The Systemic Control of Growth

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Growth is a complex process that is intimately linked to the developmental program to form adults with proper size and proportions. Genetics is an important determinant of growth, as exemplified by the role of local diffusible molecules setting up organ proportions. In addition, organisms use adaptive responses allowing modulating the size of individuals according to environmental cues, for example, nutrition. Here, we describe some of the physiological principles participating in the determination of final individual size.

GROWTH SPEED AND DURATION: TWO PARAMETERS THAT REGULATE FINAL BODY SIZE

During the development of multicellular organisms, the precise control of growth is essential for ensuring the emergence of adults with correct body sizes and proper relative organ proportions. These species-specific features condition many traits of adult life, such as fitness and survival. In most vertebrates and some invertebrate species, body size cannot be modified by environmental conditions once the adult stage has been reached, meaning that adult size is fully determined by the growth that takes place during the juvenile stages. Adulthood follows a process of sexual maturation during which organisms acquire reproductive capacities. In humans, sexual maturation, or puberty, is accompanied by an intense increase in the rate of growth (growth spurt). In contrast, in holo-

metabolous insects, such as *Drosophila*, body growth and maturation occur at distinct stages, and the beginning of maturation marks the end of the growth period. In all cases, the timing at which maturation takes place determines the final body size by constraining the length of the growth period. For example, children displaying precocious puberty are typically tall for their age because of an advanced adolescent growth spurt, but generally become shorter adults because they enter maturation and, therefore, adulthood earlier. Conversely, delayed puberty commonly leads to individuals with high stature. Similarly, artificially advancing or delaying the onset of maturation during insect development modifies the individual's final size.

During development, the activity of long-range signaling molecules—mostly hormones—is fundamental to the control of growth at the organismal scale and to the coordination of growth among developing organs. These sys-

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temic factors act as relays to adjust body growth in response to environmental changes. Indeed, although overall body size is largely fixed by internal cues, the growth of developing individuals remains plastic and can adapt to external (environmental) cues, nutrition, in particular. In their target organs, long-range signals act in concert with tissue-autonomous signals to regulate three key processes that determine the final size of tissues: (1) cell growth, which increases cell mass; (2) cell proliferation, which increases cell number; and (3) cell death, which establishes the final cell number.

In conclusion, in addition to intrinsic mechanisms, which fix organ sizes and proportions for a given species, two key parameters contribute to the determination of final body size: the speed of growth (growth rate) and the duration of growth (limited by sexual maturation and the transition to adulthood). Distinct hormonal pathways regulate these two parameters: cell and tissue growth rates are controlled by hormones belonging to the family of insulin-like peptides (ILPs), whereas developmental transitions—in particular, sexual maturation—are under the control of steroid hormones. Among the many open questions that remain relative to the regulation of body size, we will focus our review on the mechanisms that allow the growth program to adapt to environmental conditions, and on those that allow coordination between growth and developmental timing. For this, we will mainly refer to recent work performed using holometabolous insects as a model.

***Drosophila* AS A MODEL FOR STUDYING BODY GROWTH**

Insects have long served as models for the study of growth control. Two distinct approaches have been undertaken by biologists. Physiologists have been interested in understanding how hormones control the size of insects through their effect on the duration of the larval feeding period, and geneticists have explored the role of signaling pathways in controlling cell and tissue growth. Over the past 10 years, *Drosophila* has emerged as a model of choice to merge these two approaches in a unified effort to under-

stand the basic principles of body growth and size determination (Edgar 2006; Mirth and Riddiford 2007). *Drosophila*'s recent popularity is attributed to the successful combination of its relatively simple physiology and powerful genetics, together with a high level of conservation of the main hormones and signaling pathways controlling tissue growth and metabolic homeostasis.

During development, the feeding larva undergoes a remarkable increase in body mass that determines future adult mass and size. Larvae go through three instars before initiating maturation (metamorphosis) and the transition to adulthood. Two types of tissues develop in parallel in the larva. One group of differentiated tissues carries the physiological functions of the feeding larva and grows through successive rounds of genome duplication; this is the main driver behind the increase in body mass observed during larval stages. In parallel, a set of nondifferentiated imaginal tissues undergoes a fixed program of growth and proliferation before ultimately forming adult tissues. At the end of larval development, a major developmental transition takes place, allowing entry into metamorphosis. This is a phase of intensive tissue remodeling and differentiation, leading to the replacement of most larval tissues by adult structures.

As noted above, reaching the appropriate adult size requires two sets of mechanisms that control how fast tissues grow and when they stop growing. Although these regulations are complex and difficult to address experimentally in vertebrates, their study in *Drosophila* has allowed the unraveling of important principles of body growth control.

CONTROLLING THE DEVELOPMENTAL CLOCK: A CENTRAL ROLE FOR THE STEROID HORMONE ECDYSONE

The life cycle of insects is similar to that of other animal species, with four main stages: embryonic development, a juvenile growth phase, sexual maturation, and reproductive adulthood. Because each developmental transition is marked by a visible molt, insects have been

useful models for studying the molecular mechanisms that control their timing. The steroid hormone ecdysone is the master regulator of developmental transitions in insects, and pulses of ecdysone secretion drive the progression through all of the developmental stages. Ecdysone is secreted by the prothoracic gland (PG), an endocrine tissue located within the ring gland and connected to the larval brain. The control of developmental timing by the PG relies on its capacity to integrate various environmental and developmental signals. At least two checkpoint mechanisms control the timing of metamorphosis: one is mediated by nutrient availability and the metabolic status of the larva (the so-called “critical weight”), and one depends on imaginal disc growth and maturation (the growth checkpoint) (see references for detailed discussions).

In *Drosophila*, the peaks of ecdysone that occur at the end of the first and second larval instars trigger molting of the larval cuticle, accommodating the increase in animal size. Subsequently, a series of low-titer hormone pulses during the L3 stage prepare the animal for metamorphosis by inducing key behavioral and developmental changes (Warren et al. 2006), including a cessation of feeding, the onset of wandering behavior, the synthesis of glue protein in the salivary glands, and the initiation of fat body autophagy (Rusten et al. 2004; Scott et al. 2004). At the end of the third instar, a high-titer ecdysone peak triggers pupariation, growth arrest, and the onset of metamorphosis (Riddiford 1993).

Ecdysone Signaling

Like vertebrate steroids, ecdysone binds to members of the nuclear receptor superfamily, which function as ligand-dependent transcription factors (King-Jones and Thummel 2005). Ecdysone acts through a heterodimer of the ecdysone receptor (EcR) and ultraspiracle (USP) nuclear receptors (Thomas et al. 1993; Yao et al. 1993). In the absence of hormone, the same heterodimer acts as a transcriptional repressor to prevent the precocious activation of metamorphic processes (Schubiger and Truman 2000;

Schubiger et al. 2005). When activated, the EcR/USP heterodimer triggers the transcription of primary (early) and secondary (late) response genes in ecdysone-target tissues. The early gene products activate the expression of the late genes but also, simultaneously, induce the expression of early–late genes that will ultimately repress these same late genes. This complex cross talk allows the sequential activation of gene expression and the progression of the signaling cascade. The termination of the ecdysone peaks relies on feedback mechanisms that decrease the circulating levels of the hormone (Rewitz et al. 2010; Guittard et al. 2011). A similar feedback mechanism in peripheral tissues also participates in the generation of the ecdysone pulse that triggers pupariation. Specific combinations of EcR isoforms and cofactors in target tissues mediate stage- and tissue-specific responses to ecdysone (Yamanaka et al. 2013). One example of such a combination is the regulation of *ddOR*, a transcriptional activator that physically interacts with EcR (Francis et al. 2010). *ddOR* is required for maximal EcR transcriptional activity during metamorphosis. Interestingly, *ddOR* is a target of the transcription factor dFOXO, a negatively acting downstream component from IIS (see below), which is itself active during late larval development, when animals stop feeding. The specificity of cell and tissue responses to ecdysone is also illustrated by comparing its effects on endoreplicative (larval) and proliferative (imaginal) cells. Although EcR activation in larval tissues leads to their destruction and remodeling by autophagy and cell death (Baehrecke 2000; Lee and Baehrecke 2001; Rusten et al. 2004; Scott et al. 2004), it contributes to morphogenesis and differentiation of imaginal tissues (D’Avino and Thummel 2000; Schubiger et al. 2005).

Developmental Control of Ecdysone Production

During larval stages, the steroid hormone ecdysone is produced primarily in the PG by a series of enzymatic steps that converts cholesterol into ecdysone. The Halloween genes encode cytochrome P450 enzymes that catalyze the majority

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of the successive reactions of ecdysteroidogenesis (Gilbert 2004; Huang et al. 2008). Once released into the hemolymph, ecdysone is taken up by several peripheral tissues including the gut, fat body, and Malpighian tubules, where it is converted to its active form, 20-hydroxyecdysone (20E), by the P450 monooxygenase enzyme encoded by the *shade* gene (Petryk et al. 2003).

Multiple inputs converge on the PG to regulate ecdysone production, either coming from the central nervous system, from systemic cues relaying both intrinsic and extrinsic information or from autonomous feedback mechanisms (Fig. 1). These signals modulate ecdysone production by controlling cholesterol uptake, by regulating the expression of ecdysone biosynthetic enzymes (transcriptionally or posttranscriptionally), and by modifying the activity of other signaling pathways. Although the classic vision of ecdysone biogenesis has given a predominant role to the neuropeptide prothoracicotropic hormone (PTTH), on which most regulatory clues converge, recent results have highlighted new modes of regulation acting in parallel to PTTH on PG cells, where ecdysone is produced.

