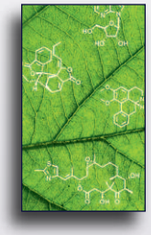
IPB Research Highlights

Understanding Biochemistry of Plant Resilience

Despite their sessile lifestyle, plants and fungi are no helpless victims of their fate. In the course of evolution, they have developed the ability to produce a variety of bioactive compounds that enable them to communicate with their environment and to defend themselves against pathogens or cope with different stresses. Research at the IPB focuses on a comprehensive molecular understanding of plants' adaptive and developmental processes. The knowledge gained paves the way to a plant-based bioeconomy: it facilitates sustainable crop production, innovative biotechnology, and drug development to improve the nutrition and health of humans, animals, and plants. We conduct research in four departments.

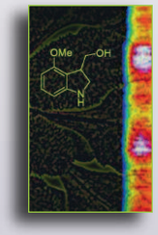
Prof. Steffen Abel
Department Molecular Signal Processing

Major areas of interconnected research activities comprise the perception of abiotic and biotic cues, the action and integration of chemical mediators, and the coordination of metabolic and cellular processes during adaptive plant development.



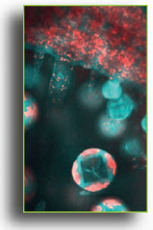
Prof. Ludger Wessjohann
Department Bioorganic Chemistry

Our primary goal is the development of new bioactive compounds and biocatalysts, inspired by the research of natural substances and processes. Such bioactives are used e.g. as active principles in medicines, for a healthier nutrition or for crop protection.



Prof. Tina Romeis,
Department Biochemistry of Plant Interactions

We investigate interactions in, of, and with plants, which guarantee the survival, growth, and yield of plants even under unfavourable environmental conditions or infestation by pathogenic microorganisms or herbivores.



Prof. Alain Tissier
Department Cell and Metabolic Biology

In our department, we aim at elucidating the biosynthesis of specialized plant metabolites, and at understanding the role they play in the life of plants. This can be in the defense against aggressors, but also in attracting beneficial partners or during development.

Program Center for Plant Metabolomics and Computational Biochemistry

As a new interdisciplinary research program, the Program Center MetaCom combines the capacities of high-performance analytical equipment at the institute and its expertise in the fields of natural product chemistry, metabolomics, and chemo- and bioinformatics. The program aims to achieve a comprehensive chemical understanding of plant resilience. With MetaCom, the IPB intends to become an international reference center for specialized natural products and thus make a visible contribution to solving current challenges such as climate change and food security.

IPB in Facts and Figures 2023

176
Employees

26
Nations

19,9 M €
Total Budget

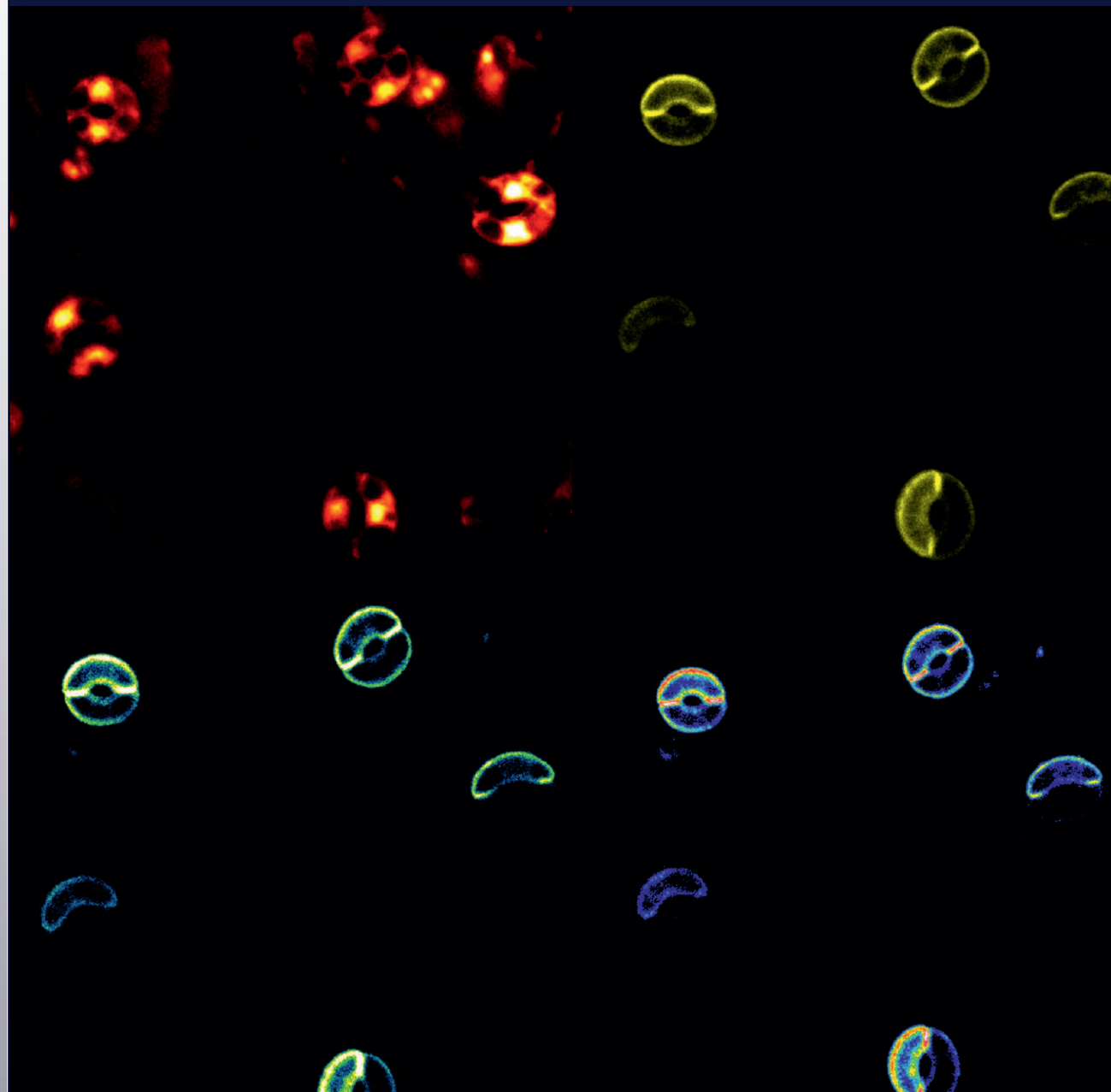
23
15
PhD Students
Defended Doctoral Theses

105
Publications

2400
Visitors

61	Interviews and Articels
126	Articles on ipb-halle.de
139	Tweets
2873	Followers on X (formerly Twitter)

New fluorescence sensor shows CDPK activation in real-time



New fluorescence sensor shows CDPK activation in real-time

Plants respond to pathogen attack, drought, nutrient deficiency, and many other challenges with a pronounced change in their intracellular calcium concentrations. This early stress signal activates calcium dependent protein kinases (CDPKs), which initiate defense cascades by phosphorylating their target proteins, leading to rapid adaptation to the changing environmental conditions. In this process, depending on the stimulus and type of stress, CDPKs coordinate different enzymes in several signaling pathways, thus enabling the plant to specifically respond to the prevailing stress in each case. One of the most exciting questions in this field is, which molecular mechanisms differentially activate the members of the CDPK family to initiate stress-specific signaling cascades. Similarly interesting is the question of how the enzymes are inactivated after the stress has subsided.

„CPKaleon is a powerful approach for visualizing real-time calcium decoding in plant cells."

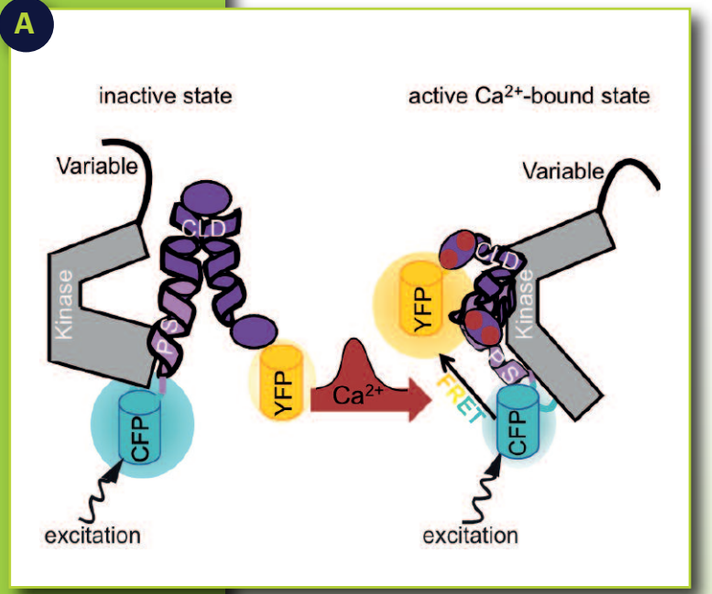
The first step for the CDPK kinase activity is a conformational change of the enzyme caused by calcium binding. We developed a FRET-based reporter that visualizes the calcium-dependent conformational changes in CDPK during its activation. To do this, we fitted two Arabidopsis CDPKs, namely the highly calcium-sensitive AtCPK21 and the rather calcium-insensitive AtCPK23 with the reporter.

