Acta Biochimica Polonica

Vol. 50 No. 2/2003

425–453

QUARTERLY

Review

Spread and survival of promiscuous IncP-1 plasmids

Małgorzata Adamczyk✉ and Grażyna Jagura-Burdzy✉

Department of Microbial Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland

Received: 28 May, 2003; accepted: 12 June, 2003

Key words: IncP-1 plasmids, stable maintenance, central control region, global regulation

Plasmids classified to the IncP-1 incompatibility group belong to the most stably maintained mobile elements among low copy number plasmids known to date. The remarkable persistence is achieved by various tightly controlled stability mechanisms like active partitioning, efficient conjugative transfer system, killing of plasmid-free segregants and multimer resolution. The unique feature of IncP-1 plasmids is the central control operon coding for global regulators which control the expression of genes involved in vegetative replication, stable maintenance and conjugative transfer. The multivalent regulatory network provides means for coordinated expression of all plasmid functions. The current state of knowledge about two fully sequenced plasmids RK2 and R751, representatives of the IncP-1α and IncP-1β subgroups, is presented.

IncP-1 PLASMIDS: DISTRIBUTION, CLASSIFICATION AND ORGANIZATION

IncP-1 plasmids (IncP in *Escherichia coli*) have been of interest to molecular, environmental and industrial microbiologists since the 1970s. Plasmids classified within the IncP-1α subgroup were originally isolated in a Birmingham hospital (U.K.) in 1969 from *Pseudomonas aeruginosa* and *Klebsiella aerogenes* strains, and found to harbor genetic information that allowed their hosts to survive contact with multiple antibiotics (penicillin, kanamycin and tetracycline). The IncP-1 plasmids can replicate and be stably maintained in almost all Gram-negative bacteria and may be transferred by conjugation to Gram-positive bacteria, yeasts and eukaryotic cell lines (Heinemann & Sprague, 1989; Waters, 2001). The IncP-1 plasmid group is an ex-

✉Department of Microbial Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5a, 02-106 Warszawa, Poland; G.J-B.: tel: (48 22) 823 7192; e-mail: gjburdzy@ibb.waw.pl; M.A.: tel.: (48 22) 659 7072 ext.: 1216; e-mail: malada@ibb.waw.pl

ample of highly potent, self-transmissible, selfish DNA molecules with a complicated regulatory circuit, which utilise very efficient strategies for stable maintenance in different host cells. Recent studies have provided evidence that plasmid maintenance mechanisms have a lot in common with the systems for chromosome segregation in bacteria. New data concerning the nature and properties of these DNA units also provide information about their hosts adaptation and versatility. Some questions about IncP-1 plasmids address their role as vehicles in the gene exchange phenomenon within bacteria, their functions in interactions between plant and animal pathogens and their hosts and the characterization of the intricate mechanisms of plasmid inheritance.

There is a growing list of naturally occurring IncP-1 plasmids identified in clinical and environmental bacterial from all over the world (Smith & Thomas, 1989). IncP-1 have been identified in soil from areas contaminated by industry, in pig manure, waste water of industrial origin, river epithelion and freshwater (Top *et al.*, 1994; Pettigrew *et al.*, 1990; Gotz *et al.*, 1996).

IncP-1 plasmids are divided into three subgroups: α, β and γ. Comparative sequence analysis of plasmids belonging to the subgroups α and β has not only shown a similar gene organisation pattern but has also facilitated the definition of the indispensable backbone, identifying so called "load" genes as well as monitoring their evolutionary pathways (Smith & Thomas, 1987).

The fully sequenced RK2 and R751 plasmids are the best-characterized members of the IncP-1α and IncP-1β subgroups, respectively (Pansegrau *et al.*, 1994; Thorsted *et al.*, 1998). Recent studies have expanded the list of members of the IncP-1β subgroup. Some of these newly identified plasmids, pADP-1, pTSA and pB4, have been sequenced and shown to be closely related to R751 (Fig. 1).

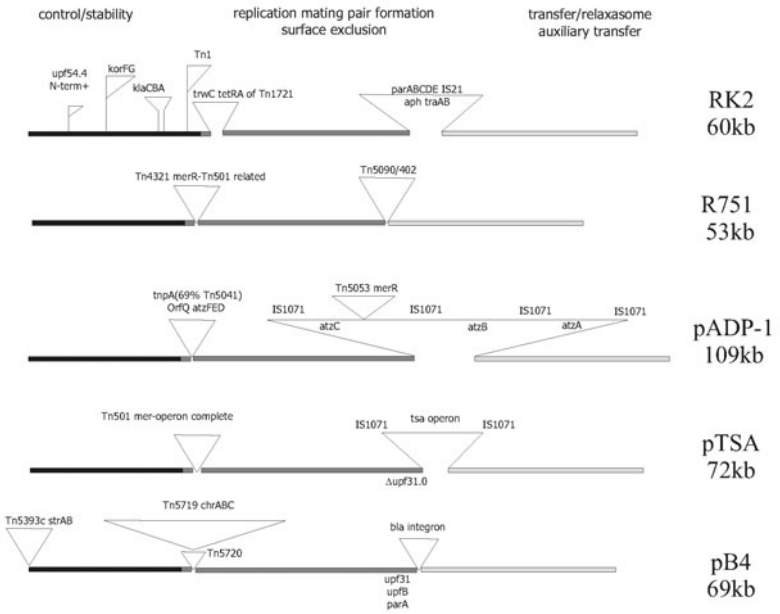

Figure 1. Schematic presentation of gene organization in the IncP-1 incompatibility group plasmids based on available sequence data.

References: RK2 (Pansegrau *et al.,* 1994), R751 (Thorsted *et al.,* 1998), pADP-1 (GeneBank accession No. U66917), pTSA (Tralau *et al.,* 2001), pB4 (GeneBank accession No. AJ431260).

Black box, control and stability functions; dark grey, replication, mating pair formation, surface exclusion; light grey, relaxosome assembly and auxiliary transfer functions. Triangles indicate positions of insertion (for detailed information see Table 1).

The plasmids assigned to the IncP-1α subgroup confer wide spectrum of antibiotic resistances (Smith & Thomas, 1989; Droge *et al.*, 2000) while IncP-1β members carry multi-antibiotic resistance determinants and degradative cassettes (Burlage *et al.*, 1990; Droge *et al.*, 2000). There are no conclusive data about resistance to inorganic mercury conferred by plasmids of the latter subgroup. All IncP-1β plasmids seem to share homology with at least part of the Tn*501*/Tn*21*-related mercury resistance operon (R751, R772, R906, pJP4, pSS50, pSS60, pBR60, pTSA, pB10, pADP-1), although some lack the resistance genes and display a mercury sensitive phenotype (R751, R772, pB12). The IR element fused to the remnants of the *merR* gene appears to be homologous to the *mer*- proximal IR in Tn*501* and Tn*21*. Such fusion of this element was recently reported for other mercury resistance operons, such as Tn*5041*, Tn*5053*, plasmid pKLH2 and Tn*5718*. In the case of R751, the sequence homologous to the IR element within transposon Tn*4321* is inverted compared to that in R906, R772 and pJP4. The presence of sequence relics of the mercury resistance transposon Tn*501* may reflect inheritance of the sequence from a common ancestor of IncP-1β plasmids.

Comparison of the physical maps and DNA sequences of IncP-1α and IncP-1β plasmids has lead to the conclusion that they are very similar and share a common structural organization (Pansegrau *et al.*, 1994; Burlage *et al.*, 1990; Martinez *et al.*, 2001). The regions of replication control and stability, and two Tra1/Tra2 regions responsible for conjugative transfer constitute the plasmid "backbone". Tra1 and Tra2 in IncP-1β plasmids are always separated by regions of clustered restriction sites, which may represent hotspots for the integration of various determinants (Thorsted *et al.*, 1998). Interestingly, a similar insertion site occurs between the *oriV* and *trfA* operons splitting the replication cassette into two parts. In IncP-1β plasmids, the backbone segments are in the same order, the only exception being R772 in which a region containing the *trb* genes (Tra2) is inverted compared to the other members of this subgroup.

The number of insertions in the "backbone" differs among IncP-1 plasmids (Fig. 1). pJP4, pSS50 and pSS60 backbones are interrupted only once to accommodate the insertion of catabolic genes, invariably located between the *oriV* and *trfA* loci. In contrast, R751 has two such interruptions. The *tsa* degradative genes present in plasmid pTSA (which shares 99% identity with the backbone of R751) are integrated within a transposon-like structure located in a region where R751 carries a transposon conferring trimethoprim resistance (Tn*402/5090*). In the case of pADP-1, the "insertions" are found between the origin of replication *oriV* and the replication activating gene *trfA*, and between the Tra1 and Tra2 regions. The only plasmid analysed to date with three insertions in the main backbone is pB4. In common with plasmids containing only one interruption it has its degradative operon placed near *oriV*. The pB4 antibiotic resistance determinants are located proximal to both ends of the Tra2 region. Even when a resistance plasmid has only one interruption, such as pJP4, it usually has clusters of restriction sites at other potential sites of integration.

The "load" of IncP-1 plasmids presented in Table 1 is a "protective arsenal" of genes carried by individual plasmids which may influence the host survival and thus benefit the plasmids indirectly. The genetic information in the backbone sequence is much more significant because it ensures the existence of the plasmids.

REPLICATION DETERMINANTS

The ability of IncP-1 plasmids to replicate efficiently in a wide range of bacterial hosts is the major property of these autonomy units. They rely on a theta replication mode. The replication of plasmid RK2 in *E. coli* is now

Table 1. Characteristics of IncP-1α and IncP-1β plasmids and their "load" genes.

References: IncP-1α and R751 (Pansegrau *et al.*, 1994; Thorsted *et al.*, 1998; Smith & Thomas, 1989; Jovanovic & Figurski, 1997), pB1-pB12 (Droge *et al.*, 2000), pSS50 (Hooper *et al.*,1989), pSS60 (Burlage *et al.*, 1990), pJP4 (Leveau & van der Meer, 1997), R906, R772 (Smith & Thomas, 1987), pTSA (Tralau *et al.*, 2001), pADP-1 (Martinez *et al.*, 2001), pPS12-1 (Beil *et al.*, 1999).

Plasmid	Size	"Load" information
IncP-1α		
RK2/RP4, R18, R68, RP1	60kb	Pn, Tc (*tet*R, *tetA*, *tcc*A C-term of TrwC relaxase/helicase R388 plasmid (72%)), Km, Tn*1* transposase
R26	?	Km, Tc, Pn, Sm, Su, Hg, Cm, Gm
R702		Km, Tc, Sm, Su, Hg
R839	?	Km, Tc, Pn, Sm, Su, Hg
R934	?	Km, Tc, Pn, Hg
R938	?	Km, Tc, Pn, Sm, Su, Hg, Cm
R995	?	Km, Tc
R1033	?	Km, Tc, Pn, Sm, Su, Hg, Cm, Gm
PUZ18	?	Km, Tc, Hg
pB5	56kb	Km, Tc, Gm, Su, aminoglycoside 3'-N-acetyltransferase *P.aeruginosa* (99%), dihydropteroate synthetase TypeI (97%)
pB11	68kb	Km, Tc, Hg, mercury operon co-regulator Tn*5053* (100%), transporter protein (99%), mercury reductase *Pseudomonas fluorescens* (100%), mercury reductase Tn*5053* (90%)
IncP-1β		
R751	53kb	Tp (Tn*5090/402*)
R772	63	Sm, Km
R906	60	Hg
pSS50	53kb	4-chlorobiphenyl degradation genes
pSS60	?	4- chlorobenzoate catabolic genes
pJP4	80kb	2,4-dichlorophenoxyacetate catabolic genes *tfdCDEF* 3-chlorobenzoate catabolic genes, Hg (Tn*501*), PMA,
pADP-1	109kb	Hg (Tn*5053*), antrazine catabolic genes *atzA,B,C,D,E,F,* transposase *tnpA* Tn*5041* (69%), putative cointegrate resolution protein OrfQ Tn*5041* (100%)
pTSA	79kb	Hg (Tn *501*), *p*- toluenesulfate degradation *tsa* operon,
pB1	61kb	Tc
pB2	51kb	Tc [Tc repressor class C pSC101 (100%)], Sm, Sp, Cm [Cm transporter *P.aeruginosa* (100%)], Su
pB3	46kb	Tc, Sm, Sp, Cm, Su
pB4	69kb	Pn, Sm, Em, Amx, DIN family DNA invertase *Rhodospirillum rubrum* plasmid pKY1 (65%); DNA invertase (*pin*) *E.coli* (72%), outer membrane protein OprJ *P.aeruginosa* (multidrug efflux system (57%)/ transmembrane protein MexY *P.aeruginosa* (48%), M protein *Eco*KI type I restriction-modification system (44%), putative superoxide dismutase [chromate resistance operon *chrABC* pMOL28; pUM505 (73%;75%)]
pB6	58kb	Tc, Sm, Sp, Cm, Su, Din family DNA invertase *Thiobacillus ferrooxidans* plasmid pTF5 (75%); DNA invertase (*pin*) *Shigella boydii* (64%)
pB8	51kb	Sm, Sp, Su, Amx, dihydropteroate synthetase TypeI (98%)
pB10	58kb	Tc [Tc repessor Tn*1721* (100%)], Sm [streptomycin phosphotransferase *strB* (97%)], Su, Amx, Hg [mercury reductase Tn*501* (83%), mercury operon regulator *P.fluorescens* (100%)]

pB12	61kb	Tc, Sm, Sp, Em, Su, dihydropteroate synthetase TypeI (91%)/ orf5 Tn*21* (100%)
pPS12-1	?	1,2,3,4,5-tetrachlorobenzene degradation genes

List of abbreviations: Amx, amoxicillin; Cm, chloramphenicol; Em, erythromycin; Gm, gentamicin; Hg, mercuric salts; Km, kanamycin; Pn, penicillin; Sm, streptomycin; Sp, spectinomycin; Su, sulphonamide; Tc, tetracycline; Tp, trimethoprim; % in brackets show the level of similarity to known proteins.

quite well understood. Some recent data emphasize the plasticity (flexibility) at the initiation step and the recruitment of host proteins in different hosts (Doran *et al.*, 1999).

