Program overview

ISFB Conference August 3rd-6th, Lady Margaret Hall, University of Oxford.
and
European Planarian meeting August 6th -7th, Lady Margaret Hall, University of Oxford,

SL= Simpkins Lee Lecture Theatre.
MR= Monson Room.

Monday August 3rd

13.00-16.30 pm Registration at LMH and put up posters (in MR).

from 15.45- coffee and cake (MR)

17.00-17.05 Welcome (SL).

Session 1 (17.05-19.40, SL) Chair: Peter Olson

17.05-17.40 Paul Brindley
"Lentivirus HIV-1 integrates widely throughout the genome of the human blood fluke *Schistosoma mansoni*"

17.40-18.05 Kathrin Geyer
"Unraveling DNA methylation in the blood fluke *Schistosoma mansoni*"

18.05-18.30 Tania Rozario
"Characterization of growth and regenerative potential of the rat intestinal tapeworm, *Hymenolepis diminuta,* cultured *in vitro"*

18.30-19.05 Tim Littlewood
"Flatworms with complex parasitic life cycles"

19.05-19.55 "optional 3 minute lightning talks for odd numbered Poster presentations"

19.55 First Poster session focus on odd numbers (drinks, canapés and food)

Tuesday August 4th

Session 2 (9.00-13.00, SL) Chair: Teresa Adell

9.00-9.35 Colette Dissous
"Venus Kinase Receptors in the control of reproduction"

9.35-10.10 Uriel Koziol
"The Wnt pathway and the evolution of tapeworm development"

10.10-10.35 Pete Olson
"Planarians to Parasitism: identifying signals and switches of development in tapeworms"

Coffee break

11.00-11.35 Peter Ladurner
“Biological Adhesion in Flatworms”

11.35-12.00 Stijn Mouton
“The influence of regeneration on ageing of the flatworm *Macrostomum lignano”*

12.00-12.25 Julian P.S. Smith III
“Functional morphology and Biomechanics of the Proboscis in Schizorhynchia”

12.25-12.50 Johannes Girstmair
“Analysing dynamics of spiral cleavage and the cell lineage of the polyclad flatworm *Maritigrella crozieri* by home-built SPIM-based microscopy”

12.50-2.00 pm LUNCH

Session 3 (14.00-18.00, SL) **Chair: Christian Petersen**

14.00-14.35 Karen Smeets
“Regenerative mechanisms: a survival kit for cancer?*”*

14.35-15.10 Bret Pearson
“The Queen is dead: understanding stem cell lineage hierarchies using freshwater planarians”

15.10-15.45 Teresa Adell
“Functional specialization of Planarian β-catenins”

15.45-16.10 Dasarideh Palakodeti
“MicroRNA, *miR-124* is essential for cephalic ganglion patterning and organization of optic chaisma during planarian regeneration”

Coffee break

Session 4 (16.30-18.30, SL) Chair: Karen Smeets

16.30-16.55 John D Chan
“Planarians (Re)generate a Way to Drug Discovery”

16.55-17.20 James Sikes
“Plasticity in axial polarity during postembryonic development in acoel flatworms”

17.20-17.45 Jason Pellettieri
“A Classroom-Based RNAi Screen for Regeneration Genes in the Freshwater Planarian *Schmidtea mediterranea”*

17.45-18.10 Christian Petersen
“Positional information and scaling in regeneration of the planarian anteroposterior axis”

18.10-18.45 Kazuya Kobayashi
"Yolk glands containing a large amount of tryptophan have a set of sex-inducing substances to fully sexualize asexual worms of *Dugesia ryukyuensis*"

18.45-19.10 Jordi Solana
"Alternative splicing in the regulation of planarian stem cells *in vivo*"

19.10-20.00 "optional 3 minute lightning talks for even numbered Poster presentations"

20.00 Second Poster Session, focus on even numbers (drinks and BBQ)

Wednesday August 5th

Session 5 (9.00-13.00, SL) Chair: Tim Littlewood

9.00-9.35 Marta Alvarez-Presas
"Biodiversity is not only a matter of the tropics"

9.35-10.10 Veronica Bulnes
"Alfa-taxonomy meets a "new" world: 10 years of research on marine microturbellarians in Argentina.

10.10-10.35 Ana Leal-Zanchet
"New triclad species questioning the status of the suborder Cavernicola"

Coffee break

10.55-11.20 Ulf Jondelius
"Phylogeny and morphology of Nemertodermatida: chimeric sequences and plastic morphology"

11.20-11.45 Tom Artois
"Phylogeny of the limnoterrestrial Rhabdocoela (Platyhelminthes), an enigmatic group of minute metazoans

11.45-12.10 Fernando Carbayo
"New incomers into Europe: the true identity of a Geoplaninae land planarian species spreading across Europe"

12.10- 12.35 Piter Boll
"Diet and food niche breadth in six Neotropical land planarians (Tricladida: Continenticola)"

12.35-13.00 Christoper Laumer
"Nuclear genomic signals of the 'microturbellarian' roots of platyhelminth evolutionary innovation"

13.00-14.00 LUNCH

Session 6 (14.00-16.00, SL) Chair: Colette Dissous

14.00-14.35 Klaus Brehm
"The unique stem cell system of the immortal larvae of the human parasite *Echinococcus multilocularis* – implications for chemotherapy and drug development"

14.35-15.00 Christoph Grevelding
"Towards an in-depth view of the reproductive biology of *Schistosoma mansoni*: from gonad isolation to sub-transcriptomics and beyond"

15.00-15.25 Hayley Bennett
"Single-cell sequencing in *Schistosoma mansoni"*

15.25-15.50 Kezia Whatley
"Development of an *in silico* pipeline for prioritizing novel *Schistosoma mansoni* drug targets"

Coffee Break

Session 7 (16.15-18.00) Chair: Stijn Mouton

16.15-16.50 Lukas Schärer
"Hypodermic insemination and correlated reproductive trait evolution in the Macrostomorpha"

16.50-17.25 Eugene Berezikov
"Genetic tools and genomic resources for the flatworm *Macrostomum lignano"*

17.25-17.50 Kira Zadesenets
"Evidence for karyotype polymorphism in the free-living flatworm, *Macrostomum lignano*, a model organism for evolutionary and developmental biology"

17.50-18.15 Juliana Bahia
"Polycladida phylogeny based on 28S rDNA challenges traditional classification systems"

Short meeting to discuss future of ISFB for those interested.

18.40 Drinks Reception (MR)

7.00 KEYNOTE SPEAKER, Phil Newmark
"Germ cell development and regeneration: from planarians to parasites."

8.00 Conference Dinner

Thursday August 6th (Checkout of rooms/remove posters)

Session 8 (9.00-13.00) Chair: Aziz Aboobaker

9.00-9.35 Francesc Cebrià
"Smed-egfr-1 controls planarian gut regeneration and homeostasis by regulating neoblast differentiation"

10.35-10.10 Eric Ghigo

“Neoblasts support "trained immunity" in the planarians”

10.10-10.35 Louise Goupil
“The role of cathepsin proteases in the free-living flatworm *Schmidtea mediterranea”*

10.35-11.00 Melanie Issigonis
“Germ cell specification from somatic stem cells in planarians”

Coffee break

11.20-11.45 Francesca Jarero
Heads or tails: investigating gene regulatory networks controlling strobilation in the model tapeworm Hymenolepis microstoma

11.45-12.10 Brigit Lengerer
Adhesive organ regeneration in *Macrostomum lignano*

12.10-12.35 Akash Gulyani
“Illuminating the landscape of neural regeneration: Evidence for multi-modal, innate light sensing and sensory processing in Planaria”

12.35-13.00 Johanna Cannon
‘Xenacoelomorpha are basal bilaterians: evidence from phylogenomics

13.00-13.25 James Gurtowski
‘The genome and transcriptome of the regeneration-competent flatworm, *Macrostomum lignano’*

END OF ISFB, delegates not attending one day planarian meeting can depart, free time for those staying.

1 Day Planarian meeting.

13.45 Sandwich Lunch for Attendees of Planarian Meeting (MR)

Delegate free time to visit Oxford during the day, football in University Parks etc.

Session 1 Chair: Dasaradhi Palakodeti

17.35-18.00 Aziz Aboobaker
"Preliminary data from establishing a X-ray shielded assay to reveal stem cell control mechanisms in planarians"

18.00-18.25 Nicky Pirotte
"The necessity of ROS signalling for successful differentiation and patterning during planarian regeneration"

18.25-18.50 Dhiru Bansal
"SMED-PABPC2 is essential for maintenance of epidermal integrity and second mitotic peak activation during planarian regeneration"

18.50-19.15 Yuliana Mihaylova
Epigenetic control of planarian stem cell potency limits stem activity and accurately defines differentiation programs

Delegate Social Time in Oxford.

Friday August 7th

Session 2 Chair Francesc Cebria

9.00-9.25 Miquel Sureda-Gómez
"Beta-catenin specifies posterior identity through a protein gradient and it is required for anterior patterning in planarians"

9.25-9.50 Takanobu Maezawa
*"*Tryptophan enhances the reproductive organ-specific expression level of an amino acid transporter homolog, Dr-SLC38A9 to promote sexual induction of the planarian *Dugesia ryukyuensis*"

9.50-10.15 José Ignacio Rojo-Laguna
"Controlling Regeneration Speed

10.15-10.40 TBC

Coffee Break

11.10-11.45 Jochen Rink
"Patterning pathways in planarian regeneration"

11.45-12.10 Nanna Nagao

“5-hydroxytryptophan induces ovaries, and knockdown of tryptophan hydroxylase homolog inhibits sexual induction in the asexual worms of Dugesia ryukyuensis.”

12.10-13.00 Open discussion regarding the next planarian meeting and potential European based initiatives.

END

KEYNOTE SPEAKER: PHILLIP A. NEWMARK

Germ cell development and regeneration: from planarians to parasites.

Jim Collins[1], Bo Wang[2], Harini Iyer, Amir Saberi, and **Phillip Newmark**

Howard Hughes Medical Institute
Department of Cell and Developmental Biology
University of Illinois at Urbana-Champaign
[1]Current address: Department of Pharmacology, University of Texas Southwestern Medical Center
[2]Current address: Department of Bioengineering, Stanford University

The regenerative prowess of planarians is based upon a population of adult stem cells, called neoblasts, that serve as the source of new tissue during regeneration and tissue homeostasis. Using the functional genomic tools available for studying planarians, we have been investigating how these stem cells give rise to the germ cell lineage and how reproductive system development and regeneration are controlled systemically. This talk will discuss how our work on planarian germ cell development led us to study the biology of schistosomes, parasitic flatworms with great significance for global health. We have shown that, like planarians, schistosomes have neoblast-like stem cells in the adult stage of the life cycle, providing one potential explanation for their longevity. Our recent work suggests that these stem cells serve to replenish the schistosome tegument, the outer surface with which the parasite interacts with the host bloodstream. Extending this work to the intramolluscan stage of the schistosome life cycle, we find that the so-called germinal cells in the sporocysts resemble neoblasts morphologically and express similar genes that are required for germinal cell proliferation and maintenance. We have used single-cell RNA sequencing to characterize the heterogeneity of the germinal cell population, and have identified co-regulated gene clusters that define distinct sub-populations of germinal cells. With these molecular markers, we can follow these lineages in space and time throughout the parasite's development in both snail and mammalian hosts. Thus, applying the lessons learned from studying planarians has provided new insights into the biology of their parasitic cousins.

EUGENE BERIZIKOV

Genetic tools and genomic resources for the flatworm *Macrostomum lignano*

Eugene Berezikov

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

Macrostomum lignano is a free-living flatworm with high regeneration capacity. Due to its small size, short generation time, amenability to genetic manipulation and easy maintenance in laboratory conditions, *M. lignano* is a potent invertebrate experimental model for stem cell research. For the last several years our laboratory has been developing molecular biology resources and tools for this model organism. Here I will present *M. lignano* genome and transcriptome assemblies and annotation and provide several examples of their use. Using *de novo* transcriptome assembly and gene expression profiling by RNA-seq in response to irradiation, we identified and validated several novel stem-cell specific genes (neoblasts markers). In another experiment, we adapted recently developed RNA tomography approach to generate gene expression profile of the animal spatially resolved along the anterior-posterior body axis, allowing identification of organ-specific genes (e.g. genes expressed only in the head region, in testes, in ovaries, etc.). Finally, we utilized draft genome assembly to identify promoter regions of genes of interest, and used promoter::GFP transcriptional fusions to develop an efficient transgenesis approach in *M. lignano*.

KLAUS BREHM

The unique stem cell system of the immortal larvae of the human parasite *Echinococcus multilocularis* – implications for chemotherapy and drug development.

Uriel Koziol[1], Andreas Schubert[1], Matt Berriman[2], **Klaus Brehm[1]**

[1]Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany
[2]Parasite Sequencing Unit, WT Sanger Institute, Hinxton, UK

The metacestode larval stage of the tapeworm *E. multilocularis* is the causative agent of alveolar echinococcosis (AE), a lethal zoonosis of the Northern Hemisphere. AE is characterized by infiltrative, tumor-like growth of metacestode tissue into host organs, leading to organ failure and death. Current therapeutic options are limited and mostly rely on chemotherapy using benzimidazoles (BZ). However, BZ chemotherapy is associated with significant adverse side effects and often has to be given life-long since it acts parasitostatic only.
We herein show that parasite development and growth within the host organs are driven by a population of neoblast-like stem cells (germinative cells; GCs) which are the only *Echinococcus* cells capable of proliferation and which give rise to all differentiated cells. GCs are homogenous in morphology but heterogenous at the molecular level since only subpopulations express homologs of *nanos* and *argonaute*. Important differences are also observed for selected stem cell markers in GCs when compared to stem cells of other flatworms, including widespread expression of some genes in *Echinococcus*which are stem cell-specific in planarians.
We developed methods for GC cultivation and for the specific depletion of GCs from parasite metacestode vesicles and used these for the characterization of the GC-specific transcriptome. Interestingly, when analyzing the expression patterns of genes for beta-tubulins, the cellular targets of BZ, we found one, tub-2, specifically expressed in GC whereas the other major beta-tubulin genes, tub-1/3, are expressed in differentiated cells. Since the tub-2 product, Tub-2, displays amino acid sequence signatures characteristic of beta-tubulins with little affinity to BZ, this could be the molecular basis for a strongly reduced sensitivity of *Echinococcus* GC to BZ, which we could observe *in vitro*. Hence, the reason for the high recurrence rates in AE patients upon discontinuation of chemotherapy could be that the parasite's decisive cell type, the GCs, specifically express a beta-tubulin isoform that is 'resistant' to BZ. For the development of novel, effective and parasitocidal drugs against AE, we therefore propose to pay particular attention to the parasite's GC population and to target factors that are important for GC function.

PAUL BRINDLEY

Lentivirus HIV-1 integrates widely throughout the genome of the human blood fluke *Schistosoma mansoni*

Paul J. Brindley

Department of Microbiology, Immunology & Tropical Medicine, and Research Center for Neglected Diseases of Poverty, School of Medicine and Health Sciences, The George Washington University, Washington, DC 20037 USA

Lentivirus-mediated manipulation offers advantages for functional genomics of the schistosome genome, to establish informative lines of transgenic schistosomes, and to elucidate gene functions of these pathogens of major neglected tropical diseases. Blood stream forms of the human schistosome, *Schistosoma mansoni*, including schistosomules and adult female and male parasites were exposed to vesicular stomatitis virus glycoprotein pseudotyped HIV-1 (strain LAI) virions. The virions bound to and transduced the parasites, after which reverse transcription of the lentiviral RNA genome proceeded, as detected by the presence of both strong-stop and positive strand cDNAs, and thereby indicating penetration and internalization of the nucleocapsid of the HIV-1 virion into the cytoplasm of the cells of the schistosome. Integration of the provirus of HIV-1 into chromosomes of the schistosomes followed, as established by anchored PCR targeting integrated provirus in the vicinity of endogenous mobile genetic elements, by high throughput sequencing of lentivirus-anchored PCR products, and by whole genome sequence analysis with the virions-exposed schistosomes. On a population scale, integration events of provirus dispersed throughout all eight pairs of chromosomes of the schistosomes. The density of integrations was at least 5 to 10 per 100 kilobase pair windows at the level of the schistosome population. Integration sites preference was biased to non-coding regions of the schistosome genome, dissimilar to that described for activated human T cells (LAI is T cell tropic). The ability of HIV-1 to complete biochemical processes essential for lentivirus development was unexpected and notable within a schistosome, a platyhelminth phylogenetically distant from the primates and other mammals that are the natural hosts of the genus *Lentivirus* occurs. The findings predict that lentivirus-based manipulation can advance functions genomics for schistosomes and related neglected tropical disease pathogens.

VERONICA BULNES

Alfa-taxonomy meets a "new" world: 10 years of research on marine microturbellarians in Argentina.

Verónica N. Bulnes

Zoología de Invertebrados I, Dpto. Biología, Bioquímica y Farmacia., Universidad Nacional del Sur, San Juan 670

Cataloguing and describing the turbellarians to species level may sound old fashioned, especially after the surprising results produced by the recent inflow of molecular data, which have radically affected the phyletic relationships of the plathyelmithes.
Nevertheless, phyletic studies and metagenetic approaches to the ecology of turbellarians (second-generation environmental sequencing) cry out after the help of taxonomists.
The Southern Hemisphere has been only partially explored. The magnitude and complexity of the marine biodiversity of this awfully broad area nowadays result in a very weak knowledge of the turbellarian diversity. In this context, an assessment of the Southern biodiversity of microturbellarians still is undefined at a range of spatial and taxonomic scales.
Traditionally, marine microturbellarians were considered as lacking dispersal stages, and their descriptions have been mostly devoid of any ecological data. This heavy shortcoming undermines the appreciation of biodiversity patterns.
For almost ten years now, an integrated approach study of this group is been carried out along the Atlantic coast of the Buenos Aires province (Argentina), to describe its almost unknown microturbellarian fauna. The concurrent collection of ecological data to be combined with the taxonomic information may lead to elucidate diverse biogeographical matters, and to detect the factors influencing some still poorly explained phenomena, such as range shifts, community changes, and local extinctions.

