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Review Article

Active partitioning of bacterial plasmids

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Introduction

Bacterial plasmids, by definition, are not essential for host cell survival except in specialized environments where plasmid-borne genes, like those for antibiotic resistance or degradative metabolic pathways, confer a selective advantage. Although some plasmids are lost quite rapidly when appropriate selective pressure is removed, others are found to be retained over many generations in the absence of any selection. This implies that many plasmids do not rely for their stability on constant selection for the genes they carry. This review concerns the mechanisms responsible for this stability and focusses on the active partition systems which may provide insights into mitotic processes in bacteria, if they exist (see Austin, 1988; Nordström & Austin, 1989, for previous reviews).

The inheritance of a plasmid on the basis of random segregation alone can be expressed by the relationship

$$P_0 = 2^{1-n}$$

where P_0 is the probability of a plasmid-free cell arising per division and n is the number of copies of the plasmid per cell at division. Plasmids isolated from nature fall into two broad categories: small, high copy number plasmids and large, low copy number plasmids. High copy number plasmids are generally inherited efficiently on the basis of random segregation alone. For example, a plasmid with 20 copies in the cell at division would be expected to give rise to a plasmid-free cell only once in over 5×10^5 divisions (ColE1 has a copy number of this order), a prediction reasonably well in line with the loss rate of high copy number plasmids which have been tested directly (Jones *et al.*, 1980; Summers, 1991). In the case of a cell with five plasmid copies at division, the probability of a plasmid-free cell arising increases to 1 in 16 per division. Yet large plasmids with this number of copies or lower, down to the limit of two per cell at division, can also be inherited stably. A mechanism or mechanisms must exist to produce the pattern of stable inheritance seen in such large plasmids, where plasmid loss is sometimes undetectable.

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Abbreviation: IHF, integrative host factor.

Plasmid stability mechanisms

The stable inheritance mechanisms so far detected fall into two groups. Those mechanisms which appear to result in a greater than random probability of each daughter cell receiving a copy of the plasmid are described in the next section. The other group consists of functions which do not improve upon the random segregation of plasmids but either ensure that it operates as efficiently as possible or minimize the effects of its failure.

Efficient control of plasmid replication is an important aid to stability for a variety of reasons. First, it sets the average copy number. For large plasmids, having a controlled low copy number restricts the metabolic burden the plasmid places on the cell so that the selective disadvantage caused by the plasmid is minimized. This reduces the rate at which plasmid-negative bacteria will take over the population due to their increased growth rate. Such a copy number effect was seen in the case of the unstable, pUB110-based vector pEB114 (Leonhardt & Alonso, 1988). This plasmid was stabilized by the *cop*-1 mutation that reduced the copy number (Leonhardt, 1990). In addition, plasmid copy number control circuits often allow stimulation of plasmid replication when copy number falls, thereby minimizing the number of cells which contain only one plasmid molecule prior to cell division. An artificial example of stabilization due to increase in copy number was seen for deletion derivatives of pAMβ1 in the heterologous host *Clostridium perfringens* (Allen & Blaschek, 1990), where a 100-fold increase in copy number stabilized the plasmid. Efficient replication of high copy number plasmids ensures that

random partitioning does not result in a high frequency of loss, which might be expected to occur if the distribution of the number of plasmid copies per cell showed a wide variation around the mean (Summers, 1991).

Plasmid multimer resolution functions maximize the number of independently segregating units at division. They are thought to be used by all plasmids which replicate via a theta intermediate. Plasmids which use rolling-circle replication have not yet been shown to possess this kind of activity. Indeed, such plasmids might not require a resolution system because the nicking process which terminates their replication may minimize the propagation of multimers.

Multimer resolution systems have been characterized on the F plasmid (the *rsfF* site and the product of the *D* gene) (O'Connor & Malamy, 1984; Lane *et al.*, 1986), the P1 plasmid prophage (the *loxP* site and Cre recombinase) (Austin *et al.*, 1981), and ColE1 (the *cer* site at which the host *xer*-encoded recombinase acts) (Summers & Sherratt, 1984; Stirling *et al.*, 1988). The *parA* gene of RP4 encodes a recombinase (Gerlitz *et al.*, 1990; Roberts *et al.*, 1990) and resolution functions are also found on CloDF13 (Hakkaart *et al.*, 1984) and the *Clostridium perfringens* plasmid pIP404 (Garnier *et al.*, 1987). The resolvases encoded by transposons on plasmids could also act in the same way (Grindley & Reed, 1985), e.g. Tn*1* on RK2. A similar mechanism is used by the 2 μ circle of yeast for resolution and to promote over-replication (Broach & Hicks, 1980; Futcher, 1986; Volkert & Broach, 1986).

Other plasmid-encoded functions act by killing or reducing the growth of plasmid-free cells which arise from a plasmid-bearing line. They are found on R1 where translation of the long-lived *hok* mRNA, which encodes a toxic product, is prevented by an unstable antisense *sok* RNA (Gerdes *et al.*, 1990*a*); an homologous system on F is termed *flm* (Loh *et al.*, 1988) or *stm* (Golub & Panzer, 1988) and several other examples are known (reviewed by Gerdes *et al.*, 1990*b*). The F plasmid *ccd* system operates in a similar way, though the toxic product and unstable 'antidote' are both proteins (Hiraga *et al.*, 1986; Tam & Kline, 1989; Bernard & Couturier, 1991). The *pem* killer system of R100/NR1 (Tsuchimoto *et al.*, 1988), known as *kid*/*kis* on R1 (Bravo *et al.*, 1987) also works this way. When its labile inhibitor decays in a plasmid-free cell, PemK kills the cell, primarily by inhibiting cell division (Tsuchimoto & Ohtsubo, 1989; compared with *ccd* by Ruiz-Eschevarría *et al.*, 1991). Plasmids which operate killer systems pay for their increased stability by a reduction in the growth rate of the host population, caused by the death of cells which have lost the plasmid (Jaffé *et al.*, 1985).

The *par* site of pSC101 (Tucker *et al.*, 1984), a site for the binding of DNA gyrase, is required for stable inheritance (Wahle & Kornberg, 1988). It might aid the separation of replicated plasmids from possible non-covalent aggregation or assist in the maintenance of a narrow variation in copy number by ensuring the correct topology of the plasmid. The latter could correlate with the involvement of integrative host factor (IHF) binding at the replication origin (Stenzel *et al.*, 1987; Biek & Cohen, 1989; Manen *et al.*, 1990). Another possibility is that a particular level of superhelicity is required for binding to some host factor or structure which leads to partition (Gustafsson *et al.*, 1983; Miller *et al.*, 1990).

Loci affecting segregational stability have also been found on a number of plasmids of Gram-positive origin. Studies with pBAA1, pLS11 and pUB110 have shown that the origin of minus strand DNA synthesis used during rolling-circle replication of these plasmids is also a stability determinant (Devine *et al.*, 1989; Chang *et al.*, 1987; Bron *et al.*, 1988). However, the two functions may be separable and both might involve membrane binding. Partition mechanisms may be present and seem to be required to account for the observed stability of low copy number Gram-positive plasmids like pPOD2000 and pBAA1 (Gleave *et al.*, 1990; Devine *et al.*, 1989). The *par* site on the corynebacterial plasmid pBY503, like pSC101 *par*, confers stability without encoding a polypeptide (Kurusu *et al.*, 1991), though interaction between host proteins and the site could effect partition.