To replicate, IncP-1 plasmids require *oriV*, from which replication proceeds unidirectionally (at least in *E. coli*) and the *trfA* gene, whose two in-frame products are essential to activate *oriV* (Pansegrau *et al.*, 1994). The host proteins: DnaA, DnaB, DnaC, DNA gyrase, DnaG primase and DNA polymerase III holoenzyme are necessary for plasmid replication (Pinkney *et al.*, 1988).

Analysis of the *oriV* nucleotide sequences from RK2 and R751 revealed conserved features in this region. The minimal DNA segment providing *oriV* activity is approximately 393bp long and consists of nine direct repeats called iterons (17-mers) to which TrfA binds, DnaA boxes and A+T-rich and G+C-rich regions (Fig. 2) (Pansegrau *et al.*, 1994).

The A+T rich repeats (13-mers) designated L, M1, M2 and R are the initial sites of helix destabilization. Iterons, to which the monomeric TrfA molecules bind are organized into three groups: containing one, three and five copies of the direct repeats, respectively (Fig. 2). Iterons 5 to 9 form a cluster upstream of the A+T region and they represent minimal origin activated by the TrfA initiation protein. TrfA exists in two forms TrfA44 and TrfA33, which are expressed from the *trfA* mRNA using separate in-frame translation start sites (Pansegrau *et al.*, 1994). The carboxyl terminus of TrfA, which constitutes the dimerization domain, is highly conserved in the form of the protein encoded by both RK2 and R751 plasmids and exhibits homology to the substrates of *E. coli* ClpX. It has been shown that the ClpX chaperone is involved in TrfA monomerization (Konieczny & Helinski, 1997a). TrfA dimers are believed to play a key role in the negative control of replication ("handcuffing model") (Toukdarian & Helinski, 1998). When present in excess, TrfA monomers bound to the iterons of the two replication *ori* interact with each other and form a sandwich-like structure, making the *ori* inaccessible for other components of the replication complex. Thus, the TrfA protein has a dual role in RK2 replication: as a positive initiator of replication and a negative regulator of plasmid copy number when in excess.

The positive action of TrfA as an initiator of replication is modulated by the DnaA protein. This factor binds to four DnaA boxes arranged as inverted pairs of a 9 bp consensus sequence separated by a few base pair which are located immediately upstream of the block of five iterons. The DnaA box cluster is located approximately 200 bp upstream of the A+T-rich region which provides the origin duplex opening site (Fig. 2). The binding of DnaA to DnaA boxes enhances or stabilizes the formation of the TrfA-mediated open complex, which requires ATP and proceeds in the presence of HU (Konieczny *et al.*, 1997). In *E. coli* a mutation in DnaA box 4 abolishes replication and disturbs DnaA binding at all four boxes, whereas inversion of DnaA box 4 has no effect on binding to the other boxes (Doran *et al.*, 1999). DnaA bound to its recognition sites interacts with the DnaB helicase to form a DnaABC complex in which DnaB is responsible for interaction between the TrfA protein and a prepriming form of the DnaBC complex

(Konieczny & Helinski, 1997b). In the case of RK2, one molecule of the DnaB helicase is loaded at the A+T rich 13-mer repeat region and replication proceeds toward the G+C-rich region. The mechanism for helicase loading on ssDNA in the open duplex region and helix unwinding are not yet well understood. The process of DnaB loading and activation at $oriV_{RK2}$ may be different in various hosts (Caspi *et al.*, 2001).

The recent observation that the four DnaA boxes are not required for the replication of RK2 in *P. aeruginosa* (Fig. 2) raises the possibility of an alternative mechanism for the stimulation of open complex formation and DnaB loading at *oriV* (Doran *et al.,* 1999). Such a mechanism might also be utilised in other hosts like *E. coli* or *Pseudomonas putida* under certain conditions. It has been shown that mutation of DnaA boxes 3 and 4 (indispensable for replication in these hosts) or deletion of all four DnaA boxes may be compensated for by over-production of the DnaA protein (Doran *et al.,* 1999).

It also remains to be determined whether the preinitiation role of the ClpX chaperone is performed by ClpX homologues in the wide range of Gram-negative bacteria that can support the replication of RK2 plasmid.

A comparison of the nucleotide sequences of *oriV* regions of RK2 and R751 revealed an overall homology of 65% (Smith & Thomas, 1985). However, all the important features of $oriV_{RK2}$ are also present in $oriV_{R751}$. In *oriV* of R751 a single copy of the iteron is found to be separated from the group of three repeats by a sequence relic of Tn*501*. This is not the case in plasmids R906 and R772 (64% overall homology) which have the single repeat adjacent to *oriV* and the other highly conserved features (Thomas & Smith, 1987).

The *trfA* gene of RK2 has been shown to activate replication from cloned *oriV* locus of R751, indicating that there is functional conservation between the two plasmids. Interestingly, while the TrfA33 protein is highly conserved in both subgroups (78% of aa identity, 91% homology), the N-terminal extensions of the TrfA44 forms are less similar (41% identity, 54% homology). Genetic drift in the N-terminus of TrfA1 is probably related to the adaptative ability of the two plasmid subgroups to specific hosts. The full length TrfA is required for replication in *P. aeruginosa* whereas TrfA33 is sufficient in *E. coli* and *P. putida* (Thorsted *et al.*, 1996).

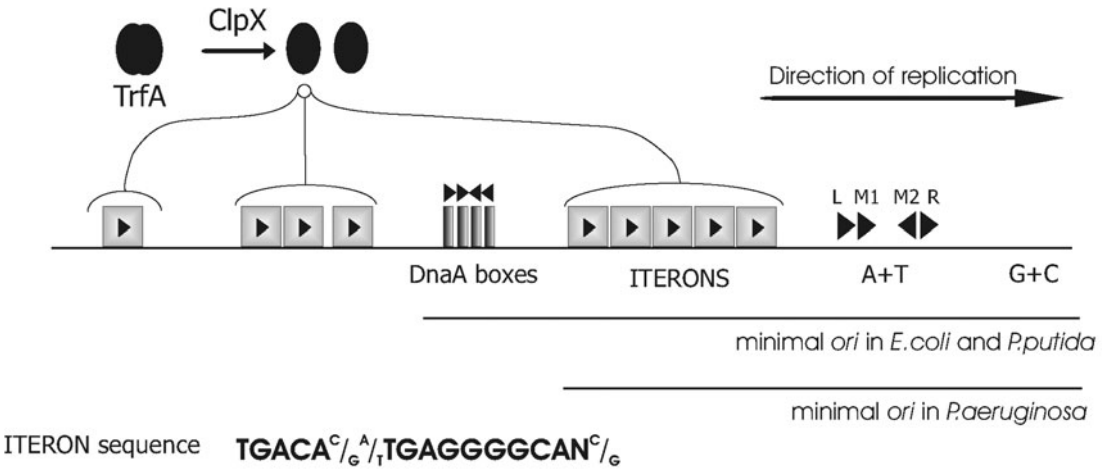

Figure 2. The *oriV* region from plasmid RK2.

TrfA (replication initiator protein) binds to directly repeated iteron sequences, ClpX (chaperone) monomerizes TrfA. The orientation of iterons, the DnaA boxes to which DnaA binds as well as orientation of 13-mers in A/T rich region are indicated by arrowheads.

THE CONTROL OF COPY NUMBER

IncP-1 plasmids exist in bacterial cells at a copy number of 5–7 per chromosome. The number of replication initiation events depends on the level of the initiation protein TrfA. The control of copy number is achieved by tight regulation of *trfA* operon expression. The *trfA* promoter is classed as a very strong promoter so it may drive expression at a sufficient level in a wide spectrum of hosts. It is regulated by at least two plasmid-encoded regulators: KorA and KorB (described in the Regulatory Network Section) which bind to sites that overlap the −10 (O_A– KorA binding site) and precede the −35 sequence (O_B – KorB binding site) of the promoter. Whereas KorA represses *trfAp* approximately 10 fold and KorB on its own 3–5 fold, acting cooperatively they decrease the activity of *trfA* promoter about 1000fold (Smith *et al.*, 1984).

The level of active TrfA is also regulated by its ability to form inert dimers. These dimmers, as mentioned previously, are dissociated into monomers active in binding to iterons and initiating replication by the action of the ClpX and ClpB chaperones (Konieczny & Helinski, 1997b; Konieczny & Liberek, 2002). While the group of five iterons is essential and sufficient for *oriV* function it has been demonstrated that the mini-replicon copy number is negatively dependent on the total number of iterons present, suggesting an accessory role of "extra" iterons in decreasing the level of TrfA available for the initiation of plasmid replication (Thomas *et al.,* 1984).

It has also been demonstrated that the mini-replicon copy number is not proportional to the increase in the concentration of TrfA (Pansegrau *et al.,* 1994). This prompted speculation concerning the role of TrfA as a negative regulator of copy number when present in excess ("handcuffing model" mentioned in the previous section).

CONJUGATIVE TRANSFER

IncP-1 plasmids have a broad-host-range with respect to their vegetative replication, which originates at *oriV* and also with respect to conjugation, which originates at *oriT*. The conjugative replication functions are different from those required for vegetative replication. Conjugative replication proceeds by the rolling circle mode. The range of recipient bacteria is broader than the host range in which the plasmids may replicate. Although conjugation is defined as bacterium-to-bacterium DNA transfer, in principle any type of cell can serve as the recipient as the process is typically dependent on factors encoded by the plasmid and expressed in the donor cell. It has been shown that plasmid RK2 mediates the transfer of DNA from bacteria to yeast (Heinemann & Sprague, 1989) and to Chinese hamster mammalian cells (Waters, 2001), although it cannot replicate in those eukaryotic organisms. The recent discovery that at least 41 genes are shared exclusively by humans and bacteria implies that some lateral transfer of DNA into the human genome may have occurred (Waters, 2001).

All IncP-1 plasmids are conjugationally highly promiscuous, with the exception of R906, isolated from *Bordetella bronchiseptica* R906. This plasmid has a reduced recipient host range although examination of its restriction profile reveals no drastic differences from other IncP-1β plasmids. The fact that IncP-1β plasmids share a common arrangement of transfer loci with the IncP-1α plasmid RK2 (Pansegrau *et al.,* 1994) supports the assumption that they rely on similar conjugational transfer mechanisms and the extensively studied RK2 may serve as a paradigm for the whole group.

Mating pair formation (Mpf) apparatus

The strategy for plasmid spread among bacteria is reliant on intimate molecular associations between the surfaces of cells, which are

plasmid-determined and in the case of IncP-1 plasmids promoted by extracellular filaments called sex pili. After initial contact has been established the DNA is thought to be transported in a complex with associated proteins through a channel at the mating bridge between the donor and the recipient cells. Formation of this channel as well as pilus assembly depends on gene products of the Mpf system (Pansegrau *et al.,* 1994; Haase *et al.,* 1995; Daugelavicius *et al.,* 1997; Eisenbrandt *et al.,* 2000).

Mpf genes (*trbB, C, D, E, F, G, H, I, J, K* and *L*) are located in the Tra2 region except for *traF,* which is located in the Tra1 region. These genes products are essential for intergeneric mating except for TrbK, the only surface exclusion protein encoded by IncP-1α subgroup plasmids (Giebelhaus *et al.,* 1996). TrbK is located in the inner membrane and probably prevents formation of the DNA entry pore in the recipient cell envelope (Haase *et al.,* 1996). The same genes are also required for phage reproduction (Haase *et al.,* 1995). Most Mpf components are likely to be integral membrane or membrane-associated proteins as indicated by their amino-acid sequences and some experimental data (Grahn *et al.,* 2000). It has been shown that over-expression of the IncP-1 Tra2 proteins increases permeability of the OM to lipophilic compounds and ATP and decreases intracellular K^+ content (Daugelavicius *et al.,* 1997). Genes from the so-called *trb* region of RK2 and R751 have counterparts in bacterial species with well-studied transport systems, e.g. the type IV secretion system engaged not only in conjugative processes but also in the transfer of virulence factors to eukaryotic cells. These include *Agrobacterium tumefaciens* which uses the T-DNA transfer machinery to deliver nucleoprotein complexes to plant cells, and human pathogens such as *Bordetella pertussis, Legionella pneumophila* and *Helicobacter pylori* that use type IV systems to export effector proteins to the extracellular milieu or the mammalian cell cytosol. Homologous genes within functional clusters are often arranged co-linearly in the respective operons (Pansegrau *et al.,* 1994; Vogel *et al.,* 1998; Krause *et al.,* 2000b).

The first gene in the *trb* operon, *trbA,* encodes a transcriptional regulator which controls expression of the *tra* and *trb* operons (see Regulatory Network). TrbA is non-essential for Mpf functions.

Six TrbB molecules associate to form a ring-shaped hexameric complex which is loosely associated with the inner membrane. There are four conserved domains in the TrbB homologues found in other DNA transport systems. These are a highly conserved Walker A box, an aspartate box, a Walker B box and a histidine box. TrbB possesses weak ATPase and kinase activities. The NTPase activity of VirB11-type protein (TrbB homologue of the Ti plasmid) (Krause *et al.,* 2000a) might play a role in a chaperone-like function during the pilus assembly process.

