5th European Meeting on Planarian Biology

2-4 October 2022 - Sant Feliu de Guíxols

ABSTRACTS BOOK

Invited Speakers:

Mansi Srivastava
(Harvard University, MA, USA)

Carrie Adler
(Cornell University, NY, USA)

Sessions on:

Regeneration & Stem Cells
Regulation of Tissue Dynamics
Regeneration & Signalling
Lineage Specification
Omics
Microbiome & Immune Defence
Evolution & Phylogeny

Organizers:

Teresa Adell
(*University of Barcelona, ES*)
Jordi Solana
(*Oxford-Brookes University, UK*)
Jochen Rink
(*MPI-CBG, DE*)

Thanks to: The Company of Biologists Novogene

EMPB2022

5th European Meeting on Planarian Biology

October 2^{nd}–4^{th}, 2022

Sant Feliu de Guíxols, Catalunya

https://compgen.bio.ub.edu/EMPB2022

#EMPB2022

Organized by:

Teresa Adell
University of Barcelona, Catalunya

Jordi Solana
Oxford-Brookes University, UK

Jochen Rink
MPI-CBG, Dresden, Germany

Edited by:

Josep F Abril
University of Barcelona, Catalunya

Contents

About the Meeting

Introduction

We are happy to welcome all of you to the 5^{th} European Meeting on Planarian Biology (EMPB2022) that takes place from October 2^{nd} to 4^{th}, 2022, at Hotel Eden Roc in Sant Feliu de Guixols, Catalunya. This meeting continues the tradition of previous meetings (Münster 2010, Kyoto 2011, Dresden 2013, Oxford 2015, and Sant Feliu 2016).

The aim of the meeting is to gather all European laboratories working on planarians to share their recent advances in all aspects of flatworm biology and regeneration as well as to strengthen the links and foster collaborations between all labs. This meeting should also promote discussions about how to improve and implement community resources that would benefit all the laboratories. In addition, the meeting is open to all research groups worldwide working on planarians as well as other Platyhelminthes aiming to continue our efforts to build up a collaborative community.

In this sense, we are really pleased to have Dr Mansi Srivastava, from Harvard University (USA) and Dr Carrie Adler, from the Cornell University (USA), as **invited speakers** as their numerous contributions have improved significantly our understanding on planarian regeneration. Furthermore, the Organizing Committee has selected 25 talks and 19 posters, for which you can find details on this abstract book. We hope you will enjoy both the scientific topics as well as the location.

Best wishes from the Organizing Committee...

About the Abstracts

The abstracts contained in this booklet should not be cited in bibliographies. Material contained herein should be treated as personal communication and should be cited as such only with consent of the author.

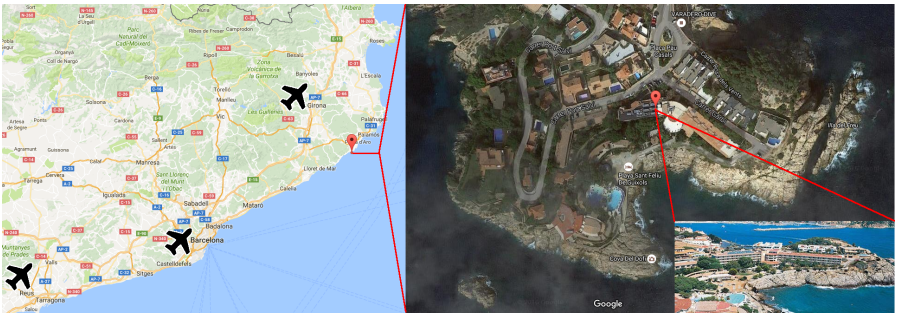
Talks abstracts follow same ordering as in the program sessions, while the posters abstracts were sorted alphabeticaly by first author surnames. You can find an index at the end of this book summarizing all the authors that were contributing to all the presented abstracts, regardless those abstracts were selected as talks or posters.

Venue Information

Hotel Eden Roc

The meeting will be held at Hotel Eden Roc, Punta de Port Salvi, Sant Feliu de Guixols (Girona), Catalunya. At a distance of only 1 km from Sant Feliu de Guixols, in a unique and quiet peninsula, is located the Hotel Eden Roc. The clear Mediterranean Sea surrounds along 360 meters the Eden Roc Resort.

+0034-972-320-100



Google Maps Coordinates: Latitude 41.772304 – Longitude 3.030225

How to Arrive

By Plane:

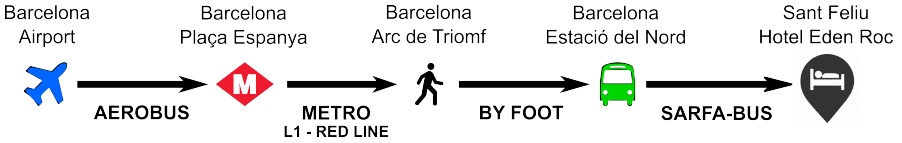
We assume you will fly to Barcelona airport, yet there are two other airports at Girona and Reus, and you will take a bus or train to reach the hotel at Sant Feliu de Guixols. A taxi from the Girona airport to Sant Feliu costs about 50 Euros (Official rates link).

By Car:

When driving from Barcelona take the exit numer 9 on the motorway A-7. When traveling from France you should take the exit number 7.

By Bus:

There is a bus company Moventis-SARFA (link to booking page) providing regular liner trips between Barcelona and Sant Feliu de Guixols. Depending on the avialability from the booking page, it can be possible to travel directly from Barcelona Airport terminals 1 or 2 to Sant Feliu, but it can also be done in two steps: first going from Barcelona Airport to Barcelona 'Estació del Nord' central bus station, and then from there to Sant Feliu. Make sure you ask for "Sant Feliu de Guixols", as there are other cities named after Sant Feliu. A PDF with the daily schedule is available from this link on the meeting web page.



Barcelona Airport – Barcelona city

You should take the 'AeroBus' bus (http://www.aerobusbcn.com/en/). It departs from Barcelona Airport Terminal 1 and 2. If you get off the Bus in Barcelona 'Plaça d'Espanya', you can take the metro to 'Arc del Triomf' (Red line), which is very close to 'Estació del Nord'.

Barcelona city - Sant Feliu

'Estació del Nord' is the central Barcelona bus station. There you can take a Bus to Sant Feliu from SARFA company. More information about this bus line at: http://compras.moventis.es/paginas/resultados_trayectos.php

By Train:

You can also take a regional regular train or a high-speed one (two RENFE options via AVE or AVLO) from Barcelona Sants station, you can find further info from this link, to the nearest train station, which is in Girona (at 30 km.). A taxi from the train station to Sant Feliu costs about 50 Euros.

Meeting Contact

You can contact Teresa Adell during the meeting at the following number:

+0034-690-618-625

Sponsors

We are grateful to the EMPB2022 sponsors:

Sessions Program

Sunday, October 2nd

13:00–15:30 Registration at Hotel Eden Roc

16:00–17:00 **Welcome and Opening Session**

Keynote Invited Talk by Mansi Srivastava
[Harvard University, MA, USA]
Mechanisms of regeneration and their evolution.

17:00–18:40 **Session I – Regeneration and Stem Cells**

Chair: *Francesc Cebrià*

17:00–17:25 Eugene Berezikov
[University Medical Center Groningen]
Analysis of cell hierarchies in the regenerative flatworm Macrostomum lignano using single-cell transcriptomics and stem cell-specific transgenic lines.

17:25–17:50 Cristina González-Estévez
[Universitat de Barcelona]
How to rejuvenate stem cells: the role of autophagy during starvation in regenerative processes.

17:50–18:15 Simon Kershenbaum
[University of Oxford]
Gene conversion may limit the impact of long-term asexuality in planarians.

18:15–18:40 Jordi Solana
[Oxford Brookes University]
Stem cells and regeneration in annelids: the single cell atlas of Pristina leidyi reveals insights into annelid cell types and their differentiation trajectories.

18:40–19:00 **"Explain your Poster" Session (odd numbers)**

19:15–20:00 *Welcome cocktail*

20:00–21:00 *Dinner (buffet)*

21:00–23:00 **Posters Session I**

Monday, October 3rd

09:00–09:45 **Keynote Invited Talk: Carrie Adler**
[Cornell University, NY, USA]
Inhibition of ATM kinase rescues planarian regeneration after lethal radiation.

09:45–10.40 **Session II – Regulation of Tissue Dynamics**

Chair: *Cristina González-Estévez*

09:45–10:10 Uri Weill
[Max Planck Institute of Multidisciplinary Sciences]
Planarian chimeras as a system to experimentally study the consequences of genetic heterogeneity.

