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Investigation of trait-based community assembly mechanisms across trophic levels in Central European wood-pastures

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This study investigated the ecological and conservation significance of Central European wood-pastures. The aim was to identify the key community assembly mechanisms operating across different trophic levels in these complex landscapes.

Wood-pastures are ancient silvopastoral systems, which, in a broader sense, are viewed as complex landscapes, as they accommodate four different habitat types on a relatively small spatial scale: grasslands, solitary trees scattered in the grassland matrix, closed forests, and forest edges. In our previous studies we have shown that each of these habitat types provide unique micro-environmental conditions and host unique communities, thus wood-pastures provide a model system to study the factors influencing trait-environment relationships and different aspects of community organization. In the present study, we focused on two ecologically prominent groups occupying different trophic levels: plants and ants. Our results revealed significant differences in taxonomic and functional composition for both groups among the different habitat types of wood-pastures. However, the underlying mechanisms driving these patterns differed between the two trophic levels. Based on RLQ and fourth-corner analyses, heterogeneity in environmental conditions mainly influenced plant compositional patterns (taxonomic and functional composition). On the other hand, for ants, we did not detect any significant direct trait-environment associations, and local environmental conditions alone failed to explain compositional patterns and trait distributions. Instead, ant community attributes (species richness and functional diversity) were indirectly affected by microclimate through its strong effect on vegetation, as shown by path analyses. This highlights that environmental heterogeneity of complex landscapes may shape functional composition and diversity metrics differently across trophic levels (i.e. predominantly environmental filtering for plants and interspecific competition for ants). As a result, the peaks of taxonomic and functional diversity of plants and ants did not align in space across different habitat types, as certain vegetation diversity metrics influenced the diversity metrics of ants, and possibly other organisms along the food-web. This spatial mismatch reinforces the "ecosystem complex" approach of heterogeneous landscapes, emphasizing that future conservation efforts should focus on the landscape as a whole, rather than individual habitat types, to maximize biodiversity conservation.

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Investigation of electrophysiological and morphological properties of cortical Interneurons and Pyramidal cells from juxtacellular recording and labelling in vivo experiments

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Cortical neuronal activity was recorded in drug-free, freely moving rats during natural sleep, and experiments were also performed in anaesthetized animals to increase the number of morphologically characterized neurons using juxtacellular recording and labeling. With this technique, the electrophysiological and morphological properties of different excitatory and inhibitory neurons can be evaluated. In addition, by applying immunohistochemical methods, additional molecular markers can be identified in the labeled neurons. In total, 6 successful in vivo freely moving and 8 in vivo anaesthetized experiments were completed. The activity of 30 pyramidal cells (3 cells labeled) and 10 interneurons was recorded. On average, recordings were obtained for at least 20 min before attempting labeling.

The main objective of this thesis is to find a correlation between the morphology of different types of supragranular pyra-

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midal cells and their firing patterns during sleep. To do this, a morphological analysis was performed on pyramidal cells that the group had previously recorded and reconstructed. On newly labelled pyramidal cells, PV immunohistochemical staining was used to investigate PV+ bouton distribution on cells closer and further from the cortical surface. During the animal adaptation periods, data recorded from previous experiments were analyzed. Oscillations in local field potentials were detected semi-automatically. Firing patterns of supragranular (layers 2 and 3) pyramidal cells were analyzed in different oscillatory events of nonREM and REM sleep. Action potential shape and firing frequency were examined at different sleep stages. Firing patterns were mapped during spindle oscillations in nonREM sleep, across spindle cycles, in the immediate vicinity of delta waves, and during theta oscillations dominating REM sleep. Observations were made to determine how certain morphological features displayed by pyramidal cells affect pyramidal cell function. Results indicate that during natural sleep, pyramidal cells in supragranular layers of the dorsal cortex show a surface-to-deep firing frequency increase. Application of dimensionality reduction methods for the identification of subclasses of pyramidal cells with different anatomical and electrophysiological characteristics is underway.

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The distribution of ion channels in parvalbumin interneurons in the human and mouse neocortex is characterised by species-specific features

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This work investigated the electrical activity of fast-spiking interneurons located in layer 2/3 of the human neocortex. Somatic whole-cell and outside-out patch-clamp recordings were used to analyze neuronal activity and its impact on input–output functions, including EPSP-to-spike conversion, somatic input resistance, input excitability, and action potential firing threshold. Fast-spiking interneurons were identified based on narrow action potentials and minimal firing frequency accommodation. Recorded cells were filled with biocytin for post hoc visualization using a streptavidin fluorophore. All cells were PV-positive by immunoreactivity or expressed PV following nucleus harvesting and transcriptomic analysis using the patch-sequencing method. Brain tissue samples were obtained from subjects undergoing surgical procedures and sectioned for slice recordings; most recordings were from frontal and temporal cortex, with additional neocortical areas also represented. For comparison, fast-spiking PV-expressing neurons were analyzed in mouse frontal and parietal cortex using PV-Cre;tdTomato labeling.

Fast-spiking parvalbumin (PV) interneurons have been identified as a critical component of inhibitory circuits in neocortical regions, thereby playing a crucial role in regulating the excitability and timing of pyramidal cells (PCs). To investigate the ion channels that are prominent in these interneurons, such as HCN channels, Nav1 channels, Kv1 channels, and Kir channels, helps to better understand synaptic integration and plasticity processes. Considering the expression of a diverse array of ion channels in fast-spiking PV interneurons helps to interpret cortical circuit dynamics. The interaction between ion currents – facilitated by HCN, Nav1, Kv1, and Kir channels – contributes to the excitability, firing patterns, and synaptic connections of these interneurons. The focus on specific ion channels in fast-spiking parvalbumin interneurons has enhanced our understanding of their functional roles in neocortical circuits. A comparison of the molecular and electrophysiological profiles of interneurons across human and rodent species reveals notable disparities, which impact the functionality of these cells, particularly with regards to the generation of action potentials and the integration of synaptic signals.

