A general repair process for DNA heteroduplexes has been detected in HeLa cell extracts. Using a variety of M13mp2 DNA substrates containing single-base mismatches and extra nucleotides, extensive repair is observed after incubation with HeLa cell cytoplasmic extracts and subsequent transfection of bacterial cells with the treated DNA. Most, but not all, mismatches are removed as well as two frameshift heteroduplexes are repaired efficiently. Parallel measurements of repair in HeLa extracts and in Escherichia coli suggest that repair specificities are similar for the two systems. The presence of a nick in the molecule is required for efficient repair in HeLa cell extracts, and the strand containing the nick is the predominantly repaired strand. Mismatch-dependent DNA synthesis is observed when radiolabeled restriction fragments, produced by reaction of the extract with heteroduplex and homoduplex molecules, are compared. Specific labeling of fragments, representing a region of approximately 1,000 base pairs and containing the nick and the mismatch, is detected for the heteroduplex substrate but not the homoduplex. The repair reaction is complete after 20 min and requires added Mg²⁺, ATP, and an ATP-regenerating system, but not dNTPs, which are present at sufficient levels in the extract. An inhibitor of DNA polymerase β, dideoxythimidine 5'-triphosphate, does not inhibit mismatch-specific DNA synthesis. Aphidicolin, an inhibitor of DNA polymerases α, δ, and ε, inhibits both semiconservative replication and repair synthesis in the extract. Butylphenyl-dGTP also inhibits both replicative and repair synthesis but at a concentration known to inhibit DNA polymerase α preferentially rather than δ or ε. This suggests that DNA polymerase α may function in mismatch repair.

Base pair mismatches arise continually in the DNA of an organism as a result of several mechanisms, including errors during replication or repair synthesis, chemical modification of bases, and recombination between homologous but nonidentical DNA sequences. The repair of such mispaired bases is essential for reducing the frequency of mutations and maintaining the integrity of the genome. Bacterial cells have evolved several distinct and well-characterized repair mechanisms to deal with mismatches (for reviews, see Refs. 1-4). In Escherichia coli, a general repair system referred to as the mutHLS, long patch, or methyl-directed mismatch repair system corrects most replication errors with efficiencies reflecting the frequency and specificity of mispair formation (5-8). More specific pathways exist which repair mismatches inefficiently repaired by the methyl-directed system (9-12) or generated by chemical modification of bases such as deamination of 5-methylcytosine to form T·G mispairs (6, 13, 14). This latter form of repair is also referred to as very short patch repair. In Streptococcus pneumoniae, a general repair pathway exists, requiring the hexA and hexB genes, which is similar to the mutHLS system but appears to use single-strand breaks as the strand recognition signal (4).

Mismatch repair in eukaryotic organisms is less well understood. In the yeast Saccharomyces cerevisiae, mismatch repair has been demonstrated in cell-free extracts (15). The PMS1 gene, whose product has been shown recently to be homologous to the bacterial MutL and HexB proteins (16), as well as the PMS2 and PMS3 genes are required for efficient repair of a number of mispairs (17-19).

In higher systems, the lack of genetic studies has slowed the progress in elucidating mismatch repair pathways. Evidence for mismatch repair in mammalian systems, based on transfection of cells with heteroduplex-containing viral or plasmid vectors, has existed for several years (20-22). A similar study demonstrated heterogeneity of mismatch repair in simian cells for a wide variety of mispairs, suggesting that a general mismatch repair pathway may exist (23). A specific repair pathway was discovered in simian cells whereby G·T mispairs were preferentially repaired to G·C (24, 25), a system analogous to the very short patch repair pathway in E. coli. This system has also been observed in human cell extracts (26). A protein has been partially purified which binds to G·T mispairs and may serve a role in initial recognition of the mismatch (27). A mismatch-specific thymine DNA glycosylase activity has been detected which removes the thymine base, and after removal of the baseless sugar phosphate, the one-nucleotide gap may be filled in by DNA polymerase β (28).

Other reports using extracts to demonstrate mismatch repair indicated that A·C and G·T mispairs are corrected in human (29) and Xenopus systems (30), suggesting a more general system operating in these cells. In the Xenopus system, mismatch-specific DNA synthesis occurred in the region of the mismatch, providing strong evidence that repair is not the result of nonspecific events such as recombination or mismatch-independent nick translation.

