Meeting report: Salamander models in cross-disciplinary biological research meeting

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Abstract
The third annual meeting on “Salamander Models in Cross-disciplinary Biological Research” took place online on August 2021, bringing together over 200 international researchers using salamanders as research models and encompassing diverse fields, ranging from Development and Regeneration through to Immunology, Pathogenesis, and Evolution. The event was organized by Maximina H. Yun (Center for Regenerative Therapies Dresden, Germany) and Tatiana Sandoval-Guzmán (TU Dresden, Germany) with the generous support of the Deutsche Forschungsgemeinschaft, the Center for Regenerative Therapies Dresden, Technische Universität Dresden, and the Company of Biologists. Showcasing a number of emerging salamander models, innovative techniques and resources, and providing a platform for sharing both published and ongoing research, this meeting proved to be an excellent forum for exchanging ideas and moving research forwards. Here, we discuss the highlights stemming from this exciting scientific event.

KEYWORDS
immunology, pathogens, regeneration, resources and workshops, salamander

1 | INTRODUCTION

The third annual meeting on “Salamander Models in Cross-disciplinary Biological Research” was originally slated to take place in 2020 but unfortunately had to be postponed due to COVID-19 with the hope of it taking place in person in 2021. Given the ongoing crisis it was not possible to safely hold an international in-person meeting, and as such it was decided to run a fully virtual meeting. The meeting was organized by Maximina H. Yun (CRTD & MPI-CBG, Germany) and Tatiana Sandoval-Guzmán (TU Dresden, Germany), and
sponsored by the Deutsche Forschungsgemeinschaft, the Center for Regenerative Therapies Dresden, Technische Universität Dresden, and the Company of Biologists. One enjoyable aspect of the online setting was that it significantly lowered the barrier of entry for people throughout the world to attend. Over 200 people had the chance to participate, which represents the largest number of attendees thus far—we certainly hope this level of accessibility is maintained in future meetings.

Diversity was undoubtedly a common theme throughout the meeting: from a multicultural assembly of researchers from all over the world, to the range of fields represented and the introduction of new salamander species as research models. This meeting facilitated the discussion of a broad range of topics relevant to salamander researchers from development, regeneration, immunology, evolution, and beyond. Moreover, even in its virtual setting, it provided an important platform for scientists to interact and share a wide range of resources, techniques, and ideas.

After some opening words from the organizers, the meeting got quickly on its way with an exciting plenary talk by Elly Tanaka (IMP, Austria). Dr Tanaka provided an exciting overview of the work currently ongoing in her lab, which scopes from exploring genome organization and size, to fundamental differences in protein, an expression behavior between regenerative salamanders and nonregenerative mammals. An interesting example of this is the extracellular release of Axolotl Marcks-like protein (MLP). While Axolotl MLP is readily secreted and an important inducer of blastema cell proliferation, mouse MLP is not secreted. Furthermore, while canonical tetrapod limb development places FGF8 expression in the AER, in salamanders this has shifted to the underlying limb bud mesenchyme. This shift in expression is observed both in the axolotl limb bud and also the regenerating blastema. Taken together, these observations provide exciting leads to explore fundamental differences in protein sequences and gene regulation between regenerative and non-regenerative species.

2 THE WONDERS OF REGENERATION

The high regenerative ability of salamanders has fascinated people for hundreds of years, which was also reflected by the emphasis on regeneration in many of the invited and selected talks. Broadly speaking, three main research themes could be identified: induction of regeneration, cell biological aspects of regeneration, and bone regeneration.

The early induction of regeneration is not only dependent on the clearing of cellular debris but also involves the dedifferentiation and recruitment of progenitor cells to the injury site. Among others, Akira Satoh (Okayama University, Japan) and Josh Currie (Wake Forest University, USA) provided excellent overviews of their research on the factors and signals needed for cells to migrate and contribute to form a blastema. Using the axolotl model, Josh Currie discussed the role PDGF signaling as a potent inducer of fibroblast migration to the injury site, a key mechanism for the establishment of the blastema, and how this can be further explored using photoactivatable transgenics. Furthermore, Akira Satoh leveraged a dermal fibroblast culture from the Iberian ribbed newt, Pleurodeles waltl, which is able to form blastema-like aggregates in response to BMP and FGF signaling. He used this system to uncover molecules involved in the control of cell plasticity of connective tissue, identifying Pde4b as a promising candidate for cell dedifferentiation.

Following on the theme of cellular plasticity, Maximina Yun presented ongoing work in her lab indicating that senescent cells, which are recurrently induced during limb regeneration, are co-opted to promote regeneration. By developing new biochemical and genetic tools to study cellular senescence in salamanders, her lab found that senescent cells impact on blastema progenitors through secretion of WNT factors, leading to the promotion of dedifferentiation during newt (Notophthalmus viridescens) limb regeneration, or proliferation of blastema progenitors in the axolotl context.

