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Identification of a Site of ATP Requirement for Signal Processing in Bacterial Chemotaxis

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In *Escherichia coli* and *Salmonella typhimurium*, ATP is required for chemotaxis and for a normal probability of clockwise rotation of the flagellar motors, in addition to the requirement for *S*-adenosylmethionine (J. Shioi, R. J. Galloway, M. Niwano, R. E. Chinnock, and B. L. Taylor, J. Biol. Chem. 257:7969–7975, 1982). The site of the ATP requirement was investigated. The times required for *S. typhimurium* ST23 (*hisF*) to adapt to a step increase in serine, phenol, or benzoate were similar in cells depleted of ATP and in cells with normal levels of ATP. This established that ATP was not required for the chemotactic signal to cross the inner membrane or for adaptation to the transmembrane signal to occur. Depletion of ATP did not affect the probability of clockwise rotation in *E. coli cheYZ scy* strains that were defective in the *cheY* and *cheZ* genes and had a partially compensating mutation in the motor switch. Strain HCB326 (*cheAWRBYZ tar tap tsr trg*::Tn*10*), which was deficient in all chemotaxis components except the switch and motor, was transformed with the pCK63 plasmid (p*tac-cheY*$^+$). Induction of *cheY* in the transformant increased the frequency of clockwise rotation, but except at the highest levels of CheY overproduction, clockwise rotation was abolished by depleting ATP. It is proposed that the CheY protein is normally in an inactive form and that ATP is required for formation of an active CheY* protein that binds to the switch on the flagellar motors and initiates clockwise rotation. Depletion of ATP partially inhibits feedback regulation of the *cheB* product, protein methylesterase, but this may reflect a second site of ATP action in chemotaxis.

Bacterial chemotaxis is an elementary sensory response system in which the reception, signal processing, and final response take place within a single cell. Considerable progress has been made toward the goal of describing the sensory transduction pathway in terms of molecular events (5, 17, 25). However, most current knowledge is of events that occur at the beginning or end of the transduction pathway. The signal produced by an attractant is transmitted by integral membrane proteins, known as transducing proteins, from the outer surface to the inner surface of the cytoplasmic membrane (Fig. 1). The best-characterized transducing proteins, the products of the *tsr*, *tar*, *trg*, and *tap* genes in *Escherichia coli*, are methylated in the process of adaptation to an attractant (20, 22, 34). There are other chemotaxis pathways in *Salmonella typhimurium* and *E. coli*, such as the pathways for oxygen and certain sugars, that are independent of transducer methylation (21, 43). The present discussion will be limited to the methylation-dependent pathways; the methylation-independent pathways will be discussed elsewhere (J. M. Smith, A. J. Wolfe, M. P. Conley, H. C. Berg, and B. L. Taylor, manuscript in preparation).

Bacteria with peritrichous flagellation swim forward when the flagellar motors rotate in a counterclockwise direction (33). In an environment to which they are adapted, the bacteria occasionally reverse the direction of flagellar rotation for a brief interval (3). This produces a momentary chaotic tumble that reorients the direction of swimming so that the bacteria move in a three-dimensional random walk through space. Chemotaxis is achieved by changing the probability of tumbling, thereby biasing the random walk (3, 17).

The methylatable transducing proteins in *S. typhimurium* and *E. coli* (Fig. 1) consist of a periplasmic domain that recognizes the attractant and a cytoplasmic domain that includes a signaling sequence that transmits a signal to the switch on the flagellar motors, thereby locking the motors into the counterclockwise (i.e., swimming) mode (13). A putative signaling sequence in the cytoplasmic domain is flanked by methylation regions. Protein methyltransferase, the product of the *cheR* gene, progressively methylates γ-glutamyl residues in the methylation regions until the attractant-induced signal is cancelled and the cell is adapted to the presence of the attractant (34, 37). If the attractant is removed or a repellent is introduced, the transducing protein sends a signal for clockwise rotation of the motors. Protein methylesterase, the product of the *cheB* gene, progressively hydrolyzes the γ-glutamyl methyl esters until the signal is again returned to the prestimulus state (42). Methylation is the principal mechanism of adaptation, although Stock and Stock (41) have observed a partial adaptation in the absence of methylation for transduction pathways that are usually regarded as methylation dependent. We have verified that observation, but the methylation-deficient bacteria adapted only to very weak stimuli (7).

