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Potentiation, desensitization, and inversion of response in bacterial sensing of chemical stimuli

(chemotaxis/*Salmonella typhimurium*/sensory transduction)

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ABSTRACT **Behavior patterns of chemotactic mutants of *Salmonella typhimurium* were compared to those of the wild type by using the quantitative tumble frequency assay. Some *cheU* mutants were completely inverted in their responses—e.g., attractants produce responses expected for repellents and repellents produce responses expected for attractants. Still others swam smoothly and did not respond to any stimuli. Mutants of other complementation groups were found to exhibit exact additivity or potentiation in response to multiple stimuli whereas the wild type showed desensitization. The results suggest that the *cheU* gene product acts as a switch at the interface between the sensing system and the motor response. The system is finely tuned so that changes in individual proteins can produce potentiation, desensitization, exact additivity, or inversion of responses.**

Bacterial chemotaxis serves as a simple model for a sensory system. Many properties of the system have been described in recent reviews (1–6). A schematic mechanism for the sensory system as revealed by a various studies is summarized in Fig. 1. An input stimulus impinges on receptors in the cell that are specific for certain chemicals, pH, temperature, etc. (7). The signal is then processed by a machinery that includes at least nine gene products common to all chemotactic signals (8–10) and several genes that appear to be common to some but not all of the receptor signals (11–15). The sensing system utilizes a rudimentary memory and has the capacity to adapt (16). It produces the behavioral response by controlling tumbling frequency (16, 17), a tumble being caused by a reversal of the flagellar motors (18, 19).

A response regulator model has been developed to explain the existence of the bacterial memory and the adaptive response of the processing system (1, 2, 16). It assumes that the level of a response regulator varies relative to a threshold such that it controls the frequency of tumbling. The response regulator level is controlled by the rate constants of the enzymatic machinery and the external stimuli impinging on the cell (1, 2, 16). This model made it possible to explain the behavior of nonchemotactic mutants (6, 20) and the effect of methionine (20, 21), even though the primary nature of the response regulator (whether a chemical, an electrochemical potential, a metal ion, or a protein complex) is not yet known.

One prediction of such a model is that altering the time constants of the system by sequential stimuli or by mutation might result in different additive properties and may cause potentiation and desensitization as observed in higher sensory systems (22). Mutants in the chemotactic machinery were therefore examined by using the quantitative tumble frequency assay (23). The results reveal that the bacterial system has the capacity to show both exact additivity in responses to stimuli and also nonadditivity such as desensitization and potentiation. Furthermore, mutants were found that have inverted responses to stimuli; that is, they migrate toward substances that are normally repellents and away from substances that are normally attractants.

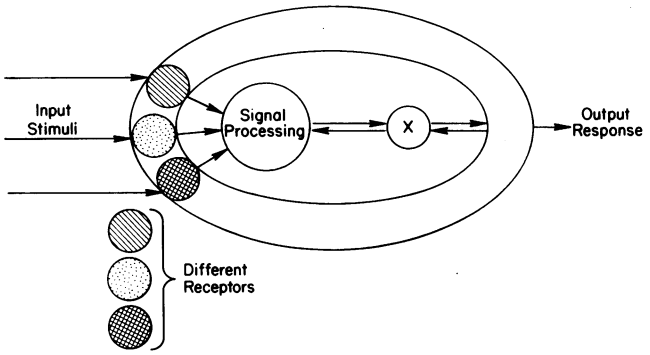

FIG. 1. Signal processing system. External stimuli activate receptors at the cell surface which then convey information to the central signal processing system. This system compares this new information to the previous state and produces alterations in levels of the response regulator, X, for any changes in state. The level of X controls the output response, the frequency of tumbling. The double arrows in the scheme indicate possibilities of feedback between output and signal processing.

METHODS

Bacterial Strains and Culture Conditions. The strains used are ultimately all derivatives of wild-type *Salmonella typhimurium* LT2, except for the *Escherichia coli* mutant RP4310 (*cheB* 274), which was obtained from J. S. Parkinson (24). ST1 was selected from LT2 as a spontaneous *his*$^-$ and *thy*$^-$ auxotroph obtained by serial selections (21). ST120, ST134, ST155, and ST171 were obtained by diethyl sulfate mutagenesis of ST23, followed by selection for their inability to migrate in a preformed liquid gradient of attractant (21). ST213 and ST221 were obtained by sodium nitrite mutagenesis of ST23 followed by the same selection procedure (8). ST313 is a transductant of SL4041 (*cheX*) (25) into the LT2 genetic background.

