Repair or regenerate—how can we tip the balance?

Will Wood & Sabine A. Eming

The fourth EMBO conference on 'The Molecular and Cellular Basis of Regeneration and Repair', held in September 2012, brought together researchers from both the regeneration and wound-healing fields. The meeting spanned a wide range of research topics from basic science to clinical application, and a veritable melting pot of model organisms and approaches resulted in an excellent fourth conference in this series.

Enrique Amaya (U. Manchester, UK), Antonio Jacinto (U. Lisboa, Portugal), Kimberly Mace (U. Manchester, UK) and Paul Martin (U. Bristol, UK) organized the fourth EMBO conference on 'The Molecular and Cellular Basis of Regeneration and Repair', held at St Catherine's College at the University of Oxford (UK) in September 2012. The conference was unique in that it was a joint meeting with the British Society for Developmental Biology, and it served as their Autumn Meeting in 2012. The conference covered an impressive array of model systems including the well-established champions of regeneration—planaria, axolotl and hydra—and those more recognizable for their use in developmental biology—zebrafish, mouse, *Xenopus* and *Drosophila*—as well as some new model systems that we discuss later. The wide variety of organisms and approaches gave rise to an exciting and stimulating meeting that, together with Professor Enrique Amaya's DJ talent and the punting excursion, kept the participants entertained for five rare sunny days in Oxford. Given the breadth of research covered in the meeting, it is impossible to cover all the work presented. This report instead attempts to highlight some of the key themes to come out of the meeting and how the field is moving forward.

Susan Bryant (U. California, Irvine, USA) opened the conference with a stunning keynote lecture in which she set the stage for many of the discussions of fundamental questions that emerged during the meeting. She emphasized the importance of combining studies in developmental biology and regenerating tissues to understand how the

body is made and remade. On the basis of findings in both fields, she presented her view of the relationship between cell cycle, morphogens and pattern formation, and the ultimate implications for evolution.

Damage-induced signalling events

What are the earliest signalling cues triggered by damage *in vivo*? Several talks highlighted work focused on the earliest events after wounding. Andrew Chisholm (U. California, San Diego, USA) presented beautiful live imaging that showed a rapid and immediate epithelial calcium wave after wounding in the worm *Caenorhabditis elegans*. He demonstrated that this early signal is important to kick-start the cytoskeletal rearrangements required for driving wound closure. He went on to show that wounding also rapidly triggers mitochondrial fragmentation in epithelial cells around the wound, and presented evidence that this early event is also important for efficient wound repair. A similar calcium wave is induced after wounding in the fly, as illustrated by some equally stunning imaging experiments from Antonio Jacinto (U. Lisbon, Portugal). He showed that laser ablation to the pupa initiates an immediate calcium wave that triggers a reciprocal wave of actomyosin contraction in the wound epithelial cells, beginning distal to and moving towards the wound. This wave of contraction culminates in the formation of the contractile actomyosin cable at the leading edge of the front row epithelial cells, a structure that ultimately drives epithelialization. Will Wood (U. Bath, UK) showed that as well as triggering the cytoskeletal rearrangements that drive epithelialization,

the early calcium flash also triggers the resulting inflammatory response in the fly. By using live imaging, he showed that the rapid increase in intracellular calcium in wounded epithelial cells stimulates the production of the potent inflammatory chemotactic hydrogen peroxide (H_2O_2) through activation of the NADPH oxidase dual oxidase (DUOX).

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On the basis of a screen searching for master regulators of appendage regeneration in tadpoles, Enrique Amaya (U. Manchester, UK) uncovered reactive oxygen species (ROS) as a pivotal signal in amputation-induced tail regeneration in *Xenopus*. Even more interestingly, he found that ROS-mediated events in regeneration are recapitulated in developing embryos. Collectively, his work suggests that ROS is a global modulator of cell proliferation, specification and patterning, required for both correct embryonic development and regeneration. The key function of ROS in regeneration was underpinned by the presentation from Brigitte Galliot (U. Geneva, Switzerland), who presented work in hydra, the model system her group uses to investigate the question of how bisecting injury induces the two distinct regenerative programmes, head- compared with

foot-regeneration, a typical feature in this organism. Interestingly, she identified production of mitochondrial ROS as an immediate injury signal that directs the induction of diverse regenerative programmes.

Immune cells: good or bad?

Damage in most systems leads to an accompanying inflammatory response, but is inflammation beneficial or harmful, and is its role in repair and regeneration the same? So far, few studies have unravelled a positive link between immunity and regeneration. Intriguingly, Nadia Rosenthal (Monash U., Melbourne, Australia) demonstrated that during axolotl limb regeneration the innate immune response is compressed, such that cytokines are all induced rapidly and turned off early when compared with the mammalian response to damage. When macrophages are depleted before amputation, limb regeneration is blocked in the axolotl and scar tissue is formed. If the amputated stump is then reamputated, but this time in the presence of a normal inflammatory response, the limb can regenerate, suggesting an instructive role for the immune response in the regenerative process. Similarly, by using a genetic mouse model of selective and inducible myeloid cell depletion, Sabine Eming (U. Cologne, Germany) presented work demonstrating that macrophages are crucial not only for the initiation and progression of the healing response in skin wounds, but also for its termination. Her group identified distinct gene-expression profiles in wound macrophages during the transition of an early-stage pro-inflammatory towards a late-stage resolution phenotype, and correlated those phenotypes to specific cell functions orchestrating induction and termination of tissue growth and differentiation.

