hMSH2-independent DNA Mismatch Recognition by Human Proteins*

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Two distinct mismatch binding activities are detected using bandshift assays with human cell extracts and DNA with mispairs at defined positions. One requires hMSH2 protein and is absent from extracts of LoVo cells, which contain a partial deletion of the hMSH2 gene. The second activity is independent of hMSH2 and is present at normal levels in LoVo and three other cell lines, which are defective in in vitro hMSH2-dependent binding. The two mismatch recognition activities are distinguished by their sensitivity to polycations and can be resolved by chromatography on MonoQ. hMSH2-independent activity has been purified extensively from wild-type cells and from a cell line deficient in hMSH2-dependent binding. The purified material preferentially recognizes A-C, some pyrimidine-pyrimidine mismatches, and certain slipped mispaired structures. Binding exhibits some sequence preferences. The similar properties of the two mismatch binding activities suggest that they both contribute to mismatch repair.

The recognition and correction of non-Watson-Crick base pairs increases the fidelity of DNA replication (1) and prevents recombination between partially diverged (homologous) DNAs (2). In Escherichia coli, a single mismatch recognition protein, MutS, initiates both the correction of DNA replication errors and the abortion of homologous recombination intermediates (3). Mismatch repair is more complex in eukaryotes, and the abortion of homeologous recombination intermediates (2). In Saccharomyces cerevisiae (3), Mismatch repair is more complex in eukaryotes, and the abortion of homeologous recombination intermediates (2). In human cells, we identified a human mismatch binding function that could be distinguished from a known GT mismatch recognition activity (15) by its apparent preference for A-C and pyrimidine-pyrimidine mismatches (16). The GT binding reaction is now known to be mediated by hMutSα, a GTBP-hMSH2 heterodimer (12, 13). Here, we describe the extensive purification of the other mismatch binding function. The activity is independent of hMSH2. It is present in several cell lines defective in hMSH2-dependent binding, including one that is homozygous for a partially deleted hMSH2 gene. Extracts of cells deficient in hMSH2-dependent binding but containing apparently normal hMSH2-independent (A-C) binding activity, are unable to repair A-C mismatches in vitro, suggesting that the A-C binding function does not simply replace hMutSα in the repair of A-C mismatches. A-C binding is carried out by a protein complex of similar size to hMutSα. The two activities are resolved following extensive purification, and the partially purified binding functions are affected differently by polycations. Preferential binding by both mismatch recognition activities is influenced to some extent by the sequence context of the mismatch. The substrate preferences of the purified A-C binding activity provide clues to its possible role in mismatch repair, and the similarities between hMSH2-dependent and independent binding suggest that the two might play complementary roles.

EXPERIMENTAL PROCEDURES

Materials—Biochemicals were obtained from Sigma except where stated otherwise. Oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer.

The generic 34-mer substrates for bandshift assays are shown below. The specific sequences of mismatched duplexes (oligonucleotides 1–7) are given in Table I.

Oligonucleotides were end-labeled and annealed as described previously to generate either paired or mismatched duplexes. For most substrates, oligonucleotides synthesized in two or more independent
batches were used. No differences in the ability to serve as substrates of Li and Kelly (19).

LoVo and SW620 cells were detached from the flasks by trypsinization. The human colorectal adenocarcinoma lines LoVo and SW620 were maintained in RPMI medium containing 5% fetal calf serum (Life Technologies). The Raji Burkitt's lymphoma, its methylation tolerant clonal derivative, RajiF12 (17), and HeLa cells were maintained in spinner culture in RPMI medium containing 5% fetal calf serum (Life Technologies, Inc.).

III. The purified linear duplexes were denatured, and transferred to Hybond N+ filters (Amersham International), which were subsequently probed with radiolabeled random-primed (Boehringer) M13 DNA. Radioactive DNA was localized by autoradiography.

The following bands were the most informative: 1) fragments of the two ends of the M13 vector, and 2) two fragments of the insert, indicating that the mismatch was located in the insert sequence.

