RNA silencing in genomics and plant biotechnology

Lullaby for a Gene

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Attempts to increase gene activity can sometimes have the opposite result: switching off the gene in question. We are now trying to exploit this phenomenon to control plant physiology

The discovery of RNA silencing seems to be one of the most spectacular and promising achievements of molecular biology at the end of the 20th century. RNA silencing is widely present in plants, animals and fungi. It can be defined as an RNA-mediated process of suppressing a gene's activity. (Just to remind you: RNA is a close relative of DNA, with more or less the same information-coding ability). This results in the selective degradation of mRNA (the messenger molecule that transfers information from the DNA to the protein) but it can lead to the chemical modification of the DNA itself.

Observations have revealed that the introduction of an additional gene copy into a plant genome often leads not to an increase in protein production, but rather to its inhibition. It has also been found that gene expression can be completely shut down by introducing a short piece of RNA, complementary to the mRNA fragment, into a cell. It has been assumed that this fragment binds to mRNA, or directly to a gene, thus preventing its activity. Moreover, the phenomenon of gene silencing was intensified when both molecules, i. e. a complementary one and one identical to the mRNA sequence, were used simultaneously as double-stranded RNA (dsRNA). There are a number of reports suggesting that RNA silencing evolved in plants as an antiviral defense mechanism (more than 95% of viruses infecting plants use RNA to code their genetic information). Consequently, the expectation was that RNA viruses had evolved some counter-defense strategies, so as to suppress the RNA silencing machinery.

Mission 1: Shut down a gene

Several projects focused on RNA silencing are currently underway in our laboratory. We are trying to develop efficient methods of gene silencing and over-expression in tobacco, barley and legumes. In parallel, we are conducting some basic studies on the mechanism of RNA silencing, and applying RNA silencing vectors (i. e. viruses) and DNA microarrays in the functional analysis of plant genomes and individual genes. And last but not least, we are focusing on the practical application of RNA silencing suppressors in plant biotechnology.

At present, three methods are most frequently used to induce RNA silencing in plants: VIGS (virus-induced gene silencing), agroinfiltration and microbombardment. All three were assessed in our laboratory with two model systems: transgenic *Nicotiana benthamiana* or *N. tabacum*, continu-

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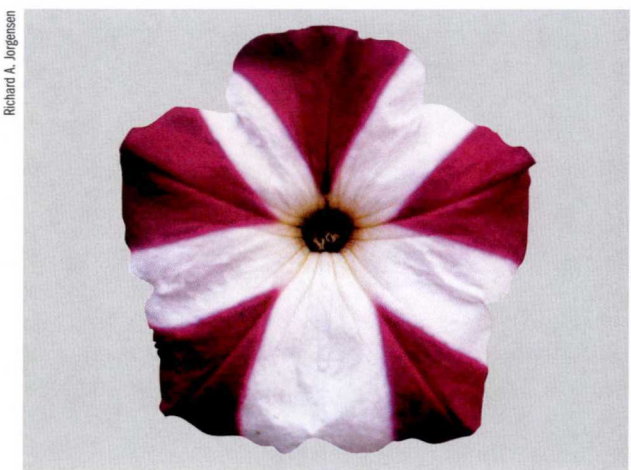

The first examples of RNA-mediated gene silencing were reported some 13 years ago. Richard A. Jorgensen inserted into purple-flowered petunias (left) additional copies of their native pigment gene. Instead of the expected more vibrantly violet flowers, he obtained blooms with patches of white (right). It appeared that an overdose of pigment genes "switches off" their activity

Richard A. Jorgensen

ously expressing green fluorescent protein (GFP) or hepatitis B virus surface protein (HBsAg) genes, respectively. The first method utilizes modified viruses as RNA silencing triggers. Our experiments involved two bromoviruses – BMV and BBMV. We found that both can function as handy RNA silencing vectors. The second method employs Ti plasmids (tumor-inducing plasmids). They are introduced into a special bacteria strain, which infects the plant and is delivered to plant tissue by means of agroinfiltration. DNA inserted into the Ti plasmid is integrated with the plant genome and serves as a template for dsRNA synthesis in the nucleus. In the third method, a linear or circular DNA template is transferred into the nucleus by microbombardment. Virial infection and agroinfiltration were found to be the most effective. Now they are being tested in the cultivable plants that are the major targets of our research, i. e. barley and lupine.

