Cell, Vol. 15, 1221-1230, December 1978, Copyright © 1978 by MIT

Sensory Adaptation Mutants of E. coli

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Summary

The ability of E. coli to adapt to constant levels of attractant and repellent chemicals was studied by examining the patterns of flagellar movement in cells subjected to abrupt concentration changes. Wild-type bacteria exhibited transient responses to such stimuli, in support of previous findings. Nonchemotactic mutants of the *cheX* class responded to both attractants and repellents, but were unable to terminate these behavioral changes as long as the stimulating chemical was present. The sensory adaptation defect of *cheX* strains may be due to an inability to methylate several cytoplasmic membrane proteins that initiate changes in flagellar movement in response to chemoreceptor signals. Based on these results, possible mechanisms of stimulus transduction and sensory adaptation during chemotaxis are discussed.

Introduction

Motile bacteria detect and respond to changes in their environment in much the same way as higher organisms (see reviews by Adler, 1975; Berg, 1975; Koshland, 1977). E. coli, for example, can swim toward certain types of chemicals and away from others, a behavior known as chemotaxis (Adler, 1966). To accomplish this feat, the bacteria must be able to detect chemical stimuli and to convert that information into signals which regulate swimming activity. These events define the basic functions of a sensory transduction system. Since bacterial chemotaxis is amenable to a variety of experimental approaches, including genetic dissection (see reviews by Parkinson, 1975, 1977), it allows the investigation of stimulus detection and signal processing at the molecular level.

Chemotaxis in E. coli is carried out by modulating the occurrence of turning movements or tumbles which ordinarily take place at frequent intervals during swimming (Berg and Brown, 1972; Macnab and Koshland, 1972). For example, when an individual happens to swim toward an attractant, its probability of tumbling decreases, which promotes further movement in the preferred direction. Such responses are triggered whenever the organism senses a temporal change in attractant or repellent concentration (Macnab and Koshland, 1972; Tsang, Macnab and Koshland, 1973; Brown and Berg, 1974). Temporal sensing can be readily demonstrated by subjecting bacteria to abrupt, spatially homogeneous concentration changes. Such stimuli elicit an immediate change in swimming behavior followed by a slow return to the unstimulated swimming pattern (Macnab and Koshland, 1972; Berg and Tedesco, 1975; Spudich and Koshland, 1975). Depending upon the nature and magnitude of the initial stimulus, the recovery phase may last as long as several minutes. The fact that the response to a single temporal stimulus is transient demonstrates the existence of a sensory adaptation system in E. coli which enables the organism to ignore constant levels of attractants or repellents effectively so that it responds only to concentration changes.

Chemical stimuli are detected by specific receptors (Adler, 1969) which appear to transmit information about their state of occupancy to the transduction machinery (Mesibov, Ordal and Adler, 1973; Berg and Tedesco, 1975; Spudich and Koshland, 1975). Since different chemoreceptors appear to use similar adaptation mechanisms, the adaptation system may be part of a shared signalling network that handles all sensory inputs (Tsang et al., 1973; Adler and Tso, 1974; Berg and Tedesco, 1975; Spudich and Koshland, 1975). Common components of the transduction machinery have been defined through studies of motile but generally nonchemotactic mutants (Armstrong, Adler and Dahl, 1967). In E. coli, mutations in eight different genes can yield such a phenotype (Parkinson, 1976, 1978; Silverman and Simon, 1977a). We discovered that one of those genes, *cheX*, may specify a component of the adaptation system. This report describes the behavior of *cheX* mutants in response to chemotactic stimuli and discusses the possible role of *cheX* function in sensory adaptation. The accompanying paper by Goy, Springer and Adler (1978) describes the biochemical basis of the *cheX* defect.

Results

Experimental Plan

Bacteria swim by rotating their flagellar filaments (Berg and Anderson, 1973; Silverman and Simon, 1974). Counterclockwise rotation corresponds to smooth swimming, whereas clockwise rotation corresponds to tumbling (Larsen et al., 1974). Flagellar rotation can be observed by tethering bacteria to a microscope slide by means of antibody molecules directed against the flagellar filament (Silverman and Simon, 1974). When subjected to various chemical stimuli, tethered cells exhibit

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changes in rotational behavior that parallel the responses observed in swimming cells (Larsen et al., 1974; Berg and Tedesco, 1975). In the experiments described below, bacteria were tethered in a chamber which allowed the introduction of various attractant and repellent solutions, and adaptation was measured by following the rotational responses of the cells to various types of stimuli. The rotational data are reported in an essentially qualitative form. Detailed quantitative treatments were not undertaken, although it is possible that additional features of *cheX* behavior might be revealed by such an analysis.

The strains used in this work are listed in Table 1. Experiments were performed with RP487, which is wild-type for chemotaxis, and with six essentially isogenic derivatives of RP487 made by transducing *cheX* mutations into the RP487 background.

