Transcriptional Organization of a Cloned Chemotaxis Locus of *Bacillus subtilis*

AAMIR R. ZUBERI, CHINGWEN YING, MICHAEL R. WEINREICH,[†] AND GEORGE W. ORDAL*

Department of Biochemistry, Colleges of Medicine and of Liberal Arts and Sciences,
University of Illinois, Urbana, Illinois 61820

Received 18 October 1989/Accepted 29 December 1989

A cloned chemotaxis operon has been characterized. Thirteen representative *che* mutations from different complementation groups were localized on the physical map by recombination experiments. The use of integration plasmids established that at least 10 of these complementation groups within this locus are cotranscribed. An additional three complementation groups may form part of the same transcript. The direction of transcription and the time of expression were determined from chromosomal *che-lacZ* gene fusions. The promoter was cloned and localized to a 3-kilobase fragment. Expression of β -galactosidase from this promoter was observed primarily during the logarithmic phase of growth. Three-factor PBS1 cotransduction experiments were performed to order the *che* locus with respect to adjacent markers. The *cheF141* mutation is 70 to 80% linked to *pyrD1*. This linkage is different from that reported previously (G. W. Ordal, D. O. Nettleton, and J. A. Hoch, *J. Bacteriol.* 154:1088-1097, 1983). The *cheM127* mutation is 57% linked by transformation to *spcB3*. The gene order determined from all crosses is *pyrD-cheF-cheM-spcB*.

Substantial recent progress has been made in understanding how chemotaxis might function in *Escherichia coli*. Six proteins are required for chemotaxis. These are encoded by the *cheA*, *cheW*, *cheZ*, *cheY*, *cheR*, and *cheB* genes (reviewed in reference 15). Chemotaxis appears to be controlled at the transcriptional level by an alternate sigma factor (2) and at the posttranslational level by both methylation and phosphorylation reactions (3, 23).

The chemotactic mechanism appears more complex in *Bacillus subtilis* than in *E. coli*. In both organisms, methyl groups are transferred from *S*-adenosylmethionine (AdoMet) to the methyl-accepting chemotaxis proteins (9, 26). In *E. coli* these groups are then directly released as methanol, but in *B. subtilis* reversible transfer occurs to other carriers before these groups are eventually released (24). Addition of attractant causes an increased flux of methyl groups through *B. subtilis* methyl-accepting chemotaxis proteins, but addition of repellent does not (24a, 25). However, methanol is evolved at a high rate in both cases until the cells have adapted to the stimulus (24).

To more fully understand how chemotaxis might function in *B. subtilis*, we isolated mutants that are defective in chemotaxis. At least 21 different complementation groups have been identified (19, 20). All mutants are defective in taxis to all attractants or repellents tested, and we have been unable to deduce likely roles based on a characterization of the mutant phenotype. The one exception has been the characterization of *cheR* methyltransferase mutants of *B. subtilis* (5). One approach we are taking is to clone and sequence chemotaxis genes so that we may possibly identify function through homology with other known proteins, or by direct experimentation on purified proteins.

Genetic experiments using the flagellum-specific generalized transducing bacteriophage PBS1 established that the *che* mutations, with the exception of *cheR*, map to a single locus on the *B. subtilis* genetic map (19). Part of this locus

has been cloned on two λ transducing phages, and 13 complementation groups have been identified (20). We have previously reported the nucleotide sequence and characterization of the *B. subtilis cheF* gene (30; GenBank/EMBL accession number, M24528). The *cheF* gene is located within this locus. Some *che* mutants are not complemented by this DNA, suggesting that *che* genes are also present on neighboring DNA fragments.

In this paper we describe the characterization of the cloned *che* locus.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* host for plasmid transformation was JM103 (17) or TG1 (Amersham Corp.). When the plasmid carried the *erm* gene, *E. coli* HB101 (4) was used as the host, since these plasmids are unstable in other strains (31).

B. subtilis OI1085 (*hisH2 metC trpF7*) has been used as a chemotactic wild type for previous biochemical and genetic studies (26). The *che* mutants used are listed in Table 1. They were all derived from mutagenesis of OI1085. The *spcB3* mutation confers resistance to spectinomycin.

