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Identification of Polypeptides Necessary for Chemotaxis in *Escherichia coli*

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Molecular cloning techniques were used to construct *Escherichia coli*-λ hybrids that contained many of the genes necessary for flagellar rotation and chemotaxis. The properties of specific hybrids that carried the classical "*cheA*" and "*cheB*" loci were examined by genetic complementation and by measuring the capacity of the hybrids to direct the synthesis of specific polypeptides. The results of these tests with lambda hybrids and with a series of deletion mutations derived from the hybrids redefined the "*cheA*" and "*cheB*" regions. Six genes were resolved: *cheA*, *cheW*, *cheX*, *cheB*, *cheY*, and *cheZ*. They directed the synthesis of specific polypeptides with the following apparent molecular weights: *cheA*, 76,000 and 66,000; *cheW*, 12,000; *cheX*, 28,000; *cheB*, 38,000; *cheY*, 8,000; and *cheZ*, 24,000. The presence of another gene, *cheM*, was inferred from the protein synthesis experiments. The *cheM* gene directed the synthesis of polypeptides with apparent molecular weights of 63,000, 61,000, and 60,000. The synthesis of all of these polypeptides is regulated by the same mechanisms that regulate the synthesis of flagellar-related structural components.

More than 25 genes necessary for bacterial flagellar structure and function have been identified in *Escherichia coli* (5). To understand the mechanisms involved in flagellar organelle assembly, energy transduction to produce flagellar rotation, and the integration of sensory information for chemotaxis, the products of these genes will have to be identified. There are no simple enzymatic activities that are known to be associated with these products; their identification, therefore, depends upon cloning the individual genes on appropriate molecular vehicles and measuring the capacity of these hybrid deoxyribonucleic acid (DNA) molecules to direct the synthesis of specific polypeptides. In initial experiments, three hybrid *E. coli* λ phages carrying flagellar genes were isolated (Fig. 1). λ*fla*1 carried the *hag* gene and was shown to direct the synthesis of flagellin (13), the major subunit of the flagellar filament. Two other phages carrying flagellar genes were isolated, λ*fla*2 and λ*fla*3. They carried the *Eco*RI endonuclease-generated fragments of *E. coli* DNA that included the *mot* and *che* genes. The phenotype associated with the *motA* and *motB* genes is paralyzed flagella (1, 4), i.e., the flagellar organelle is intact; however, it does not rotate. The phenotype associated with the *che* mutants (2) is that they are motile but do not respond to changes in attractant or repellant concentrations. *che* mutants in *E. coli* were initially isolated by Armstrong and Adler (2) and more recently by Parkinson (9). Many of these mutations map on the *E. coli* genome in the region between *motB* and *flaG*, at about 41.5 min (15). Genetic complementation tests with *che* mutants initially resolved two genes in this region. We will refer to these as the classical chemotaxis genes "*cheA*" and "*cheB*." Further genetic tests indicated extensive intracistronic complementation within both genes in *E. coli* (9). Collins and Stocker (3) mapped two regions in *Salmonella*, *cheI* and *che*II, that were homologous to the "*cheA*" and "*cheB*" genes in *E. coli*. Warrick et al. (18) studied the complementation behavior of chemotaxis mutants in *Salmonella* that carried mutations which mapped in these regions. They concluded that there were at least five distinct *che* genes. The availability of hybrid λ*fla* carrying the *E. coli* genes provided an opportunity to reexamine the *che* region in another way. Complementation tests between the phages and specific mutants indicated that λ*fla*2 carried *flaI*, *motA*, *motB*, and part of "*cheA*" (14). λ*fla*3 carried the other part of "*cheA*" "*cheB*", and the *flaG* and *flaH* genes (16). Thus, an *Eco*RI endonuclease site apparently existed in the middle of "*cheA*," and one fragment was carried by λ*fla*2 and the other fragment was on λ*fla*3. A new hybrid was prepared which restored the continuity of "*cheA*" by fusing the region on λ*fla*2 which carried one part of the gene with the region of λ*fla*3 that carried the other part of

