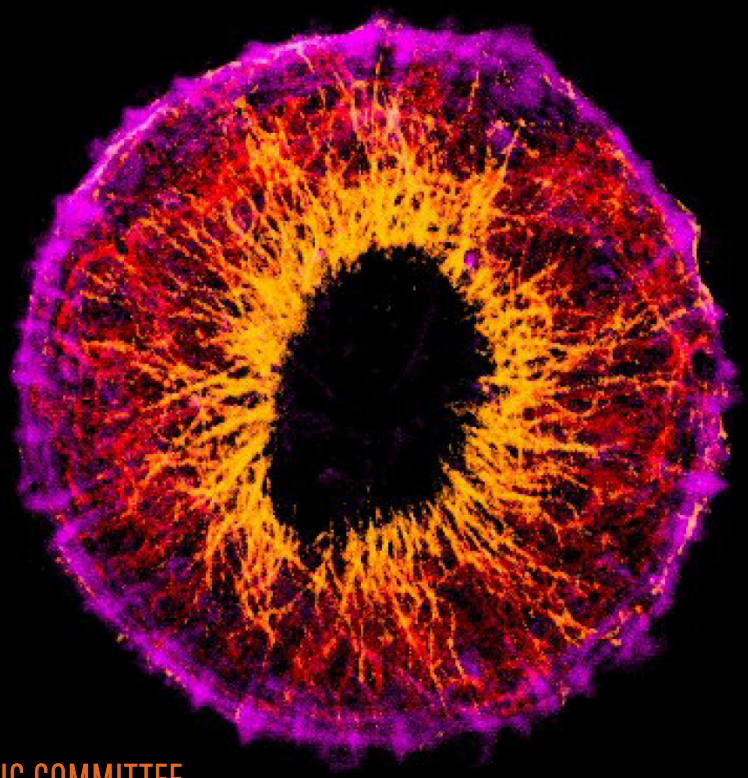
EUROPEAN INTERMEDIATE FILAMENT MEETING

24 - 27 SEPTEMBER 2025 / LYON - FRANCE



KEYNOTE SPEAKER

Roy Quinlan - *England*

SPEAKERS

Giulio Agnetti - *Italy*
Anna Akhmanova - *The Netherlands*
Pierre Coulombe - *USA*
John Eriksson - *Finland*
Daniel Garcia-Gonzalez - *Spain*
Elly Hol - *The Netherlands*
Pierre Joanne - *France*
Sarah Köster - *Germany*
Cécile Leduc - *France*
Rudolf Leube - *Germany*
Jean-Baptiste Manneville - *France*
Ohad Medalia - *Switzerland*
Milos Pekny - *Sweden*
Dolores Pérez Sala - *Spain*
Stéphanie Portet - *Canada*
Nicole Schwarz - *Germany*
Diana Toivola - *Finland*

SCIENTIFIC COMMITTEE

Pascale Bomont, *Chair - Neuromyogene Institute, Lyon*
Onnik Agbulut - *Sorbonne University, Paris*
Anne Bertrand - *Myology Institute, Paris*
Gisèle Bonne - *Myology Institute, Paris*
Sandrine Etienne-Manneville - *Pasteur Institute, Paris*

BOOK OF ABSTRACTS

ACKNOWLEDGMENTS

INSTITUTIONS & PARTNER SUPPORT

AFMTÉLÉTHON INNOVER POUR GUERIR
EURO BIOIMAGING
MARION ELIZABETH BRANCHER FONDS MEB SOUTENIR LA RECHERCHE SUR LES CELLULES SOUCHES ADULTES
Inserm La science pour la santé From science to health
Institut thématique Biologie cellulaire, développement et évolution
JCB Journal of Cell Biology
LMNA Cardiac Diseases Network
sbcf French Society for Cell Biology
sfm SOCIÉTÉ FRANÇAISE DE MYOLOGIE
SORBONNE UNIVERSITÉ
The Company of Biologists
bionomous
LABORATOIRE WATCHFROG
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Chair: Pascale Bomont

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Ohad Medalia - University of Zurich, Switzerland

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19:00 - 19:15 Vimentin intratumoral heterogeneity shapes nuclear mechanotransduction, DNA damage sensing, and glioblastoma cell survival
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- *Raphaël Crépin - Université Evry Paris-Saclay, France*
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14:00 - 16:00 POSTER SESSION 1 & Coffee break with sponsors

THURSDAY, SEPTEMBER 25TH

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- *Elisabeth Cortier - Université Claude Bernard Lyon 1, France*
- *Maria Ilomäki - Åbo Akademi University, Finland*
- *Audrey Smith - University of North Carolina, USA*
- *Maritzaida Varela Salgado - Institut Jacques Monod, France*

12:45 - 14:00 Lunch - Sponsored by LABORATOIRE WATCHFROG

FRIDAY, SEPTEMBER 26TH

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Domitille Callon - Sorbonne Université, France

09:45 - 10:00 Atlas of DES (desmin) mutations
Andreas Brodehl - University Bochum, Germany

10:00 - 10:30 Giulio Agnetti - *University of Bologna, Italy*

10:30 - 11:00 Coffee break

11:00 - 11:30 Prizes

11:30 - 12:00 Closing remarks

12:00 Departure with Lunch boxes *(on reservation when you register)*

ABSTRATCS

Opening
Keynote lecture

OPENING KEYNOTE LECTURE

KEYNOTE SPEAKER

Roy QUINLAN,

University of Durham, England

Our Intermediate Filament World and beyond...

In this plenary lecture, I will take a personal view to prepare the stage for the amazing headline acts that are to follow at this Intermediate filament conference in Lyon. I shall connect our present understanding of IFs to past milestones in the evolution of our field and the research questions that have engaged our community and others. Structure and assembly pathways have and continue to be a key area of investigation. In the last 5 years, the discoveries afforded by cryo-EM and associated computational and mathematical tools provide a solid structural basis for IF diversity and their distinct biomechanical properties. Such information gives reason to, and inspires more, detailed investigations of the cell, tissue, developmental and differentiation programmes of IF expression. It most certainly heralds a new phase of discovery and greater recognition of the importance and bioengineering potential for IFs across the natural sciences. The role of IFs in the stress response, their relative stability and integration with the rest of the cytoskeleton and with organelles and other membrane compartments means IFs are very more than just "filaments". I will explore some potential avenues from light guides to "wetware" components and a few stops in-between as we seek to understand better and promote our IF world in the cellular solar system. Exhilarating times lie ahead - of that I'm very sure!!!!

SESSION 1
Structure & Biophysics of IFs

SESSION 1 - STRUCTURE & BIOPHYSICS OF IFS

INVITED SPEAKER

Ohad MEDALIA,

Baihui Wang, Qiang Luo, Matthias Eibauer, Rajaa Patersky

University of Zurich

The molecular basis of lamin specific chromatin interactions

In the cell nucleus, chromosomes are anchored to the nuclear lamina, a structure composed primarily of lamins and their binding proteins. The nuclear lamina constitutes a fibrillar layer that is situated beneath the inner nuclear membrane. The nuclear lamina, a structural layer of proteins that bridges between the DNA and the nuclear membrane, impact chromatin organization through the interaction with lamin associated domains (LADs) within the densely packed heterochromatin regions. Employing a combination of approached allowed us to reolve the concentration of nucleosomes at the lamin-chromatin interface, at sub-nanometer resolution. TThe depletion of lamin A/C is resulting in a reduction of chromatin density at the lamina interface, suggesting the involvement of lamins in a direct interaction with chromatin. Employing cryo-electron microscopy (cryo-EM), we have identified the specific motif of lamin A tail domain that interacts with nucleosomes, distinguishing it from other lamin isoforms. Genome-wide analyses have revealed lamin-dependent macroscopic-scale alterations in gene expression and chromatin remodeling. The present findings offer insights into the dynamic and specific interplay between lamin isoforms and chromatin, thereby providing a more comprehensive understanding of the process of nuclear lamina-chromatin tethering.

SESSION 1 - STRUCTURE & BIOPHYSICS OF IFS *

Jonas RECKMANN[1,2],

Franziska Klag[1], Sabrina Voß[2], Andreas Brodehl[2], Hendrik Milting[2], Volker Walhorn[1]

1. Biophysics University of Bielefeld - Germany
2. HDZ Bad Oeynhausen - Germany

Unraveling the essential role of a highly conserved motif in Desmin's Coil 2 domain for intermediate filament assembly

Unraveling the Essential Role of a Highly Conserved Motif in Desmin's Coil 2 Domain for Intermediate Filament Assembly. A Multi-Technique Approach using Cell Transfection, Confocal Microscopy, and Atomic Force Microscopy.

Background: Cardiomyopathies are frequently caused by DES mutations (prevalence between 1 % and 2 % for DCM). The intermediate filament protein desmin, encoded by the human DES gene, forms scaffolds within skeletal and cardiac muscle cells. Data from the Human Million Exome Browser reveal a highly conserved region within desmin's coil 2 domain across multiple species. Public disease databases contain several variants of uncertain significance (VUS) within this region of desmin making pathogenetic classification difficult. Thus, the functional characterization of DES-VUS has an impact on genetic counselling and provides further mechanistic insights into the disease mechanism.

Material and Methods: Transfection experiments were performed in desmin-null SW13 cells and desmin-expressing H9c2 myoblasts. Intracellular desmin assembly was analyzed using confocal microscopy coupled with computational deconvolution. To complement cellular findings, atomic force microscopy (AFM) was used to resolve in a cell-free environment the nanoscale structural properties of different desmin mutants.

Results: Mutations in a highly conserved stretch at the C-terminus of desmin coil 2 domain disrupted filament assembly. Of the 22 VUS tested, 14 exhibited aberrant cytoplasmic aggregation in cell culture, while the remaining variants assembled into filaments resembling wild-type (WT) desmin. AFM analysis revealed that aggregation-prone variants occasionally formed proto-filamentous structures. Furthermore, homology-based 3D modeling of desmin, leveraging vimentin structural data, pinpointed a cluster of missense mutations correlated with aggregate formation.

Conclusion: The C-terminus of the coil 2 desmin domain is essential for proper filament assembly. xxx Variants of unknown significance may disturb filament formation. Results of individual desmin mutants xxx may be of impact for rational genetic counseling.
Outlook: Predicted pathogenicity by AI or in silico tools do not completely match experimental findings. Future studies should precisely identify the assembly step at which pathogenic mutations disturb desmin polymerization. Additionally, the interaction between desmin aggregates and cellular quality control pathways requires systematic investigation.

**Candidate for best oral communication award*

SESSION 1 - STRUCTURE & BIOPHYSICS OF IFS *

Camille RODRIGUEZ[1],

Hyeontae Jeong[2], Ian Y. Wong[2], Robert D. Goldman[3], Ming Guo[1]

1. Massachusetts Institute of Technology, Cambridge - USA
2. Brown University - USA
3. Northwestern University - USA

Impact of Vimentin Intermediate Filaments on 3D Collective Behavior

Collective cell migration is crucial for many biological processes and diseases. During cell migration the cytoskeleton maintains the structural and mechanical integrity of the cells. One major component of the cytoskeleton is intermediate filaments (IFs). Previous studies on 2D have shown that vimentin intermediate filaments (VIFs) form a highly stretchable network and work synergistically with the rest of the cytoskeleton to enhance cell deformability and cell migration. However, in a physiologically relevant 3D environment, the role of VIFs in collective cell migration and cell-matrix interactions remains largely unknown. Here we utilize MCF-7 human epithelial cells with controllable vimentin gene expression to study the role of mature VIFs in 3D collective cell behaviors.

We find that VIFs significantly enhance cell migration in 3D cell clusters, along with increasing cell-matrix interactions evidenced by increased cluster boundary fluctuations and gel displacements generated by these clusters. MCF-7 clusters without vimentin (uninduced) have a spherical-like morphology with minimal protrusions from the cluster; with mature VIFs in MCF-7 clusters (induced), the morphology is elongated with multiple protrusions. Furthermore, we observe enhance cell migration in the induced MCF-7 clusters with VIFs. Through traction force microscopy we find that induced clusters generate significantly larger contractile and protrusive gel displacements confirming stronger cell-matrix interactions. In addition, through transcriptomics we observe significant upregulation of gene expressions associated with cell migration and matrix-adhesion. We find an upregulation of ROBO1 and PODXL, regulating cell projections while weakening cell-cell adhesion, along with a downregulation of PKP1, associated with desmosomes.

