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Review

The control of body size in insects

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Abstract

Control mechanisms that regulate body size and tissue size have been sought at both the cellular and organismal level. Cell-level studies have revealed much about the control of cell growth and cell division, and how these processes are regulated by nutrition. Insulin signaling is the key mediator between nutrition and the growth of internal organs, such as imaginal disks, and is required for the normal proportional growth of the body and its various parts. The insulin-related peptides of insects do not appear to control growth by themselves, but act in conjunction with other hormones and signaling molecules, such as ecdysone and IDGFs. Size regulation cannot be understood solely on the basis of the mechanisms that control cell size and cell number. Size regulation requires mechanisms that gather information on a scale appropriate to the tissue or organ being regulated. A new model mechanism, using autocrine signaling, is outlined by which tissue and organ size regulation can be achieved. Body size regulation likewise requires a mechanism that integrates information at an appropriate scale. In insects, this mechanism operates by controlling the secretion of ecdysone, which is the signal that terminates the growth phase of development. The mechanisms for size assessment and the pathways by which they trigger ecdysone secretion are diverse and can be complex. The ways in which these higher-level regulatory mechanisms interact with cell- and molecular- level mechanisms are beginning to be elucidated.

Keywords: Allometry; Autocrine; Autoregulation; *Drosophila*; Ecdysone; Growth; Insulin; Juvenile hormone; *Manduca*; Nutrition *Oncopeltus*; Size; Stretch reception

Introduction

The regulation of body size and of the sizes of body parts in animals continue to be formidably vexing problems in developmental biology. The size to which an individual and its body parts grow is affected by both genetic and environmental factors that operate through complex molecular and physiological mechanisms. Much of the recent research on the regulation of growth and size has focused on the molecular mechanisms that control the growth of cells and tissues. This work has revealed much about how cytoplasmic growth and cell division are regulated, but has not yet been successful at uncovering how the final size of a cell or a tissue is established. The control of overall body size is somewhat better understood. Experimental work on the endocrine physiology of growth has revealed some of the mechanisms by which body size is assessed, as well as the mechanisms that terminate the growth phase of development. The present review focuses on the mechanisms of body size regulation that have been uncovered in various species of insects and attempts to link these findings to the molecular mechanisms that control cellular growth.

Growth, cell size, and cell number

Many authors have noted that the size of an organ, or a body, is determined by the size of the component cells, and their number (Robertson, 1959; Partridge et al., 1994; De Moed et al., 1997; Azevedo et al., 2002). The view that body (or organ) size is functionally the simple product of cell size and cell number would seem to reduce the problem of size regulation to two distinct and possibly independent problems, namely the control of cell size and the control of cell number.

The belief that the control of size may be a simple function of the control of cell size and cell number emerges from a series of experiments by Alpatov (1930) and Robertson (1955, 1959). Robertson showed that genetic differ-

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ences in wing size in different strains of *Drosophila melanogaster* were mainly due to genetic differences in cell number, cell size remaining constant or nearly so. By contrast, when a given inbred strain was reared at an elevated temperature, the adults had smaller body and wing sizes, and this difference was due to a decrease in cell size, not cell number. Thus, it appears (1) that cell size and cell number are regulated by separable mechanisms, (2) that cell size and cell number can respond independently to genetic and environmental variation, and (3) that changes in wing size can be caused by changes in either cell size or cell number. These observations have been confirmed and expanded by many subsequent studies in different laboratories. This subsequent work consists of two largely nonoverlapping approaches: studies of the molecular genetic mechanisms that regulate growth and size, and analyses of the evolutionary genetics of growth, size and reaction norms. I will briefly outline the results of each of these approaches in turn.

Molecular mechanisms

Molecular genetic approaches have focused largely on the roles of cell cycle regulators, insulin signaling, and morphogens (such as diffusible transcription factors) in the regulation of growth and size. This work has been the subject of a number of excellent recent reviews (Conlon and Raff, 1999; Edgar, 1999; Day and Lawrence, 2000; Oldham et al., 2000; Weinkove and Leevers, 2000; Leevers, 2001; Potter and Xu, 2001; Stern, 2001; Johnston and Gallant, 2002), so I will deal with it here only in summary.

It has become increasingly clear that insulin signaling is essential for normal growth, and that it functions as the mediator between nutrition and cell growth (Britton and Edgar, 1998; Edgar, 1999; Weinkove et al., 1999; Edgar et al., 2001; Britton et al., 2002; Ikeya et al., 2002; Rulifson et al., 2002). Partial loss-of-function mutations in the insulin receptor or in genes of the insulin signal transduction pathway in *Drosophila* cause a severe delay in development and a reduction in body size, resulting in a normally proportioned but small adult fly. Overexpression of one of the *Drosophila* insulin-like proteins (DILPs) causes an increase in body size associated with an increase in both cell size and cell number (Brogiolo et al., 2001; Oldham et al., 2002; Rulifson et al., 2002). It has been shown that insulin-like molecules function as growth factors for a *Drosophila* imaginal disk-derived cell line and for intact butterfly wing imaginal disks grown in vitro (Kawamura et al., 1999; Nijhout and Grunert, 2002). In each of these cases, the insulins (DILPs or bombyxins) act together with an additional factor to stimulate growth and cell division: the *Drosophila* cell line requires chitinase-derived proteins named Imaginal Disk Growth Factors (IDGFs; Kawamura et al., 1998; Bryant, 2001), whereas the butterfly wing disks require the steroid hormone ecdysone (Nijhout and Grunert, 2002).

