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# Chemotaxis and phototaxis require a CheA histidine kinase in the archaeon *Halobacterium salinarium*

**J.Rudolph and D.Oesterhelt**

Max Planck Institute for Biochemistry, D-82152 Martinsried, Germany

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**Histidine kinases are part of the two-component signal transduction system responsible for eubacterial responses to diverse environmental signals. They have recently been detected in eukaryotes but their existence in the kingdom Archaea remains uncertain. Here we report the sequence and function of a histidine kinase ($CheA_{H.s.}$) from *Halobacterium salinarium*, the first such transmitter in Archaea. The protein $CheA_{H.s.}$ (668 residues) has significant sequence identity with the CheA proteins known from eubacterial signal transduction (e.g. 34% identity with CheA from *Bacillus subtilis*). Antibodies were raised against $CheA_{H.s.}$ as expressed in *Escherichia coli* and were used in Western blotting to demonstrate the expression of $cheA_{H.s.}$ in *H.salinarium*. As has been observed for other halophilic proteins, $CheA_{H.s.}$ has a deviant electrophoretic migration, with an apparent molecular weight of 103 kDa on SDS–PAGE compared with a calculated molecular weight of 72 kDa. Deletion of a part of the $cheA_{H.s.}$ gene leads to loss of both chemotactic and phototactic responses in *H.salinarium* as measured by swarm plate assays, motion analysis and tethering experiments. This indicates that $CheA_{H.s.}$ plays a crucial role in chemical and light signal integration, presumably interacting with at least two phototransducers and a number of chemoreceptors.**

*Key words*: chemotaxis/histidine kinase/phototaxis/signal transduction

## Introduction

Signal transduction is the molecular mechanism by which cells sense and then respond to a wide variety of external stimuli. Chemical, electrical and light signals are received by receptors usually located in the cell membrane and the signals are subsequently transferred through a series of molecular interactions to their intracellular target(s). Such terminal target sites include promoters and repressors involved in regulated gene expression in a wide variety of organisms, as well as the flagellar motors in motile cells such as the chemotactic response in the eubacterium *Escherichia coli* (Parkinson, 1993). In eubacteria signal transduction is mediated by reversible phosphorylation of histidine and aspartate residues of signaling proteins. In contrast, in eukaryotic systems signal transduction occurs mainly through the reversible formation of phosphate esters of serine, threonine and tyrosine residues, although recent evidence indicates an important role for histidine kinases in *Arabidopsis* (Chang *et al.*, 1993) and yeasts (Ota and Varshavsky, 1993; Maeda *et al.*, 1994).

*Halobacterium salinarium* is an archaeon capable of both chemo- and phototaxis (Oesterhelt and Marwan, 1990). In the absence of a stimulus *H.salinarium* performs a random walk based on the spontaneous switching of the flagellar motor from clockwise (CW, forward swimming) to counter-clockwise (CCW, reverse swimming) (Alam and Oesterhelt, 1984). An attractant (e.g. increase in green light, decrease in UV–blue light, addition of histidine) prolongs a single run followed by adaptation and return to normal switching behavior (Hildebrand and Dencher, 1975; Schimz and Hildebrand, 1979). A repellant (e.g. increase in UV–blue light, addition of phenol) increases the switching frequency, again followed by adaptation and return to normal switching behavior. Overall chemo- and phototactic signal integration has been demonstrated for simultaneously applied attractant and repellant stimuli (Spudich and Stoeckenius, 1979; Hildebrand and Schimz, 1986).

Halobacteria possess both chemo- and phototactic signal receptors. Evidence for a number of chemotactic receptors exists in *H.salinarium* from detection of protein bands following electrophoresis by radiolabeling with methionine and by antigenic cross-reactivity with antisera to eubacterial transducers (Alam and Hazelbauer, 1991). However, no chemoreceptors have been isolated or characterized. The photoreceptors which are responsible for the phototactic behavior of *H.salinarium* are sensory rhodopsin I (SRI) and sensory rhodopsin II (SRII). SRII is a photophobic receptor which has been characterized extensively for its role in *H.salinarium*'s repellant response to blue light (Marwan and Oesterhelt, 1987). The gene for SRII is unknown in *H.salinarium*, but has been isolated recently along with its transducer protein from *Haloarcula vallismortis and Natronobacter pharaonis* (Seidel *et al.*, 1995). Interestingly, under conditions of intense light, bacteriorhodopsin (BR) also mediates a phototactic response by an unknown mechanism based on changes in the proton motive force (Bibikov *et al.*, 1993).

The only signal receptor for which detailed biochemical and genetic data exist in *H.salinarium* is SRI, which mediates both an attractant response to orange light and a repellant response to UV light (Spudich and Bogomolni, 1984; Schegk and Oesterhelt, 1988; Blanck *et al.*, 1989). Hydropathy analysis indicates a secondary structure of seven transmembrane helices for SRI, in analogy with BR and halorhodopsin (HR), with both of which SRI also shares significant sequence homology (14% identity). Interestingly, a similar three-dimensional topology has been characterized for SRI's functional counterpart in vertebrates, the visual pigment rhodopsin, but no significant sequence homology exists (Henderson *et al.*, 1990; Soppa, 1994a). This suggests a mechanism of light signal

transduction in Archaea similar to the G-protein-mediated pathway in eukaryotes (Hargrave and McDowell, 1992). However, the absence of large cytoplasmic interhelical loops in SRI, the regions known to be involved in the interaction between rhodopsin and its transducer protein (Hargrave and McDowell, 1992), the weak evidence for GTP-mediated signaling (Schimz and Hildebrand, 1987), and the evidence for the involvement of methylation in chemo- and phototactic signal transduction (Alam *et al.*, 1989; Spudich *et al.*, 1989; Nordmann *et al.*, 1994) suggest instead a eubacteria-like signal transduction pathway in *H.salinarium*.