More recently, using nuclear extracts of both human and Drosophila melanogaster cell lines, the first evidence was provided for a general mismatch repair system in eukaryotes which is capable of strand discrimination by utilizing a strand break to direct repair of the strand containing the nick (31). In this study, mismatch-specific DNA synthesis was also detected and localized in the region of the mismatch and strand break.

Recent studies in our laboratory have focused on measuring...
the fidelity and error specificity of DNA replication in cytologic extracts of human HeLa cells using an SV40 origin-dependent replication system (32). The observed error rates for both base substitutions and frameshifts are significantly lower than those observed for the purified replicative polymerases. One possible explanation for the lower error rates of DNA replication observed in extracts and the even lower estimated spontaneous mutation rates in vivo (33) is the action of postreplication repair processes such as mismatch repair. Our interest has been to determine whether mismatch repair is operating in these extracts and, if so, to elucidate the efficiency and specificity of this repair to assess its influence on replication errors produced by these same extracts.

We have therefore constructed heteroduplex substrates containing single-base mispairs at various sites in the target DNA used in our replication studies, the lacZα gene in bacteriophage M13mp2. In the present study, we report a versatile and efficient mismatch repair activity operating in HeLa cell extracts.

**EXPERIMENTAL PROCEDURES**

**Materials**—E. coli strains NR9099, MC1061, NR9162, and CSH50 and bacteriophage M13mp2 have been described (32, 34, 35). Mutant M13mp2 derivatives were from our collection (36, 37). Restriction enzymes and DNA ligase were obtained from New England Biolabs or International Biotechnologies, Inc. HeLa cell cytoplasmic extracts were prepared by the method of Li and Kelly (38). [α-32P]dCTP was purchased from Amersham Corp. N2-(p-n-butylphenyl)dGTP (Bu-PdGTP) was a generous gift of Dr. George Wright, University of Massachusetts Medical School.

**Preparation of Heteroduplexes**—M13mp2 single-stranded viral (+) DNA and RF I DNA were propagated in E. coli strain NR9099 and purified as described (34). Double-stranded M13mp2 DNA molecules containing defined mispairs at known positions and a nick in the minus strand at the unique AulII site (position -264, where position 1 is the first transcribed base of the lacZα gene) were prepared essentially as described (39). The (+) strand of the heteroduplex is derived from viral DNA whereas the (-) strand represents the (-) strand of RF DNA. After the hybridization step, the heteroduplex RF II DNA was purified on a 1% agarose gel followed by electrophoresis, dialysis, and etanol precipitation, with the final sample resuspended in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. For preparation of heteroduplexes with the nick sealed, 100 ng of purified nicked heteroduplex was treated with 0.2 unit of DNA ligase for 0 hr at 37 °C. Aliquots were analyzed on agarose gels in the presence of ethidium bromide. More than 80% of the DNA was converted to closed circular DNA.

**Mismatch Repair in HeLa Extracts**—The standard mismatch repair reaction (25 μl) contained 30 mM Hepes (pH 7.8); 7 mM MgCl₂; 200 μM each of ATP, GTP, and UTP; 100 μM each of dATP, dGTP, dTTP, and [α-32P]dCTP (4,000 cpm/pmol); 40 mM creatine phosphate; 100 μM/ml creatine phosphokinase; 15 mM sodium phosphate (pH 7.5); 5 ng (1 fmol) of purified heteroduplex DNA; and 75 μg of protein of HeLa cytoplasmic extract. After incubation at 37 °C for 1 hr, the reaction was terminated by the addition of proteinase K and sodium dodecyl sulfate and further incubation for 30 min. The sample was precipitated with isopropanol alcohol, extracted twice with phenol, then with diethyl ether, resuspended in 50 μl of H₂O. Transfection of E. coli strain NR9162 with 1 μl of sample was carried out using a Bio-Rad Gene Pulser electroporation system. Plating was performed as described (35, 40), and plaques were scored as colorless, blue, or mixed (blue/white) bursts. Repair efficiency is expressed in percent as 100 × (1 minus the ratio of the percentages of mixed bursts obtained from extract-treated and untreated samples).

