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Novel sensory adaptation mechanism in bacterial chemotaxis to oxygen and phosphotransferase substrates

(aerotaxis/protonmotive force/methylation-independent adaptation)

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ABSTRACT **The involvement of methylation in the chemosensory response of bacteria to many attractants has been clearly established by studies in several laboratories. It has been assumed that adaptation of *Salmonella typhimurium* and *Escherichia coli* to all attractants involves methylation of a transmembrane methyl-accepting chemotaxis protein. The methyl donor in this reaction is S-adenosyl-L-methionine, and the protein methyltransferase is the product of the *cheR* gene. In contrast, adaptation to oxygen and phosphotransferase substrates were found to be independent of this methylation system. In *E. coli* AW660 (*tsr tar trg*), which lacks the known methyl-accepting chemotaxis proteins, chemotaxis was normal to oxygen and to substrates of the phosphotransferase system such as D-mannose, D-glucose, and *N*-acetyl-D-glucosamine. When S-adenosyl-L-methionine was depleted by methionine starvation or by addition of 1-aminocyclopentane-1-carboxylic acid, methylation-dependent adaptation to serine, aspartate, and ribose was defective in wild-type *E. coli* and *S. typhimurium*. However, adaptation to oxygen and phosphotransferase substrates was independent of S-adenosyl-L-methionine and the *cheR* product. These results suggest that there are methylation-independent and methylation-dependent mechanisms for sensory adaptation in bacteria.**

The most thoroughly studied behavioral responses of prokaryotes are those of *Escherichia coli* and *Salmonella typhimurium* to serine, aspartate, galactose, and ribose (1–4). Serine and aspartate bind to transmembrane methyl-accepting chemotaxis proteins (MCPs) that are the products of *tsr* and *tar* genes, respectively (5, 6). Ribose and galactose bind to periplasmic receptors and induce a conformational change that enables the receptors to interact with a third MCP coded for by the *trg* gene (7–10). Increased binding of an effector or periplasmic receptor to a MCP initiates the behavioral response and also activates selective methylation of the MCP by a protein methyltransferase (3, 11). The period of methylation to a new level corresponds to the adaptation phase of the response. The methyl donor is S-adenosyl-L-methionine (AdoMet), and the product is a γ-glutamyl methyl ester (12, 13). The MCP can be multiply methylated, and net methylation increases until it reaches a plateau level that is a function of receptor occupancy (14–18). Adaptation to the effector stimulus is complete, and prestimulus behavior is resumed, when methylation reaches the plateau and protein methyltransferase activity is balanced by the activity of a protein methylesterase (19, 20). The protein methyltransferase and methylesterase are coded for by *cheR* and *cheB*, respectively (19, 21).

Studies of taxis to other stimuli, including extremes of temperature and pH, reveal that most of the responses involve one of the three known MCPs (22–25). Thus, the MCPs have the additional role of focusing signals from diverse stimuli. However, evidence has accumulated that stimuli from some effectors are not focused through one of the known MCPs. These effectors include oxygen, blue light, and sugars transported by the phosphotransferase system (PTS) (26–30). We have explored the alternative possibilities that sensory adaptation to these stimuli requires methylation of an additional MCP or that it is a novel, methylation-independent process. A preliminary account of some of this work has appeared (31).

MATERIALS AND METHODS

Chemicals. L-[*methyl*-^{3}H]Methionine (20 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was obtained from ICN. Cycloleucine (1-aminocyclopentane-1-carboxylic acid), *N*-acetyl-D-glucosamine, and β-D-glucose were obtained from Sigma. Ultrex grade D-mannose was obtained from J. T. Baker.

Bacteria. The *S. typhimurium* strains ST1, ST23 (*hisF thyA*), and ST1038 (ST23 *cheR*) were obtained from D. E. Koshland, Jr. (21, 32), and LJ219 (*manA54 glu-51*) was from M. Saier (33). The *metE*::TN10 insertion from *S. typhimurium* TT218 (J. Roth) was transduced with bacteriophage P22 into strain ST1038 to produce BT20 and into ST23 to produce BT21. The parent *E. coli* OW1, wild type for chemotaxis, and MCP mutants AW656 (*tar*), AW655 (*tsr*), AW701 (*trg*), AW658 (*tar trg*), and AW660 (*tar tsr trg*) were obtained from J. Adler (10). The following strains of *E. coli* were obtained from W. Epstein (28): AW582 (*ptsM tga glk nalA strA*) and AW581 (AW582 *ptsG*). Cells were grown at 30°C in Vogel–Bonner medium E (34) supplemented with the auxotrophic requirements of the strain. Glucose (1%) or glycerol (1%) was added as the carbon source.

