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Mitochondrial Genetics in Bakers' Yeast: A Molecular Mechanism for Recombinational Polarity and Suppressiveness

(mitochondrial DNA/*Saccharomyces cerevisiae*)

PHILIP S. PERLMAN AND C. WILLIAM BIRKY, JR.

Department of Genetics, The Ohio State University, Columbus, Ohio 43210

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ABSTRACT **Recombinational polarity and suppressiveness are two well-known but puzzling cytoplasmic genetic phenomena in bakers' yeast, *Saccharomyces cerevisiae*. Little progress has been made in characterizing the underlying molecular mechanisms of these phenomena. In this paper we describe a molecular model for recombinational polarity that is compatible with the available genetic evidence. The model stresses the role of small deletions and excision/repair processes in otherwise canonical recombinational events. According to the model, both phenomena require recombination and may share mechanistic elements.**

In *Saccharomyces cerevisiae*, sensitivity or resistance to a number of antibiotics is determined by alleles at genetic loci on mitochondrial DNA (e.g., C^s/C^r for chloramphenicol, S^s/S^r for spiramycin, E^s/E^r for erythromycin, O^s/O^r for oligomycin, and P^s/P^r for paromomycin). During mating, the fusion of haploid cells with genetically different mitochondria produces diploid zygotes containing both kinds of mitochondria; recombination of mitochondrial DNA (mtDNA) occurs, producing new genotypes. It is probable that the fusion of mitochondria is a necessary prerequisite for recombination events to occur. During subsequent vegetative reproduction, segregation of mitochondria (and mtDNA) results in the production of diploid cells, all of which are pure for one mitochondrial genotype or another (1–4). The percentage of cells of each genotype provides an estimate of the proportion of mtDNA molecules of the same genotype produced in the cross, and may be used to map the loci (5–7).

Slonimski and colleagues have reported (5–8), and we have verified (9, 10), that such crosses fall into two distinct classes (Table 1). Among the progeny of "homopolar" crosses (Table 1: A-1, A-2, B-1, B-2, and C-1), parental genotypes occur in roughly equal proportions while reciprocal recombinant types are equal and relatively rare; among the progeny of "heteropolar" crosses (Table 1: A-3, B-3, and C-2), parental proportions are very unequal and reciprocal recombinants are very unequal but relatively common. They proposed that a mitochondrial locus, called ω, existing in two allelic forms, ω^+ and ω^-, controls the polarity of a cross (5). Strains are identified as ω^+ or ω^- on the basis of crosses to standard tester stocks; "homopolar" crosses are $\omega^+ \times \omega^+$ or $\omega^- \times \omega^-$, while "heteropolar" ones are $\omega^+ \times \omega^-$ (5). These crosses were originally termed "homosexual" and "heterosexual" by Slonimski's group (4, 5), but the polarity phenomenon now appears to have nothing to do with sexuality.

Any proposed mechanism for recombinational polarity in heteropolar crosses must be compatible with the following experimental observations:

(*i*) Recombinational polarity is controlled by a mitochondrial locus, called ω, which exists in two allelic forms. The ω locus is closely linked to the chloramphenicol (C^s/C^r) locus (5). (*ii*) Polarity is seen only in zygotes containing both ω^+ and ω^- mitochondrial genomes. It is seen both as nonreciprocal recombination and as the preferential appearance in the progeny of mitochondrial alleles linked to ω^+ (or the loss of alleles linked to ω^-). (*iii*) Recombinational polarity is confined to markers that are linked to ω (e.g., C^s/C^r, S^s/S^r, and E^s/E^r) (5); crosses involving markers unlinked to ω (e.g., O^s/O^r and P^s/P^r) (6, 7) yield nonpolar (essentially quantitatively reciprocal) recombinant classes in heteropolar crosses. (*iv*) The extent of loss of a particular ω^--linked marker decreases with increasing distance from ω; a unidirectional gradient of marker loss decreasing in the order $\omega^- > C^s/C^r > S^s/S^r > E^s/E^r$ has been reported (5). (*v*) ω^+ and ω^- probably do not code for a protein involved in polarity because petite mutants, which are deficient in mitochondrial protein synthesis, show polarity in crosses with wild-type whenever they have retained their parental ω allele (4, 10).