**Analysis of Reaction Products of Mismatch Repair**—Reactions were performed as described above except that all dNTPs were omitted from the reaction mix except for [α-32P]dCTP; incubation was reduced to 20 min, and the reaction mix volume was increased to 100 μl. The reaction products were prepared as for transfection and then digested by HinII restriction endonuclease. The products were resolved on a 5% native acrylamide gel, which was then dried and exposed for autoradiography. HinII fragments were excised from the dried gel and their radioactivity determined by liquid scintillation counting. The net cpm for each fragment were obtained by subtracting the cpm of a background blank strip excised near the top of the gel in each lane from the total cpm of each fragment in that lane. The specific activity of each fragment was calculated by dividing the net cpm by the total dCMP residues in the fragment.

**RESULTS**

**The Mismatch Repair Assay**—A flow diagram depicting the assay used for measuring mismatch repair is presented in Fig. 1. The assay (41) is based on the analysis of plaque color phenotypes resulting from the transfection of a mutS E. coli strain (NR9162, which is defective in methyl-directed mismatch repair) with purified M13mp2 heteroduplex DNAs that have been treated with human cell extracts. The heteroduplex substrates contain base mispairs in the E. coli lacZα gene, which is capable of α-complementation and yields blue plaque plaques on host indicator plates. The composition of the mispair is such that expression of one strand yields a light or dark blue plaque phenotype whereas the other strand contains a stop codon and leads to a white plaque. Upon transfection any un repaired molecules are potentially capable of forming mixed color plaques at a certain frequency as well as pure color plaques. For un repaired molecules the ratio of mixed and pure bursts are generally constant for a given mispair but may vary among different heteroduplexes. When repair occurs, the ratios change such that mixed bursts decrease, and one or both pure bursts will increase according to the extent of repair of either strand. If repair is directed primarily to one strand, the percentage of pure bursts representing the genotype of that strand will decrease while the other pure burst frequency will increase. With proper plating conditions, the analysis of plaque phenotype ratios can be achieved with greater than 95% accuracy (41). A listing of the various mispairs used in this study is shown in Table I.

**Detection and Measurement of Mismatch Repair in HeLa Cell Extracts**—Initial mismatch repair experiments involved incubating 100 ng of heteroduplex DNA substrates with HeLa cell extracts in order to mimic DNA concentrations used in replication reactions. Negligible mismatch repair was observed (32). We considered the possibility that this may have been due to the number of mismatched molecules used relative to the potentially low concentration of mismatch repair-specific factors in the extract. We therefore began the present...
Heteroduplex Repair in HeLa Cell Extracts

study by systematically varying the ratio of extract to DNA (data not shown). From this we determined conditions that in the present study provide clear evidence for mismatch repair. For example, results (Table II) obtained by adding 5 ng of purified heteroduplex DNA to reactions with the HeLa cell extract demonstrate that both the C-A and T-G mispairs at position 87 are repaired, as indicated by a greater than 50% reduction in mixed bursts resulting from incubation in a complete reaction (see below for reaction requirements). In contrast, negligible repair occurred with the T-C mispair in the identical sequence context (Table I). This suggests that the observed repair is indeed mismatch specific rather than merely nonspecific processing of a nicked DNA substrate by the extract (e.g. by nick translation).

To determine if the inefficiently repaired T-C mispair was also a poor substrate for methyl-directed mismatch repair in E. coli, this heteroduplex was used to transfected the mismatch repair-competent strain MC1061. This heteroduplex is repaired inefficiently in E. coli, especially when compared with repair of the C-A mispair or the T-G mispair in the same sequence context, either in the extract or in E. coli (Table II).

Specificity studies were extended to other mispairs and to two frameshift heteroduplexes containing an additional nucleotide in either the (+) or (−) strand. All were tested for repair efficiency in both the HeLa extract and E. coli and the results listed in descending order of repair efficiency in the extract (Table III). In general those mispairs repaired efficiently in E. coli are repaired efficiently in the HeLa extract, though at lower levels. The C-C mispair tested was not repaired in either system although the G-G at the same site (position 88) was repaired efficiently. This further supports the notion that repair is mismatch specific. Interestingly, C-T and T-C mispairs only 2 bases apart show considerable variation in repair efficiency in the extract although both are relatively poor substrates in E. coli. Both heteroduplexes containing an extra nucleotide in one strand are repaired by the extract. For all heteroduplexes, repair predominated in the nick-containing (−) strand (seen by the relative levels of the pure burst phenotypes) to preserve the (+) strand sequence.

