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Localization of Proteins Controlling Motility and Chemotaxis in *Escherichia coli*

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Flagellar proteins controlling motility and chemotaxis in *Escherichia coli* were selectively labeled in vivo with [^{35}S]methionine. This distribution of these proteins in subcellular fractions was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. The *motA*, *motB*, *cheM*, and *cheD* gene products were found to be confined exclusively to the inner cytoplasmic membrane fraction, whereas the *cheY*, *cheW*, and *cheA* (66,000 daltons) polypeptides appeared only in the soluble cytoplasmic fraction. The *cheB*, *cheX*, *cheZ*, and *cheA* (76,000 daltons) proteins, however, were distributed in both the cytoplasm and the inner membrane fractions. The *hag* gene product (flagellin) was the only flagellar protein examined that copurified with the outer lipopolysaccharide membrane. Differences in the intracellular locations of the *che* and *mot* gene products presumably reflect the functional attributes of these components.

More than 30 genes are involved in flagellar formation and function in *Escherichia coli* (1, 7, 9, 12). Some of these genes (perhaps 11 or 12) code for polypeptides that constitute the major structural elements of the flagellar filament, hook, and basal body assembly (1). The remaining genes, however, appear to be involved in controlling flagellar biogenesis and function. Mutations that map in the *mot* genes, for example, render cells phenotypically paralyzed, though they possess an apparently anatomically complete flagellar apparatus (1). Thus, it is suspected that the *mot* gene products may somehow mediate the transfer or coupling of metabolic energy to the flagellar rotor (3, 14). Similarly, the *che* gene products have been shown to modify flagellar rotation (and therefore bacterial behavior) in response to chemotactic signals received by the chemoreceptors at the cell membrane (4). To understand motility and chemotaxis at a molecular level, it is necessary to know not only what protein components participate in these processes, but also their subcellular locations and mutual spatial dispositions in the cell. Historically, the problem of identifying the components of the flagellar system of *E. coli* was hindered because many of the genes associated with motility and chemotaxis are apparently transcribed at very low levels in vivo. Moreover, there was no specific enzymatic or other biochemical assay for these gene products. These obstacles have recently been overcome, in part, with the advent of molecular genetic cloning techniques. Silverman et al. (9) constructed hybrid lambda-*E. coli* phage carrying various combinations of flagellar genes. They used these phage to study the expression of flagellar gene activity by measuring incorporation of radioactive amino acids into proteins synthesized in ultraviolet-irradiated bacteria lysogenic for lambda. Using this technique to selectively label specific flagellar proteins in vivo, in conjunction with methods for cell fractionation, we examined the subcellular distribution of proteins controlling motility and chemotaxis in *E. coli*. The possible functional significance of our findings is discussed.

MATERIALS AND METHODS

Bacterial strain. The organism used in this study, *E. coli* 159, was made lysogenic for phage lambda (9). The cells were maintained in a minimal salts medium.

Construction and properties of hybrid lambda-*E. coli* phage. The construction of hybrid lambda-*E. coli* phage carrying various combinations of flagellar genes has been published elsewhere (8-10, 12). The hybrid phage used in this investigation were λ*fla*52, λ*fla*3Δ14 (a deletion mutant of λ*fla*3 lacking *flaG* and *flaH*), λ*fla*23, and λ*fla*91. The specific flagellar genes carried by these phage are summarized in Table 1.

Infection, labeling, and cell fractionation. Cells were grown at 37°C in one liter of RM medium (9) to about 100 Klett units (red filter) and harvested by centrifugation at 5,000 × *g* for 10 min at 20°C. The inside walls of the centrifuge bottle were wiped dry of medium before suspending the sedimented cells to 400 Klett units in a phosphate-free buffer

TABLE 1. *Composition of hybrid lambdas carrying genes for motility and chemotaxis*

Hybrid λ	Presence of flagella gene[a]:												
	motA	*motB*	*cheA*	*cheW*	*cheM*	*cheX*	*cheB*	*cheY*	*cheZ*	*flaG*	*flaH*	*hag*	*cheD*
λ*fla*52 (10)[b]	+	+	+	+	+	+							
λ*fla*23 (7, 11)				+	+	+	+	+		+	+	+	
λ*fla*3Δ14 (11)				+[c]	+	+	+	+	+	+	+		
λ*fla*91[d]													+

[a] The flagellar genes *motA* through *flaH* are one contiguous set of genes and are shown in the order of their location at about 41.5 min on the *E. coli* genome. On the *E. coli* genome, the *hag* gene is located at 42.5 min. The *cheD* gene is located at about 99 min.