Salamanders are excellent vertebrate models to apply novel tools to study cell biological aspects of regeneration. For example, the recent development of axolotl Fucci lines independently by both Duerr et al., as well as Cura Costa et al., allows for an unprecedented level of detail to understand cell cycle dynamics during axolotl tissue regeneration, particularly in combination with either live imaging or the recently developed optical clearing protocols for salamander systems. Leo Otsuki (IMP, Austria) provided an overview of how these novel imaging tools can be used to study cell cycle kinetics during axolotl spinal cord regeneration. The combination of advanced imaging and mathematical modeling by Emanuel Cura Costa and Osvaldo Chara led to the precise definition of an 800 μm zone of stem cell activation. Cell cycle kinetics and proliferative potential need to be tightly regulated to regenerate the correct amount of tissue. Distal amputations only require the regeneration of a relatively small amount of tissue compared to proximal amputations, and as such require different proliferative potential. Sandra Edwards-Jorquera (TU Dresden, Germany) is currently exploring the role that tissue stiffness plays in specifying the differences in proliferative potential between amputation planes. Edwards-Jorquera found differences in tissue stiffness between proximal
and distal limb blastemas, which correlates with differences in YAP-TAZ signaling. This could explain the differences in proliferative potential between proximal and distal amputations.

Insights into the molecular basis underlying the establishment of proximal and distal identities during regeneration were discussed by Catarina Oliveira (CRTD, Dresden), who introduced Tig1 as a new determinant of proximal identity in the salamander limb. Tig1 is a highly conserved surface protein required for a number of cellular behaviors associated with proximal determination and, strikingly, its overexpression is able to reprogram distal cells toward proximal identities. Further research will determine what mechanisms underlie Tig1’s ability to control positional identity.

Historically, skeletal structures have been used to provide easily visualized anatomical landmarks to assess regenerative fidelity. This has led to a rich context of people studying skeletal development and regeneration. Several excellent talks during the conference illustrated how both the limb and tail skeleton are easily visualized and provide distinct morphological landmarks to assess regenerative fidelity. To this day, people are studying salamander bone formation and regeneration. Nadia Fröbisch (Leibniz Institute MfN, Germany) presented her work on evolution and development of salamanders. Salamanders are the only tetrapods that exhibit preaxial polarity in limb development, but share this pattern as well as the ability to regenerate limbs with some fossil amphibian groups. It is debated whether preaxial polarity and regeneration are evolutionarily or mechanistically linked, and combined data on fossils and living salamanders can help resolve this question. Her work has shown that gene expression patterns are shared with other tetrapods in early stages of limb development, but differ in the late phases of limb development when the autopod is formed.

Camilo Riquelme Guzmán (TU Dresden, Dresden) discussed his work of the cellular basis behind skeletal regeneration. He found that the successful integration of regenerating skeletal structures is dependent on Osteoclast-mediated resorption. This resorption creates a dynamic space where blastema cells begin to accumulate. Furthermore, he showed that axolotls continue to grow during their entire life and in contrast to mammals, maintain limb bones that are never fully ossified. As an alternative to the classical injury model, Anastasia Polikarpova (IMP, Austria) established and characterized an axolotl fracture repair assay. This new injury model will allow for comparative studies between axolotl and mouse fracture healing. As a corollary to classical limb injury models, Wouter Masselink (IMP, Austria) studies vertebrae regeneration in the axolotl tail. He showed that in addition to tail amputation, the successful regeneration of a vertebrae extirpation injury model is dependent on spinal cord-derived hedgehog signaling.

Other less common injury models such as cardiac and thymus injury are now also being explored on a cellular level. A long-standing question in the cardiac repair and regeneration field is which cells provide the progenitors needed for cardiac regeneration.

Using a combination of biochemical and genetic approaches, Elif Eroglu (Karolinska Institute, Sweden) discussed her studies on the role of the epicardium during P. waltl heart regeneration. The thymus is an immunologically important organ for the production of T cells. Indeed, thymectomy is a necessary step when performing heterologous grafting experiments in xenopus. In xenopus, the thymus does not regenerate and as such permanently prevents T-cell production. Considering the importance of the immune system for regeneration, Anna Czarkwiani (CRTD, Germany) asked herself the question whether the axolotl thymus can regenerate. She could identify and visualize three pairs of thymus nodules which are all regeneration competent. Interestingly, even with complete thymectomy, the thymus effectively regenerates, providing a new de novo regeneration paradigm. She is now employing a scRNAseq approach to uncover the cellular source for thymus regeneration.

