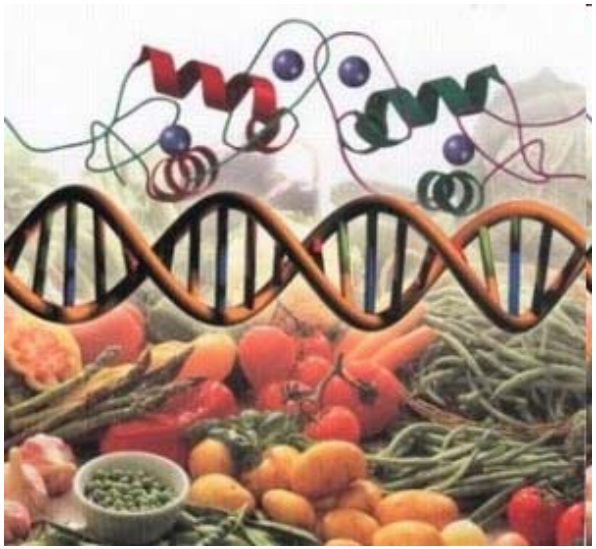
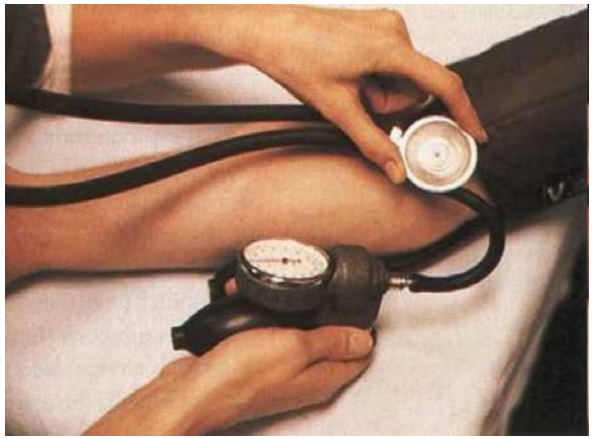
Facultad de Ciencias Experimentales