At the end of the signaling pathway, the probability of clockwise rotation of the flagellar motors is a function of binding of the *cheY* product to the motor switch (6, 14, 27, 47) (Fig. 1). The *cheZ* product is antagonistic to the action of the CheY protein and increases the probability of counterclockwise rotation (14, 47). The signaling pathway between the transducing proteins and the CheY or CheZ proteins is unknown, but it is evident from genetic studies that the *cheA* and *cheW* gene products are involved (24, 36, 47) (Fig. 1). A feedback signal regulates the *cheB* product, protein methylesterase (36). The generation of the feedback signal appears to require the CheA and CheW proteins in the signaling pathway but not the CheY protein (39).

Studies in this laboratory of the role of ATP in chemotaxis

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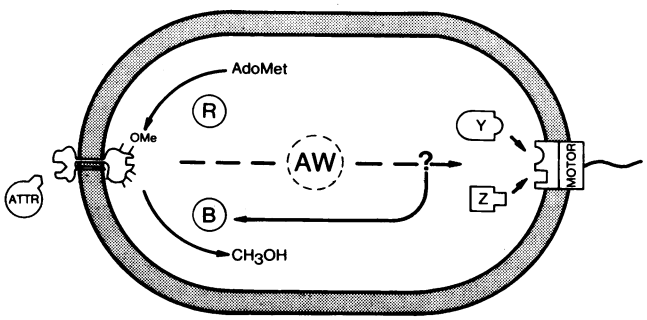

FIG. 1. Scheme for sensory transduction in methylation-dependent chemotaxis in *E. coli* and *S. typhimurium*. R, B, A, W, Y, and Z represent the *che* genes with the same letter designation. Attr, Attractant; AdoMet, *S*-adenosylmethionine; OMe, γ-glutamyl carboxymethyl ester; ?, unknown intermediate that regulates CheB protein methylesterase; - - →, order of reactions has not been established, but it is known that the CheA and CheB proteins are required.

have had the goal of elucidating some of the unknown events in the signaling pathway (9, 31, 44, 45). Earlier studies in other laboratories demonstrated that arsenate-treated *E. coli* and *S. typhimurium* lose all clockwise rotation of the flagellar motors (2, 12, 16, 35). The significance of this finding was unclear, because arsenate treatment is nonspecific in depleting the bacteria of nucleoside phosphates. We used histidine starvation of *hisF* mutants of *S. typhimurium* to specifically deplete the bacteria of ATP and to demonstrate an ATP requirement for normal clockwise rotation of the flagella (31). In the absence of exogenous histidine in a *hisF* mutant, ATP phosphoribosyltransferase is unregulated and rapidly depletes the cell of ATP (29, 30). Clockwise rotation is rapidly restored in histidine-starved mutants by addition of adenine or slowly restored by addition of guanine (9). Analysis of nucleotide pools in *S. typhimurium* showed that the ATP requirement for normal clockwise rotation cannot be satisfied with GTP (M. S. Johnson and B. L. Taylor, manuscript in preparation). The site of ATP action has not been established and is addressed in this investigation.

(Preliminary accounts of portions of this work have been presented [E. H. Rowsell, J.-I. Shioi, and B. L. Taylor, Fed. Proc. **43**:1966, 1984; J. M. Smith and B. L. Taylor, Fed. Proc. **44**:1767, 1985; J. M. Smith, A. J. Wolfe, and B. L. Taylor, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, p. 187].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used in this study are listed in Table 1. *E. coli* BT3076 and BT3077 were constructed by transformation essentially as described in Silhavy et al. (32). The plasmids pCK65 (p*tac-cheY*) and pCK63 (p*tac-cheY*$^+$) were used to transform *E. coli* HCB326, yielding the transformants BT3076 and BT3077, respectively. The plasmids pCK65 and pCK63 were the gift of D. E. Koshland, Jr. (6).

All strains were grown at 30°C in Vogel-Bonner citrate medium E (46) supplemented with auxotrophic requirements and a carbon source such as 0.6% (wt/vol) glucose or 1.0% (wt/vol) glycerol.

ATP depletion by histidine starvation and arsenate treatment. The *hisF* mutants were grown in Vogel-Bonner medium supplemented with glucose, thymine, and histidine at 30°C (31). They were then washed twice by centrifugation and suspended in the same medium without histidine. Starvation was completed by incubating the culture at 30°C for 4 h from the end of the first wash, unless otherwise noted.

