

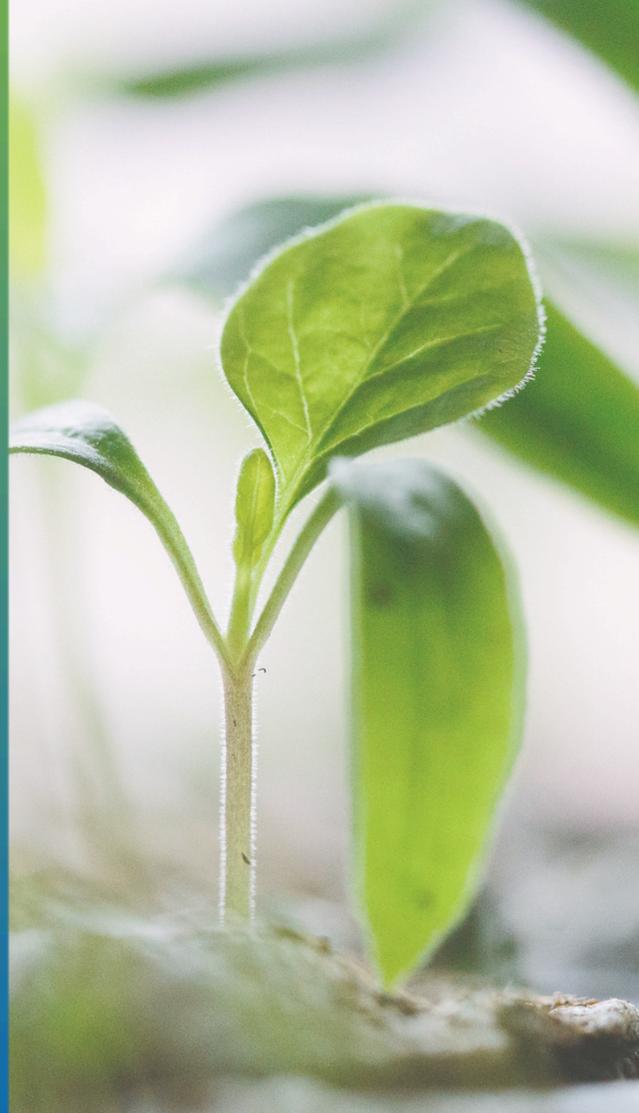
IMMUNOMETABOLISM & MICROBIAL INTERACTIONS

Insights across Kingdoms



**INTERNATIONAL
SYMPOSIUM
11.03.-13.03.2026,
COLOGNE GERMANY**

MARCH 11-13, 2026 |
BIOCENTER COLOGNE





Dear iHEAD Members and Invited Speakers,

Welcome to the International iHEAD Meeting 2025!

We are delighted to welcome you to this year's international gathering, funded by the *Ministry of Culture and Science of North Rhine–Westphalia (MKW)*. Over the past years, the iHEAD initiative has brought together diverse research groups, fostering a vibrant and interdisciplinary community dedicated to advancing our understanding of host–microbe interactions.

This meeting offers a unique opportunity to reflect on shared ideas, productive collaborations, and the significant progress we have achieved together. Your contributions—through presentations, discussions, and scientific exchange—are essential to the success of iHEAD and to the continued advancement of our research field.

As we convene for this international event, we look forward to an inspiring and stimulating meeting, enriched by your expertise and perspectives. Let us celebrate the accomplishments of the iHEAD initiative and explore new horizons for future scientific collaborations.

Cheers to an engaging and memorable meeting,
Your iHEAD Steering Committee

IMMUNOMETABOLISM & MICROBIAL INTERACTIONS

– Insights across Kingdoms –

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AGENDA

11.03.2026

WEDNESDAY

- 2pm Alga Zuccaro **Welcome & iHEAD Presentation**
- 2.30pm Xinnian Dong Yin and Yang of Central Metabolism in Regulating Plant Immunity
- 3.05pm Florian Kümmel Activation and cell death mechanism of a barley NLR targeted by a fungal nonribosomal peptide effector
- 3.30pm Arthur Matcha Towards a platform for identifying structural rewiring in immunometabolism-associated proteins.
- 4pm **Coffee Break**
- 4.45pm Corné Pieterse The Extended Host Immune System
- 5.20pm Maribel Schönewolf Infochemical production during plant immune responses by the novel root TIR protein ISI

12.03.2026

THURSDAY

- 9.30am Lena Pernas Social sensing of infection reprograms peripheral immunity in healthy mice
- 10.05am Lara Hasse Macrophages and nucleotide Metabolism
- 10.35am **Coffee Break**
- 11.15am Michael Knopp Microbiome composition (counter-)selects for antibiotic resistant strains in a personalized manner
- 11.50am Hannah Dorethy Exploiting *P. aeruginosa* Metabolism to Combat Resistance and Enhance Host Defence
- 12.15pm **Lunch Break Mensa & Poster session from 1.30pm onwards**
- 3pm Nuria Sancez-Coll Metabolic Nexus: Shaping Host-Pathogen dynamics
- 3.35pm Steven Cheng Plant disease resistance strategy using a ribosylated nucleotide signal
- 4pm Christof Domnik Application of natural immune-stimulating pRib-AMP to plants limits disease
- 4.30pm **Coffee Break**
- 5pm Marc Nishmura The Arabidopsis TIRome informs the design of artificial TIR (Toll/interleukin-1 receptor) domain proteins.
- 5.35pm Martina Feierabend Pan-genome-scale metabolic modeling to unravel metabolic functions of plant-associated Sphingomonas
- 6.30pm **Conference Dinner**

13.03.2026

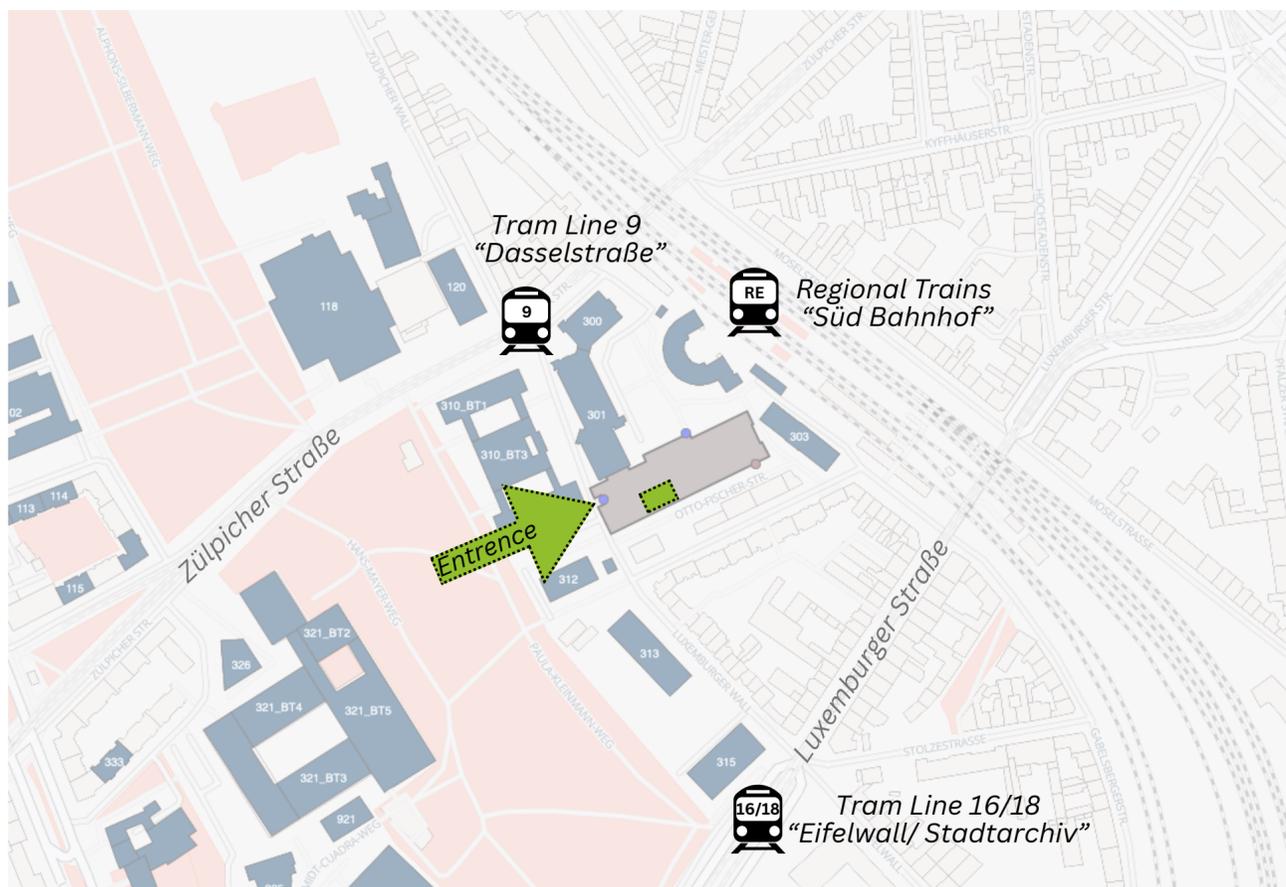
FRIDAY

- 9.30am Claus Peter-Witte There is still much to learn about (immuno)-nucleotide metabolism in plants.
- 10.05am Claus Schmitz Pathogen-Induced Modulation of Extracellular Nucleotide Signaling in Plants
- 10.35am **Coffee Break**
- 11.15am Rosa Lozano-Duran Rewiring the Host: Geminiviruses as Probes of Immunometabolic Networks
- 11.50am Raja Ganesan Metabolic Nexus: Shaping Host-Pathogen dynamics
- 12.15pm Alga Zuccaro **Closing Remarks & Poster Prize**

Location

The conference will take place at the **Biocenter (Building 304) of the University of Cologne**, located at *Zülpicher Straße 47b, 50674 Cologne*. All scientific sessions will be held in Lecture Hall 0.024, with additional space available in the foyer.

The Biocenter can be reached via various tram connections (line 9, 16 or 18) via the stops “Dasselstraße”, “Südbahnhof” or “Eifelwall/ Stadtarchiv”.



Overview of nearby tram stops providing access to the Biozentrum (Building 304), University of Cologne.