True partition systems†

In contrast to the above mechanisms, true partition actively ensures that each daughter cell receives a plasmid copy at division. These systems *decrease* the chance of a plasmid-free cell arising. Table 1 summarizes the known active partition systems. To be classified as an active partition mechanism, a system must confer better than random segregational stability on the plasmid which carries it without affecting the copy number or reducing the host growth rate by killing plasmid-free segregants. The system must be able to stabilize heterologous replicons and should act as an incompatibility determinant. In most cases, two plasmids sharing

† *Note on nomenclature.* The term '*par* system' is often used to denote true partition. Although genes in some of these systems are called *par*, other names have been used (*sop*, *stb*), and functions designated *par* include genes or sites which do not appear to be part of active plasmid partition mechanisms. For example, pSC101 *par* is a DNA gyrase binding site. *parB* of IncFII plasmids is the *hok*/*sok* killer system and *parD* is *pemK*/*I*. The *flm* system of F was originally known as *parL* (Loh *et al.*, 1986). Some Gram-positive plasmid minus strand origins are called *par*. Also, numerous host mutations affecting chromosomal segregation were originally termed *par*, though many have been reclassified as the lesions have been identified (see below). These inconsistencies in nomenclature seem likely to persist.

Table 1. *Active partition systems*

Plasmid	*trans*-acting factors		*cis*-acting site	Associated incompatibility
sop/par family	A protein	B protein		
F	SopA	SopB	Downstream *sopC*	*incD*
P1	ParA	ParB	Downstream *parS*	*incB*
P7	ParA	ParB	Downstream *parS*	*incB*
pTAR†	ParA	-	Upstream 7 bp repeats	*incP*
RK2	IncC	KorB		
pTiB6S3*†	RepA	RepB		
pRiA4b*†	RepA	RepB		
pCHL1*‡	(ORF5)	-		
pSS120*§	-	InvE		
IncFII family				
R1	*parA* products (36 kDa and 13 kDa‖)		P_{parA}	*incA*
NR1/R100	StbA	StbB	P_{AB}	None
Other systems				
RK2 *mrs/par*	ParA (resolvase), ParB, ParC, ParD		*res/par* site	Weak
Salmonella virulence plasmid	*par* products		*par*	*incR*

* Systems not yet demonstrated to be involved in partition.
† *Agrobacterium* plasmids.
‡ *Chlamydia trachomatis* plasmid.
§ *Shigella* virulence plasmid.
‖ The original paper gives a smaller size due to a sequencing error (Gerdes & Molin, 1986).

the same active partition system, or more specifically the *cis*-acting site associated with it, will interfere with each other's ability to be partitioned correctly, and thus show incompatibility towards each other (Novick, 1987; Austin & Nordström, 1990). That is, it can be demonstrated that a population of cells initially containing both plasmids will tend to segregate individuals with one or other of the plasmids, but not both. By analogy with the *cis*-acting sequences required for the segregation of eukaryotic chromosomes, the *cis*-acting plasmid sites necessary for partition are termed centromere-like sequences. The incompatibility determined by these sites is the primary evidence that plasmids exist in a free pool in the cell, from which they pair, via the centromere-like sequence, and are then separated to either side of the cell division plane. Incompatibility arises as a result of mixed pairing between plasmids with the same *cis*-acting site. The distribution of the cell's plasmid complement between the daughter cells constitutes the key requirement for a partition system able to maintain the stability of a single copy plasmid.

A partition/resolution system

The *mrs/par* locus situated between 33 and 36 kb on the RK2/RP4 map appears to be a complex hybrid of a multimer resolution system and a true partition system (Gerlitz *et al.*, 1990; Roberts *et al.*, 1990) (Fig. 1). It resides on a 2·2 kb region between the *fiwA* gene and IS*21*. A 24 kDa resolvase related to those of Tn*3* and Tn*1721* is encoded by the *parA* gene, the last of three cistrons transcribed in one direction from a bidirectional promoter. The promoter region is also the site required *in cis* for multimer resolution by the *trans*-acting *parA* product. Similar to Tn*3* *res*, the site was mapped to a 140 bp segment which includes two direct repeats of six and seven bases and two inverted repeats of six and eight bases. But resolution alone cannot account for the high plasmid stability conferred by the whole locus. A second function, which did not act by killing plasmid-free segregants, behaved like a true partition mechanism. Absence of the *parD* gene product (9 kDa) (transcribed in the other direction from the same promoter) was associated with reduction, but not complete absence, of stabilization. The *parB* product (18 kDa) was absolutely required and the *parC* product (10 kDa) might also be involved. The same region was required *in cis* for resolution and partition. This system, which functioned in several hosts, showed no homology to other partition loci and displayed only weak incompatibility, maybe as a result of close linkage between replication and pairing so

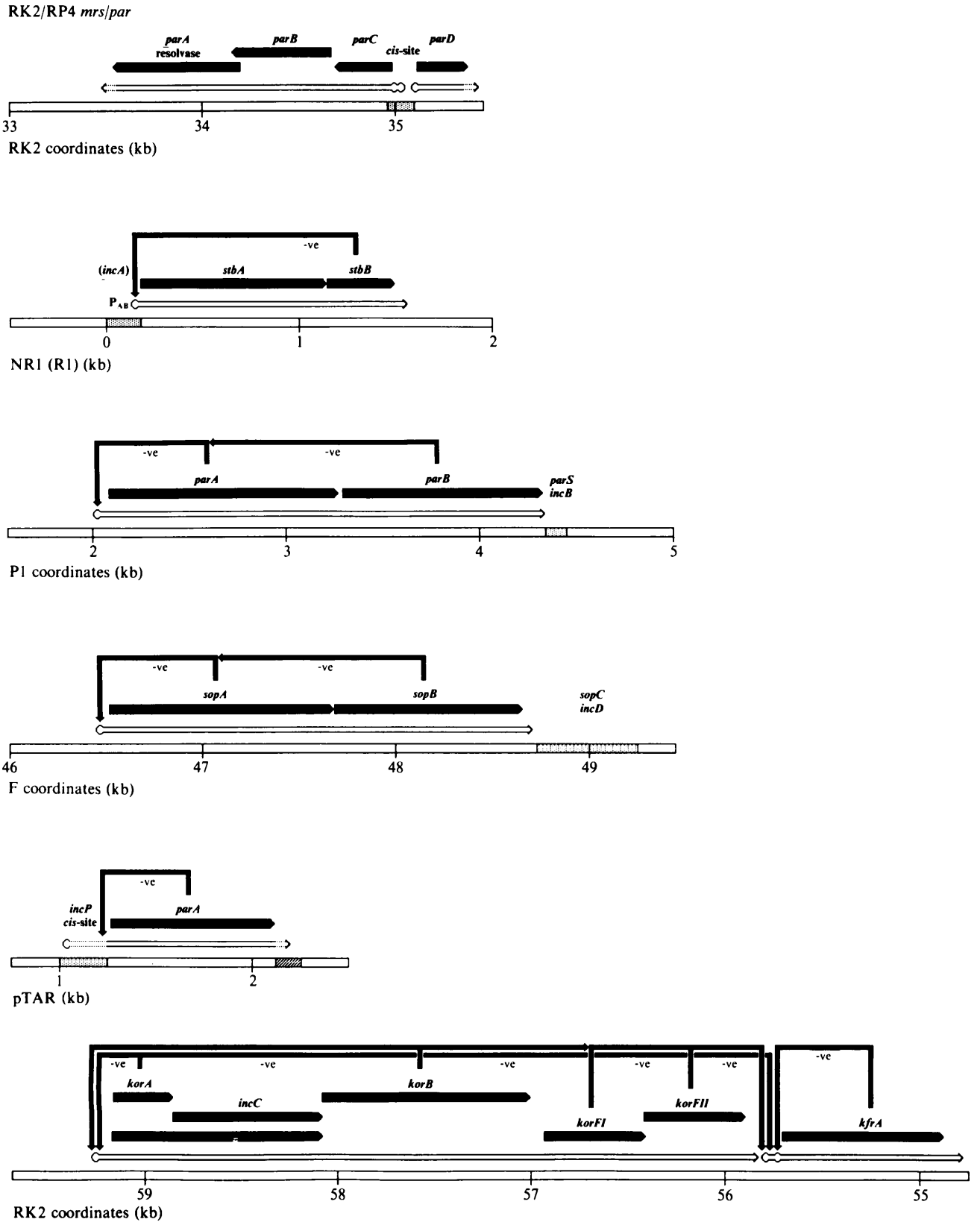

Fig. 1. Structural organization and autoregulation of some of the better characterized active partition systems (see text). Symbols are: open arrows, RNA transcripts; thick solid arrows, protein-coding regions; thin solid arrows, transcriptional control circuits; stippled boxes, centromere-like sequences; hatched box in pTAR, additional essential region.

the plasmids do not enter a free pool from which they are paired.

