Bacterial Plasmids

Secondary article

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Plasmids are carriers of accessory genetic information in bacteria and are capable of infectious spread through bacterial populations. They regulate their own replication and transmission and are largely responsible for the proliferation of antibiotic resistance during the second half of the twentieth century.

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Introduction

The bacterial chromosome is a repository of essential housekeeping genes but, in addition, many bacteria possess supplementary genomes which encode nonessential information. Much smaller than bacterial chromosomes, these plasmids control their own replication and move horizontally between and within species. In contrast to the original view of plasmids as symbiotic elements or an integral part of the bacterial genome, they are increasingly viewed as independent, even parasitic, elements. By providing a mechanism for horizontal gene transfer they allow bacterial evolution to proceed as a network rather than a conventional tree, a mechanism whose effectiveness is illustrated by the rapid spread of multiple antibiotic resistance in recent decades.

Plasmid Structure

The most intensively studied plasmids are negatively-supercoiled circles of double-stranded deoxyribonucleic acid (ds DNA) found in *Escherichia coli* (**Figure 1a**). They range in size from a few to 100 kb but even the largest *E. coli* plasmids have only 2–3% of the coding capacity of the chromosome. Small plasmids typically exhibit high copy numbers (sometimes over 100 copies per cell), while larger ones are limited to just a few copies per cell. Plasmids are not invariably circular: linear forms have been reported in many bacterial genera, including *Borrelia*, *Streptomyces*, *Thiobacillus*, *Nocardia*, *Rhodococcus* and even *Escherichia*. A linear structure raises the question of how to ensure complete replication of the plasmid and protect the ends from degradation. Some linear plasmids in *Streptomyces* species contain terminal inverted repeats and it is suggested that proteins binding the repeats protect the ends of the plasmids, bringing them together to form a 'racket frame' structure (**Figure 1b**). An alternative solution to the ends problem is found in the genus *Borrelia*, which contains linear plasmids from 15 to 200 kb. Here, the two strands of the DNA duplex are linked by a single-strand loop (**Figure 1c**).

The discovery of very large plasmids has raised the question of where plasmids stop and chromosomes begin.

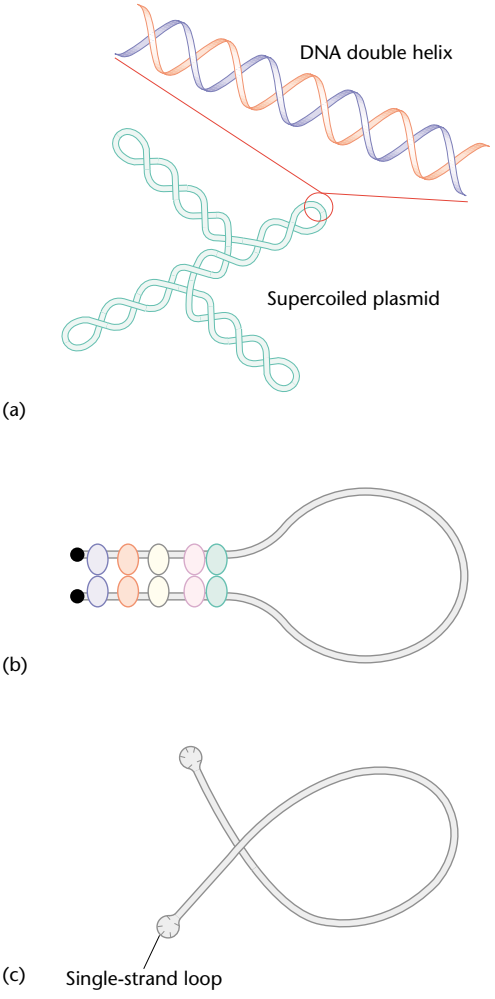

Figure 1 Alternative plasmid structures: (a) supercoiled circular plasmid; (b) linear 'racket frame' structure; (c) an 'endless linear' plasmid (5′- and 3′-DNA termini are joined by a single-strand loop).

The *Borrelia* chromosome migrates as a linear molecule of only 950 kb (compare this with the 3500-kb circular chromosome of *E. coli*), so the 200-kb linear plasmids found in this genus might reasonably be regarded as minichromosomes. The ambiguity is not confined to *Borrelia*; several genera of nonenteric Gram-negative bacteria contain huge extrachromosomal genomes. For example, *Rhizobium meliloti* harbours 'symbiotic megaplasmids' of 1.4 and 1.7 Mb in addition to a main chromosome of 3.4 Mb. Since these encode genes for essential housekeeping functions, it is probably more appropriate to classify them as chromosomes.

