1

The Function and Organization of Plasmids

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1. Introduction

In 1952, Joshua Lederberg coined the term *plasmid* to describe any bacterial genetic element that exists in an extrachromosomal state for at least part of its replication cycle ***(1)***. As this description included bacterial viruses, the definition of what constitutes a plasmid was subsequently refined to describe exclusively or predominantly extrachromosomal genetic elements that replicate autonomously. Plasmids are now known to be present in most species of Eubacteria that have been examined, as well as in Archaea and lower Eukarya ***(2)***.

Although most of the genetic material that directs the structure and function of a bacterial cell is contained within the chromosome, plasmids contribute significantly to bacterial genetic diversity and plasticity by encoding functions that might not be specified by the chromosome ***(3)*** (*see* **Subheading 3**). For example, antibiotic resistance genes are often plasmid-encoded, which allows the bacterium to persist in an antibiotic-containing environment, thereby providing the bacterium with a competitive advantage over antibiotic-sensitive species.

Under laboratory conditions, plasmids are generally not essential for the survival of the host bacterium and they have served as invaluable model systems for the study of processes such as DNA replication, segregation, conjugation, and evolution ***(3)***. Moreover, ever since their utility was evinced by the first gene-cloning experiments in the early 1970s, plasmids have been pivotal to modern recombinant DNA technology as gene-cloning and gene-expression vehicles, among other uses ***(4,5)***.

2. Basic Plasmid Characteristics

2.1. Size and Copy Number

Naturally occurring plasmids vary greatly in their physical properties, a few examples of which are shown in **Table 1**. They range in size from <2-kilobase pair (kbp) plasmids, which can be considered to be elements simply capable of replication, to

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Table 1
Examples of Plasmids with Different Physical Characteristics

Plasmid	Host	Plasmid size (kbp)	Plasmid geometry	Plasmid copy number	Ref.
pUB110	*Bacillus subtilis*	2.3	Circular	20–50	***7***
ColE1	*Escherichia coli*	6.6	Circular	10–30	***9***
lp25	*Borrelia burgdorferi*	24.2	Linear	1–2	***6***
pNOB8	*Sulfolobus* sp.[a]	41.2	Circular	2–40	***10***
F	*Escherichia coli*	99.2	Circular	1–2	***11***
SCP1	*Streptomyces coelicolor*	350.0	Linear	4	***12***
pSymA	*Sinorhizobium meliloti*	1354.2	Circular	2–3	***8***

[a]Archaea.

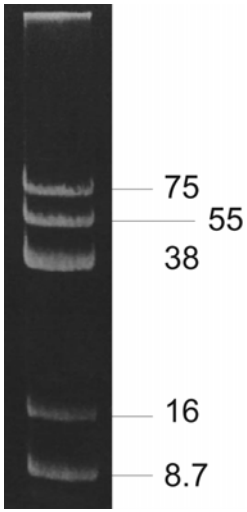

Fig. 1. Plasmid complement of a multiplasmid-containing strain of *Lactococcus lactis* analyzed by agarose gel electrophoresis. The approximate sizes of the plasmids are indicated (kbp).

megaplasmids that are many hundreds of kilobase pairs in size. At the upper end of this scale, the distinction between a megaplasmid and a minichromosome can become obscure. Some bacterial species simultaneously harbor *multiple* different plasmids that can contribute significantly to the overall genome size of the host bacterium (*see* **Fig. 1**) ***(6,13)***. As an example, the symbiotic soil bacterium *Sinorhizobium meliloti* has three replicons (3.65, 1.68, and 1.35 megabase pairs [Mbp]) in addition to its chromosome (6.69 Mbp) ***(8)***. The smallest megaplasmid, pSymA, can be cured from the host bacterium under laboratory conditions but provides nodulation and nitrogen-fixation functions that are important for the symbiotic interaction of the bacterium and its plant host.

Different plasmids have different *copy numbers* per chromosome equivalent. Some plasmids have a steady-state copy number of one or a few copies, whereas other, mainly small, plasmids are present at tens or even hundreds of copies per chromosome. The plasmid copy number is determined by replication control circuits that are discussed under **Subheading 4**, and in detail by del Solar and Espinosa ***(14)***. Therefore, the contribution of plasmid DNA to the host bacterium's genome depends on the number of different plasmids that the bacterium harbors, as well as their size and copy number.

2.2. Geometry

Although most plasmids possess a circular *geometry*, there are now many examples in a variety of bacteria of plasmids that are linear ***(15,16)***. As linear plasmids require specialized mechanisms to replicate their ends, which circular plasmids and chromosomes do not, linear plasmids tend to exist in bacteria that also have linear chromosomes ***(17)***.