10:10–10:25 Bret Pearson
[Doernbecher Children's Hospital]
Cell clearance by intestinal phagocytes in the freshwater planarian.

10:25–10:40 Daniel Moreno
[Universitat de Barcelona]
Appearance of senescent cells during planarian regeneration.

10:40–11:15 *Coffee Break*

Monday, October 3rd (Continued)

11:15–13:00 Session III – Regeneration and Signalling

Chair: *Teresa Adell*

11:15–11:40 Karen Smeets
[Hasselt University]
Redox signals: positional cues during regeneration? A spatiotemporal characterisation of redox molecules in Schmidtea mediterranea.

11:40–12:05 Daniel Font-Martín
[Universitat de Barcelona]
Inhibition of the Hippo pathway in planarians impairs proper tissue renewal through defects in cell cycle, cell polarity and transcriptional deregulation.

12:05–12:30 Martijn Heleven
[Hasselt University]
Monoaminergic neural signaling: the role of riboflavin in planarian sensing.

12:30–12:45 Azurra Codino
[Italian Institute of Technology]
Pharmacological activation of Notch signalling pathway impairs head regeneration and locomotion in the asexual Schmidtea mediterranea.

12:45–13:00 Pablo Coronel
[Universitat de Barcelona]
Genomic analyses reveal FoxG as an upstream regulator of wnt1 required for posterior identity specification in planarians.

13:00–14:30 *Lunch*

Monday, October 3rd (Continued)

14:30–16:35 Session IV – Lineage Specification

Chair: *Jordi Solana*

14:30–14:55 Jason Pellettieri
[Keene State College]
Nonsense-mediated mRNA decay controls cell fate specification in S. mediterranea.

14:55–15:20 Yarden Yesharim
[Tel Aviv University]
m6A is required for resolving progenitor identity during planarian stem cell differentiation.

15:20–15:45 M. Dolores Molina
[Universitat de Barcelona]
Smed-cbp-2 and Smed-cbp-3 genes have functionally diverged to regulate planarian survival and stem cell differentiation.

15:45–16:10 Sophie Peron
[Oxford Brookes University]
Molecular regulation of pluripotent stem cells in a highly regenerative planarian.

16:10–16:35 Elena Emili
[Oxford Brookes University]
Organization and development of neuronal cell types in the model Schmidtea mediterranea: a single-cell transcriptomics study.

16:35–17:00 *Coffee break*

Monday, October 3rd (Continued)

17:00–18:15 **Session V – Omics**

Chair: *Omri Wurtzel*

17:00–17.25 Jakke Neiro
[University of Oxford]
Identification of putative enhancer-like elements predicts regulatory networks active in planarian adult stem cells.

17:25–17:50 Jochen Rink
[Max Planck Institute of Multidisciplinary Sciences]
The new chromosome-scale S. mediterranea reference genome assembly and annotations—an update.

17:50–18:15 Omri Wurtzel / Josep F. Abril / Jordi Solana
[Tel Aviv University / University of Barcelona / Oxford Brookes University]
Single cell repositories.

18:15–18:30 Vinay K. Dubey
[Institute for Stem Cell Science and Regenerative Medicine (inStem)]
Smed-ets-1 regulates epidermal lineage landscape in a cell non-autonomous fashion via basement membrane remodelling.

18:30–19:30 **"Explain your Poster" Session (even numbers)**

20:00–21:00 *Dinner (buffet)*

21:00–23:00 **Posters Session II**

Tuesday, October 4th

09:00–10:00 **Session VI – Microbiome and Immune Defence**

Chair: *Karen Smeets*

09:00–09:25 Karolien Bijnens
[Centre for Environmental Sciences at Hasselt University]
More than a gut feeling? The composition, diversity and variability of the planarian microbiome.

09:25–09:50 Claus-D. Kuhn
[University of Bayreuth]
The role of piRNAs in planarian stem cells and their potential role in immune defense.

09:50–10:15 *Coffee Break*

10:15–11:20 **Session VII – Evolution and Phylogeny**

Chair: *Klaus Kuhn*

10:15–10:40 Miquel Vila Farré
[Max Planck Institute of Multidisciplinary Sciences]
Why some animals regenerate while others cannot: evolution of regeneration in planarians.

10.40–11:05 Marta Riutort
[Universitat de Barcelona]
Phylotranscriptomics uncovers a complex evolutionary history for the planarian genus Dugesia (Platyhelminthes, Tricladida) in the Western Mediterranean.

11:05–11:20 Martin Sacha
[First Faculty of Medicine, Charles University]
Department of Planaria, At Home.

11:20–12:30 **Where do we go & what to improve as a community?**

12:30–12:40 **Closing Remarks and Farewell**

13:00–14:00 *Lunch*

Invited Talks

Mechanisms of regeneration and their evolution

Srivastava, Mansi[1]

1. Harvard University, MA, USA.

Wound repair and regeneration are fundamental features of animal biology, yet little is known about how these pathways compare across animal lineages. The goals of my research program are: 1) to identify cellular and genetic mechanisms for whole-body regeneration, and 2) to create a framework for rigorous cross-species comparisons to understand the evolution of regeneration.

In this talk, I will discuss how we utilize a diversity of approaches including functional genomics, single-cell RNA-sequencing, and transgenesis to uncover the mechanisms of regeneration and stem cell regulation in *Hofstenia miamia*, an acoel worm. In particular, I will highlight how studying embryonic development informs these questions.

Inhibition of ATM kinase rescues planarian regeneration after lethal radiation

Shiroor, Divya A[1]; Wang, Kuang-Tse[1]; Sanketi, Bhargav D.[1]; Tapper, Justin K[1]; Adler, Carolyn E[1]

1. Cornell University, NY, USA.

Radiation exposure causes rampant double-strand breaks in DNA. Exposing planarians to radiation induces rapid loss of stem cells, completely arresting regeneration and leading to animal death. To identify regulators of radiation-induced cell death, we combined RNAi interference-based screens with radiation, and assessed the extent of stem cell survival. We focused on genes in the DNA damage response (DDR), a network of proteins that regulates how cells react to double-strand breaks—either by initiating DNA repair, cell cycle arrest, or death—. At the helm of the DDR are three PI3-like kinases that coordinate its output, including Ataxia Telangiectasia Mutated (ATM). Knockdown of ATM promotes full recovery of stem cells after lethal radiation. In this context, stem cells circumvent apoptosis, escape cell cycle checkpoints to replicate their DNA, and recover function using homologous recombination-mediated DNA repair. Amazingly, despite radiation exposure, atm knockdown animals survive long-term and regenerate new tissues. Together, our results demonstrate that ATM's primary function in planarians is to drive apoptosis, and suggest that inhibition of ATM can potentially favor cell survival after radiation without adverse effects.

Selected Talks

Analysis of cell hierarchies in the regenerative flatworm *Macrostomum lignano* using single-cell transcriptomics and stem cell-specific transgenic lines

Mouton, Stijn[1]; Ustyantsev, Kirill[1]; Wudarski, Jakub[1,2]; Berezikov, Eugene[1]

1. European Research Institute for the Biology of Ageing; University Medical Center Groningen; Antonius Deusignlaan 1, Groningen, 9714AV; The Netherlands.
2. National Institute for Basic Biology; Nishigonaka 38, Myodaiji, Okazaki 444-8585 Aichi; Japan.

Marine flatworm *Macrostomum lignano* is an excellent model organism to study the mechanisms of stem cell regulation and regeneration. This animal can regenerate most of its body parts, it is easy to culture in laboratory conditions, and it is possible to create transgenic *M. lignano* lines. We use this model to understand how stem cells (neoblasts) in this animal make all other cell types and regenerate tissues and organs. Towards this, we have generated several transgenic *M. lignano* lines labeling specifically neoblast by CRIPS/Cas9-mediated knock-in of various fluorescent protein-encoding genes in frame with a histone 2A variant gene that is expressed specifically in neoblasts. Single cell transcriptomic analysis of FACS-isolated neoblasts, as well as all cells of the animal allowed us to identify major cell types in *M. lignano* and establish relations between neoblasts and differentiated cells. These data prove instructive for generating experimental hypotheses for further in-depth investigation of stem cell regulation in *M. lignano*.

How to rejuvenate stem cells: the role of autophagy during starvation in regenerative processes

Iglesias, Marta[1]; Felix, Daniel A[1]; Sankaranarayanan, Ramya A[1]; Thems, Anne[1]; Saló, Emili[2]; Adell, Teresa[2]; González-Estévez, Cristina[1,2]

1. Leibniz Institute on Aging-Fritz Lipmann Institute (FLI); Jena; Germany.
2. Department of Genetics, Microbiology & Statistics; Institut de Biomedicina de la Universitat de Barcelona (IBUB); Universitat de Barcelona; Barcelona; Catalonia, Spain.

