

REVIEW

Current advances in tissue repair and regeneration: the future is bright

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Abstract

The fifth EMBO conference on 'The Molecular and Cellular Basis of Regeneration and Repair' took place in the peaceful coastal town of Sant Feliu de Guixols (Spain) on September 2014. The meeting was organised by Emili Saló (U. Barcelona, Spain), Kimberly Mace (U. Manchester, UK), Patrizia Ferretti (University College London, UK) and Michael Brand (Centre for Regenerative Therapies Dresden, Germany) and received the generous support of Society for Developmental Biology, The Company of Biologists, Centre for Regenerative Therapies Dresden, Garland Science and the journals *Regeneration* and *Cell Signalling*. The natural surroundings provided an inspiring setting for 185 researchers from all over the world to share their latest findings and views on the field. The conference showcased the great diversity of model organisms used for studying regeneration and tissue repair, including invertebrate and vertebrate species (Fig. 1). Importantly, this diversity in animal models allowed for a global overview of the mechanisms that promote regeneration. In addition, it highlighted some of the unique aspects that confer differences in regenerative capacities among different species. These differences might lie in each of the different steps involved in performing regeneration, including triggering the regenerative response, controlling cellular plasticity, re-establishing the correct tissue patterns, as well as determining the roles of extrinsic factors, such as the role of inflammation in regeneration. A deeper understanding of these processes in the naturally regenerating species is a prerequisite for advancing the field of regenerative medicine and tissue repair in humans.

Keywords

regeneration, ros, planaria, axolotl, newt, zebrafish, hydra

Introduction

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Signalling. The natural surroundings provided an inspiring setting for 185 researchers from all over the world to share their latest findings and views on the field. The conference showcased the great diversity of model organisms used for studying regeneration and tissue repair, including invertebrate and vertebrate species (Fig. 1). Importantly, this diversity in animal models allowed for a global overview of the mechanisms that promote regeneration. In addition, it highlighted some of the unique aspects that confer differences in regenerative capacities among different species. These differences might lie in each of the different steps involved in performing regeneration, including triggering the regenerative response, controlling cellular plasticity, re-establishing

the correct tissue patterns, and determining the roles of extrinsic factors, such as the role of inflammation in regeneration. A deeper understanding of these processes in the naturally regenerating species is a prerequisite for advancing the field of regenerative medicine and tissue repair in humans.

Setting up the Regenerative Response

The recognition of tissue injury or loss is a critical step for the promotion of wound repair or regeneration. Several speakers presented their ongoing efforts to elucidate the signaling events that mediate such recognition. Intriguingly, independent studies in different regenerative species including *Hydra* (Brigitte Galliot, University of Geneva, Switzerland) and *Drosophila* (Florenci Serras, University of Barcelona) converged on showing a prominent role for reactive oxygen species (ROS) in setting up the regenerative response. In these different models, tissue injury leads to an increased production of ROS, which in turn promotes the plasticity of the surviving cells, allowing them to undergo compensatory growth and differentiation. Importantly, Florenci Serras presented elegant genetic analysis in *Drosophila* showing that ROS control the activity of several pathways previously implicated in compensatory proliferation, including JNK, STAT and p38. Along these lines, Emili Saló highlighted a role for JNK in integrating and coordinating the apoptotic and proliferative responses both during regeneration and during body homeostasis in *Planaria*.

An important goal is to identify the molecular mechanisms linking ROS production and the activation of these pathways. Andrew Chisholm (University of California San Diego, USA) made an important step in this direction by showing that mitochondrial ROS (primarily anion superoxide) inhibit the small GTP-ase RHO-1 via a specific redox-sensitive motif, which in turn is necessary for promoting actin cytoskeleton changes and hence efficient epidermal wound repair in *Caenorhabditis elegans*. Furthermore, to identify the targets of ROS signaling, Brigitte Galliot's group performed RNA-seq analyses during consecutive steps of *Hydra* regeneration. Interestingly, this work uncovered several conserved genes involved in the innate immune response. Besides ROS, additional signals are activated upon injury in order to induce the full regenerative response. Karen Echeverri (University of Minnesota, USA) pointed to a novel mechanism that triggers neuronal progenitor cell proliferation during spinal cord regeneration in the axolotl. She showed that spinal cord injury leads to changes in the transmembrane potentials of nearby progenitors, which in turn promotes their proliferation. As there is evidence that minute currents can initiate regeneration in different contexts, it will be interesting to explore the molecular workings of transmembrane potential oscillations and progenitor differentiation.

