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DnaK, DnaJ, and GrpE Are Required for Flagellum Synthesis in *Escherichia coli*

WENYUAN SHI,[1] YANNING ZHOU,[2]† JADWIGA WILD,[2] JULIUS ADLER,[1] AND CAROL A. GROSS[2]*

Departments of Biochemistry and Genetics[1] and Department of Bacteriology,[2] University of Wisconsin—Madison, Madison, Wisconsin 53706

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The DnaK, DnaJ, and GrpE heat shock proteins are required for motility of *Escherichia coli*. Cells deleted for *dnaK* or *dnaJ*, or with some mutations in the *dnaK* or *grpE* gene, are nonmotile, lack flagella, exhibit a 10- to 20-fold decrease in the rate of synthesis of flagellin, and show reduced rates of transcription of both the *flhD* master operon (encoding FlhD and FlhC) and the *fliA* operon (encoding σ^F). Genetic studies suggest that DnaK and DnaJ define a regulatory pathway affecting *flhD* and *fliA* synthesis that is independent of cyclic AMP-catabolite gene activator protein or the chemotaxis system.

The flagellum/chemotaxis system of *Escherichia coli* consists of at least 13 operons that encode over 40 genes (18, 20, 28, 29) organized into a regulatory hierarchy (17, 21). The general organization of the flagellum/chemotaxis regulon, diagrammed in Fig. 1, is reproduced from Jones and Aizawa (18). The *flhD* operon consists of the *flhD* and *flhC* genes and is at the top of this hierarchy. Expression of this operon is required for expression of all remaining genes in the regulon. Genes in the next level of the hierarchy encode *fliA*, the flagellar sigma factor σ^F (35), as well as functions required for basal body and hook assembly, while those in the lowest tier of the hierarchy are involved in assembly of filaments, motor activity, and chemotaxis.

Certain growth conditions affect the synthesis of flagella of wild-type *E. coli* cells. Flagellum genes are not expressed in the presence of D-glucose (1) because expression of the *flhD* master operon is sensitive to catabolite repression (4, 41, 50). Mutants insensitive to catabolite repression were isolated and found to be located at the *flhD-flhC* locus (40). The synthesis of flagella is also controlled by growth temperature; cells are not flagellated at 42°C (1). The mechanism for high-temperature inhibition of flagellum synthesis is unknown; however, mutations circumventing this inhibition have been selected and map at both the *flhD* and *fliA* operons (41).

The heat shock regulon of *E. coli* consists of a group of proteins that show a transient increase in their rate of synthesis in response to stresses such as heat, ethanol, or DNA-damaging agents (33). Heat shock proteins are positively regulated by the *rpoH* gene, whose product has been shown to be an alternate sigma factor (σ^{32}) (8, 13, 14, 24). Holoenzyme containing σ^{32} ($E\sigma^{32}$) uniquely recognizes the heat-inducible promoters preceding heat shock genes (5, 15, 32).

The heat shock proteins presumably protect cells against a variety of adverse conditions. In addition, they play important roles in steady-state growth (6, 12, 51). The heat shock protein DnaK, homolog of Hsp70 (3), along with the DnaJ and GrpE heat shock proteins are involved in cell division, RNA and DNA synthesis, protein folding, secretion, λ phage replication, mini-F and P1 phage replication, and regulation of the heat shock response (9, 12, 13, 25). In this paper, we report that the DnaK, DnaJ, and GrpE proteins are also required for the synthesis of flagella.

MATERIALS AND METHODS

Bacterial strains, phages, plasmids, media, and growth conditions. Bacterial strains, phages, and plasmids are listed in Table 1.

Cells were grown at 30°C in Luria broth (31) or Vogel-Bonner medium (46) containing 10^{-1} M D-glucose to an optical density at 590 nm (OD_{590}) of 0.3 to 0.6. Thiamine (1 μg/ml) or amino acids (1 mM) were added if needed. Swarm plates were used for testing bacterial motility and chemotaxis (2). Antibiotics were added to the following final concentrations: ampicillin, 50 μg/ml; tetracycline, 20 μg/ml; chloramphenicol, 10 μg/ml; and kanamycin, 50 μg/ml. Phosphate buffer solution was prepared according to Sambrook et al. (38).

Microscopy. Bacterial motility and cell size were observed with a Zeiss microscope and recorded on videotape for study with a motion analysis system (37).

Flagella were viewed in the Hitachi S-900 LVSEM electron microscope. For these studies, cells on glass coverslips (5 by 10 mm) coated with poly-L-lysine were immersed in 2% glutaraldehyde fixative in 0.8 M sucrose and 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) for 1 h, postfixed in 0.1% osmium tetroxide for 20 min, washed in 50 mM HEPES buffer (pH 7.4), stored overnight in 30% ethanol, dehydrated in increasing concentrations of ethanol (50-70-85-95-95-100%) for 10 min at each step, critical-point dried in CO_2, ion-beam microsputtered with platinum, and then viewed.

