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Effect of landscape heterogeneity in conserving the arthropod fauna of forest-steppe in Great Hungarian Plain

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Biodiversity loss is major threat to the survival of ecosystems and considered as major driver of ecosystem changes in Hungary as well. Main cause of decrease biodiversity is habitat fragmentation, habitat loss or habitat modification. Loss of biodiversity on a local spatial scale is much more alarming than global scale biodiversity decline, as it includes regional endemic flora and fauna. Understanding the effects of different landscape in the matrix, their quality and local attributes on the biota of habitat patches is often challenging The objective of this dissertation is to improve the understanding of distribution patterns of species and their preservation in highly modified landscapes of Great Hungarian Plain.

To achieve this goal firstly we studied the biota of natural forest fragments and hypothesized that the local and landscape level variable and their interactions have different effect on forest specialist and open-habitat plant, spider and carabid species. Secondly, we focus on biodiversity in these structurally complex habitat fragments while also considering the effects of fragment size and landscape quality on the species composition (beta-diversity) of arthropods and plants. Thirdly, we try to understand the importance of semi-natural linear landscape elements such as road-verges, in maintaining the connectivity between habitat fragments of highly modified landscapes and preserving the specialist fauna of arthropods in Great Hungarian Plain.

Our sampling was conducted in forest-steppe of Kiskunság region between Tisza and Danube interfluve. Sampling included, arthropods like spiders, true-bugs, ants, carabid beetles and plants and their identification to species level. We employ various statistical methods like, counting species richness and abundance, linear mix models, beta diversity indices, multivariate scaling and functional diversity according to our questions or hypothesis.

We found that increasing forest fragment size, forest habitat amount and forest edge length (local and landscape attributes) had in general positive effects on forest species, but negative on open-habitat species varying a bit among the studied taxa. Secondly, landscape quality and habitat type drive the diversity pattern of forest-steppe fragments and lastly, road-verges act as important secondary habitats for grassland species.

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Uncovering the mitotic interactome of protein phosphatase 4

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Protein phosphatase 4 (PP4) is an essential PP2A-like Ser/Thr phosphatase that control different cellular events, such as DNA repair, differentiation, apoptosis, cell cycle and various signaling processes. However, its role in the regulation of mitosis is poorly known. To better understand this, we aimed to uncover its mitotic interactome and define how PP4 bind its substrate proteins.

To achieve this, we generated transgenic flies overexpressing different functional domains of the regulatory R3 subunit (Falafel) of PP4, namely the EVH1 and the Smk-1 domains. We collected early syncytial embryos from these flies and performed an affinity purification followed by mass spectrometric analysis. With this approach, we were able to identify several possible novel mitotic interaction partners of PP4. We validated the real physical interaction partners with *in vitro* binding experiments, using GST-tagged EVH1 or Smk-1 as bait proteins and ^{35}S-methionine labelled *in vitro* produced prey proteins, respectively. After verifying the novel binding partners (potential substrates), we narrowed down the interacting surfaces in

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order to define the substrate binding consensus motif of PP4. We found two motifs for the EVH1 domain, which could present in multiple copies within the partner proteins. With site specific mutagenesis we identified the exact residues and motifs, which are truly required for the interaction. From the Smk-1 binding partners, only one possesses such a motif, but after *in vitro* and *in vivo* experiments we could declare that the substrate binding of PP4 through the Smk-1 domain is achieved in a completely different, yet undescribed manner. We also found that a conserved leucine in Falafel's EVH1 domain is essential for substrate binding, since disrupting this amino acid would lead to the complete loss of the interaction.

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Non-canonical role of E2FB in auxin transport

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Plant development, organ growth and their shape mainly depend on the regulation and orientation of cell division. Cell number is primarily determined by genetic program, but it also could be influenced by environmental conditions. Plant growth hormone auxin plays an essential role in determining the activity of proliferation, and thus has major impact on the final size and shape of a developing organ. The temporal and spatial activity of cell proliferation is controlled by complex regulatory mechanisms, but their nature is still not fully understood. It is strongly believed that the switch from proliferation to differentiation in plants is regulated by an evolutionarily conserved transcriptional regulatory mechanism called E2F-RB pathway.

In our study we focused on E2FB, one of the activator type E2Fs in *Arabidopsis*. Previously we have seen that ectopic E2FB could stimulate cell proliferation in the absence of growth hormone auxin. That indicates regulatory role for E2FB in auxin-driven cell proliferation. We propose a model where the RBR-free form of E2FB stimulates cell division, while their complex promotes cell cycle exit. How this switch could operate during plant development and whether it depends on auxin is not known yet.

As E2FB is a transcription factor it is expected to be localized in the nucleus. Indeed, we observed E2FB in the nucleus but surprisingly in association with the plasma membrane as well. We were curious whether recruitment of E2FB by the plasma membrane influenced its activity like it was reported on other membrane associated transcription factors (MTF) or E2FB has non-canonical role in the membrane. By analyzing the interacting partners of E2FB we have found membrane associated proteins involved in vesicular trafficking and auxin transport. The auxin efflux carrier protein PIN3 was one of these E2FB interactors. By using transient transfection system E2FB and PIN3 were found to be co-localized at the membrane. In addition, auxin transport enhanced by PIN3 was repressed when PIN3 was co-expressed with E2FB. That strongly suggests that E2FB has non-canonical function by regulating auxin transport in developing organs.

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Assessment of intracellular singlet oxygen by GFP fluorescence in *Synechocystis* PCC6803

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Singlet oxygen ($^1O_2$) is a very important reactive oxygen species (ROS), it can damage a wide range of macromolecules, like lipids, carotenoids and proteins. Its formation takes place in the second photosynthetic reaction centre (PSII), during the photosynthetic reactions. In high light condition the generated triplet state chlorophyll can interact with the molecular oxygen leading to $^1O_2$ formation via energy transfer.

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This highly reactive ROS, besides its degradation effects can take part in signal transduction mechanisms and other intracellular reactions. This importance is the reason why we investigate the $^1O_2$ intracellular mechanisms. There are various $^1O_2$ detection methods, such as His mediated O_2 uptake, which allows calculating the rate of $^1O_2$ generation by the rate of O_2 consumption. However, still there is a lack in detection methods that could be used to detect the spatial distribution of $^1O_2$ generation inside intact cyanobacterial cells.

The Green Fluorescent Protein (GFP) is a very commonly used reporter protein in biological research nowadays. $^1O_2$ can damage this protein, hence quenching its fluorescence. We treated GFP producing *Synechocystis* PCC6803 cyanobacterial mutant cells with the $^1O_2$ sensitizer Rose bengal (Rb) and Methylene blue (Mb) dyes under high light conditions. We observed that the GFP fluorescence decreased suggesting that $^1O_2$ mediated degradation of GFP can be utilized for *in vivo* $^1O_2$ detection.

We investigated the specificity and sensitivity of the quenching reaction and established experimental parameters for a widely applicable *in vivo* $^1O_2$ assessment method protocol.

