

13TH SCIENTIFIC DAYS ON AUTOPHAGY

12-14 OCTOBER 2026



PROGRAM





TABLE OF CONTENTS

WELCOME	3
SPONSORS	4
GENERAL INFORMATION	5
CONGRESS VENUE	5
WELCOME DESK : OPENING HOURS	6
MEMBERSHIP AND SOCIAL NETWORKS	7
BADGES	7
AWARDS	7
SPEAKER INFORMATION	8
POSTERS INFORMATION	8
REGISTRATION GRANT RECIPIENTS	9
WIFI	9
CFATG XIII CONTACTS	9
EVENING PARTY – MEMBRANE BAND	10
LOCAL COMMITTEE	11
SCIENTIFIC COMMITTEE	12
REGISTRATION GRANT RECIPIENTS	13
PROGRAM	13
MOLECULAR REGULATION OF AUTOPHAGY – SESSION 1	17
AUTOPHAGY-INDEPENDENT FUNCTIONS OF ATG PROTEINS	23
ATGs IN IMMUNITY AND INFECTION	28
ATGs AND CANCER	33
SELECTIVE AUTOPHAGY	38
MOLECULAR REGULATION OF AUTOPHAGY – SESSION 2	43
FLASH TALKS	49
POSTERS	60
LIST OF PARTICIPANTS	96



WELCOME

Dear Colleagues,

The CFATG (Club Francophone de l'Autophagie) is delighted to welcome you to the 13th Scientific Days on Autophagy at FIAP Paris, nearly 10 years after the previous Paris edition.

Over the years, this meeting has become a key occasion for researchers from all over France, as well as several of them from Europe and North America to share their latest findings, discuss emerging challenges, and build new collaborations.

We are honored to welcome here prestigious international speakers, with the presence of **Roberto Zoncu** (UC Berkeley, USA), **Sharon Tooze** (The Francis Crick Institute, UK), **Katja Simon** (Max Delbrück Center for Molecular Medicine, Germany), **Ian Ganley** (University of Dundee, UK), **Carine Joffre** (Centre de Recherche en Cancérologie de Toulouse, France) and **Louis Lapierre** (Université de Moncton, Canada) and we would like to thank them again for their participation and enthusiastic contribution.

This year again, the CFATG proposes a very exciting scientific program. During the next three days, you will have the opportunity to attend plenary lectures, short talks, flash talks, and poster presentations. Six scientific sessions will report on the wide range of research topics and models of autophagy studies: Molecular Regulation of Autophagy, Autophagy-independent functions of ATG proteins, ATGs and Cancer, Selective autophagy and ATGs in Immunity & Infection. We also encourage you to take advantage of the many moments for informal exchange—coffee breaks, lunches, and social events—where some of the most fruitful ideas often emerge.

Tuesday evening, the Gala dinner, right here at FIAP, will be followed by a live concert by the famous **Membrane Band**, composed of four scientists: Thierry Galli (IPNP, Paris), Franck Perez (Institut Curie, Paris), Etienne Morel (INEM, Paris), and Cédric Delevoye (INEM, Paris). Don't miss it and enjoy music and lyrics!

As usual, one of the objectives of the CFATG meeting is to help promote young scientists, by giving them the opportunity to present their data in front of an expert and supportive audience. At the end of the meeting, several prizes will reward the work presented by talented young "autophagists" (PhD students and post docs). A small sticker next to their names in this abstract book indicates that they are eligible. All participants will be invited to vote via an internet application in parallel with the juries. The selection of the best posters and presentations will be based on a combined score from both the audience and the jury. We would like to thank all the people who agreed to be part of these juries.

Finally, we would like to thank you all and our financial support for making this CFATG event as pleasant and successful as possible.

Thank you for choosing us over the Céline Dion concert (though perhaps you combined the two by going before or after the conference?)

Welcome to Paris!

Aurore CLAUDE-TAUPIN, *coordinator of the CFATG-2026-Paris*

Audrey ESCLATINE, *President of the CFATG*



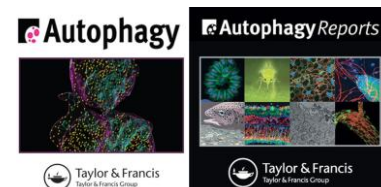


SPONSORS

INSTITUTIONAL PARTNERS



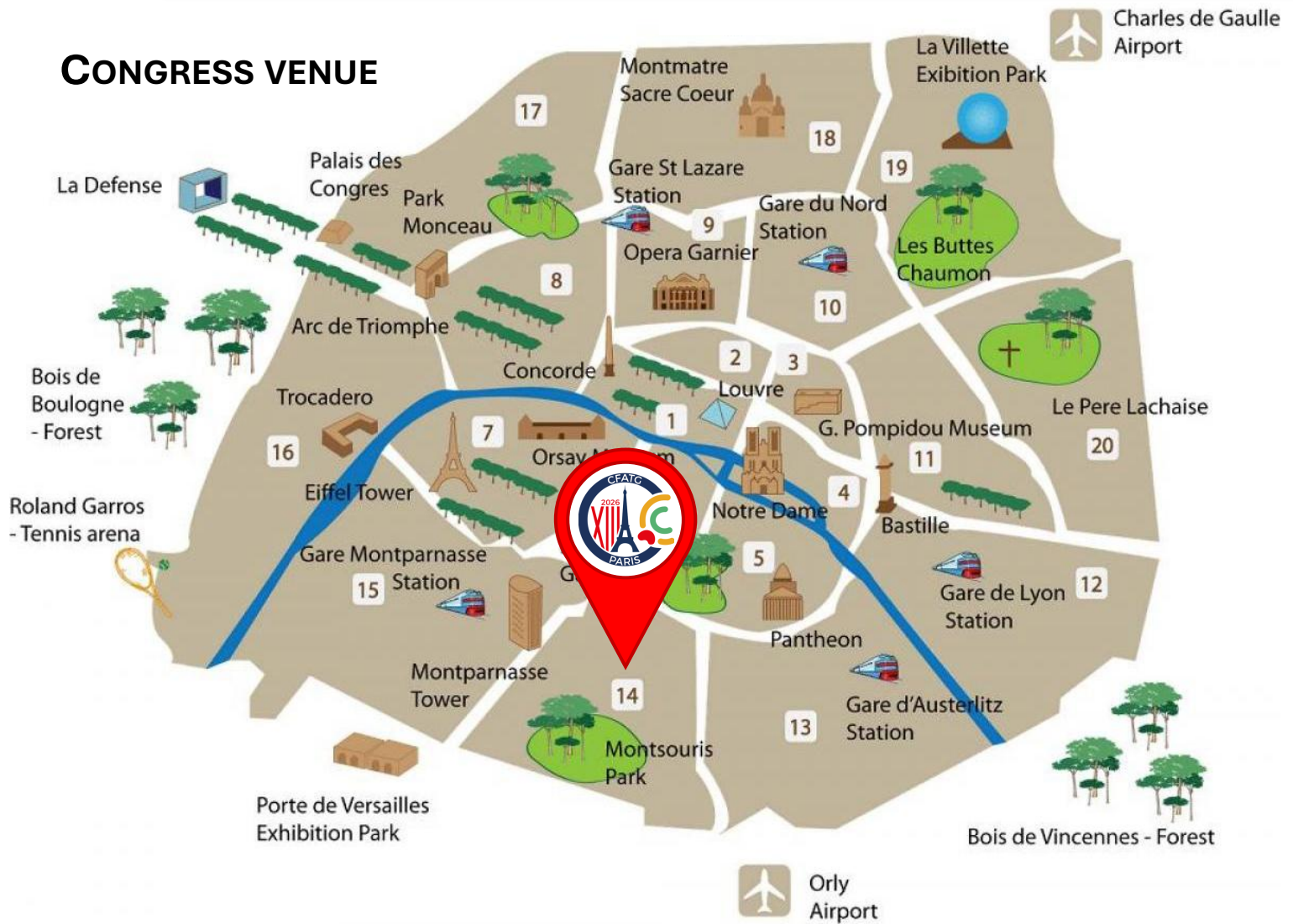
INDUSTRIAL & PUBLISHING PARTNERS





GENERAL INFORMATION

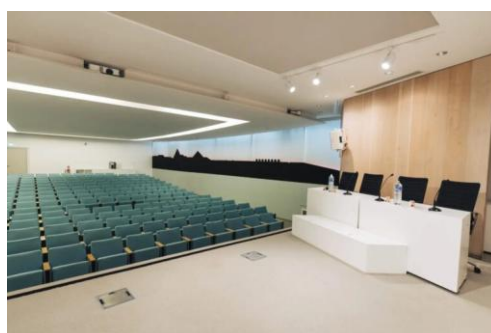
CONGRESS VENUE



FIAP Jean Monnet
30 rue Cabanis
75014 Paris

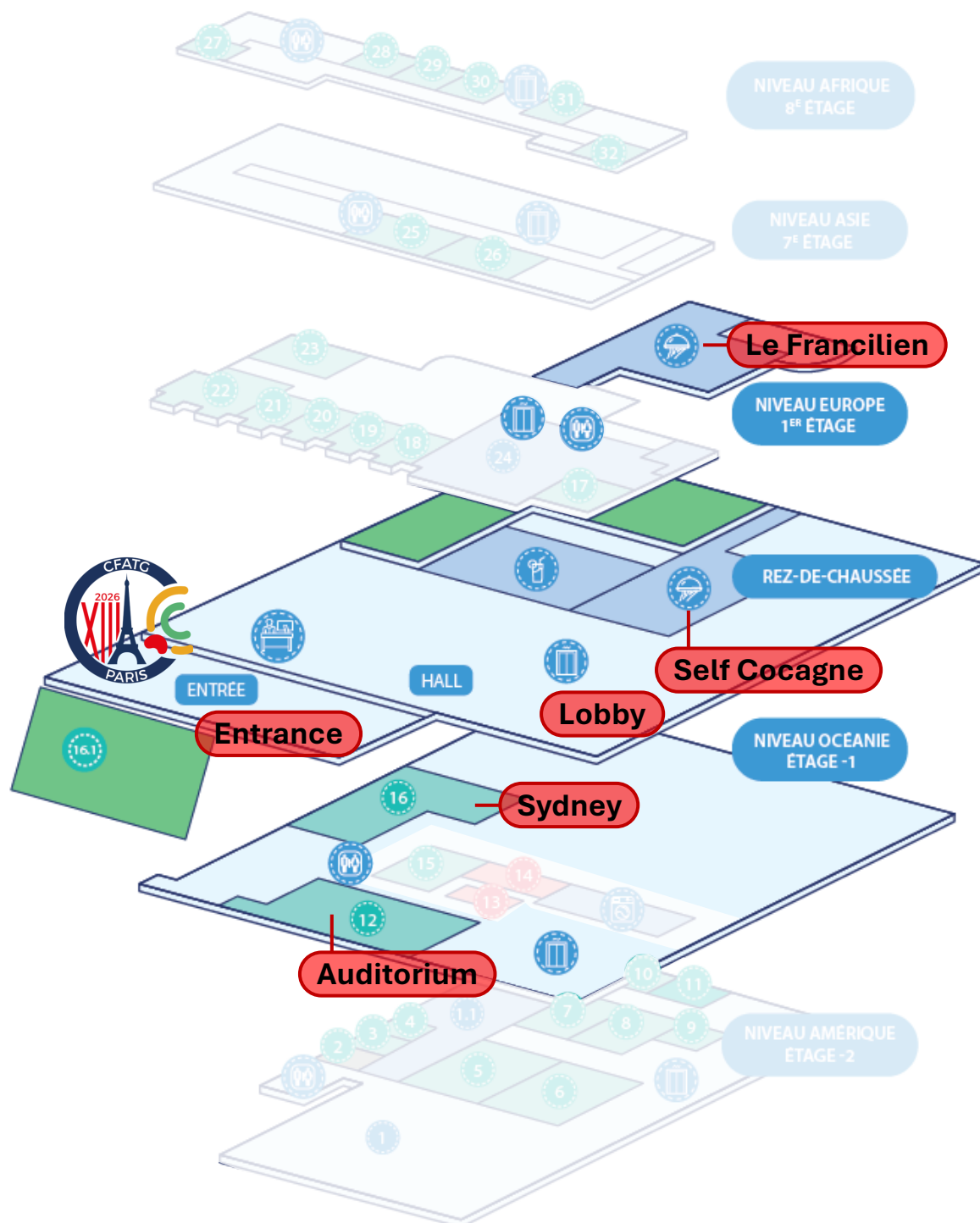
M 6 Glacière

RER B Denfert-Rochereau





GENERAL INFORMATION



WELCOME DESK : OPENING HOURS (Lobby – Level 0)

Monday	12:30 - 14:00	
Tuesday	08:00 - 09:00	12:00 - 14:00
Wednesday	08:00 - 09:00	12:00 - 14:00



GENERAL INFORMATION

MEMBERSHIP AND SOCIAL NETWORKS

You can contribute to the CFATG by subscribing to the annual membership program. You can become a CFATG member for 1 year (15 euros), 2 years (25 euros) or 3 years (30 euros). Membership fees allow you to receive the newsletter from the CFATG network, to apply for CFATG travel grant to attend to national and international meetings, to apply for poster and oral communication awards during CFATG meetings and to obtain discount on registration fees of the annual CFATG meetings.

A CFATG booth will offer you the opportunity to fill out a membership form and to give your contribution for 2027 and/or until 2029. You can subscribe also on the CFATG website: <https://cfatg.org/adherez-a-lassociation>

To check on your membership status: <https://cfatg.cyame.eu>

Follow CFATG on X: @CFATG, Instagram: cfatg_officiel, Facebook: CFATG and Linkedin: Club Francophone de l'AuTophagie (CFATG)

BADGES

All delegates and speakers are kindly requested to wear their name badges during all conference activities, even for the Gala dinner.

Organizing committee members will be identifiable thanks to their yellow badges. At any time, feel free to request any help from organizers.

AWARDS

Several awards will be offered at the end of the meeting: 2 prizes for the best **short talks**, 1 prize for the best **flash talk**, 2 prizes for the best **posters**.

Only people with the logo  in front of their name in the program and list of abstract are eligible.

All participants will be invited to vote via an online application. In parallel, a dedicated jury will evaluate the presentations and cast their votes. The awardees will be selected based on a ratio between the audience and jury votes. We would like to warmly thank the members of the jury for their time, expertise, and valuable contribution. All necessary information regarding the voting process and the weighting of the votes will be provided during the meeting.



GENERAL INFORMATION

SPEAKER INFORMATION

Oral presentations

Oral presentations will take place at the auditorium (located on Level -1).

Invited speaker lectures are scheduled for 30 min plus 15 min for discussion.

Short talk presentations are scheduled for 10 min plus 5 min for discussion.

Flash talk presentation are scheduled for 3 min without any time for discussion.

Speaker guidelines:

- Please provide your final presentation on a USB stick at least 30 minutes before the beginning of your session. Personal laptops cannot be used. Presentations should be provided in Powerpoint and/or PDF format.
- A laser pointer will be available on stage at your convenience.
- All mobile phones must be turned off during all presentations since they may cause feedback with the microphones.

POSTERS INFORMATION

Poster sessions will take place at the Lobby (Ground floor).

Presentation requirements:

- At least one of the authors must be present in front of the **poster** during the presentation session time.

Session 1 (P1 to P18) : Tuesday, October 13th • 11:30 – 12:45

Session 2 (P19 to P34) : Tuesday, October 13th • 16:30 – 18:00

- Please refer to the page in the booklet corresponding to your poster to identify the **number assigned to your poster**. (List in page 60)

Poster setup and removal:

Presenters are requested to set up their poster from 09:00 on Tuesday and to remove them before 10:30 on Wednesday.



Presenters eligible for an award will have 5 minutes to present their poster to a jury.



GENERAL INFORMATION

REGISTRATION GRANT RECIPIENTS

The following awardees were selected by the CFATG scientific committee to receive complimentary registration to facilitate their attendance and presentation at the conference.

Guilhem Cantaloube - O10
IRIM, Montpellier

Juliette Henna - FT6
UMR 1231, Dijon

Amandine Ducreux - O11
UMR PAM 1517, Dijon

Mathilde Lartigau - O13
MFP UMR5234, Bordeaux

Flavie Garampon - O14
INEM, Paris

Chloé Mercier - O25
UMR RIGHT, Besançon

WIFI

The WIFI access code will be provided at the registration desk.

CFATG XII CONTACTS

Aurore Claude-Taupin aurore.claude-taupin@inserm.fr
(in case of emergency +33 7 49 30 60 67)

Audrey Esclatine audrey.esclatine@universite-paris-saclay.fr
(in case of emergency +33 6 81 45 37 38)

Etienne Morel etienne.morel@inserm.fr
(in case of emergency +33 6 71 81 16 81)



GENERAL INFORMATION

EVENING PARTY – MEMBRANE BAND

The Gala Evening will conclude with a live concert by **Membrane Band**, featuring **Thierry Galli** (Institut de Psychiatrie et Neurosciences de Paris), **Franck Perez** (Institut Curie, Paris), **Etienne Morel** (Institut Necker Enfants Malades), and **Cédric Delevoye** (Institut Necker Enfants Malades).

More information: membraneband.com

The concert will take place in the **Sydney Room** (Level -1), following the Gala Dinner: everyone is welcome!

MEMBRANE BAND
A SELF EATING MUSICAL SHOW

13th of October 2026 9pm
Flap Jean Monnet Paris 14
fiap Paris

Cfatg
13TH SCIENTIFIC DAYS
ON AUTOPHAGY

CFATG
2026
PARIS

membraneband.com
YouTube f X

MEMBRANE



LOCAL COMMITTEE



Guillaume Beauclair
Institut Pasteur, Paris



Aurore Claude-Taupin
INEM, Paris



Audrey Esclatine
I2BC, Gif-sur-Yvette



Pierre-Emmanuel Joubert
CRC, Paris



Delphine Judith
Institut Cochin, Paris



Mojgan Djavaheri-Mergny
CRC, Paris



Etienne Morel
INEM, Paris



SCIENTIFIC COMMITTEE



Guillaume Beauclair
Institut Pasteur, Paris



Aurore Claude-Taupin
INEM, Paris



Coralie Daussy
IRIM, Montpellier



Audrey Esclatine
I2BC, Gif-sur-Yvette



Carole Kretz-Remy
PGNM, Lyon



Iban Seiliez
INRA/UPPA, St Pée-sur-Nivelle



Flavie Strappazon
PGNM, Lyon



Monday, October 12th, 2026

CFATG XIII PROGRAM

13:00 – 14:00: Participant registration and welcome (Lobby – Level 0)

14:00 – 14:15: Opening remarks by the CFATG President & Welcome address from the local organizing committee (Auditorium – Level -1)

SESSION 1 – Molecular regulation of autophagy – Part. 1

Chairs: *Carole Kretz-Rémy* (PGNM, Lyon) & *Iban Seiliez* (INRA/UPPA, St Pée-sur-Nivelle)

14:15 – 15:00: Lecture – Roberto Zoncu (UC Berkeley, CA, USA)

Decoding cholesterol-mTORC1 signaling in neuronal homeostasis and NPC pathogenesis



15:00 – 15:15: O1 Dobrawa Amiri-Czajkowski (INEM, Paris)

Dynamics of lipid transfer protein VPS13A at ER-endosome contact sites in stress response



15:15 – 15:30: O2 Antoine Dessus (MRC LMB, Cambridge)

A GABARAP–PtdIns3K–C1 feedback loop at the heart of the phagophore nucleation



15:30 – 15:45: O3 Quentin Frenger (Institut Curie, Paris)

Vimentin intermediate filaments support autophagosome biogenesis at ER–endosome contact sites in response to starvation



15:45 – 16:00: O4 Thibault Kosuth (LBM UMR5200, Bordeaux)

A singular lipid composition supports the progression of plant autophagy

16:00 – 16:15: O5 Thomas Wollert (Institut Pasteur, Paris)

Building omegasomes from scratch: WIPI2 orchestrates early membrane sources for autophagosome biogenesis

16:15 – 17:00: Coffee break (Lobby – Level 0)

SESSION 2 – Autophagy-independent functions of ATG proteins

Chairs: *Delphine Judith* (Institut Cochin) & *Pierre Lapaquette* (Institut Agro, Dijon)

17:00 – 17:45: Lecture – Sharon Tooze (The Francis Crick Institute, UK)

Molecular function of WIPI proteins in autophagy



17:45 – 18:00: O6 Eunice Cardoso Moreno (I2BC, Gif-sur-Yvette)

LC-MS/MS identification of novel LC3B-conjugated proteins and functional characterization of LC3Bylated NRBF2, a component of PI3KC3 complex I

18:00 – 18:15: O7 Elise Jacquin (CTM UMR1231, Dijon)

CASM amplifies STING-mediated NF-κB signaling in immune cells



18:15 – 18:30: O8 Maribel Lara Corona (INEM, Paris)

Fluid shear stress intensity governs the canonical and non-canonical functions of ATG9A in kidney tubular cells



18:30 – 18:45: O9 Alessia Rubiola (Institut Cochin, Paris)

VPS34 safeguards intestinal epithelial homeostasis by coordinating membrane trafficking, mitotic fidelity and cell survival

19:00: Welcome diner (Restaurant Francilien – FIAP – Level +1)



Tuesday, October 13th, 2026

CFATG XIII PROGRAM

SESSION 3 – ATGs in Immunity and infection

Chairs: *Guillaume Beauclair* (Institut Pasteur) & *Clarisse Berlioz-Torrent* (Institut Cochin)

09:00 – 09:45: Lecture – Katja Simon (Max Delbrück Center, Germany)

Autophagy in the context of immune aging



9:45 – 10:00: O10 Guilhem Cantaloube (IRIM, Montpellier)

An unexpected LC3B-positive compartment during HIV-1 cell-to-cell transmission



10:00 – 10:15: O11 Amandine Ducreux (UMR PAM 1517, Dijon)

Candalyisin dampens the autophagy machinery involved in the plasma membrane stress response to the invasion of epithelial cells by the human fungal pathogen Candida albicans



10:15 – 10:30: O12 Tanja Muralt (University of Bern, Bern)

Establishing Sensitive Autophagic Flux Assays in Patient-Derived B Cells to Investigate Immune Dysregulation in Immune-Mediated Thrombotic Thrombocytopenic Purpura



10:30 – 10:45: O13 Mathilde Lartigau (MFP UMR5234, Bordeaux)

Proximity proteomics deciphers autophagy-linked host factors at the site of AdV-induced membrane damage

10:45 – 11:00 Flash talk session 1



FT1 Hala Shalhoub (CRCT, Toulouse)

Selective targeting of VPS34-driven autophagy deregulation by p62 degrading PROTACs is a promising therapeutic strategy in pancreatic cancer



FT2 Tiffany Darbas (UMR 1308 CAPTuR, Limoges)

Autophagy and extracellular vesicle secretion in glioblastoma: a heterogeneous primary culture response to temozolomide and chloroquine

FT3 Fatima De Palma (University of Naples Federico II, Naples)

Bempedoic acid induces autophagy and improves liver cancer management



FT4 Thibaut Flipo (Institut Cochin, Paris)

Protein ATG8ylation : Development of a strategy to investigate a novel post-translational modification during viral infections



FT5 Loïs Guillemard (UMR PAM, Dijon)

Decoding Candida albicans-Autophagy interactions at the plasma membrane during invasion

11:00 – 11:30: Coffee break (Lobby – Level 0)



11:30 – 12:45: Poster session 1 – P1 to P18 (Lobby – Level 0)

12:45 – 14:00: Lunch (Self Cocagne – FIAP – Level 0)



Tuesday, October 13th, 2026

CFATG XIII PROGRAM



SESSION 4 – ATGs and Cancer

Chairs: *Elise Jacquin* (CTM, Dijon) & **Pierre-Emmanuel Joubert** (CRC, Paris)

14:00 – 14:45: Lecture – Carine Joffre (CRCT, France)

Selective autophagy as a central mechanism of therapy resistance in AML



14:45 – 15:00: O14 Flavie Garampon (INEM, Paris)

Shear stress-dependent modulation of autophagy shapes metastatic features of circulating tumor cells

15:00 – 15:15: O15 Laura Poillet (CRCT, Toulouse)

Host autophagy is required for acute myeloid leukemia growth and therapeutic resistance



15:15 – 15:30: O16 Alexandra Hamood (I2BC, Gif-sur-Yvette)

Autophagy induction by a viral protein contributes to Burkitt Lymphoma resistance to chemotherapy

15:30 – 15:45: O17 Yunus Akkoc (Koç University, Istanbul)

CTF1 is a critical cancer-derived factor regulating autophagy in the tumor microenvironment

15:45 – 16:00: Flash talk session 2



FT6 Juliette Henna (UMR 1231, Dijon)

Autophagy proteins regulate HSP110 release from immune cells



FT7 Claudia Serot (I2BC, Gif-sur-Yvette)

An annexin involved in adaptation to Heat Stress in Caenorhabditis elegans



FT8 Evalena Thore (Institut Brain, Caen)

In vivo assessment of autophagic flux using Thy1-RFP-GFP-LC3 mice following ischemic stroke



FT9 Jules Blanc (CIRI, Lyon)

Exploring the functions of selective autophagy receptors at the virus / host interface



FT10 Lucie Mazzola (INEM, Paris)

Chronic TLR9 signaling drives PLD3-dependent lysosomal lipid remodeling and dysfunction in Alzheimer's Disease Microglia

16:00 – 16:30: Coffee break (Lobby – Level 0)



16:30 – 18:00: Poster session 2 – P19 to P34 (Lobby – Level 0)

18:00 – 18:45: CFATG General Assembly (Auditorium – Level -1)

19:00: Gala diner and Membrane Band concert (Sydney – Level -1)



Wednesday, October 14th, 2026

CFATG XIII PROGRAM

SESSION 5 – Selective autophagy

Chairs: *Renaud Legouis* (I2BC, Gif-sur-Yvette) & *Flavie Strappazzon* (PGNM, Lyon)

09:15 – 10:00: Lecture – Ian Ganley (University of Dundee, UK)

Eating your mitochondria: good or bad?