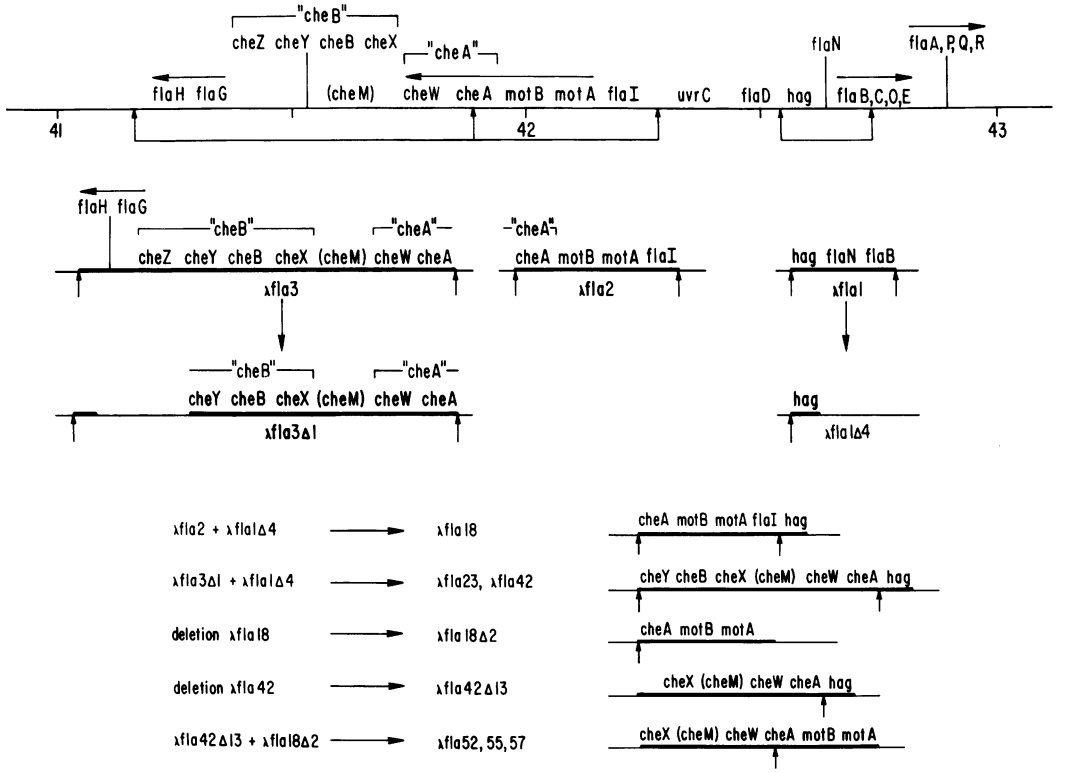

FIG. 1. *Summary of the gene distributions on the bacterial genome and on various hybrid lambdas deduced from genetic complementation tests. The top line indicates the bacterial genome, and the numbers refer to positions on the E. coli map. The vertical arrows pointing up indicate the position of EcoRI endonuclease sites. The second line shows the composition of the initial hybrid lambda phages. The heavy line represents the region of E. coli carried by the virus. The third line and the rest of the figure show schematically how the other viruses used in this study were constructed.*

the gene. When these phages were tested (15), they were found to direct the synthesis of a variety of polypeptides corresponding to the *mot* and *che* genes. *motA*, *motB*, and *cheA* were cotranscribed (the Mocha operon), starting with *motA*. The presence of the intact "*cheA*" gene led to the appearance of three new polypeptide products. The region adjacent to "*cheA*" was also responsible for the synthesis of a group of three polypeptides, which were referred to as the "triplet." Finally, the "*cheB*" region had the capacity to direct the synthesis of a number of polypeptides (16). All of these data suggested that the *che* region in *E. coli* was complex, containing more than two genes. To assign the various products to specific genes, we characterized deletions of the λ*fla* phages by complementation with a variety of Che⁻ strains obtained from J. Parkinson. By comparing the genetic activity of the deleted phages with their capacity to program polypeptide synthesis, it was possible to resolve the classical "*cheA*" and "*cheB*" region into seven genes and to identify the polypeptides whose synthesis was directed by each of these genes.

MATERIALS AND METHODS

Bacterial strains. A variety of strains from our collection and from that of J. Adler and J. S. Parkinson were used. These strains were made lysogenic for λ and the lysogens were given new strain numbers. With Che⁻ strains, it was necessary to introduce F′ *gal* to provide an attachment site for lysogenization by λ. MS5015, MS5025, and MS5014 are λ lysogens of strains carrying the mutations *flaG161*, *flaH616*, and *hag-726* (10). MS1513 and MS5039 are lysogens of *mot-797* and *mot-483* (1). All of the other tester strains are Che⁻ originally selected and described by J. S. Parkinson (9). Some are Rec⁺, whereas others carry a *recA* mutation.

Construction and properties of hybrid λ. Hybrid λ*fla*2 and λ*fla*3 were selected from a population of hybrid λ constructed by the method of Thomas et al. (17). The central dispensable portion of the cloning vehicle was replaced with *Eco*RI digestion fragments from the F′ element MSF1338, which carried

most of the genes necessary for flagellar formation and function. Typically, λ*gt*λ*c* or MSF1338 DNA at 100 to 200 μg/ml in 100 mM tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (Sigma), pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, and 0.1 mM ethylenediaminetetraacetic acid was digested with *Eco*RI endonuclease for 1 h at 37°C. The digestion was terminated by heating at 65°C for 5 min. *Eco*RI-digested DNA was diluted 1 to 5 in 10 mM Tris-hydrochloride, pH 7.5, 100 mM NaCl and stored at 0°C. Before ligation, *Eco*RI-digested λ*gt*λ*c* DNA was mixed with at least a fivefold excess of donor (MSF1338) DNA and heated at 65°C for 5 min to melt out endonuclease cleavage sites and allow for formation of hybrid structures. Samples were cooled to 0°C. Ligation was carried out for 5 h at 10°C with DNA at a concentration of approximately 20 μg/ml in ligation buffer (7) with T4 ligase (Miles). The details of this procedure and the recovery of λ phage by transfection have been reported elsewhere (13).

