DARWIN 2.0

NEW TOOLS TO GO THROUGH TIME

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Program, Abstracts and Participants

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Francesca Benzoni | University of Milano-Biococca.

Crossing the line: New perspectives on the Precambrian-Cambrian transition in the fossil record

Graham Budd l Uppsala University.

The origin of animals, apparently so suddenly recorded in the fossil record, has long excited interest and controversy. Here I review the fossil record around the Precambrian-Cambrian boundary some 540 million years ago, and present a novel hypothesis that attempts to explain the remarkable transformation that takes place during this time interval. This attempt to tie the Precambrian and Cambrian fossil records together has important new implications for body pattern transformations during basal animal evolution, especially as regards cnidarian-bilaterian homologies.

Genomic bases of key metabolic innovations in the genus Nicotiana

Emmanuel Gaquarel I Heidelberg University.

Plants are champion synthetic chemists! They take advantage of their metabolic prowess to produce an extremely large repertoire of structurally diverse natural products, also referred to as specialized metabolites (e.g. alkaloids such as nicotine). Importantly, these specialized metabolites have extensively diversified across plant taxa, thereby suggesting that particular metabolic systems have been recruited through natural selection when the set of compounds that they produce address specific ecological needs. Research in our group combines molecular and chemical analyses of specialized metabolism with respect to plant responses to insect herbivory. Specifically, our group studies species from the genus Nicotiana. This genus is ideally suited to explore the genomic bases of key metabolic innovations as it includes multiple species with well-assembled reference genomes and transcriptomes. Nicotiana spp. also have well-characterized chemical profiles as well as the capacity to deploy different defensive strategies against insect herbivores. During my presentation, I will first elaborate on recent comparative genomic efforts conducted in the context of the analysis of the Nicotiana attenuata genome (an ongoing collaboration with Dr. Shuqing Xu, Baldwin lab, MPI, Jena), one of the ecological model systems in this genus. This work aims at retracing the evolution of the biosynthetic pathway of nicotine, a Nicotiana-specific defensive metabolic innovation. How plants cope with their own defense metabolites and avoid intoxication is frequently unknown. I will discuss our current hypotheses regarding mechanisms that co-evolved with nicotine biosynthesis to reduce nicotine autotoxicity. Allopolyploidization (hybridization between two species, followed by genome doubling) is frequent in the genus Nicotiana, but its consequences for specialized metabolism evolution are largely unexplored. I will finally present evidence for the evolution of a new type of defense metabolites in one specific allopolyploid group as well as first attempts towards dissecting the underlying pathway using transcriptome and metabolite profiling.

Regulatory evolution and the diversification of pigmentation patterns in Drosophila

Nicolas Gompel | LMU München.

The typical pattern of morphological evolution associated with the radiation of a group of related species is the emergence of a novel trait and its subsequent diversification. Yet the genetic mechanisms associated with these two evolutionary steps are poorly characterized. We show that a spot of dark pigment on the wings of the fly Drosophila biarmipes emerged from the assembly of a novel gene regulatory module. In the ancestor of this species and its wing-spotted relatives, a set of pigmentation genes evolved to respond to the common transcriptional regulator Distal-less (Dll), which determines their spatial distribution. One of these pigmentation genes, yellow, is integrated into the regulatory module by a cis-regulatory element that drives its expression in the developing wing, precisely where the pigmentation spot will appear. We are dissecting this enhancer in a quantitative way to understand how the novel transcriptional activity has emerged during evolution. While Dll is necessary and sufficient to control the spot enhancer spatial activity, other sites in its sequence also determine this activity in different ways. The emergence of this novel regulatory function will be discussed.

Systems neurobiology of the Platynereis larva

Gáspár Jékely | Max Planck Institute for Developmental Biology.

Neural computations by nervous systems require proper synaptic connectivity between neurons. However, behaviour cannot be predicted from connectivity alone because a ‘chemical map’ of neuromodulation is superimposed upon the synaptic connectivity map. To understand animal behaviour the combined study of anatomical connectomes and neuromodulatory maps is needed. Currently such an integration at the whole-organism level has only been achieved in C. elegans and to some extent in Drosophila. To complement these conventional model organisms we have been developing the marine annelid Platynereis dumerilii into a powerful new experimental model. We study Platynereis from both connectomics and the neuromodulatory perspectives to understand how interactions between the nervous system and the environment shape behaviour, physiology and development. Given the phylogenetic position (within the lophotrochozoans) and ancestral neuron-type complement of annelids, by studying Platynereis we can also address key questions about nervous system evolution in a comparative framework (e.g. origin of sensory systems).

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eQTL analysis using allele-specific expression

José Jiménez-Gómez | Max Planck Institute for Plant Breeding Research Köln.

Next generation sequencing allows novel experimental designs to explore natural diversity. My lab is interested in understanding the effect of expression variation on physiological phenotypes and its implications in plant adaptation. We approach this question surveying differences in expression between Arabidopsis thaliana natural accessions using RNA-seq and performing expression QTL analysis through allele-specific expression analyses in F1 hybrid individuals. This is a powerful technique that helps us localize genes with regulatory mutations in cis and, in some cases, the causal polymorphism itself. In this seminar I will describe our experiences with this technique.

Evolution of cell and tissue coordination during fly gastrulation.

Steffen Lemke | Heidelberg University.

Well-known morphogenetic networks underlying fly development are headed by transcription factors like the helix-loop-helix transcription factor Twist, which in Drosophila melanogaster embryos orchestrates the coordinated involution of mesoderm precursors along a deep ventral fold. Presumably, these networks and the associated morphogenetic processes have been constantly optimized during the course of evolution to facilitate fast and robust development typical for Drosophila melanogaster. We are interested in this process of morphogenetic optimization, and we have quantitatively compared early embryonic development in Drosophila melanogaster with embryonic development in Chironomus riparius, a mosquito-related fly species that has retained some aspects of a more ancestral and less optimized mode of development. We find that surprisingly few changes in the developmental genetic network downstream of Twist appear to be evolutionary sufficient for substantial morphogenetic differences and account for an overall speed-up of development.

Transitions between combined and separate sexes in plants

John Pannell I Universite de Lausanne.

Most plants are hermaphroditic, with individuals transmitting their genes through both male and female sexual functions. However, separate sexes (dioecy) have evolved repeatedly form hermaphroditism, either through the spread of male- and/or female-sterility mutations, or through a gradual divergence in sex allocation towards increasing maleness or femaleness. Dioecy may also break down, with males or females re-acquiring the opposite sexual function and returning populations to a hermaphroditic state. In this seminar, I will explore several leading ideas for the drivers of these major sexual-system transitions in plants. I will then showcase research from my lab that has been testing these ideas using the plant Mercurialis annua as a model; M. annua shows remarkable variation in its sexual system and has both gained and lost separate sexes more than once.

Computational Biology as Computational Science: Challenges & Problems

Alexandros Stamatakis | HITS Heidelberg.

Initially, I will outline the technical and algorithmic challenges we had to overcome to be able to analyze large-scale transcriptomic (Misof et al., Science, 2014) and phylogenomic (Jarvis et al., Science 2014) datasets on supercomputers. I will focus on algorithmic as well as technical challenges and present them in an accessible way. Motivated by the above large data analysis projects, in the second part of my presentation I will focus on software quality issues. Because of the data deluge that was triggered by the advent of next generation sequencing technologies, evolutionary biology is experiencing a paradigm shift toward a quantitative science. To this end, practitioners have to rely on an increasing number of tools to analyze their data and publish their papers. In a recent, unpublished, study we have assessed the quality of 15 widely-used codes in evolutionary biology with over 60,000 citations in total. The results indicate that there is a huge potential for improving software quality and that the probability that the implementations are correct is not too high.

