IPB Newsletter January 2013

Editorial

Dear reader,

Last year was overabundant with extraordinary activities. The 20th IPB anniversary celebration attracted all institute members and many guests and was an all-out success. The event was fired up by the 'Rolling Mill-Stones' with drums and trumpets. Numerous guests of honor, such as Leopoldina President Jörg Hacker and Leibniz Community President Karl Ulrich Mayer, kindly attended the ceremony – surely also prompted by the simultaneous celebration of the 80th birthday of Benno Parthier, our former director and former Leopoldina president. This public function was a great reward on its own but it was getting even better: Right on time, Marcel Quint treated us to a very special anniversary gift – an article in Nature with title page and cover story. He had produced the paper in a joint effort with Ivo Große (MLU) and four PhD students in all of eighteen months and – due to high-performance computer technology – without a single lab experiment. This is what we call an unparalleled low-cost and efficient approach. Also all other IPB researchers boasted a high publication record on phytohormones, biosyntheses and further topics so that we can look back on a very busy and successful year 2012.

This shall suffice, however, in terms of our track record: We are facing a similarly exciting year with interesting challenges. I trust we are all aware of the fact that we have to and intend to give proof of our excellence, strategies and expertise in a very special manner in 2013. Another seven-year-period has elapsed: That is, the IPB will be up for re-evaluation by the Senate of the Leibniz Community (WGL) in summer this year. We are looking forward with interest to this assessment and, of course, hope for a positive outcome.

In this spirit, I would like to wish all of you a successful 2013. May it be a busy, brilliant and brightly-colored year!

Best regards,
Sylvia Pieplow

IPB Newsletter January 2013

Table of contents

Emergence into a new era

The 20th anniversary of the reestablishment of the IPB was celebrated in a joyful atmosphere and relaxed mood by all institute members on 14 September 2012. The festive event also celebrated the 80th birthday of our founding director, professor Benno Parthier, who steered the institute's fate supremely well into the new science system of the Federal Republic of Germany. While the last issue was devoted to the IPB foundation period, this newsletter issue will give a brief historic survey of structural changes and scientific highlights in the past 20 years.

The Institute of Plant Biochemistry (IPB) was re-established on 1 January 1992 with the status of a Public Law Foundation under the protection and supervision of the government of the Federal State of Saxony-Anhalt. As a member of the Blue List Association (later: WGL) it is co-financed in equal parts by the German national and federal state governments.

Benno Parthier became its first executive director and in this capacity swiftly processed all pending administrative tasks and was instrumental in further implementing recommendations given by the German Science Council. One of the initial tasks was to stipulate organizational, structural and legal details of the foundation's purpose in its statutes that was adopted in May 1993. Advisory and controlling bodies were subsequently founded, namely the Scientific Advisory Board chaired by Jozef Schell on 6 January 1994 and the Foundation Board on 7 January 1994.

◊ New structures through changing times

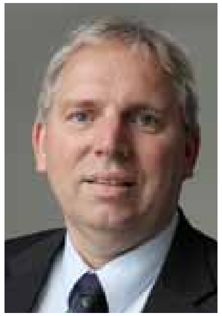
Department heads and directors

The three original scientific departments were complemented by a fourth division as recommended by the Foundation Committee. In 1993, **Dieter Strack** of Technische Universität Braunschweig was appointed by the institute as competent head of the new department **Secondary Metabolism.** Lothar Franzen took on the administrative management of the institute in the same year. In 1994, the IPB managed to entice **Dierk Scheel** of Cologne-based Max Planck Institute for Plant Breeding Research to become head of the Stress Research department – today **Stress and Developmental Biology.** He succeeded Lutz Nover who had accepted a position at Universität Frankfurt/Main in 1992.

From 1998, the institute had found in Dierk Scheel a very prudent and highly committed executive director. The organizational structure into four scientific departments was retained but there were

back to the table of contents

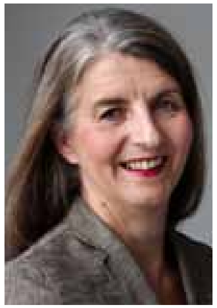
several changes in names and orientations and new appointments. Following the age-related retirement of Benno Parthier, in 1999 **Toni Kutchan** became head of the Hormone Research department, which was renamed into **Natural Product Biotechnology**. The Natural Product Chemistry department faced with a similar situation when its head Günter Adam retired in 1999.



Under the responsibility of **Ludger Wessjohann** this division gained new momentum from 2000 as **Bioorganic Chemistry** department. Toni Kutchan accepted a post in the US in 2006 and hence, held the management position only a bit over one year. Dierk Scheel was again appointed executive director and passed on this position to Dieter Strack in 2008. Responsibility for the Natural-Product Biotechnology department was temporarily assumed by **Claus Wasternack** but completely ceased

activities after his retirement. In 2009, the institute attracted **Steffen Abel** from UC Davis (US). Under his direction, the department was reoriented and renamed into **Molecular Signal Processing.** Dieter Strack

terminated his employment in October 2010 and his successor at departmental level became **Alain Tissier** of Montpellier. He extended the Secondary Metabolism department by research into Glandular Trichomes and it was renamed into **Cell and Metabolic Biology.** Ludger Wessjohann was appointed IPB executive director in October 2010.



Lothar Franzen left the institute in 2011 after long years of valuable work and was succeeded as administrative director of the IPB by **Christiane Cyron** of Hamburg in October 2011.

Junior research groups

In addition to the IPB departments, the first Independent Junior Research Group was founded in early 2007. **Marcel Quint** is heading this group, which is working on the topic of Auxin Signaling and

has established itself as a separate working group in the Molecular Signal Processing department.

Further junior research groups under **Marco Trujillo** and **Nico Dissmeyer** were established in 2011 to research into ubiquitin and protein degradation.

