



TOR de France

3rd edition

CONFERENCE PROGRAM

5-6 October 2023

Saint Paul Hotel, Nice, France

Welcome

Dear colleagues,

It is our pleasure to welcome you to the 3-d edition of the “TOR de France” meeting, the only conference in the world entirely devoted to the TOR-signaling pathway, its function and molecular mechanisms and TOR-related diseases. The “TOR de France” represents a perfect possibility for students, postdocs, researchers and clinicians to exchange and foster national and international collaborations.

In 2023 TOR de France will cover the following topics:

- Molecular mechanisms of cell growth control
- TOR pathway in development in immunity
- TOR pathway and autophagy
- Therapeutic strategies to modulate TOR pathway in human diseases

Four international keynote speakers, a number of invited talks and talks, selected from abstract as well as poster session provide the most updated picture of the TOR field.

The TOR de France is broadcasted online with interactive hybrid sessions.

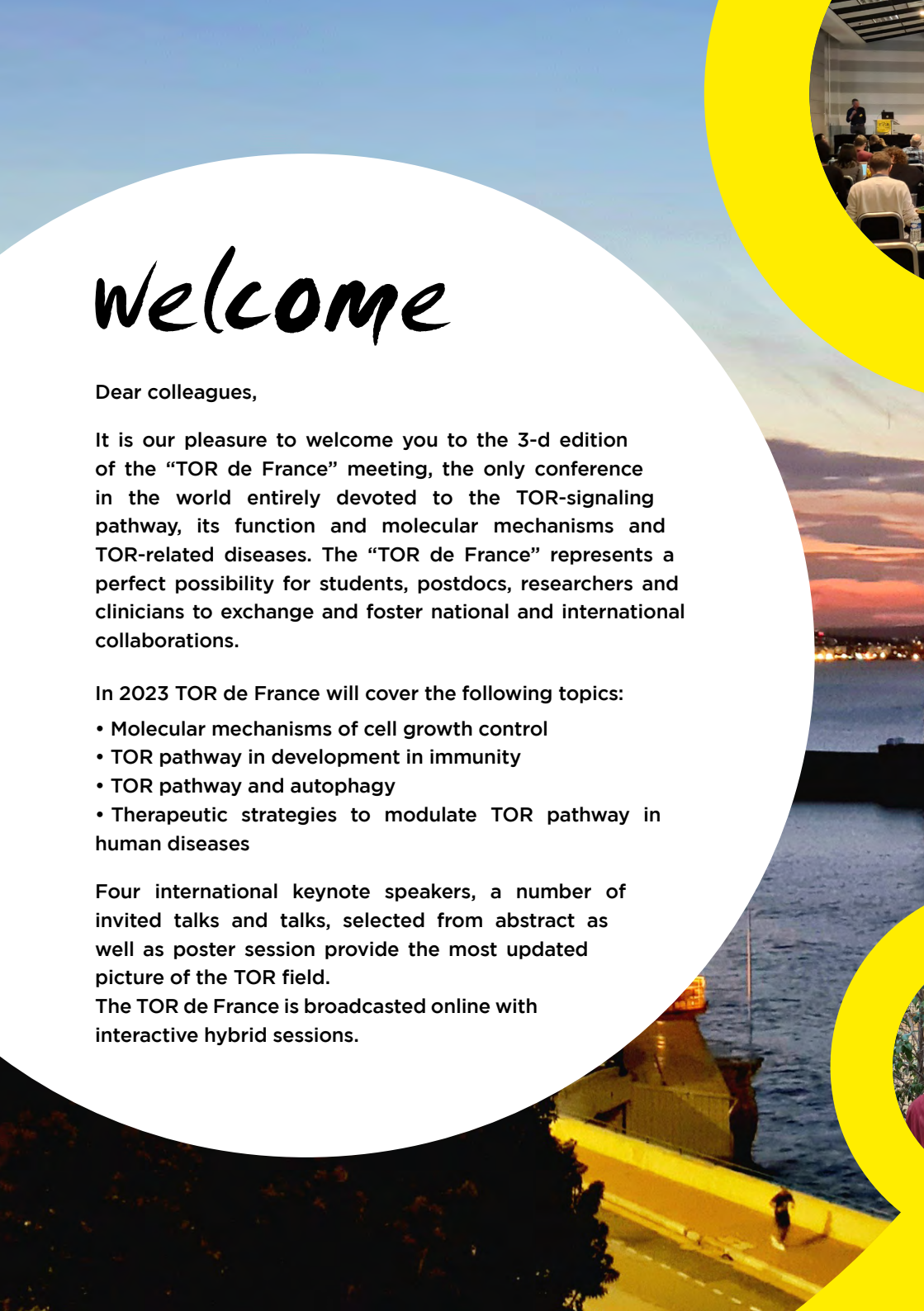




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Location

AND PRACTICAL INFORMATION



ADDRESS **OF THE CONFERENCE VENUE**

Le Saint Paul Hotel
29 boulevard Franck Pilatte
06300 Nice - France



HOW TO GET TO LE SAINT PAUL HOTEL

> By car:

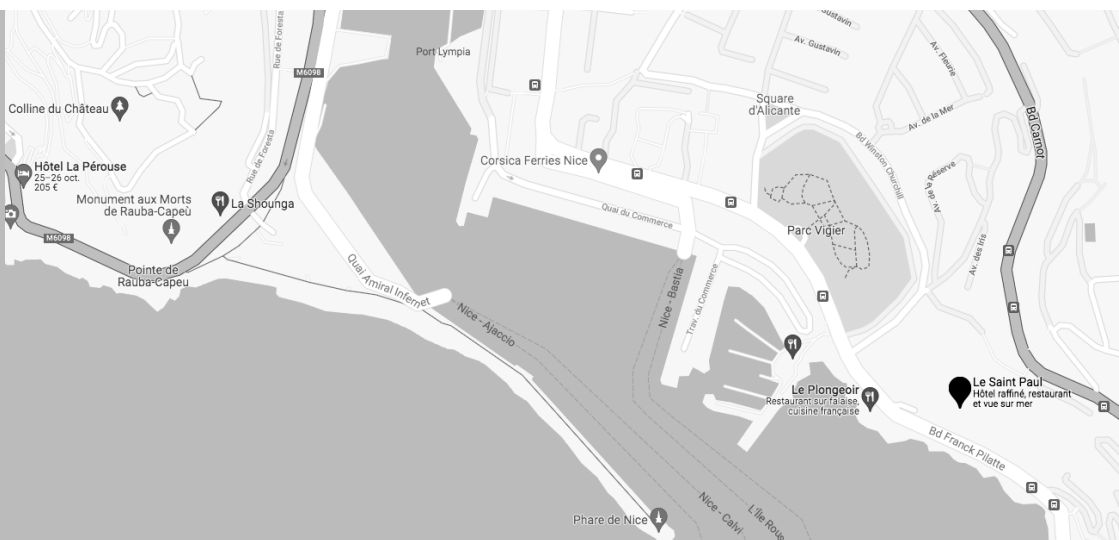
A8. Exit 50 Promenade des Anglais – towards the port
A8. Exit 55 Nice Est – towards the port

> From the train station:

Bus n° 30 (Direction Riquier). Stop *La Réserve*.

> From the airport:

Tramway to the terminus (Port), then 10min walk till the hotel





REGISTRATION

The registration desk at the entrance of the hotel where you can pick up your badge, conference booklet and goodies **will open on October 5th at 12:00.**



INTERNET ACCESS

> Wi-Fi network:

LE SAINT PAUL network

> Password:

riviera



CATERING

> Thursday, October 5th:

Coffee break, wine and cheese during poster session is offered. The lunch and dinner are not provided.

> Friday, October 6th:

Lunch and coffee breaks will be provided at the hotel. Gala dinner will be offered in Le Boccaccio restaurant (7 Rue Massena, 06000 Nice). The vegetarian menu will be available, but we can not guarantee the possibility to adjust to other dietary restrictions.



Schedule

THURSDAY, 5 OCTOBER 2023	12:00 - 14:00	Arrival and registration
	14:00 - 14:15	Opening and Welcome Members of organizing committee
	NEUROBIOLOGY OF mTOR AND NEUROLOGICAL DISEASES	
	14:15 - 15:00	Ghayda Mirzaa • Keynote Lecture
	15:00 - 15:15	Jacek Jaworski
	15:15 - 15:25	Deepika Mokkachamy Chellapandi
	15:25 - 15:40	Angeliki Louvi
	15:40 - 15:55	Stéphanie Baulac
	15:55 - 16:10	Amanda Almacellas Barbanoj
	Coffee break	
	16:40 - 17:10	Michele Studer
	17:10 - 17:25	Alice Gilbert
	17:25 - 17:35	Jan Kudlacek
	17:35 - 17:45	Steffen Syrbe
	17:45 - 17:55	Francesco Avanzi
	17:55 - 18:25	Daniela Cota
	18:25 - 20:35	Poster Session, Wine and cheese
	20:35	Free evening

mTOR AND GROWTH CONTROL IN HEALTH AND DISEASE		FRIDAY, 6 OCTOBER 2023
09:30 - 10:15	Kathrin Thedieck • Keynote Lecture	
10:15 - 10:25	Zhenrui Pan (cf. poster)	
10:25 - 10:55	Jette Lengefeld	
Coffee break		
11:30 - 12:00	Guillaume Canaud	
12:00 - 12:10	Milica Vucetic	
12:10 - 12:20	Marianna Maddaluno	
12:20 - 12:30	Khaled Tighanimine	
12:30 - 12:40	Xiaolin Zi	
12:40 - 12:55	Nitzan Samra	
12:55 - 13:05	Sophia Ladraa	
Lunch		
MOLECULAR MECHANISMS OF MTOR REGULATION		
14:15 - 15:00	Robbie Loewith • Keynote Lecture	
15:00 - 15:10	Lain Hay	
15:10 - 15:25	Constantinos Demetriades	
15:25 - 15:55	Aurelio Teleman	
Coffee break		
16:30 - 17:00	Jenna Jewell • Keynote Lecture	
17:00 - 17:15	Anders Mutvei	
17:15 - 17:25	Aishwarya Acharya	
17:25 - 17:40	Sarah Coubier	
17:40 - 17:50	Mikhail Schepetilnikov	
17:50 - 18:05	Fried Zwartkruis	
18:05 - 18:35	Gennaro Napolitano	
18:35 - 20:00	Poster awards	
20:00	Gala dinner in the Nice city center	

Keynote

SPEAKERS



JENNA JEWELL

is an Assistant Professor in the Department of Molecular Biology UT Southwestern Medical Center. Her lab focuses on how cells sense nutrients to control cell growth and metabolism, and how these processes are altered in human disease.

Originally trained in the field of chemistry, Jenna has extensive experience in biochemistry, signaling pathways, cell biology, and vesicle trafficking. Jenna joined Dr. Debbie Thurnmond's lab as a graduate student at Indiana University working on how SNARE proteins regulate glucose-stimulated insulin secretion from pancreatic β -cells and peripheral tissue glucose uptake. Then, Jenna did her postdoctoral fellowship with Dr. Kun-Liang Guan at the University of California, San Diego where she identified a new signaling cascade, which her lab at UT Southwestern Medical Center is currently working on. She received the Cancer Prevention & Research Institute of Texas (CPRIT) Recruitment of First-Time, Tenure-Track Faculty Member award, CPRIT High Impact/ High Risk Research Award, 2017 Distinguished Researcher Award, Dean's LEAD Program Capstone Project Recognition Award, Federation of American Societies for Experimental Biology (FASEB) junior investigator award, and the American Cancer Research Scholar Grant. Jenna is a member of the Hamon Center for Regenerative Science and Medicine and Harold C. Simmons Comprehensive Cancer Center.



ROBBIE LOEWITH

grew up on a dairy farm near Hamilton Ontario Canada. In 2000, after completing his BSc. at the University of Guelph, and his PhD at the University of Calgary, he went to the Biozentrum in Basel Switzerland for Post-Doctoral work in the laboratory of Prof. Michael Hall. During this time, using *Saccharomyces cerevisiae*, Robbie discovered that TOR assembles into two, distinct multiprotein complexes, broadly conserved in bigger eukaryotes, which he named TORC1 and TORC2.

In 2005, Robbie became an independent group leader at the University of Geneva. He was awarded a Professorship from the Swiss National Science Foundation in 2006, and is now a Full Professor in the Department of Molecular and Cellular Biology. He has directed / is directing the Department, the Dubochet Centre for Imaging and the Swiss National Center for Competence in Research (Chemical Biology). He was elected an EMBO member in 2021. His ERC-supported lab continues to use yeast to investigate conserved aspects of TOR signaling, recently using structural biology approaches to understand TOR signaling at near atomic resolution.



KATHRIN THEDIECK

is Professor and Institute Head of Biochemistry at the University of Innsbruck in Austria. Her research spans the field of metabolic signaling with a focus on networks converging on mTOR and PI3K.