Reaction Requirements for Mismatch Repair—Mismatch repair requires the extract, Mg²⁺, and ATP since omission of any of these results in less than 1% of the activity obtained using the complete reaction mixture containing DNA with an A-C mispair at position 148 (data not shown). Even though the standard reaction contains 4 mM ATP, an ATP-regenerating system is also required since its omission likewise abolishes repair. Repair is efficient in the absence of added dNTPs, with 80% of the activity remaining relative to the complete reaction. The repair of A-C and G-T mispairs, both at position 148, was monitored over a 2-h time course, and the maximal repair for both was achieved in 20 min (data not shown).

Mismatch Repair Requires a Nick and Is Directed toward the Nicked Strand—The percentage of pure burst phenotypes

*Position 1 is the first transcribed base of the lacZa gene.

The lower strand of each duplex is the viral (−) strand.

The lower strand of each duplex is the viral (−) strand.

TABLE I

| Position of mismatch | Mismatch with flanking sequence | Color of strands | E. coli, Me reaction requirements |
|---------------------|--------------------------------|-----------------|----------------------------------|
| 87                  | CTG A CTG 5'-GAC C GAG-3'       | W               |                                 |
| 87                  | CTG G CTC 5'-GAC T GAG-3'       | W               |                                 |
| 87                  | CTG C CTC 5'-GAC T GAG-3'       | W               |                                 |
| 88                  | TGA G TCT 5'-ACT G AGA-3'       | W               |                                 |
| 88                  | TGA C TCT 5'-ACT C AGA-3'       | W               |                                 |
| 89                  | GAC T CTT 5'-CTG G GAA-3'       | W               |                                 |
| 89                  | GAC C CTT 5'-CTG A GAA-3'       | W               |                                 |
| 89                  | GAC T CTT 5'-CTG C GAA-3'       | W               |                                 |
| 103                 | CGC T ATG 5'-GCC G TAC-3'       | MB              |                                 |
| 148                 | CGA C CGC 5'-GCT A GAA-3'       | W               |                                 |
| 148                 | CGA T CGC 5'-GCT G GAA-3'       | W               |                                 |
| 70-73               | AGC AAAA TGT 5'-TGG TTT TAC-3' | W               |                                 |
| 70-73               | AGC AAAA TGT 5'-GCG CAC-3'      | W               |                                 |

TABLE II

Mismatch repair in HeLa cell extracts and in E. coli

The heteroduplexes used all contained the mispair at position 87. Each experiment represents the average of at least two determinations, and the total number of plaques/determination was 500 or more.

| Mispair (forward/reverse) | Mean of minus extract | Mean of plus extract | Repair (%) |
|---------------------------|-----------------------|----------------------|------------|
|                          | (+) Strand            | (-) Strand          | Mixed      | (+) Strand | (-) Strand | Mixed      |
|                           | % of plaques          | %                   |            | %          | %          |
| Repair in HeLa cell extracts |                       |                     |            |            |            |
| C-A                      | 17                    | 44                   | 30         | 64         | 20         | 16         |
| T-G                      | 5                     | 48                   | 47         | 37         | 41         | 22         |
| T-C                      | 5                     | 50                   | 45         | 7          | 49         | 44         |
| Repair in E. coli        |                       |                     |            |            |            |
| C-A                      | 16                    | 75                   | 9          |            |            |            |
| T-G                      | 23                    | 68                   | 9          |            |            |            |
| T-C                      | 6                     | 61                   | 33         |            |            |            |

*The repair efficiency for heteroduplexes transfected into E. coli strain MC1061 was calculated as for the HeLa extracts using the "minus extract" mixed burst percentage obtained by transfection of NBS162 as the control.

Transfection of E. coli strain NR9162 (mutS).

