6th European Meeting on Planarian Biology

13 – 15 October 2024
Platja d'Aro — Catalonia

ABSTRACTS BOOK

Invited Speakers:

Bo Wang
(Stanford University, USA)

Oded Rechavi
(Tel-Aviv University, IL)

Sessions on:

Stem cells & Regeneration
Immune & Toxin Responses
Regeneration & Stem Cells
Beyond *S. mediterranea*
Planarian Cell Biology
New Technologies

Organizers:

Teresa Adell
(*Universitat de Barcelona, CAT*)
Jordi Solana
(*University of Exeter, UK*)
Jochen Rink
(*MPI-NAT, DE*)

Thanks to: The Company of Biologists Novogene

EMPB2024

6th European Meeting on Planarian Biology

October 13^{th}–15^{th}, 2024

Platja d'Aro, Catalunya

https://compgen.bio.ub.edu/EMPB2024

#EMPB2024

Organized by:

Teresa Adell
Universitat de Barcelona, Catalunya

Jordi Solana
University of Exeter, United Kingdom

Jochen Rink
Max Planck Institute for Multidisciplinary Sciences, Germany

Edited by:

Josep F Abril
Universitat de Barcelona, Catalunya

Contents

About the Meeting

Introduction

We are happy to welcome all of you to the 6^{th} European Meeting on Planarian Biology (EMPB2024) that takes place from October 13^{th} to 15^{th}, 2024, at Hotel Cap Roig in Platja d'Aro, Catalunya. This meeting continues the tradition of previous meetings in this series: Münster 2010, Kyoto 2011, Dresden 2013, Oxford 2015, Sant Feliu 2016, and Sant Feliu 2022. This edition will gather 70 researchers from 26 institutions from 14 countries all around the world.

In line with those previous editions, the aim of the meeting is to bring together the European planarian research community to discuss recent advances in flatworm biology, to exchange tips and tricks and 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. Talks by postdocs and graduate students will be an integral part of the program, as well as plenty of time for discussion, poster sessions, round-table sessions and simply time for socializing by the sea.

In this sense, we are really pleased to have Dr. Bo Wang, from Stanford University (USA) and Prof. Oded Rechavi, from the Tel-Aviv University (Israel), as **invited speakers** as their numerous contributions have improved significantly our understanding on evolution, regeneration, or epigenetics, among other topics. Furthermore, the EMPB2024 Organizing Committee has selected 22 talks and 30 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.

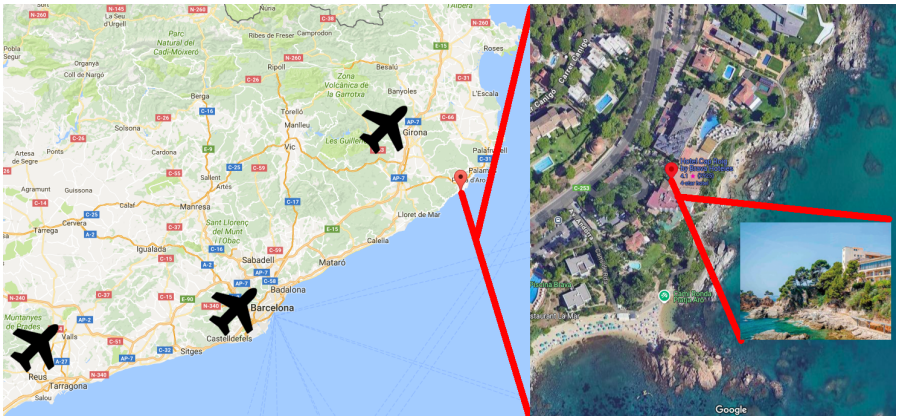
Talks abstracts follow same ordering as in the program sessions—titles on that section are hyperlinked to each abstract page—, while the posters abstracts were sorted alphabeticaly by first author surnames. Presenting author names are underlined on the abstracts. You can find an index at the end of this 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 Cap Roig

The meeting will be held at **Hotel Cap Roig**, Av. Andorra, 18, Platja d'Aro – Calonge (Costa Brava, Girona), Catalunya. At a distance of few kms from Palamós, the clear Mediterranean Sea borders the Cap Roig Resort and is surrounded by the well-known "Camí de Ronda", between pine trees, sea and rocks, on its magnificent outdoor terrace, you can contemplate the Cap Roig cove and the adjoining cliffs.

+0034-972-652-000



Google Maps Coordinates: Latitude 41.827754 – Longitude 3.084019

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 Platja d'Aro. A taxi from the Girona airport to Platja d'Aro costs about 60–80 Euros (Official rates link).

By Car:

When driving from Barcelona drive to C-33 that continues to AP-7, take the exit to C-35 on the motorway AP-7 to reach C-65 and then C-31, to finally reach GI-665 to Platja d'Aro; then, follow C-253 to Calonge. You can find Google-Maps detailed instructions from this link.

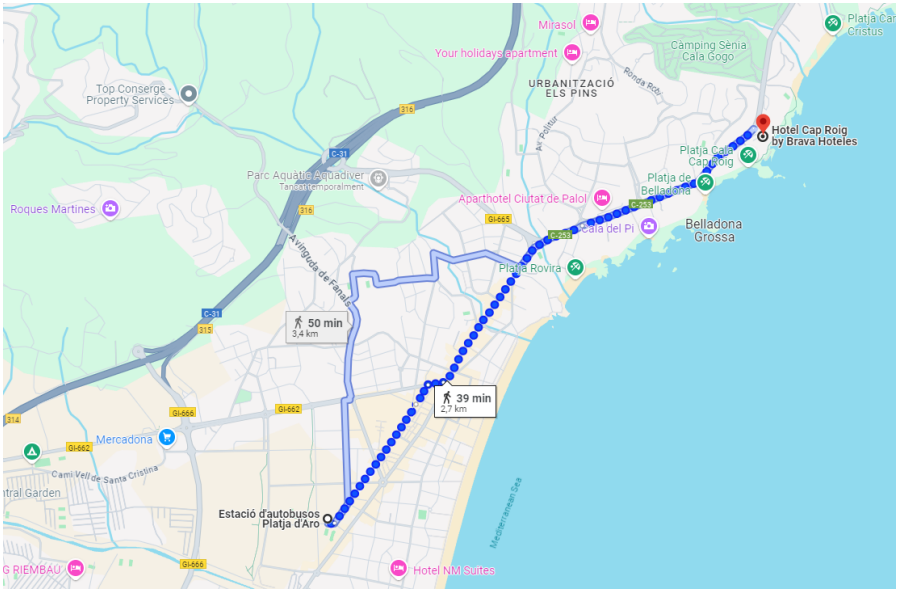
By Bus:

From Barcelona Airport to Cap Roig Hotel (Platja d'Aro):

If you arrive to Barcelona Airport (Terminal 1 or Terminal 2), you can take a regular bus. It is advisable to buy the tickets in advance, to ensure having a seat at the desired time. This is the webpage of the company:

https://compras.moventis.es/online/

There are 2 buses in the morning that will arrive on time to the Meeting in Patja d'Aro. The bus also stops in Barcelona city center (Estació del Nord), in case you are already in Barcelona. When you buy the tickets, make sure you ask for **Platja d'Aro (Sarfa-Estació)** stop. A timetable with all the buses is available from this link on the meeting web page. Once in Platja d'Aro, you can walk to the Hotel—it is around 40 minutes—, or you can take a local bus or a taxi. Here you have one walk through captured from Google Maps:



From the Cap Roig Hotel (Platja d'Aro) to Barcelona Airport:

To return on Tuesday, Oct 15th, there are different possibilities with the regular bus; here you have a link with the timetable. However, we can arrange a Bus for all participants if there is enough people. Please, you can contact tadellc at ub.edu if you would be interested in a Bus that goes directly from the Hotel to Barcelona Airport on the 15th at around 15:00h (it will arrive to the Airport at around 17:00).

Barcelona Airport – Barcelona city

You should take the 'AeroBus' bus (https://aerobusbarcelona.es/?lang=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 - Platja d'Aro

'Estació del Nord' is the central Barcelona bus station. There you can take a Bus to 'Platja d'Aro (Sarfa-Estació)' from MOVENTIS company. More information about this bus line at: https://compras.moventis.es/online/selection

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 60 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 EMPB2024 sponsors:

Sessions Program

Sunday, October 13th

13:00–15:30 Registration at Hotel Cap Roig

16:00–16:15 **Welcome and Opening Session**

16:15–17:15 **Keynote Invited Talk by Bo Wang**
[Stanford University, CA, USA]
Learning the super power of animal diversity onecell type at a time.

17:15–19:15 **Session I – Stem cells and Regeneration**
Chair: *Omri Wurtzel*

17:15–17:45 Adriaan Meiborg
[EMBL-Heidelberg]
A single-cell view of growth dynamics in S. mediterranea.

17:45–18:15 Daniel Moreno-Blas
[Universitat de Barcelona]
Cellular senescence in planarian regeneration.

18:15–18:45 Kai Lei
[Westlake University]
The role of mitochondria in planarian neoblast pluripotency and regeneration.

18:45–19:00 Alberto Perez-Posada *Short Talk*
[University of Exeter]
The regulatory logic of Planarian stem cell differentiation.

19:00–19:15 Sophie Peron *Short Talk*
[University of Exeter]
Deciphering clonal lineages using natural somatic mutations in an entire adult organism.

19:15–20:00 *Welcome cocktail*

20:00–21:30 *Dinner*

21:30– **Official Conference Opening Surprise**
(*dressed for a walk on the beach*)

Monday, October 14th

09:00–11.00 **Session II – Immune and Toxin Responses**

Chair: *Karen Smeets*

09:00–09:30 Omri Wurtzel
[Tel-Aviv University]
Elucidating the role of the immune system in Planarian regeneration.

09:30–10:00 Prasad Abnave
[NCCS Pune]
The Role of PGRPs in Planarian Immune Resilience to Bacterial Infection.

10:00–10:30 Leonard Drees
[Max Plank Institute - NAT]
Innate immunity limits transgene expression in S. mediterranea.

10:30–10:45 Guillaume Reho & Hervé Cadiou *Short Talk*
[Université de Strasbourg]
The need for nociception: planarians are no exception.

10:45–11:00 Karolien Bijnens *Short Talk*
[Hasselt University]
Planarians as an alternative in vivo model to study micro- and nanoparticle-induced neurodevelopmental toxicity.

11:00–11:15 **"Explain your Poster" Session A**

11:15–13:00 *Coffee Break*

Posters Session A

13:00–14:30 *Lunch*

Monday, October 14th (Continued)

14:30–16:30 Session III – Regeneration and Stem Cells

Chair: *Hanh Vu*

14:30–15:00 Virginia Vanni
[University of Exeter]
Single-cell characterization of planarian regeneration.

15:00–15:30 Martijn Heleven
[Hasselt University]
From Amputation to Axis formation: The role of early MAPK/ERK activation and redox signals during regeneration.

15:30–16:00 Ying Yang
[China National Center for Bioinformation]
RNA modification regulates planarian regeneration.

16:00–16:15 Daniel Font *Short Talk*
[Universitat de Barcelona]
MFN2 is Necessary for Stem Cell Proliferation During Growth but Dispensable During Starvation.

16:15–16:30 Bret Pearson *Short Talk*
[Oregon Health & Science University]
Investigating the Origins and Function of Planarian Glia.

16:30–17:00 *Coffee break* ☕

Monday, October 14th (Continued)

17:00–19:00 **Session IV – Beyond *Schmidtea mediterranea***

Chair: *Claus Kuhn*

17:00–17:30 Helena García-Castro
[University of Exeter]
Intra- and Inter-Species Cell Type Comparisons in Planarians by Single-Cell Transcriptomics.

17:30–18:00 Jochen Rink
[Max Plank Institute - NAT]
Towards planarian comparative genomics.

18:00–18:30 Gaurav Vaidya
[EMBL-Heidelberg]
Let's talk about sex: Mechanisms of reproductive switching in planarian flatworms.

18:30–18:45 Animan Tripathi *Short Talk*
[Max Plank Institute - NAT]
Understanding molecular mechanisms of head and tail regeneration in Stenostomum brevipharyngium.

18:45–19:00 Jason Pellettieri *Short Talk*
[Keene State College]
Flatworm transcriptomes reveal widespread parasitism by histophagous ciliates.

19:00–19:15 **"Explain your Poster" Session B**

20:00–21:30 *Dinner*

21:30–23:00 **Posters Session B**

Tuesday, October 15th

09:00–10:00 **Session V – Planarian Cell Biology**

Chair: *Prasad Abnave*

09:00–09:30 Andreas Pittroff
[University of Bayreuth]
Definition of m6A sites in the planarian transcriptome with single-nucleotide precision and characterization of their effects on mRNA fate.

09:30–10:00 Nikhil Kumar
[Institute for Stem Cell Science & Regenerative Medicine (INSTEM)]
Sneak peek into mechanisms regulating neoblast maintenance and differentiation.

10:00–11:00 **Keynote Invited Talk: Prof. Oded Rechavi**
[Tel-Aviv University, IL] — **Virtual Seminar**
Transgenerational Small RNA Memories.

