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The Science Behind the Next GE Revolution

This outline is a summary of anticipated opening remarks that will guide a dialogue between myself and Dr. Alison Peck.

The understanding of how DNA encodes proteins and regulates the activities of cells that unfolded during the 1960s and 70s led to the development of methods to combine DNA from different organisms, what is now described as recombinant DNA technology.

The first transgenic plants were produced in 1983 and in just over ten years the first transgenic or genetically modified (GM) crops were approved by regulatory agencies in the United States and grown by farmers.

Over the last twenty years, GM varieties have taken over much of the seed market for soybean, corn, cotton and canola in the US and in some other countries. While the initial promise that GM technology would result in a plethora of new traits that would transform agriculture, just two traits, herbicide tolerance and insect resistance, account for almost all of the acres of GM crops grown around the world.

All of the GM traits used in crop agriculture to date have relied on either a bacterium (*Agrobacterium tumefaciens*) or the "gene gun" to deliver one or two transgenes (recombinant DNA molecules) into plant cells. GM plants are then regenerated from these cells. Development of these methods was a major challenge for agricultural biotechnology. These methods remain, however, fairly crude and unsophisticated; only a small number of genes can be inserted into the genome and where these genes are inserted is random. More precise types of genetic modification are not possible using this type of gene delivery system.

The addition of new genes inserted at random locations in the genome has provided important, valuable and novel sources of genetic variation that have been useful in plant and animal agriculture. Other types of more precise genetic modification, such as targeted inactivation of individual genes, alteration of specific single nucleotides in a gene, and the replacement of one gene with another at a precise location in the genome, open up new horizons for crop improvement. These methods also bring with them many new regulatory challenges.

It is not very often that a scientific discovery reaches the covers of Science, Nature, Time, and the Economist. Dolly the sheep, the Higgs Boson and CRISPR/Cas9 have

all received that acclaim. CRISPR (I will use this abbreviation rather than CRISPR/Cas9) is a still-new technique for making precise changes to specific target genes in a genome. This has the potential to have a major impact on medicine, agriculture and several other fields.

CRISPR is a biochemical tool for editing (changing) specific genes in the genome. Similar to how the editing functions in Microsoft Word allow you to search for specific text in a document and delete, alter or replace that text, CRISPR can perform analogous functions with DNA. CRISPR uses a short RNA molecule to search for a specific DNA sequence in a target gene in the genome. Once that sequence has been located, CRISPR cuts the DNA at a precise location. The biochemical processes that repair the DNA can result in a gene that is silenced (analogous to "delete"), altered or replaced (analogous to "paste").

CRISPR is generating a huge amount of interest for its biomedical and agricultural potential. It is noteworthy that CRISPR is not the first gene editing technology to have been developed. Other targeting nucleases (zinc-finger nucleases and TALENS) were used over the last ten years to perform genome editing in plants. CRISPR, however, has several advantages over these other methods that make it much simpler to use and more widely applicable, and it appears likely that CRISPR will be the dominant genome editing technology going forward.

In plants, CRISPR has already been used successfully to silence specific genes in model species such as Arabidopsis as well as in several crop plants including maize, soybean wheat and rice. The pace of progress is very rapid. The first use of CRISPR in plants still relied on using *Agrobacterium* to insert the genes to make the CRISPR machinery into the genome of the target plant, i.e. production of a standard transgenic plant. After the desired genetic modification had been made in the genome, the CRISPR transgenes were removed by segregation after a sexual cross. Recently published protocols describe using either a transient gene delivery system, or simply delivering the RNA necessary to make the CRISPR machinery (without any DNA) into cells. In addition to being a much more rapid procedure, which has several inherent advantages, this avoids producing any typical transgenic plant material.

There are several successful examples of CRISPR genome editing in crop plants. While much of the initial research has been proof-of-concept, there have been some impressive breakthroughs that highlight the potential of CRISPR. One example is in wheat, a plant whose genetics is complicated by having the genomes of three progenitor species, and therefore six copies of every gene, in each cell. CRISPR was used to edit all six copies of a gene that is important for resistance to powdery mildew. With all six of these genes silenced by CRISPR, the wheat plants had a high level of resistance to powdery mildew. It would be close to impossible to achieve this result using either traditional plant breeding or conventional transgenic approaches.

There is no shortage of hyperbole about how CRISPR may impact plant and animal agriculture. Some of this is justified because CRISPR does offer new, simpler and elegant methods to modify the genome in ways that were inconceivable if we had to rely on simple *Agrobacterium* gene transfer technology. It is equally true that our understanding of how biological systems function is much more sophisticated now compared to 1990 when the Roundup Ready gene was first developed and transferred into soybean plants.

While CRISPR is still in its infancy, it is evident that this technology poses several novel challenges to the existing regulatory framework for GM crops. CRISPR produces new genetic variation that is in principle similar to the natural variation that crop improvers and plant breeders have been exploiting for centuries. That is likely to be the first type of application of CRISPR to be pursued in both plants and animals, mainly through the silencing of individual genes. How will these be evaluated and regulated? The use of DNA that did not necessarily come from a pathogen, yet served the same function as the pathogen-derived DNA, has also been used to circumvent some regulations. The existing framework did not anticipate these developments in genetic technologies and they need to be revised. This is long overdue.

CRISPR does have the ability to produce novel combinations of DNA sequences that are similar in nature to the transgenes used in the current generation of GM crops. As these technologies develop and evolve, different approaches must be developed to assess and regulate new crop and animal varieties. A recent review study conducted under the auspices of the National Academy of Sciences proposed a new four-tiered strategy that would focus on what was altered in the organism rather than the method used to produce the new organism. Such a framework would be more robust and durable, better able to adapt to new, as yet unforeseen technological developments that in the future may transform how plants and animals are modified for agriculture.