[b] Number in parentheses represents reference number.

[c] Hybrid λ*fla*3Δ14 carries the *cheW* gene, but it is not expressed becaused an active promoter is not present.

[d] Silverman and Simon, in press.

system containing (per liter of distilled water): 1.0 g of NH_4Cl; 2.0 g of KCl; 2.0 g of NaCl; 1.5 g of glycerol; 10^{-5} g of $FeCl_2$; 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.0. Phosphate was avoided to prevent formation of Mg^{2+} precipitates that interfered with spheroplast formation in a subsequent step. A portion of this suspension (18 ml) was removed for ultraviolet irradiation and labeling, while the remainder (ca. 282 ml) was incubated at 34°C for use at a later time. Ultraviolet irradiation, infection with hybrid lambda phage, and radiaoctive labeling of flagellar proteins with [^{35}S]methionine were carried out by the method of Silverman et al. (9). The labeled cells were chilled to 0 to 2°C and washed once with 250 ml of ice-cold 30 mM tris(hydroxymethyl)aminomethane-Cl buffer (pH 7.8) containing 0.25 mg of unlabeled methionine per ml. The unlabeled cells were treated likewise and suspended with the labeled cells in plasmolysis buffer (0.75 M sucrose and 10 mM tris(hydroxymethyl)aminomethane-Cl, pH 7.8) to an optical density (at 600 nm) of 10.0. Spheroplasts were then prepared on ice by the method of Osborn et al. (6), except that 17 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.8) was used as the diluent. The spheroplasts were sedimented by centrifugation at 20,000 × *g* for 20 min and lysed osmotically by suspension in 100 ml of 1.0 mM EDTA solution (pH 7.8). This suspension was incubated overnight at 2°C and then passed through a 20-gauge needle several times to insure maximal lysis. Unlysed bacteria were removed by centrifugation at 1,500 × *g* for 15 min. The membranes were sedimented by centrifugation at 80,000 × *g* for 90 min, suspended in 20 ml of "membrane suspension buffer" [MSB: 0.25 M sucrose, 1.0 mM EDTA, 10 mM tris(hydroxymethyl)aminomethane-Cl, pH 7.8], and dialyzed 20 h against 2 liters of 3 mM EDTA (pH 7.8). The membranes were sedimented as before 7.8). The membranes were sedimented as before and suspended in 8 ml of MSB. Inner and outer membranes were separated by isopycnic sucrose density gradient centrifugation by a modification of the method of Osborn et al. (6). The subcellular fractions collected for analyses are indicated in Fig. 1. Analytical methods, such as sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and autoradiography, were performed as previously reported (9).

RESULTS

Labeling and fractionation of cells. The protocol for labeling and fractionation of cells is outlined in Fig. 1. Briefly, ultraviolet-irradiated bacteria were infected with the appropriate hybrid lambda-*E. coli* phage (carrying only the flagellar genes in which we were interested) and their flagellar proteins selectively labeled with [^{35}S]methionine (lambda genes were not transcribed because of the presence of the C-1 repressor). Labeled bacteria were plasmolyzed in the cold in 0.75 M sucrose, and osmotically sensitive spheroplasts were prepared by lysozyme-EDTA treatment. The spheroplasts were sedimented by centrifugation at 20,000 × *g* for 20 min, and the supernatant fluid, which was enriched for periplasmic proteins, was retained and designated fraction IV. The spheroplasts in the pellet were lysed osmotically by suspension in 1.0 mM EDTA solution and fractionated by a modification of the method of Osborn et al. (6) into soluble cytoplasmic proteins (fraction V) and cell envelope vesicles [fraction VII(a)]. The latter was further separated into cytoplasmic membrane [fraction VII(b)], intermediate fraction [fraction VII(c)], and outer lipopolysaccharide membrane [fraction VII(d)] by isopycnic sucrose density gradient centrifugation (Fig. 1). Separation of the inner from the outer membrane was monitored by assaying for cytochrome content or specific enzyme markers (e.g., succinic dehydrogenase), which are known to reside exclusively in the inner membrane fraction of *E. coli* (cf. 6), and by SDS-PAGE of the membrane proteins. Based on these assays, the outer membrane fraction was seldom contaminated by more than about 5% by inner membrane material. The intermediate fraction exhibited properties of both types of membrane and was therefore presumed to be a mixture of the two.

