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Polarity of meiotic gene conversion in fungi: contrasting views

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Abstract. The frequency of meiotic gene conversion often varies linearly from one end of the gene to the other. This phenomenon has been called 'polarity'. In this review, we will primarily discuss studies of polarity that have been done in the yeast *Saccharomyces cerevisiae* (*ARG4 and HIS4* loci) and in *Ascobolus* (*b2* locus) with an emphasis on possible mechanisms. The genetic and physical data obtained at these 'hotspots' of recombination strongly suggests that the formation of a polarity gradient reflects both the frequency of heteroduplex formation and the processing of this recombination intermediate by mismatch-repair-dependent processes.
Key words. Recombination; meiosis; gene conversion; polarity; heteroduplex DNA; mismatch repair; fungi.

This article is dedicated to the memory of Seymour Fogel.

The frequency of meiotic gene conversion often varies linearly from one end of the gene to the other (reviewed in 16, 37, 47, 59). This phenomenon has been called 'polarity'. In this review, we will discuss studies of polarity that have been done in the yeast *Saccharomyces cerevisiae* and in *Ascobolus*, with an emphasis on possible mechanisms. As will be evident from this discussion, no single mechanism easily explains all the data.

I. General features of meiotic recombination in yeast and models of recombination

Most studies of meiotic recombination in *S. cerevisiae* involve tetrad analysis. Strains heterozygous for various markers (often auxotrophic mutations) are sporulated, and individual spores derived from a single ascus are micromanipulated onto a rich growth medium. After colonies are formed, they are replica-plated to medium allowing detection of the phenotype. If a diploid strain is heterozygous for a single genetic marker (alleles *A* and *a*), most unselected tetrads will yield 2*A* spore colonies and 2*a* spore colonies. Occasionally, tetrads with 3*A* spore colonies and 1*a* spore colony or 1*A* spore colony and 3*a* spore colonies are found. Such tetrads represent gene conversion events, the non-reciprocal transfer of information from one chromatid to another. The frequency of gene conversion per meiosis in yeast varies at different loci between 1% to 50% with a median value of about 4%[11,37]. In general, in *S. cerevisiae*, 3*A*:1*a* and 1*A*:3*a* conversions are equally frequent. In *Ascobolus*, strong biases in favor of the mutant or wild-type allele are often observed[47].

A second type of non-Mendelian segregation event is post-meiotic segregation (PMS). PMS events are detected by spore colonies that are sectored for the heterozygous marker[9]. Tetrads with two *A* colonies, one *a* colony and one sectored *A*/*a* colony are classified as 5*A*:3*a* tetrads (using the nomenclature borrowed from 8-spored fungi), whereas tetrads with one *A* colony, two *a* colonies, and one sectored *A*/*a* colony are classified as 3*A*:5*a* tetrads. Tetrads with one *A* colony, one *a* colony, and two sectored colonies represent aberrant 4:4 tetrads. For most alleles, PMS events are much less frequent than gene conversion events, and aberrant 4:4 tetrads are much less frequent than 5:3 or 3:5 tetrads[11,37].

A large number of genetic and physical studies suggest that PMS events reflect heteroduplex formation between DNA strands derived from the two different alleles in which the resulting mismatch is segregated into a spore without repair. Consequently, in the first mitotic division following meiosis, the two alleles segregate, generating a sectored colony. The observation that aberrant 4:4 tetrads are much rarer than 5:3 or 3:5 tetrads is interpreted as indicating that heteroduplex formation is usually asymmetric, with one allele acting as a donor of a DNA strand and a second acting as a recipient.

Several types of experiments suggest that most gene conversion events reflect heteroduplex formation between the heterozygous alleles, followed by correction of the resulting mismatch (reviewed in 11, 37). For example, if the *A* allele donates a strand to the *a* allele, and the resulting mismatch is corrected using the *A* strand as a template, then a conversion event of the 3*A*:1*a* type would be observed. If the same mismatch is corrected using the *a* strand as a template, then 2*A*:2*a* segregation would be observed; mismatch corrections that restore normal Mendelian segregation are called 'restoration' events. Later in the review, we will discuss the relative frequencies of these two repair processes. Finally, we should state that, although most gene conversion events appear to involve repair of mismatches in heteroduplexes, we cannot rule out that some conversion events are a consequence of a different type of

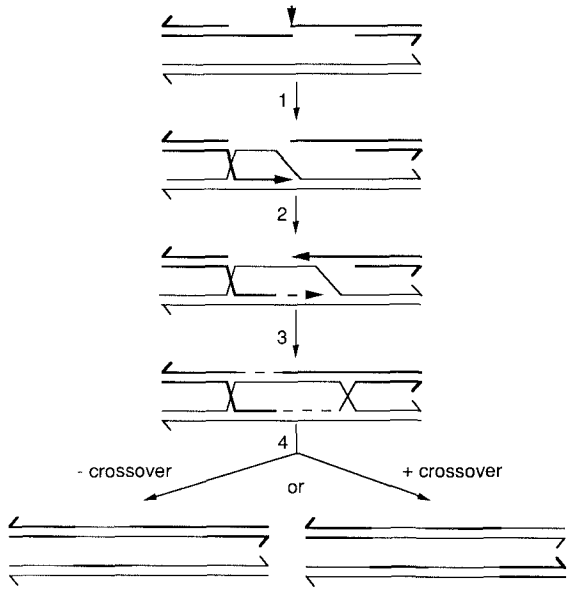

Figure 1. Revised version of the double-strand break repair model of recombination (after figure 5, ref. 51). In this model, recombination is initiated by a double-strand break. The broken ends are excised symmetrically 5′ to 3′. One 3′ end invades the other homolog forming a region of heteroduplex and acting as a primer for DNA synthesis (Step 1). The displaced strand pairs with the other broken end, which also acts as a primer for repair replication (Step 2), and continued strand displacement results in a second region of heteroduplex (Step 3). The two junctions connecting the homologs are resolved by strand cleavages (Step 4). Depending on which strands are cleaved, the resulting DNA molecules have flanking sequences located in the parental (left side of diagram) or recombinant (right side) configuration.

mechanism (for example, repair of a double-strand gap).

The observation that most mutant alleles have much higher frequencies of gene conversion than PMS suggests that most mismatches are efficiently recognized and repaired. Exceptions include single base pair changes that result in a C/C mismatch, small palindromic insertions, and certain small deletions (reviewed in 37). With these types of mutations, up to 75% of the aberrant segregations are PMS events. In strains with mutations in mismatch repair genes, relatively high frequencies of PMS are found even for alleles that usually produce well-repaired mismatches[41,60].