To determine the minimum effective concentration and thereby substrates were able to cut mismatched DNA, and each batch of mismatched substrate was therefore titrated with the appropriate enzyme prior to assay to determine the minimum effective concentration and thereby avoid over-digestion. For analysis with Xmn1, for which there are two sites in HK7 outside the heteroduplex cassette region, digestion with AlwNI was omitted. Digestion products were separated on agarose gels, denatured, and transferred to Hybond N+ filters (Amersham International).

In Vitro Mismatch Correction —The substrates for in vitro mismatch correction assays were prepared from freshly harvested cells by a modification (18) of the method of Li and Kelly (19).

Bandshift Assay—Bandshift assays were performed as previously reported (16). Briefly, cell extract (10–15 μg of protein) was preincubated at 20°C with 40 fmol of matched non-radioactive 34-mer ( oligonucleotide) I in a 20-μl reaction buffer comprised of 25 mM Hapes KOH, pH 8.0, 0.5 mM EDTA. The 0.1 mM ZnCl2, 10% glycerol, 50 μg of poly(dI-dC)poly(dI-dC). After 5 min, the reactions were supplemented with radioactive substrate (20 fmol), and incubation continued for a further 20 min. 10-μl aliquots, supplemented with bromophenol blue, were analyzed by electrophoresis on 6% polyacrylamide gels as described (16). Reaction products were detected by autoradiography. When non-radioactive competitor oligonucleotides or spermine were included, they were present during the preincubation and subsequent incubation.

In Vitro Mismatch Correction —The substrates for in vitro mismatch correction are circular M13 heteroduplexes containing a single nick. The HK7 M13 derivatives used in their construction (kindly provided by Dr. Peter Brooks), the details of the heteroduplex cassette, which places mismatches in positions that create diagnostic restriction sites after correction, and the annealing conditions are described by Varlet et al. (20). Briefly, replicative form I M13 molecules (10 μg) were linearized by digestion with Avall. The purified linear duplexes were denatured and annealed in the presence of a 10-fold molar excess of appropriate single-stranded HK7 DNA. Remaining single strands were removed by chromatography on benzoylated naphthylated DEAE-cellulose. The resulting replicative form II heteroduplex preparations were free of single-stranded DNA and generally contained about 10% linear homoduplex molecules.

The assay mixtures (20 μl) were based on that of Holmes et al. (21) and contained 20 mM Tris·HCl, pH 7.6, 5 mM MgCl2, 1 mM glutathione, 50 μg/ml bovine serum albumin, 0.1 mM each dNTP, 1.5 mM ATP, 70 mM KCl, and 10 ng of heteroduplex DNA. The reaction was started by addition of extract (70 μg) and continued for 60 min at 37°C. Reactions were terminated by the addition of 36 μl of 25 mM EDTA, 0.5% SDS, and proteinase K (50 μg/ml). After a further 15 min at 37°C, the mixture was extracted with phenol/chloroform followed by chloroform, and DNA was recovered by ethanol precipitation. Precipitated DNA was dissolved in buffer for digestion with AlwNI followed by the appropriate diagnostic restriction enzyme (at high concentrations, some diagnostic enzymes were able to cut mismatched DNA, and each batch of mismatched substrate was therefore titrated with the appropriate enzyme prior to assay to determine the minimum effective concentration and thereby avoid over-digestion). For analysis with Xmn1, for which there are two sites in HK7 outside the heteroduplex cassette region, digestion with AlwNI was omitted. Digestion products were separated on agarose gels, denatured, and transferred to Hybond N+ filters (Amersham International).

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tracts with the A
various non-radioactive competitor oligonucleotides in the
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by the extracts of wild-type Raji cells. In particular, an unla-
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are homozygous for a large deletion in the hMSH2 gene, the
and the preference for A
observations confirm the selective binding to mismatched DNA
heteroduplex were not significantly inhibitory (Fig. 1
ply, however, that this mismatch is necessarily its preferred substrate.

