Assembly of Hybrid Flagellar Filaments

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The distribution of flagellin subunits in flagellar filaments synthesized by merodiploid strains carrying two distinguishable hag loci has been examined. The filament was found to be a homogeneous co-polymer of the two subunits. This suggests that the subunits may be able to mix freely before being assembled.

Salmonella flagella are assembled from either of two flagellin molecules encoded by two genes, H1 and H2. The synthesis or assembly of these two different flagellin subunits is regulated so that only one type of homopolymeric filament, containing the product of the H1 or H2 gene, is produced. In transient merogogotes (6), resulting from the abortive transduction of a second H1 gene that codes for a flagellin subunit distinguishable from the endogenote H1 product, evidence for the presence of both flagellin molecules has been found. Koffler and his co-workers (Koffler, J. Bui, and G. Somkuti, Abstr. Annu. Meet. Amer. Soc. Microbiol. 73:34, 1973) have shown that some strains of Bacillus pumilus produce two different kinds of flagellin subunits, presumably resulting from the expression of two genes within the same cell. Furthermore, extensive complementation studies with the flagellar genes in Escherichia coli and Salmonella (5, 7) are all consistent with the assumption that there is no restriction of expression of exogenous flagellar structural genes.

Although it seems clear, therefore, that in merodiploids two different alleles can be expressed, there is very little evidence that indicates how these gene products interact in vivo. Asakura et al. (1, 2) have shown that hybrid flagellar filaments can be assembled in vitro from different flagellin subunits. The nature of the product depends on the conditions of assembly. Thus, it has been difficult to prepare flagellar filaments that contain alternating blocks of two different flagellin subunits as well as filaments that are relatively homogeneous mixtures of the two. The nature of the in vivo hybrid product might yield further information about the in vivo mechanism of assembly of flagellin.

There are three ways, a priori, in which the different subunits might be assembled in vivo. (i) All of the subunits of one type might be segregated into one filament and subunits of another type might be segregated into a separate filament; thus, the merodiploid bacteria would have two different types of flagella. (ii) The subunits could be co-polymerized in blocks. If this were the case, we would expect alternating patches of the two different flagellins in the same filament. (iii) They could be intermixed and co-polymerized continuously; thus, each filament would be a fairly homogeneous mixture of the two types of subunits.

To distinguish among these possibilities, the strains shown in Table 1 were constructed. MS1306 carries the wild-type allele of the hag locus on the endogenote and the allele that determines the straight flagellin on the exogenote. These two kinds of flagellin subunits can be distinguished by acrylamide gel electrophoresis (Fig. 1). Flagella isolated from MS1306 gave two bands on electrophoresis, one corresponding to the wild-type flagellin and the other corresponding to the flagellin that determines

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<th>Mat'ing type</th>
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<tr>
<td>W3110</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
<td>hag&lt;sup&gt;2&lt;/sup&gt;7S (straight flagella)</td>
<td>J. Adler</td>
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<tr>
<td>AB2463</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
<td>thi, thr, leu, pro, his, argE&lt;sup&gt;E&lt;/sup&gt;, str, lac, gal, ara, xyl, mtl, recA&lt;sup&gt;13&lt;/sup&gt;, hag&lt;sup&gt;2&lt;/sup&gt;07</td>
<td>D. Kingsbury</td>
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<tr>
<td>MS1032</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
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<td>MS1257</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
<td>his&lt;sup&gt;S&lt;/sup&gt;, uvr&lt;sup&gt;S&lt;/sup&gt;, hag&lt;sup&gt;2&lt;/sup&gt;07 in MS1032</td>
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<td>MS1324</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
<td>his&lt;sup&gt;S&lt;/sup&gt;, uvr&lt;sup&gt;S&lt;/sup&gt;, hag&lt;sup&gt;2&lt;/sup&gt;08 in AB2463</td>
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FIG. 1. Acrylamide gel electrophoresis of flagellins obtained by disaggregation of flagella from merodiploid cells. Gel electrophoresis was performed by the method of Davis (3). A, 25 μg of Hag207 flagellin (from AB24631); B, 25 μg of Hag207(S) flagellin (from W3110); C, 25 μg Hag207 and 25 μg of Hag207(S) flagellin mixed and electrophoresed together; D, 50 μg of flagellin from merodiploid F′ hag207(S)/hag207 (from MS1306).

