Gene Conversions and Crossing Over During Homologous and Homeologous Ectopic Recombination in *Saccharomyces cerevisiae*

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Manuscript received March 23, 1993
Accepted for publication May 5, 1993

ABSTRACT

The *pma1-105* mutation reduces the activity of the yeast plasma membrane H^+-ATPase and causes cells to be both low pH and ammonium ion sensitive and resistant to the antibiotic hygromycin B. Revertants that can grow at pH 3.0 and on ammonium-containing plates frequently arise by ectopic recombination between *pma1-105* and *PMA2*, a diverged gene that shares 85% DNA sequence identity with *PMA1*. The gene conversion tracts of revertants of *pma1-105* were determined by DNA sequencing the hybrid *PMA1::PMA2* genes. Gene conversion tracts ranged from 18–774 bp. The boundaries of these replacements were short (3–26 bp) regions of sequences that were identical between *PMA1* and *PMA2*. These boundaries were not located at the regions of greatest shared identity between the two *PMA* genes. Similar results were obtained among low pH-resistant revertants of another mutation, *pma1-147*. One gene conversion was obtained in which the resulting *PMA1::PMA2* hybrid was low pH-resistant but still hygromycin B-resistant. This partially active gene differs from a wild-type revertant only by the presence of two *PMA2*-encoded amino acid substitutions. Thus, some regions of *PMA2* are not fully interchangeable with *PMA1*. We have also compared the efficiency of recombination between *pma1-105* and either homeologous *PMA2* sequence or homologous *PMA1* donor sequences inserted at the same location. *PMA2* × *pma1-105* recombination occurred at a rate approximately 75-fold less than *PMA1* × *pma1-105* events. The difference in homology between the interacting sequences did not affect the proportion of gene conversion events associated with a cross-over, as in both cases approximately 5% of the Pma^+ recombinants had undergone reciprocal translocations between the two chromosomes carrying *pma1-105* and the donor *PMA* sequences. Reciprocal translocations were identified by a simple and generally useful nutritional test.

THE evolution of repeated gene families represents a complex balance between mutational events that create divergence and recombinational events that propel homogenization. In eucaryotes, gene conversions between members of a repeated gene family have been identified in organisms ranging from fungi to humans (ARNHEIM 1983); but it has been possible to investigate these interactions most systematically with the yeast *Saccharomyces cerevisiae*.

Gene conversions, sometimes accompanied by reciprocal crossing-over, have been described for a number of different sets of dispersed repeated genes in yeast. Ectopic gene conversions occur frequently in meiosis (as often as a few percent of meioses) but much less often in mitotic cells. These recombination events have been studied using both artificially duplicated genes placed in ectopic locations (MIKUS and PETES 1982; JINKS-ROBERTSON and PETES 1985, 1986; LICHTEN, BORTS and HABER 1987; FASULLO and DAVIS 1987; LICHTEN and HABER 1989; HABER *et al.* 1991) as well as between naturally repeated sequences (KUPIEC and PETES 1988; LOUIS and HABER 1990). In both mitotic and meiotic cells, recombination between nearly identical DNA sequences in ectopic locations occurred as frequently as when these sequences were in allelic locations. Ectopic recombination between genes only a few kilobase pairs in length were also associated with reciprocal exchange.

Gene conversions in *S. cerevisiae* have also been described between pairs of significantly divergent copies of yeast genes located at different chromosomal (ectopic) positions (ERNST, STEWART and SHERMAN 1981; BAILIS and ROTHSTEIN 1990). Similar experiments have been carried out between homeologous DNAs introduced by transformation (GOGUEL, DELAHODDE and JACQ 1992; MEZARD, POUPON and NICOLAS 1992). There have also been studies of recombination between homeologous chromosome segments of different yeast species (RESNICK, SKAANID and NILSSON-TILLGREN 1989) and of mammalian DNA (RESNICK *et al.* 1992). These studies have indicated that DNA sequence divergence markedly reduces the likelihood of their recombination. There is

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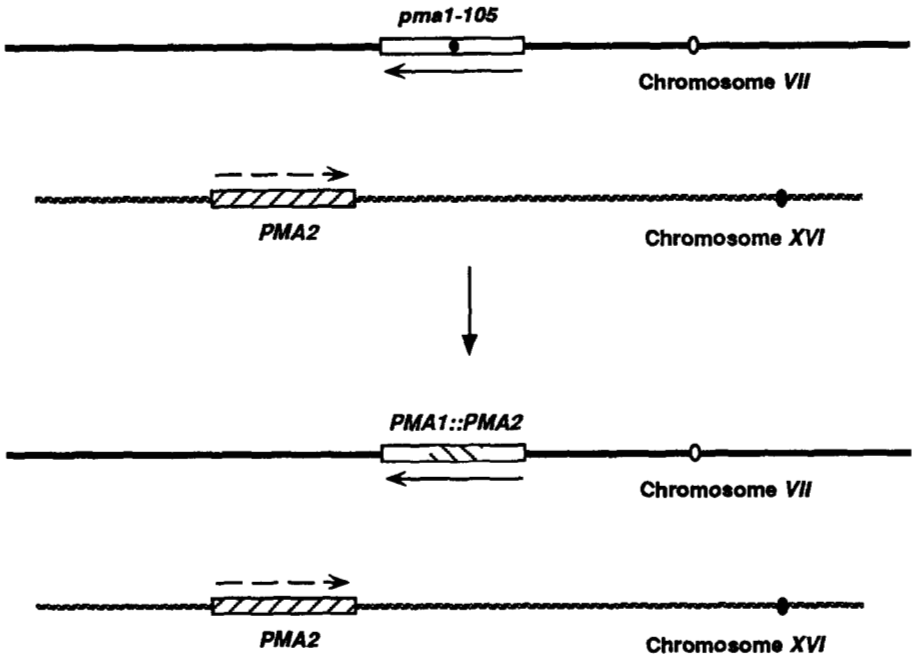

FIGURE 1.—Ectopic gene conversion between *pma1-105* on chromosome *VII* and the unexpressed *PMA2* gene on chromosome *XVI*. The *pma1-105* mutation creates low pH-sensitivity and an inability to grow on medium containing ammonium. Revertants that are selected to be either low pH-resistant or able to grow on ammonium-containing plates can arise either by mutations within the *pma1-105* gene (HARRIS *et al.* 1991) or by ectopic gene conversion between *pma1-105* and *PMA2*. The orientation of *PMA2* with respect to its centromere is not known. It is shown in the opposite orientation to *pma1-105* because no reciprocal crossovers were found among the gene convertants.

also a suggestion that highly diverged sequences may be more constrained in crossing-over accompanying gene conversion than are more completely homologous sequences (WHEELER *et al.* 1990).