Three hybrids were recovered: λ*fla*1, λ*fla*2, and λ*fla*3. Hybrid λ*fla*1 carried the *flaB*, *flaN*, and *hag* genes. Hybrid λ*fla*2 was found to complement *motA* and *motB* defects and to give recombinants with one $CheA^-$ tester strain, MS5034. Thus, λ*fla*2 carried the *motA* and *motB* genes and at least part of the *cheA* gene (14). Hybrid λ*fla*3 gave recombinants with one $CheA^-$ tester strain, MS5033, and complemented $CheB^-$ defects in two $CheB^-$ tester strains, MS5031 and MS5032. It also complemented defects in *flaG* and *flaH* tester strains. Thus, λ*fla*3 carried part of the *cheA* gene and all of the *cheB*, *flaG*, and *flaH* genes. λ*fla*2 appeared to carry the promotor-proximal portion of the *Eco*RI-fragmented *cheA* gene, and λ*fla*3 appeared to carry the promotor-distal fragment. To restore the integrity of the *cheA* gene, the *Eco*RI fragments from λ*fla*2 and λ*fla*3 were moved to λ*fla*1Δ4, which has one *Eco*RI site adjacent to the *hag* gene (Fig. 1).

Examination of the deletion mutants of a series of phages (with the relevant fragments of λ*fla*2 and λ*fla*3 transposed to λ*fla*1Δ4) showed that (Fig. 1) λ*fla*18 and λ*fla*42 had acquired the portion of the *cheA* gene in the proper orientation. With appropriate deletions, one fragment (from λ*fla*2) was fused to the left arm of λ and the other fragment (from λ*fla*3) was fused to the right arm of λ. By *Eco*RI restriction and ligation of a mixture of λ*fla*18Δ2 and λ*fla*42Δ13 DNA, a hybrid λ was selected that had acquired the left arm of λ*fla*18Δ2 and the right arm of λ*fla*42Δ13 (Fig. 1). This hybrid (λ*fla*52, λ*fla*55, λ*fla*57) now complemented all the strains carrying *cheA* defects (15). Deletions of λ*fla*52, λ*fla*55, and λ*fla*57 were selected by the method of Parkinson and Husky (8). Hybrid λ*fla*3 was unstable, presumably because the inserted piece of DNA was very large and made phage assembly difficult. Deletions were readily obtained and they were stable. Single plaques from λ*fla*3 were purified and tested, and many of the deletions were saved.

Genetic tests. The transductional crosses used to characterize hybrid λ carrying flagellar genes have been described previously (14). Briefly, the hybrid λ was cross-streaked on the surface of a motility agar plate on tester strains with defined flagellar mutations. The mutant testor strains were lysogenic for λ to prevent lysis by the transfecting hybrid λ. Recombinants were recognized as swarms emanating from the region of infection, and complementation was recognized by the presence of trails of immotile colonies in the motility agar. The trails resulted from abortive inheritance of a complementing gene on the transducing phage.

Protein synthesis and polyacrylamide gel electrophoresis. Hybrid *fla* were used to program protein synthesis in ultraviolet light (UV)-irradiated *E. coli* K-12 159 (λ) (obtained from H. Murialdo). The details of the infection and labeling of UV-irradiated cells with hybrid λ and sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been described (13). [^{35}S]methionine with a specific activity of 320 Ci/mmol (New England Nuclear Corp.) was used to label the protein synthesized in the UV-irradiated cells infected with hybrid λ.

RESULTS

The *cheA* region. A large number of deletions of λ*fla*52, λ*fla*55, and λ*fla*57 were isolated. These phages carried the intact "*cheA*" gene. They were tested against a variety of mutant tester strains, and the results are shown in Table 1. The mutant strains, initially all classified as belonging to the "*cheA*" group, appeared to break down into two distinct groups. Strains MS5034, MS5033, MS5103, and MS5109 all behave as if they were in a single functional unit, and deletions in the phage show good complementation or no complementation at all with these strains. On the other hand, MS5104 appears to represent another class of mutations. It shows complementation activity with λ*fla*57Δ21 and λ*fla*42, which do not complement the other strains. This tester strain, MS5104, defines a new complementation group *cheW*. *cheW* behaves as if it were cotranscribed with *motA*, *motB*, and *cheA*. Thus, for example, MS1513, which is a strong polar mutation caused by the insertion of the mu phage genome into the *mot* gene, is complemented by lambda phage that carry the whole operon *motA*, *motB*, *cheA*, and *cheW*, e.g., λ*fla*52 and λ*fla*52Δ1. There is no complementation when *cheW* is missing, e.g., in λ*fla*52Δ22. Furthermore, λ*fla*55Δ1, which has a deletion in the *motA* gene and therefore cannot transcribe *motA* or *cheA*, also does not have *cheW* complementation activity. Although all of these observations can be easily explained if *cheW* is part of the Mocha operon, other observations are not apparently consistent with this notion. For example, λ*fla*57Δ21 and λ*fla*42 appear to lack the promoter region of the Mocha operon and yet synthesize *cheW* activity; λ*fla*3Δ14 also lacks the Mocha promoter region and it does not synthesize *cheW* activity. The same pattern can be observed in the crosses

shown in Table 2. All of the λ*fla*42 and λ*fla*23 derivatives lack the promoter regions of the Mocha operon, but some are able to synthesize *cheW* activity. On the other hand, λ*fla*3 derivatives lacking the Mocha promoter cannot synthesize *cheW* activity. An explanation which is consistent with all of these observations is that the *mot* and *che* genes do in fact form a cotranscribed group; however, λ*fla*42 derivatives were prepared with the *hag* gene adjacent to the Mocha group. Thus, deletions of the Mocha promoter could fuse the *hag* promoter to the remaining genes, and *cheW* activity could result from reading initiated at *hag*. λ*fla*3 derivatives do not have a *hag* gene adjacent to *mot* and, therefore, promoter deletions result in the loss of *cheW* and *cheA* activity. This interpretation is consistent with all the data presented in Tables 1 and 2. We conclude that there is a group of genes that are cotranscribed with the following order: *motA*, *motB*, *cheA*, and *cheW*. Furthermore, the presence of the *hag* gene in

