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Genetics

The *Saccharomyces cerevisiae ARG4* initiator of meiotic gene conversion and its associated double-strand DNA breaks can be inhibited by transcriptional interference

(yeast/homologous recombination/hotspot)

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ABSTRACT **In the yeast *Saccharomyces cerevisiae*, as in other eukaryotes, some regions of the genome have a much higher level of meiotic gene conversion than others. Previous deletion analysis indicated that the sequence necessary for the high level of gene conversion within the *ARG4* region defined an initiation site located between positions −316 and −37 [relative to the first base pair (+1) of the *ARG4* coding sequence] of the *ARG4* promoter. To test whether this sequence is sufficient to promote gene conversion in a novel chromosomal context, we inverted on the chromosome various DNA fragments including the implicated region and the *ARG4* coding sequence. Surprisingly, these inversions resulted in the loss of the normal recombination properties and double-strand-break formation associated with this process. By Northern analysis, we found that a transcript traverses the *ARG4* initiation site in these inversion mutants but not in the wild type. When transcription through this region was prevented by a transcription terminator, the activity of the initiation site and the formation of double-strand breaks were restored. From these results and from complementary deletion analysis in the normal *ARG4* orientation, we conclude that the activity of the *ARG4* initiation site requires protection from transcriptional interference.**

Two types of meiotic recombination events have been found to occur in all eukaryotes examined, crossovers (reciprocal exchanges) and gene conversions (unidirectional transfers of information). The frequencies of both events vary within a genome (1–4). The nature of the underlying molecular processes that control the distribution of recombination events remains to be elucidated. One approach is the study of gene conversion hotspots, defined as sites or regions enhancing gene conversion in their vicinity.

In fungi, where all four products of individual meiosis remain grouped in a tetrad, gene conversion events are genetically detected when a diploid strain is heterozygous at a locus (alleles A and a) leading to a tetrad containing 3A:1a or 1A:3a products rather than the normal Mendelian segregation (2A:2a). In *Saccharomyces cerevisiae*, the frequency of meiotic gene conversion is high in the vicinity of the *ARG4* gene (5, 6). The highest frequency of gene conversion (17% of total meiosis) is found for a marker located in the *ARG4* promoter region around position −119 (Fig. 1). The frequencies decrease in a gradient, termed polarity, for markers located on either side of the *ARG4* promoter (10). Within the *ARG4* coding region, the conversion frequencies decrease from 10% (position +3) to 0.4% (position +1274). Two previous deletion analyses mapped an initiation site for meiotic gene conversion in the promoter region of *ARG4* between positions −316 and −37 (6, 7). These genetics data and the physical analysis of the DNA of this chromosomal region during meiosis, which demonstrated the occurrence of a transient double-strand break (DSB) around position −200 (8, 9, 11), strongly suggest that the implicated region includes a cis-acting initiation site which stimulates recombination in its vicinity.

To know whether all the cis-acting elements had been identified by deletion analysis, we tested whether the −319/−37 sequence was sufficient to promote gene conversion in a novel chromosomal context—i.e., when displaced so that it was adjacent to new flanking sequences. We chose to invert the *ARG4* gene on the chromosome. This "minimal displacement" was preferred to the random insertion of DNA fragments on different chromosomes, which is known to be strongly subject to position effects for recombination between *LEU2* (12) or *ARG4* (13) heteroalleles. However, a 12-kilobase (kb) *ARG4*-containing fragment inserted on a yeast artificial chromosome has the same recombinational properties as at its normal location on chromosome VIII (14).

We report here that the necessary −316/−37 cis-acting sequence previously defined by deletion analysis at the normal location is not sufficient to promote gene conversion at *ARG4* in any chromosomal context. We show that the presence of a transcriptional terminator upstream from the initiation site in these inversions is absolutely required in order to maintain both the DSB and the recombination properties at *ARG4*. We also show that high and low levels of gene conversion at *ARG4* obtained in various constructs are correlated with the presence or absence of the DSB, providing further evidence for its relevance in this recombination process.