In an effort to explore more fully recombination between divergent sequences, we have taken advantage of a pair of 85% identical genes in *S. cerevisiae*. The *PMA1* gene encoding the plasma membrane H^+-ATPase is an essential protein that is responsible for the maintenance of normal internal pH and membrane potential (SERRANO, KIELLAND-BRANDT and FINK 1984). Mutations such as *pma1-105* and *pma1-147* (MCCUSKER, PERLIN and HABER 1987; PERLIN, BROWN and HABER 1988; PERLIN *et al.* 1989; HARRIS *et al.* 1991) create mutant proteins that exhibit a reduction in both ATP hydrolysis and proton transport. As a consequence, these mutants are unable to grow under low pH or acid-loading conditions such as 0.18 M acetate, pH 4.8 (MCCUSKER, PERLIN and HABER 1987). Low pH-resistant revertants of *pma1-105* include intragenic second site alterations of the protein that can partially compensate for the original mutation, as well as full revertants resulting from mutation of the original mutation back to the wild-type protein sequence (HARRIS *et al.* 1991). In the course of this analysis, however, we discovered that nearly 90% of the full revertants did not arise by a mutation within the *PMA1* gene, but instead were the result of a gene conversion between the *pma1* mutant and a homeologous gene, *PMA2* (Figure 1). *PMA2* is a diverged gene relative of *PMA1* that is apparently unexpressed under normal physiological conditions but has nevertheless maintained an open reading frame (SCHLESSER *et al.* 1988). Except for the N-terminal 115 amino acids, *PMA1* is very similar to *PMA2*. Though the DNA sequences have diverged about 15%, there are only 31 amino acid differences between *PMA1* and *PMA2* from position 115 to the end of the protein, at amino acid 918 (SCHLESSER *et al.* 1988).

We have used this gene pair and the convenient method of selecting recombinants to analyze the mechanism of ectopic gene conversion between homeologous sequences. We were interested in several questions. First, are certain sites within the gene used preferentially as the end points of gene conversion tracts? Second, do the replacements of *PMA1* sequences occur by the insertion of a continuous tract of *PMA2* sequences, or do they consist of a mosaic of short stretches of *PMA1* and *PMA2* sequences? Such mosaics might be expected if mismatches in heteroduplex DNA are repaired independently or in short patches. Third, to what extent does the high degree of heterology between *PMA2* and *PMA1* reduce gene conversion, compared with gene conversions between identical partners? Finally, are gene conversions between homeologous DNA segments as likely to be accompanied by crossing-over as those between homologous regions?

MATERIALS AND METHODS

Yeast strains: All yeast strains used were derivatives of Y55 (*HO gal3 MAL1 SUC1*). A complete list of the yeast strains used in this work is presented in Table 1. All *pma1-105* revertants were derived from the previously described (HARRIS *et al.* 1991) haploid strain, SH91. In strain SH124, the *PMA2* coding sequence (SCHLESSER *et al.* 1988) was deleted from the chromosome by transforming an isogenic yeast strain with the *Hind*III fragment of plasmid pPSPZT-*PMA2::TRP1* (kindly provided by P. SUPPLY and A. GOFFEAU) in which the *TRP1* gene replaces the *PMA2* coding region. Strain KS1 is a segregant from a cross of strain

TABLE 1

Yeast strains

Strains	Genotypes
SH91	*MAT***a** *ho::LEU2 trp1-5 arg4-1 ura3-1 leu2-1 pma1-105*
SH124	*MATα ho::LEU2 trp1-5 lys5-2 arg4-1 ura3-1 leu2-1 pma1-105 pma2::TRP1*
KS1	*MAT***a** *ho::LEU2 trp1-5 lys5-2 arg4-1 ura3-1 leu2-1 pma1-147 pma2::TRP1*
SH134	*MAT***a**/*MATα ho::LEU2/ho::LEU2 trp1-5/trp1-5 lys5-2/lys5-2 ura3-1/ura3-1 +/arg4-1 leu2-1/leu2-1 pma1-105/pma1-105 pma2::TRP1/pma2::TRP1*
SH135	*MAT***a** *ho::LEU2 trp1-5 lys5-2 ura3-1 leu2-1 pma1-105 pma2::TRP1*
SH136	*MATα ho::LEU2 trp1-5 lys5-2 ura3-1 arg4-1 leu2-1 pma1-105 pma2::TRP1*
SH137	*MAT***a**/*MATαho::LEU2/ho::LEU2 trp1-5/trp1-5 lys5-2/lys5-2 +/arg4-1 leu2-1/leu2-1 pma1-105/pma1-105 pma2::TRP1/pma2::TRP1 ura3-1::pma1*-YIp5-*URA3/ura3-1*
SH138	*MAT***a**/*MATα ho::LEU2/ho::LEU2 trp1-5/trp1-5 lys5-2/lys5-2 +/arg4-1 leu2-1/leu2-1 pma1-105/pma1-105 pma2::TRP1/pma2::TRP1 ura3-1::pma2*-YIp5-*URA3/ura3-1*

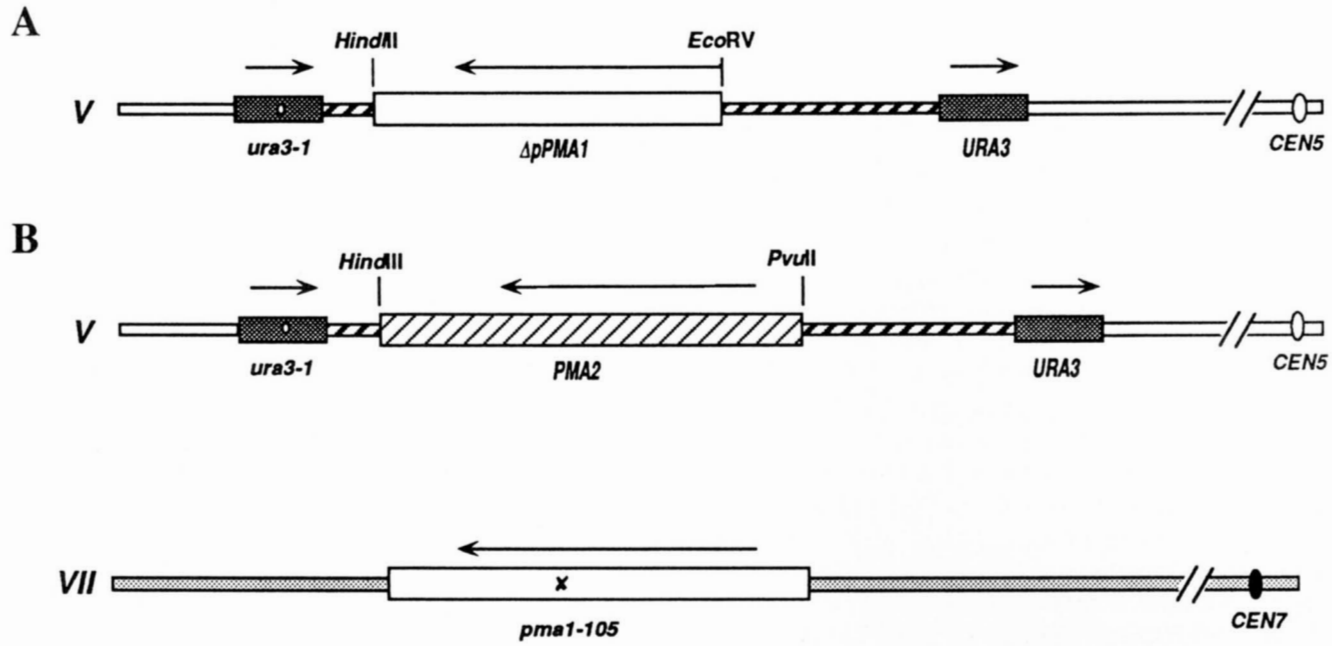

FIGURE 2.—Integration of circular pBR322 plasmids containing *URA3* and either a promoterless fragment of *PMA1* (A) or the complete, but unexpressed, PMA2 gene (B) at the *ura3-1* locus. The details of construction are presented in MATERIALS AND METHODS. The orientation of the *URA3* genes and the *PMA1* or *PMA2* genes are shown, along with the orientation of a mutant *pma1-105* gene on chromosome *VII*. pBR322 sequences are shown as a heavy diagonally striped bar.