Table 1. Rotational Patterns of Unstimulated Cells

		% Rotating Cells[a]		
Strain	*cheX* Allele	CCW Only	CW Only	Reversing
RP4332	*202*	94	4	2
RP4333	*203*	100	0	0
RP4334	*210*	99	0	1
RP4335	*239*	98	2	0
RP4336	*243*	100	0	0
RP4337	*255*	99	0	1
RP487	Wild-type	9	0	91

[a] Cells were tethered in chemotaxis buffer plus 10^{-6} M methionine. Each cell was observed for approximately 15 sec and classified as either reversing or exclusively counterclockwise or clockwise. At least 100 cells were scored for each strain. It is probable that exclusively clockwise *cheX* cells may actually be driven by a free flagellum (Parkinson, 1976).

Rotational Patterns of Wild-Type and *cheX* Strains

The rotational behavior of RP487 and its six *cheX* derivatives in the absence of chemotactic stimuli is given in Table 1. In agreement with previous work, we observed frequent reversals in the direction of rotation of wild-type cells which presumably correspond to the alternating episodes of smooth and tumbly behavior found in swimming cells. The *cheX* strains did not appear to tumble while swimming (data not shown) and rotated almost exclusively in the counterclockwise direction. In the experiments to follow, we assume that the unstimulated and adapted behavior of wild-type cells is characterized by frequent reversals, whereas the corresponding behavior of *cheX* strains is dominated by counterclockwise rotation.

The rotational responses of wild-type and *cheX* strains to various types of stimuli are summarized in Table 2. On the basis of the wild-type responses, the stimuli can be divided into two categories: those that elicit clockwise rotation and those that elicit counterclockwise rotation. Repellent increases and attractant decreases elicit clockwise rotation and are designated clockwise stimuli; repellent decreases and attractant increases elicit counterclockwise rotation are are classified as counterclockwise stimuli. The adaptation or recovery times to counterclockwise stimuli are considerably slower than to clockwise stimuli, suggesting that the mechanism of adaptation may not be the same in both cases. The basis of this difference is considered in the Discussion.

As Table 2 shows, the rotational pattern of *cheX* strains is not changed by counterclockwise stimuli. This is hardly unexpected since *cheX* strains exhibit no clockwise behavior before stimulation. However, repellent increases, which evoke clockwise rotation in wild-type, also cause clockwise or reversing behavior in *cheX* strains. Thus *cheX* mutants can detect and respond to repellent stimuli. These responses are dramatically longer than in wild-type cells and indicate that *cheX* mutants are somehow defective in adapting to clockwise stimuli. The details of this adaptation defect are examined below.

Repellent Responses of *cheX* Strains

As shown in Table 2, repellent increases elicit clockwise rotation in both wild-type and *cheX* strains. Because of the adaptation process, the wild-type response is quite brief. For example, the response of RP487 to a change from 0 to 10^{-3} M leucine, a weak repellent stimulus (Tso and Adler, 1974; Berg and Tedesco, 1975), lasted for less than 1 min (data not shown). The responses of *cheX* strains to that same stimulus were dramatically different. Three of the mutants (*X202*, *X203* and *X255*) responded and continued to respond for more than 15 min, whereas the other three mutants (*X210*, *X239*, and *X243*) failed to respond. These two classes of *cheX* strains are designated group 1 and group 2, respectively.

Responses of the group 1 mutants to 10^{-3} M leucine are shown in Figure 1. In general the responses, as evidenced by episodes of clockwise rotation or reversals, persisted for as long as the repellent was present. Removal of the leucine produced an immediate return to counterclockwise rotation, showing that the repellent had not damaged the rotational machinery (data not shown). The prolonged response indicates that group 1 mutants are defective in adapting to the leucine stimulus. This defect, however, does not appear to be absolute. For example, the duration of the reversing episodes decreased with time and the time between successive episodes increased.

Table 2. Summary of Attractant and Repellent Responses

	RP487[a]		RP487 *cheX*	
	Response	Duration	Response	Duration
"Clockwise Stimuli"				
Repellent increases				
$0 \rightarrow 10^{-2}$ M leucine	Reversing → CW	<1 min	CCW → CW	>10 min
$0 \rightarrow 10^{-2}$ M acetate				
$0 \rightarrow 10^{-3}$ M salicylate				
$0 \rightarrow 2.5 \times 10^{-4}$ M indole				
$0 \rightarrow 10^{-4}$ M Co^{++}				
Attractant decreases				
$10^{-5} \rightarrow 0$ M aspartate	Reversing → CW	<1 min	(See Figure 4)	
$10^{-5} \rightarrow 0$ M serine				
"Counterclockwise Stimuli"				
Attractant increases				
$0 \rightarrow 10^{-5}$ M aspartate	Reversing → CCW	4.3 ± 1.1 min	No response	
$0 \rightarrow 10^{-5}$ M serine	Reversing → CCW	6.7 ± 1.5 min		
Repellent decreases				
$10^{-2} \rightarrow 0$ M leucine	Reversing → CCW	1–2 min	(See text)	

[a] Data are based on observations of at least ten cells in each experiment. The criterion for termination of a response was one full revolution in the opposite direction. There was considerable cell-to-cell variation in response times, and means were determined only for the longer responses. In the case of very brief responses, some individuals probably recovered while the solution changes were being made; during this period, reliable observations were impossible due to effects of solution flow on the behavior of rotating cells.