Plasmid-encoded Phenotypes

Plasmids encode an enormous variety of functions which promote their own survival and extend the range of environments in which their host can survive (**Table 1**). One class of plasmids protects the host from the ill effects of heavy metals, toxic anions and intercalating agents and, through provision of additional repair systems, confers increased resistance to ionizing radiation. A second class extends the host's metabolic versatility. This group includes plasmids which encode enzymes for the synthesis of bioactive compounds, including colicins and antibiotics, or confer the ability to degrade recalcitrant organic molecules. Thirdly, we find plasmids which open up new environments for their bacterial host, conferring pathogenicity by encoding toxins and colonization antigens.

Despite their enormous diversity, genes on plasmids do have something in common. Apart from those which promote the replication, maintenance and proliferation of the plasmid itself (so-called plasmid-selfish genes), they adapt the bacterial host to circumstances which exist transiently or only in part of the organism's environment (Eberhard, 1990). These genes are concentrated on plasmids not through chance but as a direct consequence of natural selection. In conditions where transient-advantage genes increase the host's fitness, those on plasmids will spread more rapidly because they are capable of both vertical and horizontal transmission, while genes on the chromosome are limited to conventional vertical transmission.

Classification

Plasmids are classified into incompatibility groups. Incompatibility is the inability of pairs of plasmids to coexist stably in the same cell line; the members of such pairs are said to belong to the same incompatibility group (Bergquist, 1987). Most plasmids produce a *trans*-acting repressor of replication as part of their copy number control circuit, and crossreactivity of repressors from

Table 1 Plasmid phenotypes

1. Resistance properties
   - Antibiotic resistance
     - Aminoglycosides (e.g. streptomycin, gentamicin, amikacin)
     - Chloramphenicol
     - Fusidic acid
     - β-Lactam antibiotics (e.g. benzylpenicillin, ampicillin, carbenicillin)
     - Sulfonamides, trimethoprim
     - Tetracyclines
     - Macrolides (e.g. erythromycin)
   - Heavy metal resistance
     - Mercuric ions and organomercurials
     - Nickel, cobalt, lead, cadmium bismuth, antimony, zinc, silver
   - Resistance to toxic anions
     - Arsenate, arsenite, tellurite, borate, chromate
   - Other resistances
     - Intercalating agents (e.g. acridines, ethidium bromide)
     - Radiation damage (e.g. by ultraviolet light, X-rays)
     - Bacteriophage and bacteriocins
     - Plasmid-specified restriction/modification systems
2. Metabolic properties
   - Antibiotic production
   - Bacteriocin production
   - Metabolism of simple cabohydrates (e.g. lactose, sucrose, raffinose)
   - Metabolism of complex carbon compounds (e.g. octane, toluene, camphor, nicotine, aniline) and halogenated compounds (e.g. 2,6-dichlorotoluene, 2,4-dichlorophenoxyacetic acid)
   - Metabolism of proteins (e.g. casein, gelatin)
   - Metabolism of opines (by Ti^+ *Agrobacterium*)
   - Nitrogen fixation (by Nif^+ *Rhizobium*)
   - Citrate utilization
   - Phosphoribulokinase activity in *Alcaligenes*
   - Thiamine synthesis by *Erwinia* and *Rhizobium*
   - Denitrification activity in *Alcaligenes*
   - Proline biosynthesis by Ti^+ *Agrobacterium*
   - Pigmentation in *Erwinia*
   - Hydrogen sulfide production
   - Extracellular DNAase
3. Factors modifying host life style
   - Toxin production
     - Enterotoxins of *Escherichia coli*
     - Exfoliative toxin of *Staphylococcus aureus*
     - Exotoxin of *Bacillus anthracis*
     - δ-endotoxin of *Bacillus thuringiensis*
     - Neurotoxin of *Clostridium tetani*
   - Colonization antigens of *Escherichia coli* (e.g. K88, K99, CFAI, CFAII)

