Evidence for Negative Interference: Clustering of Crossovers Close to the am Locus in Neurospora crassa Among am Recombinants

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ABSTRACT

In response to a conflict between two mapping studies in the predicted orientation of the allele map with respect to the centromere, Fincham proposed that recombination events at the Neurospora am locus rarely have an associated crossover. Fincham considered that the elevated levels of crossing over between flanking markers in am recombinants resulted from negative interference, an increased probability of a nearby second event, and on this basis predicted a clustering of crossing over near am in these recombinants. In this article we reevaluate the data from three mapping studies of the am locus and report molecular evidence that shows crossovers to be clustered immediately proximal to am in am recombinants.

Despite the elegant simplicity of a model for meiotic recombination that accounted for both gene conversion and crossing over (Holliday 1964, 1974), a challenge to the ubiquity of a mechanistic association between gene conversion and crossing over was soon offered by Fincham (1974) in considering an apparent conflict between his (Fincham 1967) and Smyth's (1973) mapping data for the Neurospora am locus.

am+ progeny selected at random from crosses of the type P m1 m2+ D by p m1+ m2 d, where P/p and D/d are proximal and distal flanking markers, respectively, while m1 and m2 are different auxotrophic mutations within am, were examined in both mapping efforts. The relative proportions in the two classes with nonparental association of flanking markers, R1 (p m1+ m2+ D) and R2 (P m1+ m2+ d), differed between studies. Although both authors found that R1 ≈ R2 in the majority of their crosses, where there was disparity, Smyth found that R1 was larger than R2, while in certain of Fincham’s pairings, R2 exceeded R1. As the class in excess was assumed to indicate which of the two possible gene orientations minimized the number of exchange events required, this disparity led each author to propose a different orientation for the am gene with respect to the centromere. While the am data suggest that the R1:R2 disparity cannot be used to orient genetic maps (Bowring and Catcheside 1995), the “polarity” criterion that relies on the relative proportions of recombinant progeny with a parental configuration of flanking markers describes a reasonably accurate allele order (see Figure 1; Bowring and Catcheside 1995).

Fincham (1974) attempted to reconcile the conflict-
We repeated one of Smyth’s crosses and followed the segregation of both tightly linked molecular flanking markers and conventional flanking markers among am⁺ progeny (cross B163; Bowring and Catcheside 1996). Where conventional flanking markers were recombined, the majority of events were outside of the region bounded by molecular markers, and we concluded that a maximum of 7% of am conversions had a crossover that could be considered mechanically associated. While the distance to flanking markers was elevated among am⁺ progeny, the elevation was just short of statistical significance, and although an absence of molecular markers proximal to am precluded examination of the position of crossovers here, there appeared to be no clustering of crossovers in the distal interval. We now have molecular markers in the proximal interval and, in accordance with Fincham’s prediction, report a clustering of crossovers immediately proximal to am among B163 recombinants.

**MATERIALS AND METHODS**

B163 is a cross between F6325 and F11089 (Bowring and Catcheside 1996). B539 is a cross between strains B530 (a, his-3 K874, cog⁺; cyh-2, leu-5, sp B132, rec2) and B537 (A, his-3 K874, cog⁺, ad-3 K118; cot-1 C102; rec-2⁺, ure2 D74, am⁺), which was carried out at 25°C in SC medium (Davis and De Serres 1970) supplemented with 2% sucrose, histidine (0.2 g/liter), adenine (0.4 g/liter), leucine (0.2 g/liter), and alanine (0.5 g/liter). leu⁺ am⁺ recombinants from cross B539 were selected on Vogel’s N medium (Davis and De Serres 1970) lacking both leucine and alanine but with added histidine and adenosine. Glycine (1.5 g/liter) was also added to suppress leaky growth of am mutants.

