or counterclockwise past the *Hin*dIII site. In experiments with substrates 1 and 2 (Fig. 2) mismatch-correction-specific DNA synthesis was confined to the 46-bp *Hin*dIII–*Eco*RI fragment that contained the mispair (data not shown). The data presented in Table 3 and Fig. 5 have been used to calculate that the approximate length of the A·C mismatch correction associated excision/resynthesis tract is on the order of 10–20 nucleotides.†

DISCUSSION

Our results indicate that the *S. cerevisiae* cell-free mitotic recombination system developed in this laboratory (32, 34) will catalyze mismatch correction. The repair reactions were efficient, with the specific activity for repair of the A·C mispair (2 pmol/mg of protein, Table 2) representing approximately 300 repair events per cellular equivalent of protein. Two lines of evidence indicate that these repair events were catalyzed by a specific mismatch-correction system. First, the different mispairs were corrected at different rates and with different degrees of strand bias indicating that mispairs are specifically recognized. Second, the occurrence of mismatch-repair-specific DNA synthesis indicates that mispaired nucleotides direct their own repair.

The *S. cerevisiae* mismatch-correction reaction described here is similar to mismatch correction in *S. cerevisiae* and

†[54 (nucleotides per fragment) × 0.5% (net synthesis) × 2 (dNTP dilution from Table 1) × 5 (synthesis above background from Fig. 5)]/0.2 (fraction of strands containing repair tract) = 13.5.

Table 3. Distribution of mismatch-correction-specific DNA synthesis

Experiment	Substrate	Base pair	Relative incorporation* 27 bp	149 bp	230 bp
1	3	A·C	146	70	ND
	5	T·C	29	94	ND
	No mispair†	A·T	24	100	ND
2	3	A·C	193	ND	102
	5	T·C	21	ND	108
	No mispair†	A·T	16	ND	100

Mismatch correction assays using the indicated substrate DNAs and subsequent product analysis were carried out essentially as described in Fig. 5. In experiment 1, the product DNA was digested with *Hin*dIII, *Eco*RI, and *Bgl* I to produce the 27-bp and 149-bp fragments; and in experiment 2, the product DNA was digested with *Hin*dIII, *Eco*RI, and *Nar* I to produce the 27-bp and 230-bp fragments (see Fig. 1). ND, not determined.

*The relevant peaks on the densitometer tracings were cut out and weighed. In experiment 1 the values presented are relative to the value obtained for the 149-bp fragment from the A·T-base-pair containing substrate. In experiment 2 the values presented are relative to the value obtained for the 230-bp fragment from the A·T-base-pair containing substrate.

†Mutant 1 RF DNA was used as substrate in this case.

other organisms in that transition mismatches (A·C and G·T) were repaired more efficiently than transversion mismatches (14, 15, 35–37). Asymmetry of repair was observed with some mispairs, although its cause was unclear. With the symmetric A·A, T·T, and C·C mispairs it could be due to an effect of neighboring nucleotides because the repair reaction always used the complementary strand as the template. In the case of the G·T mispair, some asymmetric feature of the mispair may be recognized.

S. cerevisiae mismatch correction *in vitro* differs from *E. coli* mismatch correction *in vitro*. In the *E. coli* system the mismatch-correction-specific excision/resynthesis tracts are several thousand nucleotides long and appear to be initiated away from the site of the mispair (38). In contrast, the excision/resynthesis tracts associated with *S. cerevisiae* mismatch correction *in vitro* are 10–20 nucleotides long and are likely to be initiated near the mispair. This suggests that the mechanisms of these two reactions could be quite different. While the mechanism of mismatch correction in *S. cerevisiae* is unclear at present the *in vitro* system described here should provide an assay for use in purifying the proteins that catalyze this reaction and make it possible to elucidate the mechanism of the reaction.

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