MOLECULAR AND CELLULAR BIOLOGY, Jan. 1987, p. 218–224
0270-7306/87/010218-07$02.00/0

Vol. 7, No. 1

DNA Mismatch Repair Detected in Human Cell Extracts

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A system to study mismatch repair in vitro in HeLa cell extracts was developed. Preformed heteroduplex plasmid DNA containing two single base pair mismatches within the *SupF* gene of *Escherichia coli* was used as a substrate in a mismatch repair assay. Repair of one or both of the mismatches to the wild-type sequence was measured by transformation of a *lac*(Am) *E. coli* strain in which the presence of an active *supF* gene could be scored. The *E. coli* strain used was constructed to carry mutations in genes associated with mismatch repair and recombination (*mutH*, *mutU*, and *recA*) so that the processing of the heteroduplex DNA by the bacterium was minimal. Extract reactions were carried out by the incubation of the heteroduplex plasmid DNA in the HeLa cell extracts to which ATP, creatine phosphate, creatine kinase, deoxynucleotides, and a magnesium-containing buffer were added. Under these conditions about 1% of the mismatches were repaired. In the absence of added energy sources or deoxynucleotides, the activity in the extracts was significantly reduced. The addition of either aphidicolin or dideoxynucleotides reduced the mismatch repair activity, but only aphidicolin was effective in blocking DNA polymerization in the extracts. It is concluded that mismatch repair in these extracts is an energy-requiring process that is dependent on an adequate deoxynucleotide concentration. The results also indicate that the process is associated with some type of DNA polymerization, but the different effects of aphidicolin and dideoxynucleotides suggest that the mismatch repair activity in the extracts cannot simply be accounted for by random nick-translation activity alone.

The repair of base pair mismatches in the DNA of an organism plays an important role in reducing the frequency of mutations and in preserving the genetic integrity of the organism. Mismatches can occur in several ways. Recombination events can generate heteroduplex regions in DNA, and the process of gene conversion is thought to involve heteroduplex structures as intermediates. In DNA replication errors can occur which produce mismatched bases that must be corrected to avoid a high rate of spontaneous mutagenesis.

In *Escherichia coli* DNA mismatch repair has been studied extensively. Transfection experiments with heteroduplex bacteriophage DNA (for a review, see reference 11) have demonstrated that *E. coli* has an efficient system for mismatch repair. The power of procaryotic genetic analysis has allowed identification of mutants that are deficient in various aspects of the repair process, and this has led to an understanding of some of the mechanisms that are involved. The products of the *mutH*, *mutL*, *mutS*, and *mutU* loci all seem to play a role in mismatch repair. Mutations at these loci produce strains that undergo a high rate of spontaneous mutagenesis because they cannot repair DNA mismatches effectively (11). Experiments involving the transfection of *E. coli* with hemimethylated λ DNA coupled with the identification of mutants with altered function in the methylase encoded by the *dam* gene have led to a model of methyl-directed strand selection in *E. coli* mismatch repair (5, 8, 14, 17). The subsequent development of a system to detect mismatch repair in cell extracts of *E. coli* has greatly facilitated the study of the enzymology of this process (12).

In contrast, much less is known about the mechanisms of heteroduplex repair in mammalian cells. Microinjection experiments have demonstrated that mouse cells can efficiently correct mismatched bases in exogenously prepared heteroduplex DNA (4). Studies involving the transfection of monkey cells with hemimethylated simian virus 40 (SV40) DNA have suggested that strand selection in mismatch repair in these cells may be influenced by methylation patterns (7).

Extension of these findings by the sort of genetic analysis used with *E. coli*, however, is hampered by the difficulty of genetic manipulation of mammalian cells. As another approach, we sought to develop an assay to detect DNA mismatch repair in cell extracts of mammalian cells as a way to study the mechanisms of repair directly in vitro. Such an approach to the study of other aspects of mammalian DNA and RNA metabolism has already been described in several studies (2, 9, 10, 13, 23). We report here our work with extracts from HeLa cells in which we were able to detect repair of DNA mismatches in specifically constructed substrate heteroduplex DNA. Results of preliminary experiments indicate that this activity is dependent on ATP, deoxynucleotides, and DNA polymerization.

