BOTANY

Apomixis: The Asexual Revolution

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Sexual reproduction is intrinsic to the life cycles of most eukaryotes. Some flowering plants (angiosperms), however, can follow asexual life cycles that rely on recurrent apomixis, the formation of seeds with a set of genes identical to that of the maternal parent. If the process of apomixis can be understood and harnessed, the benefits for agriculture are potentially enormous.

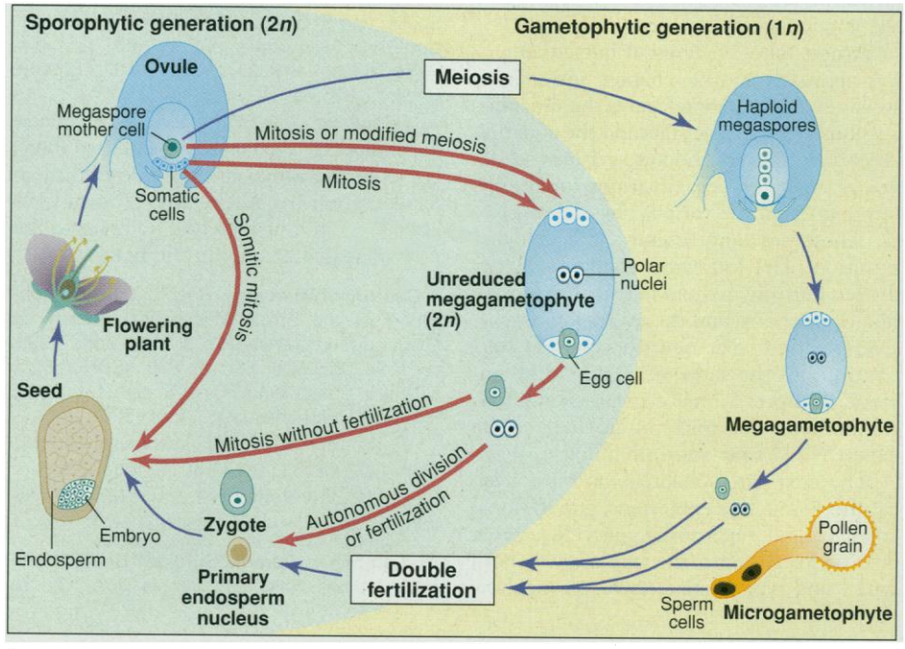
In angiosperms, sexual reproduction entails cyclic alternation of generations (see figure): the plants, which comprise the sporophyte generation ($2n$), produce specialized reproductive structures (flowers) in which genetic recombination and reduction occur during meiosis, yielding haploid microspores (male) and megaspores (female) that undergo limited mitotic development to form microgametophytes (pollen) and megagametophytes (embedded in a maternal ovule). A microgametophyte delivers two haploid sperm cells into a megagametophyte through an ovule-penetrating pollen tube. By processes still not well understood, one sperm fertilizes the central cell, which often is binucleate ($n + n$), to form the endosperm ($3n$), a terminal tissue with multiple hormonal and nutritive functions critical to the embryo; the other sperm fertilizes the egg cell (n) to form the zygotic embryo ($2n$).

Angiosperm apomixis, or asexual reproduction through seed, can occur when the sexual life cycle is short-circuited (*1*). Apomixis entails changes of ploidy in one or both generations. In nonrecurrent apomixis, haploid ($1n$) embryogenesis occurs without a preceding fertilization step. In recurrent forms of apomixis, embryogenesis arises in a cell lineage lacking both meiotic reduction and fertilization (see figure). In this second kind of apomixis there is exclusive transmission of the entire maternal genotype to the next generation. A genetically stable, seed-propagated clone is thus established, which can perpetuate itself across multiple sporophytic generations. Although certainly a challenging biotechnological endeavor, the prospect of rendering sexual plants recurrently apomictic presents opportunities so revolutionary as to justify a sustained international research effort to harness apomixis.

