X SPANISH WORM MEETING

SEPTEMBER 14 - 15, 2026

VALÈNCIA, SPAIN

BOOK OF ABSTRACTS

Spanish Worm Meeting 2026 (SWM2026)

València, Spain, 2026 September 14th - 15th
Conference organizers: Nuria Flames, Jose Perez-Martin and Rafael Vázquez-Manrique

Conference Program

September 14th - Day 1 (Monday) 1	
08:15 - 08:50	**Registration**
09:00 - 09:15	**Opening Presentation**
	Session 1 Chair: Laura Molina and Jesús Fernández-Abascal
09:15 - 09:45 1-K1	Christian Froekjaer-Jensen Evolution of mechanical chromatin insulators from selfish genetic elements
09:45 - 10:00 1-O1	Andrea del Carmen Fabregat, Nicholas Stroustrup An RNA Polymerase II disequilibrium state drives global declines in mRNA abundance in aging
10:00 - 10:15 1-O2	José Ignacio Jordá-Llorens, María Sánchez-Carcelén, Jessica Valdivia, Eduardo Leyva-Díaz CUT Homeobox Transcription Factors Regulate Neuronal Subtype Identity Through Chromatin Remodeling
10:15 - 10:30 1-O3	Vladimir Lazetic Microbiome-Derived RNA Promotes Heritable Defense Against Intracellular Pathogens in C. elegans
10:30 - 10:45 1-O4	Arturo Bujarrabal-Dueso, Björn Schumacher A Novel H2A.Z-Dependent Mechanism Underlies DREAM Complex Regulation of the DNA Damage Response
10:45 - 11:00 1-O5	Lídia Nunes-Carvalho, Jorge H. Fernandes, Bruna Ferreira-Lomba, Ana Rita Peixoto, Joana Ribeiro, Patrícia Maciel, Joana Pereira-Sousa, Andreia Teixeira-Castro Role of the serotonin receptor 5-HT3/LGC-50 in Spinocerebellar Ataxia type 3 pathogenesis
11:00 - 11:15 1-O6	Carmen Muñoz-Méndez, Patricia de la Cruz-Ruiz, David Guerrero-Gómez, Juan Cabello, Antonio Miranda-Vizuete Aggrepoptosis: Ferroptotic cell death induced by protein aggregation
11:15 - 11:45	**Coffee Break**
	Session 2 Chair: Carla LLoret and João Picão-Osório
11:45 - 12:15 2-K1	Ana Xavier Carvalho Building specialized actomyosin networks in C. elegans contractile systems
12:15 - 12:30 2-O1	Adrián Fragoso Luna, Andrea Del Valle Carranza, María Luque Jiménez, Anita G Fernandez, Marion Kennel, Bente Wohlert, Colin Morris, Bárbara Sánchez Ruiz, Marina Hernández-Ballester, Nina Mellmann, Patricia De la Cruz Ruiz, Jesús Fernández Abascal, Ángeles Bretón Robles, Cristina Ayuso García, Antonio Miranda Vizuete, Ángeles Ortega De La Torre, Rafael Vázquez Manrique, Peter Askjaer A progeria mutation in baf-1 confers neuroprotection against polyQ-based aggregates
12:30 - 12:45 2-O2	David Borrego, Adrián Fragoso-Luna, Alan Koh, Javier Rodríguez, Julia Hillung, Andre E. X. Brown, José Pérez-Martín The cell cycle regulator APC/CFZR-1 regulates Wnt-mediated fate decisions in C. elegans postembryonic development.
12:45 - 13:00 2-O3	Victoria G Castiglioni, Isaac Martínez-Ugalde, Santiago F Elena, Julie M Claycomb Understanding the dynamics of AGO proteins in the C. elegans–Orsay virus pathosystem
13:00 - 13:15 2-O4	Julia Tortajada-Pérez, Sergio Gordillo-García, Cristina Trujillo-del-Río, Maksym Kupchyk-Tiurin, Mar Collado-Pérez, Laura Cantalejo-Carrasco, Marta Roca, Agustín Lahoz, Christian Neri, José Maria Millan, Marta Artal-Sanz, Andrea del Valle Carranza, Rafael P. Vázquez-Manrique Synergistic Activation of PPARγ and AMPK: A Metabolic Approach to Improve Proteostasis and Mitochondrial Health in HD
13:15 - 13:30 2-O5	David Logan, Carlos Oliva, Lei Wang, Melisa Lamberti, Isabella Gay, Olivia White, Enrique Oliver, Jesus Fernandez-Abascal, Bianca Graziano, Laura Bianchi Glia-to-neuron serotonin signaling controls ASH-dependent nose touch in C. elegans
13:30 - 13:45 2-O6	Eric Cornes, Bernard Florian, Billiet Alexia, Shukla Akanksha Coordinated regulation of spermatogenesis by germline small RNA pathways
13:45 - 15:30	**Lunch**
	Session 3 Chair: Marta Costa and Adrián Fragoso-Luna
15:30 - 16:00 3-K1	David Vilchez, Christian Frezza, Thorsten Hoppe, Ming Yang, Agnieszka Soko, Theodoros Georgomanolis, Dimitrios Prymidis, Joana Schnubel, Murat Artan, Rute Loureiro, Dunja Petrovic, Markus Wehrmann, Seda Koyuncu, Cansu Doğan, Hyun Ju Lee Cold temperature activates a BH2-driven intertissue metabolic network that promotes longevity and proteostasis
16:00 - 16:15 3-O1	Guilherme Ferreira, Joana Vilas Boas, João Picão-Osório Uncovering the genetic architecture of pathogen avoidance behaviour in Caenorhabditis elegans
16:15 - 16:30 3-O2	Rafael Alis, Nuria Flames Cell-type-specific profiling of transcription factor binding in C. elegans

16:30 - 16:45 3-O3	María Olmedo, Francisco Javier Romero-Expósito, Almudena Moreno-Rivero, Marta Muñoz-Barrera, Nicola Gritti, Jeroen S van Zon, Francesca Sartor, Martha Merrow, Alejandro Mata-Cabana Desynchrony between events triggers a compensatory delay during C. elegans development
16:45 - 17:00 3-O4	Sergio Gordillo-García, Jesus Fernandez-Abascal, Blanca Hernando-Rodríguez, María Jesús Rodríguez-Palero, Laura Cantalejo-Carrasco, Aitor Jarit-Cabanillas, Manuel D. Martínez-Bueno, Mercedes M. Pérez-Jiménez, Enrique J. Clavijo-Bernal, Aitana Cambón, Ildefonso Cases, Nicholas E. Stroustrup, Matthias Eder, Marta Artal-Sanz The Deubiquitinase USP-48 Enables Insulin-Mediated Longevity and Modulates the Mitochondrial Stress Response
17:00 - 17:15 3-O5	Silvia Llopis, Miren Maicas, Ferran Balaguer, Nuria González, Lucía Jareño, Paula Laguna, Cristina Isabal, Empar Chenoll, Patricia Martorell , Validation of the Functionality of Heat-Inactivated Strains in Food Applications Using the Caenorhabditis elegans Model
17:30 - 18:30 public)-K1	**Nobel Prize Talk: Victor Ambros (open to public)** Chair: Nuria Flames Victor Ambros MicroRNA-mediated gene regulation and developmental robustness
18:30 - 20:00	**Poster Session**
21:00	**Gala Dinner**

September 15th - Day 2 (Tuesday) 2

Session 4
Chair: Montserrat Porta-de-la-Riva and Marcos F. Pérez

09:30 - 10:00 | Arantza Barrios
4-K1 | The flexible brain: lessons from a worm with 387 neurons

10:00 - 10:15 | Israel Campo-Bes, Alejandro Burga, Manuel Irimia
4-O1 | Recent horizontal transposon transfer boosted by virus-like proteins in nematodes

10:15 - 10:30 | Andrés Velázquez Mudarra, Carmen Martínez Fernández, Marta García García, Alba Olaso Llorca, David Aristizábal Corrales, Homa
4-O2 | Kohandelgargari, Janet Hoenicka, Francisco Palau, Julián Cerón
Modelling the clinical continuum of ATP7A-related diseases in Caenorhabditis elegans

10:30 - 10:45 | Dominik Herek, María J. Olmo-Uceda, Victoria G. Castiglioni, Santiago F. Elena
4-O3 | A Novel Role for FOG-2 in Caenorhabditis elegans Antiviral Immunity

10:45 - 11:00 | Carlos López, Anna Laromaine, Juan Pellico, Olga Zeni
4-O4 | From Field Radiation to Plastic Toxicology: C. elegans Bridges the Gap Across Stressors

11:00 - 11:15 | Silvia Romero-Sanz, Andrea Morán-Cerrro, Elena Caldero-Escudero, Paloma García-Casas, Rosalba I Fonteriz, Silvia Fernández-Martínez, Mayte
4-O5 | Montero, Javier Álvarez, Jaime Santo-Domingo, Sergio De la Fuente
Mitohormesis in action: how mild complex-I inhibition by rotenone promotes longevity in C. elegans

11:15 - 11:30 | María Pilar de Lucas, Ana Belén Cámara, Cristina Ortiz-Gutiérrez, Natasha Zarich, Berta Anta, José María Rojas-Cabañeros
4-O6 | Turning Back to C. elegans to Move Forward in Aging: A Role for SOC-2

11:30 - 12:00 | **Coffee Break**

Session 5
Chair: Andrea Millan and Eric Cornés

12:00 - 12:30 | Pablo Lopez-Jimenez, Joseph Davy, Rahul Saha, Marius Rutkauskas, Mariana Sacerdoti, Josh Prince, George Sioutas, Eugene Kim, Enrique Martinez-Pérez
5-K1 | Biophysical and in vivo approaches uncover the mechanisms by which cohesin orchestrates meiotic chromosome structure and function

12:30 - 12:45 | Ángela Metola, Eva Gómez-Orte, Rosario López, Begoña Ezcurra, Angelina Zheleva, María de Toro, Gonzalo Jiménez-Osés, Peter Askjaer, Marta
5-O2 | Artal-Sanz, Ricardo García-Muñoz, Antonio Miranda-Vizuete, Juan Cabello
The C. elegans Integrator interactome reveals its architecture and novel roles in homeostasis

12:45 - 13:00 | Laura Molina-García, Paula Mancebo-Gamella, Lucía Pérez-García, Susana Quesada-Gutiérrez, Lucía Rodríguez-Heine, Gabriela
5-O1 | Vázquez-Cartagena
Development of genetic tools to dissect the role of microbiota-derived bacterial amyloids in neurodegeneration

13:00 - 13:15 | Loreto Castro Castro, Ana María Brokate Llanos, Carlos Gomez Marín, Almudena Moreno Rivero, María Olmedo López, María de las Mercedes
5-O6 | Pérez Jiménez, Manuel Jesús Muñoz Ruiz
Sulfated Steroid Hormones Regulate Starvation-Induced L1 Arrest in Caenorhabditis Elegans

13:15 - 13:30 | Paula Soto Carmona, Belen Perales Villegas, Marta Artal-Sanz, Jesus Fernandez-Abascal
5-O5 | Neuron subtype-specific mitochondrial stress signalling engages glial responses and shapes systemic ageing trajectories

13:30 - 13:45 | Adrián Millán, Lorien Abellanas, Víctor López, Carlota Gómez, Cristina Moliner
5-O4 | Membrane disruption as a potential anthelmintic mechanism of an extract of Acmella oleracea against nematodes

13:45 - 14:00 | Eider Ciaurriz Arrastia, Maite Solas Zubiaurre, Carolina González Ferrero
5-O3 | Development of a Caenorhabditis elegans-Based Screening Platform to Identify Neuroprotective Candidates for Alzheimer's Disease

14:00h | Farewell and Picture

Session 1

1.1. Evolution of mechanical chromatin insulators from selfish genetic elements

Authors: Froekjaer-Jensen, Christian

Affiliations:

1. Bioscience Program, Biomedical Sciences Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

Abstract:

Genomes require physical boundaries to separate active and repressed chromatin, a function traditionally attributed to insulator proteins. Here, we demonstrate that genomic insulation is also a physical property encoded into the DNA polymer. In C. elegans, transcription-coupled mutational bias remodels Helitron transposon minisatellites to match the DNA helical repeat. This intrinsic curvature pins supercoiled DNA into nucleosome-depleted plectonemes, creating mechanical barriers that partition chromosomes into gene-sized regulatory domains. Constrained by DNA mechanics, this structural strategy has evolved convergently across Metazoa into a shared mechanical code at Drosophila insulators and human CTCF sites. Strikingly, active human LINE-1 retrotransposons encode a similar plectoneme structure. Thus, sequence-encoded mechanics represent a universal solution to transcription-silencing conflicts—a topological barrier harnessed by host genomes to organize chromatin, and seized by selfish elements to survive.

Program Time: Monday, September 14th at 9:15 AM

1.2. An RNA Polymerase II disequilibrium state drives global declines in mRNA abundance in aging

Authors: Andrea del Carmen Fabregat (1,2); Nicholas Stroustrup (1,2)

Affiliations:

1. Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain
2. University Pompeu Fabra, Carrer del Doctor Aiguader, 88, Barcelona, ES

Abstract:

Aging involves progressive declines in physiologic homeostasis, but the molecular mechanisms that in youth drive organisms away from equilibrium remain unclear. Using absolute transcript quantification, we identify a global collapse in cellular and organismal mRNA abundance that occurs in most cell types during invertebrate and mammalian aging. Using an interventional nematode model, we find that global declines in mRNA abundance are tightly coupled to global declines in RNA Polymerase II (RNAPolII) protein abundance. Longitudinal measurements reveal that individuals enter adulthood in a disequilibrium state, with nuclear RNAPolII abundance far in excess of the steady-state level supported by adult rates of protein synthesis and degradation. As a result, RNAPolII abundance falls during adulthood, progressively limiting mRNA synthesis. We find that even transient accelerations in the fall of RNAPolII abundance produce permanent, dose-dependent reductions in healthspan and lifespan. Conversely, modulation of insulin/IGF signaling simultaneously slows declines in RNAPolII and global mRNA abundances while increasing healthspan and lifespan. Together, our results demonstrate how an out-of-equilibrium state of RNAPolII present in early adulthood is sufficient to drive organismal aging, and highlights a new molecular target for interventions aimed at preserving mRNA synthesis capacity in aging.

Program Time: Monday, September 14th at 9:45 AM

1.3. CUT Homeobox Transcription Factors Regulate Neuronal Subtype Identity Through Chromatin Remodeling

Authors: José Ignacio Jordá-Llorens (1); María Sánchez-Carcelén (1); Jessica Valdivia (1); Eduardo Leyva-Díaz (1)

Affiliations:

1. Instituto de Neurociencias, CSIC-UMH, Av. Ramon y Cajal, s/n, San Juan de Alicante, ES

Abstract:

Neurons acquire distinct molecular identities during development through specific transcriptional programs, which must be actively maintained throughout the lifespan. Characterizing the molecular identity of fully differentiated neurons is therefore critical for understanding nervous system organization and function. This identity is encoded by the coordinated expression of neuron type–specific genes, which distinguish individual neuronal subtypes, and shared neuronal genes, which support core neuronal features and functions. In C. elegans, the expression of shared neuronal genes is regulated by the combined action of broadly expressed CUT homeobox transcription factors and neuron type–specific master regulators known as terminal selector (TS) transcription factors. Previous studies have shown that CUT homeodomain binding motifs are strongly associated with differential chromatin accessibility between induced neurons and fibroblasts, suggesting a potential pioneer factor role for CUT proteins. We hypothesize that CUT factors modulate chromatin accessibility to facilitate the binding of additional transcription factors. To investigate how CUT factors influence the neuronal chromatin landscape, we developed a method to isolate nuclei from all C. elegans neurons by FANS and profile chromatin accessibility in CUT homeobox mutants. This revealed reduced chromatin accessibility in the regulatory regions of shared neuronal genes, consistent with the established role of CUT factors in pan-neuronal gene regulation. Strikingly, we also identified changes at the regulatory regions of neuron type–specific genes, pointing to a broader role in neuronal identity. Revisiting bulk transcriptomic data from CUT mutants confirmed this observation: expression of both shared neuronal and neuron type–specific genes is reduced in CUT mutants. To directly characterize this broader contribution, we performed single-nucleus transcriptomic profiling of FANS-isolated nuclei from wild-type and CUT mutant animals, providing the first comprehensive view of CUT factor function at single neuronal subtype resolution.

Program Time: Monday, September 14th at 10:00 AM

1.4. Microbiome-Derived RNA Promotes Heritable Defense Against Intracellular Pathogens in C. elegans

Authors: Vladimir Lazetic (1)

Affiliations:

1. The George Washington University, 22nd Street Northwest, 800, Washington, US

Abstract:

The microbiome profoundly influences host physiology, including immune homeostasis and defense against infection, yet the molecular signals by which specific commensal bacteria modulate innate immunity remain poorly defined. The nematode Caenorhabditis elegans provides a powerful system for dissecting these host–microbiome interactions, as it possesses a simple and well-characterized innate immune system and a defined natural microbiome. One of the key immune programs in C. elegans is the Intracellular Pathogen Response (IPR), a transcriptional response activated by obligate intracellular pathogens of the intestine, including viruses and microsporidia. The IPR shares some similarities with the mammalian type I interferon response and serves as a framework for understanding how intestinal epithelial cells respond to infection. To identify microbiome members capable of modulating the IPR, we screened twelve native bacterial isolates for their ability to activate an IPR fluorescent reporter. We discovered that Stenotrophomonas indicatrix (JUb19) robustly induces reporter expression in multiple tissues, including the intestine, epidermis, neurons, and somatic gonad. Other Stenotrophomonas species trigger similar activation, suggesting a genus-linked feature. Notably, JUb19 resides extracellularly in the intestinal lumen, making this the first known example of a non-invasive bacterial species that activates the IPR reporter. Mechanistic analyses revealed that heat-killed JUb19 fails to induce IPR reporter expression, whereas mechanically or chemically inactivated bacteria retain this activity, indicating the presence of a heat-labile molecular trigger. Transcriptomic profiling revealed that JUb19 only partially induces the IPR, alongside broader metabolic remodeling. Nevertheless, this response confers resistance to both viral and microsporidian infections, and this protection is also inherited by naïve progeny that were never exposed to JUb19. We further show that bacterial RNA acts, at least in part, as a molecular cue underlying the transcriptional and immune phenotypes associated with JUb19 exposure. Together, these findings establish a new paradigm for microbiome-driven epithelial immunity in C. elegans, in which microbiome-derived RNA links bacterial exposure to immediate and intergenerational defense against intracellular pathogens.

Program Time: Monday, September 14th at 10:15 AM

1.5. A Novel H2A.Z-Dependent Mechanism Underlies DREAM Complex Regulation of the DNA Damage Response

Authors: Arturo Bujarrabal-Dueso (1,2); Björn Schumacher (1,2)

Affiliations:

1. Institute for Genome Stability in Aging and Disease, Medical Faculty, University and University Hospital of Cologne, Cologne, Germany

2. Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

Abstract:

The DREAM complex is a conserved transcriptional repressor of cell cycle genes that promotes quiescence and regulates the expression of germline genes. Using C. elegans we found that DREAM binds and represses multiple genes involved in the DNA damage response. DREAM-complex-deficient mutants show a remarkable resistance to, and improved repair of various DNA-damage types, during both development and aging. The mechanisms of DREAM complex regulation are not fully understood, but emerging evidence shows that this complex interacts with epigenetic modulators that could be driving its repressive function. Among them, the deposition of the H2A variant H2A.Z in gene bodies has been described as a DREAM-mediated repression mechanism in C. elegans. Using a forward genetic screen in C. elegans we found that a mutation for the gene ssl-1 (ortholog of SRCAP/EP400), involved in the maintenance and deposition of H2A.Z, confers severe sensitivity to DNA damage. Employing RNA-seq and ChIP-seq analysis on these mutants we observe a broad downregulation of DREAM-associated genes, including repair genes. Repressed genes in ssl-1 mutants are characterized by a marked reduction of H2A.Z around the promoter area, suggesting that H2A.Z deposition is required for the normal expression of DNA repair genes. Furthermore, the capacity to deposit H2A.Z is also required for the DNA damage resistance phenotype of DREAM deficient organisms. In summary, we identify a novel function for H2A.Z deposition in the regulation of DNA repair genes and DREAM target genes. Unlike previous research linking DREAM and H2A.Z, our analysis suggests that H2A.Z at the promoter region has an activating effect that is needed in both wild-type and DREAM-deficient mutants for genome maintenance.

Program Time: Monday, September 14th at 10:30 AM

1.6. Role of the serotonin receptor 5-HT3/LGC-50 in Spinocerebellar Ataxia type 3 pathogenesis

Authors: Lídia Nunes-Carvalho (1,2); Jorge H. Fernandes (1,2); Bruna Ferreira-Lomba (1,2); Ana Rita Peixoto (1); Joana Ribeiro (1); Patrícia Maciel (1,2); Joana Pereira-Sousa (1,2,3); Andreia Teixeira-Castro (1,2)

Affiliations:

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal

2. ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

3. Screen4Health, School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal

Abstract:

Introduction: Spinocerebellar ataxia type 3 (SCA3), also known as Machado–Joseph disease, is a neurodegenerative disorder caused by an unstable expansion of CAG trinucleotide repeats in the ATXN3 gene. This expansion results in an abnormally long polyglutamine (PolyQ) tract within the ATXN3 protein, which promotes protein misfolding, self-association, and the formation of intracellular toxic aggregates. These aggregates are widely considered to contribute to neuronal dysfunction and degeneration. Previous studies have demonstrated that treatment with citalopram (CIT), a selective serotonin reuptake inhibitor (SSRI), can ameliorate SCA3-related pathogenesis in both Caenorhabditis elegans and mouse models [1]. CIT binds to the serotonin transporter (SERT), inhibiting serotonin (5-HT) reuptake from the synaptic cleft and thereby enhancing 5-HT signalling via serotonin receptors (5-HTRs) [1, 2]. The therapeutic effect of CIT in SCA3 is dependent on serotonin receptor activity. Recently, the LGC-50 receptor was identified in C. elegans as a pentameric cation channel activated by serotonin [3], functionally analogous to the human 5-HT3 receptor (5-HT3R), which is also a pentameric ligand-gated ion channel composed of five subunits mediating fast excitatory neurotransmission in both the central and peripheral nervous systems [4]. Aims: The present study aimed to explore the degree of homology between LGC-50 and human 5-HT3R using detailed bioinformatic analyses and to assess the functional role of LGC-50 in the pathogenesis of SCA3 using genetically modified C. elegans models. Specifically, we sought to compare the structural characteristics of LGC-50 and human 5-HT3R subunits and to evaluate the impact of modulating LGC-50 expression on mutant ATXN3 aggregation, neuronal health, and motor dysfunction in SCA3 worm models. Methods: Bioinformatic analyses were conducted to compare the two- and three-dimensional structures of LGC-50 and human 5-HT3R subunits, using HHpred, EMBL-EBI T-Coffee and PyMOL. Genetic tools, including CRISPR–Cas9 and Mendelian breeding strategies, were employed to generate C. elegans SCA3 models with altered LGC-50 expression, including receptor deletion (LGC-50 del; AT3q130) and exclusive LGC-50 expression (LGC-50 only; AT3q130). Motor function was assessed using motility assays, and mutant ATXN3 aggregation was quantified via confocal microscopy. Results: Sequence analysis revealed high similarity and low E-values between LGC-50 and all human 5-HT3A–E receptor subunits, except for 5-HT3D. Three-dimensional structural analyses demonstrated significant homology, particularly in the 5-HT binding domain of the 5-HT3A subunit. Deletion of LGC-50 in C. elegans expressing mutant ATXN3 did not alter disease phenotypes. Unexpectedly, expression of LGC-50 alone, in the absence of other 5-HTRs, was sufficient to suppress motor deficits and neuronal aggregation associated with mutant ATXN3. Control 5-HTR-null animals also displayed partial SCA3 suppression, a finding currently under validation in additional mutant strains. Conclusion: These findings suggest that LGC-50 is a likely C. elegans homologue of human 5-HT3A. Moreover, LGC-50/5-HT3R may play a key role in mitigating SCA3 pathogenesis, supporting the importance of serotonergic signalling in the development and potential treatment of SCA3/Machado–Joseph disease.