About 50% of conversion or PMS events at a locus are associated with crossing-over between flanking markers (reviewed in 11). Thus, in current models of recombination, conversion, PMS and crossovers are intimately linked. Two types of molecular models that are consistent with most of the genetic data are shown in figures 1 and 2. In figure 1, recombination is initiated with a double-stranded break (DSB), followed by symmetric excision of the 5′ ends of the broken chromosome[51]. In this modified version of the original double-strand break repair model[52], there is extensive heteroduplex formation on both sides of the break. Whether heteroduplex formation is associated with crossovers depends on the mode of resolution of the junctions flanking the heteroduplex. In figure 2, heteroduplex formation is a consequence of a single-stranded gap on the

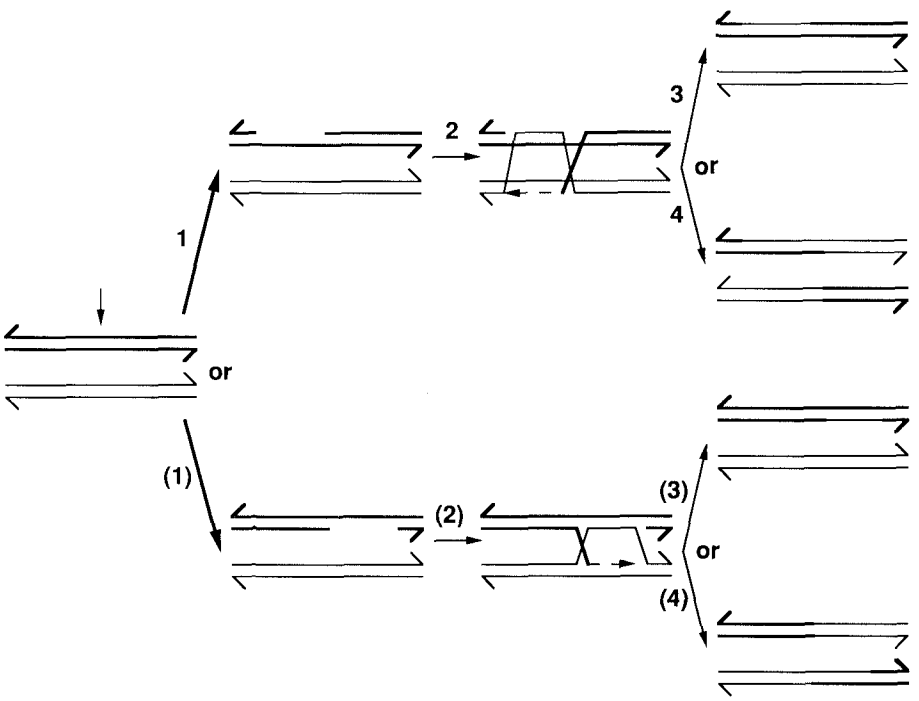

Figure 2. Modified version of Radding[39] model of recombination[38]. In this model, recombination is initiated by single-strand cleavage, followed by 5′ to 3′ degradation of the cut strand to the left (top part of diagram) or to the right (bottom part). The resulting gap is invaded by a strand derived from the other homolog [Step 2 and (2)], and the resulting intermediate is resolved by strand cleavages. Depending on which strands are cleaved, the flanking sequences are in the parental [3 and (3)] or recombinant [4 and (4)] configurations.

DNA[39]. In one version of this model, gaps are formed by 5′-3′ excision from an initiation site, and gap formation can occur on either side of the initiation site[38]. Although both of these models are consistent with most of the genetic data, thus far, physical studies have detected double-strand breaks at a number of recombination hotspots, but not large single-stranded gaps[3,51].

For most models of recombination[14,17,25,52], it has been assumed that polarity reflects specific sites for the initiation of heteroduplex formation. Thus, a marker located close to an initiation site will have a higher probability of inclusion in the heteroduplex than a marker located further away. Below, in sections IV and V, we will discuss this issue in more detail.

II. Operational definitions of polarity

In *Saccharomyces cerevisiae*, polarity has been observed for meiotic, but not mitotic, recombination events (reviewed in 37). Three types of experiments can be done to show polarity[59]. In organisms with high levels of meiotic recombination, one can analyze a series of diploid strains, each heterozygous for a mutation at a different position within the gene. Unselected tetrads are dissected and the level of aberrant segregation (gene conversion events plus PMS events) is measured in each strain. Ideally, such experiments should involve markers at positions in the gene that have been physically mapped, and all markers should be the same type of mutation (for example, markers that result in high levels of conversion relative to PMS).

A second approach is to construct strains that are heterozygous for two markers within the same gene, with the markers on opposite chromosomes. In general, recombination between such markers is a consequence of gene conversion of one of the two markers, rather than reciprocal exchange. In genes exhibiting polarity, the marker on one side of the gene (the high-conversion end) is preferentially converted. This type of analysis must be done with multiple pairs of alleles in order to demonstrate convincingly a polarity gradient. The third approach is related to the second. A strain is constructed with heteroalleles in one gene, as well as closely-linked flanking heterozygous markers. For example, one chromosome could contain the arrangement A (b1+) C and the second a (+b2) c. If a prototrophic spore derived from this strain had the arrangement of markers A (++) C, one would infer that the recombination event was a consequence of gene conversion of the b1 allele. If the prototrophic spore had the arrangement a (++) c, one would infer conversion of the b2 allele. In *S. cerevisiae*, polarity gradients are usually established using the first method.

III. General observations concerning polarity in fungal systems

Polarity was discovered at approximately the same time in *Ascobolus*[23] and *Neurospora*[26]. We will summarize some of the features of polarity that have been observed in these fungi (reviewed in 59), although it should be emphasized that some of these features may not apply to *S. cerevisiae*. First, in most genes, the frequency of conversion varies linearly from one end of the gene to the other, but two types of exceptional genes have been observed. One exceptional class is that in which both ends of the gene have high rates of conversion, whereas markers in the middle of the gene convert less frequently; the *lysF* gene of *Aspergillus* is one example of this class[36]. The other exceptional class is genes, like the *gray* locus of *Sordaria*[21], that have no obvious polarity gradient. Second, using *Neurospora* strains containing large inversions, Murray[27] showed that polarity was determined by elements located close to the gene, rather than by the orientation of the gene with respect to the centromere. Third, mutations exerting a *trans* effect on polarity have been identified in *Neurospora*. For example, mutants with the *rec-1* mutation elevated recombination at the unlinked *his-1* locus, and reversed the polarity gradient[18]. Fourth, at the *b2* locus of *Ascobolus*, the frequency of tetrads with the properties expected for asymmetric heteroduplex formation (5:3 and 3:5) have a polarity gradient that is opposite of that observed for tetrads with the properties expected for symmetric heteroduplex formation (aberrant 4:4) (reviewed in 47).

IV. Polarity in *S. cerevisiae*

Since the detection of polarity gradients usually involves extensive tetrad analysis with many strains, relatively few genes in *S. cerevisiae* have been examined extensively enough to determine whether they have such a gradient. There are reasonably clear polarity gradients for meiotic recombination in the *ARG4*[11,34], *DED81*[49], *HIS4*[6], *HIS1*[10,48], and *HIS2*[24] genes. At the *ARG4*, *DED81* and *HIS4* loci, the high-conversion end of the gene is the 5′ end, but for *HIS2*, the 3′ end is the high end. In addition, although there is a double-strand break located near the high end of the polarity gradients for *ARG4*, *DED81*[50] and *HIS4*[30], the double-strand break at the *HIS2* locus appears to be in the middle of the polarity gradient (R. E. Malone, pers. commun.). The steepness of polarity gradients shows considerable variation in these genes: from 1%–10% at *ARG4*, from 4% to 15% at *DED81*, 17%–50% at *HIS4*, 1%–3% at *HIS1*, and 5%–14% at *HIS2*. In this review, we will concentrate on the analysis of the *ARG4* and *HIS4* loci.

