AN INTEGRATIVE DEVELOPMENTAL BIOLOGY

Workshop Report

An active interchange of ideas has begun to develop in recent years between developmental biologists and physiologists interested in the development of complex traits.

It is clear that this is a time of exciting challenges for both fields, challenges that present opportunities for significant mutual illumination between them.

Members of both fields met at a workshop held in Arlington, Virginia, on November 15-16, 2004. This report presents the synthesis of ideas that emerged from a broad-ranging discussion that resulted.

INTRODUCTION

Developmental biologists have, for many years, focused their efforts to understand ontogeny by selecting a few model organisms that are genetically tractable, and that are appropriate to the study of fundamental processes of development at the genetic, molecular and cellular levels. These efforts have led to a detailed understanding of the genetic mechanisms that are involved in the control of developmental events. Many of the findings that have emerged from this work have proven remarkably transferable among the models studied.

Developmental biologists have relied on model systems with relatively little but controllable genetic variation. Consequently they have typically not studied the way developmental mechanisms differ among species, nor the variance in mechanism among individuals due to normal variation in genetic and environmental factors. Some developmental biologists have recently begun to expand their studies to include non-model species for understanding aspects of developmental processes not reflected in the models. Still others are interested in illuminating the breadth or limitations of the generalizations discovered in the model systems. Recent developments in genomic approaches have facilitated this move away from the few genetically tractable model systems.

Animal physiologists, by contrast, have been reluctant to adopt the use of a relatively small number of model species. This is in part because the physiological principles that bind the subscience cohesively, such as regulation and control of the functions required for normal operation, are known to differ between species. Thus, animal physiologists have employed a broad array of study systems, each selected for its suitability to address a specific physiological mechanism. Interestingly, some investigators have recently advocated the adoption of model systems that are genetically tractable as a means to approach questions about the genetics and evolution of physiological mechanisms, and as a means to leverage financial support of genomic approaches, which remain costly.

A deep understanding of the development and function of complex phenotypes will require integration of the cellular and organ-level approaches of developmental biology and physiology, respectively. This is not just an issue of filling in gaps that have been neglected (although these do exist), but more importantly, of synthesizing the conceptual approaches, methodologies, and analytical tools of the two disciplines. This multi-level approach to understanding development is called *Integrative Developmental Biology* (IDB).

Below we present two examples that illustrate the need to combine information from the traditions of developmental biology and physiology if we are to achieve a complete rather than patchy understanding of the development and evolution of complex traits. We follow this by outlines of the kinds of problems that must be addressed by an integrative developmental biology.

TWO EXAMPLES

1. **Sex determination** illustrates both the opportunities and the strengths of synthesizing approaches across levels of biological organization. Mechanisms of sex determination span the gamut from genetic to environmental control. Cases of strict environmental sex determination are phylogenetically widespread, as are cases of maternal control over sex determination in response to environmental cues. Even in *Caenorhabditis elegans*, one of the most thoroughly studied systems of genetic sex determination, recent studies have demonstrated that the environment can have an impact.

   The environmental cues that trigger sex determination differ widely. Sex in turtles and crocodiles is determined by incubation temperature, and in sea bass by body size; in *C. elegans* nutrition can influence sex determination and in parasitoid wasps prior infection of a host influences maternal behavior that determines the sex of the offspring she deposits within that host. Although the relevant environmental cues have been identified in each of these cases, we know little about how these cues affect developmental processes leading to sexual differentiation.

   A well-documented case that illustrates the complex interactions associated with sex determination is that of the bluehead wrasse, where aggressive behavior by the dominant male within a social group causes juveniles to develop as female. If the dominant male dies or is removed, the largest female changes sex and becomes the dominant male. The endocrinology of this system has been worked out in detail. Yet the means by which social and behavioral cues are sensed and transduced into changes in hormone titers are not known, nor are the developmental processes that respond to these changes and produce the sexual transformation in the gonads, body shape and pigmentation. A full understanding of sex determination in bluehead wrasse will require physiological and neurobiological studies to understand how social signals are transduced to the endocrine system, and molecular genetic studies to understand how hormones exert their effects on cellular processes and cause changes in gene expression. Finally, understanding how changes in gene expression lead to the development of the male form and behavior will require an understanding of a broad range of cellular mechanisms, and an understanding of how cell-level changes lead to appropriate changes in the structure and function of the nervous system, gonads and body proportions.

