# Polar Location of the Chemoreceptor Complex in the *Escherichia coli* Cell

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**The eukaryotic cell exhibits compartmentalization of functions to various membrane-bound organelles and to specific domains within each membrane. The spatial distribution of the membrane chemoreceptors and associated cytoplasmic chemotaxis proteins in *Escherichia coli* were examined as a prototypic functional aggregate in bacterial cells. Bacterial chemotaxis involves a phospho-relay system brought about by ligand association with a membrane receptor, culminating in a switch in the direction of flagellar rotation. The transduction of the chemotaxis signal is initiated by a chemoreceptor-CheW-CheA ternary complex at the inner membrane. These ternary complexes aggregate predominantly at the cell poles. Polar localization of the cytoplasmic CheA and CheW proteins is dependent on membrane-bound chemoreceptor. Chemoreceptors are not confined to the cell poles in strains lacking both CheA and CheW. The chemoreceptor-CheW binary complex is polarly localized in the absence of CheA, whereas the chemoreceptor-CheA binary complex is not confined to the cell poles in strains lacking CheW. The subcellular localization of the chemotaxis proteins may reflect a general mechanism by which the bacterial cell sequesters different regions of the cell for specialized functions.**

The bacterial cell has been viewed as a vessel encased by a membrane in which proteins of varied function are randomly distributed. The eukaryotic cell, on the other hand, is known to be highly organized, with many membrane-bound organelles performing distinct functions. It comes as no surprise that eukaryotic proteins of a given function are also found to aggregate and localize within specific regions of the cell. For example, the $Na^+,K^+$-adenosine triphosphatase (ATPase) is localized at the basal rather than the apical pole of Madin-Darby canine kidney (MDCK) cells (*1*), proteins involved in new bud formation in a/α diploid cells of the yeast *Saccharomyces cerevisiae* localize to the cell pole opposite from the bud scar left from the previous division event (*2*), and chemotaxis receptor molecules in mammalian neutrophils are positioned at the pole of the pseudopodial extensions, in the direction of cell movement (*3*).

*Caulobacter crescentus*, a bacterium that bears a single polar flagellum for a portion of its cell cycle, localizes its chemoreceptors to the flagellated pole of the cell (*4, 5*). We have now found that in *Escherichia coli* a complex of chemoreceptor proteins is also localized at the cell poles, and we suggest that polar localization of the chemotaxis machinery may be a general phenomenon in the bacterial cell and not simply a special case in an organism with distinct polar characteristics, such as *Caulobacter crescentus*.

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*Escherichia coli* monitors environmental changes in repellent or attractant concentration, and efficiently and rapidly [about 200 ms (*6*)], transmits the signal to the flagellar motors via a phospho-relay consisting of interactions among several cytoplasmic proteins. Compelling evidence for a long-lived ternary complex of the chemoreceptor MCP (methyl-accepting chemotaxis protein) and two intracellular proteins, CheA and CheW, has been provided by in vitro analysis of the interactions between the Tsr chemoreceptor and purified CheA and CheW proteins (*7–9*) and by studies of allele-specific suppressors of *tsr* mutants (*10*). The association between the chemoreceptor and CheA is greatly enhanced by CheW (*7–9, 11*).

A change in the conformation of the chemoreceptor on ligand binding or release is believed to mediate the transmission of chemotactic signals (*12*). This change, in turn, affects the CheA autophosphorylation rate, most likely as a result of a direct change in CheA conformation rather than disruption of the MCP-CheA-CheW ternary complex (*7–9*). The rate of CheA autophosphorylation increases in the presence of repellent and decreases in the presence of attractant (*7–9*). Phosphorylated CheA, in turn, phosphorylates CheB (a methylesterase important for sensory adaptation) and CheY (*13, 14*). Phosphorylated CheY interacts with the flagellar switch and causes the motor to turn clockwise (*15*). CheZ accelerates the dephosphorylation of CheY (*14*).

We have used antibodies to phospho-relay chemotaxis proteins and to *E. coli* strains with well-characterized chemotaxis defects to address several issues including (i) the spatial organization of the chemoreceptors in the *E. coli* cell, (ii) the location of the other components of the chemotaxis phospho-relay system, and (iii) whether the chemoreceptors, CheA and CheW proteins, colocalize in vivo.

**Clustering of MCPs at the pole of the cell.** We used immunoelectron microscopy and indirect immunofluorescence light microscopy to examine the spatial distribution of chemoreceptors in *E. coli*. The intracellular location of the chemoreceptors was analyzed with the use of three different antibodies, each generated against an *E. coli* chemoreceptor (Tar, Trg, and Tsr). Each of these polyclonal antibodies recognized Tar and Tsr, and because of the high sequence conservation among the chemoreceptors most likely recognize all four known chemoreceptors. With each of these antibodies we observed a clustered distribution of protein in wild-type *E. coli* cells as detected by the location of conjugated gold particles (Fig. 1A). The gold particles were

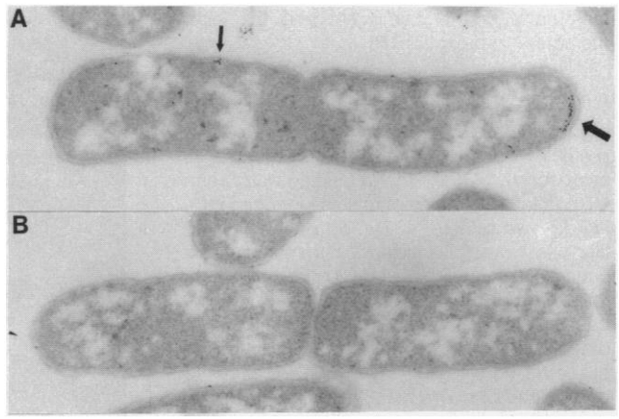


**Fig. 1.** Intracellular positioning of Tsr in septating cells by immunoelectron microscopy. Immunoelectron micrographs of longitudinal sections of (**A**) septating wild-type (RP437) or (**B**) ΔMCP (KO607) cells after incubation with anti-Tsr as described (*35*). The position of the MCP proteins is revealed by the presence of colloidal gold particles on the cell sections. Most of the MCP protein is nonuniformly distributed into one or more clusters (*16*). The clusters can be found anywhere along the cell length although polar clusters (80 percent) are the most frequent. The thick arrow identifies a polar cluster; the thin arrow identifies a nonpolar cluster.

aggregated into clusters (*16*) at the inner membrane, and most of the clusters were at the poles of the cells. In the same wild-type strain, we found that the distribution of the outer membrane protein LamB, or the inner membrane protein $II^{mtl}$ appeared all around the membrane (Fig. 2).