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Synergistic radiosensitization of gold nanoparticles and the histone-deacetylase inhibitor SAHA on 2D and 3D cancer cells

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Anti-cancer agents applied in traditional chemotherapy have often just moderate success rate and exhibit low specificity during clinical cancer treatment. Chemotherapy is mostly accomplished in multimodal manner, i.e. in combination with other treatment modalities, like radiotherapy. Radiosensitizing agents are frequently utilized upon irradiation, since these compounds are capable to increase the efficiency and decrease the severe side effects of cancer therapy exerted by irradiation and help to attenuate the disadvantageous consequences of radiation treatments on healthy tissues.

Our aim was to investigate the radiosensitizing capability and the efficiency of gold nanoparticles (AuNPs) and of a histone-deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) in combination on 2D and 3D cell cultures of A549 lung adenocarcinoma, DU-145 and PC-3 prostate cancer and MCF-7 breast cancer cell lines.

According to our experiments, treatment of cancer cells with SAHA results in a high level of acetylated histones, which feature was not affected by the presence of AuNPs when combinational treatments were applied. Thus, we can conclude that AuNPs do not influence the histone-deacetylase-inhibiting activity of SAHA. The AuNP and SAHA combinational treatment synergistically decreased the viability of A549, DU-145, PC-3 and MCF-7 cells after 2 Gy irradiation. Moreover, the colony forming capabilities of cancer cells were significantly diminished upon irradiation with 2 Gy and 4 Gy doses. The amount of irradiation-provoked DNA double strand breaks was the highest in the AuNP and SAHA double treated cells, which we have visualized by γH2AX immunostaining. Significantly more γH2AX positive cells and γH2AX foci were counted after histone deacetylase inhibitor and nanoparticle combinational treatments compared to the untreated and to the AuNP- or SAHA-treated cells after 2 Gy irradiation. After studying the effect of AuNP, SAHA and their combination after irradiation on 2D cell cultures, we investigated the impact of individual and double treatments on 3D cell cultures as well. In 3D structure - as in *in vivo* tumors - the cells often manifest increased radio-, and drug-resistance due to integrin-mediated pathways. Following combinational treatments, the number of colonies formed by living cells in 3D spheroids was significantly reduced after irradiation with 2 and 4 Gy doses, moreover, significantly more γH2AX-positive cells were counted after 2 Gy irradiation in the AuNP+SAHA-treated samples than in the control or in cancer cells receiving individual treatments.

Our results suggest that inhibition of deacetylase activity by SAHA leads to a more relaxed chromatin structure, which increases the DNA damaging effect of ionizing radiation. Furthermore, irradiation can provoke the release of reactive electrons from AuNPs, causing substantial amount of DNA double strand breaks, which leads to enhanced cancer cell death.

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Cellular and network mechanisms of physiological and pathophysiological brain states

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In the absence of sensory input, the mammalian brain exhibits a wide array of structured brain state dependent spontaneous activity as happens during relaxed wakefulness, sleep and epilepsy. In cortical areas brain state dependent neuronal activity is determined by both intrinsic and thalamocortical network interactions fine-tuned by neuromodulation.

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and the Ih current are one of the major intrinsic drivers of thalamic oscillations, but their role in generating spike and wave discharges of absence epilepsy has remained elusive. We investigated whether reducing the expression of HCN channels in the ventrobasal thalamus (VB) using shRNA could suppress absence seizures (AS). Control and Stargazer mice were injected with either HCN-targeting or non-targeting shRNA before recording. Using patch clamp recordings, we examined the electrophysiological properties of VB neurons in slices. Our data demonstrate that our HCN-targeting shRNA does selectively affect Ih-dependent membrane properties of VB neurons without altering other neuronal properties. At the end of experiments, we did immunostaining. Our data indicate that HCN-targeting shRNA-infected mice VB neurons had a low HCN immunoreactivity compared to non-targeting shRNA-infected mice. Our finding provides that block of VB neuron HCN channels prevents AS.

In addition to intrinsic properties of thalamic neurons thalamocortical states areal so profoundly influenced by the lateral hypothalamus, a brain area involved in arousal and energy control but whether and how the LH can impact the activity of neuromodulatory circuits and the downstream consequences of this modulation have remained elusive. Using a combination of anterograde and retrograde viral tracing, optogenetics, in vitro and in vivo electrophysiology we investigated the effect of LH on one of the major neuromodulatory systems. The serotonergic system implicated in many (patho)physiological functions like the regulation of brain states, mood, reward and sensory processing. Our results show that LH GABAergic projections promote arousal from NREM but not REM sleep by selectively inhibiting DRN GABAergic neurons via GABAA receptors resulting in a prominent disinhibition of DRN output neurons. Our results identify a novel long-range inhibitory projection implicated in the control of serotonergic neuromodulation and arousal.

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Characterization of plant growth-promoting activities of endophytic fungi isolated from *Sophora flavescens*.

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Several studies of endophytic fungi indicated that they are excellent producers of compounds that can be exploited for agrochemical or medicinal purposes due to their biological activity. Endophytes are beneficial to the host plants in terms of production of plant growth regulating hormones, solubilisation of minerals and their antagonistic behaviour against plant pathogens and pests. The mineral solubilisation is the indirect way for the plant growth promotion and as the direct way of the promotion, it was demonstrated that endophytic fungi can produce phytohormones, such as gibberellins and indole acetic acid, which are able to improve plant growth and reduce the adverse impacts of abiotic stresses.

The indirect plant promoting activities of the isolates were examined including phosphate mobilization and siderophore productions. It could be concluded that the siderophore production is more common within the examined isolated fungal endophytes than the phosphate solubilisation. Almost all produced siderophores in a measurable amount except for SZMC 26657 strain, while only 5 showed phosphate mobilization activities. Regarding to the IAA plate assay, each isolate proved to

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produce IAA, from which 7 strains showed production only in the presence of Trp and 2 only in the absence of Trp as well as 6 in both cultivation conditions. Therefore, 4 strains the SZMC 26659, 26661, 26651 and 26648 were positive for those three plant growth-promoting assays. The IAA production of the isolates in the ferment broth ranged from 0.02 to 1.2 μg/mL and from 0.1 to 16.0 μg/mL, in the absence and presence of Trp, respectively. Furthermore, chosen 6 isolates tested on their potential for plant growth-promoting activity on plant assay. As a consequence, the crude fungal IAA of the endophytes at concentrations of 0.1 and 1 μg/mL promoted the elongation of the lengths of all *Arabidopsis* roots significantly and the biomasses of plants were reduced due to the treatment of the fungal extracts, while the accumulation of photosynthetic pigments were increased only certain cases. Therefore, 15 isolates represented the genera of *Alternaria*, *Didymella*, *Fusarium* and *Xylogone*, which has not been previously reported as the fungal endophytes of *S. flavescens*.

