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# The *osmZ* (*bglY*) gene encodes the DNA-binding protein H-NS (H1a), a component of the *Escherichia coli* K12 nucleoid

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**Summary.** A class of *trans*-acting mutations, which alter the osmoregulated expression of the *Escherichia coli proU* operon, maps at 27 min on the chromosome in a locus we have called *osmZ*. Mutations in *osmZ* are allelic to *bglY, pilG* and *virR*, affect gene expression, increase the frequency of the site-specific DNA inversion mediating fimbrial phase variation, stimulate the formation of deletions, and influence in vivo supercoiling of reporter plasmids. We have cloned the $osmZ^+$ gene, mapped it at 1307 kb of the *E. coli* restriction map, identified its gene product as a 16 kDa protein, and determined the nucleotide sequence of the $osmZ^+$ gene. The deduced amino acid sequence for OsmZ predicts a protein of 137 amino acid residues with a calculated molecular weight of 15530. The primary sequence of OsmZ is identical to that of H-NS (H1a), a DNA-binding protein that affects DNA topology and is known to be associated with the bacterial nucleoid. Thus, *osmZ* is the structural gene for the H-NS (H1a) protein. The nucleotide sequence of *osmZ* is almost identical to that of *hns*; however, *hns* was incorrectly located at 6.1 min on the *E. coli* linkage map. Increased *osmZ* gene dosage leads to cell filament formation, altered gene expression, and reduced frequency of fimbrial phase variation. Our results suggest that the nucleoid-associated DNA-binding protein H-NS (H1a) plays a critical role in gene expression and in determining the structure of the genetic material.

**Key words:** Cell morphology – DNA topology – Gene expression – Gene mapping – Osmoregulation

## Introduction

The enteric bacteria *Escherichia coli* and *Salmonella typhimurium* can adapt to an increase in medium osmolarity by various mechanisms, one of which involves the uptake and intracellular accumulation of glycine betaine. At low substrate concentrations, uptake of glycine betaine is primarily mediated by the osmoregulated, high-affinity, binding protein-dependent transport system ProU (for an overview see Csonka 1989). An increase in the external osmolarity triggers a rapid and large increase in *proU* transcription, which is maintained for as long as the osmotic stimulus persists (Cairney et al. 1985; Dunlap and Csonka 1985; Gowrishankar 1985; Barron et al. 1986; May et al. 1986; Sutherland et al. 1986; Gutierrez et al. 1987). Mutants with altered osmoregulation of *proU* carry either *cis*-acting mutations closely linked to *proU* (Druger-Liotta et al. 1987; May et al. 1989) or *trans*-acting mutations in *topA*, the structural gene for DNA topoisomerase I, or in *osmZ* (Higgins et al. 1988).

The *osmZ* locus has been genetically mapped at 27 min on the *E. coli* linkage map and *osmZ* mutations have been shown to be allelic with *pilG* and *bglY* (Higgins et al. 1988). Mutations in *pilG* lead to a greatly increased frequency of phase variation in type I fimbriae (Spears et al. 1986), which is mediated by the site-specific inversion of a small DNA segment that carries the promoter for the *fimA* structural gene (Abraham et al. 1985). Mutations in *bglY* activate the expression of the cryptic *bgl* operon for β-glucoside uptake and metabolism (Defez and DeFelice 1981; Schnetz et al. 1987; Mahadevan et al. 1987). They also result in a mutator phenotype by increasing the frequency of deletions in various regions of the *E. coli* chromosome (Lejeune and Danchin 1990). Mutations in *osmZ* increase *proU* transcription both at low and at high osmolarity (Higgins et al. 1988) and alter the osmotically controlled expression of the *ompC* and *ompF* porin genes (Graeme-Cook et al. 1989). Furthermore, *osmZ* is equivalent to the *virR* gene in *Shigella flexneri* (Dorman et al. 1990), which affects the temperature-regulated expression of virulence genes residing on a 220 kb plasmid (Maurelli and Sansonetti 1988). A possible link between the diverse phenotypes of *osmZ* strains is provided by the finding that negative supercoiling of reporter plasmids is increased

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in these strains (Higgins et al. 1988). Since such an increase was also observed in *osmZ*$^+$ strains that were grown at high osmolarity, we previously proposed that DNA topology is an important determinant for the osmoregulated expression of *proU* (Higgins et al. 1988). To gain insight into the physiological function of the *osmZ*-encoded protein, we have cloned this gene, identified its product and determined its nucleotide sequence.

## Materials and methods

*Bacterial strains, plasmids, and bacteriophages.* The bacterial strains, plasmids, and bacteriophages used in this study are listed in Table 1.

*Media, chemicals, and growth conditions.* Rich (LB) and minimal media with 0.2% glucose as the carbon source (Minimal Medium A, MMA) were prepared as described (Silhavy et al. 1984). The osmolarity of liquid minimal media was elevated by the addition of NaCl as required. Lactose MacConkey agar plates (Silhavy et al. 1984) and BS agar plates containing bromothymolblue and salicin (Schnetz et al. 1987) were used to score Lac and Bgl phenotypes, respectively. Agar plates containing 0.1 ml of a 10 mg/ml solution of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) in dimethylformamide were used to distinguish between LacZ$^+$ and LacZ$^-$ phage plaques and colonies. Antibiotics were used at the following concentrations: kanamycin (Km), 30 mg/l; ampicillin (Ap), 50 mg/l; tetracycline (Tc), 5 mg/l; and chloramphenicol (Cm), 25 mg/l. Cells were grown at 37° C with vigorous aeration, with the exception of bacterial strains carrying the mini-Mu element pEG5005 or its derivatives, which were grown at 28° C.