Localization of the chemoreceptor clusters was confirmed by examination with immunofluorescence light microscopy with antibody to Tsr (Fig. 3B1). Areas of bright fluorescence were most often positioned at the poles of the cells although we also detected in each cell several areas of weaker fluorescence. We show, for comparison, the position of the periplasmic maltose-binding protein MBP, which is localized to the cell poles (Fig. 3A2) (*17, 18*). Using antibodies to flagella, we confirmed that the seven to ten flagella per *E. coli* cell are randomly distributed and there was no apparent concentration of flagella at the poles of the cells (Fig. 3A1). This observation is consistent with a previous study showing that MCPs did not accumulate near the flagellar basal bodies (*19*). Thus, most of the chemoreceptors are not coincident with the flagellar motors. However, the possibility that some areas of weak fluorescence correspond to the site of flagellar basal bodies cannot be excluded.

We analyzed the positioning of the chemoreceptors by counting the distribution of antibody-conjugated gold particles in sections of wild-type cells. Of the 1878 gold particles counted, 81 percent were at the membrane, and 80 percent of these were at the cell pole (Table 1). In these sections, there was an average of five gold particles per micrometer of linear membrane at the pole, whereas there was only 0.6 gold particle per micrometer of nonpolar linear membrane (Table 2). This indicates that there were nine times as many

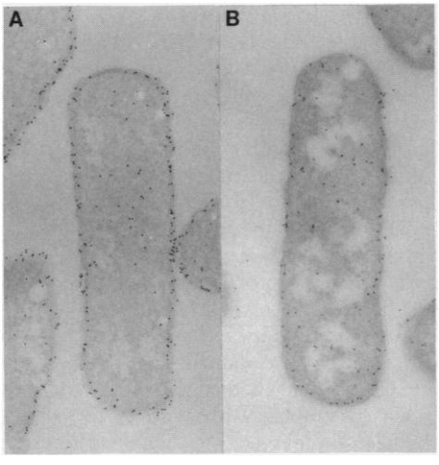


**Fig. 2.** Localization of randomly distributed membrane proteins by immunoelectron microscopy. Immunoelectron micrographs (*35*) of longitudinal sections of wild-type *E. coli* (RP437) cells reveal the random distribution of the outer membrane protein LamB (**A**) and the inner membrane protein $II^{mtl}$ (**B**).

