

port). Presumably, actin filaments and motor proteins are involved in this previously uncharacterized retrograde transport, but the possibility that receptors glide on the surface of filopodia while escaping endocytosis remains open.

Along with demonstrating signaling competence and steric compatibility of QDs conjugated to a natural ligand, Lidke *et al.* directly prove that erbB2, but not erbB3, can modulate the endocytic fate of activated receptors. This finding is relevant to attempts aimed at pharmacological blocking of erbB-expressing cancers using either monoclonal antibodies (e.g., Genentech's (S. San Francisco, CA, USA) Herceptin/trastuzumab), or low molecular weight kinase inhibitors (e.g., Astra Zeneca's (London) Iressa/gefitinib).

The report by Dahan *et al.* in *Science* demonstrates another advantage offered by QDs. Their size—smaller than latex beads, but larger than organic dye molecules—allows penetration into dense structures like neuronal synapses, whereas their photostability enables receptor tracking for long durations. Thus, using QDs conjugated to antibodies specific to the glycine receptor, a major inhibitory neurotransmitter receptor in the spinal cord, these authors were able to track the motion of individual receptors for more than 20 minutes, well beyond the few second limit of fluorescent labels.

One property of QDs that limits their use in quantitative analyses is random intermittence of their fluorescence emission (blinking). However, this property enabled Dahan *et al.* to detect and track individual QDs, and they report a signal-to-noise ratio significantly better than that obtained with fluorophores. In addition to the almost immobile population of synaptic receptors and the highly mobile extrasynaptic receptors (diffusion coefficient of $\sim 0.1 \mu\text{m}^2/\text{s}$), single QD tracking defines a third category of transitory receptors at the synapse periphery. Random and directional exchanges of glycine receptors among the three membrane sub-domains, as well as receptor endocytosis and exocytosis, are thought to underlie synaptic plasticity, a process crucial for neural functions⁸.

Although it is evident that QDs have come of age, with a constantly increasing number of applications, it is also clear that several issues still need to be addressed. Cytotoxicity is still an impediment. Because of their polyvalent surfaces, QDs induce cross-linking of surface proteins, which may interfere with biological processes. In addition, blinking and color changes currently limit analytical applications of QDs. The successful conjugation of EGF to QDs will likely be followed by tagging

QDs with other biological molecules, as well as drugs, which will require a wider range of QD cores and coatings. Nevertheless, the combination of QDs with multiphoton microscopy, fluorescence resonance energy transfer, high-throughput methodologies and optoelectronic devices will likely affect the fields of live cell imaging, as well as the imaging of animal behavior, disease diagnosis and drug delivery. More specifically, many spectrally narrow QD colors can be created to allow simultaneous analysis of multiple receptors. Thus, receptor dynamics will better interface with molecular biology, and both

will facilitate a systems biology approach to signal transduction studies.

1. Lidke, D.S. *et al.* *Nat. Biotechnol.* **22**, 198–203 (2004).
2. Dahan, M. *et al.* *Science* **302**, 442–445 (2003).
3. Bruchez, M. Jr. *et al.* *Science* **281**, 2013–2016 (1998).
4. Chan, W.C. & Nie, S. *Science* **281**, 2016–2018 (1998).
5. Watson, A., Wu, X. & Bruchez, M. *Biotechniques* **34**, 296–300, 302–293 (2003).
6. Akerman, M.E. *et al.* *Proc. Natl. Acad. Sci. USA* **99**, 12617–12621 (2002).
7. Yarden, Y. & Sliwkowski, M.X. *Nat. Rev. Mol. Cell Biol.* **2**, 127–137 (2001).
8. Gomes, A.R. *et al.* *Neurochem. Res.* **28**, 1459–1473 (2003).

The fate of transgenes in the human gut

John Heritage

Gut microbes that cannot be recovered in artificial culture may acquire and harbor genes from genetically modified plants.

Can transgenic DNA in a genetically modified (GM) crop be transferred to the people or animals that eat the crop or to their intestinal microflora (Fig. 1)? In this issue, Netherwood *et al.*¹ report that microbes found in the small bowel of people with ileostomies (that is, resection of the terminal ileum and diversion of digesta to a colostomy bag) are capable of acquiring and harboring DNA sequences from GM plants. This finding raises important questions for those charged with risk assessment of transgenic plants destined for food use.