COLETTE DISSOUS

Venus Kinase Receptors in the control of reproduction

Colette Dissous

Center for Infection and Immunity of Lille, Inserm U1019, CNRS-UMR 8204, University Lille Nord de France, Institut Pasteur de Lille, Lille, Nord, France

Venus Kinase Receptors (VKRs) are invertebrate receptor tyrosine kinases first discovered in the human parasite *Schistosoma mansoni* (Vicogne et al, 2003). They contain an extracellular Venus FlyTrap (VFT) module similar to the ligand-binding domain of G protein-coupled receptors of class C and an intracellular tyrosine kinase domain similar to that of insulin receptors. VKRs are present from cnidarians to echinoderms (Vanderstraete et al, 2013). They were shown to be activated by amino-acids and to be highly expressed in larvae and in gonads of helminths and insects (Ahier et al, 2009). In *S. mansoni*, two genes Smvkr1 and Smvkr2 encode two VKR receptors which are respectively activated by L-arginine and calcium ions. Signaling pathways analysed in *Xenopus* oocytes revealed the capacity of SmVKRs to activate the PI3K/Akt/p70S6K and Erk MAPK pathways involved in cellular growth and proliferation. Additionally, SmVKR1 induced JNK (c-Jun N-terminal kinase) pathway activation. Suppression of SmVKR expression by RNA interference provoked spectacular morphological changes in female worms with a strong disorganization of the ovary, dominated by the presence of primary oocytes, and a defect of egg formation (Vanderstraete et al, 2014). These results indicate the importance of SmVKR in gametogenesis, and particularly in oogenesis and egg formation. By eliciting signaling pathways involved in oocyte proliferation, growth and migration, these receptors can control parasite reproduction and are therefore considered as potential targets for anti-schistosome therapies. Of importance, recent works have confirmed the role of VKR in ecdysteroid production and egg formation in the *Aedes aegypti* mosquito (Vogel et al, 2015), thus providing novel opportunities to use VKR as targets for the control of other parasites and vector-borne infectious diseases.

URIEL KOZIOL

The Wnt pathway and the evolution of tapeworm development

Uriel Koziol[1, 2], Francesca Jarero[3], Peter Olson[3], Klaus Brehm[2]

[1] Sección Bioquímica, Facultad de Ciencias, Universidad de la República, Uruguay
[2] Institute for Hygiene and Microbiology, University of Würzburg, Germany
[3]Department of Life Sciences, The Natural History Museum, London, UK

The early development of flatworms is extremely diverse, and very difficult to compare between distant groups. In the case of tapeworms, this is exacerbated by their lack of endodermal derivatives and their highly derived morphology. In fact, the true orientation of the antero-posterior axis of tapeworms has been a matter of speculation since their discovery. Among tapeworms, *Echinococcus* has a highly derived larval form (metacestode) which grows as round, fluid-filled cysts, from which many scoleces (heads) develop by asexual budding.

In planarians, it has been shown that the Wnt pathway plays a key role in specifying the antero-posterior axis. Although many developmental genes and pathways have been lost in tapeworms, the Wnt pathway is conserved, with clear orthologs to planarian genes. We hypothesized that during early larval development in tapeworms, the Wnt pathway could also play a role in antero-posterior specification.

By comparative analysis of gene expression of Wnt factors and other pathway components, we have found that there is a remarkable similarity in gene expression patterns between the early stages of tapeworm larval development and planarians. We postulate this to be a phylotypic stage for flatworms, allowing for the first time direct comparisons of their development. In the case of *Echinococcus*, there is a generalized expression of posterior Wnt factors in the metacestode larva, and the asexual budding of scoleces is preceded by localized expression of Wnt inhibitors. These results indicate that the highly derived *Echinococcus* metacestode is the result of a generalized posteriorization of the larval tissue, and that only much later during development, many foci of anterior development appear. Furthermore, similarly to what has been described in planarians, we identified muscle cells as the source of Wnt factors. This provides an explanation for the evolutionary retention of a muscle layer in the immotile *Echinococcus* metacestode.

KAZUYA KOBAYASHI

Yolk glands containing a large amount of tryptophan have a set of sex-inducing substances to fully sexualize asexual worms of *Dugesia ryukyuensis*

Kazuya Kobayashi

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Metazoans occasionally switch their mode of reproduction on the basis of environmental changes, the phase in the life cycle, or both. However, the mechanisms underlying the switch from an asexual to a sexual mode of reproduction, and *vice versa*, remain unknown. In the multicellular green flagellate *Volvox*, heat shock increases the production of a sex-inducing substance. Although identification of such a chemical compound provides an insight into the mechanisms underlying the switch, there is little information on the sex-inducing substance in metazoans. It is well known that in certain planarians, asexual worms can switch to a sexual state if they are fed sexual worms; this means that sexual worms contain sex-inducing substances. There is much debate on the identity of the organs or tissues responsible for producing the sex-inducing substances.
The OH strain of the planarian *Dugesia ryukyuensis* reproduces asexually without functional reproductive organs when fed a common diet (chicken liver). Indeed, since we established the OH strain in 1984, the OH worms have never switched to sexual ones under our laboratory conditions. We found that the OH worms can be switched experimentally to a sexual one (sexual induction) when fed minced conspecific sexual worms and a xenogeneic planarian, *Bdellocephala brunnea*. We suggested that sex-inducing substances could be hydrophilic low molecular weight compounds. Recently, we have found that tryptophan (Trp) is an ovary-inducing substance, and sexual worms can pool free Trp in large amounts in yolk glands, a planarian specific reproductive organ. This finding allowed us to examine the sex-inducing activity of yolk glands. We fed the OH worms with their cocoons, collected within a day after their deposition when numerous yolk gland cells remained intact. Interestingly, the cocoons were capable of inducing not only ovaries but also all the other reproductive organs in the OH worms. We concluded that a set of sex-inducing substances to fully sexualize asexual worms was contained in yolk glands. In the present study, we carried out metabolic analysis to obtain the information of hydrophilic low molecular weight compounds rich in cocoons. Here, we will introduce putative sex (ovary**)-**inducing substances among these compounds**.**

PETER LADURNER

Biological Adhesion in Flatworms

Peter Ladurner

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Synthetic adhesives are widely used in our daily lives, in medicine and industry. These man-made glues come at a cost: they usually contain toxic or carcinogenic components. In contrast, biological adhesives produced by animals and plants are non-toxic, tissue compatible, and are able to function under wet conditions. Moreover, these natural adhesives perform extremely well in their natural environment. However, little is known about the mechanisms underlying biological adhesives. Free-living and parasitic flatworms are able to attach and detach from surfaces by means of a duo-gland adhesive system. We have started to characterize adhesion and release in our model system *Macrostomum lignano*. First, we explored in detail the morphology of the duo-gland system which consists of an adhesive-, and a releasing gland cell, and a modified epidermal cell, the anchor cell. Next; we used differential transcriptomes, in situ hybridization screening, RNA interference, combined with Mass Spectrometry, Super resolution Microscopy, and Atomic Force Microscopy to narrow down the number of adhesion- and release related genes to a handful of candidates. Furthermore, we analyze permanent adhesion in *M. lignano*, i.e. to study the secretions used to permanently attach eggs to the substrate. We are currently expanding the characterization of adhesives to other flatworm taxa, and established an additional laboratory system with the proseriate *Minona ileanae*. We aim for understanding the fundamental mechanisms that mediate adhesion and release in flatworms with the goal to generate a flatworm-derived biomimetic glue that can be applied in biomedicine and industry.

Supported by Austrian Science Fund: [P 25404-B25].

TIM LITTLEWOOD

Flatworms with complex parasitic life cycles

D Tim J Littlewood

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Estimates of cestode and trematode phylogenies rely heavily on molecular data to provide resolution, and a robust framework for evolutionary interpretation and taxonomic stability. Remarkable progress has been made in the last 20 years with taxon-rich comprehensive phylogenies now available for scrutiny. However, only a limited number of molecular markers have been employed, usually components of the nuclear ribosomal RNA gene array (fragments of 18S, 28S or ITS(1/2)) and the mitochondrial genome (usually fragments of *cox1*).

Progress towards multi-gene phylogenetic assessments have been slow, largely because few researchers have access to the taxa required for comprehensive assessments or the resources for such endeavours. A consortium of researchers focused on surveying tapeworm diversity has provided material for new multi-gene phylogenies over the last few years and has highlighted the need for additional markers to provide greater stability for cestode classification and to better understand patterns of radiation and diversification. This has provided an important framework for community-led systematics with substantial progress made in diversity discovery and molecular systematic effort. Meanwhile, next generation sequencing, and the opportunities to apply this technology to material already collected, provides options for a move towards phylogenomics and renewed efforts in taking cestode and trematode systematics still further. Here, I assess where we are with the phylogenies of flatworms with complex life cycles, and contrast progress made with flatworm phylogenetics generally.

The molecular systematic approach provides tools for diagnosticians, ecologists, pathologists, evolutionary biologists and parasitologists alike.

MARTA ALVAREZ-PRESAS

Biodiversity is not only a matter of the tropics

Marta Álvarez-Presas, Eduardo Mateos, Ronald Sluys, Laia Leria, Marta Riutort

It has been known for a long time that the number of described species on the planet is less than the number pending to be described. Another common claim is that the number of so-called missing species is much higher in the tropics than in more temperate areas. However, this does not mean that most species in temperate regions, such as Europe, have already been described and that there is no more work left to do. A clear example is formed by land planarians, a little-known group because of the difficulty in finding them and the lack of specialists on their taxonomy. However, they are interesting forest soil dwelling creatures that can provide useful information on the effects of past climatic changes as reflected in their present biodiversity distribution. For this reason, in recent years we have performed several sampling campaigns across Europe to analyse the distribution patterns of genetic diversity for the most common European species, *Microplana terrestris*. Contrary to our expectations, we found a large number of cryptic, new species. For example, in some areas such as Central Balkan National Park and surrounding landscapes in Bulgaria, we found 14 different morphotypes, of which at least 6 are potential new species, none of them corresponding to *M. terrestris*. Similarly, in forests of two National Parks in Northern Spain we found 15 different morphotypes, of which at least 6 represent new species. Thus, the number of European autochtonous known species has increased from 21 (in 2008) to now at least 34 species. The integration of molecular data with anatomical information allows us to know more about these species and to determine that the level of genetic diversity among them is quite high. Moreover, we have found a considerably enlarged distributional range for some already known species, and thus it will be possible to conduct new biogeographical studies. Although land planarians may be difficult to find in temperate regions, the study of their diversity and biogeography proves to be very interesting and fruitful. Here we will show some preliminary data shedding light on the true taxic diversity of European terrestrial flatworms.

BRET PEARSON

The Queen is dead: understanding stem cell lineage hierarchies using freshwater planarians

Bret Pearson

Assistant Professor, Dept. of Molecular Genetics, University of Toronto, Hospital for Sick Children.

Neoblasts are adult stem cells (ASCs) in planarians which sustain cell replacement during homeostasis and regeneration of any missing tissue. While numerous studies have examined mechanisms underlying neoblast pluripotency, molecular pathways driving the postmitotic fate remain poorly defined and it is unknown how many progenitor cell types exist between the stem cell and a differentiated cell type. Here we used transcriptional profiling of irradiation-sensitive and -insensitive cell populations and RNA interference (RNAi) functional screening to uncover markers and regulators of postmitotic progeny. We identified 32 new markers, which distinguish two epithelial progenitor populations, and a planarian homolog to the MEX3 RNA-binding protein (*Smed-mex3-1*) as a key regulator of lineage progression. *mex3-1* is required for generating progenitors of epithelial, eye, and neural lineages, and concomitantly restricting expansion of the stem cell compartment. We also demonstrate the utility of using *mex3-1(RNAi)* animals to identify additional progenitor markers. These results show that *mex3-1* promotes differentiation in multiple lineages, maintains the balance between ASC self-renewal and commitment, and that neoblast lineages have a shallow cellular hierarchy.

LUKAS SCHÄRER

Hypodermic insemination and correlated reproductive trait evolution in the Macrostomorpha

Lukas Schärer,

Zoological Institute, University of Basel, Switzerland
Homepage: http://evolution.unibas.ch/scharer/index.htm

Hypodermic insemination is a form of traumatic mating where sperm cells are transferred to the mating partner through the epidermis, rather than being deposited into a female genital system via a female genital opening. In my talk I explore the idea that hypodermic insemination evolves due to sexual conflicts over the fate of received ejaculate components between sperm donor and sperm recipient. Specifically, I present evidence that hypodermic insemination has evolved many times independently, not only among the Macrostomorpha as whole, but also within the genus Macrostomum, and suggest that this can go along with often drastic changes in a number of reproductive traits, including sperm morphology, male copulatory organ morphology, female genital morphology, and the mode of sexual reproduction.

KAREN SMEETS

Regenerative mechanisms: a survival kit for cancer?

Karen Smeets,

University of Hasselt, Belgium.

A delicate balance exists between the process of carcinogenesis and tissue regeneration. Regeneration-competent tissues and animals are able to prevent or counteract growth abnormalities and seem to have a low vulnerability to chemically-induced carcinogenesis. Our research focuses on the effects of carcinogenic compounds in regeneration models, such as *Schmidtea mediterranea*, to identify underlying mechanisms of regeneration-associated tumor suppression.
Although neoplastic outgrowths were occasionally reported in planarians during carcinogenic exposure, we never observed tumorigenic malformations, not even during long-term screenings to different non-genotoxic and genotoxic compounds (cadmium, chromium, methyl methanesulfonate, 4-nitroquinoline N-oxide, cyclophosphamide monohydrate, methapyrilene hydrochloride, sodium phenobarbital, estradiol, chlorpromazine hydrochloride, cyclosporine A). Despite the continuously-induced DNA damage, animals were able to regenerate and survive during prolonged exposure. Stem cells responded by altering their proliferation patterns and depending on the organism's stage of regeneration, dissimilarities in the proliferative behaviour of stem cells were detected.
On the molecular level, the processes of regeneration and cancer activate strikingly similar mechanisms, despite their different outcomes. Tumor suppressor genes were hypothesized as crucial in regenerative animals to bypass carcinogenesis, but their genetic knock down during carcinogenic exposure did not support this line of thought. With an open screen comparison (regenerating vs regenerated animals in a carcinogenic environment), we were able to expose new targets that, when knocked down, induced strong malformations in exposed planarians.

Selected ISFB Abstracts for talks in order of presentation.

Unraveling DNA methylation in the blood fluke *Schistosoma mansoni*

Authors: K. K. Geyer[1], H. M. Bennett[2], U. H. Niazi[3], J. G. Rinaldi[4], M. T. Swain[1], N. Holroyd, P. J. Brindley[4], M. Berriman & K. F. Hoffmann[1]

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Abstract:

Cytosine methylation is an epigenetic mark vital for numerous biological processes in eukaryotes, and is highly prevalent across animal phyla. Despite being dispensable in some invertebrate species, it plays an important role in others (e.g.*Apis mellifera*). We have previously demonstrated that 5-methyl cytosine (5mC) is a conserved feature across the Platyhelminthes, however we are only beginning to elucidate its biological implication in flatworms. Past research in our lab revealed that DNA methylation is developmentally regulated in the trematode *Schistosoma mansoni* and provided preliminary evidence suggesting an important role of this epigenetic mechanism during parasite reproductive events. To shed further light on the biological role of DNA methylation in the blood fluke, we performed an extensive methylated DNA immunoprecipitation sequencing (MeDIP-Seq) experiment to enable the analysis of 5mC distribution across lifecycle stages on a global scale.

DNA derived from sexually-mature adult male and female worms, as well as sexually-immature, mixed-gender schistosomula was enriched for the presence of methylated cytosines and sequenced on an Illumina HiSeq platform. Subsequent bioinformatics analyses produced DNA methylation maps covering the entire genome in ample sequence depth. In line with a previous experiment demonstrating the presence of 5mC within a repetitive region, methylated regions are primarily associated with repeats in the present study. Interestingly, approx. 70% of the total repeat MeDIP peaks occur within Class I retrotransposonable elements (mostly LINEs), whereas exons appear to be primarily depleted of 5mC. Pertinently, qRT-PCR analysis revealed that the knockdown of SmDNMT2, the only enzyme responsible for DNA methylation in *S. mansoni*, results in a significant upregulation of the LTR retrotransposon Boudicca in comparison to control cultures.

Repetitive DNA, including transposons and retrotransposons, are common targets of eukaryotic DNA methylation machineries since their subsequent silencing prevents any deleterious effects caused by active TEs. A subsequently performed RNAseq analysis of 5-AzaC (a demethylating agent) treated vs. control worms will provide further insight into the biological function of cytosine methylation in schistosomes. This study aims to characterise the 5mC pattern throughout *S. mansoni* developmental stages and to provide the first global methylome analysis of any flatworm species.

Characterization of growth and regenerative potential of the rat intestinal tapeworm, *Hymenolepis diminuta,* cultured *in vitro.*

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Abstract:
The remarkable growth potential of tapeworms has been attributed to neoblast-like stem cells resident in the germinative region (GR), which lies in between the scolex and the strobilated body. The GR serves as a growth zone from which the strobila is formed by budding of proglottids. We, and others, have found that mitotically active cells are distributed throughout the length of adult tapeworms and are not confined to the GR. This observation begs the question of whether dividing cells throughout the tapeworm have multi/pluripotent potential to mediate growth and regeneration. We have established an *in vitro* culture assay to monitor growth and regeneration of amputated fragments of the rat intestinal tapeworm, *H. diminuta*. In this context, regeneration is defined as the ability to specify and add new proglottids following amputation. As expected, amputated head fragments that retain the scolex and GR are capable of regenerating new proglottids. We show that posterior amputees also regenerate so long as the GR is retained. In fact, the GR alone is competent to regenerate. When amputation was made outside of the GR, posterior fragments were capable of growing in length and differentiating normally but could not specify new proglottids. This strongly suggests that the dividing cells outside of the GR can mediate growth but not regeneration. We are currently pursuing various regeneration assays to determine whether stem cells within the GR are intrinsically unique, or whether extrinsic cues are responsible for the differing regenerative potential we observe.

