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Preferential strand transfer and hybrid DNA formation at the recombination hotspot *ade6-M26* of *Schizosaccharomyces pombe*

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The *ade6-M26* mutation of *Schizosaccharomyces pombe* stimulates intragenic and intergenic meiotic recombination. *M26* is a single base pair change creating a specific heptanucleotide sequence that is crucial for recombination hotspot activity. This sequence is recognized by proteins that may facilitate rate-limiting steps of recombination at the *ade6* locus. To start the elucidation of the intermediate DNA structures formed during *M26* recombination, we have analyzed the aberrant segregation patterns of two G to C transversion mutations flanking the heptanucleotide sequence in crosses homozygous for *M26*. At both sites the level of post-meiotic segregation is typical for G to C transversion mutations in *S.pombe* in general. Quantitative treatment of the data provides strong evidence for heteroduplex DNA being the major recombination intermediate at the *M26* site. We can now exclude a double-strand gap repair mechanism to account for gene conversion across the recombination hotspot. Furthermore, the vast majority (>95%) of the heteroduplexes covering either of the G to C transversion sites are produced by transfer of the transcribed DNA strand. These results are consistent with *ade6-M26* creating an initiation site for gene conversion by the introduction of a single-strand or a double-strand break in its vicinity, followed by transfer of the transcribed DNA strands for heteroduplex DNA formation.

Key words: fission yeast/heteroduplex DNA/M26 hotspot/mismatch repair

Introduction

Homologous recombination contributes to genetic diversity and is essential for proper chromosome segregation in meiosis. The responsible mechanisms have been studied extensively in ascomycetous fungi amenable to classical genetics and molecular analyses. Tetrad analysis allows the fate of the four chromatids to be followed in a diploid cell undergoing meiosis. It reveals the various types of non-Mendelian segregation that have led to the proposal of mechanistic models. Some of the predicted intermediate DNA structures have been validated by physical analysis of DNA extracted from meiotic cells, reviewed by Petes *et al.* (1991). Two types of non-Mendelian (aberrant) segregations are observed. One is the non-reciprocal transfer of the information of both DNA strands of a donor chromatid to one chromatid of the homologous chromosome, producing the segregation types $6^+:2^-$ or $2^+:6^-$ (numbers referring to the eight DNA single strands in a tetrad). They will be called whole chromatid conversion (WCC). When only the information of one DNA strand is transferred to a homologous chromatid, post-meiotic segregation (PMS) occurs. PMS tetrads most frequently contain one ($5^+:3^-$ and $3^+:5^-$ segregations) or rarely two haploid spores (aberrant $4^+:4^-$) that segregate both parental alleles of a heterozygous marker in the first mitotic division after meiosis.

PMS tetrads envision the formation of heteroduplex DNA (hDNA) which can be achieved by either asymmetric transfer of one DNA strand or symmetric transfer of two DNA single strands. In *Saccharomyces cerevisiae*, the segregation patterns characteristic for symmetric hDNA formation (aberrant 4:4, aberrant 5:3 and 4:4 apparent two-strand double crossover) are rare and match the frequencies predicted for two independent asymmetric events (Fogel *et al.*, 1979, 1981; Detloff *et al.*, 1991). This implies asymmetric hDNA formation in budding yeast. So far the tetrad data from *Schizosaccharomyces pombe* (Gutz, 1971; Thuriaux *et al.*, 1980; Schär *et al.*, 1993) allow no distinction between symmetric and asymmetric hDNA formation.

Two hypotheses have been put forward to explain WCC. The first assumes hDNA formation initiated by a DNA single-strand (ss) break, followed by mismatch repair. Repair is either toward recipient chromatid information (restoration) or toward donor chromatid information (WCC) (Holliday, 1964; Meselson and Radding, 1975; Porter *et al.*, 1993). The alternative is conversion by repair of double-strand (ds) gaps with sequences copied from the homologous chromatid. The initiating lesion in this model is a DNA ds break (Szostak *et al.*, 1983). Several lines of evidence favor hDNA formation for the major part of fungal genes. First, the PMS/WCC ratio depends on the type of mismatches created at a mutant site, rather than on the position of a mutation in genes showing a gradient of gene conversion (Paquette and Rossignol, 1978; Fogel *et al.*, 1981; White *et al.*, 1985; Detloff *et al.*, 1991; Schär *et al.*, 1993). Second, the frequencies of PMS and WCC appear to be dependent. High-PMS alleles usually display increased PMS at the expense of WCC relative to nearby low-PMS alleles (Fogel *et al.*, 1979; Nag *et al.*, 1989; Lichten *et al.*, 1990; Schär *et al.*, 1993). Third, yeast strains defective in mismatch repair enzymes show an increased frequency of PMS and a decreased frequency of WCC for most of the alleles analyzed (Williamson *et al.*, 1985; Bishop *et al.*, 1989; Kramer *et al.*, 1989; Reenan and Kolodner, 1992). These observations are best explained by mismatch-specific processing of hDNA.

