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Identification, Cloning, and Expression of *bolA*, an *ftsZ*-Dependent Morphogene of *Escherichia coli*

MARTÍ ALDEA,[1] CONCHA HERNÁNDEZ-CHICO,[2]† ADELA G. DE LA CAMPA,[2] SIDNEY R. KUSHNER,[1] AND MIGUEL VICENTE[2]*

Department of Genetics, University of Georgia, Athens, Georgia 30602,[1] and Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, 28006 Madrid, Spain[2]

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A newly found morphogene of *Escherichia coli*, *bolA*, mapping at min 10 of the genetic map, was cloned in a 7.2-kilobase *Bam*HI fragment and identified by its ability to produce osmotically stable spherical cells when overexpressed. This gene codes for a polypeptide of 13 kilodaltons. Overexpression of *bolA*$^+$ was achieved in low-copy-number vectors with operon fusions to the *tet* and *lac* promoters, indicating a clockwise direction of transcription. While no modification of any of the penicillin-binding proteins was observed, morphological effects due to overexpression of *bolA*$^+$ were shown to be dependent on the presence of an active *ftsZ* gene product. Our results suggest the existence of a mechanism mediated by FtsZ for modifying the conformation of nascent murein in the early steps of septum formation.

Escherichia coli cells are rod shaped with rounded polar ends and divide by constriction. Cell shape is maintained by the covalently bonded murein layer of the cell envelope (35). Higgins and Shockman (15) postulated the existence of two different systems for murein synthesis: the cell elongation system would produce only cylindrical murein required for cell growth, and the septation system would be periodically activated to synthesize the polar caps during cell division. Penicillin-binding protein 3 (PBP 3), a protein encoded by the *pbpB* gene (33), is a transpeptidase needed only during septum formation (17). The *pbpA* gene product, PBP 2 (39), is another protein with transpeptidase activity required only to maintain the characteristic rod shape during cell wall elongation (37). Specific inhibition of PBP 2 by mecillinam produces round cells, and *pbpA* mutants show an increased resistance to this β-lactam (37). PBP 3 shows a similar affinity for penta- and tripeptide precursors for performing the murein transpeptidase reaction (34). In contrast, it seems that PBP 2 is able to use only the pentapeptide precursor, since high levels of PBP 5, a carboxypeptidase that converts pentapeptide to tetra- and tripeptide precursors, lead to round cell shape and changes in murein composition identical to those observed in the absence of PBP 2 (27).

A high level of carboxypeptidase activity has been reported to occur immediately before cell division (31, 32), and a model has been proposed in which the activity of a carboxypeptidase would play a central role in switching between cylindrical- and hemispherical-murein synthesis (27). However, the two main carboxypeptidases of *E. coli* are totally dispensable for normal growth and division.

We describe here a newly found gene, *bolA*, that produces round cell shape when overexpressed. The *bolA*$^+$ gene was cloned, and its position on the genetic map was determined. Operon fusions to the *tet* and *lac* promoters in low-copy-number and runaway vectors were designed to characterize further the morphological effects produced by *bolA*$^+$ overexpression. Possible relationships of *bolA* to the genes involved in cell morphogenesis suggest the existence of an *ftsZ*-dependent process that could alter murein conformation in the early steps of septum formation.

* Corresponding author.
† Present address: CID—Genética Molecular, Centro Especial Ramón y Cajal, 28034 Madrid, Spain.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and cell parameter measurements. The bacterial strains used and their genotypes are listed in Table 1. Luria broth and Luria agar were supplemented with the following antibiotics (in micrograms per milliliter) when required: ampicillin, 50; kanamycin, 50; tetracycline, 20; and chloramphenicol, 20. Auxotrophies were tested in M9 medium supplemented with amino acids (50 μg/ml) and vitamins (1 μg/ml) as required. The growth conditions in liquid medium have been previously described (28). Procedures for measuring optical density and cell number and for observing cells under phase-contrast optics have been published elsewhere (13).

Plasmids. The runaway plasmid pMOB45 (8) was used to increase the copy numbers of cloned inserts. pBS44 (*pbpA*$^+$ *rodA*$^+$), pBS47 (*pbpA*$^+$), and pLG346 (*rodA*$^+$) (5) were used to increase the expression of *pbpA*$^+$ and *rodA*$^+$. Plasmids obtained in this study are briefly described in Table 2, with further details in the text.

Genetic techniques. Plasmids were transformed by the method of Kushner (20) by using SK6501 (*recA56*) as the recipient strain unless otherwise indicated. Conjugation experiments and P1 *vir* transductions were carried out as described by Miller (30). Chromosomal insertions of ColE1-derivative Tet^r plasmids were obtained by transforming AB1157 *polA5* and selecting on plates with a low concentration of tetracycline (5 μg/ml). The *polA5* mutation derived from JG110 was transferred to various genetic backgrounds by using a Tn*10* insertion linked to *polA* as the selected marker. The method designed by Maloy and Nunn (25) was used to subsequently remove the Tn*10*. Transposon Tn*1000* insertions into pSC101-derivative plasmids were isolated as exconjugants of conduction experiments by the method of Guyer (14).