Periplasmic proteins were released preferentially into the supernatant fluid (fraction IV)

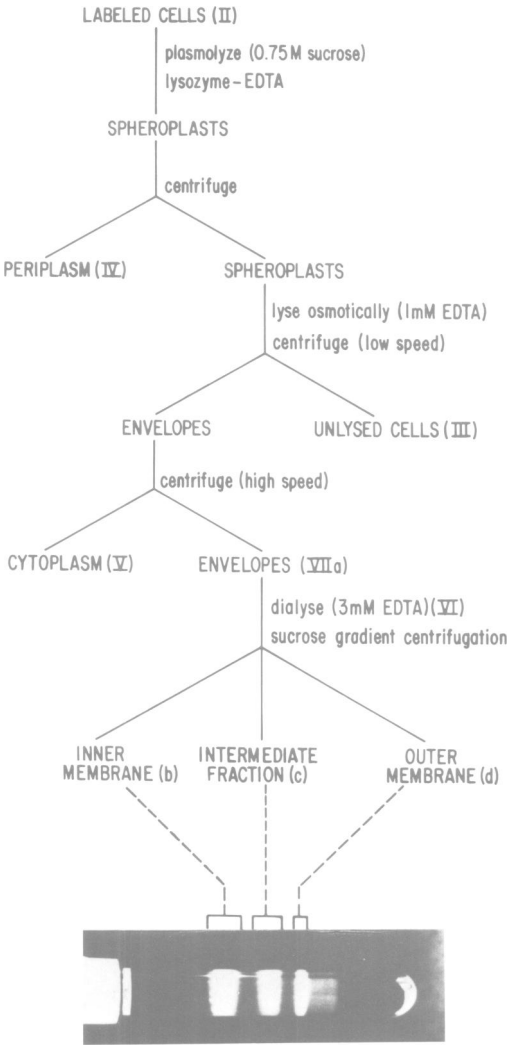

FIG. 1. *Outline of subcellular fractionation procedure for E. coli 159 λ after infection with hybrid lambda phage and labeling of flagellar proteins with [^{35}S]methionine. Fractions retained for analysis are indicated by the symbols in parentheses.*

during sucrose plasmolysis of labeled cells and subsequent formation of spheroplasts by lysozyme-EDTA treatment. Theoretically, this fraction should contain only proteins located external to the cytoplasmic membrane, assuming the absence of cell lysis. In this respect, our periplasmic fraction was analogous to that obtained by the $MgCl_2$ shock method of Nossal and Heppel (5); in fact, gel patterns obtained by either method were similar. However, since partial cell lysis was unavoidable during spheroplast formation, our periplasmic fraction invariably contained some cytoplasmic proteins (as determined by SDS-PAGE). However, it was difficult to accurately assess the extent of this contamination.

Distribution of [^{35}S]methionine in subcellular fractions. Tables 2 and 3 show quantitatively the distribution of radioactive methionine among the subcellular fractions recovered after infection with λ*fla*52 and λ*fla*3Δ14, respectively. In each case, the infected cells accumulated about 8% of the total added radioactive label (usually about 1.0 mCi). About 24% of the label that was taken up by cells infected with λ*fla*52 was incorporated into the cell envelope fraction [fraction VII(a)]. This represents about 2% of the [^{35}S]methionine initially added to the cells. The bulk of the remaining label was distributed roughly equally between the periplasmic (21%) and cytoplasmic (37%) fractions and was apparently present in a soluble form, since ultracentrifugation at 100,000 × *g* for 2 h failed to sediment the radioactivity. A small proportion of the accumulated label (about 6%) could be accounted for in cells that did not lyse during the osmotic shock ("unlysed cells," fraction III) and in the membrane dialysis supernatant fluid (about 0.54%, fraction VI). About 90% of the label accumulated by cells was recovered in subcellular fractions III to VII(a). A qualitatively similar distribution of radioactivity was observed for cells infected with λ*fla*3Δ14.

