Interactions Between Chemotaxis Genes and Flagellar Genes in *Escherichia coli*

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Escherichia coli mutants defective in *cheY* and *cheZ* function are motile but generally nonchemotactic; *cheY* mutants have an extreme counterclockwise bias in flagellar rotation, whereas *cheZ* mutants have a clockwise rotational bias. Chemotactic pseudorevertants of *cheY* and *cheZ* mutants were isolated on semisolid agar and examined for second-site suppressors in other chemotaxis-related loci. Approximately 15% of the *cheZ* revertants and over 95% of the *cheY* revertants contained compensatory mutations in the *flaA* or *flaB* locus. When transferred to an otherwise wild-type background, most of these suppressor mutations resulted in a generally nonchemotactic phenotype: suppressors of *cheY* caused a clockwise rotational bias; suppressors of *cheZ* produced a counterclockwise rotational bias. Chemotactic double mutants containing a *che* and a *fla* mutation invariably exhibited flagellar rotation patterns in between the opposing extremes characteristic of the component mutations. This additive effect on flagellar rotation resulted in essentially wild-type swimming behavior and is probably the major basis of suppressor action. However, suppression effects were also allele specific, suggesting that the *cheY* and *cheZ* gene products interact directly with the *flaA* and *flaB* products. These interactions may be instrumental in establishing the unstimulated swimming pattern of *E. coli*.

Escherichia coli and most other motile bacteria are propelled by flagellar motors that are capable of rotating in either direction: counterclockwise (CCW) rotation produces smooth-swimming "runs," whereas clockwise (CW) reversals cause abrupt turning movements or "tumbles" (2, 17, 19, 28). In the absence of chemical stimuli, wild-type cells typically reverse their direction of flagellar rotation about once per second, enabling them to move randomly through their environment (2, 5). Upon encountering an attractant or repellent gradient, the cells modulate reversal probability to bring about net movement in a preferred direction (3, 17, 18). It appears that the spontaneous flagellar reversal rate in *E. coli* is designed to optimize the efficiency of these chemotactic responses. Lower tumbling rates would result in very long runs, during which the cells would have a high probability of being knocked off course by Brownian motion or collisions with other cells (3). Thus, the organism would not be able to exert any control over its swimming direction if reversal frequency were too low. Conversely, a very high reversal frequency would lead to nearly incessant tumbling and very short runs, which would not allow sufficient time to detect meaningful concentration changes in chemical gradients (4).

Genetic studies of *E. coli* and *Salmonella typhimurium* have identified a number of genes that play a role in generating or regulating the spontaneous pattern of flagellar reversals of the cell (see reference 22 for a review). Mutants defective in any of these genes (designated *che*) have a very low reversal frequency and consequently exhibit a pronounced CCW or CW bias in flagellar rotation. Such defects lead to a general loss of chemotactic ability. Many of the *che* gene products have been identified, but in most cases their biochemical functions have not yet been established.

The most serious obstacle to a better understanding of the *che* functions is the fact that very few of them seem to have an easily assayed enzymatic activity. In fact, most *che* proteins may play purely structural roles. With this problem in mind, we have attempted to utilize genetic methods to detect functional interactions between the *che* products and other components of the chemotaxis machinery of *E. coli* (10, 21, 24). In this report, we describe the reversion analysis of *cheY* and *cheZ* mutants and show that both of these *che* products interact with flagellar components—the *flaA* and *flaB* gene products. These studies have led to a useful working model of how the spontaneous pattern of flagellar reversals is regulated in *E. coli*.

TABLE 1. Bacterial strains

Strain	Relevant genotype[a]	Comments (reference)
RP461	thr-1(Am) leuB6 his-4	Donor of eda[+] marker (20)
RP421	metF159(Am) supD60	Donor of supD marker (20)
RP437	metF159(Am) eda-50	Repository strain for che mutations; used for all reversion studies (21)
RP4182	Δ(flaD-flaP)4	Host for λfla36 phages (15)
YK410	his purC thyA	Parent of Y. Komeda fla mutants (13)
W3110	Prototroph	Parent of H. Kondoh fla mutants (15)
W3623	trpA	Parent of H. Kondoh fla mutants (15)
MS1350	his arg thyA	Parent of M. Silverman fla mutants (26)

[a] All strains are F⁻ and most carry additional markers such as sugar fermentation defects and phage or drug resistances. The complete genotypes are given in the cited references.

MATERIALS AND METHODS

Bacterial strains. The genetic backgrounds of the strains used in this work are listed in Table 1. All are derivatives of *E. coli* K-12. The mutant *cheY* and *cheZ* alleles employed in reversion studies are listed in Table 2. All were isolated in our laboratory and most have been described previously (20, 21). They are presumed to be missense mutations since they were induced by ethyl methane sulfonate, map as points, are not corrected by nonsense suppressors, and in the case of *cheY*, are clearly nonpolar in nature. In fact, most of the *cheY* mutations that were used have been transferred to λ tranducing phages and shown to make protein products of wild-type size (D. Sherris, personal communication). All of the genetic manipulations involving these mutants and their revertants were done in derivatives of strain RP437, which is wild type for chemotaxis (23).