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Investigation of the effect of triglyceride and fenofibrate treatment in volume overloaded rat heart model

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Cardiovascular diseases lead the death statistics, both domestically and internationally. During heart failure, hypertrophic changes of the heart can also be observed. The condition can also develop because of chronic volume overload. In connection with heart failure, the energetic homeostasis of the cells is also damaged due to the altered substrate preference of cardiomyocytes. AMP-activated protein kinase (AMPK), which is regarded as the central regulator of cell metabolism, stimulates the processes associated with ATP generation by phosphorylating the proteins of the catabolic pathways depending on the intracellular energy level. The metabolic state of the cells can be further modified if the condition is aggravated by hypertriglyceridemia. To understand these processes, we examined the left ventricular tissues of the hearts of untreated healthy and surgically created rats with chronic volume overload. An additional group of chronically volume-stressed animals received triglyceride feeding and, at the same time, fenofibrate, which is used in human therapy. In addition to the operated animals, we also created sham-operated controls for each group of animals. We examined AMPK at the mRNA level using RT-qPCR, and the expression of several key enzymes involved in carbohydrate and lipid metabolism under the control of AMPK in the left ventricular tissues of the animals, as well as tissue lipid level were determined. Our results indicate that although the presence of heart failure did not significantly affect the expression of the isoforms and target genes of AMPK subunits expressed in the heart, triglyceride feeding and fenofibrate used in addition to triglyceride markedly increased the expression of the catalytic subunit of AMPK. Shifting the metabolism in the glycolytic direction can have a protective effect, and the use of fenofibrate can be beneficial in the therapy of heart failure aggravated by hypertriglyceridemia.

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Investigation of insulin-immunoreactive myenteric neurons and glucagon-like peptide-1-immunoreactive enteroendocrine cells in a type 1 diabetic rat model

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Previous work reported gut region-specific damage of nitrergic myenteric neurons and regional response to immediate insulin treatment in a type 1 diabetic rat model. Insulin plays a crucial role in cell survival, but insulin presence/expression in the enteric nervous system has not been studied before. Therefore, our primary aim was to investigate the presence of insulin in myenteric neurons, especially in the nitrergic subpopulation. Because the glucagon-like peptide-1 (GLP1) as an incretin is connected to enhancement of glucose stimulated insulin secretion, we also aimed to study the gut region-specific expression of GLP1 and its receptor.

Adult male Wistar rats were randomly divided into control, streptozotocin-induced diabetic and insulin-treated diabetic groups. After 1 or 10 weeks, duodenal, ileal and colonic segments were removed and processed for fluorescent immunohistochemistry, post-embedding electron microscopy, ELISA and PCR.

We first identified the presence of insulin in myenteric neurons by fluorescent immunohistochemistry. The proportion of insulin-immunoreactive (IR) myenteric neurons was approximately 2–4% in the duodenum and 8–9% in the ileum and colon of control rats. In the duodenum, this proportion did not change either in acute or in chronic hyperglycaemia. At the same time, this proportion significantly increased in the ileum and colon of acute diabetic rats and markedly decreased in the colon of chronic diabetic rats. However, the proportion of the insulin-nNOS-IR myenteric neurons remained unchanged in all segments of chronic diabetic rats.

By electron microscopy, increased density of insulin-labelling gold particles was revealed in the duodenum in chronic diabetes. ELISA confirmed the insulin content of intestinal tissue, while insulin mRNA was not detected with PCR.

GLP1-IR enteroendocrine cells were most abundant in the ileal mucosa. In acute diabetic rats, the density of GLP1-IR cells

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did not change in any gut segments, while it increased only in the colon of chronic diabetics. In myenteric ganglia of controls the density of GLP1 receptor-labelling gold particles was the highest in the duodenum. In diabetes the density of gold particles was significantly decreased in the duodenum but did not change in other segments.

This study identified the presence of insulin in the myenteric neurons. The expression of enteroendocrine GLP1 and enteric neuronal GLP1 receptor also showed region-dependent alterations in diabetic state.

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Expressional changes of TNFR1, NFκB and Nrf2 in the myenteric plexus of type 1 diabetic rats

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We previously reported that myenteric neurons located in different intestinal segments respond differently to diabetic states. Tumour necrosis factor (TNF) alpha and one of its receptors (TNFR2) have shown intestinal segment-specific changes in type 1 diabetic rats, suggesting that TNF signalling may be involved in diabetic myenteric neuropathy. TNF receptor activation can induce the nuclear factor-kappa B (NFκB) pathway, in which NFκB and its negative regulator molecule, nuclear factor erythroid 2-related factor 2 (Nrf2) regulate the cellular responses.

Therefore, we investigated the expression of TNF receptor 1 (TNFR1), NFκB p65 subunit and Nrf2 proteins along the duodenum–ileum–colon axis in myenteric ganglia and their muscular environment of control, streptozotocin-induced diabetic and insulin-treated diabetic rats. Both the effects of acute (1 week) and chronic (10 weeks) hyperglycaemia were investigated by quantitative PCR, ELISA, fluorescent immunohistochemistry and immunogold electron microscopy.

The density of TNFR1-labelling gold particles was the highest in the ileum of controls. In the diabetic rats, the density of TNFR1-labelling gold particles did not change in the investigated gut segments compared to controls.

The level of NFκB p65 mRNA increased in all gut segments of diabetic animals compared to controls. In contrast, the amount of NFκB p65 protein increased only in the small intestine and did not change in the colon. In the acute and chronic experiments, the proportion of NFκB p65 immunoreactive myenteric neurons was higher in the distal part of the small intestine in control animals. In the acute hyperglycaemic group, this proportion increased in both the duodenum and ileum compared to controls. However, in the chronic diabetic rats, this proportion was significantly decreased in the duodenum while increased in the ileum. The density of NFκB p65-labelling gold particles remained unchanged in duodenal ganglia of diabetic rats compared to controls. In contrast, the density of particles increased in both the nuclei and cytoplasm of smooth muscle cells in the diabetic duodenum. In the ileum of diabetics, the number of NFκB p65 gold labels increased in myenteric ganglia and surrounding smooth muscle both in the nuclei and cytoplasm.