Plasmids. *B. subtilis* DNA was subcloned from λ -14.9 and λ -11.7 phages (19) into pUC18. Five derivative plasmids were subsequently recovered; they are represented diagrammatically in Fig. 1. Plasmids pGO101, pGO103, and pGO104 contain the 7.7-, 4.0-, and 10.9-kilobase (kb) *EcoRI* fragments of cloned DNA, respectively. Plasmid pAZ202 contains a 4.4-kb *BamHI* fragment from λ -14.9, and plasmid pAZ203 contains a 5-kb *PstI* fragment from λ -11.7.

Integration analysis was performed by subcloning DNA fragments from these plasmids into one of four integration plasmids. The plasmid pJH101 has been described previously (8). Other integration plasmids used were pBGSC6 (*Bacillus* Genetic Stock Center strain ECE22), pAZ100, and pAZ101. The last two plasmids are derived from pAAM74 (33). A nonessential 1.2-kb *EcoRI-XhoI* fragment was deleted from pAAM74 to generate pAZ100. pAZ101 was constructed by insertion of an *EcoRI* linker at the position of the former *BamHI* site of pAZ100. Both the *BamHI* site of

* Corresponding author.

[†] Present address: Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

TABLE 1. *B. subtilis* strains used

Strain	Genotype	Source or reference
M104	<i>cheE104 metC trpF7 hisH2</i>	20
M124	<i>cheG124 metC trpF7 hisH2</i>	20
M127	<i>cheM127 metC trpF7 hisH2</i>	20
M128	<i>cheC128 metC trpF7 hisH2</i>	20
M137	<i>cheK137 metC trpF7 hisH2</i>	20
M141	<i>cheF141 metC trpF7 hisH2</i>	20
M152	<i>cheJ152 metC trpF7 hisH2</i>	20
NI17	<i>cheA17 metC trpF7 hisH2</i>	20
NI30	<i>cheI30 metC trpF7 hisH2</i>	20
NI32	<i>cheD32 metC trpF7 hisH2</i>	20
NI37	<i>cheB37 metC trpF7 hisH2</i>	20
OI1088	<i>cheN1088 metC trpF7 hisH2</i>	20
OI1130	<i>cheL1130 metC trpF7 hisH2</i>	20
OI1137	<i>cheO1137 metC trpF7 hisH2</i>	20
OI1498	<i>pyrD1 leu spcB3</i>	This work
OI1592	<i>cheH103 metC trpF7 hisH2</i>	20
OI2373	OI1498(pCY706), Cm ^r	This work
OI2396	OI1085(pAZ226), MLS ^r	This work
OS112	<i>spcB3 str-1</i>	J. Hoch
1A6	<i>ilvA1 pyrD1 thyA1 thyB1 trpC2</i>	BGSC ^a

^a *Bacillus* Genetic Stock Center.

pAZ100 and the *EcoRI* site of pAZ101 are located close to the 5' end of the *lacZ* gene. The *lacZ* gene can be expressed in *B. subtilis* because it has the ribosome-binding site of the *spoVG* gene (32).

All plasmids were maintained in *E. coli* by selection for ampicillin resistance (Ap^r). In addition, plasmids pJH101 and pBGSC6 confer chloramphenicol resistance (Cm^r) and plasmids pAZ100 and pAZ101 confer resistance to the macrolide, lincosamide, and streptogramin B class of antibiotics (MLS^r). Other plasmids used are described below.

Media. L broth and L agar (18), supplemented with 0.1% glucose, were used for the growth of *E. coli*. Antibiotic resistance was selected by the addition to agar of ampicillin

to 50 µg/ml, tetracycline to 5 µg/ml, chloramphenicol to 5 µg/ml, and spectinomycin to 100 µg/ml. MLS^r was selected by the simultaneous presence of 1 µg of erythromycin per ml and 25 µg of lincomycin per ml.

B. subtilis strains were normally grown on tryptose blood agar base (Difco Laboratories) plates. Penassay broth (Difco) was used to grow cells for phage PBS1 transduction experiments. Cultures for β-galactosidase assays were induced to sporulate by growth in 2× SG medium (14). *pyrD*⁺ transformants were selected on appropriately supplemented minimal medium (1). All cultures were incubated at 37°C.

Transformation procedures. Plasmid transformation of RbCl-induced competent *E. coli* was carried out as described by Hanahan (10). Transformation of competent *B. subtilis* cells with chromosomal and plasmid DNA was carried out as described by Davis et al. (6) or by Warburg and Moir (28).