A formal model has been proposed by Dujon *et al.* for this recombination polarity in which polarized gene conversion occurs in heteropolar crosses beginning at or near ω^- and proceeding toward the E^s/E^r locus (7, 8). The conversion is unidirectional, converting alleles on the ω^- strand into the alleles of the ω^+ strand. This was visualized as the degradation of the ω^- molecule starting at ω^- followed by resynthesis using the ω^+ strand as template. These workers did not discuss the nature of the difference between ω^+ and ω^- or the enzymes involved in the process. To enhance the predictive value of their model, we propose the following molecular mechanism (Fig. 1), which is compatible with the foregoing observations and which may also be extended to explain the phenomenon of petite suppressiveness (Fig. 2).

We assume that ω^+ and ω^- are deoxyribonucleotide sequences differing in length, with ω^+ being shorter than ω^-; for the sake of simplicity, we assume that ω^- is a duplication of ω^+ [other possibilities, such as ω^+ being a complete deletion of ω^-, are also compatible with this model (Fig. 1A)]. Whenever a recombinational event occurs in the region containing ω, a heteroduplex is formed consisting of one ω^+ and one ω^-

Abbreviations: *C*, *S*, *E*, *O*, and *P*, genetic loci for chloramphenicol, spiramycin, erythromycin, oligomycin, and paromomycin, respectively. The superscripts s and r mean sensitive and resistant, respectively.

TABLE 1. *Genetic data illustrating homopolar and heteropolar crosses*

Parental genotypes	n	% Random diploids				% Single markers		Strains used	
								α	a
		C^tE^s	C^sE^r	C^rE^r	C^sE^s	C^s	E^r		
(A) 1. $\alpha\ \omega^+C^rE^s \times a\ \omega^+C^sE^r$	296	35.2	46.1	8.6	10.4	56.5	54.7	(32)6-2/5	(39)5D1/1
2. $\alpha\ \omega^-C^rE^s \times a\ \omega^-C^sE^r$	5831	46.4	44.0	6.1	3.3	47.3	50.1	IL16-10B	55R5-3C/221
3. $\alpha\ \omega^-C^rE^s \times a\ \omega^+C^sE^r$	1155	1.3	60.5	0.4	37.5	98.0	60.9	IL16-10B	D22 E_2^r
		C^sO^s	C^rO^r	C^rO^s	C^sO^r	C^s	O^s		
(B) 1. $\alpha\ \omega^+C^rO^r \times a\ \omega^+C^sO^s$	1065	47.1	40.6	3.1	9.0	56.1	50.2	(32)6-2/5	4810
2. $\alpha\ \omega^-C^rO^r \times a\ \omega^-C^sO^s$	957	31.4	58.2	7.7	2.8	34.2	39.1	(32)1-1/1	N123
3. $\alpha\ \omega^-C^rO^r \times a\ \omega^+C^sO^s$	1115	36.5	2.6	0.1	60.9	97.4	36.6	(32)1-1/1	4810
		O^rP^s	O^sP^r	O^rP^r	O^sP^s	O^s	P^r		
(C) 1. $\alpha\ \omega^+O^rP^s \times a\ \omega^+O^sP^r$	1065	40.4	45.0	9.2	5.2	50.2	54.2	(32)6-2/5	4810
2. $\alpha\ \omega^-O^rP^s \times a\ \omega^+O^sP^r$	1115	52.1	28.0	11.3	8.6	36.6	39.3	(32)1-1/1	4810

