the future as the strengths we already have. While being receptive to new ideas, we must not forget what we already have. We must build our future by identifying, understanding, and using our existing strengths.

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15. I thank J. G. Morone and G. Wise for their assistance.

RESEARCH ARTICLE

Separation of Signal Transduction and Adaptation Functions of the Aspartate Receptor in Bacterial Sensing

Andrew F. Russo and Daniel E. Koshland, Jr.

Abstract. *In order to investigate the functions of stimulus recognition, signal transduction, and adaptation, the aspartate receptor gene for bacterial chemotaxis in* Salmonella typhimurium *has been sequenced and modified. A carboxyl-terminal truncated receptor was shown to bind aspartate and to transmit a signal to change motility behavior. However, the truncated receptor showed greatly reduced methyl-accepting capacity, and did not allow adaptation to the sensory stimulation. The separation of receptor functions by alteration of primary structure emphasizes that the receptor is directly involved in adaptation and is not solely a device for transmitting a signal across a membrane.*

Cells can respond to external stimuli by processes initiated upon binding of an effector to a membrane-bound receptor. In many sensory systems, the cells not only respond but also adapt to the stimulus. In at least two systems, bacterial chemotaxis (*1, 2*) and vision (*3*), this adaptation is associated with covalent modification of the receptor. In a third adaptive system, the acetylcholine receptor, phosphorylation has been observed, but its function is not yet known (*4*). Thus a receptor can transduce a signal from the outside to the inside of a cell and facilitate sensory adaptation through covalent modification during signal transduction.

To understand these processes, the aspartate receptor involved in bacterial chemotaxis offers a particularly useful system to study. The receptor gene has been cloned, and the purified protein has been reconstituted into artificial membranes (*5*). The gene has been mapped and identified as the *tar* gene (*6*). The protein is known to be multiply methylated (*7*) by a transferase (*8*), and demethylated by an esterase (*9*), both of which are encoded by chemotaxis genes. The degree of receptor methylation can be followed by means of in vivo and in vitro labeling techniques (*8, 10, 11*).

It seemed that altering this receptor might clarify the relationships between its structure and the functions of stimulus recognition, signal transduction, and adaptation. Our first step was to obtain the complete sequence of the aspartate receptor from *Salmonella typhimurium*. Having the sequence, we could systematically alter specific regions of the gene to generate new receptors for study both in vivo and in vitro. The first results of our investigation are reported here.

Sequence and properties of the aspartate receptor. The receptor gene has previously been localized within an Eco RI restriction fragment cloned into pBR322 (*12*). A subclone pRK41 containing the gene on a 2.35-kilobase Cla I fragment was used to obtain unique fragments for sequence determination. The method of Maxam and Gilbert (*13*), with minor modifications (*14*), was used for sequencing. A combination of 3′- and 5′-end labeling techniques allowed complete sequence determination on both strands.

The nucleotide sequence, and the amino acid sequence deduced from it, are shown in Fig. 1. The gene contains 1656 nucleotides and codes for a 59,416-dalton protein, which agrees with the molecular weight deduced from polyacrylamide gels (*1, 2*). Since the amino terminus is blocked, the NH_2-terminal amino acid sequence could not be determined directly (*15*). The terminus shown in Fig. 1 was deduced from correlation with the purified protein amino acid composition, especially the complete absence of cysteine residues (*15*). Furthermore, upstream from the assigned amino terminus, the nucleotide sequence (from −6 to −12) closely resembles the Shine-Dalgarno ribosome binding site (*16*).

The receptor has an average hydrophobicity index comparable to that of soluble proteins (*17*) [2.4 kJ per mole of residue, calculated as described by Gilson *et al.* (*17*)]. If the receptor sequence is further examined by the method of

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Kyte and Doolittle (*18*), there are two regions (residues 7 to 30 and 189 to 212) which are highly hydrophobic, flanked by regions containing a mixture of hydrophobic and hydrophilic residues typical of a globular cytoplasmic protein (Fig. 2). Both of the hydrophobic regions are long enough to span the lipid bilayer and, as discussed below, may give some indication of the way this transmembrane protein is anchored in the membrane.