PTTH

PTTH is the primary factor that stimulates the production of ecdysone in the PG. Its production by two pairs of neurosecretory cells in the brain (Kawakami et al. 1990; McBrayer et al. 2007) is key for the generation of ecdysone pulses (McBrayer et al. 2007). Genetic ablation of PTTH cells strongly delays larval development. Surprisingly, larvae still eventually enter metamorphosis following such ablation, suggesting that PTTH is not absolutely required for metamorphosis per se, but rather that it controls final body size by regulating the timing of metamorphosis. PTTH signaling promotes ecdysone biosynthesis by promoting the expression of several Halloween genes; the expression of these genes is strongly delayed in ablated animals.

PTTH signals through the receptor tyrosine kinase Torso, which was previously identified as a master controller of terminal patterning in the embryo (Rewitz et al. 2009). Interestingly,

Trunk, the torso ligand for terminal patterning, is structurally related to PTTH, and PTTH expression in the embryo rescues terminal differentiation defects of *trunk* mutants. Torso/PTTH signaling relies on the activation of the canonical mitogen-activated protein kinase (MAPK)-signaling pathway, leading to the phosphorylation of extracellular signal-regulated kinase (ERK). The depletion of *torso* or of *ERK* in PG cells phenocopies the ablation of the PTTH neurons and can be rescued by feeding animals with 20E, confirming that the delay and increased body size are indeed caused by abnormally low ecdysone levels. Consistent with this, expressing an activated form of Ras (Ras^{v12}) in PG cells activates ecdysone synthesis and changes the timing of the larva/pupa transition (Caldwell et al. 2005).

The loss of PTTH delays not only the larva/pupa transition but also, to a lesser extent, the larva/larva molts, suggesting that PTTH plays a conserved role during all of these transitions. In addition, PTTH signaling is required for generating the small peaks of ecdysone that occur at the beginning of the third larval instar. In particular, an elegant study has shown that the nucleocytoplasmic trafficking in PG cells of the nuclear receptor DHR4, a target of the PTTH pathway, is required for these early ecdysone pulses and for the resulting changes in behavior (Ou et al. 2011). When *DHR4* expression is impaired in the PG, larvae stop feeding prematurely and enter metamorphosis following a rapid increase in ecdysone levels; this suggests that the nuclear translocation of DHR4 normally blocks the occurrence of the ecdysone pulses. Further, loss of PTTH causes DHR4 to remain in the nucleus, whereas constitutive activation of PTTH signaling maintains it in the cytoplasm. Thus, PTTH acts early in the third larval instar to promote ecdysone production by preventing DHR4 from entering the nucleus of PG cells.

PTTH expression in *Drosophila* appears to be regulated by the photoperiod (McBrayer et al. 2007). It shows a cyclic pattern with an 8-h periodicity, before increasing to 12 h before pupariation. Multiple lines of evidence suggest that the circadian clock contributes to the periodicity of *ptth* expression. For example, in animals mutant for *pdf* (a gene known to regu-

Figure 1. Environmental and developmental signals controlling steroid hormone production and the developmental timing. Developmental transitions are controlled by peaks of the steroid hormone ecdysone. Ecdysone is produced by the prothoracic gland (PG), an endocrine tissue that integrates various signals to adjust the progression through the life cycle with developmental and environmental cues. In late larval development, a sharp increase in the production of prothoracicotrophic hormone (PTTH) by two pairs of brain neurons induces the production of ecdysone by the PG. Secreted factors produced by growing or regenerating tissues inhibit the larval/pupal transition, allowing coupling tissue size assessment with the developmental program. Information on body size could also come from the levels of circulating oxygen. Finally, insulin and target of rapamycin complex 1 (TORC1) signaling in the PG coordinate steroid hormone production with nutritional conditions. IPCs, insulin-producing cells; Dilp, *Drosophila* insulin-like peptide; InR, insulin receptor; PDF, pigment dispersing factor; DHR-3/4, *Drosophila* hormone receptor 3/4; ERK, extracellular signal-regulated kinase.

late circadian rhythms), PTTH oscillations are altered and *ptth* mRNA levels are up-regulated. Furthermore, PDF-producing neurons impinge directly on PTTH neurons. PTTH levels are also modulated by two developmental checkpoints that monitor animal size, critical

weight, and the development of future adult structures (imaginal discs). The question of the developmental checkpoints controlling insect metamorphosis is addressed later in this section. It thus appears that PTTH is an important relay in the coordination of developmental

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timing with environmental and developmental cues, although very little is known about the mechanisms that regulate its levels.

Transforming Growth Factor (TGF)- β /Activin

Consistent with its playing a role in promoting ecdysteroidogenesis, the activation of TGF- β /activin signaling in the PG leads to precocious pupariation and pupal lethality (Gibbens et al. 2011), whereas inactivating this signaling pathway produces giant larvae that fail to enter metamorphosis. TGF- β /activin has a dual function in controlling ecdysone production, as they modulate both PTTH and insulin signaling by promoting the expression of *Torso* and *InR*. Consistent with this finding, the delayed metamorphosis obtained after knocking down *Smad2* in the PG can be rescued by expression of activated *Ras* (a downstream effector of PTTH/*Torso*), *InR*, or *Akt*. However, only *Ras*^{V12} expression can rescue the mRNA levels of ecdysone biosynthetic enzymes, suggesting that the two pathways act at the transcriptional and posttranscriptional stages of biosynthetic enzyme expression, respectively. Therefore, TGF- β /activin signaling acts as a competence signal that confers high responsiveness to PTTH and insulin signals to PG cells. This regulation appears to be conserved in other model systems, because in *Caenorhabditis elegans*, TGF- β /activin signaling controls the production of dafachronic acid, a functional counterpart of ecdysone (Wollam and Antebi 2011). The developmental or environmental factors that modulate TGF- β /activin in this control are currently unknown.

Nitric Oxide and E75

Nitric oxide (NO) gas acts as a short-range signaling molecule in numerous biological processes. The nuclear receptor E75, an early puff gene in the ecdysone-signaling hierarchy, was found to contain a heme and to function as an NO sensor (Reinking et al. 2005). In target tissues, NO acts by inhibiting the normally repressive activity of E75A (Johnston et al. 2011). NO synthase activity is required in the PG to induce pupariation (Cáceres et al. 2011). Molecularly,

NO reverses the ability of E75 to interfere with its partner DHR3, allowing DHR3 to activate β FTZ-F1 expression, which, in turn, promotes ecdysone synthesis. Given that E75 is a target of ecdysone signaling, and that *EcR-A* is expressed in PG cells (Talbot et al. 1993), it is likely that this NO-induced rise in ecdysone levels, in turn, negatively feeds back on the further production of ecdysone by activating E75 and, thus, down-regulating β FTZ-F1. In their study, Cáceres and colleagues point out that several cofactors required for NO synthase activity are produced on PTTH/*Torso*/*Ras* signaling. Therefore, PTTH could act through nitric oxide synthase (NOS) activation to induce the production of ecdysone. Alternatively, because NOS is activated under low oxygen (Wingrove and O'Farrell 1999), the NO pathway could serve to couple oxygen levels with ecdysone production. This would be in line with two recent reports linking oxygen levels and pupariation timing in both *Manduca sexta* and *Drosophila* models (Callier and Nijhout 2011; Callier et al. 2013). In *Manduca*, the size and internal volume of the tracheal network are fixed at the last larval molt. The drastic increase in larval volume and metabolic activity that takes place after this molt suggests that oxygen becomes limiting, and this could be a trigger for the pupal transition (Callier and Nijhout 2011). Consistent with this, *Drosophila* larvae reared in hypoxic conditions produce a peak of ecdysone at an earlier time and at a reduced mass than their normoxic controls, even though the converse condition (hyperoxic) does not lead to conclusive results (Callier et al. 2013). This, nevertheless, suggests that variations in oxygen levels could participate in the control of ecdysone production. It remains to be determined, however, whether a physiological drop in oxygen at the end of larval development constitutes a trigger for ecdysone production, and whether a NOS-dependent mechanism is involved.

SUMOylation and Cholesterol Uptake

The only *Drosophila* member of the SUMO family, Smt3, is required for ecdysone synthesis and, in particular, for cholesterol uptake. Down-reg-

ulating *Smt3* in PG cells lowers the lipid content of animals, prevents entry into metamorphosis, and produces giant larvae (Talamillo et al. 2008). *Smt3* acts by promoting the expression of the nuclear receptor *Ftz-f1*, which, in turn, activates the expression of scavenger receptors (Talamillo et al. 2013). These receptors, whose functions may be conserved in the mammalian adrenal gland, mediate the regulation of lipid uptake by *Smt3* in PG cells. Interestingly, *Smt3* and scavenger receptors carry out similar functions in the follicle cells of female ovaries, suggesting that *Smt3*, *Ftz-f1*, and scavenger receptors may constitute a common module in the steroidogenic pathway.

Mutations in either of the two human Niemann–Pick type C (NPC) genes, *NPC1* and *NPC2*, cause a neurodegenerative disease characterized by an abnormal accumulation of cholesterol in cells. In *Drosophila*, *npc1a* and *npc2a;npc2b* mutants die during larval/pupal development because of cholesterol shortage and a lack of ecdysone (Huang et al. 2005, 2007). The expression of *npc1a* is regulated by the Broad-Complex (BR-C). Indeed, the BR-Z4 isoform directly binds to a conserved *cis*-regulatory module within the *npc1a* locus, suggesting that BR-C transcriptionally controls cholesterol uptake by directly activating *npc1a* expression (Xiang et al. 2010). Interestingly, BR-C is an early target of ecdysone, and BR-Z4 is strongly up-regulated in PG cells from the wandering stage until pupariation (Zhou et al. 2004). This could constitute a positive feedback loop in the PG, in which ecdysone-mediated activation of BR-Z4 and *npc1a* would stimulate further ecdysone biosynthesis. Overall, these forms of regulation appear to participate in a permissive control mechanism that allows the PG to benefit from the right metabolic environment before intense ecdysteroidogenesis takes place.

