NOTES

Genetic Analysis of the flaA Locus of Bacillus subtilis

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We isolated two clones of recombinant lambda bacteriophage with overlapping inserts of Bacillus subtilis chromosomal DNA corresponding to part of the flaA locus. The flaA4 and flaA15 mutations were localized on the physical map by marker rescue experiments. The flaA locus and the flaB (sigD) gene were mapped in transduction crosses, and the order glnA polC flaB flaA was determined. FlaB was linked to polC in transformation crosses.

A large number of genes involved in flagellar synthesis and assembly have been described in both Escherichia coli and Salmonella typhimurium. Extensive genetic and molecular analyses have shown that about 3% of the genetic information in these bacteria is devoted to synthesis and assembly of flagella, to their functioning (proteins of the motor and of the motor switch), and to the chemotactic response (10, 11). Although Bacillus subtilis has been used as a model system to study the energetics of the flagellar motor (9, 17), little is known about the genetics of the system. Few mutants of B. subtilis affected in motility have been isolated and characterized (7). On the basis of transformation and transduction mapping, they have been assigned to four loci: flaA, flaB, flaC, and flaD (1, 15); flaA and flaB map between thyA and pyr, flaC is linked to hisA, and flaD is linked to araD. The flaD locus has been cloned (16), and flaB has recently been shown to be an allele of sigD (12). More advanced is the genetics of the chemotactic response of B. subtilis, for which a large number of mutants have been isolated and attributed to at least 21 complementation groups (13, 14, 18). A number of chemotaxis genes are linked to pyr and thus map in the same region as flaA and flaB. We isolated two overlapping clones, λUF7 and λA21 (Fig. 1A), from a λChrom 4A B. subtilis chromosomal library (2, 5). The B. subtilis strains used are described in Table 1. On the basis of restriction maps, the two clones appeared to be identical to the two clones described by Ordal et al. (13) and reported to contain most of the che genes. The two clones span the flaA locus.

Clone λUF7 contains the wild-type alleles of flaA4 and flaA15. On the basis of previous mapping experiments (6), it appeared that the B. subtilis DNA insert of λUF7 was derived from a region of the chromosome linked to the pyrD marker, i.e., not far from the flaA locus. We thus tested the lambda clone for its ability to restore the wild-type phenotype to flaA4 and flaA15 mutant strains. Marker congress experiments (Fig. 1B) showed that both flaA markers could be rescued by DNA obtained from clone λUF7. Further subcloning of the lambda insert definitely demonstrated that flaA4 and flaA15 are located in different portions of the cloned DNA (Fig. 1B). The flaA4 mutation was rescued by a DNA fragment of 2,570 bp (pUF12). We were unable to assign the position of the mutation more precisely. The shortest fragment that could restore the motility phenotype to strains with the flaA15 mutation had a length of 655 bp (carried by plasmid pUFH-B). As deduced from the nucleotide sequence of the 8.3-kb EcoRI fragment (2), the flaA15 mutation corresponds to open reading frame 2 (ORF2), whereas the flaA4 mutation corresponds to a different ORF (ORF8, ORF9, or ORF10). Mutations flaA4 and flaA15 were found to be closely linked in transformation crosses and, for this reason, were both named flaA (15). Our data show that the two mutations affect two different cistrons.

The flaA15 mutation appears to be in a gene involved in the synthesis of a switch protein, whereas flaA4 may affect the rod, the hook length, or unknown functions, depending on its exact location. We reevaluated the phenotype of strains PB5060 (flaA15), PB5070 (flaA4), and PB5071 (flaA in a different genetic background). Examination by electron microscope of samples from cultures at the end of exponential growth and in the stationary phase showed that both PB5060 and PB5070 were completely devoid of flagellar filaments. Under the same conditions, cells of control strains were surrounded by many flagellar filaments. Most strain PB5071 cells were without flagella, and less than 10% had few, bent filaments. The leakiness of the flaA4 mutation was observed on a swarm plate as well. We conclude that both flaA4 and flaA15 mutations affect the assembly of flagellar filaments.

Integration plasmids containing various DNA fragments derived from λUF7 were used in transformation experiments with selection for chloramphenicol resistance. Good transformation efficiencies were obtained with all of the integrational plasmids analyzed, and the only phenotype associated with chloramphenicol resistance was invariably the inability of the colonies to swarm on gelatin-agar plates. These results suggest the possibility that the insert of λUF7 is part of a single transcription unit involved in flagellar biosynthesis and confirm the finding of Zuberi et al. (19) that scoring for the chemotaxis deficiency phenotype showed that the transcription unit starts upstream of the 8.3-kb EcoRI fragment and ends in the 10.9-kb EcoRI fragment or downstream of it.