**Fig. 1.** A-C mismatch binding by colorectal carcinoma cell ex-
tracts. **a,** extracts (12 μg) of the human colorectal carcinoma cell lines indicated were combined with radiolabeled A-C-mismatched duplex
34-mer oligonucleotide as described under "Experimental Procedures." Bound and free oligonucleotides were separated by electrophoresis on a
6% polyacrylamide gel and located by autoradiography. Complex A is the A-C-specific complex. **b,** extracts of Raji or LoVo cells were preincu-
bated with a 30-fold excess of the non-radioactive duplex oligonucleo-
tide indicated. Radioactively labeled A-C substrate was then added, and complex A formation was analyzed by gel electrophoresis.

by the rate of migration of the respective DNA-protein com-
plexes in the bandshift assay. Extracts of several mutator human cell lines, the methylation tolerant RajiF12, the colorectal carcinoma lines LoVo and DLD1/HCT15, and the methy-
lation tolerant hamster line Chinese hamster ovary clone B, are defective in G-T binding (17, 23, 24). All contain A-C binding activity comparable to that seen in Raji (17) and the G-T binding
proficient colorectal carcinoma cell lines SW620, HRA19, and LS174T (Fig. 1, a and b and data not shown). Inclusion of various non-radioactive competitor oligonucleotides in the
binding assay indicated that the complex formed by LoVo ex-
tracts with the A-C substrate is the same as complex A formed by the extracts of wild-type Raji cells. In particular, an unla-
beled A-C heteroduplex was an efficient competitor and effec-
tively abolished binding when present in 30-fold excess, whereas the same concentrations of homoduplexes and a G-T heteroduplex were not significantly inhibitory (Fig. 1b). These
observations confirm the selective binding to mismatched DNA
and the preference for A-C over G-T mismatches. Since LoVo cells are homozygous for a large deletion in the hMSH2 gene, the data also provide direct evidence that hMSH2 is not required for A-C binding.

In Vitro Mismatch Correction and the A-C Binding Activity—To investigate if the A-C binding activity might substitute
for the G-T binding function in correction of A-C mismatches,
we measured A-C mismatch repair in an in vitro assay. Correction
of an A-C mismatch by extracts of the G-T binding-defec-
tive DLD1, LoVo, and RajiF12 cells and control HeLa, SW620,
and Raji cells was compared. Cell extracts were incubated with circular heteroduplexes that contained a single A-C mispair.
The mismatch is positioned in a heteroduplex cassette se-
quence such that correction to a G:C base pair creates an Mlu
site, whereas correction to A:T generates a unique Clal site
(Fig. 2a). Mismatch repair in vitro by human cell extracts
requires a nick in one of the DNA strands, and correction
is directed to the nicked strand (21). The A-C heteroduplex con-
tained a nick in the mismatched A-containing strand and was
efficiently repaired by HeLa, SW620, and Raji cell extracts.
After incubation with extract, substrate DNA was recovered,
linearized with AlwNI and further digested by MluI. The DNA
was cleaved into fragments of 4.2 and 3.2 kilobases, indicating
repair of the A-C mismatch to G:C. No correction to A:T (Clal
sensitivity) was detected, confirming that repair was strand-
specific (data not shown). In contrast to wild-type cells, extracts
of G-T binding-defective LoVo, DLD1, or RajiF12 cells did not
carry out detectable repair (Fig. 2b). In a second substrate, the
mismatch was inverted, and the nick was positioned in the
C-containing strand. In this case, nick-directed repair to a T:A
base pair generates an additional XmnI site (Fig. 2a), whereas
correction in the opposite orientation produces a unique EcoNI
site. The presence of fragments of 3.7 and 1.4 kilobases in XmnI
digests of substrate DNA recovered from extracts of HeLa,
SW620, or Raji cells indicated the creation of a new XmnI site
by nick-directed correction to a T:A base pair. Extracts of LoVo,
DLD1, or RajiF12 cells did not carry out detectable repair (Fig.
2b). Thus, wild-type cell extracts can correct A-C mismatches in
two different sequence contexts by a nick-directed process. The
normal levels of A-C binding activity in G-T binding-deficient
extracts do not compensate for the absence of the G-T recogni-
tion function in correction of A-C mismatches in the heterodu-
plex cassette substrate.