* * *

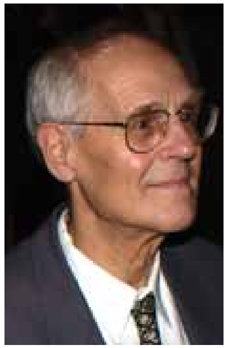
back to the table of contents

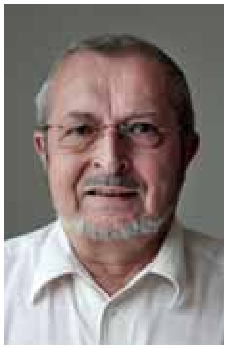
Scientific highlights - phytohormones, plant stress and natural products at focus of research

◊ **Phytohormones**

All-out research

The institute has conducted research and worked on all known phytohormones in its 50+ years of existence. The early years were devoted to ethylene and abscisic acid (ABA) and predominantly to **cytokinins** and **gibberellins.** Since the early 1980s, the institute has analyzed two new substance classes that were discussed as new phytohormones: **Brassinosteroids** and **jasmonates.** Brassinosteroid research officially ceased in 1997 with retirement of **Günter Adam** and petered out over the next few years. Molecular jasmonate research was initiated under **Benno Parthier** and was continued after his official retirement in 1997 by **Claus Wasternack.** Until his retirement in 2008, jasmonates were a top research priority at the institute, and today is dealt with by **Bettina Hause. Marcel Quint, Luz Irina A. Calderón Villalobos** and **Steffen Abel** brought the best-known phytohormones, auxins, back into the focus of research at the IPB.







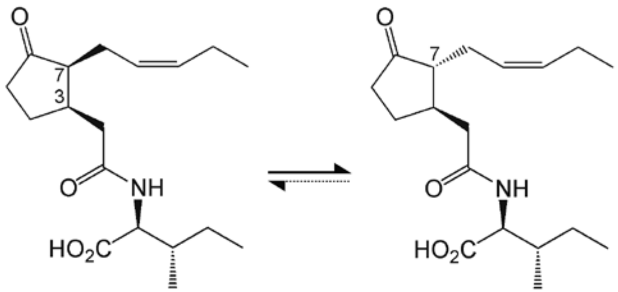
Hormone researchers in the post 1992 IPB era

Prof. Günter Adam, Prof. Benno Parthier, Prof. Claus Wasternack, Prof. Bettina Hause, Dr. Marcel Quint, Dr. Luz I.A. Calderón Villalobos and Prof. Steffen Abel (from left to right).

Jasmonates – quest for their active form

Jasmonates (JAs) are acting in a large variety of ways as all other phytohormones but play a specific role in stress defense. After wounding by plant-feeding insects, JAs induce for instance a whole battery of defense reactions, inter alia, production of a substance that causes digestion problems in insect intestines and prompts insects to let go of the plant. It was soon discovered in the more than 30 years of JA research at the institute that not only JAs proper but also their derivatives with various amino acids act as signaling substances. This finding enabled identification, isolation and characterization of many different JA derivatives with various biological activities. Much knowledge was accumulated about fine-tuning JA-regulated defense processes while the active jasmonate form – i.e. the molecule that acts as ligand of the JA receptor – remained unknown.

The underlying reason is its instability. It was not before 2009 that IPB researchers, in cooperation with teams in Madrid and Stockholm, identified the active form of jasmonate, which bonds to the relevant receptor and thus triggers the defense mechanism (*Nat. Chem. Biol.* **5**: 344-350, 2009). It is the *cis* form of the isoleucine conjugate of JA: (+)-7-*iso*-JA-Ile. The search turned out to be extremely difficult because the *trans* form the conjugate, (-)- JA-Ile, has a much higher stability and thus prevails in the *cis/trans* mixture of the two compounds. This result is the first-ever verification that only a certain stereoisomeric form of JA-Ile bonds to a JA receptor – a fundamental finding for hormone researchers.



(+)-7-*iso*-JA-L-Ile (-)-JA-L-Ile

***Jasmonate** in its active cis form (left) and inactive trans form.*

◊ Stress Research

Plant tolerance to heavy metals

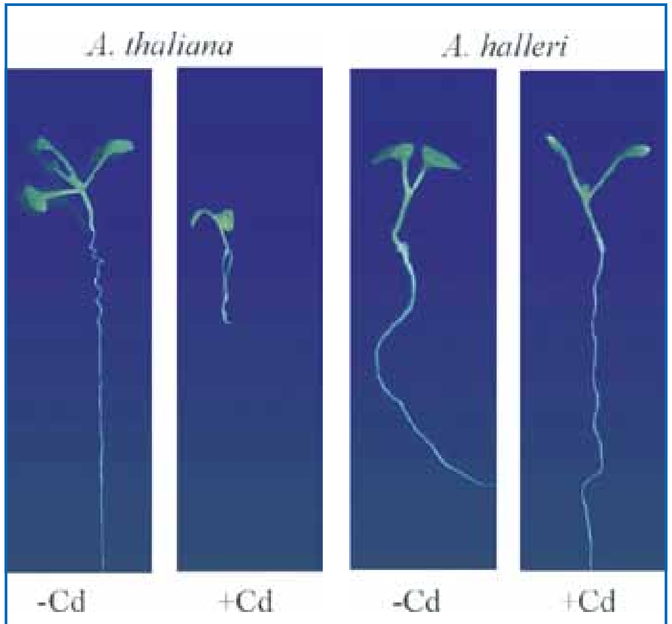
Outstanding research was performed by **Stephan Clemens** on plant metal balance and heavy-metal resistance in various plant species. Plants, like any other organism, have to protect themselves from toxic heavy metals. Plant root-based metal transporters do not specifically distinguish between harmful and useful metals; hence, often toxic metals are absorbed together with essential metals. Two examples to illustrate this fact are cadmium and zinc; two elements with strong chemical similarities, which only occur together in nature. While cadmium wreaks all-out havoc in a cell and causes enormous damage, zinc is a co-factor of many enzymes and essential for plants. Metal-binding peptides – so-called phytochelatines – enable plants to bind toxic substances, such as cadmium, arsenic, mercury and copper, and thus strip them of their reactivity as free ions. This

back to the table of contents

mechanism allows most plants to tolerate low concentrations of harmful heavy metals, which are accumulated in their roots for good.

