Integrative and Comparative Biology

Integrative and Comparative Biology, volume 54, number 4, pp. 658–666
doi:10.1093/icb/icu108

Society for Integrative and Comparative Biology

SYMPOSIUM

The Cell's View of Animal Body-Plan Evolution

Deirdre C. Lyons,[1,*] Mark Q. Martindale[†] and Mansi Srivastava[‡,2]

*Department of Biology, 4115 French Family Science Center, Duke University, Durham, NC 27708, USA; [†]Whitney Laboratory for Marine Biosciences, University of Florida, St Augustine, FL 32080, USA; [‡]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA

From the symposium "The Cell's View of Animal Body Plan Evolution" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

[1]E-mail: dclyons1@gmail.com
[2]E-mail: mansi@wi.mit.edu

Synopsis An adult animal's form is shaped by the collective behavior of cells during embryonic development. To understand the forces that drove the divergence of animal body-plans, evolutionary developmental biology has focused largely on studying genetic networks operating during development. However, it is less well understood how these networks modulate characteristics at the cellular level, such as the shape, polarity, or migration of cells. We organized the "Cell's view of animal body plan evolution" symposium for the 2014 *The Society for Integrative and Comparative Biology* meeting with the explicit goal of bringing together researchers studying the cell biology of embryonic development in diverse animal taxa. Using a broad range of established and emerging technologies, including live imaging, single-cell analysis, and mathematical modeling, symposium participants revealed mechanisms underlying cells' behavior, a few of which we highlight here. Shape, adhesion, and movements of cells can be modulated over the course of evolution to alter adult body-plans and a major theme explored during the symposium was the role of actomyosin in coordinating diverse behaviors of cells underlying morphogenesis in a myriad of contexts. Uncovering whether conserved or divergent genetic mechanisms guide the contractility of actomyosin in these systems will be crucial to understanding the evolution of the body-plans of animals from a cellular perspective. Many speakers presented research describing developmental phenomena in which cell division and tissue growth can control the form of the adult, and other presenters shared work on studying cell-fate specification, an important source of novelty in animal body-plans. Participants also presented studies of regeneration in annelids, flatworms, acoels, and cnidarians, and provided a unifying view of the regulation of cellular behavior during different life-history stages. Additionally, several presentations highlighted technological advances that glean mechanistic insights from new and emerging model systems, thereby providing the phylogenetic breadth so essential for studying animal evolution. Thus, we propose that an explicit study of cellular phenomena is now possible for a wide range of taxa, and that it will be highly informative for understanding the evolution of animal body-plans.

Introduction

Understanding how diverse body-plans evolved remains one of the most exciting and challenging goals for evolutionary and developmental biologists alike. Over the past few decades, genomic and molecular genetic approaches have uncovered gene networks that regulate tissue patterning during development. However, we are currently lacking in the understanding of how specification of cell types generates specific cells' biological properties, such as polarity, migration, and adhesion from a highly conserved set of effector proteins (such as actin, integrin, and PARs) (e.g., Beh et al. 2007; Saunders and McClay 2014). Yet, cells are the fundamental unit of all biological structures and phenomena—evolution shapes phenotypes by ultimately changing cellular characteristics. Recent technological advances in cell and molecular biology (functional approaches), microscopy (live-cell imaging), and computational biology (modeling and next-generation sequencing and bioinformatics) will likely enable comparisons of cellular behavior during development across animal species. Our symposium aimed to consider integrative approaches for understanding the evolution of animal body-plans from a cellular perspective.

Advanced Access publication August 8, 2014

The Society for Integrative and Comparative Biology (SICB) has a rich tradition of fostering integrative approaches for studying organismal evolution. Thus, SICB was a natural home for a ''cell-evo-devo'' symposium at the annual meeting in Austin, TX, USA. The research presented by symposium participants allowed us to place biological studies of cellular development in an explicitly comparative context. The symposium revealed the methodological advances that provide an opportunity to investigate how aspects of cell biology have been altered by evolution to yield varied animal forms. The many presentations at the symposium and accompanying sessions allowed us to consider major cell-biological phenomena that can be tinkered with over the course of evolution to change development, for example: (1) shape, adhesion, and movement of cells, (2) cell division and tissue growth, and (3) cell-fate specification. Additionally, investigations of regeneration offered the opportunity to study the cell-biological basis (e.g., migration and proliferation) of maintaining adult form. Several examples of these lines of inquiry are highlighted in the sections below (Fig. 1).

Shape, adhesion, and movement of cells during morphogenesis

Morphogenesis was a theme of many of the presentations, from how epithelia fold, to how tissues change shape or exchange neighbors, to how cells move individually and collectively.