Planarians to Parasitism: identifying signals and switches of development in tapeworms

Peter D. Olson

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Advances in model systems for studying parasitic flatworms have made it possible to investigate their genotype-to-phenotype map for the first time, helping to bridge the historical gulf between the fields of Development and Parasitology. New and continuously improving genomic resources combined with a recently emerged model of planarian gene regulatory networks (GRN) provide a roadmap for elucidating the genetic underpinnings of the highly derived development of the parasitic groups. We are using genomic and RNAseq profiling to identify signalling and transcription factors (TF) in the model tapeworm *Hymenolepis microstoma* and mapping their expression to body regions, organ systems or cells using in situ hybridisation. Expression during larval metamorphosis in the beetle and subsequent strobilar development in the mouse shows up-regulation of the same principal signalling systems, including Wnt, Notch and TGFB, indicating that strobilation evolved through redeployment and modification of conserved flatworm GRN. Spatial expression shows the nervous system as the source of signalling factors, whereas most TF exhibit regionalised, reproductive organ or cell-specific, expression domains. The latter are dominated by homeobox, zinc-finger and forkhead box TF with homologs in planarians, but also include novel zinc finger TF lacking homologs in other animals. Perhaps surprisingly, the diversity of developmental genes associated with proglottide maturation (including organ formation, embryogenesis and senescence) is considerably highly than that associated with larval metamorphosis and strobilation. Diversity of TF in the strobila proves to be chiefly associated with ovarian tissues, with a number of factors showing simultaneous expression in the cells innervating the genital pore. Analysis of the ‘neck’ region reveals discrete qualitative and spatial changes in gene expression that correlate to circumscribe a unique region of cellular differentiation, despite the absence of any visible boundary. Examples of tapeworm developmental gene expression and comparison with that of planarians will be presented together with results of studies employing in vitro culture and immunohistochemistry to characterise the distribution of proliferating cells (neoblasts) and organs systems in *H. microstoma*.

The influence of regeneration on ageing of the flatworm *Macrostomum lignano*

Mouton S, Grudniewska M., Berezikov E.

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

Determining the causes of ageing remains one of the central questions in biology. To answer this question, different model systems have been used. All have their advantages and disadvantages and allow focusing on specific aspects of the ageing process. Interesting non-standard models to study the role of stem cells in ageing are flatworms. They have a pluripotent population of mesodermal stem cells which are responsible for a high cellular turnover during homeostasis and often a high regeneration capacity. Some flatworms are claimed to be immortal, others are shown to age, and in different species it has been observed that repeatedly regenerated animals live longer than non-regenerated individuals. This led to the hypothesis that regeneration can induce rejuvenation, which was originally proposed by Child in 1911. However, the amount of data supporting this hypothesis is very limited and gene expression as a function of age and the effect of regeneration on it have not been studied yet.

To address these research questions, we use the flatworm model*Macrostomum lignano*, for which genome and transcriptome draft assemblies are generated, and methods for making transgenic animals are being developed. To test the effect of regeneration of *M. lignano* on ageing, we are studying the following parameters as a function of age in uncut, once cut, and repeatedly cut animals: survival, morphology, fertility and gene expression. The aim of the non-molecular parameters is to determine the fitness as a function of chronological age in the different treatment groups. At the same time, more than 90 RNA-seq libraries of worms from the different treatment groups at several ages have been made. This allows to determine a molecular ageing profile and to study the effect of regeneration on ageing. The data is currently being analyzed and reveals a role for conserved pathways previously implicated in ageing, apoptosis and cell quiescence, such as the Notch signaling pathway. This dataset will form the basis for further in depth studies. Here, we will present the progress and first findings of this project.

Functional morphology and Biomechanics of the Proboscis in Schizorhynchia

Julian P.S. Smith III, Dept of Biology, Winthrop University

Theodore Uyeno, Dept. of Biology, Valdosta State University

Turbellarians belonging to the taxon Kalyptorhynchia are characterized by an anterior proboscis that is thought to be used primarily for the capture and manipulation of prey located within the smaller interstices that are formed between the grains of sand of their meiofaunal environment. Within the sub-taxon Schizorhynchia, this proboscis takes the form of dorsoventrally paired, finger-like muscular tongues that are sometimes armed with teeth or hooks. Although it seems clear that these structures rely on a muscular hydrostatic mechanism to generate support and movement, we lack a full understanding of how the tongues' structural arrangement supports the observed forceps-like opening and closing movements. Here, we describe and model the anatomy of both armed and unarmed schizorhynch proboscides using fluorescently-labeled confocal images and both transmission and scanning electron microscopy to characterize the organization of both contractile and tensile soft tissues. The results suggest that although the tongues are indeed deformed by the contraction of muscular tissue, limitation of deformation by the tensile extracellular matrix elements described herein is also extremely important in generating the movements observed.

This presentation was supported by grants from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health (to JPSSIII) and a grant from the National Science Foundation (IOS-1354788) and Valdosta State University (FRSG-2012/13-1) (to TAU).

Analysing dynamics of spiral cleavage and the cell lineage of the polyclad flatworm *Maritigrella crozieri* by home-built SPIM-based microscopy.

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To better understand of the evolution of marine invertebrate body plans we focus on the comparative developmental biology of the Tiger flatworm *Maritigrella crozieri*.
This polyclad flatworm has a ciliated planktotrophic larval stage known as Müller's larva, which shares some morphological similarities to spiralian's trochophore larvae such as are found in marine annelids and molluscs. Despite these observed similarities, the question whether these larvae represent an ancient feature or whether they are a case of convergent evolution is still unresolved.
Polyclads are the only group within the Platyhelminthes that undergo indirect development and it has been argued that the existence of a larva in a single order of Platyhelminthes points to its convergent evolution in polyclads rather than repeated loss in the other clades. Our lab has produced a deeply sampled phylogenomic study of most platyhelminth orders which supports a relatively basal position of the polyclads within Platyhelminthes. This position means that it is reasonably parsimonious for the existence of a larva to be considered as a primitive character.
We are particularly interested in complex larval structures such as eyes, nervous system and apical organ with putative homology to similar structures in canonical annelid and mollusc trochophores. We are studying the elaboration of these larval structures during embryogenesis of *Maritigrella* with an emphasis on discovering their origins amongst the early blastomeres using cell lineage tracing. To achieve this we have built a Selective Plane Illumination Microscope (SPIM) and are using injected nuclear markers to follow cell division in vivo. Comparing the origins of these characteristic features of *M.crozieri* larvae with other spiralian primary larvae and their cell lineages is the major goal of this project.
The fact that polyclad embryonic development shows the stereotypic spiral cleavage pattern with quartets and remarkable similarities to that of other spiralian embryos further suggests that polyclads may have retained ancestral features of their phylum.

Functional specialization of Planarian β-catenins

Miquel Sureda-Gomez[1], Hanxia Su[2], Neus Rabaneda[1], Andrea Orus[1], Jose Maria Martin-Duran[3], Wei Wu[2], Emili Saló[1] and **Teresa Adell[1]**

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β-Catenin is an evolutionary conserved protein which shows a dual-function; it is involved in cell adhesion as a component of adherent junctions and in transcriptional regulation as a key target of Wnt signaling. The evolutionary conservation of the structural domains involved in each function points to its key role to integrate both cellular processes. Interestingly, a bi-functional β-catenin is not found in planarians. Two β-catenins have been reported from*S. mediterranea*, originated by genomic duplication and functional specialization. *Smed-βcatenin1* is specifically involved in transcriptional regulation upon Wnt activation, and it is essential for antero-posterior axial patterning. *Smed-βcatenin2* is dedicated to cellular adhesion.
Here we report the in deep analysis of *Smed-βcatenin1* function in antero-posterior patterning and the identification of three new planarian β-catenins (*Smed-βcatenin3/4/5*). We demonstrate the existence of the predicted gradient of nuclear *Smed-βcatenin1* from posterior to anterior, according to its role in posterior specification. Interestingly, the gradient does not include the head and brain, where *Smed-βcatenin1* is highly nuclearized and required for patterning. *Smed-βcatenin3* and *4* are also expressed in the central nervous system and are required for proper neural regeneration, being *Smed-βcatenin4* also necessary for the differentiation of photoreceptors. The structural domains of *Smed-βcatenin3* and *4*, together with its functional analysis in heterologous systems, suggest that both β-catenins could be acting as transcriptional regulators through competition with *Smed-βcatenin1*. The expression pattern and preliminary functional analysis of the fifth β-catenin, *Smed-βcatenin5*, suggest its function as transcriptional activator together with *Smed-βcatenin1*.
Its tempting to speculate that the high degree of duplication suffered within the β-catenin gene family in planarians could be related to its cellular plasticity. However, a closer look in the literature reveals that the presence of β-catenin paralogs is common in the animal kingdom, although only in few cases a functional specialization has been demonstrated. Comparative analysis of the β-catenin family within species could give light into the mechanism of gene duplication and to its evolutionary relationship with alternative splicing, the two widespread types of genetic variations that facilitate genetic diversification.

MicroRNA, *miR-124* is essential for cephalic ganglion patterning and organization of optic chaisma during planarian regeneration.

Vidyanand Sasidharan*, Srujan Marepally*, Vairavan Laxman, Praveen Vemula*, **Dasaradhi Palakodeti***

Planarians are bilaterally symmetrical fresh water animals capable of regenerating the whole body or lost tissues and organs. They have specialized cells called neoblasts, which are functionally equivalent to embryonic stem cells. MicroRNAs are small RNA species that control gene expression by modulating translation and mRNA stability. MicroRNAs have been implicated in the regulation of various cellular processes such as neurogenesis, cell cycle, stem cell function and etc. Recently, we identified several miRNAs whose expression is enriched in different neoblast subpopulations and in the regenerating tissues at different time points during planarian regeneration. Our results also revealed miRNAs, such as *sme-mir-2d-3p* and *sme-mir-124* families, whose expression is enriched in the cephalic ganglia, and in brain primodia during CNS regeneration. We were also able to knockdown *sme-miR-124c-3p* using LNA *anti-miRs* to study its function in cephalic ganglion regeneration in planarians. Knockdown of *sme-miR-124c* showed varied photoreceptor defects such as under developed eye, single eye and animals with out eyes. Further analysis of phenotype revealed disorganized optic chaisma and disrupted neuronal connections between cephalic ganglia and ventral nerve cord. Transcriptome analysis of *miR-124c* knockdown animals showed increased levels of genes involved in axon guidance cues. Thus these results suggest that *miR-124c* is a key regulator of axon guidance cues essential for proper patterning of neurons in planaria.

Planarians (Re)generate a Way to Drug Discovery

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Treatment of parasitic flatworm infections has been largely reliant on a single drug - praziquantel (PZQ) - for nearly four decades. Research into alternative agents is hindered by a variety of barriers that have impeded identification of next generation therapies and frustrated resolution of why exactly PZQ proves such an effective anti-helminthic. To bring fresh perspective to these problems, we have proposed using a free-living turbellarian species, the planarian Dugesia japonica, as a phenologous drug discovery model for revealing novel agents with efficacy against the parasitic trematode Schistosoma mansoni. Using approaches optimized in planarians, such as in vivo RNAi and phenotypically clear-cut regenerative assays, we have discovered that the historically important class of ergot alkaloid compounds show broad efficacy at miscuing regenerative outcomes (yielding 2-headed, or no-headed animals) and impairing planarian muscle function. These compounds, initially identified in free-living planarian regenerative assays, also proved efficacious as paralytics when applied to parasitic schistosomula. The broad efficacy of this compound class permit key structural activity insight as to specific pharmacophore modifications that are predictive of the drug action on either class of flatworms. These modifications are supported by structural modelling and allow the rational design of ergomimetics with penetrant action on regenerative outcomes. Intriguingly, ergot-alkaloid induced paralysis was mimicked by RNAi of a bioaminergic receptor, highlighting candidate targets for the development of new anti-schistosomal therapies. These data imply that planarian regenerative polarity and parasite neuromuscular control are regulated by a common underlying signaling pathway, and evidence an experimental approach capitalizing upon this phenology to implicate bioaminergic signaling as a crucial node in flatworm biology that can be exploited for anti-parasitic drug development**.**

Plasticity in axial polarity during postembryonic development in acoel flatworms

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Abstract: Axis polarity is specified during early embryogenesis and remains largely unaltered during the lifetime of most organisms, yet axis polarity can be re-established during regeneration and novel axes can be specified during agametic asexual reproduction during budding and fission. Acoel species in the genus *Convolutriloba* are unique among bilaterians in their ability to either transiently reverse the anterior-posterior (AP) axis during reversed polarity budding or re-specify left-right (LR) polarity during longitudinal fission. We have developed *C. macropyga* and *C. longifissura* as models for understanding the developmental modifications of body axis polarity during localized AP axis reversal and midline re-specification respectively. We have characterized a region of tissue at the transitional zone between parent and bud where AP axis reversal presumably occurs at the initiation of bud outgrowth in *C. macropyga*and have identified a zone of apoptotic activity that develops along the midline during longitudinal fission in *C. longifissura*. Chemical genetics screens along with targeted functional studies have identified signaling proteins as candidates for functioning in the disruption and re-specification of the AP and LR axes during asexual reproduction in these species. We are currently investigating the role of Hedgehog signal transduction in the disruption and modification of the AP axis during reversed polarity budding, and have identified a putative role of Bone Morphogenetic Protein (BMP) and Slit/Robo signaling in modification of LR and midline polarity that occurs as part of longitudinal fission

A Classroom-Based RNAi Screen for Regeneration Genes in the Freshwater Planarian *Schmidtea mediterranea*

Derek Starkey, Alexandra Abbate, Casey Kimball, Amber Poirier, Stuart Nelson, and **Jason Pellettieri***

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In addition to playing an increasingly important role in stem cell and regeneration research, freshwater planarians are ideally suited to inquiry-based science education projects. The rapid and easily observable regenerative response in species such as *Schmidtea mediterranea* provokes strong curiosity amongst nearly all students, regardless of their level of prior research experience, and the availability of genome sequencing information and tools like RNA interference enable high-throughput molecular genetic analyses. We took advantage of these attributes to develop a discovery-based laboratory research project for an undergraduate developmental biology course. During this semester-long research experience, each of the 16 students enrolled in the class identifies and clones at least one novel *S. mediterranea* gene and then uses RNAi to analyze possible regeneration phenotypes. To date, we have screened over 50 genes via this approach and identified 3 new genes required for blastema formation, including a *mago nashi* homolog previously shown to be expressed in the ovaries of the *S. mediterranea* sexual strain. We find that silencing of *mago nashi* in asexual animals causes head regression, ventral curling, and eventual lysis, in addition to failed regeneration. These phenotypes are all hallmarks of stem cell loss/dysfunction in planarians. Intriguingly, *Drosophila mago nashi*regulates germline stem cell differentiation in the egg chamber, and the related mouse gene *Magoh* is required for maintenance of neural progenitor cells, suggesting evolutionarily conserved functions for this gene family in stem cell regulation. We are currently using whole-mount in situ hybridization to characterize the *mago nashi*expression pattern in asexual animals, and phospho-histone H3 and TUNEL staining to address a possible requirement for *mago nashi* in planarian stem cell maintenance; results from these analyses will be reported at the meeting. Our work illustrates the simultaneous educational and research impacts that can be achieved through integration of discovery-based planarian research projects into the undergraduate biology curriculum.

Title: Positional information and scaling in regeneration of the planarian anteroposterior axis

Rachel Lander, Eric Hill and **Christian Petersen**

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Planarian regeneration perfectly reestablishes animal form from diverse injuries, suggesting the existence of robust processes controlling tissue identity and organ scaling. Through homology and expression approaches we identify cues expressed regionally along the anteroposterior axis as candidates for control of these processes. Canonical Wnt signaling and several additional factors establish identities of the termini of the anteroposterior (A-P) axis during head and tail regeneration, but comparatively little is known about how position along that axis is redefined after injury or how regeneration reestablishes appropriate organ sizes. We demonstrate that planarians perfectly restore brain:body proportion by reversible regulation of differentiated cell numbers through Notum/Wnt feedback inhibition. Analysis of this pathway identified beta-catenin-1-dependent and -independent steps in control of brain size, determined that Wnt/Notum signaling controls brain progenitor numbers rather than injury-induced cell death, and suggests that size restoration might be achieved by reaching a balance between signal activation and repression. Furthermore, we identify functions for additional A-P regionally expressed cues in controlling body-wide positional and regional identity, including a Wnt co-receptor, protein tyrosine kinase-7 (ptk7) that controls positional information within the trunk. Surprisingly, ptk7 operates early after injury and in a dose-dependent manner to position formation of trunk and posterior tissues prior to the restoration of A-P gradient gene expression domains. Clarifying the regulatory logic that enables restoration of positional information and scale to an axis truncated by amputation will be an important step in understanding regenerative abilities.

Alternative splicing in the regulation of planarian stem cells in vivo

Jordi Solana, Manuel Irimia, Salah Ayoub, Marta Rodriguez Orejuela, Benjamin Blencowe and Nikolaus Rajewsky.

Planarians are a paradigm of regeneration and stem cells. Recently, deep sequencing techniques have boosted the study of AS in many different tissues, cell types and conditions, but little is known about the role of alternative splicing (AS) in animal stem cells. Here, taking advantage of the planarian *S. mediterranea* and its abundant and experimentally amenable stem cell population, we approach this question. We computationally identified hundreds of AS events that are differentially enriched or depleted in planarian stem cells compared to differentiated cells. We experimentally validate these results. We were able to identify conserved regulatory proteins that selectively affect these splicing patterns and investigate their roles in stem cell self-maintenance and differentiation. These factors include the previously described CELF type factor *Smed-bruno-like* as well as several Muscleblind-like (MBNL) genes. We show that *Smed-bruno-like* and MBNL factors have antagonistic effects on planarian stem cells. Together, our data suggest that (a) in animal stem cells, alternative splicing is a key pathway for regulating self-renewal and differentiation (b) this pathway is largely regulated by a conserved antagonism between CELF and MBLN factors.

