Origin of programmed cell death from antiviral defense?

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COMMENTARY

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Viruses and other genetic parasites are ubiquitous in the biosphere, and virtually all cellular organisms evolved multiple defense mechanisms to cope with onslaughts of these parasites (1). In multicellular life forms, a major class of such mechanisms is programmed cell death (PCD), whereby an infected cell "commits altruistic suicide" to prevent infection of other cells (2). Many unicellular eukaryotes and prokaryotes also possess PCD pathways but, in this case, the function and, especially, the evolution of such self-destructing mechanisms remain controversial topics because it is not immediately clear how unicellular organisms would benefit from suicide (3–5). Models have been developed to account for the origin of PCD through population-level kin selection (5–7) but the controversy persists. In a PNAS article, Gao et al. (8) present observations suggesting the possibility that some forms of PCD might have evolved from antivirus defense mechanisms.

The experimental system is budding yeast sporulation that involves a meiotic division resulting in the development of spores and PCD of the remains of the mother cell (9, 10). This PCD is accompanied by fragmentation of the nuclear DNA of the dying cell that is catalyzed by the NUC1 nuclease released from the mitochondria. NUC1 belongs to the EndoG family of nucleases that is highly conserved among all eukaryotes and most prokaryotes and is responsible for DNA fragmentation during PCD in animals and at least some protists (11–13). Thus, this phenomenon is widely conserved in evolution and yet remains somewhat enigmatic because DNA fragmentation is not required for PCD that occurs normally in *NUC1*-null mutants (10).

The study by Gao et al. (8) presents a solution to this enigma, at least in the specific case of budding yeast, by showing that NUC1 targets the replication of double-stranded RNA (dsRNA) genomes of vertically transmitted yeast viruses, known as L-A and M (Killer) (14, 15). Although the antivirus activity of NUC1 has not been studied biochemically, Gao et al. (8) show that the catalytic site of the nuclease is essential, and given that EndoG family nucleases have broad substrate specificity (16), direct cleavage of virus RNA appears most likely (Fig. 1*A*).

The L-A virus is nearly ubiquitous in yeast isolates and is generally considered to be commensal, whereas M, whose reproduction depends on L-A, is relatively rare and produces a toxin that is secreted to kill virus-free cells while the virus-carrying cells are protected (14, 17). The antivirus defense pathways in yeast appear to be redundant (Fig. 1*A*), so that *NUC1*-null mutants are tolerant of the Killer but double mutants, in which a second antivirus gene, *SKI3*, is disrupted, are susceptible to the lethal effect of the killer toxin (8). Moreover, *NUC1*-null mutants of yeast strains that carry L-A virus but not the Killer M RNA produce increased amounts of the virus, in accord with early observations (18), and display respiratory defects, showing that L-A reproduction actually incurs a fitness cost on the host (8).

The dsRNA viruses of the family *Totiviridae*, which includes L-A, are widespread among unicellular eukaryotes and are also found in many plants and animals (19). An intriguing possibility is that defense against dsRNA viruses is an ancestral function of the EndoG family nucleases, at least in eukaryotes. Indeed, the role of these nucleases in PCD remains unclear, and the possibility that DNA fragmentation is only a by-product of their antivirus activity cannot be ruled out. Notably, bacterial homologs of EndoG/NUC1 nucleases are secreted into the extracellular milieu (Fig. 1*B*), where they are believed to serve nutritional purposes and, possibly, also function as bactericides (20). In a twist of the same theme, *Streptococcus* NucA nuclease is a virulence factor that facilitates bacterial evasion of the human innate immune response by degrading the DNA matrix component of neutrophil extracellular traps (21). The processes of nuclease secretion from bacteria and from mitochondria are topologically equivalent and potentially evolutionarily related. Thus, the ancestor of EndoG/NUC1, most likely, was introduced into protoeukaryotes along with the α-proteobacterial ancestor of the mitochondria and retained its role in the degradation of foreign nucleic acids (Fig. 1*B*).

