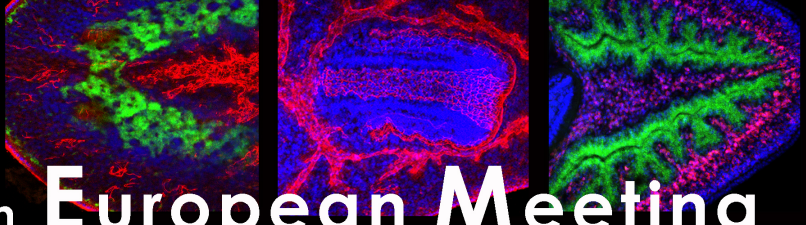

4th European Meeting on Planarian Biology

25 - 27 September 2016

Sant Feliu de Guíxols - Catalonia

Plenary lecture
Peter Reddien (HHMI)

Invited speaker
Norito Shibata (Kyoto)

Sessions on:
Stem cells
Signalling & Regeneration
Genomics & Transcriptomics
Ecology & Evolution
Parasitism

http://europlannet.org/EMPB2016

ABSTRACTS BOOK

EMBO excellence in life sciences

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Organizers:
Francesc Cebrià (UB)
Teresa Adell (UB)
Jochen Rink (MPI-CBG)

EMPB2016

4th European Meeting on Planarian Biology

September 25-27, 2016

Sant Feliu de Guíxols, Catalunya

http://europlannet.org/EMPB2016

 **#empb2016**

Organized by:

Teresa Adell
University of Barcelona, Catalunya

Francesc Cebrià
University of Barcelona, Catalunya

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MPI-CBG, Dresden, Germany

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About the Meeting

Introduction

We are happy to welcome all of you to the 4th European Meeting on Planarian Biology (EMPB2016) that takes place from September 25th to 27th, 2016, at Hotel Eden Roc in Sant Feliu de Guixols, Catalunya. This meeting continues the tradition of the previous meetings (Münster 2010, Kyoto 2011, Dresden 2013 and Oxford 2015).

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

In this sense, we are really pleased to have Peter Reddien, from MIT (USA) and Norito Shibata, from the National Institute of Technology, Tsuyama College (Japan), as **invited speakers** as their numerous contributions have improved significantly our understanding on planarian regeneration. Furthermore, the Committee has selected 24 talks and 18 posters, for which you can find details on this abstract book. We hope you will enjoy both the scientific topics as well as the location.

Best wishes from the Organizing Committee...

About the Abstracts

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

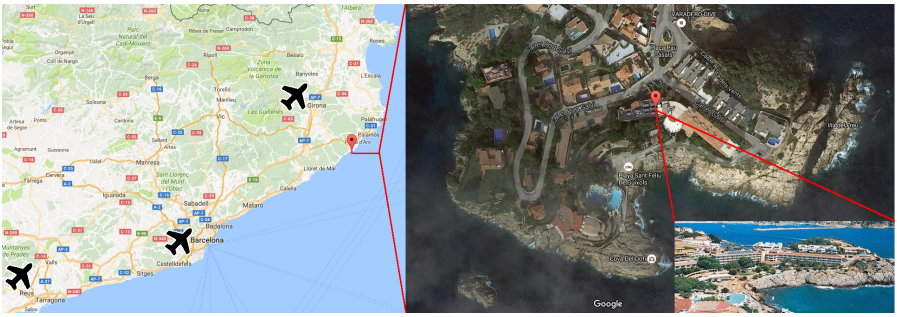
Talks abstracts follow same ordering as in the program sessions, while the posters abstracts were sorted alphabeticaly by first author surnames. You can find an index at the end of this book summarizing all the authors that were contributing to all the presented abstracts, regardless those abstracts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

Venue Information

Hotel Eden Roc

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

+0034-972-320-100



Google Maps Coordinates: Latitude 41.772304 – Longitude 3.030225

How to Arrive

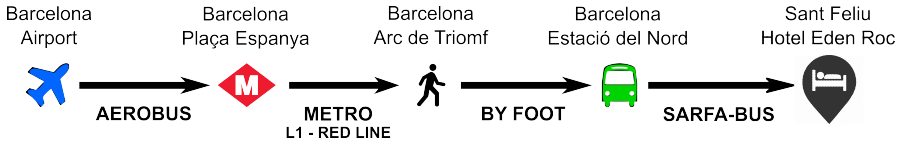
By Plane:

We assume you will fly to Barcelona airport, yet there are two other airports at Girona and Reus, and you will take a bus or train to reach the hotel at Sant Feliu de Guixols.

By Car:

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

By Bus:



Barcelona Airport – Barcelona city

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

Barcelona city - Sant Feliu

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

By Train:

The nearest train station is in Girona (at 30 Km.). A taxi from the train station to Sant Feliu costs about 46 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 EMPB2016 sponsors:

Sessions Program

Sunday, September 25th

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

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

Keynote Lecture by Peter Reddien
[Dpt. of Biology, Whitehead Institute, HHMI]
Regulation of the fates of specialized neoblasts

17:00–18:30 **Session I – Growth Control**
Chair: *Alessandra Salvetti*

17:00–17:20 Óscar Gutiérrez
[Leibniz Institute on Aging – Fritz Lipmann Institute]
Mechanisms of planarian stem cell regulation during starvation

17:20–17:40 Eudald Pascual
[Dpt. Genetics, Microbiology & Statistics, University of Barcelona]
Smed-BS is a novel secreted peptide with an essential role in the control of cell number in planarians

17:40–18:00 Albert Thommen / Steffen Werner
[MPI Molecular Cell Biology & Genetics / MPI Physics of Complex Systems, Dresden]
There and back again—towards a quantitative model of planarian growth and degrowth

18:00–18:30 **"Explain your Poster" Session**

19:00– *Welcome cocktail*
20:00– *Dinner (buffet)*

21:00– **Poster Session I**

Monday, September 26th

09:00–09:45 **Invited Speaker: Norito Shibata**
[National Institute of Technology, Tsuyama College]
Coordination of expression of genes by PIWI during differentiation of neoblasts

09:45–10.25 **Session II – Neoblasts I**
Chair: *Luca Gentile*

09:45–10:05 Sounak Sahu
[University of Oxford]
Radiation sensitivity and DNA damage response mechanism in planarian stem cells

10:05–10:25 Aziz Aboobaker
[University of Oxford]
The planarian ortholog of the tumour suppressor Mll3/4 prevents stem cell hyperplasia and controls differentiation programs

10:25–11:10 *Coffee Break* ☕

11:10–12:50 **Session III – Neoblasts II**
Chair: *Kerstin Bartscherer*

11:10–11:30 David Schmidt
[MPI Molecular Biomedicine, Münster]
Neoblast maintenance requires Integrator complex-mediated processing of Uridine-rich snRNAs

11:30–11:50 Luca Gentile
[Fraunhofer Institute for Biomedical Engineering, Sulzbach/Saar]
6/9.2 a potential surface protein marker for planarian pluripotent stem cells

11:50–12:10 John M. Allen
[San Diego State University]
A screen to identify RING E3 ubiquitin ligases involved in stem cell regulation in vivo

12:10–12:30 Stijin Mouton
[European Research Institut-Biology of Ageing, Groningen]
DUF2366 a novel gene essential for neoblast functionality

13:00–14:30 *Lunch*

Monday, September 26th (Continued)

14:30–16:10 **Session IV – Evolutionary & Phylogenetic Perspective**
Chair: *Emili Saló*

14:30–14:50 Laia Leria
[Dpt. Genetics, Microbiology & Statistics, University of Barcelona]
Why Dugesia flatworms would be Gaudi's favorite animals

14:50–15:10 Lisandra Benítez
[Dpt. Genetics, Microbiology & Statistics, University of Barcelona]
Multiple introductions of Girardia in Europe; a molecular evidence

15:10–15:30 Miquel Vila-Farré
[MPI Molecular Cell Biology and Genetics, Dresden]
Why some animals regenerate while others cannot: tracing the evolution of regeneration in planarians (Order Tricladida)

15:30–15:50 Jakub Wudarski
[European Research Institut -Biology of Ageing, Groningen]
Efficient transgenesis in Macrostomum lignano, the flatworm model organism for stem cell research

15:50–16:10 Peter D. Olson
[The Natural History Museum, London]
Strobilar development in tapeworms involves a novel intercalary process regulated by Wnt and Hedgehog

16:10–16:50 *Coffee break* ☕

Monday, September 26th (Continued)

16:50–18:10 **Session V – Signaling in the Nervous System**
Chair: *Karen Smeets*

16:50–17.10 Christian P. Petersen
[Northwestern University, Evanston]
Integrin regulates brain tissue assembly in the planarian regeneration blastema