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NGS-based method for the investigation of homologous recombination repair and for the increase of genomic integration efficiency

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The integrity of the cell's genome is indispensable for its proper function. Reactive oxygen species, ionizing and UV radiation, or chemotherapeutic agents can cause double-strand DNA breaks (DSBs) that jeopardize genome stability, as they lead to chromosome rearrangements and possibly to cell death. Consequently, living cells' DNA is inherently unstable. An extensive network of pathways has evolved for the repair of DNA damage. During the repair of DSBs, two pathways play a major role: non-homologous end joining (NHEJ) and homologous recombination (HR). Both pathways function in eukaryotic cells, but their relative contribution to damage repair as well as the speed of the process and the outcome is variable. While for NHEJ a homologous template is not required, it is essential for HR. NHEJ potentially creates indels, so it can lead to mutations; in contrast, HR is an error-free pathway. For the fast and easy examination of different known and unknown genes that can influence HR or NHEJ events, the establishment of a precise, versatile method is needed.

Therefore, the aim of the project is to establish a system through which the events of double-strand break repair (DSBR) can be analyzed at the nucleotide level by next-generation sequencing. Our goal is to increase the number of HR-related repair events and to maximize the length of the DNA that can be integrated into the genome via HR. We plan to monitor the repair of artificial DSBs induced by the CrispR-Cas9 system, through silencing and/or overexpression of putative HR/NHEJ-related genes. Changes in the ratio of these processes will be measured, and possible new members will be identified. Then, the effect of DNA length on integration into the host cell's genome through HR will be examined. The aim is to maximize the length of the donor DNA as well as the ratio of its HR-based integration. Through this project, we will have a deeper insight into the DSBR process, which may lead to more efficient genome editing procedures.

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Rapid decline of bacterial drug-resistance in an antibiotic-free environment through phenotypic reversion

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Antibiotic resistance typically induces a fitness cost that shapes the fate of antibiotic-resistant bacterial populations. However, the cost of resistance can be mitigated by compensatory mutations elsewhere in the genome, and therefore the loss of

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resistance may proceed too slowly to be of practical importance. We present our study on the efficacy and phenotypic impact of compensatory evolution in *Escherichia coli* strains carrying multiple resistance mutations. We have demonstrated that drug-resistance frequently declines within 480 generations during exposure to an antibiotic-free environment. The extent of resistance loss was found to be generally antibiotic-specific, driven by mutations that reduce both resistance level and fitness costs of antibiotic-resistance mutations. We conclude that phenotypic reversion to the antibiotic-sensitive state can be mediated by the acquisition of additional mutations, while maintaining the original resistance mutations. Detailed genetic analysis of the mar regulon also supports the phenotypic reversion hypothesis. MarR is a transcriptional regulatory protein that controls the activity of the mar regulon in *E. coli* through the repression of marA. The mar regulon participates in controlling several genes involved in antibiotic-resistance, including the AcrA/AcrB/TolC multidrug-efflux system. In response to antibiotic stresses (e.g. doxycycline or ciprofloxacin), marR is regularly mutated both in clinical and in laboratory settings, leading to increased expression of marA and other members of the mar regulon. This resistance mutation has an associated fitness cost, promoting the accumulation of further mutations. Our study indicates that this can be achieved by a compensatory mutation in the promoter region of the mar operon. This compensatory mutation increases bacterial fitness, susceptibility to multiple antibiotics alike, and restores wild-type-like membrane permeability, probably through changing the activity of the mar regulon.

Our study indicates that restricting antimicrobial usage could be a useful policy, but for certain antibiotics only.

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Investigations into the fly population of a white button mushroom (*Agaricus bisporus*) growing house in Hungary

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In Hungary over 30 000 tons of white button mushroom (*Agaricus bisporus*) is produced each year. Besides being the most popular cultivated mushroom in our country, it dominates the European and American mushroom industry. Producers face everyday challenges when it comes to protecting their crops from different pathogens. The most harmful fungal diseases of white button mushroom are green mould, dry bubble, wet bubble and cobweb, while further important pathogens involve various bacteria and viruses. Pests, such as certain flies, nematodes and mites also contribute to crop losses, either directly by crop consumption or indirectly, by acting as vectors in transmitting different diseases. Flies are very motile and could change their position fast by flying or walking on the surface of the mushroom growing material. As they move, spores, bacteria and mites might be carried on their bodies, thereby spreading them within and between mushroom growing houses.

Information about the mushroom fly populations in our country is very limited. A Hungarian mushroom growing facility, producing more than 7000 tons of white button mushrooms yearly was investigated in terms of fly populations over a 3-year period. In 2017, 2018 and 2019 deceased flies were collected mushroom growing houses, examined by microscopy and identified at the species level by the PCR amplification and sequence analysis of a fragment of the COI (cytochrome oxidase I) gene. Our results revealed the presence of members of the Sciaridae family: *Lycoriella sativae*, *L. castanescens*, *Bradysia tilicola* and *B. vagans*. In the year 2019, *Megaselia halterata*, belonging to the Phoridae family was also detected.

To investigate the attractant and repellent properties of plant essential oils to mushroom flies, experiments were designed and conducted in the same years. Fly traps containing essential oils suspended in water or beer were kept in the mushroom growing houses for 5 days. Lemon, thyme and citronella proved to be repellents while beer, cinnamon, and in most cases essential oils suspended in beer had attractant properties.

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Towards the biological control of devastating forest pathogens from the genus *Armillaria*

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Armillaria species are among the economically most relevant soilborne tree pathogens causing devastating root diseases worldwide. Biocontrol agents are environment-friendly alternatives to chemicals in restraining the spread of *Armillaria* in forest soils. *Trichoderma* species may efficiently employ diverse antagonistic mechanisms against fungal plant pathogens. The aim of this paper is to isolate indigenous *Trichoderma* strains from healthy and *Armillaria*-damaged forests, characterize them, screen their biocontrol properties, and test selected strains under field conditions.

Armillaria and *Trichoderma* isolates were collected from soil samples of damaged Hungarian oak and healthy Austrian spruce forests and identified to the species level. In vitro antagonism experiments were performed to determine the potential of the *Trichoderma* isolates to control *Armillaria* species. Selected biocontrol candidates were screened for extracellular enzyme production and plant growth-promoting traits. A field experiment was carried out by applying two selected *Trichoderma* strains on two-year-old European Turkey oak seedlings planted in a forest area heavily overtaken by the rhizomorphs of numerous *Armillaria* colonies.

Although *A. cepistipes* and *A. ostoyae* were found in the Austrian spruce forests, *A. mellea* and *A. gallica* clones dominated the Hungarian oak stand. A total of 64 *Trichoderma* isolates belonging to 14 species were recovered. Several *Trichoderma* strains exhibited in vitro antagonistic abilities towards *Armillaria* species and produced siderophores and indole-3-acetic acid. Oak seedlings treated with *T. virens* and *T. atrobrunneum* displayed better survival under harsh soil conditions than the untreated controls. Conclusions: Selected native *Trichoderma* strains, associated with *Armillaria* rhizomorphs, which may also have plant growth promoting properties, are potential antagonists of *Armillaria* spp., and such abilities can be exploited in the biological control of *Armillaria* root rot.

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Linear scale up of aflatoxin separation by centrifugal partition chromatography

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Mycotoxins are the secondary metabolites produced by certain filamentous fungi. Within these toxic compounds, aflatoxins are playing an outstanding role due to their high-level toxicity, which causes remarkable problems in food industry and agriculture. Plenty of methods are available for monitoring or measuring these compounds from various matrices, which requires relatively high amounts of pure aflatoxins.