Her team pioneers systems modeling of the mTOR network in cancer and crosstalk of mTOR with RNA-protein networks, focusing on non-canonical roles of RNA-binding proteins in membrane associated mTOR signaling. K. Thedieck studied in Münster (DE) and Strasbourg (FR) before she completed her PhD at the Helmholtz Centre for Infection Research (HZI) in Braunschweig (DE). As a postdoc she worked with Michael N. Hall in Basel (CH) and became afterwards group leader at the University of Freiburg (DE). In 2013, she joined the University of Groningen (NL) as an Adjunct Professor for metabolic signaling. In 2019, she chose to accept the chair for Biochemistry at Innsbruck University. She remains affiliated with Groningen University and the University of Freiburg. K. Thedieck received several awards including the TSC research award of the German TS Foundation. Since 2022 she holds an ERC Advanced Grant.

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DR. GHAYDA MIRZAA

is a geneticist and researcher with board certifications from the American Academy of Pediatrics and the American Board of Medical Genetics and Genomics.

Dr. Ghayda Mirzaa graduated from the Arabian Gulf University in Bahrain and then pursued pediatric residency at the University of Illinois in Chicago followed by fellowships in clinical genetics and molecular genetics at the University of Chicago and the University of Washington. She is currently an attending at Seattle Children’s Hospital who staffs the Epilepsy Genetics and Neurogenetics Clinics, program director of the Neurotherapeutic clinic and co-director of the molecular diagnostic lab at the University of Washington. Her research focuses on many aspects of human developmental disorders including understanding the causes, mechanisms and outcomes of these disorders, and associated co-morbidities including autism, epilepsy and intellectual disability, with a special focus on brain malformations.



Talks

ABSTRACTS

ANALYSIS OF MTOR-RELATED DISORDERS THROUGH DEEP GENOMIC AND FUNCTIONAL MODELS REVEALS NEW CLINICAL INSIGHTS AND DISTINCT MOLECULAR PATHOMECHANISMS

• **Ghayda Mirzaa** | *University of Washington School of Medicine, USA*

Megalencephaly (MEG), Hemimegalencephaly (HME) and focal cortical dysplasia (FCD) are neurodevelopmental disorders characterized by brain overgrowth and cortical abnormalities, associated with epilepsy, autism, and intellectual disability. Gain and loss of function mutations in the MTOR pathway have been identified in this spectrum with variable levels of mosaicism and tissue distribution of mutations, however the mechanisms underlying brain overgrowth, dysplasia, and epileptogenesis are not well understood. We aimed to better define genotype-phenotype relationships among the most common mutations in the pathway in a large cohort of patients with surgical brain tissue and modeled disease pathogenesis using stem-cell derived human cell lines. The molecular diagnostic

yield was 37% in HMEG and 12.7% in FCD. Interestingly, individuals with FCD mostly had mutations in MTOR, while those with MEG and HMEG had PIK3CA mutations. We generated iPSCs carrying the common PIK3CA and MTOR mutations and differentiated them to Neuronal Progenitor Cells (NPCs) and cerebral organoids. Preliminary results show both overlapping and exclusive cellular phenotypes in PIK3CA and MTOR mutant lines. Specifically, both mutant lines showed increased NPC proliferation and hypertrophy. Mutant organoids had an average area at least double that of controls, recapitulating MEG in vitro. In summary, our results broaden our understanding of the phenotypic spectrum of PI3K-AKT-MTOR related disorders.

TBC1D5, NOVEL MTORC1 SUBSTRATE NEEDED FOR NEURONAL DEVELOPMENT

• **Jacek Jaworski** | *Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland*

mTOR is one of the major protein kinases involved in neuronal development. Although many known and novel mTOR substrates have been demonstrated to contribute to neurogenesis, new ones are still being identified. TBC1d5 is the GAP protein for Rab7, one of the key regulators of late endosomes. TBC1d5 regulates the retromer complex, which retrieves cargo from late endosomes to either the trans-Golgi network or the plasma membrane. In our search for novel substrates of mTOR, TBC1d5 was identified as one of them. Subsequently, we identified several mTOR-dependent phosphorylation sites of TBC1d5. Therefore, our work aimed to determine the role of mTOR-dependent phosphorylation of TBC1d5 in neuron development. As a result, we show that TBC1d5

is essential for neuron migration, axono-, dendrito-, and synaptogenesis. However, its mTOR-dependent phosphorylation was found to be critical only for dendritogenesis. Subsequent experiments aimed to unravel the cellular processes regulated by mTOR via TBC1d5 at this developmental stage. We demonstrated that overexpression of TBC1d5, which is resistant to mTOR phosphorylation (TBC1d5-5A), resulted in the mislocalization of TrkB, a receptor for BDNF. However, TrkB signaling downstream was impaired only to a limited extent. Instead, overexpression of TBC1d5-5A resulted in impaired endolysosomal flow. In conclusion, we identified a novel mTOR substrate critically involved in dendritogenesis by likely controlling unperturbed endolysosomal flow.

IDENTIFICATION OF DYSREGULATED MOLECULAR PATHWAYS IN FRATAXIN DEFICIENT PROPRIOCEPTIVE NEURONS

• **Deepika Mokkachamy Chellapandi** | *Université Lyon 1, France*

Friedreich ataxia (FA) is a rare neurodegenerative disorder characterized by a mixed spinocerebellar and sensory ataxia. The disease primarily affects the proprioceptive sensory neurons (pSNs) of the dorsal root ganglia (DRG). Axonal degeneration of these neurons has been reported, but the exact nature of their selective degeneration remains poorly understood. The disease is due to reduced levels of frataxin, a mitochondrial protein involved in Fe-S cluster biogenesis. Our study aims at uncovering the molecular mechanisms involved in the neuropathophysiology of FA. As a disease model, *FxnL3/L-; Pvalb^{tm1}(Cre)Arbr/J*

conditional knock-out mouse model that has a full depletion of frataxin in the pSNs. We used single-cell RNA sequencing (scRNA-seq) to determine the transcriptomic signature of the DRG. DRGs scRNA-seq was performed at 3.5 weeks and 7.5 weeks of age, the two key stages of the disease progression. In proprioceptive neurons, we observed the down-regulation of molecular pathways involved in cellular functions such as mTOR, axon guidance and up-regulation of pathways involved in ferroptosis, autophagy implying a reduced cellular and mitochondrial function along with severe cellular stress response.

In addition, we also observed an increase in satellite cell activity and immune response in the satellite glial cells of the DRG. To-

gether, these results allow us to determine the cellular pathways dysregulated upon frataxin depletion.

HUMAN IPSC-DERIVED CEREBRAL ORGANOIDs IMPLICATE MTOR PATHWAY DYSREGULATION IN LISSENCEPHALY

• **Angeliki Louvi** | *Yale School of Medicine/New Haven, Connecticut/USA*

Lissencephaly ("smooth brain") is a severe brain malformation commonly associated with epilepsy and intellectual disability. Underlying molecular mechanisms remain largely unclear, preventing therapeutic development. Here, we demonstrate that iPSC-derived cerebral organoids harboring a recessive mutation in P53-Induced Death Domain Protein 1 (PIDD1) display neural progenitor and cortical lamination defects and recapitulate the thickened cortex typical of human lissencephaly. Single-cell transcriptomic and proteomic analyses revealed dysregulation of protein translation, metabolism, and the mTOR pathway, mechanisms not previously implicated in lissencephaly. Furthermore, cerebral or-

ganoids from a patient with Miller-Dieker Lissencephaly Syndrome (MDLS) displayed, along with cortical thickening, similar molecular perturbations, suggesting a converging mechanism in lissencephaly. Finally, an oral, brain-selective activator of mTOR complex 1 (mTORC1) prevented morphological and molecular defects in PIDD1-mutant and MDLS organoids. In summary, we have established dysregulated mTOR signaling as a molecular mechanism underlying lissencephaly of distinct genetic causation and a clinically relevant pathway for therapeutic intervention.

BRAIN MOSAICISM IN EPILEPSY

• **Stéphanie Baulac** | *Paris Brain Institute, France*

Focal Cortical Dysplasia (FCD) is the most common focal cortical malformation leading to intractable childhood focal epilepsy. In recent years, we and others have shown that FCD type II is caused by mosaic mutations in genes within the PI3K-AKT-mTOR-signaling pathway. Hyperactivation of the mTOR pathway accounts for neuropathological abnormalities and seizure occurrence in FCD. We further showed from human surgical FCDII tissue that epileptiform activity correlates with the density of mutated dysmorphic neurons, supporting their pro-epileptogenic role. The level of mosaicism, as defined by variant allele frequency (VAF) is thought to correlate with the size and regional brain distribution of the lesion such that when a somatic mutation occurs early du-

ring the cortical development, the dysplastic area is smaller than if it occurs later. Novel approaches based on the detection of cell-free DNA from the CSF and from trace tissue adherent to SEEG electrodes promise future opportunities for genetic testing during the presurgical evaluation of refractory epilepsy patients or in those that are not eligible for surgery. In utero-based electroporation mouse models allow to express somatic mutation during neurodevelopment and recapitulate most neuropathological and clinical features of FCDII, establishing relevant preclinical mouse models for developing precision medicine strategies.

GENE THERAPY FOR EPILEPSY ASSOCIATED WITH FOCAL CORTICAL DYSPLASIA TYPE II

• **Amanda Almacellas Barbanoj** | *Institute of Neurology/London/United Kingdom*

FCD type II is the commonest pathology found at epilepsy surgery in children. It is typically caused by somatic mutations resulting in the mammalian Target Of Rapamycin Complex 1 (mTORC1) hyperactivation. Epilepsy surgery is not always effective and is often precluded by proximity to eloquent brain regions. Gene therapy is thus currently the most promising candidate replacement for surgical treatment of FCD. We tested a gene therapy approach based on viral expression of a modified KCNA1 gene (which encodes the potassium channel Kv1.1), which we denote EKC (engineered K⁺ channel). We chose Kv1.1 as a therapeutic protein not only because it attenuates neuronal excitability, but also because mTOR hyperactivation is associated

with downregulation of KCNA1 expression. The therapy was tested in a mouse model of FCD type II generated by in-utero electroporation of neuronal progenitors with a constitutively active RHEB plasmid, an activator of mTORC1. A battery of molecular biology and behavioural tests confirmed that the model recapitulates the pathology seen in humans, including cognitive comorbidities. Continuous EEG recordings from mice before and after local injection of AAV9-EKC, showed a 64% decrease in seizure frequency over 4 weeks, which was not seen in mice injected with a control AAV. In conclusion, AAV9-EKC gene therapy is a promising approach for the treatment of epilepsy associated with FCD type II.

MODELING AN EMERGING NEURODEVELOPMENTAL DISORDER AFFECTING CORTICAL GYRIFICATION

• **Michele Studer** | *Institute of Biology Valrose (IBV), Univ. Côte d'Azur (UniCA), Nice, France*

The relationships between impaired brain development and consequent symptoms in neurodevelopmental disorders (NDD) are still unclear. BBSOAS is a monogenic NDD caused by the haploinsufficiency of the NR2F1 gene, a transcriptional regulator playing key roles during cortical development. Intellectual disability, autistic traits, and visual impairments are the most common symptoms affecting BBSOAS patients although with variable levels of severity. By employing complementary approaches, we have assessed the impact of the different mutations on protein stability and cell function. Nr2f1 in the mouse exerts its functions in a regionalized manner by locally control-

ling long-term self-renewal of neural progenitor cells and neuronal differentiation. Surprisingly, the PI3K/Akt/mTOR signaling is not significantly altered in these mutants despite the overgrowth of the caudal cortex; instead, cell cycle genes and key cortical development master genes regulate regionalized neurogenic activity. In humans, different NR2F1 expression levels encompass gyri and sulci that correlate with localized neurogenesis, and patients have abnormal gyral patterns, called dysgyria, in distinct cortical regions where social cognition, attention, and memory retrieval are elaborated.

In addition, reduced NR2F1 levels in human cerebral organoids affect neurogenesis. We thus propose NR2F1 as a regionalized regulator of mouse and human brain morphology and a novel causative gene of abnormal gyrification.

MTOR MOUSE MODELS TO DISSECT MECHANISMS IN CORTICAL MALFORMATIONS AND EPILEPSY

• **Alice Gilbert** | *Paris Brain Institute - ICM Paris, France*

Focal cortical dysplasia type II (FCDII) are rare cortical malformations that manifest with epilepsy and autistic spectrum disorder or intellectual disability.

FCDII is due to brain somatic mutations in mTOR pathway genes, leading to mTORC1 hyperactivity. Most patients (57%) have a variant in the MTOR gene itself. Previous *In vitro* functional assays demonstrated that the two FCDII hotspot mutations MTOR-S2215F and MTOR-T1977K induce variable mTORC1 activity levels. We hypothesize that both variants may contribute to variable phenotypes in FCDII patients.

For this purpose, we developed two mouse strains by inducing the conditional expression in *Emx1* cortical excitatory neurons of the two MTOR-S2215F and MTOR-T1977K variants: our data demonstrate that both models recapitulate key FCDII aspects, including increased cortical thickness, mTORC1 hyperactivity and spontaneous seizures. Behavioral tests on social interactions may reflect patients' co-morbidities.

