Small helpers in the genome coordinate defence strategies in plants

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Wild tobacco (Nicotiana attenuata) native to North America is one of the ecologically best characterized plant species. This picture was taken in 2006 in Utah, USA. Image: Max Planck Institute for Chemical Ecology/Baldwin

Not only are ribonucleic acids (RNA) active as transmitters of genetic information between DNA and proteins, but they also have an impact on gene expression in the form of small segments, 18-26 nucleotides long. These nucleic acids, called small RNAs (smRNAS), regulate developmental processes in animals and plants.

Scientists in Prof. Ian Baldwin's group at the Max Planck Institute for Chemical Ecology in Jena have now found that smRNAs are also involved in plant defences against herbivores. After sequencing the

whole smRNA vocabulary in tobacco plants, they found about 110,000 "words" consisting of RNAs, each with a length of 15 to 30 letters.

Using this "dictionary," they showed in subsequent experiments that the smRNA transcriptome and therefore the "word choice" changed after insect attack; as a result, certain defence genes were regulated differently. One of the main players is the enzyme RdR1, an RNA polymerase that is involved in the production of smRNAs. In a second step of the analysis, groups of specific smRNAs regulate the hormonal balance of the attacked plants; in other words, changing hormones regulate the defence.

Small RNAs (smRNAs) play different roles in organisms. Their role in defending plants against viruses is particularly important. RNA-dependent RNA polymerases (RdRs) are responsible for the formation of some small RNA segments in cells. Genes that encode these kinds of enzymes have already been identified in several plant species. The regulation of genes based on smRNA is called "RNA interference".

Shree Pandey and Ian Baldwin asked whether RNA interference played a role in plant defense against herbivores. To investigate this, they cloned RdRs from wild tobacco (Nicotiana attenuata). They discovered that after only one hour one of the three RdR-coding genes they had found, RdR1, was ten times more active than usual in the plants that had been induced with the spit of insect larvae. Genetically modified plants in which the expression of RdR1 had been deactivated were nearly defenceless against herbivore attack.

It was remarkable that these plants were strongly attacked by their natural enemies, in particular Manduca sexta and Tupiocoris notatus. This means that RdR1 plays a crucial role in plant defence against insect herbivores. The researchers' next job was to find the RdR1 products, namely certain smRNAs, in the attacked and unattacked plants, identify

their sequences, and look at how these small RNAs influence the plant defence.

The scientists sequenced a total of 110,122 different smRNAs, which they had isolated from attacked and unattacked plants. Comparing smRNAs in attacked and "healthy" plants, they found that 43% of the smRNAs found in the herbivore-attacked plants were not present in healthy plants. 1,224 smRNAs were detected in both plant groups, but in different amounts: some were more present, others less so in induced plants. The emergence of many novel smRNAs after herbivory correlates directly with the increase of RdR1 gene expression in attacked plants, an increase that was about ten times higher than usual, demonstrating that RdR polymerases are involved in the production of smRNAs.

A bioinformatics analysis of the smRNA sequences showed that some of these "words" directly influenced the expression of those genes that regulate enzymes involved in the metabolism of plant hormones, especially jasmonate. Jasmonate is a signalling compound that regulates plant defence against insect herbivores. When the RdR1 gene is switched off in transgenic plants, jasmonate metabolism genes are down-regulated. This is easily observed because unlike other plants, transformed plants are heavily attacked by insects.

"We assume that the defenceless phenotype of RdR1-silenced plants results from RNA interference in the signal transduction pathways that activate genes involved in plant defence, as previously described in humans" says Ian Baldwin, director at the Max Planck Institute. Biologists have recently found that double-stranded RNAs, precursor molecules for smRNAs, can switch on a certain gene after injection into human cells - a rather unusual effect, because up to now many smRNAs had been identified as "gene inactivators". Baldwin continues: "but there are alternative mechanisms. We also want to consider the possibility that

smRNAs produced by tobacco after insect herbivory function as defences when they are ingested by attackers. Recently it has been shown that when insect larvae ingest double-stranded RNA produced by the plants that target genes in the insect, these insect genes are silenced. In this way, the smRNAs could function post-ingestively as direct defenses that target digestion or detoxifications systems in the insect".

Citation: Shree P. Pandey, Priyanka Shahi, Klaus Gase, Ian T. Baldwin, Herbivory-induced changes in the small-RNA transcriptome and phytohormone signaling in Nicotiana attenuata, *Proceedings of the National Academy of Sciences USA*, online first, March 14th, 2008

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