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The medical use of mAbs has grown tremendously since the first mAb, Orthoclone, was approved in 1986 for treatment of transplant rejection. In addition to immunosuppressive therapy, mAbs have proven useful as treatments for rheumatoid arthritis, transplant rejection, inflammatory diseases, and a variety of cancers. The market for medical mAbs is estimated to exceed $20 billion, and >150 are in clinical development (1). Although this progress is seemingly a biotechnology success story, the costs associated with a therapeutic mAb regimen can exceed $20,000 per year, which seriously may limit their current and future clinical use (2). These high costs are attributable to a number of factors, including the inherent expense of purification and cGMP for recombinant biologics and, to a significant extent, the cost of producing the raw material containing the antibody. To date, all Food and Drug Administration (FDA)-approved mAbs are derived from mammalian cells. Because of a reliance on relatively expensive equipment, an economy of scale may not be met by mammalian cell-based fermentation technology. This realization has fueled the development of numerous transgenic technologies during the last two decades that, it was hoped, would significantly lower production costs. These transgenic systems have involved transgenic yeast, bacteria, insects, animals, and plants (3). Each method appears to offer some advantages in scalability and cost, but they have all proven to possess distinct shortcomings precluding their acceptance by the larger scientific community, the medical community, or the FDA. These shortcomings include immunogenic glycosylation, propensity for viral pathogen copropagation, developmental instability, complex genetics, environmental concerns, and, most significantly, prolonged development times. One or more of these deficiencies is associated with every transgenic system, including current mammalian cell-based techniques. In this issue of PNAS, Giritch *et al.* (4) report significant progress in remedying several of these drawbacks but, most importantly, describe a technique that drastically reduces overall development time.

Stably transformed plants, which have been touted for their potential to provide very low-cost raw material, always have suffered from very long development times because of inherently long life cycles and complex genetics associated with identifying and stabilizing transgenic lines. In the case of many commonly used plant species, such as corn, this process can take many years. In addition, the low yield of recombinant proteins observed in these systems has led to projections that commercial quantities of recombinant biomass production could be economically achieved only via large, open-field acreage, where cGMP controls are difficult to enforce and environmental containment is problematic. Several recent reports of environmental genetic contamination from recombinant crops (5–7) also may make the open-field production of biopharmaceuticals unattractive.

Table 1. Transient plant technology: The advantage of magnifection

Expression system	Time to milligrams of mAb	Time to grams of mAb
Mammalian cell culture*	2–6 months	6–12 months
Transgenic animals	>12 months	>12 months
Stable transgenic plants	12 months	>24 months
Magnifection	14 days	14–20 days

*Values are based on direct quotes from contract manufacturers.

The article by Giritch *et al.* (4) offers an entirely new methodology that attempts to address a number of these problems. Referred to as "magnifection," the technique involves transient expression and may be the most rapid path from genes to full length, assembled mAb (i.e., 14 days from gene delivery to harvested plant cells) (Table 1). Even in this early stage of development, the approach easily can accommodate gram-level production. Because antibody drug development has evolved into a highly iterative process where sequential modifications are introduced into candidate molecules and evaluated for changes in efficacy, the shortened time frame to gram-level production described in this article may accelerate this process greatly. Moreover, the ability to increase the volume of mAb-containing biomass does not appear to involve any change in growing conditions, infection procedures, or equipment. Finally, concerns about transgenic containment and environmental contamination are vastly minimized because optimum growth conditions appear to be in controlled growth rooms and there are no transgenic seeds or viable transgenic plants resulting from the process.

The fundamental discovery that enabled this progress builds on an earlier article by the same group (8) where they reported significant enhancement of *in planta*, viral-mediated expression of several recombinant proteins by reengineering the viral transcripts. Because virus replication is cytoplasmic and transcripts are commonly large and intron-free, they reasoned that adapting such genes for efficient nuclear processing would require some modification. Therefore, transcripts were optimized through the removal of eukaryotic cryptic splice sites and the addition of several introns that shortened mRNA to be more "plant-like" and compatible with the host transcription and translation machinery. Observed expression levels of several homooligomeric proteins in this system were remarkable. However, attempts to express heterooligomeric proteins, such as antibodies, met with limited success. As reported in this article, low expression of such proteins appeared linked to a distinct spatial segregation of different reporter genes [GFP and red fluorescent protein from *Discosoma* (DsRED)] when delivered in mixed populations with the same viral vector. Such interaction of plant viruses resulting in symptom suppression, or in some cases enhancement, has been observed historically for many years (9), and the phenomena has been applied practically as a method for controlling viral diseases (viral exclusion or cross-protection). The mechanism of viral exclusion is not well understood, although some instances of RNA-mediated gene silencing have been documented (10, 11). However, the authors also were aware that viral interaction may not always be suppressive or exclusionary and