Cellular reorganization

Actomyosin contractility is known to drive cellular rearrangements that can result in morphogenetic movements, including convergence and extension of tissues, formation of boundaries, and alignment of cells (St Johnston and Sanson 2011). Several speakers presented work showing developmentally and phylogenetically diverse contexts in which actomyosin operates.

Jennifer Zallen (Memorial Sloan Kettering Cancer Center) presented her research on the role of actomyosin in elongation of the fly embryo, a process that relies entirely on cellular rearrangements because proliferation of cells does not occur (Zallen and Blankenship 2008). Groups of cells exchange neighbors to form rosettes that mediate local elongation that ultimately results in elongation across the length of the entire embryo. Myosin II is enriched at the vertical interface between anterior–posterior neighboring cells, whereas Par-3 is enriched at horizontal edges, where it stabilizes adhaerens junctions and inhibits Myosin II. Thus, contraction of the myosin cables results in anterior–posterior elongation of the embryo. Activated Rho-kinase is localized in higher concentration at vertical edges and activates myosin specifically at those edges. Contraction of myosin at vertical edges results in elongation of the tissue in the anterior-posterior direction. However, the upstream processes that control Rho-kinase are unknown.

A very similar actomyosin-driven system underlies morphogenesis in vertebrates in varied contexts such as the neural epithelium and renal tubules (Wallingford 2012). John Wallingford (University of Texas at Austin) showed that convergent extension of the dorsal mesoderm in frogs also progresses in a manner very similar to the elongation of fly embryos. The planar cell polarity (PCP) pathway is required for convergent extension in frogs, with Fritz (a PCP pathway component) required for restricting septins to mediolateral cell junctions, which in turn restrict activated myosin to specific cellular junctions.

The upstream controls of actomyosin contractility in the elongation of fly embryos, and in convergent extension during vertebrate development appear to be different. PCP components, required in vertebrates, are not required for setting up the asymmetric localization of myosin activity in the elongation of fly embryos. Notably, the types of tissues involved are very different in the two contexts—mesoderm in frogs versus epithelium in flies. Uncovering the mechanisms upstream of actomyosin in the elongation of fly embryos would be in important step toward understanding the evolution of two very different processes in the context of two different types of cells (mesenchyme versus epithelium) with underlying similarities in cellular behavior in two distantly related species (frogs versus flies).

Adding phylogenetic breadth, Ed Munro (University of Chicago) described a role for Rho-kinase/Myosin II in ascidian neurulation. During this process, which is a feature of all chordates, junctions between epithelial and neural cells exchange to neural–neural and epithelial–epithelial cell junctions (Munro and Odell 2002). These exchanges progress in a posterior to anterior direction, giving the appearance of a ''zipper,'' and the force for the process is generated by myosin contractility. The Munro Laboratory is using mathematical modeling on data from laser-ablated junctions to further understand the dynamics of zippering, assessing whether the amount of force, or the release of resistance, impact the process. These computational methods can be extended to other systems to assess whether the same or different features of actomyosin

