The partitioning activity of the RK2 central control region requires only *incC*, *korB* and KorB-binding site O_B3 but other KorB-binding sites form destabilizing complexes in the absence of O_B3

D. Ross Williams,[†] Donia P. Macartney[‡] and Christopher M. Thomas

Author for correspondence: Christopher M. Thomas. Tel: +44 121 414 5903. Fax: +44 121 414 5925.
e-mail: c.m.thomas@bham.ac.uk

School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

The sector of the genome of broad-host-range IncP plasmid RK2 from kb co-ordinate 54.0 to 60.0 confers an active partitioning phenotype, increasing the segregational stability of low-copy-number unstable plasmids. This Par region encodes the central control operon (*korA*, *incC*, *korB*, *korF* and *korG*) and the associated genes *kfrA*, *upf54.8* and *upf54.4*. Each ORF in this region was knocked out in turn and it was shown that only *incC* and *korB* are needed for the stability phenotype. *incC* encodes two polypeptides from alternative translational starts. A deletion of the start of the operon showed that only IncC2, the shorter product, is essential for partitioning. Directed mutation or deletion was used to inactivate in turn each of the three KorB-binding sites (O_Bs) which were candidate *cis*-acting sequences needed for stability. Only inactivation of O_B3, which lies between *upf54.4* and *upf54.8*, resulted in an increased rate of segregational loss. However, the rate of loss was significantly higher than the rate of loss of the test plasmid carrying none of this RK2 Par region. Either inactivation of *korB* or deletion of O_B1 from this O_B3 mutant resulted in restoration of the loss rate to that expected for the unstable test plasmid alone. Thus KorB can act on O_B1 to create a complex that either inhibits replication or reduces the effective plasmid copy number, perhaps by promoting pairing between plasmid molecules. This implies that RK2 goes through a cycle of pairing and separation, akin to the mitotic cycle of eukaryotic chromosomes.

Keywords: plasmid partitioning, broad-host-range plasmid, mitotic apparatus, plasmid RK2

INTRODUCTION

The process that leads to the accurate distribution of genomes prior to cell division in bacteria is of interest because of the central role it plays in the cell cycle (Hiraga, 1992; Rothfield, 1994; Wake & Errington, 1995). The machinery involved may provide novel targets for antimicrobial agents. Studies with *Bacillus*

subtilis have identified two genes, *spoOJ* and *soj*, which have been implicated in chromosome segregation into the pre-spore during sporulation (Sharpe & Errington, 1996). This appears to be achieved by anchoring the *oriC* region of the chromosome near the cell pole so that the rest of the chromosome can be spooled into the pre-spore compartment (Wu *et al.*, 1995; Lewis & Errington, 1997). Fluorescent tagging has shown both the *oriC* region and Par proteins in polar locations during sporulation and vegetative growth (Webb *et al.*, 1997; Lin *et al.*, 1997). Defects in chromosome partitioning occur even during vegetative growth when these genes are disrupted (Ireton *et al.*, 1994). Therefore, it is proposed that Soj and SpoOJ form part of a general mitotic apparatus for directing chromosome movement

[†]Present address: Division of Biosciences, School of Natural Sciences, University of Hertfordshire, Hatfield Campus, College Lane, Hatfield AL10 9AB, UK.

[‡]Present address: Department of Paediatrics and Child Health, Division of Medical and Molecular Genetics, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Fig. 1. RK2 *ctl* region showing the location of the genes studied in this paper. This corresponds to the region from co-ordinate 54.0 kb (right end) to 60.1 kb (left end) on the complete map of RK2. The ORFs shown correspond to the following co-ordinates: *korA*, 59341–59036; *incC1*, 59354–58260; *incC2*, 59039–58260; *korB*, 58263–57187; *korF*, 57116–56595; *korG*, 56602–56075; *kfrA*, 55889–54963; *upf54.8*, 54804–54454; *upf54.4*, 54425–53079 (Pansegrau *et al.*, 1994). Most of the plasmids used included a truncated *upf54.4* terminating at approximately co-ordinate 54000 but inclusion of the whole of *upf54.4* was found not to increase the segregational stability observed. Transcriptional units identified to date run from *korAp* (solid circle) with a transcriptional start point at co-ordinate 59402, and *kfrAp* (solid circle) with a transcriptional start point at 55970. A possible third promoter prior to *upf54.8* is indicated by an open circle. A rho-independent transcriptional terminator upstream of *korAp* protects transcription into the *korA*–*korB* operon (terminators are indicated by a 't'). A second rho-independent terminator lies downstream of *korG* and before *kfrA*. A third may be situated after *kfrA* but observations during the course of this work suggest that expression of *upf54.8* and *upf54.4* may depend on transcription from *kfrAp*. The *KorB*-binding sites in this region are labelled *O_B1* (upstream of *korAp*), *O_B2* (upstream of *kfrAp*) and *O_B3* (between *upf54.8* and *upf54.4*). Construction of the mutations in each of the ORFs and at *O_B2* and *O_B3* and the deletion that removes *korA* and *O_B1* are described in Methods.

(Glaser *et al.*, 1997). Homologues of either one or both SpoOJ and Soj are encoded by all bacterial chromosomes that have been studied to date. In *Caulobacter crescentus* the homologous proteins move in the bacteria in a way that is also strongly suggestive of a mitotic cycle (Mohl & Gober, 1997). In *Escherichia coli* the Soj homologue MinD is encoded away from *oriC* but is required for correct placement of septa apparently through a recognition of key sites in the internal cellular architecture (reviewed by Lutkenhaus & Addinall, 1997). Nevertheless, *E. coli* chromosomal *oriC* regions have been observed to migrate in a fashion indicative of a cycle with similarity to that of *B. subtilis* and *C. crescentus* (Gordon *et al.*, 1997).

These chromosomally encoded proteins belong to families, designated ParA and ParB, originally discovered on bacterial plasmids and associated with genetic loci responsible for the better than random segregation of low-copy-number plasmids, a phenomenon known as active partitioning (Austin, 1988; Nordström & Austin, 1989; Williams & Thomas, 1992). Recent evidence suggests that the best studied low-copy-number plasmids F and P1 actively segregate to quarter/three-quarter positions of the *E. coli* cell rather than the poles as observed for the chromosome (Gordon *et al.*, 1997; Niki & Hiraga, 1997). Nevertheless, it seems likely that study of the plasmid-encoded ParA/ParB proteins and their associated *cis*-acting sequence, referred to as centromere-like regions, will help to uncover the nature of this bacterial mitotic apparatus (reviewed by Williams & Thomas, 1992). Sequence comparison showed that many plasmids carry related genes. The ParB proteins bind specifically to the *cis*-acting sequences (Mori *et al.*, 1989; Watanabe *et al.*, 1989), although the nature of this element seems variable in size and organization (Lane *et al.*, 1987; Martin *et al.*, 1987, 1991).

