DOES CHI GIVE OR TAKE?

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ABSTRACT

In lytic cycle crosses with Red\(^+\)Gam\(^-\)\(\lambda\) phage, particles were examined that had undergone an Int-mediated exchange. It was assumed that this exchange dimerized the circular \(\lambda\), making it packageable. Among these Int-mediated recombinants, particles were identified that had, in addition, enjoyed a close double exchange mediated by the RecBC pathway. Such close double exchanges indicate localized negative interference and are analogous to eukaryotic conversions that have retained parental configuration of flanking markers. These events are stimulated by Chi, a recombinator specific to the RecBC pathway. When Chi is present in only one parent in the cross, the complementary double exchange recombinants are Chi stimulated to the same degree. This behavior of Chi contrasts with that of characterized eukaryotic recombinators.

ALL current models for homologous genetic recombination hypothesize the loss of DNA from one chromosome and its replacement by DNA copied from the other to account for the gene conversion that frequently accompanies crossing over. The conversion-crossing over model of HOLLIDAY (1968) attributed all losses of information to mismatch-stimulated excision of DNA in heteroduplexes formed by swapping of chains of like polarity. The gain of allelic information results when the single-chain gaps are refilled by repair synthesis. The chain swapping itself was precisely reciprocal. The assumption of strict reciprocity of single-chain swapping has since been set aside (for eukaryotes) because of failures to find a variety of predicted symmetries in genetic data (see STAHL 1979 for an extended discussion.) To accommodate those data better, alternate models were put forth in which conversion was not entirely dependent on mismatch correction. These models can be classified in the following two ways:

1. The first classification is according to whether there is or is not a hybrid DNA intermediate in the formation of a 6:2 tetrad. When a hybrid DNA intermediate is presumed, the formation of a 6:2 tetrad ("full conversion") involves two kinds of DNA destruction and synthesis. The first kind is independent of genetic markers and involves the destruction of a single chain on one chromatid and its replacement by a single chain from its homologue. This

We dedicate this paper to the memory of our friend and colleague, George Streisinger.

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event, which forms hybrid DNA on one chromatid only, will produce a 5:3 tetrad ("half-conversion") if the event overlies a marked site and mismatch correction fails. If correction occurs, however, a 6:2 tetrad may result. If hybrid DNA is not presumed, then conversion must involve the loss of a duplex segment from one chromatid and its replacement by a duplex segment derived from the homologue. Hybrid DNA may bracket the event but will play a minor role in conversion if it is short compared to the donated duplex segment. We will call models of the first class "single-chain models" and those of the second class "double-chain models."

2. The second classification is according to the relative timing of the synthesis and the destruction (of the noncorrection sort). In some models the initiating event is destructive; in others it is synthetic. Among familiar models, the Aviemore model (Meselson and Radding 1975) is one in which the synthesis-destruction involves only one chain of each DNA duplex (a single-chain model) and the synthesis starts first. The sex-circle model (Stahl 1969, 1979) is a double-chain model in which the synthesis event, likewise, comes first. On the other hand, the double strand gap repair model (Szostak et al. 1983) is a double-chain model in which destruction precedes synthesis. In all of the models, intermediates are postulated that can be resolved with comparable probability to give or not to give crossing over of flanking markers.

In other places (Stahl 1979; Szostak et al. 1983) we have discussed criteria for testing the applicability of single-chain and double-chain models. In this paper we are concerned with the question of which component of conversion comes first, the destruction or the synthesis of DNA.

The impetus for these experiments comes from observations made on fungal recombinators (reviewed by Markham and Whitehouse 1982). Crosses in which only one parent carried the active recombinator were performed, and individual tetrads were analyzed for the recombinator and nearby markers whose conversion was demonstrably stimulated by the recombinator. These recombinators showed one or both of the following properties: (1) conversion to an excess of the inactive recombinator allele at a higher rate than conversion to an excess of the active allele or (2) preferential loss, through conversion, of alleles cis to the recombinator. Thus, these recombinators affect recombination through the loss of DNA linked to them. Their action is destructive. They "take" DNA from the homologue. [Markham and Whitehouse (1982) have interpreted these data to mean that recombinators invite the homologous chromosome to undertake a synthetic event that precedes the destruction cis to the recombinator. The recombinator invites the homologue to "give." Of course, the genetic observations do not distinguish these possibilities; they ask only whether the recombinator-bearing chromosome tends to donate or receive information in a conversion event.] In this paper we investigate the behavior of Chi, an E. coli recombinator active in the RecBC pathway (for review, see Stahl 1979).

