JOURNAL OF BACTERIOLOGY, Apr. 2009, p. 2307–2314 Vol. 191, No. 7
0021-9193/09/$08.00+0 doi:10.1128/JB.01353-08

Properties of HflX, an Enigmatic Protein from *Escherichia coli*▽

Dipak Dutta,[1]† Kaustav Bandyopadhyay,[1] Ajit Bikram Datta,[1]‡
Abhijit A. Sardesai,[2] and Pradeep Parrack[1]*

Department of Biochemistry, Bose Institute, P-1/12, C.I.T. Scheme VIIM, Kolkata 700054, India,[1] and Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500076, India[2]

Received 29 September 2008/Accepted 7 January 2009

The *Escherichia coli* gene *hflX* was first identified as part of the *hflA* operon, mutations in which led to an increased frequency of lysogenization upon infection of the bacterium by the temperate coliphage lambda. Independent mutational studies have also indicated that the HflX protein has a role in transposition. Based on the sequence of its gene, HflX is predicted to be a GTP-binding protein, very likely a GTPase. We report here purification and characterization of the HflX protein. We also specifically examined its suggested functional roles mentioned above. Our results show that HflX is a monomeric protein with a high (30% to 40%) content of helices. It exhibits GTPase as well as ATPase activities, but it has no role in lambda lysogeny or in transposition.

The temperate bacteriophage lambda can follow either the lytic or the lysogenic mode of development upon infection of its host bacterium, *Escherichia coli* (16, 21, 26, 56). The developmental choice is influenced by phage proteins CII and CIII, as well as by a number of host proteins (27). Initially, the two loci *hflA* and *hflB* were identified in the *E. coli* genome based on their effects on lambda lysogeny (4, 5). Subsequently, the *hflA* locus was found to comprise three genes, *hflX*, *hflK*, and *hflC* (3), while *hflB* encoded the ATP-dependent metalloprotease HflB (also known as FtsH) that acts upon λCII and drives the cell toward the lytic pathway (48, 50, 51). In addition, another *E. coli* protein that has an effect on the lysis-lysogeny switch, HflD, has been identified (33).

HflK and HflC are membrane proteins and form a membrane-bound protease complex along with HflB, and they are thought to act as modulators of the function of HflB (31, 32). The function of HflX, however, is totally unknown. Although *hflX* was first identified as an "additional gene" in the *hflA* locus (3), there is no evidence suggesting that the HflX protein has a role in λ lysogeny. It was hypothesized that HflX was required for the activities of HflK and HflC (42), but the actual involvement of HflX with any of the other Hfl proteins remains speculative. On the other hand, the *hflX* gene is widely distributed, occurring both in prokaryotes and in eukaryotes, which presumably acquired this gene from proteobacteria via the mitochondrial route (36). Like motifs in the proteins of the GTPase superfamily (7), putative GTP binding motifs have been identified in the derived amino acid sequence of HflX (42). Indeed, an "HflX family" of proteins, characterized by a distinct conserved domain with a glycine-rich segment N terminal to the putative GTP binding domain, has been postulated. This family belongs to the translation factor superfamily (TRAFAC class) of the GTPase superclass of P-loop nucleoside triphosphatases (36). Recently, a global search for host factors responsible for the modulation of the transposable elements Tn*10*, IS*103*, and Tn*552* led to a report that a reduction in the transposition frequency occurred when the *hflX* gene was found to be disrupted by insertion mutagenesis (54). *hflX* is part of a complex superoperon, *amiB-mutL-miaA-hfq-hflX-hflK-hflC*, that is located at 94.78 min in the *E. coli* K-12 genome and has a complex arrangement of genes that are cotranscribed from a series of alternating $E\sigma^{70}$ and $E\sigma^{32}$ heat shock promoters (52, 53). No independent promoter for the *hflA* region (*hflX-hflK-hflC*) has been detected, and the transcription of the *hflA* genes depends solely on the promoters upstream of the *hfq* gene. Several important cellular processes are mediated by the gene products of the superoperon (11, 14, 24, 29, 37, 40, 41, 53), but so far no definite function has been ascribed to HflX, whose cellular role remains enigmatic. Compared to the levels of expression of the upstream genes *miaA* and *mutL* or the downstream *hflKC* genes, the expression level of *hfl*X is very low. The intracellular concentration of the protein also is rather low (52). Upon heat shock, enhanced expression of HflX, HflK, and HflC has been reported (13, 45).

The HflX protein thus seems to have been implicated in such diverse functions as the lysis-lysogeny decision of λ, GTP binding and hydrolysis, and transposition. Interestingly, studies of HflX at the protein level are scarce. While this paper was being prepared, a report on the ribosome binding properties of *Chlamydophila pneumoniae* HflX (44) appeared, and we also showed a similar effect for *E. coli* HflX (28). In this paper we report purification of His_6-HflX by cloning and overexpression (as a histidine- or glutathione *S*-transferase [GST]-tagged recombinant protein) of the *E. coli hflX* gene. His_6-HflX and GST-HflX, as well as HflX, obtained by removal of the hexahistidine or GST tag, were studied with a view toward determining the cellular function of the protein. It was found that HflX exhibited both GTPase and ATPase activities. We also

* Corresponding author. Mailing address: Department of Biochemistry, Bose Institute, P-1/12, C.I.T. Scheme VIIM, Kolkata 700054, India. Phone: 91-33-25693227. Fax: 91-33-23553886. E-mail: pradeep@bic.boseinst.ernet.in.

† Present address: New York University School of Medicine, Smilow Research Center, New York, NY 10016.

‡ Present address: Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

▽ Published ahead of print on 30 January 2009.

TABLE 1. Bacterial strains, plasmids, and primers used

Strain, plasmid, or primer	Genotype, description, or sequence	Source (reference)
E. coli strains		
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	J. Gowrishankar
MG1655	F$^-$ λ$^-$ *ilvG rfb*-50 *rph-1*	J. Gowrishankar
GJ2433	(*mutL*::*tet*)	J. Gowrishankar
DH10B	F$^-$ *araD139* Δ(*ara leu*)*7697* Δ*lacX74 galU galK rpsL deoR* ϕ80d*lacZ*Δ*M15 endA1 nupG recA1 mcrA* Δ(*mrr hsdRMS mcrBC*)	J. Gowrishankar
MG1655-Δ*hfl*X- hflKC$^+$	MG1655 strain with *hflX* deleted in frame	This study
DH10BΔX	DH10B strain with *hflX* deleted in frame	This study
DH10BΔKC	DH10B strain with *hflKC* deleted	This study
AK990	Δ*hflK-hflC*::*kan*/F′ *lacI*^{q} *lacZ*Δ*M15*	Kihara et al. (31)
Plasmids		
pDX101	Kan^r; *hflX* cloned in pET28a(+)	This study
pGDX101	Kan^r; *hflX* cloned in pGEX4T1	This study
pMPMK4	Kan^r; expression vector for pBS origin, under *araBAD*	Mayer (38)
pMPMX	Kan^r; *hflX* cloned in pMPMK4	This study
pKD4	Ap^r Kan^r; FRT-flanked *kan* cassette-bearing plasmid; oriR6K origin; template for PCR primers DELHFLX1 and DELHFLX2	Datsenko and Wanner (19)
pKD46	Ap^r, λ$^-$ Red recombinase expression plasmid	Datsenko and Wanner (19)
pCP20	Ap^r Cm^r, plasmid with Ts replicon and heat-inducible Flp recombinase gene	Cherepanov et al. (12)
pRecA	Ap^r, *recA* gene cloned in pBR322	J. Gowrishankar
pNT105	Cm^r, pACYC184 derivative expressing IS*903* transposase and carrying a cryptic *lacZ* transposon with IS*903* ends	Swingle et al. (49)
pET003	Ap^r, pBR322 derivative expressing Tn*552* transposase and TnpB and carrying a cryptic *lacZ* transposon with Tn*552* ends	Coros et al. (15)
pET005	Cm^r, pACYC184 derivative expressing Tn*10* transposase and carrying a cryptic *lacZ* transposon with Tn*10* ends	Coros et al. (15)
Primers		
PPD1	5′-GCGGATGGATCCTTGTTTGACCGTTATGA-3′[a]	
PPD2	5′-GCTCTGCCTCGAGCGTTAGATGAGGTAATC-3′[b]	
DELHFLX1	5′-CGGTACTGGTACACATCTATTTTACGCAAGACAAAGATATGGAAGACCT GTGTAGGCTGGAGCTGCTT-3′[c]	
DELHFLX2	5′-CTTCCCCACGGGTCGCGGTCTTGTCCGTTATTACCGGGCTGATTCCACGC CATATGAATATCCTCCTTAG-3′[d]	