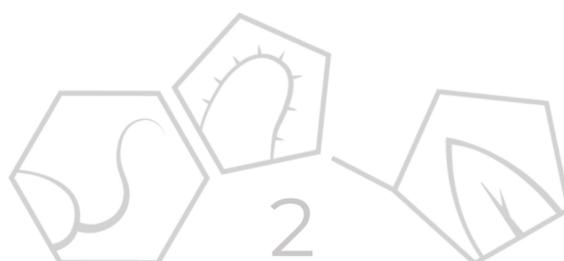
Lunch & Conference Dinner

Participants are warmly invited to join the joint lunch as well as the conference dinner on 12 March.

The conference dinner will begin at 6:30 pm and will take place at XII Apostel in Cologne (Heumarkt 68-72, 50667 Köln).

Poster Session

A poster session will be held on Thursday, 12 March, following the lunch break, in the foyer of the Biozentrum. The poster session will provide early career researchers with the opportunity to present and discuss their current research in an informal and interactive setting.



iHEAD

Introduces itself

Scientists in the Faculty of Medicine and the Faculty of Mathematics and Natural Sciences (MNF, Departments of Chemistry, Physics and Biology) at the University of Cologne (UoC) have teamed up with the Max Planck Institute for Plant Breeding Research (MPIPZ) and the Max Planck Institute for Biology of Ageing **to investigate the regulatory roles of metabolic signals and bioactive metabolites in immune systems.**

The central goal of the interdisciplinary research consortium *iHEAD* is **to establish immunometabolism as an emerging field of research in host-microbe interactions.** We hypothesize that immunometabolism is a fundamental property of immune systems across kingdoms of life that resulted in the evolution of potentially common as well as kingdom-specific infochemicals. In interactions with pathogenic microbes, such molecules connect host metabolism and immunity, underpin immune functions and regulate the host metabolism. In beneficial interactions with microbes, metabolic signals integrate host and microbiota metabolic activities to establish metabolic homeostasis, which is critical for host health. We will broaden this emerging area of research in plant-microbe interactions by investigating unexplored extracellular and intracellular metabolic signaling mechanisms driving inflammation and disease in animal-microbe interactions.



Christian Frezza



Lara Hasse

“It has been shown that infection initiate a metabolic rewiring in macrophages to clear the infection. In addition to the increase in mitochondrial metabolites with specific antimicrobial properties in macrophages during inflammation, it has been shown that other metabolic pathways such as nucleotides and cyclic nucleotides are affected. However, the role of these metabolites on macrophage function remains unclear.”

Using metabolomic approaches, we want to characterize th dysregulation of nucleotide and cyclic nucleotide metabolism in mouse macrophages. Furthermore, we aim to manipulate relevant metabolic pathways to modulate the inflammatory response in mouse macrophages to unravel the metabolic role of nucleotides in macrophages during inflammation.”

*“The dynamic interplay between hosts and pathogens represent an ongoing evolutionary battle. Hosts rely on their innate immune system to detect and counter invading pathogens, while pathogens employ an array of virulence proteins to subvert host defences. Metabolic reprogramming is essential for immune cells like macrophages to mount effective antimicrobial responses. However, certain intracellular pathogens, such as *Pseudomonas aeruginosa*, can exploit and alter host metabolic changes to promote their growth and survival, leading to biofilm formation and treatment challenges. Despite the critical role of metabolism in immune function, the mechanistic relationship between host metabolism and immunity remains poorly understood.*”

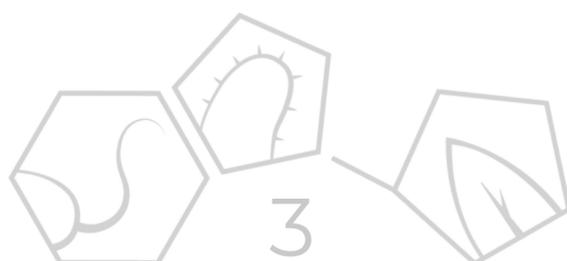


Raja Ganesan



Hamid Kashkar

Our research aims to elucidate the intricate metabolic crosstalk in host-pathogen interactions, shedding light on novel therapeutic targets and metabolic modulators for intervention strategies.”





Filipe Cabreiro



Hannah Dorethy

“The functional output of the microbiota is dynamically influenced by environmental cues, including nutrients and xenobiotics. Such tripartite interactions between host, microbes, and exogenous compounds are broadly conserved across species—from plants to invertebrates and humans. Xenobiotics can remodel microbial community composition and activity, thereby affecting host physiology either directly or indirectly. Similarly, host-derived metabolites and proteins influence microbial dynamics by serving as selective nutrients or by inhibiting essential microbial functions.”

Using integrated omics analysis combined with high-throughput Caenorhabditis elegans infection models, we aim at uncovering infochemicals responsible for host to microbe and microbe to host communication and understand how environmental cues shift the fine symbiotic balance in the context of health and disease.”

“Our research addresses immunometabolism by studying plant immunity in the ecological context of interactions between fungal pathogens, plant hosts, and the plant-associated microbiota. Focusing on the vascular wilt fungus Verticillium dahliae, we adopt a holobiont perspective in which immune responses and metabolic processes are shaped not only by direct host-pathogen interactions but also by microbial community dynamics.”

A key contribution of our group is the discovery that fungal pathogens deploy secreted effector proteins with antimicrobial activity that selectively target members of the plant microbiota. These effectors reshape microbial community composition, suppress beneficial microbes, and create a metabolic and immune environment favorable for pathogen colonization. By manipulating the microbiota, pathogens indirectly influence plant immune activation, resource allocation, and defense-associated metabolic pathways.

Through the integration of effector biology, microbiome profiling, and functional plant studies, our work highlights how immune responses are tightly coupled to metabolic state and microbial context. This framework aligns closely with the concept of immunometabolism, demonstrating that plant defense outcomes emerge from coordinated immune and metabolic processes operating across host and microbiota.”



Claus Schmitz



Bart Thomma

“Within the iHead initiative, we integrate high-throughput data generated by the consortium by combining mechanistic models with statistical and machine learning techniques. Using data-integrative genome-scale metabolic modeling, we explore host-microbe metabolic interdependencies across various consortia previously identified by the iHead initiative. Our analysis will generate quantitative predictions about metabolic pathway activities, which are then subjected to classical statistical and machine learning methods.”

This allows us to elucidate how immunity-related compounds modulate the host-microbiota metabolic system and to identify common and unique metabolic response patterns in photoautotrophic and heterotrophic host-microbe systems.

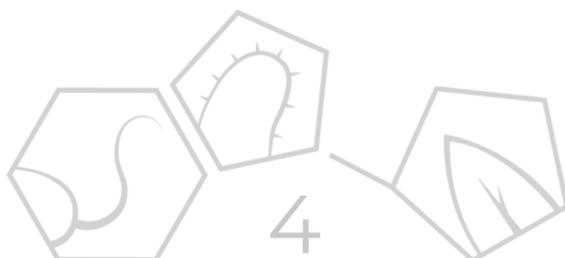
Furthermore, our models will provide experimentally testable hypotheses regarding the evolutionary interface between metabolism and immune systems, and whether they share common co-metabolism principles across different kingdoms of life or are subject to kingdom-specific solutions.”



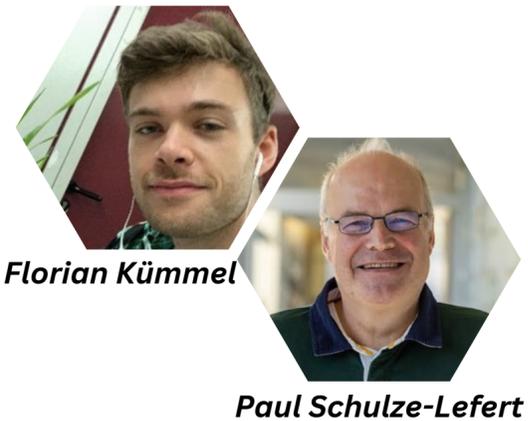
**Martina
Feierabend**



Nadine Töpfer



“The evolutionary history of plant interactions with necrotrophic pathogens that feed on dying host cells and their virulence mechanisms remains fragmentary. We isolated barley *Scs6*, which is required for the necrotrophic fungus *Bipolaris sorokiniana* isolate ND90Pr to cause spot blotch disease. *Scs6* is located at the *Mla* resistance locus and encodes an intracellular nucleotide-binding leucine-rich repeat receptor (NLR). In transgenic barley, *Scs6* is sufficient to confer susceptibility to ND90Pr in accessions naturally lacking the receptor, resulting in infection-associated host cell death. Expression of *Scs6* in evolutionarily distant *Nicotiana benthamiana* or human embryonic kidney (HEK293) cells reconstitutes a cell death to an uncharacterized non-ribosomal peptide effector produced by ND90Pr-specific non-ribosomal peptide synthetases (NRPSs) encoded at the *VHv1* virulence locus, suggesting that the effector directly activates *SCS6*. *Scs6*-mediated cell death in HEK293 cells is independent of known vertebrate cell death pathways, but dependent on extracellular calcium. *Scs6* is an allelic variant of functionally diversified *Mla* resistance genes each conferring strain-specific immunity to barley powdery mildew isolates with a matching proteinaceous pathogen effector. Domain swaps between *MLA* and *SCS6* NLRs and expression of the resulting hybrid proteins in *N. benthamiana* reveal that the *SCS6* leucine-rich repeat domain is a specificity determinant for the NRPS-derived effector to activate the receptor. Similarly, four substitutions in an *MLA* member unresponsive to ND90Pr render the receptor sensitive to the NRPS-derived effector, leading to cell death. Thus, *Mla* is subject to contrasting evolutionary selection, recognition of biotrophic pathogen effectors and evasion of targeting by a necrotrophic pathogen effector. In iHEAD, we aim to i) purify and characterize the NRPS metabolite product derived from *B. sorokiniana* ND90Pr by mass spectrometry and ii) clarify how the effector activates *SCS6* using structural biology approaches.”



Florian Kümmel

Paul Schulze-Lefert



Alga Zuccaro

Maribel Schönewolff

“Emerging metabolic signals are increasingly recognized as pivotal regulators of plant immunity and programmed cell death, shaping defense and accommodation strategies during interactions with microbial colonizers. Recent studies in our group have highlighted the role of metabolic signals in modulating plant immune responses during colonization by both beneficial and pathogenic fungi.

