

RNA silencing in Gödöllő/Hungary

The Viral Empire Strikes Back

Plants defend themselves against viral attacks by applying RNA interference mechanisms. The aggressors, however, strike back by suppressing the defence machinery of their hosts. József Burgyán and his group from the Agricultural Biotechnology Centre in Gödöllő (Hungary) investigate the viral strategies in this “small RNA war”.

Viruses like the potato viruses and the plum pox virus are the scourge of agricultural crops. By replicating in plant cells they slow down the growth rate of their hosts.

To cope, plants have developed specific defence strategies. One of them is gene silencing via RNA interference, an ancient mechanism found among all organisms from fungi to animals. It is induced by the proliferation of viral intruders in plant cells. Intermediate RNA products of the viral replication process are cleaved by plant enzymes to small RNAs of about 20 nucleotides in length. These small RNAs specifically bind certain proteins and form the so-called RNA-induced silencing complexes (RISCs), “defence machines”, which are now able to silence viral transcripts by binding to complementary sequence regions.

However, viruses do not accept this without protest. Selection pressure has caused them to develop response mechanisms to undermine the plants’ defences. These mechanisms are of special interest to biological and agricultural science. Once plant virologists understand them, this would improve their ability to use plant viruses as vectors for transgenes and, probably more importantly, even find ways to protect agronomically valuable plants from viral infection.

The first suppressor ever discovered

These are also the main reasons why József Burgyán and his group from the Agricultural Biotechnology Centre (ABC) in Gödöllő (Hungary) have been studying the molecular interactions between plants and their viral plagues for several years. “My interest came about in the late nineties,” Burgyán remembers. “It was at a time when the whole thing on interfering RNAs came up

plants.” Originally, he had focussed on plant viruses without considering the molecular level of interaction between them and their hosts. But when the principle of gene regulation by small RNAs was discovered, he realised what great potential lay in this field. As only very few people worked with plant viruses at that time, he had a good head start. “We already had a very good tool,” Burgyán says. “The Cymbidium Ringspot Tombusvirus, which is not really ecologically important but very good to handle.” Not only had his group already sequenced the genome of this model virus, they also knew



Head start in plant virology:
József Burgyán

exactly how it behaved in plant cells.

The most important advantage provided by the Cymbidium Ringspot Tombusvirus was the fact that its genome contains a small protein called p19. Burgyán *et al.* could easily knock out the protein without destroying the virus. In this way they finally discovered that p19 protects viruses from the RNA interference mechanism in plants, revealing it as the first silencing suppressor protein in plant viruses ever to be described



Plant defence screwed up:
typical Cymbidium virus damage

at molecular level. In collaboration with a lab in North California they even succeeded in crystallising p19 and describing the molecular structure of the protein in detail.

A superb Christmas gift

But that wasn’t enough. “In addition, we revealed the mechanism by which p19 confers the protective function,” says Burgyán. The silencing suppressor works as a trap for siRNAs. It sequesters them and thereby prevents their incorporation into the RISCs, the “defence machine”, which silences the replicating viral genome. In fact, p19 is very choosy, selecting only siRNAs of a certain length. “The important finding was that p19 very specifically binds small RNAs of 19 nucleotides in length, which are important for RISC formation,” Burgyán says. “The protein functions as a kind of calliper that measures the exact length of its targets.”

How does it manage this? The answer can be found after a close look at the molecular structure of the protein. It reveals a cleft into which only RNA fragments of a defined length fit. Longer or shorter ones do not bind as well. This simple complementarity between the function and structure of p19 has fascinated not only Burgyán and his colleagues but also the editors of *Cell*. They published the pioneering study in December 2003 and a model of the protein structure even made it onto the cover of the issue (*Cell* 115: 799-811). A superb Christmas gift for the group.

In the following years, Burgyán *et al.* investigated a couple of other viral suppressor proteins. They were puzzled that, although the proteins were completely unrelated to each other, their mechanisms for silencing suppression were often very similar. In one of their latest publications they described the p122 subunit of Tobacco Mo-

saic Virus (TMV) replicase as a potent silencing suppressor (*Journal of Virology* 81: 11768-80). “The main function of the replicase enzyme is to replicate the viral genome,” says Burgyán. “It is interesting that, at the same time, the protein protects its own products from being silenced by the plant defence.”

Complex interactions

This mechanism of protection is again very similar to that of p19. The p122 subunit of TMV replicase measures the length of smRNA, which would normally be incorporated into the RISCs, and sequesters them. Again, this prevents the formation of silencing machines in the plant cells. Similarly to p19, it is a trap for small RNAs that are used as templates for the silencing of the viral genome.

However, p122 does even more. Unlike p19, it additionally prevents interfering RNAs from being methylated, a process that normally protects them from degra-



dation. In this way, p122 shortens the lifetime of the molecules and thus hampers the silencing of its own replication. “This is a very interesting finding,” says Burgyán. “It shows that the molecular interactions between viruses and plants on the RNA level are very complex.”

No wonder, therefore, that over the years Burgyán and Co. came to realise that small RNAs play a crucial role in the interplay between plant viruses and their hosts. And this is not restricted to infection or defence strategies. “When a virus infects a cell, changes follow in the expression profile of many different regulatory RNAs in the plant,” says Burgyán. In order to measure such changes the researchers use a method which they invented in collaboration with the Dutch biotech company, Exiqon. It is based on short sequences of locked nucleic acids (LNAs). These chemically modified nucleic acid fragments are very robust and thus allow the formation of stable hybrids between themselves and small RNAs in plant cell sections. Labelled LNAs can subsequently be used for the detection of well-

defined sequences of small RNAs in high temporal and spatial resolution

“In the meantime, it has been discovered that small RNAs are able to regulate many genes in plants,” Burgyán says. “The question is, which ones are indeed affected by viral infections. That’s what we are investigating at the moment.” The goal is to reveal how viruses reprogramme the physiological state of their hosts by affecting the expression profiles of regulatory RNAs.

Resistant plants

In addition, the research done by Burgyán’s group opens the door for many applications. In some application-oriented projects they themselves are looking for ways to make plants resistant to other parasites with the help of viruses. One example is powdery mildew, which is caused by the fungus *Blumeria graminis* and affects cereals worldwide. “When we know how viruses escape from the plant defence mechanisms, we can use them as vectors to silence interesting plant genes,” Burgyán says. “We adopted such a system for wheat and found the genes that are important for powdery mildew resistance.” This might open the door of creating resistant wheat by RNA interference technology in the future.

Of course, opinions about the use of such methods diverge. The manipulation of genetic information in agricultural plants has little support in the European Union. “The creation of genetically modified organisms is currently not very welcome in Europe,” says Burgyán. But it might be welcome in poor Third World countries.

Grateful for support

The use of viral vectors could be fruitful in other cases, too. It might, for example, help to express pharmaceutically useful proteins in plants on “protein farms”. In plant cells high expression of transgenes is usually countered by gene silencing. It is a feedback mechanism, which has, to date, restricted satisfying expression rates. Combining transgenes with viral silencing suppressor sequences might solve this problem and enhance the crop of enzymes or other proteins which are important for industrial needs. That’s why Europe should welcome this research with open arms.

Even though Burgyán is about to move to Torino, Italy to take up a position as Director of the Istituto di Virologia Vegetale of the National Research Council (CNR), he is very grateful to the ABC making his work of the last 18 years possible.

MATTHIAS NAWRAT