Insights into evolution of eumetazoan regulatory developmental networks from the sea anemone Nematostella vectensis

Ulrich Technau | University of Vienna.

Cnidaria, the sister phylum of Bilateria, lacks a number of key bilaterian traits, such as mesoderm, a central nervous system and a clear bilaterality, yet the underlying genetic basis for these crucial differences is unknown. Genome sequencing projects have revealed that the anthozoan Nematostella vectensis displays a stunning ancestral complexity in gene repertoire, gene structure and genome organisation. Therefore, differences in the cis-transcriptional or post-transcriptional regulation as well as protein interactions may account for differences in functions of conserved genes. To this end, we mapped cis-regulatory elements (promoters and enhancers) on a genome-wide level using a combination of histone modifications and binding of Pol II and of transcriptional cofactor p300. We found that the basic logic of enhancers and promoters marked by conserved and specific combinations of histone modifications predates the origin of eumetazoans. To assess the evolution of specific gene regulatory networks we also determined all target genes of the conserved transcription factor Brachyury by ChIP-seq on a genome-wide scale and compared it with similar datasets from sea urchins and frogs. Our data reveal the evolutionary conservation and divergence of a the GRN of a conserved developmental regulator.

P 1 (short talk)

The genus Cochlearia (Brassicaceae) - phylogenomics, trancriptomics and population genomics of a cold relic in a warming world

Lua Lopez | COS, Heidelberg (Germany).

The genus Cochlearia represents an isolated evolutionary lineage that diverged from its Mediterranean sister clade during the Miocene but did not undergo any significant speciation until mid-Pleistocene. During the Pleistocene glaciation and deglaciation cycles approximately 20 taxa evolved, with most of the species being closely associated with cold-characterized habitats. Similar phenotypes of varying ploidy levels and different ecotypic adaptations emerged and are nowadays scarcely distributed all over Europe and the circumarctic region. Thanks to various naturally occurring species pairs, the Cochlearia study system provides a great opportunity to study parallel evolution in response to environmental changes.

Based on NGS data we built up a phylogeographic-evolutionary scenario that strongly supports a survival of the whole genus in the Arctic from where Europe was recolonized probably in several waves. Apparently, adaptation to different alpine habitats in Central Europe took place several times in parallel, potentially fuelled by standing genetic variation. The functional annotation of a Cochlearia transcriptome enables us to test these hypotheses on parallel evolution by identifying candidate genes for cold adaptation and by performing genome-wide dN/dS analyses aiming to detect signatures of past selection pressures. Namely, we have identified many exons related with cold adaptation and other associated processes.

As a next step, we are setting up a population-based approach to investigate, on a finer scale, the evolutionary processes behind the ecological speciation of Cochlearia. This will be addressed by using exon capture of approximately 1500 exons from the annotated transcriptome in ecological relevant populations covering its whole distribution range.

P 2 (short talk)

Cell expression atlases to study cell-type evolution

Hernando Martinez | EMBL, Developmental Biology, Heidelberg (Germany).

Cells are the biological units that compose every animal. The molecular toolkit of each of these cells might well recapitulate its evolutionary history. In the lab we make use of a cell-type approach to study core evolutionary events in bilaterians by comparing the cell-complement of distantly related body plans.
In order to compare cell-types within and across organisms, it is necessary to use the molecular information in a system-wide and unbiased manner.
We used the annelid model Platynereis dumerilii, animal for which gene expression atlases are available for highly stereotypic developmental stages (Profiling by Image Registration - PrImR). We have built on and extended this principle to less stereotypic and more complex body plans, achieving single-cell resolution using high-throughput imaging and image analysis routines. PrImR pipeline is now completely automatic and easy to customize to fit other developmental stages and/or other organisms.
It is just recently that we have been able to access the technology to study non-model organisms with cell resolution in a high-throughput way (single-cell sequencing, connectomics...). PrImR can be used to bring this information to a common atlas, rendering the location, morphology, gene expression and connectivity information for each cell within an organism.
The possibility of employing this image analysis pipeline in other organisms could permit the reconstruction of the cell-type complement of key ancestors in the evolutionary tree.

P 3

Role of AtRLP44 in brassinosteroid-mediated cell wall signaling

BORJA Garnelo Gómez I COS, Plant Cell Wall Signaling, Heidelberg.

Communication between the extracellular matrix and the cell interior is essential for all organisms as intrinsic and extrinsic cues have to be integrated to coordinate development, morphogenesis, and behavior. This applies in particular to plants, the growth and shape of which is governed by cell wall deposition and remodeling. In this context, the biophysical properties of the cell wall are constantly monitored and information must be relayed to the cell interior in order to fine-tune the physico-chemical properties of the cell wall for optimal growth responses. However, very little is known about the molecular components and signaling mechanisms involved in these processes.
AtRLP44 was identified as a key component of brassinosteroid-mediated cell wall signaling. RLP44 alone is sufficient to activate brassinosteroid-regulated gene expression. However, it is not clear how RLP44 activates signaling. Interaction with the regulatory receptor-like kinase BAK1 has been demonstrated but its functional significance is unclear. During this thesis, the effect of RLP44 on composition, motility, and signaling output of the brassinosteroid receptor complex and its downstream targets will be assessed. In addition, we aim to analyze the contribution of previously identified phosphorylation sites in the cytoplasmic tail of RLP44 on its function in cell wall signaling using transgenic complementation analysis. Taken together, these approaches are expected to reveal the mechanism by which the newly discovered RLP44-mediated cell wall signaling pathway is integrated with well-known brassinosteroid hormone signaling.

P 4

Imprinting facilitates the fast evolution of BMI1C in Brassicaceae

Chao Yang | COS Heidelberg, Biodiversity and Plant Systematics, Heidelberg (Germany).

The imprinted gene AtBMI1C, which encodes one of the PRC1 RING-finger proteins, was found functionally redundant with its homologs AtBMI1A and AtBMI1B involving in the regulation for Arabidopsis seeds and root development. The parent-of-origin effect of Ath_BMI1C is genotype independent in Arabidopsis and Capsella genus in Brassicaceae. Further studies showed that in the basal Brassicaceae lineage Aethionema, the expression of Aar_BMI1C is also limitedly expressed in the endosperm; but beyond Brassicaceae, in the out group Tarenaya hassleriana, Tha_BMI1C is expressed in all the tissues. These results indicated that the imprinted expression of BMI1C occurs only in Brassicaceae. Genealogy of BMI1C_cds showed that the three homologs of the BMI1s in Brassicaceae evolved from the same locus in ancient Angiosperms. Retention of three BMIs (γ-BMI1, γ-BMI2 and γ-BMI3) in core Eudicots is the consequence from the γ-triplication event. In Brassicaceae, γ-BMI3 was eliminated, γ-BMI1 and γ-BMI2 were maintained; after the sequential At-β and At-α WGD events, the ancient γ-BMI1 gene evolved into two copies as BMI1A and BMI1B kept in Brassicaceae genomes. Differently, the ancient γ-BMI2, even after two more polyploidy events, only one copy was kept in Brassicaceae (BMI1C). Comparing with BMI1A and BMI1B, BMI2 (BMI1C) exhibits significantly increased substitution rates in Brassicaceae. We proposed that the fast evolution worked on BIM1C locus in Brassicaceae is facilitated by the imprinted expression of this gene, considering the reduced selection pressure from the silencing of this gene during the vegetative development that allowed γ-BMI2 (BMI1C) accumulating more variation.