Planarians are able to withstand long periods of starvation while maintaining the adult stem cell population and their regenerative capabilities. Our research has found several clues on how planarians enhance stem cell function and can regenerate during starvation. Autophagy is an evolutionary conserved mechanism directly activated by suppression of mTOR signalling to maintain the homeostasis of all tissues and a candidate cellular mechanism that could also be enhancing stem cell function and regeneration during starvation. The working hypothesis is that autophagy will contribute to rejuvenate the planarian stem cell pool during starvation.

The approach is based on a RNAi screen of autophagy (atg) genes that we are functionally characterizing. Our preliminary data shows genes with expression enrichment in stem cells and stem cell-related RNAi phenotypes during starvation, which are rescued by feeding. Our results point towards a function in preventing stem cell death and increasing telomere length in the stem cell population.

Our work will investigate the function of the atg genes during planarian regeneration and homeostasis in starved conditions. We will also establish a repertoire of functional assays to detect autophagy in planarians. This has the potential to uncover novel functions of autophagy in somatic stem cells.

Gene conversion may limit the impact of long-term asexuality in planarians

Kershenbaum, Simon[1]; Griffin, Ashleigh[1]; Aboobaker, Aziz[1]

1. Department of Biology; University of Oxford; 11a Mansfield Rd, Oxford OX1 3SZ; UK.

Most multicellular organisms reproduce sexually despite the costs associated with sexual reproduction. This has traditionally been explained by suggesting that the lack of recombination in asexual species reduces their adaptability and leads to the accumulation of deleterious mutations, increasing the risk of extinction. Nonetheless, successful asexual life histories persist and explanatory mechanisms which may help limit the cost of asexuality remain enigmatic. Asexual mechanisms of recombination, such as gene conversion, have been suggested as potential mechanisms for limiting the impact of long-term asexuality, but this possibility remains largely untested. Here we compare protein coding gene sequences between the sexual and obligate asexual strains of *Schmidtea mediterranea* and find that while the asexual strain shows higher levels of deleterious mutation accumulation compared to the sexual strain, this pattern is not seen in highly conserved genes and is not as severe as in other obligate asexuals. Additionally, we find stronger signatures of gene conversion in the asexual strain compared to its sexual counterparts, and find evidence that selection is able to act on these gene conversion events to remove deleterious mutations from the population. Taken together, these findings suggest that gene conversion may be used by asexual lineages to unlink loci and limit some of the key problems facing obligate asexual species.

Stem cells and regeneration in annelids: the single cell atlas of *Pristina leidyi* reveals insights into annelid cell types and their differentiation trajectories.

Solana, Jordi[1]

1. Department of Biological and Medical Sciences; Oxford Brookes University; Headington campus, Gipsy lane, OX3 0BP, Oxford; United Kingdom.

Annelids are a broadly distributed, highly diverse, and economically and environmentally important group of animals. Most annelids can regenerate body parts, by yet unknown cellular mechanisms. Here, we perform single cell transcriptomics of 75,218 cells of the regenerating annelid species *Pristina leidyi* using ACME and SPLiT-Seq. Our results uncover regionally expressed genes in the annelid gut, and a diversity of neuronal populations, as well as muscle and epidermis specific genes. We also characterise annelid specific cell types such as the chaetal sacs and the globin producing cells. We also uncover unknown cell types of enigmatic affinity. We identify a broadly abundant cluster of putative stem cells. This population expresses well known stem cell markers such as *vasa*, *piwi* and *pumilio* homologues, but also shows a heterogeneous expression of differentiated cell markers and their transcription factors. We also find conserved expression of multiple chromatin and epigenetic regulators, revealing that this is likely a universal feature of invertebrate stem cells. Our characterisation of an annelid putative adult pluripotent stem cell population will serve as a platform for the study of annelid stem cells and their enigmatic regeneration process.

Planarian chimeras as a system to experimentally study the consequences of genetic heterogeneity

Weill, Uri[1]; Vila Farré, Miquel[1]; Rink, Jochen[1]

1. Department of Tissue Dynamics and Regeneration; Max Planck Institute of Multidisciplinary Sciences; Am Fassberg 11, 37077 Göttingen; Germany.

Stem cells are the basic self-replicating units that maintain tissue homeostasis in adult animals. But how the body coordinates the division and differentiation of its stem cell populations to maintain dynamic tissue homeostasis is still unclear. The unusual adult stem cell system of planarians exemplifies the challenge of understanding dynamic tissue homeostasis. Planarians possess pluripotent adult stem cells that continuously renew all cell types and of which a single one can make a complete animal body. The additional continuous divisions of the stem cells, extreme longevity and lack of a single cell generational bottleneck due to asexual reproduction by fission/regeneration exemplify a profound question: How do planarians maintain dynamic homeostasis in the face of inevitable genetic drift? To address this question, we experimentally induced genetic heterogeneity by tissue transplants between a diverse collection of genetically distinct *Schmidtea polychroa* strains. By following the host and donor cell populations in the resulting chimeric animals over time and space, we can distinguish different donor-host cell dynamics between specific strain combinations. These results provide first glimpses of the processes that might contribute to the maintenance of tissue homeostasis in the face of inevitable genetic drift.

Cell clearance by intestinal phagocytes in the freshwater planarian

Pearson, Bret[1]

1. Hospital for Sick Children; Program in Developmental and Stem Cell Biology; Toronto, ON M5G 0A4, Canada.

Regeneration and wound healing both require the clearance of dead cells, yet this process is often impaired in vertebrates for reasons that remain unclear. By contrast, the planarian *S. mediterranea* perfectly regenerates all organs and tissues, and efficiently resolves a body-wide apoptotic response following amputation. Here, we show that planarians are able to remove large numbers of dying cells by excreting these cells through the intestine. Intestinal phagocytes are required for this process, and likely phagocytose dying cells through a mechanism involving the conserved engulfment gene *elmo*. Our results suggest that planarians utilize a novel mechanism of cell clearance by employing intestinal phagocytes, previously thought to be involved only in digestion, in the process of cell clearance.

Appearance of senescent cells during planarian regeneration

Moreno-Blas, Daniel[1]; Font-Martín, Daniel[1]; Saló, Emili[1]; Adell, Teresa[1]

1. Department of Genetics, Microbiology & Statistics, Institute of Biomedicine (IBUB), University of Barcelona, Barcelona, Catalonia, Spain.

Cellular senescence is a cell state characterized by stable cell cycle arrest combined with a significant secretory activity that influences the function and behavior of surrounding cells. Even though senescence has been widely associated with aging and age-related diseases, recent studies have highlighted the importance of cellular senescence during tissue repair and regeneration. However, whether planarian cells can undergo senescence and whether senescent cells participate in planarian regeneration remains unknown. In this study, we explored the presence of senescent cells in planarian tissue during regeneration. We found a transient appearance of features of the senescent phenotype in planarian fragments at different days of regeneration after amputation. These results suggest the induction of cellular senescence in planarians that could play a positive role in contributing to tissue repair and regeneration.

Redox signals: positional cues during regeneration? A spatiotemporal characterisation of redox molecules in *Schmidtea mediterranea*

Jaenen, Vincent[1]; Heleven, Martijn[1]; Fraguas, Susanna[2,3]; Bijnens, Karolien[1]; Cebrià, Francesc[2,3]; Smeets, Karen[1]

1. Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium.
2. Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Catalonia, Spain.
3. Institute of Biomedicine of the University of Barcelona (IBUB), University of Barcelona, Barcelona, Catalonia, Spain.

Reactive oxygen species (ROS) are produced during wounding and amputation. ROS and redox-activated molecules interact with evolutionary conserved pathways such as the MAPK/ERK pathway, and guide stem cell proliferation, differentiation, and survival. We aimed to further unravel these interactions, and investigated how redox alterations drive regeneration and tissue patterning.

Schmidtea mediterranea was used to study the strict coordination between ROS, antioxidative molecules and MAPK signaling during tissue regeneration. After inducing regeneration via amputation, wound-oriented dependent differences in both pro- as well as anti-oxidant production were observed. A higher and more prolonged production of superoxide and hydrogen peroxide (H2O2) was induced at the wound sites of fragments that needed to regenerate anterior body parts. In addition, H2O2 kinetics differed depending on the position of the amputation site along the pre-existing anteroposterior (AP) body axis. The positional redox differences are in line with the AP-dependent activation of the MAPK/ERK pathway, and interfering with this pathway resulted in impaired anterior regeneration. This regenerative impairment could be rescued by artificially interfering with the redox status. Our exploratory study indicates that ROS and antioxidants are tightly intertwined and provide positional cues to establish correct regeneration and tissue patterning, potentially via the MAKP/ERK pathway.