Transfection of E. coli strain MC1061 (wild type).
in Table II provides evidence that the strand discrimination signal is different for mismatch repair of these heteroduplexes in the HeLa cell extract versus in E. coli. As a result of repair in the extract, the percentage of pure bursts having the phenotype of the (+) strand increases from 17 to 64% for the C·A mispair and from 5 to 37% for the T·G mispair (Table II). We interpret this to reflect repair directed by the phosphodiester bond interruption (nick) in the (−) strand of the heteroduplex. In contrast, repair in E. coli is in the opposite direction, as indicated by the increase in the percentage of pure bursts having the phenotype of the (−) strand (from 44 to 75%) for the C·A mispair, Table II. This is consistent with methyl-directed mismatch repair of an undermethylated strand, which for these artificially constructed heteroduplexes is the (+) strand. (As shown by Lu et al. (42), M13 viral DNA obtained from a dam+ strain is known to be undermethylated relative to RF DNA. The (+) strand of the heteroduplexes used in these studies is derived from viral DNA whereas the (−) strand is derived from RF DNA.)

To examine the role of the nick in defining directionality and to determine if the nick is required for efficient repair in this HeLa cell extract, covalently closed heteroduplexes were prepared by incubation of purified, nicked heteroduplexes with DNA ligase. As seen in Table IV, a heteroduplex containing a C·A mispair at position 148 was repaired when a nick was present at position −264 (the site of incision by AτII, used to construct the substrate). When the nick in these molecules was sealed, the repair efficiency was reduced almost 10-fold. A similar result was obtained when this experiment was repeated with a heteroduplex containing a T·G mispair at position 103 (Table IV). The low level of repair which still remains with both substrates may reflect the fact that a small portion of the DNA was not sealed and would remain susceptible to repair. Alternatively, the extract may contain factors capable of specifically recognizing covalently closed, mismatch-containing DNA and repair this to preserve a particular base pair, such as the pathway described for G·T to G·C repair (26, 28). There may also be a low level of nonspecific repair, perhaps caused by random nicking and synthesis.

Detection and Analysis of Mismatch Repair-specific DNA Synthesis—In order to analyze repair synthesis in HeLa cell extracts, reactions were performed without adding dNTPs except for [α-32P]dCTP. Four reactions were performed, containing either a nicked homoduplex or one of the three heteroduplexes whose repair was described in Table II (i.e., a C·A, T·G, and T·C at position 87). When aliquots from each reaction were used in transfection assays (without prior digestion), the results confirmed the repair efficiencies found previously. When a small aliquot of each of the four reactions was analyzed by incorporation of radioactivity into an acid-insoluble form, a direct correlation was noted between the amount of incorporation and the efficiency of repair (Table V).

In order to analyze the reaction products, the samples were purified and digested with the restriction endonuclease HinfI, which cleaves M13mp2 26 times (Fig. 2A). The resulting fragments were separated on a nondenaturing 5% polyacrylamide gel to give the autoradiograph shown in Fig. 2B. Most fragments are labeled to some degree in all four reactions. However, it is readily apparent that several bands are labeled preferentially in the heteroduplex substrates containing the A·C and the T·G mispairs. These include the 529-, 261-, 253-, and 137-base pair fragments. The corresponding fragments for the DNA substrates containing either the T·C mispair or no mispair (the homoduplex) were considerably lighter.

The fragments were excised from the gel and their specific activity determined as described under "Experimental Procedures." The 413-bp fragment, located on the opposite side of the M13mp2 map (Fig. 2A) and labeled to about the same extent in all reactions, was chosen as a control band. The specific activity of each band of interest was normalized to this fragment and plotted as a bar graph. The results (Fig. 2C) show preferential repair synthesis for the substrates containing mismatches relative to the homoduplex substrate. Thus, synthesis is mismatch dependent. Furthermore, the extent of preferential labeling correlates with the efficiency of repair; for both repair and preferential labeling, the order is C·A ≈ T·G > T·C. Preferential labeling occurs in the region from the nick-containing fragment (253 bp) to at least the 137-bp fragment, a distance of about 1,000 bp. The highest specific activity is for the fragment containing the nick, with the second highest value observed for the mismatch-containing fragment.