DNA manipulations and enzymes. Plasmid isolation, restriction enzyme digestion, fragment purification by electroelution, ligation, and Southern hybridizations on nitrocellulose were all performed by standard procedures (16). DNA probes were labeled by nick translation with a kit obtained from Bethesda Research Laboratories, Inc., and [³²P]dCTP purchased from ICN Radiochemicals.

Scoring the chemotaxis phenotype. The chemotaxis phenotype was scored as described previously (19, 30). Strains were tested on both tryptone broth and mannitol (0.1 mM) swarm plates. An OI1085 Che⁺ control was always included. When appropriate, chloramphenicol or erythromycin plus lincomycin were added to the swarm plates to prevent plasmid segregation in *B. subtilis* transformants. In these cases OI1085 that had been transformed to Cm^r with pSI-1 (29) or to MLS^r with chromosomal DNA from a Che⁺ Tn917-containing strain (G. W. Ordal, unpublished data) was used as the positive control.

For experiments to determine the location of *che* mutations by recombination, linear DNA restriction fragments were added to competent *B. subtilis che* mutants. The transformation mix was spotted directly onto a mannitol

FIG. 1. Extent of cloned *B. subtilis* DNA present on phages and plasmids. A restriction map of the cloned *che* locus is shown. The sizes of the three *EcoRI* fragments are indicated. Horizontal lines beneath the map represent the extent of DNA carried by each of two λ phages (λ-14.9 and λ-11.7). Also shown are the restriction fragments subcloned into pUC18 to generate the five derivative plasmids listed.

swarm plate. After overnight incubation, swarm rings characteristic of *Che*⁺ bacteria were observed moving away from the cells at the point of inoculation for some combinations of mutant and restriction fragment DNA. Controls lacking cloned DNA were included to take into account the possibility of reversion.

The gene order within the 4.0-kb *Eco*RI fragment was established after unidirectional deletions of the cloned DNA were generated in M13mp18 (11). Linearized M13 replicative-form DNAs from specific deletion derivatives were introduced into competent *B. subtilis che* mutants and spotted onto swarm plates as described above.

Preparation of cell extracts. Cultures were grown in 2× SG medium in the presence of antibiotic selection as necessary. Growth was monitored at 525 nm. At hourly intervals after the cultures had reached midexponential phase, 10-ml samples were collected and chilled to 4°C, and the cells were pelleted by centrifugation. The cells were washed twice in 50 mM sodium phosphate buffer (pH 7.0), and the pellet was stored overnight at -70°C.

The following day, cells were suspended in 1 ml of the same buffer. Lysozyme was added to a final concentration of 200 µg/ml, and the cells were incubated on ice for 30 min. They were then broken by ultrasonic treatment. The cell debris were removed by centrifugation, and the cell extract was assayed immediately for β-galactosidase activity (18).

The total protein concentration was determined by using a protein assay reagent (Pierce Chemical Co.) with bovine serum albumin as the standard.

PBS1 transductions. The PBS1 transduction procedure has been described before (19). Transductants were purified once by single-colony isolation because the presence of the phage on the original selective plates interferes with the swarm phenotype.

RESULTS

Localization of *che* mutations on the cloned DNA. Previous genetic studies have established that the cloned DNA present on λ-14.9 and λ-11.7 can complement many different chemotaxis mutants (20). The 7.7-kb *Eco*RI fragment was shown to complement the *cheE*, *cheF*, *cheG*, and *cheH* mutants. The 4.0-kb *Eco*RI fragment can complement the *cheA*, *cheB*, *cheC*, and *cheD* mutants. The 10.9-kb *Eco*RI fragment complements the *cheL*, *cheM*, *cheN*, and *cheO* mutants. In addition, it was also reported that the *cheI*, *cheJ*, and *cheK* mutants can be complemented only by a combination of the 7.7- and 4.0-kb *Eco*RI fragments. We sought to further localize these *che* genes on the cloned DNA by recombination experiments.

The data obtained are summarized in Fig. 2. Mutations within the 7.7- and 10.9-kb *Eco*RI fragments were localized to specific *Pst*I or *Pst*I-*Eco*RI fragments. We have previously reported that the 0.7-kb *Pst*I fragment of pGO101 carries the *cheF* gene (30). The *cheO1137* mutation was repaired by the 10.9-kb *Eco*RI fragment, but it could not be further localized by using *Pst*I or *Eco*RI-*Pst*I subclones (see the Discussion). The relative positions of the four mutations mapping to the 4.0-kb *Eco*RI fragment (Fig. 2) were determined by using specific deletion derivatives generated as described in Materials and Methods. The data obtained are shown in Fig. 3. The gene order was found to be *cheD-cheB-cheA-cheC*.

