

Plant power

Dr Michael Wassenegger leads a major plant research programme in Germany, investigating epigenetics and RNA interference. His discoveries are opening the door to new crop management techniques and will ultimately lead to improved plant health at a time when food supplies represent a serious concern



From what context did the AlPlanta Research Institute emerge? Who does it primarily seek to serve?

Following the reformation of the agricultural administration in Rhineland-Palatinate, RLP AgroScience was founded in January 2005. This non-profit organisation is an amalgamation of the former Centre for Green Technology, now the AlPlanta Institute for Plant Research, and the Department of Ecology of the former State Education Centre Neustadt. The Rhineland-Palatinate State is the sole owner of RLP AgroScience.

The central mission of AlPlanta is practice-orientated research in the field of small crops, mainly fruit trees and grapevines but also basic research in the field of epigenetics, the results of which might be beneficial to the economy and society in the future. Our research and development contribute to the competitiveness of the region and Germany as a whole. We promote innovation, improve state-of-the-art technologies and participate actively in the education of young academics in close cooperation with different universities, such as the Ruprecht Karls University of Heidelberg.

What is RNA interference (RNAi) and why has this become a particular focus of your work?

RNAi was detected in plants in 1990 when plant genes involved in flower pigmentation were expressed as transgenes in petunia. Transgene expression appeared to co-suppress the transgene and endogenous gene. Further analysis of co-suppression phenomena revealed that interference with gene expression was mediated by RNA, pointing to an RNA-mediated gene silencing mechanism. In 1993, it was published that in plants, transgene-induced virus resistance was based on RNA-mediated post transcriptional gene silencing (PTGS). In 1998, Andrew Fire and Craig Mello demonstrated that in the nematode *Caenorhabditis elegans*, double-

stranded RNA (dsRNA) induced a gene silencing process closely related to PTGS. They coined the term RNA interference subsuming all RNA-mediated gene silencing mechanisms that were detected in almost all eukaryotes. Members of the protein families Dicer (Dcr), which process dsRNA into small interfering RNAs (siRNAs), and Argonaute (AGO) loaded with siRNAs, represent the key components of RNAi. RNAi mainly acts in the cytoplasm and ultimately leads to the degradation or translational inhibition of RNAs sharing homology with siRNAs.

Our interest in RNAi goes back to the early 1990s when we discovered a nuclear RNAi process termed RNA-directed DNA methylation (RdDM) in tobacco. Like RNAi, RdDM is triggered by dsRNA and, among others, it is dependent on Dcr and AGO proteins. RdDM is the most prominent de novo DNA methylation (cytosine methylation) mechanism in plants and recent datasets indicate that it is also functional in mammalian cells. The RNA seems to scan the entire genome to recruit the RdDM machinery to regions that are homologous to the dsRNA. Homologous regions become specifically and densely methylated and methylation of promoter sequences generally leads to transcriptional silencing of the corresponding gene. Since DNA methylation patterns are heritable, RdDM has a strong effect on plant epigenetics.

The importance of plants

The **AlPlanta Institute for Plant Research** in Germany is using plants as model systems to analyse epigenetic phenomena, aiming to elucidate pathogenic mechanisms which will inform the development of new crop varieties and management systems

How are you testing the correlation and interaction between RNAi and epigenetic alterations using genetic and biochemical approaches?

We developed several tools to induce *de novo* methylation at a sequence specific level. Within a target sequence the methylation status of each cytosine can be monitored by applying the bisulfite sequencing technology. Using plant lines deficient in RNAi-associated enzymes – eg. Dcr (in plants Dicer-like), AGO and RDR proteins – we can easily follow the effect of such mutants on the establishment of methylation patterns.

How does your work fit into the wider research conducted at AlPlanta?

