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The *UDP Glycosyltransferase* gene *UGT73F3* is involved in root and symbiotic nodule development in *Medicago truncatula*

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Legume plants are able to establish a nitrogen-fixing symbiotic interaction with soil bacteria, named rhizobia. This association enables legume plants to grow in nitrogen-depleted soil and without the chemical fertilizers. However, our knowledge of this symbiotic interaction is still not complete and to better understand the process of nitrogen fixation, the genes involved in symbiotic nitrogen fixation is required. Symbiotic mutant plants provide an excellent tool to study the process of symbiotic nitrogen fixation. In this study, we analyzed the symbiotic mutant line T00187, which was isolated from the *Tnt1* insertion mutant collection of the model legume *Medicago truncatula*. The T00187 mutant shows an ineffective symbiotic phenotype; they have stunted growth, developed light green leaves and form small white nodules. In addition to the nodulation phenotype, T00187 mutant plants develop shorter and reduced number of lateral roots and have rigid root structures. Mutant nodules did not show the characteristic zonation of indeterminate nodules and the nodule development was arrested following the bacterial invasion of symbiotic cells.

Genetic mapping identified the symbiotic locus of T00187 on top arm of chromosome 2 of *M. truncatula*. To identify the impaired gene, the flanking sequences (FSTs) of the *Tnt1* and *Mere* retrotransposon insertions were identified with next generation sequencing. The sequence analysis defined 4 FSTs in the symbiotic locus of T00187, and one of them was found in an exon of the *UDP-glucuronosyl/UDP-glucosyltransferase* (*UGT73F3*) gene. We verified with co-segregation analysis and complementation experiments that the insertion in the *UGT73F3* is responsible for root and nodulation defects of T00187. Gene expression and protein localizations studies revealed that UGT73F3 is active in root and nodule meristem and vascular system. The analysis of the saponin and flavonoid content of T00187 roots revealed the defects in the glycosylation of saponin and saponin related compounds and identified the accumulation of a glycosylated isoflavone. The identification of the target molecule of UGT73F3, which is required for normal root and nodule development is in progress.

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Gut region-specific interleukin 1β induction and NLRP3 downregulation in the myenteric neurons of a streptozotocin-induced diabetic rat model

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Intestinal motility dysfunction is common in type 1 diabetes (T1D), which is related to enteric neuropathy caused by long-lasting hyperglycemia and the accompanying pro-oxidative and neuroinflammatory mechanisms. Our earlier studies revealed a gut segment-specific damage of the myenteric neurons in a streptozotocin (STZ)-induced T1D rat model. The pro-inflammatory cytokine interleukin-1β (IL1β) is expressed as pro-IL1β and activated enzymatically by inflammasomes. Under sufficient inflammatory and pathogenic stimuli, the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) forms an inflammasome complex leading to the activation of IL1β. NLRP3 and IL1β play critical roles in inflammatory and immune responses and may contribute to the regional diabetic myenteric neuropathy. Our aim was to investigate the effect of chronic hyperglycemia and immediate insulin treatment on IL1β and NLRP3 expression in different myenteric neuronal populations in a type 1 diabetic rat model.

For our experiments, adult male Wistar rats were randomly divided into control, STZ-induced diabetic, and insulin-treated diabetic groups. Hyperglycemia was induced by a single intraperitoneal injection of STZ (60 mg/kg). Insulin was subcutane-

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ously injected twice a day to the insulin-treated group. After the 10-week experimental period, the animals were sacrificed and different gut segments (duodenum, ileum, and colon) were dissected and processed for fluorescent immunohistochemistry, enzyme-linked immunosorbent assay, immunogold electron microscopy, and RNAscope multiplex fluorescent V2 assay.

In control rats, the proportion of IL1β-immunoreactive (IR) myenteric neurons was significantly higher in the colon compared to the small intestine. In diabetic rats, this proportion was induced in all gut segments which was prevented by immediate insulin treatment. The proportion of IL1β-IR neuronal nitric oxide synthase-expressing neurons was only increased in the colon, while the proportion of IL1β-IR calcitonin gene-related peptide-expressing neurons was only increased in the ileum of diabetics. Enhanced IL1β protein levels were also confirmed in smooth muscle/myenteric plexus-containing tissue homogenates of diabetics. IL1β mRNA induction was detected in the myenteric ganglia, smooth muscle, and intestinal mucosa of diabetics. The density of NLRP3-labelling gold particles in the myenteric ganglia was significantly decreased in the duodenum and colon of diabetics relative to controls. NLRP3 mRNA expression was specifically downregulated in the myenteric ganglia, smooth muscle, and mucosal layers of the ileum segment which was not prevented by immediate insulin replacement.

Based on these results, the cell-specific IL1β induction highlights its key role in the region-dependent and neuronal population-specific diabetic myenteric neuropathy. Moreover, NLRP3 downregulation indicates that this inflammasome-forming protein is dispensable for hyperglycemia-induced IL1β activation in the myenteric ganglia and their intestinal milieu in our T1D rat model. However, as a crucial regulator for intestinal immunity, a drastic decrease in NLRP3 may contribute to intestinal damage in T1D.

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Investigation of NMDA-induced excitotoxicity and neuromodulatory effects of kynurenic acid and its analogues using *in vivo* and *in vitro* methods on mouse brain tissue

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The main pathway of tryptophan metabolism is the kynurenine pathway. As a result, many neuroactive substances such as kynurenic acid (KYNA) are produced. KYNA has been shown to have a neuroprotective effect but cannot cross the blood-brain barrier. There are several chemically modified KYNA analogues, such as SZR72 and SZR104, which are permeable to the blood-brain barrier and have shown a protective effect in several animal models. Excitotoxicity plays a key role in many neurodegenerative diseases. Therefore, the above-mentioned neuromodulatory substances were tested in the N-methyl-D-aspartate (NMDA)-induced excitotoxicity model.

In our *in vivo* experiments, animals of different ages (3-4 and 8-10 weeks) were injected intraperitoneally with NMDA and then their brains were examined histologically. The *in vitro* experiments were performed on acute brain slice preparations from the brains of mice of different ages (1, 4, 8 weeks, and 1 year) in a small-volume incubation system to investigate the effect of KYNA and its analogues against NMDA excitotoxicity. The degree of hippocampal tissue damage was assessed using biochemical and histological methods.

