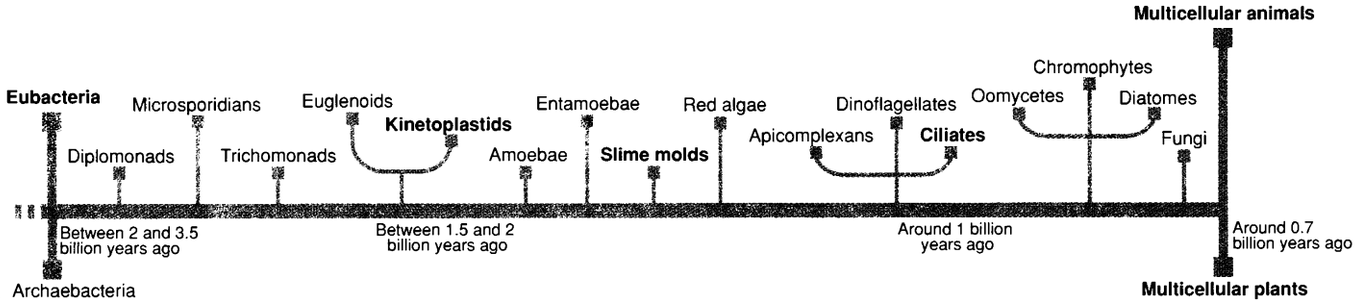

Programmed cell death and evolution. Various forms of PCD have been identified (in bold) in several branches of the phylogenic tree. No data on PCD are available for members from other branches. The tree and estimated divergence times are adapted from (*11*).

best-adapted offspring by preventing flawed offspring from competing for resources. The hypothesis that the eukaryote cell is a symbiont that arose from fusion of different bacteria species suggests that PCD may have evolved from a resolution of conflict between heterogeneous genomes within a cell, a process similar to step (i), that subsequently led to enforced cooperation.

Although such an evolutionary scenario is plausible, an alternative model would remove the need for a multistep process in the emergence of cell suicide machinery. In multicellular animals, some gene families function solely as inducers (the Ced-3/ICE/CPP32 cysteine proteases) or inhibitors (CED-9/Bcl-2/Bcl-XL) of PCD (*1*). However, most genes that control the cell cycle and cell differentiation—including proto-oncogenes, tumor suppressor genes (*1*), cyclins, and cyclin-dependent kinases (*7*, *8*,)—also participate in the control of PCD, and mitotic catastrophes resulting from uncoordinated activation of cyclins in mammalian cells and in yeast mutants have a phenotype similar or identical to apoptosis (*8*). In bacteria, the autolysins that participate in cell division can also induce self-destruction. If effectors of the cell cycle machinery can also be effectors of the self-destruction of the cell in which they operate, then the requirement for coupling cell survival to the prevention of self-destruction is as old as the origin of the cell (*10*).

The evolution of PCD would share similarities with the evolution of genetic diversity. The inability of a cell to avoid random genetic mutation has led to the selection of both DNA proofreading and repair mechanisms and the amplification of DNA diversity by genetic reassortment. The view that an intrinsic inability to avoid random self-destruction is an "original sin" of the cell, an inherent consequence of progression through the cell cycle, implies that selective pressures regulate the cell cycle machinery so that cell suicide is repressed. Such a scenario provides a simple mechanism for the selection of upstream inducers of PCD that allow enhanced fitness of the colony through the rapid dismisal of an individual once a mistake has been made during the cell cycle.

Is the origin of social control of cell survival coextensive with the origin of the cell, or have there been several parallel evolutionary attempts toward PCD? Do unicellular eukaryotes share effectors and regulators of cell suicide with multicellular animals? Are mutations that uncouple the cell suicide machinery from extracellular signals counterselected in unicellular colonies, as in multicellular animals (*1*, *7*)? The identification of the genes that regulate cell suicide in other unicellular organisms should help to address these questions.