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Kim Mace (U. Manchester, UK) highlighted the importance of a balanced pro-inflammatory versus anti-inflammatory response in successful wound healing. She found that diabetic wounds in a mouse model of type II diabetes lead to a chronic inflammatory response with an excessive number of inflammatory cells present in the

injured tissue. She showed a difference in the ratio of pro-inflammatory M1 compared with anti-inflammatory M2 macrophages in diabetic wounds, with a higher level of M1 cells. By using transfer experiments, the Mace lab has shown that this shift in balance is due both to intrinsic factors within macrophages, as well as extrinsic factors in the diabetic wound environment that seem to push transition to M1 identity. By using a combination of *in vitro* and *in vivo* models, Matthew Hardman (U. Manchester, UK) identified oestrogens as key pleiotropic regulators of cutaneous healing, specifically age-impaired repair.

Regeneration against tumorigenesis

It has long been noticed that effective wound healing after injury is essential both to restore proper tissue function and to prevent uncontrolled tissue growth and cancer formation. Recently, this observation has received new attention as mechanisms underlying the molecular link between stem cells, wound healing and cancer have begun to emerge. Several presentations highlighted that a misregulated inflammatory response is a crucial link between abnormal wound healing and cancer formation. Along these lines, Paul Martin (U. Bristol, UK) unveiled that H₂O₂ not only is an early damage signal initiating immune cell recruitment to wounds, but also serves as a key attractant enabling immune cells to sense early clones of transformed cells before they progress to cancers. By using the zebrafish as a model, his findings also suggest that exposure to wound-induced H₂O₂ makes immune cells more efficient at detecting transformed cells *in vivo*. However, when presented in excessive amounts, ROS inhibit wound healing and promote carcinogenesis, as demonstrated by the work of Sabine Werner's group (ETH, Zurich, Switzerland). Werner reported important functions of peroxiredoxin 6 in skin repair and carcinogenesis. Whereas the overexpression of peroxiredoxin 6 in mouse epidermis promotes wound healing in aged mice, epidermal carcinogenesis is inhibited.

Liliane Michalik (U. Lausanne, Switzerland) presented the diverse roles of the hormone receptor peroxisome proliferator-activated receptors (PPARs) β/δ as a regulator of skin healing and carcinogenesis. Earlier work by her group has shown that IL-1-mediated PPAR β/δ activation favours skin wound healing in mice, but she has also identified PPAR β/δ additionally as a

promoter of ultraviolet-induced epidermal tumour formation and progression, indicating that this process is closely linked to the activity of Src family kinases. Tanya Shaw (St George's U., London, UK) introduced the use of ovulation as a fascinating new model to study how repetitive injury and repair might affect cell behaviour, as well as tumour initiation and progression. Yet, the ability to regenerate tissues does not inevitably correlate with the susceptibility to cancer formation. In fact, salamanders are able to regenerate complex structures such as the limb, but are extremely resistant to tumour formation. The work of Max Yun (U. College London, UK) in salamanders suggests a pivotal role for the function and tight regulation of p53 to segregate the capability of regeneration against tumorigenesis.

Epithelialization: insights from the fly

Restoration of epithelial barrier function is essential post injury. Detailed mechanisms by which epithelial cells integrate damage signals into an effective epithelialization programme are still not completely resolved, and the identification of new elements in this process were discussed in several presentations that used *Drosophila* as a model organism. Michael Galko (U. Texas, USA) reported a new link between integrin-based cell adhesion and activated Janus kinase (JNK) signalling in wound-induced epidermal cell-cell fusion. Although *Drosophila* larval wound closure was surprisingly independent of the expression of β -integrin, integrin-linked kinase (ILK) and particularly interesting new cysteine-histidine rich protein (PINCH), epidermal cell-cell fusion was significantly increased following knockdown of these genes. Wound-induced syncytium formation was in parallel with locally increased JNK activity and loss of PINCH and ILK from epidermal cell membranes of fusing cells proximal to the wound. Local hyperactivation of JNK activity in the absence of a wound was found to drive both cell-cell fusion and relocalization of PINCH and ILK from the membrane, suggesting a mechanistic link between the loss of integrin-based adhesion, activated JNK signalling and wound-induced cell-cell fusion. Tom Millard (U. Manchester, UK) elaborated on the regulation of actin dynamics at epithelial wound edges and drew parallels between wound healing and dorsal closure in *Drosophila* embryos. He found that partitioning defective 3 (PAR3)/Bazooka-mediated accumulation of lipid

phosphatidylinositol (3,4,5)-triphosphate (PIP3) along the epithelial leading wound edge is crucial for effective epithelial wound closure. Enrique Martin-Blanco (CSIC, Barcelona, Spain) presented a genome-wide gene-expression analysis during healing in imaginal discs in *Drosophila*. He has uncovered that the chaperonin TCP1 complex affects actin synthesis and folding during wound closure.