Conserved motifs suggested that the A proteins may be ATPases (Motallebi-Veshareh *et al.*, 1990), a proposal which has since been confirmed for P1 ParA and F SopA (Davis *et al.*, 1991; Watanabe *et al.*, 1992). The ParA ATPase activity is necessary for partitioning (Davis *et al.*, 1996). However, the way that these proteins direct the better than random segregation of plasmid DNA molecules remains unclear and further analysis of individual systems is needed to build up a picture of the properties of this family of proteins.

The central control region (*ctl*) of broad-host-range IncP plasmid RK2 encodes five polypeptides of which two, IncC and KorB, show significant sequence similarity to SopA/ParA and SopB/ParB of F and P1, respectively (Motallebi-Veshareh *et al.*, 1990). Inactivation of *incC* leads to decreased stability of mini IncP plasmids, indicating that the region may encode partitioning functions similar to those of F and P1 (Motallebi-Veshareh *et al.*, 1990). However, because the region is more complex than those of F and P1 we thought its study might provide additional insights. This was particularly prompted by the presence of the *kfrA* gene encoding a DNA-binding protein with an extended coiled-coil domain reminiscent of components of force-generating motor proteins (Jagura-Burdzy & Thomas, 1992).

The *ctl* region by our definition here runs from *korAp* at the start of the *korA korB* operon to the bi-directional terminator between *upf54.4* and *traM* (Fig. 1). It contains three operons, the *korAB* operon encoding *korA*, *incC*, *korB*, *korF* and *korG*, the *kfrA* monocistronic operon and the *upf54.8*–*upf54.4* bicistronic operon (reviewed by Pansegrau *et al.*, 1994), although during the course of this work our observations sug-

gested that expression of *upf54.8* and *upf54.4* depends on transcription from *kfrAp* (D. R. Williams, unpublished), implying that *kfrA*, *upf54.8* and *upf54.4* form a single operon. KorA (101 aa) is a global regulator that binds to seven operators on the RK2 genome (Bechhofer & Figurski, 1983; Jagura-Burdzy & Thomas, 1995). Two of these KorA operators are in the *ctl* region, at *korAp* and *kfrAp*, both of which are repressed by KorA (Theophilus *et al.*, 1985; Thomas *et al.*, 1990). *incC* encodes the putative ATPase related to SopA and ParA (Motallebi-Veshareh *et al.*, 1990; Thomas & Smith, 1986), but contains two translational starts so that it produces two polypeptides, IncC1 (364 aa) and IncC2 (259 aa), both of which should have ATPase activity. KorB (358 aa), which belongs to the SopB/ParB family (Motallebi-Veshareh *et al.*, 1990), is a second global regulator, binding to 12 operators in the RK2 genome (Theophilus & Thomas, 1987; Kornacki *et al.*, 1987; Balzer *et al.*, 1992; Williams *et al.*, 1993). Three of these, O_B1, O_B2 and O_B3, are in the *ctl* region – near *korAp*, near *kfrAp* and between *upf54.8* and *upf54.4*, respectively. KorF (173 aa) and KorG (175 aa) are putative histone-like proteins (Jagura-Burdzy *et al.*, 1991). KrfA (314 aa) has been described above. Little is known about the additional two proteins encoded by *upf54.8* and *upf54.4*, although they are quite highly conserved between RK2 and R751 (Macartney *et al.*, 1997).

The purpose of the work described in this paper was to check that this region can stabilize heterologous replicons, and to determine which genes are necessary for this activity. However, the phenotypes of some of the mutants generated suggest that the multiple binding sites that exist for KorB provide a degree of complexity which may help to identify the components essential for active partitioning and their mechanism of action.

METHODS

Bacterial strains, plasmids and growth conditions. *Escherichia coli* strains used were K-12 strains C600K (*thr-1 leu-6 thi-1 lacY1 supE44 ton21 galK*), MV10 (C600 *trpRΔtrpE5*) and TG1 (*supE hsdΔ5 thi Δ(lac-proAB) F'[traD36 proAB⁺lacI^q lacZΔM15]*; Sambrook *et al.*, 1989), and *E. coli* C strain C2110 (*polA1 his rha*) (all strains except TG1 were from Professor D. R. Helinski, Department of Biology, UC San Diego, CA, USA). Bacteria were generally grown in L broth (Kahn *et al.*, 1979) at 37 °C or on L agar (L broth with 1.5%, w/v, agar) supplemented as appropriate with benzyl penicillin, sodium salt (100 µg ml⁻¹ in liquid media and 300 µg ml⁻¹ in agar plates), or ampicillin (100 µg ml⁻¹) to select for penicillin resistance. Previously described plasmids used during the course of this study were pALA1029 (Ludtke *et al.*, 1989), pOG04 derived from pALA1029 by replacing the *EcoRI* to *HindIII* *par* region by the short *EcoRI* to *HindIII* segment from pBR322 (Macartney *et al.*, 1997) and pOG4 derived from pALA1029 by replacing the P7 *par* genes with the RK2 *ctl* genes (Macartney *et al.*, 1997). The structures of pOG04 and pOG4 are shown in Fig. 2. All plasmids constructed during the course of this work are related to pOG4 or were involved in constructing derivatives of pOG4. These are described below.

Isolation, analysis and manipulation of plasmid DNA. Plasmid DNA was isolated on small and large scales by standard

Fig. 2. Shuttle vectors used to dissect the Par phenotype of the RK2 *ctl* regions. pOG04 was derived from pALA1029 (Ludtke *et al.*, 1989) by replacing the P7 *par* genes which had been inserted between *EcoRI* and *HindIII* sites by the original segment from pBR322. It thus essentially consists of pBR322 with the P7 replicon inserted on a *SalI* fragment. pOG4 was derived from pOG04 by inserting the *EcoRI*–*HindIII* fragment from pRK259.4 which carries the RK2 *ctl* region running from RK2 co-ordinates 54.0 to 60.1 kb followed by a short linker that runs to the *HindIII* site that was used to define the ends of the fragment used for subcloning the region (Smith & Thomas, 1983).

procedures (Sambrook *et al.*, 1989). Digestion of plasmid DNA with restriction enzymes was carried out under conditions recommended by suppliers and run on a 0.8 or 1% (w/v) agarose gel. Bacterial transformations were carried out using standard CaCl₂ treatment. DNA sequencing was carried out by AltaBioscience using the Dye-terminator method (derived from Sanger *et al.*, 1977) in conjunction with an ABI 373 automated DNA sequencer. Sequences were aligned and analysed using programs of the Wisconsin package (Devereux *et al.*, 1984). DNA PCR amplified fragments were labelled with terminal transferase and [α -³²P]ddATP.

