The prevalence and significance of plasmid maintenance functions in the virulence plasmids of pathogenic bacteria.

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ABSTRACT

Virulence functions of pathogenic bacteria are often encoded on large extra-chromosomal plasmids. These plasmids are maintained at low copy number to reduce the metabolic burden on their host. Low-copy-number plasmids risk loss during cell division. This is countered by plasmid-encoded systems that ensure that each cell receives at least one plasmid copy. Plasmid replication and recombination can produce plasmid multimers that hinder plasmid segregation. These are removed by multimer resolution systems. Equitable distribution of the resulting monomers to daughter cells is ensured by plasmid partition systems that actively segregate plasmid copies to daughter cells in a process akin to mitosis in higher organisms. Any plasmid-free cells that still arise due to occasional failures of replication, multimer resolution or partition are eliminated by plasmid-encoded post-segregational killing systems. Here we argue that all of these three systems are essential for the stable maintenance of large low-copy-number plasmids. Thus, they should be found on all large virulence plasmids. Where available, well-annotated sequences of virulence plasmids confirm this. Indeed, virulence plasmids often appear to contain more than one example conforming to each of the three system classes. As these systems are essential for virulence, they can be regarded as ubiquitous virulence factors. As such they should be informative in the search for new antibacterial agents and drug targets.
INTRODUCTION

Pathogenic bacteria differ from their harmless relatives in having genes for virulence factors that facilitate host invasion and infection. These factors are encoded either by the host chromosome or are carried on mobile genetic elements. The latter include transposons, viral prophages and plasmids. Mobile elements provide an extensive library of potentially useful functions that can be readily adopted for the rapid adaptation of the bacterium to its environment. As these elements are acquired from other organisms in the biosphere, the bacterium has the potential to express a wide array of virulence-associated functions without the burden of carrying all the genetic information involved. Only those virulence factors required for the current adaptation need be carried. As mobile elements are able to re-assort the functions they carry by interaction with other elements in the biosphere, the variety and assortment of functions that can be acquired by transfer of an element is virtually unlimited.

Virulence plasmids are usually transmitted between hosts by conjugation. They often carry many virulence genes in addition to functions for plasmid transmission and maintenance and are therefore large (Table 1). Large plasmids are always present in low copy numbers in their bacterial host. Otherwise, the metabolic burden of maintaining and duplicating their genomes would be excessive. This compromises their chance of being faithfully transmitted to daughter cells during cell division, because random distribution of plasmid copies cannot ensure inheritance at low copy number (51). Although acquisition and loss of plasmids is an important asset for evolution, the plasmids must be inherited stably on a shorter time scale in order to minimize random losses at cell division. This is especially important in the reservoir of infective cells outside of the infected host, where there is no selection for the virulence factors that the plasmid carries.

Three principle classes of plasmid maintenance function have been described. Multimer resolution systems resolve plasmid multimers caused by replication and recombination among sister plasmids, thereby maximizing the number of plasmid copies available for segregation (3). Post segregational killing systems kill cells that lose the plasmid, thus ensuring the continued presence of the plasmid in the bacterial population (73). Finally, partition systems actively segregate sister plasmids to daughter cells by a
process that is analogous to the mitotic segregation of chromosomes (18). As these three
types of system combine to ensure high levels of segregation fidelity (51), we argue that
all three types of system are likely to be present on all large virulence plasmids. These
systems should be critical for pathogenesis and may be informative for the development
of novel antibacterial agents.

There is no uniform nomenclature for plasmid replication or maintenance
systems. Sometimes, terms that are generally used for one type of system have been used
for another type in the case of a specific plasmid. For example, the term *par*, which is
generally used for partition systems, refers to a gene for a multimer resolution
recombinase in the case of plasmid RP4 (17) and to a post-segregational killing system
component gene in pAD1 (72).

**RESOLUTION OF PLASMID MULTIMERS**

Homologous recombination between sister plasmids during or after replication can
readily give rise to plasmid dimers or higher multimers. This decreases the number of
plasmid copies available for segregation to daughter cells. As many large plasmids are
present as only two or three copies per cell, such events would lead to frequent plasmid
loss (3, 51). In addition, the type of replication control used by many plasmids makes
them vulnerable to a cumulative effect of dimer formation termed “dimer catastrophe”
(63), further decreasing plasmid stability. For accurate segregation, it is important that
multimers are resolved to monomers prior to cell division. This is accomplished by
enzyme mediated, site-specific recombination systems. Large plasmids encode their own
recombinase systems consisting of genes for a specific recombinase and a recombination
site at which they act (Table 1). Dimers contain two such sites that are cut, exchanged
and rejoined by the protein to yield two separate circular monomers. Two families of
recombinases are represented in various plasmid species; active site tyrosine
recombinases and active site serine recombinases (62).