continued

Table 1 – *continued*

Haemolysin synthesis (e.g. in *Escherichia coli* and *Streptococcus*)
Serum resistance of enterobacteria
Virulence of *Yersinia* species
Capsule production of *Bacillus anthracis*
Crown gall and hairy root disease of plants (by Ti^+ and Ri^+ *Agrobacterium*)
Infection and nodulation of legumes (by Sym^+ *Rhizobium*)
Iron transport (e.g. in *Escherichia coli* and *Vibrio anguillarum*)
4. Miscellaneous properties
Gas vacuole formation in *Halobacterium*
Pock formation (lethal zygosis) in *Streptomyces*
Killing of *Klebsiella pneumonia* by Kik^+ IncN plasmids
Sensitivity to bacteriocins in *Agrobacterium*
Translucent/opaque colony variation in *Mycobacterium*
Rhizosphere protein by Nod^+ Fix^+ *Rhizobium leguminosarum*
R-inclusion body production in *Caedibacter*
Endopeptidase activity by *Staphylococcus*
Chemotaxis towards acetosyringone by Ti^+ *Agrobacterium*

closely related plasmids results in incompatibility. In a cell containing two compatible plasmids the repressors act independently and both plasmids maintain their normal copy numbers. When plasmids are incompatible, each produces an inhibitor which represses its own replication and that of its cohabitant so the total number of plasmids in the cell falls. Moreover, although the total copy number is regulated, the relative contribution of the two incompatible plasmids to the total plasmid population is free to drift and, in time, the loss of one or other is inevitable (**Figure 2**).

Some plasmids have more than one replicon and this can lead to ambiguous results from incompatibility tests. To avoid this difficulty an alternative classification scheme based on replicon typing has been developed. This uses a bank of cloned replicons to probe for DNA sequence homology in the test plasmid. It affords a more direct test of the relatedness of plasmids and detects the presence of multiple replicons, even when they are inactive.

Isolation and Characterization of Plasmid DNA

Density gradient centrifugation

Ultimately all techniques for plasmid purification exploit the different susceptibility to breakage of plasmids and the chromosome. Shear forces associated with cell lysis and the early stages of purification invariably fragment the large bacterial chromosome, while plasmids, by virtue of their smaller compact structures, remain intact. Purification of most plasmids thus involves the separation of a minority of plasmid circles from a majority of linear chromosome fragments. Originally this was achieved by caesium chloride–ethidium bromide density gradient centrifugation. On such a gradient the DNA migrates to the point

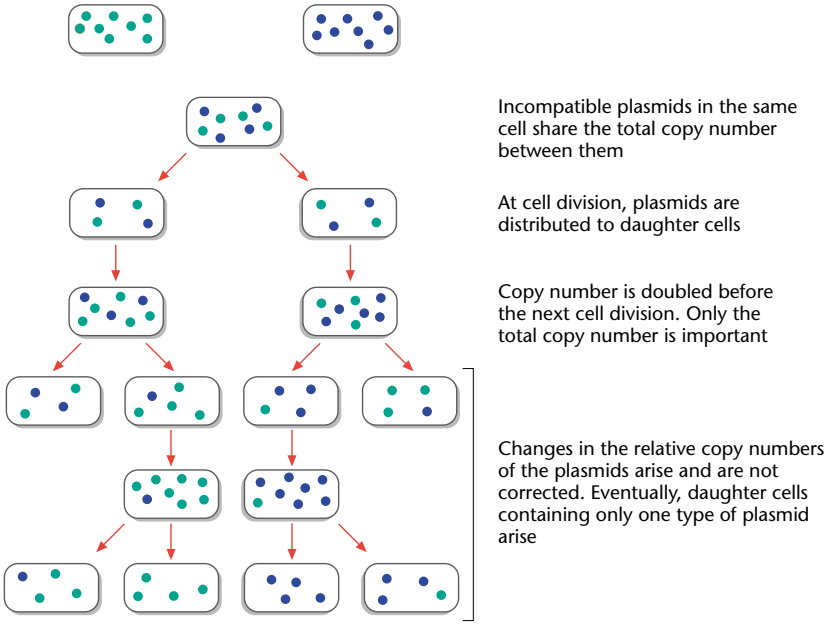

Figure 2 Segregation of incompatible plasmids.

where its density equals that of the surrounding solution. Ethidium bromide is a planar molecule which inserts between the DNA base pairs and unwinds the double helix. Linear DNA or nicked circles bind more ethidium bromide than intact circles because their ends are free to rotate. Consequently chromosome fragments bind more ethidium bromide than plasmids and the two species equilibrate at different positions on the density gradient.