Restriction-site polymorphism was detected by probing EcoRV-digested Neurospora DNA with cosmids G18A7 or G4B5, which are from the pMcoX library (Orbach 1994). Genomic DNA was prepared either as described (Bowring and Catcheside 1996) or using the mini-prep method of Irelan et al. (1993). EcoRV digestion of genomic DNA was carried out according to the manufacturer’s instructions (Bresatec, Adelaide, South Australia). Probe labeling, Southern transfer, hybridization, and membrane stripping were carried out according to protocols included with the Phototope kit (New England Biolabs, Beverly, MA). Probe was synthesized using linearized cosmID DNA as a template.

**RESULTS**

Of 205 progeny from cross B163 that experienced a recombination event in am, 21 had a crossover between HP and sp (Bowring and Catcheside 1998), markers 8.3 kb (Bowring and Catcheside 1996) and 450 kb (F. J. Bowring and D. E. A. Catcheside, unpublished results; T. Sone, J. Bok, F. J. Bowring, D. E. A. Catcheside and A. J. F. Griffiths, unpublished results) proximal to am, respectively. To probe for clustering of crossovers in these recombinants, two restriction-site polymorphisms (RSPs) were used to define three contiguous subintervals immediately proximal to am. The closest (subinterval I) is bordered by HP and G18E, which is 80 ± 10 kb proximal; the next (subinterval II) extends from G18E to G4E (at 145 ± 15 kb proximal); and the most remote, G4E to sp (subinterval III), represents the remainder of the HP to sp interval (Figure 2).

The same RSPs distinguish strain B530 from strain B537 (cross B539, Figure 2). Of 153 leu-5⁺ am⁺ recombinants from this cross, 67 were also sp⁺ and thus had a crossover between sp and am. The segregation of G18E and G4E was examined in 64 of these recombinants.
Because $HP$ was not scored in progeny from cross B539, subinterval I is 8.3 kb larger in this cross than in B163. However, as the $HP$ to $am^6$ genetic distance is estimated to be <0.09 cM (Bowring and Catcheside 1996), any effect is expected to be slight.

Figure 3 shows the position of crossovers among the subset of recombinant progeny from crosses B163 and B539 that experienced an exchange proximal of $am$ in regions I, II, and III. The progeny of cross B163, selected for conversion in $am$, have a significantly different distribution of crossovers in these intervals from the progeny of cross B539 in which $am$ conversion was not selected for ($\chi^2 = 11.0$, d.f. = 2, $P = 0.004$). Because subinterval III accounts for 57% of crossovers among progeny from both pairings, the difference appears to be wholly contained within the two subdivisions most closely proximal to $am$. In cross B163, 38% of crossovers occurred in subinterval I, immediately proximal to $am$, compared with only 11% in subinterval I for cross B539. The situation is reversed in subinterval II where, in cross B163, only 5% of crossovers occurred compared with 32% of crossovers in cross B539.

**DISCUSSION**

We compared the distribution of crossovers across three chromosome segments proximal to $am$ in two crosses. In cross B163, where we first selected for a conversion event in $am$, there was clustering of crossovers in the interval immediately proximal to $am$: 8 of 21 (38%) crossovers within the 450-kb $sp$ to $HP$ interval were between $G18E$ at $+80 \pm 10$ kb and $HP$ at $+8.3$ kb. In contrast, in cross B539, where there was no selection for conversion in $am$, only 7 of 63 (11%) crossovers occurred in the equivalent interval ($G18E$ to $am^6$ at $+15$ bp).

Although these data show a significant clustering of crossovers close to $am$ in progeny that experienced conversion in $am$, they may underestimate the degree of clustering if the number of crossovers designated as associated with conversion is an overestimate. Bowring and Catcheside (1996) assessed the likelihood of association on the basis of proximity. $HP$ and another molecular flanking marker 5.7 kb distal to $am$ ($HD$) were chosen to define an interval outside of which crossovers were thought unlikely to be associated with conversion in $am$. However, those inside this interval are not necessarily associated. For example, the principal criterion that led Fincham (1974) to consider crossovers among the R2 class as being separately initiated events was a converted segment separated from a crossover by a non-converted segment. A total of 8 of the 14 crossovers between $HP$ and $HD$ in B163 recombinants satisfy this criterion (Bowring and Catcheside 1998) and on these grounds could be considered independent. Moreover, a higher density of markers in $am$ and the associated increase in resolution could conceivably increase this number and further reduce the estimated number of crossovers associated with the conversion tract.

