3rd micerco

# BOOK OF ABSTRACTS

Milan

25th – 26th march 2025

Dear Participants,

We are pleased to welcome you to the *3.0rd Meeting of the Italian C. elegans Research Community (M.I.C.e.R.Co.)*, a valuable opportunity to connect, exchange ideas, and strengthen our scientific community.

This meeting represents a unique occasion to foster scientific exchange and promote collaboration among researchers working with *C. elegans* both in Italy and abroad. Our objectives are to facilitate sharing ideas, materials, and knowledge while introducing the latest technologies and advanced equipment available to our community. We strongly believe that working together can drive innovation and push the boundaries of our understanding in this field.

We are honored to host distinguished speakers and enthusiastic participants from diverse backgrounds, including academia, industry, and young researchers. Their contributions will undoubtedly make this event a stimulating and enriching experience.

We would like to express our sincere gratitude to our sponsors—Nagi Bioscience, Nikon, Gilson, D-Tails, Zeiss, NemaSync, Suny Biotech, and The Company of Biologists—whose generous support has made this meeting possible. A special thanks also goes to our speakers and all attendees for their valuable participation and engagement.

We look forward to two days of inspiring discussions, fruitful collaborations, and new discoveries. Thank you for being part of this exciting event!

Warm regards,
*The M.I.C.e.R.Co Organizing Committee*

# 3rd micerco Milan | 25th - 26th march 2025

# EVENT PROGRAM

## Day 1 – Tuesday, March 25, 2025

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| 12.30 - 14.00 | **Registration and Welcome Light Lunch** | |
| 14.00 - 14.10 | Opening and Welcome | ***Christodolous Xinaris**, Scientific Coordinator of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*<br>***Lulsa Diomede**, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*<br>***Elia di Schiavi**, Institute of Biosciences and BioResources (IBBR), CNR, Naples, Italy* |
| 14.10 - 14.25 | The R of replacement in animal testing | ***Giuliano Grignaschi**, General Secretary of Research4life* |
| **Session 1** | **Molecular basis of Disease** | ***Chairpersons:***<br>***Eva Trevisson and Emilio Cusanelli*** |
| 14.25 - 15.00 | Small RNAs in Epigenetic Inheritance: A Lessons from Worms | ***Germano Cecere,** Institut Pasteur, Paris, France* |
| 15.00 - 15.15 | Erucin protects against polyglutamine-induced toxicity in Caenorhabditis elegans via aak-2/AMPK and daf-16/FOXO signaling | ***Martina Balducci,** University of Bologna, Italy* |
| 15.15 - 15.30 | Identification of new SMN-independent compounds to counteract Spinal Muscular Atrophy through an in vivo semi-automated drug screening in C. elegans | ***Roberto Iacomino,** Institute of Biosciences and BioResources (IBBR), CNR, Naples, Italy* |
| 15.30 - 15.45 | Metabolic and Mitochondrial Dysregulation in AL Amyloidosis: Insights from C. elegans | ***Margherita Romeo,** Istituto di Ricerche Farmacologiche Mario Negri IRCCS , Milan, Italy* |
| 15.45 - 16.00 | Dopaminergic system dysfunction in a C. elegans model of Spinal Muscular Atrophy | ***Giada Onorato,** Institute of Biosciences and BioResources (IBBR), CNR, Naples, Italy* |
| 16.00 - 16.15 | Unraveling TBP/TBP-1 and CHIP/CHN-1 interaction in a C. elegans model of human digenic SCA17 disease: does it affect neuron function? | ***Giuliana Madonna,** Fondazione IRCCS Istituto Neurologico Carlo Besta, and University of Milano-Bicocca, Milan, Italy* |
| 16.15 - 16.30 | Unlocking new frontiers in research with SydLab™ One: the ultimate solution for compounds and genetic studies, using C. elegans. | ***Alessandro Berto,** Nagi Bioscience* |
| 16.30 - 17.15 | **Coffee Break** | |
| **Session 2** | **Mechanisms of neuronal transmission** | ***Chairpersons:***<br>***Manuela D'Alessandro and Elia di Schiavi*** |
| 17.15 - 17.50 | Identification of synaptic organizers: the power of C. elegans genetics | ***Jean-Louis Bessereau,** Melis, Universite Claude Bernard Lyon 1, Institut NeuroMyoGene, Lyon, France* |
| 17.50 - 18.05 | Biophysical models of C.elegans neurons: a tool for elucidating the nematode neuronal dynamics | ***Martina Nicoletti,** Università Campus Bio-Medico di Roma, Rome, Italy.* |
| 18.05 - 18.20 | Exploring Prohibitins in Nervous System Development and Function | ***Fernandez-Abascal Jesus,** Andalusian Centre for Developmental Biology (CABD), CSIC-Universidad Pablo de Olavide-Junta de Andalucía and Department of Molecular Biology and Biochemical Engineering, Universidad Pablo de Olavide, Seville, Spain.* |

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| 18.20 - 18.35 | A genetic tool to monitor neuromodulation in vivo | ***Ivan Gallotta,*** *University College London, UK* |
| 18.35 - 18.50 | Study of Heteromeric Acetylcholine Receptor Composition and its Regulation by TMED-3 | ***Greta Maiellano,*** *Laboratoire MeLiS, Université Claude Bernard Lyon 1, Lyon, France* |
| 18.50 - 19.05 | Exploring neuronal mechanisms and therapeutic strategies in GNAO1 encephalopathy: insights from C. elegans model | ***Martina Di Rocco,*** *Istituto Superiore di Sanità, Rome, Italy* |
| 19.05 - 19.20 | Elucidating the Pathogenic Mechanisms of GNAO1-Associated Neurological Disorders through C. elegans Calcium Imaging and Evaluating the Therapeutic Potential of Caffeine | ***Enrico Lanza,*** *D-Tails* |
| 19.20 - 19.30 | Closing | |
| 19.30 - 21.00 | **Poster session and Dinner at Mario Negri Institute** | |

## Day 2 – Wednesday, March 26, 2025

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| 9.00 - 9.20 | **Registration** | |
| **Session 3** | **Mitochondria dysfunction and aging** | ***Chairpersons:*** ***Maria Elena Regonesi and Luca Pannone*** |
| 9.25 - 10.00 | ONE HEALTH: an integrative approach to mitochondria-regulated diseases and aging | ***Natascia Ventura,*** *Institute of Clinical Chemistry and Laboratory Diagnostic Medical Faculty, Heinrich Heine University and the IUF- Leibniz Research Institute for Environmental Medicine, DÜSSELDORF, Germany* |
| 10.00 - 10.15 | C16ORF70, A Novel Gene that Regulates Aging in C. elegans | ***Valeria Morbidoni,*** *Università degli Studi di Padova e Istituto di Ricerca Pediatrica, IRP, Città della Speranza, Padova, Italy* |
| 10.15 - 10.30 | Redox-mediated link between Ferroptosis and Aging in Caenorhabditis elegans: a role for fard-1 and dhs-25 | ***Roberta Pensotti,*** *University of Milano-Bicocca, Milan, Italy* |
| 10.30 - 10.45 | Investigating the function and regulation of the telomeric repeat-containing RNA TERRA in C. elegans | ***Emilio Cusanelli,*** *Department CIBIO, University of Trento, Trento, Italy* |
| 10.45 - 11.00 | Stomatin-like protein 2 mediates mitochondrial unfolded protein response through phospholipid sensing | ***Vozdek Roman,*** *Eurac research, Institute for Biomedicine, Bolzano, Italy* |
| 11.00 - 11.30 | **Coffee Break** | |
| **Session 4** | **Predictive toxicology** | ***Chairpersons:*** ***Luisa Diomede and Ivan Gallotta*** |
| 11.30 - 11.45 | Health-Boosting Potential of Coffee Silverskin: Insights from Caenorhabditis elegans | ***Emily Schifano,*** *Sapienza University of Rome, Rome, Italy* |
| 11.45 - 12.00 | The physicochemical properties of graphene oxide drive their toxicity in C. elegans | ***Carmina Natale,*** *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy* |
| 12.00 - 12.15 | Warns from Worms | ***Kai Niklas Kremers****, University of Urbino, Italy and the IUF- Leibniz Research Institute for Environmental Medicine, DÜSSELDORF, Germany* |
| 12.15 - 12.30 | **Closing Remarks** | Organizing Committee |

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# Oral Communications

## Erucin protects against polyglutamine-induced toxicity in *Caenorhabditis elegans* via aak-2/AMPK and daf-16/FOXO signaling

**Martina Balducci**[1], Julia Tortajada Pérez[2,3], Cristina Trujillo del Río[2,3], Mar Collado Pérez[2,3], Andrea del Valle Carranza[2,3], Ana Pilar Gomez Escribano[2,3,4], Rafael P. Vázquez-Manrique[2,3,4], and Andrea Tarozzi[1,5]

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The polyglutamine (polyQ) diseases consist of a group of nine neurodegenerative diseases (NDDs) caused by expanded cytosine-adenine-guanine repeats encoding a long polyQ tract in the respective proteins. These proteins are highly prone to lose their proper folding and form neurotoxic aggregates. Natural compounds capable of improving polyQ-induced NDDs are currently of great interest. In this regard, we demonstrated that erucin, an isothiocyanate released through the enzymatic hydrolysis of its precursor glucoerucin, naturally present in rocked salad leaves, and even released by the in vivo reduction of sulforaphane, which represents its oxidized form, modulates the aggregates accumulation in *C. elegans* models expressing polyQ in different tissues. In particular, through neuronal and muscular models of polyQ toxicity in worms we proved that erucin prevention from polyglutamine-induced aggregates formation is dependent on the catalytic subunit of AMP- activated protein kinase (AMPKα2), and that, downstream in this pathway, its action mediated by daf-16/FOXO, one of the key regulators of the aging and health span processes, since loss of function mutants of aak-2/AMPKα2 and daf-16 did not respond to the treatment, respectively. Although not caused by abnormal repetition of glutamines, but by aggregates formed by the protein alpha- synuclein (α-syn), Parkinson's Disease (PD), one of the of the most pervasive diseases in the world, was considered in our study, to extend number of diseases in which this natural compound is potentially protective. Through the *C. elegans* model of PD our results showed that erucin diminishes aggregates formation induced by α-syn expression and also exerts a behavioural effect, improving the motility capacity of worms. Taken together, these results provide the justifying basis for investigating additional signalling pathways potentially involved in erucin's action and its neuroprotective potential on additional models of NDDs in *C. elegans*.