The halobacterial transducer for SRI (HtrI) was originally identified as a methylated membrane protein with an apparent molecular weight of 97 kDa whose expression correlated with SRI protein expression (Spudich *et al.*, 1988). The methylation was found to be reversible carboxymethyl esterification of glutamate residues, analagous to the chemotactic signaling proteins in *E.coli*. Methylation/demethylation is controlled by the attractant and repellent states of SRI and is responsible for light adaptation, again in analogy with chemotactic transducer proteins in *E.coli* (Alam *et al.*, 1989; Spudich *et al.*, 1989). The gene for HtrI (*htrI*) lies immediately upstream of the gene for SRI (*sopI*) and is cotranscribed (Ferrando-May *et al.*, 1993). The deduced protein sequence indicates a protein of 57 kDa, with two N-terminal transmembrane helices which anchor the protein to the membrane (Yao and Spudich, 1992). Following a long hydrophilic domain, a C-terminal region has been identified which contains significant homology (64% identity) to the signaling domains of eubacterial chemotaxis transducers (e.g. Tsr and Tar from *E.coli*). This information, along with the demonstrated functional interaction between SRI and HtrI (Olson and Spudich, 1993; Krah *et al.*, 1994), suggests a signaling pathway in Archaea based on the two-component regulatory system utilizing the CheA histidine kinase transmitter module and the CheY receiver module known from eubacterial signal transduction (see Figure 1).

In search of further components of halobacterial signal transduction, and in order to investigate where chemo- and phototactic signal integration takes place, we searched for the halobacterial equivalent of the histidine kinase CheA. This paper describes the isolation, primary structure determination and deletion analysis of the first histidine kinase from the kingdom Archaea. Also the role of CheA in signal integration is discussed.

## Results

### *Cloning of cheA$_{H.s.}$*

Degenerate primers for PCR amplification were designed based on alignments from known CheA sequences from *E.coli*, *Salmonella typhimurium*, *Bacillus subtilis* and *Myxococcus xanthus* using two highly conserved regions, the N-block and G2-block (see Figure 1). Following PCR amplification of halobacterial DNA, a 270 bp fragment with high similarity to the intervening G1-block and F-block was isolated, cloned and sequenced. A probe derived from this fragment was then used to clone the gene from two overlapping genomic fragments generated by digestion of halobacterial chromosomal DNA with *Bam*HI (1.9 kb) and *Sac*I (3.2 kb). A part of the cloned genomic sequence (2.7 of 4.5 kb) is shown in Figure 2.

An open reading frame (ORF) with the characteristic G/C bias of halobacteria (65%) was identified starting at nucleotide 104 of the *Bam*HI clone with a putative ribosomal binding site (GGAGGA) (Kagramanova *et al.*, 1982) beginning 15 nucleotides prior to the start codon. The deduced protein sequence (= CheA$_{H.s.}$) yields a protein of 668 residues (calculated $M_r$ = 71 878) with 32.8% and 34.0% overall sequence identity with CheA$_{E.c.}$ and CheA$_{B.s.}$, respectively (Figure 2). Regions of higher similarity include the phosphotransfer (1–160) and autophosphorylation (260–507) domains (Parkinson and Kofoid, 1992), with 38.0% and 39.4% identity of CheA$_{H.s.}$ compared with CheA$_{B.s.}$. The conserved His (His48 in CheA$_{E.c.}$), which is autophosphorylated in all previously characterized CheAs, is located at position 44 in CheA$_{H.s.}$. The alternative start site (Met98) yielding CheA-short in CheA$_{E.c.}$ (Wolfe and Stewart, 1993) is not present in CheA$_{H.s.}$.

The region downstream of the *cheA$_{H.s.}$* gene (Figure 2), although containing an ORF of 585 bp only 3 bp following the *cheA$_{H.s.}$* stop codon, codes for neither a CheW equivalent (as found in *E.coli*, *B.subtilis* and *S.typhimurium*) nor a CheY equivalent (as found in *M.xanthus*). No significant similarities could be detected between this ORF and any of the sequences in the EMBL database as of September 1994.

### *Deletion analysis of CheA$_{H.s.}$*

In order to test the function of CheA$_{H.s.}$, a deletion mutant which lacks the central section of the *cheA$_{H.s.}$* gene was constructed of strain S9 (BR+, HR+, SRI+, SRII+, chemotaxis+). The vector pJR3557 containing a mevinolin resistance cassette was constructed for use in the transformation procedure. The pJR3557 construct lacks the catalytic core of CheA$_{H.s.}$ (residues 193–609), which should destroy the activity of the protein, and yet sufficient nucleotides are preserved at both ends of *cheA$_{H.s.}$* to allow the homologous crossover event needed to generate the deletion strain. This construction incorporates a new *Bam*HI site which generates a characteristic 0.9 kb fragment containing the initial 576 nucleotides of the *cheA$_{H.s.}$* gene (see Figure 3). Also, the ORF which immediately follows the stop codon of *cheA$_{H.s.}$* is not altered by this insertion. Screening of transformants resulting from both single as well as double crossover events led to the isolation of the strain E4, the *cheA$_{H.s.}$* deletion strain (Figure 3).

The deletion strain E4 is incapable of chemotaxis as tested by swarm plate assays. After 4 days of growth the diameter of the swarm formed by the S9 cells was 4.3 ± 0.1 cm, whereas the diameter of the E4 swarm was only 2.3 ± 0.2 cm. Also, the S9 swarms formed a pronounced chemotactic ring which was not detected in the E4 swarms. The lack of swarming was not the result of a general immotility, as determined by visual examination of the cells. Thus the CheA deletion in *H.salinarium* leads to the same behavior on swarm plates as has been found for both *E.coli* and *B.subtilis* (Fuhrer and Ordal, 1991; Oosawa *et al.*, 1988).