Interestingly, the amino-terminal hydrophobic sequence resembles the classical signal sequence involved in protein secretion (*19*). There are two arginines at the amino end of a 30-residue stretch that contains 22 nonpolar residues. Since proteolysis of this region would remove four methionine residues, and the 20 predicted methionines were still present in the purified protein, as determined by acid hydrolysis and double labeling (*20*), it would appear that the signal-like sequence is not removed. This result is consistent with increasing evidence that insertion into the bacterial inner membrane does not require cleavage of a signal-like sequence, nor even require a hydrophobic amino terminal sequence (*21*).

Sites of receptor methylation. The sites of receptor methylation were determined by performing in vitro methylation of the receptor protein, isolating tryptic peptides and comparing the amino acid composition of these peptides with the known sequence (*15*). The sequences that contain the methylated glutamic acid residues are shown in Fig. 2. The lysine peptide is located in the middle of the sequence (residues 293 to 315) and the arginine peptide is located near the carboxyl terminus (residues 481 to 504). The number of methyl groups formed during in vitro methylation differs substantially between these two regions: the lysine peptide contains three methyl groups and the arginine peptide contains only one group under the conditions specified (*15*). The location and properties of the two methylated peptides agree with those reported for the methylated peptides of the *Escherichia coli* serine receptor (*22, 23*).

Since receptor methylation is carried out by a single transferase and demethylation is carried out by a single esterase, it is reasonable to expect similarities in the sequences surrounding the modified residues. Examination of the primary sequence confirms the expected homology between the lysine and arginine methyl-