We previously considered the possibility that at $am$, where the frequency of crossovers per kilobase is low, recombination events might extend over a large distance. If this were the case, our estimate that a maximum of 7% of conversion events in $am$ have a mechanistically associated crossover would be an underestimate. One of several reasons for our earlier rejection of this possibility was that there appeared to be no clustering of crossovers distal to $am$ among B163 recombinants (Bowring and Catcheside 1998). However, as we have now found clustering of crossovers in the proximal interval, the possibility seems worthy of further consideration.

Could this clustering be due to the spreading of recombination events initiated at $am$ to a point beyond $HP$? Indeed, Gilbertson and Stahl (1996) have proposed a model that could account for the clustering of crossovers proximal to $am$. In this model, which was formulated to account for the observed tendency of crossovers to occur on one side of the ARG4 recombination initiation site, cleavage of the two Holliday junctions comprising the hypothesized recombination intermediate is both asynchronous and strand specific. If $HP$ was contained in symmetrical heteroduplex following migration of the Holliday junction beyond this marker, the apparent separation of conversions from crossovers could be accounted for. However, as $HP$ is 8.3 kb from the peak of conversion frequency in cross B163 (Bow-
of his and Smyth’s mapping efforts, that the increase interference are both expected to increase the level of am.

have shown this to be the case (presented here and elsewhere do, however, fit Fin-

crossover is an absence of conversion-

am.

pairings. However, no such genes are known to in
tinguish between the corrected frequencies

of flanking marker exchange in Smyth’s (B) and Fin-

am.

interference by increasing the probability of additional LITERATURE CITED


of synapsis at an extended distance with the effect decreasing with distance from am and ultimately subsiding somewhere outside of the interval bounded by sp and inl.

The counting model for chiasma interference (Foss et al. 1993) also predicts clustering of crossovers about a converted locus. In this model, an organism-specific number (m) of conversion events (Co) must occur between each conversion event that is resolved as a crossover (Cx) and, based upon an estimate of \( \sim 0.3 \) for the proportion of conversion events that have an associated crossover in both Saccharomyces cerevisiae and Neurospora crassa, m was thought to be 2 in these fungi. Within the framework of the counting model, recombination in am signals the completion of one Co. Clustering results from an increased likelihood of a crossover nearby, because the number of subsequent Co’s that must occur prior to a Cx is now reduced by one. While a test of the model in S. cerevisiae failed, for Neurospora, where the model accurately accounts for certain recombination data, its applicability was not ruled out (Foss and Stahl 1995).

Clustering of crossovers in the subinterval immediately proximal to am in am+ recombinants might be driven by local synopsis or by the action of a counting machine. Indeed, this clustering is explicable within the framework of existing molecular models of meiotic recombination if extensive Holliday junction migration is invoked. However, such models have crossovers that are mechanistically associated with conversion events as a foundation, and we wonder if this foundation has been rigorously tested. Fogel et al. (1979) point out that an assumption inherent in the method used to estimate the proportion of conversion events that have an associated crossover is an absence of conversion-driven interference. While conversion-driven positive interference has been ruled out (Stadler 1973), conversion-driven negative interference has not. Indeed, whereas a mechanistic association between gene conversion and crossing over and the operation of negative interference are both expected to increase the level of flanking marker exchange about a converted locus, it would not be difficult to miss this latter phenomenon. Perhaps the possibility of conversion-generated negative interference deserves more experimental attention than it has received.

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