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Nevertheless, a role for DNA ds breaks as initiating lesions has been strengthened by the discovery of transient ds breaks in meiotic prophase of *S.cerevisiae*. They are correlated with the occurrence of aberrant segregation (Sun *et al.*, 1989, 1991; Liang *et al.*, 1990; De Massy and Nicolas, 1993; Nag and Petes, 1993; Wu and Lichten, 1994). The same studies also provided evidence for the absence of a ds gap. Exonucleolytic degradation was observed, but only on the DNA strands exposing a 5′ end, leaving long tails of ss DNA with a 3′ end. Hence, recent versions of the ds gap repair model have reduced the ds gap to a ds break and consequently explain WCC by hDNA formation and mismatch repair (Sun *et al.*, 1991; Nicolas and Petes, 1994). However, the physically detectable ds breaks cannot explain all the data in a quantitative way and suggest the existence of pathways involving ss breaks (Nag and Petes, 1993; Nicolas and Petes, 1994).

Recombination hotspots and coldspots, and gradients of gene conversion frequencies across many genes indicate non-random initiation of meiotic recombination. The currently favored idea is that initiation occurs upon the introduction of a DNA lesion at specific sites (hotspots). Hybrid DNA is then formed by single-strand transfer and expanded in one or both directions to random distances from the initiation site, resulting in gradients of aberrant segregation. Examples of extensively studied hotspots for meiotic recombination are the promoter regions of the *S.cerevisiae ARG4* and *HIS4* genes (Nicolas *et al.*, 1989; Detloff *et al.*, 1992; Nicolas and Petes, 1994). In *S.pombe*, the *ade6-M26* allele generates a hotspot for meiotic recombination. Analysis of spore tetrads from crosses with a wild-type *ade6* allele shows that *M26* undergoes gene conversion ~10 times more frequently than does the closely linked *M375* mutation (5% versus 0.5%). In contrast to *M375*, *M26* displays a marked disparity of gene conversion. Chromatids containing *M26* are converted eight times more frequently to wild type than chromatids with the wild-type gene to *M26*. *M26* stimulates co-conversion of other mutations to its right and to its left, or both. In intragenic crosses with other *ade6* alleles, *M26* recombines at a 3- to 15-fold higher frequency than does *M375* (Gutz, 1971). *M26* also stimulates crossover (Grimm *et al.*, 1994) and it is active in heterozygous and homozygous crosses (Goldman and Smallets, 1979; Ponticelli *et al.*, 1988), indicating independence from mismatch correction. Nucleotide sequence analysis revealed that *M26* is a G to T transversion mutation (Ponticelli *et al.*, 1988; Szankasi *et al.*, 1988). This single base pair (bp) change creates an opal (5′ UGA 3′) suppressible stop codon, and the heptanucleotide 5′ A̲TG-ACGT 3′ required for the hotspot activity, as shown by site-directed *in vitro* mutagenesis (Schuchert *et al.*, 1991). Proteins binding to this heptanucleotide sequence have been purified recently (Wahls and Smith, 1994).

In the present study, hDNA formation has been investigated at the *M26* recombination hotspot. We have reported previously that G to C transversion mutations give rise to high levels of PMS due to inefficient C/C mismatch correction. Those studies also revealed that the repair efficiencies of the two conjugate mismatches C/C and G/G are remarkably constant and sequence context independent in *S.pombe* (Schär *et al.*, 1993). We have exploited these findings and analyzed the aberrant segregation patterns (PMS; WCC) of a G to T and two G to C transversion mutations flanking the *M26* heptanucleotide. The results are compared with those from other G to C transversions located far downstream of *M26* and in other genes of *S.pombe*. We report evidence for extensive hDNA formation at *M26* and for preferential transfer of the transcribed DNA strands between the chromatids.

Results

PMS unambiguously indicates hDNA formation, while WCC can result from either ds gap repair or hDNA formation followed by mismatch repair. Thus, mutations displaying little PMS are not useful for the genetic investigation of recombination intermediates. The level of PMS at a mutant site depends on the frequency of hDNA formation and the efficiency of repair of the two conjugate mismatches created. In *S.pombe*, most base substitutions show very low frequencies of PMS (Schär *et al.*, 1993). This is also true for the hotspot mutation *ade6-M26*, a G to T transversion producing $<2\%$ PMS (0 PMS/52 WCC) among total aberrant tetrads in crosses with the wild-type allele (Gutz, 1971). Exceptional are the G to C transversions. They generate the conjugate mismatches C/C and G/G, and show increased PMS, most likely due to poor C/C mismatch correction (Schär *et al.*, 1993). To monitor hDNA formation around *M26*, we investigated the segregation patterns of two G to C transversions, one being 2 bp upstream (08C) and the other 6 bp downstream of *M26* (16C). As a control we used a G to T transversion (16T) changing the same position as 16C. Figure 1 illustrates the three crosses A, B and C carried out. Common to all is homozygosity for *M26* and *sup9-UGA* (Table I). *sup9-UGA* suppresses the opal codon created by the *M26* mutation and, hence, confers a wild-type phenotype (Ade$^+$, white) to the cells. This configuration allows the observation of the mutant phenotypes of the G to C (crosses A and C) and G to T (cross B) transversions of interest. For simplicity we refer to the suppressed *ade6-M26* allele as wild-type and to the adenine auxotrophic *ade6-M26/X* as mutant allele or chromatid.

