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MEETING REPORT OPEN

Comparative biology of tissue repair, regeneration and aging

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The Symposium on the Comparative Biology of Tissue Repair, Regeneration and Aging, held 26 June to 28 June 2015 at the MDI Biological Laboratory in Salisbury Cove, Maine, brought together a diverse group of biologists with a common interest in understanding why regenerative capacity varies among animal species, why it is progressively lost in senescence, and how answers obtained from studies that address those questions might be applied in regenerative medicine.

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INTRODUCTION

The goal of regenerative medicine is to restore the functionality of tissues, organs, or body parts damaged by trauma, disease or aging. For over a decade much of the field's attention has been focused on trying to unlock the therapeutic potential of stem cells, and it has become increasingly clear that the key to that lock is knowledge of the *in vivo* context required to support regeneration. It has long been known that in the animal kingdom, the capacity to regenerate lost or damaged body parts varies considerably among species, as does susceptibility to degenerative aging (senescence). Attention has thus turned to the study of animals with exceptional regenerative abilities and/or negligible senescence, in order to learn what they have that we lack. This is motivated in part by a growing awareness that evolutionarily, the ability of animals to regenerate lost or damaged body parts is the ancestral condition, which for largely unknown reasons has been lost in various lineages, including amniotes, and then (importantly) in some species regained. Thus, regenerative capacity involves both conserved and species-specific mechanisms and gene regulatory networks. In this context, key questions for regenerative medicine are: what are the determinants of regenerative capacity in animals that are able to regenerate, and how might regenerative medicine make use of that knowledge to augment human regenerative capacity? The Symposium on the Comparative Biology of Tissue Repair, Regeneration, and Aging, held 26 June to 28 June 2015 at the MDI Biological Laboratory in Salisbury Cove, Maine, brought together a diverse group of leading biologists with a common interest in trying to answer those questions.

The symposium featured plenary talks by invited speakers, short talks selected from submitted abstracts, posters, and a panel discussion on translating the results of basic research to clinical applications. The talks covered different topics relevant to regeneration and aging that are not often juxtaposed at a single meeting, ranging from the biology of proteostasis and comparative models of aging, to the determinants of regenerative capacity and comparative models of regeneration. The meeting also included a public discussion by a panel of experts in the field, who fielded questions related to the practical problem of how knowledge gained by basic research on regeneration might be translated to medical applications. Here we review the highlights of the symposium, concluding with the major questions that were raised, and which are likely to drive the field going forward.

WOUND HEALING, TISSUE REPAIR AND REGENERATION

In a joint keynote lecture Claudia Angeli (University of Lousiville and Kentucky Spinal Cord Injury Research Center, Frazier Rehab Institute) and Dustin Shillcox described one of the extraordinary successes recently achieved by regenerative medicine. Shillcox was paralysed from the chest down at age 26 years after a car accident. Classified as 'sensory and motor complete' after being injured at T5, he was told he had no chance of recovering movement. But hope that he might move his arms and legs again came from research led by Angeli in collaboration with Reggie Edgerton (UCLA) and Susan Harkema (University of Louisville). Angeli's work focuses on mechanisms by which human locomotion is controlled following neurologic injury. She has found that the use of epidural stimulation and activity-based retraining of the nervous system following spinal cord injury increases the excitability of spinal cord neurons and augments return of function.[1,2] Shillcox participated in Angeli's research as a subject and today has regained the extraordinary ability to voluntarily control his legs and toes over brief time periods.

A long-standing question in regeneration biology is why mammals, including humans, possess a relatively poor regenerative ability, while some other vertebrates and many invertebrate animals have an extraordinary capacity to regenerate even highly complex tissues. Epimorphic regeneration, by which a proliferating blastema gives rise to new tissue, is the default regeneration mode for appendage injury in non-amniotic vertebrates, such as salamanders, frog tadpoles and zebrafish. In contrast, this process is extremely rare in mammals and instead tissue injury typically stimulates scar formation. Unlike most mammals, rabbits have been known for decades to be exceptional in regenerating ear wounds by blastema formation. Research led by Ashley Seifert (University of Kentucky) has discovered a similar ability in another mammal, the African spiny mouse (*Acomys*).[3] Ear puncture wounds in *Acomys* exhibit all the hallmarks of a blastema, such as formation and maintenance of an active wound epidermis, cell cycle re-entry and cell proliferation, delayed revascularisation, and formation of a proregenerative extracellular

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matrix. Besides ear regeneration, this species has a propensity for skin tearing under very low tension due to a porous extracellular matrix rich in collagen type III. Although similar conditions exist in humans and are associated with disease, *Acomys* utilise this strategy to escape predators by shedding off their skin. Remarkably, upon skin loss (including dermis) rapid wound contraction is followed by hair follicle regeneration in dorsal skin wounds and scar-free healing. Understanding the precise mechanisms by which this vertebrate species repairs large skin wounds and completely regenerates ear puncture holes may inform development of therapies for promoting more effective wound healing and regeneration in humans.

