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Microscopy to mechanism across the scales of development:

Editorial overview

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Introduction

Developmental biology is at a turning point. Three decades of relentless and successful attack at the molecular level have arguably brought us to the point where we know most of the key molecular principles and many of the specific genes that regulate embryonic development. We know that Hedgehogs, Wnts, BMPs, FGFs, and other secreted signaling proteins allow cells to communicate; we know the receptors and signal transduction cascades that transmit information from outside to inside the cell; we have a list of the homeobox, zinc finger, basic helix-loop-helix, and other families of transcription factors that can be turned on by signaling cascades and by other transcription factors; and we know many of the downstream proteins that are induced by transcription factors to allow cells to adopt their differentiated states. But do we really understand development? Can we compute how an egg turns into an embryo using our knowledge of the genome? Do we even understand the key *principles* let alone the specific details that orchestrate development? We believe that the answer to these questions is still no.

Developmental genetics and molecular embryology have played a primary role in bringing us to our current state of knowledge about embryogenesis, but these approaches have their limits going forward, for two reasons. One is that they are victims of their own success. We have already discovered much of what there is to be discovered at the molecular level of development. Complete genomes have been sequenced. Many phenotypes have been screened to saturation in several model systems and the causative genes identified. Much of

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what is left to discover is not new principles but rather additional examples of already known principles (e.g. transcription factor X is required for the development of tissue Y). The second reason is that these approaches are bumping into the limits of the paradigm in which they work. The paradigm is that if development is the process of going from genotype (a set of genes) to phenotype (a set of traits), then it can be understood as the set of connections between single genes and single traits. This paradigm is currently dominant in developmental biology because it provides a ready interpretation for the experimental approaches we use — such as gene expression and mutant analysis — that look at 'opposite' ends of the developmental scale, from a single gene to a whole embryo, without understanding how the molecular networks, cellular dynamics, and tissue mechanics in between connect them. This paradigm also fits with the way in which developmental biology is often practiced: one gene, one mutant, one phenotype, one lab member, and one paper (with regards to Beadle and Tatum).

The molecular paradigm of development has several limitations. One is that mechanism can often not be reduced to a *single* gene. As developmental biologists, we know that you cannot really draw a straight line between genes and phenotypes. Rather, the mechanisms that control development happen through the often nonlinear interactions of *many* genes in a dynamic network. Another limitation is that currently, much of development uses only a static readout such as looking at the terminal phenotype of a mutant. Understanding function often results from seeing the *dynamics* of a process occurring over time such as protein levels in a molecular circuit going up and down as a cell fate decision is computed.

The final limitation of the current paradigm is that mechanism does not occur only at the molecular level; mechanism happens at all scales. As laid out in the review by Adams and Blanchard and echoed in a number of other reviews in this issue, there are many additional scales that are at least as important including: networks of molecules, single cell behaviors, interactions between and within groups of cells, mechanical properties of cells, and tissues, on up to the whole embryo. If we want to understand how an airplane flies, what level of physics is most useful? Fluid mechanics can help us understand how airflow around the wings causes drag and lift, electronics can help us understand how the plane is controlled, but quantum mechanics likely offers little insight. You might argue that all these higher scales can be 'deduced' from the lowest scale, but this is not necessarily true in practice, either because the data are never perfect, or we lack sufficient computing power, nor even in theory because many mechanisms occur because of the emergent properties of populations of entities (cf. statistical mechanics). The importance of these intermediate scales in development is not new. It was, in fact, appreciated before the molecular era of embryology by scientists interested in the mechanics of morphogenesis. However, without understanding the molecules below or having solid computational and theoretical frameworks above, this middle level was unable to make the sustained progress seen in molecular embryology. Fortunately, these barriers are now being overcome.

This issue of Current Opinions in Genetics & Development is focused on the role of microscopy in developmental biology because we believe imaging will play a key role in advancing our understanding of developmental mechanisms across the scales. As discussed in the invited reviews that comprise this issue, imaging can be used to watch, measure, and even perturb developmental processes at all the relevant scales: molecular, cellular, tissue, and organismal. Importantly, we wish to challenge the simplistic notion that imaging is inherently 'descriptive' while molecular approaches are inherently 'mechanistic'. As discussed above, key mechanisms and principles are found at many scales. Imaging can assay all of these scales, while molecular approaches are often irrelevant. Observing that tissue Y does not form when gene X is missing does not constitute an explanation of how tissue Y forms, nor what key principles are important in controlling its formation; it is

important information, but it is an observation that should begin a mechanistic investigation, not end one. On the other hand, careful time-lapse microscopy has the potential to reveal the dynamics of gene regulatory networks, collective behaviors of cells, and tissue mechanics that underlie the formation of tissue Y. The first review in this issue, by James Sharpe, directly addresses the question of how imaging can be used to gain mechanistic insight into development. He argues that imaging can reveal mechanism in two ways. The first is straightforward: by directly seeing how something happens. This has been done for many years, and is very powerful, but runs into trouble when the process of interest is complex. Thus the second, more recent, approach is to combine imaging with computational analysis to construct quantitative models of complex processes. This review is recommended as an extended introduction to anyone interested in the epistemological question of how imaging fits in with the science of embryology.