17:10–17:30 Kerstin Bartscherer
[MPI for Molecular Biomedicine, Münster]
Integrins are required for blastema organization and restriction of neurogenesis in regenerating planarians

17:30–17:50 Bret Pearson
[Hospital for Sick Children, Toronto]
Nu thoughts on neurogenesis in planarians

17:50–18:10 José Ignacio Rojo-Laguna
[Dpt. Genetics, Microbiology & Statistics, University of Barcelona]
WNT5-ROR2 and SLIT-ROBO-c signals generate a mutually dependent system to position the CNS along the medio-lateral axis in planarians

18:10–19:30 **Discussion in Groups**

20:00– *Dinner (buffet)*

21:00– **Poster Session II**

Tuesday, September 27th

09:00–10:00 **Session VI – Omics**
Chair: *Aziz Aboobaker*

09:00–09:20 Katja Hüttner
[MPI Molecular Biomedicine, Münster]
Translational control of regeneration initiation in planarians

09:20–09:40 Jordi Solana
[Max Delbrück Center for Molecular Medicine, Berlin]
The histone methyltransferase DOT1L has a conserved stem cell-specific alternative splicing switch in planarians

09:40–10:00 Jochen Rink
[MPI Molecular Cell Biology and Genetics, Dresden]
High quality de novo assembly of the planarian Schmidtea mediterranea genome using PacBio SMRT sequencing and in vitro long-range linkage

10:00–10:30 *Coffee Break*

10:30–11:30 **Session VII – Environmental Signals**
Chair: *Marta Riutort*

10:30–10:50 Jason Pellettieri
[Keene State College]
Light-induced depigmentation in planarians—an animal model of acute porphyrias

10.50–11:10 Andrea Degl'Innocenti
[Istituto Italiano di Tecnologia, Pisa]
Chlorophyll Derivatives Enhance Planarian Vision

11:10–11:30 Annelies Wouters
[Hasselt University]
Planarian stem cells' defence to genotoxic exposure depends on the cellular environment and stem cell subtype

11:30–13:00 **Where do we go & what to improve as a community?**

13:00–13:15 **Closing Remarks and Farewell**

13:15– *Lunch*

16:20– BUS to Barcelona from Sant Feliu Station

Invited Talks

Regulation of the fates of specialized neoblasts

Reddien, Peter W

MIT Dpt. of Biology; Whitehead Institute HHMI;
9 Cambridge Center; Cambridge, MA; USA

Planarian regeneration involves the neoblasts population, which includes pluripotent cells. Recent work has identified numerous specialized populations of neoblasts, with expression of transcription factors specifying lineage. Within a particular lineage cells can adopt different identities depending upon position. For example, the epidermis displays distinct gene expression states in a specific spatial pattern. How are these different choices of state within a single lineage, the epidermis, made? The epidermis lineage is initiated in the neoblast population with expression of *zfp-1*, producing "zeta-neoblasts". These cells progress through a series of spatiotemporal gene expression states during maturation. Essentially all cells in the lineage transition through these states, and yet, ultimately, different epidermal expression states are generated. Here we used single cell sequencing and RNAi studies to study how and when in the lineage spatial distinction in state arises.

Coordination of expression of genes by PIWI during differentiation of neoblasts

Shibata, Norito

National Institute of Technology; Tsuyama College;
624-1, Numa; Tsuyama-City; Okayama; Japan

PIWI family proteins have been well known as agents that repress transposable elements (TEs) during germ cell differentiation in many animal species. Dr. Peter Reddien first reported that SMEDWI-2 is involved in precise differentiation of neoblasts in *Schmidtea mediterranea*. Recently, we discovered and reported that DjPiwiB, a homologous protein of SMEDWI-2 in *Dugesia japonica*, is translated in neoblasts, and inherited by descendent differentiated cells of the neoblasts to repress TEs in those cells during the differentiation processes. This TE repression by DjPiwiB might ensure precise differentiation of the neoblasts. More recently, we found that DjPiwiB is also involved in controlling the expressions of genes at the several steps of differentiation from the neoblasts. In this talk, I will introduce our recent researches.

Selected Talks

Mechanisms of planarian stem cell regulation during starvation

Gutiérrez-Gutiérrez, Óscar; González-Estévez, Cristina

Leibniz Institute on Aging — Fritz Lipmann Institute (FLI); Jena; Germany

Caloric restriction is the most powerful anti-aging strategy, which is conserved throughout evolution in the animal kingdom. It is known that caloric restriction extends the life span of vertebrate and invertebrate animals and protects against some age-related diseases. Caloric restriction beneficial effects are due at least in part to an increase in stem cell function. However, little is known about the cellular and molecular mechanisms that caloric restriction uses to regulate stem cell function. To address this, we use the freshwater planarian *Schmidtea mediterranea*, an attractive model in which to study how caloric restriction regulates stem cells. Planarians are able to stand long periods of starvation without showing physiological impairment and maintaining a stable population of proliferating stem cells.

Through a RNA-seq screen we aimed to find novel genes involved in the enhancement of stem cell function during starvation. We are comparing the transcriptome of stem cells from starved and well-fed planarians. So far, we have identified the main signalling pathways involved in stem cell regulation during starvation. Currently, we are characterizing the function of the top candidate genes through RNAi experiments.

In conclusion, this study will clarify how caloric restriction regulates stem cell function and will allow a better understanding of its influence in processes such as regeneration and aging.

Smed-BS is a novel secreted peptide with an essential role in the control of cell number in planarians

Pascual-Carreras, Eudald[1]; de Sousa, Nidia[1]; Marín, Marta[2]; Eckelt, Kay[1]; Saló, Emili[1]; Adell, Teresa[1]

1. Dpt. of Genetics, Microbiology & Statistics; Universitat de Barcelona (UB); and Institute of Biomedicine of Universitat de Barcelona (IBUB); Barcelona; Catalonia; Spain
2. Norwich Research Park; Norwich NR4 7UH; United Kingdom

The final size of animal bodies depends on the size and number of their cells. Cell number relies on the tight balance between cell death and cell proliferation. The control of cell size seems largely dependent on nutrient conditions. The molecular mechanism underlying both processes and their tight relationship during development remains largely unknown. The striking plasticity of planarians, which allows them to regenerate any missing part and to continuously change their body size, offers us an ideal scenario to approach this question. Here we report the finding of a novel secreted peptide, *Blitzschnell* (*Smed-bs*), whose inhibition produces an acceleration of the regenerative response and an increase in the total cell number in homeostatic starved planarians. The phenotype comes with a higher rate of proliferation and a decrease of cell dead, while cell differentiation appears unaffected. Interestingly, the increase in cell number in starved planarians never produces bigger animals but a reduction in cell size, which leads to an increase in cell density and eventually to overgrowths. We are currently exploring the influence of nutrition supply in the cell size and number of *Smed-bs* RNAi planarians.

There and back again—towards a quantitative model of planarian growth and degrowth

Thommen, Albert[1]; Werner, Steffen[1,2]; Frank, Olga[1]; Alt, Nicole[1]; Richter, Johanna[1]; Friedrich, Benjamin M.[2,3]; Jülicher, Frank[2]; Rink, Jochen[1]

1. Max Planck Institute of Molecular Cell Biology and Genetics; Pfotenhauerstraße 108; 01307; Dresden; Germany
2. Max Planck Institute for the Physics of Complex Systems; Nöthnitzer Straße 38; 01187; Dresden; Germany
3. Center for Advancing Electronics Dresden; Technische Universität Dresden; Gunther Landgrafbau, office: 6-227, Mommsenstr. 15; 01069; Dresden; Germany

Unlike many other animals, planarians do not have a fixed body size. Instead, they continuously adjust their size to the current nutritional status. Therefore, planarians are a unique model organism for studying the mechanistic basis of the universal scaling relationship between body size and metabolic activity.

Following up on pioneering studies by Baguñà and colleagues, we established an imaging pipeline allowing us to reproducibly quantify planarian body size despite their soft and highly deformable bodies. We further developed assays to accurately measure total cell numbers depending on body size. Our robust measurements revealed an allometric scaling relationship between length and area. Further, we found an almost linear relationship between the area and the total cell number. We thus confirm that planarians grow and degrow mainly by regulating their total cell number. We further show that both, the growth and degrowth dynamics are size-dependent, suggesting a differential regulation of cell turnover in small and large planarians. Combining theoretical modeling and experiments, we are currently exploring the size dependence of metabolic rates and cell turnover and their contribution to growth and degrowth dynamics.