Regulation of Cellular Plasticity

The regulation of cellular plasticity lies at the heart of all regenerative processes. Following injury, it is critical for the generation of the progenitors of the regenerate, which arise through dedifferentiation, transdifferentiation, proliferation of resident stem cells, or a combination of these mechanisms. Afterwards, it is critical for the restitution and maintenance of the different tissues. Clearly, uncovering the mechanisms underlying the regulation of cellular plasticity is essential to our understanding of regeneration. At Sant Feliu de Guixols, several talks shed new light into this matter.

Work by Heng Wang (András Simon laboratory, Karolinska Institutet, Sweden) uncovered a new link between injury and the promotion of dedifferentiation in adult tissues. He showed that in differentiated salamander muscle cells the promotion of apoptosis—followed by its inhibition—leads to dedifferentiation of the surviving cell progeny, which can then contribute to regeneration. Furthermore, he demonstrated that this strategy can be used to generate muscle progenitors with regenerative potential in mice.

Although dedifferentiation is a key regeneration mechanism in many vertebrate systems, its role in invertebrate models is less established. Jose José García García Arrarás (University of Puerto Rico, Río Piedras, Puerto Rico) discussed recent findings from his laboratory using an invertebrate system, the sea cucumber, to study regeneration of complex structures. He showed that regeneration takes place through dedifferentiation in both intestinal muscle and radial nerve chord, and highlighted significant differences in transcriptional regulation between dedifferentiating echinoderms and cellular reprogramming in mammals. Differences aside, his laboratory found that the pluripotency factor *c-myc* is required for radial glia dedifferentiation, suggesting that its role in promoting reversal of differentiation is conserved.

Further insights into the regulation of cell plasticity came from Sophie Jarriault (IGMBC, France), who presented an epigenetic mechanism safeguarding robust cell conversion in *C. elegans*. Using genetic screens to analyze the factors involved in the natural transdifferentiation of hind-gut cells into neurons, Jarriault and co-workers found that this process requires the sequential organization of distinct histone-modifying activities at the single cell level. These epigenetic modifications are not only required for precise cell conversion; they are also critical for sheltering this process from environmental stresses to ensure its robustness. In addition, her work highlighted a role for UNC-3, a COE-type transcription factor, as a regulatory hub key to neuronal differentiation. This finding has interesting parallels with the planarian system, in which COE is required for the maintenance of tissue architecture and regeneration of the central nervous system, as discussed by Ricardo Zayas (San Diego State University, USA).