MATERIALS AND METHODS

Plasmids. All plasmids were derivatives of pNPS104, which contains the 3.3-kb *Pst* I restriction fragment of *ARG4* in the pMLC12 vector, with either the *arg4-RV* (pNPS308) or the *arg4-Bgl* (pMY232) mutation (6, 7). pMY195 and pMY197 were derived from pNPS308 and pMY232, respectively, by deletion of the 149-base-pair (bp) *Eco*47III–*Hpa* I fragment. pMY107 and pMY104 were derived from pNPS308 and pMY232 by inversion of the *ARG4* containing 2.06-kb *Hpa* I fragment, pMY138 and pMY223 by inversion of the 1.83-kb *Hpa* I–*Sna*BI fragment, and pMY159 and pMY160 by inversion of the 1.98-kb *Eco*47III–*Sna*BI fragment. pRY1 and pRY2 were obtained by deletion of the 300-bp *Hpa* I fragment of pMY159 and pMY160, respectively. The 380-bp *Sma* I–*Hin*dIII *PGK* fragment containing the transcriptional terminator (15) was inserted at the *Hpa* I site of pRY1 and pRY2 to obtain pRY3 and pRY4. Restriction enzyme digestions and ligations were performed according to the instructions of the manufacturers (Biolabs, Pharmacia, or Boehringer Mann-

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Abbreviation: DSB, double-strand break.

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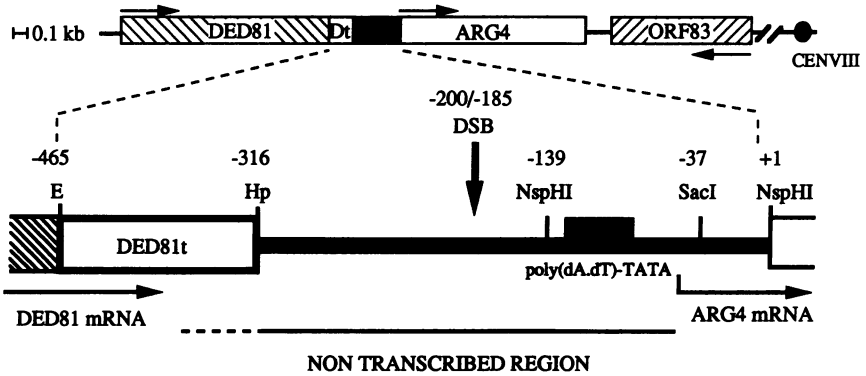

FIG. 1. Schematic representation of the *ARG4* chromosomal region. It includes three genes: *DED81* (ref. 6; C. Mezard, personal communication), *ARG4*, and *ORF83* (unpublished work). Direction of mRNA transcription is indicated by arrows. The gene conversion initiator region is composed of three regions: *Sac* I (−37) to *Nsp*HI (−139), which includes the *ARG4* mRNA start site, the TATA box, and the poly(dA·dT) promoter elements (7); *Nsp*HI (−139)–*Hpa* I (−316), in which map the site of DSBs (8, 9) and a second poly(dA·dT) promoter element (7); and the *Hpa* I (−316) to *Eco*47III (−465) region, which contains the *DED81* transcription terminator (DED81t; see text). Numbers refer to the DNA sequence, with position +1 indicating the first base of the *ARG4* open reading frame. The *ARG4* DSB is located at positions −185 to −200 (9).

heim). New DNA junctions were sequenced with the Sequenase 2.0 kit (United States Biochemical).