Motion analysis was then performed to test the phototactic behavior of E4 compared with the control strain S9. Although no selection on swarm plates was performed,

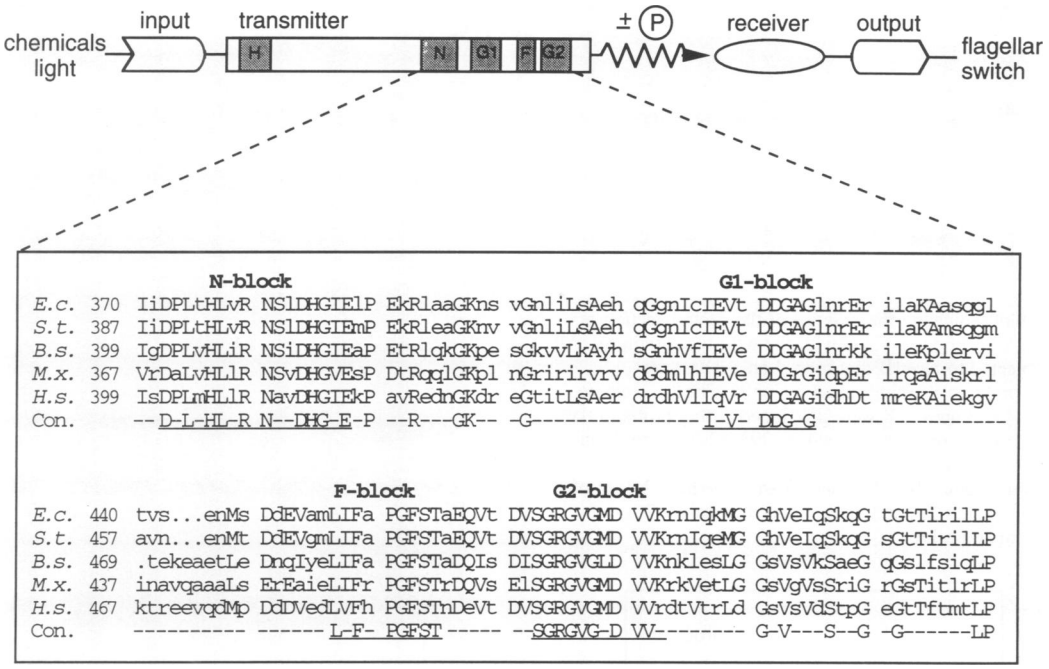


**Fig. 1.** Circuit elements in two-component bacterial signaling systems and partial alignment of highly conserved regions of known CheA protein sequences. The alignments were made using PILEUP and PRETTY from the GCG package (Genetics Computer Group, 1994): *E.coli* (Kofoid and Parkinson, 1991), *S.typhimurium* (Stock *et al.*, 1988), *B.subtilis* (Fuhrer and Ordal, 1991), *M.xanthus* (McCleary *et al.*, 1990) and *H.salinarium* (this work). Residues which are similar in four of five sequences are indicated in capital letters and the consensus (identity in all of five sequences) is also shown. The circuit element scheme and the labeling of the homology blocks is adapted from Parkinson (1993) and Parkinson and Kofoid (1992).

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                                                                -102  GGATCCCCGAGCGCGCCATCGAGACGG
 -75  GATGCGTTGACGACGTGTTGCCGGTCGATCAGCTAACTGAAGCGATCGCGGATTCGATACGGAGGACGACGTGAC

   1  ATG GAC GAC TAC CTC GAA GCG TTC GTT CGT GAA GGC GAA GAA CAC GTA ACC AGC CTC
   1   M  D  D  Y  L  E  A  F  V  R  E  G  E  E  H  V  T  S  L

  58  AAC AAC GCC CTG CTG GAG CTG GAG TCG GAC CCG GGC AAC GAG GAG GCG ATG GAC GAA  1141  GAC TTC GTC GTG GAG GGC GAC GAC GTC GAG CTC GAC CGC ACG ATC CTC ACG GAG ATC
  20   N  N  A  L  L  E  L  E  S  D  P  G  N  E  E  A  M  D  E                  381   D  F  V  V  E  G  D  D  V  E  L  D  R  T  I  L  T  E  I

 115  ATA TTC CGG ACC GCC CAC ACG CTG AAG GGG AAC TTC GGC GCG ATG GGG TTC GAG GAC  1198  AGC GAT CCG CTG ATG CAT CTG TTG CGG AAC GCG GTC GAT CAC GGG ATC GAG AAG CCG
  39   I  F  R  T  A  H  T  L  K  G  N  F  G  A  M  G  F  E  D                  400   S  D  P  L  M  H  L  L  R  N  A  V  D  H  G  I  E  K  P

 172  GCA AGC GAT CTC GCC CAC GCC GTC GAG GAT TTG CTC GAC GAG ATG CGG CAG GGC AAC  1255  GCG GTG CGC GAG GAC AAC GGC AAG GAC CGC GAG GGG ACG ATC ACG CTG TCC GCC GAG
  58   A  S  D  L  A  H  A  V  E  D  L  L  D  E  M  R  Q  G  N                  419   A  V  R  E  D  N  G  K  D  R  E  G  T  I  T  L  S  A  E

 229  CTC GAA GTG ACC TCC GAC AGG ATG GAC CGC ATC TTC GAG GGG ATC GAC GGC ATC GAA  1312  CGC GAC CGC GAT CAC GTG CTC ATC CAA GTC CGA GAC GAC GGC GCC GGC ATC GAC CAC
  77   L  E  V  T  S  D  R  M  D  R  I  F  E  G  I  D  G  I  E                  438   R  D  R  D  H  V  L  I  Q  V  R  D  D  G  A  G  I  D  H

 286  GCC TGC CTC GAC GAG ATC CAG GCC ACC GGT GAC GTG GAC CGA GAC GTC ACC GGC ACC  1369  GAC ACG ATG CGG GAG AAA GCC ATC GAG AAA GGC GTG AAA ACC CGC GAG GAG GTC CAG
  96   A  C  L  D  E  I  Q  A  T  G  D  V  D  R  D  V  T  G  T                  457   D  T  M  R  E  K  A  I  E  K  G  V  K  T  R  E  E  V  Q

 343  ATC GAG TCG GTG CGG GCT GTG CTG GAT GAG GTC GAC GGG CAC GGT GGC AGC GGC ACC  1426  GAC ATG CCC GAC GAC GAC GTC GAG GAT CTG GTC TTC CAT CCC GGG TTC TCG ACG AAC
 115   I  E  S  V  R  A  V  L  D  E  V  D  G  D  G  G  S  G  T                  476   D  M  P  D  D  D  V  E  D  L  V  F  H  P  G  F  S  T  N

