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Aberrant Cell Division and Random FtsZ Ring Positioning in *Escherichia coli cpxA** Mutants

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In *Escherichia coli*, certain mutations in the *cpxA* gene (encoding a sensor kinase of a two-component signal transduction system) randomize the location of FtsZ ring assembly and dramatically affect cell division. However, deletion of the *cpxRA* operon, encoding the sensor kinase and its cognate regulator CpxR, has no effect on division site biogenesis. It appears that certain mutant sensor kinases (CpxA*) either exhibit hyperactivity on CpxR or extend their signalling activity to one or more noncognate response regulators involved in cell division.

In dividing *Escherichia coli*, a complex of different Fts proteins directs septum formation in the middle of the cell. Early in the division cycle, the FtsZ protein assembles into a ring structure at the future division site (1, 4, 16, 24–26, 35, 40). The Min proteins are involved in directing assembly of FtsZ to the medial division sites (2, 11, 12, 41). MinC and MinD, together, block FtsZ assembly at potential polar division sites, while MinE stimulates its assembly at the medial position (5, 12). Thus, when FtsZ is excessive or MinC or MinD is insufficient, FtsZ may assemble at the polar positions, resulting in minicell formation (3, 12, 46).

The *cpxA* gene encodes the sensor kinase (27, 28) of a two-component system, with CpxR as the response regulator (15, 31, 44). Numerous phenotypic changes (7, 27, 28, 32, 33, 38), many of which are associated with membrane function, have been attributed to *cpxA** mutants (8, 10, 34). Recently, a CpxA* protein was found defective in CpxR-P phosphatase activity (39), which would explain the elevated expression of CpxR-P target operons, such as *degP*, *dsbA*, *ppiA*, and *cpxP* (8–10, 34). Here we describe the randomized location of FtsZ ring assembly and septation in *cpxA** mutants (Table 1), a cell division phenotype heretofore not reported.

Irregular septation and nucleoid inheritance in *cpxA populations.** Since *cpxA** mutations seem to impair membrane functions, we wondered whether cell morphology was also affected. Therefore, strains JP406 (*cpxA*$^+$) and JP408 (*cpxA9**) were grown exponentially (glucose medium, 37°C) and examined by electron microscopy. Unexpectedly, the mutant cells did not show uniform morphology: a minority exhibited irregular shapes and sizes which seemed to result from aberrant cell septation (Fig. 1A to D). When 250 dividing cells of each strain were surveyed, JP406 (*cpxA*$^+$) almost invariably cleaved at the cell midpoint (97% of the population). By contrast, 38% of the JP408 (*cpxA9**) cells cleaved at random positions along the cell axis and sometimes showed multiple septum formation. Phase-contrast microscopy of cells (Luria-Bertani [LB] medium, 37°C) stained for DNA by 4′-6-diamidino-2-phenylindole showed that, in contrast to wild-type JP406 (*cpxA*$^+$), mutant strains JP408 (*cpxA9**) and JP467 (*cpxA2.1**) often formed subsized cell bodies devoid of DNA (nucleoids) during division (Fig. 1E and F).

Whereas the division anomaly of JP408 (*cpxA9**) occurred at 42 and 37°C but not at 30°C (LB or minimal-glucose medium), this growth defect occurred at all three temperatures with JP467 (*cpxA2.1**). The *cpxA** mutation alone seems to account for the growth defect, since this phenotype was P1 transduced to wild-type strains. It is noteworthy that a *cpx* deletion mutant, ECL1212, divided normally.