Genetic manipulations. Transformations were performed according to Sambrook et al. (38), and P1 transductions and selections for λ lysogens were carried out according to Silhavy et al. (39). Because P1 phage grows poorly on *dnaK* mutants, P1 lysates of the *dnaK* mutants were prepared on merodiploid strains that carried a wild-type copy of *dnaK*, either on the lambda phage λ*dnaK*$^+$ *dnaJ*$^+$ (19) or on a plasmid (pNRK416). The *dnaK* and *grpE* mutant alleles were selected for Tet^r conferred by a 50%-linked Tn*10* marker, while the Δ(*dnaK-dnaJ*) and Δ*dnaJ* strains were selected by virtue of the Kan^r conferred by the Kan^r cassette that has

* Corresponding author.
† Present address: National Cancer Institute, Bethesda, MD 20892.

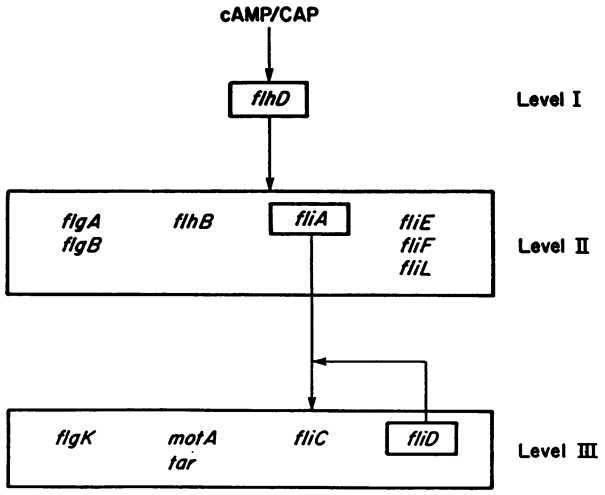

FIG. 1. Transcriptional control in the *E. coli* flagellum/chemotaxis regulon (reproduced from reference 18). Each operon is designated by the first gene transcribed in that operon. Transcription of the *flhD* operon (flagellum master operon) is activated by cAMP-CAP. Transcription of the level II operons requires the products of the master operon. Transcription of the level III operons, in turn, requires transcription of all of the level II operons; the level II product FliA is a sigma factor, σ^F, required for level III operon transcription (35), but how the effect of the other level II genes is mediated is unknown. The *fliD* operon probably codes a factor responsible for repression of all level III operons (22).

replaced these genes. Transductants containing mutant alleles were identified by screening for known mutant phenotypes (temperature-sensitive growth and inability to grow λ phage). P1 transductants containing *flhD*$^+$-*lacZ*$^+$ or *fliA*$^+$-*lacZ*$^+$ were selected with a linked Amp^r marker. Appropriate transductants were identified by examining the motility and β-galactosidase activity of the transductants.

Assays. β-Galactosidase assays were performed according to Miller (31).

Cell surface bacterial flagellin was determined by an enzyme-linked immunosorbent assay (ELISA) based on a modification of the method described by Harlow and Lane (16). One milliliter of cells was washed and resuspended in phosphate buffer solution adjusted to 5% bovine serum albumin, incubated with preabsorbed antiflagellum antibody (from rabbit) at 35°C for 1 h, washed three times in phosphate buffer solution, resuspended in the same buffer, and incubated with goat anti-rabbit alkaline phosphatase-linked antibody at 35°C for 1 h. Cells were washed three times in phosphate buffer solution and resuspended in 1 ml of Sigma104 phosphate substrate buffer (9.7% [vol/vol], diethanolamine, 3 mM NaN_3, 0.49 mM $MgCl_2$, pH 9.8) with 1 mg of *p*-nitrophenyl phosphate per ml. The reaction was stopped by centrifuging the cells when yellow color had developed. The OD_{405} of the supernatant is a measure of the amount of *p*-nitrophenol produced by the alkaline phosphatase and reflects the amount of flagellin on the cell surface.

The synthesis of flagellin was examined by pulse-labeling with L-[^{35}S]methionine for 1 min, followed by a chase with excess unlabeled L-methionine for 1 or 5 min, and was quantified by immunoprecipitation with antiflagellum antibody according to Straus et al. (43).

The amount of flagellin was determined by Western immunoblotting (38). Protein samples were precipitated with 5% trichloroacetic acid, dissolved in Laemmli buffer (23), and neutralized with 1 N NaOH. Equal amounts of protein were determined by the BCA assay (BCA is a trademark of Pierce Chemical Co. for the quantitative determination of protein by the enhanced, colorimetric detection of Cu^{1+} produced in the reaction of protein with alkaline Cu^{2+}), loaded on sodium dodecyl sulfate–9% polyacrylamide gels, and run in a Bio-Rad mini-protein II dual-slab cell. Samples were transferred to nitrocellulose paper by use of a Hoefer Transphor (TE50), probed with antiflagellum antibody, developed with goat anti-rabbit antibody coated with alkaline phosphatase by use of a 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium system (Kirkegaard & Perry Laboratories), and quantified with a Zeineh soft-laser scanning densitometer (SL-504-XL).