REGULATION OF BODY GROWTH: A CONSERVED ROLE FOR THE INSULIN/INSULIN-LIKE GROWTH FACTOR (IGF)-SIGNALING PATHWAY (IIS)

The insulin/IGF-signaling pathway (IIS) is highly conserved and regulates a number of

physiological functions, such as metabolic homeostasis, growth, and longevity. In vertebrates, the metabolic functions are mediated by insulin, whereas growth is promoted by IGFs (Nakae et al. 2001). This functional division is partly explained by the differential binding of the ligands to distinct receptors (insulin receptor [IR], IGF-I receptor [IGF-IR], and IGF-II receptor), although it is also true that IR and IGF-IR receptor activation induce common signaling pathways in many cell types. Insects possess a conserved insulin-like system, which could represent a possible ancestor of the vertebrate IIS (Wu and Brown 2006). *Drosophila* insulin-like peptides (Dilps) carry out both the metabolic functions of mammalian insulins and the growth-promoting functions of IGFs (Garofalo 2002), and activate a conserved kinase cascade.

Insulin Signaling in *Drosophila*

Activation of the insulin receptor (InR) by the Dilp ligands leads to the recruitment of Chico, the sole insulin receptor substrate (IRS)-adaptor protein in *Drosophila*, as well as to the activation of phosphoinositide-3-kinase (PI3K). Activated PI3K phosphorylates PIP2 (phosphatidylinositol-4,5-diphosphate) at the plasma membrane and produces PIP3 (phosphatidylinositol-triphosphate). This activity is reversed by phosphatase and tensin homolog (PTEN), a negative regulator of the pathway (Goberdhan et al. 1999). PIP3 induces the recruitment of Akt (a proto-oncogene also known as protein kinase B [PKB]) to the plasma membrane, leading to its subsequent phosphorylation. Chimeric receptors encoding the cytoplasmic domain of the *Drosophila* InR together with the extracellular domain of human IR bind vertebrate insulin and show tyrosine kinase activity when expressed in mammalian cells (Yamaguchi et al. 1995); this indicates that the vertebrate and invertebrate receptors share common signaling properties.

Mutations that block the activity of the pathway cause a reduction in tissue growth and lead to either lethality or severe body size reduction (Böhni et al. 1999). In the opposite direction, mutations in *PTEN* or overexpression

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of positively acting components of the pathway, such as InR, PI3K, or Akt, lead to dramatic overgrowth at both the tissue and organismal levels (Leevers et al. 1996). These results could suggest that IIS participates in the control of final body size. However, a large body of evidence indicates that IIS plays a permissive role in final size determination, coordinating the intrinsic growth program with nutritional conditions.

The Dilps

Eight ILPs have been identified to date in *Drosophila*. With the exception of the recently identified Dilp8 (Colombani et al. 2012; Garelli et al. 2012), all of the other Dilps activate the canonical fly IIS pathway and promote cell growth and anabolism. Overexpression of any of the Dilps 1–7 leads to increased larval size in an InR-dependent manner (Ikeya et al. 2002). Conversely, homozygous mutant animals presenting a deletion of the main *dilp* gene cluster (*dilp1–5*) show growth and metabolic defects (Zhang et al. 2009; Grönke et al. 2010) pointing to a functional involvement of Dilps in the regulation of growth and metabolic homeostasis.

Temporal and Spatial Expression of Dilps

Quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis on whole animals has revealed that members of the *dilp* gene family display different temporal expression patterns. Although *dilp1*, 3 and 6 are expressed at high levels during early pupal stages, *dilp2* and 5 display high levels during the larval growth period (Okamoto et al. 2009; Slaidina et al. 2009). The *dilps* are also expressed in various larval tissues. For example, *dilp1*, 2, 3, and 5 transcripts are found in two symmetric clusters of seven neurosecretory cells in the larval brain (hereafter referred to as the insulin-producing cells [IPCs]), which are functionally homologous to the vertebrate β cells (Brogiolo et al. 2001; Ikeya et al. 2002; Rulifson et al. 2002). Lineage analysis of these cells in the developing central nervous system has shown that precursor neuroblasts of both the insulin-producing cells and the glucagon (AKH in flies)-producing cells

express a transcription-factor cascade that is orthologous to that expressed in the adenohypophyseal placode of vertebrates. Therefore, a rudimentary endocrine axis might have evolved from a common ancestor of flies and vertebrates to control insulin and glucagon-like functions (Wang et al. 2007).

Although located in the same gene cluster, *dilp2*, 3, and 5 present independent regulated expression in the IPCs (Ikeya et al. 2002). Whereas *dilp3* and *dilp5* transcripts decrease on starvation, *dilp2* transcription is insensitive to nutrient levels (Ikeya et al. 2002; Colombani et al. 2003). *dilp5* is also expressed in the midgut, *dilp2* in the imaginal discs and the salivary glands, *dilp6* in the fat body, and *dilp7* in the ventral nerve cord (Brogiolo et al. 2001). Thus, although the different *dilp* genes can replace each other functionally, their specific expression patterns suggest that they carry out distinct physiological functions in vivo.

Local Signaling in the Central Nervous System

In addition to the systemic role that ILPs play, recent studies have highlighted other local, short-range functions for several of them (Fig. 2) (Cheetham and Brand 2013). At the end of embryonic development, neural stem cells enter a period of quiescence that precedes their active proliferation during the larval stages. Dilp2 and Dilp6 are found within a population of glial cells and are required to stimulate neural stem cell proliferation after the first larval feeding (Chell and Brand 2010). The activation of *dilp* gene expression in glial cells indirectly depends on dietary amino acids. This regulated expression is mediated by a target of rapamycin (TOR)-dependent-sensing mechanism that takes place in fat body cells and is relayed to the brain glia by an unknown humoral signal (Sousa-Nunes et al. 2011). A recent study has revealed that gap junctions between the glial cells that form the blood–brain barrier coordinate nutrient-dependent calcium oscillations, which are required for the local production and secretion of ILPs (Spéder and Brand 2014). Interestingly, this local release of Dilps by glial cells is incapable of regulating systemic larval body growth, and IPC-derived

Figure 2. Local and systemic modulations of insulin signaling. The tight control of insulin signaling relies on complex mechanisms of interorgan communication. The insulin-producing cells (IPCs) produce *Drosophila* insulin-like peptide (Dilp)2, 3, and 5, which represent the main systemic source of circulating insulin during the growth phases (larval stages). Several inputs affect *dilp* gene expression in the IPCs, whereas nutritional information is sensed by the fat body and relayed by humoral factors controlling Dilp secretion. Dilp6 is induced upon nutrient shortage by fat cells to sustain growth of high priority organs. Several Dilp-binding factors modulate the activity of circulating Dilps (SDR, dALS, and Imp-L2). An interplay between glial and neural cells promotes growth and proliferation of larval neuroblasts and allows them to evade nutrient control during late larval development. NBs, neuroblasts; PG, prothoracic gland; AAs, amino acids; Hh, Hedgehog; FOXO, Forkhead box-O transcription factor; PI3K, phosphoinositide-3-kinase; Upd2, unpaired 2; Imp-L2, imaginal morphogenesis protein-Late 2; dALS, *Drosophila* acid labile subunit; Alk, anaplastic lymphoma kinase; SDR, secreted decoy of InR; Jeb, Jelly belly.

Dilps in the hemolymph do not induce the re-activation of neuroblasts. Therefore, local and systemic Dilps are used to trigger separate outputs in response to nutritional status.

Functional Characterization of Dilps

The *dilp6* gene is expressed at high levels in the fat body during the late larval stage (wandering

period) and the pupal period (Okamoto et al. 2009; Slaidina et al. 2009). Functional analysis reveals that *dilp6* is induced under conditions of nutrient deprivation, caused either by developmentally induced cessation of feeding or by starvation, to promote tissue growth. Accordingly, *dilp6* mutants hatch as smaller adults and present a greater size reduction after starvation than do wild-type animals. Adipose expression

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of *dilp6* under starvation relies on *Drosophila* forkhead box-O transcription factor (dFOXO), which accumulates in an active, nuclear form upon nutrient deprivation. Induction of *dilp6* at the larval/pupal transition is independent of dFOXO and is controlled rather by ecdysone peaking before metamorphosis. On the basis of these studies and according to structural features that distinguish it from other fly insulins, Dilp6 was proposed to be an analog of vertebrate IGF-I. Indeed, IGF-I is present at high levels during puberty and is regulated by nutrition and sex steroids.

dilp2, 3, and 5 are expressed at high levels in the brain IPCs during larval stages. Ablation experiments indicate that larval IPCs are the main supply for systemic insulins and are essential for maintaining growth and carbohydrate homeostasis (Rulifson et al. 2002). Interestingly, the reduction in larval growth and adult size observed upon IPC ablation can be rescued by ubiquitous expression of *dilp2* alone, suggesting that brain-derived Dilps may be partially redundant (Rulifson et al. 2002). In line with this result, no defects in final body size are observed upon Dilp2 knockdown in the IPCs (Broughton et al. 2008). A deeper analysis of *dilp* gene function, made possible by the generation of knockout mutations (Grönke et al. 2010), has revealed complex compensatory regulations acting at the transcriptional level: *dilp5* is up-regulated in *dilp2* and *dilp2–3* mutants, and *dilp3* is up-regulated in *dilp2* and *dilp5* mutants. Individual knockouts of brain *dilps* do not affect general insulin signaling, nor do they induce organismal growth defects. However, overall insulin signaling and body size are severely reduced in *dilp2*, 3, and 5 mutants, consistent with the data from IPC ablation. Strikingly, *dilp6* expression is strongly induced in *dilp2–3*, 5 mutants, suggesting that this condition could mimic a starvation-like state.