New triclad species questioning the status of the suborder Cavernicola

Ana Leal-Zanchet[1], Arnau Poch[2], Oleguer Castillo[2], Stella Souza[1], Ana Morais[1], Rodrigo Ferreira[3] & Marta Riutort[2]

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The triclad suborder Cavernicola Sluys encompasses six species and five genera of the family Dimarcusidae Mitchell & Kawakatsu. Most cavernicolans are typical troglobitic organisms, unpigmented and eyeless, occurring in hypogean environments with a disjunct distribution. One species, however, was exclusively found in epigean habitats. In order to investigate the phylogenetical relationships of two candidate species of triclads, we phylogenetically compared their 18S and 28S rRNA gene sequences to those of other triclad species including representatives of Maricola, Cavernicola and Continenticola. The candidate species are represented by (1) epigean specimens sampled in freshwater wetlands from two localities in southern Brazil and (2) hypogean specimens sampled in freshwater lakes from a limestone outcrop in northeastern Brazil. Morphological analyses indicated that the species constitute two new taxa showing characteristics concordant with the diagnosis of the suborder Cavernicola. Both species show a common oviduct perpendicular to the bursal canal or female genital duct as well as sperm ducts which unite and form an extra-bulbar common sperm duct. The epigean specimens belong to the genus *Rhodax*, whereas the hypogean specimens represent a typical troglobitic triclad which cannot be assigned to any known genus. The phylogenetic trees obtained from 18S and 28S rRNA gene sequences show *Rhodax* sp. grouping with GenBank sequences of an undescribed species of Cavernicola. This clade constitutes the sister group of Maricola, the Continenticola being basal in the Tricladida tree. Surprisingly, the troglobitic triclad is situated deeply within the suborder Maricola, with high support. This result may have an explanation in the geologic history of the region. The sampling area in northeastern Brazil is located near the sea, at a low altitude, which has allowed different invertebrates to colonize the caves during sea level rises in the past. However, the question of the taxonomic adscription of the new troglobitic triclad remains open. It would be necessary to obtain sequences from other species of Cavernicola to check their molecular adscription, and revise the morphological characteristics of the group closest to this troglobitic triclad in the molecular trees (*Ectoplana*) in order to have a definitive picture of the adscription of all these species.

Phylogeny and morphology of Nemertodermatida: chimeric sequences and plastic morphology

Ulf Jondelius[1], Olga I Raikova[2] and Inga Meyer-Wachsmuth[1]

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The phylogeny of Nemertodermatida, a group of microscopic marine worms, was analysed using nucleotide sequences from the ribosomal LSU and SSU genes and the protein coding Histone H3 gene. All currently known species except *Ascoparia neglecta* and *A. secunda* were included in the study in addition to several yet undescribed species. Nucleotide sequences deposited in Genbank before 2013 as nemertodermatid were validated against our dataset; some of them are shown to be chimeric implying falsification of prior hypotheses about nemertodermatid phylogeny whereas other sequences should be assigned new names.

The nervous system of *Flagellophora apelti, Sterreria spp*. and *Nemertoderma westbladi* was studied using anti-tubulin, anti-5-HT and anti-FMFRamide antibodies as well as phalloidin staining.

The nervous system of *Flagellophora apelti* is composed of a large brain neuropile at the level of the statocysts with several fibres surrounding it and innervating the broom organ. *Sterreria spp*. shows a commissural-like brain and several nerve cords going frontad and caudad from this. At the level of the statocysts there is also a thicker aggregation of IR fibres. The nervous system of *N. westbladi* consists of a nerve ring lying outside the body wall musculature at the level of the statocyst and a pair of ventro-lateral nerve cords, from which extend numerous fibres innervating the ventral side of the animal.
The nemertodermatids do not display a clear common nervous system pattern. Instead correlation with our phylogenetic hypothesis reveals a remarkable degree of morphological plasticity. The nervous system may be the least conserved organ system in these animals. The ancestral state of the nemertodermatid and acoelomorph nervous system is discussed.

"Phylogeny of the limnoterrestrial Rhabdocoela (Platyhelminthes), an enigmatic group of minute metazoans"

Houben Albrecht & **Artois Tom**

Although limnoterrestrial rhabdocoels appear to be common in terrestrial environments such as mosses, moist meadows and grasslands, and leaf litter in woods, they have received very little attention from taxonomists. Therefore, these animals are poorly known in all aspects of their biology, including their phylogenetic relationships. In a recent phylogenetic analysis of Dalytyphloplanida by Van Steenkiste et al. (2013) the first semiterrestrial rhabdocoels were included in a large scale analysis. Although limnoterrestrial taxa were still very much underrepresented, the results of this analysis were rather surprising because they suggest that a terrestrial life style was addopted several times independently within Limnotyphloplanida, the clade to which almost all limnoterrestrial rhabdocoels belong. In this study we pick up the thread were Van Steenkiste et al. (2013) left it, and we will further explore the phylogenetic relationships of the limnoterrestrial rhabdocoels using the complete 18S and 28S rDNA genes, trying to solve the question of the origin of these taxa and their ancestral habitat. The results of our study shows: (1) a limnoterrestrial origin of the limnotyphloplanids, (2) multiple independent colonizations of freshwater habitats within Limnotyphloplanida, (3) that limnic thalassotyphloplanids and neodalyellids have originated from the marine/brackish environment without a limnoterrestrial intermediate phase, (4) that a rapid species radiation within the limnic environment occurred only with a limnoterrestrial ancestor, (5) several well-supported limnic and limnoterrestrial clades (i.e. Mesostominae, *Acrochordonoposthia,* *Dochmiotrema*, *Phaenocora,* *Opistomum*and *Strongylostoma*), (6) polyphyly of the traditional limnoterrestrial subfamily Protoplanellinae, (7) and, last but not least, that a major effort is needed to sample the limnoterrestrial habitat in order to further investigate the phylogeography and even α-diversity of limnoterresstrial rhabdocoels.

Reference:
Van Steenkiste, N., Tessens, B., Willems, W., Backeljau, T., Jondelius, U. & Artois, T. (2013) A comprehensive molecular phylogeny of Dalytyphloplanida (Platyhelminthes: Rhabdocoela) reveals multiple escapes from the marine environment and origins of symbiotic relationships. Plos One, 8, e59917. http://dx.doi.org/10.1371/journal.pone.0059917

New incomers into Europe: the true identity of a Geoplaninae land planarian species spreading across Europe

Fernando Carbayo, Marta Álvarez-Presas, Hugh David Jones, Marta Riutort

Since 2008 there have been many records in Europe (British Isles, Spain, France, Italy) of a large terrestrial planarian similar in the external appearance to the Brazilian species*Obama marmorata* (Schultze & Müller, 1857). This species was one of the first land planarian species described but only on its external aspect, though Froehlich (1959, BFFCL USP Zool 22) described the external features and internal anatomy. In a molecular phylogenetic approach of the Geoplaninae, Carbayo et al. (2013, Zool Scr 42) included two specimens of an externally and internally alike species, but, unsure of their identity, they just recorded it as *Obama* sp 6. Sequences ofDNA (Cox1) recently obtained from several European and Brazilian specimens indicate that those from Europe and some of the specimens collected in Brazil are of one species but other Brazilian specimens are a different species. Moreover, the phylogenetic results show that they are not even sister-species. Histological sections of both Brazilian and European specimens have allowed us to distinguish subtle morphological differences between the species. *Obama marmorata* is confined to Brazil, and the second undescribed species of *Obama*, is found in Brazil and Europe. These cryptic species may be syntopic in areas in Brazil. The new species occurs in man-modified environments both in Brazil and Europe. Based on our results, we also conclude that the specimens from Spain and Argentina identified by Lago-Barcia et al. (2015, Invertebr Syst 29) as *Obama marmorata,* using both anatomical and molecular (actually DNA sequences from only one species) evidence, belong to the new species.

Diet and food niche breadth in six Neotropical land planarians (Tricladida: Continenticola)

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Land planarians are recognized as important predators, yet studies on their feeding ecology are usually restricted to invasive species. Thus, it is difficult to determine the real ecological role of this group, which limits the understanding of the mechanisms that lead some species to become successful invaders. In this study, we analyzed the diet of six Neotropical land planarians based on experiments in the laboratory. We kept the flatworms individually in small terraria simulating their natural habitat. Twice a week, we offered a specimen of an invertebrate species as a possible prey (e.g. gastropods, earthworms, woodlice, millipedes, termites, other land planarians). Each invertebrate species was offered 15 times for each planarian species in a random sequence. We estimated prey preference by the number of attacks recorded on each consumed species, and capture success by the proportion of successful attacks. We also calculated indices of food niche breadth (Levins' Index) and food niche overlap (Pianka's Index). The diet of *Luteostriata abundans* comprises only woodlice and does not overlap with the other species. The diets of *Obama ficki* and *O. ladislavii* are composed only by gastropods, but include different species. *Paraba* cf. *gaucha* and *O. anthropophila* feed on both gastropods and other land planarians. *Obama marmorata* showed the highest food niche breadth, feeding on gastropods, planarians and earthworms, but showing a preference for the latter. We found the highest niche overlap between *O. anthropophila* and *P.* cf. *gaucha*. The results suggest that land planarians are frequent predators of woodlice and land gastropods in the Neotropical ecozone and thus are important for the maintenance of native ecosystems. Several exotic invertebrates were consumed, which indicates a possible role of Neotropical land planarians on the control of invasive species. However, some species highly adapted to human-disturbed areas could also become pests if introduced outside of their native range. The coexistence of several species of land planarians in the same habitats in the Neotropics is possible because different species use different invertebrates as main prey, which reduces interspecific competition.

Nuclear genomic signals of the 'microturbellarian' roots of platyhelminth evolutionary innovation

Christopher E Laumer, Andreas Hejnol, Gonzalo Giribet

Flatworms number among the most diverse invertebrate phyla and represent the most biomedically significant branch of the major bilaterian clade Spiralia, but to date, deep evolutionary relationships within this group have been studied using only a single locus (the rRNA operon), leaving the origins of many key groups unclear. In this study, using a survey of genomes and transcriptomes representing all free-living flatworm orders, we provide resolution of platyhelminth interrelationships based on hundreds of nuclear protein-coding genes, exploring phylogenetic signal through concatenation as well as recently developed consensus approaches. These analyses robustly support a modern hypothesis of flatworm phylogeny, one which corroborates several long-held morphological hypotheses which had not previously seen support in molecular studies (e.g. Trepaxonemata, Cercomeromorpha), several hypotheses also found in rRNA studies (e.g. Euneoophora, Adiaphanida), and several hypotheses not previously discussed (e.g. a sister-group relationship between Prorhynchida and Polycladida). Perhaps most notably, these data also introduce a novel scenario for the interrelationships between free-living flatworms and the vertebrate-parasitic Neodermata, providing new opportunities to shed light on the origins and biological consequences of parasitism in these iconic invertebrates. We also present evidence for previously unrecognized deep phylogenetic diversity within the clade Adiaphanida, showing that the enigmatic crustacean-parasitic genus*Genostoma*, previously considered a member of Fecampiida, represents its deeply branching lineage. We conclude with a synoptic discussion of the status of deep flatworm phylogeny to date, highlighting those areas of the tree most in need of continued investigation, and emphasizing the crucial role of continued morphological and developmental research in testing and ultimately conferring biological meaning to the tree topology encoded in the genomes of these remarkable invertebrate animals.

Towards an in-depth view of the reproductive biology of *Schistosoma mansoni*: from gonad isolation to sub-transcriptomics and beyond.

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Schistosomiasis, the second most devastating parasitic disease worldwide after malaria, is caused by trematodes of the genus *Schistosoma*. Schistosomes are unusual in being dioecious and because a constant pairing contact is essential for the sexual maturation of the female. Pairing induces gonad differentiation in the female and the production of eggs, which are required for maintaining the life-cycle, but they are also the cause of pathogenicity in infected humans and animals.
To better understand the reproductive biology of schistosomes we established a protocol to isolate gonads from adults. RNA of good quality was obtained, which provided a basis for RNA-Seq to generate the first organ-specific transcriptome data for ovaries and testes. We additionally investigated pairing-dependent aspects by comparing data from testis and ovary of pairing-experienced versus pairing-unexperienced worms.
Bioinformatics analyses revealed that > 7,700 genes (of a total of about 10,800 genes in *S. mansoni*) were transcribed each in testes and ovaries (RPKM > 2), of which many were significantly regulated by pairing ($\geq$ 1.5-fold difference; FDR < 0.05 for testes and < 0.005 for ovaries):

(i) 243 in testes, of which 96 were up-regulated in bT (testes from paired, bisex males) and 147 were up-regulated in sT (testes from unpaired, single sex males).
(ii) 3600 in ovaries, of which 1752 were up-regulated in bO (ovaries from paired, bisex females) and 1848 were up-regulated in sO (ovaries from unpaired, single sex females).

To gain insights into:

(i) biological processes in gonads of schistosomes in general
(ii) differences of testis versus ovary
(iii) the influence of pairing on gonads

the data were analysed for the enrichment of functional annotation categories amongst genes differentially expressed between gonad tissues from paired and unpaired worms and using paired and unpaired whole-worm data as controls. This approach led to a huge amount of novel and interesting insights with respect to evolution (hermaphroditism vs dioecy), gonad development and physiology, and testis- or ovary-specific factors. Finally, it was confirmed, also by qPCRs, that the influence of pairing is fundamental and far-reaching because many genes in testis and/or ovary are obviously under the direct control of male-female interaction.

Single-cell sequencing in *Schistosoma mansoni*

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The *Schistosoma mansoni* genome assembly and annotation provide a high quality community resource for molecular biology. However, data on the role of organism-specific genes, predicted signalling pathways and the contextual function of many genes within the whole organisim are currently sparse. Single-cell resolution sequencing provides opportunities to define cellular subtypes and investigate co-expression of genes in this important parasite.

Using staining and image analysis we estimate there to be around a thousand non-dividing cells in the larval schistosomula stage, representing divergent structures including tegument, nervous system, muscle, gut and secretory organs. After *in vitro* transformation and culture, we have devised a dissassociation protocol to obtain a cell suspension from this stage of the parasite and used flow cytometry to capture individual cells. Using the Smart-seq2 protocol and Illumina paired-end sequencing we have obtained independent transcriptomic profiles for individual cells and report our first findings from this data.

Development of an *in silico* pipeline for prioritizing novel *Schistosoma mansoni* drug targets.

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Schistosomiasis, a neglected tropical disease caused by the platyhelminth *Schistosoma sp*., currently affects ~249 million people, across 78 countries. Praziquantel (PZQ), a highly efficacious and low cost treatment, is the only current pharmaceutical intervention against Schistosomiasis. However, despite PZQs exemplary role in mass drug administration (MDA) for more than 30 years, the future of MDA with PZQ is overshadowed by concerns of developing resistance. To ensure MDA sustainability, an innovative, rapid, and low cost chemotherapeutic strategy is required for identifying next generation anti-schistosomals.

To objectively identify novel "druggable" S*. mansoni* proteins (Smps), an *in silico* pipeline was created utilising freely accessible databases, including CHEMBL and DrugBank- (both containing proteins with associated drug-like compounds), Protein Data Base (PDB) and GeneDB. Initially orthologues between the *S. mansoni* proteome and ChEMBL/DrugBank protein targets were identified using PSI-BLAST (sequence identity of >50%, alignment of >70%, and an E-value cut-off of 0.0001). This successfully identified 1999 *S. mansoni* protein orthologues as potential drug targets. Transcriptome data was then applied to refine this list of Smps further, by selecting for Smps significantly expressed in the definitive host lifecycle stages - schistosomula and adult worms. This filter gave an output of 1678 Smps. From the final 1678 Smps, only those with orthologues in the*S. japonicum* assembly and PDB databases were selected using the same PSI-BLAST criteria previously stated. A final list of 291 Smp drug targets was produced, each having an orthologue in CHEMBL, DrugBank, PDB and the *S. japonicum* genome assembly.

This *in silico* pipeline has objectively selected 291 potential anti-schistosomal targets and their respective drug-like compounds, from a proteome of 10,852 Smps, for further *in vitro* molecular and biochemical focus. In addition, each Smp target also shares >50% sequence identity with a PDB structure, facilitating further work on modelling and assessing Smp-compound binding efficiency in an *in silico* docking environment. The latter enabling further refinement of the compounds selected for high-throughput screening on schistosomula, and low-throughput screening on adult worms.

Evidence for karyotype polymorphism in the free-living flatworm, *Macrostomum lignano*, a model organism for evolutionary and developmental biology

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The free-living flatworm *Macrostomum lignano* is a model in many research areas, including development, ageing and evolution. This species is attractive as a laboratory model system because it (1) is small and transparent; (2) has clearly defined organs systems; and (3) is easily cultured in the laboratory. Additionally, it has a high regenerative potential provided by pluripotent stem cells, which allows regeneration of damaged worms and, as a result, the production of many dividing cells. This ability for regeneration has now been extensively used in karyotyping of *M. lignano*. Although initially the karyotype of *M. lignano* was described as 2n=8, with two large and six small chromosomes, we show that, even within the inbred lines DV1 and HUB1, as well as within several outbred cultures (e.g. LS1, LS2, LS3), there is substantial karyotype diversity, mainly linked to numerical chromosomal aberrations (especially, copy-number variation of the large chromosome) and in some cases also structural chromosomal aberrations. By individually karyotyping worms from a range of different inbred lines and outbred cultures we here document the presence of karyotype diversity in most lines and cultures, and find that their frequency can even vary between samples of the same culture taken at different times. In order to understand this karyotype instability, we therefore crossed parental worms (DV1 and HUB1) of known karyotype and checked their offspring's karyotype and GFP(+) expression (the HUB1 line is a transgenic line derived from DV1). We found offspring with unexpected karyotypes in many cross combinations (ranging from 2n=8 to 2n=21), even when both parents had 'normal' 2n=8 karyotypes. Furthermore, we found that the penetrance of the GFP marker was strongly influenced by the karyotype of the HUB1 parent, suggesting that at least one insertion site of the construct is on the large chromosome, so that the construct can occur in a range of different copy numbers in an individual. Individually karyotyping of freshly field-collected *M. lignano* revealed that karyotype diversity is not an artifact of rearing worms in the lab and new karyological data for other *Macrostomum* species suggests that *M. lignano* is not the only species with variable karyotypes.

