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Biochemistry

Primary structure of an archaebacterial transducer, a methyl-accepting protein associated with sensory rhodopsin I

(phototaxis/chemotaxis/signal transduction/halobacteria)

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ABSTRACT **A methylated membrane protein of 97 kDa was suggested on the basis of mutant analysis to transduce signals from the phototaxis receptor sensory rhodopsin I to the flagellar motor in *Halobacterium halobium*. Here we report isolation of the proposed transducer protein, cloning of its gene based on partial protein sequences, the complete gene sequence, and analysis of the encoded primary structure. The 1611-base-pair gene termination codon overlaps the initiator ATG of the *sopI* gene, which encodes the sensory rhodopsin I apoprotein. The predicted size of 57 kDa for the methylated protein indicates an aberrant electrophoretic migration on SDS/polyacrylamide gels, as occurs with other acidic halophilic proteins. Putative promotor elements are located in an A+T-rich region upstream of the gene. Comparison of the translated nucleotide sequence with N-terminal sequence of the purified protein shows the protein is synthesized without a processed leader peptide and the N-terminal methionine is removed in the mature protein. The deduced protein sequence predicts two transmembrane helices near the N terminal that would anchor the protein to the membrane. Beyond this hydrophobic region of 46 residues, the remainder of the protein (536-amino acid residues total) is hydrophilic. The C-terminal 270 residues contain a region homologous to the signaling domains of eubacterial transducers (e.g., *Escherichia coli* Tsr protein), flanked by two regions homologous to the methylation domains of the transducer family. The protein differs from *E. coli* Tsr in that it does not have an extramembranous-receptor binding domain but instead has a more extended cytoplasmic region. Coexpression of the methyl-accepting protein gene (designated *htrI*) and *sopI* restores sensory rhodopsin I phototaxis to a mutant (Pho81) that contains a deletion in the *htrI/sopI* region. These results extend the eubacterial transducer family to the archaebacteria and substantiate the proposal that the methylated membrane protein functions as a signal-transducing relay between sensory rhodopsin I and cytoplasmic sensory-pathway components.**

Sensory rhodopsin I (SR-I) is a retinal-containing intrinsic membrane protein that functions as a phototaxis receptor in the archaebacterium *Halobacterium halobium* (also known as *Halobacterium salinarium*) (1). SR-I controls swimming behavior of the cells by modulating the frequency of reorientation ("reversals") of their swimming direction (for reviews, see refs. 2 and 3). Orange light generates attractant signals that suppress reversals, whereas near-UV light generates repellent signals that induce reversals. Several early events in the signaling process have been elucidated. Photon absorption by SR-I (λ_{max}, 587 nm) causes isomerization around the C13=C14 double bond of the retinal chromophore (4), which is essential for receptor activation (5). The photoisomerization energy is transferred to the protein in a process requiring steric interaction between the retinal C13 methyl group and protein residues (6). These events produce in <1 msec a spectrally distinct attractant signaling state (λ_{max}, 373 nm) of the protein that decays thermally within seconds to the original unstimulated-receptor conformation (7, 8). The repellent signal is generated by absorption of a second photon by the receptor when it is in its attractant signaling conformation (9).

What is the nature of the components responsible for transmission of these signals to the flagellar motor? The gene-derived primary structure of SR-I reveals a seven-transmembrane helix protein largely embedded in the membrane that has minimal cytoplasmic loops, similar in its structure to the related proton pump bacteriorhodopsin (10). Unlike bacteriorhodopsin, SR-I is not an electrogenic ion pump (1, 11), and photoreceptor signals are not propagated as electrical impulses along the membrane (12). Fumarate (13), cyclic nucleotides and Ca^{2+} (14), and a G protein (15) have been suggested to be involved in postreceptor signaling. However, no postreceptor component capable of sensing SR-I conformations and transducing this information to modulate the motor has been demonstrated in these studies.

Phototaxis mutant analysis identified a methylated membrane protein with an apparent molecular mass of 97 kDa, expression of which tightly correlated with the SR-I protein of 25 kDa (16). The methylation linkage (reversible carboxylmethyl esterification; refs. 16 and 17) of the 97-kDa protein is of the same type as that found in the chemotaxis signal generators ("transducers") of eubacteria (e.g., *Escherichia coli*), chemoreceptor proteins that transmit signals from the membrane to a cytoplasmic sensory pathway (18–20). SR-I attractant and repellent signals modulate methyl group turnover *in vivo*, as do those from eubacterial chemotaxis transducers (21). Based on these findings the 97-kDa methyl-accepting protein was postulated to be the postreceptor transducer for SR-I signals (21). Analysis of taxis mutants and revertants (22, 23) and antigenic crossreactivity of the 97-kDa protein with eubacterial transducers (24) further strengthened the analogy to the chemotaxis system.