Chemotaxis Assays. The temporal aerotaxis assay as described (29) was modified by use of blue light to determine the end point of the response (35). Bacteria were exposed to blue light for 0.5–1.0 s; then the microscope stage was moved, and a new region of the culture was exposed to blue light. This was repeated until the bacteria were observed to tumble in response to light. Chemotaxis was assayed by rapidly mixing 1 μl of attractant with 10 μl of bacteria on the microscope slide. The end point of the chemotactic response was also estimated with the aid of blue light. *E. coli* OW1 was protected against blue light for 15 s after the addition of 20 μM L-serine, but AW655 (*tsr*) was not protected by serine.

Electrophoresis and Fluorography. Cells were grown and labeled with [^{3}H]methionine, and radioactive samples were prepared as described (36). Electrophoresis and fluorography of the labeled proteins were performed by published procedures (17, 37). The fluorograms were scanned with a Beckman DU-8 spectrophotometer.

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Abbreviations: MCP, methyl-accepting chemotaxis protein; AdoMet, S-adenosyl-L-methionine; PTS, phosphotransferase system; pmf, protonmotive force.

Measurement of AdoMet. The amount of AdoMet was measured by the method of Glazer and Peale (38).

RESULTS

Taxis to Oxygen and PTS Sugars Is Independent of Known MCPs. Aerotaxis was measured by the quantitative temporal assay of Laszlo and Taylor (29) in which a drop of bacterial culture in a microchamber is made anaerobic by equilibration with prepurified nitrogen gas. Air is reintroduced into the chamber, and the time interval during which the bacteria suppress tumbling is measured. The swimming of unstimulated wild-type *E. coli* and *S. typhimurium* is interrupted about once per second by a brief tumbling motion (39). In response to an increase in attractant concentration, the bacteria suppress tumbling until they adapt to the higher concentration of attractant.

Mutants that are deficient in MCP have a low tumbling frequency in the unstimulated state, and it is difficult to distinguish between smooth swimming (zero tumbling frequency) and normal motility. This difficulty has been overcome by using blue light to define a smooth swimming response (26, 27, 35). Both wild-type bacteria and strains deficient in MCPs tumbled on exposure to a pulse of intense blue light, except during a smooth swimming chemotactic response. After anaerobic bacteria were exposed to air, they were tested with pulses (≈1 s) of blue light. The end of the response was defined as the point where 50% of the cells tumbled in response to blue light. A similar procedure was used to measure chemotaxis to attractants other than oxygen.

E. coli tsr mutants do not respond to serine, and *tar* or *trg* mutants do not respond to effectors that interact with the corresponding altered MCP (3). In contrast, the response to oxygen was unaffected by mutations in one or all three MCP genes (Table 1). AW660 (*tsr tar trg*) gave no detectable response to serine, aspartate, or ribose but responded to oxygen and the PTS substrates D-glucose and *N*-acetyl-D-glucosamine. The response to glucose was decreased in AW660, presumably because glucose is detected by a *trg*-dependent pathway and a PTS pathway (28, 40).

Cells were exposed to [^{3}H]methionine and examined for labeled MCPs. Wild-type (OW1) cells showed the normal methylation pattern, but none of the known MCPs were detected in AW660, and no additional MCPs were methylated in response to an increase in oxygen, D-glucose, or *N*-acetyl-D-glucosamine. This suggested that aerotaxis and chemotaxis to PTS substrates might not require a MCP. To investigate this possibility, we compared the methylation dependence of aerotaxis and chemotaxis.