Control of Dilp Production in the IPCs

In mammals, insulin levels are modulated by hormones and neurotransmitters acting on its release from β cells (Drucker et al. 1987; Aspinwall et al. 1999; Adeghate et al. 2001; Dong et al.

2006; Sonoda et al. 2008). Similarly, in flies the production and release of Dilps by the brain IPCs seems to be controlled by both nutrition-dependent and independent cues (Fig. 2). We will detail the nutrition-dependent control of Dilp function below. In addition, the *Drosophila* IPCs receive many inputs from neurons producing neuropeptides or neuromediators, the function of some of which is possibly linked to the processing of nutritional information.

Drosophila short neuropeptide F (sNPF), a homolog of mammalian neuropeptide Y (NPY), controls food intake. Recent work has indicated that this peptide promotes body growth by regulating *dilp* expression (Lee et al. 2008). sNPF is expressed in adjacent neurons and signals to the IPCs through the sNPF receptor 1, inducing expression of *dilp* genes. This suggests a possible feedback control mechanism linking feeding behavior to insulin signaling, although the physiological context of such feedback has not yet been clarified. Such a mechanism would also parallel the case of rat insulinoma cells, in which NPY signaling promotes the expression of insulin in an ERK-dependent manner, suggesting a conserved regulatory mechanism.

Another neuronal mechanism regulating IPC activity involves serotonergic circuits. NS3, a *Drosophila* nucleostemin family GTPase (Kaplan et al. 2008), is required in serotonergic neurons—some of which are closely apposed through their axonal projections to the IPCs—to regulate final body size. Mutant *ns3* animals present a reduced size and an accumulation of Dilp2 in the IPCs, suggestive of a defect in Dilp release. Expression of *ns3* in these 5-HT neurons is sufficient to rescue the growth defects and the developmental delay observed in *ns3* mutant animals. However, it is not clear yet whether the lack of NS3 function leads to an increase or a decrease in serotonin signaling, what is the precise identity of the 5-HT neurons that control IPC function, and under what physiological conditions serotonin is used to control Dilp release. In line with these results, the 5-HT1A receptor is expressed in the IPCs, in which its specific knockout leads to a variety of phenotypes reminiscent of both increase

and decrease in insulin signaling (Luo et al. 2012, 2014).

The knockdown of specific receptor-encoding genes in the IPCs has revealed that other neuromodulators like GABA, tachykinin, and octopamine can also modulate IPC function (Crocker et al. 2010; Enell et al. 2010; Birse et al. 2011). After this initial phase defining the possible signaling pathways at play, the next challenge will obviously be to put these different mechanisms into their physiological context.

Drosophila miR-14 is expressed in the IPCs of adult flies, in which it promotes the expression of *dilp2*, 3, and 5 and controls metabolism (Varghese et al. 2010). The combination of expression profiling and target prediction analysis has led to the identification of *sugarbabe* as the functional target of *miR-14*. Interestingly, *sugarbabe* is down-regulated in starved animals independently of *miR-14*, meaning that its expression in the IPCs is regulated by two independent means. Overexpressing *sugarbabe* enhances the decrease in *dilps* transcript levels observed upon starvation, indicating that its down-regulation in this condition acts to limit the negative effects of nutrient deprivation on *dilps* expression.

Finally, insulin signaling in the IPCs positively regulates the secretion of systemic Dilps, linking the levels of activation of the pathway to the production of the ligand (Cheng et al. 2011). This data contrasts with the observation that, in adult flies, Dilp6 produced by fat cells increases longevity and longevity-associated metabolic phenotypes by repressing *dilp2* and *dilp5* expression in the brain IPCs (Bai et al. 2012). This suggests that in this latter case, Dilp6 might not act directly on the brain IPCs.

Nutritional Control of Body Size

Nutrition is a key environmental parameter in the determination of body size. In multicellular organisms, nutritional information is conveyed at both the cellular and the systemic levels to achieve balanced growth. The coordination of growth among different body parts according to environmental conditions relies on cross talk among different organs or tissues through the production of long-range signaling molecules

(Mirth and Shingleton 2012; Andersen et al. 2013). Among these organs, the fat body plays a key role in orchestrating organism-wide responses to nutritional status (Fig. 2). In addition to its function in accumulating energy resources that will be used during metamorphosis (by storing sugars in the form of glycogen and fat in the form of triacylglycerol [TAG]), the fat body integrates and emits signals involved in the regulation of growth and metabolism. The insulin/IGF and TOR pathways are the main players involved in sensing and relaying nutritional information to coordinate growth with nutrient availability. Information about nutritional status is sensed directly at the level of individual cells via the TOR pathway, whereas the systemic response is performed by the hormonal action of insulin.

The TOR-Signaling Pathway Acts as a Conserved Cell-Based Nutrient Sensor

The role of the protein kinase TOR in mediating cellular nutrient sensing is conserved from yeasts to mammals (De Virgilio and Loewith 2006). Both cell size and cell proliferation are regulated by TOR signaling (Zhang et al. 2000). TOR activity is controlled by the small GTPase Rheb and *Rheb* mutant flies display severe undergrowth as a consequence of reduced cell size and cell number (Saucedo et al. 2003; Stocker et al. 2003). The tumor suppressors tuberous sclerosis complex genes 1 (TSC-1) and 2 (TSC-2) act together in a complex to inhibit Rheb activity (Inoki et al. 2003a; Tee et al. 2003; Zhang et al. 2003). In *Drosophila*, clones of cells mutant for TSC-1/2 display strong overgrowth (Potter et al. 2001; Tapon et al. 2001), consistent with increased Rheb and TOR activities. The TOR kinase is found in two complexes, TORC1 and TORC2 (Loewith et al. 2002). TORC1 is the primary mediator of energy and amino-acid sensing and couples growth cues to cellular metabolism (Wullschleger et al. 2006). TORC1 promotes tissue growth by adjusting protein biosynthesis, through the regulation of the two translational regulators S6K (ribosomal protein kinase 6) and 4E-BP (initiation factor 4E binding protein). TOR-mediated inhibition

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of 4E-BP and activation of S6K enable the assembly of the translation preinitiation complex and stimulate peptide chain elongation, thereby enhancing protein synthesis (Wang et al. 2001; Holz et al. 2005). Inhibiting TORC1 function in *Drosophila* reduces body growth and causes larval lethality (Zhang et al. 2000). However, flies lacking S6K or 4E-BP activities alone display significantly milder phenotypes (Miron et al. 2001; Teleman et al. 2005), indicating that TORC1 has additional functions in vivo. Indeed, TORC1 also regulates transcription, mainly through the posttranscriptional activation of the dMyc transcription factor (Teleman et al. 2008). Consistent with a role for dMyc in promoting ribosome biogenesis (Grewal et al. 2005), the majority of TORC1-regulated genes are involved in the production of ribosomes and modulate cell growth (Guerin et al. 2006). This dual activity of TORC1 allows the rate of translation to be coupled with the amount of ribosomes.

TORC1 is modulated by cellular amino-acid levels, thereby linking nutritional status with the rate of protein synthesis (Avruch et al. 2009). The amino-acid-dependent activation of TORC1 takes place downstream of the TSC-1/2 complex and is mediated by Rag GTPase proteins. The Rag complex is required for the translocation of TORC1 to the lysosomal surface, which is a crucial step for TOR activation (Kim et al. 2008; Sancak et al. 2008, 2010). Under amino-acid deprivation, activation of mTORC1 is abolished by a negative regulator of the pathway, SH3BP4, which inhibits the formation of active Rag GTPase complexes (Kim et al. 2012). The adaptor protein p62 is also required to mediate the interaction between mTORC1 and the Rag complex in an amino-acid-dependent manner (Duran et al. 2011). Another study in *Drosophila* has proposed that MAP4K3 also participates in the regulation of Rag complex activity by amino acids (Bryk et al. 2010).

TORC1-mediated cell growth is also under the control of an energy-sensing mechanism that depends on AMPK (AMP-activated protein kinase). AMPK activity monitors the immediately available energy by measuring cellular ATP

levels (Hardie 2007) as well as reserve energy stores in the form of glycogen (McBride et al. 2009). AMPK signaling regulates growth by phosphorylating and activating TSC-2, which results in TORC1 inhibition (Inoki et al. 2003b).

Links between Insulin and TOR Pathways

The cross talk between IIS and TORC1 constitutes a connection between the systemic and the cell-intrinsic nutrient-sensing modules. However, this connection has been a matter of debate. In human and *Drosophila* cultured cells, treatment with insulin leads to rapid TORC1 activation as a result of the direct phosphorylation of TSC-1/2 and inhibition by Akt (Inoki et al. 2002; Potter et al. 2002). At the same time, in vivo studies in *Drosophila* have challenged the relevance of this link under physiological conditions. Indeed, the removal of all Akt phosphorylation sites from TSC-1 and TSC-2 does not lead to an increase in TORC1 activity, as judged by an absence of a growth increase in vivo (Dong and Pan 2004; Schleich and Teleman 2009); this result indicates that, under physiological conditions, Akt function does not contribute to TORC1 activation. More recently, the protein PRAS40 (proline-rich Akt substrate 40) was proposed to link IIS to TORC1 in cultured cells (Sancak et al. 2007; Vander Haar et al. 2007). PRAS40 binds to TORC1 and prevents it from interacting with its substrates. Phosphorylation by Akt on a single site relieves this inhibition and is necessary for the insulin-induced activation of TORC1. The identification of the *Drosophila* homolog of PRAS40 has allowed the generation of mutant animals and the study of its involvement in the regulation of TOR signaling in vivo (Pallares-Cartes et al. 2012). Consistent with a role as an inhibitor of TORC1, overexpression of *PRAS40* reduces tissue growth in an autonomous manner by reducing cell size. However, *PRAS40* null mutant flies are viable and present no obvious alterations of cell or body growth, suggesting that PRAS40 does not regulate TORC1 activity during the growth period in *Drosophila*. Further analysis of adult *PRAS40*[−] mutant females revealed that PRAS40 represses TORC1 in the ovaries but not in the adult soma. In line with

this, the ubiquitous reduction of insulin signaling decreases TORC1 activity in a PRAS40-dependent manner only in the ovaries, and removing PRAS40 in *chico* mutants rescues female fertility but not small body size. In conclusion, this in vivo study in flies shows that IIS and TORC1 are linked through PRAS40 specifically in nonsomatic issues.