Rapid plasmid purification

Since the end of the 1970s, a number of rapid plasmid preparation methods have been developed. These exploit the topological differences between plasmid circles and linear chromosomal fragments. When the hydrogen bonds between the complementary strands of circular plasmid DNA are broken by heating or by alkaline pH, the strands remain closely associated because they are linked by the intertwined backbones of the double helix. In contrast, the strands of linear or nicked DNA are free to separate completely. If a mixture of denatured plasmid and chromosomal DNA is renatured rapidly (by cooling or restoration of neutral pH), the fidelity of reassociation differs substantially for the two species. The renaturation of plasmid circles is rapid and accurate because the strands are already in close physical proximity. Linear molecules generated by random shearing of chromosomal DNA renature less accurately, forming networks of DNA which can be removed from the lysate by centrifugation. Plasmid DNA remains in solution and can be precipitated with alcohol after chromosomal DNA has been removed.

Characterization of plasmid DNA

Agarose gel electrophoresis is commonly used to analyse plasmid DNA and the size of a new plasmid can be estimated by comparison with known standards. The analysis is complicated by the fact that migration in the gel is influenced by both the size and shape of the molecule; nicked plasmids migrate much more slowly than their supercoiled counterparts. Although fresh preparations of plasmid are predominantly supercoiled, nuclease contamination can lead to the accumulation of nicked, or even linear, forms. However, as long as equivalent physical forms are compared, the rate of migration remains inversely proportional to the log of molecular weight. Conventional agarose gels cannot easily resolve molecules of greater than 20 kb but the development of orthogonal field pulsed gel electrophoresis (OFAGE) allowed resolution up to 2000 kb and led to the discovery of giant linear plasmids in *Streptomyces*. A disadvantage of the OFAGE method was the requirement for complicated gel apparatus to deliver perpendicular electric fields. A later modification overcame this problem by using conventional electrophoresis equipment with a periodically reversed field.

Plasmid Replication

It is a characteristic feature of plasmids that they control their own replication. They must coordinate their replication with the growth and division of the host cell so that, on average, they replicate once every generation. They must, however, be able to increase or decrease that rate to correct a fall or rise in their copy number. Functions involved in replication control are typically clustered within a region of 1–3 kb known as the basic replicon, which is defined experimentally as the smallest piece of the plasmid that replicates with wild-type copy number. A key component of the control system, encoded within the basic replicon, is the replication inhibitor, whose concentration reflects the plasmid copy number. The inhibitor may be a protein (as in the case of the bacteriophage lambda-derived plasmid λ-*dv*), a small antisense ribonucleic acid (RNA) (plasmids ColE1, pT181 and R1) or a set of short DNA repeats (P1 prophage and plasmid F).

Replication control by antisense RNA

Short antisense RNA inhibitors of plasmid replication are commonplace. In *E. coli* they are employed by high copy number ColE1-like plasmids (Cesareni *et al*., 1991) and by low copy number IncFII replicons, and in *Staph. aureus* they regulate the replication of the pT181 multicopy plasmid family.

ColE1 replication initiates when a transcript (RNA II) synthesized from a constitutive promoter 555 bp upstream of the origin of replication is processed to form the primer for leading strand replication. Left to its own devices, RNA II folds into an active configuration and forms a stable complex with the complementary DNA strand at the replication origin. The RNA strand in this DNA–RNA duplex is cleaved by RNAaseH, creating the primer for leading-strand synthesis. The RNA I inhibitor of ColE1 replication is complementary to the 5′-end of the RNA II preprimer and base pairing between these RNAs causes the preprimer to fold into an inactive configuration which is not cleaved by RNAaseH. RNA I is a very effective inhibitor; it has been estimated that only one in 20 RNA II transcripts is processed into a primer. An additional component of the ColE1 copy number control system is encoded by the *rom* gene. The Rom protein increases the rate at which RNA I binds to the preprimer transcript, thus improving its effectiveness.

Other examples of antisense RNA replication inhibitors are found in the multicopy *S. aureus* plasmid pT181 and the low copy number *E. coli* plasmid R1. In pT181 the inhibitor transcript forms an RNA duplex with the RepC (initiator protein) messenger RNA (mRNA). This changes the secondary structure of the mRNA so that a rho-independent terminator forms and transcription stops upstream of the start codon. The system is analogous to

attenuators that regulate amino acid biosynthesis genes in *E. coli*. In plasmid R1 the inhibitor (CopA) also regulates the production of an initiator protein (RepA) but in this case it acts indirectly by inhibiting translation of a 24 amino acid polypeptide (Tap) whose translation is coupled to that of RepA.

