Perfect Inheritance

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The study of plasmid stabile inheritance systems may open up new ways of effectively fighting bacteria that have become resistant to most known antibiotics

For some time now, world medicine has been wrestling with dangerous, hard-to-combat hospital infections. Strains of bacteria seemingly "resistant to everything" are becoming a real plague. Mankind's chief weapons against microbes, antibiotics, are in this case becoming useless. What is it that enables bacteria to grow resistant to so many antibiotics simultaneously? The answer lies in plasmids, DNA molecules that one bacterium can quickly and easily transfer to another. Moreover, plasmids are governed by refined molecular systems to ensure their stable maintenance and spread within bacteria populations.

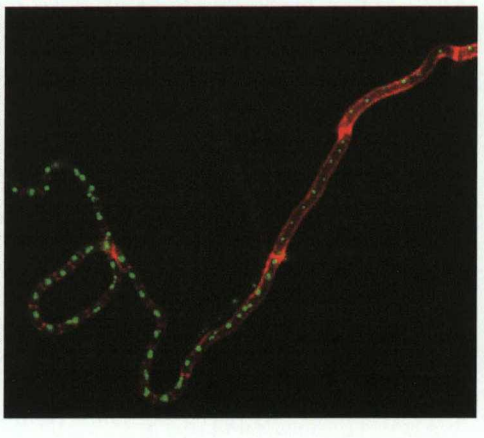
What are plasmids?

Bacterial plasmids are autonomous molecules of DNA, ranging in size from several to several thousand base pairs. By comparison, the genome of the model organism *E. coli* contains around 4.7 million base pairs. Unlike the cell chromosome or chromosomes, plasmids are not crucial for the life of a bacterial cell, but they do frequently carry genes which could prove very useful under certain environmental conditions: genes providing resistance to antibiotics or coding metabolic pathways for harnessing specific compounds. They occur in cells in a fixed number of copies (ranging from one up to several dozen).

To understand the problem of stable plasmid inheritance, one has to first realize that aside from supplying useful genes these molecules also represent a certain metabolic burden for the host cell. A small number of copies may limit that burden, but such a solution also has its drawbacks. Because plasmid molecules get randomly distributed between the daughter cells in cell division, a smaller number of plasmids in the parent cell means a greater possibility that one of the two daughter cells will inherit all of the plasmid molecules, whereas the other will inherit none. The probability of such a plasmid-less cell so arising can be calculated using the formula $P = 2^{(1-n)}$, where n represents the number of plasmid copies. For a plasmid occurring in two copies this probability is 0.25, for one with 10 copies the likelihood is 100 times less, and for a plasmid with 20 copies the probability becomes more than 100,000 times smaller. A higher likelihood of the plasmid being lost during cell division impedes its spread within the bacteria population: the number of cells carrying the plasmid does not increase, and their share in the growing population decreases.

Ensuring stable inheritance

Plasmids have developed a range of molecular mechanisms meant to ensure their survival. The most important of these is the replication system, which closely controls the number of copies present within a given cell. For low-copy plasmids, their loss is prevented by special systems facilitating their stable