MATERIALS AND METHODS

Cells. HeLa cells were obtained from P. Ghosh, Yale University (New Haven, Conn.). The construction of *E. coli* SY204 *lacZ125*(Am) *trp-49*, *hsdR2*::Tn*10* has been described previously (20). *E. coli* EG826 *lacZ125*(Am) *trp-49 hsdR2*::Tn*10 ssb-1 malE*::Tn*10* was made by Efim Golub by P1 transduction of the *ssb-1* mutation (15) into SY204. SY208 *lacZ125*(Am) *hsdR2*::Tn*10 mutH3 mutU4 strA143* was constructed from E. coli KL874 F⁻ *hisF818 leu3 lacZ498*, *rpsL143 mutH3 mutU4* by first introducing a deletion spanning the *lac* and *pro* loci by conjugation and then introducing the *lacZ125*(Am) mutation by a second conjugation, followed by introduction of the host restriction mutation *hsdR2* by P1 transduction with Tn*10* tetracycline resistance. SY209 was constructed by P1 transduction of the *recA56* mutation from *E. coli* MC16 *argH trpA36 srl-300*::Tn*10 recA56* into SY208 by contransduction of Tn*10* as a screen after first

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curing SY208 of tetracycline resistance. SY302 was constructed by P1 transduction of the *recA56* mutation, again from *E. coli* MC136, into SY204 by cotransduction of Tn*10* as a screen after first curing SY204 of tetracycline resistance.

Plasmids. Plasmid p3AC was constructed as described previously (20). Plasmids p3AC-4 and p3AC-8 differ from p3AC only in that they bear single point mutations in the amber suppressor tyrosine tRNA gene of *E. coli*, *supF* (1), rendering this gene nonfunctional. Plasmids p3AC-4 and p3AC-8 were isolated in the course of a study of mutagenesis described previously (20). The *supF* genes in these plasmids were sequenced directly from the plasmid DNA by the method of Sanger et al. (19) with a pBR322 *Eco*RI site primer (New England Biolabs, Inc., Beverly, Mass.).

Heteroduplex preparation. For the preparation of heteroduplex molecules from p3AC-4 and p3AC-8, 25 μg of p3AC-4 linearized at the *Sca*I site and p3AC-8 linearized at the *Bam*HI site were mixed in a total volume of 1 ml of water to which 110 μl of 1 N NaOH was added. After 30 min at room temperature, 110 μl of 1 M NaH_2PO_4 and 1,280 μl of deionized formamide were added. The solution was incubated overnight at 37°C, followed by overnight dialysis against 10 mM Tris and 1 mM EDTA (pH 8). The DNA in the sample was concentrated by ethanol precipitation and examined by agarose gel electrophoresis for successful generation of the nicked circular duplexes representing heteroduplex molecules, prior to use in the experiments described below.

Transformations. Bacterial transformations were done by the method of Hanahan (6). Bacteria transformed to ampicillin resistance were screened for β-galactosidase activity by growth in the presence of the chromogenic indicator, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) at 50 μg/ml and isopropyl β-D-thiogalactopyranoside at 20 μg/ml.

HeLa cell extracts. Suspension cultures of HeLa cells were grown in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum. Whole cell extracts, from 2 to 3 liters of culture containing 4.5×10^5 to 5×10^5 cells per ml, were prepared essentially as described by Manley et al. (13) with some minor modifications. Cellular material precipitated by 60% ammonium sulfate was collected and suspended in the prescribed volume of a buffer containing 50 mM Tris (pH 7.9), 6 mM $MgCl_2$, 0.2 mM EDTA, 40 mM $(NH_4)_2SO_4$, 15% glycerol, and 1 mM dithiothreitol. This solution was dialyzed for 15 h at 4°C against two 500-ml volumes of 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.9), 100 mM KCl, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 17% glycerol, and 1 mM dithiothreitol. Precipitated material was removed by centrifugation (10 min at 12,000 × *g* at 4°C), and the resulting supernatant was quick frozen in fractions and stored at −80°C. Protein concentrations of the extracts were 10 to 12 mg/ml. These extracts were also used for in vitro transcription reactions and were found to be active in this assay, producing predicted, specific, template-dependent products.

