Pleiotropic Aspartate Taxis and Serine Taxis Mutants of *Escherichia coli*

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Mutants that at one time were thought to be specifically defective in taxis toward aspartate and related amino acids (*tar* mutants) or specifically defective in taxis toward serine and related amino acids (*tsr* mutants) are now shown to be pleiotropic in their defects. The *tar* mutants also lack taxis toward maltose and away from Co^{2+} and Ni^{2+} . The *tsr* mutants are altered in their response to a variety of repellents. Double mutants (*tar tsr*) fail in nearly all chemotactic responses. The *tar* and *tsr* mutants provide evidence for two complementary, converging pathways of information flow: certain chemoreceptors feed information into the *tar* pathway and others into the *tsr* pathway. The *tar* and *tsr* products have been shown to be two different sets of methylated proteins.

INTRODUCTION

Workers in this laboratory have described bacterial mutants defective in taxis toward aspartate and related amino acids (Mesibov & Adler, 1972) and other mutants defective in taxis toward serine and related amino acids (Hazelbauer *et al.*, 1969; Mesibov & Adler, 1972). These defects were interpreted in terms of the chemoreceptor theory, which states that in chemotaxis chemicals are detected by specific receptors (Adler, 1969). Thus the aspartate taxis mutants seemed to be defective in an aspartate receptor and the serine taxis mutants in a serine receptor. Such an interpretation has proved correct for a number of specific taxis mutants; for example, galactose taxis mutants indeed lack a galactose receptor – the galactose binding protein (Hazelbauer & Adler, 1971).

Subsequently, it has been shown that the aspartate taxis mutants and serine taxis mutants have additional taxis defects (Mesibov & Adler, 1972; Tso & Adler, 1974; this report). Hence these mutants are not specific taxis mutants, and we shall refer to them as ‘pleiotropic taxis mutants’. The aspartate taxis mutants will be referred to as *tar* mutants, for taxis defects towards aspartate and certain repellents, and the serine taxis mutants as *tsr*, a name first used by Parkinson (1975), for taxis defects towards serine and certain repellents.

In this article we describe our present knowledge of these two kinds of mutants. We also present evidence that the *tar* mutants and the *tsr* mutants are each defective in one or other of two pathways through which most chemoreceptors channel their information. Each pathway converges upon a different component; these components are proteins that can be methylated, and they have been described in detail elsewhere (Silverman & Simon, 1977; Springer *et al.*, 1977).

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METHODS

Bacterial strains. The *tar* and *tsr* mutants, listed in Table 1, were derived from *Escherichia coli* K12 strains that are wild-type for chemotaxis. These parent strains have been described previously: AW405 (Mesibov & Adler, 1972); AW574 (Ordal & Adler, 1974a); and AW546, a His⁻ derivative of B275 (Hazelbauer & Adler, 1971).

The double mutants (*tar tsr*) were constructed by transferring the chemotaxis defect of the *tsr* mutant AW518 into the *tar* mutant AW539 via P1 cotransduction with *thr* from *thr*⁺ derivatives of the *tsr* mutant. Two independent double mutants having identical properties were isolated: AW565 and AW569.

Chemotaxis assays. Capillary assays (Adler, 1973) were used for positive chemotaxis. Semi-solid agar swarm plates were used for demonstrating positive chemotaxis to metabolizable chemicals (Adler, 1966). For negative chemotaxis, the chemical-in-plug assay, the chemical-in-capillary assay, the chemical-in-pond assay, and the chemical-in-plate assay (Tso & Adler, 1974) were used. Temporal assays for positive and negative chemotaxis were carried out according to the procedures described by Koshland's group (Macnab & Koshland, 1972; Tsang *et al.*, 1973), except that the bacteria and the chemical to be tested were mixed on a microscope slide. Addition of repellents or attractants resulted in periods of enhanced or suppressed tumbling, respectively. L-Methionine (10^{-6} to 10^{-4} M) was included in all taxis assays involving Met⁻ bacteria (Adler, 1973).

