Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2731–2732, March 1998

Commentary

Protecting the neighborhood: Extreme measures

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In the unending wars of organism vs. organism, the growth of bacteriophage and the defenses raised by bacteria were among the first recognized and continue to provide new variations and insights on ways to defend oneself. A paper in this issue of the *Proceedings* demonstrates that prokaryotes, like eukaryotes, have chosen proteolytic self-destruction as a route to protection from attack, albeit a protection for the community rather than for the cell under attack (1).

Unleashing a protease that is capable of degrading a vital cell protein is likely to be an irreversible process and thus a somewhat risky proposition, particularly if the vital cell protein is one involved in protein synthesis, therefore blocking new synthesis of the degraded protein. Nonetheless, it is a strategy that is proving to be common. The cascade of caspases, the proteases that lead to programmed cell death both as part of developmental programs and in response to an enormous variety of insults is one increasingly popular example of intracellular proteolysis as endgame (2). Plants resist pathogens in part through a hypersensitive response, a programmed cell death of the infected cell that presumably limits further pathogen growth, although it remains to be seen whether similar proteases participate in this process (3).

Georgiou *et al.* (1) have reconstructed *in vitro* the protease-based exclusion system that limits growth of the lytic bacteriophage T4 in certain strains of *Escherichia coli*. Protection is provided by killing the host before T4 has a chance to fully develop a burst of infecting phage. This is accomplished, in part, by cleavage of the translation elongation factor Tu (EF-Tu), essential for protein synthesis. The putative protease, Lit, is encoded by a cryptic prophage-like element called e14 found in some but not all *E. coli* strains.

Obviously, expressing both an active protease and an essential target in the same cellular compartment is likely to be incompatible with survival. In the case of the eukaryotic caspases, initial signaling events lead to proteolytic activation of otherwise inactive proteases (2). The ATP-dependent proteases found in the eukaryotic and prokaryotic cytoplasm, although capable of cleaving many peptide bonds, restrict entry of most proteins to the proteolytic cavity by their assembly into particles with limited and selective entry pores (4, 5). The proteolytic cleavage studied by Georgiou *et al.* is activated only when a short peptide, called gol (*g*rowth *o*n *L*it) is synthesized. The gol peptide is part of the most abundant of the T4 proteins, the gene 23 coat protein (gp23). In the *in vitro* system, the 29-residue gol peptide is necessary and sufficient to trigger the ability of purified Lit to cleave purified EF-Tu. As was seen *in vivo*, the cleavage within EF-Tu is at a conserved Gly-59–Ile-60 within the nucleotide binding region. It is not yet clear how the peptide functions to activate cleavage; it might serve as a general activator for the protease or, possibly more likely, associate with EF-Tu to promote either a change in conformation or protease recognition of the complex.

This demonstration of the *in vitro* components of this exclusion system, combined with previous *in vivo* experiments (6), suggests that late T4 protein synthesis leads to high levels of gp23, presumably exposing the gol peptide within gp23, resulting in activation of the proteolytic activity of Lit. Cleavage of EF-Tu and shutdown of further protein synthesis follows. The net result is a lower burst size of the phage, presumably limiting the ability of T4 to spread among other bacteria in the population. Thus, the exclusion system appears to provide a benefit for the population as a whole, and presumably as a result, helps to preserve the e14 prophages in neighboring cells. Given that the original host was destined for death within the first few moments of T4 infection, this is a relatively painless form of altruistic behavior, a last-ditch attempt to salvage the rest of the population by destruction of the co-opted infrastructure.

The ability of e14 and other phage exclusion systems not discussed here (7) to promote the death of one cell for the greater good is reminiscent of the programmed cell death systems that contribute to the maintenance of low copy plasmids in cell populations. Plasmids with relatively low copy numbers are efficiently maintained in cells by a combination of controls to ensure regulated replication and accurate segregation to daughter cells. When these fail, a final system that ensures the maintenance of the plasmid are the "addiction systems" that kill cells that have had the temerity to lose the plasmid in spite of everything (8). Addiction systems have in common two components, both plasmid encoded. The first, a toxin, is capable of efficient killing by itself, but either its synthesis or its function are blocked in cells that carry the plasmid DNA and are actively synthesizing the second component. The second component, the antidote, can be an antisense RNA that blocks toxin synthesis (9) or a protein that blocks toxin killing. These systems work because the second component is unstable while the toxin is stable. Thus, when the plasmid DNA is lost from a cell, new transcription of both toxin and antidote ceases, but the unstable antidote eventually is destroyed, leaving the toxin to kill the plasmid-less cell. Again, in this situation, one must assume that death of the plasmid-less host helps the population as a whole to survive and reproduce.