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CGTCGCGTAATAACGTTGCCGGATGGCATCGCGCGGCAATATTCACCGTTATGCTTCCGCCAGCGGTGAGAGTGCCTCTTCACCGCCCGCGCCCAGCCCCTGCATTTTGCGCCGGCATC
GCTGCGACACTGTCATTGGCAATGTCGTCTTCCGGAACAGCGTATTTCCTTTTTGCAAAAGTAAAGTTATCGCCGCAGGTGCCGATAACGTTGATAACTCGTTATCAGGAAGGTGCCTT
Met Phe Asn Arg Ile Arg Val Val Thr Met Leu Met Met Val Leu Gly Val Phe Ala Leu Leu Gln Leu Val Ser Gly Gly Leu Leu Phe  30
ATG TTT AAC CGT ATC CGC GTT GTC ACA ATG CTG ATG ATG GTG CTG GGG GTT TTC GCA CTG CTA CAG CTT GTT TCC GGT GGT TTG CTG TTT
Ser Ser Leu Gln His Asn Gln Gln Gly Phe Val Ile Ser Asn Glu Leu Arg Gln Gln Gln Ser Glu Leu Thr Ser Thr Trp Asp Leu Met  60
TCT TCA TTA CAG CAT AAC CAG CAA GGT TTT GTT ATT TCT AAC GAA TTA CGT CAG CAA CAA AGC GAA CTC ACG TCG ACA TGG GAC TTA ATG
Leu Gln Thr Arg Ile Asn Leu Ser Arg Ser Ala Ala Arg Met Met Met Asp Ala Ser Asn Gln Gln Ser Ser Ala Lys Thr Asp Leu Leu  90
CTG CAA ACG CGC ATT AAC CTG AGC CGC TCC GCC GCA CGC ATG ATG ATG GAC GCT TCT AAC CAG CAG AGC AGC GCC AAA ACG GAT TTA CTC
Gln Asn Ala Lys Thr Thr Leu Ala Gln Ala Ala Ala His Tyr Ala Asn Phe Lys Asn Met Thr Pro Leu Pro Ala Met Ala Glu Ala Ser 120
CAG AAT GCA AAA ACG ACT CTC GCA CAG GCG GCG GCG CAC TAC GCC AAT TTC AAA AAT ATG ACG CCA TTG CCA GCG ATG GCG GAG GCC AGC
Ala Asn Val Asp Glu Lys Tyr Gln Arg Tyr Gln Ala Ala Leu Ala Glu Leu Ile Gln Phe Leu Asp Asn Gly Asn Met Asp Ala Tyr Phe 150
GCG AAC GTC GAT GAA AAA TAT CAG CGC TAT CAG GCC GCA TTA GCC GAA CTT ATT CAG TTT CTG GAC AAT GGC AAT ATG GAT GCC TAC TTC
Ala Gln Pro Thr Gln Gly Met Gln Asn Ala Leu Gly Glu Ala Leu Gly Asn Tyr Ala Arg Val Ser Glu Asn Leu Tyr Arg Gln Thr Phe 180
GCC CAG CCA ACC CAG GGA ATG CAA AAC GCG TTG GGC GAG GCG CTG GGC AAT TAC GCC CGG GTG AGC GAA AAC CTG TAC CGC CAG ACA TTT
Asp Gln Ser Ala His Asp Tyr Arg Phe Ala Gln Trp Gln Leu Gly Val Leu Ala Val Val Leu Val Leu Ile Leu Met Val Val Trp Phe 210
GAT CAA AGT GCT CAT GAC TAC CGT TTT GCG CAA TGG CAA CTG GGG GTT CTT GCG GTC GTG CTG GTG CTG ATT TTG ATG GTG GTT TGG TTC
Gly Ile Arg His Ala Leu Leu Asn Pro Leu Ala Arg Val Ile Thr His Ile Arg Glu Ile Ala Ser Gly Asp Leu Ala Lys Thr Leu Thr 240
GGC ATT CGT CAT GCC CTG CTT AAC CCA TTA GCG CGA GTG ATT ACT CAT ATC CGT GAA ATT GCC AGC GGC GAT CTG GCG AAA ACG CTC ACC
Val Ser Gly Arg Asn Gly Ile Gly Glu Leu Ala Gly Thr Val Glu His Met Gln Arg Ser Leu Ile Asp Thr Val Thr Gln Val Arg Glu 270
GTC TCA GGA CGT AAT GGA ATT GGC GAA CTG GCG GGA ACG GTT GAA CAT ATG CAA CGC TCG CTG ATT GAC ACC GTA ACG CAG GTT CGT GAA
Gly Ser Asp Ala Ile Tyr Ser Gly Thr Ser Glu Ile Ala Ala Gly Asn Thr Asp Leu Ser Ser Arg Thr Glu Gln Gln Ala Ser Ala Leu 300
GGT TCG GAT GCG ATT TAT TCC GGC ACC AGT GAA ATT GCC GCC GGT AAT ACC GAC CTC TCT TCC CGT ACC GAA CAG CAG GCC TCC GCT CTG