Partition-only mechanisms

The partition-only mechanisms studied thus far fall into two main families. Those on IncFII plasmids and others which have homology to *sop* of F and *par* of P1.

The partition genes on *Salmonella* virulence plasmids may constitute a third family, or could fall into one of the above when sufficiently characterized. They do not act by multimer resolution or plasmid-free segregant killing and do not increase copy number, but were able to stabilize other replicons efficiently. The two systems investigated encode two proteins, the genes for which appear to be transcribed towards each other. One (Cerin & Hackett, 1989) or both (Tinge & Curtiss, 1990) of these proteins are required for partition. The loci also have incompatibility determinants, one of which is coincident with the site required *in cis* for partition. No similarity to other partition systems was detected by hybridization though there is clearly a functional similarity.

The IncFII partition family

The IncFII plasmids have an active partition system known as *parA* on R1 or *stb* on NR1 (R100) (Fig. 1). The partition locus was found on a region of 1·5 to 1·7 kb which was able to stabilize an unstable mini-F replicon. Though the organization of the system is superficially similar to the *sop/par* family (Tabuchi *et al.*, 1988) (Fig. 1), DNA and protein sequence comparisons reveal no significant homologies. Polypeptides of 36 kDa and 13 kDa, the products of the *stbA* and *stbB* genes respectively, are both essential for the partition phenotype, and in addition the *stbB* gene product is believed to auto-repress its own synthesis by interaction with the promoter P_{AB} region (Min *et al.*, 1988). P_{AB} was also found to be the *cis*-acting site required for partition (Gerdes & Molin, 1986; Tabuchi *et al.*, 1988). Hence, the location of the partition site differs from that of the *sop/par* family. While the locus in R1 shows incompatibility (*incA*) associated with the promoter region, the same region from NR1 lacks this property.

The *sop/par* family

The most extensively studied active partition systems are *sop* of F and *par* of P1, which are located in identical positions relative to the F and P1 primary replicons and have a similar genetic organization (Fig. 1). Each encode two *trans*-acting polypeptides A and B, and a *cis*-acting centromere-like site, all three of which are essential for partition (Ogura & Hiraga, 1983; Mori *et al.*, 1986; Austin & Abeles, 1982*a*, *b*; Friedman & Austin, 1988; Martin *et al.*, 1987). The A/B operons are auto-regulated, with the A protein acting as the primary repressor, helped by the B component (Mori *et al.*, 1989; Friedman & Austin, 1988; Abeles *et al.*, 1989). It is the B protein which binds directly at the centromere. *sop* resides on a 3 kb region of F and was found to be necessary and sufficient for stable maintenance of the plasmid, which has a copy number of one to two per cell (Austin & Wierzbicki, 1983, Mori *et al.*, 1986). The same region was able to stabilize heterologous replicons without increasing their copy number. *par* is present on a 2·1 kb segment of P1 (Abeles *et al.*, 1985) and is highly efficient giving a <1 in 10^5 per generation loss of the prophage, which is present as a unit copy plasmid. As with *sop*, the *par* system stabilized other replicons (Austin *et al.*, 1986). *sop* and *par* have both been completely sequenced and substantial biochemical investigations have been carried out. These will be described in more detail after consideration of other less well characterized members of the family. Comparisons between the amino acid sequences of the A proteins and B proteins encoded by the *sop/par* family of genes are given in Figs 2 and 3 respectively.

A partition region closely related to that on P1 was found on the P7 prophage. ParA and ParB proteins of 44 kDa and 37 kDa were encoded but the proteins for P1 and P7 were not able to cross-complement. The *parS* sequence of P7 was also distinct though similar to its P1 analogue (Ludtke *et al.*, 1989). This system shows how even extensively homologous systems (Figs 2 and 3) have evolved different specificities to ensure their own survival.

The partition system present on a 1·2 kb fragment of the *Agrobacterium tumefaciens* plasmid pTAR is interesting both for its similarities to and differences from the above systems (Fig. 1). It appears to encode only one 23·5 kDa protein, ParA, that shows homology to the A proteins of the family (Fig. 2). As well as *parA*, two regions outside the open reading frame were required for partition, an upstream region consisting of an array of 7 bp repeats at 10 bp intervals and 125 bp downstream, of unknown purpose. The upstream region is an incompatibility determinant (*incP*), the *cis*-acting partition site, and the promoter region at which ParA binds to autoregulate its own transcription (Gallie & Kado, 1987) (Fig. 1).

We have also discovered that two other *Agrobacterium* plasmids encode polypeptides with A and B protein homologies. These are the *repA* and *repB* gene products

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          1                                                   49
Con       ......... ........a. .e.a...... .....e.... ....+..r..
P1ParA    ....MSDSS QLHKVAQRAN RMLNVLTEQV QLQKDELHAN EFYQVYAKAA
P7ParA    ...MKRDYG GVGTIALRAS ALLKAMSQDI EDQRKEFNQT EYYQTFTRNA
FSopA     .....MKLM ETLNQCINAG HEMTKAIAIA QFNDDSPEAR KITRRWRIGE
RK2IncC   ......... .....MGVIH EETAYRKPVP GGDPGAGSGD AADHRDSAGR
TiRepA    VSKAAAISR NDRPSVDVTI GEHAEQLSSQ LQAMSEALFP PTSHKTLRKF
RiRepA    MAKSVLKAA PVVVGLTALM ERHADALSSQ LQAHHLKVFP PHSEKGIRTF
pTARPar   ......... ......... ......... ......... .........
C.t Orf5  ......... ......... ......... ......... .........
E.c MinD  ......... ......... ......... ......... .........

          50                                                  99
Con       .a+l...... ..-.a.+.m. ..g....++. ...gr+.a.. .qi.......
P1ParA    LAKLPLLTRA NVDYAVSEME EKGYVFDKRP AGSSMKYAMS IQNIIDIYEH
P7ParA    VAKLPKLSRR IVEQAIKEME DDGYQFNKKP QGVNEQYALT IQNVIDIYAH
FSopA     AADLVGVSSQ AIRDAEKAGR LPHPDMEIRG RVEQRVGYTI EQINHMRCDV
RK2IncC   LSRWEATGDV RNVAGTDQGR SVASGASRVG RVRGQELARG VRAGNGGSAG
TiRepA    TSGEAARLMK ISDSTLRKMT LAGEGPQPEL ASNGRRFYTL GQINEIRGML
RiRepA    GPSEASKLLG VGESYLRQTA SEMPELNVSM SPGGRRMFSI EDIHVIRKYM
pTARPar   ......... ......... ......... ......... .........
C.t Orf5  ......... ......... ......... ......... .........
E.c MinD  ......... ......... ......... ......... .........