Here we report the cloning and sequencing of the gene encoding the proposed transducer protein.* The protein primary structure and its sequence similarities to eubacterial transducers substantiate the proposal that the 97-kDa protein acts as a signal-transducing relay between SR-I and cytoplasmic sensory pathway components. In this paper we refer to the gene as *htrI* and the protein as HtrI, in reference to its function as a halobacterial transducer for SR-I. In earlier papers we referred to HtrI as methyl-accepting phototaxis protein I (MPP-I).

MATERIALS AND METHODS

Bacterial Strains. *H. halobium* Flx5R (HtrI$^+$SR-I$^+$), which produces 4-fold greater amounts of HtrI than does a wild-type

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Abbreviation: SR-I, sensory rhodopsin I.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L05603).

(25) strain was used for HtrI isolation and preparation of genomic clones. Pho81 (22) [HtrI$^-$SR-I$^-$, resulting from an insertion of the transposable element ISH2 into position 1775 of *sopI* and an upstream deletion including *htrI* (E. N. Spudich and J.L.S., unpublished work)] was used as a negative control. *E. coli* DH5α (*supE44* Δ*lacU169* (ϕ80 *lacZΔM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*) was used as the host strain for cloning. *H. halobium* DNA was cloned into the plasmid vector pBluescript II KS(+) (Stratagene).

Molecular Biological Procedures. Standard protocols were used (26). Enzymes were purchased from GIBCO/BRL, New England Biolabs, Boehringer Mannheim, Promega, and United States Biochemical. Exonuclease digestions were done by using the Erase-a-Base kit (Promega) following the manufacturer's protocol.

Protein Isolation. *H. halobium* strains Flx5R and Pho81 were grown to stationary phase at 39°C in complex medium, and sonicated membranes were isolated (16) and dialyzed against water at 4°C for 1.5–2 hr. The suspension was solubilized, and proteins were separated electrophoretically on an SDS/low-bisacrylamide/polyacrylamide gel, as described (16), except the gels were polymerized for 48 hr at 4°C, preelectrophoresed in 10 mM glutathione at 5 mA constant current for 2 hr, and electrophoresed overnight at 6 mA. The gels were stained with Coomassie brilliant blue R-250, and a small portion of the gel was subjected to autofluorography with EN^{3}HANCE (DuPont/NEN). Comparison of the autofluorogram with the stained gel and absence of the band in Pho81 identified the 97-kDa band. Slices containing protein from three to four gels were pooled, macerated with a spatula, and soaked in 0.4 mM $NaHCO_3$/2% SDS for 20 min at room temperature. Protein was electroeluted from the gel matrix for 16 hr at 50 V in 50 mM NH_4HCO_3/0.1% SDS, dialyzed for 8–12 hr against 1 liter of 5 mM NH_4HCO_3/0.01% SDS (twice), lyophilized, and resuspended in 50 μl of water. Two and one-half microliters of the resuspension was applied to a 10% acrylamide/0.27% bisacrylamide mini-gel (Hoefer) to confirm its purity and identity by autofluorography, by migration position relative to molecular weight standards (Bio-Rad), and by comparison to the [^{3}H]methylated Pho81 and Flx5R membrane proteins. The remaining 45 μl of protein suspension was precipitated with 0.475 ml of 1 mM HCl/acetone solution at −20°C for 2 hr to remove residual SDS (27). The protein pellet was dried in a Speed-Vac evaporator and stored at −20°C. Dried protein from 24 preparative gels was solubilized and pooled in 50 μl of 31 mM Tris·HCl, pH 6.8/2% SDS/5% (vol/vol) glycerol/0.001% bromophenol blue/5% (vol/vol) 2-mercaptoethanol, and the resuspension was gently sonicated 30 sec twice with ice-bath cooling for 30 sec between sonication periods (Branson model 1200 sonicator). The solubilized protein was applied to a preelectrophoresed SDS/mini-polyacrylamide gel. Separate protein samples were electroblotted onto poly(vinylidene difluoride) (Bio-Rad) and nitrocellulose (Schleicher & Schuell) membranes at 2.5 mA/cm^2 with a semi-dry electroblotter (Integrated Separation Systems, Hyde Park, MA or Bio-Rad). Membranes were stained for 1.5 min in 0.2% Ponceau S in 1% acetic acid, destained for 1 min in 1% acetic acid, and rinsed with milliQ (Millipore, Bedford, MA) water for 1.5 min. The stained protein was excised from the blot, rinsed extensively with water in a 1.5-ml microfuge tube, frozen at −20°C, and sent on dry ice to the Harvard Medical School Microsequencing Facility (Boston).