Glu Glu Thr Ala Ala Ser Met Glu Gln Leu Thr Ala Thr Val Lys Gln Asn Ala Asp Asn Ala Arg Gln Ala Ser Gln Leu Ala Gln Ser 330
GAG GAG ACG GCT GCC AGC ATG GAA CAA CTG ACG GCC ACC GTG AAG CAA AAC GCC GAT AAC GCC CGC CAG GCT TCG CAA CTG GCG CAA AGC
Ala Ser Glu Thr Ala Arg His Gly Gly Lys Val Val Asp Gly Val Val Asn Thr Met His Glu Ile Ala Asp Ser Ser Lys Lys Ile Ala 360
GCC TCC GAG ACC GCG CGT CAT GGC GGC AAA GTG GTC GAC GGC GTA GTA AAC ACT ATG CAC GAA ATT GCC GAC AGT TCG AAA AAA ATC GCT
Asp Ile Ile Ser Val Ile Asp Gly Ile Ala Phe Gln Thr Asn Ile Leu Ala Leu Asn Ala Ala Val Glu Ala Ala Arg Ala Gly Glu Gln 390
GAC ATT ATC AGC GTT ATC GAC GGT ATT GCC TTC CAG ACT AAC ATT CTG GCG CTG AAC GCG GCG GTA GAA GCG GCG CGC GCG GGA GAG CAG
Gly Arg Gly Phe Ala Val Val Ala Gly Glu Val Arg Asn Leu Ala Ser Arg Ser Ala Gln Ala Ala Lys Glu Ile Lys Ala Leu Ile Glu 420
GGG CGC GGT TTT GCG GTC GTG GCA GGC GAG GTG CGT AAT CTG GCC AGC CGC AGC GCC CAG GCG GCG AAA GAA ATA AAA GCG TTG ATT GAA
Asp Ser Val Ser Arg Val Asp Thr Gly Ser Val Leu Val Glu Ser Ala Gly Glu Thr Met Thr Asp Ile Val Asn Ala Val Thr Arg Val 450
GAT TCC GTC TCG CGT GTC GAT ACC GGT TCT GTG CTG GTG GAA AGC GCC GGG GAA ACC ATG ACT GAC ATC GTC AAT GCC GTT ACG CGC GTC
Ala Asp Ile Met Gly Glu Ile Ala Ser Ala Ser Asp Glu Gln Ser Arg Gly Ile Asp Gln Val Ala Leu Ala Val Ser Glu Met Asp Arg 480
GCG GAT ATC ATG GGC GAA ATC GCC TCC GCC TCG GAT GAG CAA AGC CGG GGT ATC GAT CAG GTC GCT TTG GCC GTT TCC GAA ATG GAT CGC
Val Thr Gln Gln Asn Ala Ser Leu Val Gln Glu Ser Ala Ala Ala Ala Ala Leu Glu Glu Gln Ala Ser Arg Leu Thr Gln Ala Val Ser 510
GTA ACG CAA CAG AAC GCC TCG CTG GTT CAG GAA TCC GCA GCG GCC GCC GCG CTG GAA GAG CAG GCC AGC CGT CTG ACC CAG GCG GTA TCG
Ala Phe Arg Leu Ala Ser Arg Pro Leu Ala Val Asn Lys Pro Glu Met Arg Leu Ser Val Asn Ala Gln Ser Gly Asn Thr Pro Gln Ser 540
GCT TTC CGC CTG GCA TCG CGA CCG CTG GCG GTA AAT AAA CCT GAG ATG CGT TTG TCA GTG AAC GCT CAG TCC GGC AAT ACG CCG CAG TCA
Leu Ala Ala Arg Asp Asp Ala Asn Trp Glu Thr Phe End
TTA GCC GCC AGG GAT GAT GCG AAC TGG GAA ACC TTC TGA TCGACGTGCGCTGTCGGTTATCGAT
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Fig. 1. Nucleotide and deduced amino acid sequence of the aspartate receptor gene. The DNA was sequenced by the technique of Maxam and Gilbert (*13*) entirely on both strands. Plasmid DNA was prepared and digested by restriction enzymes as described (*36*). The 5′ ends were labeled by polynucleotide kinase (*13*), and the 3′ ends by Klenow fragment DNA polymerase (*36*). Reaction and electrophoresis conditions resolving up to 500 nucleotides from the labeled end were performed as described (*14*). Abbreviations: A, adenine; C, cytosine; G, guanine; T, thymine; Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