          100                                                 149
Con       ....+.r-.. ...ph+rG.e .m.vivv.n. KGGvgKttts vhla......
P1ParA    RGVPKYRDRY SEA..... ..YVIFISNL KGGVSKTVST VSLAHAMRAH
P7ParA    RKIPKYRDIH KSP..... ..YVIFVVNL KGGVSKTVST VTLAHALRVH
FSopA     FGTRLRRAED VFP..... ..PVIGVAAH KGGVYKTSVS VHLA......
RK2IncC   TSGVHRPEVG SGRQEKTGNQ TMKTLVTANQ KGGVGKTSTL VHLA......
TiRepA    ARSTRGRESI EFVPHRRGSE HLQVIAVTNF KGGSGKTTTS AHLA......
RiRepA    DQVGRGN..R RYLPHRRGGE QLQVISVMNF KGGSGKTTTA AHLA......
pTARPar   ......... ......... .MPVVVVASS KGGAGKSTTA VVLG......
C.t Orf5  ......... ......... .MHTLVFCSF KGGTGKTTLS LNVG......
E.c MinD  ......... ......... MARIIVVTSG KGGVGKTTSS AAIA......

          150                                                 199
Con       q-la..+glr vl.id.ldpq .sls..lg.. ...-...... ..........
P1ParA    PHLLM.EDLR ILVID.LDPQ SSATMFLSHK HSIGIVNATS AQAMLQNVSR
P7ParA    QDLLR.HDLR ILVID.LDPQ ASSTMFLDHT HSIGSILETA AQAMLNDLDA
FSopA     QDLAL.KGLR VLLVEGNDPQ GTASMYHGWV PDLHIHAEDT LLP..FYLGE
RK2IncC   FDFFE.RGLR VAVID.LDPQ GNASYTLKDF ATGLHASKLF GAVPAGGWTE
TiRepA    QYLAL.QGYR VLAVD.LDPQ ASLSALLGVL PETDVGANET LYAAIRYDDT
RiRepA    QYLAM.RGYR VLAID.LDPQ ASLSALFGSQ PETDVGPNET LYGAIRYDDE
pTARPar   TELAH.KGVP VTMLD.CDPN RSLTIWANAG EVPENITALS DVTESS....
C.t Orf5  CNLAQFLGKK VLLAD.LDPQ SNLSSGLGAS VRSDQKGLHD IVYTSNDLKS
E.c MinD  TGLAQ.KGKK TVVIDFDIGL RNLDLIMGD. ..CERRVVYD FVNVIQGDAT

          200                                                 249
Con       .......i+p t..pgld.iP as.-...... .......-.. ..........
P1ParA    EELLEEFIVP SVVPGVDVMP ASIDDAFIAS DWRELCNEHL PGQNIHAVLK
P7ParA    ETLRKEVIRP TIVPGVDVIP ASIDDGFVAS QWKELVEEHL PGQNQYEILR
FSopA     KDDVTYAIKP TCWPGLDIIP SCLALHRIET ELMGKFDEGK LPTD.PHLML
RK2IncC   TAPAAGDGQA ARLALIESNP VLANAERLSL DDAREL.... .........FG
TiRepA    RRPLRDVIRP TYFDGLHLVP GNLELMEFEH TTPKALSDRG GRDGLFFTRV
RiRepA    QVAIERVVRG TYIPDLHLIP GNLELMEFEH DTPRALMNRK EGDTLFYGRI
pTARPar   ......... ......... ......... ......... .........I
C.t Orf5  IICETKKD.. ....SVDLIP ASFSSEQFRE LDIHRGPS.. ......NNLK
E.c MinD  LNQALIKDKR T..ENLYILP ASQTRDKDAL TREGVA.... ..........
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          250                                                        299
Con       ...id..a.d ydviviD.pp .lg..tl.al .aad.ll.p. .pe..d..s.
P1ParA    ENVIDKLKSD YDFILVDSGP HLDAFLKNAL ASANILFTPL PPATVDFHSS
P7ParA    RNIIDRVADD YDFIFIDTGP HLDPFLLNGL AASDLLLTPT PPATVDFHST
FSopA     RLAIETVAHD YDVIVIDSAP NLGIGTINVV CAADVLIVPT PAELFDYTSA
RK2IncC   ANIKALANQG FDVCLIDTAP TLGVGLAAAL FAADYVLSPI ELEATSIQGI
TiRepA    AQAFDEVGDD YDVVVIDCPP QLGFLTLSGL CAATAMVVTV HPQMLDIASM
RiRepA    SQVIEDIADN YDVVVIDCPP QLGYLTLSAL TAATSILVTV HPQMLDVMSM
pTARPar   VKTIKQHDVD GAVVIVDLEG VASRMVSRAI SQADLVLIPM RPKALDATIG
C.t Orf5  LFLNEYCAPF YDICIIDTPP SLGGLTKEAF VAGDKLIACL TPEPFSILGL
E.c MinD  KVLDDLKAMD FEFIVCDSPA GIETGALMAL YFADEAIITT NPEVSSVRDS

          300                                                        349
Con       .k.l..l.-l l+..e-.g.. .......... s+........ .s....+...
P1ParA    LKYVARLPEL VKLISDEGCE CQLATNIGFM SKLSNKADHK YCHSLAKEVF
P7ParA    LKYLTRLPEM LEQLEEEGVE PRLSASIGFM SKMTGKRDHE TSHSLAREVY
FSopA     LQFFDMLRDL LKNVDLKGFE PDVRILLTKY SNSNGSQSPW MEEQIRDAWG
RK2IncC   KKMVTTIANV RQKNAKLQFL GMVPSKVDAR NPRHARHQAE LLAAYPKMMI
TiRepA    SQFLLMTRDL LGVVREAGGN LQYDFIRYLL TRYEPQDAPQ TKVAALLRNM
RiRepA    NQFLAMTSNL LREIENAGAK FKFNWMRYLI TRFEPSDGPQ NQMVGYLRSI
pTARPar   AQSLQLIAEE EEAIDRKIAH AVVFTMVSPA IRSHEYTGIK ASLIENGVEI
C.t Orf5  QKIREFLSSV GKPEEEHILG IALSFWDDRN STNQMYIDII ESIYKNKLFS
E.c MinD  DRILGILASK SRRAENGEEP IKEHLLLTRY NPGRVSRGDM LSMEDVLEIL

          350                                                        399
Con       .......... .......... .......a.. ......a-.. +.........
P1ParA    GGDMLDVFLP RLDGFERCGE SFDTVISANP ATYVGSADAL KNARIAAEDF
P1ParA    ASNILDSSLP RLDGFERCGE SFDTVISANP QSYPGSAEAL KKARTEAERF
FSopA     SMVLKNVVRE TDEVGKGQIR MRTVFEQAID QRSSTGAWRN ALSIWEPVCN
RK2IncC   PATVGLRSSI ADALASGVPV WKIKKTAARK ASKEVRALAD YVFTKMEISQ
TiRepA    FEDHVLTNPM VKSAAVSDAG LTKQTLYEIG RENLTRSTYD RAMESLDAVN
RiRepA    FGENVLNFPM LKTTAVSDAG LTNQTLFEVE RGLFTRSTYD RALEAMNAVN
pTARPar   IEPPLVERTA YSALFQFGGN LHSMKSKQGN MAAAIENAEA FAMAIFKKLT
C.t Orf5  TKIRRDISLS RSLLKEDSVA NVYPNSRAAE DILKLTHEIA NILHIEYERD
E.c MinD  RIKLVGVIPE DQSVLRASNQ GEPVILDINA DAGKAYADTV ERLLGEERPF

          400          416
Con       ...-..+k.f w.r...
P1ParA    AKAVFDRIEF IRSN
P7ParA    TKAVFDRIEF VRGEAA
FSopA     EIFDRLIKPR WEIR
TiRepA    AEIEALIKAA WGRA
RiRepA    DEIETLIKKA WGRPT
pTARPar   EALR
C.t Orf5  YSQRTT
E.c MinD  RFIEEEKKGF LKRLFGG
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Fig. 2. Alignment of SopA, ParA and related proteins. Gaps have been introduced to optimize alignment of obvious motifs but the number of gaps in other regions has been kept to a minimum. The consensus line (Con) shows absolutely conserved amino acid residues as upper-case and consensus (> 2 out of 6 or > 3 out of 9) positions in lower-case. Conservation of either K or R is shown as + ; D or E is shown as −.

of pTiB6S3 and pRiA4b (Figs 2 and 3). Studies on these genes show that their disruption caused plasmid instability (Tabata *et al.*, 1989; Nishiguchi *et al.*, 1987). While it was reported that the instability was due to a decrease in plasmid copy number and therefore was unlikely to be the result of inactivation of partition functions, the organization of this region is not sufficiently well understood to determine the role of these genes. Therefore the instability could be a result of their involvement in partitioning.