For each cross, both parental strains were grown to logarithmic phase in a semisynthetic medium (33) containing 4% glycerol as carbon source; cells were mated in suspension (34), and prototrophic diploids were selected on minimal medium (plus dextrose) (35). Prototrophic revertants and petite mutants in the parental cultures were low enough ($<1/10^6$ and $<3\%$, respectively) to have no effect on the genetic analysis. Mitochondrial genotypes of a random sample of diploid cells issued from >1000 zygotes were scored on solid semisynthetic medium containing 4% glycerol and antibiotics as needed (chloramphenicol at 3 mg/ml, erythromycin at 1 mg/ml, and oligomycin at 1 μg/ml, all purchased from Sigma Chemical Co.). Strains used were obtained from the following workers: 55R5-3C/221 and IL16-10B from P. Slonimski; D22 E_2^r is a mutant selected by C. W. Birky in strain D22 from D. Wilkie; N123 from E. Moustacchi; 4810 from R. Kleese; (32)1-1/1 constructed by P. Perlman from D243-4A-O_4^r (R. Criddle) and IL126-1B (P. Slonimski); (32)6-2/5 constructed by P. Perlman from D243-4A-O_4^r and IL458-1A (P. Slonimski); and (39)5D1/1 constructed by P. Perlman from 55R5-3C/221 and D6 (D. Wilkie). n, number of diploids. Homopolar crosses are A-1, A-2, B-1, B-2, and C-1. Heteropolar crosses are A-3, B-3, and C-2. These data illustrate the similarity of $\omega^+ \times \omega^+$ with $\omega^- \times \omega^-$ two-factor crosses. Columns "% single markers" show the equal recovery of all markers in homopolar crosses and the unequal recovery of omega-linked markers (C and E) in heteropolar crosses. C-1 and C-2 show that markers that are unlinked to ω (i.e., O and P) are unaffected by omega alleles carried on their genomes. In cross C-2 the transmission of the ω^+ C^s alleles was highly polar (97.4% of diploids were C^s); in other words, polar and nonpolar exchanges can occur in the same cells. For a more detailed exposition of these properties of mitochondrial crosses the reader should consult refs. 5–7.

strand, containing a single-stranded region (loop) in the ω^- strand (at the ω locus) (Fig. 1B). Base pairing in regions flanking the loop will be essentially complete. An endonuclease specific for single-stranded DNA breaks the ω^- strand at the ω locus (Fig. 1C), and an exonuclease degrades a portion of the ω^- strand starting from one or both of the free ends (Fig. 1D). DNA synthesis repairs the gap, using the intact ω^+ strand as template, and ligase seals the remaining nick, thus completing the conversion event (Fig. 1E).

If the size of the excised region is invariant while the position of ω in the heteroduplex is *not*, or if the position of ω in the heteroduplex is invariant while the size of the excised region is not, a gradient of frequency of gene conversion that decreases with marker distance from ω^- is obtained. An alternative situation in which the size of the excised region *and* the position of ω in the heteroduplex are both invariant (ω being a preferential site for the initiation of recombination events) would yield a constant frequency of conversion for certain markers with a distinct boundary between polar and nonpolar markers. We, therefore, prefer either of the former possibilities. Since genetic markers are not yet known to the left of ω (as shown in Fig. 1), it is not possible to say whether the excision event is uni- or bidirectional, although bidirectional excision would result in similar conversion events in both reciprocal heteroduplexes.

So far, this model accounts for the observed strand preference of the conversion event. In order to account for the great efficiency of the conversion process (Table 1), multiple rounds of recombination and (possibly) preferential pairing at the ω locus must be assumed (7, 9). The repair of mismatched bases may occur in the mitochondrial system but is not a factor in polarity according to our model. Duplications and deletions of regions of mtDNA are known to occur in cytoplasmic petite mutants (11–18); either ω^+ or ω^- might have arisen by the same mechanism that produces petites, but with the duplicated or deleted regions not involving essential mitochondrial functions and thus not producing the usual petite phenotype.

