Intracellular localization of P1 ParB protein depends on ParA and *parS*

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The P1 partition system promotes faithful plasmid segregation during the *Escherichia coli* cell cycle. This system consists of two proteins, ParA and ParB, that act on a plasmid site called *parS*. By immunofluorescence microscopy, we observed that ParB localizes to discrete foci that are most often located close to the one-quarter and three-quarters positions of cell length. The visualization of ParB foci depended completely on the presence of *parS*, although their visualization was independent of the chromosomal context of *parS* (in P1 or the bacterial chromosome). In integration host factor-defective mutants, in which ParB binding to *parS* is weakened, only a fraction of the total pool of ParB had converged into foci. Taken together, these results indicate that *parS* recruits a pool of ParB into foci and that the resulting ParB–*parS* complexes serve as substrates for the segregation reaction. In the absence of ParA, the position of ParB foci in cells is perturbed, indicating that at least one of the roles of ParA is to direct ParB–*parS* complexes to the proper one-quarter positions from a cell pole. Finally, inhibition of cell division did not inhibit localization of ParB foci in cells, indicating that the positioning signals in the *E. coli* host that are needed for P1 partition do not depend on early division events.

Low-copy-number plasmids and bacterial chromosomes are specifically localized and oriented inside bacterial cells (1–4), and this localization is thought to be essential for faithful chromosomal inheritance by daughter cells at cell division. Much of our understanding of the segregation mechanisms in prokaryotes has arisen from studies of the partition systems of low-copy-number plasmids in *Escherichia coli*, which have led to the identification of homologous systems in a growing number of bacterial species. Partition of the P1 prophage, a unit-copy-number plasmid (5), is accomplished by two plasmid-encoded proteins, ParA and ParB, that act on a plasmid site called *parS* (6). ParB and *E. coli* integration host factor (IHF) bind to *parS* to form a partition complex (7–9), which is predicted to be the substrate for the partition localization reaction. ParA is an ATP-binding protein and weak ATPase (10, 11). ParA can assemble onto the partition complex at *parS* in an ATP- and ParB-dependent fashion (12), but its action in partition is still unclear.

Using green fluorescent protein (GFP) fused to the Lac repressor, Gordon *et al.* (2) showed that P1 plasmids, tagged with multiple copies of the *lac* operator, were specifically localized within cells. For most of the cell cycle, they were located at the one-quarter and three-quarters positions of cell length. These became the midpoint of a new cell after division, but plasmids soon moved, presumably after DNA replication, to the new one-quarter and three-quarters positions. The F plasmid in *E. coli* shows a similar distribution pattern (2, 3), which depends on its partition system (3).

The F and P1 proteins share sequence similarity with each other and with proteins encoded by other low-copy-number plasmids and several bacterial genomes. In *Bacillus subtilis*, the ParB homologue SpoOJ is required for proper chromosome partition and binds to eight copies of a specific DNA sequence in the origin region of the bacterial chromosome (13, 14). Fluorescence microscopy has shown that SpoOJ is localized to the polar regions of the cell, along with the replication origin (15, 16). The ParA and ParB homologues of *Caulobacter crescentus* are also concentrated at the cell poles (17). However, in the F plasmid system, there are conflicting reports about the location of the ParB-like protein SopB. SopB has been reported to show polar localization that is independent of *sopC*, the F partition site (18). Conversely, another study found that SopB localization depends on *sopC*; it was distributed throughout cells that harbored no *sopC*-containing plasmid but relocalized to specific positions in the presence of *sopC* (19). In both of these studies, visualization was possible only when the plasmid proteins were overexpressed. SopB and ParB also have the ability to silence genes near *sopC* and *parS*, respectively (20–23). This property has been attributed either to extensive protein binding to DNA surrounding its specific site or to sequestration away from the transcription machinery.

We have examined the intracellular location of P1 ParB by immunofluorescence and have detected the endogenous protein expressed from a unit-copy P1 plasmid. We observed that ParB formed foci whose positions coincided with the positions previously measured for P1 plasmids (2) and whose formation depended completely on the presence of *parS*. The position of ParB foci bound to mini-P1 plasmids depended on ParA. Our results indicate that an initial partition complex containing ParB and IHF assembles at *parS*, followed by extensive recruitment of most of the remaining intracellular ParB to the plasmid. Attachment of this complex to specific regions of the cell requires ParA. These observations indicate distinct and specific functions of ParB and ParA in positioning P1 plasmids in *E. coli* cells.