Types of microscopy

Imaging can be a technically complex and demanding endeavor. There is a long list of types of microscopy, all of which have different requirements, benefits, and limitations. (Un)fortunately this list keeps getting longer, making it difficult for potential users to judge what imaging modality is best for them. The next group of reviews therefore discusses the primary types of microscopy that are useful for development with an emphasis on recent improvements. We start with the smallest scale and work our way up.

An image is a set of measurements with regular ordering in space and optionally time. In everyday life we typically think of an image as just a map of how well different wavelength photons bounce off an object, but in microscopy imaging can be extended to many other types of interaction with light such as fluorescence, harmonic generation, and Raman scattering as well as other probes such as sound and mechanical probes. Kristian Franze describes how atomic force microscopy (AFM), in which a physical probe is scanned across a sample, can be used not just for acquiring ultra-resolution images but also to map out the mechanical properties of embryos.

Two reviews discuss two-photon imaging because of its importance and perceived difficulty. Supatto and colleagues provide an overview of nonlinear optical techniques including two-photon laser scanning microscopy, light sheet microscopy, and second/third harmonic generation. A key advantage of two-photon microscopy is that it uses longer wavelength light (750–1050 nm), which is able to penetrate and thus image embryos more deeply. This wavelength range is able to efficiently excite blue through green fluorophores. Mojzisova and Vermot describe how the wavelength of the Ti:Saphire lasers used for two-photon imaging can be extended through the use of an OPO (optical parametric oscillator). This now longer wavelength light can penetrate tissues even better, and can excite red fluorophores more efficiently.

In the past few years light sheet microscopy has emerged as an important and rapidly advancing approach in developmental biology. Here a sheet of light is formed within an embryo and imaged with an objective lens that is orthogonal to the excitation optics. Light sheet microscopy can capture optical sections very quickly since it is performed using wide field rather than point-scanning imaging, and it potentially offers better depth penetration. It therefore is very attractive for a range of applications across organisms, and may very well rival or even supercede laser scanning confocal based approaches for time-lapse imaging of cellular dynamics. Two reviews from pioneers in the development of light sheet microscopic systems discuss the current state-of-the-art for light sheet microscopy of embryos, one by Tomer, Khairy, and Keller and another by Weber and Huisken.

The final three imaging modalities may be less familiar to readers, yet permit data acquisition from even larger samples and greater depth penetration. Mohun and Weninger review Episcopic microscopy in which the face of a block of tissue is repeatedly imaged as it is being physically sectioned away. Episcopic imaging can provide high-resolution imaging for large fields of view and unlimited depth, but requires fixation and physical sectioning so cannot be used for time-lapse studies. Larina and colleagues review optical coherence tomography (OCT), which looks at the timing of backscattered signal to form images. This relies on the same basic concept as ultrasound imaging, but uses light instead of sound. Since the arrival time of back scattered photons from different depths is too close to be measured directly, OCT relies on detecting shifts in the phase of the light to measure the inherent scattering properties of tissues. OCT is capable of imaging live tissues to depths of several millimeters as well as measuring the speed of objects using the Doppler effect making it a unique imaging modality.

The type of light scattering detected by OCT is termed Rayleigh scattering. In the following article, Folick and Wang discuss an imaging approach based on scattering of a different type. Rayleigh scattering occurs when photons change direction without changing energy and accounts for most scattering events. In Raman scattering, both the energy and the direction of the photon change. The change in energy seen here is not the same as that seen in fluorescence, which requires the absorption and re-emission of a photon. Raman scattering is instantaneous and occurs when a photon interacts with matter as the matter is going through an energy change (e.g. vibrational or rotational). Folick, Min and Wang describe how this shift in energy can be used for imaging in coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy. Like OCT, these approaches allow for deep imaging of living, unlabeled tissue. CARS and SRS, however, detect specific molecular transitions (e.g. CH_2 stretching vibration) using narrow band light rather than the nonspecific scattering events detected by the broadband light of OCT.

