Developmental Mechanisms of Body Size and Wing-Body Scaling in Insects

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Abstract

The developmental mechanisms that control body size and the relative sizes of body parts are today best understood in insects. Size is controlled by the mechanisms that cause growth to stop when a size characteristic of the species has been achieved. This requires the mechanisms to assess size and respond by stopping the process that controls growth. Growth is controlled by two hormones, insulin and ecdysone, that act synergistically by controlling cell growth and cell division. Ecdysone has two distinct functions: At low concentration it controls growth, and at high levels it causes molting and tissue differentiation. Growth is stopped by the pulse of ecdysone that initiates the metamorphic molt. Body size is sensed by either stretch receptors or oxygen restriction, depending on the species, which stimulate the high level of ecdysone secretion that induces a molt. Wing growth occurs mostly after the body has stopped growing. Wing size is adjusted to body size by variation in both the duration and level of ecdysone secretion.

INTRODUCTION

Body size and the relative dimensions of body parts are the most characteristic attributes of species. Indeed, size and shape are the primary characteristics by which species are defined. The developmental mechanisms that control size and shape remain poorly understood in all organisms except insects. Recent research has revealed the mechanisms by which body size is sensed and how this information is transmitted to the endocrine system, which controls growth.

The final size of the body and its appendages is determined by the mechanisms that control exactly when each stops growing. The control of growth is a difficult problem. It involves control of cellular growth, DNA and protein synthesis, and mitosis and needs to be coordinated among tissues so that each grows in correct proportion to the whole. These mechanisms are controlled centrally, mostly by the brain, via the secretion of hormones and growth factors, which initiate intracellular signaling pathways that lead to tissue growth. The problem of control is how to ensure that the endocrine events that cause cessation of body growth happen at exactly the right time, regardless of variation in nutrition and growth rate.

The control of the relative sizes of appendages, such as wings, that are derived from imaginal disks presents a different problem. Most wing growth occurs after the larva has stopped feeding and the body has stopped growing, and here the problem is how to ensure wings grow to the correct proportion when body size varies due to genetics or variation in nutrition. Below we review the biological background and recent history of our understanding of the interaction among the physiological, cellular, and molecular processes that control the growth and final size of the bodies and wings of insects.

THE SIZES OF INSECTS

Adult insects vary in body size (measured as body length) over some three orders of magnitude. For instance, Collembola range from 0.1 to 10 mm, Hymenoptera from 0.15 to 60 mm, and Coleoptera from 0.25 to 180 mm (52). Adult parasitic wasps of the genus *Megaphragma* are smaller than *Paramecium* adults (78), and goliath beetles (*Goliathus*) are larger than many songbirds.

It is thought that maximum insect size is limited by the tracheal system (29). Insects breathe through a system of branching tubes, the tracheal system, that brings air directly to every cell in the body. The rate at which oxygen can be supplied in such a system is limited by diffusion, and it is generally thought that over distances greater than a millimeter or so oxygen cannot diffuse fast enough to meet metabolic needs (105). Large-bodied insects have therefore evolved mechanisms for ventilating their tracheal systems by means of collapsible air sacs and collapsible tracheae that can be compressed and dilated. The very largest insects have enormous air sacs that can fill most of their abdomens (9), so that all internal tissues remain within a short distance from fresh air.

The minimum size of insects is limited by the needs of a fully functional multicellular body. There has been a longstanding interest in miniaturization and how the sizes of organs, tissues, and cells of the very smallest insects differ from those of larger ones (82). In general, the sizes of the compound eyes often remain in the same proportion to the head but the eyes are composed of fewer ommatidia (79). In miniature beetles of the genus *Mikado*, the number of muscles is only slightly reduced relative to those of larger species, but the number of muscle fibers within a muscle is smaller (79). The consequences of miniaturization for the structure and function of the nervous system have been of particular interest (72). The size of the brain does not appear to scale isometrically with body size. Rather, in very small insects the brain is disproportionately large and, together with the subesophageal ganglion, can extend into the thorax (77, 79), much as the brain extends even into the proximal parts of the legs in some miniature spiders (17, 18). There is also an

extreme reduction in cell number in the central nervous system, as well as a reduction in the size of the cell bodies of neurons; cell body size seems to be limited by the size of the nucleus, which occupies most of the cell body volume in neurons of very small insects (45, 77). In the extreme case, neurons lose their nuclei altogether (78).

