Movement and equipositioning of plasmids by ParA filament disassembly

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Bacterial plasmids encode partitioning (par) loci that confer stable plasmid inheritance. We showed previously that, in the presence of ParB and parC encoded by the par2 locus of plasmid pB171, ParA formed cytoskeletal-like structures that dynamically relocated over the nucleoid. Simultaneously, the par2 locus distributed plasmids regularly over the nucleoid. We show here that the dynamic ParA patterns are not simple oscillations. Rather, ParA nucleates and polymerizes in between plasmids. When a ParA assembly reaches a plasmid, the assembly reaction reverses into disassembly. Strikingly, plasmids consistently migrate behind disassembling ParA cytoskeletal structures, suggesting that ParA filaments pull plasmids by depolymerization. The perpetual cycles of ParA assembly and disassembly result in continuous relocation of plasmids, which, on time averaging, results in equidistribution of the plasmids. Mathematical modeling of ParA and plasmid dynamics support these interpretations. Mutational analysis supports a molecular mechanism in which the ParB-parC complex controls ParA filament depolymerization.

In bacteria, it has been difficult to analyze how chromosomes are segregated. To gain insight into the problem, partitioning (par) loci encoded by plasmids have been used extensively as model systems. Type I par loci encode 3 components: a Walker Box ATPase (ParA), a DNA binding protein (ParB), and one or more cis-acting DNA regions where the proteins act (parC). The ParB proteins bind site-specifically to their cognate parC sites to form a “partition complex.” ParB also interacts with the cognate ParA protein and thereby functions as an adaptor between ParA and parC DNA. Thus, the parC region at which the segregation apparatus congregates is functionally equivalent of a eukaryotic centromere. Interestingly, ParA ATPases form helical structures that dynamically relocate over the nucleoid (1–6). ParA relocation but not the formation of filamentous structures depends on the presence of ParB bound to parC (1, 2, 4, 6). The presence of helical ParA structures in living cells is consistent with the ability of the proteins to polymerize in vitro (4, 6–13).

Purified ParAs of Thermus thermophilus and plasmid pSM19035 both dimerize in the presence of ATP (6, 14), whereas ParA of P1 dimerizes also without nucleotide (13). The ParA-ATP dimers bind cooperatively and nonspecifically to DNA. Thus, the in vitro DNA binding activity of ParA proteins is consistent with the nucleoid association seen in vivo (1, 8). In all cases investigated, ParB stimulates ParA ATPase activity, either on its own or in the presence of its cognate centromere site (6, 9, 11, 15).

We showed previously that the type I par locus of pB171, on average, distributes plasmids regularly over the bacterial nucleoid (7). Our observations raised the possibility that the dynamic ParA filaments generate the mechanical force that move and position plasmids within the cell.

Here we analyze the relative movements of ParA and plasmids in single cells. We find that ParA dynamics and plasmid movements are intimately correlated in a pattern indicating that the partition complex stimulates disassembly of ParA structures. Strikingly, plasmids consistently migrated in the wake of disassembling ParA in manner suggesting that retracting ParA structures move plasmids by a pulling mechanism. We used mathematical modeling of ParA dynamics and plasmid movement to see if a simple pulling model could yield the observed plasmid movements and distributions, and ParA dynamics. The modeling reliably generated the observed distributions, provided the rate of detachment of a plasmid from a filament was filament-length dependent, a prediction that we verified experimentally. In vivo data showed that perpetual cycles of ParA assembly/disassembly continuously moved plasmids relative to each other, which results in a time-average equidistribution of plasmids, as predicted by the mathematical model. Our observations and computations elucidate how the type I par locus of Escherichia coli plasmid pB171 moves and positions plasmids.

Results and Discussion

Visualization of Plasmid, ParA, and Nucleoid by a Triple Labeling System. We engineered a triple color labeling system to simultaneously analyze the subcellular dynamics of plasmids, ParA, and the bacterial nucleoid in E. coli cells. A fully functional ParA-GFP fusion was expressed at a level close to that of ParA expressed by par2 (1). For brevity, ParA will be used interchangeably with ParA-GFP in the following. Plasmids were visualized by a TetR-mCherry fusion protein that binds to an array of 120 plasmid-encoded tetO operators (16), and the nucleoid was stained with Hoechst. When TetR-mCherry was donated in trans, the plasmid of interest was visible as bright foci (Fig. 1). Plasmid stability assays confirmed that the cytological data acquired using tetO/TetR-mCherry to label plasmids were all obtained under conditions in which par2 was functional (supporting information (SI) Fig. S1C), consistent with the regular plasmid distributions shown in Fig. S1A and B.