The ultimate key in understanding tissue healing is to monitor this process in living animals. A molecule of great interest for its important functions in a variety of wound-repair processes is hydrogen peroxide (H_2O_2), which has been implicated in leukocyte migration and axon repair. Far less is known about its role in re-epithelialisation during which epidermal keratinocytes migrate over the injured area to re-establish a protective skin barrier. Research led by Sandra Rieger (MDI Biological Laboratory) utilised *in vivo* imaging of larval zebrafish to explore the dynamics of H_2O_2 during keratinocyte migration. Comparative studies with human keratinocytes uncovered a conserved role for low but not high levels of H_2O_2 in oxidative regulation of Inhibitor of kappa B kinase alpha (Ikkα). In differentiated keratinocytes Ikkα represses *EGFR* activity,[4,5] whereas oxidation leads to *EGFR* de-repression. Thus, Ikkα appears to be a redox sensor within epidermal keratinocytes to control the switch between a differentiated and migratory (EGF-dependent) cell fate after injury (S Rieger, unpublished).

The classical wound repair model describes proliferation and migration as essential steps to promote wound closure. Could alternative mechanisms have a role and if so how are they regulated? Vicki Losick (postdoctoral associate of Allan Spradling, Carnegie Institution for Science; now at MDI Biological Laboratory) asked this question. By utilising the adult fruit fly as a model for studying epidermal wound repair, her work uncovered a previously unknown repair mechanism by which cells grow to become polyploid instead of dividing, also known as wound-induced polyploidy. In this process, upon injury cells at the wound edge enlarge in size and form a very large multinucleated syncytium, which facilitates resealing of the epidermis.[6] Further investigations showed that wound-induced polyploidy is regulated by the Hippo signalling pathway to fine tune the extent of polyploidy, possibly with respect to wound size. Polyploid cells are often observed in mammalian tissues in response to injury, stress, and/or aging, and thus wound-induced polyploidy appears to be an evolutionarily conserved mechanism to compensate for cell loss.

MAINTENANCE OF CELLULAR INTEGRITY AND REGENERATIVE POTENTIAL WITH AGE: INTERSPECIES COMPARISON

In humans, the ability to replace and maintain tissues and organ systems diminishes with age following reproductive maturation, a phenomenon referred to as senescence. To study this, investigators have used animal models with attributes that make them especially well suited for the specific senescence-related phenomena under investigation. For example, in studies that aimed at investigating the physiological impact of processes that maintain health of existing cells, a frequently employed model is the small nematode *Caenorhabditis elegans*, in which there is no replacement of somatic tissues in adult animals. Thus, health of the mature animal must be maintained entirely through quality control in terminally differentiated cells. Despite this inherent limitation on regenerative capacity, modulating known longevity pathways can increase lifespan by as much as 10-fold in this organism. This demonstrates the plasticity of cellular homeostasis and indicates that potential exists for ameliorating age-related decline in the absence of tissue replacement.

One of the most intensely studied types of intracellular homeostasis is that of proteins, often referred to as 'proteostasis'. Rick Morimoto (Northwestern University) discussed his laboratory's use of *C. elegans* to investigate the role of molecular chaperones in maintaining proteostasis. A key observation from their work is that stress responses that help ensure proteostasis are blunted very early after reaching adulthood, by signals originating from reproductive tissue. In addition, they have found a critical cell-non-autonomous component of maintaining protein balance, involving signalling between tissues.[7] Proteostasis was also the focus of studies carried out by Steven Austad (University of Alabama at Birmingham), who presented work on long-lived clams, an emerging model for aging and proteostasis studies. *Arctica islandica* is the longest-lived non-colonial animal, living 500 or more years. In their study of this exceptionally long-lived clam, Austad and colleagues found that it possesses a remarkable ability to maintain proteostasis under conditions that induce protein misfolding in shorter-lived species of clam,[8] an attribute that may be a key factor underlying its exceptional longevity.