Cells were grown in Vogel–Bonner citrate medium (26) at 30° with auxotrophic requirements of 0.05 g of L-histidine and 0.03 g of thymine per liter as needed. Cultures were harvested in logarithmic phase by centrifugation at 3000 × *g* and resuspended in fresh medium with auxotrophic requirements and 0.1 g of chloramphenicol per liter.

Chemotaxis was studied by the quantitative tumble frequency assay (23), the semiquantitative tumbling assay (16), and the capillary assay (27).

RESULTS

Inverted Response Mutant. In Fig. 2 are shown the responses of the chemotactically wild-type strain, ST23, and the

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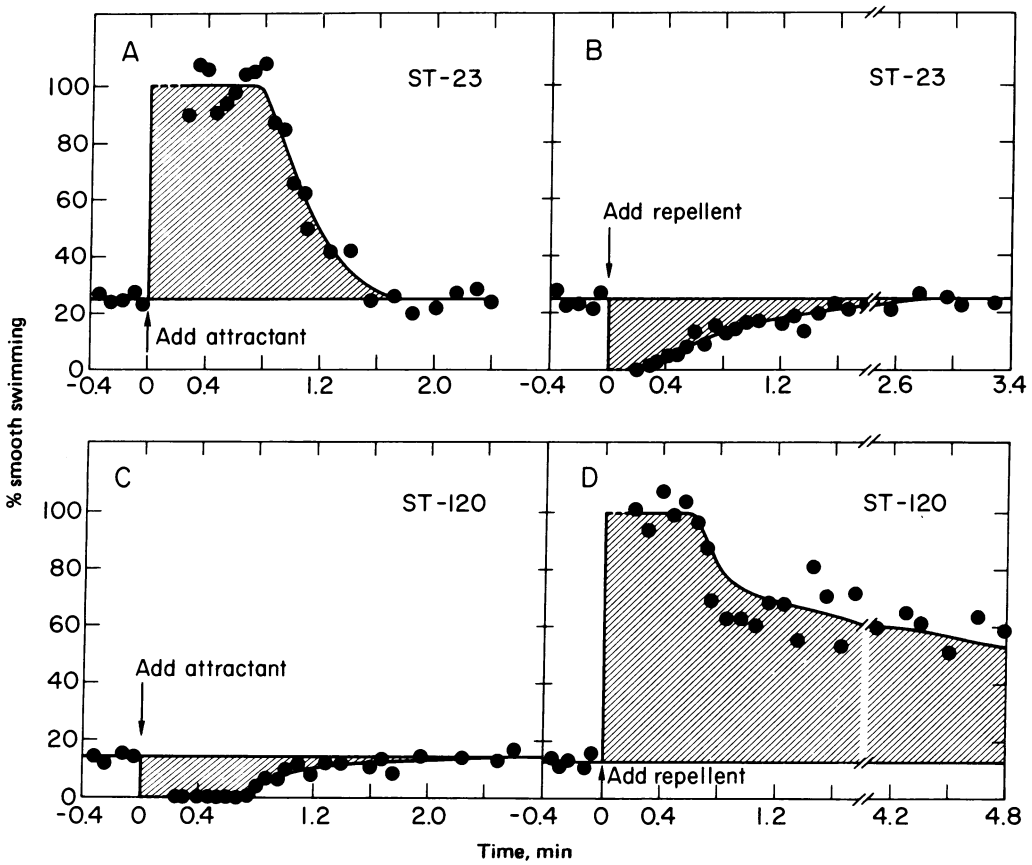

FIG. 2. Behavioral responses of wild type (ST23) and inverted response mutant (ST120) to temporal gradients of attractant and repellent. The stimuli (attractant, 0 → 1 mM L-aspartate; repellent, 0 → 5 mM phenol) were applied at zero time. The response and recovery of the populations were followed by measuring the fraction of the maximum number of tracks at each time point as described (22). The baseline, about 20% for both strains, measures the behavioral patterns in the absence of stimuli and in complete recovery from a stimulus. Data points above the baseline indicate an increase in the smooth swimming mode as compared to a nongradient situation; those below the baseline indicate an increase in tumbling.

cheU mutant, ST120. Responses were measured by the quantitative tumble frequency procedure (23) in which the percentage of cells that are smooth swimming is recorded (100%, all smooth swimming; 0%, all tumbling).