Construction of derivatives of pOG4. Mutations were introduced by a variety of strategies: exploitation of spontaneous deletions that had been defined by DNA sequence analysis; cutting at unique sites; oligonucleotide-directed mutation with the Sculptor kit from Amersham. Since the whole region being manipulated is about 5 kb we found that it was best to introduce mutations into subcloned fragments which could then be spliced back into the full context after the mutation had been obtained and checked by sequencing. Attempts to

carry out oligonucleotide-directed mutation on the full region tended to generate deletions at the single-stranded stage and subsequently required large amounts of sequencing to check for secondary mutations. In other cases unique sites were obtained in the region of interest only when a subclone was used lacking segments that had additional copies of the desired site. Construction of each mutant was quite complicated, so the intermediate plasmids are not listed in full below.

Removal of O_B1 , *korA* and the start of *incC* so that IncC1 is no longer produced was achieved by replacing the *EcoRI* (at RK2 co-ordinate 60 099 kb)–*BspEI* (in *korA*) fragment with *Tsp5091* fragments bearing the *tet* promoter from pBR322 and a transcriptional terminator (giving pOG4.100). *incC* was inactivated (so that neither IncC1 nor IncC2 was produced) by a spontaneous 70 bp internal deletion which was described previously (Thomas, 1986; Thomas & Smith, 1986). It was spliced into the *ctl* region using the *SphI* and *SacII* (*SstII*) sites that lie upstream and downstream of the deletion, respectively (giving pOG14). *korB* was inactivated by a 2 bp deletion generated by digestion with *SacII*, removal of the 5' overhang with T4 DNA polymerase and ligation (giving pOG41). *korF* was inactivated by disruption of the *AvaI* site to create a stop codon at triplet 66 within the *orf* (giving pDM204.3). *korG* was inactivated by oligonucleotide-directed mutagenesis of the first bases of triplets 20 and 21 in the *orf* to create an *XbaI* site (giving pDM205.3). A double mutant with both the *korF* and the *korG* mutations was made by cloning the appropriate *SfiI* fragment from pDM204.3 into the context of pDM205.3 giving pDM209.3. *kfrA* was inactivated by a spontaneous deletion of 351 bp that occurred between repeated sequences that included *SfiI* sites (giving pOG453). *upf54.8* was inactivated by a 4 bp deletion by digestion with *ApaI*, removal of the 5' overhang with T4 DNA polymerase and ligation (giving pOG47). O_B2 was inactivated by use of oligonucleotide-directed mutagenesis, which changed the sequence from 5' CCGCTAAAAA 3' to 5' CCGCCATTAAAA 3' (giving pOG4.020). O_B3 was inactivated by oligonucleotide-directed mutagenesis, which deleted the central region of the sequence to leave a *DraI* site (5' TTAAAA 3'; pOG4.003). The logic for nomenclature of derivatives with one of the three *KorB*-binding sites inactivated or deleted was to add .100, .020 or .003 to the name of the parent plasmid depending on which O_B was affected.

Visualization of polypeptide products by *in vitro* transcription/translation of plasmid DNA. Plasmid-encoded polypeptides were labelled with [³⁵S]methionine (DuPont NEN) using the Promega *in vitro* transcription/translation kit for circular molecules on CsCl-purified plasmid DNAs (2 µg). Polypeptides were separated by SDS-PAGE and detected by phosphorimaging.

Estimation of the ability of RK2 segments to stabilize a low-copy-number plasmid. Loss rates were determined as previously described (Macartney *et al.*, 1997). The tests employed a shuttle vector, pOG04, which was derived by deletion of the P7 *par* system from pAL1029 (Ludtke *et al.*, 1989). The vector carries both pMB1 and P7 origins and therefore replicates at high copy number in a *PolA*⁺ strain (pMB1 origin) and at low copy number in a *PolA*[−] strain (*polA*-independent P7 origin). In *PolA*[−] strain C2110 pOG04 is lost during exponential growth at the rate expected of a randomly segregating plasmid present at four to six copies per cell at division. Derivatives bearing wild-type and mutant *ctl* regions from RK2 were constructed and selected in a *PolA*[−] strain and then transformed into C2110 to test their stability. The transformants were subcultured in selective medium to an OD₆₀₀ ~ 1.0, diluted 10^{−6} into non-selective medium (*t*₀) and grown for 8 h

until they had undergone about 25 generations (*t*_E). Viable counts and the percentage of ampicillin-resistant colonies were calculated for both *t*₀ and *t*_E. The mean apparent percentage loss of plasmid was calculated using the formulae L (loss rate) = $(1 - R^{1/g}) \times 100$, where g (generations) = $1.44 \times \log_e(x_E/x_0)$ (x_0 is the viable count at the start of the experiment and x_E is the viable count at the end of the experiment), and R = % Amp^R bacteria at *t*_E/ % Amp^R bacteria at *t*₀ (*t*_E is the time at the end of the experiment while *t*₀ is the time at the beginning of the experiment).

Estimation of relative plasmid copy number. Relative plasmid copy number was estimated as follows. To extract total DNA, 0.7 ml bacterial culture and 0.7 ml aqueous phenol that had been equilibrated with 50 mM Tris/HCl, pH 8.0, 10 mM NaEDTA (TE) were mixed thoroughly for 30 s in a 1.5 ml microfuge tube. After centrifugation for 5 min in a microfuge (12 500 r.p.m.) the aqueous (top) layer was removed and mixed thoroughly with phenol/chloroform (24:1 with isoamyl alcohol) for 30 s. After centrifugation again the aqueous layer was removed, avoiding any carry-over of the phenol/chloroform layer, and then precipitated by addition of 2 vols 2-propanol, mixing and leaving at room temperature for 2 min. After spinning in a microfuge for 5 min the DNA pellet was washed with ice-cold 70% (v/v) ethanol, dried and resuspended in 50 µl TE. A standard amount of DNA was digested with *EcoRI* and run on a 1% (w/v) agarose gel, before Southern blotting and probing by standard methods (Sambrook *et al.*, 1989). A DNA fragment with part of the *trp* operon was used as the probe for chromosomal DNA while a fragment containing the *bla* gene was used as the probe for the plasmid DNA. After standard washing the filters were placed in a phosphorimager cassette which was scanned after overnight exposure. The image was analysed using Imagequant software and the pixels in the plasmid and chromosomal bands were compared to give a plasmid to chromosome ratio which was used as a measure of the relative copy number.