Complementation tests. For complementation of *tsr* mutants, F' factors carrying *tsr* were prepared as follows. The *tsr* marker was introduced into Hfr Hayes *serB*, *thr*⁺, *leu*⁺, *lac*⁺ by joint transduction (Ordal & Adler, 1974a) with *serB*⁺. The resulting *tsr*, *serB*⁺, *thr*⁺, *leu*⁺, *lac*⁺ Hfr was then mated with a *serB*⁺, *thr*, *leu*, *lac*, *recA*, *str*^R F⁻ strain in the presence of streptomycin, and *serB*⁺, *thr*⁺, *leu*⁺, *lac*⁺ bacteria were isolated. F'-carrying strains were those able to transfer *tsr*, *serB*⁺, *thr*⁺, *leu*⁺ and *lac*⁺ into F⁻ *recA* recipients.

To test for complementation, *tsr* mutants in their F⁻ *recA*, *nal*^R, *thr*, *leu*, *lac* form were mated with strains carrying an F' factor that bore one of several *tsr* loci. After selection of the *thr*⁺, *leu*⁺, *lac*⁺, *nal*^R partial diploids in the presence of nalidixic acid, complementation was assessed by their ability to carry out serine taxis on a tryptone swarm plate (Adler, 1966), modified to contain only 2 g tryptone l⁻¹ (instead of the usual 10 g l⁻¹) to increase the speed of the serine ring, 10^{-3} M-L-aspartate to retard the aspartate ring (as in Fig. 5), 1 g NaCl l⁻¹ and 2.5 g agar l⁻¹.

RESULTS

Pleiotropic aspartate taxis (tar) mutants

Positive chemotaxis properties. The first aspartate taxis mutant to be described (Mesibov & Adler, 1972), AW539, appeared to lack taxis only toward aspartate and related amino acids; taxis was evident for all other attractants that had been tested (Mesibov & Adler, 1972), including serine and α -aminoisobutyrate, ribose and galactose (Mesibov & Adler, 1972; Fig. 1, this report). Subsequently, however, taxis toward maltose was tested and found to be abnormal (Fig. 1). In addition, certain repellent responses were found to be abnormal (see below). Thus AW539 is what we shall call a 'pleiotropic aspartate taxis mutant' or a '*tar* mutant', not a specific aspartate taxis mutant.

For the *tar* mutant AW539, the defects in taxis toward aspartate and maltose were indicated on an aspartate swarm plate (Fig. 2) and a maltose swarm plate (Fig. 3), respectively. The mutant, unlike its parent, did not form a ring of bacteria showing taxis toward aspartate or maltose.

All the other aspartate taxis mutants studied so far were also found to be pleiotropic (*tar*) mutants (Table 2). All the mutants tested appeared to belong to a single complementation group: mutants 1, 5, 6, 7 and 8 failed to complement with mutation 4 carried on a lambda phage, according to studies by H. Kondoh (unpublished results).

Negative chemotaxis properties. AW539, the *tar* mutant that was tested most extensively for negative chemotaxis, was repelled normally by acetate, indole, leucine, benzoate and low pH (H⁺) (Table 3). These chemicals represent five different repellent classes (Tso & Adler, 1974). In contrast, Co²⁺ and Ni²⁺, which are members of a different repellent class (Tso & Adler, 1974), elicited little if any response in AW539 (Table 3 and Fig. 4). Thus, mutant AW539 retains certain negative taxes but is defective for others.

Pleiotropic aspartate taxis (*tar*) mutants other than AW539 were not tested extensively

Table 1. *Derivation of tar and tsr mutants*

Mutant		Parent strain*	Isolation method†	Mutagen‡
Allele no.	Strain no.			
<i>tar mutants</i>				
1	AW539	AW405	1	NTG
2	AW631	AW405	1	NTG
3	AW632	AW405	1	NTG
4	AW633	AW405	1	NTG
5	AW634	AW405	2	none
6	AW635	AW405	2	none
7	AW636	AW546	3	NTG
8	AW637	AW574	3	EMS
<i>tsr mutants</i>				
1	AW518	AW405	2	none
2	AW638	AW405	2	none
3	AW639	AW405	1	NTG
4	AW640	AW405	1	NTG
5	AW641	AW405	1	none
6	AW642	AW405	1	none
7	AW643	AW405	1	none
8	AW644	AW405	1	NTG
9	AW645	AW405	1	NTG
10	AW646	AW405	1	NTG
11	AW647	AW546	3	NTG
12	AW648	AW574	3	EMS
13	AW649	AW574	3	EMS
14	AW650	AW574	3	EMS
15	AW651	AW574	3	EMS
16	AW652	AW574	3	EMS
17	AW653	AW574	3	EMS

* See Methods.

