Precise Position of the nmfl Locus on the Genetic Map of Salmonella

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INTRODUCTION

The presence of ε-N-methyllysine (NML) was first reported by Ambler & Rees (1959) in the flagellar protein, flagellin, of Salmonella typhimurium strain sw1061. Since then, this amino acid has been found in many proteins including nuclear histones (Murray, 1964). In Salmonella flagellin, the presence or absence of NML is under the genetic control of a gene termed nmfl, which is assumed to be the structural gene for an enzyme methylating the ε-amino groups of certain lysine residues in flagellin (Stocker, McDonough & Ambler, 1961). By transductional analyses Stocker et al. (1961) demonstrated that nmfl is closely linked to HI (the determinant of phase-1 flagellar antigen, i.e. the structural gene for phase-1 flagellin) and some fla genes (a group of genes required for the production of flagella). Since their work, genetic analysis of the HI-fla region has progressed considerably and the following arrangement of genes has been established:

\[-\text{his-} \ldots-\text{flaD-flaB-flaQ-flaP-flaR-flaAIII-flaAII-flaAI-} \\
\text{HI-flaL-flaE-flaK-motA-motB-flaC-flaM-} \ldots-\text{tre-}\]

(Yamaguchi et al., 1972). All these fla and mot genes (a group concerned with motility), except for flaM, are cotransducible with HI in P22-mediated transduction. The present study was undertaken to determine the position of nmfl in this gene cluster.

METHODS

Two series of fla (non-flagellate) mutants of Salmonella were used: one derived from SJ241 fla+ nmfl+ HI-a, a phase-1 stable strain of Salmonella abortusequi (Yamaguchi et al., 1972), and the other derived from SJW1201 fla+ nmfl HI-b, a derivative of SJ241, whose nmfl and HI-b alleles were introduced from Salmonella abony sw803 by transduction.

The nutrient broth, nutrient agar and semi-solid nutrient gelatin agar were as described by Yamaguchi et al. (1972).

Transductional crosses between fla strains were carried out using P22 phage as mediator, and fla+ motile recombinants were selected in semi-solid medium: fla+ clones formed swarms in the medium. The nmfl character of the fla+ recombinants was determined by examining for the presence or absence of NML in their flagellin. Preparation of flagellin was by the method of Yamaguchi & Iino (1969). Detection of NML in flagellin was carried out by applying acid-hydrolysed flagellin to a model 034 Hitachi automatic amino acid analyser. The HI character of fla+ recombinants was determined by examining their flagellar antigens by slide agglutination.
Table 1. HI and nml character of fla^+ recombinants obtained in the reciprocal transductions between SW310 flaAI H1-a nml^+ and SW11213 flaL H1-b nml and the inferred order of flaAI, flaL, H1 and nml

<table>
<thead>
<tr>
<th>Transduction</th>
<th>Genotype</th>
<th>Crossover regions*</th>
<th>Inferred order of genes†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>Recipient</td>
<td>nml</td>
<td>H1</td>
</tr>
<tr>
<td>Cross 1</td>
<td>SW310</td>
<td>SW1213</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>+</td>
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<td>-</td>
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<tr>
<td>Cross 2</td>
<td>SW1213</td>
<td>SW310</td>
<td>+</td>
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</table>

* Figures represent the regions shown in the illustrations in the right-hand column.
† The order of genes was inferred from the assumption that each of the fla^+ recombinants obtained resulted from two crossovers.
RESULTS AND DISCUSSION

Preliminary determination of the position of nml in the H1–fia region was achieved by the following three-point cross test. Reciprocal transductions were carried out between pairs of fia nmL+ and fia nmL strains whose fia mutational sites were in neighbouring fia genes, and the fia+ transductants obtained were examined for their nml character as the unselected marker. If nml is the inside marker, transduction should produce both fia+ nmL+ and fia+ nmL recombinants regardless of the direction of the transduction, because only two crossovers are required for their production. If nml is the outside marker, it is expected that in one of the reciprocal crosses both fia+ nML+ and fia+ nmL recombinants are produced, while in the reverse cross most of the fia+ recombinants have only the recipient-type nml character unless four crossovers occur.

Among several pairs of fia mutants examined, only sjw310 fiaAI nml+ (a derivative of sj1241) and sjw1213 fiaL nml (a derivative of sjw1201) produced both fia+ nml+ and fia+ nmL recombinants in either of the reciprocal crosses. Among 37 fia+ recombinants obtained in the cross from sjw310 to sjw1213 (cross 1 in Table I), 13 clones were nml+ and the remaining 24 were nml. In the reverse cross (cross 2 in Table I), eight clones among 28 fia+ recombinants were nml+ and 20 were nml. This result clearly shows that the nml locus is located between fiaAI and fiaL.

As described in the Introduction, H1 is also located between fiaAI and fiaL. The relative order of nml and H1 in relation to fiaAI and fiaL was determined by considering the H1 character of the fia+ recombinants obtained in the above crosses. Among the four possible genotypes of recombinants, H1-a nml+, H1-b nml+, and H1-b nmL were found in both crosses, whereas no recombinant of H1-a nml type was found in either cross (Table I). Assuming that each of the recombinants obtained arose by only two crossovers, the following order of genes is obtained (Table I):

\[-fiaAI-H1-nml-flaL-\]

In the genetic map of Salmonella summarized by Sanderson (1972), nml was tentatively assigned between fiaAI and H1. The only data so far reported on the mapping of nml are those of Stocker et al. (1961). Their data demonstrated the close linkage of nml with H1 but did not determine on which side of H1 the nml locus was located. In the present study nml was assigned between H1 and fiaL. This result agrees with our previous report in which the presence of an interval of about the same length as that of the H1 gene was demonstrated between H1 and fiaL (Horiguchi et al., 1975).

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REFERENCES


