PLASMIDS

The term *plasmid* was introduced in 1952 by the American molecular biologist Joshua Lederberg to refer to "any extrachromosomal hereditary determinant The term's early usage included any bacterial genetic material that exists extrachromosomally for at least part of its replication cycle, but because that description includes bacterial viruses, the notion of plasmid was refined over time to comprise genetic elements that reproduce autonomously. Later in 1968, it was decided that the term plasmid should be adopted as the term for extrachromosomal genetic element, and to distinguish it from viruses, the definition was narrowed to genetic elements that exist exclusively or predominantly outside of the chromosome and can replicate autonomously.

A **plasmid** is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria; however, plasmids are sometimes present in archaea and eukaryotic organisms. In nature, plasmids often carry genes that benefit the survival of the organism and confer selective advantage such as antibiotic resistance. While chromosomes are large and contain all the essential genetic information for living under normal conditions, plasmids are usually very small and contain only additional genes that may be useful in certain situations or conditions. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms. In the laboratory, plasmids may be introduced into a cell via transformation.

Plasmids are considered *replicons*, units of DNA capable of replicating autonomously within a suitable host. However, plasmids, like viruses, are not generally classified as life. Plasmids are transmitted from one bacterium to another (even of another species) mostly through conjugation. This host-to-host transfer of genetic material is one mechanism of horizontal gene transfer, and plasmids are considered part of the mobilome. Unlike viruses, which encase their genetic material in a protective protein coat called a capsid, plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host; however, some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer. The size of the plasmid varies

from 1 to over 200 kbp, and the number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances.

In order for plasmids to replicate independently within a cell, they must possess a stretch of DNA that can act as an origin of replication. The self-replicating unit, in this case, the plasmid, is called a replicon. A typical bacterial replicon may consist of a number of elements, such as the gene for plasmid-specific replication initiation protein (Rep), repeating units called iterons, DnaA boxes, and an adjacent AT-rich region. Smaller plasmids make use of the host replicative enzymes to make copies of themselves, while larger plasmids may carry genes specific for the replication of those plasmids. A few types of plasmids can also insert into the host chromosome, and these integrative plasmids are sometimes referred to as episomes in prokaryotes.

Plasmids almost always carry at least one gene. Many of the genes carried by a plasmid are beneficial for the host cells, for example: enabling the host cell to survive in an environment that would otherwise be lethal or restrictive for growth. Some of these genes encode traits for antibiotic resistance or resistance to heavy metal, while others may produce virulence factors that enable a bacterium to colonize a host and overcome its defences or have specific metabolic functions that allow the bacterium to utilize a particular nutrient, including the ability to degrade recalcitrant or toxic organic compounds.[5] Plasmids can also provide bacteria with the ability to fix nitrogen. Some plasmids, however, have no observable effect on the phenotype of the host cell or its benefit to the host cells cannot be determined, and these plasmids are called cryptic plasmids.

Naturally occurring plasmids vary greatly in their physical properties. Their size can range from very small mini-plasmids of less than 1-kilobase pairs (Kbp) to very large megaplasmids of several megabase pairs (Mbp). At the upper end, little differs between a megaplasmid and a minichromosome. Plasmids are generally circular, but examples of linear plasmids are also known. These linear plasmids require specialized mechanisms to replicate their ends.

Plasmids may be present in an individual cell in varying number, ranging from one to several hundreds. The normal number of copies of plasmid that may be found in a single cell is called the Plasmid copy number, and is determined by how the replication initiation is regulated and the size of the molecule. Larger plasmids tend to have lower copy numbers. Low-copy-

number plasmids that exist only as one or a few copies in each bacterium are, upon cell division, in danger of being lost in one of the segregating bacteria. Such single-copy plasmids have systems that attempt to actively distribute a copy to both daughter cells. These systems, which include the parABS system and parMRC system, are often referred to as the partition system or partition function of a plasmid.

Plasmids may be classified in a number of ways. Plasmids can be broadly classified into conjugative plasmids and non-conjugative plasmids. Conjugative plasmids contain a set of transfer or *tra* genes which promote sexual conjugation between different cells. In the complex process of conjugation, plasmids may be transferred from one bacterium to another via sex pili encoded by some of the *tra* genes (see figure). Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids. An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence.