 400  ACC ACC AGC AGC GGC GAC GCC GGC AGC CCG GCC GGC GAC GGT GAC GTG GAC GCG ACG  1483  GAC GAG GTG ACC GAC GTC TCC GGG CGC GGG GTC GGG ATG GAC GTG GTC AGG GAT ACG
 134   T  T  S  S  G  D  A  G  S  P  A  G  D  G  D  V  D  A  T                  495   D  E  V  T  D  V  S  G  R  G  V  G  M  D  V  V  R  D  T

 457  CGG GTC GTC GAC GCG GAC ACC ATC GAC GCC GTC GAG GAC CCC GTC TAC CAC ATC CAC  1540  GTG ACG CGA CTC GAT GGG AGC GTC TCC GTC GAC AGC ACG CCC GGC GAG GGA ACG ACG
 153   R  V  V  D  A  D  T  I  D  A  A  E  D  P  V  Y  H  I  H                  514   V  T  R  L  D  G  S  V  S  V  D  S  T  P  G  E  G  T  T

 514  ATC GAC ATG GGC GAC TCC CAG ATG AAA GGC GTC GAC GGG ATG TTC GTA CTC GAG GAG  1597  TTC ACG ATG ACG CTG CCG GTG ACC GTC GCC ATC GTG AAG GTG CTG TTC GTG GAG AGC
 172   I  D  M  G  D  S  Q  M  K  G  V  D  G  M  F  V  L  E  E                  533   F  T  M  T  L  P  V  T  V  A  I  V  K  V  L  F  V  E  S

 571  GCC ACG GAG GCC TTC GAT CTG CTC GGG GCC GAG CCG TCG CCG GAC GCG ATC AAC GAC  1654  GGT GGC GAG GAG TAC GGC ATT CCG ATC AAG ACA GTC GAC GAG ATC TCG CGG ATG AAG
 191   A  T  E  A  F  D  L  L  G  A  E  P  S  P  D  A  I  N  D                  552   G  G  E  E  Y  G  I  P  I  K  T  V  D  E  I  S  R  M  K

 628  GGC GAG TAC GGT GAT GGC TTC GAA CTG GTC GTC GCG ACG CCC AGC GAC GAG GTC AGC  1711  TCC GTG AAA TCA GTC GAC GGC GAA GAG GTC ATC ACC TAC GAC GAG ACG GTG TAC CCG
 210   G  E  Y  G  D  G  F  E  L  V  V  A  T  P  S  D  E  V  S                  571   S  V  K  S  V  D  G  E  E  V  I  T  Y  D  E  T  V  Y  P

 685  GAC ACC GTG GCG GCG TTC CCC AAG CTG TCG GAC GCA ACG GTC ACC GCC GTC GGC GAC  1768  CTG GTT CGA CTC GGC GAC GCG TTG AAC GTC CCC GAC GAG ACG CGG AAC GGC GAC GGC
 229   D  T  V  A  A  F  P  K  L  S  D  A  T  V  T  A  V  G  D                  590   L  V  R  L  G  D  A  L  N  V  P  D  E  T  R  N  G  D  G

 742  GAC GAG CAC GCC CCA GAC GCC GAC AGC GGG ACC GAG GCG GAC GCC AGC GCC GAC GAC  1825  ATG CTC GTC CGG ATC CGT GAT TCG GAG CGA CAG GTC GCC GTG CAT TGC GAC GAC GTT
 248   D  E  H  A  P  D  A  D  S  G  T  E  A  D  A  S  A  D  D                  609   M  L  V  R  I  R  D  S  E  R  Q  V  A  V  H  C  D  D  V

 799  GAT GCG GAC GAT GCC GGG ACG ACC GCC GAC AGC GGG AGT TCC AGC GGC GGG TCC TCG  1882  CGC GGC CAA GAA GAG GTC GTC GTC AAG CCC TTC GAA GGC ATC CTC TCC GGG ATT CCC
 267   D  A  D  D  A  G  T  T  A  D  S  G  S  S  S  G  G  S  S                  628   R  G  Q  E  E  V  V  V  K  P  F  E  G  I  L  S  G  I  P

 856  GCG ATC GAC AAC ACG GAC ACG GAG ATC CAG TCG GTT CGC GTG GAC GTC GAT CAG CTC  1939  GGG CTG TCC GGG GCG GCC GTC CTC GGC GAG GGT GAC GTG GTG ACC ATC CTG GAC GTG
 286   A  I  D  N  T  D  T  E  I  Q  S  V  R  V  D  V  D  Q  L                  647   G  L  S  G  A  A  V  L  G  E  G  D  V  V  T  I  L  D  V

 913  GAC GAG CTC CAC GGG CTG GTC GAG CAG TTG GTC ACC ACC CGC ATC AAG CTC CGG CGG  1996  GCT ACG CTG TAA TCATGAGCACAATGATCGACATTCGGCGGTTACAGACCGTCAACGAACTCGCCCGGGAA
 305   D  E  L  H  G  L  V  E  Q  L  V  T  T  R  I  K  L  R  R                  666   A  T  L  *

 970  GGC ATG GAG GAG AGC GAC CGC GAG GTG CTC GAC GAG CTG GAC GAG CTG GAC AAG ATC  2067  GGAGCGTCGACGGTCGCCGAGAACATGAGCCAGCTGACCGGCGTCGAGACCCAGATGCAGATCACGAAGATCAAC
 324   G  M  E  E  S  D  R  E  V  L  D  E  L  D  E  L  D  K  I                  2142  GTCATCGACGTCGAGGACCTCGGCGCACACCTGGGAGCCGCCAAGCAGGTGGGCGTGAGCGTGCCGCTCAAAGAA
                                                                2217  CAGCCCTACGGGTCGGTGCTCGTGTTGTTCGACGACGAGAGCGCACGCCGTGTCGCCGGAACGATGATGGGCCGC
1027  ACC TCC AGC CTC CAG GAC ACC GTG ATG GAC ATG CGG TTG GTC CCG ATG AAG AAG ATC  2292  ATCGAGAGCGAGGGCGGCGGCTACAGCGACATGGAGCGGTCCGCGATCCGTGAGGTCGGGAACATCATGACAAGC
 343   T  S  S  L  Q  D  T  V  M  D  M  R  L  V  P  M  K  K  I                  2367  GGGTTCATCGACGGCTGGGCGAACGTGCTCGGCCGCACATCGACATCTCCACGCCACAGCTCATCCGGGCGTCC
                                                                2442  GGCGAGGACATTGCGAGTCATTGTGTCGACCCCGGCGAACACGAGATCGCGATGGTGTTCGACGCGGAGCTACAC
1084  GTC GGG AAG TTC CCC CGT CTC GTT CGG GAT CTG GCC CGC GAG CAG GAC AAG GAC ATC  2517  GCCCCAGACGCGAACGTCGAGGCGAAAATCTACTCGTTCCCCGACATCGAGGCGTTCGTCTCGATGATCAACAGT
 362   V  G  K  F  P  R  L  V  R  D  L  A  R  E  Q  D  K  D  I                  2592  ATCTAA
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**Fig. 2.** Nucleotide and encoded protein sequence of the $cheA_{H.s.}$ region. The $cheA_{H.s.}$ gene spans nucleotides 1–2007. Nucleotides 2110–2597 encode an ORF with no apparent homology to any known sequences. The sequence of the remaining 1.5 kb of cloned genomic DNA is not shown. The sequence of the entire 4.5 kb of cloned halobacterial DNA has been deposited in GenBank under accession number X82645.