TABLE 1. Strains and plasmids used in this study[a]

Strain	Relevant genotype	Reference
ECL525	MC4100 Δ(*argF-lac*)*U169 araD139* Δ*frd-101 rpsL150 relA1 deoC1 flb-5301 ptsF25*	22
JP406	ECL525 F′pOXgen	This study
ECL1212	JP406 Δ*cpxRA-2*	This study
ECL1215	JP406 *ara*$^+$	34
JP408	JP406 *zii-510*::Tn*10 cpxA9**	This study
JP466	JP406 *argE*::Tn*10*	34
JP467	JP406 *argE*::Tn*10 cpxA2.1**	34

[a] All of the strains used in this study are isogenic derivatives of ECL525. F′pOXgen (14) was used to introduce conjugative ability to ECL525. The defined deletion Δ*cpxRA-2* was constructed as described by Blum et al. (6). The 1.2-kb deletion between the *Xho*I and *Eco*RI sites in the *cpxRA* operon removed most of the coding sequence of the two genes. The deletion was confirmed by PCR. Mutant *cpxA* alleles were introduced into strain JP406 by phage P1 cotransduction of linked *zii-510*::Tn*10* or *argE*::Tn*10*. Strain JP408 received the *cpxA9** allele from strain AE2293, which was provided by P. M. Silverman (38). The *cpxA9** mutant was originally selected for amikacin resistance (38) and shown to have a Leu38→Phe (TTT) substitution in the periplasmic domain of CpxA (42). This mutation was confirmed in strain JP408 by DNA sequencing. The *cpxA2.1** mutant was isolated in a selection for increased expression of a *lacZ* transcriptional fusion to the *yajC-secDF* operon. This *cpxA* allele was sequenced and shown to have a Val20→Ala (GCG) substitution plus an insertion of Leu and Val (CTGGTG) between Ala20 and Leu21 in the first membrane-spanning segment. For other characteristics of this mutation, see reference 34. Strains with *cpxA** alleles were routinely grown at 30°C, except where indicated, to minimize reversion or suppression.

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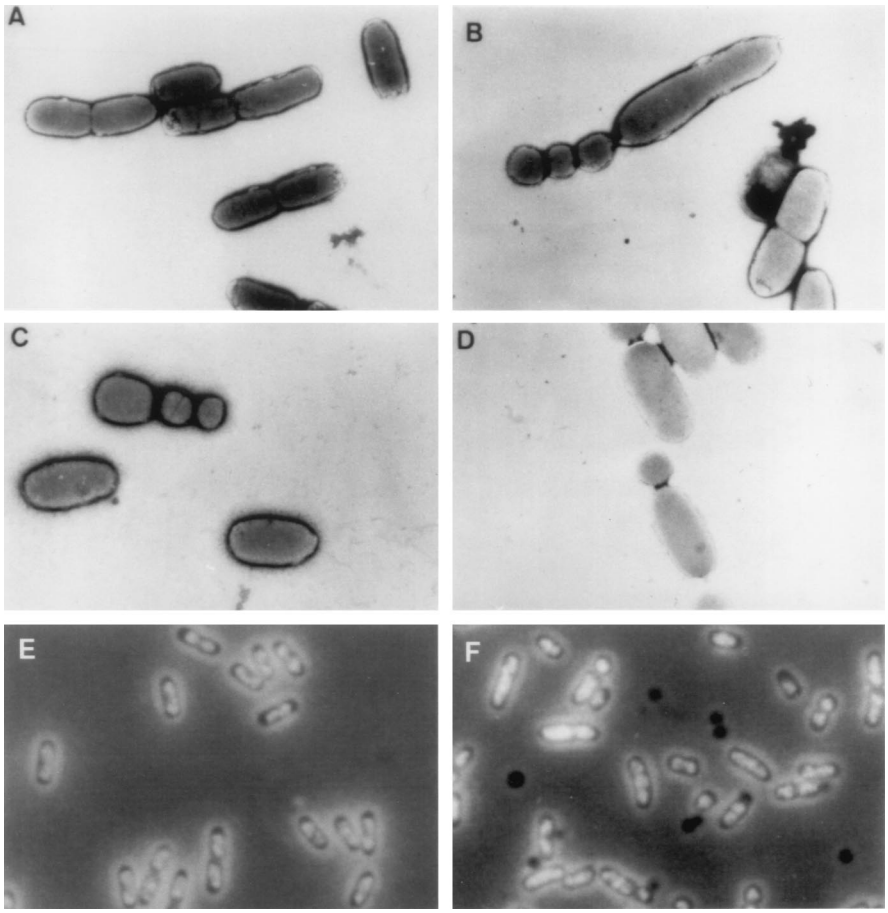