The strains without a *hisF* mutation and those containing a plasmid were grown at 30°C in Vogel-Bonner medium supplemented with the nutritional requirements, washed twice by centrifugation, and suspended in an arsenate buffer containing 10 mM PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid], pH 7.0), 0.6% glucose or 1% glycerol (depending on the carbon source that was used for growth), 1 mM sodium arsenate, 0.1 mM EDTA, 1 mM $MgSO_4$, and 1 mM $(NH_4)_2SO_4$.

ATP assays were performed by the luciferin-luciferase method as described previously (31).

Behavioral assays and tumble frequencies. Temporal assays of behavior were performed by rapidly adding a chemoeffector to cells grown and suspended in Vogel-Bonner medium as described previously (45). Tumbling frequencies were determined by the photographic method of Spudich and Koshland (38). Response times are reported as the time required for 50% of the cells to return to the prestimulus motility pattern.

Methylesterase assays. Methylesterase activity was measured by the method of Kehry et al. (11) except that Vogel-Bonner medium with 0.6% glucose was used as the primary buffer for *hisF* strains. For strains lacking a *hisF* mutation, Vogel-Bonner medium was replaced by the arsenate buffer described above. Cells (2×10^9) were washed, suspended in 1.0 ml of Vogel-Bonner medium containing 0.6% glucose, and then incubated with 0.06 mCi of [*methyl-*

TABLE 1. Strains used

Strain	Relevant genotype	Source (reference)
S. typhimurium		
ST23	*hisF8786 thyA1981*	D. E. Koshland, Jr. (1)
ST171	*hisF8786 thyA1981 cheB* (formerly *cheZ221*)	D. E. Koshland, Jr. (1, 2, 15, 40)
E. coli		
HCB326	Δ(*tsr*)*7021* Δ (*cheA-cheZ*)*2209* *trg*::Tn*10*	H. C. Berg (47)
RP437	Wild type	J. S. Parkinson (23)
RP3000	Δ(*cheY-cheZ*)*m43-13 scyA2*	J. S. Parkinson (26)
RP3001	Δ(*cheY-cheZ*)*m43-13 scyA3*	J. S. Parkinson (26)
RP3068	Δ(*cheY-cheZ*)*m43-13 scyB10*	J. S. Parkinson (26)
BT3076	HCB326 with pCK65 (p*tac-cheY*)	This work
BT3077	HCB326 with pCK63 (p*tac-cheY*$^+$)	This work

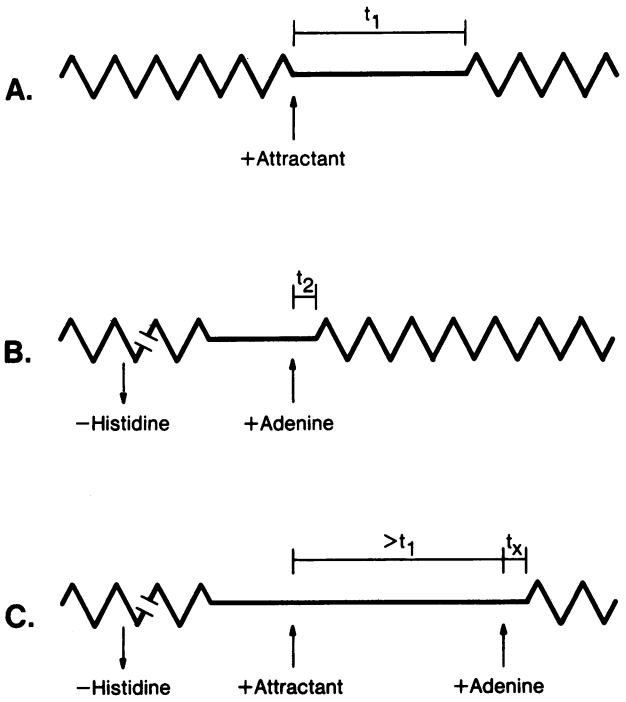

FIG. 2. Summary of the experimental strategy used to determine the effect of ATP depletion on adaptation of *S. typhimurium* ST171 to an attractant. The sawtooth pattern represents a constantly tumbling motility that is normal for ST171, and a flat line represents a smooth-swimming motility. For an explanation, see the text.

^{3}H]methionine for 30 min at 30°C with shaking. The cells were centrifuged, suspended in Vogel-Bonner medium with glucose, thymine, 0.1 mM methionine, and [^{14}C]methanol (ca. 2,000 dpm/ml), and loaded onto a filter. Medium was pumped past the cells at a rate of 0.4 ml/min. An attractant mixture (0.5 mM each serine, aspartate, and ribose) was added to the perfusing medium at fraction 8 and removed at fraction 28 (see Fig. 4). From each fraction collected (0.3 ml), 0.15-ml samples were transferred to 0.5-ml microfuge tubes. The tubes were placed in 6-ml scintillation vials containing 2.4 ml of Beta Phase (WestChem) scintillation fluid. The vials were incubated at room temperature for 10 to 12 h to allow vapor phase transfer of the methanol from the microfuge tube to the scintillation fluid. Both ^{3}H and ^{14}C in the vials were counted in a liquid scintillation counter.