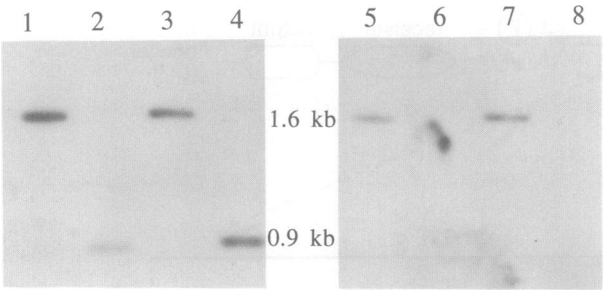


**Fig. 3.** Southern blot indicating the absence of the central section of $cheA_{H.s.}$ from strain E4 in comparison with the wild-type strain S9. All samples were subjected to *Bam*HI digestion. Lanes 1–4 were developed with a probe to the N-terminal region of $CheA_{H.s.}$ (amino acid residues 48–113). Lanes 5–8 were developed with a probe to the deleted part of $CheA_{H.s.}$ (amino acid residues 298–520). Control lanes 1 and 5 contain 7 ng vector pJR4816, control lanes 2 and 6 contain 7 ng of the deletion vector pJR3557, lanes 3 and 7 contain 1.7 µg of genomic DNA from strain S9, and lanes 4 and 8 contain 1.7 µg of genomic DNA from strain E4.

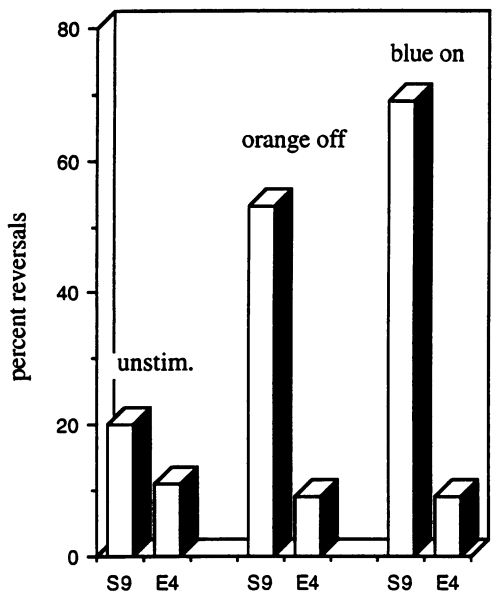


**Fig. 4.** Motion analysis of wild-type strain S9 and the $cheA_{H.s.}$ deletion mutant E4 measuring percent reversals within 4 s in response to no stimulus (unstim.), orange light off, or blue light on, as indicated. The number of individual cells observed varied from 300 to 2000 with an average of 950.

both strains were highly motile after 4 days of growth in liquid culture. The S9 strain showed significant levels of spontaneous reversals as well as responses to the repellants orange light off (SRI response) or blue light on (SRII response) (Figure 4). The deletion strain E4, however, is incapable of phototaxis. The low but significant levels of spontaneous reversals measured in E4 (11%) could not be confirmed by visual inspection and are due to tracking errors and Brownian motion leading to apparent reversals. In addition, the E4 cells could not be stimulated to a reversal event by either the repellant orange light off or blue light on (Figure 4).

To investigate the observed lack of switching by the E4 strain further, intact cells were attached to coverslips via their flagella. The direction of flagellar rotation as seen along the axis of the flagella towards the cell, CW or CCW, could then be determined by visual inspection.

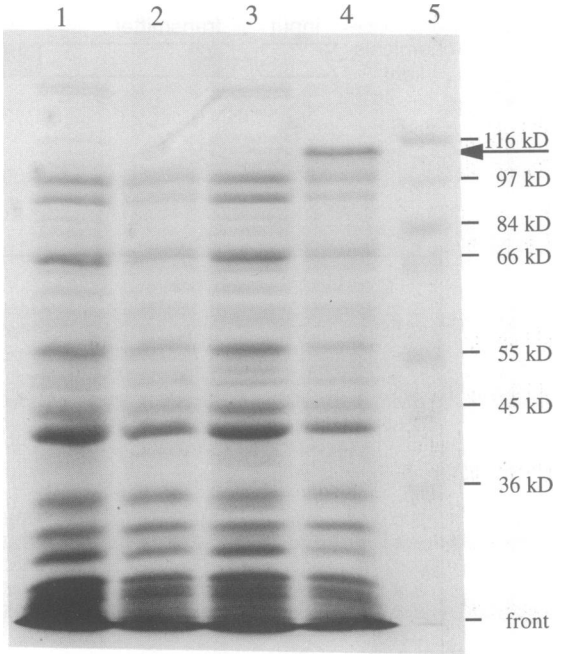


**Fig. 5.** Expression of $CheA_{H.s.}$ in *E.coli*. Each lane contains 100 mg of protein from harvested cell samples which were subjected to 3 min of boiling in Laemmli buffer. Lanes 1 and 2 are from the control strain KO685 transformed with pT7-7 and pGP1-2. Lanes 3 and 4 are from the $CheA_{H.s.}$ expression strain HSA. Samples in lanes 1 and 3 were taken prior to induction, samples in lanes 2 and 4 after induction, as described in Materials and methods. The molecular weights are shown in lane 5, and the arrow indicates the position of $CheA_{H.s.}$.