[a] The BamHI site is underlined.
[b] The XhoI site is underlined.
[c] Nineteen-nucleotide priming site (underlined) with 49-nucleotide upstream sequence from the *hflX* gene.
[d] Twenty-nucleotide priming site (underlined) with 50-nucleotide downstream sequence from the *hflX* gene.

tested recombinant *E. coli* strains lacking the *hflX* gene (prepared by using an in-frame deletion that allows expression of the downstream genes *hflK* and *hflC*), as well as strains overexpressing this gene. Our results show that the lysogenic frequency of λ or the transposition frequency of transposable elements in *E. coli* is not affected by *hflX*.

MATERIALS AND METHODS

Materials. Oligonucleotide primers PPD1 and PPD2 (Table 1) for PCR amplification of the *hflX* gene using *E. coli* DH5α genomic DNA as the template were custom synthesized by Isogen, Germany. ^{32}P-labeled GTP and ATP were obtained from the Board of Radiation and Isotope Technology, India. Ni^{2+}-nitrilotriacetic acid (NTA) agarose, a PCR product purification kit, and an agarose gel DNA extraction kit were purchased from Qiagen (Germany), prepacked GSTrap columns and thrombin were purchased from GE Healthcare (United States), and a rapid ligation kit was purchased from Roche (Germany). All other reagents were procured from various vendors, such as Sigma, E. Merck, or Qualigen.

Bacterial strains BW25113, MG1655, GJ2433, and DH10B, starter P1 lysate, and plasmids pKD4, pKD46, pCP20, and pRecA were obtained from J. Gowrishankar, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India. Strain AK990 was provided by Y. Akiyama, and phage λc$^+$Cam105 (25) was a gift from Sankar Adhya. Plasmids pNT101, pET003, and pET005 were obtained from K. M. Derbyshire. Other strains and plasmids were available in our laboratory. Details of the various bacterial strains, plasmids, and primers used are shown in Table 1.

Methods. (i) Cloning and overexpression of HflX. The *hflX* gene from the *E. coli* K-12 genome was PCR amplified using primers PPD1 and PPD2 and was cloned between the BamHI and XhoI sites of the vector pET28a(+) or pGEX4T1 (Novagen). The resulting plasmid (pDX101 or pGDX101) was used for expression of the recombinant His_6-HflX or GST-HflX protein. For purification, *E. coli* strain BL21(DE3) harboring the plasmid was grown at 37°C in Terrific broth (46) until the absorbance at 590 nm reached 0.7, which was followed by induction with 400 μM isopropyl-β-D-thiogalactopyranoside (IPTG) and growth for 8 h at 25°C. The cells were harvested by centrifugation and stored at −20°C until further processing.

(ii) Purification of HflX. For pDX101, the cell pellet was resuspended in buffer A (20 mM Tris-HCl [pH 8.0], 500 mM NaCl, 10% glycerol) containing imidazole (15 mM), phenylmethylsulfonyl fluoride (100 μg/ml), and aprotinin (2 μM) and lysed by sonication. The lysate was centrifuged at 14,500 × g for 15 min, and the supernatant was centrifuged at 100,000 × g for 45 min to remove the membrane fraction. The supernatant was passed through an Ni^{2+}-NTA affinity column preequilibrated with buffer A. After the column was washed with buffer B (buffer A plus 25 mM imidazole), His_6-HflX was eluted from the column using buffer C (buffer A plus 500 mM imidazole). The eluted protein was dialyzed against buffer D (20 mM Tris-HCl [pH 8.0], 200 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol, 2 mM EDTA), and its purity was checked by 12.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), followed by silver staining and densitometric scanning.

For pGDX101, the purification scheme was similar, except that buffer A was

used for cell suspension, sonication, and loading onto a prepacked GSTrap column. The column was washed with buffer A, and the protein was eluted with buffer A containing 10 mM reduced glutathione. The eluted protein was dialyzed against buffer D and checked for purity as described above.

Concentrations of the purified proteins were determined by the Bradford assay with bovine serum albumin (BSA) as the standard (9, 20). When necessary, the N-terminal histidine or GST tag was removed by incubating 1 mg of purified recombinant protein with 15 U of thrombin at 14°C in buffer C for 14 to 16 h, according to the protocol supplied by the manufacturer (20).

(iii) Size exclusion chromatography. Size exclusion chromatography was carried out using an ÄKTA protein purification system (Amersham Biosciences, Sweden) with a Superdex G75 column run at 0.5 ml/min with buffer D without β-mercaptoethanol. The column was calibrated with BSA (66 kDa), *E. coli* cyclic AMP receptor protein (47 kDa), soybean trypsin inhibitor (20 kDa), and lysozyme (14 kDa) as molecular mass markers. Freshly prepared His_6-HflX (500 μl, 2 mg/ml) was loaded onto the column, and elution was recorded.

(iv) Dynamic light scattering. Dynamic light scattering was performed with a Zetasizer Nano ZS dynamic light scattering-molecular sizing instrument (Malvern Instruments). The scattering for His_6-HflX in buffer D was observed by exposing the sample to a 675-nm laser beam. The scattering was observed eight times with concentrations of the protein ranging from 5 μM to 25 μM at ambient temperature (~27°C). Scattering for BSA was measured in the same buffer under identical conditions. The scattering data were analyzed using the software provided by the instrument's manufacturer to obtain average diameters for the proteins.