GROWTH AND MOLTING

Insects increase in size at each larval molt. The number of larval instars, however, is quite variable both between and within species (20, 106). The largest numbers of instars (10–15) are found among species in the Odonata, Blattidae, and Plecoptera, whereas the smallest numbers (3–7) are found in the Lepidoptera, Diptera, Hymenoptera, and Coleoptera (62, 106). As a general trend, Hemimetabola have a larger number of instars than Holometabola, which suggests that insect evolution has been accompanied by a decrease in the number of instars. A possible reason for this trend is that molts are precarious events in an insect's life, during which it is relatively defenseless and immobile, so reducing the number of molts may increase fitness.

There is no relationship between number of larval molts and adult body size: Some of the very largest insects, such as the atlas moths (*Attacus* spp.) and goliath beetles, have few larval instars (5 and 3, respectively). Small species that have many larval instars simply grow in smaller increments at each molt than larger species with fewer larval instars. For a given species growing under standard conditions, the increment of growth of the sclerotized parts of the cuticle at each larval molt is typically constant from instar to instar; this is referred to as Dyar's rule (16). The increment of growth at each molt depends on the species and on the structure. The growth ratios of head widths range from 1.1 to 1.6 in hemimetabolous insects and from 1.3 to 2.2 in holometabolous insects, with median growth ratios of 1.27 and 1.52, respectively (12).

Incremental growth by molting only applies to sclerotized parts of the body wall. The larvae of many Holometabola, for instance, Hymenoptera, Lepidoptera, Diptera, and some Coleoptera, are soft-bodied with an unsclerotized body wall. In larvae of *Manduca sexta* the endocuticle grows throughout the last larval instar (107, 108) and can increase in surface area fourfold (33). Concomitant with this growth, some of the epidermal cells become increasingly polyploid. In some regions of an abdominal segment cells remain about 4n, but in others they increase in ploidy over a four-day period, some growing to about 128n (33). *Drosophila* spp., and presumably other Cyclorrhapha, are extreme in this regard, in that larval epidermal cells never divide but become increasingly polyploid from instar to instar (19, 97), so growth of the body wall is due to endomitotic cycles at each molt.

Hormonal Control of Molting

All molts, whether they are from one larva to a larger larva or a metamorphic molt to a pupa or to an adult, are controlled by ecdysone. The general pathway is illustrated in **Figure 1**. The molt begins with the secretion of prothoracicotropic hormone (PTTH), a neurosecretory hormone produced by the medial neurosecretory cells of the brain that is released into the hemolymph via the corpora cardiaca in most insects. [In some Lepidoptera PTTH is released from the corpora allata (59), and in cyclorrhaphan Diptera it is released from the ring gland (85).] PTTH stimulates the prothoracic glands to secrete the relatively inactive prohormone ecdysone (formerly called α-ecdysone), which is converted into the active hormone 20-hydroxyecdysone (20E, formerly β-ecdysone) in peripheral tissues such as the fat body. In the remainder of this review we refer to 20E simply as ecdysone. Ecdysone acts directly on epidermal cells to stimulate the entire sequence of the molt: apolysis, secretion of molting fluid, mitosis, and synthesis of the new cuticle (62). The