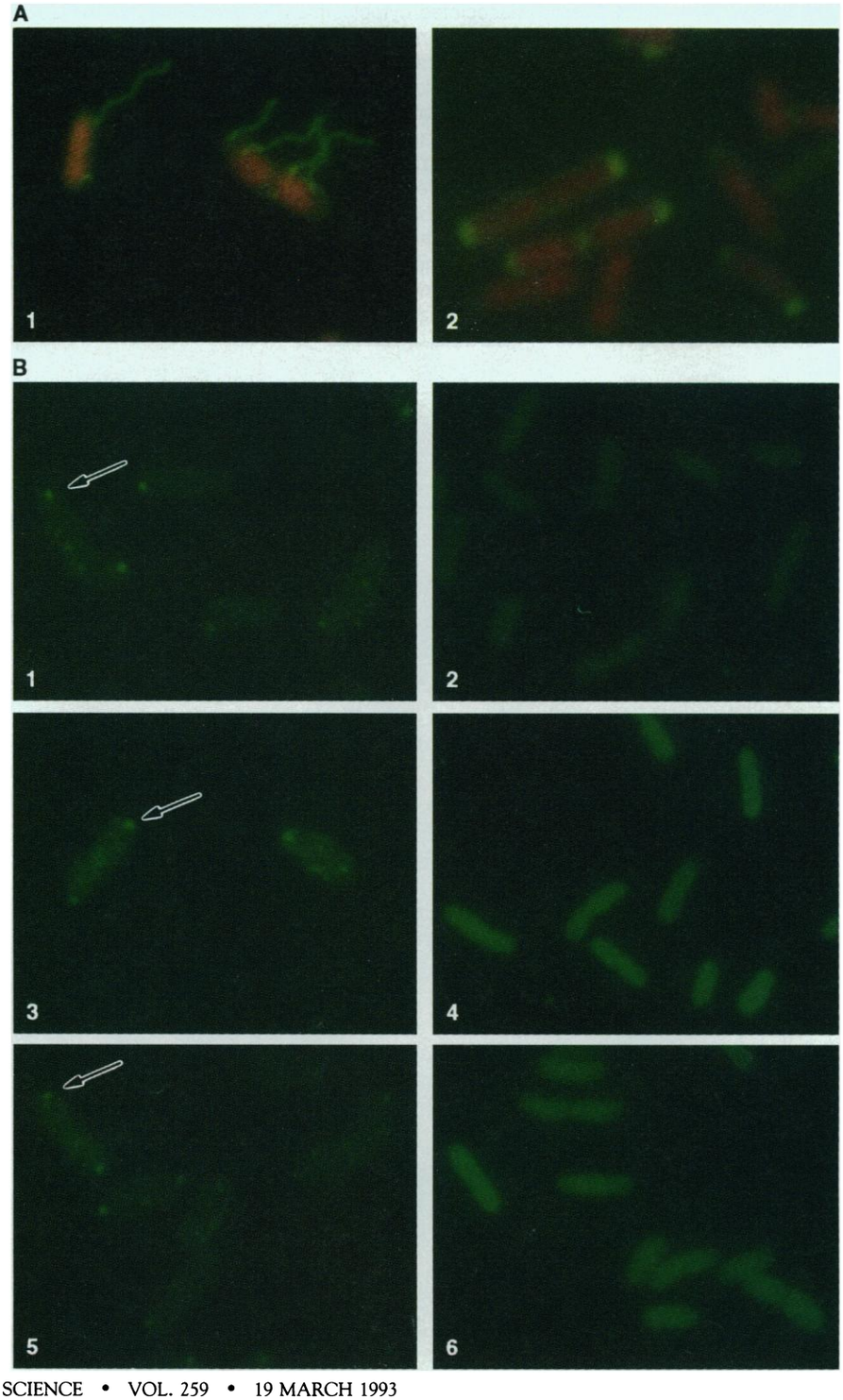


**Fig. 3.** Intracellular positioning of chemotaxis proteins by indirect immunofluorescence light microscopy. (**A**) Position of the flagella (1) and MBP (after 15 minutes of maltose induction) (2) in wild-type cells (RP437) (*36*). Antibody-reactive sites were visualized by immunofluorescence with fluorescein-labeled goat antibody to rabbit serum. The DNA can be seen (orange) because of the incorporation of propidium iodide into the DNA. (**B**) Intracellular distribution of the MCPs with anti-Tsr (1 and 2), CheA (3 and 4), and CheW (5 and 6) by immunofluorescence in wild-type (1, 3, and 5) and in an ΔMCP mutant, KO607 (2, 4, and 6). The arrows show selected foci of polar fluorescence.

gold particles (and therefore chemoreceptors) at the cell pole. Not only were most of the MCP molecules at the cell poles, but 81 percent of the polar protein appeared in tight clusters (Table 1). The observed polar distribution of gold particles was due to the positioning of the chemoreceptors and was not the result of a cross-reacting protein. In an isogenic strain (KO607) with deletions in all four chemoreceptor genes (*20, 21*), the background signal after immunoreaction with the antibodies to MCP (anti-MCP) was very low and showed no polar bias (less than one membrane-associated gold particle per cell with the antibodies to Tsr) (Table 1 and Figs. 1B and 4B).

Although most of the clustered MCPs were at the poles of the cells, a few clusters were found along the lateral membrane (Fig. 1, A and C). Of the sections of nonseptating cells examined, 42 percent contained clusters and, of these, 7 percent contained a single nonpolar cluster (compared to 76 percent with a single polar cluster) (Table 3); the remaining sections contained more than one cluster (Table 3). The distribution of clusters in sections of septating cells was similar to that of nonseptating cells although the frequency of clusters was higher (56 percent). In addition, nonpolar clusters were often associated with the site of septation.

Polar clusters contained a larger number of gold particles than nonpolar clusters (Table 4). For example, the average polar cluster contained 11 gold particles compared to 8 gold particles in nonpolar clusters (Table 4). More than 70 percent of all chemoreceptors detected by immunoelectron microscopy were in clusters, presumably reflecting the distribution of MCPs in the cell.

**Clustering of CheA and CheW at the cell pole.** CheA and CheW are soluble proteins (*22*). However, since they are able to form ternary complexes with chemoreceptors (*7–9, 10*), we examined their cellular distribution with antibodies to CheA or CheW. Immunoelectron micrographs (Fig. 4, E and I) and indirect immunofluorescence images (Fig. 3B, 3 and 5) revealed that in wild-type cells these proteins preferentially localized to the polar membrane with a pattern similar to that of the MCPs. Most of the CheA protein was associated with the inner membrane (Table 1). Sections of nonseptating cells had seven times as many membrane-associated CheA proteins at the cell poles (Table 2). The observed polar bias could be ascribed specifically to CheA protein because in an isogenic *cheA* deletion mutant (RP9535) the background reactivity attributed to the antibody to CheA (anti-CheA) was less than two gold particles per section. Most of those nonspecific gold particles (61 percent) were in the cytoplasm (Table 1).

As with the MCPs, the membrane-localized CheA was clustered (Figs. 3B3 and 4E). The immunofluorescence images obtained with anti-CheA (Fig. 3B3) were similar to those obtained with anti-MCP, cells usually had one to three localized regions of bright fluorescence, and these were most often at the cell poles. In addition, there were multiple patches of weaker fluorescence (Fig. 3B3). Counting of gold particles revealed that at the cell poles, 56 percent of the gold particles were in clusters (Table 1). Consistent with the lower frequency of gold particles, the clusters at the pole were smaller than with the MCPs, averaging only 4.3 gold particles per cluster. Although the number of clusters ob-