ARG4 locus

The *ARG4* gene is about 1.4 kb in size[2]. The 3′ end of the neighboring *DED81* gene is located at position

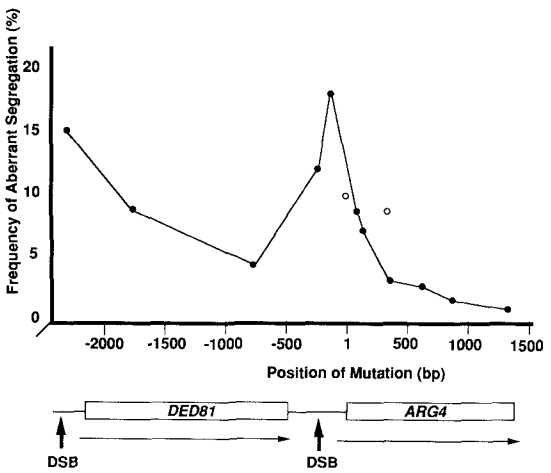

Figure 3. Polarity gradients in the *DED81-ARG4* region (data from references 11, 34, 49, 55). The frequencies of aberrant segregation were obtained by analysis of unselected tetrads and are plotted against the position of mutations. Position +1 represents the first base of the *ARG4* translation initiating codon. Dark circles correspond to well-repaired alleles giving only 6:2 aberrant segregation (conversions). Open circles correspond to the *arg4-Nsp* (position +3) and *arg4-16* (position +340) alleles giving a low level of PMS among total aberrant segregations. Arrows indicate the direction of transcription. DSB: double-strand break.

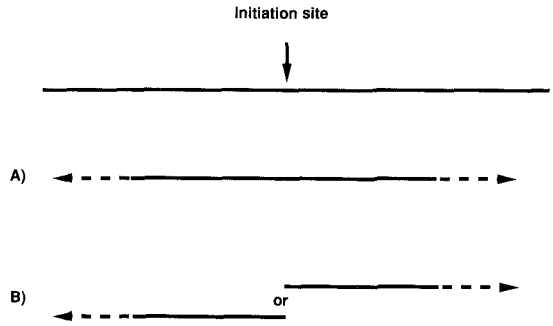

Figure 4. Two patterns of conversion flanking an initiation site. A) Conversion tracts extend in both directions as a consequence of a single initiation event (as observed for *ARG4*); B) Conversion tracts extend in both directions from a single site, but individual events are unidirectional (as observed for *HIS4*).

−491 from the *ARG4* coding region. The frequency at which mutations leading to well-repaired mismatches engage in gene conversion is dependent on their positions (fig 3). It varies from almost 10% (*arg4-Nsp* allele at position +3) to about 1% at the *arg4-Bgl* allele (position +1274)[11,34,55]. This 10-fold variation observed over about a 1 kbp region reflects a decrease from a 'hotspot' frequency of conversion to the background level observed for most mutations in the *S. cerevisiae* genome[11]. This gradient of conversion is seen for mutations of the same nature (insertions of two or four bp), eliminating the possibility of individual marker effects. Rather, it suggests that the polarity of gene conversion frequencies reflects a distance-dependent propagation from a fixed initiation site located towards the high conversion end of the gradient (5′ region of *ARG4*). Consistent with this hypothesis, bringing 3′ alleles in closer proximity to the *ARG4* promoter by interstitial deletions was found to raise their gene conversion frequencies[34].

Measurements of the distribution of gene conversion frequencies upstream of the *ARG4* locus were obtained by the introduction of restriction site polymorphisms in the *DED81–ARG4* region[49]. Unselected tetrad analysis revealed that the gradient of conversion extends from the *ARG4* promoter with a peak around position −118 (17% gene conversion). Further upstream, gene conversion frequencies decrease, resulting in a 3′ to 5′ gradient into the adjacent *DED81* gene; conversion frequencies then increase toward the 5′ end of the *DED81* gene to reach 15% for a mutation located in the *DED81* promoter region (fig. 3). Thus, there is a U-shaped gradient within *DED81* (similar to that observed for the *lysF* gene of *Aspergillus*) and a unidirectional gradient within the *ARG4* gene.

Studies of multiply-marked diploids also allowed examination of the distribution of coconversion tracts around the ARG4 peak[49]. Assuming that a gene conversion event begins at this peak, one can envisage two different types of conversion tracts. First, conversion tracts could extend in both directions from the initiation site as a consequence of a single initiation event (fig. 4A). In this case, coconversion of markers flanking the initiation site would be frequently observed. Alternatively, gene conversion tracts could extend in either direction from the peak, but only in one direction in a given meiotic event (fig. 4B). For this type of distribution, conversion would be observed on either side of the peak, but coconversion of markers flanking the peak would be more frequent than expected from coincident independent events. Such a distribution could be obtained by the presence of two closely linked but independent initiation sites that propagate tracts unidirectionally or by a single site that stimulates conversion bidirectionally, although individual events are propagated unidirectionally (see '*HIS4* locus' below). In *ARG4*, markers on both sides of the ARG4 peak frequently coconvert, consistent with the view that gene conversion events are propagated bidirectionally from a single initiation site. For tetrads with a conversion event involving a marker at the 5′ end of *ARG4* (*arg4-RV*), the percentage of coconversion with markers located about 300 bp away is very high (91%) and rapidly decreases to 10–20% for markers located above 700 bp away (fig. 5). The number of coconversion events involving the 5′ regions of *DED81* and *ARG4* was no higher than expected as a consequence of two independent conversion events, demonstrating the spatial independence of these two adjacent recombination units, landmarked by their distinct initiation sites (see below).

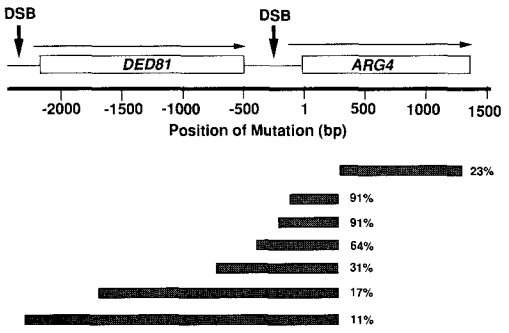

Figure 5. Percentage of coconversion of alleles in the *DED81-ARG4* region in tetrads in which the *arg4-RV* site (position +260) has been converted[49].

Given the bidirectionality of conversion events initiated at the *ARG4* and *HIS4* (see below) sites, one would expect that the high end of a conversion gradient would be at the 5′ or 3′ end of a given gene due to the proximity of an initiation site located either in its own promoter or in the promoter region of an adjacent gene. In the *HIS2* gene, however, where the high-conversion end of the gene is the 3′ end, no obvious promoter sequences were found in the 860 bp downstream of the *HIS2* coding region[24].

As mentioned above, the peak of gene conversion at *ARG4* is located around position −118. Interestingly, this position coincides with the mapping of the *cis*-acting region required for high conversion frequencies between position −465/−37 (refs 4, 34, 42, 49). A deletion of this region (*arg4-ΔEA464*) reduces the conversion frequency of the *arg4-RV* allele at least 10-fold (from 8% to less than 1%) and eliminates the polarity gradient[4]. It was also observed that an inversion of the *ARG4* coding region including the −465/+1 region retains a high level of gene conversion at the 5′ end of the gene as well as the polarity gradient, demonstrating that all the necessary *cis*-acting sequences required for these phenomena are included in the inverted 1.8 kb fragment[42]. Recent experiments also show that the high level of gene conversion at *ARG4* and the associated polarity gradient can be transplaced onto chromosome V (de Massy and Nicolas, unpubl. data) or onto a linear artificial chromosome[43]; these properties are maintained for both ectopic and allelic interactions (de Massy and Nicolas, unpubl. data), indicating that this polarity is controlled by a closely-linked *cis*-acting sequences(s).

Meiotic double-strand breaks have been detected at the 5′ end of *ARG4* and *DED81* in both plasmid and chromosomal DNA molecules[3,4,42,50,51]. Lines of evidence indicating that this lesion corresponds to the initation step of gene conversion events in *ARG4* are:

1) the breaks appear before the formation of DNA heteroduplexes and mature recombinant products during the prophase of the meiosis I division;[12]

2) the breaks are located (around positions −180/−200) in close proximity to the *ARG4* peak of gene conversion[49,51]; and

3) the amounts of the break correlate with the frequencies of gene conversion[4,42,50].