RESULTS

Signal transduction and adaptation. A strategy was devised to determine whether ATP was required for generation of the chemotactic signal in the transducing protein or for suppression of the signal to the switch by methylation of the transducing protein (Fig. 2). A constantly tumbling mutant will respond to the addition of attractant by suppressing tumbling for a time interval (t_1) and then adapt and resume constant tumbling (Fig. 2A). When these cells are depleted of ATP by starving for histidine, tumbling is also suppressed but is rapidly (t_2) recovered when ATP is restored by the addition of adenine (Fig. 2B) (9, 31). Since ATP-depleted cells are smooth-swimming, neither the smooth response to an attractant nor adaptation to the attractant can be observed directly. The experimental strategy adopted (Fig. 2C) to investigate responses to an attractant in ATP-depleted cells was to stimulate the cells by addition of attractant and then, at various time intervals after attractant addition, to add adenine and observe the interval (t_x) before the cells tumbled. The addition of adenine to ATP-depleted cells would not restore tumbling if the flagellar motors were receiving a signal for smooth swimming (counterclockwise rotation) generated by the bound attractant. Tumbling would only be restored when the attractant signal was suppressed by methylation of the transducing protein. Thus, the time required for suppression of the signal in ATP-depleted cells could be determined. If ATP were required for the signal to cross the membrane or for suppression of the signal, the ATP-depleted cells would not begin the methylation process until after adenine was added. That is, t_x would be greater than t_2 and might approach t_1 even when adenine was added after an interval equal to t_1. If, however, ATP were not required for excitation or adaptation, the cells would tumble almost immediately ($t_x \approx t_2$) when adenine was added after time t_1. An analogous procedure was used to investigate an ATP requirement for adaptation to a repellent.

The constantly tumbling mutant used in these investigations was *S. typhimurium* ST171 (*hisF thyA cheB*). Unstarved ST171 cells adapted to 1 mM L-serine in 5 min. After starvation for greater than 3 h in histidine-free Vogel-Bonner medium with glucose as the carbon source, the ST171 cells were smooth-swimming, indicating that ATP was depleted in the cells. The addition of 100 μM adenine restored tumbling in less than 3 s, which was the shortest time in which a response could be observed. When 1 mM serine was added to starved cells followed 1 min later by 100 μM adenine, tumbling did not resume until the cells had adapted to the serine (t_x = 4 min). However, when adenine was added 5 min or more after addition of 1 mM serine, tumbling occurred immediately. In strain ST23 (*che*$^+$), a tumbling response to the addition of a repellent (5 mM sodium benzoate or 250 μM phenol) could be observed directly even in cells that had been starved for 3 h. The times required for adaptation to these repellents were similar in starved and unstarved cells. It was evident then that *S. typhimurium* cells can adapt to a repellent or an attractant without ATP. Previous studies have shown that the concentration of *S*-adenosylmethionine is adequate to support a normal rate of transducer methylation in ATP-depleted cells (31). The site of the ATP requirement was concluded to be in transmission of the signal from the transducing protein to the switch on the flagellar motor or in the switch-motor complex.

Interaction of the CheY protein and switch. The CheY and CheZ proteins interact directly with the switch on the flagellar motor to regulate the direction of flagellar rotation (see Introduction). Strains of *E. coli* deleted for *cheY* and *cheZ* had signal transmission to the switch eliminated (Fig. 1) and could be used to investigate the effect of ATP depletion on the switch-motor complex (14, 47). Such mutants are smooth-swimming, but the *cheYZ* mutants used in this study had an additional second-site suppressor mutation to *cheY*, *scy*, that mapped in either the *flaAII* (*E. coli* RP3000 [*cheYZ scyA2*] and RP3001 [*cheYZ scyA3*]) or *flaBII* (*E. coli* RP3068 [*cheYZ scyB10*]) switch gene (26). The *scy* mutations slightly altered the switch, so that spontaneous tumbling was restored in the absence of CheY. This made it possible to observe any increase or decrease in tumbling frequency in strain RP3068 that resulted from depletion of ATP.