In E. coli we do not have the advantage of a process like meiosis that potentiates the recovery of all products of each exchange event. Therefore, conversion defined as nonreciprocal recombination is not open to investigation.
However, there are conversion-related aspects of recombination that are accessible. A fraction of the exchange events in bacteria and phage are paired. In the Holliday and Aviemore models, these are the strand transfers that are completed without recombination of flanking markers (the “patches” of Stahl 1979). In the sex-circle and double-strand gap repair models these are the lengths of duplex that are transferred without accompanying recombination of flanking markers. Paired events have been shown to be Chi stimulated (Stahl et al. 1982; Kobayashi et al. 1983). If Chi is present on only one of the two interacting chromosomes, we can ask whether paired events (call them patches) arise equally often on the two chromosomes or whether, on the contrary, chromosomes with Chi give and take patches at unequal rates.

We have investigated that question using Chi in λ that is mutated for its own recombination system and subject to recombination by E. coli’s RecBC pathway. The detection of patches in λ particles under these conditions is complicated by the requirement that each chromosome must splice together with another chromosome to create a dimeric packaging precursor. The event that gives a patch does not result in dimeric λ and will not be detected unless an avenue to packaging is provided. In the present study, the splice that ensures packageability is provided by the Int system operating on an especially reactive pair of attachment sites (att) (Gottesman and Weisberg 1971). The two parents differ by net deletions flanking att so that the Int-mediated crossover particles can be detected by their buoyant density. One of these att-linked deletions along with a third deletion flank a marked site, or sites. Buoyant density allows the identification of particles that, although having enjoyed a crossover at att, are parental for the latter two deletions. Within these two complementary density-identified genotypes the patches of information transferred from one parent to the other can be scored. When Chi is present in only one of the two parents, inequalities between these two classes will reveal whether Chi “gives” or “takes.”

MATERIALS AND METHODS

Phage mutations: The mutations employed are given in Table 1 (and see Figure 1). Revertant frequencies were less than $10^{-4}$ for all Pam and Oam phage stocks. Revertant frequencies for clear mutants were likewise too low to intrude into the results reported here. Desired λ genotypes were obtained by UV-stimulated in vivo crosses.

Bacterial strains: See Table 2.

Phage crosses: Cells in exponential phase (1 × 10⁸/ml) were mixed with phage at a ratio of one cell to seven of each of the two parents. Crosses with blocked DNA replication (in FA77) were not diluted following adsorption of phage to cells. Other crosses (in 594) were diluted five times after allowing 20 min for adsorption. The infected cultures were aerated for 90 min. Crosses in 594 were terminated with chloroform; FA77 crosses were terminated with chloroform plus egg white lysozyme.

Phage stocks: Phage were grown on plates from single plaques. The host was JC8679.

RESULTS

Identification of Int-mediated recombinant peaks: Our crosses were of the form shown in Figure 2. The phage progenies were banded in a cesium formate
**TABLE 1**  