In the wild-type diploid, these transiently-broken DNA molecules are heterogeneous in length and have S1-sensitive tails, indicating terminal single-stranded regions of several hundred nucleotides[50]. The single-stranded DNA on either side of the DSB is confined to opposite strands of the DNA duplex, forming 3′ overhanging DNA tails[51]. Strikingly, the variable degree of single-strandedness was found to parallel the bidirectional gradient of gene conversion frequency around the *ARG4* gene.

Altogether, these genetic and physical data are compatible with the simple view that the polarity of gene conversion at *ARG4* results from the formation of a site-specific DSB (no physical evidence of a double-stranded DNA gap has yet been obtained) and the adjacent formation of heteroduplex DNA[22], as proposed in the modified version of the DSB repair model diagrammed in figure 1 (see ref. 51). Although simple, the above view may not be fully correct. Indeed, recent data showing a weaker polarity gradient at *ARG4* in the *msh2* mutants (Alani, Reenan and Kolodner, pers. commun.) suggest the contrasting view that the gradient of conversion measured with mutations that lead to well-repaired mismatches may not primarily reflect the extension of heteroduplex intermediates, but rather a subsequent mismatch-repair-dependent process. In our description of the *HIS4* polarity gradient, this issue will be discussed further.

HIS4 locus

The *HIS4* gene is about 2.4 kb in size and encodes a protein with three enzymatic activities[8]. As a consequence of research done by G. Fink and co-workers, regulation of *HIS4* gene expression is understood in great detail. Wild-type levels of *HIS4* gene expression require a TATAA sequence located about 60 bp upstream of the mRNA start site[31]. Four transcription factors, GCN4, RAP1, BAS1 and BAS2 bind to sites located in the region 100 to 200 bp upstream of the mRNA start site[1,7,53]. The 3′ end of the neighboring *BIK1* gene is located about 200 bp from the *HIS4* mRNA start site[54].

Certain lab strains, when sporulated at 18°C, have very high rates of meiotic gene conversion at the *HIS4* locus[28]. When the frequency of recombination for mutant alleles that lead to well repaired mismatches was examined, alleles located at 5′ terminus of *HIS4* (mutations in the initiating codon) had about 50% gene conversion in unselected tetrads, the highest frequency reported in *S. cerevisiae*[5,6]. The frequency of conversion declined to about 17% near the 3′ end of the gene (fig. 6). In addition, the frequency of gene conversion was

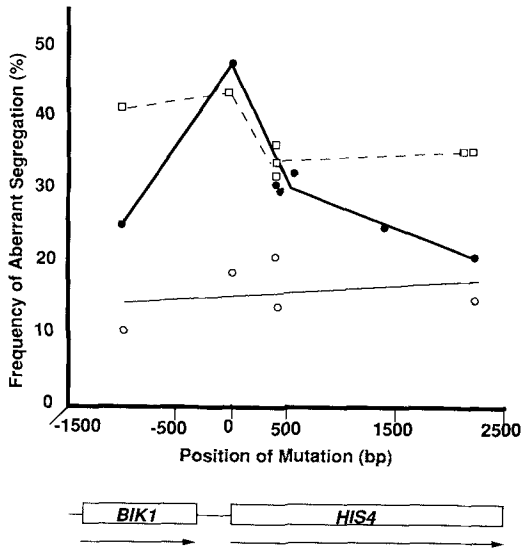

Figure 6. Polarity gradients for high- and low-PMS alleles in *HIS4* (after figure 2, reference 6). The frequency of aberrant segregation in single-point crosses for a large number of isogenic strains is summarized in this figure. The results of crosses involving low PMS alleles (alleles that lead to well-repaired mismatches) are shown by dark circles with the connecting thick solid line. Data from high PMS alleles (alleles leading to poorly-repaired mismatches) are shown by squares connected with a dashed line. The thin solid line connecting open circles reflects the aberrant segregation frequencies of alleles in strains with a deletion (*his4-Δ52*) that removes the recombination hotspot located upstream of *HIS4*: all but one of the alleles examined were low PMS alleles. The approximate positions of the alleles within *HIS4* and *BIK1* are indicated on the bottom part of the figure. Position 0 represents the first base upstream of the initiating codon of *HIS4*. Arrows indicate the direction of transcription.

about 25% in the *BIK1* gene, indicating that the peak of the conversion gradient might be located between the 5′ end of *HIS4* and the 3′ end of *BIK1*. A deletion within this region eliminated the polarity gradient in *HIS4* and reduced the frequency of recombination of a mutation located in *BIK1* (fig. 6). This result suggests the existence of a site located between *HIS4* and *BIK1* that can promote recombination in either direction[61]. Subsequent studies showed that single meiotic recombination events that were simulated by this site usually involved *HIS4* or *BIK1*, as diagrammed in figure 4A, rather both markers simultaneously[38].

Four transcription factors bind to the region upstream of *HIS4*. In experiments in which either the transcription factors or their binding sites were deleted, it was shown that BAS1, BAS2, and RAP1 were needed for wild-type recombination at *HIS4*, but GCN4 was not[56-58]. The requirement of these factors for wild-type levels of recombination is not a consequence of their effect on transcription, since a promoter deletion that reduces transcription more severely than the absence of any of the transcription factors, had no effect on meiotic recombination[56]. The current hypothesis is that the binding of these factors creates a chromatin structure that facilitates the entry of the enzymes that initiate meiotic exchange.

As described above, when the frequency of recombination of markers leading to well-repaired mismatches (low-PMS alleles) was examined, a clear polarity gradient was observed. If markers that lead to poorly-repaired mismatches (high-PMS) alleles were used, however, the polarity gradient was much more shallow (fig. 6). The gradients for the two types of markers coincide for mutations located near the 5′ end of *HIS4* and then deviate for mutations near the 3′ end of HIS4 and in *BIK1*.

For well-repaired mismatches, the frequency of aberrant segregation reflects two parameters: the frequency of heteroduplex formation (creating the mismatch) and the direction of mismatch repair (repair to generate a conversion or a restoration event). For poorly-repaired mismatches, the frequency of aberrant segregation primarily reflects heteroduplex formation. The observation that the frequency of aberrant segregation is similar for high- and low-PMS alleles near the 5′ end of *HIS4* indicates that mismatches involving low-PMS alleles at the 5′ end of *HIS4* are usually repaired by conversion-type repair[5,6,38]. To express this conclusion more rigourously, we symbolize the frequency of heteroduplex formation for high- and low-PMS alleles as H_H and H_L, respectively. The frequency of unrepaired mismatches (PMS tetrads), conversion-type repair, and restoration-type repair for high- (subscript H) and low- (subscript L) PMS alleles are symbolized by U_H, U_L, C_H, C_L, R_H, and R_L respectively. If the frequency of heteroduplex formation is the same for mismatches at the two positions, $H_H = H_L$, then $U_H + C_H + R_H = U_L + C_L + R_L$. Since we find that the total observed aberrant segregation frequency $(C + U)$ is approximately the same for high- and low-PMS alleles located near the 5′ end of *HIS4*, R_H is approximately equal to R_L. The simplest interpretation of the observation that the amount of restoration repair is independent of the type of mismatch is that there is no restoration repair for mismatches located near the 5′ end of *HIS4*; we cannot rule out, however the possibility that restoration-type repair is insensitive to the types of alterations that affect conversion-type repair. Using the above equations and data for aberrant segregation frequencies for high- and low-PMS alleles at the 3′ end of *HIS4* and different positions in *BIK1*, one can calculate that about half of the mismatches of low-PMS alleles at these positions are repaired by restoration-type repair[6,38].

