

19th IMG PhD Conference 2026

Abstract Booklet

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Research Spotlight Talks



MICAL1 interaction with phospholipids

Jonas Vlasak

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ABSTRACT ■

Precise control of membrane vesicle trafficking is fundamental to the establishment of neuronal connectivity. During axon guidance, synapse formation, and the maintenance of neuronal polarity, vesicles must be targeted to specific subcellular compartments with high fidelity. Rab GTPases serve as spatial landmarks on organelle membranes, recruiting effector proteins that couple membrane identity to downstream cytoskeletal remodeling. Among these effectors, MICAL1, a multidomain flavoprotein monooxygenase, has established roles in axon guidance downstream of semaphorin-plexin signaling and in neurite outgrowth. MICAL1 oxidizes actin methionine residues, driving local F-actin depolymerization upon activation by Rab8 family GTPases, which release the enzyme from an autoinhibited state. Whether MICAL1 can directly sense membrane lipid composition, independently of or in cooperation with Rab GTPases, remains unknown. Notably, the related family member MICAL-L1 directly binds phosphatidic acid through its C-terminus, an interaction required for its recruitment to tubular recycling endosomes. We therefore asked whether MICAL1 shares this capacity, and whether direct lipid binding constitutes a second layer of spatial control enabling MICAL1 to discriminate between organelle compartments defined by their phosphoinositide identity. Using a PIP-strip lipid overlay assay, we identified direct binding of MICAL1 to phosphatidic acid (PA), phosphatidylinositol phosphates (PIPs) a profile spanning plasma membrane and endosomal compartments central to vesicle recycling and axonal signaling. To define the structural basis and functional consequences of this interaction, we investigated MICAL1-membrane binding by lipid vesicle co-sedimentation, mapped the binding interface by hydrogen-deuterium exchange mass spectrometry (HDX-MS), and measured the effect of membrane association on MICAL1 actin-depolymerization activity. Together, we investigate MICAL1 as a direct phospholipid binding protein and propose a model in which membrane lipid engagement cooperates with Rab-mediated activation to precisely position MICAL1-dependent cytoskeletal remodeling at neuronal membrane compartments critical for connectivity.

Innate immune restriction of Syncytin fusogenic activity

Lukáš Jech

Laboratory: Laboratory of Viral and Cellular Genetics | Supervisor: Kateřina Trejbalová

ABSTRACT ■

During pregnancy and placenta development, Syncytins drive cell-cell fusion by binding to specific receptors on neighbouring cells. In humans, Syncytin-1 and -2 interact with receptors ASCT2 and MFSD2a, respectively, and together contribute to the formation and maintenance of the syncytiotrophoblast. Both proteins are encoded by endogenous retroviral envelope (Env) genes captured in the mammalian genome, and may therefore remain susceptible to cellular antiviral mechanisms. Whether interferon-stimulated restriction factors can interfere with Syncytin-mediated placental fusion, however, remains poorly understood. Here, we investigate the susceptibility of human Syncytin-mediated fusion to cellular restriction factors. We performed a preliminary screen of a selected library of interferon- α -stimulated genes for their ability to inhibit Syncytin-mediated cell-cell fusion. For this purpose, we developed a novel fusion quantification assay system based on a complementation of split NanoGlo luciferase followed by its secretion into media. This approach allows us to identify cellular factors that interfere with Syncytin-mediated fusion. Understanding how innate immune factors interfere with cell-cell fusion process may provide insight into how antiviral responses during pregnancy influence placenta formation.

Keywords Syncytin-1, Syncytin-2, fusion, placenta, syncytiotrophoblast, fusion assay, innate immunity, restriction factors

Hematopoietic stem cells undergo metabolic rewiring during the early stage of infection

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Laboratory: Laboratory of Haematooncology | Supervisor: Meritxell Alberich Jordà

ABSTRACT ■

Hematopoietic stem cells (HSCs) are precursors of all blood cell lineages. Under steady-state conditions, committed progenitors drive hematopoiesis, keeping HSCs quiescent. To safeguard their stemness, HSCs reside in a low metabolic state driven by glycolysis, which helps them avoid excessive ROS production that could impair their function. However, in response to stress (e.g. infection, injury, or cytotoxic stress), HSCs activate to produce mature blood cells. During acute infection, HSCs meet the increased demand for granulocytes through a process known as emergency granulopoiesis, supporting first-line defence against pathogens. We previously described that murine HSCs respond to infection as early as 4h after lipopolysaccharide (LPS) administration by shifting their transcriptional bias from lymphoid towards myeloid lineage to produce more granulocytes. The transcriptional rewiring during emergency granulopoiesis increases energy demands that cannot be sustained by glycolysis alone. We hypothesize that HSCs undergo metabolic reprogramming to meet energetic needs during emergency granulopoiesis, and that they rewire from a low glycolytic state towards a more active one driven by oxidative phosphorylation. Indeed, HSCs exhibit elevated glucose uptake and basal metabolism after 4h LPS treatment, showing an early metabolic response to infection. Moreover, ATP and mitochondrial ROS production were increased in HSCs and progenitor cells. We also saw a difference in mitochondrial mass and membrane potential. These results indicate that stem cells undergo metabolic rewiring towards more active and aerobic metabolism upon emergency granulopoiesis. Furthermore, we assessed how the LPS challenge affects HSC function. Interestingly, we observed that HSC function was impaired after 4h LPS treatment. Altogether, these results reveal how HSC metabolism adapts during emergency granulopoiesis and help identify metabolic targets to preserve stemness during acute infection.