TABLE 1. *Transductional crosses with hybrid λfla*

Tester strains		λ*fla* and λ*fla* deletion mutants[a]												Complementation group
Mutant	Allele	2	52	52Δ1	52Δ22	57Δ27	57Δ5	52Δ11	52Δ2	55Δ1	57Δ21	42	3Δ14	
MS5020	*797*	+r	+r	+r	+r	+r	+r	+r	0	0	0	0	0	MotA
MS5039	*483*	+r	+r	+r	+r	+r	+r	0	0	0r	0	0	0	MotB
MS5034	*e14t1*	0r	+r	+r	+r	+r	0r	0	0	0r	0	0	0	CheA
MS5033	*e14q1*	0	+r	+r	+r	+r	0	0	0	0r	0r	0r	0r	CheA
MS5103[b]	*A104*	0	+	+	+	+	0	0	0	0	0	0	0	CheA
MS5109[b]	*A131*	0	+	+	+	+	0	0	0	0	0	0	0	CheA
MS5104[b]	*A110*	0	+	+	0	0	0	0	0	0	+	+	0	CheW
MS5123[b]	*B242*	0	+	+	+	0	+	+	+	+	+	+	+	CheX
MS5124[b]	*B251*	0	+	+	+	0	+	+	+	+	+	+	+	CheX
MS5032	*e13p1*	0	0	0	0	0	0	0	0	0	0	+r	+r	CheB
MS5114[b]	*B209*	0	0	0	0	0	0	0	0	0	0	+	+	CheB
MS1513[b]	*1513*	0	+	+	0	0	0	0	0	0	0	0	0	MotA, B CheA, CheW

[a] +, Complementation; 0, no complementation. Complementation measured as described in Materials and Methods. r, Recombination formed.

[b] These strains are RecA. The rest are Rec+.

TABLE 2. *Transductional crosses with hybrid λfla*

Tester strains		λ*fla* and λ*fla* deletion mutants[a]															Complementation group
Mutant	Allele	2	52	42	42Δ9	23Δ5	23Δ6	42Δ5	3Δ14	3Δ26	3Δ1	3Δ23	3Δ16	3Δ28	3Δ15	3Δ30	3Δ11	
MS5020	*797*	+r	+r	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MotA
MS5039	*483*	+r	+r	0	0	0	0	0	0	0	0	0	0	0	0	0	0	MotB
MS5034	*e14t1*	0r	+r	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CheA
MS5033	*e14q1*	0	+r	0r	0	0	0r	0r	0r	0r	0r	0r	0r	0r	0r	0r	0	CheA
MS5104[b]	*A110*	0	+	+	0	0	+	+	0	0	0	0	0	0	0	0	0	CheW
MS5123[b]	*B242*	0	+	+	+	0	0	0	+	+	+	+	+	+	+	+	0	CheX
MS5124[b]	*B251*	0	+	+	+	0	0	0	+	+	+	+	+	+	+	+	0	CheX
MS5032	*e13p1*	0	0	+r	+r	+r	0	0	+r	+r	+r	+r	+r	+r	0	0	0r	CheB
MS5114[b]	*B209*	0	0	+	+	+	0	0	+	+	+	+	+	+	0	0	0	CheB
MS5127[b]	*B274*	0	0	+	+	+	0	0	+	+	+	+	+	+	0	0	0	CheB
MS5031	*e14n1*	0	0	+r	+r	+r	+r	0	+	+	+	+	0	0	0	0	0r	CheY
MS5115[b]	*B221*	0	0	+	+	+	+	0	+	+	+	+	0	0	0	0	0	CheY
MS5121[b]	*B234*	0	0	+	+	+	+	0	+	+	+	+	0	0	0	0	0	CheY
MS5118[b]	*B227*	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	CheZ
MS5119[b]	*B229*	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	CheZ
MS5129[b]	*B292*	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	CheZ
MS5131[b]	*M1b1*	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	CheZ
MS5015	*161*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+r	FlaG
MS5025	*616*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+r	FlaH
MS5014	*726*	0	0	+r	0	0	+r	+r	0	0	0	0	0	0	0	0	0	Hag

[a] +, Complementation; 0, no complementation. Complementation measured as described in Materials and Methods. r, Recombination formed.

[b] These strains are RecA. The rest are Rec+.

the proper orientation can provide promoter activity for the transcription of genes in this group when the Mocha promoter is deleted.