DNA isolation and cloning techniques. Restriction endonucleases, calf intestine alkaline phosphatases, T4 DNA ligase, and DNA polymerase large fragment were obtained from Boehringer Mannheim Biochemicals and were used accord-

TABLE 1. *E. coli* strains

Strain	Genotype	Source or reference
AB1157	F⁻ *ara-14 galK2 lacY1 mtl-1 xyl-5 argE3 his-4 leu-6 proA2 thr-1 thi rpsL (str-31) sup-37*(Am) *tsx-33*	1
CH931	Hfr PO1 *lysA polA1*	S. R. Kushner
D-3	As OV-2 *leu*⁺ *ftsA3*(Ts)	42
HfrH	Hfr PO1 *thi-1 rel-1*	23
JG110	F⁻ *thyA36 metE70 polA5 deoC2* IN(*rrnD-rrnE*)1[a]	CGSC[b]
JM103	F′ (*traD36 proAB*⁺ *lacI*ᵠ ZΔM15) *supE thi sbcB15 rpsL endA hsdR4* Δ(*lac-proAB*)	29
KL226	Hfr PO2A (Cavalli) *rel-1 fhuA* (*tonA22*) T2ʳ	23
LMC500	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 flb-5301 pstF25 deoC1 rbsR relA1 lysA1*	41
LMC509	As LMC500 *ftsZ84*(Ts)	41
OV-2	F⁻ *ilv his leu trp*(Am) *thyA* (*deo*) *ara*(Am) *lac-125*(Am) *galU42*(Am) *galE tsx*(Am) *tyrT* [*supFA81*(Ts)]	13
OV-25	As OV-2 *met*(Ts) *arg*(Ts) *wee*(Am)	28
SK4900	F⁻ *argG6 leu thr zgi-203*::Tn*10*	S. R. Kushner
SK6501	F⁻ *araD139 lac*(Δ*U169*) *thi rpsL recA56 mot*	F. Moreno
TOE1	F⁻ *thr leu proA his argE lac gal ara xyl mtl thi thyA rpsL ftsQ*(Ts)	4
TOE23	F⁻ *leu pbpB* [*ftsI23*(Ts)]	5
χ478	F⁻ *thi leu proC trp lys metE purE lac ara xyl mtl ton tsx rpsL*	6

[a] IN, Inversion.
[b] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

ing to the instructions of the manufacturer. RNase A and lysozyme were obtained from Sigma Chemical Co.

E. coli chromosomal DNA was isolated as described by Davis and Vapnek (11). Plasmid DNA was isolated by the method of Birnboim and Doly (7) for large-scale preparations or by the method of Ish-Horowicz and Burke (16) for rapid screenings.

To obtain an *E. coli* genomic library, OV-2 chromosomal DNA was completely digested with *Bam*HI and cloned into the unique *Bam*HI site of pLG339, a low-copy-number vector derived from pSC101 (40).

Subcloning steps were usually done by isolation of the desired fragments from low-melting-point agarose gels (22) and inactivation of the vector by calf intestine alkaline phosphatase.

Southern blot hybridizations were performed by the procedures described by Maniatis et al. (26).

Detection of plasmid-encoded proteins. Strain SK6501 (*recA56*) was transformed and used to detect plasmid-encoded proteins by the maxicell technique (36). Maxicells were labeled with L-[^{35}S]methionine (1,114 Ci/mmol, 25 μCi/ml, final concentration; New England Nuclear Corp.), and samples were analyzed in sodium dodecyl sulfate-polyacrylamide gels (21) which were then fixed, dried, and autoradiographed. Protein molecular weight standards were obtained from Pharmacia Fine Chemicals.

Analysis of β-lactam binding to PBPs. The binding of [^{14}C]benzylpenicillin was assayed as described elsewhere (37). Mecillinam affinity for PBP 2 was assayed by the procedure of Spratt et al. (39).

RESULTS

Cloning and genetic mapping of *bolA*. Plasmid pDAV54 was isolated from a genotheque of OV-2 chromosomal DNA that had been digested to completion with *Bam*HI and cloned into the low-copy-number vector pLG339. This plasmid was isolated by its ability to weakly complement a mutant, OV-25 (*wee*), in which elongation and division are uncoupled (12). This strain was able to produce only microcolonies when transformed with pDAV54 and plated directly at 42°C; these microcolonies contained cells showing an osmotically stable spherical shape. Furthermore, pDAV54 produced identical morphological changes in several wild-type strains. This alteration of the normal rod shape was also observed in liquid cultures of the strains containing pDAV54 during late-exponential and stationary growth phases. These observations suggest the presence in this plasmid of a gene involved in the morphogenetic pathways of *E. coli*.

To establish the position of the chromosomal insert carried by pDAV54 in the *E. coli* genetic map, an insertion of a plasmid containing this fragment and a ColE1 replicon into

TABLE 2. Plasmids obtained in this study

Plasmid	Description[a]	Presence of *bolA*[b]
pDAV54	7.2-kb *Bam*HI fragment from *E. coli* OV-2 cloned in pLG339 (low-copy-number pSC101 replicon)	+
pBRCI29	3.5-kb *Pst*I fragment from pDAV54 cloned in pBR322	−
pMAK542	As pDAV54, 5.9-kb *Sph*I fragment deleted, *Sph*I sites inactivated by S1 nuclease	+
pMAK543	210-bp *Bam*HI-*Pvu*II fragment containing *lacP* from pUC9 ligated to *Eco*RI + Klenow, *Bam*HI-treated pMAK542 (*lacP* in positive orientation)	+
pMAK546	2.6-kb *Pvu*II fragment containing ColE1 replicon from pBR325 cloned into *Pvu*II site of pMAK542	+
pMAK551	As pMAK543 with *lacP* in negative orientation	+
pMAK552	1.6-kb *Bam*HI fragment from pMAK551 cloned in positive orientation in *Bam*HI site of *tet* of pMOB45 (runaway R1 replicon)	+
pMAK553	As pMAK 552 in negative orientation	+
pMAK580	900-bp *Bam*HI-*Sma*I fragment of pDAV54 cloned in *Bam*HI-*Eco*RV-digested pBR325	+

[a] Positive orientation indicates that transcription of the vector and that of the insert are in the same direction; negative orientation indicates that transcription is in opposite directions. bp, Base pair.
[b] +, Presence of a *bolA*⁺ gene; −, absence of a *bolA*⁺ gene.