P 5

Order from disorder - How randomly dividing cells create a homogenous growth profile

Erika Tsingos I Centre for Organismal Studies, Developmental Biology/Physiology, Heidelberg (Germany).

Proper organ shape and function crucially depend on coordination between tissue growth and individual cell proliferation.
This delicate balance continuously takes place in the fish eye - a steadily growing organ that must maintain its structure to function.

Both the sensory retina and the surrounding retinal pigmented epithelium originate from an annular domain apposed to the lens: the ciliary marginal zone (CMZ).
Thus, the eye grows in regular concentric rings, but lineage-tracing in the fish retina revealed that some stem cells in the CMZ create disproportionally large or small progeny.

How do stem cells match their proliferative output to balance with organ growth? Vice versa, how does organ function shape the proliferative domain?
Using a computational agent-based model, I recreate eye growth and proliferation in silico from the bottom up.
The model reveals that intrinsically equal cells can nevertheless behave differently by introducing stochastic choices, such as a random probability to divide.

P 6

Unbalancing adult stem cells – assessing effects of enhanced EGFR signaling on different cellular subsets in the teleost retina

Eva M Hasel | COS, AG Wittbrodt, Heidelberg (Germany).

The adult teleost retina harbors multipotent stem cells that are active throughout the life of fish. These retinal neural stem cells (NSC) grow and differentiate in a very stereotypic manner and form Arched Continuous Stripes (ArCoS). The tight balance of retinal NSC proliferation is very stable and under- or over-growth of the adult fish retina basically never occurs. In the subventricular zone of mice, EGFR signaling maintains the balance between NSC and progenitor cells. This hints to an important function of EGFR in the adult NSCs.
We are interested in the role of EGFR signaling in the fish retina and how retinal growth is influenced by loss and gain of EGFR signaling.
Thus, we established genetic tools to study the effects of the mutated EGFR Xmrk (Xiphophorus melanoma receptor kinase) on proliferation and differentiation behavior of retinal NSCs and their progenitors. Following an initial characterization of expression patterns of the medaka (O. latipes) EGFRs and their ligands by in situ hybridization, effects of Xmrk on retinal cells will be studied in vivo. In an over-expression study we could already show that proliferative function of Xmrk-eGFP is preserved.
By Tet transactivators we can induce strong Xmrk expression and fluorescent labeling at the same time in the different subsets of cells within the ciliary marginal zone (CMZ) and study the effect of long- and short-term activation of growth signaling. In parallel, we set up a CRISPR/Cas9 based method to knock out the egfr a gene in different subsets of cells that will yield important information about the role of EGFR signaling in different compartments of the CMZ.
To address effects of enhanced EGFR signaling in single retinal clones we use Cre-lox based induction of Xmrk-eGFP expression in RSCs and progenitors. The readout will be based on an ArCoS assay, showing us if and how EGFR signaling influences proliferation and differentiation in the retina.

P 7

SMXL-sub family regulates meristem activity by altering strigolactone-dependent sugar distribution.

Eva-Sophie Wallner I Centre for Organismal Studies, AG Thomas Greb, Heidelberg (Germany).

Body shaping in multicellular organisms depends on the activity of distinct stem cell niches coordinated over long distances by nutrients and growth hormones. In plants, the strigolactone (SL) signaling pathway is crucial for the differential regulation of stem cell activity in various types of meristems. However, how tissue-specificity of SL signaling is achieved and why different meristems respond to SLs in an opposite manner is unknown.
Here we show that two members of the SL signaling-associated SUPRESSOR OF MAX2-LIKE (SMXL) gene family, SMXL4 and SMXL5, redundantly promote the activity of shoot and root apical meristems but repress cambium activity, essential for lateral stem growth. SMXL4 and SMXL5 are expressed along the whole vasculature in (pro)cambial and phloem tissues and transcriptome alterations and altered carbohydrate distribution in single and double mutants indicate a role of both genes in positively regulating sugar transport from source to sink tissues. Consistently, smxl4 smxl5 mutants exhibit an increase in SL sensitivity and defects in root growth which are both light- and sucrose-dependent. Moreover, similarly like mutations in SMAX1, the founding member of the SMXL gene family, smxl4 or smxl5 mutations are able to rescue several growth defects resulting from reduced SL signaling.

All together, we propose that SMXL4 and SMXL5 act as site-specific repressors of SL-signaling, which in turn regulates plant architecture, at least partly, by distributing primary energy metabolites over long distances.

P 8

Substrate preference in Dianthus gratianopolitanus Vill.

Florian Michling | COS, Biodiversity and Plant Systematics, Heidelberg (Germany).

We test on a large geographical scale, whether regionally limited gene flow between populations in endangered Dianthus gratianopolitanus Vill. can be interpreted to be the result of adaptation to edaphic conditions, namely parent rock type.
Our experimental setup consists of reciprocal transplantations onto substrates derived from limestone, rhyolite and serpentine rock. The experiment was carried out under common garden conditions for sixteen months in the Heidelberg Botanic Garden. We measured variables associated with reproductive fitness and growth performance. Additionally, concentrations of thirteen chemical elements were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES).
We detect significant differences in element uptake among plants originating from limestone and from siliceous bedrock only when cultivated on limestone and on serpentine soil. However, individual performance was not higher in cultivation on the native rock type and phenotypic variation was large within populations and not between.
Based on these results, we hypothesize that in the case of D. gratianopolitanus, adaptation to edaphic conditions, namely parent rock type, did not play a major role in shaping the species current day distribution range and genetic structure. It is likely that parent rock type acts only in combination with other factors, such as local climate and biological competition. Furthermore, these findings suggest that a high level of potentially adaptive variants are maintained in populations of this polyploid (2n = 6x).

P 9

Recapitulating the evolution of fly extraembryonic tissue: the role of mmp1

Francesca Caroti I COS, Heidelberg (Germany).

Evolution is characterized by changes of gene expression that lead to the generation of new morphological features between species. Here, we aim to understand such a step in change of shape by studying the formation of the fly extraembryonic tissue topology as an example.

During gastrulation, flies form a specialized extraembryonic tissue that eventually seals the embryo dorsally. In most flies and insects, this extraembryonic tissue consists of two distinct epithelia, the amnion and the serosa. In Drosophila melanogaster only a single epithelium is formed, the amnioserosa. In spite of this morphological difference, extraembryonic tissue in all flies is specified by the homeobox gene zerknüllt (zen). We are interested in the cellular and genetic mechanisms that changed during evolution and gave rise to the single epithelium observed in D.melanogaster.

To address this question we have focused our attention on the scuttle fly Megaselia abdita, which has been previously shown to develop a distinct amnion and serosa. Based on a combination of functional analyses and high resolution light-sheet in vivo microscopy, we found that the crawling of the serosa can be blocked by manipulating the expression of two factors: Mab-dorsocross (doc) and Mab-matrix metalloproteinase1 (mmp1). The knock-down of either of these two genes in M.abdita appears to recapitulate evolution, as it gives rise to a single dorsal extraembryonic tissue similar to the amnioserosa in D.melanogaster. Our results suggest that changes in the regulation of mmp1 are sufficient to explain the evolutionary transition from two to a single extraembryonic epithelium.

P 10

A proteomic and phosphoproteomic approach on early hydra head regeneration

Hendrik Petersen I University of Heidelberg, COS, Heidelberg (Germany).