**Table 1.** Cellular distribution of chemotaxis proteins detected by immunoelectron microscopy of nonseptating cells.

| Antibody* | Strain† | Gold particles (%)‡ | | | Membrane gold particles | |
|---|---|---|---|---|---|---|
| | | Cells examined | Cytoplasm | Membrane | At the pole§ | Pole in clusters‖ |
| Tsr | Wild-type (RP437) | 180 | 19 (362) | 81 (1516) | 80 | 81 |
| | ΔMCP (KO607) | 180 | 70 (268) | 30 (115) | 34 | 0 |
| | ΔCheA (RP9535)¶ | 180 | 8 (175) | 92 (2060) | 60 | 21 |
| | ΔCheW (LS436) | 180 | 10 (269) | 90 (2271) | 50 | 25 |
| | ΔCheA ⇓ CheW (RBB382)# | 180 | 38 (341) | 62 (567) | 50 | 13 |
| CheA | Wild-type (RP437) | 180 | 38 (259) | 62 (422) | 78 | 56 |
| | ΔMCP (KO607) | 360 | 68 (753) | 32 (353) | 35 | 0 |
| | ΔCheA (RP9535) | 60 | 61 (60) | 39 (39) | 49 | 0 |
| | ΔCheW (LS436) | 180 | 33 (255) | 67 (365) | 39 | 6 |
| | ΔCheA ⇓ CheW (RBB382) | 180 | 63 (88) | 37 (52) | 36 | 0 |
| CheW | Wild-type (RP437) | 300 | 48 (448) | 52 (487) | 66 | 26 |
| | ΔMCP (KO607) | 240 | 74 (588) | 26 (202) | 39 | 4 |
| | ΔCheA (RP9535) | 194 | 49 (173) | 51 (181) | 70 | 12 |
| | ΔCheW (LS436) | 180 | 52 (68) | 48 (63) | 48 | 0 |
| | ΔCheA ⇓ CheW (RBB382) | 180 | 69 (138) | 31 (63) | 59 | 0 |

*The type of antibody detected by the goat antibody to rabbit antibody conjugates of colloidal gold (*35*). †The examined strains listed by the relevant phenotypes with the strain number (*21*) indicated in parentheses. ‡The percent of gold particles in cytoplasm or associated with the membrane and the actual number of gold particles counted in parentheses. §The percent of all membrane gold particles that are at the poles of the cells. ‖The percent of membrane gold particles at the pole that are in clusters (*19*). ¶On Western blots the amount of CheW in these cells is approximately 50 percent of that in wild type. #On Western blots, the amount of CheW is reduced to less than 10 percent of that in wild type. This strain also has a twofold reduction in MCP.

**Table 2.** Polar bias of the MCP, CheA, and CheW proteins. Sections of *E. coli* cells were incubated with antibodies to Tsr, CheA, or CheW as described (*35*) and the position of the deposited gold particles was recorded. Sections of nonseptating cells are presented here although similar results were obtained by examination of septating cells. The number of sections examined is presented in Table 1.

| Antibody* | Strain† | Gold particles per micrometer of membrane‡ | | Polar bias‖ |
|---|---|---|---|---|
| | | Polar | Nonpolar | |
| Tsr | Wild-type (RP437) | 5.00 (1214) | 0.57 (302) | 8.8 |
| | ΔMCP (KO607) | 0.16 (39) | 0.14 (76) | 1.1 |
| | ΔCheA (RP9535) | 5.10 (1233) | 1.60 (827) | 3.3 |
| | ΔCheW (LS436) | 4.50 (1137) | 2.10 (1134) | 2.2 |
| | ΔCheA ⇓ CheW (RBB382) | 1.20 (286) | 0.53 (281) | 2.2 |
| CheA | Wild-type (RP437) | 1.30 (324) | 0.18 (98) | 7.3 |
| | ΔMCP (KO607) | 0.26 (124) | 0.22 (229) | 1.2 |
| | ΔCheA (RP9535) | 0.24 (19) | 0.11 (20) | 2.1 |
| | ΔCheW (LS436) | 0.59 (143) | 0.41 (222) | 1.4 |
| | ΔCheA ⇓ CheW (RBB382) | 0.08 (19) | 0.06 (33) | 1.3 |
| CheW | Wild-type (RP437) | 0.80 (324) | 0.18 (163) | 4.4 |
| | ΔMCP (KO607) | 0.24 (78) | 0.18 (124) | 1.4 |
| | ΔCheA (RP9535) | 0.49 (127) | 0.09 (54) | 5.2 |
| | ΔCheW (LS436) | 0.12 (30) | 0.06 (33) | 2.0 |
| | ΔCheA ⇓ CheW (RBB382) | 0.15 (37) | 0.05 (26) | 3.1 |

*The type of antibody detected by gold conjugated with the goat antibody rabbit antibody (*35*). †The strains examined (*21*) listed by the relevant phenotypes with the strain number indicated in parentheses. ‡The average number of gold particles found along a 1-μm length of polar or nonpolar membrane with the number of gold particles counted in parentheses. ‖Ratio of polar to nonpolar.

served with anti-CheA was lower than that for antibody to Tsr (anti-Tsr), the distribution of clusters was similar (Table 3). In both septating and nonseptating cells, most of the sections with clusters of gold particles contained a single polar cluster (65 percent and 77 percent, respectively). Nonpolar clusters were found in 15 percent of these cells. Sections with more than one cluster were less frequent although in septating cells clusters at both poles were relatively frequent (12 percent) (Table 3).

We detected a similar pattern of localized CheW protein by immunoelectron microscopy (Fig. 4I) and by immunofluorescence (Fig. 3B5). More than half of the gold particles were associated with the membrane in wild-type cells and there were 4.4 times as many membrane gold particles at the pole (Table 2). The membrane-associated CheW protein was slightly clustered with 26 percent of the polar gold particles in clusters (Table 1) and 6 percent of the nonpolar gold particles in clusters (*23*). The distribution of membrane clusters mirrored that of the MCPs and CheA (*23*). The cytoplasmic distribution of CheW was different from that of CheA in that the gold particles were not distributed randomly; many were in pairs and 8 percent were in small clusters (average size was 3.2 gold particles per cluster), suggesting that they may form aggregates (Figs. 4J and 5G).

**Table 3.** Spatial distribution of gold particle clusters. The cellular distribution of gold particles found in clusters after immunoreaction with either anti-Tsr or anti-CheA was determined in sections of both nonseptating and septating wild-type cells (*16*). The numbers are the percentages of cells having a cluster of gold particles at the indicated positions.

| Antibody* | Cell type | Position of cluster* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | One pole | Two poles | One pole + side | Two poles + side | Side | Sections with clusters (%) | Sections examined (no.) |
| MCP | Nonseptating | 76 | 9 | 6 | 1 | 7 | 42 | 825 |
| MCP | Septating | 61 | 13 | 12 | 3 | 11 | 56 | 640 |
| CheA | Nonseptating | 77 | 4 | 4 | ≤1 | 15 | 36 | 521 |
| CheA | Septating | 65 | 12 | 7 | ≤1 | 15 | 42 | 353 |

*The types of antibody detected by the gold particles conjugated to goat antibody to rabbit IgG. †The percentage of cells with a cluster (or clusters) of gold particles at the indicated positions.