2. **Body size determination** is among the oldest problems in development and is currently the subject of intense research in many laboratories. Most of this research focuses on the mechanism of action of growth hormones and insulin, and on the dissection of the signaling pathways by which these hormones achieve their cellular effect. Genetic and experimental manipulation of these hormones and their signaling pathways can result in dwarf as well as giant animals. These findings suggest that these hormones are somehow involved in growth and size regulation, but they tell us little about why animals stop growing when they have

achieved their species-specific body size. The only animals in which we know, absolutely, the mechanism that controls body size are insects of the Order Hemiptera. Larvae of these insects have abdominal stretch receptors that gradually stretch as the larva grows. When stretch reaches a critical level, the receptors signal to the brain and this signal initiates secretion of the developmental hormones that cause the cessation of growth and initiate metamorphosis to the adult stage. These stretch receptors were discovered by means of classical physiological techniques, and one can argue that this mechanism of size control would not, and could not, have been discovered by studies that focus exclusively on cellular and intracellular signaling events, nor by methods that begin with the study of gene expression and regulation. Although the mechanisms of cell growth and signaling pathways are important, they are not the locus at which "control" over body size resides. When we study regulatory processes at the tissue, organ and whole body level we leave the realm of cell biology and enter that of physiology.

These two examples illustrate the need for approaches that cross traditional disciplinary boundaries. Understanding the development of complex traits requires analyses at many levels of biological organization. It also requires integrating concepts and techniques from many different subfields of biology, such as molecular genetics, signal transduction, neurobiology, endocrinology and behavior. Investigators in each of these subfields typically do not interact effectively, but it is clear that such interactions must become the norm if we are to develop a deep understanding of the development of complex traits.

THE BIG QUESTIONS

All of development builds on pre-existing platforms of morphology and gene expression, and at each point environmental and genetic variables can have profound effects on both. The principal goal of integrative developmental biology is to understand how the progression of development, from embryo to adult, is influenced by its environment and genetic background. Achieving such an understanding not only requires both genetic and physiological approaches, but also requires investigations that are not restricted to a single model system or a narrow conceptual approach. Investigations into the developmental physiology of animals can be organized around the following major questions.

- **How are developmental and physiological systems integrated?** As development proceeds and the animal grows in size, the coordination of development of distant parts becomes increasingly a problem of physiology rather than cell biology. Integration of development must occur on a spatial scale, in that the simultaneous development of different parts must be coordinated, and also on a temporal scale, in that the developmental progression to different stages in a life cycle must be regulated. The mechanisms by which spatial and temporal coordination of development occur are largely unknown.

- **How are complex phenotypes built?** It is well known that complex phenotypes are determined through the interaction of many and diverse genetic and environmental factors whose effects accumulate over long periods of time. Yet, the development of cells and tissues are typically studied in isolation and over brief time periods. As a consequence, little is known about how the development of different cells and tissues is integrated to produce complex organs and physiological systems, and how the ever-changing organism remains functionally well-integrated throughout its development.

- **What are the developmental and physiological bases of phenotypic variation?** The genetic and environmental determinants of development and physiology naturally vary in space and time. As a consequence, phenotypic variation has both environmental and genetic causes. Although the relative effects of genes and environmental variation can be assessed statistically, the mechanisms through which normal genetic and environmental variation produce natural phenotypic variation are virtually unexplored and therefore remain poorly understood.

SPECIFIC ISSUES

The broad questions outlined above provide the context for a range of research programs designed to address specific problems in the development of complex traits.

- **Phenotypic effects of natural genetic variation.** The roles of genes in development are typically studied using artificial genetic constructs in a controlled genetic background. Very little is known about how the regulatory pathways revealed by these studies operate in the presence of normal genetic variation. How much individual variability is there in gene structure and gene regulation? How does this variation affect the operation of genetic regulatory networks? How does variation in genetic regulation become expressed as phenotypic variability?

- **Phenotypic effects of natural environmental variation.** The environment can affect development via two distinct mechanisms. The environment can have a direct effect on the physical and chemical processes that underlie development and physiology by altering the rates of reactions or by providing substrates and co-factors. The environment can also have indirect effects via its input into the central nervous system, which in turn can control the secretion of hormones and a host of physiological processes that have an impact on gene expression. From quantitative genetics we know that phenotypic variation is strongly influenced by environmental variation, but very little is known about how developmental mechanisms are affected by normal environmental variation, and how this results in phenotypic variation.