Addition of attractant (1 mM L-aspartate) to the wild type led to a suppression of tumbling and a gradual return to the random swimming pattern in a total of 1.6 min. Addition of repellent (5 mM phenol) caused an increase in tumbling and then adaptation to random behavior after a period of 3 min. In the mutant ST120, increases in the level of attractant caused tumbling and increases in the level of repellent caused smooth swimming, the reverse of the normal behavior pattern. The behavior in a spatial gradient was tested in a capillary containing chemoeffector. The mutant migrated away from L-serine (an attractant for wild type) and toward phenol (a repellent for wild type) (Fig. 3). The time courses of the migrations were similar for the two strains.

The behavior as well as the response times of ST120 were surprisingly similar to those of the wild type (Table 1). However, the response to 10 mM phenol was distinguishably different in the wild type and ST120.

This mutation in *S. typhimurium* maps in the *cheU* gene which was found (8) to be in the same locus as the *flaQ* gene previously identified by Iino (28). In *E. coli*, a parallel system exists, and Simon (29) showed that the *cheC* complementation group maps in the *flaA* gene. The same location of a chemotactic gene and a flagella gene suggests that they result from different modifications of the same protein. The *fla*$^-$ phenotype causes complete loss of flagella formation. The *che*$^-$ mutant has flagella but its response to gradients is aberrant. This

Table 1. Responses of wild type to temporal gradients of chemoeffectors (ST23) and inverted response mutant (ST120)

Strain	Nongradient behavior	Recovery time after temporal gradients				
		1 mM serine	1 mM aspartate	1 mM ribose	10 mM phenol	50 mM acetate
ST23	Random tumbling	Smooth 5 min	Smooth 1.7 min	Smooth 0.9 min	Tumbling 3.0 min	Tumbling <0.1 min
ST120	Random but more tumbling than wild type	Tumbling 4.5 min	Tumbling 1.6 min	Tumbling 0.9 min	Smooth 10 min	Smooth <0.2 min

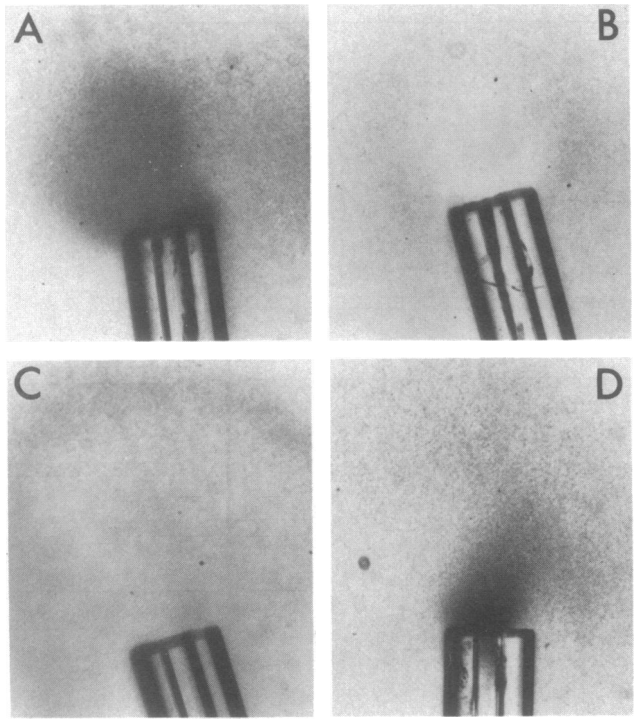

FIG. 3. Migration of wild type (ST23) and inverted response mutant (ST120) to chemoeffectors. (*A*) ST23 accumulated outside capillary containing 10 mM L-serine. (*B*) ST23 moved away from 10 mM phenol. (*C*) ST120 moved away from 10 mM L-serine. (*D*) ST120 accumulated near capillary containing 10 mM phenol. All photographs were taken at 5 min after introduction of the capillaries into the microchambers.

suggests that the more moderate mutation allows synthesis of the flagella but alters the sensing function.

