David Schubert replies:

It is indeed a fortunate facet of science that the quantitative aspect of authorship does not dictate the validity of the conclusions! The impetus for my commentary was my repeated laboratory observation that the slightest genetic modification of a cell leads to completely unpredicted phenotypes. A careful reading of the plant literature supported my conclusions from animal cells. The commentary intended to raise this concern in the context of generating further impetus for labeling and stringent testing of GM food. I had no *a priori* commitment to a particular technology, no pre-existing political ideology, and I did not deliberately ignore any pertinent published material. I do believe, however, that both replies suffer from these problems. They make claims about technology and safety-testing requirements that do not exist, use my statements out of context, and are rather adroit at the use of tenses to skirt questions regarding testing.

In the United States, the Food and Drug Administration has no mandatory safety approval regulation for GM foods and no specific testing requirements.

The issue of traditional breeding versus genetic modification has been extensively discussed. I have, however, always wondered how it can be claimed that they are the same when genes from completely different species (sometimes kingdoms!) are expressed in genetically modified (GM) food products. Although the biological world works by variation and selection, this is generally accomplished in the context of a normal complement of endogenous genes that, though perhaps different, are allelic. This is quite distinct from GM plants where many copies of a gene are introduced and integrate randomly[1]. It is my experience and that of others that the response to (trans)genes is completely unpredictable, not specific and predictable as argued. Genetic modification is also distinct from breeding two strains that have been safely consumed for extended periods of time. Parrot and colleagues cite multiple gene deletion in varieties of maize to illustrate their argument that "unintentional consequences" are much more likely to occur in breeding than in biotechnology. However, maize is unique because its genome comprises 80% retrotransposons. It has evolved to deal with redundancy; most other species have not. The fact that plants are capable of producing toxins is a very good argument for testing.

I stated that the illness caused by Shawa Denko KK (Tokyo, Japan) GM tryptophan "was highly correlated with contaminants," not that it definitively caused the disease. I used this as an argument for labeling GM material. There is certainly no good evidence in reviewed journals that it was the purification procedure that caused the problems or that only people who used this brand took larger amounts and therefore became ill. The company has destroyed the bacteria and has paid large out of court settlements.

The most important issue to me is rigorous safety testing. The statements made in the replies that address this issue are completely misleading. Although it is true that in Europe laws are being formulated that require stringent testing of GM foods, in the United States, the FDA has no mandatory safety approval regulation for GM foods and no specific testing requirements[2]. There are no all-inclusive mandatory food-safety testing requirements in the United States. The cited testing protocols are only suggestions for producers. There is, however, an effort by a consumer advocacy group, the Center for Science in the Public Interest[3], to require GM food products to obtain FDA safety approval. With respect to testing technology, Parrot and colleagues claim that "the protein produced in the new host is subjected to extensive biochemical characterization to confirm that the protein produced is the one and only one intended." However, there is no technique that can assay all cellular proteins. The best to date is 2,528 out of the rice genome of 50,000 genes (a mere 5%)[4]!

I am very pleased that both letters support rigorous testing of GM food and hopefully all involved will back efforts to hasten mandatory rules through the FDA.

1. Svitashev, K. & Somers, D.A. *Genome* **44**, 691–697 (2002).
2. http://www.cspinet.org/biotech/pbn_rule.html
3. http://www.cspinet.org
4. Koller, A. *et al. Proc. Natl. Acad. Sci. USA* **99**, 11969–11974 (2002).

Expectations and reality in gene repair

To the editor:

Gene repair by synthetic oligonucleotides, chimeric RNA/DNA oligonucleotides (RDO or chimeraplast), or single-stranded oligodeoxynucleotides produces targeted alterations in the genome of mammalian cells. Although repair frequencies as high as 40% have been reported in liver[1], the reproducibility of gene repair by RDO has been questioned because many researchers have failed to achieve a significant level of gene alteration. The expectation was that oligonucleotide-directed gene repair would be quick and easy to use in comparison to other gene-targeting strategies and would produce a high frequency of gene alteration without selection. The reality, however, is very different.

Targeted alteration of genomic DNA in mammalian cells by RDO or oligodeoxynucleotide occurs at a low frequency that is detectable only by highly sensitive assays[2–6]. Besides the quality and delivery of oligonucleotides to cells, one needs to consider many other factors to achieve a successful gene repair. Each cell type has different repair activity and needs to be tested for such activity before extensive gene-targeting experiments are undertaken[4]. The biological activities of cells (including DNA recombination and repair[4–7]) and the replication and transcription status[6] of the targeted gene also influence the process. Given the low frequency of oligonucleotide-directed gene alteration, a selection procedure is urgently required to make the gene-repair technology practical.

Work in our laboratory has focused on two aspects of research: development of reproducible assays to score the frequencies of gene repair in mammalian cells, and mechanistic studies to improve the design of oligonucleotides and identify the rate-limiting step in gene repair[2–8]. Our group has established several assay systems in which phenotypic changes can be detected upon gene correction. These include histochemical staining of cells containing a corrected alkaline phosphatase gene[8], pigmentation of cells containing a corrected mutant tyrosinase gene[2], and X-gal staining of cells containing a corrected mutant *lacZ* gene.

The mutant *lacZ* system is particularly useful because it provides an easily detectable and measurable marker for gene correction in studies using nuclear extracts, episomes, and chromosomes of mammalian cells[4–7], including mouse embryonic stem cells[9]. Using the mutant *lacZ* vector, my colleagues and I have developed an *in vitro* reaction that can measure gene-repair activity in a given cell type. This *in vitro* reaction by nuclear extracts enables the feasibility of gene targeting to be predicted in a given cell type because a good correlation exists between *in vitro* gene-repair activity and chromosomal gene-repair activity[4]. Such testing may avoid the frustrations encountered by