Suppression of food intake and growth by amino acids in *Drosophila*: the role of *pumpless*, a fat body expressed gene with homology to vertebrate glycine cleavage system

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SUMMARY

We have isolated a *Drosophila* mutant, named *pumpless*, which is defective in food intake and growth at the larval stage. *pumpless* larvae can initially feed normally upon hatching. However, during late first instar stage, they fail to pump the food from the pharynx into the esophagus and concurrently begin moving away from the food source. Although *pumpless* larvae do not feed, they do not show the typical physiologic response of starving animals, such as upregulating genes involved in gluconeogenesis or lipid breakdown. The *pumpless* gene is expressed specifically in the fat body and encodes a protein with homology to a vertebrate enzyme involved in glycine catabolism. Feeding wild-type larvae high levels of amino acids could phenocopy the feeding and growth defects of *pumpless* mutants. Our data suggest the existence of an amino acid-dependent signal arising from the fat body that induces cessation of feeding in the larva. This signaling system may also mediate growth transition from larval to the pupal stage during *Drosophila* development.

Key words: Growth, Feeding, *pumpless*, Amino acid, Fat body, *Drosophila*

INTRODUCTION

Growth and nutrient intake are functionally linked processes in development. During embryogenesis of most animals, nutrients required to support growth are mainly provided maternally. By contrast, growth and development during the postembryonic stages require the organism to actively take in nutrients from the environment. This demands that animals recognize when there is shortage or excess of nutrients in the body and to translate this information into specific alterations in feeding and other behavioral responses. The system of energy homeostasis must also cope with the changing growth demands during the different phases in the life cycle of the organism, from bringing about size increase during the juvenile stage, to initiation of major developmental transitions such as metamorphosis in amphibians and insects, and for maintaining constant size in adults. In addition, specialized regulatory responses must exist to maximize survival under different environmental conditions. During starvation, animals need to conserve energy and alter their foraging habits. In environments where animals are confronted with times of prolonged food shortage, many adopt specialized non-feeding lifestyles with alterations in body metabolism, such as diapause in invertebrates and hibernation in mammals (Wilson et al., 1978; Campbell, 1990). The molecular mechanisms that coordinate body growth pattern with food intake response in multicellular organisms are largely unknown.

The *Drosophila* larva offers several features that makes it a favorable system for studying the mechanisms underlying food intake and growth control. (1) The rapid growth rate, with the larvae growing many times its size in a few days, and continuous feeding, enable defects in feeding and growth to be rapidly monitored. (2) *Drosophila* larvae stop feeding and initiate pupation at a very specific time after hatching, and this can be used as a developmental transition point with which to assay growth pattern alterations (Riddiford, 1993). (3) A rich body of classical studies that exists for a variety of insects on the anatomic basis underlying growth control (Bodenstein, 1953; Pflugfelder, 1958). (4) The internal feeding organs are visible from the outside, making it possible to efficiently monitor food intake in live animals. We have carried out a genetic screen to isolate larval mutants that are defective in food intake. In this paper, we describe one of these mutants, named *pumpless* (*ppl*). Our results suggest that *ppl* is involved in mediating food intake suppression in response to amino acids, and may be part of a systemic signaling system that
regulates growth transition from the feeding larval to the non-feeding pupal stage of development.