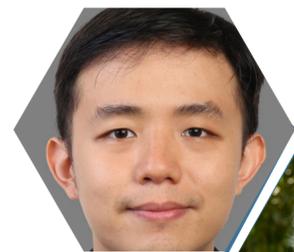
Our research in iHEAD focuses on characterizing the interference of fungal root microbiota with TIR-mediated plant immunometabolism. In particular, we study the beneficial fungal endophyte *Serendipita indica*, which employs a biphasic colonization strategy in *Arabidopsis thaliana* and barley roots. This involves an initial biotrophic phase followed by a cell death phase, confined to the epidermal and cortical root layers, which is essential for fungal accommodation.

Two fungal enzymes, *SiE5NT* and *SiNucA*, act synergistically in the apoplast to produce deoxyadenosine (dAdo), a nucleoside that triggers cell death when taken up by the plant. This process is mediated by the equilibrative nucleoside transporter *AtENT3* and a novel *Arabidopsis* TIR-NLR protein, *ISI* (Induced by *S. indica*), which modulates the plant’s immune response. The discovery that dAdo-triggered cell death involves TIR-NLR pathways establishes a novel link between fungal metabolism and plant immunometabolism, where host cell death is finely regulated to support beneficial colonization.

A key question that arises is the absence of caspases in plants and the involvement of an NLR in dAdo-mediated cell death in *Arabidopsis*, suggesting that this signaling pathway is not conserved between plants and animals and relies on different regulatory and execution mechanisms that are not yet fully understood.”



“We are studying the immune responses of *Arabidopsis thaliana* (a plant dicot model) and barley (*Hordeum vulgare*; a monocot model) cells and tissues to nucleotide-based immunostimulatory chemicals. A set of natural non-cyclic phospho-ribosylated nucleotide immunostimulatory second messengers (pRib-ADP/pRib-AMP, ADPr-ATP and di-ADPR) were discovered as enzymatic products of pathogen-induced Toll-Interleukin-1 Receptor (TIR)-domain proteins. The biochemical modes-of-action of these small molecules (SMs) as direct activators of *A. thaliana* EDS1-SAG101 and EDS1-PAD4 complexes, respectively controlling host cell death and basal immunity, was elucidated (Huang et al, 2022; Jia et al, 2022). In iHEAD, we are exploring different plant TIR-protein enzymes and the range of TIR products that accumulate in dicot and monocot backgrounds. For the analysis, *in vitro* and *in vivo* nucleotide-based infochemical profiling is being developed in collaboration with Christian Frezza and the iHEAD metabolomics platform. Together with the organic chemistry group of Stephanie Kath-Schorr at Uni. Köln, we are testing chemically synthesized natural and bioengineered SMs for immunostimulatory activities *in vitro* and *in plant* cells and tissues. We aim to track the localizations, actions and persistence of modified SM analogs inside plants to better understand their *in vivo* properties.”



Steven Cheng



Jane Parker



Stephanie Kath-Schorr



Christof Domnick

“In iHEAD, we are developing modified signaling molecules with enhanced biological properties. Recent studies by the Parker group of (Max-Planck Institute for Plant Breeding Research, Cologne) in 2022 revealed two pairs of plant TIR-catalyzed nucleotide-based small molecules (SMs) that were previously unknown in nature. Specific binding of these compounds to EDS1-PAD4 or EDS1-SAG101 dimer complexes, which are intracellular receptors for the SMs, causes an allosteric change in the dimers which promotes their association with a co-functioning nucleotide-binding leucine-rich repeat domain (ADR1 or NRG1) to induce pathogen resistance. We will investigate the action of these compounds on a molecular level. We therefore aim to probe the delivery and localization of the SMs *in vitro* and *in vivo* as well as further investigate the mechanism of the protein-protein interaction between the ADR1 or NRG1 domain with the EDS1/PAD4 or EDS1/SAG101 complexes using the natural and modified SM.”

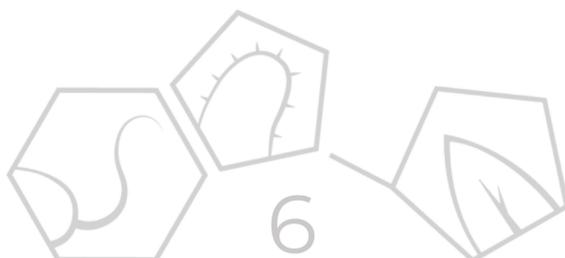
“To defend themselves against invading microbes, plants rely on either a pathogen-associated molecular pattern (PAMP)-triggered system or an effector-triggered system. The latter is mainly mediated by the class of intracellular nucleotide-binding leucine-rich repeat (NLR) receptors, which can be divided into two groups that differ in their N-terminal structural element. CC-NLRs are characterized by an N-terminal coiled-coil element and are thought to act by assembling into ion channels upon activation. In contrast, TIR-NLRs are characterized by an N-terminal Toll/interleukin-1 receptor domain and act as either NADases and/or synthetases of cNMP derivatives. For both groups, structural biology tools have provided important insights into the mechanisms of activation and activity of these cornerstone proteins of plant immunometabolism. Using cryo-electron microscopy, we aim to support other i-HEAD projects in their quest to unravel the mechanisms of plant immunometabolism and to better understand the molecular components that allow plants to differentiate between beneficial and detrimental microbial interactions.”

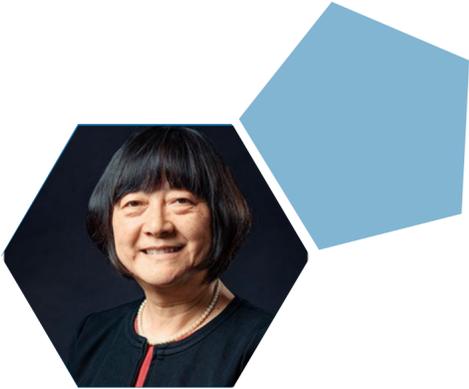


Arthur Macha



Elmar Behrmann





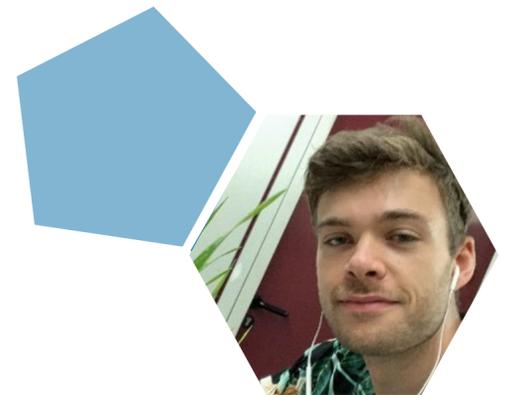
Xinnian Dong,
Duke University

Yin & Yang of Central Metabolism in Regulating Plant Immunity

Unlike animals, which rely on a wide array of specialized cells to perform distinct biological functions (e.g., T cells and B cells for immune response), plants use a small set of cell types to perform a wide range of physiological functions. This cellular “multi-tasking” ability makes plants ideal model organisms for studying regulatory mechanisms that reprogram and coordinate transcriptional and metabolic activities in response to environmental challenges, such as pathogen infection. It is well established that the success of a plant immune response is significantly impacted by environmental cues. To coordinate immune responses and other physiological activities, production of immune signals such as salicylic acid and jasmonic acid, immune output genes as well as response to immune induction are directly regulated by the circadian clock and redox rhythms. In my presentation, I will share our findings on how cellular metabolic activities directly regulate key immune regulators and shape the outcome of immune responses.

Activation and cell death mechanism of a barley NLR targeted by a fungal nonribosomal peptide effector

Virulence mechanisms of host-selective necrotrophic plant pathogens remain fragmentary. The necrotrophic fungus *Bipolaris sorokiniana* isolate ND90Pr (Bs_{ND90Pr}) requires barley Scs6 to cause spot blotch disease and associated host cell death. Scs6 is located at the Mla resistance locus and encodes an intracellular NOD-like receptor (NLR). SCS6 is activated by a nonribosomal peptide effector (NRP1) produced by Bs_{ND90Pr} to induce cell death in barley and *Nicotiana benthamiana*. Moreover, expression of barley Scs6 in human HEK293 cells followed by application of Intercellular Washing Fluid (IWF) from ND90Pr-infected barley is necessary and sufficient to reconstitute a cell death response in the vertebrate cell line, suggesting direct activation of the NLR receptor by the NRP1 effector. Scs6-mediated cell death in HEK293 cells is Ca^{2+} -dependent but independent of four characterized vertebrate cell death mechanisms. Using blue native-PAGE, oligomerization of SCS6 is detectable within 30 minutes of IWF infiltration in intact *N. benthamiana* leaves, indicating rapid effector translocation inside plant cells and intracellular NLR activation. Sensitivity of SCS6 to the NRP effector depends on four amino acids in a ‘latch’ region of the winged-helix domain. Using high-resolution mass spectrometry (MS), we identified a candidate mass of 739 Da likely corresponding to NRP1. Together, our findings indicate that Bs_{ND90Pr} employs a nonribosomal effector NRP1, which directly activates SCS6 via NLR oligomerization, enabling sustained Ca^{2+} influx and host cell death for pathogen virulence. Our next goal is to determine the nonribosomal infochemical NRP1 in its bound form to activated SCS6 with atomic resolution using cryogenic electron microscopy.



Florian Kümmel, MPIPZ

Towards a platform for identifying structural rewiring in immunometabolism-associated proteins.