Polycladida phylogeny based on 28S rDNA challenges traditional classification systems

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Polyclads are free living marine flatworms which have worldwide distribution. The order Polycladida is undisputedly divided in two suborders, Acotylea and Cotylea, based on the absence or presence of a ventral sucker. In contrast there is no consensus on family level classification. Between 1983 and 1985 Faubel and Prudhoe published two incompatible classification systems, based on different morphological characters. Superfamilies according to Prudhoe are characterized by the arrangement of eyespots on the body; while Faubel emphasized male and female reproductive structures. Not until recently molecular data was included in the study of the group with some general platyhelminth phylogenies and, in Polycladida, a phylogeny of the family Pseudocerotidae (Cotylea). Here we aim to add more data to the knowledge of the group and open a discussion about the validity of the competing, morphology-based family classification. We extracted genomic DNA from numerous samples representing 16 families of both Cotylea and Acotylea polyclads. The nuclear marker 28S was chosen as it is informative for resolving shallow and deeper polyclad relationships. We found that our maximum likelihood trees are not compatible with any traditional system, resulting in paraphyletic superfamilies. Prudhoe's Stylochoidea and Planoceroidea are divided in 2 clades each and form mixed clusters. Also, Faubel's Schematommatidae were paraphyletic and mixed with Craspedommatidae with good node support. In Cotylea the superfamilies Pseudocerotoidea and Euryleptoidea were also paraphyletic. Our still preliminary molecular tree thus indicates that both traditional Polycladida are unnatural on superfamily level, and should be reconsidered. In contrast, conventional families were well supported molecularly. Also the genera, for which we sampled more than single samples, were well supported; only in the case of the genera *Chromoplana* and *Chromyella* there is some conflict between morphological and molecular data. We expect that adding further taxa and genes to systematic study will significantly to a better understanding of the evolutionary history of Polycladida.

Smed-egfr-1 controls planarian gut regeneration and homeostasis by regulating neoblast differentiation

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Abstract:
The activation and regulation of differentiation programs in stem cells is a fundamental process during animal development and regeneration, required for proper tissue and organ formation and maintenance. Freshwater planarians are an excellent model to study the behavior of stem cells *in vivo* as they possess a population of pluripotent stem cells called neoblasts. Neoblast are a heterogeneous population containing many different lineage-specific progenitors, identified by the expression of particular transcription factors. However, little is known about how these lineage-committed neoblasts differentiate into mature cell types.
The EGFR signaling pathway has been shown to play an important role during key steps of cell differentiation and organogenesis in all studied model systems. Previous studies have showed that, in planarians, the receptors *Smed-egfr-3* and*Smed-egfr-5* are required for proper blastema and excretory system differentiation, respectively; also, *Smed-egfr-1* is required for pharynx and eye pigment cells regeneration and maintenance. Here we show that *egfr-1*, which is expressed in the digestive system, is additionally essential for correct gut regeneration and homeostasis, as *Smed-egfr-1(RNAi)* animals fail to regenerate new gut branches and dramatically lose the existing ones during homeostasis. Moreover, their gut has a very reduced lumen, aberrant tissue organization, and significantly less gastrodermal cells. Importantly, the loss of gut cells in *Smed-egfr-1(RNAi)* animals is not due to an increase in apoptotic levels in the gastrodermis, suggesting that the gut-associated phenotype is likely caused by defects in gut cell differentiation. In fact, double labeling indicates that *Smed-egfr-1* is co-expressed with the gut progenitor markers *gata4/5/6*,*hnf4* and *nkx2.2* in the mesenchyme around the gut. After silencing *Smed-egfr-1* the number of *hnf4*-positive cells increases in the mesenchyme, suggesting that the defects observed in the regeneration and maintenance of the gut could be caused by the failure of those progenitors to differentiate into mature gut cells. Therefore, the EGFR pathway would have a key role regulating the differentiation of gut cells from their specialized progenitors.

Neoblasts support "trained immunity" in the planarians

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We recently demonstrated that the planarians have an extraordinary capacity to fight a wide spectrum of pathogens that in humans cause life-threatening illnesses: pneumonia, sepsis, brucellosis, salmonellosis, meningitis, and tuberculosis, and which cause death of Drosophila and Caenorhabditis. Our findings have underscored the importance of studying the planarian immune defences in aim of identify evolutionary conserved innate immunity mechanisms and immune factors, in order to tackle the intriguing question of resistance of the host to bacterial infection. We have proposed the planarians as new invertebrate models to investigate conserved innate immune mechanisms (Cell Host and Microbe 2014). Investigating the immunity of the planarians via genetic invalidations and irradiation experiments, we have unravelled that the antibacterial response against *Staphylococcus aureus*, agent responsible for nosocomial diseases in the planarians, is not supported by intestinal phagocytes, but it is supported by stem cells. Of note in mammalian, several reports have suggested that stem cells can play an important role in anti-bacterial immunity. Stem cells have the capacity to express Toll like receptors, to secrete cytokines and to produce antimicrobial peptide such as the cathelicidin LL-37, however the immune properties of stem cells remain poorly investigated. A deep analysis has revealed the existence of a specific “trained immunity” in the planarians supported by a functionally distinct class of neoblasts, and that this mechanism requires an epigenetic programming via a specific histone methylase.

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The role of cathepsin proteases in the free-living flatworm *Schmidtea mediterranea*

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Proteases perform numerous vital functions in flatworms. Many of these proteases are conserved throughout much of the phylum*Platyhelminthes*, which contains over 30,000 different species. However, this phylum is relatively unexplored despite including many parasitic species relevant to human health. The flatworm *Schmidtea mediterranea*, or planaria*,* is a good organism for study due to its conservation of key proteases with parasitic flatworms and its amenability to techniques like RNA interference (RNAi). A survey of various planaria proteases in the *S. mediterranea* genome revealed several cysteine, serine, and metallo-proteases. Analyzing whole worm lysates revealed the major proteolytic activity corresponds to a cathepsin B-like protease. Immunohistochemistry and whole-mount *in situ* hybridization (WISH) shows that the full-length and active forms of cathepsin B are found in secretory cells surrounding the planaria intestinal lumen. This suggests that active cathepsin B is gut-associated and most likely performs a digestive function. We have also used RNAi knockdown and inhibitors of cysteine cathepsin proteases to probe the function of this protein. These studies suggest that not only is cathepsin B involved in digestion, but it may also be implicated in regeneration along with other cathepsin L-like proteases.

Germ cell specification from somatic stem cells in planarians

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Most knowledge of germ cell specification comes from model organisms (e.g., *Drosophila* and *C. elegans*) in which maternal determinants specify germ cells early in embryogenesis. However, in mice, germ cells are specified by BMP and Wnt signal-mediated induction later in development. Despite its obvious significance to human reproductive biology, much less is known about inductive specification.
Planarians possess traits that make them an attractive model for understanding germ cell biology. Similar to mammals, planarians undergo inductive germ cell specification during development. Unlike mammals, planarians can also regenerate germ cells *de novo* from pluripotent stem cells (neoblasts) in amputated fragments devoid of germ cells. Here, we use the planarian *Schmidtea mediterranea* to study fundamental questions pertaining to germ cell induction and development.
First, we used a candidate gene approach to examine the expression patterns and functions of conserved BMP and Wnt pathway members in sexual planarians. By *in situ* hybridization, we found that several factors were expressed within the planarian germline. Knockdown of individual BMP and Wnt pathway components by RNA interference (RNAi) resulted in germline defects, suggesting a role for these pathways in germ cell specification or development.
To identify novel factors important for inductive germ cell specification, we used next-generation RNA sequencing to generate a genome-wide expression profile at distinct time points throughout germ cell specification and development. Using this unbiased approach, we found thousands of genes differentially regulated during early hatchling development (when primordial germ cells are induced). Since we expected genes involved in germ cell specification to be upregulated as new germ cells are specified, we focused subsequent screening efforts on a set of ~1400 genes with increasing expression over time. *In situ* hybridization experiments uncovered many genes expressed in germ cells or in restricted cell populations nearby. In an ongoing RNAi screen, disruption of several genes has resulted in various germline defects indicating potential roles in germ cell specification and development.
By capitalizing on the unique biology of planarians to regenerate and respecify germ cells inductively, these studies will provide a comprehensive view of conserved mechanisms underlying inductive germ cell specification.

Heads or tails: investigating gene regulatory networks controlling strobilation in the model tapeworm Hymenolepis microstoma

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Tapeworms are a medically and economically important group of flatworms for which, like most parasitic animals, we remain ignorant of their basic developmental pathways. By contrast, an increasingly sophisticated and instructional model of developmental gene regulation has formed over the last decade from studies of free-living planarians. Gene regulatory networks (GRN) controlling antero-posterior patterning during planarian growth are centred on Wnt signalling and linked with the polarity gene Hedgehog. Wnt signalling is found to be regulated by Hedgehog signals, while downstream Wnt targets include Hox and other genes. In segmented animals, Notch signalling is key to segment formation, producing waves of expression driven by an oscillation clock and is also implicated in polarity during early development. We hypothesise that these three pathways will also be significant in tapeworm patterning, polarity and strobilation, and RNAseq data show that components of each are expressed throughout the life cycle. We examine quantitative and spatial expression of Wnt, Hedgehog and Notch signalling components throughout the complex lifecycle of Hymenolepis microstoma. In addition, we present on-going functional investigations of Wnt inhibition during larval development via injection of small molecule inhibitors into the haemocoel of the beetle intermediate host. Initial results of these studies indicate that tapeworm patterning, although highly modified, is controlled by the same underlying GRNs found in planarians and that they are redeployed during strobilar, adult growth.

Adhesive organ regeneration in *Macrostomum lignano*

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Understanding the mechanisms in regenerating tissues can incredibly increase our knowledge of stem cell regulatory mechanisms and the differentiation of somatic stem cells. One problem to overcome is the limited number of valuable model systems for post-embryonic organogenesis. The adhesive organs of *Macrostomum lignano* are a useful model system for*de novo* organ regeneration. The marine flatworm exhibits approximately 130 adhesive organs, to attach and stabilize itself to the substrate. In a previous study we described the morphology of these organs on ultra-structural level. One adhesive organ consists of solely three interacting cells, two secretory cells and one modified epidermal cell. When amputated the organs regenerate completely within 9 days. Using cell type specific lectin- and antibody staining, as well as transmission electron microscopy, we analyzed the morphology of the adhesive organs during different regeneration stages. We have generated a blastema-specific RNAseq data at different stages of tail regeneration. This data is currently used to identify and characterize transcripts involved in the regeneration of the adhesive system. Based on our knowledge of the morphology and the available molecular biology tools we aim to establish the adhesive organs of *Macrostomum lignano* as a new model system for post-embryonic organogenesis.

Supported by Austrian Science Fund: [P 25404-B25]. BL is a recipient of a DOC Fellowship of the Austrian Academy of Sciences.

Illuminating the landscape of neural regeneration: Evidence for multi-modal, innate light sensing and sensory processing in Planaria

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Light sensing and response is fundamental to life. However, a comprehensive and quantitative understanding of light sensing systems, widespread in nature, is very limited. Planarians are known to have a true cerebral eye linked to a well-defined brain, believed to be one of the early examples of a functional brain structure that integrates different sensory modalities. The Planarians have a cup shaped eye, with a pigment cup and photoreceptors, and is believed to be capable of sensing presence and direction of light. Using quantitative light sensing and phototaxis assays, we have examined light sensing in Planarians in greater detail. Remarkably, we found that Planarian light sensing is much more complex that previously documented, with Planarians able to resolve subtle differences in light input. Our results dramatically alter the conventional view of 'rudimentary eyes' and offer new insights into how organisms can potentially carry out complex functions with relatively simple optical systems. Since Planarians show whole-body regeneration, we have used eye and brain regeneration to demonstrate clear hierarchies in light responses, including eye-based and whole body photoreception. On the flip side, our light sensing assays offer a unique way to map the trajectories of regeneration in new ways, including temporally segregating 'processive' functions of the brain. Results from a highly collaborative, comprehensive approach to light sensing, regeneration and neural function would be discussed.

Xenacoelomorpha are basal bilaterians: evidence from phylogenomics

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Resolving the phylogenetic position of Acoela, Nemertodermatida and Xenoturbellida has important consequences for understanding early metazoan evolution. Separately and together, these animals have been hypothesized as Platyhelminthes, as basal metazoans, briefly as molluscs in the case of *Xenoturbella*, and more recently, as a monophyletic clade within deuterostomes. Phylogenomics has potential to resolve this critical open question. However, representation in phylogenomic datasets to date for *Xenoturbella* and acoelomorphs has been poor, in terms of both taxon sampling and percentage of missing data. We have generated novel Illumina transcriptome sequence data from *Xenoturbella bocki*, six acoels and four nemertodermatids. Whereas previous studies have included transcriptomic data from only higher Acoela (Convolutidae and Isodiametridae), we have sequenced two species of Diopisthoporidae, which is the sister group to all other acoels. We have additionally sequenced novel transcriptome data from 9 diverse metazoans, including Platyhelminthes and Gastrotricha. Taken in combination with publically available data, our primary data matrix contains 78 broadly sampled taxa, 212 orthologous groups, and 44,896 amino acid positions. We have additionally conducted a series of signal dissection and taxon pruning experiments to identify potential sources of conflicting phylogenetic signal. Xenacoelomorpha form a monophyletic clade at the base of bilateria in all our phylogenetic analyses, including both maximum likelihood and Bayesian analyses using the site-heterogeneous CAT+GTR model. Our results show no support for deuterostome affiliation of Xenoturbellida or Acoelomorpha. Implications for the initial acquisition of key bilaterian characters, as well as the outgroup to Platyhelminthes will be discussed.

The genome and transcriptome of the regeneration-competent flatworm, *Macrostomum lignano*

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The free-living flatworm, *Macrostomum lignano*, much like its better known planarian relative, *Schmidtea mediterranea*, has an impressive regenerative capacity. Following injury, this species has the ability to regenerate almost an entirely new organism. This is attributable to the presence of an abundant somatic stem cell population, the neoblasts. These cells are also essential for the ongoing maintenance of most tissues, as their loss leads to irreversible degeneration of the animal. This set of unique properties makes a subset of flatworms attractive organisms for studying the evolution of pathways involved in tissue self-renewal, cell fate specification, and regeneration. The use of these organisms as models, however, is hampered by the lack of a well-assembled and annotated genome sequences, fundamental to modern genetic and molecular studies. Here we report the genomic sequence of *Macrostomum lignano* and an accompanying characterization of its transcriptome. The genome structure of *M. lignano* is remarkably complex, with ~75% of its sequence being comprised of simple repeats and transposon sequences. This has made high quality assembly from Illumina reads alone impossible (N50=222 bp). We therefore generated 130X coverage by long sequencing reads from the PacBio platform to create a substantially improved assembly with an N50 of 64 Kbp. We complemented the reference genome with an assembled and annotated transcriptome, and used both of these datasets in combination to probe gene expression patterns during regeneration, examining pathways important to stem cell function. As a whole, our data will provide a crucial resource for the community for the study not only of invertebrate evolution and phylogeny but also of regeneration and somatic pluripotency.

Selected Planarian meeting abstracts for talks in order of presentation.

Preliminary data from establishing a X-ray shielded assay to reveal stem cell control mechanisms in planarians

Prasad Abnave, Ellen Aboukhatwa, Nobuyoshi Kosaka, Holy Sadler and **Aziz Aboobaker**

We have adapted the classical assay of using lead shielding to allow us to ablate neoblasts with X-rays, leaving a stripe of stem cells at chosen areas along the AP axis. Our method limits the dose of X-rays to shielded stem cells and allows us to irradiate reasonably large numbers of planarians simultaneously to allow the assay to be conveniently combined with RNAi screens of gene function.
Asking animals to regenerate from just a small stripe of stem cells is clearly a greater challenge then widely used regenerative paradigms, such as amputation into three parts. In our assay for example we have detected a role for genes in orchestrating stem cell behavior that we do not see in normal regenerative paradigms. Having established the utility of this assay we are now in the process of both candidate and expression driven RNAi screens of gene function and will present the very early data from this approach.

The necessity of ROS signalling for successful differentiation and patterning during planarian regeneration

Nicky Pirotte, An-Sofie Stevens, Susanna Fraguas, Michelle Plusquin, Andromeda Van Roten, Frank Van Belleghem, Rik Paesen, Marcel Ameloot, Francesc Cebrià, Tom Artois, and Karen Smeets.

To increase the medical application of regenerative research, it is crucial to understand all aspects of stem cell control and regeneration, including the signalling factors of the cellular environment. The importance of ROS (reactive oxygen species) as signalling molecules to induce proper tail regeneration was already addressed in *Xenopus* species and zebrafish. However, the necessity of redox signalling for anterior regeneration, and specifically regeneration of the brain, was yet to be identified. We used the planarian *Schmidtea mediterranea*, an organism with unlimited regenerative capacities, to study the cellular processes that are coordinated by ROS signalling to achieve proper regeneration. Not only did we observe the same oxidative burst at the wound site as did Love et al. (2013) and Gauron et al. (2013), we also noticed differences in the intensity of the ROS production as well as differences in vulnerability to an inhibition of this ROS burst between the amputated head, trunk and tail fragments. Diminished ROS levels caused reduced blastemas, together with smaller cephalic ganglia as well as the ectopic formation of specific neuronal cells. Although no effect of the ROS production inhibition on stem cell proliferation was observed, we did find that ROS signalling is necessary to induce early differentiation of the stem cell progeny. In addition, differences in the expression of polarity determinants show that diminished ROS levels also affect proper patterning and polarization.

SMED-PABPC2 is essential for maintenance of epidermal integrity and second mitotic peak activation during planarian regeneration

Dhiru Bansal

Cytoplasmic poly(A) binding proteins (PABPCs) have emerged as key regulators of translation initiation in eukaryotes. Recent studies in metazoans have shown multifunctional roles for PABPC-mRNA turnover, nonsense mediated decay and miRNA mediated repression. PABPCs have also been implicated in embryonic development and other cellular processes such as cell adhesion and cell migration. In recent years, planarians have become a powerful model system to study regeneration and stem cell function. In the current study, we have identified a novel Poly adenylate binding protein (cytoplasmic), SMED-PABPC2, in Schmidtea mediterranea.

The knockdown of *Smed-pabpc2* resulted in the failure of neoblast differentiation, and subsequent lysis of regenerating animals. We also observed defects in the activation of second mitotic peak near the wound region, which may have possibly led to the failure in neoblast differentiation. We found smed-pabpc2 knockdown animals showed loss of epidermal cells and disorganization of extracellular matrix. Interestingly, we didn't find gross change in subepidermal muscle cells but expression of PCGs like slit and sFRP-1 were affected. This suggests that the epidermal integrity could possibly be important for signalling neoblasts for differentiation, which is disrupted upon Smed-pabpc2 knockdown. Thus, our study highlights the novel role of poly (A) binding protein in planarian regeneration and homeostasis.