Peptide Sequencing. Ten percent of the nitrocellulose and of the poly(vinylidene difluoride)-immobilized protein was quantitated by amino acid analysis. The nitrocellulose-blotted sample was digested with trypsin, and peptides were separated by reverse-phase HPLC by a Vydac C_{18} 2.1 × 15 cm column. Sequence was obtained from two peaks as well as from the N terminus of the poly(vinylidene difluoride)-blotted protein with an Applied Biosystems 477A protein sequencer with a 120A online phenylthiohydantoin–amino acid analyzer.

Deoxyoligonucleotides and Southern Hybridization. Three fully degenerate (64-, 256-, and 512-fold) deoxyoligonucleotide mixtures were designed and synthesized (Genosys, The Woodlands, TX) based on the 28-amino acid tryptic peptide sequence (Fig. 1). The deoxyoligonucleotides were end-labeled with T4 polynucleotide kinase (GIBCO/BRL) and [γ-^{32}P]dATP (6000 Ci/mmol, DuPont/NEN; 1 Ci = 37 GBq). Restricted Flx5R genomic DNA was blotted onto Hybond-N (Amersham) by either the Southern blot capillary method or the Pharmacia VacuGene system. Southern blots were prehybridized in sealed plastic bags with 20 ml of 6× SSC/0.05 M $NaPO_4$, pH 6.8/50 mM EDTA, 5× Denhardt's solution/200 μg of denatured calf thymus DNA at 37°C for at least 4 hr. The prehybridization buffer was discarded, and 10 ml of fresh buffer containing labeled probe was added. After a 66-hr incubation at 37°C, blots were washed three times for 15 min with 150 ml of 3× SSC/0.1% SDS. Hybridization stringencies were increased by decreasing ionic strength (3×–0.1× SSC) and increased wash-solution temperature (37°C–65°C) until single hybridization signals were obtained with the 64-fold degenerate probe. The 11-kilobase-pair (kbp) *Eco*RI fragment (Fig. 2) was confirmed by its high-stringency hybridization to each of the other two probes. Blots were exposed to either Kodak X-Omat XAR film or Hyperfilm (Amersham).

Plasmid Preparation. Overnight *E. coli* cultures (1.5 ml) were grown in LB broth containing 100 mg of ampicillin per liter. Plasmids were prepared by using the Magic Mini Plasmid Prep kit (Promega). DNA was eluted from the cartridge with sterile milliQ water at 68°C, and the final volume was adjusted to 50 μl with additional milliQ water. Plasmids were denatured at room temperature for 5 min by adding 5 μl of a 2 M NaOH/2 mM EDTA solution. DNA was precipitated by adding 7.5 μl of 3 M NaOAc (pH 5.2), 7.0 μl of sterile milliQ water, and 200 μl of −20°C absolute ethanol. The preparation was incubated at −20°C for at least 20 min and centrifuged at 4°C for 15 min at 14,000 × *g*, the supernatant was aspirated, and the pellet was rinsed with 70% (vol/vol) ethanol/30% (vol/vol) Tris/EDTA, pH 8.0. The final pellet was dried in the Speed-Vac evaporator, resuspended in 25 μl of Tris/EDTA, pH 8.0; 5 μl was then used for each hybridizing reaction.

Sequencing. The labeling reactions were prepared by using a modified protocol of the Sequenase version 2.0 kit (United States Biochemical). Hybridizing reactions contained 5 μl of denatured plasmid, 2 μl of Sequenase reaction buffer, 2 μl of primer (20 ng), and 1 μl of dimethyl sulfoxide (Fisher Scientific), incubated at 65°C for 10 min and cooled immediately in an ice/slush bath. The hybridized template (2.5 μl) was added to 4 wells of a round-bottomed microtiter plate (Falcon), and the plate was incubated on a 45°C heating block. One microliter of labeling mix [1 μl of 0.1 M dithiothreitol, 1.6 μl of sterile water, 0.4 μl of dimethyl sulfoxide, 0.4 μl of dGTP or dITP sequence-labeling mix, 0.5 μl of deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate (1000–1500 Ci/mmol, DuPont/NEN), 0.25 μl of Sequenase version 2.0] was added to the side wall of each well and mixed in a Mistral 2000 centrifuge (Leicestershire, England). The dITP mix included 0.125 μl of pyrophosphatase and 1.475 μl of sterile water. After centrifugation, the microtiter plate was returned to the 45°C block for 8 min. Two microliters of each Sequenase dideoxynucleotide chain-termination mix was added to each well, centrifuged to mix, and the plate was returned to the 45°C block for 10 min. Three microliters of stop solution was added to each well and mixed; the quenched reactions were stored at

