

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 in

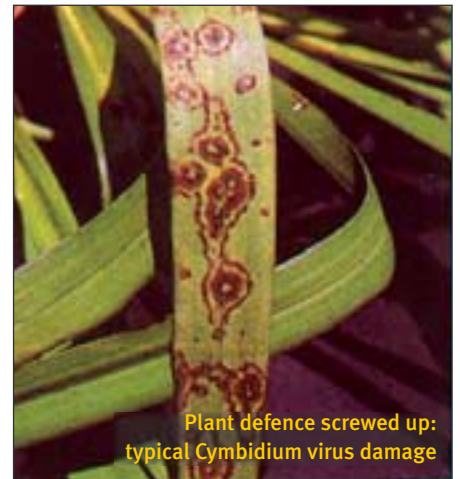
plants.” Originally, he had focussed on plant viruses without considering the molecular level of interaction between them and their hosts. But when the principle of gene regulation by small RNAs was discovered, he realised what great potential lay in this field. As only very few people worked with plant 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-