What would these opportunities be? Clonal apomictic derivatives from economically important sexual plants could facilitate the development, mass production, and maintenance of elite hybrid genotypes. Genetically pure seed lots could be produced from apomictic species without the physical isolation that is now required. Breeding and production of more hybrids will be feasible, allowing development of genotypes better adapted to abiotic and biotic environmental pressures and better tailored to end-product uses. Use of transformants will be facilitated, in that apomixis will render hemizygous transgenes capable of 100% seed transmission, obviating the need of inbreeding to obtain transgenic homozygotes. Furthermore, apomictic transformants may exhibit less sterility from tissue culture–induced somaclonal variation, which often results from disturbed meiosis. In species with high genetic load, the level of hybrid heterosis attainable under apomixis will exceed that attainable by sexual means. Once freed by apomixis from the constraints on chromosome number imposed by meiotic sterility, it will be far easier to seek genetic synergism at higher-than-diploid levels, that is, to minimize mono-allelism (homozygosity, at the diploid level) and maximize multiallelism (heterozygosity, at the diploid level). Moreover, with meiotic sterility eliminated, crops might be developed more readily from new interspecific and intergeneric hybrids.

Recurrent apomixis occurs in species of more than 40 angiosperm families, including those containing grasses, sunflowers, and roses. Its close association with polyploidy suggests possible pleiotropic recessive gametophytic lethal effects, or tight linkage with such lethals (*2*). Inheritance studies, although complicated by polyploid segregation, suggest that it is controlled by a single dominant locus (*2*). Attempts to introduce apomixis from wild relatives into pearl millet (*3*) and maize (*4*) have to date resulted only in agronomically unsuitable, partially fertile apomictic plants with added chromosomes. In *Tripsacum* (*4*), the gene or genes for apomixis map to a region densely populated with molecular markers (where polymorphisms abound or recovery of recombinants is rare or nil), suggesting potential involvement of alien chromatin, pericentromeric heterochromatin, inversion heterozygosity, or complementary gametocidal loci.

No recurrently apomictic mutants have been recovered to date in sexual species, suggesting that recurrent apomixis requires gains in function. A number of known mutants, however, cause nonrecurrent apomixis, and others affect genotypic transmission from



Sex or apomixis? The life cycle of sexual flowering plants follows the alternation of generations, in which meiosis precedes the formation of gametes, and double fertilization restores the somatic chromosome number. In species with recurrent apomixis, asexual reproduction by seed occurs when the life cycle is short-circuited, resulting in seed with a genotype identical to that of its maternal parent.

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the sporophytic to the gametophytic generation—for example, by eliminating recombination or the first meiotic nuclear division. Chemically induced *Arabidopsis thaliana* mutants (5, 6) exhibit autonomous inception of endosperm divisions in unfertilized ovules, albeit without full maturation. These and other findings imply that angiosperm reproduction can be genetically manipulated.

To harness apomixis, we will need to answer some fundamental biological questions: What factors determine the commitment of specific cells in the ovule to somatic, meiotic, or apomictic development? What are the respective roles of cell lineage and positional sensing in cell determination? How do gametes communicate during fertilization? What functional relations exist between cell cycle regulators and regulators of sexual and apomictic development? We will also need to understand reproductive development in yeast and ferns, in which mutants that mirror angiosperm recurrent apomixis have been identified (7). In angiosperms, relatively few reproductive genes have been identified and mapped, and little is known about the role of plant hormones in female reproductive development, although new techniques (8, 9) promise to reveal much about natural and induced apomictic reproduction.

The harnessing of apomixis will lead to large increases in agricultural production, perhaps even greater than those seen with the adoption of broadly adapted, semidwarf grain varieties some 20 years ago during the Green Revolution. Such an "asexual revolution" now appears to be within our scientific grasp as we approach the 21st century. The main determinant of its occurrence may be the funding and international cooperation needed to bring it about.

References

1. P. Maheshwari, *An Introduction to the Embryology of Angiosperms* (McGraw-Hill, New York, 1950).
2. G. A. Nogler, in *Embryology of Angiosperms*, B. M. Johri, Ed. (Springer-Verlag, Berlin, 1984), pp. 475–518.
3. P. Ozias-Akins, E. L. Lubbers, W. W. Hanna, J. W. McNay, *Theor. Appl. Genet.* **85**, 632 (1993).
4. D. Grimanelli, O. Leblanc, D. Gonzalez de Leon, Y. Savidan, *Apomixis Newslett.* **8**, 35 (1995).
5. A. M. Koltunow, R. A. Bicknell, A. M. Chaudhury, *Plant Physiol.* **108**, 1345 (1995).
6. N. Ohad *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5319 (1996).
7. L. E. Buckingham *et al.*, *ibid.* **87**, 9406 (1990).
8. P. S. Springer, W. R. McCombie, V. Sundaresan, R. A. Martienssen, *Science* **268**, 877 (1995).
9. J. E. Faure, C. Digonnet, C. Dumas, *ibid.* **263**, 1598 (1994).