*Genetic techniques and strain constructions.* Standard techniques were used for the growth of bacteria and bacteriophages, generalized transduction with P1*vir*, and the formation of λ lysogens (Silhavy et al. 1984). Strains RK5048 and TG1 (Table 1) were used for maxicell experiments and propagation of derivatives of M13mp18 and M13mp19, respectively. A pair of *osmZ*$^+$ and *osmZ200* strains, GM344 and GM345, was constructed by transducing the *Φ(fimA-lacZ*$^+$*)* operon fusion from strain VL386 (Table 1) into strains MC4100 and GM125 (Table 1) and selecting for Lac$^+$ colonies on lactose minimal plates. Strain GM125 is a derivative of strain BRE2071 from which the *Φ(proU-lacZ)*hyb2 fusion was removed with the aid of an *srl*::Tn*10* insertion which is linked to the *proU*$^+$ operon (May et al. 1986). Phage λpGM27 is an *imm21* derivative of the specialized transducing phage λpGM1 carrying the *Φ(proU-lacZ)*-hyb2 fusion (Barron et al. 1986), from which the *kan* gene was removed by a phage-phage cross with *λimm21*. To isolate transposon insertions in plasmid pGM7, strain MC4100 (pGM7) was infected with λ::Tn*5* or λ::Tn*5*-B20 and Km$^r$ Cm$^r$ colonies were selected on LB agar plates (de Bruijn and Lupski 1984; Simon et al. 1989). These colonies were pooled, and their plasmid DNA was extracted and transformed into a strain har-

**Table 1.** Bacteria, bacteriophages, and plasmids

| Strain | Description[a] | Origin/ Reference |
|---|---|---|
| Strains derived from *Escherichia coli* K12 | | |
| MC4100 | F$^-$ *Δ(argF-lac)U169 araD139 rpsL150 deoC1 relA1 ptsF25 flbB5301 rbsR* | Casadaban (1976) |
| VL386 | *ara Δ(lac-pro) rpsL thi Φ(fimA-lacZ*$^+$*)* (λp1(209)) | Freitag et al. (1985) |
| TG1 | *Δ(lac-pro) supE thi hsd5*/F′ *traD36 proA*$^+$*B*$^+$ *lacI*$^q$ *Δ(lacZ)* M15 | Carter et al. (1985) |
| RK5048 | MC4100 *gyrA219 non metE70 recA tonB* | Mann et al. (1986) |
| BRE2071 | MC4100 *osmZ200 Φ(proU-lacZ)* hyb2 (λp*lac*Mu15) | Higgins et al. (1988) |
| GM37 | MC4100 *Φ(proU-lacZ)* hyb2 (λp*lac*Mu15) | May et al. (1986) |
| GM125 | BRE2071 *srl*::Tn*10 proU*$^+$ | Higgins et al. (1988) |
| GM230 | GM37 *osmZ205*::Tn*10* | Higgins et al. (1988) |
| GM301 | MC4100 Mucts62 Ap$^r$; pEG5005 | This work |
| GM305 | GM125 Mucts62 Ap$^r$ (λpGM27) | This work |
| GM344 | MC4100 *Φ(fimA-lacZ*$^+$*)* (λp1(209)) | This work |
| GM345 | GM125 *Φ(fimA-lacZ*$^+$*)* (λp1(209)) | This work |
| Bacteriophages | | |
| λ::Tn*5* | *λb221 rex*::Tn*5 cI857 O*am*29 P*am*80* | De Bruijn and Lupski (1984) |
| λ::Tn*5*-B20 | *λb221 cI857 P*am*80* Tn*5*-B20 | Simon et al. (1989) |
| λpGM1 | λp*lac*Mu15 *Φ(proU-lacZ)*hyb2; Km$^r$ | Barron et al. (1986) |
| λpGM27 | λpGM1 *imm21*; Km$^s$ | This work |
| *λimm21* | | Laboratory collection |
| Plasmids | | |
| pEG5005 | minu-Mu element; Km$^r$ | Groisman and Casadaban (1986) |
| pHSG575 | cloning vector; Cm$^r$ | Takeshita et al. (1987) |
| pGM1 | *osmZ*$^+$ derivative of pEG5005; Fig. 1A | This work |
| pGM2 | *osmZ*$^-$ derivative of pGM7; Fig. 1A | This work |
| pGM7 | *osmZ*$^+$ derivative of pHSG575; Fig. 1A | This work |
| pGM10 | *osmZ*$^+$ derivative of pHSG575; Fig. 1A | This work |

[a] The symbol *Φ* indicates the presence of a *lacZ* fusion, and the abbreviation hyb indicates that the gene fusion encodes a hybrid protein. The symbols *lacZ*$^+$ and *lacZ* denote *lacZ* genes with or without translational initiation signals, respectively

bouring the *osmZ200* mutation by selecting for $Km^r$ $Cm^r$ transformants. The *osmZ200* strain exhibits a $Bgl^+$ phenotype on BS agar plates (orange colonies), and a chromosomal *proU-lacZ* fusion is expressed at low osmolarity to such a degree that the strain forms $Lac^+$ (red) colonies on lactose MacConkey indicator plates (Higgins et al. 1988). Complementation of the $Lac^+$ $Bgl^+$ phenotypes by the plasmids with transposon insertions was tested by streaking colonies on indicator plates.

For cloning of the *osmZ* gene, a gene bank was prepared in vivo after heat induction of strain GM301 (*osmZ*$^+$), which carries both a Mucts62 $Ap^r$ prophage and the mini-Mu element pEG5005 ($Km^r$) (Groisman and Casadaban 1986). The resulting mixed phage lysate was used to transduce strain GM305 (*osmZ200* Mucts62 $Ap^r$; λpGM27) to $Km^r$, and these colonies were replica-plated on BS and lactose MacConkey indicator plates containing Km to screen for $Lac^-$ $Bgl^-$ strains. Phages 4D8, 3D5, 8G4, and 5A5, which carry various segments of the *E. coli* chromosome, have been described by Kohara et al. (1987).

*Nucleic acid procedures.* Isolation of plasmid, phage λ, and chromosomal DNA and routine manipulations of nucleic acids were all as described (Maniatis et al. 1982; Silhavy et al. 1984). DNA sequencing was performed according to Sanger et al. (1977) using derivatives of phages M13mp18 and M13mp19 (Norrander et al. 1983) carrying cloned restriction fragments. The method of Southern (1975) was used to detect homology between DNA fragments and DNA probes labelled with digoxigenin-11-dUTP by means of a non-radioactive DNA labelling and detection kit (Boehringer, Mannheim).