## Identification of new SMN-independent compounds to counteract Spinal Muscular Atrophy through an in vivo semi-automated drug screening in *C. elegans*

**Roberto Iacomino**[1], Pamela Santonicola[1], Ilenia Matino[1], Giuseppina Zampi[1], Sandro Montefusco[2], Diego Medina[2,3], Ersilia Nicorvo[4], Daniela Maria Rasà[4], Serena Stanga[4], Alessandro Vercelli[4], Piera Smeriglio[5], Marina Boido[4], Elia Di Schiavi[1]

(1) Institute of Biosciences and Bioresources, IBBR, CNR, Naples, Italy; (2) Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy; (3) Department of Translational Medicine, University of Naples "Federico II", Naples, Italy; (4) Department of Neuroscience Rita Levi Montalcini, Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Turin, Italy; (5) Sorbonne Université, INSERM, Institut de Myologie, Centre de Recherche en Myologie, F-75013 Paris, France

Spinal Muscular Atrophy (SMA) is a neurodegenerative disease caused by the absence of the SMN1 protein, leading to the degeneration of motor neurons in the spinal cord, atrophy of the innervated muscles, and eventually patient death. Currently, three treatments approved by the FDA (Food and Drug Administration) and the EMA (European Medicines Agency) aim to restore the insufficient levels of the involved protein, significantly improving pathological conditions. However, these treatments have limitations, such as the patient's age and potential adverse effects. Therefore, a need for more comprehensive therapies has emerged, encompassing both SMN-dependent and SMN-independent strategies. To meet this need, we thought to use an alternative animal model to identify new small molecules that work in a SMN-independent way. We thus took advantage of the *C. elegans* SMA model we developed, where the ortholog of SMN1 is specifically silenced in motoneurons (MNs), causing an age-dependent neurodegeneration. The *C. elegans* SMA model displays several phenotypes that can partially revert to a wild-type condition when treated with candidate small neuroprotective molecules (e.g. Valproic acid), including the increase in motoneurons, made visible with GFP. This finding led to the development of a screening system based on drug repositioning, enabling an unbiased drug screening with a semi-automated high-content imaging system. With this approach we tested three FDA-approved libraries comprising 2,794 compounds, and allowed the analysis of 384 compounds per week, in triplicate, on living animals. With this strategy we identified 23 novel lead compounds that counteract smn-1 related neurodegeneration in *C. elegans*. Then, we validated the most promising compounds in a secondary screening and identified the dose-response curve and the time of action in our SMA model. Interestingly, one of the hit compounds, pimozide, has been recently published to be effective in another SMA model in *C. elegans*, thus strongly supporting the efficacy of our approach. Some of the most promising hits were also confirmed in vitro in various mammalian systems. We are now identifying the molecular pathways through which our drugs exert their function, and the tissues involved. Our results demonstrate that this system allows for the rapid identification of small molecules that suppress MNs degeneration by combining high-content imaging of living animals with drug-screening approaches.

## Metabolic and Mitochondrial Dysregulation in AL Amyloidosis: Insights from *C. elegans*

Maria Monica Barzago[1], Alessandro Corbelli[1], Fabio Fiordaliso[1], Silvia Maglioni[2,3], Natascia Ventura[2,3], Giada Andrea Cassanmagnago[1], Marco Bolis[1], Luisa Diomede[1], **Margherita Romeo**[1]

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Immunoglobulin light chain (AL) amyloidosis is a severe multisystem disorder with a global incidence of 5.1 to 12.8 cases per million person-years. It results from the deposition of misfolded immunoglobulin light chains (LCs) in tissues, leading to progressive organ dysfunction. Cardiac involvement, often manifesting as fatal cardiomyopathy, is a key determinant of patient survival. However, the mechanisms driving LC-induced cardiac damage remain poorly understood, and developing an effective animal model is crucial for advancing research in this field. To address this gap, we established a new transgenic model of *Caenorhabditis elegans*, expressing a human amyloidogenic λ LC in body-wall muscle cells, to investigate AL amyloidosis-related heart damage. The LC sequence was derived from an AL patient with cardiac involvement (MNH). These worms have functional and mitochondrial damage in the pharynx, an ancestral heart, characterized by increased superoxide production, mitochondrial disruption, and sarcomere disorganization- closely mimicking the cardiac pathology observed in AL patients. Single-cell RNA sequencing of 3-day-old transgenic worms revealed the upregulation of two pharyngeal genes, T03F1.11 and idpp-16, which are critical for organ dysfunction. Notably, RNA interference-mediated silencing of these genes fully restored pharyngeal function to levels comparable to control worms. Additionally, we observed downregulation of asg-2, encoding ATP Synthase G homolog 2- a component of mitochondrial oxidative phosphorylation- alongside a paradoxical increase in ATP levels compared to controls. Our findings provide novel insights into the molecular mechanisms underlying AL amyloidosis-related cardiac dysfunction, identifying T03F1.11 and idpp-16 as key mediators of LC-induced toxicity. The observed mitochondrial alterations suggest a complex interplay between LC toxicity and metabolic adaptation, with pharyngeal dysfunction in MNH worms potentially arising from compensatory metabolic reprogramming. This transgenic model offers a valuable in vivo platform for studying AL amyloidosis. Future research should focus on targeted therapeutic strategies to modulate these molecular pathways, aiming to reduce organ damage and improve patient outcomes.

## Dopaminergic system dysfunction in a C. elegans model of Spinal Muscular Atrophy

**G. Onorato**[1], P. Santonicola[1], F. La Rocca[1], F. Cieri[1], V. Bassareo[2], G. Zampi[1], M. Carta[2], F. Di Cunto[3], E. Di Schiavi[1]

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Spinal Muscular Atrophy (SMA) is a rare neurodegenerative disease, historically considered as a motoneuron (MN) specific disorder. However, this selectivity is in contrast with the fact that the causative gene, Smn1, is a ubiquitous gene with housekeeping functions and, in fact, SMA has been recently re-defined as a multi-system disorder. In iPSCs-derived MNs from SMA patients, in SMA mice and in *C. elegans* null mutants in the ortholog smn-1, the dopaminergic pathway resulted to be highly dysregulated at the transcriptional and post-transcriptional level. Taking advantage of multiple *C. elegans* SMA models mutated in smn-1, we investigated the unexplored connection between SMA and dopamine (DA) in vivo. We performed DA quantification by HPLC and revealed a reduction in total DA in SMA models, also detected in vivo at intracellular level in dopaminergic neurons through the formaldehyde induced fluorescence assay. The Basal Slowing Response (BSR) in *C. elegans* SMA models was found impaired, suggesting that the reduction of DA causes an alteration in dopaminergic neurons function. We also confirmed a reduction in intracellular DA and in BSR in animals silenced for smn-1 only in dopaminergic neurons, suggesting a cell-autonomous effect of smn-1. bas-1 is responsible of the conversion of tyrosine to levodopa (L-DOPA) and its expression was found reduced in SMA mutants, possibly accounting for the biochemical and behavioural defects we observed. Accordingly, overexpression of bas-1 in dopaminergic neurons rescued the behaviour defect. In further confirmation, administration of the DA precursor L-DOPA was able to rescue the reduction in intracellular DA and the behavioural defect. Interestingly, we also found that bas-1 overexpression rescued SMA-related phenotypes in SMA mutants such as the defect in the MNs viability and in the thrashing locomotion. Taken together our results point out to a dysfunction of the dopaminergic system in SMA that may account for mood alterations observed in some SMA patients. Most importantly, our results may suggest new pharmacological approaches to reduce the effects of Smn1 loss in dopaminergic neurons of SMA patients modulating dopaminergic genes like bas-1.

## Unraveling TBP/TBP-1 and CHIP/CHN-1 interaction in a *C. elegans* model of human digenic SCA17 disease: does it affect neuron function?

**G. Madonna**[1,2], P. Santonicola[3], E. Di Schiavi[3], F. Taroni[1], S. Magri[1]

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The transcription factor TATA-box binding protein (TBP) gene is characterized by a polyQ-encoding CAG/CAA repeat whose expansion causes the autosomal dominant spinocerebellar ataxia type 17 (SCA17). In particular, up to 39 repeats are wild-type, between 40 and 46 show reduced penetrance and are thus defined intermediate alleles, and ≥47 are fully penetrant pathogenic alleles. We have recently described that affected patients with intermediate alleles carried a concurrent heterozygous pathogenic variant in STUB1 gene. Therefore, we demonstrated that SCA17 is monogenic for TBP with ≥47 polyQ and digenic TBP/STUB1 for intermediate alleles (SCA17digenic). STUB1 encodes the C-terminus Hsp70-interacting protein (CHIP), an E3-ubiquitin ligase with co- chaperone activity. Mutations in STUB1 have been associated with the autosomal recessive spinocerebellar ataxia type 16 (SCAR16) and the autosomal dominant spinocerebellar ataxia type 48 (SCA48). Moreover, last year, STUB1 mutations have also been described as possible genetic modifiers in spinocerebellar ataxia type 8 (SCA8). In addition, CHIP has been found to act as a modifier in SCA1, SCA3, and Huntington disease in cellular and animal models. The molecular mechanism underlying the strong genetic interaction between CHIP and TBP in SCA17 is still unknown. To unravel the nature of this interaction, we took advantage of the nematode *Caenorhabditis elegans* whose genome harbors orthologs for both TBP and STUB1, named tbp-1 and chn-1, respectively. We have generated SCA17 animal models by pan-neuronal overexpression of the cDNA of human TBP alleles with 38 (wild-type, TBPWT), 43 (intermediate, TBPQ43), and 54 repeats (fully-penetrant, TBPQ54). We observed that only the fully-penetrant pathogenic TBPQ54 expansion caused defects in gentle touch response, suggesting an altered function of mechanosensory neurons. On the other hand, CHN-1 knockout (chn-1(by155)) compromised wild- type backward locomotion, consistent with the lower number of visible GABAergic motor neurons observed. Interestingly, the SCA17digenic model TBPQ43;chn-1(by155) showed an age-dependent defect in gentle touch response, similarly to TBPQ54 animals. Moreover, treatment of TBPQ43 animals with the proteasomal inhibitor MG132, but not with the lysosomal inhibitor chloroquine, impaired gentle touch response resulting in a defective phenotype similar to SCA17digenic model (TBPQ43;chn-1(by155)). These results support the hypothesis that CHIP/CHN-1 mediates TBP/TBP- 1 degradation by proteasomal pathway. We therefore speculate that while intermediate polyQ expansions in TBP are not sufficient to result in an altered phenotype, the absence of CHN-1 impairs TBPQ43 proteasomal degradation triggering TBP accumulation and aggregation. Further studies are required to deeply define the interaction occurring between these two proteins and to identify other players in this interaction.

## Unlocking new frontiers in research with SydLab™ One: the ultimate solution for compounds and genetic studies, using *C. elegans*

**Alessandro Berto**

Nagi Bioscience SA, EPFL Innovation Park, Rue des Jordils 1A, CH-1025 St- Sulpice, Switzerland

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- **Global Relevance**: Addresses urgent aging population challenges (WHO projections) by accelerating healthspan and drug discovery research.

## Biophysical models of *C. elegans* neurons: a tool for elucidating the nematode neuronal dynamics

**Martina Nicoletti**[1,2], Letizia Chiodo[1], Alessandro Loppini[1], Viola Folli[2,3], Giancarlo Ruocco[2], Simonetta Filippi[3]

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*Caenorhabditis elegans* is a well-established model organism in neuroscience, widely used to study neural function in both physiological and pathological contexts. Its relatively simple nervous system, combined with a fully mapped connectome and identified neuron types, provides a powerful platform for investigating neural dynamics across different scales, from single neurons to network- level interactions. However, despite recent advances in *C. elegans* electrophysiology, the cellular and biophysical mechanisms underlying individual neuron responses remain poorly understood. To address this gap, we present a biophysical modeling framework designed to accurately simulate *C. elegans* neurons activity, with the goal of uncovering novel aspects of neuronal dynamics and support electrophysiological experiments interpretation. Adapting the Hodgkin-Huxley model of neuronal responses to the *C. elegans* case, we successfully replicate the characteristic electrophysiological responses of sensory (AWC), motor (RMD, VA5, VB6, and VD5), and interneurons (AVA, RIM, AIY). Moreover, thanks to the high level of detail, our models can be used for investigating the ionic currents interplay underling the electrical activity of the nematode neurons, as well as for predicting potential unobserved responses in *C. elegans* neurons. In the case of AWC neurons, we also simulate the intracellular calcium dynamics during chemotaxis, obtaining a comprehensive model of the AWC neuron functioning. In conclusion, this computational framework serves as a valuable tool for integrating computational and experimental research, ultimately advancing our understanding of *C. elegans* neural function and providing a foundation for constructing comprehensive models of its nervous system.

