P-01 | Probing actin-tropomyosin interactions with drag force

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Keywords: Tropomyosin, Microfluidic, Force Spectroscopy, Interaction energy

Tropomyosin are central regulators of the actin cytoskeleton. They form parallel dimeric coiled coils that bind cooperatively along actin filaments and control the binding and activity of the other cytoskeleton proteins. The interactions between tropomyosins and actin filaments are particularly complex: single tropomyosin dimers bind weakly to 6-7 actin subunits but get stabilised by end-to-end attachment with other tropomyosin dimers, forming clusters which wrap around the filament. Here, we use force spectroscopy to better understand tropomyosin-actin interactions.

Various approaches have been developed to apply controlled forces to proteinprotein pairs. Each protein is typically anchored to a surface or bead, often via a DNA linker. The proteins are then put in contact and pulled apart by diverse instruments such as optical traps, atomic force microscopy and magnetic tweezers. But these methods are ill-suited for tropomyosins: a single tropomyosin dimer form a transient interaction with a filament, such that very weak forces would be required. Moreover, since tropomyosins form clusters, we cannot study single proteins, and we must consider complexes with multiple interactions.

We propose a method in which a hydrodynamic drag force is applied directly to the protein of interest, by imposing a controlled fluid flow inside a microfluidic chamber. Tropomyosin clusters are assembled on actin filaments that are anchored by one end to the bottom glass coverslip. By changing the overall flow rate, we apply a controlled hydrodynamic drag force over the tropomyosin clusters and discovered that they spontaneously detach. By applying very low forces from 0.01 to 0.1 pN per Tpm dimer, we obtained key insights into Tpm-actin interaction and dynamics, for different Tpm isoforms.

This method is also well-suited for other actin binding proteins. As an example, we quantified the effect of a drag force on the movement of myosin motors. This approach bypasses the need for surface anchoring and widens the range of proteinprotein interactions that can be studied by force spectroscopy.

P-02 | Clathrin-mediated endocytosis associates with transient clusters of branched F-actin in the activated *C. elegans* oocyte

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A key event in the embryonic development of *C. elegans* is the activation of a contractile actomyosin cortex during maturation of the oocyte before its fertilization. Recent studies have reported that this cortex activation is supported by the emergence of highly dynamic F-actin clusters on the nascent oocyte cortex. These are primarily composed of WSP-1/N-WASP, the Arp2/3 complex and branched actin filaments, and exhibit stereotypical compositional dynamics. However, the physiological role and function of these dynamic clusters remains unclear. Using high resolution time-lapse microscopy of isolated oocytes we find that the cortical actin clusters co-localize with various components involved in Clathrin-Mediated Endocytosis (CME). Furthermore, blocking CME by either genetic or chemical perturbations affects the distribution, morphology and compositional dynamics of the branched actin clusters. Altogether, our results suggest that the dynamic branched actin clusters are sites of clathrin-mediated endocytosis, and put forward a possible mechanism of how endocytosis can control the activation and remodeling of the actomyosin cortex.

P-03 | Arp2/3 regulates endothelial barrier integrity

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Keywords: Arp2/3 complex, CK666, Endothelial permeability, Actin

Compromised endothelial barrier is associated with vascular leakage and tissue edema in various diseases such as sepsis, but effective therapies stabilizing the vasculature are lacking. While much research has focused in endothelial cells on junctional adhesion molecules, like the adherens junction protein VE-cadherin, in regulating endothelial barrier permeability, the role of the underlying actin cytoskeleton in dynamic barrier regulation is less well understood. Here, we show that Arp2/3 complex, which assembles branched actin networks implicated in junction assembly, regulates endothelial permeability. In cultured endothelial cells, vascular endothelial growth factor (VEGF) and thrombin compromised the endothelial cell monolayer in an Arp2/3-dependent manner. Inhibition of Arp2/3 using CK666 or gene silencing decreased VEGF- or thrombin-induced actin stress fiber formation and VE-cadherin internalization. Importantly, CK666 prevented VEGF-induced vascular leakage in vivo and decreased lipopolysaccharide-induced leakage in murine endotoxemia. Our results suggest a model where Arp2/3 balances endothelial barrier stability v/s permeability through an interplay with other actin binding proteins and that Arp2/3 inhibition may provide means for vascular barrier stabilization.

P-04 | The role of nuclear myosin 1 in mouse brain development

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Keywords: Nuclear myosin 1; Transcription regulation; Cell identity; Brain development

Nuclear myosin 1 (NM1), one of the three alternatively spliced isoforms of the Myosin 1c gene, is a motor protein located in the cellular nucleus, serving as a global transcription regulator. Mechanistically, NM1 binds to the B-WICH subunits WSTF and SNF2h, facilitating the anchoring of the chromatin remodeler to the DNA promoter region and the recruitment of histone acetyltransferases and histone methyltransferases. These enzymes add histone modifications, allowing for chromatin opening and transcription activation. A previous study from our lab demonstrates that the depletion of NM1 results in the dysregulation of cell cycle-related genes and the accumulation of DNA damage in mouse embryonic fibroblasts. Additionally, NM1 positively regulates oxidative phosphorylation (OXPHOS) by binding to mitochondrial transcription factors; dysregulation of NM1 expression induces a metabolic switch from OXPHOS to aerobic glycolysis, thus contributing to tumorigenesis. Changes in gene expression, mitochondrial function, and metabolism also play a significant role in the regulation of stem cell fate and differentiation. In this context, our lab recently showed that NM1 is involved in hematopoiesis in the bone marrow by regulating gene programs related to the immune system, platelet activation, and osteoclast differentiation. These studies highlight NM1's potential role as a critical regulator of cell identity in both health and disease. Yet, NM1's role in neurodevelopment remains unexplored.

Our results provide evidence that NM1 regulates genes involved in hematopoiesis, vasculature, and the ECM during both embryonic and postnatal stages of brain development. Dysregulation of these processes during development leads to an overall disruption of gene programs related to various aspects of adult brain function. Notably, in the absence of NM1, we observed an increase in total brain volume in older animals (~10-12 months), highlighting the long-term impact of NM1 deficiency on brain structure and function. Preliminary behavioral studies also indicate potential motor dysfunctions in NM1 KO mice. Collectively, these findings suggest a crucial role for NM1 in mouse brain development and raise important questions of whether its role during development indirectly contributes to its involvement in central nervous system aging and degeneration.

P-05 | The actomyosin-dependent α-catenin-vinculin-VASP machinery stimulates actin polymerisation

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Keywords: adhesion, actin, myosin, mechanotransduction

Tissues maintain their integrity by adapting to intracellular and extracellular mechanical variations. This requires the establishment and maintenance of mechanosensitive cell-cell junctions. First, cells initiate contact with each other by projecting their lamellipodia, which rely on the formation of an Arp2/3-branched actin network. This process enables the formation of cadherin-based adherens junctions (AJs). As these AJs mature, remodelling of the branched actin network into a contractile actomyosin network reinforces the adhesion via the assembly of the actin-binding proteins α-catenin, vinculin and VASP. Each of these three proteins can individually influence the nucleation, elongation, and organization of actin filaments. However, it is not known whether the three proteins, α-catenin, vinculin and VASP, assemble in response to actomyosin force, and whether the resulting protein machinery regulates actin polymerisation and reorganises the Arp2/3 branched actin network.

The complex signalling environment controlling AJs justifies disentangling these pathways by isolating this machinery in an in vitro assay made of purified proteins. To do this, we have developed a minimal system whose spatial organisation mimics the 2D architecture of the apical side of epithelial tissue. This artificial structure is a micropatterned honeycomb network in which the edges of the 'artificial cells' are decorated with α-catenin. This experimental system, in which the concentration and spatial organisation of a set of known components are adjustable, makes it possible to observe by TIRF microscopy the recruitment of fluorescent proteins and their ability to regulate actin polymerisation and organisation.

Our observations show that the binding of a contractile actomyosin network to α-catenin is sufficient to induce the assembly α-catenin, vinculin and VASP. Once formed, this machinery stimulates actin assembly and inhibits the formation of an Arp2/3-banched actin network.

P-06 | The open to closed D-loop conformational switch determines length in filopodia-like actin bundles

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Keywords: antibodies, filopodia, dynamics, length regulation

Filopodia, microspikes and cytonemes are thin membrane protrusions composed of parallel bundles of actin filaments. They allow cells to sense the environment, and are important for the dissemination of morphogens, organelles and pathogens across tissues. These roles require them to be highly dynamic, with fine control over length central to their function, however it is unclear how actin bundle dynamics ultimately determines structure length.

To explore this, we combined a phage-display phenotypic screen with our adaptable cell-free reconstitution system that generates filopodia-like actin bundles from a supported lipid bilayer, identifying a set of monoclonal antibodies that lengthen these filopodia-like structures (FLS). These antibodies immunolabelled along actin bundles in both FLS and cells, and could displace the actin-binding probes LifeAct and Utrophin-CH from FLS actin bundles. LifeAct and Utrophin-CH both bind the DNase 1-binding loop (D-loop) of F-actin, a flexible region which can switch between open and closed conformations, and is important as a marker for filament age, and as a key site for actin regulatory protein binding.