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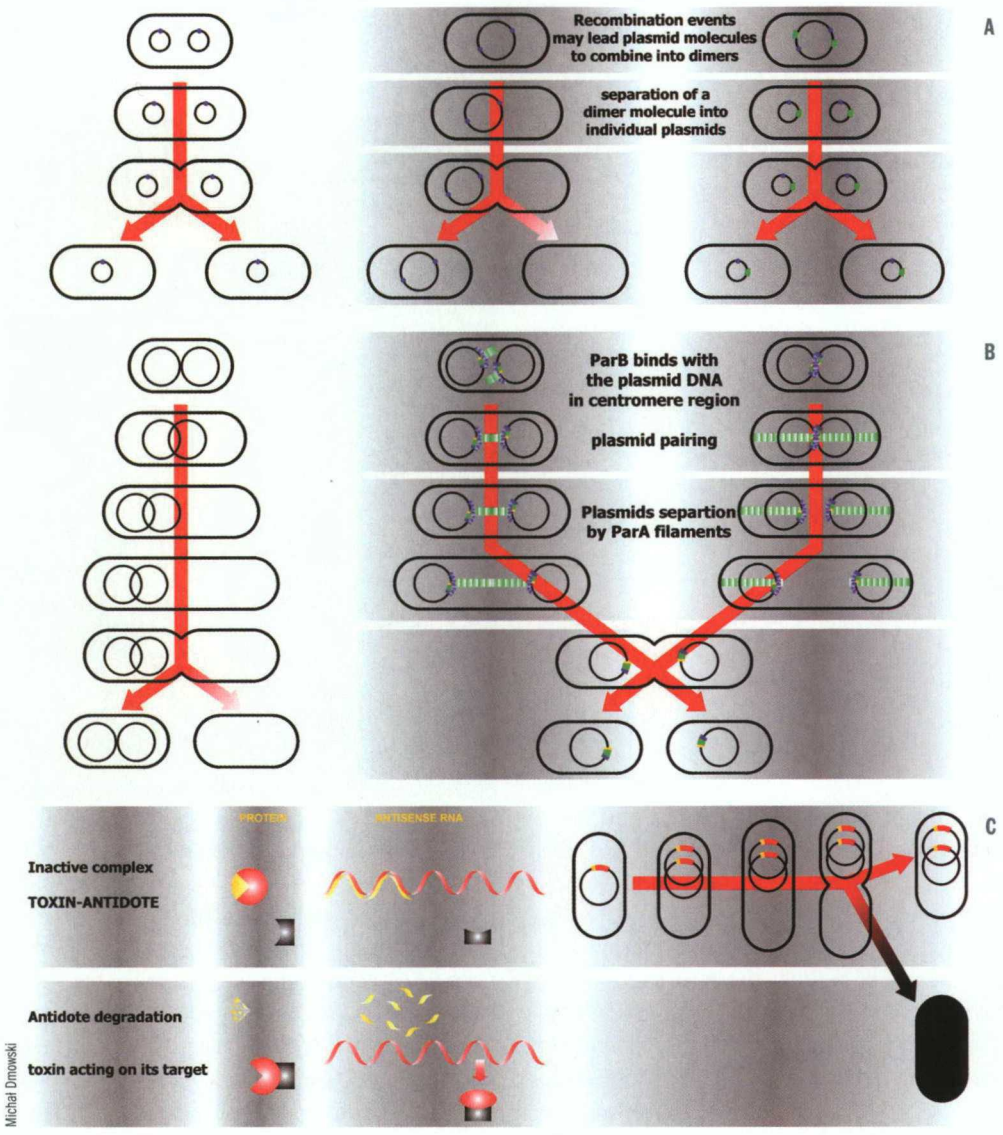
In plasmid partition systems, the key event is the creation of a segregosome, a protein complex helping plasmid copies to be placed evenly into the daughter cells. Here: the aerial mycelium of *Streptomyces coelicolor* bacteria. The green dots are segrosomes



MRS systems (A) split up dimers, enabling plasmids to migrate freely into the daughter cells. PAR systems (B) actively assist the segregation of plasmids into the future daughter cells. PSK systems (C) ensure that cells which do not inherit the plasmid are eliminated from the population

maintenance within the host cell. Certain plasmids also contain a set of genes coding for a system of conjugation or horizontal transfer, which gives them the ability to shift from one bacterial cell to another. Such systems ensuring stable plasmid maintenance within a population can be classified into 3 groups, depending on how they operate: multimer resolution systems (MRS), partition systems (PAR), and postsegregational killing systems (PSK).

Preventing dimer catastrophe

A multimer resolution system ensures plasmid inheritance on a level consistent with the number of copies. Before considering how this works, we should first describe how the numer of plasmid copies is controlled. The systems regulating their number in a sense "count off" the number of replication origin locations (known as *ori*). Because these starting points for replication occur only once on each plasmid, the *ori* number corresponds to the number of plasmid copies. Unfortunately, recombination events may lead certain plasmid molecules to combine with one another into larger structures (dimers, trimers, or tetramers). Counting off the number of *ori* points then leads to mistaken "conclusions" – since some of the molecules are composite, the overall number of molecules subject to inheritance is smaller than the *ori* number.

Such multimer structures essentially hamper the free migration within the cell of all the individual plasmid copies: for instance, following cell division one of the daughter cells might inherit a dimer, whereas the other may not inherit any copies of the plasmid at all. This disruption of normal inheritance may be further reinforced by a phenomenon known as the "dimer catastrophe": since copying of each of the *ori* occurs with equal likelihood (the choice being random), the probability that a dimer will be replicated is twice the

probability for monomers, thus giving a numerical advantage to the former.

Multimer resolution systems (MRS) work to counteract the effects of multimers and the resulting increased likelihood of plasmidless daughter cells. The main actor here is a resolvase enzyme, a locally specific recombinase that binds a specific DNA sequence (*res*). After attaching to the plasmid DNA, resolvase separates a multimer molecule back into its individual plasmids. As a result, each of them can then migrate freely into the daughter cells, enabling their random distribution.