Effect of AdoMet Depletion on Aerotaxis and Chemotaxis. A decrease in the intracellular concentration of AdoMet impairs sensory adaptation in MCP-dependent chemotactic responses (41, 42). The AdoMet concentration was lowered in *S. typhimurium* and *E. coli* by adding cycloleucine to inhibit AdoMet synthetase (43). A 70% decrease in AdoMet concentration increased by 140% the time required for *S. typhimurium* ST1 to adapt to a step increase in serine concentration from 0 to 20 μM (Fig. 1). The time for adaptation of ST1 to aspartate (20 μM) and D-ribose (20 μM) was also increased (Table 2), but the response to oxygen was not affected. To confirm that the effect of high concentrations (0.5 M) of cycloleucine on behavior was specifically related to methylation, the AdoMet concentration was also lowered by starving BT21 (ST23 *metE*) for methionine (Fig. 2). Methionine starvation sharply increased the time for adaptation to serine but did not affect adaptation to oxygen. The addition of methionine rapidly restored the serine response times to the prestarvation level. Similar results were obtained when *E. coli* was starved for methionine.

The adaptation of *E. coli* strains to D-mannose and *N*-acetyl-D-glucosamine was unaffected by cycloleucine (Table 2). The adaptation to glucose was essentially unchanged if glucose was the carbon source for growth but was increased by 92% if the galactose receptor was induced by galactose (Table 2). The cycloleucine-independent responses to mannose and glucose were abolished by mutations affecting the respective enzyme IIA receptors for the PTS. Adaptation of *E. coli* RP437 (*che*$^+$) to mannose was also unaffected by methionine starvation that impaired adaptation to serine (data not shown). In *S. typhimurium* BT21, adaptation to mannose and *N*-acetyl-D-glucosamine was independent of methionine starvation but anomalous results were obtained in the presence of cycloleucine. Adaptation times for mannose (10 mM), *N*-acetyl-D-glucosamine (10 mM), and glucose (10 mM) were increased 134%, 207%, and 141%, respectively, by the addition of cycloleucine (0.5 M); but cycloleucine also caused a response to mannose in the receptor mutant LJ219 (*manA54 glu-51*) that was not observed in the absence of cycloleucine.

Effect of AdoMet Depletion on Methylation. AdoMet depletion was assumed in the above experiments to increase adaptation times by decreasing the rate of methylation of MCP. This was confirmed for methylation of the *tsr* protein in response to a step increase in L-serine concentration from 0 to 5 mM (Fig. 3). *E. coli* AW658 strain (10) lacks the *tar* and *trg* MCPs, and all methylation of proteins with an apparent M_r between 55,000 and 68,000 was assumed to be due to methylation

Table 1. Response to oxygen and PTS substrates by *E. coli* mutants deficient in chemotaxis signaling proteins

Attractant	Strain	Genotype	Response,* s
Oxygen, 250 μM	OW1	Wild type	12.6 ± 2.7 (8)
	AW656	*tar*	15.2 ± 2.2 (8)
	AW655	*tsr*	13.1 ± 2.4 (11)
	AW701	*trg*	14.6 ± 2.5 (9)
	AW660	*tar tsr trg*	16.2 ± 3.0 (7)
Glucose, 10 mM	OW1	Wild type	30.3 ± 2.8 (7)
	AW660	*tar tsr trg*	23.5 ± 4.4 (7)
GlcNAc, 10 mM	OW1	Wild type	17.3 ± 2.2 (7)
	AW660	*tar tsr trg*	18.0 ± 2.5 (7)

* Mean ± SD with the number of determinations in parentheses.

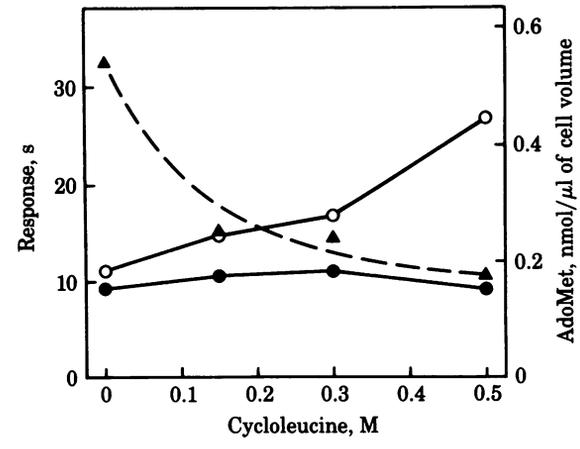

FIG. 1. Effect of cycloleucine on the intracellular concentration of AdoMet (▲) and taxis to 20 μM L-serine (○) and 250 μM oxygen (●) by *S. typhimurium* ST1. Cells were grown in medium E with 1% glucose and tested in similar medium 40 min after cycloleucine was added at the specified concentration. AdoMet was determined by the method of Glazer and Peale (38).