The pilus subunit is encoded by the gene *trbC.* IncP-1 pilin is composed of a 78-amino acid TrbC polypeptide forming a ring structure *via* an intramolecular head-to tail peptide bond (Eisenbrandt *et al.,* 1999). Three-step processing of the prepilin termini precedes the cyclization reaction. LepB, a chromosomally encoded protease cleaves 36 aa from the N-terminus of TrbC and another (so far unidentified) host-encoded protease removes 28 C-terminal residues dispensable for pilin maturation. The cyclization of TrbC occurs only in the presence of TraF. TraF protein (encoded in the *tra* region) catalyzes the intramolecular cyclization of TrbC by removing a further 4 aa from the C-terminus (Eisenbrandt *et al.,* 2000). These modifications are consistent with data obtained by mass spectrometry. The resulting pili of RP4 and R751 seen in the electron microscope appear to be very rigid, inflexible structures measuring 10 nm in diameter (Eisenbrandt *et al.,* 1999).

Recently, mating pair formation and plasmid R751 establishment in live *E.coli* cells have been visualized by fluorescence micros-

copy using donor cells containing R751::*lacO* and recipient cells expressing a GFP-LacI fusion protein (Lawley *et al.,* 2002). Evenly dispersed GFP-LacI fusion protein in the recipient forms foci upon the entry and replication (establishment) of R751::*lacO*. The localization of the GFP-LacI fusion protein enabled the authors to monitor the distribution of the plasmid in the recipient cell. Interestingly, the plasmid DNA was not randomly distributed throughout the recipient cells but rather localized either at the cell centre or at quarter positions in the cell similar to the localization observed during plasmid replication/cell cycle events (see Stable Inheritance of IncP-1 Plasmids). Another finding was that the junctions between mating cells occurred at various positions along the polar and lateral sides of both donor and recipient cells indicating that conjugative pores have no specific pre-programmed locations.

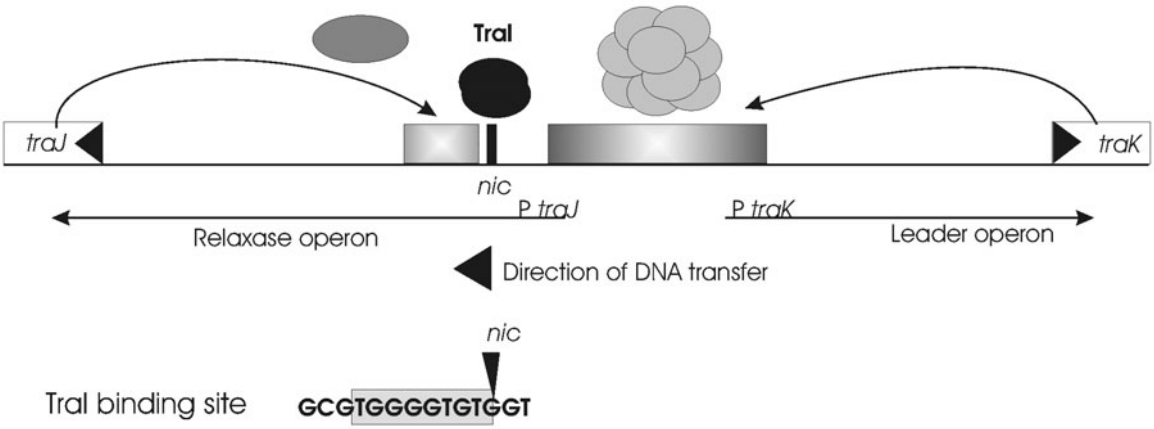

Figure 3. *oriT* of plasmid RK2.

Proteins involved in nick site recognition as well as the *nic* (cleavage site) sequence are shown. Shaded box indicates the binding site for TraI.

DNA transfer and replication (Dtr) functions

The preparation of IncP-1 plasmid DNA for transfer includes relaxosome formation and initiation of rolling circle replication. A nick within the plasmid *oriT* sequence followed by extension of the 3′ end displaces the single strand that is to be transferred into the recipient cell. The proteins encoded by the *tra* region are involved in Dtr functions.

Tra1 of IncP-1α consists of three operons: the relaxase operon containing genes *traJIH,* the primase operon of genes *traGFEDCBA* (transcribed in the same direction) and the leader operon in which genes *traK, traL* and *traM* are transcribed in the opposite direction (Pansegrau *et al.,* 1994). IncP-1β R751 lacks the two first genes of the primase operon (*traA* and *traB*) and shows poor conservation of *traE* and *traD* (59% and 56%, respectively) (Thorsted *et al.,* 1998). The greatest divergence is displayed in the *traJ* and *traK* genes encoding essential relaxosome-proteins. Yakobson & Guiney (1983) showed that although both R751 and R772 could mobilize a plasmid carrying a segment of RK2, including the *oriT* and *traHIJK* genes, they could not mobilize plasmids carrying $oriT_{RK2}$ alone. Thus, the initiation of conjugative replication *via* relaxosome binding and nicking of *oriT* is highly specific for plasmids of different IncP-1 subgroups.

The transfer origin (*oriT*) sequence of 250 bp, which is also located within Tra1, is the only *cis*-acting DNA element that is essential for transfer (Pansegrau *et al.,* 1994). The nick region is highly conserved in *oriT* of the RK2,

R751, R64 (IncI1), pTF-FC2 (IncQ) and in both the left and right T-DNA borders of the plant tumor-inducing Ti plasmids of *A. tumefaciens* (Pansegrau *et al.,* 1994; Pansegrau & Lanka, 1996). This conservation emphasizes the basic similarity between IncP-1 mediated conjugation and T-DNA transfer to plants. The transfer origin of both R751 and RK2 lies in the intergenic region between the divergently transcribed *traJ* and *traK* genes and consists of their two promoter sequences located to the one side of the nick sequence (Fig. 3) where TraK binds and 19 bp inverted repeats recognized by TraJ on the other side of the nick site. A TraI binding site overlaps the nick site. Binding of TraJ has been proposed as the first step in functional relaxosome assembly (Pansegrau *et al.,* 1994). In the second step TraI interacts with the TraJ-*oriT* DNA complex. An altered DNA conformation is thought to allow TraI to interact with the nick region. TraI access to its target site is also facilitated by TraK. TraK binds in the divergent promoter region and autoregulates the expression of both operons with the help of other relaxosome proteins (Pansegrau *et al.,* 1994; Zatyka *et al.,* 1994). *In vitro* studies on TraK-*oriT* nucleoprotein complexes have revealed that TraK induces local changes in DNA superhelicity. TraK wraps DNA and helps to melt the nick region.

The last player in the relaxosome assembly that has been identified so far is TraH protein. TraH has been proposed to have chaperone-like activity, which might stabilize the relaxosomal nucleoprotein complex by specific interactions with both TraI and TraJ. When relaxosome formation has been completed, single-strand nicking occurs within the 6 bp *nic* site and TraI becomes covalently bound to the 5′-terminal nucleotide (G) of the nicked strand (Pansegrau & Lanka, 1996). Rolling-circle replication may then start and the donor cell is ready to transfer DNA to the recipient cell.

Two products of the primase operon are essential for conjugative transfer between *E. coli* cells: TraG and TraF (previously mentioned for its involvement in Mpf apparatus formation). These proteins are highly conserved, fully exchangeable between the R751 and RK2 sytems and are thought to temporarily link the DNA processing and transfer reactions. Indeed, TraG is also known as "coupling protein". Unlike the relaxosome components, which are specific to their cognate *oriT* sequences, the coupling proteins of different conjugative plasmids belonging to distinct incompatibility groups (TrwB of IncW plasmid R388, TraD of IncF plasmids, VirD4 of Ti plasmids) show high levels of homology, suggesting that they have similar properties (Cabezon *et al.,* 1994). These proteins have transmembrane α-helices in their N-terminal regions and their C-terminal domains are cytoplasmic and all contain conserved Walker motifs, although no NTPase activity has yet been reported. Direct interactions between the coupling proteins and relaxosome components have been demonstrated, both *in vitro* and *in vivo*.

TraG of IncP-1, which was shown to localize to the cytoplasmic membrane, may also bind DNA and could be a transporter protein interacting with the Mpf apparatus (the direct interactions between TraG and Mpf proteins have been demonstrated (Grahn *et al.* 2000). It has been observed that TraG coupling protein is essential for transfer of mobilizable plasmids. *In vivo* interaction between TraG and the Mob relaxase protein of pBBR1 has been reported (Szpirer *et al.,* 2000) suggesting that the relaxosome may be delivered to the Mpf apparatus by TraG. There is also evidence that inhibition of IncP-1 plasmid conjugation and IncP-mediated mobilization of RSF1010 to cells already carrying an F factor or plasmid pKM101 is due to the action of PifC and FipA, respectively, on TraG (Santini & Stanisich, 1998).

No functions have yet been assigned to the products of *traA*, *traB*, *traD*, and *traE* which are thought not to be essential for conjugation (Pansegrau *et al.,* 1994). TraE of R751 is predicted to be a DNA topoisomerase on the basis

of sequence analysis only (Lin *et al.,* 1997; Thorsted *et al.,* 1998). Two products of the *traC* gene, TraC1 and TraC2, possess primase activity. During conjugation, TraC1 and Ssb (single strand DNA binding protein encoded by *trfA* operon) are transferred to the recipient cells as part of the DNA–protein complex (Pansegrau *et al.,* 1994).

STABLE INHERITANCE OF IncP-1 PLASMIDS

Conjugative transfer as the tool to spread IncP-1 plasmids across a wide range of bacteria and vegetative replication as the mean to establish these plasmids in new intracellular environments are accompanied by systems providing stable inheritance in the host populations.

The active partitioning mechanism

Low copy number plasmids encode functions for stable maintenance that are in many respects very similar to the strategies used by their hosts. The best example as illuminated by recent data is the active partitioning process of bacterial chromosome segregation to daughter cells at cell division. The best understood chromosomal Par systems include: *sojspoOJ* of *Bacillus subtilis* (Mysliwiec *et al.*, 1991; Ireton *et al.*, 1994; Lewis & Errington, 1997; Sharpe *et al.,* 1998), *parAB* of *Caulobacter crescentus* (Mohl & Gober, 1997; Mohl *et al.,* 2001) and *P. putida* (Ogasawara & Yoshikawa, 1992; Lewis *et al.*, 2002; Godfrin-Estevenon *et al.*, 2002). These systems share many features with the active partitioning of plasmid molecules between dividing cells (Bignell & Thomas, 2001).

All Par systems (plasmid and chromosomal) which have been studied to date require three components to drive the partitioning process: these are two *trans*-acting factors, ParA and ParB, and a *cis*-acting centromere-like site (by analogy to the role of the centromere during eukaryotic mitosis) on which a nucleoprotein complex is formed. ParB is a DNA-binding protein that recognizes and binds to the centromere-like site and then interacts with ParA whose ATPase activity (energy supply) is essential to the segregation process. The plasmid molecules may form pairs after replication that takes place at mid-cell (at the division plane). Although the consensus of opinion is that plasmid pairs have to be separated and that this is an active process, there is still controversy on the localization of the pairs as well as the timing of the whole process. Some have argued that the pairing and separation of plasmids are not strictly related to the cell cycle but that specific localization of plasmid clusters at polar or so called L̓ and l̓ positions must occur before the host cell divides. Others (Li & Austin, 2002) support the idea that the plasmid molecules are moving freely within the cytoplasm until the host cell reaches a certain stage of its cycle and then the plasmid pairs/clusters attach to the future site of division and become separated actively at cell division.

The protein components of the active partitioning process are always encoded by a single operon and are usually transcribed from an autoregulated promoter. The virulence plasmid pSLT (Cerin & Hackett, 1989; 1993) and QpHI plasmid (Lin & Mallavia, 1994) whose *parA* and *parB* genes have separate promoters are the only exceptions to date. The structure of the centromere-like site, the length and DNA sequence involved in ParB binding are specific for different plasmids. The binding sequences are repetitive and a single locus may contain many ParB-binding sites (Hayakawa *et al.*, 1985; Helsberg & Eichenlaub, 1986; Lane *et al.*, 1987) or alternatively single binding sites may occur in multiple locations as exemplified by N15 phage (Ravin & Lane, 1999; Grigoriev & Lobocka, 2001) and IncP-1 plasmids (Williams & Thomas, 1992).

On the basis of amino-acid sequence comparisons of ParA and ParB as well as centromere-like site location, the *par* loci of different

plasmids have been classified into two main groups (Gerdes *et al.*, 2000). The first group (I) consists of the *par* systems in which ParA contains typical ATPase motifs which match the Walker motifs for NTP binding. Group I has been further divided into two subtypes Ia and Ib on the basis of ParB homology as well as location of the centromere. The second group (II) consists of systems in which the ParA homologue is an actin-like ATPase (Fig. 4). The best studied representatives of type Ia *par* are the P1 and F plasmids. All putative chromosomal partitioning proteins also fall into this category. Type Ib is represented by *par* of the pTAR plasmid and type II by *par* of plasmid R1.

The IncC and KorB proteins of the IncP-1 plasmids are equivalent to ParA and ParB of type Ia plasmids. KorB and IncC are highly conserved between the IncP-1α and IncP-1β subgroups (77% and 72% nucleotide sequence identity, respectively). They are encoded by one operon, which is negatively autoregulated by the KorB protein. IncC enhances the repression exerted by KorB (Jagura-Burdzy *et al.*, 1999b) (Fig. 6).