(v) Determination of molar extinction coefficient and isoelectric points. The molar extinction coefficient for purified His_6-HflX was calculated from measurements of the concentration of the recombinant protein by using the method (9) of Bradford and absorption at 280 nm. For determination of the isoelectric points of His_6-HflX and HflX, 100-μg portions of the proteins were dialyzed against chilled double-distilled water for 2 h to completely remove buffers and salts before two-dimensional (2D) gel electrophoresis. Dialyzed samples formed precipitates, which were recovered by centrifugation at 5,000 rpm for 10 min, redissolved in 0.25 ml of rehydration stock solution containing 8 M urea, 2% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 2% Immobilon pH gradient buffer, a pinch of bromophenol blue, 8 mM phenylmethylsulfonyl fluoride, and 18 mM dithiothreitol, and then 30 μg of each protein was used for isoelectric focusing (15 min at 350 V, 4 h with a gradient from 350 V to 3,500 V, and 1 h at 3,500 V) on an IPG strip using a Multiphore apparatus (Amersham Pharmacia). For the second dimension, the IPG strip was equilibrated in sodium dodecyl sulfate (SDS) sample loading buffer for 1 h and placed on an SDS-PAGE resolving gel, which was run for 5 h at 200 V and then stained with Coomassie brilliant blue R. The proteins were detected from spots in the 2D gel, and isoelectric points were determined from the standard plot for a nonlinear pH 3 to 10 IPG strip (Immobilon DryStrip pH 3-10NL) supplied by the manufacturer.

(vi) CD. Far-UV circular dichroism (CD) spectra were recorded at 25°C with a JASCO J600 spectropolarimeter with a cuvette having a path length of 1 mm. A 4 μM protein solution was used. The spectrum was deconvoluted using *CDNN* (1, 6) for estimating the secondary structure components.

(vii) GTP-ATP binding assay. Nucleotide binding assays were performed as described by Sazuka et al. (47). Briefly, 5 μg of His_6-HflX was dissolved in 20 μl binding buffer G (20 mM Tris-HCl, 200 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol) containing 20 μCi of [α-^{32}P]GTP or [α-^{32}P]ATP (specific activities, 3,000 Ci/mmol and 4,500 Ci/mmol, respectively) and incubated at 30°C for 10 min. The samples were kept on ice and subjected to UV cross-linking with a UV cross-linker (Stratagene) for 7 min at 5 cm from the UV source, followed by 12.5% SDS-PAGE and autoradiography.

(viii) Assay for GTPase and ATPase activities. GTPase and ATPase activities of His_6-HflX were measured by determining the release of inorganic radioactive phosphates from corresponding ^{32}P-labeled nucleotide triphosphates. A 400-μl reaction mixture was prepared by mixing 100 μM GTP (or ATP) and 30 μCi of [γ-^{32}P]GTP (or [γ-^{32}P]ATP) in buffer G to which HflX was added to a final concentration of 1 μM. Immediately, the reaction mixture was incubated at 37°C for up to 80 min. Aliquots (50 μl) of the reaction mixture were removed at different time points, mixed thoroughly with 750 μl of a slurry of activated charcoal (5% charcoal in 50 mM NaH_2PO_4), and then incubated on ice for 15 min. The charcoal was pelleted by centrifugation, and the supernatants, containing liberated inorganic phosphates, were collected. Five microliters of each supernatant (in triplicate) was soaked in small pieces of Whatman blotting paper and mixed with 5 ml of scintillation cocktail, the radioactive counts of liberated phosphates were determined with a Wallac 1409 scintillation counter, and the amount of liberated phosphates was calculated from the values obtained.

(ix) Measurement of frequency of lysogenization. Bacterial cultures were grown to an A_{600} of 0.8 to 1.0 at 30°C in tryptone broth supplemented with 0.01% arabinose. Two-hundred-microliter cultures were mixed with an appropriate dilution of λc$^+$Cam105 phage stock so that the cells were infected at a multiplicity of infection (MOI) of 0.1 or 10.0, as needed. After 30 min of adsorption at 0°C, free phage (if any) were removed by centrifugation, which was followed by washing with cold 10 mM $MgSO_4$. The infected bacteria were resuspended in 1 ml LB medium and incubated for 30 min at 37°C or 42°C or for 10 min at 50°C. After appropriate dilution, 100-μl portions of cell suspensions were spread onto tryptone broth agar plates supplemented with 10 μg/ml of chloramphenicol and grown overnight at 30°C. The number of chloramphenicol-resistant colonies was counted to determine the number of lysogens. The lysogenic frequencies were determined by determining the ratio of the number of lysogens to the number of infective centers. For experiments using nutrient-depleted bacteria, cells were centrifuged before infection and were resuspended in 10 mM $MgSO_4$. They were then grown for 30 min at 37°C prior to infection so that all the remaining nutrients were used up.

(x) In vitro binding assay. In vitro pull-down assays were carried out to examine possible protein-protein interactions between HflX and HflK or HflC. Fifty micrograms of GST-HflX was incubated with 50 μl of glutathione Sepharose beads (Amersham Biosciences) preequilibrated in buffer P (20 mM Tris-HCl [pH 8.0], 100 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol). N-terminally hexahistidine-tagged HflK and HflC were overexpressed from *E. coli* BL21(DE3) cells in 25 ml LB broth. The cells were harvested and resuspended in the same buffer (buffer P), followed by sonication. The cell lysates were incubated with GST-HflX-bound glutathione beads for 1 h at ambient temperature (~28°C). The beads were then washed three times with buffer P and finally resuspended in 100 μl of the same buffer. Twenty microliters of this suspension was mixed with gel-loading dye and analyzed by 12.5% SDS-PAGE.

(xi) Construction of the Δ*hflX* strain. The *hflX* gene was deleted from the *E. coli* genome using the λ Red mutagenesis system (19). Briefly, a 1.5-kb PCR fragment was generated by performing a 30-cycle PCR with DeepVent polymerase, a pair of ~70-bp primers, DELHFLX1 and DELHFLX2 (Table 1), and purified pKD4 plasmid DNA as the template. The primers were 69 and 70 bases long, had ~50 bases that were homologous to the upstream and downstream regions of the *hflX* gene, and carried priming sequences (19 to 20 bases) from the upstream and downstream regions of an FRT-flanked selectable Kan^r gene of pKD4. Fifty microliters of electrocompetent BW25113 cells carrying pKD46, a λ Red recombinase-expressing helper plasmid, were transformed with 400 to 500 ng of PCR-amplified DNA and spread onto an agar plate to select for Kan^r (25 μg/ml) transformants at 37°C. After primary selection, cells were streaked on an LB plate at 42°C and selected for the loss of plasmid pKD46. The *hflX*::*kan* genotype was ensured by P1 transduction against the linked *mutL*::*tet* locus of strain GJ2433, as well as by PCR of the *hflX* gene.

(a) P1 transduction. The *hflX*::*kan* mutation in strain BW25113 was transduced into a DH10B or MG1655 strain by P1 transduction. Since transduction requires RecA, the *E. coli recA* gene was expressed in *trans* from plasmid pRecA (Table 1). After successful transduction, colonies were grown nonselectively at 37°C, and colonies cured of pRecA were selected. Similarly, the ΔKC genotype from AK990 was transduced into MG1655.

(b) Elimination of FRT-flanked *kan* cassette. The *kan* cassette was eliminated by using plasmid pCP20, a temperature-sensitive plasmid that codes for the Flp recombinase (12). After transformation of pCP20 into *hfl*X::*kan*-bearing DH10B or MG1655, cells were grown at 43°C, and this was followed by screening for loss of Kan^r or Ap^r.