Taken together, these data imply that the components of the ternary complex MCP, CheA, and CheW localize to discrete regions of the inner membrane. These localized domains do not appear to be coincident with the flagellar basal bodies, but are predominantly polar. Not only do the MCP, CheA, and CheW proteins localize to the poles, but they are also constrained to concentrated regions that, by immunoelectron microscopy, are identified as clusters. Clustering could be due to the intermolecular interactions between ternary complexes or a subcompartmentalization that leads to the concentration of ternary complexes.

**Location of CheA and CheW in the absence of MCPs.** To test whether the

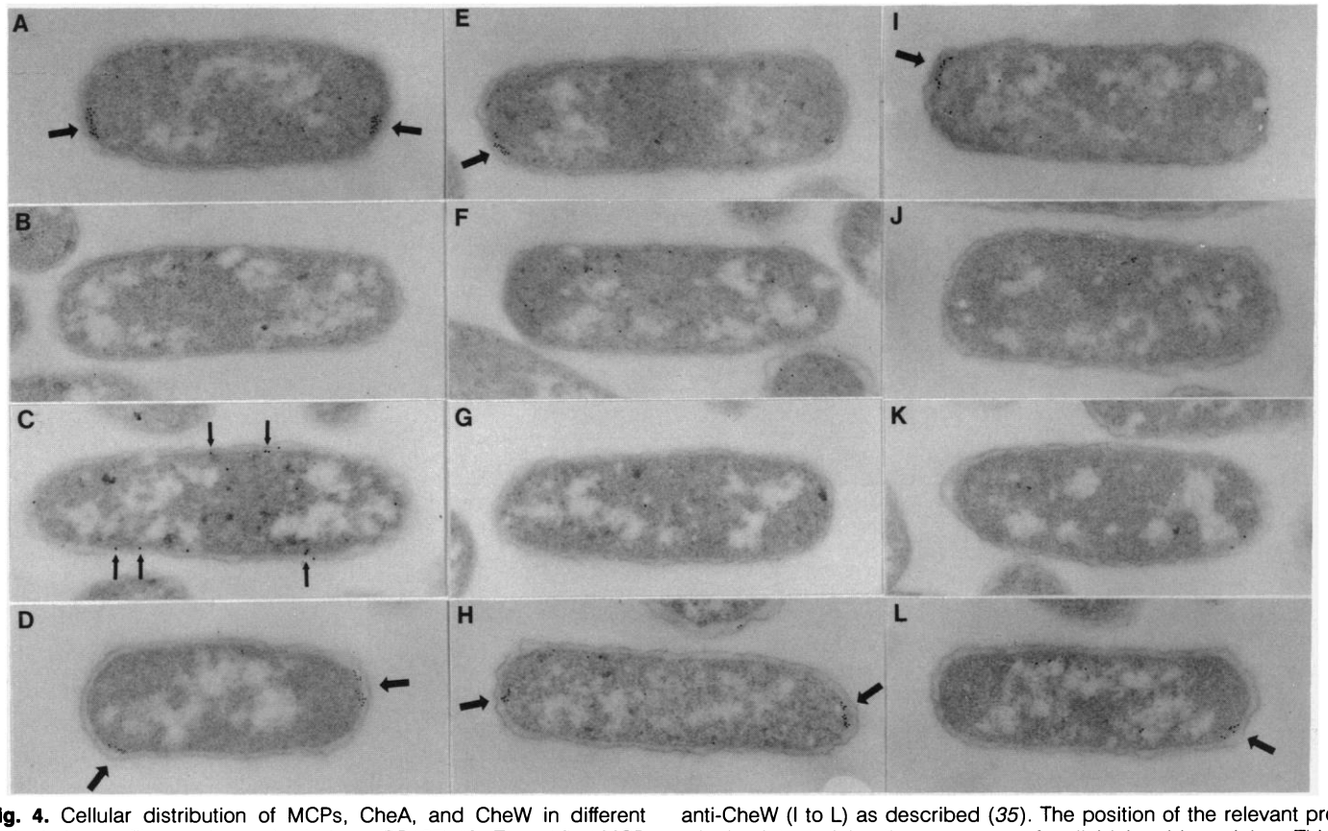


**Fig. 4.** Cellular distribution of MCPs, CheA, and CheW in different mutant strains. Thin sections of wild-type (RP437) (**A**, **E**, and **I**), ΔMCP (KO607) (**B**, **F**, and **J**), ΔCheA ⇓ CheW (RBB382) (**C**, **G**, and **K**), and ΔCheY (RP5232) (**D**, **H**, and **L**) cells were prepared and treated with appropriate dilutions of anti-Tsr (A to D), anti-CheA (E to H), or anti-CheW (I to L) as described (*35*). The position of the relevant protein is detected by the presence of colloidal gold particles. Thick arrows identify regions of colloidal gold clusters. The thin arrows in (C) indicate a few of the nonclustered membrane-associated gold particles.