*Microscopy of E. coli cells.* Cells grown in LB to mid-log phase were fixed, and their DNA was stained with a 2 μg/ml solution of DAPI (4',6-diamino-2-phenyl-indole) in water as described (Hiraga et al. 1989). Two photographs were taken of the same field using the phase-contrast or fluorescence systems (filter combination: G365/FT397/LP435).

*Radiolabelling of proteins in maxicells and SDS-polyacrylamide gel electrophoresis.* Plasmid-encoded proteins were expressed in the maxicell strain RK5048 according to Sancar et al. (1979), and radiolabelled with [$^{35}$S]-methionine (1000 mCi/mmol; Amersham). The proteins were separated electrophoretically on a 15% SDS-polyacrylamide gel (Laemmli 1970) and were visualized by autoradiography using Kodak X-omatic 100 film.

*Determination of β-galactosidase activity.* β-Galactosidase specific activity was assayed as described by May et al. (1986) and is expressed as micromoles of substrate (2-nitrophenyl-β-D-galactoside; ONPG) cleaved per min per mg of protein.

## Results

### *Cloning of the* osmZ *gene*

Mutations in *osmZ* are recessive and confer $Lac^+$ and $Bgl^+$ phenotypes on strains carrying a chromosomal

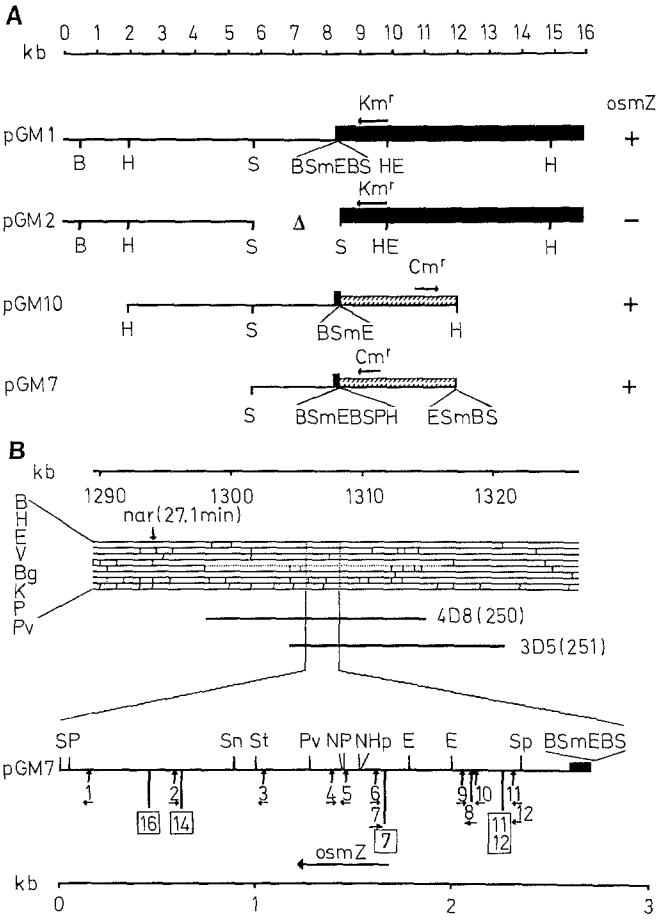


**Fig. 1 A and B.** Structure of plasmids used. **A** Plasmids pGM1 and pGM2 are derivatives of the mini-Mu element pEG5005, and plasmids pGM7 and pGM10 are derivatives of the low copy-number vector pHSG575. The cloned chromosomal DNA is represented by the *thin line*, the mini-Mu material by the *black boxes*, and pHSG575 DNA by *hatched boxes*. The symbol *Δ* represents a deletion, which was constructed by removing a *Sal*I restriction fragment from pGM7. **B** The physical structure of the chromosomal segment of pGM7 is shown together with a part of the restriction map of *Escherichia coli* from the 27 min region of the chromosome (Kohara et al. 1987). The exact position and direction of transcription of the *osmZ* gene was inferred from the nucleotide sequence. The chromosomal insert in phages 4D8 and 3D5 from the Kohara collection is indicated. *Numbers with arrows* indicate the positions of the Tn*5*-B20 insertions in pGM7 and the orientation of the *lacZ* gene carried by this transposon. The positions of Tn*5* insertions are indicated by *boxed numbers*. Tn*5*-7 and Tn*5*-14 represent two clusters of eight and three independently isolated insertions at the same loci, respectively. Abbreviations used for restriction enzmyes are: B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; N, *Nru*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sm, *Sma*I; Sn, *Sna*BI; Sp, *Sph*I; St, *Stu*I; V, *Eco*RV

*proU-lacZ* fusion (Higgins et al. 1988). Using the mini-Mu element pEG5005 (Groisman and Casadaban 1986), we prepared a gene bank in vivo of the *osmZ*$^+$ strain GM301 and used it to identify $Lac^-$ $Bgl^-$ transductants in the *osmZ200* strain GM305. Among 12000 transductants inspected, four $Lac^-Bgl^-$ colonies were found. The plasmid (pGM1) present in one of these colonies carries a chromosomal segment of approximately 8 kb, and the deletion of a 2.7 kb *Sal*I restriction fragment from pGM1 resulted in an $OsmZ^-$ phenotype (pGM2; Fig. 1 A). This fragment and a *Hin*dIII-*Bam*HI restric-