ated peptides. In each peptide there are three sites that contain adjacent glutamate or glutamine residues (or both) flanked by similar nonpolar and uncharged polar residues. In that it has been reported that some glutamine residues in the homologous serine receptor can be deamidated and then methylated (*22*), then any of the boxed residues shown in Fig. 2 are potential methylation sites.

Comparison of the aspartate receptor with the serine receptor. The *E. coli* serine receptor has now been sequenced (*23*) and it is thus possible to compare the sequence of these receptors. A significant amount of homology would be expected since both receptors react with antibodies to the aspartate receptor (*5*), and DNA hybridization indicates extensive homology among the chemotaxis receptors (*24, 25*).

Overall, 57 percent of the residues in the *Salmonella* aspartate receptor and *E. coli* serine receptor are identical. However, the similarity varies considerably in different regions of the receptors. The two strongly hydrophobic regions, which probably span the membrane, have 63 and 38 percent identity. The amino-terminal portion between these two hydrophobic stretches is only 26 percent identical, which might indicate divergence due to ligand binding specificity. In the methylation regions, the identity rises to 91 percent with both receptors maintaining the multiple glutamate pattern outlined in Fig. 2. An intriguing portion covering residues 357 to 405 shows 100 percent identity but has yet to be assigned a function. Clearly, the sequences have been preserved in several regions, and this at least partly explains the complementation and cross-reaction of *E. coli* and *Salmonella* chemotaxis gene products with both receptors (*26*). The chemotaxis receptors appear to have evolved from a common ancestor and have retained great similarity in terms of structure as well as function.

Binding and methylation properties of a truncated receptor. Selective alteration of the wild-type receptor was pursued in order to clarify its functions. A truncated receptor was constructed by digestion of the gene with the restriction enzyme Nru I, and religation into the plasmid vector DNA. In so doing, we deleted DNA coding for 35 amino acids at the carboxyl-terminal end of the receptor, and fused vector DNA encoding nine amino acids to the truncated receptor gene (legend to Fig. 2). A recombinant plasmid containing the truncated receptor gene was introduced into the mutant strain RP4372, which lacks the aspartate receptor (*26*). When broken cells were tested with antiserum to the aspartate receptor, there was essentially the same amount of reaction as with normal receptor in an Ouchterlony double-diffusion assay (*27*). The cross-reacting material was localized only in the membrane fraction, even after treatment with 2*M* KCl. Thus, the truncated receptor, like the normal receptor, is an integral membrane protein. In control experiments, normal receptor expressed at high levels was also completely localized in the membrane.

The first function of the truncated receptor to be tested was binding of the ligand. Membranes were isolated from cells containing high levels of either the normal or truncated receptor. Aspartate binding was measured by a competition centrifugation assay (*28*). The binding of aspartate to the truncated receptor ($K_D = 4 \times 10^{-6} M$) was essentially identical to that of the normal receptor ($K_D = 5 \times 10^{-6} M$). The amount of bound aspartate agreed with the amount of cross-reaction with receptor antibody, and indicated a substantial overproduction of receptor protein, about 15 times more than that of the wild type. This eliminated the possibility that only a fraction of the truncated receptors bound aspartate. These results demonstrate that the carboxyl-terminal truncation had relatively little effect on the aspartate binding site.

To test whether the truncated receptor was methylated, the in vivo level of methylation was determined by growing the cells on tritium-labeled methionine and measuring the amount of base labile protein methylation (*11, 29*). The methylation was normalized per receptor by using the total number of aspartate binding sites, as determined above. A plasmid encoding either the normal or truncated receptor gene was introduced into the receptor mutant RP4372, which itself did not have any detectable methylation. The normal receptor was extensively methylated 45 minutes after aspartate stimulation (1-2 methyl groups per receptor), which is comparable to previously reported wild-type levels of receptor