Reaction conditions. Reactions were carried out at 37°C for 2 h in a total volume of 25 μl containing 15 μl of extract (150 to 180 μg of protein), between 100 and 500 ng of DNA in 5 μl of water, and 5 μl of the appropriate buffer. The final concentrations of components in the complete reaction were 12 mM HEPES (pH 7.9); 60 mM NaCl; 60 mM KCl; 7.5 mM $MgCl_2$; 3 mM $MgSO_4$; 0.1 mM each of dGTP, dATP, dTTP, and dCTP; 0.9 mM ATP; 10 mM creatine phosphate; 10 μg of creatine kinase per ml; 0.2 mM dithiothreitol; and 10% glycerol. Reactions were terminated by the addition of 125 μl of 10 mM Tris (pH 8)–5 mM EDTA–0.1% sodium dodecyl sulfate–200 μg of proteinase K per ml. After 1 h at 37°C, protein was extracted from the samples with phenol-chloroform, and the DNA was precipitated with ethanol, redissolved, and used for bacterial transformations.

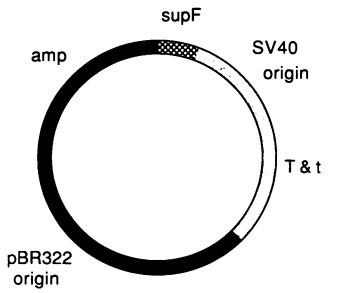

FIG. 1. Structure of the plasmid p3AC. This plasmid contains a total of 6,128 base pairs. It was constructed by ligation of a 200-base-pair *Eco*RI fragment containing the *supF* gene from the plasmid πVX into the unique *Eco*RI site of the plasmid pBR322. In addition, the 622-base-pair *Hae*II B fragment of pBR322 was deleted, and a fragment containing the *Bam*HI to *Hpa*II early region of SV40 (2,187 base pairs) was inserted at the *Cla*I site which is present in pBR322.

DNA polymerase assays. Extract reactions were carried out as described above, except that unlabeled dCTP was omitted and [α-^{32}P]dCTP was added to all reactions at a concentration of 20 μM and a specific activity of 3,000 Ci/mmol. The buffer was adjusted according to the desired experimental conditions. Just prior to the phenol extraction step, fractions of each sample either were spotted onto filter disks for measurement of radioactivity incorporated into trichloroacetic acid-insoluble material or were subjected to agarose gel electrophoresis and autoradiography.

RESULTS

Experimental design. The study of mismatch repair in mammalian cell extracts depends on a method of detecting and measuring such repair. We chose to develop a biological assay that would exploit the power of *E. coli* genetics. This entailed the generation of mutations in a gene which has a discernible phenotype in *E. coli* and the use of mutant genes to prepare heteroduplex DNA. This heteroduplex DNA was incubated in mammalian cell extracts, and the DNA was recovered from the extracts and used to transform a suitable strain of *E. coli*. Analysis of the phenotypes of the transformed *E. coli* allowed detection of heteroduplex repair. A crucial aspect of this assay was the construction of a strain of *E. coli* which was deficient in the metabolism of the heteroduplex DNA. This was needed so that the processing of the heteroduplex DNA could be attributed to reactions that occurred in the mammalian cell extracts rather than to in vivo repair in the bacterial cells.

Heteroduplex preparations. As a basis for the heteroduplex assay, we used the plasmid p3AC (Fig. 1). This plasmid contains the pBR322 origin of replication and the ampicillin resistance gene, along with the simian virus 40 (SV40) replication origin and T-antigen gene. It also contains the *supF* gene, which is an amber suppressor, tyrosine tRNA gene of *E. coli*. When a plasmid bearing a functional *supF* gene is introduced into an *E. coli* strain that has an amber nonsense mutation in the β-galactosidase gene and the resulting bacterial colonies are grown in the presence of the chromogenic β-galactosidase indicator X-gal, the colonies

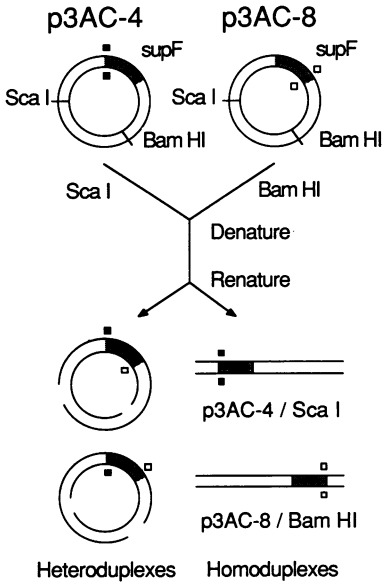

FIG. 2. Preparation of heteroduplex plasmid DNA. Plasmids p3AC-4 and p3AC-8, each bearing a single mutation in the *supF* gene as indicated by the open and closed boxes, were linearized with *Sca*I and *Bam*HI, respectively. The linearized plasmids were mixed, denatured, and renatured. In this process, circular molecules are formed which represent heteroduplexes containing two single base pair mismatches in the *supF* gene.