Yeast Strains, Media, and Genetic Analyses. All DNA constructions were introduced (16) into the two isogenic strains MGD131-2C (*MATα, arg4-Δ2060, leu2-3,112, ura3-52, trp1-289, cyhR*) and MGD131-102A (*MATa, arg4-Δ2060, his3Δ1, ade2, ura3-52, trp1-289*) by one-step replacement (17) using a cotransformation protocol (18). The *arg4-Δ2060* mutation is a 2060-bp *Hpa* I deletion of the *ARG4* gene. The expected constructions were confirmed by Southern blot analysis (19). The names of the diploids, generated by mating, are ORD149 (pNPS308 and pMY232), ORD122 (pMY107 and pMY104), ORD1132 (pMY138 and pMY223), ORD195 (pMY159 and pMY160), ORD836 (pRY1 and pRY2), ORD980 (pRY3 and pRY4), and ORD334 (pMY195 and pMY197). ORD813 derives from the previously described Δ3 (6) and has a *Bam*HI–*Hpa* I deletion upstream from the *ARG4* gene. Strains ORD307, ORD311, ORD1160, ORD839, and ORD377 are respectively identical to ORD149, ORD122, ORD195, ORD980, and ORD334 but are homozygous for the *rad50S-KI81* mutation (20). Standard media and growth conditions were used (21). Presporulation and sporulation were as described (22). *arg4* allele testing and random spore analysis for Arg^+ recombinants were done as described (6, 21).

Northern Analysis. Samples (10 ml; 2–5 × 10^7 cells per ml) were taken 8 hr after transfer into sporulation medium, washed with ice-cold water, suspended in 0.5 ml of ice-cold 10 mM Tris, pH 8.0/1 mM EDTA and immediately frozen in dry ice/ethanol and stored at −70°C. The cell walls were broken with an equal volume of glass beads and RNA was extracted (21, 23). RNA samples (20 μg per lane) were submitted to gel electrophoresis, blotting, and hybridizations as described (21, 24). The filters were probed with an RNA complementary to the *ARG4* transcript. This riboprobe was synthesized by the T7 RNA polymerase transcribing the 433-bp *ARG4 Eco*RV–*Hpa* I fragment (+260/−316) inserted in the *Hin*cII site of the pGEM-3 vector (Promega kit). RNAs were quantitated by video densitometry of autoradiograms with a CCD (charge-coupled device) camera Bio-Azur system (Orkis, Aix-en-Provence, France), coupled with a SCAN ANALYSIS (Biosoft, Cambridge, U.K.) computer program.

Analysis of Meiotic DSBs. Cells (200 ml; 2–5 × 10^7 cells per ml) were sporulated. At each time point, 25 ml was removed, mixed with 25 ml of 100% ethanol and 1.25 ml of 0.5 M EDTA, and kept at −20°C. To extract the chromosomal DNA, cells were centrifuged, washed, suspended in 0.5 ml of a spheroplasting solution [1.2 M sorbitol/0.1 M EDTA/1% 2-mercaptoethanol/0.1% Zymolyase (20,000 units)] and incubated at 37°C for 30 min. Spheroplasts were lysed in 0.5 ml of 50 mM EDTA/0.3% SDS/0.01% proteinase K at 65°C for 30 min. After addition of 0.2 ml of cold 5 M potassium acetate, the suspension was incubated on ice for 30 min and centrifuged at 10,000 × *g* for 15 min. Nucleic acid precipitation and RNase A digestion were as described (21). After restriction enzyme digestion, the DNA samples (1–3 μg) were electrophoresed (21) and the separated fragments were blotted onto Hybond N+ membranes (Amersham) with a Vacugene apparatus (Pharmacia LKB). Membranes were heated at 80°C for 2 hr. Prehybridizations and hybridizations were as described (25). The probe was a 1014-bp *Eco*RV–*Bgl* II DNA fragment of the *ARG* coding region, labeled by random priming (Boehringer Mannheim) with [α-^{32}P]dCTP (Amersham; 3000 Ci/mmol; 1 Ci = 37 GBq).