(xii) Papillation assay. The possible role of HflX in transposition was checked by performing papillation assays as described by Swingle et al. (49). *E. coli* strains DH10B, DH10BΔX, and DH10BΔKC were each transformed with plasmids pNT105, pET003, and pET005 bearing cryptic transposons carrying *lacZ* (Table 1) and plated on an LB agar medium containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (50 μg/ml) and lactose (0.05%). The plates were incubated at 30°C for 4 days, and the number of blue papillae on each colony was counted using a simple microscope.

RESULTS

HflX is monomeric. HflX, overexpressed from plasmid pDX101 as an N-terminal hexahistidine-tagged fusion protein, formed an inclusion body at 37°C. When the induction was done at 25°C, more than 95% of the protein was found to remain in the soluble fraction after the sonication and high-

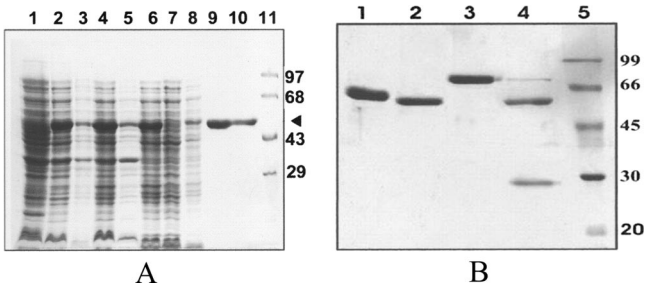

FIG. 1. (A) Purification of His_6-HflX. The 12.5% SDS-PAGE gel contained uninduced (lane 1) and induced (lane 2) cells, the pellet (lane 3) and supernatant (lane 4) obtained after sonication and high-speed centrifugation, the pellet (lane 5) and supernatant (lane 6) obtained after ultracentrifugation, the flowthrough (lane 7) and wash (lane 8) fractions from the Ni^{2+} affinity column, and purified protein bands (lanes 9 and 10, indicated by arrowhead) obtained by elution using 500 mM imidazole, along with molecular weight markers (lane 11). The numbers on the right indicate molecular masses (in kDa). (B) Recombinant proteins after removal of the tag by thrombin cleavage. The 12.5% SDS-PAGE gel contained His_6-HflX (lane 1) and GST-HflX (lane 3) and their thrombin cleavage products (lanes 2 and 4, respectively). Lane 5 contained molecular mass markers. The numbers on the right indicate molecular masses (in kDa).

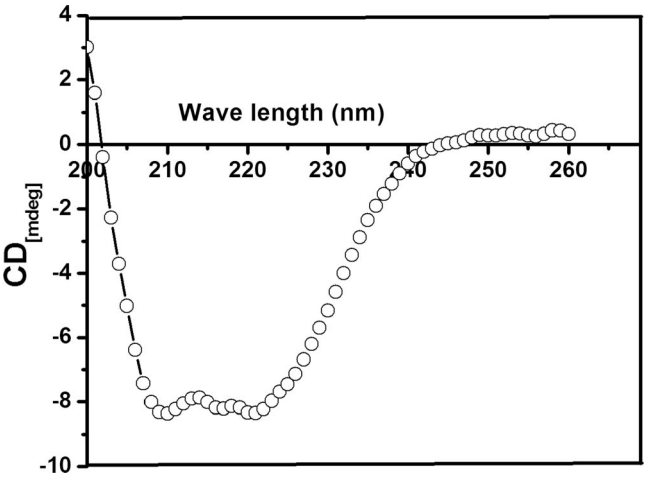

FIG. 3. Far-UV CD spectrum of HflX. The spectrum was recorded at 25°C with 4 μM of protein.

speed centrifugation steps. Ni-NTA affinity chromatography of the soluble fraction yielded a preparation containing 2.5 mg/ml with >95% purity (Fig. 1A). HflX was also purified as a GST-HflX fusion protein from plasmid pGDX101 (data not shown). The N-terminal tags were removed by cleavage with thrombin (Fig. 1B).

His_6-HflX eluted as a 54-kDa molecule in a Superdex G75 gel filtration column (calculated subunit molecular mass, 51.8 kDa), as shown in Fig. 2A, indicating that the protein was monomeric under native conditions. This result was verified by the results of dynamic light scattering experiments, which yielded an average diameter of 10.2 nm for His_6-HflX. Based on comparison with the monomeric 66-kDa protein BSA, which had an average diameter of 14.4 nm in similar studies (Fig. 2B), it is apparent that His_6-HflX is not an oligomeric protein but a monomer.

Other biophysical properties. The predicted molar extinction coefficient of His_6-HflX is 32,000 M^{-1} cm^{-1}, as determined from its sequence using the ProtParam software (http://expasy.ch/tools/protparam.html) (23). The experimentally determined value was found to be 29,982 ± 4,550 M^{-1} cm^{-1}, in good agreement with the value mentioned above. The isoelectric point of His_6-HflX was determined to be 5.7 from 2D gels, as described in Materials and Methods. This value compares well with the pI of 5.6 calculated using ProtParam (23).

The percentage of secondary structural elements of HflX predicted from its sequence and as analyzed by deconvolution of its far-UV CD spectrum (Fig. 3) shows that HflX has a large (30% to 40%) content of helices, although the amount of unstructured regions is also significant (Table 2).

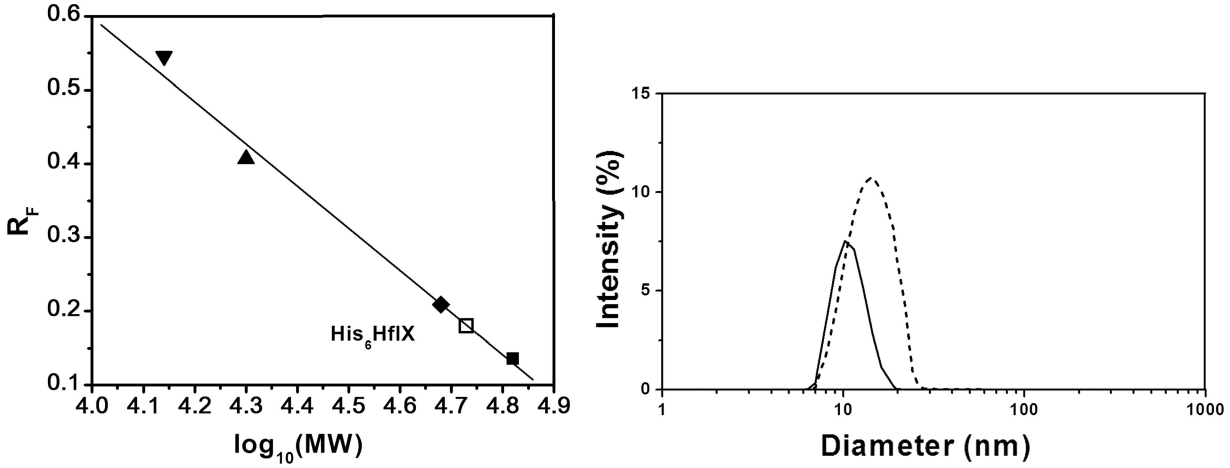

FIG. 2. (A) Gel filtration analysis of His_6-HflX: plot of R_f ($[V_e - V_0]/[V_t - V_0]$, where V_e, V_0, and V_t are the elution volume, void volume, and total column volume respectively)] versus the logarithm of molecular weight (MW) for the four standard proteins BSA, *E. coli* cyclic AMP receptor protein, soybean trypsin inhibitor, and lysozyme. The position of His_6-HflX (□), which eluted as a 54-kDa protein, is indicated. (B) Size distribution of His_6-HflX as obtained from dynamic light scattering. The average diameters obtained for His_6-HflX (solid line) and BSA (dashed line) by an analysis of scattering data at various concentrations (5 μM to 25 μM) are shown.

