suitable for amplifying all 4,000 TB genes can be obtained from a commercial supplier in about 2 months time. Within about another month all 4,000 TB genes could be amplified and organized into about forty 96-well plates, and using the LEE approach, the promoter and terminator sequences could be added to the amplified fragments. These transcriptionally active LEE fragments could be organized into 40 pools containing about 100 fragments, and each pool could be evaluated for in vivo immunologic activity. Active pools could be further segregated into smaller pools or the fragments could be individually evaluated for in vivo activity. In this way the immunologically active antigens suitable for a DNA vaccine could be comprehensively identified.

In addition to pointing the way toward an improved method for identifying immunologically active antigens in complex organisms, the LEE approach should find broader uses as a genomics tool to help elucidate the function of undefined genes. It could be used to produce antibodies against proteins even before they have been cloned and expressed. And finally, chemically modified linear-expression elements may eventually replace plasmids in synthetic gene delivery systems for many more gene therapy applications[9,10].

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Transgene escape and transplastomics

Dean Chamberlain and C. Neal Stewart, Jr.

Genetically modified (GM) food is big news at the moment, particularly in Europe. Hysteria seems to have gripped the British press (from the low-brow tabloids to the highbrow broadsheets) in a furor of at least the magnitude of *Salmonella* in eggs and BSE in beef (food scares seem to be a special favorite of Fleet Street). Press releases appear weekly with descriptions of the latest anti-GM crop activities of groups like Greenpeace, who have, for instance, deposited 4 tons of GM soybeans on Tony Blair's doorstep, and filed a lawsuit against the EPA for approving transgenic plants carrying the *Bacillus thuringiensis* toxin (see http://www.greenpeace.org for details). The UK government is reappraising its stance on commercial growing of GM crops, and Monsanto was fined in Lincolnshire, England for failing to conduct proper field trials. Clearly, the use of transgenic technology—and the perceived threat of uncontrolled transgene spread—is a hot, organically produced, nontransgenic potato.

In this issue, Scott and Wilkinson[1] assess the probability of pollen-mediated movement of transgenes from transplastomic (rather than nuclear transgenic) *Brassica napus* to its wild relative *Brassica rapa*. Proving that transgene escape from transplastomic crops poses a negligible risk would do much to support the use of this technology for containment of transgenes. But it's important to remember that for any transgene to spread (nuclear or plastomic), there must be successful hybrid formation between a sexually compatible crop plant and recipient species. The two species must flower at the same time, share the same insect pollinator (if insect-pollinated), and be close enough in space to allow for the transfer of viable pollen[2]. Thus, the transfer of transgenes will depend on the sexual fertility of the hybrid progeny, their vigor and sexual fertility in subsequent generations, and the selection pressure on the host of the resident transgene[2,3].

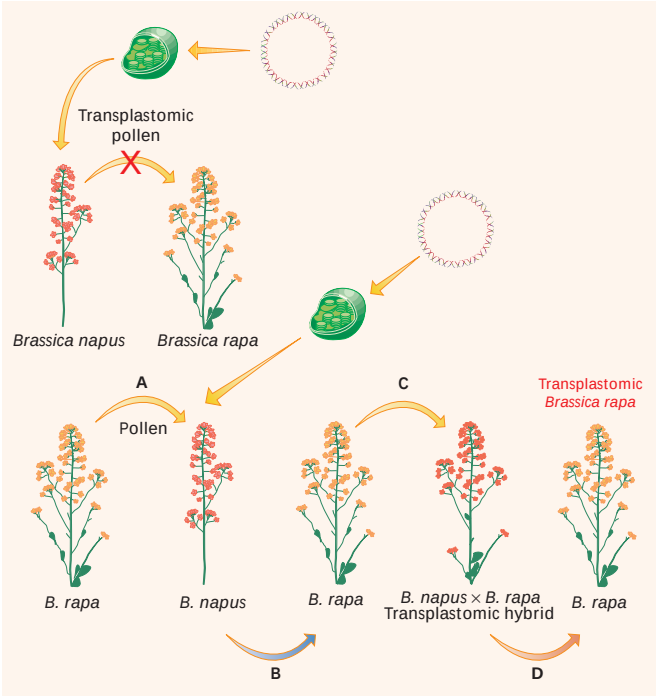

Figure. (A) Transplastomic oilseed rape (*Brassica napus*) transgenes will not flow into related weeds (e.g., *Brassica rapa*) through pollen. (B–D) If transplastomic oilseed rape served as the female parent, then transgenes could be introgressed into the weed *B. rapa*. Transplastomic oilseed rape plants might be rare in a wild *B. rapa* population and might be pollinated by wild *B. rapa* (A). Some of the progeny would be transplastomic hybrids (B). After a single backcross of the transplastomic hybrids with wild *B. rapa* pollen (C), some of the progeny would be functional transplastomic *B. rapa* (D).

