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Commentary

When developmental pathways diverge

*H. Frederik Nijhout**

Department of Zoology, Duke University, Durham, NC 27708-0325

We think of development as a process of inexorable progressive change by which a single cell is transformed into a complex multicellular organism, made up of many tissues and organs, each with a distinctive structure and function. In any given species, the progression from the simple to the complex occurs in an orderly fashion that is repeated with little or no variation in millions of individuals, generation after generation. Barring severe genetic mutations or environmental trauma, all individuals of a species develop in identical fashion and the final products, the adult animals, resemble each other to a remarkable degree.

As a rule, developmental pathways are highly resistant to genetic and environmental variation, a phenomenon that goes variously by the names of canalization (1), robustness (2), or developmental homeostasis. Superimposed on this constancy, some animals have evolved the ability to develop into drastically different adult forms by switching to one of several alternative developmental pathways, depending on certain signals from their environment. Although the general phenomenology has long been known, it is only recently that methods have been developed to study the genetic and molecular mechanisms that underlie such developmental switches (3). This work opens up an entirely new area of investigation into the control of development. This is perhaps best appreciated by briefly outlining a significant difference between the control of embryonic and postembryonic development.

There is no centralized control mechanism that coordinates embryonic development. As far as we know, each cell in the developing embryo simply responds to signals it receives from the cells that surround it. Such cell-to-cell signals regulate gene expression in the receiving cells, and altered gene expression, in turn, sets up the conditions that cause the next round of developmental events. The observation of developmental homeostasis, together with the recognition that there is no centralized control of embryonic development, suggests that embryonic development is almost entirely self-organizing.

The situation is quite different during postembryonic development. As a developing animal grows, long-range communication by cell-to-cell signals becomes increasingly inefficient. Insofar as regulatory and inductive signals can extend only to small portions of the developing animal, the regulation of development becomes largely a local affair. The regions within which cells are able to communicate with each other are called developmental fields (4), and distant developmental fields can interact only if morphogenetic movements (such as gastrulation or involution) physically bring them close together. Thus as a developing embryo grows, each of its parts becomes increasingly autonomous: in each part development proceeds at its own pace and in a direction dictated by local interactions among cells. The autonomy of emergent developmental fields creates a new problem in developmental regulation, namely, that of coordinating the development of distant parts so that the animal as a whole remains well integrated. Such coordination is particularly important in situations where the environment in which the organism develops varies from time to time or from place to place. Variation in environmental factors such as temperature or nutrient supply affects some processes more than others, and such variation therefore can affect different developmental fields in different ways. The potential for the production of developmental malformations as the result of a lack of coordination or integration among distant parts is self-evident. To coordinate the development of distant parts, it has proven advantageous to deploy some kind of long-range signaling mechanism that can range across the entire growing organism and that can differentially affect the timing and the rate of development of various parts. Hormones are almost universally used for this purpose, and the role of hormones in coordinating development has been best studied in the insects.

The most intricate instances of hormonal coordination of development occur during the events that lead up to metamorphosis. In holometabolous insects (insects with complete metamorphosis, that have distinctive larval, pupal, and adult stages) the developmental events that lead to metamorphosis begin some time during the early portion of the last larval stage and culminate during the prepupal stage (for recent reviews see refs. 5 and 6). During this interval there is a succession of hormone-sensitive periods that regulate the developmental progression of various tissues. In *Lepidoptera*, where the hormonal control of metamorphosis has been particularly well studied, there are juvenile hormone (JH)-sensitive periods early in the last larval instar that control the commitment of the imaginal disks (7), and a JH-sensitive period at the very end of the instar that controls pupal commitment of the epidermis (8). It is believed that during pupal commitment certain pupa-specific genes become somehow available for transcription while certain larva-specific genes become unavailable.

In the epidermis, the switch from larval to pupal commitment and the subsequent expression of the pupa-specific genes requires a second hormone, an ecdysteroid. A brief pulse of ecdysteroid secretion defines the timing of the JH-sensitive period, which occurs during the rising phase of the ecdysteroid pulse (9). A second pulse of ecdysteroid secretion, about 24 hr after the first, actually stimulates the novel gene expression and initiates the developmental events that lead to the formation of pupal characters in the epidermis (9). The pulses of ecdysteroid secretion are accompanied by the expression of various isoforms of the ecdysteroid receptor in the epidermis (10). Ecdysteroid receptor expression rises and falls dramatically during development in patterns that differ from tissue to tissue (11). The picture that is emerging is that of a dialogue between centrally controlled hormone secretion and tissue-specific receptor expression. Tissues are not just passive responders to the hormone, but actually vary the amount and isoforms of their receptors over time, sometimes quite abruptly (10, 11). Hormone secretion, likewise, varies in what appears at times to be a quite erratic pattern (5, 11). It seems that transient peaks of hormone secretion only affect a small number of tissues, namely those that happen to have a coincident expression of receptors. Tissues indicate their de-

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*To whom reprint requests should be addressed. e-mail: hfn@acpub.duke.edu.

velopmental stage by expressing an ecdysteroid receptor, whereas ecdysteroid secretion provides a common timing or synchronizing signal for the further progression of development.