Table 2. Effect of cycloleucine on chemotaxis of *S. typhimurium* and *E. coli* strains*

Strain	Genotype†	Attractant	Response, s: Without cycloleucine	Response, s: With cycloleucine	Increase, %
S. typhimurium					
ST1	Wild type	Ser	11.2 ± 2.0	26.9 ± 4.3	140
		Asp	28.2 ± 7.0	63.7 ± 12.3	126
		Rib	7.2 ± 1.4	27.2 ± 7.7	278
		O_2	9.4 ± 0.9	9.6 ± 2.1	2
E. coli					
OW1	Wild type	Ser	15.4 ± 1.8	44.2 ± 5.2	187
		Man	23.4 ± 2.4	25.2 ± 5.3	8
		Glc‡	30.3 ± 2.8	38.6 ± 6.0	27
		Glc§	71.9 ± 17.0	137.7 ± 20.7	92
AW660	*tsr tar trg*	Glc‡	23.5 ± 4.4	27.3 ± 6.9	16
AW582	*ptsM*	Glc‡	22.0 ± 4.0	27.5 ± 4.3	25
		Man	<5¶	<5	
AW581	*ptsM ptsG*	Glc	<5	<5	
		Man	<5	<5	
		Ser	18.5 ± 3.0	37.5 ± 9.5	103

* Cells were grown in Vogel–Bonner medium E(34) except cells for ribose chemotaxis, which were grown in tryptone broth containing 10 mM D-ribose. Unless indicated otherwise the carbon source was glycerol. Chemotactic responses to amino acids were measured in medium E and to sugars were measured in chemotaxis buffer (10 mM potassium phosphate, pH 7.0/1 mM $MgSO_4$/1 mM $(NH_4)_2SO_4$/0.1 mM EDTA). The concentrations of attractants were: oxygen, 0.25 mM; amino acids and D-ribose, 20 μM; and other sugars, 10 mM. The concentration of cycloleucine, when present, was 0.5 M. Mean ± SD from seven determinations.

† The gene products of *ptsM* and *ptsG* are the mannose enzyme II and glucose enzyme II—the PTS receptors for mannose and glucose, respectively.

‡ Carbon source: glucose (55 mM).

§ Carbon source: galactose (10 mM).

¶ Shortest measurable response was 5 s.

of the *tsr* MCP. In uninhibited cells the change in methylation after addition of serine was similar to the time course observed by other investigators (18). The motility of the stimulated AW658 was observed in the microscope, and adaptation to serine took 14 min, which was similar to the time required for methylation to reach a steady level (Fig. 3). Cycloleucine (0.5 M) decreased the rate of methylation by 65%, and the increased adaptation time (24 min) reflected the longer time required for methylation to reach a steady level.

The plateau level of methylation was 35% lower in the cycloleucine-inhibited AW658 cells compared to the uninhibited cells. Previous studies (18) found that the plateau methylation level was a function of attractant occupancy of the chemoreceptor, but the present data indicates that other factors are also involved. Multiple bands of MCP observed after $NaDodSO_4$/polyacrylamide gel electrophoresis were the result of multiple methylation of the *tsr* protein (14–17). The distribution of ^3H label in the MCP bands was comparable for cycloleucine-inhibited and -uninhibited AW658.