Circular plasmids can have more than one *topology* determined by the opposing actions of DNA gyrases and topoisomerases ***(18)***. Plasmid DNA is mostly maintained in a covalently closed circular, supercoiled form (analogous to the behavior of an elastic band that is held fixed at one position while it is twisted at the 180° position). However, if a nick is introduced into one of the strands of the DNA double helix, supercoiling is relieved and the plasmid adopts an open circular form that migrates more slowly in an agarose gel than the covalently closed circular form. If nicks are introduced at opposite positions on both DNA strands, the plasmid is linearized. In addition, the activity of DNA homologous recombination enzymes can convert plasmid monomers to dimers and higher-order species that, because of their larger size, will migrate more slowly during agarose gel electrophoresis than the monomeric forms.

3. Plasmid-Encoded Traits

Many plasmids are phenotypically cryptic and provide no obvious benefit to their bacterial host other than the possible exclusion of plasmids that are incompatible with the resident plasmid (*see* Chapter 2). However, many other plasmids specify traits that allow the host to persist in environments that would otherwise be either lethal or restrictive for growth (*see* **Table 2**).

Antibiotic resistance is often plasmid encoded and can provide the plasmid-bearing host a competitive advantage over antibiotic-sensitive species in an antibiotic-containing environment such as the soil, where many antibiotic-producing micro-organisms reside, or a clinical environment where antibiotics are in frequent use ***(35)***. Indeed, plasmid-encoded antibiotic resistance is of enormous impact to human health. The relative ease with which plasmids can be disseminated among bacteria, compared with chromosome-encoded traits, means that antibiotic resistance can spread rapidly and this has contributed to the dramatic clinical failure of many antibiotics in recent years. Furthermore, resistance genes may be located on transposable elements ***(36)*** within plasmids that can further promote the transmissibility of antibiotic resistance genes. In some instances, plasmids may harbor a number of genes encoding resistance to different antibiotics (multidrug resistance).

Table 2
Examples of Naturally Occurring Plasmids and Relevant Features

Plasmid	Host	Plasmid size (kbp)	Relevant feature	Ref.
pT181	*Staphylococcus aureus*	4.4	Tetracycline resistance	***19***
pRN1	*Sulfolobus islandicus*[a]	5.4	—	***20***
2μ	*Saccharomyces cerevisiae*[b]	6.3	—	***21***
ColE1	*Escherichia coli*	6.6	Colicin production and immunity	***9***
pMB1	*Escherichia coli*	8.5	*Eco*RI restriction–modification system	***22***
pGKL2	*Kluyveromyces lactis*[b]	13.5	Killer plasmid	***23***
pAMβ1	*Enterococcus faecalis*	26.0	Erythromycin resistance	***24***
pSK41	*Staphylococcus aureus*	46.4	Multidrug resistance	***25***
pBM4000	*Bacillus megaterium*	53.0	rRNA operon	***13***
pI258	*Staphylococcus aureus*	28.0	Metal ion resistance	***26***
pSLT	*Salmonella enterica* ssp. *typhimurium*	93.9	Virulence determinants	***27***
pMT1	*Yersinia pestis*	101.0	Virulence determinants	***28***
pADP-1	*Pseudomonas* sp.	108.8	Atrazine (herbicide) catabolism	***29***
pWW0	*Pseudomonas putida*	117.0	Aromatic hydrocarbon degradation	***30***
pBtoxis	*Bacillus thuringiensis* ssp. *israelensis*	137.0	Mosquito larval toxicity	***31***
pX01	*Bacillus anthracis*	181.7	Exotoxin production	***32***
pSOL1	*Clostridium acetobutylicum*	192.0	Solvent production	***33***
pSymB	*Sinorhizobium meliloti*	1683.3	Multiple functions associated with plant symbiosis	***34***

[a] Archaea.
[b] Eukarya (yeast).