**Effect of Polycations on Mismatch Binding**—The A-C and G-T
recognition activities could be distinguished by their differen-
tial sensitivity to polycations. When fractions enriched for both
A-C and G-T binding by successive ammonium sulfate, heparin-
Sepharose, and DNA-cellulose chromatography were assayed in
the presence of increasing concentrations of spermine, the
polycation stimulated G-T binding. In contrast, A-C binding
was progressively inhibited over the same range of spermine
concentrations (Fig. 3a) and was essentially abolished at 1 mM.
Spermine was more effective than spermidine (data not shown), suggesting that charge neutralization might underlie
the observed effects. When a similarly enriched fraction from
Raji12 cells was used, A-C binding was inhibited in an identi-
cal fashion to the wild-type fraction (Fig. 3b). As expected, no
G-T binding was observed either in the presence or the absence
of spermine. Essentially identical results were obtained using
partially purified LoVo cell extracts (data not shown). The
difference in susceptibilities of the two binding activities to
spermine provides supporting evidence that G-T and A-C bind-
ing are carried out by different proteins or complexes. The
effect of the polycation on A-C binding in F12 and LoVo further
dicates that the A-C binding activities in the variant cells
have similar properties to their wild-type counterpart.

**Fractionation of Binding Activities**—The standard assay in-
dicates that A-C, but not G-T, binding activity is recovered by
precipitation with ammonium sulfate between 5 and 35% sat-
uration. The loss of G-T binding is not due to its inactivation
since both functions are recovered in a 5-55% fraction. The A-C
and G-T binding activities in an ammonium sulfate fraction of
a wild-type Raji cell extract copurified through subsequent
consecutive heparin-Sepharose, AAc34 gel filtration, and DNA-
cellulose chromatography. Both bound to heparin-Sepharose
Fig. 2. In vitro correction of A–C mismatches. a, the substrates for the reaction were form II duplex molecules constructed by annealing viral (v) and mismatched complementary (c) strands as described under “Experimental Procedures.” Each substrate contained the mismatch shown within a heteroduplex cassette sequence. The position of the mismatch is designated 0. The numbers in parentheses indicate the distance (kilobases) measured in a clockwise direction from the mismatched site. Correction of the A–C mismatch in the nicked strand (left) generates a unique Mlu I site. Correction of the C–A mismatch in the nicked strand (right) introduces an additional Xmn I site. b, correction assay. Substrates (10 ng) were incubated with cell extract (70 μg) for 1 h at 37°C. The DNA was recovered and digested with AlwNI plus Mlu I (left panel) or with Xmn I (right panel). Digestion products were separated on agarose gels, transferred by Southern blotting, and probed with radiolabeled M13 DNA. The fragment sizes in kilobases are indicated next to the arrows.

from which they coeluted close to the end of a 0.1–1 M NaCl gradient. They were retained by double-stranded DNA-cellulose and were eluted at approximately 0.3 M NaCl (data not shown). A–C and G–T binding cochromatographed on AcA34 at the position corresponding to a monomeric Mₘ for approximately 250,000 (data not shown). The elution position was unchanged over a range of NaCl concentrations from 0.1 to 1 M, suggesting that this apparent large size was not due to nonspecific protein aggregation. A similar estimated Mₘ for both binding functions was obtained by gel filtration on Sephacryl 300. Thus, the two binding activities share several physical properties including a similar size.

A–C and G–T mismatch binding activities were resolved by chromatography on MonoQ. An extract of Raji cells enriched for both activities by sequential ammonium sulfate and DNA-cellulose fractionation was applied to a MonoQ column in buffer containing 0.1 M NaCl. The bound protein was eluted by a gradient of NaCl to 0.5 M. Fractions were assayed for both A–C and G–T mismatch binding. A–C binding activity eluted early in the gradient at approximately 0.2 M NaCl. It was detected by the formation of complex A (Fig. 4a) with an A–C substrate. No G–T binding (complex B) was observed in these early fractions. The peak of A–C binding was partially coincident with the formation of a complex that migrated close to the well of the gel. This complex was not mismatch-specific and was formed to the same extent with both A–C and G–T substrates. In fractions containing very high levels of A–C binding activity, a small amount of complex A was also formed with the G–T substrate, suggesting that while formation of this complex with A–C mismatches is highly preferred, this preference is not absolute. Similar behavior has been observed with unfractionated extracts (16). G–T binding (complex B) eluted after the A–C and this nonspecific binding activity. There was a slight overlap with the tail of the A–C binding peak (fraction 13, Fig. 4a), but the later G–T binding fractions did not bind detectably to A–C. Two other rapidly migrating complexes (C and D) were also seen. These complexes, which have been noted previously in crude extracts, are not mismatch specific and were formed to similar extents with both substrates. Thus, in addition to their differential susceptibility to spermine, the two binding activities also exhibit different behavior on ammonium sulfate fractionation and can be physically resolved on MonoQ.