SH124 with an isogenic strain carrying *pma1-147*; this segregant contains both the *pma1* mutation and the deletion of *PMA2*.

Haploid strains SH135 and SH136 are segregants from a cross between SH124 and a trp1-5 derivative of Y55 (MCCUSKER and HABER 1988). Diploid SH134 is the result of a mating between SH135 and SH136. In strain SH137, a portion of the *PMA1* coding sequence has been integrated at the *URA3* locus (Figure 2A). SH137 was created by transforming diploid SH134 with pSH79 (see below) that had been digested with *Sma*I, which cuts within the *URA3* open reading frame. In strain SH138, a portion of the *PMA2* open reading frame has been integrated at the *URA3* locus (Figure 2B). SH138 was created by transforming SH134 with pSH80 (see below) that had been digested with *Sma*I.

Diploid strains SH137 and SH138 were isogenic and were themselves derived from a set of isogenic derivatives of strain Y55 (MCCUSKER and HABER 1988). These diploids were then sporulated and dissected to obtain a series of at least 15 haploid derivatives to determine the rates of ectopic recombination and reversion of *pma1-105*. The haploid derivatives of SH137 carrying the *PMA1* donor sequences at *URA3* are designated SH137hA, SH137hB,. . .SH137hP. The haploids derivatives of SH138 carrying *PMA2* donor sequences at *URA3* are designated SH138hA to SH138hP. Another set of haploid segregants from SH138 that have no ectopic donor sequences and are Ura- are designated SH138h(Ura$^-$)A to SH138h(Ura$^-$)P. The use of 15 different segregants in a fluctuation analysis (see below) eliminated the possibility that one particular segregant of SH137 or SH138 was atypical.

Plasmid construction: pSH9 contains a 5-kilobase pair *Hind*III fragment that contains all of the *PMA1* coding sequence (SERRANO, KIELLAND-BRANDT and FINK 1984) in pGEM3 (Promega). To create pSH79, the *Eco*RV-*Hind*III fragment of pSH9 was ligated into YIp5, which had been digested with *Nru*I and *Hind*III. Thus, pSH79 contains a *pma1* allele that is missing its promoter and the first 41 amino acids of the coding sequence. To create pSH80, the *Pvu*II-*Hind*III fragment from pPSPZT-*PMA2* (SCHLESSER *et al.* 1988) was ligated into YIp5 that had been digested with *Nru*I and *Hind*III. The *PMA2* sequences in pSH80 contains

its promoter and all the coding sequence. This promoter is not expressed at detectable levels in yeast (SCHLESSER *et al.* 1988).

Selection of revertants of *pma1-105* and *pma1-147*: Strains carrying the *pma1-105* allele cannot grow on YEPD (pH 3.0) or on complete synthetic media (Com) (MCCUSKER, PERLIN and HABER 1987). The *pma1-105* mutation also causes strains to be resistant to the antibiotic hygromycin B (MCCUSKER, PERLIN and HABER 1987). To obtain revertants, isolated single colonies from *pma1-105* strain SH91 were patched to YEPD and grown overnight at 30°. The patches were replica plated to YEPD (pH 3.0) and Com plates. After 2 days, revertants arose as papillae growing on a background of dead cells. Papillae (no more than one from each patch) were then streaked for single colonies on the same type of selective plate. The set of independent revertants was replica plated to YEPD (pH 3.0) and Com, to confirm that revertants selected on one medium were resistant to the other. Revertants were also replica plated to YEPD-Hyg to determine if they were fully wild-type revertants and therefore sensitive to hygromycin B. The media used have been previously described (HARRIS *et al.* 1991).

Revertants of the *pma1-147* mutation in strain KS1 were selected for their ability to grow on YEPD plates containing 0.2 M acetate, pH 4.8 (MCCUSKER, PERLIN and HABER 1987), after UV irradiation of colonies replica plated to YEPD and grown in the dark overnight. Only one revertant was selected from any one colony.

Molecular analysis of *PMA1* revertants: Revertant alleles were cloned by gap repair (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) using plasmid pSH10 as described previously (PERLIN *et al.* 1989). The alleles were sequenced using the dideoxy method with modifications for sequencing double-stranded template with Sequenase according to the suggestions of the enzyme manufacturer (United States Biochemical Corp.) Southern blots, probed either with *PMA1* sequences or with *URA3* sequences, were carried out as previously described (HARRIS *et al.* 1991). Chromosome separation, using a CHEF gel apparatus (BioRad) was performed as previously described (LOUIS and HABER 1990).

Measurement of mitotic recombination rates between *pma1-105* and an ectopic donor: After 60 hr of growth on YEPD, isolated single colonies were resuspended in 1 ml of H_2O. Appropriate dilutions of the colonies were plated on YEPD to determine the number of viable cells and on YEPD (pH 3.0) to determine the rate of formation of pH-resistant revertants of *pma1-105*. The median number of pH-resistant colonies formed from a total of 15 individual experiments was used to determine the rate by the method of LEA and COULSON (1949).

RESULTS

Gene conversion of *pma1*-105 by ectopic recombination with *PMA2*: Revertants of *pma1-105* can be selected either by resistance to low pH or the ability to grow on NH_4^+-containing medium (HARRIS *et al.* 1991). Some revertants of strain SH91 are completely wild type (full revertants), whereas others are only partial revertants that remain resistant to hygromycin B and are often sensitive to acid loading (0.2 M acetate, pH 5.0). Most of the partial revertants have proven to be intragenic second-site mutations in *PMA1* though some were different amino acid substitutions at the original site (HARRIS *et al.* 1991). As shown in Table 2, the majority of revertants selected either by resistance to low pH or to ammonium ion proved to be full revertants. Among these full revertants, 9 of 12 examined resulted from ectopic gene conversions between *pma1-105* on chromosome *VII* and *PMA2*, located on chromosome *XVI*. That they were gene conversions was established by gap-repair cloning of the *PMA1* gene and sequencing the region embracing the *pma1-105* site (HARRIS *et al.* 1991). These gene convertants were chosen for the more complete DNA sequence analysis presented here.

TABLE 2

Phenotypic analysis of revertants of *pma1-105*

Selection media	Partial revertants Hyg^R	Full revertants Hyg^S	Total
YEPD pH 3.0	9	61	70
Complete	8	119	127

Selection of revertants of *pma1-105* can either be accomplished on low pH (pH 3.0) YEPD plates or on synthetic complete medium (Complete), containing ammonium (McCusker, Perlin and Haber 1987). All pH-resistant revertants have proven to be able to grow on medium containing NH_4 + ion, and vice versa. Partial revertants remain resistant to 300 μg/ml hygromycin B (Hyg^R) and are often sensitive to acid loading, as seen by their inability to grow on YEPD plates containing 0.2 M acetate, pH 5.0. Full revertants are indistinguishable from the wild-type parental strain and are sensitive to hygromycin B (Hyg^S).