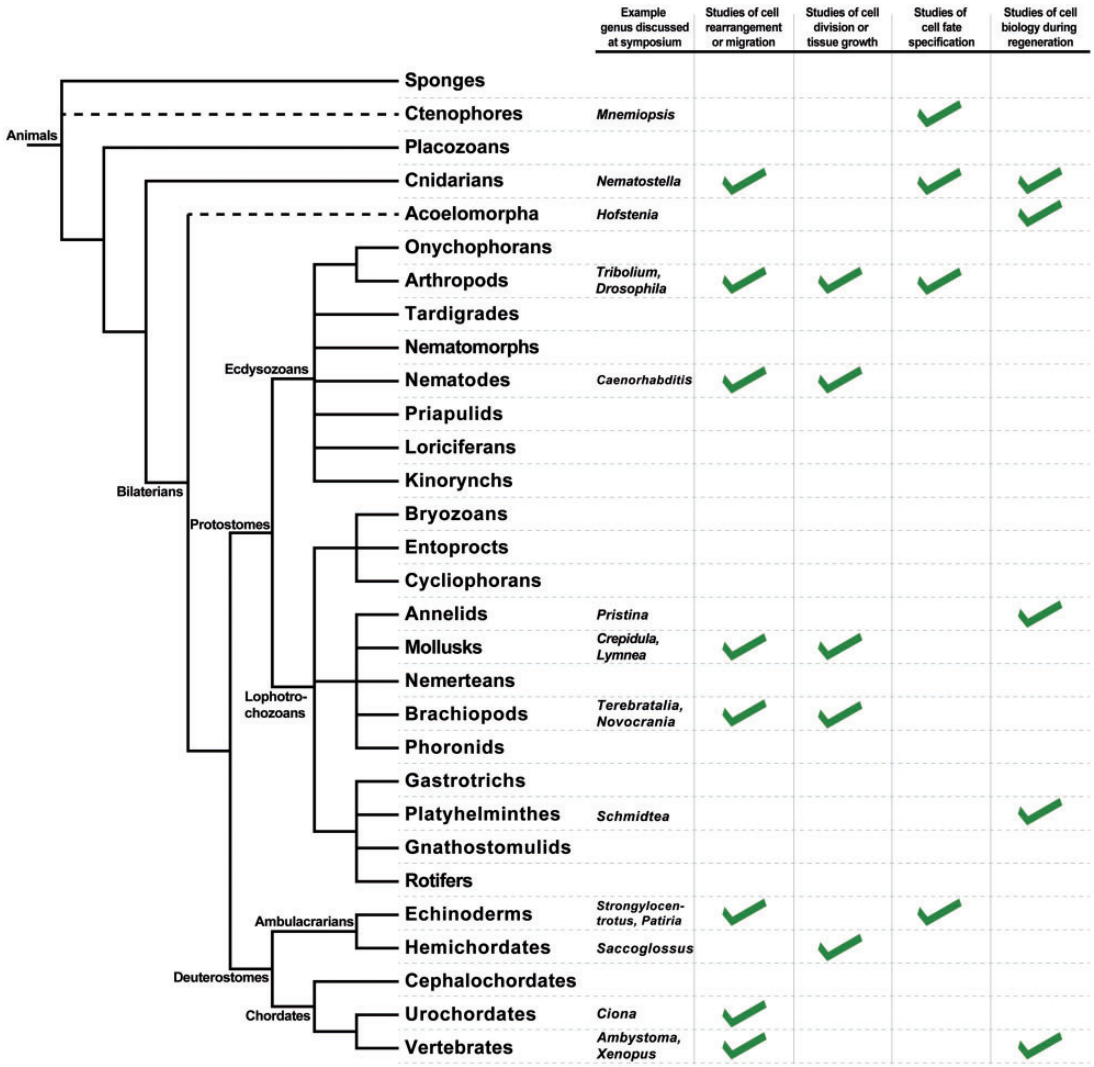

Fig. 1 Phylogenetic distribution of traditional and emerging model systems highlighted at the symposium. Schematic tree represents the phylogenetic relationships of major animal lineages. Dashed branches in the tree indicate the positions of groups whose relationships to other animals are highly debated. Examples of genera represented at the symposium are shown. Only the systems discussed in this article are indicated here, several others were highlighted in other talks and posters (see the abstracts of the meeting for more details on those species). The check marks indicate the clades in which various aspects of cell-evo-devo have been studied. The talks at the symposium represented a broad phylogenetic distribution of animal lineages.

contractility drive cell movement in the varied contexts where this system operates (Lecuit et al. 2011).

Bob Goldstein (University of North Carolina at Chapel Hill) presented work on endodermal invagination in *Caenorhabditis elegans*. The two endoderm-forming cells (Ea and Ep) have a higher concentration of activated Myosin II on their apical sides. Myosin moves toward the center of the apical surface and then the edge of the cell follows to move the cell inwards (Lee et al. 2006). The cause of the delay in the movement of the cell membrane inward is unknown, but Goldstein proposed a ''molecular clutch'' that has to engage for the myosin motor machinery to connect to the cell membrane. Newly developed methods using the CRISPR/Cas-9 system will provide transgenic *C. elegans* with tagged actin and myosin to visualize the process directly (Dickinson et al. 2013). It would be important to investigate whether the molecular clutch phenomenon turns out to be similar to other processes such as invagination of the endoderm in ascidians (Martin and Goldstein 2014).

Avenues for future research include dissecting the upstream control of actomyosin contractility in all these developmental contexts to understand how the system has been altered over the course of evolution to shape diverse forms of animals. Additionally one could expand the study of cellular

rearrangements in morphogenesis to previously understudied, yet evolutionarily informative, lineages. Matthew Gibson (Stowers Institute) spoke about his work on the emerging model sea anemone, *Nematostella vectensis*. During metamorphosis, as the planula larva settles and begins to form tentacles, the pseudostratified epithelium re-organizes to elongate the tentacles, and main body, along the oral–aboral axis. The cellular mechanisms underlying this elongation are unknown (e.g., oriented cell division versus rearrangement of cells via adhesion and alteration of shape), but mosaic labeling of clones of epithelial cells in green fluorescent protein (GFP)-tagged transgenic animals now can be used to visualize how the epithelium becomes organized/elongated (Fritz et al. 2013). Progress in transgenesis in *Nematostella* is moving at a rapid pace—stable transgenic lines are being developed (Michael Layden and Mark Martindale, Whitney Marine Laboratory) and Aissam Ikmi (Gibson Laboratory, Stowers Institute) presented a poster on developing inducible reporters. Thus, the recent development of methods for transgenesis in *Nematostella* will enable a comparison of cellular biology in epithelia between well-studied model organisms (e.g., Guillot and Lecuit 2013) to processes in this early-diverging animal lineage. This would allow us to assess whether actomyosin-based cellular rearrangement is a fundamental feature of metazoan biology.