RESULTS

Loss of the Hotspot Activity in Three Inversions. If the previously identified −316/−37 cis-acting DNA sequence (Fig. 1) is sufficient for the initiation of recombination, it should be functional in a different chromosomal context. To address this question, several chromosomal inversions containing this region as well as the whole *ARG4* coding sequence were constructed. We then generated diploids homozygous for a given inversion and heterozygous for two heteroallelic markers in *ARG4* (*arg4-RV* and *arg4-Bgl*, at positions +260 and +1274, respectively), which allowed us to measure gene conversion frequencies at both alleles by unselected tetrad analysis. Random spore analysis for the frequency of Arg^+ segregants was also performed to confirm the recombinational effects with larger samples. Previous tetrad analysis showed that in the wild type, the frequency of Arg^+ recombinants is mostly representative of gene conversion events of the 5′-located *arg4* allele (6). Details of the inverted constructions and recombination results are shown in Fig. 2. Surprisingly, in these strains (ORD122, ORD1132, and ORD836), sharing the same 5′ breakpoint (*Hpa* I site at −316) but various 3′ ends beyond the *ARG4* coding region, the conversion frequency of the *arg4-RV* marker was reduced by at least a factor of 10, down from 9.6% in the isogenic wild-type strain ORD149 to ≤1%. The low conversion frequency of the *arg4-Bgl* marker and the frequency of intragenic reciprocal exchanges (<1% of meiosis) were unchanged. Random spore analysis confirmed the loss of recombination activity. We conclude that the *ARG4* 5′ region up to −316 is not functional in these inversions.