For comparative purposes, we have considered several other models. In the first, ω^+ and ω^- are assumed to be *different* base sequences of the same length. Without additional *ad hoc* assumptions (e.g., the existence of a base sequence-specific nuclease) this model fails to yield the preferential conversion of ω^--linked markers. In the second, an analogy was drawn to the well-characterized phenomenon of restriction/modification as observed in bacteria; this model assumes that ω^+ strands are nuclease-resistant (19) while ω^- strands are progressively degraded starting from the ω^- region of mtDNA. It would also require that ω^+ code for the requisite enzyme(s). If a particular nuclear genotype were responsible for the destruction of ω^- genomes, it would have to be ubiquitous, to insure that it was always present in all homopolar crosses, including $\omega^- \times \omega^-$, where it would degrade *all* of the mtDNA in the zygotes.

Several predictions follow from our preferred hypothesis: (*i*) ω^- could mutate to ω^+ by a deletion event. It could also mutate to a modified form of ω^- by partial deletion of the additional bases. (*ii*) ω^+ could mutate to ω^- by a duplication event. Depending on the base sequence that is inserted, it could become ω^- or it could lose polarity entirely. Crosses between the latter mutant and ω^+ or ω^- would then fit the first alternative model in the preceding paragraph. (*iii*) Small deletions or duplications elsewhere in the mitochondrial genome

