Journal of Heredity 2003:94(2):181–183
DOI: 10.1093/jhered/esg020

Programmed Cell Death and Hybrid Incompatibility

S. A. FRANK AND C. M. BARR

From the Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525, USA. This research was supported by National Institutes of Health grant AI24424 and National Science Foundation grant DEB-0089741.

Address correspondence to S. A. Frank at the address above, or e-mail: safrank@uci.edu.

We propose a new theory to explain developmental aberrations in plant hybrids. In our theory, hybrid incompatibilities arise from imbalances in the mechanisms that cause male sterility in hermaphroditic plants. Mitochondria often cause male sterility by killing the tapetal tissue that nurtures pollen mother cells. Recent evidence suggests that mitochondria destroy the tapetum by triggering standard pathways of programmed cell death. Some nuclear genotypes repress mitochondrial male sterility and restore pollen fertility. Normal regulation of tapetal development therefore arises from a delicate balance between the disruptive effects of mitochondria and the defensive countermeasures of the nuclear genes. In hybrids, incompatibilities between male-sterile mitochondria and nuclear restorers may frequently upset the regulatory control of programmed cell death, causing tapetal abnormalities and male sterility. We propose that hybrid misregulation of programmed cell death may also spill over into other tissues, explaining various developmental aberrations observed in hybrids.

Natural populations with mitochondrial male sterility usually have two or more mitochondrial genotypes (Frank 2000). Each mitochondrial genotype can be repressed by its own specific set of matching nuclear restorer genes, suggesting that each mitochondrial type causes male sterility in a biochemically different way. Distinct polymorphic sets of mitochondrial and nuclear genes coexist, each set with mitochondrial destruction of pollen production and matching nuclear repression of mitochondrial action.

The matching mitochondrial and nuclear polymorphisms arise from the conflicting patterns of transmission between mitochondrial and nuclear genes (Frank 2000). Mitochondria typically transmit only through seeds and not through pollen. Matrilineally transmitted mitochondria increase their fitness by pollen abortion and enhanced seed production, whereas biparentally inherited nuclear genes favor a balance of pollen and seeds. The polymorphisms from this reproductive conflict are much like the matching polymorphisms of attack and defense that often occur in host-parasite systems.

Morphological signs of tapetal deterioration have been observed in several male-sterile species. The maize mitochondrial gene T-*urf13* expresses a protein that results in early tapetal degeneration soon after microspore meiosis (Schnable and Wise 1998). In sunflower, the mitochondrial PET1 gene causes tapetal degeneration soon after meiosis II (Schnable and Wise 1998). Similar tapetal abnormalities arise in male-sterile petunia, wheat, sorghum, and other species (Laser and Lersten 1972).

Mitochondrial genes may cause tapetal degeneration through pathways of programmed cell death (PCD). Balk and Leaver (2001) provide the clearest study relating tapetal deterioration to PCD. In their study, they analyzed morphological and biochemical aspects of male sterility caused by the PET1-CMS cytoplasm in sunflower. They observed classical signs of PCD in tapetal tissues, including cell condensation, oligonucleosomal cleavage of nuclear DNA, separation of chromatin into delineated masses, and partial release of cytochrome *c* into the cytosol of tapetal cells before the major changes associated with PCD. These characteristics of PCD in tapetal tissue are similar to apoptosis in mammals. However, tapetal deterioration lacks two attributes of apoptosis: condensation of nuclei and deterioration of cells into structures called apoptotic bodies. Thus, following Balk and Leaver, we use *PCD* as a general term for the triggered and orderly killing of cells; we reserve *apoptosis* for the subset of PCD with characteristics that have so far only been observed in animals.

Other studies have also suggested that PCD causes tapetal deterioration in mitochondrial male sterility. Induced pollen abortion in barley causes the tapetum and nearby tissue to digest its DNA into fixed size classes (Wang et al. 1999). Such DNA laddering is a hallmark of PCD (Danon et al. 2000). Some authors have suggested that maize mitochondria carrying the T-*urf13* gene cause male sterility by inducing PCD in the tapetum, but this has not yet been demonstrated directly (Wu and Cheung 2000). It has been

shown that, in the presence of a fungal toxin, maize mitochondria carrying the T-*urf13* gene experienced small ion leakage and a loss of membrane potential (Holden and Sze 1987). These symptoms of mitochondrial deterioration appear to be caused by the formation of pores in the mitochondrial membrane (Wu and Cheung 2000), a common feature of apoptosis in animals (Green and Reed 1998).

Different mitochondrial genotypes may trigger cell death in different ways by altering the complex regulatory cascade leading to PCD. Each male-sterile mitochondrial genotype has its own matching nuclear restorer genes. Thus, it appears that each mitochondrial pathway for interfering with the regulation of PCD can be blocked by a matching nuclear pathway that restores normal regulation. Normal regulation therefore arises from a delicate balance between the disruptive effects of mitochondria and the defensive countermeasures of the nuclear genes.

Hybrid crosses often produce male-sterile progeny (Laser and Lersten 1972; Schnable and Wise 1998), suggesting that hybrid incompatibilities readily disrupt the delicate mitochondrial–nuclear balance over the regulation of tapetal cell death. Such mitochondrial–nuclear imbalances may sometimes disturb the regulation of cell death in other tissues, causing diverse hybrid aberrations associated with the misregulation of PCD. Many tissues, such as xylem and leaves, use PCD as part of their normal development (Pennell and Lamb 1997). In addition, plant cells use PCD as a mechanism to limit pathogen growth at the site of infection (Pennell and Lamb 1997). Therefore, misregulation of the PCD pathway could cause defects in tissues throughout the plant.

