Plasmid 64 (2010) 51–59

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

# On the role of centromere dispersion in stability of linear bacterial plasmids

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ARTICLE INFO

*Article history:*
Received 10 February 2010
Accepted 12 April 2010
Available online 18 April 2010

Communicated by Dr. B. Funnell

*Keywords:*
Linear plasmid
Partition
Centromere
N15

ABSTRACT

The N15 prophage-plasmid is linear, and the four centromere sites that enable its active partition are scattered rather than confined to a tandem array. This unusual arrangement suggested that the two features might be linked: centromere dispersion could enable condensation of linear DNA through interaction of partition complexes and so facilitate movement of segregating plasmid molecules. The present study examines this possibility. Linear N15 derivatives varying in centromere-site (IR) position, number and spacing were constructed, and stabilization of these plasmids by N15 SopAB proteins was measured. Stabilization increased in proportion to the number of IR sites and the distance between IR sites, the result expected if condensation mediated by partition complexes (SopB-IR) improves efficiency of SopA-directed segregation. However, visualization of two IR sites on the same molecule revealed that their colocalization did not depend on SopB but resulted from spontaneous folding of the linear DNA. Segregation of these sites by SopA was limited and incomplete compared to partition of plasmid copies. These observations imply that forces responsible for intrinsic folding of linear DNA tend to counter attempts to partition centromeres *in cis* to each other. We suggest that the beneficial effects of IR number and spacing on partition stem not from condensation but from provision of more numerous and better arranged substrates for SopA action.



## 1. Introduction

Centromeres which assure the mitotic stability of low copy-number bacterial plasmids generally consist of a specific array of short sequence repeats that occupies a single locus. They act by binding the plasmid's ParB protein to form a partition complex, which then stimulates the partner ParA protein to segregate copies of the plasmid to the new cells-to-be (reviewed by Ebersbach and Gerdes (2005)). Most bacteria specify homologues of plasmid ParA and B proteins, and use them to optimize segregation of their chromosomes. In chromosomes however, the ParB binding sites (termed *parS*) are not confined to a single array; rather they are typically dispersed over ~15% of the chromosome surrounding the replication origin (Lin and Grossman, 1998; Livny et al., 2007). The distinct formats of plasmid and chromosomal centromeres suggest that they are adaptations to the partition needs of each replicon type. The atypical plasmid RK2, which has 12 scattered ParB (KorB) binding sites (Balzer et al., 1992; Smith et al., 1984), shows that the distinction is not absolute. Even so, it is not yet certain whether more than one of the sites has a genuine role in partition (Williams et al., 1998); at least some of the others appear to have regulatory functions (Bingle et al., 2005; Jagura-Burdzy et al., 1999).

The behaviour of derivatives of another atypical plasmid suggested it as a more promising model for examining the importance of centromere configuration. N15 is a temperate bacteriophage which resembles phage λ in size, morphology, proliferative properties and general

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doi:10.1016/j.plasmid.2010.04.001

regulatory format, but whose prophage is a linear plasmid with hairpin ends, maintained in that state by a recombinase ("protelomerase") that resolves the circular dimer product of replication (reviewed by Ravin (2003)). Stable inheritance of the N15 plasmid-prophage is assured by a homologue of the F plasmid partition system, which comprises an ATPase with dynamic properties, SopA, a centromere-binding protein, SopB, and a series of repeat sequences that act as the centromere, *sopC* (Ravin and Lane, 1999). The major difference between these two Sop systems is the disposition of the centromere. Whereas in F it consists of a single locus of 12 tandem repeats, each carrying the 16 bp inverted repeat that binds SopB, in N15 it is scattered over about 12 kb as four individual inverted repeats (IR1-4). This dispersal immediately suggests that the demands of linear replicon stability might have selected for centromere dispersal as an adaptation of the Sop system (Ravin and Lane, 1999). Dispersal of similar sequences in more recently analyzed linear plasmids (pY54 – (Hertwig et al., 2003); ϕKO2 – (Casjens et al., 2004)) reinforces this view. We report here an assessment of the importance of number, location and spacing of centromere units in partition of linear replicons, using the example of N15, and an analysis of how the Sop system copes with two centromere sites on a single linear DNA molecule.