The tetrad data obtained with the three crosses A, B and C are listed in Table II. The total aberrant segregation frequencies (combined total WCC + PMS) are similar in all crosses and vary between 6 and 7.5%. As expected, the two G to C transversion alleles (crosses A and C) perform similarly. They show significantly higher levels of PMS than the G to T transversion (A, 2.0% versus B, 0.41%; $P \leqslant 0.00054$). The ratios between PMS and WCC (PMS/WCC) are 0.36 for both G to C transversion alleles and 0.07 for the G to T transversion. In the following, we use these values as a relative measure for mismatch correction efficiency.

More insight emerges from the comparison of the aberrant segregation patterns revealed by the wild-type and the mutant chromatids of the individual crosses. The former produce the $2^+{:}6^-$, $0^+{:}8^-$ and $3^+{:}5^-$, and the latter the inverse $6^+{:}2^-$, $8^+{:}0^-$ and $5^+{:}3^-$ segregations. The tetrad data in Table II are grouped according to this classification. In cross A, the comparison shows that the total number of events (WCC + PMS) is about the same in both chromatids, 4.2% versus 3.3%, respectively. This indicates parity of aberrant segregation at the 16C site.

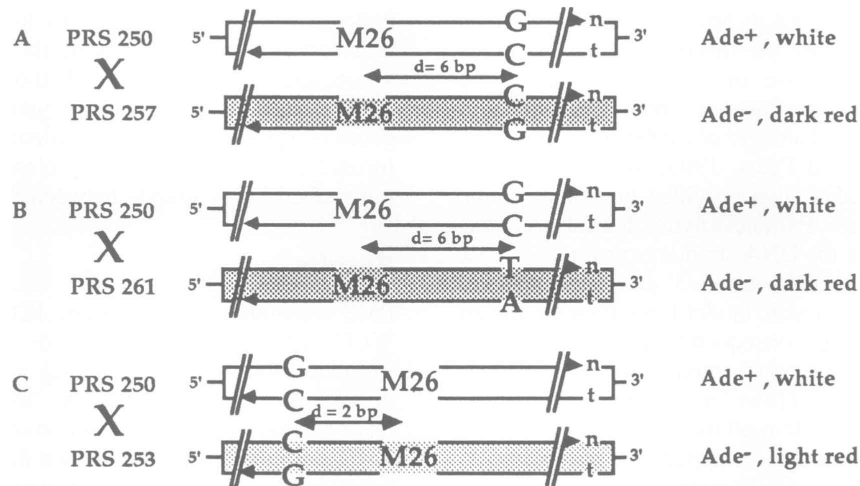

Fig. 1. Configurations at *ade6-M26* in crosses A, B and C. The *M26* region is schematically represented for each cross. The strain designations are shown on the left side and their relevant phenotypes on the right side of the boxes. The shading of the boxes corresponds to the colony phenotype displayed by the parental *ade6* alleles (white, dark red, light red). *M26* is homozygous in the crosses as indicated within the boxes. The positions of the G to C transversions (crosses A and C) and the G to T transversion (cross B) are shown within the boxes and their distance to *M26* by the distance arrows between the boxes. The transcribed and non-transcribed strands of the alleles are distinguished by the letters t and n, respectively, and their 5′–3′ polarity is indicated by arrows.

Table I. *Schizosaccharomyces pombe* strains used

Strain	Genotype	Source or reference
M26/08C	h^- *ade6-M26/08C*	Schuchert *et al.* (1991)
M26/16C	h^- *ade6-M26/16C*	Schuchert *et al.* (1991)
M26/16T	h^- *ade6-M26/16T*	Schuchert *et al.* (1991)
PRS 250	h^+ *ade6-M26 sup9-UGA ura4-D18*	this study
PRS 253	h^- *ade6-M26/08C sup9-UGA ura4-D18*	this study
PRS 257	h^- *ade6-M26/16C sup9-UGA ura4-D18*	this study
PRS 261	h^- *ade6-M26/16T sup9-UGA ura4-D18*	this study