The cheB region. Table 1 shows the mutant strains initially classified as belonging to the classical *cheB* gene. They also show differences in complementation behavior. MS5123 and MS5124 show patterns of complementation that are clearly different from those of MS5032 and MS5114. An extensive survey of λ*fla* deletions and mutant tester strains is shown in Table 2. Four distinct groups can be differentiated. We have called these *cheX*, *cheB*, *cheY*, and *cheZ*. *cheX* includes the tester strains MS5123 and MS5124; *cheB* includes MS5032, MS5114, and MS5127; *cheY* includes MS5031, MS5115, and MS5121; whereas *cheZ* includes the tester strains MS5118, MS5119, and MS5129. The phages λ*fla*3Δ14 and λ*fla*3Δ26 carry all four of the activities. These probably represent all of the genes in this region, since in tests which involve using Col E1-*fla* hybrid plasmids that carry the region (P. Matsumura, M. Silverman, and M. Simon, unpublished data) including the genes *flaG*, *flaH*, and the *cheA* and *mot* genes, no other complementation activities have been found. On the basis of these results, as well as previous deletion mapping experiments, the order of the genes in this region is *motA*, *motB*, *cheA*, *cheW*, *cheX*, *cheB*, *cheY*, and *cheZ*. It is interesting that some of the phages, e.g., λ*fla*3Δ11, appear to carry the *cheB* and *cheY* genes but show no expression in complementation tests. It is possible that there is a deletion in the *cheX* gene and that this group also forms a cotranscribed unit with a promoter at the *cheX* end and transcription proceeding through *cheX*, *cheB*, *cheY*, and finally *cheZ*. Three independent mu-induced mutations in this region were tested. All of them were complemented only by the phages λ*fla*3Δ14 and λ*fla*3Δ26, which carried all four genes (e.g., MS5131, Table 2). On the other hand, some of the phages, e.g., λ*fla*23Δ5, have *cheX* deleted and do express *cheB* and *cheY*. Again, one might argue for an explanation involving fusion to an adjacent promoter. In the λ*fla*23 and λ*fla*42 phages carrying the *hag* promoter in the proper orientation, it could be fused to the adjacent genes. The results of all of these complementation tests are shown and the steps involved in deriving the various deleted phages and newly constructed phages are schematically presented in Fig. 1.

Identification of gene products. The various lambda *fla* derivatives that were characterized by transductional crosses were tested to determine the polypeptides coded for by each of them. Figure 2 shows that the genetic activities can be accounted for by polypeptide bands on the acrylamide gels. Thus, *motA* gene activity corresponds to the capacity to direct the synthesis of a polypeptide that has an apparent molecular weight of 31,000. *motB* programs a 39,000-molecular-weight polypeptide, whereas *cheA* directs the synthesis of two polypeptides, one with an apparent molecular weight of 76,000 and another with an apparent molecular weight of 66,000. Finally, *cheW* is responsible for the synthesis of a polypeptide with an apparent molecular weight of 12,000. The 76,000- and 66,000-molecular-weight polypeptides that are derived from *cheA* are coded for by a single gene: one may be derived from the other by processing or they may be read from different starting points, in the same gene (15). A band with an apparent molecular weight of 28,000 appears to correspond to the *cheX* activity. It is present in λ*fla*42, λ*fla*57Δ21, λ*fla*52Δ2, λ*fla*52-Δ11, and λ*fla*57Δ5, but not in λ*fla*57Δ27. Furthermore, a band with a molecular weight of 12,000 corresponds to the presence of the *cheW* activity. It appears in λ*fla*42, λ*fla*57Δ21, Δ*fla*52, and λ*fla*52Δ1. Finally, λ*fla*42, which also carries *cheY* activity and *cheB* activity, shows two additional bands, one with a molecular weight of approximately 38,000 and another with a molecular weight of around 8,000. The assignment of the complementation activities to specific polypeptide bands is seen even more clearly in Fig. 3. λ*fla*3Δ30 carries the *cheX* gene activity and also has a 28,000-molecular-weight polypeptide. Again, the *cheB* activity corresponds to the 38,000-molecular-weight polypeptide found with λ*fla*3Δ28, λ*fla*3Δ23, and λ*fla*3Δ14. *cheY* corresponds to a polypeptide with an apparent molecular weight around 8,000, which is very intense and forms a band that is not as distinct as the other polypeptides. Finally, *cheZ* corresponds to the polypeptide with a molecular weight of around 24,000. λ*fla*3Δ23 also makes a polypeptide whose molecular weight is close to that of the *cheZ* gene product. However, using a gel that is capable of yielding better resolution (Fig. 3B, a 5 to 15% gradient of acrylamide and a 23-cm gel), it is quite clear that these are two different polypeptides. The *cheZ* gene product is unique and is found in λ*fla*3Δ14.