The amount of radioactive methionine incorporated into the cytoplasmic membrane and outer membrane fraction was not measured exactly, since the percent recovery of these membranes from sucrose gradients was not measured. However, comparison of the specific activities (expressed as counts per minute per milligram of protein) of the labeled flagellar proteins in these membranes clearly revealed that the inner membrane incorporated 10 to 20 times more [^{35}S]methionine than the outer membrane. This suggested that most, if not all, of the envelope-bound flagellar proteins were associated with the inner membrane.

Distribution of radioactive polypeptides in subcellular fractions. The radioactive polypeptides in each subcellular fraction were separated by SDS-PAGE and identified by autoradiography. Figures 2 and 3 show the stained gels and accompanying autoradiograms of fractions recovered from cells infected with λ*fla*52 and λ*fla*3Δ14, respectively. All of the flagellar proteins coded for by λ*fla*52 were readily identified in the well containing intact plasmolyzed cells (well no. 1, Fig. 2). Three of these proteins were confined exclusively to the inner membrane fraction. These were the 61,000-dalton

TABLE 2. *Distribution of [^{35}S]methionine label in cell fractions from E. coli 159λ infected with λfla52*

Fraction	Vol (ml)	Protein (mg)	Total cpm	Sp act[a]	Overall distribution of label (%)	% Accumulated label in each fraction
I. Medium	210		8.8×10^8		92.0	
II. Plasmolyzed cells	50	225.0	7.6×10^7	3.4×10^5	8.0	100.0
III. Unlysed cells	5	12.3	4.8×10^6	3.9×10^5	0.5	6.3
IV. Periplasmic fraction	150	45.0	1.6×10^7	3.6×10^5	1.7	21.3
V. Cytoplasmic	100	120.0	2.9×10^7	2.4×10^5	3.0	37.5
VI Dialysis supernatant fluid	25	12.0	3.6×10^5	3.0×10^4	0.04	0.5
VII.						
(a) Envelope (total membranes)	8.5	57.0	1.8×10^7	3.2×10^5	1.9	23.8
(b) Cytoplasmic membrane	2	6.6	7.1×10^6	1.1×10^6		
(c) Intermediate	2	9.4	1.5×10^6	1.6×10^5		
(d) Outer membrane	2	9.6	5.0×10^5	5.2×10^4		
						89[b]

[a] Counts per minute per milligram of protein.
[b] Percent of accumulated label recovered from subcellular fractions III to VII(a).

TABLE 3. *Distribution of [^{35}S]methionine label in cell fractions from E. coli 159λ infected with λfla3Δ14*

Fraction	Vol (ml)	Protein (mg)	Total cpm	Sp act[a]	Overall distribution of label (%)	% Accumulated label in each fraction
I. Medium	100		8.9×10^8		92.0	
II. Plasmolyzed cells	30	102.0	8.0×10^7	7.8×10^5	8.0	100.0
III. Unlysed cells	5	3.0	1.6×10^6	5.3×10^5	0.16	2.0
IV. Periplasmic	90	27.0	3.0×10^7	1.1×10^6	3.1	39.0
V. Cytoplasmic	100	55.0	3.4×10^7	6.2×10^5	3.5	44.0
VI. Dialysis supernatant fluid	30	3.8	9.9×10^5	2.6×10^5	0.1	1.3
VII.						
(a) Envelope (total membranes)	7.5	21.4	1.1×10^7	5.1×10^5	1.13	14.0
(b) Cytoplasmic membrane	2	3.1	3.6×10^6	1.2×10^6		
(c) Intermediate	2	4.6	2.4×10^6	5.3×10^5		
(d) Outer membrane	2	4.9	5.5×10^5	1.1×10^5		
						100[b]

[a] Counts per minute per milligram of protein.
[b] Percent of accumulated label recovered from subcellular fractions III to VII(a).

cheM, the 39,000-dalton *motB*, and the 31,000-dalton *motA* proteins. Although certain chaotropic agents, such as guanidine hydrochloride or urea and nonionic detergents (Triton X-100), tended to solubilize the *mot* and *cheM* proteins from inner membrane vesicles, a variety of mild washing procedures (e.g., with EDTA or salt solutions) failed to do so, suggesting that these polypeptides are integral components of the cytoplasmic membrane. On the other hand, the 66,000-dalton *cheA* and the 12,000-dalton *cheW* proteins were apparently localized totally within the soluble cytoplasmic fraction. Some of the 76,000-dalton *cheA* gene product was associated with the inner membrane fraction, but the majority of this protein appeared to be present in the cytoplasm as judged by a comparison of the relative intensities of the bands that appeared in the autoradiograms. No labeled flagella-related components were detected in the periplasmic or outer membrane fractions, despite the presence of a variety of unlabeled proteins in these fractions.