The region III flagellar mutations used for deletion mapping tests were obtained from Y. Komeda, National Institute of Genetics, Mishima, Japan; H. Kondoh, Kyoto University, Japan; and M. Silverman, University of California, San Diego (Table 2).

Media. Tryptone broth and semisolid agar and H1 salts minimal medium have been described previously (20).

Genetic analyses of the *che* **and** *fla* **mutants.** Map positions of the loci relevant to this work are shown in

Fig. 1. All manipulations of these genes (*cheY*, *cheZ*, *flaA*, and *flaB*) were accomplished by cotransduction with either the *eda* locus or the *supD* locus. Transductions were done at 37°C with phage P1 *kc* as previously described (20). Transductants inheriting the donor *supD* allele were selected as Met⁺ recombinants on H1 minimal glucose medium (the *metF* marker in RP437 is suppressible by *supD*); *eda*⁺ transductants were selected on H1 minimal medium containing 5 g of glucuronic acid per liter as a carbon and energy source. The chemotaxis phenotype of individual transductant colonies was determined by transferring them to semisolid tryptone agar plates and scoring swarm size after 8 to 16 h of incubation at 35°C. The *cheY* and *cheZ* loci are ~25% cotransducible with the *eda* marker, whereas the *flaA* and *flaB* loci are ~40% cotransducible with the *supD* marker (Fig. 1).

Complementation analyses and deletion mapping. Second-site suppressors of chemotaxis mutations were initially mapped by P1 transduction and subsequently transferred to RP437(λ) for more detailed study. Suppressors linked to the *eda* marker were tested for complementation and recombination with a series of λche22 specialized transducing phages that carry known segments of the *tar-cheZ* region (Fig. 1) as described previously (23).

Suppressors linked to the *supD* locus were analyzed by deletion mapping with the specialized transducing phage λfla36 obtained from Y. Komeda (13). Deletion

TABLE 2. The che and fla mutations

Mutant alleles	Genetic background	Source (reference)
che mutations		
cheY199, 200, 201, 207, 216, 219, 220, 225, 230	RP437 *eda*⁺; RP437 *supD*	This laboratory (21)
cheZ4, 102, 104, 105, 163, 165, 280, 281	RP437 *eda*⁺	This laboratory (20, 21)
fla mutations[a]		
flaA4160, B4126, C4127, P4144, Q4120, R4150; motD4117	YK410(λ)	Y. Komeda (12)
flaA75(Am), B4(Am), C87(Am), E112, N79(Am), 069(Am), P46(Am), Q95; flbD52	W3110(λ); W3623(λ)	H. Kondoh (15)
flaA371(Am), B111, B722(Am), C8012, E694(Am), P871, Q1083, R641, R827	MS1350(λ)	M. Silverman (26)

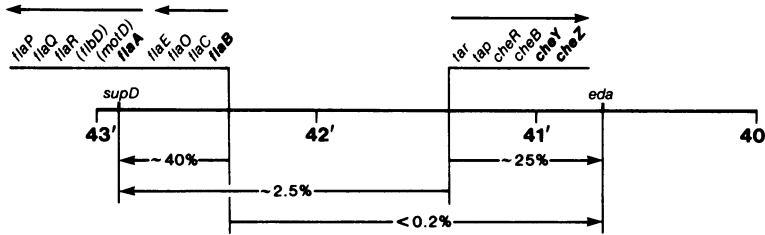[a] Gene assignments are based on our deletion mapping results (see text and Fig. 4).

FIG. 1. Linkage map of *che* and *fla* genes. The four loci shown in boldface type were studied in this work. These genes are cotranscribed with other *che* and *fla* loci, as indicated by the arrow above each operon. The *flbD* and *motD* genes are newly described loci that may be identical (see text and Fig. 5). The frequency of cotransduction between these genes and the *supD* and *eda* markers is given below the map with arrows pointing toward the selected markers. Values are taken from unpublished data and reference 20.

derivatives of λfla36 were selected as plaque formers on tryptone plates containing 0.6 to 1.0 mM EDTA at 37°C (23) and were propagated on strain RP4182 (Table 1), which carries an extensive deletion of *fla* region III and prevents the formation of wild-type λfla36 recombinants.

RESULTS

Isolation of mutations that suppress cheY defects. Mutants defective in the *cheY* function are generally nonchemotactic and cannot swarm on semisolid agar (Fig. 2). After prolonged incubation, however, *cheY* mutants give rise to chemotactic revertants (Fig. 2). As discussed below, most *cheY* revertants are not produced by back mutation, but rather through acquisition of a compensating mutation outside the *cheY* locus. We have adopted the designation *scy* (suppressor of *cheY*) to refer to the second-site muta-
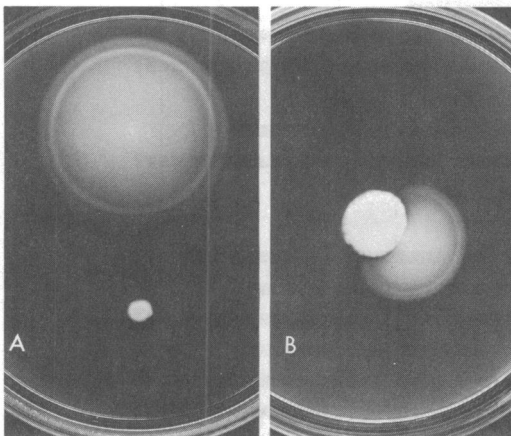