† Method 1: as in Mesibov & Adler (1972), using 3×10^{-4} M-L-aspartate for *tar* mutants and 10^{-3} M-L-serine for *tsr* mutants. Method 2: as in Armstrong *et al.* (1967). Method 3: as in Ordal & Adler (1974a), except that tryptone swarm plates (Hazelbauer *et al.*, 1969) were used.

‡ The mutagens *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) and ethyl methanesulphonate (EMS) were used as described by Armstrong *et al.* (1967) and Ordal & Adler (1974a), respectively.

for negative chemotaxis, but the available data (not shown) indicate a response pattern similar to that of AW539.

Reversion studies From mutants 2, 3 and 8 an aspartate taxis revertant was isolated from a ring that eventually formed spontaneously on an aspartate swarm plate, and from mutant 8 a maltose taxis revertant was isolated from a ring that eventually formed spontaneously on a maltose swarm plate. All these revertants showed normal taxis toward aspartate and maltose on swarm plates, judged by normal ring formation. Thus the two chemotaxis defects co-revert, indicating that the two defects result from a single mutation. Attempts to isolate revertants of AW539 were unsuccessful.

Pleiotropic serine taxis (tsr) mutants

Positive chemotaxis properties. The original serine taxis mutant (Hazelbauer *et al.*, 1969; Mesibov & Adler, 1972), AW518, appeared to be defective in taxis only toward serine and related amino acids, for example, α -aminoisobutyrate; its response to all other attractants tested was normal or above normal (Mesibov & Adler, 1972; Figs 1, 2, 3, 5 and 6, this report). However, the response of this mutant to a number of repellents was found to be abnormal (Mesibov & Adler, 1972; Tso & Adler, 1974; see below). Therefore, we shall refer to such a mutant as a 'pleiotropic serine taxis mutant' or a '*tsr* mutant', not a specific serine taxis mutant.

Fig. 1. Chemotactic responses to several attractants of the chemotactically wild-type parent AW405 (●), the *tar* mutant AW539 (△) and the *tsr* mutant AW518 (○), measured by capillary assays at a bacterial concentration of 4×10^6 cells ml^{-1} (A_{590} 0.005). Where available (Mesibov & Adler, 1972), results of assays at a bacterial concentration 20 times higher are in fair agreement with these. The bacteria were grown in glycerol-minimal medium (Adler, 1973) when they were to be tested for responses to L-aspartate and L-serine and also for D-galactose (since the strains are Gal⁻). Bacteria to be tested for responses to maltose and D-ribose were grown in the respective sugar-minimal medium (Adler, 1973). For AW405, the results for the amino acids and ribose are the average of two experiments.

We have studied 16 additional similar mutants that are attracted well by aspartate but are defective in response to serine in a capillary (Table 4) and fail to make a serine ring on a swarm plate (Hazelbauer *et al.*, 1969; Fig. 5, this report). All 17 mutants belong to a single complementation group (see below). The additional mutants have received limited study of their attraction by sugars: eleven tested were attracted into capillaries containing glucose (Table 4); two tested were attracted into capillaries containing maltose (data not shown); three tested exhibited normal responses in swarm plates containing D-fructose, D-galactose,

Fig. 2

Fig. 3

Fig. 2. Use of a swarm plate as an indication of taxis toward L-aspartate. Note the aspartate-consuming ring (as in Adler, 1966), indicative of aspartate taxis, in the parental strain AW405 (left) and the *tsr* mutant AW518 (right), and the decreased spreading and absence of a ring in the *tar* mutant AW539 (bottom). The medium contained 10^{-4} M-L-aspartate and, as an extra carbon and energy source, 10^{-3} M-glucuronate or (in other experiments) 10^{-3} M-glycerol. Other additions and procedures were as described in Adler (1966). Incubation was for 12 h at 35 °C.

Fig. 3. Use of a swarm plate as an indication of taxis toward maltose. Note the maltose-consuming ring at the edge of the swarm, indicative of maltose taxis, in the parental strain AW405 (left) and the *tsr* mutant AW518 (right), and the decreased spreading and absence of a ring in the *tar* mutant AW539 (bottom). The medium contained 2×10^{-4} M-maltose. Other additions and procedures were as described in Adler (1966). Incubation was for 20 h at 35 °C.

