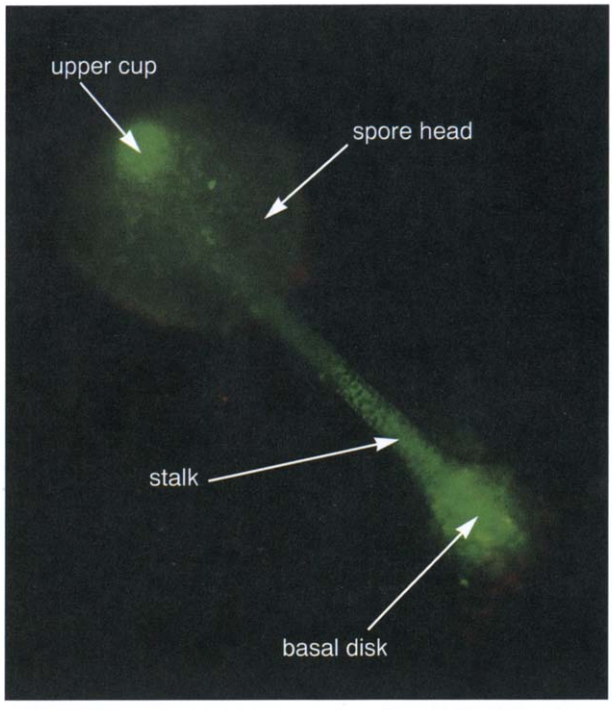

technical advances have optimized conditions for using GFP in both conventional and confocal fluorescence microscopy. GFP is an increasingly important and versatile tool for the *Dictyostelium* research community.

FIGURE 2. Cell-type-specific expression of green fluorescent protein (GFP). A mature fruiting body of AX-2 cells transformed with an *ecmB* promoter–*gfp* construct. The *ecmB* promoter is only activated in a subclass of prestalk cells, giving this distinctive pattern (viewed by epifluorescence microscopy, ×20).

References

1 Chalfie, M. *et al.* (1994) *Science* 263, 802–805
2 Abe, T. *et al.* (1994) *Cell* 77, 687–699
3 Traynor, D., Kessin, R.H. and Williams, J.G. (1992) *Proc. Natl Acad. Sci. USA* 89, 8303–8307
4 Steinberg, M.S. and Takeichi, M. (1994) *Proc. Natl Acad. Sci. USA* 91, 206–209

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GFP in plants

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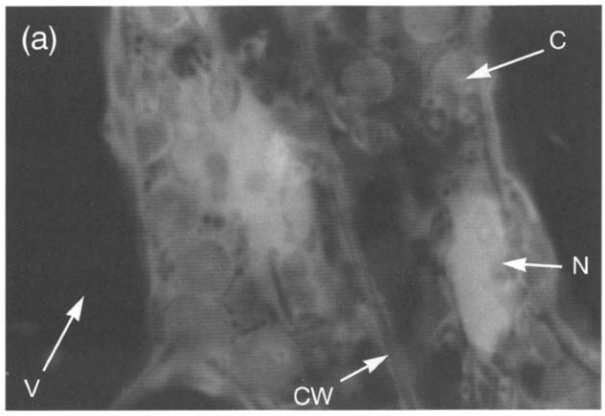
There are two major uses for green fluorescence protein (GFP) in plants: monitoring gene expression and protein localization at high resolution, and providing an easily scored genetic marker in living plants. GFP can be used as a replacement for β-glucuronidase, which is commonly used as a reporter for genetic fusions in plants. It allows direct imaging of the fluorescent gene product in living cells without the need for prolonged and lethal histochemical staining procedures[1]. In addition, GFP expression can be scored easily using a long-wave UV lamp if high levels of fluorescence intensity can be maintained in transformed plants. An assay for gene expression using fluorescence *in vivo* would be a very useful tool for plant transformation and breeding experiments.

To employ GFP successfully in plants, three major steps need to be taken: (1) The GFP apoprotein must be produced in suitable amounts in the plant cells. (2) The apoprotein must undergo efficient post-translational cyclization and oxidation to produce the mature GFP (Ref. 2). (3) The fluorescent protein may need to be suitably targeted within the cell, to allow efficient post-translational processing, safe accumulation to high levels, or to facilitate detection of expressing cells.