IncC, like other members of the ATPase superfamily, contains two motifs involved in NTP hydrolysis: the A motif (Walker A or P-loop) and the B motif (Walker B), which is always found adjacent to the former (Motallebi-Veshareh *et al.*, 1990; Koonin, 1993). IncC shows the highest similarity to ParA of the *P. putida* pM3 (IncP-9) and *Rhodococcus erythropolis* pFAJ2600 plasmids. The closest homologues of IncC among chromosomal ParA proteins are those of the Soj family of *B. subtilis* and ParA of *Mycobacterium tu-*

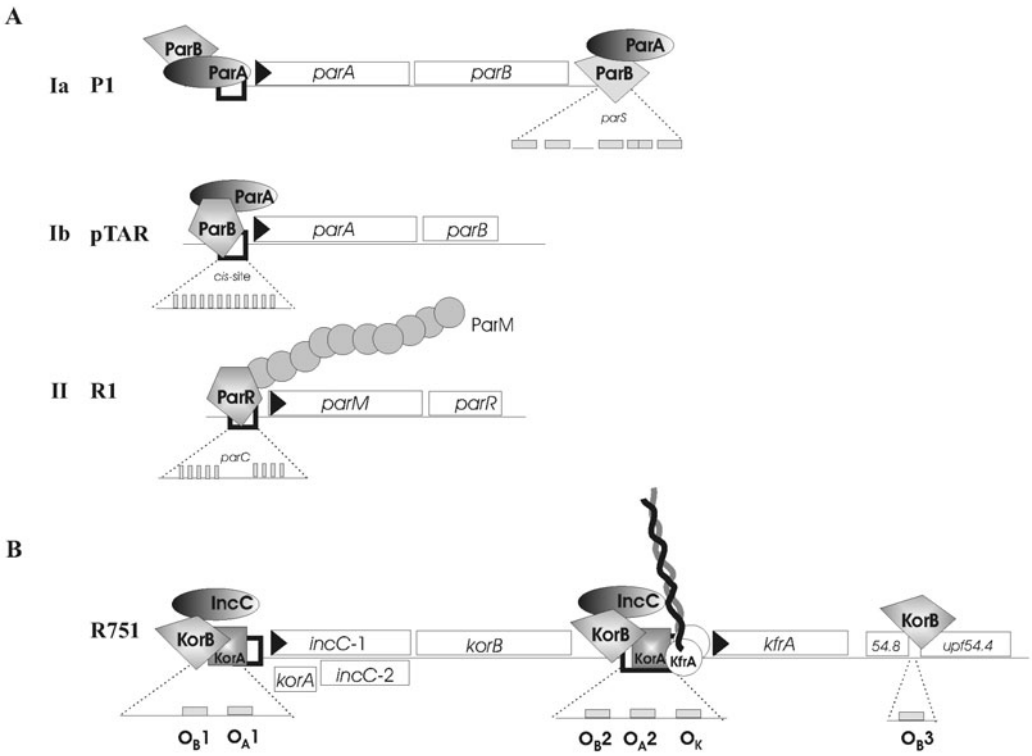

Figure 4. Comparison of the *par* loci of P1, pTAR, R1 and IncP-1 plasmids.

The proteins involved in active partitioning process and a schematic presentation of *cis*-acting sites are shown. *parS*, *cis*-site, *parC* — centromeric sequences; O_B1, O_B2, O_B3 – $KorB_{IncP-1}$ (ParB) binding sites; O_B3 – putative centromeric-like sequence; O_A1, O_A2 – KorA binding site, O_K — binding site for KfrA. Partitioning proteins ParA and ParB, global regulator KorA and the KfrA protein are shown bound with promoters. Promoters are indicated by black open squares and direction of transcription by black arrows.

berculosis, *H. pylori, P. putida*, *Streptomyces coelicolor* and *C. crescentus* (Hayes, 2000).

The partitioning model suggests that a conformational change in ParA as it converts from the ATP-bound to ADP-bound form is a key driver of the partitioning cycle (Bignell & Thomas, 2001; Watanabe *et al.,* 1992; Davey & Funnell, 1997; Bouet & Funnell, 1999; Quisel & Grossman, 2000). As the ParA proteins are known to be membrane bound, this model proposes that the ParA-ATP form promotes cell wall attachment of the partitioning factory (Lin & Mallavia, 1998) while ATP hydrolysis leads to its dissociation from the membrane.

The plasmids RK2 and R751 produce two forms of ParA-like proteins encoded by the *incC* locus. For RK2 these are IncC-1 (364 aa) and IncC-2 (258 aa) and for R751, IncC-1 (361 aa) and IncC-2 (259 aa). The IncC-2 form is sufficient for IncP-1 active partitioning (Williams *et al.*, 1998), while the N-terminally extended form IncC-1 appears to play a regulatory role by enhancing KorB-mediated repression (Jagura-Burdzy *et al.*, 1999b). Other plasmid ParA homologues are similar to IncC-1 whereas the chromosomal ParA homologues are generally shorter, like IncC-2. The helix-turn-helix motif, which is present in a number of plasmid-encoded ParA proteins (Bignell & Thomas, 2001) is missing from IncC and the protein cannot bind to DNA. The KorA protein encoded by *incC* using a different reading frame (from a start codon located 4 bp upstream of the IncC-1 start) seems to compensate for the lack of autoregulatory functions of this ParA homologue. KorA recognizes the specific O_A operator sequence in *par* promoter (Regulatory Network section). KorA homologues have recently been discovered on other plasmids unrelated to IncP-1: pM3 (Greated *et al.*, 2000), pSB102 (Schneiker *et al.,* 2001), pIP02 (Tauch *et al.,* 2002), pXF51 (GeneBank accession No. NC002490), pRA2 (Kwong *et al.,* 2000) and pRA3 (Hayes, unpublished).

IncC inactivation causes a severe instability phenotype of mutant plasmids (Bignell *et al.*, 1999; Siddique & Figurski, 2002). When IncC function was impaired *in vivo*, the loss of stability of a minireplicon, composed of the P_7 replication cassette plus the *incCkorB* partitioning cassette, was due to a defect in the separation of plasmid molecules (observed as immunofluorescent foci linked in pairs) and the mislocalization of non-separated plasmid pairs to one cell pole (Bignell *et al.*, 1999).

ParB proteins share a similar architecture and properties within the family. Proteins classified as ParB type Ia have a helix-turn-helix motif in their central region, that is likely to be involved in DNA recognition (Bignell & Thomas, 2001). These factors can bind DNA and act as transcriptional repressors and silencers. There are many experimental data demonstrating the dimerization and multimerization properties of ParBs. The dimerization domain is usually located in the C-terminus of the protein (Lobocka & Yarmolinsky, 1996; Jagura-Burdzy *et al.*, 1999a; Kim & Shim, 1999). Typical ParB, as a component of the partitioning complex, has an ability to interact with ParA. The location of the domain which interacts with ParA seems to be more varied and may be found in the N-terminal, central or C-terminal parts of the ParB proteins (Surtees & Funnell, 1999; Kim & Shim, 1999; Lukaszewicz *et al.,* 2002).

The ParB equivalent protein of IncP-1 plasmids, $KorB_{RK2}$, possesses DNA binding activity and also forms dimers and multimers. The dimerization domain has been localized to the C-terminus, with that responsible for multimerization probably located in the central part of KorB (Lukaszewicz *et al.*, 2002; Delbruck *et al.,* 2002). The central region is also responsible for interaction with IncC (Lukaszewicz *et al.* 2002).

The cellular location of IncP-1 plasmids has been studied by immunofluorescence microscopy using anti-KorB antibodies (Bignell *et al.*, 1999). KorB localization is KorB DNA binding site-dependent. The KorB foci reflected a symmetrical plasmid distribution

pattern which matches that of other type Ia plasmids (F, P1), being coupled to the replication zone in the center of the cell and then moving to the 1/4 and 3/4 positions before division (Erdmann *et al.*, 1999; Bignell *et al.*, 1999). This pattern of localization is remarkably well conserved among the Gram-negative bacteria *P. aeruginosa*, *Vibrio cholerae* and *E. coli*. One focus of RK2 represents clustered plasmid molecules (Pogliano *et al.*, 2001; Pogliano, 2002). RK2 replication is coordinated with the host growth rate, so that the number of symmetrically localized foci per cell increases with cell length and decreases when the cells enter stationary phase. The correct RK2 plasmid distribution in filamentous cells proves that cell division is not required for plasmid segregation (Pogliano *et al.*, 2001; Ho *et al.*, 2002).

Although the IncP-1 *par* system appears to be closely related to that of the type Ia plasmids on the basis of ParA/ParB homologies, the *cis*-acting centromere-like sequence has not yet been precisely identified. The 13 bp sequences (5′TTTAGCCGCTAAA3′) specifically recognised by KorB (O_B) are dispersed throughout the whole plasmid. There are twelve such candidates (O_B1–O_B12) for the partitioning site in RK2 and eleven in R751 (see Regulatory Network). O_B3 which is present in both plasmids has been suggested to function as a centromere on inspection of the available data (Williams *et al.*, 1998). Firstly, KorB may bind to all O_Bs but only binding to O_B3 is not potentiated by the presence of IncC (Kostelidou & Thomas, 2000). Secondly, the removal of O_B3, leaving O_B1 and O_B2 intact, leads to a strong destabilization effect of the test plasmid (greater than random loss) (Williams *et al.*, 1998). However, the observation that this destabilization phenotype could be reversed to the loss rate of an unstable control by the deletion of O_B1 or inactivation of *korB* indicates that the situation is more complicated and prevents the drawing of firm conclusions as to the role of O_B3 in the resolution of RK2 partitioning pairs. So the question remains, whether one particular O_B acts as the centromere site in IncP-1 plasmids or all sites are equivalent and chosen for this function at random. It is also possible that a few or all of these sequence elements participate in the formation of plasmid pairs.

The mechanism by which plasmids are transferred to the desired locations within the cell remains unknown, besides the fact that it is dependent on the ParA ATPase activity (Bignell *et al.* 1999; Erdmann *et al.*, 1999). A recent double labelling study on P1, F and RK2 subcellular positioning indicated that each plasmid possesses its own unique strategy (Ho *et al.*, 2002). The positions in which plasmids normally reside as well as the timing of segregation differ. It has been previously suggested that the timing of segregation is regulated at the stage of replication and the resolution of plasmid dimers (Austin *et al.*, 1981). Recent studies correlate plasmid active partitioning with the cell cycle (Li & Austin, 2002).

Some important features of the segregation mechanism of plasmids using the type II active partitioning system (Fig. 4) have been elucidated recently. Studies on R1 plasmid revealed that ParM, an actin-like ATPase which is equivalent to ParA, forms pole-to-pole axial filaments which facilitate R1 plasmid DNA segregation (Moller-Jensen *et al.*, 2002). Filament formation requires the ParR protein (ParB homologue) and the centromere-like sequence *parC*, located upstream of the genes encoding ParM and ParR, and containing direct repeats to which ParR binds (Dam & Gerdes, 1994). ParR binding is important both for plasmid partitioning and for autoregulation of the *par* promoter. The new model implies that the ParM ATPase generates mechanical force that drives separation and subsequent movement of plasmid molecules to opposite cell poles after replication (Moller-Jensen *et al.*, 2002; Van den Ent *et al.*, 2002). In contrast to F, P or IncP-1, R1 plasmid molecules seem to be tethered at the

cell poles rather than at the 1/4 and 3/4 positions (Jensen & Gerdes, 1999). The R1 example has provided the first evidence that active daughter plasmid segregation is reliant upon a plasmid encoded analogue of the eukaryotic mitotic spindle apparatus. The fact that the ATPase activity is essential for regular intracellular localization of R1 argues that it has analogous function to those of other *par* families.

In the light of the above data concerning R1 plasmid segregation, it seems reasonable to look for mitotic spindle structures involved in the active partitioning of type I plasmids. Studies on R751 revealed that the *kfrA-upf54.8-upf54.4* region adjacent to the active partitioning operon plays an important role in the stability of the plasmid (Adamczyk & Jagura-Burdzy, unpublished). It has been suggested previously (Jagura-Burdzy & Thomas, 1992) that KfrA of RK2 might be involved in the active partitioning process (see *kfrA-upf54.8-upf54.4* Genes as Part of the Central Control Region). $KfrA_{RK2}$ was hypothesized to provide a filamentous bridge for the plasmid to travel along toward the cell poles after paired molecules separation (Williams & Thomas, 1992). The immunofluorescence studies on R751 plasmid confirmed the existence of such structures under conditions of KfrA over-production (Adamczyk, unpublished). Further experiments are needed to visualize intracellular structures without the necessity to over-produce the KfrA protein.

The efficiency of the KorB/IncC active partitioning system is reportedly improved by functions encoded by the *klc/kle* region in at least two hosts: *P. aeruginosa* and *E. coli* (Wilson *et al.,* 1997; Thorsted *et al.,* 1998; Bignell *et al*., 1999). When the *kle/klc* region was fused to the central control region (which on its own does not confer complete stability (Macartney *et al.,* 1997) no loss of a test plasmid carrying this region was observed in *E. coli* (Thorsted *et al.,* 1998). Inactivation of *incC* in the presence of the *kle/klc* region causes more severe plasmid destabilization than when the *klc/kle* region is absent. There are some differences in the gene content within the *klc/kle* region of plasmids RK2 and R751. The *kleCD* genes, which seem to represent duplicated *kleAB* genes in RK2, are absent from R751. On the other hand plasmid RK2 apparently lacks functional *klcB*, the second gene in the *klcA* operon, due to a Tn*1* transposon insertion. The *klcA* gene product of both plasmids shows homology to the ArdB anti-restriction proteins of the IncN plasmid pKM101, suggesting a protective role after conjugative transfer (Larsen & Figurski, 1994). The KorC proteins encoded by this region (*korC* has been recently re-named *klcC*) have no assigned function apart from being regulatory factor for the *klcA, kleA* and *kleC* promoters (see Regulatory Network) (Larsen & Figurski, 1994). Recently, additional elements have been described that may contribute to the stability effect provided by the *klc/kle* region. Between the *korC* and *kleA* genes two 9 bp direct repeats (DR) have been identified, which when placed *in trans* significantly destabilize the RK2 plasmid in *P. aeruginosa*. This effect has not been observed in *E. coli.* These DR elements appear to be a target for a plasmid-encoded protein and, interestingly, the actual sequences differ between RK2 and R751 (Wilson & Figurski, 2002).