Other plasmid-encoded traits also contribute to the persistence of the host bacterium in otherwise inhospitable environments. These include resistance to metal ions such as lead, mercuric, and zinc ***(37)***, production of virulence factors that allow the bacterium to colonize hosts and survive host defenses ***(38)***, and metabolic functions that allow utilization of different nutrients. The last trait includes the plasmid-mediated biodegradation of a variety of toxic substances such as toluene and other organic hydrocarbons, herbicides, and pesticides ***(39)***. The production of plasmid-encoded bacteriocins to which other microorganisms are susceptible can give the plasmid-containing bacterium a competitive edge over other microorganisms in an ecological

niche ***(39a)***, as can plasmid-located genes for bacteriophage resistance and for the restriction of foreign nucleic acids which enter the cell. Conversely, plasmid-encoded antirestriction systems may protect plasmid DNA from degradation by host restriction enzymes when it first enters a new cell ***(39b)***. The profound effects that plasmids can exert on bacterial behavior is sharply illustrated by the recent observation that *Bacillus cereus*, an opportunistic food-borne pathogen; *Bacillus thuringiensis*, a source of commercially useful insecticidal proteins; and *Bacillus anthracis*, the causative agent of anthrax, are mainly discriminated by their plasmids ***(40)***.

4. Plasmid Replication

Plasmids, like chromosomes, are replicated during the bacterial cell cycle so that the new cells can each be provided with at least one plasmid copy at cell division ***(41)***. To this end, plasmids have developed a number of strategies to initiate DNA replication but have mostly co-opted the host polymerization machinery ***(42)*** for subsequent stages of DNA synthesis, thereby minimizing the amount of plasmid-encoded information required for their replication. Small plasmids have been identified which consist of a replicon and very little extraneous DNA sequences ***(42a)***. These, and other cryptic plasmids, can be viewed as purely selfish genetic elements as they apparently provide no advantage to their host. However, they may exclude related, invading plasmids from the host or may function as the core of lager plasmids which will evolve in the future. Large plasmids often contain multiple replicons dispersed at different locations on the plasmid or express different forms of a replication protein. These phenomena may reflect the different replication requirements of a plasmid that can exist in more than one bacterial host ***(43)***.

4.1. Iteron-Containing Replicons

The genetic organization of a stylized plasmid replicon is illustrated in **Fig. 2A**. This replicon consists of a number of elements, including a gene for a plasmid-specific replication initiation protein (Rep), a series of directly repeated sequences (iterons), DnaA boxes, and an adjacent AT-rich region. The relative positions of the operator site, iterons, AT-rich stretch, and DnaA boxes can vary between replicons ***(44)***. The numbers of iterons and DnaA boxes and the length of the AT-rich region can also differ.

Rep, which usually negatively autoregulates its own expression, binds to the iterons, which typically are 17–22 bp in length but vary in number and sequence between different replicons ***(44)***. The spacing between shorter repeats is greater than that between longer repeats so the distance between equivalent positions within adjacent iterons is always approx 22 bp, corresponding to two turns of the DNA helix. Thus, when Rep proteins bind to the iterons, they are arrayed on the same face of the DNA helix. DnaA is a protein required for initiation of replication of the bacterial chromosome. It also performs a similar function in plasmid replication by binding to the DnaA boxes in the replicon ***(45)***. The Rep-DnaA-DNA nucleoprotein complex promotes strand melting at the nearby AT-rich region to which host replication factors subsequently gain access and promote leading and lagging strand synthesis in a manner analogous to initiation of replication at the chromosomal origin, *oriC*.