References and Notes

1. M. C. Raff, *Nature* **356**, 397 (1992); D. L. Vaux, G. Haeker, A. Strasser, *Cell* **76**, 777 (1994); G. A. Evan, *T Cell Biol.* **4**, 191 (1994); H. Steller, *Science* **267**, 1445 (1995).
2. J. C. Ameisen *et al.*, *Cell Death Differ.* **2**, 285 (1995).
3. S. C. Welburn, C. Dale, D. Ellis, R. Beecroft, T. W. Pearson, *ibid.* **3**, 229 (1996).
4. S. Cornillon *et al.*, *J. Cell Sci.* **107**, 2691 (1994).
5. S. T. Christensen, D. N. Wheatley, M. I. Rasmussen, L. Rasmussen, *Cell Death Differ.* **2**, 301 (1995).
6. M. B. Yarmolinsky, *Science* **267**, 836 (1995).
7. D. S. Ucker, *New Biol.* **3**, 103 (1991); G. T. Williams, *Cell* **65**, 1097 (1991); E. A. Harrington, A. Fanidi, G. I. Evan, *Curr. Opin. Genet. Dev.* **4**, 120 (1994); J. C. Ameisen, *AIDS* **8**, 1197 (1994).
8. K. Lundgren *et al.*, *Cell* **64**, 1111 (1991); R. Heald, M. McLoughlin, F. McKeon, *ibid.* **74**, 463 (1993); L. Shi *et al.*, *Science* **263**, 1143 (1994).
10. The conserved Ced-3/ICE/CPP32 cysteine protease gene families that are considered as downstream executioners of apoptosis in multicellular animals may in fact be upstream regulators of cell suicide or may act in conjunction with regulators of the cell cycle (or both). This is consistent with findings indicating that (i) the Bcl-2 survival gene product prevents apoptosis downstream of ICE activation [M. Enari, A. Hase, S. Nagata, *EMBO J.* **14**, 5201 (1995)]; and (ii) granzyme B, which directly activates CPP32 [A. J. Darmon, D. W. Nicholson, R. C. Bleakley, *Nature* **377**, 446 (1995)], also requires cdc2 cyclin-dependent kinase activation in order to induce apoptosis (*8*).
11. M. L. Sogin, *Curr. Opin. Genet. Dev.* **1**, 457 (1991); A. H. Knoll, *Science* **256**, 622 (1992).

Infertility Treatment: A Nuclear Restorer Gene in Maize

Charles S. Levings III

The best seed corn is a true hybrid, the parents being of different and carefully selected lines. But controlling the parentage of corn requires the intervention of humans, because individual corn plants self-fertilize, serving as both the male and female parent. Historically, corn breeders had to remove the tassel from corn plants by hand to prevent self-fertilization, a tedious process at best. So the discovery that some corn plants natually have no pollen was welcomed, and these strains were adopted for use in hybridization.

But these convenient plants had a cost. Between 1969 and 1970, an epidemic of southern corn leaf blight (*1*) struck a strain of sterile U.S. maize that accounted for 85% of the U.S. hybrid maize grown for commercial seed production—the Texas male-sterile cytoplasm [cmsT (cytoplasmic male sterility–T)] system. The subsequent intense interest in the biology of cytoplasmic male sterility (CMS) and its modulators is continued in this issue of *Science*, in which Cui *et al.* (*2*) report the identity of a gene–*Rf2*, one of the restorers of fertility genes—that can inhibit CMS. *Rf2* turns out to be an aldehyde dehydrogenase.

Cytoplasmic male sterility is a maternally inherited trait that suppresses the production of viable pollen and causes sterility in male, but not female, plants (*3*). The sterility effects of CMS, mediated by mito-

The author is in the Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA.

chondrial genes (*4*), can be reversed by specific nuclear genes—the *Rf* genes. For example, in the cmsT strain, pollen restoration depends on the simultaneous activity of two restorer genes, *Rf1* and *Rf2* (*5*).