Nervous system regeneration

The regeneration of the central nervous system (CNS) following damage is of significant clinical and public interest, and is a key long-term goal for the regenerative medicine field. Karen Echeverri (U. Minnesota, USA) presented work from her lab on a comparative analysis of a regenerative model (axolotl spinal cord) and a corresponding non-regenerative system (rat spinal cord). She presented data to show that the microRNA miR-125b is highly expressed in axolotl and lowly expressed in rats after spinal cord injury, and that decreasing levels of this microRNA in axolotl impairs spinal cord regeneration. These findings identify miR-125b as a key regulator of the regenerative response in the nervous system. By using zebrafish as a model, Michael Brand (Center for Regenerative Therapies, Dresden, Germany) has been able to identify another important player in CNS regeneration. He demonstrated that after traumatic lesion, the adult zebrafish brain regenerates from radial glia-type progenitor cells, and a transcriptional analysis of these cells showed an injury-induced expression of the GATA family transcription factor Gata3. Knockdown experiments showed Gata3 to be required for regeneration not only in the brain, but also in the fin and the heart. Not to be outdone, the chick embryo can also tell us much about CNS regeneration, as it undergoes a loss of regenerative capacity during development. By comparing the response to spinal cord injury in regeneration-competent (stage E11) and regeneration-incompetent (E15) chick embryos, Patrizia Ferretti (UCL, London, UK) identified a calcium-dependent enzyme, peptidylarginine diiminase 3 (PAD3) isozyme, as a potential player in the loss of regenerative ability. PAD3

converts protein arginine residues to citrulline (citrullination), and work from the Ferretti lab shows that its expression and activity are increased following spinal cord injury in non-regenerating spinal cords. Ferretti went on to demonstrate that PAD3 acts as a mediator of cell death in the non-regenerating spinal cord and suggested that progressive upregulation during embryonic development contributes to the loss of regenerative capacity observed in the chick embryo.

New systems and translational hope

The post-natal loss of regenerative capacity in mammals is still an unresolved mystery in the field. Regeneration of finger-tips in humans and toe-tips in rodents, as well as increased regeneration capabilities in a few specific genetic strains represent exceptional examples of post-natal regeneration in mammals. The study of these tissues and organisms might have implications for the development of new strategies for inducing regeneration from non-regenerating tissue injuries. Ken Muneoka (Tulane U., USA) reported that targeted treatment of toe-tip amputation wounds in rodents with bone morphogenetic proteins stimulates a level-specific regenerative response that resembles the blastema-induced endogenous regenerative response seen in amphibians. The capability for amputation regeneration is tightly regulated in time and space during the post-amputation response, and depends crucially on a regeneration-permissive wound environment. Malcolm Maden (U. Florida, USA) introduced the African spiny mouse, whose regenerative capability is not limited to ear cartilage, as shown in the well-characterized regenerative MRL/MpJ strain, but reveals also scarless repair of skin wounds. Thus, this model might complement already known mechanisms explaining the regeneration-like behaviour in the MRL/MpJ strain. Interestingly, findings presented by Anna Jazwinska (U. Fribourg, Switzerland) suggest that transient scar formation in an injured zebrafish heart is an essential step towards providing mechanical stability of the damaged tissue and structure for the cardiomyocytes during heart regeneration.

Indeed, providing structure to induce regeneration in non-regenerating tissues was discussed as an important feature in additional presentations. Jeff Davidson (Vanderbilt U., USA) discussed the role of

the Wnt inhibitor secreted frizzled-related protein 2 (sFRP2) and transient expression of connective tissue growth factor (CCN2) in mesenchymal stem cell function, which contributes to the enhanced regenerative capacity in MRL/MpJ mice. To translate this knowledge into regenerative medicine, he introduced a new biocompatible, polyurethane scaffold for the delivery of repair agents that might recapitulate the MRL regenerative phenotype. Similarly innovative approaches were presented by Tomas Egana (Technical U., Munich, Germany), who has developed a new photosynthetic scaffold that provides oxygen to the regenerating tissue, and by Marcela Del Rio (U. Carlos, Madrid, Spain), who presented a tissue-engineered scaffold that provides a suitable milieu for keratinocyte migration and triggers granulation tissue maturation in a new skin-humanized mouse model that recreates diabetic healing impairment.

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In summary, the workshop provided deeper—and sometimes unexpected—insights into parallels and differences between regeneration and repair in diverse model systems. The presentations and discussions yielded ideas and innovative directions for how this knowledge contributes to the unravelling of one of the capital questions in the field: why some species can regenerate and others cannot. The highlighted themes provide a basis for translational hopes to resolve imperfect healing in mammals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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