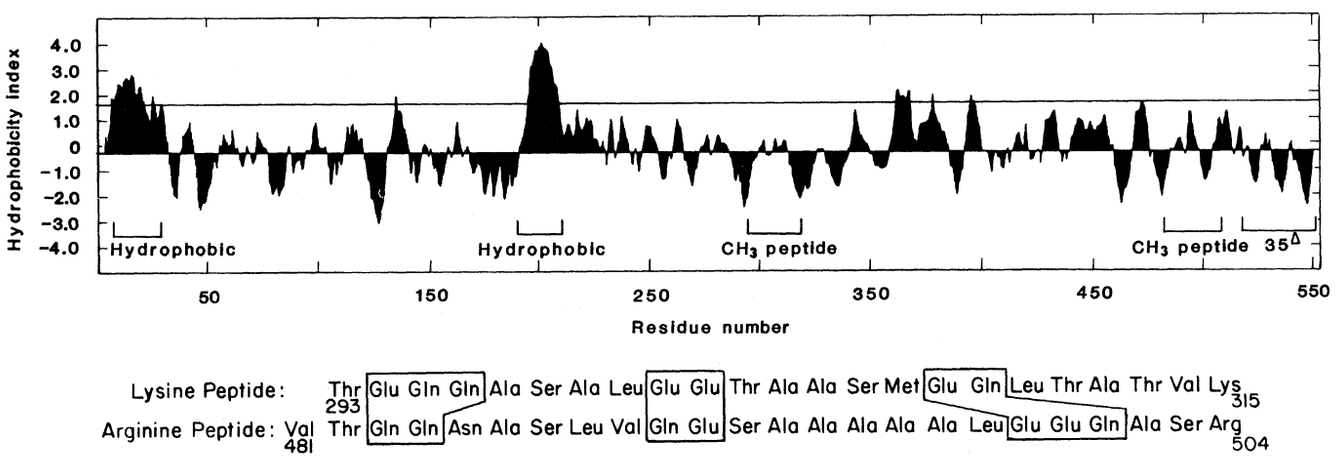

Fig. 2. Properties of the receptor protein. The hydrophobicity was predicted from the Kyte and Doolittle SOAP program (substituting the term hydrophobic for hydropathic) over a running average of seven residues (*18*). Two regions that are significantly hydrophobic (above 1.6 index) and capable of spanning the membrane (20 residues) are indicated. The location of the two methylated peptides in the middle and carboxyl-terminal end of the protein are shown. The homologous potential methylation sites are boxed in the peptide sequences below the hydrophobicity plot. The deletion that removes the 35 carboxyl-terminal amino acids but not either of the methyl regions is shown immediately under the hydrophobicity plot. This deletion was constructed by digestion with the enzyme Nru I and ligation to the Nru I site in the vector pBR322 (*36*). Since the Nru I site was preserved, the reading frame in the fused vector DNA predicts nine additional amino acids attached to the truncated receptor (*36*). From residue 517 the following amino acids would be attached: Arg-Glu-Ala-Gly-Trp-Pro-Ser-Pro-Leu.

methylation (*7, 15, 22*). The truncated receptor was found to have extremely low levels of methylation after aspartate stimulation (0.05 to 0.1 methyl group per receptor). Thus the truncated receptor is deficient in methylation despite the fact that the DNA coding for both methylation regions is present.

Signaling and adaptation properties of the truncated receptor. To determine the signaling and adaptive properties of the truncated receptor a strain containing this receptor was exposed to sudden temporal gradients of aspartate. Wild-type bacteria respond to such gradients with a period of smooth swimming followed by adaptation to the stimulus, even though the stimulus remains at a new, higher concentration (*30*). Such behavioral changes can be observed by tethering the bacteria by their flagella and monitoring the suppression of bacterial rotation reversals which corresponds to smooth swimming (*31*). This method can quantify the response by determining the recovery time required for adaptation (Fig. 3). In a cell containing wild-type levels of receptor there was a brief response upon aspartate stimulation, which persisted for 2 minutes. Cells containing high levels of normal receptor (15-fold over wild type) with only wild-type levels of transferase and esterase, had a prolonged response; however, by 40 minutes, almost all of the cells had adapted. Cells containing the truncated receptor could respond, but none of the bacteria had returned to normal behavior even after 3 hours. Thus, while high levels of the truncated receptor allowed the bacteria to respond to aspartate stimulation, these bacteria were unable to recover and adapt back to their normal behavior.