Adaptation in a Mutant Deficient in Methyltransferase. *S. typhimurium* BT20 (ST1038 *metE*) had no detectable MCP methyltransferase activity *in vivo* (data not shown). More extensive studies of ST1038 by J. Stock and D. E. Koshland, Jr. (personal communication) indicate that the *cheR* mutation is tight. The lack of methyltransferase activity did not prevent adaptation to 10 mM mannose or to 0.25 mM oxygen, and the adaptation times were unaffected by starvation for methionine

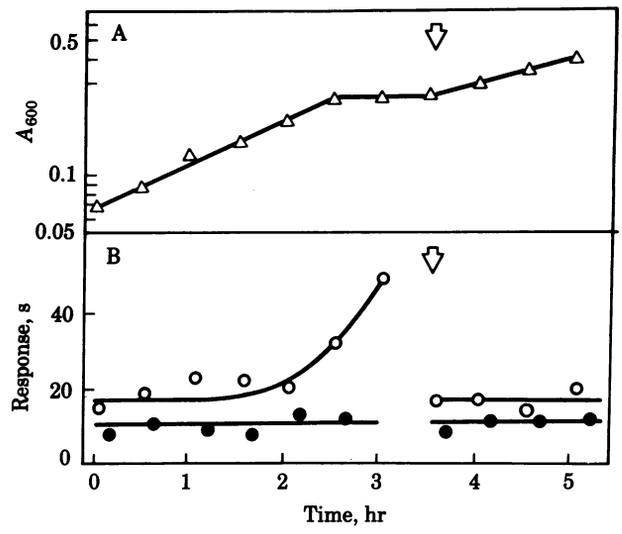

FIG. 2. Effect of methionine starvation on taxis to oxygen and serine. *S. typhimurium* BT21 (ST23 *metE*) was grown in medium E with 1% glucose and 40 μg of L-methionine per ml. The cells were washed and suspended in similar medium with 1 μg of methionine per ml 1.5 hr before the start of the experiment. At the time indicated by the arrow, 100 μg of methionine per ml was added to the medium. (*A*) Growth of BT21. (*B*) Response of BT21 to 20 μM serine (○) and 250 μM oxygen (●).

(data not shown). ST1038 also adapted to 20 μM serine, although the process was impaired when compared to wild type. The adaptation time was strongly dependent on AdoMet concentration. This unexpected observation suggested that in ST1038 there are two methyltransferase-independent adaptation mechanisms: AdoMet-independent adaptation to oxygen and a second AdoMet-dependent adaptation to attractants such as serine. Stock and Koshland (personal communication) have demonstrated that in the absence of methylation, ST1038 adapts to repellents, although adaptation is impaired.

DISCUSSION

This study used several mutants which had a low steady-state tumbling frequency that made standard assays of behavior more difficult. The use of the blue-light response to identify the end

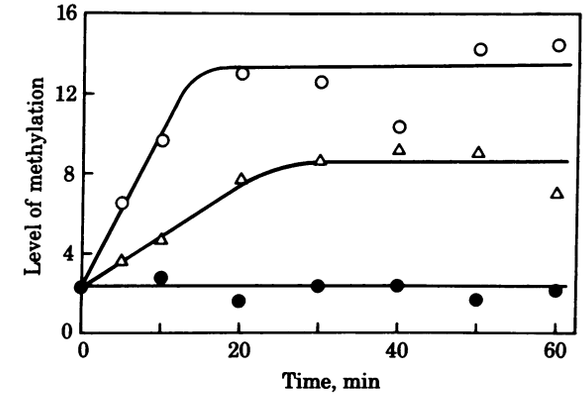

FIG. 3. Serine-stimulated methylation of *tsr* protein in *E. coli* AW658 (*tar trg*) in the presence (△) and absence (○) of cycloleucine. Conditions for cell growth are described in Fig. 1. L-[*methyl*-^3H]Methionine (5 μCi per 3×10^8 cells) was added 40 min before the start of the experiment, and 30 min later cycloleucine (0.5 M) was added to each sample except the control cells (●). Methylation shown is the integration of the densitometric scan of the fluorogram of *tsr* proteins resolved by electrophoresis.