Keywords Hematopoietic stem cells, emergency granulopoiesis, metabolism, infection

Regulation of PPM1D Stability through C-Terminal Modifications and Cell-Cycle–Dependent APC/C Activity

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ABSTRACT ■

Protein phosphatase PPM1D (WIP1) is a negative regulator of the tumour suppressor p53 involved in stress response pathways. Mutations in the last exon of PPM1D produce a truncated protein, lacking unstructured C-terminal domain. This variant retains full enzymatic activity while exhibiting abnormal stability, which leads to enhanced p53 suppression, thereby promoting genomic instability and oncogenesis. However, the precise molecular mechanisms of PPM1D turnover in human cells, and how its C-terminal region corresponds to its rapid degradation, remain unknown.

We previously established that the APC/C–Cdc20 complex degrades PPM1D during mitosis, supported by mass spectrometry data confirming PPM1D interaction with multiple APC/C subunits. Here, we demonstrate that loss of Cdh1, another APC/C coactivator, stabilizes wild-type (WT) PPM1D.

In addition to cell-cycle control, we hypothesized that the cellular redox state modulates PPM1D stability. Structural evidence confirms a nitrogen–oxygen–sulfur bridge (K336–C346) within the catalytic domain, and cysteine residues are also present in the C-terminus, including an extreme C-terminal CVC motif. Moreover, induction of oxidative stress leads to a reduction in WT PPM1D protein level.

We therefore suggest that redox-mediated modification of C-terminal cysteines acts as a regulatory switch controlling PPM1D turnover. To explore the role of this region in PPM1D degradation, we are using cell lines expressing WT and mutant variants of PPM1D and we observed that deletion of last 15 amino acids (variant Δ C15) is sufficient to enhance protein stability. However, non-reducing electrophoretic mobility assays revealed that oxidative stress induces a mobility shift in both WT and Δ C15 PPM1D, whereas the A457 variant (comprising primarily the catalytic domain) remains unaffected. This indicates an oxidative modification within the C-terminus excluding CVC motif.

Together, these findings indicate that the regulation of PPM1D stability is complex and context dependent. Clarifying these pathways is critical to explaining the pathogenic behavior of PPM1D mutants in cancer.

Keywords PPM1D, oncogenesis, degradation, cell cycle, oxidative stress

Beyond the Structured Core: Exploring Dicer's Intrinsically Disordered Regions

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Laboratory: Laboratory of Epigenetic Regulations | Supervisor: Petr Svoboda

ABSTRACT ■

Dicer is a highly conserved RNase III ribonuclease at the heart of small-RNA pathways. Yet embedded within its well-characterized structured domains are several intrinsically disordered regions (IDRs), evolutionarily dynamic sequences whose regulatory roles remain to be fully explored. Deletion of individual IDRs can reshape small-RNA profiles and alter Dicer's interaction landscape, suggesting that these regions are far more than passive flexible linkers. My PhD project focuses on two of Dicer's four IDRs, IDR2 and IDR4, whose regulatory functions remain less defined. This will be done by replacing mouse IDRs with their counterparts from representative deuterostome species to determine how these substitutions affect miRNA biogenesis and pinpoint the features underlying their regulatory function. Because IDRs can also serve as interaction platforms, I will use co-immunoprecipitation coupled with mass spectrometry to identify Dicer interaction partners whose association depends on these regions. Finally, I will ask whether individual IDRs can function as regulatory modules capable of fine-tuning Dicer activity. Together, this work aims to reveal how these evolving disordered regions can introduce new layers of regulation into an otherwise deeply conserved RNA-processing enzyme.

Keywords Dicer, small RNA biogenesis, Intrinsically Disordered Regions, Argonaute

Tracing the roots of Neural Crest in Amphioxus

Pratyasha Biswas

Laboratory: Laboratory of Transcriptional Regulation | Supervisor: Iryna Kozmikova

ABSTRACT ■

Understanding how vertebrates acquired phylum-specific features, like Neural Crest(NC) is an important question in evolutionary and developmental biology. This sheds light on the origin of vertebrates and will help us better understand the general regulatory environment underlying their development, including cell fate specification. With the recent discovery of Neural Crest-like Cells (NCC-like) in amphioxus *B. floridae* - a cephalochordate and sister group to vertebrates and tunicates, it brings up many intriguing questions, like: Where do these cells originate from in the animal and what do they lead to?

Keywords Lineage tracing, EOS, Photoconversion

Advancing Breast Morphogenesis Modelling through Novel Human Mammary Organoids

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Laboratory: Laboratory of Tissue Morphogenesis and Cancer | Supervisor: Mgr. Zuzana Sumbalová Koledová, PhD.

ABSTRACT ■

Breast morphogenesis is a complex, dynamic developmental process where epithelial structures organize into a characteristic tree-like architecture through coordinated interactions with the surrounding stroma. While historical understanding of these mechanisms stems primarily from murine models, significant physiological differences—specifically the human-specific fibrous stroma and distinct hormonal sensitivities—limit the translational value of rodent studies. This project aims to advance human breast modeling by establishing a novel 3D human mammary organoid system derived from primary human mammary epithelial cells (HMECs) and primary human breast fibroblasts. The study will optimize protocols to consistently reproduce key developmental features, including epithelial polarity, lumen formation, and branching morphogenesis. Furthermore, we will investigate the epithelial–stromal molecular dialogue, specifically evaluating ERK, YAP, and WNT signaling dynamics via advanced imaging, transcriptomics, and proteomics. By developing specialized models that recapitulate dynamic physiological states such as pubertal branching, the menstrual cycle, and lactation, this platform will enable precise identification of the cellular and molecular origins of breast pathologies, including cancer initiation.