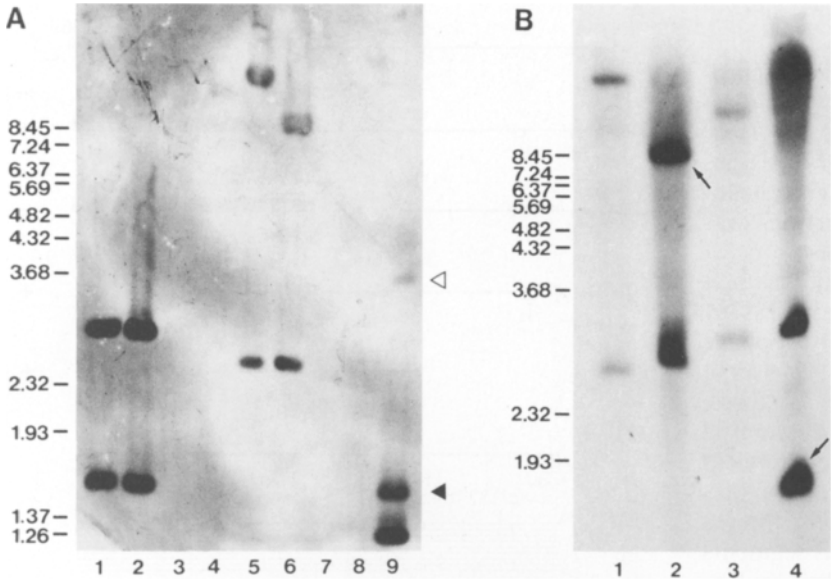


**Fig. 2A and B.** Chromosomal origin of the *osmZ* region. **A** DNA from phages 4D8, 3D5, 8G4, and 5A5 from the Kohara collection was digested with *Pst*I (lanes 1–4, 9) or *Pvu*II (lanes 5–8), electrophoretically separated on an agarose gel, transferred onto a nitrocellulose membrane, and hybridized with a *Sph*I-*Stu*I fragment from pGM7 labelled with digoxigenin-11-dUTP. Lanes 1 and 5, 4D8; lanes 2 and 6, 3D5; lanes 3 and 7, 5A5; lanes 4 and 8, 8G4; lane 9, pGM7. The internal *Pst*I fragment of the chromosomal insert from pGM7 (see Fig. 1B) is indicated by a *closed triangle*. In lane 9 the smaller hybridizing *Pst*I fragment from pGM7 represents a chromosomal segment that is incomplete in pGM7. The difference in size between the large *Pvu*II fragments from phages 4D8 and 3D5 (lanes 5 and 6) results from the fusion of chromosomal material present in phage 4D8 to vector sequences (see Fig. 1B). The weakly hybridizing 3.6 kb fragment from pGM7 (lane 9; *open triangle*) corresponds to the vector fragment; this hybridization is due to a slight contamination of the labelled probe with undigested pGM7 material. **B** Chromosomal DNA from strains GM37 (*osmZ*$^+$; lanes 2 and 4) and GM230 (*osmZ205*::Tn*10*; lanes 1 and 3) was digested with *Pst*I (lanes 3 and 4) or *Pvu*II (lanes 1 and 2) and treated as described in **A**, except that the complete chromosomal *Sal*I fragment from pGM7 (Fig. 1B) was used as a hybridization probe. The restriction fragments from the *osmZ*$^+$ DNA (lanes 2 and 4), which are altered in size by the *osmZ205*::Tn*10* insertions, are indicated by *arrows*. Size standards shown on the left of the photographs are given in kb

tion fragment from pGM1 were subcloned into the low copy-number vector pHSG575 (Takeshita et al. 1987) resulting in plasmids pGM7 and pGM10, respectively, both of which conferred on OsmZ$^+$ phenotype (Fig. 1A). Repeated attempts to subclone these fragments into pBR322 failed. A restriction map of the smaller plasmid, pGM7, is shown in Fig. 1B.

### *Chromosomal origin of the cloned* osmZ *gene*

Using three-factor crosses, we previously mapped the *osmZ* gene to the 27 min region of the *E. coli* chromosome (Higgins et al. 1988). The restriction map of pGM7 is identical to the *E. coli* chromosomal restriction map (Kohara et al. 1987) around position 1307 kb (corresponding to approximately 27 min), with the exception of a missing 220 bp *Eco*RI restriction fragment (Fig. 1B). To prove that the cloned segment in pGM7 originates from this chromosomal region, we performed Southern blot experiments with phages 4D8 and 3D5 from the Kohara collection whose inserts come from the 27 min region (Fig. 1B). DNA from these phages was digested with *Pst*I or *Pvu*II, and a *Stu*I-*Sph*I restriction fragment from the insert in pGM7 (see Fig. 1B) was used as a hybridization probe. In both cases, two restriction fragments from both phages specifically hybridized with the probe (Fig. 2A). This is expected, since the probe contains one recognition site for each enzyme (Fig. 1B). No hybridization was detected when the vector pHSG575 was used as a probe (data not shown). Furthermore, Southern blot experiments were carried out using the *Sal*I insert from pGM7 to probe chromosomal DNA prepared from the *osmZ205*::Tn*10* strain GM230 and its isogenic *osmZ*$^+$ parent GM37 and digested with either *Pst*I or *Pvu*II (Fig. 2B). In each of these digests we detected two specific fragments in the *osmZ*$^+$ strain; the sizes of one *Pst*I (1.5 kb) and one *Pvu*II (7.6 kb) fragment were altered in the DNA prepared from the *osmZ205*::Tn*10* strain (Fig. 2B). The known position of these two restriction fragments on the chromosomal map reveals an overlap of 190 bp (see Fig. 1B). It is thus clear that the Tn*10* insertion had occurred within this common segment, suggesting that the 190 bp *Pvu*II-*Pst*I fragment (positions 1.33 kb to 1.52 kb in plasmid pGM7; Fig. 1B) contains part of the *osmZ* gene. Thus, the genetic and physical mapping data for the *osmZ* gene are in complete agreement and the data presented above show unambiguously that the cloned segment in pGM7 originates from the 27 min region of the *E. coli* chromosome.