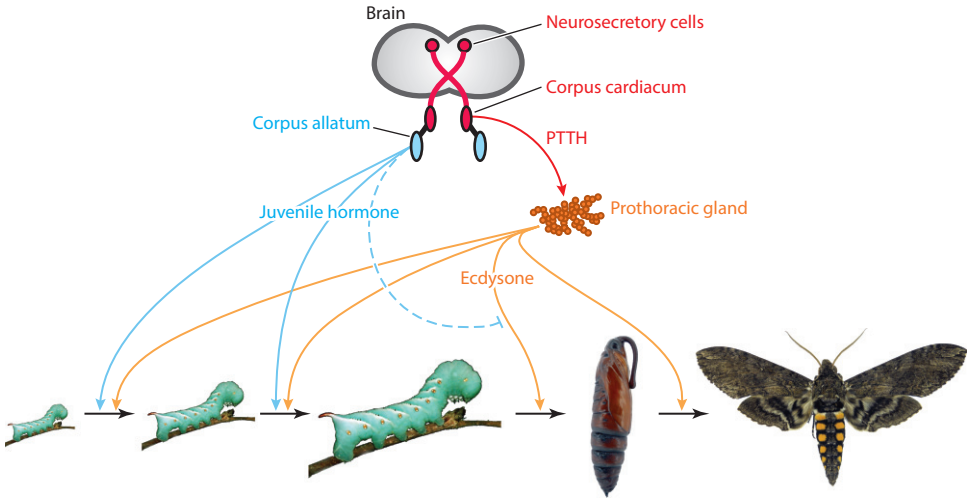

Figure 1

Control of molting and metamorphosis in holometabolous insects. Periodic pulses of ecdysone, stimulated by prothoracicotropic hormone (PTTH), a neurosecretory hormone produced by the brain and released from the corpora cardiaca, induce periodic molts. The nature of a molt, whether it is a growing molt to a larger larval instar or a progressive molt to a pupa or adult, is controlled by juvenile hormone (JH), secreted by the corpora allata. In the presence of JH, ecdysone induces a molt to a larger larva. The first molt that occurs in the absence of JH stops growth and produces the pupal stage, and the next molt results in the adult. In the last larval instar ecdysone secretion is inhibited by JH so that the molt will not occur while any JH is present.

finding that ecdysone is both necessary and sufficient to stimulate the entire sequence of events in a molt comes from in vitro studies with cultured pieces of integument (1, 22, 47). Studies in *Drosophila melanogaster* (13, 54) have found that ecdysone affects not only the timing of molting but also the growth rate.

Hormonal Control of Growth

The round of epidermal mitoses stimulated by ecdysone in effect defines the growth of the integument and the growth increment of the next instar. Thus this aspect of growth, the increment of growth from instar to instar, is controlled entirely by ecdysone. Differences in cell division patterns in individuals that are undernourished or that change shape during a molt must come about by patterned expression of the ecdysone receptor or elements of the ecdysone response pathway.

During a molt a larva increases in size by swallowing air or water. Thus, although the epidermis and integument greatly increase in surface area there is no significant increase in biomass. Growth in biomass occurs during the intermolt period. This somatic growth involves mostly the gut, muscles, and fat body. Most of our information about the control of growth of internal organs comes from cell and tissue culture studies. Insect cells and tissues generally do not grow well (if at all) in a defined culture medium. It is necessary to use a conditioned medium, in which other cells have been grown. (Typically a conditioned medium is derived from cultures of fibroblasts or hemocytes or established cultures of embryonic cells or cancer cells.) Successful cultures also typically require a medium that is supplemented with fetal calf serum or a vertebrate or insect tissue homogenate (44, 46, 48, 102). These complex additives seem to provide something essential

for normal growth, but it is not known exactly what that is. It is unlikely to be a specific growth factor, given that almost any tissue source can be an effective supplement. In a process similar to conditioning, these additives probably supplement critical nutrients, like small peptides, that are not normally present in a defined culture medium.

The Role of Insulin

In addition to these complex additives, normal growth in vitro also typically requires either ecdysone, insulin, or both. For instance, primary cell cultures of midgut cells require low concentrations of ecdysone for proliferation (25, 94), whereas cultured fat body cells typically grow well in a conditioned culture medium, though they seem to grow faster with the addition of ecdysone (55, 81). Cell lines derived from *Drosophila* imaginal disks always require insulin for normal growth (10), and intact imaginal disks from Lepidoptera require both insulin and ecdysone for normal growth in vitro (67, 70). A brain extract can substitute for the insulin, but that is because the brain contains the neurosecretory cells that produce the lepidopteran insulin (67).

The hormonal control of overall somatic growth has been best studied in *D. melanogaster*, where insulin signaling plays a dominant role. Nutrition is obviously required for growth, but nutrition acts by stimulating the secretion of insulin-like growth factors, which, in turn, stimulate cellular growth and cell division. Insulin stimulates growth by stimulating protein synthesis via the S6K kinase, the forkhead transcription factor (FOXO), and the target of rapamycin (TOR) (**Figure 2**). Insulin signaling also regulates the import of carbohydrates (36) and amino acids (32, 35, 51) into cells, and this synergizes growth. Genetic manipulations that reduce insulin production or inactivate the insulin receptor result in slower larval growth (87, 91), and overactivation of phosphatidylinositol-3-kinase (PI3K), an element in the insulin-signaling cascade, causes more rapid growth (103).