FRET measurements in tobacco and Arabidopsis recorded *in vivo* conformational activation and, after calcium signal decline, inactivation of CDPKs. In Arabidopsis, we focused on the guard cells, whereas in tobacco, we mainly observed growing pollen tubes, in which simultaneous imaged a strong calcium fluctuation occurs. Here, accordingly, only the highly calcium-sensitive CPK21-FRET responded in an oscillatory manner to the intracellular calcium fluctuation, while the less sensitive CPK23-FRET showed no changes in the fluorescence signal. In Arabidopsis stomatal guard cells, CPK21-FRET also responded to early calcium surges triggered by abscisic acid or by the flagellin peptide flg22. This suggests the involvement of AtCPK21 in pathogen defense.

With this study, we demonstrate that CDPK isoforms respond differently to calcium signals, which in tissues with pronounced calcium fluctuation leads to the activation of only the highly sensitive CDPKs and thus influences the

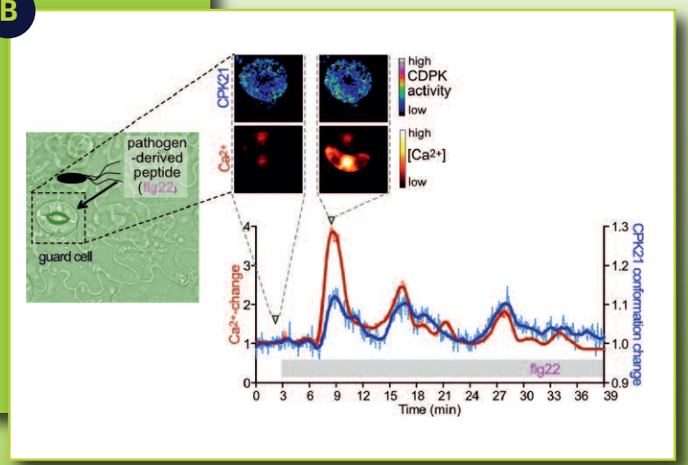
Imaging of plant calcium-sensor kinase conformation monitors calcium-dependent decoding *in planta*.

A: Schematic diagram of CPKaleon, a single molecule CPK-FRET construct encompassing the CPK variable domain, kinase domain, pseudosubstrate segment (**PS**), and a calmodulin-like domain (**CLD**) that contains four Ca^{2+}-binding sites. The regulatory Ca^{2+}-binding domain (**PS-CLD**) was positioned between cyan fluorescence protein (**CFP**) and yellow fluorescence protein (**YFP**). Ca^{2+}-binding brings CFP and YFP into close proximity, allowing the recording of this conformation change via FRET (Förster resonance energy transfer).



B: flg22-induced changes in Ca^{2+}-concentrations in Arabidopsis guard cells induce real-time conformational activation and inactivation of CPK21-FRET.

Modified from Liese et al., Plant Cell, 2023



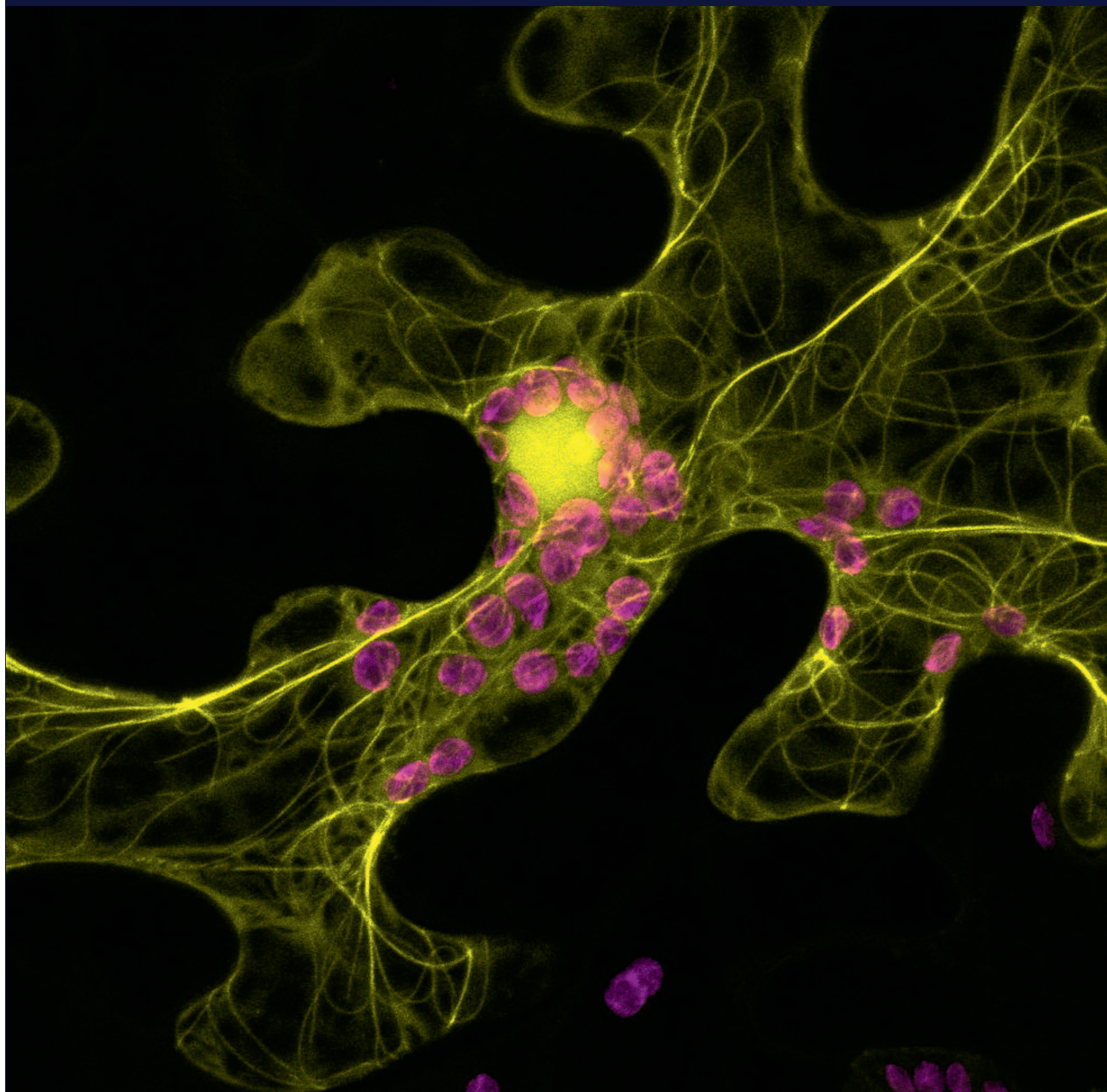
direction of the signal cascade. In addition, for Arabidopsis guard cells, we make initial statements about the type of stress that had triggered AtCPK21 activation. Based on these data, CDPK-FRET is a powerful approach for tackling real-time live-cell calcium decoding in a multitude of plant developmental and stress responses. The CDPK fluorescence sensor, named **CPKaleon**, will be used in the future to elucidate the function of additional CDPK isoforms in various signaling pathways.

Original Publication:
Anja Liese, Bernadette Eichstädt, Sarah Lederer, Philipp Schulz, Jan Oehlschläger, Susanne Matschi, José A Feijó, Waltraud X Schulze, Kai R Konrad & Tina Romeis. Imaging of plant calcium-sensor kinase conformation monitors real time calcium-dependent decoding in planta. ***Plant Cell* 2024**, DOI: 10.1093/plcell/koad196.

Fluoreszenzsensor macht Stressreaktion sichtbar

Pflanzen reagieren auf den Befall von Krankheitserregern, Trockenheit, Nährstoffmangel und viele weitere Herausforderungen mit einer starken Veränderung ihrer intrazellulären Kalzium-Konzentrationen. Dieses frühe Stresssignal aktiviert Kalzium-abhängige Proteinkinasen (CDPKs), die durch Phosphorylierung ihrer Zielproteine Abwehrkaskaden initiieren, was zu einer schnellen Anpassung an die veränderten Umweltbedingungen führt. Dabei koordinieren CDPKs je nach Stimulus und Art des Stresses unterschiedliche Enzyme in verschiedenen Signalwegen, mit denen die Pflanze ganz gezielt auf den jeweils vorherrschenden Stress reagiert. Eine der spannendsten Fragen auf diesem Gebiet ist, durch welche molekularen Mechanismen die Mitglieder der CDPK-Familie differenziert aktiviert werden, sodass sie die stressspezifischen Signalkaskaden in Gang setzen können. Erste Voraussetzung für die Kinase-Aktivität der CDPK ist eine durch die Bindung von Kalzium hervorgerufene Konformationsänderung des Enzyms. Wir haben jüngst einen fluoreszenzbasierten Sensor entwickelt, mit dem man die Kalzium-abhängigen Konformationsänderungen der CDPK während ihrer Aktivierung, in Echtzeit, sichtbar machen kann. Der CPKaleon getaufte CDPK-Fluoreszenzsensor ist demnach ein leistungsfähiges Tool zur Entschlüsselung der Kalzium-Signalwege in lebenden Zellen bei einer Vielzahl von pflanzlichen Stressreaktionen. Er kann künftig genutzt werden, um die Funktion weiterer CDPKs in verschiedenen Signalwegen aufzuklären.

Xanthomonas effector protein manipulates plant microtubules



Xanthomonas effector protein manipulates plant microtubules

Xanthomonas, as plant pathogenic bacteria, cause enormous damage to crops and ornamentals. They infect more than 400 plant species, including wheat and rice, white cabbage, walnuts, lemons, cotton and soybeans, as well as begonias, geraniums and hyacinths. Over the course of evolution, these pathogens have developed numerous strategies to successfully infect and colonize different plant species. We have taken a closer look at one of these infection strategies and gathered new insights into this particular mechanism of disease development.

