Nucleic Acids Research, 1994, Vol. 22, No. 24 5289–5295

Identification of two mismatch-binding activities in protein extracts of *Schizosaccharomyces pombe*

Oliver Fleck*, Primo Schär+ and Jürg Kohli
Institute of General Microbiology, University of Bern, Baltzer-Strasse 4, CH-3012 Bern, Switzerland

Received September 5, 1994; Revised and Accepted November 3, 1994

ABSTRACT

We have performed band-shift assays to identify mismatch-binding proteins in cell extracts of *Schizosaccharomyces pombe*. By testing heteroduplex DNA containing either a T/G or a C/C mismatch, two distinct band shifts were produced in the gels. A low mobility complex was observed with the T/G substrate, while a high mobility complex was present with C/C. Further analysis of the mismatch – binding specificities revealed that the T/G binding activity also binds to T/C, C/T, T/T, T/–, A/–, C/–, G/–, G/G, A/A, A/C, A/G, G/T, G/A, and C/A substrates with varying efficiencies, but not binds to C/C. The C/C binding activity efficiently binds to C/C, T/C, C/T, C/A, A/C, C/–, and weakly also to T/T, while all other mispairs are not recognized. Protein extracts of a mutant strain, defective in the *mutS* homologue *swi4*, displayed both mismatch-binding activities. Thus, *swi4* does not encode for either one of the mismatch-binding proteins.

INTRODUCTION

Mismatch repair is an essential cellular process protecting the genetic material against mutation. Mismatched bases arise by replication errors, chemical base alterations and during genetic recombination between homologous, but nonidentical DNA strands. In the bacteria *Escherichia coli* and the closely related *Salmonella typhimurium* several pathways for mismatch correction have been distinguished (1). The methyl-directed *mutHLS* pathway shows long repair tracts (about 1 kb) and efficiently corrects single base additions/deletions and all base–pair mismatches except C/C (1–3). Repair by the *mutHLS* pathway is dependent on the function of the *mutS*, *mutL*, and *mutH* gene products and additionally requires MutU helicase II, exonuclease I, exonuclease VII, RecJ, DNA polymerase III, SSB (single-stranded DNA-binding protein), and DNA ligase (1,4,5). The MutS protein binds to DNA mismatches (6), while MutH binds at hemimethylated *dam* sites (7). MutL mediates the interaction between MutS and MutH, leading to the incision of the unmethylated strand by MutH (1,7,8). Mismatch repair then occurs by excision of the nicked DNA strand towards the mismatch and DNA resynthesis (1). A related pathway is the *hex* system of *Streptococcus pneumoniae* (9,10). HexA is homologous to MutS (11), while HexB is homologous to MutL (12).

The VSP (very short patch repair) and the MutY systems are two additional mismatch-repair pathways of *E.coli* which differ from the *mutHLS* system by their mismatch specificities and length of repair tracts (1). Both the VSP and the MutY systems mediate only the correction of a minor subset of base–pair mismatches and show repair tracts of only about 10 bp (1,13). The VSP system requires the Vsr endonuclease and in addition MutS and MutL like the *mutHLS* pathway (14,15). In contrast the MutY pathway is independent of MutS and MutL functions (16). The VSP pathway exclusively restores the G=C match from G/T mismatches in *dcm* sites which arise spontaneously by deamination of 5-meC (1,13,14,17). The MutY system is responsible for the correction of G/A and A/C mismatches to give a G=C match (16,18,19) and is additionally involved in the repair of oxidative guanine damages (20).

Genetic and biochemical studies revealed eukaryotic mismatch-repair systems that show common features to the *mutHLS* repair pathway (21–29). MutS homologues have been identified in human, hMSH2 (30) and Duc-1 (31), mouse, Rep-3 (32), *Schizosaccharomyces pombe*, Swi4 (33), and *Saccharomyces cerevisiae*, MSH1, MSH2 (34), MSH3 (35,36). Surprisingly, three MutS homologues were found in budding yeast. MSH1 is likely involved in the repair of mitochondrial DNA (37), while the others possibly carry out different nuclear functions. The *msh2* mutant shows a mitotic mutator phenotype and an increased rate of postmeiotic segregation of genetic markers indicating a fundamental role in mismatch repair (37). This is consistent with the recent finding of a lack of a mismatch–binding activity in nuclear extracts of *msh2* mutants (38). Therefore, MSH2 might represent the functional counterpart of the bacterial MutS. The function of MSH3 is still unclear. The *msh3* mutant exhibits only a weak mutator phenotype and a slightly increased rate of postmeiotic segregation (36). Since the mismatch–binding activity is present (38), MSH3 seems to be a MutS homologue involved in a process other than general mismatch repair.

Based on genetic analyses it was recently postulated that at least two systems are responsible for mismatch correction in *S.pombe* (26,27). A major and efficient pathway was suggested to correct all mismatches, except C/C and might be the counterpart to the

*To whom correspondence should be addressed

+Present address: Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, UK

bacterial *mutHLS* pathway. Mismatch repair by this pathway is accompanied by repair tracts of about 100 bp unidirectionally. In contrast, a minor pathway is proposed to correct all mismatches including C/C. This newly described pathway is less efficient and shows short repair tracts of approximately 10 bp unidirectionally.

