Helgoland Excursion 2023 Developmental Biology & Comparative Molecular Biology of Marine Organisms Biologische Anstalt Helgoland 14th-23rd June 2023

Ernst-Martin Füchtbauer, Molecular Biology & Genetics, Aarhus University Achim Paululat / Heiko Harten, Zoology & Developmental Biology, Osnabrück University Peter Heimann, Bielefeld University

Contents

A. G	General information Addresses, tide schedule Maps Names of participants Seminar topics Literature Introduction	2 3 4 5 6 9
В.	Fertilization and embryonic development	10
B.1	Spiralia: polychete worm <i>Pomatoceros triqueter</i>	11
	Sea Urchins: <i>Psammechinus miliaris</i>	13
	Preparation of isolated cortex membrane and release of cortical granules	14
	Isolation and re-aggregation of sea urchin blastomeres	15
B.3	Chordates: Ciona intestinalis	16
	Self-fertilization	17
	Removal of egg envelopes in ascidians	18
	Experiments on cell lineage Cell ablation	19
	Separation of blastomeres	20
	Autofluorescence and AChE staining of muscle lineage	21
	Detection of AChR and tissue specific proteins	22
B.2	General experimental manipulation of the early development	24
	Influence of external ions on early development	24
	Role of maternal mRNA (Cycloheximide, Actinomycin D)	25
	Effects of cytoskeleton toxins (Colchicine, Cytochalasin D)	25
C. B	liochemical methods	26
-	Enzyme histochemistry	26
••••	AChE	26
	AP	27
C.2	Antibody staining	28
	Staining of cartilage and bone	31
C.4	Differentiation and protein pattern:1D - SDS-electrophoresis	33
C.5	Immunoblot	40
	Iolecular methods (PCR, RT-PCR, <i>in situ</i> hybridisation)	41
	DNA barcoding	42
	Analysis of gene expression by RT-PCR	42 43
	Analysis of gene expression by RNA <i>in situ</i> hybridization	5 5
2.0		01
E. P	lankton: Larval stages of vertebrates and invertebrates	59
F. A	ppendix with larval stages	71

A General Information

Mail address:

Wilhelm-Mielck-Haus Biologische Anstalt Helgoland D 27491 Helgoland

https://www.awi.de/ueber-uns/standorte/helgoland.html

Director: Karen Wiltshire, Professor, PhD

Responsible for lab courses: Herr Uwe Nettelmann Tel: 0049 171 331 3267 Administration: Kathrin Böhmer Tel: 0049 4725 819 3282

Tide table Helgoland, June 2023

Tag	Juni			
ray	HW	- Zeit	NW	- Zeit
14 Mi	9:38	22:01	3:56	16:20
15 Do	10:37	22:58	4:56	17:20
16 Fr	11:27	23:50	5:48	18:13
17 Sa		12:14	6:36	19:03
18 So 🗣	0:38	12:57	7:20	19:48
19 Mo	1:22	13:36	8:00	20:27
20 Di	2:02	14:14	8:36	21:05
21 Mi	2:40	14:50	9:12	21:41
22 Do	3:16	15:25	9:45	22:14
23 Fr	3:51	15:59	10:17	22:49



Participants, e-mails, phone numbers

Participants from Osnabrück			
Name	E-mail	Mobil phone)	
Lara Deters	lardeters@uni-osnabrueck.de	+49 1727467989	
Merle Gallus	mgallus@uni-osnabrueck.de		
Maja Ludwig	majludwig@uni-osnabrueck.de	+49 15233947456	
Bakeeran Pathmalolan	bpathmalolan@uni-osnabrueck.de	+49 1622703307	
Anna Pollinger	apollinger@uni-osnabrueck.de	+49 1711679815	
Karina Rosin	krosin@uni-osnabrueck.de	+49 1746173250	
Anita Ruf	aruf@uni-osnabrueck.de	+49 17655488166	
Luisa Vehling	lvehling@uni-osnabrueck.de	+49 15209601126	
Alea Znamiec	aznamiec@uni-osnabrueck.de	+49 1605648749	