The proteins forming the ParA group above also have homology to the IncC protein of broad host range

```
          0                                                  49
Con       .......... .......... .......... .......... ..........
ConA      .......... .......... .......... .......... ..........
InvE      .......... .......... .......... .......... MVDLCNDLLS
P1ParB    .......... .......... ....MSKKNR PTIGRTLNPS ILSGFDSSSA
P7ParB    .......... .......... ........MK KIVSRGRVLG KNSSEFARML
FSopB     .......... ..MKRAPVIP KHTLNTQPVE DTSLSTPAAP MVDSLIARVG
RK2KorB   .......... .......... .MTAAQAKTT KKNTAAAAQE AAGAAQPSGL
TiRepB    MAERQTEET  FIMSRKDAID SLFLKKQPST ERPSIDKSAV RVRTGAISAM
RiRepB    .......... .......... .......... ...MPLLGVT RKERDPATKL
ConB      .......... .......... .......... .......... r......s.l

          50                                                 99
Con       .......... .......... .......... .......... ..d.......
ConA      ........f. l...r...F. ..v....... ..tfV....N .RDQ..LT..
InvE      IKEGQKKEFT LHSGNKVSFI KAKIPHKRIQ DLTFVNQKTN VRDQESLTEE
P1ParB    SGDRVEQVFK LSTGRQATFI EEVIPPNQVE SDTFVDQHNN GRDQASLTPK
P7ParB    EGSEGTKTFT LKSGRQAKFL LTVVLSGEII SRTFVDPAVN GRDQSLLTPE
FSopB     VMARGNAITL PVCGRDVKFT LEVLRGDSVE KTSRVW.SGN ERDQELLTED
RK2KorB   GLDSIGDLSS LLDAPAASQG GSGPIELDLD LIDEDPHQPR TADNPGFSPE
TiRepB    GSSLQVMAEG AKAASRLQDQ LAAGETVVSL DPSMIDGSQI TDRLPVDVDP
RiRepB    TANIGNALRE QNDRLSRAEE IERRLAEGQA VIELDASSIE PSFVQDRMRG
ConB      .......l.. ..da....-. .......... ..-.d..... ....p.....

          100                                                149
Con       s...i..tik ..gq..P.i. .R......gr ..i..G.RR. ..........
ConA      s..di..tik ..qQf.PaiG .R......g+ IEilDGsRRR a.ai......
InvE      SLADIIKTIK L.QQFFPVIG .RE...IDGR IEILDGTRRR ASAIYAGADL
P1ParB    SLKSIRSTIK H.QQFYPAIG VRR...ATGK IEILDGSRRR ASAILENVGL
P7ParB    SVSDISRTIK L.QQFFPAIG .R...MVGER IEVLDGSRRR AACIFNETKF
FSopB     ALDDLIPSFL LTGQQTPAFG .RR...VSGV IEIADGSRRR KAAALTESDY
RK2KorB   SIAEIGATIK ERGVKSP.IS VRENQEQPGR YIINHGARRY RGSKWAGKK.
TiRepB    KFDQLEASIS QDGQQVP.IL VRPHPETTGR YQIVYGRRRL RAAANLRREV
RiRepB    DIDGLLTSIR EQGQQVP.IL VRPHPSQPGR YQVAFGHRRL RAVSELGLPV
ConB      .id.L.asI+ e.GqqvP.Il VRphpeqpGR Yqi..G.RRl Ra...lg+.v

          150                                                199
Con       ........d. ....a.q... ..q...l... .re....l.. ..........
ConA      ........-i s..-a..La. D.q.Ta..h. .re.g.rl.. .....ms..-
InvE      EVLYSKE.YI STLDARKLAN DIQ.TAKEHS IRELGIGLNF LKVSGMSYKD
P1ParB    R.VLVTDQEI SVQEAQNLAK DVQ.TALQHS IREIGLRLMR MKNDGMSQKD
P7ParB    E.ILVTKDEI SLADARQLAI DIQ.TAREHT LRELGKRFEV MYGKSMTKEE
FSopB     R.LVGELDDE QMAALSRLGN DYRPTSAYER GQRYASRLQN EFAGNIS...
RK2KorB   ..SIPAFIDN DYNEADQVIE NLQRNELTP. .REIADFIGR ELAKGKKKGD
TiRepB    SAIVRNLTDR EL.VVAQGRE NLDRADLSF. .IEKAFFALR LEEAGFDRPT
RiRepB    RAVVRELTDE QV.VVAQGQE NNEREDLTF. .IEKARFAHR LN.RQFSREI
ConB      .a.vr.ltD. -..vvaQg.E Nl.R.dLtf. .iEkA.Fa.R l...gf.+..

          200                                                249
Con       .iA.....s. a..t....a. ..P.el.... ..as...... ...l......
ConA      .iA..En.S. akvTRa.qaA ..P.e..alF p..seL...d y..L......
InvE      .IAKKENLSR AKVTRAFQAA SVPQEIISLF PIASELNFND YKILFNY.YK
P1ParB    .IAAKEGLSQ AKVTRALQAA SAPEELVALF PVQSELTFSD YKTLCAV.GD
P7ParB    .IARAENISK AKVTRAFQAA AVPDEMIAVF PVASDLALPD YQLLLQI.SE
FSopB     ALADAENIST KIITRCINTA KLPKSVVALF SHPGELSARS GDALQKAFTD
RK2KorB   .IAKEIGKSP AFITQHVTL. ...LDLPEKI ADAFNTGRVR DVTVVNELVT
TiRepB    VIAALSTDKA DLSRYIAVAK AIPLDLATQI GPASKAGRSR WVALAEGL..
RiRepB    VIAAMSIDKS NLSKMLLLVD ALPSELTDAI GAAPGVGRPS WQQLAELI.E
ConB      vIAa.s.dk. .ls....... a.pldL...I g.A...GR.r mw.lae.l..
```

```
          250                                                       299
Con       .......... ..l....... .......... ....lk.... ..........
ConA      .....k.... .......... I.-....... .....K..il ....k.....
InvE      GLEK.ANESL SSTLPILKEE IKDLDTNLPP ..DIYKKEIL NIIKKSKNRK
P1ParB    EMGN.KNLEF DQLIQNISPE INDILSIEEM AEDEVKNKIL RLITKEASLL
P7ParB    DANA.KSVPI EELVDTVRER IAET.....E GAKEIKAKIL AI.FKAESKS
FSopB     KEELLKQQAS NLHEQKKAGV IFEAEEVITL LTSVLKTSSA SRTSLSSRHQ
RK2KorB   AFKKRPEEVE AWLDDDTQEI TRGTVKL... LREFLDEKGR DPNTVDAFNG
TiRepB    ANSKAADAIG GVLESELFRT SDSDTRFSLV LKAVLKPAVK PSKSVKAWSM
RiRepB    KVSSPADVAK YAMSEEVQAL PSAE.RFKAV I.ASLKPSRV ARGLPEVMAT
ConB      ..sk.ad... ..l-.e.q.. .....rf..v l+a.Lkp... ....v.a...

          300                                                       349
Con       .......... .......... ......k... .........k ..........
ConA      ......v.s. .......... .r...+.... .........+ ..........
InvE      QNPSLKVDSL FISKDKRTYI KRKENKTNRT LIFTLSKINK TVQREIDEAI
P1ParB    TDKGSKDKSV VTELWK.FED KDRFARKRVK GRAFSYEFNR LSKRVTGRTR
P7ParB    .LKPAPVKSV VVEKLRDFSD RRQYARKKSD PKKRVVAYEF SRLPSEVQTE
FSopB     FAPGATVLYK GDKMVLNLDR SRVPTECIEK IEAILKELEK PAP
RK2KorB   QTDAERDAEA GDGQDGEDGD QDGKDAKEKG AKEPDPDKLK K.AIVQVEHD
TiRepB    P......... .......... .......... .......KGK KAAVIQKD.T
RiRepB    P......... .......... .......... .......DGT RIAQVTQS.K
ConB      p......... .......... .......... .......kgk +.A.vq....

          350                                                       399
Con       .......... .
ConA      .d........ .
InvE      RDIISRHLSS S
P1ParB    QDDCGISLER ASIKSRSLKL SPFKFHY
P7ParB    IDEAIKKIIG KMSAGE
RK2KorB   ERPARLILN. RRPPAEGYAW LKYEDDGQEF EANLADVKLV ALIEG
TiRepB    GRTA.LIFDE KLVPAFG... ........EF VADQLDRLYA QFIETTDGEK
RiRepB    AKLE.ITIDR KATPDFA... ........TF VLDHVPALYQ AYHAENQRKR
ConB      .+.a.li.d. +..Pafg... ........eF vad..d.ly. a.ie......

          400
TiRepB    LDQ
RiRepB    GE
ConB      ..
```