We have used band shift assays to detect mismatch−binding proteins in *S.pombe* and have determined their mismatch specificities. This study provides further evidence for the existence of at least two mismatch−repair systems in *S.pombe*.

MATERIALS AND METHODS

Preparation of protein extracts

The *S.pombe* strains 968 (h^{90} wild type) and LH110 (h^{90} *swi4::ura4*$^+$ *ura4-D18*) were propagated in 1 liter yeast extract liquid medium (39) to a final cell density of approximately $1.2-1.4\times10^8$ cells per ml (stationary phase). The cells were harvested by centrifugation, washed with 15 ml 25 mM HEPES (pH 7.5), 0.5 M NaCl, 1 mM EDTA, 0.5 mM spermidine, 0.1 mM spermine, 5 mM β-mercaptoethanol, 0.1 mM PMSF, 1 mM DTT and 1 g aliquots were resuspended in 2 ml of the same buffer. The cells were shock frozen in liquid nitrogen and stored at −80°C until further use. For preparation of native protein extracts, 1 g cells were mixed with an equal volume of glass beads (ø = 0.5 mm) and disrupted in a mini bead-beater by ten 30 s intervals. Proteins were separated from cell debris and lipids by two centrifugation steps (each 30 min, 13 000 r.p.m. at 4°C), and then dialyzed two times for 2 h against 300 ml 25 mM Tris−HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 10% glycerol, 5 mM β-mercaptoethanol, 0.1 mM PMSF, 1 mM DTT. Protein concentrations were measured as described by Bradford (40) using the Bio-Rad reagent.

Preparation of DNA heteroduplexes

Single-stranded oligonucleotides were synthesized using a 381A DNA Synthesizer (Applied Biosystems). Before annealing, the 'plus' strands (Fig. 1) were 5′ end-labeled by polynucleotide kinase in the presence of [γ-^{32}P]ATP. 0.4 pmol of radiolabeled plus strands were mixed with 1.6 pmol of the complementary 'minus' strands in 10 mM Tris−HCl (pH 8.0), 10 mM $MgCl_2$, 80 mM NaCl. Annealing occurred by heating the mixtures for 5 min at 90°C and slow cooling to room temperature.

Mismatch binding assay

Binding of proteins to the DNA heteroduplexes was monitored by band shift assays, which were carried out by a modified protocol of Jiricny *et al.* (41). 200 μg protein extracts were preincubated for 10 min at 4°C with unlabeled competitor DNA, if present. After addition of 40 fmol radiolabeled oligonucleotides, incubation was continued for 15 min. All reactions were carried out in 25 mM Tris−HCl (pH 7.5), 0.5 mM DTT, 4 mM spermidine, 0.5 mM EDTA, 10% glycerol, 25 mM NaCl, 25 mM KCl, 0.01 mM $ZnCl_2$, 0.125 mM dATP, 0.125 mM dCTP, 0.125 mM dGTP, 0.125 mM dTTP. Subsequently, the reaction mixtures were separated by electrophoresis (100 V, 4°C) on non-denaturing 6% polyacrylamide gels in 40 mM Tris−HCl (pH 7.5), 0.5 mM EDTA. All band shift experiments were carried out at least three times.

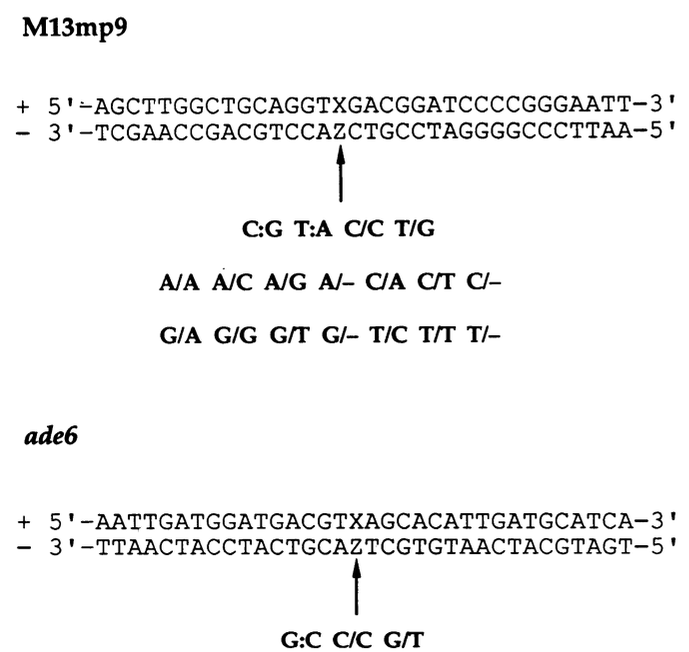

Figure 1. Substrates used for the detection of mismatch-specific protein binding. Two sets of double-stranded oligonucleotides were used. One set derives from the polylinker of phage M13mp9 (42), the second derived from the *ade6* gene of *S.pombe* (43). In both cases the upper strand is designated as plus (+) strand, the lower strand as minus (−) strand. The position containing a defined mismatch is designated with X/Z and is marked by an arrow. The matches and mismatches included in the two sets are given below the sequences.