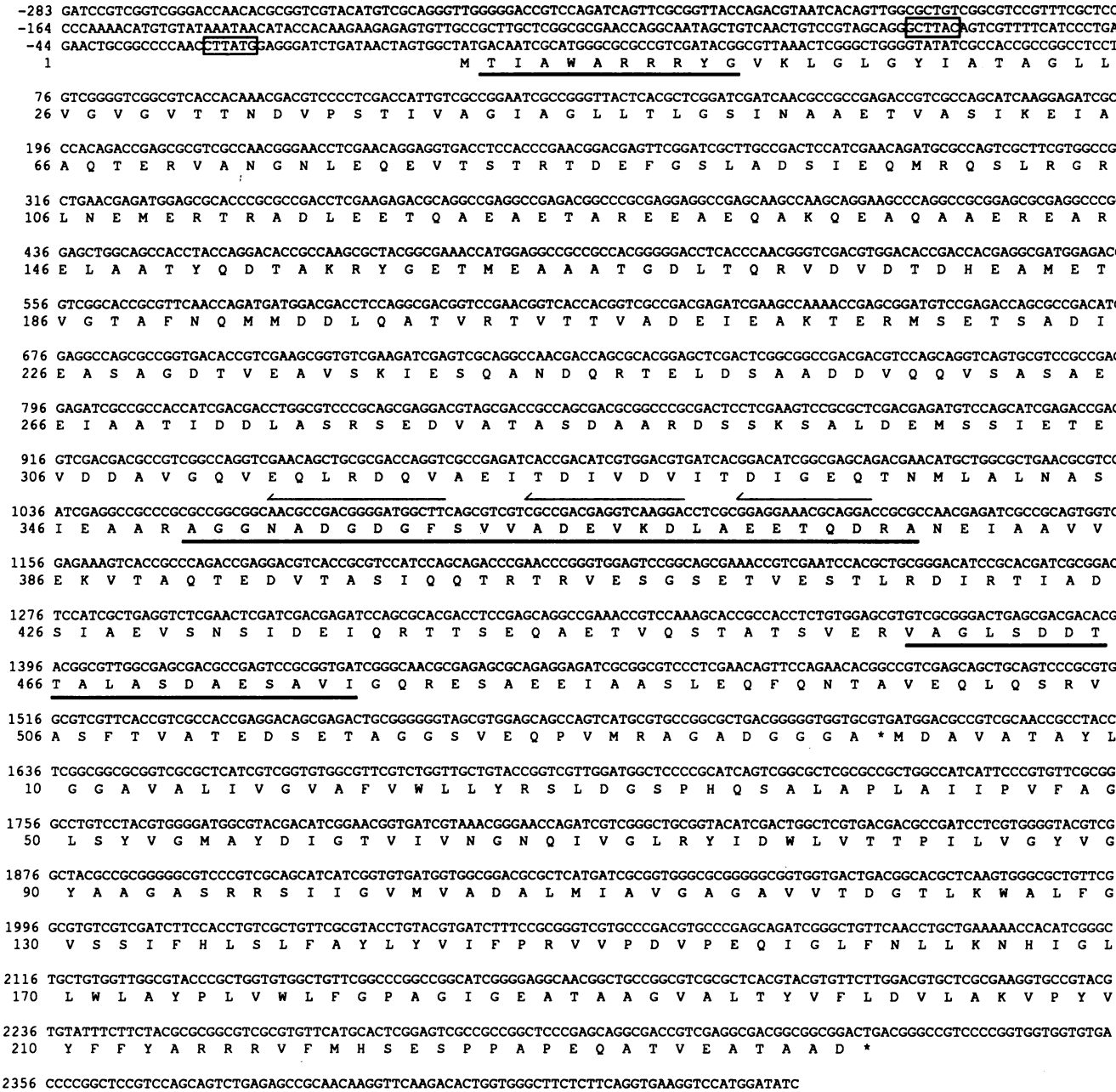

FIG. 1. Nucleotide and encoded protein sequences of the *htrI/sopI* region. The *htrI* gene spans nucleotides 1–1611, and the *sopI* gene spans nucleotides 1611–2331. Underlined amino acid residues were determined by tryptic peptide and N-terminal sequencing. The three half-arrows drawn above the DNA sequence denote regions of the peptide sequence to which fully degenerate deoxyoligonucleotide mixtures complementary to the noncoding strand were synthesized for cloning. Putative promoter sequences are boxed.