Fig. 3. Alignment of SopB, ParB and related proteins. Gaps have been introduced to optimize alignment of obvious motifs but the number of gaps in other regions has been kept to a minimum. Three consensus lines are shown: ConA is the consensus for the ParB subfamily (InvE; P1ParB; P7ParB; SopB) with upper-case being completely conserved positions and lower-case showing conservation in 3 out of 4 members; ConB is the consensus for the KorB subfamily (KorB; TiRepB; RiRepB), upper-case again represents absolute identity while lower-case represents conservation in 2 out of 3 members; Con is the consensus for the whole group, upper case being complete identity, lower case being >3 out of 7 with at least one from each subfamily.

plasmid RK2 (Thomas & Smith, 1986; Thomas, 1986) (Fig. 2), while the RK2-encoded KorB protein, which is a multifunctional regulator repressing a number of RK2 operons by interactions at several specific binding sites (Shingler & Thomas, 1984; Smith *et al.*, 1984; Schreiner *et al.*, 1985; Theophilus *et al.*, 1985; Bechhofer *et al.*, 1986; Theophilus & Thomas, 1987; Young *et al.*, 1987; Jagura-Burdzy *et al.*, 1991) (Fig.1), has homology to members of the ParB group (Fig. 3). Disruption of *incC* in plasmids which bore the *korA incC korB korF* and *kfrA* operons of RK2 resulted in destabilization of the plasmids. The same regions were able to stabilize a ColE1-derived replicon at low copy number (Motallebi-Veshareh *et al.*, 1990). Similarly the *korAB* region has been shown to stabilize mini-IncP plasmids (Schmidhauser *et al.*, 1989). Therefore, IncC and KorB are likely to be components of a partition system. How much the other RK2 genes encoded by this region are also involved in partition is unknown. However, the *kfrA* product has an unusual structure which may play a role in the process.

It is a site-specific DNA binding protein whose operator overlaps the *kfrA* promoter. It seems to consist of extensive α-helix, much of which is found in an extended domain which appears to multimerize by means of a heptad-repeat segment resulting in a coiled-coil. It has low but significant homology to eukaryotic cytoskeletal proteins which have such coiled-coil domains (Thomas *et al.*, 1990; G. Jagura-Burdzy & C. M. Thomas, unpublished). It also has similarity to the REP1 (or B) protein involved in yeast 2 μ circle partitioning, which has been found to be associated with the karyoskeleton and tubulin in yeast cells (Wu *et al.* 1987; A. Cashmore, personal communication). That the *kfrA* product may play a structural role, linking a plasmid complex to a host factor which provides the driving force for partition, seems plausible. The two additional polypeptides encoded by the *korABF* operon, KorFI and KorFII are also able to bind DNA as they act as transcriptional repressors. They appear to be histone-like and include hydrophobic domains which could provide a membrane binding component of the partition system (Jagura-Burdzy *et al.*, 1991). Which of the RK2 partition systems, *mrs/par* or *incC/korB*, is of greater importance is unknown and may vary from species to species.

The A proteins were also found (Motallebi-Veshareh *et al.*, 1990) to have homology with the putative product of a *Chlamydia trachomatis* cryptic plasmid open reading frame (ORF5) (Commanducci *et al.*, 1988) and the *E. coli* cell division inhibitor MinD (de Boer *et al.*, 1989) (Fig. 2). The B proteins have homology to InvE, produced by the *Shigella* virulence plasmid (Watanabe *et al.*, 1990). This, like KorB, is a transcriptional regulator but is not known to participate in any partition apparatus (Fig.3).

Properties of the *sop/par* components

The A proteins

SopA (43·7 kDa) has been purified and found to bind to its promoter to effect repression in a manner which is enhanced by SopB (Mori *et al.*, 1989) (Fig.1). ParA (44 kDa) behaves similarly (Friedman & Austin, 1988). The analysis of the A protein sequences revealed that they had homology to each other and to the consensus for type 1 ATP-binding motifs (Motallebi-Veshareh *et al.*, 1990). It is now known that ParA of P1 is an ATPase and that the activity is stimulated by ParB and further by DNA. Similarly, the ParA of P7 is an ATPase. Stimulation of the ATPase activity by P7 ParB occurs but not by P1 ParB, indicating a specificity in the ParA : ParB interactions (Davis *et al.*, 1991). The energy for part of the partition mechanism may be derived from ATP hydrolysis by the ParA proteins.

The B proteins

Purified SopB (35·4 kDa) and ParB (38 kDa) proteins both bind to their respective centromere-like sequences (Watanabe *et al.*, 1989; Mori *et al.*, 1989; Davis & Austin, 1988), SopB to 7 bp inverted repeats within the 43 bp direct repeats of *sopC*, and ParB to 35 bp of *parS*. Overproduction of either protein led to an incompatibility phenotype (*incG*), presumably due to disruption of the partition mechanism (Kusukawa *et al.*, 1987; Funnell, 1988*a*). The SopB protein was also found to sediment with host membrane fraction in the presence of Mg^{2+} ions (Watanabe *et al.*, 1989), so the B proteins may provide the link from the plasmid to a membrane-bound host site involved in partition.

The cis-acting sites

sopC of F consists of 12 direct repeats of a 43 bp sequence downstream of the *sopB* gene (Helsberg & Eichenlaub, 1986; Mori *et al.*, 1986). While all the repeats were required for the *incD* phenotype to be expressed by this region, only one was required for functional partition (Lane *et al.*, 1987). *parS* of P1 is located in a similar relative position as for *sopC* but its structure is quite different. It consists of an AT-rich region including a 20 bp inverted repeat. Another AT-rich inverted repeat was found at the promoter region. A discovery of great interest was that the incompatibility specificity of *parS* could be changed by deletion in experiments where the essential proteins were provided *in trans*. Thus plasmids containing the minimal site ($IncB^d$), consisting of 22 bp including half of a 13 bp palindrome, showed incompatibility towards other plasmids with the same site, but not to plasmids with the full wild-type 84 bp $IncB^+$ determinant. The full site included multiple binding sites for ParB and a site for the host protein IHF (Funnell, 1988*b*; Davis *et al.*, 1990; Martin *et al.*, 1991). The change in specificity is proposed to be due to an alteration in the conformation of the site as the loss of the IHF and ParB binding sites changes its pattern of folding, such that the mechanism for plasmid pairing fails to recognize the complete and partial sites as the same.