Epigenetic control of planarian stem cell potency limits stem activity and accurately defines differentiation programs

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

Little is currently known about how epigenetic mechanisms direct planarians stem cell fate. In order to understand if and how these are important we have looked at the role of Trithorax-related proteins known to tri-methylate histone H3 lysine residue 4 (H3K4me3) on selected gene promoters (Sedkov et al., 2003). They have also been implicated in mono-methylating H3K4 on active enhancers (Herz et al., 2012), as well as on promoters of transcriptionally repressed genes (Cheng et al., 2014). The orthologs of Trithorax-related genes (mammalian *MLL3* and *MLL4*) are frequently mutated in a plethora of cancerous tumours suggesting that mis-regulation of epigenetic events normally controlled by these proteins may underpin the formation of some cancers and cancer stem cells (Jones et al., 2012, Morin et al., 2011).

Schmidtea mediterranea's Trithorax-related complex has at its core two proteins – Trithorax-related (*Trr*) and Lost PHD domains of Trithorax-related (*LPT*). These correspond to the mammalian C- and N-terminus parts of a single protein (*MLL3*) respectively, and as in other metazoans, this protein is split into separate genes in planarians. Upon RNAi mediated knockdown, we observe defects in differentiation in multiple lineages characterised by a failure to form some differentiated cell types and both reductions and increases in some progeny cell types. Of specific interest is that in LPT knockdown particularly, we also observe stem cell over-proliferation and increased stem cell migration, together often resulting in the formation of outgrowths that lead to death of the animals. These defects correlate with a measurable global decrease in H3K4me1 in X1 sorted stem cells.

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Beta-catenin specifies posterior identity through a protein gradient and it is required for anterior patterning in planarians

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NB Planarian meeting talk.

Re-establishment of the axial identities is a main challenge during regeneration. Whole-body regenerating animals, as planarians, are an ideal system to study the molecular signals underlying axial re-specification. The canonical or beta-catenin-dependent Wnt signaling is an evolutionary conserved mechanism to specify posterior identity during embryogenesis. Through RNAi silencing we and others have demonstrated that in adult planarians beta-catenin is necessary for posterior specification, both during regeneration and during its normal homeostasis. We hypothesized a model in which a gradient of beta-catenin activity underlies planarian antero-posterior identities. To test this hypothesis we have generated an antibody against planarian beta-catenin1 protein (BCAT1) to analyze its subcellular expression along planarians AP axis. Our results show that it exist a parenchymatic gradient of nuclear BCAT1 along the animal, from the pharynx to the tip of the tail. During regeneration, BCAT1 is highly activated in every blastema, but it is higher expressed in posterior ones. Moreover, we demonstrate that planarian Wnt1 is the responsible of the accumulation of BCAT1 in posterior blastemas. Finally we show that BCAT1 is also expressed in specific organs and tissues, as the testis, the pharynx and the brain, and that it exerts an essential function during anterior regeneration, especially for brain patterning. In conclusion, we demonstrate the existence of a gradient of nuclear BCAT1 protein which drives posterior specification and we show a novel function of BCAT1 during anterior regeneration in planarians.

Tryptophan enhances the reproductive organs-specific expression level of an amino acid transporter homolog, Dr-SLC38A9 to promote sexual induction of the planarian *Dugesia ryukyuensis*.

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Certain planarians switch their reproductive modes, and can reproduce asexually or sexually, depending on environmental conditions and/or the phase of life cycle. The mechanism underlying the switch of both reproductive modes is a central issue in reproductive biology. We previously established an assay system for sexual induction by feeding asexual planarians with hermaphrodite sexual planarians containing sex-inducing substances in the planarian *Dugesia ryukyuensis*. Recently, we have isolated tryptophan (Trp) as one of sex-inducing substances. However, it is unclear how tryptophan regulates sexual induction process. In this study, we report that a neutral amino acid transporter homolog Dr-SLC38A9 is transcribed by Trp in a reproductive organs-specific manner and required for sexual induction. In situ hybridization analyses revealed that Dr-SLC38A9 was expressed in ovaries, testes and yolk glands. Interestingly, the expression in yolk glands is highly enhanced by Trp. We have found that Trp accumulates in yolk glands of sexual planarians. Since a deduced protein encoding by Dr-SLC38A9 may transport Trp, it is possible that Trp promotes its accumulation via the induction of Dr-SLC38A9 expression. Additionally, Dr-SLC38A9 knockdown during sexual induction inhibited the development of ovaries, testes and somatic reproductive organs. This result suggested that Dr-SLC38A9 is required for development of these reproductive organs. Above all, we propose that Trp activates Dr-SLC38A9 expression to enhance their own activity for sexual induction of D. ryukyuensis

Controling Regeneration Speed

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Regeneration is a natural process that has fascinated humans forever. The limitless regenerative capacity of planarians has been systematically studied since the nineteenth century, and a huge progress has been made in the last decades in the understanding of the cellular and molecular features which enable such capability. The pluripotency of planarian adult stem cells, neoblast, has emerged as the essential trait underlying planarians plasticity. Moreover, several “regeneration activators”, required for neoblast maintenance and/or proliferation, have been reported to date, which’s silencing produce a delay or impairment of planarian regeneration. The present study reports the identification of two independent genes that after knock down do not inhibit but accelerate planarian regeneration.
Smed-Blitzschnell (*Smed-BS*) is a small secreted peptide expressed in a subset of secretory cells located in the pre-pharyngeal and along the lateral dorso/ventral border. It appears to be specific of planarians since no homologous has been found in any database. *Smed-klf10/11* is the planarian homologous to the vertebrate klf10/11, a member of the highly conserved krüppel-like family of transcription factors. It is expressed in neoblast. The RNAi analysis of both genes revealed that at early regeneration stages silenced animals show a more advanced regenerative stage than controls regarding to brain, eyes and sensory cells formation. Remarkably, at later regeneration stages neither of them showed morphological defects. The analysis of the mitotic dynamics during regeneration shows an abnormal profile in both cases. However, Smed-BS RNAi produces a general increase of proliferation while Smed-klf10/11 RNAi generates an accelerated dynamics.
Our results demonstrate that the rapid and perfect regenerative response of planarians do not only require “regeneration activators” that induce neoblast proliferation but also “regeneration inhibitors” to modulate the mitotic response. Although further analyses are being performed, our present data suggests that *Smed-BS* and *Smed-Klf10/11* modulate the regenerative response through different pathways, highlighting the physiological importance of this mechanism.

“Patterning pathways in planarian regeneration”

Jochen Rink

5-hydroxytryptophan induces ovaries, and knockdown of tryptophan hydroxylase homolog inhibits sexual induction in the asexual worms of Dugesia ryukyuensis.

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To elucidate the mechanisms underlying action, we developed an assay system for sexual induction in the planarian *Dugesia ryukyuensis*. Under this assay system, the asexual worms differentiated hermaphroditic reproductive organs when fed with sexual worms of the same as well as the different species, indicating that the sexual worms contain sex-inducing substances of poor species-specificity. Recently, we have found that sex-inducing substances were contained in yolk glands, a planarian specific reproductive organ. We have also found that sexual worms can pool free tryptophan (Trp) in large amounts in yolk glands. By excessive administration of Trp under the feeding assay system, asexual worms developed a pair of ovaries and sexual worms induced supernumerary ovary pairs. This result indicates that Trp is an ovary-inducing substance.

In the present study, we carried out metabolic analysis in terms of tryptophan metabolites in yolk g asexual worms. We also found that feeding 5-HTP and Trp in combination had an additive effect of ovarian induction. It is likely that the pathways in the ovarian induction by 5-HTP and Trp are different. We will also discuss this possibility in our other presentation by Maezawa et al.

Furthermore, we isolated a tryptophan hydroxylase homolog in D. ryukyuensis, Dr-TPH. Knockdown of Dr-TPH inhibited the sexual induction in asexual worms and induced supernumerary ovary pairs in sexual worms. On the basis of an assumption that Dr-TPH acts as tryptophan hydroxylase, the results of the knockdown of Dr-TPH will be discussed.

Posters for the ISFB conference (note where poster abstract is being presented in the Planarian meeting, just the title appears below.

Poster 1

Optimization and Validation of *In vivo* Indirect Measurement of Phase I and II Enzyme Activities in the Freshwater Planarians, *Dugesia japonica*

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Different cytochrome P450 as well as other drug-metabolizing enzyme activities can be induced or inhibited by various classes of environmental pollutants and commonly used as a biomarker for exposure in wild organisms or experimental animals. Ecotoxicological studies grow interests on uses of freshwater planarians as test organisms to examine effects of various environmental toxicants. However, biochemical measurement of planarian cytochrome P450 activities had not been reported in published literature despite planarian popularity in recent toxicological studies. In order to develop planarians as an integrated toxicity bioassay, it will be desirable to measure cytochrome P450 enzyme activities in freshwater planarians to investigate different effects of environmental pollutants in aquatic animals. In this study, *in vivo* measurements of cytochrome P450 enzyme activities were firstly conducted to determine if the presence of O-deethylation activities with alkoxyresorufin or alkoxycoumarin in *Dugesia japonica*. Secondly, *in vivo* measurements of DT-diaphorase (DTD) andUDP-glucuronosyltransferase (UDPGT) enzyme activities were also determined in *Dugesia japonica* by using resourfin and 4-methylumbelliferone (4-MU) as substrates, respectively. 7-ethoxycoumarin deethylase (ECOD) activity and 7-methoxycoumarin dethylase (MCOD) can be directly measured by increasing the fluorescent intensity of reaction product from planarian culture media. On the other hand, the reaction product of 7-ethoxyresorufin O-deethylase (EROD), 7-pentoxyresorufin O-deethylase (PROD), or benzyloxyresorufin O-deethylase (BROD) activity was not detectable by measuring fluorescent intensity of reaction product from planarian culture media. In addition, DTD and 4-MU UDPGT enzyme activities were able to directly measure by disappearances of fluorescent intensity of substrates from planarian culture media. Based on the results of this study, *in vivo* measurements of Phase I and II enzyme activities in *Dugesia japonica* demonstrates its attractive features as potential test species in ecotoxicology.

Poster 2

The *Schistosoma mansoni* protein SmShb interacts with and regulates SmVKR1 signalling

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Venus Kinase Receptors (VKRs) are invertebrate Receptor Tyrosine Kinase (RTKs) formed by an extracellular Venus Fly Trap (VFT) ligand binding domain associated *via* a transmembrane domain with an intracellular tyrosine kinase domain. These structurally atypical receptors were initially discovered in the parasitic flatworm *Schistosoma mansoni*, then identified in many invertebrates (Ahier et al., 2009 ; Vanderstraete et al., 2013).

Quantitative RT-PCR on various stages and isolated organs of the sea urchin and various insects (Ahier *et al.,* 2009), together with recent studies in the parasite *S. mansoni* (Vanderstraete *et al.,* 2014), argued for a role of VKRs in embryonic development and in reproduction.

To better understand the cellular functions of VKRs, *S. mansoni* interacting partners of SmVKRs were identified by yeast-two-hybrid (Y2H) screening and SmVKR signalling pathways were characterized in *Xenopus* oocytes (Vanderstraete *et al.,* 2014).

The protein SmShb, an SH2 domain-containing protein, homologous to members of the Shb adaptor family known to act as a platform to transduce signals induced by activated RTKs, was identified during SmVKR1 Y2H screening. SmShb was shown to interact specifically with the phosphorylated form of SmVKR1. This interaction occurs between the SH2 domain of SmShb and the phosphotyrosine residue (pY979) located in the juxtamembrane region of SmVKR1. In *Xenopus* oocyte, this SmVKR1/SmShb interaction activates specifically the JNK signalling pathway. *SmShb* and *SmVKR1* transcripts were both detected by *in situ* hybridization in mature oocytes and testes of adult schistosomes. Although a clear phenotype in the ovary was not detected, RNA interference experiments in adult *S. mansoni* exhibited an accumulation of mature sperm in testes following SmShb knockdown.

To further analyze the potential importance of SmShb/SmVKR1 signalling in germinal cells, we focused on the determination of SmShb interacting partners by Y2H screening of a *S. mansoni* adult cDNA library using SmShb as a bait. Among the various potential SmShb partners, we identified two homologs of RhoU and TcTex-1, already known for their respective functions in cell migration and sperm motility.

All together, these data suggest a role of SmVKR1/SmShb pathway in maturation and migration of germinal cells.

Poster 3

Reproductive, behavioural, and ecological peculiarities of cave-dwelling planarians from Sardinia (Platyhelminthes, Tricladida) *

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Reproductive, behavioural and ecological traits of sexual and asexual populations of cave-dwelling planarians are reported from Sardinia. (A) An asexual strain of presumed Planariidae never sexualized, neither underwent spontaneous fission during long-term rearing under laboratory conditions. Unexpectedly, after two years two bipolar planarians spontaneously developed in the strain culture. Due to a paratomic process with reversed-polarity a new planarian originated from a caudal swelling of the parent worm. This peculiar heteromorphosis was the only spontaneous reproductive process observed in this strain. Experimental decapitation was the only single stimulus inducing asexual reproduction, which occurred through a very long process, involving encystment of the posterior fragment in a mucous capsule. Repeated fissions of the fragment occurred within the cyst. (B) A peculiar life style characterizes other freshwater planarians from a cave microhabitat with intermittent dripping water. Sexual anophtalmous specimens were collected during the wet season from under stones on a humid clayey subsoil. Since the animals were found in a state of strong contraction or rolled up, this may signal a capacity for burrowing and/or for modifying their body dimensions, which may have enabled them to find relatively humid refuges and thus survive as freshwater planarians in a basically terrestrial habitat. (C) As far as autochthonous land planarians are concerned, the only record from Sardinia until now concerns specimens collected from a cave in the North-West of the island. However, recent intensive samplings led to several new records of terrestrial planarians, most of which were found in caves. In some cases their presence in caves could be considered accidental, due to a probably passive transport by water flooding through limestone cracks from top ground layers. Interestingly, a persistent population of hyaline land planarians was collected very recently from a cave in South-West Sardinia.

*Research funded by RAS2012-CRP60215 “Conservazione e valorizzazione delle grotte sarde: biodiversità e ruolo socio-economico-culturale”, SYNTHESYS, the European Union-funded Integrated Activities grant (project grant: NL-TAF 3497), Master&Back (RAS), Fondazione Banco di Sardegna, and MIUR-PRIN.

Poster 4

Transcriptome-wide elucidation of RNA binding protein target sites in the *in vivo* stem cell system *Schmidtea mediterranea.*

Vera Zywitza, Marta Rodriguez-Orejuela, Salah Ayoub, Nikolaus Rajewsky and Jordi Solana.

RNA binding proteins (RBPs) are key regulators in post-transcriptional regulation (Gerstberger et al. 2014). Interestingly, all steps of RNA processing from transcription to translation or degradation have been shown to be crucial for pluripotency (Ye and Blelloch, 2014). However, insights into post-transcriptional regulation of stem cells are still scarce and were mainly obtained by studying cell culture of embryonic stem cells. To understand the function of RBPs in stem cells in their physiological environment we aim to transcriptome-wide identify and characterize RBP target genes as well as the site of binding at nucleotide resolution (Hafner et al. 2010). Thus, we are establishing *in vivo* photoactivatable- ribonucleoside- enhanced crosslinking and immunoprecipitation (iPAR-CLIP) (Jungkamp et al. 2011) in the planarian *in vivo* stem cell system *Schmidtea mediterranea.* As a proof of principle we focus on the CELF factor *Smed-bruno-like* (*bruli*), which is one of the most highly expressed RBPs in planarian stem cells (called neoblasts) (Guo et al. 2006, Onal et al. 2012). Members of the CELF/bruno-like family are highly conserved, and are known to be involved in multiple post-transcriptional processes (Dasgupta and Ladd, 2012). Hence, iPAR-CLIP of *bruli* will not only provide a basis for further iPAR-CLIP experiments but also will help to gain insights into general stem cell biology.

Poster 5

Updated inventory and distribution of Turbellarian flatworms in Tunisia

Mehrez GAMMOUDI & **Saïda TEKAYA**

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Abstract

In Tunisia, the knowledge of flatworms fauna is poorly documented compared to invertebrates with economical importance (Molluscs, Echinoderms, Annelids..). Almost all informations dealing with zoology and biology of the tunisian free living platyhelminthes are obtained from the results of our team researches. Therefore, this presentation is mainly a synthesis of our findings including some data reported in literature and a total of 29 species are signalized.

During the two last decades, our prospections of various tunisian waters localities, have allowed us to collect, describe and identify marine and fresh-water species belonging to three Free-living Platyhelminthes orders : Polycladida,Tricladida and Proseriata.

Polycladida are subdivided into Cotylea (with a sucker behind the female genital pore) and Acotylea (without sucker). 6 acotyleans and 7 cotyleans are found in marine stations located essentially in the Northeast coasts.

Tricladida are represented by 14 species : We have collected 5 marine and 2 freshwater planarians and based on bibliographic data, 7 other fresh-water triclads are added (Stocchino et al, 2009 ; Harrath et al 2011 et 2012)

2 Proseriates are found among the interstitial fauna in marine coasts.

Ecological data and illustrated description of each species are given. A map showing the distribution of all these species is established.

Key words : Tunisian fauna, free living platyhelminthes, Polyclads, Triclads, Proseriates

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Poster 6

Female gonad structure and oogenesis in *Echinoplana celerrima*Haswell, 1907 (Platyhelminthes, Polycladida)

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Abstract

The transition from the Archoophora to the Neoophora level of organization is considered an important step in the evolutionary history of Platyhelminthes "Turbellaria" (Gremigni, 1997). The distinction between the two evolution levels is based on the female gonad structure. Because of the importance of this transition, several studies have focused on the ultrastructural characteristics of the oocytes and vitellocytes in many flatworms, particularly Tricladida species (Falleni et al., 1995; Tekaya et al., 1999). However, our knowledge about ovary organization in Polyclads is still rudimentary. The present study aims to investigate for the first time the female gonad of the marine Polyclad *Echinoplana celerrima* Haswell, 1907 using both light and electron microscopy. We found that in mature specimens, the female gonad is represented by numerous ovaries scattered in the dorsal parenchyma. Each ovary shows a germinative zone and a growing zone. The former, located dorsally, includes undifferentiated young germ cells (oogonia). The growing zone occupying the ventral part, is composed of growing oocytes. Early previtellogenic oocytes are provided with cytoplasmic translucent vesicles and some eggshells granules. During maturation, their cytoplasm undergoes a notable increase in volume due to the production and accumulation of both yolk and shell globules, corresponding to the Archoophora level of the female gonad organization. Late previtellogenic oocytes reach up to 200 µm in diameter. Their cytoplasm contains a large number ofeggshells granules mainly at the cortical zone and yolk globules dispersed throughout the whole ooplasm.