Radiation sensitivity and DNA damage response mechanism in Planarian stem cells

Sahu, Sounak[1]; Kosaka, Nobuyoshi[1]; Abnave, Prasad[1]; Bird, Luke[2]; Thompson, James[2]; Higgins, Geoffrey[2]; Hill, Mark[2]; Aboobaker, Aziz[1]

1. Dpt. of Zoology; University of Oxford; OX1 3PS; United Kingdom
2. Gray Institute of Radiation oncology; ORCRB; University of Oxford; OX3 7DQ; United Kingdom

Radiotherapy using ionizing radiation (IR) is routinely used to kill cancerous cells. There is growing evidence that tumour-initiating cancer stem cells survive and adapt to repeated rounds of IR eventually leading to cancer-recurrence. However, the molecular basis underlying the variations in IR resistance of cells is not well understood. We are trying to develop a convenient *in vivo* experimental system using the planarian *S. mediterranea* to study the molecular mechanisms of radiation resistance. We have identified a non-lethal dose of irradiation (15 Gy) that leads to an initial block in mitosis and to a significant decrease in neoblasts number but can fully recover over time without any long-term physiological effects. The number of stem cells in M phase is greatly reduced as early as 1.5 hours post 15 Gy exposure due to an early DDR response causing apoptosis. The surviving stem cells stay in G1 and only start to proliferate after 72 hours post IR. Exposure to usually non-lethal IR doses following knockdown of ATR, BRCA2, Rad51 is lethal, providing a proof of principle that well known DDR genes have a role in stem cell survival post IR exposure. We are using this preliminary data to optimize the strategy for transcriptomic profiling of pASCs following non-lethal IR to identify novel genes that respond to IR and DNA damage.We hope to identify potential molecules and mechanisms that can be targeted to increase radio-sensitivity of tumour cells and to augment radiotherapy.

The planarian ortholog of the tumour suppressor Mll3/4 prevents stem cell hyperplasia and controls differentiation programs

Mihaylova, Yuliana; Kao, Damian; Hughes, Samantha; Lai, Alvina; Jaber-Hijazi, Farah; Kosaka, Nobuyoshi; Abnave, Prasad; Aboobaker, Aziz

Dpt. of Zoology; South Parks Road; University of Oxford; United Kingdom

The H3K4 histone methyltransferases proteins MLL3 and MLL4 are part of a broadly conserved set of histone methyltransferases that play key roles in epigenetic regulation. Plenty of evidence has established that mutations in MLL3 and MLL4 play important roles in cancer, but little is known about the link between their epigenetic targets and cell behaviours that underpin pathology. Planarian flatworms provide an *in vivo* system to study the link between epigenetic functions and key stem cell behaviours. We have studied the roles of the MLL3/4 orthologs in planarians to assess whether there is a deep conservation of MLL3/4 loss of function leading to aberrant stem cell behaviour and, if so, whether we can identify the epigenetic and expression changes underpinning this. We searched for MLL3/4 orthologs in planarians and found these genes are split into two genes, LPT and TRR, as is the case for many animal taxa. RNAi of these genes led to decreased stem cell differentiation in the lineages producing gut, brain and pharyngeal lineages. These defects were accompanied by increased stem cell proliferation and the formation of ectopic growths caused by this hyperplasia, reminiscent of a fundamental cancerous phenoytype. Given these effects on stem cells are likely to be caused by underlying gene expression changes mediated histone modifications across the genome we have performed CHIP-seq and RNA-seq to describe the underlying changes that may explain aberrant stem cell behaviour.

Neoblast maintenance requires Integrator complex-mediated processing of Uridine-rich snRNAs

Schmidt, David[1]; Reuter, Hanna[1,2]; Ruhe, Larissa[3]; Irimia, Manuel[4]; Solana, Jordi[3]; Bartscherer, Kerstin[1]

1. Research Group Stem Cells and Regeneration; Max Planck Institute for molecular Biomedicine; Von-Esmarch Straße 54; Münster; Germany
2. Leibniz Institute on Aging - Fritz Lipmann Institute (FLI); Beutenbergstraße 11; Jena; Germany
3. Max-Delbrück-Centrum für Molekulare Medizin (MDC); Robert-Rössle-Str. 10; Berlin; Germany
4. Centre for Genomic Regulation (CRG); C/ Dr. Aiguader, 88; Barcelona; Catalonia; Spain

How neoblasts balance self-renewal and differentiation is poorly understood. Recent studies on mammalian stem cells identified alternative splicing as a key regulator of cell fate and showed that it is regulated, in part, through the expression of different Uridine-rich small nuclear RNA (UsnRNA) variants, the RNA components of the spliceosome. Here, we characterized the role of UsnRNAs and of the splicing events catalyzed by them for neoblast maintenance and regeneration. We identified 22 genes representing major and minor spliceosomal UsnRNA types. Interestingly, 4 of them were significantly enriched in neoblasts. We induced UsnRNA loss-of-function by depleting subunits of the Integrator complex (*ints*), which is required for UsnRNA maturation. RNAi against *ints3* or *ints9* resulted in the expected accumulation of unprocessed UsnRNAs and, interestingly, caused severe defects of regeneration and tissue homeostasis. A progressive stem cell loss was observed after *ints3* and *ints9* RNAi, while proliferation and differentiation into early progeny initially was normal. Transcriptome analysis of neoblasts isolated from ints RNAi animals detected hundreds of aberrant splicing events, confirming the loss of spliceosome function. These data show that planarian neoblast maintenance requires the snRNA-processing activity of the Integrator complex. The identification of neoblast-enriched snRNA variants suggest a role for these variants in stem cell-specific alternative splicing.

6/9.2, a potential surface protein marker for planarian pluripotent stem cells

Tran, Thao[1]; Zhang, Yudong[2]; Key, Göran[3]; Gentile, Luca[1,2]

1. Cell Biology and Applied Virology; Fraunhofer IBMT; Sulzbach; Germany
2. Planarian Stem Cell Laboratory; Max Planck for Molecular Biomedicine; Münster; Germany
3. Max Planck for Molecular Biomedicine; Münster; Germany

It is well documented that planarian adult stem cells (pASCs) are heterogeneous in terms of pluripotency. We hypothesized that—as shown for mammalians—both naïve and primed states co-exist also in planarian. Currently, the only way to ascribe a molecular ID to a planarian cell is by gene expression (mRNA or protein); however, this implies the destruction of the cell. In 2012, our group discovered a surface antigen, recognized by a monoclonal antibody (6/9.2), which is roughly expressed by 2/3 of the X1 population, and correlates with the expression of early markers of epithelial commitment. In order to understand the function of the 6/9.2 antigen, here we analyzed single X1 cells and found striking correlation among morphology, gene expression and the presence of the 6/9.2 antigen. Besides the differential expression of the progeny markers, 6/9.2+ and 6/9.2− cells also differ at the functional level. Upon transplantation into lethally irradiated hosts, 6/9.2− cells have better chances to engraft and to form multiple clones than 6/9.2+ cells. Therefore, we hypothesized that 6/9.2− cells possess a ground state-like pluripotency, while 6/9.2+ cells are more primed. We also worked on the identification of the 6/9.2 antigen. A clear band at 74 kDa was detected by Western blot, and subjected to Mass Spectrometry. In summary, our study provides a unique protein marker, which possibly plays a role in stem cell signaling, that can be used for ex vivo manipulation of viable pASCs.

A screen to identify RING E3 ubiquitin ligases involved in stem cell regulation *in vivo*

Allen, John M.; Iberkleid, Ionit; Romero, Celeste; Zayas, Ricardo M

Dpt. of Biology; San Diego State University; USA

E3 ubiquitin ligases catalyze the attachment of ubiquitin polypeptides onto specific substrates, which influences a wide range of cellular processes, including mitosis, transcription, and regulated protein degradation. However, the biological roles and target substrates for many E3 enzymes remain poorly understood. We are utilizing planarians to investigate the role of E3 ubiquitin ligases in stem cell-based regeneration. Here, we present our ongoing analysis of the RING finger-domain E3 (RFE3) gene family. We performed BLAST against *Schmidtea mediterranea* transcriptomes using 300 human RFE3s and identified 164 putative planarian RFE3s. We have targeted RFE3 genes enriched in neoblasts (>30) for functional analysis using RNAi. Knockdown of a homolog of *Prpf19* resulted in ventral curling and eventual lysis during homeostasis; *MARCH5* RNAi exhibited epidermal lesions and lysis. The severity of these phenotypes indicates that these RFE3s are involved in stem cell maintenance and differentiation. We are currently investigating the cellular mechanisms underlying the RFE3 RNAi phenotypes. In our presentation, we will also discuss our approaches in analyzing changes in the ubiquitylated proteome in order to characterize the mechanistic roles of these genes and identify their target substrates. Overall, our work aims to provide insights into the function of ubiquitin signaling in tissue regeneration.

