7th Euro Evo Devo meeting: Report on the "Evolution of regeneration in Metazoa" symposium

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Running title: "**Evolution of regeneration" symposium report**

Abstract:

Regeneration, the ability to restore lost parts of the body, is a widespread phenomenon in animals. While this ability is somehow limited in classical developmental model organisms, a variety of animals are able to regenerate complex structures such as limbs or important parts of their body, upon injury. Despite the recent emergence of regenerative studies using a large variety of metazoans, we still lack a general view of the evolution of animal regeneration. In the context of the 7th EvoDevo meeting that took place in June 2018 in Galway, Ireland, the “Evolution of regeneration in Metazoa” symposium gathered scientists studying the regenerative potential of evolutionarily distant animal species.

Keywords: Regeneration**,** Evolution, Metazoan, Meeting report, EvoDevo

Introduction:

While regeneration is a well-known process that has intrigued scientists over the centuries (Réaumur, 1712), regeneration research has been limited to only a handful of systems for decades (Figure 1). Recent technological advances (*e.g.* NGS, CRISPR/Cas9) and improved experimental approaches (*e.g.* clonal analysis, live imaging) have revived the interest in regenerative biology using additional and (re)-emerging models (Figures 1, 2), enabling us today to address this phenomenon from a comparative perspective and gain insight into the evolution of regeneration within metazoans (Tiozzo and Copley, 2015; Grillo et al., 2016; Lai and Aboobaker, 2018; Ricci and Srivastava, 2018).

The 7th meeting of the European Society for Evolutionary Developmental Biology (EED), organized by Gerd Schlosser and Uri Frank from National University of Ireland, Galway, as well as the EED scientific committee, took place on June 26-29, 2018 in the beautiful city of Galway, Ireland. Since 2006, biannual EED meetings are usually organized in summer at some major European city famous for its evolutionary biology studies. The scientific program is always composed of a variety of plenary sessions, symposia, contributed talks and poster sessions. Both junior and senior scientists from all over the world have the opportunity to present their most recent research and to discuss the current state of the art and also the future directions of animal and plant Evo Devo. Among the 23 symposia of the 2018 EED meeting, we organized the “Evolution of regeneration in Metazoa” symposium aimed at uncovering general principles and/or shared genetic programs underlying this intriguing developmental trajectory. This symposium was dedicated to the morphological, cellular and molecular mechanisms underlying regeneration in a variety of models that are representative of metazoan diversity and that are each

particularly suited to address specific questions of interest (Figure 2). Beyond this phylogenetic consideration, several types of regeneration (whole-body, appendages, organ, tissue) were presented and emphasized. Specific attention was given to the identity, plasticity and differentiation potential of the cells required for the regeneration processes. In addition, the symposium encompassed different scientific approaches and techniques, such as comparative transcriptomic, morphological, molecular and functional assays, genetic lineage-tracing and live imaging, to efficiently address this regeneration topic. The “Evolution of regeneration in Metazoa” symposium featured the main session with four invited speakers (Karen Etcheverri - USA, Maja Adamska - AUS, Jochen Rink [EMBO YIP] - DEU and Uri Frank - IRL), as well as two contributed sessions with nine additional speakers. Moreover, 17 posters were selected for the poster sessions.

Nervous system

The symposium started with a presentation by **Karen Echeverry** (Woods Hole, USA) on the molecular underpinning of spinal cord regeneration in axolotl. Mining transcriptional profiling data (Sabin et al., 2015) and putative miR target sites, this study highlights a finely-regulated miR-200a-dependent FOS/JUN circuit that promotes functional spinal cord regeneration (Sabin et al., 2019). **Aniket Kshirsagar** (Galway, IRL) presented an iTRAQ-based proteomics study on spinal cord injury in *Xenopus* that revealed more than 200 differentially regulated proteins during early phases of regeneration. Interestingly, MARCKS-like protein (MLP), identified to initiate appendage regeneration in axolotl (Sugiura et al., 2016) and to be up-regulated during lungfish fin regeneration (Nogueira et al., 2016), is also significantly up-regulated during early stages of *Xenopus* spinal cord regeneration. Focusing on the well-known role of the nervous system on the regulation of the regenerative

process in various species, **Chiara Sinigaglia** (Lyon, FRA) presented her work on limb regeneration using the crustacean *Parhyale hawaiiensis* (Alwes et al., 2016). Coupling live imaging with laser nerve ablation, she showed that the absence of nerves severely impairs the regenerative process.

