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Transcriptional Control of Flagellar Genes in *Escherichia coli* K-12

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Autoregulation of the expression of flagellar genes was investigated by the technique of operon fusion. The results suggested that the *flaU* gene is a repressor and the *flaD* gene is an activator of transcription of the *hag*, *flaS*, and Mocha operons. The action of the putative *flaU* repressor appears to be masked by its interaction with other flagellar proteins during assembly; thus, repression is apparent only when the interacting proteins are absent. This hypothesis is supported by the phenotype of an unusual *flaU* mutant, which represses even though it is unable to promote flagellar assembly. Presumably, the mutant synthesizes a repressor whose activity is no longer masked by interaction with other flagellar proteins.

In an earlier report, I described the effects of *fla* mutations on the transcription of each of the flagellar operons in a series of experiments that used the technique of fusion (17). I examined whether a given Fla$^-$ mutant would permit the transcription of a specific flagellar operon, as detected by *lacZ*-directed activity. This qualitative analysis led to the hypotheses that each flagellar operon has intrinsic requirements for the products of particular genes for its transcription and that the flagellar operons can be divided into six groups with respect to transcriptional control (classified as shown in Table 1). Figure 1 depicts the hypothetical regulatory network.

In another study, (17), my colleague and I identified flagellar precursor structures in some flagellar mutants by electron microscopy. The precursor structures that accumulated in mutants with specific genetic defects allowed predictions to be made as to the roles of these genes in flagellar assembly. Flagellar assembly is assumed to proceed, step by step, from simpler structures to more complex ones (14). Mutants with defects in *hag*, *flaD*, *flaS*, *flaT*, or *flbC* had the most complex precursor, the hook-basal body structure (6, 14, 17). *flaE* mutants had an equivalent polyhook-basal body structure. Basal body structures appeared in *flaK* mutants; *flaM*, *flaU*, *flaV*, and *flaY* mutants had incomplete basal body structures (16, 17). From a comparison of these observations (17) with the sequence shown in Fig. 1, it can be seen that the operons placed in the later, or numerically higher, groups appeared to be responsible for the formation of more complex structures. Therefore, there appears to be a possibility that the sequence of transcriptional interactions (Fig. 1) (7) is coupled with the assembly of flagellar structures (11, 17). To elucidate the transcriptional control of flagellar genes, I examined the interactions between the operons in group 2 or 3 (Table 1) and the later operons by using *fla-lacZ* fusion analysis and *fla* double mutants.

MATERIALS AND METHODS

Media. The following media were used: minimal medium, L broth, L broth agar, tryptone broth, tryptone broth top agar, and MacConkey agar (Difco Laboratories) containing 1% lactose (9, 10).

Bacteria and phages. The flagellar mutants were derived from strain YK410 (F$^-$ *araD139* Δ*lacU169 rpsL thi pyrC46 gyrA thyA his*) and were described in an earlier report (10). The mutations are described in Tables 2 and 3. The lambda *fla-lacZ* fusion phages were described previously (1, 7) and are shown in Tables 2 and 3.

Isolation of *fla* double mutants. All *fla* mutants are nonflagellate. It is difficult to distinguish between *fla* single mutants and *fla* double mutants. Some of the double mutants obtained from H. Kagawa (Okayama University) were constructed by cotransduction of region I (13) *fla* genes with the *pyrC*$^+$ gene into *flaE* and *hag* mutants. Other *fla* double mutants used in this study were constructed by transduction of *fla*::Tn*10* (or *fla*::Tn*5*) mutations into *fla* mutants representing the various classes of *fla* genes. Transductions were performed as described previously (10). A P1 *kc* derivative (8) lysate was grown on each insertion mutant on L broth agar and used to transduce its drug resistance. Presumptive double mutants were examined for the existence of two mutations by use of λ *fla*$^+$ phages and P1 transduction.

Scoring of Lac phenotypes of lambda *fla-lacZ* phages on *fla* mutants. For poorly understood reasons, it proved impossible to construct a whole set of lambda *fla-lacZ* lysogens (the fusion phages appeared to move around the *Escherichia coli* genome). Accordingly, Lac phenotypes of lambda *fla-lacZ* phages (7) were examined after plating onto appropriate flagellar mutants on MacConkey-lactose agar and incubation for 20 h at 37°C. This qualitative analysis was simple and convenient for the examination of large numbers of hosts and was accurate enough to allow the classification of the hosts.

RESULTS AND DISCUSSION

Rationale. If a product of operon A is required for the transcription of operon B, there are at least three possible mechanisms that could explain this effect. The basic mechanisms are shown in Fig. 2. (i) Products of the A operon may be positive factors (activators) for transcription of the B operon (for example, the *araC* gene in the *ara* operon and the *malT* gene in the *mal* regulon) (2, 3). (ii) Products of the A operon may be responsible for assembly of products of the B operon. Without products of the A operon, products of the B operon cannot assemble, and they repress their own transcription (this mechanism is similar to the mechanism by which the T4 gene 32 protein stops its own transcription) (4).