References: 1. M Nicoletti, et al., "Biophysical modeling of *C. elegans* neurons: single ion currents and whole-cell dynamics of AWCON and RMD." PLoS ONE ,14(7): e0218738, 2019 2. M. Nicoletti et a.l, "Biophysical modeling of the whole-cell dynamics of *C. elegans* motor and interneurons families". PLoS ONE, 19(3): e0298105, 2024 3. M. Nicoletti et al. "Modeling of olfactory transduction in AWCON neuron via coupled electrical-calcium dynamics". Biomolecular Concepts, 14: 20220035, 2023.

## Exploring Prohibitins in Nervous System Development and Function

Naroa Jara Ortega, Diana Rangel Rosario, Marta Artal-Sanz and **Jesus Fernandez-Abascal**

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Increased life expectancy has dramatically raised the incidence of age-related disorders. Unfortunately, the diagnosis of such disorders is typically possible only when symptoms are evident, and the underlying damage is already advanced. Although age-related decline is a universal phenomenon, the cellular and molecular mechanisms behind it remain obscure. Interestingly, age-related decline shares early pathways involving mitochondrial stress that can serve as a potential target for understanding and potentially mitigating these effects. In mitochondria, the evolutionarily conserved mitochondrial prohibitin (PHB) complex is a key regulator of aging and metabolism. However, its role in age-related neuronal decline is poorly understood. To address this fundamental question, we conducted genetic manipulation and behavioral assays to test the function of mechanosensory neurons and the morphogenesis of several neurons and glial cells. We found that systemic and glutamatergic-neuron specific PHB knockdown causes nose touch insensitivity in both young and day 7 adults, regardless of whether PHB was depleted from egg or L4 stage. Furthermore, specific PHB depletion in GABAergic neurons causes morphological alterations that correlate with higher paralysis rates. In glial cells, lack of PHB causes several morphological defects that may impact neuronal functionality. These data suggest that PHB plays a crucial role in maintaining neuronal function and integrity, particularly in mechanosensory and GABAergic neurons. Our findings indicate that PHB depletion leads to sensory deficits and structural abnormalities in neurons. These insights into PHB's involvement in neuronal health highlight its potential as a key player in age-associated neuronal decline. Future research should focus on elucidating the precise molecular mechanisms by which PHB influences neuronal function and its interaction with other cellular pathways involved in aging.

## A genetic tool to monitor neuromodulation *in vivo*

**Ivan Gallotta**, Emma Clark, Evie Goss-Sampson, Angela Jimeno-Martín and Arantza Barrios

Department of Cell and Developmental Biology, University College London, UK

Neurons produce and release neuropeptides to communicate with one another. Neuromodulator and neuropeptide signaling throughout the brain is a key element for regulating neuronal plasticity and behavior. Altered neuropeptide and neuromodulator signaling is central to the pathogenesis or treatment of many human neurological and psychiatric disorders. Despite their importance in brain function, circuit-based mechanisms of peptidergic transmission are poorly understood, primarily due to the limited availability of tools for monitoring and manipulating neuropeptide release in vivo.
*C. elegans* is an ideal model to investigate neuromodulation in vivo, due to its fully described nervous system and conserved families of neuropeptides. Here, we are implementing a genetically based tool, called TANGO, into *C. elegans* to detect the release of endogenous neuropeptides in vivo and identify the circuits onto which they act. The TANGO tool converts transient ligand-receptor interactions into stable expression of fluorescent transgenes, allowing visualization of neuropeptide receptor activation. Specifically, we are using Pigment Dispersing Factor (PDF) and its receptor (PDFR-1) as a model system, as PDF release can produce diverse behaviors depending on the source of release and context. The fluorescent reporter transgene is activated by a signal transduction pathway, using a transcription factor that is covalently coupled to the PDF receptor via a specific tobacco etch virus (TEV) protease-sensitive cleavage site. The transcription factor is cleaved from the PDFR-1 receptor following ligand binding, by recruitment of a beta-arrestin-TEV protease fusion protein, and translocates to the nucleus where it activates the fluorescent reporter transgene Despite its sensitivity due to signal amplification, a drawback of TANGO is its background noise. We are testing the possibility that this background is due to PDF-independent activation of the receptor, by expressing the TANGO transgene in a mutant strain in which neuropeptide release is impaired. In addition, we are investigating whether this noise could be caused by an unspecific recruitment of the beta-arrestin-TEV protease fusion protein independent of the receptor activation. Finally, we are exploring ways to integrate the TANGO transgene into the genome to minimize silencing over time. Optimizing the specificity of TANGO could ultimately offer insights into the cellular and molecular mechanisms by which neuropeptides can provide functional flexibility to hard wired circuits.

## Study of Heteromeric Acetylcholine Receptor Composition and its Regulation by TMED-3

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Acetylcholine receptors (AChRs) are neurotransmitter receptors found both in the nervous system, where they modulate synaptic transmission, and at the neuromuscular junction (NMJ), where they permit muscle contraction. AChRs belong to the family of the Cys-loop receptors, which are composed of 5 subunits. AChRs can be either homo- or hetero-pentamers and their composition is regulated at many levels. Since impairments in AChRs expression and composition are associated with several neurological and muscular pathologies, we aim to characterize new regulators of their biosynthesis and composition using *C. elegans*. At the NMJ of *C. elegans* the muscle is innervated by both cholinergic and GABAergic terminals and presents three main types of Cys-loop receptors at the plasma membrane, namely the GABAergic receptors (GABAAR), the homomeric nicotine-sensitive AChRs (N-AchRs) and the heteromeric levamisole-sensitive AChRs (L-AChRs). In a forward genetic screen performed in *C. elegans*, we identified tmed-3 and sel-9 as two genes required for the expression of heteromeric L-AChRs at the NMJ. TMED-3 and SEL-9 are homologues of human TMED7 and TMED2 respectively, two members of the transmembrane emp-24 domain (TMED) proteins. TMED proteins are endoplasmic reticulum- and Golgi-resident transmembrane proteins involved in the anterograde and retrograde transport of cargos by the COPI (COatomer Protein complex I)- and COPII-coated vesicles. The TMED protein family is divided into four subfamilies (alpha, beta, gamma and delta). In *C. elegans* only one member of each subfamily is expressed in body wall muscle cells, except for the 7-subfamily with two members, TMED-3 and TMED-1. We found that in *C. elegans* TMED proteins act as a tetrameric complex that requires the presence of at least one of each subfamily member to be functional and stable. This tetramer is required for the biosynthesis of Cys-loop receptors located on the postsynaptic membrane of the muscle (L-AChR, N-AChRs and GABAARs), since knock-out of each subfamily results in an equivalent loss of receptors at the NMJ. Interestingly, TMED-3 is selective for L-AChRs and TMED- 1 can not replace its function. Recently, we have shown that there are two types of heteromeric L-AChRs at the NMJ, which differ in their composition by the presence of two alternative subunits. Intriguingly, we found that TMED-3 is differentially required for the expression of these two types of L-AChRs, thereby modulating the composition of AChRs at the NMJ. Further investigations will be addressed to define other cargoes dependent on TMED-3 and to test the conservation of TMEDs in the trafficking of AChRs in human muscle cells.

## Exploring neuronal mechanisms and therapeutic strategies in GNAO1 encephalopathy: insights from *C. elegans* model

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Dominant mutations in the GNAO1 gene cause a heterogeneous group of childhood-onset neurological disorders, characterized by developmental delay, drug-resistant seizures, and movement disorders. GNAO1 encodes the α-subunit of an inhibitory G-protein (Gαo) regulating ion channel activity, neurotransmitter (NT) release, and neurodevelopment. Effective pharmacological treatments are lacking, highlighting the urgent need for a deeper understanding of disease mechanisms. Our recent work established *C. elegans* as an informative model for studying pathogenic mechanisms underlying this condition and performing drug screening. Genetically modified animals carrying pathogenic variants in goa-1, orthologue of GNAO1, exhibit increased egg laying and are hypersensitive to aldicarb, suggesting excessive NT release by different classes of motor neurons (HSNs and ventral cord MNs, respectively). Knock-in (KI) animals also show hyperactive and uncoordinated locomotion and are resistant to low doses of levamisole. While the role of HSN neurons in inducing egg laying is well known, the specific classes of neurons involved in the other phenotypes remain unclear. To address this issue, we performed RNAi knock-down of goa-1 specifically in cholinergic, GABAergic, dopaminergic, and glutamatergic neurons. Our results demonstrated the involvement of all four neuronal classes in the hyperactive reversal behavior, with a predominant role of cholinergic neurons. Interestingly, animals exhibited hypersensitivity to aldicarb-induced paralysis following goa-1(RNAi) in both cholinergic and GABAergic neurons, indicating an unpredicted role of Gαo in GABA neurons. Unexpected results were also observed regarding the expression of GABAA receptors at the neuromuscular junctions (NMJs), which was found to be higher in mutants compared to controls, suggesting a possible link with the response to levamisole. Additionally, we have previously shown that caffeine rescues hyperactive motor behavior of goa-1 mutants, by blocking, at least in part, a putative adenosine receptor in the nematode. Here, we observed a slight but significant improvement of the locomotor phenotype in mutant strains either by knocking-down or knocking-out the expression of a subset of GPCRs playing a role upstream to stimulatory G-proteins. Finally, neuronal overexpression of the wild-type goa-1 allele in mutant worms was shown to ameliorate both aldicarb hypersensitivity and hyperactive locomotion, also in the presence of dominant-negative alleles, providing a proof-of-concept for the development of a gene therapy approach based on gene supplementation in GNAO1-related disorders. Overall, our findings establish *C. elegans* as an efficient platform for deciphering pathogenic mechanisms underlying this malady and performing chemical and genetic screens.

## Elucidating the Pathogenic Mechanisms of GNAO1-Associated Neurological Disorders through *C. elegans* Calcium Imaging and Evaluating the Therapeutic Potential of Caffeine

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De novo dominant mutations in the GNAO1 gene cause an emerging group of childhood-onset neurological disorders characterized by developmental delay, epilepsy, and hyperkinetic movement disorders, including dystonia. GNAO1 encodes the α subunit of a heterotrimeric G protein (Gαo) that negatively regulates ion channel activity and neurotransmitter release. Recently, we established *C. elegans* as a powerful model for exploring pathogenic mechanisms associated with GNAO1-encephalopaty and identifying new therapies. This study investigates the impact of GNAO1 pathogenic variants (PVs) on neurotransmission in *C. elegans*, by performing calcium imaging analysis. Specifically, animals harboring three different PVs (p.S47G, p.R209H, and p.E246K) in goa-1, orthologue of GNAO1, were crossed with a strain expressing a genetically encoded calcium indicator (GCaMP) pan-neuronally to examine the neuronal dynamics of the head ganglia neurons in vivo, using a custom-made microfluidic device. This allowed us to monitor and analyze the defect at a single- cell resolution, identifying neurons or clusters of neurons that are mainly dysregulated in the disease. In *C. elegans*, two distinct neuronal modules control forward and backward movements; while the activity within each module is highly correlated, the activity between opposite modules is anti-correlated. Mutant animals showed a frank dystonic phenotype: correlations were reduced, and neurons spent most of their time in transition between active and inactive states. Of note, each variant displayed a unique pattern of defects, which might reflect their distinctive phenotypic readout. Moreover, our findings documented the beneficial effect of caffeine to ameliorate the aberrant neuronal activation of genetically modified strains, highlighting the potential role of this molecule in controlling GNAO1-related dyskinesia. This study provides novel insights into the mechanisms underlying GNAO1-associated pathophysiology and highlights the promising therapeutic effect of caffeine in mitigating neuronal dysregulation associated with these PVs. Our findings also provide a new in vivo model to study dystonia.