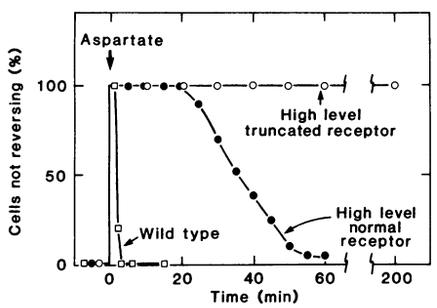

Fig. 3. Behavior of cells containing the aspartate receptor. The ability of cells to respond to aspartate stimulation by suppressing flagellar reversals and to adapt to the stimulus by returning to the prestimulus behavior was measured. The change in the percentage of cells not reversing during 1-minute intervals was determined from fields of 10 to 30 tethered bacteria after stimulation with 60 μM aspartate. The normal and truncated receptor genes were transformed (*36*) into the *E. coli* receptor mutant RP4372, which lacks the *tar*, *tsr*, and *tap* chemotaxis receptors (*24, 26*), and maintained by ampicillin (50 μg/ml) selection. Receptor functions could be most conveniently tested in this strain since a comparable background in *Salmonella typhimurium* has not yet been constructed. The behavior was compared to the wild-type RP437 containing the pBR322 vector DNA. All cells were grown under conditions that suppress flagellar synthesis and thereby facilitate tethering [Vogel-Bonner citrate (VBC) glucose 0.4 percent, 39°C] (*31*).

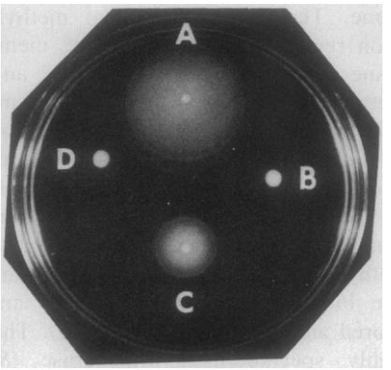

Fig. 4. Effect of receptor truncation on chemotaxis. Cells were incubated on semisolid (0.35 percent agar) LB broth (*36*) swarm plates for 15 to 30 hours at 30°C to measure their ability to migrate toward nutrients and away from repellents. Cells and swarm rates (increase in diameter) are: (A) RP437 (wild type), 4.3 mm/hour; (B) RP4372 (receptor mutant), 0.3 mm/hour; (C) RP4372pRK41 (normal receptor), 1.2 mm/hour; (D) RP4372pRK45 (truncated receptor), 0.3 mm/hour. As controls, introduction of the normal *Salmonella* aspartate receptor into RP4372 at wild-type levels, on a lambda vector, allowed swarming at approximately the same rate as wild-type cells containing the *E. coli* aspartate receptor, and introduction of pBR322 into RP437 or RP4372 did not affect the swarm rates.

Chemotaxis ability of bacteria containing truncated receptor. To test the effect of the receptor truncation on detection of gradual chemoeffector gradients, bacteria containing the truncated receptor were placed on chemotaxis swarm plates (*26*). These plates effectively measure the bacteria's chemotaxis ability, as they swarm away from repellents generated inside the colony and toward the nutrient attractants in the plate. As can be seen in Fig. 4, wild-type bacteria form a diffuse circle as they swarm from the center of the colony. The mutant strain RP4372 lacking both the aspartate and serine receptors cannot swarm. Expression of the normal receptor gene on a plasmid at 15-fold higher levels allowed chemotaxis by the receptor mutant, but the swarm rate was only about 30 percent of that of the wild type. This decreased swarm rate is probably due to an increased adaptation time which makes the response to gradients less than optimal. When the truncated receptor was expressed in the bacteria at this high level, the bacteria were unable to swarm. The inability of bacteria containing the truncated receptor to migrate on the swarm plate is consistent with the defective adaptation demonstrated by the direct tethering assay.