Differences in the ratio of conversion- to restoration-type repair were also reported at the *HIS1* locus, although this ratio did not vary linearly according to the position of the mismatch within the gene[15]. At *ARG4*, most mutations analyzed had little or no PMS; only two mutations located at the 5′ end (*arg4-Nsp* and *arg4-16*) with a substantial amount of PMS (about 20% of

aberrant segregants) have been studied[11,34]. The *arg4-16* mutation (position +340) exhibits a higher frequency of aberrant segregations (8% among total meiosis) than the closely-linked *arg4-Bcl* mutation at position +345 (3.4%), but more systematic studies in isogenic strains will be required to establish the shape of the polarity gradient for high-PMS mutations at *ARG4*.

If the direction of mismatch repair is the major influence on the polarity gradient of low PMS alleles at *HIS4*, then mutations that prevent mismatch repair should reduce or eliminate the polarity gradient. Two different types of mutations affecting mismatch repair have been examined with different results. The *E. coli* MutL homolog in yeast, *pms1*, had no obvious effect on the polarity gradient[6], whereas the MutS homolog *msh2* eliminates the polarity gradient in the predicted way[41]. At the *ARG4* locus, *msh2* and *pms1* reduce, but do not eliminate, the polarity gradient (E. Alani, R. Reenan and R. Kolodner, pers. commun.). It is not clear why *pms1* and *msh2* have different effects at *HIS4*. It is possible that the *pms1* mutation affects only mismatches destined for conversion-type repair, whereas *msh2* affects mismatches destined for either conversion- or restoration-type repair[6]. The locus-to-locus difference in the effects of *pms1* on recombination at *ARG4* and *HIS4* are not yet understood.

Although most of the results described above indicate that the *HIS4* polarity gradient for low-PMS alleles primarily reflects the ratio of conversion- to restoration-type repair, Reenan and Kolodner[41] suggested an alternative possibility: mismatches located near the 5′ end of *HIS4* are repaired exclusively by conversion-type repair, whereas mismatches located near the 3′ end of *HIS4* either cause abortion of the heteroduplex or are repaired by restoration-type repair. This heteroduplex-aborting function of the mismatch repair system is similar to that proposed by Radman[40] for certain recombination events in prokaryotes. In support of this model, E. Alani, R. Reenan and R. Kolodner (pers. commun.) found that a well-repaired mismatch located near the 5′ end of *HIS4* reduced the frequency of aberrant segregation of a poorly-repaired mismatch located near the 3′ end of the gene without affecting the efficiency of mismatch repair.

The genetic data concerning *HIS4* and *BIK1* recombination suggest the existence of a recombination initiation site located between *HIS4* and *BIK1*. The data are consistent with two molecular models[38]: one involving a double-strand break and asymmetric processing of the broken chromosome toward *HIS4* or *BIK1* (fig. 7), the other involving a single-strand gap initiating at the site and processed toward *HIS4* or *BIK1* (fig. 2). Since heteroduplex formation preferentially involves transfer of the non-transcribed strand at *HIS4*, the strand that becomes degraded at the *HIS4* side of the site is degraded 5′ to 3′ (ref. 29). Two arguments favor the

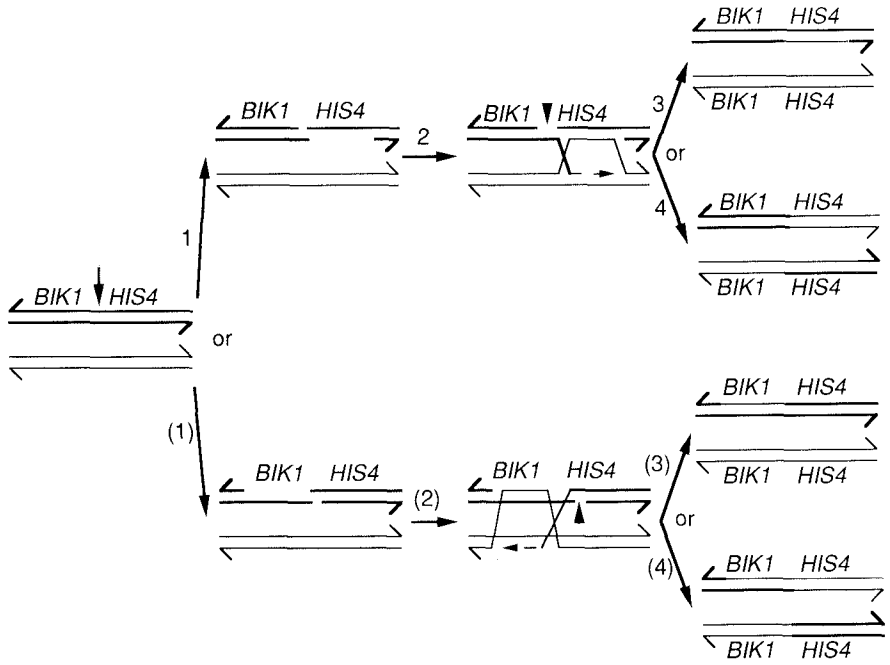

Figure 7. Model of recombination involving asymmetric processing of a double-strand break (after figure 7, reference 38). In this model, recombination is initiated by a double-strand break that is processed (by 5′ to 3′ excision) asymmetrically either toward *HIS4* (top part of figure) or toward *BIK1* (bottom part). Heteroduplexes are formed [2 and (2)] and processed by strand cleavages to yield either parental [3, (3)] or recombinant [4, (4)] configurations of flanking markers. The black triangles in the intermediate shown between Steps 2 (2) and 3 (3) indicate a single-strand nick that could be used to target an excision event during mismatch repair.

double-strand break model. First, a double-strand break located between *HIS4* and *BIK1* has been observed[30], and this break is lost in strains with a mutation in the RAP1 binding site at *HIS4* (Xu Fei and T. Petes, unpubl. data). Second, the model predicts the existence of a strand nick located near the initiation site (fig. 7). If this nick signals which strand will be excised when there is a mismatch, then one would expect that mismatches located near the initiation site will be preferentially corrected by conversion-type repair, as suggested by several experiments[5,6]. One argument against the possibility that the double-strand break is the sole initiating lesion at *HIS4* is that the amount of the double-strand break does not appear sufficient to account for the level of meiotic recombination[30]. The overall pattern of gene conversion in the *HIS4-BIK1* region is likely to represent the sum of at least two overlapping recombination units with distinct initiation sites. One of them is the site of the DSB between *BIK1* and *HIS4*; the location and the mode of initiation of the other(s) is not yet known.

V. Comparison of yeast data with data derived from *Ascobolus*

In *Ascobolus*, Lissouba and Rizet[23] found that intragenic recombination was polarized because, in heteroallelic crosses, the converted allele was always on the same side of the gene. This observation led to the earliest suggestion that recombination events may have preferred starting or ending sites. This concept defined a recombination unit (termed a 'polaron'), a length of DNA under the influence of one *cis*-acting initiation site. The *b2* gene, in which mutations exhibit the highest frequencies of aberrant segregation in *Ascobolus*, has been the most studied.