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that combinations of potato virus X (PVX) with other potyviruses can coexist in mixed infections (12, 13). This observation led Giritch *et al.* (4) to hypothesize that building heterooligomeric expression vectors on noncompeting viral strains might be the path to successful expression of antibody molecules. This article dramatically validates this approach.

The actual infection of the cell is accomplished after introducing several provectors into *Agrobacterium tumefaciens* that can deliver the viral components and the foreign genes to plant cells. In this sense, *Agrobacterium* is the vehicle for primary infection and systemic movement in the plant, whereas the ultimately recombined, functional viral replicon provides cell-to-cell spread, amplification, and high expression. None of the provectors contain plant-selectable markers (e.g., kanamycin resistance), and they are not selected for genome integration and expression (the process that can consume years). Instead, the *Agrobacterium*-delivered provectors are engineered with specific recombinase sites that, when codelivered into the cell with their counterpart enzyme (phage C31 integrase), recombine efficiently *in planta*, forming the completed viral replicon. The mixing and codelivery of multiple *Agrobacterium*-based vectors, each containing a separate component of the viral replicon, is a fast and efficient method for expressing a wide range of proteins combining different elements. The combinatorial and iterative nature of antibody research is well matched to such an approach.

In Giritch *et al.* (4), tobacco mosaic virus (TMV) and PVX transcripts, derived from their respective provectors containing promoter, recombination, and processing elements, coexist in the cells of *Nicotiana benthamiana* and efficiently transcribe their respective nonviral genes, i.e., the heavy chain (HC) and light chain (LC) of an antibody. In fact, these viruses appear to share certain virally encoded proteins useful for propagation (i.e., the TMV-encoded movement protein) and may not be competing for cellular constituents that would be required for replication and movement. This coexpression involved ≈95% of the leaf cells, indicating a very high level of *Agrobacterium*-mediated infection. When optimized for an enhanced level of expression (by reconfiguring the PVX coat protein gene), 82% of cells coexpressed each protein, which is still sufficient for high-level antibody expression.

Agrobacterium-delivered provectors are engineered with specific recombinase sites that recombine efficiently *in planta*.

Viral systems have been used for foreign gene expression in plants. In general, a serious limitation on the size of the foreign gene that could be accommodated by the virion was observed (14). When the improved viral vectors described in Giritch *et al.* (4) are delivered in *Agrobacterium*, it appears that the size limitation is increased sufficiently to allow for expression of transcripts >1,500 bases, thus permitting HC expression.

The system described in Giritch *et al.* (4) may represent a prototype that can be further optimized to increase the yield of antibody (or other heterooligomeric proteins) from the current level of 0.5 g/kg leaf. In the current methodology, multiple recombinant agrobacteria are required to initiate efficient propagation of viral transcripts and their foreign genes. Individual agrobacteria for the HC, LC, their respective TMV and PVX provectors, and an *Agrobacterium* with an integrase-expressing vector to catalyze efficient recombination of the provector components all are required in a single infection experiment. A simplified system (specifically for antibodies) that does not require an integrase or recombination may boost further the levels of expression.

Interestingly, LCs accumulated at substantially higher levels compared with HCs. This result may be due to the limiting amounts of processing machinery components. In other words, this quantity of transcripts being translated into the endoplasmic reticulum may exceed the titer of endogenous BiP-like chaperones required for efficient assembly of both chains. As suggested in Giritch *et al.* (4), these characteristics offer the opportunity to investigate the role of specific endogenous elements and their contribution to the processes of assembly, endoplasmic reticulum retention, and secretion. Of interest as well will be characterization of the glycosylation patterns that, in plants, are considered to be an important target for modification to prevent potential immunogenicity and to elicit appropriate effector functions in mammals. The techniques described in Giritch *et al.* (4) may provide important tools for these kinds of extensive modification and evaluation endeavors.

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