In our experiments in *Arabidopsis thaliana* (J. Haseloff, K. Siemering, D. Prasher and S. Hodge, unpublished), we have shown that expression of the *Aequorea victoria gfp* cDNA was curtailed by aberrant splicing, with an 84 nucleotide intron being excised efficiently from within the GFP coding sequence between nucleotides 400 and 483. A modified version of the *gfp* sequence has been constructed with altered codon usage, to mutate the cryptic splice sites and to decrease the AU content of the mRNA. We have used this modified *gfp* gene to generate transgenic lines of *Arabidopsis*, and shown that proper expression of the protein is restored and that the plants fluoresce green.

Neidz *et al.*[3] demonstrated recently the successful expression of the jellyfish *gfp* cDNA in *Citrus sinensis* protoplasts, so it is possible that the cryptic intron may not be recognized with equal efficiency in different plant species or perhaps during transient-expression experiments. However, it is probable that the modified *gfp* gene will be useful for expression studies in plants, which appear to have similar features involved in intron recognition[4]. It is also possible that aberrant RNA processing may interfere with GFP expression in other organisms.

GFP has also been expressed in plants using the potato virus X (Ref. 5) and the tobacco mosaic virus-based vectors (S.J. Casper and C.A. Holt, unpublished). The use of these cytoplasmic RNA viruses evades any problem with aberrant splicing and very high levels of GFP fluorescence have been seen in infected plants. It is clear that the GFP apoprotein can undergo maturation when produced in virus-infected, or transformed, plant cells and that the fluorescent form of the protein readily accumulates. In our experiments, transformed cells in *Arabidopsis* are often so intensely fluorescent, that they are easily detected by eye using a long-wave UV lamp. However, it proved difficult to regenerate fertile plants efficiently from the brightest transformants: the cells remained as highly fluorescent calli or masses of shoots after several months of culture. It is possible that high levels of GFP expression are mildly toxic or interfere with regeneration, perhaps because of the fluorescent or catalytic properties of the protein. In the natural situation, in jellyfish photocytes, where high levels of GFP are tolerated, the protein is found sequestered in microbody-like lumisomes. In contrast, the mature protein is found throughout the