10:00 – 10:15: O18 Krenare Bruqi (INMG - PGNM, Lyon)

Novel insights into the role of an undisclosed NDP52 phosphorylation in mitophagy

10:15 – 10:30: O19 Damien Glon (I2BC, Gif-sur-Yvette)

When SARs meet LLPS: human cytomegalovirus induces the formation of antiviral p62-bodies

10:30 – 11:15: Coffee break (Lobby – Level 0)



11:15 – 11:30: O20 Maria Ángeles Morcillo Parra (IBPC, Paris)

Exploring the role of deubiquitination in ERMES during mitophagy

11:30 – 11:45: O21 Manuel S. Rodriguez (LCC, Toulouse)

Targeting the autophagy receptor p62 for degradation to regulate proteaphagy and recover sensitivity to bortezomib in mantle cell lymphoma

12:00 – 13:30: Cocktail lunch (Lobby – Level 0)

SESSION 6 – Molecular regulation of autophagy – Session 2

Chairs: *Etienne Morel* (INEM, Paris) *Salwa Sebti* (IRMB, Montpellier)

13:30 – 14:15: Lecture – Louis Lapierre (University of Moncton, Canada)

Karyopherin-mediated regulation of autophagy



14:15 – 14:30: O22 Carla Alemany (INEM, Paris)

Stress-induced remodeling of RAB11-positive endosomes promotes autophagic adaptation through RAB30



14:30 – 14:45: O23 Cléa Villette (CRCT, Toulouse)

RNA G-quadruplexes reprogram translation to sustain autophagy and cancer cell survival



14:45 – 15:00: O24 Gwenaëlle Laurent (Institut Imagine, Paris)

Unraveling the pathophysiology of TANGO2 loss of function in rhabdomyolysis symptoms



15:00 – 15:15: O25 Chloé Mercier (UMR RIGHT, Besançon)

Post-transcriptional control of autophagy-related pathways by Nonsense-Mediated mRNA Decay

15:15 – 15:30: O26 David Israeli (Genethon UMR-S951, Paris)

When muscle meets the lysosome: Expanding the spectrum of lysosome-driven diseases

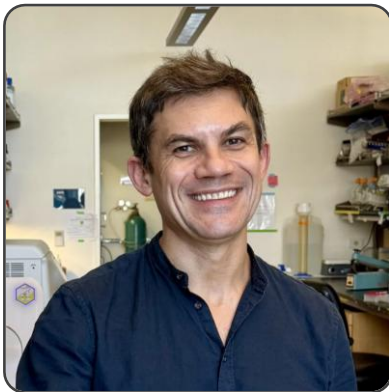
15:30 – 16:00: Award ceremony and closing remarks (Auditorium – Level -1)



Molecular regulation of autophagy – Session 1

Invited speaker

Decoding cholesterol-mTORC1 signaling in neuronal homeostasis and NPC pathogenesis



Roberto Zoncu

*Department of Molecular and Cell Biology
University of California Berkeley
United States*

Monday, October 12th • 14:15 – 15:00

The lysosome plays a central role in cellular homeostasis through its participation in autophagy and its physical and functional association with the master growth regulator, mechanistic target of rapamycin complex 1 (mTORC1) kinase. mTORC1 integrates signals from nutrients, hormones and energy to control the balance between cellular growth and repair programs. Nutrients, including cholesterol, drive the localization of the master growth regulator, mechanistic Target of Rapamycin Complex 1 (mTORC1) to the lysosomal membrane, where mTORC1 regulates its downstream programs. Cholesterol sensing by mTORC1 occurs upstream of the heterodimeric Rag guanosine triphosphatases (GTPases) via the lysosomal cholesterol sensor LYCHOS, and is negatively regulated by the cholesterol exporter, Niemann-Pick C1 (NPC1) protein. In NPC1-deleted cells, mTORC1 becomes dysregulated, leading to disruption of autophagy and mitochondrial function. These data implicate aberrant cholesterol-mTORC1 signaling as a candidate pathogenic driver in the neurodegenerative disease, Niemann-Pick type C (NPC), but the molecular mechanisms of mTORC1-dependent cellular dysfunction in NPC remain to be determined. I will present our recent effort to unravel lysosomal cholesterol sensing in neuronal models through a combination of imaging, organelle proteomics, bioinformatic analysis and functional assays. Moreover, I will discuss ongoing work leveraging selective autophagy for targeted elimination of toxic cellular substrates via the lysosome.



O1

Molecular regulation of autophagy – Session 1

Short talk

Dynamics of lipid transfer protein VPS13A at ER-endosome contact sites in stress response

Dobrawa Amiri-Czajkowski¹, Juliane Da Graça², Elena Sampanai¹, Damiana Lecoeuche¹, Etienne Morel¹

¹ Université Paris Cité, INSERM U1151 - CNRS UMR 8253, Institut Necker Enfants Malades (INEM), Paris, France

² École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Autophagy is a fundamental self-degradation process that maintains cellular homeostasis. It starts with the de novo formation of a double-membrane vesicle, the autophagosome, originating from a transient precursor membrane called phagophore.

Previous work in our lab showed that, upon nutrient deprivation, early endosome dynamically established contact sites at omegasome, a specialized ER subdomain that serves as a platform for autophagosome formation. These localized interactions generate a confined environment for Ca²⁺ release, phase transition, and the formation and fusion of nanovesicles driving phagophore biogenesis (Da Graça *et al*, 2022; Da Graça *et al*, 2025). Given the considerable lipid demand associated with membrane expansion during phagophore biogenesis, we hypothesized that lipid transfer proteins (LTPs) could sustain this process. VPS13A emerged as a strong candidate, as it interacts with the ER-resident tethering protein VAPB (Yeshaw W.M. *et al*, 2019), as well as the endosomal protein SNX5 (Tornejo Ecira A. *et al*, 2023).

Using expansion microscopy and biochemical approaches, we show that VPS13A is dynamically recruited to ER-endosome contact sites and to nascent autophagic structures following nutrient deprivation. To investigate its molecular function at these contacts, we combined in silico analysis with TurboID proximity labelling revealing a network of candidate VPS13A interactors associated with membrane contact sites and early autophagy. Importantly, proximity ligation assays together with biochemical and imaging analysis demonstrate that VPS13A depletion destabilizes ER-endosome contact sites and specifically impairs the earliest steps of autophagosome formation. Collectively, our findings identify VPS13A as a key regulator of ER-endosome contact site organization and support a central role for lipid transfer at membrane contact sites during the initiation of autophagy



O2

Molecular regulation of autophagy – Session 1

Short talk

A GABARAP–PtdIns3K-C1 feedback loop at the heart of the phagophore nucleation

Antoine N. Dessus^{1,*}, Yohei Ohashi^{1,*}, Maxime Bourguet¹, Tomos E. Morgan¹, Anastasia Nunez², Maria Manifava², Nicholas T. Ktistakis², Roger L. Williams¹

¹ MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, United Kingdom

² Signalling Programme, Babraham Institute, Babraham, Cambridge CB22 3AT, United Kingdom

* These authors contributed equally to this work

During macroautophagy in mammalian cells, autophagosomes can reach diameters of over 1000 nm within 30 min after triggering starvation, however how such substantial amounts of membranes can be synthesized within a brief time remains elusive. A protein complex central to the phagophore initiation is the lipid kinase PIK3C3-Complex 1 (PtdIns3K-C1), which produces phosphatidylinositol-3-phosphate (PtdIns3P). PtdIns3P recruits a variety of downstream proteins, among which is PtdIns3P-binding WIPI2 that facilitates lipidation of mammalian ATG8 (mATG8) family proteins on phagophores.

Here we showed that the inhibition of mATG8 lipidation in cells prevents the accumulation of WIPI2, suggesting a feedback loop between mATG8s and PtdIns3P production. The role of PtdIns3K-C1 in this feedback was demonstrated by in vitro experiments where recombinant membrane-coupled mATG8s were shown to bind to and potently activate PtdIns3K-C1, with GABARAP being the most potent activator among all mATG8s.

By a combination of cryo-electron microscopy, structural mass spectrometry, activity assays and mutagenesis, we showed that GABARAP binds two sites in PtdIns3K-C1, with one site showing an atypical bipartite interaction with the mATG8. We also confirmed both sites are essential for GABARAP to activate PtdIns3K-C1.

We propose that once GABARAP is indirectly recruited by PtdIns3P generated by basal activity of PtdIns3K-C1, a positive feedback loop is formed where PtdIns3K-C1 interacts with GABARAP and becomes activated to produce more PtdIns3P, thereby further stimulating GABARAP lipidation. This mechanism would be central for autophagosome biogenesis, where enlarged membranes need to be synthesized within a brief period.



O3

Molecular regulation of autophagy – Session 1
Short talk

Vimentin intermediate filaments support autophagosome biogenesis at ER–endosome contact sites in response to starvation

Quentin Frenger^{1,2}, Dobrawa Amiri-Czajkowski¹, Carla Alemany¹, Damiana Lecoeuche¹, Cédric Delevoye¹, Etienne Morel¹

¹ INSERM U1151, CNRS UMR8253, Institut Necker Enfants Malades, Université Paris Cité, Paris 75015, France

² Institut Curie, CNRS UMR144, Paris (Present affiliation)

Macroautophagy (autophagy) is a fundamental catabolic process requiring the biogenesis of autophagosomes to support cell survival during stress. Autophagosome biogenesis requires coordinated membrane remodeling at organelle contact sites, notably between the endoplasmic reticulum (ER) and endosomes. While actin and microtubules drive vesicle transport and autophagosome–lysosome fusion, the contribution of intermediate filaments (IFs) to the early initiation steps of autophagy remains poorly understood.

Here, we show that the type III intermediate filament vimentin is mobilized upon nutrient deprivation to support autophagosome formation. We reveal that starvation triggers a rapid (within 15 min) perinuclear compaction of vimentin filaments and increases their spatial overlap with the ER. This structural rearrangement coincides with the dynamic phosphorylation of vimentin.

Early autophagic markers, including PI3P, DFCP1 and LC3B, accumulate preferentially within ER subdomains confined by the vimentin meshwork. Perturbation of the filament network, either pharmacologically with withaferin-A or genetically via CRISPR/Cas9 knockout in HeLa cells, impairs autophagic flux. Specifically, vimentin deficiency compromises starvation-induced phosphorylation of ATG16L1 (Ser278) without perturbing upstream mTORC1 inactivation.

Proximity ligation assays (EEA1–VAP-B) demonstrate that starvation transiently stimulates early endosome–ER contact sites (EERCS), a response abolished in vimentin-deficient cells or upon withaferin-A treatment. Furthermore, silencing RNF26, an ER-anchored E3 ligase that bridges the ER, vimentin, and endosomes, phenocopies vimentin loss by preventing starvation-dependent EERCS stabilization and blocking autophagosome formation.

These findings establish vimentin intermediate filaments as structural organizers that physically constrain ER–endosome contacts, providing the localized platform necessary for autophagosome biogenesis during nutrient stress.



O4

Molecular regulation of autophagy – Session 1

Short talk

A singular lipid composition supports the progression of plant autophagy

Thibault Kosuth¹, Josselin Lupette^{1,2}, Clément Chambaud^{1,3}, Matthieu Buridan¹, Julie Castets¹, Valérie Wattelet-Boyer¹, Inés Toboso Moreno¹, Céline Yatim¹, Franziska Dittrich-Domergue¹, Valérie Gros^{4,5}, Juliette Jouhet⁴, Stéphane Claverol⁶, Su Melser⁷, Manon Genva⁸, Cécile Mirande-Bret¹, Laetitia Fouillen¹, Jean-Jacques Bessoule¹, Frédéric Domergue¹, Amélie Bernard¹

¹ Univ. Bordeaux, CNRS, LBM, UMR5200, F-33140 Villenave d'Ornon, France

² Aix Marseille Université, CEA, CNRS, BIAM, LGBP, 13009 Marseille, France

³ Bordeaux Imaging Center, Plant Imaging Platform, UAR 3420, CNRS, INSERM, Univ. de Bordeaux, INRAE, 33140 Villenave d'Ornon, France

⁴ Laboratoire de Physiologie Cellulaire et Végétale, UMR 5168 CEA, CNRS, UGA, UMR 1417 INRAE, 38054 Grenoble, France

⁵ Institut des Biomolécules Max Mousseron (IBMM), Pôle Chimie Balard Recherche, UMR 5247, CNRS, Université de Montpellier, ENSCM, Montpellier, France

⁶ Université de Bordeaux, Bordeaux Proteome, 33076 Bordeaux, France

⁷ Elysia Bioscience, Zone Actipolis, 33610 Canéjan, France

⁸ Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium

Autophagy is an intracellular degradation and recycling pathway essential for cell quality control and plant tolerance to stress. It relies on the remarkable plasticity of a specialized membrane, the phagophore, to orchestrate and support intense membrane remodeling events towards the formation of the autophagic vesicle that sequesters cargo. To resolve the elusive molecular bases of these events, we need to integrate inputs from both components of biological membranes: proteins and lipids, yet information regarding lipids is still very limited.

Here, we established a method combining cell fractionation and immuno-isolation in native conditions to purify phagophores from *Arabidopsis thaliana*. Characterization of the phagophore lipid composition showed a large predominance of glycerophospholipids, including phosphoinositides, and a particular enrichment in phosphatidylcholine and phosphatidylglycerol. Comparative analyses revealed the singularity of this lipid signature and notably identified phosphatidylglycerol as a plant-specific component of autophagic membranes. Finally, analyses of phosphatidylglycerol-deficient plants showed defects in autophagy activity and increased sensitivity to nutrient starvation thereby supporting the functional relevance of the phagophore lipid composition, particularly that of phosphatidylglycerol homeostasis.

Together, our findings place lipids as fundamental components of the autophagy molecular landscape and provide a framework to further investigate their contribution to autophagosome biology and functions in plant acclimation to environmental changes.



Building omegasomes from scratch: WIPI2 orchestrates early membrane sources for autophagosome biogenesis

Daniele Campisi¹, Giulia Bertolin², Bence Ézsiás¹, Puck N. Norell¹, Kennedy Bonjour¹, Anastasia D. Gazi³, Christine Girard-Blanc⁴, Stephane Blanchard¹, **Thomas Wollert**¹

¹ Membrane Biochemistry and Transport, Institut Pasteur, UMR 3691, Université de Paris Cité, 28 rue du Dr Roux, 75015 Paris, France

² CNRS, Univ Rennes, IGDR (Institut de Génétique et Développement de Rennes) - UMR 6290, Rennes F-35000, France

³ Ultrastructural BioImaging, Institut Pasteur, Université de Paris Cité, CNRS UMR 3528, 75015 Paris, France

⁴ Trypanosome Cell Biology Unit, Institut Pasteur, Université de Paris Cité, INSERM U1347, Paris, France

Autophagosomes arise from transient, phosphoinositide-rich membrane structures known as omegasomes, yet the origin of these compartments and the mechanisms driving their formation remain unresolved. Here, we combine advanced live-cell imaging with correlative super-resolution and electron microscopy, cryo-electron tomography, and quantitative image analysis to visualize omegasome biogenesis across scales.

Using two complementary cellular systems that capture early and stalled intermediates, we reveal an unexpected sequence of membrane transformations. Omegasome formation begins with the WIPI2-dependent recruitment of nanovesicles, which progressively assemble into interconnected membrane networks and flattened cisternae. This process requires coordinated input from ATG9A, FIP200, membrane-tethering and fusion factors, and occurs upstream of the canonical machinery that conjugates ATG8 proteins to nascent autophagic membranes. Strikingly, directing WIPI2 to an ectopic cellular location is sufficient to generate omegasome-like membrane compartments, demonstrating that WIPI2 does not merely mark pre-existing autophagic membranes but actively organizes their formation.

Our findings redefine the membrane origin of autophagosomes. Rather than emerging solely through remodeling of the endoplasmic reticulum, omegasomes are actively constructed through the WIPI2-directed recruitment and assembly of vesicular membrane precursors. By capturing this process with cutting-edge imaging, we establish WIPI2 as a central organizer that converts nanovesicles into the membrane platform from which autophagosomes emerge.



Autophagy-independent functions of ATG proteins

Invited speaker

Molecular function of WIPI proteins in Autophagy



Sharon Tooze

*The Francis Crick Institute
London
United Kingdom*

Monday, October 12th • 17:00 – 17:45

There are four human WIPIs, WIPI1-WIPI4, (WD-repeat protein Interacting with Phospholinositides), all members of the PROPPIN family. These WIPIs function in macroautophagy and selective autophagy by binding to newly synthesized PI3P in the phagophore membrane produced by activated PI3KC3-C1, to recruit autophagy proteins to the phagophore membrane. Specifically, WIPI2b/d bind the ATG16L1 subunit of the E3 ligase, and WIPI4 binds the lipid transfer protein ATG2. In addition, interactome experiments have shown the WIPI family can form hetero-oligomers but the nature and function of these interactions is unknown. We have begun characterisation of these hetero- and homo-oligomers to understand the functional protein complexes at the phagophore and the consequence of these assemblies in cell models and neuronal models. Mutations in the WDR45 gene, encoding WIPI4, cause the neurodegenerative disease BPAN. Mutations in the WIPI2 gene have also been linked to neurodevelopmental disorders, with milder phenotypes than BPAN. The molecular mechanism of how such mutations cause disease is not clear, and we aim to provide insight into the link to the WIPI function in autophagy with neuronal disease.



O6

Autophagy-independent functions of ATG proteins

Short talk

LC-MS/MS identification of novel LC3B-conjugated proteins and functional characterization of LC3Bylated NRBF2, a component of PI3KC3 complex I

Eunice Cardoso Moreno¹, David Cornu², Arnaud Moris¹, Audrey Esclatine¹, Guillaume Beauclair^{1,3}

¹ I2BC, Institute for Integrative Biology of the Cell, Université Paris-Saclay, CEA, CNRS, 91190, Gif-sur-Yvette, France

² Proteomics-Gif (SICAPS), Institute for Integrative Biology of the Cell, 91190, Gif-sur-Yvette, France

³ Université Paris Cité, Institut Pasteur, Environnement et risques infectieux, Paris, France (Present affiliation)

Autophagy is a tightly regulated degradation process in eukaryotic cells, which degrades unnecessary, damaged, organelles and proteins and which is controlled by a set of autophagy-related (ATG) proteins. In humans, the ATG8 family comprises seven members (LC3/GABARAP). To be active, these ubiquitin-like proteins need to be conjugated to a phospholipid, thanks to the E1 and E2-like enzyme ATG7 and ATG3 and E3-like ATG12-ATG5/ATG16L1 complex. This process is reversible, through the activity of the ATG4 proteases.

While LC3/GABARAP lipidation is well characterized, recent studies have revealed that these proteins can also be covalently conjugated to proteins, giving rise to a newly post-translational modification (PTM) known as protein ATG8ylation. At present, only a limited number of substrates has been identified, the conjugation machinery remains poorly characterized, and the physiological function of this PTM is largely unknown. To investigate conjugation of LC3B to target proteins, we generated LC3B mutants as molecular tools to identify new substrates and map LC3Bylation sites.

Using tandem affinity purification under denaturing conditions coupled with high-resolution LC-MS/MS, we identified proteins covalently conjugated to LC3B. Comparative proteomics analysis between conjugable and non-conjugable LC3B mutants revealed several high-confidence LC3Bylation substrates. As proof of principle, we confirmed the previously reported conjugation of LC3B to ATG3 and to ATG7. Among the candidates, we identified NRBF2, a regulatory subunit of the class III PI3K complex involved in autophagy initiation, as a novel LC3B substrate. To further characterize this modification, LC3B conjugation sites on NRBF2 are being mapped using two complementary approaches: (i) large-scale identification by mass spectrometry and (ii) targeted site-directed mutagenesis. Preliminary results indicate that LC3B is conjugated to at least three distinct lysine residues on NRBF2. Functional studies are now being performed to elucidate how this modification affects PI3KC3-C1 complex assembly, autophagosome biogenesis, and autophagic flux. Together, these studies are expected to provide new insights into the physiological role of protein LC3Bylation and uncover novel regulatory mechanisms of autophagy under basal and stress conditions.



CASM amplifies STING-mediated NF- κ B signaling in immune cells

Isis Benoit-Lizon^{1,2}, Abdelmnim Radoua¹, Cassandre Pignol¹, Juliette Henna¹, Kirsty Hooper³, Caroline Truntzer⁴, Ludivine Dal Zuffo¹, Julie Lucas⁵, Quentin Frenger⁵, Romain Aucagne^{6,7}, François Hermetet¹, Elisabeth Dubus⁸, Thomas Maldiney⁸, Sayyed Hamed Shahoei¹, Emeric Limagne⁴, Christian Poüs⁹, Pauline Soulas-Sprauel⁵, Frédéric Gros⁵, Frédérique Végran⁴, Carmen Garrido¹, François Ghiringhelli⁴, Catherine Paul^{1,2}, Oliver Florey³, Lionel Apetoh^{1,10}, **Elise Jacquin**¹

1 Université Bourgogne Europe, INSERM, CTM UMR 1231, 21000 Dijon, France

2 Laboratoire d'Immunologie et Immunothérapie des Cancers, EPHE, PSL Research University, 75000 Paris, France

3 Signalling Programme, Babraham Institute, Cambridge, United Kingdom

4 Université Bourgogne Europe, Centre de Lutte Contre le Cancer G.-F. Leclerc, Platform of Transfer in Biological Oncology, INSERM, CTM UMR 1231, 21000 Dijon, France

5 Laboratoire d'ImmunoRhumatologie Moléculaire, INSERM UMR-S1109, F-67000 Strasbourg, France; Strasbourg University, Faculty of Life Sciences and Faculty of Pharmacy, Strasbourg, France

6 Université Bourgogne Europe, CHU Dijon Bourgogne, Unit for Innovation in Genetics and Epigenetics in Oncology, INSERM, CTM UMR 1231, 21000 Dijon, France

7 INSERM UMS 58 BioSanD, CRISPR Functional Genomics (CRIGEN) Facility, Dijon, France

8 Université Bourgogne – Europe, INSERM, CTM UMR 1231, Laboratoire Biofilms et Microscopies Dynamiques du Vivant, Centre Hospitalier William Morey, 71100 Chalon-sur-Saône, France

9 INSERM UMR-S 1193, UFR de Pharmacie, Université Paris-Saclay, Orsay, France

10 Brown Center for Immunotherapy, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Stimulator of Interferon Gene (STING), a key player of antimicrobial immune responses, has also emerged as a promising target to mitigate inflammation and cancer. Following STING activation, the production and release of proinflammatory molecules and type I Interferons (IFN) facilitate the establishment of effective immune responses and adaptive immunity. While STING activation drives LC3 lipidation, the underlying mechanisms and functional consequences remain however incompletely defined. Autophagosome formation following STING activation has been proposed to act as a negative feedback mechanism that limits STING signaling. Importantly, STING activation also triggers Conjugation of autophagy related (ATG) 8 to Single Membranes (CASM) but the consequences of STING-associated CASM on immune responses remain elusive. Using innate and adaptive cells specifically inactivated for autophagy or CASM, we found that STING agonists primarily trigger CASM, with canonical autophagy occurring as a delayed and comparatively modest response. STING-driven CASM facilitates the recruitment of the kinase IKK ϵ to STING-containing protein complexes and potentiates downstream NF- κ B signaling and TNF production. Together, these findings uncover a previously unrecognized role for CASM in promoting STING-dependent inflammatory signaling and highlight the importance of considering CASM, in addition to canonical autophagy, in the design and interpretation of studies and therapeutic strategies targeting the STING pathway.



O8

Autophagy-independent functions of ATG proteins

Short talk

Fluid shear stress intensity governs the canonical and non-canonical functions of ATG9A in kidney tubular cells

Maribel Lara Corona¹, Khoulood Chekoua¹, Océane Vialle¹, Tom Vennin¹, Béatrice Durel², Catherine Durieu³, Jean-Marc Verbavatz³, Etienne Morel¹, Céline Elie-Caille⁴, Aurore Claude-Taupin¹

¹ Institut Necker-Enfants Malades (INEM), INSERM UMR-S1151, CNRS UMR-S8253, Université Paris Cité, 75015 Paris, France

² Structure Fédérative de recherche Necker, INSERM US24, 75015 Paris, France

³ Institut Jacques Monod Université Paris Cité, CNRS UMR-7592, 75013 Paris, France

⁴ Université Marie et Louis Pasteur, CNRS, Institut FEMTO-ST, 25030 Besançon, France

Fluid shear stress generated by urinary flow is an important regulator of proximal tubule function and renal reabsorption, a process requiring substantial ATP. We previously identified lipophagy as a key metabolic pathway supporting this energy requirement, activated in response to physiological fluid shear stress, which is sensed by the primary cilium. In Chronic Kidney Disease (CKD), however, elevated shear stress impairs the primary cilium-dependent activation of autophagy. To elucidate the underlying mechanisms, we employ a fluidic pump system that recreates physiological and pathological shear stress conditions in vitro.

Using mouse Kidney Epithelial Cells (KECs), we demonstrate that pathological shear stress induces plasma membrane (PM) damage, reflected by increased membrane permeability and disruption of the ultrastructure of the apical membrane, including the microvilli and primary cilium. Recently, PM repair mechanisms have been associated with non-canonical functions of ATGs, such as ATG9A-dependent recruitment of the ESCRT machinery, promoting PM budding of damaged areas and subsequent release of extracellular vesicles. As the autophagy pathway is inhibited in tubular cells subjected to pathological shear stress, we therefore investigated whether this non-canonical function of ATG9A could mediate PM repair in response to mechanical stress.

We found that ATG9A deficiency renders cells more susceptible to PM damage even at physiological shear stress. This increased membrane permeability is accompanied by increased extracellular vesicle release. Strikingly, reducing fluid shear stress decreased PM damage and restored autophagy, revealing a reversible relationship between the two processes.

Together, these findings suggest that the canonical and non-canonical functions of ATG9A are not additive but are functionally interconnected in maintaining PM integrity in response to mechanical stress.

Elucidating these repair mechanisms may reveal novel therapeutic targets for CKD, while ATG-dependent EV release may provide a potential source of biomarkers for early disease detection.



O9

Autophagy-independent functions of ATG proteins

Short talk

VPS34 safeguards intestinal epithelial homeostasis by coordinating membrane trafficking, mitotic fidelity and cell survival

Alessia Rubiola¹, Camille Boucher¹, Yomna Gharib¹, Caterina Luana Pitasi¹, Lilia Younsi², Benjamin Saintpierre², Lucie Adoux³, Franck Letourneur³, Alain Schmitt⁴, Bart Vanhaesebroeck⁵, Romain Coriat^{1,6}, Benoît Terris^{1,6}, Béatrice Romagnolo¹

1 Université Paris Cité, INSERM, CNRS, Institut Cochin, F-75014 Paris, France, Equipe Labellisée Ligue Nationale Contre Le Cancer

2 Bioinformatic Facility, INSERM, CNRS, Institut Cochin, F-75014 Paris, France

3 Genomic Facility, INSERM, CNRS, Institut Cochin, F-75014 Paris, France

4 Electron microscopy Facility, INSERM, CNRS, Institut Cochin, F-75014 Paris, France

5 Cell Signalling, Cancer Institute, University College London, London, UK

6 Department of Gastroenterology, Endoscopy, and Gastrointestinal Oncology, AP-HP, Hôpital Cochin, F-75014 Paris, France

7 Department of Pathological Anatomy and Cytology, AP-HP, Hôpital Cochin, F-75014 Paris, France

The class III phosphatidylinositol 3-kinase VPS34 (PIK3C3) is a central regulator of autophagy and endosomal trafficking, two pathways essential for epithelial homeostasis. However, its physiological and pathological roles in the adult intestinal epithelium remain unexplored.

Here, we show that inducible deletion of Vps34 in the intestinal epithelium causes severe autophagy and endocytosis defects, leading to rapid epithelial collapse, crypt loss, and lethality through the combined disruption of stem cell maintenance, mitotic fidelity, and cell survival. Vps34 deficiency resulted in the rapid depletion of Lgr5⁺ intestinal stem cells and the emergence of a fetal-like revival stem cell population associated with activation of YAP signaling. We also identified VPS34 as a critical regulator of mitosis. Loss of VPS34 impaired spindle organization and cytokinetic abscission, leading to persistent midbody remnants, genome instability, and activation of DNA damage response pathways. These defects triggered a p53-dependent senescence program but failed to engage canonical p53-mediated apoptosis. Instead, epithelial cell death occurred through the coordinated activation of extrinsic apoptosis, necroptosis, and a metabolic vulnerability consistent with disulfidptosis. Finally, pharmacological inhibition and oxidative inactivation of VPS34 demonstrated that intestinal epithelial cells, particularly Apc-mutant cells, critically depend on VPS34 activity for survival.

Together, our findings uncover previously unrecognized functions of VPS34 in safeguarding mitotic fidelity and intestinal stem cell homeostasis and identify VPS34 as a central integrator of membrane trafficking, genome stability, and regulated cell death in the intestinal epithelium, highlighting its potential as a therapeutic target in colorectal cancer.