**Phage mutations**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>b516</td>
<td>10% deletion leftward from <em>att</em></td>
<td>PARKINSON (1971)</td>
</tr>
<tr>
<td>bio1</td>
<td>Substitution rightward from <em>att</em> (5% net deletion)</td>
<td>HRADECNA and SYBALSKI (1969)</td>
</tr>
<tr>
<td>nin5</td>
<td>5% deletion in right arm</td>
<td>FIANDT et al. (1971)</td>
</tr>
<tr>
<td>Pam3</td>
<td>amber in <em>P</em></td>
<td>CAMPBELL (1961)</td>
</tr>
<tr>
<td>Pam80</td>
<td>amber in <em>P</em></td>
<td>CAMPBELL (1961)</td>
</tr>
<tr>
<td>Oam29</td>
<td>amber in <em>O</em> (left of gene <em>P</em>)</td>
<td>CAMPBELL (1961)</td>
</tr>
<tr>
<td>x*D123</td>
<td>Leftward-acting Chi, right of nin</td>
<td>STAHL, CRASEMANN and STAHL (1975)</td>
</tr>
<tr>
<td>x*C151</td>
<td>Leftward-acting Chi in <em>cII</em></td>
<td>STAHL, CRASEMANN and STAHL (1975)</td>
</tr>
<tr>
<td>CP7</td>
<td><em>cl</em> mutation</td>
<td>OPPENHEIM and SALOMAN (1970)</td>
</tr>
<tr>
<td>Ets4</td>
<td>Heat-sensitive in <em>E</em></td>
<td>BROWN and ARBER (1964)</td>
</tr>
<tr>
<td>red3</td>
<td>Unconditional Red-</td>
<td>SIGNER and WEIL (1968)</td>
</tr>
<tr>
<td>gam210</td>
<td>amber in <em>gam</em></td>
<td>ZISSLER, SIGNER and SCHAEFER (1971)</td>
</tr>
</tbody>
</table>

**FIGURE 1.**—Map of λ showing the approximate locations of relevant genes, sites and markers (also see Table 1).

equilibrium density gradient. The Int-mediated recombinants are expected to be the most dense and the least dense types, banding separately from all other genotypes (Figure 3, peaks 1 and 8). Within these two peaks, patches are detected in either of two ways: (1) In some crosses, *m1* and *m2* are mutant and wild type, respectively. In those crosses, patches are detected as *m2* particles in the heavy peak and as *m1* particles in the light peak. (2) In other crosses, *m1* and *m2* are recombinable mutations in the same gene or adjacent genes. Patches are scored as *m*+ recombinants in the heavy and light peaks. Most crosses were accompanied by Chi- controls to verify that the patches scored were, in fact, Chi stimulated.

In Figure 3, the heights of the two Int-mediated recombinant peaks are seen to differ about two-fold. However, in our experiments we often see the two peaks to be equal, and when they do differ they are as likely to do so in favor
### TABLE 2

**Escherichia coli K12 Strains**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Genotype/properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA77</td>
<td>Su' dnaBts rec+</td>
<td>STAHLE et al. (1972)</td>
</tr>
<tr>
<td>594</td>
<td>Su' rec+</td>
<td>WEIGLE (1966)</td>
</tr>
<tr>
<td>QR48</td>
<td>Su' recA</td>
<td>SIGNER and WEIL (1968)</td>
</tr>
<tr>
<td>JC8679</td>
<td>Su' recB21 recC22 sbcA23</td>
<td>STAHLE and STAHLE (1977)</td>
</tr>
<tr>
<td>JC9387</td>
<td>Su' recB21 recC22 sbcB15</td>
<td>STAHLE and STAHLE (1977)</td>
</tr>
<tr>
<td>C600(P2)</td>
<td>Su' C600 carrying prophage P2</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.**—Type of cross to ask whether Chi gives or takes patches. For a description of the markers, see Table 1 and Figure 1. Int-mediated exchange produces $b516 \text{ bio}1$ and the complement, $b516^+ \text{ bio}1^*$. Among each of these recombinants there is a class that is not recombinant for $\text{bio}1$ and $\text{nin}$. These phages, $b516 \text{ bio}1 \text{ nin}5$ and $b516^+ \text{ bio}1^* \text{ nin}5^*$ will be, respectively, the least and the most dense genotypes produced. They can be separated from all other types in a cesium formate equilibrium gradient (see Figure 3). The Red- Gam- conditions required for maximum Chi activity are achieved by the $\text{bio}1$ substitution in one parent and by red3 gam210 mutations in the other.

of one as of the other, and the difference is never more than two-fold. The differences may represent fluctuations in the efficiencies of plating of the bacterial indicator strains used. They are not large enough to endanger our primary conclusion.

The size of the Int-mediated recombinant peaks is expected to be independent of the presence of an added Chi, since Chi stimulates only generalized recombination (via the RecBC pathway). The sizes of some of the other peaks are also likely to be insensitive to an added Chi, since $\lambda \text{ bio}1$ already contains a (leftward-acting) Chi in the $\text{bio}$ substitution (HENDERSON and WEIL 1975; MCMILIN, STAHLE and STAHLE 1974; MALONE et al. 1978).