Post-segregational killing (*psk*) and multimer resolution systems (*mrs*) as accessory stabilization mechanisms

Plasmids of the IncP-1β subgroup seem to rely only on active partitioning as the stability function, while IncP-1α plasmids possess additional stability determinants that are also confusingly termed *par* region (Gerlitz *et al*., 1990). The locus occurs between the Tra1 and Tra2 gene blocks in RK2. In R751 this locus is occupied by the Tn*402* mercury resistance transposon belonging to the Tn*5053*/Tn*402* family whose members are known as *res* site hunters. "*Par*" of RK2 encompasses two dis-

tinct stability functions: post-segregational killing (*psk*) and multimer-resolution system (*mrs*), encoded by two divergently arranged operons, *parDE* and *parCBA*, respectively.

parDE operon

Deletion of the *parDE* genes was found to produce an unstable plasmid phenotype in *E. coli* and in *P. aeruginosa* (Sia *et al.,* 1995). This operon was identified as the *psk* system. The presence of *psk* results in the death of plasmid-free segregants (Roberts & Helinski, 1992). The system ensures that plasmids are maintained in their natural hosts for a number of generations in the absence of selection. The post-segregational killing effect is based on a toxin-antidote principle. The plasmid encodes two components; a stable toxin and an unstable antidote, which prevents the lethal action of the toxin by direct interaction (proteic *psk*) or by posttranscriptional inhibition of its synthesis (by anti-sense RNA). Both elements of *psk* remain in the cytoplasm of plasmid free segregants and such cells are quickly deprived of the unstable antidote leaving the active toxin behind.

The *parDE* operon of RK2 codes for a proteic *psk* system. ParD is a 9 kDa protein which forms a homodimer with two functions: inhibition of ParE toxicity and an autoregulatory function through binding to a 33 bp region overlapping the −10 sequence of the *parDE* promoter. The N-terminal part of ParD has been found to possess DNA binding properties and anti-toxic activity (Roberts *et al.*, 1993). Recent data obtained from NMR analysis have shown that the DNA-binding domain of ParD belongs to the ribbon-helix-helix fold family like the prokaryotic transcriptional repressors of *E. coli*: Arc, MetJ, Mnt and the streptococcal plasmid pMV158-encoded CopG (Oberer *et al.*, 2002). The C-terminal part of ParD is very flexible. As this region appears to be responsible for ParE binding it may become structurally ordered upon anti-toxin-toxin complex formation.

The 12 kDa ParE protein also exists in solution as a homodimer which interacts with the ParD dimer. ParE alone is unable to bind to the *parDE* promoter region but can bind there when complexed with ParD (Johnson *et al.*, 1996). The specific cellular target for ParE toxicity has been recently identified as DNA gyrase (Jiang *et al.,* 2002). Inactivation of DNA gyrase by ParE leads to DNA replication inhibition and cell filamentation. It is possible to reverse the replication arrest *in vitro* by the addition of the ParD antitoxin to the ParE-inhibited gyrase (Jiang *et al.*, 2002).

Although the ParDE system was found to act post-segregationally it could also have some pre-segregational effect by providing a homeostatic control loop between host cell and plasmid replication. In this case impaired plasmid replication could lead to the inhibition of host replication *via* the toxin activity and thus render plasmid replication more efficient.

Similar killer gene systems are widely spread among plasmids and are also found in chromosomes of *E. coli* K-12 (Masuda *et al.*, 1993), *V. cholerae* (Heidelberg *et al.*, 2000) and the plant pathogen *Xylella fastidiosa* (Frohme *et al.*, 2000). It is still unclear as to how the chromosomally encoded *psk* could benefit bacteria but a possible role in programmed cell death under stress conditions has been discussed (Engelberg-Kulka & Glaser, 1999).

parCBA operon

The *mrs* system present in almost all plasmids and chromosomes ensures that after replication or recombinational events each copy of the plasmid/sister chromosome will function as a separate entity. Theoretically, an absence of this system would cause catenation of circular molecules at each replication cycle that could lead to so-called dimer catastrophy in the case of plasmid DNA (Summers *et al.*, 1993). In IncP-1α plasmids the *parABC* operon seems to provide the *mrs* functions.

The *parA* gene codes for a resolvase which acts on the plasmid multimer resolution site (*res*) to resolve multimers. This protein functions as a site-specific recombination system homologous to the Tn*3* family of resolvases. The *res* site is located within a 100 bp region between the divergent *parABC* and *parDE* promoters. Mutational analysis within the *parABC* region indicates that the *parA* gene is absolutely essential for the stabilization of mini-RK2. Single deletions in the *parB* or *parC* genes have relatively little effect on the stabilization. However, deletion of both the *parB* and *parC* genes significantly reduces the effectiveness of the whole operon and the *mrs* function becomes strain dependent (Easter *et al.*, 1998).

ParB is a Ca^{2+}-dependent endo- and 5′-3′ exonuclease (Johnson *et al.*, 1999) with homology to certain nucleases of *Staphylococci* and RuvC of *E. coli*. ParB has been shown to be a monomer in solution and display only weak sequence specificity. It is speculated that ParB plays a role in recombination, DNA repair and conversion of catenated plasmid dimers to the monomeric form. A putative signal sequence and a cleavage site found in the N-terminus of ParB suggest localization of the protein in the cell envelope.

The function of ParC has not yet been determined.

It is worth mentioning that plasmid RK2 carries a functional Tn*1* transposon with an active ParA-like resolvase, although deletion of this transposon has little effect on the stability of the plasmid compared to deletion of the *parABC* region.

Plasmids from IncP-1β subgroup do not possess the *parABCDE* locus however their stability seems to be unaffected. The question of how the lack of the *parABCDE* locus can be compensated for by IncP-1β plasmids is still unanswered.

THE REGULATORY NETWORK OF IncP-1 PLASMIDS

IncP-1 plasmids could easily become a burden to the host metabolism unless there is some regulation of plasmid gene expression. The regulation of gene expression is achieved by multiple factors and each operon on the plasmid is controlled by more than one

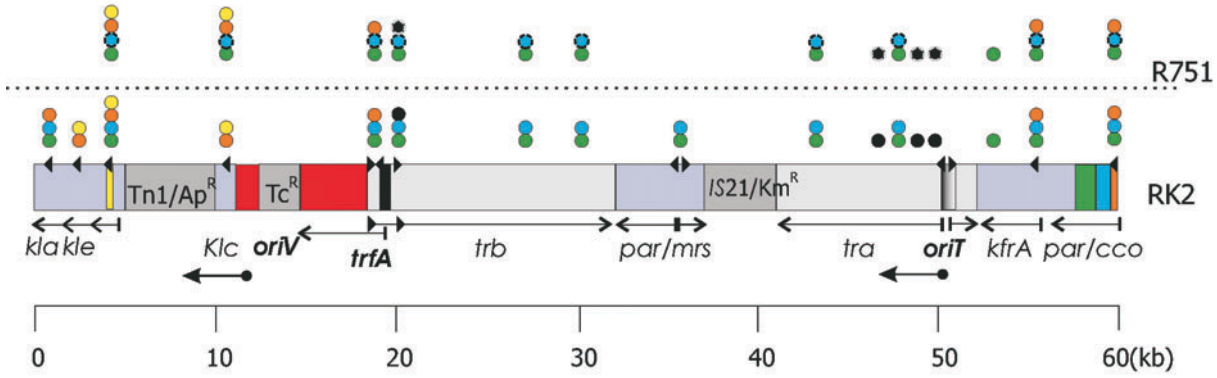

Figure 5. A comparison of regulatory networks of the RK2 and R751 plasmids.

A linear map of the RK2 plasmid. Vegetative replication functions are marked red, light grey boxes correspond to transfer functions, bluish, stability functions; dark grey, phenotypic markers and transposable elements. General transcriptional organization of the replication, transfer and stable inheritance functions are shown by arrows. The scheme shows in colours the regions from which regulators are originated: orange, *korA*, blue, *incC*, green, *korB*, black, *trbA*, yellow, *korC*. Sites of action for repressors are showed as filled circles (for R751 some aspects of the regulatory network are hypothetical, extrapolated from the experimental data for RK2 and marked here by broken external lines). The O_A and O_B operators are numbered on RK2 map from right to left.

repressor. It is possible that in this way broad-host range plasmids respond to variable concentrations of repressors in different hosts. In addition, cooperativity in the action of different repressors potentiates the regulatory effect that a single repressor can exert. Overlapping regulatory circuits provide coordination of regulatory response that is tight but sensitive.

Besides the local control circuits mentioned in the previous sections (e.g. relaxosome operon, *parABCDE*), a common feature of dimer in solution (Jagura-Burdzy & Thomas, 1995). The KorA monomer consists of three functional domains: the N-terminal region I acts as the dimerization domain (Kostelidou *et al.*, 1998), region II containing a H-T-H motif facilities binding to O_A operators (5′TTTAGCTAAA3′) (Smith *et al.*, 1984), while the C-terminal domain is required for interaction with KorB (Kostelidou *et al.*, 1999), although some data suggest that it also plays a role in dimerization (Bhattacharayya & Figurski, 2001). Regions I and II are sepa-

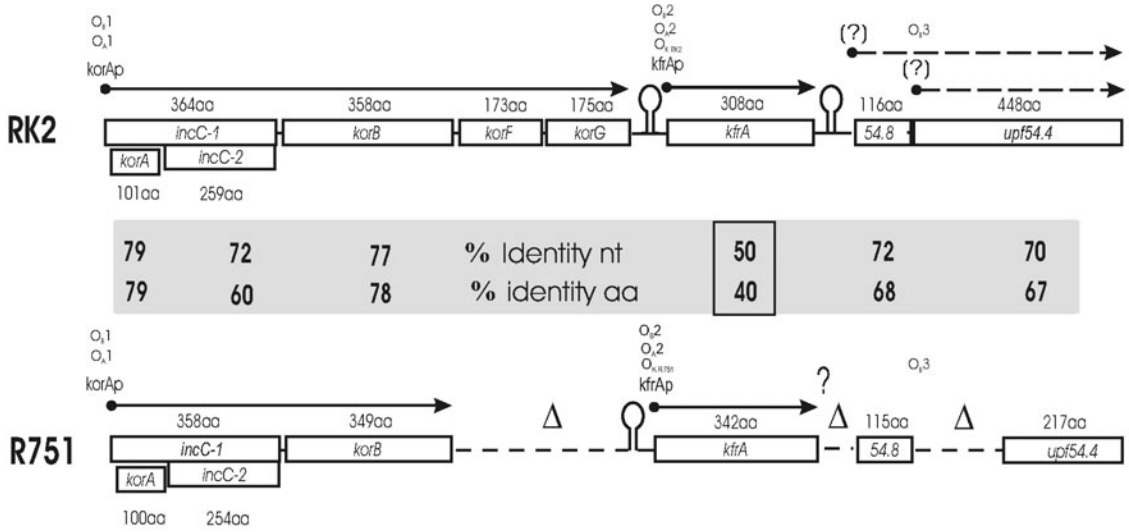

Figure 6. Comparison of the central control regions of RK2 and R751.

ORFs are shown as open boxes, deletions in R751 are indicated by the delta symbol, transcripts (continuous arrows) and putative transcripts (broken lines) are marked, Rho-independent terminators are shown as stem loops and binding sites for KorA (O_A), KorB (O_B) and KfrA (O_K) are listed. The numbers in the shaded box correspond to % nucleotide and amino acid identities between the two plasmids.

IncP-1 plasmids is the existence of regulatory circuits in which at least four global repressors KorA, KorB, KorC and TrbA are involved.

KorA and KorB are encoded by the same operon (Figs. 4 and 6), located within the replication/stability segment in a region termed the central control region (*ccr*) which was mentioned earlier (Fig. 4) due to its function in active partitioning.

The $korA_{RK2}$ gene encodes a 101 amino-acid protein ($KorA_{R751}$ – 100 aa). This is a potent global regulator that co-operates with KorB (Kostelidou *et al.*, 1999) and exists as a homo-rated from the C-terminal domain by a 4 aa linker sequence which might result in physical (spatial) demarcation in the KorA tertiary structure (Kostelidou *et al.*, 1998). There are seven KorA binding sites in RK2 and five in R751. In plasmid RK2 the O_A operators have been assigned to class I (O_A1 *korAp*, O_A3 *trfAp*, O_A7 *klaAp*) or II (O_A2 *kfrAp*, O_A4 *klcAp*, O_A5 *kleAp*, O_A6 *kleCp*) (Jagura-Burdzy & Thomas, 1995). This classification is based on the affinity of KorA binding which coincides with the localization of O_A within the promoter sequences, class I O_As overlap the –10 sequences whereas the low affinity class II

O_As precede the -35 sequence of the regulated promoters. The affinity of binding depends on how perfect the palindromic sequence is and on the nature of flanking sequences. KorA has been shown to bind to and repress all promoters in which O_A operators are present. The genes whose expression is regulated by KorA are involved in vegetative replication and stable maintenance. The strength of these regulated promoters is similar in both RK2 and R751. *korA* promoter, containing O_A1, is the most strongly KorA-repressed promoter in both plasmids (Kostelidou & Thomas, 2002). Through binding to the O_A3 operator, KorA mediates the switch in gene expression between vegetative replication and conjugation transfer that takes place at the *trfAp/trbAp* face-to-face divergent promoters. The strong transcriptional activity of *trfAp* inhibits RNA polymerase (RNAP) proceeding from *trbAp*. KorA plays a dominant role in the repression of the *trfA* promoter, causing elevation of the *trbA* promoter activity at the same time (Jagura-Burdzy & Thomas, 1994; Jagura-Burdzy & Thomas, 1995).