These new mouse models hold promise in elucidating FCDII pathophysiological mechanisms and identifying potential therapeutic targets.

NEURONS CARRYING MUTATED MTOR HAVE A CAUSAL ROLE IN ICTOGENESIS IN FOCAL CORTICAL DYSPLASIA

• **Jan Kudlacek** | *Second Faculty of Medicine, Charles University, Prague, Czech Republic*

Introduction: Focal cortical dysplasia (FCD) is a malformation of cortical development caused by mutation of the mTOR gene in few percent of neurons. It is a common cause of refractory epilepsy. We tested the role of mutated neurons in generation of pathological activity in a realistic murine model of FCD.

Methods: FCD was generated by *in utero* electroporation of mutant mTOR (p.Leu-2427Pro) in mouse embryos on E14.5. Eight weeks postnatally, the mice were implanted cranial window and EEG electrodes and AAV vector with Channelrhodopsin-2 (ChR2) was injected into the FCD. ChR2 was expressed in the mutated neurons only using Cre-loxP system. Six weeks later we applied optical stimulation.

Results: Out of eight FCD mice, four presented spontaneous seizures (2.9 ± 2.6 per day). FCD mice also presented interictal epileptiform discharges (IEDs) and high-frequency oscillations (HFOs). The activation of mutated neurons by a single optical pulse evoked a response resembling the spontaneous IEDs with HFOs. Pulse trains induced seizures in all FCD animals.

Conclusion: The data suggest that the mutated neurons are functionally incorporated in the neuronal network and they can play a causal role in the generation of seizures and interictal activity in FCD.

Supported by grants from the Czech Health Research Council of the Ministry of Health of the Czech Republic (NU21-08-00533, NU21-04-00601), Czech Science Foundation (20-25298S, 21-17564S) and Charles University grant Primus 2170011.

DE NOVO LOSS- AND GAIN-OF-FUNCTION VARIANTS IN RHEB CAUSE A SPECTRUM OF NEURODEVELOPMENTAL DISORDERS

• **Steffen Syrbe** | *Center for Pediatrics and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany*

Monogenic disorders affecting the mechanistic target of rapamycin (mTOR) pathway are a heterogeneous group of neurodevelopmental disorders (NDD). These “mTORopathies” share features of variable cerebral malformations including focal cortical dysplasia, heterotopia, and megalencephaly, commonly associated with epilepsy, intellectual disability and behavioral abnormalities. Rheb-mediated activation of mTORC1 is inhibited by the Tuberous Sclerosis Complex, a GTPase activating protein complex. To date, only few indi-

viduals with somatic and germline variants in RHEB have been identified. In this study, we assess clinical, genetic and functional data from 11 individuals with de novo germline variants in RHEB including follow up data from three previously published cases. We provide detailed epidemiologic, clinical, neuroradiologic and treatment data, expanding the number of affected individuals and define genotype-phenotype correlations.

S6 KINASE IS INVOLVED IN TSC-RELATED EPILEPSY THROUGH THE REGULATION OF TREK-1 POTASSIUM CHANNEL

• **Francesco Avanzi** | *Institut Necker Enfants Malades (INEM), Paris*

Epilepsy is the most common neurological manifestation of Tuberous Sclerosis Complex (TSC), a genetic disease caused by loss-of-function mutations on the TSC1 or TSC2 genes that results in the mTOR pathway hyperactivation. However, the molecular mechanisms that are responsible of the alterations of neuronal excitability in TSC are largely unknown. To study the role of the downstream mTOR-effector S6 Kinase (S6K), we generated a model of TSC based on AAV9-Cre-mediated deletion of the TSC1 gene in the brain of TSC1f/f and TSC1f/fS6K^{-/-} mice. Interestingly, epilepsy development and premature death of TSC mice is completely reverted upon the deletion of the S6K1/2 genes. To identify new potential downstream targets of S6Ks we performed a phosphoproteomic analysis

and we observed an enrichment of proteins known to be involved in autism and seizure with a specific phosphorylation consensus sequence for S6K (RXXS*/T*). We focused on TREK-1, a potassium channel, whose deletion increases seizure susceptibility. We demonstrated that TREK-1 is phosphorylated on Ser333 in a rapamycin-sensitive way by S6K in HEK293 cells and in primary cortical neurons upon glutamatergic stimulation. By electrophysiology recording, we observed that TREK-1 is phosphorylated and kept in a closed conformation after amino acids stimulation, while is opened in presence of rapamycin. Our work suggests a mechanism for epilepsy development and may provide new potential therapeutic approaches for TSC patients.

ROLE OF MICROGLIAL MTOR IN OBESITY

• **Daniela Cota** | *University of Bordeaux, INSERM, Neurocentre Magendie, U1215, F-33000, Bordeaux, France*

Facing an obesity epidemic, it is urgent to understand the biological mechanisms behind diet-induced changes of brain functions controlling energy balance, as those may be relevant for the identification of new anti-obesity therapies. The mechanistic target of rapamycin (mTOR) couples environmental signals, including nutrients and hormones, with the regulation of several energy-demanding cellular functions. Our work has previously shown that mTORC1 signaling regulates whole-body energy balance at the level of the hypothalamus. Beyond hypothalamic neurons, microglial cells, the resident macrophages of the brain, can also regulate food intake and body weight. In particular, microglia pro-inflammatory activation in response to

the intake of hyper-caloric food causes obesity through poorly understood mechanisms. Here I will present novel data illustrating how microglia adapt to energy surfeit. Functional kinase activity profiling of hypothalamic microglia from chow-fed and high-fat diet mice has revealed diet and sex-dependent responses, pointing to a role for mTOR signaling. Induced deletion of Raptor, an essential partner of mTORC1, in microglia protects from obesity. However, defective microglial mTORC1 signaling also increases the expression of pro-inflammatory cytokines. Thus, mTORC1 activation in microglia contributes to obesity, independently from its action on neuroinflammation. Underlying mechanisms are currently being investigated.

NEW METABOLIC SIGNALS TO MTOR: HOW CANCER CELLS DEAL WITH TRYPTOPHAN STRESS

• **Kathrin Thedieck** | *Lab for Metabolic Signaling / Institute of Biochemistry / University of Innsbruck / Austria*

Due to Tryptophan (Trp) catabolism, enhancing tumor malignancy, as well as poor vascularization, tumors are often depleted of the essential amino acid Trp. This seems to be at odds with the requirement of amino acids for protein biosynthesis in rapidly growing tumor cells; raising the question how cancer cells sustain growth when depleted of Trp. We unraveled a surprising mTOR-driven mechanism that enables tumor cells to survive and grow when Trp is scarce. We also discovered new metabolic inputs to the tuberous sclerosis protein (TSC) complex, mediated by the stress granule protein G3BP1 that anchors the TSC complex to lysosomes. Our findings unveil unexpected vulnerabilities and open new avenues for cancer therapies.

References

Pauline Pfänder*, Lucas Hensen*, Patricia Razquin Navas*, Marie Solvay*, Mirja Tamara Prentzell*, (...), Benoit J. Van den Eynde*, Christiane A*+. Opitz, Kathrin Thedieck*+ bioRxiv 2023; doi: <https://doi.org/10.1101/2023.01.16.523931>.

Prentzell MT*, Rehbein U*, Cadena Sandoval M*, De Meulemeester AS*, (...), Opitz CA*, Thedieck K+. Cell. 2021 Feb 4;184(3):655-674.e27.

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FUNCTION OF GATOR1 COMPLEX IN CISPLATIN RESISTANCE

• **Zhenrui Pan** | *CNRS UMR9018, Université Paris-Saclay, Institut Gustave Roussy, Villejuif, France*

Cisplatin is the first-line chemotherapeutic drug for a variety of epithelial cancers, especially for lung, breast and ovarian cancers. However, cisplatin resistance represents one of the most significant barriers for efficient treatment. Expression of NPRL2, NPRL3 and DEPDC5, three components of the GATOR1 complex involved in mTORC1 inhibition, is deregulated in many cancers. Low expression of NPRL2 was linked to cisplatin resistance but whether NPRL3 and DEPDC5 also involved in this resistance was unknown.

Herein, the role of three GATOR1 components in cisplatin resistance was examined in BEAS2B cells and in lung cancer cells resistant or not to cisplatin. We found that depletion of each of GATOR1 members promotes cisplatin resistance while their

overexpression makes cells sensitive to the drug. In the cells with GATOR1 depletions, less cisplatin-DNA adducts were formed. Expression of the transporters required for cisplatin efflux was increased and required for cisplatin influx was decreased upon cisplatin treatment, explaining decreased cisplatin accumulation in GATOR1 depleted cells. We also found that these cells have altered DNA damage response and exhibited compromised mitochondrial function, which is interesting given that mitochondria plays essential role in cisplatin resistance and mitochondrial functions are controlled by mTORC1. Together our data propose that modulating of GATOR1 expression might be considered as a new strategy to overcome cisplatin resistance.

CELLULAR ENLARGEMENT - A NEW HALLMARK OF AGING?

• **Jette Lengefeld** | *University of Helsinki, Finland and Center for Hematology and Regenerative Medicine, Karolinska Institutet, Sweden*

Years of important research has revealed that cells heavily invest in regulating their size. Nevertheless, it has remained unclear why an accurate size control is so important.

Our recent study using hematopoietic stem cells (HSCs) in vivo indicates that cellular enlargement is causally associated with aging. I present an overview of these findings and their implications. Furthermore, I present the

results of a broad literature analysis revealing the potential of cellular enlargement as a new aging hallmark and highlight interesting work that shows a correlation between cell size and age-related diseases.

Taken together, I present observations suggesting that deviations from the optimal cellular size are linked to aging and age-related diseases.

DRUG REPOSITIONING FOR RARE DISEASES INVOLVING RAS/PIK3CA PATHWAY

• **Guillaume Canaud** | *Hôpital Necker Enfants Malades, Université Paris Cité, INSERM U1151*

The genetic landscape of overgrowth syndromes and vascular anomalies is rapidly evolving and opens opportunities for targeted therapies. I will review different examples during this presentation.

MTORC1 SENSES IRON LEVEL AND REGULATES ANTI-FERROPTOTIC RESPONSE OF THE CELL: BETWEEN « FERRO » AND « PTOSIS »

• **Milica Vucetic** | *Department of Medical Biology, Centre Scientifique de Monaco (CSM), 98000 Monaco, Monaco*

As a major cellular hub, mTORC1 is recognized as a key sensor for various nutrients such as amino acids, lipids, and nucleotides. However, this is far from a complete picture of the sensory function of mTORC1. Previously, several studies indicated its ability to sense one of the most abundant, essential micronutrients - iron. Indeed, data from our laboratory showed that mTORC1 promptly senses any change in iron content, most likely via TSC2, a kinase sensor and negative regulator of the mTORC1. Suppression of mTORC1 following iron deprivation is quite reasonable given the essential role that iron plays in the structure and function of many proteins/enzymes involved in cellular oxygenation and metabolism. However, this seems to be only one side of

the story. Namely, in addition to being an essential element, iron can also have a very harmful effect, causing oxidative damage to the cell, which can eventually lead to cell death, known as ferroptosis. According to our results, it seems that iron-dependent regulation of mTORC1 could be a way to protect the cell from ferroptosis. Namely, we showed that mTORC1 regulates the expression of glutathione peroxidase 4 (GPx4), the main player in the anti-ferroptotic cellular defense that enables the detoxification of iron-catalyzed oxidative damage. Therefore, mTORC1 sensing of iron appears to allow cells to make the most of this essential element, while at the same time, protecting it from oxidative insults catalyzed by the same element.

ER-STORAGE RESPONSE TO PROTEIN MISFOLDING ACTIVATES ER-PHAGY VIA FAM134B TRANSCRIPTIONAL INDUCTION.

• **Marianna Maddaluno** | *TIGEM/Pozzuoli (NA)/Italy*

Protein production within the endoplasmic reticulum (ER) is essential for the development and functioning of organisms. However, the efficient removal of misfolded proteins is necessary to avoid cellular toxicity. While the ER-associated protein degradation (ERAD) pathway governs the disposal of misfolded clients through proteasomal degradation, ER-phagy directs the degradation of ERAD ineligible misfolded polypeptides within lysosomes. Nevertheless, the mechanism underlying the initiation of ER-phagy in response to misfolded protein accumulation remains unknown. Our work unveils that misfolded

proteins in the ER elicits an ER storage response mediated by the transcriptional regulation of a handful of genes, including SESTRIN2, a leucine-binding protein. Upregulation of SESTRIN2 inhibits mTORC1 kinase and consequently TFEB/TFE3 transcription factors migrate into the nucleus causing upregulation of the ER-phagy receptor FAM134B, involved in lysosomal and autophagy processes. This coordinated response effectively facilitates ER-phagy-mediated lysosomal degradation of misfolded cargoes. Moreover, we demonstrate that targeted pharmacological manipulation of this pathway enhances the clearance of

misfolded proteins in cellular models of ER storage diseases. Our study sheds light on the interplay between nutrient signaling and ER quality control, opening new potential

therapeutic implications for managing disorders associated with aberrant protein folding in the ER.