As corn growers discovered to their dismay several decades ago, cmsT maize is sensitive to the pathotoxin HmT produced by the southern corn leaf blight pathogen *Cochliobolus heterostrophus Drechsler* race T and to the pathotoxin PmT produced by the yellow leaf blight pathogen, *Phyllosticta maydis*. These pathotoxins, collectively designated T toxin, contribute to the unique susceptibility of cmsT maize to these pathogens. Maize carrying the normal cytoplasm or other male-sterile cytoplasms is insensitive to T toxins.

The mitochondrial genome of cmsT carries a unique gene, T-*urf13*, that mediates disease susceptibility (toxin sensitivity) and most likely CMS. T-*urf13* encodes URF13, a hydrophobic polypeptide of 115 amino acids (13 kD) that resides in the inner mitochondrial membrane as an oligomer (*6*). URF13 is a ligand-gated, pore-forming receptor that binds the T toxin produced by the southern corn leaf blight and yellow leaf blight pathogens and also the insecticide methomyl. When URF13 binds T toxin, it forms a pore in the inner mitochondrial membrane, resulting in dissipation of the membrane potential and uncoupling of oxidative phosphorylation (*7*, *8*). These events lead to mitochondrial dysfunction, cell death, and rapid colonization of cmsT plants by the pathogens. Transformation of *Escherichia coli*, yeast, insects, and tobacco with the T-*urf13* gene confers T toxin and methomyl sensitivity to these organisms and confirms that T-*urf13* causes toxin sensitivity (*9*, *10*).

Cytoplasmic male sterility–T cells grown in culture sometimes revert to normal (they show male fertility and toxin insensitivity); these plants have either lost the T-*urf13* gene or the T-*urf13* gene contains a mutation (*11*). Thus, CMS and toxin sensitivity are inseparable: T-*urf13* causes both toxin sensitivity and CMS, possibly by the same mechanism. Also implicating T-*urf13* in CMS is the effect of the *Rf1* restorer gene on URF13 expression. *Rf1* alters the transcript profile of T-*urf13* and reduces URF13 expression by nearly 80% (*12*); in contrast, *Rf2* does not affect URF13 expression (*13*).

Corn without pollen. Normal maize (**top**) makes abundant pollen and can self-fertilize; cmsT maize (**bottom**) has no pollen and is therefore sterile.

The new analysis by Cui *et al.* (*2*) reports the nucleotide sequence of the nuclear gene *rf2*, which together with *rf1*, can inhibit the sterility effects of T-*urf13*. The sequence of the RF2 protein is similar to that of mammalian mitochondrial aldehyde dehydrogenases (ALDH), and RF2 contains two catalytic domains commonly found in the ALDHs. The mammalian ALDH catalyzes the irreversible oxidation of many aldehydes to acids. The *Rf2*-encoded protein contains a putative mitochondrial targeting sequence, suggesting that it resides in the mitochondrion.

Cui *et al.* (*2*) have proposed two models to explain how the *Rf2*-encoded ALDH restores pollen fertility. Their metabolic model proposes that URF13 alters mitochondrial function such that additional pyruvate is shunted into fermentation, a process that can take place under aerobic conditions. Without ALDH activity, increased fermentation can cause acetaldehyde and ethanol accumulation—both phytotoxic compounds—and could lead to tapetum death and pollen abortion. The tapetal cell layer surrounds the developing pollen, supplying it with nourishment for development. In cmsT, pollen sterility occurs because of tapetal degeneration (*14*). The model predicts that RF2 ameliorates the URF13-mediated disturbance by preventing the poisoning of the tapetum by ethanol and acetaldehyde. Because fermentation takes place in the cytoplasm and the *Rf2*-encoded ALDH resides in the mitochondria, acetaldehyde probably diffuses into the mitochondrion, where ALDH oxidizes it to acetic acid, generating a chemical potential gradient. This model is attractive because the *Rf2*-encoded ALDH can play a role in metabolism, even without an URF13-mediated disturbance.