$KorB_{RK2}$ (358 aa) binds to O_Bs with different affinities (Kostelidou & Thomas, 2000). Operator O_B10 in *trfAp* is bound by KorB with the highest affinity, which implies that it is a key element in controlling replication. O_B2 and O_B6 carrying a one base pair change (A→G) at position +4 from the centre of symmetry of the operator, which disrupts the sequence symmetry compared to the O_B consensus sequence (5′TTTAGC$^{G}/_{C}$GCTAAA3′), have been shown to bind KorB with the lowest affinity. Additionally, flanking sequences modulate KorB binding to the O_B operators (Kostelidou & Thomas, 2000). Among global regulators, KorB has the most general function in the IncP-1 plasmid biology. It represses operons involved not only in the vegetative replication and stable maintenance but also those required for conjugative transfer (Motallebi-Veshareh *et al.,* 1992).

As mentioned earlier there are eleven and twelve highly conserved operator sequences recognized by KorB in the IncP-1 plasmids R751 and RK2, respectively. The location of these sequences in respect to the different promoters is the basis for the classification of $KorB_{RK2}$ operators into three classes. Class I sites (O_B1 *korAp*, O_B10 *trfAp*, and O_B12 *klaAp*) lie immediately upstream of the –35 region of each promoter, Class II sites (O_B2 *kfrAp*, O_B9 *trbBp*, O_B10 *trbAp* and O_B11 *kleAp*) are located up to 189 bp upstream or downstream of the transcriptional start

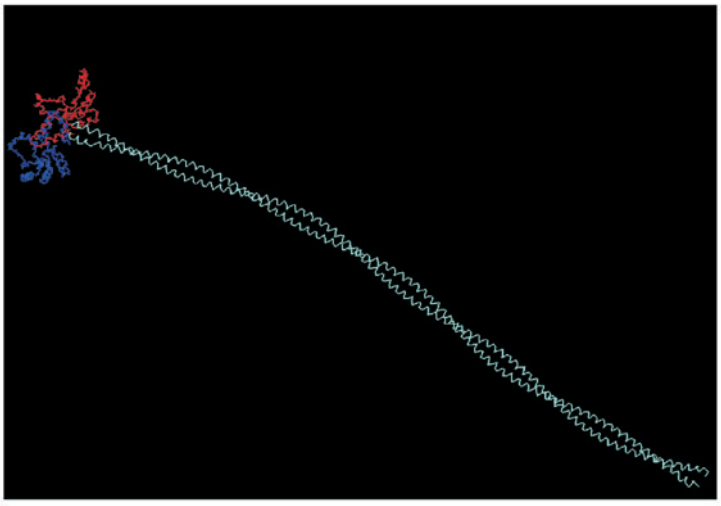

Figure 7. Predicted structure of $KfrA_{R751}$ (Dr .D. Płochocka, Dept. of Bioinformatics, Institute of Biochemistry & Biophysics, PAS).

The hypothetical dimer is modelled because α-helical proteins have a tendency to form dimeric coiled-coil structures.

points, whereas Class III is comprised of six (intergenic/intragenic) sites (O_B3 to O_B8) located at least 1 kb from the promoters (Balzer *et al.*, 1992; Williams *et al.*, 1993; Kostelidou & Thomas, 2000) (Fig. 5). It has been established that KorB represses transcription by binding to the Class I and II operators (Jagura-Burdzy *et al.,* 1999a), but not to Class III sites. In the case of Class II O_Bs, KorB acts at a distance and formation of KorB dimers/multimers is required for the repressor function (Jagura-Burdzy *et al.,* 1999a). KorB repression at a distance from the site of binding is facilitated by co-operation with other repressors KorA (Kostelidou *et al.,* 1999) and TrbA (Zatyka *et al.,* 2001).

IncC-1 which is encoded by the same operon as KorA and KorB and is involved with KorB in active partitioning as a ParA homologue, is also an important factor in the regulation of gene expression. Although it does not bind to DNA, IncC-1 enhances KorB repression at Class I and II operators. It also stimulates KorB binding and the formation of higher order complexes at all operators except for O_B3 (Jagura-Burdzy *et al.,* 1999b).

A third global regulator, KorC (85 aa) acts at three promoters, *klcAp*, *kleAp* and *kleCp* of RK2 and at two promoters, *klcAp* and *kleAp*, of R751 in the regions providing both plasmids with additional stability functions. It recognizes the palindromic O_C sequence (5′TAGGGCATAATGCCCTA3′), located at the –10 region of both promoters (Thomas *et al.,*1988; Kornacki *et al.,* 1990).

The last global repressor to be considered, $TrbA_{RK2}$ (121 aa) ($TrbA_{R751}$—120 aa), has been shown to repress transcription from promoters of operons involved in conjugative transfer: *traGp, traJp, traKp* and *trbBp* (Zatyka *et al.,* 1994). The binding site for TrbA (O_T) (consensus 5′CGATATATCG3′) is poorly conserved but is located close to the –10 region of regulated promoters (Zatyka *et al.,* 1994, Bingle & Thomas, unpublished). The C-terminal part of TrbA shares similarity with the C-terminal domain of KorA and is probably responsible for interaction with KorB. The co-operativity between TrbA and KorB in *trbAp* regulation takes place despite the significantly long distance (150 bp) between O_B and O_T operators (Zatyka *et al.,* 1994; 2001).

kfrA, *upf54.8* AND *upf54.4*, GENE, AS PART OF THE CENTRAL CONTROL REGION (*ccr*)

The organization of the essential *ccr* of plasmid RK2 and R751 is basically the same. It contains the central control operon *cco* and three genes transcribed in the same direction: *kfrA, upf54.8* and *upf54.4*. The genes encoding KorA and KorB, the key global regulators, as well as *incC* and the specific recognition sites O_A and O_B are highly conserved (Fig. 6). Clustering of the regulatory functions into a single operon is unique among plasmids. Besides *korA*, *incC* and *korB*, the *cco* of RK2 also contains two additional regulatory genes *korF* and *korG* which encode putative histone-like proteins. KorF (173 aa) and KorG (175 aa) repress transcription from *kfrAp* and *trfAp*, the two strongest promoters on plasmid RK2 (Jagura-Burdzy *et al.*, 1991). Plasmid R751 lacks the *korF* and *korG* genes.

The *ccr* region of RK2 and R751 contains three O_B sites: O_B1 (Class I) is in the *korA* promoter, O_B2 (Class II) within the *kfrA* promoter and O_B3 (Class III) in the intergenic region between *upf54.*8 and *upf54.4* (Fig. 6). O_B2 in RK2 is unique, being the only KorB binding site without the perfect consensus sequence (it has an A→G transition at +4 position) (Macartney *et al.*, 1997). In O_B2 R751 a perfect palindrome core is extended for 15 bp rather than 13 bp. The intergenic O_B3 site was postulated to play a centromere-like role in the active partitioning process (Williams *et al.,* 1998).

While the *cco* gene products are conserved between plasmids RK2 and R751 and have assigned functions, *upf54.8* and *upf54.4* are orphan genes encoding proteins of high gluta-

mine content. The Upf54.4 amino acid sequence contains the purine/pirimidine phosphoribosyl transferase signature (Pansegrau *et al.,* 1994), whereas Upf54.8 shows the highest homology (58%) to the anthranilate synthase component II (Blast search). The sequence conservation of these genes between RK2 and R751 is significantly high (Fig. 6) although Upf54.4$_{RK2}$, in comparison with Upf54.4$_{R751}$, contains an additional 231 aa at the N-terminus (Pansegrau *et al.,* 1994).

kfrA$_{RK2}$ encodes a protein of 308 amino acids whereas *kfrA*$_{R751}$ codes for a protein of 342a.a. Both have high alanine content. At the DNA level *kfrA*$_{RK2}$ and *kfrA*$_{R751}$ show only 50% identity. High G+C content and presence of G+C-rich repeats in this gene promote illegitimate recombination resulting in local deletions or duplications. Although the homology between KfrA$_{RK2}$ and KfrA$_{R751}$ is low at both the nucleotide and amino-acid levels, both proteins have a conserved tertiary structure. KfrA$_{RK2}$ was predicted to possess an unusually long α-helical tail and a globular head domain. The α-helical tails might form coiled-coil structures organized in a filamentous network, which has been hypothesized to facilitate the plasmid partitioning process during cell division (Jagura-Burdzy & Thomas, 1992; Williams & Thomas, 1992). The predicted structure for KfrA$_{R751}$ shows the same coiled-coil folding (Fig. 7).

Experimental data have revealed that both KfrA$_{RK2}$ (Jagura-Burdzy & Thomas, 1992) and KfrA$_{R751}$ (Adamczyk & Jagura-Burdzy, unpublished) repress their own transcription and that this function is dependent on the N-terminal region. Both KfrAs can form dimers and higher order complexes *in vitro*. *kfrA*$_{RK2}$ has been postulated to form a monocistronic operon (Thomas *et al.,* 1990) although there are some observations which suggest that expression of the two *upfs* in an RK2 derivative is dependent on transcription from *kfrAp* (Williams & Thomas, unpublished). Recent studies on KfrA$_{R751}$ revealed that *upf54.8* and *upf54.4* belong to the *kfrA* operon and the genes have been renamed *kfrB* and *kfrC,* respectively (Adamczyk & Jagura-Burdzy, unpublished). KfrA interacts with KfrB (Upf54.8) whereas KfrC (Upf54.4) has the ability to interact with KfrB implicating that all three proteins may form a multiprotein complex. A role for such complex in the stable inheritance of IncP-1 plasmids has been postulated (Adamczyk & Jagura- Burdzy, unpublished).

The steadily increasing volume of plasmid sequence data has shown that KfrA-like proteins are encoded by many plasmids of different incompatibility groups (Kwong *et al.,* 1998; 2000; Tauch *et al.,* 2002; Burland *et al.,* 1998; Close & Kado, 1992; Haneda *et al.,* 2001). The fact that *kfrA*-related genes are frequently found close to the *par* locus or the *oriV* region rises the possibility that the biological role of this protein is more general and important for key survival functions.

CONCLUSIONS

The IncP-1 plasmids represent extremely well organized genetic units. Their genomes consist of a basic backbone disrupted by mobile elements with different phenotypic traits. The regions coding for "survival" functions may be physically separated but the genes remain co-regulated due to the global regulatory network The network of at least four overlapping regulons is supported by autoregulatory circuits. Such multivalent regulatory system provides unique coordination of all plasmid functions: vegetative replication, stable maintenance and conjugative transfer, facilitates the fine-tuning of gene expression and at the same time creates a safety device to minimize the effects of genetic changes or cell-cycle fluctuations. Assembly of the regulatory module (central control operon coding for at least three global regulators) is the unique feature of IncP-1 plasmids. It is believed that flexibility in the replication initiation, multiple stability mechanisms and coordinate regulation of

all plasmid backbone functions guarantee the enormous adaptability and stable maintenance of the IncP-1 plasmids in such a wide spectrum of hosts.

REFERENCES

Austin S, Ziese M, Sternberg N. (1981) A novel role for site-specific recombination in maintenance of bacterial replicons. *Cell.*; **25**: 729–36.

Balzer D, Ziegelin G, Pansegrau W, Kruft V, Lanka E. (1992) KorB protein of promiscuous plasmid RP4 recognizes inverted sequence repetitions in regions essential for conjugative plasmid transfer. *Nucleic Acids Res.*; **20**: 1851–8.

Beil S, Timmis KN, Pieper DH. (1999) Genetic and biochemical analyses of the *tec* operon suggest a route for evolution of chlorobenzene degradation genes. *J Bacteriol.*; **181**: 341–6.

Bhattacharayya A, Figurski DH. (2001) A small protein-protein interaction domain common to KlcB and global regulators of KorA and TrbA of promiscuous IncP plasmids. *J Mol Biol.*; **310**: 51–61.

Bignell CR, Haines AS, Khare D, Thomas CM. (1999) Effect of growth rate and *incC* mutation on symmetric plasmid distribution by the IncP-1 partitioning apparatus. *Mol Microbiol.*; **34**: 205–16.

Bignell CR, Thomas CM. (2001) The bacterial ParA-ParB partitioning proteins. *J Biotechnol.*; **91**: 1–34.

Bouet J-Y, Funnell BE. (1999) P1 ParA interacts with the P1 partition complex at *parS* and ATP-ADP-switch controls ParA activities. *EMBO J.*; **18**: 1415–24.

Burlage RS, Bemis LA, Layton AC, Sayler GS, Larimer F. (1990) Comparative genetic organization of incompatibility group P degradative plasmids. *J Bacteriol.*; **172**: 6818–25.

Burland V, Shao Y, Perna NT, Plunkett G, Sofia HJ, Blattner FR. (1998) The complete DNA sequence and analysis of the large virulence plasmid of Escherichia coli O157:H7. *Nucleic Acids Res.*; **26**: 4196–204.

Cabezon E, Lanka E, de la Cruz CF. (1994) Requirements for mobilization of plasmids RSF1010 and ColE1 by the IncW plasmid R388: *trwB* and RP4 *traG* are interchangeable. *J Bacteriol.*; **176**: 4455–8.

Caspi R, Pacek M, Consiglieri G, Helinski DR, Toukdarian A, Konieczny I. (2001) A broad host range replicon with different requirements for replication initiation in three bacterial species. *EMBO J.*; **20**: 3262–71.

Cerin H, Hackett J. (1989) Molecular cloning and analysis of the incompatibility and partition functions of the virulence plasmid of *Salmonella typhimurium. Microb Pathog.*; **7**: 85–99.