TABLE 2. Secondary structure of HflX

Secondary structure	% Predicted from sequence[a]	% Obtained from far-UV CD data[b]
Helix	41.5–48.6	29.3
Beta strands	15.7–18.8	19.7
Beta turns	0	17.6
Random coil	32.6–40.4	35.6

[a] The ranges of values predicted by the following programs available at the ExPASy website (www.expasy.ch) are shown: JPred (17), PSIPRED (10, 39), and Prof (43).
[b] Calculated by deconvolution of CD data, using *CDNN* (1, 6).

HflX exhibits GTPase and ATPase activities. HflX has been described as a putative GTP binding protein, possibly a GTPase (42). We assayed for the GTP binding ability of His_6-HflX using [α-^{32}P]GTP and UV cross-linking, as described in Materials and Methods. GTPase proteins are known to sometimes exhibit ATPase activity (2, 34). We therefore also tested His_6-HflX for ATP binding and found that this protein binds both GTP and ATP (Fig. 4). Additionally, His_6-HflX was found to release inorganic phosphate from GTP, as well as from ATP (Fig. 5). Thus, the protein possessed both GTPase and ATPase activities. As we have shown recently (28), the GTPase activity is inhibited by ATP, but inhibition of the ATPase activity of HflX by GTP requires much higher concentrations of GTP. Apparently, GTP and ATP bind to the same site on HflX.

Effect on lambda lysogeny. To check the role of HflX in the λ lysis-lysogeny decision, specific in-frame deletion of the *hflX* gene from the *E. coli* genome was done, using the λ Red mutagenesis system of Datsenko and Wanner (19) as described in Materials and Methods. Further, a recombinant *E. coli* strain in which *hflX* could be overexpressed from plasmid pMPMX by induction by arabinose was also prepared. Lysogenic frequencies were determined at an MOI of 10 using

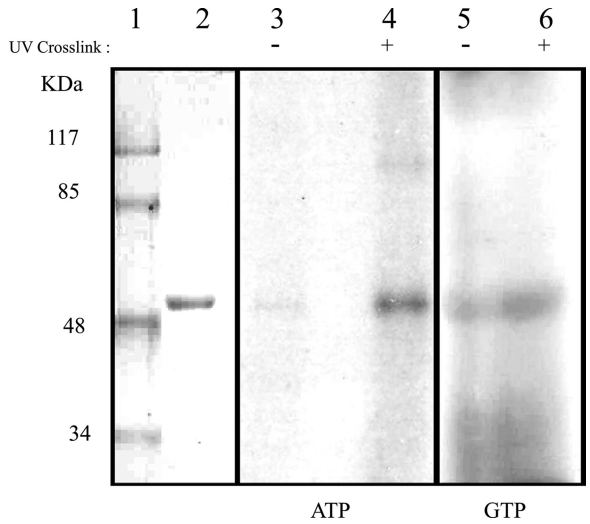

FIG. 4. GTP and ATP binding assays. Lane 1, molecular weight markers; lane 2, His_6-HflX; lanes 3 to 6, His_6-HflX incubated with [α-^{32}P]ATP (lanes 3 and 4) or [α-^{32}P]GTP (lanes 5 and 6). Samples in lanes 4 and 6 were subjected to UV cross-linking. Lanes 1 to 2 were stained with Coomassie blue, and lanes 3 to 6 were autoradiographed.

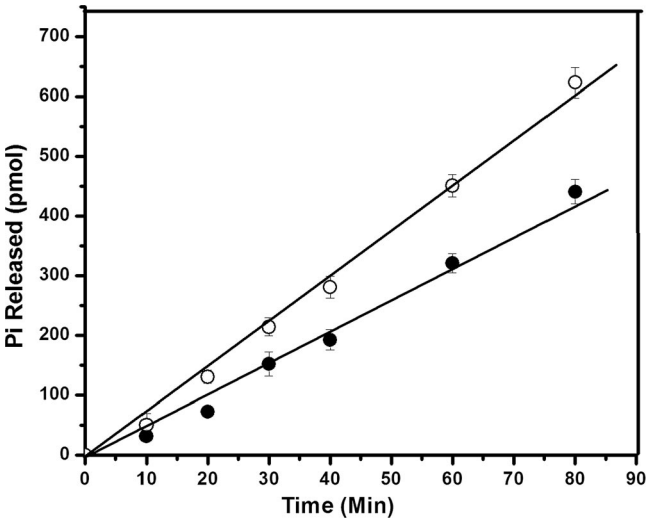

FIG. 5. GTPase and ATPase activities of HflX. The time kinetics of liberation of phosphate from [γ-^{32}P]ATP (○) and from [γ-^{32}P]GTP (●) by HflX are shown.

these strains at three different temperatures, as shown in Table 3. The two strains exhibited comparable frequencies of lysogenization indistinguishable from that observed for the control strain, MG1655 (6 to 9%). When nutrient-deficient cells were used (at an MOI of 0.1), the lysogenic frequency increased to 15 to 16% for all the strains, but there was little difference among the strains (Table 3). On the other hand, we observed that an AK990 strain carrying Δ*hfl*KC::*kan* showed a lysogenic frequency of 38% at an MOI of 0.1 as determined by similar assays. Thus, *hflX* has no effect on lysogeny of the phage and is unlikely to be responsible for the high-frequency lysogenization phenotype shown by the *hflA* locus.

HflX does not interact with HflK or HflC. Possible binding of HflX to HflK and to HflC was tested by performing GST pull-down assays, using GST-HflX and His_6-HflKC. As shown in Fig. 6, only GST-HflX was found to be associated with the GST beads, showing a lack of interaction between GST-HflX and His_6-HflK or His_6-HflC. In separate experiments, GST-HflB was found to interact with His_6-HflKC (our unpublished results), suggesting that the terminal tags did not influence these intermolecular interactions.

TABLE 3. Effect of *hflX* on the frequency of lysogenization by λc$^+$Cam105

E. coli strain	Genotype	Frequency of lysogenization (%)[a]			
		37°C	42°C	50°C	Starved cells
MG1655	Wild type	6.5	9.4	9.2	15.1
MG1655-Δ*hflX*-hflKC$^+$	*hflX* deleted in frame	6.8	7.6	8.4	15.8
MG1655(pMPMX)	*hflX* overexpressed	7.3	6.0	8.0	15.6

[a] The frequency of lysogenization at 37°C, 42°C, and 50°C was determined by counting the number of chloramphenicol-resistant clones per 100 λc$^+$Cam105-infected cells, using an MOI of 10. The frequency of lysogenization of starved cells was determined as described above using nutrient-depleted cells grown at 37°C and infected at an MOI of 0.1.