### *Complementation of OsmZ phenotypes by the* osmZ$^+$ *plasmid pGM7*

We tested the influence of the cloned *osmZ* gene on *proU-lacZ* expression in the *osmZ*$^+$ strain GM37 and

**Table 2.** Effect of the cloned *osmZ*$^+$ gene on *proU-lacZ* expression

| Strain | Chromosomal genotype | Specific β-galactosidase activity in MMA | |
|---|---|---|---|
| | | −NaCl | +NaCl |
| GM37 (pHSG575) | *osmZ*$^+$ | 0.02 | 4.09 |
| GM37 (pGM7) | *osmZ*$^+$ | 0.007 | 3.33 |
| BRE2071 (pHSG575) | *osmZ200* | 0.33 | 5.91 |
| BRE2071 (pGM7) | *osmZ200* | 0.009 | 4.70 |

Cells were grown in Minimal Medium A (MMA) to mid-log phase, with or without 0.3 M NaCl as indicated, and β-galactosidase specific activity was determined. Activity specific of β-galactosidase is expressed as μmoles *o*-nitrophenyl-β-galactoside (ONPG) cleaved per min per mg of protein. These data are the mean values of two independent experiments and a *Δlac* strain was included as a control. All strains carry the same *Φ(proU-lacZ)*hyb2 protein fusion as a single copy in the chromosome. Plasmid pGM7 carries the cloned *osmZ*$^+$ gene and plasmid pHSG575 is the vector used for its construction

its isogenic *osmZ200* derivative, strain BRE2071, when these strains were grown in media of low and high osmolarity (Table 2). As expected, *proU-lacZ* expression at both osmolarities was reduced in strain BRE2071 (pGM7), but the presence of the cloned *osmZ*$^+$ gene also reduced the level of expression of this fusion in the *osmZ*$^+$ strain GM37. These pGM7-mediated effects are not limited to *proU-lacZ* expression, but are also seen when the uptake of glycine betaine through the ProU transport system is measured (data not shown).

Mutations in *osmZ* influence the osmoregulated transcription of the *ompC* and *ompF* structural genes and lead to an increase in OmpC synthesis and a decrease in OmpF synthesis at low osmolarity (Graeme-Cook et al. 1989). We analysed the outer membrane protein profiles of strains GM37 and BRE2071 carrying either pHSG575 or pGM7 by SDS-polyacrylamide gel electrophoresis. Plasmid pGM7 nearly restored the wild-type pattern of OmpC and OmpF synthesis in the *osmZ200* strain BRE2071 but also slightly influenced porin synthesis in the *osmZ*$^+$ strain GM37. Analysis of β-galactosidase activities in *osmZ*$^+$ and *osmZ200* strains carrying *ompC-lacZ*$^+$ or *ompF-lacZ*$^+$ operon fusions demonstrated that these effects of pGM7 on porin synthesis occur at the level of transcription (data not shown).

Mutations in *pilG* (*osmZ*) lead to a large increase in the frequency of fimbrial phase variation (Spears et al. 1986; Higgins et al. 1988). A *fimA-lacZ* fusion strain is a convenient system for monitoring fimbrial phase variation, since the orientation of the invertible promoter fragment can be detected as Lac$^+$ ("ON") and Lac$^-$ ("OFF") colonies (Freitag et al. 1985). The high frequency of phase variation in *osmZ* strains results in the formation of colonies of only one type, with an intermediate Lac phenotype. Transformation of pGM7 into an

**Table 3.** Effects of the cloned *osmZ*$^+$ gene on *fimA-lacZ* expression and on the frequency of fimbrial phase variation

| Strain[a] | Phase[b] | β-Galactosidase specific activity | Frequency of phase variation (%) |
|---|---|---|---|
| GM344 (pHSG575) | "OFF" | 0.52 | 6.2 |
| GM344 (pHSG575) | "ON" | 7.88 | 3.9 |
| GM344 (pGM7) | "OFF" | 0.04 | 1.4 |
| GM344 (pGM7) | "ON" | 5.32 | 0.4 |
| GM345 (pHSG575) | – | 4.31 | – |
| GM345 (pGM7) | "OFF" | 0.007 | 0.3 |
| GM345 (pGM7) | "ON" | 2.48 | 0.3 |

Colonies showing a defined fimbrial phase were picked and grown in LB medium to mid-log phase, and the β-galactosidase specific activity was measured. Aliquots of these cultures were then plated onto lactose MacConkey indicator plates to determine the percentage of cells that had undergone phase variation. The data shown are mean values of two independent experiments

[a] Strains GM344 and GM345 are genotypically *osmZ*$^+$ or *osmZ200*, respectively, and carry the same *Φ(fimA-lacZ*$^+$*)* operon fusion as a single copy in the chromosome. [b] The designations "ON" (Lac$^+$) and "OFF" (Lac$^-$) refer to the orientation of the invertible DNA segment carrying the *fimA* promoter on which expression of the *fimA-lacZ* fusion depends. In the *osmZ* mutant strain GM345, the frequency of switching between an "ON" and "OFF" phase is so high that individual Lac$^+$ and Lac$^-$ colonies cannot be detected; colonies comprise mixed populations of cells showing both phenotypes

*osmZ*$^+$ *fimA-lacZ* strain yielded both Lac$^+$ and Lac$^-$ colonies regardless of the Lac phenotype of the colony used to inoculate the culture. However, the frequency of phase variation in the resulting transformants was greatly reduced (Table 3). As was found with the *proU-lacZ* fusion (Table 2), expression of the *fimA-lacZ* fusion was reduced in the presence of multiple copies of *osmZ* (Table 3). Transformation of pGM7 into an *osmZ200 fimA-lacZ* strain also yielded Lac$^+$ and Lac$^-$ colonies, but the same strain transformed with the vector only gave rise to colonies with an intermediate Lac phenotype (Table 3). Thus, the cloned *osmZ* gene reduces the high frequency of fimbrial phase variation characteristic for *osmZ* strains to such an extent that individual Lac$^+$ and Lac$^-$ colonies can be distinguished.