Using immunoprecipitation and ELISA assays, we determined that our antibodies selectively bound F-actin, specifically in the open D-loop conformation. In photobleaching live imaging experiments, we determined that this inhibited disassembly of the FLS bundles, and found that we could alleviate the lengthening phenotype by supplementing in either extra actin or additional cofilin to the assay. Taken together, we suggest that our antibodies bind and stabilise bundles in the open D-loop state, and that this D-loop conformational switch is important for disassembly, and thus for length regulation in filopodia-like actin bundles.

P-07 | Structure of the Huntingtin F-actin complex reveals its role in cytoskeleton organization

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Keywords: Huntingtin, Huntington's disease, actin, actin-binding protein, cryo-electron microscopy.

Huntington's disease is a neurological polyglutamine (polyQ) repeat disorder caused by an abnormal expansion of a polyQ tract in the N-terminal region of the huntingtin (HTT) protein. A repeat length of around 21 glutamines (Q21) is considered typical, whereas expansions to 78 repeats (Q78) are associated with disease onset. The longer the polyQ tract, the earlier and more severe the disease symptoms tend to be. HTT is a large, 3,142-amino acid protein organised into three major domains: an N-terminal HEAT repeat domain (N-HEAT), a C-terminal HEAT repeat domain (C-HEAT), and a Bridge domain that connects them. The precise physiological functions of HTT are not well understood, though it plays roles in a variety of cytoskeleton-related processes involving actin and microtubules, and is generally described as having scaffolding functions. However, direct molecular interactions between HTT and cytoskeletal components have remained elusive, hindering a detailed understanding of its cellular roles. Here, we show that HTT directly interacts with F-actin and organizes it into bundles. Using cryo-electron tomography (cryo-ET) and subtomogram averaging (STA), we present *in vitro* structures of full-length, HTT Q21 and Q78 bound to F-actin at subnanometre resolutions. These structures reveal that the N-HEAT and Bridge domains of HTT wrap around actin filaments, while the C-HEAT domain is not observed to bind. We further demonstrate that HTT forms dimers *via* its N-HEAT domain, which bridge parallel F-actin filaments. Together, our work unambiguously shows HTT is a novel actin-binding protein, engaging with F-actin through a distinct mode of binding. This provides a structural basis for understanding how HTT potentially organizes the actin cytoskeleton.

P-08 | Conservation of ArpC5L-mediated nuclear actin polymerization in immune cells

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Keywords: Nuclear F-actin; Arp2/3 complex; ARPC5L; immune cells

Nuclear actin polymerization in response to extracellular cues is observed in an increasing number of cell types and signaling processes. The resulting nuclear F-actin (NFA) meshworks differ in architecture and dynamics and are thought to mediate diverse functions including gene positioning in the nuclear space as well as regulating chromatin organization and gene expression. We previously identified that in CD4 T lymphocytes, engagement of the T cell antigen receptor or stimulation with PMA/Ionomycin triggers the rapid and transient formation of NFA meshwork that governs T cell helper function by controlling the expression of important cytokine genes (Tsopoulidis et al., 2019 Sci Immunol). This CD4 T cell response is triggered by nuclear calcium transients and mediated by ARP2/3 complexes containing the C5L but not the C5 subunit isoform (Sadhu et al., 2023 eLife).

To assess if this nuclear actin response and the C5L-dependant mechanism is a general feature of immune cells, we screened cells from different lineages for the ability to generate NFA upon stimulation with PMA/Ionomycin. We found that B lymphocytes, monocytes, macrophages, and dendritic cells form NFA in response to PMA/Ionomycin but failed to observe any NFA formation upon stimulation of neutrophils. The NFA meshwork formed upon stimulation in these distinct cell types differed in architecture and dynamics with a significantly more branched and dynamic filament network in dendritic cells than the other immune cells. Despite these morphological differences, depletion of C5L by targeted gene knock out revealed that NFA formation required C5L in all immune cells that responded to stimulation. C5L-mediated nuclear actin polymerization therefore is a broadly conserved immune cell response to stimulation. Comparing these cell systems allows us to study the functional consequences of morphologically distinct NFA responses induced by the same stimulus and mediated by the same actin polymerization machinery. Gene expression and chromatin accessibility studies are ongoing to define the effector functions of nuclear actin dynamics in these different immune cells.

P-09 | Differential interference with actin-binding protein function by acute Cytochalasin B

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Keywords: lamellipodium, VASP, capping protein, Arp2/3 complex, cytochalasin

Actin filament remodeling is essential for various cellular functions, including cell migration, division or intracellular transport. Cytochalasins B and –D (CB/CD), secondary metabolites derived from fungi, are commonly used to disrupt these processes. While these compounds are agreed to interfere with actin polymerization by blocking the growth of the rapidly-growing, so called barbed ends of filaments, the precise molecular consequences of their modes of action remain unclear. Cell migration at least on rigid surfaces is frequently accompanied by the protrusion of flat leaflets of plasma membrane, generally termed lamellipodia.

In this study, we are combining live-cell imaging of this frequently used model structure and the analysis of the behavior of various actin-binding proteins to acute but reversible exposure to Cytochalasin B or–D. Our results reveal that CB, despite halting lamellipodial protrusion, has unexpected and differential effects on various actin binding proteins. More specifically, we find that CB (upon local application) increases the accumulation of critical barbed end-binding proteins, such as Ena/VASP family proteins and capping protein (CP), whereas the localization of the F-actin bundling protein Fascin vanishes immediately. Similar results were obtained with CD, which on one hand more potent also appeared to behave in a slightly less reversible fashion, which is why follow-up experiments were mostly restricted to CB. As opposed to VASP and CP, other factors at the lamellipodium edge, such as the formin family member FMNL2 or the unconventional myosin MyoX were much less strongly affected by CB. When combining CB treatments with CRISPR/Cas9 genetics, we found that the surprising accumulation of Ena/VASP family members effected by CB requires the presence of CP, but not *vice versa*, and is accompanied by suppression, but not elimination of both actin and VASP turnover. These results were all incompatible with a simple competition model of binding between cytochalasins and aforementioned barbed end binding proteins *in vivo*. Finally, these results, as e.g. the accumulation of VASP following suppression of actin polymerization by CB, could be recapitulated *in vitro*, using bead motility assays with a minimal set of purified proteins, which also fits the increase of VASP dwell times at filament barbed ends upon CB binding at the single filament level.

All these data suggest a much broader range of cytochalasin activities on barbed end-binding proteins than previously anticipated, providing novel molecular insights that reshape our understanding of the impact of these and similar compounds on dynamic actin filament remodeling.

P-10 | CAP1 acts as a cytosolic brake on MRTF-SRF signaling in neurons and the mouse brain

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Keywords: CAP1, actin, MRTF, SRF, BDNF

In the developing and mature brain, the interaction between actin regulatory proteins, growth factors and transcription factors are important for shaping neuronal complexity, connectivity and function. One critical signaling module that couples cytoskeletal dynamics and growth factor signaling to gene expression is the myocardin-related transcription factor (MRTF)–serum response factor (SRF) pathway. In this study, we uncover how the actin regulator cyclase-associated protein 1 (CAP1) acts as a cytosolic repressor of MRTF-SRF signaling in mouse cortical neurons.

Using mouse primary cortical neurons deficient for key actin regulators, we show that CAP1, but not its close homolog CAP2 nor cofilin1 or actin-depolymerizing factor (ADF), plays a crucial role in regulating MRTF-SRF–dependent transcription. Loss of CAP1 leads to a robust nuclear accumulation of MRTF isoforms and a strong increase in MRTF-SRF reporter activity. Pharmacological manipulations that increase the monomeric actin pool, or inhibition of MRTF function, restore normal SRF activity in CAP1-deficient neurons, highlighting an actin-dependent mechanism driving nuclear translocation of MRTF in the absence of CAP1. In addition, the treatment of CAP1-deficient neurons with brain-derived neurotrophic factor (BDNF) resulted in robust increase of MRTF-SRF reporter activity, suggesting that CAP1 might function to some extent as a damper of BDNF signaling. Further domain-deletion and mutational analyses pinpoint two CAP1 regions, the helical folded domain (HFD) and the CARP domain, as necessary for repressing MRTF-SRF–driven transcription. In contrast, CAP1's oligomerization domain, WH2 and P1/2 domains are dispensable for its function in neuronal gene regulation. Analysis of CAP1-knockout mice cerebral cortex by RNA sequencing and mass spectrometry showed a strong and specific upregulation of MRTF-SRF targets at both mRNA and protein levels, corroborating the cell-based assays.

Overall, this work highlights CAP1 as a crucial brake on MRTF-SRF–mediated transcription in neurons. By preventing excessive actin polymerization–induced MRTF nuclear localization, CAP1 ensures balanced activation of growth factor induced signaling and the expression of cytoskeletal genes that are necessary for normal development and function of neurons.