When cells were infected with λ*fla*3Δ14, the 61,000-dalton *cheM* gene product was again found to be associated exclusively with the inner membrane fraction (Fig. 3). The 38,000-dalton *cheB*, the 28,000-dalton *cheX*, and the 24,000-dalton *cheZ* proteins behaved similarly in that they were distributed between both the inner membrane and soluble cytoplasmic fractions. The 8,000-dalton *cheY* protein, however, appeared to be localized entirely within the cytoplasm. Although some radiaoctive bands

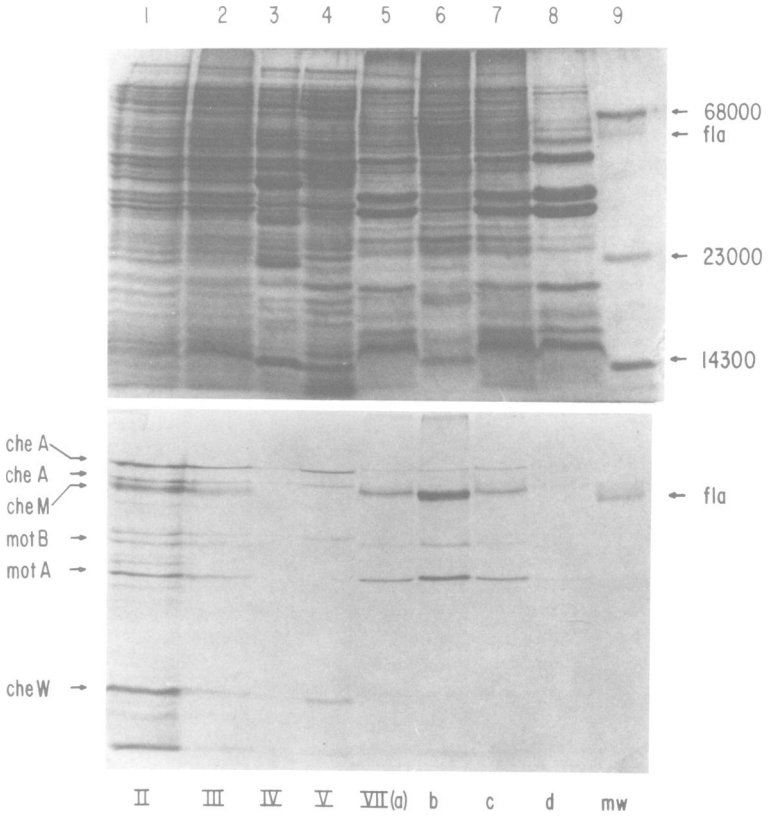

FIG. 2. *SDS-PAGE of subcellular fractions recovered from cells infected with λfla52. Upper panel is the Coomassie brilliant blue-stained gel. Lower panel is the corresponding autoradiogram. All of the flagellar gene products coded for by λfla52 are indicated in the autoradiogram. The different subcellular fractions (Table 1 and 2; Fig. 1) are indicated by symbols at the bottom of the autoradiogram. Wells 1 through 8 each contained 100 μg of protein. Well 9 contained molecular weight standards: bovine serum albumin, 68,000 daltons; ^{35}S-labeled E. coli flagellin monomer, 52,000 daltons; trypsin, 23,000 daltons; and lysozyme, 14,300 daltons.*

were visible in the outer membrane fraction, they were probably due to a slight contamination of the latter with inner membrane vesicles and showed up as a result of overexposure of the autoradiogram.

A significant proportion of the *cheB* gene product consistently appeared in the periplasmic fraction (fraction IV). However, when infected cells were extracted specifically for periplasmic proteins by the $MgCl_2$ shock method of Nossal and Heppel (5), the *cheB* protein was not detected. Therefore, it is suspected that the presence of this band in our periplasmic fraction may have been the result of contamination by cytoplasmic constitutents.

