SHORT PAPERS

A HISTIDINE OPERATOR-CONSTITUTIVE MUTATION DECREASES THE FREQUENCY OF RECOMBINATION WITHIN THE HISTIDINE OPERON OF SALMONELLA TYPHIMURIUM

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ABSTRACT

A constitutive mutation in the histidine operon in Salmonella leads to a decrease in the recombinant recovery following transduction with P22 phage. This decrease appears to be a true decrease in recombination within the histidine operon and specific to that gene region. In addition, a strain with an unlinked mutation was isolated in which an operator-constitutive mutation is lethal.

Herman (1968) presents data indicating competition between transcription and recombination in contrast to the results of Riley and Pardee (1962) and Shestakov and Barbour (1967) where no interaction is found. In further conflict, the thorough analysis of Helling (1967) shows that the frequency of recombination within the arabinose operon increases when that operon is actively transcribed. Here we examine the relationship between transcription rate and recombination frequency within the histidine operon of Salmonella typhimurium LT-2.

The histidine operon consists of an operator-promoter region and nine structural genes regulated as a unit (Brenner and Ames 1971). The gene order is hisOGDCHAFIE (Hartman et al. 1971). Strains carrying hisO1242 have constitutive histidine enzyme levels elevated by a factor of about 15 when compared with wild-type hisO+ strains grown on histidine containing medium (Roth, Anton and Hartman 1966; Fankhauser 1971). P22 phage mutants L4 (Smith and Levine 1967) were prepared as described (Hartman 1956). Overnight bacterial cultures in Difco nutrient broth were washed and resuspended in T2 buffer. Phage were mixed with bacteria at a multiplicity of 10 and incubated at 37°C for 8 min to allow adsorption. Thereafter, they were spread on plates of minimal agar enriched with 2.5% (v/v) nutrient broth and 2% glucose (EM medium) supplemented with 20 μg/ml of L-tryptophane or L-histidine when necessary. Recombinants were scored after 48 hr of incubation at 37°C. Recombinants carrying hisO1242 mutation were easily discriminated from those carrying wild-type operator by their wrinkled colony appearance on plates with 2% glucose (Roth,
Figure 1.—The histidine operon with location of the markers used in this study. Possible recombination events are indicated with dashed lines. The mutant *hisG2556* harbors also the *hisO1242* mutation.

Antón and Hartman 1966). In crosses involving phage grown on *hisG* mutants, all *his*\(^+\) recombinants must result from recombinational events within the histidine operon, i.e., between *hisF3031* and the *hisG* markers (Figure 1).

The data in the upper third of Table 1 shows that the frequency of transduction to *hisF*\(^+\) with phage grown on wild-type bacteria is decreased in strain SB1267, which is isogenic with SB1266 except for the *hisO1242* marker. Some of these recombinational events may occur outside of the histidine operon (Figure 1). The relative transduction frequency is further reduced to about 50% in crosses involving *hisG*\(^-\) donors where only recombinational events occurring within the histidine operon are examined. The reduction still occurs with phage prepared on a donor containing the *hisO1242* mutation so can not be due merely to non-homology in the *hisO* region. The data in the center of Table 1 show that recombination frequency in the unlinked tryptophane region (*Trp*\(^+\) recombinants selected on EM\(^+\) His) is not influenced by the presence of the *hisO1242* mutation. Therefore, the reduction noted in the histidine region does not extend to the entire chromosome. This makes it unlikely that high enzyme levels in the histidine operon influence recombination in general or that the peculiar mode of growth of such strains, giving rise to wrinkled colonies, indirectly causes the reduction seen. We suggest that the high rate of transcription of the histidine operon results in a reduction in recombination within the operon.

In the course of these studies we noted that strain SB2299 forms wrinkled colonies on media containing 2% glucose and that all *trp*\(^+\) recombinants are wrinkled (center, Table 1). This is not expected since *F3031* is a frameshift mutation (Oeschger and Hartman 1970) and all such mutations abolish the wrinkled phenotype which is in part due to overproduction of *hisF* protein (Murray and Hartman 1972). The data in the lower portion of Table 1 indicate that when *hisF3031* is replaced by *hisF*\(^+\), recombinants cannot grow unless *hisO1242* also is replaced by *hisO*\(^+\). That is, transduction to *hisF*\(^+\) with phage grown on wild-type bacteria is efficient since *hisF*\(^+\) and *hisO*\(^+\) are integrated together with high frequency. In crosses with phage grown on *hisG* mutants, only quadruple crossovers will yield *hisF*\(^+\) *hisO*\(^+\) (Figure 1), and when *hisO1242* is present in the donor (last column, Table 1) no recombinants are detected. The
### TABLE 1

*Frequency of hisF+ hisG+ recombinants and trp+ recombinants*

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Plating medium</th>
<th>Phage grown on:</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild type</td>
<td>G3025</td>
<td>G300</td>
<td>G2556 01242</td>
<td></td>
</tr>
<tr>
<td>SB1266 (F3031)</td>
<td>EM</td>
<td>3330±79.3 (0)</td>
<td>1050±64.9 (0)</td>
<td>900±31.0 (0)</td>
<td>1010±35.0 (3.3%)</td>
<td></td>
</tr>
<tr>
<td>SB1267 (F3031 01242)</td>
<td>EM + His</td>
<td>2321±85.9 (20%)</td>
<td>496±29.5 (90%)</td>
<td>375±21.6 (99%)</td>
<td>478±17.2 (100%)</td>
<td></td>
</tr>
<tr>
<td>SB2298 (F3031 trpC3)</td>
<td>EM + Trp</td>
<td>1264±43.3 (0)</td>
<td>1272±28.5 (0)</td>
<td>1320±38.0 (0)</td>
<td>1260±47.7 (0)</td>
<td></td>
</tr>
<tr>
<td>SB2299 (F3031 01242 trpC3)</td>
<td>EM + Trp</td>
<td>1264±49.4 (100%)</td>
<td>1160±36.5 (100%)</td>
<td>1240±56.7 (100%)</td>
<td>1200±38.0 (100%)</td>
<td></td>
</tr>
<tr>
<td>SB2298 (F3031 trpC3)</td>
<td>EM + Trp</td>
<td>3000±74.0 (0)</td>
<td>1075±54.9 (0)</td>
<td>948±60.2 (0)</td>
<td>1048±57.8 (0)</td>
<td></td>
</tr>
<tr>
<td>SB2299 (F3031 01242 trpC3)</td>
<td>EM + Trp</td>
<td>2560±80.0 (0)</td>
<td>36±3.3 (0)</td>
<td>2±0.70 (0)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The data in the table represent mean values of five independent experiments ± standard error.

Trp = supplementation with tryptophane and His = supplementation with histidine. The number of recombinants in the body of the table is the number of hisF+ hisG+ prototrophic colonies observed per 10⁷ bacteria plated. Numbers in parentheses refer to the percent of hisF+ hisG+ recombinants that form wrinkled colonies as opposed to the total number of colonies including smooth colonies.
same behaviour is noted in strain SB2298 (i.e., no wrinkled $hisF^+$ recombinants in last column, Table 1). We conclude that SB2298 and SB2299 isogenic strains except for $hisO1242$ carry a mutation unlinked with either $his$ or $trp$ that makes them hypersensitive to $F$ gene products of the histidine operon. A mutation with similar effects has been found in strain TA520 by Fankhauser (1971).

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LITERATURE CITED