Keywords morphogenesis, primary human epithelial cells, mammary gland, organoids

Molecular regulation of lactation: investigating DNA replication, transcription, and damage repair

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Laboratory: Laboratory of Tissue Morphogenesis and Cancer | Supervisor: Zuzana Sumbalova Koledova

ABSTRACT ■

Lactation is essential for providing nourishment to mammalian offspring during the early postnatal period. To sustain this, mammary epithelial cells undergo extensive proliferation, differentiation and transcriptional reprogramming while maintaining genome integrity. Cyclin-dependent kinase 12 (CDK12) is a transcription-associated kinase that supports the expression of long genes among which belong those involved in DNA replication and DNA damage response (DDR), yet its role in mammary gland development and lactation remains poorly understood. Preliminary observations from Cdk12 heterozygous female mice indicate impaired lactation and reduced alveolar development. In mammary epithelial cells, CDK12 inhibition leads to reduced binucleation and milk-protein expression, suggesting a link between CDK12 activity, cell-cycle regulation and functional differentiation. We hypothesise that CDK12 supports lactation by coordinating transcription, replication-stress responses and cell-cycle transitions needed for mammary epithelial cell differentiation. To investigate this, we combine mouse models and primary mammary organoids with transcriptional and imaging-based analyses. Lactogenic differentiation is assessed through mammary gland morphology and expression of milk-production genes, while DDR is examined using markers of genome integrity and stress signalling response. Cell-cycle progression and polyploidisation will be evaluated to determine whether altered CDK12 disrupts the acquisition of the differentiated lactating state. The work aims to define how transcriptional regulation and genome-maintenance pathways are integrated during mammary gland differentiation and to clarify the molecular basis of the lactation defect associated with reduced CDK12 activity.

Research Progress Talks



Deciphering contractile fibroblasts in the developing mammary gland

Kriti Attri

Laboratory: Laboratory of Tissue Morphogenesis and Cancer | Supervisor: Dr. Zuzana Sumbalova Koledova

ABSTRACT ■

The stromal microenvironment orchestrates epithelial morphogenesis through extracellular matrix remodeling and mechanical forces. We previously showed that mammary fibroblasts drive epithelial organoid budding via contractility, yet whether and how this mechanism operates in vivo remains unknown. Heterogeneity of mammary fibroblasts has only recently started to be investigated, but their functional specialization during development is still poorly understood. Recently, we discovered that the growing tips of mammary ducts (terminal end buds, TEBs) are surrounded by a transient niche-forming population of specialized contractile (α SMA+) fibroblasts that arise from preadipocytes. However, the signals that instruct peri-TEB fibroblast specification and coordinate their morphogenetic function have remained elusive. Our pathway analysis on scRNA-seq data from pubertal mammary fibroblasts has revealed elevated WNT/ β -catenin signaling activity specifically in peri-TEB fibroblasts. To assess its functional role, we chemically inhibited WNT signaling in vivo, which resulted in the depletion of α SMA+ peri-TEB fibroblasts, morphological alterations in TEBs, and reduced mammary epithelial outgrowth. Perturbing WNT signaling in a 3D co-culture of mammary organoids and fibroblasts also reduced epithelial organoid branching and Nestin (NES, a novel peri-TEB marker) expression. Furthermore, fibroblast (subtype)-specific WNT/ β -catenin signaling overactivation using tamoxifen- and doxycycline- inducible mouse models caused transient fibroblast activation after a short period that either returned to or declined below control levels after longer treatment durations, depending on subtype-specific activation. This suggests a lineage- and time- dependent effect of WNT/ β -catenin signaling on fibroblast subpopulations and mammary epithelial morphogenesis. Our findings, therefore, show that WNT/ β -catenin signaling in fibroblasts promotes accelerated progression through the transient, peri-TEB stromal state characterized by NES expression and an α SMA+ contractile fibroblast state required for mammary branching morphogenesis, with both reduced and excessive WNT signaling activity disrupting this process.

Keywords Mammary Gland, Fibroblasts, WNT/ β -catenin signaling

The role of cytoskeletal architecture in metastatic cell polarization

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ABSTRACT ■

Cancer remains a leading cause of death worldwide, with the vast majority of cancer-related mortality attributed to metastasis. Cancer metastasis requires circulating tumor cells (CTCs) to adapt to dynamic environments, transition between different polarized states and seed distant organs. A key hallmark of CTCs is their polarized architecture, with asymmetric accumulation of cortical actin, ezrin, adhesion molecules, and plasma membrane components at the cellular pole. This so called “single-cell (SC) polarity” in suspension enhances vascular adhesion and facilitates extravasation into underlying tissue. Therefore, understanding its molecular regulation is crucial for identifying new therapeutic strategies to target metastasis. A targeted drug screen against the cytoskeletal proteins, actomyosin network and membrane linker proteins identified plectin - a cytolinker protein, as a critical regulator of SC polarity. High-resolution microscopy further revealed that plectin localizes specifically to the pole, where it is spatially encased by both actin and ezrin. Given that plectin upregulation is associated with poor survival across multiple cancer-types, we investigated its role in regulating SC polarity. Both genetic (plectin KO) and pharmacologic inhibition with plecstatin significantly impaired pole formation and its stability. Mechanistically, loss of plectin impaired RhoA signaling, thereby leading to dysregulation of cellular contractility and perturbed SC polarity. Functional assays further revealed that plectin KO cells exhibited reduced attachment to HUVEC-grown endothelial monolayers, underscoring plectin's essential role in vascular adhesion during metastasis. These findings demonstrate the essential role of plectin in regulating SC polarity, facilitating cancer cell adhesion to endothelial cells and thus contributing to overall metastatic progression.