These effects could be due to a slight amount of leakiness or, alternatively, to a backup adaptation system that does not depend upon *cheX* function. The oscillation between exclusively counterclockwise rotation and clockwise or reversing behavior (shown in Figure 1) was not observed when stronger clockwise stimuli, such as 10^{-1} M leucine, were used (data not shown). The cause of these oscillations is not known, but the mechanism might be related to the one that causes spontaneous reversals in wild-type cells. A possible explanation of these effects is mentioned in the Discussion.

The group 2 *cheX* mutants, which failed to respond to 10^{-3} M leucine, did respond at higher concentrations. Examples of the *X243* responses are shown in Figure 2. Note that some cells responded to 5×10^{-3} M leucine and that others did not respond even to 10^{-2} M leucine. Responses in the latter individuals could usually be induced by still higher levels of leucine (data not shown). The cell-to-cell variability in initiating a leucine response may reflect individual differences in physiological condition due to leakiness. Since the response thresholds of group 2 strains are clearly greater than in wild-type, it appears that *cheX* function is somehow required in eliciting clockwise responses to leucine stimuli. Tests with other repellents demonstrated that high response thresholds were a general feature of the group 2 strains (data not shown). As shown in Figure 2, however, once a response was initiated, it tended to persist, again implying an adaptation defect.

Attractant Responses of *cheX* Strains

Although *cheX* mutants rotate counterclockwise in the unstimulated state, the fact that in wild-type conflicting stimuli are summed algebraically by the signalling system suggested a way to test *cheX* mutants for attractant responses. *cheX* strains were first presented with 10^{-2} M leucine to elicit clockwise rotation and then switched to a solution containing the same amount of leucine, but an attractant as well. An immediate reversal of the clockwise response would indicate that the attractant had been detected. We found that challenges with 10^{-5} M aspartate or 10^{-5} M serine were able to overcome the leucine response in all six of the *cheX* strains studied. The repellent response persisted, however, when 5×10^{-7} M aspartate or serine were used. Since the wild-type exhibited similar behavior, the challenge assay demonstrates that *cheX* mutants can detect attractant stimuli and that the thresholds for detection may not be greatly different from wild-type.

The relative strengths of attractant and repellent stimuli have been established by determining the dominant stimulus in various conflict experiments (see Adler and Tso, 1974). In wild-type, simultane-

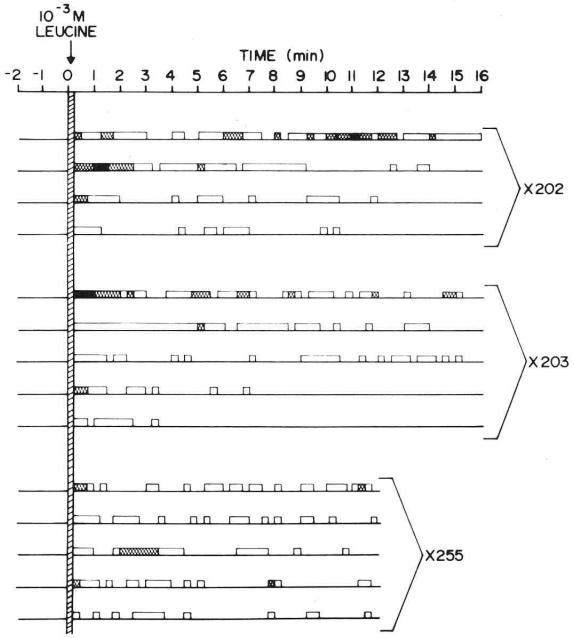

Figure 1. Responses of Group 1 *cheX* Mutants to 10^{-3} M Leucine

The rotational behavior of tethered cells in motility buffer was observed for several minutes at the beginning of the experiment. The chamber was then flushed with motility buffer containing 10^{-3} M leucine and subsequent behavior was followed. Each line represents the rotational pattern of an individual cell: a single line denotes exclusively counterclockwise rotation; open boxes denote predominately counterclockwise rotation, but reversing; shaded boxes denote predominantly clockwise rotation, but reversing; and solid boxes denote exclusively clockwise rotation.