The cnidarian freshwater polyp Hydra sp. exhibits an unparalleled regeneration capacity in the animal kingdom. Using an integrative transcriptomic and SILAC proteomic/ phosphoproteomic approach we studied stem cell based regeneration in Hydra polyps.
Our global analysis reveals two distinct molecular cascades: an early injury response and a subsequent, signaling driven patterning of the regenerating tissue with an upregulation of a large set of proteins after 0.5h and 6-12h, which is transcriptionally independent. Our data are in accord with a function of the recently discovered Wnt/STOP pathway, where canonical Wnt activity inhibits Gsk3 and subsequently proteasomal protein degradation. In our phosphoproteome analysis we identified a rapid activation/inhibition of Casein kinases 1 and 2 (CK1 and CK2) only 30 minutes after the injury stimulus. CK1 and CK2 are important regulators of the Wnt pathway acting downstream of LRP5/6 signalling.
Combining our proteome and phosphoproteome dataset, we propose a model where the injury response leads to an ligand independent activation of the Wnt pathway, potentially mediated by Ck1/2 and/or Gsk3 which then activates β-catenin dependent transcription of Wnt ligands and subsequently a ligand dependent activation of the pathway after 6-12h.

P 11

A device for imaging and asymmetric perfusion of Arabidopsis roots

Jagriti Shrivastava I Centre for Organismal Studies (COS), Heidelberg (Germany).

Plant roots are highly sensitive to changing environmental conditions such as water and nutrient availability, biotic and abiotic stresses. The response to change is observed in its phenotype and/or at molecular level which is regulated by complicated mechanisms that include perception of the signal, integration of the signal, and eventually response to environmental cues. Recently it has been reported that the long-distance communication from root to shoot is specific for salt stress perceived by roots (Choi et al., 2014). However, it is not yet fully understood how and why certain stimuli cause local, cell-autonomous responses, whilst others result in cell-cell communication and coordinated responses by tissues, organs or the entire plant.

To understand how roots perceive and process information about their change in environment we need tools that allow live imaging of roots and provide precise control over the root microenvironment. Over the past years, a number of microfluidic devices have been developed to cultivate and perfuse Arabidopsis roots at the microscope. These devices have substantially advanced experimental access to roots and some devices, e.g. the RootChip, allowed pulsed treatments (Grossmann et al., 2011). So far, in devices where roots grow in channels it has been difficult to locally apply treatments only to selected regions of the root, as the organ was usually perfused as a whole. Moreover, due to roots bending within observation chambers, an even perfusion was often challenging to achieve, thus affecting reproducibility of the treatments.

Here we present a new imaging and perfusion device for Arabidopsis roots that not only provides guidance to the root tip but also prevents bending of the root tip by centering the root within the chamber. Thus, allowing symmetric or asymmetric perfusion on either side of the root to distinguish between cell-autonomous responses and coordinated responses to changing environmental conditions.

P 12

Thrombospondin: a novel Wnt target gene in the lower metazoan Hydra.

Jennifer Strompen I COS, Molecular Evolution and Genomics, Heidelberg (Deutschland).

Among others, Wnt signaling controls initiation of neurogenesis and various studies have shown that thrombospondins (TSPs) play a major role in neurogenesis in particular in vertebrates. It has been demonstrated that immature but not mature astrocytes express thrombospondins 1 and 2 and that these TSPs promote CNS synaptogenesis in vitro and in vivo.

In Hydra, member of the phylum Cnidarians - nearest outgroup to bilateria - proteomic and genomic analysis revealed a great number of proteins with tsp-like repeats; however, only one true Thrombospondin. Hydra TSP is localized in the hypostome region of the adult polyp and emerging buds, where the head organizer is formed. Its expression is regulated by beta-catenin signaling, but its presence is not essential for organizer maintenance. We propose that Hydra TSP plays a role in inducing neurogenesis in newly forming buds and during regeneration, where it is highly upregulated.

P 13

Retinoblastoma pathway, an ancient tool to understand hierarchical shifts in development and evolution

Jubin Narendra Shah I COS, Heidelberg (Germany).

Plants and animals have evolved across millions of years to develop into present day organisms. An extremely complex network of molecular signatures underlies the developmental processes generating mechanistic diversity across both the systems. Gamete formation in animals and plants form intrinsic part of sex-specific developmental mechanisms controlled by a myriad of regulatory networks. One of the key components of these regulatory mechanisms is the RETINOBLASTOMA protein (RB), a tumor-suppressor protein known to control cell cycle and differentiation in multiple model systems. RETINOBLASTOMA-RELATED protein (RBR), a homologue of RB in Arabidopsis thaliana is known to principally follow conserved mechanism of cell cycle regulation. Recent work in Arabidopsis has also explained the role of RBR in cell differentiation and development. It remains to be explored whether or not the differentiation and cell cycle functions of RBR could be fully uncoupled. We aim to dissect the role of RBR and cell cycle-independent factors that function upstream and downstream of RBR during plant reproduction and evolution. Using both forward and reverse genetic approaches, we examine how the RBR regulatory network functions along plant evolution using extant model systems such as Arabidopsis thaliana (higher plant) and Physcomitrella patens (lower plant). Ongoing work presented here suggests the role of RBR networks (genetic, epigenetic and/or metabolic) connecting multiple signaling hubs at distinct developmental transitions. Together, combinatorial genetic, biochemical and computational approaches are anticipated to unravel evolutionary signatures of an essential signaling network.

P 14

Induction of gametogenesis in the symbiotic cnidarian Aiptasia sp., a model for coral biology

Liz Hambleton I COS, Heidelberg (Germany).

The tropical sea anemone Aiptasia sp. is a laboratory model for the endosymbiosis between reef-building corals and dinoflagellates (genus Symbiodinium spp.). Many important tools are currently available for Aiptasia and Symbiodinium, including multiple clonal lines and genomic and transcriptomic resources, thereby providing the basis for cell and molecular analysis. However, further development of the model system is currently impeded by the lack of a reliable laboratory spawning protocol. Here we identify the key environmental cues to induce reproducible spawning in Aiptasia under fully controlled laboratory conditions. We find that simulating a lunar cycle with blue-wavelength light in combination with increased temperature is necessary and sufficient to promote synchronous release of abundant gametes in well-fed animals. Sexual reproduction rates are genetically fixed and differ among strains under similar conditions. We show that, based on molecular phylogeny, the two female laboratory Aiptasia lines F003 and H2 belong to two distinct genetic networks and that they quantitatively differ in their sexual reproduction rates under similar conditions, with F003 exhibiting higher reproductive output than H2. Interestingly, we found that rates of asexual reproduction showed the opposite pattern. This study provides the requisite basis for further development of the Aiptasia model system, allowing analysis of basic molecular mechanisms in the laboratory as well as investigations of broad questions of ecological and evolutionary relevance.

P 15

Role of endogenous selection and edaphic adaptation in shaping the fitness of tetraploid Arabidopsis hybrids in Wachau suture zone

Long Li I COS, Biodiversity and Plant Systematics, Heidelberg (Germany).

The A. lyrata tetraploid hybrids in the Wachau suture zone have undergone genome duplication and have colonized a new siliceous habitat (potentially via adaptive introgression). However, tetraploid A. arenosa populations are the putative source of siliceous alleles. It thus provides an opportunity to investigate the relative contributions of endogenous vs. exogenous selection in shaping the substrate adaptation. Here, tetroploid populations from different ecotypes of each species are chosen and reciprocal crosses are performed between substrate within and between species. This design allows us to control for both substrate effects within A. lyrata (our focal species) and substrate effects between species (focusing on the changes calcareous A. lyrata populations have undergone in the colonization of siliceous substrates with or without the assistance of A. arenosa). Our common garden experiments will test the germination behavior and early seedling growth of the F1 hybrids and then evaluate their adaptive performance (relative to their parents) for substrate treatments experienced in siliceous and calcareous soils. Such a result may explain how putative adaptive introgression in the Wachau succeeded in generating stable F1 hybrids and the role of endogenous selection in shaping the local adaptation to new siliceous habitats within A. lyrata. These observations will set the basis for the prospective work and provide important information about the edaphic adaptation in Arabidopsis hybrids.