RESULTS

The RK2 *ctl* region stabilizes a low-copy-number P7 replicon

We have previously demonstrated the need for *incC* of the RK2 *ctl* region for stable inheritance of IncP replicons (Motallebi-Veshareh *et al.*, 1990). To allow easier dissection of this system we exploited a shuttle vector previously used by Austin and co-workers (Ludtke *et al.*, 1989) that consists of pBR322 joined to the replicon from phage/plasmid P7. The pBR322 section allows easy plasmid construction in a *PolA*⁺ host while the P7 replicon allows replication at low copy number in a *PolA*[−] host. However, in such a *PolA*[−] host the rate of loss is so high if the plasmid lacks an active partitioning system that selection for the plasmid favours mutants with higher copy number, which therefore rapidly dominate. Therefore, tests on the stability of derivatives of these plasmids need to be performed as soon as possible after their introduction into the *PolA*[−] strain. Our starting point was pALA1029, which possesses both the P7 replicon and the P7 active partitioning system. In pOG04 the P7 *par* is deleted and in pOG4 it is replaced by the RK2 *ctl* region (Fig. 2). After introduction into *PolA*[−] strain C2110, stability was estimated as described in Methods. The results are

Fig. 3. Segregational stability of parent plasmids and key mutant derivatives described in this paper. Loss rate was determined as described in Methods from at least three cultures inoculated from separate transformants. Error bars represent 95% confidence limits. The zones of stability designated stabilized, random (the same as the test plasmid lacking a known partitioning system) and destabilized were chosen to group apparently similar phenotypes. It should be noted that the error bars do not extend across the boundaries drawn, adding validity to these groupings.

Table 1. Relative plasmid copy numbers of the plasmids studied in this work

Plasmid*	Relative copy number†	95% confidence limits (n)‡
pALA1029 (P7 <i>par</i>)	4.96	2.58 (3)
pOG04 (unstable control)	2.09	0.19 (6)
pOG4 (RK2 <i>ctl</i>)	1.95	1.54 (3)
pOG14 (RK2 <i>ctl incC</i>)	2.66	0.29 (3)
pOG41 (RK2 <i>ctl korB</i>)	2.08	0.57 (3)
pDM204.3 (RK2 <i>ctl korF</i>)	2.21	0.69 (3)
pDM205.3 (RK2 <i>ctl korG</i>)	1.72	0.20 (4)
pDM209.3 (RK2 <i>ctl korF korG</i>)	2.32	0.43 (4)
pOG453 (RK2 <i>ctl kfrA</i>)	2.92	0.37 (3)
pOG47 (RK2 <i>ctl upf54.8</i>)	1.99	0.51 (7)
pOG4.100 (RK2 <i>ctl korA O_B1</i>)	1.85	0.68 (6)
pOG4.020 (RK2 <i>ctl O_B2</i>)	1.88	0.64 (4)
pOG4.003 (RK2 <i>ctl O_B3</i>)	1.88	0.37 (7)
pOG4.103 (RK2 <i>ctl O_B1 O_B3</i>)	1.70	0.55 (6)
pOG4.023 (RK2 <i>ctl O_B2 O_B3</i>)	2.36	0.48 (4)
pOG41.003 (RK2 <i>ctl korB O_B3</i>)	1.28	0.18 (4)

* Plasmid genotypes: pALA1029 has the P7 partitioning system; pOG4 and below have the RK2 *ctl* with mutations as indicated by the genes listed.

† Determined as described in Methods.

‡ n, Number of samples for which a copy number determination was performed.

shown in Fig. 3. The RK2 *ctl* region clearly decreases loss rate but not as much as the P7 *par*. However, to correct for plasmid copy number variation we carried out a Southern blot of *EcoRI*-digested total DNA separated by agarose gel electrophoresis and then hybridized with chromosomal and plasmid probes. Copy number was estimated by comparison with a segment of the *trp* operon. The *trp* operon is encoded at 27.5 min on the genome, close to the replication terminus, so that the total number of *trp* gene copies should approximate to the number of completed chromosomes in the bacterial cell. For exponentially growing bacteria in L broth at 37 °C the mean generation time is 25–30 min so that when the bacteria divide there should still

only be two copies of the terminus. Thus very roughly our relative copy number of two should correspond to a copy number of approximately four per cell. We found that the combination of the P7 *rep*/P7 *par* has a twofold higher copy number than P7 *rep*/RK2 *ctl* (Table 1). Although we have not measured absolute copy numbers precisely, data previously obtained for the P1/P7 replicon with an active partitioning system suggest that the copy number should be in the region of two to three prior to cell division. Our results suggest a rather higher value. A twofold decrease in copy number as observed when the P7 *par* system is removed or replaced by the RK2 system would be expected to dramatically increase the rate of plasmid loss. Thus much of the increased

stability of pALA1029 compared to pOG4 may be due to its higher copy number, which could arise from an interaction between the two related systems. Therefore, we decided that this shuttle vector demonstrated the stabilizing effect of the RK2 *ctl* region and provided an adequate system for studying the effect of mutations on this phenotype.