RESULTS

Mutations in *dnaK*, *dnaJ*, and *grpE* affect motility. Some *dnaK*, *dnaJ*, and *grpE* mutant strains grown at 30°C were nonmotile in the phase-contrast microscope, whereas their wild-type parental strains were motile under the same conditions (Table 2). Both transduction and complementation experiments confirmed that the nonmotility was caused by the mutation in *dnaK*, *dnaJ*, or *grpE*. Each of the mutant alleles showing a motility defect was transduced into three different wild-type strains (W3110, MC1061, and MG1655), and more than 70 transductants were examined for phenotypes. In each case, there was 100% coincidence between nonmotility and possession of the mutant allele. In addition, the motility defect of every *dnaK* mutant could be complemented by a wild-type copy of *dnaK* (Table 3). The motility defect of the Δ*dnaJ* strain could be complemented by λ*dnaK*$^+$ *dnaJ*$^+$ but not by *dnaK*$^+$ alone; this result indicates that a functional *dnaJ* is required for motility (Table 3). These results showed that mutation in *dnaK*, *dnaJ*, or *grpE* can result in nonmotility. Some of the *dnaK* and *grpE* point mutation strains were motile at 20°C (Table 2), and a strain (CAG9301) lacking σ^{32} (having less than 1% of DnaK and DnaJ found in the wild-type cell) (51) was also partially motile at 15°C. However, the Δ(*dnaK-dnaJ*) and Δ*dnaJ* strains were not. These results suggest that DnaK, DnaJ, and GrpE may be less important for flagellation at low temperatures, but functional DnaK and DnaJ are required for motility at all temperatures.

Some *dnaK* mutations did not result in nonmotile phenotype (Table 2); the *dnaK756* and *dnaK211** (a derivative of the *dnaK211* mutant lacking the N-terminal mutations) mutants were motile at both 30 and 35°C, while the *dnaK* (AT174) mutant was partially motile at 30°C. In addition, *groEL140* and *groES30* mutants were motile at 30 and 35°C.

The *dnaK*, *dnaJ*, and *grpE* mutants are defective in flagellar synthesis. Nonmotility could result either from paralyzed flagella or from absence of flagella on the cell surface. That the latter was the case was demonstrated by assaying the amount of flagellin on the cell surface. The nonmotile mutant strains grown at 30°C had less than 1/20 the amount of flagellin on their cell surfaces compared with the parental strains (Table 2). This level of flagellation is comparable to that of a nonmotile *flhD* strain (Table 2). Scanning electron microscope pictures of the *dnaK211* mutant (Fig. 2) confirmed the nonflagellation phenotype and also demonstrated the blocked cell division phenotype characteristic of *dnaK* mutant strains (6, 19).

The lack of surface flagella reflects a lack of total cellular flagellin in mutant cells. This result is demonstrated for the *dnaK211* mutant in Fig. 3. The *dnaK211* mutant (lanes 3 and

TABLE 1. Bacterial strains, phages, and plasmids used

E. coli strain, phage, or plasmid	Relevant genotype	Reference or source
Strain		
W3110	Motile wild type	30
MC1061	Motile wild type, parent of GD341, DN201, EK171, AT174, and CAG13211	38
MG1655	Motile wild type, parent of PK101, PK102, and PK5	51
MC4100	*flhD*	7
GD341	*dnaK* (GD341)[a]	J. Wild
DN201	*dnaK* (DN201)[a]	J. Wild
CAG13211	*dnaK211* (GD198, EK230, and C-terminal truncation)[a]	J. Wild
CAG13600	*dnaK211**, a derivative of *dnaK211* lacking the N-terminal mutations	J. Wild
EK171	*dnaK* (EK171)[a]	J. Wild
AT174	*dnaK* (AT174)[a]	J. Wild
B178*dnaK756*	*dnaK756*	44
PK101	Δ(*dnaK-dnaJ*)	19
PK102	Δ*dnaJ*	19
PK5	*grpE280*	19
CAG9301	*rpoH*	51
CAG9309	*groES30*	10
CAG9310	*groEL140*	45
YK4331	*flhD*$^+$-*lacZ*$^+$	20
YK4337	*fliA*$^+$-*lacZ*$^+$	20
JLV45-3	Δ*cya* Δ*crp cfs*	47
JLV15-4	Δ*cya cfs*	47
JLV70-2	Δ*crp cfs*	47
HCB326	MCPI$^-$, MCPII$^-$, MCPIII$^-$, MCPIV$^-$, *cheA cheW cheY cheZ cheB cheR*	49
AW901	CAG13211/pNRK416	This study
AW902	CAG13211/λ*dnaK*$^+$ *dnaJ*$^+$	This study
AW903	PK101/λ*dnaK*$^+$ *dnaJ*$^+$	This study
AW904	PK102/λ*dnaK*$^+$ *dnaJ*$^+$	This study
AW905	PK102/pNRK416	This study
AW906	PK101/pNRK416	This study
AW907	CAG13211/pLS1	This study
AW908	YK4331/*dnaK211*	This study
AW909	YK4337/*dnaK211*	This study
AW910	YK4331/Δ(*dnaK-dnaJ*)	This study
AW911	YK4337/Δ(*dnaK-dnaJ*)	This study
AW912	CAG13211/pPM61	This study
AW917	JLV45-3/Δ(*dnaK-dnaJ*)	This study
AW918	HCB326/Δ(*dnaK-dnaJ*)	This study
AW923	CAG13211/pMRG7	This study
AW947	MC1061/pPM61	This study
AW948	MC1061/pLS1	This study
AW949	MC1061/pMRG7	This study
AW950	MG1655/*fliA*$^+$-*lacZ*$^+$	This study
AW951	JLV45-3/*fliA*$^+$-*lacZ*$^+$	This study
AW952	AW917/*fliA*$^+$-*lacZ*$^+$	This study
AW953	HCB326/*fliA*$^+$-*lacZ*$^+$	This study
AW954	AW918/*fliA*$^+$-*lacZ*$^+$	This study
AW955	PK101/pMRG7	This study
AW956	PK101/pPM61	This study
AW957	PK101/pLS1	This study
AW958	CAG13600/*flhD*$^+$-*lacZ*$^+$	This study
AW959	CAG13600/*fliA*$^+$-*lacZ*$^+$	This study
AW960	GD341/pNRK416	This study
AW961	DN201/pNRK416	This study
AW962	EK171/pNRK416	This study
Phages		
λ*dnaK*$^+$ *dnaJ*$^+$	*dnaK*$^+$ *dnaJ*$^+$	19
P1 *vir*	Cml^r	31
Plasmids		
pNRK416	*lacUV5-dnaK*$^+$ Amp^r	N. Kusukawa
pLS1	*groES*$^+$ *groEL*$^+$ Amp^r	K. Tilly
pPM61	*flhD*$^+$ *flhC*$^+$ Amp^r Kan^r	4
pMRG7	*rpoD*$^+$ Amp^r	26