***key words*:** Archoophora, Polycladida, Ultrastructure , Oogenesis,*Echinoplana celerrima*

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Poster 7

MiRNA in the early intra-mammalian stages of of Schistosoma mansoni.

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Schistosomes are parasitic helminths and the causative agent of schistosomiasis, also called bilharzia, a disease affecting circa 200 million people in Africa, south East Asia and south America – some of the most underprivileged regions of the world. Schistosoma mansoni is the preferred species kept in the laboratory due to its ease of propagation both in the snails and the rodent models. It's genome, transcriptome and at a certain extent the proteome of the intra-mammalian stages of these parasites have been studied. However, the role of non-coding RNAs in the development of this parasite has been left unknown.

Non-coding RNAs (ncRNAs) include all RNA species that are not translated into a protein product. MiRNAs are ~21nt long RNA molecules specially processed to undertake key roles in regulating the availability of messenger RNAs (mRNAs). A number of them have been described in schistosomes, yet there is nothing known about their function or potential targets.
We used strand-specific RNAseq and microRNA-seq libraries from several time points of mechanically transformed schistosomula (0, 3, 6, 12, 24, 48 and 72 hours old larvae) to profile miRNAs in the invading larvae. Using both in silico and experimental methods we found novel and possibly stage specific miRNAs including a three conserved yet not previously identified miRNA species.

Poster 8

DNA barcoding of flatworms: new primers targeting digeneans and cestodes (Platyhelminthes)

Niels Van Steenkiste, Sean A. Locke, Magalie Castelin, David J. Marcogliese, Cathryn Abbott

Flatworms are among the most species-rich metazoans and digeneans and cestodes can seriously impact human health, fisheries, aqua- and agriculture, and wildlife conservation and management. DNA barcoding using the COI Folmer region could be applied for species detection and identification, but both ‘universal’ and taxon-specific COI primers fail to amplify in many flatworm taxa. We found that high levels of nucleotide variation at priming sites made it unrealistic to design primers targeting all flatworms. Monogeneans and ‘turbellarians’ were therefore excluded and digeneans and cestodes became our target taxa. New degenerate COI primers were designed to amplify across these two groups and the performance of these primers was tested on 46 specimens representing 23 families of digeneans and 6 orders of cestodes. COI sequences were obtained for all specimens. We hope that the primers and methods provided here will help to redress the current paucity of COI barcodes for these taxa in public databases. We conclude with potential promises and some future challenges of DNA barcoding in flatworms.

Poster 9

Phylogenetic relationships within the genus *Imbira* Carbayo (Tricladida: Continenticola) with the proposition of a new species

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The Geoplaninae genus *Imbira* Carbayo was proposed based on molecular analyses, being constituted by two species, *Imbira marcusi* Carbayo et al., 2013 and *Imbira guaiana* (Leal-Zanchet & Carbayo, 2001). These species occur in areas of mixed ombrophilous forest and dense ombrophilous forest from south Brazil. Specimens of *Imbira* have slender body with parallel margins, large sized-body (90 – 140 mm in lenght), monolobulated eyes, mesenchymal muscle layers of longitudinal bundles, long and branched prostatic vesicle, folded male atrium, eversible penis papilla, and female atrium presenting a lining with multilayered aspect. In the present study, we analyzed phylogenetic relationships within the genus, based on morphological characters, including an undetermined species (*Imbira* sp.) from an area of dense ombrophilous forest in southern Brazil. The cladistic analysis was done based on 55 morphological characters from a bibliographical revision, besides 46 characters selected from analyses of the internal morphology. We studied 32 terminal taxons of Neotropical land flatworms from 17 genera. The character matrix was constructed using MESQUITE Projects and Files, Version 1.12 and the phylogenetic tree was calculated by means of heuristic analyses with 1,000 replications using NONA. The strict consensus showed a robust clade (95%) constituted by *I. marcusi*, *I. guaiana* and *Imbira* sp., supported by the occurrence of longitudinal bundles of the mesenchymal musculature around intestine. *Imbira* sp. differs from other species of the genus by a combination of autapomorphic characters regarding colour pattern, type of pharynx and esophagus length. Thus, the phylogenetical analysis has been proved to be efficient in the selection of morphological characters for species definition. The phylogenetic analysis supports the monophyly of the genus *Imbira*, corroborating a previous phylogenetic analysis based on molecular data.

Poster 10

Community structure of turbellarians in different classes of palustrine wetlands in the southern Neotropical region

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Freshwater turbellarians are abundant organisms and constitute an important group in the structuring of the communities. They constitute a species-rich group in freshwater wetlands in southern Brazil. These permanent wetlands are classified, based on characteristics of aquatic plant cover, in five main classes: (1) without vegetation, (2) with submerged macrophytes, (3) with emergent macrophytes, (4) with floating macrophytes and (5) with diverse vegetation types (multi-stratified wetlands). In this study we analyzed the community structure of turbellarians among the different classes of palustrine wetlands in southern Brazil. Sampling occurred in 25 water bodies, five of each wetland class. Each wetland was sampled once, in the period between October 2013 and February 2014. A total of 1257 specimens of turbellarians was sampled, representing 62 species and 23 genera, from the orders Catenulida, Lecithoepitheliata, Macrostomida, Rhabdocoela and Tricladida. The dominant species were *Catenula lemnae* Duges, 1832 (n=320) and *Stenostomum grande* Child, 1902 (n=284). Both occurred in all wetland types, being more abundant in wetlands with floating macrophytes. Unique species occurred in all wetland types; their abundance was higher in multi-stratified wetlands as well as in wetlands with emergent macrophytes. Significant differences were found in turbellarian abundance and species richness in relation to vegetal composition ($p<0.05$). Multi-stratified wetlands, as well as wetlands with floating macrophytes had higher turbellarian abundance than the others types of wetlands ($p<0.001$). Regarding turbellarian richness, multi-stratified wetlands and wetlands with emergent macrophytes had higher values than other wetland types ($p<0.001$). Wetlands without vegetation had the lowest values of turbellarian abundance and species richness of this study ($p<0.001$). The structure of turbellarian communities was different among wetlands, being affected by temperature and dissolved oxygen. Results suggest that turbellarian assemblages are strongly related to the habitat heterogeneity. Turbellarians need various types of environment for their occurrence, thus the conservation of all types of ecosystems is necessary.

Poster 11

About turbellarians (Prolecithophora: Plagiostomidae, Multipeniatidae and Cylindrostomidae) from the Lake Shinji and the Lake Nakaumi of Japan

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Prolecithophorans are mostly small (0.5–4 mm in length), and often conspicuously colored, on hard bottoms, in gravel, and among algae. Prolecithophora comprises about 265 known species, the many of which are described from Europe, North America, and Brazil. By contrast, only a few species have previously been recorded from Japan. Most known prolecithophoran species are marine, although a few are found in brackish water. It is known that various species of organisms inhabit the brackish-water Lake Shinji and Lake Nakaumi. For the purpose to increase the knowledge concerning the diversity and ecology of brackish prolecithophorans,
I collected material at the lake Shinij and the lake Nakaumi, Japan, during 2014. In this presentation, prolecithophoran species are described from the Japanese brackish lake.

Poster 12

Next Generation Sequencing: providing new insights in the uneasy study of the evolutionary history of freshwater triclads (Platyhelminthes, Tricladida)

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Systematic studies within triclads were traditionally carried out using classical morphological techniques that are both time consuming and frequently not informative enough to infer the evolutionary history of the lineages. Moreover, they rely on the anatomical reconstruction of the reproductive system, which is absent from the fissiparous individuals, thus considerably hindering their analysis. The incorporation of molecular data has implied a step forward for this field of research: it has increased the known biodiversity of the group and opened the possibilities of also performing phylogeographical and population genetics studies. The most popular molecular markers have been the mitochondrial genes (specially the Cox1) and few nuclear genes from the ribosomal cluster (18S, 28S and ITS). Nevertheless the use of this limited set of genes is not exempt of problems, being the saturation of the mitochondrial genes and the high degree of conservation of the ribosomal genes some of them. Therefore we need new molecular markers to improve our understanding of the Tricladida systematics, particularly nuclear ones.

The main objectives of our work are:

1)To obtain complete genome data of different freshwater *Dugesia* species and populations using Next Generation Sequencing (NGS).

2) To search for regions with different levels of genetic variability along the genomes.

3)To validate their use as markers for integrative taxonomy, phylogeny and phylogeographic studies in planarians.

We will discuss the problems and potential solutions of getting genomic information from non-model organisms. Moreover, we will present a case example of how working with multiple nuclear markers can help to clarify the taxonomic status of one *Dugesia* species (*D.subtentaculata*) and to disentangle its evolutionary history.

Poster 13

Inducing Complete Fragmentation in Planaria and Malignant Cells Using a Temporally Patterned Electromagnetic Field

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The freshwater flatworm, planaria, have been the subject of intense scientific research primarily due to their apparently limitless ability to regenerate, rendering the organism ‘immortal’. Researchers have identified a cluster of undifferentiated adult stem cells, termed neoblasts, and have attributed the planarian’s longevity to the functionality of these cells. Even when placed in less than optimal conditions, such as nutrient deprivation, or tissue damage/wound induction, as seen in aging, the planarian has the ability to heal and regenerate to an entire organism. Recently, we have identified a tandem sequence of weak temporally-patterned magnetic fields that produced 100% dissolution of planarian in their native environment, despite the presence of these regenerative cells. Though magnetic field interactions within the planaria have been pursued for the last decade, many of the effects utilized non-patterned extremely low frequency (ELF) magnetic fields to *amputated* planaria. We utilized a highly specific sequence of complex, frequency modulated magnetic fields in the ultraweak intensity range (0.1-5 μT) to *intact worms*. This tandem sequence, was applied over a 5-day period, where 24 hours post-magnetic field exposure, observations of death and complete dissolution of planarian exposed to this combination of fields were unprecedented. Direct video evidence showed a protracted expansion (approximately twice the normal volume) during the first field presentation, and reduction (approximately half normal volume) and death 24- hours following the second field presentation. Contortion behaviour during the exposures suggests an interaction with contractile proteins, and changes in volume implicate changes in cell membrane permeability. Subsequently, we investigated the effect of this pair of magnetic fields on a malignant cell lines, as they are known parallel stem cells with respect to particular molecular processes. Similar to the planaria, complete dissolution of the cells were observed 24-hours post field sequence exposure. However, no deleterious effects were observed in any of the healthy cell lines used as controls. Fluorescence staining of the actin cytoskeleton was used to confirm the change in volume within malignant cells 5-days after exposure. Acridine-orange confocal microscopy was also used to confirm DNA damage 24-hours post field exposure. The selective effects reported in this study lend credence to the use of frequency modulated magnetic fields in interacting with specific molecular pathways *in vitro*, and evidence to the specificity of neoblasts within planaria and the parallels they display to malignant cells such as indefinite potential for self-renewal

Poster 14.

Light-Mediated Regulation of Long-Term Potentiation in Planaria

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The acquisition of a task, or learning, and its retention, memory, are common processes seen throughout the animal kingdom; lending support to their evolutionary significance. While the physiological mechanisms of learning and memory are still being elucidated, a process that has been strongly implicated is long term potentiation (LTP) within neuronal cells. Several *in vitro* studies have shown that chemical analogues or exposure to physical forces such as magnetic fields can alter LTP, thus affecting the organism's ability to learn and recall the information. In the present study, freshwater flatworms (*Dugesia tigrina*) were used as the model organism in the investigation of light-mediated manipulation of LTP, as their well-defined nervous system is comprised of neural molecular systems that are comparable to vertebrate neurons. This, coupled with their unique photosensitivity, makes the planarian nervous system potentially excitable under photostimulatory conditions. Photostimulation conditions were modelled after the LTP-firing patterns of neurons whereas control conditions received either no light or a sine-wave patterned photostimulation. The wavelengths of light, 430nm, 660nm and 880nm, were selected based on the physicochemical property of the proteins (tPA, BDNF, cAMP, CREB) involved with the various stages of LTP formation within the post-synaptic neurons. Results of this study show that planaria exposed to a LTP patterned light performed better than the sine-wave group in the learned task. With respect to wavelengths, planaria exposed to 430nm and 880nm light acquired the task quicker than worms placed in other conditions. Remarkably, this effect is seen only 3.5-4 hours after the 30-minute light exposure. This temporal factor may be reflective of the physiological correlates of LTP. This process can be delineated into early and late stages, with the early stage lasting up to 2 hours, and is not dependent on *de novo* protein synthesis. The effect of the present study is isolated to ~3 hours post-light exposure, suggesting involvement of late-stage LTP, which involves trafficking proteins and mRNAs from the cell soma and distal dendritic sites. We conclude that using the appropriate combination of wavelength, intensity and pattern of photostimulation, light can be used as a tool to enhance learning through the activation of the time dependent LTP process.

Poster 15

Hierarchies in Planaria light sensing response and regeneration

Nishan B.S., Rimple Dalmeida, Rohini G., Asawari Joshi, Manoj Mathew, Dasaradhi Palakodeti, Akash Gulyani

Planarians are flat worms with a true cerebral eye structure connected via axon tracts to the brain. Planarians have specialized photoreceptors cells that are implicated in light perception through the eye. Current understanding suggests that the Planarian eye is a rudimentary cup-like eye that is involved in simple light and directional sensing, mediated by through the photoreceptor cells. While Planarians have long known to avoid light (are negatively phototactic), we discovered new light-mediated behavior in the model organism *Schmidtea mediterranea.* Therefore, to better understand this light sensing, we have established quantitative assays to record specific light-induced behavior and movement in Planarians. Some of these assays would be described here in detail. Our analysis of light-induced behavior in planarians has led us to uncover dramatic light sensitivities in these flatworms, wherein worms can discriminate between multiple light inputs. Our results have challenged the conventional view of the Planarian eye as a rudimentary light detector with directional sensing. Further, we show whole body light sensing in Planarians as well. In addition to having these unique modalities of light sensing, decades of research has shown that planarians are a powerful model for studying regeneration with its remarkable whole-body regenerative capacity following a variety of injuries or naturally occurring fission. Specifically, after decapitation, Planarians can regenerate their entire cephalic ganglion (brain) as well as sensory organs, including eyes. Using unique light-induced behavior that we observed, we have established novel light assays to probe neuronal and visual center/eye regeneration. Our assays show that we can temporally delineate regeneration into critical steps that are linked to specific functional states. We hypothesize that while light avoidance may be a product of rudimentary neuronal signaling, the ability to resolve different light inputs requires finer network level processing/computing. We can now attempt to identify the precise features and determinants of the eye and the associated neuronal network that allows an organism to sense specific optical inputs

Poster 16

Investigating the growth/degrowth dynamics in planarian flatworms

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Planarian flatworms display not only remarkable regenerative abilities. In fact, also their uninjured bodies show pronounced plasticity. The body size of most planarian species varies depending on food supply. Frequent feeding results in growth whereas starvation causes planarians to shrink (degrowth). Yet, remarkably, the animals retain their external and internal body plan proportions irrespective of their size. We aim to address the question of how body plan scaling is achieved. Towards the goal of a quantitative understanding of growth/degrowth dynamics in *Schmidtea mediterranea,* we are quantifying size change dynamics across a range of body sizes. Further, we are carrying out experiments to investigate how the regulation of total cell number, cell density and cell size contributes to growth and degrowth. Our results show that growth dynamics are size-dependent. We have developed a hypothetical model linking growth rates with cell number changes and feeding status, centering on metabolic energy as a growth-limiting factor. We are currently experimentally parameterizing and validating the model and its predictions.

Poster 17

Why some animals regenerate while others cannot- Analysis of a large live collection of planarian species with differing regenerative abilities.

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The regenerative capabilities of the many hundred planarian species worldwide ranges from whole body regeneration, as in Schmidtea mediterranea or Dugesia japonica to poor, as in Dendrocoelum lacteum. As a group, planarians therefore offer a perfect system to explore why some animal species can regenerate while others cannot. In order to make available the range of regenerative abilities to experimental analysis, we are characterising a large collection of triclad species at the Max Planck Institute in Dresden (MPI-CBG). We assay species-specific regenerative abilities both along the Anterior/Posterior body axis and along the medio/lateral axis. In conjunction with genotyping of the entire collection, this enables the mapping of regenerative abilities onto planarian phylogeny. Finally, we have initiated the molecular characterisation of regeneration deficient species with the objective of understanding the molecular mechanisms that cause planarian regeneration defects. Here, we will present preliminary results of these efforts.

Poster 18

Increased TNFα expression in the hyperplasic ovary of the ex-fissiparous planarian *Dugesia arabica* compared to the normal one.

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The functional relationships between the different cell death processes is very complex, especially that between apoptosis and autophagy (Galluzzi et al. 2012). Drawing the border between autophagy and apoptosis in *Drosophila* (as an example) is very difficult. In a previous study, we have demonstrated that a complex process of early autophagy followed of apoptotic processes, occurs in the hyperplasic ovary of the freshwater planarian *Dugesia Arabica* (Harrath et al., 2014). This peculiar event represents one of the most important factors that cause ex-fissiparous planarian infertility. Based on TEM results, we have demonstrated a novel extensive co-clustering of cytoplasmic organelles, such as lysosomes and microtubules, and their fusion with autophagosomes. The immunohistochemical have shown that the proapoptotic protein bax was more highly expressed in the hyperplasic ovary compared to the normal one. TUNEL analysis confirmed that the nuclei of the majority of differentiating oocytes were TUNEL-positive.