11:00–11:30 *Coffee Break*

11:30–12:30 **Session VI – Cool tools**

Round Table: *Open Discussion - New Technologies*

12:30–13:00 **Future Directions and Concluding Remarks**

13:00–14:00 *Lunch*

14:00– Departure (*BUS to Barcelona from Platja d'Aro bus station*)

Invited Talks

Learning the Super Power of Animal Diversity One Cell Type at a Time

Wang, Bo[1]

1. Department of Bioengineering; Stanford University;
Shriram Center, 443 Via Ortega, Rm 119; Stanford, CA 94305; USA.

All animals are built from cells, which exhibit a myriad of shapes, forms, and functions. Recent technological upsurge has revolutionized our ability to analyze and manipulate cells within their tissue contexts, allowing us to systematically catalog the diversity of cell types present within individual species and compare them across organisms. In this talk, we will discuss the type of insights that may be gained from such analyses. We will delve into questions such as: How do animals like planarians prevent hormonal dysregulation in ways that surpass adaptive immunity? What cellular adaptations allow an animal to benefit from symbiosis with photosynthetic algae? How did neurons controlling behaviors like sleep, hunger, stress, and fighting evolve? Through addressing these questions, we aim to shed light on the basic principles that shape life from the cellular level.

Transgenerational Small RNA Memories

Rechavi, Oded[1]

1. Department of Neurobiology, Biochemistry & Biophysics;
Wise Faculty of Life Sciences & Sagol School of Neuroscience;
Tel-Aviv University; Tel-Aviv; Israel.

In *C. elegans*, dedicated machinery enables transmission of small RNAs which regulate gene expression across multiple generations, independently of changes to the DNA sequence. I will discuss new insights regarding the underlying mechanisms and the potential of RNAi inheritance to affect the worms' fate. I will also discuss what I think this type of inheritance allows, and what is probably impossible. Lastly, I will provide evidence that transgenerational inheritance of small RNAs is possible even in other, very different organisms... (hint: not roundworms!)

Selected Talks

A single-cell view of growth dynamics in *Schmidtea mediterranea*

Meiborg, Adriaan[1,2,★]; Palavalli, Amrutha[1,★]; Mellado-Fuentes, Ana M.[1,★]; Mirkes, Kristina[1,2]; Vaidya, Gaurav[1,2]; Werner, Steffen[3]; Dorrity, Michael[4]; Vu, Hanh Thi-Kim[1]

1. Developmental Biology Unit;
European Molecular Biology Laboratory (EMBL);
Mayerhofstrasse 1, 69117 Heidelberg; Germany.

2. Faculty of Biosciences;
Collaboration for joint PhD degree between EMBL & Heidelberg University;
Im Neuenheimer Feld 234, 69120 Heidelberg; Germany.

3. Department of Experimental Zoology;
Wageningen University & Research;
De Elst 1, 6708WD Wageningen; The Netherlands.

4. Structural & Computational Biology Unit;
European Molecular Biology Laboratory (EMBL);
Mayerhofstrasse 1, 69117 Heidelberg; Germany.

★. These authors have contributed equally to this work.

Achieving an appropriate size is critical for the development of animal species, as it has important functional consequences for survival and reproduction. How animals coordinate the growth of tissues, organs, and the entire body to reach the correct size remains a fascinating, yet largely unresolved, question in Biology. Planarian flatworms represent a unique opportunity to address this question due to their remarkable ability to adjust their body size over a wide range in response to food availability. We employed a multiplexed single-cell RNA sequencing approach to build a comprehensive whole-animal description of growth dynamics in the model planarian species *Schmidtea mediterranea*. The scalability of our methods allowed us to sample across an order-of-magnitude body length range while preserving the cellular composition of individual animals. In this presentation, I will discuss our ongoing efforts to reconstruct the molecular and cellular growth trajectories, to gain new insights into the regulatory mechanisms of growth and body size.

Cellular senescence in planarian regeneration

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

1. Department of Genetics, Microbiology & Statistics;
Institute of Biomedicine (IBUB);
Universitat de Barcelona; Catalunya; Spain.

There is increase evidence that senescent cells play an important role in wound healing and tissue regeneration in several organisms, ranging from salamanders and cnidarians to organisms with a more restricted regenerative potential such as mammals.

Planarians are one of the most well-known model animals widely studied for their extraordinary regenerative capabilities, as they can regenerate any missing or damaged body tissue, thanks to a population of self-renewing adult stem cells (neoblasts), which are abundantly present throughout the worm. This makes planarians an ideal model for studying the role of cellular senescence in tissue repair and regeneration. However, whether cellular senescence occurs during the normal physiology of these worms and whether it participates in planarian regeneration has not been systematically examined. Here, we explored the conservation of tissue injury-induced senescence in planarians and its participation in regeneration. Interestingly, amputation is associated with the appearance of senescence markers. Remarkably, treatment with drugs that target senescent cells ('senolytic' drugs) caused regenerative defects in these worms. These results suggest a role of senescence signaling in planarians regeneration and present planarians as a suitable model to unravel the link between senescence, stem cells and regeneration.

A Functional Study of Mitochondria in Planarian Regeneration

Pan, Xue[1,2,3,4,★]; Zhao, Yun[2,3,4,5,★]; Li, Yucong[2,3,4,5,★]; Chen, Jiajia[1,2,3,4]; Zhang, Wenya[2,3,4,5]; Yang, Ling[6]; Xiong, Yuanyi Zhou[2,3,4,5]; Yuqing, Ying[1,2,3,4]; Xu, Hao[1,2,3,4]; Zhang, Yuhong[1,2,3,4]; Gao, Chong[2,3,4]; Sun, Yuhan[2,3]; Li, Nan[6]; Chen, Liangyi[7,9,10]; Chen, Zhixing[7,8,10]; Lei, Kai[2,3,4,✉]

1. College of Life Sciences; Zhejiang University; Hangzhou, Zhejiang; China.
2. Westlake Lab of Life Sciences & Biomedicine; Hangzhou, Zhejiang; China.
3. Key Lab of Growth Regulation & Translational Research of Zhejiang Province; School of Life Sciences; Westlake University; Hangzhou, Zhejiang; China.
4. Inst. of Biology; Westlake Inst. for Advanced Study; Hangzhou, Zhejiang; China.
5. Fudan University; Shanghai; China.
6. HPC Center; Westlake University; Zhejiang; China.
7. Institute of Molecular Medicine; Beijing Key Lab of Cardiometabolic Molecular Medicine; Peking University; Beijing; China.
8. Peking-Tsinghua Center for Life Sciences; Peking University; Beijing; China.
9. State Key Laboratory of Membrane Biology; Peking University; Beijing; China.
10. PKU-Nanjing Institute of Translational Medicine; Nanjing; China.

★. These authors contributed equally. ✉. Lead contact.

Tissue regeneration is a multifaceted process involving extensive changes in cell proliferation, fate determination, and differentiation. While mitochondrial dynamics and metabolism play a crucial role in development and wound repair, their specific contributions to large-scale regeneration remain poorly understood. To address this gap, we utilized the planarian *Schmidtea mediterranea*, a model organism renowned for its exceptional regenerative capabilities, to investigate the role of mitochondrial dynamics in whole-body regeneration. Our study involved *in vivo* and *in vitro* examination of mitochondrial dynamics during planarian regeneration and in various cell types. We discovered that mitochondrial morphology is highly dynamic during the regeneration process. Notably, we found that deficiency in mitochondrial fusion inhibited both stem cell pluripotency and the overall regeneration process. Restoration of mitochondrial dynamics ameliorated these regenerative defects, underscoring the critical role of mitochondrial fusion in regeneration capacity. Furthermore, our results highlight the importance of a delicate mitonuclear balance and the expression of mitochondrial proteins essential for regeneration, which are regulated by mitochondrial dynamics. These findings elucidate the fundamental importance of mitochondrial dynamics in large-scale tissue regeneration and suggest potential strategies for enhancing stem cell functionality and regenerative processes.

The regulatory logic of Planarian stem cell differentiation

Pérez-Posada, Alberto[1,2]; García-Castro, Helena[1,2]; Emili, Elena[1]; Vanni, Virginia[1,2]; Arias-Baldrich, Cirenia[1]; Frölich, Siebren[3]; van Heeringen, Simon J.[3]; Kenny, Nathan[4]; Solana, Jordi[1,2]

1. Department of Biological & Medical Sciences; Oxford Brookes University; Oxford; UK.
2. Living Systems Institute; University of Exeter; Exeter; UK.
3. Department of Molecular Developmental Biology; Radboud University; Nijmegen; The Netherlands.
4. Department of Biochemistry; University of Otago; PO Box 56, Dunedin, Aotearoa; New Zealand.

Cell type identity is determined by gene regulatory networks (GRNs) formed by combinatorial, specific transcription factors (TFs) regulating target genes (TGs) via binding to open chromatin regions (OCRs). Classic approaches of GRN discovery used perturbational data to elucidate TF-TG links but they are laborious and not scalable across species. Single cell transcriptomics allows to study gene expression with cell type resolution, but incorporating perturbational data is challenging. Planarians, with their pluripotent neoblast stem cells giving rise to all cell types, are an ideal model for this integration. Here we investigate the regulatory logic of planarian stem cell differentiation by obtaining an organism-level integration of single cell transcriptomics and single cell chromatin accessibility data. We identify OCR profiles for major differentiated cell types and reveal distinct gene modules expressed in individual and combinations of cell types. Integrated analysis unveils gene networks reflecting known TF interactions in each type and identifies TFs potentially driving differentiation across multiple cell types. We validate our predictions by combining RNAi knockdown of the hnf4 TF with single cell transcriptomics, demonstrating its influence over parenchymal cells and a high overlap between predicted targets and differentially regulated genes. Our study shows the combination of single cell methods and perturbational studies will be key for characterising GRNs widely.

Deciphering clonal lineages using natural somatic mutations in an entire adult organism

Peron, Sophie[1,2]; Solana, Jordi[1,2]

1. Biosciences; Exeter University; Living Systems Institute; Streatham campus, Exeter EX4 4QD; United Kingdom.
2. Biological & medical sciences; Oxford Brookes University; Gipsy lane campus, Headington, Oxford OX3 0BP; United Kingdom.

We are developing a method of lineage tracing in an entire adult organism by cross-referencing somatic mutations and cell type information in single cell RNA-seq datasets. Our model organism is the asexual strain of the planarian *Schmidtea mediterranea*. Since that strain reproduces by fission only, mutations are not eliminated by sexual reproduction and accumulate as the stem cells divide. Those mutations can be used for lineage tracing, similarly to methods used in cell cultures and mammalian tissues. Resolving clonal lineages in Schmidtea will allow to solve the long-standing question of whether homeostasis relies on pluripotent or lineage restricted progenitors.

Planarians were irradiated to accelerate mutations generation. After 6 months of clonal expansion, they were dissociated and barcoded individually to produce a single cell RNA-seq dataset using SPLiT-Seq. We determined that: 1) we can detect a variety of somatic mutations in our datasets, 2) irradiation is an efficient method to manipulate mutation loads in our system, and 3) single cells have diverse mutation profiles and can be clustered accordingly. We are currently analysing the somatic mutations of individual animals and developing the bioinformatic pipeline for lineage tracing.

Our work will provide a method of lineage tracing using somatic mutations as natural cell barcodes for whole organisms. This will allow evaluating stem cell potency in physiological conditions without invasive experimental procedure.

Elucidating the role of the immune system in Planarian regeneration

Hendin, Noam[1]; Wurtzel, Omri[1,2]

1. The School of Neurobiology; Biochemistry and Biophysics; Tel-Aviv University; Chaim Levanon St 55, Tel-Aviv; Israel.
2. Sagol School of Neuroscience; Tel-Aviv University; Chaim Levanon St 55, Tel-Aviv; Israel.

Injury activates a series of responses aimed to seal the wound, eliminate pathogens, and restore homeostasis. In regenerative organisms injury initiates new tissue growth. A critical aspect of injury is activation of an immune response, yet despite evidence for its importance in wound response, the role of the immune system in initiating regeneration is poorly understood. Planarians are flatworms capable of whole-body regeneration using a widespread population of adult stem cells called neoblasts. Here, I analyzed planarians response to immune activation by exposing the animals to different pathogens and immune activators. My analysis indicates that planarians mount a cellular and transcriptional response to infection that recapitulates crucial aspects of the injury response, even without injury. Moreover, we found that the presence of pathogens was correlated with unfavorable regenerative outcomes, consistent with the hypothesis that immune response activation can affect tissue growth. Our work suggests that the planarian immune system mediates central aspects of regeneration, shedding light on tissue growth mechanisms in highly regenerative organisms.

The Role of PGRPs in Planarian Immune Resilience to Bacterial Infection

Kumar, Sahil[1]; Bharti, Puja[1,2]; Dudeja, Puja[1]; Deshpande, Nimish[2]; Sharma, Aman[1]; Chavan, Sakalya[2]; Abnave, Prasad[1,2]

1. Regional Centre for Biotechnology; NCR Biotech Science Cluster; 3rd Milestone, Gurgaon-Faridabad Expressway, Faridabad 121001; India.
2. National Centre for Cell Science; Savitribai Phule Pune University Campus; Pune, Maharashtra 411007; India.