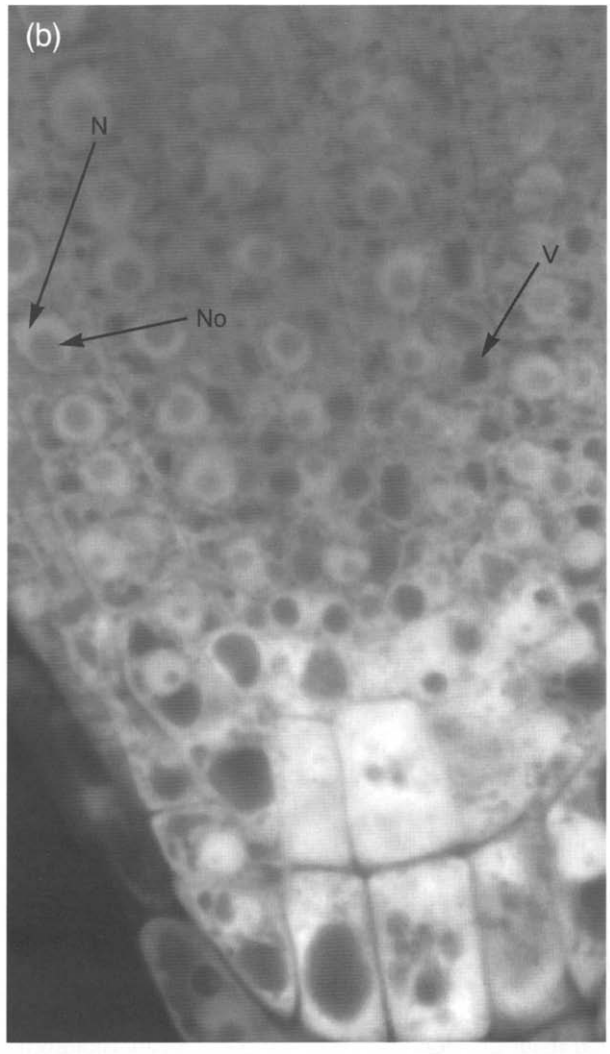


FIGURE 1. (a) Transgenic *Arabidopsis thaliana* C24 seedlings were produced by *Agrobacterium*-mediated root transformation and contain an altered form of the *Aequoria victoria* green fluorescent protein (GFP) cDNA with modifed codon usage, driven by the cauliflower mosaic virus 35S promoter. 5 d old seedlings were mounted in water and examined using a BioRad MRC-600 laser-scanning confocal microscope. A dual channel image of cotyledon mesophyll cells is shown. Standard FITC/TRITC filter sets were used. GFP fluorescence is shown in the green channel and chlorophyll derived autofluorescence is shown in the red channel. GFP is found thoughout the cytoplasm and predominates in the nucleoplasm, but is excluded from the nucleolus (see also Fig. 1b). GFP is excluded from vacuoles, components of the endomembrane system and small organelles, which appear shadowed within the cells. (b) Using the same procedure, a root tip from a living *Arabidopsis* seedling was optically sectioned to reveal structure within the meristem. Files of cells within the root tip can be clearly distinguished and subcellular detail corresponding to the distribution of GFP can be seen. GFP is found throughout the cytoplasm and at higher levels in the nucleoplasm, but appears excluded from nucleoli. Abbreviations: C, chloroplast; N, nucleus; No, nucleolus; CW, cell wall; V, vacuole.

cytoplasm and nucleoplasm in transformed *Arabidopsis* (Fig. 1). If GFP proves to be a source of fluorescence-related free radicals, for example, it might be advisable to target the protein to a more localized compartment within the plant cell.

The use of GFP to label plant cells is causing a revolution in the observation and analysis of plant ultrastructure. Living-plant-tissue whole mounts can be sectioned optically using a laser-scanning confocal microscope to allow the analysis of cellular and sub-cellular detail, despite autofluorescence, and the refractile nature and light scattering properties of plant cell walls (Fig. 1b). This avoids the need for sectioning or staining of the tissues and allows potentially the precise monitoring of dynamic events in living plants.

The use of GFP in this manner imposes greater demands on the techniques of microscopy. For example, the collection of the image shown in Fig. 1b required the use of a new type of water immersion objective (Nikon 60×, N.A. 1.2 Planapochromat) designed to minimize the effects of spherical aberration, which invariably degrades the microscope image when an oil-immersion objective is focused deep into an aqueous or cytoplasmic medium. Although when used in conjunction with such an objective and blue laser light at 488 nm, confocal microscopy clarifies the image from such thick specimens, the problems of photobleaching and phototoxicity remain.

Recently, we have been experimenting with 2-photon scanning microscopy[6] using a Coherent Innova/Mira mode-locked titanium-sapphire laser system. The method relies on the coincident absorption of two photons of red or infrared light by the fluorophore. Not only is this radiation more penetrating than the short-wave light normally used, but, because of the non-linear relationship between intensity and 2-photon absorption, the bleaching and cytotoxic effects can be restricted to the extremely thin plane of focus. We hope to extend our imaging of GFP in plant material to prolonged time-lapse studies by the use of this method.

References

1 Chalfie, M. *et al.* (1994) *Science* 263, 802–805
2 Heim, R., Prasher, D.C. and Tsien, R.Y. (1994) *Proc. Natl Acad. Sci. USA* 91, 12501–12504
3 Niedz, R.P., Sussman, M.R. and Satterlee, J.S. (1995) *Plant Cell Reports* 14, 403–406
4 Goodall, G.J. and Filipowitz, W. (1991) *EMBO J.* 10, 2635–2644
5 Baulcombe, D., Chapman, S. and Santa Cruz, S. (1995) *Plant J.* 7, 1045–1053
6 Denk, W., Strickler, J.H. and Webb, W.W. (1990) *Science* 248, 73–76

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