P-11 | Myosin-X bundles actin filaments and synchronizes barbed end growth

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Keywords: Myosin-X, filopodia, bundling, *in vitro*

Filopodia are finger-like protrusions in the cell membrane that are involved in cell migration, mechanical sensing of the cell's environment and initiating focal adhesions. They are made up of fascin-induced parallel F-actin bundles. Myosin-X is important for the initiation and the elongation of filopodia, and accumulates at their tips, throughout the protrusion's lifetime. Myosin-X is considered to be optimized for interacting with actin bundles. It can side-step to a different filament in a bundle, or may walk in a 'straddle' fashion, its two heads walking on different filaments. Most of the literature about Myosin-X describes its single-molecule walking behaviour, or its importance for filopodia in cells. However, little is known from a mechanistic perspective about the role that this unconventional myosin plays in filopodia organization.

Using TIRF microscopy with purified proteins, we show that Myosin-X bundles actin filaments *in vitro* and aligns filament barbed ends without help from other bundling proteins. Interestingly, Myosin-X walks processively on these Myosin-X-induced bundles, and accumulates at the tips of the bundles, reminiscent of what is observed in filopodia. Strikingly, this enhanced residence time at the actin barbed end is not observed on individual filaments and is therefore an emergent property that appears in bundles only. Using microfluidics, we identify the exact requirements for Myosin-X barbed end accumulation, such as the minimum number of synchronized actin filament barbed ends. We investigate how the processive motion of Myosin-X is affected by increasing concentrations of fascin.

Our results shed light on the interaction of Myosin-X with actin bundles and its cooperation or competition with other bundling factors. This will contribute to a more complete understanding of the complex machinery involved in the initiation and maintenance of filopodia.

P-12 | Decoupling the actin cortex's role in membrane electroporation

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Keywords: reconstituted actin cortex, membrane electroporation, giant unilamellar vesicles, synthetic biology.

Delivering molecular cargo into cells remains a persistent challenge in biological research. Electroporation offers a promising solution using high-voltage electric pulses to disrupt cell membranes transiently through aqueous pores. Nevertheless, the fundamental biophysical mechanisms underlying pore formation and membrane recovery remain incompletely understood, particularly regarding the role of the actin cytoskeleton. While prior work suggests actin networks influence membrane stability, the mechanistic role of cytoskeletal architecture in electroporation efficiency remains unresolved.

We propose that membrane-associated actin networks modulate electroporation outcomes by altering mechanical resistance to pore formation and slowing down resealing. To test this, we engineered a synthetic platform of giant unilamellar vesicles (GUVs) with tuneable actin cortices varying in architectural complexity, mechanical stiffness, and membrane attachment points. Membrane dynamics were quantified using a combination of high-speed imaging (10,000 fps) and fluorescence recovery assays during controlled electric pulses (0.9–3 kV/cm, 500 μs duration).

Our preliminary findings demonstrate critical differences in membrane response between actin-free and actin-containing vesicles during electroporation. Actin-containing GUVs exhibit a higher critical threshold for pore formation and maintain structural integrity under strong electric field conditions, fully recovering their original spherical shape with minimal content loss compared to actin-free GUVs. However, further refinement of experimental protocols is necessary to establish definitive correlations.

This work still aims to provide a quantitative link between actin network properties and electroporation efficiency, directly informing strategies to optimize cargo delivery. These insights may enable tailored electroporation protocols that leverage our understanding of the cytoskeleton's role to enhance gene therapeutic outcomes.

P-13 | Phalloidin and DNase I-bound F-actin pointed end structures reveal principles of filament stabilization and disassembly

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The dynamic turnover of actin filaments (F-actin) controls the shape and movement of eukaryotic cells. The two polar ends of F-actin, being the only sites for the addition or loss of actin subunits, are especially important for controlling filament dynamics. The F-actin pointed end represents the primary site of filament disassembly. However, it remains incompletely understood how toxins and actin-binding proteins structurally alter the pointed end arrangement to either stabilize the filament or trigger its depolymerization. Here, we present high-resolution cryo-EM structures of the F-actin pointed end, undecorated and in complex with the F-actin stabilizing-toxin phalloidin and the F-actin disassembly factor DNase I. While in the undecorated pointed end the two terminal subunits adopt a twisted, G-actin-like conformation, the binding of phalloidin stabilizes these subunits in a flattened F-actin arrangement. Thus, phalloidin acts as a bridge that rigidifies inter-actin subunit contacts at the filament pointed end. The DNase I-bound structures revealed that two DNase I molecules can simultaneously interact with the F-actin pointed end in a flexible manner. Their binding disrupts the packing of the two terminal actin subunits and triggers rapid filament depolymerization. Our work exemplifies how diverse actin-binding molecules affect the stability of the F-actin pointed end at the atomic level, highlighting some of the mechanisms by which the pointed end dynamics can be controlled in cells.

P-14 | The role of the actin cortex in DNA electrotransfer

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Keywords: DNA, electrotransfer, intracellular transport, actin cytoskeletal network

Understanding the mechanisms behind DNA internalization through electrotransfer is essential for advancing clinical technologies, particularly in applications such as gene therapy. By adjusting DNA size and the biophysical barrier properties of the actin cortex, this research aims to contribute valuable insights into how the cellular actin cytoskeleton is involved in fundamental processes governing DNA translocation.

To systematically explore how molecular size influences electrotransfer efficiency, we utilize DNA fragments of 100, 250, and 500 base pairs (bp) and DNA plasmids of 4713, 7614, and 9553 bp and test the electrotransfer efficiency in mammalian CHO-K1 cells. We employ fluorescence microscopy to observe the intracellular transport dynamics of DNA aggregates. We show that changes in DNA size do not significantly impact the transport behaviour of DNA within the cellular environment for the duration of our observed trajectories. Independent of DNA base pair length, all DNA fragments and plasmids form aggregates that display anomalous diffusive behaviour.

In future experiments, particular focus will be placed on two key aspects. First, we will modulate the actin cytoskeletal network density through inhibition of binding proteins to influence filament length. This variation allows us to assess whether different DNA sizes, when interacting with distinct actin networks, play a significant role in determining the movement and aggregation of DNA within cells. Second, we aim to determine whether DNA size affects actin patch formation. Fluorescent labelling of the actin cortex further enables us to examine the involvement of actin in DNA internalization by tracking actin patch formation. These actin patches, which develop at sites of DNA aggregation, serve as crucial indicators of cytoskeletal dynamics in response to DNA uptake.

Ultimately, we hypothesize that intracellular activity levels, rather than DNA size, play a more significant role in determining the observed transport behaviour. The active transport processes within different cell types appear to exert a greater influence on DNA mobility than the physical properties of the DNA itself. These findings provide valuable insights into the factors that govern DNA electrotransfer, offering potential implications for optimizing gene delivery strategies in therapeutic applications.

P-15 | Deciphering actin-riched podosome stabilization in immune cell accumulation on surface nanotopography

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Keywords: nano-topography, podosomes, THP-1 cell, cell migration, actin enrichment

The surface topography of pathogens and extracellular matrix can influence the behavior of immune cells. Podosomes are typical adhesion structures in immune cells with an actin-rich core and adhesive ring play a crucial role in immune cell migration in response to microtopographical cues. However, the effects of nano-topography surfaces to podosomes-forming cells remain unclear. Here, we fabricate nanopillars with precisely controlled dimensions to emulate *in vivo* topographical features possibly experienced by individual cells. Specifically, the nanopillars range in diameter from 100-1000 nm, overlapping with individual podosomes dimensions. Comparing the distribution of podosomes forming and non-forming cells, we observed preferential accumulation of podosomes-forming THP-1 cells on nanopillar regions. By analyzing cell migration behaviors, we found that THP-1 cells showed reduced migration speed upon encountering the pillar areas. To understand the selective accumulation of THP-1 cells, we tested the properties of podosomes on nanopillars. Spatially, we found that the core of podosome protein-actin, exhibited enrichment proximal to the nanopillars.Temorally, we observed the curvature-dependent dynamics of actin which is more stable on pillars compare to flat area. Thus, our results suggest that immune cells can selectively accumulate on nanoscale topography areas by modulating the formation of podosome related proteins.

P-16 | EB1: Structural dynamics and aggregation

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Keywords: EB1, calponin homology domain, aggregation, denaturation

EB1 (End-binding protein 1, MAPRE1) is a member of the +TIPS (microtubule plus-end tracking proteins) family. Its N-terminal calponin homology (CH) domain binds to microtubules and actin filaments, potentiating EB1 as a cytolinker. The C-terminus (EBH-tail) is a structural scaffold for dimerization and +TIP interactions. Our study aims to investigate EB1's structural properties and its capacity to form droplets via LLPS (liquid-liquid phase separation).

We assessed the protein's structural characteristics using in silico methods. As experimental approach, the aggregation index of recombinantly produced EB1 (*Drosophila melanogaster*) was derived from absorption spectra [Katayama et al., 2005] and the aggregates were detected using light microscopy and machine learning-assisted image analysis. Protein stability was investigated using fluorescence spectroscopy and differential scanning calorimetry.

In contrast to a previous study [Song et al., 2023], we have not yet observed LLPS of EB1, but aggregation. Aggregation characteristic was found to be altered by truncation and concentration of the protein. The unusual fluorescence intensity of tryptophans during chemical and thermal denaturation can be well explained by intramolecular quenching [Nanda & Brand, 2000] in its native state. The CH domain of EB1 exhibits lower thermal stability (41°C) compared to the CH domains of utrophin (57°C or 71.5°C) [Bandi, Singh, & Mallela, 2014].