Program Time: Monday, September 14th at 10:45 AM

1.7. Aggrepoptosis: Ferroptotic cell death induced by protein aggregation

Authors: Carmen Muñoz-Méndez (1); Patricia de la Cruz-Ruiz (1); David Guerrero-Gómez (1); Juan Cabello (2); Antonio Miranda-Vizuete (1)

Affiliations:

1. Instituto de Biomedicina de Sevilla (IBIS), Hospital Universitario Vírgen del Rocío, Sevilla, Spain, Sevilla, ES

2. Centro de Investigación Biomédica de la Rioja (CIBIR)

Abstract:

The successful execution of cellular processes depends on the coordinated interactions of proteins. Consequently, imbalances in protein homeostasis (proteostasis) underlie the pathogenesis of numerous human disorders including cancer, diabetes and neurodegenerative diseases. In many of these conditions, proteostasis disruption results in the aggregation of specific proteins, which constitutes the key pathogenic event. Over the last few years, our group has uncovered a novel, protective role of reduced glutathione (GSH) in yeast, Caenorhabditis elegans and mammalian cell models of proteotoxicity caused by aggregation-prone proteins. We have demonstrated that GSH modulates autophagy at multiple levels and that compromising GSH availability, either genetically or pharmacologically, severely impairs redox regulation of autophagy-dependent degradation of protein aggregates, ultimately leading to cellular and organismal death [1,2]. However, the type of cell death as well as the molecular mechanisms governing the lethal phenotype associated with exacerbated protein aggregation following disruption of GSH redox homeostasis remain unknown. In this study, we will present evidence that C. elegans expressing aggregating proteins in muscle cells rapidly die upon exposure to glutathione-depleting compounds and identify ferroptosis —a recently discovered iron-dependent cell death caused by massive plasma membrane lipid peroxidation—, as the ultimate cause of death. Our findings reveal that both the reverse transsulfuration pathway and the cystine reduction pathway are required for the survival of worms expressing aggregation-prone proteins in muscle cells [3], and that inhibition of ferroptosis, but not apoptosis or necroptosis, restores animal viability. In addition, we will discuss recent proteomic and transcriptomic analyses, as well as genetic suppressors arising from a genetic screen, to further elucidate the molecular players and pathways underlying the differential responses of C. elegans tissues and organs to protein aggregation and ferroptosis.

Program Time: Monday, September 14th at 11:00 AM

Session 2

2.1. Building specialized actomyosin networks in C. elegans contractile systems

Authors: Xavier Carvalho, Ana

Affiliations:

1. Instituto de Investigaçao e Inovaçao em Saude. Universidade do Porto. Porto. Portugal

Abstract:

Actin crosslinkers and myosins shape whether an actomyosin network is dynamic or stable, tuning it to the mechanical needs of the contractile structure it builds. I will discuss how crosslinker and myosin identity contribute to the assembly and function of contractile actin networks in C. elegans, drawing on work in contractile systems such as the spermatheca and the early zygote. I will close by presenting a newly funded COST Action on advancing nematode research through technology and collaboration across Europe, and invite the community to get involved.

Program Time: Monday, September 14th at 11:45 AM

2.2. A progeria mutation in baf-1 confers neuroprotection against polyQ-based aggregates

Authors: Adrián Fragoso Luna (1); Andrea Del Valle Carranza (2); María Luque Jiménez (1); Anita G Fernandez (3); Marion Kennel (1); Bente Wohlert (1); Colin Morris (1); Bárbara Sánchez Ruiz (1); Marina Hernández-Ballester (1); Nina Mellmann (1); Patricia De la Cruz Ruiz (4); Jesús Fernández Abascal (1); Ángeles Bretón Robles (1); Cristina Ayuso García (1); Antonio Miranda Vizuete (4); Ángeles Ortega De La Torre (5); Rafael Vázquez Manrique (2); Peter Askjaer (1)

Affiliations:

1. Andalusian Centre for Developmental Biology, Consejo Superior de Investigaciones Científicas/Junta de Andalucía/Universidad Pablo de Olavide, Seville, Spain
2. Instituto de Investigación Sanitaria La Fe, Avinguda de Fernando Abril Martorell, 106, València, ES
3. Fairfield University (USA)
4. Instituto de Biomedicina de Sevilla (IBIS), Hospital Universitario Vírgen del Rocío, Sevilla, Spain, Sevilla, ES
5. Universidad Pablo de Olavide, , Sevilla, 0, ES

Abstract:

Progerias are rare syndromes that cause accelerating ageing since early childhood, with an average lifespan close to 15 years. The aetiology lies in mutations within components of the nuclear envelope, like lamin (Hutchinson-Gilford Progeria Syndrome) and BAF (Néstor-Guillermo Progeria Syndrome). Patients suffer typical problems linked to ageing, like osteoporosis, atherosclerosis, lipodystrophy, sarcopenia, hair loss, etc. Paradoxically, it is remarkable that they do not develop neurodegeneration, a classical hallmark of ageing. Using a C. elegans model of Néstor-Guillermo Progeria Syndrome (baf-1(G12T)), we have observed a significant resistance to polyQ-associated neurodiseases like, Huntington and Joseph-Machado ataxia. The frequency of protein aggregates is reduced specifically in neurons of baf-1(G12T) mutants, accompanied by an improvement in motility and touch response. Other neurodegenerations, as Alzheimer, Parkinson and ALS are also triggered by aggregation of aberrant proteins, that interfere with the correct physiology of the cell. Specifically, many studies suggest that the main intracellular process affected is endolysosomal trafficking, key in neurons. Interestingly, our work indicates that Néstor-Guillermo Progeria Syndrome also affect endolysosomal trafficking both in C. elegans and human patient cell lines. Analysis of chromatin accessibility in neurons reveals that the progeria mutation induces opening of genes involved in proteostasis. We postulate that accelerated-ageing disorders antagonize neurodegeneration through endolysosomal trafficking

Program Time: Monday, September 14th at 12:15 PM

2.3. The cell cycle regulator APC/CFZR-1 regulates Wnt-mediated fate decisions in C. elegans postembryonic development.

Authors: David Borrego (1); Adrián Fragoso-Luna (2); Alan Koh (3); Javier Rodríguez (3); Julia Hillung (1); Andre E. X. Brown (3); José Pérez-Martín (1)

Affiliations:

1. Instituto de Biomedicina de Valencia, Carrer de Jaume Roig, València, ES
2. Centro Andaluz de Biología del Desarrollo, Avenida Rectora Rosario Valpuesta 1, Dos Hermanas, ES
3. Imperial College London, South Kensington Campus, London, SW7 2AZ

Abstract:

Cell cycle regulators have long been regarded as passive elements regulating cell fate during development. Their main role is to control the timing of cell cycle phases. This is particularly true for the mitosis-to-G1 transition, which is critical for differentiation. However, whether cell cycle regulators directly influence developmental decisions beyond their canonical functions remains largely unclear. This communication shows how the Anaphase Promoting Complex/Cyclosome (APC/C)—an E3 ubiquitin ligase involved in cell cycle regulation—together with its coactivator FZR-1, can induce the acquisition of terminal features in cellular lineages across different tissues. APC/CFZR-1 is required for the differentiation of hermaphrodite Distal Tip Cells (DTCs). Hermaphrodite DTCs arise post-embryonically following two consecutive Wnt-regulated divisions of the Z1/Z4 precursor cells in the somatic gonad primordium at the L1 stage. In this context, APC/CFZR-1 activity determines whether the descendants of the Z1.a/Z4.p cells adopt the DTC fate or the alternative Sheath and Spermatheca precursor (SS) fate. APC/CFZR-1 promotes DTC fate through proteasome-mediated downregulation of EFL-3, a transcriptional regulator that modulates the bHLH transcription factors LIN-32 and HLH-12, both required for DTC specification. The regulatory logic identified in the somatic gonad appears to be conserved in additional developmental contexts. Other Wnt-regulated postembryonic decisions, including those within the postdeirid lineage that gives rise to the PDE and PVD neurons, were examined. Consistent with previous findings, loss of FZR-1 function compromises the establishment of PDE and PVD fates, resulting in severe motility defects. Furthermore, lin-32and efl-3 seem to interact with FZR-1 to promote neurogenesis in a manner similar to the one initially described in the somatic gonad. Extending these findings, we will introduce high throughput motility analyses of fzr-1-deficient worms, which supports a broader role for APC/CFZR-1 as a regulator of terminal differentiation across diverse lineages in C. elegans .

Program Time: Monday, September 14th at 12:30 PM

2.4. Understanding the dynamics of AGO proteins in the C. elegans–Orsay virus pathosystem

Authors: Victoria G Castiglioni (1,2); Isaac Martínez-Ugalde (2); Santiago F Elena (1,3); Julie M Claycomb (2)

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Abstract:

Viruses, the most abundant biological entities on Earth, infect all known living organisms. If a virus produces double-stranded RNA during its life cycle it will often be recognised by the RNA interference (RNAi) pathway and the Argonaute (AGO) proteins, leading to its degradation. Orsay virus (OrV), a positive sense RNA virus, replicates in the intestinal cells of C. elegans. OrV is recognised and degraded by the RNAi machinery, and even though we have a general understanding of the players involved in the antiviral RNAi response, we still lack mechanistic details about the process. In particular, we still don't understand how the RNAi silencing complex is organised within cells and how dynamic the complex is throughout the infection. By using a combination of live-cell imaging and FISH, we describe a dynamic localisation pattern, with primary and secondary AGO proteins localising only to bystander cells in the phase of maximum replication, and to infected cells at later timepoints, suggesting a transient viral induced repression mechanism. This was accompanied by an absence of AGO-bound viral small interfering RNAs (vsiRNAs) at early stages, whilst vsiRNAs were bound at later timepoints. Progression of viral load in different AGO mutants indicate that these proteins do not act on viral degradation at early stages, but determine the outcome of the infection. Moreover, we describe distinct somatic AGO complexes upon infection and different sub-cellular locations at different stages of infection. Proximity interaction mapping allowed us to characterise the different complexes formed, distinguishing between common and unique interactors of AGO proteins, highlighting the diverse roles of AGO proteins and identifying unexpected interactors upon infection. Collectively, these results improve our understanding of the RNAi silencing complexes induced upon viral infection, inform on antiviral intercellular communication mechanisms and highlight the complex and dynamic nature of the interactions between viruses and the RNAi pathway.

Program Time: Monday, September 14th at 12:45 PM

2.5. Synergistic Activation of PPARγ and AMPK: A Metabolic Approach to Improve Proteostasis and Mitochondrial Health in HD

Authors: Julia Tortajada-Pérez (1,2); Sergio Gordillo-García (3,4); Cristina Trujillo-del-Río (1); Maksym Kupchyk-Tiurin (1); Mar Collado-Pérez (1,2); Laura Cantalejo-Carrasco (3,4); Marta Roca (5); Agustín Lahoz (6); Christian Neri (7); José Maria Millan (8); Marta Artal-Sanz (3,4); Andrea del Valle Carranza (1); Rafael P. Vázquez-Manrique (1,8)

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8. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, 28029, Spain.

Abstract:

Huntington's disease (HD) is a rare neurodegenerative disorder caused by expanded mutant huntingtin (mHTT) aggregation. With no disease-modifying therapies currently available, symptoms include motor and cognitive decline and psychiatric alterations. However, beyond classic proteotoxicity, systemic metabolic dysfunction is increasingly recognized as a hallmark of HD. Indeed, robust lipid dysregulation has been demonstrated across various HD models and in clinical patient data (unpublished, from our group). To address this metabolic disruption, we aimed to concurrently modulate convergent energy and lipid pathways by combining the PPARγ agonist rosiglitazone (RZ) with the AMPK activator salicylate (SAL). This strategy serves as a proof-of-concept that parallel reprogramming of the lipid-energy axis can restore metabolic equilibrium and directly enhance cellular health and induce neuroprotection. Using Caenorhabditis elegans (C. elegans) models expressing pan-neuronal 40Q::YFP or mHTT128Q::CFP prone-to-aggregation proteins, we evaluated the impact of parallel RZ/SAL co-treatment on functional outcomes and proteotoxicity. By shifting the focus to a purely metabolic perspective, we found that concurrent pathway activation significantly restored motor function, improved mechanosensory responses, and mitigated proteotoxic stress. Notably, Bliss Independence analysis confirmed true synergistic effects specifically for the reduction in the number of neuronal protein aggregates (2.43-fold above additive expectation), as well as motor rescue (3.27-fold). As an added benefit of this metabolic alignment, these protective effects were achieved using sub-effective concentrations (50 µM RZ and 5 µM SAL), entirely circumventing the need for high-dose monotherapy. Genetic loss-of-function studies demonstrated that this protection requires both AMPK (aak-2) and the PPARγ functional homolog NHR-49 (nhr-49) activation, with intestinal NHR-49 re-expression sufficient to restore efficacy. To validate results at the subcellular level, we analysed mitochondrial bioenergetics, β-oxidation, and lipid remodelling. Quantitative lipidomics demonstrated that while pan-neuronal polyQ expression

induces systemic lipid depression, parallel RZ/SAL co-treatment coordinately rewires the metabolic state. This shifts lipid flux toward cell-membrane stabilization and the adaptive synthesis of protective ultra-long-chain neutral triglycerides, which mechanistically translates into restored mitochondrial network morphology and biomass. Finally, this synergistic reduction in proteotoxicity was conserved in mammalian HEK293 cells expressing the mutant huntingtin caspase 6 fragment (mHTT121Q), confirming a maintained cross-species mechanism of action. Overall, our findings strongly suggest that the lipid-energy axis is a fundamental, targetable regulator of cellular proteostasis. By demonstrating that parallel activation of the PPARγ-like nuclear hormone receptor NHR-49 and AMPK pathways restores mitochondrial health and reduces aggregation, we show that actively reshaping specific lipid profiles is a primary determinant of proteotoxic resilience. This work highlights a distinct therapeutic framework for HD, moving beyond direct aggregate clearance toward an approach focused on metabolic reprogramming to preserve mitochondrial and cellular health.

Program Time: Monday, September 14th at 1:00 PM

2.6. Glia-to-neuron serotonin signaling controls ASH-dependent nose touch in C. elegans

Authors: David Logan (1); Carlos Oliva (1); Lei Wang (1); Melisa Lamberti (1); Isabella Gay (1); Olivia White (1); Enrique Oliver (1); Jesus Fernandez-Abascal (1); Bianca Graziano (1); Laura Bianchi (1)

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1. University of Miami, Northwest 10th Avenue, 1600, Miami, US

Abstract:

Touch is essential for survival, enabling organisms to detect and respond to environmental stimuli and generate appropriate behavioral outputs. Beyond reflexive responses, mechanosensation also contributes to complex behavioral states, yet the cellular and molecular mechanisms that shape touch sensitivity remain incompletely defined. While mechanosensory neurons are known to mediate stimulus detection, they function within a microenvironment shaped by glia; however, the contribution of glia to mechanosensory processing has remained poorly understood. Here, we identify a previously unrecognized role for glial-derived serotonin in regulating touch sensitivity in Caenorhabditis elegans. Using a combination of in vivo Ca^{2+} and serotonin imaging, fluorescence microscopy, and behavioral assays, we show that Amphid Sheath (AMsh) glia release serotonin via dense-core vesicles. This release is spatially and temporally coordinated with sensory activation, supporting a direct role for glia in modulating neuronal output. Mechanistically, glial serotonin activates the neuronal SER-5 receptor to enable robust ASH neuron responses. In parallel, serotonin engages the glial MOD-1 receptor, establishing a feedback loop that fine-tunes touch perception. This dual targeting of neuronal and glial receptors supports a model in which glia both initiate and self-regulate serotonergic signaling. Extending beyond touch, we find that serotonin signaling also modulates additional ASH-mediated aversive behaviors, including responses to high osmolarity and the repellant octanol, indicating that glial serotonin broadly influences polymodal sensory circuits. We further define a concentration-dependent mechanism through which serotonin tunes sensory responsiveness. Specifically, distinct serotonin levels differentially engage cAMP- and Ca^{2+}-dependent pathways, suggesting that neuromodulator concentration provides a graded means of controlling circuit output rather than a simple on/off signal. This mechanism offers a framework for understanding how glia dynamically adjust sensory gain under varying environmental conditions. Together with prior evidence for glial GABA release, these findings support a model in which glia actively regulate both mechanosensory and chemosensory function by providing complementary excitatory and inhibitory signals. In this context, glia emerge as integral components of sensory circuits that shape the amplitude and fidelity of behavioral responses. More broadly, these results raise the possibility that modulation of extracellular serotonin levels, such as by selective serotonin reuptake inhibitors (SSRIs), may influence glia-neuron signaling dynamics and thereby alter sensory processing.

Program Time: Monday, September 14th at 1:15 PM

2.7. Coordinated regulation of spermatogenesis by germline small RNA pathways

Authors: Eric Cornes (1); Bernard Florian (1); Billiet Alexia (1); Shukla Akanksha (1)

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Abstract:

A well-characterized function of non-coding RNAs (sRNAs) expressed in animal germ cells is the silencing of transposable elements by RNA interference. In this regulatory paradigm, sRNA molecules loaded into Argonaute proteins act as guides, targeting transposon RNAs through antisense complementarity and repressing their expression at both post-transcriptional and transcriptional levels, thereby preserving genome integrity and fertility. However, the vast majority of germline-expressed sRNAs do not target transposons, suggesting broader roles in endogenous gene regulation. By studying sRNA pathways during C. elegans germline development, we found that PIWI-interacting small RNAs (piRNAs) and 26G-RNAs, two distinct classes of germline-expressed sRNAs, directly repress the transcription of hundreds of spermatogenesis-specific genes. These two pathways exhibit distinct target specificities, each regulating a largely non-overlapping set of transcripts, and together repress nearly the entire sperm-specific gene expression program. At the cellular level, this regulatory activity controls the timing of spermatogenic differentiation and is required for proper sperm function. Genetic assays reveal synthetic sterility in mutants deficient for both pathways. This phenotype is caused by the production of non-functional sperm, confirming the cooperative function of piRNAs and 26G-RNAs during spermatogenesis. Overall, these findings reveal that transcriptional repression of spermatogenic genes is a shared function of mechanistically distinct germline sRNA pathways that together regulate endogenous gene expression programs essential for fertility.

Program Time: Monday, September 14th at 1:30 PM

Session 3

3.1. Cold temperature activates a BH2-driven intertissue metabolic network that promotes longevity and proteostasis

Authors: Vilchez, David, Frezza, Christian, Hoppe, Thorsten, Yang, Ming, Soko, Agnieszka, Georgomanolis, Theodoros, Prymidis, Dimitrios, Schnubel, Joana, Artan, Murat, Loureiro, Rute, Petrovic, Dunja, Wehrmann, Markus, Koyuncu, Seda, Doğan, Cansu, Lee, Hyun Ju

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4. Institute for Metabolomics in Ageing, Faculty of Medicine, University Hospital Cologne, Cologne, Germany
5. Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

Abstract:

Moderately cold temperatures extend lifespan and promote health across species, yet the molecular mechanisms underlying these benefits remain unclear. Here, we define a metabolite-driven, multi-tissue signaling system that links cold temperature to enhanced longevity and proteostasis in Caenorhabditis elegans. We find that moderate cold temperature upregulates the biopterin-synthesis enzyme GCH1/CAT-4, leading to increased production of the metabolite dihydrobiopterin (BH2) in muscle cells. Subsequently, BH2 is transported to distal tissues, including the intestine and neurons, where it is converted into its bioactive form, BH4. This enzymatic cofactor activates phenylalanine hydroxylase PAH-1, increasing tyrosine synthesis and thereby extending lifespan. Moreover, elevated BH2 levels prevent disease-related protein aggregation and subsequent neuronal dysfunction during aging. Notably, increasing BH2 synthesis in muscle cells is sufficient to promote longevity and suppress pathological protein aggregation in distal tissues, even at normal or elevated temperatures. In human cells, BH2 and tyrosine supplementation prevents aggregation of polyQ-expanded proteins associated with Huntington's disease, as well as ALS-causing mutant variants of TDP-43 and FUS. Together, these findings identify BH2 as a central cold-induced metabolite whose synthesis, transport, and utilization preserve organism-wide proteostasis and extend lifespan.

Program Time: Monday, September 14th at 3:30 PM

3.2. Uncovering the genetic architecture of pathogen avoidance behaviour in Caenorhabditis elegans

Authors: Guilherme Ferreira (1); Joana Vilas Boas (1); João Picão-Osório (1)

Affiliations:

1. Centre for Ecology, Evolution andEnvironmental Changes (CE3C), Global Change and SustainabilityInstitute, Department of Biology, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa,Portugal.

Abstract:

Behavioural variation is fundamental to organism survival and reproduction, mediating key ecological interactions such as foraging, mate choice, and avoidance of predators and pathogens. Despite its importance, the mechanisms underlying behavioural variation remain poorly understood. In particular, pathogen avoidance behaviour represents a critical yet underexplored adaptive trait, with existing studies largely confined to laboratory-adapted systems and non-ecologically relevant pathogens. Consequently, the natural genetic architecture of avoidance behaviour remains largely unknown. Here, we analysed natural variation in avoidance behaviours at the species-wide level to test whether there are genetic 'hot spots' for behavioural avoidance, and whether avoidance variation is pathogen specific. For this, we used world-wide natural isolates of the nematode Caenorhabditis elegans and a curated panel of naturally associated bacteria, combining quantitative behavioural approaches with a quantitative genetics framework. We quantified pre-contact (chemotaxis) and post-contact (lawn leaving) avoidance behaviours, that capture distinct sensory and behavioural processes, across 94 wild C. elegans for six bacteria: four ecologically associated pathogenic bacteria, the pathogenic Serratia marcescens (Db11), and the reference beneficial Escherichia coli strain OP50. We observed a broader natural behavioural avoidance for post-contact than for pre-contact avoidance, and, surprisingly, avoidance behaviours are not inversely correlated to bacterial virulence. Next, we performed genetic correlation analysis to test to what extent genetic variation contributes to the covariation of distinct behavioural traits. We observed an absence of genetic correlation for pre- and post-contact avoidance for a given bacterium, and for pre-contact among different bacterial strains, suggesting independent genetic basis for these traits. In contrast, our analysis reveals a shared genetic architecture for post-contact avoidance among bacterial strains by showing multiple significant positive genetic correlations. Finally, we performed genome-wide association studies (GWAS) to identify naturally segregating variants. Surprisingly, we mapped several quantitative trait loci (QTL) across the genome that were independent for post-contact avoidance among bacterial strains, and partly overlapping for pre-contact avoidance among bacterial strains and for pre- and post-contact avoidance behaviours. Interestingly, all these QTL harbour variations in genes with putative chemoreception function, representing potential candidates for future analysis. Altogether, our results show new insights of a complex natural genetic architecture of bacterial avoidance towards ecologically associated microorganisms in C. elegans. We will discuss whether these genetic architectures of avoidance behaviours are mediated by linkage, pleiotropy, selection and/or mutational input.

Program Time: Monday, September 14th at 4:00 PM

3.3. Cell-type-specific profiling of transcription factor binding in C. elegans

Authors: Rafael Alis (1); Nuria Flames (1)

Affiliations:

1. Developmental Neurobiology Unit, Instituto de Biomedicina de Valencia IBV-CSIC, Valencia, Spain.

Abstract:

Transcription factors (TFs) are the main orchestrators of gene expression. An universal feature of TFs is their pleiotropy, they are usually expressed in several cell types and tissues and their specific actions are determined by the context, mainly the combined action with other TFs and the specific chromatin landscapes of each cell type or state. Caenorhabditis elegans nervous system is composed by 118 neuron types, which are specified by combinations of TFs termed terminal selector (TS) collectives. Although at least one TS has been identified for almost every neuron type, we do not know the cell-type specific binding profiles for those TFs. In addition, a few TFs are recurrently used as TS for a broad number of different neuron types, and how they execute their different actions is now well understood. Due to their pleiotropic actions, conventional approaches, such as whole-worm chromatin immuno precipitation followed by sequencing (ChIP-seq) fail to provide cell-type specific information. We aim to overcome these difficulties by using a combination of genetic tools to obtain C. elegans strains in which a given TF will be endogenously and specifically tagged in a given neuron type and then its chromatin binding profile will be determined by CUT&RUN. We are using CRISPR to knock in a cassette that labels global endogenous TF expression with RFP fluorescence and when combined with a strain expressing a flipase recombinase in a neuron-type specific manner, the endogenous TFs switches to TF::eGFP::3xFLAG tagging only in that specific neuron type. We have already successfully labeled and recombined different TFs with this cassette. Recombined strains will be later used to profile TF binding using anti-GFP nanobodies and CUT&RUN. We are currently establishing this methodology to profile different TFs that act as TS of specific neuron types and gain insights in their mode of action.