- Establish structural biology tools to understand how plants differentiate between beneficial and detrimental microbes on the molecular level
 - Aims accomplished in regard to iHEAD project & work packages
 - We have developed an EM sample staining method that avoids the use of stains classified as radioactive materials, to enable all iHEAD groups to prepare samples in a decentralized and timely manner (Gunkel 2024).
 - Photosensitive caging groups can allow structural characterization of intermediate states to chart molecular activation pathways. For this, we have generated an optimized design of a freeze-plunger allowing for defined light-exposure of samples (Flores-Ibarra 2026).
 - Cryo-EM poses hardware and knowledge-based barriers, but we’ve overcome these by integrating local IT resources (ITCC/RAMSES) and establishing robust teaching procedures for iHEAD PostDocs, as exemplified by two recent publications (Lawson 2025, Lawson 2025).
- Future:* affinity grids - both establishing locally known designs (e.g. anti-His) and develop specific ones for iHead (e.g. immobilized nucleotides)



Arthur Macha, UoC

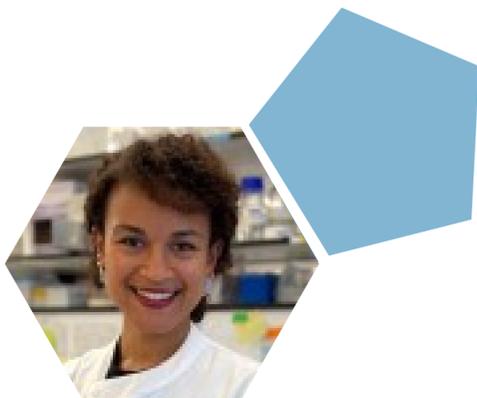


The Extended Host Immune System

Multicellular organisms depend on intimate associations with microbiota to optimize nutrient acquisition, growth, and survival. In both plants and animals, these host-associated microbial communities extend the genetic and functional capacity of their hosts, contributing not only to nutrition and development but also to health and immunity. Research by the Utrecht Plant-Microbe Interactions group has shown that plant roots secrete specialized metabolites that shape the rhizosphere microbiome, and help host plants to distinguish between beneficial and pathogenic microbes. We also showed that foliar infection in *Arabidopsis* triggers a belowground response in which roots selectively recruit beneficial microbes. This results in microbiome-mediated immune priming and the formation of a soil-borne legacy that protects future generations. We further identified specialized metabolites such as coumarins as central mediators of root-microbiome communication and immune activation. Together, these findings highlight that plant immunity is not confined to genetically encoded host responses but emerges from a dynamic dialogue between the plant and its associated microbiome. This “extended plant immune system” parallels the human extended immune system, where the host microbiome also plays a role in shaping host immunity. By connecting historical frameworks of plant immunity with contemporary microbiome research, we work on an integrated view of host defense in which microbial partners are indispensable allies. This perspective not only advances fundamental understanding of plant health but also provides a conceptual foundation for microbiome-assisted strategies in sustainable agriculture.



Corné Pieterse,
Utrecht University



Maribel Schönewolff,
UoC

Infochemical production during plant immune responses by the novel root TIR protein ISI

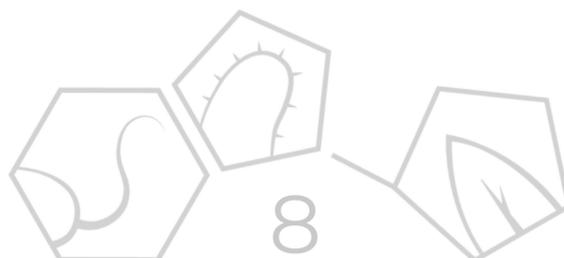
Intracellular immune receptor proteins, including members of the Toll/interleukin-1 receptor (TIR) family, perform key regulatory rolls during plant-microbe interactions by coordinating plant immune response and host cell death. A feature commonly shared amongst most TIR proteins is their ability to elicit cell death in planta by virtue of their NADase activity and through the production of immunostimulatory metabolites. A previously uncharacterized TIR-nucleotide-binding leucine-rich repeat receptor (NLR) protein, ISI (induced by *S. indica*), was shown to affect host cell death, fungal colonization, and growth promotion during colonization of *A. thaliana* roots by the beneficial fungal endophyte *Serendipita indica*. The identification of ISI as a novel TIR protein involved in coordinating host-microbe interactions begs the question of how ISI contributes to cellular outcomes of infection mechanistically, yet a biochemical characterization of ISI is missing. Preliminary data together with sequence and structural analysis suggest that ISI might function not as a NADase but rather as a nuclease and/or synthetase. To shed light on ISI's mode of action, this study aims at characterising ISI on a biochemical, biophysical and structural level. A complete picture of ISI's function will enable us to understand how this recently identified TNL in the root contributes to immune signalling and cell death during beneficial host-microbe interactions.

Social sensing of infection reprograms peripheral immunity in healthy mice

In plants and insects, social immunity enables individuals to detect infection in neighbors and mount protective, community-level responses. Whether mammals possess analogous mechanisms remains unknown. Here, we asked how the presence of sick cage-mates influences the physiology of uninfected neighbors. We found that healthy mice co-housed with conspecifics infected with the non-communicable murine pathogen *Toxoplasma gondii* undergo a shift in peripheral immune responses that establishes a primed immune state. This exposure-induced priming conferred physiological resilience to a sublethal lipopolysaccharide (LPS)-inflammatory challenge and was mediated by increased myeloid-derived IL-10 production. Blocking IL-10 signaling abrogated exposure-induced protection against a subsequent immune challenge. Thus, our findings show that immune state in healthy mammals can be shaped by exposure to infected conspecifics, hinting at social immunity-based protective mechanisms in mammals.



Lena Pernas, UCLA





Lara Hasse, CECAD

Macrophages and nucleotide Metabolism

Macrophages are part of the innate immune system and are highly responsive to their environment. When activated, they drastically alter their metabolism to fulfil the energy and biosynthetic demands required to mount an inflammatory response and eliminate pathogens. Several metabolic pathways, including glycolysis, OXPHOS, NO synthesis, have been shown to participate in this process. Yet, nucleotide metabolism is emerging as a new player in macrophage migratory capacity and phagocytosis, suggesting that this pathway could have a role in infection.

To further investigate the role of nucleotide metabolism in macrophages we performed metabolomics analyses and tracing experiments in pro-inflammatory bone marrow derived macrophages (BMDMs). We showed that upon LPS, macrophages increase de novo synthesis of pyrimidines. Interestingly, the addition of IFN γ reverses this phenotype and cells synthesise CTP and purines from the salvage pathway instead, highlighting a stimulus-dependent rewiring of nucleotide synthesis. Moreover, proteomics analysis of pro-inflammatory macrophages uncovered a decrease of RNA and DNA synthesis suggesting that the newly synthesised nucleotides fulfil other functions linked to inflammation. Consistent with this hypothesis, we found that macrophages redirect CTP synthesis towards ddhCTP, an antiviral metabolite, through RSAD2 upregulation. Overall, our study shows that pro-inflammatory stimulation with type II interferon signalling substantially modifies the nucleotide synthesis towards the salvage pathway and promotes the synthesis of antiviral molecules.

Microbiome composition (counter-)selects for antibiotic resistant strains in a personalized manner

Antibiotic resistant pathogens are a major threat to modern medicine as development of novel therapeutics is outpaced by resistance emergence and dissemination. Alternative approaches to slow down or even reverse antibiotic resistance, such as the amplification of deleterious side effects associated with resistance, are necessary to maintain antibiotic efficacy. Here, we investigate the influence of the human gut microbiome on the competitiveness of an antibiotic resistant *Klebsiella pneumoniae* strain and identify microbiome specific drivers towards a high-fitness drug-resistant variant mediated (partly) by a focal *E. coli* strain. We determined carbohydrate competition, in particular for glycerol-containing compounds, as a major driver for the selection for mutations in the Lac-type transcriptional regulator GlyR resulting in an upregulation of the glycoporin GlyP. This selective benefit is strictly microbiome specific, as higher-order community interactions can increase, or decrease, the selective advantage of GlyP upregulation in an ompK35/36 deficient background. Finally, we show that the selective pressures driving GlyR mutations are prevalent in vivo and that this niche specialization for glycerol strongly reduces competitiveness in other environments, consequently causing extinction of the niche-specialized variants. This study exemplifies, how we can utilize in vitro screens using fecal matter derived microbiomes to identify (counter-)selective pressures prevalent in vivo to uncover and amplify relevant vulnerabilities of resistant pathogens consequently reducing the likelihood of acquiring hard-to-treat infections.



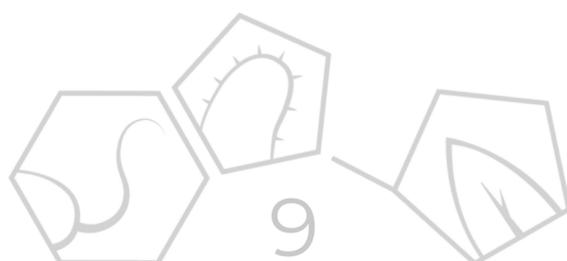
Michael Knopp, EMBL



Hannah Dorethy, CECAD

Exploiting *P. aeruginosa* Metabolism to Combat Resistance & Enhance Host Defence

Antimicrobial resistance is a global crisis with significant health and economic impacts. *Pseudomonas aeruginosa*, a pathogen particularly problematic within the nosocomial environment, has been classified as a high priority pathogen for which the R&D of new antibiotics is paramount due to staggering multi-drug resistance. Our work has discovered that the combination of a repurposed drug with the targeting of *P. aeruginosa* central metabolism results in a multifactorial bacterial response that enhances the host immune response, aids host survival and reverses antimicrobial resistance. This talk will discuss the advantages of targeting a previously overlooked system, central metabolism, and how exploiting this essential process can force bacteria to become vulnerable to our current arsenal of antibiotics.



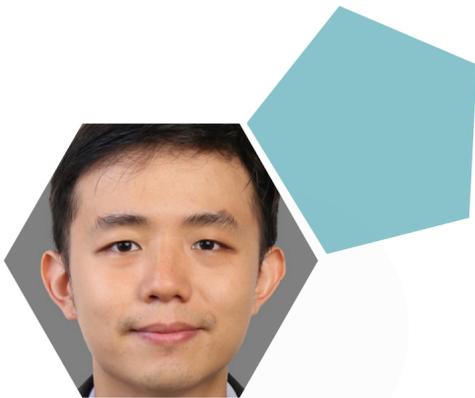
Metabolic Engineering for Vascular Pathogen Resistance

Vascular pathogens pose a serious threat to global agriculture, causing devastating diseases in a wide range of economically important crops. These pathogens employ diverse strategies to invade the xylem, where they proliferate extensively, obstruct water and nutrient transport, and ultimately cause plant wilting and death. In response, certain resistant plant varieties have evolved mechanisms to detect such infections and activate strong defense responses within the xylem. Among the most effective of these is the formation of physico-chemical barriers that block pathogen colonization and spread.

Using the tomato–*Ralstonia solanacearum* pathosystem as a robust model, our work demonstrates that resistant cultivars respond to infection by reinforcing their xylem cell walls with ligno-suberin polymers and accumulating feruloyl-tyramine, a key compound involved in shaping the defense-related metabolic network in plants. We present evidence that both genetic and pharmacological manipulation of this metabolic pathway significantly increases resistance to infection. These findings offer a promising avenue for engineering broad-spectrum resistance to vascular pathogens through precise modification of plant cell wall metabolism.