MATERIALS AND METHODS

Phenotypic and genetic characterization

Flies were allowed to lay eggs on apple juice agar plates with yeast paste containing Carmen Red dye. The stocks were maintained either over TM3,Sb or TM6B,Tb; the latter allows homozygous mutant animals to be identified at the larval stage. The fly stocks Df(3L)ME1325, Df(3L)Pc-cp1, ppl-78Cb1, ppl-78Cb3 and ppl-00217 were kindly provided by A. T. C. Carpenter. Excision of the P elements was carried out by introducing a source of transposase using standard genetic crosses. Excision of ppl-06913 did not revert the lethality, consistent with the subsequent results showing that ppl-06913 transposon is associated with a chromosomal deletion. Excision of ppl-00217 showed a 22% reversion rate (8 viable lines from 36 total excision lines). Transgenic flies carrying the rescue construct were made by cloning a 10 kb genomic fragment containing all of the GCS transcribed region into a pCaSpeR 4 vector (Thummel and Pirotta, 1992) and transforming into the fly germline (Spradling, 1996). The rescue was carried out for two ppl alleles, ppl-06913 and ppl-00217, with two independent transposon insertions, termed P(rescue), on the second chromosome. Flies of P(rescue+)/+; mutant chromosome/TM3 Sb were crossed inter se and scored for appearance of Sb+ flies. For ppl-00217, 73 Sb+ and 272 Sb flies were recovered. As the P(rescue) is homozygous viable, the expected number of Sb+ progeny is (3/4)·(272) = 102. For ppl-06913, 7 Sb+ and 436 Sb were recovered from the equivalent experiment. These Sb+ flies, however, were lethargic, did not fly about and died after a few days. We have never observed any Sb+ escapers in a stock of ppl-06913. To analyse the larval phenotype of the rescued animals more carefully, another set of rescue experiments was performed on ppl-00217. A stable stock was established with the genotype P(rescue)/P(rescue); 6913/TM3Sb and these were crossed inter se. A small number of the larvae initially showed the feeding defect but most of these could swallow the food and grew relatively normally afterwards, reaching the late pupal stage. All of these were Sb+ as could be seen through hand dissection out of the pupal cases. As with earlier rescue results, a few could eclose.

Molecular and histochemical analyses

The DNA fragment flanking the transposon ppl-00217 was isolated by plasmid rescue and mapped within the Polycomb genomic walk (kindly provided by Renato Paro). Genomic fragments from both sides of the plasmid insertion site were used to isolate cDNAs from a Stratagene 2-14 hour cDNA library, using standard molecular techniques (Sambrook et al., 1989). Both the cDNAs and the corresponding genomic regions were sequenced. For PCR mapping, approximately 70 homozygous mutant larvae were used for isolation of genomic DNAs. All primer sequences and PCR conditions are available upon request. Northern analysis was performed with 10 μg of total RNA and probed with a 1.6 kb genomic fragment containing the GCS coding sequence, a full-length Pepek cDNA, or a PCR product from exon 2 of lipase 3.

Whole-mount in situ were performed using sense and antisense RNA probes using a 1.6 kb genomic fragment used in the northern analysis (Tautz and Pfeifle, 1989). For larvae, the samples were sonicated (Patel, 1994), then processed as with the embryos. For Sudan Black staining of the fat body, larvae were dissected, fixed in formaldehyde (1:10), washed with PBS, and 50% EtOH. Sudan Black solution (0.05% Sudan Black in 70 % EtOH) was added for 2 minutes, then washed with 50% 25% EtOH, PBS, and mounted in 50 % glycerol/PBS. For fat body nuclei, the ppl-00217 line, which harbors a lacZ construct with a nuclear localization signal, was crossed to wild-type flies and the ppl-00217/+ larvae were stained with standard X-gal procedure.

RESULTS

Assay for larval feeding mutants

The foregut can be considered the larval organ of food intake (Fig. 1; Strasburger, 1932; Skaer, 1993). It consists of the pharynx, the esophagus and the proventriculus, and is innervated by the enteric nervous system (Hartenstein et al., 1994). The sequence of food intake in Drosophila larva is highly rhythmic. Food is sucked into the mouth atrium by contraction of the large dorsal pharyngeal muscles, pumped into the esophagus and carried through the proventriculus into the midgut (Fig. 1C). Our assay for isolating larval feeding mutants is to feed the animals yeast paste containing red dye and to monitor the movement of food along the foregut. With this screen, we have identified a number of genes that are required for proper food intake (Pankratz, unpublished data). These have been placed into two operationally defined classes. One class of mutants shows clear morphological defects in the feeding apparatus (Pankratz and Hoch, 1995; Tetzlaff et al., 1996). The other class, including ppl, shows no such defects.