Plasmids can also be classified into incompatibility groups. A microbe can harbour different types of plasmids, but different plasmids can only exist in a single bacterial cell if they are compatible. If two plasmids are not compatible, one or the other will be rapidly lost from the cell. Different plasmids may therefore be assigned to different incompatibility groups depending on whether they can coexist together. Incompatible plasmids (belonging to the same incompatibility group) normally share the same replication or partition mechanisms and can thus not be kept together in a single cell. Another way to classify plasmids is by function. There are five main classes:

- Fertility F-plasmids, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili.
- Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
- Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen. e.g. Ti plasmid in *Agrobacterium tumefaciens*

Plasmids can belong to more than one of these functional groups.

F PLASMID

The **fertility factor** (first named **F** by one of its discoverers Esther Lederberg; also called the **sex factor** in *E. coli* or the **F sex factor**; also called **F-plasmid**) allows genes to be transferred from one bacterium carrying the factor to another bacterium lacking the factor by conjugation. The F factor is carried on the F episome, the first episome to be discovered. Unlike other plasmids, F factor is constitutive for transfer proteins due to a mutation in the gene *finO*. The F plasmid belongs to a class of conjugative plasmids that control sexual functions of bacteria with a fertility inhibition (Fin) system.

The most common functional segments constituting F factors are:

- OriT (Origin of Transfer): The sequence which marks the starting point of conjugative transfer.
- OriC (Origin of Replication): The sequence starting with which the plasmid-DNA will be replicated in the recipient cell.
- tra-region (transfer genes): Genes coding the F-Pilus and DNA transfer process.
- IS (Insertion Elements) composed of one copy of IS2, two copies of IS3, and one copy of IS1000: so-called "selfish genes" (sequence fragments which can integrate copies of themselves at different locations).

When an F^+ cell conjugates/mates with an F^- cell, the result is two F^+ cells, both capable of transmitting the plasmid to other F^- cells by conjugation. The F-plasmid belongs to a class of conjugative plasmids that control sexual functions of bacteria with a fertility inhibition (Fin) system. In this system, a trans-acting factor, FinO, and antisense RNAs, FinP, combine to repress the expression of the activator gene TraJ. TraJ is a transcription factor that upregulates the *tra* operon. The *tra* operon includes genes required for conjugation and plasmid transfer. This means that an F^+ bacteria can always act as a donor cell. The *finO* gene of the original F plasmid (in E. coli K12) is interrupted by an IS3 insertion, resulting in constitutive *tra* operon expression. F^+ cells also have the surface exclusion proteins TraS and TraT on the bacterial surface. These proteins prevent

secondary mating events involving plasmids belonging to the same incompatibility (Inc) group. Thus, each F^+ bacterium can host only a single plasmid type of any given incompatibility group.

In the case of Hfr transfer, the resulting transconjugates are rarely Hfr. The result of Hfr/F^- conjugation is a F^- strain with a new genotype. When F-prime plasmids are transferred to a recipient bacterial cell, they carry pieces of the donor's DNA that can become important in recombination. Bioengineers have created F plasmids that can contain inserted foreign DNA; this is called a bacterial artificial chromosome.

The first DNA helicase ever described is encoded on the F-plasmid and is responsible for initiating plasmid transfer. It was originally called ***E. coli* DNA Helicase I**, but is now known as **F-plasmid TraI**. In addition to being a helicase, the 1756 amino acid (one of the largest in *E. coli*) F-plasmid TraI protein is also responsible for both specific and non-specific single-stranded DNA binding as well as catalyzing the nicking of single-stranded DNA at the origin of transfer.

Ti PLASMID

A **tumour inducing (Ti)** plasmid is a plasmid found in pathogenic species of *Agrobacterium*, including *A. tumefaciens*, *A. rhizogenes*, *A. rubi* and *A. vitis*.

Evolutionarily, the Ti plasmid is part of a family of plasmids carried by many species of Alphaproteobacteria. Members of this plasmid family are defined by the presence of a conserved DNA region known as the *repABC* gene cassette, which mediates the replication of the plasmid, the partitioning of the plasmid into daughter cells during cell division as well as the maintenance of the plasmid at low copy numbers in a cell. The Ti plasmids themselves are sorted into different categories based on the type of molecule, or opine, they allow the bacteria to break down as an energy source.