Mutagenesis of each protein-coding gene from *korA* to *upf54.8*

In the RK2 *ctl* region there are a number of cistrons, in addition to *korA*, *incC* and *korB*, which have not yet been assigned a function. Therefore, we hypothesized that the IncP *ctl* region may provide more partition functions than the F and P1/7 region and thus give greater insights into the partition process. To test this hypothesis with respect to encoded polypeptides we exploited spontaneous mutations or generated site-directed mutations in each of the ORFs as described in Methods. This was done using subcloned sections of the *ctl* region to limit the number of restriction sites present and to minimize the region that went through a single-stranded stage during *in vitro* site-directed mutagenesis. The mutagenized region was then sequenced to ensure that only the desired changes had been introduced. The mutated region was spliced back into the complete region. *In vitro* transcription/translation was performed on each mutant cloned *ctl* region to check that only the gene product targeted had been affected by the mutation (data not shown). Interestingly, the results showed clearly that the repressors synthesized by the region reach sufficient *in vitro* concentrations to inhibit further transcription of the operons present. Thus when repressor genes were inactivated, the level of the polypeptide products synthesized *in vitro* was considerably elevated compared to wild-type template. Checking the mutants in this way also proved valuable because we detected secondary mutations in a number of clones. For example, in at least one *upf54.8* mutant we discovered that IncC1 was no longer produced either. Such clones were rejected in favour of clones that exhibited the expected polypeptide profile. However, when the desired mutation had already been confirmed in the target region the easiest strategy was then to splice the desired mutation back into a known wild-type region.

incC and *korB* are the only protein-encoding genes needed for partitioning

Each plasmid into which we had introduced the specific mutations described above was then established in C2110 (PolA⁻) along with appropriate controls and the loss rate for each plasmid was determined. We found that only the mutations in *incC* and *korB* resulted in loss of the Par⁺ phenotype (Fig. 3). These mutations returned the plasmid loss rate to the region of 4–6% per generation, similar to the level of instability observed for pOG04. Mutations in *korF*, *korG*, *kfrA*, *upf54.8* and *upf54.4* had no significant effect on loss rate (data not shown). Since it was possible that *korF* and *korG* may be

able to substitute for each other we created a *korFkorG* double mutant but this did not show any increased rate of loss either (data not shown). The relative copy number of each plasmid was tested at the start of the experiment as described in Methods with the results shown in Table 1. While variation in copy number was observed between different plasmids, there was no correlation between these small variations and the rate of plasmid loss. Therefore, it appears that only two plasmid-encoded proteins are needed for the stability phenotype.

IncC1 is dispensable when IncC2 is still produced

The start of the *korA/korB* operon is complicated because *korA* and *incC* overlap. However, *incC* has two translational starts, producing IncC1 and IncC2. One start precedes the *korA* start while the other overlaps the stop codon for *korA*. To determine the importance of this part of the operon we created a deletion/fusion which removes *korAp*, most of *korA*, the start of *incC* and places the remains of the *korA* *orf* in-frame with the *tet* *orf* from pBR322. The *tet* promoter provides transcription for the operon. We found that this deletion did not result in loss of the Par⁺ phenotype (data not shown). Therefore KorA is not required, nor is IncC1. We can conclude that only IncC2 and KorB are needed for active partitioning.

cis-acting sites needed for partitioning

By analogy with the F and P1 systems we expected that the *cis*-acting sequences – the centromere-like region – should contain KorB-binding sites. In the *ctl* region there are three such sites which we have designated O_B1 (at *korAp*), O_B2 (at *kfrAp*) and O_B3 (between *upf54.8* and *upf54.4*) (Fig. 1). We determined the effect of removing each of these in turn. O_B2 and O_B3 were inactivated by site-directed mutation while O_B1 was removed by deletion along with *korAp* as described in the previous section. Again we determined the sequence of mutated regions, checked that the polypeptide profile was complete and then tested the effect on stability. We found that inactivation of O_B1 and O_B2 had no effect on the Par⁺ phenotype but that inactivation of O_B3 resulted in complete loss of the stabilizing effect conferred by the *ctl* region (Fig. 3). In fact, mutagenesis of O_B3 gave a much higher rate of appearance of plasmid-free segregants than any of the other plasmids with a single mutation. This effect was not due to an effect on copy number, which remained similar to the other plasmids. Therefore we propose that the O_B3 region is the candidate centromere-like region.

The relative orientation of *incC/korB* and O_B3 does not affect the stability phenotype

During the dissection of the RK2 *ctl* region we isolated a series of mutations arising from the insertion of the transposon Tn1723 (Km^R). This transposon has *EcoRI* sites 15 bp from each end so that it is possible to remove

the majority of the transposon but leave behind an *EcoRI* site at the original point of insertion. One such mutation was found in the *kfrAp* region. In attempting to construct a pOG4 mutant derivative of this region we generated an inversion of the *korA-korG* region which is flanked by *EcoRI* sites. Testing the stability phenotype of this region we found that it was no less stable in maintaining the plasmid than the parent from which it was derived (data not shown), indicating that the orientation of *incC* and *korB* relative to O_B3 is not important for the stability phenotype.

Suppression of the extreme instability of the O_B3 mutant

Inspection of the data in Fig. 3 shows that in contrast to the effect of inactivating *incC* or *korB*, inactivation of O_B3 results in a loss rate that is considerably greater than that for pOG4, the control plasmid that lacks a partitioning apparatus and should therefore be distributed at random. However, the apparent level of plasmid DNA in the bacteria is not reduced (Table 1). This suggests that the mutation reduces the number of physically separate units which are available to segregate prior to cell division. Since a favoured model for partitioning involves KorB-mediated pairing, it seemed possible that although only one region with a KorB-binding site should be able to promote active segregation, other binding sites may promote pairing. Applying this to the RK2 *ctl* region and the data presented so far we hypothesized that pairing occurs at O_B1 , O_B2 or O_B3 but that separation depends on O_B3 alone. In this case the absence of O_B3 would result in the plasmid becoming unstable because pairs of plasmids would be held together by KorB at O_B1 and O_B2 and thus reduce the number of segregating units. We would then expect that the high instability of the O_B3 mutant should be suppressed by deleting other O_B sites or inactivating the protein which mediates pairing. We therefore created hybrids in which the O_B3 mutant was combined with O_B1^- , O_B2^- and *korB*⁻. Starting from pOG4.003 we inactivated either O_B1 (pOG4.103) or *korB* (pOG41.003) and observed instability return to the level for the unstable vector control (Fig. 3). However, inactivation of O_B2 had little effect (pOG4.023). This correlates with our recent studies which showed that O_B2 has 10-fold lower affinity for KorB than O_B1 or O_B3 , probably due to the mismatch in one half operator site compared to the consensus sequence (C. M. Thomas, unpublished). Copy number determinations indicated that the increased stability was not the result of an increase in copy number (Table 1).

