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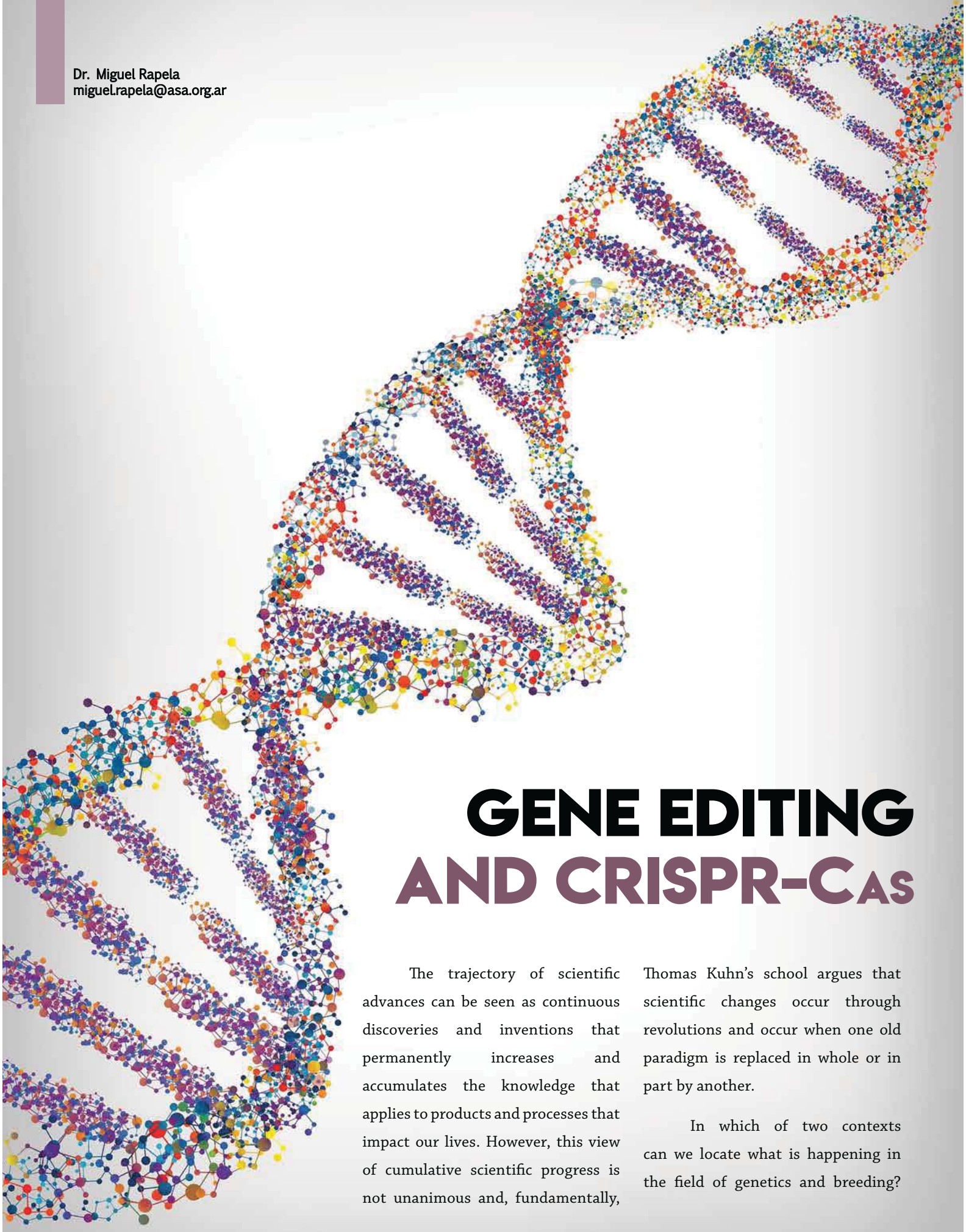


GENE EDITING AND CRISPR-Cas

The trajectory of scientific advances can be seen as continuous discoveries and inventions that permanently increases and accumulates the knowledge that applies to products and processes that impact our lives. However, this view of cumulative scientific progress is not unanimous and, fundamentally, Thomas Kuhn's school argues that scientific changes occur through revolutions and occur when one old paradigm is replaced in whole or in part by another.