Discussion

Multiple roles of receptors. One of the key properties of receptors is to relay information, most notably from outside a cell to the cytoplasm (*32*). However, some receptors have other functions as well. For receptors involved in chemotaxis and vision, covalent modification allows the signaling property of the receptor to be reset to zero, thus enhancing its ability to detect small changes in background (*1*). The receptor, therefore, plays a feedback role in addition to signal transduction. The construction of a receptor that relays a signal but is simultaneously defective in methylation and adaptation is consistent with covalent modification being essential for adaptation. It is to be noted that an increasing number of receptors are being shown to be covalently modified, including the epidermal growth factor receptor (*33*), the insulin receptor (*34*), and the acetylcholine receptor (*4*). Whether these modifications perform the same function remains to be

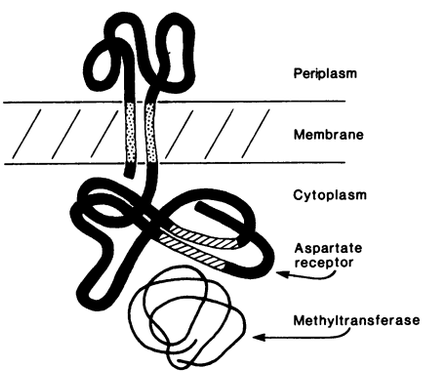

Fig. 5. Hypothetical schematic of the aspartate receptor. The amino-terminal aspartate binding domain is shown located in the periplasm and the carboxyl-terminal signaling and modification domains would be cytoplasmic. The receptor is drawn to scale, on the basis of an α-helical structure, with the width of the inner membrane representing 35 Å. The hydrophobic sections (each 23 residues) are shown in the membrane, and the methylated peptides (23 and 24 residues) are shown cross-hatched. The 31K (kilodalton) methyltransferase is depicted on the same scale as the receptor.

seen, but the correlations are intriguing.

Separable functions of receptors. The experiments presented above demonstrate that the functions of signaling and adaptation can be separated from each other by alteration of the receptor. In early studies on bacterial chemotaxis, the adaptation time was found to correlate with the time for receptor methylation (*10*). Subsequent studies have further implicated the methylation process in sensory adaptation [see (*1, 2*) for reviews]. The behavior of the truncated receptor is consistent with these previous studies and argues for the following sequence of events in signal transduction and adaptation. Upon stimulus binding, the receptor is presumably induced into a new conformation in both the signal transduction and the covalent modification regions. Gradually the modification region is methylated to a new steady-state level, which returns the signaling to its initial activity. The methyltransferase, methylesterase, and other components of the cytoplasmic signaling-processing system play a key role in adaptation since they modify the receptor and transmit the signal, but it is the conformation of the receptor itself which allows a return to the initial state. The receptor truncation apparently did not remove signal transduction, but did prevent the methylation required for sensory adaptation.

Structure of the receptor. While amino acid sequences are not reliable indicators of the three-dimensional structure of proteins, a rudimentary estimate of structure may be valuable as a working hypothesis. Such a structure is shown in Fig. 5. Except for the highly hydrophobic sequences, the remaining sequences are characteristic of soluble proteins and are therefore depicted as globular regions on alternate sides of the membrane. The carboxyl-terminal methylation region must be inside the membrane since the methyltransferase and methylesterase are in the cytoplasm. This identifies the amino-terminal region as outside the inner membrane. Moreover, removal of approximately 60 amino acids from the carboxyl-terminus eliminates signal transduction as well as methylation, but does not affect aspartate binding, suggesting a stably anchored amino-terminal region (*35*). The highly specific methyltransferase (*8*) could conceivably bind to the region of 100 percent homology between the two methyl-accepting regions. The relation between the signaling and adaptive regions, as well as other properties of the receptor, can now be explored on a receptor of known primary structure. The results so far establish that the receptor is not simply a device to transmit a signal across a membrane, but also plays a significant role in the information processing within the cell.

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