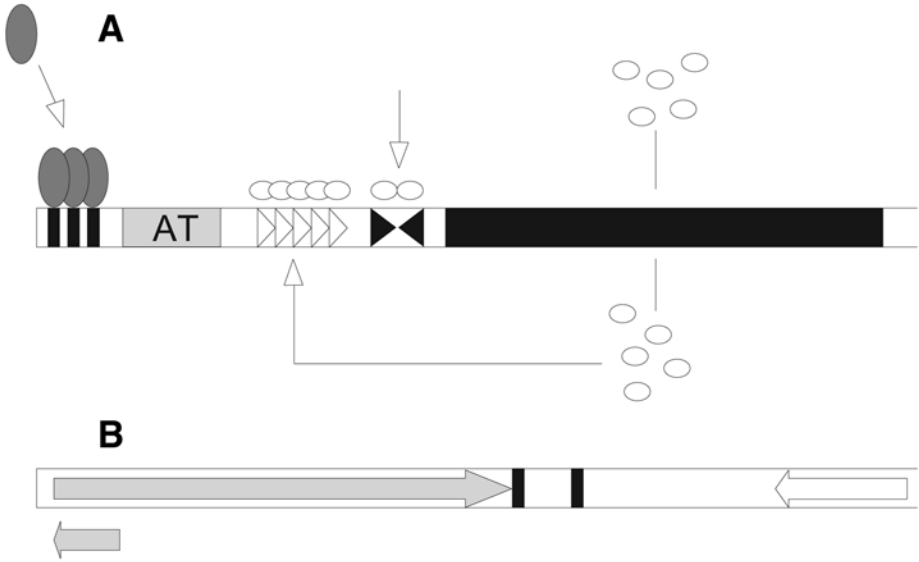

Fig. 2. The genetic organization of plasmid replicons. **(A)** The organization of a generic replicon that contains iterons. The stippled rectangle represents the *rep* gene whose protein product (ovals) binds both the directly repeated iterons (open triangles) and the operator site (filled triangles) upstream of *rep*. The filled boxes represent binding sites for host DnaA protein (shaded spheres). The AT-rich region is also indicated. **(B)** The organization of the ColE1 replicon. The leftward- and rightward-shaded arrows indicate the genes for the RNAI and RNAII transcripts, respectively. The open arrow represents the *rom* gene. The filled and hatched rectangles indicate the origin and primosome assembly sites, respectively.

Plasmid replication is a rigorously controlled process in part because plasmid overreplication would tax the metabolic capacity of the host cell and put the plasmid-bearing cell at a disadvantage compared to a plasmid-free counterpart. Plasmids control their copy number primarily at the stage of replication initiation. The frequency with which initiation of replication of iteron-containing plasmids occurs is modulated in part by sequestration of the origin region in nucleoprotein complexes and intermolecular pairing of complexes on different plasmids, which is referred to as "handcuffing" ***(14,44)***.

4.2. ColE1-Type Replicons

The replicon of the ColE1 plasmid of *Escherichia coli* is the basis for many gene-cloning and gene-expression vectors that are commonly used in current molecular biology (*see* Chapters 2 and 28). In contrast to the replication of iteron-containing plasmids, ColE1 replication proceeds without a plasmid-encoded replication initiation protein and instead utilizes an RNA species in initiation and RNA–RNA interactions to achieve copy number control (*see* **Fig. 2B**) ***(46)***.

ColE1 uses an extensive RNA primer for leading-strand synthesis. The RNAII preprimer is transcribed from the RNAII promoter by host RNA polymerase. RNAII forms a persistent RNA–DNA hybrid at the plasmid origin of replication. This hybrid is cleaved by RNase H and the resulting free 3'OH group on the cleaved RNAII acts as a primer for continuous leading-strand synthesis, catalyzed by host DNA polymerase I.

ColE1 regulates its copy number with a short RNA countertranscript, RNAI. This species is expressed constitutively from the strong RNAI promoter, is nontranslated, and is fully complementary to part of RNAII. The interaction of RNAI with RNAII results in an RNAII configuration that impairs further elongation of this transcript, thereby reducing the frequency of RNA–DNA duplex formation and initiation of replication. The RNAI–RNAII interaction is counterbalanced by the shorter half-life of RNAI compared to RNAII. The ColE1-encoded Rom protein (also known as Rop) increases the frequency of RNAI–RNAII interactions. The gene for Rom is deleted in many ColE1-based plasmid vectors, resulting in increased copy numbers compared to ColE1 itself. Perturbations of ColE1 plasmid copy number are rapidly mirrored by changes in RNAI concentration, resulting in the enhancement or suppression of replication and the maintenance of ColE1 copy number within a narrow window.

4.3. Rolling-Circle Replication

Many small (<10 kbp) plasmids of Gram-positive Eubacteria replicate by a rolling-circle mechanism, which is distinct from the replication of iteron-containing or ColE1-like plasmids (*see* **Fig. 3**) ***(47)***. Rolling-circle plasmids have also been identified in Gram-negative Eubacteria and in Archaea. Some bacteriophage, including M13 of *E. coli*, also replicate in this way.

In rolling-circle replication, binding of a plasmid-encoded replication protein to the leading-strand origin (also known as the double-strand origin) distorts the DNA in this region and exposes a single-stranded region in an extruded cruciform. A nick is introduced at this site by the replication protein and this exposes a 3'OH group from which the leading strand is synthesized by DNA polymerase III. Leading strand initiation differs between rolling circle plasmids, procaryotic chromosomes, and other plasmids, although chain elongation is similar in all systems. As the leading strand is synthesized, the nontemplate strand of the old plasmid is displaced ahead of the replication fork until, eventually, it is removed entirely. The resulting single-stranded intermediate is characteristic of rolling-circle replication and its identification provides evidence that a plasmid replicates by this mechanism ***(48)***. The lagging-strand origin (also known as the single-strand origin) is exposed on the displaced single-stranded intermediate and lagging-strand initiation commences at this origin using host replication factors. RNA polymerase synthesizes RNA primers at the lagging strand origin. DNA polymerase I initiates lagging strand synthesis from these RNA primers, after which DNA polymerase III continues elongation.