Our results suggest that VIFs play an important role in 3D multicellular migration and promote invasive-like behavior through cell-matrix interactions evident through increased protrusion and boundary fluctuations, migration, and matrix displacement.

**Candidate for best oral communication award*

SESSION 2
Mechanobiology of IFs

SESSION 2 - MECHANOBIOLOGY OF IFS

INVITED SPEAKER

Jean-Baptiste MANNEVILLE[1],

Xiuyu Wang[1], David Pereira[1], Nathan Lardier[3], Isabelle Perfettini[2], Florent Péglion[2], Emma Van Bodegraven[2], Ryszard Wimmers[3], Ananya Roy[4,5], Karin Forsberg-Nilsson[4,5], Alexandre Baffet[3], Bruno Goud[3], Sandrine Etienne-Manneville[2]

1. Laboratoire Matières et Systèmes Complexes, Université Paris Cité, CNRS UMR7057, 10 Rue Alice Domon et Léonie Duquet, F-75013, Paris, France
2. Cell Polarity, Migration and Cancer Unit, Institut Pasteur, CNRS, UMR3691, F-75015, Paris, France
3. Institut Curie, PSL Research University, CNRS UMR144, Paris, F-75246 France
4. Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden
5. Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Probing the mechanics of nuclear and cytoplasmic intermediate filaments in living cells with optical tweezers

Although extensively studied in vitro, the mechanics of intermediate filaments (IFs) is still largely unexplored in living cells. Here we probe nuclear and cytoplasmic IF mechanics using an intracellular optical tweezers-based micromanipulation technique.

In the first part of the presentation, I will give evidence for lamin-driven nuclear stiffening in glioblastoma malignancy. Glioblastoma multiforme (GBM) is the most aggressive primary brain tumour, characterised by a poor prognosis and limited treatment options. Recent studies have highlighted the role of cell mechanics in cancer progression, and particularly that of the nucleus and its lamina composed of A and B-type lamin isoforms. We first quantify lamin levels across eleven patient-derived GBM cell lines and human radial glial cells, revealing heterogeneous lamin expression patterns. We then use optical tweezers to measure the mechanical properties of the nucleus in living GBM cells and combine in vitro and in vivo analyses of GBM cell proliferation, migration, and invasion. We find that increased nuclear stiffness correlates with greater GBM proliferation and migration and propose a physical model to quantitatively describe the relationship between the expression levels of A- and B-type lamins and nuclear mechanics. Our results suggest that lamins regulate nuclear mechanics which in turn influence GBM aggressiveness, and have potential implications for therapeutic strategies targeting nuclear mechanics.

In the second part of the talk, I will show that microtubules promote the stiffening of vimentin IF bundles under repeated mechanical stress in living cells. Measuring the force-deflection curves of the filaments in live cells allows us to quantify the rigidity of vimentin IFs and microtubules and to show that microtubules have a lower lateral effective stiffness than vimentin IFs. We then apply repetitive stress on the same cytoskeletal bundle to show that vimentin IFs, but not microtubules, stiffen more than three times upon repeated deflections. In cells knockout for vimentin, the mechanical properties of microtubules are unchanged. Destabilising or stabilising microtubules or increasing microtubule acetylation do not affect the lateral stiffness of vimentin IFs. In sharp contrast, destabilising or acetylating microtubules significantly reduces vimentin IF stiffening upon repeated deflection. Our findings highlight the importance of the interactions between microtubules and IFs in cell mechanics and suggest that vimentin IFs are mechanosensitive structures which exhibit microtubule-dependant and history-dependent mechanoresponses.

SESSION 2 - MECHANOBIOLOGY OF IFS

Winnok DE VOS[1],

Sarah Peeters[1]*, Sarah De Beuckeleer[1], Hera Kim[2], Romy Timmer[2], Matthieu Piel[3], Coen Campsteijn[2°]

1. University of Antwerp
2. Oslo University
3. Institut Curie
**First Author*
° Shared senior author

An adaptive response to nuclear envelope stress controls the invasive potential of glioblastoma cells

Glioblastoma (GBM) is the most lethal primary brain tumor in adults, infamous for its cellular heterogeneity and extensive tissue infiltration. The increased stiffness and intrusive nature impose significant mechanical load onto GBM cells, which may render them vulnerable to nuclear envelope (NE) stress. To investigate this, we have quantified nuclear dynamics in a panel of GBM cell lines. We found a significant, but cell-type dependent degree of nuclear dysmorphy, which correlated with the level of nuclear deformation over time. Using live cell imaging, we found that all GBM cells experience NE ruptures, which are exacerbated under mechanical confinement. When examining GBM cell migration in 3D collagen matrices and human iPSC-derived cerebral organoids, we discovered an inverse relationship between the level of NE stress and the invasive capacity.

A targeted proximity proteomics experiment, using TurboID labelling, allowed identification of a mechanoregulatory circuit that is specifically upregulated in more invasive cells. Targeted knockdown or pharmacological inhibition of its main drivers abrogated GBM invasiveness. Thus, we conclude that GBM cells experience NE stress and identified an adaptive signalling hub that could represent a new targetable vulnerability in GBM.

SESSION 2 - MECHANOBIOLOGY OF IFS *

Giulia SULTANA,

Leila S Coelho-Rato, Arun Venu, John E Eriksson

Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, 20520 Turku, Finland

Vimentin scaffolds YAP to support directional persistence in Wound Healing

Chronic wounds, including diabetic ulcers, affect millions worldwide. Despite extensive research, the molecular mechanisms underlying wound healing remain poorly understood, particularly the dynamics of cell migration and orientation. Vimentin, a type III intermediate filament protein, plays a crucial role in orchestrating wound healing signalling and supports directional persistent cell migration, which is essential for efficient wound closure. The removal of vimentin significantly delays wound healing due to the loss of cell directionality. Additionally, YAP, a key regulator of wound healing, cellular proliferation, and migration, interacts directly with vimentin and maintains physiological cell directionality. The downregulation of either protein leads to dysregulated wound healing, which can cause non-healing wounds. The study aims to elucidate the bidirectional interplay between vimentin and YAP, mapping their molecular mechanisms and studying the effects of YAP functions through gain-of-function and loss-of-function.

In our research, we have found that YAP is downregulated in vimentin-deficient fibroblasts and directly interacts with vimentin in wild-type cells. Downregulation of YAP in wild-type cells was shown to mimic the behavior of inadequate cell directionality, as observed in the vimentin-deficient cells. Moreover, reintroduction of the YAP WT plasmid in the VIM -/- system seems to revert the phenotype of coordinated cell directionality. The novelty of this project lies in bridging mechanical cues to the intermediate filament machinery.

These preliminary results indicate that, through this interaction, YAP and vimentin cooperate to maintain physiological cell directionality, which is needed for the successful closure of a wound. Therefore, our key hypothesis is that YAP and vimentin are dependent on each other and needed to support directional cell migration, and downregulation of either protein leads to dysregulated wound healing, which in turn can cause non-healing wounds.

**Candidate for best oral communication award*

SESSION 2 - MECHANOBIOLOGY OF IFS *

Elvira INFANTE,

Emmanuel Terriac, Matthieu Gelin, Hugo Siegfried, David Pereira, Vanessa Roca, Hugo Varet, Sara Khalilian, Reinier Rietveld, Atef Asnacios, Emma J van Bodegraven, Sandrine Etienne-Manneville

Institut Pasteur - UMR 3691, France

Vimentin intratumoral heterogeneity shapes nuclear mechanotransduction, DNA damage sensing, and glioblastoma cell survival

Glioblastoma (GBM), the most common and aggressive primary brain tumour, has a particularly poor prognosis, largely due to its pronounced but poorly understood heterogeneity. Among the key contributors to this heterogeneity is vimentin, a type III intermediate filament protein traditionally linked to epithelial-mesenchymal transition, cell motility and invasion. Single-cell transcriptomic analyses of IDH-wild-type GBM specimens show a coexistence of vimentin-positive and vimentin-negative tumour cells, with vimentin expression exhibiting spatial heterogeneity. Using a multidisciplinary approach that combined single-cell RNA sequencing, biophysical techniques and 3D models, we uncovered a dual role for vimentin in GBM progression. CRISPR-mediated knockout or siRNA-mediated depletion of vimentin in GBM cell lines caused transcriptome-wide remodelling, marked by downregulation of key DNA damage response pathways, including ATM, Chk1 and H2AX. These cells displayed defective checkpoint activation, impaired γH2AX induction and enhanced cell survival.

Loss of vimentin lowers cytoplasmic stiffness, facilitates nuclear flattening and accelerates migration through tight constrictions, albeit at the cost of more frequent nuclear envelope ruptures. Strikingly, externally imposed compression phenocopied vimentin loss: histone marks associated with heterochromatin (H3K27me3, H3K9me3) and acetylation (AcH3K9) declined, DNA damage sensing proteins were downregulated and radiosensitivity was reduced. Thus, nuclear compression, whether caused by extracellular forces or by intrinsic softening due to vimentin depletion, is sufficient to reprogramme chromatin and suppress genome integrity pathways.

Together, our findings reveal an unconventional function of vimentin wherein its loss—whether intrinsic or mimicked by external compression—disrupts chromatin organisation and disables DNA damage response pathways. This mechanistic failure allows tumour cells to bypass DNA damage checkpoints and apoptosis, thereby promoting survival and contributing to therapy resistance and further intratumoural heterogeneity. Vimentin expression and mechanical stress emerge as intertwined regulators of genome integrity, highlighting vimentin as a potential target to improve GBM treatment.

**Candidate for best oral communication award*

SESSION 3
Dynamics of IFs

SESSION 3 - DYNAMICS OF IFS

INVITED SPEAKER

Cécile LEDUC,

Institut Jacques Monod, France

Continuous self-repair protects vimentin intermediate filaments from fragmentation

Intermediate filaments are key regulators of cell mechanics. Vimentin, a type of intermediate filament expressed in mesenchymal cells and involved in migration, forms a dense network in the cytoplasm that is constantly remodeling through filament transport, elongation/shortening, and subunit exchange. While it is known that filament elongation involves end-to-end annealing, the reverse process of filament shortening by fragmentation remains unclear. Here, we use a combination of in vitro reconstitution, probed by fluorescence imaging and atomic force microscopy, with theoretical modeling to uncover the molecular mechanism involved in filament breakage. We first show that vimentin filaments are composed of two populations of subunits, half of which are exchangeable and half immobile. We also show that the exchangeable subunits are tetramers. Furthermore, we reveal a mechanism of continuous filament self-repair, where a soluble pool of vimentin tetramers in equilibrium with the filaments is essential to maintain filament integrity. Filaments break due to local fluctuations in the number of tetramers per cross-section, induced by the constant subunit exchange. We determine that a filament tends to break if approximately four tetramers are removed from the same filament cross-section. Finally, we analyze the dynamics of association/dissociation and fragmentation to estimate the binding energy of a tetramer to a complete versus a partially disassembled filament. Our results provide a comprehensive description of vimentin turnover and reveal the link between subunit exchange and fragmentation.

SESSION 3 - DYNAMICS OF IFS *

Quentin FRENGER,

Etienne Morel

INSERM UMR-S1151, CNRS UMR-S8253, Institut Necker Enfants Malades, Université Paris Cité, Paris, France

The vimentin network supports autophagosome biogenesis in response to starvation

Cells depend on their endomembranes coordination and proper morphodynamics regulation to adapt to acute stress conditions such as nutrient starvation. Autophagy is a stress response mechanism by which cytosolic content is captured by double membrane vesicle termed autophagosome, which ensures transport to lysosomes for degradation and material recycling.

This process starts at the endoplasmic reticulum (ER) where membrane contact sites with several organelles provide adequate conditions for de novo autophagosome formation. Vimentin is a type III intermediate tightly associated with the ER. Knowing the putative importance of cytoskeleton, such as actin and microtubules in membrane remodeling and transport, we asked if the vimentin IF network could act as a scaffold that regulates autophagosome biogenesis and dynamics of autophagic processes. Here, we show that vimentin is quickly reorganized to the perinuclear region in response to starvation and that its interaction with the ER increases. We also show that autophagy is impaired in cells treated by Withaferin-A, a vimentin destabilization drug.

We specifically show that sites of autophagosome biogenesis sites occur at ER and vimentin overlapping regions and that the degradation of autophagic cargo is less effective in cells displaying destabilized vimentin network. These findings reveal that vimentin/ER membrane crosstalk is a key regulator of autophagosome formation and autophagic flux, highlighting its importance in cellular stress response. We now aim to decipher the molecular mechanisms behind the regulation of autophagy by vimentin.

