Precise Position of the *nml* Locus on the Genetic Map of Salmonella

By R. KONNO, H. FUJITA, T. HORIGUCHI AND S. YAMAGUCHI

Department of Biology, School of Education, Waseda University,
Nishiwaseda, Tokyo 160, Japan

(Received 23 September 1975)

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 *nml*, 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 *nml* is closely linked to *H1* (the determinant of phase-I flagellar antigen, i.e. the structural gene for phase-I flagellin) and some *fla* genes (a group of genes required for the production of flagella). Since their work, genetic analysis of the *H1-fla* region has progressed considerably and the following arrangement of genes has been established:

-his-...-*flaD-flaB-flaQ-flaP-flaN-flaR-flaAIII-flaAII-flaAI*-
H1-flaL-flaE-flaK-motA-motB-flaC-flaM...-tre-

(Yamaguchi *et al.*, 1972). All these *fla* and *mot* genes (a group concerned with motility), except for *flaM*, are cotransducible with *H1* in P22-mediated transduction. The present study was undertaken to determine the position of *nml* in this gene cluster.

METHODS

Two series of *fla* (non-flagellate) mutants of *Salmonella* were used: one derived from SJ241 *fla*⁺ *nml*⁺ *H1-a*, a phase-I stable strain of *Salmonella abortusequi* (Yamaguchi *et al.*, 1972), and the other derived from SJW1201 *fla*⁺ *nml* *H1-b*, a derivative of SJ241, whose *nml* and *H1-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 *nml* 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 *H1* character of *fla*⁺ recombinants was determined by examining their flagellar antigens by slide agglutination.

Table 1. *Hr* and *nml* character of *fla*⁺ recombinants obtained in the reciprocal transductions between SJW310 *flaAI* *Hr*-*a nml*⁺ and SW1213 *flaL Hr*-*b nml* and the inferred order of *flaAI*, *flaL*, *Hr* and *nml*

Transduction		<i>fla</i> ⁺ recombinants			Inferred order of genes†
Donor	Recipient	Genotype		Crossover regions*	
		<i>nml</i>	<i>Hr</i>	Number	
Cross 1 SJW310	SW1213	+	<i>a</i>	12	Donor: $-flaAI-Hr-a-$ + $-$ + $-$ Recipient: $\underline{(1)}$ + $\underline{(2)}Hr-b\underline{(3)}nml$ $\underline{(4)}flaL$ $\underline{(5)}$
		+	<i>b</i>	1	
		-	<i>a</i>	0	
		-	<i>b</i>	24	
Cross 2 SW1213	SJW310	+	<i>a</i>	4	Donor: $-$ + $-Hr-b-nml-flaL-$ Recipient: $\underline{(1)}flaAI\underline{(2)}Hr-a\underline{(3)}$ + $\underline{(4)}$ + $\underline{(5)}$
		+	<i>b</i>	4	
		-	<i>a</i>	0	
		-	<i>b</i>	20	

* 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*-*fla* region was achieved by the following three-point cross test. Reciprocal transductions were carried out between pairs of *fla nml*⁺ and *fla nml* strains whose *fla* mutational sites were in neighbouring *fla* genes, and the *fla*⁺ transductants obtained were examined for their *nml* character as the unselected marker. If *nml* is the inside marker, transduction should produce both *fla*⁺ *nml*⁺ and *fla*⁺ *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 *fla*⁺ *nml*⁺ and *fla*⁺ *nml* recombinants are produced, while in the reverse cross most of the *fla*⁺ recombinants have only the recipient-type *nml* character unless four crossovers occur.

Among several pairs of *fla* mutants examined, only SJW310 *flaAI nml*⁺ (a derivative of SJ241) and SJW1213 *flaL nml* (a derivative of SJW1201) produced both *fla*⁺ *nml*⁺ and *fla*⁺ *nml* recombinants in either of the reciprocal crosses. Among 37 *fla*⁺ recombinants obtained in the cross from SJW310 to SJW1213 (cross 1 in Table 1), 13 clones were *nml*⁺ and the remaining 24 were *nml*. In the reverse cross (cross 2 in Table 1), eight clones among 28 *fla*⁺ recombinants were *nml*⁺ and 20 were *nml*. This result clearly shows that the *nml* locus is located between *flaAI* and *flaL*.

As described in the Introduction, *H1* is also located between *flaAI* and *flaL*. The relative order of *nml* and *H1* in relation to *flaAI* and *flaL* was determined by considering the *H1* character of the *fla*⁺ 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 1). Assuming that each of the recombinants obtained arose by only two crossovers, the following order of genes is obtained (Table 1):

-*flaAI*-*H1*-*nml*-*flaL*-.

In the genetic map of *Salmonella* summarized by Sanderson (1972), *nml* was tentatively assigned between *flaAI* 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 *flaL*. 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 *flaL* (Horiguchi *et al.*, 1975).

The authors are indebted to Professor T. Iino and Professor T. Taira for their helpful discussions and criticism.

REFERENCES

- AMBLER, R. P. & REES, M. W. (1959). ϵ -N-methyllysine in bacterial flagellar protein. *Nature, London* **184**, 56-57.
- HORIGUCHI, T., YAMAGUCHI, S., YAO, K., TAIRA, T. & IINO, T. (1975). Genetic analysis of *H1*, the structural gene for phase-1 flagellin in *Salmonella*. *Journal of General Microbiology* **91**, 139-149.
- MURRAY, K. (1964). The occurrence of ϵ -N-methyllysine in histones. *Biochemistry* **3**, 10-15.
- SANDERSON, K. E. (1972). Linkage map of *Salmonella typhimurium*, edition IV. *Bacteriological Reviews* **36**, 558-586.
- STOCKER, B. A. D., McDONOUGH, M. W. & AMBLER, R. P. (1961). A gene determining the presence or absence of ϵ -N-methyl-L-lysine in *Salmonella* flagellar protein. *Nature, London* **186**, 556-558.
- YAMAGUCHI, S. & IINO, T. (1969). Genetic determination of the antigenic specificity of flagellar protein in *Salmonella*. *Journal of General Microbiology* **55**, 59-74.
- YAMAGUCHI, S., IINO, T., HORIGUCHI, T. & OHTA, K. (1972). Genetic analysis of *fla* and *mot* cistrons closely linked to *H1* in *Salmonella abortusequi* and its derivatives. *Journal of General Microbiology* **70**, 59-75.