Participants from Aarhus			
Name	E-mail	Mobil phone	
Eva Sofie Bovbjerg	202005057@post.au.dk	+45 25173800	
Fanny Silke Jaeger Hansen	202007046@post.au.dk	+45 52400533	
Jonas Lønskov	jonlo@biomed.au.dk	+45 41691857	
Mina Ansari-Jou Bendz	202005941@post.au.dk	+45 25173800	
Thilde Phie Hjorth	201910250@post.au.dk	+45 21620124	
Thomas Gravgaard Andersen	202007435@post.au.dk	+45 53707256	

Теа	Teacher from Aarhus and Osnabrück				
	Name	E-mail	Mobil phone		
os	Achim Paululat	Achim.paululat@uni-osnabrueck.de	+49 178 282 6833		
OS	Heiko Harten	heiko.harten@biologie.uni- osnabrueck.de	+49 174 476 2349		
AA	Ernst-Martin Füchtbauer	emf@mbg.au.dk	+45 2899 2238		
BI	Peter Heimann	peter.heimann@uni-bielefeld.de	+49 157 73824024		

Seminar topics June 2023

No	Торіс	Names	Chair
I	Fertilization		
1	Genetics of self-sterility in C. intestinalis	Thomas Gravgaard Andersen	9
2	Sperm chemosensation in sea urchin	Anita Ruf	10
3	Zona pellucida Fetuin	Karina Rosin	11
=	Development and Regeneration		
4	Muscle development in ascidian	Luisa Vehling	12
5	Eye development in C. intestinalis	Eva Sofie Bovbjerg	13
6	HH signaling in L/R asymmetry in sea urchin and amphioxus	Fanny Jaeger	14
7	Tooth regeneration in sharks	Bakeeran Pathmalolan	15
III	Molecular Biology		
8	CRISPR in C. intestinalis	Jonas Lønskov	1
9	CRISPR in <i>L. pictus</i>	Alea Znamiec	2
10	Microbiome in sea urchins	Thilde Hjorth	3
IV	Phylogeny and Evolution		
11	Noctiluca phylogenomics & symbiosis	Anna Pollinger	4
12	Phylogenetic origin of the nervous system	Mina Ansari-jou Bendz	5
13	On the origins of ion homeostasis and gas exchange in vertebrates	Maja Ludwig	6
V	Physiology		
14	Heart function in <i>C. intestinalis</i>	Lara Deters	7
15	Temperature adjustment in fish	Merle Gallus	8

The column 'Chair' indicates for which topic you should introduce the speaker, prepare questions, and lead the discussion.

Literature:

I Fertilization:

1. Self-sterility in Ciona intestinalis

Sawada H. et al. (2020). Three multi-allelic gene pairs are responsible for self-sterility in the ascidian Ciona intestinalis. *Scientific Reports* 10:2514. https://doi.org/10.1038/s41598-020-59147-4 1.

Takako Saito and Hitoshi Sawada (2022). Fertilization of Ascidians: Gamete Interaction, Self/Nonself Recognition and Sperm Penetration of Egg Coat. *Frontiers in Cell and Developmental Biology* 9. doi:10.3389/fcell.2021.827214.

Hashimoto S et al. (2022). Removal of the block to self-fertilization by low- calcium artificial seawater in the ascidian Ciona intestinalis. *Zygote* 30: 738–742. doi:10.1017/S0967199422000144.

2. Sperm chemosensation in sea urchin

Seifert, R., Flick, M., Bönigk, W., Alvarez, L., Trötschel, C.,Poetsch, A., et al. (2015). The CatSper channel controls chemosensation in sea urchin sperm. *The EMBO Journal*, 34(3), 379–392. <u>http://doi.org/10.15252/embj.201489376.</u>

Strünker T., Alvarez L., Kaupp U.B. (2015). At the physical limit - Chemosensation in sperm. *Current Opinion in Neurobiology* 34: 110-116. doi:0.1016/j.conb.2015.02.007.

3. Zona pellucida Fetuin

Dietzel, E., Wessling, J., Floehr, J., Schäfer, C., Ensslen, S., Denecke, B., et al. (2013). Fetuin-B, a liver-derived plasma protein is essential for fertilization. *Developmental Cell*, 25(1), 106–112. <u>http://doi.org/10.1016/j.devcel.2013.03.001.</u>

II Development and Regeneration

4. Muscle development in ascidian embryos

Nishida H, Sawada K. (2001). Macho-1 encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. *Nature* 409:724-9. doi:10.1038/35055568.