The gene conversion tracts replacing the *pma1-105* mutation are shown in Figure 3A. One additional gene conversion event, a partial revertant labeled PC, is discussed below. In addition to restoring the S368F mutation (phenylalanine in place of the normal serine at position 368) back to wild type, these gene conversion events replaced a varying amount of *PMA1* sequence with *PMA2* sequences. All of the gene conversion tracts were continuous, without interspersion of short patches of *PMA1* and *PMA2* sequences. The sizes of the minimum gene conversion tracts ranged from as few as 18 bp to as many as 774 bp (Table 3), with an average minimum conversion tract length of 237.5 ± 237 bp. On average, the lengths of the gene conversion tracts among the five examples selected by pH-resistance (designated P in Figure 3) are shorter (93 ± 54 bp) than the tracts of the four ammonium-resistant (designated by N) revertants (418 ± 260 bp). We do not know if the difference is meaningful, as in both cases the majority of gene conversion events resulted in identical, wild-type proteins. In every case the revertants selected for growth at low pH were also able to grow in the presence of ammonium ion, and vice versa.

The boundaries of the gene conversion tracts are presented in Table 3. Some of the boundaries end where there are as few as three or five base pairs that are identical between the *PMA1* and *PMA2* genes. The longest shared identity at any of these boundaries

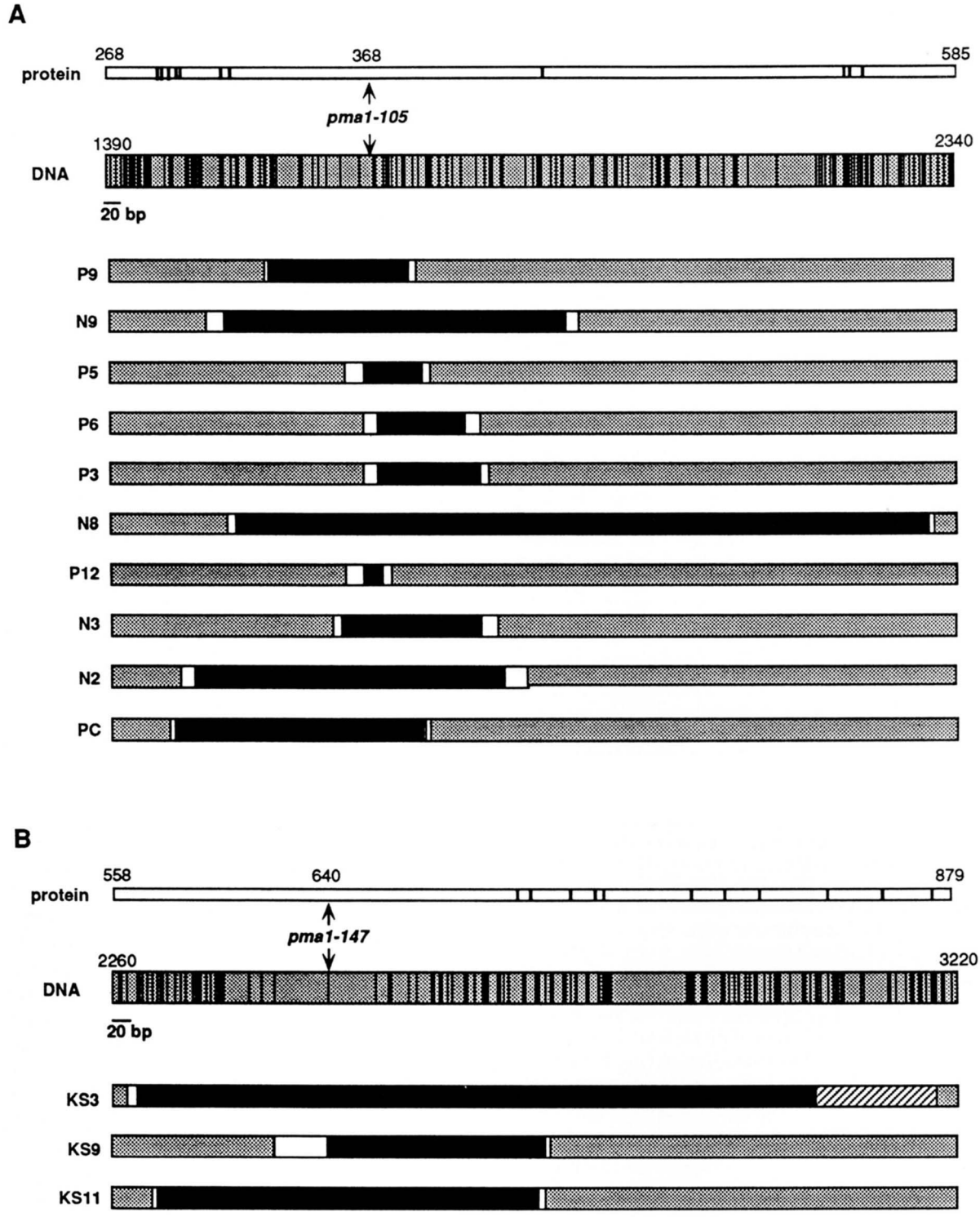

FIGURE 3.—Gene conversion tracts that restore a *pma1* mutation to wild type by replacing *PMA1* sequences with homeologous sequences from *PMA2*. (A) Gene conversions replacing the *pma1-105* (S368F) mutation (arrow). Amino acid differences in the Pma1 and Pma2 proteins are shown in the open box at the top of the figure. Base-pair differences between *PMA1* and *PMA2* DNA are shown by black lines in the gray box at the top of the figure. The *PMA1* sequence is for strain Y55, which has a few differences from that determined by SERRANO, KIELLAND-BRANDT and FINK (1984) (PERLIN *et al.* 1989). The *PMA2* sequence determined by SCHLESSER *et al.* (1988) is identical in the region shown to the sequence in the Y55 strains. The regions of *PMA2* that were inserted into different gene converted revertants of *pma1-105* are shown as black bars. The white borders of these bars illustrate the adjacent region of DNA sequence identity between *PMA1* and *PMA2* at the borders of the gene conversion. There are no amino acid differences between the *PMA1* and *PMA2* genes in this interval, from amino acids 321–430 (nucleotides 1548–1875 in *PMA1*). Most amino acid substitutions outside of this region are conservative changes. (B) Gene conversions replacing the *pma1-147* (P640L) mutation (arrow). Amino acid differences and base-pair differences between *PMA1* and *PMA2* are indicated by black lines in the two boxes at the top of the figure, as in (A). The gene conversion tracts are indicated as in (A). In one case, the precise end of the gene conversion tract was not determined. The region of ambiguity is indicated by a diagonally striped bar. *PMA1* and *PMA2* are identical in amino acid sequence from amino acids 625 to 685. There is a single nucleotide difference between the two wild-type genes in the 98 bp surrounding the *pma1-147* mutation.