The *E. coli cheYZ* mutants did not have a *hisF* mutation, so arsenate was used to deplete the bacteria of ATP (see Materials and Methods). Each of the mutant strains tested had a similar spontaneous tumbling frequency in the pres-

TABLE 2. Effect of ATP on the tumbling of mutants deleted for *cheYZ* and carrying an *scy* mutation[a]

Strain	Tumbling frequency (%)		ATP concn in presence of arsenate (%)
	Without arsenate	With arsenate	
RP437 (*che*$^+$)	40.7	15.4	2.9
RP3000 (*cheYZ scyA2*)	86.2	81.1	2.3
RP3001 (*cheYZ scyA3*)	85.3	90.5	2.7
RP3068 (*cheYZ scyB10*)	85.1	80.8	2.7

[a] Tumbling frequencies are expressed as the percentage of cells exhibiting tumbling during the 1-s photographic exposure. ATP levels are expressed as a percentage of the level before treatment with 1 mM arsenate. The values for the tumbling frequencies and ATP levels are the average of determinations made in two separate experiments.

ence and absence of arsenate (Table 2). We concluded that the switch-motor complex was not the site of the ATP requirement for chemotaxis and that ATP was not required for reversal of the flagellar motors.

The effect of depletion of ATP on tumbling induced by binding of the CheY protein to the switch was then investigated by using a "gutted" strain obtained from A. J. Wolfe and H. C. Berg. *E. coli* HCB326 (*cheAWRBYZ tar tap tsr trg*::Tn*10*) was deficient in all methylation-dependent transducing proteins and in the proteins involved in signal transmission, but had a normal switch and motor (47). The gutted strain was transformed with *E. coli*-derived plasmids containing a normal *cheY*$^+$ gene (pCK63) and a mutated *cheY* gene (pCK65) (6). The *cheY* genes were under the control of the *tac* promoter and were induced with isopropyl-β-D-thiogalactoside (IPTG). The transformants were *E. coli* BT3077 (p*tac-cheY*$^+$) and *E. coli* BT3076 (p*tac-cheY*). The latter served as a control for nonspecific effects produced by the plasmid. The tumbling frequency shown in Fig. 3 for BT3076 was obtained from a photographic assay that scored the number of cells that abruptly changed direction during a 2-min exposure (see Materials and Methods). Reexamination of the motility of this strain under the microscope revealed

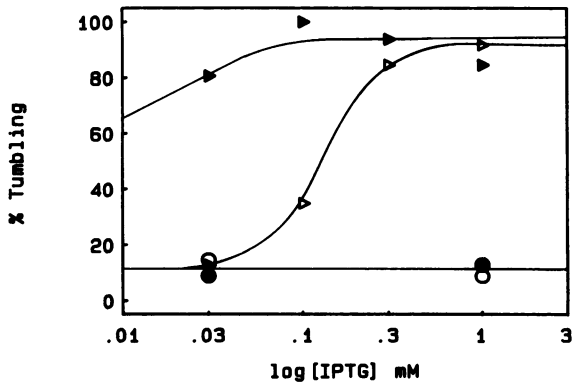

FIG. 3. Tumbling frequency of normal and ATP-depleted *E. coli* BT3076 (○) and BT3077 (△) as a function of IPTG. The cells were grown at 30°C to early log phase in Vogel-Bonner medium with glycerol and nutritional requirements. IPTG was added to the concentration indicated, and the cells were grown for an additional 30 min. The cells were then washed twice and suspended in the arsenate buffer. Tumbling frequencies of the cells before washing and 4 h after the first wash were determined by the photographic method described in Materials and Methods. Solid symbols represent cells before arsenate treatment, and open symbols represent arsenate-treated cells.

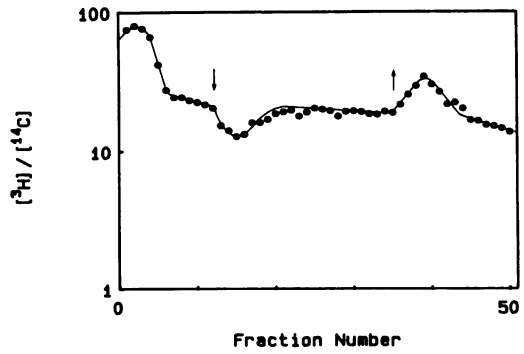

FIG. 4. Protein methylesterase activity in *S. typhimurium* ST23. Cells were prepared as described in the text. The effects of the attractant mixture were first observed after two or three additional fractions had been collected (arrows). Portions of each fraction collected were counted in a liquid scintillation counter.

that some of the cells had poorly coordinated flagellar bundles. Such cells are more susceptible to change of direction when the flagella interact with the surface of the microscope slide or with other bacteria. Extensive observations did not reveal a single tumble in free-swimming cells unless they were interacting with another surface, as would be expected for a gutted strain (see reference 47).

