

Emerging plant biotechnologies: new bugs for old

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One species of creativity is the fruitful combination of concepts from different fields. Ideas imported from without can catalyse great changes in science-physicists and chemists helped biologists to become molecular biologists, while mathematicians co-parented bioinformatics into existence. Now one agri-biotech organization wants to enliven the industry with the clever, creative combination of an updated biotechnology and a decades-old idea from computer programming.

Black boxes and building bricks

Biotechnology is the redeployment of natural systems for human ends; molecular biology is the artful arrangement of life's little black boxes. Scientists borrow systems, processes, and architecture built and honed by natural selection for millennia. We characterize their function and activity then re-arrange and re-purpose them. Genes, regulons, proteins, plasmids, chromosomes, bacteriophages, cells, tissues, organs, and whole organisms are treated as 'black boxes', i.e., modules that perform a function but for which the contents may for the moment remain mysterious. Functional modules are useful because we know what they do, whether or not we know exactly how they do it. Restriction endonucleases, for example, are treated as embodiments of their specific activity, even in the hands of a novice, EcoRI or XhoI represent black boxes which can be given inputs (DNA substrate, buffer, ions) and predictably deliver certain outputs (DNA cleaved at the EcoRI or XhoI recognition sites). Utility is, here, everything; the detail of what is inside the box is secondary and can be left to those on other grants. The boxes are also building bricks or line-replaceable components, like Legos or military aircraft parts, because they can be switched and swapped for others that behave similarly. For such swaps, each 'box' may have different contents: the isoschizomer TliI can, in most cases, replace XhoI, and many diverse polymerases are available for PCR.

Much of molecular biology (in vitro and in vivo) is like this. Research scientists open life's black boxes to find out how an endonuclease, polymerase, or T4 bacteriophage works. They often find smaller black boxes nested inside. This simplistic depiction of molecular biology is aptly reductionist; genes construct, and are themselves constructed of, switchable modules too; that is Life. This reductionism can lead to cognitive biases; it is also incontestably successful.

Agrobacterium tumefaciens, described in Science as "Nature's genetic engineer"¹, is the vehicle for the only known example of natural trans-kingdom horizontal inheritance. Widely used in agricultural biotechnology, Agrotransformation also showcases modern biotechnology as the artful placement of Nature's marvellous modules and scientific research as vigorous box-opening that provides answers and nested black boxes. Some examples:

- The fundamental conceptual breakthrough in plant genetic engineering was the realization that most of the heritable material trafficked to the plant by *Agrobacterium* was not essential to the process. Opening this particular black box (the T-DNA) revealed several components, only some of which were prerequisites. Any gene could be introduced if flanked by left and right border regions because the wild type opine synthesis genes were replaceable components.

- The limited host range of wild type *Agrobacterium* was shown to arise from its preference for (taxonomic) class-specific biochemicals released in response to tissue damage. Opening this box allowed investigators to source this particular component differently, supplying equivalent conditions to other plants and tissues in vitro and thereby increasing Agrotransformation efficiency and broadening the host range.

- Problematic manipulation of the Ti plasmid gave way to widespread use of binary vectors when research revealed more potential for swaps and shuffles. The plasmid 'black box' turned out to be made up of the T-DNA and several vir genes encoding proteins involved in its

transport; these proteins, being diffusible within the cell, function in trans, i.e., they need not be located on the same plasmid as the T-DNA. These smaller 'black boxes' (some vir protein functions are still obscure) were combinatorial. Binary vectors comprise a pair in which a partially disarmed Ti plasmid, unable to mobilize its own T-DNA, facilitates DNA transfer to the plant from a second, more manipulable vector.

The omitted box

Broothaerts and co-workers at the Centre for the Application of Molecular Biology to International Agriculture (CAMBIA) report that another element of the Agrotransformation process is replaceable². This component has been overlooked by some, perhaps due to the aforementioned cognitive biases since it is too big, and perhaps ignored by others for practical reasons. Nevertheless, it is a switchable, swappable module in the tradition of molecular biology: it is the *Agrobacterium* itself, just one more 'black box' that can be substituted by other boxes with analogous input-output characteristics. *Agrobacterium* is, it seems, unnecessary for Agrotransformation.