Keywords cancer, circulating tumor cells (CTCs), single-cell polarity, vascular adhesion, cytoskeleton, actomyosin contractility

Getting to the heart of the evolutionary origins of the vertebrate LPM

Rohin Sengupta

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ABSTRACT ■

The vertebrate lateral plate mesoderm (LPM) is a bilateral sheet-like structure that gives rise to the precursors for various different organ systems like the cardiovascular system, blood, limb fields etc. However, it is still not clearly understood how this specialized tissue came to evolve from the ancestral chordate state. The simple chordate amphioxus serves as an important model organism for us to be able to answer this question. Amphioxus, an invertebrate chordate that is very close to the base of the chordate lineage, has not undergone the two rounds of whole genome duplication that vertebrates have, and yet it shares a basic body plan with them. However, unlike in vertebrates where the somitic and LPM populations are clearly distinct even from a morphological point of view, the amphioxus LPM has been traditionally defined as the ventral part of the somites based on the expression of key LPM marker genes in vertebrates. Here, we combine gene expression data, pharmacological treatments, and bioinformatics analyses to clearly demonstrate that not only are the somitic and LPM populations in amphioxus distinct, but also that the amphioxus LPM is more regionalized than was previously assumed.

Keywords Lateral Plate Mesoderm, Somite, Heart Field, Amphioxus, Evolution, Pharmacology, In-Situ Hybridization, ScRNA-Seq

Actomyosin-driven embryonic cytokinesis

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ABSTRACT ■

During cytokinesis in animal cells, constriction of the actomyosin ring at the cell equator of the mother cell leads to formation of two daughter cells. The cortical actomyosin ring is under the control of the small GTPase RhoA, which promotes the assembly and recruitment of cytokinetic ring components like Non-Muscle Myosin II (NMY-2), Formin and Anillin. In most cases, cytokinesis requires active force generation and is dependent on NMY-2/Myosin. However, some recent studies suggest that ring contraction can also happen in the absence of NMY-2/Myosin. Moreover, in vitro studies have shown that the passive crosslinker Anillin can contract F-actin rings. In this project we combine the strength of *C. elegans* genetics with quantitative imaging and in vitro reconstitution to show that 1) cytokinesis in *C. elegans* can occur in the absence of NMY-2/Myosin and 2) NMY-2/Myosin-driven ring ingression depends on the passive cross linker Anillin. Our results thus indicate that in *Caenorhabditis elegans* embryos Anillin is able to generate forces independent of NMY-2/Myosin. We anticipate our findings to pave way for myosin-independent ring ingression mechanisms that could be insightful in other fields of study related to cytokinesis.

Emergence of actomyosin cortex terminates a burst of clathrin-mediated endocytosis during oocyte maturation

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Laboratory: Laboratory of Developmental Mechanobiology | Supervisor: Teije Corneel Middelkoop

ABSTRACT ■

In sexually reproducing animals, an unfertilized oocyte transitions to an embryo via a multi-step process involving oocyte maturation, ovulation and fertilization. In *C. elegans*, as oocytes mature, a contractile actomyosin cortex forms directly underneath the plasma membrane, which coincides with the emergence of thousands of dynamic branched F-actin condensates. Concurrently, the cell surface is biochemically remodeled via clathrin-mediated endocytosis (CME), which is important for oocyte maturation. To date, it is entirely unclear how CME, cell surface mechanics and the actin cytoskeleton are interlinked during oocyte maturation. Here, we combine high resolution time-lapse microscopy and functional endocytosis assays in isolated oocytes. We find that oocyte maturation triggers a transient wave of clathrin-mediated endocytosis. This requires the association of dynamic branched F-actin condensates which form distinct rings at clathrin coated pits. After this transient phase, a contractile actomyosin cortex emerges, and ring-like F-actin condensates become punctate. We further show that the sites of punctate condensates are incapable of internalizing vesicles. Chemical perturbation of NMY-2 activity identifies contractility as a primary mechanism to rapidly terminate endocytosis. Altogether, we have identified a novel developmentally controlled switch in endocytic behavior, which is triggered by elevated cortical-membrane tension before oocyte-to-embryo transition.

Keywords actomyosin contractility, cortical condensates, endocytosis, oocyte, *C. elegans*

Diversification of actomyosin-driven zygote polarization in nematodes

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ABSTRACT ■

Many nematodes establish the anteroposterior (AP) body axis by fertilization-triggered polarization of the zygote. This process is driven by cellular-scale flows of the actomyosin cortex, which is a thin contractile layer directly underneath the plasma membrane. Sperm entry locally inhibits the activity of Non-Muscle Myosin II (NMY-2), generating a gradient of contractility that induces posterior-to-anterior cortical actomyosin flows. We aim to investigate how actomyosin-driven cell polarization has diversified across nematode species by comparing two evolutionarily distant models: *Caenorhabditis elegans* (Rhabditidae) and *Pristionchus pacificus* (Diplogastridae). Our results reveal differences in the dynamic behavior of polarizing cortical flows, as well as in the geometrical constraints imposed by the eggshell. Moreover, by experimentally varying embryo confinement we found that embryo geometry impacts on actomyosin dynamics and viability in a species-specific manner. Together, these findings highlight species-specific polarization dynamics and demonstrate how actomyosin regulation can adapt to distinct geometrical constraints.