5. Plasmid Segregation

DNA replication produces precise plasmid copies, but plasmids must also ensure that they are distributed to both daughter cells during bacterial cell division. If the

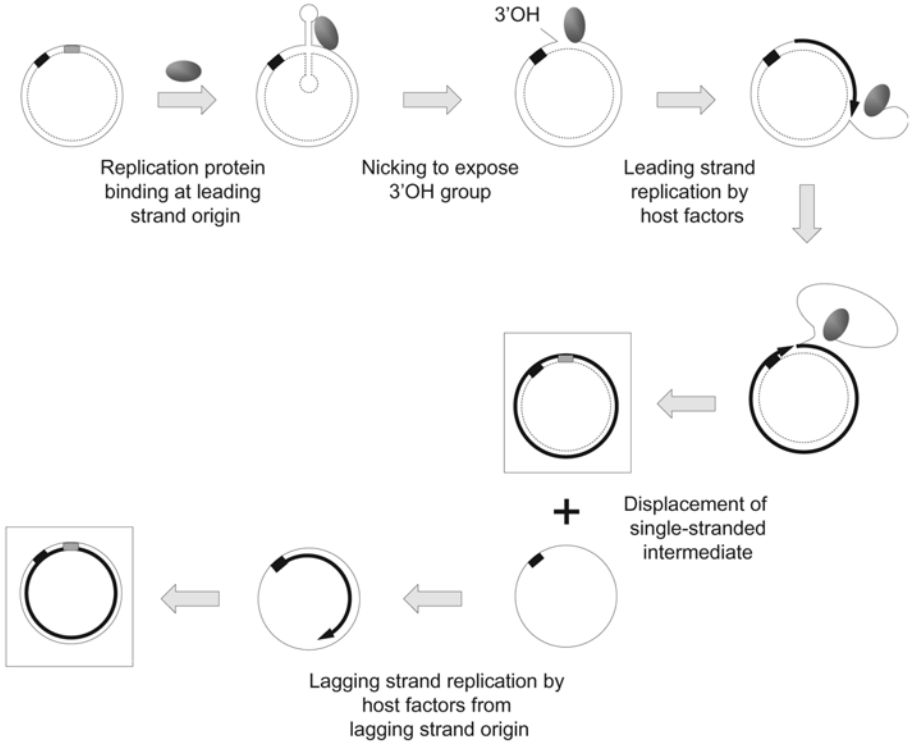

Fig. 3. Replication of rolling-circle plasmids. The two DNA strands of the plasmid are shown as solid and dotted lines. Newly replicated strands are shown as thick lines. Filled and shaded boxes represent the lagging-strand and leading-strand origins, respectively. The plasmid-encoded replication protein is shown as an oval. The replication protein nicks at a specific site in this region exposing a 3'OH group, which host replication factors use to initiate leading-strand synthesis. Synthesis of the leading strand displaces the nontemplate strand from the plasmid and forms the typical single-strand intermediate. The lagging-strand origin on this intermediate serves as an initiation site for RNA-primed synthesis of the complementary strand. The two double-stranded products of rolling-circle replication are boxed.

steady-state copy number of a plasmid is sufficiently high, it is easy to envisage how passive diffusion of these copies might be sufficient to ensure that each daughter cell acquires at least one copy of the plasmid when the cell divides. Plasmid copy number control circuits subsequently modulate the numbers of plasmid copies in the daughter cells to normal levels in preparation for the next round of cell division. Although it is still considered likely that random diffusion is sufficient for the stable inheritance of moderate- or high-copy-number plasmids, recent evidence suggests that these plasmids might not be entirely free to disperse through the cytoplasm but, instead, might be compartmentalized into subcellular regions from which the plasmids are distributed equitably ***(49)***. The mechanism for this is unknown.

In contrast to high-copy-number plasmids, plasmids with a copy number of one or a few have evolved specific strategies to guarantee their faithful inheritance, which cannot be achieved by random diffusion.

5.1. Active Partition Systems

Following plasmid replication, active partitioning systems position the plasmids appropriately within the cell such that at cell division, each of the new cells acquires at least one copy of the plasmid (*see* **Fig. 4**). The most well studied active partition system is, arguably, that of the P1 plasmid in *E. coli* ***(50,51)***. The plasmid located components of this system are organized in a cassette that consists of an autoregulated operon containing the *parA* and *parB* genes and a downstream *cis*-acting sequence, *parS*. The ParA and ParB proteins and a host protein, integration host factor, form a nucleoprotein complex at *parS* that is presumed to interact with an unknown host partitioning apparatus. This complex guides the tethered P1 plasmid copies to the one-quarter and three-quarter cell-length positions following replication at the midcell. The plasmids remain at these positions as the bacterial cell elongates. When the cell divides at its center the plasmids are again at the midpoint positions of the new cells and the cycles of replication and partition are repeated (*see* **Fig. 4**).