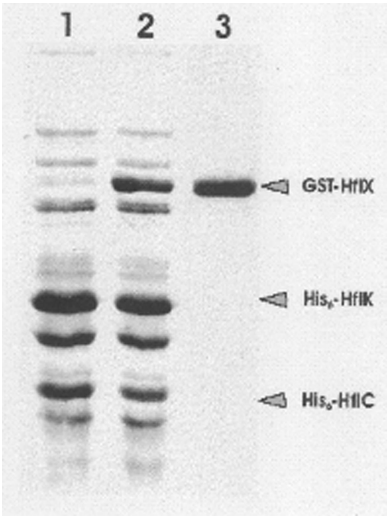

FIG. 6. GST pull-down assay for the interaction between GST-HflX and His_6-HflK or His_6-HflC. Lane 1, crude extract of cells overexpressing His_6-HflK and His_6-HflC (indicated by arrowheads); lane 2, glutathione Sepharose beads bound with GST-HflX were mixed with cell lysate overexpressing His_6-HflKC and incubated at the ambient temperature for 1 h; lane 3, preparation after the mixture described above was washed with buffer P. The samples were run on a 12.5% SDS-PAGE gel.

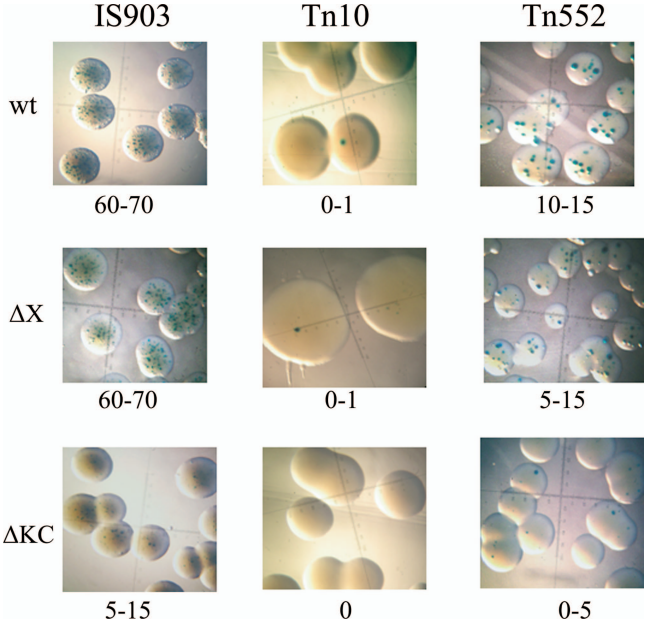

FIG. 7. Effect of HflX and HflKC on transposition, examined by the papillation assay. Wild-type (DH10B) *E. coli* cells (wt) and the mutants in which either the *hflX* gene (ΔX) or the *hflKC* genes (ΔKC) were removed by in-frame deletion (DH10BΔX or DH10BΔKC) were separately transformed with plasmids containing IS*903*, Tn*10*, and Tn*552*. Each of these plasmids bears a transposon with a cryptic *lacZ* reporter. The number of blue papillae was counted for each colony. The average number of papillae per colony is indicated below each panel.

HflX has no role in the transposition of transposable elements. As mentioned above, disruption of *hflX* and subsequent downregulation of transposition frequency have been reported previously (54). However, from these results it would be improper to conclude that *hflX* is involved in transposition of transposable elements, since the disruption also caused inactivation of the downstream genes, *hflK* and *hflC*, due to transcriptional polarity. In our experimental approach we carefully carried out in-frame deletion of the *hflX* gene without any polar effect on *hflKC* (DH10BΔX) and also prepared another strain (DH10BΔKC) in which *hflX* was intact but the downstream *hflKC* genes were deleted (described in Materials and Methods). Papillation assays were carried out using these strains, as shown in Fig. 7. The transposition frequency was calculated semiquantatively by counting the average number of papillae per colony. It is evident that the transposition frequency of DH10BΔX was similar to that of control strain DH10B, in which *hflX* was not disturbed. Only in the case of an *hflKC* mutant (DH10BΔKC) was a reduction in the transposition frequency observed. For Tn*10*, however, we observed 0 or 1 papilla per colony for the control, in contrast to 15 to 20 papillae per colony observed by Twiss et al. (54). Nevertheless, it is clear that the previously reported effect on transposition (54) is likely to have been due to the effect of one or both of the downstream genes, *hflK* and *hflC*, rather than *hflX*.

DISCUSSION

HflX has been predicted to be a GTPase based on sequence signatures, viz., the four highly conserved sequence motifs, G1 (P-loop or Walker A motif), G2, G3 (Walker B motif), and G4 (determinant of GTP specificity) (8, 18). Phylogenetically, HflX is closest to another prokaryotic protein, Obg, which is also a GTPase (36). We find that HflX is indeed a GTPase. In addition, it also exhibits ATPase activity in vitro. In this respect it differs from Obg, which has strict specificity for GTP. Insertional mutagenesis that caused polarity of *hflX* expression did not result in distinct phenotypes in *E. coli* (53). Thus, unlike Obg, HflX does not appear to be an essential gene in *E. coli*.

In our study, a portion of the overexpressed HflX protein appeared in the membrane fraction, while larger amounts fractionated with the cytoplasmic pool (our unpublished data). In *Corynebacterium glutamicum*, HflX has been found to occur equally in the cytoplasm and in the membrane fractions (22). No transmembrane region, however, could be predicted from the primary sequence of HflX, either in *E. coli* or in *C. glutamicum*. Thus, the possible interaction of HflX with the membrane is likely to be mediated by other membrane proteins. Using GST pull-down assays, no interaction between HflX and HflKC could be detected (Fig. 6). On the other hand, addition of the nonionic detergent Triton X-100 increased the ATPase activity of HflX by 35% (our unpublished data). Therefore, involvement of an unidentified protein in the membrane attachment of HflX cannot be ruled out.

The *hflX* gene was first mentioned in connection with a high frequency of lysogenization, as the designation implies. It was believed that *hflX* might have a role in the life cycle of phage λ (3). However, unlike deletion of *hflK* or *hflC*, specific deletion of *hflX* in *E. coli* did not alter the lysogenization frequency of λ (32). Our results comprehensively rule out any role of *hflX* in lysogeny, since neither deletion nor overexpression of the gene had any effect on the lysogenization frequency (Table 3).

The *hflX* gene belongs to a large superoperon, which comprises genes which have been reported to be involved in functions related to some stress condition (52). Although our experiments do not point toward a definite function of HflX, there are indications that *hflX* shows a response to stress; overexpression of the gene under heat shock conditions (13, 45), downregulation of *hflX* in the presence of ofloxacin (30), and upregulation under osmotic stress conditions (55) have been reported. Since papilla development occurs after 3 or 4 days under nutrient stress conditions (54), it was expected that *hflX* may have some effect on the response to transposition, as shown by the papillation assay. Contrary to the earlier report (54), however, we found that the inactivation of *hflKC* rather than the inactivation of *hflX* conferred downregulation of papilla development (Fig. 7).