Table 2. Chemotactic responses of *tar* mutants to various attractants

Chemotaxis was measured by the capillary assay (Adler, 1973) at a bacterial concentration of 4×10^6 cells ml $^{-1}$. The results (average of several experiments) show the mutant response compared with that of its parent at the attractant concentration which elicited the peak response from the parent. Attractant concentrations and peak responses of the parental strains AW405, AW574 and AW546 were, respectively: 10^{-3} M-L-aspartate, 120000, 120000 and 140000; 10^{-3} M-maltose, 45000, 50000 and 35000; 3×10^{-4} M (AW405) or 10^{-3} M-D-ribose, 31000, 70000 and 70000; 10^{-2} M-L-serine, 140000, 70000 and 170000. The bacteria were grown in minimal salts medium (Adler, 1973) with the attractant sugar as carbon source, or with glycerol for the amino acid attractants.

Mutants 1 and 8, the only ones tested with galactose in the capillary assay, gave responses of 90 and 35 %, respectively, of their parents.

<i>tar</i> mutant	Percentage of parental response to:			
	Aspartate	Maltose	Ribose	Serine
1 (AW539)	0.4	5	90	73
2	0.1	1	6	21
3	0.4	5	32	36
4	0.5	10	52	86
5	0.3	5	190	68
6	0.3	4	ND	57
7	0.3	5	57	85
8	0.1	1	16	51

ND, No data available.

Fig. 4. Use of the chemical-in-plate assay as an indication of negative chemotaxis to Co^{2+} . The three wells each contained a suspension of bacteria (A_{500} 0.3) in 0.2 % (w/v) agar but without CoSO_4 ; the rest of the plate (the light surface) contained 3 % (w/v) agar and 10^{-2} M- CoSO_4 , but no bacteria. Other additions and procedures were as described in Tso & Adler (1974). Note the ring of bacteria, indicative of repulsion by Co^{2+} , in the wells containing the parental strain AW405 (left) and the *tsr* mutant AW518 (right), and the abnormality of the ring in the well containing the *tar* mutant AW539 (bottom). Incubation was for 27 min at room temperature.

Table 3. *Chemotactic responses to repellents*

Chemotactic responses were assayed by the following methods: (a) Chemical-in-plug assay. Concentrations of chemicals used in the plug were: 10^{-1} M-sodium acetate, 10^{-1} M-sodium benzoate, 3×10^{-3} M-indole, 10^{-1} M-L-leucine, 10^{-3} M- NiSO_4 , 10^{-2} M- CoSO_4 .

(b) Chemical-in-capillary assay.

(c) Chemical-in-pond assay [both the centre and right panels of Fig. 3 of Tso & Adler (1974) were used in all cases].

(d) A special version of chemical-in-plug method. A plug of 3 % agar containing a centre well was embedded in 1 % methylcellulose containing bacteria; then 0.33 M- H_3PO_4 was added to the centre well.

(e) Temporal assay (Macnab & Koshland, 1972; Tsang *et al.*, 1973). Final concentration of each chemical, duration of response in the wild type (in seconds $\pm$ average deviation, with number of experiments indicated in parentheses) and duration of response in the *tar* mutant (in seconds $\pm$ average deviation, with number of experiments indicated in parentheses) were, respectively: 17 mM-sodium acetate, 16 ± 1 (2), 17 (1); 33 mM-sodium benzoate, 32.5 ± 2.5 (2), 55 ± 5 (2); 0.33 mM-indole, 30 ± 5 (6), 37 ± 5 (4); 33 mM-L-leucine, 14 ± 1 (4), 14.5 ± 0.5 (3); 0.5 mM- CoSO_4 , 25 (1), 0 (1); 0.5 mM- NiSO_4 , 30 ± 2 (4), 0 (4). In the *tsr* mutant: 0.5 mM- CoSO_4 , 50 (1); 0.5 mM- NiSO_4 , 46 ± 3 (4); data for repellents which have become attractants in *tsr* have been presented elsewhere (Muskavitch *et al.*, 1978). In the double mutant, addition of indole transiently caused a small fraction of the cells to display a slight increase in tumbling frequency; this does not represent a full repellent response.