To gain a better understanding of the mechanisms of metal absorption, transport and accumulation, Stephan Clemens and his colleagues compared gene activities of two different model plants: *Arabidopsis thaliana* (mouse-ear or thale cress) and *Arabidopsis halleri* (meadow rockcress). Arabidopsis halleri grows on abandoned mining waste tips in the Harz Mountains. In contrast to its close relative, *A. thaliana, A. halleri* tolerates cadmium in high concentrations.

The plant is a so-called **metal hyper-accumulator,** which boasts special buffer mechanisms, which permits toxic metals to be transported into its leaves for accumulation. The comparison of activities by the Halle-based researchers yielded the following: Several metal homeostasis genes are more activated in *A. halleri* than in *A. thaliana* (*Plant Journal* **37**, 269-281, 2004). Several of such activated genes are coded as



***Arabidopsis thaliana** (left) reacts sensitively to cadmium while **Arabidopsis halleri** tolerates high toxic metal concentrations.*

metal transporters or enzymes, which catalyze synthesis of metal-binding molecules, while the functions of other genes are still completely unknown. Hence, plant adaptation to extreme location conditions is not due to the existence of specific *tolerance genes* but rather to modified regulation of individual genes. Clarification of activated gene functions will provide fundamental knowledge about the molecular bases of metal balance and heavy-metal resistance.

Advanced research in this field has not been conducted at the institute since 2006. Relevant findings obtained at the IPB should be integrated, though, into developing practice-oriented remediation technologies for heavy metal-contaminated soils. Improved understanding of heavy metal tolerances could contribute to developing plants with a high biomass, fast growth rates and large heavy-metal accumulation capabilities in their leaves. Such plants would simply be sown on contaminated soils and harvested, together with highly-concentrated toxic metals.

* * *

back to the table of contents

Innate immunity in plants

Very much like animals, plants have developed in the course of evolution highly efficient defense strategies against a broad range of hostile invaders. Most plants, as all organisms, are permanently exposed to potential pathogens but rarely catch a disease. Apparently, plants – similar to animals – have a kind of basic immunity which prevents their becoming host plants for pathogenic microorganisms. The molecular bases of such non-host resistance are at the focus of IPB stress research.

A shared feature of innate immunity in animals and plants was postulated proceeding from findings on innate immunity response in vertebrates: A potential host identifies certain surface structures of a pathogen and classifies such as *alien*. This discrimination is followed at cellular level by inducing local inflammatory reactions to prevent multiplication and eventually kill the pathogen. Substances ,which are identified by a host as *alien* and fended off are fundamental molecular structures of microorganisms that are wide-spread in the microbial world and of such significance for their survival that they have hardly modified in the course of evolution. Large numbers of such *pathogen-associated molecular patterns (PAMPs)* were found in animal pathogens, including cell-wall components of fungi (such as chitin) and bacteria (such as flagellin of flagella and flagellates) but also fragments of bacterial DNA. Such molecular recognition patterns were long unknown in plant disease pathogens.

Dierk Scheel and **Thorsten Nürnberger** are among the first scientists to have discovered a PAMP structure also in a plant-pathogenic organism (*Science* **276**, 2054-2057, 1997). They found a peptide consisting of 13 amino acids **(Pep 13)** that is part of a transglutaminase on the surface of the late blight pathogen *Phytophthora infestans*. According to their findings, Pep13 fulfils all criteria defined for PAMPs (*EMBO J.* **21**, 6681-6688, 2002). Pep 13 is an exogenous molecule to plants but was verified in ten different *Phytophthora* species. In all ten analyzed species-specific transglutaminases, Pep13 consisted of exactly the same 13 amino acids – hence, it was preserved during species-to-species transition and not subject to any mutation. Replacement of a single amino acid in the peptide resulted in a functional loss of the relevant transglutaminase.

Transglutaminases are enzymes that catalyze cross-linking of certain proteins. In animals, they are involved in nerve growth, bone formation and wound healing. Their function in *Phytophthora* is still very much unclear. What has been clarified, however, is that transglutaminase triggers defense reactions in various plants even without the pathogen. And if this were not enough: Pep13, a minute enzyme component, is capable of inducing plant protection mechanisms. Injection of this peptide into parsley and potato leaves prompted multiple cellular defense responses in both plants, such as production of phytoalexins, highly-reactive oxygen compounds and other anti-microbial substances. Pep13 hence is the first PAMP for plant

back to the table of contents

pathogens. Pep 13 (synthetically produced) is still being used to study plant defense reactions, rather than pathogens proper which require cumbersome cultivation and have to be kept alive.

***Phytophthora infestans**, the late blight pathogen wreaks great havoc on potatoes and tomatoes.*

Most other pants are spared as they are not its host plants.

* * *

◊ Active substance research

New substances for the world

Resistance to antibiotics, new diseases, soil salinization, draughts, climate disasters, overpopulation, hunger, epidemics – there is a long list of challenges to be tackled. Plant, fungal and bacterial active substances have accompanied mankind since its coming into existence in combating diseases and death. Multiplication of population figures entails increasing problems – this is why the search for healing substances in nature will remain an urgent issue for relief and mitigation. The search is currently intensifying: Substances, as yet unknown but with much-needed effects. Finding active substance has always been a characteristic pillar in the institute's research profile. But apparently it is not enough to consult shamans and healers in exotic cultures for plant-based anti-flu agents. Such ethno-pharmacological projects are still being pursued at the IPB but our attention is increasingly concentrated on active substances in less-researched organisms, such as fungi and algae. Our researchers found in fungi (genus *Hygrophorus*) a highly-active antibiotic for pyogenic pathogens and promising candidates for curbing late blight in potatoes.