DUF2366, a novel gene essential for neoblast functionality

Mouton, Stijin; Beltman, Frank; Berezikov, Eugene

European Research Institute for the Biology of Ageing; University Medical Center Groningen; Groningen; The Netherlands

Understanding *in vivo* behavior of stem cells is a major goal of biomedical research. This boosts the development of different model systems, including flatworms. A flatworm model recently gaining momentum is *Macrostomum lignano*, due to the establishment of genome and transcriptome assemblies and the development of transgenic methods. We have recently identified transcriptional signatures of germline cells and somatic neoblasts in this animal, and in this project we used this information to investigate conserved genes which were previously not connected to stem cell functionality. We focused on neoblast-enriched genes that are annotated as Domains of Unknown Function (DUFs), as the function of these genes is completely unknown despite their broad conservation across the animal kingdom. Here, we will present our ongoing work on a specific example, called *DUF2366*. RNA interference (RNAi) of this gene does not result in clear phenotypes during development, homeostasis, and initial regeneration of the tail. However, repeated regeneration of the tail, regeneration of the whole body, and starvation for several weeks lead to a diminished production of new tissue and maintenance of the body. Currently, we are studying the function of *DUF2366* in more detail by performing RNA-seq during RNAi, *in situ* hybridization, and computational approaches.

Why *Dugesia* flatworms would be Gaudi's favorite animals

Leria, Laia[1]; Vila-Farré, Miquel[2]; Solà, Eduard[1]; Riutort, Marta[1]

1. Dpt. de Genètica, Microbiologia i Estadística; Facultat de Biologia; and Institut de Recerca de la Biodiversitat (IRBio); Universitat de Barcelona; Barcelona; Catalonia, Spain
2. Max Planck Institute of Molecular Cell Biology and Genetics; Dresden; Germany

The way how an organism reproduces plays a crucial role upon its genetic evolution. Sexual reproduction increases the genetic variability of the populations due to recombination and outcrossing. On the other hand, the accumulation of deleterious mutations can lead asexual populations to extinction. Freshwater flatworms of the genus *Dugesia* show a wide variety of reproductive strategies even in the same species: they can reproduce sexually, by parthenogenesis and also asexually by fission.

The main objective of this work is to investigate how genetic variability is generated by the different reproductive strategies using *D. subtentaculata* as a model species.

We have cloned three molecular markers of individuals belonging to eight natural populations having different types od reproduction and we have analyzed its genetic variability by estimating population genetic parameters.

The preliminary results show an extremely high haplotype mosaicism within individuals no matter their type of reproduction. However, the nucleotide diversity is significantly higher in individuals of mixed and fissiparous populations. Moreover, all the haplotypes of the exclusively sexual populations are private, while the rest of individuals share haplotypes between distantly distributed populations.

These data provide new insights into the evolution of the different types of reproduction, helping us to understand how asexual organisms overcome the problems associated to the absence of sex.

Multiple introductions of *Girardia* in Europe: A molecular evidence

Benítez–Álvarez, Lisandra[1]; Castillo, Oleguer[1]; Solà, Eduard[1]; Sluys, Ronald[2]; Leal-Zanchet, Ana M[3]; Riutort, Marta[1]

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The genus *Girardia*, formerly a subgenus of *Dugesia*, includes more than 40 species that, as happens for other genera of planarians, all have a very similar external appearance. The genus is distributed along the American continent. The biogeographical hypothesis for the origin of Dugesiidae genera proposes that *Girardia* split from *Dugesia* and *Schmidtea* when Gondwana broke up to give the American and African continents, having posteriorly spread over the whole America. Moreover, it has been described that a species belonging to *Girardia*, *G. tigrina*, arrived to Europe in the early years of the past century, and since then many populations of the species have been reported from many countries in Europe, rising the question whether a such rapid spread was possible or multiple introductions had occurred.

The aim of this study is to perform a first and preliminar biogeographical analysis for the genus, trying to answer (1) how the colonization of America proceeded, and (2) whether *G. tigrina* had arrived to Europe once or more times.

To full fill those objectives we have obtained molecular data from multiple populations of *Girardia* species coming both from America and Europe, and used phylogenetic inference and genetic diversity analyses. The preliminary results show the genus have more probably originated in south america and moved toward the north. Surprisingly, the populations analysed from Europe have shown that not only *Girardia tigrina* is present in that continent.

Why some animals regenerate while others cannot: Tracing the evolution of regeneration in planarians (Order Tricladida)

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Regeneration is observed throughout the metazoans phylogeny. However, regenerative abilities varies even among closely related species. This raises the question: Why can some animals regenerate while others cannot? We investigate the evolution of regeneration in planarians, that offer unique opportunities in this respect. First, they display a wide range of regenerative abilities from whole-body in *Schnmidtea mediterranea* to poor, e.g. in *Bdelloura candida*. Second, planarians are generally easy to maintain in the lab and amenable to modern molecular biology techniques, offering an ideal system to explore the molecular and evolutionary causes of changing regenerative abilities. Through systematic collecting in the wild, we have established a collection of more than 50 species of planarians at the MPI-CBG. Through the development of molecular barcoding strategies and de novo transcriptome assembly, we have constructed a multigenic phylogeny with representatives of most of the major planarian clades. Second, we have systematically assessed species-specific regenerative abilities along the anteroposterior and mediolateral body axes. We find that already our current collection encompasses the full regeneration diversity reported in the literature. Third, we are currently screening all planarians with regeneration defects to ask whether all are linked to increased *Wnt* pathway activity. Our preliminary data suggest that the defects evolved multiple times, but are still *Wnt* dependent.

Efficient transgenesis in *Macrostomum lignano*, the flatworm model organism for stem cell research

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Regeneration-capable planarian flatworms are commonly used as research models to study the mechanisms of stem cell regulation, regeneration and tissue patterning. However, the lack of transgenesis methods significantly hampers their wider use. Here we report development of the transgenesis method for *Macrostomum lignano* (Macrostomida, Rhabditophora)—a basal flatworm with excellent regeneration capacity that complements planarian models. We demonstrate that microinjection of DNA constructs into eggs followed by low dose of irradiation frequently results in random integration of the transgene in the genome and its stable transmission through germline. To facilitate selection of promoter regions for transgenic reporters, we generated RAMPAGE data for genome-wide mapping of transcription start regions. We demonstrate the efficiency of the transgenesis approach and the value of the genomic resource by generating multiple stable transgenic lines expressing fluorescent proteins under muscle-specific promoter MYH6, testis-specific promoter ELAV4 and gut-specific promoter APOB. The efficient transgenesis protocol will greatly increase the power of *M. lignano* as a model organism to study stem cells and regeneration.

Strobilar development in tapeworms involves a novel intercalary process regulated by *Wnt* and *Hedgehog*

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We recently showed that AP polarity during tapeworm metamorphosis involves canonical *Wnt* signaling, with the cyst expressing posterior *Wnt*'s and scolex formation occurring at site(s) of *Wnt* repression (Koziol *et al*, 2016, *BMC Biology*). Extending this work to the adult phase on *Hymenolepis microstoma* shows that excysted worms re-establish the larval axis, with SFRP/SFRP-like (SFL) expressed in the scolex/neck region and Wnt1/Post2 in the posterior body. Between these poles develops a transition zone in which these 'positional control genes' (PCG; Witchley *et al*, 2013, *Cell Reports*) are co-expressed, mirroring their co-expression during embryonic development and early regeneration in planarians. Strikingly, strobilation is characterized by up-regulation of a tapeworm-specific *Wnt11* paralog that forms a gradient of punctate stripes between the transition zone and the nascent 'segments'. SFL/Wnt1 in this region begin to form opposing, punctate stripes that mirror the AP polarity of the larval and pre-strobilar worm, showing strobilation to be a form of paratomy (i.e. repetition of AP axes). Unlike SFRP, SFL is also expressed in the genital primordial (GP) and is thus associated with the processes of both proglottisation and strobilation. Similarly, *Hedgehog* is co-expressed in the GP and cortical tissues where it co-localizes with synapsin in the medial nerve cords. We suggest that the novel body plan of tapeworms evolved through modification the same underlying GRN of planarians.

Integrin regulates brain tissue assembly in the planarian regeneration blastema

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Animals capable of adult regeneration require specific signaling to control injury-induced cell proliferation, specification and patterning, but comparatively little is known about how the regeneration blastema assembles differentiating cells into well-structured functional tissues. Using the planarian *Schmidtea mediterranea* as a model, we identify *β1-integrin* as a critical regulator of blastema architecture. *β1-integrin(RNAi)* animals formed small head blastemas with severe tissue disorganization, including ectopic neural spheroids containing differentiated neurons normally found in distinct organs. By mimicking aspects of normal brain architecture but lacking normal cell-type regionalization, these spheroids bore a resemblance to mammalian tissue organoids synthesized *in vitro*. We identified one of four planarian integrin alphas whose inhibition phenocopies these effects, suggesting a specific receptor controls brain organization through regeneration. Neoblast stem cells and progenitor cells were mislocalized in *β1-integrin(RNAi)* animals without significantly altered body-wide patterning. Furthermore, tissue disorganization phenotypes were most pronounced in animals undergoing brain regeneration and not homeostatic maintenance or regeneration-induced remodeling of the brain. These results suggest that integrin signaling ensures proper progenitor recruitment after injury, enabling the generation of large-scale tissue organization within the regeneration blastema.