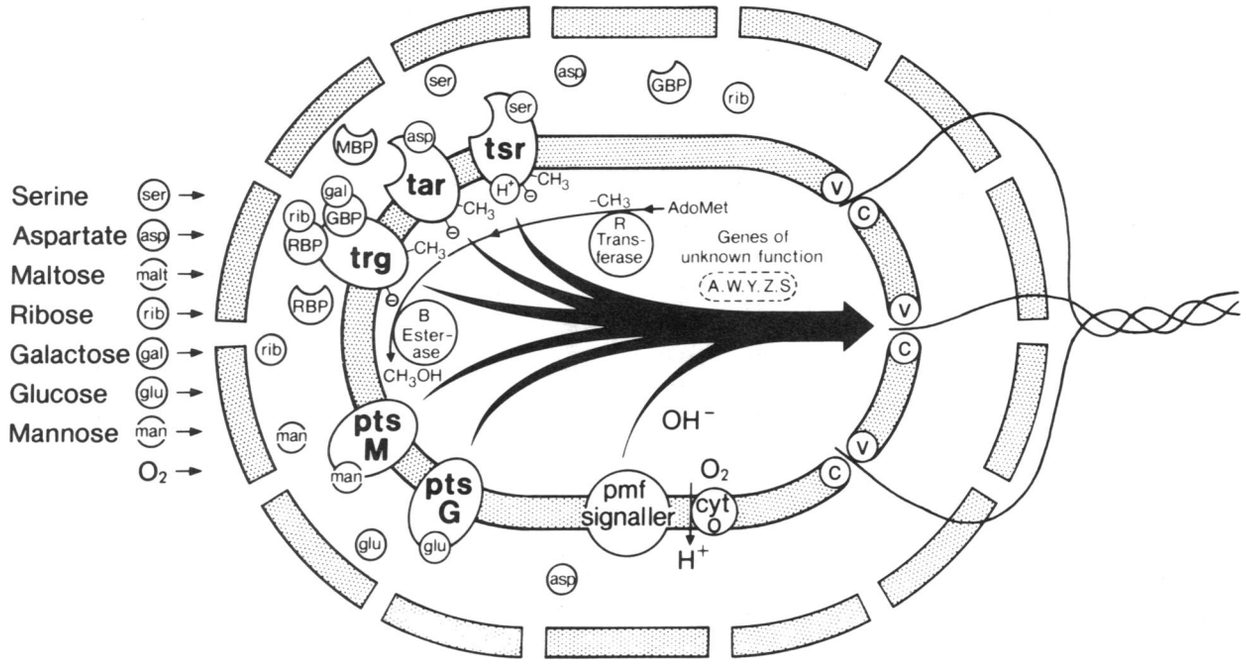

FIG. 4. Scheme to summarize sensory transduction in chemotaxis in *E. coli* and *S. typhimurium* [modified from Koshland (1)]. The chemoeffectors (serine, aspartate, etc.) can cross the outer membrane and bind to receptors in the periplasmic space (RBP, GBP, MBP) or in the membrane (ptsM, trg, cyt o, etc.). For signals processed through MCPs (tsr, tar, trg), adaptation is dependent on methylation or on demethylation catalyzed by protein methyltransferase (R) or protein methylesterase (B), respectively. For signals processed through other signaling proteins (ptsM, ptsG, pmf signaler), adaptation may be independent of the activity of R. All sensory transduction pathways converge prior to C and V, which represent the switch on the flagellar motor. A, W, Y, Z, S are chemotaxis gene products with unassigned functions in signal processing.

point in the temporal chemotaxis assay enabled measurement of adaptation in bacteria, such as MCP mutants and cells starved for L-methionine, which seldom tumble in the unstimulated state. Previously, it has not been possible to directly measure attractant responses in smooth strains.

The ability to measure the responses of such strains permitted us to identify specific impairment of methylation-dependent adaptation and revealed sensory adaptation processes that are independent of AdoMet over the range of concentrations tested. The concentration of AdoMet, the substrate for the *cheR* methyltransferase, was decreased by L-methionine starvation or by the addition of cycloleucine. Depletion of AdoMet limited the rate of the protein methyltransferase reaction (Fig. 3) and, thereby, inhibited sensory adaptation in each known MCP-dependent pathway (Table 2). AdoMet depletion also should block adaptation through any unidentified MCP that is a substrate for the *cheR* protein. The test of sensitivity to cycloleucine or methionine starvation is a procedure that could be used to characterize the adaptation requirements of other sensory transduction pathways in *S. typhimurium* and *E. coli*.