In *Ascobolus*, poorly-repaired mutations giving a large excess of PMS (5:3, 3:5 and aberrant 4:4) over conversions (6:2, 2:6) were classified as type C mutants, whereas mutations giving only conversions (less than 10% PMS) were classified as either type A (bias in favor of 6:2 over 2:6 tetrads) or B (bias in favor of 2:6 over 6:2 tetrads) mutants. In *b2*, the frequencies of aberrant segregation observed in mutant x wild-type crosses decrease from about 30% of total asci at the left end to about 10% for mutations at the right end[35,44]. The orientation of this gradient with respect to the direction of transcription of the gene is not known. No major differences were observed between type A, B or C mutations located in close proximity within intragenic groups of suppression from left to right on the genetic map (fig. 8). Small differences between closely-linked mutations can be easily accounted for by the degree of mismatch correction which varies considerably from one mutant to another in a position independent manner[47]. Based on these results, it was concluded that the gradient of

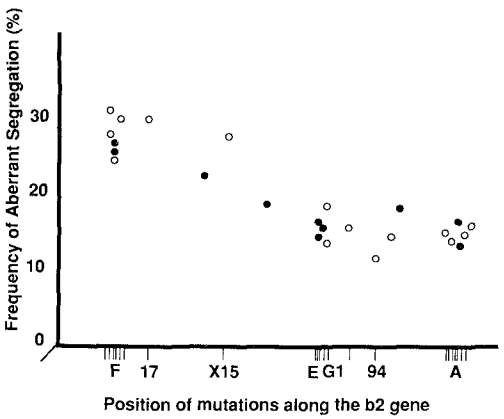

Figure 8. Polarity gradient of well-repaired (types A and B, dark circles) and poorly-repaired mutations (type C, open circles) as a function of map location within the *b2* gene of *Ascobolus* (data from references 35, 44). F, E and A correspond to the intragenic groups of suppression involving numerous frameshift mutations.

aberrant segregation along the *b2* gene is independent of the type of mutation used and primarily reflects a gradient in the formation of heteroduplex DNA. These results are different from those obtained for the *HIS4* gene in *S. cerevisiae*.

The existence in *Ascobolus* of type C mutations (large excess of PMS over conversions) also allowed the study of the distribution of heteroduplex DNA between the two interacting chromatids. Aberrant 4:4 asci reflect the presence of two heteroduplexes at the mutant site. 5:3 asci may have two possible origins. They could either be the consequence of heteroduplex formation in only one duplex (asymmetric heteroduplex DNA), or the consequence of symmetric heteroduplex DNA formation followed by mismatch correction in one of the two molecules. Since type C mutations have very low proportions of 6:2 compared to 5:3 asci, one can assume that a large proportion of 5:3 asci reflect asymmetric heteroduplex DNA formation. Along the *b2* gene, the frequency of 5:3 was found to decline towards the right end, whereas the frequency of aberrant 4:4 tetrads increased (fig. 9). The formation of heteroduplex DNA is mostly asymmetric in the left part of *b2* (90% asymmetric, 10% symmetric) and mostly symmetric in the right portion (30% asymmetric 70% symmetric).

Crosses involving two or more type C mutations (*17, G1* and *A4*) along the gene allowed the analysis of patterns of co-PMS in the transition between asymmetric and symmetric heteroduplexes[19,20,45]. Several conclusions emerged from these studies:

1) the full length of *b2* can be spanned by symmetric heteroduplex DNA, as shown by the presence of coincident aberrant 4:4 asci;

2) although many asymmetric heteroduplexes end between *17* and *G1*, some span the entire gene;

3) asymmetric and symmetric heteroduplexes are often associated in the same meiotic recombination event. In

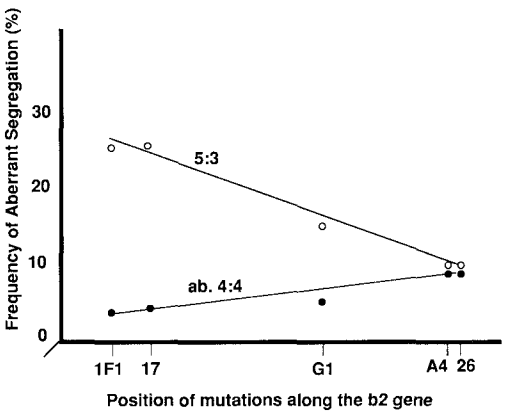

Figure 9. Percentage of 5:3 (open circle) and aberrant 4:4 (dark circle) asci among total meioses as a function of map location within *b2* for five mutations showing 80–90% PMS among aberrant segregations (after figure 14, reference 47).

such tetrads, the left site is involved in the asymmetric phase and the right site is involved in the symmetric phase;

4) finally, a minor fraction of events involves only the right end of the gene.

This fraction (called 'secondary heteroduplex') was found to increase from *G1* to *A4*, reaching 35% of total aberrant segregations. Heteroduplex DNA for this minor opposite gradient is primarily asymmetric. The distribution of the various populations of heteroduplex intermediates covering the *b2* gene is summarized in figure 10.

The direction of propagation of these heteroduplexes was addressed by using mutations that block their propagation. When heterozygous, several mutations (double-frameshifts and large insertion-deletions) were found to affect the frequency of aberrant segregations on their right but had no effect on their left (refs 13, 32; reviewed in 33). These results strongly argued that the propagation of asymmetric as well as symmetric heteroduplex DNA was rightward (indicated by arrows in figure 10), and supported the conclusion that the majority of conversion events are initiated at the high-conversion end of the polarity gradient.

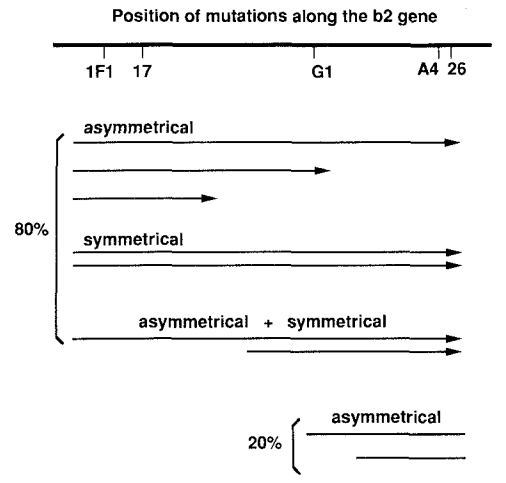

Figure 10. Distribution of heteroduplex DNAs within the *b2* gene of *Ascobolus*. The arrows indicate the direction of propagation of the heteroduplex DNA. The direction of propagation of the minor fraction of asymmetrical heteroduplex covering only the right side of the *b2* gene is not known.

A major difference between conversion events in yeast and other fungi is the absence of disparity in the direction of conversion in *S. cerevisiae*. There are strong biases in the direction of conversion toward wild-type (type A) or mutant (type B) for frameshift mutations in *Ascobolus*[47] and *Sordaria*[59]. This directionality is explained by a bias in the mismatch correction mechanism that preferentially excises the shorter strand of the heteroduplex, and copies the longer strand (reviewed in 46, 47). An intriguing aspect of conversion patterns in *b2* is the variation of the disparity ratio along the gene. Single-base-pair addition mutations show a 2:6/6:2 ratio close to 2 at the left end, but a 10-to 20-fold ratio at the right end. Rossignol and Paquette[49] explained this variation as being a consequence of the change from asymmetric to symmetric heteroduplex DNA along the gene. Since the bias in correction of this mismatch for symmetrical heteroduplex involves two heteroduplexes in the same meiosis, it will be much stronger than that observed for an asymmetric heteroduplex. The observation that the disparity ratio decreases from 10 to 4 at the right end of *b2* when symmetric heteroduplex formation is blocked[13] agrees with this hypothesis. However, the change in heteroduplex DNA distribution cannot fully explain the range of disparity ratios, since the disparity is still two-fold higher at the right end than at the left end in conditions where only asymmetric heteroduplex is formed[33,46]. This result indicates that some feature of the mismatch repair mechanism is sensitive to the position of the mismatch within the *b2* gene. It is possible that this feature of mismatch repair in *Ascobolus* is related to the gradient in conversion- to restoration-type repair observed at the *HIS4* (and, perhaps, the *ARG4*) locus.