The ParA/Res system of the broad host range, multidrug resistant plasmid RK2,
also known as RP4, is an example of a resolution system of the serine class. The ParA
recombinase is produced from a three open reading frame operon and acts at the linked
recombination site res. RK2 res has an organization similar to Tn3 family of transposons
with three inverted repeats being bound by recombinase (16, 17). It contributes greatly to
plasmid stability. The virulence plasmid of *Yersinia pestis*, pMT1, encodes a comparable
resolvase (45).

Well-characterized tyrosine recombinase systems include the Cre/loxP
recombination system of the P1 plasmid prophage (Figure 1A) (3) and ResD/rfs
resolution system of the F plasmid of *Escherichia coli* (41). These systems contribute
significantly to the stability of the plasmids and have been proven to act by resolution of
plasmid dimers that accumulate due to recombination between plasmid sisters. The
ResD/rfs resolution system is encoded within the RepFIA replication region of the F
plasmid. ResD is cotranscribed with ccdA and ccdB genes that are responsible for post
segregational killing. The target of ResD, rfs lies upstream of this gene cluster (14, 41).
An example of a related system in a virulence plasmid is the Rsd/crs system of the
virulence plasmid pSDL2 of *Salmonella enterica* serovar Dublin (39). The cis-acting
resolution site crs is located upstream of the resolvase gene rsd and contains eight direct
incomplete 17bp repeats followed by a segment of indirect repeats. This system
contributes directly to plasmid stability (39). The *E. coli* virulence plasmid pB171
encodes Rsv that is homologous to the ResD recombinase of the F plasmid (66).

Similar site-specific recombination systems are utilized by transposable elements
for integration and excision from the genome and from other mobile elements. It is likely
that dimer resolvases are derived from transposition resolvases, as is the case with the
ParA/res of RK2 that is derived from the Tn3 family of transposon resolvases (17). The
multimer resolution system of multidrug resistant plasmid pJHCMW1 appears to have
been acquired from the transposon Tn1331 (67).

Large plasmids are targets for transposition, and often contain multiple
transposons. Thus, in searching plasmid sequences for resolution systems, it is often
unclear whether candidate sequences are involved in multimer resolution or in
transposition. It seems likely that some loci serve both purposes. Also, recombination
loci are likely to be involved in inter-plasmid recombination events that re-assort gene
cassettes between heterologous plasmids. Thus, site-specific recombination systems can
be viewed as having multiple contributions to virulence. They stabilize virulence
plasmids by resolving dimers and contribute to the plasticity of the available virulence functions by facilitating the lateral transfer of virulence cassettes and their re-assortment into novel combinations.

POST-SEGREGATIONAL KILLING SYSTEMS

Post-segregational killing (PSK) systems eliminate plasmid-free cells that arise due to mis-segregation or replication errors (73). Such systems have also been termed toxin/antitoxin or plasmid addiction systems, and their activities as programmed cell death. These plasmid loci have a gene for a stable antibacterial toxin that either kills or stops cell growth when expressed, and for a short-lived product that acts as an antidote to the toxin. When the plasmid is present in the cell, the antitoxin inactivates the toxin, but when the plasmid is lost, the antitoxin is degraded rapidly and the toxin kills the cell (33).

In addition to these systems, termed type II PSK systems, there is a second class, type I that acts in a similar way. Here the “antitoxin” is an anti-sense RNA that blocks the function of the plasmid messenger RNA for a toxic protein. If the plasmid is lost, the antitoxin RNA degrades rapidly and translation of the more stable messenger RNA leads to toxin production and cell death (25, 29). Thus, only cells that successfully propagate the plasmid thrive in the population.