Is exclusion of T4 the real reason for the existence of the Lit protein? The relevant region of the T4 head protein is well conserved in both other T-even phages and even relatively distantly related "pseudo T-even" phages (10), so infection by any of them would be expected to trigger the system. Neither the activating peptide sequence nor anything closely related is found in the *E. coli* genome or in other sequences in the databank, although a definition of the minimal active peptide will be necessary before one can say that the relevant peptide is unique to T-even phages. A second strong argument in favor of an evolution of Lit specifically to attack incoming T-even phages is evidence that the Lit protein interferes with T4 growth in other ways that do not depend on the gp23 peptide (6). If Lit and T-even phages are dedicated enemies, why does the exclusion system only activate late in the T4 growth cycle, when interference with translation may not be 100% efficient and cell killing is already certain? Possibly this is simply a

0027-8424/98/952731-2$0.00/0
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mechanism for ensuring that the cell really is lethally infected and that drastic measures should be undertaken, or possibly the highly abundant gene 23 product is the only protein made in sufficient quantity to do the job. It seems possible, however, that some other damage to the cell and/or to the translation apparatus might require clearing of (damaged?) EF-Tu. In cells in which no other inhibition of translation exists and in which not all of the EF-Tu is cleaved, new synthesis would be expected to overcome any transient activation of the system. Thus, one could imagine that the Lit protein might, under some circumstances still to be defined (or under conditions found when the parental phage for e14 roamed the Earth), provide a quality-control activity rather than a cell-killing activity.

Are there advantages to targeting EF-Tu, a translation elongation factor for destruction rather than some other, possibly less abundant, protein? Cleavage occurs within a highly conserved region, near the nucleotide active site of the protein; presumably related phages may find related sequences in hosts other than *E. coli* (6). The related elongation factor G, with an identical sequence around the cleavage site, is not a substrate *in vitro*, suggesting that specificity elements that have not yet been defined are important for recognition and/or cleavage (1), and why EF-Tu rather than EF-G is a target remains mysterious. Because T4 infection rapidly causes a shutdown in host translation (11), all of the EF-Tu presumably is engaged in making T4 proteins, including the most abundant structural protein, the capsid protein gp 23 (12). Given that the trigger peptide is within gene 23 (1), one might wonder whether the translation of the peptide can cause EF-Tu cleavage *in situ*, as the gp23 protein is being synthesized. Even if this is not the case, if almost all protein synthesis is directed to making more gp23 and gp23 is causing destruction of the EF-Tu, cessation of further synthesis and therefore of further phage growth seems inevitable.

It is intriguing that translation elongation factors in eukaryotic cells with similarity to EF-Tu are the primary targets for at least two bacterial toxins. Both *Pseudomonas aeruginosa* exotoxin and Diphtheria toxin kill cells by gaining entry to the cytoplasm (a not uncomplicated process) and catalyzing the irreversible ADP ribosylation of the EF-2 elongation factor and therefore causing cessation of host translation and subsequent cell death through the apoptotic pathway (13–15); summarized in ref. 16. ADP ribosylation in both cases requires previous modification of a histidine residue within EF-2; possibly the evolution of this requirement for a uniquely eukaryotic modification acts as an additional safety valve, protecting the bacterial elongation factors from the lethal action of their own toxins, although the site of ADP ribosylation is not within the region of similarity of the bacterial and eukaryotic elongation factors. The efficient inactivation of EF-2 by toxins has been harnessed for the development of immunotoxins that target the toxin activities specifically to subpopulations of cells (16). Whether the T4/Lit system also can be used as an antibiotic, as has been suggested (7), will require significantly more information on its mode of action.

Why might a translational elongation factor be a particularly attractive target for cell killing in both prokaryotes and eukaryotes? The perfect target ought to be: (*i*) highly conserved in all possible hosts; (*ii*) accessible to attack [Proteins that are integral parts of protein assemblies may be protected; in this case, the only time for attack might be shortly after synthesis and before assembly. Therefore, we can imagine that a recycling component of the translation machinery, for instance, might be a much more accessible target than a component of the ribosome.] (*iii*) limiting [If there is a relatively high requirement for the protein, even partial inactivation/destruction may be sufficient.]; and (*iv*) unlikely to be regenerated rapidly. Destroying the translation apparatus is one sure way to prevent new synthesis of the target protein. Elongation factors would appear to fit most of these requirements, although they are clearly not the the only ones. Whether the choices of this particular protein in prokaryotes and eukaryotes are independent events remains to be seen.

I would like to thank David Fitzgerald and Robert Weisberg for discussions, advice, and comments on the manuscript, and Michael Gottesman for comments on the manuscript.

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