**Candidate for best oral communication award*

SESSION 3 - DYNAMICS OF IFS *

Leticia ARIAS[1],

Stéphanie Portet[2], Pascale Bomont[1]

1. ERC team, INMG-PGNM, Inserm U1315, Lyon University, France
2. Department of Mathematics, University of Manitoba, Winnipeg, MB, Canada

Live Dynamics of Neurofilaments

Neurofilaments (NFs) are neuronal Intermediate Filaments (IFs) that constitute the most abundant component of mature neurons. Earlier studies in mouse revealed that NFs bear essential functions in the nervous system and play critical roles in neurodegeneration (1,2). In human, beyond the genetic involvement of NF genes in various neuropathies, abnormal NF aggregation has been identified as an early pathological hallmark for most neurodegenerative diseases (3). While NF proteins have been pointed as pivotal actors in the maintenance of the nervous system, very little is known regarding their dynamics and signaling in vivo and what triggers neurodegeneration in disease.

To expand the knowledge of NF biology, our laboratory developed novel tools and methodologies in zebrafish, a species suited to simultaneously explore multiple genetic settings and live-imaging in optically transparent animals. Thus, we generated novel NF transgenic lines carrying a photoconvertible fluorophore to investigate with spatio-temporal resolution the behavior of NFs within axons. In agreement with previous studies performed in vitro, we reveal that NFs are dynamic in the developing nervous system. Investigating this in vivo, we were able to further monitor the anterograde and retrograde flux of NFs during development. Here, we will present our latest results on this novel methodology and on the mathematic modeling of NF behavior in a physiological context.

Overall, using our novel NF zebrafish lines, we aim to explore the live dynamics of a stable cytoskeleton network in vivo, to uncover its fundamental roles during development and in aging, and the pathological signaling in various diseases.

1. Van Asperen et al., Curr Opin Cell Biol. 2024
2. Bomont, Curr Opin Cell Biol. 2021
3. Kotaich et al., Front Cell Dev Biol. 2023

**Candidate for best oral communication award*

SESSION 3 - DYNAMICS OF IFS

INVITED SPEAKER

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4.University of Florida, Gainesville, Florida, USA
5. Institut Pasteur, Paris, France

Decoding the intracellular logistics of intermediate filaments

The spatial-temporal organization of intermediate filaments (IFs) is critical for cellular architecture and function. This organization emerges from a complex interplay between filament assembly/disassembly and diverse transport mechanisms.

Integrating experimental approaches, image analysis and mathematical modelling, we explore key aspects of IFs intracellular transport. Our investigations focus on how individual long filaments are moved along microtubules by opposing motor proteins, how filament ensembles behave collectively, and how cells regulate this transport. We also examine the influence of actin retrograde flow on the overall filament network. This presentation will provide an overview of our findings.

SESSION 4
Cross-talk & Signaling

SESSION 4 - CROSS-TALK & SIGNALING

Benjamin NANES[1],

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2. University of Zurich

Keratin filaments and soluble vimentin interact with isoform specificity to organize cell signaling

The keratin intermediate filament (IF) cytoskeleton provides crucial mechanical support for the epithelial tissues, but the large number of keratin genes and their context-dependent expression patterns suggest additional non-mechanical roles. How individual IF proteins might perform different non-mechanical functions while maintaining the canonical IF mechanical scaffold has not been clear. We recently found that changing keratin expression during skin wound healing triggers a myosin-activating signaling circuit, potentiating wound closure without compromising mechanical stability (Nanes et al., Dev Cell, 2024). We now report that this signal transduction pathway relies on non-filamentous small oligomers of vimentin, the principal IF protein in mesenchymal cells, to shuttle regulatory kinases into transient signaling complexes with myosin organized on keratin 6A-containing filaments.

These results highlight two mechanisms by which IF proteins differentially influence cell signaling and cell mechanics. First, through isoform specific interactions, changing keratin expression modulates recruitment of different proteins to the IF cytoskeleton, increasing the likelihood that these proteins interact. This allows the IF cytoskeleton to spatially and temporally organize signal transduction, effectively functioning as a membraneless organelle. Second, largely overlooked non-filamentous pools of even lowly expressed IF proteins can function as signaling shuttles or adapters. Context-dependent cell and tissue regulation, rather than simple mechanics, may therefore explain the size of the IF protein family.

SESSION 4 - CROSS-TALK & SIGNALING *

Lilian PATY,

Quang D. Tran, Maritzaida Varela-Salgado, Antoine Jégou, Guillaume Romet-Lemonne, Cécile Leduc

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Vimentin promotes actin assembly by stabilizing the interaction between ATP-actin monomers at the barbed end

The vimentin cytoskeleton is widely recognized as a key regulator of cell migration, which relies on the assembly and disassembly of actin filaments. While there is evidence that these two cytoskeletons interact in cells, the underlying mechanisms of this interaction are only partially understood. Actin and vimentin can interact through biochemical signaling pathways or via cross-linkers, but whether they are involved in a direct protein-protein interaction remains controversial. If such an interaction exists, it is probably weak and transient, making it difficult to characterize. By allowing direct observation of single filaments in well-controlled biochemical conditions, in vitro experiments from purified proteins represent a powerful tool to address the question of the direct actin-vimentin interaction. Using TIRF microscopy to monitor the elongation of single actin filaments, we show that vimentin promotes actin assembly at the barbed end in the absence of actin-vimentin cross-linkers.

We demonstrate that this acceleration of actin assembly results from a decrease in the off-rate of actin monomers, i.e., vimentin stabilizes the barbed end of actin filaments without preventing the arrival of new monomers. Of note, we show that this stabilizing effect arise from the vimentin subunits and thus does not require vimentin to be elongated into filaments. Strikingly, this effect depends on the nucleotide state of actin. The acceleration is only observed for the polymerization of ATP-actin, but not ADP-actin, explaining why vimentin does not stabilize aged actin filaments against depolymerization. We further show that this stabilizing effect of vimentin allows it to promote spontaneous nucleation of actin filaments. Interestingly, other type III intermediate filaments – desmin and GFAP – display a similar effect on actin assembly. Altogether, these findings reveal an unexpected new actor in the regulation of actin dynamics at the barbed end, and provide new insights to further understand the upregulation of cell migration by vimentin.

**Candidate for best oral communication award*

SESSION 4 - CROSS-TALK & SIGNALING

INVITED SPEAKER

Anna AKHMANOVA[1],

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Controlling vimentin filaments with optogenetics and chemical genetics tools

Intermediate filaments (IFs) are a key component of the cytoskeleton, essential for regulating cell mechanics, maintaining nuclear integrity, organelle positioning, and modulating cell signaling. Current insights into IF function primarily come from studies using long-term perturbations, such as protein depletion or mutation. While these studies have provided insights into the consequences of vimentin loss, they could not distinguish the impact of direct interactions and interdependencies between vimentin and other cellular structures from the long-term effects caused by the absence of vimentin and the ensuing compensatory cellular changes, including alterations in gene expression. To address this problem, we developed a method to acutely manipulate vimentin organization by recruiting microtubule motors to IFs using chemical- or light-induced dimerization. This approach enables local or global vimentin relocalization within 15–60 minutes—too short for transcriptional compensation—allowing direct analysis of IF interactions and dynamics. Our results show that clustering vimentin centrally via minus-end directed kinesins caused only mild effects on actin and microtubules, and did not disrupt cell spreading or focal adhesion number, but markedly reduced cell stiffness. Keratin-8 remained unaffected in HeLa cells, but co-clustered with vimentin in other cell lines, indicating cell-type specific IF organization. ER sheets and mitochondria relocalized together with vimentin, confirming direct coupling, while lysosomes were only mildly displaced and rapidly restored their distribution. This work introduces a generally applicable tool for acute IF manipulation, enabling dissection of their immediate roles in cytoskeletal crosstalk, organelle positioning, and cell mechanics, distinct from long-term compensatory effects.

SESSION 5
Physiological roles of IFs

SESSION 5 - PHYSIOLOGICAL ROLES OF IFS

INVITED SPEAKER

Pierre A. COULOMBE,

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Keratin 16 spatially inhibits type I interferon

The stress-induced keratin intermediate filament gene, KRT16, and K16 protein are expressed in a spatially-restricted manner in the suprabasal layers of the epidermis in psoriasis and other inflammatory disorders, and widely used as biomarkers in such settings. Pathogenic variants in KRT16 are causative for pachyonychia congenita (PC), a rare genetic skin condition in which tissue homeostasis is profoundly disrupted in palmoplantar epidermis and other KRT16-expressing epithelial appendages. How K16 impacts the cellular and molecular mechanisms underlying these conditions is poorly understood. Here we show that K16 negatively regulates type I interferon (IFN) signaling and innate immune responses in skin. Clinically, the signature palmoplantar keratoderma (PPK) lesions in individuals with PC show enhanced type I interferon signaling. In mouse skin in vivo, loss of Krt16 leads to exacerbation of imiquimod-induced psoriasiform disease and heightened recruitment of neutrophils in a phorbol ester-induced model of acute sterile inflammation. IFN signaling is amplified in KRT16 null human keratinocytes treated with the synthetic dsRNA poly(I:C). Mechanistically, K16 interacts with effectors of the RIG-I-like receptor (RLR) pathway, including 14-3-3ε, and inhibits the 14-3-3ε:RIG-I interaction upstream of IFN activation in vivo and ex vivo. Topical application of a JAKi inhibitor, Ruxolitinib, to PPK lesions of KRT16 null mice markedly reduced lesion severity. These and other findings uncover a new paradigm for keratin-dependent regulation of innate immunity, with significant implications for our understanding of inflammatory skin diseases and potential treatments for PC.

SESSION 5 - PHYSIOLOGICAL ROLES OF IFS *

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Keratin filaments regulate organization of organelles and secretory compartments

Keratins are epithelial-specific intermediate filament proteins that provide mechanical resistance in tissues such as the epidermis. Epidermolysis bullosa simplex (EBS), a skin blistering disease, is caused by mutations in keratins 5 and 14 (K5/ K14). Prior studies have reported activation of endoplasmic reticulum (ER) stress and disruption of junctional desmosomes in EBS cell culture models. However, the molecular mechanisms linking keratin dysfunction to these outcomes remain poorly understood. Using electron and fluorescence microscopy in A431 cells, we tested the hypothesis that keratin filaments and the ER are spatially integrated. We find that peripheral ER tubules are in close proximity to keratin filaments and form paired arrangements at desmosome cell-cell junctions. Focused ion beam scanning electron microscopy (FIB-SEM) revealed intricate ER-keratin associations at points of contact we term the keratin-ER contact site. Fluorescence live-cell microscopy demonstrates that keratin filaments stabilize ER membrane dynamics. Disruption of keratin filaments by an EBS-causing aggregation mutant, $K14^{R125C}$, leads to changes in ER morphology, converting ER tubules at the cell periphery to ER sheets. Further, FIB-SEM revealed that keratin filaments localize within nanometers of ER-mitochondria contact sites. We determined the effects of keratin disruption on mitochondrial organization using MitoView. In $K14^{R125C}$ cells, mitochondria are more peripheral, co-localizing with ER sheets, suggesting that ER morphological domains govern the positioning of mitochondria. Lastly, we determined the effects of keratin aggregation on the formation ER exit sites (ERES) using fluorescently-labelled Sec16A. In $K14^{WT}$ cells, ERES are dynamic and migrate along filamentous keratin. In $K14^{R125C}$ cells however, these compartments co-localize with aggregates and display stalling along the ER membrane. Our results demonstrate that keratin filaments regulate ER network stability and mitochondrial positioning in epithelial cells. Further, these studies suggest that keratin disruption attenuates the dynamics and formation of secretory compartments, potentially leading to perturbation of desmosome assembly observed in EBS.

**Candidate for best oral communication award*

SESSION 5 - PHYSIOLOGICAL ROLES OF IFS *

Diego MONEO-CORCUERA,

Dolores Pérez-Sala

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Vimentin supports primary cilium organization

Vimentin, a dynamic type III intermediate filament protein, plays multifaceted roles in cells, including mechanical and regulatory functions, maintenance of organelle positioning and interplay with other cytoskeletal networks. Vimentin can be present in various assemblies and form robust cytoplasmic filament networks, droplet-like structures, and it can also be found at the cell surface and in the extracellular space.