FIG. 2. Isolation of *cheY* revertants. Colonies of RP437 (wild type) and RP4764 (*cheY216*) were transferred to semisolid tryptone agar and incubated at 35°C for 14 h (A). After incubation for 24 to 36 h, the *cheY* mutant gave rise to a pseudorevertant swarm (B).

tion(s) responsible for restoring chemotactic ability in *cheY* pseudorevertants of this sort.

A total of 30 independent, spontaneous revertants were isolated from four *cheY* missense mutants (*cheY201*, *cheY216*, *cheY219*, and *cheY220*) and examined for the presence of *scy* mutations. To identify strains in which the reversion site was not tightly linked to the *cheY* locus, each revertant was transduced to *eda*+ with P1 grown on an *eda*+ derivative of its *cheY* parent. Since the *cheY* locus is ca. 25% cotransducible with the *eda* marker (Fig. 1), revertants with back mutations or suppressors close to *cheY* should have yielded ca. 25% nonchemotactic transductants in this test cross. Of the 30 revertants tested, only 1 produced Che⁻ transductants at the predicted frequency of 25%; the other 29 strains gave no Che⁻ transductants in the test cross and evidently contained *scy* mutations that were unlinked to the *eda* marker. The approximate map locations of the *scy* mutations in these *cheY* pseudorevertants were established by transducing various regions of the revertant genome into recipient *cheY* mutants and testing for restoration of a pseudorevertant swarm phenotype. We found that all 29 revertant strains contained *scy* mutations linked to the *supD* marker with a cotransduction frequency of ca. 40%.

Chemotaxis phenotypes of scy mutants. Each *scy* mutation was transferred into strain RP437 to assess its effect, if any, on chemotactic ability in a *cheY*+ genetic background. Derivatives of *cheY scy* strains carrying the *supD* marker were constructed and used as donors in transductional crosses to RP437, selecting for inheritance of the donor *supD* allele. Since the *scy* mutations were ca. 40% cotransducible with the *supD* locus, nearly half of the transductants from these crosses were expected to acquire the donor *scy* allele. Most of the *scy* mutations proved to cause a significant defect in chemotactic ability in the RP437 background, and in those crosses we observed transductants with obvi-
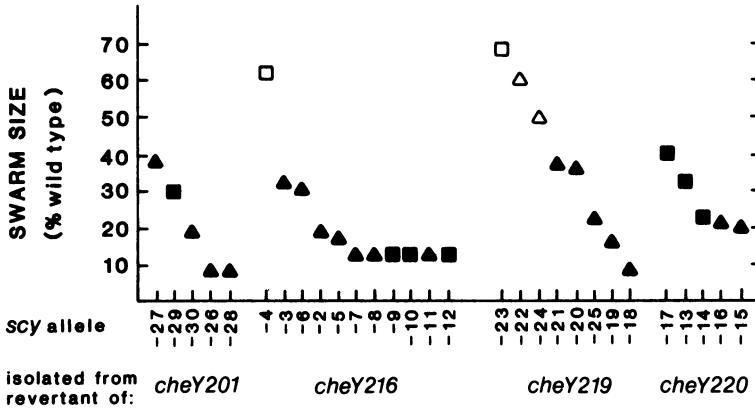
FIG. 3. Chemotactic ability of RP437 *scy* strains. Colonies were transferred to semisolid tryptone agar and incubated for 10 h at 35°C. The diameters of at least five swarms from each *scy* strain were averaged and normalized to that of an RP437 control swarm on the same plate. These *scy* strains were subsequently divided into two groups on the basis of complementation tests: *flaA* = *scyA* (△,▲); *flaB* = *scyB* (□,■). □ and △, Tentative gene assignments due to the marginal nature of the mutant phenotype in those cases.
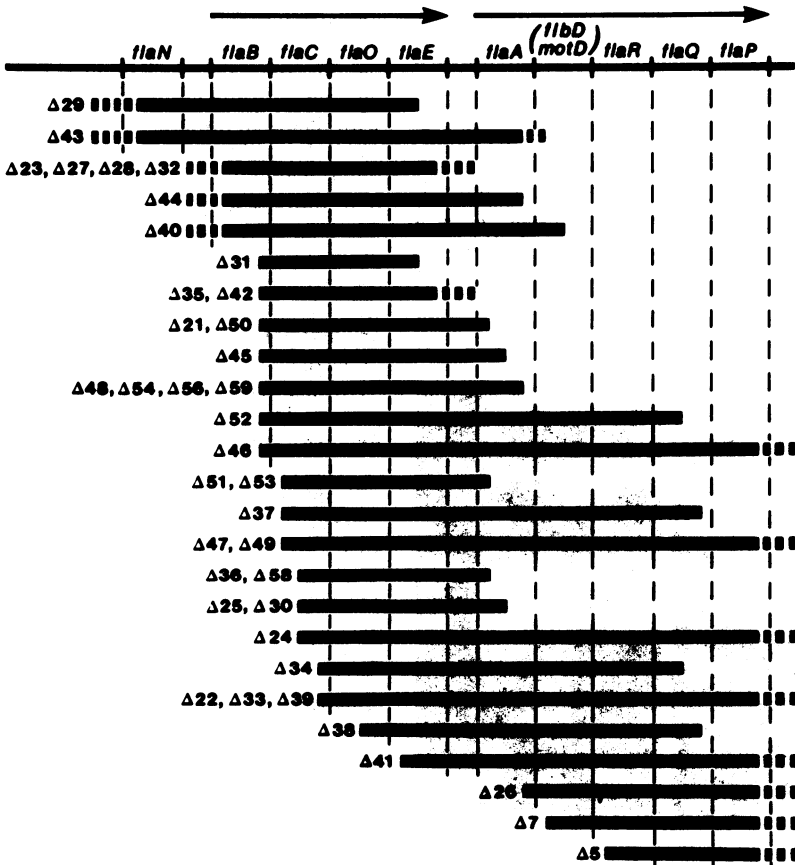

