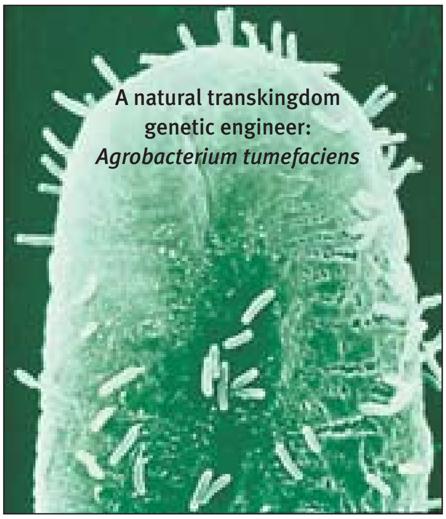
A natural transkingdom genetic engineer: *Agrobacterium tumefaciens*

Plant transformation in Vienna

Intruder Alert

Agrobacterium transforms plants, that's a well-known fact. But how exactly does the transforming T-DNA get into the plant's nucleus? Heribert Hirt and his team now know: *Agrobacterium* abuses the host's own defence response.

A plant-focused laboratory barely copes without it; every plant geneticist admires its services and those learning about this tool for the first time generally are fairly impressed. *Agrobacterium tumefaciens*, also referred to as a natural trans-kingdom genetic engineer, has made the transformation of thousands and thousands of plants possible. In its free time, the soil-borne bacterium lives as a plant pathogen that causes tumourous growth and demotes its host to a kind of bed and breakfast. In the lab it functions as a handy gene ferry, introducing scientifically relevant pieces of DNA into the plant genome. In both cases *Agrobacterium* fools the plant cell the ancient Greeks' way – an exciting and important strategy of the *Agrobacterium*-mediated transformation which was recently published by Heribert Hirt's group from the University of Vienna, Austria, and the URGV Plant Genomics Laboratory, Evry, France (*Science*, 318, 2007: 453-6).

"Botany used to be a thorn in my side. All that dissection and drawing seemed awfully boring to me," laughs Hirt at his former attitude towards plants. Hirt was born in Persia and grew up in Germany. He first studied biochemistry at the University of Cape Town in South Africa, followed by a PhD. His interest was in the medical topics of neurobiology and human genetics; later he switched to yeast as a model system for cell cycle control. "All I knew about plants was that they have RNA, DNA and proteins. Still, we decided to look for parallels in their regulation of the cell cycle compared to yeast and other eukaryotes", he says, remembering the early stages of his plant research.

Not boring at all

In the early nineties, Hirt and his team identified several homologues of regulatory components of the eukaryotic cell cycle in alfalfa (*Medicago sativa*). They found cdc2 protein kinases, phosphoprotein phosphatases, cyclins and mitogen-activated protein kinases (MAPKs). Closer investigation revealed that it was the latter that built a bridge to the regulation of stress response and tolerance in plants. MAPK-pathways are known to transfer information from sensors to responses in all eukaryotes. Now they have been shown to be involved in development, cell proliferation, and hormone physiology, as well as in biotic and abiotic stress signalling. "We looked for MAPKs as regulators of the cell cycle and we found many of them, but they were all involved in the plant stress response," Hirt explains. "This is how we finally got on that track."

Thus were green and silent plants revealed to be less boring than they appeared. As sessile organisms, plants cannot move away from adverse environmental conditions. Instead, they have to show a good sense of detection of and adaptation to perturbations. "With *Arabidopsis* we have a fascinating, complex organism and a perfect genetic model system. Yeast is a great system too but it is not that impressive in its appearance," Hirt laughs. How do plants react to too much salt and heat, to too little water and light, to the attacks of pests and pathogens? How do they communicate with the latter? Have they experienced a co-evolution with their enemies? If so, what has been the outcome?

These are the questions Hirt and his group ask every day – and which finally lead them to *Agrobacterium*. Hirt: "During our search for targets of the *Arabidopsis thaliana* MAP kinase MPK3 we repeatedly isolated the VirE2 interacting protein (VIP1). This protein was only known to be involved in *Agrobacteria*-mediated transformation; its function in plant cells had not been resolved yet."

Abuse of a transcription factor

However, let's remember the course of a typical transformation first: *Agrobacterium* introduces a partial copy of its tumour-inducing Ti plasmid into the host cell. Bacterial virulence (Vir) proteins, together with several plant proteins, stabilize this so-called transfer DNA (T-DNA) and guide it to the plant cell nucleus. The T-DNA is inserted into the plant genome where it can fulfil *Agrobacterium*'s ultimate goal of hijacking the plant's metabolic system. The result is the synthesis of T-DNA encoded opines and plant-type hormones. While the former constitute an ideal carbon and nitrogen source for the bacteria, the latter lead to a tumour-like proliferation of the plant cells – altogether creating a spacious and cosy habitat for *Agrobacterium*.