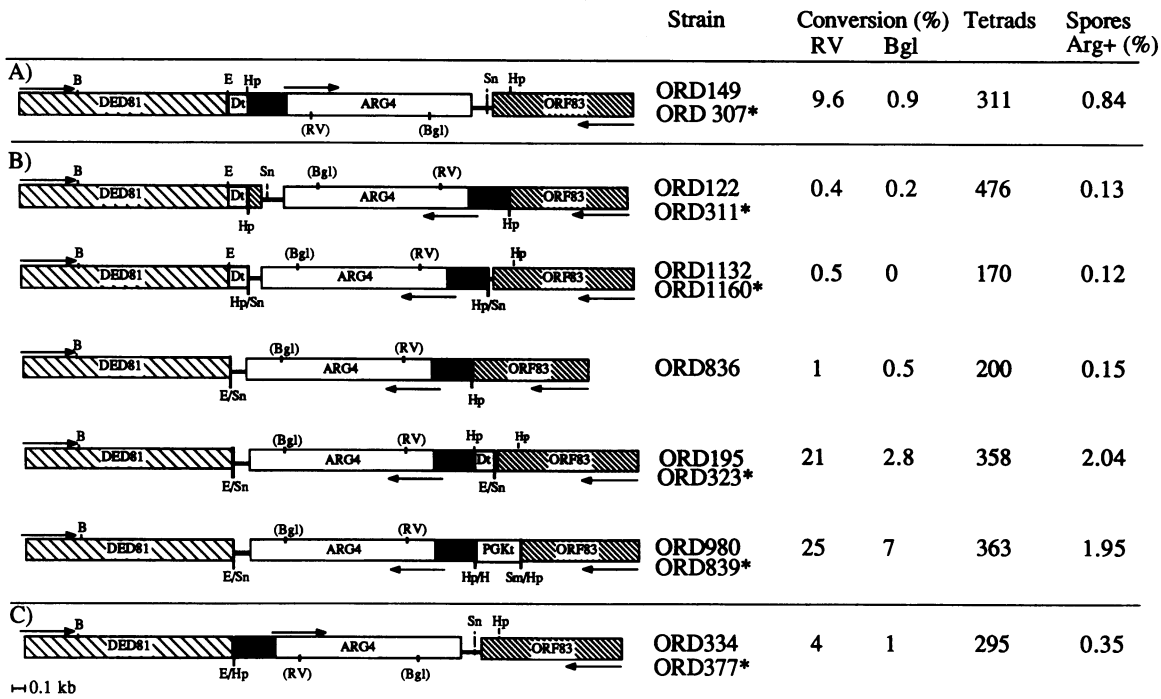

FIG. 2. Effect of various chromosomal constructions on *ARG4* meiotic recombination: Wild-type orientation (*A*); inversions (*B*); deletion in the wild-type orientation (*C*). Maps: *DED81*, *ARG4*, and *ORF83* genes are boxed. Their transcriptional orientations are indicated by arrows. The black box represents the *ARG4 Hpa* I–*Nsp*HI (−316/+1) fragment. Dt and PGKt are the transcription terminator fragments of the corresponding genes. Restriction sites are *Bam*HI (B), *Eco*47III (E), *Hpa* I (Hp), *Eco*RV (RV), *Sma* I (Sm), and *Sna*BI (Sn). Positions of the *arg4-RV* and *arg4-Bgl* mutations [(RV) and (Bgl) are indicated]. Other symbols are as in Fig. 1. Strain: Strains are diploid cells homozygous for the illustrated configuration and heterozygous for the two heteroallelic *arg4-RV* and *arg4-Bgl* mutations. Asterisks indicate names of the *rad50S-KI81* homozygous diploid derivatives. Conversion (%): Gene conversion frequencies at the *RV* and *Bgl* alleles, indicated as percentages of total meiosis. Tetrads: no. of unselected tetrads analyzed. A contingency χ^2 test for statistical significance was performed in comparing values for the *RV* marker obtained from the different strains with the values obtained for ORD149. In each comparison the *P* value was statistically significant ($P < 0.05$). Spores Arg+: percentage of arginine prototroph in random spore analysis (21).

Northern Analysis Reveals a Transcript Traversing the *ARG4* Promoter Region in the Inversions. To understand the cause of the low levels of gene conversion in these inversions, we examined the meiotic *ARG4* transcripts of diploid cells ORD122, ORD1132, and ORD836 by Northern analysis. We found two transcripts in the strains ORD122, ORD1132, and ORD836 (Fig. 3). One of these was the *ARG4* transcript (≈1.5 kb) also found in the wild-type diploid ORD149. The additional transcript of 2.8–3.0 kb, which was also detected by an *ORF83*-specific probe (data not shown), arises from the adjacent *ORF83* gene (see Fig. 2). Thus, these inversions had created a transcriptional fusion between *ORF83* and *ARG4*. Moreover, the level of the *ARG4* transcript was severely reduced in comparison to the ORD149 reference transcript, although all the promoter elements should have been included in these inversions (7). It seems likely that this reduction arose from transcriptional interference created by the fusion of the *ORF83* coding region to the *ARG4* promoter. This phenomenon has been previously described (26–28).

Restoration of the Hotspot Activity in the Presence of a Transcription Terminator Upstream of Position −316. We postulated that the existence of the *ORF83/ARG4* fusion transcript traversing the *ARG4* promoter was correlated with the loss of proper recombination function. To test this hypothesis, two constructions were made (Fig. 2). In one case, we inverted the −465/+1519 region to include most of the 3′ noncoding region of the *DED81* gene and generated the corresponding homozygous diploid (ORD195). In the other construction, we inserted the transcription terminator of the *PGK* gene at the *Hpa* I site of the inversion present in ORD836 and generated the diploid ORD980. Meiotic RNA from diploids ORD195 and ORD980 was examined. In both cases, the transcription pattern of the wild-type strain was restored, as shown by presence of an *ARG4* transcript normal in length and intensity and by the absence of the *ORF83/ARG4* fusion transcript (Fig. 3). Seemingly, in both constructions, the high conversion frequency of the *arg4-RV* marker and the decreasing gradient within *ARG4* were restored (Fig. 2). These results support our hypothesis of a correlation