In *S. typhimurium* and *E. coli*, chemotaxis to oxygen and PTS substrates was independent of *tsr*, *tar*, or *trg* proteins (Table 1). A fourth MCP has been reported in *E. coli*, but there is no evidence that methylation of the MCP is stimulated by oxygen or PTS substrates (44). Extensive studies in *E. coli* AW660 failed to detect any novel MCPs that were methylated in response to these attractants. Therefore, it was necessary to consider the possibility that a novel mechanism was involved in adaptation to oxygen and PTS substrates.

The behavior of AdoMet-depleted cells provided clear evidence of diversity in adaptation mechanisms (Fig. 1, Table 1). All the known methylation-dependent systems had impaired sensory adaptation in the depleted cells, whereas adaptation to oxygen was not changed by any of the conditions tested. The PTS attractants glucose, mannose, and *N*-acetyl-D-glucosamine also had distinctive adaptation properties. In *E. coli*, adaptation to these attractants was unaffected by cycloleucine or methionine starvation. In *S. typhimurium*, adaptation to PTS attractants was not impaired by methionine starvation but was altered by cycloleucine. The anomalous results obtained with the receptor mutant LJ219 suggested that high concentrations of cycloleucine had multiple effects on *S. typhimurium*. Consequently, the results obtained in methionine starvation are likely to be more reliable.

Depletion of AdoMet would probably have similar effects on all sensory adaptation processes that are mediated by the *cheR* protein methyltransferase. The divergent effects of AdoMet observed in this study are consistent with methylation-independent adaptation to oxygen and possibly to PTS attractants, although they do not prove it. Other possibilities include adaptation mediated by a second methyltransferase that has a higher affinity for AdoMet and is saturated by even the lowest concentrations of AdoMet achieved in these studies. AdoMet independence could also result from a change in the AdoMet K_m of the *cheR* methyltransferase when the transducing protein for oxygen or PTS attractants is the substrate, but this is less likely because BT20 (*cheR*) adapts to these attractants. More definitive experiments will be required to distinguish between these possible mechanisms.

In view of the novel adaptation mechanism for aerotaxis and chemotaxis to PTS sugars, it is interesting to note the differences between their sensory transduction mechanism and those of the MCP-dependent systems. All identified receptors for the MCP-dependent systems are MCPs or soluble periplasmic proteins that interact with a MCP (1–4). The receptors for temperature and cytoplasmic pH have not been identified, but these could also be the *tsr* protein (22–25). The chemoreceptors for PTS sugars are membrane-bound enzymes IIA of the PTS (28). In aerotaxis the binding of oxygen to the terminal oxidase of the respiratory chain stimulates electron transport and in-

creases the protonmotive force (pmf) across the cytoplasmic membrane (29, 45). An unidentified pmf sensor detects the change and initiates the behavioral response. The bacteria adapt to the new steady-state pmf without the return of the pmf to the prestimulus level (29, 46). Phototaxis (47), the blue-light response, and taxis to electron acceptors such as nitrate and fumarate also appear to be mediated by the pmf sensor (29, 45).

It seems likely that the sensory adaptation mechanism for chemotaxis to PTS substrates is different from the adaptation mechanism for taxis mediated by the pmf. The membrane-bound enzymes IIA in the PTS, or a nearby transmembrane protein, may be chemically modified in response to an increased concentration of PTS substrate. Phosphorylation by phospho*enol*pyruvate would be an attractive possibility for signal processing in this system. For aerotaxis, the pmf sensor is presumably a transmembrane protein. A model incorporating these speculations is presented in Fig. 4. Alternatively, the pmf sensor and adaptation system might be associated with the flagellar basal body because the flagellar motor is energized by the pmf, which also influences tumbling frequency in unstimulated bacteria (48).

The findings presented in this paper indicate a diversity of adaptation mechanisms not previously reported. Remarkably, in addition to protein methyltransferase-dependent adaptation there are methyltransferase-independent adaptation mechanisms. Elucidation of the novel mechanisms for adaptation will provide additional insight into the molecular mechanism of sensory transduction.

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