The Nrf2 mRNA level significantly increased in all intestinal segments in the diabetic rats, whereas the protein level decreased. In the diabetic group, the density of Nrf2 labelling gold particles increased in the myenteric ganglia and their adjacent smooth muscle cells of duodenum. Moreover, the Nrf2 density significantly increased not only in the cytoplasm but also in the nuclei of myenteric neurons.

Our results reveal a segment-specific TNFR1, NFκB p65 and Nrf2 expression in myenteric neurons and muscular environments. Based on these findings, we propose that Nrf2 nuclear translocation may inhibit NFκB signalling in the duodenum, which may contribute to the survival of myenteric neurons in the duodenal ganglia.

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Discovery and functional characterization of a new phase-separated ribosome–nascent chain complex

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At the focus of our research group's interest are assemblysomes, recently described cytoplasmic, phase-separated, membraneless organelles. In 2019, Panasenko and colleagues described Not1-containing granules with halfway translated proteasomal subunits Rpt1 and Rpt2 inside. It turned out that these granules serve as the site for co-translational assembly, and the name Not1-containing assemblysomes (NCAs) is derived from this function. Assemblysomes are ribonucleoprotein complexes (RNCs) that contain translationally stalled ribosomes with protruding nascent protein chains. They contain both small and large ribosomal subunits and are enriched in translationally stalled ribosomes with nascent chains.

We conducted a bioinformatics analysis of all protein complex subunits found in yeast, using the previously described essential properties for assemblysome formation as filters to identify additional potential proteins stored in assemblysomes. The results were enriched in DNA repair proteins, and we selected three among these for further investigation: Sgs1, Rad10 and Rad14, which play a role in protecting against stress-induced DNA damage. We examined the quantity of SGS1, RAD10, RAD14, RPT1 and RPT2 mRNAs under different stress conditions. Our findings indicate that proteotoxic stress led to an increase in the amount of mRNAs in the assemblysomes, conversely a decrease was identified under genotoxic stress.

We examined yeast cells transformed with the previously used Rpt1-RNC plasmid and the Sgs1-RNC, which we generated, with dSTORM microscopy to gain more information about the structural organization of assemblysomes. Following this, we extended our analysis to higher eukaryotes as well. For this purpose, we performed ultracentrifugation experiments on MCF7 human breast adenocarcinoma cells and then analyzed the RNA content of each fraction with sequencing. We were able to confirm that assemblysomes originate through phase separation by using 1,6-hexanediol (HEX). HEX is an aliphatic alcohol that inhibits the formation of phase separation by disrupting the weak interactions between protein and RNA molecules. HEX treatment significantly reduced the amount of mRNAs stored in assemblysomes. This was further confirmed by transmission electron microscopy (TEM) experiments, namely the number of identified ribosome clusters—presumably representing RNCs—significantly decreased after HEX treatment.

Our results suggest that phase separation plays a role in the formation of assemblysomes, which primarily store proteins with disordered N-termini involved in DNA repair and stress responses. To validate the role of assemblysomes in DNA repair processes, we examined cell viability after exposure to a 1 Gy irradiation dose, with or without HEX, in A549 cells. These results support our hypothesis, as cell viability significantly decreases following repeated irradiation in the presence of HEX.

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Interkingdom communication between *Staphylococcus aureus* and *Candida* species at the level of extracellular vesicles

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The human oral cavity is colonized by more than 700 microbes, such as bacteria, viruses, and fungi, known as the oral microbiota. As a result of environmental effects, such as smoking or infections, microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral candidiasis is mostly caused by *Candida albicans*, which can alter the bacterial diversity. To examine the nature of such fungal-bacterial interactions, we aim to investigate the interaction between *Candida* species and oral pathogenic bacteria at the level of extracellular vesicles (EV). For our experiments we used the *C. albicans* SC5314 and *C. parapsilosis* CLIB214 strains, along with *Staphylococcus aureus* as pathogenic bacterial counterpart. We optimized the fungal and bacterial EV isolation protocol from solid media. The characterization of the EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 50 and 250 nm. We examined the effects of EVs released by *C. parapsilosis* and the yeast and hyphae form of *C. albicans* on the growth and biofilm formation efficiency of *S. aureus* and *vica versa*. As a result, we found that EVs from *C. albicans* and *C. parapsilosis* had different effects on

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the growth and biofilm formation efficiency of *S. aureus*. Regarding the effect of bacteria, the *S. aureus* EV treatment can induce the hyphae formation of *C. albicans* cells. Altogether these results suggest the presence of an active interaction between fungal and bacterial cells at the level of EVs.

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Development and adaptation of synthetic biology tools for rhizobia

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Tool development was initiated for investigating gene-for-gene interactions between various *Medicago truncatula* ecotypes and *Sinorhizobium meliloti* strains. The work focuses on developing synthetic biology tools that allow both smaller and larger deletions, as well as tools that enable the creation of genomic libraries. Four projects were pursued:

"Pop-out deletion and library creation using the Lox and FRT systems". The aim is to create a genetic toolkit comprised of two types of vectors: destination vectors that contain either FRT or Lox sites and selection markers, and expression vectors that contain the genes for Cre and FLP recombinases necessary for the recombination. Using this system, it is planned to either delete genes or create genomic libraries of various *Sinorhizobium meliloti* strains.

"Retron-based mutagenesis". Retrons are prokaryotic retroelements that undergo targeted reverse transcription, producing single-stranded DNA. This DNA can then be used as a template to precisely induce or revert point mutations in genes of interest. The first demonstration task is to restore the mutant "exoB" gene of the *Sinorhizobium meliloti* strain "AK631" to its wild-type sequence. This will result in a clear phenotypic change, as the bacteria will produce exopolysaccharides again and become mucoid. With this system, the aim is to study the effect of point mutations on the phenotype of certain *Sinorhizobium meliloti* strains. Cloning for this project is now finished, and testing has begun.

"Transposon-mediated library creation" aims to create a simpler and cheaper (albeit randomly integrating) library creation tool compared to the other project ("Pop-out deletion and library creation using the Lox and FRT systems"). The operating principle is as follows: with the help of transposons, oriT, antibiotic resistance, and both rhizobial and *E. coli* origins of replication are introduced into the rhizobial genome. Then, the oriT-flanked genomic segments are mobilized into *E. coli* and later into other rhizobia. Utilizing this system, the aim is to build genomic libraries of rhizobial strains of interest. Cloning for this project is finished and it is now in the testing phase.