3 | IMMUNOLOGY

The immune system has long been understood to play an important role in generating an effective regenerative response. Changes to the immune system such as a loss of macrophages result in a loss of salamander regenerative abilities. Georgios Tsissios (Miami University, USA) presented a striking example of this phenomenon in the context of newt lens regeneration, a process which is disrupted by macrophage depletion. Additionally, he discussed the use of OCT to visualize lens regeneration in real time and in a noninvasive manner.

While most work on the salamander immune system attempts to understand the interplay of the immune system with the progenitor niche to induce an effective regenerative response, without a detailed understanding and characterization of the immune system any systematic analysis is going to be fundamentally challenged. To solve this, James Godwin (MDI Biological Laboratory, USA) presented some recent work on the characterization of the axolotl immune system. Although the axolotl was previously thought to be relatively immunodeficient, the proportions of major immune subsets in the axolotl fall within a similar range to that of nonregenerative species such as mice. This finding may point to functional differences in salamander immune
cells that support scar-free repair and regeneration. For example, unlike mammals, axolotls lack hematopoiesis in bone marrow cavities, and instead they recruit pro-regenerative macrophages from the liver.\textsuperscript{13} Along similar lines, Ed Scott (University of Florida, USA) wondered whether the axolotl hematopoietic stem cell is capable of indefinite regeneration or, like nonregenerative species, is eventually depleted with successive round of transplantsations. Interestingly, it turns out that hematopoietic stem cell depletion occurs at a similar rate in axolotl and mice, suggesting there is no fundamental differences in the maintenance of the hematopoietic niche between axolotl and mice.

4 | SALAMANDER PATHOGENS

Chytridiomycosis is a disease caused by \textit{Batrachochytrium dendrobatidis} and is thought to be a significant driver behind the worldwide decline in amphibian populations. As members of the amphibian family, Salamanders are also susceptible. Understanding this disease and the broader microbiome of salamanders has important implications for both laboratory husbandry and best practices, and the continued survival of entire ecosystems. Two presentations further delved into this topic. First, An Martel (University of Ghent, Belgium) described a second Chytrid fungus (\textit{B. salamandrivorans}), with salamanders as well as newts being particularly susceptible. Second, to better understand fungal diseases, it is essential to understand the normal microbiome of salamanders. To this end, Rakeyah Ahsan characterized the microbiome of the spotted salamander across life stages.

5 | SALAMANDER RESOURCES AND WORKSHOPS

Several talks and workshops discussed important resources that are available to the broader salamander society. Toshinori Hayashi (Hiroshima University, Japan) provided a detailed overview of several \textit{P. waltl} lines that were generated in Japan and have now been made available to the broader research community (see also the iNewt website: \url{https://www.nibb.ac.jp/imori/main/}). \textit{P. waltl} provides an interesting alternative to the more commonly used axolotl, due to its shorter generation time and their limited cannibalism. Furthermore, Tijana Vučić (University of Belgrade, Serbia) discussed emerging \textit{Triturus} newt models which can be bred in captivity and constitute valuable systems for ecological and evolutionary studies, including probing of balance lethal systems.\textsuperscript{14} Beyond new models, the meeting benefitted from the introduction of cell resources, including a new method for culturing dorsal root ganglia from axolotls, from Gürkan Öztürk (Medipol University, Turkey), enabling studies of axon formation and regeneration.

To expand the community’s training on key research tools, state-of-the-art workshops focusing on both transgenesis\textsuperscript{15} in the axolotl (Prayag Murawala, MDI Biological Laboratory, USA) as well as axolotl genomic resources\textsuperscript{16} (Sergej Nowoshilow, Boehringer Ingelheim RCV, Austria) were presented to close this successful event.

Finally, it is worth commending all the scientists who shared their work in the form of posters, covering further topics including comparative biology, DNA repair, algae–salamander symbiosis, metabolism, and immunology.

6 | CONCLUSIONS

Together these exciting presentations provide an overview of the breadth of cutting-edge research on all things salamander related. Salamanders take up an interesting phylogenetic and ecological position, which was accurately reflected in the selected presentation. Moreover, the passion and collegiality that was on display makes us think the future of salamander research is incredibly bright. We are looking forward to the fourth annual meeting on “Comparative studies and translational opportunities in salamander research” (salamander 2022. com), which will take place as a hybrid event on 22–24th of August 2022 at Medipol University in Istanbul and is kindly organized by Gürkan Öztürk.

AUTHOR CONTRIBUTIONS

Wouter Masselink: Project administration (equal); writing – original draft (lead). Tatiana Sandoval-Guzmán: Writing – review and editing (supporting). Maximina Yun: Writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.
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