Generating transgenic chloroplasts with biolistics is still difficult, as is selecting a pure population of transformed chloroplasts. In the 9 years since the first transplastomic higher plant was generated[4], stable biolistic chloroplast transformation in plants has been achieved in only one species: tobacco[5,6]. A major drawback with transforming the chloroplasts of agronomically important crops is that gramineous embryogenic plant cultures contain proplastids that are smaller than the projectiles used for biolistic plant transformation[7]. So at present, it seems unlikely that the success rate of generating transplastomic crops will ever approach that of nuclear transformation.

But if we assume that transplastomic oilseed rape is possible to produce, will use of this technology translate into transgene containment? Scott and Wilkinson describe an interesting scenario that addresses this issue. First, consider a feral wild-type population (*B. rapa*) that is contaminated

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with a few transplastomic oilseed rape plants (*B. napus*) from an adjacent field. Then consider the same situation, but substitute a nuclear transgenic oilseed rape biotype for the transplastomic biotype. Assume that the transgene (located in the chloroplast or nuclear DNA, respectively) is a *B. thuringiensis cry* gene active against certain defoliating caterpillars. Can either transgene escape? Scott and Wilkinson addressed this scenario experimentally, using naturally occurring chloroplast genes as markers for hypothetical transgene escape. They confirmed that in contrast to a nuclear-encoded trait, a plastid-encoded trait will not be transmitted from the crop to the weed through pollen (Fig. 1).

However, the oilseed rape (*B. rapa* cross occurs quite easily when *B. rapa* is the pollen donor[9], especially if oilseed rape is rare in the population and its flowers are overwhelmed with *B. rapa* pollen. If this occurs, then one hybridization event and one or two backcrosses later (with *B. rapa* again as the pollen donor), we have transplastomic *B. rapa*. (Fig. 1). As Scott and Wilkinson implied, selection pressure would favor the transgenic biotype; indeed, this has proved to be true with insecticidal transgenic oilseed rape[3]. Paradoxically, as Mikkelsen et al.[9] and Metz et al.[10] point out, nuclear transformation of oilseed rape with the transgene on the C genome (originating from *Brassica oleracea*, *CC*, 2n = 18) will not be passed to *B. rapa* (which has the A genome, *AA*, 2n = 20). Oilseed rape has both (*AACC*, 2n = 38). Therefore, in terms of transgene escape, the riskiest placement of a transgene is on the *A* genome, followed by the chloroplast genome. The safest placement is the *C* genome[9,10].

However, given that paternal chloroplast inheritance is rare, transplastomic plants may prove highly useful for transgenic crop control, but only when integrated into a strict management program. One potential strategy for tracing the movement of transgenes and transgenic plants is to tag them with a visual marker such as green fluorescent protein (GFP)[8]. This would depend, of course, on establishing that GFP is nontoxic and imposes no fitness costs on the plants. Another monitoring scheme would be to express GFP in the seed coat of transgenic seed, using seed coat–specific promoters. This would allow large-scale sorting of nontransgenic from transgenic seed, as well as tracking of seed spilled in the environment.

The next step in assessing biosafety will be to integrate transformation, gene flow, and ecology for specific transgenic events per crop. Certainly, real field experiments in nature of the type that Scott and Wilkinson perform are needed, as well as assessments of how selection pressure affects the spread of transgenes. Even if transgenic weeds are produced, the odds of creating a superweed are remote. However, this must be established empirically.

Clearly there are no hard or fast rules when it comes to transgene containment. Each system has to be considered separately. If agronomically important transplastomic crops ever become a reality, their use would have to be considered on a plant-type basis to decide if the transplastomic route gives the strongest transgene-spread protection. But the bitter truth is that no matter what safeguards are put into place, the anti-GM lobby will never be appeased.

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