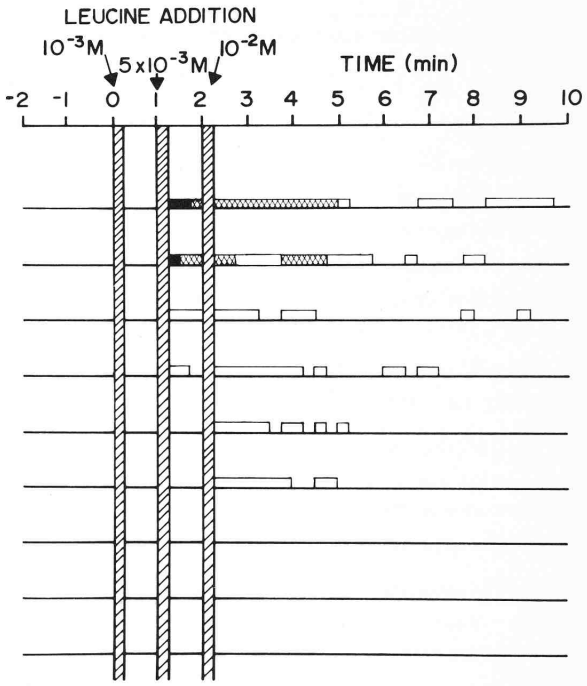

Figure 2. Responses of RP4336 Cells (*cheX243*) to Leucine Stimuli

At 0 min, the tethering chamber was flushed with motility buffer containing 10^{-3} M leucine. At 1 min, the leucine concentration was raised to 5×10^{-3} M and at 2 min to 10^{-2} M. Each line represents the rotational behavior of an individual cell as described in the legend to Figure 1.

ous presentation of an attractant and a repellent results in either clockwise or counterclockwise rotation, depending on which of the two stimuli is the stronger. For example, we found that a combination of 10^{-5} M aspartate or serine plus 10^{-2} M leucine produced a counterclockwise response in RP487, demonstrating that these attractant stimuli dominate the repellent stimulus. If the repellent is added sometime after the attractant, it should elicit a clockwise response provided that the cell has been able to adapt to the prior attractant stimulus. Thus adaptation to attractant stimuli can be monitored by testing for a response to a weaker clockwise stimulus. The results of such an experiment to measure attractant adaptation in *cheX* mutants are summarized in Figure 3. Cells were presented with either 10^{-5} M aspartate or 10^{-5} M serine and then challenged at various times with solutions containing the same concentration of attractant and, in addition, 10^{-2} M leucine. In wild-type, the leucine challenge first elicited a response approximately halfway through the adaptation period. However, all six *cheX* strains failed to respond to the leucine challenge, even at 10 min, when adaptation in the wild-type strain was complete. Repellent responses could be observed after removal of

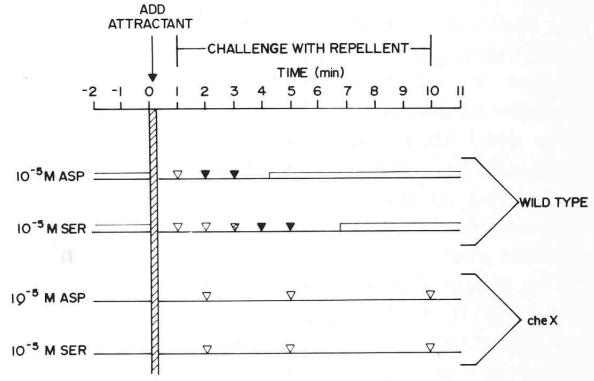

Figure 3. Results of Repellent Challenge Assay for Attractant Adaptation

At 0 min, the tethering chamber was flushed with motility buffer containing 10^{-5} M aspartate or serine. At subsequent times (indicated by the triangles), the chamber was flushed with 10^{-5} M attractant plus 10^{-2} M leucine. Open triangles indicate that no change in behavior was produced by the repellent challenge; shaded and solid triangles denote partial and full responses, respectively, to the leucine stimulus. Each challenge point represents the results of a separate experiment involving at least ten cells. The same individuals were not used in more than one experiment and all six *cheX* strains listed in Table 1 were tested. Behavior of the cells in the absence of a repellent challenge is indicated by the horizontal lines, as described in the legend to Figure 1.

the attractant, showing that the attractants had not somehow destroyed the ability of *cheX* strains to exhibit clockwise rotation (data not shown). These results demonstrate that *cheX* mutants are defective in adapting to attractant increases.

Another experiment for detecting attractant adaptation in *cheX* strains is shown in Figure 4. In this experiment an attractant, either aspartate or serine, was presented to the cells and then removed after 15 min. In wild-type, removal of the attractant elicited a brief period of clockwise rotation and was evidently perceived as a clockwise stimulus. It seems probable that this can happen only if adaptation to the initial attractant increase has taken place. If adaptation does not occur, attractant removal should simply restore the cells to their pre-stimulus condition. In this test, the *cheX* mutants again fell into two groups. The group 1 strains exhibited a few reversals upon attractant removal, indicating that some adaptation had probably occurred. As previously mentioned, this group of mutants may be somewhat leaky. Group 2 strains did not respond to attractant removal, which could be due to a more severe defect in adaptation. This test should be more sensitive than the repellent challenge experiment described above, which probably accounts for the fact that it can distinguish group 1 and group 2 strains, whereas the challenge assay did not. Both experiments, however, provide convincing evidence that *cheX* mutants have severe defects in adapting to attractant stimuli as well as to repellent stimuli.

