Systems ensuring plasmid inheritance

Epsilon vs. Zeta

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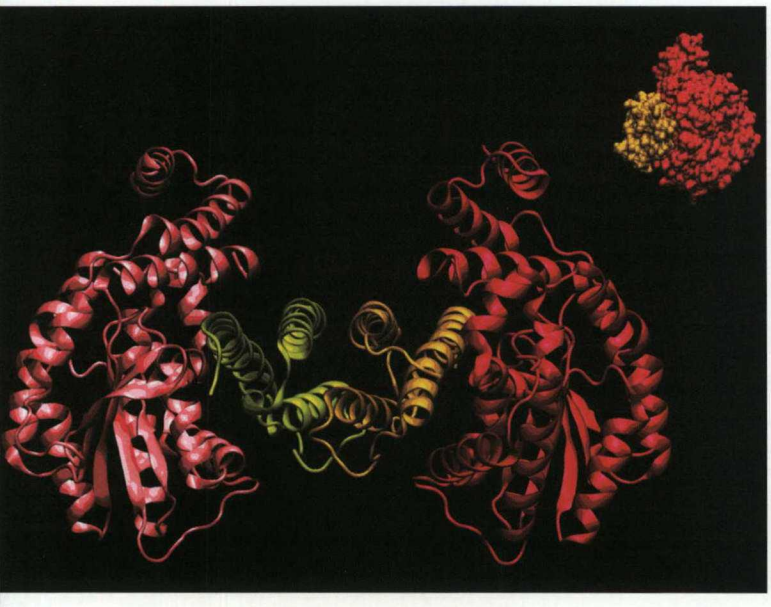
Some plasmids present inside bacteria cells encode toxic proteins designed to kill off their host cells. Paradoxically, through a delicate toxin/antitoxin balance, such mechanisms actually help plasmids to survive within bacteria populations

Many organisms produce poisons - substances that have an unfavorable or even fatal effect on other organisms. Such toxic substances make themselves felt when we contract various illnesses, suffer from food poisoning, have a wound that gets infected, or watch helplessly as one of our favorite plants withers away. Poisonous toxins may be produced by animals, plants, and pathological bacteria, but in all these cases they are produced by one organism in order to fight another.



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The Epsilon/Zeta complex consists of a tetramer, $Epsilon_2$-$Zeta_2$, shown here as a 3D ribbon model. A surface model of the Epsilon-Zeta dimer is depicted in the upper right corner

Another extraordinary kind of poison is found in what are called toxin-antitoxin systems, or TA systems. Such systems are frequently encoded by plasmids (additional, autonomous molecules carrying genetic information outside of a bacteria cell's chromosomes). Some plasmids give their hosts cells favorable characteristics, e.g. resistance to antibiotics, which may even be crucial for their survival. The poisons generated by such plasmid-encoded TA systems, rather than being released outward, have the purpose of killing the very cell that produces them. Paradoxically, this mechanism ensures the survival of the plasmids within the bacteria population.

Because such plasmids are present in small numbers of copies in each bacteria cell, symmetrical cell division might leave all of them in just one of the two halves. If that occurs, only half of all offspring would inherit the plasmid. With time, after several generations, a low-copy plasmid would gradually disappear from the bacteria population. The purpose of TA systems is to prevent that from happening.

Delayed revenge

How? A toxin-antitoxin system is a pair of closely matched proteins, a poison paired with its own antidote, each encoded by adjacent genes with jointly regulated expression (called an "operon"). In a vast majority of cases, the gene encoding the antidote comes first in the operon, preceding the gene encoding the poison. This means that expression of the poison will always be accompanied by expression of the antidote, a balance crucial for the survival of the cell carrying the plasmid.

A key feature of such a TA system is that the antitoxin is instable and short-lived, while the toxin is highly stable. When a cell carries a plasmid encoding a TA system, both genes are expressed, the toxic effect of the poison is kept in check by the rapidly-degrading but constantly-produced antidote, and the toxin and antitoxin together form an inactive complex. The same balance is sustained in successive generations, as long as the plasmid is inherited by the daughter cells. But if one of the daughter cells ends up not inheriting the plasmid, the inactive toxin-antitoxin complex will still remain present within it for a certain time after division, even though neither of the two components are still being produced. But because the antidote gets quickly broken down by specific proteases, the longer-lived poison will soon be released from the inactive complex. This eventually kills

or irreversibly inhibits the growth of the cell that failed to inherit the plasmid. Because this effect is observed after plasmid segregation, TA systems are also known as "postsegregational killing systems."