METABOLIC ADAPTATIONS OF SENESCENT CELLS

• **Khaled Tighanimine** | *Cell growth control by nutrients / Institut Necker Enfants Malades / Paris*

Cellular senescence impacts many physiological and pathological processes. A durable cell cycle arrest, inflammatory secretory phenotype, and metabolic reprogramming characterize it. Identifying common and specific metabolic liabilities in senescence provide novel inroads to exploit senescence targeting for health benefits. Here, we use dynamic transcriptome and metabolome profiling in different senescence subtypes to reveal common and specific metabolic signatures. Specifically, we pinpoint the homeostatic switch of glycerol-3-phosphate (G3P) and phosphoethanolamine (PEtn) accumulation, intimately linking lipid metabolism to the senescence

gene expression program. Mechanistically, p53-dependent glycerol kinase (GK) activation and post-translational inactivation of Phosphate Cytidyltransferase 2-Ethanolamine (PCYT2) regulate this metabolic switch, which is senogenic. Conversely, G3P phosphatase (G3PP) and Ethanolamine-Phosphate Phospho-Lyase (ETNP-PL)-based scavenging of G3P and PEtn is senomorphic. Collectively, our study ties the G3P-PEtn homeostatic switch to controlling lipid droplet biogenesis and phospholipid flux in senescent cells, providing a potential, novel therapeutic avenue for senescence targeting in pathophysiology.

THE POTENT ANTI-TUMOR EFFECTS OF RHODIOLA DRINKING ARE ASSOCIATED WITH THE INHIBITION OF THE MTOR PATHWAY AND MODIFICATION OF TUMOR METABOLISM

• **Xiaolin Zi** | *Department of Urology, University of California, Irvine, USA*

Background: SHR-5 has been used as an “adaptogen” for enhancing physical and mental performance and for fighting stress in the healthy population. The purpose of this study is to determine the chemopreventive efficacy of SHR-5 for superficial bladder cancer and to investigate the underlying mechanisms of action. Methods: UPII-mutant Ha-ras bladder-cancer-transgenic mice, that developed low-grade and noninvasive papillary transitional urothelial cell carcinoma, were fed with 1.25 and 6.25 mg/mL SHR-5 in drinking water for 6 months. The survival of the mice, obstructive uropathy, tumor burden and morphology, and proliferation were evaluated by pathological, molecular, metabolic, and statistical analyses. Results: Approximately 95% or

more of the male UPII-mutant Ha-ras mice that drank SHR-5 daily survived over 6 months of age, while only 33.3% of those mice that drank normal water survived over 6 months of age ($p < 0.0001$); SHR-5 drinking exposure also reduced tumor-bearing bladder weight and urinary tract obstruction and inhibited mTOR signaling in neoplastic tissues. Global metabolic analysis revealed that SHR-5 resulted in increased phenolic metabolites and decreased CoA, a critical metabolic cofactor for lipid metabolism. Conclusions: Our findings highlight the potential of SHR-5 as an anti-aging agent for bladder cancer prevention through reshaping tumor metabolism via the inhibition of the mTOR signaling.

NEURONAL CELL GROWTH AND TISSUE SIZE REGULATION BY MTOR UTRS

• **Nitzan Samra** | *The weizmann institute/Rehovot/Israel*

mTOR is known to promote cell growth, survival, and body mass. However, how mTOR functions in different subcellular compartments to support these functions is not clear. In previous work, we showed that mTOR is locally translated in the axons of dorsal root ganglia neurons (DRG) and its partial mis-localization results in reduced neuronal survival following nerve injury. Here, we used fluorescence in-situ hybridization (FISH) assay of a reporter transcript to identify three axonal localization elements in both 5' and 3' untranslated regions (UTRs) of mTOR, termed MLS1, 2 and 3. Affinity proteomics revealed several RNA binding proteins that interact with different MLS combinations, including nucleolin, an RBP previously shown to play a role in mRNA axonal transport and cell size regu-

lation. We generated gene-edited mice with deletions of the different MLS, alone or in different combinations. Deletion of MLS1/2 in the mTOR 5'UTR caused a general mTOR hypomorph phenotype with stunted growth, partial embryonic lethality and reduced testis size. Interestingly, these phenotypes were either partially ameliorated or fully rescued by concomitant deletion of mTOR 3' UTR. An MLS2/3 deletion mouse showed reduced mTOR translation levels in the axons but not in the cell body. Interestingly, this specific reduction in axonal mTOR caused an increase in axonal outgrowth. These findings highlight specific roles of mTOR UTRs in regulating subcellular localization, cell growth and tissue size.

PIK3CA GAIN-OF-MUTATION IN ADIPOSE TISSUE INDUCES METABOLIC REPROGRAMMING WITH THE WARBURG-LIKE EFFECT AND SEVERE ENDOCRINE DISRUPTION

• **Sophia Ladraa (online)** | *Hôpital Necker Enfants Malades, Université Paris Cité, INSERM U1151*

PIK3CA-related overgrowth syndrome (PROS) is a genetic disorder caused by somatic mosaic gain-of-function mutations of PIK3CA. Clinical presentation of patients is diverse and associated with endocrine disruption. Adipose tissue is frequently involved, but its role in disease development and progression has not been elucidated. Here, we created a mouse model of PIK3CA-related adipose tissue overgrowth that recapitulates patient phenotype. We demonstrate that PIK3CA mutation leads to GLUT4 membrane accumulation with a negative feedback loop on insulin secretion,

a burst of liver IGFBP1 synthesis with IGF-1 sequestration, and low circulating levels. Mouse phenotype was mainly driven through AKT2. We also observed that PIK3CA mutation induces metabolic reprogramming with Warburg-like effect and protein and lipid synthesis, hallmarks of cancer cells, in vitro, in vivo, and in patients. We lastly show that alpelisib is efficient at preventing and improving PIK3CA adipose tissue overgrowth and reversing metabolomic anomalies in both animal models and patients.

TOR HD – REVEALING TOR REGULATION THROUGH CRYOEM

• **Robbie LOEWITH** | *University of Geneva, Switzerland*

The Loewith group focuses primarily on understanding how various stimuli are recognized and signaled to the TOR complexes, primarily using the model eukaryote *Saccharomyces cerevisiae* (bakers' yeast, where TOR was originally discovered). Although yeast has been traditionally exploited for its facile genetics, we have recently extended our studies to include structure determinations of the highly conserved signaling nodes upstream of TORC1 and TORC2. In this talk I will present our recent work on the SEAC (GATOR) – EGOC (RAGULATOR) – TORC1 (mTORC1) axis. Elegant work by others demonstrated that the SEA complex can be functionally divided into two subcomplexes SEACAT (GATOR2, an activator of TORC1) and SEACIT (GATOR1, an

inhibitor of TORC1). To achieve this regulation, it is postulated that SEACAT inhibits the GAP activity of SEACIT towards Gtr1p (RagA/B). To understand this regulation, we solved the cryo-EM structure of the native eight-subunit SEAC and the SEAC bound to its five-subunit substrate EGOC. These structures reveal that SEACIT and SEACAT indeed form separate subcomplexes within the SEAC, but do not explain how SEACIT inhibits SEACAT GAP activity. Subsequent, structure-guided mutational and cell biology studies rather revealed that the GAP activity of SEACIT plays a relatively minor role in TORC1 regulation prompting us to hypothesize that SEACIT functions primarily by competing "active" EGOC away from TORC1, in addition to creating "inactive" EGOC.

THE ROLE OF MEMBRANE BINDING IN MTOR COMPLEX 2 SIGNALLING

• **Iain Hay** | *MRC Laboratory of Molecular Biology, Cambridge, UK*

In human cells, the mTOR kinase is a key regulator of cellular homeostasis, converging on multiple fundamental processes. Within the cell, mTOR is present in two structurally and functionally distinct complexes; mTOR complex 1 (mTORC1) and complex 2 (mTORC2). In response to nutrient abundance, mTORC1 is localised to the lysosome via the Rag/Ragulator complex, where it is activated by the small GTPase RHEB to stimulate downstream signalling. In contrast, mTORC2 is active at the plasma membrane, where it plays a key role in the PI3K signalling pathway by phosphorylating the AKT kinase 'hydrophobic motif', leading to activation of downstream signalling such as cell growth and survival. Despite this importance,

the molecular mechanism of mTORC2 membrane association and how this leads to enzymatic activation/signal propagation remains poorly understood.

My ongoing work focuses on investigating the molecular mechanisms of mTORC2 membrane association. I have reconstituted membrane binding of mTORC2 in vitro on synthetic liposomes containing various phosphoinositide (PIP_x) species. Through biochemical and kinetic analyses, I have investigated the PIP_x specificity of mTORC2 and how this binding alters enzymatic activity. Furthermore, I have determined an initial cryo-ET reconstruction of mTORC2 on PI(4,5)P₂ liposomes, allowing visualisation of membrane-bound mTORC2 for the first time.

SPATIALLY AND FUNCTIONALLY DISTINCT MTORC1 ENTITIES ORCHESTRATE THE CELLULAR RESPONSE TO AMINO ACID AVAILABILITY

• **Constantinos Demetriades** | *Max Planck Institute for the Biology of Ageing (MPI-AGE), Cologne, DE*

Amino acid (AA) availability is a robust determinant of cell growth, through controlling mTORC1 activity. According to the predominant model, AA sufficiency drives the recruitment and activation of mTORC1 on lysosomes by the heterodimeric Rag GTPases, from where it coordinates the majority of cellular processes. Importantly, however, almost 15 years after its initial discovery, the teleonomy of the proposed lysosomal regulation of mTORC1, and where mTORC1 acts on its effector proteins remain enigmatic. Here, by using multiple pharmacological and genetic means to perturb the lysosomal AA sensing and protein recycling machineries, we describe the spatial separation of mTORC1 downstream functions in mammalian cells, with lysosomal and non-lysosomal mTORC1 entities phosphorylating distinct substrates in res-

ponse to different AA sources. Moreover, we reveal that the reason that a fraction of mTOR localizes at lysosomes is due to basal lysosomal proteolysis that locally supplies new AAs, even in cells grown in the presence of exogenous nutrients. Overall, our study substantially expands our knowledge on the topology of mTORC1 regulation by AAs, and hints at the existence of distinct, Rag- and lysosome-independent mechanisms that control its activity at other subcellular locations. Given the importance of mTORC1 signaling and AA sensing for human ageing and disease, our findings open new directions towards the identification of function-specific mTORC1 regulators and effectors.

MTORC1 ACTIVITY AND TISSUE GROWTH IN VIVO

• **Aurelio Teleman** | *IGerman Cancer Research Center (DKFZ)*

I will discuss insights we have gained into how mTORC1 activity is regulated in vivo in living tissues using *Drosophila* as a model system, and how mTORC1 regulates tissue growth.

INHIBITION OF PHOSPHODIESTERASE 4D SUPPRESSES MTORC1 SIGNALING AND PANCREATIC CANCER GROWTH

• **Jenna Jewell (online)** | *UT Southwestern Medical Center*

The mammalian target of rapamycin complex 1 (mTORC1) senses multiple upstream stimuli to orchestrate anabolic and catabolic events that regulate cell growth and metabolism. Hyperactivation of mTORC1 signaling is observed in multiple human diseases; thus, pathways that suppress mTORC1 signaling may help to identify new therapeutic targets. Here, we report that phosphodiesterase 4D (PDE4D) promotes pancreatic cancer tumor growth by increasing mTORC1 signaling. GPCRs paired to Gas proteins activate adenylyl cyclase, which in turn elevates levels of 3',5'-cyclic adenosine monophosphate (cAMP), whereas PDEs catalyze the hydrolysis of cAMP to 5'-AMP. PDE4D forms a complex with

mTORC1 and is required for mTORC1 lysosomal localization and activation. Inhibition of PDE4D and the elevation of cAMP levels block mTORC1 signaling via Raptor phosphorylation. Moreover, pancreatic cancer exhibits an upregulation of PDE4D expression, and high PDE4D levels predict the poor overall survival of patients with pancreatic cancer. Importantly, FDA-approved PDE4 inhibitors repress pancreatic cancer cell tumor growth in vivo by suppressing mTORC1 signaling. Our results identify PDE4D as an important activator of mTORC1 and suggest that targeting PDE4 with FDA-approved inhibitors may be beneficial for the treatment of human diseases with hyperactivated mTORC1 signaling.