"Creating large-scale deletions in rhizobia using a compact Cascade-Cas3 system". The aim is to create a system capable of creating large-scale deletions in rhizobia, as well as other Alphaproteobacteria. It is an adaptation of a Cas3-based system already working in Gammaproteobacteria. Our goal is to use this system to delete larger genomic segments and operons in strains of interest, where the desired phenotype can only be achieved by creating larger deletions. The project is currently past its first round of testing; additional experiments are ongoing for fine-tuning.

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Summary of current scientific work: promising biocontrol effects of a native hemiparasitic plant against a non-native C4 grass

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Hemiparasitic plants are known to be able to diversify degraded grasslands by lowering the competitive power of dominant grasses. Recent research indicates that hemiparasites may also be used against invasive alien plants. We established raised flower

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beds to test the effects of *Odontites luteus*, a native European hemiparasite, on *Sporobolus cryptandrus*, a recently established and rapidly spreading C4 grass of North American origin. We found that *Odontites* considers *Sporobolus* a suitable host and reduces its biomass production (and potentially its competitive ability) by approximately 50%, equaling the effect on its major native host, *Festuca vaginata*. However, *Festuca* showed severe metabolic impairment (reduced photosynthetic capacity and increased physiological stress) under hemiparasite pressure. Thus, the application of hemiparasites is a promising biocontrol tool against *Sporobolus* (and potentially other invasive C4 grasses), but it is not a silver bullet. Full eradication of the invasive species and complete recovery of the native community are not expected; however, thinning monodominant *Sporobolus* stands to allow certain populations of native species, particularly those resistant to *Odontites*, to recover is a more realistic goal. This could lead to a partial recovery of the former species composition and an improvement of ecosystem functions, such as providing food for pollinators.

We also started a project aiming for a better understanding of the effects of solar park management (grazing, mowing, mulching, herbicide application) on species, trait-based plant and animal community characteristics, and associated ecosystem functions and services (decomposition, predation, herbivory, primary production, soil carbon sequestration, conservation). All parameters are related to adjacent grasslands representing targets in biodiversity and ecosystem service provisioning. We selected eight solar parks in Austria, Czechia, Hungary, and Slovenia. We apply a cross-sampling design with three independent design variables: management, solar park size and landscape heterogeneity. Vegetation biodiversity data were sampled, including vegetation surveys and subsequent data processing.

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Novel antifungal mechanism of *Neosartorya* (*Aspergillus*) *fischeri* antifungal protein 2 in Candida albicans

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Candida albicans is the most common opportunistic human fungal pathogen and a major contributor to drug-resistant invasive fungal infections, which have significantly increased over the past two decades. A promising therapeutic approach involves an antifungal protein, *Neosartorya* (*Aspergillus*) *fischeri* antifungal protein 2 (NFAP2). Notably, NFAP2 exhibits potent antifungal activity against both planktonic and biofilm-forming *Candida* cells *in vitro* and *in vivo*. Importantly, NFAP2 demonstrates fungal-specific activity while exhibiting no toxicity toward human cell lines, making it an attractive therapeutic candidate. However, its clinical application remains limited due to two key challenges: The precise antifungal mechanism of NFAP2 remains unresolved, and NFAP2 binds with high affinity to human serum albumin. Nevertheless, the molecular targets of NFAP2 may serve as valuable templates for the discovery of novel antifungal compounds. Recent observations indicate that NFAP2 is internalized by *C. albicans* cells, leading to a long-term reduction in growth rate, in addition to its previously characterized rapid plasma membrane-disrupting effect.

This study aims to characterize the molecular mechanism underlying the long-term, growth-slowing antifungal effect of NFAP2 in *C. albicans* at sub-inhibitory concentrations and to identify repurposed antifungal drug candidates from drug databases based on the intracellular protein targets of NFAP2. To achieve these objectives, a multidisciplinary experimental approach was employed: Comparative transcriptome analysis to elucidate physiological changes in *C. albicans* exposed to sub-inhibitory concentrations of NFAP2. *In vivo* protein-protein interaction analysis to identify the direct intracellular protein targets of NFAP2 that mediate transcriptomic alterations and growth slowing. *In silico* protein-protein docking to determine NFAP2 binding pockets on its identified targets. Structure-based virtual screening to discover repurposed antifungal drug candidates from existing drug databases.

Transcriptomic profiling of *C. albicans* treated with sub-lethal concentrations of NFAP2 revealed downregulation of genes involved in central metabolic pathways and upregulation of genes associated with the cell cycle and filamentous growth, suggesting a complex cellular response. *In vivo* protein-protein interaction analysis identified three intracellular protein targets of NFAP2 - glutamate decarboxylase (Gad1p), ATP synthase subunit alpha (Atp1p), and enolase 1 (Eno1p). These interactions likely contribute to the observed decline in metabolic activity and the long-term growth-retarding effect. Based on protein-protein docking and virtual screening experiments, dihydroergocristine and meclizine were identified as potential repurposed

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drugs for *Candida* infections. Both compounds demonstrated binding affinity to the target proteins of NFAP2 and inhibited *C. albicans* growth *in vitro*. Additionally, they exhibited synergistic interactions with amphotericin B, flucytosine, and micafungin.

Summarizing, NFAP2 exhibits a unique dual mode of action in *C. albicans*: below the minimum inhibitory concentration (<MIC), NFAP2 interacts with Gad1p, Atp1p, and Eno1p, leading to reduced metabolic activity and slow fungal growth. At the MIC, NFAP2 induces rapid plasma membrane disruption. Furthermore, repurposed drugs identified through NFAP2-interacting partners may serve as effective adjuvants to existing antifungal therapies, potentially enhancing their efficacy while minimizing side effects.

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Harnessing genomics to combat pathogenic bacteria

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In the context of critically antibiotic-resistant nosocomial pathogens, there is growing interest in bacteriophage therapy. Most bacteriophages have a narrow host range and are only effective against specific pathogen strain types, calling for precision approaches that are challenging to scale. This work examined the genomic epidemiology of pathogens to assess the feasibility and scalability of phage therapy and guide the identification of effective bacteriophages. A genomic surveillance framework was developed and applied to *Acinetobacter baumannii* and subsequently streamlined and extended to *Klebsiella pneumoniae*.