Finding active substances has changed

Nowadays one is not searching for individual substances – selected organisms are rather combed through for everything that seems to be helpful. Or even for everything that is present (or can be verified with currently available analytical methods) – as it may turn out to be helpful in the future. Modern computer technology enables comprehensive asset surveys of whole substance classes per organism. Substance libraries and databases are fed with vast numbers of small molecules and complex metabolites; albeit that very few of them in terms of their structures and functions.

Structure elucidation and synthesis

Identification of the most promising candidate substances in laborious bioactivity tests (screening) is followed by elucidation and, where possible, synthesis of their structures. Synthesis is of key importance as it preserves natural resources,

back to the table of contents

provides sufficient substances for further analyses and makes researchers more independent from climatic conditions; most fungi, e.g., are predominantly found in fall. Synthesis is also a precondition for active substance optimization. The biological effects of many natural products can be enhanced by minor modifications, such as attaching of functional groups. Targeted modification of one molecule, however, presupposes exact knowledge, which part of the molecule controls its efficacy. Structure/efficacy analyses are necessary and exciting but highly time-consuming.

One-pot evolution

IPB researchers also rely on the random principle. The multi-component reaction developed at the institute enables assembly of active substances from single modules. Minor modifications of individual modules yield multi-faceted combinations and a whole pool of chemical varieties of a given active substance, which are similar but not identical to their natural paragon. The whole process is carried out in a single synthesis cycle and in one reaction vessel. The result of this one-pot reaction is a whole library of potentially effective substances that is again combed through for the most promising candidates. There is high probability that among synthetically produced substances there are higher-activity variants with better pharmacological profiles than among the original natural active substances. And: One learns quite incidentally which structure achieves the optimal effect. Potential anti-cancer agents, such as complex-structured tubulysin were already synthesized at the IPB in multi-component reactions.

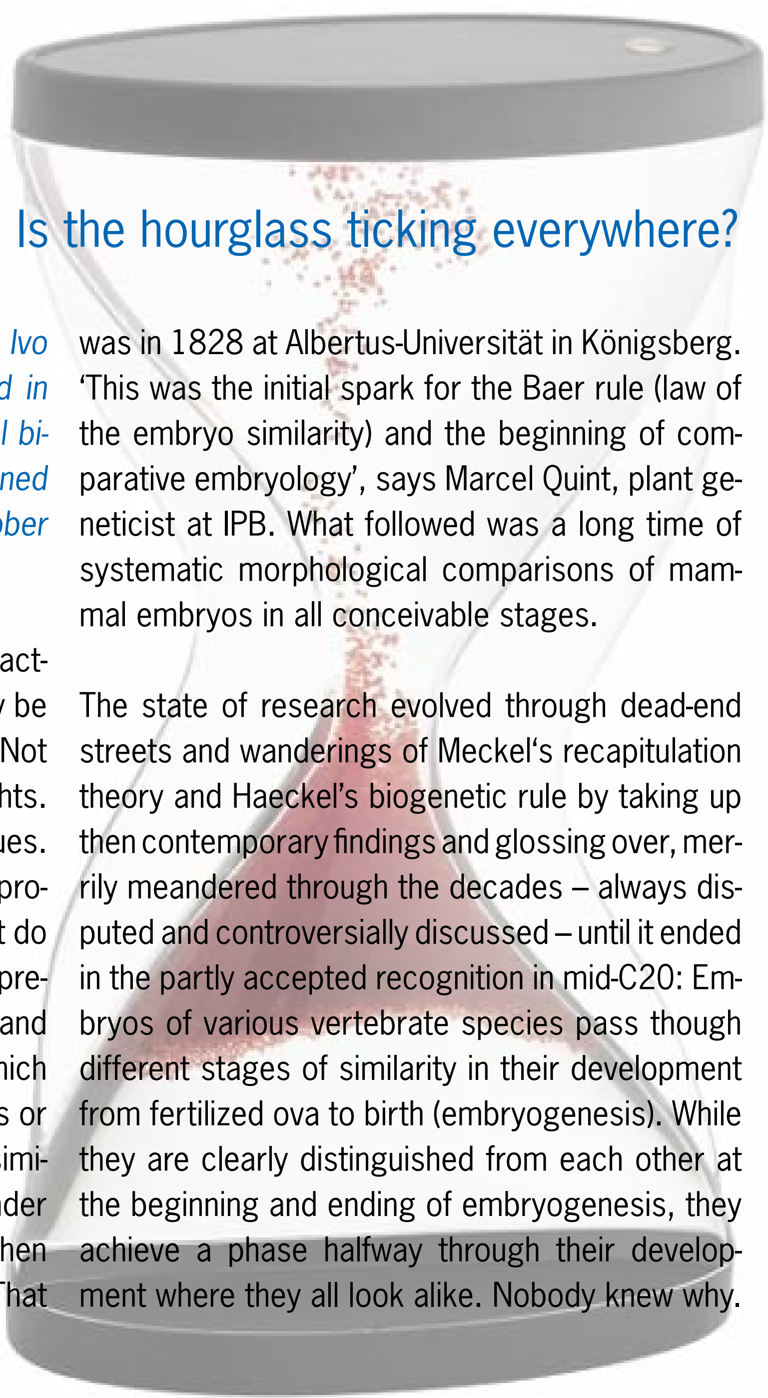
In-silico screening

The whole process is even more efficient when an active substance is initially computer-modeled to develop and optimized versions. Appropriate search criteria are defined to comb through millions and millions of molecules in virtual structure databases for suitable active substances. This may be, for instance, substances that bind to certain enzymes and inhibit or activate them. Such binding studies of virtual enzyme models limit the number of potential binding partners. Thus, several hundred thousand initial structures are analyzed to identify a maximum of one hundred candidates for review in biotests. *In-silico* screening helped IPB researchers identify promising molecules to protect plants from drought stress.