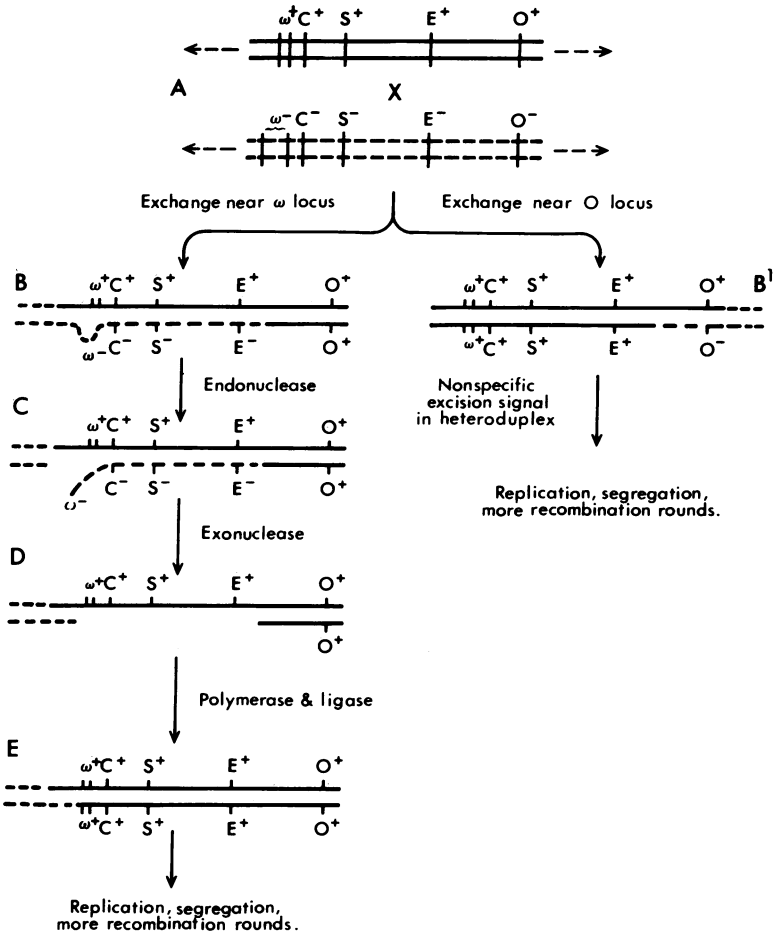

FIG. 1. Molecular model for mitochondrial recombinational polarity. The elements of our model are illustrated as a series of interactions of duplex DNA molecules. The arrangement of the loci, ω, C, S, and E, and the involvement of degradative and synthetic steps are similar to the scheme given in Fig. 2 of Dujon *et al.* (8). O is placed to the right of E for convenience since its precise map position has not been established (6). The remainder of the circular mtDNA molecule is omitted. Strands from ω^+ genomes are identified as *solid lines*, while ω^- strands are *broken lines*. Alleles are identified according to their parental origin by the superscript (+) or (−); for example, C^+ is the C allele of the ω^+ parent. The distances between markers and the size of ω^+ and ω^- regions are not intended to be accurate. In the series A through E we have shown only one of the two (possible) reciprocal recombinant structures; the other has a similar structure and will produce the $\omega^+C^+S^+E^+O^-$ recombinant type. This model does not require specifying whether interacting molecules are circular, whether concatemers are formed, or whether two or one recombinant molecules are produced. The repair of mismatched bases may occur as a consequence of heteroduplex formation; if it occurs, we would expect it to change each strand with equal frequency so that it would not contribute to unequal recoveries of alleles in populations of zygotes. Refer to the text for additional description of the events depicted in the figure.

could result in preferential conversion of linked markers that would be independent of ω^+/ω^- interactions. As new mitochondrial loci are discovered, new polarity phenomena may be found.

This model may be extended as an explanation of the suppressiveness of cytoplasmic petite (ρ^-, respiratory deficient) mutants, i.e., the production of various proportions of petite diploids in crosses of petite by wild-type (20–24). It has already been proposed that suppressiveness might result from recombination spreading of damage (e.g., nonsense sequences) in the petite genome (5). However, recent studies of petite genomes (refs. 13, 18, and unpublished) make our specific hypothesis an attractive one. At least some petites retain various portions of the wild-type genome in the form of tandem repeats (17); most of the known genetic loci can be found singly or in groups in petite mutants (ref. 18 and unpublished), and all such mutants can exhibit various degrees of suppressiveness (ref. 24 and unpublished). Additionally, petites that lack mtDNA are neutral (25) while all suppressive petites have mtDNA (26). For simplicity, we assume that all sequences in petites are derived from wild-type ones; whether petites can contain sequences that did not originate in the wild-type genome is currently controversial (11, 12, 15). Thus, suppressiveness seems to require the presence of mtDNA, and differences between DNA-containing petites that differ in suppressiveness, especially ones that have retained the same markers, must be due to the molecular arrangement of the retained sequences.

Consider three possible arrangements of the genes C^r and E^r in a petite genome containing tandem repeats as shown in Fig. 2. Since the only region of homology between the petite and wild-type mtDNA is in the region retained by the petite, recombinational events between petite A and wild-type mtDNA would not produce excisable loops except at the ω locus. Therefore, no damage would be inflicted on the wild-type genomes as a consequence of recombination with the petite; such a petite