Amino acids activate TOR (**Figure 2**), and this increases protein synthesis and cellular growth; reducing the activity of TOR reduces the growth rate (14). It turns out that reducing amino acid transporter in the fat body alone is sufficient to slow growth and reduce body size, much like the effect of poor nutritive conditions (14). The fat body responds to amino acids by secreting a factor that stimulates insulin secretion by the brain, which then stimulates growth of peripheral tissues (14, 69).

Insulin signaling is said to link nutrition and growth (4, 43), and there is evidence that variation in insulin signaling is correlated with variation in nutrition. When *Bombyx mori* larvae are starved, bombyxin (the lepidopteran insulin) levels in the hemolymph decline and the content in the brain increases, indicating that bombyxin is being stored rather than released (49). Refeeding larvae reverses this situation, and simply injecting glucose also causes bombyxin in the hemolymph to rise, suggesting that glucose is the main signal for the fed state (49). In *D. melanogaster*, starvation leads to a decrease in expression of two insulins (DILP-3 and DILP-5) but not another (DILP-2) in the brain neurosecretory cells (31). It is not known how this is reflected in the hemolymph titers, nor is it clear which one of these insulins contributes to normal growth. In normally feeding *B. mori*, insulin (bombyxin) expression in the brain and insulin levels in the hemolymph fluctuate at low levels but then rise to very high levels during the wandering and pupal stages, when no feeding takes place (56, 88). Likewise, a rise in insulin expression in the fat body occurs during the nonfeeding stage in *D. melanogaster* (93) and may be required to support tissue growth during metamorphosis. In *M. sexta*, the level of expression of insulin in the brain rises during the last larval instar, and its expression is unaffected when larvae are starved (99). In *D. melanogaster*, the fat body produces insulin (DILP6) during the nonfeeding stage in pupal and adult development, and its secretion is simulated by ecdysone (73, 74). Different tissues grow at different times and

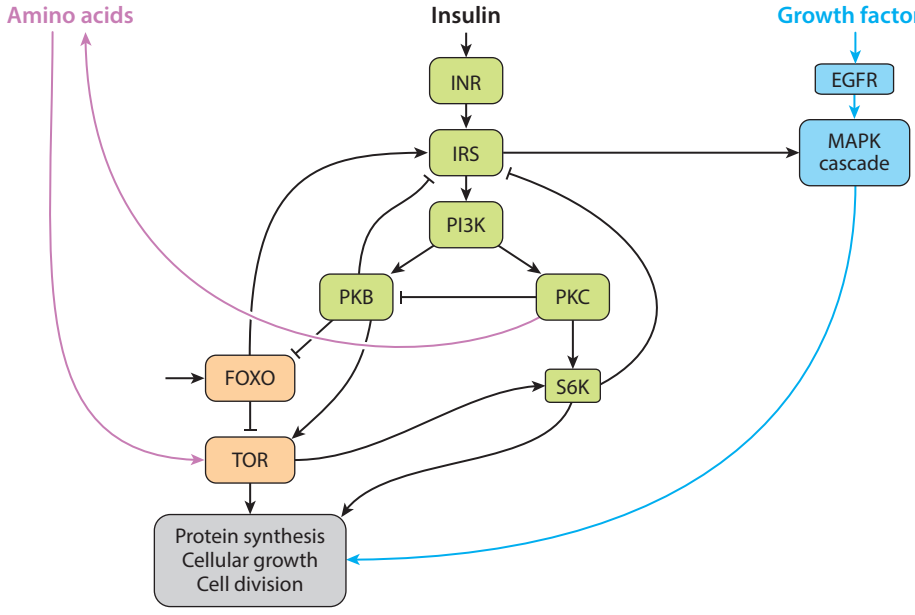

Figure 2

The insulin-signaling pathway that controls growth. Insulin stimulates protein synthesis and growth via the S6K kinase, the forkhead transcription factor (FOXO), and the target of rapamycin (TOR). Insulin also stimulates the uptake of certain amino acids, which in turn stimulate protein synthesis via TOR. Growth factors acting via the epidermal growth factor receptor (EGFR) also stimulate cellular growth and mitosis via the mitogen-activated protein kinase (MAPK) pathway (which can also be stimulated by insulin). The insulin-signaling network is complex, and experimental or genetic interference with any element can affect growth.

at different rates during late larval, pupal, and adult development, and they respond in a time-dependent manner to insulin (91), which suggests that differences in tissue growth do not come about by fluctuations in circulating insulin but are due to tissue-level differences in the response to insulin (98).