Food intake and growth defect of ppl larvae

The original ppl mutant, ppl-06913, was isolated by screening a collection of P-element-induced lines (Karpen and Spradling, 1992). In wild-type larvae, the midgut becomes clearly red after a short time of feeding on red yeast; there is no accumulation of food material in the pharynx or the proventriculus (Fig. 2A). By contrast, ppl-06913 larvae show food accumulation in the pharynx (Fig. 2B) and only very little in the midgut, indicating that the food is not passing into the esophagus. This is not due to a physical blockage in the foregut since cutting the mutant larva in half and gently agitating the head causes the food to flow into the esophagus (Fig. 2C-E). Further phenotypic analysis revealed several additional properties of ppl mutants. First, the feeding defect is observed...
starting late first instar stage. In the initial 12 hours after hatching (early first instar), no ppl-06913 mutants can be observed with food accumulation in the pharynx. In the next 12 hours (late first instar), several variations on this feeding phenotype can be seen (Fig. 2F), which most likely reflects a time course of the phenotypic progression. Larvae can be seen in which the red food is observed mostly in the midgut and some in the pharynx. The food then accumulates more in the pharynx and less in the midgut. One finally observes mutant larvae in which the food is no longer present in the midgut and are moving about with food only in the pharynx. There is some variation in the size of the ppl-06913 larvae that show these feeding defects, most likely reflecting differences in the time when the animals lose their ability to pass food into the esophagus. This feeding defect is accompanied by a drastic reduction in growth (Fig. 2G). Majority of these larvae die within about 3 days after hatching with the food still in the pharynx. About a third of the ppl-06913 with the food accumulated in the pharynx do succeed in swallowing their food, and these invariably grow slightly larger than those that have not swallowed their food; however, these also die as larvae without further growth.

Based on complementation analysis, several other alleles of ppl were identified (Russell et al., 1996). ppl-00217 shows a significant reduction in growth, with the body size being approximately the same as the bigger class of ppl-06913 mutants (Fig. 2G). ppl-00217 therefore is most likely a weaker allele than ppl-06913, ppl-78Cb3 and ppl-78Cb1 show a slight difference in the feeding defect. Both can feed upon hatching. They then gradually stop feeding, as seen by the disappearance of food in the midgut; however they do not show food accumulation in the pharynx. These variations in the phenotype may reflect differences in the strength of the alleles and the exact point in the deglutition cycle at which food intake is halted. We have focused our analysis on ppl-06913, as this represents a null allele (see below).
**ppl mutant larvae do not display a typical starvation response**

In addition to the cessation of food intake, *ppl* larvae display another interesting food response. Wild-type first instar larvae feed continuously and essentially remain buried in the food the entire time, rarely straying far from the food source; when these are deprived of food for a while and then placed near a food source, they will quickly move towards it. By contrast, *ppl* mutants move away from the food source and start wandering about (Fig. 2H); some leave the food, wander about, then return again, and this cycle can be repeated (not shown). The movement of the body *per se* is indistinguishable from wild type, and the mutants move about with the same rigor, respond to touch, and can right themselves rapidly when turned upside down as in wild-type animals (unpublished observations).

These observations suggested that *ppl* larvae, which are essentially not feeding, are not showing a typical starvation response. To further investigate this, we searched for molecular markers that would be regulated by starvation. We found two such markers, phosphoenolpyruvate carboxykinase (Pepck) and lipase 3 (Lip3). The *Drosophila* Pepck gene encodes an enzyme that is highly homologous to the mammalian PEPCK that catalyzes the rate-limiting step of gluconeogenesis (Gundelfinger et al., 1987). It is known that the PEPCK gene in mammals is regulated at the transcriptional level by nutritional signals (Hanson and Reshef, 1997). The *Drosophila* Lip3 gene shows significant homology to the acid hydrolase lipases (Pistillo et al., 1998), which is a member of a larger class of lipases that are involved in lipid metabolism (Warner et al., 1981). We first wanted to determine how these genes would be regulated upon starvation. As seen in Fig. 3A, both genes show a constant level of expression during second instar larvae, with both increasing slightly at the end of second instar; upon starvation, however, both genes were highly upregulated (Fig. 3B). We then asked how these genes would be regulated in *ppl* larvae. As shown in Fig. 3B, neither of these genes were upregulated. Taken together, these data indicate that the lack of food intake and growth in *ppl* larvae is not accompanied by a normal physiologic response to starvation.