Área de Bioquímica y Biología Molecular



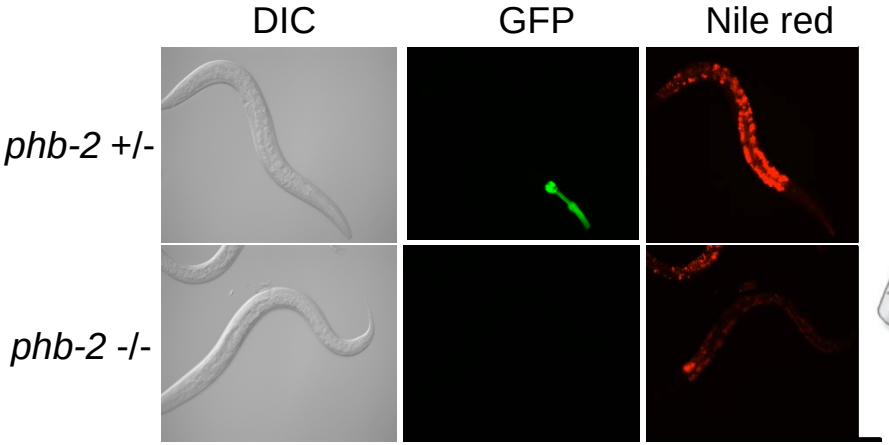


Oferta de Proyectos de Fin de Grado para el Curso 2016-2017

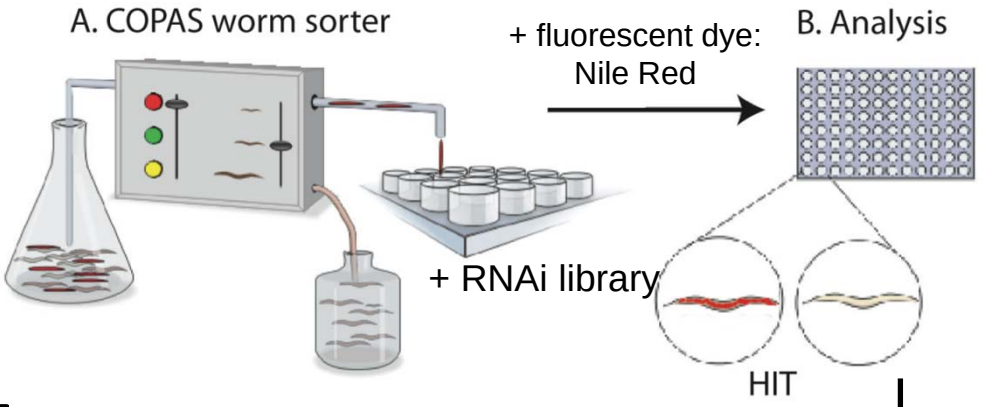
PFG_1 Interacción mitocondria-rutas de señalización celular en la regulación del envejecimiento en *C. elegans*

Identificación de rutas de señalización implicadas en la reducción del contenido de grasa causado por falta de PHB en animales salvajes y en mutantes de la ruta de la Insulina.

RNAi screen for fat content



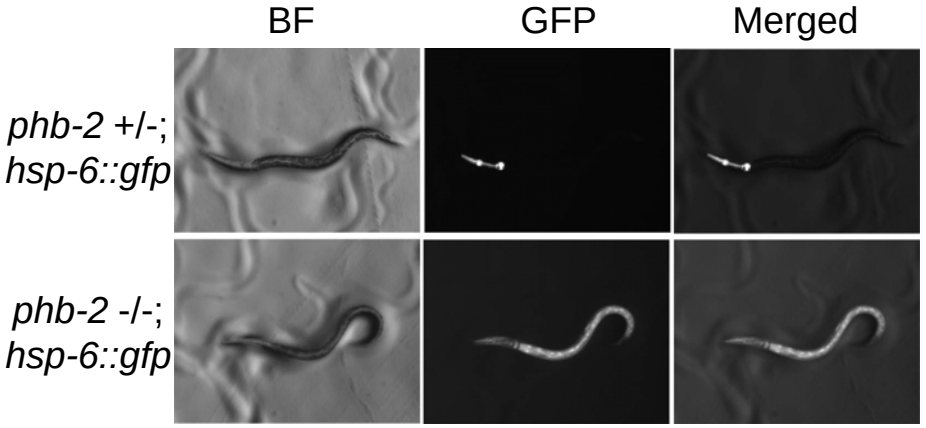
Automated sorting and analysis



Mutantes phb-2 muestran un fenotipo de reducción de grasa y ausencia de GFP en la faringe (panel izquierda). Los mutantes phb-2 se seleccionan utilizando el COPAS BIOSORT (panel derecha), en función de la ausencia de GFP, y distribuyen en placas (96 pozillos) que contienen bacteria que produce RNAi para cada gen del genoma de C. elegans y la prueba fluorescente Nile Red.

PFG_2 Escrutinios genómicos para identificar reguladores de la respuesta al estrés mitocondrial en *C. elegans*

Mitochondrial stress response



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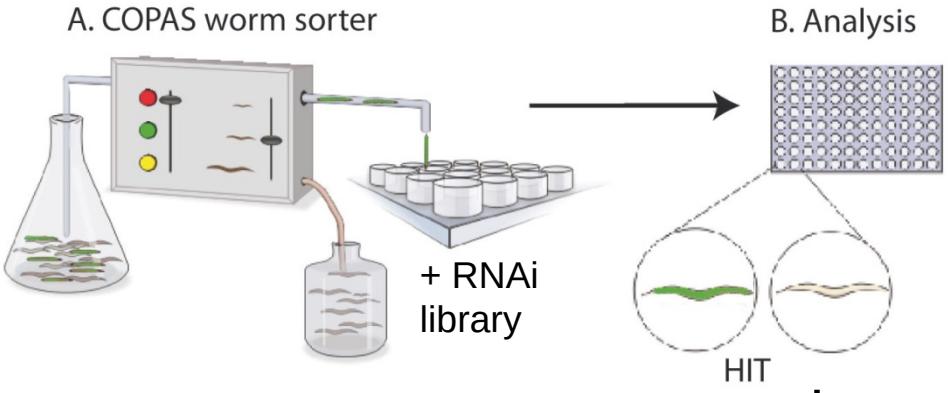