„Studying an effector protein in the context of a genus can provide important insights into its localization and activity."

In general, *Xanthomonas* bacteria gain access to plant tissue through wounds or stomata and, depending on the pathogen type, grow either in the vascular tracts or in the intercellular space between the leaf mesophyll cells. The bacteria inject type III effector proteins (T3Es) directly into the cytosol of plant cells *via* needle-like structures of the type III secretion system. Here, the effector proteins suppress the plant basal immune response. This leads to necrotic spots of dead tissue and overall wilting and rotting of leaves and stems in susceptible plants.

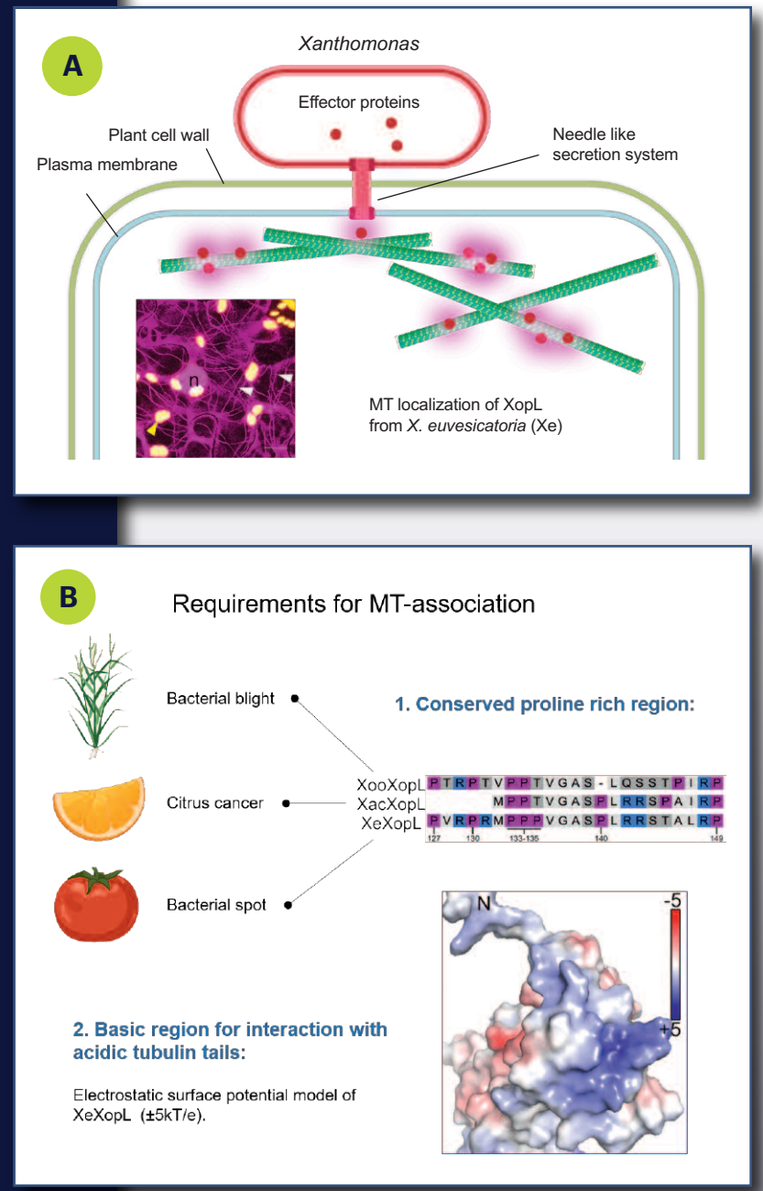
Each bacterial strain or pathovar has evolved its own arsenal of different effector proteins over the course in interaction with its host plant species. Elucidating the diverse biochemical activities of T3Es within plant cells has been the focus of plant research for the past 25 years. Some effector proteins can activate transcription in the host cell, others are able to phosphorylate or ubiquitinate host proteins; however, for many of the T3Es discovered so far, it is still unknown what they do and which plant signaling pathway they interfere with.

Although T3E arsenals are often diverse in structure and action from pathovar to pathovar, some T3Es with conserved structural traits are present in most sequenced *Xanthomonas* strains. These include *Xanthomonas* outer protein L (XopL), which is an ancestral effector contributing to the virulence of several *Xanthomonas* strains infecting different plant species. XopLs exhibit a conserved leucine-rich region and an E3 ligase activity. Despite these conserved characteristics, we have new evidence to suggest functional variation exists between the XopLs of different *Xanthomonas* species. Expression of XopL proteins in tobacco plants revealed two major findings: XopLs differ in their ability to induce plant cell death, and the *in planta* localization of XopL proteins is not conserved between *Xanthomonas* species. Our study revealed that microtubules or their associated proteins, are probably targets for the XopL li-

Xanthomonas outer protein L (XopL), a bacterial-derived effector protein mimics plant enzymatic and microtubule (MT) binding activities.

A: Effector proteins, including XopL, are secreted from Xanthomonads directly into plant cells, where they interact with and manipulate the microtubules (MT). Confocal images of *Agrobacterium*- mediated expression of fluorescently labeled XopL in a leaf cell of the model species *N. benthamiana* depicts the localization of XopL from *X. euvesicatoria* (Xe) to MTs, nucleus and cytosol (visible in magenta; chloroplasts in yellow).

B: As is the case for eukaryotic proteins, XopL requires a proline rich region as well as a stretch of basic amino acids for binding; features that are conserved among XopL homologs from many sequenced *Xanthomonas* strains including the rice pathogen *X. oryzae oryzae* (Xoo) and citrus pathogen *X. citri citri* (Xac). Modified from Ortmann et al., PLOS Pathog., 2023.



gase from three out of the four *Xanthomonas* species tested.

By comparative sequence analyses of the different XopL proteins, we could pinpoint a proline-rich and an α-helical region as the structures responsible for microtubule binding of the E3 ligases, and to link microtubule binding and cell death phenotypes. It is not yet clear exactly how these XopL-microtubule interactions lead to the initiation of plant cell death. Microtubules are thought to play a role in signal transduction of plant immune responses and also in autophagic degradation and disposal processes. Here we demonstrated in an exemplary way that studying an effector protein in the context of a genus rather than a single species can provide important insights into its localization and activity.

Original Publication:
Simon Ortmann, Jolina Marx, Christina Lampe, Vinzenz Handrick, Tim-Martin Ehnert, Sarah Zinecker, Matthias Reimers, Ulla Bonas & Jessica Lee Erickson. A conserved microtubule-binding region in *Xanthomonas* XopL is indispensable for induced plant cell death reactions. ***PLOS Pathogens* 2023**, DOI: 10.1371/journal.ppat.1011263

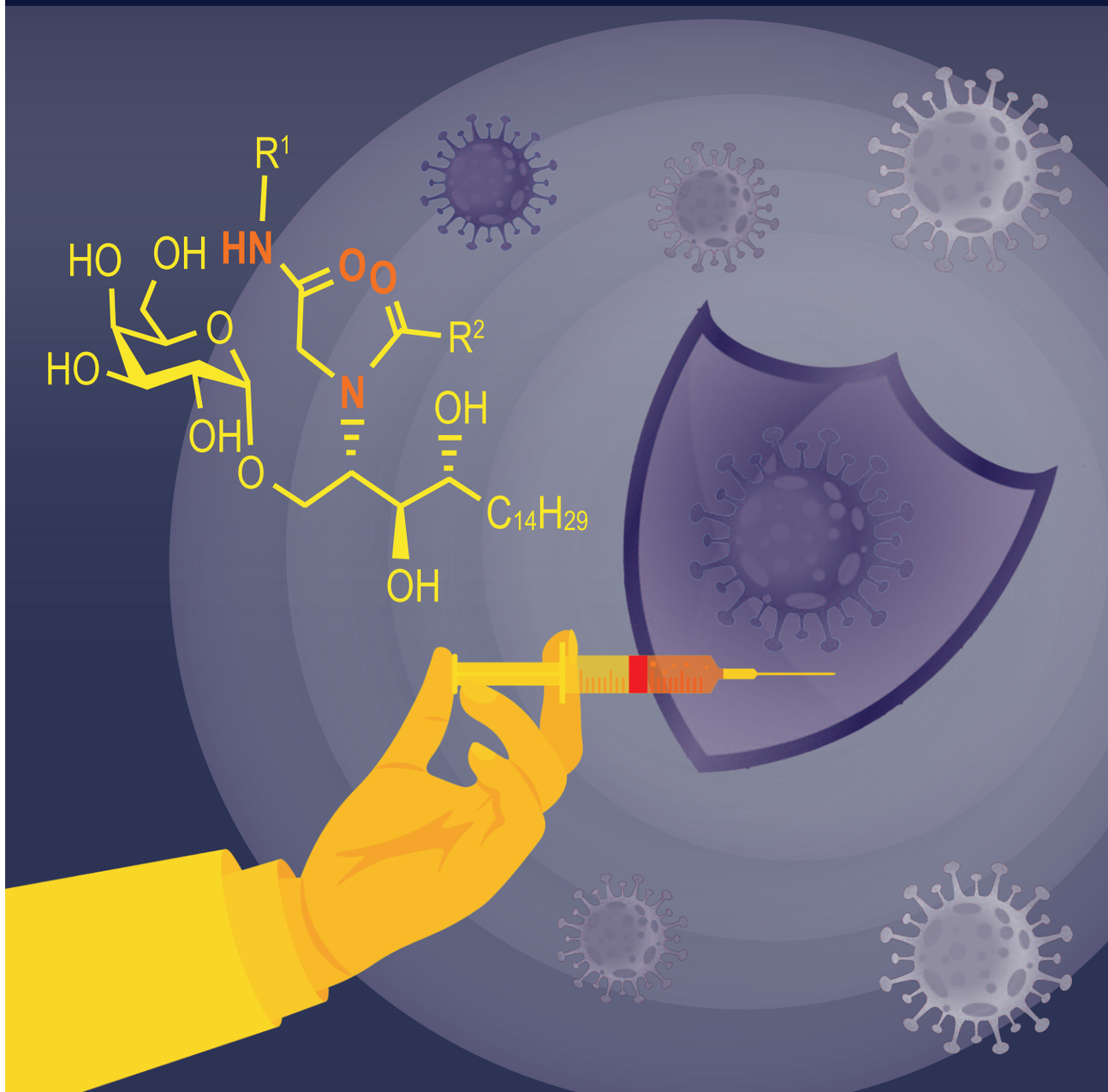
Xanthomonas-Effektorprotein manipuliert Mikrotubuli