PROLONGED DEPRIVATION OF ARGININE OR LEUCINE INDUCES PI3K/AKT-DEPENDENT REACTIVATION OF MTORC1

• **Anders Mutvei** | *UT Southwestern Medical Center*

The mechanistic target of rapamycin complex 1 (mTORC1) is a serine/threonine kinase complex that promotes anabolic processes including protein, lipid, and nucleotide synthesis, while suppressing catabolic processes such as macroautophagy. mTORC1 activity is regulated by growth factors and amino acids, which signal through distinct but integrated molecular pathways: growth factors largely signal through the PI3K/Akt-dependent pathway, whereas the availabilities of amino acids leucine and arginine are communicated to mTORC1 by the Rag-GTPase pathway. While it is relatively well described how acute changes in leucine and arginine levels affect mTORC1 signaling, the effects of prolonged amino acid deprivation remain

less well understood. Here, we demonstrate that prolonged deprivation of arginine and/or leucine leads to reactivation of mTORC1 activity, which reaches activation levels similar to those observed in nutrient-rich conditions. Surprisingly, we find that this reactivation is independent of the regeneration of amino acids by canonical autophagy or proteasomal degradation but is dependent on PI3K/Akt signaling. Together, our data identify a novel crosstalk between the amino acid and PI3K/Akt signaling pathways upstream of mTORC1. These observations extend our understanding of the role of mTORC1 in growth-related diseases and indicate that dietary intervention by removal of leucine and/or arginine may be an ineffective therapeutic approach.

MTORC1 ACTIVITY LICENSES ITS RELEASE FROM LYSOSOMES

• **Aishwarya Acharya** | *Max Planck Institute for Biology of Ageing/Cologne/Germany*

The activity of mTORC1 is tightly regulated by an elaborate machinery that conveys information about nutrient availability to trigger an appropriate cellular response. A major part of this fine-tuning is brought about by control of the sub-cellular localisation of mTORC1: in nutrient-starved conditions, mTORC1 is non-lysosomal and inactive, while in nutrient-rich conditions, mTORC1 is actively recruited to lysosomes where it is activated and subsequently phosphorylates its lysosomal as well as non-lysosomal substrates. The recruitment of mTORC1 to lysosomes is orchestrated by the Rag GTPases, the ultimate node of the upstream nutrient sensing machinery, that are in their active conformation when the cellular environment is conducive to active mTORC1. However,

for the dynamic cycling of mTORC1 on and off lysosomes in order to maintain basal activity as well as to regulate its non-lysosomal substrates, a critical point of regulation is its release from the lysosomal surface. Here we propose a newfound mechanism by which active mTORC1 licenses its own release from lysosomes and acts as a signalling checkpoint to prevent its anomalous retention. This mechanism also ensures that inactive mTORC1 complexes stay associated with lysosomes, presumably to prime them for their reactivation once fresh nutrients are produced locally. In sum, our work reveals that not only recruitment but also release of mTORC1 from the lysosomal surface is an active process mediated by the Rag GTPases.

THE ROLE OF TOR IN THE REGULATION OF GROWTH AND DEFENSE RESPONSES IN PLANTS

• **Sarah Courbier** | *Light Perception and Signalling in Plants / Albert-Ludwigs-Universität Freiburg, Institut für Biologie II / Freiburg im Breisgau / Germany*

The irreversible losses of arable land areas (due to intensive farming) forces growers to produce more yield with less land. One strategy to boost yield is to grow plants closer together. However, at such high planting densities, plants ultimately shade each other, which strongly affects plant growth and yield. Plants perceive shade via specialized photoreceptors called phytochromes allowing plants to outgrow their neighbours for light via a process called shade avoidance. In addition to eliciting growth responses, shaded plants display decreased resistance towards pathogens, via a dampening of defence hormone signalling referred to as the shade-induced susceptibility. Recently, we found that shaded plants ac-

cumulate soluble sugars, which is closely associated with faster lesion development by pathogens in plant leaves. Yet, the mechanism behind how sugars leads to enhanced pathogen susceptibility in shaded conditions remains unclear. In a recent RNA-sequencing study, we found that TARGET OF RAPAMYCIN (TOR) is upregulated in response to shade. In eukaryotes, TOR is a master growth regulator activated by glucose and auxin, both strongly elevated in shaded plants, too. I am currently investigating whether and how shade perception regulates TOR expression and TOR activity dynamics, which could in turn influence plant growth and defense responses.

SELECTIVE RIBOPHAGY IN PLANTS: UNRAVELING THE MECHANISM OF TOR-DRIVEN RIBOSOME DEGRADATION

• **Mikhail Schepetilnikov** | *IBMP UPR2357 CNRS Strasbourg France*

Deleterious stress induces persistent damage to ribosomes, which activates cytoxic stress response and promotes bulk autophagic flux as a part of cellular clearance programme. Autophagy targets detrimental or unwanted cytoplasmic content to vacuolar degradation via selective or bulk pathway, thus being a subject to tight regulation, while dysregulation has severe impact on proteostasis and cell fitness. Selective degradation of ribosomes (ribophagy) has extreme importance for translational reprogramming during stress adaptation. TOR signaling pathway controls key cellular processes such as translation, autophagy, and ribosomal biogenesis. However, relative contribution of TOR-mediated translational and degradative mechanisms to the control

of differential ribosome abundance during various stresses is poorly understood. Here we tend to uncover what makes one ribosome susceptible to degradation, while protects and excludes others (translation competent) from autophagy flux. We designed a ribosome profiling-based experimental setup to provide mechanistic insight into the failure to degrade ribosomes properly. We discovered that TOR activity promotes ubiquitin-like autophagy adaptor ATG8 binding to the ribosomes. ATG8 forms a complex with the compromised ribosome to mark it for autophagy-mediated degradation. Our biochemistry and genetics data firmly established that ATG8 binds to the ribosome and provided a potential mechanism of ATG8-dependent selective ribophagy upon stress.

MTORC1 RESTRICTS TFE3 ACTIVITY BY AUTO-REGULATING ITS PRESENCE ON LYOSOMES

• **Fried Zwartkruis** | *University Medical Center Utrecht (UMCU)*

mTORC1 is a protein complex that plays a central role in cell growth control. Basal activity of its residing kinase mTOR requires the presence of amino acids. This is relayed to mTORC1 by dimeric Rag-GTPases that associate with mTORC1 on lysosomes, where growth factor signaling can further activate mTORC1 via the small GTPase Rheb. In the absence of amino acids, GATOR1 inactivates the Rags, resulting in lysosomal detachment and inactivation of mTORC1. Here, we use pharmacological inhibitors and genome editing to demonstrate that release of mTORC1 from lysosomes critically depends on its kinase activity. In accordance with a negative feedback mecha-

nism, activated mTOR mutants display low lysosome occupancy, which causes hypo-phosphorylation and nuclear localization of its lysosomal substrate TFE3. Inactivation of GATOR1 rescued this effect by re-localising mutant mTOR on lysosomes. Surprisingly, mTORC1 activated by Rheb does not increase the cytoplasmic/lysosomal ratio of mTORC1. Based on these data, we propose the existence of separate mTORC1 pools with distinct substrate specificity. Proper control of each pool is essential to restrict activity of MiTF/TFE transcription factors that are critical in development of syndromes like Birt-Hogg-Dubé (BHD) and Tuberous Sclerosis (TSC).

SELECTIVE MODULATION OF MTORC1 SIGNALING AT THE LYSOSOME

• **Gennaro Napolitano** | *Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy*

The mechanistic target of rapamycin complex 1 (mTORC1) is a nutrient- and stress-sensing protein kinase that plays a central role in the control of cell metabolism and growth. The activation of mTORC1 requires its amino acid-dependent recruitment to the lysosomal membrane via the Rag GTPases, obligate heterodimers of RagA or B bound to RagC or D, and its growth factor-dependent activation by Rheb. Different nutritional inputs impinge on mTORC1 activation status to induce the phosphorylation of its multiple substrates. However, whether and how mTORC1 can differentially phosphorylate its substrates in response to specific stimuli has long remained elusive. We recently discovered a substrate-specific mTORC1 pathway that

controls the function of TFEB and other MiT-TFE factors, key transcriptional regulators of cell catabolism. Owing to a “non-canonical”, Rag GTPase-mediated substrate recruitment mechanism, mTORC1-mediated phosphorylation of TFEB is highly sensitive to amino acid-dependent activation of RagC/D but insensitive to growth factor-mediated activation of Rheb, whereas the “canonical” mTORC1 substrates S6K and 4E-BP1 behave in the opposite manner. This mechanism enables mTORC1 to promote selective metabolic responses to different environmental cues by differential substrate phosphorylation. I will discuss the mechanisms regulating mTORC1 substrate-specific activity and the relevance of these processes in physiology and disease.

Posters

ABSTRACTS



1

IDENTIFICATION OF A DISEASE-RELEVANT DUB THAT DIRECTLY CONTROLS MTOR UBIQUITINATION AND ACTIVITY

• **Stephanie A. Fernandes^(1,2), Jiyoung Pan^(1,2), Aurelio A. Teleman^(3,4) and Constantinos Demetriades^(1,2,5)** | ⁽¹⁾ *Max Planck Institute for Biology of Ageing (MPI-AGE), Cologne, Germany;* ⁽²⁾ *Cologne Graduate School for Ageing Research (CGA), Cologne, Germany;* ⁽³⁾ *German Cancer Research Center (DKFZ), Heidelberg, Germany;* ⁽⁴⁾ *Heidelberg University, Heidelberg, Germany;* ⁽⁵⁾ *University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany.*

Regulation of mTOR activity plays a crucial role in cellular homeostasis and is commonly dysregulated in various disease settings and in ageing. mTOR activity is regulated by upstream signaling in response to multiple stimuli and by post-translational modifications (PTMs) like phosphorylation and ubiquitination. Importantly, although its phosphorylation has been studied in detail, the role of mTOR ubiquitination is less well understood. Protein ubiquitination is a reversible process. While some E3 ligases that attach ubiquitin (Ub) molecules

on mTOR have been identified before, no deubiquitinase (DUB) enzyme that removes these PTMs is known to date. Here, through an RNAi screen for novel mTOR regulators, we have identified and characterized a disease-relevant DUB that interacts with mTOR, removes K63-linked Ub chains, and regulates its activity, both in the context of mTOR complex 1 (mTORC1) and mTORC2, thereby controlling key cellular downstream processes like protein synthesis and cell size.

2

LEC1: A NOVEL REGULATOR OF THE YEAST PI3P MEDIATED AUTOPHAGY?

• **Patricio Aguirre Pineda⁽¹⁾, Johana Chicher⁽²⁾, Lauriane Kuhn⁽²⁾, Philippe Hammann⁽²⁾, Séverine Bär^(1*) and Sylvie Friant^(1*)** | ⁽¹⁾ *Laboratoire de Génétique Moléculaire, Génomique, Microbiologie (GMGM), UMR7156, Université de Strasbourg, Centre National de Recherche Scientifique (CNRS), 67000 Strasbourg, France;* ⁽²⁾ *Plateforme Strasbourg-Esplanade, Institut de Biologie Moléculaire et Cellulaire, CNRS, Université de Strasbourg, France.*

Vps15 (Vacuolar protein sorting 15) is a protein kinase essential for autophagy. By recruiting the lipid kinase Vps34, it forms the autophagy-specific PI3P-kinase complex I, producing phosphatidylinositol-3-phosphate (PI3P), which is required for autophagy initiation. Our yeast *Saccharomyces cerevisiae* Vps15 protein interactome revealed a new partner: the Lec1 (Lipid-ergosterol cortex 1) protein, which regulates ergosterol distribution. Lec1 resides at lipid droplets-ER contact sites, and contains a PX (Phox homology) domain, which interacts with PI3P.

To study the link between Vps15 and Lec1 in autophagy, a phenotypic study under nitrogen starvation has been done. Interestingly, contrary to *lec1Δ* deletion mutants, cells overexpressing N- and

C-terminal GFP tagged Lec1 lacked autophagic bodies in their vacuolar lumen. Moreover, in nitrogen starvation, Lec1 protein levels decreased in both wild-type and *vps15Δ* cells. These data are indicative of Lec1 being a negative regulator of autophagy, maybe by regulating a step of autophagosome biogenesis linked to Vps15-PI3P production.

In *vps15Δ* cells, upon nitrogen starvation, a subset of Lec1 seems to delocalize from the cytoplasmic fraction to the vacuole membrane fraction P13 (13000 g pellet).

These data show that Lec1 is downregulated during autophagy, and this takes place in a Vps15 independent manner. Our goal is to uncover what could be Lec1's inhibitory role in autophagy and what effectors regulate its function.

3

SINGLE-CELL AND SPATIAL TRANSCRIPTOMIC PROFILING OF MTOR HYPERACTIVATION IN FOCAL CORTICAL DYSPLASIA SHEDS LIGHT ON DISEASE MECHANISMS

• Sara Baldassari⁽¹⁾, Esther Klingler^(2,3), Lucia Gomez Teijeiro⁽²⁾, Marion Doladilhe⁽¹⁾, Corentin Raoux⁽¹⁾, Sergi Roig Puiggros⁽²⁾, Sara Bizzotto⁽¹⁾, Lina Sami⁽¹⁾, Théo Ribierre⁽⁴⁾⁽⁵⁾, Eleonora Aronica⁽⁶⁾⁽⁷⁾, Mathilde Chipaux⁽⁸⁾, Homa Adle-Biassette⁽⁹⁾, Denis Jabaudon^(2,10) and Stéphanie Baulac⁽¹⁾ |

Focal cortical dysplasia type II (FCDII) is a brain cortex malformation characterized by dyslamination and cytomegalic cells. FCDII is associated with pediatric drug-resistant epilepsy subjected to neurosurgery; it is caused by somatic mutations in genes of the mTOR pathway causing its hyperactivation in a mosaic pattern, higher in cytomegalic cells. The neurodevelopmental effects of these mutations and the affected cell types remain unclear.