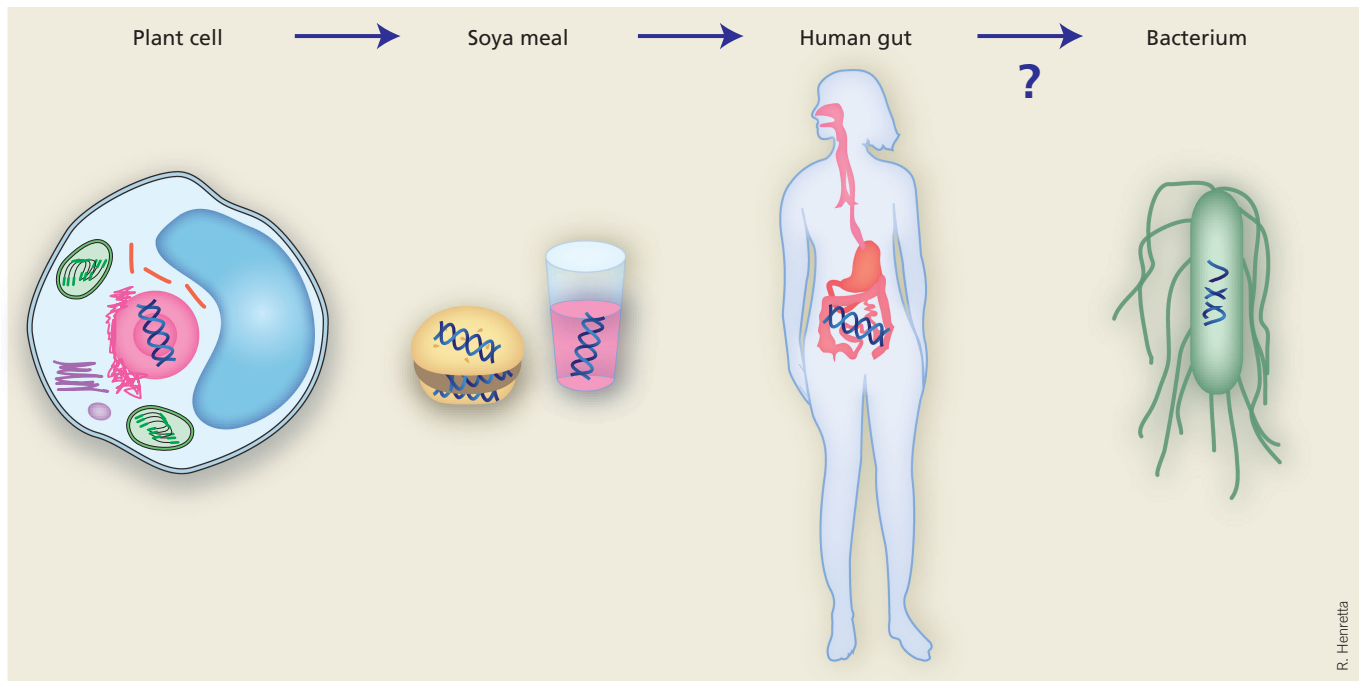
Recently, much attention has been focused on the potential of transgenes to escape from GM plants to wild relatives^{2–4}. But relatively little work has been devoted to the possibility of transgene transfer to the animals consuming the plant or to their commensal microflora. Even less work has been carried out on the fate of plant DNA when crops are eaten by humans. It has been argued that transgenes are the same as any other DNA that we consume and that if gene transfer from plants to humans or animals were commonplace, we should all be green by now. Intriguingly, it is chloroplast DNA that is found in the lympho-

cytes of animals eating both GM and conventionally bred plant material⁵, and there is evidence that bacteria in the oral flora remain competent for genetic transformation when suspended in saliva⁶.

In its 2003 GM Science Review, the UK government concluded that trans-kingdom transfer of DNA from GM plants to bacteria is “unlikely to occur because of a series of well-established barriers”⁷ and illustrated support for this position from experimental evidence in peer-reviewed literature. The review also concluded that transgenic DNA is no different from other DNA consumed as part of the diet and that it will have a similar fate. Again, these conclusions were based on a review of the published scientific evidence, which indicates that ingested DNA is subject to degradation but that the degradative process is not necessarily complete. Much of this work has been done using animal studies, and very little is known about the process in humans.

Netherwood *et al.* have taken a significant step to address the lack of human studies. They studied 12 healthy volunteers and seven ileostomists given a meal of GM soya that contained the 5-enol-pyruvyl shikimate-3-phosphate synthase (*epsps*) transgene, which encodes resistance to the herbicide glyphosate. In the healthy subjects, DNA from the transgenic plant material was degraded completely after passage through the colon.

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Figure 1 A possible route for transfer of DNA from plant cells in the human diet to bacteria. Some DNA in food is degraded during cooking and processing, but the remainder is ingested intact. Consumed DNA is largely hydrolyzed during digestion. Netherwood *et al.* provide evidence that intact transgenic DNA can be recovered in the human ileum and taken up by bacteria in this environment.

