Plasmid incompatibility:

Not all plasmids can stably coexist in a bacterial host cell. Two genetically distinguishable plasmids that cannot be stably maintained within a particular host are designated as members of the same incompatibility (Inc) group (19). Generally, if two plasmids are members of the same incompatibility group, the introduction of one of the two plasmids by conjugation, transformation, or transduction into a cell carrying the other plasmid destabilizes the inheritance of the resident plasmid. The placement of plasmids in various Inc groups has proved useful in classifying plasmid elements and identifying plasmids that are genetically related. The phenomenon of plasmid incompatibility is a consequence of two plasmids sharing common elements responsible for plasmid maintenance, namely, replication control and/or partitioning systems (63). It is also dependent on the fact that at least for those plasmids examined, representing plasmids from both gram-positive and gram-negative bacteria, the selection of individual plasmid molecules for replication and partitioning is carried out randomly from a common pool of molecules (5, 63, 82, 84).

PLASMID HOST RANGE:

Plasmids since their discovery have been detected in many different genera. Various microorganisms in which a plasmid can replicate and be maintained is called its host range. Accordingly, plasmids can be classified into narrow and broad host ranges (BHR). The first classification into these two groups was made in 1972 by (Datta & Hedges, 1972), who defined BHR plasmids as those which are able to transfer

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among *Enterobacteria* and *Pseudomonas* spp. However, according to the current understanding, BHR plasmids transfer and maintain among bacteria belonging to different phylogenetic subgroups (Top *et al.*, 1998).

Broad host range plasmids are of considerable interest because they not only play an important role in horizontal gene transfer but also their replicons can serve as good sources for vector construction. Several barriers limit plasmid transfer between unrelated bacteria: interactions at the cell surface may prevent effective mating contact, restriction systems may degrade foreign DNA, or the plasmid may not replicate in the new host. There are several reviews published on BHR plasmids (Kues & Stahl, 1989; del Solar *et al.*, 1993, 1996; Sakai & Komano, 1996). However, in the era of genomics and mobile metagenomics, it would be of immense importance to predict the host range of the plasmid based on the sequence

information. For this, it is worth listing the general features that confer broad host range properties to the plasmid. In the present review, we describe the possible reasons behind the unique capability of plasmids to replicate and maintain in varied hosts, molecular basis of host range evolution, and their application in recombinant DNA technology.

Factors affecting broad host range (BHR) plasmid replication

Some well-characterized naturally occurring BHR plasmids and their host range determined so far are shown in Table 1. There are a number of features, which determine the host range of plasmids.

Table 1

Naturally occurring broad host range plasmids described according to their sizes

Plasmid	Size (kb)	Antibiotic resistance markers	Microorganism from which isolated	Host range	Reference
pBC1	1.6	Cryptic	*Bacillus coagulans* Zu1961	*E. coli, B. subtilis, B. amyloliquefaciens, S.aureus, S. carnosus, and Lactobacillus reuteri*	De Rossi *et al.* (1992)
pEP2	1.85	Cryptic	*Corynebacterium diphtheriae*	*Corynebacteria, Mycobacteria*, and *E. coli*	Zhang *et al.* (1994)
pWVO1	2.2	Cryptic	*Lactococcus lactis* subsp. *cremoris*	*Bacilli, Lactococci, Streptococci, Clostridia* and *Staphylococci, E. coli*	Leenhouts *et al.* (1991)
pLF1311	2.38	Cryptic	*Lactobacillus fermentum* VKM1311	*E. coli, Lactobacillus, Lactococcus, Enterococcus, Bacillus*	Aleshin *et al.* (1999)
pAP1	2.4	Cryptic	*Arcanobacterium* (*Actinomyces*) *pyogenes*	*E. coli, Corynebacterium pseudotuberculosis, Arcanobacterium*	Billington *et al.* (1998)
pBBR1	2.6	Cryptic	*Bordetella bronchiseptica*	*E. coli, Bordetella pertussis, B. bronchiseptica, Vibrio cholerae, Rhizobium meliloti, Pseudomonas putida*	Szpirer *et al.* (

Plasmid copy number

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This article **may be too technical for most readers to understand**. Please help improve it to make it understandable to non-experts, without removing the technical details. *(September 2015) (Learn how and when to remove this template message)*

Plasmids must regulate their copy number (average number of plasmid copies per cell) to ensure that they do not excessively burden the host or become lost during cell division. Plasmids may be either high copy number plasmids or low copy number plasmids; the regulation mechanisms between these two types are often significantly different. Biotechnology applications may involve engineering plasmids to allow a very high copy number. For example, pBR322 is a low copy number plasmid (~20 copies/cell) from which several very high copy number cloning vectors (~1000 copies/cell) have been derived.[1]

Contents

Regulation[edit]

High copy number plasmids, also called relaxed plasmids, require a system to ensure that replication is inhibited once the number of plasmids in the cell reaches a certain threshold. Relaxed plasmids are generally regulated through one of two mechanisms: antisense RNA or iteron binding groups. Low copy number plasmids, also called stringent plasmids, require tighter control of replication.