Active partition systems are widely distributed among low-copy-number bacterial plasmids and homologous systems are likely to be implicated in chromosome partition in many bacteria ***(52)***.

5.2. Site-Specific Recombination

Many laboratory strains of *E. coli* have been mutated to be deficient in homologous recombination. This reduces the frequency with which genes cloned in multicopy plasmids undergo rearrangements in these strains. In contrast, most wild-type bacteria are recombination proficient and this is critical for bacterial DNA repair and evolution ***(53)***. As plasmid copies are identical, homologous recombination in wild-type bacteria can convert plasmid monomers to dimers or higher-order species. The complete dimerization of a plasmid population within a cell will halve the number of plasmids available for partition at cell division and thereby contribute to plasmid segregational instability. Furthermore, because dimers have two replication origins, they may be more favored for replication than plasmids with a single origin, which may further skew intracellular plasmid distribution toward dimeric forms. The formation of trimers and other multimers will have an even more profound effect on plasmid segregation ***(54)***.

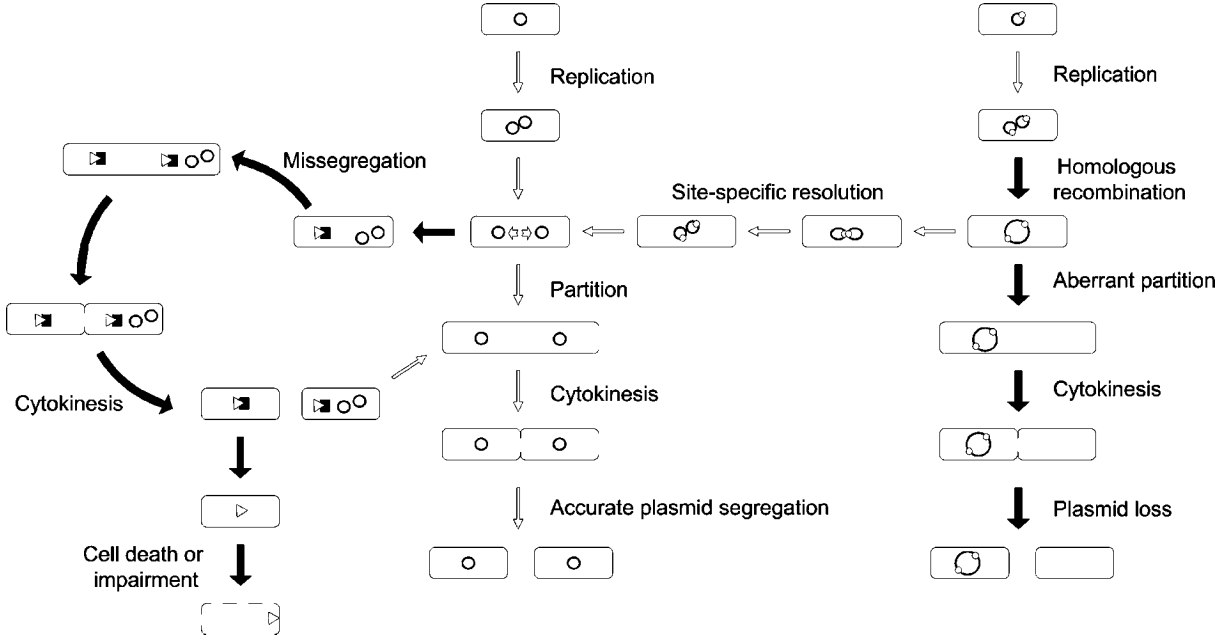

Fig. 4. The contribution of accessory stability mechanisms to plasmid maintenance. Active partitioning systems position replicated plasmids (large circles) appropriately within the elongating bacterial cell (rounded rectangles) such that each daughter cell receives a plasmid copy after cell division (**center**). If homologous recombination dimerizes two plasmid copies, the plasmids cannot be distributed equitably at cell division and this may lead to plasmid loss (**right**). However, site-specific recombination at plasmid recombination sites (small circles) can resolve the plasmid dimer to monomers that can now be partitioned accurately. If a plasmid-free cell arises because of missegregation or a defect in replication, toxin–antitoxin systems can kill or impair the growth of the plasmid-free cell specifically (**left**). The plasmid-encoded toxin (open triangle) is efficiently sequestered by an antitoxin (filled rectangle) in the plasmid-containing cell. In the plasmid-free derivative, the antitoxin is more susceptible to degradation by host enzymes than the toxin, so that the latter is eventually liberated from the former and can poison the host. Open and filled arrows indicate productive and nonproductive steps, respectively, in accurate plasmid segregation. For clarity, the host chromosome is not depicted in this representation.