We previously reported the presence of vimentin at the primary cilium [1, 2], an antenna-like organelle enriched in acetylated tubulin that extends from the surface of most resting mammalian cells. Primary cilia constitute signaling hubs that sense the environment, and participate in intercellular communication and in the response to stress. Here, we identify an important role of vimentin in primary cilium biogenesis, architecture, and signaling. Using mouse embryonic fibroblasts and A549 lung epithelial cells we have observed that vimentin filaments are closely associated with the axoneme of the primary cilium. Ablation of vimentin expression results in a reduced proportion of ciliated cells, significantly shortened cilia, and aberrant ciliary morphology, accompanied by disrupted pericentriolar architecture and disorganized basal bodies. Moreover, vimentin depletion disrupts the pericentriolar actin balance, suggesting that vimentin-actin interplay may be important for cilia development. Finally, Hedgehog signaling is compromised in vimentin depleted cells.

In summary, vimentin appears to play an important role as a cytoskeletal component, influencing cilium formation, architecture, and signal transduction.

[1] Lalioti, V., González-Sanz, S., Lois-Bermejo, I., González-Jiménez, P., Viedma-Poyatos, Á., Merino, A., Pajares, M. A., & Pérez-Sala, D. (2022). Cell surface detection of vimentin, ACE2 and SARS-CoV-2 Spike proteins reveals selective colocalization at primary cilia. Scientific reports, 12(1), 7063. https://doi.org/10.1038/s41598-022-11248-y
[2] Lalioti, V., Moneo-Corcuera, D., & Pérez-Sala, D. (2024). Key role of vimentin in the organization of the primary cilium. bioRxiv. https://doi.org/10.1101/2024.01.17.576004
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**Candidate for best oral communication award*

SESSION 5 - PHYSIOLOGICAL ROLES OF IFS

INVITED SPEAKER

Rudolf E. LEUBE,

Hoda Moazzen

Institute of Molecular and Cellular Anatomy, RWTH Aachen University

Misguided by a broken heart

Desmosomal junctions provide structural stability supporting concerted cardiomyocyte contractility. The occasionally occurring hemopericardium in murine embryos harboring desmosomal mutations was therefore interpreted as loss of cellular cohesion resulting in heart rupture. Examining murine embryos homozygous for mutations of the desmosomal transmembrane cadherin Dsg2 (Dsg2) we found reduced desmosome formation that was, unexpectedly, paired with excessive endothelial-to-hematopoietic cell transformation. The extensive intramyocardial red blood cell clusters are either completely resorbed resulting in normal further cardiac morphogenesis or give rise to compromised myocardial wall stability and subsequent lethal myocardial rupture. To find out whether this phenotype is specifically driven by Dsg2-deficiency or is a broader consequence of impaired desmosome adhesion, we studied mouse embryos lacking the desmosomal plaque protein Pkp2. In this instance, we observed a complete loss of desmosomes. In contrast to the Dsg2 mutants, however, epicardial cells appear to undergo epithelial-to-hematopoietic transition leading to even earlier embryonic lethality. In conclusion, our observations suggest that deficiencies in both Dsg2 and Pkp2 promote hematopoiesis in the developing murine heart but target preferentially different cell types, i.e. endothelial cells, which lack desmosomes, and desmosome-containing epicardial cells, respectively. Our results extend the known functions of desmosomal proteins beyond maintenance of structural integrity, providing evidence for their involvement in endocardial and epicardial morphogenesis and remodeling.

SESSION 6
From cell to organogenesis

SESSION 6 - FROM CELL TO ORGANOGENESIS

INVITED SPEAKER

Elly HOL[1],

Werner Dykstra[1], Zuzana Matusova[2,3], Rachel A Battaglia[4], Pavel Abaffy[2], Nuria Goya-Iglesias[5], Dolores Pérez-Sala[5], Henrik Ahlenius[6], Mikael Kubista[7], R Jeroen Pasterkamp[1], Li Li[8], Jianfei Chao[8], Yanhong Shi[8], Lukas Valihrach[2,7,9], Milos Pekny[10]

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8. Department of Neurodegenerative Diseases, Beckman Research Institute of City of Hope, Duarte, California, USA.
9. Department of Cellular Neurophysiology, Institute of Experimental Medicine CAS, Prague, Czech Republic.
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Mutations in GFAP Alter Early Lineage Commitment of Organoids

Glial fibrillary acidic protein (GFAP) is a type-3 intermediate filament protein mainly expressed in astrocytes in the central nervous system. Mutations in GFAP cause Alexander disease (AxD), a rare and fatal neurological disorder. How exactly mutant GFAP eventually leads to white and gray matter deterioration in AxD remains unknown. GFAP is known to be expressed also in neural precursor cells in the developing brain. Here, we used AxD patient-derived induced pluripotent stem cells (iPSCs) to explore the impact of mutant GFAP during neurodifferentiation. Our results show that GFAP is already expressed in iPSCs. Moreover, we have found that mutations in GFAP can severely affect neural organoid development through altering lineage commitment in embryoid bodies. Together, these results support the notion that GFAP plays a role as an early modulator of neurodevelopment.

SESSION 6 - FROM CELL TO ORGANOGENESIS *

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Institut Pasteur - Sorbonne University
2. CNRS

The role of the intermediate filament protein Nestin in Glioblastoma biology

Nestin is a class VI intermediate filament (IF) expressed in neuronal progenitor cells during mammalian CNS development. Following neuro- and gliogenesis, it is replaced by other IF proteins such as glial fibrillary protein for mature astrocytes. However, Nestin can be re-expressed in adults in the context of wound healing and pathological conditions. Notably, higher grade gliomas express increased levels of Nestin which correlates with lower patient survival. My PhD project, aims to study the role and the mechanisms of action of Nestin in glioblastoma cell proliferation, adhesion, migration and invasion using the GBM cell line U251. For this purpose, we used Nestin siRNA knock-down and also generated Nestin knock-out (KO) U251 cell lines by CRISPR-Cas9. Nestin KO cells show an intact IF network formed of vimentin, GFAP and Synemin. However, U251 Nestin KO cells present a disrupted retrograde flow of Vimentin which is significantly slower compared to their wildtype Ctrl and leads to the reorganization of the network into thick bundles together with an altered actin network. Interestingly, the cortical stiffness of Nestin KO cells, assessed by Atomic Force Microscopy appear significantly higher than in control cells and we hypothesize that cytoskeletal reorganization of vimentin may be responsible. Following Mass Spectrometry Analysis, I identified several actin-associated proteins that are overexpressed in our Nestin samples and that play an essential role in linking the cell membrane to the actin cytoskeleton. I am currently performing diverse experiments to define the role of Nestin in this network and the effects of Nestin down,- or upregulation on the downstream biological pathway.

Absence of Nestin also modified cell behavior. We found that the speed of 2D migration and 3D invasion is not significantly affected by Nestin depletion. In contrast cell proliferation is altered. In particular, contact inhibition of proliferation is strongly increased in Nestin depleted cells, without any perturbation of adherens protein expression. Altogether these results suggest that Nestin plays a specific role in cytoskeletal crosstalk controling cortical stiffness, which may be involved in sensing cell density.

**Candidate for best oral communication award*

SESSION 6 - FROM CELL TO ORGANOGENESIS *

Pallavi DEVRE,

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Vimentin and PKM2: Emerging Partnership to Regulate Glucose Metabolism and Respiratory Pathways

During metabolism, cells generate reactive oxygen species as a by-product. Moderate amount of ROS supports cell signalling and functions, however excessive ROS cause oxidative stress. We have found that vimentin is regulating metabolism. During cell proliferation, metabolic demand is very high, cells adapt its metabolism to support synthesis and maintain redox state. In glycolysis, pyruvate kinase activity decides whether glucose metabolites enter mitochondria to produce energy or pentose phosphate pathway to produce macromolecules and NADPH. NADPH replenish GSSG to GSH and support fatty acids synthesis. We have observed that vimentin has a key role in regulating metabolic signaling (Mohanasundaram et al. 2022) and wanted to examine the underlying mechanisms. Recently, we have discovered an important link between cytoskeletal protein vimentin and Pyruvate kinase M2 (PKM2). PKM2 is one of the key regulation enzymes in glycolysis. It is regulated by phospho-proteins; since vimentin is also a phospho-protein we performed an interactomics study indicating that vimentin and PKM2 interacts with each other, which we could confirm by several methods.

We also found that vimentin deletion markedly reduced antioxidant GSH level and glucose consumption. We observed that cells were protected against oxidative stress in presence of vimentin; short term anti-oxidative responses such as reduced glutathione were observed. Vimentin also protected the structural integrity of the mitochondria from being compromised. It can be hypothesized that vimentin regulates metabolism via PKM2, it acts as a gatekeeper to decide whether glucose enters mitochondria or pentose phosphate pathway, through which it maintains redox states and support synthesis.

**Candidate for best oral communication award*

SESSION 6 - FROM CELL TO ORGANOGENESIS

INVITED SPEAKER

Pierre JOANNE,

Team Stem Cells and Biotherapies, Unit Development, adaptation and ageing (UMR8263, CNRS), Institute of Biology Paris-Seine (IBPS), Sorbonne Université

Miniaturized engineered cardiac tissues to study the pathophysiology of desmin mutations

Cardiomyocytes derived from human induced pluripotent (hiPSC-CMs) can be used for a large variety of applications, from cell therapy to models of genetic-driven cardiomyopathy. We are using this model to assess the cellular mechanisms underlying the development of cardiomyopathy driven by mutations in DES encoding desmin, the muscle-specific intermediate filament. However, several limitations appear in the use of this model. These limitations are intrinsically linked to the cell culture method used, which consists of evaluating their cellular parameters following 2D monolayer culture. To overcome these limitations, we have generated hiPSC-CMs from different patients carrying different mutations in DES that were cultured as miniaturized engineered cardiac tissues (cardiac spheroids) in specific devices allowing high-throughput analysis of their contractility and calcium transients. We sought to determine whether the phenotypic variability observed in patients with DES mutations could be mimicked in this 3D model. Indeed, it is well known that DES mutations are pleiotropic, generating variable phenotypes, which probably results from interactions between desmin, chaperones and/or other partners. However, the factors underlying this variability are not known for the moment. Thus, we have observed important variations in functional characteristics, ie calcium transient and contractility. In addition, ultrastructural observations of spheroids, using electron microscopy, showed alterations in the distribution of mitochondrial alterations in mutant-CMs as well as morphological perturbations often related to the degradation of myofibrils. We then observed a consistent decrease in mitochondrial respiration in all mutants-CMs. Taken together, our results confirm that cardiac spheroids can mimic the phenotypic disorders of cardiomyopathy associated to DES mutations. Most importantly, structural and functional defects are variable and seem to be correlated to the localization of mutation in DES gene.

SESSION 7
Inside-out of IFs

SESSION 7 - INSIDE-OUT OF IFS

INVITED SPEAKER

Dolores PÉREZ SALA,

Centro de Investigaciones Biológicas Margarita Salas, CSIC

Regulation of vimentin remodeling by oxidative stress and pH

Vimentin plays multiple roles in cells, from mechanotransduction to modulation of cell signaling and integration of stress responses. These diverse functions are related to the numerous interactions and the versatility of assemblies that vimentin can adopt. The vimentin network is highly dynamic and can rapidly remodel in response to stimuli evolving from filaments to bundles, squiggles, biomolecular condensates and/or diffuse protein. These transitions are finely modulated by posttranslational modifications. Among them, modifications at the single cysteine residue, C328 in the human protein, can influence the morphology of vimentin assemblies in a structure-dependent manner. The susceptibility of this residue to modification could depend on its accessibility, interactions and/or reactivity. We have observed that pH variations are associated with differences in the extent of modification of C328, and this correlates with the magnitude of the remodeling. In particular, alkaline pH increases the susceptibility of vimentin to oxidants and electrophiles, whereas acidic pH displays a desensitizing effect. This modulation impacts the conversion of vimentin filaments into phase-separated biomolecular condensates under oxidative stress. The interplay between these factors: oxidative stress, pH and phase separation could have important implications in vimentin organization and cellular functions.

References: González-Jiménez (2023) Redox Biol. 64:102756; Martínez-Cenalmor (2024) Redox Biol. 75:103282; Martínez (2025) bioRxiv, doi: 10.1101/2023.12.21.572888.
PID2021-126827OB-I00, FJC2021-047028-I European Union NextGenerationEU/PRTR (MCIN/AEI/ 10.13039/501100011033, ERDF "A way of making Europe"); Grant "Astromad" from Fundación "la Caixa" LCF/PR/HR21/52410002.