Our previous *in vitro* experiments have shown that the pyramidal cell layer of the hippocampus of younger animals is more resistant to the damaging effects of NMDA. In older animals, this damage was intense. Although, after intraperitoneal administration of NMDA, we were unable to detect any damage in the hippocampus in any age group. However, behavioral changes were observed in young animals. Given the lack of brain damage, it can be said that NMDA is not suitable for testing additional neuroprotective substances *in vivo*. Based on our recent results, KYNA is protective against excitotoxicity in younger and older animals under *in vitro* conditions. Treatment with SZR104 was not able to reduce the damaging effect of NMDA. SZR72 has similar positive effects as KYNA in all tested animal groups.

As our results show, KYNA and its analogues are effective neuromodulating substances, so further preclinical testing is recommended in different animal models.

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Impact of mycoflora on mycotoxin contamination in winter wheat in Hungary

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Toxigenic fungi are among t9ö8he most significant disease-causing agents, not only due to grain yield reduction but primarily because of their toxin contamination. This study aimed to analyze wheat genotypes for fungal infection and natural multitoxin contamination in Hungary. A total of 18 winter wheat genotypes were examined for fungal contamination across three different locations (Szeged, Törökszentmiklós and Iregszemcse) in 2021 and 2022. Fourteen different mycotoxins: deoxynivalenol, aflatoxin (B_1, B_2, G_1, G_2), fumonisin (B_1, B_2), sterigmatocystin, ochratoxin A, nivalenol, zearalenon, T-2, HT-2 and diacetoxyscirpenol were analyzed using an Agilent 1260 Infinity II LC coupled with an Agilent Ultivo QQQ MS. Based on the results of the morphological and sequence based species determination, the distribution of the species responsible for fungal infection shifted significantly towards *Alternaria* species, with fewer representatives of *Fusarium*, *Aspergillus*, *Penicillium*, and *Cladosporium* species. Results showed that most samples were contaminated by one or more mycotoxins. Although mycotoxin concentrations typically remained below EU limit values, some samples exhibited higher levels, particularly concerning aflatoxins. Significant variations were observed across years, locations, and genotypes. Recent findings indicate that many toxins, beyond just DON and its masked types, can be present in winter wheat. Aflatoxins and HT-2 require special attention and further research, as they occurred in more samples at higher rate than the EU limits allow, and the four types of aflatoxin were found in nearly similar amounts in many samples. The significance of aflatoxin, in Hungarian wheat samples, particularly their pre-harvest presence, was previously unknown. Our study found that the forms AFB_1, AFB_2, and AFG_1 of aflatoxins are of similar significance, with the highest concentrations observed in AFB_1. This indicates that assessing AFB_1 alone is insufficient to fully characterize the food safety risk of aflatoxins in wheat.

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Characterization of the endophytic and rhizospheric *Bacillus licheniformis* strains isolated from sweet potato with yield enhancing and plant growth-promoting potential

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The primary aim of the present study was to identify bacterial isolates with yield-enhancing potential for application as biofertilizers in the cultivation of sweet potato. Therefore, endophytic and rhizospheric strains were isolated from sweet potato plantations in Hungary to identify bacterial strains with plant growth-promoting and antifungal activity. In total, seven *Bacillus licheniformis* strains were identified and subjected to detailed ecophysiological tests. Experiments have been carried out to investigate the tolerance of the selected strains to different limiting factors such as pH, temperature, and water activity, which affect survivability in different agricultural environments.

The majority of tested *B. licheniformis* strains exhibited plant growth-promoting potential (e.g., production of indole-3-acetic acid up to 40.42 μg mL^{-1}, production of ammonia up to 870 μg mL^{-1}, phosphorus solubilizing activity, siderophore production), with two strains (SZMC 27713 and SZMC 27715) demonstrating inhibitory activity (ranging between 7 and 38%) against plant pathogenic fungi occurring in sweet potato cultivation. Furthermore, strain SZMC 27715 induced accelerated germination and a significantly higher germination rate in tomato seeds compared to the control. In a field experiment, it was observed that strain SZMC 27715 had a prominent yield enhancing effect in sweet potato, where a significant yield per plant increase was observed in all applied treatments (1.13 kg, 1.09 kg and 1.40 kg) compared to the control plants (0.92 kg). The highest yield per plant was observed when the sweet potato cuttings were soaked combined with two additional foliar treatments.

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To the best of our knowledge, this is the first report of the successful application of the *B. licheniformis* strain as a biofertilizer for yield enhancement in the cultivation of sweet potato. Based on our results, strain SZMC 27715 has potential for utilization as a biofertilizer in sweet potato cultivation either as a standalone product or in a microbial consortium.

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Giving significance to missense variant mutations via large-scale protein structure prediction. The development and optimization of a high-throughput protein prediction pipeline on a high-performance computing cluster

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The widespread use of next-generation sequencing (NGS) technologies in clinical diagnostics has resulted in the identification of thousands of novel missense variants whose clinical significance is often unknown. To address this issue, a variety of computational tools have been developed to facilitate the interpretation of these variants of unknown significance. However, the vast range of these tools and ambiguous guidelines often make it challenging for the naive user to choose the appropriate software. In the early stages of my doctoral research, I showed that the performance of leading algorithms in the field was far from ideal, as these algorithms suffered from high sensitivity and specificity trade-offs and concluded that in their current state, they can't be utilized to make meaningful predictions on novel missense variants.

Our analysis of leading algorithms in the field revealed a severe lack in the incorporation of structural-derived features. Part of this can be attributed to the limited number of proteins whose structures have been experimentally resolved. With the development of AlphaFold, it became possible to predict both wild-type and mutated protein structures relatively quickly; however, it is still questionable if their structure can be used to improve missense variant prediction algorithms.