We discovered the concentration and truncation-dependent aggregation capability of EB1 and the unusual increase in the intensity of Trp emission during CH domain denaturation. This provides a marker-free tool for probing its structural alterations. Furthermore, we identified the EB1 CH domain's low stability. In the subsequent studies, we aim to phase-separate EB1 and investigate the parameters influencing its solubility and to explore how these structural features correlate with the activity.

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P-17 | Flightless-I and *Drosophila* LRRFIP2 work together to regulate radial growth of the sarcomeres

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Keywords: Actin, Sarcomere development, STORM, Flightless-I, Alphafold modelling

Myofibrils are composed of serially organized contractile units, called sarcomeres. The first phase of myofibrillogenesis includes the formation of premyofibrils consisting of immature sarcomeres that must undergo a substantial growth process, both in length and diameter, until they reach their final size. Despite sarcomere development is extensively studied, the mechanisms of sarcomere elongation and radial growth remained largely unknown. Flightless-I (FliI) has been identified as a key factor of muscle development in Drosophila and in mammalians as well. It's an evolutionarily conserved protein composed of leucine-rich repeats (LRR) and six gelsolin homology (GH) domains. To unravel the molecular mechanisms of FliI, we performed a detailed analysis of Drosophila FliI, using the indirect flight muscle as our major model system. In agreement with former data, we found that in the absence of FliI the myofibrils often look disorganized and they are composed of shorter and thinner sarcomeres than the wild type. Based on dSTORM measurements, the FliI protein is localized to the (+) end of the thin filaments in the Z disc, and it plays an important role in Z-disc formation and peripheral growth of the myofilaments. A structure-function analysis highlighted that the GH 1-3 domains are indispensable for FliI function, while the GH 4-6 domains are involved in the regulation of those activities. Moreover, we identified CG8578 (the Drosophila LRRFIP2 ortholog) as an important IFM-specific partner of FliI. Our results indicate that dLRRFIP2 interacts with the LRR region of FliI, and this interaction appears to play a key role in radial growth of the sarcomeres. The molecular relevance of this interaction is still unresolved. However, based on the AlphaFold structure prediction algorithm dLRRFIP2 and FliI might form a hetero-tetramer, which could serve as an actin crosslinking factor. Collectively, our observations further confirm that FliI is essential during muscle development, and we show that it is specifically required to promote myofilament incorporation at the Z-disc.

P-18 | Nuclear actin-dependent *Meg3* expression suppresses metabolic genes by affecting the chromatin architecture at sites of elevated H3K27 acetylation levels

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Keywords: Nuclear actin, chromatin remodeling, epigenetics, genome organization, long non-coding RNAs transcriptional control

Actin regulates the 3D genome architecture by controlling the level of H3K27acetylation. This, in turn, influences enhancer-dependent transcriptional regulation and plays a crucial role in driving gene expression changes observed upon compartment-switching. Using RNA-seq and qPCR analyses performed on total RNA from WT mouse embryonic fibroblasts (MEFs), β-actin heterozygous (HET) MEFs, and β-actin KO MEFs, here we demonstrate that expression of several lncRNAs, including *Meg3*, is directly affected by β-actin depletion. Results from ChIRP-seq, ChIRP-MS and f-RIP-qPCR show that in β-actin depleted cells *Meg3* becomes enriched and binds to H3K27 acetylation within gene regulatory regions. Activity by Contact (ABC) analysis based on RNA-seq, H3K27acetylation ChIP-seq, ATAC-seq and HiC-seq data in WT and β-actin KO MEFs shows that *Meg3* binding to H3K27 acetylation leads to loss of promoter-enhancer interactions. Results from metabolomics experiments in WT, HET and β-actin KO MEFs show these mechanisms contribute to the repression of genes involved in metabolic biosynthetic pathways for chondroitin, heparan, dermatan sulfate, and phospholipases, hence impacting their synthesis. We propose that at sites of actin-dependent increase in H3K27acetylation levels, *Meg3* interferes with promoter-enhancer interactions, potentially impairing local genome organization (or DNA looping), and negatively regulating gene expression.

P-19 | Endothelial-mesenchymal transition and possible role of cytokines in streptozotocin induced diabetic heart

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Although endothelial mesenchymal transition (EMT) has been characterized as a basic process in embryogenesis, EMT is the mechanism that accelerates the development of cardiovascular diseases, including heart failure, aging and complications of diabetes or hypertension as well. Endothelial cells lose their distinct markers and take on a mesenchymal phenotype during EMT, expressing distinct products.

Type 1 Diabetes mellitus (T1DM) was induced in rats with streptozotocin (STZ) by intraperitoneal injection at 60 mg/kg dose. Diabetic rats were randomly divided into two groups, namely, control and diabetic rats for 4 weeks. Heart, aorta and plasma samples were collected at the end of 4 weeks.

Biochemical parameters, cytokines, reactive oxygen species (ROS) protein expression of EMT markers (Chemokine C-X-C motif ligand-1 (CXCL-1), Vimentin, Citrullinated histone H3 (H3Cit), α-smooth muscle actin (α-SMA), Transforming growth factor beta (TGF-β) and versican), as well as components of extracellular matrix (Matrix metalloproteinase 2 (MMP-2), Tissue inhibitor of metalloproteinase-1(TIMP-1), Discoidin domain tyrosine kinase receptor 2 (DDR-2)) were detected by ELISA or Western blot, respectively.

Our findings: cytokines, ROS were increased in diabetic heart which induce partial EMT. Among EMT markers histone citrullination, α-SMA and CXCL-1 increased, and vimentin decreased in DM. Endothelial marker as endothelin-1 was significantly higher in the aorta of DM rats. Interestingly, TGF-β showed a significant decrease both in diabetic heart, plasma and aorta as well. Additionally, MMP-2/TIMP-1 levels also decreased in DM.

Conclusions: The identification of molecules and regulatory pathways involved in EMT should provide novel therapeutic approaches for cardiac pathophysiological conditions mediated by EMT.

P-20 | Large-scale imaging of cellular actin networks at single filament resolution using cryo-ET

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Keywords: Cell migration, Lamellipodia, Actin network architecture, Montage cryo-ET

Cell motility depends on the protrusive force generated by the actin cytoskeleton at the leading edge of cells. In lamellipodia, thin protrusions at the front of migrating cells, actin forms higher-order networks with versatile physical properties. These properties are attributed to the reciprocal regulation between actin binding protein activity and the underlying actin network geometry.

Cryo-electron tomography (cryo-ET) has been used to characterise actin networks in cellular protrusions at leading cell edge, including lamellipodia, but these descriptions have been mostly limited to small areas, capturing only 1-2 percent of cellular protrusions. More specifically, such characterisations only provide snapshots of defined regions. This does not allow filament tracing in its entirety, in order to establish the global architecture and connectivity of actin filament networks. Hence, we lack an understanding of how actin network geometries orchestrate cell migration.

To overcome these limitations, we have established a novel large-scale, high-resolution montage cryo-ET workflow, to image entire leading edge of migrating cells. Seamless 3D tomogram stitching is achieved by combining neural network based denoising strategies with novel optical flow-based algorithms. This allows segmentation and vectorisation of the actin cytoskeleton at the complete leading edge of a moving cell at single filament resolution, spanning areas up to 150 μm^2. Our computational analysis pipeline enabled us to vectorise more than one hundred thousand filaments, with filament lengths ranging up to 6 μm in a migrating keratocyte fragment. The obtained data provides a ground-truth understanding of how the geometrical complexity of the actin cytoskeleton steers directional cell migration and defines lamellipodial shape. Further, this method allows quantitative analysis of entire cellular actin network rearrangements associated with emergent migratory behaviours.

P-21 | Signal-regulated clustering of nuclear myosin vi at the androgen receptor promotes transcription

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Steroid hormone receptors are ligand-binding transcription factors essential for mammalian physiology. The androgen receptor (AR) binds testosterone mediating gene expression for sexual, somatic, and behavioral functions, and is involved in various conditions, including androgen-insensitivity-syndrome (AIS) and prostate cancer. Our previous work revealed the actin-dependent formation of biomolecular condensates consisting of the AR, the mammalian formin Disheveled-Associated Activator of Morphogenesis 2 (DAAM2) and active RNA Polymerase II (RNA Pol-II). Of note, highly dynamic nuclear F-actin polymerization by DAAM2, directly at the AR is essential for androgen signaling. To better understand actin-driven AR transcriptional activity we turned our interest to the unconventional Myosin VI, which was previously proposed to be involved in RNA Pol-II transcription (Hari-Gupta et al.,2022). Indeed, dihydrotestosterone (DHT)-dependent mass spectrometry of immunoprecipitated MyosinVI-GFP identified the AR as a prominent associator. Consistent with this, structured illumination microscopy (SIM) in prostate cancer cells revealed signal-dependent nuclear enrichment of Myosin VI, which localized in close proximity to AR- as well as RNA Pol-II clusters and the actin nucleator DAAM2. Pharmacological inhibition of actin polymerization or inhibition of the Myosin VI motor domain with 2,4,6-triiodophenol (TIP) disrupted the formation of AR-related transcriptional clusters. Furthermore, reporter gene analysis and proliferation assays supported a critical role for Myosin VI in AR signaling. Our findings thus uncover Myosin VI as a novel regulator for the spatial organization of androgen-dependent transcription.