Xanthomonaden verursachen als pflanzenpathogene Bakterien enorme Schäden im Kultur- und Zierpflanzenanbau. Sie befallen mehr als 400 Pflanzenarten, darunter Weizen und Reis, Baumwolle und Sojabohnen. Die Erreger infizieren die Pflanzen über Wunden und durch Spaltöffnungen und vermehren sich in den Leitbahnen oder im Interzellularraum zwischen den Mesophyllzellen der Blätter. Über nadelartige Strukturen des Typ-III-Sekretionssystems injizieren sie zudem Typ-III-Effektorproteine (T3Es) direkt ins Innere der Pflanzenzellen. Hier unterdrücken die Effektorproteine die basale Immunreaktion. Das führt bei empfindlichen Pflanzen zu nekrotischen Flecken, zu Welke und Fäule von Blättern und Stängeln.

Jeder Bakterienstamm oder Pathovar verfügt dabei über sein ganz eigenes Arsenal an verschiedenen Effektorproteinen. Die vielfältigen Aktivitäten der T3Es innerhalb der Pflanzenzellen stehen seit 25 Jahren im Fokus der Pflanzenforschung. Von vielen der bisher gefundenen T3Es weiß man noch nicht, welchen pflanzlichen Signalweg sie stören.

Trotz ihrer Vielfalt in Struktur und Wirkung gibt es einige T3Es mit konservierten strukturellen Merkmalen, wie z.B. das *Xanthomonas* Outerprotein L (XopL), das zur Virulenz verschiedener *Xanthomonas*-Pathovare beiträgt. Dennoch haben wir bei den XopLs verschiedener Xanthomonas-Arten Unterschiede in Funktion und Lokalisierung innerhalb der Pflanzenzelle gefunden. Als ein mögliches Zielprotein für XopL konnten wir die Mikrotubuli des pflanzlichen Zytoskeletts identifizieren. Mikrotubuli spielen vermutlich eine Rolle bei der Signalübertragung pflanzlicher Immunreaktionen.

New adjuvants with multicomponent reaction



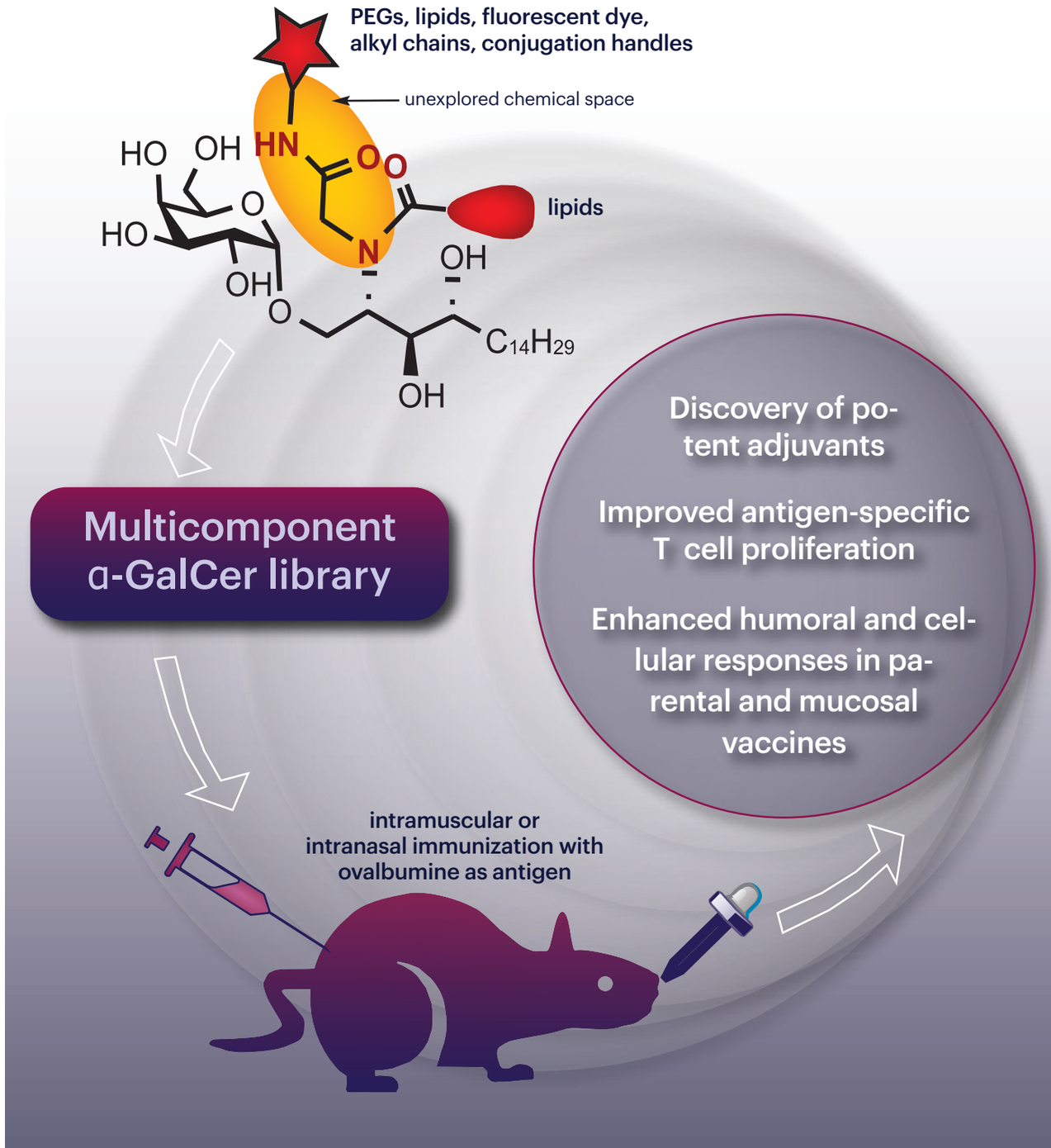
New adjuvants with multicomponent reaction

The production of effective adjuvants plays an important role in the development of new vaccines. These vaccine boosters enable a strong and targeted immune response that protects against emerging viruses and multidrug-resistant bacteria. Together with Cuban partners, we have created a library of new adjuvants and potential precursor molecules. To do this, we diversified the phytoceramide α-galactosylceramide (α-GalCer), which is already used as a vaccine booster, with various functional side chains *via* multicomponent reaction. The range of molecules attached to α-GalCer includes both hydrophobic and hydrophilic moieties, as well as reactive groups such as amines and aldehydes, which can be used for further modifications. What is unique about the new adjuvants is that, for the first time, the side chains were attached to the main α-GalCer molecule at the amide linkage - a position that previously had not been used for derivatization. Finally, we obtained more than 20 α-GalCer derivatives, whose vaccine-enhancing effect we extensively investigated together with partners from the Helmholtz Centre for Infection Research in Braunschweig, Germany.

„The development of mucosal adjuvants is of particular interest for future vaccine production."

All compounds were tested in cell-based studies and in mice for induction of both the cellular non-specific immune response by natural killer cells and the humoral immune response with the production of specific antibodies by activated B lymphocytes. Both pathways rely on the activation of various T helper cells, to whose receptors the potential adjuvants must bind in order to elicit an effective immune response. Among the α-GalCer compounds produced, derivatives were found that enhanced either the cellular or the humoral pathway, or both. Adjuvants that stimulate both immune pathways are currently in demand because they can be used to cover a broader vaccine spectrum. In addition, we analyzed all compounds for their efficacy when administered intramuscularly or nasally. Here, too, promising candidates emerged that showed good and in some cases even better efficacy than the basic adjuvant α-GalCer, especially when administered through the nasal mucosa.

The experiments were supported by modelling approaches. These virtual analyses provided initial insights into the relationship between the



structure of each derivative and its effect, laying the foundation for knowledge-based access to even better products. With our study in *Angewandte Chemie*, we have identified a new diversification hotspot of α-GalCer that is ideally suited for conjugation with other side chains and therefore has great potential to greatly expand the spectrum of future vaccine boosters. Given the great potential of mucosal vaccines, the development of mucosal adjuvants is of particular interest for future vaccine production.