Iteron control of replication

For many plasmids, including F and the P1 prophage, replication is controlled by a series of short DNA repeats (iterons) which bind the Rep protein (Nordström, 1990). Studies of the R-replicon of P1 reveal that its replication is not limited simply by the availability of the RepA initiator protein but also by whether RepA is able to gain access to the replication origin. The iteron repeats (*incA* and *incC*) flank the *repA* gene and the replication origin lies within *incC* (**Figure 3a**). It is proposed that, after replication, Rep-mediated pairing of the daughter molecules ('handcuffing') blocks further initiation, even in the presence of excess initiator protein. Eventually plasmid partition and cell division separate the plasmids, which are then free to replicate (**Figure 3b**).

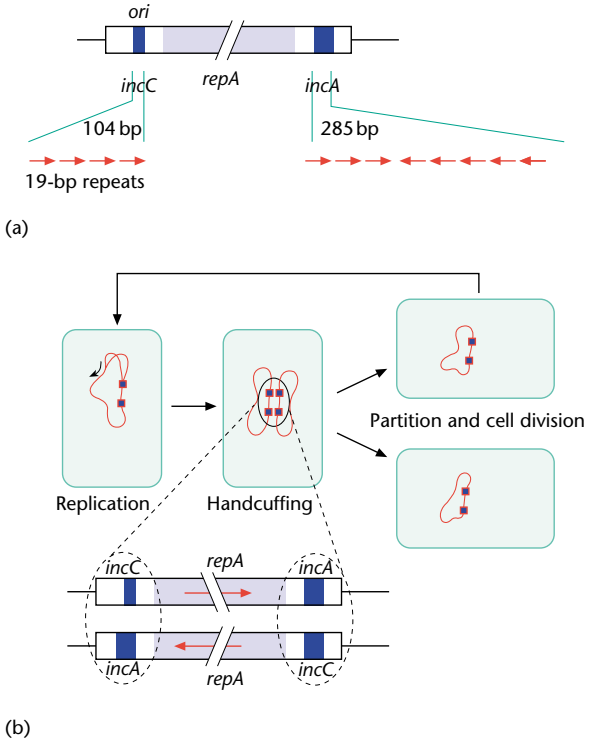

Figure 3 Replication control of the P1 prophage by plasmid handcuffing (a) the R-replicon of phage P1; iterons flanking *repA* are indicated by arrows (b) following replication, plasmid pairing is mediated by iteron-bound RepA and eventually disrupted by partition and cell division.

Plasmid Maintenance

It is essential that plasmids are distributed to both daughter cells at division. Depending on plasmid copy number, one of two distinct strategies is adopted: low copy number plasmids use active partition (Williams and Thomas, 1992), whereas high copy plasmids rely upon random distribution (Summers, 1998).

Maintenance of multicopy plasmids

Multicopy plasmids are distributed randomly to daughter cells, so the number of plasmids in the dividing cell determines the probability that one of the daughters will be plasmid-free. When a cell containing more than 20 randomly distributed plasmids divides, the probability of either daughter cell failing to receive a plasmid is very low ($< 10^{-6}$) but any event which reduces copy number (e.g. inefficient copy number control) will increase this. While the rate at which plasmid-free cells arise is determined by the dividing cell copy number, the rate at which they accumulate in culture is strongly influenced by the relative growth rate of plasmid-containing and plasmid-free cells. The growth of plasmid-containing cells is depressed by plasmid metabolic load, which results from the demands placed upon the host by the replication, transcription and translation of the plasmid genome. Load can become severe if the plasmid is used to express genes whose products are toxic or interfere with the host's metabolism.

Multimer resolution systems

Multimerization is an important cause of instability for ColE1-like plasmids because multimers have a copy number significantly lower than monomers. Even a small percentage of dimers (the most common form of multimer) has a deleterious effect upon plasmid stability because they are concentrated in a small proportion of the population from which plasmid-free cells arise at high frequency. The concentration of dimers into ghettos is a consequence of an inherent replication advantage of dimers over monomers, which leads to their rapid clonal accumulation in the descendants of the cell in which the first dimer arose. This blind spot in copy number control makes the detection and eradication of multimers of vital importance for multicopy plasmids.