Integrins are required for blastema organization and restriction of neurogenesis in regenerating planarians

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Tissue regeneration depends on proliferative cells and on instructive mechanisms that regulate differentiation and restrict tissue growth. In planarians, muscle cells express some of these instructive cues. Here we show that members of the Integrin family of adhesion molecules are required for the integrity of the regenerating tissues, including the musculature, in regeneration blastemas of planarians. Remarkably, increased numbers of proliferative cells and progenitor cell types, as well as reduced ability of stem cells to re-distribute towards wound sites, accompany this phenotype. β1-integrin RNAi planarians also form ectopic spheroid structures of neural identity in anterior regeneration blastemas. These polarized assemblies comprise a variety of neural cells and undergo continuous growth. Our study indicates that Integrins are required for the formation of an organized blastema and for restricting amputation-induced proliferation and neurogenesis during planarian regeneration.

Nu thoughts on neurogenesis in planarians

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The planarian, *Schmidtea mediterranea*, is a master regenerator with a large adult stem cell compartment. The lack of transgenic labeling techniques in this animal have hindered the study of lineage progression and has made understanding the mechanisms of tissue replacement during regeneration a challenge. However, recent advances in single cell transcriptomics and analysis methods may allow for the discovery of novel cell lineages as differentiation progresses from stem cell to terminally differentiated cell. Here, 168 single stem (X1) and progeny cells (X2) from the planarian head were subjected to single-cell-RNAseq (scRNAseq). Pseudotime analysis predicted a molecularly distinct neoblast sub-population with neural character (νNeoblasts). Using the νNeoblast markers, we demonstrate that a novel *piwi-2*+*piwi-1lo* stem cell population exists adjacent to the brain and incorporates BrdU within 4 hours, suggesting active cycling. We then performed RNAi screening and have discovered three factors required for the proper maintenance of neurons in the ventral-medial region of the CNS. Furthermore, these neurons signal back to adjacent stem cells to modulate their own rates of homeostasis. Together, scRNAseq can be used to predict tissue-specific cell lineages in planarians, which can then be tested through functional analyses. We find that despite lack of an organized neural epithelium, planarians modulate rates of neurogenesis through signals from neurons to adjacent stem cells.

WNT5-ROR2 and SLIT-ROBO-c signals generate a mutually dependent system to position the CNS along the medio-lateral axis in planarians

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The acquisition of bilateral symmetry was a key evolutionary step, allowing the development of a centralized nervous system. However, the developmental signals that position bilateral symmetric structures in relation to the midline remain poorly understood. Planarian plasticity demands continuous positional information to maintain body proportions and axis information during regeneration and homeostasis. This ability offers an ideal context to study the signals required to position the CNS in relation to the midline. Our results demonstrate that *Wnt5* and *Slit*, which are expressed in complementary domains respect to the CNS, are axon repulsive cues in planarians. We identified ROR2 and ROBO-c as WNT5 and SLIT receptors, respectively. Their co-expression in neurons suggests that both signals cooperate to guide the axonal path in relation to the midline. Furthermore, *ror2* and *robo-c* are also expressed in muscular cells that express *slit* and *wnt5*, respectively, suggesting a regulatory relationship between both signals. We are currently exploring the hypothesis that WNT5-ROR and SLIT-ROBO-c signals could conform a self-regulated system to define their expression boundaries in addition to guide the axonal path. In conclusion, WNT5-ROR2 and SLIT-ROBO-c are axon repulsive cues that define the medio-lateral position of the CNS in planarians. Their domains of expression could be mutually regulated, allowing the self-maintenance of the medio-lateral positional information.

Translational control of regeneration initiation in planarians

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The regenerative response following amputation in planarian flatworms is extremely fast and plastic indicating that processes initiating regeneration are controlled tightly and can be induced quickly. This switch from homeostasis to regeneration has been studied intensively in the last years focusing almost exclusively on transcriptional changes. However, the fast nature of regeneration initiation together with the abundance of RNA granules in planarian stem cells suggests post-transcriptional regulation to be an important player in regeneration initiation. The aim of this work is to study the importance of translational regulation in the induction of tissue regeneration. To address this question, we successfully established Ribosome profiling, an RNA-seq based method for the identification of actively translated mRNAs, for *Schmidtea mediterranea*. We applied Ribosome profiling and RNA sequencing at different timepoints of regeneration. Interestingly, we found that the transcriptional induction of hundreds of genes was not reflected on the translational level. Conversely, several hundred transcripts were differentially translated while their mRNA levels were not significantly changed. This indicates that gene expression is highly regulated on translational level during regeneration initiation and sets the ground for further investigations of the underlying regulatory mechanisms.

The histone methyltransferase DOT1L has a conserved stem cell-specific alternative splicing switch in planarians

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Alternative splicing (AS) is a crucial process for stem cells. In mammals, MBNL proteins play a key role by negatively regulating a stem cell-specific exon program that comprises a mutually exclusive exon in the transcription factor FOXP1, which is crucial for embryonic stem cell transcriptional control. We have shown that MBNL factors also negatively regulate stem cell-specific AS in planarians, placing this mechanism at the base of bilaterian evolution. Furthermore, we have shown that the CELF factor *Smed-bruno-like* antagonizes MBNL factors and positively regulates this program in planarian stem cells—the so-called neoblasts—where it is expressed. Thus, CELF/MBNL antagonism is likely a key conserved process for animal stem cells (Solana *et al*; *eLife*; *in press*). Here, we focus in one of the stem cell specific alternative splicing events that we recently identified. It affects the region coding the enzymatic core of the histone methyltransferase *Smed-DOT1L*. Orthologs of DOT1L mediate the methylation of H3 at Lysine 79. The event has extensive conservation in phylogenetically distant species. Similar to the transcription factor FOXP1 in mammals, two mutually exclusive exons encode the same alternative region of the protein and are highly regulated. One isoform is stem cell specific, while the other is specifically depleted in stem cells.

High quality *de novo* assembly of the planarian *Schmidtea mediterranea* genome using PacBio SMRT sequencing and *in vitro* long-range linkage scaffolding

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The planarian *S. mediterranea* was the first planarian genome to be sequenced a decade ago and the initial assembly revealed a highly repetitive, A/T-rich and compositionally biased genome. Recent advances in single molecule long read sequencing have allowed the generation of high quality assemblies comparable to classical Sanger-sequenced reference genomes.

Here we show that PacBio sequencing of the *S. mediterranea* genome is capable of overcoming the limitations of previous clone-based or short read sequencing approaches. The generation of our PacBio assembly involved the development of a new planarian DNA isolation protocol, which yields clean, high molecular weight DNA (>200 kb). Further, we developed new assembly strategies for uncorrected PacBio reads and efficient algorithms to tackle genome heterozygosity. In addition, we made use of the Dovetail Genomics *in vitro* long-range chromatin linkage method to validate the quality of our assembly. The fact that we only detected very few misjoined contigs demonstrates the high quality of our assembly. Furthermore, we used the linkage data to subsequently generate genome scaffolds of several megabases in length.

Overall, our PacBio-only assembly approach provides the most contiguous and complete planarian genome assembly to date, which we will make available as a community resource.

Light-induced depigmentation in planarians —an animal model of acute porphyrias—

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Porphyrias are rare metabolic disorders typically caused by inherited mutations in heme biosynthesis enzymes. The resulting buildup of heme precursors, including cyclic tetrapyrroles called porphyrins, can trigger abdominal pain, seizures, and mental changes such as anxiety or hallucinations. Because porphyrins are strongly photosensitizing compounds, many affected individuals also develop cutaneous symptoms (e.g., blistering or lesions) upon prolonged light exposure. Treatment options for severe cases, which can be life threatening, are limited. We recently reported intense visible light induces bodily depigmentation in *Schmidtea mediterranea*, and traced this response to physiological porphyrin biosynthesis in pigment cells by the first three enzymes of the heme biosynthesis pathway. RNAi knockdown of these genes inhibits porphyrin biosynthesis and protects from light-induced pigment cell loss. Remarkably, starvation is associated with increased porphyrin levels and enhanced photosensitivity, mirroring the sudden onset of disease symptoms some porphyria patients experience when dieting or fasting. Our results establish light-induced depigmentation in planarians as an animal model of 'acute' porphyrias, providing an experimentally tractable system in which to study the basic science of these diseases. We are also exploiting unique advantages of this model to screen for novel porphyria drugs, and will report preliminary results from that effort at the meeting.