Original Publication:
Yanira Méndez, Aldrin V Vasco, Thomas Ebensen, Kai Schulze, Mohammad Yousefi, Mehdi Davari, Ludger A Wessjohann, Carlos A Guzmán, Daniel García Rivera & Bernhard Westermann. Diversification of a Novel α-Galactosyl Ceramide Hotspot Boosts the Adjuvant Properties in Parenteral and Mucosal Vaccines. ***Angew Chem Int Ed 2023***, DOI: 10.1002/anie.202310983.

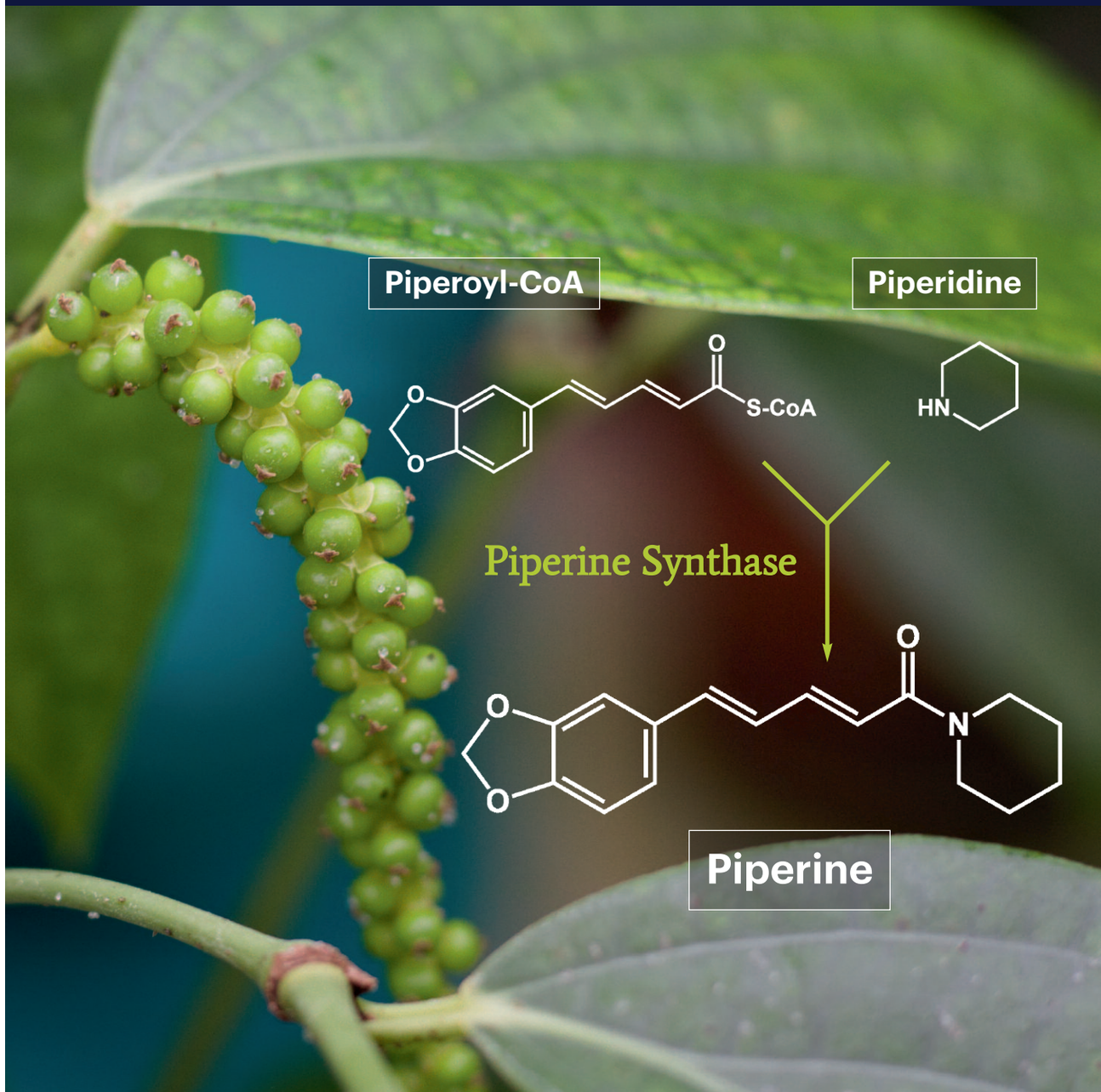
Neue Adjuvantien mit Multikomponentenreaktion

Die Erzeugung von effizienten Adjuvantien nimmt im Rahmen der Entwicklung neuer Impfstoffe einen zunehmend hohen Stellenwert ein. Die Impfverstärker ermöglichen eine starke und zielgerichtete Immunantwort gegen neu auftretende Viren und multiresistente Bakterien. Wir haben gemeinsam mit unseren kubanischen Partnern eine Bibliothek mit neuen Adjuvantien und potentiellen Vorläufermolekülen hergestellt. Dafür wurde das bereits als Impfverstärker eingesetzte Phytoceramid α-Galactosylceramid (α-GalCer) mittels Multikomponentenreaktion mit verschiedenen funktionalen Seitenketten versehen. Auf diesem Weg entstanden mehr als 20 α-GalCer-Derivate, deren impfverstärkende Wirkung wir in Kooperation mit dem Helmholtz-Zentrum für Infektionsforschung einer ausgiebigen Prüfung unterzogen.

Alle Verbindungen wurden in zellbasierten Untersuchungen und auch in Mäusen auf die Auslösung von sowohl der zellulären unspezifischen Immunantwort als auch der humoralen Immunreaktion mit der Produktion von spezifischen Antikörpern getestet. Es fanden sich Derivate, die entweder den zellulären oder den humoralen oder beide Wege verstärkten. Darüber hinaus haben wir alle Verbindungen auf ihre Wirksamkeit bei intramuskulärer oder nasaler Applikation getestet. Auch hier fanden sich aussichtsreiche Kandidaten, die vor allem bei der Applizierung über die Nasenschleimhäute gute und zum Teil sogar bessere Wirkungen zeigten als das Grund-Adjuvans α-GalCer.

Durch molekulare Dockingstudien konnten erste Erkenntnisse über den Zusammenhang zwischen der Struktur der Derivate und ihrer Wirkung gewonnen und damit der Grundstein für einen wissensbasierten Zugang zu noch besseren Produkten gelegt werden.

The biochemistry of pungency

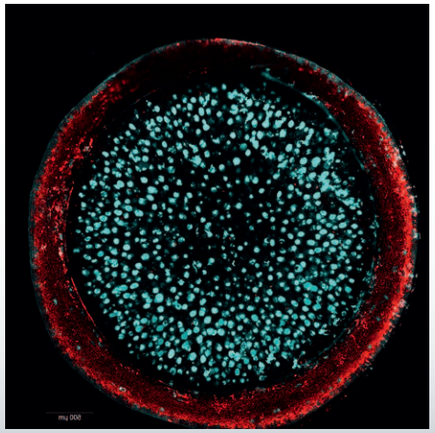


The biochemistry of pungency

Piperine, the pungent principle of black pepper (*Piper nigrum*), was identified 200 years ago, but the key enzymes and reaction steps of its biosynthesis remained obscure until recently. With the identification of three consecutive biosynthetic steps, we have made a significant contribution to the elucidation of piperine production in the pepper plant. Using a combination of RNA sequencing and LC-MS-based analysis of substrate and product profiles, we identified a cytochrome P450 enzyme that catalyzes the con- version of feruperic acid to piperic acid. The corresponding gene encoding CYP719A37 was expressed in yeast and tested for substrate specificity with various potential aromatic precursors. Results: The characteristic methylenedioxy bridge formation in the aromatic moiety of piperine is based on specific oxygenation of feruperic acid rather than feruperine. The product of the enzymatic catalysis was identified as piperic acid. The enzyme is highly active in unripe black pepper fruits.

Acyltransferases will offer new ways to produce a wide range of bioactive compounds.

Activation of piperic acid to piperoyl coenzyme A (CoA) by a more substrate tolerant piperoyl CoA ligase then delivers the substrate of piperine synthase (PS). This acyltransferase links precursors derived from two different pathways, piperoyl-CoA from phenylalanine and piperidine derived from lysine to the pungent alkaloid piperine. The enzyme and its products, mainly piperine were found to accumulate in specialized cells of the perisperm of pepper fruits. Amazingly, the berries contain piperine in molar concentrations. Piperine and related piperamides have also been found in the cortex of root cells in similar concentrations. Piperamides may serve the plant as a defense against biotic stressors.