*Intragenic crosses to detect patches:* Recombinants between markers in the same gene are frequently parental for flanking markers (see STAHLE 1979 and WHITEHOUSE 1982 for reviews). In this paper, we define such recombinants as arising...
FIGURE 3.—The progeny from a cross like that in Figure 2 was centrifuged to near equilibrium in a cesium formate density gradient. The bio1 phages (○) were detected on a P2 lysogen, C600 (P2), which fails to plate bio⁺. The bio⁺ phages (●) were detected on a recA host (QR48), upon which the bio1 phages do not make plaques. Conditions for the cross are described in Figure 6b. The numbered peaks correspond to the following genotypes:

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Host</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QR48</td>
<td>λ⁺</td>
</tr>
<tr>
<td>2</td>
<td>C600(P2)</td>
<td>bio1</td>
</tr>
<tr>
<td>3</td>
<td>QR48</td>
<td>nin5</td>
</tr>
<tr>
<td>4</td>
<td>QR48</td>
<td>b516</td>
</tr>
<tr>
<td>5</td>
<td>C600(P2)</td>
<td>bio1 nin5</td>
</tr>
<tr>
<td>6</td>
<td>C600(P2)</td>
<td>b516 bio1</td>
</tr>
<tr>
<td>7</td>
<td>QR48</td>
<td>b516 nin5</td>
</tr>
<tr>
<td>8</td>
<td>C600(P2)</td>
<td>b516 bio1 nin5</td>
</tr>
</tbody>
</table>
FIGURE 4.—Intragenic crosses to detect Chi-induced patches. The crosses were in FA77, so that DNA replication was blocked. Among total phage, genotypes are identified as described in Figure 3. The $P^+$ recombinants ($\Delta$) were detected on bacterial strain JC9387. a, $\chi^+$; b, $\chi^+D$.

from patches. When the patch covers one marked site only, then a duplex arises that is recombinant on at least one chain. If the patch involves the transfer of one chain across both marked sites, then mismatch correction at one site or the other may generate the scored recombinant. Our intragenic crosses involve two amber mutations in the $P$ gene, located between $bion$ and $nin5$. Patches are conveniently scored by plating the density fractions on a host selective for $am^+$ recombinants.

The results of such a cross, performed in the absence of Chi, are shown in Figure 4a. In that figure, those $P^+$ phages (lower curve) that result from patches in Int-mediated recombinants are identifiable as the heaviest and lightest peaks. These recombinants are about 15% of all $P^+$ recombinants and are, therefore, an even larger fraction of the $P^+$ recombinants that have had an Int-mediated exchange. This high frequency of retention of parental flanking deletions among $P^+$ phages justifies our designation of "patch."

$\chi^+D$, located to the right of $nin5$, and acting leftward, can be expected to increase the $P^+$ frequency arising by both splices and patches (STAHL et al. 1982). The results of Chi$^-$ and Chi$^+$ versions of two crosses are shown in Figures 4 and 5. The two pairs of crosses differ only in the coupling relations
between $\chi^+D$ and the two $P$ alleles. In one cross (Figure 4) the frequencies of patches in the lightest and heaviest peaks are each increased four- to five-fold by Chi. In the other (Figure 5), they are each increased ten-fold. These results imply that the chromosome carrying Chi gives and receives patches with equal frequency.

**Intergenic crosses to detect patches:** The intragenic crosses described were performed under conditions ($P^-$, dnaB-) that block DNA replication (McMILIN and Russo 1972). We presume, therefore, that the progenies examined represent immediate products of recombination. We varied the cross and conditions to test the generality of the results. In our intergenic crosses, $m_1$ and $m_2$ are still very close markers, but they are in different (adjacent) genes. The crosses were conducted with unhampered DNA replication. The results are in Figures 6 and 7. Those figures show that, in all relevant respects, save one, the results are like those obtained with the corresponding intragenic crosses. In one of the $\chi^o$ crosses (Figure 6a), the heaviest and lightest $O^+P^+$ peaks are unequal. Despite the inequality in these $\chi^o$ peaks, we can conclude that $\chi^+D$ gives and takes patches equally since it raises the light and heavy $O^+P^+$ peaks (Figure 6b) by the same factor.
FIGURE 6.—Intergenic crosses to detect Chi-induced patches. Crosses in 594, so that DNA replication was normal. Key for total phage as in Figure 3. The O\(^*\)P\(^+\) recombinants (Δ) were detected on JC9387. a, \(χ^o\); b, \(χ^+D\).

