Partition Functions of Unit-Copy Plasmids Can Stabilize the Maintenance of Plasmid pBR322 at Low Copy Number

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The maintenance of plasmid pBR322 is highly unstable in a *polA12* strain of *Escherichia coli* at 29°C due to severely reduced copy number. Under these conditions, introduction of the *par* (partition) locus of plasmid P1 or the *par* (*sop*) region of F into pBR322 stabilizes it. A region with similar activity was detected in the P7 plasmid. The activity of the P1 *par* locus was dependent on the P1 *parA* gene product and was sensitive to *par*-specified incompatibility.

Unit-copy plasmids such as F and P1 have as few as two copies per dividing cell and yet are very stably maintained (6, 14). These plasmids must make use of some highly precise partition mechanism analogous to the mitotic segregation of eucaryotic chromosomes. For both P1 and F, the plasmid-encoded partition system appears to consist of a *cis*-acting recognition sequence and two genes encoding essential protein products (1, 4, 13). It is difficult to prove definitively that these partition systems are directly responsible for active partition, but strong circumstantial evidence has been presented in the form of a demonstration that they can stabilize the maintenance of "foreign" replicons. Thus, when placed in *cis*, F *par* (*sop*) can stabilize unstable *oriC* plasmids (13) and P1 *par* can stabilize a mini-F plasmid that lacks its own partition region (2). Thus, it is tentatively concluded that these *par* regions are functional cassettes of information that can promote the equitable distribution of any DNA replicon into which they are introduced. To test this in an extreme case, we devised a method of assaying their function in a plasmid very different from the parental plasmids from which the *par* cassettes originated. Plasmid pBR322 is replicated by a ColE1-like replication system originating from plasmid pMB1. Such systems are entirely different in their structure and requirements from those of F and P1 (17). pBR322 is normally maintained as a high-copy-number plasmid and relies on diffusion for its partition. We present evidence that *par* cassettes from unit-copy plasmids F and P1, and an analogous region isolated from the P7 plasmid, can promote the partition of pBR322.

Like its close relative ColE1, plasmid pBR322 cannot replicate in the absence of DNA polymerase I (10). Thus, in the *Escherichia coli* temperature-sensitive polymerase I strain MM383 (*polA12*) (12), pBR322 is able to transform and replicate at 29°C but cannot be established or maintained at 42°C. However, even at 29°C, the polymerase I activity of this strain is not optimal for pBR322 replication because the plasmid is unstable (1). Under these conditions, pBR322 replication is limited, giving an average copy number of about three per host chromosome in the *polA12* strain at 29°C (data not shown). Subsequent loss of the plasmid is presumably due either to a complete failure of replication in some cells or to inequitable partition of the limited plasmid copies to daughter cells.

Plasmid pALA283 was constructed by inserting the *Hind*III-*Aha*III fragment of P1 *par* from pALA17 (2) between the *Hind*III and *Hinc*II sites of phage M13mp10 (11) and excising the *Eco*RI-*Bam*HI fragment and inserting it into the complementary sites of the pSC101-based vector pLG338 (16). Plasmid pALA292 was constructed by isolating the 800-base-pair (bp) *Sau*3A fragment from the P1 *par* region of pALA17 and inserting it into the *Bam*HI site of pLG338 in the orientation shown.

Plasmids pALA287 and pALA610 were isolated by cloning the relevant *Pst*I-*Eco*RI fragments of F into the complementary sites in pBR322. pALA610 shows evidence of a small (ca. 300 bp) deletion in the F insert. The plasmid is presumably identical to plasmid pBR322-A2^{del}, which contains a deletion in the *incD* region which always occurs at high frequency when this fragment is cloned (7).

To determine the kinetics of plasmid loss without selection at 29°C, overnight cultures were grown in Luria broth at 29°C with thymine (50 µg/ml) and ampicillin (100 µg/ml). After 1:50 dilution into the same medium, cells were grown for 2.5 h at 29°C, diluted 1:1,000 in Luria broth with thymine, and incubated again at 29°C. At intervals, portions were spread on antibiotic medium 2 agar plus thymine to give single colonies at 29°C. Colonies were picked with toothpicks and stabbed sequentially into antibiotic medium plus thymine agar plates with and without ampicillin (10 µg/ml). Plasmid loss at zero time at 29 or 42°C was calculated by counting the drug-resistant and drug-sensitive colonies detected on the relevant agar plates. The number of generations of growth was calculated from the viable cell count on the nonselective agar plates.