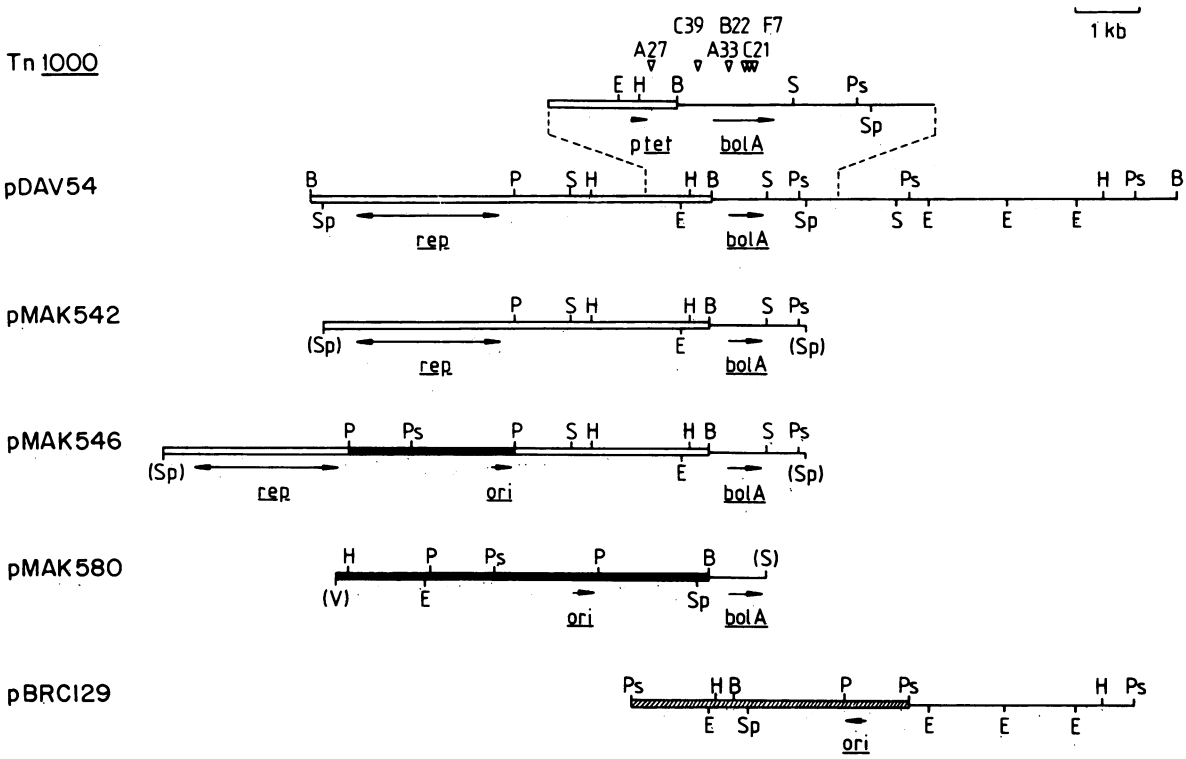

FIG. 1. Restriction maps of pDAV54, pMAK542, pMAK546, pMAK580, and pBRCI29. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pvu*II; Ps, *Pst*I; S, *Sma*I; Sp, *Sph*HI; V, *Eco*RV. Inactivated restriction sites are enclosed in parentheses. Symbols: —, *E. coli* chromosomal sequences; ▽, Tn*1000* insertions that inactivated the *bolA* overexpression phenotype; □, pLG339; ■, pBR325; ▨, pBR322.

the chromosome was attempted in a *polA* strain. A restriction map of pDAV54 is shown in Fig. 1. Attempts to clone the whole 7.2-kilobase (kb) *Bam*HI fragment in pBR322 were unsuccessful; we therefore decided to clone the *Eco*RI fragments from pDAV54 into the chloramphenicol gene of pBR325. All possible recombinants were obtained except for that carrying the 3.8-kb *Eco*RI fragment, suggesting that this fragment could not be maintained at a high copy number. To avoid this copy number effect, the 3.5-kb *Pst*I fragment from pDAV54 adjacent to the *bolA* region was subcloned into the ampicillin gene of pBR322 (Fig. 1). The resultant recombinant, pBRCI29, did not produce any effect on cell morphology or growth rate and was used to locate the insert within the *E. coli* genetic map. For this purpose a chromosomal insertion of pBRCI29 was obtained after this plasmid was transformed into AB1157 *polA5*. One of the transformants, AB1157 *polA5* CI29, showing a low tetracycline resistance level (5 μg/ml), was confirmed by hybridization against pBRCI29 as containing the correct insertion of the plasmid into the chromosome resulting from homologous recombination through the 3.5-kb *Pst*I fragment (data not shown). After this insertion was transduced into HfrH *polA5* and KL226 *polA5*, the *tet* resistance marker of pBRCI29 was located between *lac* (min 8) and the origin of transfer of KL226 (min 13). Subsequent transduction analysis with *proC* and *tsx* (Table 3) showed that the chromosomal insertion was located clockwise to these two genes, at min 10.1 on the *E. coli* genetic map (2). This genetic location was later confirmed (at min 10.0) on the restriction map of the *E. coli* chromosome published by Kohara et al. (19), thus revealing that the observed complementation of *wee* (min 67; A. G. de la Campa et al., Curr. Microbiol., in press) by pDAV54 was in fact extragenic. Consequently, we decided to designate as *bolA* this newly found gene responsible for the round cell shape produced by pDAV54.