RESULTS

Detection of two mismatch-binding activities

To detect mismatch-binding activities in *S.pombe*, we have carried out gel retardation assays as described in Materials and Methods. Cell free extracts of the wild-type strain 968 were incubated with radiolabeled oligonucleotides (34-mers) which contain a defined mismatch and the reaction mixtures were subsequently separated on non denaturing polyacrylamide gels. Binding of a protein to the 34-mer retards its migration through the gel and results in a shifted band. As probes for mismatch-specific protein binding, two sets of double-stranded oligonucleotides were used (Fig. 1). One set derives from the M13mp9 polylinker region (42). With this type of probes all possible single base-pair mismatches and the four single base loops (extra A, C, G or T) were represented and assayed. To distinguish between specific mismatch binding and general DNA binding, the two homoduplexes T=A and C=G were used as controls. The second set of substrates consists of 34-mers representing the DNA sequence flanking the *ade6-M387* mutation of *S.pombe* (26,43). Within this sequence mismatch repair has been quantified *in vivo* (26,27). This set includes the mismatches G/T and C/C as well as the G=C homoduplex.

We first analyzed the M13mp9 derived substrates containing either a T/G or a C/C mismatch. T/G is thought to be corrected efficiently by a major repair system of *S.pombe*, while C/C correction might be exclusively carried out by a postulated second pathway (26,27). Incubation of radiolabeled T=A homoduplex with protein extract, resulted in four distinct bands when no

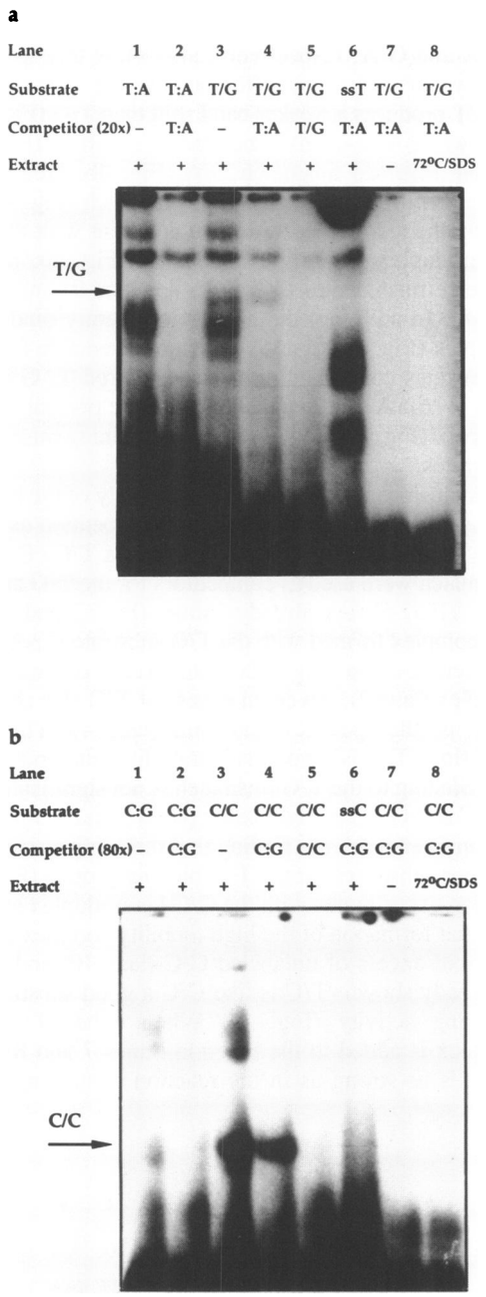

Figure 2. Detection of two mismatch-specific binding proteins. 40 fmol of radiolabeled oligonucleotides were incubated with 200 μg native proteins from extracts of the *S.pombe* wild-type strain 968. Subsequently, reaction mixtures were separated on non denaturing polyacrylamide gels. Protein–oligonucleotide complexes migrate more slowly than unbound oligonucleotides and are visualized by autoradiography. The bands at the top of the gels represent complexes which remained in the slots. (**a**) Detection of an activity, which specifically binds to T/G mismatches (lanes 3 and 4). The position is marked with an arrow. Reactions including extracts are designated with +, the reaction without extract is marked by –. In lane 8, the extract was inactivated prior to addition to the reaction mixture. Inactivation occurred by 15 min incubation at 72°C and subsequent addition of SDS to a final concentration of 1%. Reactions either contain no competitor DNA or a 20 fold excess of either unlabeled T:A homoduplex or unlabeled T/G heteroduplex. (**b**) Identification of the C/C binding activity. Unlabeled C:G or C/C containing oligonucleotides were used as competitors in an 80 fold excess with respect to the substrate. A 20 fold excess of unlabeled C/C heteroduplex does not drastically reduce specific binding to the C/C mismatch (data not shown). Therefore, in these and the following experiments, we used an 80 fold excess of competitor DNA for the analysis of the C/C binding activity.

unlabeled homoduplex was used as competitor (Fig. 2a, lane 1). When a 20 fold excess of unlabeled T=A was added to the reaction, only one of the four bands remained (lane 2). This and the other three activities (lane 1) reflect proteins that bind to the DNA substrates independent of the presence of a mismatch. When protein binding to the T/G heteroduplex was tested, an additional band appeared in the top half of the gel (lanes 3 and 4). This band is not detectable when single 'plus' strand (ssT) is used as substrate (lane 6). To show that the low mobility complex is due to mismatch binding, specific competition with a 20 fold excess of unlabeled T/G heteroduplex was carried out (lane 5). The protein–binding activity is sensitive to the T/G competitor (lane 5), but resistant to an equal amount of either homoduplex competitor (lane 4) or single 'plus' (ssT) strand (data not shown). This clearly indicates specificity for the T/G mismatch.