The S9 cells ($n$ = 48) exhibited both CW and CCW rotation when unstimulated. Surprisingly, the E4 cells ($n$ = 519) also displayed both CW and CCW rotation, ~50% of each, although variable results were obtained from one culture to another. However, while the S9 cells were seen to switch their direction of rotation spontaneously every 30–90 s, prolonged observation (up to 30 min) of the E4 cells revealed no switching behavior. In addition, the repellants orange light off or blue light on induced switching within 2 s in all the S9 cells, thereby causing the cells to change their direction of rotation. No response to either stimulus could be detected in the E4 cells. The observations made in these tethering experiments demonstrate that the CheA deletion mutant E4 is incapable of spontaneous as well as phototactic signal-induced switching.

### *Expression of $CheA_{H.s.}$ in E.coli and H.salinarium*

Given the high level of homology between $CheA_{H.s.}$ and $CheA_{E.c.}$, $CheA_{H.s.}$ was expressed in *E.coli* to test whether it could complement a *cheA* deletion of *E.coli*. $CheA_{H.s.}$ was expressed in the *E.coli cheA* deletion strain KO685 (Hess *et al.*, 1987) using the pT7-7 expression system (Tabor and Richardson, 1985) to create the strain HSA. Although the expected molecular weight of the protein is 72 kDa, heat induction led to the appearance of a protein band migrating at 103 kDa (Figure 5). In order to confirm the expression of the correct protein, the first five residues were identified by N-terminal sequencing and found to be M-D-D-Y-L, in agreement with the sequence expected from the nucleotide sequence (Figure 2). Such spurious migratory properties on SDS–PAGE are not uncommon

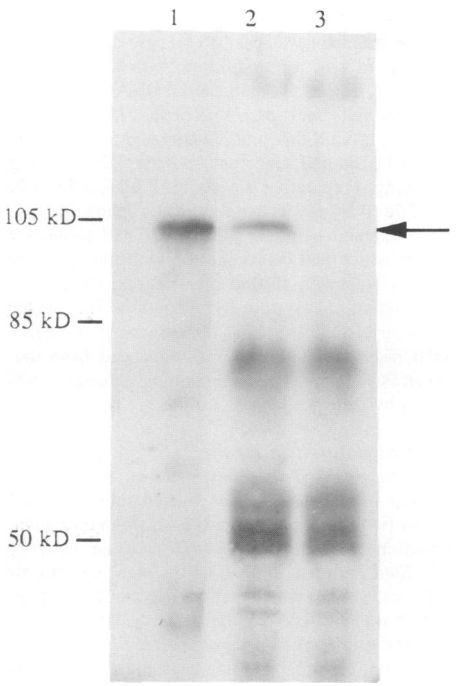


**Fig. 6.** Western blot showing expression of $CheA_{H.s.}$ in *E.coli* and *H.salinarium*. Each lane contains protein from harvested cell samples subjected to 3 min of boiling in Laemmli buffer. Lane 1 (5 mg) is from the *E.coli* $CheA_{H.s.}$ expression strain HSA. Lane 2 (100 mg) is from the wild-type *H.salinarium* strain S9. Lane 3 (100 mg) is from the halobacterial $cheA_{H.s.}$ deletion strain E4. The molecular weights indicated are derived from an overlay with the prestained protein markers from Bio-Rad and the arrow indicates the position of $CheA_{H.s.}$.

in halobacterial proteins and are attributed to the acidic nature of these proteins (Yao and Spudich, 1992).

The chemotactic properties of strain HSA were then examined by swarm plate assays and tethering experiments. The swarming behavior of HSA cells at 30°C and 37°C was indistinguishable from cells of the control deletion strain transformed only with pT7-7 (not shown). Tethered HSA cells, either induced or uninduced, were incapable of switching, and exhibited mainly CCW rotation, as expected for a CheA deletion in *E.coli* (Parkinson, 1976). Therefore, $CheA_{H.s.}$ is incapable of complementing the *E.coli* deletion, either because of its inability to interact functionally with the *E.coli* components of chemotaxis or because of its incorrect folding in the low salt environment of *E.coli* (e.g. formation of inclusion bodies containing denatured protein).

The *E.coli*-expressed $CheA_{H.s.}$ was also used to generate antibodies which were then used to test the expression of $CheA_{H.s.}$ in extracts of *H.salinarium*. Although not completely specific, the antibodies clearly identified a signal migrating at the same apparent molecular weight as the *E.coli*-expressed $CheA_{H.s.}$ (Figure 6). This band is not present in extracts from the deletion strain E4 (Figure 6), confirming the deletion of $CheA_{H.s.}$ in this strain.

## Discussion

The loss of both chemo- and phototaxis upon deletion of $cheA_{H.s.}$ indicates that $CheA_{H.s.}$ is responsible for signal integration in *H.salinarium*. Based on the work in *E.coli* (Gegner *et al.*, 1992; Schuster *et al.*, 1993) one can propose a model where a complex is formed between CheA, the various chemo- and phototactic transducers, and other possible components (e.g. CheY and CheW). The binding of ligand or the activation by light modulates the kinase activity of CheA as well as the binding of CheY. Thus the total cellular activity of CheY phosphorylation by CheA acts to integrate the signal for a single response. The observed signal integration therefore leads us to make several predictions. First, there is only one pathway for light and chemical signal transduction. This must also include the phototactic behavior mediated by BR since no switching was observed in the $CheA_{H.s.}$ deletion strain under conditions where the light conditions were intense enough to trigger this effect (Bibikov *et al.*, 1993). Whether $CheA_{H.s.}$ also mediates aerotaxis or thermotaxis, both of which have also been observed in *H.salinarium* (Bibikov and Skulachev, 1989), remains to be seen. Second, the as yet poorly characterized halobacterial chemoreceptors, of which there are at least three (Alam and Hazelbauer, 1991), must contain signaling domains which are structurally homologous to those known from eubacteria and from halobacterial HtrI for a single CheA to interact functionally with all of them. This also applies to the signaling domain of HtrII from *H.salinarium*, an expectation supported by the signaling domain sequence revealed by the recent cloning of HtrII from *Haloarcula vallismortis* and *Natronobacter pharaonis* (Seidel *et al.*, 1995).