Nat Clarke (Lowe Laboratory, Hopkins Marine Station/Stanford University) is interested in the evolution of epithelial cell-adhesion. He is applying biochemical approaches to study the functions of cell-adhesion proteins in *Nematostella*, and described his efforts in understanding whether *Nematostella* alpha-catenin can bind and bundle F-actin. Combined with cellular approaches, such as the ones employed by Matt Gibson's group, this biochemical perspective will provide a more complete picture of mechanisms for morphogenesis across animal species.

Cellular migration

Other presenters shared work on cellular migration. Sally Horne-Badovinac (University of Chicago) talked about the work in her laboratory on egg-chamber elongation in *Drosophila* (Horne-Badovinac and Bilder 2005; Horne-Badovinac 2014). The egg-chamber is composed of an outer epithelium of follicle cells that surrounds the germ-cell cluster. The egg-chamber is initially spherical, but elongates as development proceeds. Follicle cells influence elongation in two ways: (1) by collective migration that causes the egg-chamber to rotate and (2) by secreting extracellular matrices that form fibrils perpendicular to the axis of elongation of the chamber. Dr Badovinac discusses the evolutionary conservation of these mechanisms in her review in this issue (Horne-Badovinac 2014).

Megan Martik (McClay Laboratory, Duke University) talked about primordial germ cell (PGC) migration in the sea urchin *Lytechinus variegatus*. By specifically labeling the PGCs (by generating chimeras), and using time-lapse microscopy, she showed that PGCs undergo active migration during gastrulation, and find their way to the future location of the left coelomic pouch (where the adult will form following metamorphosis) by a directed homing mechanism that shares molecular characteristics with PGC migration in other systems such as *Drosophila* and vertebrates. Future work will take advantage of the well-established gene regulatory network in sea urchins to build a regulatory module for directed migration of germ cells in this basal deuterostome.

Cellular migration during gastrulation was discussed in several species, including two lophotrochozoans. These talks contributed to ongoing debates about whether deuterostomy (the anus forms from the site of gastrulation) or protostomy (the mouth forms from the site of gastrulation) is ancestral in the bilaterians (Martin-Duran et al. 2012). Dede Lyons (in collaboration with Jon Q. Henry, University of Illinois Urbana-Champaign) presented work on the process of gastrulation and axial elongation in the slipper snail *Crepidula fornicata*. Movies of embryos expressing fluorescently tagged biosensor proteins for the actin and microtubule cytoskeleton revealed the cellular behaviors during migration of the ectoderm over the yolky endoderm. Lineage-tracing illustrated the relationship of individual clones relative to the blastopore and how these contribute to the mouth and anus. Future work will focus on functional approaches to understanding the cellular and molecular control of gastrulation, and how the precursors of the mouth and anus separate during axial elongation.

Jose Martín-Durán (Hejnol Laboratory, SARS Norway) presented work on gastrulation in another branch of lophotrochozoans, the brachiopods. He compared the expression patterns of evolutionarily conserved regulatory factors known to be involved in gastrulation and in formation of the mouth and anus in both a protostomic species and a deuterostomic species. Initially these markers are expressed in a similar manner along the animal–vegetal axis in the two brachiopod species, but later, differences in expression-domains emerge, which could explain how

the different gastrulation behaviors occur. It would be important to investigate the underlying cellular movements, using techniques similar to the ones used by Lyons et al., to see how differences in gene expression ultimately control cellular behavior to yield either protostomy or deuterostomy.

These and other presentations at the symposium revealed that emerging model systems are becoming more tractable to investigations of cellular movements and the underlying cytoskeletal dynamics. Thus, study of cell migratory behaviors in a variety of developmental contexts in a broad range of species is timely, and will reveal broadly conserved mechanisms that may have been altered over the course of evolution to generate new body-plans. Furthermore, the time is right for connecting the gene regulatory networks operating in migrating cells to the specific cellular behaviors they exhibit, an exciting future direction.