## C16ORF70, a novel gene that regulates aging in *C. elegans*

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Background and Aim Our research group employs *Caenorhabditis elegans*, which combines a significant genomic evolutionary conservation with the possibility to obtain data from a multicellular model, in order to study unknown genes potentially involved in rare inherited conditions. Since many human protein-coding genes are still uncharacterized and some of them could be involved in proteostasis and aging, we started to investigate the function of C16ORF70 that we renamed MYTHO in H. sapiens and myt-1 in *C. elegans*. Methods We generated several models to functionally characterize myt-1: 1) a transgenic worm line expressing GFP under the control of the endogenous myt-1 promoter and 2) worm myt-1 knock out (KO) strains by CRISPR/Cas9 technology. Results myt-1 is mostly expressed in skeletal muscles and neurons and its expression increases in old worms, suggesting an age-related regulation. In absence of myt-1, worms show a precocious aging phenotype, with movement impairment, decreased survival upon exposure to oxidative stress and eventually reduced lifespan compared to controls. Moreover, myt-1 is involved in some well- known worm longevity pathways, suggesting its importance in longevity promotion. Mechanistically, we found that MYTHO interacts with WIPI2, allowing the recruitment of the conjugation system at the phagophore assembly site and corroborated these data by worm RNAi experiments. Conclusions We propose that MYTHO is important, possibly through its crucial role in autophagy, in promoting stress resistance to ensure healthy aging. We are currently analyzing the effect on worm lifespan and healthspan of tissue- specific overexpression of myt-1.

## Redox-mediated link between ferroptosis and aging in *Caenorhabditis elegans*: a role for fard-1 and dhs-25

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Aging is a natural process characterized by a progressive physiological decline that undermines health and well-being in the elderly population. It is widely accepted that an unbalanced redox state belongs to the hallmarks of aging, but its role as one of the main drivers of ferroptosis is quite recent. Ferroptosis is a form of iron-dependent cell death caused by massive phospholipid peroxidation. The excessive accumulation of intracellular reactive oxygen species and iron, as well as the failure of the main cellular antioxidant systems, cause ferroptotic cell death. While clear roles for ferroptosis in pathological conditions such as cancer or neurodegeneration have been described, its physiological roles and regulators are less clearly understood. Here, using *Caenorhabditis elegans* as a powerful model organism for aging studies, we uncover a role for ferroptosis in physiological aging mediated by disturbed redox homeostasis. We evaluated healthspan parameters in a *C. elegans* wild-type strain highlighting how several age-related features differentially decline during aging. A progressive loss of the capability to contrast external stressors, with an increase in hydroxyl radicals and a failure of the glutathione antioxidant system demonstrated the progressive disruption of redox homeostasis in older age. Moreover, we showed that selected genes involved in redox metabolism are downregulated with aging. Among them, mutant strains of the fatty acyl-CoA reductase, fard-1, and of the dehydrogenase, dhs-25, displayed higher sensitivity to a ferroptosis inducer, increased lipid peroxidation, anticipated drop in total glutathione and reduced lifespan. Accordingly, the expression of one of the closest mammalians dhs-25 homolog, the hydroxysteroid 17-Beta Dehydrogenase 8, was downregulated in cells which are more sensitive to ferroptosis. Our results clearly prove a causal role for ferroptosis in *C. elegans* aging driven by oxidative stress, unveiling novel genes involved in this connection that may constitute targets for possible interventions to improve healthy aging.

## Investigating the function and regulation of the telomeric repeat-containing RNA TERRA in *C. elegans*

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Telomeres are nucleoprotein structures assembled at the extremities of eukaryotic chromosomes which are essential to genome integrity. Telomeric DNA consists of tandem arrays of a short and highly conserved sequence (5'-TTAGGG-3' in vertebrates; 5' - TTAGGC – 3' in *C. elegans*) forming a double-stranded region terminating with a single-stranded 3' overhang. Telomeric sequences are bound by specialized proteins that mediate telomere functions and determine telomere structure. Telomere proteins also enable the recruitment of telomerase, a unique enzyme that synthesizes telomeric DNA by reverse transcribing its own RNA subunit. Telomeres are transcribed by RNA polII giving rise to long noncoding RNAs called TERRA, which play key roles in telomere function in humans, mice and yeasts. Importantly, TERRA also plays extratelomeric functions by controlling gene expression, and it has been shown to act as key regulator of stem cell genes in mouse pluripotent cells. Our lab is interested in studying TERRA and we have recently shown that it is expressed in *C. elegans* from multiple telomeres. Using molecular biology and cytologic approaches, we found that *C. elegans* TERRA is regulated by the telomere binding proteins POT-1 and POT-2. Notably, we detected TERRA in gonads and post-mitotic tissues by in-situ analyses. Interestingly, in germline cells only a fraction of TERRA is telomeric and it is predominantly expressed during pachytene, a stage of the meiosis when homologous recombination occurs. Using the MS2-GFP system, we studied the spatiotemporal dynamics of TERRA transcripts expressed from a single telomere in living organisms. Single particle tracking revealed that TERRA particles execute complex dynamics suggesting their association with different ribonucleoprotein complexes and/or localization to distinct nuclear compartments. *C. elegans* represents a very powerful tool to study telomere and TERRA biology as it is the only multicellular organism able to survive the absence of telomerase by spontaneously activating mechanisms of alternative lengthening of telomeres, known as ALT, which rely on homologous recombination among telomeres. However, the mechanisms that trigger ALT remain to be elucidated. In our study, we reported that TERRA expression is induced in telomerase mutant organisms before ALT induction. Interestingly, in these worms TERRA displays distinct dynamics with a higher fraction of fast-moving particles. In an unpublished work, we have generated ALT strains expressing mCherry-fused POT-1 to visualize telomeres and MS2-GFP tagged TERRA molecules. We will use these strains to study the dynamics of TERRA transcripts with respect to telomeres in the setting of ALT and during ALT activation. Finally, we would like to use the MS2-GFP system to pull down endogenous TERRA molecules and identify its RNA and proteins interactors. TERRA transcripts may represent novel key regulators of telomere function and gene expression in *C. elegans*.

## Stomatin-like protein 2 mediates mitochondrial unfolded protein response through phospholipid sensing

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Mitochondria are constantly exposed to radical agents, which can result in oxidative stress, if not prevented by repair-driven stress response mechanisms. How healthy mitochondria maintain their intrinsic stress- protective mechanisms is poorly understood. From a genetic screen in *Caenorhabditis elegans* we identified stl-1, an ortholog of human Stomatin-like protein 2 (SLP-2), as a positive regulator of the mitochondrial unfolded protein response (mtUPR) in non-stressed animals. Both *C. elegans* and human SLP-2 are proteins located at the inner mitochondrial membrane and exhibit strong lipid binding affinity to phosphatidic acid (PA). Oxidative stress alters the SLP-2 localization within the mitochondrial membrane, and triggers the mtUPR dependent on both SLP-2 and mitochondrial PA homeostasis. Our results demonstrate an evolutionarily conserved mechanism of mitochondrial protection, in which SLP-2 acts as a sensor for changes in mitochondrial membrane lipid composition through physical interaction with PA species, thereby mediating the mtUPR and enhancing stress resistance.

## Health-boosting potential of coffee silverskin: insights from *Caenorhabditis elegans*

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Coffee silverskin (CSS), a primary by-product of coffee processing, represents a valuable source of bioactive compounds, including melanoidins, polyphenols, and caffeine, with significant potential in sustainability and nutraceutical applications. This research explores the impact of CSS extracts on the physiology of *Caenorhabditis elegans*, with a particular focus on lifespan, aging, oxidative stress response, and lipid metabolism. The findings reveal that supplementation with CSS at concentrations of 25 µg/mL and 250 µg/mL provides notable benefits to nematodes, such as a 10% increase in body length and an enhancement in feeding behavior, reflected by a 10–20% rise in pharyngeal pumping rates. Importantly, aged nematodes exhibited a 57% decline in reactive oxygen species (ROS) levels, alongside the upregulation of antioxidant genes (gst-4 and sod-3), indicating that CSS helps alleviate age-associated oxidative stress via activation of the DAF-2/DAF-16 pathway. Moreover, CSS influenced lipid metabolism by reducing lipid droplet accumulation at 250 µg/mL and modulating the expression of key metabolic genes (sams-1, fat-7, acs-2, sbp- 1) under glucose stress conditions. Additionally, CSS was found to promote the growth of probiotic strains, suggesting a potential prebiotic function. Collectively, these results highlight CSS's ability to combat metabolic stress and enhance healthspan, presenting new avenues for its use in sustainable functional food and nutraceutical innovations.

## The physicochemical properties of graphene oxide drive their toxicity in *C. elegans*

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Graphene oxide (GO), with its versatility and low cost, mass production, and simple dissolution process, is widely used in various applications (i.e., catalysts, sensors, pharmaceuticals, and other fields) and is an attractive material for drug delivery and tissue engineering applications. However, the increasing production and use of this material necessitates a thorough evaluation of the potential impact on human and environmental health. In this context, our studies aim to investigate whether the different physicochemical properties of GOs developed for an extensive range of applications can influence their toxicity. Five GO types with three different oxidation levels, commonly used for industrial aims, were produced: GO1 with a high oxygen content and big flake size, GO2 with low oxygen content, and GO3 with a medium percentage of oxygen and big flake size. GO4 was prepared with the same oxidation level as GO1 but with a significantly smaller flake size, and GO-PEG with PEG-functionalized GO1. All these materials were extensively characterized to determine their physicochemical properties, and the various degrees of oxidation were corroborated by the X-ray photoelectron spectroscopy -found carbon-to-oxygen atomic ratios. Acute and sub-acute toxicity studies were performed on *C. elegans*, used as a good indicator of animal and eco- toxicological studies and providing data from a whole animal with intact and metabolically active digestive, reproductive, endocrine, sensory, and neuromuscular systems. Twenty-four hours after administration, GO1, GO3, and GO4, but not GO2 and GO-PEG, caused a dose-dependent reduction of worm viability. GO1 and GO4, the materials with the highest oxidation state, became toxic at 0.5 and 0.25 mg/mL, respectively. Interestingly, GO4, composed of medium flakes, was more toxic than GO1, composed of big flakes, and had an IC50 value about 2.8 times higher. GO3, the material with a medium oxidation state, became toxic at 0.5 mg/mL and exhibited a value of IC50, intermedia between GO1 and GO4. The toxicity of GOs did not increase with exposure time, with the worms' viability scored 48 and 72 h after the treatment, similar to that measured at 24 h. The effect of the different materials on worms' behavior, which is important for healthy survival, like feeding activity and neuromuscular function, was also investigated. GO1, GO2, and GO-PEG did not affect nematodes' pharyngeal and neuromuscular function at all doses tested, whereas GO3 and GO4 significantly affected them, indicating that these materials can also exert a sub-toxic effect. These findings pointed out that GO, depending on its oxidation state more than its flake size, can exert an in vivo acute and sub-acute toxicity in *C. elegans*, raising concern about the possible effect of accumulating this material in the environment on living organisms.