SESSION 7 - INSIDE-OUT OF IFS *

Laurie-Anne LAMIRÉ[1],

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Methodological development to identify the interactomes of IF proteins in vivo

Identifying the interactome of proteins represents a valuable tool to decipher their mode of action and functions. In the context of Intermediate Filaments (IFs), several challenges hamper the full assessment of their signaling, including the high insolubility of the filaments and the limitations of in vitro systems. In this context, our laboratory developed several animal models of diseases associated with IF alterations (1). In particular, we generated a novel knock-in and a zebrafish model for giant axonal neuropathy (GAN) (unpublished data and 2), a fatale neurological disease mutated in the pivotal regulator of IF degradation called gigaxonin (3, 4).

To further dissect the signaling of IF proteins in vivo and the pathological mechanisms underlying disease in a physiological context, we initiated the development of a proteomic methodology in animals. Here, we will present the Bio-ID methodology adapted to IFs in the zebrafish species, that will allow us to explore multiple IF types, throughout development and in adult stages, in a normal and pathological context. Starting with gigaxonin and neurofilaments (NFs), we will present the tools generated by the laboratory and preliminary results of mass spectrometry obtained during development in normal conditions.

Combining proteomics adapted to insoluble IFs to computational modeling, we aim to reveal the interactomes of IFs and their regulators in space & time, in normal and pathological conditions. Performed in vivo, we believe that this approach will not only be important to evidence key physiological players of IF dynamics but has the potential to orient targeted therapy for various human diseases.

1. Lescouzeres at al, Front Physiol 2020
2. Aribat, Mysiak et al, J Clin Invest 2019
3. Bomont et al. Nat Genet 2000
4. Mahammad et al., J Clin Invest 2013

**Candidate for best oral communication award*

SESSION 7 - INSIDE-OUT OF IFS

Florian GEISLER,

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**First Author*

Intermediate Filaments Shape Host-Microbe Interactions in C. elegans

Intestinal physiology and pathophysiology are in large part determined by the microbiome. The luminal microbes must breach the epithelial barrier to elicit cellular responses. The barrier is a multicomponent system consisting of mucus, cell-cell junctions and a subapical fibrous sheath consisting of cytoskeletal actin filaments and intermediate filaments. Comparatively little is known how the intermediate filament cytoskeleton is involved in microbe-host interactions. A major impediment has been the lack of standardized and well-defined in vivo systems. The genetically tractable model organism C. elegans offers an ideal tool to close the existing knowledge gap. It has a particularly well-developed subapical intermediate filament system. It furthermore offers the unique chance to completely remove endogenous microbes and subsequently feed the "germ-free" animals with defined microbe strains.

This allows to study microbe-specific responses and to work out mechanistic details of microbe-cytoskeletal interactions. Here we show that the responses to different microbes are affected in different ways by the absence or impairment of the intestinal intermediate filament network.

The observations suggest that intermediate filaments modulate microbial infections in a strain-specific fashion. Using RNAseq analysis we are currently trying to identify the microbial strain-specific and intermediate filament-dependent cellular pathways. Their detailed characterization will have implications for the poorly understood role of intermediate filaments in the mammalian intestine and its diseases.

SESSION 7 - INSIDE-OUT OF IFS

INVITED SPEAKER

Daniel GARCIA-GONZALEZ[1],

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2. Institut Pasteur

Magneto-active substrates for mechanobiological assays: application to cytoskeletal dynamics in glioblastoma

Progress in biomechanics and mechanobiology demands new tools to dynamically and precisely control the mechanical environment of biological systems. We present an experimental-computational framework based on magneto-active substrates enabling remote and reversible modulation of deformation modes exceeding 30%. The system integrates customized imaging and nanoindentation to quantify cellular mechanical properties during mechanical actuation. We apply this platform to glioblastoma cells to investigate how cytoskeletal dynamics shape tumor mechanics. Our results show that actin dominates mechanics in relaxed states, whereas vimentin governs responses under strain. Vimentin knockout cells exhibit pronounced softening and enhanced nuclear deformation upon substrate deformation. These findings highlight the potential of magneto-active substrates not only to probe fundamental mechanobiological processes in brain cancer but also to pave the way for predictive models and novel therapeutic strategies.

SESSION 8
Dynamics
& Regulations of IFs

SESSION 8 - DYNAMICS & REGULATIONS OF IFS *

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3. Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherland

Synemin and Microtubule Crosstalk in Glioblastoma Cell Invasion

Glioblastoma Multiforme (GBM) is the most aggressive malignant brain tumor, with limited treatment options due to its invasive nature and intra-tumoral heterogeneity. Cytoskeletal components including Intermediate Filaments (IFs) are key regulators of cell mechanics and migration. Recent findings from our laboratory reveal the importance of the IF network for GBM cell invasion. Among the GBM IF proteins, the type IV IF protein Synemin has been found to be associated with Focal Adhesion (FA) proteins, suggesting its involvement in cell adhesion and likely migration.

My project explores Synemin's role in GBM cell invasion. Synemin knock-out and knock-in GBM cells was generated. In absence of Synemin, cells display an intact IF network composed of Vimentin, GFAP and Nestin, allowing us to explore the specific role of Synemin without strong effect on the scaffolding properties of the IF network. Our experiments show that Synemin knock-out and knock-down enhance migration, and invasion. Synemin knock-out leads to reduced FA assembly and disassembly rates leading to increased FA sizes and number together with increased cell adhesion and spreading. These observations point to a possible role of Synemin in the regulation of microtubule stability which can control FA dynamics, acto-myosin-mediated traction forces and cell adhesion. In agreement with this hypothesis, our recent interactome analysis uncovered a previously uncharacterized, specific interaction between Synemin and a family of key regulator of microtubule stability, involved in cell migration and FA organization.

Thus, we propose that Synemin may interact and control the activity of these microtubule regulators to locally promote MT stabilization and thereby promoting a fast turnover of FAs, reducing GBM cell adhesion and migration. This work highlights a novel cytoskeletal crosstalk involving IFs and microtubules, and places Synemin as a new potential GBM diagnostic marker, given its variable expression across patient samples.

**Candidate for best oral communication award*

SESSION 8 - DYNAMICS & REGULATIONS OF IFS *

Marine LECONTE,

Zoheir Guesmia , Gisèle Bonne, Anne Bertrand

Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, UMR974, 75014, Paris, France

DNA damage in LMNA-related congenital muscular dystrophy

Repair of DNA double-strand breaks via the non-homologous end joining involves a cascade of histone modifications, starting with phosphorylation of H2AX (γH2AX), followed by histone H2AK15 ubiquitylation and H4K16 deacetylation that are both required for 53BP1 recruitment at DNA breaks, forming foci necessary to allow DNA repair. Recent studies have shown an accumulation of DNA breaks in a group of neuromuscular pathologies linked to mutations in the LMNA gene, and more particularly in its most severe form, the LMNA-related congenital muscular dystrophy (L-CMD). The LMNA gene codes for lamins A/C, proteins organized in a network at the nuclear membrane called the nuclear lamina, involved in nuclear resistance to mechanical stress. Lamins A/C interact with numerous proteins, including 53BP1, protecting it from degradation in absence of DNA damage and facilitating its recruitment to break sites. Although increased nuclear fragility is likely to trigger DNA damage in L-CMD, we hypothesize that the accumulation of DNA breaks might be due to DNA damage repair defect in presence of mutated lamins A/C.

Our data confirmed that primary myoblasts from L-CMD patients differentiated into myotubes display a higher number of breaks, marked γH2AX, than controls in basal conditions. Moreover, using etoposide phosphate (EP) to induce double-strand breaks on myotubes, we found that L-CMD myotubes are more sensitive to EP treatment marked by γH2AX than controls. When the 2h EP treatment are followed by 2h without treatment to allow repair, unlike control myotubes that showed a complete recovery, L-CMD myotubes are unable to repair and even evidenced increased cell death. We showed that this DNA repair defect is due to impaired formation of 53BP1 foci. Our preliminary data favour the involvement of a sequestration of 53BP1 by mutated A/C lamins in the absence of 53BP1 foci formation.

**Candidate for best oral communication award*

SESSION 9
Translational development

SESSION 9 - TRANSLATIONAL DEVELOPMENT *

Domitille CALLON[1],

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Cardiac cells with desmin gene mutations causing dilated cardiomyopathy are prone to Coxsackievirus-B infection

Sudden cardiac death (SCD) is a major public health issue. Genetic cardiomyopathies, notably dilated cardiomyopathy (DCM) and viral infections, by group B enteroviruses (EV-B), are the leading causes of SCD in the young. Little is known about the impact of EV-B infection in patients with genetic DCM, but it could explain low penetrance, clinical worsening or SCD.

To explore this hypothesis, we infected cardiomyocytes derived from human induced pluripotent stem cells carrying heterozygous DES gene mutations causing DCM, with the Coxsackievirus B3/28 (CVB3/28) strain. We compared the impact of the viral infection on control and mutant cardiomyocytes, on the desmin organization, the spontaneous contractility, the viral fitness and the innate immune response. We showed that CVB3/28 induced dramatic morphological and contractility changes in control and mutant cardiomyocytes, notably in the desmin organization. We showed that CVB3/28 replication and translation activities increased in mutant cardiomyocytes, in comparison with control. To explore the potential vulnerability of cardiac desminopathy to viral infection, we explore the antiviral immune response. We showed that interferon-beta production did not increase in infected cardiomyocytes with DES gene mutation, compare to the control cells. Finally, we found an elevation in viral (co-)receptors (Coxsackie and Adenovirus receptor and Cell-surface Vimentin) expression in infected DES-mutated cardiomyocytes, but not in control infected cells.

Together, our results show that EV-B worsened the phenotype of cardiomyocytes with a desmin gene mutation, with high viral replicative and translation activities, and a low antiviral immune response. The overexpression of viral receptors following the infection could be a leading mechanism together with a defective immune response, in the genetic susceptibility to cardiac EV-B infection. Our results open new preventive strategies of SCD, targeting at-risk populations of viral myocarditis, such as DCM patients with mutations of the intermediate filaments genes.

**Candidate for best oral communication award*

SESSION 9 - TRANSLATIONAL DEVELOPMENT *

Andreas BRODEHL,

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Atlas of DES (desmin) mutations

Background: Mutations in the DES gene, encoding the muscle specific intermediate filament protein desmin can cause severe (cardio) myopathies. With the increasing application of next-generation sequencing techniques in cardiogenetic diagnostics, a growing number of rare DES variants have been identified in cardiovascular genetics. However, their interpretation remains challenging for cardiologists, physicians and genetic counsellors due to the limited availability of functional data to most of these variants of unknown significance. Current in-silico predictions tools such as AlphaMissense or REVEL show inconsistencies and limitations.

Rationale: To address this challenge, we are developing an 'Atlas of Cardiomyopathy-Associated Desmin Variants' (ACAD) based on the functional characterization of most variants of unknown significance, which are listed in public disease databases including ClinVar and the Human Gene Mutation Database.

Material and Methods: We use cell transfection experiments, incorporating cardiomyocytes derived from induced pluripotent stem cells, in combination with confocal microscopy and deconvolution analysis to screen for pathogenic variants affecting the desmin filament assembly. Additionally, atomic force microscopy is utilized to validate detrimental effects for selected variants.

Results: We identified a hotspot of DES variants located in the border region between head and 1A domain leading to aberrant cytoplasmic desmin aggregation. Furthermore, we systematically introduced proline residues at each position within the complete 1B domain. These experiments revealed that proline residues at hydrophobic a- and d-positions significantly disrupt desmin filament assembly, whereas proline residues at other positions have frequently minor effects on filament assembly.

Conclusion: The development of an 'Atlas of cardiomyopathy associated desmin mutations' has the potential to enhance the clinical interpretation of rare DES variants in genetic diagnosis of non-ischemic cardiomyopathies. Moreover, it may serve as a valuable resource for genetic counseling of affected cardiomyopathy patients and their relatives.

**Candidate for best oral communication award*

POSTERS

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SESSION POSTER - P1 - FT6 *

Elisabeth CORTIER,

Evana Brosse, Lise Vallet, Caroline Lienard, Ryma Benlakehal, Pascale Bomont

ERC team, INMG-PGNM, Inserm U1315, Lyon University, France

Generalized disorganization of Intermediate Filaments in animal models of giant axonal neuropathy (GAN)

The steady-state of Intermediate Filaments (IFs) is controlled by several regulators. Still, gigaxonin has been identified as the unique E3 ubiquitin ligase able to degrade components of the entire IF family, through interaction with the conserved rod domain (1). Interestingly, our laboratory identified that recessive mutations in the gigaxonin-encoding gene cause giant axonal neuropathy (GAN) (2), a fatale neurodegenerative disease characterized by a broad deterioration of the nervous system and a wide aggregation of IF proteins in patients. In particular, GAN patients exhibit a massive disorganization and compaction of neurofilaments (NFs) and giant axons in the peripheral and central nervous system. While it is puzzling how the absence of a ubiquitously expressed protein can preferentially lead to neurological deficits, GAN is considered up to date as a unique biological system to investigate IF dynamics and its roles in vivo.