Many of the insulin-like peptides in insects are neurosecretory hormones (Mizoguchi et al., 1987, 1990; Nogueira et al., 1997; Brogiolo et al., 1998; Rulifson et al., 2002). Their secretion is therefore controlled by the central nervous system. This is important, because it implies that growth, and size, must ultimately be controlled by mechanisms that include the neurosecretory system of the brain. In *Drosophila*, both the central nervous system and the fat body appear to be able to respond to the level of nutrients in the hemolymph and can adjust the secretion of growth regulators (Britton et al., 1998; Edgar, 1999; Ikeya et al., 2002). The factors produced by the fat body have not yet been identified but are most likely to be either IDGFs or *Drosophila* insulin-like peptides (DILPs). The expression of three neurosecretory DILPs in *Drosophila* vary with nutrition (Ikeya et al., 2002), and may therefore act as the mediators between nutrition and growth, as the results of Britton et al. (2002) indicate.

Additional evidence that growth modulation is mediated by insulin signaling comes from experiments with *Precis coenia.* In this lepidopteran, the growth of wing imaginal disks is tightly coordinated with somatic growth. When larvae of *Precis* are starved, their wing imaginal disks cease to grow within about 4-6 h (Miner et al., 2000). When wing disks from starved larvae are put into an optimal tissue culture medium, they do not grow, unless ecdysone and bombyxin (the lepidopteran insulin-like growth factor) are added to the culture medium (Nijhout and Grunert, 2002). This suggests that the growth of disks does not respond directly to the level of nutrient in the medium, but that insulin (and possibly ecdysone) signaling is involved in the regulation of growth in response to nutritional variation. In *Manduca sexta*, the level of glucose and trehalose in the hemolymph declines sharply when the larva is starved (Dahlman, 1973). Hemolymph carbohydrates may thus be accurate indicators of the nutritional state of the larva. This hypothesis finds circumstantial support from recent experiments done with *Bombyx mori.* Starvation of *Bombyx* larvae causes a decrease in the titer of bombyxin, and injection of glucose stimulates bombyxin secretion (Satake et al., 1997; Masumura et al., 2000). In addition, injection of bombyxin causes a lowering of the carbohydrate level in the hemolymph of *Bombyx* (Satake et al., 1997). These results have been taken to indicate that bombyxin plays a role in carbohydrate metabolism, but they are also consistent with the idea that bombyxin level is a reflection of the nutritional state of the larva. It is possible, therefore, that bombyxin may have an important role in the regulation of metabolism, in addition to its effect as a growth factor for wing imaginal disks, as is the case with DILPs in *Drosophila* (Ikeya et al., 2002; Rulifson et al., 2002).

In addition to these systemic endocrine growth regulators, insects also use local autocrine and paracrine growth regulators. The secreted transcription factors wingless (Wg) and decapentaplegic (Dpp) appear at present to be the most promising candidates for a local mechanism of growth regulation. Localized overexpression of Dpp or Wg protein stimulates local cell proliferation, but the mechanism by which this stimulation occurs is not yet understood (Day and Lawrence, 2000). Although these factors are involved in the regulation of local cell proliferation, it is not clear whether they play a role

in size *regulation.* Indeed, Day and Lawrence (2000) cite evidence that suggests such a role is unlikely.

Proportional growth

Enhanced or diminished insulin signaling yields adults of larger or smaller body sizes, respectively, but these animals are of normal shape and proportions (Brogiolo et al., 2001; Ikeya et al 2002; Oldham et al., 2002; Rulifson et al., 2002). This indicates that the insulin signaling pathway somehow affects the growth of each body part in a proportional fashion. Proportional growth occurs if each tissue grows at a characteristic rate *relative* to that of other tissues. How this proportionality is achieved is not known, but because the growth signals are systemic all tissues probably experience identical concentrations of these signals, so that correct relative growth must emerge from differences at the level of the target tissues. This could be achieved either by tissue-specific levels of expression of receptors, or by tissue-specific differences in the activity of the signaling pathway downstream of the receptor.

The finding that in several species of insects the growth of one tissue can be altered by the presence or absence of another tissue suggests that different internal organs are in competition for some kind of limiting resource for growth (Nijhout and Wheeler, 1996; Nijhout and Emlen, 1998; Klingenberg and Nijhout, 1998; Stern and Emlen, 1999). It is possible that this competition is mediated by one of the circulating growth regulators (DILP, bombyxin, ecdysone, IDGF). It is not known whether these regulators occur at sufficiently low concentrations to be limiting, but insofar as normal growth can be sensitively modulated by variation in insulin-like molecules, it is possible that these may typically occur at limiting concentrations. If this is the case, then tissue-specific receptor activity could play a dominant role in controlling relative growth. Evolutionary changes in spatially patterned receptor activity then account for the evolution of body proportions and allometry. It is not clear, however, how such a mechanism could be used to control the absolute size of a tissue, or a body.

Evolutionary genetics and reaction norms

Recent statistical approaches to the genetics of growth and size have largely focused on the interaction between genetic and environmental effects. The roles of genes and environments in the determination of body size have been of particular interest to evolutionary geneticists, because of the profound effect that size has on fitness (Calder, 1984; Stearns, 1992; Roff, 1992; Schlichting and Pigliucci, 1998). That the environment can have a profound effect on body size (all other things being equal) is well known: adult insects generally are of smaller body size when larvae are reared at higher temperatures, or on lower quality diets (Atkinson, 1994; Partridge et al., 1994; Chapman, 1998). The function that describes the dependence of a phenotype (such as body size or wing size) on a particular environmental variable (such as temperature), for a given genotype, is called a reaction norm.