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TABLE 1. Classification of flagellar regulons into six groups

Group	Regulon	Member(s) of regulon
1	*flbB*	*flbB, flaI*
2	*flaU*	*flaU*
	flbC	*flbC*
3	*flbA*	*flbA, flaW, flaV, flaK, flaX, flaL, flaY, flaM*
	flaG	*flaG, flaH*
	flaD	*flaD*
	flaN	*flaN*
	flaB	*flaB, flaC, flaO, flaE*
	flaA	*flaA, flbD, motD, flaR, flaQ, flaP*
4	*flaZ*	*flaZ*
5	Mocha	*motA, motB, cheA, cheW*
	flaS	*flaS, flaT*
6	*hag*	*hag*

(iii) There may be products of another operon C, and an additional repressor. Products of the A operon are responsible for assembly of products of the C operon or, conversely, products of the C operon are required for assembly of products of the A operon, when the products of A and C operons coassemble. If the products of the A operon are not present, then the products of the C operon are not required in any way. This failure of assembly results in the accumulation of abundant free products of the C operon which, in turn, repress transcription of the B operon (this mechanism resembles the control of the synthesis of ribosomal proteins) (18). In mechanisms ii and iii, the success of assembly corresponds to the failure of repression. These mechanisms are thought to be basic, and many possible combinations may be derived from them. Since the genes in later operons (Fig. 1 and Table 1) are responsible for the formation of the most complex precursor structures, it is plausible that the completion of assembly plays a key role in transcriptional control.

Examination of double mutants. This communication focuses on the interactions between the operons in group 2 or 3 (Table 1) and the later operons (groups 4, 5, and 6). Double mutants simultaneously carrying one mutation in a group 2 operon (*flaU* or *flbC* gene) and another in one of the other operons were constructed to allow a distinction to be made between mechanism i (positive regulation) and mechanisms ii and iii (negative regulation). Initially, double mutants simultaneously carrying a mutation in the *flbC* gene and another in the region I (12, 13) operons (included in group 3) were constructed. The double mutants were used as hosts for the examination of the Lac phenotype of each lambda *fla-lacZ* phage. The phenotype of all the defects in the *flbC* gene was the expression of all the late operons. Double mutants carrying the *flbC1101* mutation had a phenotype characteristic of single mutants carrying only the second mutation (Table 2). The defects in the *flbC* gene were thus qualitatively silent in the presence of the mutations in the group 3 operons. The other *flbC* double mutants could not be constructed for practical reasons. Next, a whole set of double mutants carrying a *flaU4511*::Tn*5* mutation was constructed and examined. The phenotype of a *flaU* single mutant is shown in Table 2: all late operons are expressed. The *flaU4511-flaD*, *flaU4511-flaI*, and *flaU4511-flbB* double mutants had phenotypes typical of single mutants carrying only the second mutation (Table 2). (The *flaU4511-flaZ1118* double mutant also appeared to have the phenotype of a *flaZ* single mutant. However, mutations in the *flaZ* gene are very leaky, and I was unable to find convincing differences between the $flaZ^+$ strain and other $flaZ^-$ mutants.) In contrast, the other double mutants had the same phenotype as that of the *flaU4511* single mutant; i.e., all late operons were expressed (Table 2). These results demonstrate that the regulatory role of the group 3 operons depends on the product of the *flaU* gene. In other words, there are interactions between the product of the *flaU* gene and those of the group 3 operons (except for the *flaD* product). Defects in the *flbB* operon, whose products are themselves under the control of cyclic AMP (15), were epistatic over the *flaU4511* defect. This result conforms to mechanism i in Fig. 2 for the function of the *flbB* operon. The result with *flaD* was an exception among the results for the genes of the group 3 operons. Accordingly, the *flaD4512*::Tn*5* mutation was introduced into each *fla* mutant, and its effect was examined. The results (Table 2) showed that *flaD* activity was required for transcription of the operons in groups 4, 5, and 6. The *flaD* mutation was epistatic over the *flaU* mutation for the expression of the later operons.