As an antidote to the dangers of multimerization, natural multicopy plasmids carry recombination sites where host proteins act to convert multimers to monomers (Summers, 1998). The best-studied multimer resolution system is *cer*-Xer of ColE1. The plasmid carries a 240-bp site (*cer*), at which the host-encoded recombinases XerC and XerD and accessory proteins ArgR and PepA mediate site-specific recombination. Recombination at *cer* is under strict topological constraint; dimers are resolved to monomers but the reverse reaction is strongly suppressed.

Although dimer resolution is necessary for stable maintenance of ColE1, it is not sufficient, and the *rcd* gene, located within *cer*, is also required. Recombination at *cer* removes dimers but this process seems to be relatively slow and, if the cell divides before resolution is complete, there is a risk that a plasmid-free daughter will arise. It has been suggested that the Rcd transcript establishes a checkpoint linking multimer resolution and cell division, delaying the division of cells which contain multimers (Summers, 1998).

Maintenance of low copy number plasmids

Stable maintenance of low copy number plasmids requires their active distribution to daughter cells, although the precise mechanism is unclear. The prepairing model suggests that a monomeric protein binds to a centromere-like site on individual plasmids, subsequently dimerizing and pairing the plasmids. The dimeric protein–DNA complex then binds to the membrane on the plane of cell division. Septum formation or protein-mediated translocation separates the plasmid pair and subsequent DNA replication releases the complex from the cell membrane.

Some support for the pre-pairing model has come from examining the properties of actual partition systems. Among the best-characterized are those of plasmid F and the P1 prophage. Their genetic organization is similar; each encodes two *trans*-acting proteins (F SopA/SopB and P1 ParA/ParB) and a *cis*-acting site (F *sopC* and P1 *incB*). *sopC* binds SopB and seems to be an obvious candidate for the plasmid centromere. SopB is membrane-associated and localized to the cell poles – properties which might help to keep segregated plasmid pairs apart during septum formation. SopA is membrane-associated and belongs to a family of ATPases. It may form part of a membrane recognition site for paired plasmids or may be directly involved in the pairing process. Adenosine triphosphate (ATP) hydrolysis by the SopA–SopB–*sopC* complex could provide the driving force to separate the products of plasmid replication in a process analogous to eukaryotic mitosis.

Dimer resolution assists active partitioning

The P1 prophage is lost at less than one in 10^4 cell divisions, implying that replication and partitioning are highly efficient processes. In recombination-proficient hosts, however, some Par^+ P1 miniplasmids are lost at one in 100 cell divisions. This is due to homologous recombination between P1 monomers creating a dimer that cannot be partitioned. Unstable P1 miniplasmids lack the *lox-cre* region which encodes a site-specific recombination system. Cre recombinase mediates recombination between the directly-repeated *lox* sites in a dimer, restoring monomers, which can be partitioned successfully. Dimer resolution systems are widespread among actively partitioned plasmids.

Host killing functions

If a failure of replication control and partitioning results in the formation of a plasmid-free cell, there remains one last line of defence for low copy number plasmids. Plasmid-encoded host killing systems leave behind a stable toxin which is activated when an unstable antidote disappears following plasmid loss. Well-characterized examples include F *ccd* and a functionally-analogous but mechanistically-distinct host killing system encoded by the *parB* locus of plasmid R1. In the latter case genetic analysis has identified three genes (*hok*, *mok* and *sok*) within *parB*. Mok and Hok (the poison) are polypeptides translated from a single stable mRNA, while the *sok* gene product (the antidote) is an unstable antisense RNA transcribed from the opposite strand of the duplex and overlapping Mok-Hok mRNA by 128 bases at the 5′-end. Under normal circumstances Sok binds to Mok-Hok mRNA and prevents its translation but, if the plasmid is lost from the cell, Sok decays rapidly and translation of Mok-Hok mRNA begins, leading to the death of the plasmid-free cell.

Horizontal Transmission of Plasmids

Conjugative plasmids are capable of horizontal transmission between and within species (Day and Fry, 1992). Plasmid transfer by conjugation involves cell-to-cell contact so the nucleic acid remains within the cellular environment and is protected from degradation by extracellular nucleases and heavy metals. Transfer is associated with a round of plasmid replication, so a copy of the plasmid is left behind in the donor cell and there is a net increase in the number of plasmids in the cell population. Genes required for conjugal transfer are normally tightly repressed but when a plasmid arrives in a new host they are transiently derepressed, increasing the chance that the plasmid will transfer again to a plasmid-free neighbour. This causes an epidemic spread of the plasmid through the population, a virus-like behaviour which underlines the parasitic nature of many plasmids.