Planarians are renowned for their regenerative abilities and their unique capacity to combat various human pathogenic bacteria, a trait not seen in other invertebrates like *D. melanogaster* and *C. elegans*, which are susceptible to lethal infections. This makes planarians an invaluable model for studying antibacterial resistance mechanisms. While their immune system is not fully understood, planarians conserve several antimicrobial resistance genes and signaling pathways involved in innate immune recognition. They likely have pattern recognition receptors (PRRs) that detect microbial patterns and trigger immune responses. Therefore, we aimed to identify and characterize the role of peptidoglycan recognition proteins (PGRPs), a conserved PRR, in planarians' antibacterial resistance. Through *in-silico* analysis, we identified three secreted and four membrane-bound PGRPs in planarians, expressed in intestinal, epidermal, and stem cells. Immune challenge experiments using *Staphylococcus aureus* showed upregulation of PGRPs upon infection. RNAi-mediated silencing of PGRPs compromised planarians' immune defense, leading to increased bacterial survival. In summary, our study provides compelling evidence that PGRPs play a pivotal role in the antibacterial immune response of planarians. These findings align with observations in other animals, emphasizing the evolutionary conservation of PGRPs as integral components of innate immunity.

Components of the innate immune system limit transgene expression in *Schmidtea mediterranea*

Drees, Leonard[1]; Blösel, Daniel[1]; Kowalewski, Johannes[1]; Weill, Uri[1]; Rink, Jochen[1]

1. Department of Tissue Dynamics & Regeneration;
Max Planck Institute for Multidisciplinary Sciences (MPI-NAT);
Am Fassberg 11, 37077 Göttingen; Germany.

During the last decades, many tools and methods have been established and refined for research on planarians. Yet, the introduction of foreign genetic material and expression of transgenes remains a challenge in most planarian species. In collaboration with the Wang Lab, we have recently established a mRNA-based Nano-Luciferase (NLuc) reporter assay. With the NLuc assay as positive control, we also established proof of principle of plasmid-based transient expression in *S. mediterranea*. However, reporter expression levels remained low and transient, prompting us to explore biological factors that might limit transgene expression.

To identify candidate genes, we analysed the conservation of innate immunity pathways involved in nucleic acid sensing and used biochemical pull-down/mass spectrometry to identify DNA-binding proteins. Using RNAi-screening and the NLuc reporter as a quantitative read-out of transgene expression levels, we indeed identified a small number of gene products that significantly limit NLuc expression. RNAseq and functional experiments identified the proteins as part of an innate immunity pathway that likely triggers the removal of transfected cells and generally mediates immune and stress responses to a range of different stimuli. Our results show that innate immunity pathways limit transgene expression in *S. mediterranea* and that their further characterization will contribute to the goal of stable and high-level transgene expression in planarians.

The need for nociception: planarians are no exceptions

Reho, Guillaume[1]; Cadiou, Hervé[1]

1. CNRS UPR 3212 Institut des Neurosciences Cellulaires et Intégratives; Université de Strasbourg; Strasbourg; France.

Beyond their regeneration abilities, planarians have been widely used in a wide range of fields from pharmacology to behavior. Despite their evolutionary distance to vertebrates, their nervous system shares many similarities with the nervous structures of vertebrates, ranging from unipolar neurons to a majority of chemical synapses. This and their easy maintenance make them ideal models in the field of sensory physiology. Previous studies have shown that they are able to perceive the world using photoreception, chemoreception, electroreception and even magnetoreception. Recently, our laboratory and others have shown that they also experience nociception, i.e. the detection of stimuli which can potentially damage them. For example, we have demonstrated that well known TRPA1 agonists (a well conserved nociceptive ion channel) such as AITC, cinnamaldehyde and H2O2 induced a nociceptive scrunching gait in a dose dependant manner. This behavior was modulated by pre-exposure to common analgesics and suppressed by TRPA1 knockdown. Thermal preference tests demonstrated that planarians spend little time on the hot side ($14.0 \pm 2.6\%, n = 15, p < 0.001$) and this preference was abolished by the NSAID meloxicam. Work is currently underway in our lab to identify the cellular nociceptors.

Planarians as an alternative *in vivo* model to study micro- and nanoparticle-induced neurodevelopmental toxicity

Bijnens, Karolien[1]; Tytgat, Julie[1]; Heleven, Martijn[1]; Segnana, Charlotte[1]; Smeets, Karen[1]

1. Zoology; Biodiversity and Toxicology; Centre for Environmental Sciences; Hasselt University; Agoralaan Building D, 3590 Diepenbeek; Belgium.

Micro- and nanoparticles are present in our daily lives and form a potential threat to our health. Several of these particles have been shown to induce neurotoxicity, though the underlying mechanisms remain elusive. Understanding how particle characteristics and their uptake profiles influence toxicological responses is crucial for proper risk assessment. We studied this relationship in planarians, given their unique ability to regenerate a fully functional central nervous system. All studied particles induced neurodevelopmental toxicity, although their specific effects depend on particle characteristics and uptake profiles. Polystyrene particles of different sizes were taken up in the epidermis and intestine, and were found close to neuronal structures. The larger sizes ($1 + 2$ μm) resulted in a delay in anterior commissure formation, while the smaller particles ($50 + 200$ nm) affected the formation of the eyes and dopaminergic neurons. Silver and titaniumdioxide nanoparticles induced behavioral changes and a delayed formation of respectively the cephalic ganglia and anterior commissure, linked to altered stem cell dynamics. Currently, we focus on gaining in-depth mechanistic insights to identify adverse outcome pathways related to particle-induced neurotoxicity, providing new perspectives for risk assessment.

Single-cell characterization of planarian regeneration

Vanni, Virginia[1]; Coronel-Cordoba, Pablo[2]; Adell, Teresa[2]; Solana, Jordi[1]

1. Living Systems Institute; University of Exeter; Exeter; UK.
2. Department of Genetics, Microbiology & Statistics; Institute of Biomedicine (IBUB); University of Barcelona; Catalunya; Spain.

Planarians show enhanced regenerative abilities. Upon injury, they can re-constitute missing organs and tissues, and re-establish their physiological conditions in just 10-15 days. This complex process is sustained by neoblasts, adult pluripotent stem cells that differentiate in all the planarian cell types. Regeneration involves a plethora of regulatory mechanisms and networks that many studies in the past century have tried to unveil. However, many questions are still open, especially regarding the detailed molecular pathways followed by neoblasts and progenitors during regeneration. The development of single cell transcriptomics techniques made the study of such molecular processes at a cellular level accessible, even for organisms in which tools like transgenesis are not available. In the current work, we profiled planaria regeneration using scRNAseq, through ACME dissociation and SPLiT-seq, that allowed us to multiplex several timepoints and biological replicates. Preliminary results comprise more than 50,000 cells where all the main planarian cell types are found, including regeneration specific cell types. Future analyses will include the temporal expression of selected genes known to be involved in regeneration, sub-clustering of different cell types, weighted gene co-expression network analysis, and PAGA trajectory analysis, aiming to identify and better understand the molecular networks underlying planarian regeneration.

From Amputation to Axis formation: The role of early MAPK/ERK activation and redox signals during regeneration

Heleven, Martijn[1]; Molina, M. Dolores[2]; Jaenen, Vincent[1]; Bijnens, Karolien[1]; Cebrià, Francesc[3,4]; Smeets, Karen[1]

1. Zoology; Biodiversity and Toxicology; Centre for Environmental Sciences; Hasselt University; Agoralaan Gebouw D 3590 Diepenbeek; Belgium.
2. Department of Bioengineering; IQS School of Engineering; University of Ramon Llull; Barcelona; Catalonia; Spain.
3. Department of Genetics; Microbiology & Statistics; Faculty of Biology; University of Barcelona; Barcelona; Catalonia; Spain.
4. Institute of Biomedicine of the University of Barcelona (IBUB); University of Barcelona; Barcelona; Catalonia; Spain.

The MAPK/ERK pathway plays a crucial role in regeneration by regulating stem cell proliferation, differentiation, and survival. Previous research has suggested that this signaling cascade also influences polarity decisions, thereby initiating head regeneration in planarians.

This study aimed to further explore how the MAPK/ERK pathway drives tissue regeneration and patterning and to identify the potential involvement of upstream redox signals in this process. We show that pERK activation levels and ROS production varied depending on the wound location and orientation, with higher levels of pERK at anterior-facing wounds compared to posterior-facing wounds as early as 3-6 hours post-amputation. Correspondingly, the production of ROS in anterior-facing wounds was significantly higher compared to posterior-facing ones. We found functional and positional links between amputation-induced redox changes, pERK levels, and other redox-induced transcription factors. Furthermore, our findings suggest a feedback regulatory circuit that controls pERK activation, in which the downstream transcription factor egr-4 inhibits MKP activation. This feedback circuit is active during the first-day post-amputation and may shift as regeneration progresses.

RNA modification regulates planarian regeneration

Cui, Guanshen[1,2,3]; Zhou, Jia-Yi[1]; Ge, Xin-Yang[1,2]; Sun, Bao-Fa[1]; Song, Ge-Ge[1]; Wang, Xing[1]; Wang, Xiu-Zhi[1,2]; Zhang, Rui[4]; Wang, Hai-Lin[4]; Jing, Qing[5]; Koziol, Magdalena J.[6]; Zhao, Yong-Liang[1]; Zeng, An[7]; Zhang, Wei-Qi[1]; Han, Da-Li[1,2]; Yang, Yun-Gui[1,2,3]; Yang, Ying[1,2,3]

1. Beijing Institute of Genomics; Chinese Academy of Sciences & China National Center for Bioinformation; Beijing; China.
2. Sino-Danish College; University of Chinese Academy of Sciences; Beijing; China.
3. Institute of Stem Cell & Regeneration; Chinese Academy of Sciences; Beijing; China.
4. Research Center for Eco-Environmental Sciences; Chinese Academy of Sciences; Beijing; China.
5. Shanghai Jiao Tong University School of Medicine & Shanghai Institute of Nutrition and Health; Chinese Academy of Sciences; Shanghai; China.
6. Chinese Institute for Brain Research (Beijing); Chinese Academy of Medical Sciences; Beijing; China.
7. Shanghai Institute of Biochemistry & Cell Biology; Shanghai; Chinese Academy of Sciences; University of Chinese Academy of Sciences; Shanghai; China.

Regeneration is the regrowth of damaged tissues or organs, a vital process in response to damages from primitive organisms to higher mammals. Planarian possesses active whole-body regenerative capability owing to its vast reservoir of adult stem cells, neoblasts, providing an ideal model to delineate the underlying mechanisms for regeneration. RNA N6-methyladenosine (m6A) modification participates in many biological processes, including stem cell self-renewal and differentiation, in particular the regeneration of haematopoietic stem cells and axons. However, how m6A controls regeneration at the whole-organism level remains largely unknown. Here, we demonstrate that the depletion of m6A methyltransferase regulatory subunit wtap abolishes planarian regeneration, potentially through regulating genes related to cell–cell communication and cell cycle. Single-cell RNA-seq (scRNA-seq) analysis unveils that the wtap knockdown induces a unique type of neural progenitor-like cells (NPlike cells), characterized by specific expression of the cell–cell communication ligand grn. Intriguingly, the depletion of m6A-modified transcripts grn, cdk9 or cdk7 partially rescues the defective regeneration of planarian caused by wtap knockdown. Overall, our study reveals an indispensable role of m6A modification in regulating wholeorganism regeneration.

MFN2 is Necessary for Stem Cell Proliferation During Growth but Dispensable During Starvation

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

1. Department of Genetics, Microbiology & Statistics; Universitat de Barcelona; Barcelona, Catalunya, Spain.
2. Institute of Biomedicine (IBUB); Universitat de Barcelona; Barcelona, Catalunya, Spain.

Mfn2 is a gene required for mitochondrial fusion. Its role has been linked to the cellular energetic state, the progression of the cell cycle and also with cellular differentiation. Moreover, it has recently been suggested that *mfn2* could be operating upstream of the mTOR pathway. Here we use planarians as model system to investigate the role of *mfn2* in stem cell function. Planarians can regenerate any body part and are constantly resizing their bodies according to food availability. This active cellular turnover relies in a population of somatic stem cells capable of producing each of the cellular lineages of the animal. Recently we have showed that stem cell cycle progression has specific signaling requirements when the planarians are in starvation.

We have studied the function of *mfn2* in planarians at different metabolic contexts: feeding and starvation. In the feeding context, our results indicate that *mfn2* is necessary for the stem cell proliferation peak linked to growth after feeding through adjusting the ATP levels, since its RNAi inhibition causes planarian degrowth and a decrease in ATP. In the starvation context, *mfn2* is dispensable to maintain the basal proliferation rate of somatic stem cells but remains necessary to maintain cell identities. Both contexts alter the progression of the cell cycle but each does so by affecting different phases. Taken together, it suggests that the proliferation of somatic stem cells could have different energy requirements in different metabolic situations. We think that the study of *mfn2* function in planarian stem cells could provide new insights into the metabolic regulation of stem cells.

Investigating the Origins and Function of Planarian Glia

Lee, Chris[1]; Pearson, Bret[1]

1. School of Medicine; Oregon Health & Science University; OR, USA.

Glia, the non-neuronal cells of the nervous system, are critically involved in CNS regeneration across diverse species. Glia have been shown to facilitate regrowth of neurons in some species, but may block regeneration in others, highlighting the need for further investigation. The planarian, *Schmidtea mediterranea*, is a model organism well-suited for investigating the relationship between glial cells and neural regeneration. It has remarkable regenerative capabilities and a well-organized brain composed of a variety of neuronal subtypes as well as a recently discovered population of glia. Planarian glia express both well-conserved glial markers such as glutamine synthetase (gs) and planarian specific glial markers such as intermediate filament-1 (if-1) and calamari (cali). Despite the recent molecular characterization of the planarian glia, the function and the genes that specify the planarian glia remain unknown. We performed RNAi screening of planarian glia enriched transcription factors and identified suppressor of hairless (Su(H)) as a regulator of planarian glia. Furthermore, RNAi of other Notch pathway components ligand delta-2 and receptor notch-1 caused a similar reduction of glial marker expressions, and instead caused an ectopic expression of planarian pigment cell markers near the ventral nerve cords (VNC) of the planarians. These results show that conserved Notch signaling regulates planarian glia and may determine the fate between pigment and glial cells in planarians.