Repellent	Response			
	Wild type (AW405)	<i>tar</i> mutant (AW539)	<i>tsr</i> mutant (AW518)	<i>tar tsr</i> double mutant (AW565, AW569)
Acetate	repulsion (a, e)	repulsion (a, e)	attraction (b, e)	no repulsion (a, e*)
Indole	repulsion (a, e)	repulsion (a, e)	attraction (b, e)	slight repulsion (a, e)
Leucine	repulsion (a, e)	repulsion (a, e)	no response (b, e)	no repulsion (a, e*)
Benzoate	repulsion (a, e)	repulsion (a, e)	attraction (b, e)	no repulsion (a, e*)
H^+	repulsion (c, d)	repulsion (c, d)	no response (c, d)	ND
Co^{2+}	repulsion (a, e)	no response (a, b, e)	repulsion (a, e)	no repulsion (e*)
Ni^{2+}	repulsion (a, e)	no response (a, b, e)	repulsion (a, e)	no repulsion (e*)

ND, No data available.

* The spontaneous tumbling frequency of the double mutant was so low that it would have been hard to detect suppression of tumbling (i.e. attraction).

Fig. 5

Fig. 6

Fig. 5. Use of a swarm plate as an indication of taxis toward L-serine. Note the spreading and the serine-consuming ring at the edge of the swarm, indicative of serine taxis, in the parental strain AW405 (left) and the *tar* mutant AW539 (bottom), and the decreased spreading and absence of a ring in AW518 (right). The medium contained tryptone broth as in Hazelbauer *et al.* (1969), supplemented with 10^{-2} M-L-aspartate to retard the aspartate ring (compare with Fig. 6). Incubation was for 6.5 h at 35 °C.

Fig. 6. Use of a tryptone broth swarm plate as an indication of taxis toward L-serine and L-aspartate. Note the outer serine-consuming ring and the inner aspartate-consuming ring, indicative of serine and aspartate taxis, respectively, in the parental strain AW405 (top left). Only the aspartate-consuming ring is present in the *tsr* mutant AW518 (top right), only the serine-consuming ring is present in the *tar* mutant AW539 (bottom left), and neither is present in the *tar tsr* double mutant AW569 (bottom right). Incubation was for 6 h at 30 °C in medium containing tryptone broth as described in Hazelbauer *et al.* (1969), except that the agar concentration was 0.3 %. Photo courtesy of H. Kondoh.

Table 4. *Chemotactic responses of tsr mutants to various chemicals*

In experiments with attractants, the procedures were the same as in Table 2, except that in all glucose assays and in the serine assay for mutants 1 to 11, the bacterial concentration was 8×10^7 cells ml $^{-1}$.

In experiments with repellents, lack of repulsion is indicated by —. With acetate, there was actually attraction (see text); failure to be repelled by sodium acetate was shown by the chemical-in-plug assay or the chemical-in-plate assay or by attraction into a capillary containing sodium acetate. Defective H $^{+}$ taxis was shown by lack of attraction of bacteria at pH 5.0 into a capillary at pH 7.0. Failure to be repelled by L-leucine was shown by the chemical-in-plug assay.

<i>tsr</i> mutant	Percentage of parental response to attractants:			Response to repellents:		
	Serine	Aspartate	Glucose	Acetate	H $^{+}$	Leucine
15	0	52	ND	—	ND	—
14	0	87	ND	—	ND	—
12	0	93	ND	—	ND	—
16	0	147	ND	—	ND	—
17	0	215	ND	ND	ND	ND
13	1	72	ND	—	ND	—
1 (AW518)	1	103	267	—	—	—
11	1	109	125	ND	ND	ND
7	1	125	166	—	—	ND
3	2	52	176	—	—	—
5	2	103	142	—	—	—
10	2	115	224	—	—	ND
4	3	129	168	ND	—	—
9	4	128	150	—	—	ND
6	4	137	211	—	—	ND
2	5	95	162	ND	—	ND
8	5	145	189	—	—	ND

ND, No data available.

D-glucose, maltose or D-ribose (data not shown). In nearly all cases the response to sugars was greater for *tsr* mutants than for their parents, as was found originally with AW518 (Mesibov & Adler, 1972; Fig. 1 and Table 4, this report). Furthermore, every one of the serine taxis mutants was abnormal in negative chemotaxis (see below) and is, therefore, a pleiotropic (*tsr*) mutant.