URF13 may cause CMS because it has a major adverse effect on certain anther cells, the tapetum in particular. This negative effect may be due to the high energy requirements of maize tapetal cells—a result of their demanding job of regulating gametogenesis, which makes them particularly susceptible to mitochondrial dysfunction as induced by URF13.

Cui *et al.* (*2*) proposed a second model, in which the RF2 protein interacts (directly or indirectly) with URF13 to mollify its adverse effects. One attractive proposal for URF13-induced CMS is that the mechanisms for CMS and toxin sensitivity are the same. In this event, an anther (tapetal)–specific compound with properties similar to T toxin or methomyl could interact with URF13 to form pores in the inner mitochondrial membrane, leading to mitochondrial dysfunction, cell death, and CMS (*15*, *16*). If the endogenous compound is an aldehyde, the *Rf2*-encoded enzyme could restore pollen fertility by degrading it. Indeed, preliminary evidence shows that an anther-specific compound exists that inhibits respiration of cmsT mitochondria and *E. coli*–expressing URF13; however, it is uncertain whether the compound is an aldehyde (*13*). If the endogenous compound is not an aldehyde, then the metabolic model is favored to explain restoration.

The characterization of *Rf2* has proved only tantalizing; we still do not understand exactly how the nuclear gene *Rf2* fixes the deficit in the mitochondrial gene T-*urf13*. *Rf1* contributes to restoration of fertility by decreasing the accumulation of URF13; *Rf2*, on the other hand, encodes an ALDH whose role in restoration is still speculative. We need to learn more about the steps leading to pollen production and sterility.

References

1. A. J. Ullstrup, *Annu. Rev. Phytopathol.* **10**, 37 (1972).
2. X. Cui, R. P. Wise, P. S. Schnable, *Science* **272**, 1334 (1996).
3. D. N. Duvick, *Advances in Genetics*, E. W. Caspari and J. M. Thoday, Eds. (Academic Press, New York, 1965), vol. 13, pp. 1–56.
4. C. S. Levings III and I. K. Vasil, Eds., *The Molecular Biology of Plant Mitochondria* (Kluwer, Dordrecht, Netherlands, 1995).
5. J. R. Laughnan and S. Gabay-Laughnan, *Annu. Rev. Genet.* **17**, 27 (1983).
6. B. G. Forde and C. J. Leaver, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 418 (1980); R. E. Dewey, C. S. Levings III, D. H. Timothy, *Cell* **44**, 439 (1986).
7. C. S. Levings III, *Science* **250**, 942 (1990); ——— and J. N. Siedow, *Plant Mol. Biol.* **19**, 135 (1992).
8. D. M. Rhoads *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8253 (1994).
9. R. E. Dewey *et al.*, *Science* **239**, 293 (1988); J. Huang *et al.*, *EMBO J.* **9**, 339 (1990); F. Chaumont *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 1167 (1995).
10. K. L. Korth and C. S. Levings III, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3388 (1993).
11. W. H. Rottmann *et al.*, *EMBO J.* **6**, 1541 (1987); R. P. Wise, D. R. Pring, B. G. Gengenbach, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2858 (1987); C. M.-R. Fauron, M. Havlik, R. I. S. Brettell, *Genetics* **124**, 423 (1990).
12. R. E. Dewey, D. H. Timothy, C. S. Levings III, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5374 (1987); J. C. Kennell, R. P. Wise, D. R. Pring, *Mol. Gen. Genet.* **210**, 399 (1987); J. C. Kennell and D. R. Pring, *ibid.* **216**, 16 (1989).
13. D. Cho and C. S. Levings III, unpublished data.
14. H. E. Warmke and S.-L. J. Lee, *J. Hered.* **68**, 213 (1977).
15. C. S. Levings III, *Plant Cell* **5**, 1285 (1993).
16. R. Flavell, *Plant Sci. Lett.* **3**, 259 (1974).