TABLE 3

***PMA1/PMA2* conversion tracts**

Revertant[a]	Tract endpoints[b]		Length[c]		Flanking homology[d]	
	5′ *PMA2*	3′ *PMA2*	Min.	Max.	5′	3′
N2	1479	1839	360	400	14	26
N3	1638	1791	153	178	8	17
P12	1677	1695	18	46	20	8
N8	1536	2310	774	784	5	5
P3	1691	1809	118	139	13	8
P6	1691	1791	100	130	13	17
P5	1677	1743	66	94	20	8
N9	1518	1906	388	421	20	13
P9	1567	1728	161	172	3	8
PC	1433	1743	310	322	4	8
KS3	2278	3063–3204[e]	784[e]	937[e]	10	ND[f]
KS9	2506	2754	251	317	61	5
KS11	2304	2746	443	455	5	7

[a] Phenotypically wild-type revertants of *pma1-105* that were selected as pH-resistant (P) or ammonium-resistant (N) are indicated. The revertant marked PC is a partial revertant of *pma1-105*. Phenotypically wild type revertants of the *pma1-147* mutation are designated by KS.

[b] Tract end-points were determined from the position of the first or last *PMA2*-specific base pair in the continuous *PMA2* sequence inserted into *PMA1*. The nucleotide numbering is that of Serrano, Kielland-Brandt and Fink (1984).

[c] Minimum conversion tract lengths do not include the adjacent regions of identical DNA sequence shared by *PMA1* and *PMA2*. Maximum conversion tract lengths do include these shared regions.

[d] Number of base pairs of DNA that are identical in both *PMA1* and *PMA2* just 5′ or just 3′ of the segment of *PMA2* gene converted into *PMA1*.

[e] The 3′ boundary of this conversion tract was not determined precisely.

is 26 bases, despite the fact that there are several longer regions of shared identity in the same region (see Figure 3A). Thus, these gene conversion tracts do not begin or end at the longest available regions of identity. The lengths of gene conversion tracts are not significantly influenced by the incompatibility of the amino acid sequences of *PMA1* and *PMA2* in this interval, as most of the conversions lie completely within a region where there have been no amino acid substitutions, from amino acids 321–431 (Figure 3A).

There is in fact a boundary that limits the extent of *PMA2* gene conversions at the 5′ end of the conversion tracts. In addition to the nine full revertants, one partial revertant was discovered that also had undergone a gene conversion by *PMA2*. This revertant, designated PC, did not restore wild-type activity to the cell, which remained resistant to hygromycin B and sensitive to acid loading on 0.2 M acetate medium, pH 5.0. The only difference between revertant PC and revertant N2 are two amino acid substitutions at the 5′ end of the tract: G282V and Q283E. It appears that these substitutions are sufficient to create a *pma1* mutant phenotype, though not as severe as the original *pma1-105* mutant. Thus, there is a limit on the 5′ end of gene conversion tracts when pH-resistant or ammonium-resistant revertants are selected for full wild-type behavior. However, only one of the revertants (N2) had a conversion tract that came even close to this boundary; hence, it does not seem to have severely limited the sizes of most of the conversion tracts selected. There is clearly no boundary at the 3′ end for any of these conversions, as most of them did not extend even through the region where there are no amino acid substitutions between *PMA1* and *PMA2*. Moreover, the largest tract (N8) subtends several amino acid substitutions and retains wild-type behavior. Finally, as we present below, it is possible to obtain fully wild-type revertants in which all of the sequences 3′ to *pma1-105* are from *PMA2*. The significance of the 5′ boundary will be taken up in the DISCUSSION.

Gene conversion tracts in among revertants of *pma1-147*: A similar analysis was carried out on a smaller number of revertants of another mutation, *pma1-147*. This hygromycin B-resistant mutation is not sensitive to pH 3 nor to ammonium ion, but full revertants can be selected by their ability to grow at pH 4.8 on YEPD plates containing 0.18 M acetate (MCCUSKER, PERLIN and HABER 1987). Because the selection is less strong than for *pma1-105*, we were only able to obtain revertants after cells were UV irradiated and allowed to recover before replica plating them on to selective medium. Among six independent revertants, two proved to be simple mutational reversions of the original P640L mutation back to proline. The four others had undergone gene conversion with *PMA2*. Three of these convertants were sequenced. Their conversion tracts ranged from 251 to a minimum of 784 bp (Figure 3B and Table 3). The 3′ boundary of the largest conversion event was not precisely established; it could be as much as 141 bp longer before *PMA1* sequence resumes. These conversion tracts may be somewhat longer because they were induced by UV light rather than recovered from spontaneous mutations. Nevertheless they share the same characteristic as the spontaneous gene conversions of *pma1-105*, in that the ends of the conversion tracts share as few as 5 bp identity, even though there are substantially longer regions of sequence identity in this region. In fact, the *pma1-147* mutation sits in a region that contains only 1 mismatch of 98 bp (not counting the *pma1-147* mutation itself), but only one of the three conversion tracts begins or ends within this region. Had any of the other revertants of *pma1-147* that apparently arose by base pair substitution actually involved a gene conversion confined to this highly conserved region, we would have expected to have found the nearby single base pair difference that distinguishes *PMA2* from *PMA1*.

Rates of gene conversion of *pma1-105* by homologous (*PMA1*) and homeologous (*PMA2*) donors: To assess the effect of DNA sequence heterology on the

TABLE 4

Rates of ectopic recombination between *pma1-105* and either *PMA1* or *PMA2* sequences inserted at *URA3*

Strain	Reversion rate
SH137h (*PMA1*)	$3.7 \pm 2.5 \times 10^{-7}$
SH138h (*PMA2*)	$0.5 \pm 0.7 \times 10^{-8}$
SH138h Ura$^-$	$0.12 \pm 0.7 \times 10^{-8}$

Fifteen independent colonies of each strain were analyzed by the method of Lea and Coulson (1949) for the rate of formation of full revertants of *pma1-105* (*i.e.*, those able to grow on YEPD (pH 3.0) plates and on Complete plates, and that are also sensitive to hygromycin B).

rate of *pma1* gene conversion, haploid strains were constructed that contained either nontranscribed *PMA1* or *PMA2* donor sequences inserted at the *URA3* locus on chromosome *V* (Figure 2). In this way we could compare *PMA1* × *pma1-105 vs. PMA2* × *pma1-105* recombination in the same chromosomal context. The *PMA1* or *PMA2* donor regions were oriented so that reciprocal recombination would create viable translocations between chromosomes *V* and *VII*. In these strains, the normal *PMA2* locus was deleted (Table 1). Fifteen cultures of haploid strains carrying the homologous *PMA1* donor sequences (SH137hA to SH137hP) and 15 strains carrying the homeologous *PMA2* donor (SH138hA to SH138P) were grown as single colonies on YEPD medium and then plated, with appropriate dilutions both on YEPD plates (to measure total colony forming units) and YEPD-pH 3.0 plates. Revertants were purified by re-streaking and then tested further to establish if they were full revertants (pH-resistant and sensitive to hygromycin B) or partial revertants (low pH-resistant, but still hygromycin B-resistant). We restricted our analysis to full revertants, as we have previously shown that all partial revertants arise by intragenic second-site mutations of the *pma1-105* gene (Harris *et al.* 1991). Full revertants can arise both by gene conversion and by actual reversion of the *pma1-105* mutation (Harris *et al.* 1991). We also determined the rates of reversion for a set of Ura$^-$ derivatives that lack any *PMA1* or *PMA2* sequences inserted at the *ura3-1* locus, using strains SH138h(Ura$^-$)A to P. The rates of appearance of full revertants were then determined by the method of Lea and Coulson (1949) and are shown in Table 4.