Induction of the *cheY* gene in strain BT3077 (*cheY*$^+$) with 30 μM IPTG yielded a constantly tumbling bacterium. Treatment of the induced cells with arsenate suppressed tumbling (Fig. 3). Induction with 1.0 mM IPTG also yielded a constantly tumbling bacterium, but arsenate treatment did not suppress tumbling. At intermediate levels of IPTG, a dose-dependent change in tumbling frequency was observed (Fig. 3). It was concluded that ATP increases the effectiveness of the CheY protein for inducing tumbling in *E. coli*.

Global regulation of protein methylesterase. As discussed in the Introduction (Fig. 1), the *cheB* product, protein methylesterase, is subject to feedback regulation by an intermediate in the sensory transduction pathway (36). The effect of ATP depletion on regulation of protein methylesterase was examined. Methylesterase activity was measured in vivo in *S. typhimurium* ST23 (*hisF*), in the presence and absence of histidine, by the flow assay of Kehry et al. (11). The cells were prelabeled by incubation with [*methyl*-^{3}H]methionine. Protein methylesterase cleaves the γ-glutamyl methyl ester residues on the transducer proteins, producing [^{3}H]methanol, which is excreted by the cells (42). The amount of [^{3}H]methanol in the fractions collected from the flow cell is a measure of the in vivo methylesterase activity under the conditions of the assay. By introducing attractants or repellents to the medium flowing over the cells or by removing the same, it was possible to observe the effect of chemotactic stimuli on methylesterase activity (11).

Results of a typical flow assay in unstarved *S. typhimurium* ST23 (Fig. 4) showed a transient decrease (valley) in methylesterase activity after addition of an attractant mixture (0.5 mM each serine, aspartate, and ribose) and a transient increase (peak) in methylesterase activity after the attractant was removed. These transient changes will be collectively referred to as stimulus-altered methylesterase activity. By interpolating the steady-state baseline value of methylesterase activity and by numerical integration of the area in a valley or peak, an arbitrary magnitude was assigned to the stimulus-altered methylesterase activity.

Stimulus-altered methylesterase activity was examined in

TABLE 3. Comparison of intracellular ATP levels and magnitude of the stimulus-altered methylesterase responses in *S. typhimurium* ST23[a]

Time after histidine withdrawal (h)	ATP concn[b]	Stimulus	Methylesterase response[c]	Significance[d] (*P*)
0	100	Attractant	−1.82 ± 0.59	
		Repellent	+2.25 ± 0.59	
1	19.2	Attractant	−1.90 ± 0.80	NS
		Repellent	+2.25 ± 0.25	NS
2	9.6	Attractant	−1.32 ± 0.45	NS
		Repellent	+1.54 ± 0.15	<0.01
3	4.6	Attractant	−1.48 ± 0.25	<0.10
		Repellent	+1.73 ± 0.22	<0.02
4	3.3	Attractant	−1.32 ± 0.31	<0.05
		Repellent	+1.21 ± 0.27	<0.01

[a] Histidine was omitted from the Vogel-Bonner medium.
[b] Expressed as a percentage of the level before histidine starvation.
[c] Arbitrary units ± standard error of the mean.
[d] Obtained by using Student's *t*-test. NS, Not significant.

S. typhimurium ST23 at 1-h intervals after the commencement of starvation for histidine. Intracellular concentrations of ATP were also measured at each time interval (Table 3). A significant decrease in the magnitude of the peaks and valleys was observed as ATP was depleted. However, we were not able to eliminate attractant-altered protein methylesterase activity, even after starvation for more than 6 h. Springer and Zanolari (36) and Stewart and Dahlquist (39) have reported that a *cheA* or a *cheW* mutation impairs the feedback regulation of the methylesterase in *E. coli*; but in the absence of the CheA and CheW proteins, Borczuk et al. (4) observed transducer-mediated regulation of the methylesterase.

DISCUSSION

ATP is not required for the chemosensory signal to cross the membrane or for suppression of an attractant-generated signal by methylation of the transducing protein. *S. typhimurium* cells that were depleted of ATP showed normal times for adaptation to either attractants or repellents. The effect of ATP depletion in *E. coli* or *S. typhimurium* cells was to strongly bias the flagellar motors in the direction of counterclockwise rotation.