Chlorophyll derivatives enhance planarian vision

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Most animal species transduce light stimuli through specialized neuronal populations expressing membrane photoreceptors known as opsins. The spectral sensitivity of an opsin depends on several factors, among which is the presence of allosteric modulators. Indeed, an abyssal fish (*Malacosteus niger*) modifies the spectral tuning of its opsins by accumulating chlorophyll derivatives (CDs) within its retina. Following that natural example, a group of studies proved the efficacy of CDs in altering the spectral sensitivity of vertebrate rhodopsin, a type of opsin. However, thus far scientific investigation has focused on a few CDs, invariably in vertebrate species; also, conclusive evidence of allosteric interaction is lacking. Finally, the molecular mechanism in behind this modulation remains unexplored. Our research aims to cover these open aspects.

We assessed the effects of chlorin e6 (a CD) on the vision of the planarian *Dugesia japonica*. In particular, we evaluated whether chlorin e6-treated animals might display enhanced light avoidance with respect to controls, either in normal or in visually impaired specimens. We also plan to compare electrophysiological recordings of ocellar local field potential of CD-treated versus untreated animals. In parallel, we aim to elucidate possible mechanisms of molecular interaction via *in silico* analyses on bovine rhodopsin. CDs can enhance invertebrate vision. A putative binding pocket for CDs is present on rhodopsin.

Planarian stem cells' defence to genotoxic exposure depends on the cellular environment and stem cell subtype

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Having a detrimental role in development, tissue homeostasis and repair, stem cells possess unique stress response mechanisms. Highly regenerative organisms, such as planarians, have a low vulnerability to chemically induced carcinogenesis, making their stem cell system particularly interesting. Aiming a realistic characterization of (pluripotent) stem cell responses towards genotoxic stress, we used the planarian *S. mediterranea* as an *in vivo* system, with stem cells residing in their original niche during exposure.

We compared stem cell's (immediate and prolonged) DNA damage responses following exposure to the genotoxic carcinogen MMS during homeostasis and regeneration, both displaying distinct stem cell dynamics and cellular environments. DNA damage was observed within the stem cell population, irrespective of regeneration or exposure time; responses, however, differed. While stem cells immediately responded with a proliferation stop, the outcome after prolonged exposure depended on the cellular environment and stem cell subclass. Exposure during regeneration activated repair systems stronger compared with intact animals, where apoptosis seemed to be an important outcome. A PCA analysis revealed a co-expression of sigma-associated genes with DNA repair genes, while the more lineage-restricted zeta-class gene *zfp1* was linked with the pro-apoptotic *bax*. This is a first indication of subtype-specific damage mechanisms, but additional measurements are required.

Posters

Poster – 1

Planarians as a model to assess *in vivo* stem cell migration

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Cell invasion/migration is an important process in cancer progression and metastasis. A precise understanding of this process may have great potential to decide appropriate anticancer treatment as well as to discover novel therapeutic strategies to restrict tumour growth and metastasis. Our current understanding of cell invasion process is primarily gained from *in vitro* cell culture assays and some *in vivo* studies. Both of these methods have their own limitations and disadvantages. Here we propose an alternative model system, planarians (*Schmidtea mediterranea*) to assess 3D *in vivo* cell invasion/migration. We have developed a shielded irradiation assay that allows us to precisely monitor and quantitate the 3D *in vivo* stem cell migration in planarians. By taking advantage of this convenient model system we would like to perform single cell transcriptomic analysis of actively migrating stem cells in planarians, which will provide novel insights in understating the cell invasion process. In particular we will focus on genetic changes occurred during very early stages of cell invasion/migration that are never been looked before due to lack of appropriate model system. These findings in turn may potentially allow us to develop biomarkers and therapeutic strategies for early detection and inhibition of tumour cell invasion respectively.

Poster – 2

Diversification of the EGFR pathway in *Schmidtea mediterranea*: does it play a general role in cell differentiation?

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The epidermal growth factor receptor (EGFR) pathway plays multiple roles in many biological processes including cell proliferation and differentiation, cell survival and migration, angiogenesis and morphogenesis, among others. Also, the EGFR pathway is overactivated in many human cancers. However, very little is known of the composition and function of the EGFR pathway in most animal lineages. Recently, we have reported the presence of EGF ligands and receptors in most major animal clades in which they have experienced frequent independent expansions. In *Schmidtea mediterranea* we have identified 6 EGFR and 9 putative EGF ligands that display a wide variety of expression patterns based on *in situ* hybridizations and comparative analyses with single-cell transcriptomic data from other laboratories. Also, we have recently shown that *Smed-egfr-1* and *Smed-nrg-1* are required for gut regeneration and maintenance. Here, the role of the EGFR pathway appears to regulate the differentiation of gut progenitors into mature gut cells. Several previous studies have suggested that other EGFR genes may control also cell differentiation for cell types such as neuronal or excretory cells; however, at which step from the pluripotent neoblast to the final mature cells would the EGFR pathway exert its function is mainly unknown. Based on our results with *Smed-egfr-1* we are currently testing whether the diverse EGFR pathway has a general role in the differentiation of specialized progenitors into mature cells during planarian regeneration and homeostasis. Preliminary results suggest that a novel *Smed-egfr-4* gene might be necessary for the differentiation of the eye progenitor cells.

Poster – 3

Basal Body rotational polarity is controled by left-right asymmetric forces in *Schmidtea mediterranea*

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The flatworm *Schmidtea mediterranea* uses cilia beating at the surface of its ventral epidermis for gliding along substrates. The ventral epidermis is composed of multiciliated cells (MCCs) that are similar to the MCCs in the respiratory airways, the brain ventricles, and the oviducts in vertebrates. The coordinated beating of epidermal cilia requires the proper orientation and organisation of the basal bodies (BBs), from which cilia are assembled. The mechanism required for this polarisation is unknown but we found that depletion of Vfl1/LRRCC1, Vfl3/CCDC61 or Odf2 induce the flatworms to move sideways, suggesting that ciliary beat orientation, which depends on BB orientation, might be altered.

First, by studying the orientation of BBs in control worms, we found that BBs/cilia are aligned with the direction of locomotion only in the midline region. Away from the midline, beating direction is deviated towards the edges (i.e. anterior/central to posterior/lateral direction). The BB network thus exhibits bilateral symmetry, revealing that BB orientation is controlled along both the anterior-posterior and the medio-lateral axis.

We then analyzed Odf2, Vfl1 and Vfl3-depleted worms and observed that in the absence of Odf2, BBs are oriented towards the right side while in the absence of Vfl1 or Vfl3, they are oriented towards the left side. Our results are consistent with the respective locomotion phenotypes and furthermore reveal that BB orientation is controlled differently on the right and left sides of the animals.

Finally, we tried to determine how polarity pathways controlling axis formation or BB polarization contribute to BB orientation in planarians. Depletion of Slit, Wnt5 and BMP, which control medio-lateral patterning, did not affect cilia orientation. In contrast, depletion of the Planar Cell Polarity (PCP) proteins Dishevelled-1/2 (Dvl) and Vangl-1/2 (but not the Wnt pathway component beta-catenin) affected BB orientation, highlighting the role the non-canonical Wnt pathway in this process.

Altogether our results show that the apparent bilateral symmetry of the BB pattern in the planarian ventral epidermis likely results from left-right asymmetric forces exerted on BBs by the cytoskeleton.

Poster – 4

Towards planarian transgenesis

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Transgenesis techniques are still critically missing in the planarian toolbox. Their establishment is further hampered by a critical lack of positive controls, cell culture techniques and many unknowns in planarian biology. To nevertheless make progress in face of these issues, our approach aims to systematically evaluate methods and reagents. One focus of our work so far has been on viruses as possible planarian gene transfer vectors. The first necessary step in virus infection is capsid fusion with the cell membrane. In collaboration with the Lindemann lab on campus, we have therefore established a capsid fusion assay for planarian cells. We have also produced high titre preparations of HIV-like particles pseudotyped with VSV-G, Foamy Virus and Baculovirus also pseudotyped with VSV-G. All of the preparations displayed high fusion activity on HeLa or HT-1080 cells, but no detectable fusion with planarian cells under our experimental conditions. Therefore, our data so far suggests that neither VSV-G nor Foamy Virus Env protein are suitable coats for engineering planarian viral vectors. A second focus of our work is the introduction of recombinant *Cas9* into planarian cells. We made use of our facility's high yield protocol for the production of functional *Cas9* and we have *in vitro* validated sgRNAs against specific planarian gene loci. We are currently screening protein delivery techniques into planarian cells which will hopefully enable the genomic modification of neoblasts.