Identification of the *bolA* gene product. Since no selection for the inactivation of *bolA*$^+$ was available, 300 kanamycin- and tetracycline-resistant exconjugants from conduction cross SK6501(F'42, pDAV54 [kanamycin resistant]) × SK4900 (tetracycline resistant) were microscopically examined for the recovery of normal morphology. Recovery of normal morphology should arise as a consequence of the Tn*1000* insertions required to conduct pDAV54 from the donor into the recipient cell. Six independent clones were isolated. Restriction analysis showed that five Tn*1000* insertions (A33, B22, C21, C39, and F7) map inside a 1.4-kb *Pst*I-*Bam*HI fragment of the chromosomal DNA carried by pDAV54 (Fig. 1). This fragment is contained in the 3.8-kb

TABLE 3. Transductional linkage analysis of *bolA* linked region

Cross	Donor[a]	Recipient[a]	Marker (no. of transductants)	
			Selected	Unselected
1	HfrH CI29[b]	χ478	*proC*$^+$ (143)	*tsx* Tet^s (75) *tsx*$^+$ Tet^s (58) *tsx*$^+$ Tet^r (9) *tsx* Tet^r (1)
2	χ478 *proC*$^+$	χ478 *tsx*$^+$ CI29[b]	*proC*$^+$ (141)	*tsx*$^+$ Tet^r (73) *tsx* Tet^r (62) *tsx* Tet^s (5) *tsx* Tet^s (1)

[a] All strains were made *polA5* as indicated in the text.
[b] The chromosomal insertion of pBRCI29 was P1 transduced from the AB1157 backgrounds into HfrH and χ478, selecting for tetracycline resistance (5 μg/ml; see text).

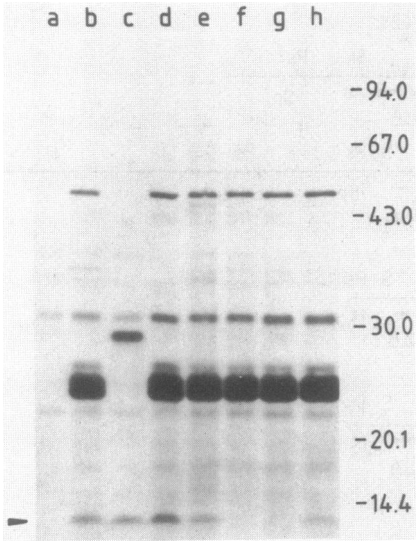

FIG. 2. Plasmid-encoded polypeptides in maxicells. Lanes: a, pLG339; b, pDAV54; c, pMAK542; d, pDAV54::A3 (used as a control); e, pDAV54::A27; f, pDAV54::A33; g, pDAV54::B22; h, pDAV54::C39. Symbol: ▶, *bolA* gene product. Protein markers (in kilodaltons) are as follows: phosphorylase *b*, 94; bovine serum albumin, 67; ovalbumin, 43; carbonic anhydrase, 30; soybean trypsin inhibitor, 20.1; and α-lactalbumin, 14.4.

*Eco*RI fragment that could not be cloned in high copy number. The other Tn*1000* insertion (A27) mapped very close to the 1.4-kb *Pst*I-*Bam*HI fragment but inside the pLG339 side of pDAV54, between the chromosomal fragment and the tetracycline promoter of the vector. These results suggest a possible location for *bolA* within the 1.4-kb *Pst*I-*Bam*HI fragment. This position of *bolA* was confirmed by deleting the 5.9-kb *Sph*I fragment from pDAV54. The deleted plasmid pMAK542 (Fig. 1) was found to produce the same spherical cell shape as pDAV54 produces.

To identify the *bolA* gene product, the polypeptides encoded by plasmids pDAV54 and pMAK542 and five of the isolated Tn*1000* insertions were analyzed in maxicells. There is only one common polypeptide encoded by both pDAV54 and pMAK542 that has a molecular size of 13 kilodaltons (Fig. 2). This polypeptide was not present in either pDAV54::A33 or pDAV54::B22. However, despite the recovery of normal morphology showed by both pDAV54::A27 and PDAV54::C39, they still coded for the 13-kilodalton polypeptide. It must be noted that any chromosomally encoded regulation of transcription should be absent in maxicells, which provides an explanation for this observation. Since the Tn*1000* insertion A27 mapped on the vector, between the tetracycline promoter and the chromosomal fragment, these results suggest that the morphological effect caused by pDAV54 depends on active transcription from the tetracycline promoter through the 1.4-kb *Bam*HI-*Pst*I chromosomal fragment. Moreover, the presence of a polypeptide corresponding to BolA in maxicells from strains containing insertions A27 and C39 suggests the existence of a natural promoter region for this gene that should be located downstream from insertion C39 (data to be published elsewhere).