The cheM gene product. The genetic activity of the different viruses correlates with the appearance and disappearance of the various bands. However, there is a set of intense bands that does not apparently correspond to a specific gene activity. The bands are seen clearly, e.g., in the polypeptide pattern obtained from λ*fla*57Δ21 (Fig. 2) and with λ*fla*3Δ30, λ*fla*3Δ28, λ*fla*3Δ23, λ*fla*3Δ14, λ*fla*42Δ5, λ*fla*23Δ6, and

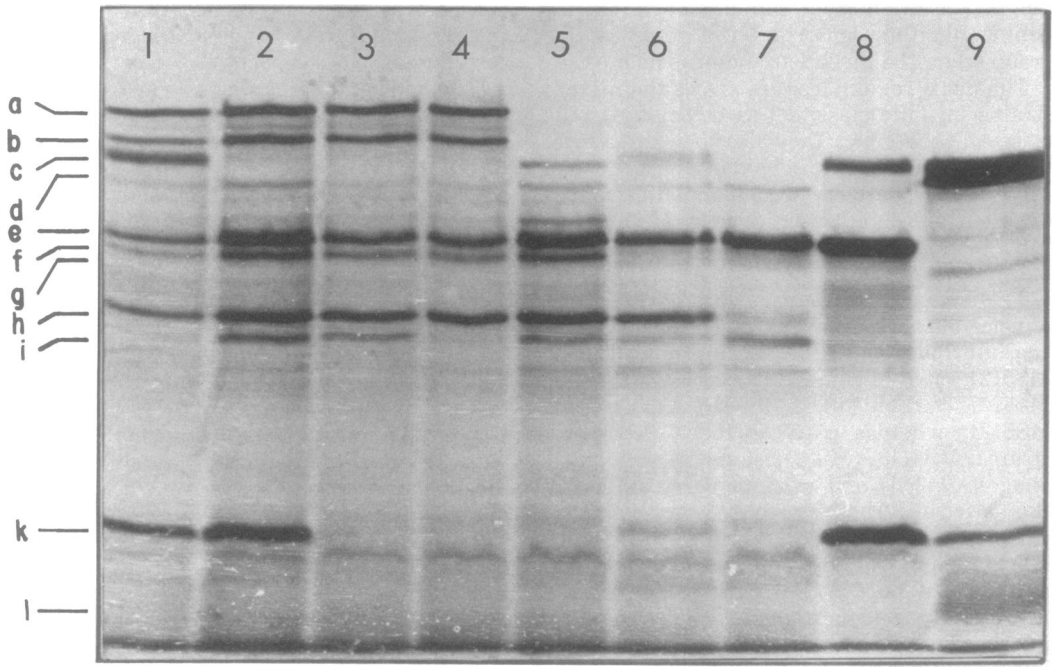

FIG. 2. *Specific protein synthesis directed by hybrid lambda carrying Mocha genes in UV-irradiated bacteria. In control experiments using an E. coli K-12 159 (λ) carrying the flaI mutation, the flagella-specific polypeptides were not observed. Polypeptides had molecular weights of (a) 76,000, (b) 66,000, (c) 61,000, (d) 54,000, (e) 41,000, (f) 39,000, (g) 38,000, (h) 31,000, (i) 28,000, (k) 12,000, and (l) 8,000. Gels: (1) λfla52 (motA motB cheA cheW cheX), (2) λfla52Δ1 (motA motB cheA cheW cheX), (3) λfla52Δ22 (motA motB cheA cheX), (4) λfla57Δ27 (motA motB cheA), (5) λfla57Δ5 (motA motB cheX), (6) λfla52Δ11 (motA cheX), (7) λfla52Δ2 (cheX), (8) λfla57Δ21 (cheW cheX), (9) λfla42 (hag cheW cheX cheB cheY).*

λ*fla*42 (Fig. 3). This banding pattern has been described before (16) and has been referred to as the triplet band pattern. It appears to correspond to the properties on acrylamide gels of the methyl-accepting chemotaxis protein (MCP) described by Kort et al. (6). Both MCP and triplet are localized in the cell membrane. The region of the *E. coli* genome that programs the synthesis of this group of polypeptides (*cheM*) has been mapped by examining the protein synthesis directed by deletion mutants of hybrid lambda phages. It is located between *cheW* and *cheX*. Thus, for example, λ*fla*52Δ1 lacks this band, whereas other λ*fla*52 derivatives have it. λ*fla*52Δ1 carries a small deletion that has been shown by heteroduplex mapping to occur in the region of DNA between the classical "*cheA*" and "*cheB*" genes (15). Furthermore, the positions of the deletions deduced from the complementation tests are consistent with the notion that the ability to direct "triplet" synthesis maps between *cheW* and *cheX* (Fig. 2 and 3). All of the "*cheA*" and "*cheB*" tester strains were examined by transductional crosses with lambda phages that carried the *cheM* region and phages that did not carry the region. The *cheM* region showed no specific complementation effect with any of these strains.

Figure 4 summarizes the information that we have on the genetic basis of flagellar structure and function. A contiguous region of the genome carries the *motA*, *motB*, *cheA*, and *cheW* genes. The *cheW* gene is followed by *cheM*, which is not included in the Mocha operon. *cheM* is next to another group of genes, *cheX*, *cheB*, *cheY*, and *cheZ*. These are followed by the *flaG* and the *flaH* genes.

DISCUSSION

The data indicate that there are at least six genes that make up the regions previous termed "*cheA*" and "*cheB*" in *E. coli*. The genetic arrangement may be similar to the arrangement in the homologous region in *Salmonella* (18).