A–C binding activity was purified from extracts of the G–T binding-defective RajiF12 cells by the same procedure. Fig. 4b shows the mismatch binding activity in MonoQ fractions. Complex A was detected with an A–C substrate in fractions 9–14 and again partially overlapped fractions forming the slowly migrating nonspecific complex. No complex B was detected in any fraction. The nonspecific binding (complex D) that partially overlapped the G–T binding activity in wild-type preparations was also observed. This serves as an internal control for these fractions. Similar data were obtained from MonoQ chromatography of extracts of LoVo cells (data not shown). Thus, the A–C binding activity, which is present at wild-type levels in G–T binding-defective cells, has the same purification properties as the wild-type A–C binding activity. This suggests that the two mismatch binding activities are independent of one another.

Purification of A–C Binding Activity—A–C binding activity was purified from the MonoQ fraction of wild-type Raji cells by further chromatography on MonoS and Superose 12. A–C binding eluted from Superose 12 slightly later than the nonspecific binding activity with which it had copurified, at a position...
indicate the positions of complexes A and B formed by partially DNA-cellulose chromatography. A by ammonium sulfate precipitation followed by heparin-Sepharose and wild-type cells. The standard bandshift assay was carried out with the corresponding to a monomeric duplex was replaced by T-C substrates, respectively. To rT-C binding activity that had been purified through the repair substrate (Fig. 2). Another striking example of sequence effects on mismatch recognition was provided by the standard 34-mer substrate. It is notable that little binding was seen with C-C or the purine-pyrimidine mismatch in the same 34-mer sequence (Fig. 7a and data not shown) nor were these duplexes efficient competitors for A-C binding (Fig. 7b). These observations agree well with the specificity previously reported for the A-C binding activity in cell extracts.

Unpurified cell extracts also form complex A on certain looped mismatched structures (23). The same 40-mer substrate containing the sequence (AT)2 in which one dinucleotide repeat is displaced from the duplex (oligonucleotide 9) was also recognized by the purified fraction, and complex A was formed (Fig. 7a). An analogous substrate (oligonucleotide 10) in which a CA loop displaced from (CA)2 replaced the AT loop was not detectably recognized by the purified A-C binding activity (Fig. 7a). This substrate was bound, however, by a late-eluting fraction from MonoQ that contained the G-T binding activity (data not shown). These data provide further evidence for the different preferences for mismatch recognition by the two binding functions and suggest that some slipped mispaired intermediates are also substrates for the A-C binding activity.

A third set of mismatched substrates was based on the heteroduplex cassette sequence in the in vitro mismatch repair assay (oligonucleotides 11 and 12). Surprisingly, the purified A-C binding activity did not efficiently recognize a 48-mer duplex containing a single A-C mismatch at the same position as the repair substrate (Fig. 2a). Complex A was formed but only to a limited extent, and binding was similar to that seen with a G-T (Table I, oligonucleotide 3) substrate (Fig. 8a). When a MonoQ fraction enriched for G-T binding, but containing a small amount of A-C binding activity (far right lane, Fig. 8a), was used with the A-C mismatched 48-mer, complex B was formed. This preferential binding by the G-T activity to the A-C mismatch in this substrate was confirmed with unfractonated extracts (data not shown). Neither binding activity detectably recognized a substrate containing an AT loop in an (AT)2 sequence placed in the heteroduplex cassette region. Thus, recognition of both single base mismatches and slipped/mispaired structures by one or either of the two mismatch binding activities exhibits some dependence on the sequence context of the mispairs.

Another striking example of sequence effects on mismatch recognition was provided by the standard 34-mer substrate. When the A-C mismatch in this substrate was inverted (Table I, oligonucleotide 7), thus altering its surrounding sequence context, the A-C binding activity no longer recognized the A-C mismatch. This mismatch was also a substrate for the partially purified GT binding activity, and complex B was formed (Fig. 8b).