Liquid-liquid chromatography, which is based on a distribution of components between two phases in a biphasic solvent system, could serve solutions in their purifications. One of the technical implementations of this technique is Centrifugal Partition Chromatography (CPC), which is the hydrostatic version of countercurrent chromatography.

Aspergillus parasiticus (SZMC 2473) was cultivated on a complete malt broth. The four main aflatoxins (AFB1, AFB2, AFG1 and AFG2) were extracted from the fermentation material. In order to have the compounds purified, a 75-minute-long method was developed and applied for the separation and purification of the aflatoxins on a 250 ml laboratory scale CPC column. The stationary phase was the lower phase of the toluene/acetic acid/water = 30/24/50 ternary system, while the elution was carried out with the organic phase. With this method, from 4,5 l fermentation material a total of 1350 mg aflatoxins could be purified with the lowest purity of 92% and with the recovery of 96%.

In order to maximize the amount of pure aflatoxins, the capacity of the 250 ml CPC column was tested and a linear scale up was achieved to a 1000 ml CPC column with the same ternary system. Several concentrations of the desired compounds were injected to both columns, to achieve the maximal capacity of the system. During the scale up, the flow rate and the injec-

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tion volume was linearly increased (quadruplicated). The same stationary/mobile phase ratio was set on the one litre column as well as on the smaller one before. Because the 1 litre column is bigger, to achieve the same gravitational field the rotation speed of the column had to be slower. With the increased application the amount of purified aflatoxins are expected to be more than quadruplicated.

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Bacterial biocontrol of *Armillaria* root rot

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The genus *Armillaria* is among the most important fungal root rot pathogens. The genus includes at least 40 species, with five morphological species being present in Europe. Members of the genus show diverse lifestyles ranging from saprotrophs to devastating tree pathogens. They cause root rot disease in both gymno- and angiosperms in more than 500 host plant species across the world.

Plant-associated biocontrol agents (BCAs) have important roles in plant growth and health. Direct plant growth promotion by microorganisms is based on improved nutrient acquisition and hormonal stimulation. Diverse mechanisms are involved in the suppression of plant pathogens which is often indirectly connected with plant growth. Bacterial BCAs may be promising alternatives to chemical pesticides for controlling *Armillaria* root rot. This study aimed the selection and characterization of potential bacterial BCAs for the control of *Armillaria* root rot. The examined BCAs (2 *Bacillus*, 11 *Paenibacillus* and 29 *Pseudomonas* strains) derived from the rhizosphere of infected trees with high presence of the fungal pathogen. The isolates were screened for plant growth promoting properties. During the experiments we examined the siderophore production with the chrome azurol S agar plate method, the phosphate mobilization on Pikovskaya agar plate method, the extracellular enzyme activities and the indole-3-acetic acid (IAA) production with colorimetric methods. Bacterial isolates were screened for their antagonistic abilities against *Armillaria* isolates *in vitro* using dual-culture confrontation test. In addition, to understand the mechanism of antagonism, the samples were examined by scanning electron microscopy (SEM). The plant growth promotion abilities of the above-mentioned BCA strains on conifer seeds were also investigated.

Most of the examined bacteria could inhibit the growth and the rhizomorph formation of all investigated *Armillaria* strains, furthermore, they were able to mobilize phosphorous and to produce siderophore, IAA and extracellular enzymes.

Based on the results, *Paenibacillus* and *Pseudomonas* strains have beneficial properties which could be exploited during the biological control of *Armillaria* root rot.

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Mass spectrometric analysis of the human urinary O-glycoproteome

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Glycosylation is among the most complex protein post-translational modifications (PTM). Its biological roles are very diverse, ranging from the controlling of protein structure to immunomodulatory functions. Our group focuses on the analysis of mucin-type O-glycosylation which is the most commonly occurring O-glycosylation type on secreted and membrane-bound proteins. Its role has been implicated in receptor activation, leukocyte extravasation and different types of cancer metastasis. All this gathered knowledge on mucin-type O-glycosylation fueled our curiosity to investigate this type of PTM in human urine.

We analyzed three urine samples from healthy donors, three samples from donors that were diagnosed with superficial

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bladder cancer and three samples from donors suffering from advanced bladder cancer. After sample preparation and mass spectrometric measurement we analyzed the data carefully with two aims. Our main goal was to determine which proteins are O-glycosylated with what type of O-glycans in urine. However, to answer this question, first we had to develop a data interpretation method that uses multiple sources of mass spectrometric information.

With the data interpretation method developed we were able to identify multiple O-glycan structures that were linked to specific proteins for the first time. With this information in hand we can compare the O-glycosylation pattern of urinary proteins from healthy and diseased individuals to find some O-glycans that may serve as biomarker candidates for bladder cancer.

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Large- and small-scale environmental factors drive distributions of ant mound size across a latitudinal gradient

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Red wood ants are keystone species of forest habitats in Europe, as being true ecosystem engineers. In their nest surroundings they affect many habitat characteristics, but also the occurrence and distribution of several plant and animal species. On the other hand, several environmental factors and habitat characteristics can affect the size of their nest mounds, an important trait being in relation to the colony size and its well-being. However, we still lack information about the distribution and environmental requirements of this species in Central Europe, mostly in the light of climate change.

In this study, we investigated the effect of large- (latitude and altitude) and small-scale environmental factors (e.g., characteristics of the forest) on the mean size of nest mounds of *Formica polyctena* in the lowland, transition and mountain areas of Central Europe (altogether 12 regions). We predicted that the change in the mound size is in accordance with the Bergmann's rule that states that the body size of endotherm animals changes along with latitude and/or altitude.

We found that the size of the mounds increased along the studied latitudinal gradient, being in accordance with Bergmann's rule. Altitude, on the other hand, did not have any effect on the size of the mounds. The irradiation was the most important environmental factor responsible for the changes in mound size, but temperature, and small-scale factors, like the diameter of the trees and their distance from the nest, were also involved.

Considering our results in the light of the global climate change, we can better understand the long-term effects and consequences of the changing environmental factors on this ecologically important ant group. This knowledge can help decision-makers and forest managers to plan forest management tactics in concordance with the assurance of the long-term survival of red wood ants.

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The effect of tight junction modulator peptide PN159 and sodium bicarbonate on human bronchial epithelial cells expressing wild-type and mutant CFTR channel

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Cystic fibrosis (CF) is a genetic disorder, caused by the mutation of the gene for cystic fibrosis transmembrane conductance regulator (CFTR) protein. The loss of CFTR function leads to dramatically impaired ion (HCO_3^-, Cl^-) and fluid movement, which produces the respiratory abnormalities that characterize CF. While the role of chloride is widely investigated, less is known about bicarbonate. Empirical observations indicate the clinical usefulness of inhaled sodium bicarbonate as an adjuvant

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therapy in CF due to its bacteriostatic and mucosolvent properties, but its direct effect has not been studied on respiratory epithelial cells. Our aim was to (i) establish and characterize co-culture models of human CF bronchial epithelial (CFBE) cell lines expressing wild-type (WT) or mutant (ΔF508) CFTR channel with human vascular endothelial cells, (ii) to investigate the response of the CFBE cell line pair to the cell penetrating and tight junction modulator peptide PN159; and (iii) to investigate the effects of sodium bicarbonate on these bronchial epithelial cells.