Plasmid R1 of Escherichia coli encodes the type I PSK system, hok/sok. The Hok toxin associates with the cell membrane and acts by destroying the membrane potential of the cell, leading to loss of energy for metabolism. The antidote, sok is a small, unstable, cis-acting antisense RNA that inhibits hok translation (23). A similar system is found in the Enterococcus faecalis conjugative virulence plasmid, pAD1. It is known as pAD1 par and encodes the messenger RNA, RNA I, for a 33 amino acid toxic peptide Fst, and an antisense RNA, RNA II (72). Fst, when translated, affects cell division and promotes membrane permeabilization, nucleoid condensation, and cell death (29). RNA I and RNA II are transcribed convergently and overlap at their 3’ bidirectional transcription termination sequence and 5’ direct repeat sequences. They form a highly stable complex via their 3’ and 5’ overlapping sequences and stop translation of fts (Figure 1B) (71).
RNA II is relatively unstable and is cleared from cells that lose the plasmid. The accumulated RNA I messenger is then free to produce the Fts toxin that kills the cells.

Well-characterized type II members include the ccd system of the F plasmid (52) and phd/doc of the P1 plasmid prophage of *E. coli* (43). In these systems, both the toxin and antitoxin are small proteins that are produced from adjacent genes on the plasmid.

The antitoxin is unstable as it is particularly susceptible to degradation by host proteases. The F plasmid *ccdA* and *ccdB* genes form an operon that is auto-regulated by its products (Figure 1B). CcdB is a stable DNA gyrase inhibitor (10). When the plasmid is present, CcdA inhibits the interaction between DNA gyrase and CcdB. In cells that lose the F plasmid, the host Lon protease rapidly degrades the CcdA pool. In absence of CcdA, CcdB binds to DNA gyrase and inactivates it, leading to DNA damage and cell death (33).

The virulence plasmid MYS6000 of *Shigella flexineri* has a type II PSK locus, *mvpA/mvpT* that has been shown to be important for plasmid maintenance (58). Type II PSK systems are characterized by two adjacent short open reading frames for the small toxin and antitoxin peptides. They have been found in large plasmids when functional tests have been used to locate them. However, they are a very diverse group and are not easy to recognize by DNA sequence alone.

Many plasmids carry specific restriction-modification (RM) loci. RM systems are composed of genes that encode a restriction enzyme that cleaves DNA in a sequence specific manner and a modification methylase that modifies the target sequence so that the host genome is protected from cleavage (38). These systems can act as post-segregational killing systems. They help in plasmid maintenance by killing plasmid free cells because, on plasmid loss, the modification activity declines before the restriction activity, and the cell are killed by digestion of their DNA (38, 50).

PSK cassettes are often found in the host chromosome. Well-characterized chromosomal PSK systems of *E.coli* include MazE/MazF (20) and RelB/RelE (24). They are activated in response to elevated levels of ppGpp that occurs as a result of amino acid starvation. These systems are probably involved in stress response.

It is possible that chromosomal PSK systems are purely selfish DNA elements that ensure their own survival by killing host cells that lose them. However, they may also serve to preserve loci to which they are linked. PSK elements linked to large islands
of virulence genes in the chromosome of a pathogen might prevent the loss of the islands as the pathogen population grows (57, 68).

**THE PARTITION (par) SYSTEMS**

Large plasmids have low copy numbers. There are sometimes only two or three copies in the dividing cell. Random distribution of these copies to daughter cells would lead to very high rates of plasmid loss (51). Under these circumstances, PSK systems would kill a large proportion of the cells in the population to the detriment of pathogen viability.

Plasmids overcome this by having plasmid partition systems that ensure that each daughter cell receives at least one copy of the plasmid DNA (30). Partition systems direct the active segregation of the plasmids to either side of the cell centre prior to cell division, minimizing the chances of plasmid loss. All low-copy-number plasmids appear to encode a partition (par) system. They consist of an ATPase or a GTPase motor protein, a specific DNA binding adaptor protein, and a *cis*-acting centromere-like site at which they act. The proteins are generally produced from an operon that is tightly autoregulated by one of the products (28).

Plasmid par systems can be classified into three types. Type I par systems encode a deviant Walker type P-loop ATPase. Type II encodes an actin/hsp70 type of ATPase, and the recently described type III par system encodes a GTPase. Type I partition systems can be further classified into Ia and Ib (28). Type Ia systems have large ATPase motor proteins that serve as the operon autorepressor. Type Ib systems have smaller motor proteins and use the DNA binding protein as an autorepressor.