[a] Designated by the single-letter code of the wild-type amino acid, followed by the substitution and the amino acid position.

TABLE 2. Motility and flagellation studied by microscopic observation and ELISA

Strain	Genotype[a]	Motility observed by microscope[b]			Flagellation at 30°C assayed by ELISA[c]
		20°C	30°C	35°C	
MC1061	Wild type	+	+	+	1.60 ± 0.08
MG1655	Wild type	+	+	+	1.48 ± 0.13
GD341	*dnaK* (GD341)	±	−	−	0.03 ± 0.01
DN201	*dnaK* (DN201)	−	−	−	0.04 ± 0.01
CAG13211	*dnaK211*	±	−	−	0.04 ± 0.02
EK171	*dnaK* (EK171)	±	−	−	0.06 ± 0.02
PK101	Δ(*dnaK-dnaJ*)	−	−	−	0.03 ± 0.01
PK102	Δ*dnaJ*	−	−	−	0.06 ± 0.03
PK5	*grpE280*	±	−	−	0.05 ± 0.02
CAG9301	*rpoH*	±	NG	NG	NG
B178*dnaK756*	*dnaK756*	+	+	+	ND
CAG13600	*dnaK211**	+	+	+	1.24 ± 0.13
AT174	*dnaK* (AT174)	+	±	−	ND
CAG9310	*groEL140*	+	+	+	ND
CAG9309	*groES30*	+	+	+	ND
MC4100	*flhD*	−	−	−	0.02 ± 0.01
AW947	MC1061/p*flhD*$^+$ *flhC*$^+$	+	+	+	1.68 ± 0.15
AW912	*dnaK211*/p*flhD*$^+$ *flhC*$^+$	+	±	−	0.78 ± 0.12
AW948	MC1061/p*groES*$^+$ *groEL*$^+$	+	+	+	1.51 ± 0.12
AW907	*dnaK211*/p*groES*$^+$ *groEL*$^+$	+	±	−	0.56 ± 0.09
AW949	MC1061/p*rpoD*$^+$	+	+	+	1.36 ± 0.14
AW923	*dnaK211*/p*rpoD*$^+$	+	±	−	0.38 ± 0.17
AW956	Δ(*dnaK-dnaJ*)/p*flhD*$^+$ *flhC*$^+$	−	−	−	ND
AW957	Δ(*dnaK-dnaJ*)/p*groES*$^+$ *groEL*$^+$	−	−	−	ND
AW955	Δ(*dnaK-dnaJ*)/p*rpoD*$^+$	−	−	−	ND

[a] p*flhD*$^+$ *flhC*$^+$, p*groES*$^+$ *groEL*$^+$, or p*rpoD*$^+$ (also called pPM61, pLS1, or pMRG7) is a plasmid carrying the wild-type *flhD* and *flhC*, *groES* and *groEL*, or *rpoD* gene, respectively.

[b] Microscopic observations were made by viewing freshly grown cells (mid-log phase) under a ×40 lens. +, cells (>90%) swimming at speeds faster than 10 μm/s; −, cells (>99.9%) showing no movement except Brownian motion; ±, less than 50% cells showing motility with a slow speed; NG, no growth.

[c] ELISAs were performed as described in Materials and Methods. The numbers listed are the averages of two readings at OD_{405} from two identical samples. ND, no data; NG, no growth.