Microscopy in use

The next group of reviews discusses how microscopy has been used to look at different scales of development in several different model organisms. Again, we work our way up the scales. Daniel Larson describes how single molecule and single cell time-lapse imaging are changing our understanding of transcription relative to bulk, *in vitro* experiments. Soroldoni and Oates extend the analysis of transcriptional networks into embryos, and describe how transgenic reporters have been used to reveal the dynamics of the somite segmentation clock. Pradeep Das reviews recent progress in understanding plant development using several types of microscopy in combination with modeling and genetics. *Drosophila* has long been a preeminent model for genetic approaches to development but many aspects of its development have traditionally been difficult to image. He, Wang, and Montell review recent progress in live imaging of *Drosophila* with an emphasis on oogenesis. Zebrafish, while easy to image at early stages, become big enough as larvae that light penetration becomes a major problem for light microscopy. Cheng and colleagues review how whole zebrafish can be imaged with high resolution all the way through adulthood by using coherent X-ray computerized tomography (microCT). As described by Sharpe, imaging can be used for both traditional hypothesis driven research, as well as for more high-throughput and quantitative approaches. The ultimate extension of this latter approach is the use of imaging for phenomics. Cheng and colleagues go on to discuss what would be needed in terms of imaging and image analysis in a proposed phenome project for zebrafish, which would digitize the phenotypes for mutants in all genes.

Because of the large quantity of images generated by modern imaging approaches as well as the desire to quantify the information contained in these images, computational image

analysis has emerged as a major challenge. Luengo-Oroz and colleagues review image analysis for integrative embryology with a focus on multi-level *in toto* reconstructions of cell lineages and gene expression in zebrafish. The authors discuss an emerging framework that illustrates the importance of quantifying development through image analysis. Specifically, the authors review the adaptation, development, and application of several custom image-processing tools for this purpose, and their application in investigating early zebrafish development. This section is wrapped up with a review by Nieman, Wong, and Henkelman. Their review discusses organismal scale imaging modalities and specifically their application to later stage mouse embryos. The authors, make it clear that the imaging techniques (e.g. MRI, ultrasound, optical projection tomography) used for these larger specimens are inherently different because of the size of the specimen, and that these modalities do not allow acquisition of single cell data. However, these approaches do allow for standardized and comprehensive phenotypic screening of mouse embryos, as discussed above for zebrafish.

Mechanics of morphogenesis

The final group of four reviews also provides exquisite examples of the use of microscopy in developmental biology, but in an area that is specialized enough that we thought it deserved a separate section. These articles all address how imaging can be used to measure, perturb, and model the mechanical properties of cells and tissues in developing organisms. The first review of this section, by Stephan Grill, paints an intuitive picture of the mechanical concepts relevant during development. It is a ‘must read’ for anyone new to the biophysics of morphogenesis. The next review, by Adams and Blanchard presents a solid framework for understanding development as a multiscale process. This framework arranges developmental mechanisms across six spatial scales, describes how influences can be propagated between scales, and describes which scales can currently be experimentally interrogated. Next, Trier and Davidson describe what the relevant viscoelastic properties of tissues are and how they can be measured. And finally, Mason and Martin describe the dynamic aspects of tissue morphogenesis focusing on ratchet-like mechanisms.

Microscopy has traditionally been thought of as a way of *observing*, but many microscopes are now capable of *perturbing* development as well. Just as microscopes can observe with high spatial and temporal resolution, they can also perturb with high spatial (submicron) and temporal (seconds) control. Several of the reviews included in this collection describe how microscopes can be used to perturb towards the middle of the scales we have discussed, such as cutting actin cables, ablating cells, and probing the tension of tissues. Recent advances that are not reviewed here are opening up the potential of microscopes to perturb development at the molecular scale as well. For example, caged ligands have recently been used to activate Cre using light [1] opening up high-resolution optical approaches to a large collection of existing loxP transgenic lines. Light can also be used to control protein function directly. Photoswitchable proteins undergo reversible conformational changes upon excitation with light. Wu et al. [2] fused a photoswitchable domain termed LOV to Rac to generate an enzyme whose activity could be precisely and reversibly controlled in living cells. This approach can potentially be applied much more broadly and has already been extended to other GTPases [3] and histidine kinases [4].

Together, this set of 20 reviews provides a thoughtful overview of both the technical limits and conceptual levels with which imaging can engage developmental biology. While we are still far from understanding how an egg turns into an embryo, and distilling down what the key principles of this process are, it is at least now becoming clear what the path to and framework for this answer might look like. It will therefore be very exciting to watch the role imaging will play in the progress of integrative developmental biology.

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