The present study aims to investigat whether or not the expression levels, of the selected gene tumor necrosis factor (TNFα) and its protein was altered in the hyperplasic ovary of the ex-fissiparous freshwater planarian *D. arabica* compared to the normal one. Using quantitative real-time PCR, we found that transcript levels of TNFa were significantly higher by 3.41-fold in hyperplasic ovary compared with the normal one (1.19). The immunohistochemical labeling supports the quantitative real-time PCR results because it has been shown that TNF α was more highly expressed in the hyperplasic ovary than in the normal one.

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Poster 19

Exome sequencing reveals differences in the repertoire of voltage gated cation channels of free living and parasitic flatworms

John D. Chan and Jonathan S. Marchant

Despite the fact that parasitic flatworms account for roughly a quarter of neglected tropical diseases (NTDs) and infect hundreds of millions worldwide, there is a dearth of drugs either on the market or in development pipelines to treat these diseases. Many conditions are entirely reliant on a single, decades old drug, praziquantel (PZQ), whose mechanism of action still remains poorly understood. The experimental intractability of parasitic flatworms (life cycles often requiring multiple host organisms, difficulty culturing long term and refractory to RNAi) may account for the lack of progress to date in developing new anthelmintics. Towards this end, free-living planarians have a role to play in adding to our knowledge of basic flatworm biology and serving as a reference to compare parasitic verses non-parasitic Platyhelminthes.

To facilitate this work, we generated a transcriptome *de novo* for the planarian *Dugesia japonica* in order to compare the voltage gated ion channel superfamily complements of free living and parasitic flatworms. Genomic sequencing of flatworms has demonstrated that the evolution of parasitism has been accompanied by a paring down of the genome, as superfluous machinery is lost and organisms more efficiently adapt to their host environment. This reduction is apparent when comparing the cation channel complements of the planarian *D. japonica* with the parasitic blood fluke, *Schistosoma mansoni*. Schistosomes have lost all low voltage activated (LVA) Ca^{2+} channels (Ca_vs), voltage gated sodium channels (Na_vs), voltage gated proton channels (H_vs), and two-pore channels (TPCs), while all of these channels are evidenced in the *D. japonica* transcriptome. Comparison of gene expression implies an even more striking reliance of parasitic flatworms on a subset of this already limited channel repertoire. The generation of this transcriptome enhances the utility of *D. japonica* as a tool to investigate basic flatworm biology, expanding access to techniques such as RNAi to assess gene function or comparative RNAseq to interrogate the effects of antihelmintics

Poster 20

Acute and Chronic Stressors Affecting Metabolic Rate in the Planarian *Dugesia japonica*

Melissa Lewallen and Warren W. Burggren, University of North Texas

Planarians live in a potentially highly variable and challenging environment, with natural stressors including hypoxia, variable light levels and temperature fluctuations. Do they respond metabolically to stressors known to alter metabolism in other invertebrates? Planarians are known to be negatively phototactic, and light conditions affect aggregation behaviors. Planarians also exhibit avoidance behavior to alarm pheromones, released through cut or homogenized planarian tissue, as an anti-predator response. Are these behaviors accompanied by metabolic rate changes? Data on metabolic rates is very limited for planarians, particularly for the common brown planarian. Furthermore, existing metabolic measurements – some nearly a century old - have not been confirmed with modern methodologies, and the metabolic scope of Planaria, as induced by stressors such as light, alarm pheromones, and temperature, is unknown. To determine the metabolic behavior of *Dugesia japonica,* oxygen consumption rates for *Dugesia japonica* at 18° C were assessed using an automated 24 well oxygen optode system, which measured the decline in oxygen over a 280 min period in a 3.3 ml well containing 3-5 planaria. Oxygen consumption was measured in varying light conditions: constant light, constant dark, and with a strobe light at 1 flash/sec. Oxygen consumption was also measured in the absence and presence of an extract made of ground planarian bodies (presumably an alarm pheromone solution), which was also tested during differences in lighting. Oxygen consumption for *Dugesia japonica* ranged from 13-20 µL/g/h at 18° C, and was not significantly affected by light conditions and/or alarm pheromones. The metabolic rates of the present study fall well within the range of recent measurements of metabolic rates at similar temperatures for relatively inactive Protostomes such as annelids, nematodes and sessile molluscs. Our measurements on *Dugesia japonica*, using modern methodologies, suggest that long-standing, historical metabolic rates for platyhelminths (e.g. Whitney, 1942) are overestimated by as much as 10-fold. Future experiments will examine how temperature differences and feeding/starvation regimens affect metabolic rate. Identification and characterization of environmental metabolic stressors will allow for subsequent cellular and molecular studies focused on stress.

Poster 21

Alternative polyadenylation in the flatworm *Schmidtea mediterranea*

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In animal cells, hundreds of RNA binding proteins (RBPs) and thousands of miRNAs bind untranslated regions downstream of the stop codon (3' UTRs) in mRNAs. Concerted binding of RBPs and miRNAs to 3' UTRs regulates mRNA turnover or translation and can even direct the subcellular localization of the encoded protein (Berkovits and Mayr 2015). Expression of 3' UTR isoforms is widespread, regulated by alternative polyadenylation (ApA), but poorly understood.

Here, we study ApA and post-transcriptional regulation in the *in vivo* stem cell system *Schmidtea mediterranea* using a 3'UTR capturing method (3'-seq) to map out the planarian 3'UTRome. As previous transcriptome annotations lack a proper definition of ORF boundaries, we designed a new pipeline to annotate gene structures on the planarian transcriptome. With this, we define a set of protein coding genes, containing >15,000 transcripts. For our analyses, we also use a set of high-quality, complete, continuously mapping transcripts, called Gold Standard (GS; n=~8,000). .

In our analysis, we recover over ~11,000 events in the GS set, comprising existing and novel cleavage sites. Further characterization of our ApA dataset show an enriched usage of proximal cleavage site in neoblast for transcripts with multiple 3'UTR, whereas distal sites are found to be preferred for differentiated cells, matching equal observations described in cell culture experiments. We also find characteristic polyadenylation cleavage signals previously described in mammalian systems 15 to 30 nucleotides upstream of our annotated cleavage sites.

In summary, we provide a more complete definition of the 3'UTRome in planaria by *in silico* annotation of ORFs boundaries as well as providing direct cleavage site evidence through 3'-seq. With this data we are able to detect thousands of existing and novel 3'UTR cleavage sites and show that differential regulation of alternative cleavage and polyadenylation in proliferating and differentiated cells is conserved throughout evolution.

Poster 22

Effects of six commonly used biocides on survival, oxidative stress, and cholinesterase activity of freshwater planarians

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Isothiazolinones, triclosan and iodopropynyl butylcarbamate (IPBC) are extensively used as biocide ingredients in our personal care products, such as shampoos, shower cleaners, detergents, lotions and cosmetics. These biocides are known to cause contact dermatitis and allergy in human; however, effects of these biocides to aquatic species are very limited. Freshwater planarians are sensitive to different types of pollutants and offer several practical and operational advantages as useful test organisms for aquatic toxicology studies. In this study, we examined effects of six commonly used biocides, including methylisothiazolinone (MIT), benzisothiazolinone (BIT), octylisothiazolinone (OIT), triclosan, IPBC, and clotrimazole, on survival, oxidative stress and cholinesterase (ChE) activity on freshwater planarians (*Dugesia japonica*). The 48-h and 96-h LC_{50} values for planarians exposed to all biocides can be ranked as IPBC > MIT > BIT > triclosan > OIT > clotrimazole. Among all biocides tested, clotrimazole was the most toxic chemical and IPBC was the least toxic chemical to planarian at each exposure period. In addition, the planarian LC_{50} of six biocides between 24 to 96 hours were all less than 5 mg/L. Interestingly and unexpectedly, the increases of ChE activities were found in planarians exposed to all biocides except BIT after 48 hour treatment. Lipid peroxidation and antioxidant enzyme activities in planarians were also measured. The relationship between oxidative stress and the increased ChE are discussed. Because of their wide use in cosmetics and personal care products,several recent studies detected the occurrence of these chemicals in effluents from wastewater treatment plants. More studies on ecotoxicological effects of these biocides are needed to provide important information to adequately assess their ecological risk to aquatic ecosystems.

Poster 23

Investigating the molecular mechanisms governing neoblast sensitivity to radiation in the planarian *Schmidtea mediterranea*

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Ionising radiation (IR) is a widely used treatment for many cancers, and recurrence of cancer after IR therapy remains a major therapeutic problem. Thus understanding how cells, especially adult stem cells, protect themselves from radiation damage is a key research priority for cancer treatment. Developing a convenient *in vivo* experimental system in which we can understand the molecular mechanisms that alter cellular responses to radiation and modulate efficacy would be of high biomedical value. The planarian *Schmidtea mediterranea* is extensively used for stem cells and regeneration studies and contains a large population of adult stem cells, known as neoblasts, which are highly sensitive to irradiation. Because of the availability of RNAi methods to study the gene function in *S. mediterranea*, it is able to undertake genome wide investigations to understand molecular basis for IR sensitivity in stem cells *in vivo*. From these facts, we hypothesized that the planarian stem cell model system can be used as a research tool to understand the radiation sensitivity in stem cells as a relevant model to cancer stem cells.
We will present our findings on the contribution of DNA damage response (DDR) pathways in *S. mediterranea* following IR exposure to prove the existence of protective mechanisms in neoblast against radiation. In prelinary experiments we identified orthologs of two key components of the DDR in *S. mediterranea,* ATR and BRCA. Although there are no effects of knock-down of ATR and BRCA in planarians in normal regenerative paradigms, we found that the survival of *Smed-ATR(RNAi)* and *Smed-BRCA(RNAi)* animals is reduced following IR exposure. Our research hopes to provides much needed investigation of the underlying mechanisms as to how the radiation-protective mechanisms, such as DDR, contribute to IR sensitivity in adult stem cells *in vivo*

Poster 24

A new Geoplaninae (Platyhelminthes: Tricladida) with an accessory musculoglandular organ

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The testes are placed differently among the planarians in general. In most planarians the testes and efferent ducts are placed at the ventral side of the body. Exceptionally in land planarians of the subfamily Geoplaninae (South America, 800 spp.) and the tribe Eudoxiatopoplanini (Southern Islands, 1 sp.) testes are located dorsally; while in the tribe Anzoplanini (Subantartic Islands, 2 spp.) testes are located dorsoventrally. Herein we describe and discuss the phylogenetic position of a land planarian from Chile which bears an exceptional combination of morphological characteristics. The land planarian specimen is about 21 mm long and presents a dark brown dorsal coloration. While bearing dorsally located testes, the copulatory apparatus is supplemented with a sophisticated musculoglandular organ and a series of glandular tampon-like muscular folds. Glando-muscular organs such as adenodactyls and adenomuralia are typical morphological characteristics of land planarians of the subfamily Rhynchodeminae, namely the tribes Caenoplanini, Anzoplanini and Eudoxiatopoplanini of the Australian regions. Furthermore, the land planarian specimen was collected in the valdivian temperate rainforest in southern Chile (40° 68` S, 72° 91` W), an ecoregion of high biogeographical interest in the context of Gondwanan connections. While morphological similarities of the land planarian fauna between the Chilean and the Notogeic regions have been briefly mentioned in the past, no musculoglandular organs of this type have been previously described in other Geoplaninae species.

Poster 25

Permanent and temporary adhesion in flatworms

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Adhesives are used in many different fields in industry, technology and medicine. Numerous synthetic materials are used in manufacturing processes and the trend for miniaturization in many fields require new innovations in adhesive technology. Man-made adhesives normally share disadvantages like poor biocompatibility or weak underwater performance. Biological systems have developed a great variety of different glues for their needs and therefore are of interest in the adhesion field.
Our investigations focus on the bioadhesive properties of flatworms. Unlike other bioadhesion model organisms like mussels, many flatworms produce two different types of adherents. A temporary adhesive is used to repeatedly adhere and detach during locomotion and permanent glue is used by the worms to adhere eggs to a substrate. In Macrostomum lignano, cement gland cells which surround the female opening are supposed to produce this permanent adhesive. By in situ hybridization, we identified 11 transcripts with expression in cement glands. Using double fluorescence in situ hybridization we were able to cluster cement gland transcripts into three distinct groups. Remarkably three large collagen alpha transcripts could be identified, each expressed in a different cell type.
In addition we will present our findings on the temporary adhesive system of the Proseriate flatworm Minona ileanae. By the use of transmission electron microscopy, we were able to morphologically characterize the Minona ileanae duo-gland adhesive system. In addition, we generated a differential transcriptome to identify tail-specific genes. This will be used as basis for an in situ hybridization screen. In future investigations we aim to characterize and compare adhesion molecules on a molecular level of several flatworm species.

Supported by the Austrian Science Foundation (FWF P25404-B25) and a PhD fellowship of the University of Innsbruck

Poster 27

Hunting for the glue: the adhesive system of *Macrostomum lignano*

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Macrostomum lignano (Rhabditophora, Macrostomorpha) possesses an elaborate adhesive system, which allows the animals to repeatedly adhere and release. The adhesive system comprises about 130 adhesive organs at the tip of the tail, each consisting of three cells: two secretory cells and one anchor cell.
To reveal the adhesion mechanisms and the adhesive and releasing factors of *M. lignano* we screened 298 mainly tail specific transcripts with whole mount *in situ* hybridization. We were able to identify 26 transcripts expressed in the adhesive organs, six of them exclusively expressed in the region of the secretory glands. A newly established double fluorescence *in situ* protocol combined with an adhesive gland cell specific lectin allowed us to assign the transcripts with expression in the adhesive organs to a distinct cell type. Furthermore, transmission electron microscopy and super resolution microscopy helped to understand the structural organization of the adhesive organs.
Functional analyses of candidate genes was done by RNA interference. We were able to obtain a non-adhesive phenotype by knocking down one transcript expressed in the adhesive gland cells. Ultrastructural analysis of RNAi worms revealed an alteration in the adhesive vesicles whereas the structure of the cells was not affected. These findings led to the suggestion that we have found an essential part of the *Macrostomum* glue.
Currently we are analyzing footprints of *M. lignano* with mass spectrometry to find other components of the adhesive material as well as the releasing factor.

Supported by FWF grant P25404-B25, PhD Fellowship Autonome Provinz Bozen

Poster 28

Role of Poly(A Binding Protein Nuclear (PABPN) in Planarian Regeneration.

Namita Mukundan, Srikar Krishna, Dasaradhi Palakodeti

The planarian Schmidtea mediterranea with its high regenerative ability is emerging as a powerful model organism. Neoblast are the adult somatic stem cells which give rise to all the cell types of the organism and these are found to be responsible for their impressive regenerative ability. Various RNA binding proteins (RBPs) play critical role in regulating gene expression patterns. Poly A binding protein nuclear (PABPN) is an RNA binding protein which interacts with polyadenylation polymerase (PAP) and Cleavage and polyadenylation specific factor (CPSF) for the polyA tail synthesis of hn mRNA. In mammals PABPN1 is widely expressed in muscles; depletion/abnormality of the protein leads to oculopharyngeal muscular dystrophy (OPMD). Transcriptomic analysis of pabpn in planaria reveals neoblast specific expression pattern. Further, RNA interference studies showed phenotypic defects like bloating, immobility and insensitivity towards light suggesting a role of the protein in regenerative processes. Immunostaining and quantitative PCR showed defects in stem cell proliferation and differentiation along with disorganization of muscle fibers, photoreceptor, protonephridia and intestinal abnormalities. These studies implicates specific role of PABPN in neoblast differentiation to specific progenitors during the course of planarian regeneration.

Poster 29

Epigenetic control of endogenous and exogenous (retro) transposable elements in *Schistosoma mansoni*

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Aiming to improve transgenesis for schistosomes, manipulations of cytosine methylation status of the genome and expression perturbation of the germ line marker *S. mansoni* vasa-like helicase were undertaken. Cytosine methylation regulates schistosome oviposition and embryogenesis. In addition, post-translational core histone modifications in epigenetic control of transcription of schistosome genes occurs, and non-coding RNAs, including epi-miRNAs that regulate expression of enzymes involved in chromatin remodeling and DNA methylation have been described. To evaluate the effect of the DNA methyltransferase inhibitor 5'-azacytidine (5'-AzaC) on both long terminal repeat (LTR)- and non-LTR retrotransposons, developmental stages of *Schistosoma mansoni*, including schistosomules, adult worms and eggs were cultured in the presence of 100 µM or 500 µM of 5'-AzaC. Expression of the endogenous *Boudicca* LTR-retrotransposon and the *SR2* non-LTR-retrotransposon were analyzed by qRT-PCR. The expression level of these retrotransposons increased 10 to 20 times in the presence of 5'-AzaC in a time- and dose-dependent manner. In addition, *Boudicca* and *SR2* elements were upregulated when the DNA methyltransferase 2 and one of the at least two *S. mansoni vasa*-like genes that belong to the PL10 clade of DEAD-box helicases, were silenced by RNAi. Notably, the expression of reporter transgenes increased in parasites transduced with pseudotyped retrovirus following culture in media supplemented with 5'-AzaC. Moreover, the copy number of integrated provirus in the transgenic worms, where the *vasa*-like gene was silenced, was almost 4-fold higher than in controls. These findings suggested that epigenetic marks regulate the activity of mobile genetic elements in schistosomes. They also indicated a central influence of methylation status on retroviral transgene activity, providing a potential approach to enhance transgenesis and other functional genomic approaches for this neglected tropical disease pathogen.

The following posters will also be presented as talks in the Planarian meeting (abstracts above).

Poster 30
Nanna Nagao
"5-hydroxytryptophan induces ovaries, and knockdown of tryptophan hydroxylase homolog inhibits sexual induction in the asexual worms of Dugesia ryukyuensis."

Poster 31
José Ignacio Rojo-Laguna
"Controlling Regeneration Speed"

Poster 32
Takanobu Maezawa
"Tryptophan enhances the reproductive organs-specific expression level of an amino acid transporter homolog, Dr-SLC38A9 to promote sexual induction of the planarian Dugesia ryukyuensis"

Poster 33
Miquel Sureda-Gómez
"Beta-catenin specifies posterior identity through a protein gradient and it is required for anterior patterning in planarians"

Poster 34
Dhiru Bansal
"SMED-PABPC2 is essential for maintenance of epidermal integrity and second mitotic peak activation during planarian regeneration"

Poster 35
Nicky Pirotte
"The necessity of ROS signalling for successful differentiation and patterning during planarian regeneration