P-22 | Mechano-induced nuclear rupture triggers nuclear actin polymerization to limit chromatin leakage in migrating cancer cells

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During confined cancer cell migration mechanical forces from the surrounding environment act onto the nucleus leading to nuclear envelope rupture (NER), chromatin leakage and genomic instability. The mechanisms that safeguard the nucleus during confined migration and NER are not well understood. Here we discovered that NER triggers dynamic nuclear F-actin formation during confined migration. We identify the mechanosensitive ATR as a formin activator which phosphorylates DIAPH3 at the autoregulatory domain to promote nuclear actin polymerization. Notably, impairment of nuclear actin assembly results in mechanically unstable nuclei and nuclear collapse during confined migration. Consistently, nuclear formin activity or actin assembly limits NER-induced chromatin leakage. Using atomic force microscopy, we demonstrate that an ATR-regulated actin nucleoskeleton promotes nuclear stiffening to support organelle integrity from within the nucleus. Our study identifies an ATR-formin module for intranuclear actin scaffolding during confined migration that could be therapeutically explored to target cancer cell dissemination.

P-23 | Novel assays determine optimal conditions for actin turnover in cell-like conditions

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A dynamic actin cytoskeleton that undergoes rapid turnover is essential for maintaining cell shape and driving intracellular transport and cellular movement. Actin turnover refers to the cyclic process of filament assembly, disassembly, and recycling of polymerizable actin monomers. While this process has been extensively studied in cells, the functions of the many actin-binding proteins (ABPs) involved in catalysing the different steps of the process remain obscure.

In this study, we have developed several novel assays to measure and compare the effect of XXX ABPs, individually or synergistically, on actin turnover. These assays, based on precise measurement of nucleotide exchange dynamics and ATP consumption, allow us to characterize the effect of ABPs over a wide range of concentrations, in bulk or in volumes similar to those found in eukaryotic cells. Overall, our work provides new methods and advances our understanding of cytoskeletal dynamics under physiological conditions.

P-24 | Nuclear Myosin 1-driven transcriptional reprogramming in adipogenesis regulates obesity in mice

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Keywords: Nuclear myocin 1, Adipogensis, metabolic reprogramming, Obesity

Adipogenesis and obesity are driven by complex metabolic and transcriptional networks. Nuclear myocin 1 (NM1) is a key regulator of cellular metabolism, influencing mitochondrial function and transcriptional networks. Our study investigates its role in adipogenesis and metabolic reprogramming. In NM1 knockout (KO) mice, we observed a significant increase in adipose tissue mass, resulting in an obese-like phenotype. RNA-seq analysis of visceral white adipose tissue from NM1 KO and wild-type (WT) mice revealed extensive differential gene expression, with 1276 genes upregulated and 845 genes downregulated ($p<0.05$, log2 fold change > 0.5 and < 0.5, respectively). These results suggest that NM1 is critical in regulating adipogenesis at the transcriptional level. Additionally, mesenchymal stem cells (mMSCs) from NM1 KO mice exhibited altered adipogenic differentiation potential, with downregulation of key adipogenic transcription factors. Our findings indicate that NM1 plays a central role in metabolic reprogramming and adipogenic gene regulation, linking mitochondrial function and glycolytic shifts to adiposity and obesity.

P-25 | Breaking hearts: FHOD3 hcm mutations disrupt cardiac sarcomere formation

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Keywords: FHOD3, actin, sarcomere, Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a heart muscle disease that can cause sudden cardiac arrest, and even death, in outwardly healthy patients. HCM is often referred to as a "disease of the sarcomere," because many cases can be traced to mutations in sarcomere proteins. Approximately 1-2% of HCM cases have been linked to mutations in Formin Homology 2 Domain Containing Protein 3 (FHOD3). FHOD3 is an actin assembly protein; it is not a structural element of the sarcomere, but it is required for sarcomere formation. However, the cellular role of FHOD3 and the molecular mechanism by which these mutations alter sarcomeres and cause disease are unknown. To better understand how these mutations lead to disease, we use an interdisciplinary approach to evaluate FHOD3 mutant behavior at multiple levels, including biochemical activity and intracellular sarcomere formation. The central hypothesis of this project is that the activity level of FHOD3 must be tightly regulated for its role in sarcomere formation. To evaluate this hypothesis, we measure FHOD3 mutant activity (1) *in vitro* with purified proteins and (2) in the context of sarcomere assembly with cardiomyocyte rescue experiments. We use *in vitro* fluorescence-based actin assembly experiments to measure the how mutations alter FHOD3's actin assembly. Then, we study how the mutant proteins affect sarcomere structure and function in cardiomyocytes. To accomplish this, we utilize cardiomyocyte-based FHOD3 knockdown and rescue experiments; changes in sarcomere structure is quantified using immunofluorescent imaging, and sarcomere function is evaluated by assessing contractility. Our previous studies showed that FHOD3-mediated actin elongation is essential in sarcomeres, while FHOD3-mediated nucleation is dispensable. However, our preliminary data show that one HCM mutant (Arg1386Gln) causes disease through a moderate increase (~30%) in actin nucleation. Our current studies of HCM mutants in the autoregulatory domains are ongoing, but we hypothesize that increasing FHOD3 actin assembly could lead to phenotypes similar to what we observed with the Arg1386Gln mutant and consistent with HCM. Through these experiments, we aim to understand how FHOD3 contributes to healthy sarcomere formation and how altered FHOD3 actin assembly disrupts cardiac muscle function, thereby providing a molecular mechanism for how FHOD3 mutations cause HCM. These studies will offer insight into how molecular changes cause higher order disease and could highlight points for therapeutic intervention in FHOD3-related HCM cases.

P-26 | Stress fiber architecture, cell migration and morphogenesis are controlled by calponin-family proteins in an isoform-specific manner

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Keywords: Calponins, α-actinin, stress fibers, dynamics, non-muscle cells

Stress fibers are the most prominent actomyosin structures in many non-muscle cells, and they have an important role in cell adhesion, migration, morphogenesis and mechanosensing. Stress fibers consist of actin, non-muscle myosin II, and a wide range of actin-associated proteins that control their assembly, maintenance and contractility. Among the abundant stress fiber components are the Calponin (CNN) family proteins, whose specific cellular functions have, however, remained unclear. Here, our primary aim was to uncover the cellular, molecular, and isoform-specific functions of Calponins. By depleting the three Calponin isoforms (CNN1, CNN2, and CNN3) individually and in combination with each other from U2OS cells, we revealed that Calponins are not negative regulators of non-muscle myosin II activity as previously suggested, but are rather vital regulators of stress fiber structure and stability. Consequently, depletion of Calponins resulted in irregular distribution of α-actinin along actomyosin bundles, reduced thickness and increased fragility of stress fibers, diminished traction forces, and defects in cell morphogenesis, migration and invasion. Interestingly, we also revealed that the non-muscle cell Calponin isoform CNN3 exhibits rapid dynamics in stress fibers, whereas the smooth muscle CNN1 displays ~10-fold more stable association with stress fibers. Consequently, expression of the smooth muscle CNN1 in non-muscle cells leads to 'smooth muscle-like' thick and static stress fibers, and inhibition of cell migration. Thus, these data elucidate the cellular functions of Calponin-family proteins and provide the rationale for the existence of smooth and non-muscle specific Calponin isoforms.

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P-27 | The nuclear import of the actin-binding moesin protein is regulated by cytoplasmic retention

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Keywords: Drosophila, nuclear import, FRAP, cytoplasmic retention

The actin-binding Moesin protein is a member of the evolutionary conserved ERM (Ezrin-Radixin-Moesin) family and the only ERM representative in Drosophila melanogaster. The cytoplasmic function of Moesin is the crosslinking of the actin cytoskeleton and plasma membrane proteins. However, Moesin is also present in the nucleus, and under certain circumstances (e.g. heat shock, ecdysone treatment) it's amount there can increase significantly. Our laboratory has shown that the nuclear activity of Moesin is pivotal in Drosophila development, but the exact mechanisms by which Moesin enters and exits the nucleus are not known.

Our goal is to elucidate the dynamics and the underlying mechanisms of the nuclear transport of Drosophila Moesin. For the investigation of the dynamics we use the fluorescence microscopy technique, Fluorescence Recovery After Photobleaching (FRAP). Our FRAP experiments revealed that in contrast to its main binding partner, actin, Moesin has a constant but weak nuclear import, and that upon induction of its import there is only a little increase in the dynamics of Moesin's nuclear entry. This suggested the existence of a mechanism that retains the protein in the cytoplasm. With the help of data available in the literature we were able to predict a 25 amino acids long motif in Moesin, which might be responsible for its cytoplasmic retention. By analyzing the nuclear import dynamics of wild type Moesin under different conditions, and by comparing the behavior of various mutant forms we showed that the motif is indeed a functional Cytoplasmic Retention Signal (CRS). Further analysis of the CRS demonstrated that the motif is able to retain even the GFP protein in the cytoplasm. Currently, we are identifying the protein that binds the CRS thus, retains Moesin in the cytoplasm.