Cell division and tissue growth

Control of cell division in early embryonic development

Control of cell division during embryonic development can modulate morphogenesis and thus changes in the regulation of the cell-division apparatus can impact the final form of the adult. A notable example of such control comes from studies of chirality (direction of shell coiling) in snails. Most snails have dextral coiling of the shell, but some species and strains exhibit sinistral coiling. This difference in chirality is first obvious at the transition from the four-cell to the eight-cell stage, when the mitotic figures of the macromeres become tilted in either a clockwise (dextral) or counter-clockwise (sinistral) direction. Reiko Kuroda (Tokyo University of Science) discussed the work in her laboratory on using embryological and molecular perturbations to understand the basis of this difference in chirality, both in terms of how positioning of the spindle is controlled and how downstream signaling-events, such as asymmetric expression of nodal signaling, are influenced by the stereotyped positioning of blastomeres (Kuroda 2014).

The positioning and control of the mitotic apparatus have likely undergone substantial change over the course of evolution as animal embryos changed size to adapt to new environments or life-history strategies. George von Dassow (University of Oregon) is using the sea star *Patiria miniata* to investigate the mechanisms that control the highly asymmetric divisions during meiosis that form the polar bodies. Using GFP-tagged biosensors, he described a wave of active Rho that precedes cytokinesis of the egg. He proposed that cortical excitability is controlled by an amplifier mechanism in large yolky eggs in which the influence of the mitotic spindle might be less reliable or more diffuse. This amplifier mechanism may be an adaptation to large cell size. Egg size varies widely between species, and so understanding how basic cellular processes such as cell division scale with changing cell size or yolk content is an important open question.

In a more explicit treatment of the evolution of the mitotic apparatus, Daniel Needleman's group (Harvard University) is studying whether standing genetic variation can explain variability in the first cleavage spindle in natural populations of *C. elegans*, and whether features of the spindle are subject to natural selection. Reza Farhadifar and Daniel Needleman (Harvard University) discussed how the mitotic apparatus scales with cell size and how this varies over evolution. They used the first division of the *C. elegans* embryo to study interspecific variation by using high-throughput microscopy to measure spindles in thousands of embryos in hundreds of lines. They measured features of the first mitotic spindle and found standing genetic variation among natural isolates of *C. elegans* for them. Future work aims to use single nucleotide polymorphisms (SNPs) associated with this variation to identify loci that control this variation. Their results indicate that there is continuous selection to scale the spindle to the size of the cell.

The nematode vulva presents another opportunity to investigate the regulation of adult form by controlling cell division. During morphogenesis of the *C. elegans* vulva, a hole in the basement membrane forms over the vulval cells, by the invasion of the anchor cell (AC). David Matus (SUNY Stony Brook) discussed his work on the role of the AC in initiating the uterine–vulval connection by breaching the basement membranes separating these tissues across approximately 20 species of rhabditid nematodes (Matus et al. 2014). All of the species examined initiate this connection through the action of an invasive AC. Matus and colleagues examined how the basement membrane gap expands following the initial breach—and found that it expands through cell division of the underlying vulval cells and then stabilizes through contact with a non-dividing cell (via integrin–laminin adhesion). In all rhabditid nematodes studied, the same homologous vulval cell has been selected to exit the cell cycle one cell division early to stabilize the gap in the basement membrane. This work highlights the advantage of examining morphogenesis across multiple species to identify

aspects of development that are crucial, and stable across hundreds of millions of years of evolution.

Control of morphogenesis through regulation of cell division can be studied in many contexts, explaining morphological evolution at many scales. The story of the nematode vulva, for example, could reveal microevolutionary trends, focusing on shorter time scales and the examination of homologous cells across the phylogeny of nematodes. Egg size and positioning of the mitotic spindle may be modified over larger time scales, explaining macroevolutionary changes.

Growth in embryonic development

Growth is a major determinant of the body-plan and is also regulated by controlling the cell cycle. One emerging theme at the symposium was a comparison of axial elongation in segmented versus non-segmented animals and the cellular and molecular mechanisms controlling growth along the anterior–posterior axis.

Ayaki Nakamoto (Nagy Laboratory, University of Arizona) talked about the cellular basis of segmentation in the beetle *Tribolium*. Although the genetic circuitry for segmentation has been studied extensively in arthropods, the process is poorly understood in terms of the underlying cellular behavior, including the relative contributions of proliferation and rearrangement. Whereas the predominant model of growth in arthropods is the addition of segments from a terminal growth zone, Nakamoto et al. used labeled clones of cells to show that cells in the posterior do not divide more frequently than cells in the anterior, but they do undergo dramatically different patterns of cellular rearrangement. They also modeled the addition of abdominal segments mathematically, and showed that segments can form by random cell movements combined with information about polarity in the absence of cell division. Thus, a cellular perspective is bringing new insights to a previously well-studied phenomenon (segmentation).