−20°C. Sequencing reactions were loaded onto a preelectrophoresed [0.5× TBE (1× TBE is 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 7.3), 30 min, 50 W] 5% polyacrylamide gel containing 0.5× TBE and 7.5 M urea and run at 50 W constant power.

RESULTS

Gene Sequence and Mapping. The *htrI* gene was identified on an 11-kbp *Eco*RI fragment of Flx5R genomic DNA by high-stringency Southern hybridization with each of the three degenerate 5′-[^{32}P]phosphorylated oligonucleotide mixtures based on an internal tryptic peptide sequence (Fig. 1). The gene was sequenced in both directions by generating overlapping subclones derived from restriction digests and exonuclease III nested deletions (Fig. 2). The gene-derived primary structure confirmed the two internal tryptic peptide sequences and the N-terminal sequence (Fig. 1).

The termination codon of the 1611-bp *htrI* overlaps the first base of *sopI*. Two putative promoter elements exhibiting close sequence identity to promoter sequences in other halobacterial genes (28) are located in an A+T-rich region upstream of *htrI* (Fig. 1).

The Encoded Protein. The *htrI* gene encodes a protein of 536 amino acids with a calculated molecular mass of 56,675 Da and a pI of 3.9. Comparison of the translated nucleotide sequence with N-terminal sequence of the purified protein shows the protein is synthesized without a processed leader peptide and the N-terminal methionine is removed in the mature HtrI. A cluster of four basic residues is present near the N terminal, and a hydropathy plot of the translated gene indicates two hydrophobic segments immediately after these

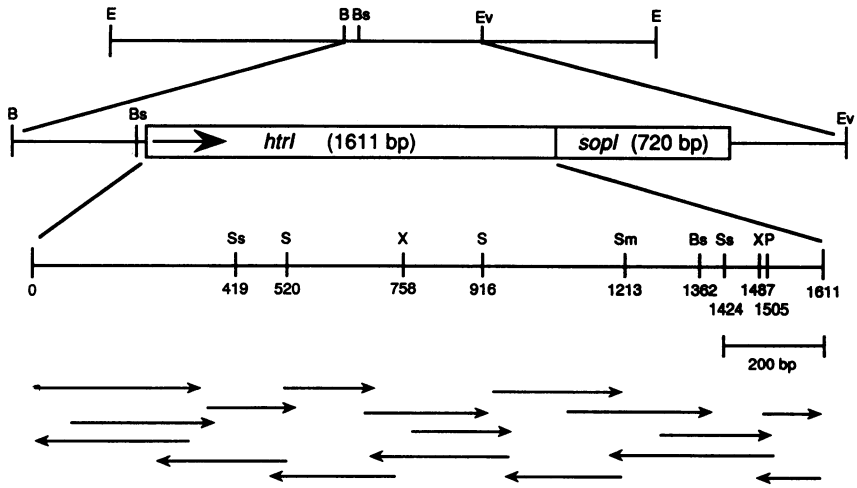

FIG. 2. Restriction map of *htrI*. Key restriction sites on the initially isolated 11-kbp *Eco*RI fragment are indicated. A 2.7-kbp *Bam*HI/*Eco*RV fragment contains the *htrI/sopI* region. The *htrI* gene was mapped by restriction analysis. B, *Bam*HI; Bs, *Bst*XI; E, *Eco*RI; Ev, *Eco*RV; P, *Pst* I; S, *Sal* I; Sm, *Sma* I; Ss, *Sst* II; and X, *Xma* III.

residues (Fig. 3*A*). These putative transmembrane regions are followed by a highly hydrophilic and highly acidic structure extending to the C terminus (Fig. 3 *A* and *B*).

Comparison of HtrI to *E. coli* Tsr. A search of the protein sequences in the GenBank and European Molecular Biology Laboratory data bases identified the highest sequence similarity of HtrI to eubacterial chemotaxis signal transducers, [e.g., *E. coli* Tsr, Tar, Trg, Tap, and related proteins in other eubacteria (18, 20)]. HtrI exhibits 64% identity and 87% similarity (Genetics Computer Group package version 7.1) to a 47-amino acid region (HtrI residues 325–371) within the signaling domain (29) of Tsr (Fig. 4). The flanking methylation domains in Tsr show a lesser degree of sequence identity to HtrI; however, the five doublet carboxylmethylation sites identified in Tsr (30) correspond to glutamate and aspartate residues in HtrI (Fig. 4), potential carboxylmethylation sites. Further, four of these potential sites in HtrI occur in doublet acidic residues, which is characteristic of eubacterial carboxylmethylation sites (30).

