A major advantage of the biolistics system is that it is useful for a wider range of cultivars than other systems currently available for stable transformation of rice. For instance, transformation and regeneration have been successful using a subsp. *javanica* cultivar, Gulfmont (W. Teng, W. G. Buchholz, and T. C. Hall, unpublished), and a subsp. *indica* cultivar, TN1 ***(7)***, by the biolistics approach. Although it is less genotype-dependent than other available methods, biolistics is still limited to those cultivars that are amenable to regeneration from tissue-culture. The major disadvantage of the biolistics system is the same as that for all other direct DNA uptake systems; fragmentation and rearrangement of input DNA leads to insertion of functionally intact genes of interest at relatively low frequencies (approx 1–2% of transformants).

Two other systems have been developed for stable transformation of rice: direct DNA uptake by protoplasts and *Agrobacterium*-mediated transformation. Protoplast transformation has received much attention and depends on physical (e.g., electroporation ***[8,9]***) or chemical (e.g., polyethylene glycol [PEG] ***[10]***) means to stimulate DNA uptake. Electroporation-mediated transformation, with which we have many years experience ***(11)***, can be an efficient way to produce large numbers of independently transformed plants with relatively low numbers of copies of the genes of interest. Similarly, PEG-mediated transformation produces many transformants, but, in our hands, the number of copies of the introduced genes tends to be very high. The primary disadvantages to protoplast systems are that they are currently limited to only a few cultivars, they are labor intensive, and the embryogenic cultures are extremely sensitive to environmental fluctuations and equipment failures. Also, depending on the culture and its age, the frequencies of cell-wall regeneration from protoplasts, plant regeneration from calli, and fertility vary widely from one experiment to the next.

Though highly sought after, *Agrobacterium*-mediated transformation of rice was not convincingly shown until recently ***(12)***. Using the same bacterial strains and plasmids as that study, we have successfully repeated transformation of rice callus tissue and have regenerated transgenic plants ***(13)***. This is an exciting development, because of the very desirable characteristic of the *Agrobacterium* system in which a discrete portion of DNA is integrated into the genome. This yields a high frequency of cotransformation of an intact copy of the gene of interest with the selectable marker. It remains to be seen whether the *Agrobacterium* system will be highly genotype dependent.

The protocol presented below, although workable and currently producing transgenic rice plants from approx 10% of the bombarded embryos, should be taken as a starting point. As with any methodology, this protocol continues to evolve as more experience is gained and as more parameters are varied and