Negative chemotaxis properties. The *tsr* mutant AW518 was repelled normally by Co^{2+} and Ni^{2+} (Table 3 and Fig. 4), but not by acetate, benzoate, indole or leucine, as previously reported (Tso & Adler, 1974) or by H^+ . Thus, certain negative taxes are retained while others are lost. A remarkable finding, reported in detail elsewhere (Muskavitch *et al.*, 1978), is that AW518 is attracted by a number of chemicals that normally repel: acetate, benzoate and indole (Table 3).

All that has been described for negative chemotaxis in AW518 is also true for the additional serine taxis mutants as far as they have been tested (Table 4). Thus, 14 of the additional serine taxis mutants have been tested and found abnormal for at least one of the negative taxes that is altered in AW518.

Reversion studies. On a tryptone swarm plate, serine taxis mutants spread abnormally owing to their failure to form a ring of bacteria showing taxis toward serine (Hazelbauer *et al.*, 1969; Fig. 6, this report). Revertants that did form such a ring were not found for AW518, but they were obtained, both spontaneously and following treatment with the mutagen ethyl methanesulphonate, for five other strains examined (mutants 12 to 16). From each of the five mutants, the revertants that had regained full serine taxis had also regained normal negative taxis away from acetate and leucine, the two repellents tested. This co-reversion indicates that the several defects in each mutant are due to a single mutation.

Complementation. The 17 *tsr* mutants studied all fall into a single complementation group: they failed to complement with F' strains carrying one of three *tsr* mutations (those of strains AW518, 12 and 14). The *tsr* mutation is not dominant: all 17 mutants did show complementation with an F' strain that carried no taxis mutation, and the parental strains showed serine taxis even when they harboured F' episomes carrying each of the three *tsr* mutations.

tar tsr double mutant

Double mutants, containing both the *tar* mutation of AW539 and the *tsr* mutation of AW518, were prepared as described in Methods. In such double mutants all chemotactic responses are missing or much reduced. Thus they behave like generally non-chemotactic mutants (*che* mutants), but differ in one important respect: their tumbling frequency is appreciable although considerably less than normal, while *che* mutants either fail to tumble or tumble incessantly (Parkinson, 1977; Warrick *et al.*, 1977).

Positive chemotaxis properties. In the double mutants, loss of aspartate and serine taxis was expected from the characteristics of the single mutants and this was found. On a tryptone swarm plate, the double mutants gave only very slight spreading (Fig. 6) like generally non-chemotactic mutants (Armstrong *et al.*, 1967); the outer, or serine-consuming, ring and the inner, or aspartate-consuming, ring produced by chemotactically wild-type *E. coli* (Adler, 1966) were missing. The double mutant was not attracted into capillaries containing aspartate or serine (data not shown).

Surprisingly, the double mutants appeared to be defective in taxis toward sugars that attracted the single mutants well. Unlike the single mutants, the double mutants made extremely small rings on D-glucose and D-ribose swarm plates (data not shown), atypical for wild-type *E. coli* (Adler, 1966). Unlike the single mutants, the double mutants were attracted little or not at all into capillaries containing D-glucose, D-galactose or D-ribose (data not shown). However, temporal stimulation by D-ribose did produce a behavioural response: addition of D-ribose suppressed the tumbling (see below) induced by indole.

Negative chemotaxis properties. As expected from the characteristics of the single mutants, the double mutants were not repelled by acetate, benzoate and leucine, or by Co^{2+} and Ni^{2+} , but addition of indole elicited a weak tumbling response (Table 3). Further, there is evidence that *E. coli* excretes a substance, as yet unidentified, that repels wild-type bacteria (Tso & Adler, 1974) and also the double mutants (H. Kondoh, unpublished observation).

DISCUSSION

We have found that the so-called aspartate taxis mutants and serine taxis mutants have additional chemotaxis defects besides the failure to be attracted to aspartate or serine and their analogues: we call them *tar* and *tsr* mutants, respectively.

The *tar* mutants lack positive taxis for aspartate and certain analogues such as α -methyl-aspartate and glutamate, but they retain positive taxis for serine and analogues such as α -aminoisobutyrate. They are highly defective in positive taxis for maltose, but other sugars remain attractants to a variable degree. They retain negative taxis for certain repellents (for example, acetate, benzoate, indole, leucine and H^+), but they have lost negative taxis for other repellents (for example, Co^{2+} and Ni^{2+}).