Conjugation in Gram-negative organisms

Conjugative plasmids from Gram-negative bacteria direct the synthesis of an extracellular pilus which has an essential role in the recognition of recipient cells and the establishment of cell-to-cell contact. Pili can be classified into two broad morphological groups: long flexible (1 μm) and short rigid (0.1 μm). Long pili, like those expressed by cells carrying the F plasmid, support genetic exchange on surfaces and in liquid media at roughly equal frequencies. Short pili, expressed by plasmids of the IncN, P and W

incompatibility groups, are 10^3–10^5 times more efficient in surface matings than in liquid. Some plasmids (Inc groups I_1, I_2, I_5, B, K and Z) encode both long and short pili.

Of the many plasmid conjugation systems in Gram-negative bacteria, those of the IncF plasmids have been studied in greatest detail. More than 30 genes are needed for conjugation and they are clustered in the 33-kb *tra* region, which constitutes about one-third of the plasmid. At least 14 genes are involved in construction of the F-pilus; a hollow cylinder of 8-nm diameter with a 2-nm axial hole. After the initial contact between the tip of the pilus and the recipient cell the pilus retracts, bringing the donor and recipient into close contact. Transfer is initiated when a single-strand nick is introduced at the plasmid origin of transfer (*oriT*) and the nicked strand is transferred to the recipient. The nontransferred strand is copied so the donor retains an intact plasmid. During transfer the nicking protein remains bound covalently to the exposed 5′-terminus. Recircularization of the transferred plasmid is *oriT*-dependent. A possible mechanism for recircularization is that the nicking complex bound to the transferred 5′-end is retained at the membrane pore, where it interacts with the trailing 3′-end and religation occurs by a reversal of the original nicking reaction.

Conjugal transfer of plasmid F can still take place after the plasmid has fused with the *E. coli* chromosome by recombination between mobile elements present in both replicons. This results in the cell-to-cell transfer of large sections of the bacterial chromosome, a process which was often used to map genes before the days of genome sequencing because the relative distance of a gene from the origin of transfer can be deduced from its time of entry into the recipient.

Mobilization

Mobilization is a process whereby plasmids that are too small to encode a full set of transfer genes can borrow gene products from a conjugative plasmid. Examples are *E. coli* plasmids ColE1 (6.6 kb) and RSF1010 (8.9 kb). The role of the conjugative plasmid is to provide pilus-mediated cell-to-cell contact, formation of a conjugation pore and related morphological functions. The mobilized plasmid must possess an active *oriT* and a small set of plasmid-specific *mob* genes whose products are responsible for nicking the plasmid at *oriT* and initiating strand transfer.

Conjugation in Gram-positive organisms

Transmissible plasmids have been reported in numerous Gram-positive genera including *Streptococcus*, *Staphylococcus*, *Bacillus*, *Clostridium*, *Streptomyces* and *Nocardia*. The early stages of the process are not well-characterized but, in contrast to Gram-negative bacteria, pili do not appear to be involved in initiating conjugation.

Conjugation among the staphylococci

Self-transmissible antibiotic resistance plasmids of 38–57 kb are found in the staphylococci and genes encoding transfer functions occupy about a third of the plasmid genome. The mechanism of conjugation in the staphylococci is not well-characterized but the relatively small amount of DNA coding for the necessary functions suggests the process may be simpler than that employed by plasmid F, which devotes twice as much DNA to its *tra* operon.

Enterococcal plasmid transfer

A feature of conjugation so far unique to the enterococci is the involvement of pheromones or clumping-inducing agents. Pheromones are hydrophobic polypeptides of 7–8 amino acids produced by potential recipient cells. Each cell may produce multiple chemical signals, inviting attention from donors containing a variety of conjugative plasmids; plasmid-free cells secrete at least five. The effect of the pheromone on the donor is to induce synthesis of a proteinaceous adhesin, which stimulates cell clumping and allows conjugation to proceed. Once a cell acquires a particular plasmid, it stops secreting the corresponding pheromone.

Conjugal transfer among *Streptomyces* spp.

In *Streptomyces* spp. conjugative plasmids carry genes which promote their transfer to other species and, in some cases, support the mobilization of chromosomal DNA and nonconjugative plasmids. Pock formation or 'lethal zygosis' is a curious and characteristic feature of *Streptomyces* conjugative plasmids that has greatly simplified their detection. Pocks are circular areas of retarded growth that appear around plasmid-containing colonies growing on a lawn of plasmid-free cells.