The fraction of time that a motor is in the clockwise mode of rotation is determined by an intrinsic stochastic process within the switch-motor complex that shifts the motor between counterclockwise and clockwise rotation and by the chemotactic signal impinging on the switch and biasing the rate constants for interconversion between the clockwise and counterclockwise states (18, 19). In the *E. coli cheYZ scy* strains, the chemotactic signal to the switch has been eliminated; clockwise rotation is determined by the intrinsic stochastic reversal generator. *cheYZ* mutants have a strong counterclockwise bias in the stochastic process, but the additional *scy* mutation in the strains used in this study introduced a small clockwise bias into the switch, so that the *cheYZ scy* strains had a random motility pattern. Since ATP depletion did not alter the tumbling frequency in *cheYZ scy* cells (Table 2), it was evident that ATP was not required by the stochastic process that generates tumbling. The alternative was that ATP was required for generation of the signal that modulates the bias of the tumble generator in wild-type cells.

In a gutted strain, *E. coli* BT3077 (*cheAWRBYZ tar tap tsr trg*[pCK63 (*cheY*$^+$)]), the only components of the methylation-dependent pathways for chemotaxis that were present were the switch, motor, and CheY protein. Induction of *cheY* on the pCK63 plasmid in BT3077 cells increased tumbling in the cells. Except at the highest levels of *cheY* induction, tumbling was abolished by treatment of the cells with arsenate (Fig. 3). Since the results in Table 2, discussed above, excluded the switch-motor complex as the site of ATP action, it is clear that ATP potentiates the interaction of the CheY protein with the switch. Unpublished observations by D. Sanders and D. E. Koshland, Jr. (personal communication) and by A. J. Wolfe and H. C. Berg (personal communication) also suggested an ATP requirement for CheY-induced tumbling.

Two models for the role of ATP in CheY-induced tumbling can be suggested. In a ternary-complex model, tumbling results from simultaneous binding of ATP and the CheY protein to the switch (Fig. 5A). In a CheY activation model, the CheY protein is activated in a reaction involving ATP to form CheY* (Fig. 5B). Tumbling results from the binding of CheY* to the switch in a binary complex.

Although the available data did not exclude the ternary-complex model, the evidence favored activation of CheY by ATP. In the wild-type cell, the CheY protein is present in a very large excess compared with the number of switch-motor complexes, yet unstimulated bacteria tumble only occasionally. This suggested that most of the CheY protein is in an inactive form. The CheY protein is the most abundant chemotaxis protein (8, 14), and it is unlikely that most of CheY is sequestered by binding to transducing proteins or other chemotaxis proteins. It is proposed that CheY is mostly free in the cytoplasm and that in response to a repellent, the chemotactic signal converts some of the inactive CheY to an active CheY* form in a reaction requiring ATP. The existence of active and inactive forms of the CheY protein is also suggested by the experiments of Ravid et al. with reconstituting tumbling in *E. coli* cell envelopes (27).

The activation of CheY occurred in the gutted strain (*E. coli* BT3077) in the absence of other components of the sensory transduction system. This might be an autoactivation of the CheY protein in the presence of ATP. Alternatively, an activation, such as a covalent modification, that is catalyzed by a chemotaxis component in Che$^+$ strains could also be fortuitously catalyzed in the gutted strain by a cellular component that is not normally important in chemotaxis but has a broad substrate specificity. Catalysis of a covalent modification by a nonchemotaxis protein would not