FIG. 1. (A to D) Electron micrographs. Cells were fixed on an electron microscopy grid with 1% phosphotungstic acid and observed at a magnification of ×5,000. Strains: JP406 (*cpxA*$^+$), A; JP408 (*cpxA9**), B to D. (E and F) Phase-contrast micrographs. Division products without DNA appear as dark bodies. Strains: JP406 (*cpxA*$^+$), E; JP467 (*cpxA2.1**), F.

Relationship between FtsZ ring formation and cell length in *cpxA mutants.** Because of the role of FtsZ in septation, the assembly of FtsZ and the positioning of the FtsZ ring during mutant cell division were compared with that in wild-type cells (Fig. 2). Consistent with the results of cell division analysis, the FtsZ ring (stained green by immunofluorescence) formed almost invariably at the cell midpoint in dividing wild-type cells, whereas the location of the FtsZ ring was random in almost half of the JP467 (*cpxA2.1**) population. The patterns of nucleoid inheritance, as revealed by DNA staining (red), confirmed the results of phase-contrast microscopy.

During steady-state growth of wild-type cells, length is an approximate indicator of age. Hence, most long cells have an FtsZ ring, whereas most short ones do not (1, 35), giving a bimodal distribution when the numbers of cells with an FtsZ ring and those without one are plotted against cell length. In the JP467 (*cpxA2.1**) population, a significant fraction of dwarf cells is present, some of which nonetheless possess the FtsZ ring. Also, a large fraction of the cells is oversized, and yet some of these lack an FtsZ ring. Consequently, the presence or absence of an FtsZ ring, and thus cell age, can no longer be statistically predicted by cell length. The results of FtsZ analysis are summarized in Fig. 3.

FtsZ levels in *cpxA mutants.** Unlike the *minB* mutants, which simultaneously produce minicells and filaments (12), *cpxA** mutants only occasionally produce cells that are moderately longer than *cpxA*$^+$ cells. The difference in length distribution between wild-type and mutant cells does not appear to be attributable to a difference in growth rate, since the doubling times of strains JP467 (*cpxA2.1**) and JP408 (*cpxA9**) are only slightly longer than that of the wild-type parent (LB medium, 37°C). The aberrant cell division phenotype of *cpxA** mutants, therefore, more closely resembles that of FtsZ overproduction. However, when rates of FtsZ synthesis and stability were measured by pulse-chase immunoprecipitation, no significant difference was found between strains JP406 (*cpxA*$^+$) and JP467 (*cpxA2.1**) (data not shown).

Normal septation in wild-type cells with NlpE-activated Cpx signal transduction. Oversynthesis of the outer membrane protein NlpE activates the Cpx pathway in wild-type cells (8, 10, 34). To determine whether this activation affects septation during cell division, strain ECL1215 was transformed with plasmid pND18, expressing *nlpE* from an L-arabinose-inducible promoter (10, 34). Cells were grown in LB medium (37°C), induced with L-arabinose, stained for DNA, and examined by phase-contrast microscopy. No cell division defects similar to those seen in *cpxA** mutants were observed.

No epistatic effect of a *degP* null mutation on *cpxA phenotypes.** Induction of *degP* in *cpxA** mutants is implicated in suppressing toxic phenotypes associated with accumulation of the LamB-LacZ-PhoA hybrid protein (8). To see whether some of the pleiotropic defects are attributable to elevated