Nuria Sánchez-Coll,
CRAG



Steven Cheng, MPIPZ

Plant disease resistance strategy using a ribosylated nucleotide signal

Diseases caused by pathogens and pests account for ~30% global losses in crop yields annually. Upon pathogen perception, seed plants generate ribosylated nucleotides pRib-AMP and pRib-ADP which activate ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)-PHYTOALEXIN DEFICIENT 4 (PAD4; EP) dimeric receptor to promote disease resistance. Broad conservation of this biochemical mode of activation between dicot *Arabidopsis* and monocot rice (*Oryza sativa*) raises questions to the potential utility of these ribosylated nucleotides in crop protection. We demonstrate that the exogenous application of pRib-AMP and engineered chemical derivatives promotes robust disease resistance with minimal growth tradeoffs. Our data provide a basis for harnessing pRib-AMP and derivatives as broadly effective immune-stimulating agents for crop protection against disease.

Application of natural immune-stimulating pRib-AMP to plants limits disease

Plants induce immunity through TIR-catalyzed ribosylated nucleotides that bind to EDS1 heterodimers. EDS1-PAD4 (EP) activation by pRib-AMP induces association with and activation of ADR1 helper NLRs (A) leading to pathogen resistance at a convergence point downstream of cell-surface and intracellular (NLR) receptor recognition.[1] Phylogenetic and functional studies revealed that pRib-AMP coordinating residues in EP are conserved between dicots and monocots. 2-cADPR, another product of TIR enzymes, was found to be a likely pRib-AMP precursor and is sufficient to activate disease resistance to bacteria when applied to leaves.[2] Here we describe experiments using pure synthetic pRib-AMP applied to plants to test pRib-AMP in vivo cellular uptake, defense-inducing bioactivity, potential mobility and defense-growth trade-offs. We show that exogenously applied pRib-AMP is likely not perceived as a MAMP or DAMP at the cell surface but activates immune transcriptional reprogramming and disease resistance through the intracellular EPA node. Establishment of pRib-AMP response assays open opportunities to develop strategies for in vivo probing to observe subcellular localization and tissue distribution. We anticipate that the TIR-catalyzed ribosylated nucleotide could play a role in cell-to-cell signalling.



Christof Domnick, UoC





Marc Nishmura,
Colorado State University

The Arabidopsis TIRome informs the design of artificial TIR (Toll/interleukin-1 receptor) domain proteins.

The plant immune system relies on NBS-LRR (NLR) immune receptors to detect the presence or activity of pathogen virulence proteins that function in the host cytoplasm. One major class of plant NLRs employs an N-terminal TIR domain to activate downstream signaling for cell death and resistance. Non-canonical TIR proteins (e.g. TIR-only, or TIR-NBS) appear to function similarly. TIR domains were recently discovered to be NADases that process NAD⁺, and related molecules, into a variety novel small molecule signals. Plant TIR domains appear to have both a spectrum of products and a wide range of cell death-triggering activity in various assays, but the diversity of products and outcomes are poorly understood. To better understand the plant immune system and TIR function we have exploited the diversity of the ~150 TIR domains in Arabidopsis to generate consensus-based synthetic TIRs to test hypotheses about the structural requirements for function. A better understanding of the determinants of TIR activity may enable rational engineering of plant immune systems via tuning of TIR domains for desired outputs.

Pan-genome-scale metabolic modeling to unravel metabolic functions of plant-associated Spingomonas

Plants and their associated microbiota form complex metabolic interactions that shape community structure and function. These microbes enhance plant fitness by promoting growth, improving nutrient acquisition, and increasing resilience to biotic and abiotic stress. Spingomonas is a highly abundant and widespread member of the plant core microbiota, often described as plant growth-promoting. However, meta-analyses show that Spingomonas is consistently depleted under biotic stress conditions. To investigate the metabolic basis of this phenomenon, we employ genome-scale metabolic modeling. This computational framework, grounded in biochemical network topologies, integrates metabolic knowledge and predicts fluxes at a large scale. Using pan-genome-scale metabolic models, we capture the collective metabolic potential of multiple Spingomonas strains while allowing strain-specific characterization.

Our work provides (i) a comprehensive set of Spingomonas network reconstructions defining the genus-level pan-reactome, (ii) a framework to study metabolic diversity within Spingomonas, and (iii) an integrated metabolic reactome as a foundation for exploring contributions to plant-microbe interactions, particularly in the context of immunometabolism.

By linking microbial metabolism with plant immune responses, this framework shall advance mechanistic understanding of cross-kingdom immunometabolism and provide insights for targeted microbiome manipulation to support sustainable agriculture.



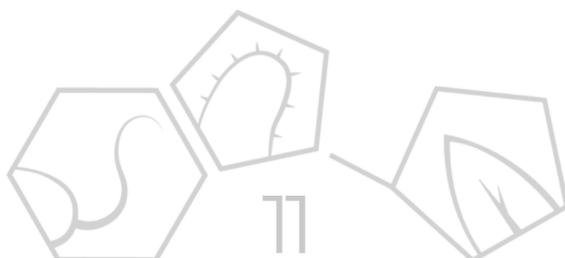
Martina Feierabend, UoC



Claus-Peter Witte,
University Hannover

There is still much to learn about (immuno)-nucleotide metabolism in plants.

The depths of the plant nucleotide metabolome have not yet been explored. In part, this is due to technical challenges of detection and quantification by LC-MS methods. Such challenges are particularly hard to overcome in plants making especially lower abundant nucleotides and derivatives undetectable. Consequently, nucleotides rarely reported in general metabolome studies. In the quest to identify and functionally characterize novel enzymes in plant nucleotide metabolism, we have greatly improved the detection of this metabolite class in plants. Here I will present some of the biological discoveries enabled by this technological advancement. In the field of immunometabolism, there has recently been an increased focus on nucleotides, prompting the need for better detection and quantification of 'immunonucleotides'.



Pathogen-Induced Modulation of Extracellular Nucleotide Signaling in Plants

Across kingdoms of life, extracellular nucleotides function as conserved metabolic signaling molecules that shape immune regulation, microbial adaptation, and community dynamics in both animal and plant host-microbe systems. Plants actively use extracellular nucleotide pools for immune and danger-associated signaling, and accumulating evidence suggests that microbes can interfere with these infochemicals, thereby reshaping immune responses and interactions with beneficial and pathogenic microorganisms. However, the molecular mechanisms by which pathogens modulate extracellular nucleotide signaling remain largely unexplored.

Building on my previous work on PorX, a nucleotide-hydrolyzing regulator of pathogenicity from *Porphyromonas gingivalis*, the keystone pathogen of human periodontal diseases, this project seeks to uncover conserved cross-kingdom principles of how pathogens exploit nucleotide modification to modulate host immunity and promote dysbiosis within host-associated microbiomes.

Focusing on the fungal plant pathogen *Verticillium dahliae*, I examine whether it modulates plant extracellular nucleotide signaling through secreted or membrane-associated hydrolases. Using genome-wide bioinformatic screening, transcription profiling during infection, in planta knock-out analyses, and an emphasis on structural and biochemical characterization of selected enzymes, I will characterize candidate hydrolases, define their molecular activities, and determine their impact on plant health and disease. Ultimately, I seek to reveal how microbial enzymes reshape extracellular metabolic signaling with downstream effects on immunity and microbial homeostasis.



Claus Schmitz, UoC

Rewiring the Host: Geminiviruses as Probes of Immunometabolic Networks

tba



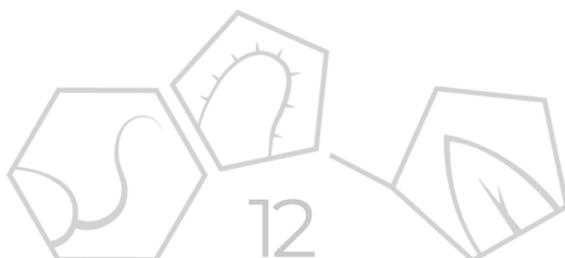
Rosa Lozano-Duran,
University Tübingen

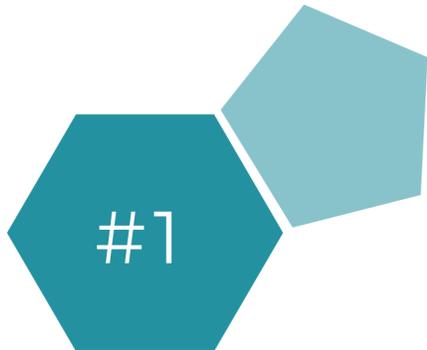
Metabolic Nexus: Shaping Host-Pathogen dynamics

Host-pathogen interactions are highly complex and dynamic, with metabolism at the core of this battle. The immune system depends on metabolic pathways to sustain its functions and mount effective responses, while pathogens have evolved strategies to exploit host metabolism for their growth and persistence. This metabolic crosstalk not only shapes infection outcomes but also drives disease progression. Among host organelles, mitochondria play a pivotal role as metabolic hubs that govern energy production, immune signalling, and cell fate. Far beyond being the “powerhouse of the cell,” mitochondria provide the plasticity needed for adaptation and survival. Given their central role, it is not surprising that many pathogens specifically target mitochondria, disrupting their dynamics and bioenergetics to evade immune defences and create favourable niches. Our work investigates how pathogen interactions induce mitochondrial metabolic rewiring, focusing on the molecular mechanisms by which bacteria manipulate mitochondrial function, and the physiological consequences of this process during infection.



Raja Ganesan,
University Clinic Cologne





Machine-learning predictions of the substrates of metabolic enzymes and transporters

Alexander Kroll, HHU

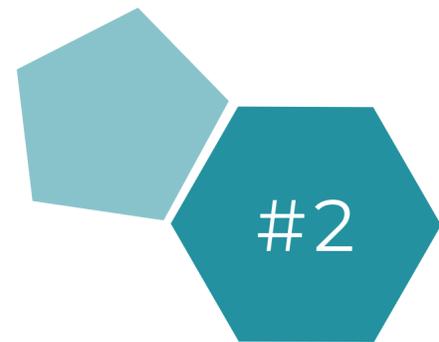
Accurately predicting which small molecules are substrates of enzymes and transporters is essential for understanding metabolism, drug discovery, and biotechnology. With ESP and SPOT, we developed the first general models for enzyme-substrate and transporter-substrate prediction. We previously improved enzyme-substrate prediction using ProSmith, a multimodal transformer that jointly encodes small molecules and protein sequences in a single input. Despite these advances, performance is still constrained by two major gaps: limited amounts of curated training data and the lack of explicit protein structural information. To address the data bottleneck, we are systematically screening the literature and protein databases to expand protein-substrate annotations, already increasing our enzyme-substrate dataset 10-fold to more than 200,000 experimentally validated pairs. To incorporate structure, we are developing a general full-atom 3D protein structure encoder designed to better represent enzyme and transporter binding sites. Together, expanded training data and structure-aware protein representations improve substrate prediction for both enzymes and transporters.