Partition systems

Partition systems (PAR), in turn, work by a mechanism that is often likened to the processes which occur when sister chromosomes in eukaryotic cells are split up during cell division. Bacterial partition systems are coded by two genes (*parA* and *parB* - a uniform nomenclature for general description), usually consisting of a single operon and having closely regulated expression. The protein products of these genes are ParA and ParB. The protein ParA is an ATPase, in other words it is capable of hydrolyzing ATP - an energy carrier in biological systems. Numerous ParA type proteins are capable of forming filaments and spiral structures within bacterial cells, which is of key importance in the process of splitting up plasmids.

Unlike ParA proteins, ParB proteins do not exhibit great homology (i.e. they have more diverse structures). The common trait of all ParB proteins is an ability to bind with DNA at the location *parS*, called a centromeric sequence or region, which is an arrangement of repeated nucleotide sequences (of varying number and length). These sequences are specific to each partition system and are only recognized by the "right" ParB protein. Some plasmid partition systems are also known in which the ParB protein binds to many locations, frequently distant from *par genes* and from one another - some examples being the pSM19035 plasmid from *Streptococcus pyogenes* bacteria (which has three sites binding ParB type proteins), the plasmid RK2 (with 12 binding sites) and the prophage N15 (with four *parS* sequences).

It appears that partition systems function in a similar way irrespective of the type of proteins coded or the genetic organization of the *par* region. The key event is the assembly of the segrosome, a nucleoprotein complex forming in the centromeric region. Dimer-forming ParB proteins bind here, and ParB interacts with the second protein in the system, ParA. Prior to cell division, the plasmids become paired off in the location where the barrier separating the daughter cells will form. Then these initially paired plasmids become split apart through their repulsion or attraction by the ParA protein. This process depends on the binding and ATP hydrolysis by ParA stimulated by the ParB protein.

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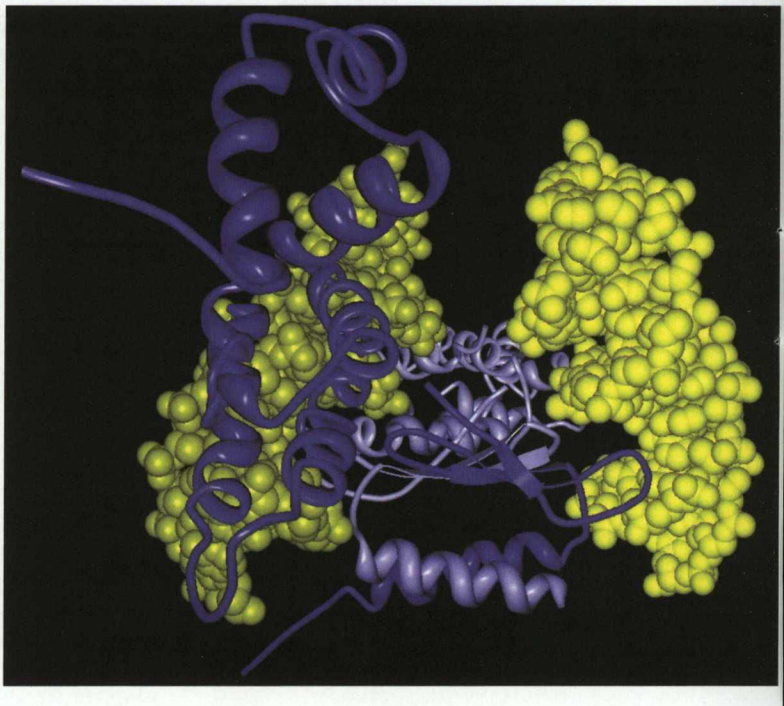

Structural model of the pairing of plasmid molecules (yellow) by the protein ParB (blue). ParB binds with the plasmid DNA in centromere regions (*parS*)

Aside from their role in regulating plasmids, PAR systems have also been discovered in bacterial chromosomes, which have numerous centromeric regions spread throughout the entire genome. Interestingly, the chromosome of the model bacteria most often used in research, *E. coli*, does not contain a partition system. In *Pseudomonas aeruginosa*, on the other hand, the protein ParA most likely also takes part in other cell processes.