In order to investigate the physiological roles of gigaxonin, our laboratory developed two animal models of the pathology: in mouse with insertion of a point mutation found in a patient in the murine GAN gene (knock-in, GAN KI) (unpublished data), and in zebrafish with (transient) deletion of the zebrafish gan gene (3). In both genetic models, we revealed robust and early-onset phenotypes, with sensory and motor symptoms reminiscent of the human pathology. Importantly, we evidenced massive aggregation and compaction of NFs in the GAN KI mouse, concomitant with increased abundance of NF subunits. Here, we will present the landscape of IF alterations in tissues in and outside the nervous system, using our novel gan zebrafish and GAN KI models. Combining these data with the study of dysfunctions at the tissular and behavioral levels, we aim to provide a template for assessing the roles of IFs in physiology and pathological contexts.

1. Mahammad et al., J Clin Invest 2013
2. Bomont et al. Nat Genet 2000
3. Aribat, Mysiak et al, J Clin Invest 2019

*Candidate for best poster award

SESSION POSTER - P2 - FT2 *

Raphaël CRÉPIN[1],

P. Joanne[2], V. Mouly[2], S. Labdi[1], O. Agbulut[2], G. Lamour[1]

1. Univ. Evry Paris-Saclay
2. Sorbonne Université

Characterizing the biomechanical alterations of human muscle cells with desmin mutations using atomic force microscopy

Desminopathies regroup genetic muscle disorders such as myofibrillar myopathy (MFM) in skeletal muscles and dilated cardiomyopathy (DCM) in myocardium. Desmin mutations greatly affect the cytoskeleton biomechanics, degrading their contractile main function. However, the interplay between desmin network destabilization and cell mechanics remains to be clarified in human cells.

Here we studied two human cell models : immortalized myoblasts fused into myotubes, and cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CM), respectively for MFM and DCM caused by desmin mutations. We used biomimetic substrates to promote organotypic and reproducible organization in both models, and then we analyzed their mechanical properties using Atomic Force Microscopy (AFM). We implemented a robust protocol that corrects the substrate effect, which is a stiffness overestimation when probing soft and thin sample on a hard substrate. That correction enables to perform deep indentations in the cell body and so reliable measurement of global cell stiffness. Our results indicate that desmin mutant myotubes (p.R350P mutation) are 19% stiffer than control myotubes. In contrast, mutant hiPSC cardiomyocytes (p.D214-E245del & p.S46Y mutations) are 66% softer than the control hiPSC-CM.

Then we developed an innovative fatigue test in which we repeatedly applied strong compressions at a fixed location of the cell for 10 minutes. Both healthy and desmin mutant myotubes exhibit a common response: linear stiffening followed by a plateau. However, the stiffening slope differs between mutant and healthy myotubes. Overall, we characterize passive stiffness and dynamic mechanical alterations induced by desmin mutations in human muscle cells.

**Candidate for best poster award*

SESSION POSTER - P3 *

Ana Maria DAZA ZAPATA[1],

Brenda Nieto[1], Amina Medyouf[1], Alexis Eschstruch[1], Cécile Leduc[2], Sylvie Schneider-Maunoury[1], Pierre-Luc Bardet[1]

1. Sorbonne univerité, Development, Adaptation & Aging
2. Intitute Jacque Monod, Université Paris Cité

Cellular behavior and Intermediate Filaments role during zebrafish neural tube remodeling

During neural tube remodeling in vertebrates, the lumen of the spinal cord shrinks to form the definitive central canal. This process involves the simultaneous extrusion of neural progenitors and the rapid extension of roof plate (RP) cells. We previoulsy have shown that lumen retraction is coupled to the extrusion of lateral progenitors.

To better understand how this process, we monitor progenitor extrusion by live time-lapse in vivo imaging on zebrafish embryo and cell cycle analysis. We then want to understand how the forces generated by these extrusion allow RP elongation. Cytoplasmic intermediate filaments (IFs) such as Nestin and glial fibrillary acidic protein (GFAP) are highly expressed in RP cells, making the RP cells an excellent model for studying the biophysical role of cytoplasmic IFs. To link the intermediate filaments' dynamics with the shape and function of the RP cells, we will express nestin-GFP protein inside the RP cells to visualize the IFs organization during RP elongation in vivo. By expressing truncated GFAP constructs acting as dominant-negative proteins, we will assess how lacking cytoplasmic IFs can interfere with RP elongation. Additionally, we will employ pharmacological treatments to accelerate RP elongation to challenge the IFs and clarify their functional role in this process. Besides, we are creating a new fish line that expresses.

This project aims to integrate mechanical regulation into current models of neural tube development, providing new insights into how IFs contribute to RP cell morphogenesis.

**Candidate for best poster award*

SESSION POSTER - P4 - FT5 *

Isa DECUYPERE[1],

Sarah Peeters[1,2], Sarah De Beuckeleer[1], Hera Jeongyeon Kim[2], Coen Campsteijn[2], Winnok H. De Vos[1]

1. Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium
2. Department of Molecular Medicine, Institute of Basic Medical sciences, University of Oslo, Oslo, Norway

Spatiotemporal-selective analysis of the NE stress response in glioblastoma cells

Glioblastoma (GBM) is the most common and aggressive primary brain tumor, with a median survival of only 15 months after diagnosis and no effective cure. While many molecular alterations in GBM have been identified, the role of altered biophysical properties remains underexplored. Notably, GBM cells are exposed to increased mechanical forces compared to non-transformed cells. Work by our group and others has shown that such forces induce nuclear envelope (NE) stress, which disrupt cellular homeostasis, cause DNA damage, and trigger inflammatory responses. As we found significant evidence of NE stress in both patient biopsies and GBM cell lines, we asked whether and how this process contributes to disease progression. To investigate this, we generated stable 1321N1 GBM cell lines expressing a SNAP-tagged NE rupture reporter (BAF or dead-cGAS, dcGAS) and to exacerbate susceptibility to NE stress, we also generated a LMNA knockout (LMNA-KO) clone of each. Under moderate mechanical confinement, we confirmed that LMNA-KO cells were more prone to rupture than wildtype cells and that BAF and dcGAS displayed differential NE mobilization kinetics. We then developed an automated microscopy protocol capable of selectively labelling cells that have undergone NE rupture allowing for time-resolved transcriptomic analysis. Through these approaches, we aim to advance our knowledge of GBM pathophysiology and uncover new therapeutic avenues for this devastating disease.

**Candidate for best oral communication award*

SESSION POSTER - P5 - FT1 *

Caroline HALDIN[1],

Petar Kosijer[1], Theresia Jansson[1], Diana M. Toivola[1], Catharina M. Alam[2]

1. Åbo Akademi University
2. Edinburgh Napier University

Keratin 18 is the sole type I keratin in pancreatic beta cells and is essential for maintaining islet mass and beta cell glucose transporter 2 plasma

Pancreatic beta cells in the islets of Langerhans control glucose stimulated insulin secretion (GSIS) and blood glucose homeostasis. Epithelial keratin (K) filaments have distinguished structural and stress-protecting cell functions, as shown in multiple animal models and cell lines, and as further demonstrated by the keratinopathies found in humans. In human beta cells, simple epithelial type II keratin K8 and type I K18 pair together producing functional keratin filaments, whereas in mice, both K8 and K7 type II keratins pair with K18. In this study we hypothesize that K18, as the only type I keratin in beta cells, has a central role in beta cell function. We utilized the K18 knock-out (K18-/-) mouse model, which deletes all endocrine keratins, to study the impact of total keratin loss on beta cell function.

Using digital histopathology analysis, the histological islet phenotype in K18-/- mice was quantified. The K18-/- islets were smaller in size, fewer, and the overall islet mass was diminished compared to K18+/+ mice. However, when analysing the total pancreas insulin content and serum insulin levels using an ELISA-assay, no difference in insulin levels was detected between genotypes. Membrane targeting of glucose transporter 2 (GLUT2) was analysed, since GLUT2 is the main glucose sensing transporter in the plasma membrane of mouse beta cells and responsible for initiating glucose stimulated insulin secretion (GSIS). Immunofluorescence staining showed that the GLUT2 localisation was decreased at the plasma membrane in K18-/- mice compared to K18+/+ mice. In conclusion, K18-/- mice have improved glucose tolerance, smaller and fewer pancreatic beta cell islets and loss of GLUT2 plasma membrane targeting, implying a role for K18 in maintaining normal beta cell islet mass and glucose homeostasis.

**Candidate for best poster award*

SESSION POSTER - P6 *

Elena HONSCHEID,

Chiara Oberwestberg, Rudolf E. Leube, Nicole Schwarz

Institute of Molecular and Cellular Anatomy (MOCA), RWTH Aachen, Germany

Keratin Network Defects: A Gateway to Lysosomal Dysfunction?

Intermediate filaments participate in various cellular processes, including differentiation, proliferation, and cell signaling, though the precise mechanisms remain unclear. Growing evidence suggests that intermediate filaments interact with organelles and contribute to subcellular organization by positioning and retaining them appropriately. Here, we show that mutations in the keratin network influence the localization and functionality of lysosomes in epidermal cells.

To investigate subcellular localization, micropatterning was used to standardize cell shape. A cathepsin B assay was performed to assess degradation activity, while lysosomal pH was measured using a ratiometric biosensor. Compared to healthy control cells, patient-derived keratinocytes carrying a Krt6aN171K or a Krt16R127C mutation showed accumulation of lysosomes at one side of the nucleus. Furthermore, lysosomes of the mutant cell lines displayed an increase in lysosomal pH and decreased lysosomal enzyme activity. Overall, these findings support a significant limitation of lysosomal function.

This study offers new perspectives on the function of intermediate filaments that go beyond their role in protecting against mechanical stress. However, the exact relationship as well as the potential binding protein has yet to be determined.

**Candidate for best poster award*

SESSION POSTER - P7 *

Yeranuhi HOVHANNISYAN,

Gabriel Friob[1], Denisa Calin[1], Gaëlle Revet[1], Alexandre Simon[1], Martin Flament[1], Alexis Canette[2], Ivan Nemazanyy[3], Vincent Procaccio[4], Anthony Béhin[5], Ekaterini Kordeli[1], Zhenlin Li[1], Helene Delanoë-Ayari[6], Karim Wahbi[7], Pierre Joanne[1], Onnik Agbulut[1]

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3. Plateforme d'étude du métabolisme SFR Necker Inserm US 24 - CNRS UAR 3633 Faculté de Médecine Paris Descartes - Paris (France)
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6. Institute of Luminescent Materials Bâtiment Léon Brillouin Universite Claude Bernard Lyon-1 - Lyon (France)
7. Université Paris Cité, Cochin Hospital, AP-HP, Cardiology Department - Paris (France)

Targeting mitochondrial dysfunction to improve function and structure of cardiomyocytes carrying different desmin mutations

Desmin is an intermediate filament that plays a crucial role in maintaining the structural integrity and function of muscle cells. Mutations in the DES gene are known to cause myofibrillar myopathy, a group of inherited muscle disorders that primarily affect skeletal muscle. Approximately 75% of affected individuals also exhibit cardiac involvement. This study aims to indentify the common impact of different human desmin heterozygous mutations located in different regions of the protein (p.S46Y, p.E245D, p.D214-E245Del, p.P419H and p.E439K) and to explore potential treatment for alleviating the associated phenotype.

In cardiomyocytes derived from induced pluripotent stem cell of patients, we demonstrated that desmin mutations are associated with significant morphological and functional abnormalities. Morphological analysis revealed severe structural alterations, including degradation or accumulation of myofibrils, desmin aggregates and disorganization of mitochondria. These abnormalities were paralleled by functional abnormalities in mitochondrial respiration, as evidenced by a significant decrease in oxygen consumption rates in all desmin mutant cardiomyocytes. Furthermore, we assessed the contractile function of cardiomyocytes in a physiologically relevant 3D model by live cell imaging. Following this evaluation, we observed an increase of duration of calcium transients and pronounced variations in contractile parameters, indicating impaired contractile function of cardiomyocytes carrying desmin mutations. Transcriptomic and metabolomic analyses revealed common alterations, particularly a downregulation of mitochondrial metabolism. These changes were correlated with a reduction in methionine cycle. Targeting this metabolic defect restored the methionine cycle, improved contractile function, reversed respiratory decline and improved mitochondrial integrity in mutant cardiomyocytes.

