Using plants to silence insect genes in a high-throughput manner

February 2 2012

Nicotine-resistant larvae of the Tobacco hornworm *Manduca sexta* have become a new tool for investigating unknown gene functions in Lepidoptera - thanks to a novel RNAi-based procedure. Credit: Courtesy of Jan-Peter Kasper, Germany

Scientists at the Max Planck Institute for Chemical Ecology, Germany, are now using a procedure which brings forward ecological research on insects: They study gene functions in moth larvae by manipulating genes using the RNA interference technology (RNAi). RNAi is induced by feeding larvae with plants that have been treated with viral vectors. This method called "plant virus based dsRNA producing system" increases sample throughput compared to the use of genetically transformed plants.

Natural toxins against herbivores

More than 200,000 insects species are herbivores. They depend on plants for food and have adapted their metabolism accordingly in the course of evolution to render plant defenses, such as the toxins plants produce to fend off herbivores, ineffective. The operating instructions of these detoxification processes are coded in different genes. Insects have evolved an enormous diversity of adaptation mechanisms; they colonize most habitats on this planet – which makes them interesting research objects in ecological studies. Which insect species attack which plants species? Which toxins or signaling substances are involved? Has the insect species adapted to one specific plant species or is it a food generalist? Interesting for agriculture: Which genes allow particular pest insects, such as the pollen beetle *Meligethes aeneus* or the Western corn rootworm *Diabrotica virgifera virgifera*, to be so destructive to crop plants? Knowing these detoxification genes and switching them off with the consequence that plant toxins are no longer effective, is currently a research subject in plant breeding. First success stories have already been reported – thanks to the use of RNAi technology.

Scientists at the Max Planck Institute for Chemical Ecology examined a well-known plant toxin: nicotine. Plants of the species Nicotiana attenuata (coyote tobacco) produce nicotine as a defensive substance against herbivores. However, it does not have any toxic effects on their worst enemy: larvae of the tobacco hornworm *Manduca sexta*. The insect is resistant against this alkaloid; genes that encode nicotine-catabolizing enzymes may be responsible for its resistance. These so called CYP genes are involved in the formation of cytochrome P450 enzymes; the expression of some of these genes is increased as soon as the insect larvae are exposed to nicotine in their food. Ian Baldwin and his team identified the DNA sequences of CYP genes in *Manduca sexta* and were able to switch off these genes using RNAi technology, but expressed in the plant.

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RNA interference (RNAi) is triggered by the production of double-stranded RNA (dsRNA) comprising about 300 base pairs in the cells of tobacco plants. If larvae feed on these plant, the RNA is released in the insect gut. In the experiments, the dsRNA harbored the sequence of the insect gene,CYP6B46, a special cytochrome P450 oxidoreductase specific for *Manduca sexta* larvae. In a next step, the dsRNA was enzymatically broken down into smaller RNA segments; a special enzyme complex called RISC (RNA-induced silencing complex), which carries several of these RNA segments, specifically binds to the messenger RNA (mRNA) of the CYP6B46 gene and disassembles the mRNA in such a way that the cytochrome P450 enzyme cannot be produced anymore. "We were impressed by the high specificity of these RNAi experiments. The analysis of mRNA transcripts of closely related CYP6 genes revealed that only the CYP6B46 gene was silenced. This means that there was no collateral damage from the procedure: the gene silencing worked on only one targeted gene," says Ian Baldwin.

The use of additional CYP RNAi probes revealed further interesting results: Young caterpillars which had ingested dsRNA of the CYP4M3 gene gained significantly less weight within 14 days in comparison to larvae reared on control plants – very likely a consequence of the nicotine and its toxic effect which had been restored by switching off the CYP gene. The RNAi experiments had been conducted using plant viral vectors. Unlike genetically transformed tobacco plants in which CYP dsRNA is produced constitutively, the virus vector-based technique provides dsRNA transiently produced in wildtype tobacco plants. Both methods worked well but the "plant virus-based dsRNA producing system" (VDPS) allows for a throughput of RNAi samples that is four times faster. Many unknown functions of different insect genes involved in the adaptation of insects to their environment can now be analyzed using the VDPS technique.

However, it is still unclear how the individual steps in the RNAi mechanism – from producing dsRNA in the plant cell via their uptake in the insect gut to the silencing of the detoxification genes – are accomplished to induce a maximum effect. One experiment provided some interesting information: If the enzymatic step which dices dsRNA into small fragments is inhibited in the experimental plants, the amount of transcripts of the detoxification gene was reduced even further. Therefore the plant mediated RNAi procedure may be more effective, if the caterpillars ingest complete dsRNA instead of smaller diced RNA segments.

More information: Kumar, P., Pandit, S.S., Baldwin, I.T.: Tobacco Rattle Virus vector: A rapid and transient means of silencing Manduca sexta genes by plant mediated RNA interference. *PLoS ONE*, DOI: 10.1371/journal.pone.0031347

Provided by Max-Planck-Gesellschaft