Inverted Response Mutant with Time Lag. In Table 2 are listed other *cheU* mutants obtained by various types of mutagenesis. ST155 showed properties significantly different from those of ST120 or ST134. In this strain there was a measurable delay period before the behavioral response to attractant ensued, but the repellent response was immediate and similar to that of ST120. Temporal increases in attractant concentrations produced no change in behavior for the first minute or longer; then tumbling began and was followed by adaptation. The L-aspartate caused no detectable change in the tumbling frequency for up to 5 min after delivery of the stimulus (Fig. 4*A*). However, the bacteria were immediately responsive to 1 mM phenol, showing transient smooth swimming (Fig. 4*B*).

The bacteria were not actually insensitive to 0.5 mM L-aspartate but they did show a modified behavior pattern. A mixture of attractant and repellent that gave a 1.4-min smooth swimming response in the wild type gave a 1.2-min tumbling response in this reversal mutant (Fig. 4*C*). Moreover, sequential addition of repellent followed by 0.5 mM L-aspartate attractant showed a potentiation effect (Fig. 4*D*). About 1 min after receiving a temporal increase in L-serine from 0 to 1 mM, the bacteria began to tumble and continue to tumble for about 5 min (a time period comparable to that in the wild type) and then adapted back to the original tumbling frequency. A similar delayed response followed a 0 → 1 mM L-aspartate addition, except that the tumbling response was much less pronounced with only slight suppression of smooth swimming.

Smooth Swimming *cheU* Mutants. Another category of mutants assigned to the *cheU* gene appears to be constantly smooth swimming. Neither attractants nor repellents produced any behavioral changes in these mutants.

Potentiation and Desensitization. The critical importance of the *cheU* gene in sensory transduction is further supported by other mutant studies. We have shown (30) that there is an exactly additive relationship in the responses of wild-type cells to stepwise increases in stimuli with the same attractant—i.e., the sum of the recovery times with stimulus from 0 → 0.02 mM serine and from 0.02 → 0.5 mM serine is equivalent to the recovery time for a single step increase of 0 → 0.5 mM serine. When such studies were applied to different types of stimuli, an interesting pattern was observed (Table 3). In wild-type cells (LT2), two different types of attractant, (for example, L-aspartate plus D-ribose) did not necessarily give an exactly additive response. In fact, there appeared to be a desensitization—i.e., the response time to combined stimuli was not as great as the sum of the response times to the stimuli when presented separately. ST171 (*cheT*), on the other hand, showed exact additivity between two different types of stimuli (e.g., an amino acid and a sugar). Moreover, in other mutants a potentiating effect was observed—i.e., the response time to the combined stimuli was greater than the sum of the response times to the component stimuli. Potentiation was observed in ST313 (Table 3) and *E. coli* RP4310. Thus, the bacterial sensing system can show exact additivity, desensitization, and potentiation.

Table 2. Behavioral response of *cheU* mutants to temporal gradients of chemoeffectors

Strain	Description	Type of mutagenesis	Nongradient behavior	Response to positive temporal gradients		Reversion frequency/ 10^9 bacteria
				Attractants	Repellents	
ST23	Wild type	—	Random tumbling & smooth swimming	Smooth period and adaptation	Tumbling period and adaptation	—
ST120	Inverted response mutant	DES	Random but more tumbling than wt	Tumbling period and adaptation	Smooth period and adaptation	3
ST134	Inverted response mutant	DES	Random but more tumbling than wt	Tumbling period and adaptation	Smooth period and adaptation	16
ST155	Inverted response mutant	DES	Random but more tumbling than wt	Time delay followed by tumbling period and adaptation	Smooth period and adaptation	0
ST213	Smooth swimming mutant	Sodium nitrite	Smooth; tumbles only rarely	No change	No change	0
ST221	Smooth swimming mutant	Sodium nitrite	Smooth; tumbles only rarely	No change	No change	100

DES, diethyl sulfate; wt, wild type.