To answer this question, we compiled three datasets containing missense variants of known clinical significance from the public repositories Humsavar and ClinVar. A strict filtering process was used to retain only pathogenic or likely pathogenic and benign or likely benign variants with evidence of their impact on protein function. As a result, 72000 pathogenic and benign variants across 2000 proteins were identified. For each genetic variant, we generated mutated amino acid sequences and used them as input for Alphafold. Additionally, we also used AlphaFold to predict the wild-type structure of the proteins in our dataset. To efficiently run AlphaFold on a large scale, we utilized a high-performance computing cluster. Proteins were divided into 13 subclusters based on their amino acid length; for instance, proteins with a length of 100 to 199 amino acids were predicted in a separate run. This separation was carried out to speed up the feature extraction process. After the clusters were generated, a period of optimization and automation was performed, and a pipeline was established to predict 65000 wild-type and mutated protein structures. Several rounds of superimposition between wild type and mutant structures were performed to ensure that the predicted structures were of high quality. In addition, we started the process of extracting sequence-based features and benchmarking potential deep learning models for our algorithm.

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Investigation of the roles of individual glutathione transferases (GSTs) under stress in tomato plants

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Glutathione transferases (GSTs), due to their broad range of activities, play a crucial role in cellular processes, including the defense under various stress conditions. To investigate their exact roles, it might be important to study these isoenzymes individually to comprehend their specific functions. My ongoing research focuses on the investigation of stress-responsive

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GSTs in the economically significant plant, tomato (*Solanum lycopersicum*). The targets of my work are certain tau (GSTU), phi (GSTF), and theta-class (GSTT) GSTs, and my research involves methods like specific enzymatic assays, gene expression analysis via qPCR, applying the CRISPR/Cas9 system, transient gene overexpression, and various bioinformatical techniques in protein modeling and substrate binding predictions.

The goal of introducing the CRISPR/Cas9 system to tomato is to induce mutation in the *SlGSTU24* gene to decrease the functionality of the enzyme. According to previous studies, the *GSTU24* gene is highly expressed in the root tip region of tomato plants, which is a crucial area for plant growth and stress response. Moreover, the GSTU24 protein plays an important role in protecting against oxidative damage caused by various environmental stressors and determining the redox state of the glutathione system. I've successfully introduced the CRISPR/Cas9 system to tomato (Moneymaker cultivar) via *Agrobacterium tumefaciens*-mediated transformation, regenerated transgenic plants, identified one insertion mutant (which results in an early stop codon due to frameshift in the *SlGSTU24* gene), and collected and planted the seeds of this mutant to the select homozygotic mutant plants for analyzing the role of this enzyme.

To investigate the role of the members of the phi class GSTs, the *SlGSTF4* gene has been selected for gene overexpression experiments. To execute this research more swiftly, the *Agrobacterium rhizogenes*-mediated transformation protocol has been optimized for tomato plants. With this technique, it is possible to generate a large amount of transgenic plant material, but only the roots of these plants carry the gene insertion. The cDNA of the *SlGSTF4* gene has been cloned into an overexpression vector (pH2GW7) via the Gateway cloning method, and several transformation experiments have been carried out.

One of our latest experiments shed light on the importance of the tomato theta class GSTs. We observed elevated expression levels of the *SlGSTT* genes along with notable glutathione peroxidase activity (GPOX) under severe osmotic stress. The GSTT enzymes are known for their strong GPOX activity which corresponds to my findings using artificial intelligence-based molecular docking predictions: the tomato GSTT enzymes might be localized in the peroxisomes and have an important role in disposal of stress metabolites, such as lipid peroxidation products.

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Investigating the kynurenine aminotransferase-2 expression under physiological and pathological conditions

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My research focuses on investigating the kynurenine system, which has been implicated in various pathological changes affecting both the central nervous system and the periphery. Diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and other neurodegenerative and neuropsychiatric disorders have been linked to alterations in this pathway. The most important metabolite in the kynurenine pathway is kynurenic acid (KYNA), which is known for its neuromodulatory effects. KYNA is produced in the largest quantities by the enzyme kynurenine aminotransferase-2 (KAT-2) in both human and rodent brain.

During my previous research, I investigated the expression of the KAT-2 enzyme in various regions of the mouse brain using fluorescent immunohistochemistry. I conducted the localization of the enzyme in those brain regions that exhibit altered KYNA levels in certain neurodegenerative diseases.

Besides the physiological condition, I also aimed to explore pathological conditions. For this purpose, I established models of MPTP-induced Parkinson's disease and 3-NP-induced Huntington's disease. Both conditions have been associated with altered KYNA levels, thus, the examination of the KAT-2 enzyme in these disease models can provide important insights into the involvement of the kynurenine system in neurodegenerative diseases.

Furthermore, I was able to extend my immunohistochemical studies from brain tissue to cell cultures. I conducted a comparative analysis of KAT-2 enzyme expression levels across astrocyte, neuron, and microglia cell cultures, making the first investigation of its kind in this field.

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Utilization of Mucoromycota hydrolases for ecofriendly production of bioactive phenolics from plant residues

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Phenolic compounds are important secondary metabolites in plants. They can exert different beneficial effects on the human body, through their antioxidant, antimicrobial and anti-inflammatory properties. However, most of these compounds are present in the plant cell wall bound to various polymers, which reduces their bioavailability. Solvent treatment is frequently used to extract phenolics from their bound form, however, this methodology often leads to quality loss in the final product and can cause environmental stress. As an alternative, treatment with enzymes can be an ecofriendly strategy to liberate these bound compounds. Hydrolases produced by microorganisms - such as cellulase, lipase, tannase and pectinase enzymes - can digest the plant cell wall and the bonds between the phenolic compound and the polymer, increasing the extractability of the phenolics. Cellulase and lipase activities of Mucoromycota fungi have been extensively studied, however, information about their tannase production is scarce.

The aim of our work was to release bound phenolic compounds from plant substrates (cereals, agricultural and food by-products) using hydrolytic enzymes produced by Mucoromycota fungi. We examined the phenolic and flavonoid contents, antioxidant activity and enzyme inhibitory properties of the extracts obtained by enzymatic treatments. Our research also included the investigation of the effect of combined treatments using enzymatic and physical, i.e., heat and microwave, extraction approaches. Tannase-producing capacity of several Mucoromycota strains has also been investigated. After testing different fermentation systems and enzyme purification strategies, the characterization of fractions with outstanding tannase activity was also performed.