In *E. coli* the classical *cheA* gene can be resolved into *cheA* and *cheW*. The *cheB* gene is resolved into *cheX*, *cheB*, *cheY*, and *cheZ*. *cheA* and *cheW* are cotranscribed with *mot*, and the

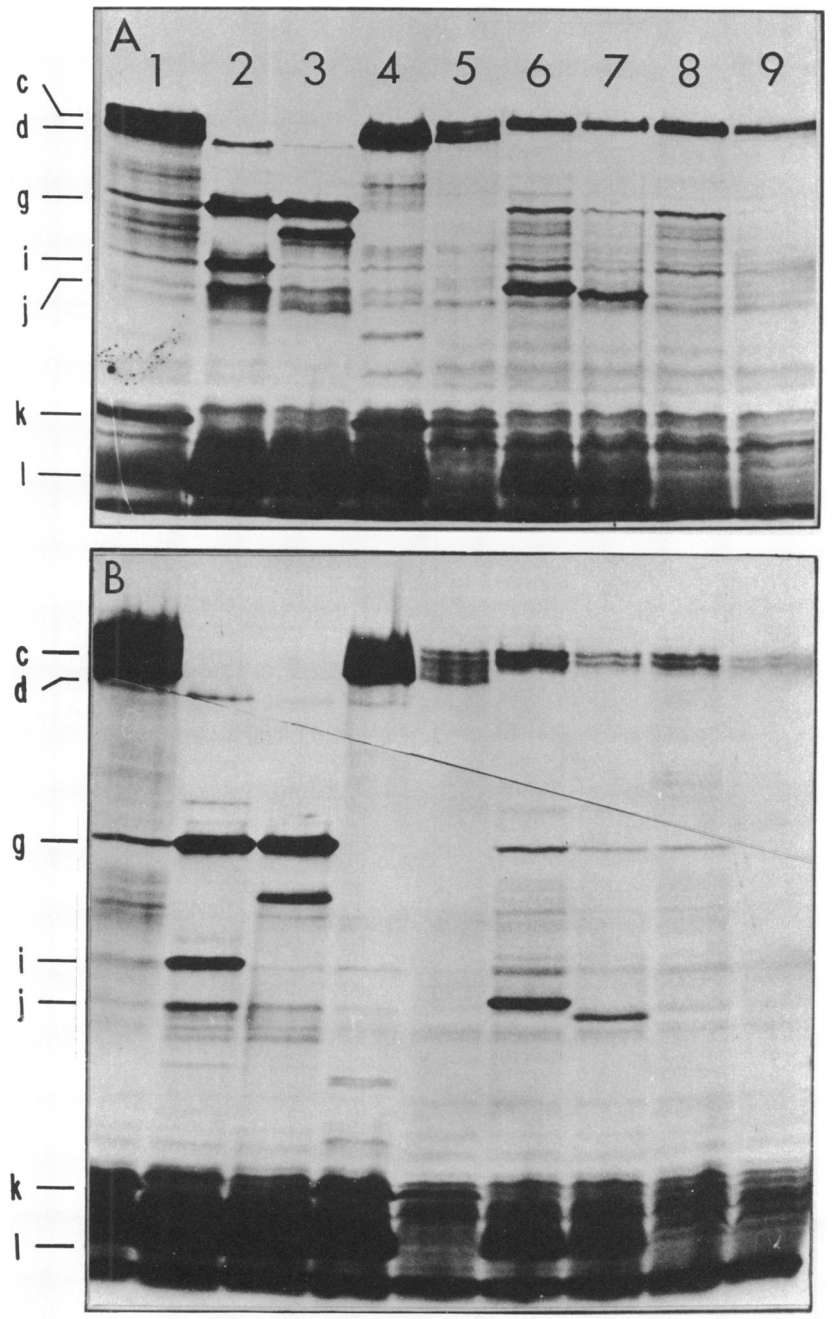

FIG. 3. *Protein synthesis directed by hybrid lambda carrying chemotaxis genes. Gels: (1) λfla42 (hag cheW cheX cheB cheY), (2) λfla42Δ9 (cheX cheB cheY), (3) λfla23Δ5 (cheB cheY), (4) λfla23Δ6 (hag cheW cheY), (5) λfla42Δ5 (hag cheW), (6) λfla3Δ14 (cheX cheB cheY cheZ), (7) λfla3Δ23 (cheX cheB cheY), (8) λfla3Δ28 (cheX cheB), (9) λfla3Δ30 (cheX). (A) Results of a 12% acrylamide-sodium dodecyl sulfate gel. The letters on the left side refer to the same molecular weights as in Fig. 2 with the addition of (j) 24,000. (B) The same samples as in (A), except that they were run on a 5 to 15% gradient gel.*