The difference of PMS between the chromatids is striking; 1.8% PMS in the mutant chromatid is significantly higher than 0.22% in the wild-type chromatid ($P \leq 0.00001$). In contrast, the WCC frequency in the mutant chromatid is slightly lower (2.4%) than in the wild-type chromatid (3.1%), presumably reflecting a compensation for the higher PMS frequency. As a consequence, the PMS/WCC ratio in the mutant chromatid (0.74) is much higher than in the wild-type chromatid (0.070), indicating differences between the two chromatids in either mismatch repair or hDNA formation. Cross B displays the segregation pattern of a G to T transversion (16T) at the same site as the G to C transversion analyzed with cross A. The total number of events (WCC + PMS) also indicates parity of gene conversion (2.5% versus 3.5%). In contrast to cross A, the comparison of the chromatid segregation patterns (WCC and PMS) reveals similarity (Table II). Almost identical values result for the PMS/WCC ratios (0.071 and 0.077). They match the wild-type chromatid value (0.070), but not the clearly higher mutant chromatid value (0.74) of cross A. Cross C again involves a G to C transversion (Figure 1). As in crosses A and B, there is no evidence for disparity of gene conversion when the total aberrant segregation frequencies are compared between the two chromatids (2.7% versus 3.3%, Table II). Yet, as in cross A there is a significant bias between the frequencies of $5^+:3^-$ (1.3%) and $3^+:5^-$ tetrads (0.32%). Hence, the PMS/WCC ratios also differ, with 0.89 for the mutant and 0.11 for the wild-type chromatid.

The overall comparison of the PMS/WCC ratios obtained for the individual chromatids of crosses A, B and C reveals two distinct classes. The first consists of the mutant chromatids of crosses A (0.74) and C (0.89), and the second of all other chromatids studied, the wild-type chromatids of crosses A (0.070) and C (0.11), and both chromatids of cross B (0.071, 0.077). Also interesting is the comparison of the distribution of $5^+:3^-$ to $3^+:5^-$ tetrads between the three crosses A (25:3), B (2:3) and C (8:2). The statistical tests reveal a significant difference between crosses A and B ($P \leq 0.032$), but identity when the two G to C transversions analyzed with crosses A and C are compared ($P = 0.5$).

Discussion

G to C transversion mutations exhibit increased levels of PMS in one-factor crosses in *S.cerevisiae* (White *et al.*, 1985; Lichten *et al.*, 1990; Detloff *et al.*, 1991) as well as in *S.pombe* (Kohli *et al.*, 1984; Schär *et al.*, 1993). We have exploited this peculiarity for the investigation of hDNA formation at the recombination hotspot mutation *ade6-M26* of *S.pombe*, and performed tetrad analyses with two G to C transversions and a control mutation (G to T) flanking the hotspot heptanucleotide. The results reveal

Table II. Tetrad analyses

Cross type	Total no. of tetrads	Aberrant events on mutant chromatid					Aberrant events on wild-type chromatid					Combined total		
		WCC			PMS	WCC + PMS	WCC			PMS	WCC + PMS	WCC	PMS	WCC + PMS
		$6^+:2^-$	$8^+:0^-$	Σ	$5^+:3^-$		$2^+:6^-$	$0^+:8^-$	Σ	$3^+:5^-$				
A	1395	30	1	34	25	59	35	2	43	3	46	77	28	105
%				2.4	1.8	4.2			3.1	0.22	3.3	5.5	2.0	7.5
(±SD)				(±0.41)	(±0.36)	(±0.54)			(±0.46)	(±0.12)	(±0.48)	(±0.61)	(±0.38)	(±0.71)
B	1205	28		28	2	30	39		39	3	42	67	5	72
%				2.3	0.17	2.5			3.2	0.25	3.5	5.6	0.41	6.0
(±SD)				(±0.43)	(±0.12)	(±0.45)			(±0.51)	(±0.14)	(±0.53)	(±0.66)	(±0.19)	(±0.68)
C	635	5	1	9	8	17	19		19	2	21	28	10	38
%				1.4	1.3	2.7			3.0	0.32	3.3	4.4	1.6	6.0
(±SD)				(±0.47)	(±0.44)	(±0.64)			(±0.68)	(±0.22)	(±0.71)	(±0.81)	(±0.49)	(±0.94)

Segregation patterns refer to each of the eight DNA single strands in the four spores. The cross types A, B and C are illustrated in Figure 1. SD, standard deviation (see Materials and methods). $8^+:0^-$ and $0^+:8^-$ segregations are considered as two WCC events. To account for undetected double WCC events in $4^+:4^-$ tetrads (2×WCC in opposite directions), the numbers of the corresponding detectable events ($8^+:0^-$ and $0^+:8^-$) are multiplied by two.

valuable insight into the *M26*-dependent gene conversion mechanism.