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Intra- and Inter-Species Cell Type Comparisons in Planarians by Single-Cell Transcriptomics

García-Castro, Helena[1]; Pérez-Posada, Alberto[1]; Salamanca-Díaz, David[1]; Kenny, Nathan[2]; Solana, Jordi[1]

1. Faculty of Health & Life Sciences; University of Exeter; Living Systems Institute, Exeter EX4 4QD; United Kingdom.
2. Department of Biochemistry; University of Otago; 710 Cumberland Street, Dunedin North, Dunedin 9016; New Zealand.

Planarians are present in a variety of ecosystems around the globe, including marine, freshwater and terrestrial environments. Moreover, they comprise multiple reproduction modes, such as sexual reproduction, and asexual reproduction by binary fission or parthenogenesis. This repertoire suggests that planarian cell types, their abundances, and gene expression profiles have diversified throughout evolution to adapt to different environments and lifestyles. To study this question, we addressed planarian cell diversity using single-cell technologies. We profiled and compared thousands of cell transcriptomes from 5 freshwater planarian species across different genera (*Schmidtea*, *Dugesia*, *Girardia* and *Polycelis*), encompassing both sexual and asexual strains, and different life stages. Our findings reveal novel planarian cell populations, intra-species variations linked to reproduction, and inter-species diversity in cell types and gene expression.

Towards planarian comparative genomics

Rink, Jochen[1]

1. Department of Tissue Dynamics & Regeneration;
Max Planck Institute for Multidisciplinary Sciences (MPI-NAT); Germany.

Let's talk about sex: Mechanisms of reproductive switching in planarian flatworms

Vaidya, Gaurav[1]; Campbell, Lahcen[2]; Ross, Eric[3]; Mellado-Fuentes, Ana M.[1]; Robb, Sofia[3]; Alvarez, Jorge[2]; Dyer, Sarah[2]; Dorrity, Michael[1]; Vu, Hanh Thi-Kim[1]

1. European Molecular Biology Laboratory (EMBL); Meyerhofstrasse 1, 69117 Heidelberg; Germany.
2. European Molecular Biology Lab – European Bioinformatics Institute (EMBL-EBI); Wellcome Genome Campus; Hinxton, Cambridgeshire, CB10 1SD; United Kingdom.
3. Stowers Institute for Medical Research; 1000 E 50 St, Kansas City, MO 64110; United States of America.

Reproduction is a key life history trait that defines fitness of the individual in a changing environment. Facultative sexuality is a form of reproduction where both sexual and asexual reproduction can occur within the same individual. In theory, this form of reproduction combines the benefits of sexual and asexual reproduction while minimizing the costs of fully investing in one strategy over the other. Yet, paradoxically, obligate sexual reproduction is the most common form of reproduction in animals. The rare incidence of facultative sexuality in animals could reflect unrecognized costs of reproductive switching. I aim to gain mechanistic insights into the causes and consequences of reproductive switching in *Phagocata morgani*, a non-model planarian species that exhibits this behaviour in response to shifts in temperature. In this talk, I will briefly present the progress of my project, including the generation of a draft genome assembly and annotation for *P. morgani*, the effects of temperature on reproductive mode, and the preliminary results of using organismal-scale single-cell transcriptomics to understand size-dependence and cell-type specific responses associated with reproductive switching. In combining novel experimental and computational approaches with traditional functional perturbations of planarians, we hope to provide new insights into the mechanisms underlying facultative sexuality.

Understanding molecular mechanisms of head and tail regeneration in *Stenostomum brevipharyngium*

Tripathi, Animan[1,2]; Ludwik, Gąsiorowski[1,3]; Rink, Jochen[1]

1. Department of Tissue Dynamics & Regeneration;
Max Planck Institute for Multidisciplinary Sciences (MPI-NAT);
Am Faßberg 11, 37077, Göttingen; Germany.

2. International Max Planck Research School for Molecular Biology;
University of Göttingen; Justus-von-Liebig-Weg 11, 37077 Göttingen; Germany.

3. Institute of Evolutionary Biology; Faculty of Biology;
University of Warsaw; Warsaw; Poland.

Catenulida, Macrostomorpha, and Tricladida are the only Platyhelminth orders capable of full-body regeneration, raising the question of whether this trait evolved independently or was an ancestral feature lost multiple times. Catenulid regeneration is particularly interesting due to their basal phylogenetic position and limited prior study. Using a fortuitous lab culture of *S. brevipharyngium* and a self-developed toolkit, we present the first comparative analysis of head and tail patterning mechanisms in Catenulida.

The Wnt signaling pathway is key for specifying the anteroposterior axis. We identified Wnt components in *S. brevipharyngium* and mapped their expression patterns via HCR v3.0. Wnt components were mainly expressed in the circular muscles of the rostrum (Notum, Wnt5a, sFRP1/2/5a/e, Frizzled5/8a/c), trunk (Wnt4b, sFRP1/2/5b/f), and longitudinal muscles (Wnt4a, Wnt11c, sFRP1/2/5c), providing insights into their roles. Notably, Wnt1 was absent in *S. brevipharyngium*, and Wnt11 may act as the posterior-specifying Wnt, though RNAi validation is needed.

We also explored early wound responses, finding upregulation of runt-1a, jun, and fos-1 after wounding, with their expression also seen during paratomy. This study marks a significant step in understanding regeneration in platyhelminths, though further research is required to fully elucidate the mechanisms of head and tail regeneration in *S. brevipharyngium* and its evolutionary implications for the phylum.

Flatworm transcriptomes reveal widespread parasitism by histophagous ciliates

Woodcock, M. Ryan[1]; Powers, Kaleigh[1]; Snead, Kirsten[1]; Pellettieri, Jason[1]

1. Department of Biology; Keene State College; 229 Main Street, Keene, NH, 03435; USA.

Planarian and flatworm meetings have historically focused on the biology of the Platyhelminthes, with other phyla generally regarded as trifling oddities unworthy of serious scientific investigation. We recently reported a bioinformatic analysis that revealed widespread parasitism by a group of organisms of underappreciated interest – histophagous (flesh-eating) ciliates. These unicellular eukaryotes can be facultative or obligate parasites with the potential to cause serious harm to their hosts (e.g., "guppy killer disease"). From over two million screened ESTs in Platyhelminthes databases, we identified nearly 6,000 that could be confidently identified as Ciliophora in origin, based in part on translation using the nonstandard ciliate genetic code. We also cultured and identified *Tetrahymena* ciliates from nine terrestrial and freshwater planarian species, including invasive members of the genera *Bipalium* and *Girardia*, as well as the widely studied regeneration models *Dugesia japonica* and *Schmidtea mediterranea*. A co-phylogenetic reconstruction provides strong evidence for extended coevolution of histophagous Ciliophora with their Platyhelminthes hosts. Together, our findings raise the possibility that invasive flatworms constitute a novel dispersal mechanism for *Tetrahymena* parasites and position the Platyhelminthes as an ideal model phylum for studying the ecology and evolution of histophagous ciliates. We will report our latest plans for this project at the meeting.

Definition of m6A sites in the planarian transcriptome with single-nucleotide precision and characterization of their effects on mRNA fate

Pittroff, Andreas[1]; Höhn, Constantin[1]; Lence, Tina[2]; Gribling, Anne-Sophie[3]; Kim, Iana[1]; Smyth, Redmond[3]; Roignant, Jean-Yves[2,4]; Kuhn, Claus-Dieter[1]

1. RNA Biochemistry; University of Bayreuth; 95440 Bayreuth; Germany.
2. Institute of Molecular Biology (IMB); 55128 Mainz; Germany.
3. Genome Architecture & Evolution of RNA viruses; Helmholtz Institute for RNA-based Infection Research; Josef-Schneider-Straße 2, 97080 Würzburg; Germany.
4. Center for Integrative Genomics; University of Lausanne; 1015 Lausanne; Switzerland.

m6A is the most abundant base modification in RNA. It is involved in several steps of mRNA surveillance, e.g. mRNA stability and decay, or alternative splicing. In a combined approach we utilized both m6A miCLIP and nanopore dRNA-seq data to identify high confidence m6A sites transcriptome-wide with single nucleotide precision. This allowed us to precisely characterize m6A in planaria, revealing several distinctions from other organisms. First, the sequence motif in which m6A is placed in planaria differed from the common DRACH motif only retaining the RAC core. Second, we found that m6A site distribution along planarian transcripts showed characteristics of both *Drosophila* and mouse – two organisms with very different m6A placement profiles. Moreover, the distribution of m6A on planarian transcripts does not follow the rules for their placement as suggested in 2022 by the Schwartz lab for all higher eukaryotes. Last, to reduce the level of m6A on planarian transcripts, we knocked down METTL14, a core component of the m6A writer complex. We then quantitatively examined the effects of this knockdown by observing differential gene and isoform expression of planarian transcripts to identify m6A-dependent isoform switches between WT and knockdown animals. These results will aid a mechanistic understanding of the role of m6A in planarian mRNA surveillance and its function for animal homeostasis and regeneration.

Sneak peek into mechanisms regulating neoblast maintenance and differentiation

Kumar, Nikhil[1]; Haroon, Mohamed Mohamed[1]; Basu, Mainak[1]; Mazumdar, Atriya[1]; Pavithran, Swathi[1]; Dubey, Vinay Kumar[1]; Vemula, Praveen[1]; Arora, Ankit[1]; Palakodeti, Dasardhi[1]

1. Institute for Stem Cell Science & Regenerative Medicine; India.

Neoblasts are adult pluripotent stem cells in planaria which also play a vital role in the organisms with remarkable regenerative capabilities. Understanding the factors governing neoblast pluripotency is crucial for deciphering the mechanisms underlying regeneration. Previous studies have established mitochondrial content as a marker for isolating distinct neoblast subpopulations. Based on these findings, we employed Single-cell RNA sequencing (scRNA-seq) to uncover novel targets and markers specific to these subpopulations. scRNA-seq analysis revealed previously uncharacterized cell types critical for the maintenance of neoblast in slow cycling state. Currently, we are working on the mechanism that signal the neoblast to maintain in a slowcycling state. In summary, our findings identified cell-types that provide extrinsic cues critical for the maintenance of indicate slow-cycling neoblasts in planarians.

Posters — Session A

Poster – A01

A comparative analysis of RNA interference mechanisms in planarian flatworms

Balaji, Kalyani[1,2]; Rink, Jochen[1]; Drees, Leonard[1]; Ditte, Peter[1]; Wurtzel, Omri[3]

1. Department for Tissue Dynamics & Regeneration; Max Planck Institute for Multidisciplinary Sciences (MPI-NAT); Am Faßberg 11, 37077 Göttingen; Germany.
2. Department of Biology; Indian Institute of Science Education & Research; Tirupati, Srinivasapuram, Andhra Pradesh 517619; India.
3. Department for Regeneration & Stem Cell Biology; Tel-Aviv University; Chaim Levanon St 55, Tel Aviv-Yafo, 6997801; Israel.

RNAi is widely used in the laboratory for loss-of-function analysis in various organisms, especially in planarians. In the model planarian species *Schmidtea mediterranea*, RNAi is highly effective and can be performed simply by mixing the dsRNA with the food. This suggests the existence of mechanisms mediating the systemic spread of RNAi, while the long duration of the knockdown response suggests the existence of a molecular memory mechanism. However, the mechanistic basis of RNAi in planarians remains poorly understood.

Previous experiments in our laboratory have shown that RNAi is ineffective in some planarian species. Each species-specific RNAi defect reflects either the loss of essential components of the RNAi machinery, the loss/inactivation of genes associated with RNAi memory, or perhaps the loss of components required for RNAi propagation. As part of a collaborative project with the Wurtzel lab, my project aims to study the RNAi mechanism in planarians using a comparative approach. I will screen different planarian species to determine whether RNA interference works in each species and then perform transcriptome comparisons between those that can and cannot perform RNA interference. This will allow us to identify candidate genes involved in the RNAi response, which can then be verified in the model species using molecular biology techniques. Altogether, my project will contribute to a better understanding of RNAi mechanisms in planarians.

Poster – A02

Gene regulatory basis of the Wnt/β-Catenin switch in planarian head/tail regeneration

Bian, Zhengyue[1]; Ivankovic, Mario[1]; Rink, Jochen[1]

1. Tissue Dynamics & Regeneration;
Max Planck Institute for Multidisciplinary Sciences (MPI-NAT);
Am Faßberg 11, 37077 Göttingen; Germany.

Mutually exclusive head and tail regeneration in planarian is switched by a β-CATENIN gradient along anterior-posterior axis (A-P axis). β-catenin, a downstream component of the Wnt signaling pathway, controls the expression of head and tail- specific genes; however, the regulatory network remains unclear. Homeodomain proteins are transcription factors involved in developmental gene regulation and are regulated by β-catenin in planarian. Our objective is comprehensively investigates the homeodomain-mediated regulatory networks governing head and tail regeneration in planarian. We will utilize RNA interference (RNAi) to functionally test homeobox gene, investigate tail/head specific genes transcriptional changes and genome accessibility changes following homeobox genes knockdown through multi-omics approach that integrates ATAC-seq, RNA-seq. ChIP-seq will enable us to map the changes in binding sites of these transcription factors, further investigating the dynamic changes in head/tail gene expression. Our study will elucidate the role of homeobox genes in regeneration and uncover the gene regulatory basis of Wnt/β-Catenin switch in planarian head/tail regeneration.