### *The cloned* osmZ *gene alters cell morphology*

The effect of plasmids pHSG575 or pGM7 on cell morphology was examined in cells whose DNA was stained with the DNA-specific fluorescent dye DAPI (4′,6-diamino-2-phenyl-indole) by phase contrast and fluorescence microscopy. Cells carrying either a chromosomal

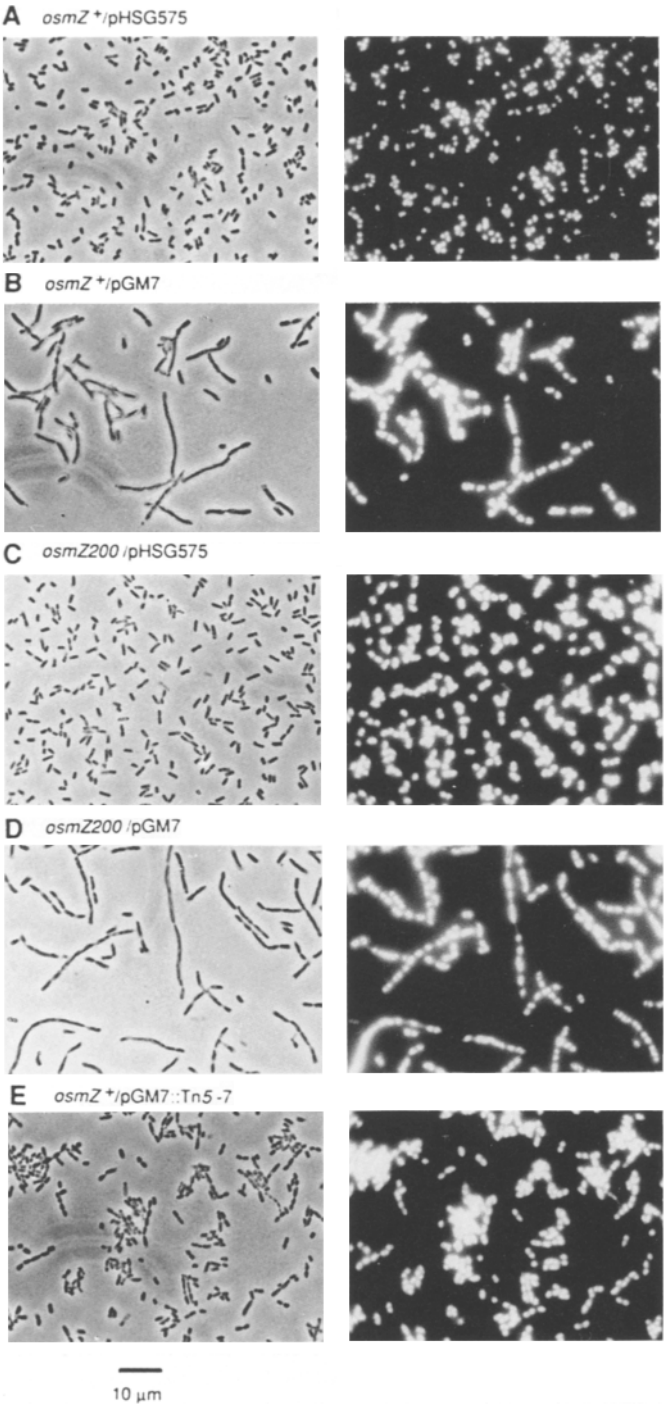


**Fig. 3A–E.** Effects of the cloned *osmZ* gene on cell morphology. Cells were grown in LB to mid-log phase and fixed; their nucleoids were then stained with DAPI. Two photographs were taken of the same field of the sample, one under phase contrast (*left*) and one under UV illumination to visualize DAPI fluorescence (*right*). All photographs were enlarged to the same magnification; the *bar* represents 10 µm. The chromosomal *osmZ* allele and the plasmids carried by the strains are indicated. **A, B**: GM37; **C, D**: BRE2071; **E**: MC4100

$osmZ^+$ or *osmZ200* allele were morphologically identical (Fig. 3A, C), indicating that *osmZ* mutations do not change cell morphology *per se*. A drastic change in cell morphology was observed when the $osmZ^+$ plasmid pGM7 was present: the cells formed long filaments containing multiple nucleoids (Fig. 3B, D). This phenotype clearly depends on the presence of multiple copies of the $osmZ^+$ gene, since an *osmZ*::Tn*5* derivative of pGM7 (see below) shows no cell filamentation (Fig. 3E). The formation of filamentous cells is not due to induction of the *E. coli* SOS system, since it also occurs in *recA* strains (data not shown).

### *Position of the* osmZ *gene in pGM7 and identification of its gene product*

To determine the position of *osmZ* within the chromosomal DNA present in plasmid pGM7, we mutagenized pGM7 with the transposable elements Tn*5* (de Bruijn and Lupski 1984) and a Tn*5* derivative, Tn*5*-B20, which can be used to isolate *lac* operon fusions (Simon et al. 1989). Plasmids with insertions located at four different positions within a 490 bp *Pvu*II-*Eco*RI fragment (positions 1.33 kb to 1.82 kb; Fig. 1B) no longer complemented the $Bgl^+$ phenotype of an *osmZ200* strain, whereas all other pGM7 derivatives still did so. We therefore conclude that this *Pvu*II-*Eco*RI fragment carries at least part of the *osmZ* gene, which is consistent with our physical mapping data for the chromosomal *osmZ205*::Tn*10* insertion. Since all Tn*5*-B20 insertions in *osmZ* conferred a $LacZ^+$ phenotype, the direction of transcription of *osmZ* could not be inferred from the orientations of the Tn*5*-B20 elements in this gene (Fig. 1B).

Proteins encoded by the cloning vector pHSG575 and the $osmZ^+$ plasmid pGM7 were synthesized in maxicells, radiolabelled, separated by SDS-polyacrylamide gel electrophoresis, and visualized by autoradiography. In addition to the vector-encoded proteins, two polypeptides with apparent molecular weights of 16000 and 38000 dalton were encoded by plasmid pGM7 (Fig. 4). To determine which of these proteins is the *osmZ* gene product, we performed an analogous experiment with all the pGM7 derivatives carrying the Tn*5* and Tn*5*-B20 insertions shown in Fig. 1B. The results of such experiments are shown in Fig. 4 for representative examples. All transposon insertions in pGM7 (Tn*5*-B20-4; Tn*5*-B20-5; Tn*5*-B20-6; Tn*5*-7) which no longer allowed the complementation of the $Bgl^+$ phenotype of an *osmZ* strain did not synthesize the 16 kDa protein; thus, this polypeptide must be the OsmZ protein. Plasmids harbouring the Tn*5*-16, Tn*5*-14, Tn*5*-B20-1, and Tn*5*-B20-2 insertions did not permit the synthesis of the 38 kDa protein, whereas Tn*5*-B20-3 allowed the synthesis of both polypeptides. Thus, the latter transposon insertion has occurred in the region between *osmZ* and the structural gene for the 38 kDa protein. All remaining transposon insertions (see Fig. 1B) did not affect the production of either protein (data not shown).