P 16

Regulation of dendritic growth relies on local and systemic signals

Aaron Ostrovsky I COS, Heidelberg (Germany).

We are interested in the mechanisms that regulate the dynamics of circuit formation in the embryo and the scaling of functional networks during post embryonic growth. Previous work has focused on fixed time points in development. In order to address the dynamic properties of growth, we have developed a system that allows us to perform intra-vital imaging of embryos and larvae during development. This system has single photon sensitivity, allowing us to image endogenous levels of synaptic proteins in the context of developing neural circuits.

We now show that in embryos, dendritic growth occurs primarily at the tips of the dendritic tree with rapid extension and retraction of filopodia (likely to be actin-rich). After hatching, growth in the dendrites changes; it now occurs through anisotropic scaling of the core (tubulin-rich) dendrites.

Previous work shows that ecdysone receptor (EcR) signaling is necessary for fast larval growth. Here we show that the RTK Alk is activated by Jelly Belly (Jeb) and is required for this prominent switch of growth behavior. We show that ALK signaling is activity independent and preliminary results indicate that it leads to the activation of the PI3K pathway, effectively overriding systemic nutritional information signaled through the insulin receptor.

To test this hypothesis we have pioneered a novel technique (dFLEX); this allows us to mutate genes in single cells or target fluorescent tags to endogenous proteins such that their trafficking and distribution can be assessed without causing artifacts common to over-expression paradigms.

P 17

The dorsal folds in Drosophila as approach to study robustness in development

Lucas Schütz I Universität Heidelberg, Centre for Organismal Studies, Heidelberg (Deutschland).

Gastrulation is a key feature of all higher animals and results in the formation of three distinct germ layers. Far, but also closely related species exhibit various climatic conditions during development; however, gastrulation seems to be robust enough to work under these conditions. Which structures exist to create or promote this robustness remains to be solved. In Drosophila melanogaster, a well described model system, almost every single of the more than 6000 cells moves during gastrulation. Muvi-SPIM microscopy shows, that these cells move coordinated in several groups. We suppose that certain mechanisms and structures, which coordinate and regulate this motion, are present in the embryo. The dorsal folds and the cephalic furrow are two of these structures to be considered for such a function. The cephalic furrow is an evolutionary novelty which is new within the Diptera, it surrounds the embryo, separates the head from the trunk region and rotates during germband extension. The dorsal folds are located on the dorsal half of the embryo; their shape changes during germband extension. Both the dorsal folds and the cephalic furrow could act as barriers within the streams of moving cells during germband extension. We hypothesize that structures like the dorsal folds and the cephalic furrow are necessary to make germband extension robust. To test this we will analyze the dorsal fold size in Drosophila species from multiple climatic regions in fixed tissue and complement this with Muvi-SPIM data to show the interaction of cells with furrows and folds of different sizes.

P 18

Roles of Cytoskeletal Components for Growth and Navigation of Axons

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During formation and regeneration of the nervous system, neurons send out axons (to interconnect with other neurons) which carry a dynamic structure at their tip, the growth cone. On its way, the growth cone senses directional cues via its plasma membrane proteins which trigger intracellular signalling cascades affecting components of the microtubule- and microfilament-based cytoskeleton which reorganizes and drives changes in growth direction. By linker proteins, the plasma membrane proteins can be also physically connected to the cortical cytoskeleton, an actin-spectrin lattice.
Using a knock out mouse lacking a microtubule-associated protein (MAP), we investigated the behaviour of retinal ganglion cell (RGC) axons in vitro and in vivo. Absence of the MAP leads to stronger lateral activities of navigating growth cones and a disturbance of advance, stop and retraction phases, indicating a role of the MAP in regulating the exploratory behaviour and travel rhythmicity of growth cones. RGC axons lacking the MAP display an aberrant navigation behaviour in the developing retina, leading to a severe routing phenotype. We are currently investigating the dynamics of microtubules and microfilaments inside w.t. and k.o. growth cones. In addition, we could show by fluorescence complementation assays that the cortical cytoskeleton beneath the cell membrane is linked to an axonal cell adhesion molecule (CAM), an integral plasma membrane molecule, via a set of linker proteins. At the moment, we are creating a biomimetic cortical cytoskeleton on a nano-patterned scaffold which enables us to analyse its molecular interactions and physical properties, for example by atomic force microscopy.

P 19

Sub-functionalization of a Nicotiana specific COI1 homolog is required for jasmonate-dependent regulation of floral maturation traits and defensive root metabolism

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Jasmonic acid and its derivatives act as central signaling compounds in many aspects of a plant's development and resistance to biotic stresses. At the molecular level, the binding of jasmonoyl-L-isoleucine to an F-box protein, CORONATINE INSENSITIVE1 (COI1), is required for most, if not all, jasmonate-dependent responses. The role of this signaling pathway in flower anthesis and pollinator attraction or root defensive metabolism is not fully understood. We identified a Nicotiana-specific duplication of COI1 which translated into the selective retention of COI2, a gene coding for a possible alternative jasmonate receptor. To dissect if this receptor is functional, and if so, what are the functional consequences of this duplication event for tissue-specific jasmonate signaling specialization, we used stable RNAi transformation to produce COI1-, COI2- and COI1/COI2-silenced Nicotiana attenuata plants. This wild tobacco species develops sympetalous flowers with complex pollination biology and employs a sophisticated set of inducible metabolic defenses regulated by jasmonate signaling. Results from combined transcriptomic, targeted metabolic, and allometric analyses highlighting synergistic controls exerted by COI1/COI2 over defensive root metabolism and flower opening will be presented.

P 20

A Time-Calibrated Road Map of Brassicaceae Species Radiation and Evolutionary History

Nora Hohmann I COS, Heidelberg (Germany).

The Brassicaceae include a number of major crop plants and important model species such as Arabidopsis thaliana, A. halleri, Brassica, Boechera, Thellungiella, Capsella, Cardamine and Arabis species. With the rapidly increasing number of diverse datasets collected from within the Brassicaceae, the entire family is becoming a model for comparative evolutionary research. However, available divergence time estimates are biased either by insufficient fossil calibration or limited DNA sequence information. We present a comprehensive time-calibrated framework with some important divergence time estimates based on whole-chloroplast sequence data for 29 Brassicaceae species. Diversification of the Brassicaceae crown group started at the transition from Eocene to Oligocene (32 million years ago, mya) in Eurasia, which demarks the onset of major environmental changes. Subsequent evolutionary splits giving rise to major evolutionary lineages are dated to about 20 mya, coinciding with the Oligocene to Miocene transition, with increasing drought and aridity and transient glaciation events. The age of the Arabidopsis crown group is 6 mya, at the Miocene and Pliocene border, and the onset of major radiation within Arabidopsis started less than 2 mya. The overall species richness of the family is well explained by high levels of neopolyploidy, but this trend is neither associated with an increase in genome size nor is there a lineage-specific constraint. Our results highlight polyploidization as an important source for generating new evolutionary lineages adapted to dramatically changing environments, and we conclude that species radiation, paralleled by high levels of neopolyploidization, follows genome size decrease, genome stabilization, and genetic diploidization.

P 21

The cephalic furrow is an evolutionary novelty of flies

Özge Akbulut I COS, Heidelberg (Germany).