Hybrids often have reduced fitness and developmental abnormalities (Burke and Arnold 2001; Waldmann 1999). However, we found surprisingly little detail in the literature about morphological and biochemical aberrations in plant hybrids. In our own work, we have observed aberrations of *Nemophila menzesii* when crossed between distant locations (Barr CM, unpublished data). The resulting progeny are stunted, have thickened and curled leaves, have aberrant petals and anthers, and make little or no pollen. Interestingly, *N. menzesii* has CMS, making it a good candidate species to analyze associations between CMS and PCD-induced aberrations in crosses between diverged populations.

We have found one case that associates hybrid aberrations with PCD. Hybrids from crosses between *Nicotiana suavolens* and *Nicotiana tabacum* had developmental abnormalities of cellular death with the classical morphological and biochemical signatures of PCD (Yamada et al. 2001). *Nicotiana* species have widespread male sterility (Nikova et al. 1999).

It is these sorts of hybrid abnormalities in *Nicotiana* and *Nemophila* that deserve closer attention. Perhaps there is a link between mitochondrial-nuclear imbalances arising from male sterility and hybrid aberrations in the regulation of PCD.

Why should the normally tapetal-specific effects of PCD in male sterility occur in other tissues in hybrids? One explanation for tapetal problems in CMS plants is that tapetal tissue experiences exceptional demand for respiration and mitochondrial performance (Hanson 1991). Thus, tapetal tissue is particularly sensitive to mitochondrial aberrations and is usually the only tissue that exceeds the threshold for showing signs of mitochondria-induced failure. If this quantitative explanation is correct, and hybrids have a lower threshold for expressing mitochondrial aberrations, then hybrids may express aberrant PCD in tissues other than the tapetum. Lower hybrid thresholds could arise from poorer physiological performance of hybrid tissues, rendering those tissues more susceptible to expressing aberrations normally masked in intraspecific crosses.

Alternatively, Balk and Leaver (2001) present evidence in favor of tapetal PCD in male sterility arising from tightly regulated, tissue-specific expression of genes. In this case, misregulation of PCD in hybrids may arise from failure of the mechanisms that confine the CMS-induced expression of PCD to the tapetum. Such failure may occur because the genes involved in causing or suppressing CMS are likely to evolve exceptionally rapidly. We expect rapid evolution because CMS arises from a conflict between cytoplasmic and nuclear genes, and antagonistic coevolution often leads to rapid evolutionary change in other systems such as host-parasite interactions (Frank 2002).

Our idea about misregulation of PCD in hybrids calls for closer attention to the nature of hybrid aberrations in plants.

References

Balk J and Leaver CJ, 2001. The PET1-CMS mitochondrial mutation in sunflower is associated with premature programmed cell death and cytochrome *c* release. Plant Cell 13:1803–1818.

Burke JM and Arnold ML, 2001. Genetics and the fitness of hybrids. Annu Rev Genet 35:31–52.

Danon A, Delorme V, Mailhac N, and Gallois P, 2000. Plant programmed cell death: a common way to die. Plant Physiol Biochem 38:647–655.

Frank SA, 2000. Polymorphism of attack and defense. Trends Ecol Evol 15:167–171.

Frank SA, 2002. Immunology and evolution of infectious disease. Princeton: Princeton University Press.

Green DR and Reed JC, 1998. Mitochondria and apoptosis. Science 281: 1309–1312.

Hanson MR, 1991. Plant mitochondrial mutations and male sterility. Annu Rev Genet 25:461–486.

Holden MJ and Sze H, 1987. Dissipation of the membrane potential in susceptible corn mitochondria by the toxin of *Helminthosporium maydis*, race T, and toxin analogs. Plant Physiol 84:670–676.

Laser KD and Lersten NR, 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. Bot Rev 38:425–454.

Nikova V, Pundeva R, and Petkova A, 1999. *Nicotiana tabacum* L. as a source of cytoplasmic male sterility in interspecific cross with *N. alata* Link & Otto. Euphytica 107:9–12.

Pennell RI and Lamb C, 1997. Programmed cell death in plants. Plant Cell 9:1157–1168.

Schnable PS and Wise RP, 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. Trends Plant Sci 3:175–180.

Waldmann P, 1999. The effect of inbreeding and population hybridization on developmental instability in petals and leaves of the rare plant *Silene diclinis* (Caryophyllaceae). Heredity 83:138–144.

Wang M, Hoekstra S, van Bergen S, Lamers GEM, Oppedijk BJ, van der Heijden MW, de Priester W, and Schilperoort RA, 1999. Apoptosis in developing anthers and the role of ABA in this process during androgenesis in *Hordeum vulgare* L. Plant Mol Biol 39:489–501.

Wu HM and Cheung AY, 2000. Programmed cell death in plant reproduction. Plant Mol Biol 44:267–281.

Yamada T, Marubashi W, and Niwa M, 2001. Facile induction of apoptosis into plant cells with temperature-sensitive lethality shown on interspecific hybrid from the cross *Nicotiana suavolens* × *N. tabacum*. Plant Cell Physiol 42: 204–213.

Received June 27, 2002
Accepted December 16, 2002

Corresponding Editor: James L. Hamrick