In *A. baumannii*, genomic analyses indicated that, among carbapenem-resistant isolates, a small number of dominant strain types accounted for most regional infections and remained relatively stable over time, supporting the feasibility of scalable phage therapy.

For the *K. pneumoniae* project, >500 genomes were collected and sequenced and molecular typing was performed on >55,000 public *K. pneumoniae* genomes. After a data-driven prioritisation procedure, 15–20 clinically relevant strain types were selected that accounted for most human-associated antibiotic-resistant infections in Europe (2018–2023). Phylogenetic trees were built for these strain types and used to guide partner strain requests to expand pathogen diversity. Using this approach, genetically diverse pathogen test sets were developed for each selected strain type. In parallel, 40–50 phages were isolated against *K. pneumoniae* strains belonging to the selected strain types, tested for phage sensitivity profiles, and the best phages were progressed for phage training. While promising phage candidates were identified against several important *K. pneumoniae* strain types, no phages were identified that infected all isolates within a given strain type. To address this gap, additional determinants of phage sensitivity are being explored through large language model–assisted analyses and approaches similar to genome-wide association studies.

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Investigation of trained immunity mechanisms against *Candida* species in human barrier cells

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Barrier tissues such as the skin, gastrointestinal tract or oral epithelium form the body's first line of defense against invading pathogens. Traditionally, the innate immune functions of barrier cells, including keratinocytes or epithelial cells, have been viewed as transient, nonspecific, and lacking memory. However, growing evidence challenges this notion, revealing that barrier cells are capable of a form of immunological "memory" known as trained immunity. Trained immunity manifests through epigenetic and metabolic rewiring of epithelial and other tissue-resident innate cells. For example, epithelial cells in the skin

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and gut can undergo chromatin remodeling upon initial microbial stimulation. This epigenetic imprinting enables faster and stronger production of cytokines, antimicrobial peptides, and chemokines during re-infections.

Candida species are a group of opportunistic fungal pathogens that are part of the normal microbiota in humans, commonly found on the skin, mucous membranes, and in the gastrointestinal tract. While typically harmless in healthy individuals, they can cause infections when the host's immune defenses are compromised or when microbial balance is disrupted. The most well-known species is *Candida albicans*, responsible for most mucosal and systemic candidiasis cases. However, non-*albicans* species, such as *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida auris*, are increasingly recognized as clinically relevant due to their rising prevalence and varying resistance profiles to antifungal drugs.

During our work we made a model system where we used healthy human skin keratinocytes (HaCaT) and healthy (OKF6/TERT-2) and cancerous (HSC-2) oral epithelial cells as the host. We pre-treated these cells with *C. parapsilosis* GA1 and CLIB214 for 24h. After that we let the cells rest for 5 days, so the gene expression levels went down to a basic level. On the 8th day we infected the cells with *C. albicans* SC5314 and WO-1. We measured cytotoxicity, cytokine production, adhesion capacities of the *C. albicans* strains and gene expression changes.

We found that the human keratinocytes were less responsive to the pre-treatments than the oral epithelial cells. Cytotoxicity, cytokine levels and the adhesion capacities of *C. albicans* strains did not change in case of the keratinocytes. In case of healthy oral epithelial cells, cytotoxicity decreased, but the cytokine levels increased due to the pre-treatments. Adhesion capacities increased. In case of cancerous oral epithelial cells, we found the opposite, cytotoxicity increased but the cytokine levels decreased. Adhesion capacities also increased. The expression levels of the investigated genes were upregulated following the pretreatments. Overall, pretreatment exerts a modest protective effect on the studied cells.

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Role of sterol C-24 reductase in ergosterol biosynthesis in *Mucor lusitanicus*

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Erg4 is in the endoplasmic reticulum and encodes the sterol C-24 reductase gene (ERG4), which catalyzes the final step in ergosterol biosynthesis. A deletion mutant in *Saccharomyces cerevisiae* has been shown to be unable to synthesize ergosterol in the absence of the gene, and the deletion significantly increases sensitivity to divalent cations and several antifungal agents. We have started to create *erg4a* single knockout mutants in *M. lusitanicus* using a CRISPR-Cas9 system.

When examining the sporulation capacity, no significant difference between the measured values was observed. The sporulation capacity of MS12, MS12+*pyrG* and MS12-*Δerg4a* strains did not differ significantly. In the germination assay, a significant decrease was observed only at the fourth hour compared to the control. The MS12-*Δerg4a* mutant showed no significant difference compared to the MS12+*pyrG* strain used as control except at the fourth hour.

The growth ability of the generated mutants was tested in the presence of six stressors. We found that MS12-*Δerg4a* can grow slower than MS12 on medium containing 0.4 M NaCl. For 1 M NaCl, we found a significant difference on days 3 and 4, where our mutant showed significantly lower growth compared to the MS12 control. On 5% glucose medium, MS12- *Δerg4a* showed a significant increase compared to MS12. This difference is even more significant on the second, third and fourth days between the two strains. The presence of SDS significantly affected the growth of mutant strains. On the second, third and fourth days, we found significant differences between MS12-*Δerg4a* and MS12 strains, with the mutant strain growing faster. Examining the effect of Calcofluor white, we found that the MS12-*Δerg4a* strain grew detectably slower compared to MS12 strains. This difference was detectable on the last day. In the presence of Triton X- 100, the MS12-*Δerg4a* strain grew detectably faster on both days 3 and 4. On medium containing Congo Red stress medium, MS12-*Δerg4a* showed significantly faster growth rate on day 2 compared to the MS12+*pyrG* control.

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The origin and composition of the "Forgotten people": genetic analysis of the Sarmatian-period population of the Carpathian Basin

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The Sarmatians were nomadic groups that expanded from the southern Ural and adjacent regions into the Pontic Steppe, and by the early centuries CE established a major presence in the Carpathian Basin.