Autophagy in the context of immune aging



Katja Simon

*Max Delbrück Center for Molecular Medicine (MDC)
Berlin
Germany*

Tuesday, October 13th • 09:00 – 09:45

Across all arms of the immune system, the process of autophagy is key in development, function, prevention of senescence and homeostasis. Particularly long-lived cells such as stem cells and memory cells require autophagy for maintenance as they divide infrequently. However, the mechanisms involved are much less clear, and encompass control of metabolism, cell survival, and selective degradation of substrates and organelles. In memory T and B lymphocytes autophagy keeps the cells young and autophagy's contribution to this process is the maintenance of mitochondrial quality and provision of energy. Our recent study demonstrates that CD8⁺ T cells partition mitochondria asymmetrically and autophagic degradation contributes to this asymmetry. We also found that memory T and B cells can be rejuvenated by replenishing the essential metabolite spermidine that is needed to signal for autophagy. I will summarise our data on autophagy's impact on the immune system and metabolism, with a particular emphasis on mitophagy, metabolism and aging in mouse and human.



O10

ATGs in Immunity and infection
Short talk

An unexpected LC3B-positive compartment during HIV-1 cell-to-cell transmission

Guilhem Cantaloube¹, Kylian Renault¹, Baptiste Pradel¹, Coralie Daussy¹, Audrey Esclatine², Arnaud Moris², Eva Hernandez Djebali², Marion Lussignol², Raphaël Gaudin¹, Lucile Espert¹

¹ Institut de recherche en infectiologie (IRIM) CNRS, Université de Montpellier, France

² Institute for integration Biology of the cell (I2BC) CNRS, CEA, Université Paris Saclay, Gif Sur Yvette, France

HIV-1, the etiological agent of AIDS, infects its target cells either as cell-free particles or through cell-to-cell transmission via a specialized structure known as the virological synapse (VS). This mode of transmission is highly efficient, as it provides a protective niche for viral particles, shielding them from neutralizing antibodies and reducing the efficacy of certain antiretroviral therapies.

My PhD project focuses on a cellular mechanism called atg8ylation, which enables cells to respond to membrane-associated stress by recruiting ATG8 proteins, such as LC3B, to cellular membranes. Although this process is well known for its role in autophagy, it is now recognized as a broader mechanism involved in membrane homeostasis. Previously, we showed that atg8ylation promotes the entry of cell-free HIV-1 into CD4⁺ T lymphocytes independently of autophagy. Since the VS is a major route of HIV-1 transmission, we investigated the role of atg8ylation in this mode of viral spread.

We identified a novel large LC3B-positive structure within target cells during cell-to-cell transmission. Interestingly, super-resolution and 3D electron microscopy revealed that this structure is a single-membrane vesicle containing a large number of enveloped HIV-1 particles. Inhibition of atg8ylation, but not autophagy, impaired the formation of this structure and reduced HIV-1 cell-to-cell transmission.

We are currently investigating whether the viruses trapped within these compartments are degraded or whether they can establish a productive infection in the target cells. Overall, our findings provide new insights into the cellular mechanisms involved in HIV-1 infection and may ultimately contribute to the development of new host-directed therapies.



O11

ATGs in Immunity and infection

Short talk

Candalysin dampens the autophagy machinery involved in the plasma membrane stress response to the invasion of epithelial cells by the human fungal pathogen *Candida albicans*

Amandine Ducreux¹, Louise Basmaciyan¹, Fabienne Bon¹, Pascale Winckler¹, Bernhard Hube², Christophe d'Enfert³, Aurore Claude-Taupin⁴, Etienne Morel⁴, Pierre Lapaquette^{1*}, Frédéric Dalle^{1*}

¹ Université Bourgogne Europe, Institut Agro, INRAE, UMR PAM 1517, Dijon, France

² Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute, Jena – Allemagne

³ Institut Pasteur, USC2019 INRAE, Paris, France

⁴ Institut Necker Enfants-Malades, INSERM U1151, CNRS : UMR8253, Université Paris Cité : UM111, Paris, France

* Equal contribution to last authorship

Candida albicans is an opportunistic fungal pathogen responsible for infections ranging from superficial to life-threatening systemic disease. In susceptible hosts, it can cross the intestinal barrier and disseminate to deeper organs. *C. albicans* hyphae can invade human epithelial cells. On the host side, autophagy is a key defense mechanism that promotes the clearance of intracellular pathogens and also regulates membrane dynamics, including repair processes following cellular damage. We previously demonstrated that several autophagy-related proteins (ATG5, ATG16L1, WIPI2, and LC3A/B) are massively recruited at sites of active *C. albicans* penetration, in association with mechanical deformation of the plasma membrane and lysosome-dependent membrane repair. Here, we aim at elucidating the molecular mechanisms by which *C. albicans* triggers and modulates host autophagy.

Using *in vitro* epithelial cell lines (Hela, HCT116 and Caco-2), autophagy-deficient cell lines and a panel of *C. albicans* mutants for genes encoding proteins involved in its ability to invade host cells, we explored in-depth, notably by immunostaining, the molecular mechanisms by which *C. albicans* modulates host autophagy. We also used synthetic candidalysin and nanobodies against it to precisely define the toxin's effects on autophagy. Our findings indicate that mechanical deformation of the host plasma membrane induced by hyphal invasion is the primary trigger of the host autophagic response. Moreover, we show that candidalysin, a pore-forming toxin secreted by *C. albicans*, dampens this protective response at the plasma membrane by markedly reducing the recruitment of ATG proteins at invasion sites. Altogether, these results suggest that *C. albicans*, through the secretion of the candidalysin toxin, is able to attenuate host autophagy at the invasion site, favoring its cellular spreading.

Keywords: *Candida albicans*, autophagy, candidalysin, epithelial cells, plasma membrane damage, lysosomal exocytosis



O12

ATGs in Immunity and infection
Short talk

Establishing Sensitive Autophagic Flux Assays in Patient-Derived B Cells to Investigate Immune Dysregulation in Immune-Mediated Thrombotic Thrombocytopenic Purpura

Tanja Muralt¹, Anna M. Schläfli², Sara M. Buonomo¹, Nikolai Engedal³, Mario P. Tschan², Monica Schaller¹

¹ Department for BioMedical Research, University of Bern, Bern, Switzerland

² Institute of Tissue Medicine and Pathology, University of Bern, Bern, Switzerland

³ Autophagy in Cancer Lab, Department of Tumor Biology, Oslo University Hospital, Oslo, Norway

Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare, life-threatening thrombotic microangiopathy characterized by a severe deficiency of a disintegrin and metalloprotease with thrombospondin type 1 motifs, member 13 (ADAMTS13), an enzyme responsible for cleaving von Willebrand factor (VWF) multimers to maintain normal hemostasis. Anti-ADAMTS13 autoantibodies cause severe ADAMTS13 deficiency leading to the accumulation of ultra-large VWF multimers, thrombocytopenia, microvascular thrombosis and ischemic organ damage, particularly affecting the brain, heart and kidneys. Despite current standard-of-care treatment including therapeutic plasma exchange and immunosuppressants, a majority of patients remain at risk for relapse. A better understanding of the underlying mechanisms driving iTTP disease pathogenesis is essential to identify novel therapeutic strategies.

Systemic lupus erythematosus (SLE) shares several pathogenic features with iTTP, including immune dysregulation leading to autoantibody production, thrombosis, and organ damage. In SLE, increased autophagy activity has been demonstrated in autoreactive B cells. Based on these findings, we hypothesize that autophagy is similarly dysregulated in B cells from iTTP patients contributing to the persistence of autoreactive B cells and ultimately production of pathogenic anti-ADAMTS13 autoantibodies and thus disease progression. To test this hypothesis, we aim to establish and optimize a robust workflow to measure autophagic flux in B cells isolated from whole blood of iTTP patients at different disease stages. Our approach combines the LDH sequestration assay, LC3B-flux analysis and complimentary assays to assess autophagy activity. As B cells constitute only 5-10% of peripheral blood mononuclear cells (PBMCs), the limited availability of patient material is a major technical challenge. We are currently adapting the assays for lower cell numbers by optimizing treatment conditions and buffers to define incubation times and increase detection sensitivity. The optimized workflow will be compared to standard methods, including immunofluorescence using autophagy markers LC3B and p62 as well as flow cytometry using Cyto-ID. Establishing sensitive and reliable methods to quantify autophagic flux in patient-derived B cells will provide important insights into the role of autophagy in iTTP pathogenesis and may identify autophagy as a promising therapeutic target to complement current treatment.



Proximity proteomics deciphers autophagy-linked host factors at the site of AdV-induced membrane damage

Mathilde Lartigau¹, Nicolas Landrein¹, Jessica Ragues¹, Muriel Faure¹, Fabienne Rayne¹, Benoit Roger¹, Axel Imhof², Harald Wodrich¹

1 Microbiologie Fondamentale et Pathogénicité (MFP) - UMR 5234 - Université de Bordeaux

2 Ludwig Maximilian University (LMU) – Munich, Germany

Adenoviruses (AdV) are highly infectious DNA viruses with a generally low pathogenic profile in humans, making them widely used as vector tools in gene therapy and as vaccine vectors. During cell entry, the viral capsid undergoes partial disassembly within the endosome, releasing the internal membrane-lytic capsid protein VI (pVI). Protein VI then interacts with the endosomal membrane and induces its rupture, allowing the virus to escape into the cytoplasm — a key step of successful cell entry.

Membrane rupture constitutes a danger signal for the cell. Damaged membranes are detected through cytoplasmic galectins, which mediate autophagy activation to remove or repair the damage. AdV takes control of this response by delaying autophagosome maturation to prevent its own autophagic clearance. However, the interplay between viral factors and the cellular machinery that detects and repairs membrane damage remains poorly characterized.

Here, we developed a novel approach to identify cellular proteins recruited to the site of membrane rupture upon AdV entry, using antibody-mediated proximity biotinylation coupled with mass spectrometry (AMPL-MS), in collaboration with Dr. Axel Imhof (LMU, Munich, Germany). This method relies on targeting protein VI at the membrane damage site with a primary antibody, which recruits a biotin ligase (Protein A-APEX2) to the site of endosomal damage. Proteins in immediate proximity are thereby biotinylated, then purified and identified by LC-MS/MS, with specificity validated using appropriate controls.

Among the approximately 4,000 proteins identified, pathway enrichment analysis revealed that a substantial proportion were linked to autophagy, consistent with its established role in membrane damage response. From this analysis, we selected 10 candidates based on statistical enrichment and biological relevance. These proteins are involved in endosomal trafficking, membrane repair, ubiquitin-mediated signalling, antiviral immunity, and stress-induced apoptosis, suggesting the coordinated activation of multiple distinct cellular pathways during adenovirus entry.

Functional validation of these candidates by siRNA depletion is currently underway. Characterizing these virus–host interactions will help us better understand adenoviral evasion strategies and membrane damage surveillance mechanisms, with perspectives for developing novel antiviral targets and improving AdV-based vector tools.



Selective autophagy as a central mechanism of therapy resistance in AML



Carine Joffre

*Centre de Recherche en Cancérologie de Toulouse
Université de Toulouse, Inserm U1037, CNRS U5071
Toulouse
France*

Tuesday, October 13th • 14:00 – 14:45

The unfavourable outcome of Acute Myeloid leukemia (AML) is primarily due to the persistence of treatment-resistant cells responsible for minimal residual disease (MRD) and the subsequent high relapse rates. Understanding the mechanisms underlying this resistance, and thereby reducing MRD, is therefore essential for developing new therapeutic strategies and improving patient survival.

Autophagy plays a central role in AML biology. Our work has significantly contributed to demonstrating its importance in AML progression, notably by showing that oncogenic signalling confers a high basal autophagy, and that autophagy sustains metabolic functions essential for leukemic cell survival (Heydt *et al.*, 2018; Larrue *et al.*, 2019; Bosc, Broin *et al.*, 2020). More recently, we demonstrated that the survival of leukemic stem cells (LSCs) depends on their ability to promote ferritinophagy, a selective form of ferritin degradation essential for iron homeostasis (Larrue *et al.*, 2024). These findings align with recent studies suggesting that autophagy is not a global, non-specific degradation process but rather a highly selective mechanism with substrate preferences.

While autophagy has emerged as a key regulator of leukemic cells, its role in AML therapeutic resistance remains limited and incomplete, particularly *in vivo*. Our goal is to investigate how selective autophagy coordinates adaptive mechanisms that support leukemic cell survival and drive relapse, thereby revealing novel therapeutic targets in AML.



Shear stress-dependent modulation of autophagy shapes metastatic features of circulating tumor cells

Flavie Garampon¹, Maribel Lara Corona¹, Clemence Barthelemy¹, Rhode Benoit¹, Sara Ceccacci², Irene Cipollone², Chiara Guerrero², Laura Salavessa¹, Cedric Delevoye¹, Etienne Morel¹, Aurore Claude-Taupin¹

¹ Institut Necker-Enfants Malades, INSERM U1151, CNRS UMR8253, Université Paris Cité, Paris, France

² Proteomic Platform Necker, INSERM US24 Structure Fédérative de Recherche Necker, Université Paris Cité, Paris, France

Metastasis accounts for nearly 90% of cancer-related deaths and relies on the ability of circulating tumor cells (CTCs) to survive in the bloodstream and colonize distant organs. During hematogenous dissemination, CTCs are exposed to fluid shear stress, a major mechanical constraint whose intensity varies throughout the circulatory system. Although both autophagy and mechanical cues have been implicated in cancer progression, whether shear stress regulates autophagy to control CTC survival and metastatic fitness remains largely unknown.

To address this question, renal cell carcinoma (RCC) cell lines with different metastatic potentials were cultured in suspension under controlled fluid flow using a microfluidic pump system generating shear stress ranging from 2 to 20 dyn/cm², representative of physiological hemodynamic conditions.

We found that shear stress intensity strongly impacts CTC survival and autophagy. High shear stress not only enhances CTC survival but also increases their post-circulatory adhesion and clonogenic capacities, which are hallmarks of metastatic fitness, whereas low shear stress promotes apoptosis. Mechanistically, surviving RCC cells exposed to high shear stress present with enlarged acidic lysosomes with reduced degradative capacity, leading to the accumulation of autophagic structures. Unbiased mass spectrometry analyses showed increased levels of mitochondrial proteins in these cells, suggesting that autophagy inhibition in surviving CTCs is associated with the accumulation of mitochondrial content. Preliminary ongoing results also indicate that cancer cells subjected to high shear stress accumulate mitochondria while exhibiting reduced mitochondrial reactive oxygen species production. Together, these findings support the hypothesis that impaired autophagy-lysosome function may promote mitochondrial preservation and support cell survival under mechanical stress. In contrast, low shear stress induced higher apoptotic rates, consistent with increased mitochondrial damage.

Altogether, these results reveal a previously underappreciated role of shear stress in modulating mitophagy, thereby influencing mitochondrial homeostasis and promoting CTC survival during metastasis.



Host autophagy is required for acute myeloid leukemia growth and therapeutic resistance

Manso Constance^{1,*}, Grignon Anaïs^{1,*}, Anaïs Chekroun¹, Nathalie Bourges Abella², Fanny Granat¹, Jean-Emmanuel Sarry¹, Carine Joffre¹, **Laura Poillet**¹

¹ Centre de Recherches en Cancérologie de Toulouse (CRCT), France

² Centre Régional d'Exploration Fonctionnelle et de Ressources Expérimentales (CREFRE), Université de Toulouse, ENVT

* Equal contribution to first authorship

Despite a high rate of complete remission after chemotherapy, the prognosis is poor in human acute myeloid leukemia (AML) due to the high frequency of relapses. These relapses are mainly caused by the growth of chemoresistant leukemic cells able to adapt their metabolism to survive. In that context, targeted therapies have been developed but relapses are still observed. Thus, additional studies are needed to understand the mechanisms involved and develop new treatments to eradicate resistant AML cells. We hypothesized that autophagy could be involved one of those mechanisms. While previous studies on autophagy and tumor growth mainly focused on tumor autophagy, host/microenvironment autophagy has also been recently implicated in tumor growth promotion by supplying important substrates. However, to date, neither its role in therapeutic resistance nor its relevance in AML development has yet been studied.

Combining complementary models: in vitro co-culture system and in vivo genetically engineered mouse models with AML cell lines and primary AML patient samples, we investigated whether host autophagy, especially bone marrow autophagy, can support AML growth and resistance and investigated the mechanisms involved.

We first showed that, host autophagy promotes AML cell growth without affecting cell homing in vivo. Similar results were obtained in immunocompetent and immunodeficient mice suggesting that immune response is not involved. We also demonstrated that loss of host autophagy sensitizes AML cells to chemotherapy as well as targeted therapy in vitro. However, in vivo preliminary data suggest that host autophagy participates in AML therapeutic resistance in an immune context dependent manner. In addition, we observed that host autophagy is regulating circulating cytokines as well as metabolites. Indeed, metabolomics analysis of autophagy-deficient and wild-type mice revealed that host autophagy regulates circulating metabolites especially pyrimidines bases and amino acids that could be required for AML growth or resistance.

Our findings establish host autophagy as a key determinant of AML progression and therapeutic resistance and identify new potential targets to eradicate AML resistant cells. Future experiments aim to better decipher the exact mechanisms involved. Thus, targeting host autophagy or the underlying mechanisms represent promising therapeutic new strategies to overcome AML patient relapses and improve their survival.



O16

ATGs and Cancer
Short talk

Autophagy induction by a viral protein contributes to Burkitt Lymphoma resistance to chemotherapy

Alexandra Hamood¹, Damien Glon¹, Guillaume Beauclair^{1,3}, Eva Hernandez¹, Joëlle Wiels², Audrey Esclatine¹, Marion Lussignol¹

¹ I2BC, Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, 91190, Gif-sur-Yvette, France

² IGR, Institut Gustave Roussy, CNRS, Université Paris Saclay, Villejuif

³ Université Paris Cité, Institut Pasteur, Environnement et risques infectieux, Paris, France (Present affiliation)

Epstein-Barr virus (EBV) is a herpesvirus that establishes lifelong latency in B lymphocytes and is associated with several cancers, including Burkitt lymphoma (BL), a highly aggressive and proliferative B-cell cancer. Several EBV proteins are known to contribute to oncogenesis and chemoresistance, but the underlying mechanisms remain poorly understood. Approximately 15% of EBV-associated BL cases display a specific latency program, with the expression of a limited set of viral proteins. These BL are characterized by enhanced tumor cell survival and resistance to chemotherapy. Here, we explored the mechanism responsible and we identified the viral protein BHRF1, a homolog of Bcl-2, as a key regulator of chemoresistance via sustained autophagy and mitophagy.

Using the EBV+ BL cell line P3HR-1, which constitutively expresses BHRF1 during latency, we found that these tumor cells are highly resistant to apoptosis inducers. BHRF1 depletion using shRNA restored the sensitivity to these treatments, supporting a central role of BHRF1 in chemoresistance. We then observed that BHRF1 expression in P3HR-1 cells sustains high levels of autophagic activity. Given the well-established role of autophagy as a pro-survival mechanism, we investigated its contribution to BHRF1-mediated chemoresistance. Silencing of key autophagy genes using shRNA restored sensitivity to apoptosis induction, demonstrating that BHRF1-mediated autophagy contributed to chemoresistance in EBV-positive BL.

Furthermore, we observed that BHRF1 induces mitophagy, as evidenced by the increase in levels of mitochondrial markers (TOMM20, TIM23, cytochrome c) upon BHRF1 depletion. BHRF1 also promotes the formation of mito-aggresomes, in which mitochondria accumulate in juxtanuclear clusters. Importantly, mito-aggresome formation depends on both LC3-mediated autophagy and DRP1-dependent mitochondrial fission, indicating the involvement of these two processes.

Finally, to map the BHRF1 interactome and define the molecular mechanism underlying BHRF1 regulation of autophagy, we performed BioID2-based proximity labeling. We identified 16 new interactors potentially linking BHRF1 to autophagy and mitochondrial dynamics.

Together, our data identify BHRF1 as a central regulator of chemoresistance in EBV-positive BL via sustained autophagy and mitophagy. Disrupting the interaction between BHRF1 and its autophagy partners could represent a promising strategy to restore chemosensitivity in Burkitt lymphoma.



CTF1 is a critical cancer-derived factor regulating autophagy in the tumor microenvironment

Yunus Akkoc^{1,2}, Dyana Sari-Turk², Joern Dengjel³, Devrim Gozuacik^{1,2}

¹ University School of Medicine, Department of Medical Biology, 34010, Türkiye

² University Research Center for Translational Medicine (KUTTAM), Istanbul 34010, Türkiye

³ University of Fribourg, Department of Biology, Chemin du Musée 10, CH-1700 Fribourg, Switzerland

Autophagy is a fundamental catabolic process that is rapidly upregulated during cellular stress, enabling cells to recycle intracellular components and maintain homeostasis. Dysregulation of autophagy plays a critical role in the pathogenesis and progression of cancer. However, the mechanisms regulating autophagy within tumor stroma interactions remain insufficiently understood.

We previously identified a cytokine, CTF1 (cardiotrophin 1), as an activator of autophagy in fibroblasts and breast cancer-derived carcinoma-associated fibroblasts (CAFs). CTF1 promotes stroma-associated breast cancer cell migration and invasion, and elevated CTF1 expression correlates with lymph node metastasis in patients.

In this study, CTF1 is shown to be a significant factor in CAF formation, with potency comparable to that of TGF β . The role of CTF1 in CAF formation and stromal autophagy was further investigated through comparative proteomic and pathway analyses of CTF1-induced CAFs. We further dissected the role of CTF1 in CAF formation and stroma autophagy. We observed that the autophagic capacity of the stroma is crucial for CAF formation and that CTF1 stimulates specific fibroblast activation-related pathways.

We have now proposed a new subtype of CTF1-induced activated fibroblasts, and our results suggest that these factors are crucial for tumor-stroma interactions and stromal fibroblast activation, and that autophagy resides at the nexus of these cancer-related events.

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) grant numbers 122Z227 and 123S660.



Eating your mitochondria: good or bad?



Ian Ganley

*MRC Protein Phosphorylation and Ubiquitylation Unit
University of Dundee
Dundee
United Kingdom*

Wednesday, October 14th • 09:15 – 10:00

Mitophagy is widely viewed as a protective pathway that clears dysfunctional mitochondria. Our work shows it can also eliminate healthy ones: mitochondria targeted by NIX-mediated mitophagy retain functional capacity, with consequences for cellular metabolism and health. This is evident in a rare and devastating mitochondrial disease termed Mitochondrial DNA Depletion Syndrome 13 (MTDPS13), for which there is no effective treatment. Here, mutations in the E3 ubiquitin ligase FBXL4 lead to the accumulation of the mitophagy receptors BNIP3 and NIX, driving excessive mitophagy and causing a profound reduction in mitochondrial content and respiratory function. We have found that small-molecule AMPK activation suppresses this aberrant mitophagy and restores mitochondrial content and respiration in MTDPS13 patient fibroblasts and an in vivo disease model, highlighting a potential therapeutic strategy.



O18

Selective autophagy
Short talk

Novel insights into the role of an undisclosed NDP52 phosphorylation in mitophagy

Krenare Bruqi¹, Anna Mattioni², Valentina Cianfanelli², Flavie Strappazon¹

¹ Université Lyon 1, CNRS, INSERM, Physiopathologie et Génétique du Neurone et du muscle, UMR5261, U1315, Institut Neuromyogène, Lyon, France

² Gynecologic Oncology Unit, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

Selective targeting of surplus or dysfunctional mitochondria for autophagic degradation is known as mitophagy. PINK1/Parkin-mediated mitophagy implicates selective autophagy receptors that are subjected to phosphorylation/dephosphorylation events to be activated in the process. An indispensable receptor of this pathway is NDP52/CALCOCO2. Upon mitochondrial/oxidative stress, NDP52 oligomerizes, an event that facilitates the recruitment of autophagy machinery to damaged mitochondria for mitophagy execution. Although NDP52 is one of the main mitophagy receptors in mammals, mitochondrial stress sensing and post-translational modifications of importance to its mitophagic function remain poorly defined.

In our work, we have firstly discovered that NDP52 is a direct substrate target of the PP2A phosphatase. Interestingly, bioinformatic predictions together with published data indicate that PP2A might target NDP52 on serine 445 (S445). In line with this, we hypothesized whether S445 phosphorylation plays role in the capacity of NDP52 to mediate the clearance of damaged mitochondria. To this end, we engineered phospho-mimetic (S445D) and phospho-dead (S445A) mutants of the receptor. Using complementation assay, we discovered that the overexpression of the phospho-dead mutant delays mitophagy in HEK293 cells treated with oligomycin and antimycin. Currently we are investigating whether S445 phosphorylation affects NDP52 protein stability and oligomerization, thereby providing further mechanistic insights into how this post-translational modification impacts NDP52 function during mitochondria selective autophagy.



When SARs meet LLPS: human cytomegalovirus induces the formation of antiviral p62-bodies

Marwan Seddiki¹, Clémence Taisne¹, Eva Hernandez¹, Audrey Esclatine¹, Marion Lussignol¹, **Damien Glon¹**

¹ Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

Selective autophagy receptors (SARs), such as p62/SQSTM1, NBR1, and TAX1BP1, can self-organize into condensates through liquid-liquid phase separation (LLPS). These condensates, known as p62-bodies, act as hubs for the autophagic degradation of ubiquitinated proteins. Whether such SAR-enriched condensates are induced by and/or modulate viral infection remains totally unexplored. Here, we investigated the formation and function of these structures during human cytomegalovirus (HCMV) infection.

Using different electron microscopy techniques (TEM, CLEM, and FIB-SEM) in HCMV-infected fibroblasts, we identified membrane-less and electron-dense cytoplasmic structures distinct from the viral assembly compartment (vAC). Correlative light-electron microscopy established that these structures correspond to a concentric accumulation of viral tegument proteins. Live-cell imaging experiments demonstrated liquid-like behavior of these previously undescribed viral structures, which we named tegument-bodies.

Strikingly, the periphery of the tegument-bodies is decorated by numerous small condensates enriched in p62, NBR1, and TAX1BP1. These condensates displayed hallmarks of LLPS, including fusion, rapid FRAP recovery, and sensitivity to 1,6-hexanediol, thereby identifying them as bona fide p62-bodies. Functionally, CRISPR knockout of SARs markedly increased viral production. Conversely, stable overexpression of SARs impaired vAC formation and reduced viral titers. Overall, our results indicate that these p62-bodies could serve as antiviral platforms against HCMV infection.

Finally, we observed recruitment of the NF- κ B adaptor NEMO to the p62-body surface, associated with increased nuclear translocation of the NF- κ B RelA transcription factor, suggesting that these structures couple SARs to innate immune signaling. Together, our data reveal that HCMV infection induces a novel class of liquid organelles, coated by p62-bodies, which act as an antiviral platform restricting productive infection. This work uncovers a previously unrecognized layer of host-virus interaction at the interface of selective autophagy and biomolecular condensates, opening new perspectives on the antiviral functions of SARs beyond their degradative role.



O20

Selective autophagy
Short talk

Exploring the role of deubiquitination in ERMES during mitophagy

Maria Ángeles Morcillo Parra¹, Laetitia Cavellini¹, Mickaël M. Cohen¹, Naïma Belgareh-Touzé¹

¹ Sorbonne Université, CNRS, UMR8226, Institut de Biologie Physico-Chimique, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, 75005 Paris

Mitochondria are highly dynamic membrane-bound organelles involved in essential cellular functions, most notably ATP production to sustain cellular energy demands. To maintain mitochondrial homeostasis, cells rely on quality control mechanisms such as mitophagy, the selective degradation of damaged mitochondria. Even though mitochondria do not form part of the secretory pathway, they depend on membrane contact sites, with other organelles, for the exchange of different molecules.