Dual Roles of Ecdysone

Growth of the epidermis and imaginal disks, and presumably other internal tissues, requires not only insulin but also ecdysone at a concentration substantially lower than the pulse of ecdysone that induces a molt. The low levels of ecdysone during the intermolt period, together with insulin signaling, are required to support tissue growth. Modulation of ecdysone levels controls both the rate and duration of cell division of wing imaginal disks (68), so it is possible that ecdysone provides the centralized control (via secretion of PTTH by the brain) over the rate of growth whereas insulin provides a permissive signal at the cellular level.

At the end of the larval feeding phase in *M. sexta* a rise in ecdysone stimulates the cessation of feeding and entry into the wandering stage in preparation for metamorphosis. In addition, the rise of ecdysone during the wandering, prepupal, and pupal stages stimulates tissue morphogenesis associated with metamorphosis. In vitro experiments have shown that low levels of 20-hydroxyecdysone (or the relatively inactive α-ecdysone) stimulate growth, whereas higher

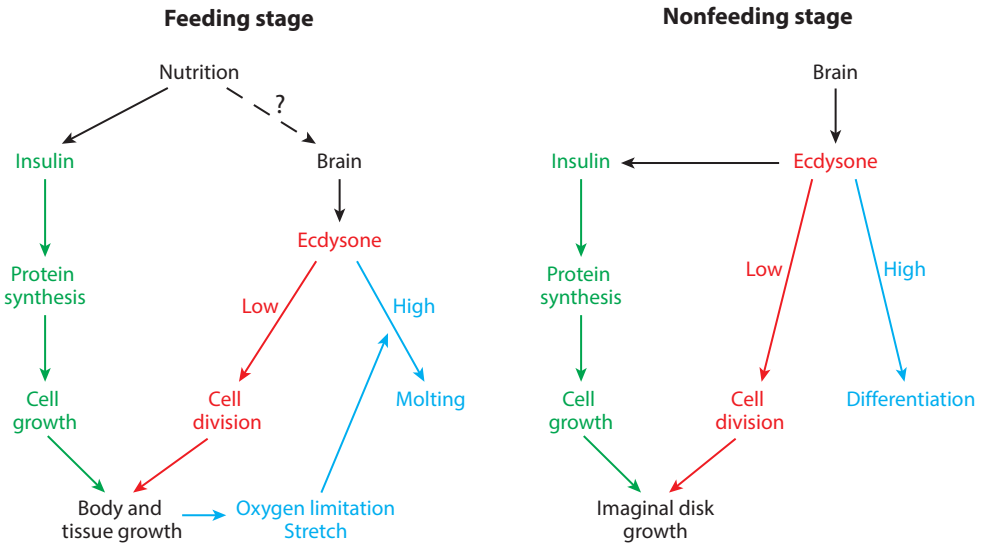

Figure 3

Control of cell growth and cell division in feeding and nonfeeding larvae. Ecdysone and insulin are believed to have distinct functions: Insulin primarily controls protein synthesis and cellular growth, whereas ecdysone primarily induces mitosis. During the feeding stage insulin secretion depends on nutrition, but during the nonfeeding stage insulin secretion is controlled by ecdysone. Both hormones are required to maintain somatic growth and growth of the imaginal disks, but whereas low levels of ecdysone stimulate growth, high levels stimulate molting and tissue differentiation during metamorphosis.

levels stimulate differentiation of cells and tissues (8, 70, 94). A low level of ecdysone stimulates growth by regulating the cell cycle and stimulating mitosis (34, 69, 80). Thus it appears that insulin signaling stimulates protein synthesis and cytoplasmic growth, whereas ecdysone acts as a mitogen (**Figure 3**). Differential actions of these two hormones could therefore regulate tissue size by independently regulating cell size and cell number. Whether a particular experimental or genetic manipulation that alters the size of an organ or appendage does so via a change in cell size or cell number (2, 50, 76, 104) may depend on how that manipulation altered the balance between ecdysone and insulin signaling. A relative reduction in ecdysone signaling or a relative increase in insulin signaling would result in relatively fewer cell divisions and growth that is dominated by an increase in cell size.

