The tools of molecular biology offer a plethora of ways to explore gene function and regulation, one of the most important being the manipulation of genomes by transformation. Integration of exogenous DNA into the nuclear genome has become routine for many plant species, and transgenic plants harboring, in their nuclear DNA, genes from other plants or from different organisms can be readily created by a number of techniques. By contrast, techniques for the transformation of plastid genomes have been developed only recently. Much of the work on plastid genome transformation has been done in the unicellular alga Chlamydomonas, stable transformation of which was reported by Boynton et al. (1988). Plastid transformation should provide essential information about how plastid gene expression is regulated as well as an important tool to investigate—and alter—the function of plastid gene products. In this issue, Staub and Maliga (pages 39–45) demonstrate that higher plant plastids can be stably transformed, paving the way for informative studies of higher plant plastid genomes.

Transformation of plastid genomes has several potential problems that nuclear transformation does not share. For one thing, it may be more difficult for DNA to cross the plastid double membrane than the nuclear membrane. In addition, plastid genomes are present in much higher copy number than nuclear genomes—the single Chlamydomonas chloroplast has 80 genome copies, and higher plants can have 100 or more plastids, each with many genome copies. For a transformed genome to replace all copies of the original genome, therefore, strong selection pressure (for prototrophic growth or for antibiotic resistance) must be applied.

The problem of getting DNA into the Chlamydomonas chloroplast was overcome by the use of particle bombardment: when DNA-coated tungsten particles are shot into cells, transformants arise at reasonable efficiency. In transformants, the donor DNA replaces homologous sequences in the recipient genome. With selection pressures, nontransformed genome copies are lost during the ensuing cell divisions. If the donor DNA corresponds to a sequence in the inverted repeat region, the other repeat copy corrects to the new sequences.

The large number of plastid genomes in higher plants posed a potential hindrance to transformation of higher plant plastids. Because Agrobacterium Ti-plasmid vectors are remarkably successful mediators of transfer of exogenous DNA to the nuclear DNA of many plant species, De Block et al. (1985) attempted to use these vectors to transfer DNA to chloroplasts. They created a chimeric marker gene in which the Agrobacterium nopaline synthase promoter is fused to the chloramphenicol acetyl transferase (cat) gene; by transforming tobacco protoplasts with a Ti-based vector containing this gene and growing calli on chloramphenicol, they were able to select transformants. Although some evidence pointed to the integration of the DNA into the chloroplast genome of chloramphenicol-resistant plants, the new DNA did not appear to be stably integrated, and these intriguing results have not been repeated.

Other approaches for introducing foreign genes into higher plant plastids have been explored. For example, several investigators have bombarded cultured tobacco cells or wheat leaf cells or calli with constructs containing the reporter genes cat or β-glucuronidase and have observed transient chloroplast expression of these genes (Daniell et al., 1990, 1991). PEG-mediated transformation of reporter genes into protoplasts may also lead to transient gene expression in chloroplasts (Spörlein et al., 1991). However, in no case has the stable incorporation of the introduced gene been demonstrated.

A different approach, and one that has been used successfully to create stably transformed tobacco plastid genomes, is that of Svab et al. (1990). These investigators used a technique similar to that used for Chlamydomonas chloroplast transformation, that is, selecting for homologous integration of DNA introduced by particle bombardment. The transforming DNA included a segment of plastid DNA that carries three markers not found in the recipient: two antibiotic resistance markers (to spectinomycin and streptomycin, each of which is caused by a different point mutation in the 16S rDNA gene, which encodes 16S rRNA) and a new RFLP marker. By bombarding tobacco leaf tissue with this DNA and selecting calli that remained green in the presence of spectinomycin, and were therefore resistant to this antibiotic, these workers isolated three independent transplastomic lines. Transformants could be distinguished from the many spontaneous spectinomycin-resistant mutants by the presence of the other two, unselected markers. The 16S rDNA gene is in the inverted repeat region of the plastid genome, and, as with homologous integration into the repeat region of the Chlamydomonas chloroplast genome, the donor sequences copy corrected into the other repeat copy.

A surprising observation to come out of this study was that, although the unselected RFLP marker was stably integrated, the unselected antibiotic marker segregated out in the next generation. This observation suggested that, unlike homologous integration in Chlamydomonas, recombination events might prevent the integration of long segments of homologous DNA in the tobacco plastid genome.

In this issue, Staub and Maliga describe the results of experiments designed to investigate in further molecular detail the nature of donor DNA integration into...
the tobacco plastid genome. By using a larger donor DNA fragment from the 75S DNA region, with six unselected markers (five RFLP markers and a streptomycin resistance marker caused by a mutation in the gene for ribosomal protein S12) in addition to the selected spectinomycin resistance marker, they have verified that a long region of homologous DNA does indeed integrate into the plastid genome by homologous recombination. The authors obtained two transplastomic lines, both of which are homoplasmic for all, or nearly all, of the donor DNA.

The results of Staub and Maliga confirm that the transformed donor sequences have copy corrected into the second copy of the inverted repeat. In addition, the absence of detectable wild-type genomes in the primary calli indicates that copy correction and sorting out of the different genomes occurred relatively soon after transformation. The transformants are stable because seed progeny from selfed transformants or from crosses in which a transformant is the female parent are resistant to both of the antibiotics. These results suggest that, despite the results of Svab et al. (1990), recombination is unlikely to eliminate newly introduced markers. Staub and Maliga speculate that the instability of the streptomycin resistance marker in the earlier study resulted from the selective advantage of rare recombinant genomes rather than from frequent recombination. Genomes that had lost streptomycin resistance may have been at a selective advantage because the two antibiotic resistance markers may be, to some extent, incompatible in the same 16S rRNA molecule.

Staub and Maliga's demonstration that intact, long segments of donor DNA can integrate stably into the plastid genome will make possible all sorts of experimental manipulations of plastid genomes. The newly introduced restriction enzyme sites in the donor DNA could, for example, serve as cloning sites for foreign DNA sequences. In addition, homologous integration offers the possibility of creating insertional inactivation mutations in open reading frames whose function is unknown. It has recently been shown that when Chlamydomonas is transformed with two markers carried on different plasmids, only one of which is selected for, the unselected marker also integrates at reasonably high frequency (Kindle et al., 1991). If cotransformation also occurs at high efficiency in higher plant plastids, this will be a valuable method for creating defined mutations in any higher plant plastid gene. The ability to create stable transgenic plastids by homologous recombination has opened the door to exciting and informative manipulations that should illuminate many aspects of plastid function in both land plants and algae.

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REFERENCES


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