DISCUSSION

In this paper we describe the mutational analysis of all the cistrons and KorB-binding sites in the *ctl* region of plasmid RK2 with respect to the ability of this region to stabilize an unstable, low-copy-number, heterologous replicon. We had expected to find that, in contrast to other plasmid systems so far characterized, the RK2

system would show a dependence on more plasmid-encoded proteins than just the ParA and ParB homologues IncC and KorB. However, the results show clearly that to achieve the degree of stability that we observe with the region cloned, we only need IncC and KorB. However, for a number of reasons we can not at this stage rule out any role for the additional proteins in this region in plasmid stability. First, it may be that the region we have been studying lacks additional functions which are needed to allow the other genes tested here to play their full role. This would be consistent with the fact that the stabilization that we see is not as efficient as that achieved by the P7 *par* system used as a positive control in these experiments. This idea would also be consistent with recent observations that overexpression of *korC* destabilizes RK2, especially in *Pseudomonas aeruginosa* (Wilson *et al.*, 1997). The *korC* regulated operons form part of a block of functions between *ctl* and *oriV* which until recently had no demonstrated function but which are conserved in many respects between IncP α plasmid RK2 and IncP β plasmid R751 (Thomas *et al.*, 1988, 1995). A role in stable inheritance would therefore make considerable sense and recent data with R751 showed clearly that inclusion of this additional region, along with the *ctl* region, results in almost no loss of a low-copy-number test plasmid (Thorsted *et al.*, 1998). Second, the observation that overexpression of *korC* has a greater destabilizing effect in *P. aeruginosa* than in *E. coli* also raises the possibility that a phenotype may be observed for some of our mutants if they were studied in species other than *E. coli*. This would imply that the system carries with it functions which are also provided by the host but where the host version can be used in some species but not others. However, to test this possibility would necessitate using a different low-copy-number plasmid than one based on the P7 replicon because this has a rather restricted host range.

The second important observation we report is that IncC2 is sufficient for partitioning, as we found previously for IncP β plasmid R751 (Macartney *et al.*, 1997). This raises the question as to the role of IncC1 but is consistent with the fact that many systems only encode a homologue of IncC2 but not IncC1. The chromosomal homologues are generally like IncC2 (for examples, Mohl & Guber, 1997; Sharpe & Errington, 1996) while among plasmids one finds one or the other but normally not both (reviewed by Williams & Thomas, 1992). The F and P1 systems are the best examples encoding a protein with the extra N-terminal region like IncC1. Our observations suggest that the N-terminal region present in IncC1 modulates KorB binding to DNA (C. M. Thomas, unpublished) and therefore this may be a feature particularly associated with the global regulation encoded in the central control region rather than with its role in active partitioning. The fact that IncC2 does function in partitioning increases its attraction as a model for the structure and function of the chromosomal homologues as compared to the F and P1 systems.

The finding that the KorB-binding site O_B3 is sufficient

Fig. 4. Model which could explain increased stability of the O_B3^- mutant of pOG4 and the suppression of this increased instability by O_B1 or $korB$ mutants. We propose that KorB can promote pairing between plasmids with primary O_B sites (rather than secondary sites like O_B2 which appears to be weaker due to a difference from the consensus KorB operator sequence). However, only protein complexes at O_B3 can achieve the active segregation. Therefore, deletion of O_B3 allows pairs to remain for longer than normal, thus reducing the number of segregating units with consequent increased appearance of plasmid-free bacteria. This additional instability resulting from inactivation of O_B3 depends on other O_B s and on KorB so inactivation of either of these results in suppression of the superinstability phenotype, as observed.

and necessary to give a Par^+ phenotype is exciting for a number of reasons. First, it confirms the expectation that a KorB-binding site is an important part of the centromere-like sequence of RK2. Second, it indicates that it is a specific site, which is important since it is difficult to conceive of a situation in which more than one site can be used unless there is co-ordination between the two sites so that they partition in the same direction. We clearly need to show that the other sites present on the complete RK2 genome (O_B4 – O_B12) are not able to substitute for O_B3 but the specificity demonstrated here fits with what one would expect: namely, that only one site can perform this role in active partitioning. Third, a role for O_B3 in active partitioning is the first demonstration of a role for one of the O_B s which does not appear to regulate transcription (see Pansegrau *et al.*, 1994). At present we do not know why O_B3 should behave differently from the other O_B sites. O_B3 is different from O_B1 and O_B2 because its two halves define a 15 bp inverted repeat which is thus longer than either of the others. However, other O_B s are of a similar length so this can not make O_B3 unique. Another possibility is suggested by the finding that IncC potentiates KorB binding at all O_B s other than O_B3 (C. M. Thomas & K. Kostelidou, unpublished). The significance of this observation awaits further investigation.

However, perhaps the most interesting observation is that inactivation of O_B3 creates a region which de-

stabilizes the unstable vector and that this destabilization is suppressed by deletion of O_B1 or by inactivation of KorB. The model that we have put forward to explain this is shown in Fig. 4. The key point is that the deletion of O_B3 decreases the number of segregating units because KorB bound at O_B1 promotes plasmid pairing but can not promote separation. A second explanation, that deletion of O_B3 results in derepression of a gene with an inhibitory product (Upf54.4), seems unlikely because it is hard to see how deletion of O_B1 or inactivation of $korB$ could suppress this effect. A third explanation might be that the conformation of the plasmid DNA is critical for the segregation process or for replication and that the complexes formed by O_B1 and KorB interfere with this. Similar explanations have been proposed for the *par* region of pSC101, which seems to work through DNA-gyrase-mediated effects on plasmid DNA conformation which affect plasmid copy number (Ingmer & Cohen, 1993; Conley & Cohen, 1995). However, this does not provide an explanation for how KorB and O_B3 actually increase the stability of the test plasmid. We also estimated changes in copy number very carefully and the data suggest that changes in copy number are not the explanation. We therefore consider the mechanism proposed in Fig. 4 as the most simple one. However, when we consider the loss rates in Fig. 3, they do not fit the predictions from the proposed changes in the copy number of segregating units. The copy number prior to cell division that is consistent with

the calculated 4% loss for pOG04 is between four and five. A reduction in copy number by twofold due to pairing would be expected to give a loss rate of between 25 and 12.5% per generation, which is much higher than the 8% loss per generation observed. This could be explained if the partitioning system is not working with complete efficiency, as is clearly the case since the complete system in pOG4 does not achieve the reduction in loss rate observed for pALA1029, which relies on the P7 system; i.e. the complexes formed may break down sufficiently frequently to prevent the effective copy number being reduced by a factor of two. However, we think that the model fits sufficiently well to justify further tests to identify pairing between plasmids mediated by the partitioning apparatus *in vitro* and *in vivo*. The same conclusions have also been reached with other plasmids, most notably plasmid R1, where recent evidence suggests strongly that pairing can be mediated by sequences which function as a centromere (Jensen *et al.*, 1998).