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Materials and Methods

Bacterial Strains, Bacteriophage, and Plasmids. *E. coli* N99 (F^- *galK*) and N99*ihfA* (F^- *galK* Δ*82ihfA*::Tn*10*; ref. 24) were the bacterial strains used in all experiments. pLG44 and pLG48 are mini-P1 plasmids (25). The Par system of pLG48, which contains *parS*, is defective because of an insertion mutation in *parA*. pBEF118 is a pBR322 derivative containing *parB* and has been described (26). The i^{21} phage λD69 (27) was the λ-vector for λ*parAB* and λ*parS*. λ*parAB* contains the *parA* and *parB* genes under the control of their natural promoter, between the P1 *Hin*dIII and *Dra*I restriction sites (6). λ*parS* contains the P1 sequences between the *Taq*I and *Eco*RV sites that span *parS*. The P1 restriction sites were changed to *Hin*dIII sites by using synthetic linkers, and the fragments were inserted into the λD69 *Hin*dIII site. The resulting recombinant phage were used to lysogenize N99, creating N99(λ*parAB*) and N99(λ*parS*).

Antibodies. Antibodies to P1 ParA and ParB were raised in rabbits as described (9, 28) and were affinity purified against pure ParA or ParB proteins (29). Cy3-coupled and FITC-coupled goat anti-rabbit IgGs (Jackson ImmunoResearch) were used at a 1:100 dilution.

Abbreviations: IHF, integration host factor; GFP, green fluorescent protein.

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Immunofluorescence Microscopy. *E. coli* cells containing different plasmids or phage were grown in Luria broth at 37°C until the culture reached an A_{600} of about 0.5. Where indicated, they were treated with cephalexin (at 10 μg/ml) for 3 h. Cells were collected and fixed either by methanol/acetic acid (30) or by glutaraldehyde plus paraformaldehyde (31). Both fixation methods gave similar results. Fixed cells were incubated with affinity purified anti-ParB or anti-ParA antibody and then with Cy3-conjugated or FITC-conjugated secondary antibody as described (30, 31). Cell preparations were examined in a Nikon Microphot FX-A microscope by using Nomarski optics and epifluorescence, and micrographs were recorded on film. For measurement of the positions of ParB foci, cells were stained with the plasma membrane stain FM4-64 (Molecular Probes), which could be visualized with FITC-stained ParB foci by using fluorescein optical sets. Measurements were taken by using SCIONIMAGE software (Scion, Frederick, MD). Negative controls for immunofluorescence were analyses of N99 cells containing no plasmids or P1 proteins or of N99 (pLG44) cells from which primary antibody was omitted. Both lacked detectable fluorescence inside the bacterial cells.

Results

ParB Protein Appears as Foci That Mark the Position of P1 Plasmids. Partition of P1 plasmids is a positioning reaction that ensures that every bacterial cell receives a copy of P1 at cell division. P1 plasmids localize to the one-quarter and three-quarters positions of bacterial cells for most of the cell cycle (2), and it is predicted that this localization is determined by the P1 partition system. We asked whether we could use immunofluorescence with Cy3-labeled antibodies to detect the P1 partition proteins in *E. coli* N99 cells containing the mini-P1 plasmid pLG44 (25). P1 ParB protein was clearly visible as discrete foci inside bacterial cells (Fig. 1 *A–C*). ParB staining appeared as bright foci whose positions correlated strongly with the position of mini-P1 plasmids as previously determined (ref. 2; see below). The presence of these foci suggested that much, if not most, of the ParB had bound to, or converged at, its binding site, *parS*. This result was intriguing, because there are several thousand molecules of ParB per cell (26).

To test the idea that ParB foci were marking P1 plasmids, we first examined their dependence on *parS.* Because mini-P1 plasmids are extremely unstable without *parS*, we used other stable episomes to provide the P1 proteins so that all cells in the population would contain the same amount of these proteins. ParB was expressed from pBEF118, a pBR322 derivative, which expresses an amount of ParB similar to that made by mini-P1 plasmids (26). ParA and ParB were expressed from a bacteriophage-λ derivative carrying *parA* and *parB* (λ*parAB*) that was integrated in single copy in the bacterial chromosome. Without *parS*, ParB staining was more diffuse throughout the cell, although not completely uniform (Fig. 2 *A–D*). The ParB staining pattern was very similar in cells containing only ParB (Fig. 2 *A* and *B*) or both ParA and ParB (Fig. 2 *C* and *D*). Therefore, this diffuse pattern of ParB was independent of ParA. In the presence of *parS*, ParB foci reappeared, and the diffuse background greatly diminished. In fact, addition of *parS* to N99 cells containing only ParB showed that foci formation did not require ParA. N99 cells with one copy of *parS* inserted in the bacterial chromosome (as λ*parS*) and pBEF118 contained foci that appeared similar to those of pLG44 (Fig. 2 *E* and *F*). These patterns were even more evident when filamentous cells were examined (Fig. 2 *M* and *N*; and see below). Therefore, ParB convergence into foci depended on the presence but not on the context of *parS*.