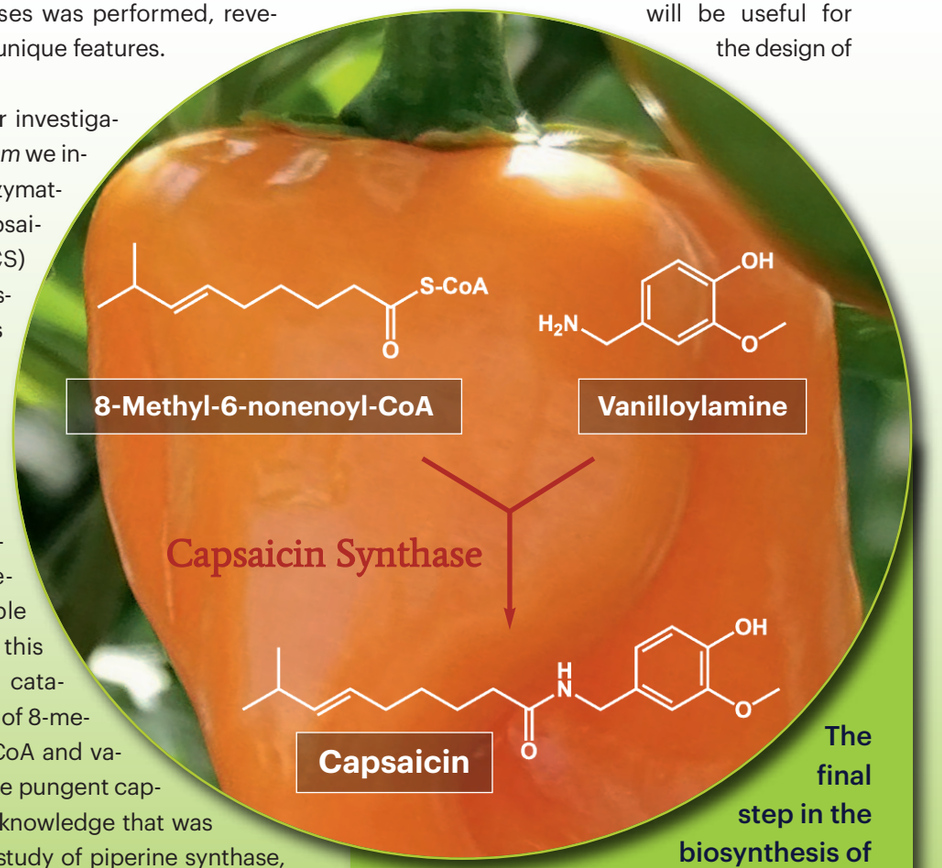


Cross-section of a 60-day-old peppercorn. Accumulation of piperine (blue fluorescence) in specialized cells of the perisperm (core of the pepper fruit).

In addition to PS we identified a closely related acyltransferase termed piper-amide synthase (PAS), in unripe black pepper fruits. This enzyme links a large variety of CoA esters to aliphatic and aromatic amines with high efficiency combined with loose substrate specificity. In addition to the identification, a detailed expression analysis and kinetic characterization of the piperine and piperamide synthases was performed, revealing a wealth of unique features.

In addition to our investigation of *Piper nigrum* we investigated the enzymatic activity of capsaicin synthase (CS) that has been described by genetics from chili pepper, *Capsicum* spec., although its enzymatic activity and catalytic properties remained enigmatic for decades. Comparable to PS and PAS, this acyltransferase catalyzes the reaction of 8-methyl-6-nonenoyl-CoA and vanilloyl amine to the pungent capsaicin. Using the knowledge that was gained from our study of piperine synthase, we succeeded in expressing capsaicin synthase in bacteria and for the first time were able to investigate the substrate specificity and additional enzymatic properties.

The study of these acyltransferases will provide the opportunity to produce a large number of medically relevant piperine and piperamide analogs by enzymatic synthesis or biotechnological means in bacteria or yeast. The lessons learned will be useful for the design of



The final step in the biosynthesis of capsaicin in hot chillies

enzymes with desired properties. Such optimized enzymes can be used to produce new bioactives. Biocatalytic syntheses like these represent a promising field of research with considerable future potential. As an alternative to petrochemical synthesis, biocatalysis can produce desired substances without the use of toxic catalysts and solvents or the formation of harmful by-products.

Original Publications:

Arianne Schnabel, Benedikt Athmer, Kerstin Manke, Frank Schumacher, Fernando Cotinguiba & Thomas Vogt. Identification and characterization of piperine synthase from black pepper, *Piper nigrum* L. ***Communications Biology* 2021**, DOI: 10.1038/s42003-021-01967-9

Raika Milde, Arianne Schnabel, Toni Ditfe, Wolfgang Hoehenwarter, Carsten Proksch, Bernhard Westermann & Thomas Vogt. Chemical synthesis of trans 8-methyl-6-nonenoyl-CoA and functional expression unravel capsaicin synthase activity encoded by the *Pun1* Locus. ***Molecules* 2022,** DOI: 10.3390/molecules27206878

Die Biochemie der Schärfe

Piperin ist die Hauptsubstanz, die den Früchten des schwarzen Pfeffers (Piper nigrum) zu ihrer charakteristischen Schärfe verhilft. Die wichtigsten Enzyme und Reaktionsschritte der Piperin-Biosynthese lagen bis vor kurzem noch im Dunkeln. Wir haben in den letzten Jahren entscheidend zur Aufklärung der Piperinproduktion in der Pfefferpflanze beigetragen.

Mit einer Kombination aus RNA-Sequenzierung und massenspektrometrischen Methoden fanden wir zunächst ein Cytochrom P450-Enzym, das die Umwandlung von Feruperin zu Piperinsäure katalysiert. Mit der Entdeckung einer, die Carbonsäure aktivierenden CoA Ligase und der Piperinsynthase ist uns dann die Aufklärung des finalen Schritts der Piperinbiosynthese gelungen. Die Piperinsynthase katalysiert die Bildung einer Amidbindung, von Piperoyl-CoA und Piperidin zum Piperin. Das Enzym und sein Produkt reichern sich in großen Mengen im Kern der Pfefferfrüchte zum Schutz des Embryos an. Piperin findet sich aber auch in der Wurzel, wo es wie in den Früchten vermutlich zur Abwehr von Fraßfeinden dient.

Erstmals konnten wir auch die enzymatische Aktivität der lange gesuchten Capsaicinsynthase aus der Chilischote Capsicum spec. beschreiben. Wie die Piperinsynthase beim Piperin, katalysiert diese Acyltransferase analog den letzten Reaktionsschritt der Biosynthese, ausgehendend vom Vanillylamin und einem aliphatischen CoA-Ester zum Scharfstoff Capsaicin.

Multicolor pigments in tobacco leaves

Multicolor pigments in tobacco leaves

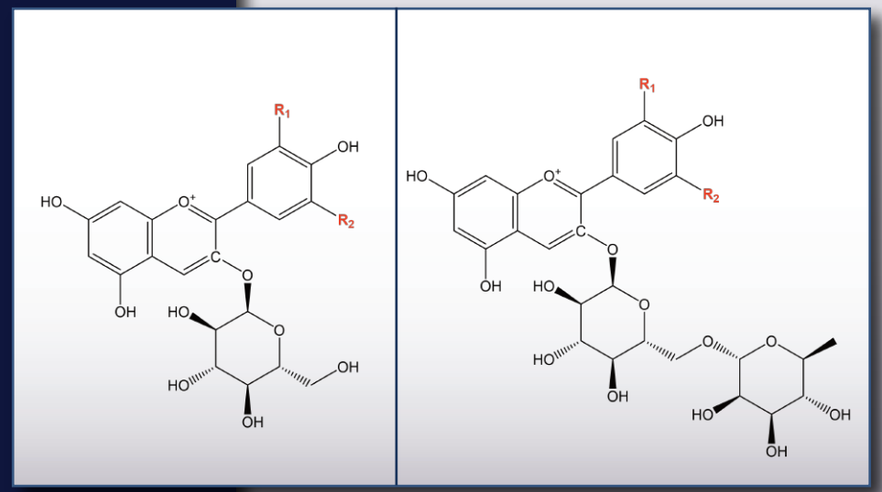
Transient expression of biosynthetic enzymes in the leaves of *Nicotiana benthamiana* is a very efficient and currently popular way to produce complex secondary metabolites quickly and in sufficient quantities. In recent years, this tobacco expression platform has contributed to the rapid elucidation of several biosynthetic pathways of complex natural products and has greatly accelerated the growth of knowledge in this field. However, this does not include anthocyanins, whose expression in *Nicotiana benthamiana* works poorly and has so far only been successful in the production of delphinidin 3-*O*-rutinoside, which is considered a precursor for some but not all complex anthocyanins. The production of other anthocyanins, however, would require the repression of certain endogenous flavonoid biosynthetic genes with simultaneous transient expression of other genes.

„The new expression platform makes it possible to elucidate the biosynthetic pathways of complex anthocyanins.“

We have now developed an *N. benthamiana* expression platform that allows the production of any desired basic anthocyanin by infiltrating a few gene constructs. For the synthesis of more complex anthocyanins, species-specific biosynthetic genes can also be introduced into the tobacco leaves. Our strategy is based on the overexpression of certain anthocyanin biosynthesis genes that are insufficiently or not at all expressed in *N. benthamiana*, while at the same time inactivating other genes that could hinder the production of certain anthocyanins. Using this platform, we were able to obtain the basic anthocyanins pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside in just a few days and in large quantities, as well as their rhamnosylated derivatives pelargonidin-3-*O*-rutinoside, cyanidin-3-*O*-rutinoside and delphinidin-3-*O*-rutinoside.

In general, compound production in *Nicotiana benthamiana* is based on cloning the required biosynthetic genes into an expression vector controlled by the robust constitutive 35S promoter of the cauliflower mosaic virus. After infiltration of the construct into the tobacco leaves by agrobacteria, the introduced genes are transiently expressed. In this process, several genes can be simultaneously transferred into the plant using different agrobacterial strains and transcribed there. This method enables the elucidation of entire metabolic pathways, even if the exact sequence of the successive enzymatic reactions is not known. The new expression platform now makes it possible to elucidate the biosynthetic pathways of complex anthocyanins and provides a possible strategy for the rapid and high-yield production of these compounds and their non-natural derivatives.

Nicotiana benthamiana wildtype leaf infiltrated with combinations of *Agrobacterium* strains containing constructs for biosynthesis of pelargonidin-3-*O*-rutinoside (left), cyanidin-3-*O*-rutinoside (center) and delphinidin-3-*O*-rutinoside (right).