FIG. 4. Genetic map of the *flaA* and *flaB* operons. The region shown is carried by λfla36. The material missing in various λfla36 deletion derivatives is indicated below the map. These deletion mutants were used to construct the map, which agrees with recent results of others (12, 14).

Phage in plate:

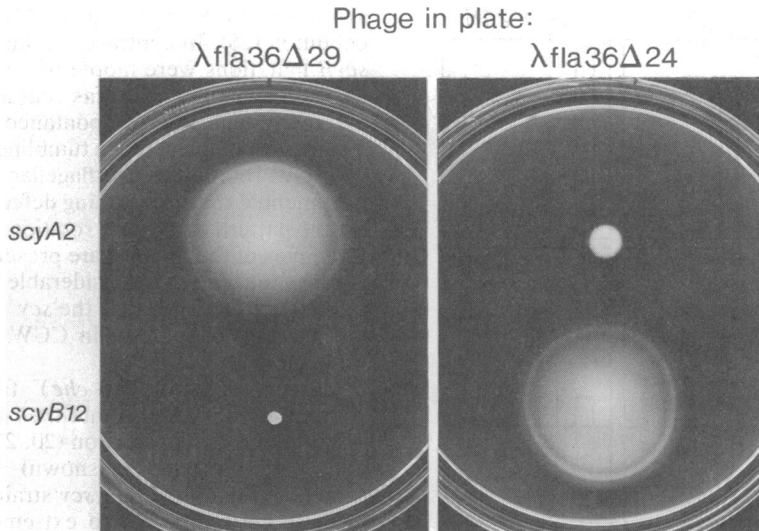λfla36Δ29   λfla36Δ24

FIG. 5. Complementation analysis of *scy* mutants. Colonies of RP4493 (*scyA2*) and RP4503 (*scyB12*) were transferred to semisolid tryptone plates containing ~10^9 particles of either λfla36Δ29 (*flaA-P*)$^+$ or λfla36Δ24 (*flaN-B*)$^+$ per ml and incubated for 16 h at 35°C. Positive complementation is indicated by the uniform spread of growth outward from the origin.

ously nonwild swarm behavior at the expected frequency of 40%. However, in four cases, the *scy* mutations only slightly impaired the chemotaxis of strain RP437. To confirm that we had, in fact, identified the *scy* derivatives of RP437, particularly in those cases where their chemotaxis phenotype was similar to that of wild type, we test crossed each of them to a *cheY* recipient to determine that they carried a *supD*-linked allele capable of suppressing the *cheY* chemotaxis defect.

In all, 29 different RP437 *scy* strains were constructed in this manner; their chemotactic ability is summarized in Fig. 3. Although the sample size is rather small, there do not appear to be any systematic differences in the chemotaxis defects of *scy* mutants derived from different *cheY* parents. All but four of these *scy* mutations caused a significant reduction in the swarm size of strain RP437 and were readily amenable to complementation analysis, as detailed below.

Complementation analysis of *scy* mutants. Several clusters of flagellar (*fla*) genes are linked to the *supD* locus with cotransduction values of ca. 40% (Fig. 1). The region containing those *fla* genes is carried by λfla36, a specialized, nondefective transducing phage (12, 13). Since the *supD* linkage of these *fla* loci is similar to that of our *scy* mutations, we tested λfla36 for the ability to complement the chemotaxis defect of RP437 *scy* strains. Of the 25 *scy* strains that had an obvious mutant swarm phenotype, all were clearly complemented by λfla36. The four re-

maining *scy* strains, which were only slightly defective in chemotaxis, also appeared to be complemented by λfla36; however, these tests were difficult to score unambiguously.