Simply rearing *Drosophila* at different temperatures for a number of years resulted in a heritable change in body size (Partridge et al., 1994). Lines reared at a high temperature had a significantly smaller body size (measured as thorax length and wing area) than lines reared at a low temperature. That these differences were due to genetic differentiation was demonstrated by rearing each strain at a common temperature, showing that the lines maintained at lower temperatures were genetically larger than the lines maintained at higher temperatures. Evidently small-bodied flies had higher fitness at high temperatures, and large-bodied flies at low temperatures. Interestingly, this fitness cline coincides with the reaction norm for temperature, suggesting that the reaction norm may be adaptive (Partridge et al., 1994; Morin et al., 1997). The increase in wing area at lower temperatures was largely due to an increase in cell size, so that in this regard the genetic response to selection by different temperatures mirrors the direct effect of temperature on cell size and body size observed by Alpatov, (1930), Robertson (1955, 1959), De Moed et al. (1997), and Azevedo et al. (2002). De Moed et al. (1997) have shown that different genetic lines of *Drosophila* can have significantly different reaction norms for a given environmental variable. Genetic changes in size can also be obtained by active artificial selection. McCabe et al. (1997) selected different lines of *Drosophila* for large or small wing areas. They found that the evolutionary responses in wing size were entirely due to changes in cell number, not in cell size.

Although few studies have been done on the cellular makeup of other tissues (e.g., Azevedo et al., 2002), these indicate that the cell size and cell number in other tissues generally follow the same pattern of association with body size as those of the wing. At lower rearing temperatures the increase in the size of body parts is consistently due to a change in cell size, not cell number, but evolutionary (genetic) changes in body size can be associated with both changes in cell size or cell number, depending on the tissue and on the genetic strain of *Drosophila* (Zwaan et al., 2000; Azevedo et al., 2002).

A matter of scale

The results of developmental and evolutionary studies on size regulation in *Drosophila* demonstrate that the size of the wing is largely independent of the number of cells or the size of cells that make up the wing (Neufeld et al., 1998; Vervoort et al., 1999; Azevedo et al., 2002). These findings suggest that it is not possible to understand the determination of overall organ size exclusively in terms of the control of cell growth, or of cell size, or of cell number. Mechanisms that operate at the level of the cell or below cannot be used to address questions about the regulation of organ size or body size, because they do not operate at the appropriate scale.

The mechanisms that regulate organ size (or body size) somehow incorporate information about the physical dimensions or mass of the organ (e.g., Potter and Xu, 2001). Although any mechanism that regulates organ or body size must ultimately exert its effect by altering cell growth or cell division, the locus of control cannot reside at the cellular level. Thus, although genetic or experimental alterations of a cellular mechanisms that controls cell division, or cell size, can alter organ or body size, this does not imply that this mechanism *controls* organ or body size, as is often suggested. Such a cellular mechanism must be a downstream component of a regulatory cascade whose control (that is, the origin of the difference that determines whether to grow or stop growing) resides at a higher level.

The locus of this control has never been investigated, but a number of models have been proposed of how this might be done. The "entelechia" model of Garcia-Bellido and Garcia-Bellido (1998) proposes a system of local interactions and an activating mechanism that arises from compartment boundaries, in which cell division is controlled by a balance among interacting transcription factors that affect the expression level of a hypothetical regulatory gene. Computer simulations show that this model can account for many of the growth and regeneration properties of imaginal discs that have been surgically or genetically manipulated. Other local models that attempt to explain the control of tissue size in terms of a changing balance between the rate of cell growth and the rate of cell differentiation have been proposed by Van der Have and De Jong (1996) and Arendt (2000).

A higher-level mechanism of size regulation that gathers information over a scale that approximates the size of the structure that is regulated has been proposed by Day and Lawrence (2000). These authors suggest that gradients of morphogens, emanating from compartment borders, could be used as a size-sensing mechanism. If the two ends of such a gradient were maintained at fixed levels, and if the gradient was linear, then the slope of the gradient would be inversely proportional to its length, and the slope would therefore be a measure of the distance between the two ends of the gradient. They proposed that size regulation could occur if cells could sense the steepness of such a gradient and stop growing (or dividing) when the steepness of the gradient dropped below some critical level. Day and Lawrence (2000) cite several lines of evidence that support such a mechanism, and several kinds of experimental evidence that are inconsistent with it, and conclude that the evidence is equivocal. One of the counterarguments to a gradient hypothesis is that no morphogen has yet been identified that has the requisite range. The Dpp signal, for instance, extends only over 5-10 cell diameters (Nellen et al., 1996; Lecuit et al., 1996; Burke and Basler, 1996), which is too short for it to act as a regulator of overall wing size. The Wg signal may range somewhat farther, but still not over the several hundred (in *Drosophila*) or several thousand (in larger insects) cell diameters that would be required to span a substantial portion of the wing. Another difficulty with a gradient-sensing mechanism is that cells must be able to sense and respond to very small concentration differences across their diameter, and that cells at all locations along the gradient must be able to sense its steepness, independent of its actual concentration. Finally, such a model would be sensitive to cell size, since, for a given gradient, a large cell would sense a greater difference across its diameter than a small cell.

An alternative mechanism that circumvents these problems is suggested by the observation that many cells and tissues secrete their own growth regulators (Kawamura et al., 1999; Casci and Freeman, 1999; Mesnier et al., 2000). Although autocrine/paracrine regulatory loops usually act locally, in the open circulatory systems of insects these secreted regulators can become blood-borne and could circulate throughout the body. On the surface, it would seem a bad idea for cells to produce their own growth stimulators, because this would constitute a positive feedback system and would result in runaway growth (as indeed happens in some cancers), unless there exists an additional mechanism that curtails such growth. If a tissue that produces its own growth stimulator also produces a growth inhibitor, then the interaction between stimulation and inhibition can lead to limited growth. The operation of such an autoregulation mechanism, in which the inhibitor simply inactivates the growth activator (by catabolism or sequestration), is shown in Box 1. With this mechanism, a cell only needs to be able to respond to the concentration of a growth factor, as most cells do. We see that the concentration of growth factor gradually declines as the tissue grows and the level of inhibitor rises. The tissue initially grows exponentially, but the growth rate rapidly diminishes as the inhibitor eliminates an increasing fraction of the secreted activator. The final size of the tissue is determined by the relative rates of activator and inhibitor production and breakdown, and by the rate of growth activator-stimulated growth. Differences in the growth rate and final sizes of different tissues can be due to tissue-specific differences in the rates of synthesis or breakdown of the growth activator and inhibitor.