## Warns from Worms

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*C. elegans* is often cultivated on nematode growth media, solid agar plates, spread with E. coli bacteria as food source. Most commonly used E. coli are OP50 and HT115, the latter routinely employed for RNAi- mediated gene knock down. While researchers often use these bacteria independently to fulfill specific project requirements, it soon become clear that the different bacterial media composition (including antibiotics) lead to different readouts even when used in the exactly same experimental conditions. To minimize this problem OP50 has been also engineered to express dsRNA-expressing vector (pL4440), allowing to use at least identical media composition. Nonetheless, a growing body of evidence is releveling intrinsic differences in OP50 and HT115 composition may also lead to divergent experimental readouts. In some cases, the underlying molecular culprits have been elucidated, such as secreted tryptophan-related metabolite differentially affecting life and health-span outcomes or Vitamin B12 levels differentially affecting *C. elegans* response to bacterial infection. While it will be interesting to identify the exact bacterial component and/or metabolite responsible for different experimental outcomes in specific contexts, it is obviously of critical importance to maintain experimental conditions as consistent as possible to allow appropriate interpretation of results. Here I present a subset of assays were we systematically compared and revealed differences in experimental readouts induced by OP5O vs. HT115. Specifically, the expression of GFP fluorescent reporters in wild-type and mitochondrial disease model (e.g., gst-4::GFP, unc-17::GFP), thermal and oxidative stress tolerance, and aldicarb-induced paralysis to assess synaptic transmission, were analyzed to investigate the broader impact of different E. coli strains (OP50 vs. HT115) as a food source on *C. elegans* physiology. In my PhD project I will specifically investigate whether some of the observed differences are ascribed to mitochondria-regulated B-Vitamins or lipid metabolism.

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# Posters

## Effects of downregulation of the rad-51/FANCR gene on the choice of DNA damage repair pathway in the *C. elegans* fcd-2/FANCD2 mutant

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Fanconi anaemia (FA) is a rare genomic instability syndrome associated with congenital anomalies, bone marrow failure and cancer predisposition. FANCD2, a key regulator of the Fanconi anaemia pathway, promotes double-strand break (DSB) repair by precise homologous recombination (HR) and blocks the error- prone non-homologous end-joining (NHEJ) pathway. RAD51/FANCR, another FA pathway factor, plays a key role in homologous strand exchange during recombination. By analysing the *C. elegans* rad-51(A-) mutant, in which the expression of the rad-51 gene is reduced, compared to the wt organism, we found a reduction in NHEJ repair and an increase of HR. The activity of these processes is the exact opposite of that observed in the absence of fcd-2 /FANCD2 gene expression, which is associated with increased NHEJ activity and decreased HR repair. We generated and analysed a fcd-2;rad-51(A-) double mutant, in which the expression of the rad-51 gene is reduced in an fcd-2 mutant. The fcd-2;rad-51(A-) double mutant revealed the rescue of FA phenotypes associated with a block in NHEJ: decrease in egg laying per worm, increase in the frequency of larval arrests and defective adults in the progeny. Furthermore, in the fcd-2;rad-51(A-) worms in which the homologs are not available for recombination (rad-51(A-),syp-2;fcd-2 and rad-51(A-),msh-4; fcd-2 worms), we observed the loss of oocytes with non-covalent aggregation of non-homologous chromosomes compared to syp-2;fcd-2 and msh-4;fcd-2 double mutants, suggesting that NHEJ is not up-regulated in the latter condition. Furthermore, we obtained a similar inhibition of NHEJ after partial downregulation of rad-51 by RNA interference (RNAi) in syp-2;fcd-2 and msh-4;fcd-2 worms. Our data suggest that modulation of RAD51 could represent a new strategy to prevent or postpone the FA defects.

## Erucin protects from the toxicity of protein aggregates under heat stress conditions in *Caenorhabditis elegans*

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Even if of different nature and causative factors or genes, protein aggregates could be referred as the main source of neurotoxicity common to many neurodegenerative diseases (NDDs). Among all the potential environmental causative factors that raise the highest interest in the global warming scenario, increased temperatures and heat waves are the most dangerous for protein function, conformation and stability. Indeed, when the unfolded protein response clearance capacity of the organism is exceeded, misfolded proteins form neurotoxic aggregates. This occurs in NDDs caused by protein aggregates of various origin and nature, as represented by α-synuclein (α-syn) aggregates in Parkinson's Disease (PD) and by polyglutamine (polyQ) aggregates falling in coding regions of DNA typical of polyQ disorders. Moreover, the onset and progression of many NDDs has been related to heat stress through several processes such as oxidative stress, excitotoxicity and neuroinflammation. In parallel, plants, in response to high temperature, activating their defense response, produce secondary metabolites, bioactive compounds presenting a well-documented antioxidant capacity and neuroprotective potential in humans. In this regard our study was focused on erucin (ERN), an isothiocyanate released by the enzymatic hydrolysis of glucoerucin, its precursor molecule, naturally contained in rocked salad leaves, and even released by the in vivo reduction of its oxidized form, sulforaphane, typical of broccoli. Our results showed that ERN explained neuroprotective capacity under heat stress conditions on *C. elegans* models of polyQ diseases. Particularly, ERN provided a recovery of the neuronal function by restoring the touch response both in the N2 wild-type strain and in the polyQ strain carrying a constitutive impairment in the mechanosensory neurons. Moreover, ERN reduced polyQ-induced toxicity under heat stress conditions, diminishing the accumulation of toxic protein aggregates in muscle cells of worms. Finally, our results demonstrated that the treatment with ERN diminished α-syn aggregation and slightly restored motor capacity under heat stress conditions on a *C. elegans* model of PD. These findings support more trials on additional models of NDDs in *C. elegans* in order to investigate ERN potential neuroprotective activity under heat stress conditions and even under the risk posed by other environmental factors.

## Exploitation of the effect of Extracellular Vesicles (EVs) from different sources on oxidative stress and neurodegeneration.

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Extracellular vesicles (EVs) are secreted by all cell-types into extracellular space for intercellular communication. The EVs deliver cargo from the secreting cell into the cytoplasm of target cells influencing their function. Different EVs can be classified by their size, cargo and target cells. We aimed to investigate what are the effects of EVs from different sources on animal physiology by using *C. elegans*. To address this question, we tested the effect of EVs isolated from microalgae, from human cells (mesenchymal stromal/stem cells, red blood cells and sperm) and from mouse cell cultures (myoblast and glioblastoma) on animal movement by performing a thrashing assay. The results showed an increase in animal locomotion after treatment with all the EVs tested, except glioblastoma, myoblast and sperm EVs. Interestingly, EVs from myoblast at different stage of differentiation and from sperm of different human samples have different effects. In *C. elegans*, the muscular function is affected by redox imbalances, and it is strongly reduced when oxidative stress levels are high or can be increased when worms are treated with antioxidative molecules. We confirmed the impact of an antioxidative molecule, N-Acetyl-Cysteine (NAC), on animal movement by observing an increase in animal thrashing after treatment. To investigate whether the EVs effect was linked to their putative antioxidative properties, we took advantage of the fact that the skn-1/Nrf2 pathway is the main antioxidative pathway and thus we decided to test all the EVs on a skn-1 loss-of-function strain. EVs from microalgae, mesenchymal stromal/stem cells and red blood cells loose their effect on animal movement when tested on the skn-1 loss-of-function strain, thus suggesting an antioxidative effect of these EVs that might be related to the increase in locomotion mediated by the skn-1/Nrf2 pathway. Notably, EVs from mouse myoblast cell cultures also showed a neuroprotective effect by decreasing the number of degenerating GABAergic motoneurons in a Spinal Muscular Atrophy model strain. We are now investigating whether the antioxidative and neuroprotective effects of these EVs are synergistic or independent, and whether also neuroprotection is exerted through the skn-1/Nrf2 pathway.

## Characterization of GABAergic neurotransmission in GNAO1-related diseases using *C. elegans*

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Dominant mutations in GNAO1 cause a group of neurological disorders that begin in childhood. These disorders are characterised by developmental delay, movement disorders, drug-resistant seizures, intellectual disability and hypotonia. GNAO1 encodes the α-subunit of an inhibitory GTP/GDP-binding protein that regulates neurotransmitter release and ion channel activity. The underlying pathogenic mechanisms of GNAO1-related disorders remain largely unexplored and no effective treatments are available. The nematode *Caenorhabditis elegans* is a powerful tool for modelling neurological disorders due to the simplicity of its nervous system and the power of the genetic tools available. In *C. elegans*, the gene goa-1 is the orthologue of GNAO1. In neurons, GOA-1 signalling inhibits the release of neurotransmitters. Simone Martinelli's (SM) team has recently used *C. elegans* to characterise the functional consequences of four pathogenic GNAO1 variants that display a typical goa-1 loss-of-function phenotype, including hyperactive locomotion and hypersensitivity to aldicarb, an acetylcholinesterase inhibitor that causes accumulation of acetylcholine in the synaptic cleft of the neuromuscular junction (NMJ). To go one step further, SM has established a collaboration with the laboratory of Jean-Louis Bessereau, who has been using the *C. elegans* NMJ as a model system to analyse synapse formation and maintenance for more than 20 years. Our preliminary data show that the amount of GABAARs at the NMJ is twice as high in goa-1 mutants as in controls. A similar phenotype has never been seen before. We are using the model organism *C. elegans* to analyse the link between aberrant GNAO1 function and GABAARs at the NMJ by characterising the neuromuscular system in goa-1 mutants. In particular, we have labelled cholinergic and GABAergic NMJs with presynaptic markers and quantified the number and size of presynaptic boutons. We also used fluorescently labelled knock-ins of other post-synaptic receptors to test the effect of GNAO1 on the distribution and levels of these receptors. Finally, we performed tissue-specific degradation of GOA-1 to identify the source of synaptic GOA-1 that causes the aberrant GABAAR phenotype. We used CRISPR-Cas9 to fuse an auxin- inducible degron linked to GFP into an internal loop of the alpha-helical domain of GOA-1 and expressed the TIR-1 E3 ligase under different tissue-specific promoters, such as muscle, cholinergic and GABAergic promoters. This approach will also allow us to knock down GOA-1 at specific times during the development of the worm and assess whether this protein plays a role in the establishment and/or maintenance of GABAAR clusters. We believe that dissecting the molecular mechanism underlying the atypical GABAAR phenotypes induced by goa-1 mutations may help in the discovery of the molecular events leading to GNAO1- associated pathologies.