ParA Recruits par2-Carrying Plasmids to the Nucleoid. Using our new plasmid labeling system, we analyzed plasmid localization in cells treated with nalidixic acid. This antibiotic, which inhibits DNA gyrase, leads to the formation of filamentous cells with nonsegregated nucleoids. Foci of the R1 control plasmid without the par2 system localized almost exclusively to cytosolic regions (Fig. 1A). By striking contrast, foci of the par2-carrying plasmid colocalized with the nucleoid (Fig. 1B). Moreover, a plasmid carrying an in-frame deletion in parA had a localization pattern indistinguishable from
that of the par- plasmid, showing that ParA is required for plasmid recruitment to the nucleoid. We also investigated if purified ParA would bind DNA nonspecifically. Gel-shift analysis showed that ParA indeed has nonspecific DNA binding activity in vitro (Fig. S2). Thus, all our observations are consistent with the proposal that the nucleoid functions as a scaffold for ParA assembly. Together, these findings suggest that ParA recruits par2-carrying plasmids to the nucleoid, consistent with previous observations (2).

**Plasmid Movement and ParA Dynamics Are Intimately Connected.** Next, we analyzed plasmid and ParA dynamics in a cell with one plasmid focus and one nucleoid (Fig. 1C and Movie S1). Plasmid localization relative to the entire cell was obtained by phase contrast microscopy (Fig. 1Ca), and to the nucleoid by labeling with Hoechst (Fig. 1Cf). We observed that the plasmid focus and ParA oscillated back and forth over the nucleoid (Fig. 1C a, b, and f).

Initially, the plasmid was located at midcell, with an assembly of ParA to the right (Fig. 1Cc, 0°). The plasmid then moved rightward while the ParA-GFP signal closed to the focus disassembled. The region behind the plasmid was left almost devoid of Par-A-GFP signal (Fig. 1Cc, 0°–8°). Simultaneously, ParA assembled in the left half of the nucleoid. Eventually, the left assembly of ParA reached the plasmid that was now located close to the right nucleoid pole (Fig. 1Cc, 8°). At this stage (Fig. 1Cc, blue arrowheads), a new dynamic event was triggered—the newly generated assembly of ParA retracted leftward and again the plasmid focus followed the retracting ParA-GFP signal. During this retraction, the region behind the plasmid was again left devoid of Par-A-GFP signal (Fig. 1Cc, 8°–17°). Once more, a Par-A-GFP assembly was initiated on the other (right) half, which eventually reached the plasmid focus (Fig. 1Cc, 18°–28°). The combined trajectories of plasmid and ParA movements show that the plasmid consistently moved toward regions of high Par-A-GFP signal (Fig. 1Cg). Two-dimensional deconvolution of the cytological recordings resolved the Par-A-GFP signal into filamentous structures colocalizing with the nucleoid (Fig. 1C d–f). It is obvious from these images that the filamentous structures reversed from growth to shrinkage when they reached the plasmid focus (Fig. 1C, yellow arrowheads), and that the focus then started to move in the direction of the retracting ParA structures, staying close to the filament end.

ParA assembly consistently initiated away from plasmid foci and gradually continued to extend toward the nucleoid poles (Fig. 1Cf, Figs. S3 and S4, and Movie S2). When the growing filaments reached a focus, they reversed into disassembly. This pattern was general for cells with one focus (see further examples in Figs. S3 and S4). The oscillation frequency and amplitude of Par-A-GFP and plasmid foci varied considerably from one cell to another and also in one cell over time: the plasmid foci shown in Fig. 1C and Figs. S3 and S4 moved over most of the nucleoid. In other cases, the foci tended to oscillate around midnucleoid whilst trailing retracting ParA (Fig. S5). Importantly, the plasmid foci consistently followed retracting ParA filaments. These observations unequivocally show that ParA dynamics and plasmid segregation inherently go hand in hand.