Program Time: Monday, September 14th at 4:15 PM

3.4. Desynchrony between events triggers a compensatory delay during C. elegans development

Authors: María Olmedo (1); Francisco Javier Romero-Expósito (1); Almudena Moreno-Rivero (1); Marta Muñoz-Barrera (1); Nicola Gritti (2); Jeroen S van Zon (2); Francesca Sartor (3); Martha Merrow (3); Alejandro Mata-Cabana (1)

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Abstract:

Robust development requires the precise temporal coordination of parallel biological processes, yet how multicellular organisms detect and correct timing mismatches between developmental events remains poorly understood. During Caenorhabditis elegans postembryonic development, larval molts and stage-specific cell divisions proceed in parallel and are influenced by environmental cues such as nutrient availability. Although these processes often appear tightly synchronized, their underlying timers are mechanistically distinct, raising the question of how temporal coherence is maintained. We have identified a previously unrecognized and highly variable pause at the beginning of larval stages revealed by continuous single-animal luminometry. This lag, which we term L2lag, occurs immediately after ecdysis and before the onset of the next intermolt and is strongly enhanced under reduced insulin signaling in daf-2 mutants. Importantly, L2lag is not a trivial consequence of slow development: its duration is largely uncorrelated with that of other larval stages and persists even when canonical insulin-dependent developmental delays are genetically suppressed. By combining high-resolution time-lapse microscopy reporting molting and seam cell divisions in the same animal, we show that L2lag arises from a desynchrony between the timing of cell divisions and ecdysis. Reduced insulin signaling delays seam cell divisions more strongly than ecdysis, creating a mismatch. Larvae entering L2lag display incomplete seam cell divisions, and experimentally advancing or delaying divisions relative to ecdysis respectively shortens or prolongs the lag. These findings demonstrate that L2lag reflects a compensatory delay triggered by unfinished stage-specific cellular events. Together, our results suggest a checkpoint-like mechanism at larval stage boundaries that prevents the propagation of timing errors across development by delaying the initiation of the next stage until key events are complete. This work reveals how developmental timing remains robust despite variability and perturbations, and positions C. elegans larval transitions as a powerful system to dissect the principles of temporal coordination in development.

Program Time: Monday, September 14th at 4:30 PM

3.5. The Deubiquitinase USP-48 Enables Insulin-Mediated Longevity and Modulates the Mitochondrial Stress Response

Authors: Sergio Gordillo-García (1,2); Jesus Fernandez-Abascal (1,2); Blanca Hernando-Rodríguez (1,2); María Jesús Rodríguez-Palero (1,2); Laura Cantalejo-Carrasco (1,2); Aitor Jarit-Cabanillas (1,2); Manuel D. Martínez-Bueno (1,2); Mercedes M. Pérez-Jiménez (1,2); Enrique J. Clavijo-Bernal (1,2); Aitana Cambón (1,2); Ildefonso Cases (1,2); Nicholas E. Stroustrup (3); Matthias Eder (3); Marta Artal-Sanz (1,2)

Affiliations:

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2. Department of Molecular Biology and Biochemical Engineering, Universidad Pablo de Olavide, Seville, Spain
3. Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain

Abstract:

The conserved mitochondrial prohibitin (PHB) complex is crucial for maintaining mitochondrial homeostasis. However, its depletion produces an opposing effect on lifespan, shortening it in wild-type C. elegans while extending it in insulin/IGF-1 receptor daf-2 mutants. This differential outcome is associated with a blunted mitochondrial unfolded protein response (UPRmt) in long-lived mutants. In order to identify regulators underlying this context-dependent stress response and lifespan modulation, we performed the first genome-wide double RNAi screen in C. elegans. We identify the ubiquitin-specific peptidase USP-48 as a novel modulator of the UPRmt, which is especially required for longevity under reduced insulin signalling. Transcriptome analysis reveals that daf-2 and usp-48 mutants share a large number of differentially expressed genes, suggesting a functional overlap. USP-48 regulates mitochondrial structure and function and interacts with the transcription factor DVE-1 to regulate the UPRmt largely independently of ATFS-1. Mechanistically, our data suggests that USP-48 acts through histone H2B deubiquitination to influence stress-responsive gene expression. Our findings demonstrate the utility of double RNAi genetic screens for essential genes and highlight ubiquitination as a new epigenetic determinant of metabolism-mediated longevity.

Program Time: Monday, September 14th at 4:45 PM

3.6. Validation of the Functionality of Heat-Inactivated Strains in Food Applications Using the Caenorhabditis elegans Model

Authors: Silvia Llopis (1); Miren Maicas (1); Ferran Balaguer (1); Nuria González (1); Lucía Jareño (2); Paula Laguna (2); Cristina Isabal (2); Empar Chenoll (1); Patricia Martorell (1)

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Abstract:

Caenorhabditis elegans (C. elegans) is a valuable preclinical model for evaluating the health benefits of probiotics (live strains) and postbiotics (heat-inactivated strains) due to its genomic similarity to mammals and ease of cultivation. ADM® utilizes this model in its research to support the incorporation of functional postbiotics into food applications for both humans and animals. The inherent stability and safety of postbiotics can help to maintain product quality and shelf life, while preserving taste and texture. This study aimed to validate the functionality and stability of heat-inactivated microbial strains in final food and feed formulations using the C. elegans model. Two recently commercialized heat-inactivated strains from ADM® were evaluated: PRIOME® Joint Health (Lacticaseibacillus rhamnosus), developed to support canine joint health, and Lactobacillus gasseri CP2305, which has been shown to promote mental well-being and sleep quality in humans. To conduct this study, both heat-inactivated strains were exposed to industry-relevant conditions specific to their intended food applications, including high temperature, high pressure, pH variations, Brix levels and food preservatives. After these treatments, samples were stored at room temperature for 12 months (the products' expected shelf life). In addition, postbiotics were incorporated into their respective food matrices—such as extruded dry kibbles as pet food for heat-inactivated PRIOME® Joint Health and in gummies, chocolate and cereal bars as food products for heat-inactivated L. gasseri CP2305 and tested. The postbiotic-containing food matrices were then subjected to the relevant industrial processing conditions and subsequently stored at room temperature for 4 months (dry kibbles) or 12 months (gummies, chocolate and cereal bars). C. elegans were fed with the corresponding samples containing postbiotics to evaluate their respective functionality; PRIOME® Joint Health was assessed for its capacity to reduce fat accumulation in the nematode, as obesity is a well-established contributor to the degeneration of joint structures. The heat-inactivated L. gasseri CP2305 was tested for its antioxidant effect by measuring worms' survival following induction of acute oxidative stress (with hydrogen peroxide), since attenuation of oxidative stress is known to exert beneficial effects on sleep quality. Results indicate that the functionality of the heat-inactivated strains was preserved after exposure to the different industry conditions at time 0 and remained stable for up 12 months of storage, compared with the corresponding heat-inactivated strains not subjected to industrial processing condition (not significant differences between conditions). Moreover, these functional properties were preserved when incorporated into the specific final food applications at time 0, with functionality remaining stable after 4 months for the heat-inactivated PRIOME® Joint Health and after 12 months for the heat-inactivated L. gasseri CP2305 (not significant differences in comparison to heat-inactivated strains not included in the food matrix). In conclusion, C. elegans is a suitable model for evaluating the functionality and stability of food applications containing heat-inactivated strains. Moreover, this study demonstrated that both heat-inactivated strains retained the functional properties evaluated, supporting their potential for incorporation into a variety of final food applications.

Program Time: Monday, September 14th at 5:00 PM

Nobel prize talk: Victor Ambros (open to public). Plenary

1. MicroRNA-mediated gene regulation and developmental robustness

Authors: Ambros, Victor

Affiliations:

1. University of Massachusetts Chan Medical School Worcester, USA

Abstract:

MicroRNAs, together with their Argonaute partner proteins, exert post-transcriptional regulation of gene expression via complementary base pairing between the microRNA and mRNAs. Genes that encode microRNAs function as negative regulators of hundreds of other genes, primarily by inhibiting the translation and/or stability of target mRNAs. MicroRNAs exhibit remarkably versatile regulatory roles within genetic regulatory networks (GRNs) – for example, to coordinate multigene functional modules, dampen gene expression noise, and/or conduct developmental switches. Genetic analysis of model organisms reveals that the function of microRNAs can be conditional – wherein a microRNA gene is required under certain environmental or physiological conditions, but relatively dispensable under other conditions. It is not uncommon for microRNA loss-of-function mutants to display emergent developmental or physiological defects when subjected to biological stresses and challenges that fall within the normal range of experience of the animal. These include temperature fluctuations within the animal's thermotolerant range, exposure to pathogens or toxins endemic to the animal's normal environment, or routine regeneration of tissues after injury. The observation that microRNA genes exhibit stress-dependent conditional phenotypes is interpreted to suggest prominent roles for microRNAs in enabling developmental and homeostatic processes to weather the contingencies of everyday life.

Program Time: Monday, September 14th at 5:30 PM

Session 4

4.1. The flexible brain: lessons from a worm with 387 neurons

Authors: Barrios, Arantza

Affiliations:

1. Dept Cell and developmental Biology University College London, London, UK.

Abstract:

Brains process information in a very plastic manner so that animals never respond to an identical stimulus in the same way twice. This is the basis of learning and decision-making, and what enables animals to respond to their environment according to their ever-changing needs. In the Barrios lab we investigate the mechanisms that provide brains with this flexibility in function. I will present our work on how previous experiences, internal state, and biological sex shape neural circuit function and behaviour to meet animal needs. We use a combination of genetics, molecular biology, neuronal recordings and behavioural analysis.

Program Time: Tuesday, September 15th at 9:30 AM

4.2. Recent horizontal transposon transfer boosted by virus-like proteins in nematodes

Authors: Israel Campo-Bes (1,2); Alejandro Burga (3); Manuel Irimia (1,2)

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1. Department of Medicine and Life Sciences (MELIS), Universitat Pompeu Fabra, Barcelona, Spain

2. Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain

3. Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), 1030 Vienna, Austria.

Abstract:

Horizontal transfer of transposable elements (HTT) is increasingly recognized as an important force shaping animal genome evolution, yet the ecological and molecular factors promoting these events remain poorly understood. Our previous discovery that Maverick transposons can capture host genes and transfer them between distantly related nematode species provided evidence that virus-like transposable elements can act as vectors of horizontal gene transfer in animals. Building on this finding, we sought to determine how widespread recent HTT is across nematodes and to identify the evolutionary, ecological, and molecular factors associated with these transfers. We performed a large-scale analysis of recent HTT across 198 nematode species with available whole-genome sequences. Using a conservative comparative genomic approach focused on protein-coding transposable elements, we identified 1,389 high-confidence HTT events involving 134 species, demonstrating that recent horizontal transfer is widespread across the phylum Nematoda. HTT was strongly influenced by phylogenetic distance, with closely related species displaying higher transfer rates. Ecological similarity also played a major role: most transfers occurred between species sharing the same lifestyle, either free-living or parasitic, whereas transfers between species with different lifestyles or host types were rare. Notably, approximately 60% of all HTT events involved just three transposable-element families: DNA-Maverick, LTR-Pao, and LTR-Gypsy. These families encode viral-like proteins, including capsid-like and fusogen-like or envelope proteins, suggesting that their ability to form viral-like particles may facilitate transmission between species. Together, our findings establish nematodes as a powerful system for investigating the mechanisms and evolutionary consequences of HTT in animals and support a prominent role for transposable elements with viral properties in mediating gene flow across species boundaries.

Program Time: Tuesday, September 15th at 10:00 AM

4.3. Modelling the clinical continuum of ATP7A-related diseases in Caenorhabditis elegans

Authors: Andrés Velázquez Mudarra (1); Carmen Martínez Fernández (1); Marta García García (1); Alba Olaso Llorca (1); David Aristizábal Corrales (1); Homa Kohandelgargari (1); Janet Hoenicka (2); Francisco Palau (2); Julián Cerón (1)

Affiliations:

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2. Laboratory of Neurogenetics and Molecular Medicine, Center for Genomic Sciences in Medicine, Institut de Recerca Sant Joan de Déu, Barcelona, Spain.

Abstract:

Genetic variants in genes involved in rare diseases are understudied because their low prevalence leads to insufficient support from funding agencies and the pharmaceutical industry. Menkes Disease (MD) is a rare multisystemic disease caused by mutations in the ATP7A gene that result in the inability to distribute the copper absorbed in the intestine throughout the organism. This X-linked genetic disorder has an incidence of around 1 in 300.000 male births, with a life expectancy of less than 3 years. Other mutations in ATP7A can also cause milder diseases like Atypical Menkes Disease (AMD), Occipital-Horn Syndrome (OHS), or X-linked distal Spinal Muscular Atrophy type 3 (SMAX3). Interestingly, mutations in ATP7B, a paralog of ATP7A sharing ~65% sequence identity, can cause Wilson Disease (WD), another rare copper-related disease due to copper accumulation and toxicity, primarily affecting the liver and brain. In Caenorhabditis elegans, cua-1 is the common ancestral ortholog gene for human ATP7A and ATP7B copper-transporting P-type ATPases, with a similarity of 45% in the protein sequence. Notably, many of the key amino acids involved in the diseases are conserved in C. elegans. Using CRISPR-Cas genome editing, we have mimicked several ATP7A and ATP7B missense mutations in cua-1, allowing the modelling of at least five distinct diseases using a single gene. Mutations in cua-1 produce a spectrum of phenotypes, ranging from severe larval lethality to reduced brood size. Interestingly, the penetrance of these phenotypes can be modulated by regulating copper bioavailability. Additionally, we show that Elesclomol, a drug in a clinical trial to treat Menkes Disease, partially rescues the most severe phenotypes in our models. Together, we are implementing C. elegans as a rapid and accurate model for functional studies of ATP7A and ATP7B variants to exploit it in clinical diagnosis and drug screens for different copper-related rare diseases.

Program Time: Tuesday, September 15th at 10:15 AM

4.4. A Novel Role for FOG-2 in Caenorhabditis elegans Antiviral Immunity

Authors: Dominik Herek (1); María J. Olmo-Uceda (1); Victoria G. Castiglioni (1); Santiago F. Elena (1,2)

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1. Institute of Integrative Systems Biology, Carrer del Catedràtic Agustín Escardino Benlloch, 9, Paterna, ES
2. Santa Fe Institute

Abstract:

The fog-2 (Feminization Of Germline) gene is involved in Caenorhabditis elegans reproductive development as a key regulator of sperm production in the L4 hermaphrodite germline. Loss-of-function mutants of fog-2 display a gonochoric phenotype, with XX animals (females) producing only oocytes and XO animals (males) producing only sperm. Interestingly, FOG-2 has been shown to have a role in immunity with fog-2 mutants being more resistant to bacterial infection. Orsay virus (OrV), a natural viral pathogen of C. elegans, is a positive sense single-stranded RNA virus related to the Nodaviridae family. It infects C. elegans through the oro-fecal route with viral replication occurring in the intestinal epithelial cells and usually being limited to just one cell per animal. To test whether the observed role of FOG-2 in immunity extends to other immune pathways, we tested its role in OrV infection. We quantified viral load of mutant animals, and show that FOG-2 is necessary to mount an efficient antiviral response and to ultimately clear infection. This mechanism was sex independent, specific to the germline spermatogenesis pathway, and not a general feature of gonochorism in C. elegans. It was accompanied by a multi-cellular infection phenotype. In rare cases, fog-2 animals showed signs of possible OrV infection in tissues other than the intestine. Consequently, OrV displayed significantly increased virulence in fog-2 mutants in general, however, the effect was stronger in females than males. We also performed a transcriptomic analysis at early, intermediate and late infection timepoints, which revealed differences in the host response to OrV infection and its change through time between wild-type and fog-2 mutant animals. Together, these results demonstrate that FOG-2, and other genes involved in reproductive development are necessary for antiviral immunity with mutant animals displaying a phenotype similar to mutants of key immunity genes, such as drh-1, pointing to broader interactions between the immune and reproductive systems necessary for their proper functioning.

Program Time: Tuesday, September 15th at 10:30 AM

4.5. From Field Radiation to Plastic Toxicology: C. elegans Bridges the Gap Across Stressors

Authors: Carlos López (1); Anna Laromaine (1); Juan Pellico (1); Olga Zeni (2)

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1. Instituto de Ciencia de Materiales de Barcelona (ICMAB-CSIC), Campus UAB, Bellaterra, 08193, ES
2. Istituto per il rilevamento Elettromagnetico dell'ambiente (IREA-CNR)

Abstract:

The increasing development of nanomaterials for biomedical, environmental, and food-related applications requires efficient and physiologically relevant models for safety and functionality assessment. Owing to its transparent body, short life cycle, low maintenance costs, and significant genetic homology with humans, the nematode Caenorhabditis elegans serves as a powerful in vivo model. By focusing on its well described physiology, we have used as a subject in toxicological studies for a variety of potential stressors, ranging from electromagnetic radiation to plastic nanoparticles. We have also further expanded upon traditional methods of experimentation, which are widely spread and successful, but can be enhanced with the recent advent of high-throughput technologies. Here, we also present an integrated platform based on C. elegans and the SydLab ONE™ microfluidic screening system, which we have used for the assessment of materials at the bio–nano interface. We exposed wild-type (N2) and cuticule-sensitive (CB6055) worms to continuous electromagnetic field radiation (RF-EMF). To mimic Wi-Fi exposure in humans, the exposure was set at 26.5 GHz, maintaining a specific absorption rate (SAR) of 1 W/kg, adapted to worm size and volume. We compared various organismal health endpoints, between exposed and non-exposed worms across two generations, and our findings reported a notable absence of effects due to RF-EMF exposure in parameters such as survival, egg hatching, length, motility, and reproductive toxicity (Figure 1A, 1B, 1C, 1D, 1E). Further studies, at the cellular level, observed oxidative damage by assessing the antioxidant capacity of exposed worms, which was not determined to be depleted by ROS production. Lastly, delving deeper into the genetic expression, functional enrichment analysis of exposed worms failed to show activation of stress-response genetic pathways that could indicate detrimental effects. Switching focus to the potential of high-throughput technology to assess nanoparticle toxicity, the SydLab ONE™ device enables automated, high-content screening of up to 64 experimental conditions in a single run. Combined with real-time data acquisition and AI-driven image analysis, the system allows rapid and quantitative assessment of growth, survival, motility, reproduction, and stress-related endpoints, while also significantly increasing throughput and reproducibility compared with conventional assays. This approach provided a framework for assessing emerging contaminants prevalent in our diets, such as nanoplastics, which have been linked to oxidative stress, neurotoxicity, reproductive impairment, and metabolic dysregulation. Polylactic acid (PLA) and polytetrafluoroethylene (PTFE) nanoparticles of varying sizes and concentrations have been used to study overall organismal health. While initial studies were not indicative of any effects caused by nanoplastic exposure, further work still needs to be performed. The platform was also applied to evaluate other plastics, such as polylactic-co-glycolic acid (PLGA) nanocapsules, in trial for their potential in drug-delivery. These were assessed for their biocompatibility with N2 C. elegans larvae during the developmental stages. The worms were exposed at 0.75, 0.5 and 0.25 mg/ml for 120 hours, with no significant alterations identified in their survival or development at any of the aforementioned concentrations, demonstrating favorable biocompatibility. Overall, by integrating advanced microfluidics, automated data-collection, and a versatile biological model, this approach establishes a scalable and cost-effective strategy for accelerating nanomaterial safety assessment and the development of next-generation health-related nanotechnologies.

Program Time: Tuesday, September 15th at 10:45 AM

4.6. Mitohormesis in action: how mild complex-I inhibition by rotenone promotes longevity in C. elegans

Authors: Silvia Romero-Sanz (1); Andrea Morán-Cerrro (1); Elena Caldero-Escudero (1); Paloma García-Casas (1); Rosalba I Fonteriz (1); Silvia Fernández-Martínez (1); Mayte Montero (1); Javier Álvarez (1); Jaime Santo-Domingo (1); Sergio De la Fuente (1)

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Abstract:

Mitochondrial dysfunction is a hallmark of aging, yet mild mitochondrial stress can promote longevity through hormetic mechanisms [1]. Here, we investigated the role of respiratory chain complex-I inhibition in aging using Caenorhabditis elegans. We found that low dose of rotenone (1 µM) significantly promoted healthy longevity. Genetic analyses indicated that this effect is at least partially dependent on complex-I function, as rotenone-induced longevity was partially abolished in nuo-6 mutants (MQ1333 strain). To explore the mechanisms underlying this phenotype, we first evaluated whether low-dose rotenone engages caloric restriction-related pathways. Although 1 µM rotenone significantly reduced both basal and maximal respiration, it did not alter AMPK or mTOR signaling activity. Nevertheless, rotenone-treated worms displayed a significant reduction in body size, suggesting metabolic adaptation independent of canonical nutrient-sensing pathways. Ongoing studies are assessing the effect of rotenone in AMPK-deficient worms and under caloric restriction conditions. We also examined whether rotenone-induced longevity is associated with activation of proteostatic stress responses. Using fluorescent reporter strains for endoplasmic reticulum, mitochondrial, and cytosolic unfolded protein responses (UPR), including SJ4005 (hsp-4::GFP), GL347 (hsp-6p::GFP), and TJ375 (hsp-16.2p::GFP), we observed that rotenone consistently activated the mitochondrial UPR at both day 1 and day 5 of treatment. Interestingly, rotenone selectively induced the cytosolic stress response in a restricted subset of pharyngeal muscle cells located in the terminal bulb, identified as pm7 cells. Together, our findings support the idea that mild complex-I inhibition promotes longevity through adaptive mitochondrial stress signaling rather than through canonical caloric restriction pathways.

Program Time: Tuesday, September 15th at 11:00 AM

4.7. Turning Back to C. elegans to Move Forward in Aging: A Role for SOC-2

Authors: María Pilar de Lucas (1); Ana Belén Cámara (1); Cristina Ortiz-Gutiérrez (1); Natasha Zarich (1); Berta Anta (1); José María Rojas-Cabañeros (1)

Affiliations:

1. Unidad de Biología Celular, Unidad Funcional de Investigación en Enfermedades Crónicas (UFIEC), Instituto de Salud Carlos III (ISCIII), 28220 Majadahonda, Madrid, Spain.

Abstract:

Aging is a natural process characterized by the progressive development of physical, psychological, and behavioral changes over time. This process is influenced by multiple factors, including genetic, environmental, social and behavioral components, which interact in a complex manner. From a genetic perspective, research has focused on elucidating the role of specific molecular pathways and genes involved in regulating aging. The RAS/RAF/MEK/ERK signaling pathway is an evolutionarily conserved cascade that regulates key cellular processes in metazoans, including proliferation, development, metabolism and differentiation. Mutations in this pathway are well known drivers of cancer initiation and progression; however, it is also implicated in several hallmarks of aging, such as dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and chronic inflammation. In C. elegans, evolutionarily conserved RAS–ERK signaling components were originally identified through EMS genetic screens affecting vulval cell fate determination, including ksr-1 and soc-2. In addition, regulation of MPK-1 (ERK) has been extensively studied, particularly in the germline, where it controls processes such as mitotic proliferation and apoptosis. This pathway has also been linked to proteostasis, immunity, and lifespan regulation, highlighting parallels with aging-related processes described in mammals. In mammals, null mutations in core components of the RAS–ERK pathway are embryonic lethal. Thus, conditional or inducible knockout models are required to investigate tissue-specific or systemic functions. In the case of SHOC2, a conserved positive modulator of RAS–RAF signal transduction, complete loss of function results in embryonic lethality. Therefore, to characterize the specific aging processes regulated by SHOC2, we turned to C. elegans, where this scaffold protein was originally identified, null mutants remain viable and aging research is well established. So far, we have observed that soc-2 knockdown shortens lifespan, alters a proteostasis aggregation reporter and induces mitochondrial dysfunction. These phenotypes are associated with gene expression changes affecting pathways such as the unfolded protein response (UPR) and extracellular matrix components, including structural collagens. On the other hand, we are also analyzing phenotypes of a truncated mutant (n1774 allele), which may represent a partial loss-of-function allele, and those of a null mutant (tm5133 allele). soc-2 mutants exhibit decreased body size and aged-associated motility decline, upregulation of stress markers and reduced brood size and lifespan.

Program Time: Tuesday, September 15th at 11:15 AM

Session 5

5.1. Biophysical and in vivo approaches uncover the mechanisms by which cohesin orchestrates meiotic chromosome structure and function

Authors: Lopez-Jimenez, Pablo, Davy, Joseph, Saha, Rahul, Rutkauskas, Marius, Sacerdoti, Mariana, Prince, Josh, Sioutas, George, Kim, Eugene, Martinez-Pérez, Enrique

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2. Max Planck Institute of Biophysics, Frankfurt am Main, Germany
3. Imperial College Faculty of Medicine, London, United Kingdom

Abstract:

Cohesin is an essential component of mitotic and meiotic chromosomes due to its ability to control the topology of DNA. During the mitotic cell cycle, cohesin has essential roles in chromosome segregation by providing sister chromatid cohesion (SCC), in DNA damage repair, and in gene regulation by controlling 3D genome organization. The core cohesin complex includes two structural maintenance of chromosome proteins plus a kleisin that recruits additional subunits that regulate the binding and activity of cohesin on DNA, including the HAWKS (Heat repeat proteins Associated With Kleisin) proteins SCC-2, SCC-3 and PDS5. During meiosis, chromosomes undergo large structural changes driven by cohesin that are required to ensure the accurate formation of haploid gametes from diploid germ cells. These changes include the formation of axial elements that promote pairing and recombination between homologous chromosomes, leading to the formation of crossover events, which together with SCC, ensure correct chromosome orientation on the first meiotic spindle. The successful execution of these events requires the assembly during early meiosis of chromosomes containing cohesin complexes with meiosis-specific kleisins: REC8 and RAD21L in mammals and REC-8 and COH-3/4 in C. elegans. Work from our group and others shows that REC-8 complexes provide SCC and contribute to axial assembly, whereas COH-3/4 complexes promote axis assembly and higher-order chromosome organisation. However, how kleisin identity determines the function of cohesin complexes during meiosis remains unclear. We are combining functional in vivo studies exploiting the advantages of the C. elegans germline with biochemical and single molecule imaging approaches to uncover the molecular mechanisms by which variant cohesin complexes control the structure and function of meiotic chromosomes. Our work sheds light on how variant cohesin complexes control meiotic chromosome organization to ensure fertility.