Although the direct interplay between TOR and the insulin pathway has been challenged in the context of autonomous cell growth control, it is clear that functional interactions between these pathways play key roles in the regulation of systemic growth.

The Fat Body as the Main Nutrient-Sensing Organ

In both flies and mammals, nutrient deprivation leads to growth retardation as a consequence of reduced IIS signaling. It has been proposed that specific organs, called “sentinel tissues” (Arsham and Neufeld 2006), could act as specific nutrient sensors that would systemically modulate the levels of insulin/IGF signaling and thereby adjust body growth in response to nutrient availability. A genetic screen in *Drosophila* for growth-related genes identified *slimfast* (*slif*), a genetic locus encoding a conserved amino-acid transporter that mediates arginine and lysine uptake in various tissues (Colombani et al. 2003). Chronic depletion of Slif specifically in the fat body mimics the effects of amino-acid starvation, provoking general growth defects and lethality. A mild down-regulation of *slif* function allows the eclosion of adults with reduced size and body weight but with normal body proportions, indicating that the fat body acts as a nutrient-sensing tissue that coordinates the harmonious growth of all body parts. The sensor mechanism in fat cells relies on TORC1, because depleting fat cells of amino acids leads to a reduction in adipose TORC1 activity, and reducing TORC1 function specifically in this tissue mimics the systemic growth defects observed on *slif* knockdown. This study also suggested the existence of humoral signals produced by fat cells that systemically control organ growth (see below). A large part of the

response to TOR signaling in the fat body is mediated by dMyc (Teleman et al. 2008), a transcription factor that promotes protein synthesis via the regulation of ribosome biogenesis (Grewal et al. 2005) and which was found to be a common target of TOR and insulin signaling (Teleman et al. 2008). The control of systemic growth by adipose TOR signaling also involves the inhibition of the repressor Maf1, leading to the activation of RNA polymerase III and tRNA synthesis (Marshall et al. 2012; Rideout et al. 2012), as well as the expression of *dref*, a gene encoding a transcription factor that controls the expression of ribosome biogenesis genes (Killip and Grewal 2012).

Remote Control of Dilps Secretion

The first humoral relay connecting the fat body to peripheral tissues involves the Dilps themselves (Fig. 2). During larval development, diet modulates the circulating levels of brain Dilps (Géminard et al. 2009). Upon full starvation, Dilp2 and Dilp5 are retained and accumulate in the IPCs; subsequently, refeeding larvae with amino acids only, but not sugar or lipids, releases the secretion block. Similarly, silencing *slif* or inhibiting TOR signaling specifically in the fat cells induces Dilp retention. In the other direction, activating TOR signaling in the fat body of underfed animals is sufficient to stimulate Dilp2 secretion. Therefore, TOR signaling in the fat body integrates nutritional inputs and controls the release of Dilps from the IPCs. Ex vivo coculture experiments further showed that the fat body secretes one or more humoral factors that remotely control insulin secretion from the brain IPCs depending on the nutritional state (Géminard et al. 2009).

The cytokine unpaired 2 (Upd2) has recently been identified as a fat body-derived factor that promotes insulin secretion from the IPCs in both larvae and adults (Rajan and Perrimon 2012). In fed animals, Upd2 produced by fat cells navigates through the hemolymph and activates JAK/STAT signaling in a few GABAergic neurons located in the brain in close proximity to the IPCs. JAK/STAT activation in these neurons relieves the GABA-dependent tonic inhibi-

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tion of the IPCs and, as a consequence, stimulates Dilp secretion. In adult flies experiencing prolonged starvation, adipose *upd2* expression is strongly reduced, leading to Dilp retention and decreased levels of stored TAG (lipid storage). In contrast, acute starvation does not inhibit *upd2* expression, indicating that the Upd/JAK/STAT pathway participates in a metabolic adaptation to a prolonged starved state. Upd2 presents structural similarities to leptin, a mammalian cytokine produced by adipose cells under conditions of nutritional surplus. Interestingly, Rajan and Perrimon report that expressing human leptin in *Drosophila* fat cells rescues the lipid metabolism defects observed in *upd2* mutant flies. This provides evidence for an evolutionarily conserved role for adipose cytokines in orchestrating metabolic responses to nutrient availability. Fat Upd2 is produced in response to dietary lipids and sugar, but does not respond to amino acids (Rajan and Perrimon 2012). Thus, additional humoral signals produced by fat cells presumably act to regulate Dilp secretion in response to amino acids and TOR signaling. Taken together, these studies establish a model whereby the global nutritional state is relayed by multiple factors originating in the fat body to modulate metabolic homeostasis in part by controlling Dilp production and release.

Larval versus Adult IPCs

Even though some of the studies described here indiscriminately used adults or larvae for their analyses, functional differences exist between the insulin-producing cells at these two stages, pointing to fundamental differences in their respective physiologies. As an example, the larval IPCs do not express two major components of the glucose response, namely, the Kir6 and Sur1 subunits of the K_{ATP} channels (Kim and Rulifson 2004); not surprisingly, these cells do not directly respond to carbohydrates in ex vivo-cultured brains (Géminard et al. 2009). This contrasts with adult IPCs, in which it has been reported that *Sur* is expressed (Fridell et al. 2009), that glucose is responsible for direct excitability (Krénisz et al. 2010), and that Glut1

function is required for Dilp secretion (Park et al. 2014). Therefore, in contrast to larval IPCs, adult IPCs seem to share some fundamental glucose response properties with pancreatic β cells. Although a link between adult IPC excitability and Dilp secretion has not yet been established, this finding suggests that changes in IPC function precede major physiological differences between the larval and adult stages. Specifically, during the larval stage, when organismal growth takes place, larval IPCs mainly provide Dilps to couple tissue growth with nutrient availability. In this context, carbohydrate and lipid homeostasis are maintained by a glucagon-like hormone called AKH that is produced in the corpus cardiacum, where cells express bona fide *Sur* and *Kir* and sense glucose (Kim and Rulifson 2004). In contrast, adults do not grow, and therefore no longer use Dilps to promote tissue growth. Accordingly, adult IPCs undergo a functional switch that allows metabolic homeostasis to be controlled in part through the expression of a set of specialized enzymes that directly sense nutrients.

In addition to intracellular sensors directly linking insulin production to the availability of nutrients, insulin secretion by mammalian β cells is regulated by enteroendocrine hormones, called incretins, and by paracrine signals like somatostatin from adjacent islet δ cells. In adult flies, the corpora cardiaca is comprised of enteroendocrine cells that secrete AKH in response to fasting, and that directly contact IPCs. In addition, studies by the Kim group now show that the adult corpora cardiaca also produces peptides that regulate insulin production and secretion by IPCs during fasting and refeeding (Alfa et al. 2015). Thus, flies may use hormonal signaling with features resembling the enteroinsular axis in mammals to control insulin output in dynamic nutrient states. Whether this freshly added mechanism also participates in systemic growth control through Dilp secretion during larval development is currently unknown.

Modulating the Activity of Circulating Dilps

In vertebrates, IGF-1 accumulates in body fluids and associates with molecules referred to as

IGF-binding partners (IGFBPs). These factors play important roles in the control of the biological activity of IGF-1 (Duan and Xu 2005). *Drosophila* Imp-L2 shows sequence homology with mammalian IGFBP-7 and was the first insulin-binding protein characterized in invertebrates (Honegger et al. 2008). Imp-L2 is a secreted protein that inhibits growth nonautonomously. It acts by binding directly to Dilp2 and inhibiting its activity. Accordingly, ubiquitous overexpression of *Imp-L2* reduces adult size, whereas *Imp-L2* mutant animals are bigger than normal. Under starvation conditions, *Imp-L2* expression is induced in the fat body and is required to adjust systemic levels of insulin signaling. *Imp-L2* expression is down-regulated in *dilp2-3, 5* mutants (Grönke et al. 2010), suggesting the existence of a feedback mechanism between the Dilps and their repressor. Dilp2 and Imp-L2 are found in a ternary complex with the fly ortholog of acid-labile subunit (ALS), a scaffold molecule involved in the stabilization of the IGF/IGF-BP complexes in mammals (Domené et al. 2009). As in mammals, the recruitment of dALS to the complex in *Drosophila* requires the prior formation of a dimeric Dilp/Imp-L2 complex, indicating that Imp-L2 serves as a molecular bridge between dALS and Dilp2. dALS is produced in the fat body and is secreted into the hemolymph, where it antagonizes Dilp function for the control of growth and metabolism (Arquier et al. 2008). Surprisingly, when nutrients are limiting, increased *dALS* expression rescues the larval growth defect, whereas *dALS* silencing enhances it (Arquier et al. 2008). Therefore, although dALS antagonizes Dilps function in well-fed animals, it is limiting for proper insulin signaling under conditions of nutritional stress. Such a dual effect can be interpreted in light of the complex functions that ALS has in mammals, as it both stabilizes circulating IGF-I complexes and inhibits IGF-I function by preventing binding to its receptor.