Treatment of the cells with PN159 peptide for 30 minutes caused the full opening of the barrier showed by the lower resistance-, higher permeability values and the changes in immunostaining of junctional proteins. However, this opening effect was reversible after 24-hour recovery. These data indicate that PN159 peptide efficiently and reversibly opened the junctions in CFBE cells similarly to our previous studies on models of the intestinal epithelium and the blood-brain barrier. There were no differences between the reaction of the CFBE cell line pair to the peptide.

The presence of vascular endothelial cells induced tighter barrier properties in bronchial epithelial cells: the resistance was higher and permeability values were lower. Sodium bicarbonate treatment slightly reduced the viability of WT-CFBE cells but not that of the mutant CFBE cells. Sodium bicarbonate (100 mM) significantly decreased the more alkaline intracellular pH of the mutant CFBE cells to the level of WT-CFTR CFBE cells, while the barrier properties of the models were only minimally changed by the treatment. Our observations indicate that sodium bicarbonate is beneficial to mutant CFBE cells suggesting that it may have a therapeutic effect on bronchial epithelium in CF.

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The role of heat shock protein 27 in the regulation of neuroinflammation

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Hsp27 is a member of the small heat-shock protein family and has an important role in the maintenance of normal cellular protein homeostasis, which is disturbed in case of inflammation. Neuroinflammation has been described as a common characteristic of most brain diseases, however, it is crucial for both neuronal tissue damage and healing following CNS injury. According to earlier results, Hsp27 can regulate the release of various inflammatory factors, proposing that it is involved in the regulation of this immune response, but its exact role is still poorly understood.

Therefore, we investigated the role of Hsp27 in acute neuroinflammatory processes in an Hsp27-overexpressing transgenic mouse model. We treated 7 day-old wild-type and Hsp27 transgenic mice with ethanol to induce neuroinflammation. Following ethanol treatment, the gene expression level of certain inflammation-related cytokines was significantly higher in Hsp27 transgenic mice compared to wild-type ones. The enhanced cytokine production was accompanied by apoptosis and morphological changes of microglia, followed by astrogliosis. The level of gliosis was also higher in certain brain regions of the transgenic animals compared to those of wild-type littermates, whereas Hsp27 overexpression slightly moderated the level of apoptosis. We also established primary astrocyte, microglia, and neuronal cell cultures from wild-type and Hsp27 transgenic animals, which were subjected to ethanol and cytokine treatment. After treatments, we quantified the concentrations of released Hsp27 and TNFα in the supernatants as well as the level of Hsp27 within the cells. We could not detect Hsp27 in the supernatants of any of the cell cultures, which suggests that intracellular Hsp27 was responsible for the immunoregulatory effects. Astrocytes showed the highest Hsp27 expression under inflammatory conditions, but in neurons it was also upregulated. Interestingly, microglia from transgenic animals released a significantly higher amount of TNFα compared to wild-type microglia in response to cytokine treatment, although it did not show Hsp27 expression. We assume that the remaining transgenic astrocytes in the primary microglia culture could be responsible for this, as these glial cells can activate each-other in several ways. Taken together, Hsp27 overexpression resulted in a more intense inflammation, but it also protected against apoptosis, proposing a balancing role for Hsp27 in neuroinflammation.

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The role of Ring1 and YY1 binding protein in the retinoic acid signalling pathway regulation during neural differentiation of mouse embryonic stem cells

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Ring1 and Yy1 Binding Protein (RYBP) is a core member of the non-canonical Polycomb Repressive Complexes 1 (ncPRC1s), which are essential epigenetic regulators of cell identity. We have previously reported that RYBP is important for central nervous system (CNS) development in mice and that *Rybp null* mutant (*Rybp*$^{-/-}$) mouse embryonic stem (ES) cells has an impaired differentiation ability in vitro as they form more progenitor and less terminally differentiated neural cells. Moreover, the increased neural progenitor pool formation was linked with the elevated level of the neural progenitor marker gene *Pax6* (Paired Box 6).

Since *Pax6* stands under the regulation of the retinoic acid (RA) signalling pathway, I have investigated whether *Rybp* can regulate the RA pathway with special focus on *Pax6* during the time curse of *in vitro* neural differentiation of mouse ES cells. The results showed that all the examined RA signaling pathway members were overexpressed in the *Rybp*$^{-/-}$ neural cultures and the rate of neural progenitor formation in the *Rybp null* mutants after RA induction was increased. By utilising luciferase reporter assays, we also demonstrated that RYBP was able to repress the *Pax6* gene through its *P1* promoter, and that the repression is attenuated when the other ncPRC1 member, Ring Finger Protein 1 (RING1) was present, suggesting that RYBP repress *Pax6* in a Polycomb dependent manner, via the ncPRC1. We also demonstrated that there were an increased rate of apoptosis and necrosis in the neural cultures when *Rybp* was absent. *Rybp* can function both as a pro and anti-apoptotic gene, depending on the given cell type and context. Our results suggest that *Rybp* exerts an anti-apoptotic function during neural differentiation and that in the lack of *Rybp* neural progenitors are prone to die, which is likely lead to the impaired terminal differentiation.

Taken together, in my PhD work I demonstrated that RYBP regulate the gene expression of *Pax6* via ncPRC1, and in the lack of *Rybp* the RA pathway increased, due to cells undergo apoptosis and necrosis, which can be one of the causatives of the impaired terminal differentiation towards neuronal lineages from progenitors.

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Expansion of genetic resources of national maize breeding materials with innovative methods

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The importance of corn in the world economy is growing year by year: The growing population of the Earth requires more and more food produced on continuously decreasing agricultural lands. Extensive production is being replaced by intensive crop production that is dependent on highly productive hybrids. In order to maintain a competitive status for maize among crops there is a need for adaptive potential to intensive cultivation systems and to extreme environmental conditions generated by climate change. These requirements can be interpreted in terms of genetic background, insuring adaptation to weather and soil conditions. It is important to note that these changes are not spontaneous in nature and in agriculture, but their result from long and persistent work by breeders.

This dissertation deals with the further development of Hungarian maize lines using the modern, precision breeding techniques, developing future-oriented and competitive technologies. In addition to shortening the time, the goal is to generate genetic variance for special traits as efficiently as possible, regardless of genotype. Having haploid maize plants, our goal was to develop an *in-planta* method for oligonucleotide-directed mutagenesis (ODM) using a recessive, albino mutational marker. By introducing the oligonucleotides into the meristem, we were able to induce a point mutation at a targeted site of the phytoene desaturase gene (PDS) in the genome with strong phenotypic feedback. Our technology development program is currently a pioneer of Hungarian precision maize breeding, and our stated intentions and long-term objectives include the routine application of the methods developed here at the forefront of national maize breeding. Precision breeding methods can provide

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a solution for breeding corn cultivars with yield stability, improved seed quality, efficient use of water and nutrition, or high adaptation capability. It is very important to emphasize that the technologies we use and develop are genotype independent and do not require the incorporation of a foreign gene. Eliminating these two factors is a key goal that has so far been the biggest barrier to molecular breeding of corn.