Poster – A03

Impact of *EGR2* and *RERE* in Anteroposterior Regeneration in Planarians

Cuadrado, Daniel[1]; Adell, Teresa[1]; Saló, Emili[1]

1. Department of Genetics, Microbiology & Statistics;
Institute of Biomedicine (IBUB);
Universitat de Barcelona; Catalunya; Spain.

Specification of polarity during planarian regeneration depends on the modulation of the Wnt signaling pathway. The main objective of this study is to further understand posterior specification through the identification of new elements related to Wnt1 activation. Through a previous genomic analysis, the *EGR2* and *RERE* genes were identified as candidates for playing early roles in the regenerative process. In this study, we show that RNAi inhibition of *EGR2* caused significant alterations in both anterior and posterior regeneration, leading to a loss of midline identity, tail bifurcation, and disconnection of brain lobes. *RERE* RNAi inhibition also disrupted anterior and posterior regeneration, impairing proper regeneration of the brain, eyes, and dorsalization, resulting in phenotypes such as absent eyes and cyclops. Recent studies identify *RERE* as part of the retinoic acid signaling pathway, whose deregulation could underlie the defects observed in RERE RNAi animals. Our results suggest a role for *EGR2* in regeneration and midline organization, as well as a role for *RERE* in mediating the effects of retinoic acid, a key regulator in cellular regeneration.

Poster – A04

In-situ Hybridization Chain Reaction (HCR) optimization in *S. mediterranea*, simplifying multi color FISH in planaria

Dreier, Fabienne[1]; Maisl, Annabel[1]; Rink, Jochen[1]

1. Department of Tissue Dynamics & Regeneration;
Max-Planck-Institute for Multidisciplinary Sciences (MPI-NAT);
Am Fassberg 11 37077 Göttingen; Germany.

The visualization of gene expression patterns is a powerful tool towards understanding mechanisms of whole-body regeneration. The analysis of multiple genes within the same sample is often required to understand the interplay and regulation of different genes and pathways. The standard approach in the field until now is multi-color fluorescence *in-situ* hybridisation (FISH), which involved multiple time-consuming development steps. *In-situ* hybridization chain reaction (HCR) is a newer technique that promises the simultaneous detection of multiple different transcripts in fixed samples. Whereas conventional FISH uses antibodies to detect hapten-labelled probes, HCR uses short DNA fragments linked to fluorophores that oligomerize on specific targets, thus offering potentially increased signal specificity and tissue penetrance. While HCR is by now well established in several model organisms including mice and *Drosophila*, the existing protocols perform poorly on whole mounted planarians. Here, we systematically optimized the HCR protocol to specifically adapt it to planarians. We find that Proteinase-K treatment is essential for robust HCR signals and, in combination with the DEEP clear method, can produce fluorescent gene expression patterns in whole-mount specimens *en par* with traditional FISH. Overall, our protocol adaptations make HCR a useful addition to the planarian tool kit, particularly for multiplex gene expression analysis.

Poster – A05

A genetic and microscopy toolkit for manipulating and monitoring regeneration in *Macrostomum lignano*

Hall, R. Nelson[1]; Li, Hongquan[2]; Chai, Chew[1]; Vermeulen, Sidney[3]; Bigasin, Robin R.[1]; Song, Eun Sun[4]; Sarkar, Souradeep[1]; Gibson, Jesse[1]; Prakash, Manu[1]; Fire, Andrew Z.[3,5]; Wang, Bo[1]

1. Dept. of Bioengineering; Stanford University; Stanford, CA; USA.
2. Dept. of Electrical Engineering; Stanford University; Stanford, CA; USA.
3. Dept. of Genetics; Stanford University School of Medicine; Stanford, CA; USA.
4. Dept. of Applied Physics; Stanford University; Stanford, CA; USA.
5. Dept. of Pathology; Stanford University School of Medicine; Stanford, CA; USA.

The regenerative flatworm *Macrostomum lignano*, with its optical transparency and genetic tractability, offers a unique opportunity to study dynamic processes such as wound healing and tissue regeneration *in vivo*. We present a comprehensive toolkit for live imaging of tissue regeneration, including a high-throughput cloning pipeline, targeted cellular ablation, and advanced microscopy techniques. By combining tissue-specific reporter expression with long-term live imaging, we explore how different tissues regenerate. Using a custom luminescence/fluorescence microscope to overcome stress-induced autofluorescence, we reveal the limited regenerative capacity of neurons and their essential role in wound healing, in contrast to the rapid recovery of muscle cells after ablation. Using an open-source tracking microscope, we continuously image freely moving animals throughout the week-long regeneration process, quantifying wound healing, nerve cord repair, body regeneration, and behavioral recovery. Our findings show that severed ventral nerve cords extend linearly and adapt their extension rate in response to asymmetric injuries. Additionally, β-catenin RNAi experiments reveal that ventral nerve cord reconnection is independent of posterior regeneration, revealing separable wound healing and regenerative processes. This toolkit highlights the potential of *Macrostomum lignano* as a powerful genetic model for studying the dynamics of wound healing and regeneration.

Poster – A06

Examining the Impact of m6A loss on the Planarian Transcriptome

Höhn, Constantin[1]; Pittroff, Andreas[1]; Gribling, Anne-Sophie[2]; Smyth, Redmond[2]; Kuhn, Claus-Dieter[1]

1. RNA Biochemistry; University of Bayreuth; 95440 Bayreuth; Germany.
2. Genome Architecture & Evolution of RNA viruses; Helmholtz Institute for RNA-based Infection Research; Josef-Schneider-Straße 2, 97080 Würzburg; Germany.

RNA modifications play a crucial role during the mRNA life cycle in various organisms. Over 150 post-transcriptional modifications of RNA have been identified, with m6A (N6-methyladenosine) being the most abundant on mRNAs. It is enriched in terminal exons without coding potential, 3'-UTRs and within atypically long internal exons. Thereby, m6A is placed on mRNAs in context of a consensus motif. However, only $1 - 5\%$ of all consensus sites are methylated. This suggests that deposition determinants other than the consensus sequence itself exist. In mammals, the presence of the EJC was found such determinant. Its presence at splice junctions precludes m6A deposition. In *S. mediterranea*, approximately 24% of all transcripts carry m6A modifications. In this study, I investigated the impact of m6A loss on the planarian transcriptome. To significantly reduce m6A placement on planarian mRNAs, I knocked down METTL14, a core component of the m6A writer complex, using RNAi, followed by m6A mass spectrometric analysis and Nanopore sequencing. These experiments are intended to elucidate the role of m6A in alternative splicing and broader transcriptional regulation. Understanding the effects of m6A loss on alternative splicing in *S. mediterranea* will provide insight into the fundamental role of m6A in the regulation of gene expression. By knocking down the EJC via RNAi I plan to decipher whether splice-site proximal regions in planarians prohibit m6A placement as found in other organisms.

Poster – A07

Investigating the relation between regeneration and sexual strategy in two planarian species: *Dugesia subtentaculata* and *Dugesia vilafarrei*

Lorenzo, Marina[1]; Rink, Jochen[1]; Vila-Farré, Miquel[1]

1. Department of Tissue Dynamics & Regeneration;
Max Planck Inst. for Multidisciplinary Sciences; Göttingen; Germany.

Regeneration varies among metazoans, even between closely related species, raising the question: Why do some animals regenerate under natural selection pressure while others do not? To answer this, we must combine laboratory research with studies in natural environments. Planarian species show a range of regenerative abilities, from whole-body regeneration in *Schmidtea mediterranea* to almost none in *Bdelloura candida*. We hypothesize that this variability is influenced by reproductive modes, ecological niches, and Wnt signalling. We propose a trade-off in Wnt signalling between asexual reproduction via fission/regeneration and sexual reproduction, as both strategies place opposite demands on Wnt pathway activity.

To explore this, we will study two Dugesid sister species: sexually reproducing *Dugesia vilafarrei* and *Dugesia subtentaculata*, which reproduces sexually, asexually or both. According to our hypothesis, head regeneration may be less efficient in *D. vilafarrei*, and possibly more efficient in asexual than in sexual lineages of *D. subtentaculata*. We will therefore collect individuals across the Iberian Peninsula, barcode them, characterise their reproductive modes, examine their ploidy, and quantify head regeneration ability. We also plan to monitor populations in the field for two years to gather data in natural environments.

In conclusion, our study aims to elucidate the relation between regeneration and sexual reproduction in planarians.

Poster – A08

GTSF1 boosts the catalytic activity of PIWI proteins: Does planarian GTSF1 do the same?

Mautner, Melinda[1]; Fassold, Franziska[1]; Eckert, Simon[1]; Leitner, Alexander[2]; Kuhn, Claus-Dieter[1]

1. RNA Biochemistry; University of Bayreuth; Universitaetsstrasse 30, 95448 Bayreuth; Germany.
2. Department of Biology; Institute of Molecular Systems Biology; ETH Zurich, 8093, Zurich; Switzerland.

The piRNA pathway comprises PIWI proteins that utilize a group of small non-coding RNA, called PIWI-interacting RNAs (piRNAs). The small RNAs serve as guides to silence transposable elements and mRNA transcripts via sequence complementarity. Silencing is achieved by cleavage of the target RNAs, an activity termed "slicer activity". Whereas PIWI's slicer activity needs to be high *in vivo* to counteract transposable elements, PIWI proteins show only weak endonuclease activity *in vitro*. However, recently, an auxiliary factor to PIWI proteins, a small zinc finger protein termed GTSF1, was discovered that was found to boost the catalytic activity of PIWI proteins. The focus of my project lies in the characterization of the two GTSF1 homologs, GTSF1.1 and GTSF1.2., which were identified in the genome of *Schmidtea mediterranea*. While GTSF1.1 is found in neoblasts, pluripotent stem cells that render planarians highly regenerative, GTSF1.2 expression is restricted to the epidermis. By employing RNA interference, I aim to elucidate the biological function of the planarian GTSF1 homologs whose phenotypes were lethal. Furthermore, I investigate the mechanistic roles of GTSF1.1 and GTSF1.2 in modulating the slicer activity of all planarian PIWI proteins utilizing *in vitro* assays. With the use of *in vivo* and *in vitro* experiments, I would like to gain a better understanding of the interplay between the GTSF1 homologs and the PIWI proteins as well as the impact on the planarian piRNA pathway.

Poster – A09

Investigating the Role of the ECM in Regulating Size-Dependent Growth

Mirkes, Kristina[1]; Vlachonikolou, Maria[1,2]; Bormann, Eric[1,2]; Meiborg, Adriaan[1]; Stein, Frank[3]; Rettel, Mandy[3]; Vu, Hanh Thi-Kim[1]

1. Developmental Biology, Vu Lab; EMBL Heidelberg; Meyerhofstr. 1 69117 Heidelberg; Germany.
2. Faculty of Biosciences; University of Heidelberg; 69120 Heidelberg; Germany.
3. Proteomics Core Facility; EMBL Heidelberg; Meyerhofstr. 1 69117 Heidelberg; Germany.

Body size is a fundamental trait in animals, influencing their physiology, ecology, and evolution. However, the mechanisms that govern species-specific body size remain largely unknown. The planarian flatworm *Schmidtea mediterranea* serves as an excellent model for addressing this question, as it can dynamically alter its adult body size in response to nutritional availability and exhibits size-dependent growth rates.

This study aims to investigate the mechanisms controlling size-dependent growth in *S. mediterranea*, focusing on three primary objectives. First, we characterize the growth dynamics of *S. mediterranea* and the underlying cellular processes, such as cell proliferation and cell death, that regulate its size.

Second, we seek to identify the molecular mechanisms modulating these cellular processes. To this end, we investigate the size-dependent expression of genes and proteins, particularly those involved in the extracellular matrix (ECM).

Lastly, we apply functional perturbations to target specific ECM components and regulators. I will present preliminary results showing that genetic perturbation of the ECM via RNA interference affects growth, along with future perspectives on investigating how the ECM regulates cellular behavior.

Poster – A10

Functional analysis of a Z-DNA Binding Protein in Planaria

Naskar, Somdatta[1,2]; Drees, Leonard[1]; Bradauskaitė, Aida[1]; Bickmeyer, Iris[1]; Kagan, Ferenc[1]; Rink, Jochen[1]

1. Department for Tissue Dynamics & Regeneration; Max Planck Institute for Multidisciplinary Sciences (MPI-NAT); Am Faßberg 11, 37077 Göttingen; Germany.
2. Department of Biology; Indian Institute of Science Education & Research, Pune; Dr. Homi Bhabha Rd, Pashan, Pune, Maharashtra 411008; India.