Others presented studies of "segmentation genes" in unsegmented animals. Jens Fritzenwanker (Lowe Laboratory, Stanford University) discussed posterior elongation of the primary axis in an unsegmented worm, the hemichordate *Saccoglossus kowalevskii*. Their approach focuses on the role of Fgf and Wnt signaling (as well as on transcription factors such as Eve, Caudal, and Brachyury), since these pathways play a central role in axial growth and segmentation in arthropods and vertebrates. Bruno Vellutini (Hejnol Laboratory, SARS Norway) talked about the roles of "segmentation genes" in the larvae of the unsegmented brachiopods. Studying the functions of segmentation genes in unsegmented contexts will reveal the broader functional roles of these genes and possibly shed light on how they function in segmentation. Combined with future cell-biological studies of posterior growth and segmentation in segmented and unsegmented phyla, this work could reveal mechanisms for regulating growth and thus explain the trend for increased body size in some animal lineages.

Cell-fate specification and the origin of novel cell types

A major modifier of animal form is cell-fate specification—cells may be specified in new positions, and new types of cells with novel functions may emerge over time. Dave McClay (Duke University) talked about the work in his laboratory on sea urchins, which are a model for understanding how gene regulatory networks control cell specification. He talked about an evolutionary novelty of echinoids, the sea urchin primary mesenchyme cell lineage, which builds the larval skeleton. The shape of the skeleton is determined by the overlying ectoderm and investigators in his laboratory have been working to understand how these signals direct the shape of the skeleton, which varies between species (Lyons et al. 2014).

Ajna Rivera's group (University of the Pacific) is studying the origin of complex structures by exploiting the sexual dimorphism of the eyes of the ostracod, *Euphilomedes*. In this species, males make complex image-forming eyes with ommatidial structures, whereas females make simple eyes (Rivera and Oakley 2009). Dr Rivera is exploring the origins of the complex cellular organization of the eyes of males relative to the simpler organization of the eyes of females by studying differences in gene expression during embryonic development. Gene expression in eyes is very similar between males and females during early development, and Dr Rivera has hypothesized that just a few changes in expression may explain this major difference in the form of the eye between male and female ostracods.

Leslie Babonis (Martindale Laboratory, University of Hawaii, and Whitney Laboratory) talked about the evolution of a novel organelle, the cnidocyst, which gives the cnidarian cnidocyte cell its stinging properties. This cell type originates from progenitors that also make neurons, and not surprisingly cnidocytes express neuronal markers. Babonis described recent work using comparative tissue transcriptomics in *Nematostella* to identify the genes specifically

expressed in cnidocysts and used this cell type as a model for studying the evolution of novelty at the cellular level (Babonis and Martindale 2014).

Antje Fischer (in collaboration with Mark Q. Martindale and Jonathan Q Henry) discussed work examining how very early cleavages in the ctenophore *Mnemiopsis* segregate determinants of cell-fate. Started by students in the Marine Biological Laboratory Embryology class (Woods Hole MA), Fischer and co-workers demonstrated that factors affected by the cytochalasin-D-sensitive actomyosin cytoskeleton are causally involved with the spatial distribution of factors responsible for the formation of two distinct types of cells, light-producing photocytes (derived from the M lineage) and motile comb plate cilia (derived from the E lineage) into the appropriate cells at subsequent cell divisions (Fischer et al. 2014). Furthermore, although these treatments halted all cytokinesis, they did not affect the timing of nuclear divisions. Fisher and colleagues went on to demonstrate that cell-type-specific differentiation markers appeared at the same time as untreated controls, indicating that these embryos "count" cell-division cycles. These data illustrate the dynamic interplay between spatial and temporal components of developmental programs.

The interplay of cell specification and cellular behavior, both in terms of how specified cell-fates launch cellular properties (e.g., secretion of the larval skeleton in urchins, organization of ostracod eyes, and formation of stinging cells in cnidarians) and of how cellular behaviors control cell-fate specification (e.g., formation of photocytes and comb plate cilia in ctenophores by regulation of cell division) will be essential to an understanding of the evolution of animal body-plans.

Cell biology in regeneration of adult forms

Most animals are able to repair tissue upon injury, and many are able to regenerate virtually any missing tissue. Regeneration requires mechanisms for re-establishing the form of the animal and therefore presents another opportunity to study cellular processes for the evolution of animal body-plans. Additionally, regenerative capacity is broadly distributed across animal lineages, raising the possibility that regeneration is a fundamental feature of metazoan biology (Bely and Nyberg 2010). Therefore, we aimed to gather researchers studying regeneration in diverse species to enable an explicit comparison of underlying molecular, genetic, and cellular processes.