Adaptation Mutants of S. typhimurium

The chemotactic behavior of Salmonella typhimurium appears to be quite similar to that of E. coli (Koshland, 1977). Generally nonchemotactic mutants of Salmonella have been isolated (Aswad and Koshland, 1975a; Collins and Stocker, 1976), and nine complementation groups have been defined (Warrick, Taylor and Koshland, 1977). Interspecies crosses have demonstrated that E. coli and Salmonella *che* functions are generally interchangeable, and the correspondence between many of the *che* genes has been determined (A. DeFranco, J. S. Parkinson and D. E. Koshland, manuscript in preparation). The *cheX* gene of E. coli is functionally homologous to the *cheR* gene of S. typhimurium, suggesting that *cheR* mutants might also be defective in sensory adaptation.

Like *cheX*, *cheR* mutants exhibit a low frequency of spontaneous tumbling behavior and the bacteria swim smoothly. We examined the behavioral responses of one *cheR* strain by subjecting swimming cells to temporal stimuli in the manner described by Goy et al. (1978). The results of those experiments are summarized in Table 3 and indicate that the *cheR* strain which we studied resembled group 2 *cheX* mutants in its behavior. For example, it did not respond to 10^{-3} M leucine, but did respond to a variety of stronger repellent stim-

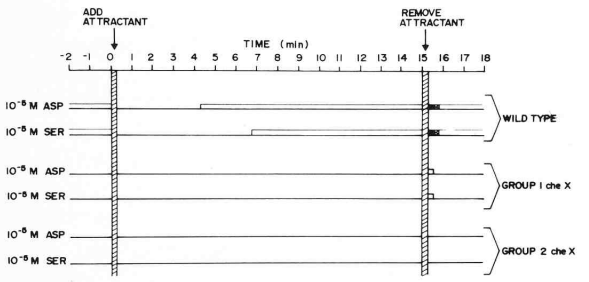

Figure 4. Responses of RP487 and RP487 *cheX* Strains to Attractant Decreases

At 0 min, the tethering chamber was flushed with motility buffer containing 10^{-5} M aspartate or serine. At 15 min, the chamber was flushed with motility buffer alone to remove attractant. Each line summarizes the rotational behavior of many cells, following the conventions described in the legend to Figure 1: wild-type (RP487), 22 cells; group 1 *cheX* mutants (RP4332, RP4333, RP4337), 43 cells; group 2 *cheX* mutants (RP4334, RP4335, RP4336), 47 cells.

Table 3. Behavior of S. typhimurium *cheR* Mutant[a]

Stimulus	Swimming Response	Duration
Repellents		
Leucine		
$0 \rightarrow 10^{-3}$ M	No response	
$0 \rightarrow 10^{-1}$ M	Smooth → tumbly	~5 min
Acetate		
$0 \rightarrow 10^{-3}$ M	Smooth → tumbly	<0.5 min
$0 \rightarrow 10^{-1}$ M	Smooth → tumbly	>5 min
Indole		
$0 \rightarrow 10^{-4}$ M	Smooth → tumbly	>5 min
Phenol		
$0 \rightarrow 10^{-3}$ M	Smooth → tumbly	>10 min
Mixed Stimuli		
10^{-4} M indole + 10^{-5} M serine	No response	
10^{-4} M indole + 10^{-5} M aspartate	No response	
Repellent Challenges[b]		
10^{-5} M serine → 10^{-4} M indole	No response	
10^{-5} M asparate → 10^{-4} M indole	No response	

[a] Bacteria were grown and tested as described by Goy et al. (1978).

[b] Attractants, at the final concentration indicated, were added at 0 min. At 10 min, cells were challenged with a mixture of repellent and attractant to maintain constant attractant levels throughout.

uli. The tumbly swimming induced by these stimuli persisted for a relatively long time, demonstrating a defect in adaptation. The *cheR* strain also appeared to detect attractant increases, as was shown by using a combination of attractant and repellent stimuli, but repellent challenge assays indicated that the *cheR* mutant could not adapt to attractant stimuli. These findings suggest that the adaptation machinery of Salmonella and E. coli is similar.

Discussion

Chemotaxis in E. coli involves modulation of the direction of flagellar rotation by chemoreceptor signals (Larsen et al., 1974). Since different sensory inputs can be integrated by the transduction machinery (Tsang et al., 1973; Adler and Tso, 1974; Berg and Tedesco, 1975; Spudich and Koshland, 1975), flagellar rotation is in effect controlled by one signal which is regulated by all chemoreceptors. The apparent behavior of that signal in response to attractant and repellent stimuli, based on the studies cited above and confirmed in the present work, is summarized in Figure 5. For purposes of this discussion, we assume that the signal produces clockwise rotation whenever it exceeds a critical threshold value; when signal level falls below threshold, counterclockwise rotation is observed. To account for the frequent flagellar reversals exhibited by wild-type cells in the absence of stimuli, we imagine that transitions from one mode of rotation to the other occur frequently when signal level is near threshold. The cause of these reversals could be either spontaneous fluctuations in signal level or variations in the sensitivity of the rotational machinery to the signal.