When these workers introduced a typical binary plasmid system into three isolates of plant-associated bacteria, all proved capable of transforming tobacco. All were nodulating symbionts associated with legume root systems, namely the promiscuous nodulator *Rhizobium* NGR234, the alfalfa symbiont *Sinorhizobium meliloti*, and *Mesorhizobium loti*. Collectively, the inventors refer to these species as TransBacter. Full genome sequences are available for *Sinorhizobium*, and *Mesorhizobium*³. The binary-equipped *Sinorhizobium* species was also used successfully to transform *Arabidopsis* (via floral dip) and rice callus. For the first time, molecular evidence of trans-kingdom horizontal transfer mediated by non-*Agrobacterium* bacteria is available. These bacterial species are not all obvious choices: *Rhizobium* is con-generic (some claim con-specific) with *Agrobacterium* but the others are much more distantly related. For the removal of doubt, constructs used in non-*Agrobacterium* species were designed to be diagnostically different in restriction analysis and sequence-specific PCR, while the bacteria themselves were genotyped and maintained under selective conditions to prevent *Agrobacterium* contamination.

This paper is just a preliminary report. A scant three plant species were transformed and only *Sinorhizobium* could transform all three; yet note that these three plant species are two excellent dicot models for further technique refinement and one monocot that is the dietary staple of over two billion people. Only a few transformants were produced, handfuls among hundreds; yet note that early success rates for new protocols are rarely impossible to improve. The idea itself is not novel and the authors faithfully report that transfer of (Ti plasmid-mediated) virulence *A. tumefaciens* to avirulent *Agrobacterium* species was first published over half a century ago⁴; yet note that they present the first unambiguous molecular evidence of a functioning and modular system ready to be taken up by biotechnologists everywhere. Lastly, a mere two generations of transgenic plants have been followed; yet note that the expected and heartening Mendelian segregation of the transgenes has occurred in all three plant species.

Free, as in speech

This breakthrough is more than a demonstration of academics at their most artful playing with nature's parts list, or confirmatory evidence of a fifty year old research paper. The authors state early on that one reason for the work was that "the complexity of the patent landscape has created both real and perceived obstacles to the effective use of [Agrotransformation] technology for agricultural improvements." Their paper is the sketch of a map for a path around these obstacles, a path still a little overgrown and as yet unpaved. The obstacles consist of complex contracts and licenses for the use of patents held on Agrotransformation methods, for the binary vector concept, and covering many other components of the process as commonly practiced. CAMBIA is offering alternatives to what some have seen as an oligopoly in key agricultural biotechnologies.

Their Biological Innovation for Open Society (BIOS)⁵ initiative seeks to create a biotechnology commons, a suite of tools and information not bound by contractual arrangements currently offered by holders of strategic intellectual property in agricultural biotechnology. Researchers and companies may join the associated community, known as BioForge, and access TransBacter species and other technologies held in commons. To do so, community members abide by an unusual contractual relationship modelled on the computer programming community's long-

established open source licenses such as the 'copyleft' system. These are justly celebrated as having facilitated the development of huge, dispersed, successful projects like the GNU-Linux operating system and Wikipedia⁶. Software developed and shared under such licenses is 'free', where 'free' is clarified by their catchphrase 'free as in speech, not free as in beer'.

BIOS is copyleft's biological orthologue. Members of BioForge may use the technology commons, even sell the resulting products, without paying fees or royalties to the inventors. They may even patent any improvements they make, but, akin to programming copyleft, BIOS members are obligated to make any such improvements, patented or not, available to other members of the community and also share data to assist regulatory or biosafety approvals. The analogy with copyleft and similar arrangements does not survive CAMBIA's insistence on a Technology Support Fee payable to BIOS, although it should be noted that this will be a subscription-like annual payment waived for or payable in kind by non-profit organizations or those headquartered outside the OECD.

The BIOS initiative and the (relatively) free way in which TransBacter is being made available to the world are fragments of a quiet but profound revolution in science. Recent years have seen increased scientific openness; greater efforts at long-overdue public education; wider engagement with the social impacts of science; a renewed and deserved prominence of ethics, accountability, and downstream consequences; and the provision of experimental data and peer-reviewed publications through open access publishing⁸. Conversely and concurrently, the escalating use of strong intellectual property protection massively affects the way the industry develops, from entrepreneurial academics worried about the applicability of the experimental use exception, to opposing transnational companies litigating for decades⁷. All of these factors have forced or are forcing changes on academic research; industry, as always, is downstream. Creative ideas such as the technology commons are now arriving at industry's door with as much or more potential for change. These are exciting times.

References

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8. Good introduction to OA at <http://www.earlham.edu/~peters/fos/overview.htm>

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