CELL BIOLOGY

Hats Off to the Tricorn Protease

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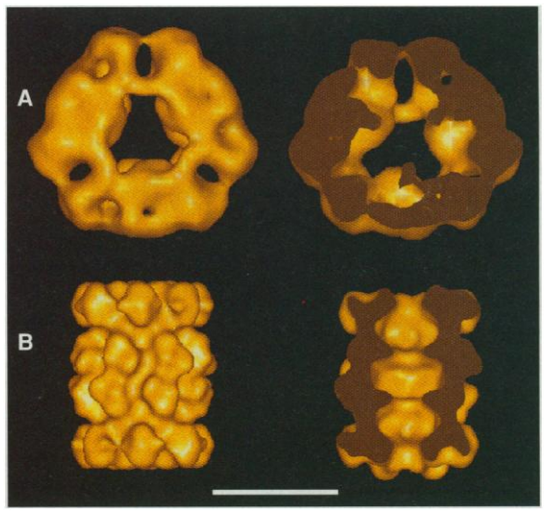
Proteolysis, the last step in the life of a protein, is essential for the maintenance of cellular homeostasis (*1*). Proteases remove damaged or denatured proteins, recycle their amino acids, and are important for cell regulation. But how do cells tame the potentially destructive activities of these enzymes to ensure that they act only where needed? One solution—used by archaebacteria, eubacteria, and eukaryotes—has been to compartmentalize proteolytic enzymes within so-called molecular organelles (*2*), ring-shaped protein assemblies in which the proteolytically active center is sequestered from the rest of the cytosol. The paradigm is the proteasome, a cylindrical multimeric protein complex with a central proteolytic chamber, to which access is carefully controlled (see figure, part B) (*1*, *3*). This ancient organizational principle has been extremely successful. Indeed, most protein degradation in the cytosol of archaebacteria and of eukaryotic cells is thought to be proteasome-mediated. But now on page 1385 of this issue, Tamura *et al.* (*2*) report the surprising discovery of a new proteasome-like particle, a large tricorn complex (TRI) abundant in the thermophilic archaebacterium *Thermoplasma acidophilum* (see figure, part A). A comparison of TRI and the proteasome reveals both interesting parallels and differences.



Destructive siblings? Structures of (**A**) TRI and (**B**) the proteasome from *Thermoplasma acidophilum* at a resolution of 20 Å. Bar, 10 nm. [Figure courtesy of T. Tamura and W. Baumeister]

As beautifully documented by a three-dimensional reconstruction of the complex from electron microscopic images, the shape of the new protease is reminiscent of a tricorn, a hat with the brim turned up on three sides. Unlike the *Thermoplasma* proteasome, which is composed of four heptameric rings (two containing α and two containing β subunits), TRI consists of six identical subunits of 120 kD. The TRI complex is a trimer of dimers forming a toroid traversed by a channel along its threefold axis (part A of the figure shows the end-on view of the toroid on the left and a horizontal cut through the middle of the complex on the right). The quaternary structure of TRI is strikingly similar to that of the Gal6 or bleomycin hydrolase from *Saccharomyces cerevisiae* (*4*). Both proteins are hexamers with a central channel. In TRI the maximum width of the channel, thought to harbor the proteolytic sites, is 8 nm, whereas the proteolytic chamber of the yeast complex is only 4.5 nm, close to that of the *Thermoplasma* proteasome (5.3 nm). However, the sequence of the *tri* gene, also reported by Tamura *et al.* (*2*), is not related to Gal6 or bleomycin hydrolase, nor are any other TRI homologs found in the databases. The only detectable homology to other proteins occurs in a stretch of ~160 residues at the COOH-terminal of the TRI subunit. This segment is related to several COOH-terminal processing proteases of bacterial and eukaryotic origin and may form a domain that participates in substrate binding.

What is known about the function of TRI? Unlike the proteasome from the same organism, which has chymotrypsin-like cleavage specificity, TRI cleaves with a specificity more like trypsin, as established with model peptides. It is inhibited by tosyl-L-phenylalanine chloromethyl ketone, suggesting the involvement of a histidine residue in the active center [the proteasome has a threonine in the active site (*3*)]. Neither the natural substrate proteins of TRI

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