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They should be addressed to:
Correspondence
Nature Biotechnology
345 Park Avenue South
New York, NY 10010-1707, USA
or sent by e-mail to biotech@natureny.com
Please include your telephone and fax numbers.

GFP movement between chloroplasts

To the editor:

In your September issue (*Nat. Biotechnol.* **17**, 906), we described the presence of green fluorescent protein (GFP) in multiple chloroplasts in a single cell following injection of a *gfp* gene construct into a single chloroplast[1], and suggested that GFP was able to move through stromules (interconnecting stroma tubules)[2] from the injected chloroplast to adjacent chloroplasts. In an accompanying Research News and Views article, Henry Daniell[3] drew attention to the lack of evidence for stromules containing GFP in transplastomic tobacco and rice plants expressing a *gfp-aadA* gene-fusion following microprojectile bombardment[4] and observed that stromules had not yet been experimentally demonstrated in transplastomic plants expressing *gfp* stably integrated within the chloroplast genome. He also raised the question whether movement of proteins through stromules occurred naturally in plants.

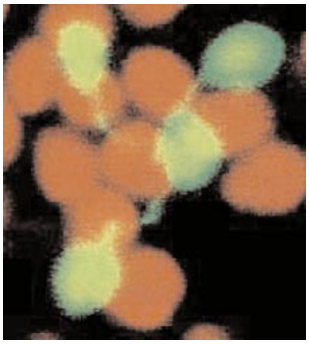

Figure 1. GFP in stromules interconnecting chloroplasts in a tobacco leaf epidermal cell. The *gfp* gene, under the control of the tobacco chloroplast *rrn* promoter and the *psbA* 3′ region, was inserted in the *rps12/trnV* intergenic region in the plasmid pNtcZ7 (ref. 5) and introduced into tobacco leaf protoplasts in the presence of polyethylene glycol[6]. Transplastomic plants were selected and regenerated on spectinomycin (500 mg/L)[6], and GFP observed with a Leica confocal scanning microscope[5]. All chloroplasts containing GFP are located in a single epidermal cell; red-fluorescing organelles are chloroplasts in an underlying mesophyll cell.

We now report that stromules containing GFP can be observed in transplastomic tobacco plants produced by polyethylene glycol-mediated introduction of pNtcZ7 (ref. 5) into tobacco leaf protoplasts and selection on spectinomycin[6]. Figure 1 shows GFP fluorescence in stromules interconnecting chloroplasts in an epidermal cell of a chimeric leaf sector (the chloroplasts in the underlying mesophyll cell are not expressing GFP). These stromules are easily visible by confocal microscopy and are dynamic structures showing rapid extension and retraction, as reported previously[2,7]. The diameter of the stromules (0.35–0.85 μm) is similar to that reported previously[2,7] and is large enough for the passage of large macromolecules, including ribosomes. Further work is needed to determine under what conditions macromolecules are able to pass between plastids and if there are exclusion limits on molecular trafficking through stromules.

John C. Gray
Julian M. Hibberd
Philip J. Linley
University of Cambridge, Cambridge, UK
jcg2@mole.bio.cam.ac.uk
Bert Uijtewaal
Nunhems Zaden BV, 6080 AA Haelen, The Netherlands

1. Knoblauch, M. et al. *Nat. Biotechnol*. **17**, 906–909 (1999).
2. Köhler, R.H. et al. *Science* **276**, 2039–2042 (1997).
3. Daniell, H. *Nat. Biotechnol.* **17**, 855–856 (1999).
4. Khan, M.S. & Maliga, P. *Nat. Biotechnol*. **17**, 910–915 (1999).
5. Hibberd, J.M. et al. *Plant J*. **16**, 627–632 (1998).
6. ONeill et al. *Plant J*. **3**, 729–738 (1993).
7. Tirlapur, U.K. et al. *Eur. J. Cell Biol*. **78**, 233–240 (1999).

Creativity and peer review

To the editor:

An unbiased ombudsman was suggested for the NIH study sections to thwart economic, biased rejections of innovative research grants that threaten panelists' self-interests[1]. In addition, the editorial emphasized that the present peer review system was hardly designed to encourage maximally innovative ideas. Bruce Alberts, president of the National Academy of Sciences, criticized the NIH peer review system as suffering from "conservatism, risk aversion, and nit picking."[2]

A far more common detrimental behavior to innovations is from study section panelists who exceed the limits of their expertises in their evaluations of research proposals in NIH grant applications. This is difficult to document by those who have had rejected grant proposals; however, a recent rejection of a Nebraska cancer grant application (NE LB 506) by national panelists of an NIH-styled review process clearly illustrates that the reviewers of this grant application had limited or no scientific expertise in the grant's discipline of photobiology. They rejected this grant because of the standard FDA regulatory methodology used in testing sunscreens. In addition, they also suggested changes in the FDA's sunscreen methodology that were pathophysiologically unsound. The FDA's approved method for sunscreen testing was developed by photobiologists in order to assure the safety and efficacy of evaluating and comparing sunscreens.

In the rejection of an NIH research proposal, the role of an ombudsman requires investigation and judgmental resolution of the conflict. This requires scientific expertise of the polar views. In the present system, the ombudsman has only the negative expertise of the section panelists. In order to validate the grant's scientific concept, the applicant could supply in his grant application at least one expert reviewer's appraisal. If the grant is rejected, the ombudsman can arbitrate a second review of the application after receiving a rebuttal from the applicant. Thus, the ombudsman would have available the polar scientific expertise in order to help the panelists with a decision regarding the funding of the grant proposal. Creativity would be fostered by this altered review process in that the panelists of the study section are held accountable and must defend their initial decision or alter it to the satisfaction of the ombudsman.

Ramon M. Fusaro
Departments of Preventive Medicine & Public Health and Internal Medicine
Creighton University and Univ. of Nebraska Medical Center
Omaha, NE 68198-4360
rmfusaro@Creighton.edu

1. Editorial. *Nat. Biotechnol.* **16**, 395 (1998).
2. Greenberg, D. *Lancet* **354**, 577 (1999).

Erratum

The editors regret editing errors in the October Correspondence, "Anti-Gal antibodies—where's the beef?" (*Nat. Biotechnol.* **17**, 938, 1999). In paragraph 3, regarding the putative anti-Gal reaction in vivo, citations were omitted from the last sentence: ". . .should be routinely detectable as complexes in vivo[8,9] at baseline. . .". In paragraph 4, an incorrect antibody drug name was listed. The third sentence should begin: "Similarly for Synagis[12] and ABX-IL8 (ref. 13). . ." Finally, the affiliation wrongly listed Harvard Medical School in Harvard, MA—it should be Boston, MA—and Dr. Junghans' email address is correctly: *junghans@hms.harvard.edu*.