Thus, the biological role of HflX remains elusive. The only definitive property that could be confirmed for HflX is that it is a P-loop GTPase and ATPase. Generally, P-loop nucleoside triphosphatases comprise 10 to 18% of all gene products in most cellular organisms (35). HflX proteins from *E. coli* and *C. pneumoniae* both bind to the 50S ribosomal subunit of *E. coli* (28, 44). Nevertheless, the function of this protein remains enigmatic. Paradoxically, HflX is apparently dispensable in *E. coli*, although the expression of *hflX* in wild-type bacteria is under tight regulation (3, 13). The presence of transcript processing sites inside *hflX* mRNA (52) and the presence of UUG rather than AUG as the initiation codon in the *hflX* gene (42) may restrict its expression. It is possible that HflX plays an active role in *E. coli* cells under special stress conditions. The search for the function of an enigmatic protein like HflX must begin with a proper examination of its proposed functions. This paper is a first attempt in that direction.

ACKNOWLEDGMENTS

We thank J. Gowrishankar of the Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India, for help in carrying out in-frame deletion of the *hflX* gene; Anjan Dasgupta (Calcutta University) for help with dynamic light scattering experiments; and Sujoy Dasgupta (Bose Institute) for his assistance with determining the isoelectric point of HflX. Various phages, bacterial strains, and plasmids were provided by N. C. Mandal (Bose Institute), J. Gowrishankar (Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India), Sankar Adhya (National Cancer Institute, NIH), Y. Akiyama (Kyoto University), and K.M. Derbyshire (Wadsworth Center, Albany, NY).

K.B. acknowledges CSIR, India, for a fellowship.

REFERENCES

1. **Andrade, M. A., P. Chacon, J. J. Merelo, and F. Moran.** 1993. Evaluation of secondary structure of proteins from UV circular dichroism spectra using an unsupervised learning neural network. Protein Eng. **6:**383–390.
2. **Arvidson, C. G., and M. So.** 1997. Isolation and biochemical characterization of the PilA protein of *Neisseria meningitides.* Arch. Biochem. Biophys. **348:** 357–362.
3. **Banuett, F., and I. Herskowitz.** 1987. Identification of polypeptides encoded by an *Escherichia coli* locus (*hflA*) that governs the lysis-lysogeny decision of bacteriophage lambda. J. Bacteriol. **169:**4076–4085.
4. **Banuett, F., M. A. Hoyt, L. Mcfarlane, H. Echols, and I. Herskowitz.** 1986. HflB, a new locus regulating lysogeny and the level of bacteriophage lambda CII protein. J. Mol. Biol. **187:**213–224.
5. **Belfort, M., and D. L. Wulff.** 1971. A mutant of *Escherichia coli* that is lysogenized with high frequency, p. 739–742. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
6. **Bohm, G., R. Muhr, and E. Jaenicke.** 1992. Quantitative analysis of protein far UV circular dichroism spectra by neural networks. Protein Eng. **5:**191–195.
7. **Bourne, H. R., D. A. Sanders, and F. McCormick.** 1990. The GTPase superfamily: a conserved switch for diverse cell functions. Nature **348:**125–132.
8. **Bourne, H. R., D. A. Sanders, and F. McCormick.** 1991. The GTPase superfamily: conserved structure and molecular mechanism. Nature **349:**117–127.
9. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
10. **Bryson, K., L. J. McGuffin, R. L. Marsden, J. J. Ward, S. Sodhi, and D. T. Jones.** 2005. Protein structure prediction servers at University College London. Nucleic Acids Res. **33:**W36–W38.
11. **Caillet, J., and L. Droogmans.** 1988. Molecular cloning of the *Escherichia coli miaA* gene involved in the formation of delta 2-isopentenyl adenosine in tRNA. J. Bacteriol. **170:**4147–4152.
12. **Cherepanov, P. P., and W. Wackernagel.** 1995. Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene **158:**9–14.
13. **Chuang, S. E., and F. R. Blattner.** 1993. Characterization of twenty-six new heat shock genes of *Escherichia coli.* J. Bacteriol. **175:**5242–5252.
14. **Connolly, D. M., and M. E. Winkler.** 1989. Genetic and physiological relationships among the *miaA* gene, 2-methylthio-N^6-(delta 2-isopentenyl)-adenosine tRNA modification, and spontaneous mutagenesis in *Escherichia coli* K-12. J. Bacteriol. **171:**3233–3246.
15. **Coros, A. M., E. Twiss, N. P. Tavakoli, and K. M. Derbyshire.** 2005. Genetic Evidence that GTP is required for transposition of IS*903* and Tn*552* in *Escherichia coli.* J. Bacteriol. **187:**4598–4606.
16. **Court, D. L., A. B. Oppenheim, and S. L. Adhya.** 2007. A new look at bacteriophage λ genetic networks. J. Bacteriol. **189:**298–304.
17. **Cuff, J. A., M. E. Clamp, A. S. Siddiqui, M. Finlay, and G. J. Barton.** 1998. Jpred: a consensus secondary structure prediction server. Bioinformatics **14:**892–893.
18. **Daigle, D. M., L. Rossi, A. M. Berghuis, L. Arvind, E. V. Konnin, and E. D. Brown.** 2002. YjeQ, an essential, conserved, uncharacterized protein from Escherichia coli, is an unusual GTPase with circularly permutated G-motifs and marked burst kinetics. Biochemistry **41:**11109–11117.
19. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
20. **Dutta, D.** 2006. Studies on the protein HflX from *Escherichia coli*: searching for its function. Ph.D. dissertation. Jadavpur University, Kolkata, India.
21. **Echols, H.** 1986. Bacteriophage λ development: temporal switches and the choice of lysis or lysogeny. Trends Genet. **2:**26–30.
22. **Engels, S., C. Ludwig, J. E. Schweitzer, C. Mack, M. Bott, and S. Schaffer.** 2005. The transcriptional activator ClgR controls transcription of genes involved in proteolysis and DNA repair in *Corynebacterium glutamicam.* Mol. Microbiol. **57:**576–591.
23. **Gasteiger, E., C. Hoogland, A. Gattiker, S. Duvaud, M. R. Wilkins, R. D. Appel, and A. Bairoch.** 2005. Protein identification and analysis tools on the ExPASy server, p. 571–607. *In* J. M. Walker (ed.),The proteomics protocols handbook. Humana Press, Totowa, NJ.
24. **Geissmann, T. A., and D. Touati.** 2004. Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator. EMBO J. **23:**396–405.
25. **Herman, C., T. Ogura, T. Tomoyasu, S. Hiraga, Y. Akiyama, K. Ito, R. Thomas, R. D'Ari, and P. Bouloc.** 1993. Cell growth and λ phage development controlled by the same essential *Escherichia coli* gene, *fts*H/*hfl*B. Proc. Natl. Acad. Sci. USA **90:**10861–10865.
26. **Herskowitz, I., and D. Hagen.** 1980. The lysis-lysogeny decision of phage λ: explicit programming and responsiveness. Annu. Rev. Genet. **14:**399–445.
27. **Hoyt, M. A., D. M. Knight, A. Das, H. I. Miller, and H. Echols.** 1982. Control of phage lambda development by stability and synthesis of cII protein: role of the viral cIII and host hflA, himA and himD genes. Cell **31:**565–573.
28. **Jain, N., N. Dhimole, A. R. Khan, D. De, S. K. Tomar, M. Sajish, D. Dutta, P. Parrack, and B. Prakash.** 25 December 2008, posting date. *E. coli* HflX interacts with 50S ribosomal subunits in presence of nucleotides. Biochem. Biophys. Res. Commun. doi:10.1016/j.bbrc.2008.12.072.
29. **Kajitani, M., A. Kato, A. Wada, Y. Inokuchi, and A. Ishihama.** 1994. Regulation of the *Escherichia coli hfq* gene encoding the host factor for phage Q beta. J. Bacteriol. **176:**531–534.
30. **Kaldalu, N., R. Mei, and K. Lewis.** 2004. Killing by ampicillin and ofloxacin induces overlapping changes in *Escherichia coli* transcription profile. Antimicrob. Agents Chemother. **48:**890–896.
31. **Kihara, A., Y. Akiyama, and K. Ito.** 1996. A protease complex in the *Escherichia coli* plasma membrane: HflKC (HflA) forms a complex with FtsH (HflB), regulating its proteolytic activity against SecY. EMBO J. **15:**6122–6131.
32. **Kihara, A., Y. Akiyama, and K. Ito.** 1997. Host regulation of lysogenic decision in bacteriophage λ: transmembrane modulation of FtsH (HflB), the cII degrading protease, by HflKC (HflA). Proc. Natl. Acad. Sci. USA **94:** 5544–5549.
33. **Kihara, A., Y. Akiyama, and K. Ito.** 2001. Revisiting the lysogenisation control of bacteriophage λ. Identification and characterization of a new host component, HflD. J. Biol. Chem. **276:**13695–13700.
34. **Klinger, C., M. Rossbach, R. Howe, and M. Kaufmann.** 2003. Thermophile-