**Effect of DNA Polymerase Inhibitors on DNA Replication**

**TABLE III**

| Specificity of heteroduplex repair | The total number of plaques/determination was 500 or more. |
|-----------------------------------|----------------------------------------------------------|
| Content of heteroduplexes | Repair |
| (++)(−) | HeLa extract | E. coli |
| G·T | 89 | 70 | 78 |
| C·A | 87 | 59 | 77 |
| G·G | 88 | 87 | 83 |
| A·C | 54 | 57 | 83 |
| T·G | 87 | 53 | 81 |
| C·T | 47 | 47 | 81 |
| —·A | 70–73 | 46 | 72 |
| T·— | 70–73 | 34 | 73 |
| T·C | 87 | 2 | 97 |
| C·C | 88 | ≤1 | ≤1 |

**TABLE IV**

| Mismatch repair with nicked versus covalently closed substrates | The average of two determinations is shown. Total number of plaques/determination was more than 500 for all determinations. |
|-----------------------------------|----------------------------------------------------------|
| Mispair (+)(−) | Minus extract | Plus extract |
| | (+) Strand | (−) Strand | Mixed | (+) Strand | (−) Strand | Mixed | % of plaques | % |
| A·C | 148 | | | | | | | |
| Nicked heteroduplex | 8 | 60 | 32 | 33 | 47 | 20 | 37 |
| Ligated heteroduplex | 9 | 66 | 25 | 20 | 56 | 24 | 4 |
| G·T | 103 | | | | | | | |
| Nicked heteroduplex | 14 | 53 | 33 | 54 | 26 | 20 | 39 |
| Ligated heteroduplex | 15 | 52 | 33 | 23 | 45 | 29 | 12 |
and Mismatch Repair Synthesis—Based on these results, at least one step in the process of mismatch repair involves DNA synthesis over a relatively large region surrounding the mispair. Initial insight into the mechanism of repair may be gained by elucidating the polymerase(s) responsible for this synthesis. We decided to test specific inhibitors of DNA polymerases in the mismatch repair reaction to determine if mismatch-specific DNA synthesis was modulated. The effect of these inhibitors on DNA replication in the same HeLa extract was tested in parallel. Since one or more polymerases could potentially serve a role in both repair and replication, it was of interest to determine whether the polymerase inhibitors had similar effects on the two processes. As shown in Table VI, ddTTP, an inhibitor of DNA polymerase $\beta$ (43), had minimal effect on repair synthesis or replication as measured by incorporation of radioactivity into an acid-insoluble form. When aliquots of the mismatch repair reactions were digested with the restriction endonuclease HinfI and XmnI and run on a 5% acrylamide gel to analyze the products further (Fig. 3), the intensity of the mismatch-containing (529-bp) and nick-containing (253-bp) fragments relative to the intensity of the 413-bp fragment was not diminished by the addition of ddTTP (Table VI). The results suggest that DNA polymerase $\beta$ is not responsible for the majority of the mismatch-specific repair synthesis.

Aphidicolin, an inhibitor of replicative DNA polymerases (44, 45), abolished both repair and replicative synthesis (Table VI and Fig. 3). Consistent with results obtained by Holmes et al. (31), this suggests the involvement of either DNA polymerase $\alpha$, $\delta$, and/or $\epsilon$ in mismatch repair (based on both biochemical and genetic data), the $\delta$ class of eukaryotic DNA polymerases has been divided recently into two classes, $\delta$ and $\epsilon$ (46)). In an attempt to distinguish among these possibilities, we used a specific inhibitor of DNA polymerase $\alpha$, BuPdGTP (47, 48). Consistent with previous results in permeabilized human cells (49, 50), this compound inhibits replication by about 50% at 25 $\mu$M, as seen in Fig. 4. Repair synthesis is more sensitive to the presence of BuPdGTP, being reduced by 75% at 10 $\mu$M. With either 10 or 25 $\mu$M BuPdGTP in the repair reactions, the relative specific activity of the mismatch- and nick-containing fragments diminish from 2.5 to values less than 1 (Figs. 3 and 4). Thus, at these concentrations, the remaining labeled material represents nonspecific incorporation, and essentially all mismatch-specific synthesis is inhibited. As indicated in Fig. 4, these concentrations inhibit HeLa DNA polymerase $\alpha$ very effectively but are only marginally inhibitory for DNA polymerase $\delta$ and $\epsilon$ (51). These results suggest that mismatch repair synthesis may be performed by DNA polymerase $\alpha$.