Determination of operon structure. Integration plasmids have been used extensively to characterize transcription units on the *B. subtilis* chromosome (21). Integration plas-

FIG. 2. Localization of representative *che* mutations. A physical map of the DNA is shown. The relative location of each mutation was determined by recombination, as described in the text. Lines below the restriction map correspond to the region of DNA that can repair the named mutation. The sizes (in kilobases) of the *Eco*RI fragments are indicated.

mids replicate in *E. coli* but not in *B. subtilis*; consequently, they can transform *B. subtilis* only if the plasmid contains cloned DNA. The whole plasmid then integrates into the chromosome at the region of homology.

Fragments of cloned *B. subtilis* DNA were subcloned into one of the integration plasmids pJH101, pBGSC6, pAZ100, or pAZ101. The plasmids (Fig. 4) were introduced into competent OI1085 cells, and the *Che* phenotype was scored. If the plasmid carries cloned DNA that is internal to a transcription unit, upon integration into the chromosome that transcription unit is disrupted and the transformant possesses a mutant phenotype (in our case, *Che*⁻). If, on the other hand, the plasmid contains at least one end of a transcription unit, that transcription unit is regenerated following integration. This results in a transformant that is phenotypically wild type.

All of the *che* genes located within the 7.7-, 4.0-, and 10.9-kb *Eco*RI fragments appeared to lie within a single transcription unit, since all integration plasmids conferred a *Che*⁻ phenotype (Fig. 4). To verify that integration had occurred via a single crossover event, we analyzed the

FIG. 3. Determination of gene order in the 4.0-kb *Eco*RI fragment. The ability of the 4.0-kb *Eco*RI fragment and deletion derivatives to repair four *che* mutants is shown. Symbols: +, the fragment was able to repair the corresponding mutation by recombination; -, no repair was observed. The orientation of the fragment with respect to neighboring DNA can be deduced from the position of the *Bam*HI site.

FIG. 4. Insertional inactivation of the *che* transcription unit by use of integrational plasmids. A restriction map of the *che* locus is shown. Below the map are listed named integration plasmids and the extent of cloned DNA they contain. The vectors used are pJH101 (A), pBGSC6 (B), pAZ100 (C), and pAZ101 (D). All plasmids listed confer a *Che*⁻ phenotype in *B. subtilis*.

chromosomal DNA from a representative transformant from each cross on a Southern blot. *EcoRI*-digested chromosomal DNA was probed with pGO101, pGO103, or pGO104 as appropriate. For all cases except one, integration appeared to have occurred as expected (data not shown). The one cross in which the Southern blot showed an unexpected pattern was in pAZ235-containing transformants. This plasmid contains a 3.8-kb *PstI* fragment subcloned into pJH101 (Fig. 4). The Southern blot showed the presence of three instead of two chromosomal fragments hybridizing to pGO104. There appears to have been a duplication event that occurred upon integration. The 3.8-kb *PstI* fragment was subcloned into plasmid pBGSC6, the derivative plasmid was introduced into OI1085, and the Southern blot was repeated. The same apparent duplication event was observed. Because of this abnormal integration, we are unable to ascertain whether the transcription unit extends through this region of DNA.

The direction of transcription of this operon was determined after a transcriptional *che-lacZ* fusion was generated on the chromosome. The 4.0-kb *EcoRI* fragment was subcloned from pGO103 in both orientations into pAZ100. Each derivative plasmid confers a *Che*⁻ phenotype in OI1085, but transformants from only one cross could express β -galactosidase, as determined on plates containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal). From the orientation of insertion of the cloned 4.0-kb *EcoRI* fragment into the vector, we can infer that transcription in this *che* operon proceeds from the 7.7-kb *EcoRI* fragment through the 4.0-kb fragment and into the 10.9-kb fragment. Furthermore, from the results with the integration plasmids (Fig. 4), we can deduce that the promoter for this transcription unit is located upstream from the 7.7-kb *EcoRI* fragment. The terminator for this transcript may be located within the 3.8-kb *PstI* fragment present in pAZ235 (Fig. 4) or termination may occur downstream of the 10.9-kb *EcoRI* fragment. We have established that the direction of transcription at the distal end of the 10.9-kb *EcoRI* fragment is the same as in the 4.0-kb *EcoRI* fragment (data not shown). The integration

plasmid pCY706 (Fig. 4) contains DNA from the extreme promoter-distal end of the 10.9-kb *EcoRI* fragment and generates a *Che*⁻ phenotype upon integration. Since the integration of the plasmid occurred as predicted, this suggests that there are additional chemotaxis genes located immediately downstream of the 10.9-kb *EcoRI* fragment.

