An intoxicating switch for plant transgene expression

Christiane Gatz

Turning the expression of transgenes either on or off with an otherwise inactive chemical has long been the dream of plant molecular biologists. In this issue, Caddick et al.[1] demonstrate that a simple molecule like ethanol can be used to specifically induce high expression levels of a transgene in plants. Such a system has the potential to develop into one of the most broadly applicable regulatory systems for plant gene expression currently available.

Chemically inducible promoters provide an essential tool for the study of gene regulation and function in various biological processes. The tetracycline-inducible and dexamethasone-inducible promoters established previously have already proven to be extremely useful, as they allow the regeneration of transgenic plants encoding genes that interfere with growth when highly expressed[2,3]. However, these chemicals are not suitable for biotechnological applications outside the laboratory. In contrast, the ethanol-inducible system described by Caddick et al.[1] has the potential to control at will agricultural traits of crop plants in the field.

The system described by Caddick et al.[1] is based on the regulatory elements of the *Aspergillus nidulans alcA* promoter, which is strongly inducible by ethanol[4]. It is the most widely used promoter for overexpressing proteins in *A. nidulans* and other filamentous fungi both for fundamental research and applied aspects in biotechnology. The transcriptional activator AlcR, a DNA-binding protein belonging to the C6 zinc binuclear cluster family, binds only to its target sequences within the *alcA* promoter when cells are grown in the presence of ethanol, the gratuitous inducer ethyl methyl ketone, or other alcohols/ketones.

To make this system work in plants, the *alcR* coding region was placed under the control of the constitutive cauliflower mosaic virus (CaMV) *35S* promoter, which provides high and reliable expression levels in transgenic plants. The target promoter contains the TATA-box as well as upstream sequences of the *alcA* promoter fused to position -23 of the CaMV *35S* promoter. When stably transformed into tobacco, these constructs mediate ethanol-dependent expression of transgenes.

The authors do not limit themselves to the description of the system using the chloramphenicol acetyl transferase reporter gene (*cat*); they also use it for understanding carbon metabolism in transgenic plants by conditionally expressing cytosolic invertase.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Figure 1. "Refreshment in a cornfield," by Charles Thomas Burt (English, 1823–1902). Farmers may soon be finding refreshing agricultural uses for liquor other than slaking their thirst.

Constitutive expression of high levels of cytosolic invertase renders plants unable to mature because of strong chlorosis developing in the sink leaves, whereas plants conditionally expressing invertase grow normally until induced with ethanol. Only four days after induction, the phenotype of the youngest leaves was severely affected. The authors use this experiment to demonstrate that the ethanol-inducible promoter is suitable to resolve the time course of the noxious effects of high levels of invertase in mature plants.

When describing a regulated promoter, one of the most often asked questions concerns the expression window: How leaky is the promoter in the absence of the inducer, and how strong is it in the presence of the inducer? In this case, the promoter activity in the absence of the inducer amounts to 1% of the induced promoter activity, a value very similar to the background activity of the tetracycline-inducible promoter[5]. Whether this leakiness is due to the *cis*-elements of the target promoter or whether it is caused by residual binding activity of the *trans*-activator remains to be shown.

Preliminary results suggest that promoter strength in the induced state may be 50% of the CaMV *35S* promoter activity, but a more extensive analysis with more than one transgenic plant per construct will be required for a solid judgment. It seems, provided that these first results are substantiated in broader studies, that the expression window is in the same range as that of the available regulated expression systems.

The toxicity and ease of application of the inducer is another important issue. Though spraying of ethanol appears to work (conditions and data not shown in the paper), the favorite means of application seems to be feeding ethanol through the roots. To monitor the activity of the reporter *CAT*, plants were treated with 0.1% ethanol. One percent ethanol was used to induce invertase. Having cultured tobacco plants in 0.1% ethanol myself, I would be surprised that this concentration would not affect at least root growth.

A promoter system that works in the field is much needed. The system described by Caddick et al. could be extremely useful for breeders who desperately need an inducible male sterility system. It might also be useful for producing in plants high levels of recombinant proteins that interfere with growth and biomass production. In addition, an inducible promoter might be used for resistance management. Conditional expression of resistance genes reduces the evolution of adaptive mechanisms of the pathogen[6].

Whether spraying or drenching with ethanol is practical for agricultural purposes remains to be seen. However, considering that AlcR responds to other alcohols and ketones, I consider it feasible that inducers less volatile than ethanol might be found. The use of the ethanol regulon of *Aspergillus* in plants thus has tremendous potential to take plant gene expression to an intoxicating new high.

Christiane Gatz is professor at the Albrecht von Haller Institute for Plant Sciences, University of Gottingen, Untere Karspule 2, 37073 Gottingen, Germany (cgatz@gwdg.de).

1. Caddick, M.X. et al. 1998. *Nature Biotechnology* **16:**177–180.
2. Gatz, C. 1997. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48:**89–108.
3. Aoyama, T. 1997. *Plant J.* **11:**605–612.
4. Felenbok, B. 1991. *J. Biotechnol.* **17:**11–18.
5. Gatz, C., Frohberg, C., and Wendenburg, R. 1992. *Plant J.* **2:**397–404.
6. Fischhoff, D.A. 1992. *Science* **255:**903–904 .