To locate more precisely the *scy* loci, we isolated and mapped a series of deleted λfla36 derivatives (Fig. 4). The *scy* mapping results are summarized in Fig. 3, and examples of the complementation results are shown in Fig. 5. We found that 20 of the *scy* mutations mapped in the *flaA* gene and 9 mapped in the *flaB* gene (Fig. 3). These two groups have been designated *scyA* (*flaA*) and *scyB* (*flaB*).

Gene order in *fla* region III. Several recent studies have indicated that the gene order in the *flaA* operon is not consistent with the original genetic analysis of this region (12, 14). Our mapping results confirmed the revised gene order (Fig. 4). Moreover, there appears to be at least one new gene in this operon, located between the *flaA* and *flaR* loci. Komeda et al. (12) designated the new gene *motD* because the mutant representative in their collection had a Mot$^-$ phenotype (i.e., nonmotile but with paralyzed flagella rather than no flagella). Kondoh (14), based on work with a different mutant that had a Fla$^-$ phenotype, designated the new gene *flbD*. We used both of these mutants in our mapping tests but were unable to distinguish the two mutations by either complementation or recombination tests with λfla36 deletions. We confirmed, however, that the two mutants did have different phenotypes: the Komeda strain was agglutinated by antiflagellar serum, whereas
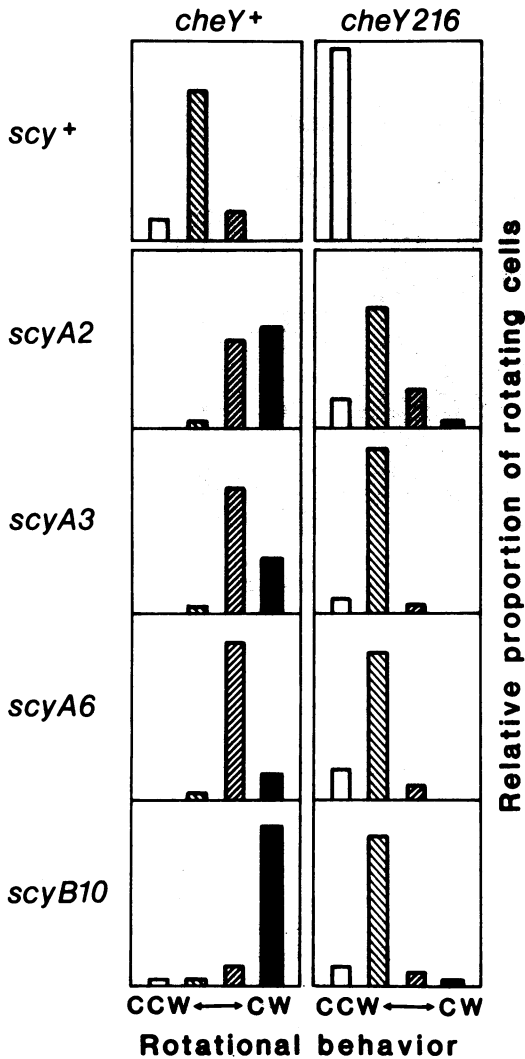
FIG. 6. Flagellar rotation patterns of *scy* strains and *cheY* pseudorevertants. Strains were grown, tethered, and analyzed as previously described (20). At least 100 rotating cells from each strain were examined and classified into four categories (20): exclusively CCW; predominantly CCW but reversing; predominantly CW but reversing; or exclusively CW. These four categories are listed from left to right in the bar graphs. Each panel presents the behavior of a single strain containing the indicated combination of *cheY* and *scy* alleles.

the Kondoh strain was not. It seems likely that both mutations are alleles of the same gene but with different phenotypic consequences. Only complementation tests between the two mutants themselves can provide an unambiguous answer to this question.

Phenotypic basis of *scy* action. Mutations at the *flaA* and *flaB* loci typically prevent flagellar

synthesis or assembly and result in a nonmotile condition (26). In contrast, strains with *scyA* or *scyB* mutations were motile but proved to have aberrant swimming patterns characterized by an extremely high rate of spontaneous directional changes or tumbles. Since tumbling is associated with CW rotation of the flagellar filaments, we documented these swimming defects by examining the pattern of flagellar rotation in *scy* strains. Examples of the findings are presented in Fig. 6. Each *scy* strain had a considerable CW bias in its rotation pattern, whereas the *scy*⁺ control spent the majority of its time in CCW rotation with frequent reversals.

Mutants defective in *cheY* function swim without tumbling and rotate their flagella exclusively in the CCW direction (20, 21). The swimming patterns (data not shown) and rotational behavior (Fig. 6) of *cheY scy* strains were intermediate between the two extremes caused by the component mutations separately and similar to those of wild type, indicating that these mutations produce opposing, but additive, effects on the pattern of flagellar rotation. Since modulation of tumbling rate in response to stimuli produces chemotactic movements, the more normal tumbling rate exhibited by *cheY scy* strains is probably, at least in part, responsible for their chemotactic ability.