## Utilizing High-throughput platform combined with high-Content Screening in *C. elegans* model of AD for drug discovery

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In the struggle against neurodegenerative diseases, the need for reliable and cost-effective pathology models has become a pressing issue. The nematode *C. elegans* is being highly regarded as a model for in vivo high throughput screening of compound libraries due to its advantages in terms of low housing costs, great experimental turnout and ease of genetic manipulation. Unfortunately, manipulating *C. elegans* necessitates labour intensive and often the experimental evaluation is prone to operator biases. The desire to overcome these technical difficulties has led to the development of many automated platforms for worm tracking and phenotypical analysis. Among these, WF-NTP (Wide-Field of view Nematode Tracking Platform) stands out as a high-throughput tool as it allows a full phenotypical characterisation of the fitness by measuring diverse worm behavioral parameters (for example maximal and average speed, size and bends per minute) at the same time on large samples of animals. This platform generates highly statistically robust data and is therefore able to accurately measure even subtle changes in the worm's phenotype, making it particularly suitable to study multifactorial complex diseases like Alzheimer's disorder. Here we exploited the WF-NTP potential to evaluate in vivo the neuroprotective effect of a novel diaryl-methane compounds using the *C. elegans* transgenic strain GMC101, an established model of Alzheimer's disease characterized by the accumulation of $A\beta_{1-42}$ aggregates in body-wall muscle cells that leads to progressive paralysis. The compounds were administered under both a preventive and treatment regimen to assess their capability to delay the pathology onset or ameliorate the fitness of diseased worms. Following compound exposure, worms were subjected to high-content screening using High Content Confocal Analyses. Exploiting the fluorescence of the GFP-tagged Aβ1-42, it was possible to physically characterize the effects of compounds on the quality and quantity of the amyloid aggregates. This approach allowed us to integrate behavioral in vivo data with high quality automated fluorescence microscopy imaging providing an accurate assessment of differences in biological processes associated with neurodegeneration. The combination of the large output of data obtained from the WF-NTP platform and the high-content screening accelerates the drug discovery process, allowing for more efficient identification of promising drug candidates

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## A new *C. elegans* model to study LDL-Related Proteins involvement in Alzheimer's disease and the possible role of small extracellular vesicles

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The Alzheimer's disease (AD) is the most common neurodegenerative disorder and its symptoms include dementia, loss of memory and motor dysfunction. The main neuropathologic hallmarks of AD are the extracellular deposits of β-amyloid (Aβ) plaques, insoluble aggregates of beta-amyloid peptide, and the intraneuronal fibrillary tangles, resulting from the hyperphosphorylation of the microtubule associated protein Tau. These aggregates both contribute to the loss of synapse functions and the consequent neuronal death and small extracellular vesicles (sEVs) have been recently implicated in AD progression by facilitating the spread of pathological proteins. The familiar form of AD (FAD), characterized by an early onset, is correlated with mutations in the APP gene or in the γ-secretase components of presenilins (PSEN-1 and PSEN- 2) which cleave APP protein. Most AD cases are sporadic and no mutations in APP, PSEN-1 or PSEN-2 have been found. The major genetic risk factor in sporadic AD is represented by the E4 isoform of Apolipoprotein E (ApoE) and ApoE expression has a negative impact on sEVs production, both in human brain and in humanized mouse model, thus suggesting a possible connection between ApoE and sEVs in AD. ApoE is the ligand of the Low-Density Lipoprotein-Related Proteins Receptors (LRPs) and LRPs intramembrane proteolysis is regulated by the γ-secretase cleavage similarly to the β-amyloid peptide. Considering the lack of information about LRPs and its involvement in AD and in sEVs mediated neurodegeneration, we propose a new *C. elegans* model to study the correlation of human LRPs and sEVs to APP and Tau. We generated transgenic worms expressing in all neurons human LRP8, the ApoE receptor, and analyzed their phenotypes to determine whether huLRP8 overexpression caused any defect similar to the one caused by Tau hyperphosphorylation or APP overexpression. We demonstrated that huLRP8 overexpression affects nematode lifespan, locomotion, that progressively worsen in aged worms, and the response to mechanical stimuli. Moreover, we found that the overexpression of huLRP8 causes neuronal abnormalities, such as the loss of motor neurons, that is not development-related, and axonal defects in touch receptor neurons. To evaluate whether huLRP8 cleavage is regulated by the γ-secretase complex, we crossed huLRP8 expressing animals with a loss of function mutant of the presenilin ortholog sel-12. Our preliminary results suggest that huLRP8 and sel-12 could act in the same pathway in *C. elegans*. All these results allow us to hypothesize the involvement of huLRP8 in neuronal functions/survival, with an involvement of the presenilin pathway. We are currently investigating huLRP8 protein processing in vivo, its connection to sEVs secretion and the effect of its overexpression in *C. elegans* AD models (Aβ and Tau)

## Phenotypic screening of SG-compounds as autophagy promoters in a *C. elegans* model of AD: a multiparametric analysis

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Neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's disease, are characterized by progressive neuronal dysfunction and degeneration, mainly due to protein aggregates accumulation and induced cytotoxicity. Autophagy is a cellular mechanism leading to damaged cytoplasmatic components removement and can be exploited to prevent neurodegeneration. *Caenorhabditis elegans* has emerged as a powerful in vivo model for studying these diseases exploiting its well-conserved neuronal pathways, transparency, and rapid lifecycle. Here, we leverage a high-throughput phenotypic screening platform (Wide Field of View Nematode Tracking Platform, WF-NTP) to systematically analyze neurodegeneration-related phenotypes in transgenic *C. elegans* models expressing human disease- associated proteins. This high-content screening system allows for the rapid identification of genetic and pharmacological modifiers of neurodegeneration, providing valuable insights into disease mechanisms and potential therapeutic targets. In 2015 we synthesized a novel series of diarylmethane derivatives, namely SG- compounds, which displayed a consistent neuroprotective activity both in in vitro and in vivo models. In this study we compared three derivatives SG2, SG22 and SG23 on *C. elegans* model of AD. Phenotypic screening results demonstrated a restoration of the worm's fitness and motility. Furthermore, PCR analysis confirmed that the improved health of *C. elegans* is, at least partially, attributed to the promotion of autophagy. The overall results strongly indicate that SG-compounds exhibit neuroprotective activity in vivo by stimulating the activation of the autophagy-lysosomal pathway.

## Effects of microgravity on Beta-amyloid aggregates using transgenic lines of *C. elegans*

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With the increase of global events such as pollution, overpopulation and climate change, humankind may be forced to undertake long journeys into space in the near future. In this context, environmental factors such as microgravity and cosmic radiation could have significant impacts on human physiology. Although space biomedicine has made notable progress, the effects of these factors on pathophysiology, particularly in the presence of neurodegenerative diseases, are still underexplored. In particular, microgravity can alter the physical and chemical properties of proteins, affecting their structure and function. Studies conducted in simulated microgravity environments will provide new insights into how the space environment modifies the aggregation process of beta-amyloid, a key pathogenic marker in Alzheimer's disease. In particular, using *C. elegans* as an experimental model to investigate the effects of microgravity on genetic and physiological responses might prove crucial to understanding the interplay between environmental factors and genetic predispositions in neurodegeneration. These studies not only offer insights into the neurological health of astronauts during extended space missions but could also lead to new therapeutic strategies for treating Alzheimer's on Earth, with potential implications in both space and clinical settings. The ultimate goal is to develop a rapid and effective screening system for the validation of novel antibodies with potential therapeutic roles. To analyze how microgravity modifies the structural dynamics of beta-amyloid aggregates, we leveraged transgenic *C. elegans* strains, which constitutively express the human beta-amyloid protein (Aβ1-42) in neurons or muscle cells. Our data show that both neuronal and muscle expression of β-amyloid causes locomotion defects compared with isogenic controls, while only muscle expression reduces pharyngeal pumping. While neuronal Aβ1-42 expression reduces life expectancy compared to its control, the muscle-expressing line shows increased lifespan, probably due to reduced caloric intake as a consequence of reduced pharyngeal pumping. By immunofluorescence analysis, we observed β-amyloid deposits in target tissues. Finally, we set up a NASA-developed Rotary Cell Culture System (RCCS) to conduct experiments in simulated microgravity from the young adult stage to 12-day-old adults. This system will enable an accurate reproduction of space conditions and help assess how microgravity influences protein aggregation and neurodegenerative processes. Overall, the collected data offer robust phenotypic insights into the effects of microgravity, aiming to clarify its potential role as a risk factor for neurodegenerative diseases. Additionally, they facilitate the investigation of the relationship between protein aggregates and anti-β-amyloid antibodies, paving the way for evaluating the feasibility of biological therapies under altered gravity conditions.

## Exploring the neuroprotective effects of REST inhibition in a *Caenorhabditis elegans* model of Huntington's disease

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Neurodegenerative diseases are a major concern in the aging population, yet therapeutical curative approaches remain unmet. Huntington's Disease (HD) is an autosomal dominant progressive brain disorder caused by an expansion of polyglutamine (polyQ) in the N-terminus of the huntingtin (HTT) protein, leading to the formation of misfolded aggregates. As a result, patients suffer from symptoms such as cognitive impairment, psychiatric abnormalities, and motor dysfunction, worsening their lifespan expectancy. Repressor element 1-silencing transcription factor (REST), known as a master regulator of neuronal development and synaptic plasticity, appears to be involved in the pathophysiology of HD. Herein, we studied the inhibition of REST in a *C. elegans* model of HD (EAK102 and EAK103 strains), both characterized by YFP- fused mutant human HTT protein (Htt513) and differed by the polyQ length (Q15 and Q128, respectively). Based on the polyQ length-dependent toxicity, the EAK103 strain presents reduced motility and lifespan, caused by Htt513 (Q128) aggregates. In this study, we demonstrated an increased thrashing number and lifespan and a reduction of toxic aggregates after the inhibition of REST in the EAK103 strain. Both N2 (wild type) and EAK102 strains were used as controls. Our results suggest *C. elegans* as a possible model organism for studying the effect of pharmacological REST inhibition and promote future research on REST regulatory mechanism as a novel target for developing therapeutic drugs and treatments for HD. This study was supported by the Ministerio de Economía, Industria y Competitividad (Agencia Estatal de Investigación, AEI) and Fondo Europeo de Desarrollo Regional (MINECO-FEDER) (PID2022-139016OA-I00), Generalitat de Catalunya (2021 SGR 00357). This study was co-financed by Secretaria d'Universitats i Recerca del Departament d'Empresa i Coneixement de la Generalitat de Catalunya 2023 (Llavor 0007; to CGF). A. I. thanks to Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) for her FI Joan Oró predoctoral fellowship (2023 FI-1 00944).