Next, we examined the frequency and position of ParB foci in N99 cells containing pLG44. The majority of cells contained two foci, and the number of foci correlated with cell length in that longer cells had more foci than short ones (Fig. 3*A*). The cells with only one focus tended to be the smallest cells (as newborn cells are expected to be). To measure the position of ParB foci, we costained the cells with the fluorescent membrane stain FM4-64 and FITC-labeled antibodies. This costaining allowed discrimination of the cell (marked in orange) from FITC-stained foci (as green dots) by using a single microscope filter (FITC; Fig. 1 *D* and *H*). The majority of foci in cells that contained two foci were positioned close to the one-quarter and three-quarters positions (Figs. 3*B* and 4*A*). (Our measurements were actually closer to 30% from each end of the cell, but given the small size of these cells, it is impossible to determine whether this measurement is significantly different than one-quarter and three-quarters positions.) In cells with one focus, it was located at or near the midpoint of the cell (Fig. 3*B*). In cells with four foci, the foci were approximately evenly distributed along the length of the cell, in positions that we presume would become one-quarter and three-quarters positions of a daughter cell if the cells were still growing. These patterns parallel the behavior of P1 plasmids measured by GFP–*Lac*I tagging of these plasmids (2). Therefore, we conclude that the position of ParB foci corresponds to the position of *parS* and thus of pLG44.

We next looked at ParB staining in *E. coli* IHF mutants, in which ParB binding to *parS* is weakened. *E. coli* IHF binds to and greatly increases ParB's affinity for *parS* (7, 9). *In vivo*, however, IHF is only an accessory factor; P1 plasmids are only slightly less stable in IHF mutants than in wild-type cells. In N99*ihfA* cells with pLG44, ParB foci were barely visible, and the background diffuse staining was brighter (Fig. 5). Foci (or brighter centers) were still present and easiest to detect when the pictures were underexposed (Fig. 5*D*). Therefore, only some of the intracellular pool of ParB had associated with *parS* in the absence of IHF. Nevertheless, because pLG44 is relatively stable, these foci are sufficient to promote partition of most of the plasmids (see *Discussion*).

P1 Distribution Is Not Blocked by Inhibition of Cell Division. We examined the localization of ParB and P1 plasmids when bacterial cell division was disturbed by the inhibitor cephalexin. The target of cephalexin is FtsI (32), and this inhibitor causes extensive filamentation of the bacterial cells. Previous P1 plasmid localization experiments had indicated that P1 plasmids did not distribute along cell filaments that resulted from cephalexin treatment (2). This result contrasted the behavior of the F plasmid, which did distribute along filaments of cephalexin-treated *E. coli*. Surprisingly, we observed that in our strains, ParB foci were relatively evenly spaced along the length of the bacterial filament (Fig. 1 *I–L*), indicating that *parS* and thus the mini-P1 plasmids were properly distributed along the filaments. Identical results were obtained when we examined ParB localization in *E. coli* ftsZ84 mutants at nonpermissive temperatures (data not shown). In the absence of *parS*, ParB staining was diffusely distributed along filaments as it was in individual cells (Fig. 2 *I–L*). Therefore, ParB distribution was independent of FtsI and FtsZ, which both act early in cell division (32, 33). These results strongly argue that P1 plasmids are properly distributed in filamentous cells.

Proper Distribution of ParB Foci Depends on ParA. We asked whether ParA was required for ParB foci formation and position within the cell. pLG48 is an unstable mini-P1 derivative because of a large insertion mutation in *parA*, which eliminates expression of both *parA* and *parB* (25). pLG48 contains *parS* and is stable in the presence of an exogenous source of ParA and ParB. Addition of ParB only (from pBEF118) did not stabilize pLG48, and about half the cell population contained no plasmid, even when the cells were grown under selective conditions. When N99 cells containing pLG48 and pBEF118 were stained for ParB, about