**DISCUSSION**

The complexity of mismatch repair in eukaryotic cells is reflected in the abundance of different human mismatch repair proteins. Since there are at least four MutS homologs in yeast, some diversification of mismatch recognition is also likely in human cells. It is interesting to note that the constituents of hMutSα, hMSH2 and GTBP, are both MutS homologs (12, 13) and so each may contribute to mismatch recognition. The A-C binding activity may be an example of the diversity in mismatch recognition.
Not all human mismatch recognition proteins are necessarily involved in mismatch repair. Mg\textsuperscript{2+} and ATP-independent mismatch binding detected by bandshift assays is associated with mammalian DNA topoisomerase I (25). Topoisomerase I can be excluded as a candidate for the A\textsuperscript{z}C binding activity on the basis of its size and its substrate preferences. It is a single polypeptide of \( M_r \approx 595,000 \), which is sometimes purified as a proteolysis product of approximate \( M_r \approx 580,000 \), whereas the \( A\textsuperscript{z}C \) binding activity is purified as an apparently stable complex of estimated \( M_r \approx 250,000 \). Topoisomerase I acts on all single base mismatches, whereas the purified \( A\textsuperscript{z}C \) binding complex is more fastidious in its recognition. Two other human proteins that bind to mismatches and can be detected by the bandshift assay have been described. One of these, hMutY, the human homolog of the E. coli MutY protein, is a DNA glycosylase that removes A residues from A-G, A-7,8-dihydro-8-oxoguanine, and, with 30-fold less efficiency, A-C mismatches (26). hMutY and the recently described deoxyinosine 3'-endonuclease (27) can be excluded as candidates for the A-C binding activity both on the basis of their size (65 and 25 kDa, respectively) and of their substrate preferences. In initial fractionation studies of human cell extracts, A-C binding activity was associated with a protein, or complex, of approximately 100 kDa (16). It is possible that this reflects an autonomous binding activity of the smaller component of an A-C complex dissociated at relatively low protein concentrations.

Our data suggest that the A-C binding complex is a dimer of proteins of approximately 110 and 140 kDa. This composition is similar to that of the GT binding complex of hMSH2 and GTBP (12, 13) (104 and 160 kDa). Despite this similarity in size, the complexes formed with A-C and G-T substrates are resolved in the bandshift assay. The faster migration of the A-C complex suggests that the A-C binding proteins may be more acidic. A difference in surface charge is consistent with separation of the A-C and G-T binding activities on MonoQ and may also underlie the different effect of spermine on A-C and G-T binding. Polyamines can affect protein-DNA interactions in several ways, including inducing DNA bending (28) and altering the site

**Fig. 4.** Separation of A-C and G-T binding activities on MonoQ. a, wild-type cells. Mismatch binding activity purified by ammonium sulfate precipitation and DNA-cellulose chromatography was applied to MonoQ. Elution conditions were as described under "Experimental Procedures." Each fraction was assayed for A-C (left lane for each fraction) and G-T (right lane) binding. Complexes A and B are the A-C and G-T complexes, respectively. Complexes C and D are nonspecific complexes. b, As in a with material from RajiF12 cells.

**Fig. 5.** Size determination of A-C binding activity. MonoQ fractions containing A-C binding activity were applied to a Superose 12 column. Each fraction was assayed for A-C binding (complex A). The fractions corresponding to the elution position of dextran blue (\( V_o \)), ferritin (440 kDa, \( F \)), and catalase (232 kDa, \( C \)) are indicated.

**Fig. 6.** Proteins involved in A-C binding. Two independent preparations of A-C binding activity from Raji cells were analyzed by SDS-polyacrylamide gel electrophoresis followed by silver staining. The positions of the molecular weight standards are indicated on the right side of the figure. The arrows on the left side indicate the positions of the two most prominent silver-stained bands that were reproducibly observed in several preparations. Lane 1, Raji activity purified by ammonium sulfate (5-55%) fractionation followed by successive double-stranded DNA-cellulose, MonoQ, MonoS, and Superose 12 chromatography as described under "Experimental Procedures." Lane 2, Raji activity purified by an identical procedure except that a 5-35% ammonium sulfate fractionation was used, and the MonoS chromatography step was omitted.
occupancy by binding proteins (29). A contribution of one or more of these other properties to the different effect of sperm-ine on A-C and G-T binding cannot be excluded.