Detect me if you can: Rare nucleotides in plants as potential signals for stress

Marco Herde, Leibniz University Hannover

Nucleotides perform numerous essential functions in living cells. While recent studies have highlighted their roles in signal transduction, this major class of metabolites remains comparatively underexplored, particularly in plants. Both nucleotides and nucleosides are central to primary metabolism, with a canonical set of these compounds serving as key carriers of energy and genetic information. Beyond these classical roles, biotic and abiotic stresses strongly influence the abundance of non-canonical nucleotides. These molecules, which lie outside the core of primary metabolism, arise through enzymatic or non-enzymatic modifications of standard nucleotides and nucleic acids or from the formation of (cyclic) nucleotide oligomers.

The low abundance of non-canonical nucleotides, their instability, and their chemical properties together with the typical high complexity of plant metabolite extracts pose a significant challenge for their detection and quantification. We developed a sensitive analytical method that quantifies nucleotides and nucleosides from plants including many non-canonical nucleotides. This technique was instrumental for the detection of the non-canonical nucleotide inosine triphosphate (ITP) and allowed the functional characterization of an enzyme (ITPA) that sanitizes the nucleotide pool by dephosphorylation of ITP. ITPA suppresses the incorporation of ITP and dITP into RNA and DNA, respectively. We investigated the metabolic origin of ITP and showed that non-enzymatic processes, which are enhanced by abiotic stress and enzyme promiscuity, can result in the formation of this non-canonical metabolite. Interestingly, ITPA mutant plants had elevated amounts of salicylic acid and specifically induced the expression of defence genes. Thus, in line with numerous studies in mammalian, bacteria and plants there is a great potential for rare nucleotides to serve in plant stress signalling. We currently attempt to identify such nucleotide-derived signals using a non-targeted metabolism approach that uses our analytical workflow in combination with various techniques for enriching nucleotides allowing their detection by mass-spectrometry.





#3

Infochemical production during plant immune responses by the novel root TIR protein ISI

Maribel Schönewolff, UoC

Intracellular immune receptor proteins, including members of the Toll/interleukin-1 receptor (TIR) family, perform key regulatory rolls during plant-microbe interactions by coordinating plant immune response and host cell death. A feature commonly shared amongst most TIR proteins is their ability to elicit cell death in planta by virtue of their NADase activity and through the production of immunostimulatory metabolites. A previously uncharacterized TIR-nucleotide-binding leucine-rich repeat receptor (NLR) protein, ISI (induced by *S. indica*), was shown to affect host cell death, fungal colonization, and growth promotion during colonization of *A. thaliana* roots by the beneficial fungal endophyte *Serendipita indica*. The identification of ISI as a novel TIR protein involved in coordinating host-microbe interactions begs the question of how ISI contributes to cellular outcomes of infection mechanistically, yet a biochemical characterization of ISI is missing. Preliminary data together with sequence and structural analysis suggest that ISI might function not as a NADase but rather as a nuclease and/or synthetase. To shed light on ISI's mode of action, this study aims at characterising ISI on a biochemical, biophysical and structural level. A complete picture of ISI's function will enable us to understand how this recently identified TIR in the root contributes to immune signalling and cell death during beneficial host-microbe interactions.

Role of persulfidation during plant-microbe interactions

Shivam Bady, UoC

Plants are exposed to a wide range of environmental challenges arising from both abiotic and biotic factors. To adapt to fluctuating environments, biological systems have evolved sophisticated intracellular signalling mechanisms to coordinate internal responses to external stimuli. Among these mechanisms, Post-Translational Modifications (PTMs), such as the persulfidation of cysteines (RSH → RSSH), serve as crucial regulators of protein function and cellular networks. Persulfidation has been implicated in a range of physiological processes (Wang et al., 2023), and emerging evidence suggests that persulfidation contributes to both fungal pathogenicity and host immune responses (Sueiro-Olivares et al., 2021). However, its specific role in the context of plant-microbe interactions requires further investigation. In our study, we aim to investigate the role of cysteine persulfidation in *Arabidopsis thaliana* during interactions with biotic stressors including *Pseudomonas syringae*, *Verticillium dahliae*, and *Serendipita indica*. To achieve this, we have optimised established methodologies, including both LC-MS-based (qPerS-SID) (Longen et al., 2016) and gel-based (Dimedone-Switch) techniques, to enable precise quantification of persulfidated proteins.

Preliminary data suggests that, during pathogenic challenge, the persulfidation status of various key proteins associated with photosynthesis, protein metabolism, and amino acid metabolism are differentially regulated. These findings point to a previously unrecognised layer of regulation within plant defence responses. A comprehensive analysis of this novel process may shed light on how persulfidation modulates the interplay between plant and microbial interactions.



#4



#5

Metabolic Nexus: Shaping Host-Pathogen dynamics

Raja Ganesan, UoC/ University Clinic Cologne

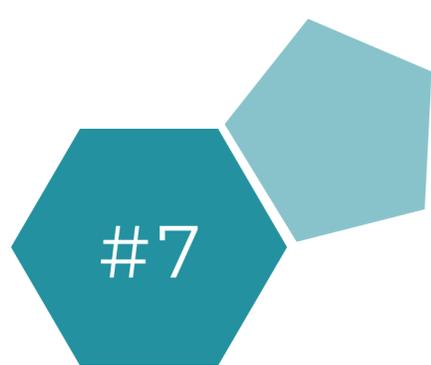
Host-pathogen interactions are highly complex and dynamic, with metabolism at the core of this battle. The immune system depends on metabolic pathways to sustain its functions and mount effective responses, while pathogens have evolved strategies to exploit host metabolism for their growth and persistence. This metabolic crosstalk not only shapes infection outcomes but also drives disease progression. Among host organelles, mitochondria play a pivotal role as metabolic hubs that govern energy production, immune signalling, and cell fate. Far beyond being the “powerhouse of the cell,” mitochondria provide the plasticity needed for adaptation and survival. Given their central role, it is not surprising that many pathogens specifically target mitochondria, disrupting their dynamics and bioenergetics to evade immune defences and create favourable niches. Our work investigates how pathogen interactions induce mitochondrial metabolic rewiring, focusing on the molecular mechanisms by which bacteria manipulate mitochondrial function, and the physiological consequences of this process during infection.



Redox rhythm regulates immune-induced cell death distinctly from genetic clock

Sargis Karapetyan, Duke University

The physiological output of the non-transcriptional redox rhythm, first discovered in anucleate red blood cells has long been unknown. Furthermore, the tentative redox-rhythm regulated genes identified in genetic clock-defective mutants did not show oscillations in the WT background. Through an alternative approach of using a long-period Arabidopsis mutant, we disentangled the redox and genetic rhythms with distinct period lengths and transcriptional targets. Analysis of the target genes indicated regulation of the immune-induced programmed cell death (PCD) by the redox rhythm. Genetic and chemical perturbations indicated that PCD is gated by the redox rhythm through jasmonic acid/ethylene defense hormone pathways. Further imaging using redox-sensitive roGFP reporters revealed reprogramming of chloroplast and mitochondria activities, changing their redox status and triggering oxidative burst in chloroplasts immediately preceding the cell death. We hypothesize that compared to robust genetic clocks, the sensitive circadian redox rhythm serves as a signaling hub in regulating incidental energy-intensive processes, such as immune-induced PCD, to provide organisms a flexible strategy to coordinate metabolic activities during stress responses.



Interference of fungal root microbiota with TIR-mediated plant immunometabolism

Nick Dunken, UoC

We recently showed that a TIR-NLR protein is involved in accommodation of beneficial fungi in the roots of *A. thaliana* by mediating a response to deoxyadenosine (dAdo), an active metabolite produced by the synergistic activity of fungal enzymes during root colonization (Dunken et al., bioRxiv 2023). The contribution of a TNL in dAdo-mediated fungal accommodation in Arabidopsis suggests that this NLR might be guarding a protein targeted by dAdo. Alternatively, as mentioned in IP 4, it is possible that dAdo is converted to a plausible substrate of TNLs related to either nicotinamide adenine dinucleotide (NAD) or to the non-canonical cyclic nucleotide monophosphate 2',3'-cAMP (Horsefield et al., Science 2019; Wan et al., Science 2019; Yu et al., Cell 2022). We were able to show that this response is independent of EDS1, which raises the question of a new, EDS1-independent branch of immune metabolism in roots. Recently, it was shown that plant TIR proteins are not only NADases but also act as 2',3'-cAMP/cGMP synthetases by hydrolysing RNA/DNA. Mutations that specifically disrupt synthetase activity abolish TIR-mediated cell death in *N. benthamiana*, demonstrating an important role for these cNMPs in TIR signaling (Yu et al., Cell 2022). The accumulation of extracellular 3',5'-cAMP upon colonization with *S. indica* and treatment with dAdo links to cyclic nucleotide monophosphates (Dunken et al., bioRxiv 2023). In this IP we want to characterize the metabolism of dAdo in plant cells and how intracellular dAdo leads to TNL activation in Arabidopsis. Since NLRs of the TIR domain proteins are also found in animals, our results open the possibility to further investigate the role of TIR domain proteins in cell death triggered by dAdo in plants and beyond. In animal systems, it has been shown that dAdo-mediated toxicity following import of dAdo into macrophages involves conversion of dAdo to dAMP through the activity of deoxycytidine kinase (DCK) and adenosine kinase (ADK) and signaling via subsequent conversion to the corresponding di- and tri- phosphates by nucleotide kinases and activation of caspase-3-induced apoptosis (Winstel et al., mBio 2018). An open question here is: The absence of caspases in plants and the implication of an TNL in dAdo-mediated cell death in Arabidopsis strongly suggest that this part of the signaling pathway is not conserved between plants and animals and relies on different regulatory and execution mechanisms that need further investigation.