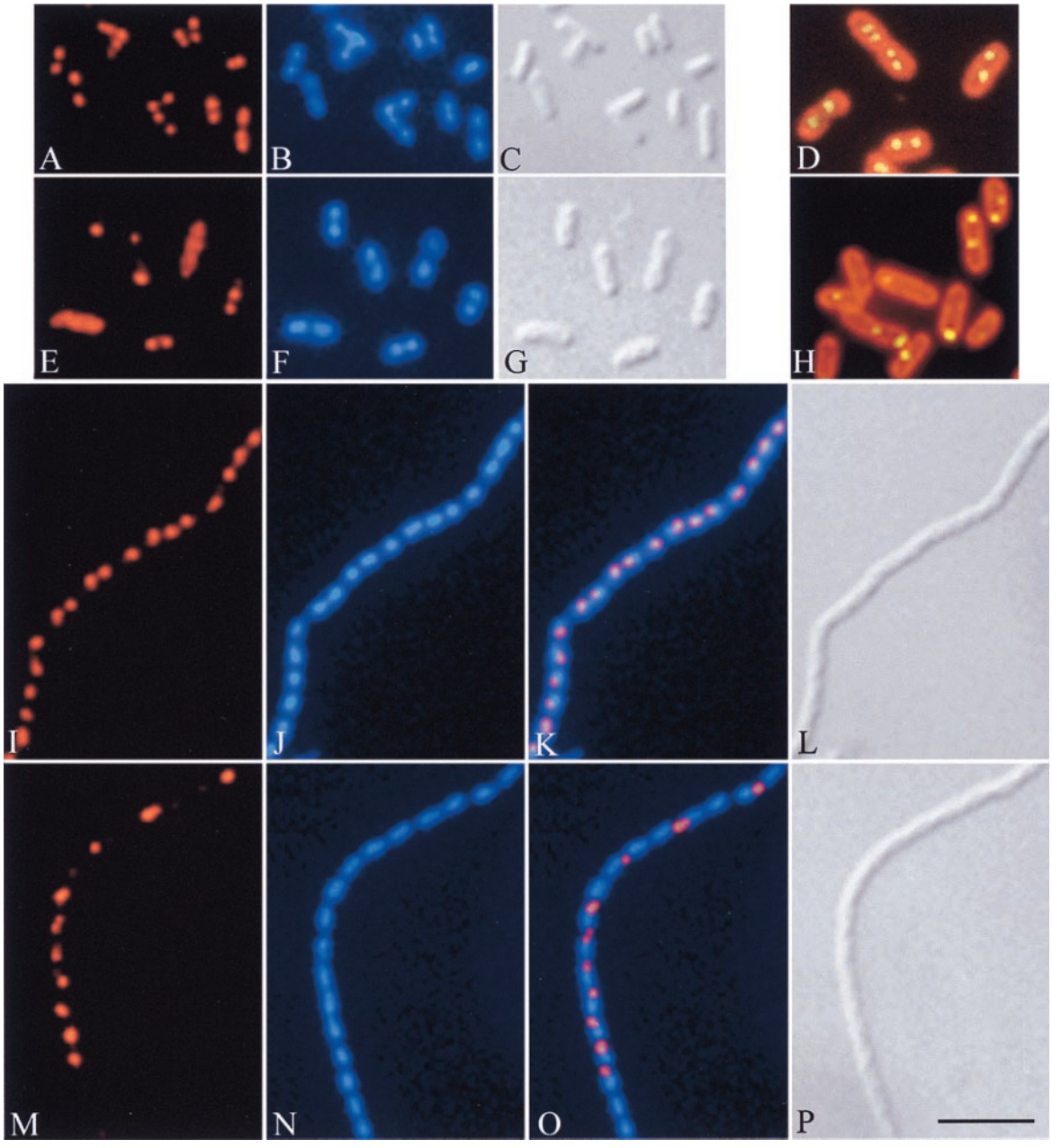

Fig. 1. Visualization of P1 ParB by immunofluorescence. *E. coli* N99 cells containing the Par$^+$ mini-P1 plasmid pLG44 (+ParA and ParB) or the Par$^-$ pLG48 derivative (*parS*$^+$) in the presence of a source of only ParB (pBEF118) were examined. (*A–D*) Log-phase N99(pLG44) cells. (*E–H*) Log-phase N99(pLG48, pBEF118) cells. (*I–L*) Cephalexin-treated N99(pLG44) cells. (*M–P*) Cephalexin-treated N99(pLG48, pBEF118) cells. The red panels at the left (*A*, *E*, *I*, and *M*) show ParB visualized by Cy3-labeled secondary antibodies. The blue panels (*B*, *F*, *J*, and *N*) show the 4′,6-diamidino-2-phenylindole (DAPI) staining to visualize nucleoids. The gray panels (*C*, *G*, *L*, and *P*) are the Nomarski images of the cells. The Cy3 and DAPI images for the filaments were overlaid in *K* (pLG44) and *O* (pLG48). (*D* and *H*) FM4-64 and FITC-ParB stained foci in N99(pLG44) (*D*) and N99(pLG48, pBEF118) (*H*) cells. Note that pLG48 is unstable without ParA; thus, the populations in *E–H* contain cells with pLG48 and cells without pLG48 (plasmid-free segregants). (Bar = 5 μm.)