Keywords Actomyosin Cortex, Developmental Biology, Live-cell Imaging, Evolution, Biomechanics, Nematodes.

The 5' Methylation Barcode: A Molecular Checkpoint Linking snRNP Biogenesis to Innate Immune Sensing

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ABSTRACT ■

snRNA biogenesis involves complex maturation pathway under strict checkpoints in the nucleus and cytoplasm. A key quality control point is Sm-ring assembly, which absence leads to significant instability and degradation. Less is known about how cells distinguish between wild-type snRNA and truncated, mutated, or pseudogene-derived variants, and what role methylation at the 5' end plays in the biogenesis process. During the biogenesis, snRNA undergoes several steps of methylation and demethylation. The first methylation occurs during transcription and involves the addition of the 5' 7-methylguanosine (m7G) cap and 2'-O-methylation to the first transcribed nucleotide, which in the case of snRNA is always adenine. In the nucleus, the first adenine is further methylated by PCIF1, which produces m6Am. This methylation is only transient and is removed by the demethylase FTO, probably before the export of snRNA to the cytoplasm. During the cytoplasmic phase of biogenesis, snRNA undergoes several additional methylations at the 5' end, such as 2'-O-methylation at the second transcribed nucleotide by CMTR2 and hypermethylation of m7G by TGS1 and the formation of a 2,2,7-trimethylguanosine cap. Our data suggest that truncated snRNA is a substrate for binding to IFIT proteins, a component of the innate immunity. We believe that this interaction is caused by the fact that truncated snRNA exhibits a distinct methylation "barcode" at the 5' end, which may lead to the activation of innate immunity. In this study, we focused on this 5' end methylation "barcode" and its role in snRNA biogenesis. Overexpression of CMTR2 and PCIF1 led to a significant reduction in the interaction between truncated snRNA and IFIT proteins. These findings suggest that methylation of the 5' end may serve as a quality control signal that monitors snRNP biogenesis. The inability to complete individual steps of biogenesis due to inappropriate length, structure, or sequence may lead to the activation of an innate immune response, which may result in the exclusion of such defective snRNAs from the biogenesis pathway. Our results provide new insights into how RNA-modifying enzymes function as guardians of the transcriptome and directly link RNA processing defects to innate immune sensory pathways.

Keywords snRNA, Cap, Methylation, innate immunity

Single-cell Profiling of Nasal Mucosa Lymphocytes in Respiratory Infections

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ABSTRACT ■

Bordetella pertussis (B.p.), the causative agent of pertussis, remains a major public health challenge despite widespread acellular vaccines. In 2024, over 37,000 cases were reported in the Czech Republic. The nasal mucosa is the primary site of B.p. infection, yet the immune landscape of the upper respiratory tract remains poorly characterized. We performed single-cell RNA sequencing and flow cytometry to investigate nasal lymphocyte populations in convalescent pertussis patients and healthy controls. Convalescent pertussis was associated with a reduced abundance of CD8⁺ T cells expressing Killer-cell Immunoglobulin-like Receptors (CD8⁺ KIR T cells), an innate-like lymphocyte subset. Downstream analyses revealed downregulation of Toll-like receptor signaling and interferon-response genes, suggesting diminished immune activation during convalescence. To generate a broader nasal lymphocyte atlas, we integrated our dataset with publicly available nasal datasets from COVID-19 cohorts. In contrast to convalescent COVID-19 and pertussis samples, acute COVID-19 samples exhibited a strong type I interferon response across both T- and B-cell compartments. Integration of datasets generated from different anatomical sites further revealed marked differences in nasal immune cell composition, with nasopharyngeal samples enriched for CD4⁺ T cells and B cells with mainly naïve or central memory phenotype, and mid-turbinate samples enriched for CD8⁺ T cells with predominantly tissue resident phenotype. Importantly, CD8⁺ KIR T cells were enriched in mid-turbينات compared with the nasopharynx. Together, our results reveal site-specific heterogeneity of upper respiratory tract lymphocytes and demonstrate how nasal immune profiles differ following bacterial and viral respiratory infections. These findings provide new insights into mucosal immunity and may inform the development of next-generation mucosal vaccines.

Keywords Respiratory Infections, Mucosal Immunity, Adaptive Immunity, T cells

Uncovering the magic of memory CD8+ T cells

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Laboratory: Laboratory of Adaptive Immunity | Supervisor: Ondřej Štěpánek

ABSTRACT ■

Cytotoxic CD8+ T cells generate long-lived memory cells following encounters with pathogens or vaccinations, providing long-lasting protection to the host. After an infection peak, antigen-specific memory T cells outnumber naive T cells with the same antigen specificity, providing a quantitative advantage during subsequent cognate challenges. In our study, we aimed to elucidate qualitative differences between memory and naive CD8+ T cells following antigen-specific infection. Previous research indicated that memory CD8+ T cells confer superior protection against *Listeria* compared to their naive counterparts on a per-cell basis; however, the underlying molecular mechanisms remain poorly understood. We hypothesized that the production of effector molecules such as granzyme B and IFN γ , or the trafficking potential of memory cells to the infection site mediated by Core 2 O-glycosylation via *GCNT1*, could be implicated in this enhanced protection. Our findings revealed that direct cytotoxicity of highly proliferative memory CD8+ T cells plays an important role in the rapid protection against *Listeria* infection.