DISCUSSION

In the *htrI/sopI* region, the only putative promoter sequences with strong identity to the *H. halobium* consensus promoter sequence (28) reside upstream of *htrI* (Fig. 1). Two putative promoter elements with weaker sequence identity to an archaebacterial consensus promoter sequence were identified within 33 bp upstream of *sopI* (10). To study expression of *htrI* and *sopI* in *H. halobium*, we used a selectable vector (M. P. Krebs, R. Mollaaghababa, and H. G. Khorana, personal communication) derived from a described plasmid (31) combined with the mevinolin-resistance gene (32) to transform strain Pho81. SR-I expression was not detected from the *Bcl* I–*Sal* I fragment (from 986 to 34 bp downstream of bp 2437, Fig. 1), which contains the suggested *sopI* promoters. However, expression of both HtrI and SR-I was obtained when this fragment was extended upstream to the *Bam*HI site (−283) to include *htrI* and its putative promoters (Fig. 1), suggesting *htrI* and *sopI* are cotranscribed. Pho81 transformants containing the *htrI/sopI* region exhibited normal SR-I photochemical activity, as assessed by flash photolysis and complete restoration of SR-I-mediated phototaxis responses *in vivo* (unpublished work).

Several *H. halobium* genes exhibit at −7 to −12 a sequence complementary to the 3′ end of the *H. halobium* 16S RNA (ribosome-binding site) (33). Such a properly positioned ribosome-binding site is not evident upstream of *htrI*, although a possible site occurs at −24 to −20. The importance of ribosome-binding sites in *H. halobium* is not clear because there are several *H. halobium* genes that appear to lack them (33).

The apparent relative molecular mass of 97,000 Da for HtrI on SDS/polyacrylamide gels is higher than the 56,675 Da

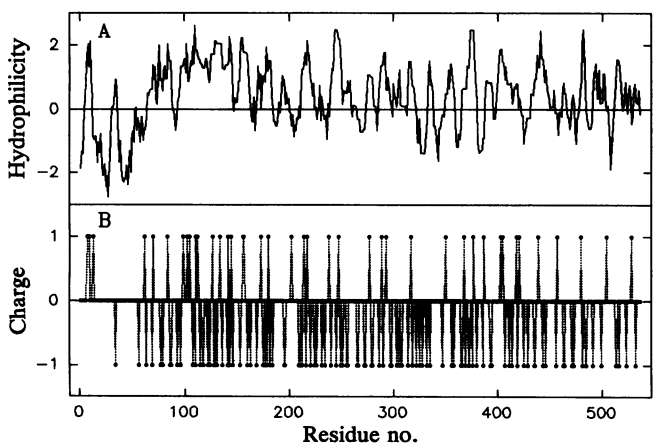

FIG. 3. (*A*) Kyte–Doolittle hydropathy plot (Genetics Computer Group sequence analysis software package, version 7.1, Madison, WI). (*B*) Linear charge distribution of HtrI (acidic residues, −1; basic residues, +1; neutral residues, 0).

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HtrI  .....ADDVQQVSASAEEIAATIDDLASRSEDV.ATASDAARDSSKSALD  297
Tsr   GASEIATGNNDLSSRTEQQAASLEETAASMEQLTATVKQNAENARQASHL
                          *     *     *
HtrI  EMSSIETE......VDDAVGQVEQLRDQVAEITDIVDVITDIGEQTNMLA  341
Tsr   ALSASETAQRGGKVVDNVVQTMRDISTSSQKIADIISVIDGIAFQTNILA

HtrI  LNASIEAARAGGNADGDGFSVVADEVKDLAEETQDRANEIAAVVEKVTAQ  391
Tsr   LNAAVEAARAGE..QGRGFAVVAGEVRNLAQRSAQAAREIKSLI......

HtrI  TEDVTASIQQTRTRVESGSETVESTLRDIRTIADSIAEVSNSIDEIQRTT  441
Tsr   .EDSVGKVDVGSTLVESAGETMAEIVSAVTRVTDIMGEIASASDEQSRGI

HtrI  SEQAETVQSTATSVERVAGLSDDTTALASDAES.........AVIGQRES  482
Tsr   DQVGLAVAEMDRVTQQNAALVEESAAAAAALEEQASRLTEAVAVFRIQQQ
                              *     *
HtrI  AEEIAASLEQFQNTA..  497
Tsr   QRETSAVVKTVTPAAP*
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FIG. 4. Primary-sequence comparison of HtrI residues 254–497 (Fig. 1) to the methylation and signaling domains of *E. coli* Tsr (29). Stars identify carboxylmethylated glutamyl residues in mature Tsr (30).