half the cells had foci, and the remainder were labeled diffusely (Fig. 1 *E–G*). The latter pattern was identical to that of N99(pBEF118) cells alone (Fig. 2*A*); thus, these cells likely represent ones that have lost mini-P1. Of cells that contained ParB foci, most contained only one. The average size of cells with one focus was not smaller or significantly different than the size of cells with two foci. The distribution of foci was not random, however. The foci were most often in the center or at the ends of the cell (Fig. 4*B*), which corresponded to the intracellular locations not occupied by the nucleoid. Over half of the cells with two foci contained one at the pole and one at midcell; the remainder showed both at the poles, both at midcell, or mixed between a pole or midcell and a one-quarter site. This type of distribution has been reported for other plasmids and/or their bound proteins (19, 34) and has been attributed to the foci occupying the cytosolic space inside the bacterial cell.

When cell division was inhibited by cephalexin, ParB foci were irregularly spaced along the filaments and located in the spaces between nucleoids (Fig. 1 *M–P*). No filaments showed the diffuse staining pattern seen in some of the log-phase cells, presumably because all filaments had at least a few mini-P1 plasmids to soak up the ParB. Finally, addition of both ParA and ParB to pLG48

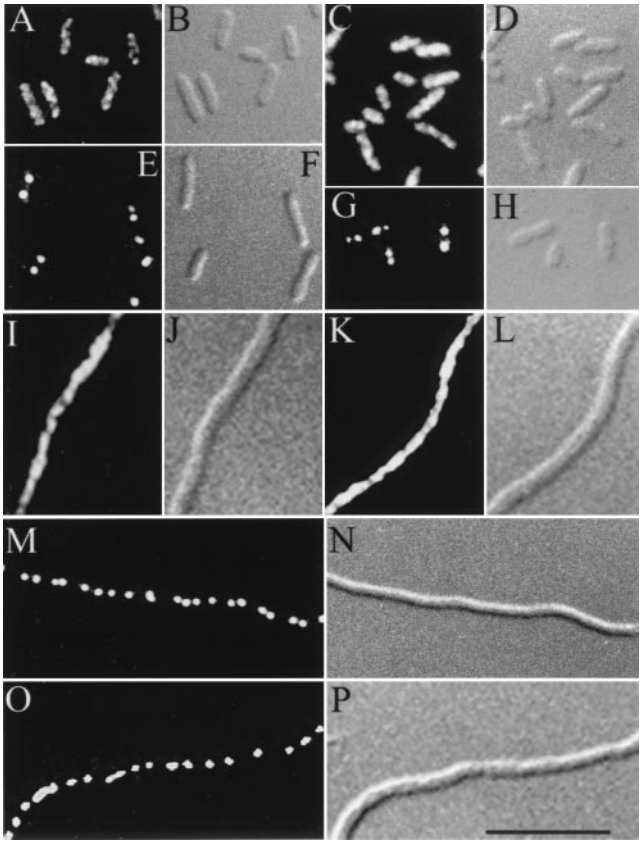

Fig. 2. ParB staining in various cells. In each pair of panels, *Left* is Cy3 fluorescence of antibodies against ParB, and *Right* is the Nomarski image of log-phase cells (*A–H*) and cephalexin-treated cells (*I–P*). (*A*, *B*, *I*, and *J*) Cells containing only ParB but no *parS* [N99(pBEF118)]. (*C*, *D*, *K*, and *L*) Cells containing ParA and ParB and no *parS* [N99(λ*parAB*)]. (*E*, *F*, *M*, and *N*) Cells containing ParB and a copy of *parS* inserted in the bacterial chromosome [N99(λ*parS*, pBEF118)]. (*G*, *H*, *O*, and *P*) Cells containing pLG48 with ParA and ParB [N99(λ*parAB*, pLG48)]. (Bar = 5 μm.)