Interestingly, however, transgenic DNA sequences were recovered by PCR from the digesta of all seven ileostomists. In six of these subjects, a PCR product spanning the entire gene was detected. In one case, it was estimated that nearly 4% of the transgenic DNA in the test meal was recovered from the ileostomy fluid. Thus, quite large fragments of DNA can survive passage through the stomach—if shown only for people with a gastrointestinal pathology.

Ileostomists clearly may not be representative of the general population as they have suffered a disease requiring radical surgery. Nevertheless, they have been validated previously as a faithful model for studying other aspects of digestion. Further research will be needed to determine the relevance of the new results for the population at large.

Perhaps a more significant and puzzling finding of this work is evidence that transgenic DNA had crossed kingdom barriers from a GM plant source to intestinal microflora, even before the subjects' participation in the study. The transgenic sequences were not detected in the original samples of ileostomy fluid taken before consumption of the GM soya meal, but they could be detected at very low copy number once bacteria in these same samples were enriched through serial passage in Luria broth.

Great care was taken to avoid sample contamination; although contamination cannot be ruled out, the pattern of results makes this explanation unlikely. The *epsps* gene used in GM soya has a bacterial origin, but the sequence was modified to optimize expression in plants. Sequence analysis of the PCR products revealed that the target amplified from cultivated digesta was identical to the plant *epsps* gene rather than to its bacterial counterpart.

Microbiological experiments, including differential cultivation, provided good evidence that in all probability the bacterium harboring the transgenic *epsps* sequence is indifferent to the presence of oxygen in the culture medium and is dependent for its nutrition upon another organism present in the sample and subsequent liquid subcultures. Is it likely that a bacterium that cannot be isolated in pure culture is capable of acquiring transgenic DNA and harboring it through several passages in liquid culture, albeit at very low frequency? It should be remembered that conventional culture techniques cannot recover more than a tiny minority of the microbes in the gastrointestinal tract. We are only just beginning to learn how to characterize such organisms.

Netherwood *et al.* could have done more to characterize the putative trans-kingdom transfer. They do not report attempts to see

whether the transgenic DNA present in cultured digesta, and presumably found in the bacterium that will not grow in pure culture on a solid medium, was being expressed—although no full-length genes were amplified from these samples. This could have been accomplished with relative ease using RT-PCR.

There is also no description of the sequences flanking the *epsps* gene, so it is impossible to determine the context in which the PCR target is found in these cultures. Nevertheless, on balance, the data presented in the paper support the conclusion that gene flow from transgenic plants to the gut microflora does occur. Furthermore, because transfer events seem to have occurred in three of the seven subjects examined, it may be that trans-kingdom gene transfers are not as rare as suggested by the UK GM Science Review Panel⁷. This observation is significant, and it is imperative that the transfer events be characterized more fully, particularly with a view to understanding the stability in cultivated ileal digesta of plant transgenes and native genes, the context in which these genes are found and their ability to be expressed.

Should the findings of Netherwood *et al.* influence risk assessment of GM crops? I believe that the authors strike exactly the right position here. They propose that the gene transfer events from transgenic plants to gut

microflora for which they provide evidence are highly unlikely to alter gastrointestinal function or endanger human health. I would conclude, however, that whereas this may be true for the construct examined by Gilbert's group, it may not be true in other cases, such as genes that encode resistance to antibiotics used in human medicine.

Netherwood *et al.* call on risk assessors to consider the possibility of trans-kingdom gene flow in the future safety assessment of GM foods¹. I endorse that conclusion and would extend it by adding that every case must be considered on its own merits.

Label-free detection becomes crystal clear

Paul S Cremer

Liquid crystalline platforms offer fast, simple and inexpensive detection of incoming analytes on two-dimensional fluid biomembrane mimics.

The binding of ligands to cell membrane-bound receptors plays a critical role in a wide range of fields from cell-cell signaling and lymphocyte trafficking to the immune and inflammatory responses^{1–2}. For example, the initial step in pathogen attack on a human host usually involves the binding and rearrangement of cell surface moieties by the incoming virus or protein toxin. Moreover, it is generally estimated that half of prescription drugs target membrane-bound receptors³. Only a handful of widely applied techniques are available for monitoring ligand-receptor binding. Most of them either require a label to be attached to the incoming analyte or involve complex optical and surface patterning methods for readout. This has motivated the search for label-free assays to rapidly detect protein-membrane interactions without requiring special optics, temperature control or thin film metal surfaces. As a step toward this goal, Brake *et al.*⁴ report in *Science* the use of a thermotropic liquid crystal, 4'-pentyl-4-cyanobiphenyl, as a responsive support for phospholipid monolayers.