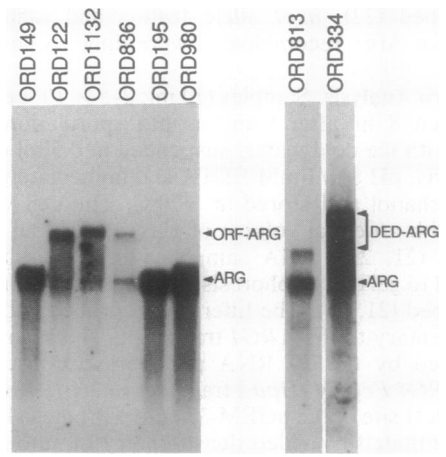

FIG. 3. Northern blot analysis of *ARG4* meiotic transcripts. RNAs extracted from the various strains were hybridized to a riboprobe complementary to the *ARG4* transcript. No hybridization was detected with a riboprobe from the opposite strand. Readthrough transcripts from *ORF83* and *DED81* were confirmed using *ORF83*- and *DED81*-specific riboprobes (data not shown). Ratios of readthrough mRNA to *ARG4* mRNA are as follows: 9.2 for *ORF83/ARG4* in ORD122, ORD1132, and ORD836; 0.2 for *DED81/ARG4* in ORD813; 4.5 for *DED81/ARG4* in ORD334.

between the level of gene conversion in *ARG4* and the presence or absence of an *ORF83/ARG4* fusion transcript. In these inversions, the level of gene conversion at *arg4-RV* is higher than in the normal orientation, reaching 25% of meiosis. This position effect may reflect either the stimulation in the inverted orientation or the repression in the normal orientation by as yet unidentified cis-acting elements.

***ARG4* Meiotic DSBs.** During meiosis, a DSB occurs in the 5′ region of the *ARG4* gene (around position −200, Fig. 1) and is a likely landmark of the gene conversion initiation process (8, 9, 11). We therefore tested the presence of the *ARG4* DSB in the various constructs. DSBs are detected as transient and heterogeneous DNA fragments in wild-type *RAD50* strains but accumulate as discrete bands in *rad50S-KI81* mutant strains (11). We constructed *rad50S* derivatives of our various diploids (Fig. 2) and analyzed the *ARG4* DSB (Fig. 4). We found a readily detectable DSB in the *ARG4* promoter of diploid ORD307 (wild-type orientation) and diploids ORD323 and ORD839 (functional inversions) but no detectable DSB in diploids ORD1160 or ORD311 (data not shown). Within the limits of our resolution (±50 bp) the location of the *ARG4* DSB in the functional inversions (ORD323 and ORD839) is the same as in the wild-type configuration (ORD307). This implies that the −465/+1519 fragment contains the necessary and sufficient cis-acting element(s) controlling the occurrence of the DSB and that the loss of recombination due to transcriptional interference is probably due to inhibition of the activity of the initiation site.

Requirement of the −465/−317 *ARG4* Region in the Wild-Type Configuration. An important conclusion of these inversion studies is that the −465/−317 region is necessary for the activity of the initiation site (compare ORD195 and ORD1132, Fig. 2). This is in contrast with the conclusion of the previous deletion analysis (6) performed with the wild-type configuration, which determined that this region was not necessary, based on the absence of effect of the deletion Δ3 (−1745/−317). To clarify this discrepancy, we constructed the complete deletion of the −465/−317 region in the normal orientation (ΔEH149) and measured *arg4-RV* and *arg4-Bgl* gene conversions in the homozygous deletion diploid (ORD334). Tetrad analysis revealed a 2-fold decrease of meiotic gene conversion at the *arg4-RV* marker (Fig. 2), indicating that this region is required for a normal level of gene conversion at *ARG4*. We then examined the *ARG4* transcripts of diploids ORD813 (Δ3) and ORD334 (ΔEH149). In both cases, at least two transcription products were detected (Fig. 3). One was the normal *ARG4* transcript. The other transcript was a *DED81/ARG4* fusion RNA that hybridized with a *DED81*-specific probe (not shown) and had the expected size (3.6–3.8 kb in ΔEH149 and 2.0–2.2 kb in Δ3. These results indicate that, in both deletions, transcription traverses the *ARG4* promoter region. However, two differences should be emphasized. One is the existence of additional transcripts in ΔEH149. They may correspond to shorter fusion transcripts due to precocious termination, alternative processing or, for some of the larger ones, activation of cryptic promoters. The second difference concerns the ratio between the *DED81/ARG4* fusion transcript(s) and the proper *ARG4* transcript, which is higher in ΔEH149 than in Δ3. These observations may aid in the interpretation of the difference in the level of gene conversion between Δ3 and ΔEH149 (see below).