In previous studies, we have shown that various fragments containing the *par* region of the P1 plasmid have a considerable stabilizing effect on the pBR322 clones that contain them (1). In the present studies, the loss of the *par*⁺ plasmid pALA17 (Fig. 1) and pBR322 from growing populations of *polA12* cells was followed for 10 generations at 29°C (Fig. 2). Plasmid pALA17 was considerably more stable under these conditions than its pBR322 parent, although not quite as stable as pBR322 in a *polA*⁺ strain (Fig. 2). At 42°C, a temperature at which the pBR322 origin cannot function, both plasmids were rapidly lost, confirming that *par* function is dependent on the pBR322 origin for replication. These results suggest that the P1 partition function is active in pBR322. We assume that since the copy number is too low

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under these conditions for random diffusion to be efficient, the active partition function of the P1 insert ensures an equitable distribution of the few copies available. However, it could be argued that some other property of the P1 insert is responsible for the increased stability of these plasmids. Perhaps, for example, a change in the pattern of gene expression or the physical state of the plasmid causes an increase in the frequency or regularity of replication under conditions of limited DNA synthesis. Therefore, we set out to show that the P1 *par* region responds to mutation, complementation, and incompatibility in the same way when stabilizing pBR322 as it does when stabilizing its parental P1 miniplasmid.

Plasmid pALA17 includes the *incB* region and the two large open reading frames for the ParA and ParB proteins that are essential for normal *par* activity (1). We repeated the stability tests in the *polA12* strain with pALA17 and a version of pALA17 (pALA104) that carries the *parA17* mutation (1, 3). This mutation eliminated the stabilizing effect of the *par* region (Table 1). An additional set of

FIG. 1. Plasmids containing P1 and F *par* sequences. The map of P1 *par* was constructed from data reported by Abeles et al. (1), which also described the construction of pALA17 and pALA104 and the conventions for the kilobase coordinates. Broad open bars, P1 or F DNA; narrow open bars, pLG338 DNA; single horizontal lines, pBR322 DNA. Abbreviations: H, *Hind*III; X, *Xho*I; S, *Sau*3A; A, *Aha*III; R5, *Eco*RV; R1, *Eco*RI; K, *Kpn*I; B, *Bam*HI; Bg, *Bgl*II; Hc, *Hinc*II; P, *Pst*I; bla, β -lactamase gene. F1 and F2 are open reading frames for the essential F *par* proteins. *tet* is the gene encoding tetracycline resistance. *incD* and *incB* show the location of the incompatibility determinants. The scale above the F plasmids shows the size and location of the cloned fragment in F coordinates (7).

FIG. 2. Kinetics of plasmid loss without selection at 29°C. Symbols: ○, strain W3550(pBR322); ●, strain MM383 *polA12*(pBR322); —, strain MM383 *polA12*(pALA17). Strain MM383 was described by Monk and Kinross (12) and is *polA12*(Ts) *thyA36 rha-5 lacZ53 deoC2 rpsL151*. Strain W3550 (2) is *polA*⁺.

experiments used versions of MM383 that carried the pSC101-based vector pLG338 (which is compatible with pBR322) or its *parA*⁺ *parB*⁺ derivative pALA283 as host strains (Fig. 1). The results (Table 1) show that the *parA17* defect in pALA104 was complemented when the ParA protein was supplied in *trans* from pALA283. The stability of normal P1 miniplasmids is also blocked by the *parA17* mutation (1) and can also be restored by supplying ParA in *trans* from pALA283 (data not shown). Thus, the activity of *par* in stabilizing pBR322 shows the same requirement for ParA protein as its activity in P1-driven plasmids.

TABLE 1. Effect of *par* regions on stability of pBR322 in the *polA12 sup*⁺ strain MM383^a

Plasmid	% Loss of plasmid after 10 generations	
	Alone	With P1 ParA protein supplied in <i>trans</i>
pBR322	94	98
pALA17 (P1 <i>par</i> ⁺)	6	5
pALA104 [<i>parA17</i> (Am)]	93	8
pALA287 (F <i>par</i> ⁺)	10	NT ^b
pALA610 (F <i>par</i>) ^c	92	NT
pDL80	14	NT

^a Tests were carried out as described in the text, except that tetracycline (20 μ g/ml) was substituted for ampicillin when relevant. Parallel tests carried out at 42°C showed a rapid loss of all test plasmids (>99% loss in 10 generations). The ParA protein was supplied in *trans* from plasmid pALA283 (Fig. 1), which is compatible with pBR322.

^b NT, Not tested.