**Table 4.** Distribution of MCP as determined by the number of gold particles in clusters in nonseptating cells. The distribution of gold particles in clusters due to the antibody to Tsr in various strain backgrounds was determined.

| Strain* | Sections with clusters† | Polar clusters‡ | Size of polar clusters§ | Nonpolar clusters‖ | Size of nonpolar clusters¶ |
|---|---|---|---|---|---|
| Wild-type (RP437) | 42 | 356 | 11.0 (±6.5) | 53 | 7.8 (±3.0) |
| ΔMCP (KO607) | 0 | 0 | 0 | 0 | 0 |
| ΔCheA (RP9535) | 24 | 43 | 6.0 (±2.7) | 2 | 4 (±0) |
| ΔCheW (LS436) | 32 | 55 | 5.3 (±2.6) | 21 | 4.5 (±0.9) |
| ΔCheA ⇓ CheW (RBB382) | 4 | 7 | 5.1 (±2.0) | 0 | 0 |

*The relevant phenotypes of the strains (in parentheses) examined. †The percentage of sections containing a cluster (*16*) is indicated. ‡The total number of polar clusters examined. §The relative average size of the polar clusters. ‖The total number of nonpolar clusters examined. ¶The relative average size of the nonpolar clusters.

polar clustering of CheA and CheW might be dependent on the presence of membrane-bound MCPs, we examined the intracellular distribution of CheA and CheW in a strain deleted for all four MCPs (KO607). Whereas in wild-type cells both CheA and CheW were predominantly associated with the membrane, these proteins were cytoplasmic in strain KO607 (Fig. 3B, 4 and 6, and Fig. 4, F and J). The loss of subcellular localization of these proteins is best illustrated by the immunofluorescence images (Fig. 3B, 4 and 6). With antibodies to CheA or CheW, quantitation of gold particles deposited on sections of cells lacking all chemoreceptors revealed that most of the CheA and CheW was cytoplasmic (Table 1). There was no polar bias of membrane-associated gold particles in either case (Table 2). Thus, the polar membrane association of CheA and CheW depends on the presence of MCPs.

To determine whether the cytoplasmic CheA and CheW proteins in KO607 would change their position in the cell on expression of a chemoreceptor, we introduced pNT201 (*8*), a plasmid containing the *tar* gene under control of the inducible $p_{tac}$ promoter. Without induction, both CheA and CheW were predominantly cytoplasmic. However, after 30 minutes of induction, when most of the Tar chemoreceptor was clustered at the poles of the cells, both CheA and CheW were also sequestered to the cell pole. With prolonged induction (90 minutes), a significant amount of chemoreceptor was detected in the lateral membrane as well as the polar region. Despite the apparent saturation of polar MCP sites, both CheA and CheW remained tightly associated with the pole of the cell, although the extent of clustering of these proteins was slightly reduced under these conditions. These data reveal that factors other than the MCPs, likely contribute to the polar distribution of CheA and CheW.

These results suggest that the MCPs, CheA, and CheW form aggregates at the poles of the cells. To determine whether the association of these proteins requires that the chemoreceptor be inserted in the polar membrane, we examined the spatial distribution of each in an MCP deletion strain containing a plasmid-borne *tsr* gene in which both of the transmembrane domains are deleted (*24*). In this strain, the Tsr protein formed cytoplasmic inclusion bodies (Fig. 5A). Using anti-CheA and anti-CheW, we found that the CheA and CheW proteins were located in these inclusion bodies (Fig. 5, B and C). We examined the positioning of LamB, and $II^{mtl}$ (*6*) and CheZ (Fig. 5D) in this strain. None of these proteins associated with the inclusion bodies. Thus, association of the Tsr, CheA, and CheW proteins in the inclusion bodies appears to reflect a specific interaction of the Tsr chemoreceptor with CheA and CheW, and this interaction is independent of the membrane association of the complex.

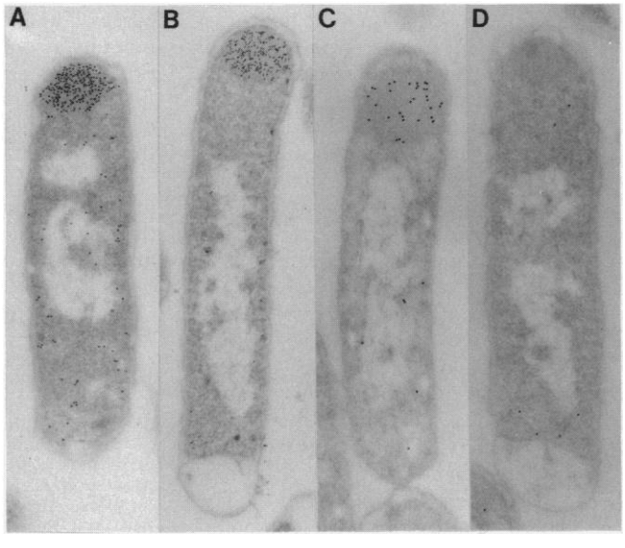


**Fig. 5.** The Tsr, CheA, and CheW proteins co-localize in inclusion bodies caused by the expression of a plasmid-borne *tsr* gene lacking both transmembrane domains. The ΔMCP strain CC602 (*21*), containing plasmid pLF306 (*24*) was prepared for immunoelectron microscopy. This strain is slightly filamentous and contains inclusion bodies. At the appropriate dilutions of anti-Tar, anti-CheA, and anti-CheW used (*35*), Tsr (**A**), CheA (**B**), and CheW (**C**) are sequestered to the inclusion bodies. CheZ (**D**) is not sequestered to the inclusion bodies.

**Requirement for cytoplasmic chemotaxis proteins in polar clustering.** The polar localization and clustering of the MCPs were reduced in RP9535, a *cheA* deletion strain (*21*) (Tables 1, 2, and 4). Although the average number of gold particles per micrometer of polar linear membrane was comparable to that in wild-type cells, there were more gold particles associated with nonpolar membrane, thus reducing the polar bias to 40 percent (Table 2). Of those gold particles at the pole, only 21 percent were in clusters (Table 1) and the size of these clusters was reduced (Table 4). Therefore, MCP molecules in this *cheA* deletion strain were not as tightly associated at the poles as in wild-type cells. These data suggest that CheA plays a role in the efficiency of polar localization and clustering of the chemoreceptor. However, this *cheA* deletion has an effect on *cheW* expression, reducing the CheW protein level by half (*23*). This experiment, therefore, does not rule out the possibility that the reduced polar localization of the chemoreceptors in RP9535 was due to the reduction in the levels of CheW rather than the absence of CheA.