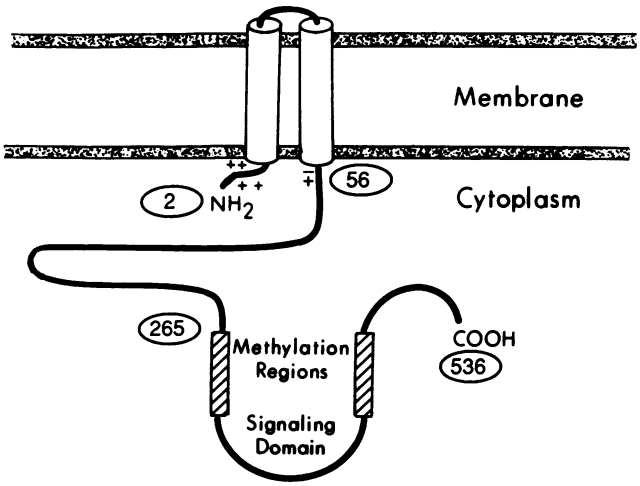

FIG. 5. Schematic model of HtrI. The N-terminal residue is indicated as the second of the 536 encoded residues in accord with N-terminal sequence data (Fig. 1). The 56th residue corresponds to the end of the two hydrophobic segments, and the 265th residue corresponds to the beginning of the region homologous to Tsr.

calculated for the mature protein from the translated gene sequence. Anomalously slow migration in an SDS/polyacrylamide gel is expected from the highly acidic nature of the protein and has been observed in the acidic *H. halobium* ATPase α and β subunits (34) and cell-surface glycoprotein (35). This effect may be sufficient to explain the slow migration; however, we have not ruled out that the molecular mass is influenced by posttranslational modification, dimerization, or binding of an additional component resistant to SDS and reducing agents.

A common motif found in diverse eubacterial signal transducers is a highly conserved signaling domain in the C-terminal portion flanked by two methylation regions that function in adaptive attenuation of the signal (18, 20). Existence of this motif in HtrI extends the family of eubacterial signal transducers to the archaebacteria, indicating an early origin for this type of signal transducer. In eubacterial chemotaxis, the signaling domain controls a two-component regulatory system that is a member of a widespread family of cytoplasmic phosphotransfer systems (19, 20). Such a cytoplasmic transduction system has not been demonstrated in archaebacteria, but the existence of a similar signaling domain on HtrI suggests that this system may occur. In this regard, phenotypes of phototaxis and chemotaxis mutants of *H. halobium* exhibit similarities to first- and second-component mutants in *E. coli* chemotaxis (22, 23).

A model for HtrI can be constructed based on sequence similarity to eubacterial transducers, hydropathy-plot analysis, and the requirement of a cytoplasmic location of the methylation and signaling domains (Fig. 5). The distribution of charges surrounding the putative transmembrane segments is as expected from the view that charged residues act as a stop transfer signal (36) and from the presence of a transmembrane electrical potential in *H. halobium* that stabilizes positive and negative charges at the intracellular and extracellular membrane surfaces, respectively. HtrI differs from *E. coli* Tsr in that HtrI does not have an extracellular-receptor binding domain but, instead, has a more extended cytoplasmic region. This structural distinction fits the different functions of the two transducers. Tsr is a chemoreceptor interacting with extracellular effectors, whereas HtrI must sense the conformational states of SR-I.

Physical proximity of HtrI to the retinal-binding site of SR-I has been suggested by a possible migration of [^{3}H]retinal from SR-I to HtrI during a borohydride-reduction procedure (16). An attractive possibility is that the cytoplasmic loop (residues 56–265) is positioned at the cytoplasmic surface of the SR-I protein. Physical proximity would concur with preliminary results from SR-I expression in the absence of HtrI that indicate HtrI influences proton-transfer reactions at the chromophore-attachment site essential in the transitions between the SR-I attractant signaling conformation (S_{373}) and the prestimulus state (SR-I_{587}) (37).