Archaeological research on the Sarmatian Period in Hungary dates to the early 20th century. The vast number of findings, including burials and settlement traces, highlight not only the cultural significance of the Sarmatians but also their potential impact on the population history of the Carpathian Basin. The extensive body of archaeological literature has enabled high-resolution sampling across distinct periods associated with Sarmatian occupation. To clarify the origins and genetic relationships of the Carpathian Basin Sarmatians and to explore their connections to other populations from the Eurasian Steppe, as well as to local groups from preceding and succeeding periods, genome-wide data were generated by sequencing 156 genomes from the Carpathian Basin and surrounding regions, spanning the Sarmatian and subsequent Hun periods.

Traditional genome-wide variance analyses were used to address broad questions, and genetic relatedness was further explored using refined identity-by-descent (IBD) fragment detection. Distant genealogical relationships were identified with high confidence, including long-distance kinship ties. Results indicate that the Carpathian Basin Sarmatians are descendants of Sarmatians from the Ural and Kazakhstan regions, who migrated via the Carpathian foothills (present-day Romania). Furthermore, descendants of the substantial Carpathian Basin Sarmatian population formed a significant portion of the population during the subsequent Hun era.

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Phage hunting guided by genomics to combat high-risk *Klebsiella pneumoniae* clones in Europe

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The global rise of multidrug-resistant (MDR) *Klebsiella pneumoniae*, particularly high-risk clones such as ST258, ST147, and ST307, poses a significant threat to public health due to their resistance to last-resort antibiotics and their association with severe hospital-acquired infections. This project aims to develop phage-based therapeutic strategies against these high-risk clones through the isolation, characterization, and optimization of lytic bacteriophages. We work with an extensive panel of clinical *K. pneumoniae* isolates collected from hospitalized patients across 12 European and neighboring countries: Serbia, Hungary, Romania, Greece, Germany, Italy, France, Montenegro, Turkey, Ukraine, Kosovo, and the United Kingdom. These isolates encompass a wide range of sequence types, resistance profiles, and capsular types representative of the epidemiological diversity circulating in the region.

To identify effective bacteriophages, we conduct host range profiling and characterize phage virulence-related traits. Importantly, we carry out experimental evolution to improve the infectivity of initially less efficient phages, enhancing key traits such as adsorption rate, burst size, and host range breadth to better suit therapeutic applications. In parallel, we investigate the genetic determinants that influence phage susceptibility in *K. pneumoniae*, focusing on surface structures such as capsules and outer membrane proteins. Resistance mutations emerging during phage exposure are characterized for their impact on bacterial fitness, including potential trade-offs like reduced virulence or increased antibiotic sensitivity.

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By integrating phage biology, bacterial genomics, and evolutionary experimentation, this project aims to rationally design potent, broad-coverage phage cocktails targeting clinically relevant MDR *K. pneumoniae* strains. Our work supports the advancement of precision phage therapy tailored to combat infections caused by high-risk clones across European healthcare systems.

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The effect of prolonged DNA quadruplex stabilization on the functions of human cells

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Guanine-rich sequences in DNA can fold into G-quadruplex (G4) structures, which are implicated in gene expression regulation, chromatin architecture, replication initiation, telomere organization, etc. and represent promising anticancer drug targets. However, the physiological impact of long-term G4 stabilization on normal human cells remains largely unexplored. In this study, we modelled a potential therapeutic scenario by treating non-cancerous human cell line (hTERT-immortalized RPE-1 cells, TP53-/-) with a potent G4-stabilizing ligand PhenDC3 in a cyclic manner to mimic a potential cancer treatment in a patient, and examined three key aspects: (i) nuclear genome integrity, (ii) global transcriptomic changes, and (iii) mitochondrial gene expression and quality control. Whole-genome sequencing revealed no significant increase in single-nucleotide variants (SNVs), small insertions/deletions or large-scale structural rearrangements in treated vs control cells, indicating no significant effect on genome stability under our experimental conditions. By contrast, RNA-seq analyses revealed pronounced and persistent transcriptional changes: 4,612 genes were differentially expressed after treatment (~2,316 up-; ~2,296 down-), and although ~74% of gene expression returned to baseline within 48 hours of recovery, ~26% of transcripts remained altered ("no-rescue" or "partial-rescue" groups). These persistent changes were enriched for genes involved in transcription, translation, DNA repair and ubiquitination, and intriguingly ~50% of the permanently upregulated genes overlap with those upregulated in various human cancers. In the mitochondrial genome, all 13 protein-coding mitochondrial genes exhibited altered expression following treatment, and none fully recovered after the recovery time. Addressing mitochondrial quality control, we show that PhenDC3, but not all G4-stabilizing ligands, induces mitophagy in both a human mt-Keima reporter cell line and in epithelial cells of Drosophila melanogaster larvae. Together, these findings indicate that prolonged G4 stabilization in otherwise healthy cells does not provoke genome instability per se, but elicits persistent transcriptional reprogramming and triggers mitophagy, effects that may constitute off-target risks in a therapeutic context. We therefore recommend that candidate G4-stabilizing drugs undergo rigorous evaluation for mitophagy induction and long-term transcriptomic changes in appropriate model systems before clinical application.

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Investigation of assemblysomes and protein aggregation in breast cancer

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This research investigated the role of Not1-containing assemblysomes (NCAs) in cancer, especially in relation to resistance to neoadjuvant chemotherapy. In addition, we evaluated whether RPB1 protein aggregation could serve as a predictive marker of response to neoadjuvant chemotherapy in breast cancer. NCAs are liquid–liquid phase-separated granules containing ribosomes with protruding nascent peptide chains stalled in translation and bound to mRNA molecules, with an approximate size of 100 nm. NCAs can be isolated by ultracentrifugation of cell lysates through a 60% sucrose cushion containing EDTA. EDTA disconnects the small and large ribosomal subunits; in its presence polysomes are disrupted and cannot travel through the sucrose cushion, enabling purification of NCAs.