In which of two contexts can we locate what is happening in the field of genetics and breeding?

What we observe is that, after a beginning based on empiricism and the selection of the best individuals, these activities have been transformed into scientific disciplines at the limits of human knowledge. Domestication, selection of the best specimens, crossbreeding, introgression of genes from other species, enhancement of pure lines, hybrids, induced (random) mutagenesis, molecular markers, and transgenesis became part of the vocabulary that allowed crop yields, make them more resistant to insects/diseases and improve their industrial qualities.

Nothing in this area of knowledge remains stable for long. Deciphering the human genome in 2003 required 13 years of work, greater scientific collaboration and more than three billion dollars of public investment funds. This same job today can be done in one day at a cost of less than $1,000. But what marks this abrupt change is that today there is a good knowledge of the sequence information and functioning of the genomes of organisms like never before.

In this context, CRISPR-Cas technology has opened up an avenue of possibilities of such magnitude that it is no longer a question of figuring out what new foods we might have, but how extensive it will be in our lives, as their scope extends to the treatment of genetic disorders in humans, animal health and the bio-industry sector.

Photos, reports from Joseph Tohme and Paul Chavarriaga (CIAT)

In vitro Cassava plants (*Manihot esculenta*). Left side picture showing a plant developed with CRISPR-Cas9 to silence the phytoene enzime gene, wich causes the inability for the leafs to synthesise clorophile and, thus, become bleached. The picture on the right shows a normal plant.

During the 6th Congress of the Seed Association of the Americas (SAA), held in Cartagena de Indias, Colombia, in September 2017, the gene editing and, in particular, CRISPR-Cas technology was the main focus of debate and discussion in terms of technical aspects, as well as their scope, use, regulation and intellectual property of the new products obtained through it.

CRISPR-Cas

The CRISPR-Cas system was discovered and described in bacteria by the Spanish scientist Francisco Mojica (University of Alicante, Spain) and functions in these organisms as an inheritable immunodefensible mechanism. After this discovery, a series of investigations involving Argentinean Luciano Marrafini (Rockefeller University), Emmanuelle Charpentier (Max Planck Institute), Jennifer Doudna (University of California, Berkeley), and Chinese-American Feng Zhang (Largo Institute, USA), allowed to transform the system into a precise tool of gene editing, able to be applied in any type of organism.

The great success of the technology is based on its simplicity, speed, precision, repeatability and low cost, compared to everything that is known until now. The editing of genes, from an idea, has become a concrete tool available to laboratories of molecular biology of medium complexity.

The power of the technology is based on its ability to target the Cas protein (which cleaves the DNA strand in one or both strands) to the precise cleavage site using a guidewire RNA derived from CRISPR and containing a sequence of approximately 20 nucleotides which fits with the target DNA sequence of the recipient organism.

The DNA cut in the precise place is recomposed by the natural system of cell repair and this can cause insertions or deletions of bases that can alter the function of the genes. The more developed CRISPR-Cas systems do not leave the changes at random and work with DNA models that establish exactly what the base replacement should be. This substitution may be one or more bases, or even entire genes. In addition, it is not necessary to make one change at a time, the genome can be edited in several places simultaneously.

CRISPR-Cas technology access

Despite its potential, there are questions about access to technology due to a conflict related to their ownership. CRISPR and Cas can not be patented since they are pre-existing components in nature. What can be patented is its process to apply technology in organisms where these components do not exist, as well as the entire development of RNA-Guides.