Inhibition of the Hippo pathway in planarians impairs proper tissue renewal through defects in cell cycle, cell polarity and transcriptional deregulation

Font-Martín, Daniel[1,2]; González-Estévez, Cristina[1,2]; Adell, Teresa[1,2]

1. Genetics, Microbiology & Statistics; Universitat de Barcelona; Diagonal 643, 08028, Barcelona; Catalonia, Spain.
2. Institut de Biomedicina de la Universitat de Barcelona (IBUB); Universitat de Barcelona; Diagonal 643, 08028, Barcelona; Catalonia, Spain.

The Hippo signaling pathway has been found deregulated in a myriad of tumoral processes. However, its connection with abnormal tissue growth remains elusive. Today, it is seen as a complex pathway that controls cellular behavior in response to the physical environment. To better understand this relation, we study its function in planarians, flatworms that constantly renew their tissues thanks to their constant supply of adult stem cells.

In a previous study we characterized the formation of overgrowths in planarians after Hippo inhibition. This correlated with a decrease in cell death, an arrest in mitosis and the increase in cell plasticity. In the present work we study the function of 3 putative hippo target genes (*syne1*, *ccdc175*, and *med17*) found deregulated in the transcriptome of hippo RNAi planarians. Here we show that RNAi inhibition of the candidates phenocopy the overgrowths found in hippo RNAi animals. They also reveal that *syne1* and *ccdc175* RNAi could trigger this phenotype through disruptions in the cytoskeleton, whereas *med17* RNAi would cause it through the deregulation of RNApolII activity.

All and all, the differences in molecular function between these effectors highlight the diversity of processes controlled by hippo and reinforces the deregulated complexity found in dedifferentiation processes associated with tumorigenesis. They also put planarians on the spot as a model of choice to understand the fine tune of cell renewal in adult organisms.

Monoaminergic neural signaling: the role of riboflavin in planarian sensing

Heleven, Martijn[1]; Jaenen, Vincent[1]; Bijnens, Karolien[1]; Smeets, Karen[1]

1. Centre for Environmental Sciences; Hasselt University; Agoralaan gebouw D; Belgium.

Planarians have various neuronal populations defined by monoaminergic neurotransmitters, including dopamine, octopamine, and serotonin. However, the functional role of monoaminergic signaling in planarian sensing is still unknown. We performed an in-depth characterization of a monoaminergic neural system, prominently abundant both ventral and dorsal of the bi-lobed ganglia of the planarian ***Schmidtea mediterranea***. Different stressors were used to indicate that these neurons are involved in multiple facets of **planarian sensing**, orchestrated via **monoaminergic neurotransmission** such as dopamine. More specifically, an increased mitochondrial activity was observed after exposure to **light, chemical and physical stimuli**. Targeting mitochondria by modulating riboflavin conversion and neuronal mitochondrial transport negatively affected sensing and planarian physiological responses. To further characterize the role of flavins in monoaminergic neurons, we performed in-depth spectral analyses, rendering us the possibility to do **live imaging** to study mitochondrial transport and the functional role of riboflavin during planarian neural signaling.

Pharmacological activation of Notch signalling pathway impairs head regeneration and locomotion in the asexual *Schmidtea mediterranea*

Codino, Azzurra[1]; La Rosa, Letizia[1]; Pisano, Claudio[1]; Gustincich, Stefano[1]; Pandolfini, Luca[1]

1. Center for Human Technologies, Central RNA Lab; Italian Institute of Technology (IIT); Genoa 16152; Italy.

The evolutionary conserved Notch signalling pathway is a key regulator of animal neurogenesis, but its role in planaria nervous system regeneration has been poorly explored. Here, we employ a pharmacological approach to study the role of Notch signalling during the early phases of head regeneration. We treated planaria with a known Notch agonist, Yhhu-3792 (Yh), 30 hours post decapitation (hpd), during neoblasts proliferation and prior to neural fate committment. We found that Yh-treated worms had significantly smaller heads and eyes, compared to control animals, 5 days post decapitation (dpd). Moreover, Yh-treated worms showed an impaired exploratory behaviour. To investigate the molecular mechanisms underlying these macroscopic defects, we analyzed the transcriptomic profile of the neoblast-enriched G2 cells from anterior blastemas of control and Yh-treated animals, 18 hours after Yh/DMSO administration (at 2 dpd). We identified over 250 down-regulated and 1000 up-regulated genes, among which many known neural markers, such as *Stoned B* and *Synaptotagmin1*. We also identified a subset of transcripts with unknown function and predicted neural expression pattern: among them, *dd_Smed_v6_1440_0_1* is exclusively present within Tricladida and thus could be key for proper head regeneration, a unique feature of these flatworms. Further work will be required to elucidate its function and its link with the Notch pathway in the context of planaria head regeneration.

Genomic analyses reveal FoxG as an upstream regulator of *wnt1* required for posterior identity specification in planarians

Coronel-Córdoba, Pablo[1,2]; Pascual-Carreras, Eudald[1,2]; Marín, Marta[1,2]; Castillo-Lara, Sergio[1,2]; Magri, Marta[3]; Abril, Josep F[1,2]; Gomez-Skarmeta, José Luís[3]; Saló, Emili[1,2]; Adell, Teresa[1,2]

1. Department of Genetics, Microbiology & Statistics; University of Barcelona; Avinguda Diagonal, 643; Barcelona, Catalonia, Spain.
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Specification of the primary body axis requires the formation of an organizer. The features of organizers during adult regeneration remain unstudied. To that aim, we study planarians regeneration. The cWNT pathway specifies the anterior-posterior axis in planarians, since inhibition of *wnt1* or *notum* produces a switch in the antero-posterior identity. During the first 12h after amputation both *wnt1* and *notum* are expressed in any wound, but 36h later they become restricted to posterior and anterior facing wounds, respectively, forming the organizers. To decipher the mechanism that restricts the expression of *wnt1* to the posterior tip, we performed ATAC-Seq, ChIPmentation and RNA-Seq analysis of regenerating blastemas of wild-type and *wnt1* (RNAi) planarians. We found that already at 12h of regeneration the accessible CREs in posterior and anterior blastemas have changed, indicating that specific posterior chromatin changes induced by amputation occur much earlier than the formation of the organizers. We also found presence of the transcription factor FOXG motif in the first intron of *wnt1*, and demonstrate that this region have enhancer capabilities. Importantly, silencing of foxG inhibits the early phase of *wnt1* expression and phenocopies the *wnt1* (RNAi) phenotype, indicating its early role in specifying posterior identity. Finally, the *wnt1* intronic enhancer region could be evolutionary conserved, since this intron maintains a conserved position and contains FoxG binding sites.

Nonsense-mediated mRNA decay controls cell fate specification in *S. mediterranea*

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Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that degrades mutant transcripts harboring premature termination codons (PTCs). Recent studies show NMD also regulates up to a quarter of the wild-type transcriptome, though its developmental roles are not as widely studied as the quality control function for which it was named. To investigate NMD functions in *S. mediterranea*, we used RNA-Seq to characterize changes in gene expression following RNAi knockdown of the core effector *SMG1*. Established NMD targets in other systems were among the most upregulated transcripts. We also observed broad changes in gene expression consistent with altered cell fate specification – intestinal markers were increased, while markers for epidermal progenitors were reduced. The latter changes were associated with reduced cell density in the epidermis and constitutive activation of injury responses. Potentially explaining these results, expression of the gut transcription factors *GATA4/5/6* and *HNF4* was elevated in *SMG1(RNAi)* animals, and over half of corresponding ESTs in public databases include PTC-generating retained introns, making these transcripts logical targets for regulation via NMD. We hypothesize that degradation of *GATA4/5/6* and *HNF4* by NMD in non-gut lineages constitutes a novel and possibly conserved molecular switch controlling developmental cell fate specification. We will present results from experiments testing this model at the meeting.

m6A is required for resolving progenitor identity during planarian stem cell differentiation

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Planarians, are capable of regenerating any part of their body using adult stem cells called neoblasts. Although planarian regeneration has been studied for centuries, the regulation of new tissue formation and differentiation of cellular lineages during regeneration remains poorly understood. N6-methylation of adenosine (m6A) is a widespread modification to mRNAs, which is conserved from yeast to mammals. We found that m6A is highly enriched in neoblasts and is required for proper cell fate choice and cellular maturation. Using single cell RNA sequencing, following inhibition of the pathway, we discovered that m6A negatively regulates transcription of histone variants, and that m6A inhibition resulted in accumulation of undifferentiated cells throughout the animal in an abnormal transcriptional state. Analysis of >1000 planarian gene expression datasets revealed that the inhibition of the chromatin modifying complex NuRD had almost indistinguishable consequences, unraveling an unappreciated link between m6A and chromatin modifications. In our study, we characterized the roles of m6A pathway in planarian tissue maintenance and regeneration, and demonstrated that m6A is necessary for gene expression regulation of the neoblast cell cycle.