The three crosses A, B and C illustrated in Figure 1 are homozygous for the *M26* mutation and the *sup9-UGA* allele. This allows the observation of *M26*-stimulated aberrant segregation at the close G to C and G to T transversion sites. The frequencies of total aberrant segregation are similar in all three crosses and vary between 6.0 and 7.5%. We also notice parity of gene conversion, although different segregation patterns (PMS/WCC ratios) are displayed by the crosses, as well as by the individual chromatids of a cross (Table II). *M26* in heterozygous crosses with a wild-type strain has previously been shown to give rise to aberrant segregation frequencies between 3.8 and 6.5% (95% confidence limits) (Gutz, 1971). Of these, 3.2–5.8% occur in the *M26*-carrying chromatid and only 0.1–1% in the wild-type chromatid (indicating the *M26*-independent level of gene conversion at the *ade6* gene). This corresponds to an average 8-fold disparity of *M26* conversion to wild-type. Our results strengthen earlier evidence (Goldman and Smallets, 1979) that homozygosity of *M26* in a cross has an additive effect on the aberrant segregation frequencies and also restores parity of gene conversion. The frequencies of aberrant segregation for the individual chromatids of all three crosses (Table II) fit well with the confidence limits given above for the conversion of *M26* to wild-type. However, the frequencies obtained for the mutant chromatids in crosses B and C appear to be slightly lower than those of all other chromatids. This trend is expected and confirms the previously observed minor effects of the 16T and 08C mutations on *M26* activity (Schuchert *et al.*, 1991). We suggest therefore that in crosses homozygous for *M26*, aberrant segregation in both chromatids is stimulated to the level of the *M26*-carrying chromatid in heterozygous crosses. This is consistent with the idea that *M26* acts as a recombinator *in cis* and therefore can be used homozygously to enhance gene conversion symmetrically.

Hybrid DNA formation at M26

Crosses A and C involve G to C transversion mutations and exhibit increased levels of PMS. The PMS/WCC ratios, expressing a relative measure of mismatch repair efficiency, are identical (0.36) for both alleles and remarkably similar to the values obtained for all other G to C transversion mutations studied so far in *S.pombe* (*ade6-M387* = 0.38, *sup3-UGA* = 0.36, *sup3-UGA,CA52* = 0.35) (Schär *et al.*, 1993). These similarities are most easily explained by uniform repair efficiencies for identical mismatches, as well as constant ratios of hDNA versus ds gap formation at the sites studied. Minor changes in either or both of the parameters would cause a considerable divergence of the PMS/WCC ratios measured at different sites. For instance, an increase in mismatch repair efficiency would decrease the PMS/WCC ratio and the same holds for an increase of ds gap formation at the expense of hDNA formation. Theoretically, the stability of the PMS/WCC ratios can also be achieved if both parameters are variable but the changes cancel each other. However, this is a less parsimonious explanation than constant mismatch repair efficiencies and hDNA versus ds gap formation. Given this, it is most interesting to observe the near identity of the PMS/WCC ratios at *ade6-M26* and at *ade6-M387*, a G to C transversion 1135 bp downstream of *M26*. This implies that there is no difference in hDNA versus ds gap formation between sites located very close to an initiation region of gene conversion and other sites far away. This finding is incompatible with extension of ds breaks at or near *M26* to gaps reaching into the *ade6* gene, as this would lower the PMS/WCC ratios for mutations located close to the recombination initiation site.

This leaves us with two questions. (i) Does the initiating event stimulated by *M26* take place at or around the *M26* site, or is it so far away that a presumed ds gap would never reach the *M26* mutation? (ii) Is the initiating lesion a double- or single-strand break? Early genetic data already indicated a polarity of gene conversion in two-factor crosses of *M26* against other *ade6* mutations. *M26* was the more often converted allele in all crosses analyzed (Gutz, 1971). This defined an initiation point in the 5′ region of the *ade6* gene. Later, the physical analysis of gene conversion tracts at *ade6* confirmed the earlier observations and, more importantly, made the possibility

of initiation far outside in the 5′ region of the gene highly unlikely (Grimm *et al.*, 1994). All in all, the genetic and physical data are consistent with the concept that the initiating event occurs close to *M26*. Our data do not allow any statement about the nature of the initiating lesion. Various attempts to identify ds or ss breaks at *M26* in DNA extracted from meiotic cells failed (Bähler *et al.*, 1991). As the predicted frequency of DNA breaks at *M26* is close to the detection limit of the methods applied, either or both types of lesions may be produced. However, by the reasoning given above, even if ds breaks occur close to *M26*, they are not extended to gaps reaching the position of *M26*. Hence, ds gaps cannot account for the gradient observed with *M26*-stimulated aberrant segregation.

Preferential strand transfer at M26

The total numbers of aberrant segregations in the mutant and wild-type chromatids are similar and indicate parity of gene conversion (Table II). Yet, a significant bias shows when the PMS frequencies in the different chromatids are compared. The crosses involving G to C transversions (A and C) show a significant excess of $5^+:3^-$ over $3^+:5^-$ PMS tetrads. No such bias is seen in the control cross B (G to T transversion). As the difference in the distribution of $5^+:3^-/3^+:5^-$ between the crosses A (25:3) and B (2:3) is significant ($P \leqslant 0.032$), a mutant chromatid-specific mismatch repair deficiency, or any other chromatid-specific bias, is unlikely to account for the divergence. Thus, the phenomenon depends on the presence of a G to C transversion and is therefore mutation specific. We have previously reported on the special behavior of G to C transversions in one-factor and intragenic two-factor crosses, and argued that it is a consequence of poor repair of C/C mismatches, while repair of the conjugate G/G mismatches occurs with high efficiency (Schär and Kohli, 1993; Schär *et al.*, 1993). Given this and the observations that (i) an equal number of events are initiated in the mutant and wild-type chromatids (parity of aberrant segregation), (ii) mismatch repair efficiencies are site independent (constant PMS/WCC ratios for identical mutations at various places) and (iii) the bias of $5^+:3^-/3^+:5^-$ is mutation and not chromatid or DNA strand specific (no bias in the control cross), the best explanation is preferential asymmetric transfer of the transcribed DNA strand from the donor chromatid. As illustrated in Figure 2, this preferentially produces C/C mismatches in the mutant chromatid which, since poorly repaired, produce a high level of $5^+:3^-$ PMS tetrads, and G/G mismatches in the wild-type chromatid only rarely giving $3^+:5^-$ tetrads due to efficient correction.