Program Time: Tuesday, September 15th at 12:00 PM

5.2. The C. elegans Integrator interactome reveals its architecture and novel roles in homeostasis

Authors: Ángela Metola (1); Eva Gómez-Orte (1); Rosario López (2); Begoña Ezcurra (1); Angelina Zheleva (1); María de Toro (1); Gonzalo Jiménez-Osés (3); Peter Askjaer (4); Marta Artal-Sanz (4); Ricardo García-Muñoz (1); Antonio Miranda-Vizuete (5); Juan Cabello (1)

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2. Scientific Computation Research Institute (SCRIUR). University of La Rioja, Spain
3. Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Spain.
4. Andalusian Centre for Developmental Biology, Consejo Superior de Investigaciones Científicas (CSIC), Universidad Pablo de Olavide, Sevilla, Spain.
5. Redox Homeostasis Group, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain.

Abstract:

The Integrator complex is a conserved multi-protein assembly that binds to RNA polymerase II and regulates its activity in two different scenarios. On the one hand, it processes small nuclear RNAs (snRNAs), which are essential for the splicing of messenger RNAs (mRNAs). On the other hand, it regulates the expression of certain genes in response to specific growth factors. Mutations in the Integrator complex correlate with the appearance of tumors in various tissues. Our group has characterized the complete interactome of the Integrator complex in C. elegans, revealing unexpected functions, such as the regulation of mitochondrial activity and the response to DNA damage. These findings contribute to a better understanding of the evolutionary conservation and functional diversity of gene regulatory mechanisms in eukaryotes. Our results define a homeostatic feedback loop in which Integrator promotes mitochondrial activity, while mitochondria-derived signals modulate the Insulin/IGF-1 signaling (IIS) pathway to tune the cellular transcriptome. These findings expand the functional repertoire of the Integrator complex beyond the nucleus and establish it as a critical node in the coordination of nuclear gene expression and mitochondrial metabolism.

Program Time: Tuesday, September 15th at 12:30 PM

5.3. Development of genetic tools to dissect the role of microbiota-derived bacterial amyloids in neurodegeneration

Authors: Laura Molina-García (1); Paula Mancebo-Gamella (1); Lucía Pérez-García (1); Susana Quesada-Gutiérrez (1); Lucía Rodríguez-Heine (1); Gabriela Vázquez-Cartagena (1)

Affiliations:

1. National Center for Biotechnology (CNB-CSIC). Dpt. of MIcrobial Biotechnology. Madrid. Spain.

Abstract:

The intestinal microbiota modulates many aspects of human physiology, including brain function. Recently, correlations have been described between microbiota composition and certain neurodegenerative diseases (e.g., Alzheimer's and Parkinson's). A hallmark of these diseases is the aggregation of amyloid proteins. Amyloid aggregates self-propagate by converting soluble molecules of the same protein into the aggregated state through protein–protein interactions (self-nucleation). However, what triggers this aggregation cascade remains unknown. Since different members of the microbiota produce functional amyloids, it has been proposed that these may initiate the aggregation of human amyloids through in vivo cross-seeding, thereby causing disease. Supporting this idea, bacterial amyloids such as curli from E. coli, FapC from Pseudomonas, and amyloid sequences from BAP proteins of S. aureus increase the aggregation of human amyloids in animal models of neurodegeneration. Moreover, it has recently been shown in C. elegans that intestinal curli can reach neurons to mediate this effect. However, it remains unknown: (i) whether in vivo cross-seeding (protein–protein interaction) between bacterial and human amyloids is the underlying mechanism driving this increased aggregation (studies investigating in vivo cross-seeding rely on fluorescence colocalization, but colocalization does not necessarily imply interaction); (ii) whether such interactions are what initiate human amyloid aggregation; (iii) whether other bacterial amyloids also reach neurons to modulate human amyloid aggregation; and (iv) whether this occurs in a protein-specific manner to promote particular diseases. To address these questions, we are developing a bimolecular fluorescence complementation (BiFC)-based system in C. elegans to determine if, when, and where direct interactions between different human and bacterial amyloids occur, using a combinatorial approach that will allow us to uncover the protein–protein specificity of these interactions. Using this approach, we have tested the interaction between ingested CsgA and neuronal a−syn. Using fluorescence microscopy we detected reconstituted Venus fluorescence in neurons of 71% (8 days adult) transgenic C. elegans producing a−syn-CtVenus (N2, Ex[a−syn-CtVenus; rol-6]) fed with E. coli BW25113 DcsgA producing CsgA- NtVenus. Importantly, Venus fluorescence was not detected in C. elegans producing a−syn-CtVenus fed with E. coli BW25113 DcsgA or WT worms fed with E. coli BW25113 DcsgA producing CsgA-NVenus. Remarkably, despite a-syn is expressed pan-neuronally, we only observed reconstituted Venus signal in a small number of neurons. Together, our results demonstrate that cross-seeding of gut bacterial and neuronal host amyloids do occur in vivo and suggests that CsgA-a-syn interactions may only occur in specific neurons that we are currently characterising. Another open question in the field is whether CsgA alone can be neurotoxic once it reaches the nervous system. To address this question, we generated a transgenic C. elegans expressing panneuronally an optogenetic switch that allows light-induced oligomerization of CsgA in neurons and we performed survival assays under light and dark conditions. Preliminary results suggest internal hatching in CsgA-CRY-mCherry animals exposed to light. These findings could indicate neurotoxicity, particularly affecting neurons controlling egg-laying behaviour, which we are currently investigating.

Program Time: Tuesday, September 15th at 12:45 PM

5.4. Sulfated Steroid Hormones Regulate Starvation-Induced L1 Arrest in Caenorhabditis Elegans

Authors: Loreto Castro Castro (1); Ana María Brokate Llanos (1); Carlos Gomez Marín (1); Almudena Moreno Rivero (2); María Olmedo López (2); María de las Mercedes Pérez Jiménez (1); Manuel Jesús Muñoz Ruiz (1)

Affiliations:

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2. Universidad de Sevilla, CL/Prof. García González 1, E-41012 Sevilla, ES

Abstract:

Animals must adapt to fluctuations in nutrient availability through mechanisms that coordinate survival and developmental recovery. In Caenorhabditis elegans, newly hatched larvae can survive prolonged starvation by entering L1 arrest, providing a valuable model for studying communication mechanisms under nutrient-deprived conditions [1] [2]. Here, we investigated whether sulfated steroid hormones function as extracellular signals during L1 arrest. Previous observations revealed that recovery after L1 arrest is influenced by larval density. We observed that mutants with altered levels of sulfated steroid hormones [3] appear to exhibit distinct recovery capacities. We therefore explored the potential role of sulfated C19 androgen steroid hormones in mediating adaptive responses to starvation and developmental recovery. Our findings support a previously unrecognized role for sulfated steroid hormones in the regulation of starvation-associated responses. These molecules may act as inter-individual chemical cues that contribute to the coordination of physiological and developmental adaptations to nutrient deprivation in this organism.

Program Time: Tuesday, September 15th at 1:00 PM

5.5. Neuron subtype-specific mitochondrial stress signalling engages glial responses and shapes systemic ageing trajectories

Authors: Paula Soto Carmona (1); Belen Perales Villegas (1); Marta Artal-Sanz (1); Jesus Fernandez-Abascal (1)

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Abstract:

Ageing is not only driven by cell-autonomous decline but also by how stress signals are coordinated across tissues. Mitochondria are central to this process, yet whether specific neuronal populations actively instruct organismal adaptation—and how glia interpret these signals—remains unresolved. Here, we identify a neuron subtype-specific signalling axis that propagates mitochondrial stress systemically and rewires ageing trajectories in Caenorhabditis elegans. Disrupting mitochondrial prohibitins (PHB-1/2) in neurons is sufficient to trigger a robust organism-wide mitochondrial stress response, with strong activation in distal tissues such as the intestine. However, this signalling is not uniform: its strength depends on neuronal identity, with cholinergic and serotonergic neurons acting as dominant drivers of systemic UPRmt activation. Strikingly, this inter-tissue communication is not intrinsic but conditional. While neuronal mitochondrial stress induces a sustained systemic response during ageing at 20°C, this signalling collapses under thermal stress. This switch-like behaviour predicts organismal outcomes: conditions that support sustained signalling promote longevity, whereas its failure correlates with reduced lifespan. These results argue that ageing trajectories are not simply dictated by mitochondrial dysfunction per se, but by the ability to propagate and maintain stress signals across tissues. At the molecular level, neuronal mitochondrial perturbation does not accelerate canonical ageing but instead diverts it into an alternative trajectory. Quantitative proteomics reveals selective engagement of stress-response, immune, and trafficking pathways, alongside repression of mitochondrial and RNA-processing functions, indicating a systemic reprogramming of proteostasis networks. Unexpectedly, glial cells do not mount a canonical UPRmt despite receiving neuronal stress signals. Instead, we observe nuclear accumulation of the stress-responsive transcription factor DVE-1 in a subset of glia, revealing a non-canonical response that is uncoupled from classical reporters. This suggests that glia act as selective interpreters of neuronal mitochondrial stress, potentially gating its propagation or transformation into downstream signals. Functionally, mitochondrial perturbation impacts specific neuronal circuits and induces structural remodelling in glia, supporting a coordinated but cell-type-specific response to stress. Moreover, neuronal mitochondrial dysfunction differentially modulates proteotoxicity, alleviating aggregation in α-synuclein models and extending survival in an ALS model, while leaving other proteotoxic paradigms unaffected. Together, these findings support a model in which neuronal mitochondrial stress is not merely a local defect but a regulated signal whose propagation depends on neuronal identity, environmental context, and glial interpretation. This framework positions neuron–glia communication as a key determinant of systemic ageing and proteostasis.

Program Time: Tuesday, September 15th at 1:15 PM

5.6. Membrane disruption as a potential anthelmintic mechanism of an extract of Acmella oleracea against nematodes

Authors: Adrián Millán (1,2); Lorien Abellanas (1); Víctor López (1,2); Carlota Gómez (1,2); Cristina Moliner (1)

Affiliations:

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2. Instituto Agroalimentario de Aragón, IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Abstract:

Introduction: The incidence of anisakiasis, a parasitic disease caused by Anisakis simplex following the consumption of raw or undercooked fish, has increased considerably in recent years. Given the limited efficacy of currently available treatments, the identification of new anthelmintic agents remains a priority. In this context, Acmella oleracea (L.) R.K. Jansen is of particular interest, as it is traditionally used to treat intestinal parasitic infections in humans and animals. However, its potential nematicidal activity and underlying mechanism of action remain poorly understood. Methods: The nematicidal activity of a N-alkylamide-enriched supercritical CO2 extract of A. oleracea was evaluated against Anisakis simplex L3 larvae. To investigate its possible mechanism of action, the effects on survival were assessed in Caenorhabditis elegans wild-type N2 worms and in the drug-resistant strains CB193 (levamisole-resistant) and JD608 (ivermectin-resistant) by using the WMicroTracker One system. In addition, effects on cuticle permeability and intestinal barrier integrity were also examined in N2 worms, and the in vitro acetylcholinesterase inhibitory activity of the extract was determined. Results: The extract exhibited nematicidal activity against A. simplex after 48 h of exposure over the concentration range of 31.25 to 1000 µg/mL(IC50 130 ± 30 µg/mL). In C. elegans, the highest concentrations tested (1000–500 µg/mL) markedly reduced worm viability in N2 strain (IC50 489 ± 106 µg/mL), while the drug-resistant strains showed even more susceptibility, IC50 133 ± 38 µg/mL and 257 ± 43 µg/mL for JD608 respectively, which suggests that the extract does noy share the targets of levamisole and ivermectin. Moreover, treatment was associated with a trend toward increased cuticle permeability and impaired intestinal barrier integrity, while no in vitro acetylcholinesterase inhibitory activity was detected. Conclusions: A. oleracea extract showed nematicidal activity against A. simplex and C. elegans. The results suggest a mode of action distinct from those of levamisole and ivermectin, possibly involving membrane disruption. These findings support the use of C. elegans as a surrogate model for mechanistic studies in Anisakis and highlight A. oleracea as a promising source of alternative anthelmintic compounds.

Program Time: Tuesday, September 15th at 1:30 PM

5.7. Development of a Caenorhabditis elegans-Based Screening Platform to Identify Neuroprotective Candidates for Alzheimer's Disease

Authors: Eider Ciaurriz Arrastia (1,2); Maite Solas Zubiaurre (2); Carolina González Ferrero (1)

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2. Universidad de Navarra (UNAV). Departamento de Ciencias Farmacéuticas

Abstract:

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of dementia worldwide, affecting more than 55 million people. Despite extensive research efforts, effective preventive and therapeutic strategies remain limited. In recent years, increasing evidence has highlighted the role of the gut–brain axis in neurodegeneration, revealing that gut microbiota composition and activity can influence neuroinflammation, oxidative stress, and amyloid-β (Aβ) pathology. In this context, probiotics and postbiotics have emerged as promising functional ingredients with potential neuroprotective properties. However, the identification of microbial candidates requires robust, rapid, and cost-effective screening platforms. In this context, the nematode Caenorhabditis elegans represents a valuable in vivo model for studying AD-related processes due to its short lifespan, well-characterized genetics, and the availability of transgenic strains expressing human Aβ peptides. The hypothesis of this work is that functional ingredients based on probiotics and/or postbiotics can exert neuroprotective effects that contribute to the prevention of AD, and these effects can be effectively evaluated using C. elegans. Therefore, the objective of this study is to develop and validate a screening platform based on C. elegans for the identification and selection of postbiotic candidates with potential neuroprotective activity. To achieve this goal, novel methodologies were developed and optimized using the transgenic AD strains CL4176 and CL2355. The established assays evaluated key AD-related phenotypes, including paralysis, Aβ aggregation, chemotaxis, reactive oxygen species (ROS) levels, motility, and survival. Several compounds previously described as neuroprotective agents, including epicatechin, resveratrol, caffeic acid, and sodium butyrate, were used to validate the methods and to select the most suitable positive controls for each endpoint. The results obtained demonstrated successful optimization and validation of the screening assays. Among the compounds tested, epicatechin and caffeic acid showed the most consistent and robust neuroprotective effects, supporting their selection as positive controls. These findings confirm the reliability of the platform, which is now ready for the evaluation of bacterial-derived functional ingredients.

Program Time: Tuesday, September 15th at 1:45 PM

Posters

1. Decoding the regulatory logic of a dual-coding splicing switch in C. elegans neurons

Authors: Naim Martín (1); Eduardo Leyva Diaz (1)

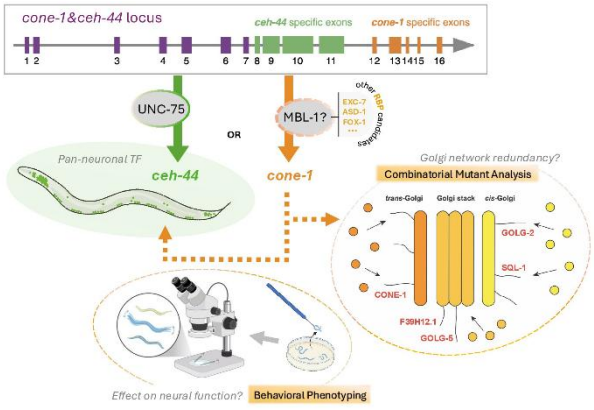
Affiliations:

1. Instituto de Neurociencias, CSIC-UMH

Abstract:

The establishment and long-term maintenance of neuronal identity requires the continuous execution of tightly regulated, multi-layered gene expression programs. While the transcriptional networks defining specific neuronal fates are increasingly well characterized, the post-transcriptional logic that further sculpts the functional neuronal proteome remains less understood. In Caenorhabditis elegans, the highly conserved RNA-binding protein (RBP) UNC-75 acts as a master post-transcriptional regulator, governing a vast network of alternative splicing required for proper synaptic transmission and structural plasticity. A one-of-a-kind molecular node governed by UNC-75 is the deeply conserved cone-1&ceh-44 locus. While alternative splicing typically generates closely related protein isoforms, this locus acts as a radical binary switch to bifurcate a single genomic unit into entirely unrelated protein families, directly mirroring the vertebrate Cux1/CASP locus. Specifically, UNC-75 actively promotes the expression of the pan-neuronal transcription factor CEH-44 while simultaneously repressing CONE-1, a structurally unrelated Golgi-tethering protein. The loss of UNC-75 results in ectopic neuronal CONE-1 expression, implying an antagonistic network of RBPs that continuously pushes for the non-neuronal transcript configuration. To decode this combinatorial splicing logic, we are pursuing two parallel aims. First, we aim to identify the specific RBPs that counteract UNC-75 at this locus. Among other candidates, we are focusing on MBL-1, a well-known C. elegans MBNL family splicing factor, as a potential antagonistic regulator. Second, while CONE-1 is actively excluded from neurons, its homology to the golgin family and broad non-neuronal expression suggest a putative role in intra-Golgi transport. However, extensive functional redundancy among Golgi proteins has thus far masked its precise contribution. Through combinatorial mutant analysis involving CONE-1 and other known Golgi-tethering proteins (e.g., GOLG-5, SQL-1), we are conducting comprehensive behavioral phenotyping to uncover potential non-cell-autonomous effects of CONE-1 loss on neuronal function. Together, this work highlights how neuronal function is shaped both by neuron-intrinsic alternative splicing networks, and by non-cell-autonomous Golgi-dependent pathways operating in adjacent tissues.

Graphical Abstract / Poster:



2. Inhibition of a Neuronal Transcriptional Repressor Ameliorates Mutant Protein–Induced Toxicity in C. elegans

Authors: Alba Irisarri (1); Gabriella Cheikho (1); Christian Griñán-Ferré (1,2); Aina Bellver-Sanchis (1)

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Abstract:

Diseases of the central nervous system (CNS) are a major cause of disability and mortality, and for most of them there is a lack of curative therapeutic approaches. A specific transcriptional repressor, which acts through epigenetic remodeling to silence neuron-specific genes in non-neuronal cells and to fine-tune synaptic plasticity in mature neurons, appears to be involved in the pathophysiology of different CNS conditions. Here, we evaluated the inhibition of this transcriptional repressor in transgenic Caenorhabditis elegans models EAK102 and EAK103, both expressing a YFP-fused mutant human huntingtin fragment (Htt513) and differing in polyQ length (Q15 and Q128, respectively). Due to polyQ length-dependent toxicity, the EAK103 strain shows reduced motility and lifespan caused by Htt513 (Q128) aggregates. In this study, we demonstrated an increase in thrashing rate and lifespan, as well as a reduction in toxic aggregates, after inhibition of the transcriptional repressor in the EAK103 strain. N2 (wild type) and EAK102 strains were used as controls. Our results support C. elegans as a suitable model organism for studying the effects of pharmacological inhibition of this repressor and encourage future research on its regulatory mechanisms as a potential target for the development of novel therapeutic strategies.

3. Addressing the roles of the cell cycle regulator APC/CFZR-1 during the development of neuronal lineages in Caenorhabditis elegans.

Authors: David Borrego (1); Marta Rodríguez-Navarro (1); Julia Hillung (1); José Pérez-Martín (1)

Affiliations:

1. Instituto de Biomedicina de Valencia

Abstract:

In metazoan organisms, cell cycle regulators not only drive cell division but also play critical roles in cell fate specification, differentiation, and morphogenesis. These regulators often utilize non-canonical mechanisms, independent of their primary cell-cycle functions, to coordinate developmental processes. The APC/C ubiquitin ligase complex, in association with its activator FZR-1, exemplifies such regulation. Previous studies from our research group have shown that APC/CFZR-1 is essential for establishing the correct cell fates of daughter cells produced by Wnt-mediated asymmetric division during somatic gonad formation in Caenorhabditis elegans. Building on these findings, the present study examines whether APC/CFZR-1 similarly influences the development of postembryonic neuronal lineages. Loss of APC/CFZR-1 activity, due to a loss-of-function allele of fzr-1, resulted in alterations in at least two distinct neuronal cell lineages regulated by Wnt signaling: the C. elegans postdeirid, which comprises two morphologically and functionally distinct sensory neurons (PVD and PDE), and the lineage responsible for ray development in the male tail. We have found that elements previously described in somatic gonadal morphogenesis, such as the transcriptional regulator EFL-3, a known target of APC/CFZR-1, are also implicated in the effects of APC/C on the neuronal lineages described above. This observation suggests the existence of a common regulatory module. These findings will be discussed in detail in this communication.

4. Exploring the gut microbiome's role in lipid metabolism in Caenorhabditis elegans

Authors: Leticia* Orti (1); Elisabet* Terrado (1); Carlos Mora (1); Veronica Tolosa (1); Sergio Romera (1); Yolanda* Sanz (1)

Affiliations:

1. Microbiome Innovation in Nutrition & Health Research Unit (INNOBIOME), Institute of Agrochemistry and Food Technology, Spanish National Research Council (IATA-CSIC), Center of Excellence Severo Ochoa, Valencia, Spain.

Abstract:

The WHO recognizes obesity as one of the major global health problems due to its high prevalence and its association with chronic diseases such as type 2 diabetes and cardiovascular disorders. Therefore, there is a growing need to develop new strategies to prevent and treat obesity-related conditions, in which the gut microbiota plays a key role in metabolic regulation. Western diets contribute to obesity and alter the composition and function of the gut microbiota, as well as communication among the intestine, metabolic organs, and the brain, thereby promoting weight gain. In this context, C. elegans is being used as a model organism to assess the effects of a specific bacterium on obesity-related outcomes. The objective of our study was to optimize the use of C. elegans to evaluate the impact of whole fecal microbiota transplants from healthy, normal-weight individuals compared to obese individuals. This model is particularly suitable due to the traceability of lipid storage, which allows identification of potential therapeutic targets for obesity. After synchronizing C. elegans eggs and staining with Oil Red O, quantitative analysis revealed significant differences between the two experimental groups, with higher lipid accumulation observed in nematodes fed fecal extract from obese donors. These results demonstrate that the microbiota from obese patients can transfer the phenotype of obese human subjects, promoting increased fat storage in vivo. These results support C. elegans as a robust platform for screening microbiome-mediated effects and identifying molecular metabolic targets associated with obesity.

5. POST-TRANSLATIONAL MECHANISMS OF NEURONAL DIFFERENTATION IN CAENORHABDITIS ELEGANS

Authors: Sandra Pérez García (1,2); Andrea Millán Trejo (1,2); Nuria Flames Bonilla (1,2)

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2. Valencia Biomedical Research Foundation, Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to the Instituto de Biomedicina de Valencia (IBV), Valencia, Spain

Abstract:

Neuronal differentiation is a protracted process. Before becoming fully mature, neurons undergo a series of tightly regulated developmental stages, including progenitor specification and proliferation, generation of postmitotic neuroblasts, migration to target locations, and terminal differentiation. Numerous factors involved in proliferation, migration and differentiation have already been identified. However, the mechanisms regulating the transitions between these different developmental stages remain less well understood. Elucidating how progenitor cells cease dividing or migrating and initiate differentiation is crucial both for fundamental biology and for understanding the molecular basis of neurodevelopmental disorders. In C. elegans (as well as in mammals), neuronal differentiation generally occurs shortly after or even concomitant to neuroblast migration, making the transition from immature migratory cells to mature neurons difficult to investigate. However, the bilateral serotonergic HSN neurons constitute a unique exception. After reaching their final position at embryonic stages, these neurons remain in an undifferentiated state throughout most of the larval stages and only acquire their mature morphology and function at L4 stage. This prolonged developmental pause provides an exceptional model for studying the temporal regulation of neuronal differentiation. One of the mechanisms currently proposed to be critical in transitions between cellular states, such as the exit from quiescence, is translational regulation. Importantly, egl-45, a translation initiation factor, exhibits defects in the temporal regulation of HSN differentiation. Our preliminary data suggests that egl-45/EIF3A affects the overall translation rates in HSN neurons, and regulates the levels of the transcription factors that act as their terminal selectors. Given that the serotonergic transcriptional regulatory program appears to be conserved between nematodes and mammals, the knowledge generated through this project could be applicable to more complex organisms.