**Molecular characterization of ppl**

The *ppl* locus was initially mapped using deficiency chromosomes to a region near the *Polycomb* locus at chromosomal position 78C on the left arm of the third chromosome (Russell et al., 1996). Two overlapping deficiencies, *Df(3L)ME1325* and *Df(3L)Pc-cp1* (Fig. 4A), in *trans*-heterozygotes as well as over *ppl-06913*, showed the same larval feeding phenotype as *ppl-06913* homozygotes (data not shown), thus delimiting the *ppl* locus to a 15 kb region. The P-element line *ppl-00217* maps within this 15 kb region and is lethal over *ppl-06913*. Excision of the *ppl-00217* transposon reverted the lethality, indicating that the P element is responsible for the lethal phenotype. Two different classes of cDNAs were isolated flanking the *ppl-00217* insertion point (Fig. 4A), one encoding acetyl coA synthetase (AcS) (Russell et al., 1994) and other encoding a glycine cleavage system subunit (GCS). *ppl-00217* has inserted between the two genes, 32 bp upstream of one of the alternatively spliced transcripts of AcS and 451 bp downstream of GCS. Molecular mapping with PCR using a series of primers derived from this genomic region (Fig. 4B–E) indicated that the original *ppl-06913* mutation deletes both the AcS and GCS transcription units. Another allele, *ppl-78Cb1*, has a chromosomal inversion whose proximal breakpoint was previously mapped to this genomic region (Russell et al., 1996). This breakpoint was narrowed further by PCR mapping to a 400 bp region at the 5′ end of the GCS gene (Fig. 4B–E). A 10 kb genomic fragment containing the GCS gene rescues the *ppl* phenotype and its associated lethality (Fig. 4F; see also Material and Methods). Taken together, these results indicate that the GCS gene corresponds to the *ppl* locus. The GCS gene encodes a protein of 165 amino acids (Fig. 5A) which has high homology to the vertebrate protein H subunit of the glycine cleavage system (Yamamoto et al., 1991; Fujiwara et al., 1991). This enzyme complex is involved in the breakdown of glycine into ammonia, carbon dioxide and one-carbon unit (Mathews and van Holde, 1990), suggesting that *ppl* functions in amino acid metabolism.

**ppl is expressed specifically in the fat body**

In situ hybridization shows that *ppl* is expressed exclusively in the fat body of developing embryos and larvae (Fig. 5B–E). The fat body is the major organ of metabolic regulation and energy storage in insects and can be viewed as performing the combined function of the liver and adipocytes of mammals (Rizki, 1978). *ppl* expression level increases during the first and second instars, but decreases near the end of third instar (Fig. 5F). We did not detect any expression of *ppl* in *ppl-06913* mutants (Fig. 5G), further indicating that this is a null allele. As many genes are downregulated during starvation, and since *ppl* larvae are not feeding, there was the possibility that our

![Fig. 3. The starvation marker genes Lip3 and Pepck are not upregulated in ppl mutants.](image-url)
inability to detect ppl transcripts was due to its downregulation. Although ppl was indeed downregulated upon starvation of wild-type larvae, we could still detect significant ppl expression (Fig. 5G).

Since ppl was expressed specifically in the fat body, we examined ppl mutants for defects in this organ. The morphology of the fat body is highly dependent on the nutritional state of the larva. In normal well-fed larva, the fat body is enriched in lipids, as seen by Sudan Black histochemical staining (Fig. 6A,C). When larvae are starved, lipid becomes undetectable in the fat body (Fig. 6B,D). In ppl mutant larvae, one can still detect lipids in the fat body (Fig. 6E,F). It should be noted that, since the mutant larvae are not feeding, they have less fat than wild-type larvae of the same age. In ppl larvae that have grown slightly bigger because they were successful in swallowing food (see Fig. 2G), there is a corresponding increase in the lipid staining of the fat body (Fig. 6G, arrowhead). The breakdown of lipids can be prevented by addition of sugar (Fig. 6H), and since larvae grown on sugar do not grow in size (see Fig. 7A; Britton and Edgar, 1998), these were also used to compare with ppl larvae. These larvae showed comparable staining in the fat body as some of the ppl mutants (Fig. 6G,H). Furthermore, when ppl mutants are completely starved for food by removing them to a wet filter paper, they eliminated their lipid contents (not shown). It should be noted that despite the breakdown of lipids during starvation, the fat body itself remains intact as can be seen by staining of the fat body nuclei (Fig. 6I,J). These results indicate that ppl larvae still have fat bodies, and can accumulate and breakdown lipids depending on nutritional conditions.

