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Construction and Behavior of Strains with Mutations in Two Chemotaxis Genes

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Double mutants have been constructed by transducing each of the six *che* genes from the main *che* gene cluster into the *cheC* mutant with reversed behavior. The behavioral properties of these double-mutant strains were examined. The results are interpreted in terms of a model based on the *cheC* gene product being the component of the flagellar basal body that generates tumbling or smooth swimming in response to changes in the level of the response regulator. The properties of the double mutants can then be explained in ways which provide further understanding of the bacterial sensing system.

Early studies on the mechanism of bacterial chemotaxis led to a model whereby the behavior of the bacterium was related to the level of a response regulator (5, 7). The level of this parameter was postulated to change depending upon the concentration of chemoeffectors in the environment surrounding the cell and also on the length of time those stimulants had been present. Adaptation to the chemoeffectors over time was postulated to cause a return of the response regulator to its prestimulus level. This model has received support from a wide variety of data (6, 9; 12) and particularly from the discovery of strains with mutations in the *cheC* gene that responded to stimuli in an inverse manner, i.e., swam away from "attractants" and towards "repellents" (11).

Since other mutations in the *cheC* gene can lead to a nonmotile phenotype (16, 17), it seemed likely that the product of this gene was a component of the flagellar basal body and, furthermore, that this protein was the sensor which determined the direction of flagellar rotation (3, 9, 11). The finding that clockwise rotation causes an associated helical transition of the flagellum to the curly form (the inverse mode [8]) was a key component in explaining the apparently anomalous behavioral responses of the *cheC* mutants. By postulating (3) that the *cheC* mutation changed the affinity of the detector for the response regulator, the reversed responses to attractants and repellents were explained as a function of a normal level of response regulator coupled with an abnormal affinity of the sensor protein. Since the normal and inverse swimming modes can be distinguished under the proper conditions, it was possible to verify this explanation experimentally (3).

To clarify the behavioral processing system, we introduced other *che* gene mutations into the *cheC* strain with the inverted behavior by using the Tn*10* selection techniques of Kleckner et al. (4). The behavior of the resulting double mutants is examined in light of the previous information obtained about the *cheC* defect.

MATERIALS AND METHODS

Bacterial strains. Table 1 gives the bacterial strains used in these experiments and the intermediate strains used in their construction.

Construction of *cheC* double mutants. Selected *che* double mutants were constructed by introducing a Tn*10* insertion near the main *che* gene cluster of *Salmonella typhimurium*. This selectable marker was introduced next to a *che* mutation by transducing a strain with that mutation (selected on Luria broth plates containing 25 μg of tetracycline per ml) with P22 *int4* phage grown on ST314, a strain carrying the tetracycline insertion (4). The desired strain was obtained from about 10 of the transductants tested on tryptone swarm plates (1% tryptone, 0.5% NaCl, 0.3% agar) for the Che$^-$ phenotype (1), indicative of retention of the *che* mutation. This strain was then used to transduce ST120 (*cheC70*) to tetracycline resistance. The other *che* mutation was chosen as representative of its class and showed no dominance in complementation tests. Again, several transductants were tested to obtain the desired double mutant. Each of the transductants to be tested was used as a donor in abortive transduction complementation tests (2). A transductant that was unable to complement either of the parental *che* strains was selected as the double mutant.

Microscopic observation of bacterial behavior. Bacteria were observed with a dark-field microscope, and the behavioral response to attractants or repellents was observed by the temporal gradient method of Macnab and Koshland (7, 13). The double mutants were grown to mid-log phase in Vogel-Bonner citrate medium (15) supplemented with 1% glycerol and histidine. The cells were washed by centrifugation into this

TABLE 1. Double mutants and strains used in their construction[a]

che allele	Strain containing *che* allele (reference[b])	Strain containing *che* allele and Tn*10*	Double mutant containing *che* allele, Tn*10*, and *cheC70*
cheR57	ST107 (2)	ST337	ST342
cheZ221	ST171 (2)	ST339	ST346
cheY62	ST176 (2)	ST338	ST343
cheA52	ST172 (2)	ST336	ST345
cheW302	ST202 (16)	ST340	ST344
cheB111	SL4041 (14)	ST341	ST347

[a] Other strains used were ST120, whose relevant genotype was *cheC70* (2), and ST314, whose relevant genotype was *zea-2*::Tn*10* (A. L. DeFranco, Ph.D. thesis, University of California, Berkeley, 1979).
[b] Reference describing specific mutant.

same medium at about 10^8 cells per ml. The bacteria were mixed with the chemoeffector in a test tube, and the cells were rapidly transferred to the microscope slide. Responses of less than 0.3 min would have been scored as no response owing to the interval between the addition of the stimulant and the first observation.