In recent years, Z-DNA, a left-handed form of dsDNA, has emerged as a regulator of cell death, stress, the innate immune response and general transcription. These mechanisms are triggered by Z-DNA binding proteins. To explore whether Z-DNA might play an important role in planarian tissue turnover or regeneration, we searched the *S. mediterranea* genome for Z-DNA binding domains. We identified a single protein with a Z-DNA-binding domain that is highly conserved among planarians, which we have named planarian Z-DNA-binding protein (PZBP). Interestingly, we found that PZBP is critical for the survival of *S. mediterranea*, as knockdown leads to progressive lesions and death. However, RNAi-mediated knockdown of PZBP did not result in an increase in TUNEL-positive cells, nor in a depletion of neoblasts or their cell division rate. Stainings using a custom-made antibody together with whole-mount fluorescence *in situ* hybridisation revealed that the PZBP transcript is ubiquitously expressed, but interestingly, PZBP protein staining is highly enriched at lateral membranes and intracellular structures of epidermal cells. Ongoing experiments include the development of Z-DNA visualisation approaches in planarians, proteomics and RNA-seq analysis of PZBP RNAi worms, and pull-downs of PZBP followed by mass spectrometry. Overall, I hope that my experiments will clarify the mechanistic basis of the PZBP requirement in planarians and whether or how Z-DNA plays a role in this.

Poster – A11

First record of the land planarian species *Rhynchodemus sylvaticus* in a nature of Czechia

Plšková, Karolina[1]

1. biolab, brmlab z.s., Pod kapličkou 20 1300 Prague, Czechia.

Rhynchodemus sylvaticus (Leidy, 1852) is a small species of land planarian with a cosmopolitan distribution. It is a successful invasive species with an unclear origin - several possible centers of origins are known, specifically the Neotropical and Indonesian regions. The Nearctic region, where *Rhynchodemus sylvaticus* was described, is less rich on members of this genus. European origin of this species was also considered by some authors. The current distribution in Europe is known from various countries, mainly from citizen science-based reports. Despite its wide distribution in Europe and its generic cosmopolitan characteristics, this species has not been reported in the wilderness of Czechia yet. Only mentions of *Rhynchodemus sylvaticus* are older reports from Prague and Chotěboř, which contains greenhouse findings. Closest report in non-anthropogenic environment is located in Dunajské luhy area in Slovakia. A set of twelve specimens with pair of ocelli, pointy head, uniformly brown base color with darker longitudinal stripes accompanied by saddle-like patterning above the pharyngeal region in larger animals and a milky ventral side was found in the Úpor-Černínovsko natural reserve in the Central Bohemian region during fieldwork in summer 2023. 2D reconstructions of the sexual apparatus were processed. Eight juveniles showed various levels of sexual maturation. No animal was confirmed as male mature.

Poster – A12

Wired for Healing: exploring neuronal control of intestinal regeneration in planaria

Segnana, Charlotte[1]; Boesmans, Werend[2,3]; Smeets, Karen[1]

1. Zoology; Hasselt University; Agoralaan; Belgium.
2. Biomedical Research Institute; Hasselt University; Agoralaan; Belgium.
3. Department of Pathology; GROW-School for Oncology & Reproduction; Maastricht University Medical Center; The Netherlands.

Planarians exhibit remarkable regenerative capabilities, rapidly rebuilding their nervous and intestinal systems through signals that guide stem cell differentiation and organ patterning. Stem and progenitor cell self-renewal and differentiation are controlled by niche-supplied signals including those from neurons, suggesting a diverse role in regeneration. However, neurotransmitter contributions in invertebrates like planarians remain underexplored. We investigated how serotonergic signaling influences the migration, proliferation, and differentiation of intestinal progenitor cells during gut regeneration. *S. mediterranea* were exposed to serotonergic neurotoxins, fluoxetine and ondansetron (a 5-HT3 receptor antagonist) at various regeneration stages. Blocking the 5-HT3 receptor reduced homeostatic and regenerative stem cell proliferation. Preliminary data reveal that neurotoxin exposure alters gene expression and disrupts gut progenitor markers, impairing regeneration, which suggests a critical role of serotonin in maintaining gut homeostasis and repair. Neurotransmitters like acetylcholine and serotonin are vital in regeneration and wound healing, primarily through modulating cellular activities and inflammation. The planarian model offers an *in vivo* window into intestinal regeneration, expanding knowledge beyond traditional cell and animal models.

Poster – A13

Searching for efficient anesthetic substances for electrophysiological studies in planaria

Shomrat, Tal[1]; Benita, Orel[2]; Nesher, Nir[1]

1. Faculty of Marine Sciences; Ruppin Academic Center; Michmoret; Israel.
2. Department of Neurobiology; Hebrew University; Jerusalem; Israel.

Due to its remarkable regenerative capabilities, the planaria is a leading model organism for regeneration and developmental biology research. The planaria hold the potential to become a valuable reductionist model for neurobiology, as well. It possesses a primal brain which integrates set of sensory information and execute relatively complex behaviors. Furthermore, their brain exhibits neuronal characteristics reminiscent of those found in vertebrates and its exceptional regeneration capabilities allow neurobiological experiments not possible in any other models. A major obstacle in conducting neurophysiological studies in planaria, is the lack of efficient anesthetic and restraining protocols that enable fine electrophysiology. Many protocols have been attempted to date with the goal of immobilizing the planaria, mainly for surgical procedures and microscopy. However, efficient anesthetic for electrophysiology, should completely immobilize the worm without abolishing the brain activity. In the present work we examined 11 drugs as potential anesthetic for electrophysiological experiments tested through three criteria: 1/ Complete immobility; 2/ Reversibility and minimum side effects; and, 3/ Substances that met the first two criteria underwent electrophysiological examination to assess their impact on brain activity. From all substances, Linalool seems to be the most efficient and together with mechanical restraint by fine dissection pins, allowed experiment on large worm.

Poster – A14

Establishing a minimal promoter sequence required for proliferation-specific expression in the flatworm *Macrostomum lignano*

Stranges, Mattia[1]; Reinoite, Filipa[1]; Wurdaski, Jakub[1]; Ustyantsev, Kirill[1]; Berezikov, Eugene[1]

1. European Research Institute for the Biology of Ageing; University Medical Center Groningen; University of Groningen; Groningen, 9700AD; The Netherlands.

The free-living flatworm *Macrostomum lignano* has impressive regenerative properties, conferred by its stem cells (neoblasts) that can continuously replace damaged cells and drive the whole-body regeneration. We identified the histone variant h2a.x as one of the genes expressed exclusively in *M. lignano* cycling cells. We generated several h2a.x knock-in transgenic lines that allow *in vivo* visualization of proliferating cells. Nevertheless, the molecular mechanisms that confer proliferation-specific expression of h2a.x are unknown. The goal of this study is to deepen our understating of the h2a.x gene regulation. To do so, three different linear DNA constructs, each containing mNeonGreen under the control of a gradually shorter version of the upstream promoter region of h2a.x will be injected into single cell stage embryos to obtain stable transgenic lines. These lines will be then compared between each other and to the knock-in lines to determine which regions are important for driving its specific expression in proliferating cells. Once the minimal promoter region is established, bioinformatics analysis will be performed to determine which transcription factors are capable of binding and therefore drive proliferative cell specific expression. Finally, the functionality of the binding sites will be tested by generating transgenic lines with specific binding site deletions in the promoters. This is an ongoing project, and the progress update will be presented at the meeting.

Poster – A15

Establishing *Camerata robusta* as a model organism for regeneration and embryogenesis

Weiss, Philipp[1]; Bradauskaitė, Aida[1]; Rink, Jochen[1]

1. Department of Tissue Dynamics & Regeneration;
Max Planck Institute for Multidisciplinary Sciences (MPI-NAT);
Am Fassberg 11, 37077 Goettingen; Germany.

The ability to regenerate highly varies even between related planarian species. While *Schmidtea mediterranea* is the master of regeneration, little is known about the regenerative capabilities in other flatworm species and their embryonic development. We use *Camerata robusta* from our planarian species collection at MPI-NAT due to their regenerative defects and high abundance of fertile eggs.

We are investigating the regeneration mechanisms, stem cell dynamics, and embryogenesis in *C. robusta*, aiming to uncover conserved aspects of planarian biology. With this objective, we assessed the head and tail regenerative potential of *C. robusta* across different body sizes. While head regeneration was never observed under our cutting paradigm, tail regeneration was possible except for individuals in the larger size class, thus suggesting that body size or age may influence regenerative capacity. This offers an opportunity to identify novel regulators of regeneration by comparing differently-sized worms. Further, the species' high reproductive output allows us to explore its embryonic development. Through *in situ* hybridisation, we aim to pinpoint key developmental markers to map critical stages of early embryogenesis and track cellular differentiation and morphogenesis. Complemented by single cell sequencing experiments, we expect that the abundant availability of *C. robusta* embryos will provide new insights into the unusual embryogenesis of planarian flatworms.

Posters — Session B

Poster – B01

Towards a three dimensional Gene Regulatory Network to Integrate Multi-Omics Time Series of Regeneration Dynamics

Bavafaye Haghighi, Elham[1]; Brochier, Thomas[1]; Brand, Jeremias[1]; Rink, Jochen[1,2]

1. Department of Tissue Dynamics & Regeneration;
Max Planck Institute for Multidisciplinary Sciences; Göttingen; Germany.
2. Faculty of Biology & Psychology;
Georg-August-University Göttingen; Göttingen; Germany.

Objectives: In light of the increasing availability of next-generation sequencing data, this study describes our latest effort to develop a three-dimensional gene regulatory network (3D-GRN) that integrates time-series multi-omics data from *S. mediterranea* regeneration.

Methods: Time-series bulk-tissue RNA-seq and ATAC-seq data were collected simultaneously at head and tail regeneration time points. Differentially and generally accessible chromatin regions within promoters and intronic sites of significantly regulated genes were integrated at each time point and condition to recognize enriched motif sets. Potential target genes of the selected TFs were identified for specific conditions and time points. Furthermore, delayed regulations between GRNs were uncovered, leading to the construction of a unified 3D-GRN for each condition.

Results: The efforts so far highlight multiple challenges, including the often unclear orthology between planarian and human TFs. The inferred GRNs are relatively shallow due to the presence of multiple overlapping feedback loops. A large number of homeodomain TFs stand out, highlighting the need for further investigation into the specific dimerization of homeobox and other TFs. Further development of our 3D-GRN holds the potential to identify key initiators, hub genes, feedback cycles, and the general temporal progression of different regeneration paradigms as an important step in understanding the gene regulatory basis of planarian regeneration.

Poster – B02

The role of the ECM in controlling the growth of *Schmidtea mediterranea*

Bormann, Eric[1,2]; Mirkes, Kristina[1]; Vu, Hanh Thi-Kim[1]

1. Developmental Biology; EMBL Heidelberg; Meyerhofstraße 1, 69117 Heidelberg; Germany.
2. Faculty of Biosciences; Ruprecht-Karls-Universität Heidelberg; Grabengasse 1, 69117 Heidelberg; Germany.

The body size of *Schmidtea mediterranea* can vary by more than 40-fold in length, with this change in size primarily reflecting differences in cell number. Regulation of mitosis and apoptosis, the two key processes of cellular turnover, is therefore crucial in controlling growth. Extracellular environments have been shown to play key roles in regulation cellular behavior and mechanisms. The overall goal of my project is to investigate the role of the extracellular matrix (ECM) in controlling growth and, ultimately, body size in *S. mediterranea*. To test if the ECM is essential for growth in planarians, pharmacological inhibition and genetic perturbation utilizing RNA interference has been utilized to disrupt the function of ECM regulator proteins involved in the degradation, synthesis, and crosslinking of core ECM components. Alongside these functional studies, work has been ongoing to establish a reliable imaging protocol to visualize collagen fibers to allow for the characterization of potential structural changes in the ECM during growth or in response to specific treatments. In my poster, I will present preliminary findings and discuss future directions for the project. Ultimately, through the systematic functional characterization of the ECM in planarians, we hope to gain new insights into the mechanisms that regulate growth and ultimately control body size in these organisms.

Poster – B03

Identification and characterization of planarian multinucleated cells

Dutta, Annasha[1]; Samelak-Czajka, Anna[1]; Trybus, Magdalena[1]; Zaremba, Anastasiia[1]; Jackowiak, Paulina[1]

1. Laboratory of Single Cell Analyses;
Institute of Bioorganic Chemistry Polish Academy of Sciences;
Zygmunta Noskowskiego 12/14, Poznań - 61-704; Poland.

Multinucleated cells are present across a wide range of species and can form in both physiological and pathological contexts—as stress-resilient cell types, in processes related to organismal growth and early development and, in response to immune activation. In planarians, multinuclearity has been observed only during sexual development and in cases of externally induced mitotic inhibition. In the present study, we have identified and characterized a stable pool of multinucleated cells in the asexual *S. mediterranea*, that are consistently present under wildtype and regenerating conditions. To achieve this, we have developed innovative staining and gating strategies utilising various flow-cytometry platforms, for the targeted identification of multinucleated cells from a diverse pool. Additionally, we have morphologically characterized them based on cell size and the number and size of nuclei per cell. By analysing the expression of specific marker genes indicative of varying differentiation potentials, we determined that these cells are undifferentiated. Moreover, both irradiation and H2B knockdown-mediated depletion of neoblasts revealed that the population containing these multinucleated cells was less affected than the neoblasts themselves, indicating a non-neoblast cell type. Altogether, the study suggests that these stably present multinucleated cells might represent certain stem/progenitor cell types which are potentially crucial for planarian viability.