The *tsr* mutants are highly defective in positive taxis for serine and analogues such as α -aminoisobutyrate, but aspartate and its analogues and various sugars remain attractants. Certain chemicals (for example, Co^{2+} and Ni^{2+}) remain repellents, but the mutants have lost negative taxis for other repellents (for example, acetate, benzoate, indole and leucine), and instead the bacteria are attracted to nearly all these chemicals (Muskavitch *et al.*, 1978).

Complementarity of tar and tsr pathways. A comparison of the properties of the *tar* and *tsr* mutants reveals a complementarity: (i) aspartate and maltose fail to attract the *tar* mutants but do attract the *tsr* mutants; (ii) serine fails to attract the *tsr* mutants normally but does attract the *tar* mutants; (iii) Co^{2+} and Ni^{2+} fail to repel the *tar* mutants but do repel the *tsr* mutants; (iv) acetate, benzoate, indole and leucine fail to repel the *tsr* mutants (indeed they attract) but do repel the *tar* mutants. This complementarity indicates that certain attractants and repellents are detected by mechanisms that pass information through a component defective in the *tar* mutants, while other attractants and repellents use a pathway that leads into a component defective in the *tsr* mutants. Figure 7 illustrates this view of two complementary pathways of information flow – the *tar* and the *tsr* pathways.

As shown in Fig. 7, the situation with regard to serine is somewhat more complex than indicated above. Chemotactically wild-type bacteria appear to have two chemoreceptors for serine: a high-affinity system that detects both serine and α -aminoisobutyrate, and a low-affinity system that detects serine but not α -aminoisobutyrate (Springer *et al.*, 1977). In the *tar* mutants, the high-affinity system is functional since these mutants respond to α -aminoisobutyrate (Mesibov & Adler, 1972; Springer *et al.*, 1977), but the low-affinity system is defective which results in the somewhat decreased response to serine observed in the *tar* strains (Mesibov & Adler, 1972; Springer *et al.*, 1977; Fig. 1 and Table 2, this report). In the *tsr* mutants, the high-affinity system is non-functional since α -aminoisobutyrate fails to attract the bacteria (Mesibov & Adler, 1972; Springer *et al.*, 1977), but the low-affinity system is still active, accounting for the residual response to serine seen at high concentrations in both capillary and temporal assays (Mesibov & Adler, 1972; Springer *et al.*, 1977). This is not evident in Fig. 1 because of the scale used on the ordinate.

As might be expected, double mutants that contain both the *tar* mutation and the *tsr* mutation are defective in all the chemotaxes defective in the single mutants. Some tactic responses to a few chemicals do remain in the double mutants. It appears now that certain sugar receptors (for example, galactose and ribose) use an additional pathway different from the *tar* and *tsr* pathways (Kondoh *et al.*, 1979). It seems likely that the response to ribose and the negative chemotaxis that is retained in the double mutants is due to this, or another, additional pathway. The expression of additional pathways is somehow largely blocked

Fig. 7. Proposed mechanism for flow of sensory information from the various chemoreceptors through two components via two complementary routes – the *tsr* and *tar* pathways. (AiBu, α -aminoisobutyrate.)

in the double mutants used here but not in other double mutants (Kondoh *et al.*, 1979. *Methyl-accepting chemotaxis proteins*. Kort *et al.* (1975) discovered the methylation of a cytoplasmic membrane component, the methyl-accepting chemotaxis protein (MCP), and showed its involvement in chemotaxis. Attractants increase the methylation level of MCP, while repellents decrease it (Goy *et al.*, 1977). Recently it has been demonstrated that this material consists of several polypeptide components (Silverman & Simon, 1977; Springer *et al.*, 1977). Methylation of certain polypeptides of MCP, the MCP I group, does not occur in the *tsr* mutants, while methylation of other polypeptides of MCP, the MCP II group, does not occur in the *tar* mutants; methylation of both groups of MCP polypeptides does not occur in the *tsr tar* double mutants (Silverman & Simon, 1977; Springer *et al.*, 1977). Thus it has been possible to identify chemically the two components through which sensory information flows from the *tsr* and *tar* pathways (Fig. 7).