Mission 2: Let the gene speak

Another subject of intensive study at our laboratory are RNA silencing suppressors that enable us to overcome RNA silencing. They are highly varied in size and structure, and target different steps of the RNA silencing pathway. So far, our studies involve three virus-derived suppressors: the HC-Pro protein encoded by potato virus Y, which inhibits the so-called Dicer enzyme and prevents small interfering RNA formation and/or accumulation; the p25 protein encoded by potato virus X – a factor which most likely slows down dsRNA production; and the 2b protein encoded by the cucumber mosaic virus, which probably interferes with the mobile silencing signal and thus inhibits systemic silencing.

To test the biological activity of RNA silencing suppressors, we use transgenic tobacco plants transformed with the HBsAg gene. Some of these plants have been successfully applied as a model for future production of edible vaccine to mediate antiviral response in humans and animals. However, there are also plants which do not express HBsAg (or express it to a very low extent) although two or more copies of the viral gene have been inserted. Our data indicate that the absence of viral protein in plant cells might result from the RNA-mediated HBsAg mRNA degradation. To check the possibility of inhibiting the latter process, RNA silencing suppressors were transiently expressed in these plants. As a result, a very high accumulation of HBsAg was observed (8-10 times higher than in regular transgenic plants expressing hepatitis B virus protein).

The sequencing of several complete genomes and the development of DNA microarray (DNA chip) technology laid proper ground for the development of modern functional genomics. Now RNA silencing will help us to determine the function of numerous genes. We will be able regulate plant properties which are desirable or undesirable from the biotechnological standpoint. Suppressors of the RNA silencing system, on the other hand, offer us hope for genetically modified plants expressing foreign genes to a very great extent. ■

Further reading:

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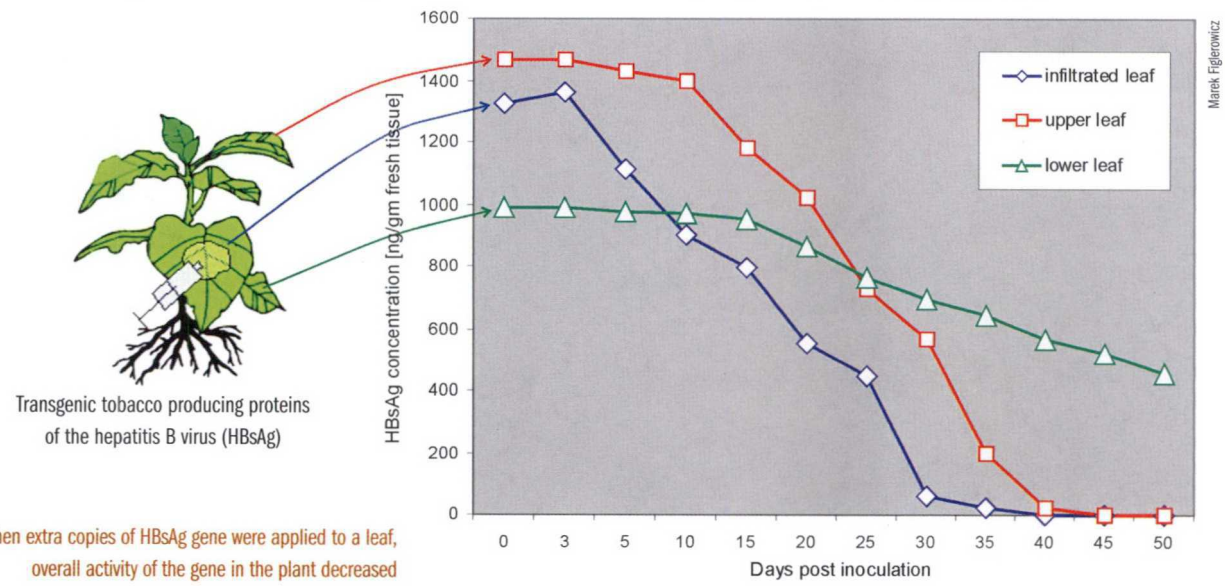

Marek Figlerowicz

Transgenic tobacco producing proteins of the hepatitis B virus (HBsAg)

When extra copies of HBsAg gene were applied to a leaf, overall activity of the gene in the plant decreased