In the reaction mixture containing the C/C heteroduplex, a mismatch-specific binding complex with distinct mobility appeared in the bottom half of the gel (Fig. 2b, lanes 3 and 4). This band shift is absent in the controls with C=G homoduplex (lanes 1 and 2), and with single-stranded substrate (lane 6). The formation of this band shift is abolished by specific competition with unlabeled C/C heteroduplex (Fig. 2b, lane 5), but is unaffected when homoduplex (Fig. 2b, lane 4) or single 'plus' (ssC) strand (data not shown) are used as competitors. Both mismatch specific bands are absent when the reactions are carried out either without extracts (Fig. 2a, lane 7 and Fig. 2b, lane 7) or with inactivated extracts (Fig. 2a, lane 8 and Fig. 2b, lane 8). Thus, the two shifts are due to mismatch-specific binding of proteins present in the extracts. Their different mobility in the gels and their distinct binding specificities indicate that they might represent different mismatch recognition proteins.

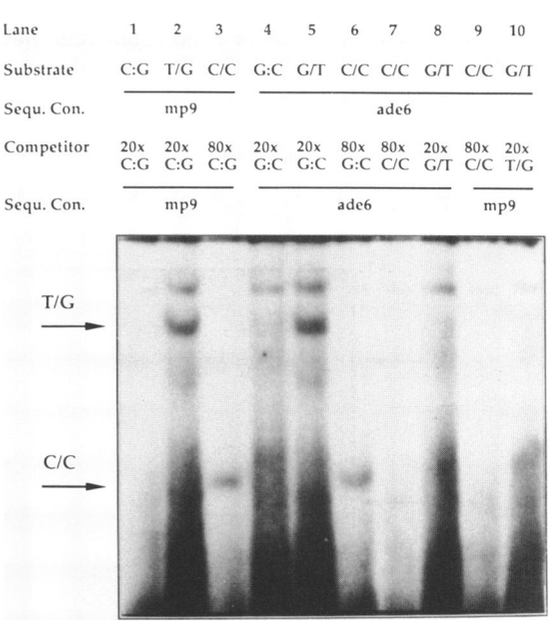

Figure 3. Binding of the mismatch-specific proteins to substrates with different sequence contexts (Sequ. Con.). Oligonucleotides derived from M13mp9 are marked by mp9, those with the sequence from the *ade6* gene are marked with ade6 (Fig. 1). The origin of the competitor DNA is indicated by the same symbols. The positions of the mismatch-specific bands are marked by arrows.

The two activities also recognize heteroduplexes with another sequence context

The initial experiments have revealed two mismatch-binding activities, one recognizes the T/G mismatch but not C/C, the second recognizes the C/C mismatch but not T/G (Fig. 2). To test whether these types of mismatches are also recognized when present in a different sequence context, we assayed the second set of oligonucleotide duplexes representing sequences from the *ade6* gene (Fig. 1). As shown in Figure 3 both activities appear in these assays. The activity binding to T/G (Fig. 2a; Fig. 3, lane 2) also binds to the G/T containing substrate derived from the *ade6* sequence (Fig. 3, lane 5) but not to C/C from either M13mp9 (lane 3) or *ade6* (lane 6). The high mobility complex found for the C/C mismatch within the M13mp9 sequence (Fig. 2b; Fig. 3, lane 3) is also present for the C/C substrate with the *ade6* context (lane 6) but is not formed with the G/T substrate (lane 5). Both specific band shifts are absent for the G=C homoduplex control (lane 4). The band shift found for the G/T probe is sensitive to competition with unlabeled G/T heteroduplex derived from *ade6* (lane 8) as well as to competition with T/G heteroduplex derived from the M13mp9 polylinker (lane 10). Similarly, competition with unlabeled C/C heteroduplex derived either from *ade6* or from the M13mp9 polylinker prevents the formation of the C/C specific band shift (lanes 7 and 9).

Specificity of the mismatch-binding activities

To further characterize the two activities, we determined their spectrum of mismatch recognition. All single base-pair mismatches and the four possible substrates containing a single base loop were tested (Fig. 4). The activity binding to T/G (Fig. 4a, lane 2; marked by the arrow above) also shows high to intermediate affinity to the mismatches T/C, T/−, A/−, G/−, G/G (Fig. 4a, lanes 3−7), C/−, T/T and A/A (Fig. 4b, lanes 4−5, 7). Binding to C/A and G/A substrates might be weaker (Fig. 4b, lanes 3 and 6), but there is no significant binding to C/C (Figures 2 and 3; Fig. 4b, lane 2). The heteroduplexes G/T, C/T, A/C and A/G, exposing the inversed mismatches of T/G, T/C, C/A and G/A, respectively, also show this specific band shift. However, there are differences in the intensity of some bands. G/T produces a weaker band shift than T/G (Fig. 4a, lanes 8 and 2), while A/G seems to be a better substrate (Fig. 4b, lane 9) than the inversed mismatch G/A (Fig. 4b, lane 6).