4) has no flagellin, unlike the parental strain (lane 7), unless it is complemented with *dnaK*$^+$ (lane 5). Lack of flagellin could result from a decrease in its synthesis or a decrease in its stability. Immunoprecipitation analysis of pulse-labeled cells indicated that the absence of flagellin resulted from a 10- to 20-fold decrease in the rate of flagellin synthesis in the mutant strains (Table 4). Further evidence that the flagellin in mutant cells was stable once synthesized comes from the observation that the amount of flagellin synthesized in a 1-min pulse with [^{35}S]methionine was the same whether the time of chase with unlabeled L-methionine was 1 min or 5 min (Table 4).

TABLE 3. Complementation experiments[a]

Strain	Genotype	Motility at 30°C observed by microscopy
AW902	*dnaK211*/λ*dnaK*$^+$ *dnaJ*$^+$	+
AW903	Δ(*dnaK-dnaJ*)/λ*dnaK*$^+$ *dnaJ*$^+$	+
AW904	Δ*dnaJ*/λ*dnaK*$^+$ *dnaJ*$^+$	+
AW960	*dnaK* (GD341)/p*lacUV5-dnaK*$^+$	+
AW961	*dnaK* (DN201)/p*lacUV5-dnaK*$^+$	+
AW901	*dnaK211*/p*lacUV5-dnaK*$^+$	+
AW962	*dnaK* (EK171)/p*lacUV5-dnaK*$^+$	+
AW906	Δ(*dnaK-dnaJ*)/p*lacUV5-dnaK*$^+$	−
AW905	Δ*dnaJ*/p*lacUV5-dnaK*$^+$	−

[a] See Table 2, footnote *b*, for details of the microscopy study. λ*dnaK*$^+$ *dnaJ*$^+$ is a lambda phage carrying the wild-type *dnaK* and *dnaJ* genes; p*lacUV5-dnaK*$^+$ (also called pNRK416) is a plasmid encoding *dnaK*$^+$ gene under control of the isopropylthiogalactopyranoside-inducible *lacUV5* promoter.

The *dnaK211* and Δ(*dnaK-dnaJ*) mutants are defective in transcribing the flagellum-regulatory genes. The inhibitory effect of mutations in *dnaK* and *dnaJ* on flagellum synthesis would be explained if they inhibited expression of the master regulators FlhD and FlhC (encoded by the *flhD* operon) and/or the flagellum-specific sigma factor, σ^F (encoded by *fliA*). To determine whether this was the case, we measured synthesis of β-galactosidase in *dnaK* mutant strains carrying transcriptional *flhD*$^+$-*lacZ*$^+$ or *fliA*$^+$-*lacZ*$^+$ fusion. The *dnaK211* mutation resulted in more than a twofold reduction of β-galactosidase activity from the *flhD*$^+$-*lacZ*$^+$ fusion and an eightfold reduction from the *fliA*$^+$-*lacZ*$^+$ fusion, while the Δ(*dnaK-dnaJ*) mutation had an even more severe effect on synthesis of β-galactosidase from the transcriptional fusions (Table 5). As expected, the *dnaK211** mutation, which permitted motility, also exhibited full expression of β-galactosidase from the *flhD* and *fliA* fusions (Table 5).

The *flhD* operon is regulated by cyclic AMP (cAMP)-catabolite gene activator protein (CAP) and therefore subject to catabolite repression. Cells growing in D-glucose or strains lacking adenyl cyclase (Δ*cya*) or CAP (Δ*cap*) are nonflagellated because of decreased expression of the *flhD* and *fliA* operons (41). Cells grown in the presence of 10^{-1} M glucose were nonmotile, lacked flagella, and had reduced total cellular flagellin (data not shown), although the glucose effect on expression of these operons was less severe than that exhibited by the *dnaK* mutations; growth in 10^{-1} M D-glucose caused less than a twofold reduction of β-galac-