* * *

back to the table of contents

From Arabidopsis to Zebrafish: Is the hourglass ticking everywhere?

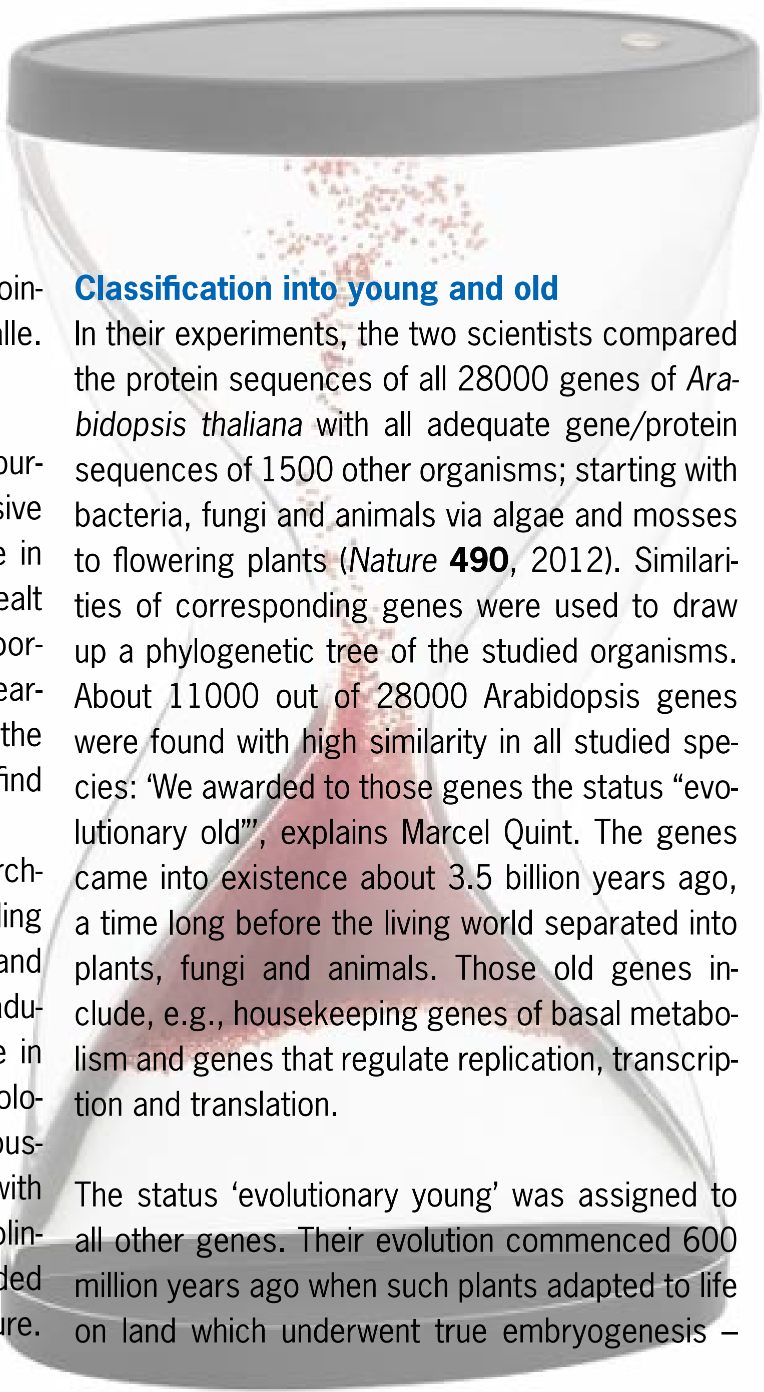


Marcel Quint, in excellent cooperation with Ivo Große of Martin-Luther-Universität, succeeded in elucidating an evolutionary and developmental biology phenomenon of plants. The paper earned the authors the cover story in the Nature October 2012 issue.

This story started with an error. Or more exactly, a bit of negligence to which people may be susceptible in the frenzy of their zest for action. Not rarely, such minor slips give rise to major insights. Provided such are not brushed off as side issues. Karl Ernst von Baer, a German zoologist and a progressive thinker in his times, apparently did not do the latter when he took down one day: 'I had preserve embryos of two species in ethyl alcohol and forgotten to label the jars. Now I cannot tell, which jars holds which species. They could be lizards or little birds, or even mammals.' The apparent similarity of his two preparations made him wonder and maybe caused annoyance or doubt – but then he started to get to the bottom of the matter. That was in 1828 at Albertus-Universität in Königsberg. 'This was the initial spark for the Baer rule (law of the embryo similarity) and the beginning of comparative embryology', says Marcel Quint, plant geneticist at IPB. What followed was a long time of systematic morphological comparisons of mammal embryos in all conceivable stages.

The state of research evolved through dead-end streets and wanderings of Meckel's recapitulation theory and Haeckel's biogenetic rule by taking up then contemporary findings and glossing over, merrily meandered through the decades – always disputed and controversially discussed – until it ended in the partly accepted recognition in mid-C20: Embryos of various vertebrate species pass though different stages of similarity in their development from fertilized ova to birth (embryogenesis). While they are clearly distinguished from each other at the beginning and ending of embryogenesis, they achieve a phase halfway through their development where they all look alike. Nobody knew why. Still, a technical term was swiftly coined: The phase of maximal morphological similarity henceforth was termed phylotypic stage. In the early 1980s, the term *hourglass model of embryogenesis* was established as the phylotypic stage was symbolized by the narrow passage at the centre of an hourglass. It was only in 2010 that the molecular cause of this phenomenon was found and the morphologically-based development model was provided with a genetic foundation. It was verified both in zebrafish and fruit fly that in the middle phase of embryogenesis only the old, less modifiable (highly conserved) genes are active (Kalinka et al & Domazet-Loso *et al; Nature* **468**, 2010). The old genes have hardly changed for at least a billion years and, hence, are almost identical in all species: Accordingly, their embryos are alike also morphologically in shape and structure; they form a kind of original-type embryo. 'It was only those genetic correlations that brought acceptance among experts of the hourglass model as fundamental developmental-biology pattern in mammals

back to the table of contents



and insects', says Ivo Große, professor of bioinformatics at Martin-Luther- Universität in Halle.