The simplest explanation of these data is that *flaU* codes for a repressor of the operons in groups 4, 5, and 6 and that it interacts with the products of the group 3 operons, which affect its repressor activity. The product of the *flaU* gene is free to repress group 4, 5, and 6 operons if there are mutations in group 3 operons (except for *flaD*, *flaE*, and *motD* genes). If, however, a *flaU* mutation exists that leads to an absence of repressor molecules, group 4, 5, and 6 operons are transcribed, even if there is a mutation in any of the genes of the group 3 operons (except for *flaD*). *flaD* is exceptional among the genes of the group 3 operons in that it appears to code for a positive factor for operons in groups 4, 5, and 6. Although the product of the *flaU* gene represses the operons in groups 4, 5, and 6, the expression of *flaD* is not repressed. Therefore, the product of the *flaU* gene is not a direct repressor of the *flaD* gene. The suggested roles of *flaD* and *flaU* conform to the mechanisms shown in Fig. 2: the *flaD* gene corresponds to operon A in mechanism i or ii, and the *flaU* gene corresponds to operon C in mechanism iii.

Repressor gene. As already mentioned, all of the products of the *fla* genes are (by definition) required for the appearance of a flagellum (14). The product of *flaU* should, therefore, be not only a genetic repressor but also a positive factor for flagellar structure and assembly. Suzuki and Komeda (17) found that *flaU* mutants formed a structure that was a precursor to a flagellum. The product of the *flaU* gene may be a constituent of the next, most complex precursor, or it may be a catalyst for its formation, as well as acting as a repressor. A mutation may occur which causes a defect in only one of these two functions. Since all of the *fla* genes have so far been identified by isolation of nonflagellate mutants, this hypothetical mutant may have a novel repres-

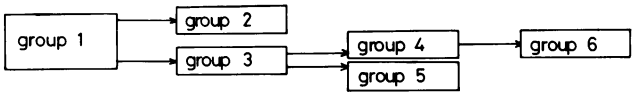

FIG. 1. Transcriptional interation of flagellar regulons demonstrated in an earlier study (7). The components of each group are shown in Table 1. The arrows show the requirement for the products of earlier genes by the operons.

TABLE 2. Expression of *fla-lacZ* fusion phages in double mutants

Host	Lac phenotype for λ yk phage(s) in indicated group[a]:					
	2 (yk11, yk101)	3 (yk21, yk51, yk81, yk111, yk121, yk131)	4 (yk31)	5 (yk41, yk61)	1 (yk71)	6 (yk95)
fla$^+$	+	+	+	+	+	+
flaU4122	+	+	+	+	+	+
flbC1101	+	+	+	+	+	+
flaI4136	−	−	−	−	+	−
flbB4116	−	−	−	−	+	−
Mutations in group 3[b]	+	+	−	−	+	−
flbC1101-mutations in group 3	+	+	−	−	+	−
flaU4511::Tn*5*	+	+	+	+	+	+
flaU4511-flaD4181	+	+	−	−	+	−
flaU4511-flaI4136	−	−	−	−	+	−
flaU4511-flbB4116	−	−	−	−	+	−
flaZ1118	+	+	±	+	+	−
flaU4511-flaZ1118	+	+	±	+	+	−
flaU4511-other mutations[c]	+	+	+	+	+	+
flaD4512::Tn*5*	+	+	−	−	+	−
flaD4512-flaU4122	+	+	−	−	+	−
flaD4512-flaI4136	−	−	−	−	+	−
flaD4512-flbB4116	−	−	−	−	+	−
flaD4512-flaZ1118	+	+	−	−	+	−
flaD4512-other mutations[c]	+	+	−	−	+	−

[a] After infection of each lambda *fla-lacZ* phage with each flagellar mutant, the Lac phenotype was examined as described in the text. The promoter carried on each lambda *fla-lacZ* phage is one of the regulons of the operon groups described previously (7). Symbols: +, red color development in the plaque; ±, reduced red color development in the plaque; −, no red color development in the plaque.

[b] The members of the group 3 operon are shown in Table 1, and the representative mutations used were the same as those described previously (7). In the operons in group 3 there are two exceptions, *flaE* and *motD*, described previously (7). The *flaE* mutation partially permitted the expression of the later operons, and the *motD* mutation permitted the expression of all of the operons (7).

[c] These mutations include those in one of the following operons: group 3 (other than *flaD*), group 5, or group 6 (Table 1). The mutations used were the same as those described previously (7).

sor function. As a result of these considerations, all of the *flaU* mutants were examined for the capacity to support the expression of *lacZ* after infection by lambda *fla-lacZ* (Table 3). Out of five *flaU* mutants, strain YK4435 differed from the rest. Like the others, it had incomplete flagella, but it did not permit the transcription of group 4, 5, and 6 operons. *flaU* insertion mutants (strains YK4511 and YK3440) were presumed to possess a null phenotype and did permit the transcription of group 4, 5, and 6 operons. Strain YK4435 therefore had a defect in flagellum formation, yet it retained repressor function. *flaU4435-flaB* double mutants did not permit the transcription of operons in groups 4, 5, and 6; other double mutants carrying defects in operons in group 3 behaved similarly. The *flaU4435- flaD4512*::Tn*5* double mutant did not promote the transcription of later operons on lambda *fla-lacZ* phages, as expected. The existence of the mutant strain YK4435 favors the hypothesis that the product of the *flaU* gene has a dual role. All 21 *flaD* mutants examined showed the same phenotype: they did not permit the transcription of operons in groups 4, 5, and 6. In summary of the above results, a scheme of flagellar gene interaction is presented in Fig. 3.