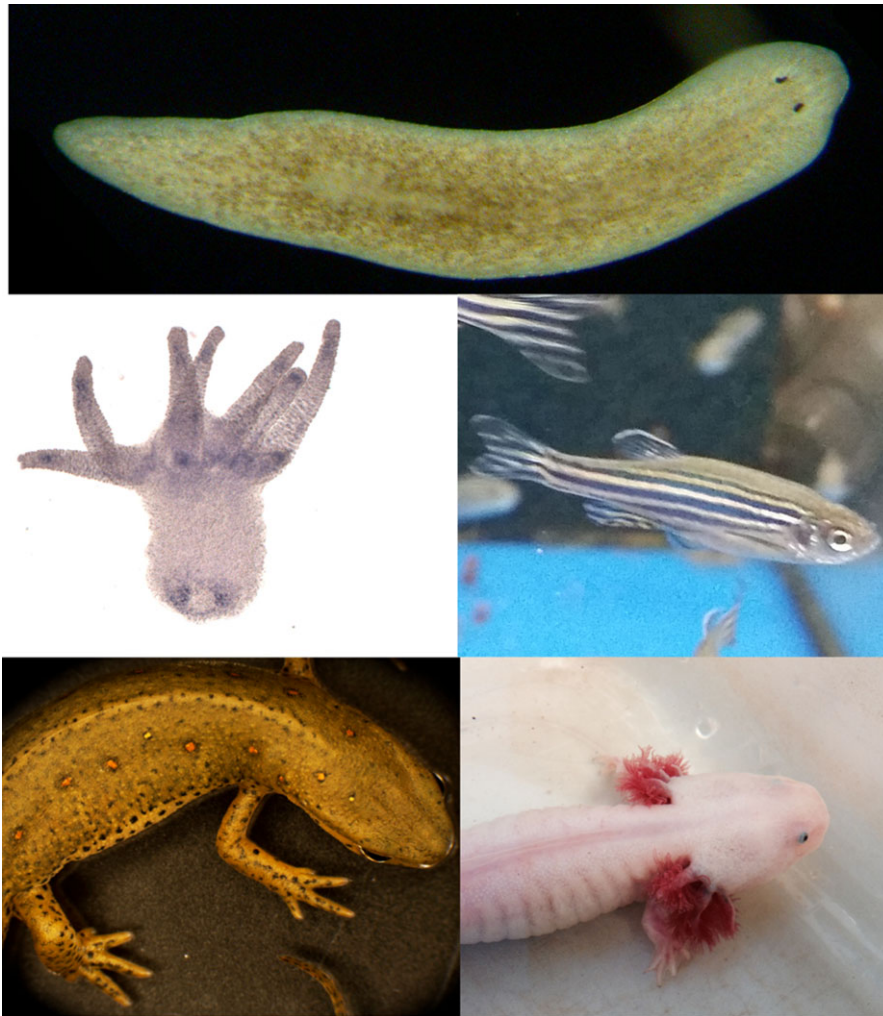


Figure 1. Classic models for regeneration studies. From top, clockwise: a planaria, *Schmidtea mediterranea* (courtesy of Teresa Adell); zebrafish, *Danio rerio*; an axolotl, *Ambystoma mexicanum*; a red-spotted newt, *Notophthalmus viridescens*; a freshwater hydrozoan polyp, *Hydra vulgaris* (courtesy of Brigitte Galliot).

The importance of epigenetic factors for reprogramming events in regeneration was also highlighted by Catherine Pfefferli (Anna Jazwinska laboratory, University of Friburg, Switzerland), who discussed the functions of the NuRD complex in zebrafish fin regeneration. She showed that the inhibition of key NuRD components impaired blastema cell proliferation as well as osteoblast redifferentiation, providing further evidence for the roles and relevance of epigenetic modifiers in regeneration. However, the work of Jelena Mann (University of Newcastle, UK) suggests that epigenetic reprogramming can be a double-edged sword. Her group had previously identified an epigenetic mechanism by which systematic fibrotic injury in previous generations can decrease the offspring's predisposition to liver fibrosis in rats. However, they subsequently found that

the same mechanism also decreases wound repair responses in skin. This important set of findings demonstrates the existence of epigenetic transmission of wound-healing properties, which allows for rapid adaptation to environmental stresses—though not without its trade-offs.

Re-establishing Patterns

The regeneration of functional structures would be impossible without the activation of a coordinate system that imparts positional information, patterning the regenerate into tissues of the right identity, morphology and size. What are the molecular components underlying positional information and patterning during regeneration? Rachel Lander (Northwestern University, USA) identified a central role for ptk7, a cell surface kinase-dead protein that modulates Wnt

signaling, in the establishment of the anteroposterior axis during organ regeneration in planarians. Her double RNAi experiments identified a network of genes involved in anteroposterior identity alongside *ptk7*, such as *WntP2*. The role of Wnt family members in controlling different aspects of patterning was also highlighted by Teresa Adell (University of Barcelona, Spain). She demonstrated how *Wnt5* positions neural tissues mediolaterally by acting as a repelling axonal cue that is sensed through Ror receptors in planarian neurons. *Wnt5* is expressed in a complementary domain to *Slit*, another repellent axonal cue that acts via Ror. This led her to propose a model whereby *Wnt5* and *Slit* cues are integrated in a self-regulated system that positions neural tissues by restricting their expression boundaries.

The restoration of identity along the proximo-distal axis is critical, in particular during regeneration of appendages. Sumihare Noji (University of Tokushima, Japan) discussed the role of planar polarity/Hippo pathway genes in positional identity and size determination during leg regeneration in crickets. He demonstrated that the classical leg gap genes *Dachshund* and *Distal-less* are involved in the determination of the length of leg segments through regulating cell proliferation, downstream of the *Dachsous/Fat (Ds/Ft)* signaling pathway. Furthermore, he highlighted the importance of epigenetic regulation for pattern formation. Finally, he integrated his results into a steepness model, supported by both *in silico* and *in vivo* data, whereby a gradient of *Ds/Ft* heterodimers along the cells determines polarity and growth during cricket leg regeneration.