Our results highlight the potential of various fungal hydrolytic exoenzymes to increase the extractable phenolic content of various plant residues, including sorghum, oat, barley and grape pomace samples. Many bioactive properties, e.g., antioxidant and enzyme inhibition potential, of the residues have increased in parallel to the phenolics yield. Physical pre-treatments with different parameters had a positive effect on the total phenolic content, with a concomitant positive effect on the antioxidant activity. High tannase yield was obtained in solid-state fermentation conditions from Mucoromycota isolates, and after appropriate extraction and purification steps, fractions with outstanding tannase activity were obtained. Temperature tolerance of the purified tannase was also studied.

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Investigation of the function of Snx21 in *Drosophila melanogaster*

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The sorting nexin (SNX) family contains proteins with a wide variety of domain structure, so their functions are diverse - they are involved in the function of membrane transport pathways, signaling processes, and the movement of organelles. Sorting nexins are characterized by the presence of the phospholipid-binding PX domain, which typically binds phosphoinositides, most commonly the early endosome-enriched phosphatidylinositol 3-monophosphate. Exact functions of many Snx proteins are still unknown, for example only one publication on human SNX21 provides experimental data concerning its endosome-associated scaffold function: in mammalian cells SNX21 recruits the huntingtin (Htt) protein to early endosomes and interacts with several members of the septin family through its C-terminal PX-associated B domain.

To explore the function of Snx21 in Drosophila, I performed loss-of-function experiments using two independent RNA-interference constructs in garland cells and salivary glands. The endosomal system of garland nephrocytes represents an excellent experimental model to study proteins with putative endosomal functions. Using immunofluorescence, we found that *snx21* knockdown leads to a disordered endosomal pattern in garland cells. In the salivary glands, after the secretion of the adhesive material stored in the so-called glue granules, a portion of these granules remain in the cytosol and fuse with lysosomes to become crinosomes (a degradative compartment). Interestingly, knockdown of *snx21* causes a minor degradation

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defect because of a potential fusion defect between glue granules and lysosomes.

To further analyze Snx21 function, we generated a null mutant allele as well as the GFP- and GST-tagged Snx21-constructs. Mutants show decreased lifespan and locomotor activity, and abnormal protein accumulation in the brain. With GST-tagged Snx21 we performed lipid flotation assay and found the lipid interactor partners of Snx21. Snx21-GFP localizes to early and late endosomes in garland nephrocytes and to glue granules in salivary glands. Immuno-precipitation followed by mass spectrometry showed the interaction of Snx21 with Flotillin 1 and Flotillin 2, proteins that are found in lipid rafts, specialized regions of the cell membrane involved in cell signaling and membrane trafficking. To further investigate the possible Snx21-Flotillin interaction we carry out colocalization and genetic interaction tests.

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The role and significance of cyclic electron transport in microalgae

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Photosynthesis, the conversion of light energy into chemical energy, involves reducing atmospheric CO_2 to organic compounds in plants, algae, and certain bacteria. This process, comprising light-dependent and light-independent phases. PSII and PSI utilize light energy to drive linear electron transport (LEF), yielding NADPH and ATP, which is utilized for CO_2 reduction via the Calvin Benson Bassham (CBB) cycle. Additionally, cyclic electron flow (CEF), involving only PS I, plays a crucial role in energy balancing by producing surplus ATP and safeguarding PSI during stress conditions.

We employed microalgae from the *Symbiodiniaceae* family, a highly diverse group of unicellular, dinoflagellate, phototrophic organisms crucial for biogeocycling in freshwater and marine habitats. They form endosymbiotic relationships with corals, aiding in meeting their energy needs. However, coral reef ecosystems are threatened by rising global temperatures, which disrupt the symbiotic relationship, ultimately leading to mass coral bleaching. To explore the photosynthetic efficiency and the physiological role of alternative electron transport pathways in different *Symbiodiniaceae* species, crucial for understanding and potentially mitigating the impacts of coral bleaching, we aimed to investigate, i) the changes occurring in various photosynthetic parameters, under acute heat stress, ii) the mechanism of electron transport under stress condition in *Symbiodiniaceae* using different inhibitors, and iii) the involvement of different pathways of cyclic electron transport such as PGR5/PGRL1 pathway and NDH-2 pathway.

We evaluated cultures of *Symbiodiniaceae* (strains CS156, 2465 and 2467) during their logarithmic growth phase, subjecting them to different heat stress conditions: acute heating (1-hour incubation at varying temperatures) and gradual heating (temperature increased and decreased by 2 °C every 24 hours). Monitoring the photosynthetic electron transport chain involved recording flash-induced chlorophyll fluorescence relaxation and fast fluorescence induction (OJIP) curves, alongside various other parameters such as electron transport rate, activities of Photosystem I and II, non-photochemical quenching (NPQ), post illumination chlorophyll fluorescence transient and P700 redox kinetics. Significant alterations were observed in these parameters under heat stress, with some evident strain-specific responses. A characteristic transient wave like fluorescence decay due to oxidation and reduction of plastoquinone (PQ) pool was observed in *Symbiodinium* under heat and microaerobic condition. This wave like decay pattern has been used as marker for alternate electron transport previously in other microalgae such *Chlamydomonas* and *Synechocystis*. We observed increased P700+ reduction rates under heat stress, indicating downregulation of CBB. The wave depicted intricate interplay of electron transfer from PSII to PSI and cycling back to PQ pool, which was confirmed using inhibitors such as DCMU, DMBQ and MV. NDH-2 was observed as a potential mediator in cycling the electrons back PQ pool under stress conditions. Currently we are exploring the details of photosynthetic mechanisms of *Symbiodinium* using antisense oligonucleotide-mediated gene silencing, and also trying to develop a fluorescence marker for intact coral to detect early signs of stress, facilitating the design of protective strategies within their natural environment.

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Bacterial cell division: New perspectives

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Cyanobacteria, photosynthetic Gram-negative bacteria, serve as model organisms in this study and are gaining prominence in various biotechnological applications. Research on *Synechococcus elongatus* PCC7942 revealed that batch population cells undergo a sequential differentiation from exponential to late stationary phases. The stationary state appears to be inevitable and is not attributed to nutrient deprivation, toxins, or contact inhibition. The model of perpetual growth and death was dismissed, suggesting that what was previously believed to be a death phase is a misconception. The colony forming unit (CFU) indicates the presence of cells capable of division; however, late stationary phase (designated as G0 state) cells are incapable of division. The concentration of these G0 cells increases over time within the population, resulting in a significant decrease in CFU. Notably, the late stationary G0 cells remain alive, and the total cell concentration remains relatively stable. Potential factors that may slow the growth rate are being investigated.