And what about plants?

'That was exactly the question we asked ourselves', says Marcel Quint. Their comprehensive literature research about the phylotypic stage in plants failed to yield results. 'Either no-one dealt with this problem yet or it was not deemed important and sunk into oblivion', opines the 38-year-old. 'But we were fascinated by the news of the hourglass in animals. We urgently wanted to find out more about embryogenesis in plants.'
This project brought the two Halle-based researchers together again to continue their long-standing cooperation. Ivo Große, a graduate physicist and now bioinformatician, and Marcel Quint, a graduate horticulturist, holder of a doctoral degree in agronomics who is currently working as a biologist. This fluctuation between disciplines obviously helped them enormously to communicate with each other. The efforts entailed by an interdisciplinary approach have paid off – and were rewarded after 18 months of work by publication in Nature.

Classification into young and old

In their experiments, the two scientists compared the protein sequences of all 28000 genes of *Arabidopsis thaliana* with all adequate gene/protein sequences of 1500 other organisms; starting with bacteria, fungi and animals via algae and mosses to flowering plants (*Nature* **490**, 2012). Similarities of corresponding genes were used to draw up a phylogenetic tree of the studied organisms. About 11000 out of 28000 Arabidopsis genes were found with high similarity in all studied species: 'We awarded to those genes the status "evolutionary old"', explains Marcel Quint. The genes came into existence about 3.5 billion years ago, a time long before the living world separated into plants, fungi and animals. Those old genes include, e.g., housekeeping genes of basal metabolism and genes that regulate replication, transcription and translation.

The status 'evolutionary young' was assigned to all other genes. Their evolution commenced 600 million years ago when such plants adapted to life on land which underwent true embryogenesis – multi-cellular and differentiated. The classification into young and old was followed by comparison of genetic activities of all 28000 Arabidopsis genes in seven different embryonic stages, staring with zygotes *via* hearts and torpedoes to mature embryos. 'We were truly amazed by our findings', admits Ivo Große. What was found, as in animals, was that the evolutionary young genes were purposefully deactivated in the middle embryogenesis, in the torpedo stage, and later re-activated. The old genes, in contrast, remain active through all stages of development.

'We found all necessary sequencing and expression data in publicly accessible databases of the Scientific Community', says Große. It took a high-performance computer one week to compute all data – about 420 billion sequences had to be compared to each other. The computer is made up of 2000 processors which are stacked like pizza boxes in meter-high cubicles in the EDP centre at Halle Universität. 'An ordinary computer would have needed years', adds the 42-year-old bioinformatician. There was, of course, a lot of

back to the table of contents

front-end work. Several gigabytes of sequence data had to be correctly downloaded from the Internet and validated. Databases were established, scripts written and parallelized. ‘Everybody who is making alignments knows: Alone comparison of all of two gene sequences can be problematic’, says the Berlin-born scientist. The program – either permitted to perform liberal or conservative comparisons – may show wrong positive homologies or no hits although such are there.

‘We started off by reproducing Drosophila and zebrafish data’, explains Große. ‘Then we tweaked the parameters of the analysis pipeline to adopt it to plants. Thus, we gradually entered new territory.’ The main work was performed by two excellent students, hand-picked by Große and Quint and encouraged to join the project. ‘As a result, Hajk-Georg Drost and Alexander Gabel authored to outstanding bachelor’s theses with Nature publication – not a bad entry into a scientific career’, is their comment with a wink.

***The Green Hourglass** decorated the cover of the leading scientific journal Nature (4 October 2012 Vol. 490, No. 7418).*

Design: Sisters of Design GbR, Halle

How to interpret the data?

The findings of the Halle-based researchers again evidence a convergent progression of evolution. 'Albeit that embryogenesis came only into existence after the separation of fauna and flora, its principle – the hourglass principle – has remained the same in the two kingdoms', says Marcel Quint. 'What is unclear, though, is why evolution yielded the same result in two different ways and why the hourglass was established in the first place.' The middle embryonic development phase in animals is obviously the phase where all important organs are forming. Mutations establish easier in young genes; hence, deactivation of such modifiable genes could cause a very stringent sequence of the genetic program and make it inaccessible for alterations. Under the strict rule of the old genes, cells are forming body axes and organ combinations at appropriate times and in appropriate places. The structure has to be complete before it is species-specific and individually clad by mean of the young genes. 'This kind of quality control could be one cause of the hourglass principle', says Marcel Quint. 'It is not clear, however, whether the genetic checkpoint can also explain the hourglass in plants'. Plant embryos, with hypocotyl and two cotyledons, are less complicated structured than animal embryos. In addition, the most significant plant organs, such as roots, leaves and blossoms, are only formed after "birth" and not during embryogenesis. 'This is why we are interested to find out what is happening in the whole plant after germination. Are old or young genes activated or de-activated?' It was found in zebrafish that genetic activity of young and old genes changes again at high age, shortly before death. Young genes are de-activated also in this stage, while old genes remain active. Nobody knows why, and neither whether the same is happening in plants. 'There are still many fascinating problems waiting to be solved', the two scientists agree. And all this was sparked off because Karl Ernst von Baer once forgot to label some jars?