![Fig. 2. Detection and analysis of mismatch repair-specific DNA synthesis in a HeLa cell extract.](image-url)

**TABLE V**

| DNA substrate | dCMP incorporated$^a$ | Repair$^b$ |
|---------------|-----------------------|-----------|
| C-A           | 1995                  | 55%       |
| T-G           | 1332                  | 45%       |
| T-C           | 638                   | 6%        |
| Homoduplex    | 743                   |           |

$^a$ The results shown were obtained by subtracting a background of 50 cpm, obtained from an unincubated control reaction. The actual dNTP concentration in the reaction is not known since except for [a-32P]dCTP, the dNTPs were supplied by the extract (see "Results" and Table IV). Thus, the specific activity of the dCTP is not known, and the results are expressed simply as cpm for an equivalent amount of each reaction.

$^b$ Results were obtained as shown for the data in Table II.
**Homoduplex Repair in HeLa Cell Extracts**

**Heteroduplex Repair in HeLa Cell Extracts**

Incorporation is expressed as the percent of the value obtained with no inhibitor added, which was 6,830 cpm (152 pmol of nucleotide in the entire 25 μl reaction) for replication and 10,971 cpm for repair.

| Inhibitor   | Concentration | Incorporation Replication | Incorporation Repair | Relative specific activity* |
|-------------|---------------|---------------------------|----------------------|---------------------------|
| None        |               | 100                       | 100                  | 25/413                    |
| ddTTP       | 500 μM        | 86                        | 89                   | 3.2/413                   |
| Aphidicolin | 50 μg/ml      | 3                         | 3                    | 2.8/413                   |

* Reaction conditions were the same as described in Ref. 32 with additions as indicated.

**DISCUSSION**

We have presented the initial characterization of a general mismatch repair system in an extract of human HeLa cells which has been shown previously also to be replication competent (32). This mismatch repair activity is most active with heteroduplex molecules containing a nick (Table IV), and repair is directed to the strand containing the nick (Table II). Repair is mismatch specific (Tables II and III), and repair synthesis is mismatch dependent (Table V and Fig. 3C). The repair patch is approximately 1,000 bp when measured with substrates containing a nick 350 base pairs to the left of the mismatches.

Overall, the repair system we have described is somewhat similar to the methyl-directed repair system in E. coli, with one obvious difference being the signal for strand discrimination. Both systems show a broad specificity and are similarly efficient or inefficient in repair of some of the same mismatches in the same sequence context (Table III). The repair patch sizes are also similar in both systems. The system we describe here is also very similar to that reported recently by Holmes et al. (31) and substantiates their finding of a strand-specific mismatch correction pathway operating in human cells. In both systems, mismatch-dependent repair synthesis was demonstrated. In this study, dNTPs could be omitted, presumably because sufficient levels of dNTPs are present in the extract. It is noteworthy that these two studies employed very different assays for measuring mismatch repair but led essentially to very similar observations. It is also interesting that the overall repair efficiencies of the two systems are similar, considering the contrasting sources of extract, nuclear in the previous report (31) and cytoplasmic in this work. This suggests that all of the necessary factors can potentially be labeled.

In panel B, after autoradiography, fragments were excised and their specific activity determined as described under "Experimental Procedures." The specific activity of each fragment was normalized to the 413-bp fragment chosen as a background band. Those fragments that were clearly resolved on the gel are displayed on the bar graph, including the nick-containing 253-bp fragment (NICK) and the mismatch-containing 529-bp fragment (MM) as well as three unresolved doublets in which at least one of the fragments in the doublet is within 1,000 bp of the mismatch. The 261- and 274-bp fragments could not be resolved for accurate determination of specific activity and are not included in the graph. However, it is apparent from the autoradiograph that between these two fragments, which have similar dCMP content, the 261-bp fragment, which is adjacent to the mismatch-containing fragment, is labeled to a higher specific activity. The identity of these fragments was confirmed by digestion with the restriction endonuclease XmnI, which incises the 274-bp fragment. Closed bars, C-A mispair; diagonal line bars, T-G mispair; stippled bars, T-C mispair; open bars, homoduplex.