1. Netherwood T, *et al.* *Nat. Biotechnol.* **22**, 204–209 (2004).
2. Wilkinson, M.J. *et al.* *Science* **302**, 457–459 (2003).
3. Anon. *Phil. Trans. R. Soc. Lond B* **358**, 1775–1913 (2003).
4. Andow, D.A. *Nat. Biotechnol.* **21**, 1453–1454 (2003).
5. Einspanier, R. *et al.* *Eur. Food Res. Technol.* **212**, 129–134 (2001).
6. Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A. & Flint, H.J. *Appl. Environ. Microbiol.* **65**, 6–10 (1999).
7. UK GM Science Review Panel. *GM Science Review. First Report. An Open Review of the Science Relevant to GM Crops and Food Based on Interests and Concerns of the Public* (UK Government, London, 2003). Available online (<http://www.gmsciencedebate.org.uk/report/pdf/gmsci-report1-full.pdf>). Last accessed 22 December 2003.

as continuous monitors of biological warfare agents. The advantage of these systems lies in their maintaining many of the properties of natural cellular membranes without the complications arising from using whole cell assays. One of the most important properties that phospholipid bilayers and monolayers share with whole cells is their two-dimensional fluidity. Individual lipid molecules are free to diffuse around the surface and membrane-bound ligands can reorganize in the presence of a protein, viral or bacterial analyte that contains multiple binding sites. Indeed, such multivalent ligand-receptor binding is a dominant motif of protein-cell membrane interactions^{6–8}.

Brake *et al.* have combined such phospholipid monolayers with a thermotropic liquid crystal to create a device that can rapidly detect incoming analytes. Their liquid crystal belongs to the same class of molecules that are found in digital watches and liquid crystal displays. The liquid crystal sensor device directly transmits information on protein binding from the thin phospholipid film to the outside world. The method works by reorienting the liquid crystal molecules beneath the portion of the monolayer at which proteins bind (see Figure 1).

In their report, the authors demonstrate that assays based on liquid crystals can easily be used to detect ligand-receptor interactions. For example, the binding of phospholipase A₂ (PLA₂) to phospholipid membranes was fol-

Phospholipid monolayers and bilayers are becoming ubiquitous for modeling the complex biophysical mechanisms of protein-cell membrane interactions⁵. They have significant potential for use as sensor devices to detect pathogens in clinical settings or even

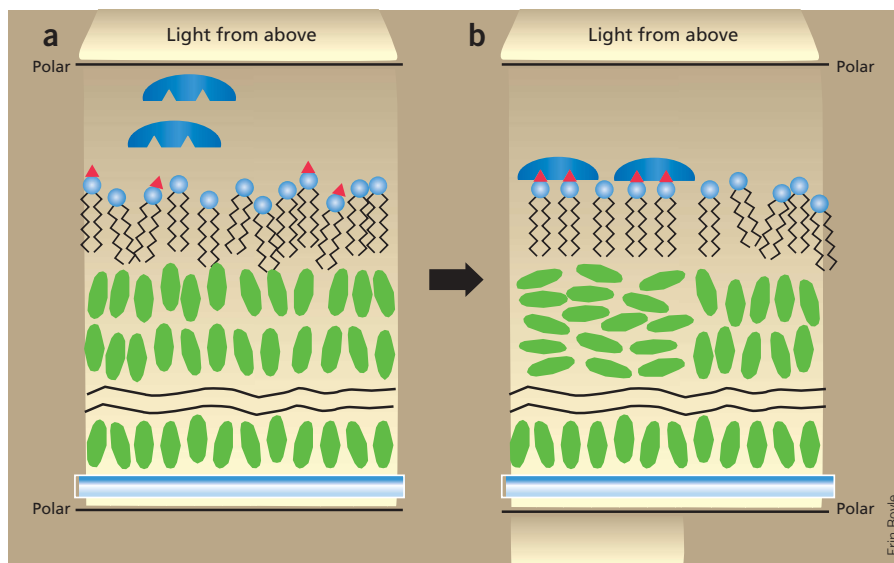


Figure 1 Molecular interactions at a phospholipid coated surface on a liquid crystal. (a) Proteins approach a surface containing a thermotropic liquid crystal reservoir coated with a lipid monolayer. (b) The membrane contains specific ligands (shown in red) that specifically bind to the incoming protein (blue). This causes the liquid crystal molecules beneath the bound proteins to realign. Light sent through crossed polars passes through the region where the realignment took place, whereas the areas containing the vertically aligned species remain dark.

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