Feeding high levels of amino acids can lead to phenocopy of ppl growth and feeding defects

As ppl encodes an enzyme that may be involved in glycine catabolism, we asked whether feeding high levels of glycine to normal larvae would also lead to similar growth and feeding defects as ppl mutants. Therefore, we fed wild-type larvae food mixed with different concentrations of glycine. At 0.1 M added...
glycine, no effect on growth was observed, whereas 0.4 M glycine led to significant growth retardation (Fig. 7A). Larvae fed high glycine diet also pupariated at significantly later times (Fig. 7B), which also depended on the concentration of glycine. Eclosion was also delayed corresponding to the delay in pupariation, indicating that the retardation of growth occurs during the larval stage. These effects were completely reversible, since taking the animals out of high glycine food significantly improved growth to nearly wild-type levels within two days (Fig. 7C).

**Fig. 5.** *ppl* encodes a glycine cleavage system subunit and is specifically expressed in the fat body of embryos and larvae. (A) Amino acid sequence of *pumpeless* gene product derived from cDNA and genomic sequences; the protein has high homology to human and chick glycine cleavage system subunit H. The stars above indicate residues which are identical in fly, human and chick sequences. (B-E) In situ hybridization pattern of *ppl*, showing exclusive staining in the fat body (arrowheads) in stage 15 (B,C; different focal planes) and stage 17 (D) wild-type embryos, and larva (E). (F) Developmental northern analysis from whole larvae probed with *ppl* gene; *ppl* expression decreases in the third instar stage (103 hours); the numbers on top indicate the age of the larvae in hours after egg laying. (G) *ppl* expression in starved wild-type larvae and in *ppl*-06913 mutant larvae: the expression is decreased in starved larvae and is absent in *ppl*-06913.

**Fig. 6.** Sudan Black lipid staining in the larval fat body of *ppl* mutants. (A) 66 hour wild-type larvae; note the dark blue staining in the fat body (arrowhead). (B) 66 hour wild type after starvation for 24 hours (i.e., the animals were starved starting at 42 hours); there is no staining above background. (C) Same condition as A but at larger magnification. (D) Same condition as B but at larger magnification; the darker blue staining structure is brain and the ventral cord. (E) *ppl* larvae (42 hours), arrowhead points to fat body staining. (F) Close up of E in the position of the arrowhead. (G) *ppl* larvae (66 hours) that have swallowed their food and have become slightly bigger. (H) Wild-type larvae (66 hours) grown with only saline and sugar for 24 hours (i.e., the animals were placed in sugar solution starting at 42 hours); these do not grow, but still keep lipid contents in the fat body (arrowhead). (I) X-gal staining of larvae (42 hours) carrying one copy of the *ppl*-00217 transposon; the staining is nuclear due to a nuclear localization signal in the *lacZ* gene. (J) Same as I, but after 24 hour starvation; note the difference in texture where the starved fat body cells appear tauter and thinner. In this case, the total age of the animal is 66 hours, as compared to 42 hours shown in I. This was done to keep the overall size of the larvae the same.
into normal food resulted in resumption of the normal growth rate (data not shown). These results indicated that high levels of dietary glycine can significantly reduce growth.

We showed earlier that Pepck and Lip3 are upregulated in starved wild-type larvae, whereas no upregulation was observed in ppl mutants. We therefore asked whether glycine or other nutrient components would affect the transcription of these genes. When wild-type larvae were raised in saline solution plus sugar, Lip3 was downregulated completely (Fig. 7C). Pepck expression, on the other hand, was downregulated but not to the same extent as that of Lip3. Adding glycine plus sugar resulted in a greater suppression than sugar alone, restoring Pepck expression almost to the basal level. Glycine alone had a similar effect as sugar alone. This indicated that Lip3 expression is completely dependent on sugar, whereas Pepck expression is dependent on both sugar and glycine.