To further confirm the locations of the *cheM, cheB, cheX, cheW,* and *cheY* proteins, cells were infected with λ*fla*23 and fractionated. In addition to the above genes, λ*fla*23 carries the *hag* gene that directs the synthesis of flagellin, the major structural protein of the flagellar filament. The *cheM, cheB, cheX, cheY,* and *cheW* polypeptides were distributed as previously observed in cells that had been infected with λ*fla*52 and λ*fla*3Δ14 (Fig. 4). Interestingly, the *hag* gene product, unlike any of the other flagellar proteins examined, copurified with the outer membrane fraction. In addition, a substantial amount of flagellin was released into the periplasmic and cytoplasmic fractions. This probably results from flagellar filament shearing during lysis and fractionation.

Kort et al. (2) have described a protein in the inner membrane of *E. coli* that is methylated in response to the chemoeffectors. It is called the methyl-accepting chemotaxis protein and is composed of two gene products, those of *cheM* (61,000 daltons) and *cheD* (64,000 daltons) (M. Silverman and M. Simon, Proc. Natl. Acad. Sci. U.S.A., in press). Cell fractionation

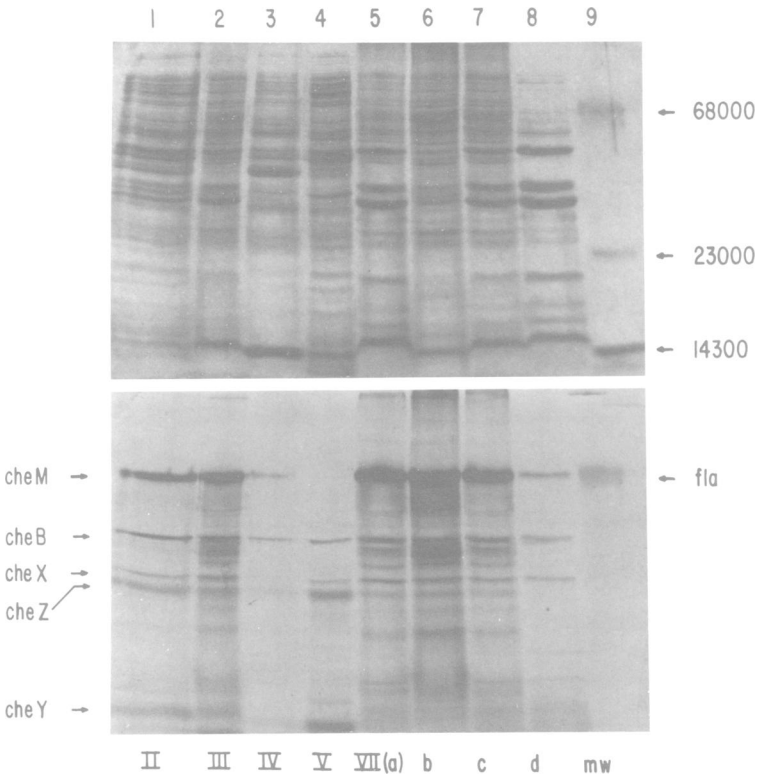

FIG. 3. *SDS-PAGE of subcellular fractions recovered from cells infected with λfla3Δ14. See legend to Fig. 1 for details.*

procedures identical to those described for the localization of the [^{35}S]methionine-labeled *mot* and *che* gene products were used to localize the [^{3}H]methyl-labeled methyl-accepting chemotaxis polypeptides. In agreement with the results of Kort et al. (2), these [^{3}H]methyl-labeled polypeptides were found only in the inner membrane fraction (data not shown). Furthermore, [^{35}S]methionine-labeled *cheD* gene products were localized exclusively in the inner membrane fraction by using a *cheD* hybrid λ, λ*fla*91 (Silverman and Simon, in press). Thus, it is apparent that both the *cheM* and *cheD* gene products are located in the inner membrane.

DISCUSSION

One assumption implicit in our localization studies is that polypeptides whose synthesis is directed in ultraviolet-irradiated bacteria by flagellar genes introduced on hybrid lambda-*E. coli* deoxyribonucleic acid behave normally with respect to intracellular localization and function. Silverman et al. (10, 11) infected mutant strains of *E. coli* 159 λ with viruses carrying *mot* and *che* gene activities and subsequently examined them microscopically for restoration of motility or normal tumbling behavior. The kinetics of onset of motility in Mot$^-$ mutants was found to be the same in irradiated or unirradiated bacteria. Likewise, CheA$^-$ mutants regained the ability to tumble within 25 min after infection with λ*fla*52. These observations suggest that the *mot* and *che* gene products are indeed functionally active and properly distributed spatially in the cell.