The rise in ecdysone at the end of the last larval instar in effect terminates growth and is, indeed, the universal mechanism that stops growth in insects. Only a relatively slight rise in ecdysone is required to stimulate the cessation of growth. Hence, experiments that simulate growth of the prothoracic glands (53) or that stimulate increased ecdysone production (42) have the effect of prematurely stopping growth, resulting in a smaller adult body. Conversely, experiments that decrease the production of ecdysone tend to prolong larval growth and result in adults of larger body size (42).

In some tissues growth is also controlled by signaling through the epidermal growth factor receptor (EGFR), which activates the mitogen-activated protein kinase (MAPK) cascade (89). Insulin and MAPK signaling can interact, as shown in **Figure 2**. This threefold regulation of growth by insulin, ecdysone, and MAPK signaling provides a flexible and adaptable mechanism for the control of tissue growth.

METAMORPHOSIS

Whether an insect is large or small, growth stops when the larva begins the metamorphic molt. Adult insects do not grow, so adult body size is in effect determined by the size at which a larva begins metamorphosis. Therefore, the mechanisms that control exactly when the metamorphic molt will occur exercise ultimate control over body size.

Hormonal Control of Metamorphosis

A metamorphic molt, just like a larval molt, is controlled by ecdysone, as outlined above. But whether a given molt is a larval molt, producing a larger additional larval instar, or whether it is a metamorphic molt, which stops growth and begins the transformation into an adult (in hemimetabolous insects) or a pupa (in holometabolous insects), depends on another hormone, juvenile hormone (JH).

JH is secreted by the corpora allata, a pair of small glands behind the brain, and its secretion is controlled by both stimulatory hormones (allatotropins) and inhibitory ones (allatohibins or allatostatins) produced by the brain. The levels of JH in the hemolymph are high throughout larval life, and in *M. sexta* and *B. mori* they drop slightly before the molt to the last larval instar before rising again (21, 23, 57). Sometime during the early part of the last larval instar JH begins to decline and gradually disappears. The decline of JH is due in part to the cessation of synthesis by the corpora allata but also to the action of JH esterase, an enzyme in the hemolymph that efficiently degrades JH (3, 5, 26, 95).

The decline in JH at or just before or the beginning of the last larval instar is sufficient to switch the developmental fate of the imaginal disks so they become committed to pupal development (39–41). Pupal commitment of the epidermis, by contrast, does not occur until the rise of ecdysone that stops growth at the end of the last larval stage (83, 84).

JH also plays an important role in controlling the timing and size at which a larva stops growing. During the last larval instar JH inhibits the secretion of PTTH and ecdysone, so the secretion of these hormones cannot occur until JH has been cleared (71, 86). This inhibition only occurs in the last larval instar and is thought to be a safety mechanism that prevents initiation of the final molt until all JH has been cleared (63, 64). A molt that occurs while there is still some JH present results in a nonviable, monstrous, larval-pupal intermediate (41). Once the JH has been cleared, this inhibition is relieved and the brain becomes competent to secrete PTTH. The secretion of PTTH is gated by the photoperiod and can only occur during a relatively brief window that repeats each day (100, 101).

The metamorphic molt is the first molt that occurs after JH has disappeared. Because the metamorphic molt also marks the end of the growth period, we can say that body size is controlled both by the mechanisms that cause JH to disappear and by those that cause ecdysone to be secreted. Both these categories of mechanisms, in turn, depend critically on mechanisms that assess body size and ensure that the endocrine events that terminate growth will occur at the correct body size for the species.

SENSING SIZE

Most insects grow to a species-characteristic size, independent of their growth rate during larval life: Slow-growing individuals simply take longer to get there than do fast-growing individuals. This general observation implies that insects are somehow able to assess their size and stop growing (i.e., begin metamorphosis) when a rather specific body size has been attained. It turns out there

are several different mechanisms by which body size is sensed, and the size triggers for molting are different from the size triggers for metamorphosis. In the Hemiptera, size is sensed by abdominal stretch receptors (11, 60, 61). In the bloodsucking *Rhodnius prolixus* and *Dipetalogaster maximus* this stretch is achieved rapidly by a single blood meal, whereas in the herbivorous *Oncopeltus fasciatus* critical degree of stretch is achieved gradually.

Critical Weight

Last-instar larvae of *M. sexta* begin the physiological process that leads to a molt when they pass a well-defined critical weight. The critical weight is operationally defined as the size of the instar after which feeding and nutrition are no longer required for a normal time course to metamorphosis. In other words, larvae that are starved after they have reached the critical weight metamorphose at the same time (albeit at a smaller size) as larvae that are allowed to continue feeding.