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We investigated the effects of epirubicin, an anthracycline commonly used in neoadjuvant chemotherapy of breast cancer, in MCF7 cells. An appropriate epirubicin dose was selected using MTT assays; at 20 ng/ml, ~20% cell death was observed, and this concentration was used for downstream analyses. MCF7 cells were treated with 20 ng/ml epirubicin for 24 hours, then harvested, lysed, and ultracentrifuged. Total lysate, pellet (containing polysomes and NCAs) and EDTA pellet fractions (enriched in NCAs) were collected from treated and control cells. Total RNA was isolated from these fractions for sequencing. After preparation of cDNA libraries of polyA-tailed RNAs, sequencing was performed on an Illumina MiSeq instrument. Pellet and EDTA pellet fractions were also analysed by protein mass spectrometry (HPLC–MS/MS). The RNA and protein content of these fractions revealed close to 200 genes affected at both RNA and protein levels with respect to their distribution across fractions. These findings will be validated by qPCR and Western blotting.

RPB1 protein aggregation was also investigated in clinical biopsy samples from breast cancer patients. A characteristic aggregation pattern was previously associated with response to neoadjuvant chemotherapy, and the model was tested by categorizing biopsy samples of unknown therapeutic response into regression, partial regression, or non-regression groups. Out of 13 samples examined, 10 were correctly classified. These results support the potential clinical utility of RPB1 aggregation as a predictive marker of therapy response in neoadjuvant chemotherapy for breast cancer.

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Effect of redlight night break on the plant defence mechanisms against fungal pathogen *Botrytis cinerea*

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Red light is known for its defense-enhancing effects, an especially relevant feature in greenhouse cultivation, where fungal pathogens such as *Botrytis cinerea* cause substantial pre- and postharvest damage to tomato (*Solanum lycopersicum*) crops. Since pathogens are most infectious in the dark, red-light treatment in the middle of the night could promote plant resistance.

The aim was to test whether nocturnal red-light treatment enhances tomato immunity and to characterize associated molecular responses. First, the effective length of red-light irradiation starting from midnight was determined. Responses induced by red light were analysed right after the light exposure ended, and 3 hours later at dawn. Measurements included the activity of key antioxidant enzymes (e.g. catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX)), their gene expression levels, and the accumulation of reactive oxygen species (ROS) as signaling molecules (e.g. superoxide and hydrogen peroxide). In parallel, native gel electrophoresis was used to assess the activity of NADPH oxidase, a membrane-bound enzyme responsible for a significant portion of ROS generation. RNA-Seq was performed to further characterize red-light–induced changes and results were validated with qRT-PCR. Several differentially expressed genes were identified, mostly related to circadian rhythm; DEGs also included MYB and DOF transcription factors. Key defense-related hormones such as salicylic acid, jasmonic acid, and abscisic acid were also examined. JA levels increased right after treatment, whereas ABA showed significant changes at dawn. Overall, 30 min of red-light irradiation applied at midnight was sufficient to trigger biochemical, transcriptional, and hormonal changes that may enhance resistance against the necrotrophic pathogen *B. cinerea*.

Based on the short-term results, long-term effects (one week of nocturnal red-light treatment) were also determined: elevated expression was observed for tomato SOD and APX genes, while other enzyme transcripts showed no changes. Regarding enzyme activities, increases were detected for SOD, CAT and APX, while NADPH oxidase activity did not change significantly. After one week of nocturnal red-light pretreatment, detached tomato leaves were infected with *B. cinerea*, and fungal lesion size was significantly reduced compared to controls. These results indicate that red light can enhance tomato defence against *Botrytis*.

To explore the role of ethylene, experiments were repeated using the Never-ripe (Nr) ethylene receptor mutant tomato line. Although SOD and POD activities increased, CAT and APX did not respond, yet lesion size was still reduced. Further work will examine the role of phytochromes using mutant lines.

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Revealing the mechanisms of low-resistance antibiotics against Gram-negative pathogens

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Although drugs that engage multiple bacterial targets are generally believed to slow the onset of resistance, the real-world effectiveness of this strategy has not been rigorously tested, especially against the most critical Gram-negative ESKAPE pathogens: *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Here, we show that while simply having two targets is helpful, it is not enough: antibiotics must also disrupt the bacterial membrane to robustly prevent resistance.

We systematically compared antibiotics with varying mechanisms and target counts, including several in preclinical and clinical trials, using laboratory evolution, whole-genome sequencing, and fitness assays. We measured how quickly and by what routes resistance arose (point mutations, gene amplification, or horizontal transfer), and we evaluated how effectively each compound eradicated bacterial populations.

Our findings revealed that three preclinical antimicrobial agents with dual modes of action—SCH79797, Tridecaptin M152-P3, and POL-7306—exhibited significantly lower resistance emergence across all pathogens examined. Despite varying mechanisms of action, each of these antibiotic candidates disrupted the bacterial outer membrane. While specific evolved lines displayed heightened resistance to these drugs, they carried a substantial fitness cost and the extent of cross-resistance among currently employed antibiotics was limited. These results indicate that membrane disruption is essential alongside a second target to outpace bacterial adaptation. Designing future antibiotics with this dual-mode approach may provide durable therapies against multidrug-resistant Gram-negative infections.

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Characterization of *Lepidium crassifolium* and *Arabidopsis thaliana* stress regulstory genes

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Small Paraquat Resistance (SPQ) is a novel 69-amino acid protein identified in *Lepidium crassifolium* that confers paraquat resistance and plays pleiotropic roles in herbicide resistance, hormone signaling, and drought tolerance. This research aims to characterize the biological and molecular function of SPQ genes in both *Arabidopsis thaliana* (model) and *L. crassifolium* (halophyte).

For functional characterization, the *Arabidopsis* and *Lepidium* SPQ genes were cloned and overexpressed in *Arabidopsis*. Two mutant lines—spq1 (a T-DNA insertion mutant) and spq2 (a CRISPR/Cas9-edited line producing a truncated SPQ protein)—were developed for functional studies. Both spq mutants displayed smaller rosette size, reduced seed size, and altered embryonic development, evidenced by shifts in embryo developmental stages. They demonstrated increased sensitivity to paclobutrazol during germination and delayed flowering, which was molecularly associated with increased expression of MAF5 and FLC, and decreased expression of GA3OX3, a key gene in GA biosynthesis. These results suggest a role for SPQ in gibberellin signaling, involvement in developmental timing and morphology, as well as flowering regulation via hormonal and genetic pathways.