We then wanted to see whether the growth-retarding effect could be brought about by other amino acids. As shown in Fig. 7D, lysine had an even greater effect on growth retardation than glycine, whereas serine, alanine and proline had less of an effect. Lastly, we asked whether we could also phenocopy the specific food intake defect of ppl-06913 larvae in addition to the growth defects. Of the five amino acids tested, we could phenocopy with one of these, lysine, the characteristic accumulation of food in the pharynx (Fig. 7E). The fact that we could phenocopy the specific pharynx phenotype with lysine but not with glycine may be due to the fact that exogenously feeding high amino acids leads to similar but not identical outcomes as endogenously preventing amino acid breakdown. In this respect, we do not know if, in ppl mutants or in wild-type animals fed high amounts of amino acids, there is a steady-state accumulation of glycine or other amino acids in the body; it could also be that they are rapidly converted to another metabolite. A detailed biochemical characterization of ppl mutants, such as measuring the amino acid levels in the larvae, should provide insights into this issue. Taken together, these data indicate that, although the ppl mutation appears to be affecting glycine metabolism, the ability to depress growth and food intake is not glycine specific but is shared by different amino acids.

**DISCUSSION**

The mechanisms that coordinate food intake with organismal growth are poorly understood. We have provided evidence that mutation in ppl results in cessation of food intake in Drosophila larvae, ppl is expressed specifically in the fat body and the gene product shows homology to vertebrate enzymes that catabolize the amino acid glycine. In general, mutants that
do not feed are expected to have growth defects and an animal may not feed for a variety of reasons. Therefore, it was important to discern whether the feeding defect of ppl mutants was a primary effect, or whether it was a secondary effect due to a general ill-health of the mutant. A major criterion that we have used to distinguish between these possibilities was to ask when the feeding phenotype can be first observed relative to the other behavioral abnormalities that might be apparent in the mutants. We can summarize the progression of the ppl mutant phenotype as follows. There is at the beginning no discernable defect in feeding, and it is impossible to distinguish the wild type from mutants shortly after hatching. During late first instar, the feeding phenotype is apparent as seen by the accumulation of food in the pharynx. As the food intake defect becomes more prominent, the mutant larvae begin leaving the food and start wandering about. These observations indicate that the food intake defect in ppl mutants precedes the appearance of general lethargic characteristics and suggest a primary defect in the feeding response.

Central versus peripheral relay of nutritional signals controlling growth

The path from intake of nutrients to alterations in feeding response requires the coordinate functioning of many organ systems. The fact that ppl is exclusively expressed in the fat body suggests that the fat body may be an important relay point in conveying nutritional signals that regulate food intake. What other organs might be involved? It is helpful to address this issue in light of classical studies performed on various insects (Bodenstein, 1953; Pflugfelder, 1958). These studies have provided a conceptual framework in which interacting central and peripheral organ systems communicate through humoral factors to regulate growth and development: neurosecretory cells of the brain send signals to the endocrine organs, causing them to release hormones that act on target tissues. The key point for our current analysis is that the activity of the neurosecretory cells in the brain, and thus the triggering of the relay system, is dependent on nutritional cues. For example, it has been shown in Rhodnius (see Bodenstein, 1968 for summary) that molting occurs at a specific time after a blood meal and that only one meal is needed for each stage. If the brain is removed, molting took place only when a certain time had passed since the feeding. Interestingly, it has been recently reported that a fat body derived mitogenic signal which is dependent on amino acids controls neuroblast proliferation in the brain (Britton and Edgar, 1998). Thus, the effect of amino acids on food intake that we observe may also be mediated through the brain (Fig. 8).

These findings can be seen in the context of molecular studies in rodents on feeding and body weight regulation. The product of the mouse obese gene, leptin, is secreted by the adipocytes and acts on the brain as an afferent signal to regulate food intake (Flier, 1995; Friedman, 1997). Although there is no evidence that a leptin homolog exists in Drosophila, the underlying logic in the interplay of central and peripheral organs in the production and relay of nutrient-dependent signals may share similarities. In this respect, it has been shown that extirpation of the ring gland (the master neuroendocrine organ of Diptera insects) in Drosophila hydei resulted in increased size of the fat body (Vogt, 1947). This result is strikingly reminiscent of studies in mammals in which destruction of specific part of the hypothalamus led to obesity (see Friedman, 1997).