The rates of reversion for SH137h derivatives, where *PMA1* donor sequences are inserted at *ura3-1*, is 3.7×10^{-7}, 300 times higher than the reversion rate for the Ura$^-$ controls lacking donor sequences (0.12×10^{-8}). Approximately 92% (103/112) of the pH-resistant revertants of SH137 were in fact full revertants (*i.e.*, they were also hygromycin B-sensitive). In contrast, the rates of reversion for strains SH138h carrying a *PMA2* donor (0.5×10^{-8}) were only about 4 times greater than those for controls lacking any donor sequence. Thus, homeologous *PMA2* sequences were used as donors in gene conversion approximately 75-fold less frequently than homologous *PMA1* sequences. Among the original set of *pma1-105* revertants that were selected (Harris *et al.* 1991), nine were actually ectopic gene conversions involving the *PMA2* gene located on chromosome *XVI* and three were simple mutations of F368 back to serine. This proportion is entirely in agreement with the fourfold difference in the rates of gene conversions and mutations for SH138h and SH138(Ura$^-$).

Association of gene conversion and crossing-over: Among those events that occurred by gene conversion, it is possible to ask if gene conversion was accompanied by crossing-over, which would produce a reciprocal translocation between chromosomes *V* and *VII*. To determine if there had been a crossing-over, we used a simple assay illustrated in Figure 4. After a *URA3* plasmid has been inserted at *ura3-1*, a "pop-out" recombination between the flanking *URA3* sequences will (sometimes) restore a Ura$^-$ cell. Such events can be detected as papillations on a plate containing 5-fluoro-orotic acid (5-FOA) (Boeke, Lacroute and Fink 1984). However, if a translocation has accompanied gene conversion, then the two *URA3* sequences will be located on separate chromosomes and cannot readily give rise to Ura$^-$ colonies. An example of this difference is shown for several Pma$^+$ gene convertants for SH137h, in Figure 5A. We used Southern blots of *Sma*I-digested DNA, probed with a *PMA1* fragment, to confirm that the full revertants which gave rise to many FOA-resistant papillae maintained the parental configuration of *URA3* genes, whereas those that yielded few, if any, papillae did indeed contain the rearranged restriction fragments expected for a translocation (data not shown). Finally, the chromosomal DNA of these same strains was analyzed by CHEF gel electrophoresis (see MATERIALS AND METHODS). As shown in Figure 5B, the strains that failed to papillate on 5-FOA medium lack the normal-sized chromosome *V* and *VII* bands, but contain two different-sized bands expected for a reciprocal translocation.

Among full revertants of the *pma1-105* × *URA3::PMA1* strain SH137h, 4/103 (4%) proved to have reciprocal translocations. For the homeologous *pma1-105* × *URA3::PMA2* recombination events, we found 9/149 (6%) of the revertants were associated with reciprocal exchange of flanking markers. These two values are statistically indistinguishable (Sokal and Rohlf 1969).

DISCUSSION

Gene conversion between *pma1* mutants and *PMA2* provides a convenient system to analyze many aspects

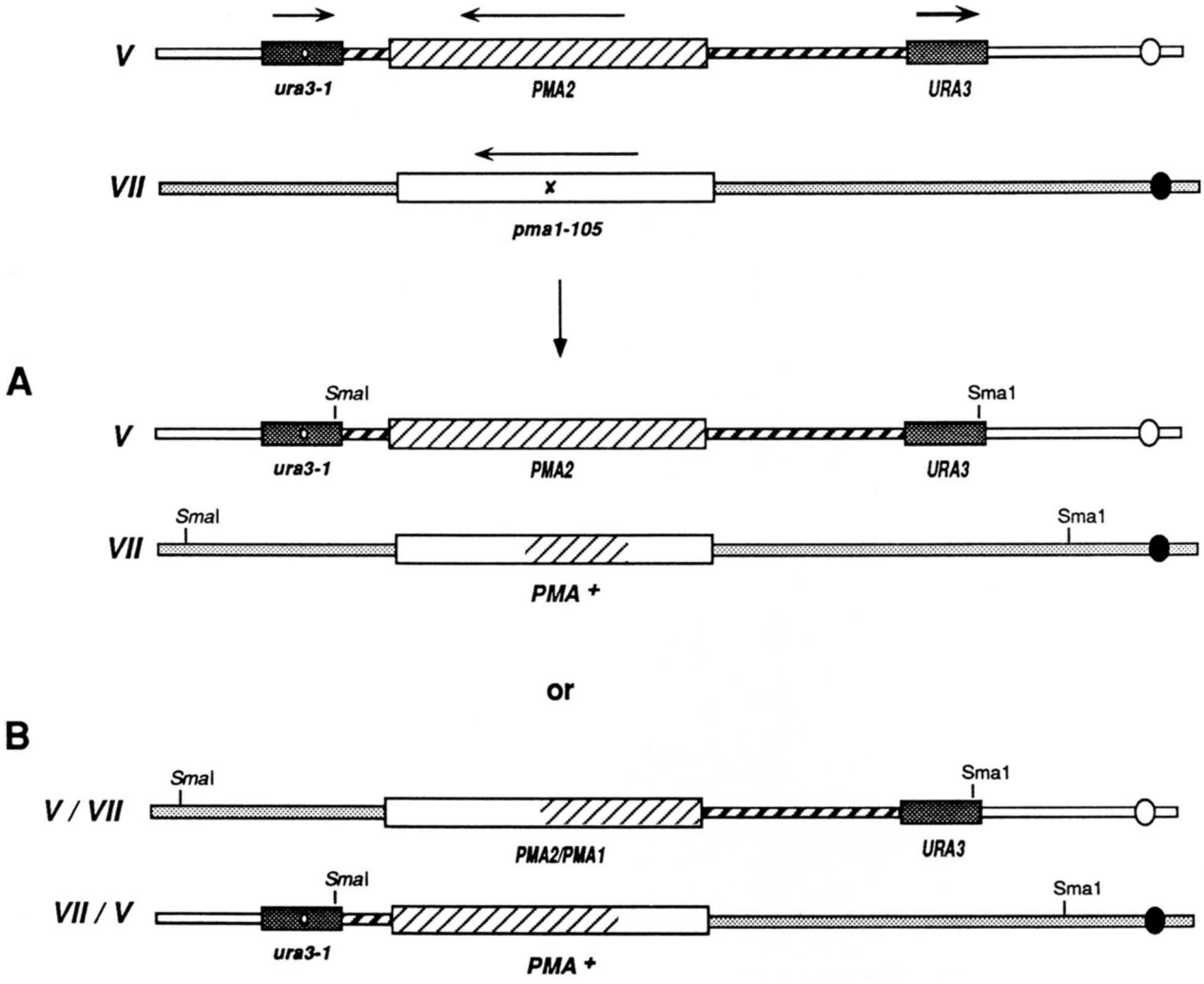

FIGURE 4.—Gene conversion of *pma1-105* by *PMA2*. Two different outcomes are possible. Gene conversion may occur without any crossing-over between the donor and recipient sequences (A). In this case, the flanking *ura3-1* and *URA3* genes are on the same chromosome and can readily recombine to eliminate the inserted DNA and leave only *ura3-1* behind (the excised DNA may be removed by a nonconservative mechanism (SUGAWARA and HABER 1992) or by a "pop-out" recombination to produce a circular product that lacks the necessary sequences for DNA replication; in either case cells will become Ura$^-$. Such Ura$^-$ cells are readily selected by growth on 5-FOA (BOEKE, LACROUTE and FINK 1984). If gene conversion is accompanied by crossing-over (B), a reciprocal translocation is created. This can be readily detected by changes in chromosome size on chromosome separating gels, but is easily scored by the very inefficient production of Ura$^-$ cells on 5-FOA medium. Here, the *ura3-1* and *URA3* genes are on different chromosomes and cannot readily recombine to produce Ura$^-$ cells.

of ectopic recombination between divergent DNA sequences. Some of our results support previous studies on related events in yeast, but other results are quite different from what have been previously inferred.