Gastrulation describes an early, dynamic stage of embryonic development, during which embryonic form arises with the generation of the three germ layers. This process differs between fly species, and thus provides a well-suited experimental system to first identify the genetic bases for morphological divergence. One particular aspect of fly gastrulation in Drosophila melanogaster is the formation of a temporary head fold (cephalic furrow). This fold is a novel feature that originated during fly evolution since it doesn't exist in mosquitoes or other, more basal insects. In D. melanogaster, positioning of cephalic furrow is defined by the overlapping expressions of gap gene button head (btd) and the anterior-most stripe of the pair-rule gene even-skipped (eve), and it has been shown that in flies mutant for either one of these two genes the cephalic furrow doesn't form during gastrulation. Preliminary data has suggested that eve and btd do not overlap in the mosquito-related midge Chironomus riparius. The lack of eve/btd co-expression and the absence of a cephalic furrow in C. riparius led me to hypothesize that ectopic co-expression of btd and eve genes in C. riparius may result in cephalic furrow or cephalic furrow-like structure. I am currently testing this hypothesis during my MSc thesis.

P 22

Phenotyping 2.0 - (new) tools to quantify the evolution of shape.

Paula Gonzalez I COS, Heidelberg (Germany).

Current studies to understand the evolution of shape have been pushed by parallel advances in genetic tools to manipulate the genome together with imaging methods to evaluate the effect of the genetic modifications performed. The results usually consist of a considerate amount of image data from which specific phenotypes have to be defined despite the omnipresence of biological variability. This new phenotyping challenge has to be addressed by creatively combining image and data analysis tools to find ideal measures that describe and classify our observations.

We were faced by this challenge in the context of studying the evolution of morphology in early fly development. Here, we present the analysis and visualisation language that arose from the necessity to quantify morphological differences of mesoderm invagination between species and the effects of specific gene manipulations.

P 23

Introducing Aiptasia as laboratory model to analyze cnidarian endosymbiosis

Philipp A Voss I Heidelberg University, Centre for Organismal Biology, Heidelberg (Germany).

Coral reefs are the most biodiverse marine ecosystems and of high ecological as well as economic importance. Corals (phylum Cnidaria) thrive in very nutrient-poor waters and their immense productivity strictly depends on a functional symbiosis between corals and dinoflagellates of the genus Symbiodinum. The photosynthetic symbionts reside within the gastrodermal host cells and transfer energy to the host. Despite the importance of coral reefs we only have limited knowledge about the cellular basis of symbiosis establishment, maintenance and breakdown (also known as “coral bleaching”). The lack of cellular information about the ecologically critically symbiosis is mainly due to the fact that corals are not suited as laboratory model organisms allowing cellular and molecular analysis.

Here we introduce Aiptasia, a marine sea anemone, as an emerging laboratory model organism to analyze cnidarians endosymbiosis. Aiptasia, just as many reef-building corals, acquires symbionts during planula larval stages from the environment anew each generation. To develop Aiptasia into a model system allowing analysis at the cellular and molecular level, a detailed description of the cellular processes involved in symbiosis establishment and maintenance as well the development of functional tools are required. To date, we have generated a detailed description of Aiptasia embryonic development and different planula larval stages. Moreover, we have analyzed when and where symbionts are intracellularized and find that Aiptasia larvae take up symbionts at a constant rate as early as two days 2 post fertilization (pf) until ten days pf, but uptake efficiency increases thereafter. Accordingly, the region of efficient symbiont uptake changes over time. Initially (day 2-day 8 pf), symbionts are predominantly taken up in the aboral region of the gastric cavity, while later the uptake region expands to the more oral areas of the cavity. Moreover, we have developed in situ hybridization protocols to analyze gene expression in Aiptasia planula larvae and are currently developing further techniques to extend the tool-kit for cellular and molecular analysis.

P 24

Analysis of the Optic Fissure Closure

Priska Eckert I University of Heidelberg, COS, Heidelberg (Germany).

During vertebrate eye development, a fissure (optic fissure) is established at the ventral pole of the eye. This optic fissure is an important migration route for mesenchymal tissue into the eye. The fissure, however, is only transient and must be closed as development proceeds. If the closure fails a Coloboma occurs, which is a cause for blindness in children. Different processes have to be coordinated to allow the fissure to close, the hyaloid vessels have to be removed from the fissure, the basal membrane of the optic fissure margins has to be dissolved and the fissure margins have to meet and eventually fuse. In this study we want to shed light on the mechanisms underlying the optic fissure closure. We used zebrafish (danio rerio) as a model organism to address with 3D time-lapse imaging and immunohistochemical stainings the processes during fissure closure. We found that the basal membrane is degraded shortly before the fissure margins meet. Furthermore we found that the fusion is initiated by cells of the future retinal pigment epithelium (RPE). We also observed the fusion in close proximity to the hyaloid vasculature. Based on our findings we hypothesize that the cells, which get in touch first during fusion, use the hyaloid vessels as a scaffold to come closer to each other. Finally we found that the differentiation process in the fissure takes at least 48 hours longer than in the remaining retina. In future analyses it will be important to further follow the cells which facilitated the fusion to study their fate. Our findings help us to understand the physiological processes of optic fissure closure in more detail. Furthermore they enable us to address coloboma phenotypes appropriately. Our findings might even be important for closure defects in other tissues e.g. cleft lips/ palates.

P 25

Multi-parameter analysis to distinguish between different ways of communication in Arabidopsis roots.

Rik Brugman I COS, Grossmann Lab, Heidelberg (Germany).

Plant roots are exposed to continuously changing environmental conditions and have to adjust their physiology, development, growth rate and direction accordingly. The wide range of environmental changes that a root has to deal with can be divided into biotic and abiotic stimuli. To achieve efficient responses to both classes of stimuli plants cells have developed means to sense signals and communicate within and across tissues. While some responses might require immediate action from the whole plant, other responses act locally and only involve few cells or a single tissue. This heterogeneity in responses calls for different ways of communication between cells. Calcium is a ubiquitous second messenger in plants. Many stimuli lead to a transient rise in intracellular calcium concentrations. While it is still unknown how signals are communicated between cells, these rises in calcium concentration can be used as readout to unveil different communication strategies within the roots. We dissected the calcium communication in roots by breaking it down into measurable parameters like the starting point of the signal, the velocity with which it travels, the cell types involved and the frequency and duration of the signal. We use these parameters to distinguish between different modes of communication in roots upon stimulation with different stresses.

P 26

Ectopic expression of Fog and T48 in mosquitoes mimics D.melanogaster-like cell behaviour of coordinated tissue involution

Silvia Urbansky I Centre for organismal Studies, Heidelberg (Germany).

During gastrulation of Drosophila melanogaster, the presumptive mesoderm involutes as a coherent sheet along the ventral midline. Here pulsed apical constriction initiates cells shape changes of mesoderm cells, which acquire a wedge shape and form a coordinated furrow, which deepens and closes to a tube. The transcription factors Twist and Snail control the contraction of the apical actin network, by activating GPCR-signaling via folded gastrulation (Fog), promoting an apical localisation of RhoGEF2 via concertina and T48.
This mode of mesoderm internalization through epithelial involution is not conserved throughout flies. In mosquitoes, the mesoderm has been shown to invaginate without a defined ventral furrow, and specifically in the nematoceran midge Chironomus riparius we could show that mesoderm cells ingress individually and in a stochastic fashion. To identify the genetic basis for the observed morphogenetic difference in mesoderm formation in D. melanogaster and C. riparius, we performed a systematic assessment of expression profiles of the respective signalling pathway components. We found that fog and T48 were expressed differently between D.melanogaster and C.riparius, and by ectopic over-expression of C.riparius fog and T48 orthologues in blastoderm embryos we were able to alter the invagination behaviour of mesoderm cells from stochastic ingression towards a coordinated involution. Using quantitative image analysis we could show that mesoderm cells show a more D.melanogaster-like behavior after fog and/or T48 over-expression in that (i) cell organisation was more coordinated and (ii) the mesodermal tissue invaginated deeper into the embryo. Our results suggest that tissue level organisation during mesoderm internalisation in flies originated by two simple modifications of a known and otherwise highly conserved developmental regulatory network.