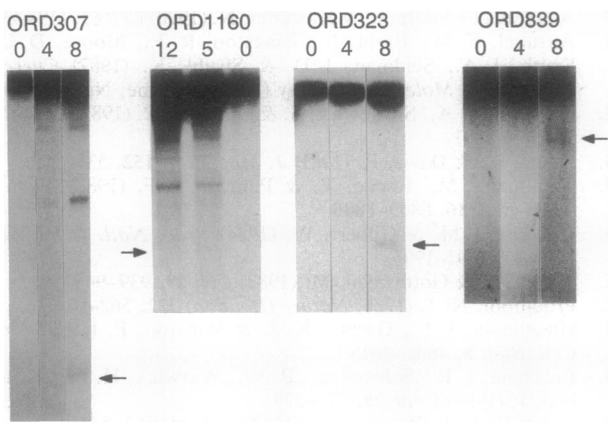

FIG. 4. Southern blot analysis of meiotic DSBs in the *ARG4* region. DNA samples extracted at various times (indicated in hours above each lane) during sporulation were digested with restriction enzymes as follows: ORD307, *Sna*BI; ORD1160, *Bam*HI; ORD323, *Eco*RI; ORD839, *Pst* I. In each lane, the upper intense band corresponds to the parental fragment. Other bands correspond to DSBs occurring in this interval. The expected *ARG4* DSB position is indicated by an arrow. In strain ORD1160, no *ARG4* DSB is detected. The experimental threshold of detection is around one-fourth the level detected in ORD307.

DISCUSSION

Our results bear on several aspects of the understanding of the *S. cerevisiae ARG4* gene conversion hotspot: the identification of the necessary and sufficient cis-acting element(s), the chromosomal context effects, and the relationship between the levels of meiotic gene conversion and DSBs. The previous deletion analyses (6, 7) defined the limits of the necessary cis-acting region between positions −316 and −37, based on the endpoints of two deletions (Δ3 and Δ11, respectively) which do not affect the conversion frequency of the allele tested. The purpose of the present study was to determine whether this region was sufficient to promote a high level of gene conversion at *ARG4*. We found that this was not the case. The functional transplacement by inversion of the *ARG4* hotspot requires the inclusion of the −465/−317 region.

Functional Role of the Intergenic −465/−317 Region. The DNA sequence of the 5′ *ARG4* chromosomal region reveals that the −465/−317 region is located between the *ARG4* promoter elements [positions −259 (see Δ136, ref. 7) to −57 (mRNA start site); see Fig. 1] and the 3′ end of the *DED81* coding region located at position −496 (ref. 29; C. Mezard, personal communication). As expected from its location in the 3′ noncoding region of the *DED81* gene, it contains a transcription termination signal, since its absence leads to readthrough transcription into *ARG4* from the adjacent *DED81* and *ORF83* genes, in the normal and inverted orientations, respectively (see Fig. 3). Although we did not find the TAG . . . TATGTA . . . TTT consensus for transcription termination proposed by Zaret and Sherman (30), we found two presumptive polyadenylylation sites (CAAA) at positions −327 and −450, as well as a TATATA sequence at −455 that appears to enhance 3′ endpoint formation of the *CYC1* mRNA in *S. cerevisiae* (31).