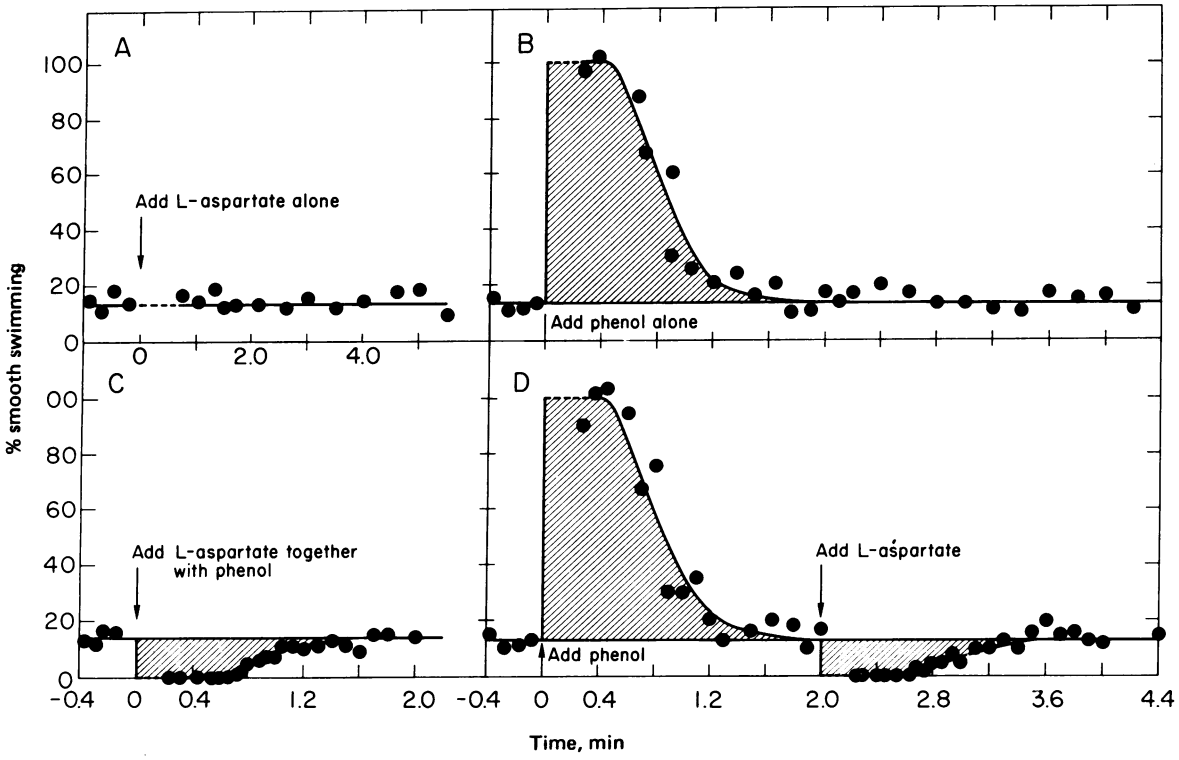

FIG. 4. Behavioral responses of inverted response mutant ST155 to chemoeffectors. Application of 0 → 0.5 mM L-aspartate at zero time produced no measurable behavioral change (*A*); 0 → 1 mM phenol produced a transient smooth swimming period (*B*). When 0 → 0.5 mM L-aspartate and 0 → 1 mM phenol were added together, a 1.2-min tumbling response was produced in ST155 (*C*) and a 1.4-min smooth swimming response was produced in the wild type (data not shown). When 1 mM phenol was applied at zero time and then, upon full recovery at 2.0 min, 0.5 mM L-aspartate was added (*D*), a 1.5-min tumbling period was observed, demonstrating a potentiation effect of prior incubation with repellent.

DISCUSSION

The above results can be summarized as follows. (*i*) A mutant has been found that has inverted responses to attractants and repellents. (*ii*) The inverted response mutant exists in a complementation group with other mutants, of which one exhibits a time lag with inverted responses and others produce no responses to either attractants or repellents. (*iii*) Other mutants and the wild type were shown to give exact additivity, desensitization, and potentiation in responses to multiple stimuli.

These results not only give further evidence for the similarity of bacterial systems to higher sensing systems but also emphasize the great importance of temporal processing in the bacterial sensing system. The presence of chemical effectors by themselves is not the only factor in bacterial behavior; the time sequence of stimuli also is a key element in the behavioral pattern. These behavioral patterns can also be considered in the light of the observation by Berg and Tedesco (31) that there is an overshoot phenomenon as in the action potentials of neurons (32).