This explanation supposes that hDNA formation in *S.pombe* is asymmetric. Assuming symmetric hDNA formation, the explanation of the excess of $5^+:3^-$ over $3^+:5^-$ needs more complicated assumptions. Either preferential transfer of the transcribed strands coupled with a strong bias of G/G correction towards restoration in the wild-type chromatid, or preferential transfer of the non-transcribed strands with a strong bias of G/G repair towards conversion in the mutant chromatid, could then account for the bias. We consider these explanations highly unlikely.

Given the above reasoning, the extent of the strand transfer bias can be estimated by calculation of the overall mismatch repair efficiencies for each chromatid and comparison with the previous estimates for C/C and G/G repair efficiencies. Earlier we expressed the compound repair efficiency for both conjugate mismatches as R = 100 (2WCC/2WCC + PMS) (Schär *et al.*, 1993). Applied to the tetrad data in Table II, 85% overall repair efficiency results for crosses A and C, and 96% for cross B if the events of both chromatids are taken into account. These results fit perfectly with the correction efficiencies obtained from other crosses involving G to C transversions (*sup3-UGA* = 84%, *sup3-UGA,CA52* = 84%, *ade6-M387* = 84%) and non-G to C transversion mutations (96–98%) (Schär *et al.*, 1993). As predicted from the PMS/WCC ratios, the individual repair efficiencies calculated for the mutant and wild-type chromatids of cross B are identical (96%), while they clearly differ between the mutant and wild-type chromatids of the crosses A (73% versus 97%) and C (68% versus 95%). The mutant chromatid repair efficiencies in the latter crosses are remarkably close to the previous estimates for C/C mismatch correction in *S.pombe* (73%), and the wild-type chromatid efficiencies are in the range estimated for well-repaired mismatches (>95%) (Schär *et al.*, 1993). This strongly suggests that >95% of the mismatches generated in the mutant chromatids are C/C, while in the wild-type chromatids >95% are G/G. Thus, the preference in *M26*-dependent recombination is >9:1 for transfer of the transcribed strand of the donor chromatid.

Preferential strand transfer in meiotic recombination has also been found at two loci of *S.cerevisiae*. The *arg4-nsp* mutation, a G to C transversion in the start codon of the *ARG4* gene, shows a 3- to 4-fold excess of $3^+:5^-$ over $5^+:3^-$ segregations, indicating preferential transfer of the non-transcribed strands between the chromatids (Lichten *et al.*, 1990). Nag and Petes (1990) analyzed palindromic insertion mutations in the promoter region of the *HIS4* gene, producing very poorly repaired conjugate mismatches in hDNA. With an experimental set up that allows the identification of the transferred strand on the basis of spore germination, they found that the non-transcribed strands were transferred with a frequency of 0.83 and the transcribed strands with a frequency of 0.17. It seems that preferential transfer of one DNA strand in the vicinity of recombination initiation sites is a common observation. However, it is not yet clear whether the opposite strand transfer preference observed in fission and budding yeast is due to an overall difference of recombination mechanisms in the two yeasts, or whether it simply reflects the view on different mutational positions relative to the initiating lesions (see below).

Implications for the mechanism of M26 recombination

As we do not know the nature and position of the recombination-initiating lesion at *M26*, several possibilities can be considered for the mechanistic explanation of the observed strand transfer preference. The basic idea is outlined in Figure 3. The most straightforward explanation is a DNA strand-specific mechanism for initiation of hDNA formation in the acceptor chromatid. Both a ss break introduced in the transcribed strand or a ds break without degradation of the non-transcribed strand are