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1. Bogomolni, R. A. & Spudich, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6250–6254.
2. Spudich, J. L. & Bogomolni, R. A. (1988) *Annu. Rev. Biophys. Biophys. Chem.* **17**, 193–215.
3. Oesterhelt, D. & Marwan, W. (1990) *Symp. Soc. Gen. Microbiol.* **46**, 219–239.
4. Tsuda, M., Nelson, B., Chang, C.-H., Govindjee, R. & Ebrey, T. G. (1985) *Biophys. J.* **47**, 721–724.
5. Yan, B., Takahashi, T., Johnson, R., Derguini, F., Nakanishi, K. & Spudich, J. L. (1990) *Biophys. J.* **57**, 807–814.
6. Yan, B., Nakanishi, K. & Spudich, J. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9412–9416.
7. Bogomolni, R. A. & Spudich, J. L. (1987) *Biophys. J.* **52**, 1071–1075.
8. Yan, B. & Spudich, J. L. (1991) *Photochem. Photobiol.* **54**, 1023–1026.
9. Spudich, J. L. & Bogomolni, R. A. (1984) *Nature (London)* **312**, 509–513.
10. Blanck, A., Oesterhelt, D., Ferrando, E., Schegk, E. S. & Lottspeich, F. (1989) *EMBO J.* **8**, 3963–3971.
11. Spudich, E. N. & Spudich, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4308–4312.
12. Oesterhelt, D. & Marwan, W. (1987) *J. Bacteriol.* **169**, 3515–3520.
13. Marwan, W., Schafer, W. & Oesterhelt, D. (1990) *EMBO J.* **9**, 355–362.
14. Schimz, A. & Hildebrand, E. (1987) *Biochim. Biophys. Acta* **923**, 222–232.
15. Schimz, A., Hinsch, K.-D. & Hildebrand, E. (1989) *FEBS Lett.* **249**, 59–61.
16. Spudich, E. N., Hasselbacher, C. A. & Spudich, J. L. (1988) *J. Bacteriol.* **170**, 4280–4285.
17. Alam, M., Lebert, M., Oesterhelt, D. & Hazelbauer, G. L. (1989) *EMBO J.* **8**, 631–639.
18. Hazelbauer, G. L. (1988) *Can. J. Microbiol.* **34**, 466–474.
19. Stock, J. B., Stock, A. M. & Mottonen, J. M. (1990) *Nature (London)* **334**, 395–400.
20. Bourret, R. B., Borkovich, K. A. & Simon, M. I. (1991) *Annu. Rev. Biochem.* **60**, 401–441.
21. Spudich, E. N., Takahashi, T. & Spudich, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7746–7750.
22. Sundberg, S. A., Bogomolni, R. A. & Spudich, J. L. (1985) *J. Bacteriol.* **164**, 282–287.
23. Sundberg, S. A., Alam, M., Lebert, M., Spudich, J. L., Oesterhelt, D. & Hazelbauer, G. L. (1990) *J. Bacteriol.* **172**, 2328–2335.
24. Alam, M. & Hazelbauer, G. L. (1991) *J. Bacteriol.* **173**, 5837–5842.
25. Spudich, E. N., Sundberg, S. A., Manor, D. & Spudich, J. L. (1986) *Proteins* **1**, 239–246.
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
27. Stone, K. L., LoPresti, M. B., Crawford, J. M., DeAngelis, R. & Williams, K. R. (1989) in *A Practical Guide to Protein and Peptide Purification for Microsequencing*, ed. Matsudaira, P. T. (Academic, San Diego), pp. 31–47.
28. Zillig, W., Palm, P., Reiter, W.-D., Gropp, F., Puhler, G. & Klenk, H.-P. (1988) *Eur. J. Biochem.* **173**, 473–482.
29. Boyd, A., Kendall, K. & Simon, M. I. (1983) *Nature (London)* **301**, 623–626.
30. Rice, M. S. & Dahlquist, F. W. (1991) *J. Biol. Chem.* **266**, 9746–9753.
31. Krebs, M. P., Hauss, T., Heyn, M. P., RajBhandary, U. L. & Khorana, H. G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 859–863.
32. Lam, W. L. & Doolittle, W. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5478–5482.
33. Jones, J. G., Young, D. C. & DasSarma, S. (1991) *Gene* **102**, 117–122.
34. Ihara, K. & Mukohata, Y. (1991) *Arch. Biochem. Biophys.* **286**, 111–116.
35. Lechner, J. & Sumper, M. (1987) *J. Biol. Chem.* **262**, 9724–9729.
36. von Heijne, G. (1984) *EMBO J.* **3**, 2315–2318.
37. Spudich, E. N., Krebs, M. P., Khorana, H. G. & Spudich, J. L. (1992) *Biophys. J.* **61**, 531 (abstr.).