Another assumption is that our fractionation procedures do not introduce artifacts in localization. Some of the *che* proteins were found to be distributed in both the inner membrane and soluble cytoplasmic fractions. It is possible that these polypeptides may be only transiently associated with the inner membrane in vivo. This could, for example, be the case for the methyltransferasse recently characterized in *Salmonella typhimurium* (13). The protein identified as the *cheR* gene product is apparently soluble and presumably localized in the cytoplasm, yet is believed to be involved in the methylation of a membrane-bound component of the chemotaxis system in that organism. In *E. coli*, the *cheX* gene product (28,000 daltons) has been shown to be required for methylation

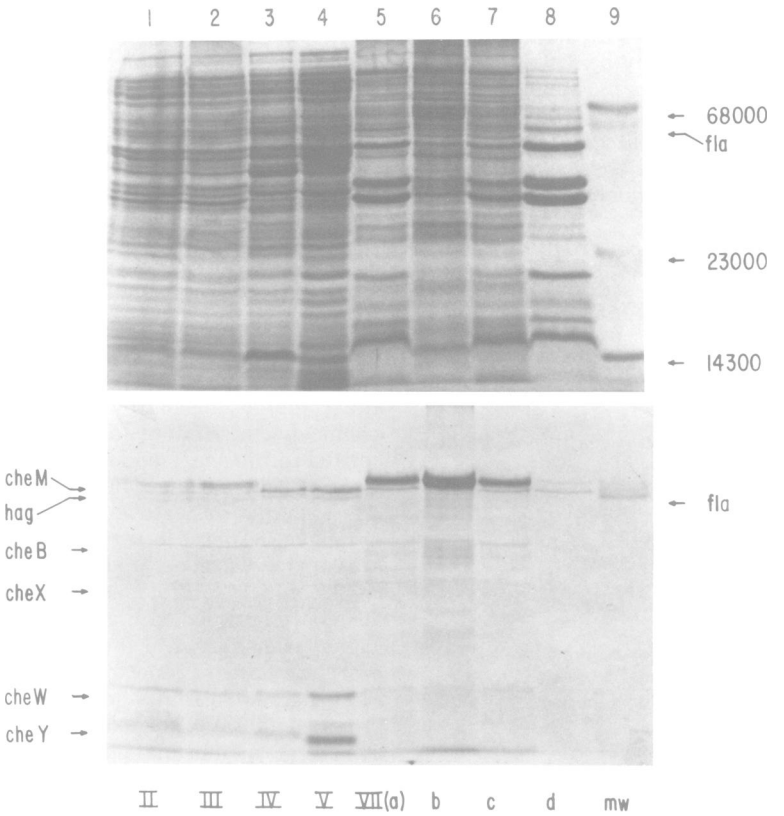

FIG. 4. *SDS-PAGE of subcellular fractions recovered from cells infected with λfla23. See legend to Fig. 1 for details.*

of the *cheM* and *cheD* proteins (Silverman and Simon, in press; M. S. Springer, M. F. Goy, R. W. Reader, and J. Adler, Proc. Natl. Acad. Sci. U.S.A., in press). The *cheM* and *cheD* genes code for membrane-bound polypeptides that undergo attractant-stimulated methylation. The biphasic distribution of *cheX* in the membrane and cytoplasmic fractions may indicate a labile or ephemeral association of this protein with components present in the cytoplasmic membrane that are involved in the methylation reaction. Alternatively, our experimental conditions may selectively release (solubilize) certain *che* proteins from the cytoplasmic membrane that would normally remain attached in the intact cell. Although there is no simple, direct way available to analyze this problem, it may be possible to modify the ionic conditions during cell lysis or to employ specific, divalent cross-linking reagents to "trap" *che* proteins on the plasma membrane.

We conclude that, with respect to localization, there are three classes of polypeptides. *cheM*, *cheD*, *motA*, and *motB* are integral membrane components. *cheW*, *cheY*, and *cheA* (66,000 daltons) are found exclusively in the cytoplasm. *cheB*, *cheX*, *cheZ*, and *cheA* (76,000 daltons) are distributed to different extents between the membrane and cytoplasmic fractions. The relative distributions of these proteins probably reflects their role in chemotaxis and motility.