Effect of *bolA*$^+$ gene dosage on cell viability. To elucidate whether the presence of *bolA*$^+$ in high-copy-number plasmids affects cell viability, a variable-copy-number plasmid was constructed by inserting the 2.6-kb *Pvu*II fragment from pBR325, which contains its *polA*-dependent replicon and the ampicillin gene, into the unique *Pvu*II site of pMAK542. The ligation mixture was used to transform CH931 (*polA1*) by selecting for both ampicillin- and kanamycin-resistant clones, so that the resultant recombinant, pMAK546 (Fig. 1), would be maintained at a low copy number by using its pSC101 replicon. In *polA*$^+$ strains, this plasmid would nevertheless be present at an even higher copy number than that of pBR325 because of the absence of the *rop* gene (10).

Plasmid pMAK546 showed frequencies of transformation similar to those of pLG339 in CH931 (*polA1*) when selection for either kanamycin or ampicillin resistance was used. When pMAK546 was used to transform a *polA*$^+$ strain, SK6501, no transformants were obtained when ampicillin selection was used. In contrast, transformation for kanamycin resistance occurred at frequencies similar to those obtained with CH931 (*polA1*). Furthermore, despite the presence of the ampicillin resistance gene in pMAK546, kanamycin-resistant transformants containing this plasmid in strain SK6501 were unable to grow on plates containing 50 μg of ampicillin per ml. Plasmid pMAK546 produced spherical cells in both *polA*$^+$ and *polA* transformant colonies.

These results indicate that a moderately high *bolA*$^+$ gene dosage is not lethal by itself to *E. coli* cells but produces a high sensitivity to ampicillin, thus explaining why the first attempts to clone the *bolA* gene in pBR322 or pBR325 (i.e., selecting for ampicillin resistance) were unsuccessful. However, the *Bam*HI-*Sph*I fragment containing *bolA* could be successfully cloned, inactivating the *tet* marker of PBR325, when selection for chloramphenicol instead of ampicillin was used. Higher *bolA*$^+$ gene dosage and expression in pMAK553, which contains a runaway replicon, were nevertheless lethal after 90 min of incubation at 42°C.

Effect of *bolA*$^+$ overexpression on cell morphology. To determine whether the presence of the tetracycline gene promoter plays an important role in the morphological changes induced by pDAV54 and presumably by pMAK542, plasmids pMAK543 and pMAK551 (Fig. 3) were constructed. Plasmid pMAK543 was derived from pMAK542 by substituting the *lac* promoter for the *tet* promoter. Another plasmid, pMAK551, containing the *lac* promoter in the other side of the chromosomal fragment and transcribing in the opposite direction, was also obtained. Figure 3 shows the cell morphology of strain JM103 transformed with plasmids pLG339, pMAK542, pMAK543, and pMAK551 in late-exponential-phase cultures with or without 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). As expected, pMAK543 produced IPTG-dependent round cell shape. In contrast, after induction of the *lac* promoter in pMAK551 to counteract transcription from the *tet* promoter, cell morphology was almost normal. These results indicate that not only a moderately higher gene dosage but also a higher level of transcription is sufficient for *bolA*$^+$ to induce the spherical cell shape.

To further determine the *bolA* gene dosage effect on cell morphology, the runaway plasmid pMOB45 was used to construct pMAK552 and pMAK553 (Fig. 4). In exponentially growing cultures at 30°C, plasmid pMOB45 is maintained at nearly 15 copies per chromosome equivalent, increasing to nearly 50 copies after 90 min at 42°C (8). Plasmids pMAK552 and pMAK553 were obtained by inserting the 1.6-kb *Bam*HI fragment containing *bolA*$^+$ in pMAK551 (Fig. 3) into the unique *Bam*HI site of the *tet* gene in pMOB45. The *tet* promoter and the chromosomal fragment in pMAK552 are, as in pMAK542, in the same orientation, whereas they are opposed in pMAK553. After 90 min at 42°C, SK6501 cells containing pMAK552 were all round,