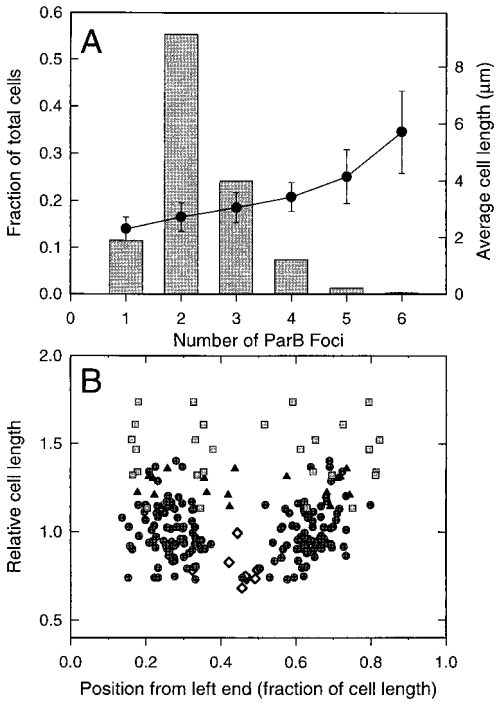

Fig. 3. Position and frequency of ParB foci in N99 (pLG44) cells. (*A*) Histogram showing the frequency (left axis) and average length (right axis) of cells with different numbers of discrete ParB foci ($n = 625$ cells). (*B*) The positions of ParB foci measured in cells of different length. The cells were oriented such that the left end was the end nearest a focus. Relative cell length refers to the individual cell length relative to the average size of cells that contain two foci. Foci from cells with one focus (◇), two foci (●), three foci (▲), or four foci (■) are shown.

[in N99(λparAB) cells] restored the pattern of ParB foci to that of pLG44 (Fig. 2 *G*, *H*, *O*, and *P*). Therefore, proper ParB localization requires ParA, indicating that ParB cannot interact stably with its host localization signals without ParA.

When *parS* was in the bacterial chromosome (as λ*parS*), the pattern of ParB foci in the absence of ParA (Fig. 2 *E* and *M*) was very similar to that of cells containing pLG44 (Fig. 1 *A* and *I*). Because the bacterial chromosome is specifically oriented inside cells (1, 2, 4), we assume that ParB foci at λ*parS* indicate the location of the λ-attachment site, as positioned by the chromosomal partition machinery, and thus would be independent of ParA.

ParA Shows More Dispersed Staining. We also examined the pattern of ParA staining in N99 cells containing either pLG44 or λ*parAB*. In both cases, the staining appeared throughout the cells and did not show the bright foci seen with ParB (Fig. 6).

Discussion

P1 plasmids are specifically localized within *E. coli* cells (2). Here, we show that P1 ParB protein is also specifically localized as foci that coincide with the position of P1 plasmids. To avoid confusion and to distinguish localization within the cell from localization into foci at *parS*, we use "localization" to mean the former and "convergence" to mean the latter. The convergence of ParB into foci depends on *parS* but not ParA; however, proper localization of foci depended on both partition components. Therefore, ParB foci are a good indicator of plasmid position, and proper positioning of P1, as seen by fluorescence, depends on its partition system.

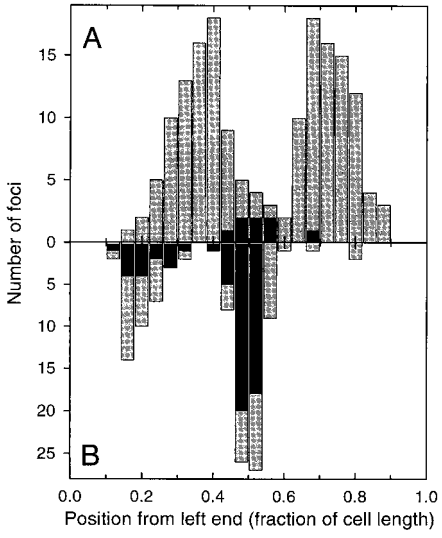

Fig. 4. Effect of ParA on the position of ParB foci. The histograms show the position of foci, as a fraction of cell length, for pLG44 (with ParA) (*A*) and pLG48 plus pBEF118 (no ParA) (*B*). For simplicity, only cells containing one or two foci were included. The cells were oriented such that the left end was nearest a focus. The position of foci from cells with one focus is indicated in black, and that from cells with two foci is indicated in gray.