- Characterization of novel nucleotide-based molecules involved in TIR-NLR-mediated immunity in *A. thaliana* roots during accommodation of beneficial and pathogenic fungi.
- Identification of new active metabolites affecting fungal accommodation.



Pan-genome-scale metabolic modeling to unravel metabolic functions of plant-associated Sphingomonas

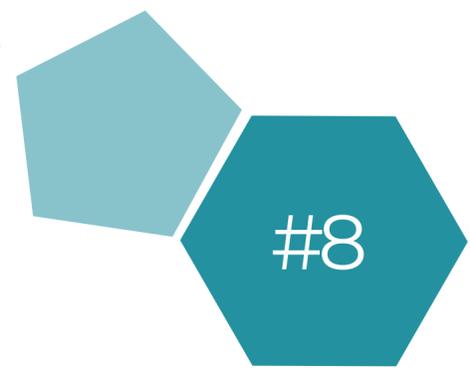
Martina Feierabend and Nadine Töpfer, University of Cologne, Cluster of Excellence on Plant Sciences (CEPLAS), Institute for Plant Sciences, Cologne/Germany

Plants and their associated microbiota form complex metabolic interactions that shape community structure and function. These microbes enhance plant fitness by promoting growth, improving nutrient acquisition, and increasing resilience to biotic and abiotic stress. Sphingomonas is a highly abundant and widespread member of the plant core microbiota, often described as plant growth-promoting. However, meta-analyses show that Sphingomonas is consistently depleted under biotic stress conditions.

To investigate the metabolic basis of this phenomenon, we employ genome-scale metabolic modeling. This computational framework, grounded in biochemical network topologies, integrates metabolic knowledge and predicts fluxes at a large scale. Using pan-genome-scale metabolic models, we capture the collective metabolic potential of multiple Sphingomonas strains while allowing strain-specific characterization.

Our work provides (i) a comprehensive set of Sphingomonas network reconstructions defining the genus-level pan-reactome, (ii) a framework to study metabolic diversity within Sphingomonas, and (iii) an integrated metabolic reactome as a foundation for exploring contributions to plant-microbe interactions, particularly in the context of immunometabolism.

By linking microbial metabolism with plant immune responses, this framework shall advance mechanistic understanding of cross-kingdom immunometabolism and provide insights for targeted microbiome manipulation to support sustainable agriculture.



Pathogen-Induced Modulation of Extracellular Nucleotide Signaling in Plants

Claus Schmitz, UoC

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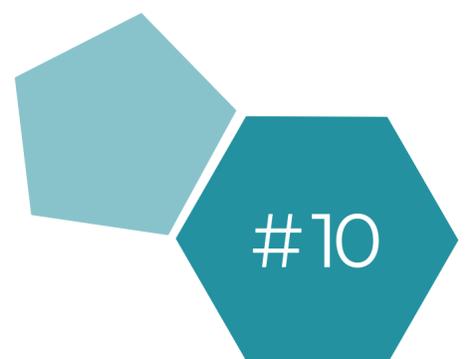
Building on my previous work on PorX, a nucleotide-hydrolyzing regulator of pathogenicity from Porphyromonas gingivalis, the keystone pathogen of human periodontal diseases, this project seeks to uncover conserved cross-kingdom principles of how pathogens exploit nucleotide modification to modulate host immunity and promote dysbiosis within host-associated microbiomes.

Focusing on the fungal plant pathogen Verticillium dahliae, I examine whether it modulates plant extracellular nucleotide signaling through secreted or membrane-associated hydrolases. Using genome-wide bioinformatic screening, transcription profiling during infection, in planta knock-out analyses, and an emphasis on structural and biochemical characterization of selected enzymes, I will characterize candidate hydrolases, define their molecular activities, and determine their impact on plant health and disease. Ultimately, I seek to reveal how microbial enzymes reshape extracellular metabolic signaling with downstream effects on immunity and microbial homeostasis.

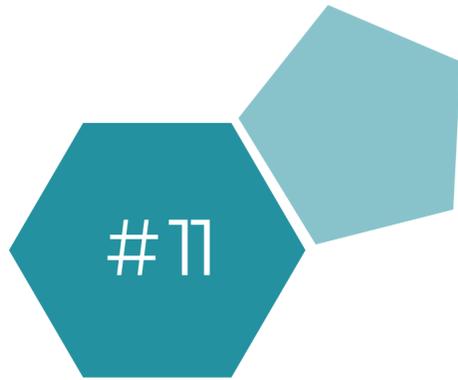
Application of natural immune-stimulating pRib-AMP to plants limits disease

Julian Strippel, UoC

Plants induce immunity through TIR-catalyzed ribosylated nucleotides that bind to EDS1 heterodimers. EDS1-PAD4 (EP) activation by pRib-AMP induces association with and activation of ADR1 helper NLRs (A) leading to pathogen resistance at a convergence point downstream of cell-surface and intracellular (NLR) receptor recognition. Phylogenetic and functional studies revealed that pRib-AMP coordinating residues in EP are conserved between dicots and monocots.



2'cADPR, another product of TIR enzymes, was found to be a likely pRib-AMP precursor and is sufficient to activate disease resistance to bacteria when applied to leaves. Here we describe experiments using pure synthetic pRib-AMP applied to plants to test pRib-AMP in vivo cellular uptake, defense-inducing bioactivity, potential mobility and defense-growth trade-offs. We show that exogenously applied pRib-AMP is likely not perceived as a MAMP or DAMP at the cell surface but activates immune transcriptional reprogramming and disease resistance through the intracellular EPA node at a minimal dose of 10 μ M. Development of pRib-AMP response assays opens opportunities to explore the chemical space of pRib-AMP for derivatization-Also, we anticipate that application of pRib-AMP- based formulations to monocot and dicot crops could be an effective strategy for disease protection and complementary to receptor engineering approaches.



Activation and cell death mechanism of a barley NLR targeted by a fungal nonribosomal peptide effector

Florian Kümmel, MPIPZ

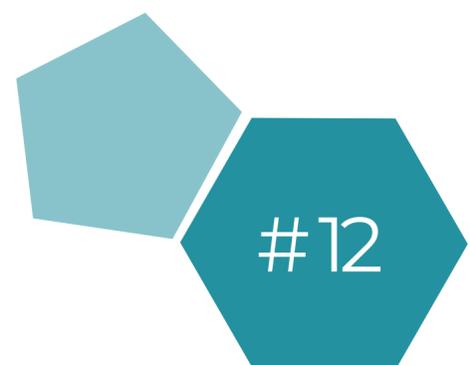
Virulence mechanisms of host-selective necrotrophic plant pathogens remain fragmentary. The necrotrophic fungus *Bipolaris sorokiniana* isolate ND90Pr ($B_{S_{ND90Pr}}$) requires barley *Scs6* to cause spot blotch disease and associated host cell death. *Scs6* is located at the *Mla* resistance locus and encodes an intracellular NOD-like receptor (NLR). *SCS6* is activated by a nonribosomal peptide effector (NRP1) produced by $B_{S_{ND90Pr}}$ to induce cell death in barley and *Nicotiana benthamiana*. Moreover, expression of barley *Scs6* in human HEK293 cells followed by application of Intercellular Washing Fluid (IWF) from ND90Pr-infected barley is necessary and sufficient to reconstitute a cell death response in the vertebrate cell line, suggesting direct activation of the NLR receptor by the NRP1 effector. *Scs6*-mediated cell death in HEK293 cells is Ca^{2+} -dependent but independent of four characterized vertebrate cell death mechanisms. Using blue native-PAGE, oligomerization of *SCS6* is detectable within 30 minutes of IWF infiltration in intact *N. benthamiana* leaves, indicating rapid effector translocation inside plant cells and intracellular NLR activation. Sensitivity of *SCS6* to the NRP effector depends on four amino acids in a 'latch' region of the winged-helix domain. Using high-resolution mass spectrometry (MS), we identified a candidate mass of 739 Da likely corresponding to NRP1. Together, our findings indicate that $B_{S_{ND90Pr}}$ employs a nonribosomal effector NRP1, which directly activates *SCS6* via NLR oligomerization, enabling sustained Ca^{2+} influx and host cell death for pathogen virulence. Our next goal is to determine the nonribosomal infochemical NRP1 in its bound form to activated *SCS6* with atomic resolution using cryogenic electron microscopy.

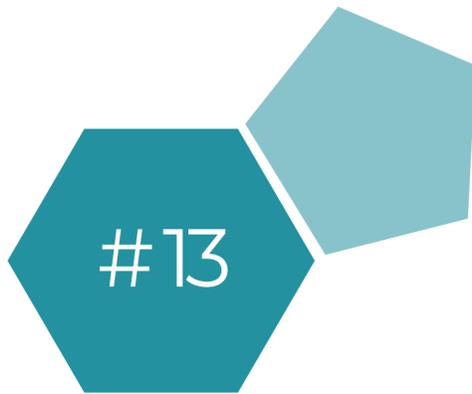
Towards a platform for identifying structural rewiring in immunometabolism-associated proteins.

Arthur Macha, UoC

- Establish structural biology tools to understand how plants differentiate between beneficial and detrimental microbes on the molecular level
- Aims accomplished in regard to iHEAD project & work packages
- We have developed an EM sample staining method that avoids the use of stains classified as radioactive materials, to enable all iHEAD groups to prepare samples in a decentralized and timely manner (Gunkel 2024).
- Photosensitive caging groups can allow structural characterization of intermediate states to chart molecular activation pathways. For this, we have generated an optimized design of a freeze-plunger allowing for defined light-exposure of samples (Flores-Ibarra 2026).
- Cryo-EM poses hardware and knowledge-based barriers, but we've overcome these by integrating local IT resources (ITCC/RAMSES) and establishing robust teaching procedures for iHEAD PostDocs, as exemplified by two recent publications (Lawson 2025, Lawson 2025).