An intriguing extension of the role of Imp-L2 in regulating IIS recently came up from the independent work of two groups demonstrating that this IGF-BP is produced at high level by fly tumors to induce wasting of nontumorous tis-

sues. Indeed, although organ growth and homeostasis are influenced by systemic signals under normal physiological conditions, abnormal tissue growth can alter these signals, leading to pathological conditions and eventual death. Bilder and colleagues show that malignant fly tumors transplanted into adult *Drosophila* induce a dramatic loss of adipose and gonadal mass, along with compromised muscle function, before the death of the host. The investigators identify Imp-L2 as a tumor-produced secreted factor that drives wasting by interfering with insulin signaling (Figueroa-Claevega and Bilder 2015). Perrimon and colleagues also report that localized overproliferation induced by activation of Yorkie, the Yap1 oncogene ortholog, in adult intestinal stem cells, causes wasting of ovary, fat body, and muscle. This wasting phenotype is associated with reduction of systemic insulin/IGF signaling via secreted Imp-L2, which is specifically elevated in the overproliferating gut. Expression of the glycolytic enzymes and the insulin/IGF pathway components are up-regulated in the overproliferating gut, suggesting a mechanism for this tissue to evade the action of Imp-L2 (Kwon and Perrimon 2015). Interestingly, these wasting phenotypes have resemblances to human cancer cachexia and may provide a simple invertebrate model for aspects of this devastating and difficult-to-treat condition.

An additional, new type of Dilp-interacting molecule has recently been identified that shows a different strategy for controlling Dilp function (Okamoto et al. 2013). The secreted decoy of insulin receptor (secreted decoy of InR [SDR]) is structurally similar to the extracellular domain of the *Drosophila* insulin receptor (InR), and binds to circulating Dilps in the hemolymph. Experimental data reveal that SDR serves as a sink, preventing Dilps from interacting with their cognate receptor and inhibiting general insulin signaling. Interestingly, SDR and Imp-L2 compete for binding to Dilp2, suggesting that they form independent complexes with Dilp2. SDR is produced by glial cells and the knockdown of SDR in the glia is sufficient to increase animal size, whereas constitutive overexpression reduces it. Intriguingly, SDR expres-

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sion is independent of the nutritional status of the larvae, raising the question of what is the physiological context of its action.

Other Nutrient-Sensing Organs

The gut is directly exposed to the ingested diet and is, therefore, expected to play important functions in sensing and transducing nutritional information (Fig. 2). Indeed, pioneering work on the “incretin effect” in mammals has revealed that glucose is more potent at triggering insulin release when ingested than when injected into the blood (McIntyre et al. 1964). The last decade has seen the identification of a number of epithelial receptors and transporters similar to the ones found in the external sensing organs (tongue, nose) and able to transduce signals controlling general metabolism and feeding in response to fat, amino acids, and carbohydrates (Miguel-Aliaga 2012). Despite the high level of both anatomical and functional conservation between the vertebrate and invertebrate intestinal systems, the identification of these receptors and transporters and the genetic analysis of their function in the invertebrate gut await experimental testing. Recent work by the Eaton group has shed new light on the role of Hedgehog (Hh), a molecule with recognized functions in tissue morphogenesis, as a metabolic hormone produced by the gut. Hh produced by midgut enterocytes can be found in the hemolymph attached to lipoproteins. Its production increases in response to nutritional deprivation, and circulating Hh is required for normal survival under starvation conditions. Circulating Hh signals to the fat body to decrease larval growth and promote TAG mobilization. It also signals to the PG to inhibit ecdysone production and delay pupariation (Rodenfels et al. 2014). These provocative findings implicate for the first time in the fly model the gut as a nutrient-sensing organ directly involved in adapting metabolism to the nutritional environment. Future work should decipher the mechanism of nutrient sensing taking place in gut cells leading to Hh production, as well as the role of Hh signaling in endocrine tissues relaying the nutritional input.

During recent years, skeletal muscle has also been recognized as a secretory organ, responding to changes in metabolic status by expressing myokines that communicate with and modify the status of other important metabolic tissues, like adipose, liver, β cells, or epithelia (Pedersen and Febbraio 2012). In flies, genetic interventions on skeletal muscles modify organismal growth and aging. Muscle-targeted inhibition of IIS, for instance, reduces muscle fiber size, but also organismal size (Demontis and Perrimon 2009). The possible myokines involved in this systemic control are not known. Recent work by the Perrimon group has identified myoglianin, a myokine with homology to human GDF11 and myostatin that remotely controls rRNA levels and the size of the nucleolus in other tissues. In adults, increased expression of myoglianin in skeletal muscles leads to p38 MAPK activation, reduced nucleolar function in fat body cells and a general increase in lifespan, whereas reduction of myoglianin expression has opposite effects (Demontis et al. 2014). Whether this myokine could participate in systemic growth control during larval stages is currently unknown.

The brain is also an important sensor of nutrients and the main site for the so-called “homeostatic control of food intake,” matching the quality and quantity of the food sources with organismal levels. In mice and flies, specific brain neurons evaluate various types of nutrients and energy balance through the use of sensory receptors, and control feeding behavior according to this nutritional information. So far, two types of internal sensors have been described in the fly brain. The gustatory receptor Gr43a senses both dietary fructose through taste neurons and circulating fructose in the hemolymph through a population of neurons present in the adult brain (Miyamoto et al. 2012). By sensing fluctuating fructose levels, the brain neurons promote feeding in hungry flies and suppress feeding in satiated ones. Besides carbohydrates, adult flies also sense amino acids and a deprivation of amino acids induces a change in their feeding preference (Ribeiro and Dickson 2010; Vargas et al. 2010; Toshima and Tanimura 2012). In larval brain, a specialized

circuitry consisting of three dopaminergic neurons senses hemolymph content in essential amino acids. Upon feeding on an imbalanced diet lacking essential amino acids, this dopaminergic circuitry is activated by a GCN2-dependent molecular mechanism. This activation, in turn, triggers feeding arrest, therefore allowing animals to seek a better food source (Bjordan et al. 2014).

Finally, recent work reveals a major role for the symbiotic gut flora in controlling organismal growth. In poor nutrient conditions, commensal flora becomes limiting and its depletion causes further reduction in larval growth rate through TORC1 inhibition in the fat body (Shin et al. 2011; Storelli et al. 2011). A reduction of ecdysone production by the PG is also observed, thereby affecting the duration of larval growth (Storelli et al. 2011). The nature of the signal linking the activity of the gut flora and TORC1 signaling in the fat body/PG axis is not known yet, but the undergoing systematic analysis of the microbiome should reveal specific bacterial genes that are beneficial in this symbiotic interaction.

Uncoupled Growth and Organ Sparing

Phenotypic plasticity refers to the ability of a single genotype to generate different phenotypes according to the environmental conditions. This plasticity is based on the differential responses of various tissues to environmental changes. During development, although changes in nutrient availability affect global body size, specific organs can be spared with respect to the rest of the body. Cheng et al. (2011) analyzed the growth response of various organs and tissues under nutrient restriction over the last 36 h of larval development (not affecting pupariation time). This study revealed that the larval body and endoreplicative tissues (fat body, salivary glands) do not grow during fasting, in contrast to the wing imaginal discs, which retain some growth activity, albeit to a lesser extent than under normal conditions. Finally, the central nervous system appears to be fully spared and to grow to normal size despite restrictive food conditions. Another study, using forced varia-

tions in global insulin signaling as a proxy for variations in nutritional conditions, revealed differential responses among imaginal discs, perturbing the normal allometric relationships that exist among organs (Shingleton et al. 2005). For example, whereas the wings and maxillary palps were reduced upon InR inhibition, the genital arches showed limited response. As a consequence, the inhibition of insulin signaling gave rise to animals with larger genitals relative to their wings and overall body size. Insulin sensitivity in different discs depends on the tissue-intrinsic levels of dFOXO activity. Low dFOXO activity in the genital disc could be responsible for minimizing reductions in organ growth on nutrient restriction or reduced insulin signaling in comparison to other organs (Tang et al. 2011).

How can insulin signaling still activate growth in these so-called “critical organs” upon nutrient deprivation when circulating Dilp levels are dramatically decreased? One hint could come from the finding that one of the Dilps, called Dilp6, is produced by the fat body in response to starvation (Slaidina et al. 2009). Up-regulation of Dilp6 by fat cells does not contribute to autocrine activation of IIS, because autophagy remains high in the fat body of starved animals. A deeper analysis revealed the presence of higher intrinsic dFOXO levels in fat cells than in wing imaginal discs, correlating with the increased sensitivity of this tissue to starvation. In addition, while decreasing Foxo dosage in fat cells increases their sensitivity to starvation, increasing Foxo in wing discs makes them more responsive to nutrient deprivation (M Slaidina, R Delanoue, and P Léopold, unpubl.). Together, these observations suggest a model whereby, on starvation, Dilp6 produced by fat cells compensates for the reduced secretion of brain Dilps and activates IIS in individual organs or tissues according to their intrinsic levels of dFOXO (Fig. 2).