The division process of *S. elongatus* is also being studied at the molecular level. Various FtsZ-GFP constructs have been developed to replace the endogenous FtsZ, providing a new tool for examining FtsZ ring assembly. Different deletion constructs of FtsZ are utilized to elucidate the roles of FtsZ domains in the cells. Other GFP-labeled proteins involved in bacterial cell proliferation and cytokinesis, such as MinD, CDV3, and MreB, are also being studied. MinD has shown interaction with FtsZ, and CDV3-GFP is associated with the Z-ring.

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Effects of lifestyle modification on adipokine pattern and lipid peroxidation in a stroke-prone spontaneously hypertensive rat model

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A high-fat diet and lack of physical activity can have significant detrimental effects on the body, particularly on the cardiovascular system and metabolism. High-fat diets increase cholesterol levels, particularly low-density lipoprotein (LDL) cholesterol, leading to plaque buildup in the arteries (atherosclerosis), which restricts blood flow to the heart and causes hypertension, thereby increasing the risk of heart disease and stroke. Conversely, a sedentary lifestyle poses significant risks to cardiovascular health and contributes to obesity, whereas physical exercise improves blood pressure and cholesterol levels and promotes healthy blood glucose levels. Adipokines, such as leptin, adiponectin, chemerin, and omentin, are signaling molecules produced by adipose tissue that play crucial roles in regulating various physiological processes, including metabolism, inflammation, immune function, and maintaining overall metabolic homeostasis.

The aim of this study was to examine the impact of a high-fat diet and physical exercise on the adipokine profile and lipid peroxidation in stroke-prone spontaneously hypertensive (SHRSP) rats. In our study, Wistar-Kyoto and SHRSP rats were utilized. Based on diet and physical exercise, SHRSP animals were divided into four groups: standard chow, high-fat diet with 40% fat content, standard chow with running, and high-fat diet with 40% fat content with running. The experiment lasted 12 weeks, during which the animals were subjected to their respective diets and exercise regimens. At the end of the 12 weeks, the animals were euthanized, and adipose tissue and serum samples were collected to determine the adipokine profile and lipid peroxidation (malondialdehyde, MDA content).

The results showed that a high-fat diet is associated with an altered leptin-adiponectin ratio, decreased omentin concentration, and increased chemerin concentration and MDA content. However, 12 weeks of exercise positively influenced these altered parameters.

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Our findings confirm the interaction between lifestyle changes and the altered adipokine pattern as well as lipid peroxidation in SHRSP rats.

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Enhancing biogas production through Direct Interspecies Electron Transfer (DIET) in Microbial Electrochemical Cells (MECs)

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Biogas, primarily composed of methane and carbon dioxide, is a renewable energy source derived from an aerobic decomposition of organic matter. The potential to replace conventional energy sources has increased in significance. However, optimizing biogas production processes remains a challenge. Direct Interspecies Electron Transfer (DIET), a microbial interaction mechanism, has emerged as a promising approach to enhance biogas production.

In DIET, microorganisms directly transfer electrons between different species, bypassing the need for intermediate compounds. This process allows for a more efficient degradation of complex organic matter and enhances methane production in anaerobic environments. To harness the potential of DIET, a specialized Microbial Electrolysis Cell (MEC) system was developed and its effect on biogas production was evaluated.

Our study tested the effects of DIET on biogas production in MECs. The initial results indicated that the presence of non-inert materials significantly hindered the DIET process, negatively affecting the biogas yield. However, through controlled mixing, a substantial improvement in biogas production rates was observed, with methane production doubling compared with non-mixed reactors.

Furthermore, increasing the surface area within the MECs resulted in lower carbon dioxide concentrations in the produced biogas, suggesting enhanced methane purity. These results emphasize the importance of optimizing the reactor design and operational parameters to maximize biogas quality and yield.

Currently, ongoing testing is being conducted to assess the long-term capabilities of the DIET-enhanced MEC systems. In addition, we investigate the resilience of the system under various Hydraulic Retention Times (HRTs) to determine the optimal operating conditions and identify potential limitations.

In conclusion, our research highlights the potential of DIET to revolutionize biogas production processes. By leveraging microbial interactions and innovative reactor designs, we aim to contribute to the advancement of sustainable energy technologies and address challenges in transitioning towards a low-carbon future.

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Identification and detailed molecular characterization of novel circadian clock mutants in *Arabidopsis thaliana*

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The circadian clock (circa diem - about a day), a system that works in all living creatures is to aid adaptation of a wide range of organisms to the daily changes in environmental conditions. The daily regulation of gene expression, biochemical and physiological processes by the circadian clock can save considerable energy, as opposed to continuous operation. Since our understanding of circadian systems is still not complete, we performed a large-scale mutant screening project that identified several independent novel clock mutants with aberrant circadian phenotypes. We focused our efforts on a particular period mutant, which had a period 2 h shorter than the wild type. This was caused by an amino acid substitution in the *UBP12* gene and resulted in a new missense allele (*ubp12-3*). *UBP12* encodes a ubiquitin protease that cleaves ubiquitin from other proteins, thus altering their stability/function.

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The mutation affects the evolutionarily conserved amino acid serine 327, which is in the active center of the protein and is replaced by the non-phosphorylatable phenylalanine. The mutation has no effect on *UBP12* transcription or UBP12 protein stability.

UBP12 was shown to affect the clock through the GIGANTEA (GI) and ZEITLUPE (ZTL) proteins, by increasing their stability and levels. Interestingly, in our conditions neither the T-DNA insertional null mutant *ubp12-1* nor our missense mutant *ubp12-3* showed alterations in the level of ZTL. This indicates that UBP12 must have alternative routes to modulate the pace of the oscillator.

However, UBP12 has other targets besides these clock proteins. One of these is the transcription factor MYC2, which plays an inhibitory role in jasmonic acid-induced gene expression. Our results suggest that the *ubp12-3* mutant is hypo-sensitive to jasmonic acid, indicating increased de-ubiquitination of MYC2, suggesting hyper-activation of UBP12 activity.