A focus often detached before the ParA assembly completely disassembled. After detachment, ParA continued to disassemble, leaving the focus behind until reached by a new ParA assembly (Fig. 1D and Figs. S3 and S4). Fig. 1D is a 3D surface intensity plot of the dashed region in Fig. 1Cc (15°–29°) showing that the focus detached at 16° but ParA-GFP continued to disassemble, leaving the focus behind where it remained until at 27° when it was moved by a new ParA assembly. These data suggest that once ParA disassembly has been initiated by ParBparC, a continuous interaction with the ParB-bound plasmid is seemingly not required for ParA filament depolymerization.

**ParA Moves Plasmids by a Pulling Mechanism.** When a ParA filament reached a plasmid focus, it consistently reversed from growth (interpreted as polymerization) to retraction (interpreted as depolymerization) (Fig. 1C). We find it reasonable to interpret retraction of ParA filaments as filament depolymerization for 3 main reasons: (i) ParA forms filaments in vitro (7), consistent with the filamentous structures we see in vivo; (ii) the observation that ParA filaments shrink from the focus/filament boundary leaving the space behind
the focus devoid of ParA-GFP signal; and (iii) the observation that ParA in elongated cells accumulates over one nucleoid simultaneously with its disappearance from another when pulling a plasmid (Fig. S6 and Movie S3). The consistent migration of plasmid foci at the boundaries of retracting ParA filaments suggests that ParA moves plasmids by a pulling mechanism in which depolymerization of ParA generates the mechanical force for plasmid migration. The assertion that ParA generates force on the plasmid foci was further strengthened by the fact that ParA could transfer plasmids between separate nucleoids, not only in cephalaxin-treated cells (Fig. S6) but also in dividing cells.

**Single Amino Acid Change in the ParB N Terminus Abolishes ParA Dynamics.** The components encoded by type Ib parFGH locus of TP228 has been extensively analyzed biochemically. Importantly, the N terminus of ParB (ParB homolog) stimulates the ATPase activity of ParF (ParA homolog) (11). Arg-19 in the N terminus of ParG is essential for this stimulation. Alignments of ParG and ParB sequences showed that Arg-19 of ParG corresponds to Arg-26 of ParB (Fig. S7). We mutated 2 arginines in the N terminus of ParB (Arg-12 and Arg-26) to either lysine or alanine (Fig. S7). Plasmid segregation assays showed that par2 carrying parBP26K exhibited a 3-fold reduced activity, whereas par2 parBP26A exhibited an almost complete par-deficient phenotype (Fig. S7B). By contrast, mutational changes of Arg-12 had no measurable effect on plasmid segregation by par2.

We then performed time-lapse microscopy of ParA in the context of parBP26A (Fig. S7C). In this case, ParA still localized to the nucleoid. However, ParA dynamics occurred in 15% of the cells, only, in contrasted to the WT case in which virtually all cells showed ParA movement. Thus, the N terminus (Arg-26) of ParB is essential for both ParA dynamics and par2 activity. Because the corresponding arginine (Arg-19) of ParG is responsible for stimulation of the ATPase activity of ParF in vitro, and the fact that Arg-26 of ParB is essential for ParA oscillation and plasmid stability, our observations suggest that ParB similarly stimulates the ATPase activity of ParA and that this stimulation is a prerequisite for ParA dynamics by means of regulating ParA depolymerization (see text following).

To further substantiate that the par loci of plasmids pB171 and TP228 function by similar mechanisms, we tagged ParF with a fluorescent protein and analyzed the subcellular localization and dynamics of the fusion protein. As for ParA, ParF also localized to the nucleoid (Fig. S7D). Most importantly, time-lapse experiments showed that, in the presence of the entire par system (including ParG and parH centromere), ParF oscillated over the nucleoid (Fig. S7E). In the absence of ParGparH, no oscillation of ParF was observed (Fig. S7F). These observations further indicate that the 2 par loci function by similar, if not identical, molecular mechanisms.