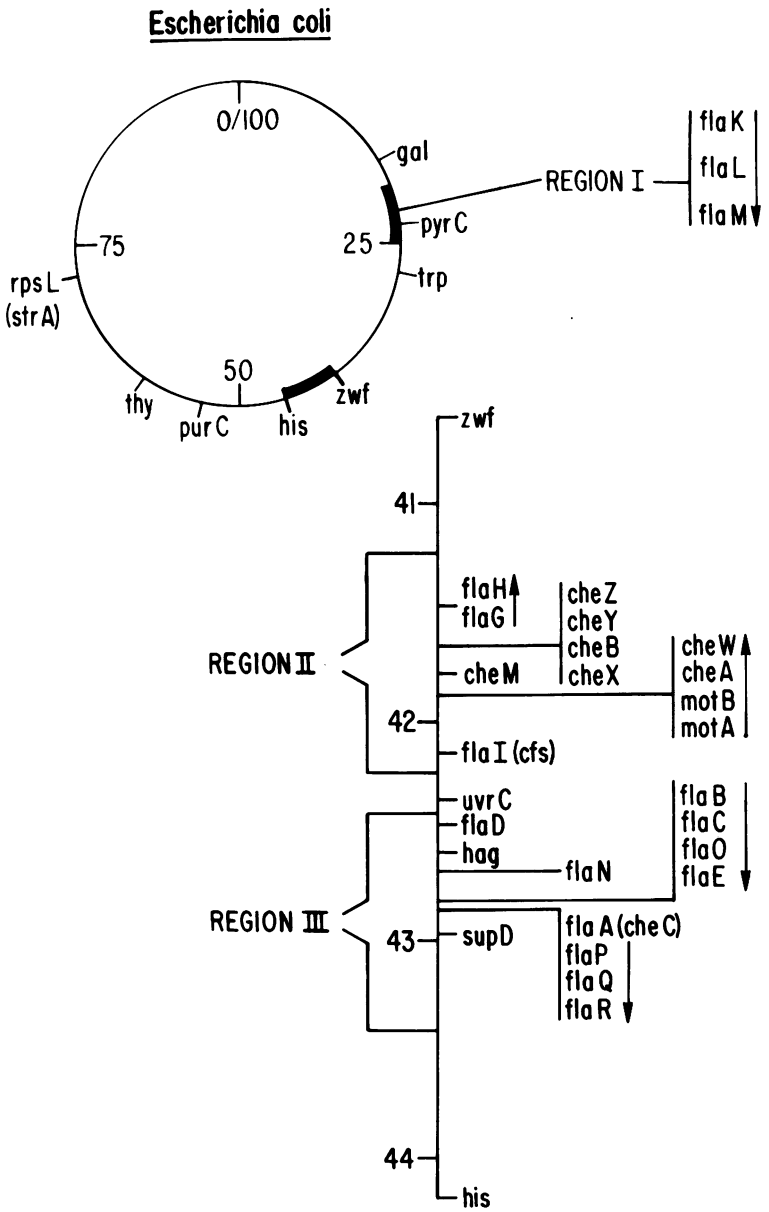

FIG. 4. *Summary of the disposition of the genes controlling flagellar structure and function on the E. coli genome.*

cheA gene directs the synthesis of two products of molecular weight 76,000 and 66,000, whereas *cheW* directs the synthesis of a 12,000-molecular-weight polypeptide. It is interesting that the *cheA*, *cheW*, and *mot* gene products are in the same cotranscribed unit. There may be some direct interaction among these products, and cotranscription would maintain the relative amounts of the proteins at the same level. The products of the *cheB* region, i.e., the polypeptides corresponding to *cheX*, *cheB*, *cheY*, and *cheZ*, may also be part of the same cotranscribed unit. However, the evidence thus far does not exclude the possibility of separate transcription of each of these genes.

All of the genes that we have cloned onto lambda and tested for protein synthesis require the presence of an intact *flaI* gene, either *cis* or

trans, (12) for expression. Thus, the initiation of synthesis of these polypeptides probably involves a specific cytoplasmic signal and an appropriate operator region at the beginning of each of the cotranscribed units that binds the regulatory protein. Each gene grouping may require the presence of the *flaI* gene product, or this product may stimulate the synthesis of other regulatory polypeptides and groups of genes could be turned on sequentially. In any event, different groups of genes synthesize different levels of protein in the λ-infected system. The *mot* and *che* genes make levels of protein that are easily detectable, whereas *flaI* and some of the other *fla* genes do not make detectable products. The *fla* genes can be shown to be functional when they are introduced into UV-irradiated cells (14), since they lead to flagellar synthesis. We have not yet found polypeptides that correspond to *flaI*, *flaG*, or *flaH*. It may be possible to increase the levels of these gene products by fusing them to the appropriate outside promoters.

On the basis of the behavior of the deleted λ phages in directing protein synthesis, we have been able to assign the region of the DNA between *cheW* and *cheX* to *cheM*. However, thus far, no mutants have been found to map in *cheM*. The "triplet" protein, which is specified by this region, is a major component of the inner membrane of the cell, and it appears to be the same as the MCP described by Kort et al. (16). The deleted lambdas may be used to prepare strains that carry deletions of *cheM*. The characteristics of such strains may reveal the role that the *cheM* gene plays in the mechanism of chemotaxis.

The λ hybrid phages have allowed us to define the products of the genes that control flagellar structure and function. Some of these products, e.g., *motA*, *motB*, and *cheM* (14, 16) are integral membrane proteins. Others appear to be in the cytoplasm but may be transiently associated with the plasma membrane (H. Ridgway, M. Silverman, and M. Simon, unpublished observations). The assembly and function of the flagellar organelle involves components associated with all of the compartments of the cell. The further description of the mechanisms involved in the interaction of these polypeptides and their products will give us insight into how they function to make the cells motile and chemotactic.

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