Both high- and low-copy-number plasmids commonly solve this problem by using site-specific recombination to resolve dimers to monomers (*see* **Fig. 4**). This process involves site-specific recombinases that bind to specific recombination sites on both monomer copies within the plasmid dimer and form a synaptic complex in which the two recombination sites are brought in to close proximity ***(55)***. The site-specific recombinases cleave DNA strands within this complex and promote strand exchange between the two sites and results in the monomerization of the plasmid dimer. The XerC and XerD site-specific recombinases encoded by most bacterial chromosomes are involved in the resolution of dimeric forms of many plasmids, including the ColE1 plasmid, and bacterial chromosomes ***(56)***.

5.3. Toxin–Antitoxin Systems

An additional mechanism which plasmids use to favor their maintenance in bacterial populations involves the killing or growth impairment of cells that fail to acquire a copy of the plasmid. This has variously been referred to as postsegregational cell killing, plasmid addiction, or toxin–antitoxin systems ***(57–60)***. This mechanism involves a plasmid-encoded protein toxin and antitoxin. The antitoxin, which may be either a protein or a nontranslated RNA, neutralizes the toxin by either binding to the toxin protein or by inhibiting its translation. The antitoxin is more susceptible to degradation by host enzymes than the toxin and, thus, replenishment of the antitoxin levels by the presence of plasmid is required to prevent toxin action. When a plasmid-free derivative arises (e.g., as a result of a replication or partitioning defect), the toxin is subsequently liberated to interact with an intracellular target and cause either death or a growth disadvantage of the plasmid-free cell (*see* **Fig. 4**). In the case of the CcdAB toxin–antitoxin system encoded by the F plasmid, the toxin (CcdB) is a DNA gyrase poison. It both entraps a cleavage complex between gyrase and DNA and associates with DNA gyrase to produce a complex that is impaired in supercoiling activity ***(61)***. These combined effects are lethal for *E. coli*.

A variety of different toxin–antitoxin systems are widely disseminated on bacterial plasmids, although the intracellular targets for the toxin components of these systems probably differ. Toxin–antitoxin homologs have also been identified on many bacterial chromosomes, where they might function as bacterial programmed cell death systems during periods of nutritional and other stresses ***(57)***.

Large, low-copy-number plasmids often utilize partition, recombination, and toxin–antitoxin systems to promote segregational stability. The segregational maintenance of these plasmids is achieved through the activity of all three mechanisms.

6. Plasmid Dissemination in Bacterial Populations

Certain bacterial species can achieve a state of natural competence for the uptake of naked plasmid DNA (transformation) ***(62)***, or can acquire DNA that has been packaged into a bacteriophage head and is injected into the host (transduction) ***(63)***. However, the conjugative transfer of DNA between donor and recipient cells is probably the most common mechanism by which plasmids are disseminated in bacterial populations ***(64,65)***. A wide variety of phenotypes can be conferred by conjugative

plasmids, including antibiotic resistance, bacteriocin production and immunity, and catabolic functions.

Conjugative plasmids have been identified in most major groups of Eubacteria, and more recently in Archaea ***(66)***. Furthermore, conjugative plasmid transfer is not limited to closely related bacteria but has also been demonstrated between evolutionary–divergent Gram-negative and Gram-positive Eubacteria ***(67)***, and from Eubacteria to yeast ***(68)***. The T-DNA region of the Ti virulence plasmid of the Gram-negative bacterium *Agrobacterium tumefaciens* is also transferred by a conjugation-like process to susceptible plant hosts, where it integrates in the plant genome and induces the formation of crown gall tumors ***(69)***.

Conjugation is mediated by cell-to-cell contact between the donor and recipient. Plasmid DNA is usually transferred through a tube-like structure known as a pilus, which is extruded by the donor and physically connects to the recipient cell. In the Gram-positive bacterium *Enterococcus*, this cell-to-cell contact is promoted by plasmid-encoded aggregation substances that are induced in response to sex pheromones excreted by the recipient cell ***(70)***. As a large number of genes may be required for the conjugation process and these genes reside on the conjugative plasmid itself, small plasmids are usually not self-transmissible. Nevertheless, small plasmids that encode relaxase enzymes, which perform the initial nicking reactions at their cognate plasmid origins of transfer (*oriT*), can undergo conjugative mobilization if other conjugation functions are provided *in trans* by a helper plasmid within the cell ***(71)***.