Involvement of host genes in plasmid partition

Information concerning which host-encoded products are required for plasmid partition is quite limited. IHF is known to be involved in the folding of the P1 centromere-like sequence *parS* (Funnell, 1988*b*). Mini-P1 plasmids show slight instability in IHF mutants, but mini-F stability is unaffected. By contrast, the small histone-like protein of *E. coli*, HU, encoded by the *hupA*

and *hupB* genes, was required for the establishment of mini-F and mini-P1 plasmids. Mini-F replication was only partially inhibited when the *hupB* gene alone was defective, whereas *oriC* plasmids were unaffected (Ogura *et al.*, 1990*a*). Mutations in DNA gyrase which affect DNA supercoiling were found to disrupt partitioning of mini-F plasmids. This may be due to aberrant regulation of the *sopB* gene as an increased IncG incompatibility, consistent with overproduction of *sopB* (Kusukawa, *et al.*, 1987), was seen (Ogura *et al.*, 1990*b*). A similar effect is seen in nucleoid segregation where the *minB* mutation results in altered supercoiling and, as with DNA gyrase mutants, chromosome partitioning is defective (Mulder *et al.*, 1990). Mutants in the *ugpA* gene of *E. coli* also destabilized mini-F plasmids (Ezaki *et al.*, 1990). The product is one of the genes in an operon responsible for glycerol phosphate uptake. Though its exact function is unknown, its requirement by F for stability could be at the level of partition. Since the transport system spans the host membrane, it could provide a site for attachment of the plasmid or the partition complex to the host cell.

Partition models

By definition, a true active partitioning apparatus must increase the probability of each daughter cell receiving a copy of a plasmid DNA molecule to a value above that expected from random segregation alone. Ultimately, the probability must approach unity to be able to stabilize low copy number plasmids effectively.

If we consider a unit copy plasmid present at two copies in a cell about to divide, then active partition must at least temporarily result in one copy of the plasmid being bound to a site on either side of the cell division plane. This could depend on diffusion of individual DNA molecules followed by collision with a specific partitioning site. Such an inheritance mechanism could stabilize a unit copy plasmid only if the number of unoccupied sites formed in each division cycle were strictly limited to two, one in each half of the dividing cell. Otherwise, both plasmid molecules could bind in the same half-cell. A high degree of specificity and very tight regulation of their production and location would be required for such sites to be effective. Moreover, since no partition-related incompatibility is observed between low copy number plasmids which encode different partition systems, even if they are closely related (as with P1 and P7), the sites used by each system would have to be different and specific for each one. The observed change in incompatibility specificity accompanying the deletion of part of the *parS* sequence (Davis *et al.*, 1990) suggests that, at least for P1, competition for a limited number of host sites is not the basis for incompatibility as it seems unlikely that removal of part of a site would, by chance, allow binding to a different pair of specific host sites in the correct position to confer stability.

A more likely sequence of events is that the plasmids pair specifically, at random, from a pool of unpaired molecules via their common *cis*-acting sites (Austin, 1988) and subsequently bind to a paired host structure which could be used by all plasmids. In this case incompatibility would arise when a second plasmid bearing the same *cis*-acting centromere-like sequence forms mixed pairs. This would tend to segregate each plasmid to a different cell. The altered specificity of the truncated *parS* centromere would then be explained by an inability of the protein complexes at the full and deleted sequences to pair.

To effect partition, plasmid-encoded functions present in the paired plasmid complex must link to some part of the host cell which will supply the necessary movement for separation towards the cell poles. It has been suggested that plasmids attach to the bacterial nucleoid and effectively 'hitch a ride'. Indeed, plasmid F has been found to co-sediment with the folded chromosome in gently lysed cells (Miller & Kline, 1979). By associating with a specific part of the unreplicated chromosome, a paired plasmid complex would be well placed to exploit separation of the daughter nucleoids once replication has occurred. However, recent studies with *muk* mutants, which are defective in chromosome partition, have shown that F stability is not affected by a decrease in chromosome stability (Hiraga *et al.*, 1989), suggesting that such a hitch-hiking model might not apply. Nevertheless, it cannot be ruled out for some plasmids.

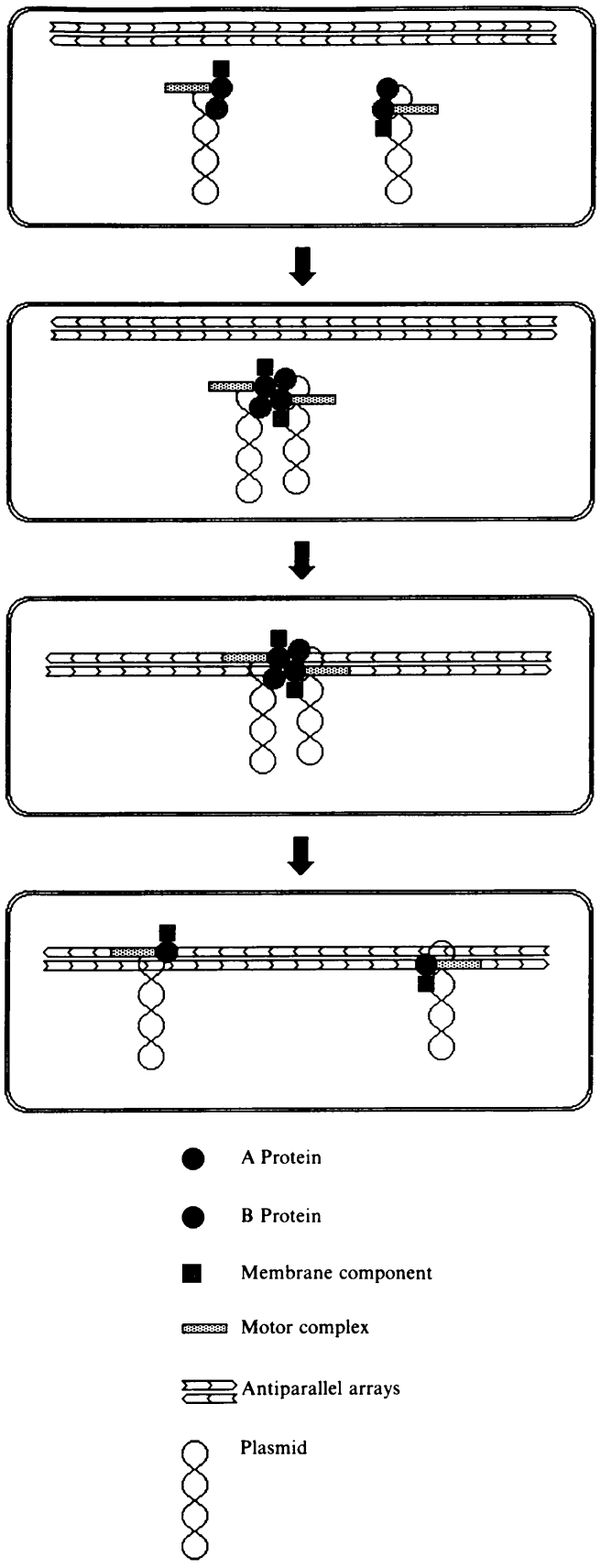
Alternatives to this model, therefore, are that plasmids use the same apparatus as the chromosome, behaving as if they were secondary mini-chromosomes, or that they use a different system but with the same result. Since there appears to be more than one class of partitioning system it is possible that more than one pathway or mechanism for partitioning exists. The extent to which each plasmid system depends on host-encoded proteins may also vary greatly. Whatever the mechanism of the pSC101 *par*-conferred stability, it apparently does not depend on plasmid-encoded proteins (see above). Other loci encode multiple proteins, e.g. the *mrs/par* system of RK2. The degree of dependence on host proteins may determine the range of species in which each system functions. For example, the *mrs/par* system of RK2 stabilizes plasmids in many species where the F *sop* system is found to be ineffective (Roberts *et al.*, 1990). For the *sop/par* family, which has been studied in most detail, a fundamental similarity between plasmid and chromosome partition is suggested by the homology of MinD to the A proteins (Motallebi-Veshareh *et al.*, 1990) (Fig. 2) and the circumstantial evidence that *minD*

mutants not only affect cell division but also chromosome partition (Mulder *et al.*, 1990). What could this common mechanism be?