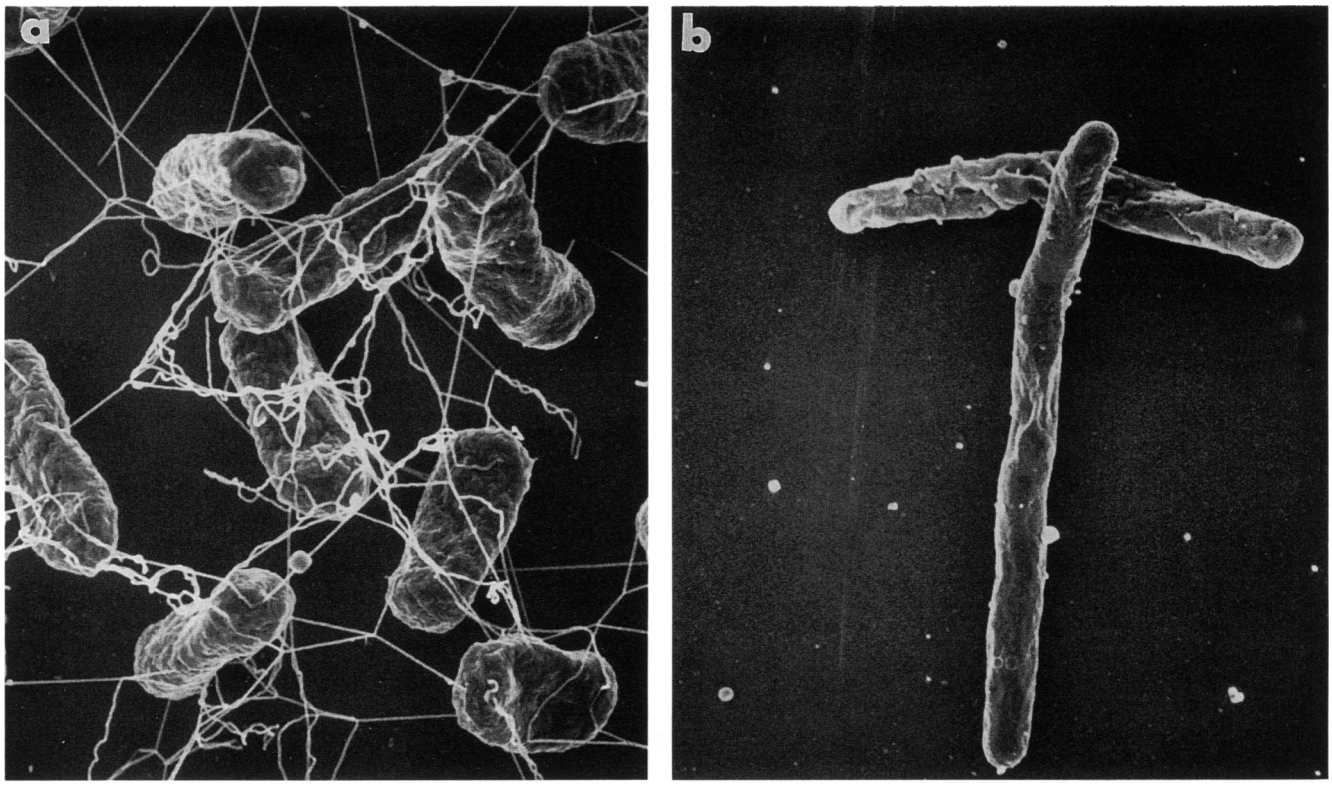

FIG. 2. Scanning electron microscopy of motile wild-type strain MC1061 (a) and the *dnaK211* mutant grown in Luria broth at 30°C (b), showing that the *dnaK211* mutant failed to make flagella at 30°C. See Materials and Methods for experimental procedures.

tosidase activity from the *flhD-lacZ* fusion and a threefold reduction from the *fliA-lacZ* fusion (Table 5). These data indicate that the reduced transcription of the *flhD* and *fliA* operons exhibited by *dnaK* and *dnaJ* mutations is sufficient to explain their nonflagellation phenotype.

The phenotype of *dnaK* and *dnaJ* mutants could be explained if these mutations induce a state of severe catabolite repression resulting in decreased expression of *flhD*. To test this possibility, we examined the effect of the Δ(*dnaK-dnaJ*) mutation on flagellar synthesis in a *cfs* (constitutive flagellum synthesis) strain. *cfs* mutations have been selected as second-site suppressors of the nonmotile phenotype of Δ*cya* or Δ*cap* strains, map to the *flhD* operon, and revert both the motility and transcriptional defects of D-glucose and the Δ*cya* or Δ*cap* mutation (41, 47). However, the *cfs* mutation did not revert either the motility defect (AW917) or the transcription defect (AW952) caused by Δ(*dnaK-dnaJ*) (Table 6). Two other *cfs* strains (JLV15-4 and JLV70-2) were also nonmotile in the presence of the Δ(*dnaK-dnaJ*) mutation (data not shown). Thus, these data suggest that DnaK and DnaJ define a regulatory pathway affecting *flhD* and *fliA* synthesis that is independent of cAMP-CAP.

A previous report suggested that the chemotaxis system itself may have a feedback effect on flagellum synthesis (48).

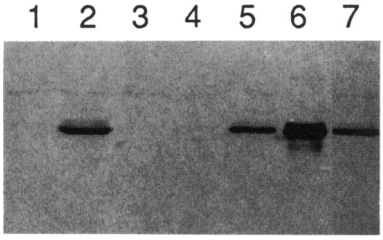

FIG. 3. Western blot of flagellin of whole cells. Cells were grown in Luria broth at 30°C. Lanes: 1, MC4100 (*flhD*); 2, AW912 (*dnaK211*/p*flhD*$^+$ *flhC*$^+$); 3 and 4, two independently prepared samples from CAG13211 (*dnaK211*); 5, AW901 (*dnaK211*/p*lacUV5-dnaK*$^+$); 6, purified flagellin; 7, MC1061 (wild type). A Zeineh soft-laser scanning densitometer was used to quantitate the amount of flagellin; for lanes 1 to 7, the values are 0, 39, 0, 0, 34, 165, and 44 arbitrary units, respectively.