Allele specificity of *scy* action. A number of different *scy* mutations were transduced into a series of *cheY* recipients, and the chemotactic ability of the resulting double mutants was assessed on tryptone swarm agar (Fig. 7). These data clearly show that a particular *scy* mutation does not suppress different *cheY* alleles with the same efficiency. A suppressor which proves effective in one background (e.g., *scyA6* in *cheY220*) may be totally ineffective in a different background (e.g., *scyA6* in *cheY201*). Moreover, *cheY* alleles (e.g., *cheY220* and *cheY225*) that respond the same to most *scy* mutations may still respond very differently to others (e.g., *scyA20*). Since the behavior of any given *cheY scy* strain cannot be predicted from the observed effects of the same mutations in other combinations, the interaction of *cheY* and *scy* must be allele specific. This implies that the *cheY* and *scy* gene products themselves interact in a stereospecific, presumably direct manner.

Reversion analysis of *scyA* and *scyB* mutants. We examined 20 independent revertants from each of three different *scyA* mutants and three different *scyB* mutants for second-site suppressor mutations. Unlike *cheY* revertants, the majority of *scy* revertants contained reversion sites in or near the original mutant locus. It is not yet clear whether all such revertants represent back mutations or whether some are due to functional interaction of the *scyA* and *scyB* loci with one
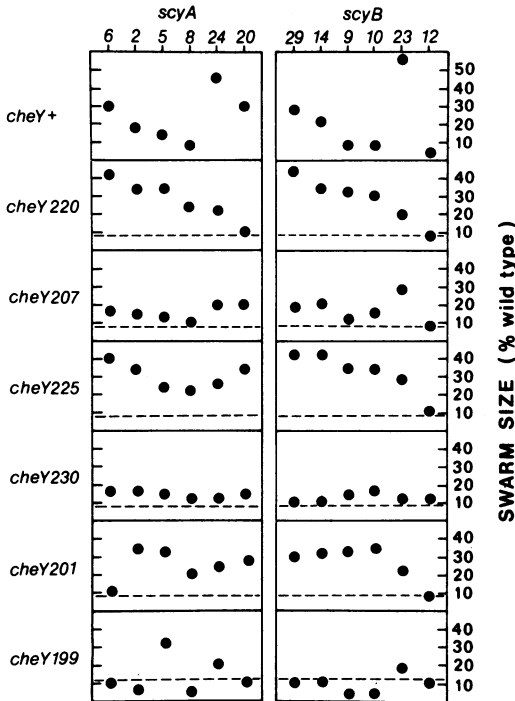
FIG. 7. Allele specificity of *cheY-scy* interactions. A series of *scyA* and *scyB* alleles were crossed into various *cheY* backgrounds, and swarm sizes of the resulting double mutants were measured on tryptone swarm agar as explained in the legend to Fig. 3. ------, Swarm size of the *cheY* parent strain. The suppressors were ordered with respect to effectiveness in the *cheY220* background to facilitate comparisons with other backgrounds.

TABLE 3. Complementation analysis of *eda*-linked mutations obtained from *scy* revertants

Allele	No. of revertants tested[a]	No. of *eda*-linked reversion sites	
		cheY	Other
scyA2	20	3	1 (unclassified)[b]
scyA5	20	5	0
scyA8	20	2	0
scyB9	20	3	1 (*tar* polar)
scyB10	20	4	0
scyB12	20	3	0

[a] All revertants were of spontaneous origin.
[b] This suppressor did not cause a sufficiently defective chemotaxis phenotype for complementation testing.

another or with other *fla* genes nearby. Approximately 10 to 20% of the *scy* revertants were found to contain *eda*-linked reversion sites (Table 3). Upon further analysis, all but two of these mutations mapped to the *cheY* locus and produced a typical *cheY* mutant phenotype. Of the other two mutations, one produced a nearly wild-type swarm and could not be classified by complementation analysis; one proved to have a polar defect in the *tar* gene. Since *tar* polar mutations cause a CCW rotational bias owing to decreased expression of the *cheRBYZ* operon, they might be able to restore normal swimming patterns to CW-biased *scy* mutants. It is not entirely obvious why this sort of phenotypic interaction would lead to the restoration of chemotactic ability, although it seems possible that, if the polar effect were not too strong, there might be sufficient *cheRBYZ* expression for chemotaxis once the swimming pattern is restored to normal.

Reversion analysis of *cheZ* mutants. We showed previously that nontumbling *cheC* mu-

tants could be functionally suppressed by *cheZ* mutations, which cause high tumbling rates (24). Since *cheC* mutations are known to be specific alleles of the *flaA* gene (20, 27), it appears that this gene product interacts with both the *cheZ* and *cheY* products. It seemed plausible, therefore, that, like *cheY*, *cheZ* mutants might also be suppressible by *flaB* mutations. To test this notion, we examined revertants from a number of different *cheZ* mutants (Table 4). Of 7 *cheZ* mutants tested, 6 gave rise to revertants carrying *supD*-linked reversion sites; 20 suppressors proved to be *flaA* mutations, 1 was defective in *flaB* function, and 1 could not be complementation tested. The frequency of such suppressors (designated *sczA* and *sczB*, respectively) among *cheZ* revertants was not very high (22/157), and it appears that the most probable course of *cheZ* reversion is by back mutation or by acquisition of a closely linked suppressor mutation.