"The process of transporting the T-DNA from the plasma membrane to the nucleus is all but trivial", Hirt stresses. "It is comparable to an asylum-seeker who tries to take on the hurdles of bureaucracy." The protein VIP1 had already been known to facilitate T-DNA transport by acting as an adaptor between T-DNA and the plant's nuclear import


Heribert Hirt (l.) and his "Trojan horse-hunters"

machinery. The discovery of its interaction with the MAPK pathway now sheds light on its actual function in the plant cell.

In fact, this very MAPK pathway had already been shown to react on plant pathogens. “This definitely was contradictory. All previous work on and with *Agrobacteria* assumed that the pathogen isn’t recognized by the plant at all, thus enabling it to perform the infection in the first place,” the scientist says. Hirt and his group questioned these assumptions and finally found that the bacteria do activate the defence pathway. Furthermore, they showed a specific phosphorylation of VIP1 by MPK3, which appeared to be the crucial factor for VIP1 localization in the cell. Wild-type VIP1 was detected in the cytoplasm as well as in the nucleus. However, the mutated form of VIP1, mimicking a constitutively phosphorylated protein, was predominantly localised in the nucleus. Above this the localisation of wildtype VIP1 could be shifted to the nucleus by treatment with *Agrobacteria*. Over-expression of wild-type VIP1 increased transformation efficiency. However, over-expression of a version of the protein not phosphorylated by MPK3 did not.

Hirt: “We could identify VIP1 as a transcriptional factor playing a general role in the host defence response. It activates the expression of the pathogenesis-related PR1 gene, a function which is dependent on its phosphorylation and nuclear import.” Taking all this together it was obvious to the scientists that *Agrobacterium* simply abuses this mechanism in order to bring its T-DNA to the nucleus; a strategy compared by the scientists to the use of the famous Trojan Horse. VIP1 has to enter the nucleus as the host defends itself but at the same time drags the pathogen along, shuttling it to its destination. Therefore the bacterium has to be recognized by the plant to be able to transform it.

How to outflank monocots?

So *Agrobacteria* managed to adapt to their hosts in a fascinating and efficient way. Why does the plant put up with that? Shouldn’t they adapt as well? “These are our next questions and hypotheses,” Hirt agrees. A great number of plants cannot be transformed by *Agrobacteria* at all. Monocot species, for example, are not susceptible; a quite unwelcome fact to crop plant scientists. Hirt, “We have two explanations for that: on the one hand the bacteria might be able to inject the T-DNA, but do not activate the pathogen defence of those plants. Therefore the T-DNA never gets into the nucleus. On the other hand this procedure might take place, but then is overwhelmed by a strong line of defence.”

The new knowledge of *Agrobacterium*´s malicious cunning could be the solution to such problems. The scientists fancy a transformation system which simply includes a constitutively active VIP1. They are even thinking of expansion into other kingdoms; the use of *Agrobacteria* to transform other bacteria, yeast or human cells. “Especially the transformation of human cells very often has a catch. Viral systems may provoke diseases, for example. It surely would be good to have an alternative,” Hirt explains.

A shuttling scientists

Considering the facilitated transformation of crop plants makes scientist similarly excited. He points to the treatment of grapes with herbicides and pesticides, which already borders on poisoning. “If we weigh this up against genetically modified and therefore resistant plants, to me the current European policy seems quite short-sighted,” he says. Quick adaptation to artificially improved plants on the part of the pathogens, as just explained for *Agrobacterium*, would not be a problem. “The introduced resistance only has to address an essential factor that can’t be ignored that easily. Such resistance will remain for quite a while.”

About nine months ago Hirt joined the URGV (Unité de Recherche en Génomique Végétale) as the new director of this plant genomics institute in Evry, France. He still has a laboratory at the University of Vienna, which means a lot of travelling. Hirt says, “In Paris we are nine scientists; in Vienna I still have a group of twelve people. A month has to be divided into one week in Vienna, two weeks in Paris and another week somewhere else.” Nevertheless, Hirt seems to be content. Recent years have brought a new trend towards quantitative analysis and modelling; not excluding the Hirt lab, of course. The scientists focus on phosphoproteomics, which means collecting data on a large scale and assembling the information like a box of building blocks. “The URGV is a genome research institute with many high-throughput facilities and we are in contact with a number of excellent mathematicians and physicists,” Hirt describes enthusiastically.

In other words, the perfect place to improve one’s knowledge of the complex interactions outside and inside a plant.

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