6. Establishing a Cell-Type-Specific Massively Parallel Reporter Assay in Caenorhabditis elegans

Authors: Thi Minh Tam Nguyen (1,2); Rafael Alis (1,2); Nuria Flames (1,2)

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2. Valencia Biomedical Research Foundation, Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to the Instituto de Biomedicina de Valencia (IBV), Valencia, Spain

Abstract:

Understanding how cis-regulatory elements (CREs) control gene expression during development and in response to environmental triggers is a central challenge in biology. Although massively parallel reporter assays (MPRAs) have become powerful tools for studying regulatory DNA at scale, their application in whole organisms with cellular resolution remains limited. We aim to establish a cell-type-specific MPRA platform in Caenorhabditis elegans to investigate the regulatory mechanisms underlying neuronal specification and neuronal homeostasis in vivo. C. elegans is an attractive model for this purpose because its nervous system comprises 118 distinct neuron types with well-defined identities. However, implementing cell-type-specific MPRA in this organism presents a major challenge: the activity of CRE candidates must be linked to the specific cell types in which they are active. To address this, we are developing a dual-barcoding strategy in which 5'-barcoded transcripts driven by cell-type-specific promoters provide cell-identity information, while 3'-barcoded transcripts driven by CRE candidates encode regulatory-element identity. These transcripts are subsequently joined through RNA transcript reassembly approaches, generating single RNA molecules that contain both barcodes. Sequencing of the reconstituted transcripts will enable quantitative measurement of CRE activity across multiple cell types in parallel and in vivo. As a proof of concept, we evaluated transcript reassembly using split fluorescent reporters in transgenic worms. We observed reporter reconstitution through mRNA trans-splicing in neurons, demonstrating that transcript assembly can occur in vivo. Ongoing work focuses on enhancing transcript reassembly and developing sequencing pipelines to identify and quantify cell-type and CRE barcodes. Together, these efforts provide the foundation for implementing cell-type-specific MPRA in C. elegans and for dissecting the regulatory grammar that governs neuronal identity and function. Keywords: Massively Parallel Reporter Assay (MPRA); Caenorhabditis elegans; cis-regulatory elements; neuronal specification.

7. Novel in vivo transcription factor activity reporters

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1. Instituto de Biologia Molecular de Barcelona (IBMB - CSIC)
2. Universitat de Barcelona

Abstract:

Transcription factors (TF) play a critical role controlling gene expression in animals in order to orchestrate development and maintain homeostasis. Consequently, their misregulation is often associated with disease. Nonetheless, TF activity is a cryptic trait – it is hard to quantify and existing methods for measuring TF activity encounter obstacles due to the complexity of TF interaction networks, among other reasons. To address these challenges, we are building in vivo TF activity reporter strains in Caenorhabditis elegans based on two novel strategies. A major mechanism of regulation of TF activity is nuclear translocation upon post-translational modification (e.g. phosphorylation of DAF-16). The first strategy takes advantage of a split fluorescent protein tag which complements a nuclear fragment to produce fluorescence only when the tagged TF translocates to the nucleus. We are currently developing genètic constructs for effective and exclusive nuclear localisation of the large split fluorescent protein subunit.The second method, which we call TRACeR, introduces a single exogenous target gene – an in silico optimised ATP-independent NanoLuciferase - to measure expression induced by upstream TF binding. Cassettes consisting of repeats of the known TF motif can be knocked in upstream of a minimal promoter to generate an activity reporter for any TF. Our goal is to generate customisable tools for the C. elegans community to illuminate cryptic TF activity in this powerfully tractable model organism. These two complementary strategies will ultimately allow the identification of new regulators of TF activity: from environmental factors to genomic variability.

8. Mechanisms of Serotonergic Phenotype Plasticity in Nematodes

Authors: María Constanza Silvera (1,2); Inés Carrera (3); Nuria Flames (1,2)

Affiliations:

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2. Valencia Biomedical Research Foundation, Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to the Instituto de Biomedicina de Valencia (IBV), Valencia, Spain.
3. Universidad de la República, School of Chemistry, Pharmaceutical Sciences Department, Pharmacology Area. Uruguay.

Abstract:

Serotonin is an ancient signalling molecule that predates the evolution of nervous systems and is found in plants, bacteria, fungi, and animals. Although it functions as an antioxidant and environmental signal, it is best known as a neurotransmitter that regulates essential physiological and behavioral processes, including mood, appetite, sleep, and stress responses. Because alterations in serotonergic signalling are associated with disorders such as depression and anxiety, understanding the mechanisms that regulate serotonin plasticity is of broad biological and biomedical interest. The serotonergic system is highly conserved across bilaterian animals. In the nematode Caenorhabditis elegans, a powerful model organism for studying gene-environment interactions, we have identified an unusual form of neurotransmitter plasticity. VC4 and VC5 are cholinergic motor neurons that participate in the egg-laying circuit. Under standard laboratory conditions, these neurons express the vesicular monoamine transporter VMAT/CAT-1, but do not express the serotonin reuptake transporter SERT/MOD-5, and therefore lack detectable serotonin. Previous work from our laboratory demonstrated that exposure to high environmental levels of serotonin can induce a serotonergic phenotype in VC4 and VC5 neurons. This transition appears to be controlled by epigenetic mechanisms: H3K9 methylation represses the mod-5 locus under normal conditions, whereas relieving this repression enables serotonin uptake and acquisition of the serotonergic state in VC4 and VC5. This PhD project aims to uncover the molecular, cellular, and evolutionary mechanisms underlying this neuronal plasticity. First, we will investigate how environmental serotonin regulates mod-5 expression and whether this process involves epigenetic remodelling. Second, we will examine how serotonin-rich environments affect VC4 and VC5 neuronal morphology using confocal microscopy and correlative electron microscopy, and how gene expression is modified through whole worm RNA sequencing. Finally, we will take a comparative evolutionary approach by studying related nematode species that exhibit either constitutive or plastic VCs serotonergic phenotypes. Together, these studies will provide insight into how environmental signals shape neuronal identity and may reveal conserved mechanisms regulating serotonin reuptake transporter plasticity across evolution. Between 160 - 600 words. Now: 321

9. LMP-1-dependent lysosomal integrity is an essential component of the DAF-16-regulated survival program during L1 quiescence in C. elegans

Authors: Almudena Moreno Rivero (1); María Olmedo López (1); Alejandro Mata-Cabana (1)

Affiliations:

1. Universidad de Sevilla

Abstract:

The role of DAF-16 in promoting survival during L1 starvation is well established; however, the specific cellular processes through which this transcription factor sustains viability during quiescence remain elusive. To address this question, we performed a transcriptomic analysis that identified the lysosome as one of the most significantly affected functional categories in the absence of DAF-16. Based on this finding, we focused on lmp-1, the C. elegans homolog of mammalian LAMP-1/2, whose loss of function produces viable but functionally compromised lysosomes, providing a suitable model to investigate the role of lysosomal integrity during starvation. Our results show that daf-16 and lmp-1 act within the same genetic pathway to maintain survival during L1 starvation, with intestinal expression of lmp-1 being sufficient to fully restore the survival of lmp-1 mutants. The regulation between DAF-16 and LMP-1 is reciprocal. DAF-16 is necessary for LMP-1 expression during extended starvation, and LMP-1 is required to maintain the nuclear localization of DAF-16 during L1 arrest. Furthermore, we demonstrate that loss of lmp-1 function alters the dynamics of autophagic markers during starvation, affecting both autophagosome formation and the degradation of selective autophagic substrates. Together, these findings identify lysosomal function as an essential component of the DAF-16-regulated survival program and establish the intestinal lysosome as a key determinant of cellular homeostasis during starvation-induced quiescence.

10. A Conserved Logic for Neuronal Identity: Cross-Metazoan Discovery of Terminal Selectors

Authors: Antonio Jordan-Pla (1); Adrian Tarazona (1); Yaiza Dominguez (1); Rafael Alis (1); Nuria Flames (1)

Affiliations:

1. Valencia Biomedical Research Foundation, Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to the Instituto de Biomedicina de Valencia (IBV), Valencia, Spain

Abstract:

During larval development, post-mitotic neurons maintain their identity while undergoing extensive functional and morphological maturation. Terminal selectors (TSs), master transcription factors that establish and maintain neuron type identity, are central to this process, yet the genetic programs they drive to govern nervous system maturation remain incompletely characterized Leveraging publicly available single-cell RNA-seq datasets spanning different larval stages of C. elegans [1], we analyzed 73 known TSs across the worm's 118 neuron types and found that the vast majority display highly restricted expression, active in fewer than 40% of cell types. Within their cognate neuronal contexts, we observed that TSs are expressed at significantly higher levels than both TSs outside their active neurons and non-TS TFs. This constitutes a specificity signature that distinguishes TSs from the broader TF landscape. Consistently, known TSs described in the literature are strongly enriched among the top-ranked TFs within each neuron type, a pattern that extends beyond the homeobox family to encompass diverse TF families. Applying this restricted-high expression logic across embryo, L2, and L4 stages, we defined a "stable" TF set (factors maintaining top-ranked, restricted expression throughout development), which, after excluding known TSs, conformed a prioritized list of novel TS candidates. Preliminary experimental validation of a subset of these candidates reveals a TS phenotype in approximately 70% of the TF–neuron pairs tested, supporting the predictive power of this computational pipeline and leading to the description of previously uncharacterized terminal selectors. To uncover conserved principles beyond a single-species snapshot, we extended this framework to a cross-metazoan analysis spanning C. briggsae [2], Drosophila melanogaster [3], Mus musculus [4], and Homo sapiens [5], utilizing publicly available longitudinal single-cell RNA-seq datasets and high-confidence orthologs. Intersecting species-specific candidate lists against the ground-truth baseline of known C. elegans TSs reveals significant cross-species overlaps, reflecting a deeply conserved evolutionary logic underlying the terminal selector model. This cross-metazoan-validated pipeline yields a prioritized candidate set for future functional work.

11. Spatiotemporal characterization of C27D8.4 as a predicted Retinol Dehydrogenase essential for C. elegans embryonic survival and reproductive health

Authors: Angelina Zheleva (1); Begoña Ezcurra Ezcurra (1); Juan Cabello (1)

Affiliations:

1. Centro de Investigación Biomédica de la Rioja (CIBIR)

Abstract:

While retinoid signaling has been heavily studied in vertebrates regarding embryonic development and ocular health, its specific function in Caenorhabditis elegans (C. elegans) remains poorly characterized. Recent work by Banse et al. (2024) demonstrated that retinol (vitamin A) and its highly bioactive metabolite, all-trans retinoic acid (atRA), modulate longevity, metabolic programming, and oxidative stress resistance in C. elegans. In this study, we combined whole-genome sequencing (WGS) with Hawaiian variant mapping to identify a point mutation responsible for an embryonic-lethal phenotype. We discovered a t1881 mutant allele of C27D8.4 (NAD-dependent epimerase/ dehydrogenase domain-containing protein). C27D8.4 is predicted to enable oxireductase and all-trans-retinol dehydrogenase (NADP+) activity, suggesting a potential role in endogenous vitamin A metabolism. To better understand its role in C. elegans embryonic development, we chracterized the dynamics of endogenously tagged C27D8.4::GFP. Consistent with these results, single-cell transcriptomic analysis suggests that C27D8.4 plays two vital roles: one in early embryonic development and another in adult reproductive health.

12. Ginsenosides as potential neuroprotectors in C. elegans models of Huntington's disease

Authors: Mar Collado-Pérez (1,2,3); Julia Tortajada-Pérez (1,2,3); Cristina Trujillo-Del Río (1,2); Ana Pilar Gómez-Escribano (4,5,6); Rafael Vázquez-Manrique (1,2,4); Andrea del Valle Carranza (1,2)

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4. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28029, Madrid, Spain
5. Cellular and Organic Pathophysiology of Oxidative Stress and Rare Diseases Group
6. Clinical University Hospital of Valencia (INCLIVA), 46010, Valencia, Spain

Abstract:

Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG triplet expansion in the huntingtin gene, resulting in the mutant huntingtin protein. This protein is prone to misfolding and aggregation, leading to impaired proteostasis and oxidative stress. Ginsenosides, the bioactive compounds of Panax ginseng, exhibit neuroprotective, antioxidant, and autophagy-activating properties, making them promising candidates for therapeutic interventions. The aim of this work is to perform a screening of five different ginsenosides on protein aggregation and neuronal functionality using C. elegans models of HD and to explore their mechanism of action. A primary screening was conducted using a C. elegans strain expressing pan-neuronal polyglutamine (polyQ) tracts, quantifying neuronal aggregates at the day-one adult stage. Our results identified several biologically active compounds capable of significantly reducing aggregate formation. These candidate compounds were further characterized through touch sensitivity assays to assess mechanosensory function, as well as evaluations of their autophagy induction capability. Some of them not only reduced protein aggregation but also improved worm movement and mechanosensory activity, both of which are typically impaired in aggregation-related proteopathies. We hypothesize that this effect is driven in some cases by the activation of autophagy, leading to enhanced clearance of misfolded proteins. These findings support further research into natural compounds as an effective therapeutic strategy to mitigate the progression of HD.

13. A Species-Specific Framework for GRN Inference in C. elegans

Authors: Adrián Tarazona-Sánchez (1); Antonio Jordán-Pla (1); Nuria Flames (1)

Affiliations:

1. Developmental Neurobiology Unit, Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain

Abstract:

Gene regulatory networks (GRNs) govern cell-type specification and maintenance through coordinated transcription factor (TF) activity acting on cis-regulatory modules. In Caenorhabditis elegans, the invariant developmental lineage, well-characterized neuronal identities, and extensive transcriptomic resources make it an ideal system for studying gene regulation. Despite recent advances in GRN inference methodologies, accessible and species-adapted frameworks for C. elegans transcriptomic data remain limited, particularly for users lacking computational expertise. To address this, we developed a species-specific GRN inference framework for C. elegans, developed by extending the Motif Activity Response Analysis (MARA/ISMARA) [1] [2] approach to support both bulk and single-cell RNA-seq data. To improve regulatory inference in nematodes, we integrated multiple layers of species-specific regulatory information, including nascent RNA sequencing data for accurate transcription start site and promoter annotation, transcription factor DNA-binding preferences from JASPAR and CisBP, and evolutionary conservation of regulatory regions across Caenorhabditis species. This strategy addresses organism-specific challenges, such as trans-splicing, while improving the definition of functional cis-regulatory modules. To maximize accessibility, the framework is implemented as an interactive web platform with hosted computation, enabling users to upload transcriptomic datasets, perform analyses, and explore regulatory predictions without programming expertise or dedicated computational infrastructure. Results can be interactively explored online and downloaded for downstream analyses. Preliminary benchmarking using publicly available bulk RNA-seq datasets shows strong agreement between MARA-derived TF activity predictions and those inferred by CelEST [3], while recovering experimentally validated tissue-specific regulators. For example, intestinal regulators ELT-2 and ELT-7 are preferentially inferred in intestinal samples, CEH-43 activity is specifically detected in neuronal tissues, and PAT-9 in muscle. Compared to CelEST, our framework shows improved tissue specificity in several contexts, reducing spurious TF activity predictions across unrelated tissues. By combining biologically informed regulatory modeling with an accessible analysis environment, this framework provides the C. elegans community with an easy-to-use resource for GRN inference across diverse applications, including neuronal differentiation, development, mutant analysis, aging, and disease models.

14. Cohesin/Nibpl Dysfunction in Cornelia de Lange Syndrome: A C. elegans Approach

Authors: Isabel Reillo (1); Kalyan Ghadage (2); Peter Meister (2); Nuria Flames (1); José Pérez (1); Ethel Queralt (1)

Affiliations:

1. Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain
2. Institute of Cell Biology, University of Bern

Abstract:

Cornelia de Lange Syndrome (CdLS) is a rare multisystem developmental disorder caused predominantly by mutations in cohesin subunits and their regulators. Approximately 70% of patients carry heterozygous mutations in NIPBL, the cohesin-loading factor. Although cohesin was first characterized for its role in sister-chromatid cohesion and chromosome segregation, accumulating evidence indicates that CdLS phenotypes arise mainly from disrupted chromatin organization and transcriptional regulation rather than overt mitotic defects. To dissect these mechanisms in vivo, we use Caenorhabditis elegans, where conserved cohesin pathways and powerful genetics are complemented by a natural separation of cohesin functions across paralogous complexes. C. elegans encodes five Rad21/Scc1-family kleisins (SCC-1/COH-2, COH-1, COH-3, COH-4 and REC-8): SCC-1 mediates mitotic and meiotic sister-chromatid cohesion, COH-3, COH-4 and REC-8 act in meiosis, whereas COH-1 is the predominant somatic, interphase cohesin and functions in gene regulation rather than mitosis. Notably, unlike mammals - where cohesin is the major loop extruder - condensin I is the primary long-range loop extruder folding the C. elegans genome, while cohesinCOH-1, acts locally: it is preferentially loaded near active enhancers, at sites bound by the NIPBL ortholog, to drive loop-extrusion "fountains". Cleavage of COH-1 collapses these fountains and de-represses fountain-proximal genes - preferentially neuronal genes - with measurable behavioral consequences. This clean uncoupling of cohesin's segregation and gene-regulatory roles makes C. elegans an ideal system to study the transcriptional basis of cohesinopathies. To model CdLS in nematodes, we introduce disease-mimetic point mutations into the orthologs of human NIPBL, RAD21 and SMC1A targeting conserved residues, thereby reproducing disease-associated molecular alterations in vivo. We characterize these models through genetic and phenotypic analyses, RNA-sequencing transcriptomic profiling, and CUT&RUN chromatin-occupancy mapping, to define developmental defects, transcriptional programs sensitive to cohesin dysfunction, and how altered cohesin loading affects genome organization, including enhancer-fountain integrity. Overall, this work aims to clarify how cohesin-mediated control of chromatin architecture and gene expression contributes to normal development and CdLS, advancing our understanding of cohesinopathies.

15. Silencing of Calreticulin Restores Cytosolic Calcium Homeostasis and Promotes ß-amyloid Clearance in a C. elegans Model of Alzheimer's Disease

Authors: Elena Caldero-Escudero (1); Andrea Morán-Cerro (1); Paloma García-Casas (1); Silvia Romero-Sanz (1); Silvia Fernandez-Martinez (1); Rosalba I Fonteriz (1); Mayte Montero (1); Javier Alvarez (1); Sergio De la Fuente (1); Jaime Santo-Domingo (1)

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Abstract:

The calcium hypothesis of Alzheimer's disease (AD) proposes that disruption of Ca2+ homeostasis constitutes a central pathogenic mechanism. AD Ca2+ dyshomeostasis involves organelle Ca2+ overload, enhanced oxidative stress, and impaired proteostasis driven by ß-amyloid (Aß) toxicity. Calreticulin (CRT-1) is a conserved ER-resident protein with Ca2+-buffering and molecular chaperone functions, suggesting a potential modulatory role in AD pathology. Our previous work studied for the first time CRT-1 in C. elegans models of muscle Aß-induced proteinopathy. crt-1 silencing improved healthspan and reduced exacerbated mitochondrial metabolic activity and ROS production. Importantly, crt-1 silencing markedly reduced Aß oligomer accumulation unaltering its constitutive expression, suggesting enhanced activation of Aß clearance mechanisms [1]. To further elucidate the mechanisms underlying the protective effects of crt-1 silencing, we are investigating both Ca2+ storage and chaperone functions of calreticulin. We found that crt-1 RNAi knockdown significantly decreased cytosolic Ca2+ transients and mitigated cytosolic and mitochondrial stress induced by Aß accumulation. In parallel, we observed crt-1 silencing reduced total amount of ubiquitinated protein, and increased the expression of proteasome genes, consistent with enhanced proteostasis capacity. Notably, crt-1 expression was elevated in Aß-expressing worms and was restored upon crt-1 knockdown, thereby preserving sufficient chaperone activity. Thus, we propose a dual mechanism whereby crt-1 knockdown limits cytosolic Ca2+ transients, attenuating Ca2+-mediated toxicity, while simultaneously mediates Aß oligomer clearance, potentially through proteasome activity.

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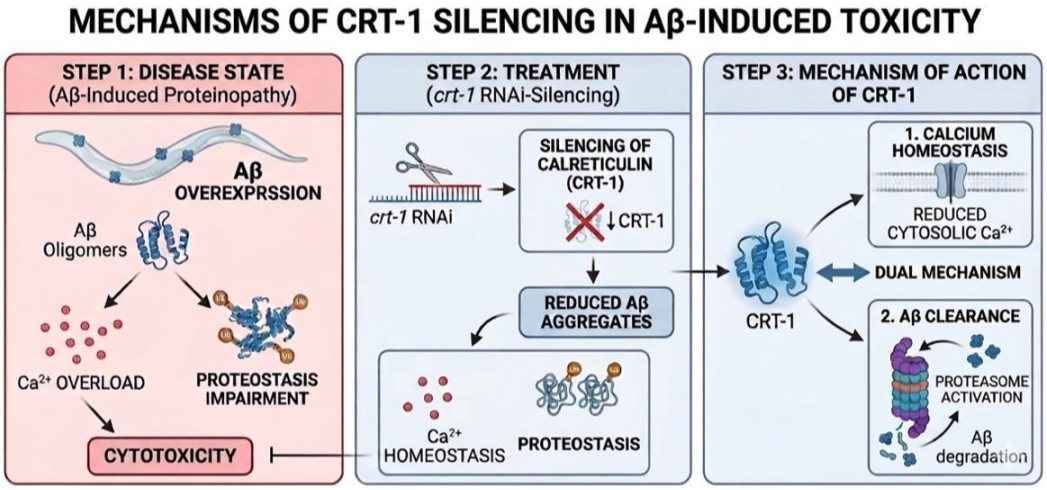

16. Development of Novel Caenorhaditis elegans Strains to Study the Role of ER-mitochondria Contact Sites in Aging and Neurodegeneration

Authors: Andrea Morán Cerro (1); Elena Caldero Escudero (1); Silvia Romero Sanz (1); Sergio de la Fuente (1); Jaime Santo Domingo (1); Mayte Montero (1); Javier Alvarez (1); Rosalba I Fonteriz (1); Paloma García Casas (1)

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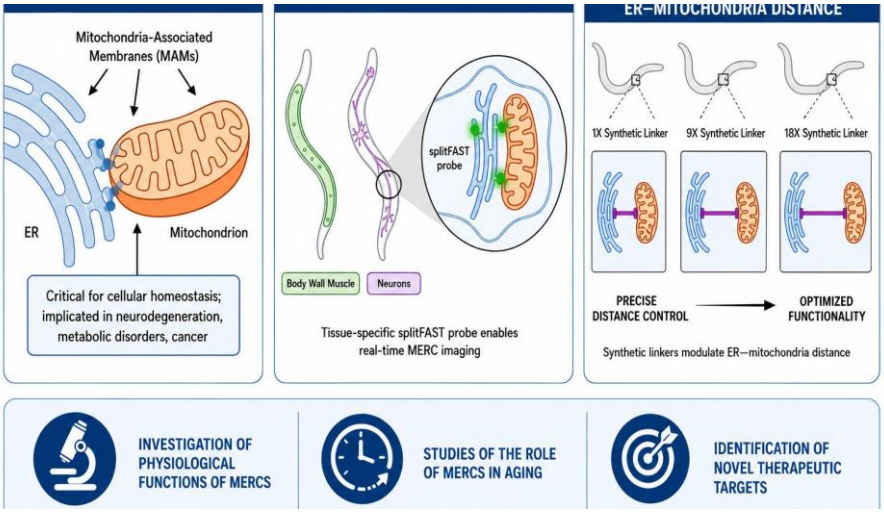
Abstract:

Domains of close proximity between the endoplasmic reticulum (ER) and the mitochondria (mitochondria-associated membranes, MAMs) are critical regulators of cellular homeostasis and participate in essential physiological pathways altered in neurodegeneration, metabolic disorders and cancer. Understanding the assembly and remodelling of ER–mitochondria contact sites (MERCs) is therefore fundamental to elucidate their contribution to disease progression. However, studying MERCs dynamics in vivo remains challenging due to the limited availability of tools with sufficient spatiotemporal resolution to monitor these interactions in living organisms. Recent advances, including the splitFAST fluorescent system, have enabled the visualization of MERCs remodelling while preserving high-resolution imaging capabilities. Here, we describe the development of two complementary sets of Caenorhabditis elegans strains designed to evaluate MERCs dynamics and function during aging and neurodegeneration. First, we generated tissue-specific strains expressing the ER–mitochondria splitFAST probe either in body wall muscle cells or neurons, allowing the analysis of MERCs remodelling in vivo. Using the pHX6743 strain, which expresses the ER-Mit splitFAST probe in body wall muscle cells, we observed an age-dependent decrease in MERCs abundance. In parallel, we are developing a second subset of tissue-specific C. elegans strains expressing synthetic ER–mitochondria linkers of different lengths (1X, 9X and 18X), designed to experimentally modulate the distance between both organelles. These strains will enable the evaluation of how alterations in ER–mitochondria spacing influence cellular homeostasis, aging and neurodegeneration. Overall, we present a novel collection of C. elegans models that provides versatile in vivo tools to study both MERCs remodelling and the functional consequences of modifying ER–mitochondria distance in physiological and pathological contexts.