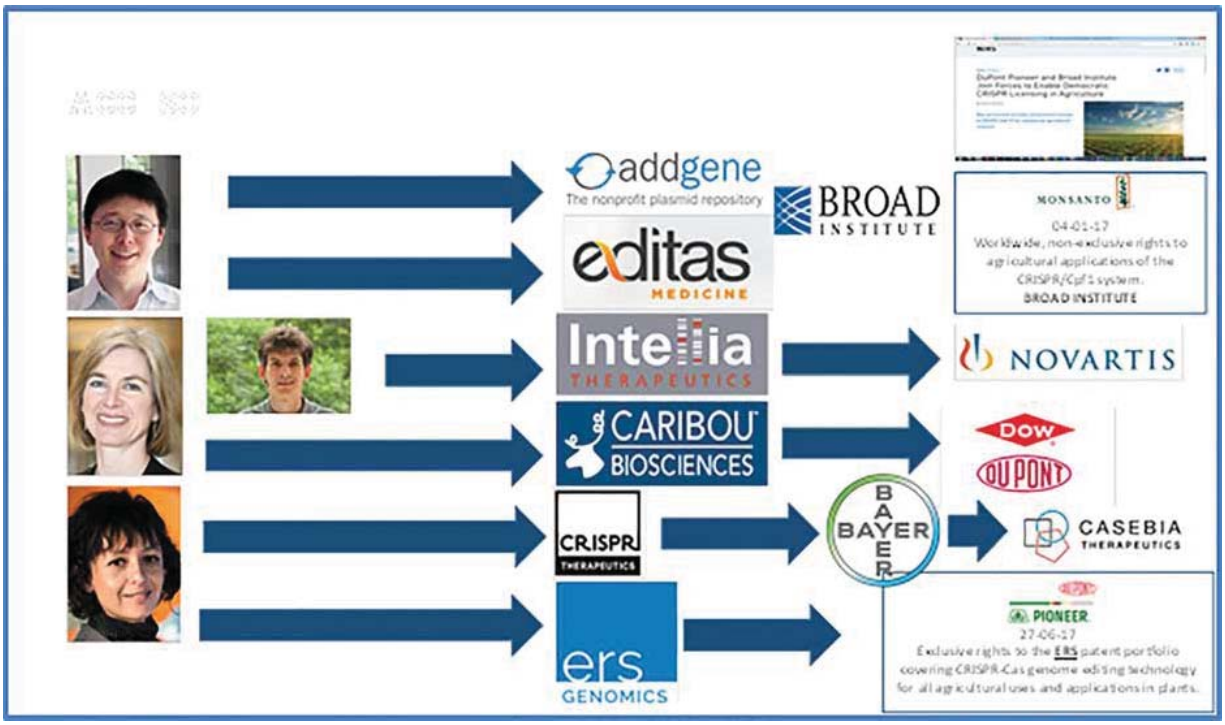
The University of California at Bekeley, the Broad Institute (under MIT and Harvard University), and Rockefeller University to a lesser extent, entered a patent dispute that originated in the United States, and now has extensions to the Union Europe and Asia. In the only decision known, the US Court ruled in favor of the Broad Institute and the patent granted to Feng Zhang, but there was an appeal. It is difficult to predict the development of these events, as they may also have different solutions in different continents.

While these disputes and their side effects continue their course, the main actors have created companies through which they are licensing their processes. The following is a summary:

• Feng Zhang created Editas Medicine, where, in turn, he placed his processes in Addgene, which is a repository of plasmids to which all researchers have access. The licenses, in turn, are also channeled through the Broad Institute.

• Jennifer Doudna and Luciano Marrafini created Intellia Therapeutics.

• Jennifer Doudna with



another group of researchers, created the Caribou Biosciences.

• Emmanuelle Charpentier created CRISR Therapeutics and, together with Bayer, created Casebia Therapeutics. The same scientist founded ERS Genomics.

• All major companies have signed non-exclusive licenses with these companies.

The solution to the patent controversy is immersed in a problem of magnitude with few precedents. Until the beginning of this year, more than a million patent applications related to CRISPR-Cas were detected, 62% belonging to academic institutions. All of this is another no less important change in the new paradigm. The academy once again played a leading role in history, but now academics are also central stars in the marketing aspects of their technologies.