### *The* osmZ *gene encodes the DNA-binding protein H-NS*

The complete nucleotide sequence of a 780 bp segment, which extends from the *Eco*RI restriction site at position

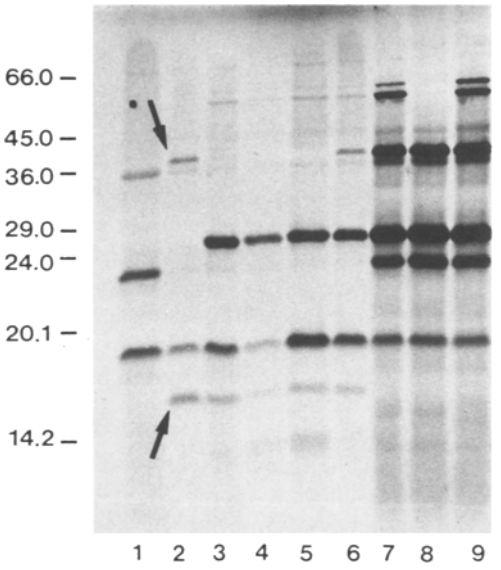


**Fig. 4.** Proteins encoded by plasmid pGM7 and its derivatives. The proteins encoded by the vector pHSG575 and by plasmid pGM7 and its derivatives carrying transposon insertions were expressed in maxicells, radiolabelled, electrophoresed on a 15% SDS-polyacrylamide gel, and visualized by autoradiography. Lane 1, pHSG575; lane 2, pGM7; lane 3, pGM7::Tn*5*-B20-1; lane 4, pGM7::Tn*5*-16; lane 5, pGM7::Tn*5*-14; lane 6, pGM7::Tn*5*-B20-3; lane 7, pGM7::Tn*5*-B20-4; lane 8, pGM7::Tn*5*-B20-5; lane 9, pGM7::Tn*5*-B20-6. The positions of the OsmZ protein (16 kDa) and the 38 kDa protein are indicated by *arrows* (lane 2). Molecular weight standards in kDa are shown at the left

2.0 kb almost to the *Stu*I restriction site at position 1.0 kb in plasmid pGM7 (Fig. 1 B) and encompasses the *osmZ* gene, was determined on both strands. The DNA sequence revealed an open reading frame (ORF) of 411 bp, which predicts a polypeptide consisting of 137 amino acid residues with a calculated molecular weight of 15530. This value is in excellent agreement with the molecular weight estimated for the OsmZ protein (16 kDa) from its electrophoretic mobility on SDS-polyacrylamide gels. Since all transposon insertions in pGM7 that result in an OsmZ$^-$ phenotype map within the region spanning the ORF (Fig. 1 B), we conclude that this ORF encodes the OsmZ protein. We found that the first 20 amino acid residues of the OsmZ protein are identical to the N-terminal amino acid sequence of the 16 kDa protein (H1a) from *E. coli* (Laine et al. 1984). Furthermore, the primary sequence of OsmZ is identical, with the exception of the N-terminal Met residue, to that determined by Falconi et al. (1988) for a 15 kDa *E. coli* protein termed H-NS. Biochemical evidence has been presented that the 16 kDa protein (H1a) and H-NS are most probably identical (Spassky et al. 1984; Rimsky and Spassky 1986; Gualerzi et al. 1986). These proteins (H1a, H-NS) have been characterized as DNA-binding proteins associated with the *E. coli* nucleoid.

We do not present the nucleotide sequence of *osmZ* because it is identical to that of *hns* (Pon et al. 1988) with the following exceptions. Within the coding region we find a T at position 969 and an A at position 1032, instead of C and G residues, respectively. These differences do not change the deduced amino acid sequence and our sequence at these positions is identical to the *hns* sequences from *Proteus vulgaris* and *Serratia marcescens* (La Teana et al. 1989). Upstream of the coding region we find a T instead of a C at position 824 in a non-conserved region of the *hns* genes. In the −10 region we find a T instead of a C at position 819; this T residue is conserved in *P. vulgaris* and *S. marcescens* (La Teana et al. 1989). Using the algorithm of Mulligan et al. (1984), which allows the estimation of promoter strength, we found a homology score of 40% for our sequence and a homology score of 28% for the *hns* promoter sequence determined by Pon et al. (1988).

Pon et al. (1988) suggested that the *hns* gene is located at 6.1 min on the *E. coli* linkage map, mainly based on their finding that two overlapping clones (8G4 and 5A5) from the Kohara phage collection, whose inserts span the chromosome from approximately 6.0 to 6.2 min, hybridize in plaque blots to an internal restriction fragment of *hns*. This is in contrast to our previous genetic mapping data for the *osmZ* gene (Higgins et al. 1988) and the physical mapping data presented here, which place *osmZ* unambiguously at 27 min of the linkage map. We note that Pon et al. (1988) did not provide any genetic mapping data for *hns*. To resolve this discrepancy, we digested the DNA of the phages 8G4 and 5A5 with *Pst*I or *Pvu*II and hybridized the restriction fragments to a labelled *Stu*I-*Sph*I fragment from pGM7, which contains *osmZ* (Fig. 1 B). No hybridization was detected (Fig. 2 A), while restriction fragments from phages 4D8 and 3D5, which originate from the 27 min region (see Fig. 1 B), yielded the expected hybridization signal (Fig. 2 A). Thus, there is no evidence to support placement of *hns* at 6.1 min on the *E. coli* chromosome. The data presented here allow the unambiguous conclusion that *osmZ* is the structural gene for the DNA-binding protein H-NS.