ColE1 derived plasmids: Antisense RNA[edit]

In ColE1 derived plasmids, replication is primarily regulated through a small plasmid-encoded RNA called RNA I. A single promoter initiates replication in ColE1: the RNA II promoter. The RNA II transcript forms a stable RNA-DNA hybrid with the DNA template strand near the origin of replication, where it is then processed by RNaseH to produce the 3' OH primer that DNA polymerase I uses to initiate leading strand DNA synthesis. RNA I serves as a major plasmid-encoded inhibitor of this process whose concentration is proportional to plasmid copy number. RNA I is exactly complementary to the 5' end of the RNA II (because it is transcribed from the opposite strand of the same region of DNA as RNA II). RNA I and RNA II first form a weak interaction called a kissing complex. The kissing complex is stabilized by a protein called Rop (repressor of primer) and a double-stranded RNA-I/RNA-II RNA duplex is formed. This altered shape prevents RNA II from hybridizing to the DNA and being processed from RNaseH to produce the primer necessary for initiation of plasmid replication. More RNA I is produced when the concentration of the plasmid is high, and high concentration of RNA I inhibits replication, resulting in regulation of copy number.[2][3]

R1 and ColIb-p9 Plasmids: Antisense RNA[edit]

Most plasmids require a plasmid-encoded protein, usually called Rep, to separate the strands of DNA at the origin of replication (*oriV*) to initiate DNA replication. Rep binds to specific DNA sequences in *oriV* which are unique to a plasmid type. The synthesis of Rep protein is controlled in order to limit plasmid replication and therefore regulate copy number. In R1 plasmids RepA can be transcribed from two different promoters. It is made from the first promoter until the plasmid reaches its copy number, upon

which the protein CopB represses this primary promoter.[3] RepA expression is also regulated post-transcriptionally from the secondary promoter by an antisense RNA called CopA. CopA interacts with its RNA target in the RepA mRNA and forms a kissing complex and then a RNA-RNA duplex. The resultant double stranded RNA is cleaved by RNase III, preventing synthesis of RepA. The higher the concentration of the plasmid, the more CopA RNA is produced and the less RepA protein can be synthesized, increasing inhibition of plasmid replication.[4]

Col1b-P9: Antisense RNA[edit]

Replication of the low-copy-number ColIb-P9 depends upon Rep, which is produced by expression of the *repZ* gene. *repZ* expression requires formation of a pseudoknot in the mRNA. *repZ* is repressed by a small antisense Inc RNA, which binds to *repZ* mRNA, forms an Inc RNA-mRNA duplex, and prevents formation of the pseudoknot to inhibit *repZ* translation into Rep. In this event, replication can no longer occur.[5]

pSC101: Iteron plasmid[edit]

Iteron plasmids, including F and RK2-related plasmids, have oriV regions containing multiple (~3-7) repeats of 17-22 bp iteron sequences.[3] pSC101 represents a simple model of an iteron plasmid. Iteron plasmids control copy number through two combined methods, suitable for low copy number stringent plasmids. One method is control of RepA synthesis. RepA is the only plasmid-encoded protein required for replication in pSC101. RepA protein represses its own synthesis by binding to its own promoter region and blocking transcription of itself (transcriptional autoregulation). Thus, the more RepA is made, the more its synthesis is repressed, and subsequently limiting plasmid replication.[3] The coupling hypothesis proposes that the second method is coupling of plasmids through the Rep protein and iteron sequences. When the plasmid concentration is high, RepA plasmids bound to iterons form dimers in between two plasmids, "handcuffing" them at the origin of replication and inhibiting replication.[6]

Incompatibility[edit]

Plasmids can be incompatible if they share the same replication control mechanism. Under these circumstances, both plasmids contribute to the total copy number and are regulated together. They are not recognized as distinct plasmids. As such, it becomes much more likely that one of the plasmids may be out-copied by the other and lost during cell division (the cell is "cured" of the plasmid).[3] This is particularly likely with low copy number plasmids. Plasmids can also be incompatible due to shared partitioning systems.