The effects on the positioning of CheW in the *cheA* deletion strain were minimal. Although we detected fewer gold particles (due to reduced levels of CheW protein in this mutant), their distribution was similar to that observed in wild-type cells; half of the gold particles were associated with the inner membrane (Table 1), and the membrane-associated gold particles were biased to the pole (Table 2). The frequency of polar clustering was reduced by half (Table 1). As in wild-type cells, gold particles in the cytoplasm were often found in pairs, and 10 percent of the cytoplasmic gold particles were in clusters. It therefore appears that the polar localization of CheW is more dependent on the presence of the chemoreceptor than on the presence of CheA in the complex. These data imply that the MCP-CheW binary complex forms polar clusters.

To test whether CheW might be required for polar localization and clustering of the MCPs and CheA, we examined an isogenic *cheW* mini-Kan insertion mutant (LS436) (*22*). In this strain, the polar bias

of the MCPs was 25 percent of wild type (Table 2). The number of clusters and their sizes were reduced. However, there was an increase in the distribution of lateral clusters in the absence of CheW (Table 4). These data indicate that CheW is required for polar localization of the MCPs and that it plays some role in the aggregation of the MCPs. In the *cheW* deletion mutant, the CheA protein was randomly distributed around the inner membrane (Tables 1 and 2) suggesting that in vivo CheA associates with the MCPs in the absence of CheW.

To examine the distribution of MCPs in the absence of both CheA and CheW, we analyzed an isogenic *cheA* deletion strain (RBB382) that has a strong effect on *cheW*. The amount of CheW in this strain is only 10 percent that of wild type (*23*, *25*), and CheW in this strain was undetectable by immunofluorescence microscopy (*23*). Some CheW was detected in RBB382 by immuno–electron microscopy, but most of that CheW was cytoplasmic (Table 1); 12 percent of the cytoplasmic gold particles were in clusters. However, the few membrane-associated gold particles had a polar bias (Table 2).

The distribution of MCPs was examined in RBB382. In this strain, the MCPs were only slightly biased to the polar membrane and clustering of the gold particles was almost abolished. As was observed in the CheW deletion mutant, LS436, there was a dramatic reduction in polar bias (Table 2). Among sections of nonseptating cells, only 4 percent contained a cluster, with an average size of only five gold particles (Table 4). The clustering was more severely reduced in this strain than in the isogenic CheW deletion strain. These results suggest that in the absence of both CheA and CheW, the MCPs are randomly distributed around the inner membrane.

To determine whether other known components of the phospho-relay system are required for the polar clustering of the MCP, CheA, and CheW proteins, we examined the intracellular distribution of these proteins in isogenic deletion mutants of both *cheY* (RP5232) (Fig. 4D) and *cheZ* (RP1616) (*23*). The intracellular distribution of MCP, CheA, and CheW were indistinguishable from wild-type cells in these mutants. Therefore, it appears that these components of the phospho-relay system are not required for the polar clustering.

The polar clustering of MCPs, CheA, and CheW most likely reflects the aggregation of ternary complexes. The clustering did not require either CheY or CheZ, consistent with the observation that, in vitro, the association of the ternary complex only requires the three proteins and is not affected by addition of CheY (*7*). It has been shown that, in vitro, CheW interacts with MCP's in the absence of CheA, although with a reduced efficiency (*7*). We found that the MCP-CheW complexes formed polar clusters in vivo and that CheA was not absolutely required for polar localization or clustering of this complex. We also found that MCP-CheA clusters in vivo are randomly distributed.

The presence of aggregates of MCP, CheA, and CheW is consistent with the in vitro data concerning ternary complex longevity. CheW incorporates into complexes with a half-life of 17 minutes, and CheA into complexes with a half-life of 7 minutes (*7*). These data suggest that the chemotaxis signal is transduced through conformational changes in the ternary complex rather than de novo association of the complex. This view is supported by the observations that the association of these proteins is not changed by repellents or attractants (*7*).

We observed that polar clusters are more frequent and larger than nonpolar clusters. We envision two models to explain the distribution of clusters. In one, aggregates of CheW associate with randomly distributed MCP protein at the inner membrane and then with CheA to form a ternary complex. The aggregate complex, through lateral membrane diffusion, would eventually reach the cell pole where it would be held by interactions with other polar components. In the other model, the chemoreceptor initially goes to the cell poles. Random association with cytoplasmic CheW aggregates and CheA would then form the functional ternary complex and stabilize the polar localization. The few lateral complexes that we observed would then be a result of inappropriate dislodging and subsequent redistribution of these complexes.

The role of the chemotaxis machinery is to sense chemical gradients and to modify swimming behavior accordingly. Several lines of evidence indicate that the cells detect temporal changes in ligand concentrations (*26*, *27*). It has been predicted that the most efficient chemosensing apparatus would be distributed randomly around the cell surface (*27*). However, given the small size of wild-type bacteria and the range of the internal signal (*28*), the localized clusters of chemoreceptor complexes described in this article are consistent with a temporal chemotaxis sensing mechanism. The clustering of the chemotaxis relay complex may be instrumental in the ability of the cell to send an integrated signal to the flagella. The limiting factor in the speed of the chemotaxis response is the diffusion of the ligand through the outer membrane and periplasm to the receptor (*29*). Three of the four transducers (Tar, Tap, Trg) can sense chemoeffectors by associating with ligand-bound periplasmic binding proteins. The dissociation constants for these protein-protein interactions are of the order of 100 to 1000 μM (*30*). One mechanism by which the cell may overcome these low affinities may be to co-localize the proteins within the cell. Although we do not know the intracellular position of most of the periplasmic binding proteins, maltose-binding protein (MBP) at least, appears to co-localize with the ternary complex. The majority of the periplasmic MBP is specifically at the pole of the cell (Fig. 3A2) (*17*). The polar distribution of MBP was broader than that of the chemoreceptors, suggesting that MBP may be constrained by a polar barrier such as the periseptal annulus (*31*). It is possible that the polar positioning of the MCP-CheA-CheW complex is due to the interactions of the chemoreceptor and its cognate polar periplasmic binding protein. The signal relay might then be facilitated by constraining all of the components to the same region.