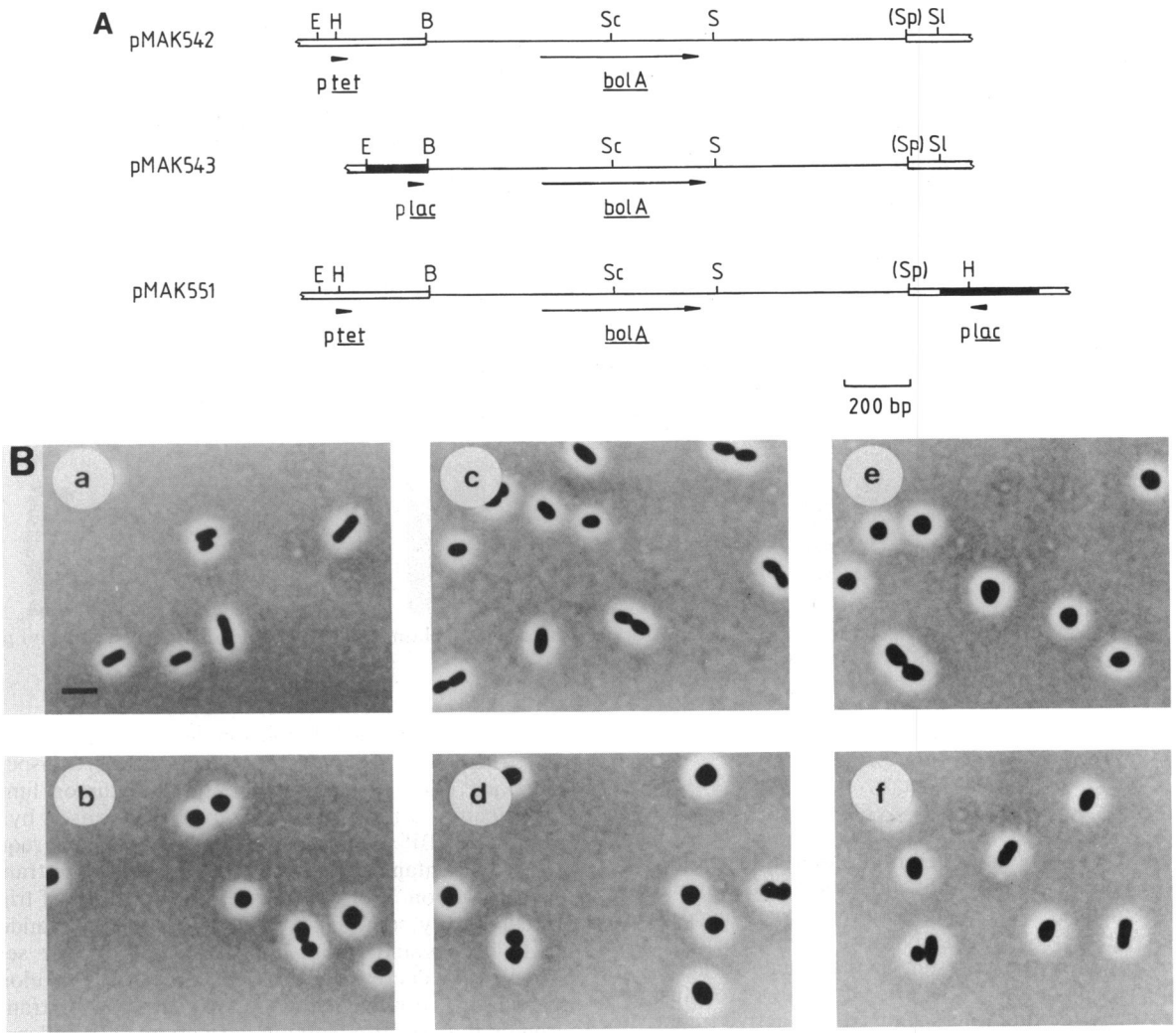

FIG. 3. Cell shapes produced by *bolA*$^+$ in low-copy-number vectors. (A) Location of the *tet* and *lac* promoters in pMAK542, pMAK543, and pMAK551. Abbreviations: Sc, *Sac*I; Sl, *Sal*I; others, as described for Fig. 1. (B) Cell morphology in late-exponential-phase cultures of SK6501 transformed with pLG339 (a), pMAK542 (b), pMAK543 (c), pMAK543 with 1 mM IPTG (d), pMAK551 (e), and pMAK551 with 1 mM IPTG (f). Bar, 2.5 μm.

whereas pMAK553 produced a mixed population with some ovoid cells (Fig. 4). However, after 120 min at 42°C, cells containing pMAK553 were also all round, indicating that readthrough transcription from the *tet* promoter is not required to produce round cells when the *bolA*$^+$ gene dosage is as high as 50 copies per chromosome.

Cell elongation, cell division, and *bolA*$^+$ overexpression. A possible relationship between the morphological effects caused by *bolA*$^+$ overexpression and genes involved in the cell elongation system was first tested by transforming SK6501(pMAK580) (*bolA*$^+$) (Fig. 1) with pBS44 (*pbpA*$^+$ *rodA*$^+$), pBS47 (*pbpA*$^+$), and pLG346 (*rodA*$^+$). The presence of *pbpA*$^+$, *rodA*$^+$, or both in higher gene dosages was not able to cancel the *bolA*$^+$ overexpression effects on cell morphology. In addition, round cells produced by *bolA*$^+$ overexpression did not acquire a higher resistance to mecillinam, which is a characteristic property of both *pbpA* and *rodA* mutants. Moreover, these round cells (strain SK6501 containing pMAK552 after 90 min at 42°C) showed no alteration in the ability of their PBPs to bind [^{14}C]benzylpenicillin; PBP 2 affinity for mecillinam remained also unaffected (data not shown).

Alternatively, *bolA*$^+$ overexpression effects on cell morphology could depend on genes involved in cell division. Accordingly, the runaway plasmid pMAK553 (*bolA*$^+$) was transformed into strains LMC509 [*ftsZ*(Ts)], TOE1 [*ftsQ*(Ts)], D-3 [*ftsA3*(Ts)], and TOE23 [*pbpB*(Ts)], and cells were examined at intervals by phase-contrast microscopy after they had been shifted to 42°C. The absence of functional *ftsA* or *ftsQ* gene products did not prevent the formation of a round cell wall (Fig. 5), thus producing a cell shape resembling that exhibited by *pbpA ftsA* double mutants (3). A *pbpB*(Ts) mutant showed an identical cell shape change at the restrictive temperature when transformed with pMAK553. In contrast, the effects of *bolA*$^+$ overexpression on cell shape were suppressed in the absence of an active *ftsZ* gene product, so that rod-shaped filaments were formed (Fig. 5), their appearance similar to but not exactly like that of *ftsZ* filaments. The pMAK553 (*bolA*$^+$) transformants obtained in isogenic *fts*$^+$ strains always showed rod-shaped cells at 30°C and round cells when shifted to 42°C and when reaching the stationary phase of growth (data not shown). Therefore, spherical-murein synthesis due to high BolA