TABLE 4. Synthesis rates of flagellin

Strain	Genotype	Relative synthesis rate of flagellin[a]	
		1-min chase	5-min chase
MC1061	Motile wild type	1	0.94
PK101	Δ(*dnaK-dnaJ*)	0.05	0.06
DN201	*dnaK* (DN201)	0.05	0.06
CAG13211	*dnaK211*	0.10	0.07
AW947	Wild type/p*flhD*$^+$ *flhC*$^+$	1.86	ND
AW912	*dnaK211*/p*flhD*$^+$ *flhC*$^+$	0.65	ND
AW949	Wild type/p*rpoD*$^+$	1.53	ND
AW923	*dnaK211*/p*rpoD*$^+$	0.38	ND
AW948	Wild type/p*groES*$^+$ *groEL*$^+$	1.02	ND
AW907	*dnaK211*/p*groES*$^+$ *groEL*$^+$	0.65	ND

[a] Determined by immunoprecipitation of flagellin from cells pulse-labeled with L-[^{35}S]methionine as described in Materials and Methods. Each value is an average of duplicate samples, normalized to synthesis of flagellin by motile wild-type MC1061 at 30°C. ND, no data.

TABLE 5. Effects of *dnaK* mutations on activities of transcriptional fusions of flagellum genes with *lacZ* genes[a]

Strain	Genotype or growth condition	β-Galactosidase activity (Miller units)	
		flhD-lacZ	*fliA-lacZ*
YK4331	*flhD*$^+$-*lacZ*$^+$	317 ± 12	
	Grown in 10^{-1} M D-glucose	197 ± 17	
AW908	*dnaK211*/*flhD*$^+$-*lacZ*$^+$	134 ± 13	
AW910	Δ(*dnaK-dnaJ*)/*flhD*$^+$-*lacZ*$^+$	69 ± 05	
AW958	*dnaK211**/*flhD*$^+$-*lacZ*$^+$	289 ± 21	
YK4337	*fliA*$^+$-*lacZ*$^+$		212 ± 24
	Grown in 10^{-1} M D-glucose		74 ± 13
AW909	*dnaK211*/*fliA*$^+$-*lacZ*$^+$		28 ± 07
AW911	Δ(*dnaK-dnaJ*)/*fliA*$^+$-*lacZ*$^+$		23 ± 04
AW959	*dnaK211**/*fliA*$^+$-*lacZ*$^+$		223 ± 17

[a] Bacteria were grown at 30°C in Luria broth except for the D-glucose repression studies that utilized Vogel-Bonner medium. Data are averages of determinations of two duplicate samples. For each strain, more than 10 independent colonies were examined, with similar results.

In this study, we have also ruled out the possibility that the motility defect of Δ(*dnaK-dnaJ*) strains results from a repression signal from the chemotaxis machinery. HCB326 is a strain which is motile but has no methyl-accepting chemotaxis proteins (MCPs) or chemotaxis (*che*) proteins (49). The presence of the Δ(*dnaK-dnaJ*) mutation in this strain also leads to nonmotility even though this strain cannot transmit chemotaxis signals (owing to lack of MCPs and *che* proteins) (Table 6). Thus, nonmotility is not related directly to the chemotaxis system.

We examined whether overexpression of FlhD and FlhC, GroES and GroEL, or RpoD (σ^{70}) could suppress the motility defect of *dnaK* mutant strains. In each case, the flagellation defect of the *dnaK211* mutant was partially suppressed at 30°C (Table 2 and Fig. 3, lane 2) but not at 35°C (Table 2). However the flagellation defect of the Δ(*dnaK- dnaJ*) mutant was not affected by overexpression of FlhD and FlhC, GroES and GroEL, or RpoD (Table 2). Pulse-labeling experiments showed that overproduction of FlhD and FlhC or RpoD increased the synthesis rate of flagellin both in the wild type and in the *dnaK211* mutant, while overproduction of GroES and GroEL increased the synthesis rate of flagellin in the *dnaK211* mutant but had no effect in a wild-type background (Table 4).

Isolation and mapping of motile revertants from a Δ(*dnaK-dnaJ*) strain. We have undertaken a genetic approach to study the role of DnaK, DnaJ, and GrpE in flagellum synthesis. Ten independent motile revertants were isolated from a Δ(*dnaK-dnaJ*) mutant strain (PK101) by using the swarm plate method (2). These revertants were motile and had normal transcription of the *fliA* gene at 30°C even though they still carry the Δ(*dnaK-dnaJ*) mutation and exhibited other mutant phenotypes of the original Δ(*dnaK-dnaJ*) mutant strain (temperature-sensitive growth and failure to grow λ phage). Thus, these suppressors are specific for the motility defect of the Δ(*dnaK-dnaJ*) mutant strain. Each of the suppressors mapped near *uvrC*::Tn*10* located at 42 min (near *flhD*) on the *E. coli* genetic map and exhibited 100% cotransduction with the *flhD*$^+$-*lacZ*$^+$ fusion among 20 transductants examined.