Premature cessation of food intake and precocious initiation of wandering response in ppl mutants

Although ppl mutants are not feeding, they do not display the typical response characteristics of wild-type animals that have been deprived of food. When wild-type larvae are starved specific behavioral and physiologic responses are invoked, including the transcriptional upregulation of Pepck and Lip3. This is accompanied by a drastic reduction in the size of the fat body since the stored lipid is broken down. In addition, when larvae that have not been fed are now presented with a food source, these animals will move towards the food source and remain there, ppl mutants, although they do not feed, are not growing and die about the same time as starved larvae, do not show these response. Pepck and Lip3 genes are not upregulated, the fat body still stores lipids and the mutant larvae wander away from the food source. This indicates that the starvation response is suppressed in ppl mutants. Although we do not know how this could be effected, the wandering movement that ppl larvae display provides a hint, in that the behavior is reminiscent of wild-type larvae at late third instar larvae. Wild-type larvae essentially feed continuously up until the late third instar stage. A short time before pupariation, however, in a process that is dependent on the hormone ecdysone, they leave the food and start wandering about the surrounding in what is termed the wandering stage (Riddiford, 1993). During this period, they empty their gut and do not feed, and the lipids in their fat body are not broken down. These observations reveal that ppl mutants, as first instar larvae, share several characteristics of non-feeding third instar wild-type animals and suggest that some aspects of the ppl mutant phenotype may be due in part to a premature activation of a developmental program that is normally activated shortly prior...
to pupariation. Therefore, ppl gene may be involved in mediating the normal sequence of events, including those that are responsive to ecdysone, that lead to pupariation.

**ppl gene and size control**

Under optimal conditions, *Drosophila* larvae pupariate within a narrow range with respect to both time and body size. However, restricting food intake during the larval stage can greatly alter the timing of pupariation as well as the size of the pupa and adult (Beadle et al., 1938). In addition, removal of the corpora allata (a major endocrine organ which, in *Drosophila*, is part of the ring gland) during earlier larval stages in a variety of insects leads to premature pupariation, and, correspondingly, very small pupae and adults (Bodenstein, 1953; Pflugfelder, 1958). These classical studies suggest that systemic signals can regulate body size in response to nutrient availability. Interestingly, heteroallelic combinations of insulin receptor mutants (Chen et al., 1996), as well as mutants of the *chico* gene, which encodes a homolog of a vertebrate insulin receptor substrate (Böhni et al., 1999), show eclosion delay and have small body size. Furthermore, the fat body produces growth factors that act with insulin to promote proliferation of imaginal disc cells (Kawamura et al., 1999). Since ppl gene appears to function in the fat body in the production of a systemic signal that affects food intake and timing of pupariation, it is possible that ppl interacts with genes in the insulin signaling system to regulate body size.

**Amino acids as regulators of feeding behavior and growth**

As protein biosynthesis drives growth (Hartwell, 1994; Nasmyth, 1996), it is not surprising that amino acids regulate nutrient intake in a variety of organisms. In yeast, amino acids have been shown to control diverse cellular processes that have direct connection to nutrient availability, such as autophagy, pseudohyphal formation and cell growth (Gimeno et al., 1991; Noda and Ohsumi, 1998; Schmidt et al., 1998). In *Dictyostelium*, ammonia, which is a common catabolic product of all amino acids, is known to be a signaling molecule that controls food response behavior and development (Schindler and Sussman, 1977; Davies et al., 1993). In hydra, which possess a nerve net that is concentrated around the mouth, specific feeding response can be elicited by the amino acid tyrosine (Blanquet and Lenhoff, 1968). Amino acids have also been implicated in appetite suppression. It has been shown in rats, for example, that direct injection of amino acids into the hypothalamus reduced food intake (Panksepp and Booth, 1971). In *Drosophila* larvae, which are continuous feeders and may not be subject to short term, periodic feeding controls, amino acids could act as a nutrient signal for progressing to the non-feeding pupal stage. As amino acids have such diverse biochemical roles in the body (Fig. 8), we do not know which pathway might be involved in regulating feeding response. However, these considerations bring to mind the discussion on the origin of hormones and intercellular signaling by Tomkins (1975), who suggested that neurotransmitters, which are in many cases amino acid derivatives or are themselves amino acids, may have initially evolved for transducing information related to amino acid accumulation.

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**REFERENCES**