- **Developmental homeostasis and canalization.** Development proceeds along predictable trajectories even in the presence of significant genetic and environmental perturbations. It is therefore believed that mechanisms exist that somehow buffer the phenotype against genetic and environmental variation. Although homeostatic mechanisms are well understood in physiology, little is known about the mechanism by which developmental homeostasis is achieved. Is developmental homeostasis strictly analogous to physiological homeostasis? Does robustness to environmental variation produce robustness to genetic variation? Are developmental homeostatic mechanisms diverse?

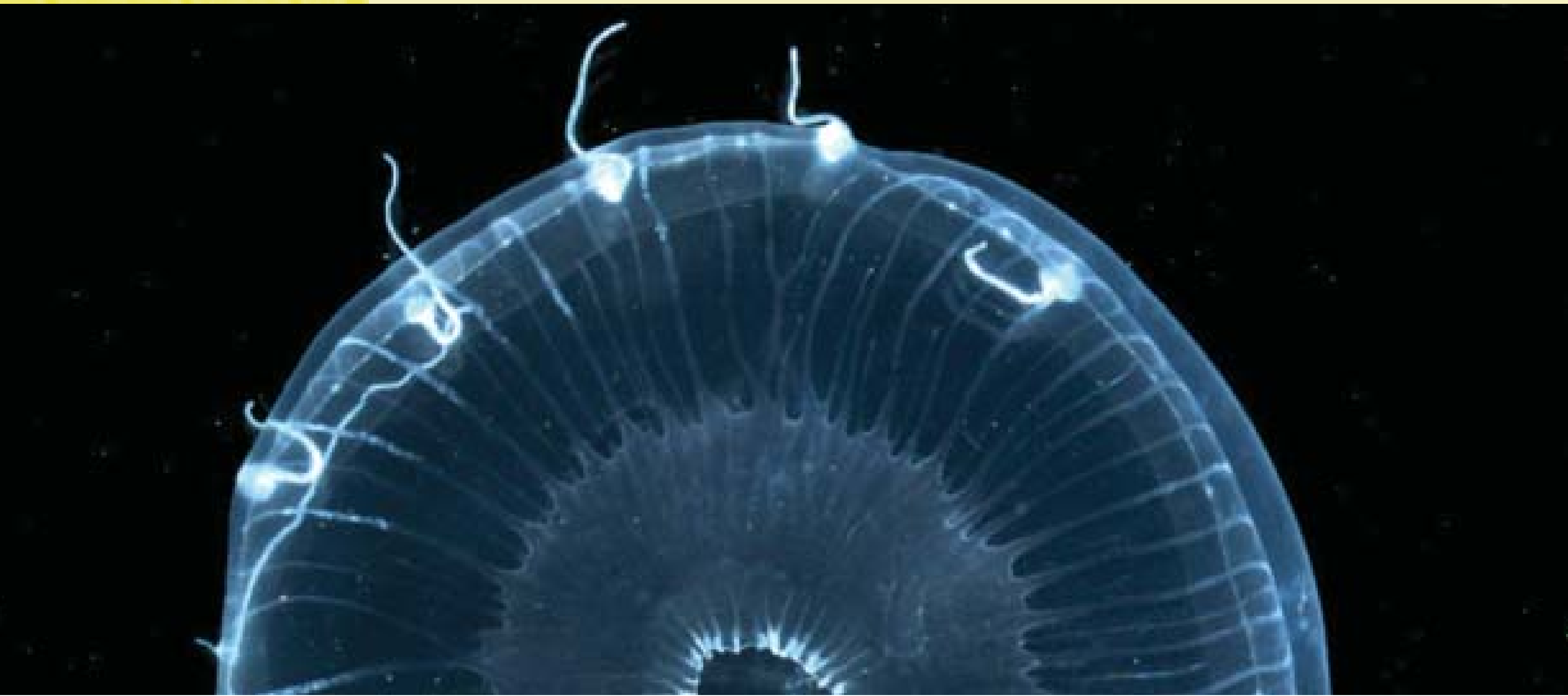
- **Adaptive phenotypic plasticity.** Many animals are able to develop alternative phenotypes that are adapted to alternative environments. Metamorphosis, polyphenisms, and environmentally controlled sex determination are highly evolved adaptations to different environments at different stages in the life cycle. Adaptive phenotypic plasticity requires highly organized and predictable changes in gene expression in response to environmental signals. How are environmental signals received and transduced to the genome? How do changes in gene expression lead to alternative phenotypes? How is adaptive phenotypic plasticity related to developmental homeostasis?