Our plant expression platform is currently used to express high amounts of single-chain antibodies for diagnostics and antibody fragments for Hodgkin's disease therapeutics. RNAi-inducing transgene constructs were introduced into tomato and grapevine to render these plants resistant against viroids and viruses, respectively. Viral silencing suppressor proteins are used to immunoprecipitate sRNAs, including microRNAs (miRNAs), from total RNA fractions enabling the enrichment of sRNAs. Since several human diseases are associated with altered miRNA composition, analysis of miRNA profiles became an innovative tool in diagnostics. Efficient isolation of sRNA populations will be a prerequisite of sensitive miRNA profiling.

Finally, what ultimate impact do you hope this work will have?

The next-generation sequencing technologies we are using will deliver data which will allow a better understanding of how plants react to environmental biotic and abiotic stresses and enable much faster breeding processes. This will allow the development of varieties which will be better suited to deliver the high yields we will need to nourish the growing world population.

PLANTS PLAY A vital role in nature. Without them, there could be no life on Earth, with photosynthesis and respiration helping to maintain the Earth's natural balance of oxygen, carbon dioxide and water. Humans have been harnessing the power of plants for thousands of years, depending upon them for medicine, fuel, shelter, and perhaps above all, food. With the world's population growing and its climate changing, there are increasing pressures upon the Earth's resources, meaning research into plants has never been so important.

The AlPlanta Institute for Plant Research was established in 1996 in the German state of Rhineland-Palatinate to help fulfil this crucial requirement within the country. As one arm of the not-for-profit, state-owned RLP AgroScience, AlPlanta is now an internationally recognised partner of research institutions and industry all over the world, active in basic and applied research and also offering services in different fields of plant biotechnology. AlPlanta is organised around three research priority programmes: fruit diseases and phytoplasmas; grapevine biotechnology; and RNA-mediated gene regulation. The Institute is currently running six research and technology platforms using state-of-the-art methods and approaches which contribute to these areas.

FRUIT CROP AND GRAPEVINE DISEASES

Phytoplasma diseases of pome and stone fruits cause major yield losses in fruit production areas in Germany, and growers attach considerable importance to managing these diseases. In the short- and medium-term, AlPlanta's aim is to fight disease vectors, with a long-term aim of developing tolerant or resistant cultivars and rootstocks. Current projects are addressing apple proliferation disease and the European Stone Fruit Yellowing disease, as well as studies on pear decline disease. The group is also investigating virus diseases, and in particular Sharka disease, with a focus on the breeding of Sharka-resistant plum cultivars using genetic engineering.

Grapevine (*Vitis vinifera*) is one of the major horticultural crops grown in temperate climates, and in Germany viticulture covers approximately 100,000 hectares. In addition to this agricultural importance, the landscape characterised by viticulture and the associated infrastructure is a highly attractive draw for tourists. The protection of this delicate vine is therefore a concern for a range of stakeholders. In the area of grapevine biotechnology, AlPlanta is focusing on elucidating the epidemiology of various economically relevant diseases such as the bacterial disease Mauke and the fungal disease Esca, in order to develop sustainable and environmentally friendly management

strategies. A further aim is to establish grapevine plants with biotic and abiotic stress tolerance.

RNA-MEDIATED GENE REGULATION

Underpinning these programmes, AlPlanta's third research priority is the issue of how gene activities are regulated. The Institute's aim here is to improve understanding of the mechanisms of plant diseases and plant-pathogen interactions, and ultimately the establishment of new management systems. In plants, reversible gene inactivation mechanisms can be transmitted to the progeny. Thus, progeny plants do not bear genetic but epigenetic changes. In mammals and plants, these changes are generally associated with hypermethylation of the corresponding genes. Pathogens such as viruses use suppression of plant RNA silencing for survival, carrying genes encoding silencing suppressor proteins which can either bind small RNAs (sRNAs) or interfere with enzymes of the silencing machinery. Similar to initiation of RNA interference (RNAi), double stranded RNA (dsRNA) plays a key role in the establishment of new epigenetic markers. Moreover, in several organisms, RNAi deficiency mutants exhibit epigenetic anomalies, proving a direct correlation between RNAi and epigenetic alterations. Dr Michael Wassenegger is leading AlPlanta's research in this area and is aiming to elucidate the interaction between RNAi and epigenetic processes by applying genetic and biochemical approaches