RESULTS

Construction and behavior of double mutants. The availability of a selectable marker near the main cluster of *che* genes (Tn*10* insertion *zea-2*) made it possible to construct strains containing two *che* mutations, one from the *che* gene cluster and *cheC70*, a mutation which seems to alter the detector of the response regulator (3, 11). These double mutants were constructed as described in Materials and Methods, and their behavior was examined (Table 2).

In every case, the behavior of a *che* mutant was reversed by the addition of the *cheC70* mutation. Thus, the *cheR* mutant, which was smooth swimming in the absence of a gradient, became tumbly when the *cheC* deficiency was added to that of the *cheR*. Similarly, *cheY*, *cheW*, and *cheA*, which were initially smooth swimming, became tumbly upon addition of the second lesion. The opposite situation occurred for *cheZ* and *cheB*; normally tumbly as single mutants, they became smooth swimming in the doubly mutated strains.

These changes in unstimulated behavior can be explained readily by using the simple schematic model shown in Fig. 1, which is based on the relationship of behavioral mode to the level of the response regulator (3). The affinity of the signal detector for X was presumed to change owing to different point mutations in the *cheC* gene. As the affinity of the detector for X decreased, there was less detector X complex, so the concentration of X was too low to generate smooth swimming of the counter-clockwise rotation type. The normal smooth swimming of the strains with single mutations in *cheR*, *cheY*,

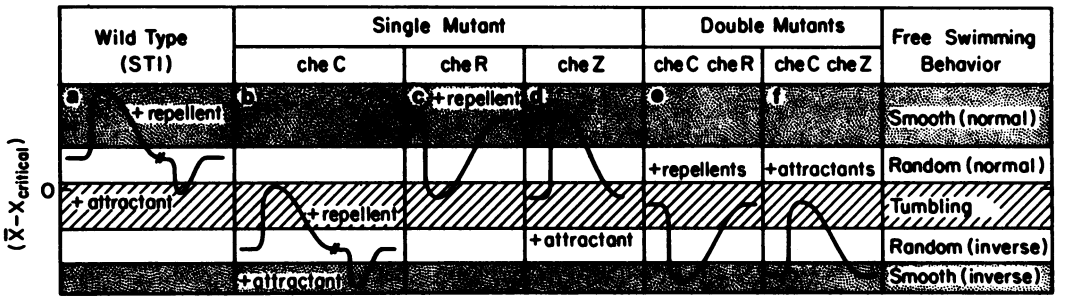

FIG. 1. Effect of *che* gene mutations on relative level of response regulator. The model of the control of motility behavior by the response regulator (3, 5, 7) is applied to the double mutants constructed here. The shifts in behavior caused by introducing the *cheC* mutation into other *che* mutants can be understood by postulating that the *cheC* mutation changes the affinity of the detector for the response regulator, thus changing the X_{crit} value. Mutations in other *che* genes are postulated to change the level of $\bar{X}$, the average level of the response regulator. The double mutants have a new level of $(\bar{X}\text{-}X_{crit})$ which is predicted by the changes in these two parameters caused by the individual mutations. For example, *cheR* raises the $\bar{X}$ value, and *cheC* raises the X_{crit} value. A *cheR cheC* double mutant (e) had a value of $(\bar{X}\text{-}X_{crit})$ intermediate between that of *cheR* (d) and that of *cheC* single mutants (b). Similarly, *cheZ* (d) lowers the level relative to wild type (a), so that the double mutant (f) has an $(\bar{X}\text{-}X_{crit})$ value lower than either. The effect of attractants and repellents on the change in $\bar{X}$ in a response remains the same, but the initial value starts from a different level of $(\bar{X}\text{-}X_{crit})$.

TABLE 2. Behavior of double mutants and their parents

Relevant *che* mutation	Parent single mutant[a]		Double mutant	Response of double mutant with *cheC* to addition of:			
	Prestimulus behavior	Response[b] to stimulus	Prestimulus behavior	Serine (10 mM)	Serine (0.1 mM)	Aspartate (5 mM)	Phenol (4 mM)
Wild type	Swimming and tumbling (normal)	Attractants n → s Repellents n → t					
cheR57	Smooth swimming	Repellents s → t	Constant tumbling	0	NT[c]	0	t → s 4 min
cheY62	Smooth swimming	0	Constant tumbling	0	NT	0	0
cheW302	Smooth swimming	0	Constant tumbling	0	NT	0	0
cheA52	Smooth swimming	0	Constant tumbling	0	NT	0	0
cheZ221	Constant tumbling	Attractants t → s	Smooth swimming	s → t 3.5 min	s → t 0.7 min	s → t 2 min	0
cheB111	Constant tumbling	Attractants t → s	Smooth swimming (with infrequent tumbles)	s → t 6 min	0	s → t 2 min	0
cheC70	Reversed normal	Attractants n → t Repellents n → s					

[a] Data from references 2 and 16.