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Creation of an overexpression strain collection and searching for putative virulence related genes *in Candida parapsilosis*

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C. parapsilosis is a commensal of the human skin, however also recognized as an opportunistic fungal pathogen. It is particularly associated with low birth weight neonatal and nosocomial infections claiming for extensive survey of the virulence factors of *C. parapsilosis*.

We aimed to create and characterize an overexpression (OE) mutant strain collection involving ORFs that are thought to be involved in the pathogenesis of the fungus. We successfully generated 37 barcoded OE mutant strains by using Thermo Fisher Gateway™ cloning method.

Six mutants showed growth disabilities in the presence of cell wall or membrane perturbants, like SDS, Caffeine, Calcofluor White and Congo Red in comparison with the control. Besides that, altered level of phagocytosis was established in the case of six OE strains, respectively. One mutant strain was less effective in forming a biofilm compared to the control. Three mutants were found to be more virulent than the control strain, while one mutant hasn't got any effect to the mortality of the larvae in *G. mellonella* model. We selected two strains (one with lower and another one with higher killing efficiency in insect model) for mice infection experiments in order to determine the fungal burden in the brain, spleen, liver and kidneys. Both mutants showed higher fungal burden in the brain, and lower fungal burden in the spleen. Only one mutant showed increased and decreased CFU levels in the kidney and the liver. In conclusion out of the 37 mutants we had generated, 11 showed alteration in at least one assay. Two of these (OE_CPAR2_109520, OE_CPAR2_302400) showed difference in their virulence compared to the control strain. The ortholog of CPAR2_109520 in *C. albicans* is *TUP1*, a transcriptional corepressor involved in filamentous growth, while CPAR2_302400 is the ortholog of *MGT1* from *Saccharomyces cerevisiae* that encodes a methyltransferase involved in protection against DNA alkylation damage.

According to our results, several mutants from our OE collection showed a phenotype different from the control under conditions that are thought to impact the pathogenicity of this species.

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A specific acetylation mimicking point mutation of H3.3A ameliorates Huntingtin induced phenotypes in a fly model

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Huntington's disease (HD) is an inherited neurological disorder with fatal consequences. HD is caused by a dominant muta-

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tion – expansion of a CAG trinucleotide repeat – in the huntingtin gene resulting in an expanded polyglutamine domain in the Huntingtin (Htt) protein. Mutant Htt forms intracellular aggregates and abnormal interactions with other proteins, for example histone acetyltransferase (HAT) enzymes. While reduced activity of HATs enhances mutant Huntingtin toxicity, inhibition of histone deacetylases ameliorates HD phenotypes. As disturbed histone acetylation and consequent transcriptional dysregulation might be partially responsible for the manifestation of HD, we aimed to identify epigenetic marks that play a role in this process.

To investigate the effects of specific histone modifications on HD pathogenesis we generated point mutations of H3.3A histone transgenes mimicking different post-translational modifications (PTM) of lysine (K) residues, arginine (R) mimicking unmodified, glutamate (Q) mimicking acetylated, while methionine (M) mimicking methylated lysine (K9R, K9Q, K9M, K14R, K14Q, K27R, K27Q and K27M). We investigated the effects of these transgenes in a *Drosophila* model of HD by analyzing changes in Huntingtin induced phenotypes, such as lethality, early death, neurodegeneration, motor impairments and daily activity.

According to our results altering the acetylation status of K14 lysine has promising effects. K14Q ameliorated, whilst K14R exacerbated HD phenotypes. Using K14Q we observed improvement in all investigated phenotypes such as eclosion rate, lifespan, neuronal survival and climbing ability, moreover hyperactivity observed in HD flies was decreased. Our findings suggest H3K14 as potential therapeutic target in HD.

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RAD18-dependent activation of FAN1 by PCNA to rescue DNA inter-strand crosslinks

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The Rad18 DNA damage tolerance (DDT) pathway can resolve stalled replication forks during DNA replication. Upon DNA lesion, PCNA acts as central hub, which can be monoubiquitylated by Rad18/Rad6, and translesion synthesis (TLS) polymerases are recruited by the monoubiquitinated PCNA. The Fanconi anaemia (FA) pathway is one of the main processes responsible for the repair of DNA interstrand crosslinks (ICL) at the S/G2 cell cycle checkpoint. In the FA pathway, FANCD2/FANCI-associated nuclease 1 (FAN1) can be activated via its UBZ domain, and it can digest the ICL-neighbouring region due to its structure-specific endonuclease activity thus facilitating the bypass of the lesion. Surprisingly, depletion of FAN1 affects neither ICL-induced double-strand DNA break formation nor leads to the development of FA. Rather, germline FAN1 mutations cause caryomegalic interstitial nephritis. FAN1 also contains an uncharacterized PIP domain. DNA repair proteins are often recruited to specific sites of DNA damage through protein-protein interactions. Therefore, the research purpose of this study is to ask whether FAN1 interacts with ub-PCNA via its PIP domain or UBZ domain. We are also going to illustrate the role of Fan1 in the Rad18 DDT pathway. Since the recruitment of TLS polymerase eta (Pol η) is the key step to repair DNA damage, we are going to examine the crosstalk between FAN1 and Pol η.

Previously, we proved that FAN1 is indeed regulated by the Rad 18 DDT pathway *in vivo* and colocalizes with PCNA. In our current study, FAN1 was expressed in yeast with an engineered N-terminal GST tag and purified. I demonstrated *in vitro* that the nuclease activity of FAN1 can be enhanced by PCNA. The PIP-domain-mutated FAN1 shows deficient nuclease activity in the presence of PCNA, which indicates that PCNA can stimulate the nuclease activity of FAN1 via its PIP domain. I could also demonstrate that the digestion specificity of FAN1 could be altered in the presence of PCNA. PCNA is able to position FAN1 to cut a specific site on the 5' flap substrate. I synthesized a DNA molecule mimicking an ICL replication fork substrate, which FAN1 is able to digest in a nuclease assay, due to PCNA, and the gap is filled by Pol η specifically, not by other TLS polymerases (Pol ι and Pol κ). To sum up, our current results indicate that FAN1 does not only play a role in the FA pathway, but it also participates in the Rad6/Rad18 DDT pathway upon stalled replication. FAN1 might be a crosstalk protein, which regulates these two pathways in different cell cycles.

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Analyzing the importance of ubiquitin-dependent selective autophagy in *Drosophila*

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Autophagy is an evolutionarily conserved intracellular degradation process of cellular self-eating and the major pathway for degradation of cytoplasmic material by the lysosomal machinery. Selective autophagy receptors can recognize ubiquitinated aggregates and bind to Atg8/LC3 proteins to ensure the capture of cargo into autophagosomes. In *Drosophila*, only one autophagy receptor for ubiquitinated protein aggregates is known: p62/Ref(2)P. p62 possesses a C-terminal ubiquitin-binding domain (UBA), an N-terminal PB1 domain to mediate aggregate formation, and a LIR (LC3-interacting region) motif in an unstructured region, which is responsible for LC3/Atg8a binding on autophagic membranes.