are blue. When the *supF* gene is absent or is nonfunctional, the colonies that are formed are white. We isolated derivatives of the plasmid p3AC in which single point mutations were introduced into the *supF* gene, eliminating its suppressor activity. The sequences of the mutant *supF* genes were determined, and two plasmids with mutant *supF* genes were chosen for this study. The mutation contained in the *supF* gene in plasmid p3AC-4 is a T to C transition, while that in p3AC-8 is a C to T transition at a site in the gene that is 61 base pairs away.

From p3AC-4 and p3AC-8 heteroduplex molecules each containing two single base pair mismatches within the *supF* gene were constructed. The scheme for construction of the heteroduplexes is shown in Fig. 2. The circular plasmids were separately converted to linear molecules by digestion with different restriction enzymes which cut each plasmid only at one site. The linear molecules were mixed, denatured, and allowed to renature. In the renaturation step, some strands from p3AC-4 annealed to strands from p3AC-8, because they were homologous except for 2 of about 6,200 base pairs. Because the plasmids were linearized at different sites, annealing between strands from the different plasmids yields linear molecules with large, complementary, single-stranded overhangs. These single-stranded regions can anneal among themselves, combining either with the complementary single strand on the same molecule or with one from another molecule, yielding nicked circular molecules or multimers, respectively. In contrast, reannealing of strands from the same plasmid regenerates the original linear molecules.

The results of the hetroduplex preparation from p3AC-4 and p3AC-8 are illustrated in Fig. 3, which shows an analysis by agarose gel electrophoresis of the steps in this process. Lanes 1 and 2 show the uncut plasmids, while the plasmids digested with their respective restriction enzymes are shown in lanes 3 and 4. As a control p3AC-8 was linearized with

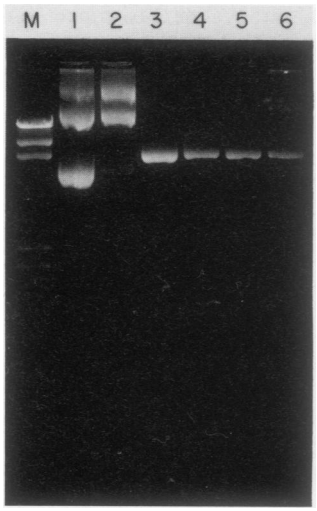

FIG. 3. Analysis by agarose gel electrophoresis of the preparation of heteroduplex plasmid DNA. M, λ-*Hin*dIII size markers; lane 1, p3AC-4; lane 2, p3AC-8; lane 3, p3AC-4–*Sca*I; lane 4, p3AC-8–*Bam*HI; lane 5, p3AC-8-*Bam*HI denatured and renatured by itself; lane 6, p3AC-4–*Sca*I and p3AC-8–*Bam*HI mixed, denatured, and renatured, as illustrated in Fig. 2.

*Bam*HI, denatured, and allowed to renature by itself in the absence of p3AC-4 (lane 5). The results of the heteroduplex preparation, in which the two linearized plasmids were denatured and renatured together, is presented in lane 6. The new band of reduced mobility (relative to the linear molecules) in lane 6 represents the nicked, circular heteroduplex molecules. The nicked, circular molecules are similar to plasmid p3AC, except for the presence of two single base pair mismatches within the *supF* gene. Because these molecules are formed by the combination of either of the two strands in p3AC-4 with its complement in p3AC-8, there are two possible heteroduplex molecules (Fig. 4), each with two mismatches, that are presumably formed in equal amounts. In either case note that both strands bear a base change that inactivates the *supF* gene. Semiconservative replication of the unrepaired heteroduplexes would yield the original mutant plasmids with defective *supF* genes. In the absence of postreplicative recombination, it is only by repair of one or both strands to the normal base prior to replication that a functional *supF* gene can be generated.