Ectopic gene conversion between *pma1-105* or *pma1-147* and *PMA2* produces continuous gene conversion patches. This result is consistent with a previous study of mitotic gene conversions between the iso-1 and iso-2 cytochromes c genes (*CYC1* and *CYC8*) in yeast (ERNST, STEWART and SHERMAN 1981). This result suggests that, if the intermediate in gene conversion includes long regions of heteroduplex DNA, the mismatch repair mechanism must act in a long and processive fashion, rather than correcting small patches at one time. Alternatively, there might be no mismatch correction at all and the heteroduplex regions would become "fixed" by chromosome replication. It is also possible that a pair of converging mismatch repair enzyme complexes might act on a region of heteroduplex DNA and create a double-strand break or gap, the repair of which would perforce create a long, continuous conversion tract. This kind of mismatch repair-induced recombination has been documented in meiotic gene conversion (BORTS and HABER 1987; BORTS *et al.* 1990). Gene conversions between somewhat more diverged genes have also been obtained after transformation of a linearized fragment with an overlapping homeologous fragment (GOGUEL, DELAHODDE and JACQ 1992; MEZARD, POUPON and NICOLAS 1992). In these cases, too, most of the conversion tracts were continuous and terminated in short stretches of identical sequences; however in 4 of 29 cases studied by GOGUEL, DELAHODDE and

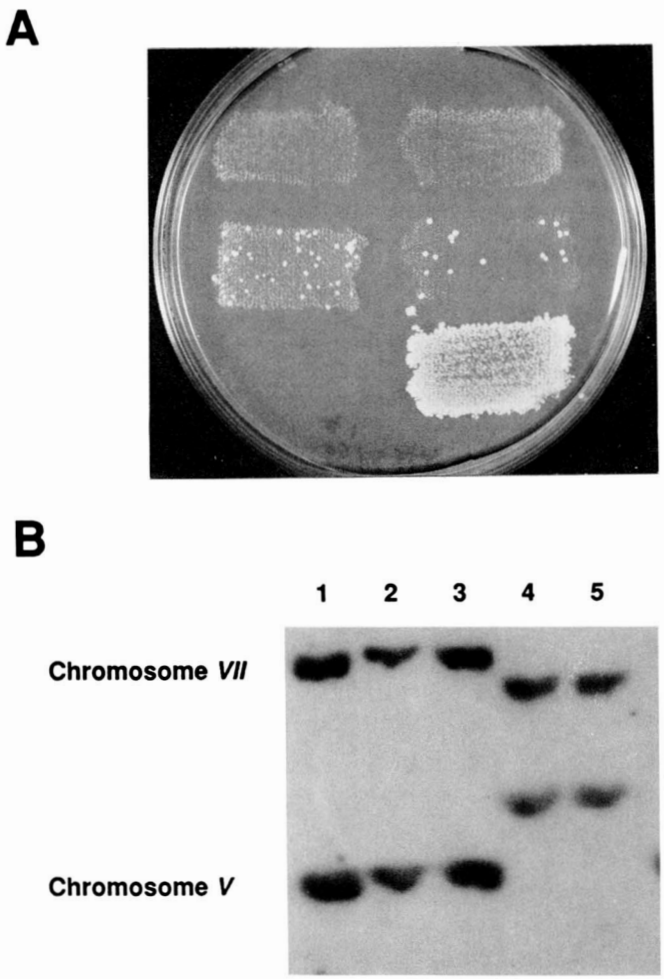

FIGURE 5.—Detection of translocations by a nutritional test. (A) Papillation of Pma$^+$ recombinants on 5-FOA plates. Two revertants that proved to have reciprocal translocations and that do not papillate are shown in the top row. The two revertants in the middle row yield many papillae and proved to have gene converted *pma1-105* to Pma$^+$ without an accompanying crossing-over. A Ura$^+$ strain does not papillate readily, whereas a Ura$^-$ strain grows well on this medium (bottom row). (B) CHEF gel analysis of ectopic gene conversions between *pma1-105* on chromosome *VII* and an ectopic copy of a promoterless *PMA1* gene fragment integrated on chromosome *V* that create translocations. A Southern blot of chromosomes separated on CHEF gels (see MATERIALS AND METHODS) was probed with the *Hind*III fragment of the *PMA1* gene. In the parental strain SH137hA, two *PMA1*-homologous chromosomes (*V* and *VII*) are identified (lane 1). In two cases where the Pma$^+$ recombinants were able to papillate efficiently on 5-FOA plates, the Southern blot shows that the same two chromosomes hybridized to the probe (lanes 2 and 3). In two cases where poor papillation on 5-FOA suggested that the cells harbored a reciprocal VII:V translocation, the *PMA1* probe hybridzed to two different-sized chromosome bands, as expected for such a translocation. Similar results were obtained for *PMA2* × *pma1-105* recombinants (not shown).

JACQ (1992), there were interspersed blocks of donor and recipient sequences.

We also find, as did ERNST, STEWART and SHERMAN (1981), that the boundaries of gene conversion events are not clustered in regions of greatest shared identity between the interacting genes. This same conclusion can be drawn from the gene conversions of homeologous *P450* and *ARG4* genes studied by MEZARD, POUPON and NICOLAS (1992), where the boundaries of gene conversion terminated in regions of between 4 and 21 nucleotides. A very different conclusion was inferred from studies of S-adenosyl methionine synthetase (*SAM*) gene conversions by BAILIS and ROTHSTEIN (1990), who concluded that the boundaries of most of the gene conversions were located in regions of greatest DNA identity. However, these investigators did not sequence the gene conversion tracts but instead used four restriction endonuclease sites to infer the location of the ends of gene conversion tracts. It is possible that the actual boundaries were not located precisely in those shared regions. It is also possible that their results are different in that the gene conversions of *SAM2* involved replacing a 4-bp insertion in the gene rather than a simple base pair substitution in the study of *PMA* or *CYC* genes.

It must be stressed that the fact that gene conversions appear to end at short regions of identity shared by the homeologous sequences does not mean that the formation of heteroduplex DNA either began or ended at these boundaries of the gene conversion tract. What we observe is the consequence of both heteroduplex DNA formation and mismatch correction. One cannot rule out that strand exchange preferentially began or ended at the longest regions of shared DNA identity, but that regions close to the ends of the heteroduplex region were preferentially restored, while the region in the middle was corrected to give a gene conversion. Thus, it would be inappropriate to conclude that recombination actually occurred at the short regions of identity.