The processes that eventually lead to the secretion of ecdysone and the cessation of feeding begin at the critical weight. These include the cessation of JH secretion and upregulation of JH esterase, a catabolic enzyme that breaks down JH. The elimination of JH and its remnant effects takes some time; afterwards secretion of PTTH and ecdysone is disinhibited, and these hormones are secreted during the next photoperiodic gate. The interval between attainment of critical weight and cessation of feeding is called the terminal growth phase. Its duration is temperature dependent but is independent of nutrition (15). In *M. sexta* there are also critical weights for the earlier instars when the decision to molt is made (6).

In *D. melanogaster* there is a phenomenon that has also been called the critical weight, though its underlying physiology is quite different from that of *M. sexta*. The critical weight is best associated with a change in growth trajectory (96), and it also corresponds closely to the minimum viable weight. There is no role for JH in *D. melanogaster*, unlike *M. sexta*, and *D. melanogaster* larvae starved at or above the critical weight have a reduced terminal growth phase and actually accelerate the timing of metamorphosis. The latter is an interesting phenomenon we have called the bailout response (99) and suggests that simply running out of food can be a trigger for molting and metamorphosis, much as it is in the dung beetle *Onthophagus taurus* (90). The bailout trigger for metamorphosis would be expected to occur in species that live on a limited or an ephemeral food resource and cannot hope to find another source should the first one run out. The underlying mechanism that accelerates the molt has not been elucidated.

The critical weight of *Manduca* is achieved in about the middle of the last larval instar. In each instar *Manduca* increases in weight almost tenfold (because of Dyar's rule; see above), so at the critical weight the larva is only about half its final weight. Low nutrition and slow growth rate reduce the critical weight slightly (15), because, given sufficient time, the secretion of ecdysone becomes independent of the brain (7). But reduced nutrition and growth rate have a great effect on the final size the larva will reach, because the duration of the terminal growth phase is fixed and independent of nutrition.

In *Manduca* the critical weight is always almost exactly 4.8 times the initial weight of the instar, both among the different instars within a genetic strain and among the final larval instars of genetic strains of very different adult body sizes (6, 65). The critical weight is thus a relative measure, relative to something set at the beginning of each instar.

Oxygen Limitation Defines Critical Weight

Just as the exoskeleton of insects increases in discrete steps at each molt, so does the tracheal system. The tracheal system is a system of cuticle-lined, air-filled tubes that take air directly to

every cell in the body (9). At each molt the tracheal system increases in length and volume, but it remains constant during the growth phase between molts. Thus, as the larva grows and requires an ever-increasing amount of oxygen, the tracheal system eventually becomes unable to meet the demand (24). In each instar the rate of oxygen consumption rises with body mass but levels off and remains constant after the larva reaches the critical weight, indicating that the tracheal system is delivering oxygen at the maximal rate possible (6). Growth still continues, but the rate now gradually declines. The overall growth trajectory of the larva is a sigmoid curve, with the inflection point at the critical weight (65). When larvae are grown in an atmosphere with reduced oxygen, the critical weight is lower and the larvae metamorphose at a smaller body size (7).

The effect of oxygen on body size has also been extensively studied in *D. melanogaster* (28, 30) and other insects (27). Hypoxia always results in an extended growth phase and a smaller body size, whereas growing larvae under hyperoxia results, in some cases, in a larger adult body. Thus, oxygen limitation appears to be a general mechanism for size sensation and size regulation during larval growth of insects.

Threshold Size

The critical weight controls the size at which a molt will occur, but because it recurs in each instar, the critical weight does not directly control the final size of an insect. That is determined by the mechanism by which an insect assesses which will be the last larval instar. Most insects that are not growing under constant conditions have a variable number of larval instars, so the biological problem is when and how insects determine which instar is to be the last. In *Manduca* the decision about which is to be the final larval instar is made at the time of the molt. Larvae that molt above a rather well-defined threshold size enter the last larval stage; those that are below the threshold size continue to grow and molt until they reach the threshold (58).

In *M. sexta* the threshold size can be measured in two ways: the mass of the larva at the time of the molt or the head-capsule width at the beginning of the instar. Larvae with a head capsule larger than 5 mm, or an initial weight greater than 600 mg, are in the last larval instar; smaller ones are not (37, 58). The threshold size is independent of the prior growth history of the larva and seems to be an absolute size, not clearly relative to anything else. The mechanism by which threshold size is assessed remains unknown.