FIG. 2. Electron microscope examination of anti-Hag207 antibody bound to flagella. A, MS2463 (Hag207 flagella) at right shows coated flagella, whereas MS1032 (Hag208 flagella) at left displays very little antibody coating flagella. Bar = 1.0 μm. B, At higher magnification antibody binding to Hag207 flagella (both almost vertical) is observed as an increase in the apparent width of the flagellum with respect to the Hag208 flagellum (almost horizontal in B). Bar = 0.2 μm. C, MS1257 after addition of anti-Hag207 antibody. The flagella are homogeneously coated with antibody. D, MS1306, the merodiploid strain shows straight flagella that result from the introduction of the gene coding for straight flagella.

straight flagellar filaments. The introduction of the episome carrying the mutant hag allele rendered the recipient nonmotile. Upon examination by electron microscopy, all of the merodiploid cells were found to have almost straight flagella (Fig. 2D). The filaments show a slight helicity, suggesting a very low amplitude and a short wavelength. There was no distribution in the shapes of filaments, i.e., all of them were identical. These results argue strongly against the assembly of homopolymeric flagellar filaments. They suggest that the subunits are co-polymerized, either in blocks or in continuous heteropolymers.

To further pursue this analysis, merodiploids
carrying genes that determine antigenically distinct flagella were prepared. hag207 is the wild-type allele, and flagellar filaments isolated from bacteria carrying this gene react with anti-Hag207 antiserum. The reaction is inhibited by flagellin subunits derived from these filaments. hag208 refers to a mutation that results in serologically distinct flagella that react with anti-Hag208 antibody, and the reaction is specifically inhibited by the flagellin subunits. Figures 2A and B show that the antisera coat the appropriate filaments specifically. In the hybrid strain (Fig. 2C), all of the flagella are uniformly coated by anti-Hag207. They are also all coated by anti-Hag208. Thus, we conclude that all of the flagella are relatively homogeneous mixtures of both types of subunits.

Figure 3 shows quantitatively that both antisera react with flagella derived from the hybrid strain. These data are also most clearly consistent with the conclusion that there is mixing of different gene products in the same flagellar structure. The in vivo formation of similar co-polymer filaments of phase 1 and phase 2 flagellins has been detected by T. Iino (personal communication) in the transitory stage of phase variation in Salmonella.

The mixing of flagellin subunits may not be entirely unrestricted, since the quantitative results (Fig. 3) suggest that the Hag207 flagellin content is about two-thirds and Hag208 is one-third. To check this result further, the experiment was repeated by depolymerizing the filaments (4) and measuring specific flagellin subunits by their ability to inhibit the flagella-antiflagella reaction. When the hag207 allele is on the exogenote, it results in 71% of the total flagellin activity, whereas it represents 57% of the total when it is on the endogenote. There appears, therefore, to be a slight preference for Hag207 subunits, irrespective of whether the gene is on the chromosome or on the episome. It is not clear whether this reflects the relative rates of protein synthesis or some restrictive mechanism in the assembly process.

The results of these experiments strongly support the conclusion that flagellar filaments in vivo can be made up of a relatively homogeneous mixture of differing gene products. It is clear that there is no gross asymmetric distribution of the subunits within the filament both in hybrids between straight and wild-type subunits and between antigenically different subunits. One simple explanation of this distribution is that there exists a small intermediate pool from which subunits are drawn for subsequent assembly. Attempts in this laboratory to identify such a pool suggest that if it exists it is generally maintained at a very low intracellular level. Its concentration may be regulated by the rate of flagellar assembly.

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**LITERATURE CITED**