## 2. Methods

### 2.1. Bacterial strains and growth conditions

*Escherichia coli* K12 strain DH10B (Grant et al., 1990) was used as a transformation recipient and for plasmid stability measurements, and a derivative of it (DLT1899) rendered constitutive for arabinose inducer import by introduction of the Δ*araFGH* and Ω*Pcp18*::*araE*533 mutations was the host for centromere localization experiments. Cultures were incubated with aeration at 37 °C, unless otherwise indicated, in LB medium supplemented as appropriate with antibiotics (μg/ml) ampicillin (100), kanamycin (50), chloramphenicol (20), tetracycline (20) and spectinomycin (100).

### 2.2. Plasmids

An outline description of the three lines of plasmid construction follows; details are provided in supplementary information.

(i) Linear mini-N15 plasmids with two visualizable IR centromere sites (Fig. S1). In one branch of the construction, two sets of tandem sites (5× UAS) for the yeast GAL4 regulator were inserted into a derivative of the pBluescript vector (Alting-Mees and Short, 1989) and a duplex oligonucleotide containing IR3 was inserted between them. In the other, an array of 59 TetR binding sites (*tetO*) was abutted to the 64 $O_L$ ($CI^{\lambda}$-binding) site array in pRFB122 (Srivastava et al., 2006). The UAS–IR–UAS module above was inserted on each side of the joined *tetO*–$O_L$ arrays, and the arrays separated by insertion of a 22 kb fragment consisting largely of the late operon of bacteriophage λ. The IR–*tetO*–λ–$O_L$–IR ensemble was then inserted into a linear mini-N15 (pG595, see below) carrying the $repA_{ts52}$ allele (Ravin et al., 2003), to create pDAG711. Parallel steps were taken in constructing the equivalent single-IR and no-IR plasmids, pDAG720 and 721.

(ii) Plasmids enabling assessment of IR centromere-site activity. The circular mini-N15, pNC10, and plasmids that provide N15 SopA and SopB, pDAG216 (mini-F-$sopOPAB^{N15}$) and pDAG242 (low-copy pSC101-$pLtetO\text{-}sopAB^{N15}$), have been described (Ravin and Lane, 1999; Ravin, 2003); pDAG713, which provides SopB only, was made by in-frame deletion between the *Pac*I and *Nsi*I sites in *sopA* of pDAG242 followed by blunt-ending with T4 DNA polymerase and ligation. For inducible visual localization of IR sites, the *Ssp*I fragment of pRFG115 (Srivastava et al., 2006) containing $para_{BAD}\text{-}cI^{\lambda}$::*ecfp* was inserted in tandem to $para_{BAD}$-*tetR*::*yfp* at the HindIII site in pLAU48 (Lau et al., 2003) to create pDAG709.

(iii) Linear mini-N15 plasmids with variable IR site number and placement (Fig. S2). The parent of the series, pG591, is a linear plasmid with hairpin ends which contains the essential left-hand portion of N15, including the *telN* protelomerase, *repA* replication regulator and *cB* transcription repressor genes, joined to the kanamycin resistance gene of Tn903 and vestiges of the N15 *sopAB* operon. The mutant IR1 (5′-GgGCcACgACaGTaGCgC, mutant bases in small letters) was substituted to make pG595. Fragments were inserted into pG591 and pG595 to place one IR3 site in the left half (pG750, 751, 752) or at the right end (pG593, 598, 751; in 599 and 752 as two copies) of the molecules. Long versions of each plasmid (denoted pG591L, etc.) were made by inserting the 22 kb λ late operon fragment into the *Bgl*II site in the residual *sopA* sequence.

### 2.3. Visualization of IR sites

Strain DLT1899 carrying pDAG709 and either pDAG242 (*sopAB*), pDAG713 (*sopB*) or pDAG196 (no sop genes) was transformed to kanamycin resistance with pDAG711, pDAG720 or pDAG721. Cultures were grown with kanamycin at 30 °C to $OD_{600} \sim 0.2$ and fluorescent protein synthesis was induced by addition of arabinose to 0.0005–0.005%. Induction was stopped after 30 min by addition of glucose to 0.2% and incubation continued for two hours or, where mini-N15 plasmids were required at single copy, without kanamycin for four hours (pDAG711) or one hour (pDAG720, 721). Samples were concentrated 6-fold by centrifugation and resuspension in LB, sometimes with FM4-64 to stain membranes, and 2–3 μL were applied to a layer of 1% agarose, 0.1× LB on a glass slide under a coverslip. Cells were examined at 100× by phase-contrast and fluorescence microscopy using an Olympus IX81 microscope. Images were captured with a Coolsnap HQ camera and processed using Metamorph Imaging System v 6.3. Positions of foci were determined relative to the cell pole closest to a focus.