Smed-cbp-2 and *Smed-cbp-3* genes have functionally diverged to regulate planarian survival and stem cell differentiation

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Cell state transitions are associated with epigenetic changes that alter chromatin structure and gene expression. However, how this process is regulated genetically and epigenetically during neoblast self-renewal and differentiation is still not well-understood. In other models, histone acetylation is known to correlate with open chromatin structure and increased gene expression during cell differentiation. The conserved CBP/p300 gene family of acetyltransferases act as transcriptional co-activators regulating gene expression by acetylating histone and non-histone proteins. Recently, we and others identified 5 members of the *cbp* gene family in *S. mediterranea*. Interestingly, our results uncovered complementary roles for two of the orthologs: *Smed-cbp-2* seems essential for stem cell maintenance and survival, while neoblasts fail to differentiate and accumulate in the absence of *Smed-cbp-3*. As a further step to better understand the function of planarian *cbp* genes, we have recently performed high-throughput experiments after *cbp-2* and *cbp-3* silencing based on ATAC-Seq and RNA-Seq. Our current results suggest that the impact of these planarian *cbp* genes on neoblast biology might relate to their functions in the progression of the cell cycle and the composition of the neoblast niche. This approach will allow us to determine the function of *cbp-2* and *cbp-3* in neoblast biology and to better understand the regulation of planarian stem cells.

Molecular regulation of pluripotent stem cells in a highly regenerative planarian

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Pluripotent stem cells give rise to all cell types of the adult body and play a key role in regeneration. Their regulation at the molecular level is still poorly understood, especially in highly regenerative invertebrates. Planarians of the species *Schmidtea mediterranea* possess a pluripotent stem cell population (called neoblasts) in the adult stage. Those cells constantly proliferate and differentiate as part of homeostasis, and fuel the regeneration process. By generating single cell datasets after knockdown of genes involved in neoblast proliferation and differentiation, we are investigating their role in homeostasis and regeneration.

After RNAi treatment of five selected genes, including chromatin and cell cycle regulators, we observed phenotypes consistent with neoblasts defects, as well as a delay or arrest of regeneration. Cytometry analyses reveal drastic changes of the proportion of G1-G2 cell populations. We generated single cell datasets using SPLiT-Seq, allowing direct comparison between treatments. Preliminary analyses confirm a decrease of differentiated cells following inhibition of genes involved in differentiation, and a decrease of proliferating cells after knockdown of genes required for cell cycle progression.

Current analyses focus on the differentiation trajectories and a finer comparison of the cell types and states following the RNAi treatments. Our data will give insight into the molecular regulation of adult stem cells in regenerating animals.

Organization and development of neuronal cell types in the model *Schmidtea mediterranea*: a single-cell transcriptomics study

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Single-cell expression profiling technologies allow disentangling neuronal heterogeneity to investigate the complexity of nervous system development. Cellular specialization is incredibly prominent in the nervous system, where even in a single brain region, individual neurons may differ deeply in their molecular, anatomical, and functional features. Platyhelminthes like *S. mediterranea* continuously turn over all their tissues, including the brain, and can regenerate a perfectly organized and functional central nervous system de novo from a small body section within just a matter of days. Such extraordinary tissue plasticity finds its source in a population of somatic pluripotent adult stem cells: the neoblasts. In this work, we have profiled >200k cells in different condition of both wild-type and multiple knock-down animals, investigating the genetic networks that drives the differentiation of planarian adult stem cells into the neural lineage. We uncovered neuronal cell populations differentially abundant and explored the transcriptional control of neural differentiation. Finally, we discuss transcriptome-based linage trajectory prediction to reconstruct relationship between neuronal differentiation stages. Our work paves the way for studying neuronal diversity across the animal kingdom using single cell transcriptomics.

Identification of putative enhancer-like elements predicts regulatory networks active in planarian adult stem cells

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Planarians have become an established model system to study regeneration and stem cells, but the regulatory elements in the genome remain almost entirely undescribed. Here, by integrating epigenetic and expression data we use multiple sources of evidence to predict enhancer elements active in the adult stem cell populations that drive regeneration. We have used ChIP-Seq data to identify regions with histone modifications consistent with enhancer identity and activity, and ATAC-Seq data to identify accessible chromatin. Overlapping these signals allowed for the identification of a set of high confidence candidate enhancers predicted to be active in planarian adult stem cells. These enhancers are enriched for predicted transcription factor (TF) binding sites for TFs and TF families expressed in planarian adult stem cells. Foot-printing analyses provided further evidence that these potential TF binding sites are potentially occupied in adult stem cells. We integrated these analyses to build testable hypotheses for the regulatory function of transcription factors in stem cells, both with respect to how pluripotency might be regulated, and to how lineage differentiation programs are controlled. We found that our predicted GRNs were independently supported by existing TF RNAi/RNA-Seq data sets, providing further evidence that our work predicts active enhancers regulating adult stem cells and regenerative mechanisms.

The new chromosome-scale *S. mediterranea* reference genome assembly and annotations—an update

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The *S. mediterranea* genome harbours significant assembly challenges, such that even the current reference assembly based on PacBio long-read sequencing remains fragmented into 400 contigs. Combining high-accuracy PacBio-CCS sequencing with Hi-C scaffolding, we have succeeded in obtaining a chromosome-scale and haplotype-phased *S. mediterranea* assembly. Further, we have functionally annotated the genome using a mixed Oxford Nanopore/short read Illumina high fidelity transcriptome assembly. In their talk, Jochen Rink and Luca Pandolfini will focus on detailing the improvements over existing community resources and remaining limitations regarding the new reference genome and annotations, as well as providing a brief outlook on ongoing planarian genomics research.

Smed-ets-1 regulates epidermal lineage landscape in a cell non-autonomous fashion via basement membrane remodelling

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6. Equal Contribution.

Extracellular matrix (ECM) is an important component of stem cell niche. Remodelling of ECM mediated by ECM regulators such as MMPs plays a vital role in stem cell function. However, the mechanisms that define the function of ECM regulators in the stem cell niche is understudied. Here, we explored the role of transcription factor, ETS-1 in regulating the expression of the ECM regulator, *mt-mmpA* thereby, modulating basement membrane thickness. In planarians, basement membrane around gut/inner parenchyma is thought to act as pluripotent stem cell niche. It has been shown that the early epidermal progenitor migrate outward from this region and progressively differentiate to maintain terminal epidermis. Our data shows thickening of BM in the absence of *ets-1* results in defective migration of stem cells progeny. Furthermore, the absence of *ets-1* led to a defective epidermal progenitor landscape, inspite of its lack of expression in those cell types. Together, our results demonstrates the active role of ECM remodelling in regulating stem cell function in non stem cell autonomous manner.

More than a gut feeling? The composition, diversity and variability of the planarian microbiome

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Many animals live in close association with complex microbial communities. The so-called microbiome includes bacteria, fungi and viruses, which often contribute to the hosts physiology and health. Recently a functional link between endogenous bacteria, planarian physiology and the regeneration process was described. Disturbances of the microbiome resulted in tissue lesions and degeneration, although the exact function of the associated bacteria for planarian physiology remains unknown. Therefore, in this study, we investigated the planarian microbiome more in detail to gain insight into the composition, diversity and variability. We found that bacteria were present at the epidermis and in the gut of *Schmidtea mediterranea*. Via 16S rRNA sequencing, we showed an overall abundance of Betaproteobacteriales in diverse conditions, although at lower taxonomic levels variation between individuals and conditions was observed. The microbiome was sensitive to external stressors, especially after exposure to silver nanoparticles. Finally, our results also suggested fluctuations in the planarian microbiomes during the different phases of the regeneration process. Together, these findings form a solid basis to understand the nature and variability of microorganism associations to further elucidate their role in planarian physiology.