If the model is correct then it illustrates the advantage of the IncP system with multiple O_B s which allow the separation of pairing and segregation steps in the prokaryotic equivalent of the mitotic cycle. We do not wish to imply that pairing at O_B sites other than the one involved in provision of centromere-like activity is an essential part of partitioning – it clearly is not since the normal degree of stabilization is observed when only O_B3 is present. Nor do we wish to imply that it is therefore an artefact. Rather, we suggest that it could be an event that is not essential for partitioning but may play a role in the control of expression of genes for replication, transfer and stable inheritance so that recently replicated molecules are reduced in activity until they have undergone the partitioning event. In this way the global regulation and active partitioning activities of KorB could be intermeshed.

The fact that inactivation of *incC* does not result in increased instability suggests that, in the absence of IncC, the process does not get stuck at the proposed intermediate ('paired') stage. This could be because in the context studied here, IncC is needed to form the stable complexes which cause the instability observed. This would be the case either if the putative ATPase activity of IncC is required to promote formation of the complexes that destabilize the plasmid, or if IncC is simply a structural component of the complex, which breaks down more rapidly in its absence and therefore does not result in an effective copy number decrease. Further studies involving point mutations to inactivate the ATP binding and ATPase active sites are under way to test these possibilities.

ACKNOWLEDGEMENTS

D.R.W. was supported by project grants from the MRC (G9231237CB). D.P.M. was supported by an MRC Studentship for training in research methods. Automated DNA sequencing was performed in Alta Bioscience using an ABI373 machine purchased with a grant from The Wellcome Trust

(038654/Z/93). The phosphorimager was purchased with grants from The Wellcome Trust (037160/Z/92) and the MRC (G9216078MB).

REFERENCES

- Austin, S. (1988). Plasmid partitioning. *Plasmid* **20**, 1–9.
- Balzer, D., Ziegelin, G., Pansegrau, W., Kruff, V. & Lanka, E. (1992). KorB protein of promiscuous plasmid RK2 recognises inverted sequence repetitions in regions essential for conjugative transfer. *Nucleic Acids Res* **20**, 1851–1858.
- Bechhofer, D. H. & Figurski, D. H. (1983). Map location and nucleotide sequence of *korA*, a key regulatory gene of promiscuous plasmid RK2. *Nucleic Acids Res* **11**, 7453–7469.
- Conley, D. L. & Cohen, S. N. (1995). Effects of the pSC101 partitioning (*par*) locus on *in vivo* DNA supercoiling near the plasmid replication origin. *Nucleic Acids Res* **23**, 701–707.
- Davis, M. A., Martin, K. & Austin, S. (1991). Biochemical activities of the P1 partition proteins. *Plasmid* **25**, 247–248.
- Davis, M. A., Radnedge, L., Martin, K. A., Hayes, F. & Austin, S. J. (1996). The P1 ParA protein and its ATPase activity play a direct role in the segregation of plasmid copies to daughter cells. *Mol Microbiol* **21**, 1029–1036.
- Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Glaser, P., Sharpe, M., Raether, B., Perego, M., Ohlsen, K. & Errington, J. (1997). Dynamic mitotic-like behaviour of a bacterial protein required for accurate chromosome partitioning. *Genes Dev* **11**, 1160–1168.
- Gordon, G. S., Sitnikov, D., Webb, C. D., Teleman, A., Straight, A., Losick, R., Murray, A. W. & Wright, A. (1997). Chromosome and low copy plasmid segregation in *E. coli*: visual evidence for distinct mechanisms. *Cell* **90**, 1113–1121.
- Hiraga, S. (1992). Chromosome and plasmid partitioning in *E. coli*. *Annu Rev Biochem* **61**, 283–306.
- Ingmer, H. & Cohen, S. N. (1993). The pSC101 *par* locus alters protein-DNA interactions *in vivo* at the plasmid replication origin. *J Bacteriol* **175**, 6046–6048.
- Ireton, K., Gunther, N. W. & Grossman, A. D. (1994). SpoOJ is required for normal chromosome segregation as well as initiation of sporulation in *Bacillus subtilis*. *J Bacteriol* **176**, 5320–5329.
- Jagura-Burdzy, G. & Thomas, C. M. (1992). *kfrA* gene of broad host range plasmid RK2 encodes a novel DNA binding protein. *J Mol Biol* **225**, 651–660.
- Jagura-Burdzy, G. & Thomas, C. M. (1995). Purification of KorA protein from broad-host-range plasmid RK2: definition of a hierarchy of KorA operators. *J Mol Biol* **253**, 39–50.
- Jagura-Burdzy, G., Ibbotson, J. P. & Thomas, C. M. (1991). The *korF* region of broad-host-range plasmid RK2 encodes two polypeptides with transcriptional repressor activity. *J Bacteriol* **173**, 826–833.
- Jensen, R. B., Lurz, R. & Gerdes, K. (1998). Mechanism of DNA segregation in prokaryotes: replicon pairing by *parC* of plasmid R1. *Proc Natl Acad Sci USA* **95**, 8550–8555.
- Kahn, M. R., Kolter, R., Thomas, C., Figurski, D., Meyer, R., Remault, E. & Helsenki, D. R. (1979). Plasmid cloning vehicles derived from plasmids ColE1, R6K and RK2. *Methods Enzymol* **68**, 268–280.
- Kornacki, J. A., Balderes, P. J. & Figurski, D. H. (1987). Nucleotide sequence of *korB*, a replication control gene of broad host range plasmid RK2. *J Mol Biol* **198**, 211–222.