One common element in many models for partitioning is membrane attachment, as proposed in the surface attachment model of the replicon hypothesis (Jacob *et al.*, 1963). Indeed, membrane-associated proteins appear to be involved in both plasmid and chromosomal partition (Hiraga *et al.*, 1989; Watanabe *et al.*, 1989) and in some cases association of plasmid DNA with cellular fractions having the properties of the outer bacterial membrane is dependent on possession of an active partition system (Gustafsson *et al.*, 1983). The *mukA* mutation of *E. coli*, which affects partition but not chromosome replication or cell division, is in *tolC*, which encodes an outer-membrane protein (Hiraga *et al.*, 1989). The ability of mini-F plasmids to partition in such mutants defective in membrane proteins might be explained by the use of a different host membrane protein (Ezaki *et al.*, 1990) or by the plasmid's provision of its own membrane-association component. Indeed, the SopB protein of F was found to sediment with the host membrane fraction in the presence of Mg^{2+} ions (Watanabe *et al.*, 1989). Thus, for F, the B proteins might provide a link to a membrane-bound host protein complex. In the case of the related set of genes from broad host range plasmid RK2, the membrane-associated KorF proteins might provide such a connection (Jagura-Burdzy *et al.*, 1991; G. Jagura-Burdzy & C. M. Thomas, unpublished). Nevertheless, it appears that membrane growth is unlikely to be the force driving chromosome segregation. Nucleoids in dividing cells do not shift position slowly as the cell grows, but move rapidly from the centre of the cell to the 1/4 and 3/4 positions (Donachie & Begg, 1989; Hiraga *et al.* 1990). In addition, bacterial membrane growth is thought not to occur exclusively at the central plane as would be needed to drive separation of chromosomes or plasmids (Green & Schaechter, 1972; Lin *et al.*, 1971).

Further clues as to the possible nature of partitioning processes comes from study of other mutants defective in chromosome partition, which fall into two classes. Those in the first class are affected in replication or decatenation and include primase, gyrase and topoisomerase mutants (Orr *et al.*, 1979; Steck & Drlica, 1984; Norris *et al.*, 1986; Hussain *et al.*, 1987*a, b*; Kato *et al.*, 1988, 1989, 1990; Mulder *et al.*, 1990). While there is a clear need for proper replication and maturation of the DNA, such processing does not appear to provide a clue to the nature of mitotic processes in bacteria. More helpful in this respect is one of the second class of mutants (*muk* mutants; Hiraga *et al.*, 1989), which produce cells that appear to replicate their DNA and divide normally but cannot segregate their chromosomes, with the result that anucleate cells are produced. The *mukB* mutation is in a gene whose product has homology to cytoskeletal proteins (Niki *et al.*, 1991), including the microtubule-associated mechanochemical enzyme dynamin (Obar *et al.*, 1990). MukB has a globular N-terminal domain, a central coiled-coil domain and a possible DNA binding, 'zinc-finger'-like C-terminal domain. Another *E. coli* protein has been discovered which cross-reacts with antibodies to yeast myosin heavy chain (Casaregola *et al.*, 1990). The discovery of these proteins with similarity to eukaryotic motors (for example, Gibbons *et al.*, 1991; Ogawa, 1991) suggests that they may drive nucleoid movement along an as yet undiscovered system of filaments bound to or associated with the cell membrane. Eukaryotic motors move unidirectionally on polar filaments (actin or microtubules) (Vale & Goldstein, 1990). Since partitioning is an inherently symmetrical process, the proposed bacterial filaments would have to be oriented in both directions if they are analogous to those known in eukaryotes. Attachment of replicated chromosomes to each of a pair of filaments would allow daughter nucleoids to move in opposite directions.

Such a model could also be applicable to some of the plasmid systems described above. This mechanism for segregating daughter molecules would provide a simple way of processing the paired plasmids proposed as the initial step for *sop/par* family partition. Assuming that paired plasmids could associate with the antiparallel filaments at almost any point along their length, then it should be possible for a large number of different plasmids to utilize the same apparatus simultaneously without interfering with each other. Association of the plasmid complexes with the filaments might occur in a number of ways and could depend on host proteins in addition to those that constitute the filaments themselves. Thus the host may provide the motor which drives movement while the plasmid simply provides the basis for the pairing/association/separation cycle. The link into the host system might vary. In the case of broad host range plasmid RK2, KfrA with its cytoskeletal-protein-like structure could provide a bridge onto a host structure at a point different from that used by F or P1. After association with antiparallel filaments, the paired plasmids could be separated by a conformational change in the complex as a result of phosphorylation catalysed by the ATPase activity of the A proteins. Movement along the filaments would involve either a motor moving relative to the filament or a filament which moves relative to the cell (e.g. by polymerization at one end and depolymerization at the other). Fig. 4 provides a schematic representation of the motor-driven model. A model with similarities to ours has been presented by Hiraga *et al.* (1991) for chromosome partition.



A measure of support for such a model of bacterial plasmid partition comes from studies on the yeast 2 μ circle plasmid partition system. This is responsible for the high mitotic stability of 2 μ and involves three *trans*-acting factors and a *cis*-acting site (Cashmore *et al.*, 1986, 1988; Dobson *et al.*, 1988). One factor, the product of the *REP1* gene, like KfrA, has a degree of homology to myosin heavy chain and the intermediate filament protein vimentin and also has DNA-binding activity (Wu *et al.*, 1987). It is envisaged either as the link between plasmid and a component of the spindle machinery, in a similar way to the model described above, or as part of the nuclear karyoskeletal network such that bound plasmid is evenly distributed, and hence stably inherited by daughter cells, in which case it is not a true active partition system (the association of REP1 with tubulin supports the former idea; A. M. Cashmore, personal communication). Another yeast protein responsible for mitotic stability of plasmids (and also for transcriptional silencing), SIR4, also has the heptad repeat structure that forms coiled-coil interactions, as identified for KfrA of RK2 (Diffley & Stillman, 1989).

Conclusions

A number of low copy number plasmids encode sets of genes which appear to fulfil the criteria required of an active partition system, which allows a mitotic process to direct daughter molecules to each side of the cell division plane. Hints from a range of experimental evidence and sequence comparisons suggest that partition in prokaryotes and eukaryotes may operate through basically similar mechanisms. The apparent similarity of some plasmid-encoded systems and chromosomal partition functions suggests that plasmids may be studied as a rather general model system. Finally, we have developed some highly speculative ideas with the purpose of stimulating a search for new genes and gene products which may provide information regarding the mechanism of this fundamental aspect of cell biology. Some of these ideas have already been presented elsewhere (Thomas & Jagura-Burdzy, 1991).

Fig. 4. A model for plasmid partition. The partition complex consisting of the A protein and B protein, possibly attached to a membrane component and a host or host/plasmid-encoded motor complex, initiates specific pairing between plasmid molecules. The paired plasmids are then bound to antiparallel filaments or arrays anchored in the cell membrane. ATP hydrolysis by the A protein causes separation of the plasmids which are then driven to opposite halves of the cell. The temporal sequence of binding the components of the complex could vary without affecting the final result.

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