In the yeast *Saccharomyces cerevisiae*, the ERMES (Endoplasmic Reticulum-Mitochondria Encounter Structure) complex mediates the physical connection between the endoplasmic reticulum (ER) and mitochondria. ERMES has been implicated in multiple mitochondrial functions, including lipid exchange, mitochondrial dynamics, protein import, and mitophagy. Increasing evidence suggests that ubiquitination play an important role in regulating mitochondrial quality control pathways. Our laboratory has demonstrated that ubiquitination of ERMES components Mdm34 and Mdm12 is crucial for efficient mitophagy. However, it remains unclear how this ubiquitination can affect mitophagy.

To unravel how deubiquitination regulates mitophagy, we analysed the involvement of deubiquitinating enzymes (DUBs) in the regulation of ERMES during this process. By identifying DUBs affecting ERMES components and characterizing their impact on ubiquitin dynamics during mitophagy, we aim to better understand how deubiquitination contributes to the coordination of mitochondrial quality control.



Targeting the autophagy receptor p62 for degradation to regulate proteaphagy and recover sensitivity to bortezomib in mantle cell lymphoma

Corentin Bouvier¹, Hala Shalhoub², Juliette Michaut¹, Lola Dumortier^{1,2}, Andrea Bertran³, Chahinez Chouib¹, Christophe Menendez¹, Camille Richagneux¹, Kevin Antraygues¹, Anne-Marie Caminade¹, Cedric-Olivier Turrin¹, Karine Reybier⁴, Gaël Roué⁵, Carles Galdeano³, Julie Guillermet-Guibert², **Manuel S. Rodriguez**^{1,4,6}

¹ Univ Toulouse, CNRS, LCC, France

² Centre de Recherche en Cancérologie de Tou-louse (CRCT), Inserm, France

³ Institute of Biomedicine (IBUB), University of Barce-lona, Barcelona, Spain

⁴ PharmaDev, UMR 152, Université de Toulouse, IRD, France

⁵ Josep Carreras Leukaemia Research Institute, Badalona, Spain

⁶ BMolecular, Centre Pierre Potier, Toulouse, France

Previous studies using ubiquitin traps (TUBEs) combined with mass spectrometry revealed that in Bortezomib (BTZ) resistant Mantle Cell Lymphoma (MCL) cells, a selective autophagy mechanism, known as proteaphagy, was a main mechanism eliminating the proteasome and reducing its sensitivity to BTZ (1). The proteaphagy receptor p62 plays a crucial role in driving the proteasome to degradation (1). In order to understand the recruitment of p62 into autophagosomes and the regulation of proteaphagy we developed molecular traps and TPD approaches. Here, we will present the development and characterisation of p62 PROTACs using a previously reported p62 binder YOK-2204 and a IMiD derivative to recruit the ubiquitin ligase CRBN. From the synthesised PROTACS, we have selected three, named Yalk6-OA, Yalk10-OA and Yalk8-A, showing the best capacity to bind and degrade p62. The fluorescent PROTAC Yalk8-A was used to confirm cell permeability, stability and colocalization with its target p62. The basal level of p62-CRBN interaction was improved in the presence of Yalk8-A and Yalk10-OA together with p62 ubiquitylation at early times after treatment. PROTAC-mediated degradation of p62 can be blocked with MLN4924 (pevonedistat) or competed with a molar excess of lenalidomide indicating the ubiquitin-ligase dependency of this proteolytic event. Altogether our data indicates that the p62 PROTACs developed here, can be used as a novel approach to explore the role of p62 in diseases where this autophagy adaptor is dysregulated.

Understanding the key molecular mechanisms acting at distinct levels will be crucial to develop new combined therapies with the ability to overcome BTZ refractoriness or in general regulating selective autophagy events in distinct pathologies.

References

1 Quinet et al, *Cancers* (2022) Feb 12;14(4):923. doi: 10.3390/cancers14040923.

2 Bouvier et al, *Cell Death Discov.* (2025) Mar 17;11(1):108. doi: 10.1038/s41420-025-02355-6.



Molecular regulation of autophagy – Session 2

Invited speaker

Karyopherin-mediated regulation of autophagy



Louis Lapierre

*Centre de Médecine de Précision du Nouveau-Brunswick
Département de Chimie et Biochimie, Université de Moncton
Moncton, NB
Canada*

Wednesday, October 14th • 13:45 – 14:30

Aging is characterized by a progressive decline in proteostasis, leading to protein misfolding and aggregation. Another emerging aspect of the age-related collapse of the proteostasis network is the loss of subcellular spatial distribution and dynamic transport of proteins across the nuclear pore. With the help of Ran GTPase, karyopherins (importins, exportins and biportins) mediate the transport of large proteins across the nuclear pore by recognizing localization motifs. During aging, changes in karyopherin levels and activity modify nuclear pore function and changes subcellular protein distribution, which in turn alters phase condensation and aggregation dynamics. Genetic and pharmacological modulations of key karyopherins, such as the exportin XPO1 impacts proteostasis by not only re-distributing a subset of proteins, but also via transcriptional activation of the autophagy-lysosomal pathway by enriching the autophagy transcription factor HLH-30/TFEB in the nucleus. In addition, subcellular protein redistribution from XPO1 inhibition leads to major changes in nucleolar size dynamics, which consequently alters ribosomal biogenesis and translational efficiencies resulting in longer lifespan in *C. elegans*. Work in human cancer cells mirrors changes observed in *C. elegans* on TFEB and nucleolar modulation, and XPO1 inhibition in a model of Alzheimer's disease in mice improves spatial cognition. Altogether, karyopherin modulation is emerging as a novel point of entry to modulate proteostasis and autophagy against the progressive proteostatic decline in aging.



O22

Molecular regulation of autophagy – Session 2

Short talk

Stress-induced remodeling of RAB11-positive endosomes promotes autophagic adaptation through RAB30

Carla Alemany¹, Camille Poulard¹, Anaëlle Kalubi Kabambi¹, Ida Chiara Guerrero², Maryse Romao^{3,4}, Graca Raposo^{3,4}, Juliane Da Graça^{1,5}, Aurore Claude Taupin¹, Virginie Stevenin⁶, Cédric Delevoye¹, Etienne Morel¹

¹ Université Paris Cité, INSERM UMR-S1151, CNRS UMR-S8253, Institut Necker Enfants Malades, F-75015 Paris, France

² Proteomic Platform Necker, INSERM US24 Structure Fédérative de Recherche Necker, Université Paris Cité, Paris, France

³ Institut Curie, PSL Research University, CNRS, UMR144, Structure and Membrane Compartments, 75005 Paris, France

⁴ Institut Curie, PSL Research University, CNRS, UMR144, Cell and Tissue Imaging Facility (PICT-IBISA), 75005 Paris, France

⁵ Interfaculty Institute of Bioengineering and Global Health Institute, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

⁶ Institute of Pharmacology and Structural Biology (IPBS), 205 Route de Narbonne, 31400, Toulouse, France

RAB11-positive recycling endosomes are emerging as dynamic membrane reservoirs that contribute to multiple cellular processes, including autophagy. However, how these membranes are mobilized to support autophagic responses during acute cellular stress remains poorly understood.

Here, we investigated RAB11-dependent membrane remodeling during nutrient deprivation and mechanical shear stress, two physiological conditions that induce autophagy. We show that RAB11 is required for efficient autophagic responses and cell survival under stress, as depletion of RAB11 impairs the formation of autophagic structures and increases cell death. Live-cell imaging reveals extensive remodeling of RAB11-positive membranes, characterized by increased RAB5/RAB11 colocalization and enhanced RAB11 tubulation. This remodeling is accompanied by a local shift in phosphoinositide composition, with increased PI3P and decreased PI4P, suggesting coordinated changes in membrane identity during the autophagic response.

To identify the molecular machinery underlying this remodeling, we combined TurboID-based proximity labeling with quantitative proteomics. Stress selectively reshaped the RAB11-associated proteome, revealing the recruitment of membrane-remodeling factors, including annexins and the small GTPase RAB30. RAB30 relocates from the Golgi/TGN to RAB11-positive endosomes and is required for efficient autophagic responses and cell survival. RAB30 recruitment depends on PI3P and is necessary for ATG9 association with RAB11-positive endosomes, linking stress-induced phosphoinositide remodeling to the recruitment of autophagic machinery.

Together, our findings identify RAB11-positive recycling endosomes as stress-responsive membrane reservoirs and reveal a coordinated RAB11–phosphoinositide–RAB30 axis that promotes their remodeling and engagement with the autophagic machinery. These findings uncover a mechanism by which recycling endosomes may be mobilized to support autophagic adaptation and cell survival during cellular stress.



O23

Molecular regulation of autophagy – Session 2

Short talk

RNA G-quadruplexes reprogram translation to sustain autophagy and cancer cell survival

Alice Desprairies^{1,*}, Pauline Herviou^{1,*}, **Cléa Villette**^{1,*}, Claire Medale-Giamarchi^{1,2}, Sauyeun Shin¹, Armelle Choquet³, Estelle Espinos^{1,2}, Stéphane Manenti^{1,2}, Alexandre David^{3,4}, Erik Dassi⁵, Stefania Millevoi^{1,2}, Anne Cammas^{1,2}

1 Cancer Research Center of Toulouse, INSERM, CNRS, UMR 1037, 31037 Toulouse, France

2 Université Toulouse III Paul Sabatier, 31330 Toulouse, France

3 IRCM, Université de Montpellier, INSERM, Montpellier, France

4 IRMB-PPC, Université de Montpellier, INSERM, CHU Montpellier, CNRS, Montpellier, France

5 Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, 38123 Trento (TN), Italy

* Equal contribution to first authorship

Autophagy enables cancer cell survival and resistance during therapy, yet the molecular mechanisms enabling autophagy-related mRNAs to escape the therapy-induced translational inhibition and achieve selective expression remain largely unexplored.

Here, we identify RNA G-quadruplexes (RG4s)—non-canonical RNA structures—as key orchestrators of autophagy during genotoxic stress. We demonstrate that RG4s, bound by the helicase DDX3X and novel regulators PRPF8 and EFTUD2, serve as critical modulators of autophagy-related mRNA translation, maintaining autophagic protein synthesis despite therapy-induced global translational suppression. This RG4-dependent process sustains prosurvival autophagy in glioblastoma, driving chemotherapy resistance. Importantly, pharmacological stabilization of RG4s enhances tumor cell sensitivity to treatment and decreases resistant cell survival, unveiling a previously unrecognized vulnerability that could be exploited to improve therapeutic outcomes.



O24

Molecular regulation of autophagy – Session 2

Short talk

Unraveling the pathophysiology of TANGO2 loss of function in rhabdomyolysis symptoms

Gwenaëlle Laurent¹, Straube Marjolène³, Renault Solène³, Huver Sophie⁴, Tomasetto Catherine-Laure⁴, Kabashi Edor¹, Oury Franck³, de Lonlay Pascale^{2,3}, de Calbiac Hortense³

¹ Translational Research for Neurological Disorders, Institut Imagine, INSERM UMR 1163, Université Paris Cité, F-75015, Paris, France

² Reference Center of Inherited Metabolic Diseases, Hôpital Universitaire Necker-Enfants Malades, AP-HP, Institut Imagine, Filière G2M, MetabERN, F75015, Paris, France

³ Neurobiologie Intégrative, Institut Necker Enfants Malades, INSERM UMR 1151, Université Paris Cité, F-75015 Paris, France

⁴ Biologie moléculaire et cellulaire des cancers du sein, IGBMC, CNRS UMR 7104, Inserm U1258, Université de Strasbourg, Illkirch, France

TANGO2 Deficiency Disorder (TDD) is characterized by life-threatening metabolic crises like rhabdomyolysis triggered by metabolic stress such as fasting. In primary myoblasts from TANGO2 patients, we observed a marked reduction in autophagy flux and LC3 levels under starvation, indicating defective autophagy. Similar results were found in tango2 knockdown (KD) zebrafish, which also showed locomotor defects worsened by atorvastatin exposure, a drug known to cause rhabdomyolysis. Recent studies further suggest that TANGO2 depletion disrupts membrane and cellular lipid composition, and that TANGO2 may participate in ER–Golgi trafficking. Altogether, these findings indicate that TANGO2 is a key player in the cellular response to metabolic stress, and that its loss increases muscle vulnerability to fasting. Taking advantages of TANGO2 patient myoblasts and tango2 KD zebrafish, we investigated autophagy transcriptional regulation, and we found decreased expression of autophagy-related genes in both models. In patient myoblasts, starvation failed to induce proper translocation of transcription factor EB (TFEB) from cytoplasm to nucleus, essential for autophagy activation, and TFEB as well as its regulator and target gene PPARGC1A were downregulated. Overexpression of human TFEB rescued locomotor defects in tango2 KD zebrafish, suggesting that the phenotype is not solely due to impaired autophagy initiation. Resveratrol treatment also restored the phenotype and TFEB translocation, opening new therapeutic avenues. Finally, collaborative work revealed reduced ER–endosome contact sites, known as autophagosome biogenesis sites, in TANGO2-depleted cells validating impaired autophagy initiation and autophagy insufficiency.



O25

Molecular regulation of autophagy – Session 2

Short talk

Post-transcriptional control of autophagy-related pathways by Nonsense-Mediated mRNA Decay

Chloé Mercier¹, Annick Fraichard¹, Aurélie Baguet¹, Michaël Guittaut¹

¹ Université Marie et Louis Pasteur, EFS INSERM UMR1098 RIGHT, F-25000 Besançon, France

Autophagy is a major cellular homeostasis pathway involved in stress adaptation, protein turnover, immunity and cancer. Its activity relies on a tightly controlled network of autophagy-related proteins, including members of the ATG8 family. Among them, GABARAPL1 is an important regulator of autophagy, but the post-transcriptional mechanisms controlling its expression remain poorly understood.

We investigated the contribution of Nonsense-Mediated mRNA Decay (NMD), a translation-dependent RNA surveillance pathway, to GABARAPL1 mRNA regulation. Comparison with the closely related GABARAP transcript showed that GABARAPL1 sensitivity to NMD depends on its 3' untranslated region (3'UTR). A conserved region within the GABARAPL1 3'UTR was sufficient to confer NMD sensitivity. Structural analyses identified a conserved stem-loop, while RNA-protein interaction experiments demonstrated direct binding of the NMD factors UPF1 and eIF4AIII, supporting a 3'UTR-dependent post-transcriptional regulation by NMD.

We then investigated whether chemical inhibition of NMD could modulate autophagy-related genes and associated stress pathways in mouse DC2.4 dendritic cells. Cells were treated with the NMD inhibitors 1a and 2a, using KVS0001 as a reference compound. Direct long-read RNA sequencing revealed modulation of several autophagy- and stress-related transcripts, including increased GABARAPL1 expression following NMD inhibition.

At higher concentrations, 1a and 2a induced NRF2-associated stress responses, with increased expression of Slc7a11, Sqstm1/p62 and Hmox1. SQSTM1/p62 is of particular interest as a selective autophagy receptor linking autophagy and oxidative stress signaling. In contrast, at lower concentrations, NMD inhibition was maintained without activation of the p62/NRF2 axis, revealing a pharmacological window in which NMD inhibition can be dissociated from broader stress responses.

Together, these results identify NMD as a post-transcriptional regulator of the autophagy-related gene GABARAPL1 and highlight a previously underexplored connection between RNA surveillance, autophagy and cellular stress responses.



When muscle meets the lysosome: Expanding the spectrum of lysosome-driven diseases

Abbass Jaber¹, Elise Lachiver¹, Isabelle Richard¹, **David Israeli**¹

¹ Progressive Muscular Dystrophy unit Genethon, INSERM UMR-S951 Evry University, Paris-Saclay University

Duchenne muscular dystrophy (DMD) is an X-linked muscle disorder caused by loss of dystrophin, a protein essential for sarcolemmal integrity. Dystrophin stabilizes myofibers by linking the cytoskeleton to the extracellular matrix through the dystrophin-associated glycoprotein complex (DGC). While DMD pathophysiology has historically been framed in structural and mechanical terms, earlier reports suggested lysosomal perturbations, although the underlying mechanisms remained unclear.

In 2021, we identified alterations in cholesterol metabolism in dystrophic muscle, which, by analogy to neurodegenerative diseases, prompted us to hypothesize that lysosomal dysfunction contributes to DMD pathogenesis. Using the lysosomal damage marker Galectin-3 (Gal3), we identified focal puncta indicative of lysosomal membrane damage in dystrophic muscles from DMD patients and animal models. Notably, similar lysosomal alterations were observed across multiple forms of muscular dystrophy.

We next assessed whether gene therapy could reverse lysosomal damage. Therapeutic-level expression of micro-dystrophin only partially reduced Gal3 puncta, whereas full-length gene replacement in LGMDR5 using wild-type γ -sarcoglycan resulted in near-complete correction. These findings suggest that truncated micro-dystrophin lacks essential domains required for the complete restoration of lysosomal integrity.

To pharmacologically target lysosomal dysfunction, we identified trehalose, a clinically established disaccharide with lysosome-enhancing properties. Trehalose treatment reduced lysosomal damage and myofiber necrosis and showed synergistic effects when combined with micro-dystrophin gene therapy. Hyperactivation of TFEB signaling further implicated lysosomal stress in disease progression.

Collectively, our data demonstrate that lysosomal damage is an intrinsic, early, persistent, and conserved feature of Duchenne muscular dystrophy and identify lysosomal restoration as a promising therapeutic target. Importantly, these findings reveal a previously underappreciated pathogenic mechanism that extends beyond the canonical structural role of dystrophin deficiency.

Our findings support the concept that muscular dystrophies, in addition to lysosomal storage disorders and neurodegenerative diseases, constitute a broader class of disorders characterized by lysosomal dysfunction, thereby expanding the spectrum of pathologies in which impaired lysosomal homeostasis is a central pathogenic driver



Flash talk session 1

Tuesday, October 13th, 2026 • 10:45 – 11:00



FT1 Hala Shalhoub (CRCT, Toulouse)

Selective targeting of VPS34-driven autophagy deregulation by p62 degrading PROTACs is a promising therapeutic strategy in pancreatic cancer



FT2 Tiffany Darbas (UMR 1308 CAPTuR, Limoges)

Autophagy and extracellular vesicle secretion in glioblastoma: a heterogeneous primary culture response to temozolomide and chloroquine

FT3 Fatima Domenica Elisa De Palma (University of Naples Federico II, Naples)

Bempedoic acid induces autophagy and improves liver cancer management



FT4 Thibaut Flipo (Institut Cochin, Paris)

Protein ATG8ylation : Development of a strategy to investigate a novel post-translational modification during viral infections



FT5 Loïs Guillemard (UMR PAM, Dijon)

Decoding Candida albicans-Autophagy interactions at the plasma membrane during invasion

Flash talk session 2

Tuesday, October 13th, 2026 • 15:45 – 16:00



FT6 Juliette Henna (UMR 1231, Dijon)

Autophagy proteins regulate HSP110 release from immune cells



FT7 Claudia Serot (I2BC, Gif-sur-Yvette)

An annexin involved in adaptation to Heat Stress in Caenorhabditis elegans



FT8 Evalena Thore (Institut Brain, Caen)

In vivo assessment of autophagic flux using Thy1-RFP-GFP-LC3 mice following ischemic stroke



FT9 Jules Blanc (CIRI, Lyon)

Exploring the functions of selective autophagy receptors at the virus / host interface



FT10 Lucie Mazzola (INEM, Paris)

Chronic TLR9 signaling drives PLD3-dependent lysosomal lipid remodeling and dysfunction in Alzheimer's Disease Microglia



Selective targeting of VPS34-driven autophagy deregulation by p62 degrading PROTACs is a promising therapeutic strategy in pancreatic cancer

Hala Shalhoub¹, Selin Bilge¹, Romina D'Angelo¹, Chahiniz Chouib², Corentin Bouvier², Angelina Acier², Manuel S. Rodriguez², Julie Guillermet-Guibert¹

¹ Centre de Recherches en Cancérologie de Toulouse (CRCT), Inserm U1037, CNRS, Université Toulouse III, Toulouse, France

² Laboratoire de Chimie de Coordination (LCC), Université de Toulouse, Toulouse, France

Autophagy has both a tumour suppressor and promoting role. The class III PI3K VPS34 controls several steps of autophagy process, possibly specifically involved in selective autophagy during cancerogenesis. In our laboratory, we demonstrated that the partial inactivation of the catalytic activity of VPS34 in a KRAS-driven mouse model accelerates pancreatic cancer (PDAC) development. We questioned whether the selective VPS34-driven autophagy could lead to specific vulnerabilities that could be targeted by novel therapeutic degrading agents.

To study this, we designed and optimized a unique protocol to analyse autophagosomal cargo in pancreatic cancer cell cultures from genetically engineered mutated KRAS-driven pancreatic tumours in which VPS34 catalytic activity was or not partially inactivated (PANC128 vs. A338 cells) using LC3 interacting region (LIR)-based molecular traps. Pulldowns of conjugated autophagosomes were analysed by LC-MS/MS.

In both cell cultures, we identified over 1000 proteins at measurable levels in LC3/GABARAP-dependent autophagosome cargos that were statistically enriched in proteins involved in cell-cell interaction/dialog molecules, mitochondrial and translational machinery. VPS34 activity deregulation led to statistically differential levels of 21 proteins that belonged to apical junction, epithelial-mesenchymal transition and apoptosis processes, such as GPX1. Associated with these changes in LC3/GABARAP cargos, PANC128 cells displayed increased levels of p62/SQSTM1 compared with A338 cells, consistent with reduced autophagic flux caused by partial VPS34 inactivation. We next targeted p62 using two newly developed PROTACs (Bouvier C. et al). The two synthetic molecules induced the degradation of p62. Besides, p62-targeting PROTAC induced cell death in both A338 and PANC128 cells, with PANC128 showing greater sensitivity. The remaining tolerant cells displayed a change in morphology and in cell /cell interaction.

We here identified a strong translational potential of the p62 degrading PROTACs in pancreatic cancer, given that the autophagy receptor p62 is correlated with poor prognosis.



Autophagy and extracellular vesicle secretion in glioblastoma: a heterogeneous primary culture response to temozolomide and chloroquine

Tiffany Darbas¹, Gabrielle Perrolan¹, Julie Kurkowski¹, Amy Gateau¹, Léa Decoux¹, Amandine Rovini¹, Barbara Bessette¹, Fabrice Lalloue¹, Elise Deluche¹, Mireille Verdier-Sage¹

¹ UMR 1308 CAPTuR, Faculté de médecine, Institut Oméga-health

Introduction. With a median overall survival of approximately 2 years, glioblastoma remains the primary brain tumour with the poorest prognosis. Treatment mainly relies on surgery, radiotherapy, chemotherapy (temozolomide) and, more recently, tumour-treating fields. The role of autophagy in carcinogenesis remains debated, with unresolved evidence for either a pro- or anti-tumoural effect. Several clinical trials have evaluated chloroquine, with heterogeneous results and limited clinical benefit to date. In this pilot study, we aimed to characterise the tumour cell response to temozolomide and/or chloroquine, focusing on autophagy and on extracellular vesicle (EV) secretion, a recognised mechanism of intercellular communication and treatment resistance.

Methods. Three glioblastoma primary cultures were grown in defined medium to enrich for stem-like cells. Cell viability was assessed by luminescence (CellTiter-Glo® 3D). Chloroquine dose (10 μ M) was selected based on the literature. Necrosis and apoptosis were quantified by ELISA Cell Death. Autophagy markers expression (BECLIN 1, LC3B I/II, p62) and tetraspanins (CD63, CD9, CD81) were evaluated by western blot. Small EVs (<200 nm) were isolated by differential ultracentrifugation, with size distribution confirmed by nanoparticle tracking analysis (NTA), and protein content was determined using the PIERCE BCA assay. Statistical analyses were performed using GraphPad PRISM V9.0.

Results. The three primary cultures (P12, P19, P25) showed distinct IC50 values for temozolomide (450, 1500 and 1850 μ M, respectively). Temozolomide reduced autophagy markers (BECLIN 1, p62) only in P12. Chloroquine blocked autophagic flux in all three lines, consistent with its known mechanism of action. The temozolomide-chloroquine combination was associated with increased apoptosis only in P12, suggesting line-dependent sensitivity rather than a widespread effect. Small EV protein content also varied by line and condition: it increased under temozolomide in P12 and P25, but decreased under chloroquine in P12 and P19.

Conclusion. These preliminary findings, from a limited number of primary cultures, illustrate the heterogeneity of glioblastoma cell responses to autophagy-modulating stress. Whether autophagy blockade directly drives the observed changes in apoptosis and small EV secretion remains to be established, ideally through genetic autophagy inhibition.



Bempedoic acid induces autophagy and improves liver cancer management

Fatima Domenica Elisa De Palma^{1,2}, Sarah Adriana Scuderi², Angelica Di Matteo², Maria Perez-Lanzon², Guido Kroemer², Maria Chiara Maiuri^{1,2}, Jonathan Pol²

¹ Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples (Italy)

² Metabolism, Cancer & Immunity, Centre de Recherche des Cordeliers U1138, Inserm, Université Paris Cité, Sorbonne Université, Paris

Hepatocellular carcinoma (HCC) is the third leading cause of global cancer-related deaths. Current advanced HCC treatments mainly rely on combinations of immunotherapies and anti-angiogenic agents, or targeted therapies. Their efficacy is limited, necessitating innovative strategies.

Recent preclinical studies indicate that boosting tumor immunogenicity via autophagy induction can enhance chemo-immunotherapeutic effectiveness. This can be achieved by preconditioning tumor-bearing hosts with caloric restriction (CR) or CR-mimicking compounds (CRM). However, no study has evaluated the interest of such combinations in HCC.

Bempedoic acid (BA), a newly approved drug for hypercholesterolemia, inhibits ATP citrate lyase (ACLY) in the liver, a key enzyme participating in lipid synthesis, which is a mechanism of action shared with some CRMs. However, BA role in sensitizing immunochemotherapy as a CRM in HCC has never been investigated.

To address this issue, we studied whether BA induces autophagy and could represent a novel CRM. Then, we explored the effectiveness of BA in enhancing immunochemotherapeutic strategies for HCC in vivo.

Here, we identified BA as a non-toxic inducer of autophagic flux that acts on human and mouse tumor cells in vitro, as well as in murine tissues. Finally, the administration of BA to HCC tumor-bearing mice improved the efficacy of immunogenic anticancer chemotherapy.

In summary, our research reveal that autophagy-inducing interventions could improve liver cancer management.



FT4 (P11) Autophagy-independent functions of ATG proteins

Flash Talk

Protein ATG8ylation : Development of a strategy to investigate a novel post-translational modification during viral infections

Thibaut Flipo¹, Margaux Versapuech¹, Lucas Lasnel¹, Marjory Palaric¹, Clarisse Berlioz-Torrent¹

¹ Institut Cochin, Université de Paris, INSERM, CNRS

ATG8ylation was initially described as the process leading to the covalent conjugation of ATG8 family proteins to lipids on autophagosomal membranes. This modification is essential for autophagosome biogenesis, selective cargo recruitment, and autophagosome-lysosome fusion during the autophagy process. More recently, several studies have shown that ATG8 family proteins (LC3A-C and GABARAP/L1/L2), which belong to the ubiquitin-like protein family, can also be covalently conjugated to cellular proteins, thereby revealing a new post-translational modification called protein ATG8ylation. To date, the mechanisms controlling this process, the nature of its substrates, and its physiological role remain largely unknown, particularly in the context of viral infections.

Recent work from our laboratory has shown that proteins of the GABARAP subfamily plays an essential role in the encapsidation of the HIV-1 genome, independently of canonical autophagy. These observations suggest that ATG8 proteins could perform new functions during the viral cycle and led us to hypothesize that the protein ATG8ylation contributes to the remodeling of cellular functions induced by the infection.