P-28 | The roles of *Arabidopsis thaliana* actin filaments in the regulation of chromatin organization

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Keywords: Actin depolymerizing factor, *Arabidopsis thaliana*, Chromatin structure, Nucleus

Actin filaments (AFs) play important roles in intercellular transport, cellular shape determination, and cell polarity in the cytoplasm. The regulation of AF organization and dynamics involves various actin-binding proteins (ABPs). ACTIN DEPOLYMERIZING FACTOR (ADF), one of the ABPs, is involved in depolymerization at minus ends and severing of AFs. The *Arabidopsis thaliana* genome encodes 11 *ADF* genes, which are classified into four subclasses based on similarities in their amino acid sequences. Subclass I *ADF*s, including *ADF1*, *-2*, *-3*, and *-4*, are expressed in all vegetative tissues, such as leaves and roots. We have previously shown that *A. thaliana* subclass I ADFs regulate various physiological processes in plants, such as pathogen response (Inada et al. 2016 Plant Physiology), plant growth (Inada et al. 2021 Journal of Plant Research), and leaf senescence (Matsumoto et al. *in press*). We also showed that subclass I ADFs function in the regulation of chromatin organization and gene expression. *A. thaliana ADF4* knockout mutants (*adf4*) and transgenic lines in which the expression of all members of subclass I *ADF*s was downregulated (*ADF1-4Ri*) exhibit a reduction in heterochromatin size and changes in expression of many genes (Matsumoto et al. 2023 Plant and Cell Physiology). However, the mechanisms by which ADFs regulate chromatin organization and gene expression are unclear.

In mammalian cells, both cytoplasmic AFs (Wiggan et al. 2017 Scientific Reports) and nuclear AFs (Baarlink et al. 2017 Nature Cell Biology) regulate the chromatin organization. Cytoplasmic AFs are connected with chromatin through interaction with proteins embedded in the nuclear envelope and nuclear lamina (Rothballer and Kutay 2013 Chromosoma). In this study, we first examined the effects of mutations in various ABPs and nuclear envelope proteins on the heterochromatin number and size. Nuclei were visualized by staining fixed leaves with 4’,6-diamidino-2-phenylindole (DAPI) and observing them with a confocal laser scanning microscope. In ABP mutants, the number of heterochromatin regions located at the nuclear periphery was altered compared to wild type. Quantification of microscopic images revealed that the size of heterochromatin at the nuclear periphery was altered in nuclear envelope protein mutants. The morphology of the nuclear envelope was comparable between Col-0 and *ADF1-4Ri*. These results suggest that cytoplasmic AFs play a role in the regulation of chromatin organization via the nuclear envelope proteins. Regarding nuclear AFs, we are currently establishing *A. thaliana* lines to visualize nuclear AFs and to examine the function of ADF in the regulation of nuclear AFs. We discuss the function of both cytoplasmic and nuclear AFs in the regulation of chromatin organization in plants.

P-29 | Molecular mechanism of actin filament elongation by formins

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Formins control the assembly of actin filaments (F-actin) that drive cell morphogenesis and motility in eukaryotes. However, their molecular interaction with F-actin and their mechanism of action remain unclear. In this work, we present high-resolution cryo-electron microscopy structures of F-actin barbed ends bound by three distinct formins, revealing a common asymmetric formin conformation imposed by the filament. Formation of new intersubunit contacts during actin polymerization sterically displaces formin and triggers its translocation. This "undock-and-lock" mechanism explains how actin-filament growth is coordinated with formin movement. Filament elongation speeds are controlled by the positioning and stability of actin-formin interfaces, which distinguish fast and slow formins. Furthermore, we provide a structure of the actin-formin-profilin ring complex, which resolves how profilin is rapidly released from the barbed end during filament elongation.

P-30 | Nuclear actin: Shaping genome and sensing forces

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Actin cytoskeleton is critical for maintaining cellular structure and enabling processes such as migration, and signal transduction. Emerging evidence highlights the role of the cytoskeleton in mechanosensing (the ability of cells to perceive and respond to mechanical cues). In particular, much is known on outside-in signaling orchestrated by the external cues. However, recent findings shed light on the importance of intrinsic nuclear mechanosensing proteins. Nuclear β-actin is one such candidate, seeing its role in the 3D genome organization at compartment level and its impact on gene expression by regulating promoter-enhancer interactions. Here, we used mouse embryonic fibroblasts isolated from a β-actin KO mouse embryo to study nuclear actin contribution to chromatin dynamics and cellular responses to mechanical stress. We used a novel confinement device to impose controlled mechanical constraints, enabling us to study gene transcriptional responses under varying confinement conditions. Microscopy data obtained under extreme confinement revealed a significant increase in nuclear F-actin localization in the WT compared to the β-actin KO condition and reintroduction of an NLS-tagged β-actin the KO background partly rescued the KO phenotype. These preliminary results suggest that in the absence of the nuclear β-actin pool the nucleus is more protected from deformation. Notably, similar results were obtained in WT and β-actin KO mouse embryonic stem cells. Taken together, these preliminary results suggest that β-actin plays a role in the maintenance of genome integrity and organization under conditions of extreme confinement similar to those observed in compact tissues such as solid tumors. We propose a novel conserved role for the nuclear F-actin pool in mechanosensing.

P-31 | Efficient migration of *Toxoplasma gondii*-infected dendritic cells is driven by host actin-myosin network and parasite mechanical resilience

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Toxoplasma gondii has evolved efficient mechanisms to spread within the host, reaching nearly every tissue, including immunoprivileged sites such as the eye, placenta, and brain. This widespread distribution is achieved by utilizing migratory immune cells as shuttle carriers. These immune cells are specialized for traveling long distances, navigating, and crossing complex biological barriers of varying physical and nanometric scales to reach any cell within the host. However, using immune cells as a mechanism for dissemination involves a delicate balance between maintaining parasite replication and the migratory fitness of the infected immune cell, which carries an increasingly large parasite "cargo." Although molecular biological data on *Toxoplasma* hijacking of cells is well-studied, the biophysical mechanisms underlying this Trojan-horse migration remain unresolved.

In this study, we use advanced microscopy techniques, custom-designed microchannels, 3D matrices, and parasite and immune cell mutants to investigate the biophysical principles of immune cell locomotion with parasite cargo. Our results demonstrate that infected immune cells are capable of maintaining migration, pathfinding, and navigation properties even through extraordinarily tight constrictions, despite carrying large parasite "cargo." Host-Toxoplasma adaptations involve a dynamic actin network encased in a cage-like host microtubule network, ensuring an optimal balance between the host's migratory properties and the mechanical resilience of the parasitophorous vacuole under extreme deformation, caused by passage through narrow and rigid constrictions

P-32 | Exploring the localization and dynamics of the actin cytoskeleton in *Ca.* Lokiarchaeum ossiferum

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Keywords: Cytoskeleton, Asgardarchaea, Evolution

In recent years, Archaea have played a central role in understanding the transition from prokaryotic to eukaryotic cells, evidenced by the discovery of proteins once thought exclusive to eukaryotes (Eukaryotic signature proteins) within Asgardarchaeota. Notable examples are components of the Ubiquitin machinery, ESCRT III family, vesicle trafficking machinery and their regulators (small GTPases) and an Actin-based cytoskeleton. The presence of these proteins in several Asgard clades, particularly Lokiarchaeota, underscores their significance as model organisms for studying the transition from pro- to eukaryotes.

Recently our lab succeeded in cultivating *Ca. Lokiarchaeum ossiferum*, which has opened new avenues for studying their cell biology. These cells form diverse shapes, with cell bodies typically 1µm in diameter, from which several micron long protrusions extend, reminiscent of eukaryotic filopodia. Filopodia are involved in cell migration, chemokine gradient sensing, cell-cell interactions and rely on a dynamic Actin cytoskeleton. Accordingly, the protrusions of *Ca. Lokiarchaeum ossiferum* are filled with filaments of Lokiactin. Besides Lokiactin, three other Actin homologues and Profilins and Gelsolins, known regulators of Actin polymerization dynamics, are expressed. The presence of these proteins suggests a dynamic regulation of Lokiactin, which potentially is the foundation of an ancient mechanism of directed cell migration towards potential interaction partners. Understanding this is of particular interest in hindsight of the current model of eukaryogenesis, where an Asgard archaeon and an Alphaproteobacterium merge to form LECA (Last Eukaryotic Common Ancestor).

Ca. Lokiarchaeum ossiferum represents a promising model organism for studying a basic form of Actin-based cell migration, lacking the complex actin regulation machinery found in higher eukaryotes.

To understand the role of Lokiactin, we study its localization and dynamics by combining microscopy of Lokiactin in fixed and live cells with *in vitro* assays of purified Lokiactin. Initial results from immunofluorescence experiments confirm that Lokiactin forms filaments in protrusions, whereas it assembles into a dome-like structure surrounding the DNA in the cell body.

In the absence of interaction partners, protrusions are evenly dispersed in all directions. When potential symbionts are nearby, the protrusions seem to be directed towards them, suggesting a sensing mechanism. Furthermore, we successfully purified recombinantly expressed Lokiactin, enabling a detailed biochemical characterization of the protein.