Keywords Memory CD8+ T cells, naive CD8+ T cells, per-cell differences, protection, infections

Poster Abstracts

Mapping Yolk Sac Hematopoiesis Reveals a Common Hemato-Endothelial Progenitor

Michaela Šímová

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ABSTRACT ■

During mouse embryogenesis, blood cell production is sustained by three independent hematopoietic waves. The first two originate in the yolk sac (YS), while the third arises intra-embryonically and is driven by hematopoietic stem cells (HSCs), which later establish lifelong hematopoiesis. However, HSCs are dispensable prenatally, highlighting the importance of YS-derived progenitors for early development and potential prenatal therapies. Our study aims to map YS-derived hematopoietic lineages, investigate their origins, and assess their contribution to myeloid cell production. Using single-cell RNA sequencing (scRNA-Seq), we identified a common hemato-endothelial ancestor for YS-derived waves. We show that the first wave generates cells with megakaryocyte-erythrocyte (MkE) potential, while the second wave, driven by erythro-myeloid progenitors (EMPs), gives rise to both MkE progenitors and the first myeloid cells. To further characterize EMP-derived blood production, we identified differentially expressed genes and searched for potential surface marker candidates. Notably, we found complement receptor CD88 to be a marker of the early EMP subset, which could enhance flow cytometry-based identification. Additionally, we are developing a novel lineage-tracing model to distinguish between hematopoietic waves across different niches. Our findings suggest that YS-derived waves share a hemato-endothelial ancestor and that EMPs are responsible for the first myeloid cells in embryogenesis. Ongoing in vivo validation of this model will provide deeper insights into early hematopoietic development and its clinical implications.

Keywords Embryonic Hematopoiesis, Yolk sac, Mouse model

Probing the Role of IDR3 in the Vertebrate Dicer–Argonaute 2 Interaction

Zuzana Granec

Laboratory: Laboratory of Epigenetic Regulations | Supervisor: Petr Svoboda

ABSTRACT ■

In vertebrates, the endoribonuclease Dicer has adapted to microRNA (miRNA) biogenesis, yet the precise mechanism by which processed RNA is handed off to Argonaute (AGO) for RISC assembly remains unclear. Previous evidence implicates Dicer's intrinsically disordered regions (IDRs) in this process. Here, we investigated Dicer's IDR3, a 153-amino-acid long, strongly negatively charged region implicated in the Dicer–AGO2 interaction. Using co-immunoprecipitation and Western blot analysis, we found that deletion of IDR3 abolished this interaction. Cross-species co-immunoprecipitation experiments further linked interaction with AGO2 to the presence of an extended, negatively charged IDR3; these features were conserved among the Dicers of the tested jawed vertebrates. Targeted alteration of charged residues in mouse IDR3 showed that charge reversal strongly disrupted the interaction, whereas charge neutralization weakened it. However, any negative charge was not sufficient: replacing mouse IDR3 with the strongly negatively charged IDR2 from *Esox lucius* Dicer failed to restore AGO2 interaction. Together, these findings show that strong negative charge contributes to the Dicer–AGO2 interaction but does not fully account for it, suggesting that additional sequence-specific features may also be involved.

Keywords Dicer, small RNA biogenesis, Intrinsically Disordered Regions, Argonaute

Characterization of the Slug piRNA Machinery

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ABSTRACT ■

The PIWI-interacting RNA (piRNA) pathway is an adaptive germline defense system that initiates and maintains post-transcriptional and transcriptional repression of transposable elements (TEs). The pathway relies on small RNA guides (typically 24-31nt long) loaded on PIWI proteins, which mediate sequence-specific recognition and silencing of TEs. While the canonical architecture of PIWI proteins is well-characterized in established models, their evolutionary divergence, structural adaptations, and somatic functions in mollusks remain poorly investigated. Here, we present a characterization of the piRNA pathway machinery in the slug species *Deroceras laeve*, *Deroceras invadens*, and *Arion vulgaris*. We have particularly focused on PIWIa and PIWIb, two PIWI paralogs in the three species. Using protein structure prediction, we identified the catalytic domains of these PIWI proteins and analyzed the structural differences in their endonuclease catalytic activity center. Subsequently, we discuss how differences in PIWI proteins and their potential interacting factors relate to piRNA activity across these species. Furthermore, we investigated the differential localization of the PIWI proteins in somatic and germline tissues using immunohistological staining. The results suggest differential expression of PIWIa and PIWIb between male and female germ cells. Our findings show the evolutionary adaptation of PIWI proteins and provide a novel model for researching the functional divergence of the piRNA pathway across gastropods.

Keywords Genome defence, Small RNA, piRNA, mollusc

Molecular Analysis of RNA Splicing in Inherited Retinal Dystrophies

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ABSTRACT ■

Retinitis pigmentosa (RP) is an inherited retinal disease characterized by progressive photoreceptor degeneration. Mutations in approximately 150 genes have been associated with RP, with variants in Rhodopsin (RHO) and Pre-mRNA Processing Factor 31 (PRPF31) representing two of the most common causes of the autosomal dominant form of the disease. This study aims to understand the effects of RP-associated mutations on splicing of these genes and evaluate the potential of antisense oligonucleotide (ASO)-based therapy to correct splicing defects. Seven RHO mutations and seven PRPF31 mutations, classified as pathogenic or of uncertain significance and associated with RP or other retinal dystrophies, were selected from the ClinVar database. The mutations were introduced into reporter constructs and transfected into HEK cells to assess their effects on splicing. Among the seven RHO mutations, three disrupted splicing, including two located at canonical splice sites and one located in intron 4. Similarly, three of the seven PRPF31 mutations were found to disrupt splicing, with two affecting canonical splice sites and one located in intron 10. Following confirmation of splicing disruption, ASOs were designed to restore normal splicing. Future work will focus on evaluating the efficacy of these ASOs in RHO and PRPF31 reporter systems to determine their potential to restore normal splicing.