Poster – B04

Smed-cbp-3 is essential for progenitor cells lineage progression and parenchymal cells gene regulation in planarians

Guixeras, Anna[1,2]; Molina, M. Dolores[3]; Abril, Josep F.[1,2]; Cebrià, Francesc[1,2]

1. Department of Genetics, Microbiology & Statistics; Universitat de Barcelona; Barcelona, Catalunya, Spain.
2. Institute of Biomedicine (IBUB); Universitat de Barcelona; Barcelona, Catalunya, Spain.
3. Department of Bioengineering; IQS School of Engineering; Universitat Ramon Llull; Catalunya, Spain.

Characterizing how planarian stem cells self-renew and differentiate into all cellular lineages is one of the main questions of the field. CBP (CREB-binding protein)/p300 proteins have an acetyltransferase activity and belong to a conserved gene family which functions as transcriptional co-activators regulating gene expression. In different organisms, CBP/p300 proteins have been involved in stem cell proliferation and differentiation. Several CBP homologues have been identified in planarians and functional analyses have uncovered an important role for *Smed-cbp-3* in stem cell differentiation. Ongoing RNA-seq analyses have identified specific biological processes and cell populations affected after silencing *cbp-3*. Remarkably, many genes expressed in parenchymal cells appear up-regulated after *cbp-3* RNAi. Also, from the identified transcription factors miss-regulated after silencing *cbp-3*, most of them are expressed in neoblasts or progenitor cells. In addition, our recent work has shown that stem cells and progenitors accumulate in the absence of *cbp-3* due to its inability to pursue differentiation, while mature differentiated cells of several cellular lineages are reduced, both under regeneration and homeostatic conditions. This inability might be caused by cell cycle alterations, as cycling cells were found to be accumulated. All these data should allow to better characterize the role of *cbp-3* in the regulation of stem cell differentiation.

Poster – B05

Autofluorescence, more than a challenge: The role of riboflavin in planarian sensing

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

1. Zoology; Biodiversity & Toxicology; Centre for Environmental Sciences; Hasselt University; Agoralaan Gebouw D 3590 Diepenbeek; Belgium.

The redox balance is crucial during planarian regeneration, as reactive oxygen species are essential for cellular repair and tissue development. Metabolically active tissues, such as nervous tissue, are especially vulnerable to redox imbalances. The redox-sensitive metabolite riboflavin (Vitamin B2) has shown the potential to promote (nerve) regeneration in several other organisms.

In this study, we localized and characterized riboflavin-containing tissues in the planarian *Schmidtea mediterranea*. Using real-time *in vivo* imaging and in-depth spectral analyses, we identified that flavins, which exhibit autofluorescent properties, are predominantly localized in neuron-like structures ventral to the bi-lobed ganglia, and in the intestinal tract. The riboflavin transporter was found to be highly expressed in neural tissues and localized in smedwi-positive cells, suggesting riboflavin's role in both neuronal function and regeneration.

To further investigate the role of flavins, various stressors were applied, indicating that riboflavin-containing neurons are involved in multiple aspects of planarian sensing, mediated via monoaminergic neurotransmission such as dopamine. Specifically, increased mitochondrial activity in riboflavin-containing neurons was observed following exposure to light, chemical and physical stimuli. Lastly, targeting riboflavin conversion and uptake negatively affected sensing and the regeneration of neuronal cells.

Poster – B06

Reconstruction of positional information during homeostasis and regeneration from the spatial transcriptomics of *Schmidtea mediterranea*

Leung, Casey Ka Wun[1]; Vu, Hanh Thi-Kim[2]; Goyal, Sidhartha[1]

1. Department of Physics; University of Toronto; 60 St George St, ON M5S1A1 Toronto; Canada.
2. Developmental Biology Unit; European Molecular Biology Laboratory (EMBL); Meyerhofstraße 1, 69117 Heidelberg; Germany.

Organ or whole body regeneration requires both positional and directional information to regulate the fate of undifferentiated cells. Positional information can be encoded by a set of genes, as demonstrated by morphogens in several species. In planarians, positional control genes (PCGs) are spatially restricted genes, many of which play important roles in patterning the planarian body plan during regeneration. Muscle polarity, on the other hand, is thought to provide directional information. Historically, it has been technically challenging to systematically investigate the genetic basis of this information. The recent revolution in RNA sequencing methods with higher spatial resolution, such as Visium 10x Genomics, offers a novel approach to identify genes that carry positional information in an unbiased manner. We use published spatial transcriptomic data from *Schmidtea mediterranea* regeneration to develop computational methods for identifying genes that have the potential to provide positional/directional information and drive regeneration in planarians. Our statistical results show that genes expressed in neoblasts and muscles exhibit distinct dynamic changes in positional information during regeneration. Gene ontology analysis further indicates that distinct gene functionalities are associated with changes in positional information at specific time points. Overall, we hope that our approach will provide new insights into positional information and regeneration.

Poster – B07

Investigating the Role of Activin Signaling in the Reproductive System of Sexual *Schmidtea mediterranea*

Mandal, Arkadeep[1,2]; Brochier, Thomas[2]; Rink, Jochen[2]

1. Department of Biology; Indian Institute of Science Education & Research, Pune; Dr. Homi Bhabha Road, Pune, 411008; India.
2. Tissue Dynamics & Regeneration; Max Planck Institute for Multidisciplinary Sciences; Am Fassberg 11, 37077 Göttingen, Germany.

Planarians possess a unique ability to scale their body size depending on nutrient availability, dynamically adjusting body size through growth when fed and shrinkage when starved. This plasticity is associated with size-dependent physiological processes, including energy metabolism, growth and de-growth rates, and the size-dependent formation of the reproductive system. In the sexual strain of the species *Schmidtea mediterranea*, reproductive tissues fully develop only at a body length of around 6 mm, implying the existence of a mechanism that links it to body size. In planarians, activin signaling is known to influence crucial processes like axis patterning, growth, and regeneration. Interestingly, activins have been identified as key regulators of gonadal function and spermatogenesis in other model organisms. Given the size-dependent appearance of sexual organs in sexual planarians, we hypothesize that activin signaling might have a dual role, coordinating and linking growth with reproductive function. Through expression analysis of activin pathway components, functional studies using RNA interference, and size-based comparisons, we aim to explore further the role of activins and elucidate the rate-limiting steps and molecular pathways involved in the formation of the reproductive system from pluripotent stem cells. This will uncover critical insights into the regulation of size-dependent tissue development and reproductive organogenesis in planarians.

Poster – B08

Establishment of an ultra-scalable multiplexed single-cell RNA sequencing protocol for planarian flatworms

Mellado-Fuentes, Ana M.[1]; Palavalli, Amrutha[1]; Vu, Hanh Thi-Kim[1]

1. Developmental Biology Unit;
European Molecular Biology Laboratory (EMBL);
Meyerhofstraße 1, Heidelberg; Germany.

Single-cell combinatorial indexing RNA sequencing (sci-RNA-seq) integrates in situ molecular indexing with a 'split-pool' strategy, enabling the unique labelling of an exponentially scalable number of cells or nuclei using distinct combinations of nucleic acid barcodes. This approach offers a highly cost-effective and multiplex solution for profiling tens of thousands to millions of nuclei across hundreds of samples in a single experiment, using only standard molecular biology laboratory reagents and equipment. In this poster, I will present an adapted sci-RNA-seq protocol designed for planarians, building on established combinatorial indexing methods. I will emphasise key modifications we have made to enhance the sensitivity and robustness of the protocol, as well as share practical tips for setting up this technique in your lab. I hope this refined method provides an ultra-scalable, reliable, and affordable approach for the community for studying gene expression at the single-cell level in planarians.

Poster – B09

Size-dependence of neoblast abundance and activity in *S. mediterranea*

Moroni, Maria Lina[1,2]; Rink, Jochen[1]

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

2. Göttingen Graduate School for Neurosciences & Molecular Biosciences (GGNB);
Georg-August-Universität Göttingen;
Wilhelmsplatz 1, 37073 Göttingen; Germany.

The planarian flatworm *S.mediterranea* can reversibly scale its body size depending on food availability; this intriguing ability is rooted in the presence of neoblasts, an abundant population of adult pluripotent stem cells. During starvation, neoblasts proliferate at a basal level to maintain tissue turnover; feeding transiently boosts their proliferation, leading to an increased addition of new cells and thus to overall growth of the animal. Both growth and degrowth rates correlate inversely with size: this suggests that the fractional abundance and/or activity of neoblasts are themselves size-dependent. I used piwi-1 FISH and H3P/PIWI-1 immunostaining on cell suspensions from worms of different sizes to measure the ratio of neoblasts to total cells, their proliferative activity and their tendency to self-renew or differentiate. In starved worms I measured a fractional abundance of neoblasts that increases with size, while their proliferative activity remains constant. During the feeding response, large worms have only one short proliferation wave, while small ones have also a second, longer proliferation wave, accompanied by a 2-fold increase in the fractional abundance of neoblasts. Overall, my data so far suggest that a transient increase in symmetric self-renewing neoblast divisions contributes to the prolonged feeding response in small animals, and that modulating neoblast division outcomes may contribute to the size-dependence of the growth rate at the cellular level.

Poster – B10

Inference of planarian life history strategies from organismal-scale single-cell transcriptomics

Palavalli, Amrutha[1]; Mellado-Fuentes, Ana M.[1]; Meiborg, Adriaan[1]; Dorrity, Michael[1]; Werner, Steffen[2]; Vu, Hanh Thi-Kim[1]

1. Developmental Biology Unit;
European Molecular Biology Laboratory (EMBL);
Meyerhofstraße 1, 69117 Heidelberg; Germany.

2. Experimental Zoology; Wageningen University & Research;
Droevendaalsesteeg 4, 6708 PB Wageningen; Netherlands.

All organisms perform multiple tasks such as growth, homeostasis and reproduction. As resources are finite, each organism must allocate its resources into tasks that maximize its fitness (i.e. reproductive success) in a specific ecological niche, resulting in trade-offs. Over many generations, such trade-offs result in species with a collection of life history traits optimised for their environment. However, the mechanistic understanding of life history traits and trade-offs remains incomplete, mainly due to the reliance on correlative studies focused on a limited number of easily measurable traits. Multicellular systems are composed of many morphologically and functionally specialised cell types that uniquely influence an organism's ability to perform tasks. Thus, analysing cellular composition at the organismal scale could reveal key biological tasks associated with life history traits and putative trade-offs. Here, I will discuss our efforts to use a concept from engineering and economics—Pareto optimality theory—to explore the possibility of inferring life history traits, their trade-offs, and potential regulatory mechanisms from high-dimensional organismal-scale single-cell transcriptomics of planarian flatworms, as well as the prospect of using the extensive functional genomics toolkit of planarians to directly test predicted cause-effect relationships.

Poster – B11

Smed-spag1 is required for motile cilia function, while *Smed-polr2k* is essential for planarian regeneration and maintaining body homeostasis

Rabiasz, Alicja[1]; Ziętkiewicz, Ewa[1]

1. Institute of Human Genetics; Polish Academy of Sciences; Poznan; Poland.

SPAG1 (sperm-associated antigen 1) is one of over 50 genes whose pathogenic variants cause primary ciliary dyskinesia (PCD), an inherited disease affecting the function of motile cilia. Two pathogenic variants predominate: a nonsense mutation in exon 16, and a large deletion encompassing parts of *SPAG1* and the upstream *POLR2K* (RNA Polymerase II, I And III Subunit K). The large deletion was found in compound heterozygosity in 30 of 60 *SPAG1*-PCD patients; interestingly, no homozygotes with this variant were identified. We suspected that this reflected lethal effect of the loss of a functional POLR2K rather than of SPAG1 protein. To examine our hypothesis we decided to compare the effect of knockdown of both genes in *Schmidtea mediterranea*, which is an attractive model organism for studying the functionality of embryonic lethal genes, and for analyzing cilia-related genes.

Knockdown of *Smed-spag1* led to characteristic changes in the planarian' locomotion pattern and speed, while silencing of *Smed-polr2k* caused severe body defects in worms, and ultimately led to their death. Despite the enormous regenerative capacity of planarians, *Smed-polr2k* deficiency resulted in disruption of the regeneration process. Our findings supported our hypothesis that the lack of homozygous patients with the large deletion reflected the lethal effect of the loss of POLR2K.

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Poster – B12

TRPA1 agonists-induced nociceptive responses in planarians

Morana, Rémy[1]; Darbon, Bénédicte[1]; Herrmann, Lalee[1]; Menger, Yannick[1]; Reho, Guillaume[1]; Cadiou, Hervé[1]

1. CNRS UPR 3212 Institut des Neurosciences Cellulaires et Intégratives; Université de Strasbourg; Strasbourg; France.

Nociception is defined as "the neural process of encoding noxious stimuli" by the International Association for the Study of Pain (IASP). Nociception relies on detecting noxious stimuli arising from a potentially or actually tissue-damaging event via specialized cells called nociceptors. In planarians, nociceptive behavior is often indicated by a 'scrunching' gait, in contrast to the usual gliding behavior displayed in normal conditions. Previous work carried by our team has shown that mustard oil induced a scrunching behavior in *Dugesia dorotocephala* in a dose-dependant manner mediated by the nociceptive ion channel TRPA1. Cinnamaldehyde, the main volatile compound from cinnamon, is also a TRPA1 agonist in vertebrates. Addition of 400 μM of cinnamaldehyde induced a strong scrunching behavior ($64.5 \pm 5.6\%, n = 12$). This effect also followed a dose-response curve, was mediated by TRPA1 (abolished by RNAi Gd-TRPA1 knockdown) and was reduced by the addition of morphine or meloxicam. H_2O_2, another TRPA1 agonist, produced similar effects. In conclusion, planarians are a suitable animal model to study TRPA1-mediated nociception.