The role of piRNAs in planarian stem cells and their potential role in immune defense

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PIWI proteins utilize small RNAs called piRNAs to silence transposable elements, thereby protecting germline integrity. In planarian flatworms, PIWI proteins are essential for the animals' fantastic regenerative abilities, which depend on their abundant stem cell population, termed neoblasts. We previously characterized planarian piRNAs and examined their role in conjunction with their PIWI binding parterns in neoblast biology. We found the planarian PIWI proteins SMEDWI-2 and SMEDWI-3 to cooperate in degrading active transposons via the ping-pong cycle. Moreover, we unexpectedly discovered an additional role for SMEDWI-3 in planarian mRNA surveillance. Here, I will report on our latest findings on planarian piRNAs with a particular focus on their potential role in immune defense and on mRNA surveillance in the planarian epidermis.

Why some animals regenerate while others cannot: evolution of regeneration in planarians

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Regeneration is widespread throughout the metazoans but varies considerably even among closely related species. This raises the question: Why can some animals regenerate while others cannot? We investigate the evolution of regeneration in planarian flatworms. Planarians display a wide range of regenerative abilities, from whole-body regeneration in the model species *Schmidtea mediterranea* to comparatively poor regeneration, e.g., in *Dendrocoelum lacteum*. Easy to maintain in the lab and amenable to molecular laboratory techniques, planarians offer an ideal system to explore the underpinning of the evolution of regeneration.

Our group has established a collection of 50 planarian species collected in the wild. Through de novo transcriptome assembly, we have constructed a multigenic phylogeny with the major planarian clades. This, combined with an assessment of species-specific head regeneration ability, led to the identification of multiple independent potential events of loss of head regeneration. Furthermore, we have probed the role of Wnt signalling in these regeneration defects and found that elevated levels of Wnt signalling are associated with regeneration defects across several planarian lineages. Based on this, and on the planarian ecology, we propose a multi-level model for the evolution of regeneration in planarians that link βCatenin-1 regulation, planarian reproductive strategy and the planarian habitat.

Phylotranscriptomics uncovers a complex evolutionary history for the planarian genus *Dugesia* (Platyhelminthes, Tricladida) in the Western Mediterranean

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The Mediterranean is one of the most biodiverse areas of the Paleartic region and it houses an important number of *Dugesia* species. Based on large data sets of single-copy orthologs obtained from transcriptomic data, we investigated the evolutionary history of the genus in the Western Mediterranean area. The results corroborated that the complex paleogeological history of the region was an important driver of diversification for the genus. These processes led to the differentiation of three main biogeographic clades. The internal relationships of these major clades were analysed with several representative samples per species. The use of large data sets regarding the number of loci and samples, as well as state-of-the-art phylogenomic inference methods, allowed us to answer different unresolved questions about the evolution of particular groups, such as the diversification of *D. subtentaculata* in the Iberian Peninsula and its colonization of Africa. Finally, we analysed here for the first time a comprehensive number of samples from several asexual Iberian populations whose assignment at the species level has been an enigma through the years. The phylogenies obtained with different inference methods showed a branching topology of asexual individuals at the base of sexual clades. We hypothesize this unexpected topology is related to long-term asexuality, rendering this lineage a potential model to study the genetic and evolutionary consequences of fissiparity.

Department of Planaria, At Home

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What it takes to start a Planaria (Tricladida) laboratory at your own home? Possibilities, roadblocks and DIY solutions regarding collection, transportation, identification and general well-being of freshwater species will be discussed as well as a progress from generic photoshooting, over histology high-resolution digitalisation, towards the opposite side of the technology spectra - preparation of animals to be scanable by high-tech preclinical instruments, be it Fluorescence camera (BRUKER Xtreme) or 7T Magnetic Resonance Imager (MR Solutions). Results obtained during last few years of our mostly free-time work will be presented together with plans for future steps.

This work has been supported by Czech Union for Nature Conservation (ČSOP), in particular by grants no. 122123 "Mapování ploštěnky *Crenobia alpina* v okolí Hojné vody" and no. 122210 "Mapování ploštěnky *Dugesia gonocephala* v povodí Svinenského potoka" and by European Regional Development Fund (Project No. CZ.02.1.01/0.0/0.0/18_046/0016045).

Posters

Poster – 1

Oxford Nanopore Sequencing for Single Cell Analysis: a Missing Piece of the Planarian Regeneration Puzzle?

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Tissue regeneration has long been fascinating the research world, however the era of **SCA** is shedding a new light on our understanding of molecular interactions at a cellular level. Of particular interest are planarians, animals which can regenerate into a complete worm, starting from almost any body part. This capacity is given by specialised **PSC**s, called neoblasts.

Alternative splicing and post-transcriptional regulation were shown to be of paramount importance in stem cell mechanisms. It was described that conserved alternative splicing pathways in planarian **PSC**s are regulated by **CELF** and **MBNL** proteins in an antagonistic manner. One member of the **CELF** family, *Smed-bruno-like*, or the bruli factor, was shown to substantially impact regeneration.

We therefore aim to investigate alternative splicing events in planarians, at single cell resolution. We hope to unravel a deeply conserved mechanism, not just in planarians, but within multiple species. We will start this journey by focusing on bruli, creating knockdown animals which we will sequence and compare to the whole worm.

Technologically, while Illumina platforms are more established in **SCA**, **ONT** sequencers are of great promise, as a key advantage is that they produce considerably longer reads. Alongside our industrial partner we aim to optimise a **SPLIT-Seq** protocol using the **ONT** platforms.

Poster – 2

The RNA polymerase II transcription initiation landscape of *Schmidtea mediterranea*

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The flatworm *Schmidtea mediterranea* has become an established model organism for the study of the molecular basis of whole-body regeneration. This process requires precise spatiotemporal control of gene expression in order to reform the appropriate missing tissue and organs. In analogy to development, is likely that also regeneration proceeds via the organization of key genes into gene regulatory networks (GRNs) controlled by transcription factors binding to Transcriptional Regulatory Elements (TREs) such as promoters and enhancers. Despite recent advances in planarian genome assemblies and the development of genome-wide assays probing various aspects of chromatin state, the functional annotation of TREs remains a challenge. Towards this goal, we have adapted protocols to isolate and sequence nuclear, short, unspliced and 5' capped RNA species characteristic of RNA polymerase II activity at TREs. We show that this assay is able to reveal active TREs possessing chromatin features of both promoters and enhancers. Applying our assay to the differential analysis of the sexual and asexual strains of *S. mediterranea* allowed us to identify TREs specifically associated with sexual reproduction, as well as the associated transcription factor binding motifs.

Poster – 3

The influence of epidermal piRNAs on the immune defense in *Schmidtea mediterranea*

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PIWI proteins and their co-bound small RNAs, piwi interacting RNAs (piRNAs), are widely known for silencing transposable elements both transcriptionally and post-transcriptionally. The primary source of piRNAs are piRNA clusters that house remnants of transposable elements and coding genes as potential targets for silencing. However, recent studies revealed additional functions of piRNAs in a wide variety of animals, such as koalas or mosquitoes, by establishing their link to innate immunity. As the TRAF gene family (important transducers of innate immunity) is greatly expanded in the planarian flatworm *S. mediterranea* and represents a prime targets of piRNAs, we hypothesized that there might also exist a connection between epidermal piRNAs and the innate immune response in S. mediterranea. To that end I characterized the planarian epidermal piRNA and mRNA response in response to immune stimuli, and I studied the TRAF expansion in S. mediterranea in more detail. Taken together, my experiments uncover a potential role of piRNAs in planarian innate immune system, a connection that is likely present in the entire order Tricladida.

Poster – 4

The role of Elac2 ribonuclease in regeneration and small non-coding RNA metabolism in *Schmidtea mediterranea*

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Planarian species, *S. mediterranea*, possess a remarkable capability of regenerating missing tissues, and hence is considered as one of the best model organisms to study regeneration in-depth. In planarians and in many other organisms, miRNAs have gained recent limelight for being involved in the regulation of regeneration. Accordingly, our goal was to explain the involvement of other cellular non-coding (nc) RNAs in regeneration. To do that, we decided to disrupt the expression of RNases having all of the following 3 characteristics: known to be involved in ncRNA processing and maturation; their disruption in humans is implicated in phenotypically manifested Mendelian diseases; and, have known planarian homologs. Elac2, a mitochondrial RNase involved in mainly tRNA, but also other ncRNA, maturation processes—was one of the targets with all the desired features. Therefore, in the current research we were motivated to describe effects of disrupted Elac2 expression in planarians, phenotypically and transcriptomically, via RNAi and next-generation sequencing (NGS). Our preliminary results show a delay in the usual course of development, especially at 5 days post amputation in regenerating Elac2 knockdown worms. NGS was performed on small and long RNA fractions and the results indicated an uneven accumulation of tRNA fragments and downregulation of genes related to cell division and neuronal function—which might have a cause-effect relationship as per previous reports.