Embryonic development *vs* regeneration, regeneration gene regulatory network (GRN), signaling

Two studies addressed the long-standing question in regenerative biology about the molecular relationship between embryonic development and regeneration (Morgan, 1901). Using the brittle star *Amphiura filiformis*, **Paola Oliveri** (London, GBR) compared the regulatory programs governing bio-mineralization of the skeleton in the developing embryo/larva and adult arms. Using candidate gene and genome-wide differential expression approaches, combined with pharmacological FGF signaling perturbation experiments, this study revealed substantial conservation of the molecular program underlying these two developmental contexts (Czarkwiani et al., BioRxiv). **Hereroa Johnston** (Nice, FRA) compared the genetic networks underlying embryonic development and whole body regeneration in the sea anemone *Nematostella vectensis*. This study compared two extensive RNAseq datasets for these two developmental trajectories in this species (Warner et al., 2018) as well as the GRNs downstream of MEK/ERK signaling deployed during embryogenesis (Amiel et al., 2017) and regeneration. Doing so, this study revealed that regeneration uses a partial and rewired redeployment of the embryonic GRN, with regeneration-specific modules (Warner et al., BioRxiv) and a regeneration-specific network logic. **Mansi Srivastava** (Cambridge, USA) focused on the molecular program in response to injury another whole-body regeneration model, the acoel *Hofstenia miamia*. By combining transcriptional and chromatin profiling, as well as functional studies, this

work led to the reconstruction of the GRN underlying the wound response program, highlighting the essential role of a homologue of the early growth response family, *egr*. The latter could play a conserved regulatory role for early stages of (whole body) regeneration in bilaterians (Gehrke et al., 2019).

Matthias Vogg (Geneva, CHE) presented recent data on head regeneration in the classical whole body regeneration model, *Hydra*. In particular, this study focused on the identification of the yet to be determined head inhibitor that counterbalances the head activator activity of Wnt3. By crossing spatio-temporal information of gene expression in *Hydra* with RNAseq data obtained after β-catenin knockdown in planarians, combined with functional assays, this work identified the transcription factor *Sp5* as a head inhibitor in the Wnt3/β-catenin/Sp5 feedback loop at the eumetazoan scale (Vogg et al., 2019). Similar to cnidarians, sponges also possess extreme regenerative capacities and some of them are even able to re-aggregate after cell dissociation. In order to gain insight into the cellular and molecular mechanisms involved in sponge regeneration, **Maja Adamska** (Canberra, AUS) presented a transcriptomic and spatial gene expression screen in *Sycon* species, indicating that the TGF-β signaling is involved in coordinated cell movements during regeneration, while taxonomically-restricted genes appear involved in re-aggregation.

Cellular aspects of regeneration

The annelid *Platynereis dumerilii* efficiently regenerates its posterior part. **Eve Gazave** (Paris, FRA) presented a study that combined morphological, cellular and molecular analyses during this process, laying down a detailed blueprint of posterior *Platynereis* regeneration. This study further highlighted the involvement of dedifferentiation and the requirement for cell proliferation in this rapid regeneration