To investigate the role of p62/Ref(2)P, we replaced two previously characterized key amino acids within the LIR motif: a tryptophan and an isoleucine to alanines by editing the endogenous gene using CRISPR. We generated *Drosophila* lines carrying a p62-LIR mutation, which disrupted the autophagic degradation of p62 and ubiquitinated cargo.

Flies homozygous for the mutation are viable and fertile, and accumulation of p62 and ubiquitinated protein aggregates was observed in various tissues in homozygous and heterozygous animals. We observed age-dependent accumulation of the p62/Ref(2)P, with an increased accumulation in older flies. The LIR mutants had a similar lifespan as WT flies under normal conditions and during complete starvation. To test their oxidative stress tolerance, we fed LIR mutant and control flies with agents that induce oxidative stress (e.g., paraquat). Interestingly, LIR mutant flies are less sensitive to paraquat treatment, and express higher levels of oxidative stress response genes like glutathione-S-transferase. LIR-mutant flies have fewer mitochondria containing reactive oxygen species, while basal mitochondrial degradation by mitophagy remains similar. However, if mitophagy is induced by an iron chelator, we observe a mitophagy defect in these flies. This leads us to think that there are other mitophagy receptors in flies independent of mitochondrial ubiquitination that function under basal conditions. Lastly, autophagy was earlier shown to be critical for neuronal function, supposedly via the continuous selective turnover of ubiquitinated cargo. Accordingly, *Atg* mutant animals show developmental delay, increased stress sensitivity, ataxia, neurodegeneration, and as a consequence, shortened lifespan. None of these phenotypes are due to the loss of selective ubiquitin-dependent autophagy based on analyses of our p62-LIR mutants.

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Analysis of genes encoding possible spore coat-like proteins in opportunistic human pathogenic fungi

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Members of the order Mucorales can be agents of life-threatening, opportunistic human infections, known as mucormycosis. Because of the devastating outcome of this disease, which has been observed despite the current treatment options, it is urgent to identify the possible virulence factors. CotH proteins are widely present in Mucorales and previous studies showed the importance of one of these proteins in the pathogenicity of this fungal group.

A new type of spore coat-like protein family and represents five possible spore-coat proteins encoding genes (i.e. *cotH1-cotH5*) of the β-carotene producing filamentous fungus, *M. circinelloides* disrupted individually by a recently developed *in vitro* plasmid-free CRISPR-Cas9 method. Pathogenicity of the *cotH3*, *cotH4*, and *cotH5* gene disrupted and *cotH4* gene complemented mutants was tested in *D. melanogaster* and MS12-*ΔcotH3+pyrG* and MS12-*ΔcotH4+pyrG* mutant strains resulted in a lower mortality rate. The MS12- *ΔcotH3+pyrG* and MS12-*ΔcotH4+pyrG* mutant strains exhibited markedly reduced virulence in a murine model. The spore coat of *cotH4* gene disrupted mutant spores presented several deviations from what was observed with the wild-type

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strain: the thickness of the spore wall differs significantly from the control based on our TEM results. Staining with the anionic dye, calcofluor white (CFW) that binds to the chitin in the fungal cell wall, a change the intensity of the dye was observed.

This study was created to assess the importance of possible spore-coat proteins in the biological processes of *M. circinelloides*. Understanding CotH proteins as possible factors in fungal pathogenicity are crucial for developing new antifungal strategies.

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Development of high throughput mass spectrometric methods for the analysis of selected groups of primary and secondary metabolites

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The microbial metabolites could be divided into two groups. The primary metabolites directly involved in normal growth, development and reproduction, while secondary metabolites play role in the other functions of the organisms. From the quantity of primary metabolites different biological processes could study for example the energy level. Meanwhile, the high throughput quantifications of certain secondary metabolites, such as mycotoxins, are crucial for food safety and human health. The high speed and accurate quantification of metabolites simultaneously can be only provided with the novel sophisticated mass spectrometric (MS) techniques such as instruments containing Orbitrap mass analyzers.

In my research work two multiplexing based LC-MS method were developed and validated to the measurements of various metabolites from different biological samples.

In the first method, the primary metabolites of the central biochemical pathway were investigated represented the members of the tricarboxylic acid cycle and related molecules. During this method development, new approaches were applied specialized to multiplex acquisition mode resulting the complete workflow for this type of analyses. It was certified that the method possesses proper sensitivity for quantification of the selected metabolites in various biological matrices. A secondary metabolite, the European Union regulated mycotoxins were also examined applying a multiplexing based LC-MS method. According to the lower limit of quantifications of the tested compounds, the method was sensitive enough for detection of mycotoxins at the levels of their maximum limits in the applied matrices except for HT2 toxin. Furthermore, the performance parameters were also over the limits of the acceptable criteria providing an applicable technique for the routine analysis.

Finally, it could be concluded that the application of multiplex acquisition mode has remarkable advantages and could serve rapid simultaneous quantification possibilities for the defined group of analytes.

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CRISPR/Cas9 and IS element-mediated engineering of prokaryotic genomes

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Phage engineering project: Using phage therapy to fight bacterial infections has a renaissance in the age of rapidly emerging antibiotic-resistant pathogens. However, we have limited knowledge about the cell biology and immunology fundamentals of phage therapy necessitating more investigations using *ex vivo* infection models. *E. coli* K1 is a nosocomial pathogen responsible for urinary tract infections, neonatal meningitis and sepsis. To aid studies concerning the treatment of *E. coli* K1 infections using phage K1F, we have applied the CRISPR/Cas system to engineer K1F phage tagged with GFP. Our collaborators at the University of Warwick used the green fluorescent phages with red fluorescent bacteria to study the phage-mediated eradication of *E. coli* from a urinary epithelial cell culture. Importantly, this research demonstrated that phages can enter urinary epithelial

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cells by phagocytosis independently from bacteria, and K1F phages can kill *E. coli* inside the eukaryotic cells. This result was published in Scientific Reports. Currently, we are working on the deletion of an essential phage gene to make the phages replicable only in hosts that provide this protein in trans. This way, the engineered phages will not amplify in the patient, making the phage count controllable similarly to the dosage of any conventional pharmaceutical.

E. coli engineering project: E. coli is a popular host for the production of valuable metabolites by expression of complete metabolic pathways. Using episomal (plasmid) DNA is convenient but has drawbacks including increased metabolic burden, the requirement for selection in the form of antibiotics, instability and gene copy number variation. Chromosomal integration offers a stable and selection-free alternative to using DNA plasmids for expression of foreign proteins and metabolic pathways. Requirement of landing pads is usually a limiting factor for chromosomal integration. We introduce the use of IS (Insertion sequences) elements as landing pads which are segments of bacterial DNA, able to move within bacterial genome and allow the amplification of transgenes. We applied IS elements to integrate a 7.5 kb long 5 gene operon, responsible for violacein production, into the *E. coli* chromosome. We got success in IS*3* as well as IS*1*-mediated integration of violacein pathway. Further we succeeded in IS3-mediated copy number amplification of violacein pathway. IS*1* mediated amplification experiments are in progress.