Theory on Curing of Plasmids.

Plasmids are defined as extrachromasomally replicating molecules of DNA. They are different from the chromosomal DNA and are present in bacteria. Bacteria are a large group of single-celled microorganisms, many of which cause infections and disease in animals and humans. For example, the Enterobacteriaceae are a large family of bacteria, which occur both commensally and pathogenically in the intestines, causing wide –spread disease. Most of the essential genes in a bacterium that are required for survival under normal or optimum conditions are contained in the chromosome. However, many bacteria also carry a variety of Mobile Genetic Elements (MGE) that can contribute significantly to their diversity and adaptability. These MGEs do not carry anything essential for the survival under non-stressful circumstances, but may be important for specialized functions, such as the ability of the bacterium to form biofilms or to be resistant to antibiotics. Plasmids are the most easily identified MGE, because they are physically separate from the chromosome and can be the visualized by lysis and electrophoretic separation of the released DNA molecules. It is expected, therefore, that in any growing population of plasmid carrying bacteria, plasmidless segregants will occasionally be produced as the result of an error in the process of plasmid replication or partitioning to daughter bacteria. The survival of such bacteria indicated that the plasmid lost does not encode functions vital for growth under the prevailing environmental conditions. Furthermore the bacteria can only regain the lost functions by acquiring, the necessary genes from an external source. Thus the instability of bacterial property, either a common or uncommon feature of that species, can be an indicator of plasmid involvement.

Bacterial plasmids are known to harbor genes for: (i) resistances to antibiotics and metals, (ii) catabolic pathways such as lactose utilization and degradation of hydrocarbons (iii) biosynthesis of certain antibiotics, etc. In many cases the characteristics of the host organism conferred by the plasmids remain elusive, and such cryptic plasmids are abundant in nature. Curing of this cryptic plasmid from a bacterial strain is a method to substantiate the relationship between a genetic trait and carriage of that specific trait in the plasmid. Various methods involving chemical and physical agents have been developed to eliminate plasmids. Protocols for curing plasmids consist frequently of exposure of a culture to sub-inhibitory concentrations of some chemical agents, e.g. acridine orange, acriflavine, and sodium dodecyl sulfate or to a super-optimal temperature followed by selection of cured derivatives.

In the instances where the plasmid is stable or the loss of property difficult to determine, the bacteria can be treated with curing agents. These include chemical and physical agents, some of which can mutate DNA, interfere specifically with its replication, or affect particular structural components or enzymes of the bacterial cell. Protocols for curing plasmids consist frequently of exposure of a culture to sub-inhibitory concentrations of some chemical agents, e.g. acridine orange, acriflavine, and sodium dodecyl sulfate or to a super-optimal temperature followed by selection of cured derivatives. The DNA intercalating agents such as Acridine orange and ethidium bromide are the most commonly used because they are found to be effective against plasmids in a wide variety of genera. Although all of these agents have been used to enhance the recovery of plasmid less derivatives of various bacteria, they are individually effective only against some plasmids and their likely response is unpredictable. The elimination of a plasmid (curing) from a bacterial culture is the best method to substantiate the relationship between a genetic trait and carriage of specific plasmid by the culture as the phenotypic characters which are associated with the plasmid are not expressed in cured derivatives but on the re-introduction of the plasmid in to the cured strain the lost phenotype is re appeared. The efficiency of curing can also vary widely depending on the plasmid and the particular bacterial host carrying it. In most instances, the underlying mechanism of curing is not known. The agent may interfere directly with plasmid replication as occurs with the heat induced curing of certain temperature sensitive palsmid or curing of them by acridines or ethidium bromide. Alternatively, curing may interfere with the growth of plasmid carrying bacteria thereby allowing spontaneously arising plasmidless segregants to become predominant. This occurs in certain instances of curing by acridines, sodium dodecyl sulphate and urea. Curing by MitomycinC is also effective. Curing experiments are usually performed under conditions similar to those used for the routine culture of the bacteria unless the limitation of an essential component is specifically intended. When treatment is to be for a continuous period, the agent is used at concentration just less than that required to inhibit growth, and the number of bacteria initially is small. If acridine is used, the culture is maintained at pH 7.6 and incubated in the dark.