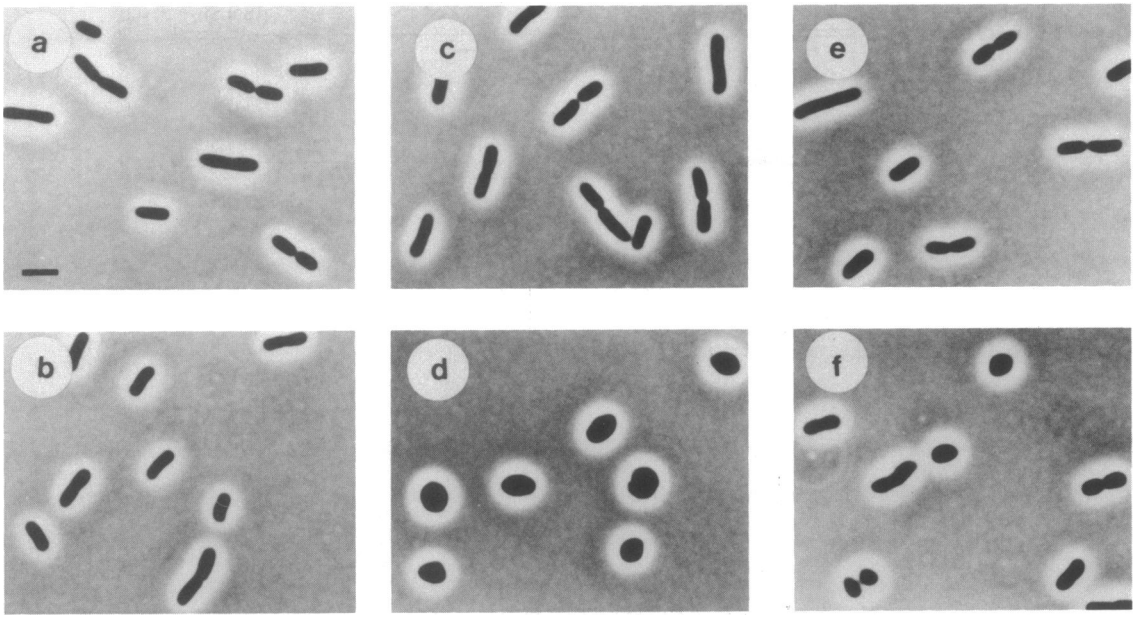

FIG. 4. Cell shapes in exponential cultures of SK6501 transformed with runaway plasmids (each at 30 and 42°C, respectively) pMOB45 (a and b), pMAK552 (c and d), and pMAK553 (e and f). Bar, 2.5 μm.

levels depends on the cell division machinery, particularly on the initiation steps of septum formation.

DISCUSSION

A newly found gene involved in the morphogenetic pathways of *E. coli* has been identified and located at min 10 on the genetic map (2). This gene, which we have named *bolA*, has been characterized by its ability to produce osmotically stable round cells when present at high gene dosages and codes for a polypeptide of 13 kilodaltons. Operon fusions with the *tet* and *lac* promoters showed that active transcription from a strong promoter is required for *bolA* to produce round cells when present in a low-copy-number vector. From these results and by comparing the restriction data obtained from pDAV54 with the *E. coli* restriction map (19), a clockwise direction of transcription for *bolA* was deduced. When present in high-copy-number vectors, *bolA*$^+$ does not need an upstream strong promoter to produce its characteristic morphological effects, while maxicell experiments have shown that BolA synthesis regulated by its own promoter is almost as efficient as it is when regulated by the *tet* promoter, suggesting that *bolA* could be tightly repressed under normal conditions during exponential growth.

Moderate overproduction of BolA did not affect cell viability but rendered the cells very sensitive to ampicillin. Although sensitivity to ampicillin is characteristic of some round-cell mutants that map at min 14 (*pbpA* and *rodA*) (18, 39), the round cells induced by *bolA*$^+$ overexpression were normally sensitive to mecillinam and their PBP patterns were unaffected. Furthermore, an increase in the *pbpA* and *rodA* gene dosages did not suppress the *bolA*$^+$ overexpression effects on cell morphology. Thus, the appearance of round cells due to *bolA*$^+$ overexpression is not likely to be caused by a negative modulation of either expression or activity of the *pbpA* and *rodA* genes.

Overexpression of *dacA*, the gene coding for PBP 5, also causes round cells, producing a decrease of pentapeptide side chains in newly inserted murein (27). PBP 3 is an essential enzyme for cell division, and it can use both penta- and tripeptide precursors as acceptors for transpeptidase reactions (34). Under pentapeptide precursor limitation, cells acquire a roundness similar to that caused by a deficiency in PBP 2 (34), suggesting that PBP 2 requires a complete pentapeptide precursor to perform the transpeptidation reaction. Thus, an increase of the levels of tripeptide precursors by some carboxypeptidase activity unidentified up to now would stimulate cross-linking by the septation system and decrease cross-linking by the cell elongation machinery, resulting either in the initiation of transversal growth required for septum formation (27) under normal conditions or in the induction of spherical cells when the elongation machinery is altered. In accord with this hypothesis, a high level of carboxypeptidase activity has been observed during cell division (31, 32). However, the two main carboxypeptidases of *E. coli*, PBP 5 and PBP 6, are not required for cell division, since mutants lacking these PBPs grow and septate normally (9, 38).