PROPORTIONAL GROWTH OF THE WING

It is not sufficient for a body to grow to a species-characteristic size; the appendages and other body parts must also grow to be in correct proportion to the body as a whole. In holometabolous insects this is a particularly interesting problem, because appendages grow from imaginal disks that grow mostly during metamorphosis, after the body has stopped growing.

Body size is not genetically determined but results from a complex set of physiological interactions, outlined above. Although body size is relatively invariant under constant environmental conditions, body size can vary greatly under variation in temperature and nutrition. Thus the challenge is to understand how appendages like the wings grow to the correct proportion with the body when the body varies plastically according to environmental variation.

When larvae are starved at the critical weight they produce adults of almost exactly half the normal size, with wings half the normal size, made up of half the normal number of cells of an adult wing (68). Half the number of cells means there is on average one less cell division in the smaller wing. How are the wings instructed to grow more or less, depending on body size? And when during wing growth is the adjustment to body size made?

Between the cessation of feeding and the fully developed adult wing there are about six rounds of cell division, three in the wandering stage and three in the pupal stage. In wings that are half the size there is one cell division missing in the wandering stage (68). This difference is controlled by ecdysone. There is a peak of ecdysone secretion during the wandering stage, and this ends one day earlier in small-bodied animals than in normal-sized ones, thus abbreviating the period of cell division and growth. In addition, the level of ecdysone is higher, above the optimum for cell division, and together these two effects account for the missing cell division. Thus, the adjustment of wing growth to body size is made quite early, during the wandering stage. The body somehow is able to assess its own size and regulates both the timing and level of ecdysone secretion so that the right number of cell divisions take place in the wing. The mechanism by which the wandering-stage larva assesses its body size is still unknown.

There is a close coordination of growth among organs and appendages during development. Imaginal disks are in competition with each other for limiting resources for growth, so that removal of one disk can cause excessive growth in others (38, 66). In *Drosophila* the coordination of growth of internal organs is also mediated by ecdysone (75), and the mechanism by which the correct proportionality of organs is sensed, established, and maintained remains an area of investigation.

FUTURE DIRECTIONS AND CHALLENGES

Although we have learned much about the hormones and pathways that control growth, there is still much to be learned about how final size is controlled. To control size it is necessary to have a mechanism that can assess size (or some accurate proxy of size), and that mechanism needs to be somehow linked to the signaling pathways that control growth.

Understanding the control of absolute size, such as the species-specific body size, is particularly challenging, but even the processes that control the relative sizes of body parts to the body as a whole, or the relative dimensions of a body part that we recognize as a characteristic shape, are largely unexplored. Genetic or molecular intervention studies that alter size or shape by interfering with signaling pathways or transcriptional regulators tell us who the necessary players are but say little about the normal mechanisms the organism uses to control size or shape: Those must operate at a higher level, the level at which size is monitored and assessed.

The work presented here shows that in insects there is not a single mechanism for the control of body size: Some use stretch receptors, others oxygen tension, and yet others the supply of food. The mechanism by which body size is sensed in the control of proportional growth of appendages is still unknown. We do not know the scope or diversity of these higher-level mechanisms. Some of them are surely adaptations to particular life histories, and all of the ones discovered so far are entirely unexpected, and nonmolecular. The challenges for the future are to elucidate how the known size-sensing mechanisms are linked to the mechanisms that stimulate growth so that growth stops when the right size is achieved, to determine how widespread the known mechanisms are, and to discover hereto unknown mechanisms by which insects assess their size and by which the proportional sizes of body parts are regulated.

SUMMARY POINTS

1. Body size is sensed and regulated at the organismal level, not the molecular level, although molecular mechanisms are involved.
2. Growth is regulated in concert by insulin and ecdysone.

3. Size is determined by the critical weight (a relative measure), which determines when a larval molt will occur, and the threshold size (an absolute measure), which determines what instar will be the last.
4. Different organs grow at different times. Correct proportions of body parts require a centrally controlled regulatory mechanism. This is provided by the central nervous system, which controls ecdysone and insulin secretion in response to nutrition and receives feedback from mechanisms that monitor size.

DISCLOSURE STATEMENT

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