REVIEW OF MODERN PLANT BIOTECHNOLOGY

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Abstract

The imminent prospect of the first approval of a plant-made pharmaceutical (PMP) for human use could herald a new era for applied plant science, after a decade of public backlash against genetically modified crops, particularly in Europe. Yet, the general resistance to genetically modified organisms might have done plant biotechnology a favour in the long run, by forcing it to adopt more-rigorous procedures for efficacy and safety in line with the pharmaceutical industry. This could, in turn, lead to renewed vigour for plant science, with the promise of developing not only food crops that deliver benefits to consumers and producers, but also a wide range of new pharmaceuticals.

This is certainly the view of David Aviezer, CEO of Protalix, an Israeli company that has developed what could become the first recombinant therapeutic protein from plants to treat Gaucher disease. The protein is called taliglucerase alpha; it is a recombinant human form of the enzyme glucocerebrosidase that is produced in genetically engineered carrot cells. This enzyme has a crucial role in the breakdown of glycolipids in the cell membrane and is either used to provide energy or for cellular recognition. Deficiency of this enzyme causes accumulation of lipids with a variety of effects including premature death.

“My feeling is that there is a dramatic change in this area with a shift away from the direction where a decade ago biotech companies like Monsanto and Dow went with growing transgenic plants in an open field, and instead moving this process into a more regulatory well-defined process inside a clean room,” Aviezer said. “Now the process is taking place in confined conditions and is very highly regulated as in the pharmaceutical industry.”resistance to genetically modified organisms

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He argues that this is ushering in a new era for plant biotechnology that could lead to greater public acceptance, although he denies that the move to clean-room development has been driven purely by the environmental backlash against genetically modified organisms in the late 1990s and early 2000s. “That was one aspect, but I think the move has been coming more from an appreciation that biopharmaceuticals require a more regulatory defined setting than is achieved at the moment with transgenic plants.”

Interest in deriving pharmaceuticals from plants, known colloquially as ‘pharming', first took off in the 1990s after researchers showed that monoclonal antibodies could be made in tobacco plants (Hiatt et al, 1989). This led to genetic engineering of plants to produce vaccines, antibodies and proteins for therapeutics, but none gained regulatory approval, mostly because of safety concerns. Moreover, the plants were grown in open fields, therefore attracting the same criticisms as transgenic food crops. In fact, a recent study showed that the views of the public on pharming depended on the product and the means to produce it; the researchers found increasing acceptance if the plants were used to produce therapeutics against severe diseases and grown in containment (Pardo *et al*, 2009).

However, it was the technical challenges involved in purification and the associated regulatory issues that really delayed the PMP field, according to George Lomonossoff, project leader in biological chemistry at the John Innes Centre for plant research in Norwich in the UK, part of the Biotechnology and Biological Sciences Research Council (BBSRC). “Extraction from plants required the development of systems which are not clogged by the large amounts of fibrous material, mainly cellulose, and the development of GMP [good manufacturing practice; quality and testing guidelines for pharmaceutical manufacture] compliant methods of purification which are distinct from those required from, say, mammalian cells,” said Lomonossoff. “All this is very time consuming.”

“Secondly there was no regulatory framework in place to assess the risks associated with proteins produced in plants, and determining how equivalent they are to mammalian-cell-produced material and what kind of contaminants you might have to guard against,” Lomonossoff added. “Again, attempting to address all possible concerns is a lengthy and expensive process.” Yet recent work by Protalix and a few other companies, such as Dow Agrosciences, has given grounds for optimism that purification and GMP-compliant methods of production have finally been established, Lomonossoff added.

The first important breakthrough for PMPs came in 2006, when Dow Agrosciences gained regulatory approval from the US Department of Agriculture for a vaccine against Newcastle disease, a contagious bird infection caused by paramyxovirus PMV-1. “Though the vaccine, produced in tobacco-suspension culture cells, was never deployed commercially, it showed that regulatory approval for a plant-made pharmaceutical can be obtained, albeit for veterinary use in this case,” Lomonossoff said.

As approval is imminent for taliglucerase alpha for human use, it is natural to ask why plants, as opposed to micro-organisms and animals, are worth the effort as sources of vaccines, antibiotics or hormones. There are three reasons: first, plants can manufacture some existing drugs more cheaply; second, they can do it more quickly; and third, and perhaps most significantly, they will be able to manufacture more complex proteins that cannot be produced with sufficient yield in any other way.

An important example in the first category is insulin, which is being manufactured in increasing quantities to treat type 1 diabetes and some cases of type 2 diabetes. Until the arrival of recombinant DNA technology, replacement insulin was derived from the pancreases of animals in abattoirs, mostly cattle and pigs, but it is now more often produced from transgenic *Escherichia coli*, or sometimes yeast. Recently, there has been growing interest in using plants rather than bacteria as sources of insulin (Davidson, 2004; Molony et al, 2005).

SemBioSys, a plant biotechnology company based in Calgary, Canada, is now developing systems to produce insulin and other therapeutic proteins in the seeds of safflower, an oilseed crop (Boothe et al, 2009).