Wassenegger's work has produced important results that may have implications for human as well as plant health. These include the discovery that RNA mediates *de novo* DNA methylation (RdDM) and thereby RNA triggers epigenetic changes. The group is also accumulating evidence that RNA has an impact on the establishment of epigenetic markers in mammals: "The fact that numerous human diseases, including cancer, genetic disorders and paediatric syndromes as well as contributing factors in autoimmune diseases and ageing, are associated with epigenetic abnormalities may emphasise how important it is to understand mechanisms involved in epigenetic changes," Wassenegger explains, adding that it is reasonable to assume that RNA-mediated manipulation of the human epigenome will enter therapeutics.

PLANTS AS MODEL SYSTEMS

Plant systems are ideal for analysing RNAi processes as well as epigenetic phenomena for a number of reasons. All RNAi mechanisms act on the transcriptional, post-transcriptional and translational level and share common components. However, in most cases, transcriptional gene silencing, post-transcriptional gene silencing

ALPLANTA INSTITUTE FOR PLANT RESEARCH

EPIGENETICS DEPARTMENT

OBJECTIVES

The Epigenetics Department aims to elucidate the interaction between RNAi and epigenetic processes. The Department's interest is not only to illuminate the underlying mechanisms but to also develop strategies to exploit them for plant adaption to environmental changes. Importantly, we propose that results gained from studying plants can be transferred to animal systems and humans.

PARTNERS

University of Heidelberg, Germany • Max-Planck-Institute for Developmental Biology, Germany • CNRS Institute of Plant Molecular Biology, France • CSIRO, Australia • FORTH, Greece • ABC, Hungary • ARO – Volcani Centre, Israel • Biodiversity and Environmental Research Centre, Palestine

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and translational gene silencing require specific members of enzyme families. These classes of enzymes mainly comprise variants of the Dicer or Dicer-like, Argonaute and RNA-directed RNA polymerase (RDR) proteins. Plants possess a complete set of these enzymes. In addition, plants accumulate all types of small regulatory RNAs, eg. small interfering RNAs, microRNAs and trans-acting small interfering RNAs.

Another advantage of using plants as model systems is based on the knowledge of plant viruses. Most of them are coding for proteins that inactivate RNAi. These silencing suppressor proteins are useful tools to analyse defined RNAi processes and can help to develop new strategies for the production of virus resistant plants. Finally, single plant cells can easily be produced and cultivated enabling investigations and manipulations of single cells. Equally, single cells can be regenerated into normal plants.

ADDRESSING SCEPTICISM

Based on the ongoing debate about genetically modified food, AlPlanta scientists postulate that their novel biotechnological approaches are also likely to be regarded by the public as problematic technologies. In 2011, AlPlanta held a workshop on the development of strategies for successful communication of novel technologies in the food sector in order to improve public opinion on food biotechnology. Wassenegger says that if scientists only offer facts and do not address the value laden structures of the public's perception

as well, communication will fail: "Central to communication with the public is trust. Therefore, building trust is of utmost importance," he adds. Although Wassenegger suspects that the GM debate may already be lost, he believes that similar social failings with regard to other novel biotechnologies could be prevented by adopting new communication strategies that AlPlanta are helping to develop.

THE POWER OF PLANT SCIENCE

Wassenegger is passionate about the future of plant research and its implications: "Plant research significantly contributes to the understanding of general biological processes. The last several years have seen revolutionary advances in DNA sequencing technologies with the advent of next-generation sequencing (NGS) techniques," he details. NGS methods now allow millions of bases to be sequenced in one round, at a fraction of the cost relative to traditional sequencing. As techniques and datasets continue to improve and grow, research is rapidly moving to the point where every organism, not just select model organisms, is open to the power of NGS. "In plant science, NGS will bring huge progress in the fields of molecular marker development, hybridisation and introgression, transcriptome investigations, phylogenetic and ecological studies, polyploid genetics, and applications for large genebank collections," Wassenegger adds – tools which may be invaluable in responding to the challenges that the growing world population faces.