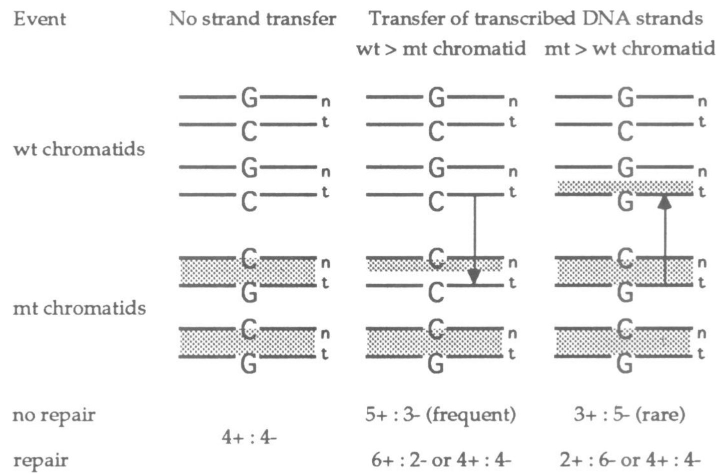

Fig. 2. The transcribed strand at *M26* is preferentially transferred. The eight DNA strands present in the four chromatids of a cross between a wild-type (white) and a G to C transversion (shaded) *ade6* allele are drawn. The transcribed and non-transcribed strands are distinguished by t and n, respectively. No transfer of the DNA strands produces Mendelian segregation (left tetrad). Transfer of the transcribed strand from a wild-type (wt) to a mutant (mt) chromatid (arrow, tetrad in the middle) produces C/C mismatches in the mutant chromatid. Transfer of the transcribed strand from a mutant to a wild-type chromatid shown in the tetrad on the right side produces G/G mismatches in the wild-type chromatid. Since C/C mismatches are less efficiently corrected than G/G mismatches, more spores containing hDNA emerge from the former situation than from the latter. Thus, more 5^+:3^- than 3^+:5^- tetrads are produced, as indicated in the bottom part of the figure.

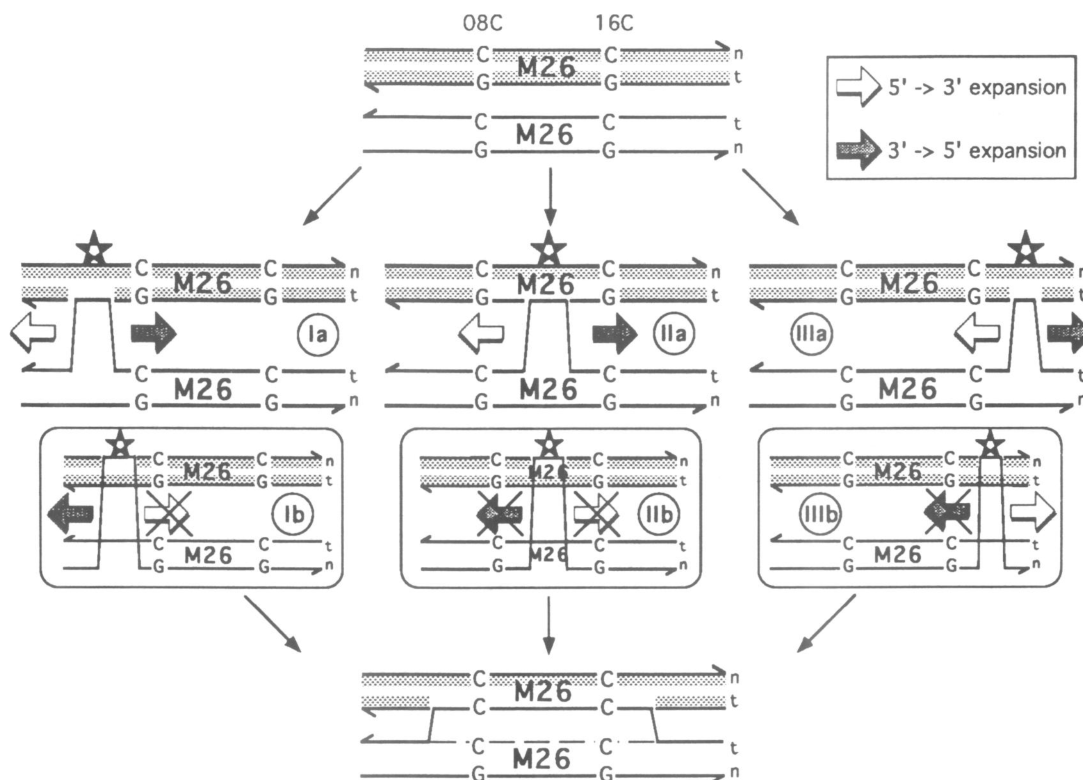

Fig. 3. Possibilities of strand transfer at *M26*. The *M26* heptanucleotide flanked by the G to C transversion sites (on top) can be covered by hDNA in different ways to produce the strand preference indicated at the bottom (see also Discussion and Figure 2). The initiating event (stars) can be a ss nick or a ds break at three different locations relative to the heptanucleotide (I = upstream, II = within, III = downstream). (a) shows the transfer of the transcribed strand (t) and (b) the transfer of the non-transcribed strand (n) from the wild-type (unshaded) to the mutant (shaded) chromatid. Strand transfer can be preferential (a only) or random (a or b) coupled with fixed directions for hDNA expansion. Preferential transfer of the transcribed strand does not restrict hDNA propagation (Ia, IIa, IIIa). If transfer of both strands is possible, hDNA is allowed to expand in either the 3′–5′ direction (Ib) or in the 5′–3′ direction only (IIIb). Random strand transfer initiated within the heptanucleotide (IIb) is not allowed as the direction of hDNA expansion required for preferential C/C formation at one G to C transversion site leads to G/G formation at the other. 5′→3′ and 3′→5′ hDNA expansion directions are indicated by unshaded and shaded arrows, respectively, and refer to the polarity of the transferred strand. X indicates directions not compatible with experimental results.