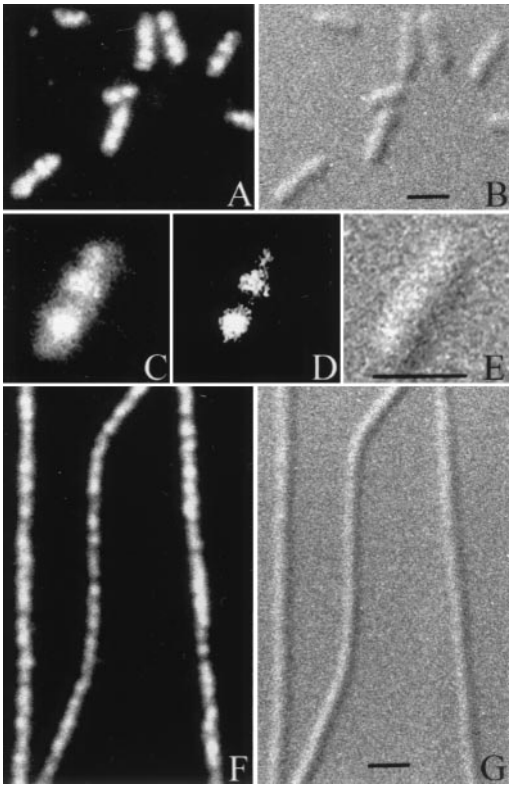

Fig. 5. ParB in N99*ihfA* cells containing the mini-P1 plasmid pLG44. Cy3-stained ParB is shown at *Left* (*A*, *C*, *D*, and *F*), and Nomarski images are shown at *Right* (*B*, *E*, and *G*). (*A–E*) N99*ihfA*(pLG44) cells. (*F* and *G*) Cephalexin-treated filaments. *D* is an underexposed version of *C*. (Bar = 1 μm.)

It is intriguing that much of the ParB in the cell is recruited to these foci at *parS* sites. That less recruitment occurs in IHF mutants argues that not all ParB must be bound to the plasmid for the plasmid to be localized. Although the minimal amount of ParB required for partition has not been measured, these experiments argue that the partition system can distinguish between ParB bound to *parS* from free ParB. This result rules out a model in which ParB converges at *parS* to prevent free ParB from competing for attachment to some cellular structure present at the one-quarter and three-quarters positions.

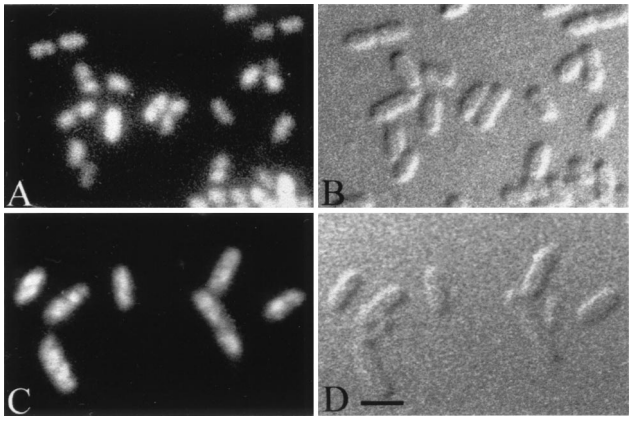

Fig. 6. Immunofluorescent visualization of ParA. (*A* and *C*) Cy3 fluorescence of secondary antibody after treatment of fixed cells with anti-ParA antibodies. (*B* and *D*) Nomarski images of cells. (*A* and *B*) N99 (λparAB). (*C* and *D*) N99 (pLG44). (Bar = 1 μm.)

Localization of ParB foci to the one-quarter and three-quarters sites requires ParA (Fig. 4). ParA is an ATPase (10), and its interactions with ParB in the partition complex require ATP (12); however, its mechanistic role in localization is not understood. Roles for ParA in both attaching and detaching plasmids from the host have been proposed. Indeed, *in vitro*, ParA can both assemble onto the partition complex (at high ParB concentrations) or hinder ParB assembly at *parS* (at low ParB concentrations; ref. 12). A direct role might be a tethering activity to bacterial "receptors" at the one-quarter and three-quarters sites. Alternatively (or in addition), ParA could be required to prevent association with or dissociate ParB from midcell (and/or polar) sites. We favor the idea that the one-quarter and three-quarters locations represent the sites of specific host receptors, because other cellular components are similarly localized (see below). ParA would thus be required directly or indirectly to allow attachment of ParB and P1 to these sites. The pattern of ParB foci without ParA was not completely random (Fig. 4*B*). It may represent random localization of foci in nonrandomly distributed cytosol or alternatively indicate that there is some affinity of ParB for midcell and polar sites without ParA. The answers to these problems await identification of bacterial factors involved in plasmid localization.

In contrast to ParB, the intracellular pool of ParA did not converge into foci, indicating that most of the ParA molecules are not attached to P1 plasmids. This result is consistent with our biochemical observations that ParA association with ParB is transient and weak (11, 12). Alternatively, fixation techniques may have disturbed the localization patterns of ParA. Detection of specific localization of Soj, a ParA-like protein from *B. subtilis*, was possible with GFP-fusion proteins but not by immunofluorescence (35).