Mutantes phb-2 muestran una fuerte inducción de hsp-6::GFP, una chaperona que responde al estrés mitocondrial (panel izquierda). Mutantes phb-2 se seleccionan utilizando el COPAS BIOSORT (panel derecha), en función de la presencia de GFP en todo el cuerpo, y distribuyen en placas (96 pozillos) que contienen bacteria que produce dsRNA para cada gen del genoma de C. elegans. Animales que expresan GFP sólo en la faringe son eliminados (botella).

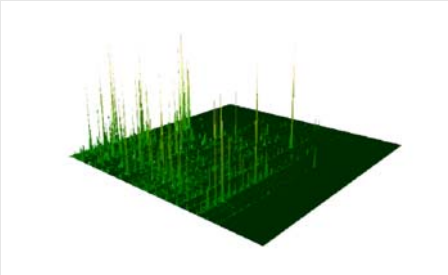
PFG_3 **Metabolomica y envejecimiento en *C. elegans***

Consecuencias Metabólicas de la falta de prohibitinas mitocondriales en animales salvajes y en mutantes de la ruta de la Insulina

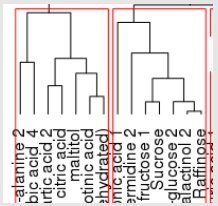
Metabolite Extraction from *C. elegans*

Metabolite profiling on complementary analytical platforms

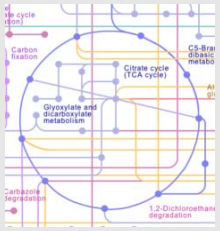
GC-EI-MS
LC-QqTOF-MS
LC-IT-MS
LC-FT-MS



Multivariate statistics, pathway analysis & biomarker search

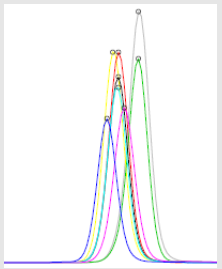


Metabolic signature



Pathway analysis

- KEGG
- metacyc
- WormBase



New Biomarkers of Aging

PFG_4: Complementación bimolecular de fluorescencia (BiFC) para estudiar las interacciones entre proteínas

We have identified the protein kinase VRK-1 as an essential regulator of nuclear envelope dynamics during cell division. However, *vrk-1* is also expressed in post-mitotic cells and *vrk-1* mutants show specific defects in development of the uterus. To understand the molecular mechanisms underlying VRK-1 function we have performed a proteomics analysis of potential interaction partners. This has provided a list of interesting candidates that we are currently validating by yeast two-hybrid experiments. As an independent method to investigate these interactions, we propose to use bimolecular fluorescence complementation (BiFC). BiFC is a powerful imaging technique to analyse protein-protein interactions *in vivo*. A fluorescent protein (typically YFP) is split in two non-fluorescent parts (let's call them "Y" and "FP") that are fused to two test proteins ("A" and "B"). If A and B are in close proximity, Y and FP may interact and restore fluorescence. We have already performed initial tests but the student taking on this project will be responsible for development of novel and more precise tool.

Skills that will be acquired in this project:

- Molecular cloning (PCR, cloning by restriction enzyme and Gibson assembly strategies, agarose gel electrophoresis, plasmid DNA purification).
- Maintenance and genetic crosses of *Caenorhabditis elegans*.
- Transgenesis by microinjection into *C. elegans*.
- Live microscopy and quantitative image analysis.

PFG_5 ¿Cómo es CN-5/NUP107 LINCed posicionamiento nuclear?