Poster – 5

Towards genome-wide discovery of transcription factor binding sites contributing to planarian regeneration

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Planarian stem cells produce every cell type in the organism. The selection of cellular identity is driven by transcription factors (TF), which activate cell-type specific gene expression programs. Many lineage specifying TFs are known in planarians, yet their direct targets, which are likely essential for cellular differentiation and lineage production, are unknown. The lack of *in vivo* tools in planarians for analyzing TF binding sites, has further limited progress in TF target discovery. Here, we optimized an *in vitro*-based DNA-Seq approach for identifying potential TF binding sites (DAP-Seq), previously developed for use in plants (Bartlett et al. 2017), and applied in pilot experiments to several TFs. This *in vitro*-based approach allows an antibody-independent discovery of potential binding sites, which together with a computational framework that integrates available ATAC-Seq and scRNA-Seq data, facilitates prediction of potentially *bona fide* direct regulation in planarian stem cell lineage specification.

Poster – 6

Smed-cbp-3 is required for planarian stem cell differentiation and cell cycle progression

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Some of the main questions in planaria regeneration are focused on deciphering how their pluripotent stem cells differentiate into all cellular lineages. CBP (CREB-binding protein)/p300 proteins belong to a conserved gene family which functions as transcriptional co-activators regulating gene expression by acetylating histone and non-histone proteins and providing transcription factors scaffold. In different models, CBP/p300 shows important roles in a wide range of cellular processes, such as stem cell proliferation, differentiation, cell death, DNA damage response and tumorigenesis. Several CBP homologues have been identified in planarians and functional analyses have allowed to identify an important role for *Smed-cbp-3* in stem cell commitment and differentiation during regeneration. Our recent work suggests that, in a sublethal irradiation context, planarian stem cells and specialized progenitors accumulate in the absence of *cbp-3* due to its inability to pursue differentiation. This inability might be caused by cell cycle alterations, as cycling cells were found to be accumulated in G2-phase. In addition, *piwi1* mRNA negative / PIWI1 protein positive cells were found to perdure longer in the absence of *cbp-3*, which suggests that *cbp-3*(RNAi) planarians retain cells in the last steps of differentiation. Overall, these results improve our current knowledge on *cbp-3* function in neoblast biology and differentiation.

Poster – 7

Screening Setup for *Schmidtea Mediterranea*

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Over the past 20 years, research has established *Schmidtea mediterranea* as a versatile model system for regeneration, stem cell function, and other intriguing phenomena. Experiments are still primarily done in individual culture dishes, which limits experimental conditions.

Towards the goal of increasing the experimental throughput capacity of the model system, we have developed a modular workflow involving the independent cultivation of different experimental groups and phenotype documentation. For animal cultivation, we adopted a drip system design developed by the Stowers Institute in Kansas City, USA. It can accommodate 125-240 experimental groups at a time, depending on the size of the cultivation chambers. We use an in-house manufactured light stand and a commercial DSLR camera with a macro-objective for the phenotype documentation. The co-imaging of unique QR codes for each experimental group allows the automatic storage and retrieval of image files via a database.

As proof of principle, we have recently performed a trial RNAi experiment of 86 different target genes and controls. Although some details still need to be improved, our set-up now allows the parallel analysis of many RNAi conditions or other experimental variables.

Poster – 8

The cellular bases of tissue turnover in the planarian epidermis

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Planarian flatworms maintain high rates of tissue turnover in an organismal level. The adult pluripotent stem cells which are widely distributed throughout the body proliferate continuously to replace the dying of old cells. Interestingly, the planarian maintains tissue homeostasis in a dynamic way. The size of the planarian varies in response of food availability due to the change of cell number, making the planarian a unique model for studying tissue turnover. In this study, we aim to identify the cellular underpinnings of tissue turnover in the planarian epidermis. First, we built an imaging pipeline to quantify the turnover rate of epidermis and uncovered that there are regional differences of turnover rates in epidermis along the dorsal-ventral and anterior-posterior axes and that the turnover of epidermis is sensitive to the nutritional status. Furthermore, we also intended to understand how epidermal cells are removed by developing a live-imaging protocol and the results showed that epidermal cells undergo basal extrusion and enter the interior of the animal. Taken together, our study elucidates how epidermal turnover is maintained in the planarian.

Poster – 9

How does dorso-ventral signaling shape bilateral symmetry in Planarians?

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Bilateral symmetry of the body plan is the defining feature of a large class of animals. The establishment of bilateral symmetry remains a fascinating challenge that has so far been mainly studied in developmental systems. The ability of Planarians to regenerate their bilaterally symmetric body from asymmetric amputation fragments provides new opportunities for probing the mechanistic and conceptual underpinnings of the phenomenon. In embryonic development, the signaling processes which establish the different body axes are intertwined. But how does dorso-ventral signaling shape bilateral symmetry during regeneration in Planarians? BMP4/ADMP signaling has been shown to be the major conserved dorso-ventral patterning regulator. Our approach relies on the quantification of BMP4 signaling activity in planarian tissues. We aim to identify the BMP4-specific receptors and extracellular signaling modulators by performing quantitative Western blotting of animal extracts treated with RNAi against different TGF-beta pathway components. As a next step, we will investigate their role in regeneration of bilaterally symmetric body parts. By documenting the expression of pathway regulators and by testing cause/consequence relationships and cross-talk with other pathways via RNAi, we hope to shed light on the mechanisms of midline maintenance and *de-novo* establishment during regeneration.

Poster – 10

The evolution of size-dependent gene regulatory networks in planarians

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Size is one the most important characteristics of an organism. It determines the physical forces that govern its life, its metabolism, its predation pressure, and by virtue of these properties, greatly impacts its life history and ecological niche. Despite the importance of size, we know little about the mechanisms determining species-specific size. Planarians are a great model to investigate this due to their unique size biology. They are incredibly plastic in their body size, being able to reversibly grow and degrow over several orders of magnitude. Moreover, size also varies tremendously across the planarian clade, ranging from a few millimeters up to a meter. As changes in gene regulatory networks (GRNs) are key drivers of phenotypic evolution, we need to understand the logic of the GRNs governing this trait to understand the evolution of species-specific body size. The comparative power of planarians will then allow us to track the evolution of these networks and shed light on the evolution of planarian body size. The overarching goal of my PhD project is thus two-fold: first, to determine size-dependent GRNs in the model species *S. mediterranea*. Second, to investigate how size-dependent GRNs have evolved in different planarian species and if these changes underlie planarian body size diversification. In this poster, I will discuss my current effort in developing single cell combinatorial indexing RNA and ATAC sequencing in planarians, a key approach for my project.

Poster – 11

Knockdown of *Schmidtea mediterranea* homolog of the human huntingtin gene affects animal reactions to environmental stimuli

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Phylum Platyhelminthes, containing free-living planarian, are the simplest organisms in the phylogenetic tree of life which developed the body plan with bilateral symmetry enabling cephalization and centralization of their nervous system. A planarian brain can be divided into four functionally and structurally different domains responsible for sensing various external signals. To better understand the mechanisms underlying these processes we silenced in *Schmidtea mediterranea* the candidate genes shown to be important for nervous system development and functioning in other animals. One of these genes was *Smed-htt* the planarian homolog of the human huntingtin gene. Huntingtin is protein found in many cells, with the highest levels of activity in the brain cells. In human this gene is linked to Huntington's disease, a neurodegenerative disorder characterized by loss of striatal neurons.

To silence the *Smed-htt* gene we applied standard RNAi technology. The level of gene silencing was examined by qPCR. In addition, *Smed-htt* mRNA accumulation and distribution in *S. mediterranea* cells was determined using whole-mount in situ hybridization. Finally, the reaction of planarians lacking the Smed-htt protein to typical environmental stimuli was tested. As a result, we found that *Smed-htt* gene silencing caused in planarian a dysphagia and loss of photophobia. The important implications of our findings for the understanding of central nervous system evolution are discussed.

Poster – 12

Ex-fissiparous strain of *Girardia tigrina* from Central Bohemian region

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Girardia tigrina—alien flatworm species from North America—is mostly fissiparous in Europe, including widespread distribution in Czech water bodies. Rare cases of so called ex-fissiparous animals were documented in Italy, Spain and England.

Preliminary results of another case of fertile flatworm of *G. tigrina* will be presented. Animals were found in Central Bohemian region during late May and June 2022. Three localities with suspiciously large specimens of stripped or spotted phenotype were found. The said areas are old sand quarries currently used for recreational purposes.