## The synergistic effect of NN-DMT and bioactive compounds in Alzheimer's Disease: Insights from the *C. elegans* model

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Alzheimer's disease (AD) is a chronic and complex neurodegenerative disorder characterized by progressive cognitive decline, memory loss, and irreversible impairment of brain functions. The etiology of AD is multifactorial, involving a complex interplay of genetic, environmental, and physiological factors, including the aggregation of Amyloid-β (Aβ) and oxidative stress (OS). Notably, the role of OS is particularly significant in AD pathogenesis, given that an imbalance between oxidants and antioxidants promotes cellular damage, exacerbates Aβ deposition, and leads to cognitive deterioration. Despite extensive research, current therapeutic strategies have largely failed, likely due to the use of single-target drugs unable to halt the multifactorial progression of the disease. However, recent studies have identified plant-derived bioactive compounds, such as Withanone, Apigenin, Bacoside A, Baicalin, and Thymoquinone, with antioxidant properties, as promising candidates for counteracting the key pathogenic processes in AD, including Aβ aggregation. Additionally, psychedelic substances, including N,N-dimethyltryptamine (NN-DMT), have emerged as potential therapeutic agents due to their neuroplastic and immune-modulatory effects. Despite the numerous studies conducted on NN-DMT, no observable oxidative effects have been reported. Consequently, the present study aims to evaluate the therapeutic potential of bioactive compounds, both individually and in combination with NN-DMT, on Aβ deposition and OS using a transgenic *Caenorhabditis elegans* model. The results of the behavioral and molecular analyses indicated that the combination therapy exhibited a higher efficacy than the monotherapies, leading to a significant reduction in age-related motility defects in the AD model. Furthermore, the combination treatment resulted in a substantial reduction in Aβ plaque burden, an enhanced survival following OS insult, and demonstrated a synergistic effect in mitigating AD-related hallmarks. Taken together, these findings support the potential of combining NN-DMT with specific bioactive compounds as a promising multi-target therapeutic approach for AD.

Funding: This study was supported by the Ministerio de Economía, Industria y Competitividad (Agencia Estatal de Investigación, AEI; to CGF and MP) and Fondo Europeo de Desarrollo Regional (MINECO-FEDER) (PID2022-139016OA-I00, PDC2022-133441-I00; to CGF and MP), Generalitat de Catalunya (2021 SGR 00357;
to CGF and MP). This study was co-financed by Secretaria d'Universitats i Recerca del Departament d'Empresai Coneixement de la Generalitat de Catalunya 2022 (Producte 0092; Llavor 005 and 007; to CGF)

## The surface charge impacts the internalization and safety of polystyrene nanoparticles in vivo and in vitro

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Micro- and nano-plastics (MNPs)'s environmental persistence generates a relevant alarm. This concern is amplified for non-biodegradable materials like polystyrene (PS). It is known that MNPs easily penetrate cells and accumulate in vital organs. Despite the great interest, a univocal idea about MNPs' toxicity is still lacking. In this study, PS-nanoparticles (PS-NPs) were employed as prototypic material. We focused our attention on the role played by the external surface in the internalization and toxicity of amine- and carboxylate-modified fluorescent PS-NPs with different Z-potentials. After a physico-chemical characterization of the PS-NPs, *C. elegans* and human embryonic kidney (HEK) 293 cells have been employed to model the effects of these particles in vivo and in vitro. Impact of both positive and negative PS-NPs was evaluated through several parameters in vivo: survival, biodistribution, alteration in reproduction and larval development, and locomotor activity. The Z-potential of PS-NPs affected their toxicity: positive NPs, but not negative NPs, were able to cause a dose-related decrease of *C. elegans* viability and defects in motility, pharyngeal function, reproduction, and development. The effect of these NPs was also assessed in vitro: both positive and negative PS-NPs entered cells primarily by clathrin-mediated endocytosis and were rapidly entrapped into lysosomes. However, amine-modified positive NPs had the higher rate of uptake and caused a dose-dependent decrease in cell growth and viability in HEK 293 cells. The effect of the formation of the protein corona on NPs internalization has been also evaluated through microscopy studies, resulting in a reduction of the internalization rate both for positive and negative PS-NPs. These results underline the crucial role of the surface charge of PS-NPs in their interaction with *C. elegans* in vivo, and with cell membranes in vitro and the consequent biological effects.

## Phenotypic assessment of p.C215Y variant in Gαo/GNAO1 associated with late-onset dystonia and response to caffeine in a new *C. elegans* model

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First identified in 2013, dominant mutations in the GNAO1 gene cause a pattern of severe and early-onset neurological manifestations, including epilepsy, movement disorders, developmental delay, and cognitive decline. A different class of variants has recently been associated with a milder phenotype characterized by adolescent-onset dystonia. GNAO1 encodes the α subunit of a heterotrimeric G protein (Gαo) that negatively regulates ion channel activity and neurotransmitter release, but positively controls cAMP production. Pathogenic mechanisms are still far from being fully elucidated and there are no effective therapies. Recently, we established *Caenorhabditis elegans* as a valuable in vivo model to investigate the pathogenic mechanisms underlying GNAO1-related disorders and perform genetic and chemical screens. In this study, we generated a CRISPR/Cas9-genetically-modified strain harboring a variant associated with late-onset dystonia (p.C215Y) in goa-1, the nematode orthologue of GNAO1. Phenotypic assessment showed that homozygous goa-1[C215Y] animals are characterized by hypersensitivity to aldicarb, an acetylcholinesterase inhibitor, increased egg-laying, and hyperactive locomotion, indicating excessive release of neurotransmitters by different classes of motor neurons. These findings demonstrate a loss-of-function effect of p.C215Y on Gαo signaling. The severity of the identified phenotypes, however, was milder than those observed in *C. elegans* strains harboring variants underlying the canonical form(s) of the disease, resembling the relatively mild clinical features of patients harboring the p.C215Y substitution. Analysis of heterozygous strains allowed to demonstrate a cell-context dominant-negative behavior for the p.C215Y allele, but also that goa-1 is a haploinsufficient gene for some of the tested phenotypes. Similarly to our previous observations, caffeine reduced the hyperkinetic behavior of goa-1[C215Y] animals, confirming that its beneficial effect appears to be variant-independent. Overall, these findings highlight possible application of rapid CRISPR-Cas9 goa-1 gene editing to define the clinical significance and output of variants of uncertain significance affecting GNAO1

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## An in-vivo evaluation of biomimetic nanoparticles and their stimuli-responsive therapeutic attitude: diving into the behaviour/study of *C. elegans*

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Nanomedicine has revolutionized cancer research and treatment, providing innovative solutions for early detection, targeted drug delivery, and precision therapies that minimize side effects. Among recent advancements, nanoparticles that are inherently safe but can become toxic when activated by external stimuli have garnered considerable attention. In this context, engineered zinc oxide nanocrystals (ZnO NCs) serve as stimuli-responsive core, encapsulated in a biomimetic lipid shell (L-ZnO) that mimics natural extracellular vesicles. This core-shell hybrid structure combines excellent biocompatibility and hemocompatibility. By exploiting ultrasonic (US) stimulation, these hybrid nanoparticles can be precisely activated, facilitating controlled therapeutic effects at specific sites. This approach enhances the specificity and efficacy while reducing off-target damage. Despite these advances, evaluating the effects of nanoparticle and ultrasound-based approaches remains challenging, as even the most advanced cellular models fail to accurately predict outcomes in humans. In the present study, we utilized *C. elegans* to investigate in vivo behaviour of ZnO NCs, their lipid-encapsulated counterparts, and ultrasound treatment (as individual treatment and in combination with NCs). This simple yet highly informative model provides critical insights into the safety and efficacy of stimuli-responsive nanoparticles, bridging the gap between in vitro studies and more complex systems. Regarding investigations on toxicological effects, results demonstrated the biosafety of L-ZnO compared to their naked counterparts, consistent with our previous cellular studies. Furthermore, behavioural and functional assays, including motility and brood size evaluations, revealed no significant effects in worms treated with the nanoparticles alone. The effect on viability and mobility of *C. elegans* following US stimulation was investigated, demonstrating a clear correlation between increased power density and decreased viability. An increment in body bends following ultrasound stimulation was also evaluated, demonstrating a correlation between increased US stimulation time and increased body bends. Finally, a combined treatment involving the incubation of worms with L-ZnO nanoparticles followed by ultrasound delivery demonstrated a significant increase in mortality in the combined treatment group compared to those treated with ultrasound alone, highlighting the enhanced effect when both treatments are used together. These findings underscore the importance of combining behavioural and functional studies with toxicological evaluations to comprehensively assess the biocompatibility of stimuli-responsive treatments, paving the way for their safe and effective use in therapeutic applications.

Acknowledgments. This project received funding from European Union – NextGenerationEU

## Proteome and metabolome remodeling in *C. elegans* strains expressing different isoforms of amyloidogenic human β-2microglobulin

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β2-microglobulin (β2 m), the light chain of the class I major histocompatibility complex, is a well-known amyloidogenic protein in humans. The wild type (WT) form of β2 m generates amyloid deposits in long term hemodialyzed patients, when its serum concentrations reach values higher than normal; otherwise, β2 m genetic variants, such as D76N β2 m, can cause amyloid deposition although plasma levels are within the normal range. Despite the progress achieved in elucidating the general mechanism of β2 m amyloidogenesis, a detailed understanding of proteotoxicty pathways remains a challenging issue. We have therefore exploited two *Caenorhabditis elegans* (*C. elegans*) transgenic strains: the first one expressing human WT β2 m at high concentrations, mimicking the condition that underlies dialysis related amyloidosis and the other one expressing the D76N β2 m variant at lower concentrations1. Both strains exhibit pathological phenotypes and show a significant remodeling of proteome and metabolome profiles, being more pronounced in the presence of higher levels of WT β2 m. The organism proteostasis is challenged by the expression of β2 m, inducing higher levels of molecular chaperones and various proteins involved in protein degradation. A redox imbalance and a strong alteration in amino acids metabolism have emerged. Furthermore, alterations in oxidative phosphorylation, fatty acids degradation and Krebs cycle were observed, thus suggesting an impairment of the mitochondrial aerobic metabolism and a shift to anaerobic metabolism. This first characterization of proteomic and metabolomic alterations linked to the expression in *C. elegans* of proteins responsible for systemic amyloidosis in humans, provides important clues on the molecular basis of amyloid cytotoxicity. Furthermore, the exposure of worms to hypoxic condition reveals how lack of oxygen could enhance amyloid toxicity.

## Exploring novel dual epigenetic treatment strategy for Alzheimer's Disease using the *Caenorhabditis elegans* model

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Alzheimer's disease (AD) is the most common cause of dementia. AD affects an estimated more than 800,000 people in Spain, and worldwide, at least 50 million people, making the disease a global health crisis that must be addressed. Until now, none of the approved AD treatments turned out to be a total success. Therefore, it is important to develop treatments aimed at alleviating or delaying the symptoms of AD to improve patients' quality of life. The cause of AD is not known but likely involves a combination of genetic, biochemical, and environmental factors. Thus, interventions targeting epigenetic regulation might be effective in treating age- related neurodegenerative disorders. Therefore, we selected a novel chemical scaffold designed to modulate epigenetic pathways. This study aimed to perform an in vitro characterization of these hit compounds and an initial in vivo approach using the *Caenorhabditis elegans* CL2006 strain, a well-established model for Alzheimer's disease. Results demonstrated a high potency in inhibiting our epigenetic targets and excellent blood-brain barrier (BBB) permeability in in vivo studies. Furthermore, these compounds were shown to reverse β-amyloid pathology and improve the CL2006 strain motility.

Acknowledgements: This study was supported by the Ministerio de Economía, Industria y Competitividad (Agencia Estatal de Investigación, AEI) and Fondo Europeo de Desarrollo Regional (MINECO-FEDER) (PID2022- 139016OA-I00), Generalitat de Catalunya (2021 SGR 00357). This study was co-financed by Secretaria d'Universitats i Recerca del Departament d'Empresa i Coneixement de la Generalitat de Catalunya 2021 (LLavor 0086) and 2023 (Producte 0092; Llavor 005). Grant PID2022-138079OB-I00 funded by MICIU/AEI/10.13039/501100011033 and by "ESF Investing in your future".