***E. coli* strains.** Our experimental goal was to use hetroduplex DNA bearing mismatches in the *supF* gene as a

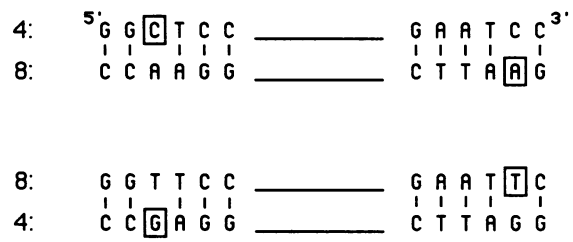

FIG. 4. Partial sequence of the heteroduplex plasmids made from p3AC-4 (designated 4) and p3AC-8 (designated 8) showing the base pair mismatches. The nucleotides indicated by the squares represent mutations from the wild-type *supF* sequence. The two mismatches within each molecule are separated by 61 base pairs. The strands in the heteroduplexes originating from either p3AC-4 or p3AC-8 are indicated.

TABLE 1. Phenotypes of colonies formed by transformation of *E. coli* mutants with heteroduplex plasmid DNA

E. coli strain	Relevant genotype	No. blue	Total	% blue
SY204	Wild type	166	8,350	2.00
EG826	*ssb-1*	17	1,970	0.86
SY302	*recA56*	24	2,661	0.90
SY208	*mutH3 mutU4*	118	5,730	2.06
SY209	*mutH3 mutU4 recA56*	37	22,235	0.17

substrate for assaying mismatch repair in mammalian cell extracts. The repair of one or both of the mismatches in the heteroduplex plasmid DNA could be detected by using the DNA, after incubation in the extracts, to transform an *E. coli* strain bearing an amber mutation in the β-galactosidase gene to ampicillin resistance in the presence of X-gal. The appearance of blue colonies would indicate a newly generated functional *supF* gene. *E. coli*, however, is able to repair mismatches in DNA. In addition, replication of unrepaired heteroduplex molecules followed by recombination between the resulting mutant plasmids could generate plasmids with functional *supF* genes. Hence, transformation of *E. coli* with heteroduplex DNA in the absence of prior incubation with cell extracts could yield a significant proportion of blue colonies because of metabolism within the bacteria. Because mismatch repair and recombination in *E. coli* are efficient processes, the effect of a mammalian cell extract on the heteroduplex DNA might be too small to detect above the background in the assay from bacterial processing of the heteroduplex.

To circumvent the background problem, we constructed several *E. coli* strains with mutations in some of the genes that are thought to play a role in mismatch repair, recombination, or both. It was necessary that all strains also contain amber mutations in the β-galactosidase gene to be useful in the assay for *supF* activity. These strains were transformed to ampicillin resistance with heteroduplex DNA, and the number of blue colonies as a percentage of the total in each case was determined. Table 1 gives the results of this experiment, along with the relevant genotypes of the strains studied. A mutation in the gene for the single-stranded binding protein of *E. coli*, as in EG826, has a measurable effect on the metabolism of heteroduplex plasmids in *E. coli*. A similar effect was seen as a consequence of a mutation in the *recA* gene, as in SY302. In contrast, the presence of mutations in both the *mutH* and *mutU* genes, as in SY208, has no detectable effect on the outcome of this assay relative to the outcome in the wild type. The presence of an additional mutation in the *recA* gene along with mutations in the *mutH* and *mutU* genes, as in SY209, however, reduces the percentage of blue colonies that are produced by a factor of 12 relative to both the wild type and the *mutH mutU* double mutant and by a factor of about 5 relative to the *recA*-deficient strain.

These results suggest that the *recA* protein has a significant role in the processing of heteroduplex plasmid DNA, probably in postreplicative recombination, but perhaps also in the mismatch repair process itself. The *mutH* and *mutU* gene products also play a role in the processing of the heteroduplex plasmid DNA, leading to the generation of functional *supF* genes, but in this assay their role is manifest only in the presence of a mutation in the *recA* gene. It should be noted that this heteroduplex DNA is derived from plasmids grown in *E. coli* SY204, which is a wild type with respect to the *E. coli* DNA methylation systems. Hence, the heteroduplex DNA is fully methylated (according to the *E. coli* pattern) on both strands. Because the mismatch repair system in *E. coli* that is related to the *mutH* and *mutU* genes is normally guided by differences in the methylation patterns on the strands of a heteroduplex, as in newly synthesized, hemimethylated DNA, the limited effect of the *mutH* and *mutU* mutations in this assay might be related to the fact that the heteroduplex DNA is fully methylated. Nonetheless, SY209, because it produces few blue colonies on transformation with heteroduplex DNA, proved useful as a host for a biological assay of mismatch repair in mammalian cell extracts.