Graphical Abstract / Poster:



17. Optogenetic Control of Bacterial CsgA Amyloid Aggregation in Caenorhabditis elegans Neurons as a Model to Study its Potential Neurotoxic Effects

Authors: Lucía Pérez-García (1); Lucía Rodríguez-Heine (1); Gabriela Vázquez-Cartagena (1); Laura Molina-García (1)

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Abstract:

Several bacteria from the human gut microbiota produce functional amyloids, which share structural and biophysical properties with human amyloids involved in neurodegenerative diseases [1]. These bacterial amyloids, such as curli (CsgA) from Escherichia coli, can reach neurons where they promote α-synuclein aggregation in a Caenorhabditis elegans Parkinson's disease model [2,3]. Furthermore, curli has been shown to exacerbate α-synuclein-induced motor and intestinal dysfunction in mouse models [4]. However, the neurotoxic effects of CsgA alone once it reaches the brain remain unknown. To address this question, we generated a transgenic C. elegans model expressing a csga::cry-mcherry under the pan-neuronal promoter rab-3, allowing CRY-dependent light-induced oligomerization of CsgA in neurons. The CRY domain is a modified version of the light-sensitive CRY2 protein from Arabidopsis thaliana which oligomerizes upon blue light [5, 6], enabling temporal control of CsgA aggregation in vivo. Our lab previously validated that fusing CRY-mCherry to CsgA does not alter its amyloid assembly capabilities using a Congo red (CR) assay. CR is an amyloid-binding dye that detects β-sheet-rich structures, indicating amyloid formation. We observed red colonies in the BW25113 E. coli ΔcsgA strain producing the fusion protein with CR, indicating that CsgA-CRY-mCherry undergoes light-dependent aggregation. Next, we confirmed neuronal expression of the construct in C. elegans by fluorescence microscopy. To evaluate the physiological effects of neuronal light-dependent aggregation of CsgA in our C. elegans model, we performed survival assays under light and dark conditions. Preliminary results suggest internal hatching in CsgA-CRY-mCherry animals exposed to light. Internal hatching, in which eggs are retained and hatch within the parental body, can lead to early death and reflects egg-laying defects and possible neuromuscular dysfunctions. These findings could indicate neurotoxicity, particularly affecting neurons controlling egg-laying, which we are currently investigating.

18. In vivo BiFC-based visualization of CsgA/α-syn interactions linking microbiota to neurodegeneration in C. elegans

Authors: Paula Mancebo Gamella (1); Susana Quesada Gutiérrez (1); Laura Molina García (1)

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1. Centro Nacional de Biotecnología (CNB-CSIC)

Abstract:

Neurodegenerative diseases are associated with amyloid protein accumulation, such as α-synuclein in Parkinson´s disease. Amyloid aggregation follows the seeding-nucleation polymerization model, in which oligomers act as nuclei inducing the aggregation of additional similar or different amyloid proteins, causing cytotoxicity. The human microbiome produces functional bacterial amyloid proteins (FBAPs) such as Curli in Eschericia coli. Previous studies observed that FBAPs increase host amyloid aggregates [1-3]. Co-localization studies [4] suggest cross-seeding between both amyloids is the mechanism implicated in human aggregation increase, but the protein interaction between them is not demonstrated yet. To study interspecies amyloid protein-protein interactions and their specificity, we used a Biomolecular Fluorescence Complementation (BiFC) system in Caenorhabditis elegans [5] due to its transparency, well-characterized nervous system, and ease of microbiota manipulation. BiFC allows to visualize interaction between proteins by the reconstitution of the fluorescent protein (Venus) whose N-ter and C-ter fragments are respectively linked to the studied proteins. We generated transgenic C. elegans (MOP-1) using a pan-neuronal rab-3 promotor to express α-synuclein linked to C-ter fragment of the Venus split reporter in neurons. MOP-1 worms were fed with E.coli strain producing CsgA fused to N-ter Venus, and we examined reconstituted Venus's fluorescence in neurons using confocal microscopy. We observed signal at 2 and 8 days of adulthood in few neurons, suggesting anatomic specificity of this interaction that we are currently characterizing. Furthermore, we also tested negative controls (WT worms fed with and without CsgA producing bacteria and MOP-1 fed with a non-producing CsgA E. coli) without observing Venus's fluorescence in any of them. These results demonstrate cross-seeding of gut bacterial and neuronal host amyloids occur in vivo. We are now using our BiFC system to characterize the specificity of a wide variety of host-bacteria amyloid interactions to reveal novel links to disease.

19. Conserved gene regulatory networks driving hierarchical cellular diversification

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Abstract:

During development, a single genome is interpreted in a context-dependent manner to generate cell-type specific transcriptomes. Single-cell technologies have revealed that this diversification is hierarchically organized: broad cell classes are progressively subdivided into finer types and subtypes, generating the full spectrum of cellular identities. However, the mechanisms that establish, maintain, and evolve these hierarchies remain largely unknown. Here, we address this question using the Caenorhabditis elegans nervous system, one of the best-defined systems with the highest cellular diversity resolved at single-cell resolution. We uncover an evolutionarily conserved role for the T-box transcription factor TBX-2 as a subtype identity selector, acting primarily through direct repression of hierarchically proximal alternative fates. We find that TBX-2 expression is highly restricted to specific neuronal subtypes, including the lateral IL2 neurons, posterior dopaminergic neurons, and subsets of I and M pharyngeal neurons. Through gain- and loss-of-function approaches, we show that TBX-2 is both necessary and sufficient to establish and maintain lateral IL2 subtype identity. Mechanistically, TBX-2 acts primarily by directly repressing alternative, dorsoventral-specific gene programs, thereby preventing a switch toward other IL2 subtype fates. Preliminary evidence suggests a similar role in shaping dopaminergic subtype diversity. Remarkably, this mechanism appears conserved in mammals. While most vertebrates possess a single class of mechanosensory hair cells in the inner ear, mammals have evolved distinct hair cell subtypes. Notably, mouse inner hair cells specifically express TBX2, where it represses expression of outer hair cell genes1,2, likely mirroring the acquisition of TBX-2 expression in C. elegans lateral IL2 neurons. Moreover, additional transcription factors involved in inner versus outer hair cell specification are orthologous to components of the gene regulatory network that controls lateral versus dorsoventral IL2 diversification in C. elegans. Together, our findings establish TBX2/TBX-2 as a conserved transcriptional repressor that acts as a subtype selector within hierarchical differentiation programs. More broadly, our work suggests that similar gene regulatory network architectures may have been repeatedly co-opted during evolution to achieve fine-grained cellular diversification.

20. Phospholipase C–mediated calcium signalling regulates proteostasis in C. elegans

Authors: Cristina Trujillo del Río (1); Julia Tortajada Pérez (1); Mar Collado Pérez (1); Howard Baylis (2); Andrea Carranza (1); Rafael Vázquez Manrique (1,3)

Affiliations:

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Abstract:

Proteostasis is vital for maintaining cellular function, and its collapse is a hallmark of neurodegenerative diseases. In polyglutamine (polyQ) disorders, pathogenic CAG repeats expansions generate mutant proteins that misfold and aggregate, producing progressive neuronal decline. In this study, we examined the role of phospholipase C (PLC)–inositol 1,4,5-trisphosphate (IP3) signalling in polyQ aggregation toxicity, using the power of genetics, in C. elegans. Disruption of individual PLC isoforms via structural loss-of-function alleles, increases polyQ inclusions bodies in body wall muscles and impair motor function. This work shows that all PLC isoforms contribute to proteostasis maintenance in body wall muscles through both cell-autonomous and non-autonomous mechanisms, as not all are expressed within this tissue. Then, we choose the IP3 orthologue of PLCε, plc-1, to further dissect the IP3/calcium signalling pathway. Ablation of plc-1 severely affects aggregation and impairs neuronal function. Mechanistically, inducible expression of an IP3-binding "sponge" reproduced the effects of PLC-1 ablation, whereas the only orthologue of the IP3 receptor in C. elegans, itr-1, shows to be downstream of this effect. Furthermore, plc-1 loss enhanced α-synuclein aggregation, suggesting effects in general proteostasis that extends beyond polyQ models. Our results suggest that PLC modulates proteostasis via both cell-autonomous and non- autonomous mechanisms in C. elegans. Notably, we pinpoint PLC-1 as a pivotal and evolutionarily conserved nodal regulator within this proteostatic network.

21. From yeasts to worms: exploring the conservation of checkpoint-regulated meiotic chromosome trajectory motifs

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Abstract:

Meiotic chromosome movements promote homologue pairing and recombination, but whether their trajectory patterns encode DNA repair status across species remains unknown. In Schizosaccharomyces pombe, we identified Motif C, a slow, checkpoint-regulated trajectory perturbation induced by persistent meiotic double-strand breaks. Motif C requires Spo11-dependent damage, the ATR/Rad3 checkpoint pathway, and an actin-dependent cytoskeletal programme, and is associated with successful meiotic progression under repair-stress conditions. We now aim to determine whether a Motif C-like response is conserved in Caenorhabditis elegans. In this organism, meiotic chromosome movements are driven by dynein-dependent pairing-centre patches at the nuclear envelope and regulated through CHK-2/PLK-dependent phosphorylation of SUN-1, providing a mechanistically distinct but conceptually comparable checkpoint–LINC–cytoskeleton axis. Using live imaging, chromosome/pairing-centre tracking, and machine-learning-based trajectory decomposition, we will search for recurrent trajectory motifs enriched in conditions of persistent meiotic DNA damage. Candidate Motif C-like signatures will be defined by altered directionality, persistence, autocorrelation, or movement intermittency, and tested for dependence on checkpoint signalling and association with synapsis, repair progression, and meiotic outcome. This project will establish whether checkpoint-dependent modulation of chromosome trajectories represents a conserved meiotic repair strategy or a lineage-specific innovation of yeast. More broadly, it will test the hypothesis that meiotic chromosome movement is not merely mechanical, but also informational: a dynamic readout and effector of genome surveillance.

22. Toxicity of the Bacillus thuringiensis-based bioinsecticides to the non-target organism Caenorhabditis elegans

Authors: Carlos Muñoz Jiménez (1); Yolanda Bel (1); Baltasar Escriche (1)

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Abstract:

The increasing incorporation of bioinsecticides based on Bacillus thuringiensis (Bt) in agriculture highlights the need for ecotoxicological evaluation of their potential toxic effects on non-target soil organisms. In this study, the nematode Caenorhabditis elegans was employed as an in vivo model, as certain Bt toxins have been reported to exhibit toxicity against this organism. The toxicity of the commercial product Delfin and two novel formulations, BLB1 and LIP, was evaluated. These formulations are characterised by the expression of Cry1A and Cry2A δ-endotoxins, which have been demonstrated to be effective against leaf-eating moth caterpillars. The novel formulations were developed within the framework of the SAFWA project, part of the European PRIMA program. Synchronised young adult nematodes were exposed to a concentration of 0.75 mg/mL of Delfin, BLB1 or LIP, corresponding to the recommended field application of Delfin and used as the reference concentration for all treatments. Bioassays were conducted for a total duration of 72 hours. Mortality was monitored every 24 hours. Nematodes exhibiting no movement were gently stimulated with a platinum wire pick, and those failing to respond were scored as dead. S medium was used as the negative control, while Cry5Ba protoxin, known to be toxic to C. elegans, at an equivalent quantity of protein was employed as the positive control. No significant differences in nematode mortality were detected between the three Bt-products tested and the negative control. In contrast, all treatments showed significantly lower mortality than the Cry5Ba positive control. Overall, these results indicate that the formulated Bt-based products Delfin, BLB1 and LIP do not present detectable toxic effects on the non-target soil nematode C. elegans at standard field application rates, supporting their environmental safety with respect to beneficial soil nematodes. Nevertheless, the mechanisms by which nematicidal Cry proteins interact with C. elegans are still not fully understood. Further research is required to identify the key determinants responsible for the differences in toxicity among Cry proteins and to better define their mode of action.

23. Establishing complex microbial communities in C. elegans to dissect the role of microbiota in neurodegeneration

Authors: Susana Quesada-Gutiérrez (1); Paula Mancebo-Gamella (1); Laura Molina-García (1)

Affiliations:

1. National Center for Biotechnology (CNB-CSIC). Dpt. of MIcrobial Biotechnology. Madrid. Spain.

Abstract:

Differences in the gut microbiota have been observed between healthy individuals and patients with neurodegenerative diseases (ND), including altered microbial community structure and changes in the relative abundance of specific bacterial taxa. ND involve the progressive loss of neuronal structure and function and are associated with the accumulation of amyloid proteins. Alterations in gut microbiota could increase the severity of ND by promoting amyloid aggregation1,2. Bacterial amyloids (BA) produced by the gut microbiota may trigger human amyloids (HA) aggregation through cross-seeding3 . Supporting this, some BAs enhance HA aggregation and co-localize with them in animal models suggesting BA-HA interaction3,4. However, this phenomenon has only been studied in germ-free animals fed with mono-culture bacterial species, remaining unclear if and how these interactions occur in a complex microbial community4 . This study aims to establish complex microbial communities to investigate whether and how these amyloid interactions change in this context. To address this, we optimized a protocol to establish and recover a complex microbiota in the bacterivorous nematode Caenorhabditis elegans. This community consist of 12 bacterial strains representing its core microbiota called CeMbio5 . We successfully cultured all strains individually and as a mixed inoculum, without affecting worm development as previously described. Bacteria were recovered from the worm microbiota through lysis and validated by 16S rRNA sequencing. Once established, we will combine it with a genetic tool developed in our laboratory based on BiFC system which allows visualization of BA-HA interactions in vivo by confocal microscopy. Neuronal BiFC patterns will be compared between worms fed with a single amyloid-producing bacteria and those fed also with CeMbio inoculum. This system will dissect BA-HA interactions in complex microbiota communities, offering a powerful platform to discover links to microbiome-dependent ND progression.

24. Nuclear Hormone Receptor-1 regulation can modulate proteostasis in C. elegans

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Abstract:

There are at least 9 neurodegenerative diseases caused by CAG repeats that encode polyglutamine, including Huntington's disease, Louisian Palerubrodental Atrophy, and six autosomal forms of spinocerebellar ataxia[1]. Proteins with abnormal polyglutamine expansion will undergo structural transitions that ultimately form inclusion bodies in neurons. The loss of the natural conformation leads to the acquisition of toxic function[2], which will ultimately produce neurodegeneration. In healthy cells, this aggregation is prevented by natural protein homeostasis mechanisms that ensure proteins are properly expressed, folded and localized, and the misfolded ones are removed. Numerous pathways and molecules are involved in maintaining a correct proteostasis, but despite extensive efforts, there is not a complete understanding of all the molecular components yet. There is growing evidence that alterations in lipid metabolism are strongly related to disruptions in proteostasis[3]. Using Caenorhabditis elegans models expressing pan-neuronal 40Q::YFP, it has been shown that the loss of function of Nuclear Hormone Receptor 1 (NHR-1), encoded by nhr-1 produces a significant decrease in polyglutamine aggregation. This nuclear receptor also controls the expression of some fat metabolism enzymes [4] in particular tissues, which in turn are essential for maintaining protein homeostasis. Mutations may cause tissue-specific effects on gene expression, which are difficult to assess by bulk whole-animal transcriptomics. For this reason, a tissue-specific RNA polymerase DamID [5] (RAPID) was performed to identify differentially expressed genes in neurons and intestine and muscle in wild-type and nhr-1(vlt16) mutants. A large number of genes were detected as differentially expressed in the three tissues. Among them, genes involved in the protein folding response, elimination processes such as autophagy, and fat metabolism were identified. The identification of downstream modified genes of nhr-1, involved in the modulation of protein aggregation, will enable us to learn more about the mechanisms behind the development of disease and, most importantly, suggests the possibility of finding pharmaceutical targets for the development of new therapies.

25. Role of the Scaffold Protein LET-413 in Aging and Frailty through Modulation of RAS–MAPK Signaling in C. elegans

Authors: Cristina Ortiz-Gutiérrez (1); Ana Belén Cámara (1); Natasha Zarich (1); Berta Anta-Félez (1); María Pilar de Lucas (1); José María Rojas-Cabañeros (1)

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Abstract:

Aging is a complex and natural process characterized by the progressive decline of biological functions over time, leading to reduced ability to maintain homeostasis, increased susceptibility to diseases, and a higher risk of death, influenced by a combination of multiple factors. It involves changes at the molecular, cellular, tissular and organismal levels. At the molecular level, aging is associated with tissue dysfunction, stem cell exhaustion, cellular senescence, and reduced physiological resilience. To investigate these processes, animal models are essential tools for understanding underlying mechanisms and developing strategies to improve lifespan and healthspan. Many aging-related processes are conserved across species, making animal models highly valuable for human aging research. Within this context, Caenorhabditis elegans is one of the most widely used model organisms in aging research, as it combines biological relevance with experimental simplicity. RAS-MAPK signaling is an evolutionarily conserved pathway that plays a crucial role in regulating various cellular processes associated with aging, including nutrient sensing, mitochondrial function, and intercellular communication. However, the specific contribution of scaffold proteins that regulate this pathway remains poorly understood. Scaffold proteins organize and assemble signaling components into functional complexes, thereby ensuring the efficiency and specificity of cellular signaling pathways. LET-413 is the ortholog of the human scaffold protein Scribble and acts as a regulator of cell polarity and junctions, being required for proper epithelial tissue organization and development in C. elegans. It helps establish and maintain the distinction between apical and basolateral domains of cells, a process that is known to deteriorate during aging. On the other hand, although direct evidence is lacking in C. elegans, studies in other systems have shown that the Scribble protein can modulate MAPK/ERK signaling. To analyze LET-413 role in aging and frailty in C. elegans, we generated let-413 RNAi feeding bacteria to reduce its expression levels. We have observed that let-413 knockdown shortens nematodes lifespan and combination with soc-2 RNAi does not further result in an additional reduction. These findings suggest that LET-413 and SOC-2 may function within the same pathway, supporting the possibility that the Scribble–SHOC2 interaction in RAS–MAPK signaling is conserved in C. elegans. Ongoing experiments aim to further characterize the aging phenotype associated with LET-413 and its potential relationship with MAPK signaling.

26. Protective Effects of Lavandula latifolia Essential Oil in HaCaT Keratinocytes and Caenorhabditis elegans

Authors: Pilar Cebollada (1); Adrián Millán-Laleona (1,2); Javier Cano-Lou (1); Víctor López (1,2)

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Abstract:

Lavandula latifolia Medik., also known as spike lavender, is a Mediterranean aromatic plant traditionally used in perfumery and cosmetics because of its characteristic fragrance. It is also valued in phytotherapy because of its reported bioactive properties. This study aimed to evaluate the protective potential of L. latifolia essential oil using HaCaT keratinocytes as an in vitro model and Caenorhabditis elegans as an in vivo model. Cytotoxicity in HaCaT cells was assessed using the MTT assay, and a scratch wound-healing assay was performed. In parallel, the toxicity of the essential oil was evaluated in C. elegans. The protective effect in this model was assessed after UV exposure followed by movement analysis or Hoechst staining. Different experimental conditions were studied to distinguish between protection associated with the presence of the essential oil during irradiation and protection derived from previous exposure of the nematodes to the essential oil. In addition, the inhibitory activity of L. latifolia essential oil against enzymes involved in the endocannabinoid system and inflammatory pathways was evaluated. The essential oil composition was also determined using gas chromatography–mass spectrometry (GC-MS). GC-MS analysis revealed linalool (32.87%), 1,8-cineole (31.26%) and camphor (12.09%) as the major compounds. The essential oil showed no toxicity in either HaCaT cells or C. elegans within the tested concentration range. Moreover, L. latifolia essential oil showed significant differences compared with the control in the scratch wound-healing assay, promoting faster closure of the scratched area. In C. elegans, treatment with the essential oil at 31.25 µg/mL reduced fluorescence intensity after UV exposure and Hoechst staining, suggesting reduced cuticle permeability compared with the control. Furthermore, the essential oil inhibited fatty acid amide hydrolase (FAAH) and 5-lipoxygenase (5-LOX) activities, although its effect on monoacylglycerol lipase (MAGL) was less pronounced. Overall, these results suggest that L. latifolia essential oil may exert protective effects in models based on keratinocytes and C. elegans, supporting its potential bioactivity in cosmetic contexts. Its inhibitory effects on fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) and 5-lipoxygenase (5-LOX) also suggest a possible modulation of endocannabinoid pathways related to pain and eicosanoid pathways involved in inflammatory responses.

27. Using C. elegans to Study Conserved Ciliome Gene Expression Mechanisms

Authors: Laura Chirivella (1); María Luisa Franco (1); Marçal Vilar (1); Nuria Flames (1)

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Abstract:

Cilia, either motile or non-motile, are complex evolutionarily conserved eukaryotic structures composed of hundreds of proteins required for their assembly, structure and function that are collectively known as the ciliome. Ciliome gene mutations underlie a group of pleiotropic genetic diseases known as ciliopathies. Proper cilium function requires the tight coregulation of ciliome gene transcription, which is only fragmentarily understood. RFX transcription factors (TF) have an evolutionarily conserved role in the direct activation of ciliome genes both in motile and non-motile cilia cell-types. In vertebrates, FoxJ1 and FoxN4 Forkhead (FKH) TFs work with RFX in the direct activation of ciliome genes, exclusively in motile cilia cell-types. In a previous work, we described FKH-8, a FKH TF, as a direct regulator of ciliome genes in sensory ciliated neurons in Caenorhabditis elegans. FKH-8 and DAF-19 (C. elegans RFX) physically interact and synergistically regulate ciliome gene expression. C. elegans FKH-8 function can be replaced by mouse FOXJ1 and FOXN4 but not by other members of mouse FKH subfamilies. These data support a model of FKH and RFX TFs working together in the direct regulation of ciliome genes both in sensory and motile ciliary cell types, suggesting an ancestral role for both TFs in ciliary function. Nevertheless, the molecular mechanisms of RFX and FKH cooperativity is still unknown and mammalian FKH TFs controlling ciliome expression in sensory ciliated cells have not been described. Here we propose to use C. elegans and in vitro cell culture to explore the structural basis of DAF-19 and FKH-8 interactions and synergistic functions. We are currently performing in vitro experiments with different truncated forms of DAF-19 and FKH-8 to study the specific domains implicated in the interaction. In parallel, we are carrying out in vivo rescue experiments with different mammalian FKH members to better understand what are the common features of FKH TFs that act as ciliogenic factors. We aim to apply this strategy to identify FKH TF candidates to participate in ciliome gene expression in mammalian sensory ciliated cell types. Our results could also help us better understand the genetic bases of orphan ciliopathies.

28. Manipulation and interrogation of dopaminergic circuits using photopharmacology

Authors: Susana Colinas-Fischer (1); Daria Roman (1); Brugjilda Kami (2); Sara Redondi (2); Alice Agnelli (2); Pau Gorostiza (1); Carlo Matera (2); Michael Krieg (3); Montserrat Porta-de-la-Riva (3); Galyna Maleeva (1)

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Abstract:

Dopamine is a neuromodulator involved in many key functions of the nervous system, including learning, motivation, reward, and movement. Furthermore, disruption of dopaminergic systems is central to numerous disorders, including addiction, depression, Parkinson's Disease and schizophrenia. While dopamine receptor (DAR) agonists have proven to be valuable therapeutic agents for alleviating disease symptoms, their broad expression across the nervous system results in significant off-target side effects. Furthermore, although the differential expression of DAR subtypes (e.g., D1- and D2-like receptors) contributes to specificity in endogenous dopaminergic modulation of neural circuits, a precise understanding of how dopamine acts extrasynaptically to influence such a broad range of circuits and behaviours remains lacking. Thus, there is a strong therapeutic and research need for tools that allow manipulation of dopaminergic circuits with high temporal and spatial precision. Photopharmacology offers a great tool to address these questions by allowing light-based modulation of dopaminergic transmission. A photoswitchable dopamine agonist is only active when exposed to light of the appropriate wavelength. Thus, photopharmacology provides a unique advantage over optogenetics in that it allows manipulating precisely the activation of specific endogenous receptors, rather than activating the neuron through heterologous expression of light-activated channels. This allows for addressing of novel and interesting questions, such as: 1) isolating the contribution that activation of, for example, D1-like dopamine receptors, has on a given neuron or circuit; and 2) proof-of-principle studies to develop therapeutic agents, given that genetic manipulation is not required. In this project we set out to 1) develop and validate photoswitchable compounds that can selectively activate specific DAR subtypes, 2) test these compounds in Caenorhabditis elegans, allowing both in-vivo validation of the compounds and functional mapping of dopaminergic circuits and 3) conduct a proof-of-principle study to test the potential of photoswitchble DAR agonists in treating Parkinson's Disease. Currently we have validated photoswitchable dopamine receptor agonists in C. elegans using behavioural tests and generated C. elegans strains that will allow simultaneous recording of neuronal activation and identification of D1-receptor positive neurons.