- Lane, D., Rothenbuehler, R., Merrilat, A. M. & Aitken, C. (1987). Analysis of the F plasmid centromere. *Mol Gen Genet* **207**, 406–412.
- Lewis, P. J. & Errington, J. (1997). Direct evidence for active segregation of *oriC* regions of the *Bacillus subtilis* chromosome and co-localization with the SpoOJ partitioning protein. *Mol Microbiol* **25**, 945–954.
- Lin, D., Levin, P. & Grossman, A. (1997). Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **94**, 4721–4726.
- Ludtke, D. N., Eichorn, B. G. & Austin, S. J. (1989). Plasmid partition functions of the P7 prophage. *J Mol Biol* **209**, 393–406.
- Lutkenhaus, J. & Addinall, S. G. (1997). Bacterial cell division and the Z ring. *Annu Rev Biochem* **66**, 93–116.
- Macartney, D. P., Williams, D. R., Stafford, T. & Thomas, C. M. (1997). Divergence and conservation of the partitioning and global regulation functions in the central control region of the IncP plasmids RK2 and R751. *Microbiology* **143**, 2167–2177.
- Martin, K. A., Friedman, S. A. & Austin, S. J. (1987). Partition site of the P1 plasmid. *Proc Natl Acad Sci USA* **84**, 8544–8547.
- Martin, K. A., Davis, M. A. & Austin, S. (1991). Fine-structure analysis of the P1 plasmid partition site. *J Bacteriol* **137**, 885–890.
- Mohl, D. A. & Gober, J. W. (1997). Cell cycle-dependent polar localisation of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* **88**, 675–684.
- Mori, H., Mori, Y., Ichinose, C., Niki, H., Ogura, T., Kato, A. & Hiraga, S. (1989). Purification and characterization of SopA and SopB proteins essential for F plasmid partitioning. *J Biol Chem* **264**, 15535–15541.
- Motallebi-Veshareh, M., Rouch, D. & Thomas, C. M. (1990). A family of ATPases involved in active partitioning of diverse bacterial plasmids. *Mol Microbiol* **4**, 1455–1463.
- Niki, H. & Hiraga, S. (1997). Subcellular distribution of actively partitioning F plasmid during the cell division cycle in *E. coli*. *Cell* **90**, 951–957.
- Nordström, K. & Austin, S. (1989). Mechanisms that contribute to the stable segregation of plasmids. *Annu Rev Genet* **23**, 37–69.
- Pansegau, W., Lanka, E., Barth, P. T. & 7 other authors (1994). Complete nucleotide sequence of Birmingham IncP α plasmids: compilation and comparative analysis. *J Mol Biol* **239**, 623–663.
- Rothfield, L. I. (1994). Bacterial chromosome segregation. *Cell* **77**, 963–966.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. M. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.
- Sharpe, M. & Errington, J. (1996). The *Bacillus subtilis* *soj-spo0J* locus is required for a centromere-like function involved in pre-spore chromosome partitioning. *Mol Microbiol* **21**, 501–509.
- Smith, C. A. & Thomas, C. M. (1983). Deletion mapping of *kil* and *kor* functions in the *trfA* and *trbB* regions of broad-host range plasmid RK2. *Mol Gen Genet* **190**, 245–254.
- Theophilus, B. D. M. & Thomas, C. M. (1987). Nucleotide sequence of the transcriptional repressor gene *korB* which plays a key role in regulation of the copy number of broad-host-range plasmid RK2. *Nucleic Acids Res* **15**, 7443–7450.
- Theophilus, B. D. M., Cross, M. A., Smith, C. A. & Thomas, C. M. (1985). Regulation of the *trfA* and *trfB* promoters of broad-host-range plasmid RK2. Identification of sequences essential for regulation by *trfB/korA/korD*. *Nucleic Acids Res* **13**, 8129–8142.
- Thomas, C. M. (1986). Evidence for the involvement of the *incC* locus of broad host range plasmid RK2 in plasmid maintenance. *Plasmid* **16**, 15–29.
- Thomas, C. M. & Smith, C. A. (1986). The *trfB* region of broad host range RK2: the nucleotide sequence reveals *incC* and key regulatory gene *trfB/korA/korD* as overlapping genes. *Nucleic Acids Res* **14**, 4453–4467.
- Thomas, C. M., Ibbotson, J. P., Wang, N., Smith, C. A., Tipping, R. & Loader, N. M. (1988). Gene regulation on broad host range plasmid RK2: identification of three novel operons whose transcription is repressed by both KorA and KorC. *Nucleic Acids Res* **16**, 5345–5359.
- Thomas, C. M., Theophilus, B. D., Johnston, L. J., Jagura-Burdzy, G., Schilf, W., Lurz, R. & Lanka, E. (1990). Identification of a 7th operon on plasmid RK2 regulated by the *korA* gene product. *Gene* **89**, 29–35.
- Thomas, C. M., Smith, C. A., Ibbotson, J. P., Johnston, L. & Wang, N. J. (1995). Evolution of the *korA-oriV* segment of promiscuous IncP plasmids. *Microbiology* **141**, 1201–1210.
- Thorsted, P. B., Macartney, D. P., Akhtar, P. & 9 other authors (1998). Complete sequence of the IncP β plasmid R751: implications for the evolution and organisation of the IncP backbone. *J Mol Biol* **282**, 969–990.
- Wake, R. G. & Errington, J. (1995). Chromosome partitioning in bacteria. *Annu Rev Genet* **29**, 41–67.
- Watanabe, E., Inamoto, S., Lee, M. H., Kim, S. U., Ogura, T., Mori, H., Hiraga, S., Yamasaki, M. & Nagai, K. (1989). Purification and characterisation of the *sopB* gene product which is responsible for stable maintenance of mini-F plasmid. *Mol Gen Genet* **218**, 431–436.
- Watanabe, E., Wachi, M., Yamasaki, M. & Nagai, K. (1992). ATPase activity of SopA, a protein essential for active partitioning of F plasmid. *Mol Gen Genet* **234**, 346–352.
- Webb, C. D., Teleman, A., Gordon, S., Straight, A., Belmont, A., Chi-Hong, D., Grossman, A. D., Wright, A. & Losick, R. (1997). Bipolar localisation of the replication origins of chromosomes in vegetative and sporulating cells of *B. subtilis*. *Cell* **88**, 667–674.
- Williams, D. R. & Thomas, C. M. (1992). Active partitioning of bacterial plasmids. *J Gen Microbiol* **138**, 1–16.
- Williams, D. R., Motallebi-Vershareh, M. & Thomas, C. M. (1993). Multifunctional repressor KorB can block transcription by preventing isomerization of RNA polymerase-promoter complexes. *Nucleic Acids Res* **21**, 1141–1148.
- Wilson, J. E., Sia, E. A. & Figurski, D. H. (1997). The *kilE* locus of promiscuous IncP α plasmid RK2 is required for stable maintenance in *Pseudomonas aeruginosa*. *J Bacteriol* **179**, 2339–2347.
- Wu, L. J., Lewis, P. J., Allmansberger, R., Hauser, P. M. & Errington, J. (1995). A conjugation-like mechanism for pre-spore chromosome partitioning during sporulation in *Bacillus subtilis*. *Genes Dev* **9**, 1316–1326.

Received 7 July 1998; revised 24 August 1998; accepted 3 September 1998.