Cloning of the chemotaxis promoter. Chromosomal DNA from an OI1085 transformant containing pAZ205 (Fig. 4) was digested with *SstI*. A unique *SstI* site is present within pAZ205 in the *lacZ* gene. The DNA was religated at low DNA concentration and used to transform *E. coli* to Ap^r. A plasmid, designated pAZ210, was recovered that carried 5.7 kb of *B. subtilis* DNA (Fig. 5). Restriction analysis of the plasmid showed that we had cloned an additional 4.2-kb *EcoRI-SstI* fragment upstream from the 7.7-kb *EcoRI* fragment.

Because pAZ210 still contains the *erm* gene, it has all of the properties of an integration plasmid. OI1085 was transformed to MLS^r with pAZ210, and all six transformants tested were observed to be phenotypically *Che*⁺. We deduce that the 4.2-kb *EcoRI-SstI* fragment contains the promoter for the *che* operon.

The promoter was further localized. Two derivative subclones of pAZ210 were constructed, and the apparent phenotype conferred by each integration plasmid was determined in *B. subtilis*. Plasmid pAZ212 contains a 1.6-kb *BglII-HindIII* fragment subcloned in pJH101 and generates *Che*⁻ transformants in *B. subtilis*. Plasmid pAZ213 is a *BglII-BamHI* deletion of pAZ210, and *B. subtilis* transformants containing this plasmid are *Che*⁺. We deduced that the promoter is located within a 3-kb *BglII-SstI* fragment (Fig. 5). We verified the chromosomal integration event in each case by probing chromosomal DNA from one representative transformant from each cross with pAZ210 (data not shown).

Time of expression of the *che* operon. The 3-kb *BglII-SstI* fragment of pAZ210 was subcloned into the *BamHI* site of pAZ100 by use of DNA linkers. A plasmid, designated pAZ226, was recovered in which β -galactosidase expression was dependent upon transcription from the *che* promoter.

FIG. 5. Cloning and localization of the *che* promoter. The map is a linear representation of pAZ210. Symbols: □, location of the *bla* and *erm* genes present on the plasmid; ■, *B. subtilis* DNA. Relevant restriction sites are indicated. Stippled areas below the map indicate the extents of DNA present on integration plasmids as described in the text. The Che phenotype of *B. subtilis* transformants is shown.

OI1085 *MLS^r* transformants containing a single copy of pAZ226 on the chromosome are all phenotypically *Che⁺*, as expected, and all express β -galactosidase. One transformant was designated OI2396.

The time course of β -galactosidase expression in OI2396 was determined. Maximal accumulation of β -galactosidase occurred approximately 30 min before t_0 , the time at which exponential growth ceases (Fig. 6). This suggests that the *che* operon is expressed primarily during the logarithmic phase of growth.

Genetic mapping of the cloned *che* genes. Previous genetic studies (19) had shown that the cloned *che* genes are linked by PBS1 transduction to *pyrD* (34%) and *thyA* (10%). We sought to refine the genetic linkage by reexamining the linkage of *che* mutations to *pyrD* and to determine the linkage to *spcB3*. The implied gene order from three-factor crosses is *pyrD-cheF-spcB* (Table 2). The linkage of *cheF141* to *pyrD* (81%) is much higher than the 34% linkage previously reported (19). We have confirmed this apparent high linkage between *cheF141* and *pyrD* by repeating the transduction experiment with 1A6 as a recipient. This was the recipient used in the transduction experiments published earlier (19). In this cross we found that *cheF141* is 70.5% (72 of 102 transductants screened) linked to *pyrD*. This data changes the relative position of the *che* locus on the published genetic map (22).