P 27

Hydra Head regeneration and the Impact of Wnt-Signaling

Stefanie Höger I COS Heidelberg, Molecular Evolution and Genomics, Heidelberg (Germany).

The cnidarian freshwater polyp Hydra sp. exhibits an unparalleled regeneration capacity in the animal kingdom. Using an integrative transcriptomic and SILAC proteomic/ phosphoproteomic approach we studied stem cell based regeneration in Hydra polyps.
Our global analysis reveals two distinct molecular cascades: an early injury response and a subsequent, signaling driven patterning of the regenerating tissue. A key factor of the initial injury response is a general stabilization of proteins and a net upregulation of transcripts, which is followed by a subsequent activation cascade of signaling molecules. As major contributors to head regeneration we identified genes that are involved in cell cycle and cell death regulation, pathways involved in MAP kinase signaling and proteolysis and members of the Wnt-pathway. The first Wnt ligand that increased was HyWnt3 followed by other Wnts. We also found essential members of the Wnt pathway that have not been identified so far in hydra, e.g. the LRP5/6 co-receptor, which is funneling a number of different Wnt ligands, and several orthologous antagonists and agonists of Wnt signaling, that are all involved in the long-range control of Wnt gradients and in Wnt-dependent patterning. Analysing the transcriptome of a transgenic Wnt3-overexpression line solidified these data.
Remarkably, we also found an enrichment of evolutionarily more recent genes in the early regeneration response and genes specific to the early injury response were enriched in transposon insertions. Genetic dynamicity and taxon-specific factors might therefore play an important role in activating a conserved patterning cascade in Hydra regeneration.

P 28

The Evolution of Mechanosensation: Transient Receptor Potential (TRP) Channels in the Lower Metazoan Hydra

Suat Özbek I Heidelberg University, COS, Molecular Evolution and Genomics, Heidelberg (Heidelberg).

TRP channels are a superfamily of cation channels that play critical roles in sensory physiology, contributing to processes as diverse as vision, olfaction, pain, mechano- and thermosensation. They are activated by a variety of stimuli and enable cells and organisms to adapt to their local environment. More than 50 TRP channels have so far been identified throughout the metazoan kingdom reaching from yeast to mammals. We have previously shown that in cnidarians, basal metazoans that evolved the first nervous system, TRP channels are unusually diverse. This offers the opportunity to study TRP molecules both on the level of structure/function analysis and comparative evolution in a simple model system. In cnidarians, prey capture by the characteristic stinging cells (nematocytes) is facilitated by a highly sophisticated mechanosensory apparatus, the cnidocil. Nematocyte discharge, which is triggered by mechanical and chemical stimuli, is accompanied by a calcium influx that is very likely induced by a TRP channel associated with the cnidocil. In this project we want to characterize the TRP channels responsible for nematocyte development and function. We additionally screen for novel chemical TRPN channel effectors in cooperation with the institute of organic chemistry, KIT. The characterization of the Hydra TRP channel will provide answers about the function and evolution of mechanosensation in early metazoans. As the cnidarian cnidocil represents a prototypic hair cell it will furthermore have important implications for TRP functions in vertebrates and genetic disease models.

P 29

Exploring olfactory learning and Mushroom Bodies function in the marine Annelid Platynereis

Thomas Chartier | EMBL, DB Unit, Heidelberg (Germany).

Mushroom Bodies, a brain structure supporting memory and associative learning in Insects, are not only found in other Arthropods like Chelicerates, but also as far as in distantly-related marine Annelids such as the Polychaete Platynereis. In these marine predatory worms, whose evolutionary split from Arthropods dates back to more than 500 million years ago, Mushroom Bodies show a strikingly similar morphology with those of Insects, and recent molecular evidence have even revealed some resemblance with the Vertebrate pallium. Even though Annelid Mushroom Bodies were described more than one century ago, their function however was never investigated, and behavioral studies in Polychaetes overall are scarce. For the purpose of comparative studies, there is thus a strong interest in developing behavioral protocols in Polychaetes to test the repeatedly suggested role of this brain structure in learning and memory, and help understanding the ancestral state of a putative learning center in early Protostomes animals. Our animal model, the lab-friendly ragworm Platynereis, has available transcriptomic resource, and is amenable to classical anatomical studies, but also to a variety of functional neurobiological approaches including live calcium imaging, laser ablations, pharmacological treatments, gene knock-outs and knock-downs. The aim of my PhD project is to establish a quantitative assay for olfactory conditioning in Platynereis, and to conduct a functional study of Mushroom Bodies.

P 30

Targeting of the V-ATPase in Arabidopsis Thaliana

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The vacuolar H+-ATPases (V-ATPases) are multisubunit complexes that are responsible for the acidification of various cellular compartments in all eukaryotic cells. The localization of this multifaceted enzyme is determined by the isoforms of the membrane integral subunit, VHA-a. The incorporation of VHA-a1 targets the V-ATPase to the trans-Golgi network/early endosome (TGN/EE) whilst the incorporation of VHA-a2 and VHA-a3 targets the V-ATPase to the tonoplast. The molecular mechanisms responsible for differential V-ATPase trafficking have not yet been determined. In order to discern the elusive targeting signal in VHA-a1, we made use of chimeric constructs which consisted of increasing lengths of the VHA-a1 N-terminus fused to decreasing lengths of the C-terminal domain of VHA-a3. By this approach we have narrowed the responsible region down to 50 base pairs. In our quest to uncover the targeting signal in VHA-a3, we have initially focused on two conserved cysteine residues in the N-terminus of all VHA-a3-related sequences. We could show that these residues are the target of the S-acylation of VHA-a3, however we found that S-acylation is not responsible for tonoplast targeting. We are thus now investigating the alluring role of S-acylation in the regulation of the tonoplast V-ATPase activity and membrane subdomain partioning.

P 31

A high-resolution expression map of the inflorescence stem – insights into the development of a differentiated plant organ

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Even though plant stems contribute fundamentally to the variation in architecture and growth of plant bodies, the molecular regulation of stem development has hardly been explored. An essential piece of knowledge in this context is information about genome-wide profiles of gene expression in individual stem tissue types. Similarly as for high-resolution expression maps of the root, this knowledge would be key for the formulation of global concepts in developmental and physiological terms. However, strong cell walls in mature stems hamper efficient protoplast-based profiling as done previously during similar approaches for shoot and root apical meristems. Therefore, we have initiated a project employing fluorescence-based nucleus sorting in order to get access to tissue-specific mRNA from Arabidopsis stems. By using a comprehensive series of tissue-specific promoters driving the expression of a nucleus-targeted fluorescent protein (H4-GFP) and subsequent nucleus sorting, we are now able to determine the transcriptomes of all major stem tissues. As a proof of principle, by taking advantage of the APL promoter, we identified the transcriptome of the stem phloem. From 12,000 genes being expressed in the phloem we classified 335 genes as being predominantly active in this tissue. We expect that the analysis of transcriptome remodelling during stem thickening will help deciphering cell fate acquisition and physiological adaptations during postembryonic growth processes in plants.