In summary, the distribution of conversion frequencies along the *b2* gene reflects at least two overlapping recombination units. The conversion gradient mostly reflects asymmetrical heteroduplexes propagating from the high-conversion end which either stop or isomerize to the symmetrical form in accordance with the Aviemore model[25]. This steep gradient is made more shallow toward the low-conversion end by a minor gradient of asymmetric DNA that has the opposite polarity. Intriguingly, a gradient in the disparity of the 6:2/2:6 ratio is seen along the gene for well-repaired mutations.

VI. Summary of conclusions and future areas of investigation

It is hard to find a single mechanism that is consistent with all of the genetic and physical data obtained in fungi. In *S. cerevisiae*, at the *ARG4* locus, there is a double-strand break located near the high end of the conversion gradient[50] and there is a good correlation between the level of gene conversion and the amount of single-strand excision[51]. Although these results suggest that the polarity gradient of *ARG4* might reflect only heteroduplex formation, E. Alani, R. Reenan and R. Kolodner (pers. commun.) have found that the steepness of the polarity gradient at *ARG4* is substantially reduced by a mutation affecting mismatch repair. At the *HIS4* locus, the conversion gradient for high PMS alleles is much less steep than that observed for low PMS alleles, and the gradient is nearly eliminated by one of the mutations affecting mismatch repair[41]. These results indicate that mismatch repair strongly influences the shape of the conversion gradient at this locus.

It is possible that shallow conversion gradients, such as the one observed at *HIS4*, are primarily a consequence of mismatch repair, whereas steeper gradients, such as the *ARG4* gradient, reflect both a gradient of heteroduplex formation at different sites and mismatch repair. The rate of strand excision will presumably control the shape of the gradient. Excision events may be much faster at *HIS4* than at *ARG4*, leading to more extensive lengths of heteroduplex and, consequently, a shallower gradient. Genetic studies indicate that mutations located 1.8 kb apart at *HIS4* show coconversion or co-PMS much more frequently than mutations in *ARG4* that are located closer together[5,38,49].

Useful future studies would be to replicate those experiments done with *ARG4* with *HIS4* and vice versa. For example, it would be useful to examine the rate at which the double-strand break is processed to a single strand at the *HIS4* locus using the same techniques that were applied to the *ARG4* locus. It would also be important to examine the frequency of aberrant segregation of high-PMS alleles located near the 3′ end of *ARG4*, as was done at *HIS4*. In addition, to prove that the differences detected between polarity gradients at *HIS4* and *ARG4* represent locus-specific differences, one should perform studies on both *HIS4* and *ARG4* in the same genetic background. Despite a few caveats, it seems fairly clear that the formation of a polarity gradient in *Saccharomyces cerevisiae*, in at least some genetic backgrounds is due to parameters other than the frequency of heteroduplex formation.

Current progress in the identification of initiation sites of gene conversion in yeasts together with extensive physical analysis of recombination intermediates in synchronous meiosis should help to thoroughly describe and decipher the molecular basis of the various layers of polarity phenomena discussed in this review.

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1 Arndt, K., Styles, C. S., and Fink, G. R., Multiple global regulators control *HIS4* transcription in yeast. Science *237* (1987) 874–880.
2 Beacham, I. R., Schweitzer, B. W., Warwick, H. M., and Carbon, J., The nucleotide sequence of the yeast *ARG4* gene. Gene *29* (1984) 271–279.
3 Cao, L., Alani, E., and Klechner, N., A pathway for generation and processing of double-strand breaks during meiotic recombination in *Saccharomyces cerevisiae*. Cell *61* (1990) 1089–1101.
4 De Massy, B., and Nicolas, A., The control *in cis* of the position and the amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae*. EMBO *J. 12* (1993) 1459–1466.
5 Detloff, P., Sieber, J., Petes, T. D., Repair of specific base pair mismatches formed during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Molec. cell. Biol. *11* (1991) 737–745.
6 Detloff, P., White, M. A., and Petes, T. D., Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. Genetics *132* (1992) 113–123.
7 Devlin, C., Tice-Baldwin, K., Shore, D., and Arndt, K., RAP1 is required for BAS1/BAS2 and GCN4-dependent transcription of the yeast *HIS4* gene. Molec. cell. Biol. *11* (1991) 3462–3651.
8 Donahue, T., F., Farabaugh, P. J., and Fink, G. R., The nucleotide sequence of the *HIS4* region of yeast. Gene *18* (1982) 47–59.
9 Esposito, M. S., Postmeiotic segregation in *Saccharomyces*. Molec. gen. Genet. *111* (1971) 297–299.
10 Fogel, S., and Hurst, D. D., Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics *57* (1967) 455–481.
11 Fogel, S., Mortimer, R. K., and Lusnak, K., Mechanisms of meiotic gene conversion, or "wanderings on a foreign strand", in: The Molecular Biology of the Yeast *Saccharomyces*, vol. 1, pp. 289–339. Eds J. R. Broach, E. W. Jones, and J. N. Strathern. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1981.
12 Goyon, C., and Lichten, M., Timing of molecular events in meiosis in *Saccharomyces cerevisae*: stable heteroduplex DNA is formed late in meiotic prophase. Molec. Cell. Biol. *13* (1993) 373–382.
13 Hamza, H., Haedens, V., Mekki-Berrada, A., and Rossignol, J. L., Hybrid DNA formation during meiotic recombination. Proc. natl Acad. Sci. USA *78* (1981) 7648–7651.
14 Hastings, P. J., and Whitehouse, H. L., K., A polaron model of genetic recombination by the formation of hybrid deoxyribonucleic acid. Nature *201* (1960) 1052–1054.
15 Hastings, P. J., Measurement of restoration and conversion: its meaning for the mismatch repair hypothesis of conversion. Cold Spring Harb. Symp. quant. Biol. *49* (1984) 49–53.
16 Hastings, P. J., Conversion events in fungi, in: Genetic Recombination, pp. 397–428. Eds R. Kucherlapati and G. Smith. American Society for Microbiology, Washington, D. C. 1988.
17 Holiday, R., A mechanism for gene conversion in fungi. Genet. Res. *5* (1964) 282–304.
18 Jessop, A. P., and Catcheside, D. G., Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. Heredity *20* (1965) 237–256.
19 Kalogeropoulos, A., and Rossignol, J.-L., Hybrid DNA tracts may start at different sites during meiotic recombination in gene *b2* of *Ascobolus* EMBO J. *7* (1988) 253–259.
20 Kalogeropoulos, A., and Rossignol, J-L., Non random transition from asymmetrical to symmetrical hybrid DNA during meiotic recombination. Genome *32* (1989) 414–419.
21 Kitani, Y., and Olive, L. S., Genetics of *Sordaria fimicola*. VI. Gene conversion at the *g* locus in mutant x wild-type crosses. Genetics *57* (1967) 767–782.