Localization to specific regions of the inner membrane of bacteria does not occur only with the chemoreceptor complex of *E. coli*. In most photosynthetic purple bacteria the photosynthetic apparatus is localized to intracytoplasmic membranes (ICMs). The ICMs are formed by continuous invagination of the cytoplasmic membrane, yet these membrane regions are not homogeneous in protein composition. For example, in *Halobacterium halobium*, bacteriorhodopsin forms a large crystalline array positioned specifically in the ICM (*32*). There is increasing evidence that in *E. coli* some receptor proteins are subcompartmentalized. The guanosine triphosphate (GTP)–binding protein FtsZ, which is essential for bacterial cell division, is specifically concentrated in an annular ring at the septation site (*33*). This cytoplasmic protein is presumably positioned through interactions with an unknown membrane "receptor" protein. Another putative GTP-binding protein essential for cell growth, Era, is clustered at a few positions proximal to the inner membrane apparently due to interactions with a membrane receptor protein (*34*). The nonrandom cellular localization of the chemotaxis machinery, photosynthetic proteins, and proteins involved in cell division may reflect a common mechanism by which bacterial cells circumvent their simple architecture to specialize domains of the cell for particular functions.

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35. Overnight cultures of cells were grown in MA + glucose [J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1977), p. 432] at 32°C for a minimum of 3 hours before fixation with 3 percent formaldehyde and 0.1 percent glutaraldehyde in 30 mM phosphate buffer (pH 7.0) for 1 to 2 hours on ice. The samples were washed three times with phosphate buffer and treated with 1 percent sodium metaperiodate for 15 minutes. The cells were washed once with phosphate buffer and quenched for 15 minutes in 50 mM $NH_4Cl$. The cells were washed in water, dehydrated in a graded ethanol series, and embedded in LR White resin in gelatin capsules at 47°C for 2 days. Sections of 80 to 100 nm were cut, placed on sticky nickel grids [(R. Wright and J. Rine, *Methods Cell Biol.* **31**, 473 (1989)]. For immunoreactions, all solutions not containing antibodies were made with MilliQ-grade water and then filtered through a 0.22-μm filter. Antibodies were diluted in phosphate-buffered saline + Tween (PBST) and bovine serum albumin (BSA) (140 mM NaCl, 2 mM KCl, 8 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, 0.05 percent Tween 20, and 2 percent BSA. The sources of the antibodies were: anti-Tsr, P. Ames and J. Parkinson; anti-Tar, H. Biemann and D. E. Koshland, Jr.; anti-Trg, G. Hazelbauer; anti-CheA, B. Bourret and M. Simon; anti-CheW, C. Amsler and P. Matusumura; anti-Fla, J. Parkinson; anti-$Il^{mtl}$, J. Reizer and M. Saier; anti-MBP, New England Biolabs; and anti-LamB, J. Carlson and T. Silhavy. The anti-MCP, anti-CheA, and anti-CheW were diluted and absorbed for 15 minutes on ice with acetone powders [E. Harlow and L. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988), p. 633] prepared from KO607 (ΔMCP), RBB382 (Δ*cheA*), and RP1078 (Δ*cheW*) strains, respectively. Nickel grids were submerged in PBST and BSA for 15 to 30 minutes and then in the diluted antibodies in a humidity chamber for 1 to 2 hours. The grids were washed in PBST and then blocked in PBST plus BSA. The grids were then incubated in a 1/30 dilution of 10-nm colloidal gold particles conjugated to goat antibody to rabbit immunoglobulin G (Jackson Immunoresearch) for 1 to 2 hours. The grids were washed in water and post-stained with a 1 percent uranyl acetate solution and examined on a Phillips 300 electron microscope at 80 kV.
36. *Escherichia coli* cells were fixed and washed as described (*35*). For maltose induction, cells were grown in MA medium and glycerol, washed, transferred to MA and 1 mM maltose for 15 minutes, and fixed. After fixation, the cells were washed in cold buffer and resuspended in GTE (50 mM glucose, 20 mM tris-HCl, pH 8.0, 10 mM EDTA) with lysozyme (2 mg/ml). Multisample slides (101206, Carlson Scientific, Peotone, Ill) were cleaned and coated with 0.1 percent polylysine and cells were deposited. The slides were washed in PBST, dipped in ice-cold methanol for 5 minutes and then in acetone for 30 seconds. The cells were rehydrated in PBST and blocked in PBST with 2 percent BSA for 15 minutes. The cells were incubated with primary antibody for 1 to 2 hours at room temperature. The cells were washed, blocked, and incubated with the secondary antibody (fluorescein-conjugated to goat antibody to rabbit immunoglobulin G) and propidium iodide for 1 hour in the dark. The samples were washed in water and sealed under cover glasses in mounting medium [J. R. Pringle *et al.*, *Methods Cell Biol.* **31**, 357 (1989)]. Samples were viewed and photographed at 1000× on a Zeiss Axiophot microscope.
37. We thank J. Parkinson, C. Manoil, and B. Bourret for strains and antibodies, for discussions, and for critical reading of the manuscript; P. Matsumura, C. Amsler, H. Biemann, J. Reizer, and J. Carlson for strains or antibodies; D. Kaiser, M. Singer, A. Spormann, I. Keseler, and members of the Shapiro laboratory for the evaluation of the manuscript. Supported by NIH grant GM32506 and an NIH postdoctoral fellowship (GM13929) (J.M.).

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