possible. In both cases, the non-transcribed strand in the acceptor chromatid anneals with the transcribed strand from the donor chromatid. This model allows hDNA to propagate bidirectionally (5′-3′ and 3′-5′ direction of the transferred strand), independent of the location of the initiating lesion (Figure 3; Ia, IIa, IIIa). Alternatively, the observed bias can be explained by non-preferential transfer of either of the donor DNA strands and unidirectional hDNA formation across *M26* in the acceptor chromatid. This can be achieved by ss breaks in either strand or ds breaks without the formation of a ds gap in the acceptor chromatid. However, to explain our tetrad data, this model requires restrictions on hDNA formation and the position of the initiating lesion. A break in either strand upstream of the M26 heptanucleotide requires a 3′–5′ (polarity of the transferred strand) directional expansion of hDNA. This provides that the G to C transversion sites are covered by hDNA only when the transcribed strand is transferred with the consequence that C/C mismatches occur. Transfer of the non-transcribed strand leads to hDNA formation upstream of the M26 region (Figure 3; compare Ia and Ib). The opposite prediction follows if the initiating lesion is downstream of the M26 heptanucleotide. hDNA is allowed to propagate in a 5′–3′ direction only to produce the excess of C/C mismatches at the G to C transversion sites (Figure 3; compare IIIa and IIIb). Initiation within the M26 heptanucleotide with random strand transfer is clearly contradicted by our data. Since the heptanucleotide is framed by the two G to C transversions (Schuchert *et al.*, 1991), a fixed direction of hDNA formation preferentially produces C/C mismatches on one side and G/G mismatches on the other side of the lesion (Figure 3; compare IIa and IIb). Unlike observed, tetrads would then show an excess of $5^+:3^-$ over $3^+:5^-$ for one G to C transversion and the opposite for the other.

Considering all possibilities and the genetic and physical data on *M26*-dependent gene conversion, we prefer the simplest explanation for the *M26* marker effect and suggest that *M26* stimulates cutting of the transcribed single strand in its vicinity, and then hDNA formation with transfer of the transcribed strand from the donor chromatid. Mismatch repair then leads to WCC or restoration, depending on which of the two mispairing bases is excised, while failure of repair leads to PMS. Further information on the nature and position of the initiating DNA lesion at *M26* is awaited, and will allow a more detailed dissection of the mechanism of *M26* recombination.

Material and methods

Strains and media

Schizosaccharomyces pombe strains and their genotypes are given in Table I. They are all derived from the original wild-type strain of Leupold (Gutz *et al.*, 1974). PRS 253, PRS 257 and PRS 261 were constructed by crossing PRS 250 with M26/08C, M26/16C and M26/16T, respectively, and the required genotypes isolated after tetrad dissection. Tetrads showing four red colonies (non-parental ditypes) were selected and the recombinant spore clones of the type *ade6-M26/X sup9-UGA* identified by their ability to segregate white Ade^+ recombinants (*ade6-M26 sup9-UGA*) in backcrosses with *ade6-M26*. The standard media YEA (yeast extract agar), MEA (malt extract agar) and the general genetic methods are described elsewhere (Gutz, 1971). The analyses with PRS 253 required the substitution of Difco yeast extract by Bio Merieux yeast extract to intensify this strain's red color phenotype on YEA. The minimal medium (MMA) consists of 0.67% Difco yeast nitrogen base without amino acids, 1% glucose and 1.8% agar.

Tetrad analyses

Aberrant segregation frequencies were evaluated by tetrad analysis of crosses heterozygous for the *ade6* locus and homozygous for *sup9-UGA* and *ura4-D18*. Mendelian segregation results in two white and two red colonies on a medium with limiting adenine concentration (YEA). The white colony phenotype is due to efficient suppression of the 5′ UGA 3′ stop codon created by the *ade6-M26* mutation, while the red colony phenotype is caused by the presence of the unsuppressible second site mutations in the vicinity of *M26* (Figure 1). Thus, aberrant tetrads are readily detected by non-2:2 segregation of colony color. PMS tetrads contain one red and white-sectored colony.

All spore colonies from aberrant tetrads (and from PMS colonies both re-isolated sectors) were checked for adenine prototrophy and auxotrophy. This excludes new mutations in early genes of the adenine pathway producing white auxotrophs. Furthermore, we checked for regular mating-type segregation ($2\ h^+:2\ h^-$) and for ploidy on the basis of cell size (microscopic inspection) and dark red staining on complete medium containing Phloxin B (20 mg/l).

Statistical methods

The statistical methods applied are described in Sokal and Rohlf (1981). The percentages of aberrant segregations are given with SDs. The values are calculated according to: $SD = \sqrt{p(100-p)/n}$, with p = proportion of aberrant segregation type (%) and n = total number of tetrads analyzed. Statistical independence of segregation patterns was tested by 2×2 tables and G-tests including Williams correction.

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