[b] s → t, Smooth swimming to tumbling upon addition of stimulant; t → s, tumbling to smooth swimming upon addition of stimulant; n → s, t, normal behavior (swimming + tumbles) to swimming or tumbling; 0, no change in behavior.

[c] NT, Not tested.

cheW, or *cheA* was postulated to be due to an excessive concentration of the response regulator X. Introduction of the mutated *cheC* gene caused a decrease in the affinity for X and changed the behavior from the smooth zone shown at the top of Fig.1 to the tumbly zone shown in the middle. Similarly, single mutations in the *cheZ* or *cheB* genes decreased X, resulting in an ($X-X_{crit}$) value which placed these mutants in the middle tumbling zone. The addition of the *cheC* mutation in the double mutants further lowered the ($X-X_{crit}$) value to the lower inverse smooth-swimming zone.

This explanation was straightforward. The next step was to explain the responses of the double mutants to chemoeffectors, and this was no longer as simple. The explanation of *cheR* nongradient behavior would predict that attractant added to the *cheR cheC* double mutant would change it from the tumbly zone to the normal smooth-swimming zone or possibly to the normal random zone. No such response was observed with either high concentrations of serine or saturating concentrations of aspartic acid. On the other hand, phenol, a repellent, did elicit an expected response which pushed the response regulator level to the low value, giving inverse smooth swimming. Although the *cheR cheC* double mutant did not respond detectably to attractants, it is not blind to them, since addition of 10 mM serine or 5 mM aspartic acid eliminated the response to phenol, even if the attractants had been present for a full 30 min before the repellent was added (data not shown). The most likely explanation for these observations is that the normal smooth-swimming zone is unattainable in strains with the *cheC70* mutation. Indeed, we have never encountered a situation in which this behavioral mode was expressed in such strains. This behavioral zone would be unattainable if it required a value of X that was greater than some practical limit (such as exceeding the solubility of X) or conversely below some practical limit (such as X = 0).

The responses of the *cheZ* and *cheB* double mutants with *cheC* to attractants and repellents is easily explained. The responses to attractants in these double mutants are what might be expected, a movement from the lowest inverse smooth-swimming zone to the tumbly zone. Such an increase in the level of the response regulator on exposure to attractant was also observed in the single mutants, which changed from the tumbly zone to the smooth-swimming zone (16). However, no response was seen upon addition of phenol. This might be explained by the fact that the cell was already near the bottom zone and it may be difficult to detect an increase in inverse smooth swimming when an organism is mostly swimming smoothly initially.

Mutations in *cheY*, *cheW*, and *cheA* cause smooth swimming in a $cheC^+$ background. In a *cheC70* background, each of these mutations caused constant tumbling, and no responses to attractants or repellents could be demonstrated in these strains. This suggests that the *cheW*, *cheY*, and *cheA* genes each may be required for responses to chemoeffectors, and the level of response regulator cannot be altered in their absence.

DISCUSSION

Double mutants were constructed that were mutated in the *cheC* gene and in one of six other *che* genes. These strains clarified the effect of the *cheC* mutations on the chemotactic system and also the role of the other *che* mutations. The swimming behavior of each double mutant was completely consistent with the idea that the *cheC* mutant is altered in its detection of the response regulator. This model was derived from analysis of the rotational properties of the flagella of this mutant by Kahn et al. (3), who showed that smooth swimming could result from rotational behavior that normally causes tumbling (CW rotation), and hence that the real cause of tumbles is a reversal of rotation direction rather than the direction of rotation itself. Furthermore, the smooth swimming observed in strains with the reversed *cheC* mutations (ST120, etc.) was inverse, the result of CW flagellar rotation.

This explanation for the nature of the *cheC* mutants also makes it possible to understand the behavior of the double mutants. In each of the six *che* mutants that were used to donate the second *che* mutation into ST120, the relative level of the response regulator was altered, because the detector had a change in affinity. In *cheR*, *cheY*, *cheA*, and *cheW*, it was relatively higher than normal, causing constant smooth swimming. In *cheB* and *cheZ*, the level of the response regulator was lower than normal, causing constant tumbling. When the former mutations were introduced into the *cheC* background, the resulting double mutants all tumbled incessantly. Thus, the higher level of response regulator placed them in the constant tumbling zone rather than the zone of inverse swimming intermixed with occasional tumbles of the parental *cheC* mutant. In none of these mutants could normal swimming be observed, nor could it be elicited with attractant stimuli. This could be explained either by the altered *cheC* affinity for the response regulator, or it could be that the level of the response regulator cannot rise high enough (or low enough) to effect this state.

These studies indicate that double mutants can be used to probe behavioral systems to

clarify interrelationships between genes. They also can be used as a valuable experimental tool to make the system subject to new tests. For example, a smooth-swimming mutant which cannot be tested with attractants can be altered to a tumbly mutant by insertion of the *cheC* gene. This shift in detector level allows attractants which generate smooth swimming to be evaluated.

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