We combined single-cell and spatial transcriptomic and genotyping approaches in surgical samples from FCDII patients with somatic mTOR-activating mutations.

We identified cell-type specific transcriptional changes in FCDII samples, affecting synaptic signaling and development in glutamatergic neurons and astrocytes. We uncovered

an unexpected distribution of the somatic mutations across cell types, suggesting that FCDII mutational events occur earlier than previously hypothesized. Cytomegalic cells (thought to be the main carriers of the mutation) represent a minor fraction of mutated cells in the tissue, highlighting an incomplete penetrance of the cell overgrowth phenotype. Finally, we identified novel disease biomarkers indicating a significant role of altered metabolism and mitochondrial function in FCDII pathogenesis.

We highlight an unforeseen diversity of cell types affected by mTOR pathway mutations in FCDII and provide a resource to better understand FCDII-related epilepsy and identify biomarkers for targeted therapeutics.

4

THE MOLECULAR MECHANISMS OF THE MTOR PATHWAY IN NEURONAL AND GLIAL DEVELOPMENT

• **Alexia Bankolé, Ayush Srivastava, Delphine De Villeneuve, Francesco Avanzi, Stefano Fumagalli, Nathalie Spassky, Mario Pende** | *INEM (Institut Necker Enfants Malades)*

The mammalian Target Of Rapamycin (mTOR) kinase is a master regulator of cell growth present in eukaryotic cells. Through the phosphorylation of substrates, mTOR coordinates growth and metabolism depending on nutrient availability. Genetic mutations in Tuberous Sclerosis Complex (TSC) upregulate the mTOR activity and predispose to malformations of cortical development and subependymal nodules, which are associated with epilepsy, intellectual disability and autism. Little is known on the molecular targets downstream of mTOR that transduce the aberrant signals in disease conditions. Furthermore, the perturbations of the neural cell development in TSC is still enigmatic. The aim is to understand how mTOR signaling perturbations regulate

the development of progenitor cells in the subependymal region. In collaboration with Nathalie Spassky's Team at the Ecole Normale Supérieure, we showed that mTOR and S6K1 favor the differentiation of ependymal cells. Moreover, in collaboration with Cell Signaling Technology, we performed phosphoproteomic analysis which revealed that the majority of the rapamycin sensitive targets were involved in microtubule dynamics and cell cycle control. Indeed, the investigation of cell cycle markers showed that rapamycin promotes cell cycle exit and a quiescence state before the G1 progression. These results could have implications in the understanding and treatments of TSC brain manifestations, such as subependymal nodules and giant cell astrocytomas.

5

THE YEAST PROTEIN KINASE SCH9 FUNCTIONS AS A CENTRAL NUTRIENT-RESPONSIVE HUB THAT INTEGRATES NITROGEN, CARBON, AND PHOSPHATE CUES TO ADAPT CELL GROWTH

• **Marco Caligaris⁽¹⁾, Raffaele Nicastro⁽¹⁾, Marie-Anne Deprez⁽²⁾, Joëlle Rosseels⁽²⁾, Zehan Hu⁽¹⁾, Benjamin Pillet⁽¹⁾, Farida Tripodi⁽³⁾, Belém Sampaio-Marques^(4,5), Sabine Rospert^(6,7), Riko Hatakeyama⁽⁸⁾, Paula Ludovico^(4,5), Paola Coccetti⁽³⁾, Jörn Dengjel⁽¹⁾, Joris Winderickx⁽²⁾, and Claudio De Virgilio⁽¹⁾** | ⁽¹⁾ Department of Biology, University of Fribourg, Fribourg, Switzerland; ⁽²⁾ Functional Biology, KU Leuven, Heverlee, Belgium; ⁽³⁾ Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy; ⁽⁴⁾ Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; ⁽⁵⁾ ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Braga, Portugal; ⁽⁶⁾ Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Freiburg, Germany; ⁽⁷⁾ Institute of Biochemistry and Molecular Biology, Faculty of Medicine, University of Freiburg, Freiburg, Germany; ⁽⁸⁾ Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, United Kingdom.

6

IMPLICATION OF REDD1 IN THE REGULATION OF CGAS/STING PATHWAY

• **Raphaëlle Clary, Karine Dumas, Jerome Gilleron, Jennifer Jager, Ines Mucel, Brice Angot, Mireille Cormont, Jean-François Tanti, Sophie Giorgetti-Peraldi**
| *Université Côte d'Azur, Inserm, C3M, Team «Molecular and Cellular Physiopathology of Obesity and Diabetes», 06204 Nice, France.*

Obesity is characterized by the expansion of the adipose tissue. This leads to low-grade inflammation and the production of cytokines involved in the development of insulin resistance and type 2 diabetes.

The stress-induced protein REDD1 (Regulated in Development and DNA damage responses-1) is a known regulator of the mTORC1 signaling pathway. We previously shown that REDD1 expression is dysregulated in liver and AT during obesity, and that REDD1 regulates insulin signaling pathway through mTORC1 dependent mechanisms.

We have investigated the effect of a decrease in REDD1 expression on cGAS-STING signaling pathway, which is has been described to be associated with obesity.

Downregulation of REDD1 in 3T3-L1 adipocytes using specific siRNA leads to an

increase in cGAS and STING expression, an increase in TBK1 phosphorylation, and the activation of oxidative stress revealed by p38MAPK phosphorylation and NRF2 expression.

In mice with a decrease in REDD1 expression (heterozygous mice, REDD1+/-), phosphorylation of insulin receptor, Akt or GSK3 is decreased in adipose tissue and muscle compared to wild-type littermates, suggesting that REDD1+/- mice are insulin resistant. This insulin resistance is accompanied by an increase in oxidative stress markers such as p38MAPK and NRF2, and an increase in cGAS and STING expression.

Our results suggest that REDD1 regulates the cGAS-STING pathway, and this could participate in the establishment of insulin resistance observed during obesity.

7

CDK5RAP3 MODULATES MTORC1 ACTIVITY THROUGH MACROPINOCYTOSIS

• **Adna Curukovic** | *UT Southwestern Medical Center*

The mammalian target of rapamycin (mTOR) is a conserved kinase that senses multiple upstream stimuli to regulate cell growth. mTOR is the catalytic component of a multi-protein complex called mTOR complex 1 (mTORC1), and mTORC1 hyperactivation is seen in multiple human diseases. Deciphering the molecular mechanisms by which mTORC1 is regulated is crucial to treat mTORC1-mediated disease. mTORC1 lysosomal localization and activation is promoted by amino acids. Multiple amino acids filter through the Rag GTPases to activate mTORC1. We previously found that glutamine and asparagine activate mTORC1 through a Rag GTPase-independent manner. This pathway requires the small G-protein ADP-ribosylation factor 1 (Arf1) and lysosomal glutamine and asparagine

transporter SNAT7. Specifically, SNAT7 increases mTORC1 lysosomal localization and activation after extracellular protein, such as albumin, is macropinocytosed in pancreatic cancer cells. Through mass spectrometry experiments we identified CDK5 kinase regulatory subunit-associated protein 3 (CDK5RAP3) as the SNAT7 and mTORC1 interacting protein. CDK5RAP3 is a lysosomal localized protein that interacts with the N-terminus of SNAT7 and mTORC1 after macropinocytosis. Importantly, depletion of CDK5RAP3 increases albumin-induced mTORC1 activation. Thus, our research reveals CDK5RAP3 as a crucial player in mTORC1 lysosomal localization and activation in pancreatic cancer cell growth.

8

PROLONGED DEPRIVATION OF ARGININE OR LEUCINE INDUCES PI3K/AKT-DEPENDENT REACTIVATION OF MTORC1

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The mechanistic target of rapamycin complex 1 (mTORC1) is a serine/threonine kinase complex that promotes anabolic processes including protein, lipid, and nucleotide synthesis, while suppressing catabolic processes such as macroautophagy. mTORC1 activity is regulated by growth factors and amino acids, which signal through distinct but integrated molecular pathways: growth factors largely signal through the PI3K/Akt-dependent pathway, whereas the availabilities of amino acids leucine and arginine are communicated to mTORC1 by the Rag-GTPase pathway. While it is relatively well described how acute changes in leucine and arginine levels affect mTORC1 signaling, the effects of prolonged amino acid deprivation remain

less well understood. Here, we demonstrate that prolonged deprivation of arginine and/or leucine leads to reactivation of mTORC1 activity, which reaches activation levels similar to those observed in nutrient-rich conditions. Surprisingly, we find that this reactivation is independent of the regeneration of amino acids by canonical autophagy or proteasomal degradation but is dependent on PI3K/Akt signaling. Together, our data identify a novel crosstalk between the amino acid and PI3K/Akt signaling pathways upstream of mTORC1. These observations extend our understanding of the role of mTORC1 in growth-related diseases and indicate that dietary intervention by removal of leucine and/or arginine may be an ineffective therapeutic approach.

9

DOES MTOR HYPERACTIVITY DIRECTLY OR INDIRECTLY AFFECT GABAERGIC CELLS IN FOCAL CORTICAL DYSPLASIA?

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Focal cortical dysplasia (FCD) is a developmental malformation wherein the lamination of the cortex is focally disrupted. FCD patients can display developmental delays and focal epilepsy. Notably, FCD type II represents the most common cause of drug-resistant epilepsy in children. FCDII lesions include, besides focal cortical dyslamination, abnormal cytomegalic cells such as dysmorphic neurons and balloon cells. Over the last five years, a clear causal link between the emergence of these abnormal cells and the presence of somatic mTOR mutations has been established in FCDII lesions. In the field, it has been considered that these somatic mutations arise in progenitors of the dorsal telencephalic lineage. However, this hypothesis does not explain

the differences in interneurons cell size, distribution, or number described in FCDII lesions.

Therefore, we aim to investigate whether these interneuronal defects can be caused by mutant dorsal telecephalic progenitors (via non-cell autonomous effects) or require a mutation in the ventral interneuron lineage (cell autonomous). For this purpose, we generated two DEPDC5 assembloid models containing the DEPDC5-/- mutation in the dorsal or ventral forebrain parts, respectively. We study (1) the mTORC1 activity and cell size using immunostaining, (2) the migration kinetics using live imaging, and (3) the network-like activity using calcium imaging of the gabaergic cells in both contexts at different developmental stages.

10

ROLE OF S6 KINASE IN TUBEROUS SCLEROSIS COMPLEX-RELATED EPILEPSY.

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Tuberous Sclerosis Complex (TSC) is a genetic disease caused by loss-of-function mutations in either Tsc1 or Tsc2, whose gene products associate to inhibit mTOR complex 1 (mTORC1) kinase. The loss of inhibition of mTORC1 leads to the formation of benign tumors in brain. We have previously established a postnatal mosaic model of TSC in brain using stereotactic injections in ventricles with AAV9-Cre-GFP or AAV9-GFP that develops lesions which are relatively similar to the human disease. We have shown in vivo that S6 kinase (S6K) activity plays a role in epileptogenesis and

cortical neuronal cell enlargement.

To get further knowledge, we took advantage of a mouse line already well described with neuronal-specific deletion of Tsc1 during early development with severe phenotype. To do so, TSC1^{f/f} mice are being crossed with SynCre mice and then crossed with our S6K1;2^{-/-} mice. mTORC1 activation, cell size, survival and epilepsy phenotype will be investigated in 4 different genotypes. This study will uncover the role of S6K and some of its potential targets in mechanism of physiopathology of TSC disease in brain.

11

ACETOGENINS PROMOTE APOPTOSIS AND AUTOPHAGY INDUCTION THROUGH MITOCHONDRIAL DYNAMICS DYSREGULATION IN COLORECTAL CANCER.

• **Izamary Delgado-Waldo^(1,2), Yahir Alberto Loissell-Baltazar⁽¹⁾, Nadia Jacobo-Herrera⁽²⁾, Carlos Perez-Plasencia⁽³⁾, Svetlana Dokudovskaya⁽¹⁾** | *Laboratory «Genetics and physiopathology of epilepsy», Institute du Cerveau (ICM), Paris, France.*

Natural products have provided a rich source of chemicals for the development of anti-cancer treatments. For example, Alkaloids and Annonaceous acetogenins (AAs) are metabolites secondaries found in the Annonaceae family, which are plants employed in traditional medicine in Central America, Africa and India for the treatment of cancer and various other diseases. The most prominent biologic activity of this compounds is inhibition of the mitochondrial Complex I, a process that is closely related to tumor metabolism, cell death, apoptosis, and autophagy. Mitochondria have a central role in cancer cellular metabolism. When mitochondria are irreversibly damaged, they are degraded by mitophagy. This process is regulated by the PI3K/AKT/mTOR pathway, which

also controls proliferation, differentiation, survival, metastasis.