specific proteins: the gene product of *aq_1292* from *Aquifex aeolicus* is an NTPase. BMC Biochem. **4:**12.
35. **Koonin, E. V., Y. I. Wolf, and L. Arvind.** 2000. Protein fold recognition using sequence profiles and its application in structural genomics. Adv. Protein Chem. **54:**245–275.
36. **Leipe, D. D., Y. I. Wolf, E. V. Koonin, and L. Arvind.** 2002. Classification and evolution of P-loop GTPases and related ATPases. J. Mol. Biol. **317:**41–72.
37. **Lieb, M., and S. Rehmat.** 1995. Very short patch repair of T:G mismatches in vivo: importance of context and accessory proteins. J. Bacteriol. **177:**660–666.
38. **Mayer, M. P.** 1995. A new set of useful cloning expression vectors derived from pBlueScript. Gene **163:**41–46.
39. **McGuffin, L. J., K. Bryson, and D. T. Jones.** 2000. The PSIPRED protein structure prediction server. Bioinformatics **16:**404–405.
40. **Mellon, I., and G. Champe.** 1996. Products of DNA mismatch repair genes mutS and mutL are required for transcription-coupled nucleotide-excision repair of the lactose operon in *Escherichia coli.* Natl. Acad. Sci. USA **93:** 1292–1297.
41. **Modrich, P.** 1995. Mismatch repair, genetic stability and tumour avoidance. Phil. Trans. R. Soc. Lond. B **347:**89–95.
42. **Noble, J. A., M. A. Innis, E. V. Koonin, K. E. Rudd, F. Banuett, and I. Herskowitz.** 1993. The Escherichia coli *hfl*A locus encodes a putative GTP-binding protein and two membrane proteins, one of which contains a protease-like domain. Proc. Natl. Acad. Sci. USA **90:**10866–10870.
43. **Ouali, M., and R. D. King.** 2000. Cascaded multiple classifiers for secondary strucure prediction. Protein Sci. **9:**1162–1176.
44. **Polkinghorne, A., U. Ziegler, Y. Gonzalez-Hernandez, A. Pospischil, P. Timms, and L. Vaughan.** 2008. *Chlamydophila pneumoniae* HflX belongs to an uncharacterized family of conserved GTPases and associates with the *Escherichia coli* 50S large ribosomal subunit. Microbiology **154:**3537–3546.
45. **Richmond, C. S., J. D. Glasner, R. Mau, H. Jin, and F. R. Blattner.** 1999. Genome-wide expression profiling in *Escherichia coli* K-12. Nucleic Acids Res. **27:**3821–3835.
46. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., p. A.1-A.2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
47. **Sazuka, T., Y. Tomooka, Y. Ikawa, M. Noda, and S. Kumar.** 1992. Drg: a novel developmentally regulated GTP-binding protein. Biochem. Biophys. Res. Commun. **189:**363–370.
48. **Shotland, Y., S. Koby, D. Teff, N. Mansur, D. A. Oren, K. Tatematsu, T. Tomoyasu, M. Kessel, B. Bukau, T. Ogura, and A. B. Oppenheim.** 1997. Proteolysis of the phage lambda CII regulatory protein by FtsH (HflB) of *Escherichia coli.* Mol. Microbiol. **24:**1303–1310.
49. **Swingle, B., M. O'Carroll, D. Haniford, and K. M. Derbyshire.** 2004. The effect of host-encoded nucleoid proteins on transposition: H-NS influences targeting of both IS903 and Tn10. Mol. Microbiol. **52:**1055–1067.
50. **Tomoyasu, T., K. Yamanaka, K. Murata, T. Suzaki, P. Bouloc, A. Kato, H. Niki, S. Hiraga, and T. Ogura.** 1993. Topology and subcellular localization of FtsH protein in *Escherichia coli.* J. Bacteriol. **175:**1352–1357.
51. **Tomoyasu, T., T. Yuki, S. Morimura, H. Mori, K. Yamanaka, H. Niki, S. Hiraga, and T. Ogura.** 1993. The *Escherichia coli* FtsH protein is a prokaryotic member of a protein family of putative ATPases involved in membrane functions, cell cycle control, and gene expression. J. Bacteriol. **175:**1344–1351.
52. **Tsui, H. C., G. Feng, and M. E. Winkler.** 1996. Transcription of the *mutL* repair, *miaA* tRNA modification, *hfq* pleiotropic regulator, and *hflA* region protease genes of *Escherichia coli* K-12 from clustered $E\sigma^{32}$-specific promoters during heat shock. J. Bacteriol. **178:**5719–5731.
53. **Tsui, H. C., H. C. Leung, and M. E. Winkler.** 1994. Characterization of broadly pleiotropic phenotypes caused by an hfq insertion mutation in Escherichia coli K-12. Mol. Microbiol. **13:**35–49.
54. **Twiss, E., A. M. Coros, N. P. Tavakoli, and K. M. Derbyshire.** 2005. Transposition is modulated by a diverse set of host factors in *Escherichia coli and* is stimulated by nutritional stress. Mol. Microbiol. **57:**1593–1607.
55. **Weber, A., A. S. Kogl, and K. Jung.** 2006. Time-dependent proteome alterations under osmotic stress during aerobic and anaerobic growth in *Escherichia coli.* J. Bacteriol. **188:**7165–7175.
56. **Wegrzyn, G., and A. Wegrzyn.** 2005. Genetic switches during bacteriophage λ development. Prog. Nucleic Acid Res. Mol. Biol. **7:**1–48.