**Type Ia Partition systems.**

Well-studied type Ia partition system is represented by the par system of the P1 plasmid of *E. coli* (44). It consists of a two-gene operon encoding the ParA ATPase, followed by a gene for the DNA binding adaptor protein, ParB (Figure 1C). The operon is auto-regulated by ParA binding to its own promoter. Repression is further aided by binding of ParB to ParA (13, 34). The centromere-like *parS* site is located immediately downstream of the par operon. It consists of two types of repeat motif for recognition of ParB (64),
and an IHF site that binds the *E. coli* integration host factor (6, 12). Binding of IHF induces a bend in the DNA that facilitates ParB binding. Further copies of the ParB protein can load at the parS site, spreading out onto the surrounding DNA sequences (6, 56). *In vivo* studies of fluorescently labeled P1 plasmids show that they move apart to become roughly evenly distributed throughout the length of the cell. This process is aided by the pairing and active separation of copies that lie close to each other (59). The properties of the ParA protein suggest that ParA forms dynamic gradients in the cell that direct plasmid movement. This involves the complex interplay between the affinity of the ATP bound ParA for non-specific host DNA and the ability of parS bound ParB to stimulate ATPase activity of ParA, thereby releasing it from the host DNA (69).

The F plasmid of *E. coli* has a Type Ia partition system, sop (Figure 1C). This is similarly organized to P1 par, but the partition site (sopC) consists of twelve 48 bp repeats and has no IHF site (49). Type Ia partition systems are widely distributed among virulence plasmids in gram negative pathogens. Those closely related to P1 par are particularly prevalent. Examples include pMT1 par of *Yersinia pestis* (74) and pWR100 par of *Shigella flexneri* (60).

**Type Ib partition systems.**

The type Ib systems have a small ParB DNA binding protein and a different DNA organization from that of Type Ia. The type species of such loci is the par2 locus of *E. coli* virulence plasmid pBI71 (Table 1) (19). The region upstream of the operon contains both the partition site parC and the operon control locus at which ParB acts as an autoregulator (Figure 1C). ParA nucleates on to the ParB/parC DNA complex forming filamentous polymers. When two plasmids are close to each other, the complexes interact, causing disassembly of the ParA filaments that lie between them. This causes the plasmids to move outward, following the retreating edge of the ParA filament field. The end result is a roughly equal distribution of the plasmids along the long axis of the cell (55). The principle difference between the latest models for type Ia and type Ib plasmid movement by ParA action lie in the proposal that ParA acts as a dynamic concentration field in the former (69) and as filaments in the latter (55). The models
otherwise have key similarities, and it seems likely that the mechanisms differ in detail but are otherwise basically the same (36).

The pAD1 plasmid of *Enterococcus feacalis* is a virulence plasmid that has a type Ib partition system. pAD1 is a low-copy-number, pheromone responsive plasmid that produces a cytolytic exotoxin that enhances virulence (9). The partition protein RepB, an ATPase of ParA family, is followed by RepC. The partition site lies upstream of repB and contains a group of twenty-five 8-bp direct repeats (21). RepB (33-kDa) and RepC (14.4 kDa) were both shown to be required for maximal stabilization. RepC binds to the iteron region, and RepB can then interact by a RepB/RepC contact. Although this system shows some differences in organization to that of pB171 of *E. coli*, the sequence similarity and indications of a common mechanism show that the type I partition family spans both gram positive and gram negative pathogens. Similar systems are to be found in several other gram-positive species, suggesting that the type Ib systems are broadly distributed (28, 32).

**Type II plasmid partition systems.**

Type II *par* systems contain a member of the actin/hsp70 super family of ATPases (5) as exemplified by *par* locus of antibiotic resistance plasmid R1. The *parMRC* locus of R1 encodes an actin homologue ParM, a DNA binding adaptor protein ParR, and an upstream centromere like region *parC* (Figure 1C) (26, 27). The promoter of the operon lies within *parC* and is auto-regulated by cooperative binding of ParR (11, 37). ParM, in presence of ParR and *parC*, forms dynamic actin-like filaments (22, 47, 48). ParM filaments grow by insertion of ParM-ATP molecules at ParR/*parC* site. ParR/*parC* complex stabilizes the ParM filament (22). *In vivo* studies have shown that ParM filaments force plasmid pairs rapidly to opposite cell poles. After reaching the poles, the ParM filaments rapidly depolymerize and the segregated plasmids resume random diffusive motion. Plasmids can undergo several rounds of segregation in a single cell cycle (7).