Cerin H, Hackett J. (1993) The *parVP* region of the *Salmonella typhimurium* virulence plasmid pSLT contains four loci required for incompatibility and partition. *Plasmid.*; **30**: 30–8.

Close SM, Kado CI. (1992) A gene near the plasmid pSa origin of replication encodes a nuclease. *Mol Microbiol.*; **6**: 521–7.

Dam M, Gerdes K. (1994) Partitioning of plasmid R1. Ten direct repeats flanking the *parA* promoter constitute a centromere-like partition site *parC* that expresses incompatibility. *J Mol Biol.*; **236**: 1289–98.

Daugelavicius R, Bamford JK, Grahn AM, Lanka E, Bamford DH. (1997) The IncP plasmid-encoded cell envelope-associated DNA transfer complex increases cell permeability. *J Bacteriol.*; **179**: 5195–202.

Davey MJ, Funnell BE. (1997) Modulation of the P1 plasmid partition protein ParA by ATP, ADP, and P1 ParB. *J Biol Chem.*; **272**: 15286–92.

Delbruck H, Ziegelin G, Lanka E, Heinemann U. (2002) An Src homology 3-like domain is responsible for dimerization of the repressor protein KorB encoded by the promiscuous IncP plasmid RP4. *J Biol Chem.*; **277**: 4191–8.

Doran KS, Helinski DR, Konieczny I. (1999) Host-dependent requirement for specific DnaA boxes for plasmid RK2 replication. *Mol Microbiol.*; **33**: 490–8.

Droge M, Puhler A, Selbitschka W. (2000) Phenotypic and molecular characterization of conjugative antibiotic resistance plasmids isolated from bacterial communities of activated sludge. *Mol Gen Genet.*; **263**: 471–82.

Easter CL, Schwab H, Helinski DR. (1998) Role of the *parCBA* operon of the broad-host-range plasmid RK2 in stable plasmid maintenance. *J Bacteriol.*; **180**: 6023–30.

Eisenbrandt R, Kalkum M, Lai EM, Lurz R, Kado CI, Lanka E. (1999) Conjugative pili of IncP plasmids, and the Ti plasmid T pilus are composed of cyclic subunits *J. Biol Chem.*; **274**: 22548–55.

Eisenbrandt R, Kalkum M, Lurz R, Lanka E. (2000) Maturation of IncP pilin precursors resembles the catalytic dyad-like mechanism of leader peptidases. *J Bacteriol.*; **182**: 6751–61.

Engelberg-Kulka H, Glaser G. (1999) Addiction modules and programmed cell death and antideath in bacterial cultures. *Annu Rev Microbiol.*; **53**: 43–70.

Erdmann N, Petroff T, Funnell BE. (1999) Intracellular localization of P1 ParB protein depends on ParA and *parS. Proc Natl Acad Sci U S A.*; **96**: 14905–10.

Frohme M, Camargo AA, Heber S, Czink C, Simpson AJ, Hoheisel JD, de Souza AP. (2000) Mapping analysis of the *Xylella fastidiosa* genome. *Nucleic Acids Res.*; **28**: 3100–4.

Gerdes K, Moller-Jensen J, Bugge JR. (2000) Plasmid and chromosome partitioning: surprises from phylogeny. *Mol Microbiol.*; **37**: 455–66.

Gerlitz M, Hrabak O, Schwab H. (1990) Partitioning of broad-host-range plasmid RP4 is a complex system involving site-specific recombination. *J Bacteriol.*; **172**: 6194–203.

Giebelhaus LA, Frost L, Lanka E, Gormley EP, Davies JE, Leskiw B. (1996) The Tra2 core of the IncP(alpha) plasmid RP4 is required for intergeneric mating between *Escherichia coli* and *Streptomyces lividans*. *J Bacteriol.*; **178**: 6378–81.

Godfrin-Estevenon A-M, Pasta F, Lane D. (2002) The *parAB* gene products of *Pseudomonas putida* exhibit partition activity in both *P. putida* and *Escherichia coli. Mol Microbiol.;* **43**: 39–49.

Gotz A, Pukall R, Smit E, Tietze E, Prager R, Tschape H, van Elsas JD, Smalla K. (1996) Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR. *Appl Environ Microbiol.*; **62**: 2621–8.

Grahn AM, Haase J, Bamford DH, Lanka E. (2000) Components of the RP4 conjugative transfer apparatus form an envelope structure bridging inner and outer membranes of donor cells: implications for related macromolecule transport systems. *J Bacteriol.*; **182**: 1564–74.

Greated A, Titok M, Krasowiak R, Fairclough RJ, Thomas CM. (2000) The replication and stable-inheritance functions of IncP-9 plasmid pM3. *Microbiology.*; **146**: 2249–58.

Grigoriev PS, Lobocka MB. (2001) Determinants of segregational stability of the linear plasmid-prophage N15 of *Escherichia coli. Mol Microbiol.;* **42**: 355–68.

Haase J, Lurz R, Grahn AM, Bamford DH, Lanka E. (1995) Bacterial conjugation mediated by plasmid RP4: RSF1010 mobilization, donor-specific phage propagation, and pilus production require the same Tra2 core components of a proposed DNA transport complex. *J Bacteriol.;* **177**: 4779–91.

Haase J, Kalkum M, Lanka E. (1996) TrbK, a small cytoplasmic membrane lipoprotein, functions in entry exclusion of the IncP alpha plasmid RP4. *J Bacteriol.*; **178**: 6720–9.

Haneda T, Okada N, Nakazawa N, Kawakami T, Danbara H. (2001) Complete DNA sequence and comparative analysis of the 50-kilobase virulence plasmid of *Salmonella enterica* serovar *Choleraesuis*. *Infect Immun.;* **69**: 2612–20.

Hayakawa Y, Murotsu T, Matsubara K. (1985) Mini-F protein that binds to a unique region for partition of mini-F plasmid DNA. *J Bacteriol.*; **163**: 349–54.

Hayes F. (2000) The partition system of multidrug resistance plasmid TP228 includes a novel protein that epitomizes an evolutionarily distinct subgroup of the ParA superfamily. *Mol Microbiol.;* **37**: 528–41.

Heidelberg JF, Eisen J A, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L, Gill SR, Nelson KE, Read TD, Tettelin H, Richardson D, Ermolaeva MD, Vamathevan J, Bass S, Qin H, Dragoi I, Sellers P, McDonald L, Utterback T, Fleishmann RD, Nierman WC, White O. (2000) DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature.*; **406**: 477–83.

Heinemann JA, Sprague GF Jr. (1989) Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature.*; **340**: 205–9.

Helsberg M, Eichenlaub R. (1986) Twelve 43-base-pair repeats map in a *cis*-acting region essential for partition of plasmid mini-F. *J Bacteriol.*; **165**: 1043–5.

Ho TQ, Zhong Z, Aung S, Pogliano J. (2002) Compatible bacterial plasmids are targeted to independent cellular locations in *Escherichia coli. EMBO J.;* **21**: 1864–72.

Hooper SW, Dockendorff TC, Sayler GS. (1989) Characteristics and restriction analysis of the 4-chlorobiphenyl catabolic plasmid, pSS50. *Appl Environ Microbiol.*; **55**: 1286–8.

Ireton K, Gunther NW, Grossman AD. (1994) Spo0J is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J Bacteriol.*; **176**: 5320–9.

Jagura-Burdzy G, Ibbotson JP, Thomas CM. (1991) The *korF* region of broad-host-range plasmid RK2 encodes two polypeptides with transcriptional repressor activity. *J Bacteriol.*; **173**: 826–33.

Jagura-Burdzy G, Thomas CM. (1992) *kfrA* gene of broad host range plasmid RK2 encodes a novel DNA-binding protein. *J Mol Biol.*; **225**: 651–60.

Jagura-Burdzy G, Thomas CM. (1994) KorA protein of promiscuous plasmid RK2 controls a transcriptional switch between divergent operons for plasmid replication and conjugative transfer. *Proc Natl Acad Sci U S A.*; **91**: 10571–5.

Jagura-Burdzy G, Thomas CM. (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, Macartney DP, Zatyka M, Cunliffe L, Cooke D, Huggins C, Westblade L, Khanim F, Thomas CM. (1999a) Repression at a distance by the global regulator KorB of promiscuous IncP plasmids. *Mol Microbiol.*; **32**: 519–32.

Jagura-Burdzy G, Kostelidou K, Pole J, Khare D, Jones A, Williams DR Thomas CM. (1999b) IncC of broad-host-range plasmid RK2 modulates KorB transcriptional repressor activity *in vivo* and operator binding *in vitro*. *J Bacteriol.*; **181**: 2807–15.

Jensen RB, Gerdes K. (1999) Mechanism of DNA segregation in prokaryotes: ParM partitioning protein of plasmid R1 co-localizes with its replicon during the cell cycle. *EMBO J.*; **18**: 4076–84.

Jiang Y, Pogliano J, Helinski DR, Konieczny I. (2002) ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of *Escherichia coli* gyrase. *Mol Microbiol.*; **44**: 971–9.

Johnson EP, Strom AR, Helinski DR. (1996) Plasmid RK2 toxin protein ParE: purification and interaction with the ParD antitoxin protein. *J Bacteriol.*; **178**: 1420–9.

Johnson EP, Mincer T, Schwab H, Burgin AB, Helinski DR. (1999) Plasmid RK2 ParB protein: purification and nuclease properties. *J Bacteriol.*; **181**: 6010–8.

Jovanovic OS, Figurski DH. (1997) A potential new gene (*tccA*) on IncP plasmid RK2 and transposon Tn*1721*: relationship of its product to the TrwC relaxase/helicase of IncW plasmid R388. *Plasmid.*; **38**: 220–3.

Kim SK, Shim J. (1999) Interaction between F plasmid partition proteins SopA and SopB. *Biochem Biophys Res Commun.*; **263**: 113–7.

Konieczny I, Helsinki, DR. (1997a) Helicase delivery and activation by DnaA and TrfA proteins during the initiation of replication of the broad host range plasmid RK2. *J Biol Chem.*; **272**: 33312–8.

Konieczny I, Helsinki DR. (1997b) The replication initiation protein of the broad-host-range plasmid RK2 is activated by the ClpX chaperone. *Proc Natl Acad Sci U S A.*; **94**: 14378–82.

Konieczny I, Doran KS, Helinski DR, Blasina A. (1997) Role of TrfA and DnaA proteins in origin opening during initiation of DNA replication of the broad host range plasmid RK2. *J Biol Chem.*; **272**: 20173–8.

Konieczny I, Liberek K. (2002) Cooperative action of *Escherichia coli* ClpB protein and DnaK chaperone in the activation of a replication initiation protein. *J Biol Chem.*; **277**: 18483–8.

Koonin EV. (1993) A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. *J Mol Biol.*; **229**: 1165–74.

Kornacki JA, Burlage RS, Figurski DH. (1990) The *kil-kor* regulon of broad-host range plasmid RK2: nucleotide sequence, polypeptide product, and expression of regulatory gene *korC*. *J Bacteriol.*; **172**: 3040–50

Kostelidou K, Jagura-Burdzy G, Thomas CM. (1998) Mutational analysis of the global regulator KorA of broad-host-range plasmid RK2. *J Mol Biol.*; **281**: 453–63.

Kostelidou K, Jones AC, Thomas CM. (1999) Conserved C-terminal region of global repressor KorA of broad-host-range plasmid RK2 is required for co-operativity between KorA and a second RK2 global regulator, KorB. *J Mol Biol.*; **289**: 211–21.

Kostelidou K, Thomas CM. (2000) The hierarchy of KorB binding at its 12 binding sites on the broad-host-range plasmid RK2 and modulation of this binding by IncC1 protein. *J Mol Biol.*; **295**: 411–22.

Kostelidou K, Thomas CM. (2002) DNA recognition by the KorA proteins of IncP-1 plasmids RK2 and R751. *Biochim Biophys Acta.*; **1576**: 110–8.

Krause S, Barcena M, Pansegrau W, Lurz R, Carazo JM, Lanka E. (2000a) Sequence-related protein export NTPases encoded by the conjugative transfer region of RP4 and by the *cag* pathogenicity island of *Helicobacter pylori* share similar hexameric ring structures. *Proc Natl Acad Sci U S A.;* **97**: 3067–72.

Krause S, Pansegrau W, Lurz R, de la Cruz CF, Lanka E. (2000b) Enzymology of type IV macromolecule secretion systems: the conjugative transfer regions of plasmids RP4 and R388 and the *cag* pathogenicity island of *Helicobacter pylori* encode structurally and functionally related nucleoside triphosphate hydrolases. *J Bacteriol.*; **182**: 2761–70.

Kwong SM, Yeo CC, Chuah D, Poh CL. (1998) Sequence analysis of plasmid pRA2 from *Pseudomonas alcaligenes* NCIB 9867 (P25X) reveals a novel replication region. *FEMS Microbiol Lett.*; **158**: 159–65.

Kwong SM, Yeo CC, Suwanto A, Poh CL. (2000) Characterization of the endogenous plasmid from *Pseudomonas alcaligenes* NCIB 9867: DNA sequence and mechanism of transfer. *J Bacteriol.*; **182**: 81–90.

Lane D, Rothenbuehler R, Merrillat AM, Aiken C. (1987) Analysis of the F plasmid centromere. *Mol Gen Genet.*; **207**: 406–12.

Larsen MH, Figurski DH. (1994) Structure, expression, and regulation of the *kilC* operon of promiscuous IncP alpha plasmids. *J Bacteriol.*; **176**: 5022–32.

Lawley TD, Gordons GS, Wright A, Taylor DE. (2002) Bacterial conjugative transfer: visualization of successful mating pairs and plasmid establishment in live *Escherichia coli*. *Mol Microbiol.*; **44**: 947–56.