Table 4 summarizes the subcellular locations of the proteins controlling motility and chemotaxis in *E. coli*. Although it is clear that the *motA*, *motB*, *cheD*, and *cheM* gene products are integral components of the cytoplasmic membrane, it is not known whether they are distributed uniformly over the cell membrane or are localized near the flagellar basal body assembly. Based on the relative intensity of Coomassie brilliant blue staining in polyacrylamide gels, the *motA* band (31,000 daltons) represents about 0.5% of the total inner membrane protein of *E. coli* (unpublished data). This translates into about 1,000 copies of the *motA* gene product per cell based upon measurements of the total membrane protein. If these proteins are clustered around the base of the flagellar structure, then each basal struc-

ture would have about 100 to 200 copies of the protein associated with it. On the other hand, the *mot* gene products may be distributed uniformly around the cytoplasmic membrane. The distribution of the *mot* gene products in the inner membrane is reasonable in view of recent results that suggest that these proteins may function as membrane ionophores that control flagellar rotation (14).

The *cheM* and *cheD* gene products in inner membrane preparations could be identified on Coomassie brilliant blue-stained polyacrylamide gels, and the relative intensity of staining indicated that there were at least as many molecules of these polypeptides as the *motA* gene product in the inner membrane. The *cheM* and *cheD* gene products act as transmembrane signaling elements. They collect information from chemoreceptors and transmit signals to other components of the chemotactic apparatus (Silverman and Simon, in press). The *mot* gene products function to energize flagellar rotation (3, 10). Most *che* gene products appear to be involved in regulating the direction of flagellar rotation, which, in turn, determines the cell swimming behavior (4). The localization of the *mot* and *che* gene products suggests the route of transmission of chemotactic signals in the cell. Chemoeffector molecules interact with receptor proteins in the periplasmic space or inner membrane of the cell. The binding of chemoeffectors induces a change that is transmitted to the *cheM* and *cheD* gene products in the inner membrane. They collect and transmit the signal to other *che* gene products in the cytoplasm. These, in turn, regulate the activities of the *mot* gene products and rotor components, which control flagellar rotation. These ideas are outlined in Fig. 5. The nature of the signal transmitted to the flagellar rotor is not known, and we cannot rule out the possibility that signal transmission occurs solely in the inner membrane, for example, via a depolarization of membrane potential (14). Further localization of the *che* and *mot* proteins will depend on the application of more refined techniques, such as immuno-electron microscopy of frozen, thin sections. However, the preparation of specific antibody for these and other studies will hinge on the development of methods for isolating relatively large quantities of highly purified flagellar gene products.

TABLE 4. *Locations of proteins controlling motility and chemotaxis in E. coli*

Gene	Mol wt of protein	Location
cheA	76,000	Cytoplasm, inner membrane
	66,000	Cytoplasm
cheD	64,000	Inner membrane
cheM	61,000	Inner membrane
cheB	38,000	Cytoplasm, inner membrane
cheX	28,000	Cytoplasm, inner membrane
cheZ	24,000	Cytoplasm, inner membrane
cheW	12,000	Cytoplasm
cheY	8,000	Cytoplasm
motB	39,000	Inner membrane
motA	31,000	Inner membrane
hag	54,000	Outer membrane

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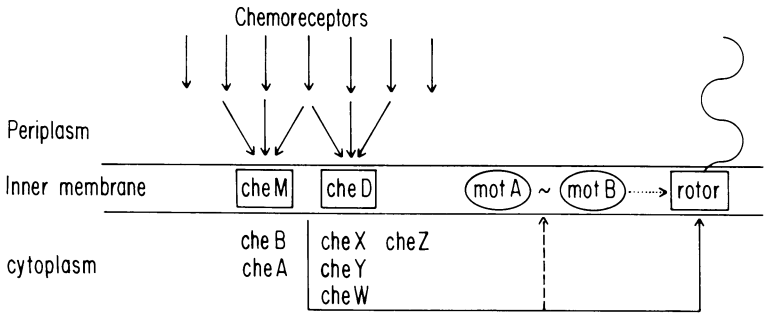

FIG. 5. *Diagrammatic representation of pathway for the flow of chemotactic information in E. coli.*

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