TABLE 2. Mismatch repair and DNA polymerase activity in HeLa cell extracts

Extract reaction conditions	Mismatch repair				DNA polymerase (% max [α-^{32}P]dCTP incorporation in DNA polymerase[c])
	Phenotype distribution of SY209 colonies[a]			% max[b]	
	No. blue	Total	% blue		
Complete	186	15,639	1.19	100	100
Without ATP, creatine kinase, and creatine phosphate	13	3,124	0.42	25	3
Without deoxynucleotides	11	3,158	0.35	18	63
With aphidicolin	16	4,805	0.33	16	2
With dideoxynucleotides	43	9,771	0.44	26	103
With dideoxynucleotides and without deoxynucleotides	9	2,988	0.3	13	NT[d]
With dideoxynucleotides and aphidicolin and without deoxynucleotides	8	3,950	0.2	3	NT
No extract	37	22,235	0.17	0	NT

[a] *E. coli* SY209 was transformed with heteroduplex DNA to yield ampicillin-resistant colonies of the given phenotype.
[b] Calculated by taking the percentage of blue colonies produced in each case, subtracting the percentage of blue colonies produced when no extract was used, and then normalizing to the value for the complete reaction.
[c] Calculated by subtracting the background counts and normalizing to the value for the complete reaction; 100% is equivalent to 24,034 cpm.
[d] NT, Not tested.

Activity in HeLa cell extracts. Heteroduplex DNA was added to cell extracts from HeLa cells that were prepared as described above. The incubation of the DNA in the extracts was carried out either under the conditions of the complete reaction as described above or with various experimental modifications. These modifications included the following: (i) no added ATP, creatine phosphate, or creatine kinase; (ii) no added deoxynucleotides; (iii) the addition of aphidicolin at 120 μM; (iv) the addition of dideoxynucleotides as follows: dideoxyguanosine triphosphate at 40 μM, dideoxyadenosine triphosphate at 40 μM, dideoxythymidine triphosphate at 80 μM, and dideoxycytidine triphosphate at 20 μM; (v) the addition of dideoxynucleotides at the concentrations given above in the absence of added deoxynucleotides; (vi) the addition of aphidicolin and dideoxynucleotides in the concentrations given above but without added deoxynucleotides; (vii) no incubation of the heteroduplex in the extract. The DNA was recovered from the extracts and used to transform SY209 to ampicillin resistance in the presence of X-gal, and the percentage of blue colonies was determined.

The results of these experiments are presented in Table 2. Preincubation of the heteroduplex in the complete extract prior to transformation of SY209 resulted in 1.19% blue colonies, whereas direct transformation of SY209 with the untreated heteroduplex yielded only 0.17% blue colonies. This sevenfold increase above the background value was statistically significant ($P < 0.001$), and it indicates that these extracts metabolize the heteroduplex DNA in some way. In the absence of exogenously added energy sources, the activity of the extracts above that of the background was reduced by about 75%. A similar effect was seen in the absence of added nucleotides, resulting in a reduction of extract activity by a factor of five. In the presence of aphidicolin or dideoxynucleotides, the activity of the extracts was again significantly reduced, by about 85 and 75%, respectively. When dideoxynucleotides were added in the absence of added deoxynucleotides, there was even less detectable activity, and when aphidicolin was also added with dideoxynucleotides in the absence of added deoxynucleotides, there was little if any effect above that of the background.

These results indicate that the process being measured in the extracts is energy dependent, is enhanced by the addition of deoxynucleotides, and is sensitive to aphidicolin and dideoxynucleotides, which are agents known to inhibit DNA polymerases (3, 16, 21, 22). Taken together, these results suggest that at least one aspect of the activity in the extracts involves DNA polymerization. To investigate this correlation further, extract reactions were set up under the same set of conditions as described above except that no unlabeled dCTP was added to any of the reactions but, instead, all reactions (including the reaction without deoxynucleotides) received [α-^{32}P]dCTP at a concentration of 20 μM (specific activity, 3,000 Ci/mmol). The proportion of radioactivity incorporated into trichloroacetic acid-insoluble material was determined in each case as one measure of DNA polymerization. This incorporation was compared with that found in the complete reaction, and in Table 2 the relative incorporation under each of the given conditions tested is expressed as a percentage of that in the complete reaction. There was little or no correlation between the effect of a given reaction modification on the mismatch repair assay as compared with the assay for dCTP incorporation, especially in the case of the addition of dideoxynucleotides alone (Table 2).