In this project, we developed an experimental strategy to stabilize and enrich the ATG8ylated proteins. This is based on the expression of different mutated forms of LC3B and GABARAPL1, combined with the use of cells deficient in ATG4 proteases to limit substrate deconjugation. ATG8 proteins immunoprecipitation followed by biochemical and proteomic analyses using mass spectrometry, made it possible to enrich the ATG8ylated proteins and compare ATG8ylation profiles obtained in different stress conditions, particularly during an infection by HIV-1.

Ultimately, this approach will be used to map ATG8ylated proteins during viral infections, evaluate the specificity of this process within the LC3 and GABARAP subfamilies, and identify new cellular effectors potentially involved in viral replication. This work should provide the first comprehensive information on the dynamics of protein ATG8ylation during viral infection and open up new perspectives on the non-canonical functions of ATG8 proteins.

This project is supported by the ANRS.



Decoding *Candida albicans*-Autophagy interactions at the plasma membrane during invasion

Loïs Guillemard¹, Amandine Ducreux¹, Lisa Degache¹, Bernhard Hube², Christophe D'enfert³, Sophie Bachelier-Bassi³, Elisabeth Werkmeister⁴, Sébastien Janel⁴, Frank Lafont⁴, Pierre Lapaquette^{1,*}, Frédéric Dalle^{1,*}

¹ Université Bourgogne Europe, L'Institut Agro, INRAE, UMR PAM 21000, Dijon, France

² Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute, Jena – Allemagne

³ Institut Pasteur, USC2019 INRAE, Paris, France

⁴ Institut Pasteur de Lille, F59000 Lille, France

* Equal contribution to last authorship

Invasive infections caused by *Candida albicans* represent a major public health concern, particularly in immunocompromised patients, due to their high mortality rate and the persistent gaps in understanding the mechanisms underlying host invasion. Translocation of *C. albicans* across the intestinal epithelial barrier is a critical step in systemic dissemination and begins with the invasion of host epithelial cells. In this context, autophagy, traditionally known for its role in lysosomal degradation, is increasingly recognized as a key regulator of membrane dynamics, participating in cellular defense mechanisms against pathogen invasion.

This thesis project aims to decipher the interactions between *C. albicans* and the autophagy machinery at the plasma membrane during epithelial cell invasion. Published data of our group demonstrate a rapid recruitment of autophagy-related proteins (ATG5, ATG16L1, LC3) at fungal entry sites, suggesting a protective role in maintaining plasma membrane integrity.

We hypothesize that these proteins actively contribute to the repair of membrane damage induced by hyphal growth and thereby limit fungal invasion.

To address this hypothesis, the project is structured around three main objectives: (i) to characterize the kinetics of autophagy machinery recruitment and to identify the fungal determinants involved in modulating it; (ii) to define the key molecular players involved in this response using both targeted approaches and proximity-based proteomics; and (iii) to evaluate how environmental factors (metabolic stress, pollutants, and co-infections) modulate this cytoprotective response.

To identify the fungal factors involved in the recruitment of autophagy-related molecules, we analyzed this process using a series of *Candida albicans* mutants. These mutants exhibit either defects in hyphal formation or elongation—processes responsible for the deformation of the epithelial cell plasma membrane—or alterations in the secretion of virulence factors, particularly those involved in epithelial damage. The initial results obtained will be presented.

Overall, this work will provide fundamental insights into non-canonical functions of autophagy and may open new therapeutic perspectives aimed at enhancing host resistance to fungal infections.



Autophagy proteins regulate HSP110 release from immune cells

Juliette Henna¹, Abdelmnim Radoua^{1,2}, Imene Boukriba¹, Mathis Rabut¹, Carmen Garrido¹, Gaëtan Jégou¹, Elise Jacquin¹

¹ Université Bourgogne Europe, INSERM, Centre de Recherche Translationnelle en Médecine Moléculaire (CTM), UMR 1231, équipe HSP-Pathies, 21000 Dijon, France

² INSERM, UMRS1138, CRC Métabolisme Cancer et Immunité, 75006 Paris, France

Autophagy-related pathways are increasingly recognized as key regulators of unconventional protein release. Beyond their canonical degradative functions, autophagy proteins can contribute to vesicular trafficking and immunomodulatory protein release through autophagosome formation or unconventional functions such as Conjugation of Atg8 to Single Membranes (CASM). The role of these pathways in the extracellular release of heat shock proteins (HSPs) remains unknown.

HSP110 is a stress-induced molecular chaperone frequently overexpressed in cancers and associated with tumor progression. Beyond its intracellular functions, HSP110 can also be released into the extracellular space, where it has been reported to modulate immune responses and contribute to immunosuppression. However, the mechanisms underlying its release remain largely unknown.

To address this question, we generated immune and colorectal cancer cell lines incompetent for canonical autophagy (ATG13 knockout) or CASM (ATG16L1 K490A mutation). We found that, upon inflammasome activation, dendritic cells release HSP110 but not its cochaperone HSP70, revealing an unexpected contribution of this protein in immune responses. Notably, HSP110 release was significantly reduced in both ATG13-deficient and ATG16L1 mutant dendritic cells compared to wild-type controls, suggesting that both canonical autophagy and CASM contribute to its extracellular release. In parallel, the release of the pro-inflammatory cytokine IL-1 β , a well-characterized cargo of unconventional release pathways, was also altered in autophagy and CASM-deficient cells. This supports a broader role for autophagy-related mechanisms in the regulation of immunomodulatory protein release. In contrast, disruption of canonical autophagy or CASM in colorectal cancer cells did not affect HSP110 release, suggesting the existence of an immune-cell-specific mechanism of release.

Together, our findings identify a role for autophagy proteins in the unconventional release of HSP110 by immune cells, and highlight these pathways as potential targets to modulate immune responses in cancer.



FT7 (P27)

Molecular regulation of autophagy – Session 2

Flash Talk

An annexin involved in adaptation to Heat Stress in *Caenorhabditis elegans*

Claudia Serot^{1,2,4}, Melina Gallopin³, Guillaume Sgard^{1,4}, Céline Largeau¹, Mickael Briens⁴, Alain Lescure⁵, Renaud Legouis^{1,2}

¹ Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

² Inserm

³ Université Paris-Sud

⁴ Adisseo France SAS

⁵ Institut de biologie moléculaire et cellulaire

When temperatures exceed the optimal range for an organism or a cell, heat stress (HS) triggers several processes that contribute to the maintenance of homeostasis. Adaptation involves evolutionarily conserved regulatory mechanisms acting at both the organismal and cellular levels, including the heat stress response and autophagy, which contribute to the maintenance of proteostasis. However, adaptation and resilience can vary substantially between individuals.

We have previously shown that *C. elegans* is a relevant model for investigating this inter-individual variability. This project aims to better characterize the mechanisms underlying resistance and adaptation to heat stress in *C. elegans*.

First, using a descriptive approach based on Gaussian mixture models, we identified that the variability can be modeled into 2 subpopulations after HS, that differ in developmental recovery, fertility, longevity, and hormetic response.

Then, we investigated the contribution of differentially regulated genes to the stress response, using RNAi and deletion mutants. By combining linear mixed-effects models with mixture models, we characterized how gene depletion affects each subpopulation. Interestingly, this approach identified annexin genes as potential contributors to adaptation to HS, particularly NEX-2, whose depletion is associated with an impairment of autophagic flux.



In vivo assessment of autophagic flux using Thy1-RFP-GFP-LC3 mice following ischemic stroke

Evalena Thore¹, Pauline Marie¹, Denis Vivien^{1,2}, Eloise Lemarchand¹, Benoit D. Roussel¹

¹ Normandie University, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders (PhIND), GIP Cyceron, Institut Brain, Blood & Memory @ Caen-Normandie (BBM@C), France

² CHU Caen, Clinical Research Department, CHU de Caen Côte de Nacre, Caen, France

Stroke is one of the leading causes of death and long-term disability worldwide. It comprises two major subtypes: hemorrhagic and ischemic, with ischemic strokes accounting for approximately 85% of all cases. Ischemic stroke results from the occlusion of a cerebral artery, leading to a reduction or complete interruption of cerebral blood flow. To date, intravenous recombinant tissue-type plasminogen activator (r-tPA) remains the only approved pharmacological treatment for the acute treatment of ischemic stroke. Ischemic stroke induces profound cellular stress disrupting neuronal homeostasis and triggering autophagy, a process that can either promote cell survival or contribute to neuronal death depending on the context. We previously demonstrated that neuronal exposure to tPA inhibits the autophagy through activation of the PI3K/AKT/mTOR pathway, thereby promoting neuroprotection.

In this study, we investigated the effect of ischemic stroke and tPA mediated thrombolysis on neuronal autophagy in vivo using transgenic mice expressing the tandem fluorescent autophagy reporter mRFP-eGFP-LC3 protein, under the neuron-specific Thy1 promoter. This model, enables real time visualization and quantification of neuronal autophagic flux by distinguishing autophagosomes from intermediate and fully acidified autolysosomes following thromboembolic stroke. High resolution confocal microscopy combined with three-dimensional image reconstruction and analysis allowed us to characterize neuronal autophagic flux at 4 and 24 hours after ischemia. This approach provides a spatial and temporal analysis of autophagy at a single-neuron resolution by discriminating autophagosomes, partially acidified autolysosomes and fully acidified autolysosomes. Our preliminary results show that ischemic stroke dynamically remodels neuronal autophagic flux over time. Ongoing analysis will determine how r-tPA-mediated thrombolysis influence these autophagic responses after stroke. Unlike conventional approaches based on static markers such as LC3 or p62, our strategy tracks the successive stages of autophagosome formation and maturation in individual neurons, thus providing a dynamic assessment of the autophagic flux in vivo. A better understanding of neuronal autophagy following ischemic stroke may uncover novel therapeutic targets and supports the development of innovative neuroprotective strategies.



FT9 (P28)

Selective autophagy
Flash Talk

Exploring the functions of selective autophagy receptors at the virus / host interface

Jules Blanc¹, Hanane El Manssouri¹, Chloé Fournié¹, Laura Dejardin¹, Samir Merabet², Arnaud Moris³, Audrey Esclatine³, Marie Galloux⁴, Chloé Journo¹

- ¹ Centre International de Recherche en Infectiologie (CIRI), Retroviral Oncogenesis, Inserm U1111-Université Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, Université Lyon, Lyon, France
- ² Institut de génomique fonctionnelle de Lyon (IGFL), Ontogenesis and Molecular Interactions, CNRS, UMR5242, Université Claude Bernard Lyon 1, Ecole normale Supérieure de Lyon, Université Lyon, Lyon France
- ³ Institut de Biologie Intégrative de la Cellule (I2BC), Autophagy and antiviral immunity, CEA, CNRS, UMR 9198, Université Paris-Saclay, Gif-sur-Yvette, France
- ⁴ Unité de Virologie et Immunologie Moléculaires (VIM), Molecular biology of Pneumovirus, INRAE, UVSQ, UMR 0892, Université Paris-Saclay, Jouy-en-Josas, France

Selective autophagy receptors (SARs) stand at the crossroad of cell-intrinsic antiviral immune processes: while they can directly address specific viral components to autophagic degradation, they are also modulators of innate signalling pathways, through both autophagy-dependent and -independent mechanisms that are still to be explored.

In this ongoing project, we posit that the central position of SARs in anti-viral processes may have selected them upon evolution to be commonly hijacked by various viruses, and that, in turn, investigating how various viruses manipulate SARs could help us broaden our understanding of SARs' functions. More specifically, this project explores how Epstein-Barr Virus (EBV)-encoded BHRF1, Respiratory Syncytial Virus (RSV)-encoded nucleoprotein, Human T-cell Leukemia Virus type 1 (HTLV1)-encoded Tax proteins, all three proteins previously linked to SARs' functions, shape the signalling landscape of SARs.

Using co-immunoprecipitation and bimolecular fluorescent complementation assays (BiFC), we first showed that these three viral proteins are physically interacting with all tested SARs (p62, OPTN, TAX1BP1, NDP52 and NBR1). We are currently aiming at identifying the cell compartments or organelles in which these interactions take place, and how the organisation of these compartments could be changed upon interaction. In parallel, we have set up an *in situ* proximity-labelling interactomics approach to determine how these viral proteins are changing the SARs' interactomes, leading to possible viral manipulation of SARs' functions.

Collectively, this ongoing project contributes to the exploration of SARs' immune functions and could help establish them as key factors at the host / pathogen interface.



FT10 (P20)

Molecular regulation of autophagy – Session 2

Flash Talk

Chronic TLR9 signaling drives PLD3-dependent lysosomal lipid remodeling and dysfunction in Alzheimer's Disease Microglia

Lucie Mazzola¹, Moise de Lavaissière de Lavergne¹, Bénédicte Manoury¹

¹ Institut Necker Enfants Malades, INSERM U1151-CNRS UMR 8253, Université Paris Cité, Faculté de Médecine Necker, Paris, France

TLR9 is a lysosomal innate immune receptor that senses double-stranded DNA (dsDNA). Its acute activation has been linked to Alzheimer's disease (AD) pathology, though the underlying mechanism has remained unclear.

In the APP23 mouse model of AD, we show that dsDNA accumulates near amyloid plaques together with phospholipase D3 (PLD3), a nuclease that cleaves nucleic acids, and reactive microglia, stimulating TLR9, which drives microglia toward a pro-inflammatory state and causes lysosomal damage. These effects are absent in APP23-TLR9-deficient (A9KO) mice, which are protected from cognitive decline, neuronal loss, and amyloid- β pathology.

Mechanistically, TLR9 signaling upregulates PLD3, which generates the lysosomal lipid bis(monoacyl)glycerophosphate (BMP). Elevated PLD3 and BMP levels correlate with impaired lysosomal fitness in APP23 microglia and are both reduced in A9KO mice. In human HMC3 microglia, chronic but not acute TLR9 stimulation increases PLD3, drives BMP accumulation, and triggers lysosomal membrane permeabilization, TFEB nuclear translocation, and alters lysosome number and size. TLR9 inhibition reverses PLD3 upregulation.

Together, these findings establish PLD3 as a key effector linking chronic TLR9-dsDNA sensing to lysosomal lipid remodeling and dysfunction, ultimately reducing amyloid- β clearance, a hallmark of AD.



POSTERS – SESSION 1



P1 Claudia Alarcón Torrecillas

Macrophage phagocytic functions are regulated by O-GlcNAcylation, a post-translational modification

P2 Naïma Belgareh-Touzé

ERMES's ubiquitination and its unrevealed role in mitophagy

P3 Hala Shalhoub (FT1)

Selective targeting of VPS34-driven autophagy deregulation by p62 degrading PROTACs is a promising therapeutic strategy in pancreatic cancer



P4 Vincent Boni

Autophagy-related-vesicles at the immune synapse: An expansion microscopy study



P5 Asma Boukhalfa

A cardiac formin-profilin-ATG16 complex is required for PI3P-dependent autophagosome initiation and extracellular vesicle release



P6 Guillaume Camus

Virulence state of Bordetella pertussis induces distinct intracellular degradation mechanisms in alveolar macrophages

P7 Sabine Chapuy-Regaud

The capsid protein of the hepatitis E virus traffics in LC3B-I and EDEM1-positive compartments

P8 Tiffany Darbas (FT2)

Autophagy and extracellular vesicle secretion in glioblastoma: a heterogeneous primary culture response to temozolomide and chloroquine

P9 Fatima Domenica Elisa De Palma (FT3)

Bempedoic acid induces autophagy and improves liver cancer management



P10 Samy Dehissi

Regulation of anti-tumour immune responses by lysosomal stress

P11 Thibaut Flipo (FT4)

Protein ATG8ylation : Development of a strategy to investigate a novel post-translational modification during viral infections



P12 Jean-Charles Fouillard--Uzel

Isolation of autophagic vesicles from Thy1-RFP-GFP-LC3 mice in stroke

P13 Loïs Guillemard (FT5)

Decoding Candida albicans-Autophagy interactions at the plasma membrane during invasion



P14 Morgane Louessard

Autophagy and endothelial activation in human iPSC-derived brain endothelial cells under inflammatory and ischemia-like stresses



P15 Dejun Kong

Deciphering the non-autophagy functions of LC3/GABARAP during C. elegans development



P16 Rajni Kumari

Understanding the role of Rer1 in regulating autophagy and UPR for cell survival

P17 Pierre Lapaquette

From proof of concept to large-scale screening of autophagy-stimulating food-grade ferments



P18 Céline Largeau

Caracterization of Heat Stress in Caenorhabditis elegans using Focused Ion Beam Scanning Electron Microscopy (FIB-SEM)



POSTERS – SESSION 2

P19 Juliette Henna (FT6)

Autophagy proteins regulate HSP110 release from immune cells

P20 Lucie Mazzola (FT10)

Chronic TLR9 signaling drives PLD3-dependent lysosomal lipid remodeling and dysfunction in Alzheimer's Disease Microglia



P21 Valentin Meire

Functional characterization of autophagosome subpopulations by in vivo single-event analysis



P22 Laura Merlet

A centriolar satellite-microtubule axis coordinates endolysosomal homeostasis in glioblastoma stem-like cells

P23 Hang Nguyen

Physical activity activates intestinal autophagy and counteracts the deleterious effects of high-fat diet and adherent-invasive E. coli

P24 Hang Nguyen

Western diet-induced autophagy dysfunction drives disrupted intestinal homeostasis, intestinal inflammation and AIEC expansion



P25 Anna Schläfli

Oncogenic DMTF1 β promotes cancer cell motility by regulating autophagy

P26 Iban Seiliez

Loss of chaperone-mediated autophagy uncouples hepatic and systemic glucose metabolism in rainbow trout

P27 Claudia Serot (FT7)

An annexin involved in adaptation to Heat Stress in Caenorhabditis elegans

P28 Jules Blanc (FT9)

Exploring the functions of selective autophagy receptors at the virus / host interface



P29 Maxime Sorro

Impact of increased basal autophagy on age-related decline in skeletal muscle regenerative capacity



P30 Léa Tantet

Dissecting MHC-II-restricted endogenous antigen presentation using viral proteins: the influence of HTLV-1 Tax



P31 Audrey Thiebaut

A novel in vitro model of intracerebral hemorrhage reveals autophagy-dependent neuronal death and a time-dependent neuroprotective role of tPA

P32 Evalena Thore (FT8)

In vivo assessment of autophagic flux using Thy1-RFP-GFP-LC3 mice following ischemic stroke



P33 Diana Tomsa

Exploring mitophagy regulation under distinct metabolism conditions



P34 Yetong Wei

Impact of dietary fatty acids on retinal autophagy: focus on saturated fatty acid intake in a low-fat diet



P1

ATGs in Immunity and infection

Poster



Macrophage phagocytic functions are regulated by O-GlcNAcylation, a post-translational modification

Claudia Alarcón Torrecillas¹, Stéphanie Carvalho¹, Tarik Issad¹, Florence Niedergang¹

¹ Université Paris Cité, CNRS, Inserm, Institut Cochin, Paris 75014, France

O-GlcNAcylation is a reversible post-translational modification that regulates the activity of intracellular proteins according to glucose availability and its metabolism into the hexosamine biosynthetic pathway. This modification is involved in the regulation of several innate immune cell functions by modulating transcription factor activity, signaling pathways, and protein-protein interactions.

Our previous results showed that O-GlcNAcylation have a protective effect against excessive inflammation in macrophages. More specifically, macrophages are specialized phagocytic cells of the immune system that engulf harmful microorganisms and destroy them in phagosomes. The destruction process depends mainly on the production of large amounts of reactive oxygen species (ROS). Several studies indicate that O-GlcNAcylation plays an important role in regulating the expression and activity of proteins involved in the oxidative stress response. However, the relationship between mitochondrial O-GlcNAcylation and ROS production upon macrophage activation have never been explored.

The aim of this study is to evaluate the consequences of manipulating O-GlcNAcylation on macrophage phagocytic functions. This study was performed on murine and human primary culture of macrophages. We used pharmacological inhibitors of O-GlcNAcylation in human macrophages in order to manipulate the levels of O-GlcNAcylation, and a murine model knockout for the enzyme that O-GlcNAcyates proteins in order to reduce the O-GlcNAcylation levels. We performed phagocytosis assays with different target particles as opsonized particles, zymosan and bacteria to explore the involvement of this modification in this macrophage function. In conclusion, our study indicates that the levels of O-GlcNAcylation can regulate the macrophages phagocytic functions.

1. M. Depierre, Jacquelin, L., Niedergang, F., *Phagocytosis. Encyclopedia of Cell Biology (Second Edition)*, Academic Press, Editor(s): R.A. Bradshaw, G.W. Hart, P. D. Stahl, Pages 286-295, (2023).

2. E. Naik et al., *Mitochondrial reactive oxygen species drive proinflammatory cytokine production. J Exp Med* 208, 417-420 (2011).

3. E. Shekhova, *Mitochondrial reactive oxygen species as major effectors of antimicrobial immunity. PLoS Pathog* 16, e1008470 (2020).

4. K. M. M. Fahie et al., *Integration of OGlcNAc into Stress Response Pathways. Cells* 11 (2022).

ERMES's ubiquitination and its unrevealed role in mitophagy

Oznur Ozturk¹, Diana Tomsa², Laetitia Cavellini¹, Manuel Rodroquez², Mickaël M. Cohen¹, **Naïma Belgareh-Touzé¹**

¹ CNRS/SU, UMR8226, Institut de Biologie Physico-Chimique, 75005, Paris

² Laboratoire de Chimie de Coordination (LCC) - CNRS, UPS, 31400, Toulouse

MERCS (Mitochondria ER-Contact sites) are involved in essential functions such as lipid transfer, calcium homeostasis or mitophagy. At MERCS the mitochondrial outer membrane (MOM) and the endoplasmic reticulum (ER) are physically connected by protein complexes called “tethers”. In the yeast *S. cerevisiae*, the ERMES (ER-Mitochondria Encounter Structure) complex is one of these tethers.

ERMES is composed of the ER integral membrane protein Mmm1, the two integral MOM proteins Mdm34 and Mdm10, and the soluble protein Mdm12. This complex has been shown to be essential for many functions, others than lipid transfer. The deletion of ERMES genes thus leads to dissociation of the ER from mitochondria, as well as defects in mitochondrial morphology, mitochondrial import, growth on respiratory media, mitophagy etc.

Mitophagy, the selective autophagy of mitochondria, is necessary for the degradation of damaged or excess mitochondria. In mammalian cells, two pathways mediate mitophagy. The first involves the ubiquitination of MOM proteins ; these ubiquitinated proteins are recognized by soluble adapters, which subsequently bind Atg8/LC3 on the autophagosomal membrane. The second pathway relies on mitophagy receptors embedded in the MOM, which are directly recognized by Atg8/LC3 upon mitophagy induction.

In yeast, only one pathway has been described to date. This pathway requires the MOM receptor Atg32. Following mitophagy induction, a phosphorylation cascade triggers the interaction of Atg32 with Atg8/LC3 on the autophagosomal membrane, leading to mitochondrial engulfment by autophagosomes. While ERMES has been implicated in mitophagy, its exact biochemical role in this process remains unclear. Mitophagy defects have been observed in ERMES-deleted strains but also in ERMES mutants that can no longer undergo ubiquitination, highlighting a functional role for ERMES ubiquitination during mitophagy.

Indeed, Mdm34 and Mdm12 have been shown to be ubiquitinated by the E3 ligase Rsp5. Under mitophagy-inducing conditions, a non-ubiquitinatable mutant version of MDM34 (*mdm34[AY]*) exhibits a detectable and specific mitophagy defect. However, how Mdm34 and Mdm12 ubiquitination triggers mitophagy remains completely elusive.

In order to address this question, we decided to dissect the impact of ERMES and its ubiquitination on mitophagy by identifying the binding partners of Mdm34 and its mutant form. We present here the technical approach undertaken and the first results obtained.



Selective targeting of VPS34-driven autophagy deregulation by p62 degrading PROTACs is a promising therapeutic strategy in pancreatic cancer

Hala Shalhoub¹, Selin Bilge¹, Romina D'Angelo¹, Chahin Chouib², Corentin Bouvier², Angelina Acier², Manuel S. Rodriguez², Julie Guillermet-Guibert¹

¹ Centre de Recherches en Cancérologie de Toulouse (CRCT), Inserm U1037, CNRS, Université Toulouse III, Toulouse, France

² Laboratoire de Chimie de Coordination (LCC), Université de Toulouse, Toulouse, France

Autophagy has both a tumour suppressor and promoting role. The class III PI3K VPS34 controls several steps of autophagy process, possibly specifically involved in selective autophagy during cancerogenesis. In our laboratory, we demonstrated that the partial inactivation of the catalytic activity of VPS34 in a KRAS-driven mouse model accelerates pancreatic cancer (PDAC) development. We questioned whether the selective VPS34-driven autophagy could lead to specific vulnerabilities that could be targeted by novel therapeutic degrading agents.

To study this, we designed and optimized a unique protocol to analyse autophagosomal cargo in pancreatic cancer cell cultures from genetically engineered mutated KRAS-driven pancreatic tumours in which VPS34 catalytic activity was or not partially inactivated (PANC128 vs. A338 cells) using LC3 interacting region (LIR)-based molecular traps. Pulldowns of conjugated autophagosomes were analysed by LC-MS/MS.

In both cell cultures, we identified over 1000 proteins at measurable levels in LC3/GABARAP-dependent autophagosome cargos that were statistically enriched in proteins involved in cell-cell interaction/dialog molecules, mitochondrial and translational machinery. VPS34 activity deregulation led to statistically differential levels of 21 proteins that belonged to apical junction, epithelial-mesenchymal transition and apoptosis processes, such as GPX1. Associated with these changes in LC3/GABARAP cargos, PANC128 cells displayed increased levels of p62/SQSTM1 compared with A338 cells, consistent with reduced autophagic flux caused by partial VPS34 inactivation. We next targeted p62 using two newly developed PROTACs (Bouvier C. et al). The two synthetic molecules induced the degradation of p62. Besides, p62-targeting PROTAC induced cell death in both A338 and PANC128 cells, with PANC128 showing greater sensitivity. The remaining tolerant cells displayed a change in morphology and in cell /cell interaction.

We here identified a strong translational potential of the p62 degrading PROTACs in pancreatic cancer, given that the autophagy receptor p62 is correlated with poor prognosis.



P4

Autophagy-independent functions of ATG proteins

Poster

Autophagy-related-vesicles at the immune synapse: An expansion microscopy study

Vincent Boni¹, Julie Lesieur¹, Clarisse Berlioz-Torrent¹, Delphine Judith¹

¹ Université de Paris, Institut Cochin, INSERM, CNRS, laboratoire « Interactions Hôte-Virus », F-75014 PARIS, France

The immune synapse (IS) is a highly organized interface formed between T cells and antigen-presenting cells, which supports T cell activation and the initiation of immune responses. Like neuronal synapses, it relies on a precise spatial organization of receptors, adhesion molecules, and cytoskeletal networks to enable efficient intercellular communication. While the roles of endocytic and secretory pathways in IS membrane remodeling are well established, the contribution of autophagy-related vesicles to this architecture and nanoscale organization of this interface remains largely unexplored.

The molecular architecture of the immune synapse remains challenging to resolve with conventional fluorescence microscopy, as it lacks the nanoscale resolution required to accurately define the organization and the positioning of closely apposed components.

Expansion microscopy (ExM) overcomes some of these limitations by physically enlarging biological samples, enabling nanoscale visualization with standard fluorescence microscopy. By separating fluorescent signals, ExM makes closely spaced structures easier to distinguish, even when they are below the resolution limit of conventional light microscopy. This allows fine details to be visualized with a standard fluorescence or confocal microscope, reaching effective resolutions of a few tens of nanometers. Compared with high-resolution methods such as STED or PALM/dSTORM, ExM uses a simpler sample preparation workflow and is compatible with standard immunostaining and multicolor imaging.