The presence of this Ti plasmid is essential for the bacteria to cause crown gall disease in plants. This is facilitated via certain crucial regions in the Ti plasmid, including the *vir* region, which encodes for virulence genes, and the transfer DNA (T-DNA) region, which is a section of the Ti plasmid that is transferred via conjugation into host plant cells after an injury site is sensed by the bacteria. These regions have features that allow the delivery of T-DNA into host plant cells, and can modify the host plant cell to cause

the synthesis of molecules like plant hormones (e.g. auxins, cytokinins) and opines and the formation of crown gall tumours.

Because the T-DNA region of the Ti plasmid can be transferred from bacteria to plant cells, it represented an exciting avenue for the transfer of DNA between kingdoms and spurred large amounts of research on the Ti plasmid and its possible uses in bioengineering.

The replication of the Ti plasmid is driven by the RepC initiator protein, which possesses two protein domains: an N-terminal domain (NTD) that binds to DNA and a C-terminal domain (CTD). Mutational analyses have shown that without a functional RepC protein, the Ti plasmid is unable to replicate. Meanwhile, the *oriV* sequence is around 150 nucleotides in length and is found within the *repC* gene. Laboratory experiments have shown that the RepC protein binds to this region, suggesting its role as the origin of replication[16]. Therefore, while the complete process behind the replication of the Ti plasmid has not been fully described, the initial step of replication would likely depend on the expression of RepC and its binding to *oriV*. Of note, the RepC protein only acts in *cis*, where it only drives the replication of the plasmid it is encoded in and not any other plasmid also present in the bacterial cell.

The Ti plasmid is maintained at low copy numbers within a bacterial cell. This is partly achieved by influencing the expression of the replication initiator RepC. When bound to ADP, RepA is activated to work with RepB, acting as a negative regulator of the *repABC* cassette. The levels of RepC is therefore kept low within a cell, preventing too many rounds of replication from occurring during each cell division cycle. Furthermore, there is a small RNA known as RepE encoded between *repB* and *repC* that lowers the expression of *repC*. RepE is complementary to RepC and will bind with the *repC* mRNA to form a double-stranded molecule. This can then block the translational production of the RepC protein[.

Separately, the expression of the *repABC* cassette and hence the copy number of the Ti plasmid is also influenced via a quorum sensing system in *Agrobacterium*. Quorum sensing systems respond to bacterial population densities by sensing a molecule, known as an autoinducer, that is produced by the bacterial cells at low levels and would build up to a threshold level when there is a high density of bacteria present[. In this case, the autoinducer is the N-3-oxooctanoyl-L-homoserine lactone (3-O-C_8-AHL) molecule, which is sensed by a regulator known as TraR. When activated, TraR will bind to regions known as *tra* boxes in the *repABC* gene

cassette's promoter regions to drive expression. Therefore, a high level of population density increases the number of plasmids present within each bacterial cell, likely to support pathogenesis in the plant host[

COLICINOGENIC PLASMID

One class of plasmids, colicinogenic (or *Col*) factors, determines the production of proteins called colicins, which have antibiotic activity and can kill other bacteria. Another class of plasmids, R factors, confers upon bacteria resistance to antibiotics.

A **colicin** is a type of bacteriocin produced by and toxic to some strains of *Escherichia coli*.[1] Colicins are released into the environment to reduce competition from other bacterial strains. Colicins bind to outer membrane receptors, using them to translocate to the cytoplasm or cytoplasmic membrane, where they exert their cytotoxic effect, including depolarisation of the cytoplasmic membrane, DNase activity, RNase activity, or inhibition of murein synthesis.

Virtually all colicins are carried on plasmids. The two general classes of colicinogenic plasmids are large, low-copy-number plasmids, and small, high-copy-number plasmids. The larger plasmids carry other genes, as well as the colicin operon. The colicin operons are generally organized with several major genes. These include an immunity gene, a colicin structural gene, and a bacteriocin release protein (BRP), or lysis, gene. The immunity gene is often produced constitutively, while the BRP is generally produced only as a read-through of the stop codon on the colicin structural gene. The colicin itself is repressed by the SOS response and may be regulated in other ways, as well.