This simple mechanism can also account for the observed competition between different imaginal disks (Nijhout and Emlen, 1999), if those disks share the same growth regulatory mechanism, and will also reproduce the size regulation of disk fragments, when parts of a disk are removed during its growth phase. This autoregulatory mechanism can, however, not account for allometry (the differences in the relative sizes of body parts with variation in overall body size; Nijhout and Wheeler, 1996), because the model contains no term for overall body size. In order to account for proportional growth and allometry (which in developmental terms can be defined as a systematic violation of proportional growth), it is necessary either to have a mechanism that makes one or more of the parameters sensitive to body size, or to have an additional control mech-

Box 1.

A simple model for size regulation by coupled autoactivation and autoinhibition. Assume an imaginal disk produces its own secreted growth stimulator, and also secretes a product that destroys or sequesters this activator (an inhibitor). Cell division and growth are entirely due to the activator, and can be described by the equation for exponential growth,

$$\frac{dN}{dt} = k_1 A N ,$$

where N is cell number, A is the concentration of the growth activator, and k_1 is a rate constant. If we assume that all cells of the disk produce the activator at a constant rate, and that the activator is removed at a rate proportional to its current concentration and that of the inhibitor, this can be expressed by the following equation:

$$\frac{dA}{dt} = k_2 N - k_3 I A,$$

where the first term on the right-hand side describes the synthesis of the activator that is proportional to cell number (or the size of the tissue), the second term describes the breakdown of the activator, and k_2 and k_3 are rate constants. Finally, we assume that each cell of the disk also produces the inhibitor at a constant rate:

$$\frac{dI}{dt} = k_4 N - k_5 I,$$

where k_4 is the rate constant for inhibitor expression and k_5 is the rate of inhibitor breakdown. The growth trajectory of the disk is then determined by the values of the five parameters (the rate constants) and the initial conditions (the starting values of cell number (N_0), and the initial activator (A_0) and inhibitor (I_0) concentrations).

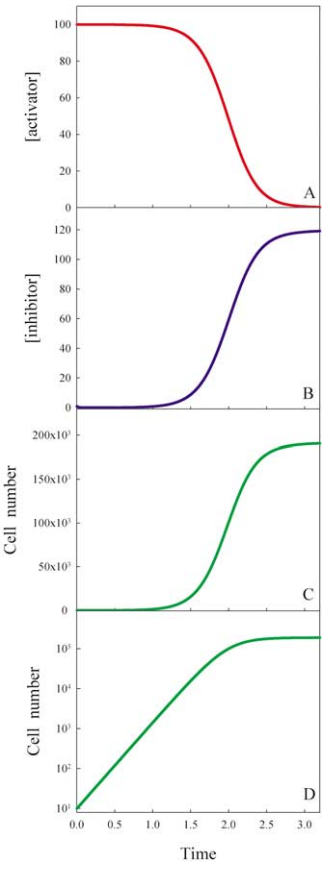

Figure 1

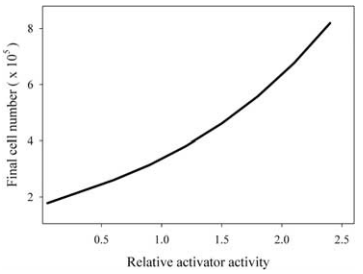

Figure 2

Figure 1 illustration of the concentration profiles for activator (A) and inhibitor (B), and the size trajectory (C) of the disk for the following parameter values and initial conditions: $k_1 = 1.0$, $k_2 = 0.9$, $k_3 = 0.0001$, $k_4 = 0.5$, $k_5 = 800$, $N_0 = 10$, $A_0 = 100$, $I_0 = 1$. The units are arbitrary in this illustration, but they can be interpreted as: concentration = nM, and time = days, for instance. The graph in (D) is a semilogarithmic plot of the same data as (C) and shows that growth is exponential during most of the growth period.

Mutations and environmental variables such as nutrition and temperature have their effect on size by altering the values of one or more of the rate constants. Variation in the rate of activator expression, for instance, affects the size at which growth stops, with increased signaling resulting in an increased final cell number (Figure 2).

When a large fraction of the disk is removed during its growth phase, the disk can "regenerate" to almost the correct number of cells (Figure 3).

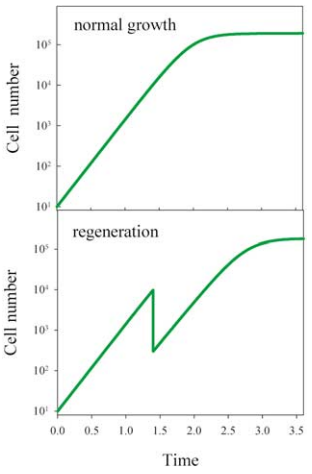

Figure 3

anism that is sensitive to body size and that can modify the autoregulatory mechanism. As noted above, it appears that some tissues in insects require the simultaneous action of two different growth regulators. If the second growth regulator is independently controlled, it could provide a mechanism that can adjust the final size of a tissue to the overall body size of the insect.

Body size

Just as the control of the size of a tissue or organ cannot be explained solely in terms of the control of cell size and cell number, the mechanism that regulates body size is more than simply the sum of the mechanisms that regulate the sizes of internal organs and appendages. The regulation of body size must either contain a mechanism that responds to information that is generated all over the body, or it must be sensitive to the size of a particular body part that acts as a proxy for the body as a whole.

Several authors have pointed out that the control of size is not so much a control of growth but a control of when to stop growing (Nijhout, 1994; Conlon and Raff, 1999; Stern and Emlen, 1999). This is particularly so for the many insects whose size increases exponentially with time. Under exponential growth, small errors in the timing of cessation of growth can have large consequences for the final size because the decision to stop growing is made at the time the animal has achieved its highest growth rate. Thus, it is likely that whatever size-monitoring mechanism is used, it must be sensitively connected to the mechanism that controls the cessation of growth.