Another way in which organs can escape starvation-induced growth inhibition is by switching their growth program from nutrient-dependent to nutrient-independent control. In young larvae, brain growth relies on the activities of TOR and InR signaling, and a

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restricted diet induces a drastic reduction in neuroblast proliferation and brain size. Once a developmental threshold called critical weight (see below) has been passed, however, nutrient restriction no longer delays the transition from larva to pupa and, interestingly, does not affect brain development and growth anymore. Normal brain development and growth is also observed in *tor* mutants, under conditions of limited amino-acid transport (inactivation of *slif*), or when systemic insulin levels are reduced. However, in these cases, downstream effectors of these pathways like PI3K/Akt and S6K/4E-BP are still required to control brain growth. Experiments have revealed that another membrane receptor with high sequence similarity to InR, called anaplastic lymphoma kinase (Alk), is highly expressed in the central nervous system and is responsible for activating PI3K signaling and phosphorylating 4E-BP during late larval stages (Cheng et al. 2011). During this period, Alk activity suppresses the requirement for TOR and InR, rendering neuroblast growth and proliferation independent of nutrients. Jeb (Jelly belly), the ligand for Alk, is highly expressed in glial cells, and the specific knockdown of Jeb in glial cells reduces the growth and proliferation of neuroblasts. The glial niche, therefore, provides short-range signaling that protects the neural lineage from nutrient deprivation. Interestingly, this study highlights functional parallels in the strategies used by developing organs and by tumors to evade nutrient limitation. Indeed, activating mutations in ALK have been found in childhood neuroblastomas, and IIS and constitutive PI3K activities have been found to be independent in cases of nutrient resistance in human cancers (Kalaany and Sabatini 2009).

INTERPLAY BETWEEN TISSUE GROWTH AND DEVELOPMENTAL TIMING

Final body size is determined both by the rate at which the constituting tissues grow and by the duration of their growth period. These two parameters need tight linkage to ensure that organisms reach their proper final size. As shown earlier, these parameters are controlled

at the organismal level by hormones, meaning that one prerequisite for understanding the determination of final size is to understand the functional interplay between these hormonal systems.

Critical Weight for Metamorphosis

Because *Drosophila* adult size is determined by the extent of growth that has been reached by the end of the larval period, the control of the transition from larva to pupa is critical for size determination. Work on bigger insects like the moth *Manduca sexta* has paved the way in this area, yielding interesting concepts concerning the control of this transition. In *Manduca*, a developmental threshold defines the weight at which starvation no longer delays entry into pupal development. Reaching this so-called “critical weight” (CW) constitutes an important but poorly understood transition that occurs soon after ecdysis marking the transition into the last larval instar (Davidowitz et al. 2003). If animals encounter poor nutrient conditions before reaching the CW, their development stalls and subsequent ecdysone pulses are delayed until an appropriate size is reached. Subsequently, when starved larvae are returned to food, pupariation is delayed, giving rise to pupae and adults of normal size. In contrast, once the CW has been passed, metamorphosis can no longer be delayed, and larvae exposed to limiting nutrients therefore develop into small adults. These findings obtained on the *Manduca* model have been supported by studies on the temporal requirements for insulin signaling in *Drosophila*. For example, using a temperature-sensitive allele of *InR* to switch general IIS on or off at different developmental timepoints, Shingleton et al. (2005) observed a developmental threshold beyond which IIS is no longer required to time the transition into metamorphosis. This threshold is analogous to the CW of *Manduca* in that it defines a larval size above which nutrition-responding pathways no longer control the timing of metamorphosis. In *Drosophila*, attempts to define a CW for molting have long been unsuccessful, owing to several differences with the *Manduca* model. First, the term

“critical weight” has often been given different meanings, because of the fact that it takes place concomitantly with other developmental checkpoints, like the minimal viable weight (a size below which animals cannot survive complete food withdrawal and never pupariate). Second, when starved above this developmental threshold, *Drosophila* larvae do not maintain a constant time to pupariation, but rather accelerate their development (Beadle et al. 1938; Stieper et al. 2008). Therefore, starvation modifies the time to pupariation both before and after the CW is reached and cannot be used to determine when the decision to pupariate is made. More recently, a graphical method called the “breakpoint method,” based on linear regressions of curves describing the time to pupariation as a function of weight, has been used to define the CW in *Drosophila* with good accuracy (Stieper et al. 2008). Under standard breeding conditions at 25°C, the CW (~0.9 g) is reached 8 h after the L2-L3 molt and coincides with a first low titer peak of ecdysone during the L3 instar. However, the mechanism by which this weight threshold is sensed and translated into hormonal outputs remains unknown. In the moth *Manduca sexta*, before the attainment of the CW, the secretion of PTTH and ecdysone is inhibited by the juvenile hormone (JH). Once CW is reached, however, the levels of JH esterase, an enzyme that inactivates JH in the hemolymph, increase abruptly. The titers of JH then decline, allowing the production of PTTH and ecdysone (Browder et al. 2001). In *Drosophila*, it is known that ablation of JH-producing cells is not sufficient to advance pupariation, nor can exogenous JH suppress it (Riddiford et al. 2010), indicating that JH is not involved in timing the onset of metamorphosis.

In *Manduca*, the CW of each instar is proportional to the initial weight of the same instar, suggesting that the size-sensing mechanism is related to a property set at the beginning of each instar (Nijhout et al. 2006). Interestingly, the volume of the tracheal network increases abruptly at each molt and is not modified among molts (Callier and Nijhout 2011). Although the respiration rate initially increases linearly with body size, it stays constant after

larvae have reached the CW. This suggests that the size of the tracheal system that is established during the preceding molt could become limiting during further body mass increase. The CW could therefore correspond to a body mass beyond which the oxygen supply becomes limiting for the tissue respiration rate. Consistent with this possibility, general hypoxia causes a reduction of the CW, although raising larvae in hyperoxic conditions is not sufficient to increase it (Callier and Nijhout 2011). Could a similar hypoxic response be involved in triggering pupariation in *Drosophila*? Interestingly, low oxygen conditions activate the production of NO (Wingrove and O’Farrell 1999), which is required for pupariation by promoting ecdysone biosynthesis in the ring gland (Cáceres et al. 2011). It is therefore interesting to speculate that, in *Drosophila* as in *Manduca*, the CW corresponds to a physiological threshold beyond which the respiration rate becomes limiting, resulting in the turning on of NO production and, consequently, the activation of ecdysone biosynthesis in the PG (Fig. 1). It should be noted that, in both *Manduca* and *Drosophila*, the CW is reached long before the larva attains its final size, and in *Manduca* it occurs even before the imaginal discs have started growing. Therefore, the CW is not likely to be a reliable indicator of final organ size, but might rather reflect a metabolic/energy threshold that larvae must reach to make it safely through the nonfeeding pupal period.

Insulin Signaling Promotes Ecdysone Synthesis in the Prothoracic Gland

Although the connection between CW and pupariation has not yet been clarified, several studies have highlighted a key role for insulin signaling in the regulation of ecdysone production in the PG (Fig. 3) (Caldwell et al. 2005; Colombani et al. 2005; Mirth et al. 2005). For example, ring gland-specific expression of an activated form of PI3K increases ecdysone levels and leads to animals with reduced size. Conversely, reducing PI3K activity in PG cells inhibits ecdysone production and increases larval growth. Although several groups have reported related ef-

In parallel to its role in promoting ecdysone synthesis, IIS promotes the growth of PG cells. This observation led to a proposal according to which the size of the PG serves as a coupling mechanism between body growth and the timing of metamorphosis (Mirth et al. 2005)—only above a certain size would the PG be competent to produce the peak of ecdysteroids that is required for pupariation. This view is challenged by multiple observations. For example, although overexpressing dMyc or cyclin D/Cdk4 in the PG increases its size, it does not affect the timing of pupariation (Colombani et al. 2005). In addition, overexpressing *bantam* in the PG leads to larger glands that produce less ecdysone because of the inhibitory role of *bantam* on ecdysone biosynthesis (Boulant et al. 2013). It, therefore, seems more plausible that IIS activates ecdysone production in the PG independently of its growth-promoting activity. At the same time, modifying IIS in the PG seems to affect the CW (Mirth et al. 2005), suggesting that IIS could participate in translating the assessment of CW into ecdysone production and pupariation.

Rather than participating in size sensing, IIS could coordinate the production of ecdysone with the availability of nutrients in the PG. In line with this idea, limiting food conditions rescues the effects of IIS inhibition in the PG. In the other direction, the body size reduction that is normally induced by forcing expression of IIS in the PG can be rescued by raising animals on rich food (Mirth et al. 2005). What is the physiological role of insulin signaling in this coordination, and how is it linked to the CW? In *Bombyx mori*, the expression of *torso*, the receptor for PTTH, is controlled by nutrients (Young et al. 2012). *torso* is expressed at high levels early in the last instar, and its expression drops when animals reach the CW (Saha et al. 2009). A similar decrease in *torso* expression at the CW has been observed in *Drosophila* (McBrayer et al. 2007). *torso* expression could then rely on insulin signaling until the CW is reached. Therefore, rather than timing pupariation, the insulin signal could act as a permissive factor for PG cells, making them responsive to PTTH. This possibility is supported by the

observation that, after the CW has been achieved and the endocrine cascade initiated, insulin is no longer required for ecdysone production and metamorphosis (Shingleton et al. 2005).