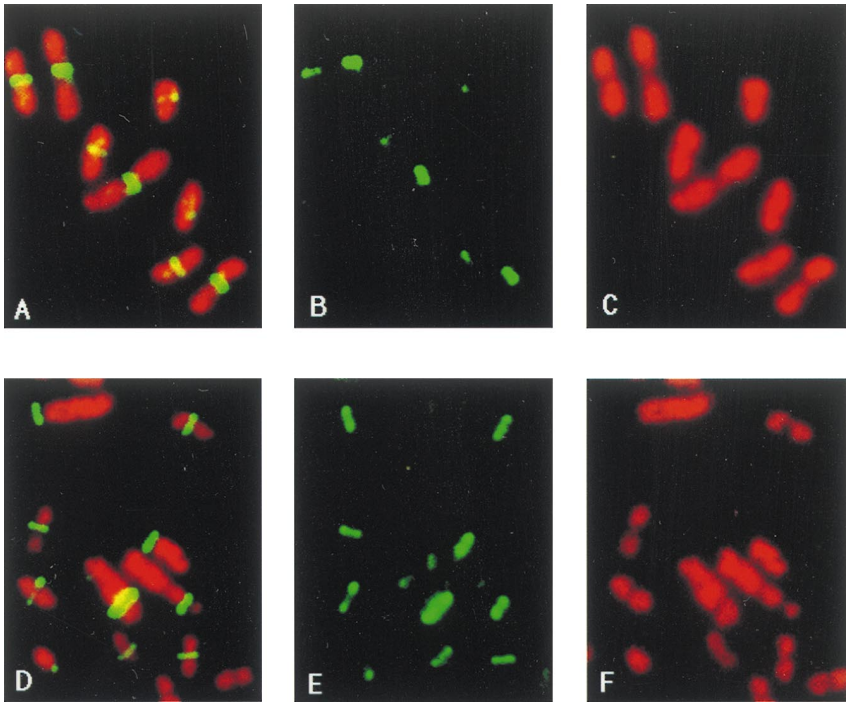

FIG. 2. Immunolocalization of FtsZ in fixed samples of strains JP406 (*cpxA*$^+$) and JP467 (*cpxA2.1**). Cultures growing exponentially in LB medium at 37°C were harvested and processed for microscopy essentially as previously described (36). Photographs of the same visual field were then taken with two different filter sets, one for visualizing the green-fluorescent antibodies against the FtsZ protein (B and E) and one for the red-stained DNA (C and F). A composite picture of both FtsZ and DNA stained was obtained by sequentially exposing the same frame of film to light emitted by each fluorophore (A and D). A to C, strain JP406 (*cpxA*$^+$); D to F, strain JP467 (*cpxA2.1**). Separate panels of the FtsZ rings and the nucleoids are presented because their superimposition rendered some of the individual images obscure.

levels of the DegP protease, causing nonspecific damage to cell envelope proteins, strain JP467 (*cpxA2.1**) was compared with a *cpxA2.1* degP*::Tn*5* double mutant. In particular, the growth phenotypes on succinate and serine as the sole carbon source, resistance to low levels of amikacin, and aberrant cell division were analyzed. Abolition of *degP* function had no effect on the *cpxA** phenotypes tested.

How CpxA* may affect cell division. Since CpxA* probably enhances CpxR-P levels (39), we attempted to identify CpxR-P target operons associated with cell division. By using the proposed CpxR-P-binding consensus 5′-GTAAAN$_{5-7}$GTAAA-3′ (34), we found no obvious candidates among the known cell division genes, including *ftsQAZ* (17, 43), *ftsYEX* (18), *minCDE* (12), and *zipA* (19). However, the *fic* gene, implicated in cell division (23), is preceded by *ppiA*, a CpxR-P-controlled gene. Whether *fic* expression is altered in *cpxA** mutants remains to be determined.

It is also possible that CpxA* cross-phosphorylates one or more noncognate two-component regulators that have a role in cell division. In *Bacillus subtilis*, a two-component signal transduction system directly regulates the localization of the division site during sporulation (21, 25). Two-component signal transduction systems also play essential roles in cell cycle control in *Caulobacter crescentus* (13, 20, 29, 30, 37, 45, 47, 48). Further characterization of the *cpxA** cell division phenotype may therefore cast new light on control of cell division in *E. coli*.