In insects, the immediate cause of the cessation of growth has long been known. Growth stops episodically whenever a larva molts, and it stops finally when metamorphosis begins. In both cases, the cessation of growth is caused by the secretion of ecdysteroids, the steroid hormones that initiate the molting cycle (Nijhout, 1994), and can be artificially induced by injection or infusion of ecdysone. Adult insects do not grow, so the size of an adult insect is determined entirely by the size at which the last-instar larva secretes ecdysteroids and begins metamorphosis. Thus, the mechanism that controls the timing of ecdysteroid secretion in effect controls body size.

The secretion of ecdysteroids is itself the culmination of a cascade of events. The immediate cause is the secretion of a brain neurosecretory hormone, the prothoracicotropic hormone (PTTH). PTTH activates the prothoracic glands via a calcium/calmodulin -cAMP/protein kinase signaling pathway (Smith and Gilbert, 1989), and causes them to secrete ecdysteroids. The control of PTTH secretion is complex and diverse. The actual stimulus for PTTH secretion is known only for several species of the order Hemiptera (the true bugs). In these insects, PTTH secretion is controlled by stretch receptors in the abdomen that are activated when the animal reaches a particular critical size (Nijhout, 1979, 1981). In *Oncopeltus fasciatus*, the milkweed bug, this size-monitoring mechanism can be fooled by artificially expanding the abdomen with an injection of saline (Nijhout, 1979). In the last-instar larva, such an injection causes the animal to secrete ecdysteroids and initiate a premature metamorphosis, resulting in a miniature adult. Thus, in this species, we have complete control over the first step in the mechanism that regulates adult size. Under normal growth, the abdominal stretch receptor is not activated until the larva has accumulated a critical amount of body mass and is thus determined by the quantity and quality of nutrition. In bloodsucking Hemiptera, like *Rhodnius prolixus* and *Dipetalogaster maximus*, the requisite abdominal stretch is achieved by a single large blood meal (Wigglesworth, 1934; Nijhout, 1984; Chiang and Davey, 1988). Larvae of these species feed only once during each instar, and molt (or metamorphose) a characteristic number of days after a meal.

Abdominal stretch reception does not appear to control PTTH secretion in any group of insects outside the Hemiptera, so it is not a general mechanism for size assessment. In the beetle *Onthophagus taurus*, for instance, PTTH and ecdysone secretion are induced by removal of the larva from its food supply (Shafiei et al., 2001). In nature, this would occur when a larva exhausts the ball of food provisioned by its mother. In other holometabolous insects, the secretion of PTTH is controlled by a much more complex mechanism. In *M. sexta*, the tobacco hornworm, the secretion of PTTH and ecdysteroids in the last larval instar are under inhibition by the juvenile hormone (JH). If the corpora allata (the glands that secrete JH) are removed early in the instar, the larvae secretes PTTH and ecdysone prematurely and metamorphoses into a miniature adult. Conversely, if additional JH is injected, PTTH secretion is delayed in a dose-dependent manner and metamorphosis begins at a much larger body size than normal (Nijhout and Williams, 1974; Rountree and Bollenbacher 1986). The inhibition of PTTH by JH only occurs in the last larval instar and appears to be part of a safety mechanism that prevents the secretion of these molt-stimulating hormones until all JH has been cleared from the hemolymph. The reason for having such a mechanism is obvious, because if a metamorphic molt occurs in the presence of JH, it results in an animal that is a mosaic of larval/pupal or larval/adult traits (Wigglesworth, 1940; Williams, 1961; Nijhout, 1983, 1994). The disappearance of JH during the middle of the last larval instar thus disinhibits the secretion of the molt-stimulating hormones.

These findings reduce the problem of the control of PTTH secretion to two independent questions, namely, what causes JH secretion to stop, and what finally stimulates PTTH secretion? The cessation of JH secretion is tightly associated with the attainment of a critical weight. This critical weight is determined by the weight of the larva at the outset of the last larval instar (Nijhout, 1981). Circulating JH is broken down by JH esterase (Hammock, 1985). The activity of this enzyme in the hemolymph increases

gradually in the course of the last larval instar, and this increase in activity has been shown to be essential for the effective clearance of JH (Hammock, 1985; De Kort and Granger, 1996; Browder et al., 2001). The level of JH esterase is strongly affected by nutrition, and its activity drops to zero almost immediately if a larva is starved (Browder et al., 2001). It is likely that, in normal life, variation in nutrition modulates JH esterase activity as well as the secretion of JH, and the consequent persistence of JH accounts for the delay in PTTH secretion in animals that grow slowly or are periodically starved.

Once disinhibited by the disappearance of JH, the timing of secretion of PTTH is controlled by a photoperiodic clock (Truman, 1972; Truman and Riddiford, 1974). PTTH secretion can only occur during a relatively brief "photoperiodic gate" that recurs daily. If the secretion of PTTH becomes disinhibited while this photoperiodic gate is open, PTTH secretion begins immediately, followed by ecdysone secretion and the cessation of growth. Otherwise, PTTH secretion is delayed until the next day's photoperiodic gate opens (Truman and Riddiford, 1974), and during this delay period, the larva continues to feed and grow, and can gain a significant amount of extra size.

The size of a *Manduca* larva at metamorphosis, and therefore the size of the adult insect, is determined by five factors: (1) the critical weight, which initiates the disappearance of JH, (2) the PTTH delay time, which is the time required for JH to disappear and for PTTH secretion to be disinhibited, (3) the timing of the photoperiodic gate for PTTH secretion, (4) the (exponential) growth rate during the last larval instar, and (5) the initial size of the final instar. Quantitative genetics have shown that there is additive genetic variance for each of these factors (Davidowitz et al., 2003). Evolution of body size in *Manduca* has been shown to be due to genetic changes in three of these five factors: the critical weight, the PTTH delay time, and the growth rate (D'Amico et al., 2001).