Conjugative transfer of the F Plasmid is one of the best-characterized conjugation processes. In this system, the propilin protein encoded by the *traA* gene is processed by host-encoded leader peptidase into the pilin product. The latter is inserted into the inner cell membrane with the aid of a transfer-specific chaperone protein, TraQ ***(71a)***. Pilin in the membrane is organized into the extracellular pilus filament through the action of a number of assembly proteins encoded by the F plasmid. Plasmid DNA conjugation involves the transfer of only one strand of the plasmid DNA between the donor and recipient cells. Following transfer, the two single strands act as templates for synthesis of the complementary strands by the DNA replication machinery in both donor and recipient cells. In the case of the F plasmid, a relaxase enzyme, TraI, nicks one DNA strand in the relaxosome complex assembled at *oriT*. TraI is also a helicase which unwinds the two strands after nicking. The nicked strand is transferred through the pilus to the recipient cell where its ends are religated. Following F plasmid transfer, the plasmid-specific TraT and TraS proteins inhibit a second transfer event to the recipient by impeding mating pair stabilization (surface exclusion) and by preventing DNA transfer (entry exclusion), respectively.

7. Plasmid Evolution: *Plasmids Are Modular Elements*

Whole genome and plasmid-specific sequencing projects have recently begun to provide fascinating glimpses into the genetic organization and evolution of plasmids. These studies have revealed that plasmids, particularly large plasmids, are commonly constructed in a modular fashion by the recombination activities of transposons, insertion sequences, bacteriophages, and smaller plasmids ***(72)***. For example, the backbone

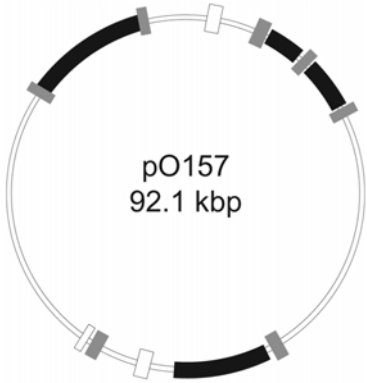

Fig. 5. Simplified representation of the relative distribution of transposable elements (gray boxes) and putative virulence genes (filled arcs) on the pO157 virulence plasmid of *E. coli*. Replicons, one of which is apparently interrupted, are shown as white boxes. For clarity, the locations of partition, conjugation and other genes are not shown. (Adapted from **ref. *73***.)

of the 92-kbp virulence plasmid of *E. coli* O157 bears a striking resemblance to that of the F plasmid. However, this backbone is interrupted by a number of regions containing putative virulence genes (*see* **Fig. 5**) ***(73)***. These virulence patches are framed by intact insertion sequences or insertion-sequence remnants, suggesting that an ancestral plasmid related to F was colonized successively by a number of mobile elements conferring different virulence functions. Similarly, the mosaic structure of the 46.4-kbp multidrug-resistance plasmid pSK41 from *Staphylococcus aureus* suggests that this plasmid has acquired its many resistance determinants by the insertion of both transposable elements and smaller plasmids into a conjugative progenitor plasmid ***(25)***. A large pathogenicity island bounded by insertion-sequence elements represents one-quarter of the 181.7-kbp virulence plasmid, pXO1, of *B. anthracis* and appears to have been acquired through transposition ***(32)***. This plasmid also harbors numerous other insertion sequences indicative of a highly recombinogenic history. These and many other recent examples indicate that large plasmids have evolved by accumulating additional genetic functions through successive, independent recombination events that are frequently mediated by transposable elements. The serial acquisition of virulence, antibiotic resistance and other determinants by plasmids allows the hosts that harbor them to invade and persist in increasingly hostile niches.

Other examples of the modular organization of plasmids include the frequent close association of plasmid replication and maintenance cassettes and the clustering of genes for conjugation functions in specific plasmid regions. Over time, common control circuits have developed in some plasmids that coordinate these core activities of replication, maintenance, and transfer ***(74)***. The continued molecular dissection of plasmids, both at the genomic level and in finer detail concerning the molecular function of specific systems, will undoubtedly prove as exciting and informative in the immediate future as the analysis of these versatile elements has proven in the last half century.

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