Another target is histone H2A. When H2A is ubiquitinated, chromatin is decondensed and the expression of certain genes is intensified. Compared to the wild type, the *ubp12-3* mutant showed a decrease in transcription of these genes, indicating increased de-ubiquitination of H2A and thus hyper-activation of UBP12. Collectively these results suggest that *ubp12-3* is a gain-of-function allele.

We replaced serine 327 with non-phosphorylatable and phosphomimicking amino acids and tested the effect of these on the ubiquitin protease activity of UBP12 in a bacterial expression system. The results indicated that phosphorylation of serine 327 abolishes the activity of UBP12. Thus, we propose that the *ubp12-3* mutation (Ser327Phe) cause hyper-activation of UBP12 by preventing inhibitory phosphorylation.

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Exploring the function of the MtnodGRP3C gene in the development of nitrogen fixing nodules

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Symbiotic nitrogen fixation takes place in the root nodules of legumes, in which Rhizobium bacteria transform into nitrogen-fixing bacteria capable of reducing the atmospheric nitrogen gas to ammonia. In the nodules of the inverted repeat-lacking clade (IRLC) of legumes, hundreds of plant peptides are produced in the symbiotic cells, most of them belonging to the NCR family, which play a role in the terminal differentiation of bacteroids. Another gene family coding for nodule-specific glycine-rich proteins (nodGRPs), has also co-evolved with NCRs in the IRLC plants. The first nodGRPs were described in *M. sativa*. The genome sequence of the model legume *M. truncatula* made possible to identify the entire set of *nodGRP* genes which similarly to the *M. sativa nodGRPs* exhibited nodule-specific expression.

In our work, we focused on MtnodGRP3C (Medtr2g069245) which is highly expressed in zone II of the nodule, where bacteroid differentiation begins, and at lower levels in the interzone, where the main differentiation of bacteroids takes place. Silencing of MtnodGRP3C in transgenic plants resulted in the formation of small and white nodules. Elongation of bacteroids occurred but they were unable to fix nitrogen, and the incomplete differentiation of bacteroids and symbiotic cells led to premature nodule formation. Constructs overexpressing the MtnodGRP3C gene from different constitutive promoters were also generated. Their potential effects on symbiosis have been tested in transgenic hairy roots. In parallel, generation of stable transgenic plants have also been initiated and currently several independent transgenic lines are ready for detailed microscopic and molecular analysis.

GRP3C promoter expression is unique among GRP family members because it is the only one that is expressed in the root too, according to our data. By analyzing the GRP3C promoter region we identified the minimal promoter required for proper expression and searching for transcription factors are that bind to the minimal promoter sequence and control GRP3C expression in the root and in zone II of the nodule.

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Effect of *Pseudomonas* strains on growth and AFB1 production in *Aspergillus flavus*

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Aspergillus flavus causes significant crop losses and reduces crop quality by contaminating seeds with aflatoxin in the field and during post-harvest processes. Various pre-harvest strategies have been implemented to control *A. flavus* infection, such as field management practices, development of resistant crop varieties, application of synthetic chemicals, and utilization of competitive atoxigenic strains of *A. flavus*. The use of microorganisms or enzymes to combat some plant diseases is known to be a promising and reliable alternative to reduce the need for agrochemical pesticides. Several *Pseudomonas* species isolated from almond fruits, maize rhizosphere, rice grains and wheat soil proved to be efficient biocontrol agents against *A. flavus*.

Our aims were (i) to isolate pseudomonads from corn rhizosphere and (ii) to characterize their antagonistic activity against the growth and toxin production of *A. flavus* in both solid and liquid co-cultures. Moreover, (iii) we aimed to investigate the mechanisms involved in controlling of aflatoxin production through the identification of major transformation products by HPLC-HRMS method.

Fifty *Pseudomonas* strains were isolated and purified from corn rhizosphere samples. Examination of the sequences of the 16S *rRNA*, *rpoD* and *rpoB* genes revealed that 45 bacterial strains can be classified into five species groups: *P. fluorescens*, *P. putida*, *P. chlororaphis*, *P. jessenii*, and *P. koreensis* species groups, while 5 strains did not relate to any known species groups. Upon assessing the antagonistic capabilities of the *Pseudomonas* strains, it was observed that three strains affiliated with the *P. fluorescens,* and *P. chlororaphis* species group completely inhibited the production of AFB1 toxin or any AFB1-related compounds in both solid and liquid co-cultures with minimal impact on fungal growth. Since neither AFB1 nor any precursors or derivatives of AFB1 were detected in the co-cultures, the antagonistic *Pseudomonas* strains supposedly act through by releasing diffusible or volatile compounds that blocks the entire toxin biosynthetic pathway of the fungus. The rest of the *Pseudomonas* strains affected AFB1 production at varying levels. Three strains triggered overproduction of AFB1, and 19 strains modulated fungal enzyme production or metabolism.

The five strains with unique 16S *rRNA*, *rpoD*, and *rpoB* sequences seemed to be isogenic and represent a novel *Pseudomonas* species. The entire genome of one strain was sequenced, and its average nucleotide identity (ANI) was compared to known reference genomes of *Pseudomonas* species. The ANI value was below 86% compared to any of that of NCBI reference sequences. To characterize this strain, phenotypic analyses, growth tests and enzyme activity tests on various substrates, fatty acid methyl ester composition, and scanning electron microscopy were carried out.

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Different roles of phytochrome B and D photoreceptors in heat and light perception

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Light is one of the environmental factors that has maybe the greatest influence on plant growth and development. It is important not only as a signal to guide plant development and growth, but also it is essential for energy and biomass production. Plants can detect a broad spectrum of electromagnetic radiation (λ = 280-750 nm) reaching the Earth's surface, through their photoreceptors. The red (λ_{max} = 660 nm) and far-red (λ_{max} = 730 nm) parts of the spectrum are sensed by five members of the phytochrome photoreceptor family (phytochrome A, B, C, D and E (phyA-E) in the model plant *Arabidopsis thaliana*). These proteins are produced in an inactive conformer (Pr) and upon light irradiation they undergo a conformational change resulting in the biologically active form (Pfr). The role of *phyA* and *phyB* (major phytochromes), expressed at high levels, has long been

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investigated but much less work has focused on the function of *phyC*, *phyD* and *phyE* (minor phytochromes) that are expressed at lower amounts. The aim of our research is to elucidate the mechanisms of *phyC* and *phyD* signaling, their biological roles and their interactions, so that we can better understand the basis of plant light perception.