The presence of A-C binding activity in RajiF12, LoVo, and DLD1 extracts, all of which have no detectable G-T binding, indicates that A-C binding is a separate function. This is supported by our partial purification of the A-C binding activity from RajiF12 and LoVo cells and the demonstration that its properties are indistinguishable from the wild-type activity. Since LoVo and DLD1 contain mutations in hMSH2 (14, 30) and GTBP (31), respectively, both of which inactivate G-T binding, it appears that neither hMSH2 nor GTBP is required for the A-C binding reaction.

Although we have no direct evidence that the A-C binding activity participates in mismatch correction, their similarities in size and general properties suggest that the G-T and A-C binding complexes may play complementary roles. We previously reported that the A-C binding activity in unfractonated cell extracts efficiently recognizes T-C and T-T mismatches but binds to C-C only poorly (16). These substrate preferences are apparent in the purified A-C binding complex. Since all single base mismatches, C-C mispairs are corrected least efficiently in vitro and in vivo (21, 32), these observations are consistent with an involvement of the A-C binding activity in mismatch repair. The purified binding complex also recognizes AT dinucleotides displaced from a (AT)₅ sequence. This ability to bind to displaced dinucleotides is analogous to the recognition of displaced CA dinucleotides by the G-T binding function (23) and suggests that the A-C binding activity might also be involved in stabilizing microsatellite sequences.

Recognition by the A-C mismatch binding activity does not exhibit an absolute dependence on DNA sequence context because the A-C complex is formed on A-C mispairs and AT loops in completely unrelated sequences. The context of the mismatch can, however, influence its recognition. We observed that neither A-C mismatches nor AT loops in a third, unrelated, sequence were recognized by the purified A-C binding complex. Instead, these mismatches were substrates for the G-T binding activity. Several other observations are also consistent with a degree of overlap in mismatch recognition by the A-C and G-T binding activities. At high concentrations, the purified A-C complex binds to G-T mismatches. This can be seen in the MonoQ fractions that are highly enriched for A-C binding activity. In addition, purified hMutSα binds (albeit rather poorly) to A-C mismatches (22). The rules that govern recognition cannot be inferred from these few data, but local sequence determinants may contribute to the probability that mismatches are recognized by one or other of the binding heterodimers.

Data from the in vitro mismatch correction assays indicate, however, that mismatches cannot simply be divided into two groups, one comprising substrates for hMutSα and the other the mismatches recognized by the A-C complex. LoVo cell extracts cannot repair several mismatched structures in vitro, implicating hMSH2 in their correction. Our data indicate that the repair deficiency of LoVo extends to A-C (and C-A) mismatches, at least in the particular context of the heteroduplex cassette. The inability of LoVo, DLD1, and RajiF12 cell extracts to correct A-C mismatches in the in vitro assay is paralleled by the absence of recognition by the A-C binding activity of A-C mismatches in the heteroduplex cassette sequence. A-C mis-
match recognition in this particular context is mediated by the G-T complex, which is absent from these cells. hMutS\(\alpha\) and the A-C mismatch binding activity may turn out to have complementary but partially overlapping roles in mismatch recognition.

The in vitro assay provides a good indication as to whether particular mismatches are repaired. These assays are building up a picture of the requirements for hMutS\(\alpha\) in the correction reaction. A caveat should be added, however. The assays provide a strand discrimination signal for correction in the form of a nick in the substrate DNA. The possibility that the mismatch binding activities participate in strand selection during correction of their preferred mispairs has not been ruled out. Provision of a pre-incised substrate may obviate the need for this selective recognition and may miss an important property of the mismatch recognition complexes.

Many DNA repair functions are partially duplicated by back-up activities with similar specificities. The A-C binding activity might be one of these and may serve a complementary function to the hMutS\(\alpha\) complex in mismatch recognition and initiation of repair.

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