Poison vs. antidote

The third type of stable plasmid inheritance systems - postsegregational killing systems (PSK), also known as toxin-antitoxin (TA) systems - are most often coded by an operon in which the first gene encodes an unstable antidote, the second a stable poison. Toxin-antitoxin systems are classified depending on the type of antidote they code for: the antidote

may be RNA complementary to the mRNA of the toxin (such as the *hok-sok* of plasmid R1), or it may be a protein like the poison, in which case the two together form an inactive complex (such as *kid-kis* of plasmid R1).

The toxins are most frequently inhibitors of gyrases (enzymes crucial for DNA replication) or RNases inhibiting the translation of mRNA. In a cell carrying a plasmid utilizing a TA system, both the poison and its antidote are produced and thus the poison is unable to attack the cell target. Whenever one of the daughter cells resulting from cell division fails to receive the plasmid, the mRNA of poison and antidote (in type I systems) or the proteins of poison and antidote (in type II systems) will continue to be present in the daughter cell, although no longer in active production. Next, in type I systems the RNA of the antidote becomes degraded enabling the mRNA of the poison to undergo translation, whereas in type II systems the less stable antidote becomes degraded and the poison protein gets released. In both types, the toxin becomes able to attack the plasmid-less cell, resulting in cell death or growth inhibition.

Toxin-antitoxin systems have likewise been identified in bacterial chromosomes (e.g. *mazE-mazF* of *E. coli*). Their presence here might seem senseless, since a cell deprived of its chromosome is doomed to death anyway. However, it is possible that in this case the function of the TA system is to regulate growth, e.g. to temporarily inhibit it under conditions of starvation stress (scarce nutrients will thus be consumed by only a small portion of the population, enabling it to survive). Another hypothesis postulates that the RNase toxins act as gene expression regulators. Alternatively, chromosomal TA systems might serve to maintain genomic parasites like conjugational transposons or bacteriophages, or may quite simply represent hard-to-eliminate "genetic trash" adopted from plasmids.

Finding an 'off' button?

The ideal, global system for maintaining the stable presence of a plasmid within a bacterial population is therefore three-stage. Firstly, a multimer resolution system enables the optimal number of plasmid molecules to be supplied for distribution. In the second stage, a partition system causes the plasmids to be precisely and symmetrically divided between the future daughter cells. Finally, in the event these systems fail and a daughter cell is left without the plasmid, a postsegregational killing system will eliminate the plasmid-less cell from the population, ensuring that only plasmid-inheriting offspring will grow.

An interesting case of the integration of plasmid stabilization systems is found in pSM19035 plasmid from *Streptococcus pyogenes*. Its PAR and PKS systems and the gene *copS* regulating the plasmid copy number have a common transcription regulator, the protein Omega, which also acts as partition protein ParB. The Omega protein is coded by gene ω, which forms a single operon with genes ε and ζ, encoding the PSK system. Another notable example comes from the RK2 plasmid, whose partition protein KorB coordinates the systems of replication, partition, and conjugational transfer.

Overall, it is such refined systems ensuring the stable maintenance of plasmids in bacterial populations that are to blame for hard-to-combat infections seen in clinical practice. Such in-depth study of how they function may help us develop methods for deactivating them. ■

Further reading:

Hayes F. (2003). Toxins-antitoxins: plasmid maintenance, programmed cell death, and cell cycle arrest. *Science, 301*, 1496-9.

Hayes F., Barilla D. (2006). The bacterial segrosome: a dynamic nucleoprotein machine for DNA trafficking and segregation. *Nat. Rev. Microbiol., 4*, 133-43.

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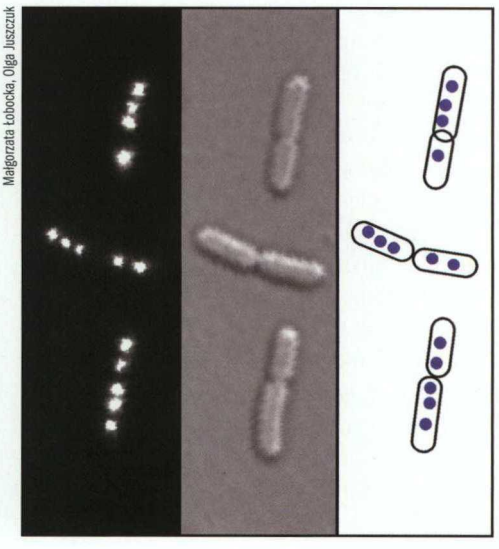

Using GFP (Green Fluorescent Protein) to fuse with ParB in bacterial cells (here *E. coli*) enables visual identification of the complexes of partition protein bound to plasmid DNA in the *parS* centromeric region