The band shift found for the C/C mismatch (Fig. 4b, lane 2; marked by the arrow below) is also apparent with the T/C and C/A heteroduplexes (Fig. 4a, lane 3 and Fig. 4b, lane 3) and their inverse mismatches C/T (Fig. 4a, lane 9) and A/C (Fig. 4b, lane 8). In addition, the activity efficiently binds the C/− heteroduplex (Fig. 4b, lane 4) but is not detectable for the other three substrates containing either an unpaired T, G or A (Fig. 4a, lanes 4−6). A faint band shift might be present for the T/T heteroduplex (Fig. 4b, lane 5), but all remaining mismatches are not substrates.

The conclusions about mismatch specificity of the two binding activities were strengthened by specific competition experiments. The four heteroduplexes containing either a T/G, C/C, T/C or G/A mismatch were used as competitors for the T/G and the C/C substrates (Fig. 5). As already shown (Fig. 2 and 3) the low mobility complex formed with the T/G substrate is not detectable in the reaction containing a 20 fold excess of unlabeled T/G heteroduplex (lane 2). An equal excess of T/C also abolishes the formation of the specific band shift (lane 4). This result is expected since T/C is a good substrate for this binding activity (Fig. 4). Binding to the T/G mismatch is not significantly altered by the C/C and G/A heteroduplexes (lanes 5 and 3, respectively). This is consistent with the finding that these mismatches are not or only poor substrates for the T/G binding protein (Fig. 4). The competition experiments with the C/C heteroduplex as substrate revealed that formation of the high mobility complex is sensitive to an 80 fold excess of unlabeled C/C (lane 10) and T/C (lane 9). As already shown, T/C is like C/C a good substrate for the C/C binding activity (Fig. 4). When either T/G or G/A heteroduplex is added to the reaction (lanes 7 and 8), the C/C band shift is as strong as in the reaction containing the C=G

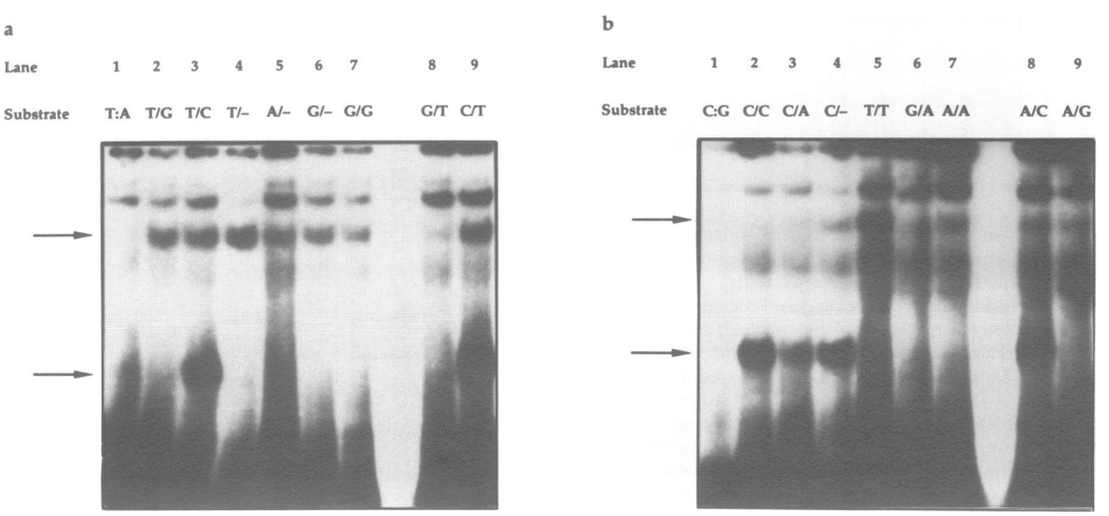

Figure 4. Mismatch specificity of the two identified activities. All possible single base-base mismatches and the four substrates with one unpaired base in the plus strand were tested. Reactions contained a 20 fold excess of unlabeled T:A homoduplex as competitor. (**a**) Substrates T:A, T/G, T/C, T/−, A/−, G/−, G/G, G/T and C/T (lanes 1−9). (**b**) Substrates C:G, C/C, C/A, C/−, T/T, G/A, A/A, A/C and A/G (lanes 1−9). The arrow above indicates the position of the low mobility complex, first detected for T/G; the arrow below indicates the position of the high mobility complex, first found for C/C (Fig. 2).

homoduplex as competitor (lane 6). This is consistent with the observation that the high mobility complex is absent when either T/G or G/A are used as substrates (Fig. 4).

The mismatch-specific activities are also present in crude extracts of a *swi4* mutant

In the *mutHLS* pathway of *E.coli* and *S.typhimurium* recognition and binding to mismatches is carried out by the MutS protein (1,3,4,6). In order to identify the genes encoding the mismatch-binding activities in *S.pombe* extracts, we started a search for mutants, in which one or both band shifts are abolished. One candidate is *swi4*, which was recently identified as a MutS homologue (33). Extracts of the *swi4* disruption mutant LH110 were incubated with substrates containing either the mismatches T/G, T/C, C/A or C/C and, as controls, with homoduplexes (Materials and Methods). Both mismatch-specific band shifts were found. With respect to wild type, no differences in gel mobility of the complexes, and specificity for the tested mismatches were detectable (data not shown). Thus *swi4* function is not necessary for formation of either of the two mismatch-specific complexes. One explanation for this result is that *swi4*, although homologous to MutS, is not involved in general mismatch repair.