DNA recovered from the extract reactions carried out in the presence of [α-^{32}P]dCTP was analyzed by agarose gel electrophoresis and autoradiography. The results (data not shown) were consistent with the measurements of trichloroacetic acid-insoluble counts and demonstrated that the only labeled high-molecular-weight DNA species were those corresponding to the input DNA. No complex, reduced mobility forms that might indicate ongoing DNA replication were present, and no discrete degradation products were noted.

Recombination in HeLa cell extracts. An investigation of the activity of the HeLa cell extracts with regard to recombination was done as a control for the mismatch repair assay. These experiments are similar in design to those of Kucherlapati et al. (9) and are similar in concept to those of Darby and Blattner (2). Instead of forming heteroduplex molecules from p3AC-4 and p3AC-8, the two plasmids were both added directly to the extracts in either the circular or linear form. The DNA recovered after incubation in the extracts was used to transform SY209, as described above, and the percentage of blue colonies that was produced, which is indicative of the formation of a functional *supF* gene, was determined in each case (Table 3). No blue colonies were detected when circular plasmids were used to transform SY209, whether or not the mixed plasmids were preincubated in the extracts. When both plasmids were first linearized, very few colonies were produced at all, and none were blue. Only when p3AC-4, which was present in its circular form, was mixed with the linear form of p3AC-8 were any blue colonies detected, with there being 0.044% blue colonies after extract incubation and about eightfold fewer, or 0.0062% blue colonies, with no extract treatment. These results, like those reported previously (2, 9), suggest that there is some activity in the mammalian cell extracts which complements the RecA deficiency in SY209. Note that a much lower percentage of blue colonies was produced when the mixture of plasmids was used as a substrate as opposed to when heteroduplex prepared from the two mutant plasmids was used, and so the results with the heteroduplex DNA in the extracts cannot be attributed to recombination events alone.

TABLE 3. Recombination in HeLa cell extracts

DNA substrate[a]	Extract[b]	Phenotype distribution of SY209 colonies[c]		
		No. blue	Total	% blue
4 and 8	+	0	39,800	0
4 and 8	−	0	50,000	0
4 and 8, *Bam*HI	+	16	36,200	0.044
4 and 8, *Bam*HI	−	4	64,000	0.006
4, *Sca*I and 8, *Bam*HI	+	0	0	0
4, *Sca*I and 8, *Bam*HI	−	0	50	0

[a] Abbreviations: 4, p3AC-4; 8, p3AC-8.

[b] Either the complete extract and reaction conditions were used or the samples were incubated in buffer without HeLa cell extract.

[c] *E. coli* SY209 was transformed with DNA recovered from the extract reactions to yield ampicillin-resistant colonies of the given phenotype.

DISCUSSION

We set up a biological assay in which repair of heteroduplex plasmid DNA by human cell extracts could be subsequently detected by screening bacteria that were transformed with the plasmid DNA recovered from the extracts. The success of this assay depended on the construction of an *E. coli* strain in which mismatch repair and postreplicative plasmid recombination were minimal so that the background in the assay was low enough to measure the effect of the human cell extracts on the heteroduplex plasmid DNA. The HeLa cell extracts used in these experiments were essentially similar to the in vitro transcription extracts first described by Manley et al. (13), and they have been used in our laboratory for the study of transcription as well as mismatch repair (18).

In the assay the percentage of blue colonies produced by the transformation of SY209 with the heteroduplex DNA recovered from the extracts is taken as a measure of mismatch repair. The production of blue colonies depends on the conversion of one or both of the single base pair mismatches in the *supF* gene to the normal *supF* base pair. A single correction gives a blue colony if it is followed by plasmid replication, because in this case semiconservative replication generates a plasmid with a wild-type *supF* gene in addition to one with a mutant gene. Some events, however, may convert the mismatches to the mutant base pairs, but these events, because they would yield white colonies, are not specifically counted in this assay. These events appear among the many white colonies that arise from the introduc-

tion of unrepaired heteroduplex into the SY209 cells. Hence, the assay underestimates the frequency of match events. It might be expected that conversion of the mispair to either the wild-type or the mutant sequence is equally likely, as there were no particular differences between the strands of the heteroduplexes in these experiments, and so the actual frequency of repair events might be at least twice the observed value.