In this project, we combine ExM with CRISPR-Cas9 technology to define the spatial organization and functional contribution of autophagy-related vesicles at the IS. Beyond elucidating how these vesicles shape the immune synapse architecture, this work will establish ExM as a powerful tool for investigating immune interfaces at nanoscale resolution, thereby extending its application beyond conventional super-resolution microscopy.

This work is supported by ANR and ANRS



P5

Molecular regulation of autophagy
Poster



A cardiac formin-profilin-ATG16 complex is required for PI3P-dependent autophagosome initiation and extracellular vesicle release

Asma Boukhalfa¹, Sarah N. Powers¹, Dawn M. Meola², Ada Yu¹, Greg Martin¹, Hushan Yuan³, Robert M. Blanton¹, Vicky K. Yang², Lakshmi Pulakat¹, Gordon S. Huggins¹, Howard H. Chen¹

¹ Molecular Cardiology Research Institute, Tufts Medical Center, Boston, MA, USA

² Cummings School of Veterinary Medicine, Tufts University, Grafton, MA, USA

³ Gordon Center for Medical Imaging, Massachusetts General Hospital, Boston, MA, USA

Introduction: Autophagosome biogenesis and extracellular vesicle (EV) formation are two fates of the endomembrane system, joined by the endolysosomal pathway, yet what sets the balance between them is unknown. Actin remodelling shapes both. Formins nucleate and elongate unbranched actin filaments, but their contribution to autophagy initiation is undefined. We used FHOD3-V1151I, a naturally occurring substitution in the formin homology domain that is tolerated in ~1 in 3 people, as a molecular tool to uncouple formin function from autophagy.

Methods: FHOD3-WT or the variant V1151I were expressed in H9C2 cells, and the homologous substitution was knocked into mice by CRISPR. Protein complexes were mapped by co-immunoprecipitation and Duolink proximity ligation, membranes by immunofluorescence, and respiration by Seahorse. Completed autophagic flux was read with the Autophagy-Detecting Nanoparticle (ADN), a ferumoxytol core whose dextran coat carries cathepsin-cleavable polyarginine-Cy5.5 peptides useful to track autophagic flux in vivo and in vitro. Phosphatidylinositol 3-phosphate (PI3P) was added back for rescue. EVs (50-150 nm) were purified by size-exclusion chromatography and characterized by dynamic light scattering, immunoblot and transmission electron microscopy. Multiplex immunoassay using NanoFCM we were able to measure same time: size, concentration and multiple fluorescent protein markers on single EVs.

Results: FHOD3 was found in a complex with profilin-4 and ATG16 confirmed by co-immunoprecipitation and Duolink; the complex was lost with V1151I in cells and in knock-in hearts. LC3 no longer associated with PI3P-positive membranes, phenocopying the initiation inhibitors 3-MA and spautin but not in chloroquine condition, and ADN activation was reduced in variant V1151I cells and hearts. Exogenous PI3P restored both the complex and autophagy flux. Blocked initiation had metabolic consequences: lipid droplets increased 5-fold, respiration and ATP production fell ($p < 0.0001$, $n = 7$). EV secretion decreased ($n = 4$), with reduced CD9 and ALIX and altered vesicle size and charge.

Conclusions: A formin-profilin-ATG16 module couples the actin machinery to PI3P-dependent autophagosome initiation with EV release reduced in the same cells and hearts, identifying a point of convergence between degradation and secretion that is under shared upstream control.



P6

ATGs in Immunity and infection

Poster



Virulence state of *Bordetella pertussis* induces distinct intracellular degradation mechanisms in alveolar macrophages

Guillaume Camus¹, Rachel Lauron¹, Fethi Khiter¹, Nicolas Barois², Carine Rouanet¹, Nathalie Mielcarek¹

¹ Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 9017 – CIL – Center for Infection and Immunity of Lille, F-59000 Lille, France

² Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41 - UAR 2014 - PLBS, F-59000 Lille, France

Bordetella pertussis is the main causative agent of whooping cough, a highly contagious respiratory disease that can be fatal in infants. Alveolar macrophages play an important role in the early stages of infection, acting as innate immune sentinel cells in the lungs. Although macrophages are key actors in the efficient bacterial clearance, a limited number of studies suggest that *B. pertussis* may be able to persist or even replicate within these cells. This seemingly contradictory findings highlight gaps in our understanding of the intracellular fate of *B. pertussis* in alveolar macrophages. In this study, we investigated the involvement of a previously unexplored mechanism of bacterial degradation during *B. pertussis* infection: the autophagy.

We infected MPI (Max Planck Institute) cells, a cellular model phenotypically and functionally very close to alveolar macrophages, with *B. pertussis*. Autophagy induction was assessed by western-blot analysis of LC3-conjugated form (LC3-II) involved in the formation of the autophagosome. *B. pertussis*-infected cells displayed an increased accumulation of LC3-II, compared with non-infected cells. In addition, using confocal microscopy, we observed colocalization of LC3 with intracellular bacteria. Electron microscopy further showed the bacteria engulfed in double-membrane vacuoles consistent with autophagosomes, supporting the involvement of canonical autophagy. Together, these results indicate that *B. pertussis* infection is associated with activation of LC3-dependent pathways. Using atg5^{-/-} macrophages to impair autophagy, we showed that this pathway contributes to intracellular *B. pertussis* clearance 24h and 96h post-infection. Interestingly, confocal microscopy revealed a differential recruitment of LC3 in cells infected with an avirulent *B. pertussis* strain, compared to a fully virulent one. Furthermore, Galectin-3 staining highlighted a potential vacuole rupture specifically in cells infected with the virulent strain.

We provided evidence that autophagy contributes to the intracellular degradation of *B. pertussis* inside alveolar macrophages. Moreover, our data suggest that bacterial virulence factors may promote evasion of intracellular degradation, leading to differential intracellular fates of virulent and avirulent strains.

The capsid protein of the hepatitis E virus traffics in LC3B-I and EDEM1-positive compartments

Claire Allioux¹, Olivia Paronetto¹, Emma Bordes¹, Sophie Allart¹, Isabelle Da-Silva², Théophile Couteau¹, Baptiste Rigaud¹, Marion Porcheron², Florence Abravanel^{1,2}, Sébastien Lhomme^{1,2}, Jacques Izopet^{1,2}, **Sabine Chapuy-Regaud**^{1,2}

¹ Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity), Université de Toulouse, INSERM, CNRS, UPS, Toulouse, France

² CHU Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France

The hepatitis E virus (HEV) is the cause of the most common acute viral hepatitis infection worldwide. HEV genotypes 1 and 2 can cause severe hepatitis in pregnant women. HEV infection by genotypes 3 or 4 can evolve into chronic hepatitis in immunosuppressed patients. HEV is a positive single-strand RNA virus infecting epithelial cells such as hepatocytes, with a preferential release at the apical side. We previously found that the ORF2 capsid protein contained a potential LIR (LC3 interacting region) domain, described for its specific recognition of the autophagy central protein LC3B. Using cultures of the polarized HepG2/C3A/F2 hepatocarcinoma cell line, we showed that ORF2 and LC3B colocalized in intracellular vesicles. But ORF2 interacted with the cytoplasmic LC3B protein form, LC3B-I. LC3B-I is known to be involved in ER-budding vesicles participating in the regulation of ER quality control and labeled with EDEM1. By immunofluorescence and analysis with the Imaris® software, we found that ORF2 was found closed to EDEM1-positive vesicles. Here, we aimed to confirm the role of the ORF2-LC3B-I interaction, and the implication of EDEM1+ positive-compartments in the HEV cycle. We performed cellular fractionation and found the cofractionation of ORF2, LC3B-I and EDEM1. We found that mutations in the LIR domain decreased ORF2 amounts and HEV RNA production. Taken together, this supports the role of LC3B-I in ORF2 trafficking and in the propagation of HEV infection.



Autophagy and extracellular vesicle secretion in glioblastoma: a heterogeneous primary culture response to temozolomide and chloroquine

Tiffany Darbas¹, Gabrielle Perrolan¹, Julie Kurkowski¹, Amy Gateau¹, Léa Decoux¹, Amandine Rovini¹, Barbara Bessette¹, Fabrice Lalloue¹, Elise Deluche¹, Mireille Verdier-Sage¹

¹ UMR 1308 CAPTuR, Faculté de médecine, Institut oméga-health

Introduction. With a median overall survival of approximately 2 years, glioblastoma remains the primary brain tumour with the poorest prognosis. Treatment mainly relies on surgery, radiotherapy, chemotherapy (temozolomide) and, more recently, tumour-treating fields. The role of autophagy in carcinogenesis remains debated, with unresolved evidence for either a pro- or anti-tumoural effect. Several clinical trials have evaluated chloroquine, with heterogeneous results and limited clinical benefit to date. In this pilot study, we aimed to characterise the tumour cell response to temozolomide and/or chloroquine, focusing on autophagy and on extracellular vesicle (EV) secretion, a recognised mechanism of intercellular communication and treatment resistance.

Methods. Three glioblastoma primary cultures were grown in defined medium to enrich for stem-like cells. Cell viability was assessed by luminescence (CellTiter-Glo® 3D). Chloroquine dose (10 μ M) was selected based on the literature. Necrosis and apoptosis were quantified by ELISA Cell Death. Autophagy markers expression (BECLIN 1, LC3B I/II, p62) and tetraspanins (CD63, CD9, CD81) were evaluated by western blot. Small EVs (<200 nm) were isolated by differential ultracentrifugation, with size distribution confirmed by nanoparticle tracking analysis (NTA), and protein content was determined using the PIERCE BCA assay. Statistical analyses were performed using GraphPad PRISM V9.0.

Results. The three primary cultures (P12, P19, P25) showed distinct IC50 values for temozolomide (450, 1500 and 1850 μ M, respectively). Temozolomide reduced autophagy markers (BECLIN 1, p62) only in P12. Chloroquine blocked autophagic flux in all three lines, consistent with its known mechanism of action. The temozolomide-chloroquine combination was associated with increased apoptosis only in P12, suggesting line-dependent sensitivity rather than a widespread effect. Small EV protein content also varied by line and condition: it increased under temozolomide in P12 and P25, but decreased under chloroquine in P12 and P19.

Conclusion. These preliminary findings, from a limited number of primary cultures, illustrate the heterogeneity of glioblastoma cell responses to autophagy-modulating stress. Whether autophagy blockade directly drives the observed changes in apoptosis and small EV secretion remains to be established, ideally through genetic autophagy inhibition.

Bempedoic acid induces autophagy and improves liver cancer management

Fatima Domenica Elisa De Palma^{1,2}, Sarah Adriana Scuderi², Angelica Di Matteo², Maria Perez-Lanzon², Guido Kroemer², Maria Chiara Maiuri^{1,2}, Jonathan Pol²

¹ Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples (Italy)

² Metabolism, Cancer & Immunity, Centre de Recherche des Cordeliers U1138, Inserm, Université Paris Cité, Sorbonne Université, Paris

Hepatocellular carcinoma (HCC) is the third leading cause of global cancer-related deaths. Current advanced HCC treatments mainly rely on combinations of immunotherapies and anti-angiogenic agents, or targeted therapies. Their efficacy is limited, necessitating innovative strategies.

Recent preclinical studies indicate that boosting tumor immunogenicity via autophagy induction can enhance chemo-immunotherapeutic effectiveness. This can be achieved by preconditioning tumor-bearing hosts with caloric restriction (CR) or CR-mimicking compounds (CRM). However, no study has evaluated the interest of such combinations in HCC.

Bempedoic acid (BA), a newly approved drug for hypercholesterolemia, inhibits ATP citrate lyase (ACLY) in the liver, a key enzyme participating in lipid synthesis, which is a mechanism of action shared with some CRMs. However, BA role in sensitizing immunochemotherapy as a CRM in HCC has never been investigated.

To address this issue, we studied whether BA induces autophagy and could represent a novel CRM. Then, we explored the effectiveness of BA in enhancing immunochemotherapeutic strategies for HCC in vivo.

Here, we identified BA as a non-toxic inducer of autophagic flux that acts on human and mouse tumor cells in vitro, as well as in murine tissues. Finally, the administration of BA to HCC tumor-bearing mice improved the efficacy of immunogenic anticancer chemotherapy.

In summary, our research reveals that autophagy-inducing interventions could improve liver cancer management.



P10

ATGs and Cancer
Poster

Regulation of anti-tumour immune responses by lysosomal stress

Samy Dehissi^{1,2,3}, Lucille Ferret^{1,2}, Jonathan Pol^{1,2}, Jean Guillon⁴, Guido Kroemer^{1,2,5}, Mojgan Djavaheri Mergny^{1,2}

1 Centre de Recherche des Cordeliers, Inserm UMRS 1138, Sorbonne Université, Université Paris Cité, Équipe labellisée par la Ligue contre le Cancer, Institut Universitaire de France, Paris, France

2 Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France

3 Faculté de Médecine, Université Paris-Saclay, Paris, France

4 Faculté de Pharmacie, Université de Bordeaux, ARNA, UMR CNRS 5320, Inserm U1212, Bordeaux, France

5 Department of Biology, Institut du Cancer Paris CARPEM, Hôpital Européen Georges Pompidou, AP-HP, Paris, France

Emerging evidence indicates that cancer cells are particularly vulnerable to lysosomal perturbation, highlighting lysosomal stress as a promising therapeutic approach. In particular, cationic amphiphilic drugs (CADs) have demonstrated potent tumor growth inhibition across diverse preclinical models. However, the mechanisms by which lysosomal stress regulates immunogenic cell death and anti-tumor immunity remain poorly understood.

My thesis project aims to elucidate the ability of some novel CADs to stimulate anti-tumor immune responses, either directly or through the induction of immunogenic cell death (ICD) in cancer cells, using complementary in vitro and in vivo approaches.

We first performed a screening of the NCI mechanistic library (comprising more than 800 compounds) to identify new CADs capable of inducing lysosomal membrane permeabilization (LMP). To this end, we used the human osteosarcoma cells (U2OS) expressing Galectin-3 tagged to the fluorescent protein mCherry as tool to detect lysosomal damage. We further expanded the identification of new candidates to a series of synthetic derivatives of chloroquine, designed by our chemist collaborator. To date, several of these compounds were found to induce LMP to a significant extent.

The effects of newly identified CADs on lysosome-related processes (e.g., autophagy, CASM, TFEB) and on the expression of immune checkpoint proteins are currently being investigated in cancer cells. Their ability to induce immunogenic cell death is also being assessed.

Depending on the results obtained, we will further assess the immunomodulatory effects of selected CADs in fibrosarcoma models in immunocompetent mice.

Protein ATG8ylation : Development of a strategy to investigate a novel post-translational modification during viral infections

Thibaut Flipo¹, Margaux Versapuech¹, Lucas Lasnel¹, Marjory Palaric¹, Clarisse Berlioz-Torrent¹

¹ Institut Cochin, Université de Paris, INSERM, CNRS

ATG8ylation was initially described as the process leading to the covalent conjugation of ATG8 family proteins to lipids on autophagosomal membranes. This modification is essential for autophagosome biogenesis, selective cargo recruitment, and autophagosome–lysosome fusion during the autophagy process. More recently, several studies have shown that ATG8 family proteins (LC3A-C and GABARAP/L1/L2), which belong to the ubiquitin-like protein family, can also be covalently conjugated to cellular proteins, thereby revealing a new post-translational modification called protein ATG8ylation. To date, the mechanisms controlling this process, the nature of its substrates, and its physiological role remain largely unknown, particularly in the context of viral infections.

Recent work from our laboratory has shown that proteins of the GABARAP subfamily plays an essential role in the encapsidation of the HIV-1 genome, independently of canonical autophagy. These observations suggest that ATG8 proteins could perform new functions during the viral cycle and led us to hypothesize that the protein ATG8ylation contributes to the remodeling of cellular functions induced by the infection.

In this project, we developed an experimental strategy to stabilize and enrich the ATG8ylated proteins. This is based on the expression of different mutated forms of LC3B and GABARAPL1, combined with the use of cells deficient in ATG4 proteases to limit substrate deconjugation. ATG8 proteins immunoprecipitation followed by biochemical and proteomic analyses using mass spectrometry, made it possible to enrich the ATG8ylated proteins and compare ATG8ylation profiles obtained in different stress conditions, particularly during an infection by HIV-1.

Ultimately, this approach will be used to map ATG8ylated proteins during viral infections, evaluate the specificity of this process within the LC3 and GABARAP subfamilies, and identify new cellular effectors potentially involved in viral replication. This work should provide the first comprehensive information on the dynamics of protein ATG8ylation during viral infection and open up new perspectives on the non-canonical functions of ATG8 proteins.

This project is supported by the ANRS.



P12

Molecular regulation of autophagy
Poster

Isolation of autophagic vesicles from Thy1-RFP-GFP-LC3 mice in stroke

Jean-Charles Fouillard--Uzel¹, Evalena Thoré¹, Violina Dorogan², Denis Vivien^{1,3}, Vassiliki Nikolettou², Morgane Louessard¹, Benoit D. Roussel¹

¹ Normandie University, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders (PhIND), Centre Cyeron, Brain, Blood and Memory @ Caen Normandie (BBM@C), France

² Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland

³ CHU Caen, Clinical Research Department, CHU de Caen Côte de Nacre, Caen, France

Abstract : Cerebral ischemia, caused by the occlusion of a cerebral artery, is a leading cause of disability and death worldwide. Alterations in autophagy play a critical role in ischemic brain injury (Thiebaut *et al.*, 2019). Furthermore, Kallergi *et al.*, 2023) demonstrated that the brain's autophagic cargo, or "degradome", is highly dynamic and context-dependent. While clinical reperfusion relies on the thrombolytic agent rtPA, its specific impact on the remodeling of autophagic profiles remains largely unexplored due to the technical challenge of isolating intact endogenous vesicles.

To overcome this, we established a robust biochemical pipeline using an *in vivo* stroke model (Orset *et al.*, 2007). Cerebral ischemia was induced in TRGL (Thy1-RFP-GFP-LC3) transgenic mice, followed by longitudinal MRI monitoring. These fluorescent reporters allow for precise tracking of autophagic dynamics. We adapted a three-step density gradient ultracentrifugation protocol (Kallergi *et al.*, 2023) to isolate endogenous vesicles from ischemic brain tissue. Fraction enrichment was rigorously validated by Western blot against core autophagic and endosomal markers (Atg5, Atg13, LC3B, and Rab7). Subsequently, we performed a GFP-targeted immunoprecipitation to specifically isolate the neuronal autophagic vesicles, with purity confirmed by p62 and LC3B immunoblotting.

As a next step, we plan to perform quantitative proteomics comparing GFP-immunopurified fractions to negative controls (IgG-IP) as a proof of concept to confirm the enrichment of the autophagic machinery. Once this pipeline is fully validated via mass spectrometry, our immediate perspective will be to characterize the ischemic degradome signature. Ultimately, applying this strategy to rtPA-treated cohorts will reveal how thrombolysis modifies the autophagic cargo, potentially unveiling new therapeutic targets to limit post-ischemic brain damage.

Keywords : Cerebral Ischemia, Autophagy, Degradome, Autophagic Vesicle Isolation, Thrombolysis.

Acknowledgements : The authors gratefully acknowledge the laboratory of Dr. Vassiliki Nikolettou (University of Lausanne) for their hospitality and invaluable training regarding the autophagic vesicle extraction and immunoprecipitation protocols. This work was supported by funding from the Fédération pour la Recherche sur le Cerveau (FRC), and this presentation was made possible thanks to a travel grant provided by the Club Francophone de l'Autophagie (CFATG).

Decoding *Candida albicans*-Autophagy interactions at the plasma membrane during invasion

Loïs Guillemard¹, Amandine Ducreux¹, Lisa Degache¹, Bernhard Hube², Christophe D'enfert³, Sophie Bachelier-Bassi³, Elisabeth Werkmeister⁴, Sébastien Janel⁴, Frank Lafont⁴, Pierre Lapaquette^{1,*}, Frédéric Dalle^{1,*}

¹ Université Bourgogne Europe, L'Institut Agro, INRAE, UMR PAM 21000, Dijon, France

² Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute, Jena – Allemagne

³ Institut Pasteur, USC2019 INRAE, Paris, France

⁴ Institut Pasteur de Lille, F59000 Lille, France

* Equal contribution to last authorship

Invasive infections caused by *Candida albicans* represent a major public health concern, particularly in immunocompromised patients, due to their high mortality rate and the persistent gaps in understanding the mechanisms underlying host invasion. Translocation of *C. albicans* across the intestinal epithelial barrier is a critical step in systemic dissemination and begins with the invasion of host epithelial cells. In this context, autophagy, traditionally known for its role in lysosomal degradation, is increasingly recognized as a key regulator of membrane dynamics, participating in cellular defense mechanisms against pathogen invasion.

This thesis project aims to decipher the interactions between *C. albicans* and the autophagy machinery at the plasma membrane during epithelial cell invasion. Published data of our group demonstrate a rapid recruitment of autophagy-related proteins (ATG5, ATG16L1, LC3) at fungal entry sites, suggesting a protective role in maintaining plasma membrane integrity.

We hypothesize that these proteins actively contribute to the repair of membrane damage induced by hyphal growth and thereby limit fungal invasion.

To address this hypothesis, the project is structured around three main objectives: (i) to characterize the kinetics of autophagy machinery recruitment and to identify the fungal determinants involved in modulating it; (ii) to define the key molecular players involved in this response using both targeted approaches and proximity-based proteomics; and (iii) to evaluate how environmental factors (metabolic stress, pollutants, and co-infections) modulate this cytoprotective response.

To identify the fungal factors involved in the recruitment of autophagy-related molecules, we analyzed this process using a series of *Candida albicans* mutants. These mutants exhibit either defects in hyphal formation or elongation—processes responsible for the deformation of the epithelial cell plasma membrane—or alterations in the secretion of virulence factors, particularly those involved in epithelial damage. The initial results obtained will be presented.

Overall, this work will provide fundamental insights into non-canonical functions of autophagy and may open new therapeutic perspectives aimed at enhancing host resistance to fungal infections.



P14

Molecular regulation of autophagy
Poster

Autophagy and endothelial activation in human iPSC-derived brain endothelial cells under inflammatory and ischemia-like stresses

Morgane Louessard¹, Valerie Roussel¹, Jean-Charles Fouillard-Uzel¹, Charlotte Lechevallier¹, Denis Vivien¹, Eloise Lemarchand¹, Benoit D. Roussel¹

¹ Normandie University, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders (PhIND), Centre Cyron, Brain, Blood and Memory @ Caen Normandie (BBM@C), France

Background : Ischemic stroke induces a cascade of deleterious events driven by oxygen and glucose deprivation followed by reperfusion, leading to neurovascular dysfunction, blood–brain barrier (BBB) disruption, inflammation, and sustained endothelial activation. Autophagy is increasingly recognized as a central stress-response pathway regulating cellular adaptation under ischemic and inflammatory conditions; however, its dynamic regulation in human cerebrovascular cells remains incompletely understood. Brain endothelial cells are key components of the neurovascular unit and play a central role in BBB integrity and inflammatory signalling. We hypothesized that modulation of autophagic pathways may contribute to endothelial adaptation or dysfunction under stress conditions.

Methods: We first investigated autophagic responses (WB LC3/p62) in human induced Pluripotent Stem Cell (hiPSC)-derived brain endothelial cells exposed to pro-inflammatory stimulation (TNF α and IFN γ), across three independent hiPSC lines with biological replicates (n=2–3 per line). Autophagic flux was further assessed using bafilomycin A1 and the autophagy inducer Tat-Beclin. Endothelial activation was evaluated through endothelial adhesion molecules expression (VCAM-1, E-selectin, PECAM-1, ICAM-1 and P-selectin). In parallel, we performed preliminary experiments under oxygen-glucose deprivation (OGD) followed by reperfusion (0h, 6h, 24h) in two hiPSC lines (n=1), to explore ischemia-like stress-induced modulation of autophagy.

Results: Inflammatory stimulation induced robust endothelial activation, together with altered autophagic flux reflected by LC3 and p62 accumulation following bafilomycin A1 exposure. Preliminary OGD/reperfusion experiments revealed dynamic modulation of both autophagy and endothelial activation markers across deprivation and reoxygenation time points. To further establish the functional and causal link between autophagy and endothelial/BBB dysfunction, ongoing work includes shRNA-mediated genetic modulation of autophagy, immunofluorescence microscopy, fluorescein-based permeability assays, and TEER measurements.

Conclusions: Together, these data provide robust evidence for inflammation-induced modulation of autophagic flux in human brain endothelial cells, and preliminary insights into autophagy dynamics under ischemia-like conditions, supporting further investigation of autophagy–endothelial activation crosstalk in neurovascular dysfunction.



P15

Autophagy-independent functions of ATG proteins

Poster

Deciphering the non-autophagy functions of LC3/GABARAP during *C. elegans* development

Dejun Kong¹, Céline Largeau¹, Audrey Sagot¹, Christophe Lefebvre¹, Renaud Legouis¹

¹ I2BC, CNRS, Avenue de La Terrasse, 91198 Gif sur Yvette Cedex, France

Autophagy is a highly conserved degradative process that promotes lysosomal degradation through the formation of double-membrane autophagosomes. The ubiquitin-like ATG8 proteins, represented in humans by 6 genes forming the LC3 and GABARAP subfamilies, are involved in multiple steps during autophagy, including initiation, cargo recognition and engulfment, as well as vesicle closure. Beyond its classical roles in autophagy, the ATG8 family also mediates diverse autophagy-independent processes through conjugation of ATG8 family to membranes or proteins, also named ATG8ylation.

Our previous work in *C. elegans*, a suitable model organism with only two ATG8 homologs (LGG-1/GABARAP and LGG-2/LC3), revealed that LGG-1 contributes to cellular and developmental processes independent of autophagy. Notably, *lgg-1* mutants exhibit both strong embryonic and larval lethality. Time-lapse imaging of *lgg-1* mutants reveals similar morphogenetic defects during embryogenesis. In contrast, *lgg-2* mutants show no observable phenotype. Double *lgg-1*; *lgg-2* mutants are completely lethal, suggesting that the two genes function redundantly. Interestingly, we identified an extragenic mutation that can rescue several *lgg-1* lethal mutants. I will present preliminary data using this genetic suppressor, which reveals the developmental functions of LGG-1 and LGG-2.



P16

Molecular regulation of autophagy
Poster

Understanding the role of Rer1 in regulating autophagy and UPR for cell survival

Rajni Kumari¹, Pranab Paul¹, Shruti Umarvaish¹, Shivani Bajaj¹, Rishana Farin², Hrudya Mohan¹, Varun Chaudhary¹, Wim Annaert²

¹ Indian Institute of Science Education and Research, Bhopal, India

² VIB - Centre for Brain and Disease Research, KU-Leuven, Belgium

Cells rely on tightly regulated homeostatic mechanisms to maintain protein quality and overall cellular function. Under conditions of stress such as nutrient deprivation, oxidative damage, or the accumulation of misfolded proteins, cells activate adaptive signaling pathways to restore balance. To maintain homeostasis, they engage coordinated signaling networks, including the unfolded protein response (UPR) and autophagy. Emerging evidence, from *Drosophila* models, suggests that UPR and autophagy are integrated components of a broader homeostatic network and these pathways can be activated in parallel or in a context-dependent manner. Depending on the type of stress, UPR signaling can promote autophagy as a protective mechanism, while in other cases, autophagy may be activated independently or in parallel to buffer proteotoxic stress. These interactions are especially relevant in processes such as cell competition, where cells experiencing higher stress or impaired proteostasis are selectively eliminated as “loser” cells to preserve tissue integrity.