DISCUSSION

The band shift assays have revealed the formation of two mismatch dependent complexes upon incubation of oligonucleotide heteroduplexes with protein extracts of *S.pombe* wild-type cells. Both are not detectable with homoduplex or single-stranded substrates (Fig. 2). In addition, competition with unlabeled homoduplex or single-stranded oligonucleotides do not prevent formation of the specific complexes. The two binding activities show differences in gel mobility and mismatch specificity. One activity, forming a low mobility complex, binds with varying efficiencies to T/G, T/C, C/T, T/T, T/−, A/−, C/−, G/−, G/G, A/A, A/C, A/G, G/T, G/A, and C/A mismatches in oligonucleotide duplexes derived from the M13mp9 sequence (Fig. 1). This activity does not bind to the C/C mismatch (Fig. 4). The second activity, observed as high mobility complex, strongly binds to C/C, T/C, C/T, C/A, A/C and C/−. Weak binding is also observed for the T/T mismatch, but no band shift is discernible with all other mismatches (Fig. 4). Thus this complex is preferentially formed with cytosine-containing mismatches and represents the first activity reported which is able to bind efficiently to C/C mismatches.

Both complexes were found with two different sets of oligonucleotide substrates representing distinct DNA sequences (Fig. 3). These data indicate that the mismatch-binding specificity of the activities is independent of sequence context. However, the intensity of the band formed by the low mobility complex is sensitive to the inversion of some mismatches within the same sequence context. In the M13mp9 sequence context, T/G and A/G seem to be better substrates than G/T and G/A, respectively (Fig. 4). These data suggest that the affinity of the respective binding activity to the mismatches may be influenced by flanking nucleotides.

The discovery of two mismatch specific band shift patterns raises the following question. Do they represent distinct complexes formed by the heteroduplex substrates and distinct, mismatch specific factors or do identical proteins form different complexes in a mismatch dependent fashion? Our data strongly support the former possibility. The two complexes do not only show different gel mobility but also clearly differ in mismatch specificity (Fig. 4). This is best supported by the competition experiment with several mismatch-containing oligonucleotides. Formation of the low mobility complex is not abolished by competition with C/C containing oligonucleotides, while the high mobility complex is resistant to competition with T/G containing oligonucleotides (Fig. 5).

The activity, forming the low mobility complex, seems to depend on a general mismatch-binding protein, with a similar recognition pattern as the bacterial MutS (1−3). Recently, a protein with a similar mismatch specificity was detected in nuclear extracts of *S.cerevisiae* (38). This activity is absent in extracts of *msh2* mutant cells, and might therefore be encoded by the MutS homologue *MSH2* (38). This finding is consistent with previous studies (21,25,29,37), implying that *S.cerevisiae* contains a mismatch-repair system which is similar to the bacterial *mutHLS* pathway. An equivalent mismatch-repair system was postulated for *S.pombe*. Based on tetrad data with various defined mutations, it was calculated that all mismatches, except C/C, are repaired by a major mismatch-repair system with an efficiency close to 100% (26,27). The mismatch specificity of the low mobility complex is consistent with this model and may classify the respective protein as a component of an *S.pombe* repair system homologous to the bacterial *mutHLS* pathway (1−3) and its related pathway in *S.cerevisiae* (25,29,37,38).

The genetic data also imply the presence of a second repair system, which is less efficient but is able to correct C/C mismatches (26,27). The high mobility complex is formed efficiently with C/C mismatches, suggesting that it may contain a component of the postulated minor pathway. While the tetrad analyses revealed repair efficiencies of mismatches during meiotic recombination, the band shift assays detected mismatch-binding proteins in extracts of vegetatively grown cells. Although the two approaches are quite different, they both indicate the existence

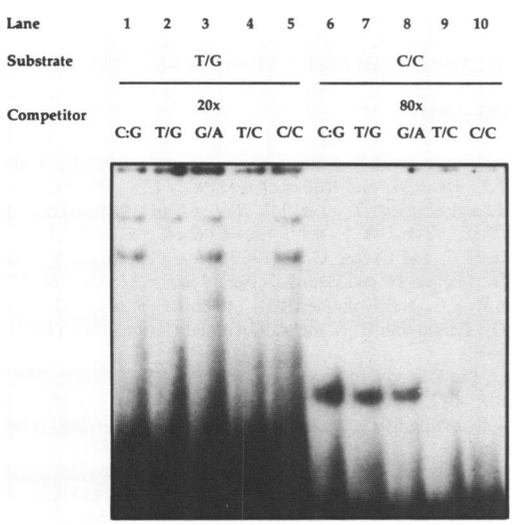

Figure 5. Specificity of the T/G and C/C binding activities determined by competition with different heteroduplexes. As competitors the C:G homoduplex (control) and heteroduplexes with one of the mismatches T/G, G/A, T/C or C/C were analyzed. A 20 fold excess was given to the reactions containing the T/G substrate (lanes 1−5), while an 80 fold excess was given to reactions with the C/C substrate (lanes 6−10).

of at least two mismatch-repair systems with different specificities (most pronounced for the C/C mismatch).