The results demonstrate that the HeLa cell extracts have a significant effect on the heteroduplex DNA. When the heteroduplex DNA was used to transform SY209 directly, only 0.17% of the colonies were blue. In contrast, incubation of the heteroduplex plasmid DNA in the extracts prior to transformation of SY209 produced 1.19% blue colonies, a sevenfold increase. Part of the assay, however, involved propagation of the DNA in *E. coli* before the final results could be determined, and so it is not known if all the steps involved in the repair of the mismatches are carried out and completed in the extracts. A significant part of the metabolism of the mismatched bases must occur in the extracts, because the results are dependent on the addition of ATP, creatine phosphate, creatine kinase, and deoxynucleotides to the extracts. The marked reduction in the activity of the extracts when these species were omitted indicates that some sort of energy-requiring enzymological process which needs a sufficient supply of DNA precursors is involved.

The exact nature of the activity in the extracts is not clear. Theoretically, some type of random nick-translation activity might be invoked to account for the repair of mismatches. Evaluation of the data, however, suggests that this explanation cannot account for all of the observed repair activity. The results show that the mismatch repair activity in the extracts is significantly reduced by the addition both of aphidicolin and dideoxynucleotides. Only aphidicolin, however, had an effect on the incorporation of labeled dCTP into the plasmid DNA. In fact, aphidicolin completely blocked [α-^{32}P]dCTP incorporation, whereas dideoxynucleotides had no measurable effect at all. The incorporation of [α-^{32}P]dCTP in these extracts is one measure of DNA polymerase activity. It is also an index of random nick-translation activity, because nick translation is a process that is associated with the incorporation of labeled nucleotides into DNA. Semiconservative DNA replication would also contribute to the results, but analysis of the reactions by gel electrophoresis showed that the pattern of complex, slowly migrating forms indicative of DNA replication was absent. Studies of in vitro replication have shown also that replication of plasmids, such as p3AC, which contain SV40 origins of replication, is dependent on exogenously added T-antigen protein, which was not added in these experiments. It is reasonable to assume, therefore, that the assay used to measure DNA polymerization in this study is essentially an assay for nick translation. Repair synthesis specifically associated with mismatch repair may also account for some [α-^{32}P]dCTP incorporation, but to the extent that it does contribute, the trivial explanation of random nick translation cannot be invoked. The data indicate that the presence of dideoxynucleotides in the extract reactions has no effect on the measured nick-translation activity but still reduces the apparent mismatch repair activity by about 75%. This suggests that some part of the observed mismatch repair activity that is sensitive to inhibition by dideoxynucleotides is unrelated to random nick translation. Similarly, in the absence of exogenously added deoxynucleotides, nick translation in the extracts was reduced to 63% of normal, while the apparent mismatch repair activity was just 18% of normal, again demonstrating an incomplete correlation between these activities in the extracts. Thus, although random nick translation cannot be ruled out as a partial explanation for the observed repair of mismatches, it does not appear to be associated with all of the mismatch repair activity in the extracts.

The activity that we observed may involve some nonspecific repair of mismatches which does not entail any particular strand selectivity. Bona fide mismatch repair, as it is understood in *E. coli*, involves a mechanism for strand discrimination so that repair can be directed toward the parental sequence. Based on the data from this study, we are unable to say whether or not the observed activity might involve some strand selectivity. The factors that affect strand selectivity in mammalian cells are not known for sure, but there has been one report (7) that when the substrate DNA is hemimethylated, there is an apparent bias toward the methylated strand in mismatch correction in vivo in monkey cells. We are currently investigating the effect of methylation on strand selection in vitro.

ACKNOWLEDGMENTS

We thank K. B. Low, E. Golub, R. D'Aquila, S. J. Baserga, and W. P. Summers for help with this study.

This study was supported by Public Health Service grants CA06519, CA13515, and CA16038 from the National Institutes of Health. P.M.G. was supported by Medical Scientist Training Program grant GM07205 from the National Institutes of Health.

ADDENDUM IN PROOF

Muster-Nassal and Kolodner (Proc. Natl. Acad. Sci. USA **83**:7618–7622, 1986) have detected mismatch correction in cell-free extracts of *Saccharomyces cerevisiae*.

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