## Discussion

Mutations in *osmZ* (*pilG, bglY, virR*) in *E. coli*, *S. typhimurium*, and *S. flexneri* affect a variety of important cellular functions, such as gene expression, site-specific inversion of a DNA segment, the formation of deletions, and the control of virulence factors (Defez and DeFelice 1981; Spears et al. 1986; Maurelli and Sansonetti 1988; Higgins et al. 1988; Graeme-Cook et al. 1989; Lejeune and Danchin 1990; Dorman et al. 1990). We have previously shown that *osmZ* mutations lead to increased supercoiling of reporter plasmids and have suggested that this topological change links the highly pleiotropic effects of such mutations (Higgins et al. 1988). In this paper we demonstrate that *osmZ* is the structural gene for H-NS (H1a), a DNA-binding protein known to be associated with the *E. coli* nucleoid (Jacquet et al. 1971; Cukier-Kahn et al. 1972; Laine et al. 1984; Spassky et al. 1984; Gualerzi et al. 1986; Rimsky and Spassky 1986). Consequently, our results connect the physico-

chemical properties of purified H-NS observed in vitro with the effects of mutations in its structural gene on cell physiology.

The primary sequence of OsmZ, as deduced from the nucleotide sequence, is identical to that determined for the H-NS protein (Falconi et al. 1988). Furthermore, its amino-terminus and amino acid composition match that of a 16 kDa DNA-binding protein (H1a; Laine et al. 1984), demonstrating the identity of OsmZ with H-NS and H1a. Our mapping data position *osmZ* at 1307 kb on the restriction map of *E. coli* (Kohara et al. 1987) and allow us to determine that *osmZ* is transcribed counterclockwise on the chromosome. Our attempts to clone *osmZ* onto the multicopy plasmid pBR322 were unsuccessful. We attribute these difficulties to the morphological changes (Fig. 3) and reduced cell viability (unpublished results) that can be observed in cells carrying the intact *osmZ* gene on a low copy-number plasmid.

Several studies have indicated that the DNA in the bacterial nucleoid is segregated into about 50 topologically independent chromosomal domains (Worcel and Burgi 1972; Drlica 1987). A small number of proteins, loosely categorized as histone-like proteins, have been reported to be associated with the nucleoid and have been implicated in the folding and condensation of the chromosomal DNA (Drlica and Rouviere-Yaniv 1987; Pettijohn 1988). Of these proteins, H-NS (H1a) has the strongest affinity for double-stranded DNA. H-NS (H1a) is a neutral, heat-stable protein, which exists predominantly as a dimer in solution due to strong hydrophobic interactions. Its amount varies with the growth phase and accumulates to approximately 20000 monomers per cell in the late stationary phase (Spassky et al. 1984; Rimsky and Spassky 1986; Gualerzi et al. 1986; Falconi et al. 1988). Purified H-NS (H1a) has effects on DNA structure and gene transcription which have so far not been correlated with any in vivo role, since mutants with alterations in its structural gene were not available. Mutations in *osmZ* now allow this correlation.

Binding of H-NS (H1a) to a restriction fragment carrying the *lac*($L_8$UV5) promoter inhibits initiation of transcription in vitro by reducing the rate of open complex formation (Spassky et al. 1984). The influence of *osmZ* mutations or increased *osmZ* gene dosage on the expression of several *E. coli* genes is consistent with this inhibitory effect. Mutations in *osmZ* result in increased transcription of *proU*, *bgl*, and *ompC*, while increased *osmZ* gene dosage reduces the expression of these genes below the wild-type levels. These alterations in gene expression indicate that the intracellular amount of H-NS (H1a) is a critical determinant of proper expression of a number of chromosomal genes.

H-NS (H1a) binds to both linear and supercoiled double-stranded DNA and upon binding strongly compacts it in vitro (Spassky et al. 1984; Rimsky and Spassky 1986). The influence of H-NS on DNA structure is of prime importance for the genetic stability of the *E. coli* genome, since mutations in *bglY* (*osmZ*) strongly increase the frequency of spontaneous deletions, while the rate of point mutations is not affected (Lejeune and Danchin 1990). These authors have speculated that *bglY* mutations lead to reduced rigidity of the genomic DNA thereby facilitating "looping out" of DNA single strands. Deletions could then occur after pairing of short regions of homology and subsequent errors in DNA replication. This hypothesis is consistent with the finding that H-NS (H1a) compacts DNA in vitro. The influence of H-NS (H1a) on DNA structure also explains why mutations in *pilG* (*osmZ*) strongly increase the frequency of a site-specific inversion event, which controls fimbrial phase variation (Spears et al. 1986; Higgins et al. 1988). The alternating expression of the *fimA* structural gene is mediated by the inversion of a promoter-carrying DNA segment flanked by inverted repeats of 9 bp (Abraham et al. 1985). This event is sensitive to topological changes in the DNA (Glasgow et al. 1989), and our result that increased *osmZ* gene dosage reduces the frequency of fimbrial phase variation can be explained by the effect of H-NS on DNA rigidity. We would therefore not be surprised if other site-specific inversion events (Glasgow et al. 1989) were also affected by *osmZ* mutations.

The H-NS (H1a) protein clearly affects the physical packaging of the DNA, but it exhibits only a small effect on DNA superhelicity in vitro (Spassky et al. 1984; Rimsky and Spassky 1986). Therefore the changes in plasmid supercoiling observed in *osmZ* mutants in vivo (Higgins et al. 1988) are most likely the result of an indirect effect. It remains to be elucidated whether these changes are mediated by alterations in the synthesis or function of DNA topoisomerase I or DNA gyrase, which determine the level of DNA supercoiling, by alterations in the expression of the genes for other histone-like proteins, or by abnormal interactions of the proteins associated with the bacterial nucleoid. Our finding that *osmZ* encodes a DNA-compacting protein lends further support to our suggestion (Higgins et al. 1988) that DNA topology is an important determinant for *proU* regulation.

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**Note added in proof**

After this manuscript was submitted, Göransson et al. (Nature 344:682–685; 1990) published a report on the thermoregulation of transcription of pilus-adhesin determinants (*pap* genes) from uropathogenic *E. coli* strains. They identified a gene, *drdX*, which maps at 27 min and affects thermoregulation of *pap* expression; this gene encodes the H-NS protein. The *drdX* and *osmZ* genes are therefore identical.