The Immune System: More Often a Friend Than a Foe

Tissue loss leads to the accumulation of cellular debris. Hence, macrophages might play a beneficial role in regeneration by clearing out cell waste, allowing for a more efficient re-growth. At the same time, macrophages could create an inflammatory environment, suppressing regeneration and contributing to pathological scarring. Finally, macrophages could secrete factors that support proliferation and/or differentiation during regeneration. Are macrophages a friend or a foe in regeneration? Addressing this question is not trivial due to the complex nature of the interactions among the various types of immune cells and the profound importance of macrophages for metabolic control and homeostasis of the organism. In addition, macrophages come in two flavors, pro-inflammatory (M1s) and anti-inflammatory (M2s), and they can have radically different effects on wound repair and regeneration. This was exemplified by Kimberly Mace (University of Manchester, UK), whose work offered new insights on macrophage polarization. She showed that, during early wound healing in diabetic mice, macrophages are polarized towards both M1 and M2 types. However, while in normal

mice there is a switch to M2 macrophages at later wound healing stages, the macrophages found in diabetic wounds are predominantly M1. What is responsible for this inability to switch to a type 2 response? Mace and co-workers found multiple levels of *C/EBP α* deregulation in diabetic macrophages and propose that this is controlled, in part, by epigenetic changes, such as histone deacetylation, promoted by the diabetic environment.

Yet, manipulating a specific macrophage type during regeneration remains challenging using the currently available tools. Nevertheless, there are certain means that allow for systemic depletion of all macrophages. For example, clodronate liposome (CL) injection into the circulation allows for temporally controlled depletion of macrophages. Taking advantage of this technique, several speakers explored the role of macrophages in regeneration. Mathieu Levesque (Jeff Mumm laboratory, Johns Hopkins University, USA) used CL to show that, in zebrafish larvae and adults, ablation of macrophages slows down the regeneration of both the fin and the pancreatic beta-cells. He proposed that macrophages might secrete factors that facilitate the differentiation of pancreatic progenitors into beta-cells. In addition to this study, James Godwin (Australian Regenerative Medicine Institute, Australia) characterized the role of macrophages in heart regeneration in the axolotl, following on from previous work demonstrating the requirement for macrophages in limb regeneration. In line with the conclusions of Mathieu Levesque, macrophage depletion prior to heart injury led to failure of regeneration, probably due to defects in extracellular matrix remodeling, indicating that macrophages do play a beneficial role in regeneration.

How could macrophages promote proliferation and/or differentiation during the regeneration in these models? An intriguing insight came from the work of Paul Martin (University of Bristol, UK), who used live imaging in zebrafish larvae to dissect out the role of immune cells in tumor growth. He showed that KRAS-induced epithelial tumors grew at a slower rate when immune cells were depleted, indicating that the immune cells provide factors that sustain tumor growth. Namely, immune cells deliver prostaglandins to the transformed cells, which in turn promote their proliferation. In light of these data, it will be interesting to explore whether prostaglandins secreted by immune cells are also responsible for promoting regeneration in the zebrafish pancreas and the axolotl heart. Can immune cells also regulate differentiation during regeneration? Important answers to this question came from Michael Brand (Centre for Regenerative Therapies Dresden, Germany), whose group has pioneered methods for studying the cellular and molecular control of adult zebrafish brain regeneration after traumatic brain injury. Using cell transplantations and elegant genetic-lineage tracing techniques, it became possible to show that regeneration of the brain involved neurogenesis

from a defined stem cell population. Strikingly, these stem cells required inflammatory signals in order to undergo proliferation and differentiation. In particular, inhibition of leukotriene receptor signaling suppressed regeneration, whereas addition of a leukotriene, LTC₄, induced neurogenesis in the absence of injury. Since synthesis of leukotrienes and prostaglandins share arachidonic acid (AA) as a common precursor, it will be interesting to explore whether AA metabolism is indeed required for both tumor growth and neurogenesis in zebrafish. An additional insight into the importance of inflammation for tissue repair was presented by Shawn Burgess (National Human Genome Research Institute, USA). His group used an unbiased forward genetic approach in zebrafish in order to screen for genes that modify the process of hearing regeneration. This screen found several unique genes required for supporting hearing regeneration, and interestingly one of the genes identified (*hspd1*) could be responsible for initiating the inflammatory response after tissue damage. Altogether, the session on immunity made an important point of the beneficial role of immune cells in regeneration, suggesting that developing strategies to fine-tune the immune response at key stages is likely to advance the current efforts for therapeutic regeneration.