Each of the five factors in this size-regulating cascade has complex genetic underpinnings that are still far from being fully elucidated. The PTTH delay time, for instance, is determined in large part by the expression level of JH esterase, a product of the fat body. The expression of JH esterase may be under feedback control by JH and is also affected by neurosecretory factors from the brain that may, in turn, be influenced by nutrition, but the details of this regulation are still not fully worked out. The growth rate is also controlled by nutrition (Davidowitz et al., 2003) and is most likely mediated by insulin signaling as it is in *Drosophila*, and in a Lepidopteran epidermal cell line (Mesnier et al., 2000), although this still needs to be critically demonstrated in intact *Manduca*. The manner in which the circadian clock stimulates PTTH secretion likewise remains unknown. The critical weight is a function of the initial size of the instar, so it appears to involve an internal relative measure, but what exactly is being measured is unclear.

Although we know that these five factors completely account for the regulation of body size in *Manduca*, much works remains to be done to elucidate the exact physiological and molecular mechanism that underlie each of them. We also do not know whether the mechanism that operates in *Manduca* occurs in other species. The inhibitory role of JH on PTTH and/or ecdysteroid secretion appears to be widespread in the holometabola, and the circadian control of hormone secretion is also a fairly general feature of insect life cycles.

But just as the Hemiptera have evolved what may be a unique stretch reception mechanism to adjust their developmental timing to body size, it is not unreasonable to assume that the holometabolous insects have evolved many variations on the much more complex theme we observe in *Manduca*. In *Drosophila* and in certain moths, for instance, metamorphosis can be delayed by partial ablation of the wing imaginal disks (Madhavan and Schneiderman, 1969; Rahn, 1972, Simpson and Schneiderman, 1975; Simpson et al., 1980). It appears that, in these species, growing and regenerating imaginal disks somehow inhibit PTTH secretion (Sehnal and Bryant, 1993; Zitnan et al., 1993), and it is the normal cessation of imaginal disk growth that signals the onset of metamorphosis. Exactly how the neurosecretory system of the brain becomes aware that the imaginal disks have stopped growing is not known, but the size control model described above (Box 1) suggests that this could be readily achieved by monitoring the disappearance of growth activator in the hemolymph (Edgar and Nijhout, 2003).

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References

Alpatov, W.W., 1930. Phenotypical variation in body and cell size of *Drosophila melanogaster*. Biol. Bull. 58, 85–103.

Arendt, J.D., 2000. Allocation of cells to proliferation vs. differentiation and its consequences for growth and development. J. Exp. Zool. (Mol. Dev. Evol.) 288, 219–234.

Atkinson, D., 1994. Temperature and organism size: a biological law for ectotherms. Adv. Ecol. Res. 25, 1–58.

Azevedo, R.B.R., French, V., Partridge, L., 2002. Temperature modulates epidermal cell size in *Drosophila melanogaster*. J. Insect Physiol. 48, 231–237.

Britton, J.S., Edgar, B.A., 1998. Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. Development 125, 2149–2158.

Britton, J.S., Lockwood, W.K., Cohen, S.M., Edgar, B.A., 2002. *Drosophila's* insulin/P13-kinase pathway coordinates cellular metabolism with nutritional conditions. Dev. Cell 2, 239–249.

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., Hafen, E., 2001. An evolutionarily conserved function for the *Drosophila* insulin receptor and insulin-like peptides in growth control. Curr. Biol. 11, 213–221.

Browder, M.H., D'Amico, L.J., Nijhout, H.F., 2001. The role of low levels of juvenile hormone esterase in the metamorphosis of *Manduca sexta*. J. Insect Sci. 11, 1–5.

Bryant, P.J. 2001 Growth factors controlling imaginal disk development in *Drosophila*, in: The Cell Cycle and Development: Novartis Foundation Symposium 237, Wiley, New York, pp. 182–199.

Burke, R., Basler, K., 1996. Dpp receptors are autonomously required for cell proliferation in the entire developing *Drosophila* wing. Development 122, 2261–2269.

Calder, W.A.I., 1984. Size, Function and Life History. Harvard Univ. Press, Cambridge, MA.

Casci, T., Freeman, M., 1999. Control of EGF receptor signaling: lessons from fruitflies. Cancer Metastasis Rev. 18, 181–201.

Conlon, I., Raff, M., 1999. Size control in animals. Cell 96, 235–244.

Chapman, R.F., 1998. The Insects: Structure and Function. Cambridge Univ. Press, Cambridge.

Chiang, G.R., Davey, K.G., 1988. A novel receptor capable of monitoring applied pressure in the abdomen of an insect. Science 241, 1665–1667.

Dahlman, D.L., 1973. Starvation of the tobacco hornworm, *Manduca sexta*. Changes in hemolymph characteristics of 5th-stage larvae. Ann. Entomol. Soc. Amer. 66, 1023–1029.

D'Amico, L.J., Davidowitz, G., Nijhout, H.F., 2001. The developmental and physiological basis of body size evolution in an insect. Proc. R. Soc. Lond. B Biol. Sci. 268, 1589–1593.

Davidowitz, G., D'Amico, L.J., Nijhout, H.F., 2003. Critical weight in the development of insect body size. Evol. Dev. 5, 188–197.

Day, S.J., Lawrence, P.A., 2000. Measuring dimensions: the regulation of size and shape. Development 127, 2977–2987.

De Kort, C.A.D., Granger, N.A., 1996. Regulation of JH titers: the relevance of degradative enzymes and binding proteins. Arch. Insect Biochem. Physiol. 33, 1–26.

De Moed, G.H., De Jong, G., Scharloo, W., 1997. Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. Genet. Res. Camb. 70, 35–43.

Edgar, B.A., 1999. From small flies come big discoveries about size control. Nat. Cell Biol. 1, E191–E193.