Future: affinity grids - both establishing locally known designs (e.g. anti-His) and develop specific ones for iHead (e.g. immobilized nucleotides)





Cytosolic sodium is a pathogen-derived danger signal to activate the NLRP3 inflammasome

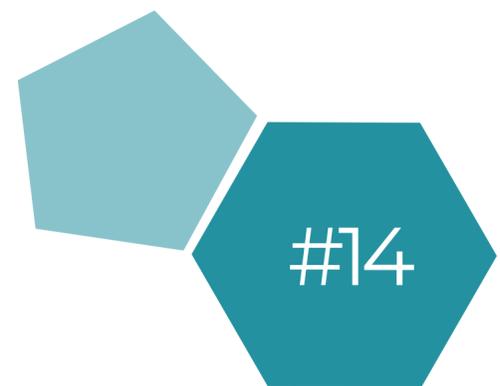
Matthew Mangan, University Clinic Cologne

Cells must sense pathogens and pathogenic molecules within the endo/lysosomal system to mount an effective cellular defense. Here, we demonstrate that release of endo/lysosomal sodium to the cytosol is a critical cellular danger signal to detect endo/lysosomal perturbation. Large clostridial toxins, including Toxin B from *Clostridioides difficile*, as well as MSU and silica crystals, trigger sodium release from endo/lysosomes. This rapidly elevates cytosolic sodium, creating an ionic imbalance that triggers activation of the NLRP3 inflammasome. Strikingly, sodium influx is also essential for NLRP3 activation by non-particulate stimuli including nigericin, which initiates sodium influx by triggering potassium efflux. These ionic perturbations converge on NLRP3 to drive its cellular relocalisation, a decisive step for its activation. These findings establish cytosolic sodium as a sensor of endo/lysosomal perturbation and a unifying signal that links diverse ionic perturbations to NLRP3 inflammasome, with implications for inflammatory disease and host defense.

Transcriptional response of *Magnaporthe oryzae* towards barley microbiome derived bacteria

Komal Pervaiz, University of Bonn INRES

The composition of the plant microbiome is shaped not only by the host plant and abiotic environmental factors, but also by inter-microbial cooperation and competition. Plant-colonizing microbes, including plant pathogens, therefore must remain competitive within the plant microbiome in order to establish themselves within their host niche. *Magnaporthe oryzae*, the blast-disease causing ascomycete fungus, is able to infect economically important hosts including rice, barley and wheat. We sought to identify barley associated bacteria able to antagonize *M. oryzae* and characterize any antimicrobial self-defense mechanisms induced in the fungus. From a library of barley-associated bacteria, two strains were identified as strong antagonists. Through RNA-seq following bacterial confrontation, we demonstrate large-scale transcriptional changes in *M. oryzae*, with both common, and strain-specific profiles. Common responses included an over-representation of genes encoding drug efflux transporters, hydrolases, secondary metabolism, DNA repair and oxidative stress responses. These findings indicate *M. oryzae* priorities stress adaptation and detoxification as a common response. We did not observe a significant increase in secreted proteins common to both strains. However, significant strain-specific changes were observed, indicating specific microbial antagonists have the potential to alter the secretome profile independent of the plant host. Understanding these resistance strategies could provide insights into antimicrobial resistance in plant pathogenic fungi.



Characterization of an Early-Expressed *Ustilago maydis* Effector Family with Copy-Number Variation in Smut Fungi

Riaz Tabassum, University of Bonn INRES

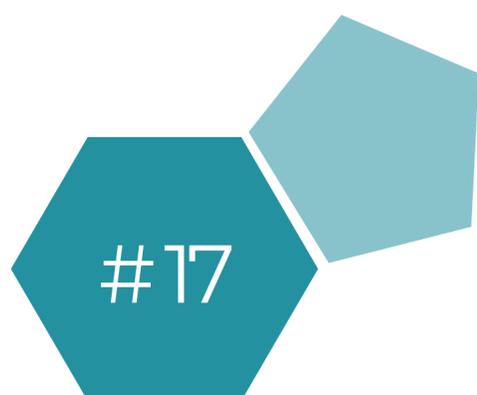
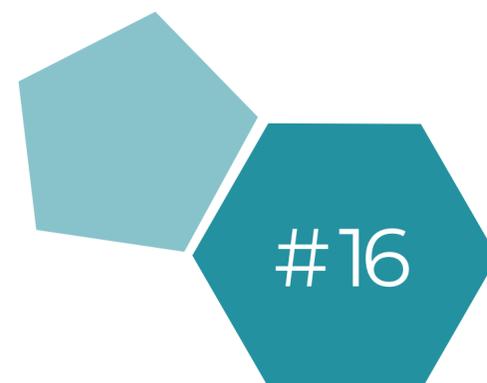
Ustilago maydis is a biotrophic smut fungus that infects aerial organs of maize. The fungus manipulates host immunity by the secretions of many effector proteins. We identified three putative effector gene paralogues (Rhi1/Rhi2 and Rhi3). Rhi1 and Rhi2 seem to have undergone evolutionary recent gene duplication and share identical coding sequences with a single synonymous SNP. Rhi3 exhibits sequence divergence yet retains conserved domains with Rhi1/Rhi2. Expression analysis indicates high expression during initial infection, which is then suppressed from two days post fungal inoculation on maize. Comparative genomic analysis across smut fungi revealed copy-number variation, with most species harboring at least one homologue, yet *U. maydis* harbors three copies – the most of any species assessed. This indicates a potential evolutionary adaptation in *U. maydis*. Several conserved motifs and domains, including a putative nuclear localization signal, were also identified. To functionally characterize Rhi1/Rhi2/Rhi3, expression was induced in *Arabidopsis* seedlings which revealed a significant increase in root hair growth, indicating a potential role in plant development modulation. We then sought to determine their subcellular localization in *Nicotinia benthamiana*, with early results supporting nuclear localisation. Future experiments will seek to assess their role in fungal virulence and assess any distinct roles during early infection.



Unravelling Fungal Antimicrobial Defence as a Driver of Niche Adaptation in *Magnaporthe oryzae*

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Magnaporthe oryzae, the causal agent of rice blast, is a major threat to global food production and a model for studying fungal adaptation in microbially complex environments. In the barley root microbiome, the fungus encounters diverse bacteria that produce antimicrobial compounds to secure ecological niches. These antagonistic interactions have driven the coevolution of fungal antimicrobial defence mechanisms, yet the genes enabling *M. oryzae* to tolerate microbiome-derived antimicrobials remain poorly understood. This project aims to elucidate the molecular basis of fungal self-protection and niche adaptation by combining microbial confrontation assays, genome-wide mutagenesis, and transcriptomic analyses. Confrontation assays with barley root-associated bacteria reveal inhibitory and non-inhibitory interactions that structure the ecological context of fungal defence. To identify the underlying genes, a transposon-based mutagenesis system is employed in which a dual-resistance cassette flanked by inverted terminal repeats serves as the substrate for transposition. Upon transient delivery of purified PiggyBac or Sleeping Beauty transposases into fungal protoplasts, single transposition events randomly relocate the cassette, generating unique insertion mutants. These transformants will be pooled into a genome-wide mutant library and subjected to negative-selection screening to identify strains sensitive to antimicrobial challenge. Insertion-pool sequencing (iPool-Seq) will map insertion sites and quantify mutant abundance, while complementary transcriptomic analyses will reveal defence-related gene expression during microbial confrontation and plant infection. This integrative approach will reveal key genes and pathways that enable *M. oryzae* to withstand antimicrobial stress and adapt within competitive plant-associated microbiomes.



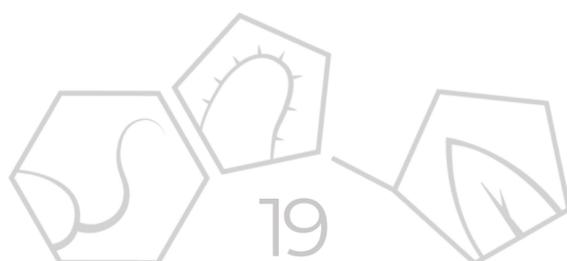
Mapping Microbial contribution to Aging: A Longitudinal Atlas of Host Physiology

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The gut microbiota plays a fundamental role in maintaining host homeostasis, and its dysregulation has been linked to multiple age-related disorders. However, the precise role the microbiota plays throughout life of its host remains unclear. In this study, we are currently conducting omics analysis of tissues from conventionally raised (Conv-R) and Germ-Free (GF) at three key life stages: young (20 weeks), adult (50 weeks), and old (100 weeks). The tissues examined include brain regions (cerebellum, hippocampus, prefrontal cortex, and striatum), spinal cord, liver, kidney, lung, muscle (heart, diaphragm, gastrocnemius, tibialis anterior), bones, and from the gastrointestinal (GI) tract segments (duodenum, jejunum, ileum, proximal colon and distal colon).

Here, we focus on the molecular and pathophysiological changes that GI tissues undergo with age in the presence or absence of microbes. Proteomic analysis of GI tissues showed a clear functional separation between groups according to both age and microbial status (GF vs SPF), revealing significant differences in the expression of proteins involved in epithelial integrity, immune function, and metabolism. Further, histological analyses of the ileum and colon revealed progressive structural alterations associated with aging in both groups. Notably, the absence of microbiota intensified or altered specific histological features, highlighting a critical interaction between aging and microbial colonization.

Our findings underscore the importance of the gut microbiota as a key modulator of intestinal aging and demonstrate how its absence impacts host physiology at multiple levels. This study will provide new key insights into the interplay between microbiota and aging, offering potential avenues for microbiota-targeted therapeutic strategies.



A delicate balance - fine tuning plant Topless corepressors while suppressing immune responses

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The conserved Topless corepressors are key regulators of auxin and other hormonal responses in plants. Within this context, it is unsurprising that numerous secreted effector proteins produced by a taxonomically-broad range of microbial pathogens were recently identified which target Topless as a means to facilitate infection. From the maize pathogen *Ustilago maydis* alone, at least 10 effectors have been shown to target Topless. These effectors elicit distinct, as well as seemingly redundant plant hormonal responses not only to auxin, but also jasmonic acid/ethylene and salicylic Acid. Phenotypically, Topless-interacting-protein (TIP) effectors induce growth abnormalities such as callus and gall formation, but some also trigger programmed cell death (PCD), a potent immune response to contain biotrophic pathogens such as *U. maydis*. These, data indicate a balance and possible interplay between individual TIP effector responses is achieved during successful infection by the pathogen. Our current research has identified *U. maydis* effectors which can delay or suppress PCD specifically triggered by (other) TIPs. This provides a potential mechanism explaining the apparent need for these redundant proteins. The ongoing functional research related to the interplay between Topless and the respective TIP effectors in the finetuning of hormonal and immune responses will be presented.