This type II mechanism clearly differs from that of the type I systems. Rather than involving disassociation of a ParA structure from the region between plasmids, the ParA structure grows between them and forces them apart. The final distribution of
copies is not even along the cell length. Rather, most copies end up at the cell poles. This is because the rigid filaments often continue to push the copies apart until they reach the ends of the cells (47).

Type II systems appear to be less prevalent in nature than type I systems. Interestingly, the *E. coli* virulence plasmid pB171 contains both a type II and a type Ib partition system and both have proven to be active in partition (19).

**Type III partition systems.**

The large virulence plasmid pXO1 of *Bacillus anthracis* encodes a RepX protein that was originally thought to be part of the replication machinery. RepX is distantly related to the bacterial cell division protein FtsZ. It is a GTPase and has a motif that is common to tubulins (65). RepX forms GTP-dependent filamentous structures both *in vitro* as well as *in vivo* and shows non-specific DNA binding. It is likely that it is involved in plasmid partition (1, 2). The pBtoxis plasmid of *Bacillus thuringiensis* encodes a RepX homolog, TubZ that is required for plasmid stability. It assembles into dynamic filaments *in vivo* (42). An upstream DNA binding protein, TubR, regulates TubZ expression. TubR binds to a cis-acting sequence of four repeats that lie upstream of the genes for the protein components (Figure 1C). TubZ forms dynamic filaments that elongate at one end and retract at the other. By associating with these filaments, the plasmid/TubR complex is presumed to move with the progressing filament to achieve segregation (42). In this novel class of elements, termed type III, the GTPase (RepX, TubZ) appears to play the role of a ParA analog, and TubR the role of ParB.

**Partition of linear plasmids.**

Very little is known about the partition system of linear virulence plasmids. Bacteriophage N15 of *E. coli* is maintained as a linear plasmid molecule with covalently closed ends. It is stably maintained at 3-5 copies per bacterial chromosome. Stable inheritance is ensured by plasmid encoded *sop* locus that is homologous to F *sop*. The locus consists of a two-gene operon, *sopA* and *sopB*, which are similar to F *sop*. But unlike F plasmid, the centromere sequence of N15 consists of four individual inverted repeats scattered over 12kb N15 genome (54). Stabilization of the linear plasmid
depends on the number and the position of the centromere site (15). Similar partition systems have been identified in other linear plasmids such as pY54 of *Yersinia enterocolitica* (35) and φKO2 in *Klebsiella oxytoca* (8). Unlike circular plasmids, scattered centromere-like sites appear to be pre-requisite for stable inheritance of linear plasmids.

**HOW PLASMID MAINTENANCE FUNCTIONS COMBINE TO PRODUCE HIGH LEVELS OF PLASMID STABILITY**

Cells containing low-copy-number plasmids often encounter environments where the resident plasmid is dispensable for growth. Thus, the plasmid must be stably maintained to prevent its loss from the population. In practice, naturally occurring plasmids are highly stable with loss rates that are hardly measurable over many generations. Plasmid replication and maintenance systems combine to achieve this (Figure 2). Replication doubles the number of plasmids in each generation. Some of these copies form dimers by recombining with each other. These dimers are efficiently reduced to monomers by the multimer resolution system. Plasmid monomers are actively partitioned to opposing cell halves by the action of the partition system. Cell division then produces new cells, each of which contains a plasmid. This basic plasmid cell cycle is prone to occasional errors that lead to the production of a few plasmid-free cells (Figure 2). These cells are subject to post-segregational killing that removes them from the population. All three of the plasmid maintenance systems are essential for physiological levels of segregational stability (51) (Figure 2). Without multimer resolution, insufficient plasmid molecules will be present in many cells for proper segregation. Without active plasmid partition, too many cured cells will be produced. Killing many cells would severely compromise the viability of the population. Without post-segregational killing, the inevitable mistakes made by the replication and partition and multimer systems of the plasmid will allow the gradual accumulation of plasmid-free cells that will eventually take over the population. Thus, all three types of system are important for physiological stability and all three are likely to be found on each naturally occurring plasmid type (51).
In practice, plasmids like P1 that have the very low copy numbers illustrated in figure 2 have error rates for replication, multimer resolution, and partition that combine to produce about one plasmid-free cell per thousand new-born cells per generation under laboratory conditions. Post segregational killing of the plasmid-free cells reduces this to less than one cell in $10^8$ (58). Most large plasmids are likely to have somewhat higher copy numbers. In these cases, the rate of loss will be further reduced due to the fact that extra copies reduce the probability that replication or segregation errors will give rise to plasmid-free cells.