Additional crosses were performed to determine how the different cloned *EcoRI* fragments were physically organized on the chromosome with respect to *pyrD*. pCY706 (Fig. 4) confers *Cm^r* when integrated into the genome of *B. subtilis*. These transformants are phenotypically *Che⁻* on tryptone broth swarm plates but *Che⁺* on mannitol swarm plates. The *CheF* mutant is *Che⁻* on both plates. Therefore, it is possible to score the chemotaxis defects due to either *cheF141* (*Che⁻*) or integration of pCY706 (*Cm^r*) separately. The implied gene/fragment order from a three-factor cross is *pyrD-cheF* (7.7-kb *EcoRI* fragment)-pCY706 (10.9-kb *EcoRI* fragment) (Table 2).

We confirmed this order by examining the cotransformation linkage between two *che* mutations and *spcB3*. M127

FIG. 6. Activity of β -galactosidase (○) expressed from a *che-lacZ* fusion during growth and sporulation. Strain OI2396 was induced to sporulate by nutrient exhaustion in 2 $\times$ SG medium. Growth was monitored as *A*₅₂₅ (●) and is shown on a logarithmic scale. The time at which exponential growth ceased was designated t_0 . The time t_n refers to n h before and after t_0 . The specific activity of β -galactosidase is expressed as nanomoles of *o*-nitrophenol produced per minute per milligram of protein (U/mg). The maximal endogenous β -galactosidase activity in OI1085 was 5 U/mg.

(*cheM127*) and M141 (*cheF141*) were transformed to spectinomycin resistance by OS112 (*spcB3*) chromosomal DNA, and the Che phenotype was scored. The data suggest that *cheM127* and *cheF141* are 57% (28 of 49) and 52% (47 of 90) linked, respectively, to *spcB3*. From the known locations of the two *che* mutations used (Fig. 2), and from the data shown in Table 2, we deduce that the 10.9-kb *EcoRI* fragment is located between *spcB* and the 7.7-kb *EcoRI* fragment. In separate experiments we have established that the 10.9-kb *EcoRI* fragment does not repair the *spcB3* mutation. The implied gene order from all of our crosses is *spcB-cheM-cheF-pyrD*.

TABLE 2. Determination of gene order by PBS1 transduction

Expt	Recombinant phenotype ^a				Implied order
	Pyr	Che	Spc/Cm ^b	No.	
1 ^c	D	D	D	66	<i>pyrD-cheF-spcB</i>
	D	D	R	11	
	D	R	D	1	
	D	R	R	17	
2 ^d	D	R	R	48	<i>pyrD-cheF-Cm^r</i>
	D	R	D	0	
	D	D	R	3	
	D	D	D	51	

^a D, Donor phenotype; R, recipient phenotype.

^b Spc for experiment 1; Cm for experiment 2.

^c The donor was M141 (*cheF141*), and the recipient was OI1498 (*pyrD1 spcB3*).

^d The donor was M141 (*cheF141*), and the recipient was OI2373 (*spcB3 pyrD1 Cm^r*). The *Cm^r* was due to the presence of the integration plasmid pCY706 on the chromosome.

DISCUSSION

We have characterized a chemotaxis operon of *B. subtilis*. Evidence from the use of integration plasmids and recombination experiments shows that at least 10 chemotaxis genes are cotranscribed. Another two chemotaxis genes (*cheL* and *cheN*) may also be part of the same transcript. We are unable to confirm this at present because of the observation that plasmid pAZ235 did not integrate into the *che* locus as predicted from a single crossover recombination event.

The *cheO* mutation must be located close to one of the *Pst*I sites within the 10.9-kb *Eco*RI fragment. None of the *Pst*I or *Eco*RI-*Pst*I fragments from pGO104 are able to confer a Che⁺ phenotype upon OI1137 in recombination experiments, even though pGO104 could repair the mutation when used directly.

The *cheI*, *cheJ*, and *cheK* mutants can be complemented only when both the 7.7- and 4.0-kb *Eco*RI fragments are present together (20). The three mutations lie within different complementation groups, suggesting different genes. The *cheI* and *cheJ* mutations are both located within the 7.7-kb *Eco*RI fragment. We were unable to determine the location of the *cheK* mutation. The M137 mutant was found to revert to Che⁺ at a high rate that precluded the physical mapping of the corresponding mutation within the cloned DNA. It is likely that all three mutations map to the same gene; the apparent complementation reported earlier (20) may be due to intracistronic complementation.