Poster – 5

Ornithine decarboxylases are regulators of planarian epidermal differentiation

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Polyamines are small charged molecules, essential for cell growth from bacteria to mammals. An increasing body of evidence indicates that a number of signaling pathways converge on polyamines. However, the mechanisms of action of these mediators is still poorly understood, therefore we decided to undertake a study on polyamine function in planarians, a model system in which cell proliferation and differentiation can be studied *in vivo*.

Six homologous genes to human ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis, are present in both the planarian species *Dugesia japonica* and *Schmidtea mediterranea*. ODCs are expressed in both differentiated tissues and X-ray sensitive neoblast daughter cells, resembling the so called "epidermal-committed late neoblast progeny". Silencing of two ODC genes led to severe defects in both regenerating fragments and intact organisms, with dramatic impairment of epidermis, basal lamina and sub-epidermal muscles. RNAi animals activated abnormal apoptotic waves upon adequate insult, for example when pricked with a needle, leading to a rapid regression of the pricked body region. Subsequently, the dramatic activation of apoptosis spread all over the body, causing animal death. Work is in progress to understand whether such misregulated apoptosis is a consequence of epidermal damage, or ODC-positive cells play a more direct role in modulating apoptosis after injury.

Poster – 6

Probing molecular and evolutionary mechanisms of regeneration defects in *Cura sp.*

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Many planarian species are able to regenerate any amputated body part; however, some species have limited regeneration abilities. Our lab is systematically exploring the molecular and evolutionary mechanisms of regeneration defects using a large collection of different planarians species. The *Wnt* signalling pathway has emerged as a key determinant of differences in regenerative abilities amongst planarians, with excessive pathway activity the likely molecular cause of head regeneration defects. In *D. lacteum*, defective head regeneration is associated with failed activation of the *Wnt* inhibitor *notum*. To determine whether *notum* misregulation is generally associated with regeneration defects, we have begun to molecularly characterise other regeneration-deficient species. *Cura sp.* is a comparatively close relative of *S. mediterranea* collected from southeastern Australia. *Cura*'s defect can also be rescued by *βcatenin-1* RNAi, indicating a highly conserved role of *Wnt* signalling; however, strikingly, preliminary data suggest that, in contrast to *D. lacteum*, *notum* is still expressed at wounds that fail to regenerate a head. Ongoing work will determine whether the defect-causing *Wnt* activity is due to *notum* misregulation after all, or because of another, *notum*-independent mechanism. By understanding differences in regeneration abilities amongst planarians, we hope to provide insights into the bigger question of why, in the face of "survival of the fittest", few animals regenerate.

Poster – 7

The role of the Tudor domain-containing proteins (TDRDs) in maintaining planarian neoblast pluripotency

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Neoblasts contain structures called chromatoid bodies (CBs) that closely resemble nuage found in metazoan germlines. Orthologs of several genes that encode nuage components, such as *piwi* and *vasa*, are required for planarian neoblast function. The presence of CBs in both the metazoan germline and somatic stem cells suggests a role for these structures in maintaining stemness, and implies the existence of a 'germline multipotency program'.

Recent studies in metazoan model systems have linked Tudor biology to the PIW-piRNA pathway as Tudor domains can bind symmetrically dimethylated arginine residues in PIWI proteins. However, the precise role of Tudor domain-containing proteins (TDRDs) in regulating the PIWI-piRNA pathway and their role in adult stem cells requires investigation.

We have identified orthologs of TDRDs that have a function in metazoan germline stem cells, as well as planarian-specific TDRDs that are highly expressed in the neoblasts of *S. mediterranea*. An antibody against one of these TDRD genes, *Smedtud-1*, has shown it to be specifically expressed in the CBs of neoblasts. Preliminary steps have been made to establish the binding partners of SMEDTUD-1. Ultimately, we will use a combination of RNAi, transcriptomics, and proteomics to investigate the role of *Smedtud-1* and other TDRDs in maintaining neoblast pluripotency and their function, if any, in regulating the PIWI-piRNA pathway.

Poster – 8

Hippo signaling controls cell death, cell cycle and differentiation in planarians

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Growth control is an open basic question in developmental biology. The Hippo signaling emerges as an essential pathway in the organ size control for its unique ability to simultaneously regulate cell proliferation and apoptosis. Although the core of the signaling pathway is well understood, the downstream transcriptional regulation and the main biological processes regulated remain still obscure. We have studied the function of the Hippo elements in planarians, which cellular plasticity provides us an ideal scenario to understand the molecular mechanism underlying growth control. Our results show that Hippo silencing in planarians does not result in an increase of organ size neither in cell number but impairs tissue differentiation and results in the formation of overgrowths. Cellular analysis demonstrates that in planarians Hippo regulates apoptosis, cell cycle and cell differentiation. Hippo inhibition could enable post-mitotic cells to regain proliferative activity.

Poster – 9

Dugesia hepta and *D. benazzi*: Two sympatric species having casual sex?

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D. hepta and *D. benazzii* share part of their distribution dwelling together in at least 4 rivers, the first habits only the north of Sardinia while *D. benazzii* has a wider distribution. *Dugesia hepta* Pala, Casu and Vacca, 1981 was described as a new species basing in that it presents an haploid chromosome number of $n = 7$, while the rest of European *Dugesia* have either $n = 8$ or $n = 9$. It was considered however that it was very similar to *D. benazzii*, although some peculiar features for *D. hepta* have been found. Molecular studies including a few individuals showed they also are genetically different. This situation poses some interesting questions about the origin of *D. hepta* and the evolution of both species: Did *hepta* originate once and spread over the same area than *benazzii* or there have been multiple origins for the species? Are they able to cross?

To try to answer these questions we sampled both species along their distribution, obtained sequences of mitochondrial and nuclear genes and performed phylogenetic and population genetic analyses. The results from different genes are not totally congruent, however some important conclusions can be drawn. They show a single origin for *D. hepta* from a common ancestor with *D. benazzii* and not from a specific locality of this species, later a parallel spread of the two species must have occurred. Moreover, some individuals from populations in which the two species cohabit show the molecular signature of hybridization.

Poster – 10

Metabolic regulation of planaria cell turnover

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Planaria flatworms steadily renew all of their tissues due to continuous neoblast divisions and controlled death of differentiated cells. Food uptake elicits a rapid increase in neoblast divisions, leading to a burst of growth of the entire animal. Starvation shifts the turnover balance towards a net loss of cells and consequent de-growth of the animal, even though neoblasts continue to divide. Currently, very little is known about how planarian cell turnover is regulated. My thesis project will explore the hypothesis that turnover is integrated at the metabolic level.

I am therefore investigating whether different diets (i.e. enriched or depleted in particular nutrients) or specific energy stores (i.e. glycogen and lipids) affect stem cell proliferation as measured by BrdU incorporation. My initial results indeed suggest that diets with higher fat elicit increased neoblast proliferation rates and I am currently in the process of elucidating the relative contributions of higher food uptake, longer storage, or specific lipophilic metabolites. Furthermore, I have initiated deep sequencing time course experiments in order to obtain an overview of food-induced transcriptional changes. By linking food composition to switches in transcriptional programs, I hope to obtain mechanistic insights into the metabolic regulation of planarian cell turnover.

Poster – 11

Transcriptional signatures of somatic neoblasts and germline cells in *Macrostomum lignano*

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Regeneration-capable flatworms are attractive models to study biology of stem cells *in vivo*. In particular, planarian flatworms, such as *Schmidtea mediterranea*, are extensively used for dissecting mechanisms of regeneration and stem cell regulation. A more recent addition to flatworm model organisms is *Macrostomum lignano*, a representative of the basal flatworm clade Macrostomorpha. *M. lignano* offers an advantage of a genetically tractable model with developed transgenesis methods. Here we present a first comprehensive characterization of gene expression in the proliferating cells of *M. lignano*, which in adult animals consist of somatic stem cell, called neoblasts, and germline cells. We generated de novo transcriptome assembly of *M. lignano* based on extensive RNA-seq dataset and used a combination of two approaches to enrich for genes expressed in either somatic neoblasts, germline cells or differentiated cells: depletion of proliferating cells by irradiation and isolation of cells in different phases of cell cycle from adult animals, juveniles, and amputated heads by fluorescence-activated cell sorting. We identified ∼2200 genes enriched in germline and a smaller set of ∼350 genes enriched in somatic neoblasts. The neoblast signature genes include the majority of know planarian neoblast markers, and almost half of the genes are conserved in mammals. Knockdown of a selected set of novel neoblast genes by RNAi confirmed their crucial role for the functionality of somatic neoblasts during homeostasis and regeneration. The generated *M. lignano* transcriptome assembly and gene expression signatures of somatic neoblasts and germline cells will be a valuable resource for future molecular studies in *M. lignano*.