Structure of anthocyanins pelargonidin-3-*O*-glucoside (R1=R2=H), cyanidin-3-*O*-glucoside (R1=OH, R2=H) and delphinidin-3-*O*-glucoside (R1=OH, R2=OH) shown on the left, and of their rutinoside derivatives on the right.

Anthocyanins are water-soluble pigments that occur in the majority of higher plants and give many flowers and fruits their beautiful red, purple, or blue colors. They are produced in the cytosol by several enzymatic reactions from the precursor molecule phenylalanine and are stored in the vacuole. The first colored and stable products are the basic compounds pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, and delphinidin 3-*O*-glucoside. In many plants, these basic anthocyanins undergo further modifications, such as glycosylation, acylation, and methylation, which affect the color and stability of the pigments. Anthocyanins are used as food coloring for confectionery, jam, canned fruit, and baking products. The colors show antioxidative, anti-inflammatory, and vasoprotective effects.

Original Publication:
Ramona Grützner, Kristin König, Claudia Horn, Carola Engler, Annegret Laub, Thomas Vogt & Sylvestre Marillonnet. A transient expression tool box for anthocyanin biosynthesis in *Nicotiana benthamiana*. ***Plant Biotechnology Journal* 2023** Doi:10.1111/pbi.14261

Multicolor-Pigmentproduktion in Tabakblättern

Ein sehr effizienter und zurzeit gern beschrittener Weg, komplexe Farb- und Naturstoffe schnell und in großen Mengen herzustellen, ist ihre Produktion in den Blättern der Tabak-Art *Nicotiana benthamiana*. Das gilt jedoch nicht für Anthocyane, deren Erzeugung in *N. benthamiana* schlecht funktioniert und bisher nur für die Herstellung der Vorläufersubstanz Delphinidin-3-*O*-Rutinosid erfolgreich war.

Wir haben jüngst eine Methode entwickelt, mit der man in *N. benthamiana* jedes gewünschte Anthocyanin durch Infiltration von wenigen Biosynthesegenen herstellen kann. Unsere Strategie beruht auf der Überproduktion von verschiedenen Vorläufermolekülen, die in *N. benthamiana* nur unzureichend oder gar nicht hergestellt werden, während gleichzeitig andere Gene inaktiviert werden, die die Produktion von bestimmten Anthocyanen behindern könnten. Mit Hilfe dieser Produktionsplattform ist es uns gelungen, die Basisanthocyanine Pelargonidin-, Cyanidin- und Delphinidin-3-*O*-Glucosid in wenigen Tagen und in großen Mengen zu gewinnen.

Anthocyane sind wasserlösliche Pigmente, die in allen höheren Pflanzen vorkommen und vielen Blüten und Früchten eine intensive dunkelrote, violette oder blaue Färbung verleihen. Die Verbindungen werden als Lebensmittelfarbstoff für die Färbung von Süßwaren, Marmelade, Obstkonserven und Backmitteln verwendet. Anthocyane wirken antioxidativ, entzündungshemmend und gefäßschützend.

Ferrooxidase arms plants against phosphate deficiency

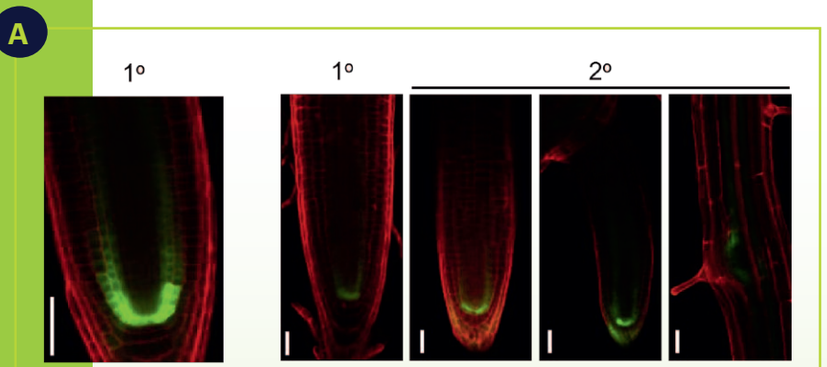
Ferrooxidase arms plants against phosphate deficiency

Phosphate (Pi) plays a central role in the energy metabolism of all living organisms and ensures the stability of genetic material. However, this essential macronutrient is not always readily available to plants, as it forms poorly soluble complexes with its antagonistic counterpart, iron, in the soil, which makes it difficult to absorb phosphate *via* roots. In phosphate-poor and often iron-rich soils, plants are therefore forced to adapt, which they usually do by stopping the vertical growth of their primary roots and forming more lateral roots instead. This increase in root surface area, especially in the phosphate-rich upper soil layers, leads to increased uptake of the coveted macronutrient. The research group Nutrient Sensing investigates the molecular mechanisms underlying this adaptation of root architecture to phosphate deficiency and examines how plants recognize the external Pi status.

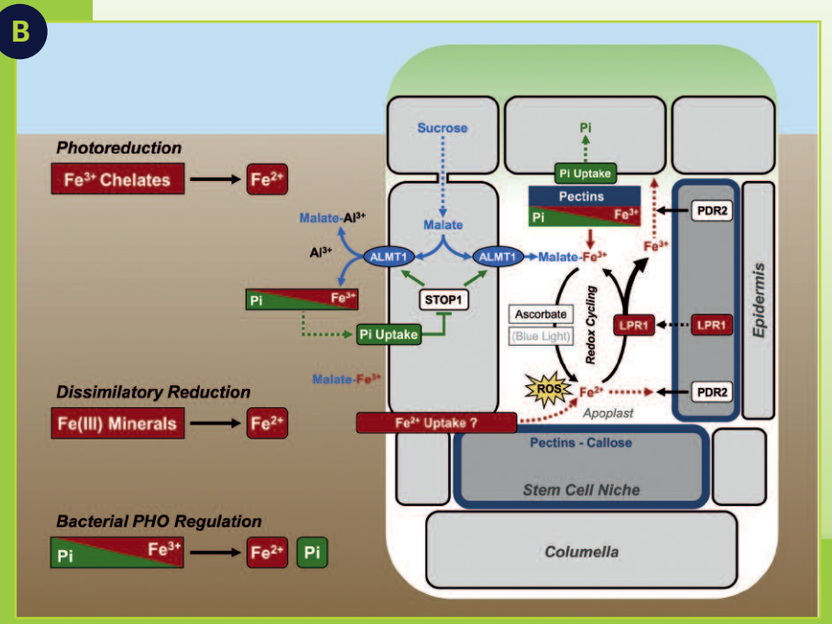
> „The acquisition of bacterial LPR1 may have enormously promoted the terrestrial colonization of plants."

The protein LOW PHOSPHATE ROOT 1 (LPR1), which the research group identified as a novel ferro-oxidase, plays a key role in this scenario. LPR1 binds Fe^{2+} with high specificity and affinity and oxidizes it to Fe^{3+}. This redox cycling of iron ions in the intercellular spaces of the root tip generates reactive oxygen species, which in turn lead to sealing callose deposits in the root tip meristem. As a result, the growth of the primary root is blocked and there is increased formation of lateral roots in the upper root area. The activity and function of LPR1 are determined by the availability of the Fe substrate in relation to Pi. Thus, plant adaptation to phosphate deficiency occurs *via* LPR1-driven perception of subtle differences in external Fe concentrations.

Interestingly, LPR1 shows strong similarities to members of bacterial Fe-oxidizing multicopper families. Phylogenetic analyses of multicopper oxidases and LPR1-like enzymes from plants, animals, bacteria and archaea led to the hypothesis that plant LPR1 may have been transferred from a soil bacterium to a land plant progenitor by horizontal gene transfer. This is supported by the presence of LPR1-related genes in some algae, which are considered sister clades of land plants. Presumably, a precursor of the LPR1 ferrooxidase evolved during the early bacterial colonization of the mainland. The acquisition of the bacterial ferrooxidase by embryophyte precursors may have enormously promoted the terrestrial colonization of plants.



A: Expression of LPR1pro:GFP in primary (1°) and lateral (2°) root meristems of wild-type seedlings germinated on +Pi medium for 6 days (left panel) and 12 days (right panels).



B: Model of LPR1 function. ALMT1-mediated malate release into the rhizosphere and root apoplast chelates toxic Al^{3+} in soil and mobilizes Pi from Fe–Pi complexes by Fe^{3+}-chelation. Ascorbate reduces Fe^{3+}-malate, stimulating ROS formation by Fenton chemistry. LPR1-dependent Fe^{2+} oxidation attenuates ROS production and presumably ROS signaling in the stem cell niche. Fe^{2+} substrate availability tunes LPR1 ferroxidase activity. PDR2/AtP5A counteracts LPR1 function by maintaining Fe homeostasis in root tips. Fe^{2+} generation in the SCN apoplast, and possibly Fe^{2+} uptake from the rhizosphere into the columella/SCN apoplast, constitute a local cue for external Pi availability monitored by the PDR2-LPR1 module. In Pi limiting and sterile laboratory conditions, UV or blue light illumination stimulates ROS formation and root growth inhibition. In such settings, Fe^{3+} photoreduction likely mimics the impact of soil bacteria, which mobilize redox-active Fe^{2+} from Fe(III) oxide minerals by various metabolic processes.