Regenerating the Central Nervous System

An understanding of how the central nervous system (CNS) regenerates can provide us with guidelines to bring about regeneration in a variety of human neurological disorders. In the past years, much research has been devoted to addressing several aspects of this process, resulting in important findings that are driving the field forwards.

In most systems, CNS repair relies on neural stem cells (NSC) for the production of new neuronal cell types. NSCs are able to initiate a regenerative program upon injury by responding to external signals that affect their proliferation, self-renewal and commitment. This raises the questions of whether and how the specialized stem cell niche affects NSCs in response to injury or homeostasis. Isabel Fariñas (University of Valencia, Spain) unraveled a novel, functional interaction between endothelial cells and NSCs in the subependymal zone of the mouse brain. She identified NT3, a nerve growth factor related neurotrophic factor, as a pro-quiescence signal secreted by the endothelial cells which penetrates the choroid plexus barrier through transcytosis and acts on NSCs by promoting endothelial Nitric Oxide synthase (eNOS) activation and NO production, thereby defining a novel stem cell niche interaction critical for NSC self-renewal.

Further insights into neurogenesis came from studies in zebrafish by Jovica Ninkovic (Helmholtz Zentrum München and University Munich, Germany). With the help of two-photon microscopy and retroviral labeling, he traced—for the first time—the response of neural progenitors and stem cells to injury at the single cell level. His data revealed

injury-induced changes in the division mode of radial glia-like cells that lead to the generation of a transit amplifying progenitor population, which then engages in the repair process. Moreover, he proposed a role for the evolutionarily conserved aryl hydrocarbon receptor pathway in the promotion of astrocyte dedifferentiation and proliferation that can act in the mammalian brain, which could constitute a novel strategy to enhance mammalian CNS regeneration.

Lastly, Ryoji Amamoto (Paola Arlotta laboratory, University of Harvard, USA) introduced a new system in which to study CNS regeneration: the axolotl pallium. He presented the first map of resident neuronal subtypes within this structure, built from immunofluorescence and in situ hybridization based on murine markers and circuit tracing, as well as evidence that neuronal diversity is maintained during pallium regeneration. This work prepares the ground for further studies on CNS regeneration using this interesting model.

Advances in Organ Regeneration

This session was opened with a plenary lecture by Kenneth Poss (Duke University Medical Center, USA). His group is studying the intricate process of adult heart regeneration in zebrafish. Since the adult heart in zebrafish appears to lack a resident stem cell population, myocardial regeneration relies on the dedifferentiation and subsequent proliferation of pre-existing cardiomyocytes. Following up on previous studies in mouse, pointing to a role for the growth factor neuregulin 1 (NRG1) in mouse heart proliferation, his group performed state of the art genetic manipulations to unequivocally show a prominent role of Nrg1 in inducing cardiomyocytes to proliferate. Nrg1 was induced shortly after heart injury and its activity was necessary to sustain the proliferation of the surviving cardiomyocytes. Notably, genetic upregulation of Nrg1 signaling in the absence of injury was sufficient to induce robust growth of the heart in adults. Thus, Nrg1 might provide a potential pro-regenerative signal for translational approaches in human heart regeneration. Further insights into heart regeneration came from Nadia Mercader (Centro Nacional de Investigaciones Cardiovasculares Carlos III, Spain), who used a cryoinjury approach for inducing heart injury in zebrafish, based on the application of a liquid-nitrogen-cooled probe to the heart, which results in rapid induction of cell death due to tissue freezing—thawing. In contrast to partial resection, cryoinjury causes generalized cell death and the formation of fibrotic tissue, resembling more closely human heart infarction. Remarkably, the zebrafish heart has the capacity to resolve tissue fibrosis leading to heart regeneration without a scar. Manipulating the pathways operating in zebrafish might instruct the development of therapies for heart regeneration after infarction.