P-33 | Borg3 controls septin polymerization for primary cilia formation

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Keywords: Septin, Primary Cilium, Cdc42EP/BORG, Cdc42

Septin GTPases, often referred to as the fourth component of the cytoskeleton, assemble into hexa- or octameric rods that polymerize into higher order structures, playing a role in the organization and functionality of the cytoskeletal network. They interact directly with actin filaments, microtubules, and cellular membranes, playing essential roles in critical cellular processes such as cell division and polarization. Furthermore, septins are involved in the formation of primary cilia, which serve as cellular signaling hubs. We investigate the oligomeric composition, assembly, and regulatory mechanisms of septins within the confined ciliary compartment. Using cultured cells, we demonstrate that septins enter cilia as octamers and require polymerization for ciliary enrichment. Our findings reveal that the localization of septin filaments within cilia depends on BORG3 (Cdc42EP5), which we identify as an essential component of primary cilia. Knockout of BORG3 as well as dysregulation of Cdc42 impairs septin dynamics and their enrichment in cilia. The localization of BORG3 is regulated by the cycling of the Rho-GTPase Cdc42 between its inactive and active states at the ciliary base. These insights enhance our understanding of septin function and regulation in ciliary biology and cytoskeletal organization.

P-34 | Disordered region in an actin-binding protein regulates its nuclear import

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Keywords: ERM, IDR, nuclear import, Drosophila

Drosophila Moesin, a key protein linking actin filaments to the plasma membrane, predominantly adopts a closed, non-phosphorylated conformation when imported into the nucleus. Our study investigates the regulatory role of its intrinsically disordered region (IDR) in nuclear import, as the IDR is not phase-separating, but is spatially located near the nuclear localization signal (NLS) when the protein is in closed conformation. Although actin-binding ERM proteins, including *Drosophila* Moesin, are highly conserved, their IDRs do not show any evolutionary conservation at the sequence level. However, a patch of acidic amino acids is found in the IDRs of all examined ERM proteins, at nearly the same position. We show that the acidic residues within the IDR are critical for efficient nuclear import, which is reminiscent of the mechanism previously described in the ERM-related Protein 4.1. If we replace the IDR of *Drosophila* Moesin with that of a closely related species or even human ERMs, the nuclear import of chimera proteins is weakened but preserved. These findings underscore the cooperative regulatory functions of the NLS and the IDR sequence in governing intracellular localization and highlight evolutionary differences between species-specific IDRs. Our further investigations aim to delineate the mechanistic basis for these regulatory interactions and their evolutionary conservation.

P-35 | CP/V-1 antagonism governs muscle hypertrophy by regulating the number of myofilaments

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Keywords: actin cytoskeleton, CP, V-1/myotrophin, sarcomeres, myofibrillogenesis

Cells contain a diverse range of actin-based structures, each tailored to a specific function and defined by its unique architecture, size, lifespan, and associated actin-binding proteins. Among the key regulators of these structures are actin nucleators—such as the Arp2/3 complex and formins—and the capping protein (CP), also known as CapZ. CP is an evolutionarily conserved heterodimer that binds tightly to the barbed ends of actin filaments, effectively preventing the exchange of actin subunits. Although its fundamental biochemical role seems simple, CP plays a complex part in shaping actin architecture by both competing with and indirectly cooperating with other actin-binding proteins. While CP's function in non-muscle cells, particularly within dendritic actin networks, is increasingly well understood, its role in specialized structures like sarcomeric actin arrays remains largely unexplored. In a genetic screen aimed at uncovering molecular players in the assembly of sarcomeric thin filament arrays, we discovered that the *Drosophila* orthologues of CapZ and V-1/myotrophin act in opposition during myofibrillogenesis. Our findings indicate that these proteins control the number of thin filaments—and, consequently, the diameter of myofibrils and overall muscle mass. Specifically, knockdown, overexpression, and mutagenesis experiments showed that CapZ functions as a negative regulator of myofilament number, whereas V-1/myotrophin, by sequestering CapZ, positively regulates the number of thin filaments and, indirectly, the number of thick filaments.

P-36 | SPEEDFix: Rapid fixation of migrating cells for network analysis

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Keywords: cryo-ET, Cell migration dynamics, On-demand fixation, Actin network architecture, Live-cell fluorescence microscopy

A complex interplay of actin and actin-binding proteins orchestrates the formation of dynamic protrusions at the cell leading edge, such as lamellipodia. Migration direction and speed hinge upon rapid changes in actin network architecture that control lamellipodial protrusion and retraction, yet the specific organisational changes that underlie these dynamics remain poorly understood. In particular, how the transitions between fast- and slow-moving regions in close proximity are reflected at the molecular level is not fully resolved.

In recent years, cryo-electron tomography (cryo-ET) has emerged as a powerful tool for visualising the organisation of actin networks *in-situ*, providing high-resolution insights into network geometries and architecture. However, conventional cryo-ET sample preparation approaches lack the temporal context necessary to comprehensively understand these inherently dynamic systems. As a result, they cannot reveal migration direction or identify which regions of the lamellipodia were actively protruding or retracting at the moment of vitrification. To our knowledge, no existing methodology enables controlled, robust, and reproducible on-demand fixation of migratory behaviour for downstream cryo-ET imaging.

To address this gap, we developed **SPEEDFix** (**S**tructural **P**roteomics of **E**phemeral **E**vents by **D**irect **Fix**ation), a rapid fixation method integrated with live-cell fluorescence microscopy. This system enables extraction and fixation of cells on cryo-EM grids within seconds of observing a specific migratory event, at the press of a button, preserving the native state of transient structures at the moment of interest. By combining SPEEDFix with cryo-tomography and large-scale single-filament network analysis, we can map the network architecture and transitions within protrusions. This also reveals cell-wide network arrangements, offering a powerful framework to uncover the structural correlates of emergent migratory behaviours. This approach provides new quantitative spatial insights into the cytoskeletal mechanisms that drive cell migration.

P-37 | Structure-function coordination in the SALS protein

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Keywords: actin, SALS, IDR, IDP, WH2

SALS (sarcomere length short) is a Drosophila-specific sarcomere regulatory protein that contributes to the structural and functional properties of the sarcomeric actin filaments in a complex manner (Bai et al., 2007; Shwartz et al., 2016). Overexpression of SALS results in the formation of actin-based aggregates (Farkas et al., 2024). A similar phenotype, so-called nemaline bodies, has been described in the striated muscle tissue of patients with nemaline myopathy linked to *Leiomodin* (*Lmod*) gene mutations. Although there is no genetic link between Lmod and SALS proteins from an evolutionary point of view, *in vivo* studies suggest that they may be functional homologs.

Two Wiskott-Aldrich syndrome 2 homology 2 (WH2) domains of a few tens of amino acids have been identified in SALS (SALS-WH2). Our *in silico* analysis suggests that SALS-WH2 is an intrinsically disordered region (IDR) of the protein. Our functional analysis indicates that SALS-WH2 has an actin-binding property. However, its biochemical activities do not reconstitute the biological function of the protein (Tóth et al., 2016). This suggests the need for further investigation into additional domains or sarcomeric partner proteins that may fine-tune SALS's function.

In our work, we experimentally verified the IDR nature of SALS-WH2 using fluorescence spectroscopy and thermal analysis. Based on sedimentation and fluorescence spectroscopy, our studies iden4tified a novel actin-binding region of SALS at the protein's N-terminus.

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P-38 | Selective recruitment of tropomyosin isoforms to actin filaments

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Keywords: Tropomyosin, single actin filaments, in vitro, microfluidics

Within a single cell, the actin cytoskeleton forms diverse networks of filaments, implicated in many fundamental functions. These actin networks are assembled from a common pool of actin and regulatory proteins, but how network differentiation is regulated has remained a very challenging question for decades. Recently, a family of actin binding proteins emerged as a key to elucidate this question: tropomyosins.

In every cell, multiple tropomyosin isoforms are coexpressed and have been shown to spatially segregate to specific networks. Tropomyosins has been proposed to give filaments an identity by regulating the recruitment and activity of the other cytoskeletal proteins. However, the mechanisms controlling tropomyosin localization are still unknown.

Our main hypothesis is that each tropomyosin isoform recognizes specific biochemical signatures/cues, that determine to which actin network and filament they get recruited. Such signatures include actin isoforms, post-translational modifications, other tropomyosins and actin binding proteins.

Experiments are performed with an in vitro reconstituted system, on purified and fluorescently labelled proteins. We take advantage of a microfluidic setup, designed in the lab, to assemble single actin filaments along with regulatory proteins of interest (nucleators, side binding proteins, enzymes). Filaments are then exposed to tropomyosins, and we quantify the assembly and disassembly of tropomyosin clusters, depending on the precise biochemical conditions.

Our results reveal a new recruitment mechanism mediated by another actin binding protein, shedding light on previously misunderstood observations in cells.