Flatworms were taken to our lab and kept in de-chlorinated tap water (12 °C) enhanced with antibiotics (gentamicine 1:2000 solution) and fed with beef liver paste once per week. First cocoons appeared shortly after the start of culturing, hatchlings appeared circa a month later. Animals—adult nor juveniles—did not carried any signs of fission. Basic histology examination was done. Further histology examination, karyotype evaluation and test of fertility per cocoon is ongoing.

Poster – 13

Which are the most useful nuclear and mitochondrial genes for molecular systematics in marine flatworms?

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Increases in the diverse use of molecular methodologies in ecology and systematics have driven the necessity for a more comprehensive understanding of the benefits and limitations of different genetic markers employed. Specifically, given the multiple existing approaches for the study of the taxonomy and systematics of Polyclad flatworms and the increasing use of molecular tools in the research of their phylogeny, the objective is to evaluate the usefulness of a range of nuclear ribosomal (18S rDNA, 28S rDNA) and mitochondrial ribosomal (16S rDNA, *Cytb*, *Cox1*) genetic markers. We estimated the rates of substitutions of the studied markers, finding *Cytb* the most variable and 18S rDNA the least variable among them. We identified the transition to transversion (Ti/Tv) ratio of the different genes, concluding that overall, the number of transversions is higher than that of transitions in this taxon. Mutation rates and Ti/Tv ratios of the different genetic markers were assessed in polyclad flatworms for the first time. Lastly, the results show that the third codon position of the studied protein-codifying genes was highly variable, observing this position is saturated in the *Cox1* marker but not for *Cytb*. Future studies should focus on the use of mitochondrial genes (*Cytb* and *Cox1*) when analysing phylogenetically closely related species and the 28S rDNA nuclear marker for resolving higher taxonomical clades.

Poster – 14

Improving planarian transcriptomic data using Oxford Nanopore Technologies sequencing techniques

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Planarians are model organisms in the study of regeneration and adult tissue renewal. Despite its importance their genomic and transcriptomic diversity is not completely understood. In this work we propose a new approach to obtain full length transcripts using the sequencing methods of Oxford Nanopore technologies (ONT) for the first time in *Schmidtea mediterranea*. Using ONT we could successfully assemble a new transcriptome and correct the errors derived from the technique using the software ESTscan. We also demonstrated that data derived from ONT sequencing technology is useful to perform differential gene expression. The DGE analysis between different physiological states in the planarian allowed us to undercover key genetic pathways to understand how planarian control regeneration and body size. In conclusion, in this work we established ONT as a reliable method for de novo transcripts assemblies and DGE analysis in *Schmidtea mediterranea*.

Poster – 15

Transcriptional control of planarian stem cell differentiation

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Research on mechanisms involved in pluripotent cell differentiation into several cell lineages are crucial in developmental biology. Previous studies have demonstrated transcriptions factors (TFs), together with epigenetic regulators, are key actors in stem cell differentiation. However, only few mechanisms of pluripotent cell regulation have been studied thoroughly in a few model species.

Recently, methods involving single cell RNA sequencing (scRNA-Seq) have democratized the analyses of cell populations and differentiation trajectories in less conventional model organisms. At the same time, detecting transcriptional changes in single cell assays is challenging, as experiments including multiple conditions together with biological and technical replication are costly and prone to batch effects. Here, using a novel scRNA-Seq approach that allows to combine RNAi conditions in replicates, we performed differential expression analysis of several TFs with single cell resolution. We report the first whole organism identification of factors involved in the gene regulatory changes that underlie all major cell lineage stem cell differentiation of planarian stem cells to all major planarian cell lineages. Altogether, this will allow us to describe the transcriptional landscape of planarian stem cell differentiation.

Poster – 16

The role of acetylcholine signaling in intestinal regeneration

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As a crucial barrier with the outside world, the intestinal epithelium is in a constant regenerative state, which is supported by a vast pool of local stem cells. The **neural network**, which controls intestinal functions, has been shown to determine intestinal stem cell fate and regulate growth and repair of the intestinal epithelium. Moreover, Neural control elements are highlighted as being essential for both regeneration and the integration of environmental signals with intestinal homeostasis. However, the tissue dynamics during **intestinal regeneration** are still poorly understood, especially the communication and coordination of regenerative cues in this context. Using *Schmidtea mediterranea*, we investigate the role of **acetylcholine**, in intestinal regeneration, in order to show how enteric neurons signal to intestinal progenitor cells to migrate and differentiate as response to injury and during homeostasis. I will discuss preliminary data regarding transcriptional knock-down (RNAi) of choline acetyltransferase, as well as effects of exposure to acetylcholine receptor antagonists, such as atropine and hexamethonium. To assess intestinal function we have developed a live *in vivo* assay based on fluorescent dextrans. Closely examining the mechanisms of intestinal tissue remodeling, will improve our understanding of gut regeneration and de novo organogenesis, and allows us to develop new approaches to direct the morphology of post-embryonic tissues or organs.

Poster – 17

Impaired (neuro)regeneration in *Schmidtea mediterranea* after exposure to micro- and nanoplastics

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Plastics are indispensable in our daily lives. They are used in various applications and are found in many places like hospitals, cars and in homes. Due to a lack of proper waste management and disposal, plastic degrades to micro- and nanoplastics (MNPs), less than 5 mm and 100 nm respectively, and ends up in the aquatic environment. So far, we know that MNPs are potentially harmful but detailed knowledge on the effects of specific subfractions is missing, especially for developing organisms. We use the regenerative capacity of the planarian model system, *Schmidtea mediterranea*, as a proxy to study developmental toxicity. Our main aim is to link physicochemical properties with induced effects, to properly define MNP-specific adverse outcome pathways in the function of new risk assessment strategies. To this end, *S. mediterranea* was exposed to carboxylated, polystyrene particles of 50 nm, 200 nm, 1 µm and 2 µm. All particles were taken up through the epidermis and intestine and impaired regeneration. More specifically, eye development and the formation of the anterior commissure were delayed. To further understand these results, we are focusing on the effects of both micro- and nanoparticles on stem cell and redox dynamics.

Poster – 18

The transcription factor *Mlig-hesl1* is essential for stem cell maintenance and regeneration in the flatworm *Macrostomum lignano*

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Conventional neoblast-specific genes identified in asexual planarians mark both somatic neoblasts and germline in the hermaphroditic marine flatworm *Macrostomum lignano*. This hinders targeted isolation of somatic neoblast populations as well as identification of specific molecular pathways implicated in pluripotency maintenance during development and regeneration in the worm.

Based on the scRNA-Seq data, we tested several candidate genes with a specific expression pattern in putative pluripotent and/or somatic neoblasts of the worm. RNAi knockdown of one of the genes, *Mlig-hesl1* (a homolog of *Hes1-like bHLH* family of transcription repressors – a Notch pathway effector), resulted in a clear phenotype in the neoblast-marked transgenic lines of *M. lignano*. *Mlig-hesl1* RNAi leads to delay or termination of regeneration. Strikingly, the phenotype is preceded by a significant decrease in the number of somatic neoblasts during homeostasis while the germline remains intact. After amputation, the depletion is even more pronounced, while their migration to the wound site remained unaffected.

Mlig-hesl1 can delineate somatic pluripotent neoblasts from the dividing germline of *M. lignano*. Therefore, *Mlig-hesl1* may serve as a powerful somatic neoblast marker to study regeneration mechanisms in the worm. Importantly, our results pinpoint a crucial role of Notch signaling in stem cell maintenance, differentiation, and regeneration in *M. lignano*.

Poster – 19

Schmidtea mediterranea regeneration is affected by a disruption of small non-coding RNA pool after PARN silencing

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Small non-coding RNAs (sncRNAs) are crucial participants in almost all biological processes, including regeneration. To study regeneration on molecular level, planarians are powerful model organisms. Here, we investigated the role of sncRNAs in *S. mediterranea* regeneration. For this aim, we examined the effect of RNA metabolism disorder upon ribonuclease (RNase) depletion. We selected several candidates, homologues of human RNases whose deficiencies lead to Mendelian diseases. One of them is a planarian homolog of PARN, an exoribonuclease that cleaves poly(A) tails of mRNAs and affects the stability of a variety of sncRNAs. Our research showed that the knockdown of PARN caused delayed regeneration, mainly characterised by photoreceptor abnormalities, such as cyclopia and eyelessness. To identify sncRNAs, including RNA-derived fragments that are accumulated during regeneration, high throughput sequencing was performed. We discovered that there was a loss of several types of sncRNAs in knockdown planarians. Therefore, we hypothesize that the reduction of miRNA and RNA-derived fragments from tRNA, rRNA and snoRNA, associated with knockdown of PARN, might affect the regeneration process. Hence, more research into the role of sncRNAs in regeneration is important.

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