Leveau JH, van der Meer JR. (1997) Genetic characterization of insertion sequence ISJP4 on plasmid pJP4 from *Ralstonia eutropha* JMP134. *Gene.*; **202**: 103–14.

Lewis PJ, Errington J. (1997) Direct evidence for active segregation of *oriC* regions of the *Bacillus subtilis* chromosome and co-localization with the Spo0J partitioning protein. *Mol Microbiol.*; **2**: 945–54.

Lewis RA, Bignell CR, Zeng W, Jones AC, Thomas CM. (2002) Chromosome loss from *par* mutants of *Pseudomonas putida* depends on growth medium and phase of growth. *Microbiology.*; **148**: 537–48.

Li Y, Austin S. (2002) The P1 plasmid in action: time-lapse photomicroscopy reveals some unexpected aspects of plasmid partition. *Plasmid.*; **48**: 174–8.

Lin Z, Mallavia LP. (1994) Identification of a partition region carried by the plasmid QpH1 of *Coxiella burnetii*. *Mol Microbiol.*; **13**: 513–23.

Lin Z, Mallavia LP. (1998) Membrane association of active plasmid partitioning protein A in *Escherichia coli*. *J Biol Chem.*; **273**: 11302–12.

Lin Z, Hiasa H, Kumar U, DiGate RJ. (1997) The *traE* gene of plasmid RP4 encodes a homologue of *Escherichia coli* DNA topoisomerase III. *J Biol Chem.*; **272**: 19582–7.

Lobocka M, Yarmolinsky M. (1996) P1 plasmid partition: a mutational analysis of ParB. *J Mol Biol.*; **259**: 366–82.

Lukaszewicz M, Kostelidou K, Bartosik A, Cooke GD, Thomas CM, Jagura-Burdzy G. (2002) Functional dissection of the ParB homologue (KorB) from IncP-1 plasmid RK2. *Nucleic Acids Res.*; **30**: 1046–55.

Macartney DP, Williams DR, Stafford T, Thomas CM. (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**: 2167–77.

Martinez B, Tomkins J, Wackett LP, Wing R, Sadowsky MJ. (2001) Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *J Bacteriol.*; **183**: 5684–97.

Masuda Y, Miyakawa K, Nishimura Y, Ohtsubo E. (1993) *chpA* and *chpB*, *Escherichia coli* chromosomal homologues of the *pem* locus responsible for stable maintenance of plasmid R100. *J Bacteriol.*; **175**: 6850–6.

Mohl DA, Gober JW. (1997) Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell.*; **88**: 675–84.

Mohl DA, Easter J, Gober JW. (2001) The chromosome partitioning protein, ParB, is required for cytokinesis in *Caulobacter crescentus*. *Mol Microbiol.*; **42**: 741–55.

Moller-Jensen J, Jensen RB, Lowe J, Gerdes K. (2002) Prokaryotic DNA segregation by an actin-like filament. *EMBO J.*; **21**: 3119–27.

Motallebi-Veshareh M, Rouch DA, Thomas CM. (1990) A family of ATPases involved in active partitioning of diverse bacterial plasmids. *Mol Microbiol.*; **4**: 1455–63.

Motallebi-Veshareh M, Balzer D, Lanka E, Jagura-Burdzy G, Thomas CM. (1992) Conjugative transfer functions of broad-host-range plasmid RK2 are coregulated with vegetative replication. *Mol Microbiol.*; **6**: 907–20.

Mysliwiec TH, Errington J, Vaidya AB, Bramucci MG. (1991) The *Bacillus subtilis spo0J* gene: evidence for involvement in catabolite repression of sporulation. *J Bacteriol.*; **173**: 1911–9.

Oberer M, Zangger K, Prytulla S, Keller W. (2002) The anti-toxin ParD of plasmid RK2 consists of two structurally distinct moieties and belongs to the ribbon-helix-helix family of DNA-binding proteins. *Biochem J.*; **361**: 41–7.

Ogasawara N, Yoshikawa H. (1992) Genes and their organization in the replication origin region of the bacterial chromosome. *Mol Microbiol.*; **6**: 629–34.

Pansegrau W, Lanka E. (1996) Mechanisms of initiation and termination reactions in conjugative DNA processing. Independence of tight substrate binding and catalytic activity of relaxase (TraI) of IncPα plasmid RP4. *J Biol Chem.*; **271**: 13068–76.

Pansegrau W, Lanka E, Barth PT, Figurski DH, Guiney DG, Haas D, Helinski DR, Schwab H, Stanisich VA, Thomas CM. (1994) Complete nucleotide sequence of Birmingham IncP alpha plasmids. Compilation and comparative analysis. *J Mol Biol.*; **239**: 623–63.

Pettigrew CA, Breen A, Corcoran C, Sayler GS. (1990) Chlorinated biphenyl mineralization by individual populations and consortia of freshwater bacteria. *Appl Environ Microbiol.*; **56**: 2036–45.

Pinkney M, Ramon D, Lanka E, Thomas MC. (1988) Replication of mini RK2 plasmid in extracts of *Escherichia coli* requires plasmid-encoded protein TrfA and host-encoded proteins DnaA, B, G DNA gyrase and DNA polymerase III. *J Mol Biol.*; **203**: 927–38.

Pogliano J, Ho TQ Zhong Z, Helinski DR. (2001) Multicopy plasmids are clustered and localized in *Escherichia coli. Proc Natl Acad Sci U S A.*; **98**: 4486–91.

Pogliano J. (2002) Dynamic cellular location of bacterial plasmids. *Curr Opin Microbiol.*; **5**: 586–90.

Quisel JD, Grossman AD. (2000) Control of sporulation gene expression in *Bacillus subtilis* by the chromosome partitioning proteins Soj (ParA) and Spo0J (ParB). *J Bacteriol.*; **182**: 3446–51.

Ravin N, Lane D. (1999) Partition of the linear plasmid N15: interactions of N15 partition functions with the *sop* locus of the F plasmid. *J Bacteriol.*; **181**: 6898–906.

Roberts RC, Helinski DR. (1992) Definition of a minimal plasmid stabilization system from the broad-host-range plasmid RK2. *J Bacteriol.*; **174**: 8119–32.

Roberts RC, Spangler C, Helinski DR. (1993) Characteristics and significance of DNA binding activity of plasmid stabilization protein ParD from the broad host-range plasmid RK2. *J Biol Chem.*; **268**: 27109–17.

Santini JM, Stanisich VA. (1998) Both the *fipA* gene of pKM101 and the *pifC* gene of F inhibit conjugal transfer of RP1 by an effect on *traG*. *J Bacteriol.*; **180**: 4093–101.

Schneiker S, Keller M, Droge M, Lanka E, Puhler A, Selbitschka W. (2001) The genetic organization and evolution of the broad host range mercury resistance plasmid pSB102 isolated from a microbial population residing in the rhizosphere of alfalfa. *Nucleic Acids Res.*; **29**: 5169–81.

Sharpe ME, Hauser PM, Sharpe RG, Errington J. (1998) *Bacillus subtilis* cell cycle as studied by fluorescence microscopy: constancy of cell length at initiation of DNA replication and evidence for active nucleoid partitioning. *J Bacteriol.*; **180**: 547–55.

Sia EA, Roberts RC, Easter C, Helinski DR, Figurski DH. (1995) Different relative importance of the *par* operons and the effect of conjugal transfer on the maintenance of intact promiscuous plasmid RK2. *J Bacteriol.*; **177**: 2789–97.

Siddique A, Figurski DH. (2002) The active partition gene *incC* of IncP plasmids is required for stable maintenance in a broad range of hosts. *J Bacteriol.*; **184**: 1788–93.

Smith CA, Shingler V, Thomas CM. (1984) The *trfA* and *trfB* promoter regions of broad host range plasmid RK2 share common potential regulatory sequences. *Nucleic Acids Res.*; **12**: 3619–30.

Smith CA, Thomas CM. (1985) Comparison of the nucleotide sequence of the vegetative replication origins of broad host range plasmids R751 and RK2 reveals conserved features of probable functional importance. *Nucleic Acids Res.;* **13**: 557–72.

Smith CA, Thomas CM. (1987) Comparison of the organisation of the genomes of phenotypically diverse plasmids of incompatibility group P: members of the IncP beta sub-group are closely related. *Mol Gen Genet.*; **206**: 419–27.

Smith CA, Thomas CM. (1989) Relationships and evolution of IncP plasmids. In *Promiscuous plasmids of gram-negative bacteria*. Thomas CM, ed. Academic Press, London.

Summers DK, Beton CW, Withers HL. (1993) Multicopy plasmid instability: the dimer catastrophe hypothesis. *Mol Microbiol.*; **8**: 1031–8.

Surtees JA, Funnell BE. (1999) P1 ParB domain structure includes two independent multimerization domains. *J Bacteriol.*; **181**: 5898–908.

Szpirer CY, Faelen M, Couturier M. (2000) Interaction between the RP4 coupling protein TraG and the pBHR1 mobilization protein Mob. *Mol Microbiol.*; **37**: 1283–92.

Tauch A, Schneiker S, Selbitschka W, Puhler A, van Overbeek L S, Smalla K, Thomas CM, Bailey MJ, Forney LJ, Weightman A, Ceglowski P, Pembroke T, Tietze E, Schroder G, Lanka E, van Elsas JD. (2002) The complete nucleotide sequence and environmental distribution of the cryptic, conjugative, broad-host-range plasmid pIPO2 isolated from bacteria of the wheat rhizosphere. *Microbiology.*; **148**: 1637–53.

Thomas CM, Cross MA, Hussain AA, Smith CA. (1984) Analysis of copy number control elements in the region of the vegetative replication origin of broad host range plasmid RK2. *EMBO J.*; **3**: 57–63.

Thomas CM, Ibbotson JP, Wang NY, Smith CA, Tipping R, Loader NM. (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**: 5345–59.

Thomas CM, Smith CA. (1987) Incompatibility group P plasmids: genetics, evolution, and use in genetic manipulation. *Annu Rev Microbiol.*; **41**: 77–101.

Thomas CM, Theophilus BD, Johnston L, Jagura-Burdzy G, Schilf W, Lurz R, Lanka E. (1990) Identification of a seventh operon on plasmid RK2 regulated by the *korA* gene product. *Gene.*; **89**: 29–35.

Thorsted PB, Shah DS, Macartney D, Kostelidou K, Thomas CM. (1996) Conservation of the genetic switch between replication and transfer genes of IncP plasmids but divergence of the replication functions which are major host-range determinants. *Plasmid.*; **36**: 95–111.

Thorsted PB, Macartney DP, Akhtar P, Haines AS, Ali N, Davidson P, Stafford T, Pocklington MJ, Pansegrau W, Wilkins BM, Lanka E, Thomas CM. (1998) Complete sequence of the IncPbeta plasmid R751: implications for evolution and organisation of the IncP backbone. *J Mol Biol.*; **282**: 969–90.

Toukdarian AE, Helinski DR. (1998) TrfA dimers play a role in copy-number control of RK2 replication. *Gene.*; **223**: 205–11.

Top E, De Smet I, Verstraete W, Dijkmans R, Mergeay M. (1994) Exogenous isolation of mobilizing plasmids from polluted soils and sludges. *Appl Environ Microbial.*; **60**: 831–9.

Tralau T, Cook AM, Ruff J. (2001) Map of the IncP1beta plasmid pTSA encoding the widespread genes (*tsa*) for p-toluenesulfonate degradation in *Comamonas testosteroni* T-2. *Appl Environ Microbiol.*; **67**: 1508–16.

Van den Ent F, Moller-Jensen J, Amos LA, Gerdes K, Lowe J. (2002) F-actin-like filaments formed by plasmid segregation protein ParM. *EMBO J.*; **21**: 6935–43.

Vogel JP, Andrews HL, Wang SK, Isberg RR. (1998) Conjugative transfer by the virulence system of *Legionella pneumophila*. *Science.*; **279**: 873–6.

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**: 249–352.

Waters VL. (2001) Conjugation between bacterial and mammalian cells. *Nat Genet.*; **29**: 375–6.

Williams DR, Motallebi-Veshareh M, Thomas CM. (1993) Multifunctional repressor KorB can block transcription by preventing isomerization of RNA polymerase-promoter complexes. *Nucleic Acids Res.*; **21**: 1141–8.

Williams DR, Macartney DP, Thomas CM. (1998) The partitioning activity of the RK2 central control region requires only *incC*, *korB* and KorB-binding site O(B)3 but other KorB-binding sites form destabilizing complexes in the absence of O(B)3. *Microbiology.*; **144**: 3369–78.

Williams DR, Thomas CM. (1992) Active partitioning of bacterial plasmids. *J Gen Microbiol.*; **138**: 1–16.

Wilson JW, Sia EA, Figurski DH. (1997) The *kilE* locus of promiscuous IncP alpha plasmid RK2 is required for stable maintenance in *Pseudomonas aeruginosa*. *J Bacteriol*.; **179**: 2339–47.

Wilson JW, Figurski DH. (2002) Host-specific incompatibility by 9-bp direct repeats indicates a role in the maintenance of broad-host-range plasmid RK2. *Plasmid*.; **47**: 216–23.

Yakobson E, Guiney DG. (1983) Homology in the transfer origins of broad host range IncP plasmids. Definition of two subgroups of P plasmids. *Mol Gen Genet*.; **192**: 436–8.

Zatyka M, Jagura-Burdzy G, Thomas CM. (1994) Regulation of transfer genes of promiscuous IncP alpha plasmid RK2: repression of Tra1 region transcription both by relaxosome proteins and by the Tra2 regulator TrbA. *Microbiology*.; **140**: 2981–90.

Zatyka M, Bingle L, Jones AC, Thomas CM. (2001) Cooperativity between KorB and TrbA repressors of broad-host-range plasmid RK2. *J. Bacteriol*.; **183**: 1022–31.