In conclusion, our results indicate that impaired mitochondrial respiration and reduced bioenergetic capacity represent a common consequence of diverse desmin mutations. Therapeutic targeting of mitochondrial dysfunction resulted in structural and functional recovery, highlighting mitochondrial-directed therapy as a promising treatment approach.

**Candidate for best poster award*

SESSION POSTER - P8 - FT7 *

Maria ILOMÄKI[1,2],

Ekta Kotharkar[1,2], Jannika Rovapalo[1], Noora Lehtonen[1], Emelie Lassas[1], Anne Nikkonen[3], Rebecka Ventin-Holmberg[3], Kaija-Leena Kolho[3], Lauri Polari[1,2], Diana Toivola[1,2]

1. Cell Biology, Biosciences, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland
2. InFLAMES Research Flagship Center, Åbo Akademi University, Turku, Finland
3. Department of Pediatrics, Children's Hospital, University of Helsinki and HUS, Helsinki, Finland

Elevated Intestinal Keratin Concentrations in Stool are Indicative of Inflammatory Bowel Disease

Ulcerative colitis and Crohn's disease are autoimmune diseases grouped under the name Inflammatory Bowel Disease (IBD). They are characterized by chronic inflammation of the gut along with major changes in the intestinal epithelium. Our recent study identified that keratin 7, generally found in ductal and glandular epithelia, is focally expressed de novo in colonocytes of patients with IBD. In an earlier mouse study, we have furthermore shown that fecal keratin content mirrors changes in keratin expression in the intestinal epithelium. Based on these findings, we hypothesized that fecal keratins could be utilized as non-invasive biomarkers for IBD. This study aimed to evaluate whether keratins are detectable in patient stool, either on an mRNA or protein level, and whether fecal keratins can stratify IBD patients from control patients. Proteomic analysis, qPCR, and Western blotting were used to identify keratins in stool and study their associations with other potential intestinal epithelial-derived biomarkers in patients with IBD and controls. Preliminary results indicate that intestinal epithelial keratins are measurable in stool in patients with IBD, both at mRNA and protein levels. Specifically, keratin 7 protein is a promising marker for the noninvasive detection of IBD. These findings warrant additional studies with a larger cohort to confirm whether the increased fecal keratin concentrations are specific for IBD, along with further studies into the mechanism behind the elevated keratin levels.

**Candidate for best oral communication award*

SESSION POSTER - P9 *

Ekta KOTHARKAR[1,2],

Lauri Polari[1,2], Diana M. Toivola[1,2]

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2. InFLAMES Research Flagship Center, Åbo Akademi University, Turku, Finland

Uncovering Spatial Gene Expression Landscapes of Keratin 7 in Intestinal Inflammation

Keratins, a family of intermediate filament proteins, exhibit altered expression patterns across a variety of epithelial pathologies. These changes are associated with cellular stress, injury, or differentiation, yet the molecular mechanisms regulating keratin expression, including the role of transcription factors and other signaling molecules, remain poorly understood. Keratin 7 (K7) is a notable example, as it is absent in healthy colonic epithelium, but de novo expressed in inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD). K7 expression is typically confined to areas of epithelial injury, such as ulcers and crypt loss, suggesting a role in epithelial remodeling and disease-specific tissue responses. While K7 does not appear to be expressed in specific cell types in the colonic crypt, preliminary observations indicate that colonocyte K7 is associated with differences in cell morphology. The aim of the study is to identify the mechanisms inducing K7 expression in colonic epithelium of IBD patients. Using spatial transcriptomic analysis of colonic tissue, profiling with the Visium HD platform will be used to compare K7-positive and K7-negative epithelial regions, enabling the identification of gene expression patterns linked to K7 induction. The findings of this study are expected to improve understanding of K7 regulation in intestinal inflammation and provide new insights into the molecular context of K7 expression in IBD and disease pathogenesis.

**Candidate for best poster award*

SESSION POSTER - P10

Giovanna LATTANZI,

Elisa Schena, Camilla Cerchier, Cristina Capanni, Chiara Peres, Andrea Cantelli, William Blalock

CNR Institue of Molecular Genetics «Luigi Luca Cavalli-Sforza» Unit of Bologna

Cross-talk of lamin A with known and new regulators of adipose tissue plasticity

Lamin A mutations cause a number of diseases featuring adipose tissue dysfunction. It has been demonstrated that lamin A interacts with transcriptional regulators and chromatin modifiers that affect adipogenic differentiation. However, a comprehensive view of how lamin A modulates fate and development of adipose tissue precursors is still missing. We recently discovered new mechanisms involved in laminopathic lipodystrophies and new lamin A interactors as the mineralocorticoid receptor and other unexpected binding partners that contribute to adipose tissue plasticity and are involved in the pathomechanisms of LMNA-linked lipodystrophies .

SESSION POSTER - P11 - FT3*

Mira M.E. MINKKINEN[1],

Guillaume Jacquemet[1,2], Diana M. Toivola[1,2]

1. Åbo Akademi University, Cell Biology
2. InFLAMES Research Flagship

Identifying the keratin 8 interactome in colorectal cancer cells - proximity labeling reveals links to cell cycle and mitosis

Colorectal cancer is a common cancer type originating from the epithelial cells of the colonic mucosa. Keratins (Ks), namely K8 (type II), K18, K19 and K20 (type I), are intermediate filament proteins that form a part of the cytoskeleton in said epithelial cells. According to numerous studies, K8 is important for colonic homeostasis, including the integrity of the epithelial barrier and maintaining balanced proliferation. Loss of K8 in the colonic epithelium leads to epithelial hyperproliferation and increased susceptibility to tumor development in mice. However, even though the characteristics of K8 loss are well described in vivo, the exact molecular mechanisms behind the tumor suppressive role of K8 and its local interactions in the colon remain unclear. The aim of this study was to establish a comprehensive K8 interactome by utilizing an unbiased enzyme-catalyzed proximity labeling approach to identify direct binding partners and adjacent proteins of K8 in colorectal cancer cells. A biotin ligase enzyme (BioID) capable of rapidly biotinylating proximal proteins was fused to K8, the construct transfected to HCT116 colorectal cancer cells, and the cells treated with biotin for 24 hours. The biotinylated proteins were subsequently purified and identified via mass spectrometry. From the K8 interactome list, we have determined that the top enriched proteins and their related pathways are related to cell cycle and mitosis regulation. Additionally, many of the K8-interacting proteins have been reported to localize to the centrosome, the main microtubule organizing center. This has prompted us to further study K8 and its relationship with centrosomes in cell cycle regulation and mitosis, with a focus on identifying the conditions of the interactions. K8's involvement in regulating centrosomes could explain its described tumor suppressive role in the colon, where establishing the correct mitotic index and orienting the mitotic spindle are crucial for colonic homeostasis.

**Candidate for best poster award*

SESSION POSTER - P12 *

Mayank MODI[1,2],

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The role of vimentin in fibroblast-mediated remodeling of the extracellular matrix

Vimentin is a cytoskeletal protein known for its adaptability under mechanical stress, increasing its stiffness under high strains and undergoing structural changes, enabling cells to sense and respond to mechanical signals such as matrix stiffness and shear stress. As our recent studies have shown that vimentin is essential for maintaining the migrational directionality of fibroblasts, we wanted to study whether it could be involved in structuring ECM during fibroblast migration. While normal fibroblasts structured the ECM they are migrating on, Vim-/- fibroblasts completely disrupted the ECM they are migrating on, exhibiting disrupted ECM organization and lost alignment. The migration behavior of fibroblasts on ECM is consistent with our previous results of disrupted directionality and speed of migration. Furthermore, vimentin determined the composition of the ECM, especially in terms of the ratio between collagen type 1 and fibronectin. Additionally, we observed major differences in the proteomic profile of ECM proteins derived from WT and Vim-/- fibroblasts. Vimentin was demonstrated to be important for the distribution of traction force over the whole cell area, as traction-force microscopy revealed that Vim-/- cells showed significantly reduced distribution of the traction. Our results show that vimentin is required for maintaining ECM structure and organization by fibroblasts, as well as for the proper distribution of traction forces.

**Candidate for best poster award*

SESSION POSTER - P13 *

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Molecular determinants for the mechanical properties of the mouse embryo extraembryonic membranes

The development of mammalian embryos depends on protective envelopes at the interface between mother and fetus, that provide nutrition, gas exchange, and support. The cytoskeleton, composed of microfilaments, intermediate filaments, and microtubules, plays a key role in regulating cells' mechanical properties. Intermediate filaments are known for their tensile strength, elasticity, and flexibility. The lab has previously identified a role for high levels of keratin intermediate filaments in the expansion of mesoderm-derived extraembryonic tissues.

We aim to investigate how cytoskeletal components help extraembryonic cells adapt to mechanical challenges and protect the embryo. Using 2D culture of mesoderm explants and stem cells-derived germ layers models, as well as live imaging and multi-omics analysis of mouse embryos, we analyze cytoskeletal structure and dynamics in extraembryonic tissues in native and stretch conditions.

Understanding cytoskeleton dynamics in extraembryonic mesoderm may help design new embryo models to explore embryo implantation and placental defects.

**Candidate for best poster award*

SESSION POSTER - P14

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Interplay between pH and redox regulation of type III intermediate filaments

Type III intermediate filaments play key roles in cell architecture, mechanotransduction, organelle position and homeostasis, as well as in the integration of cellular responses to stress. In previous work, we have characterized the modulation of the type III intermediate filaments vimentin, GFAP and desmin, by modifications of the single cysteine residues present in their monomers. This modulation is particularly important in the cellular response to oxidative and electrophilic stress. Perturbations at the single cysteine site elicit structure-dependent reorganizations of the network that impact cytoskeletal crosstalk. Here, we will present our recent work in this line, illustrating the cysteine-dependent reversible remodeling of vimentin into biomolecular condensates under oxidative stress. Also, we will discuss the role of pH in the modulation of vimentin susceptibility to modification and reorganization by reactive species. Our results suggest that other type III intermediate filaments may share these features. Therefore, we hypothesize that cellular or subcellular redox and pH fluctuations could contribute to the fine tuning of type III intermediate filament organization.

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SESSION POSTER - P15 *

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Role of Intermediate Filaments in Modulating Host-Microbe Interactions in *C. elegans*

Intermediate filaments (IFs) are essential cytoskeletal components that preserve intestinal epithelial integrity, a critical barrier against microbial invasion. Their specific role in host-microbe interactions, however, remains insufficiently defined. Using Caenorhabditis elegans as a model, we examined two IF mutants: ifb-2(kc14), which lacks an IF network, and ifd-2(bz187), which has an altered IF network. These were compared to a control strain with intact IF network. Worms were exposed to CeMbio kit microbes, with E. coli OP50 as a control. Our observations suggest that disruption of the IF network may influence lifespan, microbial colonization, innate immune activity, and IF protein expression, pointing to a possible role of IFs in modulating host–microbe interactions, potentially through effects on gut barrier integrity. Ongoing analyses include gut permeability assays and extended microbial exposures to assess structural and functional consequences for intestinal morphology. Ultimately, this work will help elucidate the molecular pathways by which IFs contribute to gut integrity, providing insights that could inform strategies for maintaining gastrointestinal health in more complex organisms.