P 32

Principles of Crucifer Evolution

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Brassicaceae (Cruciferae) is an important model family for evolutionary and molecular research, comprising 49 tribes, 321 genera, and about 3700 species. It comprises not only the most important model for molecular plant biology (Arabidopsis thaliana) but also important crop plants such as rapeseed and cabbage. We will focus on the correlations between environmental and evolutionary changes within the family (e.g. radiation, speciation rates, polyploidization, distribution range dynamics, etc.). Evolutionary processes can only be understood if the correlations between environmental dynamics and evolutionary changes are known within a well-resolved temporal context. Backbone phylogenetic reconstructions will be performed using whole chloroplast genomes to minimize the bias caused by the choice of molecular markers. Then, a reliable time scale of Brassicaceae evolution will be generated by combining evolutionary rates, fossil calibration, and phylogeographic constraints. Based on previous backbone time scale, every tribe in the family will be analyzed separately based on ITS datasets for more than 50% of all 3700 species. Furthermore, recurrent polyploidization has played an important role in the evolution of Brassicaceae. Chromosome number change will be inferred based on the tribe level phylogenetic trees, helping us to explain the haploid numbers observed today. The Brassicaceae is thought to have originated in the Irano-Turanian region, where the highest species diversity is found, and originated as a tropical/subtropical family. Reconstructions of ancestral areas will be performed, combining the WWF Ecoregions. We try to provide insights about the origin of Brassicaceae, and correlate evolutionary processes in space and time.

P 33

β-Catenin and Bmp signalling induce the semi-centralized nervous system in the sea anemone Nematostella vectensis

Hiroshi Watanabe I Centre for Organismal Studies, Department of Molecular Evolution & Genomics, Heidelberg (Germany).

The emergence of condensed nervous systems such as bilaterian central nervous system (CNS) is one of the key events in animal evolution. It is yet unknown how the basic mechanisms of the formation of a CNS evolved. We addressed this question in Nematostella vectensis (Cnidaria), the ancient sister group of bilaterians. We found that β-Catenin signalling is crucial for the development of the orally condensed nervous system, semi-centralized nervous system (semi-CNS), during early embryogenesis. β-Catenin activity induces Bmp signalling, which, at later larval stages, becomes indispensible for the maintenance and asymmetric patterning of the semi-CNS along the secondary (directive) axis of the larvae. We hypothesize that the consecutive and functionally linked involvement of β-Catenin and Bmp signalling in the formation of the cnidarian semi-CNS reflects an ancestral mechanism that evolved before the cnidarian/bilaterian split.

P 34

Wnt signalling in fate determination of retinal precursors

Eva Möller I COS Heidelberg, Heidelberg (Germany).

Wnt signalling has been proposed to direct cell division planes in vitro and that way potentially directly governs cell fate decisions. The fish retina provides an ideal experimental environment with continuous, life-long proliferation and differentiation within a domain expressing all components of the canonical Wnt signalling pathway. We target single cells in the fish retina to directly address the role of Wnt in directing cell fate decisions with a cell biological readout. Our questions relate to the instructive input of a localized Wnt source directing asymmetric divisions of retinal precursors. We aim to understand how canonical Wnt signalling downstream of the receptor mediates the signals that ultimately result in an asymmetric distribution of cell fates. To this end, we specifically study the role of Wnt signalling in the differentiating lineage of retinal progenitors. We applied our conditional, Cre/loxP based, tools to trigger expression of dominant negative Wnt signalling components in individual retinal precursors to address competence and concentration dependent fate decisions. Our data suggests that upregulation of Wnt signalling drives retinal stem cells into dormancy & progenitor cells into proliferation. Furthermore, depending on the progenitor state/position within the CMZ the differentiation potential may be limited to a subset of retinal cell fates. However, the different progenitor cell populations and the specific role of Wnt signalling within those populations remain elusive.

P 35

Integration of Cell Wall Signalling and Growth Regulation in Arabidopsis thaliana

Eleonore Holzwart I COS, AG Wolf, Heidelberg (Germany).

The growth of plant cells fundamentally differs that of animals. Almost all plant cells are encapsulated by a rigid, yet dynamic, extracellular structure, the cell wall. In the absence of cell migration, plant cells grow essentially by modifying the properties of their walls, which allows the intracellular turgor pressure to displace cell wall polymers, resulting in irreversible strain. After completion of the expansion, cell wall integrity is consolidated through deposition of new cell wall material and again, the activity of cell wall remodelling enzymes. The expression and activity of these enzymes are controlled by growth promoting hormones such as auxin, gibberellic acid (GA), and Brassinosteroids (BRs). Because of this intricate mechanism of growth, it has long been assumed that the state of the cell wall is monitored to relay information to the cytosol and to elicit an adequate compensatory response when cell wall integrity is compromised. Recently, such a surveillance mechanism was shown to be triggered by modification of the pectin component of the cell wall, leading to an activation of the BR signalling pathway. As cell wall modifying enzymes are overrepresented among BR targets, this signalling module was suggested to function in ensuring cell wall homeostasis during growth. Cell wall-mediated activation of BR signalling depends on the presence of receptor-like protein 44 (RLP44), which interacts with the BR receptor complex and is sufficient to activate BR signalling. Thus, integration of cell wall and BR signalling most likely occurs at the level of the BR receptor complex. Cross-talk with BR signalling has been demonstrated for other signalling pathways before, however, in these cases signalling converged mostly on downstream components such as transcription factors. Combining biochemical, genetic, structural and physiological approaches, this project aims at understanding how signalling from the cell wall is processed by intracellular growth regulation and to reveal the mechanism of signalling integration with the BR pathway. BR hormones are perceived by the receptor-like kinases BRI1 and BAK1, both of which were shown to interact with a crucial component of the recently identified cell wall signalling pathway. Here, we want to study dynamics and regulation of complex formation between the relevant proteins during development, as well as reveal the consequences of these interactions on the activity of the BR receptors. In addition, the contribution of cell wall and BR signalling integration to the control of plant growth through gene regulation and cell wall modification will be assessed.

P 36

A novel regulator of beta-Catenin-dependent organizer formation and maintenance in Hydra

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The organizer has a crucial role in setting up the body axis. In Hydra, the hypostomal head organizer acts as a signalling center that maintains and initiates the primary body axis in steady state polyps and during budding or regeneration. Secreted factors of the Wnt protein family act as primary signalling cues controlling this process.

It is commonly accepted that Hydra's head organizer is responsible for pattern formation by providing positional information to the epithelial cells of the body column. According to the Gierer-Meinhardt theory of de novo pattern formation, a diffusible long-range inhibitor is crucial in maintaining this gradient by interacting with the autocatalytic activation center. An autocatalytic feedback-loop based on TCF binding sites in its promotor region has been clearly demonstrated for Hydra Wnt3 that acts as the earliest Wnt ligand in a cascade of Wnt signalling activity during organizer formation.

Although several candidates like Hydra Dkk1/2/4 are discussed as possible antagonists, there is no complete picture of the molecular players involved in the proposed "activator-inhibitor system" in Hydra. In the present application, a novel factor is presented that could play a crucial role in this process. A metalloproteinase of the astacin family, designated as Hydra NAS-15-like, has been identified in a screen for Hydra Wnt3 processing factors. NAS-15-like expression is shown to be beta-Catenin-dependent and, in steady-state polyps, to form a gradient reflecting the proposed gradient of beta-Catenin activity in Hydra's body.

We propose that Hydra NAS-15-like acts downstream of beta-catenin as a negative feedback regulator of Wnt activity on the protein level providing a limitation of high canonical Wnt activity to the hypostomal organizer.

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