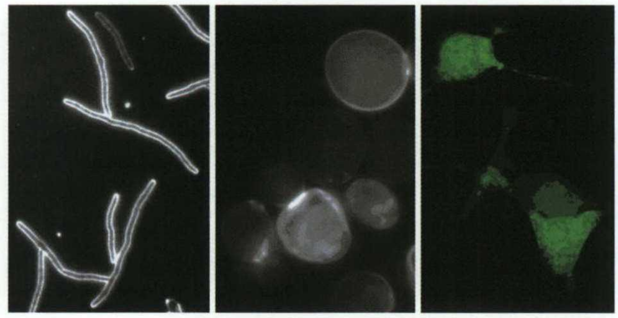
Such systems may figuratively be described as a kind of "delayed revenge." In effect, the plasmid kills off a cell when it is no longer present. Although that might seem senseless, it is in fact a very well crafted strategy. Two different, frequently opposite interests are at odds within the bacteria cell: the plasmid's interest to successfully maintain its presence, and the bacteria's interest to multiply its own population numbers as quickly as possible. While plasmids may be useful to a given population, they are not necessarily so since they do place a certain burden on a cell. By managing to kill off cells that do not contain them, plasmids can make sure a population will be dominated by cells that do.

We can explore how such a toxin-antitoxin complex functions in more detail based on the example of the proteins Zeta and Epsilon, encoded by plasmid pSM19035 isolated from pathogenic *Streptococcus pyogenes* bacteria (which renders the bacteria resistant to antibiotics from the macrolide group, such as erythromycin). Despite the plasmid's low number of copies in the cell (5 per chromosome), it is able to maintain a stable presence in successive generations largely due to this TA system.

Well-chosen pair

The crystal structure of the Epsilon/Zeta protein complex was solved by Prof. Saenger's laboratory in 2003. The complex consists of two Epsilon-Zeta halves, with the Epsilon molecules tucked in between two Zeta monomers. The Epsilon protein monomer (10.7 kDa) curls into three helixes, while the Zeta protein monomer (32.4 kDa) forms 6 chains ("β-sheets") surrounded by α-helices. The α-helices, positioned at one of the far ("N-terminal") ends of the Zeta monomer, form an indentation to which the helix of the Epsilon protein binds. The closure of that surface by the antidote blocks the ATP/GTP binding that is essential for the action of the toxin. As a result, the toxin Zeta remains inactive when it forms such a complex with the antidote Epsilon.

Compared to well-known TA systems, this system from the plasmid pSM19035 has been found to have many exceptional properties. It is unique in that it is an operon that consists of three genes: the genes encoding the antidote and the poison (ε and ζ, respectively), plus also a regulator gene ω simultaneously involved in other aspects of the plasmid's function. The poison protein Zeta is extraordinarily large compared other toxins (287 vs. around 100 amino acids) and with the exception of its frequently-encountered nucleotide binding motif, it does not show any similarities to any known proteins.



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Due to their potential use as a tool to target and eliminate unwanted bacteria or cancer cells, TA systems are attracting great research interest. From left to right: bacteria, yeast, and HeLA cells subjected to the Zeta toxin

While studying the functioning of the Epsilon/Zeta system we observed that it can also function in cells other than the Gram-positive bacteria from which the pSM19035 plasmid derives. The Zeta protein causes Gram-negative bacteria (*Escherichia coli*) to cease growing and form long cells with many chromosomes, clearly unable to divide. Similarly, when the toxin is introduced into yeast cells it changes their morphology and halts budding, and in large quantities it causes death. Human cancer cells also die from the toxic effect of the Zeta poison.

Toxin as a route to healing?

The genes of the ω-ε-ζ system are widespread among Gram-positive pathogenic bacteria, effectively contributing to maintaining populations of these antibiotic-resistant microorganisms. The molecular mechanism by which the Zeta toxin operates has not yet been discovered. The spatial structure of the complex of these proteins, completely dissimilar to other known structures, unfortunately offers no cues for possible avenues of research.

More generally, TA systems are now known to be widespread among all the prokaryotes, not only on the plasmids but also on the chromosomes, frequently in multiple copies. Some toxic proteins have been shown by in vitro tests to be specific endoribonucleases (enzymes that cut RNA molecules), although their role in bacterial physiology remains enigmatic.

Both the intriguing principles by which TA systems operate and the effectiveness of the toxins themselves have sparked huge interest in these systems as possible tools for targeting and eliminating undesirable cells - not just bacteria but also cancer cells. ■

Further reading:

Zielenkiewicz U., Cegłowski P. (2005). The toxin-antitoxin system of the streptococcal plasmid pSM19035. *J. Bacteriol., 187*, 6094-6105.

Magnuson R.D. (2007). Hypothetical functions of toxin-antitoxin systems. *J. Bacteriol., 189*, 6089-6092.