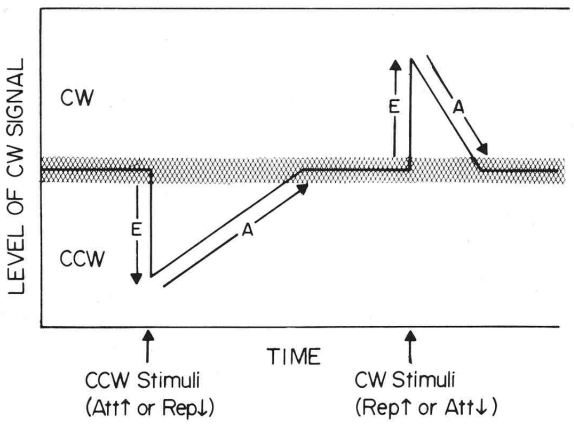

Figure 5. Signal Diagram of Wild-Type Behavior

The direction of flagellar rotation is determined by the level of a signal that is regulated by the chemoreceptors during excitation (E) and by the adaptation system (A). The shaded area indicates the transition region, or the range of signal values that result in frequent flagellar reversals. See the text for details.

As shown in Figure 5, there are two phases of signalling during a chemotactic response. The first phase, called excitation, produces a rapid increase or decrease of signal level in response to a change in chemoreceptor occupancy and initiates the flagellar response. Following excitation, the adaptation phase restores signal level to the threshold or pre-stimulus value. The purpose of the adaptation system is to maintain signal level within the transition region, ensuring that the organism is always poised to respond to either clockwise or counterclockwise stimuli in an effective way. The duration of the flagellar response is determined both by the magnitude of the initial signal change produced by excitation and by the rate of adaptation. As mentioned in the Results, the rate of adaptation to clockwise stimuli is considerably faster than to counterclockwise stimuli of similar strength.

We have shown that *cheX* mutants of E. coli have the following properties: exclusively counterclockwise rotation in the unstimulated state; the ability to detect attractant increases; the ability to detect repellent increases, but apparently with higher than normal thresholds; and an inability to adapt to either type of stimulus. Variation among strains and among individuals of the same strain is probably due to different degrees of leakiness. The behavior of *cheX* mutants can be explained in terms of a signal diagram as illustrated in Figure 6. We propose that *cheX* mutants are defective in the adaptation system and are therefore unable to maintain the level of clockwise signal within the transition range. We further assume that the production of clockwise signal by the adaptation system requires *cheX* function so that in the unstimulated state the level of clockwise signal lies below the transition value in *cheX* strains. It should be pointed out, as Goy et al. (1978) have also emphasized, that the failure of *cheX* mutants to exhibit clockwise rotation in the "unstimulated" state might actually be due to an inability to adapt to

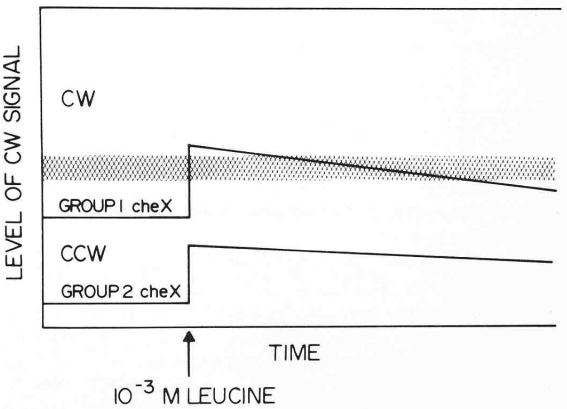

Figure 6. Signal Diagram of *cheX* Behavior

See the text for details.

various counterclockwise stimuli, such as oxygen or K^+ ion, that are present in motility buffer. It is not clear, therefore, that in the complete absence of chemotactic stimuli, *cheX* function would be required for clockwise rotation.

The fact that *cheX* mutants can respond to attractant and repellent stimuli demonstrates that *cheX* function is not directly involved in the excitation process. As indicated in the model of Figure 6, however, the response thresholds to clockwise stimuli should be greater than in wild-type because the basal signal level in *cheX* mutants is below the transition value. The threshold differences observed in group 1 and group 2 strains could reflect different basal signal levels due to variable extents of leakiness. The leaky nature of group 1 mutants might also explain the oscillations noted in their response to weak clockwise stimuli. After stimulation, the clockwise signal should slowly return toward the basal or unstimulated level as shown in Figure 6. This slow decline in signal level also implies a slow traverse through the transition region, producing an extended period of flagellar reversals as the signal decays. The time of onset of these oscillations should depend upon the magnitude of the initial stimulus, and in fact, we observed that stronger repellents elicited a much more dramatic clockwise response in group 1 *cheX* strains.

The signal that controls the direction of flagellar rotation may be generated by the products of two genes, *tar* and *tsr*, which have an important role in both the excitation and adaptation processes (Silverman and Simon, 1977b; Springer, Goy and Adler, 1977a). Mutants lacking either component are unable to respond to a variety of chemoreceptor inputs, and it seems probable that all receptors may use one or the other of these gene products during the excitation phase (Springer et al., 1977a). Springer et al. (1977a) and Silverman and Simon (1977b) found that response times in *tar* and *tsr* mutants were somewhat longer than in wild-type, indicating that *tar* and *tsr* function might be involved in adaptation as well as excitation.