In addition to proteostasis, aging also involves changes in RNA metabolism. Jarod Rollins (postdoctoral associate of Aric Rogers, MDI Biological Laboratory) showed that lifespan extension resulting from dietary restriction in *C. elegans* alters splicing and nonsense-mediated decay in a manner important for the effect on longevity. Specifically, he found diminished translation of genes encoding ribosomal subunits concurrent with an increase in ribosomal transcript variants bearing single retained introns near the 5′ end of the message. Because the retained introns contain premature stop codons, they would normally be subject to nonsense-mediated decay. However, Rollins and colleagues observe a concurrent decrease in nonsense-mediated decay rates, allowing some of these messages to escape this messenger RNA quality control and even associate with polysomes.

Other model systems exhibit a distinct lack of cellular senescence and display a superior ability to regenerate lost tissues. As discussed by Andrea Bodnar (Bermuda Institute of Ocean Sciences), in multiple species of sea urchins, gene expression analysis suggests that cellular mechanisms contributing to energy metabolism, protein homeostasis, and tissue regeneration are maintained with age.[9] The cnidarian *Hydra oligactis* also demonstrates remarkable maintenance of regenerative capacity under normal conditions and lacks signs of aging. However, cold induces sexual differentiation that leads to rapid aging and death.[10] Brigitte Galliot (University of Geneva) and colleagues recently found that loss of epithelial plasticity upon cold-induced sexual differentiation ultimately leads to loss of regenerative capacity that is associated with the onset of aging in this animal.

Vertebrate models, while closer to humans in an evolutionary context, can be challenging for research on aging because of the relatively long period required for breeding and assays of longevity. Regenerative capacity is also often quite limited in these models. However, different vertebrate fish models demonstrate remarkable regenerative capacity together with highly variable longevity. Among the shortest-lived vertebrates is the African turquoise killifish, an emerging model for aging research that lives only a few months. In work from the laboratory of Anne Brunet, Itamar Harel (Stanford University) discussed their recent development, sequencing, and use of the African turquoise killifish as a genomic model for rapid exploration of aging and age-related diseases in vertebrates.[11] Although this model is still in an early phase of characterisation, its utility was demonstrated in a small screen which showed that Telomerase-deficient fish display rapid onset of pathologies typically associated with aging in humans.

STEM CELLS AND PLURIPOTENCY

Phil Newmark (University of Illinois at Urbana-Champaign) gave an update on his work with schistosomes, addressing how these parasitic flatworms have deployed neoblast-like stem cells termed germinal cells to evolve their complex life cycle.[12,13] Schistosomiasis affects over 200 million people worldwide, and is a parasitic infection considered second only to malaria in its devastating socioeconomic consequences. Even though schistosomes require a snail host to proliferate, adult parasites can be reproductively active in their human host for over 30 years. To gain insight into their incredibly long life span and their ability to regenerate damaged tissue,[14] Newmark's group asked whether these parasitic flatworms contain somatic stem cells (neoblasts) like their free-living flatworm cousins. Like planarians, they found that schistosomes also have neoblasts—cells that express similar pluripotency factors and have the ability to differentiate.[15] Similarities between free-living and parasitic flatworms suggest that planarians can be used as a genetic model to understand schistosomiasis.

Several talks covered the latest research on muscle stem cells (or satellite cells). Satellite cells are primed to develop into muscle through activation of the transcription factor MyoD.[16] Eric Olson (University of Texas Southwestern) described how another bHLH protein called Twist2 antagonises MyoD activity and muscle differentiation.[17] Surprisingly, Twist2 appears to mark a specific, non-satellite cell population of myogenic progenitor cells. Thomas Rando (Stanford University) discussed the ability of satellite cells to remain in a non-cycling, quiescent state, only to become activated when neighbouring muscle cells are injured. When this happens, these quiescent cells become 'alert', increasing their size and cellular ATP levels to a primed state for muscle regeneration.[18] The Rando group discovered that the ability to enter the alert state requires activation of Hepatocyte Growth Factor to stimulate mTORC1 through PI3K-Akt signalling, filling in the gap of events that happen between muscle injury and repair.