Pourquie O. (2001). Developmental biology. A macho way to make muscles. *Nature* 409:679-680. doi:10.1038/35055657.

Meedel T.H., Farmer S.C., Lee J.J. (1997). The single MyoD family gene of Ciona intestinalis encodes two differentially expressed proteins: implications for the evolution of chordate muscle gene regulation. *Development* 124:1711-21. doi:10.1242/dev.124.9.1711.

5. Eye development in C. intestinalis

D'Aniello S, D'Aniello E, Locascio A, Memoli A, Corrado M, Russo MT, Aniello F, Fucci L, Brown ER, Branno M. (2006). The ascidian homolog of the vertebrate homeobox gene Rx is essential for ocellus development and function. *Differentiation*. 74(5):222-34. doi:10.1111/j.1432-0436.2006.00071.x.

6. HH signaling in L/R asymmetry in sea urchin and amphioxus

Warner JF, Miranda EL, McClay DR. (2016). Contribution of hedgehog signaling to the establishment of left-right asymmetry in the sea urchin. *Dev Biol*. 411(2):314-324. doi: 10.1016/j.ydbio.2016.02.008.

Guangwei Hu‡, Guang Li‡, Hui Wang* and Yiquan Wang (2017). Hedgehog participates in the establishment of left-right asymmetry during amphioxus development by controlling Cerberus expression. *Development* 144, 4694-4703 doi:10.1242/dev.157172.

7. Tooth regeneration in sharks

Rasch, L. J., Martin, K. J., Cooper, R. L., Metscher, B. D., Underwood, C. J., & Fraser, G. J. (2016). An ancient dental gene set governs development and continuous regeneration of teeth in sharks. *Developmental Biology*, 415(2), 347–370. http://doi.org/10.1016/j.ydbio.2016.01.038.

III Molecular Biology

8. CRISPR in C. intestinalis

Gandhi, S., Haeussler, M., Razy-Krajka, F., Christiaen, L., & Stolfi, A. (2017). Evaluation and rational design of guide RNAs for efficient CRISPR/Cas9-mediated mutagenesis in *Ciona*. *Developmental Biology*, 425(1), 8–20. <u>http://doi.org/10.1016/j.ydbio.2017.03.003.</u>

9. CRISPR in *L. pictus*

Vyas, H., Schrankel, C.S., Espinoza, J.A. et al. (2022). Generation of a homozygous mutant drug transporter (ABCB1) knockout line in the sea urchin *Lytechinus pictus*. *Development*, 149(11):dev200644. doi:10.1242/dev.200644.

10. Microbiome in sea urchin

Carrier T.J. et al. (2021). Microbiome reduction and endosymbiont gain from a switch in sea urchin life history. *PNAS* 118 (16), https://doi.org/10.1073/pnas.2022023118.

IV Phylogeny and Evolution

11. Noctiluca Phylogenomics and Symbiosis

Cooney, E.C., Leander, B.S., Keeling P.J. (2022). Phylogenomics shows unique traits in Noctilucales are derived rather than ancestral. *PNAS Nexus* 22;1(4): pgac202. doi:10.1093/pnasnexus/pgac202.

12. Phylogenetic origin of the nervous system

Ryan FJ (2014). Did the ctenophore nervous system evolve independently? *Zoology*, http://dx.doi.org/10.1016/j.zool.2014.06.001.

Pennisi E. (2019). Did neurons arise from an early secretory cell? *Science* 363 (6424) 212-213, doi:10.1126/science.363.6424.212.

Ryan JF, Chiodin M. (2015). Where is my mind? How sponges and placozoans may have lost neural cell types. *Phil. Trans. R. Soc. B* 370: 20150059. http://dx.doi.org/10.1098/rstb.2015.0059.

Babonis LS (2018). Integrating embryonic development and evolutionary history to characterize tentacle-specific cell types in a ctenophore. *Mol. Biol. Evol.* 35(12):2940–2956, doi:10.1093/molbev/msy171.