**Mathematical Model Describing the par Mechanism.** Although it is intuitively clear that a pulling-like mechanism will generate single-cell kymographs similar to those seen in Fig. 1C, it is far from obvious that such a mechanism can generate the regular focus distributions obtained from many cells (Fig. S1A and B). We therefore constructed a simple, stochastic computational model that could predict plasmid focus distributions and ParA dynamics. The model consisted of randomly nucleated ParA filaments that grew stochastically until they reached a plasmid, after which they

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**Fig. 2.** Simulated kymographs and plasmid foci distributions generated by mathematical modeling. (A–C) Simulated kymographs of ParA/focus dynamics for (A) 1, (B) 2, and (C) 3 foci cases, a movie of (C) is presented in Movie S7. (D) Simulated kymograph of one focus splitting into 2 and being segregated. (E–G) Simulated foci distributions for (E) 1, (F) 2, and (G) 3 foci cases. For all simulated foci distributions, the distributions were built up over 7,500 min of simulated time, with sampling every 7.5 min, and means and error bars constructed from 40 independent runs. (H) Plasmid travel distance is ParA filament length dependent. (Ha) Schematics showing how the focus travel distance was measured relative to initial ParA filament length. (Hb) The plot shows the focus travel distance, Δa, as function of initial ParA filament length, a. (Hc) The plot shows the ratio between focus travel distance and initial ParA filament length, Δa/a, as function of initial ParA filament length, a.
Fig. 3. Perpetual cycles of ParA assembly/disassembly move and position plasmids. (A–C) Time lapses showing the subcellular localization of ParA-GFP and par2<sup>+</sup> plasmids (A and B) in a cephalexin-treated cell. Numbers on the side indicate minutes in the time lapse. Experimental setup as in Fig. 1C. Videos of the time lapse in (A–C) are presented in Movies S4, S5, and S6, respectively. (Aa, Ba, and Ca) Overlay of phase-contrast images and TetR-mCherry (red). (Ab, Bb, and Cb) Intracellular localization of ParA-GFP. (Ac, Bc, and Cc) Overlay of ParA-GFP (green) and TetR-mCherry (red). (Ad) Nucleoid stained with Hoechst. (Ae) 3D surface intensity plot of (Ac). (Af) Kymograph of plasmid (red) and maximum intensity of ParA-GFP signal (green) in (Ac). Dotted blue lines indicate the average foci positions during the time lapse. (Bd and Cg) Kymograph of plasmid (red) and highest-intensity ParA-GFP signal in (Bb) and (Cb). (C) Focus splitting and segregation by ParA filaments. (Ce) Overlay of deconvolved images of ParA-GFP. (Cf) Overlay of deconvolved ParA-GFP (green) and TetR-mCherry (red). (Cf) Overlay of deconvolved ParA-GFP (green) and TetR-mCherry (red) and nucleoid (blue).
switched to depolymerization, during which the plasmid was “dragged” along with the shrinking filament before being released. Full details of the model are given in SI Text.

In our initial modeling, a plasmid focus detached from a ParA filament only after the filament had completely disassembled. This assumption predicted a roughly flat plasmid focus distribution across the nucleoid with peaks toward the poles (Fig. S8). When compared with our experimental data (Fig. S1 A and B, second panels), this distribution clearly had qualitatively the wrong form, a problem that persisted even with a constant nonzero probability of detachment per depolymerization step (see SI Text). To correct this problem, we implemented a revised rule that a focus would detach from a filament during depolymerization with a probability that depended on the current filament length, with longer filaments having a reduced probability of focus detachment. Simulations generated by this revised model produced kymographs and plasmid distributions that agreed well with our observations in the single focus case (cf. Fig. 2A with Fig. S1A, and Fig. 2E with Fig. S1B). In Fig. 2D we show simulated kymographs for the case where one focus separates into 2 foci, which are then rapidly pulled apart and subsequently move over separate nucleoid halves.

**Testing the Model: Plasmid Detachment Rates.** In cells with one focus, we measured experimentally the absolute distance traveled by the focus relative to the initial length of the ParA filament pulling the focus (Fig. 2H). The focus typically detached before the filament completely depolymerized (Fig. 1D), regardless of filament length. From the measurements it is evident that long ParA filaments pull the plasmid a longer distance than shorter filaments (Fig. 2Hb). Furthermore, a long filament moved a plasmid longer relative to its initial length compared to short filaments (Fig. 2Hc). Hence, the longer distances traversed by a focus attached to longer filaments was not only due to the filament itself being longer, but is also a consequence of the focus detachment rate being lower for longer filaments. Therefore, the data in Fig. 2H supported the revised rule of the mathematical model.