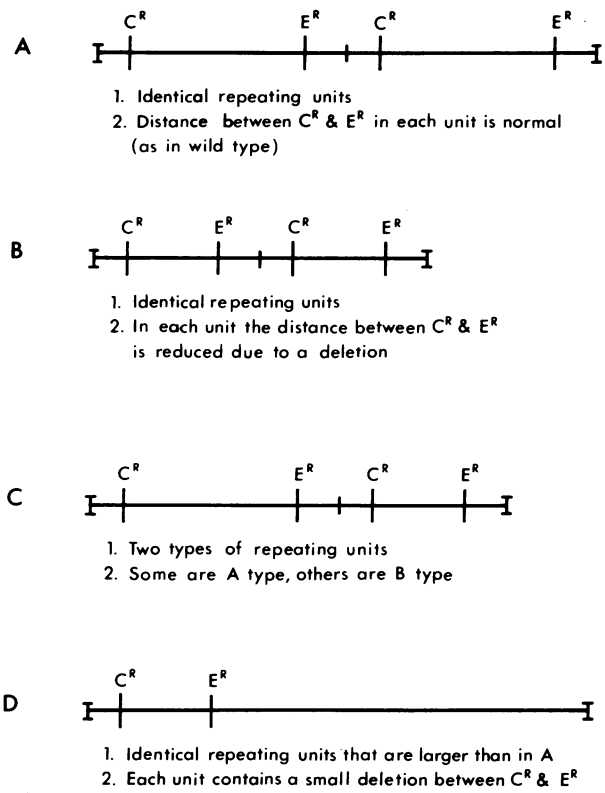

FIG. 2. Some possible arrangements of mitochondrial genes in petite mtDNA. In the figure we have depicted one or two repeating units in molecules that repeat the basic unit(s) as shown. Distinguishing features of each case are indicated in the figure, and the predicted behavior in crosses with wild-type is discussed in the *text*. For clarity we have not shown the *S* locus between C^r and E^r; Deutsch *et al.* have shown that C^rE^r petites have usually retained the *S* locus as well (18).

would be neutral or only weakly suppressive because any respiratory-deficient cells produced by mitochondrial segregation grow more slowly than respiratory-sufficient (wild-type) cells. Petite B, however, would yield heteroduplex regions in which a single-stranded loop of the wild-type strand would be found; the loop would then be sensitive to the excision process that we have proposed. Every recombinational event between the wild-type and petite B would result in the deletion of essential wild-type information, thus converting wild-type into petite; we would predict that petite B would be highly suppressive. In case C, some events of types A and B would occur and the degree of suppressiveness would depend on the ratio of the two types of C^rE^r regions. Another mtDNA arrangement that would yield an intermediate level of suppressiveness is indicated as case D. Here the frequency of conversion of wild-type to petite is reduced (relative to case B) by the presence of additional nondeleted regions of wild-type base sequences in the petite genome.

Unidirectional gene conversion, in response to deletion and duplication mutants of precisely the sort postulated here, has been demonstrated recently in the *rII* and *e* genes of bacteriophage T4 (27, 28). In crosses with wild-type, deletion mutations ranging in size from two bases to about half the *rIIB* cistron were preferentially recovered; conversely, insertion frameshift mutations of one, two, four, or five bases were preferentially lost. Heterozygote formation is lower for *rII* deletions than for point mutations (29), suggesting that deletion loops in heteroduplexes can serve as more efficient signals for excision and repair than single mismatched bases. The situation may be different for meiotic recombination in eukaryotic chromosomes. Deletions in the *His4* region in the yeast nucleus are converted to wild type, as well as vice versa (30). In *Ascobolus*, data obtained with spore-color mutants have been interpreted as indicating preferential conversion of deletion frameshifts to wild-type and of wild-type to insertion frameshift (31). This conclusion, however, is based on unproved assumptions about the action of mutagens. Since mitochondrial genomes are more closely analogous in their structure to those of phage than to eukaryotic chromosomes, we believe that their recombination mechanisms will also prove to be analogous to those of prokaryotes. In addition, postulating preferential conversion of wild-type to deletion has the advantage of requiring only enzymes specific for the excision of single-stranded DNA, and such enzymes are already known (32), while no enzymatic mechanism has been discovered for the reciprocal conversion.

In summary, we have presented a molecular model for mitochondrial recombinational polarity in bakers' yeast that is compatible with all available genetic evidence on the phenomenon. It stresses the role of small deletions (and excision/repair processes) in otherwise canonical recombinational events and it makes testable predictions with a minimum of *a priori* assumptions. According to the model, recombinational polarity and suppressiveness of petites may share mechanistic elements. If our hypothesis is correct, a study of these phenomena in yeast mitochondria may shed light on recombinational processes involving frameshifts, duplications, and deletions in prokaryotes and in eukaryotic nuclei.

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