SESSION POSTER - P16 - FT8 *

Audrey SMITH,

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A humanized mouse model of Giant Axonal Neuropathy with early functional phenotypes

Giant axonal neuropathy (GAN) is a hereditary neurodegenerative disease characterized by progressive axonal degeneration affecting the peripheral and the central nervous system. Our goal was to develop a clinically relevant mouse model to advance mechanisms and enable preclinical therapeutic testing for GAN. Specifically, we sought a model to capture the early-onset and progressive disease course observed in GAN patients carrying loss-of-function mutations in the KLHL16 (GAN) gene. Previously we discovered uniquely human features of the 3'UTR region within the KLHL16 mRNA. Therefore, we replaced the 1.2kb mouse Klhl16 3'UTR with the 13kb human KLHL16 3'UTR. This resulted in the generation of phenotypically normal control humanized mice (hGAN mice). KLHL16 encodes gigaxonin, a ubiquitin ligase adaptor that promotes the clearance of intermediate filament (IF) proteins, which accumulate in GAN cells. Most pathogenic variants are gigaxonin missense mutations. Therefore, we also introduced a known GAN-causing missense mutation (G332R) together with the human 3'UTR (hGAN-G332R mice). We then compared 3-12 month-old hGAN and hGAN-G332R mice using functional, histologic and biochemical assessments. The hGAN-G332R mice showed complete gigaxonin loss compared to hGAN and wild-type C57BL/6 control mice, similar to GAN patient neurons with the G332R mutation. The hGAN-G332R mice had increased IF protein levels and displayed giant swollen axons with densely packed IFs. At 3 months of age, the hGAN-G332R mice had significantly decreased activity in an open field test ($p=0.0203$), decreased center time ($p=<0.0001$) and decreased vertical rears ($p=0.0003$). Grip strength was normal at 3 months but significantly decreased at 12 months in hGAN-G332R mice ($*p<0.05$). Ongoing studies are aimed at translating targeted therapies from cell-based models to in vivo. In conclusion, the hGAN-G332R mice model the progressive motor deficits seen in GAN patients and will help clarify the involvement of the gigaxonin mRNA and IF accumulation in disease onset and progression.

**Candidate for best poster award*

SESSION POSTER - P17 *

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Vimentin Network Architecture

The assembly dynamics of individual vimentin intermediate filaments from their subunits to mature filaments are relatively well understood. However, in contrast to other cytoskeletal networks, so far, we have not been able to reproduce vimentin network architectures in vitro. In order to understand how vimentin network architectures are organized and maintained and how cells adapt their vimentin networks to changing cellular states and external cues, it is necessary to close the knowledge gap on how vimentin networks are formed. Here, we used a cell extract-based approach, allowing us to partially preserve the intracellular environment, to reconstitute vimentin networks and investigate their architecture in detail. This approach allows us for the first time to study cell-like vimentin networks outside the cell. Interestingly, we observed that Vimentin forms a cell-like network in the extract. In this study we investigate how this network changes with respect to varying concentrations of vimentin and cell extract, filament length and different cell types.

**Candidate for best poster award*

SESSION POSTER - P18 *

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University of Tartu

Methyltransferase HEMK2 is involved in the regulation of tubulin isotypes and microtubule dynamics

HemK methyltransferase 2 (HEMK2) is an evolutionary conserved methyltransferase which has been associated with different pathological conditions, such as diabetes, various types of cancers and neurological malfunctions. While many of these diseases have been attributed to HEMK2 role in regulating gene expression, the exact molecular pathways and cellular processes which HEMK2 is associated with have not yet been fully described. Our analysis of HEMK2-depleted cells suggests that HEMK2 is involved in the regulation of the cytoskeleton and in particular microtubule dynamics in cancer cells. In cells depleted of HEMK2, the levels of different tubulin isotypes were altered with a notable downregulation of a tumour prognostic marker β-tubulin 3 (TUBB3). Furthermore, live-cell imaging combined with automatic particle tracking analysis (PTA) revealed that HEMK2 depletion might cause changes in microtubule dynamics by increasing their growth velocity and decreasing their growth duration. This was further validated by compensation cell lines expressing recombinant functional and methyltransferase-activity deficient HEMK2. The results showed that the methltransferase activity of HEMK2 is necessary for it to induce changes in microtubule dynamics. Taken together this points to HEMK2 role in the regulation of microtubule dynamics, but further studies are needed to determine whether these changes 1) result from alterations in tubulin isotype composition or 2) from the disruption of other processes governing microtubule dynamics. Understanding the mechanisms through which HEMK2 regulates microtubule dynamics, and potentially the entire cytoskeleton, could be used to better discern diseases associated with HEMK2.

**Candidate for best poster award*

SESSION POSTER - P19 - FT4

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Spatial organization of intermediate filaments in astrocytes driven by other cytoskeletal fibers

The cytoskeletal networks of intermediate filaments, actin microfilaments, and microtubules interact dynamically, with each component influencing the organization and behavior of the others. In particular we have previously described the role of both actin retrograde flow and microtubule-dependent transport in the dynamic rearrangement of intermediate filaments (Leduc et al. JCB 2017). In the present work, our goal is to determine the relative contribution of actin retrograde flow and microtubule-dependent transport in the stereotypic organization of the intermediate filament network controlled by cell adhesion to the substrate. To tackle this question, we use cells cultured on micropatterned substrates of circular or square geometry to precisely and reproducibly control the distribution of focal adhesion, cell shape and cell size. This allows us to investigate actin, microtubule and intermediate filament organization dynamics and at steady state and quantify the effects of specific cytoskeletal drugs -namely the use of small inhibitors to affect specific processes related to cytoskeleton assembly and dynamic- on intermediate filament organization. These data are integrated into mathematical models of intracellular flows (Park et al. PRE 2021) and spring-like molecules dynamics (Portet et al. J. Theor. Biol. 2022) to generate predictive simulations of cytoskeletal organization under specific perturbations.

SESSION POSTER - P20 *

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Pathophysiological effects of novel Charcot-Marie-Tooth mutations on motor and sensory neurons and the neurofilament network

Hereditary motor and sensory neuropathies, better known as Charcot-Marie-Tooth (CMT) disease, encompass a heterogeneous group of disorders caused by genetic mutations in a variety of genes, leading to axonal degeneration and/or demyelination. In this study, we investigate the pathological mechanisms of two newly identified CMT-associated mutations. Given the frequent disruption of the neurofilament network in CMT, we focus specifically on how these mutations affect neurofilament organization. Our first gene of interest is frataxin. While intronic repeat expansions in the frataxin gene are known to cause sensory neuropathy as part of the multisystem disorder Friedreich's Ataxia (FA), a recently described homozygous point mutation (R165C) in a Turkish family initally diagnosed with CMT has been shown to cause motor neuropathy as well. This finding suggests a genetic and pathological link between CMT and FA. Our second target is a gene previously associated with CMT through point mutations, in which a novel gene duplication was identified. To model these disease variants, we employ both overexpression and knock-in strategies in induced pluripotent stem cell (iPSC) and zebrafish models. We assess the impact of these mutations on motor and sensory neuron physiology and analyze alterations in the neurofilament network.

*Candidate for best poster award

SESSION POSTER - P21 - FT9*

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Stretching vimentin intermediate filaments using microfluidics

Intermediate filaments (IFs) are a key component of the cytoskeleton, notable for their exceptional stretchability compared to other cytoskeletal elements. Unlike actin filaments and microtubules, which are mechanically fragile and prone to breakage or depolymerization under moderate strain, IFs assemble into more stable, long-lived networks resistant to significant deformation (1,2). These properties are essential for preserving cellular integrity under stress (3).

Previous studies have used optical traps to examine the response of individual intermediate filaments under strain (4, 5). In order to deepen this understanding and enable high-throughput analysis, we have developed an bottom-up reconstitution assay that allows for the simultaneous observation of multiple filaments, using vimentin intermediate filaments (VIFs) as a model system. Here, I present a microfluidic system in which VIFs are stretched by mechanical stress induced by fluid flow. Based on fluorescence measurements, this system also enables us to study local IF deformations under strain and how these deformations may be influenced by factors such as filament composition and subunit exchange. A deeper understanding of IF mechanics will provide valuable insights into their role in the cellular response to mechanical stress.

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*Candidate for best poster award

SESSION POSTER - P22 *

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Polyglutamylation as a novel Post-Translational modification of Intermediate Filaments

Polyglutamylation, catalyzed by members of the tubulin tyrosine ligase-like (TTLL) family, is a conserved post-translational modification found at the unstructured tubulin C-terminal tails. This modification is highly abundant in neurons, cilia, and centrioles, and its disruption has been linked to various pathologies, including ciliopathies, neurodegeneration, and cancer. Although TTLL enzymes primarily target tubulin, it is hypothesized that additional TTLLs' physiological substrates exist. By combining biochemical in vitro assays with cell-based experiments, we have recently identified intermediate filaments as TTLL substrates. These findings could open new avenues for understanding the broader functional implications of cytoskeletal protein polyglutamylation, including intermediate filaments.

**Candidate for best poster award*

SESSION POSTER - P23

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Detection of intermediate filament-binding proteins using atomic force microscopy.

Intermediate filaments (IF) bind to various proteins and regulate cell function in the cytoplasm. We suppose that IF regulates gene expression by acting as capture scaffolds for transcription-related proteins and preventing them from translocating into nucleus. Immunostaining, a common method for analyzing protein-protein interactions, may result in loss of molecular interactions because of treatments with detergents. Therefore, a method to analyze the interaction between IFs and transcription-related proteins in cells, where the interaction is maintained, is necessary. In this study, we focused on IF vimentin to mechanically detect transcription-related proteins trapped by the filaments.

First, vimentin binding proteins were searched from protein-protein interaction database. Prohibitin 2 (PHB2), a transcription-related factor, was selected as a candidate protein. Next, we performed mechanical detection for PHB2 using atomic force microscope and anti-PHB2 antibody-modified nanoneedle in vimentin-expressing mouse breast cancer cells (FP10SC2) and vimentin knockout cell (VK0). Significantly larger unbinding forces were detected in FP10SC2 compared to those in VK0. The result suggests that this method is useful for detecting IF binding proteins.

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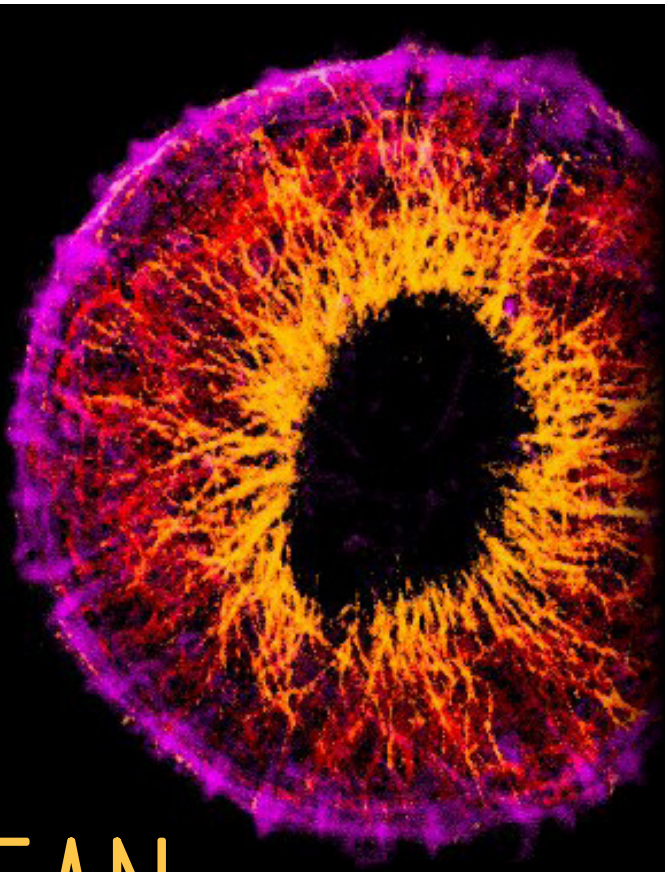

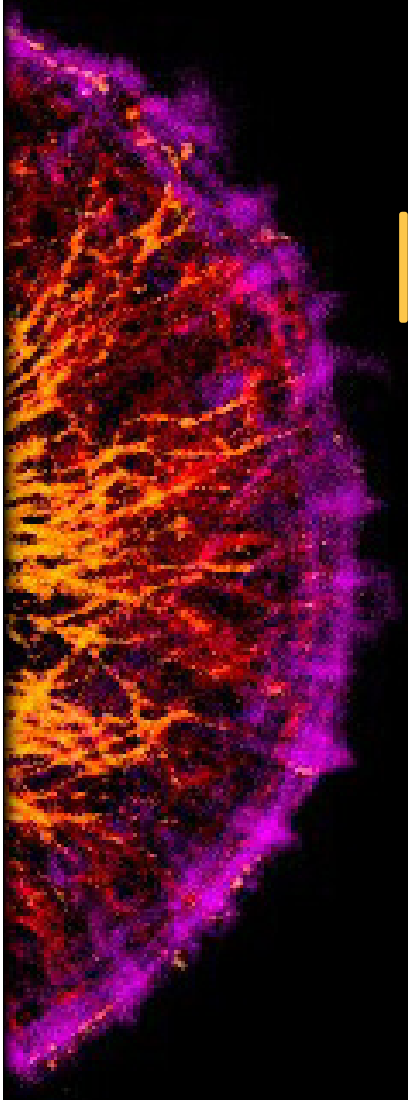

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