Keywords Retinitis Pigmentosa; Splicing defects; ASOs

The BBSome Coordinates Intraflagellar Transport and the Tubulin Code to Maintain Ciliary Integrity

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ABSTRACT ■

Primary cilia are microtubule-based sensory organelles that coordinate signaling pathways essential for development and tissue homeostasis. Genetic disruption of ciliary components, including the BBSome cargo adaptor complex, causes multisystemic ciliopathies such as Bardet-Biedl syndrome (BBS), characterized by retinal degeneration, obesity, cognitive impairment, kidney disease, and skeletal abnormalities. The BBSome regulates selective ciliary trafficking in coordination with the intraflagellar transport (IFT) machinery, while emerging evidence highlights the axonemal Tubulin Code as an additional regulator of transport dynamics and microtubule stability. Our work and that of others demonstrate that BBSome disruption impairs ciliary cargo export, destabilizes the axoneme, and disrupts ciliary length homeostasis. However, how BBSome dysfunction affects the interplay between IFT and the Tubulin Code to maintain axonemal integrity remains poorly understood. Our data show that BBSome loss alters axonemal acetylation, glutamylation, and polyglutamylation in both nascent and mature cilia. Increasing intraciliary IFT restored acetylation and glutamylation in mature cilia, suggesting that BBSome deficiency reduces the basal IFT levels required to maintain the axonemal tubulin code. However, despite restoration of tubulin modifications, IFT particles exhibited increased velocity and more error-prone transport, indicating that the BBSome regulates not only IFT abundance but also IFT fidelity. Together, our findings establish a functional connection between the BBSome, IFT, and the tubulin code, revealing axonemal regulation as a potential therapeutic avenue to partially restore ciliary function in BBS.

Keywords Cilia, PTMs, IFT

Disrupting the olfactory interface reshapes neuroinvasion dynamics and attenuates neuropathology in SARS-CoV-2 infected mice

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ABSTRACT ■

SARS-CoV-2 infection has been associated with a wide range of neurological manifestations, yet the mechanisms underlying viral neuroinvasion remain incompletely understood. Although the olfactory route has been proposed as a pathway by which the virus reaches the central nervous system (CNS), its contribution to viral dissemination and neuropathology remains debated. Here, we investigated the role of the olfactory epithelium during SARS-CoV-2 neuroinvasion using Rosa26CreERT2/hACE2 transgenic mouse model. The olfactory epithelium was selectively ablated with methimazole before intranasal infection. Viral dissemination, neuropathology, neuroinflammation and peripheral immune cell populations were analysed during early infection. Disruption of the olfactory epithelium altered the regional distribution of viral RNA within the brain, reducing viral burden in frontal and dorsal regions, while lung viral loads remained unaffected. This was accompanied by milder neuropathological lesions, particularly near the olfactory lobe, and decreased expression of microglia-associated activation markers. Only limited differences were observed in circulating immune cell populations, suggesting that the main effects of olfactory epithelium ablation occurred within the CNS. Together, our findings show that disruption of the olfactory epithelium influences SARS-CoV-2 dissemination within the brain and is associated with reduced disease severity, neuropathology and neuroinflammation. These results support an important role for the olfactory epithelium in SARS-CoV-2 neuroinvasion and show how the route of viral entry can shape the regional outcome of CNS infection.

Keywords SARS-CoV-2, Neuroinvasion, Olfactory epithelium, Neuroinflammation, Neuropathology

LRATD2 Deficiency Impairs Lysosomal Function and Triggers Retinal Degeneration

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ABSTRACT ■

LRATD2 is an incompletely characterised protein. Despite reports associating its overexpression with breast, oesophageal and prostate cancer, its function is still unclear. *Lratd2*^{-/-} mice display age-related retinal degeneration, characterised by global retinal thickness reductions, regional retinal layer disorganisation and distortion of retinal structure, alongside the development of drusen-like deposits at the RPE-Bruch's membrane interface. Consequently, these morphological abnormalities lead to diminished electroretinography amplitudes in response to light stimulation. We hypothesised that this phenotype could be due to impaired autophagic and lysosomal degradation pathways. In line with this, we detected an increased presence and spatial association of autophagosome and lysosome markers in the retinas and RPE of LRATD2-deficient mice, suggesting expansion of degradative compartments or enhanced autophagic trafficking. Further, LRATD2^{-/-} RPE1 cells harboured an increased number of autophagosomes and autolysosomes with reduced LC3B degradation rates compared to wild type following autophagy induction, which may be attributed to reduced lysosomal degradative capacity during increased autophagic load. Finally, we identified a considerable overlap between LRATD2's proximal interactome and proteins known to regulate autophagic vesicle dynamics. Overall, our works suggests that LRATD2 regulates vesicle dynamics to coordinate degradative efficiency in the retina.