In most cell types, the nucleus is positioned non-randomly within the cell. This is particularly evident in many neurons and muscles and perturbation of nuclear position can lead to diseases. So far, nuclear positioning has been attributed to SUN domain proteins in the inner nuclear membrane and KASH domain proteins on the outer nuclear membrane: together they form the LInker of Nucleoskeleton and Cytoskeleton (LINC) complex. However, we have obtained two lines of evidence, which suggest that nuclear pore protein NPP-5 (NUP107 in vertebrates) may be involved in nuclear positioning. First, nuclei in the hypodermal syncytium aggregate in aged *npp-5* mutants. Second, *npp-5* enhances the nuclear positioning defects of SUN domain *unc-84* mutants and *npp-5 unc-84* double mutants have elevated embryonic lethality. However, further genetic studies are required to determine the specificity and mechanisms. The student involved in this project will investigate if NPP-5 is required for correct nuclear positioning in other tissues and will be responsible for testing the interaction with of other SUN/KASH proteins.

Skills that will be acquired in this project:

- Molecular cloning (PCR, cloning by restriction enzyme and Gibson assembly strategies, agarose gel electrophoresis, plasmid DNA purification).
- Maintenance and genetic crosses of *Caenorhabditis elegans*.
- Transgenesis by microinjection into *C. elegans*.
- Genetic and biochemical interaction approaches.
- Live microscopy.

PFG_6

El papel de N-acetiltransferasa 10 (NATH-10) en la estructura de la envoltura nuclear y el envejecimiento

Several features are altered in cells from old individuals compared to cells from young individuals. Interestingly, this is largely recapitulated in cells from progeria patients when compared to cells from healthy donors. Among these features are nuclear morphology and heterochromatin distribution. We have initiated studies on N-acetyltransferase 10 (NATH-10), which was recently identified as a putative key player in these processes. Because of its rapid life cycle, the nematode *Caenorhabditis elegans* is an attractive model to dissect pathways involved in physiological and premature aging. In this project we would like to investigate if and how NATH-10 influences aging. Specifically, the student will be responsible for analysis of *nath-10 loss of function* phenotypes both in terms of lifespan and in the behaviour of nuclear envelope proteins, such as EMR-1, LEM-2 and BAF-1. Moreover, the student will use CRISPR technology to generate tools required for characterization of NATH-10 expression and subcellular localization.

Skills that will be acquired in this project:

- Molecular cloning (PCR, cloning by restriction enzyme and Gibson assembly strategies, agarose gel electrophoresis, plasmid DNA purification).
- Maintenance and genetic crosses of *Caenorhabditis elegans*.
- Transgenesis by microinjection and CRISPR into *C. elegans*.
- Lifespan analysis.
- Live microscopy.

PFG_7

Identificación de nuevos genes implicados en los procesos metastáticos.

Los mecanismos genéticos y moleculares que regulan la capacidad de las células tumorales de convertirse en invasivas están aún por descubrir. En este proyecto se propone el uso de *Drosophila*, y en particular el intestino de la larva, como sistema modelo para aislar nuevos genes inductores de metástasis. La ventaja de usar animales modelos es que permite tener una fuente ilimitada de material asi como un análisis in vivo y temporal del proceso tumoral. Así mismo, permite manipular a nivel genético la célula tumoral y analizar las implicaciones de estas manipulaciones, lo que nos permite elucidar mecanismos moleculares y celulares de la metástasis. Recientemente, hemos generado células tumorales en el intestino de la larva, mediante la expresión de una forma oncogénica del gen Ras, Ras^{V12}. Asimismo, expresando en este fondo genético una colección de RNAis para genes implicados en señalización celular, hemos aislado una serie de genes que cuando eliminados convierten las células tumorales Ras^{V12} en células metastáticas. Entre estos genes se encuentran algunos que ya se sabía que promovían metástasis cuando mutados, como los genes de polaridad scribble or Apc1/2, y otros cuyo papel en metástasis es desconocido, como espectrinas (reguladores del citoesqueleto de actina) o el represor caupolican (caup). En este proyecto utilizaremos técnicas de genética de *Drosophila*, microscopía confocal, análisis *in vivo* y técnicas de biología molecular para analizar el papel de los genes aislados en el proceso metastático.