Our immunofluorescence assay has the advantage that we have examined the native forms of the plasmid proteins at their endogenous levels, something that has been difficult in other systems (18, 19). In our hands, however, overproduction of these proteins by about 5-fold yielded the same localization patterns (data not shown).

Host Factors, Cell Division, and P1 Partition. The identity of the host "receptor" is still a mystery. It might be a factor that marks the one-quarter and three-quarters positions as cell division sites for the next generation, but it must be present before FtsZ and FtsI act in the cell division pathway. Recently, components of the replication machinery in *B. subtilis* (PolC) and in *E. coli* (SeqA) have been localized to the cell one-quarter and three-quarters positions (36, 37), and it is attractive to speculate that the plasmid partition proteins are attaching to components of a localized replication apparatus. However, it should be noted that Par$^-$ plasmids, such as pLG48, are not attached (Fig. 1); however, they still replicate, and their replication depends on host replication proteins.

Using GFP–LacI bound to P1, Gordon *et al.* (2) saw that P1 distribution was inhibited in cephalexin-treated cells, whereas we have observed the opposite result. Because ParB foci form at *parS* in untreated cells, it is difficult to imagine that ParB foci are distributing in filaments without the plasmids. If the difference reflects the different mini-P1 plasmids or bacterial strains used, it would be interesting to examine those differences as a probe for the bacterial localization signals. For example, a small effect of GFP–LacI on partition in normal cells may be exaggerated when cell division is inhibited by cephalexin.

Localization of Other Plasmid Partition Proteins. The localization of SopB, the ParB-like protein of the F plasmid, has been measured with conflicting results (18, 19). Our data for P1 ParB are consistent with the observation that SopB localizes with (or its localization depends on) *sopC*, the F plasmid partition site (19).

However, a SopB–GFP fusion was observed to be concentrated close to the cell poles independently of *sopC* (18). In both F studies, the plasmid proteins were overexpressed. One possible reason for the difference is that the SopB–GFP bound to *sopC* may not be detectable over the amount that is not bound. Our experiments with IHF mutants suggest that not all ParB must reside in a focus for partition to occur, because mini-P1 plasmids are relatively stable in IHF mutants (7). The SopB–GFP fusion used was functional for gene silencing (see below), but its function in partition was not reported (18), raising the possibility that the GFP moiety has partially interfered with a SopA-dependent relocalization of F and SopB. In addition, differences in affinity among ParB, SopB, and SopB–GFP for their specific host receptors could influence the amount of localized protein; such subpopulations may sometimes be visible and sometimes be hidden, depending on the protein and its context in the cell. It should also be noted that the P1 and F partition systems are compatible and thus do not compete with each other, which may reflect different modes of action of ParB and SopB.

The ability to silence genes adjacent to its DNA binding site is a property shared by P1 ParB and F SopB (18, 20–22). The silencing effects of ParB have been detected in plasmids (20) and in bacterial chromosomes (21) containing *parS*. Two models have been proposed to explain this phenomenon. In one, protein recognition of the *par* site promotes extensive binding along the adjacent DNA, and this protein prevents access of RNA polymerase. The second model proposes that sequestration of these sequences to a specific region of the cell makes them inaccessible to the transcription machinery. Our data favor the first model. The location of ParB foci is altered by ParA and not by IHF. In P1 at least, the opposite relationship is observed for the silencing effect; it is independent of ParA (20) but is reduced in IHF mutants (21). Our results indicate that ParB foci occupy different locations in the cell depending on ParA and the context of *parS*. Finally, the appearance of ParB in IHF mutants shows a reduced ability to bind extensively to *parS* and correlates with a reduction in silencing.

The R1 plasmid partition system is an analogous but nonhomologous system that requires two plasmid proteins, ParR and ParM. ParR binds the partition site and mediates a pairing reaction between two plasmids via this site (38). Pairing is stimulated by ParM, the ATPase. Interestingly, and in contrast to P1, ParM forms foci that colocalize with the plasmid, whereas ParR does not (34). These observations may indicate that the mechanisms of partition will turn out to be very similar between these types of plasmid systems but that the roles of the two plasmid proteins are shuffled with respect to the two proteins of the P1 or F plasmid-like systems.

Partition in many plasmid systems is a coordinated effort of two plasmid proteins that act on an as-yet undefined host signal. The visualization of P1 proteins shows that the action of its two partition proteins in this positioning reaction can be discriminated via effects on the formation and position of ParB foci. The localization of ParB should provide an invaluable tool to probe further the mechanism and components of prokaryotic partition *in vivo*.

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