There is evidence that MCP I and MCP II interact with each other. Attractants which still attract *tsr* mutants elicit higher responses in these mutants than in their parent (Mesibov & Adler, 1972; Springer *et al.*, 1977; Fig. 1 and Table 4, this report), as though the absence of MCP II allows more effective functioning of the MCP I present. Conversely, certain chemicals which attract or repel *tar* mutants do so more effectively than in wild-type bacteria (Springer *et al.*, 1977). Certain *tar* mutants (2, 3 and 8) are somewhat defective in *tsr* function (Table 3, this report). It is as if a defective MCP I can interfere with the functioning of MCP II, and vice versa. Possibly MCP I and MCP II may occur together in a complex, and in this way they can influence each other's functioning.

It is not yet clear how the output from the *tsr* and *tar* components, MCP I and MCP II, is integrated to produce a response; yet this convergence is necessary since ultimately the flagella must be caused to rotate either clockwise or counterclockwise (Berg, 1974; Berg & Anderson, 1973; Silverman & Simon, 1974) in response to sensory stimuli (Larsen *et al.*, 1974).

Types of mutants. Chemotaxis mutants can now be classified into three types (see Fig. 7).

'Specific mutants' lack taxis only to one chemical or a group of chemicals that are structurally closely related; these mutants lack one chemoreceptor. 'General mutants' are defective in all chemotactic responses, both positive and negative (Armstrong *et al.*, 1967; Tso & Adler, 1974). These generally non-chemotactic (*che*) mutants lack the ability to control the methylation level of MCP (Kort *et al.*, 1975; Silverman & Simon, 1977; Springer *et al.*, 1977) or they lack proper functioning of a final common pathway through which information from MCP must flow. 'Pleiotropic mutants', such as those described in this report, have lost several, but not all, taxes. Another example of the pleiotropic type are the signalling (*trg*) mutants (Ordal & Adler, 1974*b*; Strange & Koshland, 1976), which do not show taxis to ribose and galactose because they lack a component, MCP III, shared by the ribose and galactose receptors (Kondoh *et al.*, 1979).

Since they have multiple taxis defects, it is unlikely that the pleiotropic aspartate taxis mutants and the pleiotropic serine taxis mutants lack an aspartate receptor or a serine receptor; rather the output from these and other receptors fails to be registered (or in some cases is wrongly registered). Chemoreceptors generally are part of transport systems (Adler, 1975; Hazelbauer & Adler, 1971), and thus specific mutants, lacking receptors, might be expected to show defects in transport. However, eight *tar* mutants and twelve *tsr* mutants tested were indistinguishable from their parents in the transport of 10^{-8} to 10^{-6} M L-aspartate or L-serine, respectively (Mesibov & Adler, 1972; R. W. Reader, unpublished data). Thus the work described in this paper demonstrates that what we thought were receptor mutants (Mesibov & Adler, 1972) are not. The failure so far to find any specific aspartate or serine taxis mutants is probably due to the multiplicity of transport systems (and hence chemoreceptors) for these amino acids (Oxender, 1972; Schellenberg & Furlong, 1977; Springer *et al.*, 1977).

The chemoreceptors for amino acids can be divided into two types: those functional in the *tsr* mutants and those functional in the *tar* mutants. However, the classification of numerous amino acid attractants into only two chemoreceptors (the 'aspartate receptor' and the 'serine receptor') (Mesibov & Adler, 1972) must now be considered an oversimplification, in that it was based on the use of pleiotropic mutants. On the other hand, results on the basis of a different criterion – the competition experiment (Adler, 1969; Mesibov & Adler, 1972) – were in agreement with this classification. (In such an experiment one attractant is placed in a capillary that is inserted into a suspension of motile bacteria, while a second attractant, at a relatively high concentration, is placed both in the capillary and in the bacterial suspension. If the two attractants are detected by the same chemoreceptor, the second attractant should saturate the receptor and in this way prevent taxis toward the first. If the two attractants are detected by different chemoreceptors, taxis toward the first attractant should still take place.) However, competition may not have been at the receptor level, but rather at the level of later components, i.e. those involved in the pleiotropic mutants.

A more accurate, detailed classification of the amino acid attractants into chemoreceptor classes must await the isolation of specific taxis mutants.

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