Phytochromes regulate the expression of about 10-15% of the *Arabidopsis* genome. These approximately 3000 genes are identified so far, affecting almost all aspects of plant life. Our research focuses on the regulation of photomorphogenesis, *i.e.* light-regulated growth and development, following germination. Our work is based on the observation that in phytochrome-deficient transgenic plants, overexpressed *phyD*, together with endogenous *phyC*, can regulate efficient photomorphogenic responses, whereas the endogenous *phyD* and *phyC* cannot. This phenomenon is temperature dependent, and can be observed at 17-22 °C but not at 27 °C.

For more in-depth investigations our group developed a unique experimental system, based on Arabidopsis mutants and transgenic lines that express the phytochromes individually or in different combinations. We used the following methods:

- RT-qPCR and western blot measurements were used to investigate the mRNA and protein levels underlying the observed phenotype (hypocotyl length shortening).
- Confocal laser scanning microscopy was used to investigate the effect of the putative protein interaction on the efficiency of signal transduction.
- Transcriptome analysis was used to test whether different phytochromes activate the same signaling pathways.
- *In vivo* spectrophotometry and specific light treatments were used to explore how phytochromes complement each other during thermomorphogenesis.

Our data shows that under certain conditions (continuous red light, low temperature), overexpressed *phyD* is a very efficient substitute for phyB in the presence of active *phyC*. This offers the possibility to fine-tune not only photomorphogenesis but thermomorphogenesis as well. The fact that *phyD* can take over the role of *phyB* in red light-regulated photomorphogenesis under the right conditions is somewhat surprising as it is generally accepted that *phyB* dominates red light signaling in light-grown plants.

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Sexdependent effects of hyperlipidemia and HSPB1overexpression on cardiac function in a mouse model of obesity

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Obesity is a complex disease involving abnormal fat accumulation and impaired glucose homeostasis causing several cardiometabolic problems. However, heat-shock proteins can maintain protein homeostasis and are also involved in several processes related to cardiovascular diseases. Therefore, we studied the effects of hHSPB1 in an obesity model, namely in the APOB-100-overexpressing mouse strain (APOB).

For this, the model animals were crossed with hHSPB1-overexpressing mice (APOB/HSP). The wild type (WT) and the HSPB1 controls were normal chow-fed mice, while the APOB and the APOB/HSP mice got a high-fat diet (HFD) for 7 months.

Transthoracic echocardiography was performed to study the cardiac morphology and function, and relative gene expression changes in the heart were investigated by RT-PCR. Body weight and serum glucose concentration were monitored during the experiment, and it was found that HSPB1overexpression caused further weight gain in female APOB mice without insulin resistance, however in the males the elevated bodyweight appeared with impaired glucose homeostasis. Cross-sectional diastolic inferior wall thickness was significantly increased, while left-ventricular end-diastolic diameter was significantly decreased in APOB males compared to the WT suggesting obesity-induced hypertrophy. Parallel in females, these symptoms were not so severe, the wall thickness was not influenced by obesity, while left-ventricular end-diastolic diameter was significantly decreased in the APOB group compared to the WT. HSPB1 overexpression resulted in hypertrophy regardless of the sexes with

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preserved ejection fraction and without the elevation of inflammatory cytokines gene expression (*Tnfα; Il1β*).

According to our data, male APOB mice show more severe metabolic disturbances after 7 months of HFD compared to females. HSPB1 may be involved in the regulation of HFD-induced processes, by influencing weight gain and inducing physiological hypertrophy in healthy animals.

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Effects of nickel oxide nanoparticles (NiO NPs) on cell wall structure, root anatomy, and nitrosative signaling in different ecotypes of Ni hyperaccumulator plant *Odontarrhena lesbiaca*

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In my study, I explored the effects of nickel oxide nanoparticles (23.2 ± 8.3 nm, specific surface area: 76.397 m^2/g) on NP uptake, distribution, biomass production, and reactive nitrogen/oxygen species levels in three ecotypes (Ampeliko, Loutra, and Olympos) of the Ni-hyperaccumulator *Odontarrhena lesbiaca*. Applying NiO NPs at varying concentrations (0, 250, 500 mg/L) for 5 days, I found that NiO NPs were detected in the root cell walls of all three ecotypes, albeit less evident in hypocotyl parts. I supported these findings with TEM and LIBS analyses. NiO NP treatments slightly reduced root/shoot lengths and fresh/dry weights in Ampeliko and Loutra ecotypes, while positively influencing these parameters in Olympos seedlings. I measured Ni concentrations in dried *O. lesbiaca* plant samples (root and shoot separately) by using ICP-MS. The adverse impacts on biomass production were greater with the free salt form of Ni compared to the nanoform. I examined cell wall defenses, peroxidase activity, and flavonols microscopically. I assessed reactive molecule signaling intensity using fluorescent probes, observing elevated levels of nitric oxide (NO) and hydrogen peroxide (H_2O_2) in all ecotypes' roots post-NiO NP exposure. Peroxynitrite ($ONOO^-$) levels remained unchanged in Olympos roots, while superoxide anion radical ($O_2^{\bullet-}$) and glutathione levels were impacted by the NiO NPs. I observed changes in the abundance and functionality of *S*-nitrosoglutathione reductase (GSNOR) protein induced by NiO NPs, suggesting their post-translational regulation. Additionally, I found that NiO NPs marginally heightened protein tyrosine nitration, with ecotype-specific differences correlating with biomass production in their presence.

My findings demonstrate for the first time that the tolerance of *O. lesbiaca* ecotypes against NiO NPs manifests at cellular (binding of NPs by the root cell wall due to compositional modification), tissue (root anatomical changes), organ/organism (slight modifications in biomass production) and molecular (changes in RNS metabolism and induced nitrosative protein modification) levels.