Here we studied a role on mitochondrial function of two substances extracted from the seeds of *Annona cherimolia*: alkaloid liriodenine –a DNA intercalant– and annonaceous acetogenin laherradurine –an inhibitor of NADH-ubiquinone oxidoreductase or complex I–. We found that treatment with both components induce autophagy. In addition, we observed an increase in the mitochondrial membrane potential and glycolysis. We also determined the treatment with induce mitochondrial fission. And these results are related to the reduction of the mTOR signaling pathway. These findings indicate that the compounds inhibit the mTOR axis and induce apoptosis and autophagy.

12

ALPHA-KETOGLUTARATE AND ATP CONTROL YEAST TORC1 ACTIVATION UPON GLUCOSE STARVATION OF NITROGEN-DEPRIVED CELLS

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Although TORC1 has been initially identified in yeast, the mechanisms of TORC1 control in this model organism remain only partially known. When yeast cells grown on rich nitrogen source are subject to glucose starvation, TORC1 is inhibited. Interestingly, in yeast growing under poor nitrogen supply conditions, acute glucose deprivation triggers rapamycin-sensitive phosphorylation of Npr1, a kinase regulated by TORC1 via the Tap42-PP2A branch. Further investigation of this TORC1 activation revealed that it

responds to alpha-ketoglutarate and the energy status of the cell. Furthermore, it is under the control of Snf1, the yeast homolog of mammalian AMPK. Our results suggest that when nitrogen-deprived cells are subject to acute glucose starvation, their energy metabolism rapidly switches from fermentation to respiration. This results in an increase ATP production and a drop of alpha-ketoglutarate (consumed via the TCA cycle) which both contribute to an increase of TORC1 activity towards Npr1.

13

ROLE OF RTS3 IN THE REGULATION OF THE PHOSPHATASE 2A-LIKE SIT4 PATHWAY

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Rts3 is a putative component of the protein phosphatase 2A (PP2A) complex in budding yeast. In our study, we revisit the role of Rts3 in the regulation of the PP2A-like Sit4 pathway that functions downstream of TORC1. We observed that the RTS3 expression is strongly TORC1-dependent and is regulated mainly at the transcription level through Gln3 and Gat1, transcription activators of nitrogen catabolite genes. We performed stable

isotope labeling by amino acids in cell culture (SILAC)-based phosphoproteomic experiments comparing *rts3Δ* with wild-type cells and analyzed the proxisome of Rts3 using biotin proximity labeling – mass spectrometry. Our biochemical and genetic analyses indicate that Rts3 regulates the activity of Sit4 via interaction with Sap185 that forms a complex with Sit4 and is required for its function.

TARGETING CELLULAR SENESENCE IN MOSAIC FOCAL CORTICAL MALFORMATIONS

• **Marion Doladilhe, Théo Ribierre⁽¹⁾, Alexandre Bacq⁽¹⁾, Sara Baldassari⁽¹⁾, Mathilde Chipaux⁽²⁾, and Stéphanie Baulac⁽¹⁾** | ⁽¹⁾ Sorbonne Université, Institut du Cerveau - Paris Brain Institute - ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris, France; ⁽²⁾ Department of Pediatric Neurosurgery, Rothschild Foundation.

The human brain is a genetic mosaic resulting from postzygotic somatic mutations that arise during cortical development. Cortical malformations such as focal cortical dysplasia (FCDII) are caused by brain somatic mutations in genes within the PI3K-AKT-mTOR-signaling pathway, which produce abnormal cytomegalic cells, namely dysmorphic neurons and balloon cells.

We discovered that abnormal FCDII cells display signatures of cellular senescence as shown by immunohistochemistry and electron microscopy. We next showed that cellular senescence is present in two distinct mouse models of mTORopathy and FCDII,

allowing to test the efficiency of senolytics, drugs that specifically eliminate senescent cells, to control epileptic seizures. In vivo, on an in utero electroporation-based FCDII mouse model that express a somatic MTOR mutation, we reported that acute senotherapy was sufficient to reduce the density of pathogenic cells, leading to a significant reduction of epileptic seizures. These findings pave the way towards the development of precision medicine using innovative therapeutic strategies selectively clearing mutated cells with a senescent phenotype in a mosaic neurodevelopmental disorder.

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NON-CANONICAL MTORC1 SIGNALING IN FOLLICULAR LYMPHOMA

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mTORC1 acts as a central signaling node that integrates multiple environmental cues to regulate cell metabolism and growth. Ground-breaking work has shown that the nutrient-dependent mTORC1 activation is mediated by Rag GTPases, obligate heterodimers of RagA or RagB bound to RagC or RagD. However, how mTORC1 complex senses and integrates such a variety of upstream signals to induce selective responses was not elucidated. Recently, we have identified the presence of a “non-canonical” mTORC1 signaling pathway, mediated by RagC/D, which specifically controls the function of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy. De-regulation of this pathway is highly relevant in several types of cancer,

in which the concomitant and paradoxical activation of both mTORC1 and TFEB drives tumorigenesis. Whole exome sequencing data in patients with follicular lymphoma (FL), one of the most frequent forms of non-Hodgkin lymphoma, identified recurrent mutations in specific subunits of the v-ATPase, an essential mTORC1 activator. However, the mechanism by which these mutations contribute to lymphomagenesis is still poorly understood. Here we show new data indicating that dysregulation of non canonical mTORC1 signaling is a key feature of v-ATPase-associated follicular lymphoma. These new findings have the potential to unravel novel pathogenic mechanisms in FL, thus leading to the identification of new therapeutic targets for this incurable disease.

16

NON CANONICAL ACTIVATION OF MTORC1 BY IL-15 AND IL-18 IN PRIMARY NK CELLS

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Natural Killer (NK) cells are cytotoxic innate lymphocytes important in the response against intracellular pathogens and tumor cells. Their activity is controlled by different environmental factors such as cytokines. We have shown that two of these cytokines, IL-15 and IL-18, activate the mTORC1 complex in a synergic manner in primary NK cells. This activation is essential for proliferation, metabolism increase and protein synthesis by the cell. The use of molecular inhibitors coupled with KO experiments by CRISPR-Cas9 on primary cell, allowed us to show that these two cytokines activate mTORC1 independently of the classic Tuberous Sclerosis Complex (TSC), but by two original and

complementary pathways based on the MAPK ERK for IL-15 and p38 for IL-18. We also highlighted the importance of the MK2 protein downstream of p38 in the activation of mTORC1 by IL-18 and we are currently studying the molecular mechanisms involved in the activation of mTORC1 by MK2. This work reports a new pathway of activation of the mTORC1 complex in primary NK cells, independent of the classic activation pathways of this protein and to our knowledge not described yet in the literature. These findings allow a better understanding of the activation of NK cells by cytokines and are useful for the optimization of methods of culture and pre-activation of NK cells used in therapy.

17

EXPLORING THE IMPACT OF THE TOR PATHWAY ON G1/S TRANSCRIPTION FACTORS DYNAMICS AND CELLULAR RESPONSES TO VARIOUS STARVATION CONDITIONS

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Cellular processes are coordinated by the interplay between intricate molecular networks. Central to this coordination is the Tor1 protein, the key component of the TORc1 complex that regulates cellular growth and environmental responsiveness, among other things. How mechanistically Tor1's multifaceted interactions contribute to so many functions, including transcription, remain elusive. Many co-activators influence TOR activity in diverse contexts. Using Number and Brightness (N&B) quantitative microscopy analysis, we found Tor1 to be present at very low levels (10-15 nM). This poses an intriguing question: how can such low Tor1 levels interact with numerous co-activators?

We specifically investigated the role of TORc1 on G1/S transcription in yeast. We found that under nutrient deprivation, G1/S transcription factors (TFs) levels increased significantly, and their dynamics are altered. This response diminished in the absence of cellular components downstream of TORc1, highlighting Tor1's involvement in mediating TF levels under nutrient stress. To explore this further, we deleted TOR1 and measured G1/S TFs expression and dynamics under various starvation conditions. Our findings reveal a link between levels of the Swi6 TF and Tor1 in response to nutrient starvation, linking nutrient sensing to the cell division cycle.

18

TRYPTOPHAN STRESS ACTIVATES EGFR-RAS-SIGNALING TO MTORC1 AND P38/MAPK TO SUSTAIN TRANSLATION AND AHR-DEPENDENT AUTOPHAGY

• **The full author list will be presented during the conference. Pauline Pfänder^(1,2*), Lucas Hensen^(3*), Patricia Razquin Navas^(3*), Marie Solvay^(4,5*), Mirja Tamara Prentzell^(1,2*), Benoit J. Van den Eynde^(4,5,6,7#), Christiane A. Opitz^(1,8#,9), Kathrin Thedieck^(3,9,10#,9*) |**

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Limited supply and catabolism restrict the essential amino acid tryptophan (Trp) in tumors. How tumors sustain translation under Trp stress remains unclear. Unlike other amino acids, Trp stress activates the EGFR, which enhances RAS signaling to the MTORC1 and p38/MAPK kinases, sustaining translation. The AHR forms part of the Trp stress proteome and promotes autophagy to sustain Trp levels, and ceramide biosynthesis. Thus, Trp restriction elicits pro-translation signals enabling adaptation to nutrient stress, placing Trp into a unique position in the amino acid-mediated stress response. Our findings challenge the current perception that Trp restriction inhibits MTORC1 and the AHR and explain how both cancer drivers remain active. A glioblastoma patient subgroup with enhanced MTORC1 and AHR displays an autophagy signature,

highlighting the clinical relevance of MTORC1-AHR crosstalk. Regions of high Trp or high ceramides are mutually exclusive, supporting that low Trp activates the EGFR-MTORC1-AHR axis in glioblastoma tissue.

Reference

Pauline Pfänder*, Lucas Hensen*, Patricia R. Navas*, Marie Solvay*, Mirja T. Prentzell*, (...), Benoit J. Van den Eynde#, Christiane A. Opitz#+, Kathrin Thedieck#+
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MECHANISM OF CELL SIZE REGULATION BY S6 KINASE 1

• **Vonda Koka, Orestes Lopez Ortega, Nicolas Kuperwasser, Matt Stokes, Stefano Fumagalli, Mario Pende.** | *Institut Necker Enfants Malades, U1151.*

mTOR (mammalian Target of Rapamycin) is a serine/threonine kinase activated by nutrients and is a master regulator of cell growth, proliferation, senescence, and cell metabolism.

The deletion of one of the major substrates of mTOR, S6 kinase 1 (S6K1) leads to a cell size defect. S6K proteins are encoded by two different genes RPS6KB1 (S6K1) and RPS6KB2 (S6K2). S6K1 and S6K2 have more than 80% of sequence homology. S6K phosphorylates proteins involved in protein and nucleotide synthesis, RNA splicing, insulin sensitivity and metabolism.

The objective of our study is to identify novel selective substrates of S6K1 and S6K2 and also to characterize the molecular mechanisms involved in the cell size control by S6K1.

For this purpose, we generated by genome editing using CRISPR/Cas9 technology in MCF7 cells, KO cells for S6K1 and the homologous protein S6K2. We performed on these mutants phosphoproteomic analysis in collaboration with Cell Signaling Technology. I will present the data on the phosphoproteome and the strategies of identification of new S6K substrates.

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RAG-ULATING ARGININE METABOLISM

• **Tshering Dolma Lama Sherpa, Jenna Jewell.** | *University of Texas Southwestern Medical Center.*

Cells sense environmental amino acids and respond by adjusting anabolic and catabolic processes to best synchronize cell growth with nutritional status. The mammalian target of rapamycin (mTOR), a Ser/Thr kinase that is part of the mTOR complex 1 (mTORC1), coordinates cell growth with nutritional status. Amino acids are essential for mTORC1 activation, and mTORC1 senses amino acids through the heterodimeric Rag GTPases. Whether or not the Rag GTPases regulate other essential cellular processes is currently unclear. We utilized liquid chromatography mass spectrometry to analyze differential expression of metabolites in Rag GTPases knockout cells. We found that cells depleted of the Rag GTPases have a

significant increase in arginine metabolism and significantly elevated expression of the mitochondrial Arginase 2 (Arg2) which converts arginine to ornithine. Using isotope labelling of Arginine, we identified that in absence of Rag GTPases, arginine fluxes through ornithine to generate proline, which is well established to support cell proliferation. These results suggest that metabolic alterations may sustain mTORC1 activation in absence of Rag GTPases. Additionally, we found that the highly divergent homeobox 1 (HDX1) transcription factor upregulates Arg2 expression via the Rag GTPases. Our results uncover a new role for the Rag GTPases in regulating arginine metabolism.

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PI3KCA-HYPERACTIVATION MODULATES SECRETOME IN OVERGROWTH SYNDROME.