In the following plenary lecture, Thomas Reh (University of Washington, USA) shifted the focus from the heart to

the retina. He first gave a detailed historical perspective on retinal development and the associated changes in cellular potency. More recently, his laboratory has been focusing on exploring the potential of a population of cells called Müller glia (MG) to turn into photoreceptors in mice. Previous studies in zebrafish have shown that, upon retinal damage, MG cells dedifferentiate into retinal progenitor-like cells, as evidenced by them re-expressing key progenitor markers such as *Ascl1*. These dedifferentiating cells can further re-differentiate into new photoreceptors, contributing to retinal regeneration. Thomas Reh asked whether experimental *Ascl1* re-expression in adult mouse MG could induce their capacity for neogenesis of photoreceptors. Notably, *Ascl1* overexpression was sufficient to induce the differentiation of functional neurons from MG *in vitro*. However, *in vivo* *Ascl1* failed to trigger photoreceptor differentiation from MG, indicating that the *in vivo* environment imposes reprogramming blocks. Overcoming these blocks will be a necessary step for exploring the regenerative capacity of MG cells as a translational approach for retinal regeneration. Promisingly, *Ascl1* was sufficient to induce cell cycle re-entry of MG *in vivo*, which is probably the first step in MG dedifferentiation, suggesting that overcoming the reprogramming roadblock might be just one step away. An alternative approach for retinal regeneration in humans would be the direct transplantation of retinal cells into the eye in order to produce new photoreceptors. This could provide a treatment for many blinding degenerative diseases which affect the photoreceptor cells but leave the inner retinal cells and the optic nerve intact. Jane Sowden (University College London, UK) presented her ongoing efforts using this approach. She and co-workers at University College London have shown that retinal cells expressing the key photoreceptor fate markers *Nrl* and *Crx* can differentiate into new photoreceptors in the adult environment following subretinal transplantation. By differentiating embryonic stem cells into optic cups through a protocol previously established by the late Yoshiki Sasai, she was able to isolate sufficient numbers of these photoreceptor precursors that could be transplanted and tested in a mouse model of retinal degeneration. The transplanted embryonic stem cell derived photoreceptors made connections with the host inner retinal neurons and formed outer segments. An important challenge in this field is to define specific means for promoting maturation and survival of more rod and cone photoreceptor cells.

In contrast to mammals, the newt exhibits the capacity to undergo naturally full retina regeneration following removal. Moreover, the newt is unique in its capacity to regenerate the lens throughout its lifespan. The group of Panagotis Tsonis (University of Dayton, USA) focuses on lens regeneration as a model to understand the mechanisms that govern cellular plasticity within an organ. It is well established that lens regeneration takes place exclusively via transdifferentiation

from the pigment epithelial cells of the dorsal iris, while the ventral iris cells are not capable of regenerating a lens. This juxtaposition of regenerative and non-regenerative cells provides a unique experimental model for understanding the molecular factors that confer competence for regeneration. To this end, Tsonis and co-workers performed next generation RNA-seq in order to define differentially regulated genes in the ventral and the dorsal epithelium. Further functional characterization of the candidate genes should provide insights into why some cells keep their capacity for regeneration while others lose it even within the same tissue. With regard to differences among individual cells of the same type, Nikolay Ninov (Centre for Regenerative Therapies Dresden, Germany) presented work from his group showing that individual beta-cells in the same pancreatic islet exhibit very distinct capacities for growth during development. Moreover, using *in vivo* drug screening in zebrafish for beta-cell proliferation, he identified specific metabolic pathways that could help in establishing the differences in beta-cell plasticity. Whether these metabolic differences also play a role in establishing subpopulations with distinct regenerative capacities remains to be elucidated. Altogether, the session on organ regeneration showcased important advances whilst also pointing to some of the outstanding challenges in the field.

Why Can They Do It, While We Cannot?

The availability of high throughput technologies combined with a wide range of model organisms has opened the door to tackling major outstanding questions in the field, such as what mechanisms underlie the loss of regenerative potential during ontogeny and aging, why does the ability to regenerate and repair tissues vary across species, and what are the evolutionary origins of regeneration.

While wound healing and regeneration capacities decrease with aging in mammals, they remain intact in other species. Jeffrey Corwin's work (University of Virginia, USA) suggests that stabilization of the differentiated state and signaling at cell junctions could be important factors. When studying regeneration in hair cell epithelia of young and old mice, his laboratory found a striking thickening of F-actin belts in adult supporting cells (hair cell progenitors) upon maturation, which correlates with reduced cell spreading and proliferation. In contrast, such changes do not take place in species that readily regenerate hair cells through their lifespan. His results suggest that cytoskeletal thickening upon aging constitutes a barrier for cell rearrangement and dedifferentiation, and may limit critical signaling events during regeneration processes.