Specific stem cell populations require a niche to self-renew and maintain pluripotency. Leanne Jones (UCLA) is investigating the molecular signals that maintain that niche in *Drosophila* intestinal stem cells.[19] In the intestine of older flies, cells expressing stem cell markers begin to accumulate, some undergoing initial stages of differentiation. Coincident with this accumulation, tight junctions and the barrier function of the intestinal epithelia becomes compromised. The Jones lab has discovered that compromised barrier function naturally triggers intestinal stem cell proliferation to repair cells. Stem cell proliferation in older animals is the readout of tight junctions that are falling apart. She is now trying to understand the local signalling pathways that respond to compromised barrier function and trigger intestinal stem cell proliferation within the niche. In *C. elegans*, germline stem cells are maintained within a niche at the distal tip of the gonad.[20] Dustin Updike (MDI Biological Laboratory) described the role of germ granules, which reside at the cytoplasmic surface of the nuclear periphery, in maintaining germ cell pluripotency. When germ granules are depleted, the gonad niche is compromised; this is followed by aberrant differentiation of germ cells.[21] The Updike lab is taking several approaches to determine whether germ granules act as a safety net to ensure that transcripts encoding somatic differentiation factors remain untranslated in the germline. This work underscores the role of cytoplasm in maintaining cellular pluripotency within the niche. Kailin Mesa (Yale University), a graduate student in Valentina Greco's lab, described another stem cell niche within the hair follicle. In this niche, stem cell pools expand tenfold when a new hair shaft is made. When the growth phase completes, the stem cell pool is reduced by apoptosis.[22] Interestingly, Mesa is finding that the dermal papilla of the mesenchyme functions to regulate both expansion and retraction of this pool of stem cells within the niche.

CARDIAC REPAIR, AND PANEL DISCUSSION ON TRANSLATING RESULTS TO CLINICAL APPLICATIONS

Cardiovascular disease is the leading cause of mortality in the Western world. Stimulating new heart muscle formation from progenitor cells in the injured adult heart has been a major focus of cardiovascular regenerative medicine. Jonathan Epstein (University of Pennsylvania) described his laboratory's work investigating the role of Hopx, a homeodomain transcriptional repressor that marks committed but undifferentiated cardiac progenitor cells, termed cardiomyoblasts. Hopx expression in cardiomyoblasts is both sufficient and necessary to promote myogenesis through integration of Bmp and Wnt signalling.[23] The use of stem cells was also a theme in the laboratory of Steven Houser (Temple University). Here he described his on-going work on cortical-bone-derived stem cells (CBSCs) as a therapeutic source for improving heart function after an ischemic event. The Houser lab presented a comparative study of three different populations of stem cell isolated from the Goettingen miniswine. When compared with cardiac-derived stem cells and mesenchymal stem cells, CBSCs demonstrated a greater capacity for proliferation, migration and survival when cultured.[24] Given previous work that demonstrated the enhanced restorative capacity of CBSCs in adult murine ischemic injury models, CBSCs represent an intriguing alternative progenitor population to the current catalogue of stem cells within the cardiac regenerative toolkit.

A unique aspect of the symposium was a public discussion of the state of regenerative medicine by a panel composed of Jonathan Epstein, Rick Morimoto, Eric Olson, Tom Rando and Nadia Rosenthal (Imperial College, Australian Regenerative Medicine Institute at Monash University, and The Jackson Laboratory). Although there is little doubt that regenerative medicine holds tremendous promise to improve diseased and injured tissue health through stimulating endogenous repair mechanisms, the panel emphasised that to realise that potential we need to better understand the biology. Stem cells, for instance, have been advocated as a potential Holy Grail to curing everything from heart disease to muscular dystrophy and neurological disorders. But as Eric Olson noted, 'Regenerative medicine is more than stem cells. Unfortunately, there was a push to deliver stem cells for a variety of therapeutic indications without deep understanding of the biology.' In particular, the clinical trials for improving heart function after a heart attack have proven to be disappointing. Rosenthal noted that the current approach with stem cells for heart repair is akin to 'dropping someone with a parachute into the Gobi Desert without a compass or iPhone. I don't think the cells know what to do.' While the application of stem cells in the heart has fallen short of expectations, there are examples of tremendous success in other applications. Bone marrow transplant is a shining example of stem cells working well.

A key component to understanding the biology of regenerative biology is to elucidate the influence of the microenvironment on stem cell behaviour. The interplay between stem cells and the resident environment is critical for successful application of stem cells into injured tissue. As we age, the environment enforces genetic brakes that suppress stem cell activity. We know very little 'about what we are fighting against,' cautioned Rando. Understanding these factors will be critical to reveal the true potential of stem cells in therapy. It will likely take time to see the promise come to fruition. 'We may not be able to declare a day when regenerative therapy is a complete success; but it will be a gradual process of small victories,' noted Epstein.