While the *mutHLS* pathway of *E.coli* and its related pathway in *S.cerevisiae* are not able to repair C/C mismatches (1–3,21,25,44), there is evidence that *S.cerevisiae* has an independent C/C correction system. *In vitro* assays revealed C/C mismatch repair accompanied by repair tracts of only 10–20 nucleotides (45). Both, the capability to correct C/C mismatches and the existence of short repair tracts are common with the features of the minor repair system proposed for *S.pombe* (26,27).

Two mismatch-specific activities have been described in nuclear extracts of humans. One protein, termed A/C binding activity, specifically binds to the mismatches A/C, T/C, C/T, T/T and weakly to C/C (46). Obviously, this human mismatch-binding protein has a recognition pattern similar to that of the C/C binding activity of *S.pombe* (Fig. 4). Both recognize a subset of mismatches, with a preference to those containing a cytosine. The second human binding activity first appeared to be specific for G/T mismatches (41), but after purification it turned out to bind also most of the other mismatches (24). Both human mismatch-binding activities were tested for their presence in mutant cell lines showing a mutator phenotype. While the A/C binding activity is present as in wild type, the G/T binding activity is not detectable (47). These results show a clear correlation between the lack of a general mismatch-binding activity and a mutator phenotype. It has been proposed that the G/T binding activity represents a human MutS homologue (24). Two possible candidates for this G/T binding activity are the proteins encoded by the *mutS* homologous genes *hMSH2* and *Duc-1* (30,31). While no function has been assigned to the *Duc-1* gene product, the *hMSH2* gene product seems to be an important suppressor of hereditary nonpolyposis colon cancer development (30,48,49).

The *mutS* homologue of *S.pombe, swi4* (33), was identified as a gene involved in mating-type switching (50,51). A *swi4* disruption mutant (33) was tested for the presence of the two mismatch-specific band shifts. Both activities were found as in wild type. In addition, the *swi4* mutant neither shows a mitotic mutator phenotype, nor an increased level of postmeiotic segregation of a genetic marker (P.Schär, O.Fleck and J.Kohli, unpublished results), two characteristics displayed by mutants defective in mismatch repair. The biochemical and genetic data imply that Swi4 is a MutS homologue which is not involved in general mismatch repair. With this aspect Swi4 resembles MSH3 of *S.cerevisiae* (36,38). MSH3 and Swi4 may be members of a subgroup of MutS proteins, required for cellular processes other than mismatch repair. Our data further suggest that, in analogy to *S.cerevisiae* and humans, *S.pombe* may have more than one MutS homologue.

Present work is directed to the isolation of additional MutS homologues of *S.pombe*. Cell extracts of the respective mutants can then be tested for the presence of the mismatch-specific activities. On the other hand, purification of the second activity, which preferentially binds to cytosine-containing mismatches should allow the identification of the respective gene and further characterization of the novel mismatch–repair pathway.