Edgar, B.A., Britton, J., de la Cruz, A.F.A., Johnston, L.A., Lehman, D., Martin-Castellanos, C., Prober, D., 2001 Pattern- and growth-linked cell cycles in *Drosophila* development in: The Cell Cycle and Development: Novartis Foundation Symposium 237, Wiley, New York, pp. 3–18.

Edgar, B.A., Nijhout, H.F., 2003. Growth and cell cycle controls in *Drosophila*, in: Hall, M., Thomas, G., Raff, M. (Eds.), Control of Cell Growth, Cold Spring Harbor Laboratory Press, in press.

Garcia-Bellido, A.C., Garcia-Bellido, A., 1998. Cell proliferation in the attainment of constant sizes and shapes: the Entelechia model. Int. J. Dev. Biol. 42, 353–362.

Goss, R.J., 1964. Adaptive Growth. Logos Press, London.

Hammock, B.D., 1985. Regulation of juvenile hormone titer: degradation, in: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology, 7, Pergamon Press, New York, pp. 431–472.

Ikeya, T., Galic, M., Belawat, P., Nairz, K., Hafen, E., 2002. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. Curr. Biol. 12, 1293–1300.

Johnston, L.A., Gallant, P., 2002. Control of growth and organ size in *Drosophila*. BioEssays 24, 54–64.

Kawamura, K., Shibata, T., Saget, O., Peel, D., Bryant, P.J., 1999. A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disk cells. Development 126, 211–219.

Klingenberg, C.P., Nijhout, H.F., 1998. Competition among growing organs and developmental control of morphological asymmetry. Proc. R. Soc. Lon. B Biol. Sci. 265, 1135–1139.

Lecuit, T., Brook, W.J., Ng, M., Calleja, M., Sun, H., Cohen, S.M., 1996. Two distrinct mechanisms for long-range patterning by decapentaplegic in the *Drosophila* wing. Nature 381, 387–393.

Leevers, S.J., 2001. Growth control: invertebrate insulin surprises. Curr. Biol. 11, R209–R212.

Madhavan, K., Schneiderman, H.A., 1969. Hormonal control of imaginal disk regeneration in *Galleria mellonella* (Lepidoptera). Biol. Bull. 137, 321–331.

Masumura, M., Satake, S., Saegusa, H., Mizoguchi, A., 2000. Glucose stimulates the release of bombyxin, an insulin-related peptide of the silkworm *Bombyx mori*. Gen. Comp. Endocrinol. 118, 393–399.

McCabe, J., French, V., Partridge, L., 1997. Joint regulation of cell size and cell number in the wing blade of *Drosophila melanogaster*. Genet. Res. Camb. 69, 61–68.

Mesnier, M., Partiaoglou, N., Oberlander, H., ad Porcheron, P., 2000. Rhythmic autocrine activity in cultured insect epidermal cells. Arch. Insect Biochem. Physiol. 44, 7–16.

Miner, A.L., Rosenberg, A.J., Nijhout, H.F., 2000. Control of growth and differentiation of the wing imaginal disk of *Precis coenia* (Lepidoptera: Nymphalidae). J. Insect Physiol. 46, 251–258.

Mizoguchi, A., Hatta, M., Sato, S., Nagasawa, H., Suzuki, A., Ishizaki, H., 1990. Developmental changes of bombyxin content in the brain of the silkmoth *Bombyx mori*. J. Insect Physiol. 36, 655–664.

Mizoguchi, A., Ishizaki, H., Nagasawa, H., Kataoka, H., Isogai, A., Tamura, A., Suzuki, A., Fujino, M., Kitada, C., 1987. A monoclonal antibody against a synthetic fragment of bombyxin (4K-prothoracicotropic hormone) from the silkmoth *Bombyx mori*: characterization and immunohistochemistry. Mol. Cell. Endocrinol. 51, 227–235.

Morin, J.P., Moreteau, B., Pétavy, G., Parakash, R., David, J.R., 1997. Reaction norms of morphological traits in *Drosophila*: adaptive shape changes in a stenotherm circumtropical species. Evolution 51, 1140–1148.

Nellen, D., Burke, R., Struhl, G., Basler, K., 1996. Direct and long-range action of a DPP morphogen gradient. Cell 85, 357–368.

Neufeld, T.P., de la Cruz, A.F., Johnston, L.A., Edgar, B.A., 1998. Coordination of growth and cell division in the *Drosophila* wing. Cell 93, 1183–1193.

Nijhout, H.F., 1979. Stretch-induced moulting in *Oncopeltus fasciatus*. J. Insect Physiol. 25, 277–281.

Nijhout, H.F., 1981. Physiological control of molting in insects. Am. Zool. 21, 631–640.

Nijhout, H.F., 1983. Definition of a juvenile hormone-sensitive period in *Rhodnius prolixus*. J. Insect Physiol. 29, 669–677.

Nijhout, H.F., 1984. Abdominal stretch reception in *Dipetalogaster maximus* (Hemiptera: Reduviidae). J. Insect Physiol. 30, 629–633.

Nijhout, H.F., 1994. Insect Hormones. Princeton University Press, Princeton, NJ.

Nijhout, H.F., Emlen, D., 1998. Competition among body parts in the development and evolution of insect morphology. Proc. Natl. Acad. Sci. USA 95, 3685–3689.

Nijhout, H.F., Grunert, L.W., 2002. Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. Proc. Natl. Acad. Sci USA 99, 15446–15450.

Nijhout, H.F., Wheeler, D.E., 1996. Growth models of complex allometries in insects. Am. Nat. 148, 40–56.

Nijhout, H.F., Williams, C.M., 1974. Control of moulting and metamorphosis in the tobacco hornworm, *Manduca Sexta* (L.): cessation of juvenile hormone secretion as a trigger for pupation. J. Exp. Biol. 61, 493–501.