The swimming behavior and flagellar rotation patterns of RP437 derivatives carrying *scz* mutations were also examined. All of the strains exhibited a pronounced CCW bias in rotation and swam smoothly, with very few tumbling episodes. The swimming patterns of *cheZ scz* double mutants generally resembled the wild-type behavior (data not shown), indicating that *cheZ* and *scz* mutations have opposing and additive effects on flagellar rotation, just as *cheY* and *scy* mutations do.

DISCUSSION

The findings of our reversion studies are summarized in Fig. 8. Mutations in the *cheY* and *cheZ* genes produce, respectively, an extreme CCW or CW bias in flagellar rotation and cause a general defect in chemotactic ability. Such defects can be phenotypically suppressed by certain mutations in the *flaA* and *flaB* genes. In a wild-type background, these suppressor mutations also cause an aberrant pattern of flagellar rotation and often lead to generally nonchemo-

TABLE 4. Complementation analysis of *supD*-linked mutations obtained from *cheZ* revertants

Allele	Revertants tested		No. of *supD*-linked reversion sites		
	No.	Source	*flaA*	*flaB*	Unclassified
cheZ4	11	EMS[a]	1	0	0
cheZ104	12	EMS	3	0	1[b]
cheZ105	32	Spontaneous, EMS	6	1	0
cheZ163	20	Spontaneous	0	0	0
cheZ165	20	Spontaneous	2	0	0
cheZ280	20	Spontaneous	1	0	0
cheZ281	42	Spontaneous, EMS	7	0	0

[a] EMS, Ethyl methane sulfonate.
[b] This suppressor did not cause a sufficiently defective chemotaxis phenotype for complementation testing.

tactic behavior. In the cases studied here and previously (24), chemotactic revertants of such *fla* strains can arise through compensating mutations in the *cheY* or *cheZ* gene. Thus, two mutations (e.g., *cheY* and *scy*) which each alter flagellar rotation and chemotactic behavior can in combination interact productively to restore chemotactic ability. In every case, the initial nonchemotactic mutation and its suppressor produce opposing biases in the direction of flagellar rotation and appear to interact in a roughly additive fashion. Restoration of a wild-type swimming pattern is not a sufficient condition for chemotaxis, however, because some *cheC cheZ* or *cheC cheY* double mutants exhibit "normal" swimming behavior but are still nonchemotactic (9, 24). The allele-specific nature of these suppression effects indicates that the *cheY* and *cheZ* gene products probably interact directly with the *flaA* and *flaB* products.

The interactions summarized in Fig. 8 are clearly not the only ones in which these gene products take part. For example, evidence from intraspecific and interspecific complementation tests strongly suggests that the *cheY* and *cheR* gene products interact and that the *cheZ* and *cheB* products interact (10, 21). Since these genes are tightly linked, interactions among them would not have been identified in the present study. Similarly, it seems likely that the *flaA* and *flaB* proteins interact with other *fla* gene products and perhaps with one another. If the loci involved were tightly linked, we would have overlooked those interactions as well.

Mutations in the *flaA* and *flaB* genes can lead to a variety of mutant phenotypes. Most *flaA* mutants are nonmotile and have no flagella (Fla⁻), indicating that the *flaA* product could be an essential structural component of the flagellar basal complex (12, 26). Some *flaA* mutants, presumably with specific sorts of mutational defects, are motile but nonchemotactic and have excessively CW or CCW flagellar rotation patterns. Smooth-swimming *flaA* mutants, like the *sczA* strains described in this report, are also

called *cheC* mutants and have been known for quite some time (1, 20, 27). Tumbly *cheC* strains have been described recently by Kondoh (14), and the *scyA* mutants isolated in the present report appear to have defects of this type. The majority of *flaB* mutants are also nonflagellate, which suggests that, like *flaA*, the *flaB* gene product may be a structural component of the flagellum (12, 26). The *scyB* and *sczB* alleles of *flaB*, described in this report, cause either a CW or a CCW flagellar bias and a generally nonchemotactic phenotype.

An even greater variety of mutant phenotypes has been observed for the *flaA* and *flaB* counterparts in *S. typhimurium*. The *flaA* analog in *Salmonella* spp. is called *flaQ*, and it also gives rise to both nonflagellate and generally nonchemotactic mutants (16). Nonchemotactic *flaQ* mutants with either CW or CCW rotational patterns were originally designated *cheU* (29) but are now called *cheC* mutants after their *E. coli* counterparts (8). The *flaAII* gene in *Salmonella* spp. appears to be homologous to the *flaB* gene of *E. coli* (16), although there is still some doubt about this correspondence (7). Collins and Stocker (6) have described a smooth-swimming *flaAII* mutant of *Salmonella* spp. (called *cheV*) whose phenotype resembles that produced in *E. coli* by the *sczB* allele of *flaB*. Recently, Dean et al. (7) described a *flaAII* mutant with a temperature-sensitive flagellar paralysis. At the permissive temperature, this mutant exhibited a CW rotational bias similar to that in *scyB* mutants of *E. coli*. Since generally nonchemotactic *flaB* mutants of *E. coli* have not been previously described, we propose that they be designated *cheV* by analogy with the corresponding mutation in *Salmonella* spp.