13. On the origins of ion homeostasis and gas exchange in vertebrates

Sackville, M.A., Cameron, C.B., Gillis, J.A., Brauner, C.J. (2022). Ion regulation at gills precedes gas exchange and the origin of vertebrates. *Nature* 610 (7933):699-703. doi:10.1038/s41586-022-05331-7.

V Physiology

14. Heart function in C. intestinalis

Anderson, H.E., & Christiaen, L. (2016). *Ciona* as a simple chordate model for heart development and regeneration. *Journal of Cardiovascular Development and Disease* 3(3). <u>http://doi.org/10.3390/jcdd3030025.</u>

Waldrop, L.D., Miller, L.A. (2015). The role of the pericardium in the valveless, tubular heart of the tunicate *Ciona savignyi*. *Journal of Experimental Biology* 218: 2753–2763. <u>http://doi.org/10.1242/jeb.116863.</u>

15. Temperature adjustment in fish

Morgan, R., Andreassen, A.H., Åsheim, E.R., Finnøen, M.H. et al. (2022). Reduced physiological plasticity in a fish adapted to stable temperatures. *PNAS* 31; 119(22):e2201919119. doi:10.1073/pnas.2201919119.

Introduction: What the course is about

The course "Helgoland: Developmental Biology and Comparative Molecular Biology of Marine Organisms" is intended to be a lab course rather than an excursion aimed at ecological observations. However, if we are still allowed, we will spend some time on the rock shore, which is unique in Germany and Denmark except for Bornholm. During low tide, we can see the organisms in their natural habitat and collect specimens for cell biological, biochemical, and molecular analysis in the lab. We should take the time to enjoy nature on Helgoland, especially the spectacular sea bird colony that is unique to Central Europe.

We will be guests of the Biologische Anstalt Helgoland, which belongs to a federal institute, the Alfred Wegener Institute at Bremerhaven, on the mainland. The BAH is rather unique in the world in that we do not have to pay a fee for the use of the laboratory facilities. In return, we are asked to exert great care with the instrumentation and to leave the course room in a perfectly clean state, when we depart.

In terms of taxonomy, marine organisms, and specifically marine animals, represent a much wider scope than freshwater species. Important taxa such as sponges, Hydrozoa, Polychetes, Cephalopods, Ascidians, and Echinoderms are preferentially or exclusively found in the marine environment. Basic research on marine organisms has made important contributions to general biology: Think of fertilization and the significance of the nucleus and chromosomes for development (Theodor Boveri), the potency of blastomeres in development (sea urchin, Ascidians), the mechanism of nerve conduction (giant axon of the squid), or, in more recent years, factors involved in of cell sorting (sponges) and the neurobiochemistry of simple forms of learning (conditioning of the gill withdrawal reflex, *Aplysia*) – you may like to recall your basic textbook knowledge on these findings and connect them to famous names of researchers and Nobel prize winners!

Nearly all marine metazoa have a severe drawback for modern molecular analysis of development: Genetic analysis based on breeding in the laboratory is impossible, very difficult or at least extremely slow. This is a big difference to *Caenorhabditis*, *Drosophila* and mouse! However, in the time of genome sequencing, knock down methods, and particularly CRISPR/Cas, these drawbacks have been partially overcome and echinoderms and tunicates now entered the 'molecular age'.

In our course we will try to repeat, with simple methods, some findings of classical and modern developmental biology for which marine organisms have turned out to be particularly useful. Furthermore, we will perform experiments that are applicable to any animal species and relate to tissue differentiation and evolution of genes.

One difficulty of the course is that there is no clear fixed course plan. You will see, that such a plan is almost impossible to make because many factors are unpredictable beforehand. The course thus builds much more on your own initiative than you are used to from other courses. It is your own responsibility to make sure that you can get introduced to all organisms, but you will not be able to do all experiments. This might look like a limitation, but it also gives you the freedom to choose and to focus on those aspects you consider most interesting. The more active you are the more you will enjoy the course! We will try hard to help you with all the practical and theoretical questions you might have.

Ernst-Martin, Achim, Heiko and Peter June 2019

Acknowledgement: We thank Harald Jokusch, who initiated and organized the first course on "Molecular and Developmental Biology of Marine Organisms" on Helgoland 1984.