Table 3. Behavorial responses to multiple stimuli

Salmonella strain	Temporal stimulus (0 indicated mM)	Recovery time, min: Observed	Recovery time, min: Expected, based on exact additivity	Property
LT2	1.0 D-Rib	0.33		
	0.01 L-Ser	0.58		
	1.0 D-Rib + 0.01 L-Ser	0.67	+0.91	Desensitization
	0.01 L-Asp	0.40		
	1.0 D-Rib + 0.01 L-Asp	0.53	+0.73	
ST171	0.05 L-Ser	1.02		
	1.0 L-Asp	1.74		
	0.05 L-Ser + 1.0 L-Asp	2.84	+2.76	Exact additivity
	0.2 D-Rib	0.95		
	0.2 L-Asp	1.20		
	0.2 D-Rib + 0.2 L-Asp	2.21	+2.15	
ST313	10 L-Ser	4.72		
	1.0 D-Rib	0.10		
	10 L-Ser + 1.0 D-Rib	8.52	+4.82	Potentiation
	1.0 L-Asp	0.22		
	1.0 L-Asp + 1.0 D-Rib	2.31	+0.32	

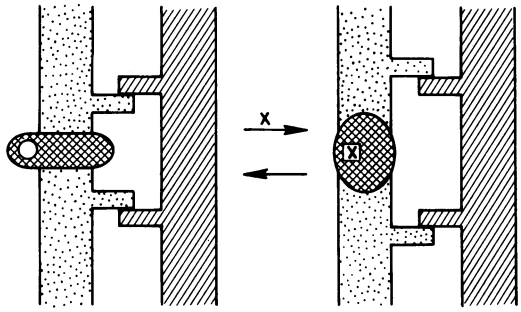

FIG. 5. Model for the *cheU* gene product. Signal effector, X, produced by the sensory system binds to the *cheU* gene product, inducing a conformational change in the latter which regulates the response by changing the flagellar motor from rotation in one direction to rotation in the opposite sense, producing smooth swimming. Mutations in *cheU* may affect the affinity for and rates of interaction with X as well as the interaction and relationship of the switching elements of the motor to the *cheU* gene product.

According to our model (16), the level of the response regulator, X, is altered by stimuli that modify the rate constants in the production and degradation of X. Both covalent changes such as methylation and mutations that change the properties of individual proteins would be expected to alter the time characteristics of sensing and have been found to do so.

Although it is relatively easy to explain additivity, sensitization, and desensitization by such a model, the inverted response mutant poses a more difficult problem. Two alternatives to explain the behavior of the *cheU* mutants are a mechanical defect and a sensory defect in a switch. By a mechanical defect we may envision the *cheU* gene product to be outside the sensory system, simply interpreting the normal signal in an inverse manner. (A gear shift in which reverse and forward have been interchanged would be an analogy.) A sensory defect in the switch, on the other hand, would be considered to be properly conveying information in regard to rotation of the flagellar motor but having defective interaction with the sensory output signals or defective feedback to the sensory system.

The evidence presented here suggests that the *cheU* gene product is involved in the sensory and not merely the mechanical aspects of the switch governing the direction of flagellar rotation. In the first place, the inverted responses of the mutant ST120 are not precisely comparable to the wild-type responses in time duration. The phenol response was 3 min in the wild type and 10 min in ST120. Second, both the time lag preceding the response to attractants of ST155 and the potentiation effect are difficult to explain by a mechanical defect.

Fig. 5 shows a model that can correlate some of these findings. In this model, X induces a conformational change in the *cheU* gene product and shifts the switching elements from one rotational mode to another. Parkinson (10) has syggested a switch role for the *cheC* gene of *E. coli*. Mutations in the *cheU* protein would then produce various effects because these act at the interface of the mechanical and sensing systems. In support of this interpretation we have found mutants in *cheU* having either altered affinity for X or altered signal transduction properties. In addition, we have found a mutant in *cheU* with an altered rate of interaction, as exemplified by the mutant with time-lag properties. Because the *cheU* mutations map in the *flaQ* locus, a gene product receiving sensory signals in the flagellar machinery is logical. As discussed above, mutants in other genes also produce changes in the time constants of the sensory system. Because some of the mutations are probably point mutations, based on the experience with diethyl sulfate mutagenesis (33) and the frequency of reversion, a single amino acid change can produce profound changes in behavioral responses. It is intriguing to note that the same types of structural changes can be produced by covalent modification such as methylation. Thus, a finely tuned sensory system can be selected for optimization of function by small changes in its proteins.

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