Cell competition is a process by which cells compare their relative fitness with their neighbors. Cells that are less fit, referred to as “loser” cells are selectively eliminated, while more fit “winner” cells expand. In *Drosophila*, cell competition has been extensively studied and is known to involve key factors such as impaired proteotoxic flux, reduced translation and defective autophagy. Previously we have shown that cells under loss of *rer1* undergo loser cell status and gets eliminated by wild type cells. Rer1 (Retention in endoplasmic reticulum 1) is a key component of the early secretory pathway that ensures proper protein quality control by retrieving misfolded or unassembled proteins. It has also been shown that loss of *rer1* triggers proteotoxic stress by activating UPR. Further, we want to understand how Rer1 influences stress-responsive pathways, with a particular focus on autophagy. We aim to address whether Rer1 loss induces autophagy and how this response is coordinated with different branches of the UPR, and whether autophagy contributes to the elimination or survival of loser cells using *Drosophila* as a model organism. By placing Rer1 within the broader framework of stress-mediated homeostatic signaling and cell competition, this work seeks to provide new insights into how cells integrate proteostasis, stress



From proof of concept to large-scale screening of autophagy-stimulating food-grade ferments

Marie-Agnès Bringer¹, Simon Manceau², Vanessa Ferreira de Souza^{1,2}, Jana Al Azzaz², Bénédicte Buteau¹, Lil Proukhnitzky^{1,2}, Amaury Aumeunier², Luis G. Bermúdez-Humarán³, Florian Chain³, Elisabeth Werkmeister⁴, Catherine Daniel⁴, Elise Jacquin⁵, Zandile Mlamla⁶, Jean-Paul Pais de Barros⁶, Julia Novion Ducassou⁷, Yohann Couté⁷, Guilhem Faure^{8,9}, Niyazi Acar¹, Aurélie Rieu², **Pierre Lapaquette²**

¹ « Eye & Nutrition » Research Group, Université Bourgogne Europe, Institut Agro, CNRS, INRAE, UMR CSGA, 21000 Dijon, France

² Université Bourgogne Europe, Institut Agro, INRAE, UMR PAM, 21000 Dijon, France

³ Laboratory of Commensals and Probiotics-Host Interactions, Université Paris-Saclay, INRAE, Micalis Institute, AgroParisTech, Jouy-en-Josas, 78350, France

⁴ Univ. Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 – CIL Center for Infection and Immunity of Lille, F-59000 Lille, France

⁵ Université Bourgogne Europe, INSERM UMR1231 Center for Translational and Molecular Medicine, 21000 Dijon, France

⁶ DiviOmics Platform, UMS 58 BioSand - Université Bourgogne Europe, Dijon, France. LipSTIC Labex, Dijon, France

⁷ Univ. Grenoble Alpes, INSERM, CEA, UA13 BGE, CNRS, CEA, UAR2048, 38000 Grenoble, France

⁸ Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

⁹ McGovern Institute for Brain Research at MIT, Cambridge, MA 02139, USA

Autophagy-related processes are essential for maintaining cellular homeostasis in eukaryotic organisms. Dysregulation of autophagy has been linked to various human diseases, including inflammatory bowel diseases and age-related disorders. Conversely, moderate long-term stimulation of autophagy appears to be generally well tolerated in model organisms and has been associated with lifespan extension in several experimental models. Emerging evidence suggests that the gut microbiota or its metabolites can modulate host autophagy both at the intestinal mucosa and in peripheral organs.

In this study, we investigated the potential of bacterial strains commonly used in food fermentation (ferments) to modulate autophagy, both in vitro and in vivo. Eleven lactobacilli and bifidobacteria strains were included in a preliminary in vitro screening to evaluate their ability to induce autophagy in human epithelial cells. Among them, *Lactobacillus helveticus* VEL12193, isolated from cheese, emerged as the most effective. Long-term dietary supplementation with this strain in mice confirmed its pro-autophagic effects, not only in the gut mucosa but also in the retina, a distant organ. Furthermore, we identified membrane vesicles derived from *L. helveticus* as key components involved in the induction of autophagy in both epithelial and immune cells.

These findings suggest that specific food-grade bacterial strains and their components may serve as promising tools to beneficially modulate host autophagy and promote healthy aging. Building on these results, we are currently developing a high-throughput screening workflow to characterize the autophagy-inducing potential of 100 food-grade bacterial and yeast strains isolated from fermented plant-based foods.

Characterization of Heat Stress in *Caenorhabditis elegans* using Focused Ion Beam Scanning Electron Microscopy (FIB-SEM)

Céline Largeau¹, Claire Boulogne¹, Baptiste Roelens¹, Emmanuel Culetto¹, Christophe Lefebvre¹, Renaud Legouis¹

¹ I2BC, CNRS, CEA, Université Paris-Saclay, Inserm

Our aim is to understand the mechanisms underlying the elimination of cellular components in *C. elegans*, by autophagy, during responses to stress. For that, we developed several electron microscopy approaches to obtain further ultrastructural information for *C. elegans* model. We also developed a new paradigm for studying the adaptation of *C. elegans* larvae to acute heat-stress (aHS), that induces a strong developmental defect but no lethality or sterility. This stress results in various cellular defects and mitophagy and ERphagy events.

Transmission Electron Microscopy (TEM) is a powerful tool for morphological analyses. Combined with other approaches, TEM participated to describe how autophagy facilitates mitochondrial reconstruction after aHS (1) and how reticulon protein mediates aHS adaptation (2).

More recently, we were interested in the interaction between the key autophagy protein LGG-2 and PDZD-8 which is able to transport lipids. We wanted to test whether PDZD-8 could control lipid transfer during autophagosome biogenesis and/or fusion between autophagosome and lysosome under heat stress. For that we have started to implement the Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) to characterize the autophagosome contents and their interactions with other organelles.

We have optimized *C. elegans* sample preparation and image acquisition to obtain high-resolution movies (8nm) in the three dimensions (X, Y, Z). Here I present a progress report on 3D reconstruction and the use of artificial intelligence tools for segmentation of organelles.

(1) Chen et al. (2021). Autophagy facilitates mitochondrial rebuilding after acute heat stress via a DRP-1-dependent process. *The Journal of cell biology*

(2) Claudia Serot *, Vincent Scarcelli * et al. (2025). Reticulon-dependent ER-phagy mediates adaptation to heat stress in *C. elegans*. *Current Biology*

(3) Alexandre Pouget et al. (2026). PDZD-8 interacts with LGG-2/LC3 to promote autolysosome formation. *bioRxiv*

Autophagy proteins regulate HSP110 release from immune cells

Juliette Henna¹, Abdelmnim Radoua^{1,2}, Imene Boukriba¹, Mathis Rabut¹, Carmen Garrido¹, Gaëtan Jegou¹, Elise Jacquin¹

¹ Université Bourgogne Europe, INSERM, Centre de Recherche Translationnelle en Médecine Moléculaire (CTM), UMR 1231, équipe HSP-Pathies, 21000 Dijon, France

² INSERM, UMRS1138, CRC Métabolisme Cancer et Immunité, 75006 Paris, France

Autophagy-related pathways are increasingly recognized as key regulators of unconventional protein release. Beyond their canonical degradative functions, autophagy proteins can contribute to vesicular trafficking and immunomodulatory protein release through autophagosome formation or unconventional functions such as Conjugation of Atg8 to Single Membranes (CASM). The role of these pathways in the extracellular release of heat shock proteins (HSPs) remains unknown.

HSP110 is a stress-induced molecular chaperone frequently overexpressed in cancers and associated with tumor progression. Beyond its intracellular functions, HSP110 can also be released into the extracellular space, where it has been reported to modulate immune responses and contribute to immunosuppression. However, the mechanisms underlying its release remain largely unknown.

To address this question, we generated immune and colorectal cancer cell lines incompetent for canonical autophagy (ATG13 knockout) or CASM (ATG16L1 K490A mutation). We found that, upon inflammasome activation, dendritic cells release HSP110 but not its cochaperone HSP70, revealing an unexpected contribution of this protein in immune responses. Notably, HSP110 release was significantly reduced in both ATG13-deficient and ATG16L1 mutant dendritic cells compared to wild-type controls, suggesting that both canonical autophagy and CASM contribute to its extracellular release. In parallel, the release of the pro-inflammatory cytokine IL-1 β , a well-characterized cargo of unconventional release pathways, was also altered in autophagy and CASM-deficient cells. This supports a broader role for autophagy-related mechanisms in the regulation of immunomodulatory protein release. In contrast, disruption of canonical autophagy or CASM in colorectal cancer cells did not affect HSP110 release, suggesting the existence of an immune-cell-specific mechanism of release.

Together, our findings identify a role for autophagy proteins in the unconventional release of HSP110 by immune cells, and highlight these pathways as potential targets to modulate immune responses in cancer.



Chronic TLR9 signaling drives PLD3-dependent lysosomal lipid remodeling and dysfunction in Alzheimer's Disease Microglia

Lucie Mazzola¹, Moïse de Lavaissière de Lavergne¹, Bénédicte Manoury¹

¹ Institut Necker Enfants Malades, INSERM U1151-CNRS UMR 8253, Université Paris Cité, Faculté de Médecine Necker, Paris, France

TLR9 is a lysosomal innate immune receptor that senses double-stranded DNA (dsDNA). Its acute activation has been linked to Alzheimer's disease (AD) pathology, though the underlying mechanism has remained unclear.

In the APP23 mouse model of AD, we show that dsDNA accumulates near amyloid plaques together with phospholipase D3 (PLD3), a nuclease that cleaves nucleic acids, and reactive microglia, stimulating TLR9, which drives microglia toward a pro-inflammatory state and causes lysosomal damage. These effects are absent in APP23-TLR9-deficient (A9KO) mice, which are protected from cognitive decline, neuronal loss, and amyloid- β pathology.

Mechanistically, TLR9 signaling upregulates PLD3, which generates the lysosomal lipid bis(monoacyl)glycerophosphate (BMP). Elevated PLD3 and BMP levels correlate with impaired lysosomal fitness in APP23 microglia and are both reduced in A9KO mice. In human HMC3 microglia, chronic but not acute TLR9 stimulation increases PLD3, drives BMP accumulation, and triggers lysosomal membrane permeabilization, TFEB nuclear translocation, and alters lysosome number and size. TLR9 inhibition reverses PLD3 upregulation.

Together, these findings establish PLD3 as a key effector linking chronic TLR9-dsDNA sensing to lysosomal lipid remodeling and dysfunction, ultimately reducing amyloid- β clearance, a hallmark of AD.



P21

Molecular regulation of autophagy
Poster

Functional characterization of autophagosome subpopulations by in vivo single-event analysis

Valentin Meire¹, Allan Sauvat², Mojgan Mergny², Manuel Rodriguez³, Renaud Legouis¹, Christophe Lefebvre¹

¹ Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

² Centre de Recherche des Cordeliers, Equipe Labellisée par la Ligue contre le Cancer, Université de Paris, Sorbonne Université, INSERM U1138, Institut Universitaire de France, Paris

³ Laboratoire de Chimie de Coordination (LCC), CNRS, Toulouse, France

Macroautophagy is the main lysosomal degradation process to recycle component of the cell. It occurs both under physiological and stress conditions. It's an essential mechanism to counteract the effect of stress condition such as nutrient starvation, chemicals stresses or heat stress. This process involves membrane nucleation leads to vesicles biogenesis called autophagosomes which engulf cytoplasm fraction to be degraded. One of key proteins involved in autophagy called ATG-8, discovered first in yeast, is anchored to the membrane of autophagosomes and contribute to selective autophagy. In *C. elegans*, there are 2 homologues of ATG-8, LGG-1 and LGG-2. These proteins are essential and play multiple roles during the autophagic process and define 3 autophagosomes subpopulations, single positive for LGG-1 or LGG-2 and double positive vesicles. The aim of the project is to decipher the autophagosome subpopulations biogenesis and their roles. Autophagosomes biogenesis is investigated using microfluidic system and 4D microscopy approaches. Autophagosome molecular traps are used to isolate autophagosome subpopulation to analyse the content by mass spectrometry and identify potential specific cargoes in it, according to LGG-1 / LGG-2 positive autophagosomes. We initially developed a microfluidic approach in the laboratory to efficiently immobilize the worms for timelapse autophagosome dynamics tracking. From strain expressing GFP::LGG-1/mCherry::LGG-2, our preliminary results show that we are able to track the dynamics of an autophagosome and monitoring the behavior of LGG-1 and LGG-2.



P22

Molecular regulation of autophagy
Poster

A centriolar satellite-microtubule axis coordinates endolysosomal homeostasis in glioblastoma stem-like cells

Laura Merlet¹, Quentin Roux, Fiorella Spinelli, Clémence Bodard, Kilian Trillet, Clotilde CN Renaud, Steven Nedelec, Rosalie Moreau, Tiphaine Douanne, Nicolas Bidère, Julie Gavard

¹ CRCI2NA, Inserm, CNRS, Université de Nantes, Nantes, France

² Equipe Labellisée Ligue Nationale contre le Cancer, Paris, France

³ Nantes Université, CHU Nantes, CNRS, Inserm, BioCore, US16, SFR Bonamy, Nantes, France

⁴ Integrated Center for Oncology, ICO, St. Herblain, France

Centriolar satellites (CS) are membrane-free cytoplasmic granules that gravitate around the centrosome, moving along microtubules. The scaffolding protein PCM1 is essential for their organization, integrity and function. CS play critical roles in various cellular processes, including ciliogenesis, centrosome proteostasis, and microtubule dynamics. However, their involvement in tumor plasticity and intracellular trafficking remains poorly understood.

Glioblastoma Stem-like Cells (GSCs) are key drivers of glioblastoma initiation, progression, and therapeutic resistance, and critically rely on efficient intracellular trafficking and lysosomal function to adapt to cellular stress. Given the intimate relationship between intracellular trafficking, lysosomal homeostasis, and autophagy, we investigated whether CS contribute to the regulation of these processes in GSCs.

Patient-derived GSCs displayed typical CS organized around the centrosome and scaffolded by PCM1. Expansion microscopy revealed a heterogeneous and complex organization of these structures. PCM1 silencing by siRNA altered microtubule dynamics and reduced tubulin deetyrosination, supporting a role for CS in the spatial organization of the microtubule network. Interestingly, PCM1 depletion also altered the distribution and organization of endo-lysosomal compartments, accompanied by changes in their maturation and a reduction in extracellular vesicle release. In contrast, CS depletion did not significantly affect autophagic activity, indicating that the observed alterations in the endo-lysosomal system are not associated with a major impairment of autophagy. These findings suggest that CS may preferentially regulate specific branches of intracellular trafficking rather than global lysosomal degradative pathways.

Overall, our findings identify centriolar satellites as regulators of intracellular organization and endo-lysosomal trafficking in GSCs, while revealing a dispensable role in autophagy. By modulating the organization of the endo-lysosomal system and extracellular vesicle release, CS may contribute to the balance between intracellular degradation and secretion, a key determinant of how GSCs interact with and remodel their tumor microenvironment. This balance may ultimately influence intercellular communication and the aggressive behavior of glioblastoma.

Physical activity activates intestinal autophagy and counteracts the deleterious effects of high-fat diet and adherent-invasive *E. coli*

Hang T. T. Nguyen¹, My Hanh Thi Hoang¹, Anaëlle Tassini¹, Annaëlle Couvert², Cyril Rozotte¹, Guillaume Dalmaso¹, Lydie Combaret³, Vincent Martin², Nathalie Boisseau²

¹ M2iSH (Microbes, intestine, inflammation and Susceptibility of the Host), UMR 1071 Inserm, University of Clermont Auvergne, INRAE USC 1382, Clermont-Ferrand, France

² AME2P (Laboratoire des Adaptations Métaboliques à l'Exercice en conditions Physiologiques et Pathologiques), Université Clermont Auvergne, Clermont-Ferrand, France

³ University of Clermont Auvergne, INRAE, UNH UMR 1019, CRNH Auvergne, Clermont-Ferrand, France

Autophagy is crucial for maintaining intestinal homeostasis and host defense to pathogen infection. Dysregulated autophagy, high-fat (HF) diet and abnormal colonization of adherent-invasive *E. coli* (AIEC) are risk factors for Crohn's disease (CD). Physical activity (PA) has been associated with a lower risk of developing several chronic diseases, including CD. Here, we aimed to investigate the impact of spontaneous PA on autophagy activity in intestinal epithelial cells (IECs), and assess its potential beneficial effects on intestinal functions under HF consumption or AIEC infection. For that, wild-type mice were fed control or HF diet for 12 weeks, and subsequently given access to running wheels (PA) or blocked wheels for the same duration. We observed that HF-fed mice exhibited substantial weight gain, increased total fat mass and mesenteric adipose tissue weight, along with reduced fat-free mass, as assessed by EchoMRI. PA effectively reduced weight gain, fat mass, and mesenteric adipose tissue weight, while increasing fat-free mass in HF-fed mice. HF diet consumption decreased autophagy activity in IECs, whereas PA restored autophagy levels. PA also mitigated the deleterious effects of HF diet on several intestinal functions regulated by autophagy, including production of antimicrobial peptides and pro-inflammatory cytokines and chemokines. PA enhanced autophagic response to AIEC infection *in vivo*, reducing intestinal AIEC colonization and intestinal inflammation. In conclusion, PA counteracts HF diet-induced dysregulation of autophagy and enhances autophagy response to AIEC infection. These results suggest that combining spontaneous PA with other therapeutic interventions may represent a promising strategy for CD patients with impaired autophagy.



Western diet-induced autophagy dysfunction drives disrupted intestinal homeostasis, intestinal inflammation and AIEC expansion

Hang T. T. Nguyen¹, My Hanh Thi Hoang¹, Angéline Duval¹, Anaëlle Tassini¹, Eugénie Cros¹, Cyril Rozotte¹, Guillaume Dalmasso¹

¹ M2iSH (Microbes, intestine, inflammation and Susceptibility of the Host), UMR 1071 Inserm, University of Clermont Auvergne, INRAE USC 1382, Clermont-Ferrand, France

Background and Aims: Crohn's disease (CD) is a chronic inflammatory bowel disease, in which dysregulated autophagy, Western diet and abnormal colonization of adherent-invasive *E. coli* (AIEC) have been identified as risk factors. Here, we investigated (i) the effect of high fat, high sugar (HFHS) diet on autophagy, (ii) impact of combined HFHS diet and dysregulated autophagy on intestinal homeostasis, and (iii) develop a microbiota-based strategy that activates autophagy, exerting beneficial effects on intestinal functions.

Methods: Mice deficient for autophagy specifically in intestinal epithelial cells (Atg16l1flox/floxCreVillin) or wild-type mice were fed a control or HFHS diet for 16 weeks and infected with the AIEC LF82 strain. Levels of autophagy markers (LC3-II, SQSTM1/p62, autophagy-related mRNAs) and anti-microbial peptides (AMPs) were assessed by Western blot, qRT-PCR and immunofluorescent staining. *E. coli* strains were isolated and characterized for AIEC phenotype.

Results: In wild-type mice, HFHS diet induced autophagy defect in IECs, as shown by decreased LC3-II and accumulated SQSTM1/p62 protein levels, decreased autophagy-related mRNA levels, and lack of autophagy induction upon AIEC LF82 infection. Combination of HFHS diet and intestinal autophagy deficiency decreased AMP levels, increased intestinal inflammation, enhanced expansion of *E. coli* strains with AIEC characteristics while decreasing abundance of several bacteria that can activate autophagy. This combination also increased host susceptibility to AIEC LF82 infection.

Conclusion: HFHS diet induced dysregulated autophagy, and combination of defective autophagy and HFHS diet caused abnormalities in IEC functions, promoted AIEC emergence, reduced autophagy-activating bacteria, and increased host susceptibility to AIEC LF82 infection, thereby exacerbating intestinal inflammation.



P25

Molecular regulation of autophagy
Poster

Oncogenic DMTF1 β promotes cancer cell motility by regulating autophagy

Anna M. Schl fli¹, Igor Tokarchuk^{1,2}, Magali Humbert¹, Federico La Manna³, Bich Vu⁴, David Maul¹, Ramin Radpour^{5,6}, Marianna Kruithof-de Julio³, Inti Zlobec¹, Joern Dengjel⁴, Bruce E. Torbett^{7,8,9}, Mario P. Tschan^{1,2}

¹ Institute of Tissue Medicine and Pathology, Division of Experimental Pathology, University of Bern, Switzerland

² Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

³ Department of Biomedical Research, Division of Urology, University of Bern, Switzerland

⁴ Department of Biology, University of Fribourg, Switzerland

⁵ Department of Biomedical Research, Division of Tumor immunology, University of Bern, Switzerland

⁶ Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Switzerland

⁷ Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA, USA

⁸ Department of Pediatrics, School of Medicine, University of Washington, Seattle, WA, USA

⁹ Center for Immunity and Immunotherapies, Seattle Children's Research Institute, Seattle, WA, USA

DMTF1 is an established tumorsuppressor. Far less is known about its splice variant, DMTF1 β , a truncated isoform that acts as an oncogene in breast cancer. How this variant actually drives cancer progression, however, has remained an open question.

To address this, we profiled DMTF1 β protein levels across a panel of breast cancer lines and found higher DMTF1 β expression in more aggressive and metastatic breast and prostate cancer cell lines. Experiments reinforced this link: Silencing DMTF1 β in invasive MDA-MB-231 breast and PC3M-Pro4 prostate cancer cells substantially impaired both cancer cell migration and invasion.

To identify the pathway underlying this phenotype, we combined interactome profiling of DMTF1 β -overexpressing cells with transcriptomic analysis of knockdown cells. Both approaches found the autophagy-lysosome recycling pathway, amongst others, as a downstream effector of DMTF1 β . We experimentally validated these findings by showing (1) that DMTF1 β interacts with ULK1 and ATG13 and (2) that DMTF1 β positively regulates autophagic flux. Cycloheximide chase assays further revealed that ULK1 protein stability is decreased upon DMTF1 β depletion. This potentially explains the lower autophagy levels in DMTF1 β knock-down cells. Interestingly, DMTF1 β -mediated migration is dependent on ULK1 and VPS34, suggesting a link between DMTF1 β induced autophagy and migration. In contrast, inhibiting autophagosome-lysosome fusion with Bafilomycin A1 or by knocking-down LAMP2 did not impact on DMTF1 β -mediated migration. We therefore speculate that degradative autophagy is not crucial for the migratory and invasive properties of DMTF1 β over-expressing cells. Furthermore, we found DMTF1 β in the supernatant of A549 cells. Preliminary data suggest that its actively secreted in rather than passively released as a byproduct of basal cell death, such as LDH. Thus, DMTF1 β may act as both a regulator and a cargo of secretory autophagy. Current research focuses on deciphering the mechanism connecting DMTF1 β , autophagy and cell motility.

Loss of chaperone-mediated autophagy uncouples hepatic and systemic glucose metabolism in rainbow trout

Steffi Reji¹, Emilio J. Vélez², Kanchan Phadwal³, Daniel Macqueen³, Karine Dias¹, Vincent Véron¹, David Bartrès Gordils¹, Jessica Calo Rodriguez¹, Alexandre Stella^{4,5}, Odile Burlet-Schiltz^{4,5}, Lisa M Radler⁶, Stéphane Panserat¹, Florian Beaumatin¹, Beth Cleveland⁶, **Iban Seiliez**¹

¹ Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, UMR1419 Nutrition Métabolisme et Aquaculture, Saint-Pée-sur-Nivelle, France, 64310

² Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain

³ The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, Scotland

⁴ Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France

⁵ Infrastructure Nationale de Protéomique, ProFI, FR 2048, Toulouse, France

⁶ National Center for Cool and Cold-Water Aquaculture, ARS/USDA, 11861 Leetown Rd, 25430 Kearneysville, WV, USA

Chaperone-mediated autophagy (CMA) is a selective lysosomal degradation pathway that regulates proteostasis and metabolic adaptation, yet its systemic role in vertebrate nutrient handling remains incompletely defined.

Here, we investigated the physiological consequences of complete CMA ablation in rainbow trout (RT, *Oncorhynchus mykiss*), a naturally glucose-intolerant vertebrate species, by generating a double knockout line lacking both Lamp2a isoforms encoded by the two salmonid-specific lamp2 paralogs. Using comparative proteomics of CMA-competent lysosomes, we defined the first CMA degradome in a non-mammalian vertebrate, identifying a conserved set of substrates related to glycolysis, lipid metabolism, mitochondrial function, and growth signaling pathways. A substantial fraction of substrates exhibited Lamp2a-dependent lysosomal degradation and were enriched for KFERQ-like motifs, supporting conservation of CMA substrate recognition mechanisms between distant vertebrate groups. Functionally, CMA deficiency improved systemic glucose tolerance and enhanced growth under dietary carbohydrate excess. This occurred despite marked suppression of hepatic glucose utilization, characterized by reduced glycogen accumulation, lower lactate production, and coordinated downregulation of lipogenic and carbohydrate catabolic pathways. Proteomic analyses further revealed a metabolic shift of the liver, with increased signatures of fatty acid oxidation, mitochondrial metabolism, and oxidative stress responses, concomitant with repression of anabolic carbohydrate-driven programs.

Together, these findings demonstrate that CMA acts as a key regulator of tissue-specific nutrient partitioning in RT, uncoupling hepatic glucose utilization from whole-body glucose handling. This study establishes RT as a comparative model for CMA biology and reveals an evolutionarily conserved role for CMA in coordinating systemic metabolic adaptation to carbohydrate availability.

An annexin involved in adaptation to Heat Stress in *Caenorhabditis elegans*

Claudia Serot^{1,2,4}, Melina Gallopin³, Guillaume Sgard^{1,4}, Céline Largeau¹, Mickael Briens⁴, Alain Lescure⁵, Renaud Legouis^{1,2}

¹ Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

² Inserm

³ Université Paris-Saclay

⁴ Adisseo France SAS

⁵ Institut de biologie moléculaire et cellulaire

When temperatures exceed the optimal range for an organism or a cell, heat stress (HS) triggers several processes that contribute to the maintenance of homeostasis. Adaptation involves evolutionarily conserved regulatory mechanisms acting at both the organismal and cellular levels, including the heat stress response and autophagy, which contribute to the maintenance of proteostasis. However, adaptation and resilience can vary substantially between individuals.

We have previously shown that *C. elegans* is a relevant model for investigating this inter-individual variability. This project aims to better characterize the mechanisms underlying resistance and adaptation to heat stress in *C. elegans*.

First, using a descriptive approach based on Gaussian mixture models, we identified that the variability can be modeled into 2 subpopulations after HS, that differ in developmental recovery, fertility, longevity, and hormetic response.

Then, we investigated the contribution of differentially regulated genes to the stress response, using RNAi and deletion mutants. By combining linear mixed-effects models with mixture models, we characterized how gene depletion affects each subpopulation. Interestingly, this approach identified annexin genes as potential contributors to adaptation to HS, particularly NEX-2, whose depletion is associated with an impairment of autophagic flux.

Exploring the functions of selective autophagy receptors at the virus / host interface

Jules Blanc¹, Hanane El Manssouri¹, Chloé Fournié¹, Laura Dejardin¹, Samir Merabet², Arnaud Moris³, Audrey Esclatine³, Marie Galloux⁴, Chloé Journo¹

¹ Centre International de Recherche en Infectiologie (CIRI), Retroviral Oncogenesis, Inserm U1111-Université Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, Université Lyon, Lyon, France

² Institut de génomique fonctionnelle de Lyon (IGFL), Ontogenesis and Molecular Interactions, CNRS, UMR5242, Université Claude Bernard Lyon 1, Ecole normale Supérieure de Lyon, Université Lyon, Lyon France

³ Institut de Biologie Intégrative de la Cellule (I2BC), Autophagy and antiviral immunity, CEA, CNRS, UMR 9198, Université Paris-Saclay, Gif-sur-Yvette, France

⁴ Unité de Virologie et Immunologie Moléculaires (VIM), Molecular biology of Pneumovirus, INRAE, UVSQ, UMR 0892, Université Paris-Saclay, Jouy-en-Josas, France

Selective autophagy receptors (SARs) stand at the crossroad of cell-intrinsic antiviral immune processes: while they can directly address specific viral components to autophagic degradation, they are also modulators of innate signalling pathways, through both autophagy-dependent and -independent mechanisms that are still to be explored.