Poster – 12

Nanoparticles: how safe are they? *Schmidtea mediterranea* as a model organism for toxicity assessment

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Up until now, the negative impact of the ever-increasing use of manufactured nanoscale materials is unclear. Although considered safe for prolonged internalization in cells, several studies indicate cytotoxic effects of several nanoparticles (NP). On the subject of genotoxicity, carcinogenicity and stem-cell-related toxicity, conflicting results are reported. We focus on the *in vivo* toxicity and biocompatibility of silver NP (AgNP) and silica NP (SiNP) on stem-cell-specific activities, making *S. mediterranea* an ideal model organism. Stem cells are an important target in several applications of AgNP and SiNP.

In our *in vivo* model system, we observed the uptake of AgNP in stem cells via transmission electron microscopy. Stem cells responded by a proliferation decrease, measured via histon H3 immunostaining. We are currently looking into the underlying cause of this decrease, as the genotoxic character of this element is still under debate. At the phenotypic level, we observed concentration-dependent behavioral effects, including C-like movements and curling. The excessive mucus production induced by increasing exposure concentrations indicates another defense strategy as compared to ionic metal exposure. Exposure to a range of SiNP concentrations showed no clear phenotypical or proliferation effects. This is a promising result for the further use of SiNP, but extra single cell studies and research on the uptake and the effect of coating are needed to confirm initial result.

Poster – 13

In vivo prediction and discrimination of carcinogenic compounds using *Schmidtea mediterranea*'s stem cell proliferation patterns

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Accurate carcinogenicity assays are crucial, as cancer risks are directly associated with the properties of a compound. The prediction of non-genotoxic carcinogens poses a challenge for the development of alternative methods. The variety of non-genotoxic cancer pathways complicates the search for suitable parameters expressing their carcinogenicity.

The presented assay enables a simple, fast and inexpensive prediction and discrimination of both genotoxic and non-genotoxic carcinogens by means of flatworm stem cell dynamics. After exposure to carcinogenic compounds during the animal's regeneration process, the most striking differences between non-genotoxic and genotoxic carcinogen-induced proliferative responses were detected during the initial stages of the regeneration process, i.e. the moment stem cells proliferate. We present a two-step-approach that combines *in vivo* adult stem cell proliferation patterns and phenotypic appearances. Based on differences in stem cell dynamics, discrimination between genotoxic and non-genotoxic carcinogens in a selected group of compounds was possible. Genotoxic carcinogens resulted in a significant drop of mitotic cells after 3 days exposure compared to 1 day while, on the contrary, non-genotoxic carcinogens were characterized by a significant rise of these cells. The ability to distinguish between genotoxic and non-genotoxic compounds makes this approach unique and with significant added value to current research and drug development.

Poster – 14

The translational landscape of regenerating *Schmidtea mediterranea*

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Transcription control of gene expression is of central importance during regeneration. Less is known about the role of mRNA translation as an additional control level in this process. Therefore we set out to investigate regulation of mRNA translation during regeneration in *Schmidtea mediterranea* using ribosome profiling. This technique relies on the purification of ∼30-nt mRNA regions (RPFs) that are protected from nuclease digestion by ribosome-binding. Purified RPFs are then deep sequenced, and the position of the ribosomes on translated mRNAs are determined by mapping sequences to the reference transcriptome. After establishing ribosome profiling for planarians and sequencing millions of RPF reads from regenerating planarian fragments, we applied a custom binning strategy to analyze ribosome density along the transcripts. In brief, the open reading frame (ORF) of each transcript was divided in bins of equal length and the total number of RPFs mapping to each bin was normalized by the average amount of RNA-seq reads mapping in each bin of the corresponding ORF. This allowed for the identification of translational changes along the ORFs and putative regulatory elements in the mRNAs that may be employed by RNA-binding proteins to control translation of key proteins required for regeneration.

Poster – 15

Using ribosome profiling data to refine gene models and identify novel ORFs for *Schmidtea mediterranea*

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High-throughput sequencing techniques and gene prediction algorithms have enabled the generation of annotated genomes and transcriptomes to understand the biological and functional aspects of genes at system level. Currently for *Schmidtea mediterranea* (`Smed`), a planarian species with extraordinary regeneration abilities, several de novo assembled transcriptomes exist but often they are incomplete i.e. they lack open reading frames (ORFs) or their transcripts are not full-length, lacking either the true start or stop codon or both. When we used Transdecoder, a bioinformatics tool to identify the potential protein coding ORFs from the Dresden transcriptome (`smed_dd_v6`), it predicted around 74% of the transcriptome has protein coding ORFs with min. length of 100 amino acids, of which around 28% are incomplete. Here, we will use sequencing reads from ribosome profiling experiments to complement and refine `smed_dd_v6`-based gene models. Ribosome profiling is a new and highly quantitative RNA-seq-based method, which relies on the protection of 30-nt stretches of mRNA by translating ribosomes. Mapping these reads and `smed_dd_v6` onto the `Smed` genome (`Smed_Asxl` v1.1) will therefore allow for the identification of novel ORFs and for the refinement of previously identified transcripts. This will enable the improvement of gene models for better analyses of transcriptome and translatome sequencing data.

Poster – 16

Role of the Wnt-βcatenin signaling during anterio-posterior axis specification and brain regeneration in planarians

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The multiple context-dependent roles of the βcatenin-Wnt pathway hinder the understanding of specific roles in embryonic and, especially, in adult tissues. Planarians, flatworms with the striking ability to regenerate and continuously change their size, offer us an ideal system to approach this issue. Planarians plasticity is based in the maintenance of a totipotent population of adult stem cells, which in turn demands the constitutive activation of the intercellular signaling mechanisms (ie. Wnt, Hh, BMP). Previous results demonstrate that βcatenin-1 is required to specify posterior identity and predict the existence of a Wnt-βcatenin activity gradient underlying the specification of the antero-posterior axis. Through the generation of a βCATENIN-1 specific antibody, we demonstrate the existence of a βCATENIN-1 activity from the pre-pharyngeal region to the tip of the tail. However, βCATENIN-1 is also highly nuclearized in the head region where it is required to pattern the central nervous system. In addition, we have identified a genetic βcatenin duplication (βcatenin-4), which is expressed in the neural tissues, where it could function as a βcatenin-1 inhibitor, particularly during the specification of the photoreceptors. Altogether, our findings provide the first direct evidence of an antero-posterior nuclear βCATENIN-1 gradient in adult planarians and uncover novel context-dependent roles for βcatenin-1 in neural tissues.

Poster – 17

6/9.2, a potential surface protein marker for planarian pluripotent stem cells

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It is well documented that planarian adult stem cells (pASCs) are heterogeneous in terms of pluripotency. We hypothesized that—as shown for mammalians—both naïve and primed states co-exist also in planarian. Currently, the only way to ascribe a molecular ID to a planarian cell is by gene expression (mRNA or protein); however, this implies the destruction of the cell. In 2012, our group discovered a surface antigen, recognized by a monoclonal antibody (6/9.2), which is roughly expressed by 2/3 of the X1 population, and correlates with the expression of early markers of epithelial commitment. In order to understand the function of the 6/9.2 antigen, here we analyzed single X1 cells and found striking correlation among morphology, gene expression and the presence of the 6/9.2 antigen. Besides the differential expression of the progeny markers, 6/9.2+ and 6/9.2− cells also differ at the functional level. Upon transplantation into lethally irradiated hosts, 6/9.2− cells have better chances to engraft and to form multiple clones than 6/9.2+ cells. Therefore, we hypothesized that 6/9.2− cells possess a ground state-like pluripotency, while 6/9.2+ cells are more primed. We also worked on the identification of the 6/9.2 antigen. A clear band at 74 kDa was detected by Western blot, and subjected to Mass Spectrometry. In summary, our study provides a unique protein marker, which possibly plays a role in stem cell signaling, that can be used for ex vivo manipulation of viable pASCs.

Poster – 18

Making the midline: A central problem in planarian regeneration

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The midline is a defining trait of the Bilateria and thus of a major group of animals. Anatomically, the midline comprises specific cell populations that extend all the way along the anteroposterior axis. Midline formation during embryogenesis is a complex process with considerable mechanistic variation between animal lineages. Planarians have the extraordinary ability to regenerate their entire body from almost any tissue fragment, including the ability to restore the midline. De novo midline formation and the regeneration of bilateral symmetry therefore provide a unique experimental paradigm for exploring the mechanistic basis of the bilaterian body plan. In this study, we aim to i) systematically characterize the molecular and cellular identities of the planarian midline; ii) establish how midline formation is coordinated between the anteroposterior and dorsoventral body axes; and iii) investigate how de novo midline formation is orchestrated during regeneration.

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