## *C. elegans* as a NAM model for micro- and nano-plastics research

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Plastic materials are ubiquitously used in human daily life and are therefore also released into the biosphere. After entering the environment, they can degrade into micro- and nano-plastics (MNPs), smaller plastic fragments with an average size of <5 mm and <100 nm, respectively. MNPs are persistent environmental contaminants that have colonized every ecological niche. Their widespread presence in the environment is an emerging risk that has raised public concern for their potential hazard to human health and ecosystems. Although there is limited information on the potential toxicity of these particles, these could involve oxidative stress, inflammatory reactions, DNA damage, and metabolism disorders. New Approach Methodologies (NAMs) are one of the first choices to generate the information to support chemical risk assessment by informing on the hazard and exposure of a chemical without the use of vertebrate animal testing. This study aims to use the nematode *Caenorhabditis elegans*, included in NAMs by EFSA Supporting publication 2024, as a 3Rs-compliant model to evaluate the biological effect of environmentally relevant concentrations of Polystyrene Nanoplastics (PS-NPs) of two different sizes. Nematodes were exposed to different concentrations of 100- and 20-nm PS-NPs, from the L1 larval stage to the young adult stage. The effect of PS- NPs was evaluated in terms of locomotor behavior in both solid and liquid medium, and ROS production. Our data show a dose-dependent reduction in the number of body bends and thrashes per minute, suggesting a toxic effect of PS-NPs on neuromuscular function. An increase in ROS production was also observed following exposure to all concentrations tested. As expected, smaller MNPs had a more severe effect than larger MNPs on all phenotypes. The effect of PS-NPs will be soon compared with that observed with a biodegradable plastic, i.e., polycaprolactone 100 nm in size (PCL). In addition, since several studies have demonstrated that toxic effects of PS-NPs in *C. elegans* can be transferred to subsequent generation and epigenetic regulations might be involved in the NPs-induced trans-generational inheritance of toxicity in *C. elegans*, the effects of multi- (P0-F4) and trans-generational (F5-F7) exposure to MNPs will be investigated. Given the amount of data available in the literature, PS-NPs serve as an informative model for methodological studies. Similarly,
*C. elegans* is a well-established model in toxicology research, offering detailed insights into biological responses to pollutants in an in vivo system. Furthermore, its use aligns with the 3R principles by partially replacing standard animal experiments, thus promoting more ethical research practices. This study aims to contribute to the development and standardization of reliable NAMs suitable for the assessment of human health risk resulting from exposure to MNPs present in food chain, in a "One Health" perspective.

## Revealing probiotic benefits for healthy aging through *Caenorhabditis elegans*

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Over the last 20 years, scientific research has increasingly focused on the beneficial effects of probiotics, particularly in maintaining a healthy intestinal microbiota and their potential clinical benefits. Conventional experimental models, such as in vitro systems, cell cultures, and mouse models have provided valuable insights into the beneficial properties of probiotic strains; however, each of these models present inherent technical, economic or ethical limitations. Here, we employed the powerful model organism *Caenorhabditis elegans* to investigate and compare the effects of different probiotic strains, owned by SynBalance S.r.l., on aging. Specifically, we fed a N2 wild-type strain with probiotics from the first day of adulthood and behavioral assays were performed at the middle of the lifespan (11th day of adulthood) to assess their potential anti- aging effects. Firstly, we screened strains for their capability to enhance either maximum lifespan and/or movement. Four lactic acid bacteria (LAB), two bifidobacteria (BB) and four Akkermansia muciniphila (Ak) strains at OD=1 were used as food source, while E. coli OP50 strain was used as a control diet. Based on improvements of these parameters, we select probiotics for further anti-aging characterization. Among the LAB strains, two significantly extended maximum lifespan by five days, with one of these also enhancing locomotor capacity, increasing the body bends number by 19%. The same LAB strain also reduced ROS levels of about 60%. Among the BB strains, only one was selected for its capability to enhance mobility by 21%, although no ROS reduction was observed. Additionally, we evaluated the effects of probiotic administration on synaptic transmission: neither LAB nor BB strains exhibited significant improvement in this parameter. Conversely, among Ak, three out of four strains ameliorated locomotion, with a maximum increase of 55% in body bend number. However, no significant effects on lifespan extension or ROS reduction were observed. Unlike LAB and BB strains, all Ak strains significantly improved synaptic transmission. This study highlights *C. elegans* as an effective model for screening probiotic anti-aging effects, identifying specific LAB, BB, and Ak strains that enhance lifespan, mobility, and synaptic transmission. The findings suggest distinct molecular mechanisms underlying these benefits. While some strains showed no ROS reduction or synaptic improvements, future studies using mutant *C. elegans* will elucidate the pathways involved, providing insights into probiotics' potential for promoting healthspan.

## Screening of I2-IR ligands in *C. elegans* models of Alzheimer's and Huntington's disease

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Imidazoline receptors are a family of non-adrenergic binding sites, with high affinity for compounds with an imidazole ring in their structure. There are three subtypes: I1-IR, I2-IR, and I3-IR, being the I2-IR widely distributed in the brain. Selective ligands for the imidazoline I2 receptors (I2-IR) have attracted much attention in recent times, because of their neuroprotective effect. Compounds such as 2-BFI, MCR5, LSL60101, BU224 have demonstrated a potential pharmacological and therapeutic effect in improving cognition and reducing neuroinflammation and in mice transgenic models of Alzheimer's disease (AD) and with antinociceptive properties. Recent studies have demonstrated that new bicyclic α-iminophosphonates as BIN02, BIN05, and B06-red, showed promising activities as I2-IR ligands in human brain tissues and good BBB permeation capabilities and exerted neuroprotective activity in *C. elegans*. Considering all this, in the present study, we performed a general screening of several I2-IR ligands such as Idazoxan, CR4056, LSL60101, among others in CL2006, a well-characterized transgenic AD strain of *C. elegans* and EAK103 a transgenic strain of Huntington's Disease (HD). First, we evaluated the safety of these compounds (in doses between 0.1 µM and 10 µM), measuring their effect on food consumption in *C. elegans* N2 (Wild-Type) (N2-WT) observed as the variation in optical density (OD595) of bacteria over time, expecting a normal development and wellbeing of animals in each dose tested. The results show that all the compounds could be classified as safe. Then, to establish a dose-response profile, the effect of the compounds was evaluated in a movement assay with the CL2006 strain, observing an increase in the motility of most of the I2-IR ligands at the 0.5 µM dose compared to the CL2006 worms that had not received treatment. Considering that, we measure the effect of all I2-IR ligands in amyloid beta (Aβ) and huntingtin (Htt-513) aggregation, with a reduction of Aβ spots and Huntingtin aggregates in several I2-IR I2 ligands. With these promising results, we can consider this the future candidate for evaluating its molecular effects and the effect in AD and HD mice models.

## Characterization of novel pediatric ultrarare genetic disorders to advance diagnosis and optimize treatment

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Validation of genetic variants and of their effects is still challenging mostly because the biological functions of many protein-encoding genes still need to be deciphered. This is particularly relevant for rare diseases where the lack of a molecular diagnosis often impedes personalized clinical management and treatment, as well as an appropriate counseling for patients and their families. In vivo models, such as *Caenorhabditis elegans*, allow modeling and validating rare variants and investigating the function of uncharacterized genes; remarkably they represent a prerequisite for drug discovery and for testing etiological treatment. We are now analyzing the effects of genetic variants detected in the KIF3B gene (MIM#603754) in a newborn patient affected by a severe primary ciliopathy that shows a pattern of inheritance compatible with an autosomal recessive condition. This gene had been previously associated with two distinct autosomal-dominant phenotypes; however, no autosomal recessive forms of KIF3B-related ciliopathy have been reported so far. We are generating a *C. elegans* model harboring the novel variants identified in the patient to explore their pathogenicity and their consequences on cilia structure and function in vivo. We also aim at deciphering the role of LARP1B (MIM#620467), a gene with unknown function not associated with a human disorder so far, and its involvement in a congenital disorder affecting one male patient at birth and characterized by severe symptoms such as coarctation of the aorta and other cardiac defects, cleft of soft palate, brain ventriculomegaly and dysmorphisms. For this purpose, we have already modeled the human LARP1B variants in the corresponding worm orthologue and we have started the phenotypic characterization of worm mutant lines in order to assess the pathogenicity of the variants. We will also employ a larp1b knock out model of Zebrafish to investigate if this gene is involved in the development of heart and cranial structures during embryogenesis. Finally, Aromatic L-Amino Acid Decarboxylase (AADC) deficiency is an early onset autosomal recessive disease caused by mutations in DDC (MIM#107930) and characterized by deficiency of neurotransmitters dopamine and serotonine. The clinical presentation of this disorder is marked by severe motor impairment, developmental delay, oculogyric crises, along with sleep and gastrointestinal problems. Typically, this infantile Parkinsonism proves fatal within the first decade of life. We have generated *C. elegans* mutant lines that carry some mutations found in patients in the corresponding worm bas-1 gene, in order to obtain an in vivo model of AADC deficiency that will be used not only to investigate genotype-phenotype correlations, but also to test compounds in order to optimize therapeutic intervention.

## High-content longitudinal imaging of *C. elegans* for biological age prediction

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Understanding how an organism remains healthy later in life is an important goal of aging research. Transcriptomic clocks are refined tools that enable prediction of biological age based on molecular data with remarkable accuracy in multiple organisms, including *C. elegans*. Yet, in *C. elegans*, these clocks require the sacrifice of the worms and thus hamper longitudinal measurements.
Here, we present a non-invasive phenotypic aging clock for C. elegans that enables the quantification of biological age at the individual worm level throughout its lifespan.
Using the automated SydLab platform, we tracked close to 4500 worms at 6h intervals throughout their whole lifespan generating a comprehensive dataset of 29 phenotypic features related to aging, such as motility, morphology, and reproductive parameters. These data were used to develop an aging clock able to predict biological age of individual worms with a median R2 of 0.84 (0.79-0.86; interquartile-range, IQR). While accurately predicting biological age at single timepoints, the clock also provides an unprecedented vision of aging trajectories at the individual level.
By day 12, biological age predictions from our aging clock distinguish interventions that extend lifespan from those with no effect with 80% accuracy and 91% specificity. This is accomplished in less than half the time required for a full lifespan assay and using as few as 40 worms per condition. Moreover, when performing a complete lifespan assay, our approach can be combined with standard survival measurements, helping to dissociate healthspan from lifespan effects.
To validate our approach, we conducted a preliminary study with several mutant strains (the long-lived slcf-1(tm2258) and the short-lived daf-18(e1375)) comparing a transcriptomic clock (BiT age) with our phenotypic clock. While the two clocks showed a strong agreement in their predictions for slcf-1 and daf-18, the double mutant daf-18(e1375);slcf-1(tm2258) revealed discrepancies between transcriptomic age and phenotypic age. This may suggest additional complexities in the coupling between transcriptional changes and functional biomarkers in worm aging.
Our results highlight the value of real-time functional phenotyping for aging research in C. elegans, complementing existing molecular-based methods. Thanks to its image-based nature, our approach is inherently scalable and adaptable to multiple experimental designs, offering potential for early drug screening and investigations that require repeated measurements of the same animals. Future work will aim on integrating tissue-specific phenotypes, fluorescence data and construct organ-specific clocks, ultimately bridging the gaps between biological scales.

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