Changes in regenerative ability are not only observed during aging but also at key developmental transitions in

many species. Patrizia Ferretti (University College London, UK) focused on early responses to spinal cord injury at regenerative and non-regenerative stages of chick development. She discussed the key role for calcium-dependent peptidylarginine deiminases (PADs) in mediating injury-induced apoptosis in non-regenerative stages. She showed that PAD3 promotes calcium-dependent cell death following injury through citrullination of cytoskeletal components and apoptosis inducing factor. Importantly, PAD3 inhibition reduces apoptosis and cavity formation after injury in non-regenerative spinal cords, and is able to promote human NSC growth and increase their survival following Ca^{2+} -induced apoptosis. These results associate PAD3 with the loss of regenerative ability during vertebrate development and suggest that targeting PAD3 could promote neuroprotection in a therapeutic setting. An alternative approach to tackling these issues was presented by Takashi Takeuchi (Tottori University, Japan), who focused on cell cycle regulation during developmental transitions and across species. While embryonic cardiomyocytes are able to proliferate upon heart injury in mice, their adult counterparts are unable to do so. Takeuchi and co-workers showed that downregulation of cyclin D1, which occurs during development, and inhibition of M-phase progression promote complete cell cycle exit in adult cardiomyocytes. Furthermore, he highlighted differences in transcriptional regulation of cyclin D1 between mice and newts, the latter being able to regenerate their hearts throughout lifespan, and discussed promising new tools to investigate this further in salamanders.

The ability of differentiated adult cells to re-enter the cell cycle is central to the differences in regenerative abilities among species. Through large-scale expression cloning, Elly Tanaka (Centre for Regenerative Therapies Dresden, Germany) identified a novel factor that is able to promote cell cycle re-entry of salamander myotubes. It is upregulated early following amputation of the axolotl tail and is necessary for proliferation and blastema formation. Furthermore, addition of exogenous protein is able to trigger cell cycle re-entry in uninjured mature tissues. Although this protein is present in other vertebrates, it is only found in a secreted form in the axolotl. This suggests that the protein may have acquired a novel function during evolution, in the promotion of cell cycle re-entry of mature cells, a fundamental aspect of the regenerative response. This is particularly interesting in the light of recent findings that report contributions of taxon-specific elements to the process of regeneration, an emerging topic at this meeting. One example of such an element is the salamander three-finger protein *Prod1*, which has been associated with the determination of proximo-distal identity during urodele limb regeneration. Recent data presented by Anoop Kumar (Jeremy Brockes laboratory, University College London, UK) suggests that *Prod1* plays a quite different

role during limb development, by controlling digit formation. Interestingly, this process is different in salamanders than in other tetrapods, as the former show pre-axial dominance (where the second digit develops prior to the first one, in contrast to post-axial dominance) in digit development. Hence, *Prod1* seems to be required for salamander-specific features of development as well as regeneration. Further insights into evolutionary aspects of regeneration came from Thomas Holstein (University of Heidelberg, Germany), who used an integrative transcriptomic/proteomic approach to study regeneration in *Hydra*. His data revealed two distinct regeneration phases, each with characteristic molecular signatures: an early response to injury and a late tissue-patterning phase. Remarkably, there is an enrichment of novel—evolutionarily more recent—genes during the early response stage, while evolutionarily ancient genes are enriched in the late phase of regeneration. Hence, it is possible that taxon-specific genes play a role during *Hydra* regeneration as well. Certainly, future research will focus on how these taxon-specific players contribute to regeneration and the way they interact with the conserved molecular network that underlies this process. In connection with this, the analysis of evolutionary aspects of regeneration is set to take a step forward with availability of full genomes in regenerating organisms such as the axolotl. In the final talk Randall Voss (University of Kentucky, USA) discussed the advances in sequencing the gigantic axolotl genome and revealed that the first two chromosome assemblies should be available at the end of the year. In addition he reviewed his extensive comparative approaches to investigating transcriptional changes during limb and tail regeneration, defining key transition points during these processes.

Together this body of work, and the new techniques emerging therein, will contribute to addressing the key question of how regenerative abilities are gained or lost during evolution, with important implications for the study of regenerative processes in different animal systems as well as for the design of approaches directed towards the promotion of regeneration in organisms with limited capacities, such as humans.

Conclusion

Together, this impressive set of presentations showcased an inspiring collection of insights which, taking advantage of both old and new models and technologies, brings us a step closer towards understanding the molecular and cellular basis of tissue repair and regeneration. Both from a basic as well as a therapeutic standpoint, the future is bright.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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