ACKNOWLEDGEMENTS

We thank Shaun Griffin for critical reading of the manuscript and him and Mike Baur for helpful discussions. This work was supported by the Swiss National Science Foundation. O.F. was additionally supported by a short term fellowship from the European Molecular Biology Organization, and subsequently by a Forschungsstipendium of the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Modrich,P. (1991) *Annu. Rev. Genet.*, **25**, 229–253.
2. Dohet,C., Wagner,R. and Radman,M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 503–505.
3. Su,S.-S., Lahue,R.S., Au,K.G. and Modrich,P. (1988) *J. Biol. Chem.*, **263**, 6829–6835.
4. Lahue,R.S., Au,K.G. and Modrich,P. (1989) *Science*, **245**, 160–164.
5. Cooper,D.L., Lahue,R.S. and Modrich,P. (1993) *J. Biol. Chem.*, **268**, 11823–11829.
6. Su,S.-S. and Modrich,P. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 5057–5061.
7. Welsh,K.M., Lu,A.-L., Clark,S. and Modrich,P. (1987) *J. Biol. Chem.*, **262**, 15624–15629.
8. Grilley,M., Welsh,K.M., Su,S.-S. and Modrich,P. (1989) *J. Biol. Chem.*, **264**, 1000–1004.
9. Claverys,J.-P., Mejean,V., Gasc,A.-M. and Sicard,A.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 5956–5960.
10. Lacks,S.A., Dunn,J.J. and Greenberg,B. (1982) *Cell*, **31**, 327–336.
11. Priebe,S.D., Hadi,S.M., Greenberg,B. and Lacks,S.A. (1988) *J. Bacteriol.*, **170**, 190–196.
12. Prudhomme,M., Martin,B., Mejean,V. and Claverys,J.-P. (1989) *J. Bacteriol.*, **171**, 5332–5338.
13. Lieb,M., Allen,E. and Read,D. (1986) *Genetics*, **114**, 1041–1060.
14. Lieb,M. (1987) *J. Bacteriol.*, **169**, 5241–5246.
15. Sohail,A., Lieb,M., Dar,M. and Bhagwat,A.S. (1990) *J. Bacteriol.*, **172**, 4214–4221.
16. Lu,A.-L. and Chang,D.Y. (1988) *Genetics*, **118**, 593–600.
17. Zell,R. and Fritz,H.J. (1987) *EMBO J.*, **6**, 1809–1815.
18. Radicella,J.P., Clark,E.A. and Fox,M.S. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 9674–9678.
19. Lu,A.-L. and Chang,D.Y. (1988) *Cell*, **54**, 805–812.
20. Michaels,M.L., Cruz,C., Grollman,A.P. and Miller,J.H. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 7022–7025.
21. Bishop,D.K., Andersen,J. and Kolodner,R.D. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 3713–3717.
22. Fang,W. and Modrich,P. (1993) *J. Biol. Chem.*, **268**, 11838–11844.
23. Holmes,J.J., Clark,S. and Modrich,P. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5837–5841.
24. Hughes,M.J. and Jiricny,J. (1992) *J. Biol. Chem.*, **267**, 23876–23882.
25. Kramer,B., Kramer,W., Williamson,M.S. and Fogel,S. (1989) *Mol. Cell. Biol.*, **9**, 4432–4440.
26. Schär,P. and Kohli,J. (1993) *Genetics*, **133**, 825–835.
27. Schär,P., Munz,P. and Kohli,J. (1993) *Genetics*, **133**, 815–824.
28. Varlet,I., Radman,M. and Brooks,P. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7883–7887.
29. Williamson,M.S., Game,J.C. and Fogel,S. (1985) *Genetics*, **110**, 609–646.
30. Fishel,R., Lescoe,M.K., Rao,M.R.S., Copeland,N.G., Jenkins,N.A., Garber,J., Kane,M. and Kolodner,R. (1993) *Cell*, **75**, 1027–1038.
31. Fujii,H. and Shimada,T. (1989) *J. Biol. Chem.*, **264**, 10057–10064.
32. Linton,J.P., Yen,J.-Y., Selby,E., Chen,Z., Chinsky,J.M., Liu,K., Kellems,R.E. and Crouse,G.F. (1989) *Mol. Cell. Biol.*, **9**, 3058–3072.
33. Fleck,O., Michael,H. and Heim,L. (1992) *Nucl. Acids Res.*, **20**, 2271–2278.
34. Reenan,R.A.G. and Kolodner,R.D. (1992a) *Genetics*, **132**, 963–973.
35. Valle,G., Bergantino,E., Lanfranchi,G. and Carignani,G. (1991) *Yeast*, **7**, 981–988.
36. New,L., Liu,K. and Crouse,G.F. (1993) *Mol. Gen. Genet.*, **239**, 97–108.
37. Reenan,R.A.G. and Kolodner,R.D. (1992b) *Genetics*, **132**, 975–985.
38. Miret,J.J., Milla,M.G. and Lahue,R.S. (1993) *J. Biol. Chem.*, **268**, 3507–3513.
39. Gutz,H., Heslot,H., Leupold,U. and Loprieno,N. (1974) In King,R.C. (ed.), Handbook of Genetics. Plenum Press, New York, London. Vol. I pp. 395–446.
40. Bradford,M. (1976) *Anal. Biochem.*, **72**, 248–254.
41. Jiricny,J., Hughes,M., Corman,N. and Rudkin,B.B. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8860–8864.
42. Messing,J. and Vieira,J. (1982) *Gene*, **19**, 269–276.
43. Skankasi,P., Heyer,W.-D., Schuchert,P. and Kohli,J. (1988) *J. Mol. Biol.*, **204**, 917–925.
44. Fox,M.S., Radicella,J.P. and Yamamoto,K. (1994) *Experientia*, **50**, 253–260.

45. Muster-Nassal,C. and Kolodner,R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 7618–7622.
46. Stephenson,C. and Karran,P. (1989) *J. Biol. Chem.*, **264**, 21177–21182.
47. Branch,P., Aquilina,G., Bignami,M. and Karran,P. (1993) *Nature*, **362**, 652–654.
48. Leach,F.S., Nicolaides,N.C., Papadopoulos,N., Liu,B., Jen,J., Parsons,R., Peltomäki,P., Sistonen,P., Aaltonen,L.A., Nyström-Lahti,M., Guan,X.-Y., Zhang,J., Meltzer,P.S., Yu,J.-W., Kao,F.-T., Chen,D.J., Cerosaletti,K.M., Fournier,R.E.K., Todd,S., Lewis,T., Leach,R.J., Naylor,S.L., Weissenbach,J., Mecklin,J.-P., Järvinen,H., Petersen,G.M., Hamilton,S.R., Green,J., Jass,J., Watson,P., Lynch,H.T., Trent,J.M., de la Chapelle,A., Kinzler,K.W. and Vogelstein,B. (1993) *Cell*, **75**, 1215–1225.
49. Parsons,R., Li,G.-M., Longley,M.J., Fang,W.-h., Papadopoulos,N., Jen,J., de la Chapelle,A., Kinzler,K.W., Vogelstein,B. and Modrich,P. (1993) *Cell*, **75**, 1227–1236.
50. Egel,R., Beach,D.H. and Klar,A.J.S. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3481–3485.
51. Gutz,H. and Schmidt,H. (1985) *Curr. Genet.*, **9**, 325–331.