Springer, Goy and Adler (1977b) have also shown that methionine is required for sensory adaptation in E. coli presumably as a donor of methyl groups, since protein synthesis is not required. Attractant stimuli have been found to enhance the methylation of several membrane proteins of E. coli (Kort et al., 1975) which were subsequently shown to be the products of the *tar* and *tsr* genes (Silverman and Simon, 1977b; Springer et al., 1977a). Goy, Springer and Adler (1977) studied the relationship between sensory adaptation events and the methylation of the *tar* and *tsr* proteins. They found that following an attractant stimulus, the level of methylation increased and then remained constant at the higher value. The time course of these changes corresponded to the duration of the behavioral response. Moreover, the new level of methylation was directly proportional to the absolute concentration of the attractant chemical. Repellent stimuli, on the other hand, produced a rapid decrease in methylation. Goy et al. (1977) suggested that adaptation to counterclockwise stimuli, such as attractant increases, is accompanied by the addition of methyl groups to the *tar* and *tsr* proteins, whereas adaptation to clockwise stimuli is accompanied by the removal of methyl groups from those proteins.

The possible role of the *tar* and *tsr* proteins in signalling is shown schematically in Figure 7. It seems probable that these proteins can exist in two alternative states, one of which corresponds to a clockwise rotational signal. This form probably represents the active configuration because *tar tsr* double mutants are defective in carrying out clockwise rotation (Silverman and Simon, 1977b; J. S. P., unpublished observations). In the absence of stimuli, clockwise signal is generated through methylation, which is controlled by the adaptation system and maintained at the transition level required for flagellar reversals. Upon an increase of repellent, additional clockwise signal is produced from the unmethylated form, perhaps by direct interaction with repellent receptors. Conversely, an attractant increase might convert a portion of the methylated clockwise form to an inactive state, producing a counterclockwise response. Adaptation to these stimuli is then achieved through the addition or removal of methyl groups in the remain-

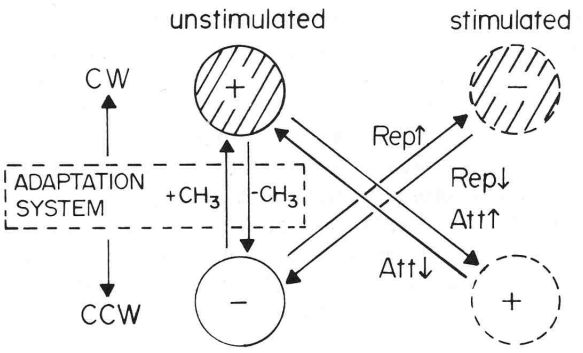

Figure 7. Model of the Signalling Process during Excitation and Adaptation

Circles represent the combined products of the *tar* and *tsr* genes. The amount of clockwise (shaded) form determines the direction of flagellar rotation. In the absence of stimuli, the clockwise form is generated through methylation controlled by the adaptation system. The overall amount of clockwise form is changed by stimuli to initiate flagellar responses. The broken circles represent *tar* and *tsr* proteins that have been acted on by repellent or attractant stimuli; these changes are reversed upon removal of the stimulus. Adaptation is achieved by methylation or demethylation of the unstimulated proteins to restore the proper level of clockwise signal.

ing supply of unstimulated proteins to restore the proper level of clockwise signal. Once adaptation is complete, removal of the stimulus reverses the initial sequence of events. The difference in response times to clockwise and counterclockwise stimuli is explained by the fact that demethylation can occur much more rapidly than methylation (Goy et al., 1977).

In the accompanying paper, Goy et al. (1978) demonstrate that *cheX* mutants are defective in methylating the *tar* and *tsr* proteins. Although it is difficult to demonstrate directly the presence of unmethylated *tar* and *tsr* proteins in *cheX* strains, it seems probable that this is the case based both on genetic evidence, which shows that *cheX* is distinct from the *tar* and *tsr* genes (Silverman and Simon, 1977a), and on the fact that excitation seems to occur normally in *cheX* strains. The behavioral properties of *cheX* mutants are also quite similar to those of wild-type strains that are unable to methylate due to a lack of methyl donors (Armstrong, 1972a, 1972b; Aswad and Koshland, 1974, 1975b; Springer et al., 1975). Like *cheX* mutants, such cells do not exhibit spontaneous tumbles, and although they can respond to certain stimuli, they do not appear to undergo adaptation. These findings imply that the behavior observed in *cheX* strains is caused by an inability to methylate the *tar* and *tsr* proteins. From previous discussion, it is clear that such a defect could readily account for the seemingly pleiotropic nature of the *cheX* behavioral phenotype.

