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Programmed Cell Death in Bacterial Populations

Michael B. Yarmolinsky

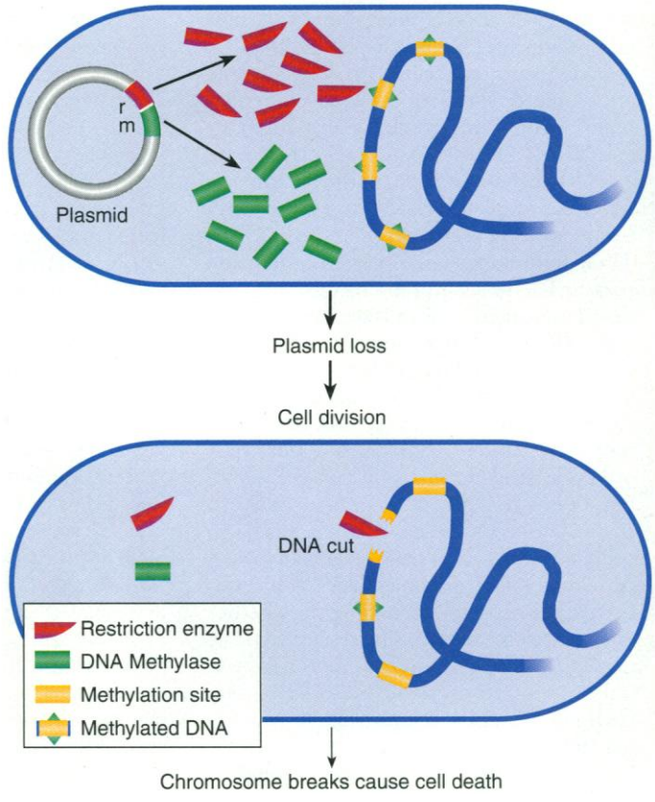
Multicellular organisms benefit not only from the death of competitors, but often, quite dramatically, from the programmed death of specific subpopulations of their own cells. Popular opinion to the contrary, programmed cell death is also alive and well in the microbial world. A striking report by Naito *et al.* in this issue of *Science* (*1*) is a case in point.

If bacteria are to profit from the death of their own kind, they must be heterogeneous. Indeed, temporal and positional variation of cell type in clonal populations is the norm, not the exception. Switching among genetic and epigenetic states in response to cell density, nutrient supply, substratum surface, plasmid burden, incident radiation, viral infection, or the passage of time accounts for such effects as the variety of colonial forms and, among pathogens, the defeat of the host's immune response (*2*, *3*). Unexpected differentiated states have been found even in thoroughly studied *Escherichia coli* (*4*). It has also been proposed that starvation induces in this bacterium the appearance of a hypermutable state in a moribund subpopulation (*5*). Moribund subpopulations, whose members are not recoverable as colony-formers, may readily escape notice. They are associated not only with mutability, but also with the carriage of another major source of bacterial adaptability, plasmids.

A growing number of large plasmids have been shown to program the death of plasmid-free segregants (*6*). This strategy prevents the survival of bacterial mutants disabled for plasmid retention that would otherwise overgrow the plasmid carriers. The set of genes carried by a plasmid that is responsible for the lethal consequences of plasmid withdrawal can be viewed as an "addiction module"; it renders the bacterial host addicted to the continued presence of the "dispensable" genetic element it harbors.

A plasmid addiction module makes a simple time bomb: the charge, a stable toxin; the timer, a labile antidote. Detonation occurs when the ratio of antidote to toxin becomes too low. In the plasmid-free cell, neither antidote nor toxin is replenished and the antidote is eliminated more rapidly than the toxin, leaving the latter to exert its lethal potential. In some cases, dilution alone may suffice to eliminate antidote function before dilution has rendered the toxin innocuous. Several familiar type II restriction enzymes and their cognate methylases are plasmid-encoded and all of them are toxin-antidote pairs, the DNA methylase offering protection from endonucleolytic attack by the separate restriction enzyme. It is easy to see how the progeny of a cell that managed to rid itself of a r^+m^+ plasmid (one that carries restriction-modification genes) might retain insufficient methylase to protect itself from the remaining restriction enzyme and so be killed (see figure). Enhancement of the apparent stability of a plasmid that encodes a type II restriction enzyme and its cognate methylase is precisely what Naito *et al.* (*1*) observed with both Pae R7 and Eco RI rm genes. Examples will surely multiply.

Toxin lethality is normally held in check in a variety of ways (*6*). DNA methylases modify restriction enzyme targets (specific DNA sequences) so as to render them unassailable. In the family of toxin-antidote pairs to which toxins Gef and Hok belong, binding of antisense RNA to a long-lived precursor of the messenger RNA that encodes the toxin prevents toxin synthesis. In



Plasmid loss avenged. Bacterial death programmed by a plasmid-encoded restriction-modification system.