Josien van Wolfswinkel (Reddien Laboratory, Whitehead Institute) presented her work on single-cell analysis of planarian neoblasts, the adult pluripotent stem cells that endow planarians with their storied ability to regenerate any missing tissue. Over the past several years, studies of regeneration in the planarian, *Schmidtea mediterranea*, have revealed many proteins required for wound signaling, maintenance and differentiation of stem cells, and patterning of new tissue. However, the large number of neoblasts present in each animal has been treated as a homogeneous population in most studies. Dr van Wolfswinkel described her efforts in studying these cells using single-cell analyses through multiplex quantitative polymerase chain reaction (qPCR), revealing considerable heterogeneity (van Wolfswinkel et al. 2014). This is likely the beginning of major efforts in understanding the diverse types of cells that migrate, divide, and differentiate to mediate planarian regeneration and will provide a new, cellular view of regeneration. Dr van Wolfswinkel explores her ideas about the molecular determinants of pluripotency of adult stem cells in diverse animal species in her review in this issue of ICB (van Wolfswinkel 2014).

Alexa Bely (University of Maryland, College Park) studies regeneration in annelids, the group that includes segmented worms and for which the term neoblasts was originally coined (Randolph 1892). Harriet Randolf described neoblasts as cells that migrated to wounds and considered them to be embryonic cells that were set aside to form regenerating tissue. Dr Bely's group has devised a method for long-duration live imaging of naid oligochaete annelids that allows observation and quantification of different migrating cell-types during regeneration (Bely 2014). Combined with gene expression analyses of known regenerative genes, this work will generate a greater depth of understanding about the cell biology of regeneration in annelids.

Comparisons of cellular behaviors during regeneration in annelids and planarians will reveal which processes have been shaped to mediate regeneration in these two distantly related groups. Talks during companion sessions offered an even broader perspective on the evolution of regeneration—ongoing work in cnidarians, acoelomorph worms, annelids, salamanders, and mice was presented by several speakers. For example, Mansi Srivastava (Reddien Laboratory, Whitehead Institute) shared her work on comparisons of cell signaling pathways during regeneration in acoels and planarians, which raises the possibility that some aspects of regeneration may have been conserved over the course of bilaterian evolution

(Srivastava et al. 2014). Yale Passamaneck (Martindale Laboratory, University of Hawaii) showed how development of EdU-labeling methods and transcriptional profiling through next-generation sequencing are revealing cellular and gene-expression dynamics during regeneration in the sea anemone, *N. vectensis* (Passamaneck and Martindale 2012). Duygu Ozpolat (Bely Laboratory, University of Maryland) is using molecular labels to understand the regeneration of the enigmatic lateral line in annelids. Saori Haigo (Reiter Laboratory, UCSF) described how multi-photon imaging can be used to visualize cellular movements in deep tissue during regeneration in vertebrates, thereby allowing lineage-tracing of transgenically labeled cells for direct observation of cellular migration and differentiation (Khattak et al. 2013). These efforts in varied regenerative animals are essential for understanding whether regenerative mechanisms are broadly conserved or independently evolved in different animal lineages.

Conclusions

The work presented at the symposium demonstrated varied approaches to test hypotheses at multiple levels of biological organization, ranging from systems-level studies of gene regulatory networks for cellular behaviors, to modeling cytoskeletal dynamics that drive tissue morphogenesis, to single-cell analysis to uncover molecular heterogeneity in populations of cells. The presentations highlighted various new techniques such as next-generation sequencing, single-cell analysis, transgenesis, genome editing, and mathematical modeling that are speeding up discoveries in standard models as well as in traditionally understudied phyla. These tools will be essential for moving forward—the more systems we can survey, the more detailed a picture we can build about the pliability of cellular behaviors across taxa (Fig. 1). The symposium encouraged cross-fertilization of ideas among speakers and introduced the SICB audience to novel methods and research questions that will inform their own research programs.

Acknowledgments

The organizers thank Chris Lowe and Billie Swalla for their help and support, as well as Jon Harrison and Brian Tsukimura. We thank Dave Matus and Antje Fischer for helpful comments on this meeting report. Finally, we thank all the participants in the symposium, only some of whose work could be discussed here.

Funding

The authors thank the funding sources that made this symposium possible, especially SICB divisions DEDB, DIZ, DPCB, DNB, and DVM. Travel support for students and post-doctoral fellows came from The Society for Developmental Biology, the Company of Biologists, the American Microscopical Society, GeneTools, and Carl Zeiss.

References

Babonis LS, Martindale MQ. 2014. Old cell, new trick? Cnidocytes as a model for the evolution of novelty. Integr Comp Biol 54:714–22.

Beh J, Shi W, Levine M, Davidson B, Christiaen L. 2007. Foxf is essential for fgf-induced migration of heart progenitor cells in the ascidian *Ciona intestinalis*. Development 134:3297–305.