P-39 | Topographical cues orchestrate actin nucleation via N-WASP–FBP17 nano-condensation

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Membrane surface topography plays a critical role in guiding local actin remodeling, which underpins numerous signaling pathways in cells. However, the spatiotemporal coordination between membrane deformation and actin nucleation remains poorly understood. By employing nanolithography to precisely manipulate membrane curvature, we developed an in vitro reconstitution system to study actin polymerization on membranes with defined nanoscale features. Our results show that curved membrane sites promote localized actin nucleation through the synergistic action of N-WASP, FBP17, and Cdc42, mediated by multivalent interactions that drive nanoscale clustering. When Cdc42 is globally distributed, localized curvature cues selectively initiate N-WASP clustering through its interactions with the curvature-sensing BAR-domain protein FBP17. We further demonstrate that the enhancement of actin nucleation is sensitive to the stoichiometry between FBP17 and N-WASP, which is modulated according to the curvature radius. These findings uncover how nanoscale membrane geometry can differentially regulate global versus localized actin assembly, providing insight into the coordination of membrane shape and cytoskeletal dynamics during complex cellular functions.

P-40 | Czech-BioImaging: Infrastructure dedicated to users for 10 years

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Czech-BioImaging is the national research infrastructure advancing biological and medical imaging in the Czech Republic. Since its launch in 2015, it has provided open access to cutting-edge imaging technologies and expert support for researchers across the scientific community. In 2025, Czech-BioImaging celebrates 10 years of providing open access to imaging services for the scientific community.

The infrastructure connects 16 core facilities in five major Czech cities, offering a broad portfolio of imaging techniques to users from academic, medical, and private sectors. Technologies include light and fluorescence microscopy, super-resolution and electron microscopy, correlative light and electron microscopy (CLEM), and preclinical and clinical medical imaging—supported by comprehensive image processing and data analysis tools. Researchers are guided throughout the entire workflow, from experimental design to data interpretation, across fields such as cell and molecular biology, genetics, physiology, parasitology, tumor biology, neuroscience, developmental biology, and pathology.

As host of three Euro-BioImaging Nodes, Czech-BioImaging is part of the European research infrastructure landscape, fostering international collaboration and scientific excellence. Its distributed model ensures regional accessibility and interdisciplinary cooperation nationwide.

Beyond technology access, Czech-BioImaging supports the community through training courses, workshops, user project support and its annual conference, and collaboration with instrumentation manufacturers, contributing to both education and innovation in imaging.

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P-41 | The impact of toxofilin$_{69\text{-}196}$ fragment on the disassembly of actin

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Toxofilin is a small actin-binding protein secreted by the intracellular pathogen *Toxoplasma gondii* during host infection. Many effects of this 27 kDa protein were previously studied on actin, focusing mainly on acting monomers. In this work we were studying the effects of toxofilin on existing filaments and on each step of filament formation with the application of differential scanning calorimetry (DSC) and fluorescence spectroscopy techniques. Our results suggest that the 69-196 residues of toxofilin carries all functions and effects of the full-length one. The presence of this truncated protein significantly increases the critical concentration of actin and decreases the polymerization rate of actin. The DSC measurements show effects of depolymerization induced by the binding of toxofilin on preexisting actin filaments. Temperaturedependent fluorescence spectroscopy measurements revealed that toxofilin causes conformational changes in the actin subdomain 1. Our results provide a more comprehensive picture of the molecular changes in the cytoskeleton during parasite infection.

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P-42 | Structural insight into small-molecule resistance in profilin and actin mutant malaria parasites

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Plasmodium spp. are intracellular parasites responsible for malaria, a disease that caused over 608 000 deaths in 2022. With rising resistance to frontline antimalarials, new treatments are urgently needed. *Plasmodium falciparum* (*Pf*) has two actin isoforms: actin 1, crucial for motility and invasion throughout the life cycle, and actin 2, expressed only during sexual stages. *Pf* actins are about 80% identical to human actins but form shorter, more dynamic filaments. Profilins, key regulators of actin dynamics, are structurally conserved but have low sequence identity across species. *Pf* profilin has a typical profilin core fold with a unique βhairpin arm participating in actin binding.

We have characterized a set of compounds from the Medicines for Malaria Venture (MMV) that block red blood cell invasion by targeting *Pf* actin 1 and profilin. These MMV020291 series compounds inhibit actin polymerization and enhance profilin's ability to sequester actin monomers, disrupting parasite invasion. Mutations conferring resistance were found in both *Pf* actin and profilin. The MMV020291-resistance-causing mutations K124N and N154Y do not affect the folding of *Pf* profilin but they decreased its actin 1 sequestering effect. We have recently determined the structure of the actin 1 mutant (M356L) and now aim to study further the structural basis for the MMV compound resistance and the interface between *Pf* actin1 and profilin in the presence and absence of the MMV020291 compounds.

P-43 | The solenoid protein GAC provides momentum for the actin-myosin motor in Apicomplexa

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Within the inner membrane complex of apicomplexan parasites exists a machinery, named glideosome, constituted by an actin-myosin motor, which is assisted by several associated proteins. The glideosome enables parasites to invade host cells and is essential for parasite survival. The glideosome-associated connector (GAC) was identified as a connector between the parasite plasma membrane and actin. GAC is a unique protein, conserved across apicomplexan parasites and indispensable for gliding motility. To date, GAC, with approximately 280 kDa, is the largest armadillo-repeat protein for which a structure is known and is also the largest armadillo-repeat protein in apicomplexan parasites. GAC has two-fold conformational flexibility: (i) The C-terminal pleckstrin-homology (PH) domain functions as an anchor, locking GAC in a compact conformation, and may function as a switch between open and closed states upon lipid sensing. (ii) The giant solenoid domain confers spring-like flexibility, which could provide elasticity, store mechanical energy, and accelerate/amplify the movement generated by the myosin power stroke during gliding motility. We have determined the structures of full-length and several truncated versions of GAC using X-ray crystallography and cryo-EM. Our structural studies are complemented by biochemical and biophysical approaches to understand which parts of this solenoid protein interact with actin and to shed light on its role in parasite gliding motility.

P-44 | Identification of novel importins responsible for the nuclear transport of actin

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It is now well known that actin is also present in the cell nucleus and is an important participant in all fundamental nuclear functions. The dynamic balance between cytoplasmic and nuclear actin pools is crucial for its nuclear activity, however, our knowledge about the mechanisms that establish this balance are still controversial. In mammalian cells, Importin 9 (Ipo9) has been identified as a key nuclear import factor of actin but there are data to suggest that this is not a universal phenomenon.

To address this problem, we developed a modular genetic system in *Drosophila melanogaster* to investigate the robustness of actin's nuclear localization and its biological significance in the fly. We generated transgenic lines expressing actin tagged with a 15 amino acid long nuclear export signal (NES) to force its removal from the nucleus, and monitored the viability of the animals. We also disrupted the sumoylation site at K285 previously implicated in actin nuclear retention, examined the role of Ipo9 by its deletion, and screened for additional actin importins by *in vitro* and *in vivo* assays.

Our results showed that NES-tagging reduces nuclear actin levels by 25% however, this reduction did not affect viability and did not cause detectable phenotypic changes. Our experiments demonstrated that K285 sumoylation does not play a role in nuclear retention of actin, but deletion of *Ipo9* led to an 18% reduction in nuclear actin levels and a 33% reduction in viability. Interestingly, when *Ipo9* deletion was combined with NES-tagging of actin, we observed 67% lethality, indicating that the two effects act synergistically.

As the amount of nuclear actin was only partially reduced even in the complete absence of Ipo9, this clearly indicated the presence of parallel actin import mechanisms. Therefore, we performed protein-protein interaction studies and identified four β-importins that are able to bind actin, providing evidence for redundant nuclear import of actin. Of the four novel actin importin candidates, we have so far focused on further investigation of Cadmus (Ipo13 in mammals). Our modular genetic system has revealed that reducing Cadmus levels in combination with nuclear actin export enforced by the NES tag does not impair viability, but causes 100% male sterility. In the testes of affected flies, we detected defects in spermatid elongation, disrupted actin cones and abnormal nuclear morphology.

Collectively, our findings highlight the complexity and redundancy of nuclear actin import mechanisms and provide direct evidence that multiple pathways ensure the nuclear localization of actin. Furthermore, our results suggest a potential link between nuclear actin regulation and the process of spermatogenesis, and provide a basis for further investigation of the regulatory role of nuclear actin and its transport mechanisms.

P-45 | Evolution of actin assembly in endocytosis via WDS proteins

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Branched actin networks generated by the Arp2/3 complex are crucial for endocytic membrane invagination in many eukaryotes. The WISH/DIP/SPIN90 (WDS) family of proteins are the major candidates to be responsible for initiation of actin assembly by activation of Arp2/3 complex. Studies on Dip1 in *S. pombe* have supported this mechanism but whether WDS proteins possess such activities across species is not clear. WDS proteins have different temporal kinetics in actin assembly. Additionally, the molecular and structural understanding of how WDS proteins bind and activate Arp2/3 complex to assemble actin network is incomplete. In my PhD work, I will address these questions by biochemical characterization of WDS family protein members across representative species. I will employ single-molecule imaging of reconstituted purified proteins to study the activity of WDS proteins and role of other endocytic proteins on its activity. Using this system, I will also screen potential regulators of WDS proteins and test their effects on actin assembly *in vivo*. In addition, I will attempt to understand the structural basis of actin nucleation by these proteins. This study will allow us to understand the evolutionary differences and similarities in mechanisms of temporally regulated actin network formation at sites of endocytosis.