ILLUSTRATION: C. FABER SMITH

The author is in the Laboratory of Biochemistry, National Cancer Institute, Building 37, Room 4D-15, National Institutes of Health, Bethesda, MD 20892–4255, USA.

other plasmid addiction systems, complex formation between an antidote protein (for example, the CcdA protein of plasmid F) and its coordinately synthesized toxin (CcdB, in this case) can prevent or even reverse toxin activity.

The toxins themselves that are implicated in plasmid addiction are diverse in structure and mode of action as well. The toxins mentioned so far act exclusively within the bacterial cells that produce them. Others, such as colicins or microcins, act only after release from producer cells and subsequent penetration of their (nonproducer) target cells. This diversity is indicative of a recurring event in evolution. Diverse lethal agents may target similar sites. Colicin E1 and its relatives kill by membrane depolarization (*7*), as do the intracellular toxins of the Gef (alias Hok) family (*6*). DNA is the target of Pae R7 and Eco RI, of microcin B17, and of CcdB. Whereas the restriction enzymes cleave DNA directly, microcin B17 and CcdB convert DNA gyrase into a DNA-damaging agent (*8*). The DNA degradation and cell death that follow have been likened to apoptosis (*9*).

Programmed cell death has a considerably larger role in the life of bacteria than its role in plasmid maintenance. Many bacteria with a feast-and-famine way of life respond to repeated stresses with offensive and defensive measures that involve self-sacrifice. For example, carriers of any of various colicinogenic plasmids respond to DNA damage by the lethal synthesis of a colicin. Ironically, the inducing damage can be intrinsically reparable. Among the bacilli, streptomyces, and myxobacteria, terminal differentiation of one portion of the population (mother cells, aerial hyphae, or fruiting bodies, respectively) helps to generate and disseminate members of a complementary portion (spores) that have a greater potential for survival (*10*). The spore is, in effect, a germline cell. In bacilli, the spore develops entirely within a larger cell. This outer or mother cell, having accomplished its maternal tasks, is lysed. In streptomyces, long chains of spores develop from specialized aerial hyphae that themselves appear to subsist mainly upon their own vegetative mycelium. Alternative microbial competitors may be discouraged by the simultaneously produced antibiotics for which the streptomyces are famous. In myxobacteria, extensive cell lysis may occur during the cellular aggregation that precedes fruiting body formation and in the nascent fruiting body itself. The myxospores are borne aloft on a structure consisting largely of dead cells, like the acorns on a spreading oak tree. Sacrificed portions of these bacterial populations may be "cannibalized." In eukaryotes, phagocytosis by neighboring cells is a hallmark of apoptosis.

Viral infection commonly triggers a protective apoptotic response in plants and animals. Some viruses retaliate with pro-life antidotes. Comparable altruistic subversions of viral development are not alien to bacteria, nor are the countermeasures (*11*). An example exploited by founding fathers of molecular genetics (*12*) is the restriction of bacteriophage T4rII and other phages by the Rex proteins of λ prophage. In response to growth of the infecting phage, Rex proteins can damage the cell so as to abort the infection. The nature of the damage and the mechanism whereby the rII^+ function of wild-type T4 subverts this defense are still unclear. Two other suicidal modes of T4 exclusion are better understood. Strains of *E. coli* that carry the gene cluster *prr* encode an anticodon nuclease that, when activated by a 26-residue polypeptide product of T4, can cleave a transfer RNA important for lysine incorporation into protein. The nuclease does not abort viral replication because T4 has evolved a pair of enzymes (otherwise inessential) that undo the damage. Strains of *E. coli* that carry defective prophage e14 accomplish exclusion by cleavage of elongation factor Tu, inhibiting translation globally. The cleavage involves an interaction of an e14-encoded protein with a short RNA or polypeptide sequence encoded within the major T4 head protein gene. The inevitable winner in these relentless battles between host and parasite or between parasites for the same host is—life.

Naito *et al.* (*1*) suggest that the proliferation of restriction enzymes, including rare cutters unlikely to damage an infecting parasite, may be ensured by the selfishness of their genes. Selfishness has previously been invoked to connect the behavior of other plasmid addiction genes to that of certain eukaryotic genes that paradoxically ensure their spread by harming their hosts (*13*). Selfishness also offers an explanation for the existence of bacterial genes that show sequence similarity to certain plasmid addiction genes and that have addiction potential when cloned into unstable plasmids (*14*). Alternatively, attention may be focused on the genome pairs subject to enforced cohabitation. As a host-parasite complex there may be more potential for evolution in the direction of enhanced fitness than for the host alone (*15*).

Did mechanisms of programmed cell death in eukaryotes and prokaryotes evolve from common antecedents? This we cannot answer, but it does appear safe to say that similar purposes are served.

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