The lack of apparent bias seen in the rotation of tethered cells with a $cheA_{H.s.}$ deletion is in contrast to the bias seen in the *cheA* deletions of *E.coli* and *B.subtilis*, where a strong CCW bias (>95%) and CW bias (88%) are observed, respectively (Parkinson, 1976; Fuhrer and Ordal, 1991). This lack of rotational bias in halobacteria has been observed previously (Marwan and Oesterhelt, 1987), and is of particular interest in that halobacteria swim smoothly no matter which direction the flagellar motor turns, in contrast to both *E.coli* and *B.subtilis*. A four-state model has been proposed to account for the observed switching behavior of halobacterial cells (Marwan and Oesterhelt, 1987). In this model, CheA phosphorylation of CheY would promote a switching event, be it from CCW to CW or vice versa. In eubacteria, on the other hand, the binding of CheY-phosphate to the switch component FliM appears to mediate CW rotation, a transient state which is returned to default CCW rotation upon dephosporylation and release of CheY (Welch *et al.*, 1994). A simple two-state model, however, does not explain the observed influence of CheY on the duration of both the CW and CCW states, and therefore a four-state model has also been proposed for eubacteria (Kuo and Koshland, 1989). In *H.salinarium* the discovery of CheA (and the now-expected existence of CheY) along with its observed behavior necessitates an explanation in which both CW and CCW rotation are possible in the absence of CheY-phosphate. The elucidation of the similarities and differences in the molecular details of switching between eubacteria and halobacteria will require further investigations.

The high similarity between the eubacterial and the archaeal *H.salinarium* CheA sequences coupled with the recent evidence that fumarate plays a role in signal transduction in eubacteria as well as *H.salinarium* is also

intriguing. Fumarate was shown to be released from an intracellular store in *H.salinarium* upon stimulation of SRII by blue light (Montrone *et al.*, 1993). In *E.coli* and *S.typhimurium*, cell envelopes completely devoid of cytoplasmic components are capable of motor switching only in the presence of both CheY and fumarate (Barak and Eisenbach, 1992). This brings into question whether the observed release of fumarate is the result of a separate non-Che signaling pathway or whether it is also mediated by CheA. These data also raise the question of the cooperativity which apparently occurs between CheY and fumarate in bringing about a switching event. The exact roles of the histidine kinase $CheA_{H.s.}$, the putative halobacterial CheY analog, and fumarate in the signal transduction pathway of *H.salinarium* are under investigation.

In conclusion, we have identified and isolated the first histidine kinase of a two-component system from the kingdom Archaea. Although these histidine kinase transmitters are well known in eubacteria and have recently been shown to exist in eukaryotes as well, their existence in Archaeae had not previously been demonstrated. Given the increasing importance of the histidine kinase two-component system in eukaryotes (Swanson and Simon, 1994), the study of $CheA_{H.s.}$ in a system which is easily switched by light stimulation should provide further molecular details of this type of signal transduction.

## Materials and methods

### Materials

*Taq* and Vent polymerases were obtained from Perkin-Elmer Cetus and New England Biolabs, respectively. Protein molecular weight standards and Freund's complete and incomplete adjuvants were purchased from Sigma. XL-Blue cells and pBluescript were from Stratagene whereas pGEM-3 and shrimp alkaline phosphatase were from US Biochemical. All restriction endonucleases and DIG-UTP (digoxigenin-UTP) were purchased from Boehringer Mannheim. T4 DNA ligase and the 1 kb DNA molecular weight ladder were obtained from Gibco-BRL.

### Genomic amplification of a $cheA_{H.s.}$ fragment

Standard molecular biological methods were performed, if not otherwise indicated, according to Sambrook *et al.* (1989). The following degenerate primers to the N-block and G2-block region of CheA (see Figure 1) were designed and synthesized using the codon usage tables for halobacteria (Soppa, 1994b): tca ggt acc CAC CTS VTS CGS AAC and cta gga tcc CGY TTS ACS ACG TCC ATC CC. Specific restriction sites for *Asp*718I and *Bam*HI were incorporated at the ends of the N-block and G2-block primers, respectively. These primers were used to amplify by PCR genomic DNA from halobacterial strain D2 as isolated by the method of Rosenshine *et al.* (1987). Standard reaction conditions contained 10 mM Tris−HCl (pH 8.3 at 25°C), 50 mM KCl, 0.15 mM $MgCl_2$, 0.001% gelatin, 0.1 mM dNTPs, 50 ng of template DNA, 0.5 μM of each primer and 5 U *Taq* polymerase. Cycling conditions on a MiniCycler (MJ Research) were 45 s at 95°C, 45 s at 37°C, ramp to 72°C at 0.2°C/s, 2 min at 72°C (5×) followed by 45 s at 95°C, 45 s at 52°C, 2 min at 72°C (30×). The 270 bp band, as visualized on a 2% agarose gel by ethidium bromide staining, was isolated using a Prep-A-Gene kit (Bio-Rad). Re-amplification of the DNA in this band under the same reaction conditions using the same primers on a DNA Thermal Cycler (Perkin-Elmer Cetus) with the cycling conditions 30 s at 96°C, 30 s at 50°C, 2 min at 72°C (30×) generated sufficient quantities of DNA for cloning of the fragment into pGEM-3 following digestion of the fragment and the vector by *Asp*713I and *Bam*HI. Six of the 10 clones sequenced by the dideoxy chain termination method (Applied Biosystems) contained an identical sequence showing significant homology to the sequences of the known CheAs in the region between the N-block and the G2-block, in particular the G1-block and the F-block (see Figure 1). There was no evidence for any additional *cheA*-like products.