"We have developed technology that combines the high-capacity, low-cost production of therapeutic proteins in seeds with a novel technology that simplifies downstream purification," said Joseph Boothe, vice president of research and development at SemBioSys. "The target proteins are engineered to associate with small, oil-containing structures within the seed known as oilbodies," Boothe explained. "When extracted from the seed these oilbodies and associated proteins can be separated from other components by simple centrifugation. As a result, much of the heavy lifting around the initial purification is accomplished without chromatography, providing for substantial cost savings."

The second potential advantage of PMPs is their speed to market, which could prove most significant for the production of vaccines, either against emerging diseases or seasonal influenza, for which immunological changes in the virus mean that newly formulated vaccines are required each year. "In terms of a vaccine, I think influenza is very promising particularly as speed is of the essence in combating new strains," Lomonossoff said. "Using transient expression methods, you can go from sequence to expressed protein in two weeks." Transient gene expression involves injection of genes into a cell to produce a target protein, rather than permanently incorporating the gene into a host genome. This is emerging as a less technically difficult and faster alternative to developing stable cell lines for expressing bioengineered proteins. The process of injecting the desired gene into the target genome, known as transfection, can be effected not only by viruses, but also by non-viral agents including various lipids, polyethylenine and calcium phosphate.

The last of the three advantages of plants for pharmaceutical production—the ability to manufacture proteins not available by other means—is creating perhaps the greatest excitement. The Protalix taliglucerase alpha protein falls into this category, and is likely to be

followed by other candidates for treating disorders that require enzymes or complex molecules beyond the scope of bacteria, according to Aviezer. “I would say that for simpler proteins, bacteria will still be the method of choice for a while,” Aviezer said. “But for more complex proteins currently made via mammalian cells, I think we can offer a very attractive alternative using plant cells.”

Indeed, plants can in principle be engineered to produce any protein, including animal ones, as Boothe pointed out. “In some cases this may require additional genetic engineering to enable the plant to perform certain types of protein modification that differ between plants and animals,” he said. “The classic example of this is glycosylation. With recent advances in the field it is now possible to engineer plants to glycosylate proteins in a manner similar to that of mammalian cells.” Glycosylation is a site-directed process that adds mono- or polysaccharides to organic molecules, and plays a vital role in folding and conferring stability on the finished molecule or macromolecule. Although plants can be engineered to perform it, bacteria generally cannot, which is a major advantage of plant systems over micro-organisms for pharmaceutical manufacture, according to Aviezer. “This enables plant systems to do complex folding and so make proteins for enzyme replacement or antibodies,” Aviezer said.

In addition to plants themselves, their viruses also have therapeutic potential, either to display epitopes—the protein, sugar or lipid components of antigens on the surface of an infectious agent—so as to trigger an immune response or, alternatively, to deliver a drug directly into a cell. However, as Lomonossoff pointed out, regulatory authorities remain reluctant to approve any compound containing foreign nucleic acids for human use because of the risk of infection as a side effect. “I hope the empty particle technology [viruses without DNA] we have recently developed will revive this aspect,” Lomonossoff said. “The empty particles can also be used as nano-containers for targeted drug delivery and we are actively exploring this.”

As pharmaceutical production is emerging as a new field for plant biology, there is a small revolution going on in plant breeding, with the emergence of genomic techniques that allow simultaneous selection across several traits. Although genetic modification can, by importing a foreign gene, provide instant expression of a desired trait, such as drought tolerance, protein content or pesticide resistance, the new field of genomics-assisted breeding has just as great potential through selection of unique variants within the existing gene pool of a plant, according to Douwe de Boer, managing director of the Netherlands biotech group Genetwister. “With this technology it will be possible to breed faster and more efficiently, especially for complex traits that involve multiple genes,” he said. “By using markers it is possible to combine many different traits in one cultivar, variety, or line in a pre-planned manner and as such breed superior crops.”

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Genomic-assisted breeding is being used either as a substitute for, or a complement to, genetic-modification techniques, both for food crops to bolt on traits such as nutrient value or drought resistance, and for pharmaceutical products, for example to increase the yield of a desired compound or reduce unwanted side effects. Yet, there is more research required to make genomic-assisted breeding as widely used as established genetic-modification techniques. “The challenge in our research is to find markers for each trait and as such we extensively make use of bio-informatics for data storage, analysis and visualization,” de Boer said.

The rewards are potentially enormous, according to Alisdair Fernie, a group leader from the Max-Planck-Institute for Molecular Plant Physiology in Potsdam, Germany. “Smart breeding will certainly have a massive impact in the future,” Fernie said. “The application of genomics technologies and next generation sequencing will surely revolutionize plant breeding and will eventually allow this to be achieved with clinical

precision.” The promise of such genomic technologies in plants extends beyond food and pharmaceuticals to energy and new materials or products such as lubricants; the potential of plants is that they are not just able to produce the desired compound, but can often do so more quickly, efficiently and cheaply than competing biotechnological methods.

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