Keywords autophagy; degradation efficiency; interactome analysis; LRATD2; lysosome; neurodegeneration; retina; vesicle trafficking

The role of flagellum distal tip proteins in axonemal microtubule assembly and length regulation in *Trypanosoma brucei*

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ABSTRACT ■

The motile eukaryotic cilium, also known as the flagellum, is an evolutionary well-conserved organelle enabling cells to swim or to move fluid across their surfaces. The cytoskeletal core of the organelle is the microtubule-based axoneme. Axonemal assembly and length regulation primarily occur at its distal tip, but due to limited knowledge of proteins residing at this site, the molecular mechanisms of these processes are poorly understood. Thanks to the high experimental tractability of the human protozoan parasite *Trypanosoma brucei*, and the unique genome-wide protein localization resource available for this organism, our laboratory was able to identify the complete set of proteins localizing to the distal tip of its flagellum. A subsequent RNAi-mediated depletion screen identified distal tip proteins involved in the axonemal assembly and length regulation. To gain a mechanistic insight into their function, we have prepared cell lines overexpressing fluorescently tagged variants of ten of these proteins. We have been characterizing the effect of overexpression on the flagellum length, morphology of its distal tip, as well as cell morphology. Furthermore, we have been using these overexpression cell lines for preparation of cell lysates for in vitro microscopy-based reconstitution assays aimed at characterization of biochemical activities of these proteins towards microtubules. Findings of this project will provide a mechanistic understanding of how individual proteins regulate the construction of the trypanosome axoneme and will contribute to understanding the fundamental biology of this widely present organelle.

Keywords flagellum, *trypanosoma brucei*, axoneme construction, flagellum distal tip proteins

MICAL1 interaction with phospholipids

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ABSTRACT ■

Precise control of membrane vesicle trafficking is fundamental to the establishment of neuronal connectivity. During axon guidance, synapse formation, and the maintenance of neuronal polarity, vesicles must be targeted to specific subcellular compartments with high fidelity. Rab GTPases serve as spatial landmarks on organelle membranes, recruiting effector proteins that couple membrane identity to downstream cytoskeletal remodeling. Among these effectors, MICAL1, a multidomain flavoprotein monooxygenase, has established roles in axon guidance downstream of semaphorin-plexin signaling and in neurite outgrowth. MICAL1 oxidizes actin methionine residues, driving local F-actin depolymerization upon activation by Rab8 family GTPases, which release the enzyme from an autoinhibited state. Whether MICAL1 can directly sense membrane lipid composition, independently of or in cooperation with Rab GTPases, remains unknown. Notably, the related family member MICAL-L1 directly binds phosphatidic acid through its C-terminus, an interaction required for its recruitment to tubular recycling endosomes. We therefore asked whether MICAL1 shares this capacity, and whether direct lipid binding constitutes a second layer of spatial control enabling MICAL1 to discriminate between organelle compartments defined by their phosphoinositide identity. Using a PIP-strip lipid overlay assay, we identified direct binding of MICAL1 to phosphatidic acid (PA), phosphatidylinositol phosphates (PIPs) a profile spanning plasma membrane and endosomal compartments central to vesicle recycling and axonal signaling. To define the structural basis and functional consequences of this interaction, we investigated MICAL1-membrane binding by lipid vesicle co-sedimentation, mapped the binding interface by hydrogen-deuterium exchange mass spectrometry (HDX-MS), and measured the effect of membrane association on MICAL1 actin-depolymerization activity. Together, we investigate MICAL1 as a direct phospholipid binding protein and propose a model in which membrane lipid engagement cooperates with Rab-mediated activation to precisely position MICAL1-dependent cytoskeletal remodeling at neuronal membrane compartments critical for connectivity.

BBSome dysfunction in cilia rewires SHH and WNT signaling to disrupt mesenchymal lineage commitment

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ABSTRACT ■

Bardet–Biedl syndrome (BBS) is a multisystemic ciliopathy caused by dysfunction of the BBSome, a protein complex essential for primary cilia-mediated regulation of developmental signalling pathways, including Sonic Hedgehog (SHH) and WNT. BBS is associated with skeletal abnormalities and hypothalamic dysfunction-driven obesity, suggesting defects in mesenchymal lineage regulation. We investigated the role of the BBSome in controlling signalling pathways governing lineage commitment of bone marrow mesenchymal stromal cells, which are critical for osteogenesis and adipogenesis. Our data shows that BBSome deficiency impairs SHH pathway activation and enhances basal activation of the canonical WNT cascade, including non-phospho- β -catenin and phospho-DVL2 accumulation. The role of canonical WNT signalling is to suppress adipogenesis and promote osteogenesis. We found that the adipo-linked genes *Adipoq* and *Ppar γ* are suppressed at the basal state, in line with the elevated canonical WNT activity in the BBS condition. Wnt3a dependent activation of the WNT resulted in increased β -catenin translocation to the nucleus but has lost the capacity of efficient adipogenic gene suppression. Adipogenic differentiation assay showed weaker activation of *Adipoq*, *Fabp4*, *Ppar γ* and absence of contact inhibition during whole differentiation process in BBS condition. Our data indicate that BBSome loss dysregulates dynamic cellular responses to ligand induced activation of signalling pathways, such as WNT. Within this project, we aim to further determine how altered SHH and WNT signalling interact with insulin-driven adipogenic commitment and to define transcriptomic changes underlying these defects. Our work will uncover how cilia dysfunction in stromal cells may contribute to obesity in BBS and potentially identify targets for combination therapies.

Keywords Cilia, Ciliopathy, BBSome, MSCs, obesity, WNT, SHH