In this ongoing project, we posit that the central position of SARs in anti-viral processes may have selected them upon evolution to be commonly hijacked by various viruses, and that, in turn, investigating how various viruses manipulate SARs could help us broaden our understanding of SARs' functions. More specifically, this project explores how Epstein-Barr Virus (EBV)-encoded BHRF1, Respiratory Syncytial Virus (RSV)-encoded nucleoprotein, Human T-cell Leukemia Virus type 1 (HTLV1)-encoded Tax proteins, all three proteins previously linked to SARs' functions, shape the signalling landscape of SARs.

Using co-immunoprecipitation and bimolecular fluorescent complementation assays (BiFC), we first showed that these three viral proteins are physically interacting with all tested SARs (p62, OPTN, TAX1BP1, NDP52 and NBR1). We are currently aiming at identifying the cell compartments or organelles in which these interactions take place, and how the organisation of these compartments could be changed upon interaction. In parallel, we have set up an *in situ* proximity-labelling interactomics approach to determine how these viral proteins are changing the SARs' interactomes, leading to possible viral manipulation of SARs' functions.

Collectively, this ongoing project contributes to the exploration of SARs' immune functions and could help establish them as key factors at the host / pathogen interface.



P29

Molecular regulation of autophagy
Poster

Impact of increased basal autophagy on age-related decline in skeletal muscle regenerative capacity

Maxime Sorro¹, Xurde Menendez Caravia², Natalia Alvarez-Darias¹, Laureen Albertos¹, Salwa Sebti¹

¹ Institute of Regenerative Medicine and Biotherapy (IRMB), University of Montpellier, INSERM, U1183, Montpellier, France

² IBBTEC 39011 Santander Spain - Universidad de Cantabria

Skeletal muscle undergoes profound changes during aging, characterized by a progressive decline in muscle mass, function, and regenerative capacity. This deterioration is associated with impaired cellular homeostasis, accumulation of damaged components, and reduced efficiency of repair mechanisms. Among the key processes involved in maintaining muscle integrity, autophagy plays a central role. In skeletal muscle, autophagy is essential for maintaining mitochondrial homeostasis, muscle fiber integrity, and muscle stem cell function. Conversely, its decline and dysregulation contribute to impaired muscle maintenance and regeneration associated with aging and in several muscle disorders.

In this context, previous studies have shown that autophagy is required for muscle stem cell homeostasis and that restoring autophagy in old stem cells improves their regenerative potential. However, whether increased basal autophagy *in vivo* is sufficient to preserve the regenerative capacity of aged skeletal muscle remains unknown.

To address this question, we used Beclin-1 F121A knock-in (KI) mice, a genetic model of constitutively enhanced autophagy in which the inhibitory interaction between Bcl-2 and Beclin-1 is disrupted throughout life. Acute skeletal muscle injury was induced in aged KI and wild-type (WT) mice and regeneration was analysed 21 days post-injury by histological and immunohistochemical approaches.

Our preliminary results suggest that genetically enhanced autophagy improves muscle regeneration in aged skeletal muscle with larger CSA, indicating that autophagy promotes regeneration and maturation of newly formed myofibers. Further analysis will thus be performed to better characterise muscle structural remodelling as well as functional recovery.

Moving forward, we aim to elucidate how autophagy orchestrates muscle regeneration at the cellular and molecular levels. We are currently implementing high-resolution transcriptomic approaches, including single-nucleus RNA sequencing (snRNA-seq), to define how enhanced autophagy reshapes the transcriptional landscape of the distinct cell populations within the regenerative niche.



P30

Selective autophagy
Poster

Dissecting MHC-II-restricted endogenous antigen presentation using viral proteins: the influence of HTLV-1 Tax

Léa Tantet¹, Gabriela Sarango¹, Annika Nelde², Emelyne Le Lay¹, Damien Glon¹, Clémence Richetta³, Audrey Esclatine¹, Hélène Dutartre⁴, Juliane Walz⁵, Bénédicte Manoury⁶, Chloé Journo⁴, Arnaud Moris¹

1 Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

2 Department of Peptide-based Immunotherapy, University and University Hospital Tübingen, 72076, Tübingen, Germany

3 LBPA, ENS-Paris Saclay, CNRS UMR8113, Université Paris Saclay, Gif-sur-Yvette, France

4 International Center for Research in Infectiology, Retroviral Oncogenesis Laboratory, INSERM U1111 - Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Université Lyon, Lyon, France

5 Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), Department of Internal Medicine, University Hospital Tübingen, Tübingen, Germany

6 Institut Necker Enfants Malades, INSERM U1151-CNRS UMR 8253, Faculté de médecine Necker, Université de Paris, Paris, France

CD4⁺ T cells, key determinants of cellular and humoral immunity, recognize peptides presented by MHC-class-II (MHC-II) molecules. These peptides derive from exogenous and endogenous antigen degradation pathways involving autophagy-dependent and independent mechanisms. In particular, selective autophagy receptors (SLRs) such as p62, TAX1BP1, OPTN, NDP52, and NBR1 regulate autophagy and vesicular trafficking, modulating MHC-II-restricted antigen presentation. Remarkably, the HTLV-1 oncoprotein Tax induces a signalosome at peri-Golgi structures of infected T cells through interaction with IKK γ (NEMO), leading to constitutive NF- κ B activation. This process depends on Tax specific domains' ubiquitination and on its interaction with SLRs. Thereafter using Tax, we investigated whether Tax might interfere with endogenous MHC-II-restricted antigen presentation, focusing on the role of SLRs.

We confirm that, in antigen-presenting cells (APCs), Tax expression induces relocation of SLRs and IKK γ into Tax-derived cytoplasmic condensates forming the signalosome. We next asked whether Tax affects antigen presentation. APCs expressing Tax and a model antigen (HIV-Gag) were co-cultured with Gag-specific CD4⁺ T cells. Tax expression reduces the capacity of APCs to activate CD4⁺ T cells, although this effect is not significantly altered by SLRs knockout. Immunopeptidome analysis revealed that Tax modifies the repertoire of MHC-II-presented peptides without significantly affecting their binding affinity. Mutational analysis identified lysines 4–6 as critical ubiquitylation sites required for Tax-mediated inhibition of CD4⁺ T cells activation. Additionally, Tax increased cathepsin B/L and S activity, suggesting altered vesicular pH in the MHC-II loading compartment.

Future work will assess signalosome formation in SLR knock-out cells and validate these findings in dendritic cells using CMV-specific CD4⁺ T cell responses.



P31

Molecular regulation of autophagy
Poster

A Novel *in vitro* model of intracerebral hemorrhage reveals autophagy-dependent neuronal death and a time-dependent neuroprotective role of tPA

Audrey Thiebaut¹, Denis Vivien¹, Benoit D. Roussel¹, Richard Macrez¹

¹ Normandie Université, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders (PhIND), GIP Cyeron, Institut Blood and Brain @ Caen-Normandie (BB@C), 14000 Caen, France

Intracerebral haemorrhage (ICH) is a devastating condition affecting over 3 million people annually worldwide, characterized by haematoma formation and severe secondary brain injury, including excessive peri-haematoma autophagy. Updated 2022 guidelines advocate for minimally invasive surgery (MIS) using thrombolytics (tissue type plasminogen activator, tPA or urokinase, uPA) for haematoma evacuation to reduce mortality. As previous *ex vivo* studies demonstrated that these drugs can diffuse through the porous haematoma into surrounding tissues, understanding their impact on ICH-induced neuronal autophagy is critical.

Current *in vitro* models relying on isolated toxic factors (e.g., thrombin, heme, iron) fail to capture the complex toxicity of the haematoma microenvironment. To address this translational gap, we developed a novel *in vitro* model utilizing the complex exudate released from clinical-size human haematomas (clotted *ex vivo* for 16h). Primary cortical neurons were exposed to varying concentrations of this exudate (2%, 5%, 10% v/v) to evaluate cell viability and autophagic flux. We subsequently investigated the specific impacts of tPA and uPA co-treatments.

Exposure to 5% and 10% exudate, but not 2%, induced neuronal death accompanied by autophagic flux. This was evidenced by a significant accumulation of LC3-II and degradation of p62 initiating as early as 3 hours and persisting at 24 hours. Crucially, pharmacological blockade of autolysosomal degradation using E64d/Pepstatin A significantly reduced 10% exudate-induced neuronal death at both 6h (20.1% vs. 49.8%, $p < 0.001$) and 24h (56.9% vs. 76.1%, $p < 0.001$), confirming an autophagy-dependent cell death mechanism. Having established this autophagy-dependent toxicity, we then investigated thrombolytic interventions. A 6-hour co-treatment with tPA (37.1% vs. 47.5%, $p = 0.0007$), but not uPA (40.8% vs. 46.5%, ns), significantly decreased exudate-induced cell death. However, when tPA administration was delayed by 3 hours, after the autophagic cascade was already initiated, its neuroprotective effect was lost, though it did not exacerbate toxicity.

Our novel *in vitro* model successfully recapitulates autophagy-dependent neuronal death observed in ICH. We demonstrate that tPA, unlike uPA, exerts a specific neuroprotective effect against exudate toxicity. However, its therapeutic window is highly time-dependent and requires administration before the irreversible commitment to autophagic cell death.



In vivo assessment of autophagic flux using Thy1-RFP-GFP-LC3 mice following ischemic stroke

Evalena Thore¹, Pauline Marie¹, Denis Vivien^{1,2}, Eloise Lemarchand¹, Benoit D. Roussel¹

¹ Normandie University, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders (PhIND), GIP Cyceron, Institut Brain, Blood & Memory @ Caen-Normandie (BBM@C), France

² CHU Caen, Clinical Research Department, CHU de Caen Côte de Nacre, Caen, France

Stroke is one of the leading causes of death and long-term disability worldwide. It comprises two major subtypes: hemorrhagic and ischemic, with ischemic strokes accounting for approximately 85% of all cases. Ischemic stroke results from the occlusion of a cerebral artery, leading to a reduction or complete interruption of cerebral blood flow. To date, intravenous recombinant tissue-type plasminogen activator (r-tPA) remains the only approved pharmacological treatment for the acute treatment of ischemic stroke. Ischemic stroke induces profound cellular stress disrupting neuronal homeostasis and triggering autophagy, a process that can either promote cell survival or contribute to neuronal death depending on the context. We previously demonstrated that neuronal exposure to tPA inhibits the autophagy through activation of the PI3K/AKT/mTOR pathway, thereby promoting neuroprotection.

In this study, we investigated the effect of ischemic stroke and tPA mediated thrombolysis on neuronal autophagy in vivo using transgenic mice expressing the tandem fluorescent autophagy reporter mRFP-eGFP-LC3 protein, under the neuron-specific Thy1 promoter. This model, enables real time visualization and quantification of neuronal autophagic flux by distinguishing autophagosomes from intermediate and fully acidified autolysosomes following thromboembolic stroke. High resolution confocal microscopy combined with three-dimensional image reconstruction and analysis allowed us to characterize neuronal autophagic flux at 4 and 24 hours after ischemia. This approach provides a spatial and temporal analysis of autophagy at a single-neuron resolution by discriminating autophagosomes, partially acidified autolysosomes and fully acidified autolysosomes. Our preliminary results show that ischemic stroke dynamically remodels neuronal autophagic flux over time. Ongoing analysis will determine how r-tPA-mediated thrombolysis influence these autophagic responses after stroke. Unlike conventional approaches based on static markers such as LC3 or p62, our strategy tracks the successive stages of autophagosome formation and maturation in individual neurons, thus providing a dynamic assessment of the autophagic flux in vivo. A better understanding of neuronal autophagy following ischemic stroke may uncover novel therapeutic targets and supports the development of innovative neuroprotective strategies.



P33

Selective autophagy
Poster



Exploring mitophagy regulation under distinct metabolism conditions

Diana Tomsa^{1,2}, Mickaël M. Cohen, Naïma Belgareh-Touzé, Manuel S. Rodriguez

1 UPR8241 - Laboratoire de chimie de coordination 205 Route de Narbonne 31077 TOULOUSE CEDEX 4

2 UMR8226/CNRS/SU, 13 rue Pierre et Marie Curie, 75005, Paris

Autophagy is a conserved cellular degradation pathway that maintains cellular homeostasis through the recycling of damaged organelles, protein aggregates, and other cytoplasmic components. Among its selective forms, mitophagy specifically targets dysfunctional or excess mitochondria for degradation and is essential for preserving mitochondrial quality and function. The physiological importance of mitophagy is highlighted by its involvement in various pathological conditions, including neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, as well as cancer.

In mammals, mitophagy can occur through two major mechanisms: receptor-dependent (ubiquitin-independent) or ubiquitin-dependent pathways. In the receptor-dependent pathway, mitochondrial outer membrane (MOM) proteins, carrying LC3/ATG8-interacting region (LIR) motifs, including NIX/BNIP3L (BCL2 interacting protein 3 like), BNIP3, and FUNDC1 (Fun14 domain containing 1).

NIX/BNIP3L is required for mitophagy during neuronal differentiation, whereas BNIP3 and FUNDC1 primarily mediate hypoxia-induced mitophagy. These proteins function as mitophagy receptors by directly binding to LC3/GABARAP proteins on the growing autophagosome membrane.

In contrast, the ubiquitin-dependent pathway is initiated by the phospho-ubiquitination of MOM proteins. The phosphorylated ubiquitin chains are recognized by soluble autophagy binding adaptors, primarily OPTN (opineurin) and NDP52 as well as p62/SQSTM1, TAX1BP1, and NBR1. These adaptors possess both ubiquitin-binding domains and LC3-interacting region (LIR) motifs, enabling them to bridge ubiquitinated mitochondria to LC3-family protein on the autophagosomal membrane, thereby promoting mitochondrial sequestration into autophagosomes.

In the budding yeast *Saccharomyces cerevisiae*, mitophagy is mediated by the receptor Atg32 (Autophagy related protein 32). Although Atg32 is currently the only established mitophagy receptor in yeast, it remains unclear whether additional receptors contribute to the process. Unlike in mammals, the existence of a canonical ubiquitin-dependent mitophagy pathway has not yet been demonstrated.

The aim of the study is to investigate and identify if there are other mitophagy receptors other than Atg32, and demonstrate the existence or not of a ubiquitin-dependent pathway. Given that Atg8/LC3 is a molecular hub through which mitophagy receptors recruit the autophagosomal membrane, LC3-traps were used to characteriz



P34

Molecular regulation of autophagy

Poster

Impact of dietary fatty acids on retinal autophagy: focus on saturated fatty acid intake in a low-fat diet

Yetong Wei¹, Jean-Baptiste Bizeau^{1,2}, Bénédicte Buteau¹, Lucy Martine¹, Stéphane Grégoire¹, Laurent Leclère¹, Elisabeth Dubus^{3,4}, Alain M. Bron⁵, Niyazi Acar⁶, Marie-Agnès Bringer¹

¹ « Eye and Nutrition » Research Group, Université Bourgogne Europe, Institut Agro, CNRS, INRAE, UMR CSGA, 21000 Dijon, France

² Université Paris Cité, CNRS, Inserm, Institut Cochin, F-75014 Paris, France (Present affiliation)

³ ChemoSens Facility, Université Bourgogne Europe, Institut Agro, CNRS, INRAE, UMR CSGA, 21000 Dijon, France

⁴ INRAE, PROBE Research Infrastructure, ChemoSens Facility, Dijon, France

⁵ « Eye and Nutrition » Research Group, Université Bourgogne Europe, CHU Bourgogne, Department of Ophthalmology, Institut Agro, CNRS, INRAE, UMR CSGA, 21000 Dijon, France

⁶ « Eye and Nutrition » Research Group, Université Bourgogne Europe, CHU Bourgogne, Research and Public Health Division, Institut Agro, CNRS, INRAE, UMR CSGA, 21000 Dijon, France

The retina is the light-sensitive tissue lining the back of the eye that converts light into neural signals. It is characterized by a high content of polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid, an omega-3 PUFA essential for retinal homeostasis and vision. Dietary fatty acids play a key role in retinal physiology. While the benefits of omega-3 PUFA-rich fish consumption are well established, high-fat diets (HFDs) have been shown to impair retinal homeostasis. However, the effects of dietary saturated fatty acids (SFAs) within a low-fat diet (LFD) remain poorly understood.

Autophagy is a fundamental cellular process that maintains homeostasis and whose regulation and machinery depend on lipids. It is highly responsive to dietary lipid composition, including HFDs, and plays a critical role in preserving retinal structure and function. Impaired autophagy has been linked to physiological retinal aging and age-related macular degeneration, a leading cause of irreversible central vision loss in older adults in high-income countries.

Here, we investigated whether varying the proportion of SFAs in an LFD influences retinal autophagy and age-related retinal changes, and whether omega-3 PUFA supplementation can prevent these alterations. One-month-old C57BL/6J mice were fed for 9 or 18 months with three isocaloric diets containing 5% fat and an omega-6/omega-3 PUFA precursor ratio of 5:1, differing only in SFA and long-chain n-3 PUFA content. Autophagy was assessed by the analysis of autophagic flux (ex vivo flux inhibition and Western blot), autophagy-related gene expression (RT-qPCR), and protein analysis (Western blot). Retinal fatty acid composition (GC-FID), function (electroretinography), and inflammation (RT-qPCR and immunostaining) were also evaluated. Our findings demonstrate that dietary SFA and omega-3 PUFA levels modulate retinal autophagy and influence retinal physiology during aging.



LIST OF PARTICIPANTS

LAST NAME	First Name	Professional e-mail	ST/PT/Poster
ACIER	Angéline	angelina.acier@lcc-toulouse.fr	FT1, P3
AKKOC	Yunus	yakkoc@ku.edu.tr	O17
AKROUF	Ines	ines_fatma.akrouf@etu.sorbonne-universite.fr	
ALEMANY	Carla	carla.alemany@inserm.fr	O3, O22
AMIRI-CZAJKOWSKI	Dobrawa	dobrawa.amiri@inserm.fr	O1, O3
BEAUCLAIR	Guillaume	guillaume.beauclair@u-pariscite.fr	O6, O16
BELGAREH-TOUZÉ	Naïma	belgareh@ibpc.fr	O20, P2, P33
BERLIOZ-TORRENT	Clarisse	clarisse.berlioz@inserm.fr	FT4, P4, P11
BIZEAU	Jean-Baptiste	jean-baptiste.bizeau@inserm.fr	P34
BLANC	Jules	jules.blanc1@ens-lyon.fr	FT9, P28
BOIZIAU	Claudine	claudine.boiziau@inserm.fr	
BONI	Vincent	vincent.boni@inserm.fr	P4
BOUCHER	Camille	camille.boucher@inserm.fr	O9
BOUKHALFA	Asma	boukhalfa.asma@mayo.edu	P5
BOUKRIBA	Imene	imeneboukriba@gmail.com	FT6, P19
BRINGER	Marie-Agnès	marie-agnes.bringer@inrae.fr	P17, P34
BRUQI	Krenare	krenare.bruqi@univ-lyon1.fr	O18
CAMUS	Guillaume	guillaume.camus.etu@univ-lille.fr	P6
CANTALOUBE	Guilhem	guilhem.cantaloube@irim.cnrs.fr	O10
CARDOSO MORENO	Eunice	eunice.cardoso-moreno@i2bc.paris-saclay.fr	O6
CHAPUY-REGAUD	Sabine	chapuy-regaud.s@chu-toulouse.fr	P7
CLAUDE-TAUPIN	Aurore	aurore.claude-taupin@inserm.fr	O8, O11, O14
DA COSTA	Anaëlle	anaelle.dacosta@thxpharma.com	
DARBAS	Tiffany	tiffany.darbas@chu-limoges.fr	F2, P8
DAUSSY	Coralie	coralie.daussy@irim.cnrs.fr	O10
DE PALMA	Fatima	fatimadomenicaelisa.depalma@unina.it	F3, P9
DEHISSI	Samy	samy.dehissi@inserm.fr	P10
DESSUS	Antoine	adessus@mrclmb.ac.uk	O2
DJAVAHERI-MERGNY	Mojgan	djavamojgan@gmail.com	P10, P21



LIST OF PARTICIPANTS

LAST NAME	First Name	Professional e-mail	ST/PT/Poster
DUCREUX	Amandine	amandine.ducieux@ube.fr	O11, FT13
ESCLATINE	Audrey	audrey.esclatine@universite-paris-saclay.fr	O6, O10, O19, FT9, P28, P30
FAURE	Mathias	mathias.faure@inserm.fr	
FLIPO	Thibaut	thibaut.flipo@inserm.fr	FT4, P11
FOUILLARD--UZEL	Jean-Charles	fouillarduzel@cyceron.fr	P12, P14
FRENGER	Quentin	quentin.frenger@curie.fr	O3
GALLOUX	Marie	marie.galloux@inrae.fr	FT9, P28
GANLEY	Ian	i.ganley@dundee.ac.uk	Lecture
GARAMPON	Flavie	flavie.garampon@inserm.fr	O14
GLON	Damien	damien.glon@i2bc.paris-saclay.fr	O16, O19, P30
GREGOIRE	Sylvie	sylvie.gregoire@i2bc.paris-saclay.fr	
GUILLEMARD	Loïs	lois.guillemard@u-bourgogne.fr	FT5, P13
GUYARD	Valentin	valentin.guyard@unifr.ch	
HAMOOD	Alexandra	alexandra.hamood@i2bc.paris-saclay.fr	O16
HENNA	Juliette	juliette.henna1@orange.fr	O7, FT6, P19
HERNANDEZ	Eva	eva.hernandez@universite-paris-saclay.fr	O6, O16, O19
ISRAELI	David	israeli@genethon.fr	O26
IZQUIERDO BERMEJO	Sara	sizqui03@ucm.es	
JACQUIN	Elise	elise.jacquin@inserm.fr	O7, FT6, P17, P19
JOFFRE	Carine	carine.joffre@inserm.fr	Lecture, O15
JOUBERT	Pierre-Emmanuel	pierre-emmanuel.joubert@sorbonne-universite.fr	
JOURNO	Chloé	chloe.journo@ens-lyon.fr	FT9, P28, P30
JUDITH	Delphine	delphine.judith@inserm.fr	P4
KERMORGANT	Stephanie	s.kermorgant@qmul.ac.uk	
KONG	Dejun	dejun.kong@i2bc.paris-saclay.fr	P15
KOSUTH	Thibault	thibault.kosuth@u-bordeaux.fr	O4
KRETZ-REMY	Carole	carole.kretz@univ-lyon1.fr	
LAPAQUETTE	Pierre	pierre.lapaquette@ube.fr	O11, FT5, P13, P17



LIST OF PARTICIPANTS

LAST NAME	First Name	Professional e-mail	ST/PT/Poster
LAPIERRE	Louis R.	louis.rene.lapierre@umoncton.ca	Lecture
LARGEAU	Céline	celine.largeau@i2bc.paris-saclay.fr	FT7, P15, P18, P27
LARTIGAU	Mathilde	mathilde.lartigau@u-bordeaux.fr	O13
LAURENT	Gwenaëlle	gwenaelle.laurent@institutimagine.org	O24
LEGOUIS	Renaud	renaud.legouis@i2bc.paris-saclay.fr	FT7, P15, P18, P21, P27
LIANG	Yulan	yulan.liang@i2bc.paris-saclay.fr	
LIU	Ying	ying.liu1@pasteur.fr	
LOUESSARD	Morgane	louessard@cyceron.fr	P12, P14
LUSSIGNOL	Marion	marion.lussignol@i2bc.paris-saclay.fr	O10, O16, O19
MANOURY	Bénédicte	benedicte.manoury@inserm.fr	FT10, P20, P30
MARIBEL	Lara Corona	maribel.lara-corona@inserm.fr	O8, O14
MAZZOLA	Lucie	lucie.mazzola@inserm.fr	FT10, P20
MEIRE	Valentin	valentin.meire@i2bc.paris-saclay.fr	P21
MERCIER	Chloé	chloe.mercier02@edu.univ-fcomte.fr	O25
MERLET	Laura	laura.merlet@etu.univ-nantes.fr	P22
MORCILLO PARRA	Maria Ángeles	morcillo@ibpc.fr	O20
MOREL	Etienne	etienne.morel@inserm.fr	O1, O3, O8, O11, O14, O22
MORIS	Arnaud	arnaud.moris@i2bc.paris-saclay.fr	O6, O10, FT9, P28, P30
MULLER	Sylviane	sylviane.muller@unistra.fr	
MURALT	Tanja	tanja.muralt@unibe.ch	O12
NGUYEN	Hang	hang.nguyen@uca.fr	P23, P24
POILLET	Laura	laura.poillet@inserm.fr	O15
PRIGENT	Magali	magali.prigent@i2bc.paris-saclay.fr	
RADOUA	Abdelmnim	abdelmnim.radoua@inserm.fr	O7, FT6, P19
ROBIN-ENNESSER	Mathilde	mathilde.robinennesser03@gmail.com	
RODRIGUEZ	Manuel S.	manuel.rodriguez@lcc-toulouse.fr	O21, FT1, P3, P21, P33
ROGER	Benoit	benoit.roger@u-bordeaux.fr	O13



LIST OF PARTICIPANTS

LAST NAME	First Name	Professional e-mail	ST/PT/Poster
ROMAGNOLO	Béatrice	beatrice.romagnolo@inserm.fr	O9
ROUSSEL	Benoit	broussel@cyceron.fr	FT8, P12, P14, P31, P32
ROZIERES	Aurore	aurore.rozieres@inserm.fr	
RUBIOLA	Alessia	alessia.rubiola@inserm.fr	O9
SALLE	Emylie	emylie.salle@etu.u-bordeaux.fr	
SCHLÄFLI	Anna	anna.schlaefli@unibe.ch	O12, P25
SEBTI	Salwa	salwa.sebti@inserm.fr	P29
SEDDIKI	Marwan	marwan.seddiki@i2bc.paris-saclay.fr	O19
SEILIEZ	Iban	iban.seiliez@inrae.fr	P26
SEITE	Fanette	fanette.seite@i2bc.paris-saclay.fr	
SEROT	Claudia	claudia.serot@i2bc.paris-saclay.fr	FT7, P27
SHALHOUB	Hala	hala.shalhoub@inserm.fr	O21, FT1, P3
SIMON	Katja	katja.simon@mdc-berlin.de	Lecture
SORRO	Maxime	maxime.sorro@inserm.fr	P29
STRAPPAZZON	Flavie	flavie.strappazzon@univ-lyon1.fr	O18
TANTET	Léa	lea.tantet@i2bc.paris-saclay.fr	P30
THIEBAUT	Audrey	thiebaut@cyceron.fr	P31
THORÉ	Evalena	thore@cyceron.fr	FT8, P12, P32
TOMSA	Diana	diana.tomsa@lcc-toulouse.fr	P2, P33
TOOZE	Sharon	sharon.tooze@crick.ac.uk	Lecture
VERDIER	Mireille	mireille.verdier@unilim.fr	FT2, P8
VILLETTE	Cléa	clea.villette@inserm.fr	O23
WEI	Yetong	weiyetong811@gmail.com	P34
WODRICH	Harald	harald.wodrich@u-bordeaux.fr	O13
WOLLERT	Thomas	thomas.wollert@pasteur.fr	O5