As noted above, the symposium highlighted diverse animal systems that are endowed with remarkable regenerative capacity, raising the question of whether a conserved genetic circuit could serve as a foundation for tissue regeneration. Benjamin King

(MDI Biological Laboratory) presented the Comparative Models of Regeneration Database (RegenDB), a searchable systems-level examination of tissue regeneration models aimed at addressing this need. A key component of RegenDB is the inclusion of microRNA expression and target gene prediction data sets across axolotls, zebrafish and mouse systems. This effort is part of the larger research program ongoing in the laboratory of Viravuth P Yin (MDI Biological Laboratory) to identify conserved genetic circuits that define tissue repair and regeneration during evolution.

STRESS AND THE IMMUNE SYSTEM IN REGENERATION, AND CONCLUSION

Stress and the physiological response thereto is a common theme linking regeneration and aging. Nadia Rosenthal discussed the role of the immune system in regulating inflammation and tissue regeneration following injury. Her laboratory's work with axolotls showing that macrophages are essential for limb regeneration[25] highlights the critical importance of inflammation and its resolution in the regenerative response, and suggests that interventions designed to facilitate resolution may also promote tissue regeneration in adult mammals.[26] To address the problem in a mammalian model, she and her colleagues at the Jackson Laboratory are using the variability in cardiac recovery following infarct injury among inbred mouse strains to identify genetic interactions that have a key role in regulating and resolving inflammation, with an eye toward discovering new therapeutic targets.

There is accumulating epidemiological evidence that chronic stress and adversity early in life correlates with the development of inflammatory conditions and degenerative diseases of aging later in life, a phenomenon commonly referred to as developmental programming.[27,28] James Coffman (MDI Biological Laboratory) presented the data showing that zebrafish embryos treated with the stress hormone cortisol develop into adults with reduced regenerative capacity, as indicated by morphological defects, aberrant macrophage behaviour and altered gene expression during tailfin regeneration. On the basis of the global patterns of gene expression measured by RNA-Seq in the treated larvae, as well as measurements of select genes in regenerating fins of adults, it was hypothesised that the cortisol-treated fish develop an adult phenotype wherein inflammation is poorly resolved, which interferes with regeneration. As discussed by Jennifer Simkin (University of Kentucky), a postdoctoral associate of Ashley Seifert, the resolution of inflammation is orchestrated by a progressive shift in macrophage polarisation, from the proinflammatory M1 subtype to the proregenerative M2a and inflammation resolving M2c subtypes.[29] Simkin's research reveals that this shift occurs more robustly in the African Spiny Mouse (*Acomys caharinus*) than in the common laboratory mouse (*Mus musculus*), which could account for the much greater capacity of the former to heal cutaneous wounds without scarring.

Finally, Jeremy Ng (Australian Regenerative Medicine Institute at Monash University), a graduate student of Peter Currie, discussed his work that asks whether regenerating neurons in a zebrafish retinal injury model follow the normal developmental sequence that generates all cell types, or specifically regenerate only the cell type that was ablated. His data indicate the latter, suggesting that regeneration of retinal nerves entails a somewhat different mechanism than retinal development, and is guided by environmental cues characteristic of the specific injury.

The context dependency of regeneration was perhaps the overriding message of this meeting: animals that regenerate well do so because the cells that mount a regenerative response are provided with microenvironments (extra- or intracellular) and signals that promote and regulate that response. In contrast, animals that do not regenerate may lack the requisite microenvironmental milieu, or the capacity to construct it owing to compromising genetic or epigenetic factors. A central theme in both regeneration and aging is that defense systems, particularly the immune system, have a key role in establishing a proregenerative milieu. In animals that undergo degenerative aging (senescence), those systems break down with age (leading, for example, to loss of proteostasis and increasing levels of unresolved tissue inflammation), whereas in animals with negligible senescence those systems are maintained or augmented with age. The challenge of regenerative medicine is to learn to provide injured and/or degenerating tissues not only with stem or progenitor cells, but with a tissue-level context conducive to regeneration. A take-home message of the symposium was that the requisite contextual understanding can be achieved through comparative studies that make use of judiciously chosen animal models with differing regenerative abilities and susceptibilities to senescence.

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CONTRIBUTIONS

All authors contributed to the writing of the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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