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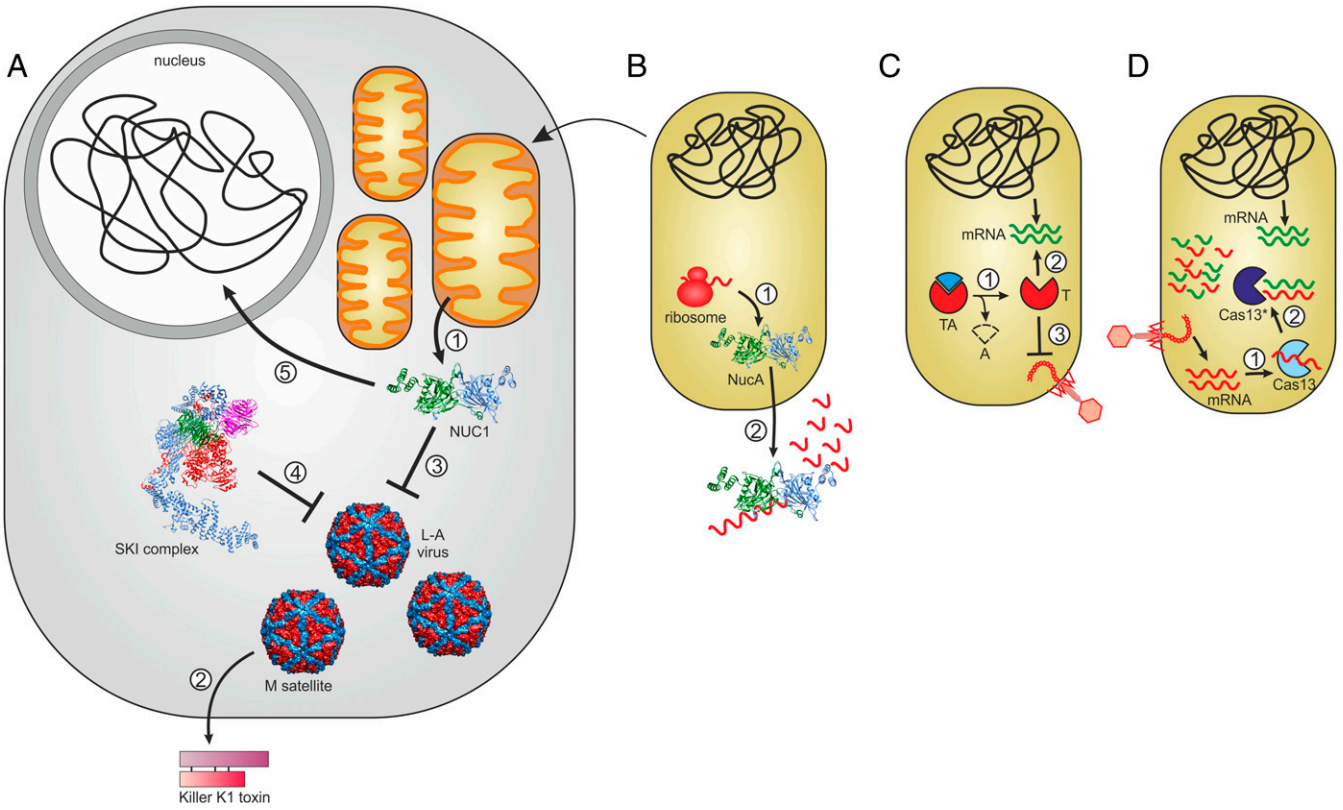

Fig. 1. Connections between immunity and programmed cell death. (*A*) Model of the NUC1-dependent antiviral defense. (1) NUC1 is secreted from mitochondria. (2) M satellite of totivirus L-A produces the secreted toxin K1 which kills M-free yeast species. (3) NUC1 controls the propagation of L-A virus and M satellite. (4) NUC1-mediated defense is redundant with the antiviral activity of the SKI complex (Ski2-3-8). (5) Concomitantly with the programmed cell death, NUC1 fragments the nuclear DNA. (*B*) Bacterial NUC1 homologs are dimeric secreted nucleases (1) involved in degradation of extracellular nucleic acids (2). The arrow connecting *A* and *B* signifies the evolution of mitochondria from an α-proteobacterial ancestor and the likely inheritance of the NucA-like nuclease. (*C*) Role of toxin-antitoxin (TA) systems in programmed cell death and antiviral immunity. (1) Inactivation of labile antitoxin (A). (2) Upon virus infection, the toxin (T) is activated and induces cell dormancy or death by different mechanisms, for example degradation of host mRNAs depicted in the figure. (3) As a result of toxin-induced cell dormancy/death, the virus cannot reproduce and spread in the population. (*D*) Cas13-mediated virus immunity and cell dormancy. (1) Upon virus infection, cognate CRISPR spacers targeting viral mRNA activate promiscuous RNase activity of Cas13. (2) Activated Cas13* indiscriminately degrades viral and cellular transcripts, leading to cell growth arrest, so that the virus cannot reproduce and spread in the population.

The connections between PCD and other forms of antivirus defense that can be generally described as immunity are multifaceted and tight (22) (Fig. 1). In prokaryotes, in particular, abortive infection systems that abrogate virus infection are closely related to toxin–antitoxin (TA) modules which cause PCD or dormancy (Fig. 1*C*). Moreover, the most common form of prokaryotic innate immunity, restriction-modification systems, also possess TA properties and can cause PCD (23, 24). Toxin components of TA modules were recruited to function within the CRISPR-Cas systems of prokaryotic adaptive immunity (25).

Notably, in all these cases, homologous nucleases, such as those of the HEPN RNase superfamily or the restriction endonuclease superfamily, participate in both PCD and immune response. A striking case of a combination of antivirus and toxic activity leading to growth arrest is presented by type III and type VI CRISPR-Cas systems (Fig. 1*D*). In each of these systems, recognition of a virus transcript via cognate CRISPR spacers activates promiscuous RNase activity of a HEPN-containing Cas protein (Csm6 and Cas13, respectively) which causes cell growth arrest and prevents virus reproduction (22, 26, 27). In the light of these close immunity–PCD links, the involvement of NUC1 in both antivirus defense and PCD seems to reflect a general, fundamental trend.

Gao et al. (8) speculate that PCD evolved from prosurvival defense mechanisms, expanding on a previous model under which PCD evolves as an initially maladaptive by-product of prosurvival defense mechanisms but is subsequently co-opted as an altruistic strategy, via kin selection (5). This does not necessarily have to be the case if PCD evolved at an early stage in the evolution of life as an altruistic defense mechanism, via population-level kin selection that would promote primitive forms of multicellularity. Mathematical models of the evolution of defense systems suggest that such a scenario is realistic (6). Moreover, given the tight links between PCD and immune mechanisms, it seems plausible that these defense strategies evolved concomitantly such that PCD is turned on when immunity fails (22). Further experimental study of the immunity–PCD coupling should shed light on the evolution of various forms of antiparasite defense that are intrinsic to life.

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