BAILIS and ROTHSTEIN (1990) found that *SAM1* × *SAM2* recombination was approximately 20-fold less frequent than *SAM1* × *SAM1* events. A similar result was obtained comparing homologous and 73% identical cytochrome P450 genes cotransformed into yeast (MEZARD, POUPON and NICOLAS 1992). Our results are quite similar: *PMA2* acts as a donor in mitotic gene conversions of *pma1-105* about 75-fold less often than a *PMA1* donor. It is not known how recombination between divergent sequences is discouraged. BAILIS and ROTHSTEIN (1990) found that a deletion of the mismatch repair gene *PMS1*, which is involved in the repair of single mismatches in both meiotic (WILLIAMSON, GAME and FOGEL 1985; BORTS *et al.* 1990) and mitotic recombination (RAY, WHITE and HABER 1991), did not increase homeologous *SAM1* × *SAM2* recombination relative to a *SAM1* × *SAM1* control. The recent discovery of additional homologues of *PMS1* (M. LISKAY, unpublished data) and of several yeast genes homologous to the *Escherichia coli mutS* gene (REENAN and KOLODNER 1992; NEW, LIU and CROUSE 1993) suggests that there may be a divi-

sion of labor among mismatch-recognizing gene products in yeast, wherein some are designed to correct small numbers of mismatches in a region whereas others may be involved in a more general surveillance of mismatched, recombining DNA. We await further experiments to learn if some of these mismatch repair gene homologues antagonize yeast recombination in the way that the *mutL* and *mutS* genes discourage homeologous recombination in bacteria (SHEN and HUANG 1986; RAYSSIGUIER, THALER and RADMAN 1990).

When *PMA2* sequences were located only at their normal position on chromosome *XVI*, none of the *pma1-105* × *PMA2* recombinants we analyzed were associated with a reciprocal exchange of flanking chromosome arms. Possibly, the arrangement of the two genes on their respective chromosomes was such that any crossing-over would have produced inviable translocation products: a dicentric chromosome and an acentric fragment. When the genes were arranged by targeted insertion into chromosome *V* so that reciprocal products could be recovered, we were able to demonstrate that approximately 5% of the gene conversion events were associated with crossing-over. This proved to be true both for *PMA1* and *PMA2* donor sequences. Thus, the frequency with which these mitotic gene conversions were associated with crossing-over does not depend on the degree of sequence divergence between the two recombining partners. The low proportion of gene conversions that were associated with crossing-over is not especially unusual; the association of crossing-over with gene conversion is generally much lower for spontaneous mitotic gene conversions than in meiosis (JINKS-ROBERTSON and PETES 1986; LICHTEN, BORTS and HABER 1987; LICHTEN and HABER 1989; also reviewed in PETES, MALONE and SYMINGTON 1992). Another possibly significant difference between gene conversions in mitosis and meiosis may be the effect of sequence divergence on crossing-over. In meiosis, WHEELER *et al.* (1990) found that short homeologous gene conversions were rarely associated with crossing-over, while in general crossovers accompany meiotic gene conversions 30–50% of the time. However, they did not carry out an appropriate homologous control for the gene conversions of mouse H-2 sequences that were studied; hence, it could be that the constructs themselves did not permit much exchange. In any case, there is no such discrimination in spontaneous mitotic recombination between homologous or homeologous *PMA* genes. This question was not addressed in the earlier studies of either the *SAM* or the *CYC* genes.

There does seem to be one notable difference between homologous and homeologous gene conversions, namely, the length of the gene conversion tracts. The average lengths of the spontaneous gene conversion tracts that replaced *pma1-105* was approximately 235 bp. Similar-sized tracts were found in other homeologous systems in different yeast strains both in spontaneous and double-strand break induced events (ERNST, STEWART and SHERMAN 1981; BAILIS and ROTHSTEIN 1990; MEZARD, POUPON and NICOLAS 1992); however, in none of these cases were conversion tracts measured for a homologous control marked by just a few polymorphisms. In one study of essentially homologous gene conversion tract lengths in mitotic cells, JUDD and PETES (1988) found that conversion tracts were on average very large, covering several kilobases. It is possible that multiple mismatches discourage the formation of extensive regions of heteroduplex DNA, though this did not affect the proportion of events associated with crossing-over.

A region of *PMA2* that does not confer wild-type function in place of *PMA1*: Our results also have some significance in understanding the structure and function of the *PMA1* gene product. Our results demonstrate that some hybrid *PMA1::PMA2* proteins can substitute for *PMA1*. The fact that we can recover *PMA1::PMA2* reciprocal recombinants argues that the entire carboxy terminal 2/3 of the *PMA1* protein, from position 368 to the end (918) can be substituted by *PMA2*. A similar conclusion has been reached by P. SUPPLY and A. GOFFEAU (unpublished data). However, it appears that some regions of *PMA2* cannot substitute for *PMA1*. First, the partial revertant PC (Figure 2) differs from the full revertant N2 only by the presence of two amino acid substitutions, G282V and Q283E. These two amino acid changes are apparently sufficient to create a partially inactive Pma1 mutant protein that is still resistant to low pH but exhibits the hygromycin B resistance and acid-loading sensitivity of many other *pma1* mutants (MCCUSKER, PERLIN and HABER 1987).

Further evidence that some parts of *PMA2* may not fully substitute for *PMA1* has come from a series of analogous reversion experiments for two mutations in the amino terminus of the protein (A135V and G158D) (NA *et al.* 1993; also S. Anand and J. E. Haber, unpublished data). We have failed to recover gene conversions between *PMA1* and *PMA2*, despite the fact that the sequences in this region are no more divergent than in the more carboxy-terminal regions described in this paper. Given the average size of the gene conversion tracts that replace *pma1-105* or *pma1-147*, one might have expected to find short (<100 bp) *PMA2* replacements among the revertants of A135V or G158D. This suggests that there are one or more incompatible amino acid substitutions close to these two sites in the first two transmembrane helices. Consistent with these results is the recent finding that when *PMA1* is deleted and replaced by the complete

PMA2 protein, transcribed from the *PMA1* promoter, cells exhibit low-pH and acid-loading sensitivity (SUPPLY, WACH and GOFFEAU 1993). Part of this acid sensitivity could be explained by the substitutions at positions 282 and 283. Additional changes in the region of the first two transmembrane domains may also be of importance. Further investigation of the incompatibility in these regions may reveal some explanation for the functional differences between *PMA1* and *PMA2*.

AMY RUN YAN WANG carried out the fluctuation tests comparing *PMA1* or *PMA2* × *pma1-105*, under the guidance of WAI-YING LEUNG. We are grateful to A. GOFFEAU for providing plasmids containing *PMA2* and a *TRP1*-marked deletion of *PMA2*. S.H. was supported as a USPHS Trainee of Genetics Training Grant GM07122. SUSAN LOVETT and EVGENYI IVANOV offered excellent suggestions on the manuscript. The initial stages of this work, as it related to the selection of revertants of the *pma1* mutants, were supported by National Institutes of Health grant GM39739. The studies on ectopic recombination and translocations were supported by National Institutes of Health grant GM29736.

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Communicating editor: S. JINKS-ROBERTSON