We examined one *cheR* mutant of S. typhimurium and found that it exhibited adaptation defects comparable to those observed in *cheX* strains, suggesting that the mechanism of adaptation may be the same in both organisms. This conclusion is in disagreement with a report by Springer and Koshland (1977) which stated that *cheR* mutants showed wild-type response times to the repellent phenol. We have no explanation for the difference in results except to note that Springer and Koshland's experiments were performed in growth medium which may have contained attractant substances that could have influenced behavioral responses if *cheR* mutants were unable to adapt to them.

Springer and Koshland (1977) showed that *cheR* strains lack a cytoplasmic activity required for methylation of membrane proteins presumably analogous to the *tar* and *tsr* products in E. coli. The *cheX* product also appears to be a cytoplasmic protein (Ridgway, Silverman and Simon, 1977). It is not yet clear whether the *cheX* and *cheR* products are methylating enzymes. It is conceivable, for example, that they are only required for methyltransferase activity. Perhaps they impart specificity to the methylation system by enabling a nonspecific enzyme to recognize the *tar* and *tsr* proteins as substrates. Additional in vitro studies along the lines of those by Springer and Koshland (1977) should soon resolve this point.

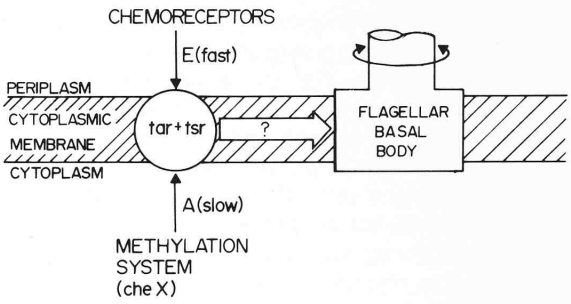

Figure 8. Summary of Sensory Transduction in E. coli

The *tar* and *tsr* proteins are located in the cytoplasmic membrane and may interact with chemoreceptors located in the periplasmic space and with a methylation system in the cytoplasm. Changes in these proteins in some way control the direction of flagellar rotation.

A summary of sensory transduction in E. coli is presented in Figure 8. The *tar* and *tsr* proteins reside in the cytoplasmic membrane and appear to be influenced both by chemoreceptors and by the methylation system. These events determine the level of a signal that modulates the direction of flagellar rotation. Many aspects of this process remain a mystery. For example, we do not yet know the form of the final signals or how they control flagellar rotation. Among the possibilities are changes in membrane potential (Szmelcman and Adler, 1976) and changes in the level of a cytoplasmic protein (Parkinson, 1977). Another unanswered question is how the adaptation system knows when to begin adding or removing methyl groups. In this regard, the *cheX* mutants may prove quite valuable in understanding the workings of the adaptation process. We are presently studying revertants of *cheX* strains in an attempt to identify possible targets and regulators of the methyltransferase activity.

Experimental Procedures

Bacterial Strains

RP487 (RP477 *metF*) is wild-type for chemotaxis (Kort et al., 1975) and has the genotype F⁻ *thi thr leu his metF* Δ(*gal-att*λ) *eda strA*. RP487 *cheX* derivatives were constructed by introducing various *cheX* alleles into RP487 by P1 co-transduction with the *eda* locus as described by Parkinson (1976). The isolation and genetic properties of *cheX* mutants are described elsewhere (Parkinson, 1976, 1978).

ST36 is a Salmonella typhimurium strain obtained from A. DeFranco and D. E. Koshland; it carries the *cheR57* mutation in a *his recA* background.

Cell Tethering

Bacteria were grown at 35°C in H1 salts-glucose medium (Adler, 1973) as previously described (Parkinson, 1976). Cells were washed twice by centrifugation at room temperature with equal volumes of 10^{-2} M potassium phosphate (pH 7.0), 10^{-4} M potas-

sium ethylene diamine tetracetate (motility buffer) containing 10^{-6} M methionine, and were then tethered to microscope coverslips with anti-flagellar antibodies (Parkinson, 1976).

Temporal Stimulation

The basic experimental design followed that of Berg and Tedesco (1975). Cells tethered to coverslips were placed in a sealed observation chamber that had inlet and outlet ports for changing solutions rapidly. Test solutions were drawn into the chamber by means of a peristaltic pump connected to the outlet side using a flow rate of approximately 2.2 ml/min. Under these conditions, a pumping time of 15 sec produced a change of concentration within the chamber of about 10^5 fold. All test chemicals were dissolved in motility buffer containing 10^{-6} M methionine.

Data Analysis

Observations were made with an inverted phase-contrast microscope connected to a video monitor and recorder. Data were transcribed by following individual cells and noting the direction of rotation and reversals with a manually operated event recorder. The resulting strip chart record was divided into 15 sec intervals, and the overall rotational behavior in each interval was classified as either reversing or exclusively clockwise or counterclockwise.

Acknowledgments

We thank M. Goy, M. Springer and J. Adler for many constructive discussions of this work and manuscript, and A. DeFranco and D. E. Koshland for the Salmonella *cheR* strain. This research was supported by a grant to J. S. P. from the National Institute of General Medical Sciences.

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Received July 24, 1978

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