GFP-tagged SPQ lines revealed protein localization predominantly in the endomembrane system, especially the vacuole, corroborated by western blot and confocal microscopy data. GUS-tagged constructs of AtSPQ are currently being developed to investigate promoter activity patterns. Immunoprecipitation of HA-tagged AtSPQ and LcSPQ proteins and MALDI TOF mass spectrometry identified several putative interacting partners, providing mechanistic insights into SPQ's regulatory network and possible involvement in phosphorylation-related signaling. Validation of these interactions is underway.

Furthermore, SPQ-expressing constructs (HA-tagged and untagged) from both *Arabidopsis* and *Lepidium* were introduced into *Nicotiana tabacum* and *Brassica napus* to explore SPQ function in a non-native species. Preliminary observations indicate enhanced drought tolerance, supporting a conserved role for SPQ in abiotic stress resistance. RNA-Seq analysis is in progress

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to profile transcriptomic differences in spq mutants and SPQ overexpressors, with the goal of uncovering downstream targets and pathways regulated by SPQ. Together, our findings establish SPQ as a key integrator of drought tolerance, ABA signaling, flowering time, and gibberellin-mediated growth, offering potential strategies for crop improvement.

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Development of a multi-patch clamp system for phenotyping and monitoring the communication between neuron cells using artificial intelligence

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This project aims to develop a versatile platform that combines automated patch-clamp electrophysiology with image-guided single-cell extraction for studies in complex 3D cell models. Key achievements include expanding the original AutoPatch system from a single-cell setup to an eight-pipette configuration, integrating an ultrasonic pipette cleaning module, and implementing advanced 3D cell segmentation for target identification.

The original AutoPatch system (previously limited to patching one cell at a time) has been extended to support simultaneous patch-clamp recordings with up to eight pipettes. This multi-pipette upgrade increases experimental throughput, enabling parallel recordings from multiple cells and facilitating network-level electrophysiological studies in vitro. The control software and hardware were upgraded to coordinate multiple micromanipulators, ensuring stable seal formation and maintenance across all eight channels concurrently. In addition, a piezo-based ultrasonic pipette cleaning system (Luigs & Neumann's LN-PCS) was integrated into the platform. Together, these enhancements establish a robust high-throughput patch-clamp system capable of performing recordings sequentially or in parallel with minimal human intervention.

Current work focuses on system refinement and validation, including calibration of multi-pipette coordination and optimization of seal success rates. Each component of the platform (from the robotic patch-clamp sequence to the imaging and extraction subsystems) is being rigorously tested to ensure reliable and reproducible performance. These tests will establish performance metrics and guide final adjustments.

The platform is designed for organoid-based applications, enabling parallel patch-clamp recordings in 3D models and subsequent image-guided extraction of individual cells or nuclei for post-recording single-cell sequencing, thereby linking electrophysiological phenotypes to molecular signatures.

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Hairy root transformation using *Rhizobium rhizogenes* as a tool for monitoring redox homeostasis and exploring redox-specific gene functions in *Arabidopsis*

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The production of hairy roots, developed after *Rhizobium rhizogenes*-plant interaction, offers an excellent research tool for analyzing redox homeostasis in response to environmental stressors or for functional analysis of various genes. To develop an efficacious *R. rhizogenes*-mediated transformation technology on *Arabidopsis thaliana* ecotype Col-0, horizontal gene transfer was directly visualized using fluorescent tracking of an mCherry-tagged tonoplast marker in root cells. I analyzed the combined effects of various environmental, nutritional, and procedural factors on transformation efficiency. Approximately 80% of *Arabidopsis* seedlings developed transgenic fluorescent roots using my optimized transformation protocol. This method was applied to introduce the cytosolic Grx1-roGFP2 (redox-sensitive green fluorescent protein) in plants for *in vivo* monitoring of the glutathione redox state. The fluorescent redox probe was expressed either by *Agrobacterium tumefaciens* or by *R.*

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rhizogenes-mediated transformation, and the influences of the two different transformation techniques on the redox potential were compared. Dynamic changes in the redox state were measured in wild-type (WT, ecotype Col-0), glutathione reductase 1 (*gr1*), and dehydroascorbate reductase 2 (*dhar2*) mutant *Arabidopsis* plants using confocal microscopy over a 60-min period following treatment with 150 mM NaCl or 300 mM mannitol. Measurements were performed in both primary and adventitious root zones. The *gr1* and *dhar2* mutants showed more positive redox potential than WT plants, which was further increased by additional stress treatments. *In situ* visualization of superoxide anion (O_2^-) and detection of intracellular hydrogen peroxide (H_2O_2) level confirmed that the two different transformation methods similarly affect the formation of reactive oxygen species in root cells**.** Furthermore, I aimed to provide a Gateway cloning-compatible *R. rhizogenes*-mediated transformation system that facilitates fast and reliable cloning of genes of interest in *Arabidopsis* plants. In my cloning experiments, I selected and cloned some tomato (*Solanum lycopersicum* L.) genes (dehydroascorbate reductase *SlDHAR1*, glutathione reductase *SlGR1*, glutathione synthetase *SlGSH1*) into overexpression binary vectors. These genes encode key enzymes involved in plant redox regulation. Using my optimized *R. rhizogenes*-mediated transformation protocol, I successfully introduced and expressed these tomato genes in *Arabidopsis* roots. Transformation success was confirmed by the appearance of transgenic roots on the selective medium within two weeks of transformation, and expression of genes of interest was also validated via real-time qPCR. Overexpression of tomato genes significantly enhanced the plant vitality and reduced the accumulation of reactive oxygen species (ROS) in the transformed *Arabidopsis* root cells under salt stress. The orthologous *SlDHAR1* and *SlGR1* genes were introduced into *Arabidopsis dhar2* and *gr1* mutants, respectively, for functional analysis. Microscopic measurements using the roGFP2 confirmed the restoration of redox homeostasis in the mutant lines. Elucidating the function of additional genes using *R. rhizogenes*-mediated transformation could provide a more comprehensive understanding of intracellular biological processes and highlight their potential application for molecular plant breeding.

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