It is not known why longer filaments have lower detachment rates than shorter filaments. One possibility is that ParB/ParC-carrying plasmids contact bundles of ParA protofilaments rather than a single filament, and that long bundles consist of a larger number of protofilaments than short ones. The parC1 and parC2 regions of par2 consist of 17 and 18 ParB dimer binding sites, respectively, and deletion of parC2 reduced the efficiency of par2 (1, 17). These observations are consistent with the proposal that multiple ParA protofilaments can simultaneously contact the partition complex of a par2-carrying plasmid, and that filament bundling reduces the detachment rate.

**Equidistribution of Plasmids by Perpetual Cycles of ParA Assembly/Disassembly.** Next we generated simulated kymographs for multifoci cases (Fig. 2B and C). Here, the model predicted much more complex ParA dynamics than in the single focus case, with cycles of ParA polymerization/depolymerization in between plasmids moving relative to each other (Fig. 2B and C), which on time averaging resulted in equipositioning of plasmids (Fig. 2F and G) similar to our experimental data (7) (Fig. S1 A and B).

Analysis of ParA and plasmid dynamics was difficult in cells with multiple foci. Thus, to analyze the multiple foci case, we treated cells with cephalaxin to obtain elongated, nondividing cells (Fig. 3A and Movie S4). Strikingly, as predicted by the mathematical modeling, ParA dynamics was much more complex in the multifoci cases (Fig. 3Ab) than the simple pole-to-pole oscillation seen in cells with a single focus. Instead, ParA exhibited continuous rounds of assembly/disassembly in between plasmid foci (Fig. 3Ac and e). Similar to cells with one focus, when assembling ParA structures reached a focus, assembly was reversed to disassembly and the focus followed behind the retracting ParA-GFP signal (Fig. 3A a–d; focus 3, 0′−10′). Concomitantly, a new ParA assembly was initiated elsewhere and the cycle was repeated. Often, a ParA assembly contacted foci at both ends. This was consistently accompanied by bipolar disassembly of ParA and movement of the 2 foci toward each other (Fig. 3Ac, 14′−19′, and Fig. S6A). The fact that disassembling ParA can move 2 plasmids toward each other is in further support of a system where ParA moves plasmids by a pulling mechanism. This perpetual cycle of ParA assembly/disassembly between foci moved and positioned plasmids relative to each other, resulting in a time-averaged equidistribution of the foci in single cells (Fig. 3A e and f) and at the level of the cell population (Fig. S1A and B). Fig. 3Af shows kymographs of plasmid foci and peak ParA-GFP dynamics. Dotted blue lines indicate the average foci positions during the time lapse. It is evident that the foci continuously move around these average positions. As a further characteristic example, we include a case in which one plasmid focus was separated by cycles of ParA-GFP assembly/disassembly into 4 foci that eventually became distributed throughout the cell (Fig. 3B and Movie S5).

We also analyzed a focus splitting event. When separation of plasmids occurred, in some instances both plasmids were pulled in the same direction (Fig. 3C, 12′−19′, and Movie S6). In other examples, after splitting, only one focus moved with ParA, and the other focus stayed behind, resulting in focus separation (Fig. 3C, 26′−29′, and Figs. S3 and S4). ParA filaments then contacted the plasmid which had been left behind (Fig. 3C, blue arrowheads, and Figs. S3 and S4), seemingly triggering movement of the plasmid toward the other half of the cell, thus resulting in the 2 plasmids being localized away from each other with the region in between devoid of ParA-GFP signal (Fig. 3C, green arrowheads). Again we see good general agreement between our mathematical simulations and experiments (cf. Figs. 2D and 3C, and Figs. S3 and S4).