The variety of mutant phenotypes that can be generated by *flaA* and *flaB* mutations (and their *Salmonella* counterparts) suggests that these gene products play important roles in controlling flagellar motion. Both proteins are clearly required for flagellar assembly, but neither has yet been identified among the components of the
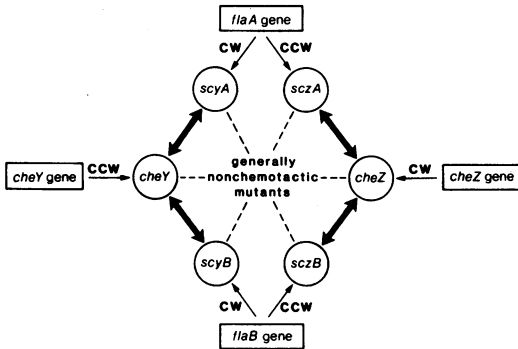
FIG. 8. Summary of known interactions between che and fla gene products. The four genes shown (flaA, cheZ, flaB, cheY) each give rise to motile but generally nonchemotactic mutants with CW or CCW biases in flagellar rotation. Certain combinations of these defects (↔) interact productively to restore the normal swimming pattern and some measure of chemotactic ability. As discussed in the text, the Che⁻ mutants of flaA have been given the general designation cheC; the Che⁻ mutants of flaB have been given the general designation cheV.
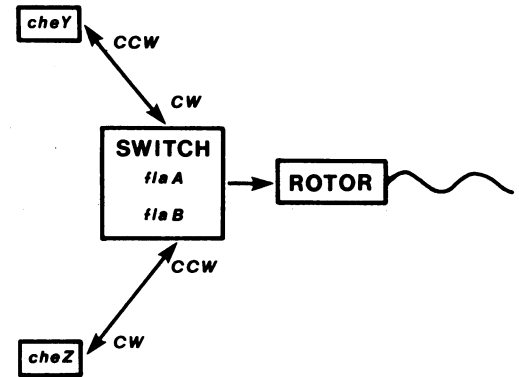

FIG. 9. Possible role of che-fla gene interactions in controlling spontaneous flagellar reversals. See text for details.

purified flagellar basal body. It may be that the flaA and flaB gene products are membrane proteins associated with the basal complex. Because some flaA and flaB mutants have aberrant rotation patterns, their primary function may be to regulate or initiate changes in the direction of flagellar rotation. However, unlike all other che functions, specific flaA and flaB defects can lead to either a CW or a CCW bias in rotation pattern, implying that they have direct control over rotational changes. For these reasons, the flaA and flaB products seem likely to comprise some sort of "switch" that modulates flagellar rotation. This switch might receive and act on input signals from the sensory receptors of the cell, as suggested previously (11, 25), but this is not an essential feature of our model.

A possible role for the cheY and cheZ gene products in controlling flagellar rotation is presented in Fig. 9. We propose, based on the genetic evidence discussed in this report, that the cheY and cheZ products interact directly with the flagellar switch composed of the flaA and flaB products. These interactions may be readily reversible ones, because the cheY and cheZ proteins are found in the cytoplasmic fraction of E. coli, whereas the flaA and flaB products are likely to be membrane associated. Moreover, the cheY and cheZ gene products appear to have opposing effects on the switch. We propose that interaction of cheY protein with the switch enhances CW rotation, whereas interaction of cheZ protein with the switch enhances CCW rotation. It is conceivable that both pro-

teins bind to similar or overlapping sites on the switch so that their interactions are mutually exclusive events. Thus in wild-type cells, the relative levels of cheY and cheZ protein may establish the steady-state switching rate of the flagella. In this regard, it is important to note that a fixed relative stoichiometry of these two gene products is normally assured by cotranscription. It will be interesting to alter this stoichiometry by individually subcloning each gene: an excess of cheY product should cause enhanced CW rotation; an excess of cheZ product should have the opposite effect.

This model readily accounts for the suppression patterns described in this report. We suggest that the primary role for the interactions between the cheY and cheZ products and the flagellar switch is to establish the optimum frequency of spontaneous flagellar reversals required for chemotactic behavior. An alteration in any one of these interacting components that results in a CCW or CW rotational bias produces an aberrant swimming pattern and a general loss of chemotactic ability. Defects of this sort can be phenotypically suppressed by mutations that affect the gene product interactions in a way that restores the wild-type swimming pattern. For example, missense defects in the cheY product probably decrease its affinity for the switch and thereby lead to a CCW rotational bias. Mutations of flaA or flaB that enhance affinity for the mutant cheY protein (or that reduce affinity for the normal form of cheZ protein) should result in a more balanced pattern of flagellar rotation and a restoration of chemotactic ability. The fact that all mutant alleles of cheY, cheZ, flaA, and flaB capable of functional interaction have opposing and additive effects on flagellar rotation provides strong support for this type of suppression mechanism.

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