Professor Ivo Große *(left) and* ***Dr. Marcel Quint*** *discover the hourglass in plants.*
Photo: Maike Glöckner (Universität Halle)

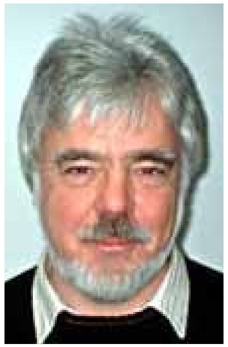
* * *

back to the table of contents

News from science

◊ **Phytohormones**

Auxin acts through a co receptor

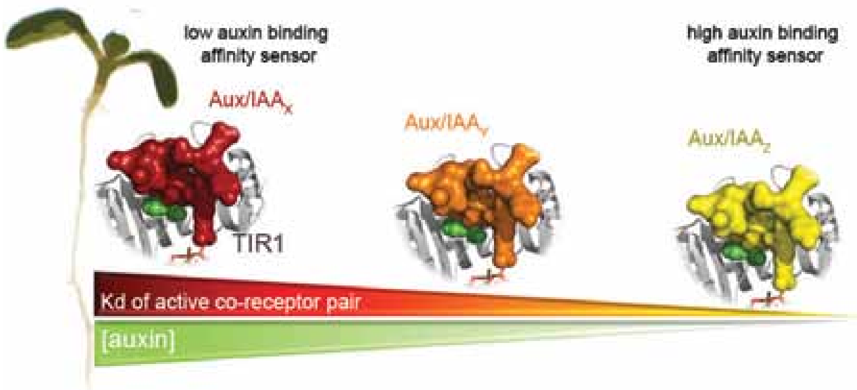


Luz Irina A. Calderón Villalobos presented a comprehensive and highly ambitious study on fine-regulation of auxin efficacy at the *10th International Congress on Plant Molecular Biology* in Korea in October 2012. The results were published in co-operation with other scientists in the renowned journal *Nature Chemical Biology*. Experimental work supporting the paper was performed in Mark Estelle's lab at *University of California* in San Diego. At the IPB, Ms. Calderón Villalobos teamed up with Wolfgang Brandt and they reaped the harvest together. Auxin, the longest-known and probably best-studied phytohormone, time and again is baffling academia even after 60 years of intensive research. Its effects on stretching growth, flower formation, fructification, aging and a variety of other processes make auxin a participant in almost all plant processes of cell division and cell growth.

The mode of action of phytohormones has given rise over time to a notional model system, which is now confirmed or refuted by experimental findings. Accordingly, phytohormones are acting as parts of a close-meshed signal network of many diverse molecular components, which interact with each other. The task at hand is to identify those components and clarify their interplay. The most interesting feature in a signal chain is always the first step: Binding of a hormone to its receptor and the triggered cellular reactions.

The auxin receptor was first identified in 2005 (*Nature* **435**, 446-451). Yet, although identification of the receptor was deemed a milestone in auxin

One puzzle stone
to our knowledge about the efficacy of auxins and other phytohormones was contributed by Luz Irina Calderón Villalobos.
Graph: *José Andres Archila Castaño*



Three potential binding combinations of Aux/IAA repressors with TIRI repressors. Auxin (green) couples the two partners into a receptor complex. High- or low-affinity complexes are formed, depending on the respective binding partners.

research, it was established that the found TIR1 receptor binds auxin at such low affinity that only a minor efficacy was expected. Elucidation of this curiosity was now provided by Calderón Villalobos et al. with her contribution in *Nature Chemical Biology*. For the first time in the history of phytohormones she was able to verify that auxin does not bind to a single receptor protein but rather functions as a link between two components of one receptor complex. These two components, TIR1/AFB receptor and Aux/IAA repressor are located in the cell nucleus, directly on the DNA, in the regulatory region of several genes that are activated by auxin. This would suggest that auxin binds to the receptor complex and causes degradation of the Aux/IAA repressor. This repressor previously blocked the following genes, which are free now and can be transcribed. And if this were not enough: Arabidopsis cells have six different TIR1 receptors and, in addition, 29 variants of Aux/IAA repressors. Hence, it was logical to assume that there is not *the* Auxin receptor but that combination of the two binding partners generates various auxin receptors which help plant cells to react to different challenges.

The authors carried out comprehensive binding studies, completed by 3D models of co-receptors, and were able to verify that formation of different receptor complexes is biochemically possible through combination of their two individual components. In addition, different co receptor pairings had different affinities to their auxin substrate. Some bound the hormone better and at lower concentrations than others. This – according to the authors– could enable a staggered regulation of auxin efficacy to enable a plant to react to different stimuli both quantitatively and qualitatively.

TIRI was previously only known for its function as an F-box protein, i.e. an enzyme that binds other proteins and thus marks them for degradation. The discovery that a TIRI-like F-box protein, in conjunction with small molecules like auxin, functions as part of a receptor complex is a novel finding. And: This principle seems to be of a general nature. A similar principle of binding to a co receptor system was recently also found for jasmonates. The authors have managed with their publication to contribute a significant puzzle stone to the overall understanding of auxin and phytohormone effects.

* * *

back to the table of contents

◊ **Biosynthesis**

Fragrant raw material in two steps

Biosynthesis of ***cis*-abienol**, a constituent of Virginian tobacco (*Nicotiana tabacum*), has now been fully elucidated. Alain Tissier and Romy Töpfer recently published their biochemical genetic analyses of *cis*-abienol biosynthesis in various tobacco varieties in *The Plant Journal*. The publication, prepared in cooperation with French and Swiss scientists, gives a comprehensive report of all aspects of abienol synthesis in *N. tabacum* which prompted the journal editors to choose it as featured article with a presence on the cover page. According to the authors, *cis*-abienol is produced in two synthesis steps from a primary metabolism product, geranylgeranyl pyrophosphate (GGPP), and exclusively in glandular trichomes of Virginian tobacco. Genes coding for the two enzymes were very elegantly isolated and characterized. Subsequently, 157 different tobacco varieties were analyzed for presence and functional efficiency of the two identified biosynthesis genes of *cis*-abienol. The result: All studied varieties have biosynthesis genes but only 50% of the analyzed varieties are capable of producing *cis*-abienol.