Overexpression of the *bolA*$^+$ gene does not cause an increase in PBP 5 or PBP 6 and produces a PBP 3-independent spherical cell shape. If the roundness of cells that overproduce BolA can be ascribed to the synthesis of septal murein, this shape should depend on a different mechanism which, as PBP 3 mutants still exhibit some degree of constriction (F. B. Wientjes, B. L. M. de Jonge, F. Driehuis, M. Aarsman, J. T. M. Wouters, and N. Nanninga, 1987 EMBO Workshop: Molecular Basis of Bacterial Growth and Division, Segovia, Spain, p. 166), could be related to the earliest steps of septation.

To produce round cells when overexpressed, *bolA*$^+$ requires the presence of an active *ftsZ* gene product, which plays a central role in cell division (24, 43) and may control the earliest step of septation (3). This fact suggests a role for BolA in cell division, providing that a mechanism exists by which FtsZ could modify the conformation of the nascent murein in these early steps of septum formation. Particularly, BolA could be directly involved in the FtsZ-dependent

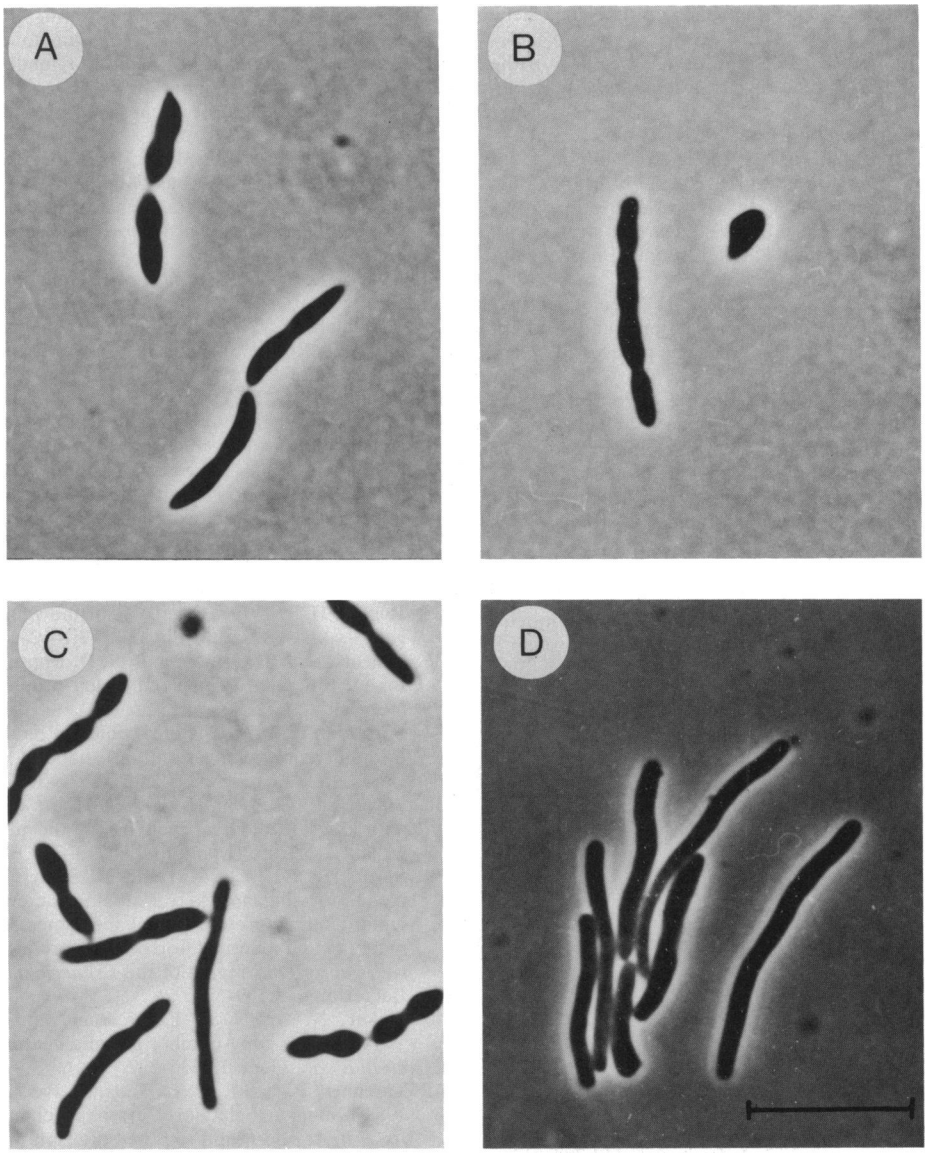

FIG. 5. Cell shapes produced by *bolA*$^+$ overexpression in different cell division mutants. Cells growing exponentially at 30°C were shifted to 42°C. Samples were photographed as described in the text. Strains used and time of sampling (in minutes) after the temperature shift (dependent on the doubling time of each strain) were as follows: (A) TOE23(pMAK553) 280; (B) TOE1(pMAK553), 90; (C) D-3(pMAK553), 60; and (D) LMC509(pMAK553), 60. Bar, 10 μm.

increase in carboxypeptidase activity that takes place immediately before cell division (31, 32).

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