**FIG. 3. Effect of DNA polymerase inhibitors on mismatch repair-specific DNA synthesis in a HeLa cell extract.** Reactions to analyze mismatch repair synthesis were performed as described under "Experimental Procedures" with the addition of inhibitors as indicated. dTTP was present at 500 μM and aphidicolin at 50 μg/ml. The heteroduplex used contained a G-G mispair at position 88. An aliquot of each sample (adjusted so that equal cpm were processed for each sample except for aphidicolin, for which the entire sample was used) was digested with HindIII plus XmnI and resolved on a 5% acrylamide gel, which was then dried and exposed for autoradiography. The 529- and 253-bp fragments containing the mismatch (MM) and nick, respectively, are indicated, as is the 413-bp fragment used as a control band. The 261-bp fragment is also indicated and is noted because with the two-enzyme digest this fragment is resolved from the 274-bp fragment observed in Fig. 2B. The 261-bp fragment is between the mismatch fragment and 137-bp fragment on the M13mp2 map (Fig. 2A) and likewise would be expected to show preferential labeling if the repair patch extends beyond to the 137-bp fragment. It is apparent from this figure that the 261-bp fragment is labeled specifically in the positive control reaction, especially when compared with the 413-bp control band, and decreases in intensity with increasing BuPdGTP. The origin of the bands at the top of the gel in the lanes containing the BuPdGTP-treated samples is unknown. Because larger volumes of sample were loaded in the 10 and 25 μM BuPdGTP lanes to adjust for lower overall incorporation, these high molecular weight bands are more intense in these lanes.
fractionated using either extract. We chose the cytoplasmic extracts for our mismatch repair studies to follow closely reaction conditions used in our in vitro replication system (52) and thus assess the contribution mismatch repair may make to the overall fidelity of DNA synthesis by the replication system.

Although the enzymology of heteroduplex repair in the HeLa system remains to be determined, the requirement for large amounts of ATP suggests that a helicase may also be involved. In a similar study but with HeLa cell nuclear extracts, relatively high concentrations of ATP were also required (31), but unlike our studies, an ATP-regenerating system was not required. In contrast, neither the yeast system (15) nor the Xenopus system (30) for mismatch repair requires a large amount of ATP. In these latter systems, repair patch size is also smaller than in HeLa cell extracts; for yeast it is apparently less than 20 nucleotides, and for the Xenopus system it is around 150 bp. For the yeast and Xenopus systems, the nature of the strand discrimination signal has not yet been described.

The system we describe here is clearly different from that reported by Wiebauer and Jiricny (26, 28), which involves specific repair of G·T to G·C base pairs in nuclear extracts of HeLa cells. It is quite possible that in the present assay the nick overrides any other recognition factor and masks the specific repair pathway. It is also possible that factors involved in the specific repair system are not present or only in reduced quantities in our extracts. More information will be needed to assess adequately the similarities and/or differences among the several eukaryotic mismatch repair systems that have now been described.

The ability to measure both mismatch repair and repair synthesis provides two assays with which to purify factors involved in mismatch repair and possibly to reconstitute such a system, as has been accomplished in E. coli (52). The mismatch repair–competent HeLa cell extract used in this study has been fractionated previously for reconstitution of a replication system from purified components (53). It is likely that some of the same proteins involved in replication are also involved in mismatch repair, such as DNA polymerases, DNA-binding proteins and helicases. If this repair system has a similar function in preventing fixation of replication errors like the E. coli system, then replication and repair may well be coupled such that nicks or chain termini on the newly replicated strand are recognized and utilized efficiently as strand discrimination signals before they are ligated. An initial effort toward identifying the DNA polymerases required for repair is the utilization of DNA polymerase inhibitors. It is clear that ddTTP, an inhibitor of DNA polymerase β, has no effect on repair or repair synthesis in the HeLa system (Ref. 31, Table VI, and Fig. 3). The inhibition of repair and repair synthesis by aphidicolin (Ref. 31, Table VI, and Fig. 3) implicates one or more of the replicative polymerases as serving a role in repair. The results obtained in Table VI and Figs. 3 and 4 suggest that DNA polymerase α is at least partly responsible for the repair synthesis provoked by DNA mismatches in HeLa extracts. By identifying the polymerase(s) involved in repair, any known accessory proteins for that polymerase become logical candidates for mismatch repair.

Another approach is the search for mismatch repair proteins with properties analogous to E. coli counterparts. Three candidates for the mammalian equivalent of the mismatch-binding MutS protein (54) have already been identified (55–57).

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Heteroduplex Repair in HeLa Cell Extracts

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