Flagellar morphogenesis. In the scheme of regulation in Fig. 2, mechanisms ii and iii predict that the failure of assembly is a prerequisite for repression. Since the existence of mechanism iii for the apparent positive control by the products of group 3 operons upon later operons has been postulated, one point should be mentioned. In a previous study (7), the *flaE* and *motD* genes were considered to be exceptional in group 3, because they permitted the expression of later operons (groups 4, 5, and 6). The defects in these two genes permitted the assembly into flagellar precursors (17) and were easily recognized as derepressors of later operons.

In conclusion, I would like to correlate transcriptional control (Fig. 3) with the assembly pathway of a flagellum as

TABLE 3. Expression of *fla-lacZ* fusion phages in various *flaU* mutants

Mutation	Lac phenotype for λ yk phage(s) in indicated group[a]:					
	2 (yk11, yk101)	3 (yk21, yk51, yk81, yk111, yk121, yk131)	4 (yk31)	5 (yk41, yk61)	1 (yk71)	6 (yk95)
flaU4122	+	+	+	+	+	+
flaU4186	+	+	+	+	+	+
flaU4435	+	+	−	−	+	−
flaU4511::Tn*5*	+	+	+	+	+	+
flaU3440::Mu dl	+	+	+	+	+	+

[a] See Table 2, footnote *a*, for details.

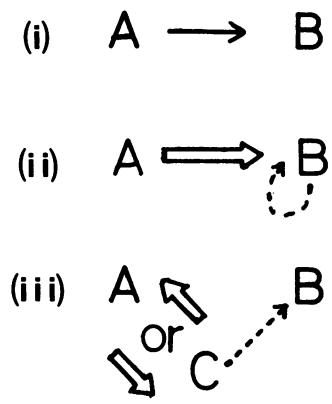

FIG. 2. Schematic description of three mechanisms for apparent positive control. Symbols: →, induction of transcription,⇢, repression of transcription;⟹, interaction during assembly.

visualized by electron microscopy (17). When products of the genes of the operons in group 3 start to assemble into a flagellum, the product of the *flaU* gene (in the group 2 operon) is used either as a catalyst or as a component of flagellar assembly. If this assembly proceeds successfully, the product of the *flaD* gene (whose transcription has already been induced by the action of the *flbB* and *flaI* genes) in turn induces the transcription of the operons in groups 4, 5, and 6 (*hag*), at which point flagellar assembly is finally completed. If there is a defect in any of the genes in the operons in group 3, such that the product of the *flaU* gene is not used for assembly, the abundant product of the *flaU* gene represses the operons in groups 4, 5, and 6. Electron-microscopic observations (17) predicted that the product of the *flaU* gene is required before the steps in which the products of the *flaY*, *flaV*, *flaK*, and *flaE* genes function. If there is a defect in one of the latter genes, transcription of the operons in groups 4, 5, and 6 might be prevented in the following way. If the assembly of flagellar precursors proceeds as a close-to-equilibrium process, the blockage of one reaction in the sequence will bring earlier steps to a halt. Accordingly, a mutation in the *flaY*, *flaV*, *flaK*, or *flaE* gene will produce the same effect as a mutation in genes whose site of action precedes that of *flaU*, namely, a buildup of the product of the *flaU* gene, which then acts as a repressor. An exceptional mutation, *flaU4435*, affected only the process of flagellar assembly and still retained its ability to repress the operons in groups 4, 5, and 6. It is assumed that the mutated protein of the *flaU4435* mutation is not correctly incorporated into a flagellar precursor and is therefore free to repress.

The product of the *flbC* gene has been suggested to be used for assembly or excretion of flagellin molecules or both (5, 14).

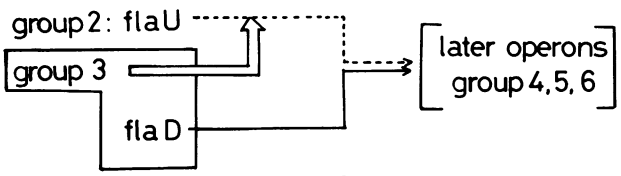

FIG. 3. Transcriptional control of flagellar genes. Symbols are the same as in Fig. 2.

To elucidate these processes in molecular terms, it will be necessary to identify all of the products of the *fla* genes and to isolate regulatory mutations in *flaD* and *flaU*.

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