CRISPR-Cas Intellectual protection and product regulation

Both are extremely important issues and involve some difficult questions to answer: How is a gene copy protected? Are the edited products genetically modified?

The first of these intellectual property issues raises the fact that, in most legislation, pre-existing products in nature are not a patentable subject. When it comes to transgenesis, the answers are relatively easy. For example, a genetic construct developed in a laboratory and not pre-existing in nature is considered to be patentable, as long as, all other patentability assumptions are met. But what happens if you edit a pre-existing gene in nature? Is the edited gene patentable? The discussion is relevant, since the potential response to Breeder's Rights would be the solution, nor is it completely satisfactory. If a gene is edited to produce an existing phenotype, the doctrine derived from UPOV Acts is not sufficient, since the plant variety is defined by the expression of the characters of a genotype. Two varieties of identical expression, for UPOV, are identical.

Photos and reports from Dr Carlos Pérez and Hugo Permingeat, BIOHEURIS, Rosario, Argentina

BIOHEURIS research team.

As regarding the second question, the difficulties are not minor. Are the edited products genetically modified? According to the definition of the Cartagena Protocol, a genetically modified organism is one that contains a new combination of genetic material. At present, Argentina, Chile, Israel and some other countries in the final stage of development are adopting the criterion that a "new combination of genetic material" is the stable and joint insertion in the genome of one or more genes or sequences of DNA, which are part of a defined genetic construction. Clearly, according to this criterion, the editing of a gene to modify base sequences, as it occurs in nature and without the insertion of one or more genes, is not a genetically modified organism and, therefore, a product edited through CRISPR -Cas of such characteristics is identical to a conventional organism.

The implications of this criterion are transcendent, both in its positive and negative aspects. As for the former, many of the products derived from CRISPR-Cas would not have to go through the regulatory system because they are conventional and present no different risks from products that result from all known techniques of plant breeding. If this criterion is accepted, regulatory costs will drop significantly, reducing the

Cabbage seedling under a breeding program

time to market it. However, negative effects may also occur if different countries adopt different exclusion criteria, running the risk of a global implementation of an asymmetric decision system with unpredictable business consequences.

Of course, CRISPR-Cas technology has all the potential for transcendent results in growing cultivars. At the same time, however, this remarkable breakthrough has introduced a number of questions about access to technology and the protection and regulation of derivative products. It will require an urgent and intelligent discussion to enable them to reach their final recipients.

Regional Development Examples

ARGENTINA: BIOHEURIS is a "StartUp" of Argentine scientists and businessmen based in the city of Rosario. Their work in soybean aims to edit proteins from three modes of action of independent herbicides, using CRISPR-Cas, in order to facilitate the use of multiple herbicides. This would allow to reduce the doses of each of them and to delay the appearance of resistant weeds. For this, they associated a technological package in which the herbicides are supplied by the company ROTAM and the germplasm of soybean by the company SANTA ROSA SEEDS.

In order to determine whether the products, under development, would be considered "GMOs", Bioheuris made a Prior Consultation for the Biotechnology Division of the Ministry of Agriculture. After assessing the information presented, the regulatory authorities considered that the product under development is not considered as a GMO. The classification of this technology as "non-GMO" facilitates its access to the market, reducing the cost of development and the time of market entry.

COLOMBIA: The International Center for Tropical Agriculture (CIAT) in Colombia is using CRISPR-Cas technology as an editing tool to improve rice, cassava and bean crops, seeking to improve agronomic attributes and/or validate candidate genes. In rice, the focus is on white-leaf virus (HBRV) resistance genes, as well as erosion genes, Xanthomonas resistance and elimination of selection markers on iron rich transgenic lines. In cassava, CIAT works to obtain waxy varieties, resistance to herbicides and generation of haploids. On the other hand, in beans, CIAT is focusing on nutritional quality, eliminating antinutritional compounds. They also extended the use of the tool to detect pathogens, based on the SHERLOCK system developed by the Broad Institute (MIT) and Harvard.