A Nutrient-Sensing Mechanism in PG Cells

In parallel to the role played by insulin signaling, an additional mechanism involving TORC1 directly couples nutrient sensing in the PG with ecdysone production and entry into metamorphosis (Fig. 1) (Layalle et al. 2008). Indeed, mild TORC1 inhibition in the PG leads to animal overgrowth as a consequence of prolonged larval development, whereas the growth rate of the animals remains unchanged. This indicates that the activation of TOR signaling is required for the production of ecdysone at the larval/pupal transition, a finding that had been suggested in early experiments measuring the activity of S6-kinase, a target of TOR, in purified ring glands of *Manduca sexta* (Song and Gilbert 1994, 1997). In the other direction, the activation of TOR signaling in the PG rescues ecdysone production as well as the developmental delay observed under poor nutrition. This contrasts with the effects that are observed on growth rates after manipulating IIS in the PG under similar conditions (Colombani et al. 2005). Therefore, although they are closely linked in their actions as growth inducers, the IIS and TOR-signaling pathways participate in distinct physiological responses of PG cells to environmental signals controlling ecdysone production. Under low nutrients, reduced TOR signaling in fat cells leads to low insulin signaling and a reduction in the general growth rate. As a consequence, the CW is reached only after a delay. Subsequently, once the CW has been passed, IIS no longer affects the developmental timing, but the reduced TOR activity in the PG cells prevents ecdysone production, further extending the larval growth period. Intriguingly, complete food withdrawal after the CW accelerates metamorphosis (Beadle et al. 1938; Stieper et al. 2008), indicating that, once the CW has been passed, other mechanisms act to advance pupariation under complete starvation conditions.

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The Systemic Inhibition of Animal Growth Rates by Ecdysone

A mild inhibition of IIS in the PG reduces the basal levels of ecdysone and leads to bigger larvae (Colombani et al. 2005). This overgrowth is accompanied by a reduction in the expression of ecdysone biosynthesis genes and can be rescued by feeding animals 20E, confirming that the phenotype is caused by reduced ecdysone levels. A close examination of larval growth parameters indicates that the basal levels of ecdysone do not act on the time of pupariation, but rather modulate body growth rate during larval development. Interestingly, general IIS activity is reduced when animals are fed ecdysone (Colombani et al. 2005). This inhibition of IIS can be rescued by silencing the ecdysone receptor (EcR) in fat cells (but not in any other larval tissue), indicating that the main target tissue of ecdysone signaling for systemic growth inhibition is the fat body (Fig. 3). In this tissue, activation of EcR signaling inhibits dMyc activity, a potent activator of systemic growth (Delanoue et al. 2010). The general activation of IIS that is observed upon adipose EcR inhibition suggests that there is an increase in circulating Dilp levels. Indeed, dMyc activity in the fat cells remotely controls Dilp release from the brain IPCs, although the molecular mechanism underlying this control remains unknown (Parisi et al. 2013). Therefore, the activation of ecdysone production by IIS in the PG could be part of a feedback mechanism that permits the integration of nutritional and metabolic inputs from the fat body. With such feedback, the attainment of a storage/metabolic threshold by fat cells would increase Dilp release from the brain by a mechanism involving fat body-derived factors (see above). This, in turn, would activate IIS in PG cells, making them competent to receive the PTTH signal and induce ecdysone production. In turn, high 20E at the end of larval development would feed back on the fat body, in which it would repress dMyc expression and inhibit systemic growth. Such a mechanism could contribute to the systemic growth arrest that is observed at the end of larval development. Because dMyc also acts downstream of

TOR signaling in the fat body (Teleman et al. 2008), both nutritional signals and ecdysone appear to converge on the regulation of dMyc in fat body cells to adjust organismal growth in response to both developmental and environmental signals.

Another form of cross talk between ecdysone and insulin signaling involves the miRNA *miR-8* (Fig. 3). *miR-8* is highly expressed in fat cells, in which it promotes IIS through the inhibition of its target *u-shaped* (*ush*), thereby influencing body growth rates (Hyun et al. 2009). Interestingly, the human miR-200 family, which is functionally homologous to the *Drosophila* *miR-8*, targets FOG2, a vertebrate ortholog of *ush*. Like USH in *Drosophila*, FOG2 prevents the activation of PI3K in human cells treated with IGF-1 by directly binding to p85 α , the regulatory subunit of PI3K, and by interfering with the formation of a functional PI3K complex. In flies, *miR-8* is repressed by ecdysone signaling, and its expression decreases during the third larval instar to reach a minimum level at the larval-pupal transition. Consequently, *ush* transcripts gradually accumulate during development (Jin et al. 2012). Perturbations of *miR-8* and *ush* expression abolish the ecdysone-mediated inhibition of body growth rates. In addition, the *miR-8/ush* module is required for ecdysone signaling to antagonize insulin signaling. These studies therefore provide a molecular mechanism by which ecdysone can suppress insulin signaling in fat cells. The miR-200 family of miRNAs seems to be also controlled by steroids in mammals, raising the possibility that the regulatory axis of steroid hormone/miR-200/insulin signaling is conserved throughout evolution. However, mild, “physiological” manipulations of IIS in the *Drosophila* fat body do not affect larval growth rates or pupal size (Colombani et al. 2003; Delanoue et al. 2010). In addition, restoring normal PI3K activity in fat cells does not rescue the size defects observed after manipulating EcR signaling in this tissue (Delanoue et al. 2010). This indicates that, in contrast to dMyc, IIS does not appear to be a relevant target of EcR signaling in fat cells for systemic growth control. This is somehow contradictory with the notion that *miR-8* controls

systemic growth downstream from ecdysone signaling by modulating adipose IIS (Hyun et al. 2009). Therefore, the most plausible role for IIS in the fat body would be to translate nutrient sensing into a general flux of metabolites, by regulating their storage and release from fat cells. Whether adipose IIS also participates in systemic growth control remains under debate.

A Growth/Maturation Checkpoint in Imaginal Tissues that Controls the Timing of Metamorphosis

Both developmental (maturation of imaginal tissues) and environmental (nutrition) cues feed into ecdysone production and determine the duration of larval growth (Fig. 1). Early experiments established that metamorphosis is delayed when imaginal tissues are damaged by genetic- or X-ray-induced cell death (Russell 1974; Simpson and Schneiderman 1975; Simpson et al. 1980; Parker and Shingleton 2011), or when imaginal disc growth is slowed down by reducing ribosomal protein content (Stieper et al. 2008; Parker and Shingleton 2011). This delay in development allows damaged tissues to regenerate and to grow to the appropriate size after injury. However, the elimination of entire imaginal discs does not alter the timing of metamorphosis (Simpson et al. 1980), suggesting that discs that have not completed a certain amount of growth inhibit the transition to maturation, and that in the absence of discs this transition can be timed by a distinct, redundant feedback mechanism. Importantly, pupariation is also inhibited under mutant conditions in which discs do not stop growing at the end of the larval stage and instead overgrow beyond normal size (Sehnal and Bryant 1993). Both regeneration and overgrowth generate extra cell proliferation, suggesting that proliferating disc cells produce a signal that prevents ecdysone release. Thus, there is a direct relationship between the growth of the imaginal discs and the timing of pupariation. In one study, close examination revealed that damage induced in first or second instar larvae does not delay early molts (Halme et al. 2010). This observation

revealed the existence of a developmental checkpoint acting during the third-instar stage, which reflects the varied abilities of damaged discs to regenerate during larval development. Damage inflicted before the checkpoint induces tissue repair, whereas lesions occurring in imaginal tissues after the checkpoint no longer repair and give rise to adults with reduced organ size and patterning defects. Mutations in genes encoding components of the retinoid biosynthetic pathway can partially rescue this damage-induced delay and restore the normal timing of *ptth* transcription, indicating that retinoids or their metabolites could function as a signal for this mechanism (Halme et al. 2010).

Two recent studies have identified Dilp8, a previously uncharacterized secreted factor related to the ILP family, as a signal produced by growing discs that inhibits entry into metamorphosis (Colombani et al. 2012; Garelli et al. 2012). In the first study, a disc-related neoplastic growth-induced developmental delay was used in a genome-wide screen to identify genes whose disc-specific knockdown could rescue the delay at pupariation. Examining microarrays from various eye tumors, Garelli and colleagues found *dilp8* as a highly enriched gene encoding a secreted peptide. Dilp8 is produced by imaginal disc cells in response to tissue damage or growth perturbation. It is not clear yet how Dilp8 acts to block pupariation. PTTH expression is only slightly delayed by Dilp8 overexpression, although the ecdysone response is abolished. This suggests that Dilp8 could act at the level of PTTH translation, release by PTTH neurons, and/or ecdysone production in PG cells. The elucidation of this mechanism will require the identification of a bona fide Dilp8 receptor. Normal *dilp8* mRNA levels drop in mid-third instar, which is likely a prerequisite for the transition to pupal development, as overexpressing *dilp8* in imaginal discs is sufficient to delay pupariation and to produce bigger adults. *dilp8* loss of function, however, does not lead to early metamorphosis. These observations indicate that the down-regulation of *dilp8* expression constitutes a permissive event for the occurrence of metamorphosis,

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but that the onset of this process requires additional signals.

One striking observation comes from older regeneration and transplantation studies. When growth is impaired in a subset of imaginal discs, intact discs do not generate organs of larger size, even though they benefit from an extended period of growth (Simpson et al. 1980). Therefore, additional mechanisms must be at work, which act to maintain the correct final proportions among organs emerging from damaged or undamaged discs. A recent reevaluation of these early experiments has revealed that unaffected discs are able to align their growth rates to the slower ones (Parker and Shingleton 2011). Under these conditions, animals fed with ecdysone present a reduction of the developmental delay and a disruption of disc growth coordination, suggesting that ecdysone plays a role in this process. Interestingly, ectopic expression of Dilp8 in discs reduces their growth rate, suggesting that Dilp8 could play a role there as well. One possible scenario could be that, on perturbed growth, Dilp8 produced by slow discs inhibits the production of ecdysone, which is needed at basal levels for optimal disc growth (Nijhout and Grunert 2002; Delanoue et al. 2010). As a consequence of the loss of ecdysone, general disc growth would be reduced and coordination could occur. Alternatively, Dilp8 could serve as an endocrine growth inhibitor produced by slow-growing tissues, acting remotely on intact tissues independently of ecdysone. Again, a better understanding of these fascinating coordination mechanisms will benefit from the identification of a Dilp8 receptor and its downstream signaling pathway.

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