Retaining the colicin plasmid is very important for cells that live with their relatives, because if a cell loses the immunity gene, it quickly becomes subject to destruction by circulating colicin. At the same time, colicin is only released from a producing cell by the use of the lysis protein, which results in that cell's death. This suicidal production mechanism would appear to be very costly, except for the fact that it is regulated by the SOS response, which responds to significant DNA damage. In short, colicin production may only occur in terminally ill cells. The Professor Kleanthous Research Group at the University of Oxford study colicins extensively as a model system for characterising and investigating protein-protein interactions and recognition

R PLASMID

Resistance transfer factor (shortened as R-factor or RTF) is an old name for a plasmid that codes for antibiotic resistance. R-factor was first demonstrated in *Shigella* in 1959 by Japanese scientists.[1] Often, R-factors code for more than one antibiotic resistance factor: genes that encode resistance to unrelated antibiotics may be carried on a single R-factor, sometimes up to 8 different resistances. Many R-factors can pass from one bacterium to another through bacterial conjugation and are a common means by which antibiotic resistance spreads between bacterial species, genera and even families.[2] For example, RP1, a plasmid that encodes resistance to ampicillin, tetracycline and kanamycin originated in a species of *Pseudomonas*, from the family Pseudomonadaceae, but can also be maintained in bacteria belonging to the family Enterobacteriaceae, such as *Escherichia coli*.

2 µm plasmid of yeast

The **2** µm **plasmid** is a benign parasitic **plasmid** harbored in the nucleus of most strains of the budding **yeast**, Saccharomyces cerevisiae (For review see [1] and [**2**]). Despite providing no selective advantage to the host, the **plasmid** is faithfully maintained at ~60 copies per cell The 2 micron plasmid of Saccharomyces cerevisiae is a relatively small multi-copy selfish DNA element that resides in the yeast nucleus at a copy number of 40-60 per haploid cell. The plasmid is able to persist in host populations with almost chromosome-like stability with the help of a partitioning system and a copy number control system.

Yeasts naturally harbour various plasmids. Notable among them are 2 µm plasmids—small circular plasmids often used for genetic engineering of yeast—and linear pGKL plasmids from *Kluyveromyces lactis*, that are responsible for killer phenotypes.

Other types of plasmids are often related to yeast cloning vectors that include:

- *Yeast integrative plasmid (YIp)*, yeast vectors that rely on integration into the host chromosome for survival and replication, and are usually

used when studying the functionality of a solo gene or when the gene is toxic. Also connected with the gene URA3, that codes an enzyme related to the biosynthesis of pyrimidine nucleotides (T, C);

- *Yeast Replicative Plasmid (YRp)*, which transport a sequence of chromosomal DNA that includes an origin of replication. These plasmids are less stable, as they can *get lost* during the budding.

Plasmid incompatibility

Plasmid incompatibility is usually defined as the failure of two co-resident plasmids to be stably inherited together in the absence of external selection. In simpler terms, if the introduction of a second plasmid negatively effects the inheritance of the first, the two are considered to be incompatible.

Plasmids can be seen as selfish entities in evolutionary terms. Having gained territory in a bacterial cell, they will try to prevent any other plasmid co-residing with them. In other words, they want the bacterial cell all for themselves! Plasmid incompatibility is a very effective strategy for protecting territory within the bacterial cell.

As we've discussed throughout this series, the number of plasmids in a cell is governed by elements encoded within the origin of replication (*ori*). It is not possible to maintain two different plasmids that use the same mechanism for replication in a single cell. Therefore, plasmids fall into compatibility groups base on their replication strategy and you cannot use two plasmids in the same cell system if the plasmids belong to the same compatibility group. Incompatibility groupings are a popular way of classifying plasmids and the current list of groupings can be found in a recent review.

It is widely accepted that competition for replication factors leads to competition between plasmids. Plasmids with inherent growth advantages such as faster replication (due to their smaller size) or less toxicity have the potential to rapidly outgrow other plasmids in the cell. This is even more likely to occur when the plasmid copy number is low. Taking into account the number of cell division cycles occurring in a single overnight culture, even small differences in competitive advantage are thought to be able to lead to rapid dominance of a culture by a single plasmid.

PLASMID AMPLIFICATION

Plasmid amplification is provided in Escherichia coli bacteria cells. **Plasmid** linearization by restriction cleavage can be ordered as a follow-up service. Such an operation is recommended especially when the **plasmid** is used as a PCR standard. **Amplified plasmids** are delivered either in midiprep or maxiprep quantities.

Plasmid curing

Plasmid curing occurs naturally through cell division or by treating the cells with any chemical or physical agents (Elias et al., 2013). The inhibition of conjugational transfer of antibiotic resistance **plasmid** can be used to decrease the spread of antibiotic resistance **plasmid** in the environment.

Regulation of copy number

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.

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.