Original Publication:
Christin Naumann, Marcus Heisters, Wolfgang Brandt, Philipp Janitza, Carolin Alfs, Nancy Tang, Alicia Toto Nienguesso, Jörg Ziegler, Richard Imre, Karl Mechtler, Yasin Dagdas, Wolfgang Hoehenwarter, Gary Sawers, Marcel Quint & Steffen Abel. Bacterial-type ferroxidase tunes iron-dependent phosphate sensing during Arabidopsis root development. ***Current Biology* 2022**, https://doi.org/10.1016/j.cub.2022.04.005.

Ferrooxidase wappnet Pflanzen gegen Phosphatmangel

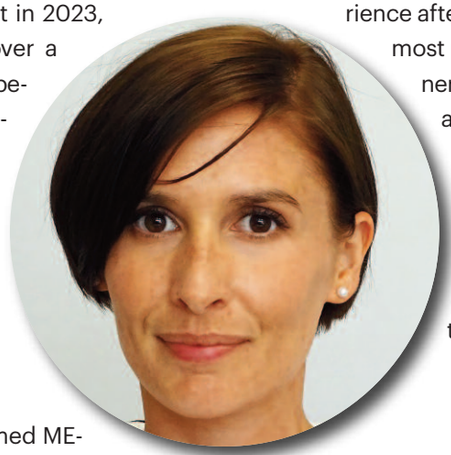
Phosphat (Pi) spielt eine zentrale Rolle im Energiestoffwechsel aller Lebewesen und sorgt für die Stabilität der Erbsubstanz. Eine gute Verfügbarkeit dieses essenziellen Makronährstoffs ist für Pflanzen jedoch nicht immer gegeben, da es im Boden schwer lösliche Komplexe mit seinem antagonistischen Gegenspieler Eisen (Fe) bildet, was seine Aufnahme über die Wurzeln erschwert. In phosphatarmen und meist eisenreichen Böden sind Pflanzen daher zur Anpassung gezwungen, die sie in der Regel bewältigen, indem sie das Tiefenwachstum ihrer Primärwurzeln einstellen und stattdessen vermehrt Seitenwurzeln bilden. Diese Vergrößerung der Wurzeloberfläche vor allem in den phosphatreichen oberen Bodenschichten führt zur gesteigerten Aufnahme des begehrten Makronährstoffs. Die Arbeitsgruppe Nährstoffperzeption erforscht die molekularen Mechanismen, die dieser Anpassung der Wurzelarchitektur an Phosphatmangel zugrunde liegen und geht zudem der Frage nach, wie Pflanzen den externen Pi-Status erkennen.

Eine Schlüsselrolle in diesem Szenario spielt das Protein LOW PHOSPHATE ROOT 1 (LPR1), das wir als neuartige Ferrooxidase identifizieren konnten. LPR1 bindet mit hoher Spezifität Fe^{2+} und oxidiert es zu Fe^{3+}. Durch dieses Redoxcycling der Eisen-Ionen in den Zellzwischenräumen der Wurzelspitze entstehen reaktive Sauerstoffverbindungen, welche wiederum zu abdichtenden Kallose-Ablagerungen im Wurzelspitzenmeristem führen. Im Ergebnis wird das Tiefenwachstum der Primärwurzel blockiert und es kommt zur vermehrten Bildung von Seitenwurzeln im oberen Wurzelbereich. Die Anpassung der Pflanze an Phosphatmangel erfolgt demnach über die LPR1-gesteuerte Wahrnehmung von subtilen Unterschieden der externen Fe-Konzentrationen.

Interessanterweise weist LPR1 große Ähnlichkeiten zu Vertretern von bakteriellen Fe-oxidierenden Multikupferfamilien auf. Unsere phylogenetischen Analysen von Multikupferoxidasen und LPR1-ähnlichen Enzymen aus Pflanzen, Tieren, Bakterien und Archaeen münden in der Hypothese, dass das pflanzliche LPR1 durch horizontalen Gentransfer von einem Bodenbakterium auf einen Vorläufer der Landpflanzen übertragen worden sein könnte. Diese Übernahme der bakteriellen Ferrooxidase durch Vorläufer der Landpflanzen hat wahrscheinlich deren Landgang enorm befördert.

ERC Consolidator Grant for Debora Gasperini

IPB group leader Debora Gasperini has been awarded the European Research Council (ERC) consolidator grant in 2023, worth nearly €2 million, over a period of five years. Dr Gasperini is one of 321 elite researchers across all scientific disciplines to benefit from funding of €657 million from this ERC grant, which is part of the EU's Horizon Europe program.



ERC Consolidator grants are designed to assist excellent scientists who have 7 to 12 years experience after their PhDs, to pursue their most promising ideas. Grant winners will carry out their projects at universities and research centres in 18 EU Member States. The grants will create around 1950 jobs for postdoctoral fellows, PhD students, and other staff at the host institutions.

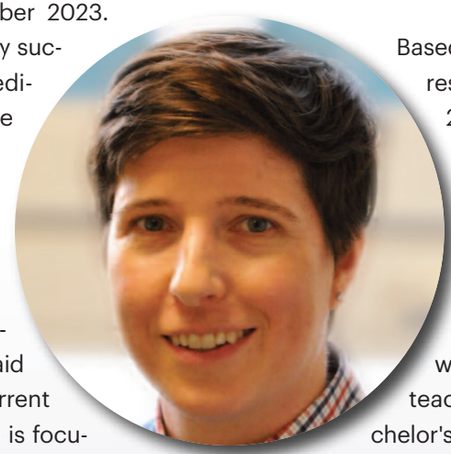
The awarded project is named MECHANOJAS, and aims to investigate biophysical signals governing the production of the plant hormone jasmonate (JA). JA is synthesized from polyunsaturated fatty acids residing in plant-specific plastidial membranes, and is essential to protect plants against numerous biotic and abiotic challenges including insect herbivory and temperature extremes. Despite the vital roles of JA in sustaining plant fitness and although its biosynthesis and perception are well characterized, it is still unknown how damage signals are transmitted to plastids to initiate phytohormone production and what is the nature of the transmitted signal(s). MECHANOJAS intends to fill this critical gap in knowledge by using high-risk, high-gain interdisciplinary aproaches, and provide the basis for future opportunities in improving plant health under changing environments.

ERC Consolidator Grant für Debora Gasperini

Debora Gasperini, Leiterin der Forschungsgruppe *Jasmonate-Signaling*, hat den ERC Consolidator Grant erhalten. Sie überzeugte in einem mehrstufigen Auswahlverfahren mit ihrem Forschungsvorhaben "MECHANOJAS". Darin will sie biophysikalische Auslöser für die Produktion des Pflanzenhormons Jasmonat untersuchen und so zum besseren Verständnis pflanzlicher Abwehr- und Anpassungsprozesse beitragen. Die renommierte Förderlinie des Europäischen Forschungsrates unterstützt exzellente Wissenschaftler, sich als führende Köpfe auf ihrem Gebiet zu etablieren. Mit der ERC-Förderung von bis zu 2 Mio. Euro kann Debora Gasperini ihre eigene unabhängige Forschung vorantreiben.

Katharina Bürstenbinder advanced to Professor at the University of Marburg

Katharina Bürstenbinder took up a professorship in cell biology at the University of Marburg in November 2023. This marks the end of a very successful and remarkably dedicated time at IPB for the head of the Cellular Coordination group. Her IPB period started in 2010, when the PhD graduate of Kiel University moved to the department Molecular Signal Processing, where she laid the foundation for her current research. In particular, she is focusing on the characterization of IQD proteins, a protein family that is apparently involved in numerous plant growth and development processes, but their specific task in the cellular framework was yet to be discovered. Elucidating the function of these poorly studied proteins was a stated goal of Dr. Bürstenbinder, which she pursued successfully. Some of the IQDs play a major role in cell division and control the shape of leaf epidermal cells. The absence of these IQDs in mutant plants results in abnormally formed cells. This deviation from the norm in cell architecture initially was a subjective impression and could only be proven by laborious measurements of single cells including their lobes and gaps. A time-intensive task and not objective enough, Dr. Bürstenbinder found. Thus, together with MLU scientists, she developed PaceQuant - an open-source program that automatically records and evaluates the specific parameters of a hundred epidermal cells simultaneously.



Based on her excellent research results, K. Bürstenbinder was 2022 included in AcademiaNet - an expert database for outstanding female scientists. In addition to her scientific milestones, she has been Speaker of the Scientific Institute Council was also readily engaged in teaching courses for both bachelor's and master's students.

Katharina Bürstenbinder ist Professorin an der Universität Marburg

Katharina Bürstenbinder hat im November 2023 eine Professur für Zellbiologie an der Universität Marburg angetreten. Die Leiterin der Arbeitsgruppe Zelluläre Koordination kam 2010 ans IPB, wo sie sich mit der Charakterisierung der bisher wenig erforschten IQD-Proteine befasste, die an zahlreichen pflanzlichen Wachstums- und Entwicklungsprozessen beteiligt sind. Für ihre ausgezeichneten Forschungsergebnisse wurde Frau Bürstenbinder 2022 ins AcademiaNet - eine Expertendatenbank für herausragende Wissenschaftlerinnen - aufgenommen. Ihr Engagement am Institut erfolgte auf zahlreichen Ebenen. Sie war Sprecherin der Wissenschaftlichen Institutsrats und hat sich aktiv an Lehrveranstaltungen für Bachelor- und Masterstudenten beteiligt.

Impressum

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Titelbild

Ein vom Pflanzenpathogen Xanthomonas abgesondertes Effektorprotein (gelb) lagert sich an das Mikrotubulinetzwerk der Pflanzenzelle an und zerstört es. Die Chloroplasten sind in magenta eingefärbt. **Foto: Jessica Erickson, IPB**