process (Planques et al., 2019). Regeneration in the colonial hydrozoan species *Hydractinia echinata* involves i-cells (hydrozoan stem cells), which after head amputation migrate towards the amputation site and act as progenitor cells for the regenerating tissue (Bradshaw et al., 2015). **Uri Frank** (Galway, IRL) investigated the cellular processes underlying regeneration of tissue fragments lacking i-cells that can nonetheless regenerate missing body parts. Interestingly, this latter process results in the formation of *de-novo* i-cells, through a trans-differentiation mechanism that may involve cellular senescence markers as trigger. Using the agamid lizard *Pogona vitticeps*, **Lotta Salomies** (Helsinki, FIN) analyzed the cellular mechanisms involved in tooth regeneration. Histological, molecular and cellular analyses revealed the presence of a successional dental lamina (SDL) that possesses distinct niches for putative stem cell populations. Further experiments indicate that each niche plays a distinct role in SDL regulation and tooth replacement involving cellular migration.

Intra-phylum comparison of regeneration

While planarians are well-known whole body regeneration models, certain species, mostly marine, lack this capacity (Liu et al., 2013; Sikes & Newmark, 2013, Umesono et al, 2013). **Jochen Rink** (Dresden, DEU) has established a unique live laboratory culture of >60 planarians species to decipher the gene network changes and ultimately gain insight into the evolution underlying the natural selection process in regenerating and regeneration-deficient species. Among the molecular examples that this has uncovered, the Wnt pathway appears to be a universal driver of regeneration in planarians. Indeed, differences in this signaling network can explain the natural variations of the regenerative capacity in planarians. Finally, **Vivien Bothe** (Berlin, DEU) presented a morphological and histological analysis of imperfect fin regeneration in lungfishes, gar and paddlefish (vertebrates) - which together

occupy a pivotal phylogenetic position - to understand the evolution of fin regeneration in vertebrates. Combining data from serial sections, 3D reconstructions and x-ray microtomography, this study revealed regeneration anomalies in all species, suggesting ancient but imperfect regenerative capacities in Sacropterygii and Actinopterygii.

Conclusions:

Regeneration has for a long time attracted interest in the fields of both biology and medicine. This process has so far been partially explored in a handful of species, but there is still a need for powerful new and complementary models to study regeneration. This stimulating symposium on the “Evolution of regeneration in animals” was particularly diverse in term of biological models, type of regeneration and technical aspects; generating cross-talk, interdisciplinary exchanges and synergy among scientists of various horizons (Figure 3).

Recent technical and conceptual advances together mean that now is an ideal time to study regeneration and to address long-standing questions in the field. Indeed, with the generation of a multiplicity of genomic and transcriptomic data of regeneration in various species, the time is ripe to use these vast resources, notably in a rigorous comparative manner. This requires a detailed descriptive and understanding of the various regeneration processes, to efficiently compare shared/different elements (*i.e.* immediate stress response, proliferation-dependent or -independent wound-healing, or the onset of proliferation, cell migration, morphogenesis, etc.). In the future, comparative analyses of regeneration will surely reveal either conserved or phylum-, taxon- or species-specific regenerative mechanisms, and will offer new insights into regenerative and stem cell biology, as well as opportunities for novel regenerative medicines. Importantly, this broad

comparative approach, using a large spectrum of metazoan lineages with different regenerative potential, is crucial to address the fundamental and yet unresolved question of why certain animals possess high regenerative capacities, while others, including humans, lack such abilities.

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Figure legends:

Figure 1: Number of publications related to regeneration from 1946 to nowadays based on PubMed data counts per model. Since 2005, the number of published papers in the regeneration field has drastically increased. Each color represents a major taxon (or a species) used for regeneration studies.

Figure 2: Simplified phylogenetic tree illustrating the evolutionary distant species and topics addressed during the "Evolution of Regeneration in Metazoa" symposium (oral presentations and poster sessions). The number after the names indicates the amount of presentations/posters involving the research model. * Indicates novel or (re)-emerging regeneration model. ** Phylogenetic position still under debate.

Figure 3: “Evolution of Regeneration in animals” symposium’s Word cloud. The word cloud was randomly generated with Nuagedemots.fr using keywords selected from the abstracts of the oral presentations. The size of each word's type is proportional to the word's frequency in the abstracts, the different colors are for aesthetic purposes only.