29. MTP302: A Novel Multitarget-Directed Ligand Candidate for Huntington's Disease

Authors: Mireia Toledano-Pinedo (1); Isabel Iriepa (2,3); Tina Spalholtz (4); Marketa Benkova (5); Lenka Pulkrabkova (5); Jiri Janousek (5); Martina Hrabinova (6); Rudolf Andrýs (7); Hildegard Colino-Lage (8,9); Mercè Pallás (8,9,10); Christian Griñán-Ferré (8,9,10); José Marco-Contelles (1); Ondrej Soukup (5,6); Abdelouahid Samadi (11)

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9. Institut de Neurociències, Universitat de Barcelona (NeuroUB), Barcelona, Spain
10. Spanish Biomedical Research Center in Neurodegenerative Diseases (CIBERNED)-Instituto de Salud Carlos III, Madrid, Spain
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Abstract:

Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) are neurodegenerative disorders characterized by the loss of neuronal populations and the progressive decline of cognitive and/or motor functions. Despite their distinct clinical manifestations and genetic backgrounds, these disorders share several common pathological features, including protein misfolding and aggregation, mitochondrial dysfunction, oxidative stress, impaired proteostasis, neuroinflammation and synaptic degeneration. Given the multifactorial nature of these diseases, they may require a polypharmacological approach with multitarget-directed ligands (MTDLs) rather than drug combinations to avoid metabolic interactions and improve patient compliance. In this study, we investigated MTP302 (2-(3-(4-(Prop-2-yn-1-yl)piperazin-1-yl)propyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole) as a new multitarget ligand for treating neurodegenerative disorders. Our results suggested that MTP302 has a high potential to cross the blood–brain barrier (BBB) and reach the central nervous system. We have confirmed the compound's safety profile using cytotoxicity assays in cultured cells. Additionally, enzymatic inhibition studies revealed that MTP302 preferentially inhibited cholinesterases over monoamine oxidases. This selectivity may be advantageous in the development of MTDLs aimed at ameliorating cholinergic deficits associated with neurodegenerative disorders. In vivo studies using transgenic Caenorhabditis elegans strains showed that MTP302 decreased polyglutamine aggregates and improved locomotor deficits in the HD model strain EAK103. However, MTP302 did not affect the locomotor impairments in the Aβ-expressing strain CL2006 or the PD disease model strain BR3579. In summary, MTP302 is an innovative therapeutic candidate for Huntington's disease, validating the potential of the MTDL strategy in the medicinal chemistry of neurodegenerative diseases.

30. Neuronal Autophagy role in Behavioral Adaptation to Nutrient Availability – insights from egli-1 in C. elegans

Authors: Jorge H. Fernandes (1,2); Jorge Diogo Da Silva (1,2); Marta D. Costa (1,2); Stéphanie Oliveira (1,2); Joana Pereira-Sousa (1,2); Andreia Teixeira-Castro (1,2); Patrícia Maciel (1,2)

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Abstract:

Sensing and responding to food is essential for animal survival, and many of the underlying regulatory mechanisms are evolutionarily conserved. Dissecting the cellular and neuronal processes that control feeding behavior is therefore crucial for understanding metabolic disorders. However, the complexity of mammalian systems poses major experimental challenges. The nematode Caenorhabditis elegans offers a powerful alternative, combining a fully mapped nervous system with diverse, quantifiable food-related behaviors. We previously identified the C. elegans gene egli-1 as a key regulator of food-dependent behavioral adaptation. Loss-of-function mutants fail to adjust to food deprivation and instead behave as if persistently well-fed. Notably, the mammalian orthologue of egli-1, TMEM41B, has been implicated in autophagy, a conserved intracellular nutrient-sensing pathway. Here, we provide in vivo evidence that egli-1 regulates autophagy in C. elegans, using fluorescent reporters and western blot analyses that reveal aberrant autophagic responses in egli-1 mutants. Behavioral analyses further demonstrate that neuronal-specific knockdown of egli-1 disrupts multiple food-related behaviors, including, food chemotaxis (reduced ability to navigate towards food sources); enhanced slowing response (inability to modulate locomotion speed upon food encounter) and egg-laying regulation (disrupted reproductive strategies in response to nutritional status). Importantly, targeted activation of neuronal autophagy restores wild-type behavioral responses. These findings support a model in which neuronal autophagy acts as a molecular switch enabling behavioral flexibility in response to nutritional state. By defining the subcellular mechanisms linking autophagy to sensorimotor integration, this work highlights conserved pathways with potential relevance to human metabolic disorders.

31. Age-Dependent Immune Heterogeneity and Transmission Dynamics Shape Orsay Virus Infection in Caenorhabditis elegans

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Abstract:

Host age is a critical determinant of immune competence and physiological resilience, serving as a powerful selective force that drives the evolution of viral exploitation strategies. In Caenorhabditis elegans, distinct life stages introduce heterogeneity into this selective landscape, a dynamic that is particularly relevant when viral transmission is assortative within specific cohorts. Our aim is to explore how host development and advanced aging influence viral fitness and virulence. Using C. elegans and its natural intracellular pathogen, the Orsay virus (OrV), we characterized viral load kinetics during the first three days post-inoculation starting at distinct life stages: larval (L1 and L4), reproductive, and post-reproductive nematodes. To quantify the physiological cost of infection, we assessed traits such as intestinal permeability, area of infection, and host movement. In addition, stage-to-stage transmission assays were performed. Finally, we leveraged transcriptomic profiling to disentangle the age-dependent genetic pathways targeted during viral infection. Our findings demonstrate that specific host age classes impose restrictive barriers to viral infection, establishing this model as a valuable experimental system to study virus ecology and evolution across the lifespan of an organism.

32. Antidiabetic and anti-obesity potential of Mangifera indica leaf extract and its main compound mangiferin in two different models: Caenorhabditis elegans and HepG2 cells

Authors: Sonia Nuñez Alonso (1,2); Henar Rojas-Marquez (1); Pilar Braulio Lasierra (1); Maria Soria Hernando (1); Lucía Ventura-Serrano (1); Carlota Gómez-Rincón (1,2)

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Abstract:

Obesity and type 2 diabetes are among the most prevalent metabolic disorders worldwide and are closely associated with oxidative stress, chronic inflammation, insulin resistance, and dysregulated glucose and lipid metabolism. The limitations of current therapeutic approaches have increased interest in the identification of natural bioactive compounds with potential metabolic benefits. Among these, compounds derived from Mangifera indica (mango), particularly leaf extracts rich in mangiferin, have attracted considerable attention due to their antioxidant, anti-inflammatory, antidiabetic, and lipid metabolism-regulating properties. The present work evaluated the effects of M. indica leaf extract and mangiferin using complementary experimental models. In the nematode Caenorhabditis elegans, obesity-like metabolic alterations were induced through glucose supplementation, and the effects of treatment were assessed by analysing lipid accumulation, feeding behaviour, and markers of cellular stress and aging. Additionally, the antidiabetic potential of M. indica leaf extract was investigated in HepG2 cells through the evaluation of glucose metabolism-related pathways and key molecular targets involved in glucose homeostasis. The results demonstrated that glucose exposure induced an obesogenic phenotype characterized by increased lipid accumulation and metabolic dysfunction. Treatment with both M. indica leaf extract and mangiferin reduced lipid deposits, decreased lipid droplet accumulation, improved metabolic parameters, and attenuated cellular stress, as evidenced by lower lipofuscin accumulation and reduced feeding frequency. Furthermore, M. indica extract exhibited significant inhibitory activity against several diabetes-related targets and modulated the expression of genes involved in hepatic glucose transport and gluconeogenesis. Preliminary findings also suggested the activation of AMPK-related pathways, supporting a role in the regulation of energy homeostasis. Overall, these findings indicate that bioactive compounds from M. indica, particularly mangiferin, may exert beneficial effects against obesity- and diabetes-associated metabolic alterations through coordinated actions on lipid accumulation, glucose metabolism, and cellular energy regulation. These results support the potential of M. indica as a promising source of natural compounds for the prevention and management of metabolic disorders.

33. Consensus Pharmacophore-Based Virtual Screening and Phenotypic Validation Identify Novel Brain-Penetrant Inhibitors for Neurodegenerative Disorders

Authors: Marta Ribalta-Vilella (1); B. Raziel Cedillo-González (2); Belén Pérez (3); Antón Leandro Martínez Rodríguez (4); Jose Brea (4); Mercè Pallàs (1,5); José L. Medina-Franco (2); Christian Griñán-Ferré (1,5); Aina Bellver-Sanchis (1)

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Abstract:

Histone lysine methyltransferases serve as crucial epigenetic regulators of gene expression and are promising targets for treating central nervous system (CNS) disorders. By altering histone methylation, these enzymes affect chromatin structure, neuronal plasticity, and cellular responses linked to neurodegeneration and cognitive issues. Although progress has been made in developing methyltransferase inhibitors, challenges such as limited scaffold variety and poor brain penetration hinder clinical application. To address this, we used a consensus pharmacophore-based virtual screening approach to find new brain-penetrant inhibitors that could be useful for CNS conditions. We generated pharmacophore models from seventeen protein–ligand crystal structures, combined them into a consensus model, and optimized into thirty sub-models for large-scale screening. This process, involving multiple commercial databases, physicochemical filtering, blood–brain barrier permeability prediction, and compound prioritization, narrowed an initial pool of 57,891 molecules down to 13,810 candidates. Eleven commercially available compounds with diverse chemical scaffolds were selected for experimental testing. Biochemical assays revealed three active compounds—CAM3, CAM5, and CAM12—with micromolar inhibitory activity. Membrane permeability assays confirmed favorable brain penetration profiles for the most promising candidates. Additionally, treatment of a transgenic C. elegans model of protein aggregation improved disease-related phenotypes, supported by biochemical evidence of target engagement. Molecular docking provided structural insights into how these compounds interact with the enzyme's catalytic domain. Overall, these results highlight the effectiveness of consensus pharmacophore-based virtual screening in discovering novel methyltransferase inhibitors and identify promising lead compounds for developing epigenetic therapies for CNS disorders.

34. Context-Dependent Activity of LAG-1/RBPJ

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Abstract:

Transcription factors (TFs) are known to be pleiotropic, regulating distinct cell type-specific gene expression profiles across diverse cell types and tissues. The regulatory outcome of TF activity whether acting as a transcriptional activator or a repressor is frequently governed by cellular context. Throughout evolution, this functional plasticity has been achieved primarily through the diversification of TF families, allowing paralogs to interact with distinct chromatin remodeling complexes while conserving sequence-specific DNA-binding affinities. This project investigates the context-dependent functionality of the CSD-family transcription factor LAG-1/RBPJ across distinct tissue types in Caenorhabditis elegans. As the primary downstream effector of the highly conserved Notch signaling pathway, LAG-1 dynamically switches between transcriptionally repressive and activating states to control cell-fate decisions. Interestingly, emerging evidence indicates that LAG-1/RBPJ also exerts regulatory functions independently of canonical Notch signaling. This study aims to elucidate the molecular mechanisms by which these divergent, context-specific features are encoded within a single genetic locus to drive diverse developmental programs.

35. EXPLORING THE SYNERGISTIC EFFECT OF A DUAL G9a/HDAC6 INHIBITOR IN A CAENORHABDITIS ELEGANS MODEL OF AUTISM SPECTRUM DISORDER

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Abstract:

Autism Spectrum Disorder (ASD) is a complex and severe neurodevelopmental condition that currently affects more than 1% of children worldwide. The causes of ASD are multifactorial, involving genetic and epigenetic alterations, environmental influences, and molecular dysregulation. Several molecular pathways are disrupted in ASD, including those related to chromatin remodeling, RNA transcription and splicing, and gene expression. Notably, histone-modifying enzymes, such as G9a and HDAC isoforms, are elevated in individuals with ASD, suggesting that alterations in chromatin structure may play a key role in the development of the disorder. G9a methylates histone H3 at lysine 9 (H3K9me2), thereby repressing the expression of genes involved in learning and memory formation as well as social interaction. HDAC6 is a class IIb histone deacetylase that deacetylates non-histone proteins such as α-tubulin and synaptic proteins. Overexpression of G9a and HDAC6 has been associated with cognitive and synaptic impairment, as well as oxidative and inflammatory damage, whereas their inhibition has shown neuroprotective effects. We examined the effects of dual G9a/HDAC6 inhibition and compared them with the effects of each enzyme's inhibition alone in two Caenorhabditis elegans models of ASD (DA609 and TV13570). DA609 has the npr-1(ad609) X loss-of-function mutation, which modifies the worms' social behavior by shifting their feeding from solitary to social, leading to increased aggregation. Conversely, TV13570 carries the nrx-1(wy778) V mutation, a null mutation in the neurexin gene that models NRXN1-associated ASD risk in humans. This mutation impacts both the social behavior of the worms and the presynaptic architecture of neurons. These strains were treated with UNC0642, a selective G9a/GLP inhibitor; Vorinostat, a histone deacetylase inhibitor; and compounds X and Y, our dual G9a/HDAC6 inhibitors. Our aim was to evaluate whether dual inhibition produces a synergistic effect compared with individual inhibition of each target, particularly in modulating worm motility and aggregation behaviors. Overall, this study aims to validate C. elegans as a valuable model for investigating the therapeutic potential of G9a/HDAC6 inhibition and to provide further insight into the role of these epigenetic regulators in ASD, paving the way for the development of novel therapeutic strategies.

36. HLH-30/TFEB maintains cell quiescence and promotes chromatin reorganization during L1 starvation.

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Abstract:

Cellular quiescence is a reversible non-proliferative state that preserves cellular integrity and regenerative potential during adverse environmental conditions. In Caenorhabditis elegans, hatching in the absence of food triggers L1 developmental arrest, providing a powerful in vivo model to investigate the molecular mechanisms that maintain quiescence. The transcription factors DAF-16/FOXO and HLH-30/TFEB are both known regulators of L1 starvation survival [1, 2]. These transcription factors have been functionally related [3], but their combined contribution to the L1 starvation response has not been previously tested. Here, we investigated the functional interplay between DAF-16 and HLH-30 during L1 starvation using genetic, transcriptomic, and imaging approaches. We found that both transcription factors cooperate to promote survival under nutrient deprivation and reciprocally influence each other's activation. Loss of HLH-30 reduced DAF-16 nuclear localization and activity, whereas DAF-16 deficiency enhanced HLH-30 nuclear accumulation and target gene expression, revealing a dynamic regulatory relationship between these factors during L1 arrest. Transcriptomic profiling of starved larvae demonstrated that HLH-30 exerts broad transcriptional control upon entry into L1 arrest. We found that HLH-30-regulated genes were enriched for chromosome organization and cell-cycle regulation categories, suggesting functions beyond its established roles in lysosomal biogenesis and metabolic adaptation. Consistent with these findings, analysis of chromatin architecture revealed that HLH-30 is required for the large-scale chromatin reorganization into concentric rings typically induced by fasting in intestinal cells [4]. We further uncovered a critical role for HLH-30 in maintaining cell-cycle quiescence. Starved hlh-30 mutants displayed aberrant seam-cell divisions, reduced expression of the cyclin-dependent kinase inhibitor CKI-1, and increased activation of the S-phase marker PCN-1. These phenotypes were exacerbated in daf-16;hlh-30 double mutants and were suppressed by inhibition of RNA polymerase II activity, indicating that inappropriate transcriptional activation underlies the loss of quiescence. Transcriptomic and regulatory network analyses revealed that hlh-30 mutants acquire transcriptional features of fed, developmentally progressing larvae despite the absence of nutrients. Notably, the pioneer transcription factor BLMP-1 was overactivated in HLH-30-deficient animals, leading to increased expression of the developmental regulator lin-4. The loss of BLMP-1 partially restored proper cell-cycle arrest, indicating that HLH-30 preserves quiescence in part by repressing blmp-1 expression during starvation. Together, these findings identify HLH-30/TFEB as a central regulator linking nutritional status to chromatin architecture, transcriptional regulation, and cell-cycle arrest during starvation. Our work expands the known functions of HLH-30 beyond metabolic adaptation and highlights conserved mechanisms through which TFEB-family transcription factors coordinate genome organization and cellular quiescence, processes with broad relevance to aging, stem-cell biology, and disease.

37. The role of GABA B receptor in the mechanism of action of sul-2

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Abstract:

Aging is a degenerative process that has been identified as a major risk factor for disease and death. Inhibition of the Caenorhabditis elegans steroid sulfatase, sul-2, by mutation or by treatment with STX64 raises the pool of sulfated steroid hormones, increases longevity and ameliorates the symptoms of diseases related to protein aggregation. This improvement is also observed in mouse models of Alzheimer's disease [1]. Preliminary data from our laboratory indicate that inhibition of steroid sulfatase activates the cholinergic response through the GABA B receptor (gbb-1). Loss of function of the gene gbb-1 in C. elegans, not only exhibits longevity, as previously observed [2], but also confers neuroprotection in Alzheimer's and Parkinson's disease models in C. elegans. Neither these phenotypes, nor the increased cholinergic activity are additive to steroid sulfatase inhibition, suggesting that the steroid sulfatase and the GABA B receptor are involved in the same pathway. We suggest that loss of sul-2 function or treatment with STX64 raises the pool of sulfated steroid hormones, which may inhibit the GABA B receptor directly or indirectly, which enhances cholinergic activity and improves the symptoms of neurodegenerative diseases. Our results advance the understanding of the mechanism of action of the drug STX64, which facilitate its potential therapeutic application in humans.

38. Steroid Sulfatase Inhibition Enhances Cholinergic Signaling to Promote DAF-16-Mediated Longevity and Amyloid-β Resistance.

Authors: Javier Valle-Galisteo (1,2); Cristina García-Gutiérrez (2); Juan Antonio Fernández-Cabrera (1,3); Elena Rodríguez-Sandoval (1,3); Ángel Manuel Carrión (1,3); Mª Mercedes Pérez-Jiménez (1,2); Manuel Jesús Muñoz (1,2)

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3. Universidad Pablo de Olavide, Department of Physiology, Anatomy and Cellular Biology, Seville, Spain

Abstract:

The amyloid hypothesis of Alzheimer's disease (AD) states that progressive neurodegeneration is driven by amyloid-β (Aβ) accumulation and toxicity, ultimately leading to cholinergic neuron loss [1]. Previously, we demonstrated that pharmacological inhibition or loss-of-function of steroid sulfatase (sul-2 in Caenorhabditis elegans) confers DAF-16/FOXO-dependent lifespan extension and resistance to Aβ proteotoxicity in both C. elegans and mouse models [2]. In this study, we elucidate the molecular mechanisms underlying this protective effect. We show that sul-2 inhibition promotes cholinergic neurotransmission by enhancing the expression of genes involved in acetylcholine (ACh) biosynthesis and vesicular transport, likely through the inhibition of the GABA-B receptor. By employing unc-17 C. elegans mutants, which are defective in vesicular ACh transport, we demonstrated that ACh is required for the nuclear translocation of the DAF-16/FOXO transcription factor in the intestine. Furthermore, we established that direct pharmacological activation of ACh receptors is sufficient to drive the nuclear translocation of DAF-16/FOXO in the intestine even in an unc-17 mutant background, indicating that ACh receptor activation alone is sufficient to regulate DAF-16 activity. Consistent with these findings, we also showed that ACh release is necessary for sul-2 inhibition to exert its protective effects on both lifespan extension and resistance to Aβ toxicity. These results reframe the amyloid hypothesis by suggesting an active, protective role for increased cholinergic signaling in Alzheimer's disease, rather than cholinergic depletion being merely a downstream consequence of it. If conserved in humans, cholinergic enhancement via steroid sulfatase inhibition emerges as a promising disease-modifying therapeutic strategy for age-related neurodegenerative disorders characterized by cholinergic deficits, most notably Alzheimer's disease.

39. Cholinergic activity in long-lived mutants and conditions that affect lifespan

Authors: Silvia Etayo Escanilla (1,2,4); Loreto Castro Castro (1,2,4); Javier Valle Galisteo (1,3); Cristina García Gutiérrez (1,2); María de las Mercedes Pérez Jiménez (1,2,3); Manuel J. Muñoz Ruiz (1,2,3)

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4. Equal contribution

Abstract:

Aging is the main risk factor for the development of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, which are characterized by a progressive loss of proteostasis, accumulation of toxic aggregates, and neuronal dysfunction1,2. In addition, these disorders exhibit a gradual degeneration of cholinergic neurons and a decline in acetylcholine (ACh) neurotransmission3. Previous studies have shown that inhibition of steroid hormone sulfatase (sul-2) extends lifespan and increases neuroprotection of neurodegenerative models in Caenorhabditis elegans4. Unpublished data from our laboratory show that this mutant, affected in the gene encoding the sulfatase of steroid hormones, exhibits increased cholinergic activity and that cholinergic function is required for the increase of longevity. In this context, we propose to determine whether cholinergic neurotransmission is affected d in a variety of long-lived mutants and under conditions that affect lifespan. Interestingly, all the mutants tested showed alterations in cholinergic function, suggesting that ACh may be a mechanism underlying their extended lifespan. However, these alterations are diverse while some mutants show changes in presynaptic cholinergic activity, others do in postsynaptic activity, and some in both. Overall, this study highlights that cholinergic signalling is altered in all the conditions or mutants affecting lifespan. However, its causal role is not obvious, probably because of the complex interplay of hormonal, neuronal, and environmental signals that affect aging.

40. The Linker Histone his-24 Participates in the sul-2 Steroid Sulfatase Signaling Pathway and Modulates the Response to Proteotoxicity in Caenorhabditis elegans

Authors: María Rivero Portal (1,2); Cristina García Gutiérrez (1,2); María de las Mercedes Pérez Jiménez (1,2,3); Manuel Jesús Muñoz Ruiz (1,2,3)

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Abstract:

Aging is a complex biological process that increases susceptibility to neurodegenerative disorders, including Parkinson's disease, which are associated with loss of proteostasis and the accumulation of protein aggregates. Previous studies have shown that inhibition of steroid hormone sulfatase activity extends lifespan and alleviates neurodegeneration-related phenotypes in Caenorhabditis elegans [1]. In addition, our laboratory has obtained evidence indicating that reduced sulfatase activity enhances cholinergic neurotransmission (unpublished data). To better understand the molecular mechanisms by which sul-2 regulates longevity and proteostasis, we performed an RNA-seq analysis (unpublished data). Using WormExp program (https://wormexp.zoologie.uni-kiel.de/wormexp/), which compare expression pattern with different conditions and patterns, we identified his-24 as exhibiting a gene expression profile similar to that observed in sul-2 mutants, suggesting a potential functional interaction between these genes. In this context, the present study investigates the role of his-24 as a potential mediator of the phenotypes associated with reduced sul-2 activity. We show that loss of his-24 function extends lifespan, improves locomotor performance in a C. elegans model of Parkinson's disease, increases sensitivity to aldicarb, and does not significantly alter the response to levamisole, indicating a predominantly presynaptic effect on cholinergic neurotransmission. Those phenotypes are similar to the ones observed in sul-2 mutants Furthermore, the absence of additive effects between his-24 and sul-2 supports the hypothesis that both genes act within a shared molecular pathway andsupport the existence of a common regulatory mechanism. Overall, these findings implicate his-24 as a relevant component of the sul-2 pathway and suggest that epigenetic regulation may underlie the protective effects associated with steroid sulfatase inhibition.

41. Caenorhabditis elegans as a Model to Assess the Effects of Selective I2-Imidazoline Receptor Ligands on Amyloid Aggregation Dynamics in the Nervous System

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2. Spanish Biomedical Research Center in Neurodegenerative Diseases (CIBERNED)-Instituto de Salud Carlos III, Madrid, Spain

Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-β (Aβ) aggregation, neuroinflammation, and synaptic dysfunction. Despite extensive research, no treatment is currently available that can reverse disease pathology, highlighting the need to explore alternative therapeutic strategies. In this context, the pharmacological properties of I2-imidazoline receptors (I2-IR) have gained increasing interest. These receptors are widely distributed in the central nervous system, with predominant localization in glial cells. Their activation has been associated with modulation of neuroinflammation and potential neuroprotective effects, although the underlying molecular mechanisms remain unclear. In this study, we evaluated the effects of selective I2-IR ligands (2-BFI and idazoxan) using a Caenorhabditis elegans model of amyloid aggregation. This transgenic strain allows panneuronal expression of the human Aβ1–42 peptide fused to the fluorescent reporter wrmScarlet, providing an in vivo system to visualize and quantify amyloid aggregation in the nervous system. As a control, animals express panneuronal wrmScarlet alone under the same promoter, enabling clear discrimination between peptide-driven aggregation and background fluorescence. Worms were cultured on nematode growth medium agar and exposed to 2-BFI, idazoxan, or vehicle control throughout development. Exposure continued until day 4 and day 7 of life, enabling assessment of compound effects at early and mid-adult stages. Amyloid pathology was assessed using complementary fluorescence-based approaches, including intensity quantification and fluorescence lifetime imaging microscopy (FLIM). In parallel, neuronal function was evaluated using behavioural assays such as thrashing and chemotaxis, as indicators of motor and sensory performance.