Unlike the situation in the epidermis, the JH-sensitive periods for the imaginal disks do not appear to be associated with a pulse of ecdysteroid secretion. A subthreshold level of JH during a critical period early in the larval instar is all that appears to be required for the developmental switch to occur (8). Unfortunately, the receptor for JH remains elusive, so it is not yet possible to determine whether here, too, the timing of the hormone-sensitive period is a direct consequence of the timing of receptor expression.

Two interesting developmental events happen at metamorphosis. The best known of these is a dramatic transformation of morphology as a grub-like larva in a few relatively quick steps becomes an elegant winged adult. Less well-known, but arguably even more interesting, is the fact that upon metamorphosis many species of insects are able to switch between alternative developmental pathways. Many insects have evolved the capacity to express very different alternative adult phenotypes (a phenomenon called polyphenism), the "choice" of phenotypes being determined by the environment the larva experiences during a critical time in its development. The alternative adult phenotypes are adaptations to specific alternative lifestyles. Examples of such polyphenisms are the seasonal forms of many insects (the differences between seasonal forms can be so great that some initially were described as different species), and the various castes of social insects. In ants and termites, for instance, any larva can develop into either a worker, a soldier, or a queen, depending on environmental stimuli it receives during certain critical periods in its development. In all cases where the mechanism that controls a developmental switch has been studied, it has proven to be mediated by a hormone acting during a relatively narrow hormone-sensitive period (6, 12). The hormones that most often are involved in this control are exactly the same ones that control other aspects of metamorphosis, JH and ecdysteroids (12–14).

In this issue of the *Proceedings*, Evans and Wheeler (3) study the mechanism that controls whether a honey bee larva will develop into a queen or into a worker. It has long been known that queen and worker bees differ in many characteristics. A queen bee is larger than a worker and has a functional reproductive system (the worker is sterile), but she has a substantially smaller brain, and her appendages, mouth parts, and stinger have a simpler anatomy than those of a worker. The developmental switch is controlled by the nutrition a larva receives. Larvae that are fed royal jelly, a secretion from the mandibular (salivary) glands of worker bees, during the latter portion of their larval life will develop into queens, whereas larvae that are fed only pollen and nectar during this period develop into workers. The difference in food quality alters the pattern of hormone secretion, so that queen larvae have a higher titer of JH than worker larvae during a JH-sensitive period that occurs during the fourth and fifth larval instars (15). A presumptive worker larva can, in fact, be induced to develop queen characters by a simple topical application of JH during this sensitive period (16).

Previous studies on the control of caste determination in honey bees have focused on the role of royal jelly and on the timing of the JH-sensitive period, whereas analyses of the consequences of the developmental switch have focused largely on descriptions of the morphological and molecular differences between adult workers and queens. Until now, information about the molecular changes that accompany the developmental switch has been restricted to studies on differences in transcriptional activity between worker and queen larvae (17). The work of Evans and Wheeler (3) is the first to specifically identify the nature of the proteins that are differentially expressed at the time of the developmental switch; in fact, it is the first specific information we have on the molecular events that accompany a developmental switch between alternative pathways in insects in general.

Evans and Wheeler (3) used a suppressive subtraction method to identify genes that are differentially expressed in presumptive workers and in presumptive queens at the time of the developmental switch. Their most interesting finding is that more genes are expressed uniquely or more strongly in larvae that switch to the worker pathway than in those that will become queens. Seven genes showed a particularly consistent pattern of caste-biased expression. Some of these, like the queen-specific gene for a storage protein, are almost certainly an effect rather than a cause of caste determination. Others are more intriguing. For instance, one of the worker-biased genes appears to code for a protein with considerable sequence similarity to retinoic acid binding proteins of vertebrates and a fatty acid binding protein of the moth *Manduca sexta*. This finding is significant because JH has a close structural similarity to retinoic acid. Retinoic acid has a juvenilizing effect on many insects (18), and the JH analog methoprene can stimulate retinoic acid-responsive transcription factors in vertebrates (19). Indeed, some strategies for identifying the JH receptor have taken retinoic acid receptors as a starting point for investigation (20). As I noted above, no JH receptor has yet been identified, although JH is known to bind to a variety of proteins. It is possible that JH does not act in a manner of a standard hormone, by binding to a receptor, but instead acts by binding to proteins and altering their activity in the cell, or by binding to the ecdysteroid receptor and altering its properties as a transcription factor. Thus finding putative JH-binding proteins that are expressed at critical times in development gives us a toehold on the investigation of the still mysterious molecular mechanism of JH action.

The finding that most differentially expressed genes are worker-biased or worker-specific is in good accord with the observation that many (but not all) features of worker morphology and behavior are more complex than those of a queen, and therefore may require more specialized gene expression. Alternatively, it is possible that queens and workers do not differ so much in the types of genes that they express but in the relative timing of their expression. When a gene is expressed in a different cellular context it can have a dramatically different effect on the phenotype. Whatever the mechanism, we now have a proven technique that can be used to delve into the details of what goes on at the molecular level when developmental pathways diverge. Interestingly, the genes identified on this first pass do not correspond to any of the genes known to control embryonic development, so it is possible that alternative-pathway-switching uses entirely novel genetic mechanisms.

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