Our observation that the loss and restoration of gene conversion at *ARG4* are correlated with the presence or absence of the −465/−317 region suggests that, in this interval, the element important for recombination is the transcription terminator signal. The fact that it can be functionally replaced by the *PGK* transcriptional terminator is consistent with this simple hypothesis. A different, but not necessarily exclusive, hypothesis is that the *DED81* and *PGK* 3′ gene regions contain a cis-acting element(s) other than a transcription termination signal (or in addition to it) which plays a direct or indirect role in stimulating recombination.

How Is Recombination Inhibited by Transcriptional Interference? The results shown here suggest a correlation between high or low frequency of gene conversion in *ARG4*, the presence or absence of a meiotic DSB (which provides a strong argument for its relevance in this recombination process), and transcriptional interference(s) primarily mani-

fested by the presence or absence of a transcript traversing the *ARG4* promoter. It is known that transcription can interfere with other cellular processes. In prokaryotes as well as in eukaryotes, transcriptional interference between promoters has been demonstrated (26, 27) but its molecular basis is not known. In yeast, genetic evidence for promoter competition has been reported (28), and traversing transcription was found to inhibit *ARS* (origin of replication) and *CEN* (centromere) functions (32, 33). To explain the inhibition of the *ARG4* initiator, one might envision that the transcriptional inhibition of recombination is directly related to the absence of the DSB event. For example, traversing transcription can inhibit the binding or the cutting by a nuclease or, as proposed in the cases cited above, interfere with specific protein binding or with some structural feature of the 5′ *ARG4* region, such as the DNA topology and/or chromatin organization, which might be important for the function of the proper DSB cis-acting element(s). Alternatively, the inhibition of recombination and DSB may be an indirect effect of the reduced or altered activity of the *ARG4* promoter. In support of this hypothesis, we observed that the decreasing level of gene conversion at *ARG4* in deletion Δ3 (no effect), in ΔEH149 (2-fold effect), and in all inversions (at least 10-fold effect) was correlated with an increasing ratio of fusion transcript (*DED81/ARG4* or *ORF83/ARG4*) over proper *ARG4* transcript. Thus, *ARG4* promoter activity might be important for the activity of the initiation site, although no satisfactory correlation has been found in a series of small deletions removing specific promoter elements (7). A correlation between high levels of transcription and stimulation of mitotic recombination has been found for the *HOT1* element (34) and for GAL10 tandem duplications (35). At the *HIS4* locus, another meiotic hotspot of recombination, a promoter deletion reducing the level of *HIS4* mRNA reduces the rate of mitotic, but not meiotic, recombination (36). For *ARG4*, further experiments are needed to clarify the precise relationship between meiotic recombination and transcription signals which overlap in the 5′ region of *ARG4*. An understanding of chromosomal context effects would also aid in the comparison of the various constructs.

Concluding Remarks. We have defined a minimal DNA fragment that contains the *ARG4* initiation site for meiotic gene conversion and is capable of functioning in a novel chromosomal context. This 1.98-kb (*Eco*47III–*Sna*BI) fragment is composed of three parts: the *ARG4* coding region, which is not essential (Δ14 in ref. 6) but was used to genetically measure gene conversion frequencies; the previously defined −316/−37 region, which includes the initiation site for gene conversion; and the −465/−317 region, which contains a transcription terminator signal that appears to be required depending on the location of the −316/−37 region relative to other transcribed regions. Further experiments should determine whether this potential cassette is sufficient to promote gene conversion in other chromosomal regions. On a different level, an important implication of the observation discussed here concerning the inhibition of recombination by transcriptional interference at *ARG4* is that natural hotspots of meiotic recombination in *S. cerevisiae* and perhaps in other organisms might be preferentially localized in intergenic/promoter regions. In favor of this hypothesis, it has generally been observed that the highest frequencies of gene conversion within genes are located near one end (reviewed in refs. 1 and 4) and that the other known natural meiotic DSBs in *S. cerevisiae* map in promoter regions (8).

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