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Overgrowth syndromes (OS) are characterized by global or local disproportionate growth associated with other anomalies, including neurological and visceral disorders. Somatic mutations in PIK3CA gene, as well as in additional elements of the mTOR pathway, are responsible of this syndrome. As the lesions in the patients are often of mesenchymal origin, we transduce fibroblasts with hyperactive mutants as cellular model of the disease. The aim is to define the humoral components that may explain the non-cell-autonomous overgrowth observed in patients with low genetic mosaicism. To understand paracrine communication, we infected fibroblasts with Ad-PI3KCA or Ad-GFP and tested the cell activation

by analyzing p-S6 in neighboring cells. The infection with Ad-PI3KCA produces an increase in p-S6 signal in adjacent cells. Supernatant from Ad-PI3KCA or Ad-GFP infected cells was used to treat wild type control fibroblasts. We noted that the conditioned media from Ad-PI3KCA-mutated cells promote the activation of S6 in not mutated cells. In order to determine the presence of proteins in the conditioned-media, we performed a mass spectrometry analysis, revealing the presence of angiogenic factors and ECM-remodeling proteins, that may be crucial for the development of OS. Functional studies will be presented to evaluate individually the role of these humoral factors in the progress of OS.

22

REGULATION OF BUDDING YEAST TORC1 WITH OPTOGENETICS

• **Andriana Koutsoumpa and Andreas Milias-Argeitis** | *Molecular Systems Biology, Biomolecular Sciences and Biotechnology Institute, University of Groningen, the Netherlands*

The two major upstream regulators of budding yeast TORC1 are the Rag GTPases Gtr1 and Gtr2 and the phosphoinositide-binding protein Pib2. Together, the Rag GTPases and Pib2 activate TORC1 on the vacuole and signaling endosomes in response to different nitrogen and carbon sources by monitoring intracellular metabolite levels.

The study of the TORC1 regulation is complicated by the fact that cells can compensate for the absence of either the Rag GTPases or Pib2, while simultaneous loss of both regulators is lethal. Moreover, homeostatic feedback loops formed between downstream effectors and upstream regulators of TORC1 can mask the effects of deletions and mutations. To study the dynamics of the TORC1 signaling network, disentangle the roles of the two

upstream branches and distinguish direct from feedback-related effects, targeted and controllable perturbations would be ideal.

To address this need, we are developing several different optogenetic constructs that reversibly delocalize the EGO complex and Pib2 from the vacuolar surface in response to low-intensity blue light with the help of the LOVTRAP heterodimerization system. Three of our optogenetic designs show growth recovery in the dark and growth defects upon light exposure, demonstrating that the LOVTRAP system is functional. Our results suggest that reversible and dynamic TORC1 (in) activation via optogenetics is indeed feasible, and will be a useful tool for studying upstream TORC1 regulation in budding yeast.

23

TORC1 REGULATES ITS ASSOCIATION WITH THE EGO C VIA AUTOPHOSPHORYLATION AND STABILIZATION OF ITS INTERFACING SUBUNIT TCO89

• **Raffaele Nicastro, Marie-Pierre Péli-Gulli, Malika Jaquenoud, Benjamin Pillet and Claudio De Virgilio** | *Department of Biology, University of Fribourg, Fribourg, Switzerland.*

The Target of Rapamycin Complex 1 (TORC1) plays an essential role in most eukaryotes in integrating pro-proliferation stimuli and fostering processes required for cell growth and division. In spite of the vast conservation of the TORC1 components, specialized subunits exist. For example, the Tco89 subunit identified in budding yeast lacks an ortholog in higher eukaryotes, and its role in TORC1 signaling is still poorly understood.

Previously, we identified several TORC1 phosphorylation sites on Tco89, and we now demonstrate that TORC1 multisite phosphorylation antagonizes Tco89 degradation. Moreover, the increased abundance and nitrogen-starvation resistance of Tco89 in cells devoid of Gtr1/Gtr2 reveal the continuous switching

of these GTPases from the TORC1-activatory to the TORC1-inactivatory state, even in nutrient rich conditions. Importantly, AlphaFold predictions show that 4 α -helices of Tco89, which constitute the only structured domain of the protein, intertwine Gtr1/Gtr2 and the TORC1 subunit Kog1. Strikingly, we show that this domain of Tco89 alone is sufficient to maintain a wt-level basal activity of TORC1. On the other hand, mutations in these α -helices disrupt the Gtrs-TORC1 signaling axis, likely by abolishing physical interactions.

Altogether, these findings support a model where TORC1 promotes its association with Gtr1/Gtr2 by phosphorylating its subunit Tco89, which is on the contrary degraded when nitrogen is scarce and TORC1 activity has to be kept low.

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METABOLIC TREATMENT OF TSC-RELATED EPILEPSY ALLEVIATES SEIZURES IN MOUSE MODEL

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Tuberous sclerosis complex (TSC) is a neurodevelopmental disorder manifesting with various symptoms among which epilepsy affects the majority of TSC patients. The underlying mechanisms linking TSC gene mutations to epilepsy remain incompletely understood, hindering the development of effective therapeutic interventions.

In this study, we determined transcriptomic and metabolic changes in a mouse model in which neuron-specific deletion of TSC1 results in epilepsy and consequently SUDEP. Subsequently, we performed a multi-omics analysis to identify early affected pathways during epileptogenesis. Lastly, we attempted to rescue metabolite levels affected by loss of TSC1 and suppress epilepsy via single amino acid supplementation as well as mTOR inhibitors.

Our findings revealed that supplementation of single amino acids such as glutamine ameliorated seizure activity in TSC mice, suggesting a potential therapeutic avenue for TSC-associated epilepsy. However, this rescue does not compare to mTOR inhibitors in its efficacy. These results highlight the strength of multi-omics approach in identifying disease related pathways in relevant tissue and add to the emerging evidence that metabolic dysregulation plays a crucial role in the pathogenesis of TSC-associated epilepsy. Further research should elucidate the precise molecular mechanisms underlying the observed effects and explore additional metabolic targets for therapeutic intervention in TSC.

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SINGLE-CELL ANALYSIS OF THE SWITCH FROM VEGETATIVE TO FILAMENTOUS GROWTH IN *S. CEREVISIAE*

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Opportunistic pathogenic fungi pose a great threat to human health, particularly to immune compromised individuals. In the pathogenic fungi like *C. albicans*, filamentation is associated with host-cell attachment, tissue invasion and virulence. Thus, there is a need to understand the molecular machinery causing the switch from vegetative to filamentous growth.

In the model organism *S. cerevisiae*, exposure to low-nutrients can trigger evolutionarily conserved signaling pathways that lead to the formation of filament-like structures called pseudohyphae. This growth pattern characterized by unipolar attached growth of elongated cells can be compared to filamentation in other fungi.

Most of our knowledge on this process comes from agar invasion assays and colony morphology analysis. These studies allowed to identify the main players that control this morphological transition.

However, we still lack a clear understanding of how the signaling pathways such as TOR, f-MAPK, PKA and SNF, interact to promote this cell-fate transition. Therefore, our objective is to develop a microscopy assay where we have a synthetic approach to the dynamic transition from vegetative to filamentous growth. When combined with specific gene deletions, these synthetic reporters will help us to understand the contribution of individual signalling pathways to cell fate decision.

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AN AFFINITY TOOL FOR THE ISOLATION OF ENDOGENOUS ACTIVE MTORC1 FROM VARIOUS CELLULAR SOURCES

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The mechanistic target of rapamycin complex 1 (mTORC1) is a central regulator of mammalian cell growth that is dysregulated in a number of human diseases, including metabolic syndromes, aging, and cancer. Structural, biochemical, and pharmacological studies that have increased our understanding of how mTORC1 executes growth control often relied upon purified mTORC1 protein. However, current immunoaffinity-based purification methods are expensive, inefficient, and do not necessarily isolate endogenous mTORC1, hampering their overall utility in research. Here we present

a simple tool to isolate endogenous mTORC1 from various cellular sources. By recombinantly expressing and isolating mTORC1-binding Rag GTPases from *Escherichia coli* and using them as affinity probes, we demonstrate that mTORC1 can be isolated from mouse, bovine, and human sources. Our results indicate that mTORC1 isolated by this relatively inexpensive method is catalytically active and amenable to scaling. Collectively, this tool may be utilized to isolate mTORC1 from various cellular sources, organs, and disease contexts, aiding mTORC1-related research.

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NUTRIENT-DEPENDENT ACTIVATION OF MTORC1 AS A DRIVER OF DILATED CARDIOMYOPATHY

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Cardiovascular disease remains the number one cause of mortality in the Western world. The remodeling of the heart plays a central role in the development of cardiac pathologies, having a particular importance the dysregulation of cell growth processes, including atrophy or hypertrophy. Recent studies have established an involvement of the Rag GTPases as prime controllers of cardiovascular development and function. Particularly, a somatic S75Y mutation in RagC was found in a patient who died prematurely with dilated cardiomyopathy. We are studying cardiac physiology in a mouse model that expresses endogenously a S75 single amino acid variant. Surprisingly,

a mild increase in mTOR activity conferred by endogenous expression of the mutation elicited several pathological phenotypes in the heart consistent with abnormal heart development. Specifically, young RagC mutant mice present decreased heart size, fibrotic deposition, aortic insufficiency and diastolic dysfunction. Moreover, structural abnormalities consistent with dilated cardiomyopathy were evident in aged animals. We are currently trying to determine the connections between the structural rearrangements and functional alterations in the heart, to understand how an alteration in the sensing of nutrient inputs drives dilated cardiomyopathy.

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MOLECULAR CORRELATION IN PEDIATRIC PATIENTS WITH DRUG-RESISTANT FOCAL EPILEPSY AND STRUCTURAL LESION RELATED TO MTORPATHIES

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THE STRESS GRANULE PROTEIN G3BP1 CONTROLS LYSOSOMAL TSC COMPLEX RECRUITMENT IN BREAST CANCER

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The tuberous sclerosis protein (TSC) complex acts as a relay for anabolic signaling and as a tumor suppressor. These functions are mediated via the inhibition of the metabolic master regulator mTORC1 (mechanistic target of rapamycin complex 1) at its central signaling platform – the lysosomes. We recently discovered that the stress granule protein G3BP1 (Ras GTPase-activating protein-binding protein 1) anchors the TSC complex to lysosomes [1]. We will present new findings on the G3BP1-TSC axis in breast cancer and discuss the implications for therapy.

References:

[1] Prentzell MT, Rehbein U, Cadena Sandoval M, De Meulemeester A-S, Baumeister R, Brohée L, et al. G3BPs tether the TSC complex to lysosomes and suppress mTORC1 signaling. *Cell*. 2021;184(3):655-74.e27.

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STRUCTURAL INSIGHTS INTO TORC1 REGULATION BY THE SEA COMPLEX

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The Seh1-associated complex (SEAC; GATOR in mammals) plays an important role in the regulation of TORC1 by nutrient signals. The SEAC has been functionally divided into two subcomplexes: SEACIT, which acts as an inhibitor of TORC1 and has GAP activity towards Gtr1, and SEACAT, which antagonizes SEACIT in vivo and has been proposed as an inhibitor of SEACIT. Despite its importance in TORC1 regulation, structural data

has been lacking. Here, I will present structures of natively purified SEAC, alone or in complex with its substrate, the EGO complex. Unexpectedly, structural and biochemical data point towards a mode of regulation that is not completely dependent on the regulation of the GAP activity of the SEACIT. Rather, it appears that regulation of TORC1 by the SEAC, as well as the regulation of SEAC itself, is more complex than previously thought.

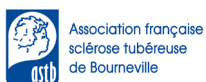
GGA1 REGULATES MTORC1 LYSOSOMAL LOCALIZATION AND ACTIVATION

• **Shweta Tiwary, Jenna Jewell** | *UT Southwestern Medical Center*

The mammalian target of rapamycin (mTOR), essential for nutrient sensing and cell growth, is a kinase that is the main component of mTOR complex 1 (mTORC1). mTORC1 senses multiple upstream stimuli like amino acids, growth factors, stress, and energy status. Elevated amino acid levels promote mTORC1 lysosomal localization and activation. Most amino acids activate mTORC1 through the Rag GTPases, however; glutamine and asparagine activate mTORC1 through a Rag GTPase-independent pathway which requires ADP-ribosylation factor 1 (Arf1). We recently reported that SNAT7, a lysosomal glutamine and asparagine transporter activates mTORC1 after extracellular protein, such as albumin, is macropinocytosed. Furthermore, mTORC1 is recruited to the lysosome

where it interacts with the N-terminus of SNAT7 in presence of glutamine and asparagine. Mass spectrometry experiments were utilized to investigate how mTORC1 is recruited to lysosome in response to glutamine and asparagine. We have identified, a vesicle coat protein Golgi-localized, gamma adaptin ear-containing, Arf-binding protein (GGA1) that complexes with SNAT7 and mTORC1. Depletion of GGA1 increases albumin-induced mTORC1 lysosomal localization and activation. Moreover, GGA1-mediated mTORC1 activation is crucial for KRAS-driven pancreatic cancer cell proliferation. Thus, our study identifies GGA1 as a critical regulator of macropinocytosis mediated mTORC1 lysosomal localization and activation in pancreatic cancer cell growth.

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