The reason for missing abienol in these plants is a mutation in one of the two synthesis genes. The very comprehensive study was prompted by application-related aspects. *cis*-Abienol, a bicyclic diterpene, is a labdan-like compound whose name is derived from **labdanum**, an aromatic resin that was produced from Mediterranean gum rockrose already in ancient times. Labdan-like compounds are currently used as starting materials to produce ***Ambrox®*** – a precious fragrant that unfolds its bouquet in many well-known perfumes **(see p.18)**. *cis*-Abienol may be the next potential starting material for semi-synthesis of *Ambrox®*. To date, *Ambrox®* is predominantly produced from sclareol, another labdan-like compound, that is extracted from clary sage (*Salvia sclarea*). Sage is very sensitive to climate changes, which is why there is a high fluctuation of sclareol yields from year, to year which makes an impact on world market prices. Tobacco is much more robust than sage plus has a higher biomass. Hence, production of an *Ambrox®* raw material from tobacco leaves would be safer from fluctuations and more effective. *cis*-Abienol, however, makes up less than ten per cent of the dry mass of a tobacco leaf and thus it would be desirable to breed new varieties with an increased percentage of the coveted raw material. Yet, this will only be possible with profound knowledge of biosynthesis and genetic constellations of individual tobacco varieties. If the genes were better known, production of *cis*-abienol could be shifted to bacteria or yeasts. The biotechnological production of this fragrant raw material is now at the focus of further research projects at the IPB.

* * *

News Ticker: Research

From Ambergris to Ambrox® - The secret of Chanel and Davidoff

The Chinese took it for 'dragon spit' spouted out by drooling marine iguanas while sleeping on rocks. Egyptian merchants, on the other hand, believed the matter grows like mushrooms on the bottom of the sea and is washed ashore by occasional storms. Only after the flourishing of the American whaling industry in the 18th century it could be proven beyond doubt that ambergris – that mysterious fragrance from the sea – hails from the bowels of sperm whales. But it would an oversimplification to despicably term it whale feces. Ambergris, a wax-like substance is produced by sperm whales as an antibiotic wound closure after mechanical injuries of their intestines. The coprolites weight up to 100 kilograms and get into the sea through excretion or whale death. Its low density makes ambergris float on the sea surface – where the dark-gray matter with fecal odor is converted through photochemical degradation and oxidation into a bright-gray, highly-esteemed raw material for the perfume industry. This process takes years to even decades. The most significant olfactory constituent of ambergris is ***(-)-Ambrox®***. It combines, according to manufacturers, *a small of wet mossy forest soil, strong tobacco and balsamic sandalwood, mixed with a warm animal musk note.* Already in the ancient world, ambergris was much-coveted fragrant in Arabian cultures. From the 10th century, matured whale feces were traded in the entire Mediterranean region and later all over Europe. On pound of ambergris was worth its weight in gold or cost the equivalent of three slaves. Sperm whales are protected today under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the fragrant are semi-synthetically produced. *Ambrox®* is found in small, albeit insufficient quantities, in Virginia tobacco *(Nicotiana tabacum)*, clary sage *(Salvia sclarea)*, gum rockrose *(Cistus labdaniferus)* and Mediterranean cypress *(Cupressus sempervirens)*. The first successful semi-synthesis of *Ambrox®* was made in 1950 from the diterpene sclareol (clary sage). Water vapor distillation of 100 kg inflorescence and growing tips yielded some 800 grams clary sage oil which – depending on its cultivation region and climate conditions – contains a certain percentage of sclareol. Sclareol is still produced from sage through similar extraction. Annual global production of sclareol is between 50 and 150 metric tons. Alternatively *Ambrox®* is produced from *cis*-abienol that is extracted from resin of the Canadian balsam fir (*Abies balsamea)*. Several other semi- and total syntheses have been continually developed over time; however, their full-scale industrial application has failed due to low yields. One alternative may be production of *cis*-abienol as starting material of *Ambrox®* synthesis from tobacco. *Ambrox®* is the base substance for *Chanel No.5®* and *Davidoff Cool Water®*. Annual global production of this sought-after fragrant is slightly more than 30 metric tons. The price of one kilogram Ambrox® is ca. 1000 USD.

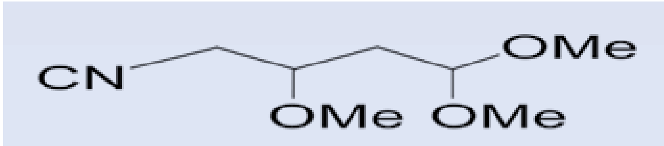
Source: *Schäfer, B. Ambrox®. Chemie in unserer Zeit 2011,* ***45****, S. 374-388.*

News Ticker: Research

◊ New Reagent

IPB substance for one-pot synthesis

IPB is the name of a new reagent that was developed by our chemists. The abbreviation stands for *4-**i**socyano**p**ermethyl**b**utane-1,1,3-triol.* This substance belongs to the group of convertible isonitriles. IPB is capable of inserting carboxylic acid groups (-COOH) or amides and esters into other molecules. Thus, IPB can be used in so-called one-pot or multi-component reactions for assembling complex molecules. The new reagent is easier and cheaper produced than previous substances of this type. It has a higher stability, dissolves better and has a higher reactivity than its predecessor molecules. At the IPB institute, IPB is used to synthesize natural products and modified peptides (protein fragments).



*__IPB__, the new reagent of our chemists, stands for 4-**i**socyano**p**ermethyl**b**utane-1,1,3-triol.*

* * *

Imprint:

The IPB Newsletter is published twice a year. Any further use of photos and contents is subject to approval by the editors.
January 2013

Editor:
Leibniz Institute of Plant Biochemistry
Weinberg 3
06120 Halle
Germany
www.ipb-halle.de

All photos by IPB, except:
Hourglass (p. 12/13): Stefan Muemmler, www.wissenswertes.biz
Puzzle (p. 15): José Andres Archila Castaño

German Texts:
Sylvia Pieplow

English translation:
inlingua®

Composition and layout:
Sylvia Siersleben