^c F *par*, Partial deletion.

The P1 *incB* locus is downstream from the two *par* open reading frames and is contained within a region of less than 170 bp (1). A pBR322 plasmid containing an isolated *incB* sequence caused displacement of a resident P1 miniplasmid when introduced into the host by transformation. The locus has the same effect on the stability of composite plasmids that consist of the F replication and P1 *par* functions (2). We have proposed that *incB* is the *cis*-acting recognition site for partition and that, when cloned separately, it can displace a resident P1 by competing with it in the partition process.

To have *incB* in a vector that was compatible with pBR322, we cloned an 800-bp *Sau*3A fragment containing *incB* into the pSC101-based vector pLG338 to form pALA292 (Fig. 1). This plasmid exerted the same *incB* incompatibility effect on P1 miniplasmids as pBR322-based *incB* clones do (data not shown). The stability of pALA17 in the *polA12* strain at 29°C was sensitive to the presence of the *incB* sequence in pALA292 (84% loss in 10 generations) but was not affected by the vector (6% loss in 10 generations). The *incB* sequence in pALA292 had no effect on the high frequency of loss of pBR322 or pALA104 (*parA17*).

The responses to both mutation and incompatibility define the phenotype of the P1 *par* region in its original context. Since these responses can be demonstrated in the pBR322 system, we conclude that the P1 partition function can act on pBR322.

The *par* region (or *sop* genes) of the mini-F plasmid lies within the 46.4- to 49.5-kilobase (kb) coordinates of the F physical map (13). This region is not homologous to the P1 *par* region. We introduced the F *par* region, present on a *Pst*I-*Eco*RI fragment (coordinates 45.8 to 49.5 kb in F) into the complementary sites in pBR322 to form pALA287 (Fig. 1). A similar plasmid (pALA610) was constructed that lacks part of the F1 protein that is essential for *par* function (4) and has a small deletion in the *incD* region of *par*. The stability of these plasmids was tested in the *polA12* strain at 29°C. Although pALA610 was no more stable than the parental pBR322, pALA287 was relatively stable (Table 1). Its stability was similar to that of P1 *par* constructions.

P7 is a plasmid prophage that is similar in sequence and properties to P1 (8, 9). We inserted a 4-kb fragment of P7 that has properties in the stabilization assay similar to those of the *par* regions of P1 and F into pBR322 (Table 1). The construct (pDL80) was relatively stable in strain MM383 at 29°C. Thus, the region may include an active partition function. As the plasmid could not be maintained at 42°C, an alternative explanation, that the region contains a *polA*-independent replication origin, can be ruled out.

Although the precise endpoints of the insert in pDL80 are yet to be determined, the fragment includes the *Bam*HI-*Bgl*II sequences at 62 kb on the P7 physical map (9). P7 and P1 are 92% homologous and show similar genetic organization (9). Alignment of the maps shows that the *Bam*HI-*Bgl*II region of P7 aligns well with the *par* region of P1. However, there is a segment of nonhomology between P1 and P7 of approximately 3 kb in the region that encompasses the P7 *Bam*HI-*Bgl*II sequences (9). Thus, it is possible that pDL80 contains an active P7 *par* region that is equivalent in position to that of P1 but is nonhomologous to it.

pBR322 makes use of a different DNA polymerase and requires none of the replication-specific proteins of host or plasmid origin that are known to be essential for P1, P7, and F replication. In addition, replication controls of pBR322, which are very similar to those of ColE1, are based on negative regulation via a small RNA effector (17), whereas the unit-copy replicons appear to be regulated by modulation

of the concentration of a positive initiator protein (5, 15, 18). Since the *par* genes act to segregate both of these replicon types, we think it is unlikely that their stabilizing effects are due to some specific interaction with the replication machinery. It could still be argued that the stabilizing effect of *par* on pBR322 is due to some replication advantage conferred by an active *par* region, perhaps some change in the cellular location of the DNA. However, we feel that the data presented here provide strong support for the concept that *par* acts by promoting the ordered movement of plasmid copies to daughter cells after replication as we suggested previously (2).

Vectors related to pBR322 are a common choice for the construction of plasmids that overproduce commercially or experimentally valuable protein products in *E. coli*. The bulk culture of some of these strains is problematic because the active plasmids reduce cell growth and viability and plasmid-free cells tend to overgrow the culture. It is possible that the introduction of unit-copy plasmid *par* sequences into these plasmids could improve yields from bulk culture by reducing the number of plasmid-free cells produced at cell division.

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