Nogueira, B.V., Muehleisen, D.P., Whisenton, L.R., Gray, R.S., Bollenbacher, W.E., 1997. Life cycle expression of a bombyxin-like neuropeptide in the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 43, 47–53.

Oldham, S., Böhni, R., Stocker, H., Brogiolo, W., Hafen, E., 2000. Genetic control of size in Drosophila. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355, 945–952.

Oldham, S., Stocker, H., Laffargue, M., Wittwer, F., Wymann, M., Hafen, E., 2002. The Drosophila insulin/IGF receptor controls growth and size by modulating $PtdInsP_3$ levels. Development 129, 4103–4109.

Partridge, L., Barrie, B., Fowler, K., French, V., 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution 48, 1269–1276.

Potter, C.J., Xu, T., 2001. Mechanisms of size control. Curr. Opin. Genet. Dev. 11, 279–286.

Rahn, P., 1972. Untersuchungen zur Entwicklung von Ganz- unt Teilimplanten der Flügelimaginalscheibe von *Ephestia kühniella* Z. Wilhelm Roux's Arch. 170, 48–82.

Robertson, F.W., 1955. The ecological genetics of growth in Drosophila. 2. Selection for large body size on different diets. Genet. Res. Camb. 1, 305–318.

Robertson, F.W., 1959. Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. Genetics 44, 869–896.

Roff, D.A., 1992. The Evolution of Life Histories. Chapman and Hall, New York.

Rountree, D.B., Bollenbacher, W.E., 1986. The release of prothoracicotropic hormone in the tobacco hornworm, *Manduca sexta*, is controlled intrinsically by juvenile hormone. J. Exp. Biol. 120, 41–58.

Rulifson, E.J., Kim, S.K., Nusse, R., 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296, 1118–1120.

Satake, S., Masumura, M., Ishizaki, H., Nagata, K., Kataoka, H., Suzuki, A., Mizoguchi, A., 1997. Bombyxin, an insulin-related peptide of insects, reduces the major storage carbohydrates in the silkworm *Bombyx mori*. Comp. Biochem. Physiol. 118B, 349–357.

Schlichting, C.D., Pigliucci, M., 1998. Phenotypic Evolution. A Reaction Norm Perspective. Sinauer, Sunderland, MA.

Sehnal, F., Bryant, P.J., 1993. Delayed pupariation in *Drosophila* imaginal disc overgrowth mutants is associated with reduced ecdysteroid titer. J. Insect Physiol. 12, 1051–1059.

Shafiei, M., Moczek, A.P., Nijhout, H.F., 2001. Food availability controls the onset of metamorphosis in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabeidae). Physiol. Entomol. 26, 173–180.

Simpson, P., Schneiderman, H.A., 1975. Isolation of temperature sensitive mutations blocking clone development in *Drosophila melanogaster*, and the effects of a temperature sensitive cell lethal mutation on pattern formation in imaginal discs. Wilhelm Roux's Arch. Dev. Biol. 178, 247–275.

Simpson, P., Berreur, P., Berreur-Bonnefant, J., 1980. The initiation of pupariation in *Drosophila*: dependence on growth of the imaginal disks. J. Embryol. Exp. Morphol. 57, 155–165.

Smith, W.A., Gilbert, L.I., 1989. Early events in peptide-stimulated ecdysteroid secretion by the prothoracic glands of *Manduca sexta*. J. Exp. Biol. 252, 264–270.

Stearns, S.C., 1992. The Evolution of Life Histories. Oxford Univ. Press, Oxford.

Stern, D.L., 2001. Body size evolution: how to evolve a mammoth moth. Curr. Biol. 11, R917–R919.

Stern, D.L., Emlen, D.J., 1999. The developmental basis for allometry in insects. Development 126, 1091–1101.

Truman, J.W., 1972. Physiology of insect rhythms. I. Circadian organisation of the endocrine events underlying the moulting cycle of larval tobacco hornworms. J. Exp. Biol. 57, 805–820.

Truman, J.W., Riddiford, L.M., 1974. Physiology of insect rhythms. III. The temporal organisation of the endocrine events underlying pupation of the tobacco hornworm. J. Exp. Biol. 60, 371–382.

Van der Have, T.M., De Jong, G., 1996. Adult size in ectotherms: temperature effects on growth and differentiation. J. Theor. Biol. 183, 329–340.

Vervoort, M., Crozatier, M., Valle, D., Vincent, A., 1999. The COE transcription factor Collier is a mediator of short-range Hedgehog-induced patterning of the *Drosophila* wing. Curr. Biol. 9, 632–639.

Weinkove, D., Leevers, S.J., 2000. The genetic control of organ growth: insights from *Drosophila*. Genet. Dev. 10, 75–80.

Weinkove, D., Neufeld, T., Twardzik, T., Waterfield, M., Leevers, S., 1999. Regulation of imaginal disk cell size, cell number and organ size by *Drosophila* class I_A phosphoinositide 3-kinase and its adaptor. Curr. Biol. 9, 1019–1029.

Wigglesworth, V.B., 1934. The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and "metamorphosis." Q. J. Microsc. Sci. 77, 191–222.

Wigglesworth, V.B., 1940. The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). J. Exp. Biol. 17, 201–222.

Williams, C.M., 1961. The juvenile hormone. II. Its role in the endocrine control of moulting, pupation, and adult development in the cecropia silkworm. Biol. Bull. 121, 572–585.

Zitnan, D., Sehnal, F., Bryant, P.J., 1993. Neurons producing specific neuropeptides in the central nervous system of normal and pupariation-delayed *Drosophila*. Dev. Biol. 156, 117–135.

Zwaan, B.J., Azevedo, R.B.R., James, A.C., Van't Land, J., Partridge, L., 2000. Cellular basis of wing size variation in *Drosophila melanogaster*: a comparison of latitudinal clines on two continents. Heredity 84, 338–347.