Poster – B13

Deciphering the evolutionary conservation of Planarian neural regeneration: A Genetic Screening Approach in Mammalian Cells

Spagnoletti, Luca[1,2]; Codino, Azzurra[1]; Gustincich, Stefano[1]; Pandolfini, Luca[1]

1. Center for Human Technology; Italian Institute of technology; Via Enrico Melen 83 Genova; Italy.
2. The Open University; Walton Hall, Kents Hill, Milton Keynes MK7 6AA; UK.

Planarians possess a unique ability to regenerate their central nervous system, a capability lacking in mammals. Despite many conserved signaling pathways, planarian neoblasts express hundreds of genes with unknown function and lacking any close orthology in other species.

Our work aims to screen planarian genes enriched in neoblasts during neural regeneration within mammalian cells. We successfully cloned the entire PolyA+ transcriptome of planarian (neural) neoblasts into a plasmid library for mammalian expression, as confirmed by long-read nanopore sequencing.

Using SOX1-GFP mouse embryonic stem cells (mESCs) as a model for mammalian neurogenesis, we developed a protocol for screening the library for neural-inducing genes. This involves dendrimer-mediated transfection and differential representation analysis of planaria genes in GFP+ (neural committed) versus GFP- mESCs via nanopore sequencing of the corresponding ds-cDNA.

Identified genes will undergo targeted analyses to determine their ability to sustain neurogenesis under various conditions as well as the molecular determinants involved.

This research aims to bridge the gap between planarian and mammalian neural regeneration, providing insights for neuroregenerative medicine and evolutionary developmental biology.

Poster – B14

How do planarian flatworms colonise the land?

Vlachonikolou, Maria[1]; Lewin, Thomas[2]; Campbell, Lahcen[3]; Jarreta, Jorge Alvarez[3]; Sitnik, Vasily[3]; Ross, Eric[4]; Robb, Sofia[4]; Stoga, Daniel[1]; Cleland, James[1,5]; Dyer, Sarah[3]; Luo, Yi-Jyun[2]; Vu, Hanh Thi-Kim[1]

1. European Molecular Biology Laboratory; Heidelberg; Germany.
2. Biodiversity Research Center; Academia Sinica; Taipei; Taiwan.
3. Wellcome Genome Campus;
European Molecular Biology Laboratory–European Bioinformatics Institute;
Hinxton; UK.
4. Stowers Institute for Medical Research; Kansas City; USA.
5. German Cancer Research Center; Heidelberg; Germany.

The transition from water to land is one of the most remarkable evolutionary events in the history of life on Earth. In order to thrive in terrestrial environments, organisms had to evolve new strategies to adapt to the vastly different physiological and structural requirements of life on land. Planarian flatworms represent one of the first animal groups to successfully colonize and diversify on land. However, the mechanisms underlying the terrestrialization of planarians remain largely unexplored. The aim of my project is to address this gap using the terrestrial planarian *Obama nungara* as a model species. In this presentation, I will first highlight our ongoing efforts to establish *O. nungara* as a new model species, including updates on the first genome assembly and annotation for the species, as well as the first comprehensive single-cell atlas of *O. nungara* from hatching to adult. Next, I will discuss preliminary findings on potential molecular and cellular innovations that drove the evolutionary transition of *O. nungara* to land, and outline future directions for the project. By uncovering the mechanisms that enabled planarians to adapt to terrestrial environments, this study will deepen our understanding of the early stages of land colonization by animals.

Poster – B15

A comprehensive pipeline for genome annotation: a case study on *Schmidtea mediterranea*

Zaremba, Anastasiia[1]; Marszałek-Zeńczak, Małgorzata[1]; Dutta, Annasha[1]; Samelak-Czajka, Anna[1]; Jackowiak, Paulina[1]

1. Laboratory of Single Cell Analyses;
Institute of Bioorganic Chemistry Polish Academy of Sciences;
Zygmunta Noskowskiego str. 12/14, 61-704 Poznań; Poland.

Genome annotation is crucial for understanding genetic information and plays a key role in exploring species diversity. However, for non-classical model organisms like *Schmidtea mediterranea*, current genome annotations are inaccurate and incomplete. Here, we introduce a comprehensive genome annotation pipeline that can be applied across different species, using both reference-guided and de novo strategies. By combining long-read sequencing (PacBio) with short-read sequencing (Illumina), we improved transcriptome assembly and gene prediction. This approach allowed us to correct genome annotations and predict new isoforms with potential biological significance. To ensure the accuracy of our results, we tested 34 different configurations using Spike-In RNA Variant Controls. Our best method, which merges reference-based and de novo approaches, achieved 81.08% prediction accuracy. Furthermore, through the prediction of non-coding RNAs and isoforms analysis, we demonstrated that our pipeline offers more accurate and comprehensive annotation than those currently available. Our framework is versatile and can be adapted for other species, offering a powerful tool for genome annotation in a variety of biological systems.

Participants

Abnave, p.b.
National Centre for Cell Science; *Savitribai Phule Pune University; IN*
✉ prasadabnave
@ gmail.com

Abril, j.f.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ jabril
@ ub.edu

Adell, t.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ tadellc
@ ub.edu

Balaji, k.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ kalyani.balaji
@ mpinat.mpg.de

Bavafaye Haghighi, e.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ elham.bavafaye
@ mpinat.mpg.de

Bian, z.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ zhengyue.bian
@ mpinat.mpg.de

Bijnens, k.
Zoology: Biodiversity and Toxicology; *Centre for Environmental Sciences; Hasselt University; BE*
✉ karolien.bijnens
@ uhasselt.be

Bormann, e.j.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ eric.bormann
@ embl.de

Cadiou, h.
Institut des Neurosciences Cellulaires et Intégratives; *Université de Strasbourg; FR*
✉ cadiou
@ inci-cnrs.unistra.fr

Cebrià, f.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ fcebrias
@ ub.edu

Cuadrado, d.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ cuadradopm
@ hotmail.com

Drees, l.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ leonard.drees
@ mpinat.mpg.de

Dreier, f.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ fabienne.dreier
@ mpinat.mpg.de

Dutta, a.
Laboratory of Single Cell Analyses; *Institute of Bioorganic Chemistry; Polish Academy of Sciences; PL*
✉ adutta
@ ibch.poznan.pl

Fassold, f.
RNA Biochemistry; *University of Bayreuth; DE*
✉ franziska.fassold
@ uni-bayreuth.de

Font-Martín, d.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ dfontmar7
@ ub.edu

García Castro, h.
Faculty of Health and Life Sciences; *University of Exeter; UK*
✉ h.garcia-castro
@ exeter.ac.uk

González Estévez, c.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ crisgonzalez
@ ub.edu

Goyal, s.
Department of Physics; *University of Toronto; CA*
✉ sidhartha.goyal
@ gmail.com

Guixeras Fontana, a.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ aguixerasf
@ ub.edu

Hall, n.
Department of Bioengineering; *Stanford University; CA, USA*
✉ nh2894
@ stowers.org

Heleven, m.
Zoology: Biodiversity and Toxicology; *Centre for Environmental Sciences; Hasselt University; BE*
✉ martijn.heleven
@ uhasselt.be

Höhn, c.
RNA Biochemistry; *University of Bayreuth; DE*
✉ Constantin1.Hoehn
@ uni-bayreuth.de

Kauffmann, b.
Zoology: Biodiversity and Toxicology; *Centre for Environmental Sciences; Hasselt University; BE*
✉ bob.kauffmann
@ uhasselt.be

Kuhn, c.
RNA Biochemistry; *University of Bayreuth; DE*
✉ claus.kuhn
@ uni-bayreuth.de

Kumar, n.
Institute for Stem Cell Science & Regenerative Medicine; *IN*
✉ nikhilkk
@ instem.res.in

Lei, k.
School of Life Sciences; *Westlake University; CN*
✉ leikai
@ westlake.edu.cn

Leung, c.
Department of Physics; *University of Toronto; CA*
✉ casey.leung
@ mail.utoronto.ca

Lorenzo, m.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ marina.lorenzo
@ mpinat.mpg.de

MANDAL, A.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ arkadeep.mandal
@ mpinat.mpg.de

MARTIROSYAN, H.
RNA Biochemistry; *University of Bayreuth; DE*
✉ heghnar.martirosyan
@ uni-bayreuth.de

MAUTNER, M.
RNA Biochemistry; *University of Bayreuth; DE*
✉ melinda.mautner
@ uni-bayreuth.de

MEIBORG, Y.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ adriaan.meiborg
@ embl.de

MELLADO FUENTES, A.M.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ ana.mellado
@ embl.de

MIRKES, K.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ kristina.mirkes
@ embl.de

MORENO-BLAS, D.
Genetics, Microbiology & Statistics Dept; *Universitat de Barcelona; CAT, ES*
✉ dmoreno
@ ub.edu

MORONI, M.L.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ maria.moroni
@ mpinat.mpg.de

NASKAR, S.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ somdatta.naskar
@ mpinat.mpg.de

PALAVALLI, A.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ amrutha.palavalli
@ embl.de

PANDOLFINI, L.
Center for Human Technology; *Italian Institute of technology; IT*
✉ luca.pandolfini
@ iit.it

PEARSON, B.P.
School of Medicine; *Oregon Health & Science University; OR, USA*
✉ pearsobr
@ ohsu.edu

PELLETTIERI, J.
Department of Biology; *Keene State College; NH, USA*
✉ jpellettieri
@ keene.edu

PÉREZ-POSADA, A.
Living Systems Institute; *University of Exeter; UK*
✉ a.perez-posada
@ exeter.ac.uk

PERON, S.
Living Systems Institute; *University of Exeter; UK*
✉ s.peron
@ exeter.ac.uk

PITTROFF, A.
RNA Biochemistry; *University of Bayreuth; DE*
✉ andreas.pittroff
@ uni-bayreuth.de

Plšková, k.
Hackerspace Prague; *brmlab z.s.; CZ*
✉ sumie-dh
@ sumie-dh.cz

Rabiasz, a.
Dep. of Molecular & Clinical Genetics; *Inst. of Human Genetics; Polish Academy of Sciences; PL*
✉ alicja.rabiasz
@ igcz.poznan.pl

Rechavi, o.
Dept. of Neurobiology; *Sagol School of Neuroscience; Tel-Aviv University; IL*
✉ odedrechavi
@ gmail.com

Reho, g.
Institut des Neurosciences Cellulaires et Intégratives; *Université de Strasbourg; FR*
✉ greho
@ unistra.fr

Riaz, m.r.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ muhammadrizwan.riaz
@ mpinat.mpg.de

Rink, j.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ jochen.rink
@ mpinat.mpg.de

Sacha, m.
Center for Advanced Preclinical Imaging; *1st Faculty of Medicine, Charles University; CZ*
✉ martin.sacha
@ lf1.cuni.cz

Segnana, c.
Zoology: Biodiversity and Toxicology; *Centre for Environmental Sciences; Hasselt University; BE*
✉ charlotte.segnana
@ uhasselt.be

Shomrat, t.
Faculty of Marine Sciences; *Ruppin Academic Center; IL*
✉ tal.shomrat
@ mail.huji.ac.il

Smeets , k.
Zoology: Biodiversity and Toxicology; *Centre for Environmental Sciences; Hasselt University; BE*
✉ karen.smeets
@ uhasselt.be

Solana Garcia, j.
Living Systems Institute; *University of Exeter; UK*
✉ j.solana
@ exeter.ac.uk

Spagnoletti, l.
Center for Human Technology; *Italian Institute of technology; IT*
✉ luca.spagnoletti
@ iit.it

Stranges, m.
University Medical Center Groningen; *University of Groningen; NL*
✉ mattiastranges1
@ gmail.com

Tripathi, a.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ animan.tripathi
@ mpinat.mpg.de

VAIDYA, G.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ gaurav.vaidya
@ embl.de

VANNI, V.
Living Systems Institute; *University of Exeter; UK*
✉ v.vanni
@ exeter.ac.uk

VILA FARRÉ, M.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ mvilafa
@ mpinat.mpg.de

VLACHONIKOLOU, M.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ maria.vlachonikolou
@ embl.de

VU, H.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ hanh.vu
@ embl.de

WANG, B.
Dept. of Bioengineering; *Stanford University; CA, USA*
✉ wangbo
@ stanford.edu

WEISS, P.
Department of Tissue Dynamics & Regeneration; *Max Planck Institute for Multidisciplinary Sciences; DE*
✉ philipp.weiss
@ mpinat.mpg.de

WURTZEL, O.
School of Neurobiology, Biochemistry & Biophysics; *Tel Aviv University; IL*
✉ owurtzel
@ tauex.tau.ac.il

YANG, Y.
Beijing Institute of Genomics, Chinese Academy of Sciences; *China National Center for Bioinformation; CN*
✉ yingyang
@ big.ac.cn

YAVRU, D.
Developmental Biology Unit; *EMBL-Heidelberg; DE*
✉ yavrudnz
@ gmail.com

ZAREMBA, A.
Laboratory of Single Cell Analyses; *Institute of Bioorganic Chemistry; Polish Academy of Sciences; PL*
✉ azaremba
@ ibch.poznan.pl

Authors Index

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M

N

P

R

S

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Numbers in **bold** for presenting author contributions.

Notes