THE CASE FOR UBIQUITY OF THE THREE TYPES OF PLASMID MAINTENANCE FUNCTION

We have argued that all three types of plasmid maintenance system are essential for the stable inheritance of large plasmids in the absence of strong selections for their presence. Most pathogenic bacteria have opportunities to grow in the environment outside of the host in which virulence functions give them a selective advantage, so that this rule should apply to the virulence plasmids that they carry. As the genomes of virulence plasmids are large and complex, exhaustive analysis of gene function is usually lacking. The most complete information available is for the virulence plasmids of gram-negative enteropathogens (Table 1). Here, all three types of system are present. In addition, sporadic information from other classes suggests that at least one member of all three of these classes is present on any given virulence plasmid. Indeed, virulence plasmids often show evidence of more than one copy of each type. For example, the annotated sequence of the large virulence plasmid of *Shigella flexneri*, pWR501(70) (Table 1) indicates the presence of two plasmid partition systems; homologues of the P1 *parA*/*parB* (type Ia) and R1 *stbA/stbB* (type II) *par* systems respectively. It contains at least two toxin/anti-toxin systems, one related to *F ccdA/ccdB* and another to pMYS6000 *mvpA/mvpT*. It also has a homologue of RelB, a toxin encoded by the *relB/relE* PSK locus (31). Plasmid pWR501 also carries a sequence similar to ColE1 *cer* site (61) that is involved in plasmid multimer resolution, and there are several putative transposases and resolvases included in the annotated sequence. Cursory examination of the sequences of many other
virulence plasmids suggests that similar patterns are frequent. Naturally, it cannot be
discounted that unknown types of plasmid maintenance systems are yet to be discovered,
but the obvious problems that threaten plasmid maintenance appear to be handled very
efficiently by the combination of the three system types described here.

WHY ARE MULTIPLE SYSTEMS OFTEN PRESENT?

In practice, the plasmid maintenance functions of a given virulence plasmid are not
limited to one of each class. There is no obvious reason why two or more dimer
resolution systems should interfere with each other, and the error frequency of resolution
should be reduced if more than one is present on a given plasmid. The mechanisms
involved in plasmid partition are also tolerant of having more than one partition system
on the same plasmid (4). Presumably, having two such systems can also improve fidelity
by minimizing failures. Finally, multiple post segregational killing systems are often
found on a single plasmid. Again the fidelity of plasmid maintenance should be
increased because the efficiency of killing of any plasmid-free cells should be increased.
Loss of the plasmid should trigger the action of the toxin associated with each PSK
system present, giving an additive effect for killing efficiency. Thus, the presence of
multiple elements of different specificities for each of the three major classes of plasmid
maintenance element is not unexpected and is likely to be beneficial for the overall
fidelity of plasmid maintenance.

THE PRACTICAL CONSEQUENCES

We propose that large, low copy number virulence plasmids encode at least one copy of
all three of the major types of plasmid maintenance system. This is likely to apply to all
such plasmids that are circular and replicated in the usual theta mode. As virulence
plasmids encode virulence functions, and maintenance of the plasmid is important for
virulence, the three types of plasmid maintenance system can themselves be regarded as
ubiquitous virulence factors. When analyzing new virulence plasmids, the presence of
such systems can be anticipated. Moreover, their presence and properties could have consequences when searching for novel therapies for infectious disease.

Plasmid partition systems are unique to bacterial plasmids and to the chromosomes of some bacterial species. Although targeting these is a rather indirect way to prevent infection, it is nevertheless possible that drugs designed to target them will prove useful, and some efforts along these lines are underway.

Post segregational killing systems offer a different potential for therapy. Each of these systems produces a small peptide toxin capable of targeting some vital target of the host. First, although peptides do not in general make good drugs, it is conceivable that drugs could be modeled on them. Second, these killer systems can be regarded as very successful selfish elements that rely on targeting host functions in order to survive. Thus, it can be argued that the host targets are vital and not easily mutated to resist the toxin. Such targets could prove to be ideal for the design of novel antibacterial drugs.

Knowledge of plasmid maintenance systems is also important for the design of stable plasmid vectors used for medical purposes. Attenuated *Shigella* and *Salmonella* strains have been used as carriers for delivering foreign vaccine antigens to mammalian cells. One of the major problems associated with the use of live bacterial carriers is the instability of the plasmids used to encode the antigen. Knowledge of the functional interplay of plasmid maintenance functions should prove useful here, and incorporation of PSK and partition functions into the vectors has already given positive results (46).