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The effect of FOXO transcription factor in the Q-system based *Drosophila* Huntington's disease model

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Huntington's disease (HD) is a severe neurodegenerative disease caused by a dominant mutation in the *Huntingtin (HTT)* gene. My research investigated the effect of the FOXO transcription factor in a *Drosophila* model of HD using transgenic *Drosophila* strains in which the N-terminal fragment of the human huntingtin protein carries 120 (Q120) glutamine repeats in diseased individuals and 25 (Q25) glutamine repeats in healthy individuals. A common method to regulate transgene expression is the use of two-element expression systems. The GAL4/UAS/GAL80ts system is widely used in *Drosophila*. However, with this system it is not possible to drive two transgenes with different expression patterns; furthermore, when two transgenes are driven simultaneously, the GAL4 transcription factor is shared between transgenes containing UAS, which can lead to mis-

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leading results in genetic interaction assays. To overcome this problem, the alternative Q-system has been developed, which works very similarly to the GAL4/UAS system, but there is no cross-reactivity between the two systems, so they can be used simultaneously. For the genetic interaction studies, we have established a HD model system that allows the *HTT* transgene to be driven by the QF/QUAS system, while the induction of the other transgene under interaction is still regulated by the GAL4/UAS system. Genetic interaction studies of *Huntingtin* were performed with the FOXO transcription factor, which is known from literature data to have a lifespan-stretching effect. In our experiments, we seek to answer the question whether FOXO overexpression can attenuate mutant *HTT* induced pathologies in a Q system-based *Drosophila* Huntington's disease model. We performed viability, longevity, locomotion and neurodegeneration experiments with two different FOXO strains. First, we used the pUASP-*foxo*, a lower expressing construct, which showed positive results. Using RT-qPCR with primers specific to the pUASP-*foxo* transgene, we confirmed that the transgene is expressed, but found that it only slightly elevates *foxo* mRNA levels. Therefore, I generated the pTWHattB-*foxo* transgenic strain, in which *foxo* is more highly expressed. The results show that the increase in expression levels is toxic during early development. However, at adult age, it extended the lifespan of animals older than 24 days. In conclusion, low levels of expression of the FOXO transcription factor have a better effect on the phenotype of HD animals than high levels of expression, i.e. the principle of less is sometimes more. RNA-sequencing of animals from interaction crosses was performed, and transcriptomic data showed that in healthy QQ25 individuals, genes involved in the dysregulation of stress, heat, and stimulus responses, as well as protein folding, and polytene chromosome rearrangement biology were overrepresented among genes showing expression changes in response to the FOXO transcription factor. In QQ120-expressing, HD muslicles, transcription of genes involved in response responses to heat shock, stress and abiotic stimuli was altered. Apparently, protein folding/refolding and chaperone-mediated protein folding are also dysregulated here. Essentially, genes involved in very similar biological pathways were enriched in response to mutant *Huntingtin* and *FOXO*, suggesting that *FOXO* may partly escape for this reason. In HD animals, *FOXO* overexpression results in altered expression of genes involved in membrane assembly and assembly and in the organization of extracellular matrix and structure. In these genotypic animals, the pathways that showed enrichment in response to huntingtin alone and *FOXO* alone were unaffected, presumably because *FOXO* restored the gene expression changes in Huntington's disease.

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Morphological and functional characterization of a new human lab-on-a-chip system by the co-culture of a blood-brain barrier model with brain organoids

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The functional basis of the blood-brain barrier (BBB) is formed by brain endothelial cells. These together with the cell types of the neurovascular unit form a protective interface between the systemic circulation and the brain. Microfluidic chip devices allow the more complex and physiological modelling of the BBB. The iPSC (induced pluripotent stem cell) technology enabled the generation of human brain spheroids and organoids that provide a simplified 3D model of the central nervous system including different types of neurons and glial cells. The aim of the study was to (1) examine the interactions between a human BBB model and midbrain organoids using a static setup and (2) to develop a microfluidic device to co-culture BBB cells and brain organoids for morphological and functional experiments. Human cord-blood derived endothelial cells and brain pericytes were co-cultured to establish the BBB model. Brain organoids were differentiated from healthy (WT) and Parkinson disease patients' (PD) iPSCs. To examine the barrier integrity, we performed transendothelial electrical resistance (TEER) measurement in the case of the static culture inserts, impedance measurement for the dynamic biochips, permeability measurements for sodium fluorescein and Evans-blue labelled albumin and immunostaining for tight junction proteins. The presence of brain organoids did not influence the barrier integrity – TEER, permeability and claudin-5 immunostaining – of the human BBB model in static conditions, which proved the co-culture to be suitable for further experiments. We successfully integrated the brain organoids into the biochips and characterized both the BBB model and the brain organoids (βIII-tubulin, MAP-2,

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GFAP immunostaining). A BBB-brain organoid co-culture model with good barrier integrity was established and tested for nanoparticle permeability. We successfully developed a new, complex microfluidic device and co-cultured a human BBB model and human midbrain organoids together for the first time in a dynamic setup. This complex organ-on-a-chip system can be a valuable tool for further drug permeability testing and pathology investigations.

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Srr1, a conserved transcription factor regulates postmeiotic spore morphogenesis and ballistospory in mushroom-forming fungi

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Spore formation is the most widespread means of reproduction and dispersal in fungi. In the Basidiomycota, spores also have industrial importance, and their active discharge represents the highest known acceleration in nature. In this study, we characterized a highly conserved C_2H_2-type zinc finger transcription factor *srr1* (sporulation-related regulator), which we identified based on its high expression in gills of multiple mushroom-forming fungi. The Δ*srr1* mutant of *Coprinopsis cinerea* showed a spore-less white cap phenotype, with no significant influence on mycelium growth or the fruiting body developmental process. The knock-down of *srr1* ortholog in *Pleurotus cornucopiae* had spore-poor phenotype, indicating the conserved role of *srr1* among Agaricomycetes. Spore development was arrested when spore initials emerge at the sterigma tips after meiosis. RNA-seq revealed 154 up- and 559 down-regulated genes in Δ*srr1*, suggesting the *srr1* is mostly an activator. The motif was inferred as the binding site of srr1 among 260 direct targets. Taken together, *srr1* has a conserved role in sporulation and ballistospory in mushroom-forming fungi, its target genes can be potential candidates in further studies of sporulation and the mushroom industry.

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