42. Biological Effects of Thinned Apple (Malus domestica Borkh.) Extracts: Toxicity, Antioxidant Activity, and Lifespan in C. elegans

Authors: Javier Cano-Lou (1); Cristina Moliner (1); Adrián Millán-Laleona (1); Carlota Gómez-Rincón (1,2); Ana Pina (2); Víctor López (1,2)

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Abstract:

Thinned apples from Malus domestica Borkh., an abundant agricultural by-product routinely removed during pruning processes, have gained increasing attention as a promising source of phenolic compounds which are bioactive compounds that through pleiotropic actions can help to prevent or treat a wide range of disorders [1]. This study aimed to characterize the phenolic composition of thinned apples from different M. domestica cultivars and to assess their toxicity, antioxidant activity, and impact on lifespan in Caenorhabditis elegans. In this study, thinned apples from the commercial cultivars Reineta and Verde Doncella, together with the autochthonous cultivar Amarilla de Octubre, were extracted with ethanol by ultrasound-assisted extraction, and their phenolic profiles were characterized by HPLC–MS/MS [1]. In addition, toxicity was evaluated in N2 (wild type) L1 and L3 worms, and antioxidant activity was assessed using juglone-induced oxidative assay using the WMicroTracker One system [2]. Lifespan was further evaluated in liquid medium using a WMicroTracker Arena with SS104 glp-4 (bn2) L3 worms [3]. The most abundant phenolic compounds quantified in the thinned apple by-products were chlorogenic acid, quercitrin and rutin. All extracts showed low toxicity in L1–L3 larvae at 100–500 µg/mL, except for Amarilla de Octubre, which showed high toxicity at 500 µg/mL in L1 worms. Furthermore, Amarilla de Octubre showed significant antioxidant potential at 25–100 µg/mL, followed by Verde Doncella and Reineta at 100–250 µg/mL compared with the control treatment in N2. Treatments with 50 and 100 µg/mL of all extracts significantly increased the mean lifespan of the worms, with the greatest effect observed for Amarilla de Octubre at 100 µg/mL. Overall, these results highlight the potential of thinned apples, particularly Amarilla de Octubre, as a sustainable source of bioactive phenolic compounds with antioxidant and health-promoting properties.

43. Targeting Machado-Joseph Disease through Small-Molecule Combination Therapy

Authors: Inês Margarida Lopes (1,2,3); Cármen Vieira (1,2); Daniela Monteiro-Fernandes (1,2); Jorge Humberto Fernandes (1,2); Lídia Nunes-Carvalho (1,2); Mariana Tavares Viana (1,2); Marta Daniela Costa (1,2); Patrícia Maciel (1,2); Andreia Teixeira-Castro (1,2); Sara Duarte-Silva (1,2)

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Abstract:

Machado–Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), is the most common dominantly inherited ataxia worldwide. It is caused by an abnormal expanded CAG repeat in the ATXN3 gene, leading to selective neuronal degeneration and progressive motor dysfunction. Despite advances in understanding disease mechanisms, effective disease-modifying therapies remain unavailable. Previous studies identified selective serotonin reuptake inhibitors (SSRIs) and the bile acid tauroursodeoxycholic acid (TUDCA) as promising therapeutic candidates in MJD models. While SSRIs reduce motor deficits and mutant ATXN3 aggregation through modulation of serotonergic signalling, TUDCA promotes neuroprotection through mechanisms involving glucocorticoid receptor signalling. Transcriptomic analyses of TUDCA-treated MJD mice also revealed alterations in genes associated with the serotonergic system, suggesting a potential crosstalk between these pathways. To investigate the relationship between serotonergic and glucocorticoid receptor-mediated pathways and to evaluate the therapeutic potential of their combined modulation, we used a transgenic Caenorhabditis elegans model of MJD. We first assessed the toxicity profile of S-citalopram and TUDCA chronic treatments, individually and in combination, across a range of concentrations. Subsequently, the effects of single and combined treatments was studied on disease-relevant phenotypes. In parallel, genetically modified C. elegans strains were used to explore the pharmacogenetic mechanisms underlying the interaction between serotonergic and glucocorticoid receptor-associated pathways. Preliminary results indicate that co-administration of S-citalopram and TUDCA is well tolerated in C. elegans, supporting further investigation of this combinatorial strategy. Ongoing studies are assessing treatment effects on motor dysfunction, protein aggregation and neuronal integrity, as well as the molecular mechanisms contributing to therapeutic response. These findings establish C. elegans as a valuable platform to investigate combinatorial therapeutic approaches in MJD and may provide new insights into the functional interaction between serotonergic and glucocorticoid receptor signalling pathways.

44. Standardized 4-hour C. elegans oxidative stress assay for screening plant infusions

Authors: Vanesa Sánchez-Martín (1); Mladen Grbušić (1); Saray Marquez Rivera (1); María Dolores del Castillo (1)

Affiliations:

1. Consejo Superior de Investigaciones Científicas (CSIC)

Abstract:

Rapid, standardized in vivo assays are needed to evaluate oxidative stress modulation by bioactive samples in Caenorhabditis elegans. Here, we developed a standardized 96-well fluorimetric assay that adapts a cell-based ROS workflow to whole worms and defines the key experimental conditions required for reproducible measurements. Using synchronized L4-arrested animals, we established chemical controls to exclude fluorescence interference, selected non-toxic working concentrations for samples and tert-butyl hydroperoxide (tBOOH), compared 4 h and 24 h readouts, defined the order of addition of probe, samples and oxidant, and normalized fluorescence to worm number. This step was important because the number of animals per well could be counted and used to improve inter-well comparability. Assay validity was further supported by the expected response of reference antioxidants, including chlorogenic acid, gallic acid and vitamin C. Under the standardized conditions, coffee leaf, cocoa bean and hemp leaf infusions tested at non-toxic, non-interfering concentrations significantly reduced tBOOH-induced oxidative damage compared with the pro-oxidant control ($p<0.05$). ABTS values were consistent with the in vivo results. Overall, this work provides a fast, cost-effective and standardized C. elegans assay for functional screening of oxidative stress modulators in complex plant matrices.

45. Host–Environment Interplay as a Determinant of Microbiota Assembly and Physiological Outcomes in C. elegans

Authors: Miguel C. Santos (1); Giorgia Ligestro (1); Mónica V. Cunha (1); Artur B. Lourenço (1)

Affiliations:

1. CE3C- Centre for Ecology, Evolution and Environmental Changes & CHANGE - Institute for Global Change and Sustainability, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

Abstract:

The assembly of the Caenorhabditis elegans gut microbiota arises from a dynamic interplay between host genetics, microbial physiology, and environmental conditions. While laboratory C. elegans are typically maintained on Escherichia coli OP50, wild nematodes feed on, and harbour in their gut, a distinct and core microbial community. Recent efforts to reconstruct this natural community have produced simplified, yet ecologically grounded, microbiota resources, including the 12-member CeMbio and the expanded CeMbio43 collections. Despite these advances, the mechanisms by which host-derived factors and abiotic environmental variation jointly shape microbiota assembly remain incompletely understood. In this project, we investigate how environmental parameters and host immune regulation influence both microbiota composition and host physiology. We first perform detailed physiological profiling of CeMbio bacterial isolates under ecologically relevant abiotic conditions, with a particular focus on pH variation. Our analyses reveal that pH exerts strong and differential effects on bacterial growth, suggesting that environmental heterogeneity, whether due to external pH or reflecting the pH conditions encountered within the worm gut, may act as a bottleneck during microbiota assembly. Building on these findings, we examine how specific CeMbio strains (wild-type N2 and pmk-1 mutant animals) grown under defined pH conditions affect C. elegans physiology, including food preference and lifespan. Young adult worms exhibit clear bacterial preferences, consistently distinguishing between more and less preferred food sources. This overall preference pattern is largely maintained across the pH conditions tested and in both genetic backgrounds. However, for certain bacterial strains, food choice is modulated by environmental pH and/or PMK-1 activity, indicating that both abiotic factors and innate immune signalling can influence the worm's feeding decisions. Interestingly, wild-type worms show a strong preference for one of the examined bacterial sources and, although this preference appears unaffected by external pH variation, the longevity of worms fed on this bacterium is differentially affected by changes in environmental pH. Altogether, this work provides a baseline for establishing an experimental and conceptual framework to understand how host–environment interactions shape natural microbiota assembly in C. elegans. By linking microbial traits, abiotic constraints, and host physiological outcomes, our study offers new insight into the ecological and mechanistic forces that govern host-associated microbial communities.

46. In vivo evaluation of filter-brewed cocoa beverages in a C. elegans glucose-induced lipid accumulation model

Authors: Vanesa Sánchez-Martín (1); Saray Marquez Rivera (1); María Dolores del Castillo (1)

Affiliations:

1. Consejo Superior de Investigaciones Científicas (CSIC)

Abstract:

Glucose-induced lipid dysregulation is relevant to obesity- and prediabetes-associated metabolic dysfunction. Here, we performed a preliminary in vivo evaluation of filter-brewed cocoa beverages prepared from ground cocoa beans used unroasted or subjected to mild roasting, with the aim of exploring cocoa as a filter-brewed beverage. Beverage preparation was standardized by particle-size selection, and the resulting samples were characterized by total phenolic content, ABTS antioxidant capacity and UV-Vis profiling. Lipid accumulation was quantified in Caenorhabditis elegans by Nile Red staining under high-glucose conditions (20 mM), using metformin as a positive control. Glucose supplementation significantly increased lipid accumulation, validating the model. Cocoa beverages reduced fat accumulation under these conditions. Physicochemical characterization supported the maintenance of relevant antioxidant-associated features after mild roasting, consistent with the comparable in vivo biological activity observed in beverages. Overall, this preliminary study supports the use of C. elegans to relate cocoa beverage processing to metabolic readouts and supports further investigation of cocoa as a filter-brewed beverage format.

47. Phytochemical Modulation of Protein Aggregation: Bridging In Vitro Assays and Caenorhabditis elegans Models of Neurodegeneration

Authors: Pedro Martínez-Rodríguez (1); Samanta Hernández-García (1); Rubén Sáez-Verdú (1); Amaya Belando-Llinás (1); M. Alejandra Guerrero-Rubio (1); Fernando Gandía-Herrero (1)

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1. Universidad de Murcia

Abstract:

Protein misfolding and aggregation are central pathological events in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. In recent years, plant-derived phytochemicals have emerged as promising modulators of these processes due to their structural diversity and bioactivity. Here, we present an integrated approach combining in vitro aggregation assays with in vivo validation in Caenorhabditis elegans models to evaluate the anti-aggregation potential of selected plant metabolites, with a particular focus on structurally related compounds. Building on our previous works demonstrating the ability of phytochemicals to interfere with oxidative stress pathways, we systematically analysed their effects on the aggregation kinetics of disease-relevant proteins, including α-synuclein and β-amyloid peptide. Spectroscopic and biochemical assays revealed significant inhibition of fibril formation and modulation of aggregate morphology, suggesting direct molecular interactions between the compounds and aggregation-prone intermediates. To assess biological relevance, the most active compounds were tested in transgenic C. elegans strains expressing human aggregation-prone proteins. Treatment resulted in reduced proteotoxicity, delayed onset of paralysis phenotypes, and improved organismal viability, supporting the translational potential of these molecules. Overall, our results highlight the complementarity between in vitro mechanistic studies and in vivo functional models, reinforcing the value of C. elegans as a versatile platform for neurodegeneration research. This combined strategy provides new insights into the anti-aggregation mechanisms of plant-derived compounds and supports their potential development as neuroprotective agents.

48. Novel genetic modifiers of SCA3/MJD: an EMS screening in a C. elegans model of the disease.

Authors: Marta Daniela Costa (1); Daniela Vilasboas-Campos (1); Jorge H Fernandes (1); Cármen Vieira (1); Lídia Nunes (1); Joana Pereira Sousa (1); Andreia Teixeira Castro (1); Patricia Maciel (1)

Affiliations:

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal.

Abstract:

In Spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD), an abnormal CAG repeat expansion in ATXN3 gene causes cerebellar ataxia of late-onset. Considering that the CAG repeat size explains about half of the disease heterogeneity, additional modifier loci must predict the remaining phenotypic variability verified in SCA3/MJD, namely at the age at onset (AO), clinical presentation and disease severity. The identification of novel modifier genes, potentially amenable to be targeted pharmacologically, is therefore crucial to propose effective therapies for this disease. With that purpose, we developed an EMS-based screening in a C. elegans model of MJD/SCA3 to identify modifiers of the animals' motor dysfunction, a key feature of the disease. The model was subjected to EMS treatment to introduce random mutations in its genome. Mutants with improved motor phenotype were isolated from the descendant F2 population. Various motor behavioral assays were conducted to assess the impact of the EMS-induced mutations on motor function and mutant ATXN3 aggregation, leading to the identification of promising screening hits. Among these candidates, clh-2, which encodes a chloride channel in C. elegans, emerged as a particularly relevant genetic modifier of the disease phenotype. Importantly, clh-2 is orthologous to the mammalian CLCN2 gene, which encodes the voltage-gated chloride channel ClC-2. The identification of clh-2/CLCN2 highlights a conserved molecular pathway that may influence neurodegeneration and disease progression in SCA3/MJD. Given the known roles of ClC-2 channels in neuronal excitability, ion homeostasis, and brain function, CLCN2 represents a strong candidate for further validation in patients. Identifying genetic modifiers of SCA3/MJD offers significant promise in uncovering new genetic factors that influence the disease. Most importantly, these genetic pathways can serve as potential targets for novel drug development, opening up new therapeutic possibilities for patients affected by this debilitating disorder.

49. C. elegans as a Nestor Guillermo Progeria Syndrome Model

Authors: Raquel Romero-Bueno (1,2); Adrián Fragoso-Luna (1); Sophia Breusegem (3); Cristina Ayuso (1); Nina Mellmann (1); Alan Kavsek (4); Christian G Riedel (4); Jordan D Ward (5); Delphine Larrieu (3); Peter Askjaer (1)

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5. Department of Molecular, Cell, and Developmental Biology, University of California-Santa Cruz, USA

Abstract:

Nestor-Guillermo Progeria Syndrome (NGPS) is a premature ageing illness that affects a variety of tissues, leading to growth retardation, severe skeletal defects and scoliosis. The syndrome is caused by a single amino acid substitution (A12T) in BAF1 (Barrier to Autointegration Factor 1). BAF1 is a highly conserved chromatin binding protein implicated in nuclear envelope (NE) breakdown, assembly and repair as well as chromatin compaction. Its NE localization is interdependent of lamins and LEM-domain proteins (LAP2, emerin, and MAN1) and contributes to chromatin organization although BAF1 is also present in the nucleoplasm. We have modified the baf-1 locus in Caenorhabditis elegans to mimic the human NGPS mutation (baf-1(G12T)) to elucidate why a mutation in an essential protein expressed throughout development triggers the appearance of symptoms ~2 years after birth. Tissue-specific chromatin binding and transcriptome analyses showed reduced BAF-1 association in most genes deregulated by the baf-1(G12T) mutation, suggesting that altered BAF chromatin association induces NGPS phenotypes via altered gene expression. We report that NE levels of lamin/LMN-1 and emerin/EMR-1 are reduced in baf-1(G12T) mutants, whereas errors in chromosome segregation are increased. The baf-1(G12T) mutation reduces fertility and lifespan and accelerates age-dependent nuclear morphology deterioration. Moreover, we found that baf-1(G12T) mutants are hypersensitive to NE perturbations, particularly to modifications affecting lamin/LMN-1. Like other progerias, NGPS-derived fibroblasts feature malformed nuclei. Importantly, a set of genes whose depletion alleviates the nuclear associated defects was unveiled by CRISPR-mediated gene knockout in NGPS fibroblasts. When orthologs were silenced by RNAi in C. elegans, several reduced the embryonic lethality of sensitized baf-1(G12T) mutants. In ongoing work, we further identify genetic and physical interactions between BAF-1 and factors involved in genome stability maintenance. Together, our findings provide mechanistic insight into NGPS pathogenesis and uncover candidate modifiers with potential for therapeutic exploration. This represents a first and encouraging list of candidate genes to be further explored for the development of NGPS therapies.

50. Protective effects of anthocyanins on proteotoxicity in amyloid-β Caenorhabditis elegans models

Authors: Rebeca de la Cruz-Sánchez (1); Lidia Garzón-García (1); Begoña Ayuda-Durán (1); Susana González-Manzano (1); Celestino Santos-Buelga (1); Ana M. González-Paramás (1)

Affiliations:

1. Grupo de Investigación en Polifenoles (GIP/USAL), Unidad de Nutrición y Bromatología. Universidad de Salamanca (Spain)

Abstract:

Dietary bioactive compounds have been associated with a lower risk of neurodegenerative disorders such as Alzheimer's disease (AD). A key pathological hallmark of AD is the accumulation of amyloid-β (Aβ) peptide, which aggregates into neurotoxic oligomers and extracellular plaques contributing to neuronal dysfunction and disease progression. In this context, anthocyanins, a subclass of polyphenols abundant in berries, have attracted particular interest due to their antioxidant, anti-inflammatory and neuroprotective properties. The present study investigates the effects of different concentrations (25–200 μM) of three anthocyanin—pelargonidin 3-O-glucoside (Pg3G), cyanidin 3-O-glucoside (Cy3G) and malvidin 3-O-glucoside (Mv3G)—on Aβ-induced toxicity using the nematode Caenorhabditis elegans as an in vivo model. Two transgenic strains were used to assess distinct manifestations of Aβ toxicity. The CL2355 strain, which expresses Aβ in neurons, was employed to evaluate sensory function through chemotaxis assays, whereas the CL4176 strain, expressing Aβ in muscle cells, was used to determine proteotoxicity by monitoring paralysis. Treatment with the three anthocyanins significantly improved chemotaxis behaviour compared with untreated controls, with optimal effects observed at 150 μM for Pg3G and 100 μM for both Cy3G and Mv3G. In paralysis assays, all compounds reduced Aβ-induced proteotoxicity, with effects observed at compound-specific concentrations. These findings suggest that Pg3G, Cy3G and Mv3G may attenuate both neuronal dysfunction and proteotoxicity associated with Aβ expression in C. elegans. Overall, the results support the potential of these dietary polyphenols as candidates for nutritional strategies targeting AD-related pathology.

51. Modulation of β-amyloid Toxicity of an Anthocyanin-rich Cherry Extract in Transgenic Caenorhabditis elegans Models

Authors: Debora Caushi (1); Lidia Garzón-García (2); Pedro Zamorano-Aguilar (2); Rebeca de la Cruz-Sánchez (2); Begoña Ayuda-Durán (2); Susana González-Manzano (2); Ana M. González-Paramás (2); Celestino Santos-Buelga (2)

Affiliations:

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2. Grupo de Investigación en Polifenoles (GIP/USAL), Unidad de Nutrición y Bromatología. Universidad de Salamanca (Spain)

Abstract:

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, memory loss and impairment of executive functions. One of AD's hallmarks is the accumulation of amyloid-β (Aβ) peptide which causes neuronal damage at synaptic level, compromising neuronal communication. Several studies have demonstrated that dietary intake of anthocyanins is associated with health benefits and preventive effects, mainly related to their antioxidant and anti-inflammatory properties. This study evaluates the potential neuroprotective effects of a cherry extract rich in anthocyanins, particularly glycosylated cyanidin derivatives, using transgenic strains of Caenorhabditis elegans expressing the Aβ peptide. For this purpose, nematodes were cultured on NGM plates supplemented with different concentrations of the extract (100, 200 and 300 µg/mL). Results showed that the cherry extract significantly improved the chemotaxis index in CL2355 (Aβ expression in neurons) at 100 and 200 µg/mL, suggesting its potential to ameliorate Aβ-associated neuronal dysfunction. Furthermore, treatment with 100 and 200 µg/mL of the extract delayed Aβ-induced paralysis in the CL4176 strain (Aβ expression in muscle cells) compared with the control group 38 h after Aβ expression induction. In contrast, the highest concentration (300 µg/mL) showed adverse effects in both assays, suggesting a possible hormetic response to the extract components, a phenomenon previously described for phenolic compounds. In conclusion, these findings suggest that cherry extract rich in cyanidin derivatives may modulate β-amyloid toxicity in C. elegans models of AD.

52. Adenosine receptor ligands as new therapeutic targets against Alzheimer Disease

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Affiliations:

1. Instituto de Investigación Sanitaria La Fe

Abstract:

Introduction: Alzheimer's disease (AD), a neurodegenerative disorder defined by a progressive cognitive decline, loss of memory, and impairment of daily function [1]. AD is characterized by accumulation of amyloid beta (Aβ) plaques, Tau agregation and neuroinflammation among others [2]. However, currently there is no definitive and accessible treatment for AD. In this sense, some adenosine receptors (AR) are emerging pharmacological targets for AD treatment, representing promising biomolecules. Methodology: C. elegans CCL2122 as wild-type (WT) and CL2355 as transgenic strains (expressing human pan-neuronal Aβ) were tested in chemotaxis assays. For this, A2AAR antagonist (ZM241385, ANR94, ANR322, ANR325, and ANR474) and agonists (CGS21680 and VT7) on plates seeded with Escherichia Coli OP50. Each plate was divided into four quadrants with 2 of them with chemoattractant agent benzhaldehyde 0.1% 15 age-synchronized worms were placed at the center of each plate and the distribution in each quadrant was recorded every 15 minutes over a period of 1 hour. Results: Untreated WT showed a higher percentage of worms in the chemoattractant area (65%) respect to Aβ peptide strain (44%), moreover DMSO did not show toxic effect for these strains maintaining the significance. Agonists did not show significant effect in WT, but a significant chemotactic increase in Aβ strain. Among the antagonists tested, ANR 325 showed the best significant increase in the chemotaxis index, in a dose-dependent manner. Conclusions: preliminary studies in vivo evidence for the neuroprotective potential of A2AAR ligands in a C. elegans models of AD. Among all the tested compounds, the A2AAR antagonist ANR 325 emerged as the most potent and selective agent, significantly restoring chemotactic behaviour in Aβ peptide worm al low micromolar concentrations.

53. A direct metabolic role for histone lysine methyltransferases in fatty acid oxidation

Authors: Giovanni Lerussi (1,2); Gisela Barragan Gallardo (1); Marcos Francisco Perez (1)

Affiliations:

1. Instituto de Biologia Molecular de Barcelona (IBMB - CSIC)
2. Universitat de Barcelona

Abstract:

Epigenetic modifications are deeply entangled with cellular metabolism — and this relationship is not unidirectional. Beyond their epigenetic role in directing gene regulation, histone (lysine) methyltransferases (HMTs) are the sole known enzymes capable of generating trimethyllysine (TML), the obligate precursor for carnitine biosynthesis. Since carnitine is essential for mitochondrial fatty acid oxidation, we hypothesize that HMTs contribute directly to energy metabolism through this biosynthetic link — a role entirely independent of their transcriptional functions. Using C. elegans as a model, we established that the carnitine pathway is functionally required for survival under metabolic stress. Knockdown of gbh-2 and gbh-1, encoding the first and last enzymes of carnitine synthesis, significantly reduced survival during starvation, while disruption of CPT-1 (carnitine palmitoyl transferase I), both with RNAi and pharmacological inhibition, produced similar phenotypes. Additionally, both conditions led to increased lipid staining relative to control during starvation, suggesting that endogenous carnitine synthesis is necessary to appropriately utilize fat reserves under nutrient stress. Experiments in gbh-2/1 knockdown animals are currently underway to determine whether exogenous carnitine supplementation can rescue these phenotypes. To identify which proteins serve as the primary TML source, we performed multiplexed Western blot analysis using panKme3 and H3 antibodies — and found that only ~1.4% of total lysine trimethylation in C. elegans resides on histone H3, with ~97% concentrated on two as-yet unidentified proteins. Mass spectrometry of immunoprecipitated Kme3- containing proteins is ongoing to identify these candidates and the HMTs responsible for their modification.

54. Developing a toolbox of optogenetic technologies for C. elegans

Authors: Montserrat Porta de la Riva (1); Michael Krieg (1)

Affiliations:

1. ICFO - The Institute of Photonic Sciences

Abstract:

Luciferases are widely employed as biosensors because of their low background activity, which provides unprecedented sensitivity in biological systems. The advantages of bioluminescence, such as low phototoxicity, no background and no autofluorescence, make luciferases very attractive for non-invasive, longitudinal in vivo observations without the need of an excitation light source. However, the use of bioluminescence in microscopy is limited, due to the low photon yield of the enzymes and suboptimal imaging technology. Current improvements in the chemical and bioengineering of enzymes and their substrates, together with advances in microscopy, are enabling increasingly sensitive, spatially resolved, and quantitative imaging of biochemical activity in living cells. In the last years we have developed a series of bioluminescent reporters that not only expand from short wavelengths to photon emission above 600 nm but also display high dynamic ranges suitable for assessing calcium fluctuations both in body wall muscle and neurons in C. elegans. In addition, we have developed PhAST (Photon-Assisted Synaptic Transmission), an all-optical synaptic engineering technology in which we used these calcium-sensitive luciferases as endogenous, non-invasive light sources to activate colour matching light-gated ion channels and control neuronal activity. By means of the PhAST technique we have successfully restored impaired neuronal communication, silenced existing and created new ones between naturally unconnected neurons. PhAST is easily adaptable and our streamlined toolbox requires minimal interventions and technology.

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