- **Proportional growth, allometry, and size regulation.** Each species has a characteristic body size and characteristic proportions of different body parts. Understanding how growth and size are regulated is complicated by the fact that different body parts grow at different rates, and often only during restricted times in development. Hormones and growth factors play a major role in the regulation of growth, but the mechanisms that control the timing of onset and cessation of growth, and those that control the actual rate of growth of different structures, are largely unknown. Because control of growth and size resides at both the cellular and organismal levels, a full understanding of proportional growth and size regulation requires an integration of cellular and physiological approaches.

- **Structure and organization of control systems.** Regulation occurs at all levels of organization, from the biochemical to the ecological. Are there common themes or patterns in regulatory processes at different levels of organization? Are some regulatory processes unique to a particular level of organization? Do regulatory systems at different levels in a hierarchy interact?

- **Evolution of cellular and physiological regulation in development.** The genetic and physiological regulatory processes of development differ from species to species. Although species-specific differences in genetic and physiological regulation have been extensively documented, little is known about the degree to which such differences are cause or consequence of divergence of form and function. Do differences in genetic regulation cause differences in physiological regulation, or vice versa? Are the differences in regulatory mechanism between species qualitative or quantitative? How many fundamentally different regulatory mechanisms are there? Are different degrees of evolutionary divergence associated with particular changes in genetic or physiological regulation?

To successfully investigate these problems will require an integration of the concepts and methods from traditionally separate subfields of biology. Molecular-genetic approaches to development are well suited to uncovering details of cellular regulatory mechanisms, but they are typically not suited to elucidate mechanisms that operate at the level of tissues, organs and whole animals. Physiological approaches, by contrast, can elucidate higher-level regulatory mechanisms but are traditionally non-genetic. Both approaches are advanced by an increasingly powerful array of genomic techniques that provide outstanding opportunities to address important issues of long-standing interest to both physiologists and developmental biologists.



RECOMMENDATIONS

Embryonic development is traditionally studied at the cellular and subcellular level, because regulation is largely local and communication is accomplished by diffusion of gene products over short distances. The regulation of postembryonic development, by contrast, occurs over greater distances and often relies on endocrine and nervous signals. Embryonic development has, therefore, typically been studied by the methods of molecular genetics, whereas postembryonic development has been most effectively studied by the methods of physiology. There are, however, broad areas of overlap, where the methods of physiology can give insight into mechanisms of early embryonic development, and where genetic and molecular approaches are critical for understanding aspects of postembryonic development.

It is clear that molecular and physiological approaches to development are complementary. Each approach produces critical insights that simply cannot be obtained by using the concepts and methodology of the other. A deep understanding of development, arguably the most complex problem in all of biology, will therefore require research programs that integrate molecular, cellular and physiological approaches.

There are three challenges in building research programs that integrate genetic and physiological approaches: (1) raising awareness and interest in such integrative approaches; (2) facilitating the transfer of technology, expertise, and information among scientists belonging to traditionally separate research communities; and (3) establishing sources of financial support for research and for graduate and post-doctoral training.

As a first step towards reaching these goals, we recommend the following:

- Publication of review articles that articulate a vision for Integrative Developmental Biology, providing illustrative examples and outlining a set of questions and issues that need to be addressed.

- A series of symposia at national conferences that focus attention on Integrative Developmental Biology within the disparate communities that contribute to it, including neurobiology, developmental biology, and physiology. These symposia will illustrate success stories and identify challenges in understanding the development of complex traits.

- Creation of a "cyber community" that provides a forum for exchanging ideas, asking advice and posting methods. This community should also develop a database of willing expert advisors (and potential collaborators) who can help investigators incorporate new approaches in their research program.

In the longer term, it will be important to provide financial and logistic support for research and training. As Integrative Developmental Biology grows and matures as a field, we anticipate that the disciplinary programs at NSF will likewise grow and adapt to accommodate the new opportunities for research and scholarship in this changing field.

For the immediate future we recommend:

- Establishing a program to support post-doctoral training in interdisciplinary research by young investigators. These postdoctoral fellows can then act as bridges between more traditionally-oriented laboratories.

- Establishing a program of mid-career sabbaticals for established investigators who want to develop a more integrative or synthetic research program and need to gain expertise with relevant methods of analysis.

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Picture credits: Doug Emlen, Eric Greene, John Godwin, Jim Hanken, Sonke Johnsen, Mimi Koehl, Fred Nijhout, Katie Peichel.

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