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Multidisciplinary research of natural and anthropogenic mummies – Multidisciplinary research of the mummies curated in the Egyptian collection of the Hungarian Natural History Museum

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During history, people wanted to preserve their body mostly for religious reasons for the beyond of afterlife or the future. Nowadays the researchers investigate these bodies with modern technological methods to gain information on the diseases of the past, traumatic injuries, eating habits and general health status of the people lived ones. Our research goal was to conduct the most comprehensive series of studies as many as possible. As the primary consideration was to preserve the remains, especially in case of the fragmentary and incomplete specimens of bad preservation, the sampling numbers were minimal. The biological anthropological investigation methods helped estimate the sex and the age at death of the specimens. The paleopathological studies lighted that the individuals, once lived in Egypt, what kind of diseases suffered from.

The research team has experts from various fields, chemists, physicists, radiologists, molecular biologists and a-DNA specialists, therefore we were able to apply physical, chemical, medical diagnostic methods, imaging technologies (ICP-OES, GC-MS, FAAS, FTIR, X-ray, CT, SEM and Keyence 3D microscope) in the multidisciplinary study.

Mostly non-invasive techniques were used. The invasive technique was applied only in case of the radiocarbon dating; but it was necessary, as the age of some mummies were disputed.

In the new phase of the project, the aim is to identify the mummification process itself and determine the materials used during the mummification process. To ascertain the social status of the mummies and the region of the mummification process organic chemistry analyses were performed. There are specimens form the Gamhud site in Vienna, in Prague and in Warsaw as well. The mummified remains curated in the Kunsthistorisches Museum, Vienna were investigated in 2017 as part of a short study tour. As a reference material, a comparative analysis with an Egyptian mummy collection of the same age in Mannheim, Germany was performed as well.

Future plans include C14 and isotope analysis of the entire Egyptian collection of the Hungarian Natural History Museum and to compare our material to others stored in other foreign collections.

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Drosophila alternative linker histone BigH1 enables rapid nuclear divisions during early development

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Linker histone H1 proteins are essential for the formation of higher-order chromatin structure. Their role is to seal the nucleosome by binding to the entry and exit site of nucleosomal DNA, thus stabilizing the structure. Linker histones have many tissue- and developmental stage-specific variants. In many organisms, the initiation of embryogenesis requires an alternative linker histone, often called oocyte-specific or embryonic linker histone variant. In *Drosophila*, BigH1 has been described as the early embryo-specific alternative linker histone, however its role during embryogenesis remains elusive.

Our work is centred on investigating why BigH1 is required during early development. For this purpose, we generated mutant fly lines in which the coding sequence of BigH1 is exchanged completely or partially with the coding sequence of somatic H1, resulting in domain switches between the two linker histone types. Analysis of the fly lines revealed that somatic H1 can completely replace BigH1 during early embryogenesis at normal temperature, as mutant flies are viable and fertile without developmental defects. We have also discovered that the N-terminal region of BigH1 is required for normal expression of the protein, however the exchange of the N-terminal domain does not impact development. Further experiments revealed that the C-terminal and globular domains of BigH1 are essential for proper nuclear divisions at low temperature, as replacing these domains with that of H1 results in severe mitotic defects and embryonic lethality. We have found that the complete replacement of BigH1 with H1 leads to enlarged nuclei, indicating altered chromatin organisation. Our experiments on the stability of nucleosomes and on their association with linker histones demonstrate that BigH1 performs its specific function in early embryogenesis by being a more dynamic linker histone that facilitates fast nucleosome exchange in S-phase during the rapid nuclear divisions in the early embryo, while providing greater stability to nucleosomes compared to somatic H1.

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Investigation of the effects of *Candida* on the progression of oral squamous cell carcinoma

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A large number of commensal microbial species reside in the human body that have co-evolved with the human genome and adopted to the host immune system. Previously it has been shown that defects in regulatory processes or alterations in the composition of microbiome can lead to various diseases, including cancer. A previous study has shown that the number of *Candida* cells in this particular niche is significantly higher in patients with oral squamous cell carcinoma (OSCC) compared to healthy individuals. Our aim is to examine how the fungal cells affect the progression of OSCC.

In order to investigate the effects of heat-killed and live *Candida* cells on the metastatic activity of the cancerous cells we used a metastatic (HO-1-N-1) and a non-metastatic (HSC-2) OSCC cell line. Cell migration, matrix metalloproteinase (MMP), proliferation activity and 3D tumor spheroid formation of HSC-2 and HO-1-N-1 OSCC cells were investigated after fungal stimuli.

The migration capacity of HO-1-N-1 cells was significantly higher if we treated the tumor cells with heat-killed *Candida* compared to the untreated samples. Prominent MMP activity and larger spheroid formation were detected after 24h pre-incubation with heat-killed *C. albicans* and *C. parapsilosis*. Both cell lines showed increased proliferation activity upon treatments, which clearly indicates that the presence of heat-killed fungi can accelerate cancer cell proliferation. In contrast to these results, live *Candida* treatment resulted in reduced migration activity and increased MMP activity of OSCC cells. No proliferation activity change was obtained.

Our *in vivo* experiments showed the tumor was growing faster in SCID mice if we pre-incubated the OSCC cells with *Candida*

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parapsilosis before the tumor cell injection to the tongue of the mice. Furthermore, we set up an *in vivo* experimental model for investigation of the effect of oral candidiasis on the OSCC in immunosuppressed wild type mice.

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A human BBB model in an organ-on-a-chip device and the effect of fluid flow

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Blood brain barrier-on-a-chip model are cutting edge microengineered devices, but only a few combine the crucial parameters. Our laboratory developed an organ-on-a-chip device to study the blood brain barrier (BBB), which enables visual observation, transendothelial electrical resistance (TEER) and permeability measurements. The objective of our study was to optimize a human BBB cell culture model in the organ-on-a-chip and determine the effect of the fluid flow in the barrier properties of the BBB model.

The device was built up from a porous cell culture membrane sandwiched between two layers of PDMS and a top and bottom plastic slide coated with gold electrodes. A peristaltic pump was used to circulate the cell culture medium to mimic the blood flow. The stem cell derived $CD34^+$ human endothelial cells in co-culture with bovine pericytes were cultured in the device, as a human BBB model.

We optimized the co-culture of stem cell derived $CD34^+$ human endothelial cells with bovine pericytes in the device. The resistance was measured in real time, and the flow conditions elevated the TEER significantly, which was also confirmed by ZO-1 and β-catenin immunostainings. To investigate the differences after fluid flow condition a gene expression study was performed. This organ-on-a-chip device for study a human BBB model, provides users with a standardized and reliable platform to perform pathology and pharmacology experiments. Our device is a cutting-edge invention in the barrier-chip field.

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