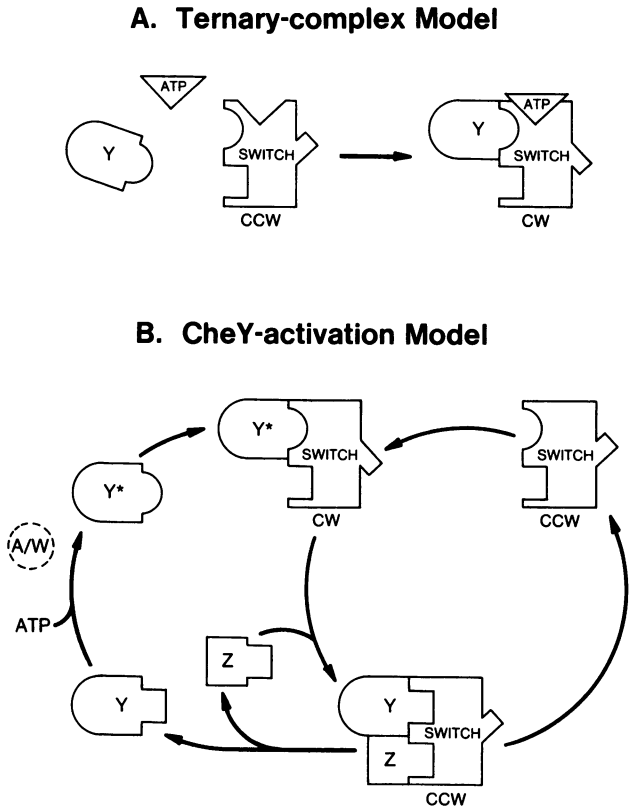

FIG. 5. Models for the role of ATP in signal transduction. (A) Ternary-complex model, in which ATP and the CheY protein (Y) must both bind to the switch to produce clockwise (CW) rotation. (B) CheY activation model, in which the CheY protein is activated by ATP to form an activated CheY (Y*). CheY* binding to the switch produces clockwise rotation. The CheY* protein is deactivated by the CheZ protein (Z) when CheZ is bound to the switch, and the switch returns to the counterclockwise (CCW) conformation. It is proposed that CheA (A) and/or CheW (W) is involved in activation of CheY.

have to be efficient, since CheY was presumably induced to very high levels. Using the same plasmid (pCK63) in *E. coli* RP1091 Δ(*cheA-cheZ*), Kuo and Koshland (14) determined the CheY level after induction with various concentrations of IPTG. At 30 μM IPTG, the level of CheY was approximately 10 times the level in wild-type cells. In our studies, we used concentrations of IPTG between 30 μM and 1.0 mM, so that CheY concentrations would have been even higher.

The CheY activation model could account for the observed constant tumbling in ATP-depleted BT3077 cells at the highest concentration of CheY by either of two mechanisms. If there is an equilibrium between CheY and CheY*, and if clockwise rotation is regulated by the absolute level of CheY* and not by the CheY*-CheY ratio, there might be sufficient CheY* in ATP-depleted cells, at very high levels of total CheY, to induce constant tumbling. Alternatively, the switch might be placed in the clockwise state by the binding of unactivated CheY but have a low affinity for unactivated CheY, so that CheY would fully activate the switch only at very high concentrations of CheY.

In wild-type cells, the concentration of CheY is much lower than in the IPTG-induced BT3077 cells and chemotaxis is achieved by modulating tumbling frequency (14). The CheY activation model assumes that the sensory transduction pathway modulates the activation of CheY. This might be catalyzed by the CheA or CheW protein or both. The only evidence from this study relating to the role of CheA and CheW is indirect. Feedback regulation of the CheB protein methylesterase, which requires CheA and CheW, was also impaired by ATP depletion (Table 3). Evidence from Wolfe et al. is consistent with deactivation of CheY* catalyzed by the CheZ protein when both proteins are bound to the switch (47).

Recently, Wolfe et al. (47) proposed a model for the signal transduction pathway in bacterial chemotaxis. In that model, repellent-activated transducers catalyzed the conversion of a low-molecular-weight precursor into an activated intermediate in a reaction that required ATP and CheA, CheW, or both. The intermediate, in turn, activated the CheY protein so that it bound to the switch complex and promoted clockwise rotation. The model accounts for the signal amplification seen in chemotaxis (28). After the present investigation was completed, it was reported that the purified CheA protein is autophosphorylated by ATP and that the phosphorylation is reversed by the CheY or CheZ proteins (10). The autophosphorylation of CheA is apparently an ATP-requiring reaction in signal transduction that is different from the ATP-requiring activation of CheY proposed in this study. It is possible that the inhibition of stimulus-induced changes in methylesterase activity in ATP-depleted cells (Table 3) reflects the requirement of ATP for phosphorylation of CheA instead of the requirement of ATP to activate CheY. Feedback regulation of the methylesterase requires the *cheA* but not the *cheY* gene (39).

Ongoing investigations of the interaction of ATP with the CheY protein in this laboratory have demonstrated the binding of 8-azido-[α-^{32}P]ATP and 8-azido-[γ-^{32}P]ATP to CheY (L. S. Wong, M. S. Johnson, and B. L. Taylor, unpublished observation). The binding of azido-ATP was inhibited by unlabeled ATP. The CheY protein was neither autophosphorylated nor adenylated in the presence of ATP. We conclude that there are apparently two sites of ATP interaction in the chemotaxis pathway.

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