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Genetic mapping of the flaA and flaB loci. The flaA locus was mapped by transduction crosses between pyrD and thyA (15). We performed transduction experiments aimed at finer mapping of flaA in regard to the markers glnA and polC. To facilitate the genetic mapping, we used integrational vector pJH101 (4), whose chloramphenicol resistance determinant could be employed as a useful selective marker. All integrational derivatives of clone λUF7 were nonmotile and thus not suitable for transduction mapping with flagellum-tropic bacteriophage PBS1. Zuberi et al. (19) showed that an integrational vector carrying a 2-kb PstI-EcoRI fragment at the end of the insert of phage λ14.9 (corresponding to our phage clone λA21) could generate motile B. subtilis transfectants. We transformed our parental strain, PB1424, with a similarly constructed plasmid (pJH2000) and confirmed that the Cm' transfectants were motile. One such transfectant was used as the donor in transduction experiments. The chloramphenicol resistance determinant (cat) was taken as a genetic marker of flaA. The recipient was PB1814, and selection was for GlnA+, chloramphenicol resistance, and Ts" (the polC allele dnaF69 of strain PB1814 confers a temperature sensitivity phenotype). The results of the three-factor crosses (Table 2 and Fig. 2) are consistent with the order glnA polC cat (flaA). This order is in agreement with the previous mapping of flaA of Pooley and Karamata (15) and with the more recent data of Zuberi et al. (19), who located cheF and cheM, two genes of the flaA locus, between spaB and pyrD. According to the data in Table 2, the flaA locus is 87% linked to polC in transduction. The flaA15 marker was not linked to polC in transduction.

![FIG. 1. (A) Physical organization of B. subtilis DNA cloned in phage lambda. (B) Localization of flaA4 and flaA15 mutations. The locations of the two mutations were determined by marker congression in transformation. The plasmids were all derivatives of pGEM-4Z (Promega Biotec, Madison, Wis.). Symbols: +, the fragment gave motile transfectants; −, no motile transfectants were observed.]()
should be noted that the strong linkage observed in transduction was between polC and the cat gene of insertional plasmid pJH2000. The plasmid carried a DNA fragment derived from downstream of the flaA locus and, according to the genetic data of Zuberi et al. (19), nearest to the polC gene. We thus tested for linkage in transfection between polC and the chloramphenicol resistance determinant of pJH2000. Chromosomal DNA isolated from strain PB5077 (flaA::pJH2000) was used to transform strain PB1669 to chloramphenicol resistance. Of 72 chloramphenicol-resistant transformants tested, 21 (29%) were found to be Ts 

The flaB2 mutation has also been mapped between thyA and pyrD (15), but the mutation could not be rescued by DNA from λUF7 and λA21 (data not shown). In transformation experiments, we failed to observe linkage between flaB2 and flaA15. This is in agreement with the observations of Pooley and Karamata (15), who could not find a linkage in transformation between flaA4 and flaB2.

It was recently shown that flaB is an allele of gene sigD, which codes for transcriptional factor σ70 (12). By using integrational plasmid pLM3, containing the 3' end of gene sigD (12), we mapped the sigD (flaB) gene with regard to polC. The downstream integrants obtained with plasmid pLM3 have only slightly reduced levels of flagellin expression and are motile (12). We used one such integrant as the donor in PBS1 transduction experiments; the results of the three-factor crosses (Table 3 and Fig. 2) suggested the order glnA polC sigD (flaB). Thus, the loci flaB and flaA map on the same side with respect to polC. The 90% linkage between sigD and polC prompted us to check for linkage of the two markers in transformation. Chromosomal DNA isolated from the pLM3 integrant was used to transform strain PB1669 to chloramphenicol resistance. Of 150 chloramphenicol-resistant transformants tested, 146 (97%) were Ts 

We thank H. M. Pooley and D. Karamata for kindly providing strains and M. J. Chamberlin for providing plasmid pLM3.

P.M.H. was the recipient of a scholarship from the Ministero degli Affari Esteri (Italy). This work was partially supported by CNR and MURST (Rome).

### REFERENCES


5. Galizzi, A. Unpublished data.


7. Grant, G. F., and M. I. Simon. 1969. Synthesis of bacterial flagella. II. PBS1 transduction of flagella-specific markers in pLM3 have only slightly reduced levels of flagellin expression and are motile (12). We used one such integrant as the donor in PBS1 transduction experiments; the results of the three-factor crosses (Table 3 and Fig. 2) suggested the order glnA polC sigD (flaB). Thus, the loci flaB and flaA map on the same side with respect to polC. The 90% linkage between sigD and polC prompted us to check for linkage of the two markers in transformation. Chromosomal DNA isolated from the pLM3 integrant was used to transform strain PB1669 to chloramphenicol resistance. Of 150 chloramphenicol-resistant transformants tested, 146 (97%) were Ts 

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### TABLE 3. Mapping of flaB (cat): three-factor* transduction cross involving glnA100 and polC (dnaF69)

<table>
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<tr>
<th>Selected marker</th>
<th>Phenotype</th>
<th>No. of recombinants</th>
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<tr>
<td></td>
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<td>DnaF</td>
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<tr>
<td>DnaF' (Ts') (A)</td>
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<td>D</td>
</tr>
<tr>
<td>DnaF' (Ts') (B)</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>DnaF' (Ts') (C)</td>
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<td>R</td>
</tr>
<tr>
<td>DnaF' (Ts') (D)</td>
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<td>D</td>
</tr>
<tr>
<td>cm' (cat) (A)</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>cm' (cat) (B)</td>
<td>D</td>
<td>D</td>
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<tr>
<td>cm' (cat) (C)</td>
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<td>D</td>
</tr>
<tr>
<td>glnA'</td>
<td>D</td>
<td>R</td>
</tr>
</tbody>
</table>

* Denoted order: glnA polC cat (flaB).

The donor was PB5079 (flaB::cat), and the recipient was PB1814 (glnA100 dnaF69). dnaF69 is a temperature-sensitive allele of polC.

D, donor; R, recipient.