Broad host range conjugative plasmids

Broad host range or promiscuous plasmids can transfer between bacteria from different species and are maintained stably within them. Plasmids of the IncP, W and Q incompatibility groups can enter and persist in almost all Gram-negative bacteria. Broad host range plasmids have been shown to mobilize DNA from Gram-negative bacteria to a number of Gram-positive species and even to yeast, demonstrating the possibility of horizontal gene transfer between genera and kingdoms. The best studied of the promiscuous plasmids belong to the IncP group; in particular the IncPα plasmid (known variously as RP1, RP4, RK2, R18 and R68) has received attention in many laboratories. The presence of a complex series of coregulated operons suggests that there is a sophisticated and coordinated system of control over plasmid replication, maintenance and transfer functions. The plasmids are self-transmissible at high frequency on a solid substrate but,

characteristically for plasmids which encode a rigid sex pilus, mating in liquid is inefficient. Despite the greater host range of IncPα plasmids, the mechanism of transfer is probably analogous to that of F and its relatives; the amount of DNA devoted to *tra* functions (approximately 25 kb) is certainly similar.

Restriction-modification systems present a major threat to the physical integrity of plasmids which transfer across species boundaries. The susceptibility of conjugative plasmids to host restriction is extremely variable. Selection pressure may have led to the eradication of restriction enzyme recognition sites from promiscuous plasmids, and some plasmids encode functions which inactivate the defences of the new host. An example of the latter strategy is provided by the *ard* (*a*lleviation of *r*estriction of *D*NA) genes of conjugative plasmids ColIb-P9 and pKM101, which are located close to the origin of transfer. Their products, synthesized in the recipient very early in conjugation, protect against type I restriction-modification systems.

Promiscuous plasmids encode additional functions which assist their establishment in a new host. These include the Sog proteins (DNA primases) which are synthesized in the donor and injected into the recipient, where they initiate complementary strand synthesis independently of the host cell primases. Another class of protein (PsiB), whose role is less well understood, appears to inhibit the induction of the bacterial SOS response during conjugation.

Mechanisms of Change in Plasmid Structure

Plasmid evolution is a saltatory phenomenon, proceeding by the gain and loss of functional modules rather than the gradual processes of mutation and selection. Two classes of elements largely responsible for plasmid structural fluidity are transposons and integrons. One of the most dramatic and well-documented consequences of the rapid pace of plasmid evolution is the spread of multiple antibiotic resistance since the late 1950s. This is a continuing process and the most pessimistic scenario for the future envisages a 'postantimicrobial era' in which clinicians face a return to the conditions in the 1930s, when hospital wards were filled with patients suffering from bacterial infections. It is easy to forget that before the advent of antibiotics the majority of such patients died from the disease or from associated complications.

Plasmids, transposons and antibiotic resistance

Antibiotic resistance genes are often found within transposable elements which are capable of movement from one plasmid to another or between plasmids and the chromosome (Sherratt, 1995). Although most transposons are unable to transfer resistance genes between cells (the exception being conjugative transposons found in the enterococci), they can effectively mobilize genes by moving them to a transmissible plasmid. Since promiscuous plasmids have host ranges which include bacteria from different genera, transposon-borne resistances have the potential to be disseminated throughout a very wide variety of organisms. Being part of a transposon can give genes a broader host range than the plasmids on whose backs they travel. It is quite possible for a plasmid to conjugate successfully into a new host but then to find that it is unable to replicate. If a resistance gene on such a plasmid is part of a transposable element it can be rescued by transposition to a native replicon.

Integrons and antibiotic resistance

Drug resistances on plasmids or transposons can be reshuffled by rearrangements within genetic cassettes known as integrons (Hall and Collis, 1995). Integrons contain three discrete regions: a 5′-common segment, a central variable region and a 3′-common segment. The variable region contains one or more resistance genes which lack their own promoters and are transcribed as an operon from a promoter at the inner boundary of the 5′-common segment. Thus integrons act as natural expression vectors for resistance gene cassettes. Integration or excision of cassettes involves site-specific recombination mediated by an integron-encoded recombinase (Int). Recombination sites recognized by Int separate the resistance gene cassettes, which can be mobilized independently. Int-mediated excision of a cassette generates a circular intermediate that can reintegrate in the original element or in a new integron by recombination with a core sequence.

The pinnacle of genetic flexibility must surely have been reached by a gene which is part of an integron, located in a transposon, on a broad host range plasmid.

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