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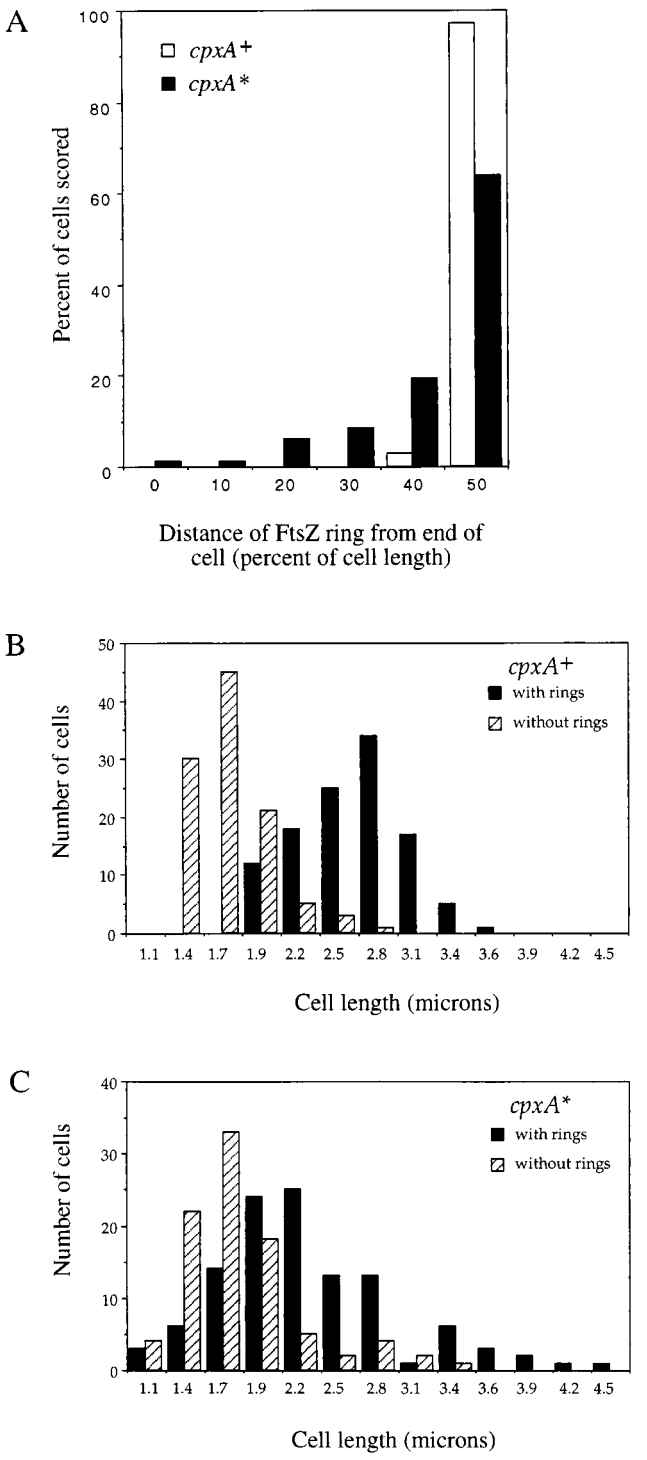

FIG. 3. Position analysis of the FtsZ ring in wild-type and mutant cells. (A) Percentage of cells containing an FtsZ ring (ordinate) versus the distance between the FtsZ ring and the nearest cell end (abscissa). Scoring for the presence of the ring and precise measurements of cell length and FtsZ ring position were carried out on photographic films on which the cell envelope is visible (from the samples shown in Fig. 2). The distance between the FtsZ ring and the nearest cell end was divided by the total cell length and multiplied by 100. Hence, the FtsZ ring in the midpoint of the dividing cell is given a value 50. Open bars, results from 220 cells of JP406 (*cpxA*$^+$); solid bars, results from 207 cells of JP467 (*cpxA2.1**). (B and C) Presence or absence of the FtsZ ring as a function of cell length. The number of cells with (solid bars) or without (hatched bars) the ring is plotted versus cell length. Strains: JP406 (*cpxA*$^+$), B; JP467 (*cpxA2.1**), C.

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