Bely AE. 2014. Early events in annelid regeneration: a cellular perspective. Integr Comp Biol 54:688–99.

Bely AE, Nyberg KG. 2010. Evolution of animal regeneration: re-emergence of a field. Trends Ecol Evol 25:161–70.

Dickinson DJ, Ward JD, Reiner DJ, Goldstein B. 2013. Engineering the *Caenorhabditis elegans* genome using cas9-triggered homologous recombination. Nat Methods 10:1028–34.

Fischer AH, Pang K, Henry JQ, Martindale MQ. 2014. A cleavage clock regulates features of lineage-specific differentiation in the development of a basal branching metazoan, the ctenophore mnemiopsis leidyi. Evodevo 5:4.

Fritz AE, Ikmi A, Seidel C, Paulson A, Gibson MC. 2013. Mechanisms of tentacle morphogenesis in the sea anemone *Nematostella vectensis*. Development 140:2212–23.

Guillot C, Lecuit T. 2013. Mechanics of epithelial tissue homeostasis and morphogenesis. Science 340:1185–9.

Horne-Badovinac S. 2014. The *Drosophila* egg chamber—a new spin on how tissues elongate. Integr Comp Biol 54:667–76.

Horne-Badovinac S, Bilder D. 2005. Mass transit: epithelial morphogenesis in the drosophila egg chamber. Dev Dyn 232:559–74.

Khattak S, Schuez M, Richter T, Knapp D, Haigo SL, Sandoval-Guzman T, Hradlikova K, Duemmler A, Kerney R, Tanaka EM. 2013. Germline transgenic methods for tracking cells and testing gene function during regeneration in the axolotl. Stem Cell Rep 1:90–103.

Kuroda R. 2014. How a single gene twists a snail. Integr Comp Biol 54:677–87.

Lecuit T, Lenne PF, Munro E. 2011. Force generation, transmission, and integration during cell and tissue morphogenesis. Annu Rev Cell Dev Biol 27:157–84.

Lee JY, Marston DJ, Walston T, Hardin J, Halberstadt A, Goldstein B. 2006. Wnt/frizzled signaling controls *C. elegans* gastrulation by activating actomyosin contractility. Curr Biol 16:1986–97.

Lyons DC, Martik ML, Saunders LR, McClay DR. 2014. Specification to biomineralization: following a single cell type as it constructs a skeleton. Integr Comp Biol 54:723–33.

Martin AC, Goldstein B. 2014. Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. Development 141:1987–98.

Martin-Duran JM, Janssen R, Wennberg S, Budd GE, Hejnol A. 2012. Deuterostomic development in the protostome *Priapulus caudatus*. Curr Biol 22:2161–6.

Matus DQ, Chang E, Makohon-Moore SC, Hagedorn MA, Chi Q, Sherwood DR. 2014. Cell division and targeted cell cycle arrest opens and stabilizes basement membrane gaps. Nat Commun 5:4184.

Munro EM, Odell GM. 2002. Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord. Development 129:13–24.

Passamaneck YJ, Martindale MQ. 2012. Cell proliferation is necessary for the regeneration of oral structures in the anthozoan cnidarian *Nematostella vectensis*. BMC Dev Biol. 12:34.

Randolph H. 1892. The regeneration of the tail in *lumbriculus*. J Morphol 7:317–44.

Rivera AS, Oakley TH. 2009. Ontogeny of sexual dimorphism via tissue duplication in an ostracod (crustacea). Evol Dev 11:233–43.

Saunders LR, McClay DR. 2014. Sub-circuits of a gene regulatory network control a developmental epithelial–mesenchymal transition. Development 141:1503–13.

Srivastava M, Mazza-Curll KL, van Wolfswinkel JC, Reddien PW. 2014. Whole-body acoel regeneration is controlled by wnt and Bmp–Admp signaling. Curr Biol 24:1107–13.

St Johnston D, Sanson B. 2011. Epithelial polarity and morphogenesis. Curr Opin Cell Biol 23:540–6.

van Wolfswinkel JC. 2014. Piwi and potency: PIWI proteins in stem cells and regeneration. Integr Comp Biol 54:700–13.

van Wolfswinkel JC, Wagner DE, Reddien PW. 2014. Single cell analysis reveal heterogeneity in planarian neoblasts. Cell Stem Cell published online (doi: 10.1016/j.stem.2014.06.007).

Wallingford JB. 2012. Planar cell polarity and the developmental control of cell behavior in vertebrate embryos. Annu Rev Cell Dev Biol 28:627–53.

Zallen JA, Blankenship JT. 2008. Multicellular dynamics during epithelial elongation. Semin Cell Dev Biol 19:263–70.