### Isolation of the $cheA_{H.s.}$ gene

The cloned PCR fragment was used to prepare a DIG-labeled probe by PCR under standard reaction conditions with the addition of 35 μM DIG-UTP. Genomic digests of halobacterial DNA with various restriction enzymes (*Pst*I, *Bam*HI, *Sac*I, *Asp*718I, *Eco*RI, *Hin*dIII, *Sph*I), followed by Southern blotting with this probe, generated a single signal for each digest. The 1.6 kb *Bam*HI fragment and the 3.2 *Sac*I fragment were chosen for cloning. Preparative digests (125 μg DNA) of halobacterial DNA were followed by 1% agarose gel electrophoresis and isolation of the correct size fragment using Prep-A-Gene. The fragments were ligated into pBluescript which had been digested with the appropriate enzyme and dephosphorylated with shrimp alkaline phosphatase. Clones were screened by standard colony blot hybridization. The plasmid DNA of positive clones, pJR1626 for the 3.2 kb *Sac*I insert and pJR3190 for the 1.9 kb *Bam*HI insert, was isolated and sequenced from the vector and from the known PCR fragment. The remaining sequence, on both strands, was determined by gene walking.

### Construction of a deletion strain

The deletion vector pJR3557 was constructed as follows. First, the 1.9 kb *Bam*HI fragment from pJR3190 was ligated into the *Bam*HI-digested vector pJR1626 (with removal of the 0.7 kb fragment) and checked for proper orientation to generate pJR4816 and an intact $cheA_{H.s.}$. A 2.9 kb *Pvu*II−*Sph*I fragment containing the $mev^R$ cassette from pJR100 (unpublished construction) which confers mevinolin resistance (Lam and Doolittle, 1992) was ligated into the vector pJR4816 which had been digested with *Stu*I and *Sph*I to generate pJR3557.

Transformation of pJR3557 into halobacterial strain S9 was performed as described by Cline *et al.* (1989). Clones were initially screened by colony hybridization using a probe to the deleted region. Colonies with weak signals compared with wild-type were further screened by Southern blotting following a *Bam*HI digest of genomic DNA using probes to both the deleted region and the upstream region which was retained.

### Analysis of chemotactic behavior

The control strain S9 and the deletion strain E4 were tested for their ability to perform chemotaxis on peptone swarm plates (0.25% agar). Equal cell numbers ($5\times10^5$) were applied just below the surface to the center of each plate and allowed to grow at 37°C for 3–4 days. The plates (three or four replicates) were then evaluated by both circle diameter and formation of chemotactic rings.

### Analysis of phototactic behavior

The control strain S9 was compared with its deletion strain E4 for its ability to respond to light signals by motion analysis as described by Marwan *et al.* (1990), except that the cells were not selected on swarm plates prior to motion analysis as this would lead to a biased comparison versus the non-swarming E4 strain. The cells, after 4 days of growth at 100 r.p.m., 40°C, were suspended in normal growth medium plus 0.1% (w/v) arginine (pH 7.5). Measurements were made at 40°C using infrared light for observation. The percent reversal in a 4 s interval was determined following either no stimulation (spontaneous reversal), a step down in orange light (100 W mercury lamp off, OG 570 nm filter), or a step up in blue light with a constant orange background (removal of orange filter from mercury lamp) using computer assisted motion analysis. Cells were allowed to readapt to orange light for 30 s between measurements.

### Tethering of halobacterial cells

Following 4 days of growth at 100 r.p.m., 40°C, 1 ml of cells was vortexed in the presence of 1 g of glass beads (125–200 μm, Serva) for 2 min. After allowing the glass beads to settle for 3 min, an aliquot of the cells was placed under a raised coverslip on a slide which then was incubated upside down for 30 min at room temperature. The untethered cells were removed by gentle aspiration of the liquid from the chamber and fresh medium was added. Following incubation at 40°C for 10 min, the cells were examined by infrared microscopy. Light repellants were applied as described for the motion analysis.

### Expression of $CheA_{H.s.}$ in E.coli strain KO685

The $CheA_{H.s.}$ expression strain HSA was constructed as follows. First, a 0.9 kb fragment of $cheA_{H.s.}$ in pJR4816 was amplified by standard PCR with Vent polymerase using the primers GAC GAA TTC ATA TGG ACG ACT ACC TCG AAG CG and AGC TCG TCG AGC TGA TCG. The first of these introduces an *Eco*RI site at the 5′-end of the fragment and an *Nde*I site at the start codon of the gene. Digestion of both pJR4816 and the PCR product with *Eco*RI and *Stu*I, followed by ligation of the 0.5 kb fragment into the 6.8 kb vector, yielded the

intermediary plasmid pJR47x1. The amplified region was resequenced to ensure that no errors had been incorporated. Digestion of pJR47x1 with *Nde*I and *Pvu*II and of pT7-7 (Tabor and Richardson, 1985) with *Nde*I and *Sma*I, followed by ligation of the 2.2 kb fragment into the 2.4 kb vector yielded the expression vector pJR4717. Sequential transformation of the helper plasmid pGP1-2 and pJR4717 into the *E.coli cheA*-negative strain yielded the λpL inducible expression system for $CheA_{H.s.}$ denoted HSA.

The chemotactic behavior of *E.coli* was examined by peptone swarm plate (0.2%) assays and by tethering experiments as described by Parkinson (1976).

Induction of $CheA_{H.s.}$ was performed as follows. Strain HSA was grown in Luria broth containing 50 μg/ml ampicillin and 50 μg/ml kanamycin at 30°C (doubling time = 1.1 h) until the $OD_{600}$ was 0.5. Following a heat shock of 20 min at 42°C and the addition of 200 mg/ml rifampicin, the cells were shaken at 37°C for an additional 2 h. Inductions were followed by SDS−PAGE using Coomassie staining.

Antibodies to $CheA_{H.s.}$ were generated by subcutaneous injection of SDS−PAGE slices containing 50 μg of protein mixed with Freund's complete adjuvant into a Chinchilla bastard rabbit (Johnstone and Thorpe, 1987). After 4 weeks, booster shots were given containing the same amount of protein mixed with Freund's incomplete adjuvant. The rabbit was bled 10 days after the booster shots. After clotting and centrifugation the serum was collected and used without further purification. Western blotting following SDS−PAGE was performed by standard procedures using a semi-dry blotting apparatus (Hölzel), Towbin buffer (Towbin *et al.*, 1979), a 1:2000 dilution of antibody, and the ECL detection kit from Amersham.

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