Vaccine antigens, naked DNA antigens, and therapeutic agents are increasingly made from plasmids in producer bacteria in liquid culture. The maintenance stability of the plasmid vectors can be a frequent problem, especially in continuous flow culture (40, 53). Here again, detailed knowledge of the interplay of the three maintenance system types should prove useful in vector design.

**ACKNOWLEDGMENTS**

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<table>
<thead>
<tr>
<th>Organism and Plasmids</th>
<th>Size (bp)</th>
<th>Partition System</th>
<th>par Type</th>
<th>Post-segregation killing system (PSK)</th>
<th>Multimer Resolution System</th>
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<tr>
<td>Enteropathogenic E.coli (EPEC) pB171</td>
<td>68,817</td>
<td>ParA/ParB, ParR/ParM</td>
<td>Ib, II</td>
<td>CcdA/CcdB, RsvA, RsvB</td>
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<tr>
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<td>II</td>
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<td>ResD</td>
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<td>Ia</td>
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<td>RsdB</td>
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<td>Ia</td>
<td>CcdA/CcdB, RsdB</td>
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<td><em>Salmonella dublin</em> pOU1115</td>
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<td>ParF/ParG</td>
<td>Ib</td>
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<tr>
<td><em>Shigella flexneri</em> pWR501</td>
<td>221,851</td>
<td>ParA/ParB, StbA/StbB</td>
<td>Ia, II</td>
<td>CcdA/CcdB, MvpA/MvpT, RelB</td>
<td>TnpR, cer</td>
</tr>
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</table>

1 All information compiled from ncbi site for bacterial plasmid sequences.
2 Compiled from (19)  
3 Homologous to the F plasmid PSK system, CcdA/CcdB (10)  
4 Homologous to the pMYS6000 PSK system, MvpA/MvpT (58)  
5 Tyrosine recombinase system components (62)
**FIGURE LEGENDS**

**Figure 1**

A. The Cre/loxP multimer resolution system of P1 has a loxP site 434 bp upstream of cre gene (green box). It consists of two inversely oriented 13 bp recombinase binding motifs (red solid arrows) flanking an asymmetrical 8 bp sequence.

B. Genetic organization of PSK loci.

The pAD1 par system consists of the fst gene (yellow box) encoding RNAI. RNA II is transcribed convergently and shares a bidirectional terminator sequence (gray solid arrows) and direct repeats at 3' and 5' ends (blue arrow heads). RNA II and I (dashed arrows) form a stable pair and inhibit fts translation. In plasmid free cells, RNA II degrades rapidly causing expression of toxic Fst protein and results in cell death.

The ccd system of F plasmid consists of two genes, ccdB, encoding the killer and ccdA encoding the antidote. CcdA binds to CcdB and inhibits cell killing. In the case of plasmid loss, CcdA is rapidly degraded causing CcdB mediated cell death. The operon is autoregulated by the protein complex.

C. Genetic organization of the different types of partition systems found in plasmids. The green boxes and the orange boxes represent centromere-binding protein and the motor protein respectively. The centromere sequence has been marked by purple boxes. Red arrows mark the direction of transcription. Transcription repression and centromere binding have been marked in each case.

**Figure 2.** How plasmid maintenance systems combine to achieve stable plasmid inheritance.

The cell cycle of typical cells (blue cells) containing a low-copy-number plasmid is represented on the left. Plasmid replication doubles the number of plasmids in each cell. Some cells contain plasmid dimers formed by generalized recombination. These are reduced to monomers by the multimer resolution system. The replicated plasmids are subjected to active partition to opposite cell halves, ensuring that cell division produces two cells, each of which contains at least one plasmid copy.

The gray cells represent those rare cells where the replication, multimer resolution, or partition system of the plasmid has failed to function properly. In each case, cell division produces one plasmid-containing cell that is returned to the general population, and one that has no plasmid copy. Post segregation killing is triggered in the latter cells, killing them and thus ensuring that all viable cells in the population retain the plasmid.
A. Genetic organization of cre-loxP multimer resolution system of P1

B. Genetic organization of PSK systems

C. Genetic organization of partition systems

Type Ia (P1 and F)

Type Ib (pB171 par2)

Type II (R1)

Type III (pBtoxis)