In general, integration plasmid insertions into the *che* operon lead to very defective swarm phenotypes on both mannitol and tryptone broth swarm plates. However, integration of pCY706 into the distal end of the 10.9-kb *Eco*RI fragment generates transformants that are Che⁺ on mannitol swarm plates. They are still phenotypically Che⁻ on tryptone broth swarm plates. This suggests that proteins expressed from genes downstream of the 10.9-kb *Eco*RI fragment are utilized primarily in response to amino acid taxis and not to sugar (mannitol) taxis.

Preliminary results suggest the presence of internal promoters located within the *che* operon. One of these is likely to be regulated by SigD-containing RNA polymerase and has been mapped to the 4.0-kb *Eco*RI fragment (M. Chamberlin, unpublished results). Our results suggest that these internal promoters are not able to complement the mutant phenotype generated as a result of insertional plasmid mutagenesis. The major promoter present on pAZ210 is required for normal expression of the *che* operon. We are attempting to characterize these minor promoters further. The pattern of β -galactosidase expression observed from OI2396 suggests that the major promoter is expressed in vegetative cells and that no net accumulation of β -galactosidase occurs after t_0 . Transcription from this promoter is likely to cease after the cells reach late-log phase and is not dependent upon the product of the *sigD* gene (C. Ying, unpublished results). This pattern of expression is commonly found in promoters that are regulated by the major vegetative SigA-containing form of RNA polymerase (27).

All attempts to directly visualize the RNA encoded by the *che* locus have proved unsuccessful. The predicted RNA is quite long (at least 15 kb), and the specific activity of β -galactosidase that we observed from OI2396 suggests that the level of expression is moderate under the conditions used. Both of these factors would make it extremely difficult to characterize the RNA directly. Other long RNA transcripts have also been found in vegetative cells of *B. subtilis* (7, 12, 13).

We are presently studying the regulation of the *che* operon characterized here and are sequencing the chemotaxis genes.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI20336 from the National Institutes of Health and National Science Foundation grant DCB85-01604.

We thank David Bischoff and Tamma Kaysser for helpful comments during the preparation of the manuscript. Douglas Fuhrer contributed some restriction data. Plasmids pAZ100 and pAZ101 were constructed by A.R.Z. in the laboratory of R. H. Doi.

LITERATURE CITED

1. Anagnostopoulos, C., and J. Spizizen. 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. 81:741-746.
2. Arnosti, D. N., and M. J. Chamberlin. 1989. Secondary σ factor controls the transcription of flagellar and chemotaxis genes in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 86:830-834.
3. Bournett, R. B., J. F. Hess, K. A. Borkovich, A. A. Pakula, and M. I. Simon. 1989. Protein phosphorylation in chemotaxis and two-component regulatory systems of bacteria. J. Biol. Chem. 264:7085-7088.
4. Boyer, H. W., and D. Rolland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. 41:459-472.
5. Burgess-Cassler, A., and G. W. Ordal. 1982. Functional homology of *Bacillus subtilis* methyltransferase II and *Escherichia coli* *cheR* protein. J. Biol. Chem. 257:12835-12838.
6. Davis, R. W., D. Botstein, and J. R. Roth (ed.). 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. Ebbole, D. J., and H. Zalkin. 1987. Cloning and characterization of a 12-gene cluster from *Bacillus subtilis* encoding nine enzymes for *de novo* purine nucleotide synthesis. J. Biol. Chem. 262:8274-8287.
8. Ferrari, F. A., A. Nguyen, D. Lang, and J. A. Hoch. 1983. Construction and properties of an integrable plasmid for *Bacillus subtilis*. J. Bacteriol. 154:1513-1515.
9. Goldman, D. J., S. W. Worobec, R. B. Siegel, R. V. Hecker, and G. W. Ordal. 1982. Chemotaxis in *Bacillus subtilis*: effects of attractants on the level of methylation of methyl-accepting chemotaxis proteins and the role of demethylation in the adaptation process. Biochemistry 21:915-920.
10. Hanahan, D. 1985. Techniques for transformation of *E. coli*, p. 109-135. In D. M. Glover (ed.), DNA cloning, vol. 1. IRL Press, Washington, D.C.
11. Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28: 351-359.
12. Henner, D. J., L. Band, G. Flaggs, and E. Chen. 1986. The organization and nucleotide sequence of the *Bacillus subtilis* *hisH*, *tyrA* and *aroE* genes. Gene 49:147-152.
13. Henner, D. J., L. Band, and H. Shimotsu. 1984. Nucleotide sequence of the *Bacillus subtilis* tryptophan operon. Gene 34: 169-177.
14. Leighton, T. J., and R. H. Doi. 1971. The stability of messenger ribonucleic acid during sporulation in *Bacillus subtilis*. J. Biol. Chem. 246:3189-3195.
15. Macnab, R. M. 1987. Motility and chemotaxis, p. 732-739. In F. C. Niedhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington D.C.
16. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. Messing, J., R. Crea, and P. H. Seeberg. 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. 9:309-321.
18. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. Ordal, G. W., D. O. Nettleton, and J. A. Hoch. 1983. Genetics of