22 Lichten, M., Goyon, C., Schultes, N. P., Treco, D., Szostak, J. W., Haber, J. E., and Nicolas, A., Detection of heteroduplex DNA molecules among the products of *Saccharomyces cerevisiae* meiosis. Proc. natl Acad. Sci. USA *87* (1990) 7653–7657.
23 Lissouba, P., and Rizet, G., Sur l'existence d'une unite génétique polarisée ne subissant que des échanges non réciproques. C. R. Acad. Sci., Paris *250* (1960) 3408–3410.
24 Malone, R. E., Bullard, S., Lundquist, S., Kim, S., and Tarkowski, T., A meiotic gene conversion gradient opposite to the direction of transcription. Nature *359* (1992) 154–155.
25 Meselson, M. S., and Radding, C. M., A general model for genetic recombination. Proc. natl Acad. Sci. USA *72* (1975) 358–361.
26 Murray, N. E., Complementation and recombination between methionine-2 alleles in *Neurospora crassa*. Genetics *48* (1960) 1163–1183.
27 Murray, N. E., Polarized intragenic recombination in chromosome rearrangements of *Neurospora*. Genetics *58* (1968) 181–191.
28 Nag, D. K., White, M. A., and Petes, T. D., Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature *340* (1989) 95–98.
29 Nag, D. K., and Petes, T. D., Genetic evidence for preferential strand transfer during meiotic recombination in yeast. Genetics *125* (1990) 753–761.
30 Nag, D. K., and Petes, T. D., Physical detection of heteroduplexes during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Molec cell. Biol. *13* (1993) 2324–2331.
31 Nagawa, F., and Fink, G. R., The relationship between the "TATA" sequence and transcription initiation sites at the *HIS4* gene of *Saccharomyces cerevisiae*. Proc. natl Acad. Sci. USA *82* (1985) 8557–8561.
32 Nicolas, A., and Rossignol, J. L., Gene conversion: Point mutation heterozygosities lower heteroduplex formation. EMBO J. *2* (1983) 2265–2270.
33 Nicolas, A., and Rossignol, J.-L., Intermediates in homologous recombination revealed by marker effects in *Ascobolus*. Genome *31* (1989) 528–535.
34 Nicolas, A., Treco, D., Schultes, N. P., and Szostak, J. W., An initiation site for gene conversion in the yeast *Saccharomyces cerevisiae*. Nature *338* (1989) 35–39.
35 Paquette, N., and Rossignol, J.-L., Gene conversion spectrum of 15 mutants giving post-meiotic segregation in the *b2* locus of *Ascobolus immersus*. Molec. gen. Genet. *163* (1978) 313–326.
36 Pees, E., Genetic fine structure and polarized negative interference at the lys-51 (FL) locus of *Aspergillus nidulans*. Genetica *38* (1967) 275–304.
37 Petes, T. D., Malone, R. E., and Symmington, L. S., Recombination in yeast, in: The Molecular and Cellular Biology of the Yeast *Saccharomyces*, vol. 1, pp. 407–521. Eds J. R. Broach, E. W. Jones, and J. R. Pringle. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1991.
38 Porter, S. E., White, M. A., and Petes, T. D., Genetic evidence that the meiotic recombination hotspot at the *HIS4* locus of *Saccharomyces cerevisiae* does not represent a site for a symmetrically-processed double-strand break. Genetics *134* (1993) 5–19.
39 Radding, C. M., Homologous pairing and strand exchange in genetic recombination. A. Rev. of Genet. *16* (1982) 405–437.
40 Radman, M., Mismatch repair and genetic recombination, in: Genetic Recombination, pp. 169–192. Eds R. Kucherlapati and G. Smith. American Society for Microbiology, Washington, D.C. 1988.
41 Reenan, R. A. G., and Kolodner, R. D., Characterization of insertion mutations in the *Saccharomyces cerevisiae MSH1* and *MSH2* genes: evidence for separate mitochondrial and nuclear functions. Genetics *132* (1992) 975–985.
42 Rocco, V., de Massy, B., and Nicolas A., The *Saccharomyces cerevisiae ARG4* initiator of meiotic gene conversion and its associated double-strand DNA breaks can be inhibited by transcriptional interference. Proc. natl Acad. Sci. USA *89* (1992) 12068–12072.
43 Ross, L., Treco, D., Nicolas, A., Szostak, J. W., and Dawson D., Meiotic recombination on artificial chromosomes in yeast. Genetics *131* (1992) 541–550.
44 Rossignol, J.-L., and Paquette, N., Disparity of gene conversion in frameshift mutants located in locus *b2* of *Ascobolus immersus*. Proc. natl Acad. Sci. USA *76* (1979) 2871–2875.
45 Rossignol, J.-L., and Haedens, V., Relationship between asymmetrical and symmetrical hybrid DNA formation during meiotic recombination. Curr. Genet. *1* (1980) 185–191.
46 Rossignol, J.-L., Nicolas, A., Hamza, H., and Langin, T., Origin of gene conversion and reciprocal exchange in *Ascobolus*. Cold Spring Harbor Symp. quant. Biol. *49* (1984) 13–21.
47 Rossignol, J.-L., Nicolas, A., Hamza, H., and Kalogeropoulos, A., Recombination and gene conversion in *Ascobolus*, in: The Recombination of Genetic Material, pp. 23–72. Ed. K. B. Low. Academic Press, Inc., New York 1988.
48 Savage, E. A., and Hastings, P. J., Marker effects and the nature of the recombination event at the *his1* locus of *Saccharomyces cerevisiae*. Curr. Genet. *3* (1981) 37–47.
49 Schultes, N. P., and Szostak, J. W., Decreasing gradients of gene conversion on both sides of the initiation site for meiotic recombination at the *ARG4* locus in yeast. Genetics *126* (1990) 813–822.
50 Sun, H., Treco, D., Schultes, N. P., and Szostak, J. W., Double-strand breaks at an initiation site for meiotic gene conversion. Nature *338* (1989) 87–89.
51 Sun, H., Treco, D., and Szostak, J. W., Extensive 3'-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell *64* (1991) 1155–1161.
52 Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J., and Stahl, F. W., The double-strand-break repair model for recombination. Cell *33* (1983) 25–35.
53 Tice-Baldwin, K., Fink, G. R., and Arndt, K. T., BAS1 has a Myb motif and activates *HIS4* transcription only in combination with BAS2. Science *246* (1989) 931–935.
54 Trueheart, J., Boeke, J. D., and Fink, G. R., Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. Molec. cell. Biol. *7* (1987) 2316–2328.
55 White, J. H., Lusnak K., and Fogel, S., Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate. Nature *315* (1985) 350–352.
56 White, M. A., Wierdl, M., Detloff, P., and Petes, T. D., DNA-binding protein RAP1 stimulates meiotic recombination at the *HIS4* locus in yeast. Proc. natl Acad. Sci. USA *88* (1991) 9755–9759.
57 White, M. A., Detloff, P., Strand, M., and Petes, T. D., A promoter deletion reduces the rate of mitotic, but not meiotic, recombination at the *HIS4* locus in yeast. Curr. Genet. *21* (1992) 109–116.
58 White, M. A., Dominska, M., and Petes, T. D., Transcription factors are required for the meiotic recombination hotspot at the *HIS4* locus in *Saccharomyces cerevisiae*. Proc. natl Acad. Sci. USA *90* (1993) 6621–6625.
59 Whitehouse, H., Genetic Recombination, John Wiley & Sons, Ltd., New York, 1982.
60 Williamson, M. S., Game, J. C., and Fogel, S., Meiotic gene conversion mutants in *Saccharomyces cerevisiae*: I. Isolation and characterization of *pms1-1* and *pms1-2*. Genetics *110* (1985) 609–646.