**Molecular Model Explaining Plasmid Movement and ParA Relocation.** The cytological observations presented here, together with previous biochemical data obtained with other type I par loci components (6, 9, 11, 15, 17−19) and our mathematical modeling, all support a simple picture of how the perpetual cycles of ParA filament growth/shrinkage may generate the force that moves and positions plasmids over the nucleoid (Fig. 4). In step 1, ParA2-ATP filaments polymerize and contact a nucleoid DNA, leading to large ParA filaments. This contention is supported by in vitro data obtained with 2 type I ParAs (Soj and ParA of pSM19035) (6, 14). Formation of filaments begins with a nucleating core from which rapid polymerization proceeds. In step 2, a growing filament contacts a plasmid via ParB bound to parC. In step 3, ParBs bound to parC on the plasmid stimulate the ATPase activity of ParA2-ATP at the end of the filament. This step is supported by the fact that ParG of TP228 stimulates the ATPase activity of ParF, that ParF of TP228

![Molecular model showing how plasmid movement is generated by dynamic ParA filaments. See Discussion for a detailed description of the molecular model.](image-url)
The N terminus of ParB was required for ParA dynamics (Fig. S7 and 2H) or continue (step 4) to be attached to the end of the depolymerizing ParA filament. In steps 4 and 4', the ParA filament continues to depolymerize until it completely disappears (Fig. 1 C and D). In step 5, the plasmid has been released and ParA-ATP subunits have assembled into a new filamentous structure away from the plasmid. Eventually, the released plasmid interacts with a new ParA filament approaching from the opposite side. When contact is made, this filament will move the plasmid in the opposite direction. Finally, in step 6, free ParA-ADP is rejuvenated to ParA-ATP (6). This perpetual cycle of ParA relocation converts energy in the form of ATP to mechanical force that powers plasmid movement.

**Comparison with Related Systems.** The type I par locus of plasmid F (sopABC) has also been investigated with respect to SopA and plasmid dynamics in living cells (3). The observed patterns of plasmid movement and localization were similar to those described here. However, SopA-GFP dynamics differed from that of ParA-GFP. First, SopA-GFP formed a big focus that oscillated from one end of the nucleoid to the other. While oscillating, the F plasmid focus followed the SopA focus, but there was no apparent contact between the SopA and plasmid loci. Second, SopA-GFP also formed a static filamentous structure that spanned the entire length of the nucleoid without being shortened as the plasmid migrated. Based on these observations, Hatano et al. (3) suggested that the SopA focus redistributes within the stationary filament while maintaining an overall filament structure. Importantly, it was not explained how the sop system positioned plasmids relative to each other. Furthermore, the nucleoid did not appear to be essential for SopA oscillation. In contrast, our data clearly show that the nucleoid plays an important role for par2-mediated plasmid partitioning.

Most bacterial chromosomes encode type I par loci (20) that have been proposed to be involved in chromosome segregation (21–24). More direct evidence for this notion has come from *Caulobacter crescentus* (23) and *Vibrio cholerae* (24). In particular, ParA1 encoded by *chl1* of *V. cholerae* exhibited a disassembly pattern suggesting that it generates force and sequesters ChlI origins by a pulling mechanism (24).

The minCDE locus of *E. coli* enables oscillating and filament-forming MinD that, in combination with MinC and MinE, prevents FtsZ-ring formation at the cell poles (25). The similarities between the *E. coli* minCDE system and type I par loci are striking. ParA and MinD are both “diviant” Walker A Box ATPases that form dynamic patterns on a cellular surface (nucleoid and cell membrane, respectively) (1, 25). Both ParA and MinD form cytoskeletal filaments that interact with their surfaces as ATP-bound dimers (6, 14, 26). The nucleoid state determines the cellular location of ParA and MinD, where ATP hydrolysis releases the proteins from their surface-bound states (6, 14, 27). The ATPase activities of the proteins are stimulated by the N termini of their dimeric partner proteins (ParB and MinE, respectively) (6, 11, 14, 15, 27). Furthermore, filament dynamics functions to position another cellular structure (FtsZ ring or plasmid, respectively) (ref. 25; this work). These observations reveal that evolution has solved 2 very different spatial problems related to cell division (DNA segregation and septum placement) by related molecular mechanisms.

**Materials and Methods.**

A functional ParA-GFP fusion was constructed as described previously (1). Plasmids were visualized by binding of TetR-mCherry fusion protein to an array of 120 plasmid-encoded tetO operators on the plasmid of interest (16). Microscopy was performed essentially as described previously (2, 16) with a few changes described in SI Text. Microscopy was performed in a DeltaFCS strain lacking poly(A) polymerase. Mathematical modeling, additional materials and methods, tables, and movie legends are presented as SI Text. Strains and plasmids are listed in Table S1.

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