- Bacillus subtilis* chemotaxis: isolation and mapping of mutations and cloning of chemotaxis genes. *J. Bacteriol.* **154**:1088–1097.
20. Ordal, G. W., H. M. Parker, and J. R. Kirby. 1985. Complementation and characterization of chemotaxis mutants of *Bacillus subtilis*. *J. Bacteriol.* **164**:802–810.
 21. Piggot, P. J., C. A. M. Curtis, and H. De Lencastre. 1984. Use of integrational plasmid vectors to demonstrate the polycistronic nature of a transcriptional unit (*spoIIA*) required for sporulation of *Bacillus subtilis*. *J. Gen. Microbiol.* **130**:2123–2136.
 22. Piggot, P. J., and J. A. Hoch. 1985. Revised genetic linkage map of *Bacillus subtilis*. *Microbiol. Rev.* **49**:158–179.
 23. Russell, C. B., R. C. Stewart, and F. W. Dahlquist. 1989. Control of transducer methylation levels in *Escherichia coli*: investigation of components essential for modulation of methylation and demethylation reactions. *J. Bacteriol.* **171**:3609–3618.
 24. Thaelke, M. S., W. A. Bedale, D. O. Nettleton, and G. W. Ordal. 1987. Evidence for an intermediate methyl-acceptor for chemotaxis in *Bacillus subtilis*. *J. Biol. Chem.* **262**:2811–2816.
 - 24a. Thaelke, M. S., J. M. Casper, and G. W. Ordal. 1990. Methyl group turnover on methyl-accepting chemotaxis proteins during chemotaxis by *Bacillus subtilis*. *J. Biol. Chem.* **265**:1928–1932.
 25. Thaelke, M. S., H. M. Parker, E. A. Ordal, and G. W. Ordal. 1988. Rapid attractant-induced changes in methylation of methyl-accepting chemotaxis proteins in *Bacillus subtilis*. *Biochemistry* **27**:8453–8457.
 26. Ullah, A. H., and G. W. Ordal. 1981. In vivo and in vitro chemotactic methylation in *Bacillus subtilis*. *J. Bacteriol.* **145**:958–965.
 27. Wang, L. F., and R. H. Doi. 1987. Promoter switching during development and the termination site of the σ^{43} operon of *Bacillus subtilis*. *Mol. Gen. Genet.* **207**:114–119.
 28. Warburg, R. J., and A. Moir. 1981. Properties of a mutant of *Bacillus subtilis* 168 in which spore germination is blocked at a late stage. *J. Gen. Microbiol.* **124**:243–253.
 29. Yansura, D. G., and D. H. Henner. 1983. Development of an inducible promoter for controlled gene expression in *Bacillus subtilis*, p. 249–263. In A. T. Ganesan and J. A. Hock (ed.), *Biology and biotechnology of the bacilli*, vol. 1. Academic Press, Inc., New York.
 30. Ying, C., and G. W. Ordal. 1989. Nucleotide sequence and expression of *cheF*, an essential gene for chemotaxis in *Bacillus subtilis*. *J. Bacteriol.* **171**:1631–1637.
 31. Youngman, P., J. B. Perkins, and R. Losick. 1984. Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. *Plasmid* **12**:1–9.
 32. Zuber, P., and R. Losick. 1983. Use of a *lacZ* fusion to study the role of *spo0* genes of *Bacillus subtilis* in developmental regulation. *Cell* **35**:275–283.
 33. Zuberi, A. R., A. Moir, and I. M. Feavers. 1987. The nucleotide sequence and gene organization of the *gerA* spore germination operon of *Bacillus subtilis* 168. *Gene* **51**:1–11.