DISCUSSION

In this report, we show that the DnaK, DnaJ, and GrpE heat shock proteins are required for motility of *E. coli*. Cells deleted for *dnaK* or *dnaJ*, or with some mutation in the *dnaK* or *grpE* gene, are nonmotile, lack flagella, exhibit a 10- to 20-fold decrease in the rate of synthesis of flagellin, and show reduced rates of transcription of both the *flhD* master operon (encoding FlhD and FlhC) and the *fliA* operon (encoding σ^F). The nonflagellation phenotype caused by catabolite repression results from reduced transcription of the *flhD* and *fliA* operons. Since *dnaK* and *dnaJ* mutants show a reduction in *flhD* and *fliA* transcription greater than that caused by catabolite repression (Table 5), the decrease of *flhD* and *fliA* transcription exhibited by the mutant strains is sufficient to explain the nonmotility phenotype of these mutant strains; however, the fact that overproduction of FlhD and FlhC failed to restore the motility of the Δ(*dnaK-dnaJ*) mutant suggests that there are additional roles for DnaK, DnaJ, and GrpE in flagellation, e.g., affecting flagellar protein folding and/or assembly (see below).

We do not yet know how DnaK, DnaJ, and GrpE are involved in transcription of the *flhD* and *fliA* operons. Our results suggest that several potential regulatory mechanisms are unlikely. (i) The Δ(*dnaK-dnaJ*) mutant does not work via the cAMP-CAP pathway of catabolite repression. *cfs* mutations permit *flhD* transcription in the absence of cAMP and CAP but do not restore *flhD* transcription in the Δ(*dnaK-*

TABLE 6. Evidence that repression of flagellation caused by the Δ*dnaK dnaJ* mutation is not directly related to cAMP-CAP or the chemotaxis system[a]

Strain	Genotype	Motility observed by microscopy		β-Galactosidase activity (Miller units) of *fliA-lacZ*	
		−Glucose	+Glucose	−Glucose	+Glucose
MG1655	Motile wild type	+	−		
AW950	MG1655/*fliA*$^+$-*lacZ*$^+$			243 ± 11	74 ± 13
JLV45-3	Δ*cya* Δ*crp cfs*	+	+		
AW951	JLV45-3/*fliA*$^+$-*lacZ*$^+$			198 ± 10	193 ± 04
AW917	JLV45-3/Δ(*dnaK-dnaJ*)	−	−		
AW952	AW917/*fliA*$^+$-*lacZ*$^+$			26 ± 05	ND
HCB326	MCPI$^-$ MCPII$^-$ MCPIII$^-$ MCPIV$^-$ *cheA cheW cheY cheZ cheB cheR*	+	−		
AW953	HCB326/*fliA*$^+$-*lacZ*$^+$			288 ± 07	57 ± 06
AW918	HCB326/Δ(*dnaK-dnaJ*)	−	−		
AW954	AW918/*fliA*$^+$-*lacZ*$^+$			29 ± 04	ND

[a] See Table 2, footnote *b*, for details of the microscopy study. For *fliA-lacZ* data, see the footnote to Table 5. D-Glucose was added at a concentration of 10^{-1} M. ND, no data.

dnaJ) mutant. (ii) Although the chemotaxis system itself may have some kind of feedback effect on flagellum synthesis (48), the nonflagellation of the Δ(*dnaK-dnaJ*) mutant is not related to chemotaxis signals. A motile nonchemotactic strain is rendered nonmotile when the Δ(*dnaK-dnaJ*) mutation is introduced. (iii) Inhibition of cell division prevents motility (34). However, it is unlikely that the cell division defect of the mutant strains is responsible for their nonmotility, since overexpression of the *ftsZ*$^+$ or *dks*$^+$ gene restores cell division (6, 19) but not motility (38a).

We do not know whether the defect in flagellum synthesis of the *dnaK*, *dnaJ*, and *grpE* mutants is related to the inability of cells to make flagella at a high temperature (42°C) (1). However, this regulatory interaction between the heat shock system and the flagellum/chemotaxis system did open the possibility that inhibition of motility at 42°C is related to induction of the heat shock response.

How might the DnaK, DnaJ, and GrpE heat shock proteins be involved in regulating transcription of the *flhD* operon? These proteins function as chaperones and have been shown to be involved in protein folding and unfolding in many systems (11, 27, 36, 42). It is likely that their involvement in *flhD* transcription is another example of such an activity. A number of possible mechanisms could be envisioned. DnaK, DnaJ, and GrpE might be required for the proper folding or activity of an unknown positive regulator of the *flhD* operon. One particularly appealing possibility is that FlhD and FlhC themselves positively regulate the *flhD* operon. In that case, DnaK, DnaJ, and GrpE could regulate the folding, translational efficiency, degradation, or activity of these proteins. Alternatively, DnaK, DnaJ, and GrpE may be required for some step in the assembly of flagella. The accumulated precursors in the mutant strains could then exhibit inhibitory effects on *flhD* transcription or other participants in the regulatory cascade. Finally, the effect on flagellum synthesis may be an indirect consequence of one of the many cellular changes in *dnaK*, *dnaJ*, and *grpE* mutant strains. Analysis of the location and effects of the motile revertants of a Δ(*dnaK-dnaJ*) strain should lead to a molecular understanding of the roles of DnaK, DnaJ, and GrpE in flagellum synthesis.

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