One-factor cross to detect patches: \(χ^+D\) increases splice formation as far away as the \(cl\) gene (LAM et al., 1974). Thus, we may expect to see stimulation of patches at \(cl\) by \(χ^+D\). Our cross (Figure 8) had a \(cl\) mutation in one parent, whereas the other parent was \(cl^+\). We enumerated patches as clear plaques among a large majority of turbids in one Int-mediated peak and as turbids among a majority of clears in the other. Although the data are few, it is apparent that the two \(cl\) patches are stimulated by \(χ^+D\) and by about the same factor.

A cross in which Chi is the marker: SUSAN ROSENBERG (Eugene) suggested to us that an inequality in the rates of giving and taking might be detected if we used a marker closer to the Chi locus. \(χ^+C\), a Chi site in the \(cl\) gene, can be used simultaneously as a Chi recombinator and as a plaque morphology marker (clear plaque) to ask whether Chi gives or takes patches. The results are shown in Figure 9. Since the marker and the Chi are inseparable, we cannot perform the \(χ^o\) control, limiting the conclusions that can be drawn. The permissible conclusion is that, if \(χ^+C\) does stimulate the formation of patches involving itself, which seems likely (STAHL, CRASEMANN and STAHL 1975; STAHL and STAHL 1975), then \(χ^+C\) gives and takes equally in its immediate vicinity.
DISCUSSION

Our results fail to show any inequality in the rate at which chromosomes carrying Chi give or take patches. This observation suggests that Chi functions differently from characterized eukaryotic recombinators, all of which preferentially take (see Markham and Whitehouse 1982 for review). Previous work (Stahl et al. 1980) had led us to conclude that Chi makes splices nonreciprocally. The nature of the nonreciprocity suggested that the chromosome carrying Chi was damaged from its own locus to the point at which the splice occurred. A single-chain gap was the obvious possibility. It seemed likely that the apparent nonreciprocity in splicing would be echoed in a nonreciprocality of patching and that we would find that Chi preferentially takes patches. Such a finding would support the idea of a Chi-initiated gap, and it would align Chi with the characterized eukaryotic recombinators. However, the companion paper (Kobayashi et al. 1984) demonstrates that the evidence for nonreciprocity of Chi-induced splices was misinterpreted. Thus, splices and patches both appear to be produced reciprocally or, at least, without regard to which parent is $\chi^+$. This conclusion, in turn, is compatible with a previously proposed (Faulds et al. 1979; Stahl et al. 1980) role for Chi in the stimulation of

![Diagram of phage titer vs fraction number for intergenic crosses to detect Chi-induced patches. The positions of the O and P markers are reversed from those in Figure 6. Key as in Figures 3 and 4. a, $\chi^+$; b, $\chi^+D$.](image-url)
FIGURE 8.—One-factor cross to detect Chi-induced patches. Crosses were performed in 594. Clear (CP7) and turbid plaques were scored in the heavy peak on QR48 (○) and in the light peak on C600(P2) (○). In each peak the points for the minority class have error bars representing 95% Poisson confidence limits. a, χ⁺; b, χ⁺D. The bio1 parent carried an Ets marker (in the left arm) allowing the exclusion of that parent from the light peak. In the χ⁺ cross (b), platings in the light peak were at 40°. All other platings were at 32°, the permissive temperature for Ets.
recombination—Chi may facilitate the resolution of a recombinational intermediate, such as a Holliday junction. Any nonreciprocity in a given junction (such as "asymmetric hybrid DNA") would bear no relation to which parent carried Chi. Thus, no matter what the mechanism by which Chi resolved the junction, Chi would be seen to give and take patches equally.

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LITERATURE CITED


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